Dynamics of progesterone receptor interactors in breast cancer cells upon hormone exposure

Jofre Font Mateu

TESI DOCTORAL UPF / 2016

DIRECTOR DE LA TESI

Dr. Miguel Beato

Chromatin and gene expression laboratory

GENE REGULATION, STEM CELLS AND CANCER PROGRAM

CENTRE DE REGULACIÓ GENÒMICA (CRG)

Acknowledgments / Agraïments

Durant tots aquest anys al CRG he conegut molta gent, i de tots n'hi ha una mica en aquesta Tesi, però hi ha gent a la que m'agradaria mencionar especialment.

Primer de tot, el Miguel. Recuerdo la primera vez que nos entrevistamos para el trabajo de técnico en tu laboratorio, me dijiste dos cosas importantes; la primera, me preguntaste qué planes tenía para el futuro, y la segunda, que si me veía capaz de hacer el trabajo, el puesto era mío. Creo que hemos superado con creces, tanto mis planes como los tuyos para conmigo, y te tengo que agradecer que me apoyaras y creyeras en mí en todas las distintas etapas que hemos recorrido juntos, sobretodo cuando me propusiste hacer la Tesis, y el respaldo durante todo esto tiempo y la tenacidad para que entendiera lo que estábamos haciendo en cada momento. Pero los planes nunca terminan! En segundo lugar estamos los viejos del lugar, Guille y Silvina, con los que hemos vivido ya muchas cosas, y lo que nos venga. Guille, muchas gracias por tu esfuerzo en hacer de mi un pequeño investigador, y por tenerme siempre en cuenta para resolver problemas técnicos que me han hecho sentir valorado y "prolijo", lo que sea que signifique. Sin tu ayuda esto habría sido bastante más difícil. Gracias Silvina. Seguramente la frase que mas he pronunciado durante años, siempre atenta y con una sonrisa, y con células de sobras, y con un poquito de buffer, y con una maestría admirable. Ya sabéis, si Silvina lo hace así, así es como se hace. And Roni, I don't know how to thank you all the help you have provide me, without expecting any reward. You have been a very important piece in the consecution of this project that is my thesis. I have learned a lot with our discussions, not to mention your friendship and help in these last though times. También François, quién sabe mas de lo que cuenta, aunque no te importa compartir todo ese conocimiento que tienes por ahí guardado. And from more recent times, I enjoyed a lot the presence and particular point of view of Andy, with the beginnings of a project shared with Michael, y de la inesperada amistad surgida con Alejandro (un abrazo transoceánico). I also want to thank the collaboration and fruitful discussions with Priyanka, and the good help received from Antonios in a multitude of problems I have encountered during my writing, i per aquells petits moments de desconnexió. I would like to thank people that arrived later to the lab, but that with their presence make us all better, including Roberto and Lara, i també en Quique i en Xavi, que m'han aportat un manera de fer les coses que jo desconeixia, i

bones converses a l'estona de dinar, i sobretot en Xavi que m'ha ajudat molt amb els anàlisis.
Voldria també agrair al membres del meu comitè de Tesi, Juan Valcárcel, Luciano Di Croce i Laia de Nadal, el suport, la paciència i les suggerències que m'han ajudat a portar aquest projecte endavant. En especial quería agradecer a Juan que confiara en mí para su laboratorio hace ya mucho tiempo, eso significó para mí entrar en la parte científica del CRG de la mano de uno de los científicos más transversales del instituto.
I voldria recordar una mica als que hi érem al principi de tot, en especial en Jaume, de qui la seva passió per la ciència és inigualable, i la Roser, que no entenc mai si hi ets o no, però quan hi ets és com si tornés la filla prodiga. També l'Albert i la seva colla, amb qui vam fer un bon grup. Voldria també agrair els companys de quan el CRG era una petita família i hi cabíem tots a la sala de seminaris. D'aquell temps tinc especial record del Luis i l'Anna, amb qui amb el temps hem mantingut una bona amistat, i m'han ajudat amb la moral per fer la tesi. Amb companys més recents també hem tingut grans experiències, tant científiques com personals. I would like to thank João, for his excellent advices, and friendship, he helped me in focusing my will for science. También a Ramón, por su pasión por la ciencia y sus incalculables gestos de amabilidad, conmigo y con todos alrededor, and Panagiotis, one of the most clever colleagues I had. També vull agrair a en Jordi, amb qui és un plaer parlar del que sigui, últimament només de la tesi però, y a Elena, que junto con Jordi son mis hijitos a la vez que hermanos en el CRG. I a tota aquella gent que fa que el CRG funcioni, en especial a la Carla i la Roxanna, i també a l'Aitor, en David Vidal, en David Rodríguez i en Sergi, i molt especialment a l'Imma, que tant suport ens dóna tots els estudiants. També haig d'agrair molt especialment tota l'ajuda de la gent de Proteòmica, en especial de la Cristina, que m'han guiat cap l'immens món del mass-spec.
Però també vull agrair a molta gent de fora del CRG el seu suport durant aquests anys, sobretot a les meves germanes Laia i Ariadna a qui no tinc el plaer de veure tant com voldria, i a les meves tietes Montse, Assumpta i Mercè (ja veieu que aquells bitxets amb els que treballo al final m'han donat algun resultat) i al meu oncle Josep, que no ha dubtat mai que ho aconseguiria.
Vull tenir un especial record per el meu oncle i padrí Quim Mateu, a qui més il·lusió li va fer quan va saber que començava la tesi doctoral, i que per desgràcia no l'ha pogut veure acabada. *Aquest tesi està en part dedicada a la seva memòria*.

També vull agrair tot el suport de l'altra part de la meva família, sobretot a l'Aurora que tant es preocupa per nosaltres i ens cuida.
També vull agrair a l'Institut Bonanova, en especial a l'Àfrica, la gran formació que em van donar i que m'ha ajudat a arribar fins aquí, a les meves primeres 'jefas' al laboratori, la Maite i la Maria Antònia, que em van saber transmetre valors i esperit científic, i als meus companys durant la carrera, que em van ajudar i em van donar la seva amistat, especialment en Dani, l'Anna, l'Àlex i la Sara.
I per últim, als meus amics, aquells amb els qui no passen els anys de tants que en fa que ens coneixem, sobretot en Quim, la Tana i en Roger, que sé que em troben a faltar, però no més que jo a ells.
I a tota aquella gent, que tant dins com fora del CRG m'han ajudat a que aquest camí fos més planer, que segur que me'n deixo molts, moltes gràcies.

Aquesta tesi està dedicada als meus pares Francesc i Anna Maria,
per tot, segur que n'estarien molt orgullosos.
Els trobem molt a faltar.

I també al meus fills, Arlet i Guillem,
que espero algun dia puguin valorar l'esforç,
i perdonar l'absència.

Per a la Maria

Summary

Progesterone receptor is a key regulatory element in hormone-dependent breast cancer cells proliferation. The mechanism of action of PR has played an important role in solving the molecular mechanism of transcription regulation. However, it has not been a thorough study of its interactors in response to hormone. In this work we have identified by RIME (Rapid Immunoprecipitation Mass spectrometry of Endogenous proteins) 315 high confidence PR interactors in breast cancer cells exposed to the potent progesterone agonist R5020 for 0, 1, 5, 15, 30 and 60 minutes. We have identified 20 known PR interactors and 295 new ones. The found PR interactors form 4 dynamic clusters; Basal cluster, 66 proteins present at similar level at all time points; Cluster 1, 41 proteins decreasing their interaction after hormone; cluster 2, 115 proteins increasing their interaction rapidly after hormone; and cluster 3, 93 proteins increasing their interaction steadily over time. PR interactors form functional complexes involved in transcriptional regulation, chromatin remodelling, mRNA processing, DNA damage repair, proteosomal degradation, protein stability and nuclear structural proteins. Exposure of cells to progesterone partial antagonist RU486 maintain the majority of PR interactors, but loses the interactors related to transcription regulation. This study set the bases for analyses of new functions of progesterone receptor in breast cancer cells.

Resum

El receptor de la progesterona és un regulador clau per la proliferació de les cèl·lules de càncer de mama dependents d'hormona. El mecanisme d'acció del PR ha tingut un paper important en la resolució del mecanisme molecular d'activació de la transcripció. No obstant això, no hi ha hagut un estudi a fons de les seves interaccions en resposta a hormona. En aquest treball s'han identificat per RIME (immunoprecipitació ràpida per l'espectrometria de masses de proteïnes endògenes) 315 interactors d'alta confiança del PR en cèl·lules de càncer de mama exposades a la potent agonista de la progesterona R5020 durant 0, 1, 5, 15, 30 i 60 minuts. Hem identificat 20 interactors coneguts del PR i 295 de nous. Els interactors del PR trobats formen 4 grups dinàmics; El grup basal, 66 proteïnes presents en nivells similars en tots els temps; grup 1, 41 proteïnes que disminueixen la seva interacció després de l'hormona; grup 2, 115 proteïnes que augmenten la seva interacció ràpidament després de l'hormona; i el grup 3, 91 proteïnes que tenen un augment de la seva interacció constant amb el temps. Els interactors del PR formen complexes funcionals que intervenen en la regulació transcripcional, remodelació de la cromatina, el processament de l'ARNm, reparació de l'ADN danyat, la degradació proteosomal, proteïnes estabilitzadores i proteïnes de l'estructura nuclear. L'exposició de cèl·lules a l'antagonista parcial de la progesterona RU486 manté la majoria dels interactors del PR, però perd els relacionats amb la regulació de la transcripció. Aquest estudi estableix les bases per a l'anàlisi de les noves funcions dels receptors de progesterona en cèl·lules de càncer de mama.

Table of contents

1. INTRODUCTION

1.1 Nuclear Receptors and their ligands

Nuclear receptors are transcription factors characterized by their ligand-dependent transcription activation. This superfamily is typically subdivided in three main families (Chawla *et al.* 2001), that include: the steroid receptor family; thyroid / retinoid family, (including the thyroid receptor (TR), vitamin D receptor (VDR), retinoic acid receptor (RAR), peroxisome proliferator-activated receptor (PPAR); and lastly the orphan receptor family, defined by a set of proteins identified by comparative sequence analysis as belonging to the nuclear receptor superfamily, but for which the activating ligands remain unknown. The work of described here, focuses on a member of the steroid hormone receptor family.

1.1.1 Steroid hormone receptor Tissue Expression

The Steroid hormone receptor (SHR) family is consists of the Glucocorticoid receptor (GR), Mineralocorticoid receptor (MR), two Estrogen receptors (ERα, and ERβ), Androgen receptor (AR) and Progesterone receptor (PR).

Nuclear receptor differential tissue expression is the first level of steroid hormone regulation. As shown Figure 1 protein levels of each hormone receptor are tissue specific. Expression varies between receptors, GR the most abundantly expressed in all types of tissues. AR is also expressed in a variety of tissues, being mostly enriched at male reproductive tissues, but also in breast or endometrium. ER and PR are more regulated in this sense, being solely found at protein level in female reproductive tissues, including breast, endometrium, fallopian tube or cervix. PR is only expressed in the first three. ER and PR are also annotated to be expressed in smooth muscle, where it has been suggested that PR regulates arterial smooth muscle cell proliferation (W. S. Lee *et al.* 1997), but this tissue also includes endometrial vascular smooth muscle cells (Rogers *et al.* 1996), where steroid hormones play a role during menstruation and pregnancy (Figure 1). At the RNA level, expression of ER and PR is also found in variety of tissues, including brain or bone, expanding the possible roles for these hormones.

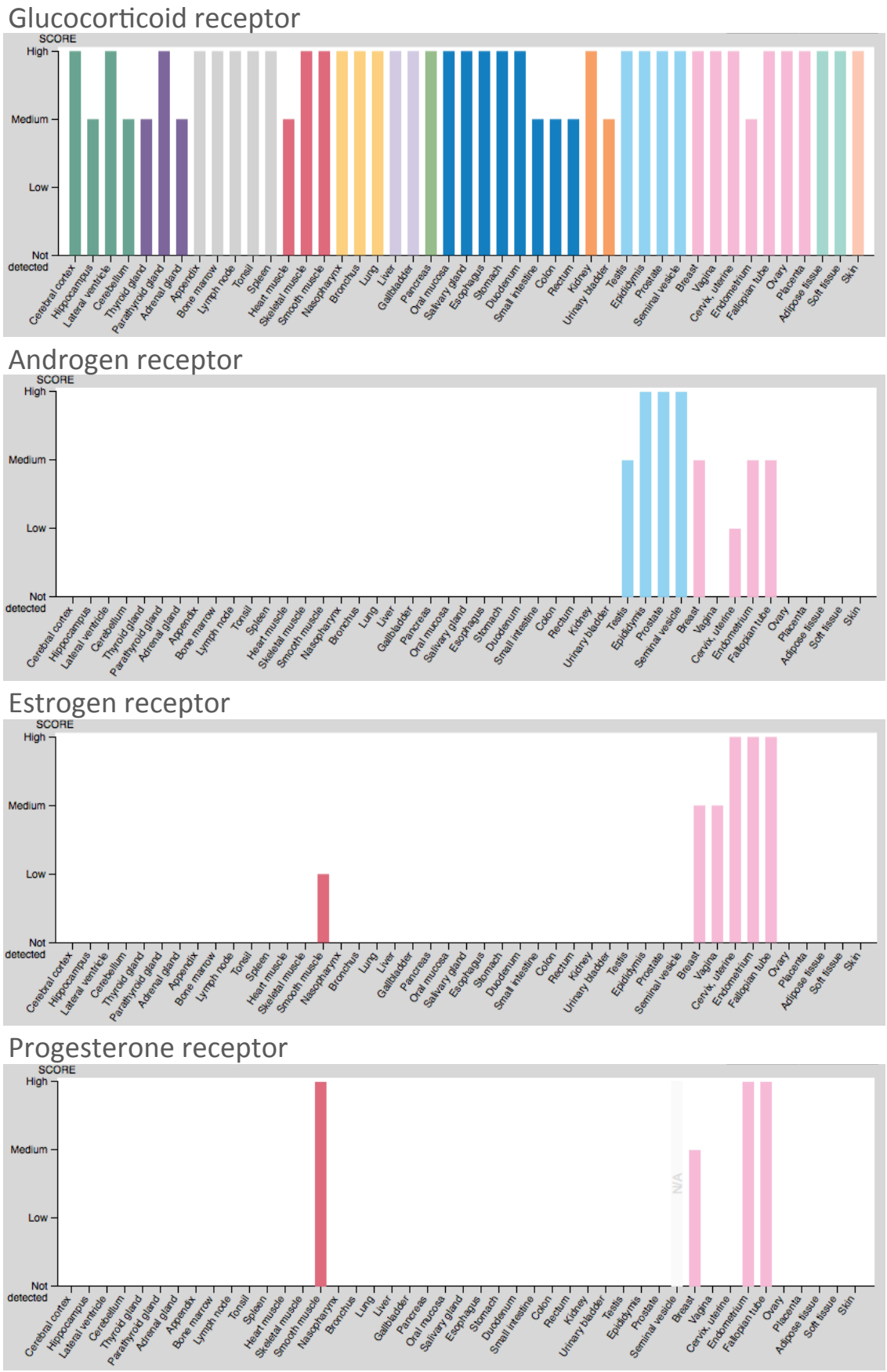


Figure 1: Steroid hormone receptors tissue expression. Protein levels of the SHR in different tissues. Adapted from *The human protein atlas* database.

1.1.2 Steroid hormones synthesis and functions

The gonads and adrenal glands produce five major groups of steroid hormones (SHs): estrogens, progestins, androgens, glucocorticoids and mineralocorticoids. Natural steroid hormones are synthesized from cholesterol, and are small lipophilic molecules that go through the cell membrane by simple diffusion and bind to steroid hormone receptors (SHRs). They have important regulatory roles in a wide variety of biological processes including reproduction, differentiation, development, cell proliferation, apoptosis, inflammation, metabolism, homeostasis and brain function (Tsai *et al.* 1994, Mangelsdorf *et al.* 1995).

They can be classified also in two groups, corticosteroids, as glucocorticoids and mineralocorticoids (typically synthesized in the adrenal cortex) and sex steroids, as androgens, estrogens and progestins (typically synthesized in the gonads or placenta). The sex steroids participate in the development of the reproductive system in embryonic stages as well as they regulate secondary sexual characteristics. Reproductive behavior in the adult remains also under the control of sex hormones through the effects that they exert over the brain (Evans 1988).

1.1.3 Steroid receptor structure and subcellular localization

Steroid hormone receptors contain two structural subunits: a C-terminal domain containing the Ligand Binding Domain (LBD, E at Fig. 2) and a central domain containing the DNA-binding domain (DBD, C at Fig. 2), separated by the hinge region (D at Fig. 2).

The LBD has a number of critical functions. Firstly, it contains an interior binding pocket specific for its corresponding hormone. Secondly, the LBD contains a ligand-regulated transcriptional activation function (AF-2) necessary for recruiting co-activating proteins. These coactivators interact with chromatin remodeling proteins and the transcriptional machinery, to affect gene expression. Finally, together with the DBD, the LBD contributes to the homodimerization necessary for DNA response element binding (V. Kumar *et al.* 1988).

The DBD is essential for binding to the hormone response elements located in regulated promoters or enhancers, and it is the most conserved region (Helsen *et al.* 2014). The DBD is also an allosteric transmitter of information to other regions of the receptor molecule (Beato *et al.* 2000). It is connected to the LBD via a short amino acid sequence termed the hinge region. The complete functional properties of the hinge are still unclear, although it can be phosphorylated and this phosphorylation is coupled to increased transcription activation (Knotts *et al.* 2001, Vicent *et al.* 2006). It also

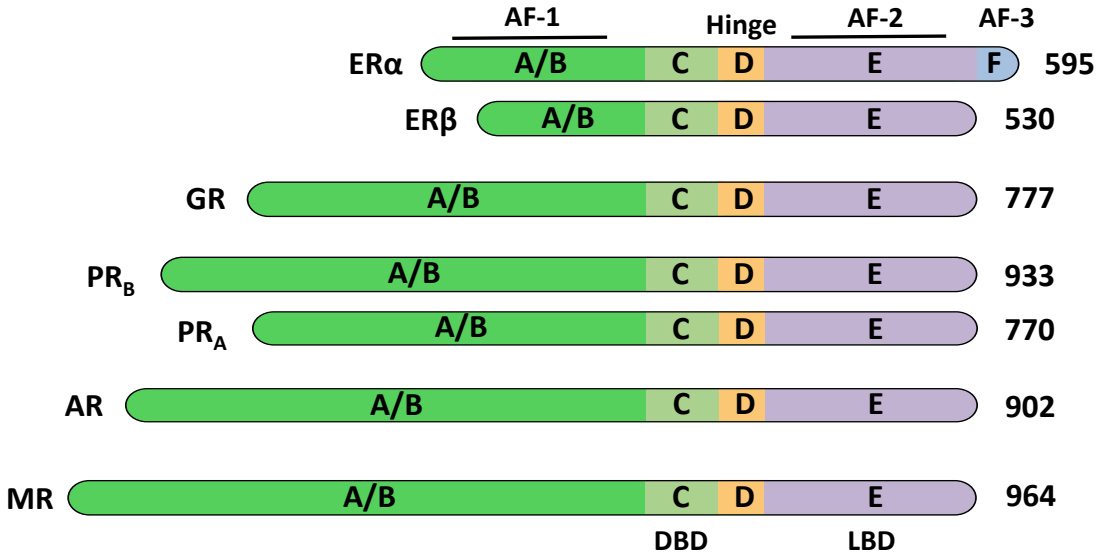


Figure 2: Atomic structure of the nucleosome particle. Each strand of DNA is shown in blue. The DNA makes 1.7 turns around the histone octamer to form an overall particle with a disk-like structure. Histones are coloured as in figure 5). Adapted from (Khorasanizadeh 2004).

influences intracellular trafficking and distribution, and modulates the interaction between the nuclear receptor and the DNA (Daniel *et al.* 2010). Although this region has a low level of conservation in terms of sequence, several common features such as its enrichment in basic residues are conserved among different nuclear receptors (Khorasanizadeh *et al.* 2001).

The amino acid sequence N-terminal to DBD contains a transcriptional activation function termed AF-1 (A/B at Fig. 2). AF-1 sequence shows weak conservation between the SHRs family members. This could explain how closely related SHRs can bind to similar response elements in vitro, but differentially regulate gene promoters containing those sequences in vivo (Takimoto *et al.* 2003).

Subcellular Localization

While the majority of SHRs in the unliganded state are localized in the cytosol, ERα and PR in breast cancer cells are also localized inside the nucleus already prior to hormone stimulation (Fig. 3). In an unliganded state, SHRs are in a constant equilibrium between nuclear and cytoplasmic localization, regulated by the ratio of nuclear import and export (Maruvada *et al.* 2003). Upon hormone exposure, steroid receptors are translocated to the nucleus were they exert their function as nuclear receptors (Dean *et al.* 2001).

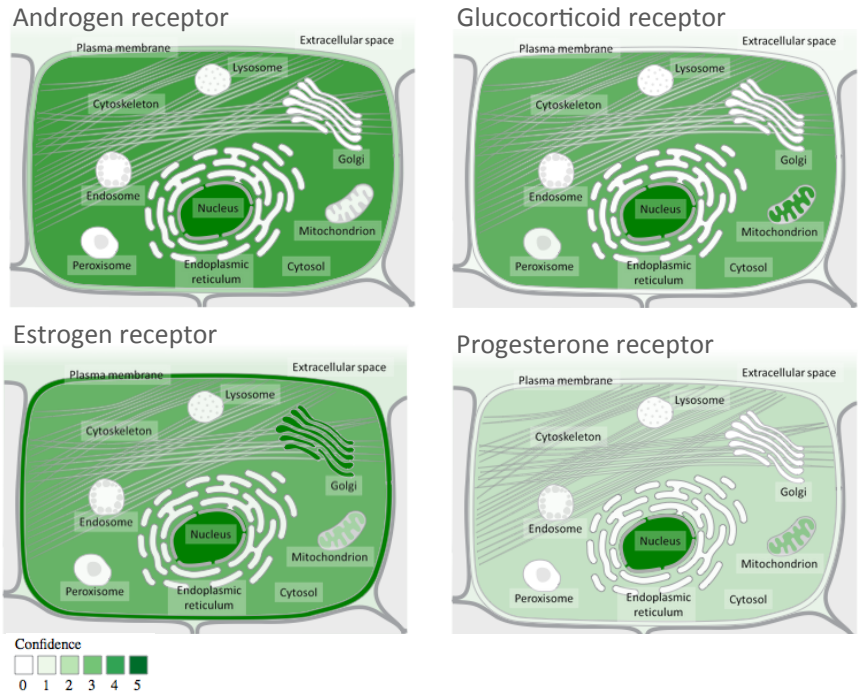


Figure 3: Subcellular locations of steroid hormone receptors, extracted from www.genecards.org, based on COMPARTMENTS (Binder *et al.* 2014)

SHR are also found at the cell membrane, as ER (Razandi et al. 2003, Pedram et al. 2007) and AR (Migliaccio et al. 2000) There is also an small fraction PR at the cellular membrane, <1% bound by pamitoylation. It has several regulating roles. Including regulation of cytochrome P450, steroidogenesis, vesicle trafficking, progesterone signaling and mitotic spindle and cell cycle regulation (Migliaccio *et al.* 1998, Cahill *et al.* 2016)

1.1.4 Steroid receptors activation

Unliganded SHRs are associated with a large multiprotein complex of chaperones that keep the SHRs properly folded, in order to enable ligand binding and repress SHRs activation of the unliganded receptor (Pratt 1993, Smith 2000). As shown in in vitro studies with PR, the receptor is in a steady state of assembly/disassembly cycle with HSP90, in which hormone exposure prevents the assembly of an intermediate HSP70 containing complex, releasing PR from the loop and leading to pathways activation (Smith 1993).

Upon hormone activation, SHRs act as transcription factors and modulate gene expression. A more detailed description of the complete mechanism of receptor activation is explained within the example of progesterone receptor later (section 1.3.5).

1.1.5 Cancer implications

Sex steroid hormones play a central role in the development and progression of hormone dependent cancers, such as prostate cancer for androgen and breast cancer for estrogens and progesterone. Major advances have been made in the identification and characterization of possible downstream targets of the steroid hormone receptors in order to block their proliferative effects in cancer cells (Y. J. Ko *et al.* 2004). Several cofactors has been identified to play a key role in the progression and expansion of cancer cells (L. Wang *et al.* 2016). Some cancers like hormone-dependent breast cancers are commonly treated with steroids antagonist to minimize steroid-dependent proliferation (Hagan *et al.* 2014, Diep *et al.* 2015). However, this treatment very often leads to hormone resistant growth of the tumors. Further research is still required in order to understand the mechanism of action of hormone and to be able to prevent hormone resistant tumor growth. One of the aims of this work is to identify and elucidate the mechanism of action of steroid receptors co-regulators in order to understand more fully cancer disease progression.

1.2 Chromatin

1.2.1 Basic chromatin structure

In eukaryotic cells, DNA is organized in the cell nucleus forming a nucleoprotein complex called chromatin. This organization has mainly two goals: first, to fit the 2 meters long genomic DNA into a 10 µm diameter nucleus, and second and probably even more important, to have another level of regulation of the information encoded therein, as chromatin structure could selectively control access of transcription factors to their binding sequences on DNA. In addition chromatin protects the DNA for environmental and endogenous damaging agents.

Each chromosome is composed by one long molecule of double-stranded DNA wrapped around core histone proteins, forming a so-called "beads-on-string" structure or the 11 nm fiber. These beads of nucleosome core particles are further compacted by the linker histones of the H1 family that favor the formation of a 30 nm fiber. The 30 nm fiber folds to form larger structures, such as chromatin loops, which are further compacted in mitosis and form chromosomal bands visible in the light microscope (Fig. 4).

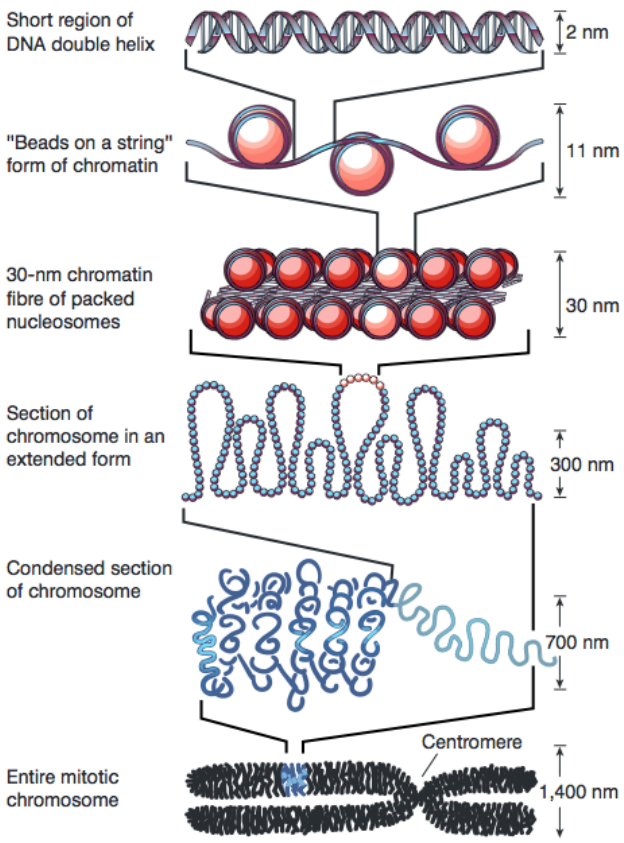


Figure 4: Chromatin structure. (Felsenfeld *et al.* 2003)

These different levels of folding the DNA create the problem of DNA accessibility: for expressing or replicating the information contained in the DNA double helix, many molecular complexes have to first "open" the chromatin fiber, and this must be a highly controlled process.

It has recently found that chromatin is distributed and organized in a superior level in what it has been called topologically associating domains (TADs) of an average size of 1Mb in human cells, which are separated by boundaries enrich in binding sites for CTCF and cohesins (Nora *et al.* 2012). TADs represent epigenetically homogeneous domains within which there are more contacts than with neighboring regions of the genome. They contain genes that are often regulated in a coordinated way and are conserved in different cell types (Le Dily *et al.* 2014). For this reason, the higher levels of chromatin organization, such as TADs, are exceptionally important and have received a lot of attention over the last few years (Gonzalez-Sandoval *et al.* 2016).

1.2.2 The Nucleosome

The nucleosome core particle is the basic unit of chromatin. Each nucleosome core particle consists in 147 base pairs of DNA, wrapped as a left-handed superhelix around a symmetric core histone octamer. Each nucleosome core particle is composed of two copies of each of the core histones H2A, H2B, H3 and H4 (Fig. 5). Each nucleosome core particle can bind on histone H1 isoform that interact with the DNA at the nucleosome dyad axis and at with the linker DNA at the entry and exit sites protecting 20 additional base pairs of linker DNA from nuclease digestion. The nucleosome is made of the nucleosome core particle and the histone H1 with its associated linker DNA. The length of the linker DNA is variable in different cell types results in variable nucleosome ladders in different cells (Khorasanizadeh 2004).

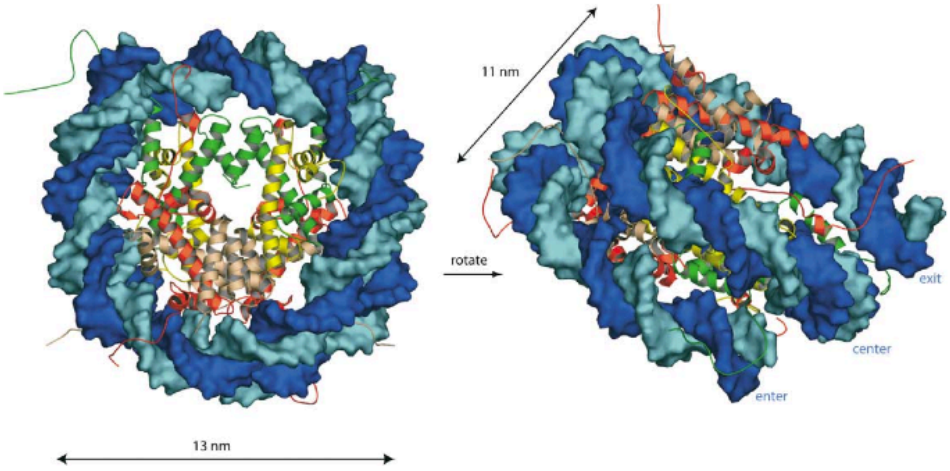


Figure 5: Atomic structure of the nucleosome core particle. Each strand of DNA is shown in blue. The DNA makes 1.7 turns around the histone octamer to form an overall particle with a disk-like structure. Histones are colored as in figure 5). Adapted from (Khorasanizadeh 2004)

Histones are highly conserved basic proteins (White *et al.* 2001). They share a structure, called the globular domain, consisting of a conserved core, formed by a long central α-helix flanked on either side by a loop and a short α- helix. These domains interact with each other to form higher order structures, which are the H2A/H2B and H3/H4 heterodimers (Arents *et al.* 1991). Two H3/H4 dimers form a stable tetramer, capable of organizing approximately 120 bp of DNA (Hayes *et al.* 1991). Dimer of H2A/H2B can bind to each side of the h3/H4 tetramer in a process facilitated by the histone chaperone NAP1 (D'Arcy *et al.* 2013) (Fig. 6).

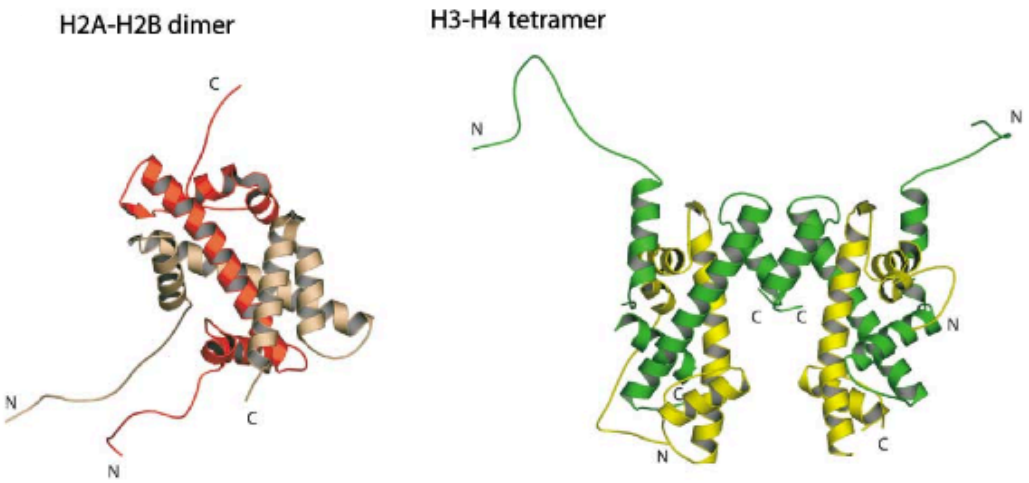


Figure 6: Atomic structure of the core histones. Dimer of H2A (red) and H2B (pink) (left). Tetramer of H3 (green) and H4 (yellow) (right). Adapted from (Khorasanizadeh 2004)

Apart from the globular domain, core histones have long C- and N-terminal tails rich in basic residues that protrude from the nucleosome, and have no secondary structure (Luger *et al.* 1997). A similar situation applies for the many isoforms of linker histones, which have particularly long C and N terminal unstructured regions. These regions particularly the C- terminal region of H1 and N-terminal tails of core histones, are responsible for the great majority of the post-translational modifications (PTMs), which are very important for the structure and dynamics of chromatin. In addition, there are multiple core histone variants, particularly of H3 and H2A, that imposed different stability on nucleosomes Therefore, nucleosomes are dynamic structure, an their PTMs and substitutions of variants can lead to differential local compaction of chromatin that determines accessibility of the DNA information (Becker *et al.* 2013).

1.2.3 Histone post-translation modifications

The N-terminal tails of core histones can be post-translationally modified in a variety of ways: acetylation, methylation, phosphorylation, ubiquitination, SUMOylation, ADP ribosylation, deamination, proline isomerization or citrullination (Kouzarides 2007).
According to the "histone code" theory, firstly postulated by Strahl and Allis (Strahl *et al.* 2000), combinations of histone PTMs may act as important docking sites for transcriptional regulators and chromatin remodelers. These proteins are also called "readers", as they bind specifically to their target modification. Cross-talk mechanisms have been described between histone PTMs (Kouzarides 2007), increasing the complexity and giving rise to a whole subset of possible regulations.

1.2.3.1 Histone acetylation

Histone acetylation is the histone PTM more related to chromatin opening and transcription activation (Kuzmichev *et al.* 2001). This modification is regulated by the actions of histone acetyl-transferases (HATs) and histone deacetylases (HDACs) (Bannister *et al.* 2011). Acetyl-modified histones can act as a docking site for proteins with acetyl-binding bromo domains (Fig. 7). Apart from this important function, acetyl modification neutralizes the overall positive charge of histone lysines, weakening the histone-DNA interaction, leading to a more relaxed state of the chromatin, enabling access to transcription factors to their DNA target sites (Workman *et al.* 1998).

1.2.3.2 Histone methylation

Histones can also be methylated on basic residues (lysines and arginines). This modification itself has a number of different conformations, as lysines can be mono-, di- or trimethylated, and arginines can only be mono- and dimethylated, but dimethyl can be in a symmetric and asymmetric form. All this variations add another degree of complexity, as different methyl-modifications are "written" and "read" by different proteins (Bannister *et al.* 2005, Jenuwein 2006). Methyl groups on histone tails are recognized by several different domains, chromo-, tudor domains or PHD fingers (Kouzarides 2007) (Fig. 7). Contrary to acetyl groups, histone methylation can act in opposite directions in terms of transcriptional regulation, depending not only on the N-terminal of which histone is deposited but also depending on which residue is modified. There are marks associated to transcriptional activation as trimethylation of lysine 4 or 36 on histone H3, while trimethylation of lysines 9 or 27 on the same histone tail are markers for repression (Bannister *et al.* 2005).

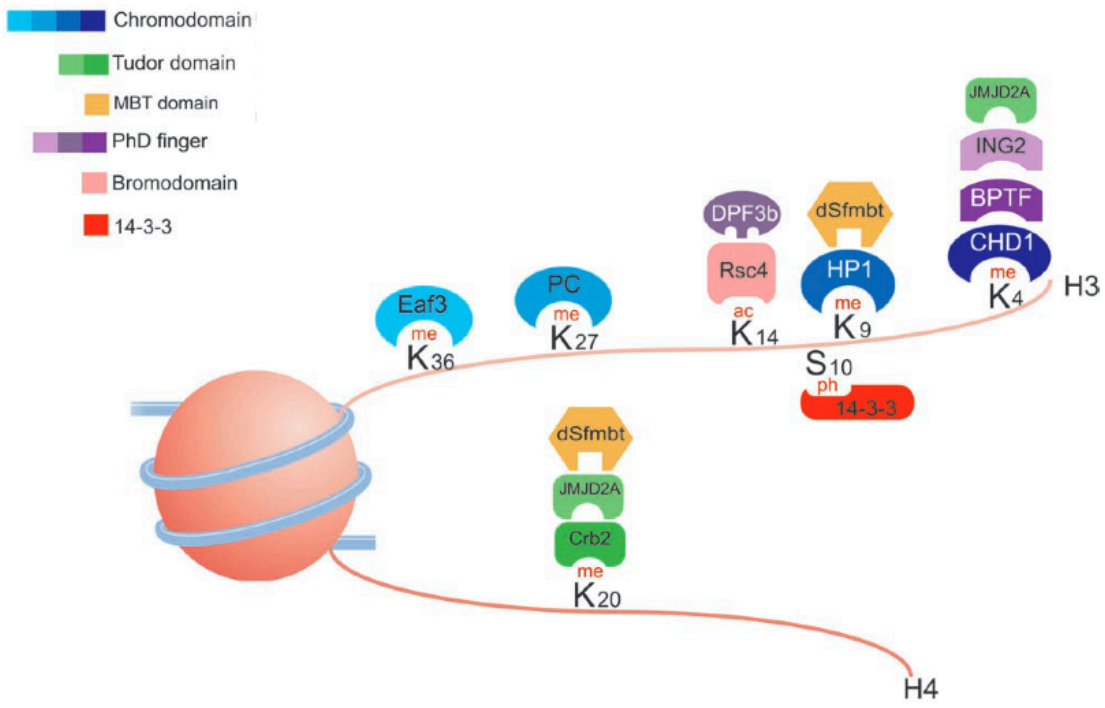


Figure 7: Domains binding modified histones. Examples of proteins with domains that specifically bind to modified histones as shown. From (Bannister *et al.* 2011).

1.2.3.3 Histone phosphorylation

Phosphorylation on histones is one of the major histone post-translational modifications for the cross-talk between PTMs. Apart from serving as a docking site for phospho-specific binding proteins as 14-3-3 (Winter *et al.* 2008), it can generate the displacement of adjacent methyl-binding proteins, as phosphorylation on H3S10 can displace HP1 from binding to H3K9me3 (Fischle *et al.* 2005). Histone phosphorylation is the major histone modification responsible for PTMs crosstalk.

1.2.3.4 Histone variants

As nucleosomes are a dynamic structure, the existence of core histone variants takes an important role in gene regulation. Their different conformation to the canonical isoform lead to differential regulation among them. The most studied variants are those from H3 and H2A. These include H3.3, which acts as a transcriptional activator (Ahmad *et al.* 2002), H2A.Z, which has several roles including gene regulation (Allis *et al.* 1986) or macroH2A, which is linked to transcriptional repression (Angelov *et al.* 2003).

1.2.4 ATP-dependent chromatin remodelling

Even after PTM of histones the nucleosomes represent a physical barrier for transcription factors and the machinery that transcribes DNA. For this reason, the cell has evolved chromatin remodelers that use the energy of ATP hydrolysis for mobilizing nucleosomes by weakening the interaction of histones with DNA.

Chromatin remodeling is a key process for gene regulation and transcription activation. It is described as a dynamic rearrangement of the chromatin architecture in order to either allow or deny access to transcription factors and other machineries as the ones related to DNA damage repair. ATP-dependent remodeling is usually associated to histone PTMs and involves different families of enzymatic activities, characterized by the central role of a helicase-related ATPase, with a variable number of associated subunits, forming different complexes (Mohrmann *et al.* 2005). There are 4 main families of ATP-dependent chromatin remodelers, the SWI2/SNF2 family, containing a bromodomain; the ISWI family, containing a SANT domain; the CHD family, containing a cromodomain and a DNA-binding motif; and the INO80 and SWR1 families, without an additional domain (Mohrmann *et al.* 2005) (Fig. 8).

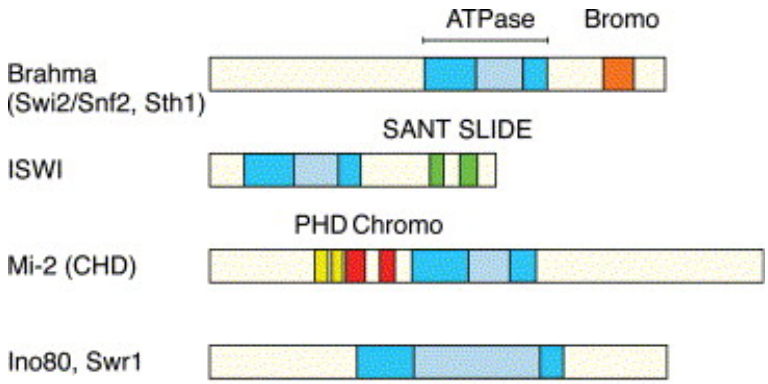


Figure 8: ATPases of the four main families of ATP-dependent Swi2/Snf2-related chromatin remodeling complexes: SWI/SNF, ISWI, Mi-2 and Ino80. Each family is defined by the presence of a distinct ATPase containing signature structural domains and a unique subunit composition. From (Mohrmann *et al.* 2005).

Chromatin remodelers can function as direct co-regulators for SRs. Both SWI/SNF-type and ISWI-type complexes were found to potently co-activate SRs. In case of SWI/SNF both ER and PR were reported to directly interact with BRG1-associated factor 57 (BAF57) (Belandia *et al.* 2002, Vicent *et al.* 2009), while for ISWI the NURF subunit BPTF was found to be a hormone-dependent interactor of PR (Vicent *et al.* 2011). The nucleosome remodeling and histone deacetylation (NuRD) complex, a representative of Mi2-type complexes, acts as a co-repressor complex for ER via direct interaction of the receptor with the MTA1 or the MTA2 subunit (Mazumdar *et al.* 2001, Cui *et al.* 2006). Each family consists in a number of different remodeling complexes, which depending on its subunits will be capable of binding to certain histone PTMs and act co-ordinatedly with the modifying enzymes. As an example, Fig.9 shows the well-conserved ISWI family of chromatin remodelers with its central helicase, SNF2H (Fig. 9).

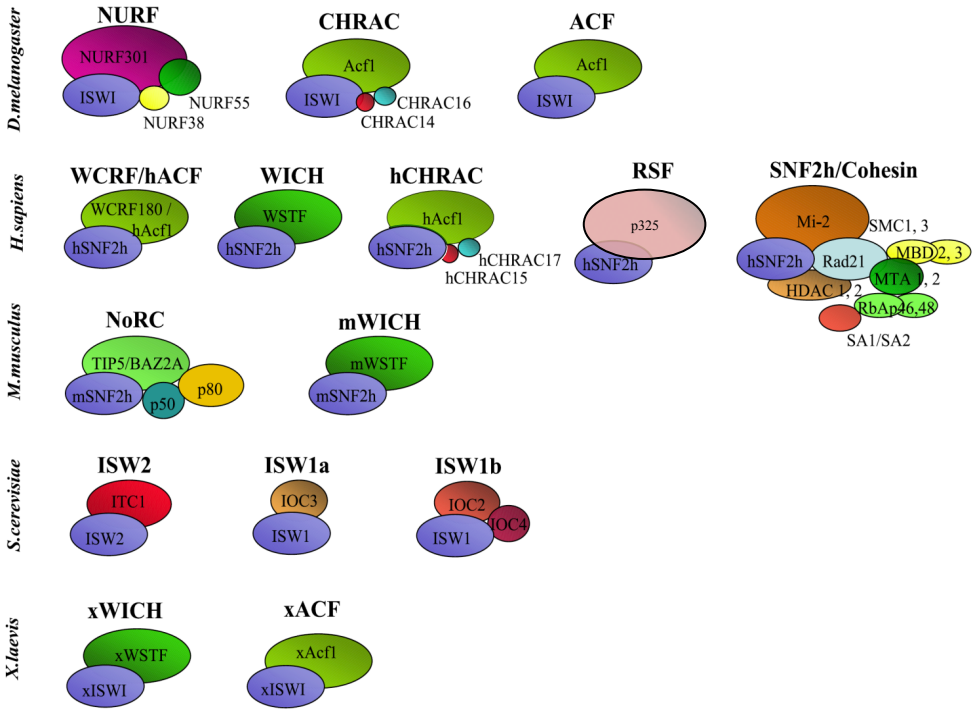


Figure 9: ISWI family of chromatin-remodelling complexes. Adapted from (Corona *et al.* 2004, Khorasanizadeh 2004)

1.3 Progesterone receptor

Progesterone receptor (PR) is a nuclear steroid receptor encoded by a single gene. PR was the first SHR shown to exist in two isoforms generated by differential promoter usage (Kastner *et al.* 1990). One promoter initiates transcription at positions +1 and +15 of the Pgr gene that gives the longer human isoform, PRB with 933 amino acids and a MW of 116 KDa. The second promoter initiates human PR transcripts between +737 and +842 encoding the shorter human isoform, PRA, which lacks 164 amino acids at its N-terminal domain (769 amino acids and 90 KDa). There is also a third isoform, named PRC, which only contains the hinge region and the LBD (Condon *et al.* 2006). PRA and PRB are typically expressed in equimolar ratios (Fig. 10, right panel) and function as ligand-activated transcription factors, whereas expression of PRC is limited and may serve largely to sequester ligand, as it is incapable of binding DNA. PR is mainly located in the nucleus, prior and after hormone induction (Fig. 10, left panel).

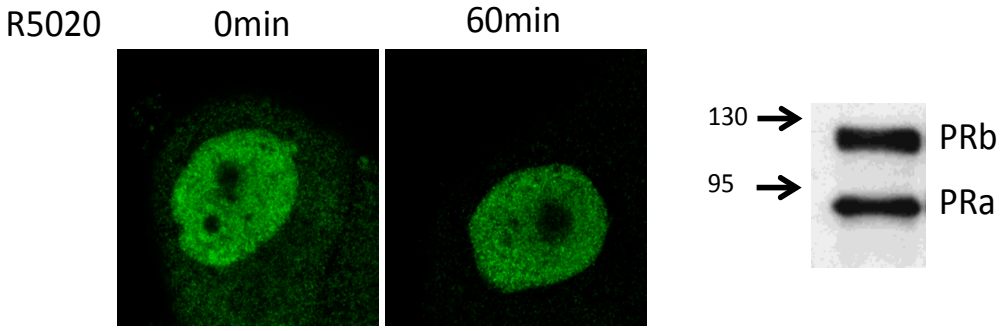


Figure 10: PR immunofluorescence for T47D cells, comparing PR localization without hormone and at 60 minutes after hormone exposure (left panel). Western-blot for PR from T47D cells extract. Note that PR antibody recognizes both isoforms (right panel). PR antibody used was the same for both IF and WB (PR, H-190).

1.3.1 PR structure

Like all nuclear receptors, PR is composed of the N-terminal domain, the DBD linked by the hinge region to the LBD. The N-terminal domains of both PR isoforms, PRA and PRB, are identical except that PRA lacks 164 amino acids contained at the N-terminal end of PRB. This B-upstream segment (BUS) contains a transcription activation function, AF-3 (Sartorius *et al.* 1994). Biochemical analyses of PRA and PRB isoforms

N-terminal domains have shown that there are differences at the level of secondary or tertiary structure. This could explain why PRB is stabilized in a more functionally active conformer that PRA (Bain *et al.* 2000, Bain *et al.* 2001).

Although LBD section of PR structure had been resolved (Williams *et al.* 1998) (Fig. 11), the full length proteins structure has not been resolved, may be due to its dynamic disordered nature of its N-terminal domain.

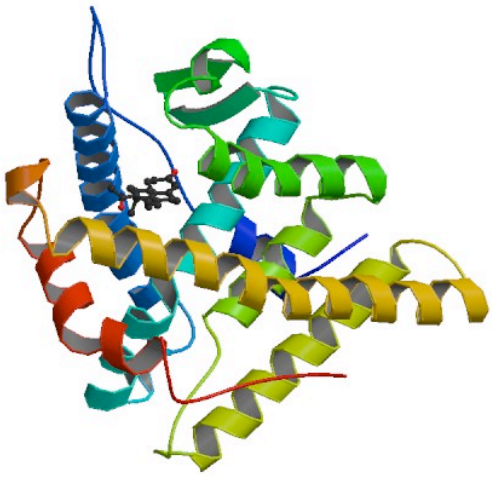


Figure 11: Hormone-bound human progesterone receptor ligand-binding domain (aa 708-932), PDB database, 1A28.

1.3.2 PR Ligand-Binding Domain (LBD)

Progesterone binds in the distal half of the LBD, forming highly specific hydrogen bonds and van der Waals contacts. Additional hydrophobic interactions between the ligand and the walls of the binding pocket contribute to the stability of the binding reaction (Williams *et al.* 1998). LBD structure it is shown necessary for binding co-activating proteins after ligand binding (Tanenbaum *et al.* 1998). The LBD includes a ligand-dependent activation function (AF-2) which is able to recruit proteins such as the steroid receptor co-activator (SRC) family (Xu *et al.* 2003).

1.3.3 PR DNA-Binding Domain (DBD)

The PR DBD folds into a globular domain made up of two different zinc-finger structures. Four cysteine residues coordinate each zinc atom, which are necessary to stabilize the structure and function because (Fig. 12); removal of the zinc ion leads to

protein unfolding and loss of DNA-binding activity (Freedman *et al.* 1988). PR was shown to binds specific palindromic sequences of the sequence TGTTCTnnnAGAACA (named hormone responsive element, HRE, or progesterone responsive element, PRE) that are also recognized by GR and AR (Scheidereit *et al.* 1983, von der Ahe *et al.* 1986, Beato 1989). DNA binding of the DBD of PR to a palindromic site induces dimerization. The residues that define the dimer interface are located into the C-terminal zinc-finger and constitute the D-Box. The sequence-specific DNA binding residues are

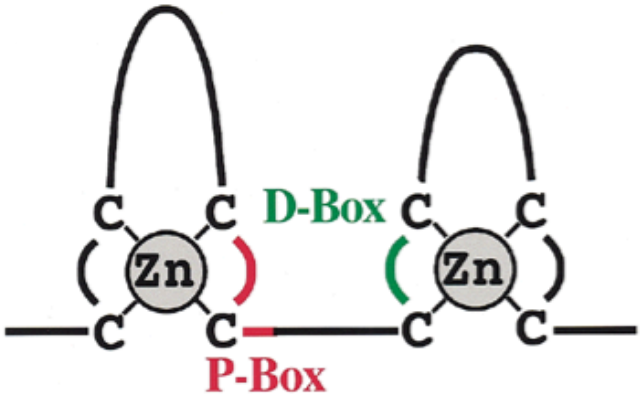


Figure 12: SHR-specific zinc fingers of the PR DBD. The DNA binding domain of human PR is characterized by the presence of two SHR-specific zinc fingers. Four cysteines each tetrahedrally coordinate two zinc ions *(grey)*. The proximal box (P-box), responsible for specific DNA recognition is shown in *red*; and the distal box (D- box), mediating DBD dimerization is shown in *green*.

defined as the P-Box (Beato *et al.* 2000). It is also shown that PR can bind to half palindrome HREs (Ballare *et al.* 2013), as a homodimer in a head-to-head orientation. Analysis of the protein-DNA interaction reveals that amino acid chain contacts with bases in the major groove are almost identical to those defined for GR and AR and are all located in one side of the double helix, allowing binding to HREs organized in nucleosomes, if the major groove is properly oriented (Pina *et al.* 1990).

1.3.4 N-terminal domain

Regarding the N-terminal domain, remarkably the DBD can stabilize and influence N-terminal structure. PR fragments lacking the DBD are immediately degraded by proteases (Bain *et al.* 2000, Bain *et al.* 2001).

Upon binding to a palindromic response element, PRA and PRB N-terminal domains undergo conformational changes. These changes were localized into the AF-1 region, but also into the hinge, demonstrating that DBD allosterically transmits structural

transitions. These changes seem to be necessary for recruitment of co-activators to the target promoter, allowing access to previously hidden binding sites by PR conformational changes (R. Kumar *et al.* 2005).

In addition to its relevance in functional differences between PRB and PRA, N-terminal half of PRB is important in the progesterone cytoplasmic-signaling mediated effects. An interaction between PRB and ERα has been identified in breast cancer cell lines (Migliaccio *et al.* 1998). PRB interacts with ERα through two domains located in the N-terminus of PRB, ERID I and ERID II (ER interaction domains I and II), that are required for the interaction with the LBD of ERα and for efficient activation of the Src / Ras / Erk cascade (Ballare *et al.* 2003).

1.3.5 PR post-translational modifications

Progesterone receptor can be post-translationally modified. To date, 17 post-translational modifications have been identified on PR that alter its transcriptional activity, including: phosphorylation (S294, S345, S81, and S400), SUMOylation (K388), acetylation (K183, K638, K640, and K641), and ubiquitylation (Lange et al. 2000, Pierson-Mullany et al. 2004, Daniel et al. 2007, Faivre et al. 2008, Daniel et al. 2009, Beleut et al. 2010, Daniel et al. 2010, Hagan et al. 2011, Knutson et al. 2012, Chung et al. 2014, Dressing et al. 2014) (Fig. 13). Post-translational modifications,

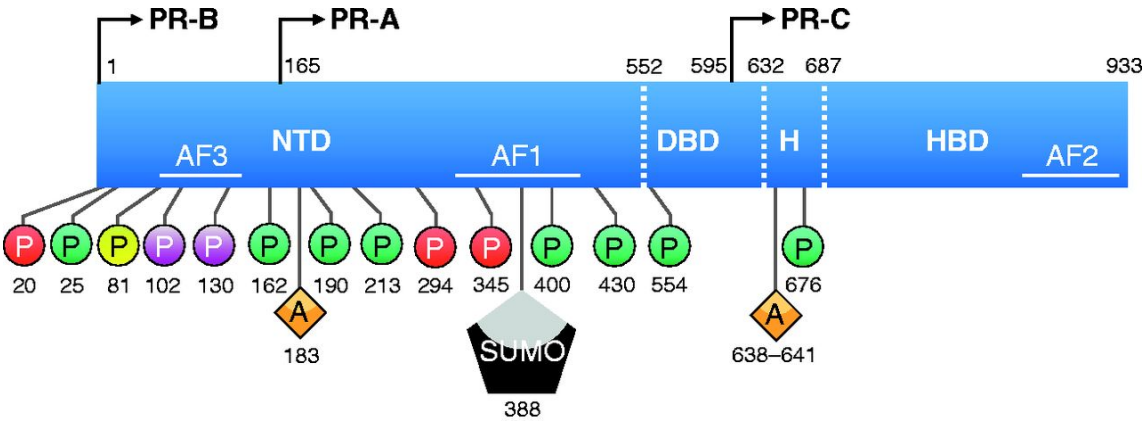


Figure 13: PR PTMs. The colour of phosphorylation sites is associated with the following: red, MAPK; green, CDK2; yellow, CK2; purple, unknown kinases. PR, PR protein isoforms A, B, or C; NTD, N-(amino)-terminal domain; DBD, DNA-binding domain; H, hinge region; HBD, hormone-binding domain; AF, activation function 1–3; P, phosphorylation; A, acetylation; SUMO, small ubiquitin-like modifier (SUMOylation). From (Diep *et al.* 2015)

basal or in response to ligand binding, affect PR transcriptional activity (H. A. Abdel-Hafiz *et al.* 2014, Diep *et al.* 2015).

The most studied PTM on PR is phosphorylation. For PR several phosphorylation sites have been identified, which are catalyzed by different kinases including extracellular signal regulated kinase (ERK) 1/2, cdk2 and CKII. Apart from S676, which is located in the hinge region, all other sites are present in the N-terminal domain of PR (Lange 2004). S81, S162, S190, and S400 are already phosphorylated in the absence of hormone (Pierson-Mullany *et al.* 2004), but their phosphorylation level increases when exposed to hormone. On the contrary, S102, S294, and S345 are hormone-induced sites, which are non-phosphorylated in the absence of hormone (Lange *et al.* 2000). Notably, some of these phosphorylation sites are in the B-upstream segment, which is only present in the PRB isoform (Fig. 13). Phosphorylation of PR in S294 is mediated by activated MAPK kinases cascade (Shen *et al.* 2001), creating conformational changes on the receptor structure (Y. Zhang *et al.* 1995), leading to chaperones dissociation and increasing its transcriptional activity (Vicent *et al.* 2006), while at the same time enhances its ubiquitin dependent degradation after 6h of hormone exposure (Lange *et al.* 2000).

SUMOylation of lysine 388 has been shown to have a regulatory role, reducing PR transactivation (H. Abdel-Hafiz *et al.* 2002). But SUMOylation requires a complex cascade of events, involving E1- E2- and E3-SUMO activating enzymes. At the last step of this cascade there are E3-ligases, including PIAS (Protein inhibitor of activated STAT) family (Kahyo *et al.* 2001), shown to modulate progesterone-dependent transcription by destabilizing its retention to the nucleus (Kotaja *et al.* 2000, Man *et al.* 2006).

Ubiquitin-dependent turnover was found to be an essential feature for the cyclical transcriptional activation by SRs (Metivier et al. 2003, Reid et al. 2003). PR is a well-known ubiquitin substrate, as part of the crosstalk with phosphorylation by MSK1 (Lange *et al.* 2000), and triggers ligand-dependent proteasomal degradation (Nardulli *et al.* 1988).

1.3.6 PR hormonal response

Unliganded SHR are bound to a number of molecular chaperones, that actively keep their structure, and protect them from unfolding or degradation, and maintain them for

an eventual hormone entering the cell (Pratt et al. 1997). Among them there are HSP90, HSP70 (Pratt *et al.* 1997), and some co-chaperones as STIP1 (Weaver *et al.* 2000) or DNAJ/Hsp40 (Johnson *et al.* 1994), as well as the immunophilins FKBP4 and FKBP5 (Smith *et al.* 1992, Smith *et al.* 1993). HSP90, together with other co-chaperones are found to be important for nuclear-shuttling of PR upon hormone induction (Elbi *et al.* 2004). It has been shown to regulate disassembly of transcription complexes (Freeman *et al.* 2002).

Upon hormone induction, the small population of PRA associated with the cell membrane in complex with ERα activates the SRC/RAS/MEK/ERK/MSK1 pathway (Migliaccio *et al.* 1998), (Boonyaratanakornkit *et al.* 2001, Ballare *et al.* 2003). The hormone also binds the major population of intracellular PR and leads to its dissociation from its chaperones and formation of PR homodimers. The PR homodimer translocates to the cell nucleus as a complex with ERK/MSK1 and is phosphorylated by ERK1/2 at S294. This ternary complex is recruited to PR binding sites within chromatin, where MSK1 phosphorylates histone H3 at S10 (Vicent et al. 2006) (Fig. 14). It has been shown by ChIP-seq analysis that in response to hormone optimal PR binding and function requires nucleosome positioning (Ballare *et al.* 2013). Other targets of the activated kinases cascades may be transcription factors and co-regulators involved in DNA synthesis and cell proliferation.

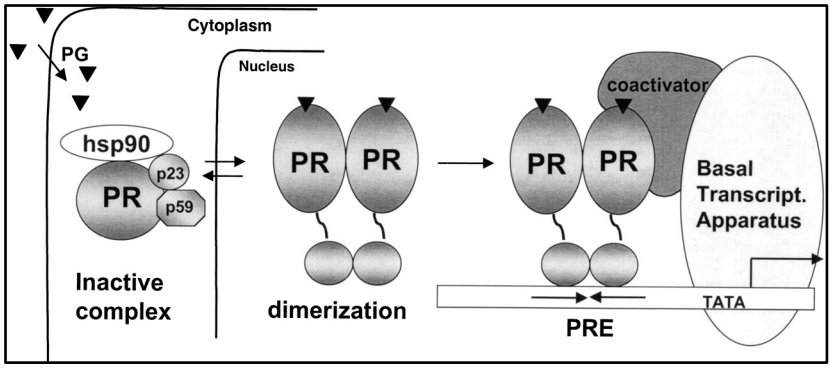


Figure 14: Progesterone activation of PR. Binding of progesterone to the inactive receptor complex induces a conformation change which leads to immunophilins and hsp dissociation, receptor dimerization, DNA binding, and recruitment of coactivators to facilitate communication with the basal transcription apparatus. From (Leonhardt *et al.* 2003).

A repressive function for unliganded PR has also been described. It has been shown that PR interacts with repressive complex containing HP1γ (heterochromatin protein 1-γ), KDM1A/LSD1 (lysine-specific demethylase 1), HDAC1/2, CoREST (corepressor for REST), KDM5B, and the non-coding RNA SRA (steroid receptor RNA activator). This

complex is recruited by unliganded PR to hormone-inducible genes, keeping them silenced prior to hormone treatment. Upon hormone exposure the kinase MSK1 associated with activated PR phosphorylates histone H3 at S10 and the repressive complex is rapidly displaced and replace by activated PR and its associated co-activators (Vicent *et al.* 2013).

1.3.7 Progesterone agonists and antagonists

In studies with cells in culture, the steroid hormone progesterone is very metabolized and it is not widely used. Instead, the potent synthetic analogue R5020 (also called Promegestone) is commonly used to induce PR activation and cell proliferation in PR positive cells. In some publications, both progesterone and R5020 have been used, however in order to achieve similar results in terms of the magnitude of gene expression changes and cell proliferation progesterone must be used at a 10 times higher concentration as R5020 (Mohammed *et al.* 2015). Also relevant for the study of PR mechanism of action is the study of progesterone antagonists or partial agonists. There are a number of progesterone antagonists (Klijn *et al.* 2000), but the most studied is RU486, also known as Mifepristone (Fig. 15). In medicine, is used to bring about an

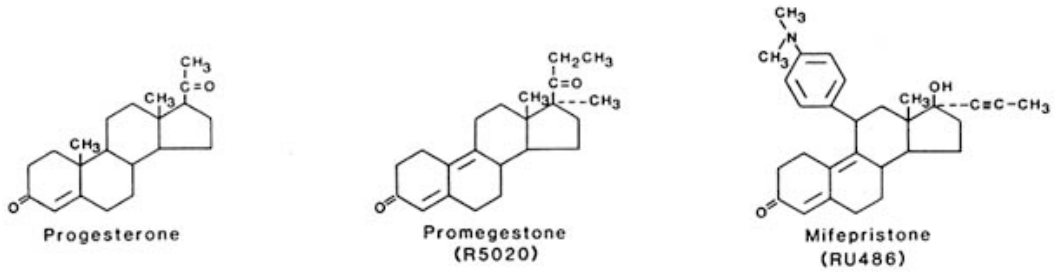


Figure 15: Molecular structure of Progesterone, R5020 and RU486. Adapted from (Weigel 1993)

abortion among the first 50 days of pregnancy, or used as a contraceptive pill (Baird *et al.* 2003). Its mechanism of action is not well known, but is that it binds to DNA but does not activate transcriptional regulation. There are indications that it may increase the residence time of receptor on DNA target sites (Pandit *et al.* 2002). Especially relevant is that when SHRs are occupied by hormone antagonists they recruit transcriptional co-repressors. Tamoxifen (antagonist of estrogens) and antiprogestin RU486 can act as partial agonist / antagonists or complete antagonists. Both steroid analogs inactivate AF-2, whereas the partial agonist activity is mediated by AF-1 (Leonhardt *et al.* 2002). Based on DNA footprinting assays, PR was found to make

identical base specific contacts with the HRE of mouse mammary tumor virus (MMTV) in the presence of agonist and RU486 (Leonhardt *et al.* 2003). In other preliminary results from our lab, it has been seen by PR ChIP-seq in T47D cells exposed to RU486 that it displays an increase DNA at the same sites than upon R5020 exposure (unpublished data). In order to elucidate possible mechanisms of transcription activation and active repression, mass spec analysis was done after PR immunoprecipitation on cells treated with RU-486. In the figure 14 is shown the molecular structure of progesterone, the agonist R5020 and the antagonist RU486.

1.3.8 PR co-regulators

These regulatory proteins come in two types, coactivators and corepressors that respectively enhance or diminish transactivation activity through various enzymatic activities, including acetylating, deacetylating, methylating, ubiquitinating, and kinase activity.

1.3.8.1 Co-activators

Co-activators recruited by ligand-bound PR include chromatin remodeling complexes, such as BAF or pBAF complexes, as well as members of the steroid receptor coactivators family (SRC) (Onate *et al.* 1995).

The SRC family consists of three members: SRC-1 (or NCoA-1), SRC-2 (or NCoA-2) and SRC-3 (or NcOA3). SRC family proteins directly bind activated SRs via three LXXLL motifs that interact with the AF-2 region in the LBD (Xu *et al.* 1998, Xu *et al.* 2009). C-terminal domains of SRC-1 and -3 contain histone acetyltransferase (HAT) activity although it is much weaker than those in CBP, p300 or PCAF enzymes (Spencer *et al.* 1997). SRC pre-existing complexes with CBP, p300, PCAF, CARM-1 and PRMT-1 are recruited to chromatin by ligand-triggered interaction between SHRs and SRCs (Xu *et al.* 2003).

Chromatin remodelers (discussed earlier) can function as direct coregulators for SRs. Both SWI/SNF-type and ISWI-type complexes were found to potently coactivate SRs. In case of SWI/SNF, PR was reported to directly interact with BRG1-associated factor 57 (BAF57) (Vicent *et al.* 2009), while for ISWI the NURF subunit BPTF was found to be a PR hormone-dependent interactor (Vicent *et al.* 2011).

During the activation of progesterone target genes, the Cdk2/cyclinA kinase complex, which directly interacts with PR, phosphorylates SRC1 (Narayanan *et al.* 2005). Phosphorylation of SRC1 is both needed for effective PR association as well as its HAT activity, indicating an essential role of the phosphorylation event in the transactivation of PR target genes. Apart from SRC1, Cdk2 also phosphorylates the Poly-ADP-ribose (PAR) polymerase 1 (PARP-1), an ER and PR coregulator that catalyzes the transfer of ADP-ribose chains onto acceptor proteins, including histones and transcription factors. Cdk2 activity enhanced PARP-1 dependent displacement of histone H1 at PR target sites resulting in increased transcriptional activation in response to progesterone (Wright *et al.* 2012).

All in all, PR transcriptional co-activation is a sequential cascade of different steps ultimately leading to RNA PolII recruitment to target sites, were many factors are implicated in a highly dynamics process aiming at allowing chromatin access for other transcriptional cofactors (Vicent *et al.* 2011) (Fig. 16).

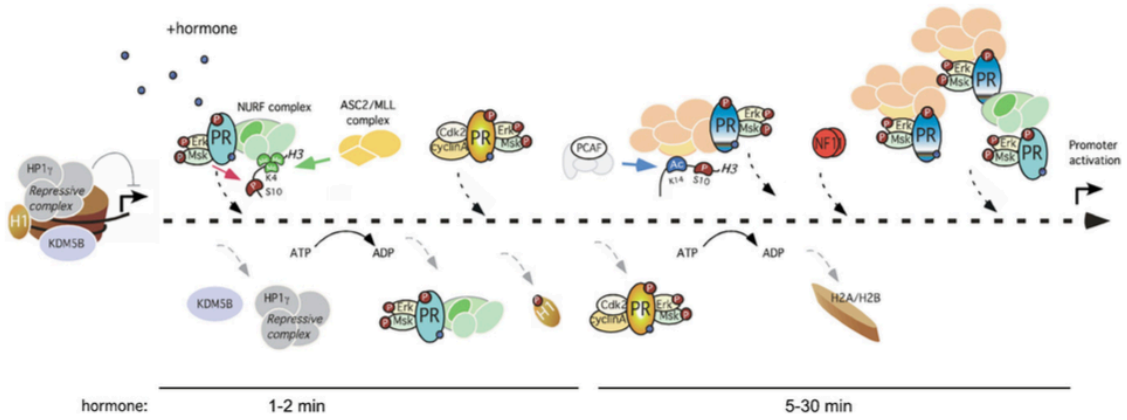


Figure 16: PR transcription activation it is shown to be a very complex cascade of events in the dynamic range of minutes after hormone exposure. From (Vicent *et al.* 2011).

1.3.8.2 Co-repressors

The focus on ligand-dependent action of PR and other SHRs has for a long time focused only on gene expression activation, however several repressive complexes has also been identified to be activated in a ligand dependent manner. The first two nuclear receptor corepressors identified were the Nuclear receptor CoRepressor (NCoR) and the Silencing Mediator of Retinoic acid and Thyroid receptor (SMRT) (J. D. Chen *et al.* 1995, Horlein *et al.* 1995), found to repress unliganded receptors. Corepressors are also shown to interact with PR in a ligand-dependent manner, mediating active down-

regulation of target genes. NCoR interaction with ER in a ligand-dependent manner activates repression mediated by the recruitment of HDACs (Fernandes *et al.* 2003). Remodeling complexes have an active role in transcription repression upon hormone induction. The Mi-2/CHD4 containing nucleosome remodeling and histone deacetylation (Mi-2/NuRD) complex, which also contain HDACs, methylated DNA binding proteins (MBDs) and histone H4-interacting proteins (retinoblastoma associated proteins PbAp 46/48), acts as a corepressor complex for ER via direct interaction with the MTA1 or the MTA2 subunits (Mazumdar *et al.* 2001, Cui *et al.* 2006).
Recently published results from RNA-seq in T47D breast cancer cells exposed to R5020 for 6h showed that more than one thousand genes that are up-regulated, and more than 600 are down-regulated (Nacht *et al.* 2016). A fraction of the down-regulated genes revealed a novel repression mechanism involving the interaction of the histone demethylase KDM1A/LSD1 and the H3K9me3 binding protein CBX3/HP1g with hormone activated PR. This complex recruits BRG1, the ATPase of the BAF complex, to the promoters of hormone-repressed genes, and facilitates the deposition of linker histone H1.2 and compaction of chromatin.

1.3.8.3 Co-regulators regulation

PR co-regulators themselves are regulated by direct interaction with sequestering proteins or by PTMs. In many cases, enzymes catalyzing these reactions can modify histones as well as non-histone proteins. For instance, it has been shown that SUMOylation of SRC1 at K732 and K774 enhances PR-transcriptional activity, by increasing PR-SRC-1 interaction and prolonging SRC-1 retention in the nucleus (Chauchereau *et al.* 2003).

1.3.8.4 In vitro transcription

Our knowledge of the mechanism of PR transactivation originated in part from a cell-free system using reconstituted minichromosomes containing the MMTV promoter and assembled preblastodermic DRosophila embryo EXtracts (DREX) (Bonte *et al.* 1999) The MMTV plasmid encompasses 6 positioned nucleosomes (Richard-Foy *et al.* 1987). The first nucleosome upstream the TATA box, called Nucleosome B, contains the 5 hormone responsive elements (HREs), and a palindromic NF1 binding site (Truss *et al.*

1995). Although only the HRE I is a perfect palindrome with 3 bp spacing, PR binds to all the HREs as a homodimer (Chalepakis et al 1988).

This system was used to demonstrate SR binding on HREs assembled in nucleosomes (Willmann *et al.* 1986), to set up a cell-free system for PR-dependent transcription activation (Kalff *et al.* 1990), to show synergistic activation between PR and NF1 (Bruggemeier *et al.* 1991), to elucidate the mechanism by which the H2A/H2B dimers are displaced from the nucleosome prior to transcription activation (Vicent *et al.* 2004), and more recently to show and characterize the HREs required for synergism between PR and NF1 (Vicent *et al.* 2010) (Fig. 17).

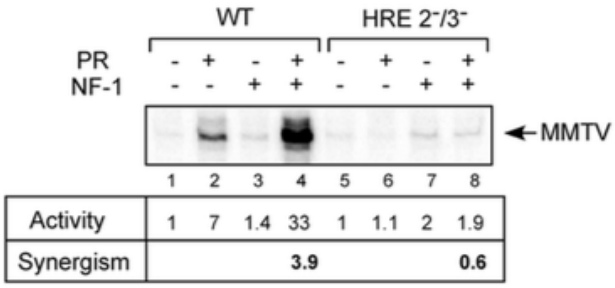


Figure 17: Figure from (Vicent *et al.* 2010), showing PR synergism with NF1 being impaired by the deletion of HRE2/3 at MMTV promoter, done in DREX assembled minichromosomes.

However the DREX system is not well characterized and we attempted to develop a biochemically defined minichromosomal transcription system following protocols from the group of James Kadonaga (Bulger *et al.* 1995) in order to define all the components required for functional transcription control.First, MMTV promoter containing minichromosomes were assembled with a circular plasmid containing the MMTV promoter driving a reporter gene, purified commercial HeLa core histones, the recombinant histone chaperon NAP1, the ACF complex, as ATP-dependent chromatin remodeler, PR and NF1 all expressed in the baculovirus system (BVES). The biochemically defined MMTV-minichromosome contained positioned nucleosomes (Fig. 18) were used for binding experiments and for in vitro transcription using HeLa cell nuclear extracts, but the transcriptional efficiency was low.

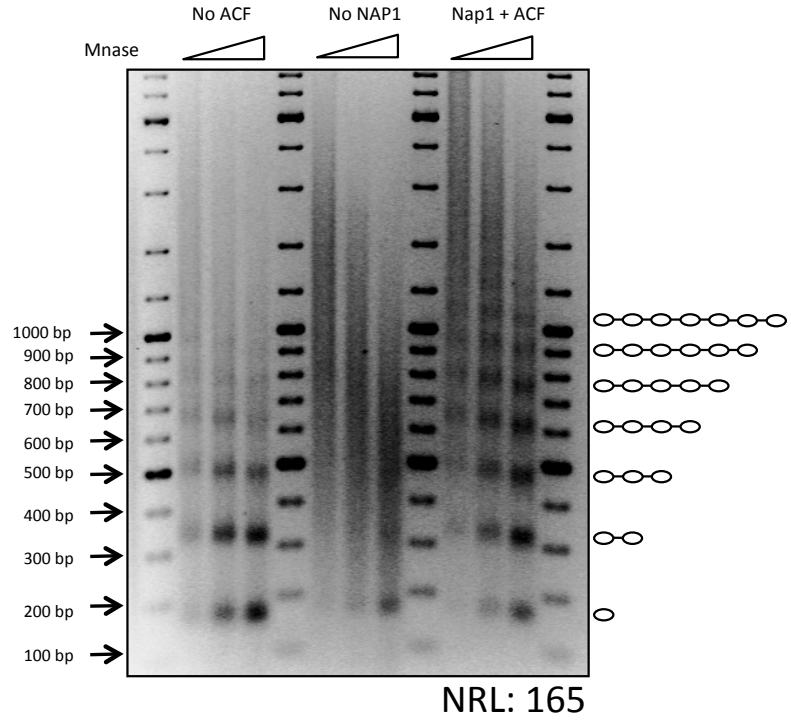


Figure 18: MNase digestion of minichromosomes assembled with the biochemically defined system, comparing the action of the chromatin remodeler ACF complex and the histone chaperone NAP1 (own work). At the left, numbers represent base pairs. At the right the scheme of the number of nucleosomes wrapped at each band.

In subsequent experiments purified calf thymus histone H1 or recombinant H1 variants were added which was shown to enhance PR synergistic activation (Koop *et al.* 2003), but still transcription was very inefficient. This was not unexpected, as we knew that additional factors are required for PR-dependent gene activation. We added different PR co-activators such as purified NURF, HATs or CDK2, but we failed in reaching the transactivation observed in DREX, indicating that the system was more complex than originally expected. It was therefore decided to identify all potential PR interactors and regulators by the use of mass spectrometry in nuclear extracts.

1.4 Progesterone receptor interactors

1.4.1 Known PR interactors

There are a number of known and characterized PR interactors, most of them implicated in the receptor transcriptional activation.

We used the iRefWeb database, which integrates protein-protein interactions (ppi) of 10 different databases, to find annotated interactors for steroid hormone receptors derived from experimental evidences. Of all steroid hormone receptors, PR the smaller number of interactors, namely 72, while there where 223 for GR, 305 for AR and 599 for ER (Fig. 19). A similar number of PR interactors, 68, is found at the STRING database (Fig. 20).

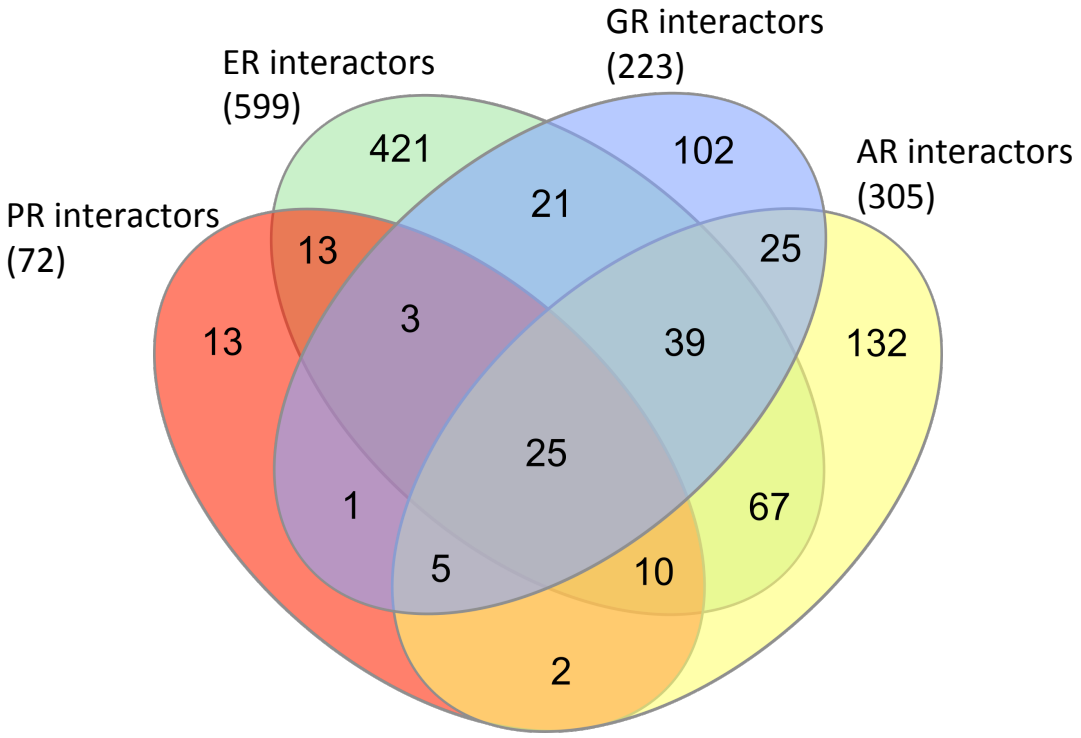


Figure 19: Venn diagram showing overlap between steroid hormone receptors annotated interactors at iRefWeb database.

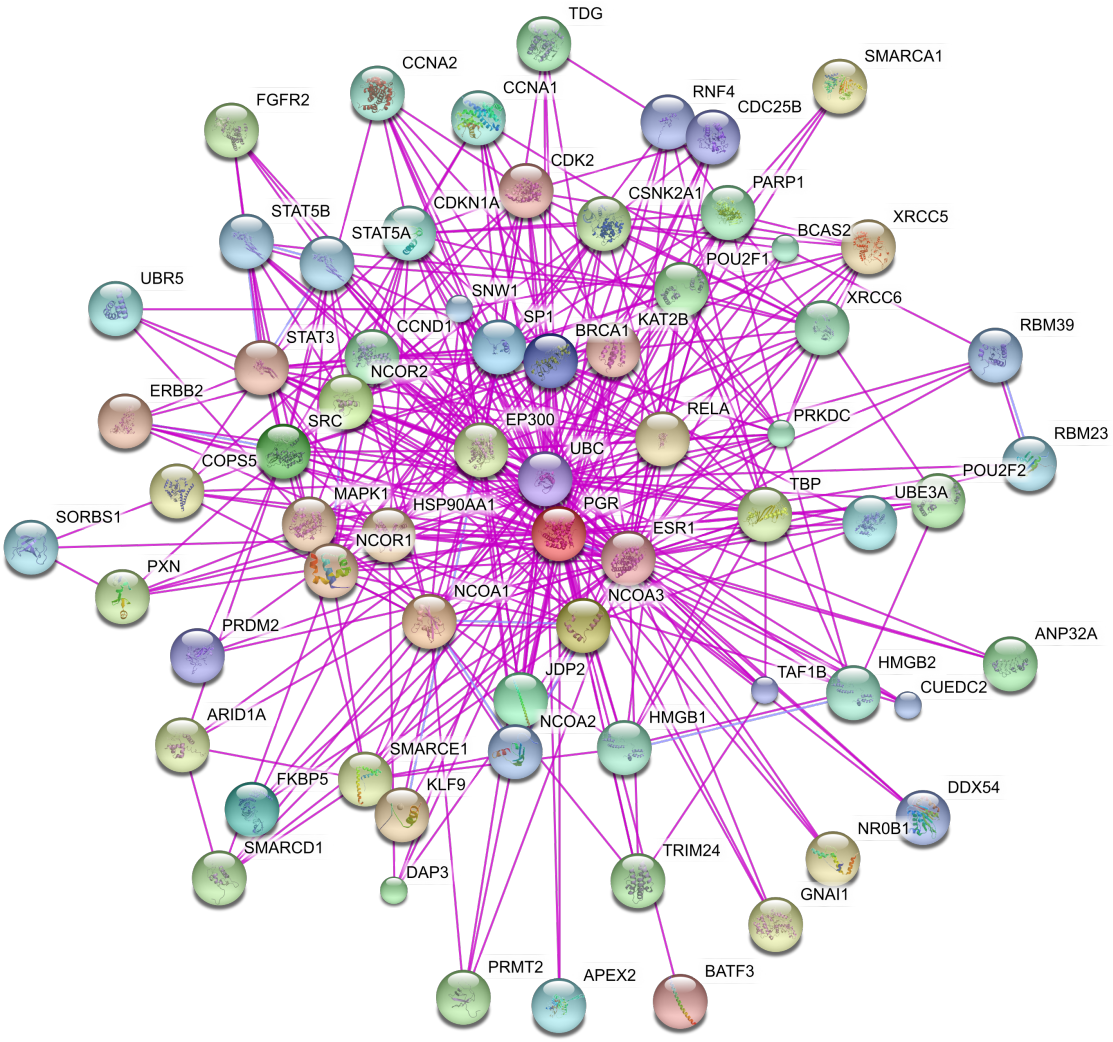


Figure 20: PPI among PR annotated interactors based on experimental evidence at STRING database (v. 10.0). PR is depicted in red.

1.4.2. Prior attempts of PR interactome

The group of Jason Carroll found in the first RIME study (Mohammed *et al.* 2015) using SILAC and exposure to hormone for 4h found only ERa as a PR interactor that increased after hormone exposure. It is difficult to compare both data sets, as mass-spec data should be normalized, and similar filtering analyses are needed. That data could be used although as longer time points of exposition compared to shorter times of our time course.

Previous attempts in our lab using the T47D-Y - a T47D-derived breast cancer cell line that does not express PR, transfected with a TAP-tag PRb were not successful. We also tested a new approach (Roux *et al.* 2012) that creates chimeras between the protein of interest and the promiscous biotin-ligase (BirA) that biotinylates proteins that come in vicinity of the target protein. Upon biotin immunoprecipitation with streptavidin, and trypsin digestion, mass spectrometry analysis should yield the interacting proteins. But the method did not work with PR in T47D cells.

For this reason, we turn to RIME (Rapid immunoprecipitation mass spectrometry of endogenous proteins), firstly published by the group of Jason Carroll (Mohammed *et al.* 2013), for finding new interactors of PR in different situations related to hormone exposure, taking the advantage of a good antibody for our protein of interest, using immunoprecipitation of the endogenous protein.

1.5 Mass spectrometry

Mass spectrometry (MS) is an analytical technique that ionizes chemical species and sorts the ions based on their mass to charge ratio. It determines the mass to charge ratio of a given sample, what is called the mass spectrum.

The basic technique consists in a tube in vacuum were the sample is vaporized, an electron gun which ionizes the sample, negatively charging it, and thanks to an electric

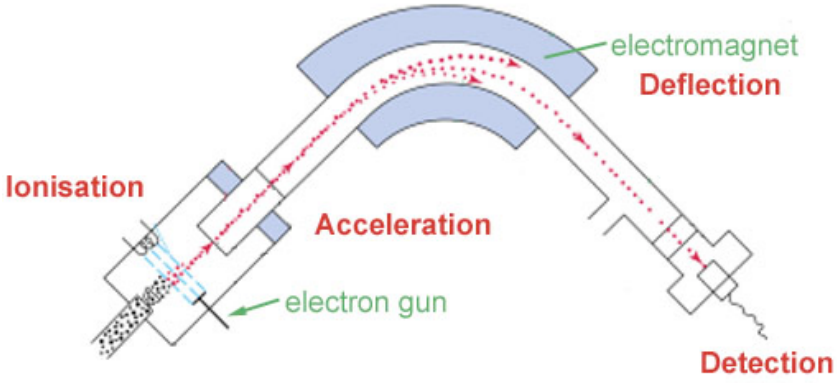


Figure 21: Scheme of a spectrophotometer. Adapted from http://scienceaid.co.uk

field, ionized samples are accelerated through the tube to the deflector. This is typically a magnet, which deflects the trajectory of the sample by its mass, the extent of the deflection depends on the mass of the ions, the greater its mass, the less it is deflected (Fig. 21). Finally, sample arrives to the detector, which originally has been a photographic plate or a phosphoscreen, but modern detectors record a current every time a cation reach the detector, calculating its mass/charge ratio (m/z).

MS/MS: Modern mass spectrometry has evolved to a much more complex system, in

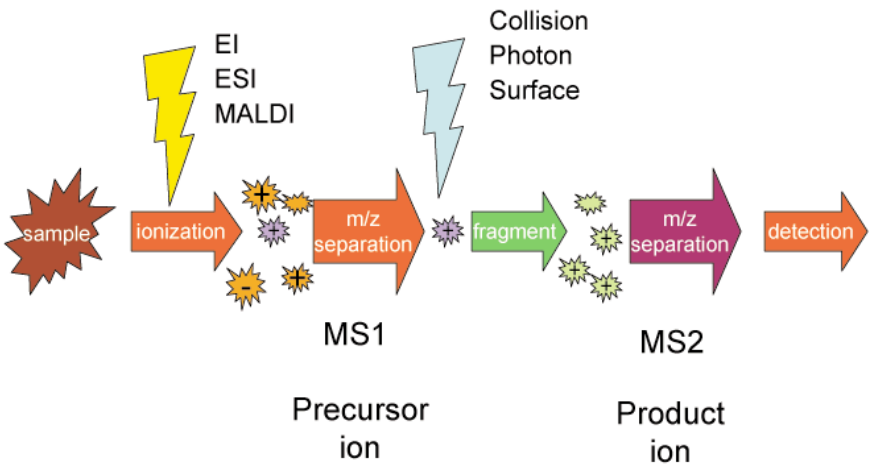


Figure 22: Scheme of MS/MS. By K. Murray (Kkmurray) - Own work, CC BY-

which samples, or in this case peptides, are fragmented by collision or other techniques between the two consecutives m/z separations (Fig. 22). Considering a given peptide, which mass is identified and isolated after the first MS, collision leads to its fragmentation (Fig. 23).

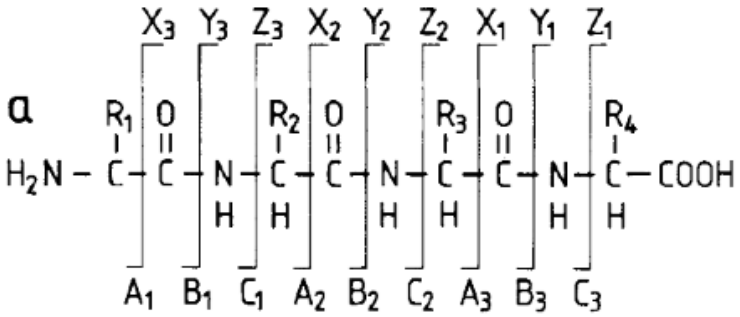


Figure 23: Nomenclature proposed for the different observed peptides, from (Roepstorff *et al.* 1984)

Fragmentation is typically happening at the peptide bonds, although secondary breaks can also happen. The random dissociation of the peptide into multiple two halves result in an spectrum of peptides, each with its own m/z ratio, and by calculating what are the masses each of the consecutive peptides detected it is possible to asses which is the order of the aminoacids, which in turn allows the identification of the protein (Fig 24).

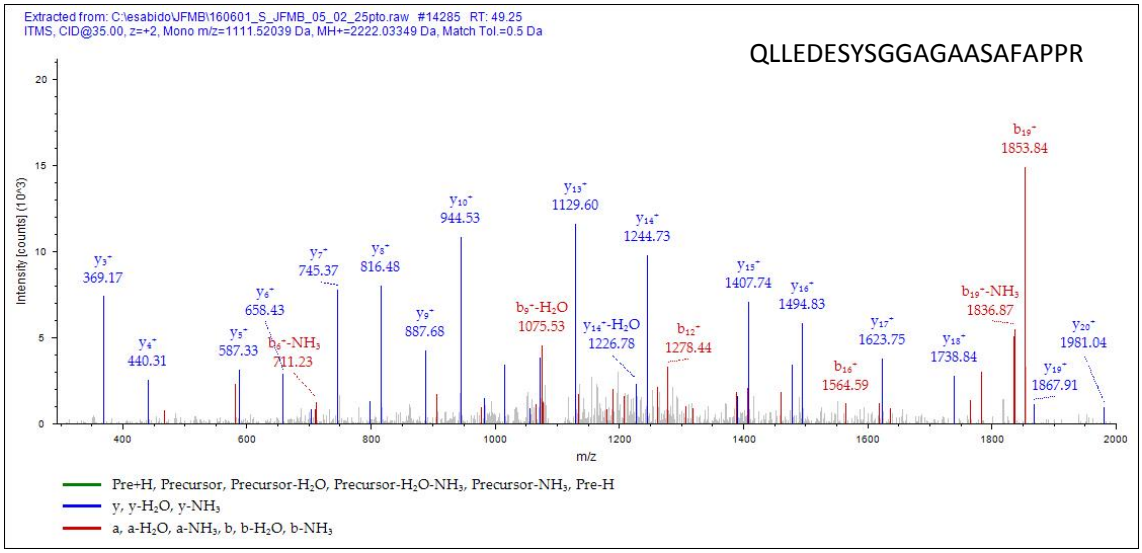


Figure 24: MS2 of a PR unique peptide fragmentation. Sequence of the identified peptide depicted inside the box.

More recently, this technique has been coupled to sample fractionation prior to injection to the analyzer. Commonly, protein samples are fractionated by running it on an acrylamide gel, cutting the lane to analyze in several bands and extract proteins in each cut for tripsinization into peptides prior to injection. With modern analyzers, a liquid chromatography device is included, and fractionation of the sample is done by automatization, in a system called LC-MS, for liquid-chromatography mass-spectrometry. A later approach for mass spec is the identification of proteins that were isolated by immunopurification, technique that is called affinity purification mass spectrometry or AP-MS.

The MS analyzer used in this thesis is LTQ-Orbitrap Velos Pro mass spectrometer (Thermo Fisher Scientific, San Jose, USA) coupled to a nano-LC (Proxeon, Odense, Denmark). It is based on the Orbitrap system invented by Makarov (Makarov 2000), in which ions are sequestered in an orbital ion trap, a central spindle electrode. Mass/charge values are measured from the frequency of harmonic ion oscillations, along the axis of the electric field, undergone by the orbitally trapped ions.

A big effort is underway to characterize interactions between proteins, and how these interactions or their loss of interaction can help elucidate their functions. It has recently published a systematic MS analysis of the human interactome, characterizing by over 2500 affinity purification experiments with different baits on HEK293 cells, over 7680 proteins, and their interactions, in a project called BioPlex Network (Huttlin *et al.* 2015). There are also several databases that report MS found protein-protein interactions, being IntAct one the best exponents (Kerrien *et al.* 2012).

The ball is now on the field of dynamics of complexes analysis and networks, as both direct and indirect interactions are similarly represented, the completion of complexes analysis after MS.

1.6 RIME protocol

Rapid Immunoprecipitation Mass spectrometry of Endogenous protein (RIME) (Mohammed *et al.* 2016) is a method that allows the study of protein complexes in chromatin, in a rapid and robust manner by mass spectrometry (MS). The method can be used in parallel with chromatin immunoprecipitation–sequencing (ChIP-seq) experiments to provide information on both the cistrome and interactome for a given protein. The method uses formaldehyde fixation to stabilize protein complexes that are immunoprecipitated using antibodies against the endogenous target. The obtained cross-linked complexes attached to beads are rigorously washed, and digested on-beads into peptides, thus avoiding the elution step, and analyzed by MS (Fig. 25).

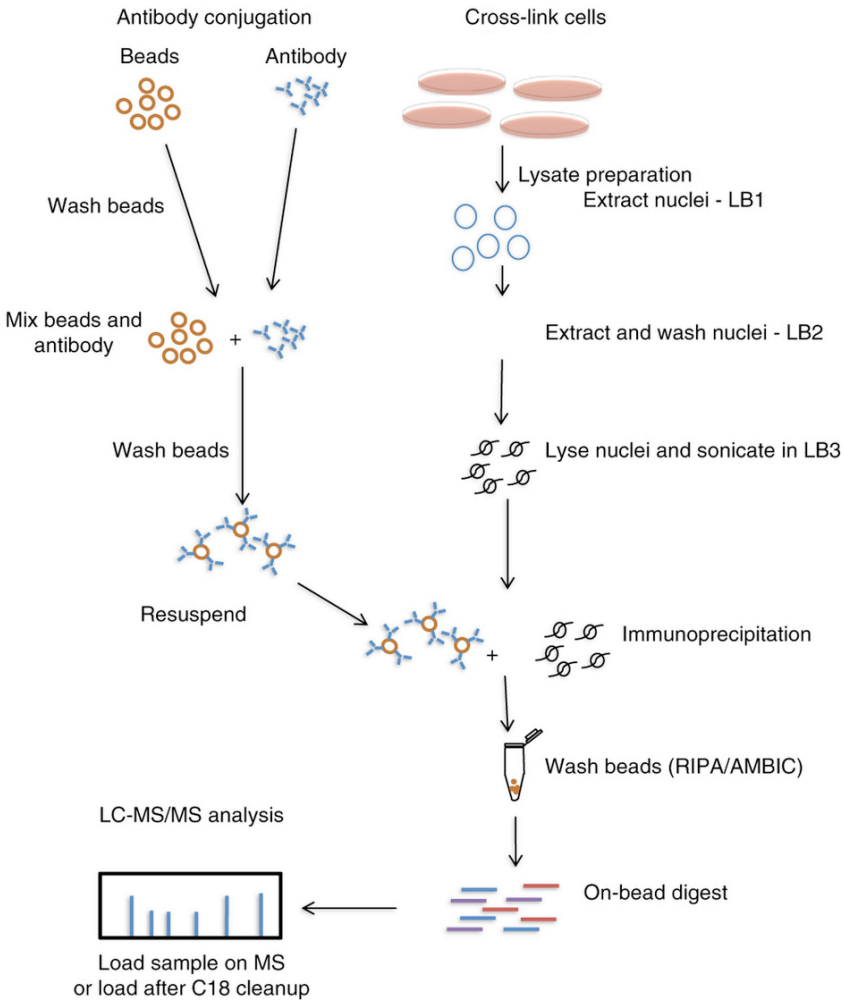


Figure 25: Scheme of the RIME procedure. Adapted from (Mohammed *et al.* 2016)

RIME can be used to identify protein complexes from limited amounts of starting material using as few as 1×10^6 cells. It is rapid, and it enriches for protein complexes that are endogenous, without the need to engineer cells either at the DNA or protein level. This final feature of RIME also eliminates artefactual interactions or perturbations as a result of overexpressed protein levels. Protein complexes can be cross-linked and purified from primary tissue and cell lines, and when coupled with ChIP-seq experiments they provide complementary results.

Although RIME has enabled better insight into several protein complexes and has the potential to be applied to immunoprecipitation of other protein classes from cellular organelles, the method does have limitations. The first limitation is the reliance on high-affinity and high-specificity antibodies. The second limitation is the current inability to discriminate multiple complexes. A single transcription factor may have thousands of genomic binding sites each with its unique combination of regulatory proteins. RIME purifies all these in a single experiment, resulting in a loss of resolution of the subtle differences in complexes at each site. Furthermore, no information is extracted about the detail of the protein assembly, and an inability to distinguish direct from indirect interactions. Another disadvantage of the methodology is the need for formaldehyde cross-linking that can result on false positives due to the known caveats of the use of formaldehyde (Poorey *et al.* 2013). The use of negative IgG controls, large number of technical and biological replicates and stringent statistical filters reduces ambiguity remains regarding the specificity of the identified interactors.

2. OBJECTIVES

1. Identification of progesterone receptor nuclear interactors in breast cancer cells in response to the potent progesterone agonist R5020 exposure.

2. Characterization of the dynamics of PR interactors in response to hormone.

3. Identification of functional protein complexes interacting with PR.

4. Identification of the differences between the PR interactors in response to progestins and to the partial agonist of progesterone RU486.

3. Materials and methods

3.1 Materials

All plates and pipettes used for culturing cells were from Falcon (Corning).

1.7ml siliconized tubes were from Sorenson, #11720.

Cell line used for all experiments is a clone of the T47D breast cancer cell line (ATCC number HTB133). This clone is named 3/17, and encompasses a single copy insertion of the MMTV promoter (Truss *et al.* 1995).

Red-RPMI 1640 was from Gibco (Life technologies), # 42401-018.

White-RPMI 1640 was from Gibco (Life technologies), # 32404-014.

Penicillin/streptomycin was from Gibco (Life technologies), # 15070-063.

Glutamine was from Gibco (Life technologies), # 25030-024.

Tripsin-EDTA for cell release was from Gibco (Life technologies), # 25300-054

Fetal Bovine Serum was from Gibco (Life technologies), # 10270.

Insulin was from Lilly, # 917476.7

Charcolized Fetal Bovine Serum was from HyClone, # 30068.03 (ThermoFischer).

R5020 was purchased from PerkinElmer, Life Sciences.

RU-486 was from Sigma.

16% formaldehyde electron microscopy (EM) grade was purchased from Tebu-bio # 18814-20.

Sonication-suitable polystyrene 15ml tubes were from Falcon (Corning), #352095.

Protein A magnetic beads were from Dynabeads, #10001D from Novex.

Trypsin for peptide digestion was purchased from Promega, Sequence grade, #V5111.

Endopeptidase LysC was purchased from Wako, # 129-02541.

Ultra-Micro spin columns, solid-phase extraction cartridges for sample desalting (Harvard Apparatus, cat. no. 747206)

PAGE loading buffer was from Roth, Roti-Load1, 4x concentrated, # K929.1.

Pre-cast gels were from Invitrogen, # NP0335BOX (Life technologies).

Ammonium bicarbonate (ABC), Iodoactamide (IAA), DL-Ditiothreitol (DTT), phenylmethylsulfonyl fluoride (PMSF), Sodium orthovanadate (Na_3VO_4) and Formic acid were from Sigma.

Protein inhibitor cocktail (PIC) was from Roche; cOmplete, EDTA free, #05056489001

Antibodies

Progesterone receptor (H-190) was from Santa Cruz, sc-7208. All immunoprecipitations were done with the same lot, #J2513.

PR s294 phosphorylation: from Abcam, ab61785

Rabbit control IgG: from Abcam, ab46540

3.2 Western-blotting

For western-blotting analyses, same protocol for immunoprecipitation was done as for RIME protocol (section 3.3), with the exception that after extensive washes, beads were resuspended in 1X SDS Loading buffer (Roth) and boiled for 10 minutes. Appropriate amount of eluate was loaded on NuPAGE 4-12% gels for the different time points, as well as for IgG control, along with 5% input and molecular weight markers (PageRuler Prestain or Spectra for high molecular weight, Life Technologies), and fractionated typically at 150V for 1hr. After proteins were separated by size, they were transferred to a nitrocellulose membrane (Bio-Rad) in a wet transfer system (BioRad, MiniTransBlot cell) with transfer buffer (25 mM Trizma base, 192 mM glycine, 20% methanol) at 90V for 90 minutes. After transfer, membranes were blocked with 5% skim-powder milk (Sigma) diluted in TBS-0.1% Tween (T-TBS) at room temperature for 1 hour. Incubation with primary antibodies was done either for 1 hour at room temperature or over-night at 4ºC. The concentration used of the different primary antibodies varied between them, being diluted in 2.5% skim milk in T-TBS in a concentration according the manufacturer's instructions. After primary antibody incubation, membranes were washed three times for 10 minutes in T-TBS and incubated 1 hour with secondary antibody (NA931V for mouse/NA934V for rabbit, GE healthcare) diluted 1:4.000 in 2.5% skim milk in T-TBS, excited with ECL reagent, exposed for different times with ECL membranes and developed.

3.3 RIME (*R*apid *I*mmunoprecipitation *M*ass spectrometry of *E*ndogenous proteins)

RIME protocol used is an adaptation of previous publications (Mohammed *et al.* 2013, Mohammed *et al.* 2016) to our model system, and to previous experiments from the lab obtained for ChIP-seq, to be able to match results from both protocols. It was also adapted for obtaining a broader set of interactors, which thanks to several replicates and time points end up with a big amount of high confidence interactors.

3.3.1 Cell culture and hormone treatment

Cells were grown and expanded in Red-RPMI 1640, 1% Glutamine, 1% Pen-Strep and 10% FBS and 100 ul insulin.

For induction experiments, cells were seeded at 70%, typically 5x10*6 cells per p150 in RPMI without phenol red (white), 1% Glutamine, 1% Pen-Strep, 100 ul insulin and 10% charcolized FBS.

After 48h in hormone-depleted media, cells were grown without serum by changing media to serum-free white media (starvation) for 16h (over night).

R5020 stock ($2x10^{-3}$ M) was diluted 1:20 in 100% ethanol for a final concentration of 10^{-4}. This dilution can be kept at -20ºC for further use.

10^{-4} stock was diluted with white media (no FBS) 1/100, for final 10^{-6}.

From this, it was diluted 1/100 to the medium contained at the plate, for a p150, 20 ml of media and 200 ul of 10^{-6} dilution of R5020, for a final 10^{-8} (10nM).

For RU-486 experiments, protocols and concentrations are equivalent, final concentration is 10^{-8} (10nM).

Cells were induced for the desired time and media was aspirated, followed by the addition of 11ml of cross-linking solution. Cross-linking solution is a mix of 69% of 16% HCOH with 31% of Hepes pH 8.0 50mM, NaCl 100mM, EDTA 1mM and EGTA 0.5mM. This solution is at the same time diluted with white serum-free media in a ratio 1:11. Final concentration of formaldehyde is 1%.

3.3.2 Extract preparation and immunoprecipitation

Cross-linking was stopped after 8 minutes by adding a final 200mM glycine and incubating at RT for 5 minutes. Plates were kept on ice and washed twice with cold

PBS. Cells were scrapped in ice-cold PBS with inhibitors and collected in a 15 ml tube suitable for sonication (BD Polyestyrene, 352095). Cells were centrifuged at 4000 rpm for 5 minutes and washed twice with cold PBS. All buffers contain freshly added inhibitors in the following concentration: cOmplete EDTA-free as recommended by manufacturer (1 tablet for 50ml), PMSF at final 10uM, Na_3VO_4 at final 10 uM.

10 ml of lysis buffer 1 (Hepes pH 7.5 50mM, NaCl 140mM, EDTA 1mM, Glycerol 10%, NP-40 0.5%, Triton X-100 0.25%) was added to each sample, and incubated on ice for 10 minutes. After centrifugation, pellet was resuspended in 10 ml lysis buffer 2 (Tris pH 8.0 10mM, NaCl 200mM, EDTA 1mM, EGTA 0.5mM) and incubated on rotation for 5 minutes at 4ºC and centrifuged again. Pellet was then resuspended in 400 ul of lysis buffer 3 (Tris pH 8.0 10mM, NaCl 100mM, EDTA 1mM, EGTA 0.5mM, Na-deoxycholate 0.1%, N-lauroylsarcosine 0.5%) by carefully pipetting up and down for ten times. Reuspended extracts were sonicated in a Bioruptor (Diagnode) at 4ºC, for 9 cycles of 30 seconds on/30 seconds off, at High output. After sonication, sample was transferred to a 1.7 ml siliconized tube, and 10% triton X-100 was added. Lysates were centrifuged and supernatant was added to the beads-antibody conjugation.

Antibody binding to the beads was done typically, for 10^7 cells, with 100ul of Protein A magnetic beads washed once in PBS, resuspended in 500 ul of LB3, with the appropriate amount of antibody or IgG (75ul of H-190 for PR, at 0.2ug/ul, or 12 ul of Rabbit IgG), incubated for 3 hours at 4ºC and wash with LB3 twice, 500 ul each.

After over-night incubation, beads were washed 10 times with RIPA buffer (Tris pH 7.4 50mM, NaCl 150mM, Na-deoxycholate 0.5%, NP-40 1%, SDS 0.1%) and 2 times with 100mM ammonium hydrogen carbonate (AMBIC) solution. For the second wash, beads were transferred to new 1.7 ml tubes.

3.3.3 Tryptic digestion

The sample was reduced by adding 10 µl of 10 mM DTT in 100mM ABC buffer (1h, 37ºC) and alkylated by adding 10 µl of 20 mM IAA in 100mM ABC (30 min, RT, avoided from light). The digestion was done in two steps; a first digestion was carried out by the addition of 1 ug of Endopeptidase LysC, incubated over night at 37º, 700 rpm. Second, 1 ug of sequencing grade Trypsin was added and incubated for 8 hr at 37ºC, 700rpm. The digestion reaction was stopped with formic acid (5% final

concentration). Supernatant was taken and tryptic peptides were desalted with C18 columns, dried in a Speed-vac and re-suspended in 10 µL of H2O + 0.1% formic acid.

3.4 Mass spectrometry

The proteomics analyses were performed in the CRG/UPF Proteomics Unit. The CRG/UPF Proteomics Unit is part of the Spanish Platform of Molecular and Bioinformatics Resources (ProteoRed), Instituto de Salud Carlos III (PT13/0001).

From the resuspended sample, 4.5 µL of each peptide mixture was analyzed using a LTQ-Orbitrap Velos Pro mass spectrometer (Thermo Fisher Scientific, San Jose, USA) coupled to a nano-LC (Proxeon, Odense, Denmark) equipped with a reversed-phase chromatography 2-cm C18 pre-column (Acclaim PepMap-100, Thermo; 100 µm i.d., 5 µm), and a 25-cm C18 analytical column (Nikkyo Technos, 75 µm i.d., 3 µm). Chromatographic gradients started at 3% buffer B with a flow rate of 300 nL/min and gradually increased to 7% buffer B in 1 min and to 35% buffer B in 60 min. After each analysis, the column was washed for 10 min with 90% buffer B (Buffer A: 0.1% formic acid in water; Buffer B: 0.1% formic acid in acetonitrile). The mass spectrometer was operated in positive ionization mode with nanospray voltage set at 2.5 kV and source temperature at 200 °C. Ultramark 1621 was used for external calibration of the FT mass analyzer prior the analyses. The background polysiloxane ion signal at m/z 445.1200 was used as lock mass. The instrument was operated in data-dependent acquisition (DDA) mode, and full MS scans with 1 microscan at resolution of 60 000 were used over a mass range of m/z 350−1500 with detection in the Orbitrap. Auto gain control (AGC) was set to 106, dynamic exclusion was set at 60 s, and the charge-state filter disqualifying singly charged peptides for fragmentation was activated. Following each survey scan, the 10 most intense ions with multiple charged ions above a threshold ion count of 5000 were selected for fragmentation at normalized collision energy of 35%. Fragment ion spectra produced via collision-induced dissociation (CID) were acquired in the linear ion trap, AGC was set to 3·104 and isolation window of 2.0 m/z, activation time of 30 ms, and maximum injection time of 250 ms were used. All data were acquired with Xcalibur software v2.2.

Data Analysis: Acquired data were analyzed using the Proteome Discoverer software suite (v1.4, Thermo Fisher Scientific), and the Mascot search engine (v2.5, Matrix Science) was used for peptide identification. Data were searched against the human

protein database derived from the SwissProt database plus common contaminants (April 2016; 20,200 sequences). A precursor ion mass tolerance of 7 ppm was used, and up to three missed cleavages were allowed. The fragment ion mass tolerance was set to 0.5 Da, and oxidation (M), and acetylation (Protein N-term) were defined as variable modifications, whereas carbamidomethylation (C) was set as fixed modification. The identified peptides were filtered by FDR < 0.01 (1%). Proteome Discoverer gives an approximate estimation of protein amount with the parameter "Area Under the Curve" (AUC) which is the average peak area of the 3 top peptides for a given protein.

3.5 Bioinformatic analysis

3.5.1 SAINT (*S*ignificance *A*nalysis of *INT*eractome) analysis

Protein-protein interactors (ppi) were assessed with the SAINT software (H. Choi *et al.* 2011). SAINT utilizes a semi-supervised mixture model of the spectral count distribution of each protein across the negative control runs and provides probability values that each bait-prey interaction is real (Skarra *et al.* 2011), and it has been widely spread and revised in the field of MS data analysis. The spectral counts obtained by MS are normalized by the length of the proteins and to the total number of spectra in the purification, in our case, considering (Pardo *et al.* 2012)each time point replicates. The method then models distributions for true and false interactions, compared with IgG negative controls, and calculates the probability of genuine ppi. As an output, SAINT provides a Bayesian Fold Discovery Rate (hereafter FDR), an IgG fold change (IgG FC) and a SAINT score.

FDR is the probability to a given identified protein to be a false positive. IgG FC is the enrichment of a given protein compared to the same identified protein at the IgG control. SAINT score is a probability for a given interacting protein to be true, in an inversely relation with FDR.

After obtaining a given FDR and IgG FC values for every protein and every time point, Fisher's combined probability test (Fisher 1925) was used for computing 1 single FDR for every identified protein, fusing data from all time points.

For filtering results, FDR was used. Proteins with at least 2 time points with FDR < 0.05 (95% confidence) and a compiled FDR < 0.01 (99% confidence) were selected as **High**

Confidence list, with the addition of any protein with at least 1 time point with an FDR < 0.005 (99.5% confidence).

A second list namely Moderate Confidence list was set up by any protein with at least 1 FDR < 0.2 (80% confidence). This list of proteins was solely used for expanding possible components of complexes identified.

IgG fold change was used for exploring the dynamics of the interaction of a given protein with progesterone receptor. A variation of this value over time of exposure to hormone was considered a variation of the interaction of the protein with PR. It was also used as a relative value of intensity of the interaction, or 'amount' of protein found, although with label-free proteomics is difficult to assess the relative quantities of different proteins in an AP-MS experiment.

3.5.2 M-Fuzz clustering

Additionally, in order to select those proteins which show a substantial change upon hormone treatment, proteins with an IgG FC lower or equal than 1.50 were selected as proteins with an stable interaction with PR (n=66). It was only retained for the clustering proteins in which the ratio between its maximum and minimum value was greater than 1.50 (n=249 proteins).

To these, per-protein normalization of the RIME values was applied, that is, each data point of a given protein was subtracted by the protein mean value and then divided by the standard deviation of the protein values. This ensures that the RIME time profiles are comparable between proteins despite the broad range of the IgG FC values.

Clustering was applied to the filtered normalized profiles to find groups of proteins that follow a similar time dynamics. Because hard clustering, i.e. each protein is assigned uniquely to a cluster, has some limitations (http://mfuzz.sysbiolab.eu/), soft clustering was used, as implemented in the R Mfuzz package (L. Kumar *et al.* 2007). Mfuzz uses two parameters: the fuzzification parameter (m) and the number of clusters (c). While Mfuzz deterministically estimates the value of m from the data, there may be a range of c values that result in biologically meaningful number of clusters. To find an optimal range of c values for the dataset, the number of non-empty values for increasing values of c was calculated; the rationale behind is that increasing c is useless when the proportion of non-empty clusters starts to decay. For optimal range of c it was obtained

the clusters profile and decision on which number of clusters to use was taken according to biologically meaningful aggrupation (see Results section).

3.5.3 Enrichment analyses

For Gene Ontology terms and pathways enrichment analysis a web-based tool called *Enrichr* (E. Y. Chen *et al.* 2013) was used. Enrichr implements four scores to report enrichment results: *p-value*, *q-value*, *rank (Z-score)*, and *combined score*.

The **p-value** is computed using a standard statistical method used by most enrichment analysis tools: Fisher's exact test or the hypergeometric test. This is a binomial proportion test that assumes a binomial distribution and independence for probability of any gene belonging to any set.

The **q-value** is an adjusted p-value using the Benjamini-Hochberg method for correction for multiple hypotheses testing.

The **rank score** or **z-score** is computed using a modification to Fisher's exact test in which it is computed a z-score for deviation from an expected rank.

Finally, the **combined score** is a combination of the p-value and z-score calculated by multiplying the two scores as follows:

```
c = log(p) * z
```

Where `c` is the combined score, `p` is the p-value computed using Fisher's exact test, and `z` is the z-score computed to assess the deviation from the expected rank. The combined score provides a compromise between both methods.

Enrichr provides all four options for sorting enriched terms, and for the analyses, terms with p-value < 0.05 were shown, sorted by maximum combined score.

3.5.4 CORUM database

CORUM stands for the *CO*mprehensive *ResoUrce* of *M*ammalian protein complexes, and it is database that provides a manually curated repository of experimentally characterized protein complexes from mammalian organisms, mainly human, but also from mouse and rat (Ruepp *et al.* 2008). It provides the method of evidence for the complexes described, as well as publication record. It is being kept updated every year, and the version used in this present work was downloaded on 22 December 2015. It is available freely at http://mips.helmholtz-muenchen.de/genre/proj/corum/index.html.

Many CORUM annotated complexes allow the presence of one of two or more related proteins; this was taken into account for percentage of complexes found analysis.

3.5.5 Cytoscape

Cytoscape (Shannon *et al.* 2003) is a popular software for biological network visualization. It is an open-source platform for network visualization, integrating state measurements and analysis of networks. In Cytoscape, biological entities like proteins, complexes or genes are represented as a node. They are connected with edge representing protein-protein interaction or protein-DNA interactions. Either node or edge can be further described by attributes. A key feature of Cytoscape is to create a "data-to-visual mapping" according to attributes: a gradient style for numeric attributes, or discrete style for non-continuously attribute. This feature visual mapping style allows multiple information overlay in single network and gives biologist a synoptical view. What makes Cytoscape so popular is that is a open source software with an accessible application programming interface (API) using Java programming language. Software developers build extensions called plugins to add new features to Cytoscape. The version used in this thesis is 3.4.0.

The application used in this present work is Clustermaker (J. H. Morris *et al.* 2011), a plugging that implements several clustering algorithms and provides network, dendrogram, and heat map views of the results. Among the available algorithms at this plugging, Community Clustering (or GLay) was used: this algorithm is generally used for finding modules and complexes within protein-protein interaction networks and for identifying functionally related groups of proteins within large protein-protein similarity networks (Newman *et al.* 2004, Su *et al.* 2010).

3.5.6 Venn diagrams

Three-way Venn diagrams were generated with the venn2 package (https://pypi.python.org/pypi/matplotlib-venn) for Python.

For 4-ways Venn diagram it was used InteractiVenn, a web-based tool (Heberle *et al.* 2015).

3.5.7 Wordclouds

Wordclouds were made with the online tool Wordle.net, which allow sizing the words and color-coding.

3.5.8 STRING complexes

Complexes figures were created using STRING on-line database, version 10.0 (STRING consortium 2016).

Identifiers for the complexes components were uploaded for annotated with experimental evidences ppi search, and the resultant figure was manipulated for color-coding using Inkscape.

For proteins with known 3-D structure solved, it is depicted inside the balloon representing the protein, otherwise balloon is smaller.

4. RESULTS

4.1 Model system: T47D breast cancer cell line

All the experiments were performed with the T47D breast cancer cell line (ATCC number HTB133), which was originally obtained from a patient with duct breast carcinoma (Keydar *et al.* 1979). It is categorized as Luminal A type and expresses high affinity receptors for progesterone (PR), estradiol (ERα), androgen (AR) and glucocorticoid (GR) hormones, but not HER2, and have low expression of Ki67 (Holliday *et al.* 2011). It has an aneuploidic karyotype, showing 66 chromosomes (Keydar *et al.* 1979) (Fig. 26).

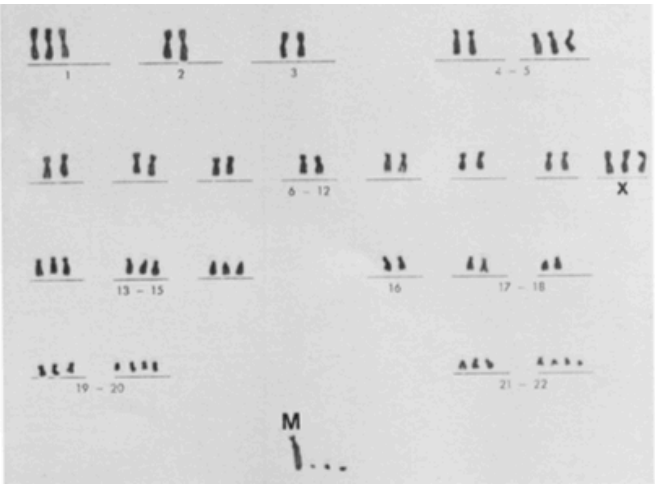


Figure 26: T47D karyotype. A representative karyotype of a T47D cell showing 62 chromosomes including markers (M) (Keydar *et al.* (1979)

In T47D in the absence of hormone the levels of PR are higher than those of ER. They express both isoforms of the receptor, PRA and PRB, with an equimolar ratio (Fig. 27).

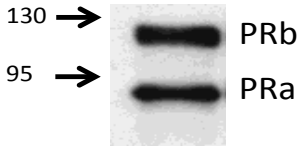


Figure 27: Western blot for PR from T47D cells.

For most experiments, cells were cultured for 48 hours in the absence of phenol red and with hormone-depleted serum (charcoal-treated serum), followed by 16 hours of serum starvation, in order to obtained a population highly enriched (close to 80%) in cells in G0/G1 (Fig. 28).

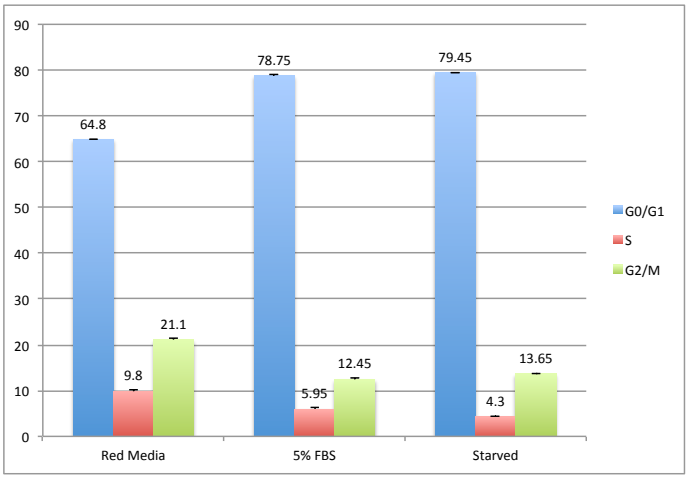


Figure 28: Cell cycle analysis of T47D in different growing conditions. T47D cells were grown in normal expanding media (Red media), in 5% hormone-depleted fetal bovine serum without phenol red (5% FBS), or after 16hr in serum-free media (Starved) and subjected to FACS analysis. Compared to cells in red media, cells 5% FBS white medium of serum starved had less cells in S-phase and G2.

Even under these conditions, not all cells respond to progestins (Wright *et al.* 2016). Previous work using ChIP-seq has shown that there are approximately 25,000 PR binding sites in starved T47D cells exposed to hormone for 30 to 60 min, and that these sites are referentially organized in nucleosomes (Ballare *et al.* 2013). Moreover, it is known that following PR binding there is extensive remodeling of nucleosomes containing the PR binding sites, that requires PR associated kinases, histone modifying enzymes and ATP-dependent chromatin remodeling enzyme complexes (Vicent *et al.* 2006, Vicent *et al.* 2009, Vicent *et al.* 2010, Vicent *et al.* 2011). To get insight into this complex process and after trying several approaches we decided to use RIME, a proteomic protocol for identifying PR interacting proteins in chromatin (Mohammed *et al.* 2015).

4.2 RIME Method validation

RIME stands for *Rapid Immunoprecipitation Mass spectrometry of Endogenous proteins*, and it was first used by the group of Jason Carroll (Mohammed *et al.* 2013). Its results depend on the affinity and the specificity of the antibody for the protein of interest. In a fast and processive manner, nuclear extracts are obtained, sonicated and centrifuge and the soluble fraction is immunoprecipitated with antibody bound to magnetic dynabeads. The beads are stringently washed with RIPA buffer, and subjected to tryptic digestion, and the supernatant is prepared for mass spec analysis. Since there is no elution step, the reproducibility and the recovery are high.

We did a first test with the RIME protocol comparing starved T47D cells not exposed to hormone (Time 0) and of cells exposed to 10 nM R5020 for 30 minutes (T30). The mass-spec results from the proteomic facility detected 40% of the possible PR peptides in samples immunoprecipitated with the PR antibody (PR H-190), and no PR peptides of were found at IgG control sample. After these results, we prepared samples for two more replicates for each time point (T0 and T30), in order to have triplicates. Proteins found in these triplicates were analysed by MS-Stats (MS-stats is an R package for statistical relative quantification of proteins and peptides in global, targeted and data-independent proteomics) to filter out proteins that were not relevant compared with IgG data. Proteins present in 2 of 3 replicates with the PR antibody but not in the IgG controls were analysed by MS-stats, and filtered by proteins with an adjusted p-value of 0.05. Among these proteins, we found some annotated PR interactors, such as PARP1, NF1, LSD1, FKBP4, HP1γ, HDAC1, SRC1, p300 or subunits of the BAF complex (BAF170 or BAF60B) (Peattie *et al.* 1992, Onate *et al.* 1995, Z. Liu *et al.* 1999, Vicent *et al.* 2010, Wright *et al.* 2012, Nacht *et al.* 2016).

We also found proteins related to splicing (SRSF1, SRS10, SRSF2, SRSF7, SRSF9, SF01 or U2AF1), DNA repair (MSH2, RAD50, MRE11 or XRCC6), putative pioneer factors (GATA3, FOXA1, AP2A, GTF2I or SOX13) and components of the Mediator complex, along with several ribonucleoproteins components (HNRDL, HNRPF, HNRH1, HNRH3, HNRPK, HNRPL, HNRPM, HNRPU, HNRL1, ROA2), RNA binding proteins (RBMX, RBM10, RBM14, RBM27, EWS) and several ribosomal proteins.

We have previously shown very rapid (1-5 min) effects of progestins on linker histone H1 displacement, followed by slower (10-30 min) displacement of histones H2A/H2B (Vicent *et al.* 2011). Therefore, we decided to do a more detailed time course of the hormone induction changes in RIME, including samples expose to hormone for 1 minute (T01), 5 minutes (T05), 15 minutes (T15) and 60 minutes (T60). The final set of data used for our analysis included 6 replicates for T0 and T30, 5 replicates for T01, T05, T15 and T60 and 8 replicates for the IgG controls.

To demonstrate the need of experimental replicates, we plotted Venn-diagrams with 4 random replicates for MS identified proteins from T30 experiments data before filtering. In Figure 29 is shown that between 59-69% of the proteins from each list are present in the rest of the replicates (highlighted with a blue circle). Including the 6 replicates, this number is reduced to 454.

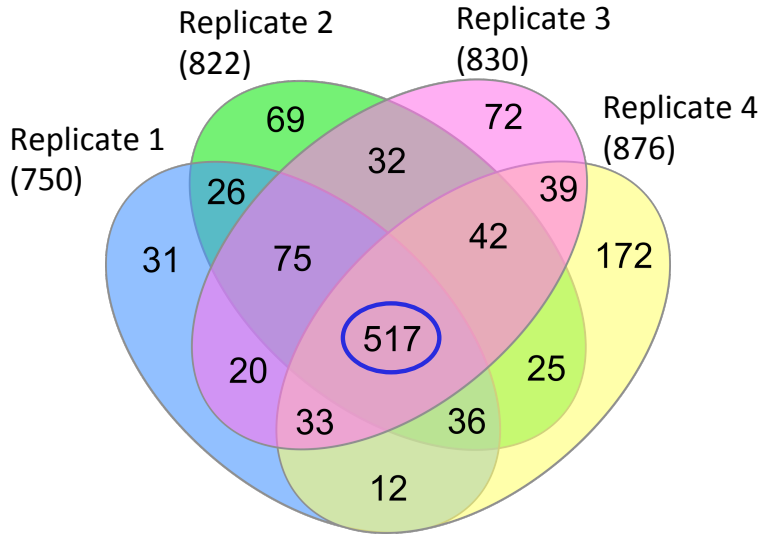


Figure 29: Venn diagram comparing non-filtered identified proteins from 4 different T30 replicates.

4.3 Mass spec data analyses

We performed Principal Component Analysis (PCA) with area under the curve data from the MS results to detect batch effect on the samples (Fig. 30). We found that no matter which batch is, IgG MS appear clustered at the same point (in red), differentiated from the rest of points with PR immunoprecipitation. Apart from this, the MS analyses done at the same time exhibit a tendency to cluster together, but in general it can be concluded that the results are independent of batch.

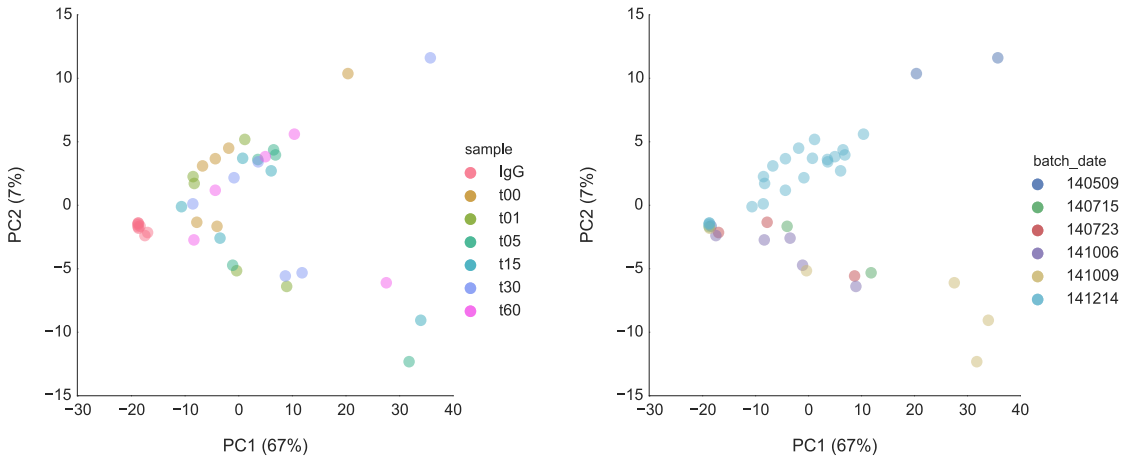


Figure 30: Principal Component Analysis of Mass-spec data. Area Under the Curve data was used from all replicates including IgG immunoprecipitated samples for PCA analysis. Left panel: distribution by Time points. Right panel: distribution by date of experimental replicate.

With the entire time course analyzed, spectral counts (mass-spec area) for PR added to $3x10^9$, reaching 72.4% peptide coverage of the protein sequence. Other previous RIME publications reached 40% coverage of the bait protein (Mohammed *et al.* 2013, Mohammed *et al.* 2015). Peptides of PR were found in all the domains of the protein, including PRB Upstream Sequence (BUS) at the N-terminus (Sartorius *et al.* 1994) (Fig. 31). However, in this study we could not distinguish between the two isoforms PRA and PRB as the antibody (PR H-190) we used recognizes both isoforms (Jacobsen *et al.* 2012).

Figure 31: Schematic representation of progesterone receptor identified peptides. Upper panel shows distribution of regions identified by mass spec (in green). Lower panel depicts schematic representation of PR with its domains. Peptides were identified for all the domains.

In total, we identified 2,345 proteins, including possible contaminants like keratins or trypsin. An aliquot of each sample was used for western-blot analysis and showed an efficient PR immunoprecipitation, and activation of PR, shown by phosphorylation of serine 294 (Fig. 32).

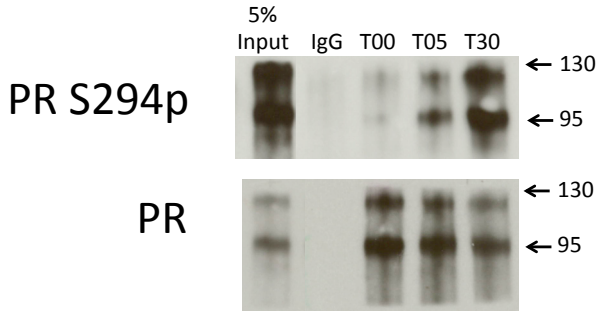


Figure 32: Western-blot for PR 294 phosphorylation for different time points (upper panel). Western-blot for PR immunoprecipitation (lower panel). At the right is depicted the MW.

4.3.1 Filtering mass spec results

After testing several possible ways of filtering the results (as MS-Stats), we choose the SAINT algorithm (H. Choi *et al.* 2011), explained in the Methods section. Briefly, this method analyses the MS data for every peptide obtained in all the replicates for each time point (PR immunoprecipitation), and compares it with the data obtained with the negative IgG control for every protein. As a result, one unique fold change over the IgG and one unique False Discovery Rate (FDR) is obtained for every protein at every time point. FDR is used for filtering confident data and IgG fold change is used to measure the dynamics of the association of each protein with PR.

Proteins with at least 2 time points exhibiting a FDR lower than 0.05 (confidence higher than 95%) and a combined FDR lower than 0.011 (99% confidence), with the addition of proteins with 1 time point exhibiting a FDR smaller than 0.005 (confidence higher than 99.5%), were chosen as **High Confidence interactors (HCI)**. Combined FDR adds data from all the time points of the time course experiment to compute a single, summary FDR for every protein, using Fisher's combined probability test (Fisher 1925). With this filtering, **315 proteins** (Table 1 for complete list) were considered as high confidence hits from a total of 2,345 proteins identified. We also set a less stringently filtered set of **Moderate Confidence Interactors** (MCI, 527 proteins), with at least 1 time point exhibiting a FDR smaller than 0.2 (Table 2 for complete list), which was

only used to find additional components of the possible complexes identified in the stringent data set.

4.3.2 Whole list overview

A correlation matrix was constructed with the 315 high confidence hits using ProHitstool (Knight *et al.* 2015) to search for similarities between time points (Fig. 33). This analysis showed that the most similar time points are T05 and T15, followed by T30 and T60. T01 had slightly more correlation with T05 and T15 than with T00, and the latter has very low correlation with the rest of the time points.

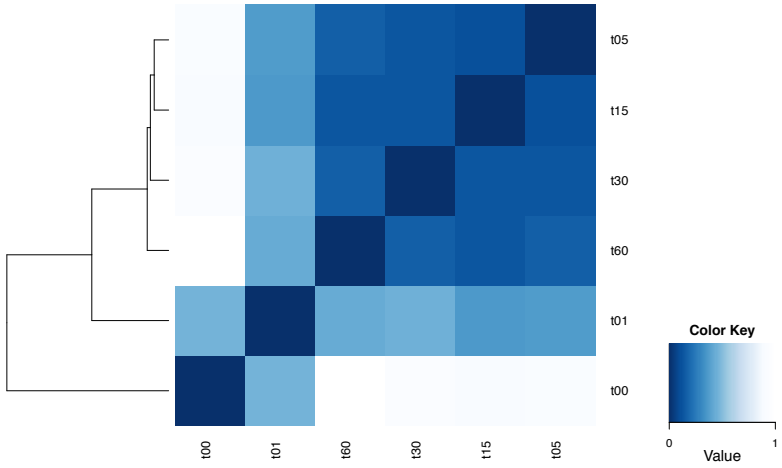


Figure 33: Heat map comparing time points. Heat map was done using standardised IgG to characterize the degree of similarity between time points. The most similar are T05 and T15, followed by T30, T60 and T01. Time 0 is the least similar to the rest of the time points.

At a first glance, we observed that within the 315 high confidence proteins, the Fold Change over the IgG (IgGFC) for a given protein varies from a minimum of 3.2 to a maximum of 326 FC. At the wordcloud all HCI are depicted with proportional size to IgGFC (Fig. 34). Although is difficult to compare two or more proteins in label-free proteomics, this value can be considered as an indicator for enrichment analysis. Log_2 of the maximum IgGFC from each protein is used at the *Enrichr* web tool (E. Y. Chen *et al.* 2013) (Fig. 36) for enrichment analyses. These values can be used to obtain Gene Ontology enriched terms for the whole list and for each kinetic cluster (see below).

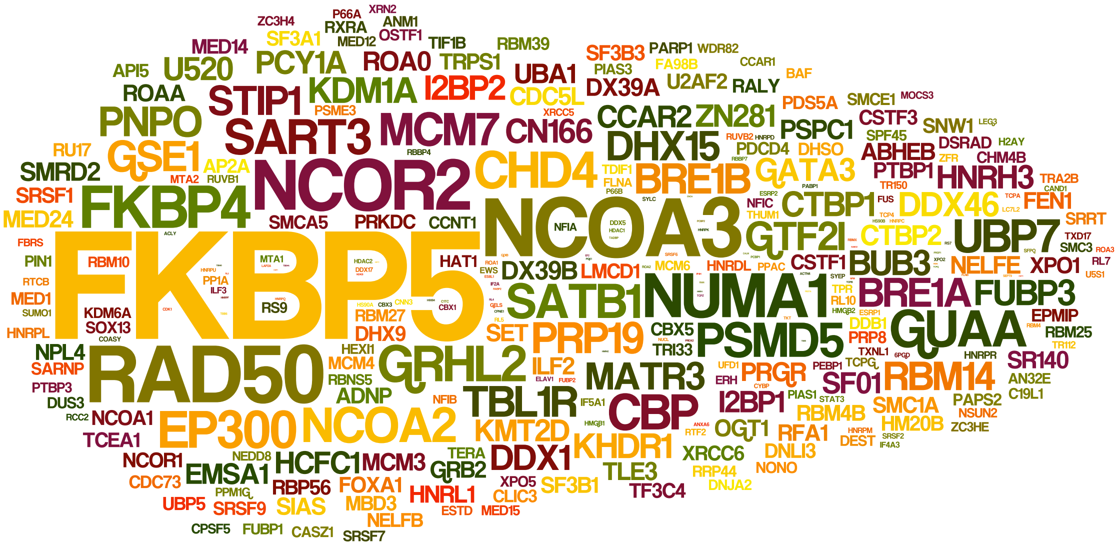


Figure 34: Wordcloud of the whole high confidence list of proteins. Size represents the fold change (IgGFC) of each protein.

When we focus on the change of enrichment of each protein as a function of the time of hormone exposure, we get the *kinetic Fold Change* (kFC, obtained by dividing the maximum IgGFC by its minimum value). The maximum kFC value was found for a well-known PR co-activator, NcOA3, which reaches 172 kFC. Moreover, when the proteins were plotted in a wordcloud by their kFC, we obtained a figure where only a few of the 315 high confidence proteins are visible (Fig. 35), reflecting those that change more significantly during the analyzed time period. There are many proteins whose kFC is very low, meaning that their interaction with PR is relatively stable and does not change significantly over time.

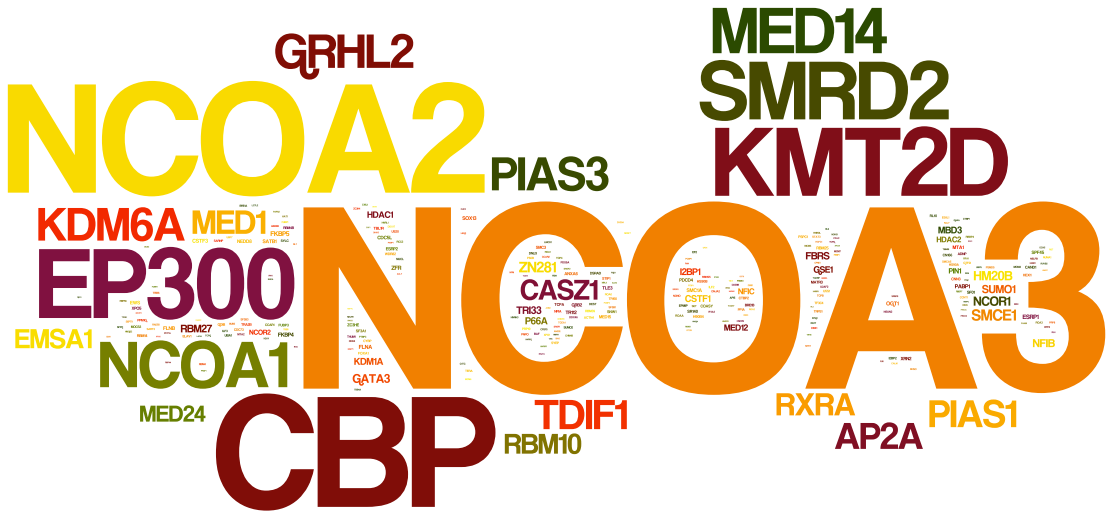


Figure 35: Word cloud of the whole high confidence list of proteins relative to kinetic fold change. Size represents kinetic Fold Change (kFC) of each protein.

4.3.2.1 Pathway analysis

To explore general features of the PR interactors we analyzed the high confidence list of proteins using *Enrichr*, a gene set enrichment analysis tool (E. Y. Chen *et al.* 2013).The whole list of high confidence proteins was uploaded, taking into consideration the Log_2 of the IgG Fold Change (IgGFC) for each protein.

First, we compared the results with the Cancer Cell Line Encyclopedia (CCLE). Among the 20 most enriched cell lines we found, there were 10 Breast Cancer cell lines, including T47D, MCF7, KPL1 and BT-474 (Fig. 26). Thus, this analysis shows that the interactors are common proteins of Breast Cancer cells.

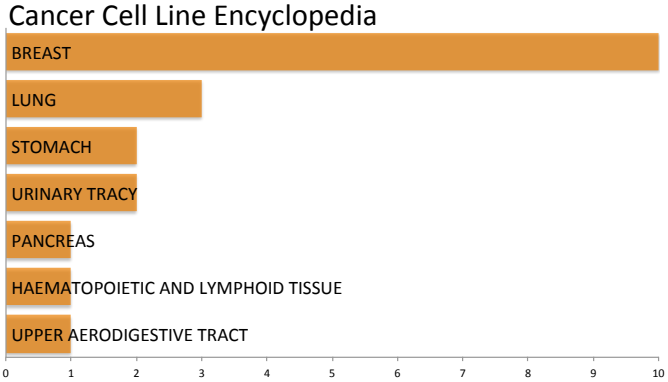


Figure 36: Bar diagram showing enrichment in cells from different tissues. Numbers depict the times of cells identified at CCLE for each tissue.

The high confidence interactors were then analyzed against pathways databases to find enriched pathways. Using the Kyoto Encyclopedia of Genes and Genomes (KEGG), we found that the most enriched pathways were related to splicing (Fig. 37). Signaling, response to stimulus, transcription, and DNA replication and repair were also enriched.

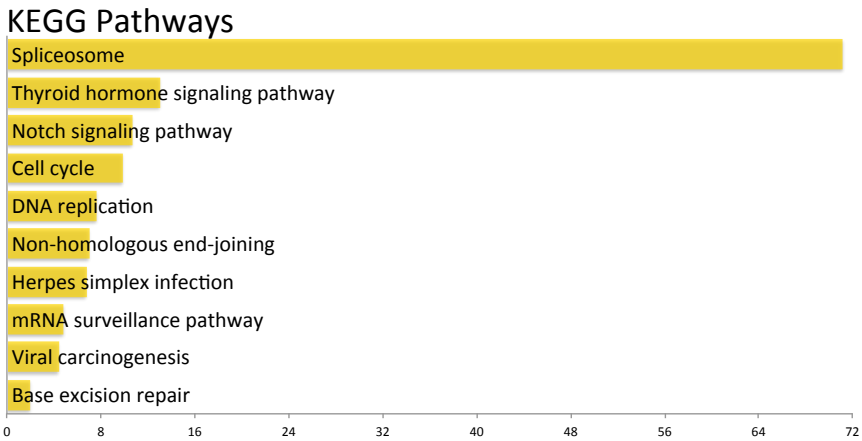


Figure 37: KEGG pathways enrichment analysis. High confidence interactors data set was compared to KEGG pathways database for enrichment analysis. The 10 most enriched terms are shown.

The data was also analyzed using another pathways database, Reactome (2016), and the most enriched pathways were gene expression, splicing, chromatin organization and chromatin modification (Fig. 38). The analysis coincide with the KEGG database, in this case including chromatin organization and most probably chromatin remodeling.

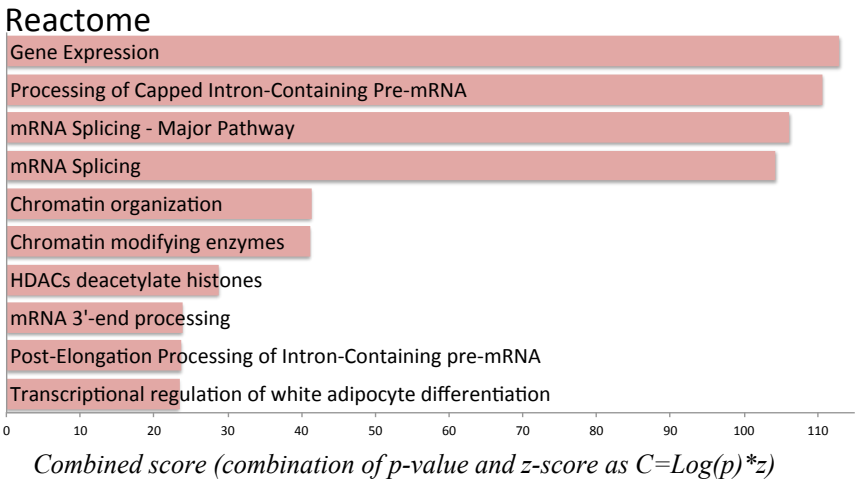


Figure 38: Reactome pathways enrichment analysis. The high confidence data set of interactors was compared with Reactome pathways database for enrichment analysis. The 10 most enriched terms are shown.

4.3.2.2 Gene ontology enrichment

We next analyzed the enrichment of the dataset for gene ontologies, starting with the enrichment for Cellular Component GO terms. We found that the most enriched terms were related to nucleus, splicesosome, transcription and chromatin (Fig. 39).

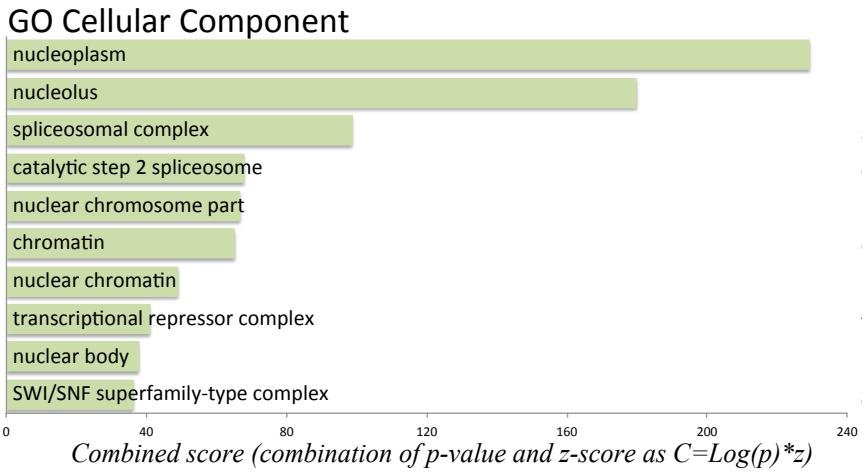


Figure 39: GO cellular component enrichment analysis. High confidence interactor data set was compared by *Enrichr* webtool with GO terms related to Cellular Component for enrichment analysis. The 10 most enriched terms are shown. Values are Combined score (combination of p-value and z-score as C=Log (p)*z).

For the Molecular Function GO, we found enriched terms related to chromatin and DNA binding, ATP-dependent complexes, transcription and hormone receptors (Fig. 40).

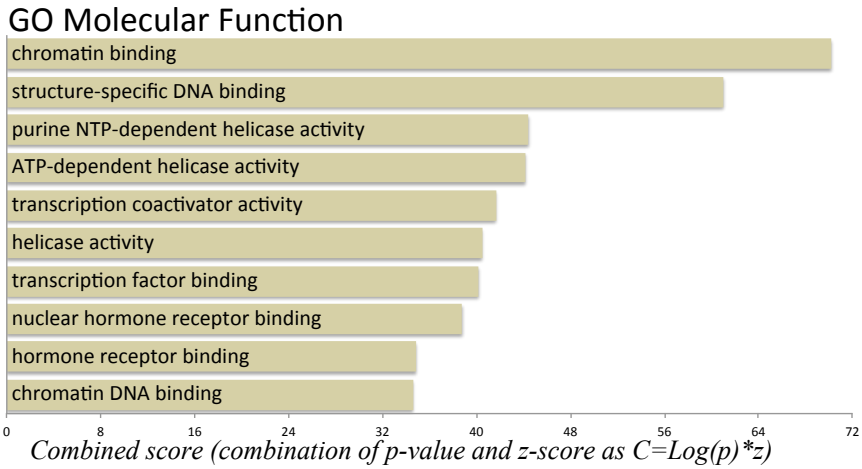


Figure 40: GO molecular function enrichment analysis. High confidence interactor data set was compared by *Enrichr* webtool with GO terms related to Molecular Function for enrichment analysis. The 10 most enriched terms are shown. Values are Combined score (combination of p-value and z-score as C=Log (p)*z).

In terms of the Biological Process GO terms we found a high enrichment in proteins related to splicing, gene expression and chromatin modification (Fig. 41).

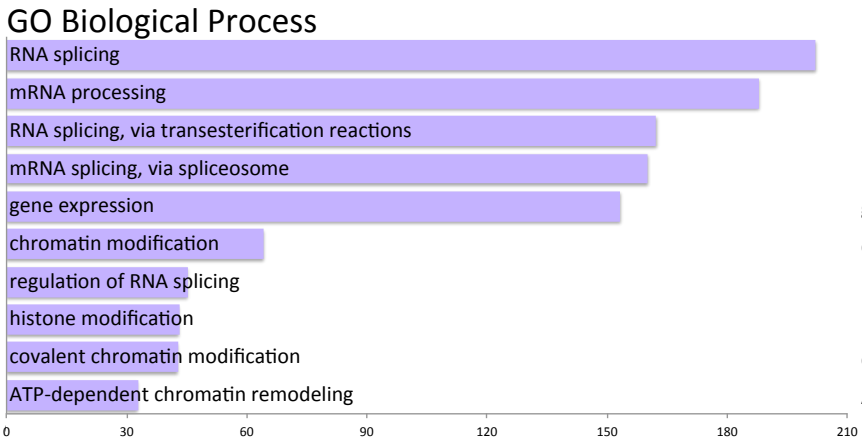


Figure 41: GO biological processess enrichment analysis. High confidence interactors data set was compared by *Enrichr* webtool with GO terms related to Biological Processess for enrichment analysis. The 10 most enriched terms are shown. Values are Combined score (combination of p-value and z-score as C=Log (p)*z).

In general, PR interactors include proteins overexpressed in Breast Cancer cell lines, that are mainly nuclear and related to chromatin, both at the structural and functional level, including histone modifiers, to splicing regulators, and to modulators of gene expression and transcription.

In the following sections I will describe how the kinetics of changes of these proteins following hormone exposure can be used to define clusters with different functional relevance.

4.3.3 Definition of kinetic clusters

We attempted to cluster PR interacting proteins according to their dynamic behavior in response to hormone. We excluded from the clustering proteins which interaction with PR did not change significantly at different time points after hormone exposure and exhibited a kFC lower or equal to 1.5. For such proteins the change of interaction over time of exposure to hormone is not significant and, as a result, their dynamic patterns are noisy and can mislead the interpretation of the clustering (data not shown). Instead, these proteins were considered as a single group (hereafter referred to as "basal cluster") for comparison with the inferred clusters.

M-fuzz R-package (L. Kumar *et al.* 2007) was used for soft dynamic clustering as explained in the Materials and Methods section. Prior clustering, IgGFC values for all proteins were normalized by per-protein normalization. Each data point of a given protein was subtracted by the protein time course mean value and then divided by the standard deviation of the protein values. This ensures that the kinetic time profiles are comparable between proteins despite the broad range of the IgGFC values.

Unlike hard clustering, in which proteins would be assigned to a unique cluster, soft clustering estimates for each protein the level of membership to each of the *c* clusters explored in the analysis and then assigns the protein to the cluster with the maximum membership.

Although *M-fuzz* allows imposing a minimum membership to include a protein in any of the clusters, we did not use it. This has the advantage that in this way we were able to assess the quality of the assignment of a given protein as well as that of the cluster as a group. We observed that there are proteins in which their memberships to two different clusters are very close. Those are usually proteins with a low induction fold change, or with one of the values outlaying the dynamics.

To determine the optimal number of clusters (*c*), we plotted the number of clusters against the number of non-empty clusters. Empty clusters start appearing for *c*>5 and at some point, for *c*≈50 all clusters have at least one protein assigned again (not empty), which probably indicates over fitting of the data (Fig. 42). Therefore, for the coming analyses we explored values of *c* in the range between 2 and 5.

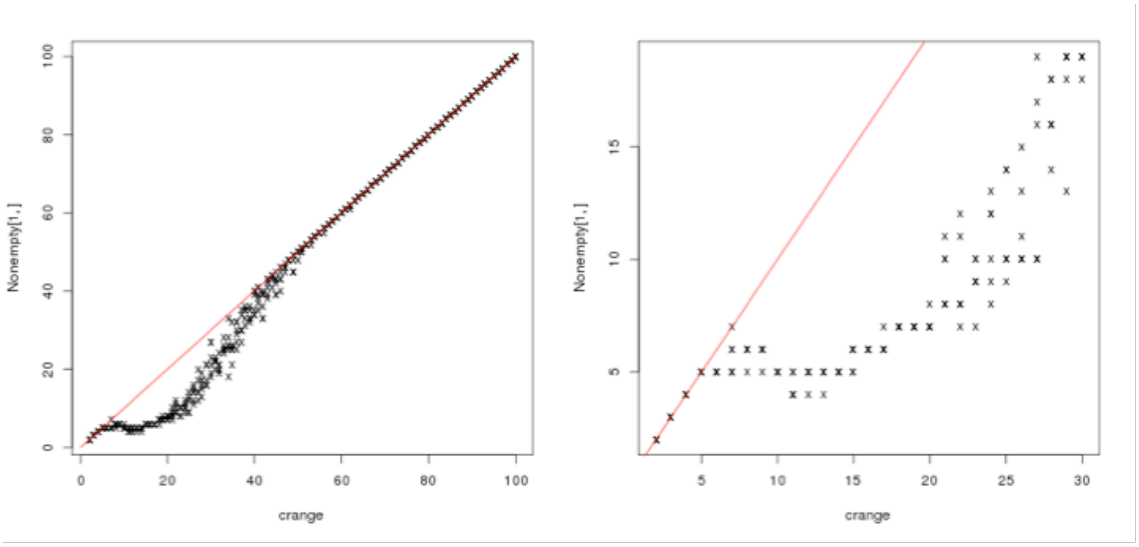


Figure 42: Definition of number of kinetic clusters. Plot comparing the number of given clusters to number of empty clusters. Optimal number of clusters is when there are no empty clusters. Left panel shows up to 100 clusters. Right panel shows a zoom of up to 30

Empty clusters start appearing for *c*>5 and at some point, for *c* ≈ 50 all clusters have at least one protein assigned again (not empty), this probably indicates over fitting of the data. For the coming analysis it was used the set-specific optimal *m* (maximum membership) and *c* up to 8 in all cases. As shown in the figure, the optimal number of clusters varies between 2 and 5.

Choosing 2 clusters divided the sample in proteins increasing and proteins decreasing their interaction with PR after induction. Membership was high but the information extracted was simple. For 3 clusters, proteins increasing their interaction with PR were subdivided in early interactors and late interactors. For 4 and 5 clusters, subdivisions of those two clusters were generated, diluting the information and the membership was drastically reduced. For cluster plots for *c* = 2, 4 and 5, see appendices section. We finally decided to use *c* = 3 because GO term enrichment was done for *c* = 3 and *c* = 4 and the aggrupation of similar functional proteins was optimal for 3 clusters (Fig. 43).

With 3 clusters -4 including the basal cluster- the dynamic patterns observed are: (i) proteins with an stable interaction with PR, both before and after hormone induction (Basal cluster); (ii) proteins that are interacting with the receptor before hormone stimulation and decrease their interaction upon hormone exposure (Cluster 1); (iii) proteins that are rapidly recruited to PR after hormone (Cluster 2); (iv) and proteins that have a constant increase of interaction with PR over time, with their peak of interaction at longer time points (Cluster 3). For complete information about clusters membership see Table 3 at appendices section.

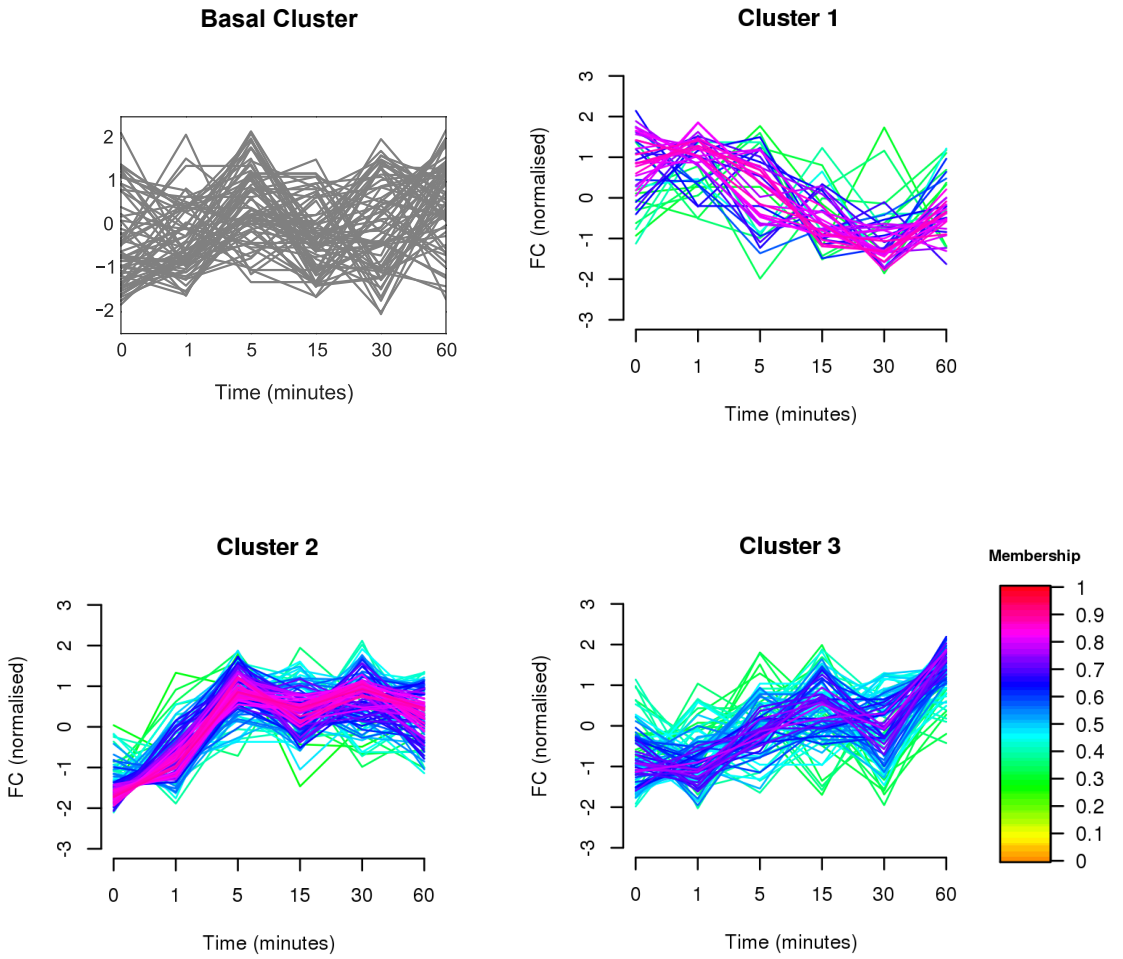


Figure 43: Kinetic clusters. Proteins were characterized by their dynamic behaviour over time of exposure to R5020. Basal cluster was created artificially with proteins with an induction Fold Change lower than 1.5. Cluster 1 groups proteins displaying a decrease of interaction with PR upon hormone exposure. Cluster 2 groups proteins with a rapid recruitment to PR after hormone. Cluster 3 groups proteins that increase their interaction with PR less rapidly. Clustering was done using the R-package M-fuzz. IgGFC values were normalized by Z-score prior clustering.

4.3.4 Characterization of kinetic clusters

4.3.4.1 Basal cluster (66 proteins):

We detected a group of proteins that interacts with PR already at time 0, before hormone induction. Some of them lose their interaction with PR upon hormone exposure (see Cluster 1), while others keep their PR interaction. The Basal cluster includes all proteins which interaction does not change more than 1.5 fold after hormone. It has been named basal cluster, as it is formed by proteins that are bound to PR at all time points, even though some exhibit a mild but not significant dynamic change, as seen in the trend line (Fig. 44). At the wordcloud are depicted all the proteins of the cluster, where colour scale is related to IgGFC and size is related to kFC. In this cluster, as expected there are no size variations. Pr itself is depicted in dark green.

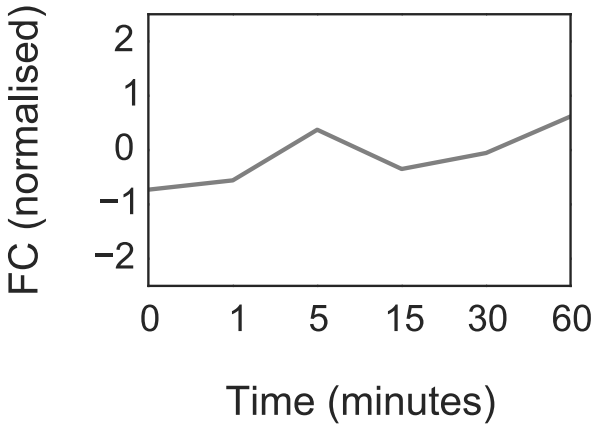


Figure 44: Basal cluster trendline. Average values of all the proteins from basal cluster. Values correspond to normalized IgG fold changes over time.

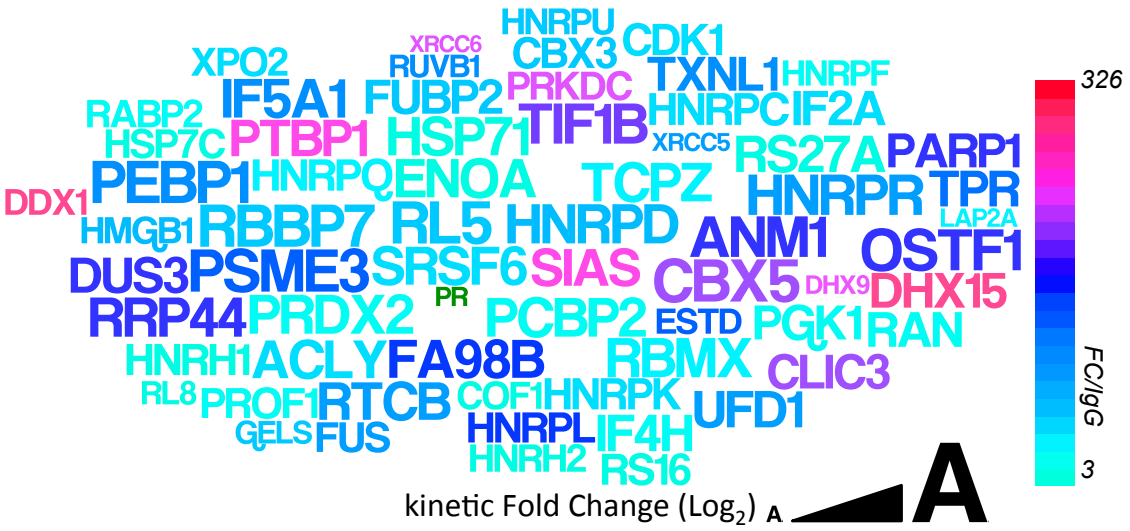


Figure 45: Wordcloud with basal cluster interactors. Representation of PR interactors at basal cluster. Colour code represents maximum IgGFC of each protein, which is a value of estimated amount of protein. Size code represents the kFC, obtained dividing each protein maximum by its minimum value of fold change over IgG. At basal cluster, most proteins have a similar size, as they are selected to have a kFC lower than 1.5.

For networks analyses, it is important to consider these proteins as interacting with PR at all time points.

According to Gene Ontology terms enrichment, in this cluster there are proteins related to Splicing (mainly due to the presence of most of the hnRNP proteins of the dataset in this cluster), DNA structure and stress response (Fig. 17).

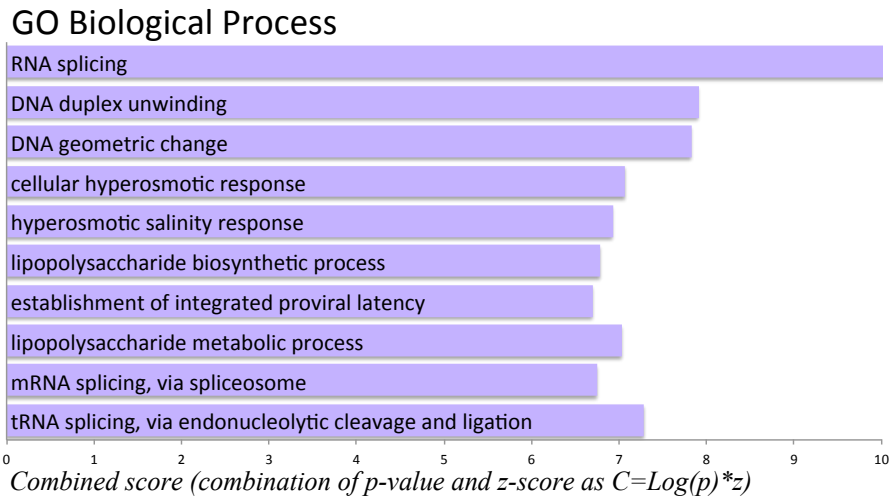


Figure 46: GO biological processes Basal cluster. Weighted list of proteins enclosed at basal cluster were compared to Biological processes GO terms for enrichment analyses. Weight was to given to each protein by its maximum IgG fold change (in Log_2). 10 most enriched terms are shown.

According to KEGG pathways enrichment analysis, the only pathways significantly enriched are related to DNA damage repair and splicing.

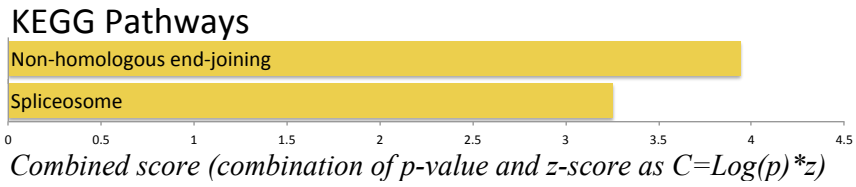


Figure 47: KEGG pathways Basal cluster. Weighted list of proteins from basal cluster were compared to KEGG pathways database for enrichment analysis. Proteins were given weight before analysis with their maximum IgGFC (Log_2). Only significant terms are shown (p-value<0.05).

DNA damage related proteins:

PARP1 (Poly [ADP-ribose] polymerase 1): Also known as ADPRT1, it catalyzes the poly-ADP-ribosylation of a number of proteins, including PARP1 itself, histones and transcription factors. It is involved in DNA damage repair, chromatin structure and in transcriptional regulation (Kraus 2015). Its interaction with PR is very constant, with a

peak at T60. It is a known PR interactor, it has been shown to be required for hormonal gene regulation, after its activation by CDK2 (Wright *et al.* 2012). Its activation modulates a number of proteins, including DNA-damage related response (Krietsch *et al.* 2012). It also has been suggested its role in alternative splicing regulation (Matveeva *et al.* 2016) and in nuclear energy availability (Wright *et al.* 2016).

XRCC5/Ku86 (X-ray repair cross-complementing protein 5): One of the proteins with a lower kFC (1.19). Together with PARP1, XRCC6 and PRKDC, it is known to interact with the DNA binding domain of PR (Sartorius *et al.* 2000).

XRCC6/Ku70 (X-ray repair cross-complementing protein 6): Has the lowest kFC of the whole dataset (1.17). Its interaction with PR is constant and high (IgGFC: 44). It has been described to interact with PR (Sartorius *et al.* 2000).

XRCC5/XRCC6: Single-stranded DNA-dependent ATP-dependent helicase (Tuteja *et al.* 1994). Has a role in chromosome translocation. The DNA helicase II complex binds preferentially to fork-like ends of double-stranded DNA in a cell cycle-dependent manner. It works in the 3'-5' direction. Involved in DNA non-homologous end joining (NHEJ) (Roberts *et al.* 2010), and required for double-strand break repair and V(D)J recombination. The XRCC5/6 dimer acts as regulatory subunit of the DNA-dependent protein kinase complex DNA-PK by increasing the affinity of the catalytic subunit PRKDC to DNA by 100-fold.

PRKDC: Serine/threonine-protein kinase that acts as a molecular sensor for DNA damage. Involved in DNA non-homologous end joining (NHEJ) (Soubeyrand *et al.* 2003) required for double-strand break (DSB) repair and V(D)J recombination. Must be bound to DNA to express its catalytic properties (Yavuzer *et al.* 1998). Its low kFC and its high IgGFC (42.1) may indicate a high and constant interaction with PR of this DNA-dependent protein kinase involved in DNA damage detection and repair. It may also has a role in RNA PolII pausing at DNA breaks (Pankotai *et al.* 2012).

RUVB1 (RuvB-like 1): Component of the chromatin remodeling complex INO80, which is involved in transcriptional regulation, DNA replication and DNA repair.

HMGB1 (High mobility group protein B1): Is one of the major chromatin-associated non-histone proteins and acts as a DNA chaperone involved in replication, transcription, chromatin remodeling, V(D)J recombination, DNA repair and genome stability (Y. Zhang *et al.* 2005). It has been described to interact and enhance PR binding activity to DNA (Boonyaratanakornkit *et al.* 1998).

Transcription related proteins:

CDK1/CDC28 (Cyclin-dependent kinase 1): Plays a central role in cell cycle progression and mitosis. Also shown required for full phosphorylation of C-terminal domain-Ser5 on RNA PolII, for efficient transcription (Chymkowitch *et al.* 2012). Among the proteins known to be phosphorylated by CDK1 there are Histone H1, MCM2, MCM4, NFIC, NPM1, NCL, NONO/p54NRB, STIP1 or beta-tubulins. Also known to regulate epigenetic silencing by regulating phosphorylation state of Polycomb proteins (S. Chen *et al.* 2010).

PRMT1 (Protein arginine N-methyltransferase 1): Also known as Histone-arginine N-methyltransferase, ANM1. First identified as a histone methyltransferase, it has ben shown capable of methylation of a number of other non-histone proteins, like STAT1, PIAS1, HNRNPA1, HNRNPD, SUPT5H, TAF15, EWS and ESR1 (Le Romancer *et al.* 2008). Mediates Histone H4 R4 methylation, a specific mark for transcription activation (Strahl *et al.* 2001, H. Wang *et al.* 2001). Also shown to regulate specific ER-target genes together with SET protein (see cluster 3) (Wagner *et al.* 2006).

TIF1B/TRIM28 (Transcription intermediary factor 1-beta): E3 SUMO-protein ligase, it is known to be involved in multiple cellular processes, like cell growth, apoptosis or DNA repair (Iyengar *et al.* 2011). Its retention at damage sites is mediated by SET proteins (see cluster 3) (Kalousi *et al.* 2015). Active SUMOylated TRIM28 binds damaged chromatin, associated with HP1/CBX. It has recently been described as a novel transcriptional elongation factor, in regulating Pol II pausing and pause release (Bunch *et al.* 2014). It is rapidly phosphorylated at S824 upon stress signal, regulated by PRKDC, and this correlates with RNA PolII progression. Its IgGFC is relatively high (36.6).

RAN (GTP-binding nuclear protein Ran, Androgen receptor-associated protein 24): Involved in nuclear transport, also have a role in AR-mediated transactivation (Hsiao *et al.* 1999).

ENOA/MBP1 (Alpha-enolase / C-myc promoter-binding protein): Possible role as tumor suppressor (Jeyabalan *et al.* 2010).

RBBP7 (Histone-binding protein RBBP7): Core-histone binding subunit of a number or complexes, including Cohesin complex, CBP containing complexes which lead to transcription activation or HDAC-containing complexes, which can lead to transcriptional repression (Q. Zhang *et al.* 2000).

Transcription repression:

CBX3/HP1-γ (Chromobox protein homolog 3): Heterochromatin protein that recognizes and binds histone H3 tails methylated at K9, leading to epigenetic repression. May contribute to the association of the heterochromatin with the inner nuclear membrane through its interaction with lamin B receptor (LBR) (Ye *et al.* 1997). Is part of the LSD1 repressive complex, shown to actively repress genes upon hormone induction (Nacht *et al.* 2016).

CBX5/HP1α (Chromobox protein homolog 5): Component of the heterochromatin, interacts with methylated K9 of H3, leading to repression. It also has been shown to bind Lamin B receptor, contributing to the association of heterochromatin to the nuclear membrane (Dawson *et al.* 2009). Has an IgGFC of 33.

Splicing related proteins:

Almost half of the proteins described as being part of the spliceosome are found in the whole dataset, and although most of them are in cluster 2 or 3, a few are also in the Basal cluster, mostly hnRNP proteins. It has been described for those type of proteins to be a common contaminant of mass-spec (Jurica *et al.* 2003), and the fact that their interaction is in all the time points, although they pass the FDR filter after the SAINT analysis, raise the suspicion of being false positives.

Post-transcriptional processes:

DDX1 (DEAD-box helicase DDX1): ATP-dependent RNA helicase, interacting with hnRNPK (also in this cluster) (H. C. Chen *et al.* 2002). Together with FAM98 and **C14orf166** (cluster 3), are suggested to act as tRNA splicing factors, acting additionally to an **RTCB** containing complex (Popow *et al.* 2014). It has a very constant interaction with PR, and a high value of interaction (IgGFC: 77).

FAM98B: Component of the tRNA-splicing ligase complex, together with DDX1 (Popow *et al.* 2011).

HnRNPK (Heterogenous Ribonucleoprotein K): Splicing related protein, it is also decribed to have other functions, as being target for GranzymeA (van Domselaar *et al.* 2012), at the telomerse transcriptase regulation system (Kang *et al.* 2009) or as transcriptional silencing by SETDB1 coordinator (Thompson *et al.* 2015).

FUBP2 (Far upstream element-binding protein 2): May be involved in mRNA trafficking, also described to interact with single-stranded DNA from the far-upstream element (FUSE) (Davis-Smyth *et al.* 1996). May activate gene expression (Min *et al.* 1997).

DUS3 (Dual specificity protein phosphatase 3): Specifically dephosphorylates ERK1/2 (Todd *et al.* 1999). Maximum IgGFC of 33.6.

Translation related proteins:

Only 10 proteins from the ribosomal complex are found in the high confidence dataset, half of them with a kFC lower than 1.5. Having a look at the moderate confidence data set, over 45 ribosomal proteins are found in total, also with a low kFC.

RRP44/DIS3: Apart from being described as part of the ribosomal complex, it also has been described as a putative catalytic component of the RNA exosome complex, which is involved in proper maturation of stable RNA species such as rRNA, snRNA and snoRNA, in the elimination of RNA processing by-products and non-coding 'pervasive' transcripts, such as antisense RNA species and promoter-upstream transcripts (PROMPTs), and of mRNAs with processing defects. It is the ribosome related complex with the highest IgGFC (34). Known to interact with RAN (in this cluster) in yeast (Noguchi et al. 1996).

RL5: 60S ribosomal protein L5.

RL8: 60S ribosomal protein L8.

RS27A: Ubiquitin-40S ribosomal protein S27a.

RS16: 40S ribosomal subunit.

IF4H and **IF5A1**: Eukaryotic translation initiation factors.

Proteasome related proteins:

PSME3/PA28gamma (Proteasome activator complex subunit 3): Proteasome regulator, associates with CCAR2 (at cluster 3 with low kFC) in a DNA damage regulated manner, in the apoptosis pathway of p53 (Magni *et al.* 2014).

UFD1 (Ubiquitin fusion degradation protein 1 homolog): Essential component of the ubiquitin-dependent proteolytic pathway that degrades ubiquitin fusion proteins.

Structural related proteins:

PROF1 (Profilin-1): Known to bind actin and affect cytoskeleton structure. This same binding is suggested to affect AR transactivation, and its overexpression reduces the aggregation of polyglutamine-expanded Huntingtin (HTT) and AR peptides (Shao *et al.* 2008). It's a direct target of the kinase ROCK1, and a known target of PP1 (Shao *et al.* 2012).

TPR (Nucleoprotein TPR / Megator): Component of the Nuclear Pore Complex, modulates the nucleocytoplasmic transport of activated MAPK1/ERK2 and huntingtin/HTT (Vomastek *et al.* 2008).

XPO2 (Exportin-2): Mediates importin-alpha re-export from the nucleus to the cytoplasm after import substrates have been released into the nucleoplasm.

GELS (Gelsolin): Low kFC (1.24), Calcium-regulated, actin-modulating protein. It has actin-severing capacity, which can inhibit tumor cell motility (Marino *et al.* 2013).

HSP71 and **HSP7C**: As part of the Heat shock protein family of chaperones, their interaction decreases after hormone, but as their kFC is lower than 1.5, it is considered stable. This stable binding to PR may be due to the fact that a fraction of the cells do not respond to hormone.

PGK1 (Phosphoglycerate kinase 1): Apart from its role in glycolysis, it may have a role as a DNA Polα during replication (Jindal *et al.* 1990).

4.3.4.2 Cluster 1 (41 proteins):

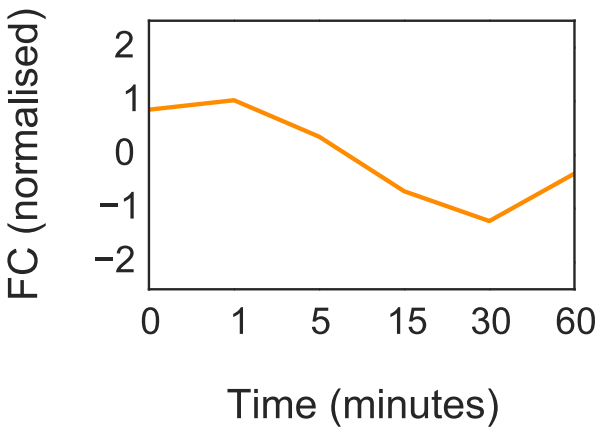


Figure 48: Cluster 1 trendline. Average values of all the proteins from cluster 1. Values correspond to normalized IgG fold changes over time.

This cluster contains proteins that decrease their interaction with PR after hormone exposure (Fig. 48). One of the proteins in this cluster is the most enriched in total numbers, with an IgGFC of 326 (FKBP5, at the wordcloud), although the maximum kFC of the cluster is 4.54. This may reflect the fact that not all cells in the population respond after hormone exposure (Wright *et al.* 2016). In the wordcloud there are depicted PR interactors from cluster 1 (Fig. 49). In this case, most of the IgGFC indicate lost of interaction with PR after hormone. There is not a clear relation between 'amount' of protein identified (IgGFC) and the kFC, in this cluster decrease fold change, as there are dark blue and reddish small proteins, and also big proteins in light blue color.

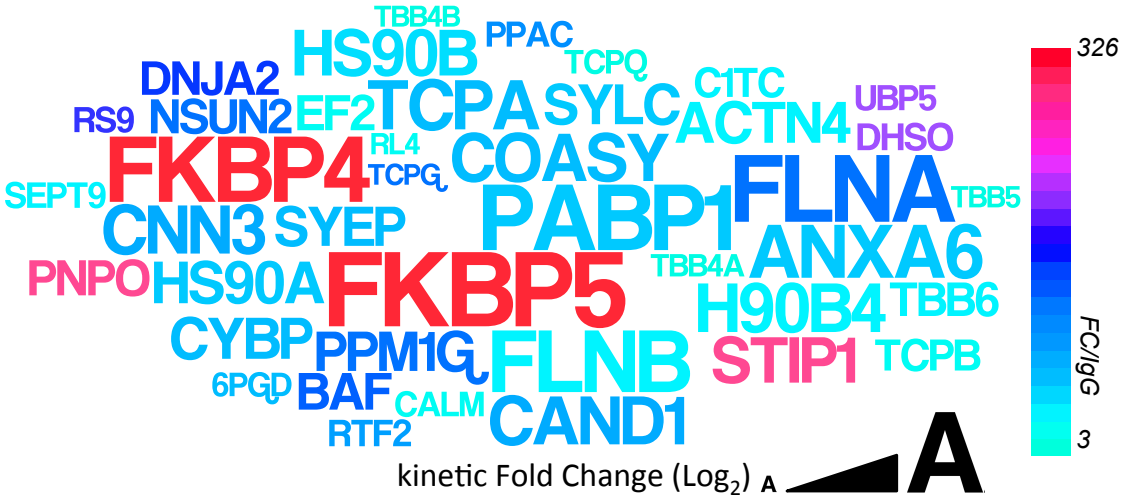


Figure 49: Wordcloud with Cluster 1 proteins. Representation of proteins found at Cluster 1. Colour code represents maximum IgGFC of each protein, which is a value of estimated amount of protein. Letter size represents the kFC, obtained dividing each protein maximum by its minimum value of IgGFC (in Log_2).

The cluster is composed mainly by proteins related to chaperones and protein folding, as indicated by the GO analysis. Other terms significantly enriched are related to RNA catabolism and translation. In Figure 50, the top 10 enriched GO terms are shown.

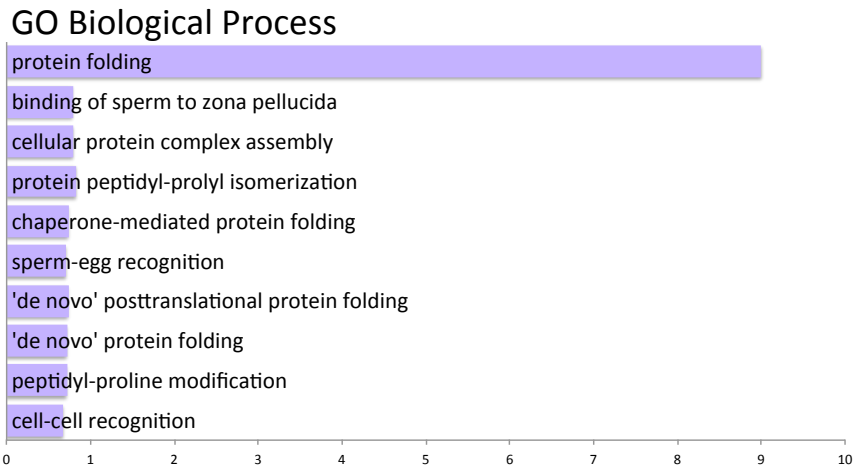


Figure 50: GO biological processes cluster 1. Weighted list of proteins enclosed at cluster 1 were compared to Biological processes GO terms for enrichment analyses. Weight was to given to each protein by its maximum IgG fold change (in Log_2). 10 most enriched terms are shown.

KEGG pathways enrichment analysis showed only 2 pathways significantly enriched, Estrogen signaling, due to chaperones, and vitamin B6 signalling (Fig. 51).

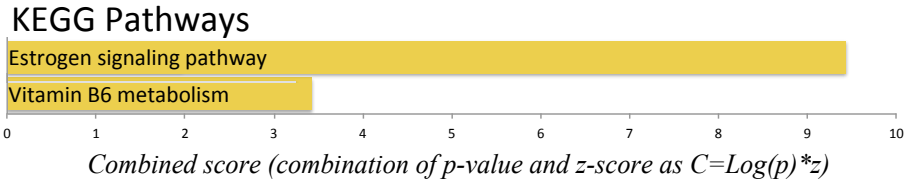


Figure 51: KEGG pathways cluster 1. Weighted list of proteins from cluster 1 were compared to KEGG pathways database for enrichment analysis. Proteins were given weight before analysis with their maximum IgGFC (Log_2). Only significative terms are shown (p-value<0.05).

Chaperones:

FKBP5: Known PR chaperone, it dissociates from the receptor upon hormone induction (Smith *et al.* 1993). It is the protein with the highest IgGFC (relative to amount), but its kFC is slightly higher than 4 (Sinars *et al.* 2003).

FKBP4: Another well-known PR chaperone, together with FKBP5 form a chaperoning complex for PR (Schulke *et al.* 2010).

STIP1: Known PR chaperone (Weaver *et al.* 2000), is a stress induced phosphoprotein, and acts as a co-chaperone with HSP90 and HSP70.

HSP90 (Heat-shock protein 90): Known PR chaperone. Subunits α and β are found in this cluster, having its maximum value at T00, and rapidly decreasing its interaction with PR.

DNAJA2: A protein chaperone known to interact with other chaperones in this cluster (HSP90 and STIP1) (Skarra *et al.* 2011). Another member of its family (DNAJA1) is known to act as anti-stress factor.

TCPA/**TCP1**: The subunit that gives name to the **TCP1 complex**. Has a more variable profile than the rest of the subunits, with a kFC of 2.73, having its lowest values at T05 and T30. Also known as Chaperonin Containing TCP1 complex (CCT), or as the TCP1 ring complex (TRiC). We also find in this cluster another 3 subunits of the complex (**CCT2**, **CCT3** and **CCT8**). 7 out of 9 components of the complex are found in the moderate confidence list. It is involved in protein folding several of actin and tubulin.

Structural proteins:

Tubulins: 4 different isoforms of β-tubulin are found in our dataset (**TBB4A**, **TBB4B**, **TBB5** and **TBB6**), and all are present in cluster 1. The first idea is that these are possible contaminants from the cytosolic fraction, but the presence of tubulins in the nucleus in a non-polymerized form has been reported (Akoumianaki *et al.* 2009) and a functional interaction of tubulin with AR has also been described (M. L. Zhu *et al.* 2010). It has to be considered that 13% of the cells are in G2/M phase upon serum starvation and we know that PR interacts with the mitotic spindle (unpublished data).

FLNA (Filamin A): Upon hormone exposure, its binding to PR decreases up to 3.5 times. It is known to be localized to the nucleus to repress AR and its coactivator functions (Loy *et al.* 2003).

FLNB (Filamin B): Has the same behavior than Filamin A.

BAF (Barrier-to-autointegration factor): Plays fundamental roles in nuclear assembly, chromatin organization and gene expression. Has a kFC close to 2, and its dynamics are not standard for cluster 1, as it has a peak of interaction at T05. Plays a role in linking nuclear lamina and DNA, interacting with LAP2A, which we found in the basal cluster with a kFC of 1.19 (Shumaker *et al.* 2001).

SEPT9 (Septin 9): Filament-forming cytoskeletal GTPase.

ACTN4 (Actinin-4): Has its maximum at T01, and decreases its interaction upon time after hormone exposure. Combined with CART complex is associated with membrane

receptors endocytosis and recycling (Yan *et al.* 2005). It is also known to interact and enhance transcription modulated by nuclear receptors (Khurana *et al.* 2012).

CNN3 (Calponin-3): Filament associated protein, capable of binding to actin and calmodulin.

ANXA6 (Annexin-6): Member of the calcium-dependent membrane binding annexin family, and a known progesterone target gene (Kester *et al.* 1997). It also acts as a scaffold/targeting protein for several signaling proteins (Enrich *et al.* 2011). We find some other annexins in the moderate confidence list (Table 2 for complete list).

CALM (Calmodulin): Mediates the Ca^{2+}-dependent control of a large number of enzymes, ion channels, aquaporins and other proteins.

CaCYBP: involved in Ca^{2+}-dependent ubiquitination and subsequent proteasomal degradation of target proteins (Santelli *et al.* 2005). Increase in CacyBP/SIP expression during development of breast cancer (Kilanczyk *et al.* 2014).

MCM4: A member of the MCM complex, with a kFC of 1.90; is the protein with most similar membership to the 3 clusters, being 0.33, 0.36 and 0.31 for cluster 1, 2 and 3, respectively. Has its peak of interaction at T15, and the rest of the time points have similar interaction values. Other members of the complex are found in the dataset.

Translation proteins:

EF2 (Elongation factor 2): Catalyzes the coordinated movement of the two tRNA molecules, the mRNA and conformational changes in the ribosome.

NSUN2: RNA methyltransferase that methylates tRNAs, and possibly RNA polymerase III transcripts. Decreases interaction with PR by 2 fold.

SYEP: kFC of 2.22, minimum value at T30. Bifunctional glutamate/proline—tRNA ligase.

SYLC: kFC of 2.25, minimum value at T30. Leucine—tRNA ligase.

RS9 (40S ribosomal protein S9) and **RL4** (60S ribosomal protein L4).

Other relevant proteins:

PPM1G (Protein phosphatase 1G): Known to have a role in several cellular processes as alternative splicing (Allemand *et al.* 2007), histone dephosphorylation and exchange (Kimura *et al.* 2006) or DNA damage response, together with TR150 (cluster 3) (Beli *et*

al. 2012). Its maximum interaction time point is T05, and keeps it high for the rest but for T30, where it drops (kFC: 2.23).

PNPO (Pyridoxine-5'-phosphate oxidase): Binds to the 3'-UTR region of mRNAs, stabilizing them. Known to be ubiquitinated in HEK293 and U2Os (Danielsen *et al.* 2011). Known to interact with ELAV1 (Cluster 3; kFC=2.23), as part of the stress response. Has a IgGFC of 90.

UBP5/USP5 (Ubiquitin carboxyl-terminal hydrolase 5): Involved in the final steps of ubiquitin-mediated proteasomal degradation of proteins (Dayal *et al.* 2009).

COASY: Acetyl CoA synthase, mitochondrial protein.

PABP1 (The poly-(A)-binding protein): May be involved in cytoplasmic regulatory processes of mRNA metabolism and in pre-mRNA splicing.

DHSO: Known to interact with DDX39A, a spliceosomal protein necessary for mRNA export from the nucleus, which associates with the nuclear membrane and is found in at cluster 2 with a kFC of 1.6. Also interacts with TCEA1, an elongation factor found in cluster 3 with a kFC of 1.83.

CAND1 (Cullin-associated NEDD8-dissociated protein 1): Key assembly factor of SCF (SKP1-CUL1-F-box protein) E3 ubiquitin ligase complexes that promotes the exchange of the substrate-recognition F-box subunit in SCF complexes, thereby playing a key role in the cellular repertoire of SCF complexes (Pierce *et al.* 2013). Has a peak of interaction at T05 with a kFC of 2.66.

4.3.4.3 Cluster 2 (115 proteins):

This is the largest cluster, and contains the proteins that increase their interaction with PR as early as 5 minutes after hormone exposure. It contains the proteins with the highest kFC, because some of these proteins show no interaction with PR at T0 prior to hormone exposure, and their interaction reach high levels already at 5 minutes after hormone exposure Fig. 52). At the wordcloud it can be observed by the amount of interactors in big letters the fact that there are big increases of interaction with PR after hormone (Fig. 53). The reddish colour indicates interactors identified with high values of IgGFC.

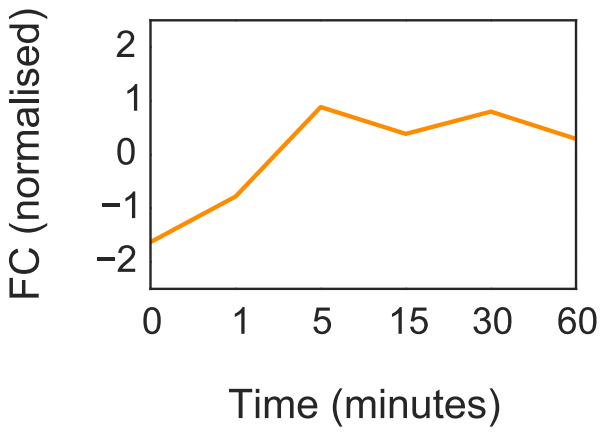


Figure 52: Cluster 2 trendline. Average values of all the proteins from cluster 2. Values correspond to normalized IgG fold changes over time.

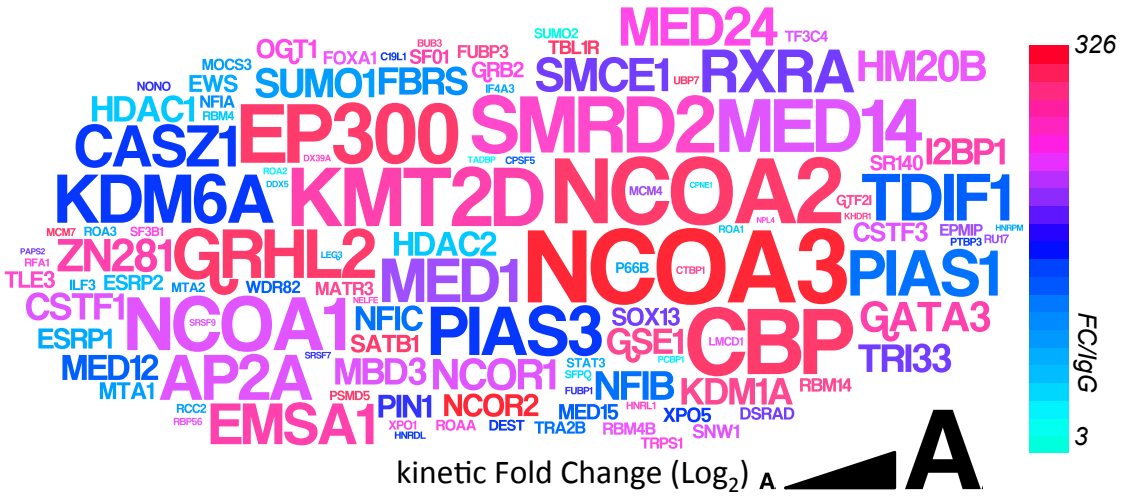


Figure 53: Wordcloud with Cluster 2 PR interactors. Representation of PR interactors at Cluster 2. Colour code represents maximum IgGFC of each protein, which is a value of estimated amount of protein. Size code represents the kinetic Fold Change, obtained dividing each protein maximum by its minimum value of fold change over IgG.

In the GO enrichment analysis we observe an increased enrichment of terms related to chromatin modification, gene expression and mRNA processing, including splicing related proteins. There is also an enrichment in transcription related terms (Fig. 54).

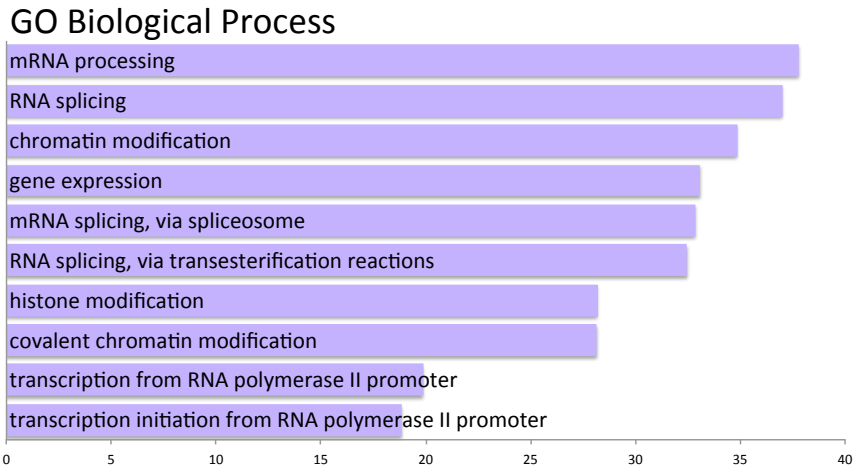


Figure 54: GO biological processes cluster 2. Weighted list of proteins enclosed at cluster 2 were compared to Biological processes GO terms for enrichment analyses. Weight was to given to each protein by its maximum IgGFC (in Log_2). The 10 most enriched terms are shown.

In the KEGG pathways enrichment analysis, we find an enrichment in hormone related pathways, and in pathways related to virus infection, which also involves transcription and chromatin modification (Fig. 55).

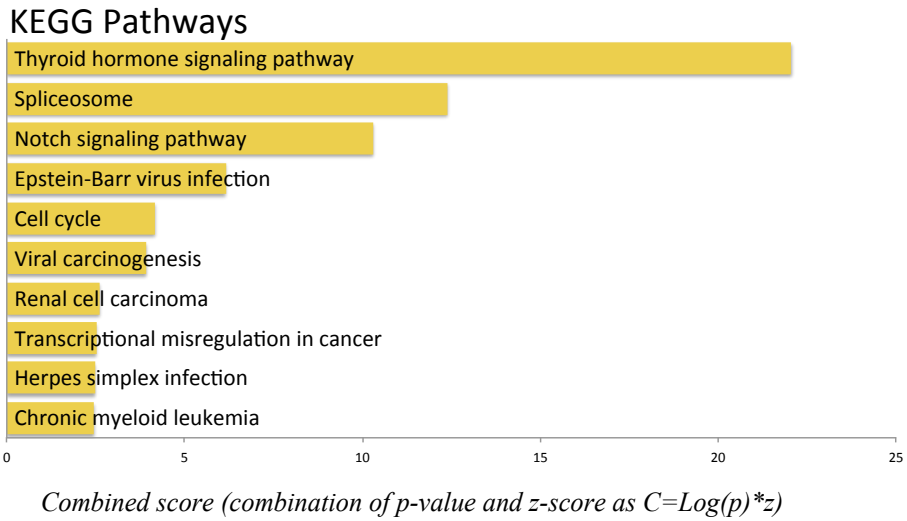


Figure 55: KEGG pathways cluster 2. Weighted list of proteins from cluster 2 were compared to KEGG pathways database for enrichment analysis. Proteins were given weight before analysis with their maximum IgGFC (Log_2). The 10 most enriched terms are shown.

Histone modifying enzymes:

Acetyl-transferases:

The p160 family of steroid receptor coactivators, are part of the nuclear receptor coactivators family, that contain LXXLL binding motifs, which can interact with ligand-bound nuclear receptors and recruit other histone acetyltransferases and methyltransferases to hormone responsive elements (HRE), facilitating chromatin remodeling, assembly of general transcription factors and transcription of target genes (Onate *et al.* 1995, York *et al.* 2010). Three members are found in this cluster.

NcoA1 (Nuclear receptor coactivator-1): It has a maximum interaction with PR after 5 minutes of hormone exposure (kFC: 44), and already at T15 the interaction decreases.

NcoA2 (Nuclear receptor coactivator-2): It increases its interaction with PR at 5 minutes after hormone exposure (kFC: 104), it is maintained until T30, and at T60 starts decreasing (kFC: 66). It is a known PR interactor (An *et al.* 2006).

NcoA3 (Nuclear receptor coactivator-3): It is the protein with the highest kFC of the whole dataset (176). Its pattern is similar to NcoA2. They are the clearest example of this cluster. A known PR interactor, among other SHRs (Giangrande *et al.* 2000).

P300/CBP coactivator family:

Composed by these two closely related transcriptional coactivator proteins, is also part of the nuclear receptor coactivator family, containing LXXLL motifs. They have intrinsic acetyltransferase activity for both histone and non-histone proteins, and also can act as scaffold to stabilize the transcriptional machinery (X. Liu *et al.* 2008).

CBP (CREB-binding protein) and **P300** (Histone acetyltransferase p300): They both exhibit a very similar dynamic pattern of interaction with PR. They reach their maximum after 5 minutes, have a mild decrease at T15, top again at T30 and decrease already at T60. Both proteins have a similar IgGFC (107 for CBP and 112 for p300).

HAT1 (Histone acetyltransferase 1): It is not clustered with the other histone acetyltransferases, we find it cluster 3, but its low kFC (1.8) and its dynamic pattern of interaction makes it worth mentioning. This protein already increases its interaction with PR 1 minute after hormone exposure, and oscillates over the rest of the time course, having its maximum values at T15 and at T60. It has not been identified to interact with nuclear receptors (Sterner *et al.* 2000).

Histone methylation proteins:

KDM6A/UTX (Lysine-specific demethylase 6A): Is a histone demethylase that specifically demethylates trimethylated K27 of histone H3, deactivating transcriptional silencing by Polycomb complexes (Rocha-Viegas *et al.* 2014). Also known to regulate, coordinately with MLL2, transcriptional programs for invasiveness and cell proliferation in breast cancer (J. H. Kim *et al.* 2014).

KMT2D/MLL2 (Histone-lysine N-methyltransferase 2D): Methylates K4 of histone H3 (H3K4me), activating transcription. It is shown to be an activator of ERα dependent transcription (Mo *et al.* 2006). Known to be phosphorylated by PLK1 in an estrogen-dependent manner (Wierer *et al.* 2013).

WDR82: Facilitates histone H3 K4 methylation via recruitment of the SETD1A or SETD1B to the S5 phosphorylated C-terminal domain (CTD) of RNA polymerase II (J. H. Lee *et al.* 2008). Known to associate with several distinct histone H3 K4 methyltransferase complexes, protein phosphatase 1 (PP1)-associated proteins and a chaperonin-containing Tcp1 complex (J. H. Lee *et al.* 2010).

KDM1A/LSD1 (Lysine-specific histone demethylase 1A): Demethylates K4 at H3, removing the activation mark (M. G. Lee *et al.* 2005). Shown to activate androgen receptor-dependent transcription by this mechanism (Metzger *et al.* 2005).

Histone deacetylation proteins:

HDAC1 (Histone deacetylase 1): Known transcription repressor, it deacetylates lysine residues of core histone N-terminal tails, removing the activation mark. Forms part of a number of repressive complexes, including Mi2/NuRD complex (Hassig *et al.* 1998, X. Shi *et al.* 2006). Has a kFC of 6.2, with a peak at T15 and at T60. It also has been described as part of activating mechanisms (Qiu *et al.* 2006).

HDAC2 (Histone deacetylase 2): Responsible for deacetylation of lysine residues at the core histone N-terminal tails. Deacetylation is a mark for repression, and this protein, together with its most known interactor, HDAC1, is part of a number of complexes, as will be discussed in chapter 4.4. It is recruited to genomic sites by PR (Vicent *et al.* 2013). It increases its interaction at T05 (kFC: 5.5) and peaks again at T60.

EMSA1 (ELM2 and SANT domain-containing protein 1): Suggested to act as scaffold for HDAC complexes (Bantscheff *et al.* 2011).

GSE1 (Genetic suppressor element 1): May be a component of a BHC histone deacetylase complex (Hakimi *et al.* 2003).

Other histone modification related proteins:

OGT1 (O-GlcNAc transferase): Glycosylates a large number of proteins, including H2B, AKT1, EZH2 or MLL5. It is suggested to regulate their functions by crosstalk between phosphorylation and glycosylation, or by affecting their degradation.

Plays a key role in chromatin structure by mediating O-GlcNAcylation of S112 of histone H2B: recruited to CpG-rich transcription start sites of active genes via its interaction with TET proteins (TET1, TET2 or TET3) (Fujiki *et al.* 2011).

MBD3 (Methyl-CpG-binding domain protein 3): Acts as transcriptional repressor and plays a role in gene silencing, as part of the Mi2/NuRD complex (Saito *et al.* 2002). It regulates expression of 5-hydroxymethylcytosine marked genes in embryonic stem cells with the same complex (Yildirim *et al.* 2011). It is shown to be localized at promoters, gene bodies and enhancers of active genes (Shimbo *et al.* 2013).

Transcription associated proteins:

Mediator complex: Known as the central integrator of transcription. All the subunits found in the high confidence proteins list are found in this cluster, and they all have their maximum interaction with PR between 5 and 15 minutes after hormone exposure, although their kFC varies from 3 to 44. Its presence clearly indicates the start of transcription at this early time point after hormone induction. **MED1; MED12; MED14; MED15; MED24** (Allen *et al.* 2015).

GTF2I(General transcription factor II-I): Interacts with the basal transcription machinery by coordinating the formation of a multiprotein complex at promoters, and linking specific signal responsive activator complexes.

Transcription activators:

NF1 (Nuclear factor 1): Recognizes and binds the palindromic sequence 5'-TTGGCNNNNNGCCAA-3' present in cellular promoters. In vitro, it synergizes with PR for transcription activation of MMTV promoter (Koop *et al.* 2003). The three isoforms are found in this cluster (**NFIA**, **NFIB**, **NFIC**).

GRHL2 (Grainyhead-like protein 2 homolog): Plays an important role in the regulation of the human telomerase reverse transcriptase, forming a complex with MSH2, hnRNPD and hnRNPK (X. Kang *et al.* 2009). It has a role in breast carcinogenesis (Werner *et al.* 2013).

TDIF1(Deoxynucleotidyltransferase terminal-interacting protein 1): Increases DNTT terminal deoxynucleotidyltransferase activity (in vitro) (Yamashita *et al.* 2001). Also acts as a transcriptional regulator, binding to the consensus sequence 5'-GNTGCATG-3' following an AT-tract. Associates with RAB20 promoter and positively regulates its transcription. Binds DNA and nucleosomes and may recruit HDAC1 complexes to nucleosomes or naked DNA.

RBM14 (isoform 1): May function as a nuclear receptor coactivator, enhancing transcription through other coactivators such as NCOA6 and CITED1 (Iwasaki *et al.* 2001).

SNW1 (SNW domain-containing protein 1): Its interaction with PR increases at T05, and decreases thereafter. Gives name to a complex with splicing related proteins and nuclear matrix proteins, possibly related to receptor-mediated transcription (C. Zhang *et al.* 2003). Its depletion in breast cancer cell lines promotes apoptosis (Sato et al. 2015). Shown to interact with PR at the signaling level (Edwards *et al.* 2002).

STAT3 (Signal transducer and activator of transcription 3): Mediates cellular responses to interleukins and other growth factors. It is a known PR intercator (Proietti *et al.* 2011). PR has been shown to help in the assembly of a transcriptional complex containing STAT3, along with AP1 and ErbB2 (Diaz Flaque *et al.* 2013).

GRB2 (Growth factor receptor-bound protein 2): Adapter protein that provides a critical link between cell surface growth factor receptors and the Ras signaling pathway (Byrne et al. 1996), and to regulate STAT3 activation (T. Zhang et al. 2003)

RXRA (Retinoic acid receptor RXR-alpha): Regulate gene expression of its target genes by binding to their response elements in response to ligand exposure (Gorla-Bajszczak *et al.* 1999). Strongly interacts with PR at T05 (kFC: 21.6) but at T60 the interaction is down to 30%.

RABP2 (Cellular retinoic acid-binding protein 2): Regulates the access of retinoic acid to the nuclear retinoic acid receptors.

CASZ1 (Zinc finger protein castor homolog 1): Transcription factor, known to bind histones and capable of recruiting NuRD complex (Z. Liu *et al.* 2015).

RBP56 (TATA-binding protein-associated factor 2N/RNA-binding protein 56): May play specific roles during transcription initiation, regulated by PRMT1 methylation (Jobert *et al.* 2009). It has a k kFC of 1.68.

TRIM33 (Tripartite motif-containing protein 33): Also known as TIF-1, E3 ubiquitin-protein ligase, may act as a transcriptional activator. Has a PHD finger known to bind H3 acetylated at K9, thus displacing the repressive factor HP1-γ, recruiting p300/CBP to genes that are repressed and enabling the recruitment of RNA PolII (Xi *et al.* 2011). It has a kFC of 7.2, with a clear peak at T05.

TF3C4 (General transcription factor 3C polypeptide 4): Part of the RNA polymerase III transcriptional machinery.

FUBP1 (Far upstream element-binding protein 1): May act both as activator and repressor of transcription (Duncan *et al.* 1994). Also shown to have a role in splicing (Jacob *et al.* 2014). It has a low kFC of 1.69, and its homolog FUBP2 is in the basal cluster.

DDX5 and **DDX17** (Probable ATP-dependent RNA helicase DDX5/17): Have multiple functions described; are involved in both pre-mRNA and pre-rRNA processing (Kar *et al.* 2011); known to synergistically interact with CBP/P300 (Rossow *et al.* 2003). Also shown to have a co-activation role with ER and to interact with HDAC1 for a promoter-dependent co-repression (Wilson *et al.* 2004). DDX17 is shown to regulate estrogen dependent transcription and cell growth upon hormone induction (Wortham *et al.* 2009).

Pioneer transcription factors:

Pioneer factors are transcription factors that can bind directly to condensed chromatin, and modulate transcription. They can also recruit other transcription factors or histone modification enzymes (Zaret *et al.* 2011, Jozwik *et al.* 2012). Some have been found in our dataset, all of them in this cluster.

AP2A (Transcription factor AP-2-alpha): The first to interact with PR, it reaches its maximum kFC at T05 (25.1) and starts decreasing thereafter. It interacts with p300/CBP (Braganca *et al.* 2003).

GATA3 (Trans-acting T-cell-specific transcription factor): High binding to PR, but it changes less over time of induction with hormone (kFC: 8.2). Reaches its top values at T05, and it stays interacting for the whole time course (Sasaki *et al.* 2013).

FOXA1 (Forkhead box protein A1): One of the most known pioneer factors (Cirillo *et al.* 2007). Has a kFC of 2.55, reaching its maximum at T30 and drops at T60 tot 56%. Both GATA3 and FOXA1 have been shown to mediate ER binding to DNA (Theodorou *et al.* 2013). It is a key determinant in ER-mediated estrogen response (Hurtado *et al.* 2011), and also associates with PR DNA-target sites that are down-regulated after hormone stimulation in breast cancer cells (Nacht *et al.* 2016).

SOX13 (SRY (Sex determining region Y-box 13): Part of the SOX proteins family. Shown to modulate Wnt activity, with interaction with TCF complex (Marfil *et al.* 2010). Its only peak of interaction is at T30 (kFC: 3.5).

Transcriptional repressors:

NCOR1 (Nuclear receptor co-repressor 1) (N-CoR1): Is part of a number of known repressive complexes. At least for one of them it has been shown to be recruited by PR to progesterone-regulated promoters (De Amicis *et al.* 2009). Also shown to form a SUMOylated-complex with NCOR2 and HDAC3, upon SUMOylation of GR in mice (Hua *et al.* 2016).

NCOR2 (Nuclear receptor co-repressor 2): It also forms part of several repressive complexes.

MTA1 (Metastasis-associated protein 1): Transcriptional regulator that can act in both activation (Gururaj *et al.* 2006) and repression as a component of HDAC-contining complexes (H. J. Kang *et al.* 2014). Its activity can at the same time be regulated by PTMs (Cong *et al.* 2011). Is part of the NuRD complex (Molli *et al.* 2008).

MTA2 (Metastasis-associated protein 2): Together with MTA1 forms part of a number of complexes with several different functions, depending of the interactors. They are some of the most upregulated genes in cancer, and correlate with aggressive phenotypes and poor prognosis (R. Kumar *et al.* 2016).

P66B / GATAD2B (Transcriptional repressor p66-beta): Is part of the Mi-2/NuRD complex, mediating MBD proteins and histone interaction (Brackertz *et al.* 2002).

TLE3 (Transducin-like enhancer protein 3): Transcriptional co-repressor that binds to a number of transcription factors, including FOXA1 in MCF7 cells, in correlation with HDAC2; maintains basal state of acetylation low, preventing ER and RNA PolII binding (Jangal *et al.* 2014).

NELFE (Negative elongation factor E): Part of the NELF complex, which represses RNA Pol II elongation (Yamaguchi *et al.* 1999).

TRPS1 (Zinc finger transcription factor Trps1): Co-repressor of GATA-mediated transcription, through its own SUMOylation by UBC9 (Kaiser *et al.* 2007).

I2BP1 (Interferon regulatory factor 2-binding protein 1): Act as transcriptional co-repressors in an IRF2-dependent manner, independently of histone-deacetylase activities (Childs *et al.* 2003). **I2BP2** is found in cluster 3, and both have a high IgGFC (65 and 74 respectively).

SATB1: Transcriptional repressor controlling nuclear and viral gene expression in a phosphorylated and acetylated status-dependent manner, by binding to matrix attachment regions (MARs) of DNA and inducing a local chromatin-loop remodeling. Acts as a docking site for several chromatin-remodeling enzymes and also by recruiting corepressors (HDACs) or coactivators (HATs) directly to promoters and enhancers (Yasui *et al.* 2002). Has a role in reprogramming chromatin organization and the transcription profiles of breast tumors to promote growth and metastasis (Han *et al.* 2008).

CTBP1: Acetylation-dependent interaction of SATB1 and CtBP1 mediates transcriptional repression by SATB1 (Purbey *et al.* 2009). It has NAD-dependent hydrolase activity (V. Kumar *et al.* 2002). It has a role in tumorigenesis by transcriptional attenuation (Nibu *et al.* 1998). It has a low kFC (1.71).

ZN281 (Zinc finger protein 281): Transcription repressor that plays a role in regulation of embryonic stem cells (ESCs) differentiation by association of NANOG protein to its own promoter and recruitment of the NuRD complex, which deacetylates histones. Represses the transcription of a number of genes (X. Zhang *et al.* 2003).

LMCD1 (LIM and cysteine-rich domains protein 1): Transcriptional cofactor that restricts GATA6 function by inhibiting DNA-binding, resulting in repression of GATA6 transcriptional activation of downstream target genes (Rath *et al.* 2005).

Chromatin structure related proteins:

SMRD2 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 2, BAF60B): Part of BAF complex. Has a very high increased interaction with PR at T05 (kFC: 56).

SMCE1 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1, BAF57): Part of the BAF complex, the PBAF complex and the LARC complex. Recruited by PR to hormone-regulated promoters (Vicent *et al.* 2004).

HM20B (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1-related, BRAF35): Part of the LSD1 repressive complex. Increases the interaction at T05 (kFC: 9.00), and maintains it high for the rest of the time course.

MCM7: Part of the MCM complex, with an important role in DNA replication licensing (Blow 2005). The whole complex is found, and their kFCs are always lower than 2.

DBHS proteins:

The DBHS (Drosophila Behavior/Human Splicing) protein family is composed by three members, with a wide range of functions, including transcription co-repression and co-activation, mRNA co-transcriptional processing, mRNA retention and double strand break repair (Knott *et al.* 2016).

NONO (Non-POU domain-containing octamer-binding protein (p54nrb)): Forms a complex with **SFPQ** (Splicing factor, proline- and glutamine-rich) and **PSPC1** (Paraspeckle component 1), found in cluster 3. The three of them have a kFC lower than 2, being NONO the one with the highest IgGFC (33). Recruits the exonuclease XRN2 (in cluster 3) to facilitate pre-mRNA 3' processing and transcription termination (Kaneko *et al.* 2007). Shown to be phosphorylated by CDK1 (in basal cluster) and to interact with PIN1 due to this modification (Proteau *et al.* 2005). Also shown to have transcriptional modulating activity on PR (Dong *et al.* 2009) and AR (Dong *et al.* 2007). PARP1 activation can modulate NONO action in the DNA-damage response (Krietsch *et al.* 2012). Has been described to be regulated by PP1 activity (also in cluster 3) (L. Liu *et al.* 2011). Shown to be methylated by CARM1, reducing its mRNA affinity (Hu *et al.* 2015), antagonized by citrullination (Snijders *et al.* 2015).

MATR3 (Matrin-3): May associate with other matrix proteins. Shown to interact with NONO-SFPQ heteromer for defective RNAs retention (Z. Zhang *et al.* 2001), also shown to participate in the DNA damage response (Salton *et al.* 2010). It has been shown to contribute to ER gene regulation (Skowronska-Krawczyk *et al.* 2014).

PIN1 (Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1): Enhances ligand-dependent binding of ERα activated by estrogen but not by Tamoxifen to its DNA binding sites (Rajbhandari *et al.* 2015). Reach its maximum kFC at T15.

DNA damage related proteins:

ILF3 / **NF90** (Interleukin enhancer-binding factor 3): Together with **ILF2**/**NF45** (at cluster 3), is known to be part of the PRKDC complex (Ting *et al.* 1998). They are suggested to modulate PRKDC activity, and to participate in DNA break repair via non-homologous end joining (Shamanna *et al.* 2011). Both have a similar pattern although are not in the same cluster, with a first peak at T15.

RPA1 (Replication protein A 70 kDa): Binds and stabilizes single-stranded DNA intermediates, formed during DNA replication or upon DNA stress (He *et al.* 1995, Lin *et al.* 1998), works together with PRP19 (see cluster 3) to sensor DNA damage (Marechal *et al.* 2014). Increases the interaction with PR by 1.8 fold already at T05.

Ubiquitin and SUMO (Small ubiquitin-related modifier) related proteins:

SUMO and ubiquitin modifications have always been considered, although similar in structure, different type of functional modifications, being SUMO more related to regulation of transcription, chromatin structure, and DNA repair. In the other hand, ubiquitin modifications are related to proteasomal pathways, although it also has nonproteolytic functions (Gill 2004).

Proteasome related proteins:

CPNE1 (Copine-1): One of the proteins with the lowest kFC of the cluster together with NPL4. It is a calcium-dependent phospholipid-binding protein that plays a role in calcium-mediated intracellular processes (Tomsig *et al.* 2004). Represses NF-kappaB transcription by endoproteolysis of p65 (Ramsey *et al.* 2008).

NPL4: Is involved in the proteasomal ubiquitin-dependent pathway. The NPLOC4-UFD1L-**VCP** complex (the latter in cluster 3) regulates spindle disassembly at the end of mitosis and is necessary for the formation of a closed nuclear envelope (Botta *et al.* 2001).

TBL1R: F-box-like protein involved in the recruitment of the ubiquitin/19S proteasome complex to nuclear receptor-regulated transcription units, playing a role in transcription activation mediated by nuclear receptors (Perissi *et al.* 2004).

PSMD5 (26S proteasome non-ATPase regulatory subunit 5): Acts as a chaperone in the assembly of the proteasome complex (Roelofs *et al.* 2009).

UBP7 (Ubiquitin carboxyl-terminal hydrolase 7): Hydrolase that deubiquitinates target proteins such as FOXO4 and p53/TP53 among others (van der Horst *et al.* 2006). Has a strong interaction with PR (IgGFC: 100) already at 1 minute after hormone induction with a low kFC (1.71) that decrease after 5 minutes.

NEDD8 (Neddylin or Neural precursor cell expressed developmentally down-regulated protein 8): An ubiquitin-like protein, it is known to covalently link to Cullins. They are not found in the high confidence list, but Cullin4 A and B are both among the moderate confidence proteins (Hori *et al.* 1999). Has a peak of interaction at 1 minute and again at T60, with a kFC of 3.36.

UBA1 (Ubiquitin-like modifier-activating enzyme 1): It catalyzes the first step of ubiquitin-mediated protein degradation. It also has been shown to have a role in the response to DNA damage (Moudry *et al.* 2012).

SUMO related proteins:

PIAS1 (Protein inhibitor of activated STAT protein 1): Functions as an E3-type small ubiquitin-like modifier (SUMO) ligase. Also shown to SUMOylate MTA1, member of Mi2-NURD complex, regulating its activity (Cong *et al.* 2011). It interacts and coregulates AR (J. Tan *et al.* 2000).

PIAS3 (Protein inhibitor of activated STAT protein 3): Also an E3-type small ubiquitin-like modifier (SUMO) ligase, shown to stimulate PR sumoylation, which represses its transactivation (Man *et al.* 2006). Both PIAS1 and PIAS3 have a high increase of interaction at T05 (kFC of 26 and 32, respectively), but PIAS1 decreases already at T30, while PIAS3 stays high to decrease at T60.

Splicing related proteins:

Although most of the proteins related to splicing found in our high confidence data set have a relatively low kFC, they all increase their interaction with PR after hormone induction. Splicing related proteins found in this cluster are characterized by the decrease in its interaction at T60, even though more than half of them have a kFC lower than 2, and most of the splicing related proteins are in cluster 3. Here are mentioned a few splicing related proteins with additional functions.

XPO5 (Exportin-5): kFC: 2.74. Mediates the nuclear export of proteins bearing a double-stranded RNA binding domain (dsRBD) and double-stranded RNAs (cargos).

Mediates nuclear export of ADAR/DSRAD in a RanGTP-dependent manner (Fritz *et al.* 2009).

DSRAD/ADAR1 (Adenosine deaminase acting on RNA 1): Catalyzes the hydrolytic deamination of adenosine to inosine in double-stranded RNA (dsRNA), referred to as A-to-I RNA editing. This may affect gene expression and function in a number of ways that include mRNA translation, pre-mRNA and RNA (W. Yang *et al.* 2005).

KHDR1/**SAM68**: Recruited and tyrosine phosphorylated by several receptor systems, having a function in signal transduction cascades (Lukong *et al.* 2005). It is also acetylated by CBP, enhancing its RNA binding activity (Hong *et al.* 2002), and regulates alternative splicing (Paronetto *et al.* 2010).

CSTF1 and **CSTF3** (Cleavage stimulation factor subunit): Factors required for polyadenylation of pre-mRNAs.

4.3.4.4 Cluster 3 (93 proteins):

Most of the proteins enclosed in this cluster have their maximum interaction after longer time points, T30 or T60 and they are characterized by their constant increase of the interaction with PR (Fig. 56), although more than 55% of them have a kFC lower than 2. This is seen at the wordcloud, where most of the proteins are in small letters (Fig. 57). Nevertheless, still a big amount of them are coloured in pink/reddish, indicating high values of IgGFC.

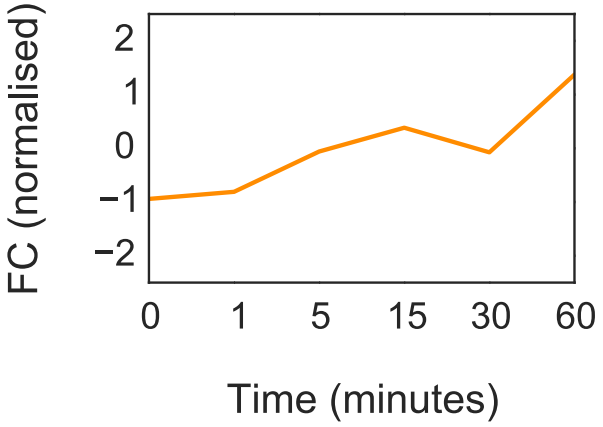


Figure 56: Cluster 3 trendline. Average values of all the proteins from cluster 3. Values correspond to normalized IgG fold changes over time.

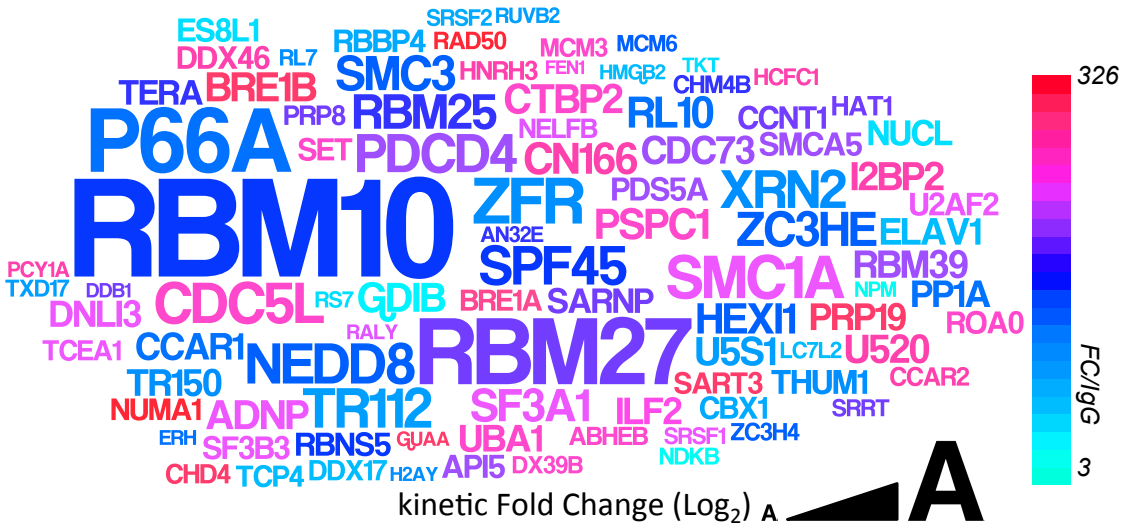


Figure 57: Wordcloud with Cluster 3 proteins. Representation of proteins found at Cluster 3. Colour code represents maximum IgGFC of each protein, which is a value of estimated amount of protein. Size code represents the kFC, obtained dividing each protein maximum by its minimum value of IgGFC.

Proteins related to RNA splicing and processing dominate this cluster representing almost 40 % of all the proteins according to GO enrichment analysis (Fig. 58). Other terms enriched in the analysis are related to chromatin modification, gene expression and DNA damage repair.

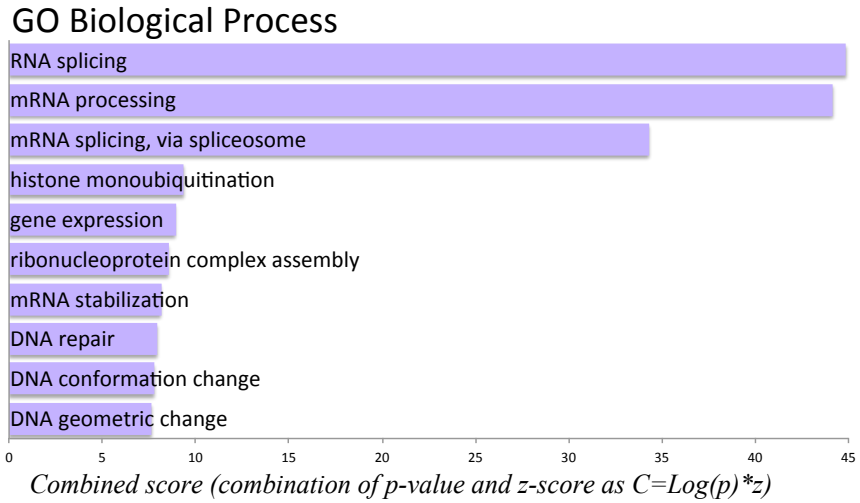


Figure 58: GO biological processes cluster 3. Weighted list of proteins enclosed at cluster 3 were compared to Biological processes GO terms for enrichment analyses. Weight was to given to each

On KEGG pathways enrichment analyses, Spliceosome is the most enriched term. Another 3 terms are significantly enriched, and they are related to DNA damage repair and replication (Fig. 59).

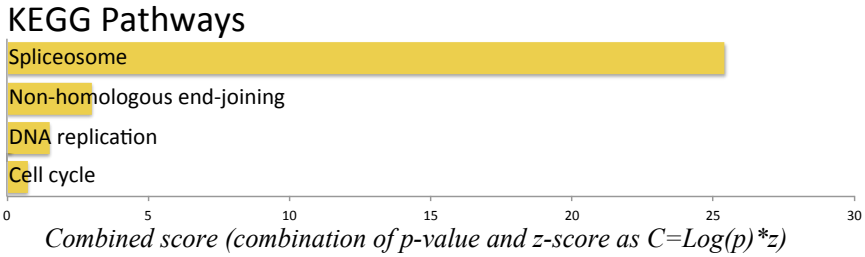


Figure 59: KEGG pathways cluster 3. Weighted list of proteins from cluster 3 were compared to KEGG pathways database for enrichment analysis. Proteins were given weight before analysis with their maximum IgG FC (Log_2). Only significative terms are shown (p-value<0.05).

Splicing related proteins:

Spliceosomal proteins[1]:

The spliceosome is a macromolecular highly dynamic machinery, responsible for the removal of introns of nascent RNA transcripts. Nearly 40% of the spliceosome is found in the data set, where most of the proteins are located in this cluster, including 3' splice site recognition proteins (**U1snRNP** or **U520**), several Heterogeneous Nuclear RibonucleoProteins (HNRPs), RNA Binding proteins (RBMs) or splicing factors like, **SPF45** or **U2AF**[65]. Among them, **RBM39** (also known as CAPERα) is a known PR coactivator (Dowhan *et al.* 2005). SPF45 has been described to have a role in DNA repair (Chaouki *et al.* 2006).

PRP19 (Pre-mRNA-Processing factor 19): Has one of the highest IgGFC, and kFC of 2.24 at longer time points. Is part of the spliceosome, but has other functions related to DNA damage response. Has ubiquitin ligase activity and promotes RPA1 (found in cluster 2) ubiquitylation in DNA damage dependent manner, in complex with **CDC5L** also found in this cluster (Marechal *et al.* 2014).

ELAV1 (ELAV-like protein 1 / Hu-antigen R): Suggested to mediate polyadenylation coupling to transcription (Hilgers 2015) and to function as an alternative pre-mRNA splicing regulator in exon definition (Izquierdo 2008).

DDX39A and **DDX39B**: These ATP-dependent RNA helicases are involved in nuclear export of spliced and unspliced mRNA. Both have a high kFC (over 50) and a similar kinetic pattern, although DDX39Bs interaction with PR increases a bit later, at T15 instead of at T05.

Splicing factors:

RBM10 (RNA-Binding protein 10): Has a role in regulation of alternative splicing (Inoue *et al.* 2014). Its interaction with PR exhibits a constant increase with a highest kFC (19.2) at T60. It also has tumor suppressor properties (Hernandez *et al.* 2016). Together with **RBM27** and **CSTF1**, is one of the few splicing related proteins found with an kFC higher than 5.

SART3 (Squamous cell carcinoma Antigen Recognized by T-cells): Functions as a recycling factor recruiting deubiquitinases to its targets including splicing factors (Song

[1] CORUM annotated core spliceosomal components

et al. 2010) and histones, like free ubH2B (Long 2014). It has a very high IgGFC (106), but a low kFC of 1.89.

SARNP (SAP domain-containing ribonucleoprotein): Known to interact and enhance **DDX39A**, and to be part of the mRNA export complex TREX (Dufu *et al.* 2010).

Transcription associated proteins:

Co-activators:

SET: Multitasking protein involved in apoptosis, transcription and nucleosome assembly. Interacts with **HMGB2** in the SET complex, together with NDKA and AN32E, which facilitates the nucleosome assembly, transcriptional activation, and DNA repair functions of SET (Fan *et al.* 2002). It inhibits PP2A phosphatase activity (M. Li *et al.* 1996).

AN32E: Histone chaperone that specifically removes H2A.Z from chromatin (mainly present in heterochromatin) (Obri *et al.* 2014). Exhibits a peak of interaction at T05 and again at T60. Is part of the SET complex.

HMGB2 (High Mobility Group protein B2): As its homolog HMGB1 (found in the basal cluster) has an IgGFC of 15, but a slightly higher kFC of 1.5, due to a lower value at T01.

ADNP (Activity-Dependent NeuroProtector homeobox protein): Contains one homeobox and nine zinc finger domains, suggesting a functions astranscription factor. Its PR interaction peaks at T15 (kFC: 2.4).

BRE1A and **BRE1B** (RNF20/40 ubiquitin-protein ligase complex): Both have a strong PR interaction (IgGFC ~90), and a kFC between 1.8 and 2.4. Are part of a complex responsible for H2B ubiquitylation, which stimulates transcript elongation (Pavri *et al.* 2006).

HAT1 (Histone AcetylTransferase 1): Has the same pattern as other histone acetyltranferases, with an increase at T60, but its kFC is low (1.80).

CAR1 (Cell Cycle and Apoptosis Regulator protein 1): Promotes and stabilizes AR binding to chromatin by favoring GATA2–AR association (W. Y. Seo *et al.* 2013). It facilitates recruitment of MED1 and RNA PolII to AR-binding sites (Mizuta *et al.* 2014). Has a kFC of 2.23, with two peaks, at T15 and at T60.

TR150 (Thyroid hormone Receptor-associated protein 3): Has a role in transcriptional activation, alternative splicing and DNA damage response, being recruited to DNA damage sites (Beli *et al.* 2012). Interacts with SFPQ (Heyd *et al.* 2010).

CDC73 (Parafibromin): Tumor suppressor probably involved in transcriptional and post-transcriptional control pathways. As part of the PAF1 complex, has a role in transcriptional elongation (J. Kim *et al.* 2010). It has 3 peaks of interaction, at T01, T15 and T60 (kFC: 2.28).

TCEA1 (Transcription Elongation factor A protein 1): Also found in PAF1 complex. Exhibits a more stable PR interaction, with a kFC of 1.83.

HCFC1 (Host Cell Factor 1): Has a strong PR interaction. May be involved in transcription activation (Vogel *et al.* 2000), participates in assembly of histone modifying complexes (Wysocka *et al.* 2003) and enables transcription of viral genes upon infection (S. S. Mahajan *et al.* 2000).

XRN2 (5'-3' exoribonuclease 2): Has a role in transcription termination (West *et al.* 2004). Exhibits peaks of PR interaction at T30 and T60. Is recruited by the NONO complex to splicing sites.

TCP4 (Activated RNA polymerase II transcriptional coactivator p15): General coactivator that functions cooperatively with TAFs and mediates functional interactions between upstream activators and the general transcriptional machinery (Kretzschmar *et al.* 1994).

CCNT1 (Cyclin-T1): Is part of the positive transcription elongation factor B (P-TEFb) complex (Z. Yang *et al.* 2005). It has been shown to interact and co-localize with ERα at target sites (Wittmann *et al.* 2005).

HEXI1/**EDG1** (Protein HEXIM1 / Estrogen Down-regulated Gene 1 protein): It acts as a growth inhibitor downregulated by estrogens and co-localizes with ER alpha in breast tissue (Wittmann *et al.* 2003). It has been shown to inhibit P-TEFb (CDK9/Cyclin T) kinase and RNA polymerase II coordinated with 7SK snRNA (Yik *et al.* 2003) and to modulate the functional interaction between ERα and CCNT1 (Wittmann *et al.* 2005).

TR112 (Multifunctional methyltransferase subunit TRM112-like protein): Participates in the methylation of tRNA and in ribosomal assembly (Liger *et al.* 2011). Has its peak of interaction at T60 (kFC: 3.30).

RUVB2 (RuvB-like 2): Together with its homolog RUVB1 (found in the basal cluster) forms part of a number of complexes that play a role in transcription activation by

histone acetylation (NuA4 complex) (Gnatovskiy *et al.* 2013), DNA repair (Utley *et al.* 2005) and H2A.Z removal (Mizuguchi *et al.* 2004). It has a low kFC of 1.59.

PDCD4 (Programmed Cell Death protein 4): Inhibits translation initiation by binding to eIF4A (found in cluster 2) (Suzuki *et al.* 2008). It increases its PR interaction at T15, and rises at T60 with a kFC of 3.17.

NDKB/NME2 (Nucleoside Diphosphate Kinase B): Major role in the synthesis of nucleoside triphosphates other than ATP. NDKA is also found in the moderate confidence data set, with a similar interaction pattern. It has been suggested as tumor suppressor (Postel *et al.* 1993). Binds Gelsolin (found in the basal cluster). They have been suggested as predictors of pathological complete response to neoadjuvant chemotherapy, together with PR, ER, HER2, Ki-67 and TopoIIα (X. R. Li *et al.* 2011).

DDX17 (DEAD box helicase 17): has RNA-dependent ATPase activity. Is involved in transcriptional regulation, and plays a role as transcriptional coactivator of ER (Wortham *et al.* 2009).

Co-repressors:

CBX1 (ChromoBox protein homolog 1) (HP1 beta): With a kFC close to 2, increases its PR interaction at T05 and maintains it high.

CTBP2 (C-Terminal-Binding Protein 2): Co-repressor targeting several transcription regulators (Mendez *et al.* 2008).

CHD4/Mi-2: Part of the Mi-2/NuRD complex, which participates in the remodeling of chromatin by deacetylation of histones (Tong *et al.* 1998). It also has a role in DNA repair regulation (Pan *et al.* 2012). It has a high IgGFC (110), and a low kFC (1.8), with relatively constant interaction with the receptor that reaches its maximum at T15.

SMC1A (Structural Maintenance of Chromosomes protein 1A): Is involved in the cohesion of chromosomes during cell cycle and DNA repair, as part of the Cohesin complex. It has the higher KFC of the cluster (3.6), and its maximum values are at T15 and T60.

SMC3 (Structural Maintenance of Chromosomes protein 3): Also part of the cohesin complex.

SMCA5/SNF2H (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 5), along with RBBP7 (found in the basal cluster) and

some other proteins from other clusters ia part of the cohesion complex. Their kinetic patterns are very similar to that of SMC1A.

PDS5A (Sister chromatid cohesion protein PDS5 homolog A): May stabilize cohesin complex association with chromatin (Terret *et al.* 2009). It follows the same kinetic pattern as SMC1A.

P66A (Transcriptional repressor p66-alpha): (GATAD2A). Forms part of Mi2-NuRD repressive complex.

RBBP4 (Histone-binding protein RBBP4): Part of the Mi2/NuRD complex, together with RBBP7 (at the basal cluster). kFC < 2.

H2AY (Core histone macro-H2A.1): It replaces H2A in a subset of nucleosomes involved in repressing transcription. It is the only core histone that appears in the high confidence data set. Has a stable kinetic pattern, andone of the lowest kFC of the cluster (1.53), which peaks at T05, T15 and T60.

NELFB (Negative ELongation Factor B /Cofactor of BRCA1): Essential component of the NELF complex, which represses RNA PolII transcription (Yamaguchi 1999 (Yamaguchi *et al.* 1999)). Has two peaks of PR interaction at T05 and at T60, and its kFC is 1.83, with a strong PR affinity (IgGFC: 42).

PP1A (Serine/threonine-protein phosphatase PP1-alpha): Known to regulate over 200 proteins. Has its maximum interaction at T60 (kFC: 2.23).

DNA damage repair related proteins:

DNA damage has been coupled to some events during transcription. It also has been suggested to have a link with hormone signaling pathways (Schiewer *et al.* 2016).

RAD50 (DNA repair protein RAD50): Exhibits a strong PR interaction (IgGFC: 172), which oscillates during early time points and rises at T60, with a kFC of 1.69. Plays a central role in DNA double-strand break repair, as part of the MRN complex (Carney *et al.* 1998) and is the only component of the complex found in the high confidence data set; the other two are found in the moderate confidence data set.

FEN1 (Flap EndoNuclease 1): Is involved in DNA replication and repair (Guo *et al.* 2008). Exhibits a strong and stable PR interaction, except for T01, and exhibits the lowest kFC of the cluster.

Lig3 (DNA ligase 3): May act as DNA strand break sensor (Abdou *et al.* 2015). Has a mild increase of PR interaction at longer time points.

NPM (Nucleophosmin): Is involved in ribosome biogenesis, protein chaperoning and as DNA damage sensor. Its histone chaperone activity enhances acetylation-dependent transcription (Swaminathan *et al.* 2005). It is recruited to DNA damage sites by ubiquitin conjugates (Koike *et al.* 2010) and mediates resistance to retinoic acid-induced transcription (Found in the moderate confidence dta set) (Nichol *et al.* 2016).

DDB1 (DNA Damage-Binding protein 1): In complex with Cul4 and RBX1 (found in the moderate confidence data set) forms a DNA damage sensor complex, which ubiquitinates histones at the damage site (kFC: 1.54).

CCAR2/**DBC1** (Cell Cycle and Apoptosis Regulator protein 2 / Deleted in breast cancer gene 1 protein): It is modified by SUMO2 in response to DNA damage (Park *et al.* 2014). Has a kFC lower than 2, but its IgGFC is high (66).

Structural proteins:

Other components of the MCM complex are found in this cluster, **MCM3** and **MCM6**, both with kFC between 1.5 and 2.

NUMA1 (NUclear Mitotic Apparatus protein 1): Has the same pattern as RAD50, with high PR interaction (IgGFC: 133) and low kFC (1.92). It is a highly abundant protein, is part of the nuclear matrix, and relocates to the spindle poles in mitosis. Its phosphorylation may affect cell proliferation (Toughiri *et al.* 2013).

NUCL (Nucleolin): Highly abundant nuclear protein, especially in the nucleolus, it has been shown to form part of a transcription factor binding component (Hanakahi *et al.* 1997). Has a role in the ribosome assembly (Ginisty *et al.* 1998) and in nucleosomes remodeling (Angelov *et al.* 2006) and has histone chaperone activity (Gaume *et al.* 2011). Its interaction with PR increases at T60, with a kFC of 2.12.

PCY1A/**CCTa** (Choline-phosphate CytidylylTransferase A): Is a phosphatidylcholine synthase. Its phosphorylation by ERK in the oxysterols signaling pathway decreases its activity (Agassandian *et al.* 2005). Has a strong interaction with PR (IgGFC: 68.8) with a mild increase at T60 (kFC: 1.59).

4.4 Dynamics of functional complexes

Many of the proteins identified by mass spec as PR interactors are annotated to belong to a number of functional complexes. In this section it will first described those protein known to form a functional complex according to CORUM and will follow the dynamic changes of the complexes upon hormone exposure.

4.4.1 Annotated complexes

CORUM stands for *Comprehensive Resource of Mammalian protein complexes* and it's a database that provides a resource of manually annotated protein complexes from mammalian organism. It provides the proteins forming the complex and the publications that described it for the first time. As a difference to other databases such as the STRING database, CORUM focus on annotated functional complexes, but not on protein-protein interactions.

From all the interactors identified in the high confidence data set, more than 40% are not included in any CORUM annotated complex, and 35% of them are part of 1 to 3 different complexes (Fig. 60 left panel). In the STRING database, only slightly more

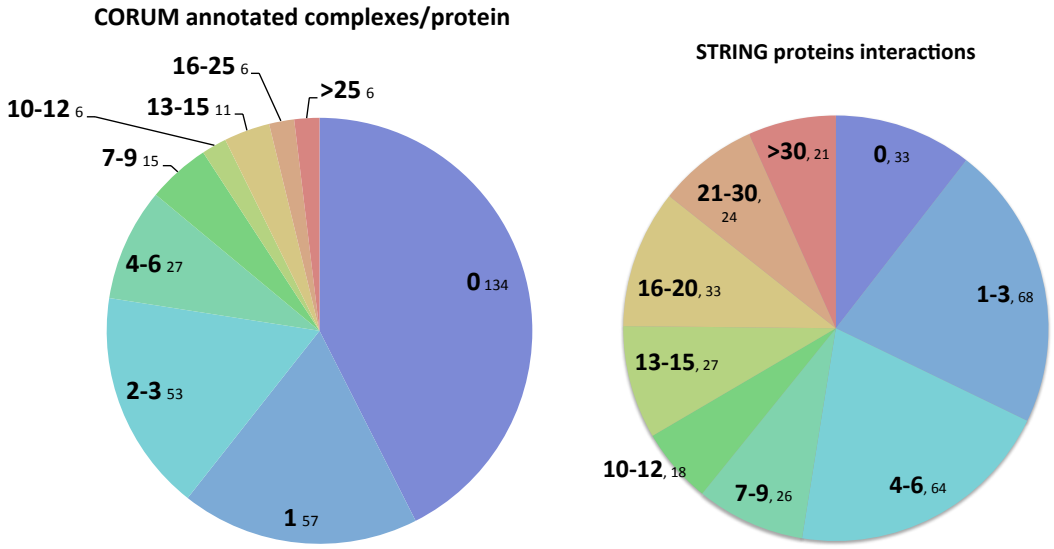


Figure 60: Annotated interaction of proteins in the high confidence data set. Left pie chart, shows number of CORUM annotated complexes (large numbers) to which each protein (small numbers) belongs. Right pie chart, shows the number of protein-protein interactions (large numbers) annotated in STRING database for each protein (small numbers).

than 10% of the identified interactors do not have annotated protein-protein interaction.

At the top of this list, there are proteins that are annotated to form part of more than 25 complexes, being the most abundant HDAC1 and HDAC2, with more than 50 complexes each, and RBBP4 and RBBP7, with 48 and 34 complexes respectively. One needs to consider that some complexes are duplicated in the database, with different names but with the same components. The name of the first identified complex is selected for the analysis.

Even so, HDAC1 and HDAC2 are really part of a big number of transcription repressive complexes. RBBP4 and RBBP7 also form part of a number of complexes with both activating and repressing functions, but they are core histone binding proteins and may be the components that link the complex with chromatin.

In the PR interactors data set we found a number of complexes annotated in the CORUM database. For our analysis, only complexes that include 2 or more PR interactors are considered.

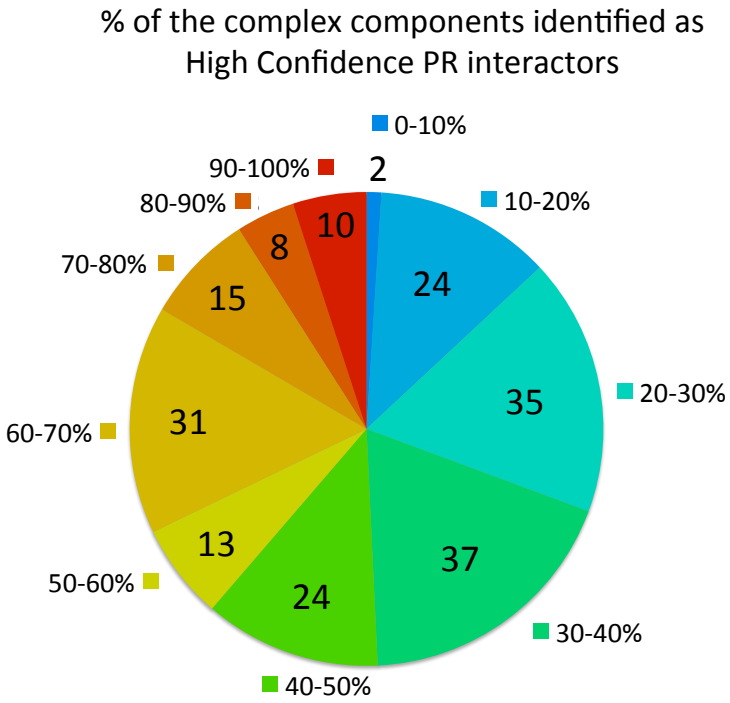


Figure 61: Pie chart of percentage of the complex identified among proteins characterize in the high confidence data set. Showing only CORUM annotated complexes. Inside the chart, the numbers of interactors for each part.

Taking into account the complexes that include at least 2 high confidence interactors (HCIs) we find 199 complexes, and 98 of them, less than 40% of the complex components are found. In another 37 complexes 40-60% of the complex components

are HCIs. In another 46 complexes 60-80% of the complex components are HCIs, and in the top 18 complexes 80% to 100% of the complex components are HCIs (Fig. 61). If we include moderate confidence interactors (MCIs), these percentages of complex components that are PR interactors increase considerably. Therefore, and taking into account that not all proteins are identified equally by mass spec, we decided to include MCIs for the identification of relevant functional complexes including PR interactors. In this case, the total number of complexes that include at least 2 PR interactors increases to 275. For 70 of the PR interactors (high and moderate confidence added) represented 70% of the complex components, and 120 of them, more than half of the complex components were PR interactors (Fig. 62).

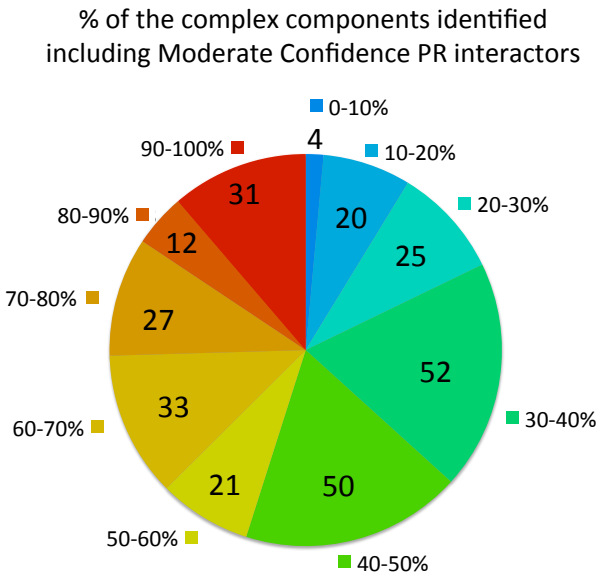


Figure 62: Pie chart of percentage of the complex identified among proteins characterize at moderate confidence data set. Showing only CORUM annotated complexes.

One should keep in mind that considering only complexes with at least 60% of their components being PR interactors, large complexes like the Spliceosome with 143 components, will be excluded. In this case, 42% of the complex components - 60 out of 143 proteins - are PR interactors. Most of the complexes described here are annotated at CORUM, otherwise it will be stated.

About the dynamics of the complexes, it has to be taken into consideration that some of the components may be part of several complexes, and this may make their pattern of interaction with PR blurrier than it would be expected for a certain complex alone.

4.4.1.1 Transcription related complexes

Many of the complexes found in the dataset are related to transcription, both activation and repression. Some of them are tightly related to nucleosomal remodeling, for both opening of the chromatin for activation or repressing transcription by compacting chromatin. In fact, it has been suggested that for a global maintenance of the chromatin state, several chromatin remodeling complexes need to act in collaboration (S. A. Morris *et al.* 2014). They mostly have a rapid interaction with progesterone receptor, indicating a fast start of both transcription activation and active repression. Here it will be described some of the complexes found and their dynamic characteristics.

Transcription activation:

SRC-1 complex:

Three subunits of this co-activation complex are high fidelity PR interactors, NCOA1, NCOA2 and CBP, and their dynamic pattern is very similar, all of them are found in the kinetic cluster 2 (Fig. 63). Although TROVE2 (or RO60) is described as part of the complex, it is not described as interacting with the rest of the complex in the STRING database.

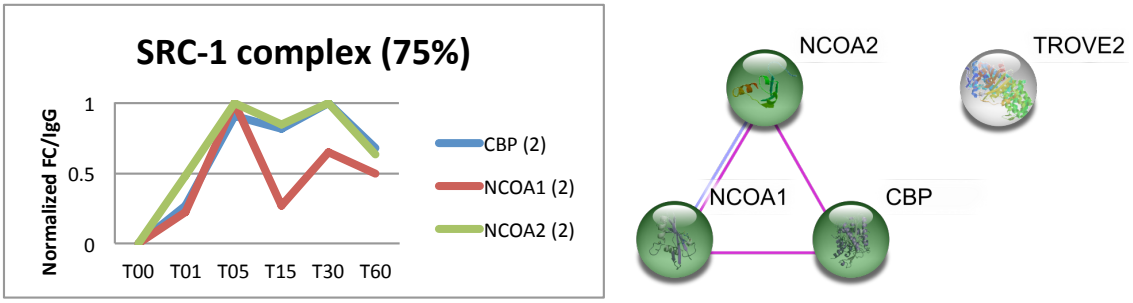


Figure 63: SRC-1 complex. Left panel: Line chart with identified proteins from the complex, normalized IgGFC values. Dynamic cluster (4.4.4) is shown in brackets (). Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high fidelity PR interactors (green) and moderate fidelity PR interactors (yellow); proteins that did not pass FDR filtering or that were not identified (light grey).

hNURF complex:

Widely known ATP-dependent chromatin remodeler complex, described as a regulator of Engrailed expression (Barak *et al.* 2003). The 3 PR interactors in the complex follow a similar kinetic pattern (Fig. 64 left panel). The biggest subunit, the ATPase BPTF, it is not even found in the medium confidence dataset. It has been shown to be recruited by PR upon hormone induction to PR target sites, where it facilitates the PR-dependent recruitment of activated CDK2, responsible for the H1 displacement in the very first minutes after hormone exposure (Vicent *et al.* 2011).

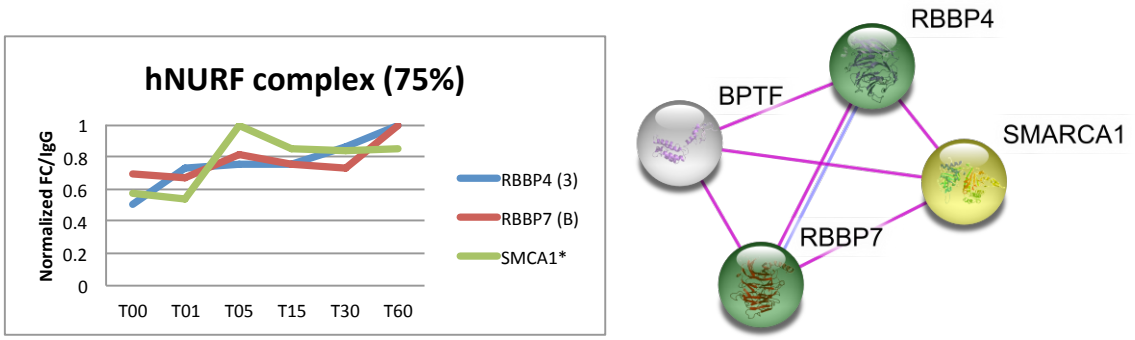


Figure 64: hNURF complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

Multisubunit ACTR co-activator complex:

ACTR is a histone acetyltransferase complex, only missing one of the subunits, which did not pass any of the FDR filters. It was described to directly bind nuclear receptors and stimulate their transcriptional activity (H. Chen *et al.* 1997). All 3 high fidelity PR interactors in the complex follow very similar kinetic pattern (Fig 65 left panel). As they postulate, nuclear receptors activation involves the recruitment of at least three classes of histone acetyltransferases that may act cooperatively as an enzymatic unit to reverse the effects of histone deacetylase shown to be part of the nuclear receptor corepressor complex.

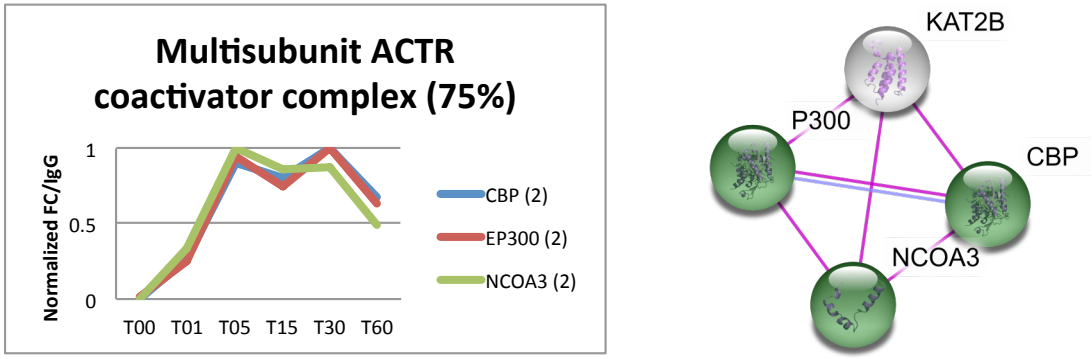


Figure 65: Multisubunit ACTR coactivator complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green); light grey, proteins that did not pass FDR filtering or that were not identified.

TRAP complex:

Contains eight subunits of the Mediator Complex and it is described as a thyroid hormone receptor-associated proteins (TRAP). It functions as co-activator of nuclear receptors (Fondell et al. 1996), and several identical subunits are shared with other described complexes, indicating that unique classes of transcription factors may be sharing common subsets of co-factors. Five of the eight components are high fidelity PR interactors, that follow a similar kinetic pattern upon hormone exposure (Fig. 66 left panel). More recently it has been shown to be recruited to Estrogen receptor target genes, where it is required for ER-mediated transcription and estrogen-dependent breast cancer cell growth (X. Zhang et al. 2005).

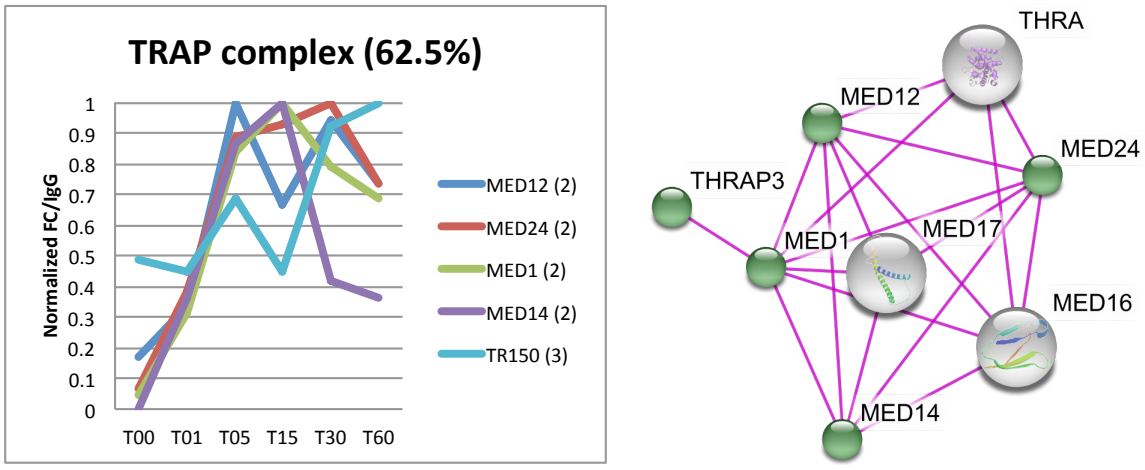


Figure 66: TRAP complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green); light grey, proteins that did not pass FDR filtering or that were not identified.

BRD4 complex:

This complex is another example on how transcription factors associate with common components of the transcription machinery. In this case, Brd4 interacts with cyclinT1 and Cdk9 that constitutes the core positive transcription elongation factor b (P-TEFb), and in association with mediator components forms the BRD4 complex (Jang *et al.* 2005). In this complex only CCNT1 and the mediator components are high confidence PR interactor, while BRD4 and CDK9, although identified by MS, doesn't pass the FDR filters. In this case, components of the mediator complex are recruited rapidly, while CCNT1 reaches its maximum interaction at later time points (Fig. 67 left panel).

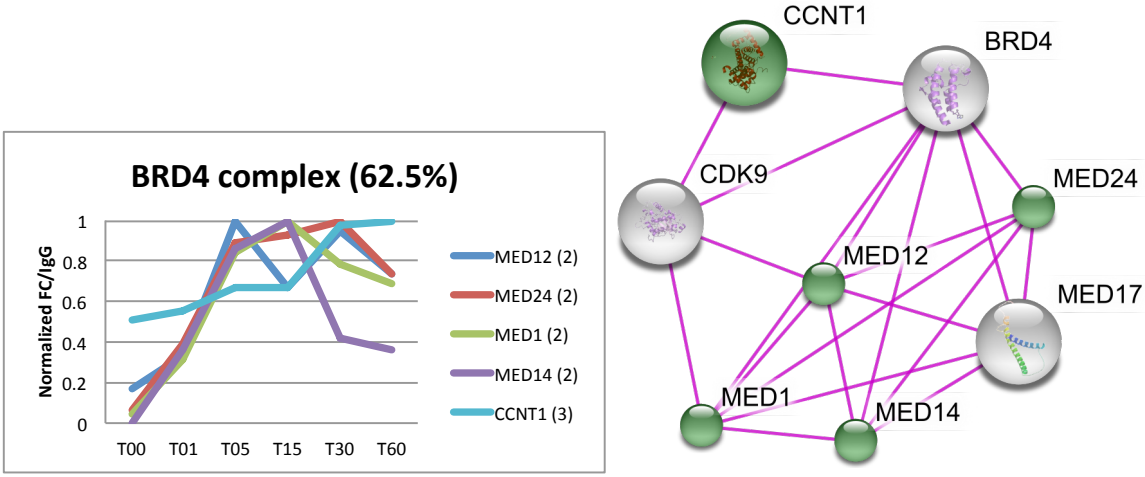


Figure 67: BRD4 complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

SNW1 complex:

Firstly described by (C. Zhang *et al.* 2003), represents components of the spliceosome, as well as other nuclear matrix-associated proteins. It is suggested to couple Vitamin D-mediated transcription and RNA splicing. It has been shown to interact with the Ligand Binding Domain of PR in a hormone dependent manner (Edwards *et al.* 2002). But it also has been shown to have dual roles, in a cell type-dependent manner, it can interact with CBP co-activator complexes, or with co-repressor/HDAC complexes. It contains 18 components of which 9 are high fidelity PR interactors and 4 are moderate fidelity PR interactors (Fig. 68). The complex here described, highlights the role of SNW1 as a link between transcription and splicing.

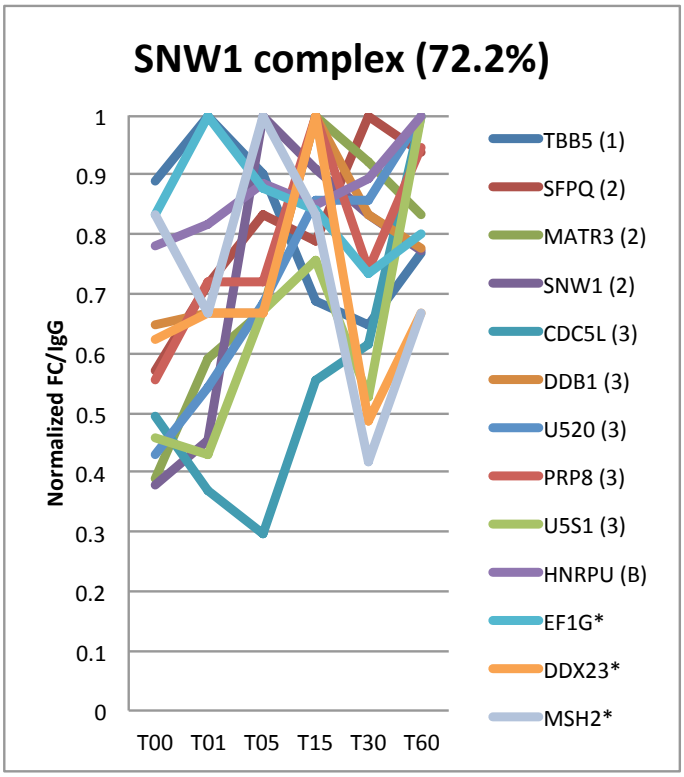


Figure 68: SNW1 complex. Top panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Bottom panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified

PBAF complex:

Firstly described as a complex distinct from the BAF complex (W. Wang *et al.* 1996). This complex contains 10 components among them the Polybromo 1 protein, found as moderate confidence PR interactor along with 3 other components, whereas SMARCE1 and SMARCD2 are high fidelity PR interactors (Fig. 69). It has been described as a chromatin-remodeling complex that facilitates gene activation by assisting transcription machinery to gain access to targets on the chromosome. As additional members of the complex the authors mentioned actin and beta-tubulin and they showed that a reconstituted complex potentiate transcription in an in-vitro system (Lemon *et al.* 2001).

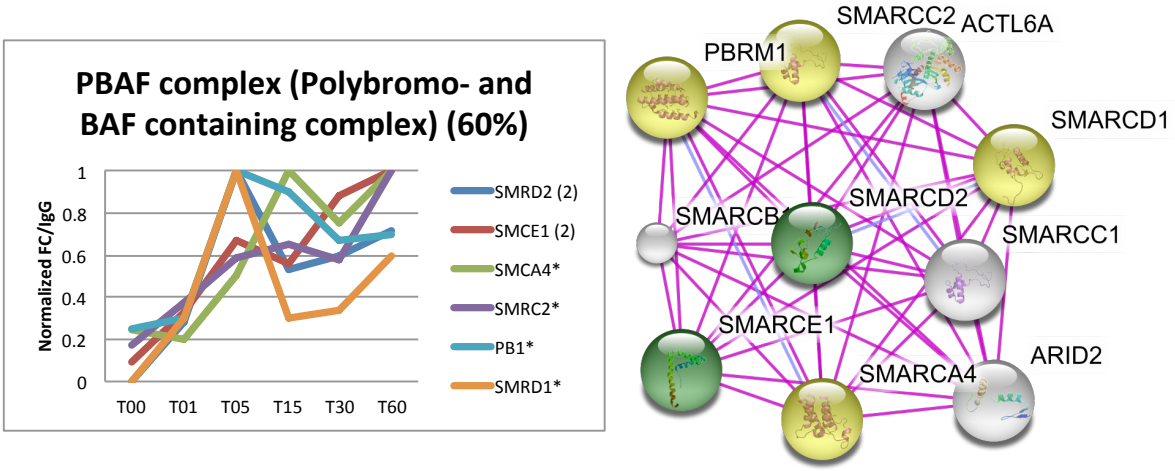
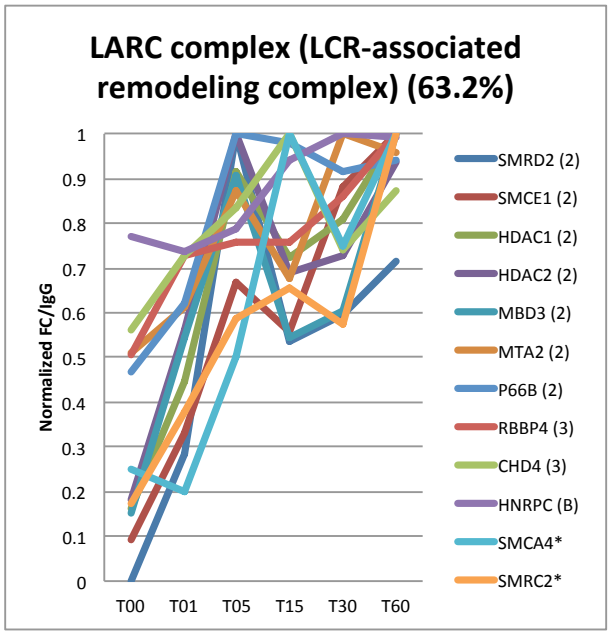


Figure 69: PBAF complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

LARC complex (LCR-associated remodeling complex):

Locus control region (LCR)-associated remodeling complex, composed by several chromatin remodeling components, it binds to specific sequences and remodels nucleosomes as a homogenous single complex. It contains HNRNPs, components of SWI/SNF and of MeCP1 complexes (M. C. Mahajan *et al.* 2005). It has been shown that controls accessibility of regulatory DNA sequences situated many kilobases away from their cognate promoters that Androgen receptor and FOXA1 can co-occupy (Robinson *et al.* 2014). Also shown to prevent transcriptional silencing by binding to Rep-P replicator protein (L. Huang *et al.* 2011). Most of the 11 PR interactors of the complex belong to cluster 2, showing rapid interaction with PR (Fig. 70).



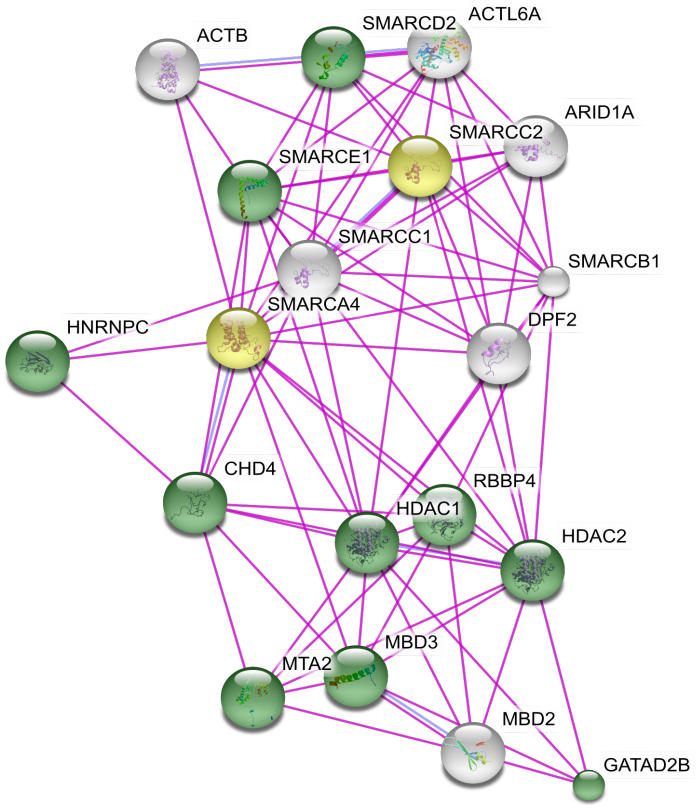


Figure 70: LARC complex. Line chart with identified proteins from the complex, normalized IgG FC values. Dynamic cluster (4.4.4) is shown in brackets, (*) indicates moderate confidence (upper panel). STRING PPI graph with complex components, depicting experimentally found interactions (lower panel). Proteins identified as high (green) and moderate (yellow) confidence data sets; light grey, proteins that did not pass FDR filtering or that were not identified.

PIAS3-SMAD3-P300 complex:

Described as a complex associated with TGF-beta activation (Long *et al.* 2004). The show association of PIAS3 with P300/CBP through its RING domain enhances the transcriptional activity of SMAD proteins upon TGF-beta treatment (Fig. 71).

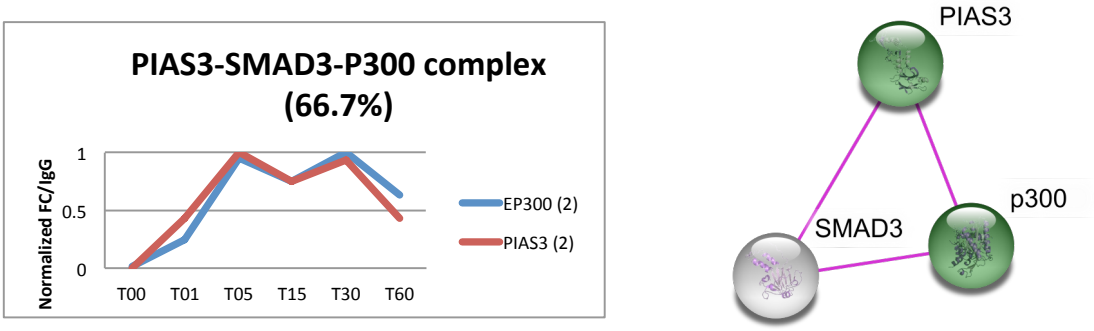


Figure 71: PIAS3-SMAD3-P300 complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

RNF20-RNF40-UbE2E1 complex:

This complex is involved in the crosstalk of H2B monoubiquitination and H3 K4 and K79 methylation, where it couples both histone marks with transcription activation (B. Zhu *et al.* 2005). The mechanism of this crosstalk between these histone modifications is still debated (Soares *et al.* 2013) (Fig. 72).

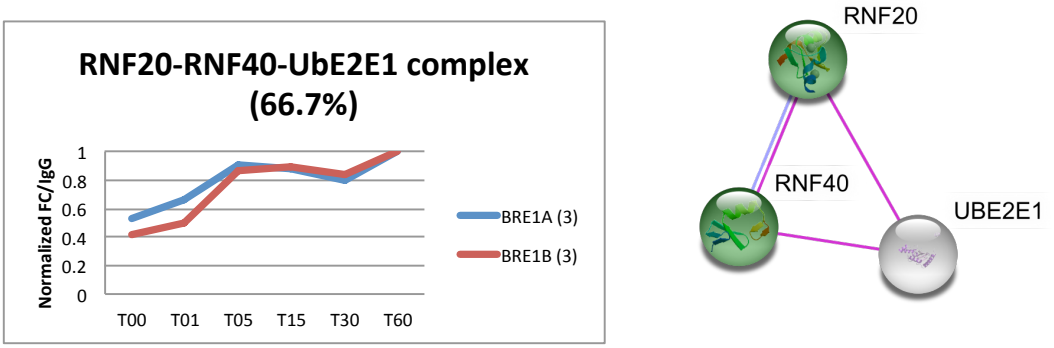


Figure 72: RNF20-RNF40-UbE2E1 complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

Transcription repression:

We found several repressive complexes, some of them with little variations on the components, and only a few do not include histone deacetylases HDAC1 and HDAC2.

Mi-2/NuRD complex:

First described by the group of Danny Reinberg (Y. Zhang *et al.* 1998), who highlighted the importance of finding Mi2/CHD4 and MTA-2 in an HDAC1/2-containing complex, establishing a connection between nucleosomal remodeling and metastasis. An interaction with steroid hormone receptors has not been reported, but all its seven components are high confidence PR interactors, and are recruited to PR within 5 min of hormone exposure and are members of the kinetic cluster 2 with the exception of CHD4 that is part of the cluster 3 (Fig. 73). The complex regulates higher-order chromatin structure and cohesin dynamics (Fasulo *et al.* 2012). Component CHD4 interacts with Poly-(ADP-ribose) (Silva *et al.* 2016) and component MTA1 participates in the DNA damage response (D. Q. Li *et al.* 2010), suggesting a role of this complex in DNA repair. A role in transcription elongation has also been proposed (Bottardi *et al.* 2014). On the other hand, NuRD complexes containing MBD3 regulate expression of genes marked with 5-hidroxymethylcytosine in embryonic stem cells (Yildirim et al. 2011). Three components exhibit a kFC higher than 2: HDAC1 (6.16), HDAC2 (5.49) and MBD3 (6.49), while CHD4 itself exhibits a high IgGFC of 110.

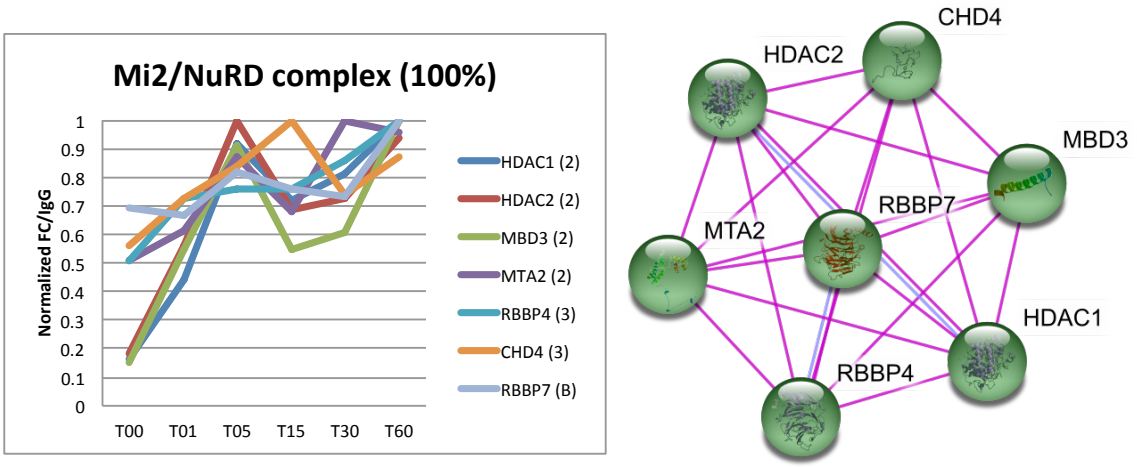


Figure 73: Mi-2/NuRD complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

MeCP1 complex:

This complex was described (Feng *et al.* 2001) and characterized as a repressor of transcription through preferential binding, remodeling, and deacetylation of methylated nucleosomes (Feng *et al.* 2002). It contains 9 components including GATAD2b (or P66B) and MBD2, which did not pass FDR filters. The other seven components are high fidelity PR interactors belonging mostly to the kinetic cluster 2 (Fig. 74).

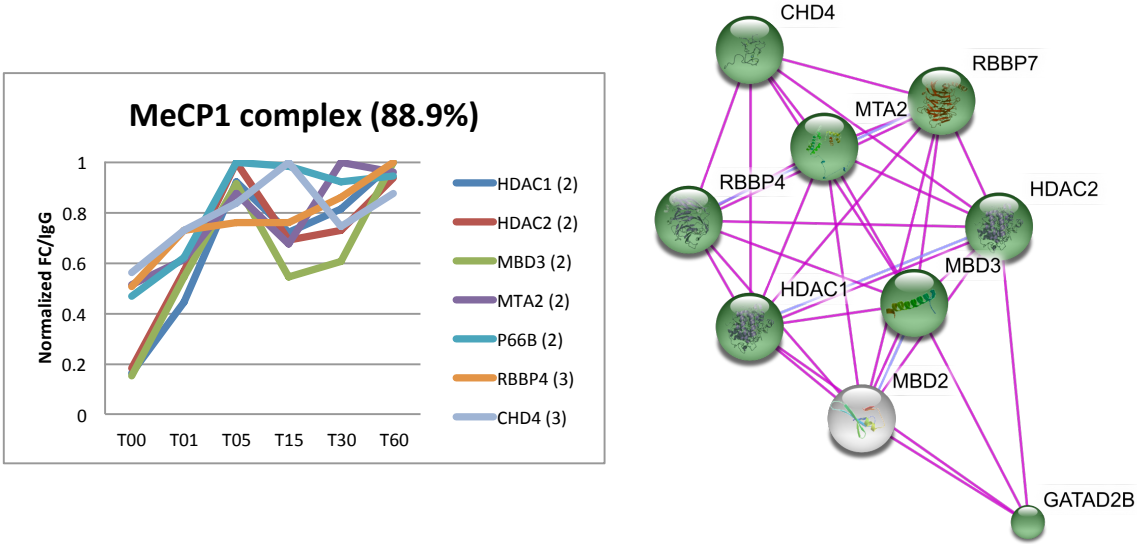


Figure 74: MeCP1 complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified

NRD complex:

The Nucleosome Remodeling and Deacetylation complex was described as the first ATP-dependent nucleosome remodeling activity that included human histone deacetylases (Tong *et al.* 1998). The ATP-dependent remodelers are the CHD3/4 proteins, which have a helicase/ATPase domain. Another characteristic of this complex is the presence of KDM1A/LSD1, a histone demethylase known to demethylate H3K4. Of the seven components of the complex six are high fidelity PR interactors, and only CHD3 is a moderate confidence interactor (Fig. 75). The complex is attached to PR already 5 minutes after hormone exposure is maintained high until T60.

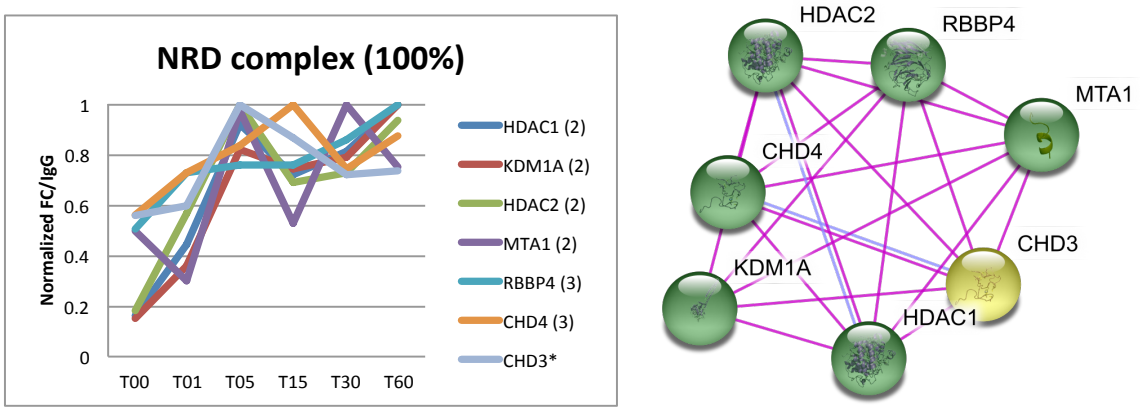


Figure 75: NRD complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified

HDAC1-associated protein complex:

This complex differs from the above describe repressive complexes by the presence of a SANT domain in the RCOR1/CoREST protein, which may play a role in the complex assembly and target recognition (Humphrey *et al.* 2001). It also has been shown to directly interact with SUMO2, contributing to gene-specific repression (Ouyang *et al.* 2009). It encompasses 9 components of which 6 are high fidelity PR interactors and 2 are moderate confidence interactors, all of then recruited to PR early after hormone exposure (Fig. 76).

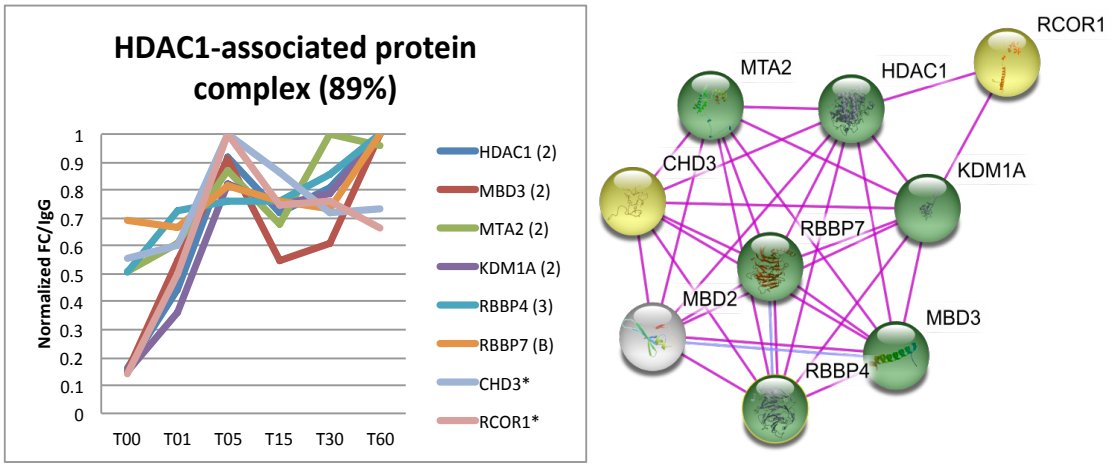


Figure 76: HDAC1-associated protein complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified

CtBP core complex:

CtBP complex is described to target genes and coordinately modify histones, allowing for the effective repression of genes targeted by CtBP (Y. Shi *et al.* 2003). Of its 9 components, 5 are high fidelity PR interactors and 1 is a moderate interactor all being recruited to PR 5 min after hormone exposure (Fig. 77). In the expanded CtBP complex there is a subunit (LCoR) shown to interact with PR (Palijan *et al.* 2009), which is not found in our data set, but is known to be recruited to progesterone up-regulated genes, and recruit the repressive complex.

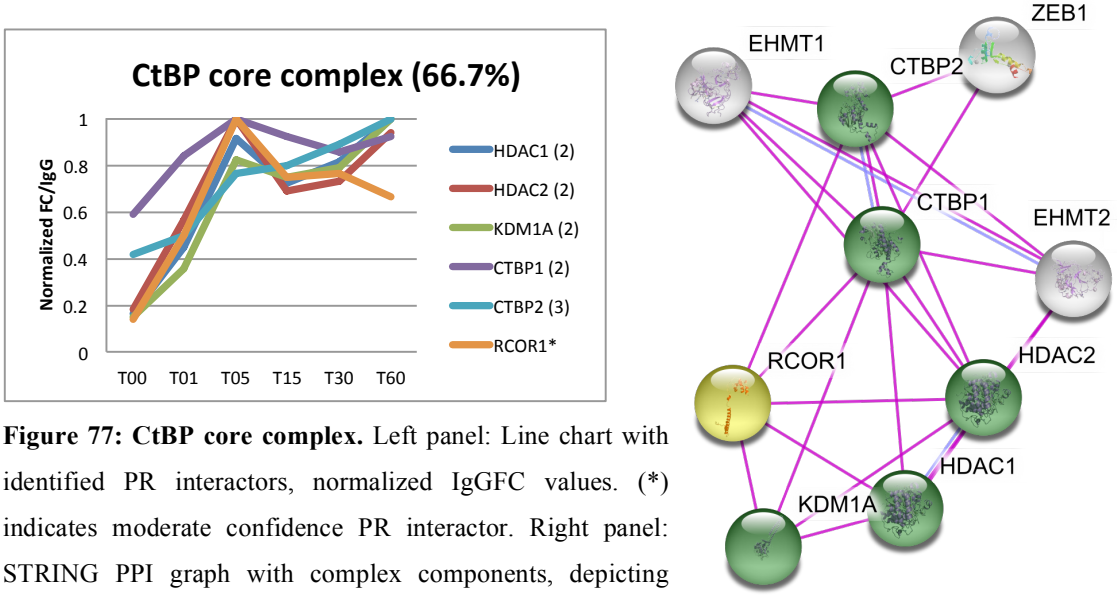


Figure 77: CtBP core complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

NELF complex:

The Negative ELongation Factor complex it is not a bona fide repression complex but it is described as an RNA PolII repressor (Yamaguchi *et al.* 1999). In cooperation with other proteins like DSIF and DRB strongly repress RNA PolII elongation. Of its 4 components 2 are high fidelity PR interactors (though NELFB did not past the FDR filter) and one is a moderate confidence interactor (Fig. 78).

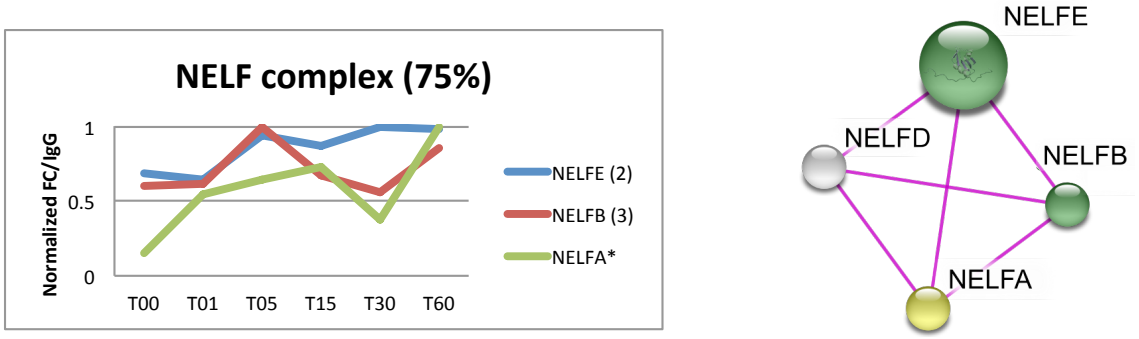


Figure 78: NELF complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified

TLE corepressor complex:

This repressive complex has 8 components and is modulated by a Ca^{2+}-dependent kinase (CaMKIIdelta) that activates PARP1 by phosphorylation, mediating dismissal of the corepressor complex from repressed promoters, activating transcription (Ju *et al.* 2004). Some of the components are also described to have a role in DNA damage response, as PARP1, RAD50 or NoNO. Of the 8 components, 5 are HCI and 1, TLE1, is a MCI. TOP2B did not pass the FDR filtering. At the complex graph, some of the components are not connected, meaning that it has not been annotated direct interaction at the experimental evidences level at STRING database (Fig. 79).

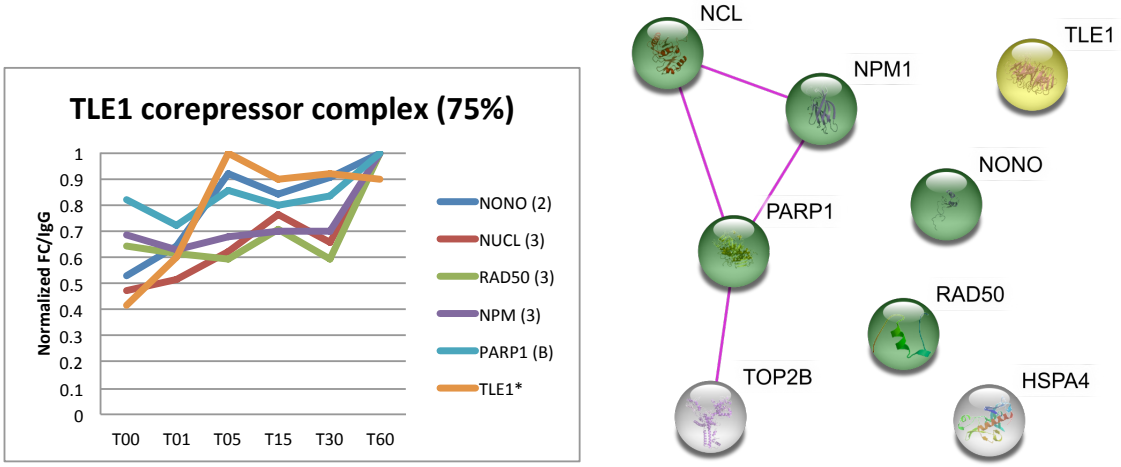


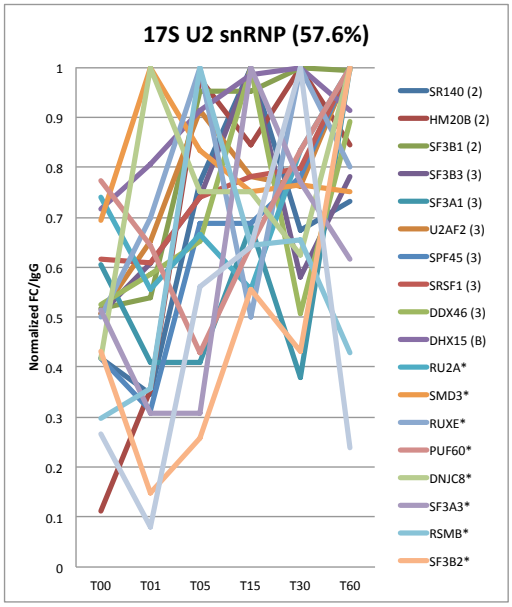
Figure 79: TLE1 corepressor complex. . Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

4.4.1.2 Post-transcriptional RNA processing

The largest complex found in our data set is the Spliceosome, but only 40% of its components are PR interactors. As an example of the many spliceosome-related complexes, I choose just one that is worth commenting, even though les than 60% are PR interactors.

17S U2 SNRP complex:

This complex plays an important role in pre-mRNA splicing, acting during or prior to pre-spliceosome assembly. Spliceosome assembly is initiated by the interaction of the U1 snRNP with the 5' splice site, forming the E complex, which includes the 17S U2 snRNP. At this stage the 17S U2 snRNP complex associates via a non-base pairing interaction. In a subsequent ATP-dependent step, the U2 snRNA base pairs with the branch site of the pre-mRNA leading to stable association of the U2 snRNP and formation of the so-called A complex or pre-spliceosome. Among the 33 proteins identified of the complex, 10 are high confidence PR interactors and 9 are moderate confidence interactors.



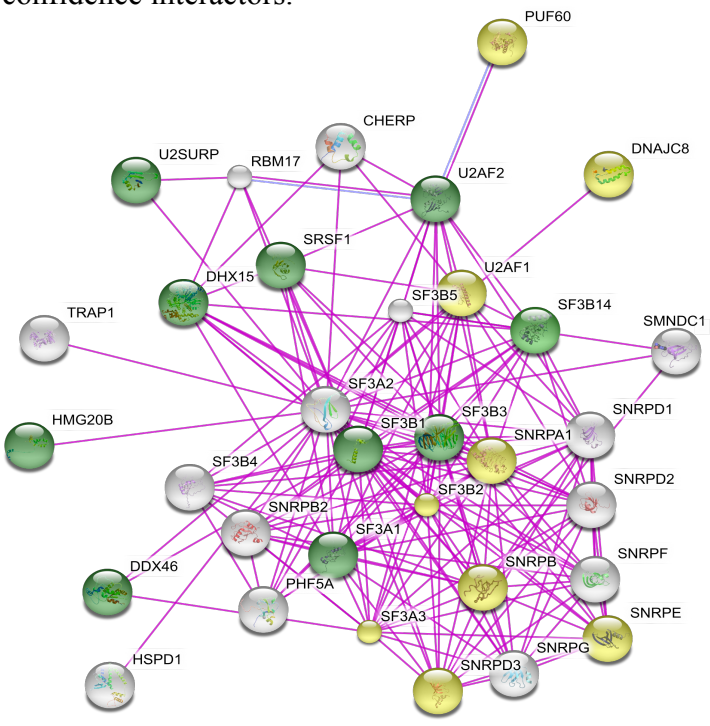


Figure 80: 17S U2 SNRP complex. Upper panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Lower panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified

Large DROSHA complex:

This complex was firstly described as part of the microRNA processing machinery, but later categorized as having only weak pre-miRNA processing activity and being involved in pre-ribosomal RNA processing (Gregory *et al.* 2004). It has been shown to be involved in RNA cleavage-independent regulation of human gene expression (Gromak *et al.* 2013). Of its 20 components, 15 are high fidelity PR interactors and 1 a moderate confidence interactor (Fig. 81).

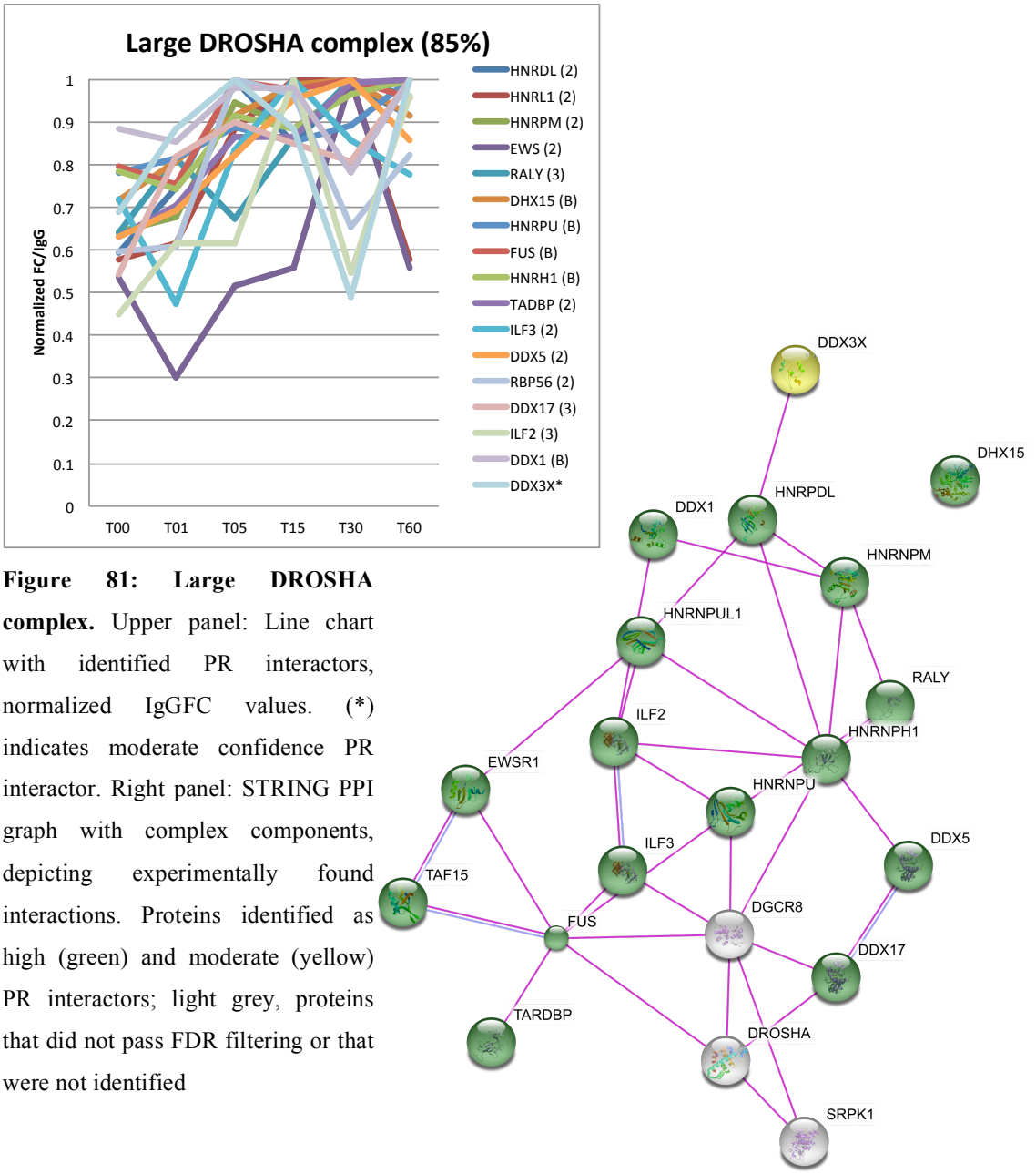


Figure 81: Large DROSHA complex. Upper panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified

DGCR8 multiprotein complex:

First identified together with the Drosha complex (Gregory *et al.* 2004), it was later characterized in detail (Shiohama *et al.* 2007). It is involved in RNA processing and transportation and controls the stability of mature small nucleolar RNA (snoRNA) transcripts independently of the Drosha complex (Macias *et al.* 2015), with which it shares 7 subunits. Of its 11 components 9 are high confidence PR interactors (though one did not pass de FDR filters) (Fig. 82), and the main component DGCR8 is missing, and was not identified by mass spec. Most of the identified interactors are in the basal cluster, except for DDX proteins, nucleolin and ILF3.

It is remarkable that both DDX5 and DDX17 are RNA helicases reported to be master regulators at ER and AR signaling pathways, controlling transcription and splicing both upstream and downstream of the receptors. Both helicases are required downstream for the splicing regulation of some target genes, and they act upstream by controlling expression at the splicing level of several regulators of ER and AR (Wortham *et al.* 2009, Samaan *et al.* 2014).

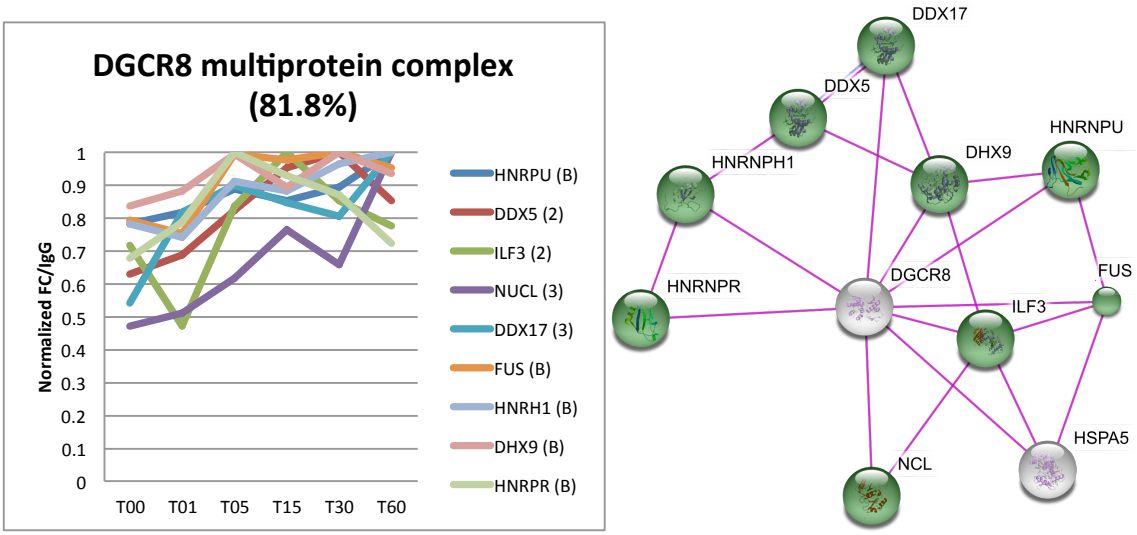


Figure 82: DGCR8 multiprotein complex. . Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

CDC5L core complex:

The CDC5L complex incorporates into the spliceosome in an ATP-dependent step. It is required for the second catalytic step of pre-mRNA splicing (Ajuh *et al.* 2000). It containing CDC5L and at least five additional protein factors, 3 of which are high confidence PR interactors, exhibiting stable interaction with PR. GCN1 is a moderate confidence interactor (Fig. 83).

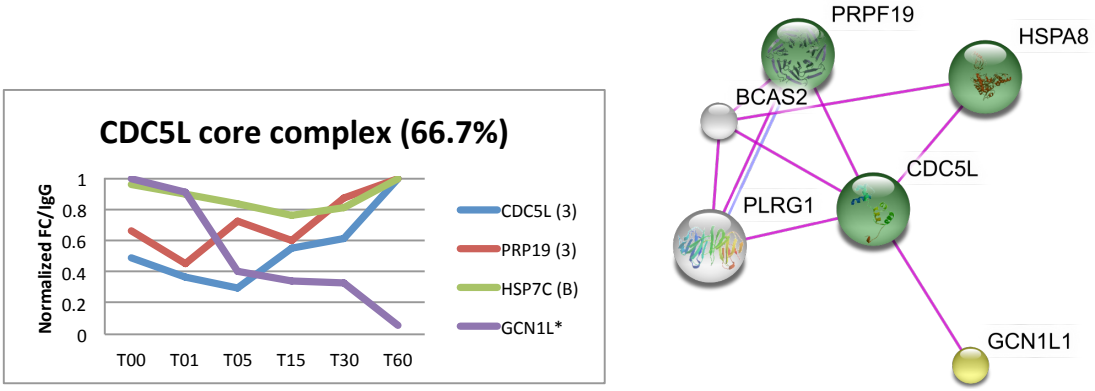


Figure 83: CDC5L core complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

SRm160/300 complex:

A splicing related complex, shown to play a role in exon recognition and in the assembly of adjacent complexes (Eldridge *et al.* 1999). Three of its 5 components are high fidelity PR interactors (though one did not pass the FDR filters) and one is a moderate confidence interactor (Fig. 84).

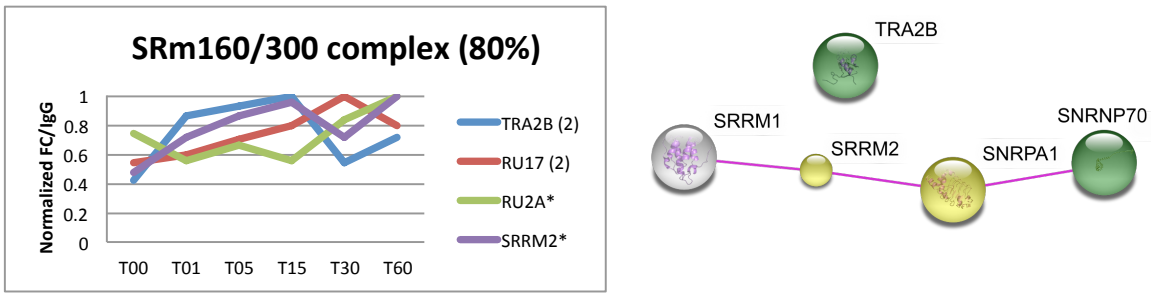


Figure 84: SRm160/300 complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

p54(nrb)-PSF-matrin3 complex:

This complex functions as a surveillance of hyperedited RNAs, binding specifically to inosine-containing RNAs, and keeping them from being exported. It is anchored to the nuclear matrix (Z. Zhang *et al.* 2001). All its 3 components are HCI PR interactors (Fig. 85).

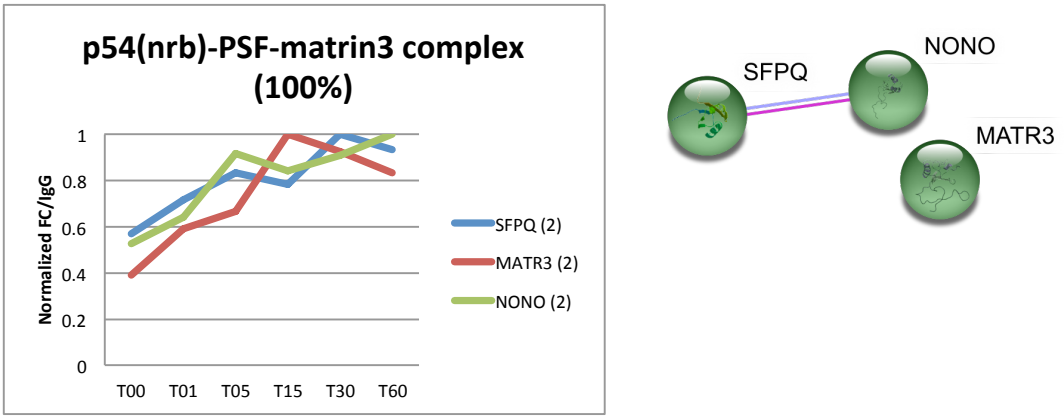


Figure 85: p54(nrb)-PSF-matrin3 complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

4.4.1.3 DNA repair complexes

Many proteins related to DNA damage repair have been found in our data set but some of them are not found in any annotated complex. Here I will describe those that are part of annotated complexes.

NCOA6-DNA-PK-Ku-PARP1 complex:

The NCOA6 component of this complex was shown first described as co-activator of thyroid hormone receptor that interacts with DNA-PK after hormone exposure and activates the kinase (L. Ko *et al.* 2003). All 5 component of this complex are PR interactors, although NCOA6 is a MCI that increase interaction with the receptor 5 min after hormone exposure (Fig. 86). The rest of the components of the complex are found in the basal cluster.

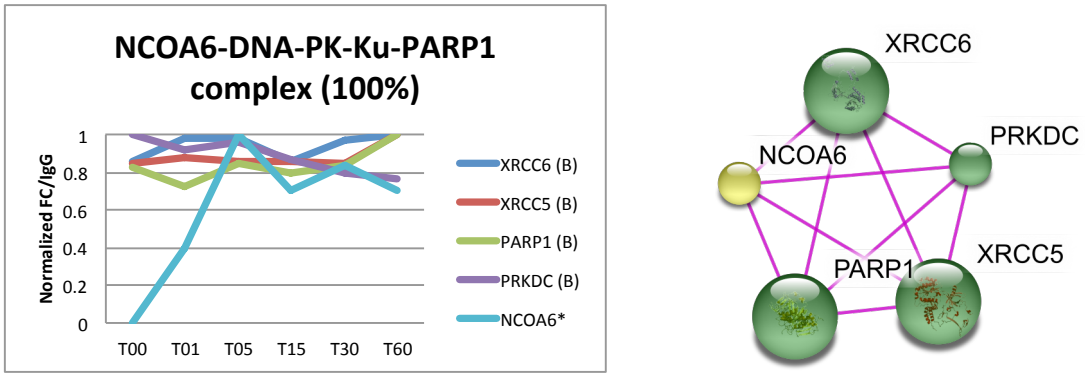


Figure 86: NCOA6-DNA-PK-Ku-PARP1 complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

DNA double-strand break end-joining complex:

This complex is important for non-homologous end-joining (J. Huang *et al.* 2002). Three of its 7 components are high fidelity PR interactors found in the basal cluster and 2, Nibrin and MRE11, are moderate confidence interactors that peak at T15 (Fig. 87).

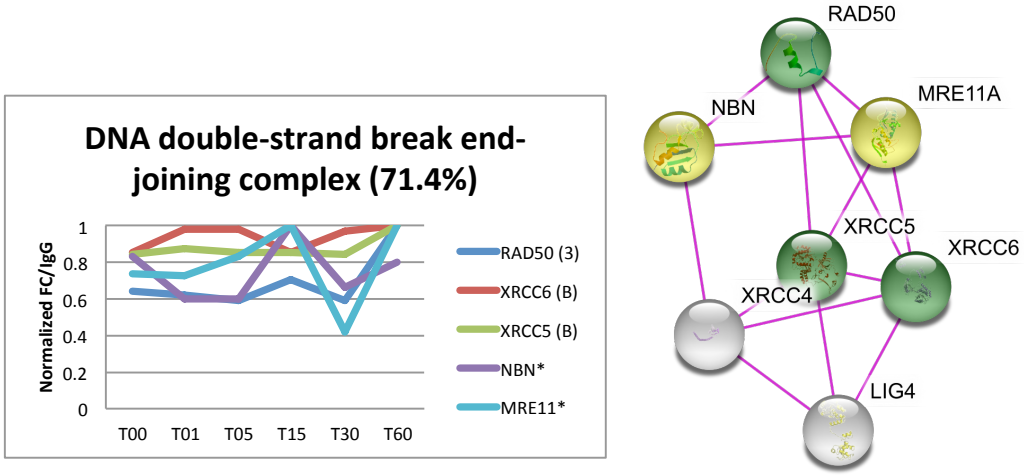


Figure 87: DNA double-strand break end-joining complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

PTIP-DNA damage response complex:

In cells not treated with DNA damage agents, PTIP/PAXIP1 associates with transcription related proteins, including MLL2, RBBP5 or NCOA6 (Cho *et al.* 2007), but in response to DNA damage it can form complexes with DNA-damage response proteins, such as RAD50, a high fidelity PR interactors, and MRE11A, NBN and TP53BP1, all of which are moderate confidence PR interactors (Fig. 88).

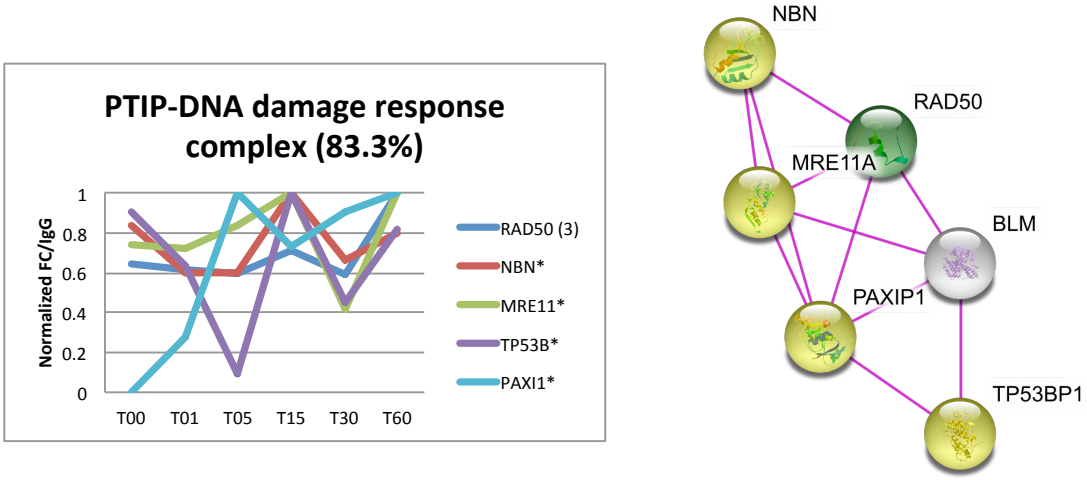


Figure 88: PTIP-DNA damage response complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

BRCA1-RAD50-MRE11-NBS1 complex:

This complex requires the presence of BRCA1, which is a known interactor of PR (Calvo *et al.* 2011), but did not pass the FDR filters. BRCA1 was shown to co-localize with the other components and mediate their DNA damage response (Zhong *et al.* 1999). The other 3 components are PR interactors also found in the PTIP complex (Fig. 89).

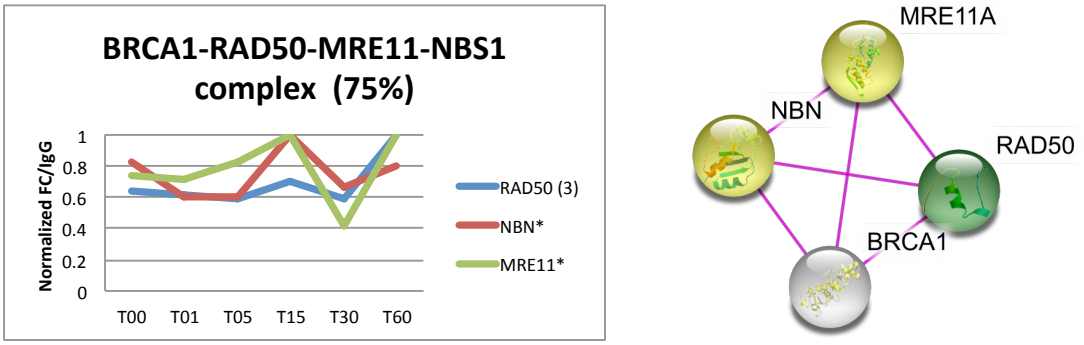


Figure 89: BRCA1-RAD50-MRE11-NBS1 complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

NONO-SFPQ-Ku complex:

This complex is not annotated at CORUM. It includes several components implicated in the non-homologous end-joining, and also some novel proteins that may be stimulating the recombination in cooperation with the rest of the machinery, all of which are high confidence PR interactors (Bladen *et al*. 2005) (Fig. 90).

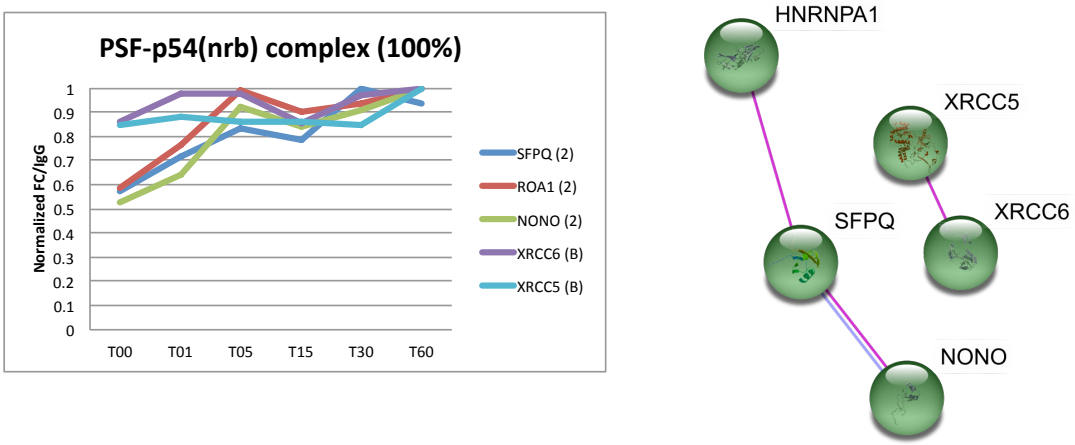


Figure 90: PSF-p54(nrb) complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

Ubiquitin E3 ligase:

Many proteins related to the proteasome and to ubiquitination have been identified in our data set, however, only this complex is significantly enriched, and has a role in the DNA damage response. Ubiquitin E3 ligases covalently attach ubiquitin to a lysine residue on a target protein. Polyubiquitination marks proteins for degradation by the proteasome. CUL4A/B-DDB2-ROC1-mediated H3 and H4 ubiquitylation was shown to facilitate cellular response to UV damage by affecting nucleosome stability (H. Wang *et al.* 2006). Four of the 5 components of the complex are PR interactors (Fig. 91).

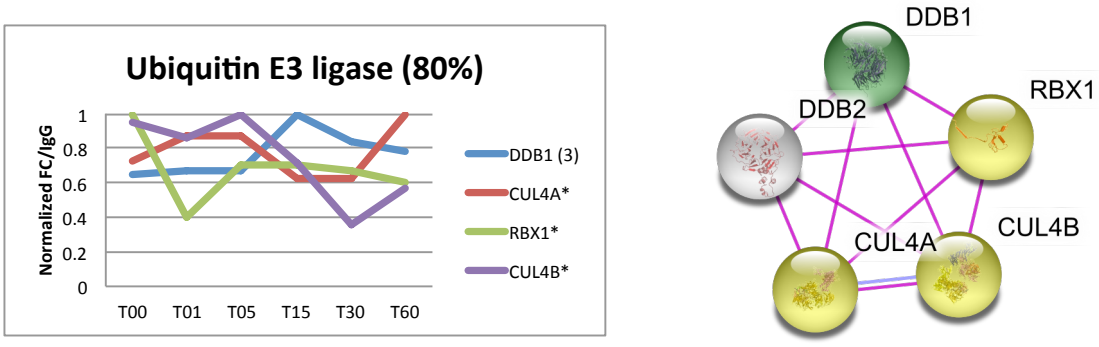


Figure 91: Ubiquitin E3 ligase. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

4.4.1.4 Structural complexes

Many structural proteins are identified in our data set, some of them are found in annotated functional complexes mostly related to chromatin structure.

MCM complex:

The Minichromosome Maintenance complex binds histone proteins and is important for chromosome replication (Ishimi *et al.* 1996). It also contributes to tumor progression for and is a potential target for cancer therapy (Lei 2005). Of its 6 components 4 are high confidence PR interactors and 2 are moderate confidence interactors (Fig. 92).

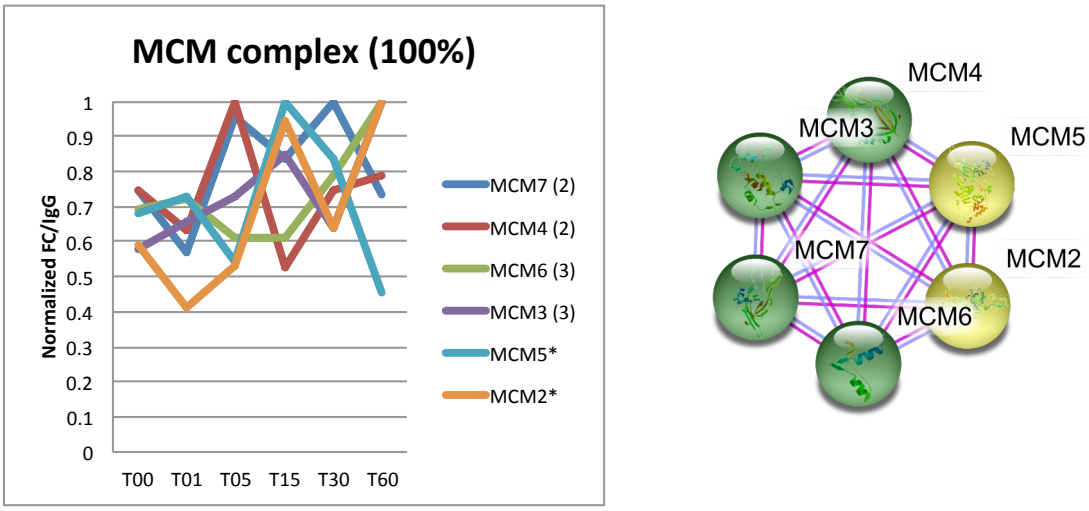


Figure 92: MCM complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

SET complex:

The SET complex was discovered as a Granzyme A (GzmA) target in cells undergoing caspase-independent T cell-mediated death. It is not annotated in CORUM data base. It contains two nucleases, NME1 and TREX that are activated by the cleavage of the inhibitor SET protein, causing DNA damage (Beresford *et al.* 2001). It has been shown to be implicated in the regulation of acetyltransferases and transcription (S. B. Seo *et al.* 2001), and more recently to act as a co-regulator of DNA-bound ERα (Schultz-Norton *et al.* 2008). Of its 6 components, 3 are high confidence PR interactors and one, NME1, is a moderate confidence interactor (Fig. 93).

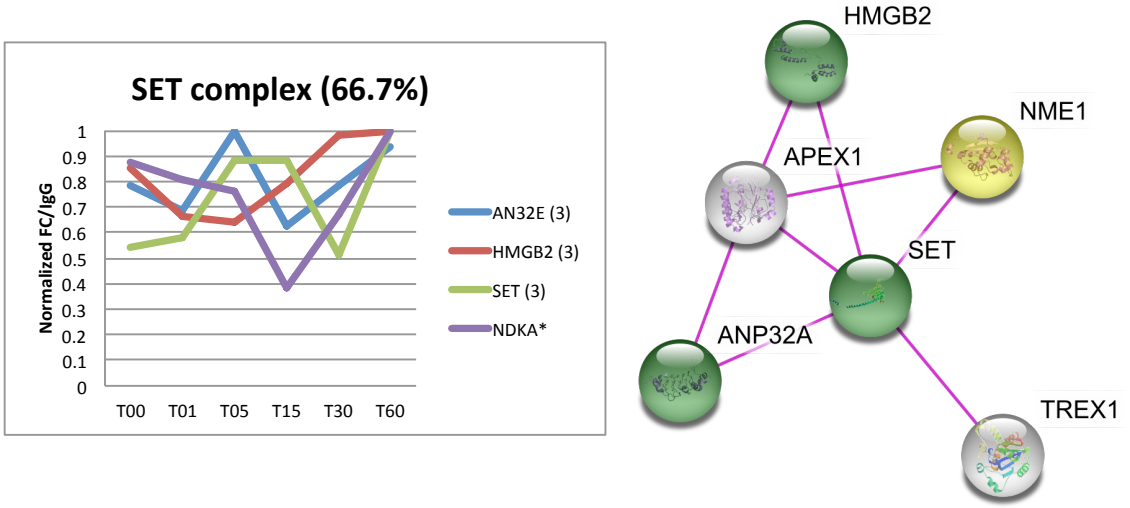


Figure 93: SET complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

Sororin-cohesin complex:

Was initially described as a mediator of the sister-chromatid cohesion (Bottardi *et al.* 2014). Although Sororin/CDC5a is not found in our data set, cohesins subunits are identified as string high confidence PR interactors and could play a role in the hormonal regulation of chromatin folding (Le Dily *et al.* 2014) (Fig. 94).

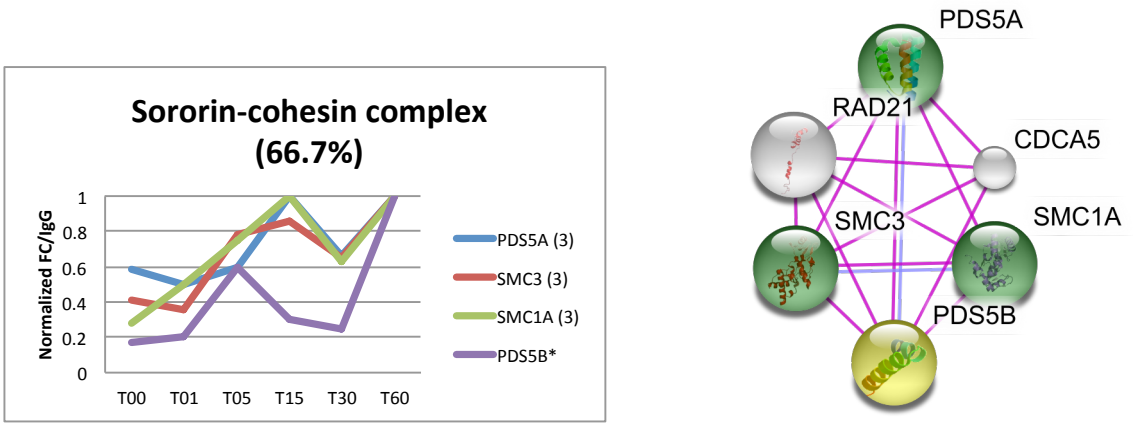


Figure 94: Sororin-cohesin complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

Snf2h/Cohesin complex:

This large complex integrates a cohesin complex and an HDAC-containing complex, supporting the idea that the cohesin complex is loaded onto DNA by the chromatin remodeling part of the complex (Hakimi *et al.* 2002). The function of the complex system depends on the action of the ATPase Snf2h/SMARCA5. Of the 15 components of the complex 11 are high confidence PR interactors (Fig. 95).

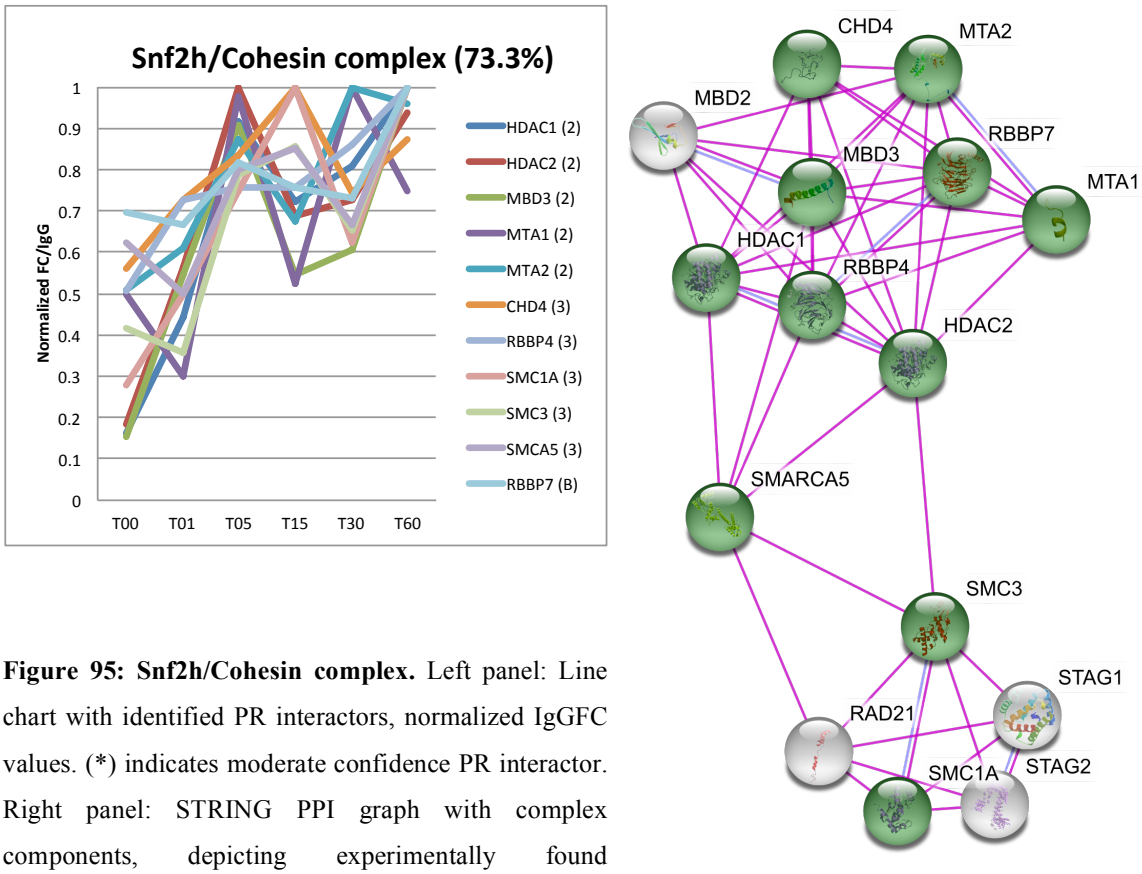


Figure 95: Snf2h/Cohesin complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

4.4.1.5 Chaperones

TCP1 complex:

This complex assists the folding of proteins using the energy of ATP hydrolysis, and it was described as a cytosolic chaperoning complex responsible for the proper folding of tubulin (Yaffe *et al.* 1992). It has also required for the biogenesis of functional PLK1 (X. Liu *et al.* 2005), and to modulate the folding and activation of STAT3 (Kasembeli *et al.* 2014). Of its 9 components, 5 are high confidence PR interactors and 2 are moderate confidence interactors (Fig. 96). Most of the complex if found in the kinetic cluster 1, showing its interaction with unliganded PR and its depletion upon hormone induction. Is not annotated in the CORUM database.

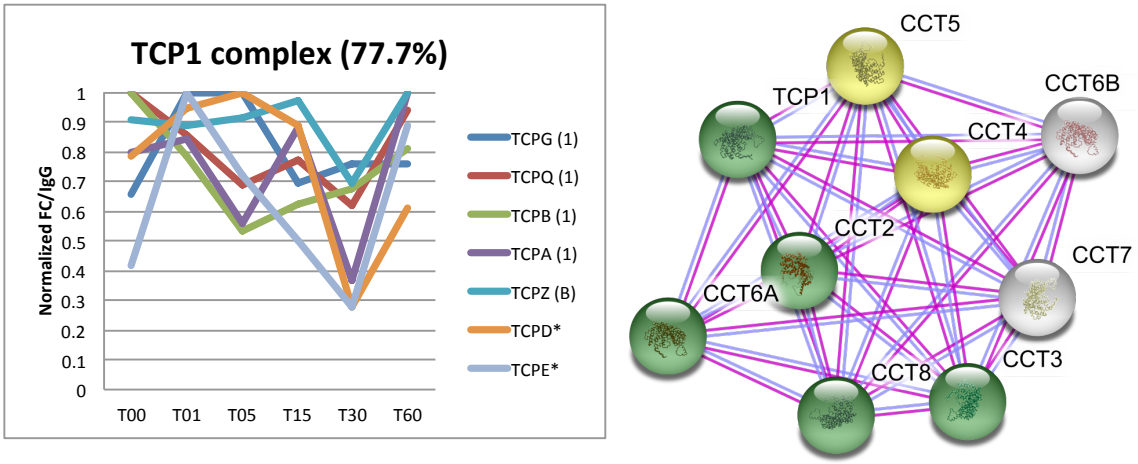


Figure 96: TCP1 complex. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

Chaperones complex:

This complex is not annotated in the CORUM database, but exemplifies how the chaperones or chaperone-related proteins identified by mass spec interact among them. There is a clear interaction with components of the TCPI complex, but also with other chaperone related proteins exposure (Knee *et al.* 2013). All its 17 components interact with PR, 13 as high confidence interactors and 4 as moderate confidence interactors (Fig. 97). Most of the components interact with PR before hormone exposure and decrease their interaction with PR upon hormone.

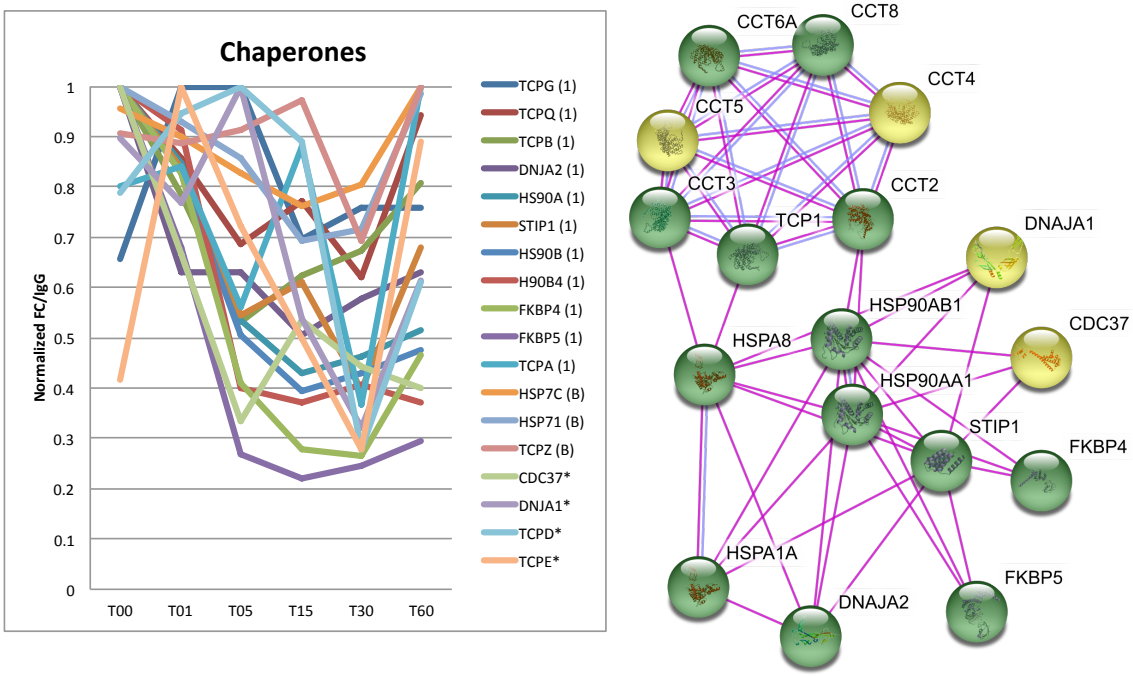


Figure 97: Identified chaperones. Left panel: Line chart with identified PR interactors, normalized IgGFC values. (*) indicates moderate confidence PR interactor. Right panel: STRING PPI graph with complex components, depicting experimentally found interactions. Proteins identified as high (green) and moderate (yellow) PR interactors; light grey, proteins that did not pass FDR filtering or that were not identified.

4.5 RU486 treated cells

R5020 is a progesterone agonist widely used in the study of hormonal response of PR positive breast cancer cells as T47D. There are also described a number of progesterone antagonists (Klijn *et al.* 2000), the most studied being RU486, also known as Mifepristone. In medicine, is used to induce abortion during the first 50 days of pregnancy. In research RU486 is used as a partial antagonist of progesterone, as it binds to PR with relatively high affinity. The exact mechanism of action of RU486 is not known, but it is suggested to increase the residence time on DNA target sites of RU486 induced GR (Pandit *et al.* 2002), while blocking transcriptional control. In ChIP-seq experiments in our lab we found that RU486 causes an increased binding of PR to genome sites compared to R5020 stimulation (data not shown). In order to elucidate possible mechanisms of transcription activation and active repression, mass spec analysis was done after PR immunoprecipitation on cells treated with RU486.

4.5.1 RU486 T47D cells stimulation

Cultured cells were treated with the progesterone antagonist RU486 at 10 nM concentration. Samples were treated the same way of that with R5020, and send for mass-spec analysis. For technical reasons, only two time points, T05 and T30, were suitable for analysis with three replicates each. The results of these 2 time points were compared with the IgG replicates and the data from unliganded PR (T00) by SAINT analysis software and filtered by the FDR. Following a similar filtering system than the one used for R5020 treated samples, proteins that did have FDR higher than 0.005 at both T05 and T30 were considered not present in the RU486 treated samples. From a total of 1257 proteins identified, including possible contaminants, proteins with at least one of the 2 FDRs lower than 0.005 were considered as present in the RU486 treated hits list. In total 379 proteins were identified as PR interactors at T05 or T30 after exposure to RU486. This list contains more proteins than R5020 high confidence list (315 hits), but it has to be considered that proteins present at high confidence in R5020-treated cells had passed high stringency filtering stringency (5 replicates for T05 and 6 replicates for T30, and 8 IgG replicates), whereas proteins identified at RU486-treated

samples were filtered with a lower stringency, as there are less replicates for each time point and they had been compared to less IgG-immunoprecipitated controls (3 replicates in each case).

RU486-induced PR interactors were compared to high and moderate confidence data sets from R5020 experiments (Fig. 98). The majority of RU486 induced interactors (248, 64%) are enclosed in the R5020 high confidence list. Interestingly, 67 proteins (21%) characterized as high confidence interactors at R5020 treatment, were not shown as interactors for RU486 stimulated samples (section 4.6.2). We found also a portion of proteins present at RU486 treated samples that are not at the HCI for R5020 treated samples (131). From those, some are included in the MCI list of R5020 (73, 18.8%), while the rest (58, 15%) were found interacting with PR only when cells were induced with RU486.

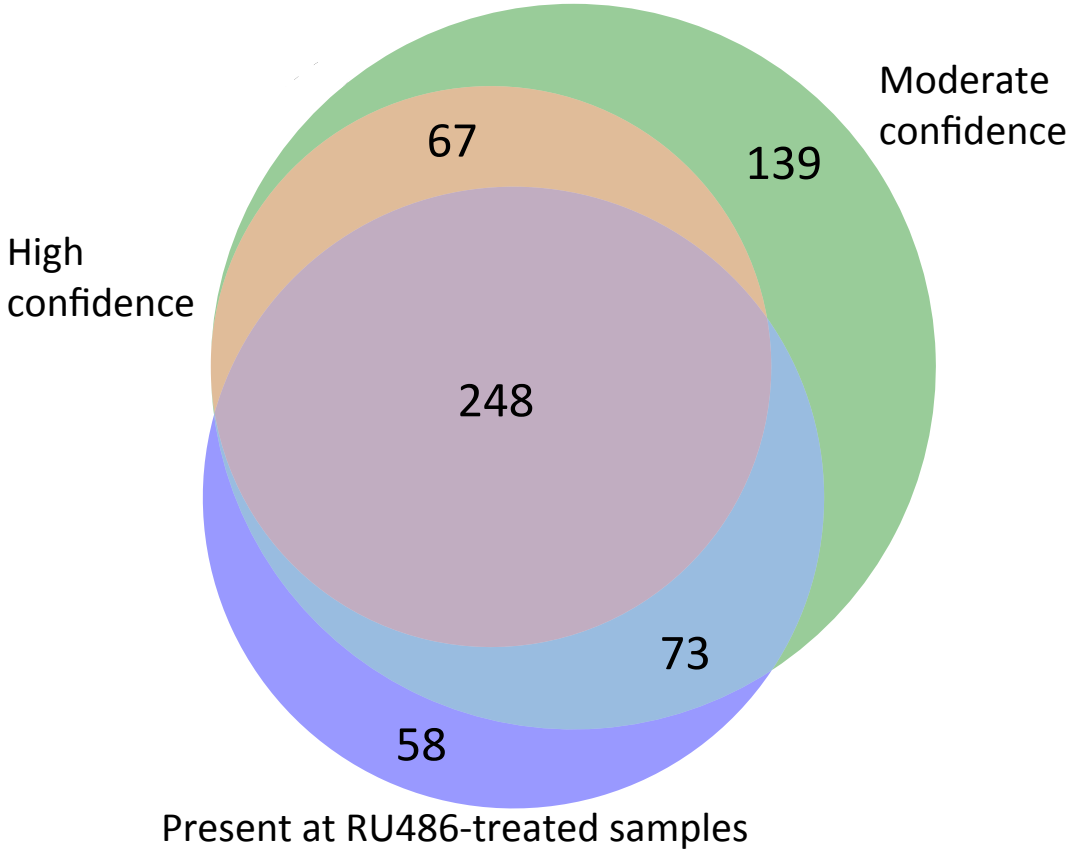


Figure 98: Venn diagram comparing R5020 vs. RU486 treated samples. Venn diagram compares R5020 treated samples, classified as high (orange) and moderate (green) confidence vs. RU486 treated samples (purple).

For proteins at HCI and present at RU486 samples, we performed a T-test comparing the two populations (at R5020 or at RU486) for T05 and T30, considering change significant for p-value < 0.05. At the same time we did a ratio comparing RU486-treated versus HCI from R5020-treated samples, obtaining a theoretical fold change between treatments. The ratio for T05 and T30 for proteins identified was plotted with the result from the T-test in a volcano plot (Fig. 99). Proteins with a fold change higher than 2 and a p-value < 0.05 were considered as stronger interactors at each time point when cells are exposed to RU486 (1 for T05, 20 for T30). In the other side, proteins with a fold change lower than -2, and with a p-value < 0.05 were considered as weaker interactors (2 for T05 and 1 for T30).

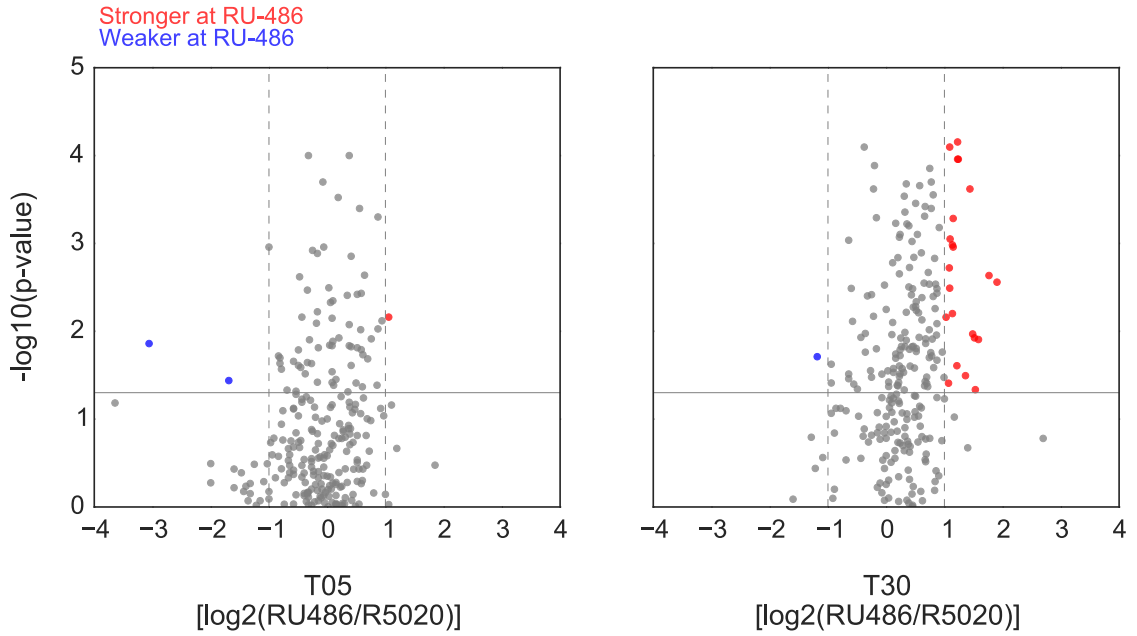


Figure 99: Comparison of RU486-treated vs. R5020-treated samples. R5020 and RU486 values for T05 and T30 were compared for proteins from R5020 high confidence data set passing the FDR filter for RU-treated samples. Dashed grey lines highlight +2/-2 FC threshold (in Log_2 scale), as considering change between treatments, solid line marks p-value = 0.05

4.5.2 High confidence interactors for R5020 not found or with weaker interaction at RU486 exposed cells

4.5.2.1 Not found at RU486 samples

There are 67 proteins from the high confidence data set that are not found at RU486 treated samples.

At the GO cellular component level there is enrichment in nucleoplasm but instead of Spliceosome compartment found with R5020, the Mediator complex appears as the dominant compartment, showing the absence of all the proteins related to the mediator complex interacting with PR when inducing with RU486 (Fig. 100).

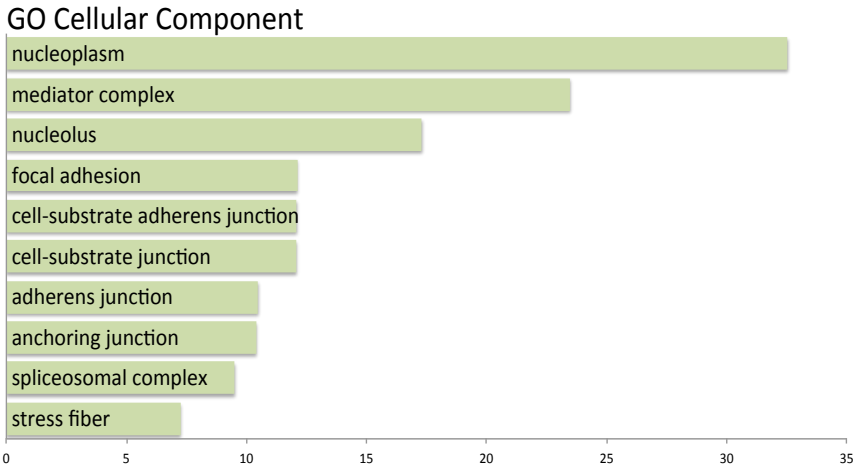


Figure 100: GO cellular component for RU486 non-interacting proteins. Proteins from high confidence data set not interacting with PR at RU486 induce samples were compared by *Enrichr* webtool with GO terms related to Cellular Component for enrichment analysis. 10 most enriched terms are shown.

As for the GO molecular function, the most enriched term is related to Vitamin D receptor binding, but the proteins enclosed in that term are again related to mediator complex (Fig. 101). The rest of the enriched terms are mostly related on ligand-dependent transcription activation, RNA polymerase II activation and hormone receptor binding. It shows enrichment in hormone activation proteins.

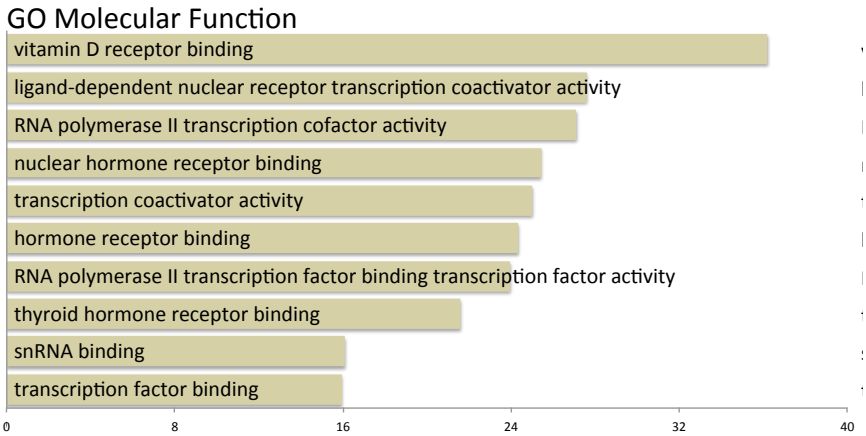


Figure 101: GO molecular function for RU486 non-interacting proteins. Proteins from high confidence data set not interacting with PR at RU486 induce samples were compared by *Enrichr* webtool with GO terms related to Molecular Function for enrichment analysis. 10 most enriched terms are shown.

As for the GO biological processes terms, the most enriched terms are again related with gene expression, steroid hormones receptor signaling pathways and transcription initiation (Fig. 102).

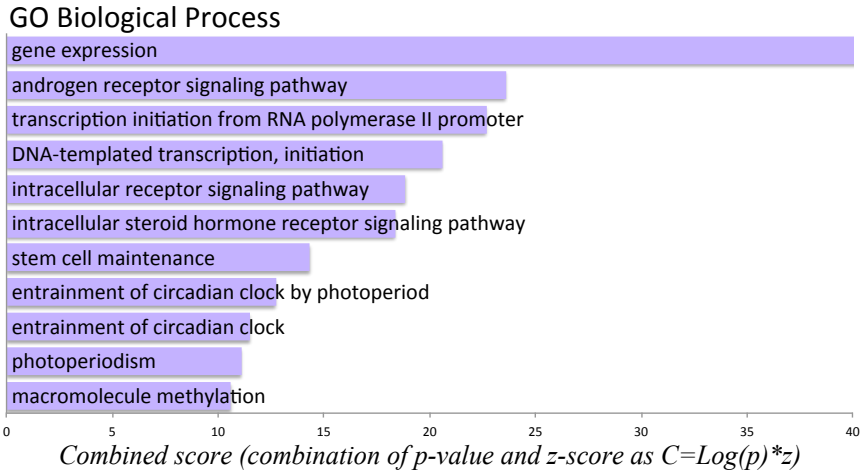


Figure 102: GO Biological Processes for RU486 non-interacting proteins. Proteins from high confidence data set not interacting with PR at RU486 induce samples were compared by *Enrichr* webtool with GO terms related to Biological Processes for enrichment analysis. 10 most enriched terms are shown.

This enrichment is understood also when the distribution by clusters of proteins not interacting with progesterone receptor at RU486-treated samples is analyzed. There is a clear increase of proteins from Cluster 2, displaying more than 40% of these proteins, which is a cluster related to transcription activation. The less enriched is the basal

cluster, and surprisingly also some proteins from cluster 1 show no binding with PR when inducing with the antagonist.

Among proteins that decrease their interaction after R5020 exposure, have even lower interaction when cells are treated with RU486 at T05 and T30, it can be found tubulins and filamins. For tubulins, some are not identified at RU486 treated samples. It has been shown that there is a fraction of them present in the nucleus in a non-polymerized form (Akoumianaki *et al.* 2009), and it also has been published a possible role as a steroid receptor regulator (M. L. Zhu *et al.* 2010). For Filamins, it has also been described a role as a repressor of androgen receptor transactivation (Loy *et al.* 2003). Other known nuclear receptor co-activators, as ACTN4 (Khurana *et al.* 2012), Calcium-regulated proteins, like Annexin-6, known to act as scaffold for several signaling proteins (Enrich *et al.* 2011) or CaCYBP, from the proteasomal system (Santelli *et al.* 2005) are not found at RU486 exposed samples.

There is an overrepresentation of known ligand-dependent transcription activators at proteins not found at RU486, as they can be NCoA1, NCoA2 and CBP, but also RXRa, KMT2D/MLL2, NF1c or KDM6A/UTX, other transcription activation related proteins. Together with activators, also the entire Mediator complex-related proteins found (5 in total) loose their PR interaction at RU486 treated data set. Other transcription-related proteins from cluster 2 that are not found when treated with the progesterone antagonist are TDIF1, CASZ1 or PIN1, which is known to enhance binding of ER to target sites when cells are induced with estrogen, but curiously, not when cells are treated with the antagonist Tamoxifen (Rajbhandari *et al.* 2015).

Other proteins related to transcription are not found at RU486-treated samples, as CCNT1, part of the elongation complex PTEFb, or Hexim1, which a known transcription elongation repressor, that acts by sequestering CCNT1 and CDK9 from the pTEFb complex (Yik *et al.* 2003), and to modulate their functional interaction with ERα (Wittmann *et al.* 2005).

Also found not to interact with PR is the protein SET, which forms a complex with HMGB proteins, and NDK/NME proteins that facilitates nucleosome assembly, transcriptional activation and DNA repair functions. Also NDKb has its interaction with PR impaired at RU486-treated samples.

Also not present at RU486 treated samples there is PIAS1, a SUMO ligase that is known to regulate androgen receptor activity (J. Tan *et al.* 2000), and also to regulate by

SUMOylation MTA1 (Cong *et al.* 2011), the only member of the Mi2/NuRD complex also not present at RU486 treated samples.

About splicing-related proteins, there are only a few that loose that interaction when cells are treated with the antagonist including RBM4, U1snRNP70, DSRAD or DDX39B, involved in mRNA export and RBM10, which apart from its role in alternative splicing it is also shown to have tumor suppressor properties (Hernandez *et al.* 2016) and is the protein with the highest induction fold change of cluster 3.

From proteins related to DNA damage repair, the only one that does not interact with PR upon induction with RU486 is Ligase 3, which is suggested to act as DNA strand break sensor (Abdou *et al.* 2015).

There are only 3 proteins that, although were identified at samples exposed to RU486, display a weaker interaction than t R5020, and they are the transcription factor GRHL2, DDX39A, (homolog of DDX39B) and an epidermal growth factor receptor kinase substrate (EPS8L1).

4.5.3 Interactors increased or found only in cells exposed to RU486

There are proteins that are only identified at RU486 treated samples (58) or that display a stronger interaction at samples exposed to this ligand (30).

From the ones only seen at RU486 samples, there are 13 that display a similar IgGFC than at T00 and 4 that has it even lower, depicting a constant interaction with PR prior and after RU486 exposure. Among those proteins, there are proteins from the chaperoning system (DNAJC7 or components of the TCP1 complex), proteins related to the proteosomal system as PSMA5 or PSMC6 (which is lower at T05 but higher at T30) or related to chromatin condensation as RCC1.

From proteins that only pass FDR filtering at RU486 samples, or that display a significant interaction higher than 2 FC at RU486 (62).

From these, 25 show an IgGFC higher than T00 at both time points, while there are 2 only higher at T05 and 14 only higher than T00 at T30.

From proteins also identified at R5020 samples (21), only 1 has a stronger interaction at T05, while the rest have a significant increase of the FC only at T30.

We have done an enrichment analysis for proteins only identified at RU486 or that display an increase of the interaction compared to R5020, and at GO Biological processes terms, RNA splicing related proteins are the most enriched. Gene expression, proteins related to chaperones and ubiquitination are the other terms enriched (Fig. 103).

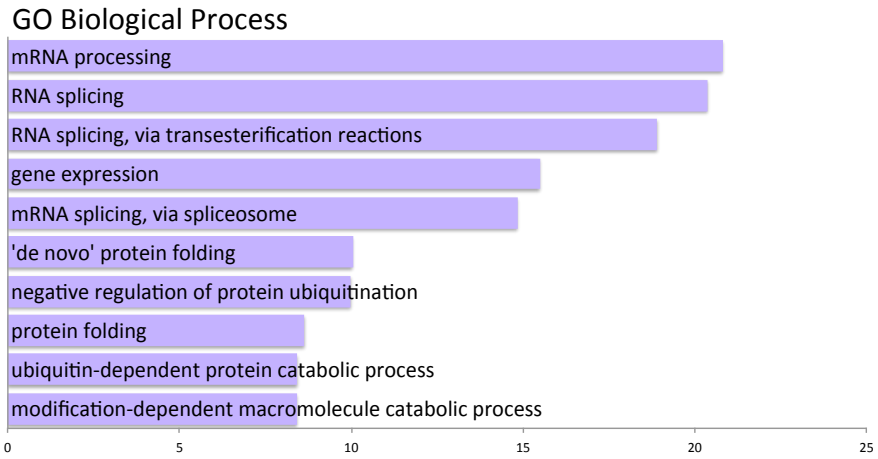


Figure 103: GO biological processes for RU486 only or stronger interacting proteins. Proteins with a stronger interaction with PR or only interacting at RU486 induce samples were compared by *Enrichr* webtool with GO terms related to Biological Processes for enrichment analysis. 10 most enriched terms are shown.

For the molecular function enriched terms, the most enriched term is *Unfolded protein binding*, showing a clear enrichment in proteins related to chaperones, The rest of the enriched terms are related to Retinoic Acid-binding (led by an RA chaperone) and to helicase activity, typical from mRNA processing (Fig. 104).

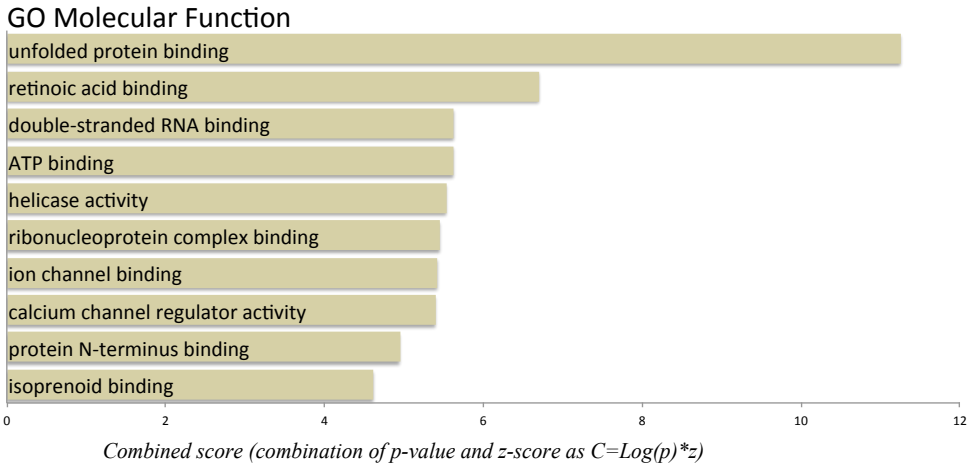


Figure 104: GO molecular function for RU486 only or stronger interacting proteins. Proteins with a stronger interaction or only interacting at RU486 induce samples were compared by *Enrichr* webtool with GO terms related to Molecular Function for enrichment analysis. 10 most enriched terms are shown.

Proteins present at HCI with R5020 with stronger interaction at RU486-exposed samples are characterized by the presence of several chaperones that depict an increased interaction at longer time points as T30, as components of the TCP1 complex. In this group there are as well proteins related to DNA damage as DDB1, or proteins from repressive complexes as RBBP7 or NCOR1, especially the latter with an increased binding at T30 four times higher than at R5020.

From the proteins only identified at RU486 exposed samples, there is enrichment in proteins related to the proteasomal system as PSMB7, ECM29, PSMC6, UBR5, ubiquitin itself or HLTF, shown to have E3-ligase capacity (Unk *et al.* 2008), but also shown to be involved in chromatin remodeling (Sheridan *et al.* 1995).

Another functional group enriched in this samples is proteins related to chaperones, as HSPD1 (from HSP family) and PPIH, from the cis-trans isomerizes proteins, also shown to have a role in spliceosome assembly.

5. Discussion

Progesterone receptor plays an important role in gene expression regulation upon hormone induction. This takes a special relevance in cases where cells had undergone hormone-dependent tumoral growth, where ligand-dependent gene regulation is key to cell proliferation.

Nevertheless, PR has been the least studied steroid hormone receptor. This is more evident to the level of annotated interactors, where PR has the least annotated known.

We have search for previously known annotated SHR interactors at iRefWeb tool that compiles information from 10 public databases (BIND, BioGRID, CORUM, DIP, IntAct, HPRD, MINT, MPact, MPPI and OPHID) in a unique one (Turner *et al.* 2010). It was selected to find only curated experimental interactions for steroid receptors. We searched for curated experimental interactors for estrogen receptor (ER), glucocorticoid receptor (GR), androgen receptor (AR) and progesterone receptor (PR).

This database corroborates that PR is the less studied of the 4 nuclear receptors, being the one with the least interactors annotated. Only 72 unique proteins were found at iRefWeb database, compared to over 200 of GR, over 300 of AR or almost 600 interactors for ER, as shown in the introduction.

To this end, we set up a protocol for identification of PR interactors at different states of hormonal activation of T47D breast cancer cell line. We obtained MS data for unliganded cells, and for 5 different early time points of hormone exposure, from 1 minute to 1 hour. We provided with up to 6 replicates for every time point, and 8 negatives controls (IgG IP). As shown, the need of replicates is indispensable to have confident results. For the analysis of MS data we used SAINT algorithm, which can discriminate with a high level of accuracy false positive results, especially when IgG controls are used. After filtering the results, we have identified 315 high confidence PR interactors, and 527 proteins adding the interactors identified with moderate confidence.

We compared data from ER, GR and AR interactors to our list of 527 MCI, in order to find possible overlapping (Fig. 105).

As shown in the Venn diagram, 72% of the PR interactors we found with moderate confidence after R5020 exposure are not shared by other steroid hormone receptors, while 21 (4%) are common to all four steroid receptors. For the rest, 75 (14.2%) are shared only with the ER, 14 (2.6%) only with AR, 10 only with GR (1.9%), and the rest (4.5%) are shared with a combination of two other SHRs.

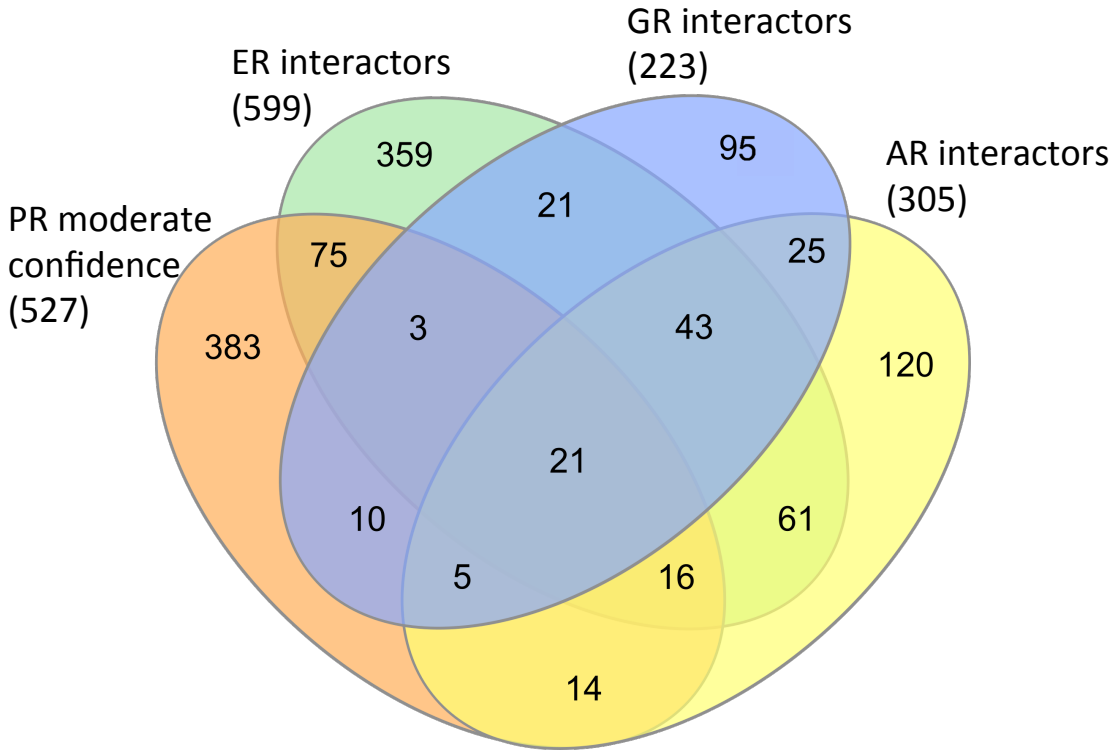


Figure 105: Comparison between known steroid receptor interactors and identified PR interactors with moderate confidence. iRefWeb annotated steroid receptors interactors were compared for overlapping with identified PR interactors after R5020 treatment with moderate confidence.

The same analysis was performed with the 315 PR HCI, and the results were similar (Fig. 106); 5.7% were common to all steroid hormone receptor, 13.3% were shared only with the ER, 3.1% only with AR and 1.9% only with GR. We conclude that the different hormone receptor share some common interactors but also have many receptor-specific interactors.

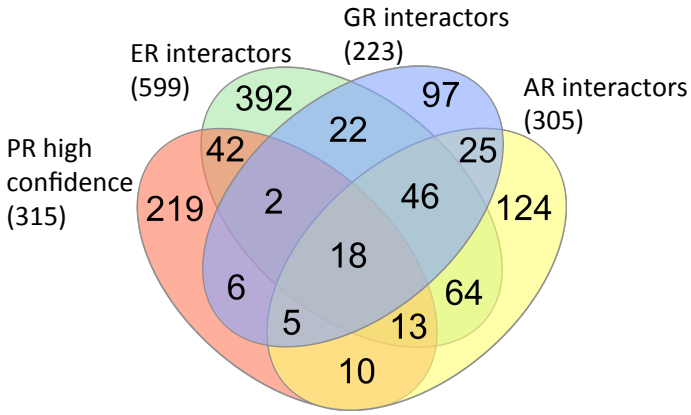


Figure 106: Comparison between known steroid receptor interactors and identified PR interactors with high confidence. iRefWeb annotated steroid receptors interactors were compared for overlapping with identified PR interactors after R5020 treatment with high confidence.

Of our 315 PR HCI, 20 (6.3%) had been previously described as PR interactors (Fig. 107). In contrast, of the additional 212 proteins detected with moderate confidence, only 3 (1.4%) had been previously described as interacting with PR.

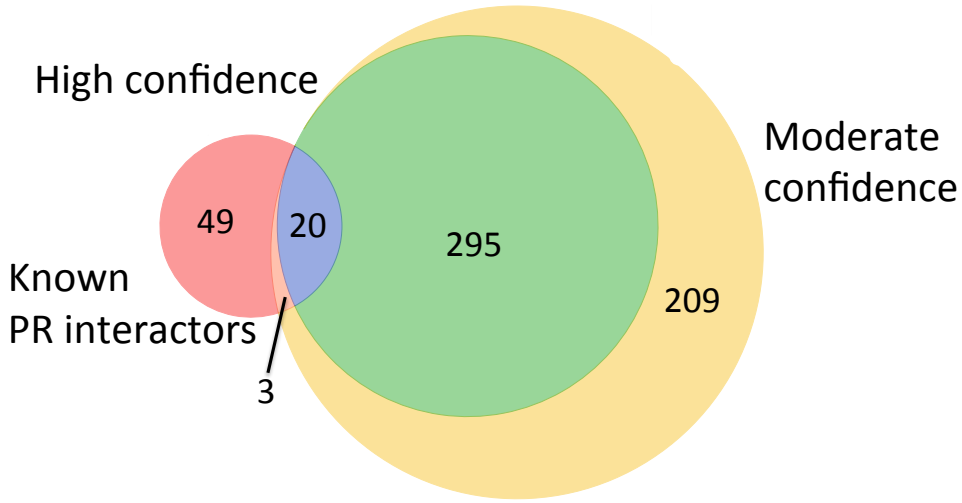


Figure 107: Overlapping of previously known PR interactors with R5020-induced identified interactors.

It has to be remembered that mass spectrometry is a sampling technique, and there are variations in peptide identification between proteins due to their aminoacid content and 3D structure. It also has to be considered that most of the already published PR interactors are not MS-based experiments.

Among the 20 already known PR interactors we find the well-known PR coactivators, such as the NCoA family, CBP/P300 proteins, other transcription regulators, such as SNW1, ANP32E or RBM39/CAPERα, and proteins related to DNA damage and signaling, such as PARP1, XRCC5/XRCC6 and PRKDC. Among chromatin-associated proteins we find HMGB1/2, and chromatin remodeling factors such as SMARCE1, or with moderate confidence SMARCA1 and SMARCD1. Also some proteins related to transcriptional repression, such as NCOR1 and NCOR2, and well-known chaperones, such as FKBP5 or members of the Heat Shock Proteins family that were previously known PR interactors (Fig. 108).

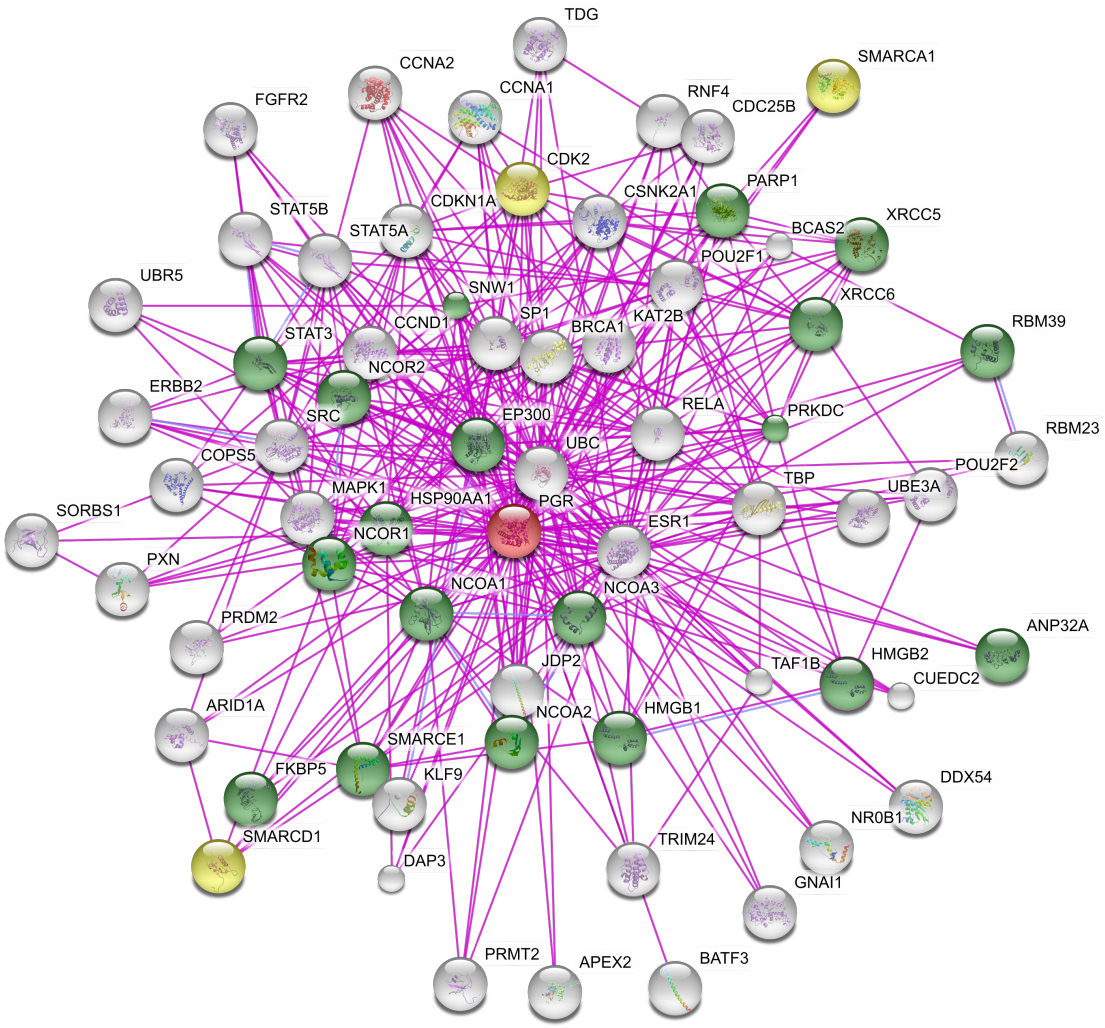


Figure 108: Progesterone receptor known interactors. STRING PPI graph with annotated progesterone receptor interactors. Proteins identified as high (green) and moderate (yellow) confidence data sets; light grey, proteins that did not pass FDR filtering or that were not identified. PR is depicted in red.

Remarkably, we elucidated the dynamics of those interactions in relation of the hormone treatment of the breast cancer cell model used.

Data from the different dynamic patterns for the interactors were presented in a soft clustering mode, but prior to that, it was set a threshold by which we consider the differences of interaction for each protein significant to 1.5 fold change. Below this number (proteins with a kinetic Fold Change lower than 1.5) we consider these proteins to be in constant interaction with PR. From the rest of the proteins, those that have a significant fold change over different times of exposure to hormone, 3 clusters were identified. The first is for proteins loosing their interaction with PR after hormone, the

second is for early interactors, which reach high percentage of interaction already after 5 minutes of hormone stimulation, and the third is for proteins that increase their interaction with PR steadily over time, reaching their maximal values at later times. Proteins enclosed in each cluster and their dynamics had been described, as well as complexes identified as PR interactors.

Among them there are proteins related to a variety of functions, including transcription activation and repression, DNA damage repair, splicing or the proteosomal degradation pathway.

We can conclude that RIME method is optimal for MS experiments with endogenous proteins, especially with the addition of negative controls.

Chaperones

Chaperones are proteins known to help in the maintenance of the structure and conformation of proteins, preventing their degradation. Proper folding of the protein is also required for proper ligand binding and activation. All chaperones identified as PR interactors display a decrease of interaction after hormone exposure and from the dynamic cluster 1. This may reflect the chaperone complexes being displaced from PR after ligand binding, as published for other chaperones (Peattie *et al.* 1992, Pratt *et al.* 1997, Paul *et al.* 2014), allowing PR homodimerize for transactivation. Most of the chaperones identified have a low induction fold change, may be due to the fact that not all cells are responding to hormone stimulus (Wright *et al.* 2016) and thus, some of the immunoprecipitated receptor is still in its unliganded conformation. Some of the chaperones identified were known PR interactors (Weaver *et al.* 2000, Sinars *et al.* 2003), while others were not described to interact with the receptor, like the TCP1 complex. Chaperones are found to interact with other proteins like tubulins, which are also found to loose their interaction with the receptor upon hormone exposure.

Structural proteins

Nuclear envelope and lamina

This group includes mainly tubulins, actin, filamins and proteins of the nuclear envelop and the nuclear lamina.

For tubulins, apart from their structural function, it is known that they mediate translocation of AR (M. L. Zhu *et al.* 2010), and the presence of several tubulins at HCI

may suggest a possible role for tubulins in the PR translocation, supported by the fact that tubulins PR interactions decreases after hormone exposure. Another protein identified as PR interactor and with a described role in SHRs translocation is RAN (Hsiao *et al.* 1999). In a similar manner, it has been identified a hormone-regulated actin network in breast cancer cells nuclei, regulated by ER (Ambrosino *et al.* 2010). In our PR interactor dataset, we found one actin-related protein, with the same dynamics as tubulins. Some actin-depolymerizing factors are also identified, but they increase the interaction or don't change it after ligand exposure.

Related to actin, filamins (known as actin-binding proteins) are identified to behave in a similar manner, loosing the interaction after hormone induction. It has been shown that Filamin A co-localizes with AR and represses androgen transactivation by competing with NCoA2 binding (Loy *et al.* 2003). Data here shown may indicate a possible similar role for filamins in PR, as they have inverted dynamics with NCoA proteins, including the slight recovery of filamins binding and lost of interaction of NCoAs at T60 (Fig. 109).

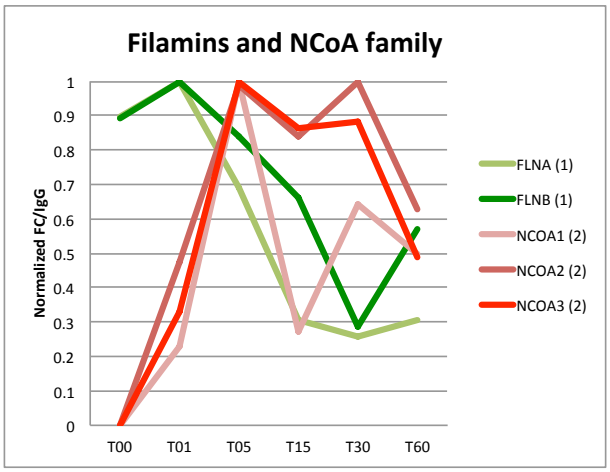


Figure 109: Dynamics of PR interaction of Filamins and NCoA family members.

Within the structural group we include proteins related to the nuclear envelope and the lamina identified as PR interactors, such as TMPO (or LAP2A). Lamins are known to interact with chromatin and with other chromatin remodelers, such as BAF, or with CBX proteins, both of them found as PR interactors. Their interaction with PR may suggest a role in localizing heterochromatin within the periphery of the nucleus, mediated by PR and heterochromatin proteins (Fig. 110).

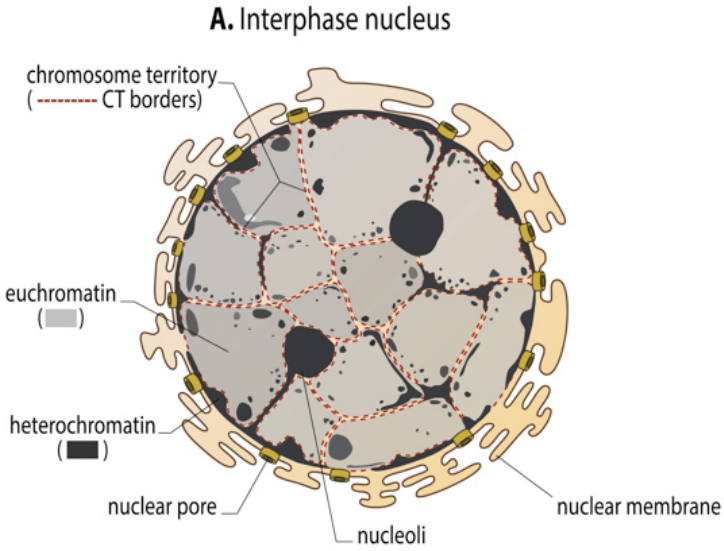


Figure 110: Scheme of a cell nucleus in interphase. From www.mechanobio.info

An interesting protein identified is BAF, which although it belongs to cluster 1, it increases its interaction with PR with a maximum at T05, for decreasing in subsequent time points. This dynamic pattern, and the fact that this protein has been suggested to mediate binding of lamins to chromatin (Furukawa 1999), but also to bind with to other chromatin-related proteins such as histones, or the PR interactors RBBP4 and proteins related to DNA-damage as PAPR1 or DDB1 (Jamin *et al.* 2015), suggests another

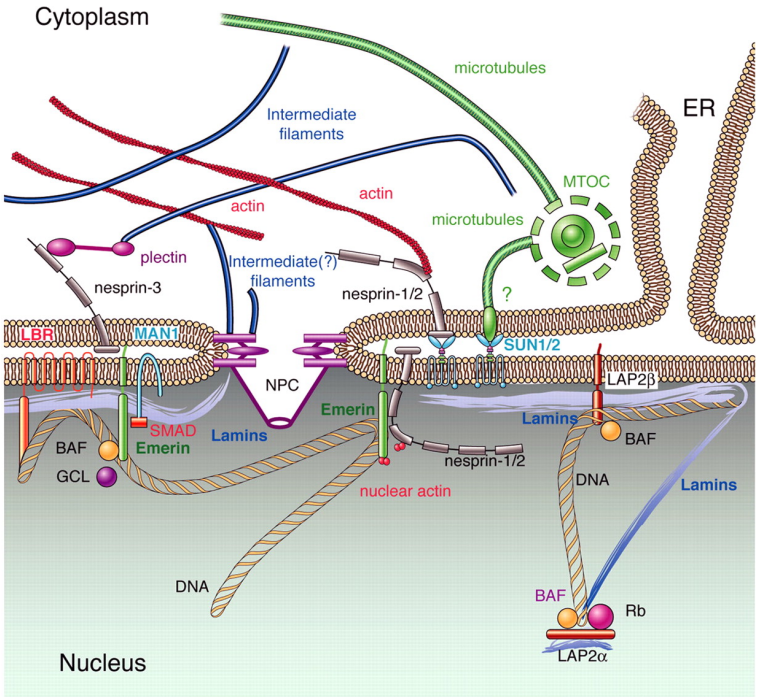


Figure 111: Model of the location of nuclear lamins and their interactions with nearby proteins. There are also depicted BAF, nuclear actin and DNA. From (Broers *et al.* 2006)

possible functional role other than attachment to the nuclear lamina (Fig. 111). Further experiments would be needed to explore the possibility.

Nuclear matrix

Other structural proteins identified as PR interactors, like the MCM helicase complex, regulate DNA replication (Lei 2005), although their dynamic changes are variable, their interaction with PR increase after hormone exposure.

Highly abundant nuclear proteins are identified as PR interactors, as NUMA1, Nucleophosmin or Nucleolin. All three have a similar pattern of interaction with PR, increasing at T60. Although they are shown to have structural roles, they may be also implicated in chromatin structure rearrangement. NUMA1 has been recently linked to DNA damage repair by recruitment of SNF2H-containing complexes to damage sites (Vidi *et al.* 2014), and to regulate ER and PR intranuclear mobility in an ATP-dependent manner (Matsuda *et al.* 2008). From other ongoing projects in our group we know that NUMA1 it is phosphorylated upon hormone exposure (data not shown). Its interaction with PR suggests its implication in PR induced 3D structural changes upon hormone exposure (Le Dily *et al.* 2014). Nucleophosmin has been shown to be involved in histone chaperone activity, enhancing acetylation-dependent transcription (Swaminathan *et al.* 2005). It also have a role in DNA damage recognition (Koike *et al.* 2010) and regulates Retinoic acid-induced transcription (Nichol *et al.* 2016). Therefore, it could be implicated in PR transactivation. Nucleolin is another highly abundant protein in the nucleus, mainly located at the nucleolus. It has roles in transcription factor binding (Hanakahi *et al.* 1997), nucleosome remodeling (Angelov *et al.* 2006) and histone chaperone activity (Gaume *et al.* 2011). Its interaction with PR may be required for in progesterone-dependent chromatin rearrangement and transcription activation.

MATRIN3 is a nuclear matrix protein also described to have other functions. It has been shown to interact with NoNO/PSF proteins for splicing regulation, and with CDC5L, DDX5/DDX17, XRCC5/XRCC6 or several HnRNP proteins (Coelho *et al.* 2016). The fact that most of those proteins also interact with PR, may suggest a functional role in relation to hormonal activation. These findings point for a functional role of the nuclear matrix that provide a structural framework for nuclear activities but also play direct functional roles in these activities.

Cohesins

Cohesins are known for their role in the separation of sister chromatids during cell division, but they also exert a role in chromosomal rearreangement during transcription activation and repression. They have been linked to chromatin remodeling factors like the HDAC-containing NuRD complex, through the ATPase SNF2H (Hakimi *et al.* 2002). More recently they have been postulated a key factor in the formation of chromatin by the process of "loop extrusion" (Fudenberg *et al.* 2016). The identification of these proteins as PR interactors suggests their role in chromatin 3D rearrangement upon hormone activation (Le Dily *et al.* 2014).

Transcription regulation

Transcriptional activation requires chromatin remodeling and transcription factor with their associated factors targeting. Also these two processes are intimately connected, in this section I will divide the PR interactors in two functional groups: factors that catalyzed changes in post-translational modifications and transcription factors with their associated ATP-dependent chromatin remodeling enzymes. An additional part will be devoted to RNA processing enzymes.

Post-translational modification enzymes

PR is a transcription factor and its role in transcription activation has been widely studied. Proteins that help PR to regulate transcription are known as co-regulators. Most of the known co-regulators participate as co-activators in transcriptional induction and are part of the early PR interactors increasing dramatically 5 minutes after hormone exposure. To this class belong the histone acetyltransferases (HATs) of the NCoA family or the related CBP/p300 proteins. Their pattern of PR interaction exhibits a slight decline after 30 minutes of hormone exposure, suggesting that activity is only transiently required. HAT1 interacts also with PR, but it exhibits less change as a function of time of exposure to hormone.

Part of the proteins that form the Mediator complex interact with PR with a time pattern similar to HATs, like GTF2I and TF3C4, a member of the RNA PolIII transcriptional machinery, which plays a role in the regulation of RNA PolII transcribed genes (Roberto Ferrari, personal communication).

Histone lysine methyl transferases (HKMTs) were also identified as PR interactors. As an example of the duality of this modification, KMT2D/MLL2, a H3K4 methyl

transferase participates in transcriptional activation (Mo et al., 2006; Wierer et al., 2013), and KDM6A/UTX, a H3K27 histone demethylase functions as co-activator (M. G. Lee *et al.* 2007). UTX has been shown to interact with MLL2 in coordinating proliferation and invasiveness of breast cancer cells (J. H. Kim *et al.* 2014), but is also known to interact with repressive complexes containing LSD1 and HDAC1, to repress epithelial–mesenchymal transition (EMT) of Breast-cancer stem cell (CSC) (H. J. Choi *et al.* 2015). We find UTX as strong ligand-dependent PR interactor, increasing its binding already at 5 minutes after hormone.

We also identified WDR82, a component of the methyltransferse complex SETD1A, as a PR interactor, which is responsible for the recruitment of the complex to RNA Pol-II at the TSS of target genes and mediates H3K4 trimethylation.

Arginine methyl transferases are also part of the PR interactome. We identified PRMT1, which methylates histone H4 at R3 as a PR interactor, and this PTM represents an early promoter event in ER-regulated genes (Wagner *et al.* 2006). But it has to be considered that PRMT1 can methylate other proteins, having an important role in general gene control (Le Romancer *et al.* 2008). As an example, it methylates the TATA box binding protein RBP56, which is involved in transcription initiation and was itself identified as PR interactor (Jobert *et al.* 2009).

The N-acetylglucosamine transferase OGT1 was also found as PR interactor. It modifies a number of proteins, including H2B, possibly contributing to its monoubiquitination, a mark for transcription activation (Fujiki *et al.* 2011). OGT1 can also modify RNA Pol-II and other transcription factors in the context of transcription down-regulation (X. Yang *et al.* 2002). OGT1 collaborates with TET proteins in CpG-rich TSS for proper H3K4 trimethylation trough SET1 complex (Deplus *et al.* 2013). Its role in chromatin structure for transcription regulation in response to progestins remains to be elucidated.

Another PR interactor related to transcription is SUMO, a post-transcriptional mark that can activate or repress a number of proteins. In the case of PR, it has been shown to inhibit its transactivation (Man *et al.* 2006). We identified as strong and rapid PR interactors several E3-SUMO-ligases (PIAS1 and 3, and TRIM33 and 28), which catalyze the final step for the modification to occur. TRIM33 has been shown to displace HP1-γ from repressed sites by its H3K9me3 binding capacity, and to recruit p300/CBP mediating transcription activation (Xi *et al.* 2011), and therefore it could mediate gene activation by activated PR.

Transcription factors

Some pioneer factors have been found as PR interactors in response to hormone, including AP2A, GATA3 and FOXA1. They are all known to be implicated in transcriptional activation. GATA3 and FOXA1 have been suggested to mediate ER-DNA interaction (Theodorou *et al.* 2013). For the latter, our group has shown that it interacts with PR in co-immunoprecipation experiments, marking the promoters of genes repressed upon hormone exposure (Nacht *et al.* 2016).

Other transcription related proteins have been shown to increase their interaction with PR after hormone. Some of them have been already described PR interactors, shown to mediate PR-dependent transcriptional regulation, including STAT3, SNW1 or RMB39/CAPER (Edwards *et al.* 2002, Dowhan *et al.* 2005, Proietti *et al.* 2011). Also two homologous proteins that are described as splicing factors DDX5 and DDX17, have been described as SHR coactivators and corepressors (Wilson *et al.* 2004, Wortham *et al.* 2009), and a complex level of regulation through splicing has been suggested (Samaan *et al.* 2014). Their hormone-dependent interaction with PR suggests that they are regulated by hormone. For instance, CCAR1 was previously described to enhance GATA2-AR interaction and to facilitate MED1 recruitment to AR-regulated sites (Mizuta *et al.* 2014). Its identification here as a PR interactor, suggests a similar role in breast cancer cell lines exposed to progestins.

Among the transcription-related proteins not previously described as SHR interactors, we want to highlight GRHL2, known to be important in beast carcinogenesis (Werner *et al.* 2013), and CASZ1, shown to be involved in NRD complexes recruitment (Z. Liu *et al.* 2015), both exhibiting a high dynamic change in PR interaction upon hormone exposure. It may be worthwhile to explore their function in progestin gene regulation.

Transcription elongation

Interestingly, transcription related proteins identified as late PR interactors, are involved in transcription elongation and termination. We identified the RNF20/RNF40 (BRE1A/B) complex, which is responsible for H2B ubiquitylation and transcription elongation control. This complex clusters together with CDC73 and TCEA1, which are part on the PAF1 complex, also involved in regulating elongation. Related to this group is CCNT1, part of transcription elongation complex P-TEFb, and Hexim1, suggested to inhibit P-TEFb (CDK9/Cyclin T) kinase and RNA polymerase II (Yik *et al.* 2003).

Hexin1 modulates the functional interaction between ERα and CCNT1 and co-localizes with ER in breast tissue (Wittmann *et al.* 2005). We identified all these proteins as PR interactors, and this indicates a possible role for PR also in elongation control. Transcription elongation pausing could be also hormone regulated as we identified the NELF complex proteins as PR interactors. Some proteins described to have a role in transcription termination were also identified as PR interactors, as XRN2 or PDCD4.

Chromatin remodelers in gene activation

Chromatin remodeling is a key event in the regulation of transcription in general, and in hormone-dependent transcription in particular. In addition to the post-translational modifications (treated above), ATP-dependent nucleosomes remodeling is needed for access of transcription factors and RNA PolII machinery to regulatory information needed for modulating the transcription rate.

ATP-dependent chromatin remodeling complexes

From the many ATP-dependent remodeling complexes, we have identified as PR interactors components of BAF and pBAF complexes. In our lab we have shown that the BAF complex is needed for opening repressed chromatin in hormonal gene induction and functions as a PR co-activator progesterone-dependent transcription during the first minutes of hormone exposure (Vicent *et al.* 2004, Vicent *et al.* 2011). The identification of BAF and PBAF components as PR interactors in RIME experiments further support this conclusion.

Chromatin remodeling in gene repression

Closed compacted chromatin state is characterized by the absence of acetylated histones and the presence of selected methylation, in particular trimethylated of H3K9 and of H3K27.

Nucleosome Remodeling and Deacetylation (NuRD)-related complexes

It has been found that a large number of ATP-dependent chromatin remodelers encompass both an ATPase helicase and a histone deacetylase activity. In a few of these complexes all their complexes components are PR interactors, suggesting that upon hormone exposure there is a need for rearrangement of chromatin, in genes down-

regulated after hormone. Some of these interactors have not been previously reported, including the Mi-2/NRD complex. Intriguingly, Mi-2/CHD4 interacts with Poly-(ADP-ribose) (Silva *et al.* 2016) and MTA1 and participates in the DNA damage response (D. Q. Li *et al.* 2010), again pointing to the relationship between transcriptional control and DNA damage repair (Beato *et al.* 2015).

CtBP containing complexes

CtBP proteins are NAD-dependent hydrolases that are targeted to SATB1 (a DNA-binding protein) marked sites, and help recruiting HDAC1/2 and KDM1A (Purbey *et al.* 2009), leading to histone deacetylation and H3K4 demethylation and ultimately repressing transcription. At the same time, SATB1 is caspase cleaved by PIAS1 SUMOylation-mediated degradation (J. A. Tan *et al.* 2010), adding another level in transcriptional control. One of the components of the complex is a known SHRs interactor (LCoR), although was not identified in our dataset; the presence of the rest of the components indicates a SATB1- and NAD-dependent active transcription repression upon hormone exposure.

TLE co-repressor complex

This repressive complex displays direct binding to transcription factors (Jangal *et al.* 2014), preventing their transactivation, which can be blocked by PARP1 activation (Ju *et al.* 2004).

Histone variant exchange

It is intriguing that we identified as PR interactors several proteins implicated in H2A.Z dynamic exchange, including AN32P from the SET complex (Obri *et al.* 2014), RUVB1 and 2 (Mizuguchi *et al.* 2004) and the HMGB proteins. It has been recently shown that H2A.Z is enriched at ERα-regulated enhancers, functioning as a marker of enhancer activation (Brunelle *et al.* 2015). It is possible that progestin-regulated enhancer activity, requires H2A.z exchange.

RNA processing

Splicing factors are one of the most enriched proteins in our analyses, but many splicing factors have additional biological functions and splicing and transcription are coupled processes (Listerman *et al.* 2006, Luco *et al.* 2011, Hilgers 2015, Martinez-Rucobo *et al.* 2015).

Several known spliceosomal proteins are identified as PR interactors, and they are often related to exon recognition and spliecosome assembly, the first steps of the processes. Among them, some important exon-recognition proteins are found, such as U520, SPF45 or U2AF^{65}. A role for PR in alternative splicing has been previously described, with nucleosome positioning being an important factor (Iannone *et al.* 2015), along with role for the hormone-induced hnRNP AB, identified as PR interactor in our dataset with one of the highest IgGFC from all the hnRNPs, thus supporting a ligand-induced regulation.

Master regulators

Among the proteins we identified as PR interactors, there is a group that is difficult to categorize. Those are proteins described to be involved in a variety of functions that can range from transcription to DNA damage machinery. We have already mnetioned PRMT1 for instance. In this case is important to highlight the identification of CDK1 (and CDK2 with moderate confidence) as PR interactors, and with a constant interaction with the receptor. From CDK2, it is known that its functions with PR and as an important role in transcription regulation in cooperation of PARP1 (Wright *et al.* 2012). It has been recently published CDK2 and DNA-PK interact with PR and modulate PR transactivation (Trevino *et al.* 2016). In that respect, there is a debate as to what extent CDK1 and CDK2 have been properly discriminated in previous publications (Sakurikar *et al.* 2016).

In this group of proteins we are including HMGB proteins, that although have a role in enhancing PR activity (Boonyaratanakornkit *et al.* 1998), they have been described to function in a variety of chromatin-associated processes (Y. Zhang *et al.* 2005). Their presence in our PR interactor data set confirms their PR-related functions.

Phosphatases are known to regulate the phosphorylation status of hundreds of proteins, and the identification of some of them as PR interactors indicates that a fine-tuning of phosphorylations is required in ligand-mediated processes.

Another promiscuous group of proteins is the one formed by NoNO, SFPQ and PSF described to act in multiple processes (Knott *et al.* 2016), and also to modulate PR transcriptional activity (Dong *et al.* 2009), in contact with PARP1, CDK1, Matrin3 or PIN1, all of them found also as PR interactors.

Signaling

We identified as PR interactors some proteins with roles in signalling cascades activation (GRB2, FUBP3, KHDRBS1/SAM68, DUSP3 or Nucleoprotein TPR), reinforcing the known role for PR in the signaling cascades pathway. But it has to be pointed out that, although they were identified, none of the MAPK kinases passed our FDR filtering. MAPK14 and MAPK38 (with FDR 0.25 at early time points) and ERK and MSK1 (with a few peptides also at early time points) are some of them. One of the reasons may be that the interactions of PR with kinases are very transient and occurs simultaneously in very few cells.

In this context, Estrogen Receptor peptides were also identified, but the protein did not pass FDR filtering, and so we did not considered it in our dataset as a PR interactor. It is worth mentioning again that Mass spectrometry has it own limitations.

Proteasomal proteins

The identification of proteins related to the proteasome degradation pathway as PR interactors is not surprising, as it has been shown how PR itself is targeted to degradation upon activation after hormone exposure (Lange *et al.* 2000). It has also been postulated that transcription repressive proteins are degraded for proper transcription activation (Vitari *et al.* 2011). There are PR interactors from all the phases of the proteasome regulation, from ubiquitin ligases to proteins in charge of recruiting to nuclear receptor-regulated transcription units the proteosomal proteins, or proteins involved in the final steps of ubiquitin-mediated proteosomal degradation. Curiously, also a Ubiquitin hydrolase, in charge of removing ubiquitin from proteins, was found in our data set with very rapid increase interaction with PR after 1 minute of hormone exposure.

DNA damage repair

The coupling of DNA damage and transcription is a focus of debate in the field (Beato *et al.* 2015). It has been suggested to have a link with hormone signaling pathways (Schiewer *et al.* 2016). We identified many DNA damage related proteins as PR interactors, some of them in all time points studied, including T0. Among them we find PARP1, XRCC5/XRCC6 or PRKDC. Some of them are previously shown PR interactors, but its constant interaction during hormonal activation was not described. Some of these proteins are DNA damage sensors, as RPA1, DNA ligase 3 or DBC1, whose binding to PR during the whole time course may make possible a faster recruitment of the repair machinery to damage sites as soon as the receptor is localized at the DNA. Proteins from the nuclear matrix are also annotated to be implicated in DNA repair, such as Matrin-3 or Nucleophosmin, and chromatin proteins as HMGBs or RUVB-like proteins. We want to highlight that the 2nd highest PR interactor we found was RAD50, which plays a central role in DNA double-strand break repair, and displays a relatively constant interaction with PR. Proteins from the three DNA repair pathways are identified (homologous recombination -HR-, Non-homologous end joining -NHEJ- and base excision repair -BER-), although these pathways have multiple interconnections.

RU-486 treated samples

The mechanism of action of this partial agonist of the PR is not known. From our data, the clearest result upon treatment with RU-486 is that most of the highly transcription-related proteins found upon exposure to R5020, show a reduced interaction with PR upon exposure to RU486. This group include NCoA proteins, CBP, the entire mediator related proteins, MLL2 and UTX, PRMT1, PIAS1, RXRa or CASZ1. All these are related to transcription regulation, and its lack of interaction may explain the low agonistic role of RU-486. It is worth mentioning that PIN1, which is implicated in ligand-dependent ERα-DNA interaction when induced with estrogen but not with its antagonist, is behaving in the same manner in the case of PR when compared the interaction after R5020 and RU-486. Another protein that is losing the interaction seen with R5020 is the Nucleoside diphosphate kinase B (NDKB), described to be a metastasis suppressor gene (Youn *et al.* 2008), and part of the SET complex, also implicated in transcription activation and SHR binding to DNA. From the proteins

found with increased interaction when cells are exposed to the antagonist, the most remarkable is the increase in chaperoning proteins and proteins from the proteasomal system.

6. Conclusions

1. The RIME (Rapid Immunoprecipitation Mass spectrometry of Endogenous proteins) approach provided a reliable identification of endogenous progesterone receptor interactors in breast cancer cells exposed to the potent progestin R5020.
2. We identified 315 interactors of which 20 were already known and 295 are new high confidence PR interactors.
3. The variation of PR interaction as a function of time upon hormone exposure allow to distinguished 4 dynamic clusters; Basal cluster, 66 proteins present at similar level at all time points; Cluster 1, 41 proteins decreasing their interaction after hormone; cluster 2, 115 proteins increasing their interaction rapidly after hormone; cluster 3, 93 proteins increasing their interaction steadily over time.
4. PR interactors form functional complexes involved in: transcriptional regulation, chromatin remodelling, mRNA processing, DNA damage repair, proteosomal degradation, protein stability and nuclear structural proteins.
5. Proteins related to the chaperoning system identified decrease their interaction after hormone.
6. Transcription activation-related PR interactors are the ones with the maximal increase in PR interaction after hormone.
7. Repressive complexes are identified to interact with PR upon hormone exposure, indicating evidences in ligand-dependent active transcription repression.
8. Proteins involved in DNA damage repair as PR interactors indicates a possible coupling between transcription regulation and DNA repair.
9. We have not identified some known PR interactors like known kinases and ATP-dependent remodelers likely because their interaction is transient or due to limitations in peptide identification.
10. Exposure of cells to progesterone partial antagonist RU486 maintain the majority of the interactors, but loses the interactors related to transcription regulation.

7. Bibliography

Abdel-Hafiz, H., G. S. Takimoto, L. Tung and K. B. Horwitz (2002). "The inhibitory function in human progesterone receptor N termini binds SUMO-1 protein to regulate autoinhibition and transrepression." J Biol Chem **277**(37): 33950-33956.

Abdel-Hafiz, H. A. and K. B. Horwitz (2014). "Post-translational modifications of the progesterone receptors." J Steroid Biochem Mol Biol **140**: 80-89.

Abdou, I., G. G. Poirier, M. J. Hendzel and M. Weinfeld (2015). "DNA ligase III acts as a DNA strand break sensor in the cellular orchestration of DNA strand break repair." Nucleic Acids Res **43**(2): 875-892.

Agassandian, M., J. Zhou, L. A. Tephly, A. J. Ryan, A. B. Carter and R. K. Mallampalli (2005). "Oxysterols inhibit phosphatidylcholine synthesis via ERK docking and phosphorylation of CTP:phosphocholine cytidylyltransferase." J Biol Chem **280**(22): 21577-21587.

Ahmad, K. and S. Henikoff (2002). "The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly." Mol Cell **9**(6): 1191-1200.

Ajuh, P., B. Kuster, K. Panov, J. C. B. M. Zomerdijk, M. Mann and A. I. Lamond (2000). "Functional analysis of the human CDC5L complex and identifcation of its components by mass spectrometry." EMBO J **19**(23): 6569-6581.

Akoumianaki, T., D. Kardassis, H. Polioudaki, S. D. Georgatos and P. A. Theodoropoulos (2009). "Nucleocytoplasmic shuttling of soluble tubulin in mammalian cells." J Cell Sci **122**(Pt 8): 1111-1118.

Allemand, E., M. L. Hastings, M. V. Murray, M. P. Myers and A. R. Krainer (2007). "Alternative splicing regulation by interaction of phosphatase PP2Cgamma with nucleic acid-binding protein YB-1." Nat Struct Mol Biol **14**(7): 630-638.

Allen, B. L. and D. J. Taatjes (2015). "The Mediator complex: a central integrator of transcription." Nat Rev Mol Cell Biol **16**(3): 155-166.

Allis, C. D., R. Richman, M. A. Gorovsky, Y. S. Ziegler, B. Touchstone, W. A. Bradley and R. G. Cook (1986). "hv1 is an evolutionarily conserved H2A variant that is preferentially associated with active genes." J Biol Chem **261**(4): 1941-1948.

Ambrosino, C., R. Tarallo, A. Bamundo, D. Cuomo, G. Franci, G. Nassa, O. Paris, M. Ravo, A. Giovane, N. Zambrano, T. Lepikhova, O. A. Janne, M. Baumann, T. A. Nyman, L. Cicatiello and A. Weisz (2010). "Identification of a hormone-regulated dynamic nuclear actin network associated with estrogen receptor alpha in human breast cancer cell nuclei." Mol Cell Proteomics **9**(6): 1352-1367.

An, B. S., D. M. Selva, G. L. Hammond, A. Rivero-Muller, N. Rahman and P. C. Leung (2006). "Steroid receptor coactivator-3 is required for progesterone receptor trans-activation of target genes in response to gonadotropin-releasing hormone treatment of pituitary cells." J Biol Chem **281**(30): 20817-20824.

Angelov, D., V. A. Bondarenko, S. Almagro, H. Menoni, F. Mongelard, F. Hans, F. Mietton, V. M. Studitsky, A. Hamiche, S. Dimitrov and P. Bouvet (2006). "Nucleolin is a histone chaperone with FACT-like activity and assists remodeling of nucleosomes." EMBO J **25**(8): 1669-1679.

Angelov, D., A. Molla, P. Y. Perche, F. Hans, J. Cote, S. Khochbin, P. Bouvet and S. Dimitrov (2003). "The histone variant macroH2A interferes with transcription factor binding and SWI/SNF nucleosome remodeling." Mol Cell **11**(4): 1033-1041.

Arents, G., R. W. Burlingame, B. C. Wang, W. E. Love and E. N. Moudrianakis (1991). "The nucleosomal core histone octamer at 3.1 A resolution: a tripartite protein assembly and a left-handed superhelix." Proc Natl Acad Sci U S A **88**(22): 10148-10152.

Bain, D. L., M. A. Franden, J. L. McManaman, G. S. Takimoto and K. B. Horwitz (2000). "The N-terminal region of the human progesterone A-receptor. Structural analysis and the influence of the DNA binding domain." J Biol Chem **275**(10): 7313-7320.

Bain, D. L., M. A. Franden, J. L. McManaman, G. S. Takimoto and K. B. Horwitz (2001). "The N-terminal region of human progesterone B-receptors: biophysical and biochemical comparison to A-receptors." J Biol Chem **276**(26): 23825-23831.

Baird, D. T., A. Brown, L. Cheng, H. O. Critchley, S. Lin, N. Narvekar and A. R. Williams (2003). "Mifepristone: a novel estrogen-free daily contraceptive pill." Steroids **68**(10-13): 1099-1105.

Ballare, C., G. Castellano, L. Gaveglia, S. Althammer, J. Gonzalez-Vallinas, E. Eyras, F. Le Dily, R. Zaurin, D. Soronellas, G. P. Vicent and M. Beato (2013). "Nucleosome-driven transcription factor binding and gene regulation." Mol Cell **49**(1): 67-79.

Ballare, C., M. Uhrig, T. Bechtold, E. Sancho, M. Di Domenico, A. Migliaccio, F. Auricchio and M. Beato (2003). "Two Domains of the Progesterone Receptor Interact with the Estrogen Receptor and Are Required for Progesterone Activation of the c-Src/Erk Pathway in Mammalian Cells." Molecular and Cellular Biology **23**(6): 1994-2008.

Bannister, A. J. and T. Kouzarides (2005). "Reversing histone methylation." Nature **436**(7054): 1103-1106.

Bannister, A. J. and T. Kouzarides (2011). "Regulation of chromatin by histone modifications." Cell Res **21**(3): 381-395.

Bantscheff, M., C. Hopf, M. M. Savitski, A. Dittmann, P. Grandi, A. M. Michon, J. Schlegl, Y. Abraham, I. Becher, G. Bergamini, M. Boesche, M. Delling, B. Dumpelfeld, D. Eberhard, C. Huthmacher, T. Mathieson, D. Poeckel, V. Reader, K. Strunk, G. Sweetman, U. Kruse, G. Neubauer, N. G. Ramsden and G. Drewes (2011). "Chemoproteomics profiling of HDAC inhibitors reveals selective targeting of HDAC complexes." Nat Biotechnol **29**(3): 255-265.

Barak, O., M. A. Lazzaro, W. S. Lane, D. W. Speicher, D. J. Picketts and R. Shiekhattar (2003). "Isolation of human NURF: a regulator of Engrailed gene expression." EMBO J **22**(22): 6089-6100.

Beato, M. (1989). "Gene Regulation by Steroid Hormones." Cell **56**: 335-344.

Beato, M. and J. Klug (2000). "Steroid hormone receptors: an update." Hum Reprod Update **6**(3): 225-236.

Beato, M., R. H. Wright and G. P. Vicent (2015). "DNA damage and gene transcription: accident or necessity?" Cell Res **25**(7): 769-770.

Becker, P. B. and J. L. Workman (2013). "Nucleosome remodeling and epigenetics." Cold Spring Harb Perspect Biol **5**(9).

Belandia, B., R. L. Orford, H. C. Hurst and M. G. Parker (2002). "Targeting of SWI/SNF chromatin remodelling complexes to estrogen-responsive genes." EMBO J **21**(15): 4094-4103.

Beleut, M., R. D. Rajaram, M. Caikovski, A. Ayyanan, D. Germano, Y. Choi, P. Schneider and C. Brisken (2010). "Two distinct mechanisms underlie progesterone-induced proliferation in the mammary gland." Proc Natl Acad Sci U S A **107**(7): 2989-2994.

Beli, P., N. Lukashchuk, S. A. Wagner, B. T. Weinert, J. V. Olsen, L. Baskcomb, M. Mann, S. P. Jackson and C. Choudhary (2012). "Proteomic investigations reveal a role for RNA processing factor THRAP3 in the DNA damage response." Mol Cell **46**(2): 212-225.

Beresford, P. J., D. Zhang, D. Y. Oh, Z. Fan, E. L. Greer, M. L. Russo, M. Jaju and J. Lieberman (2001). "Granzyme A activates an endoplasmic reticulum-associated caspase-independent nuclease to induce single-stranded DNA nicks." J Biol Chem **276**(46): 43285-43293.

Binder, J. X., S. Pletscher-Frankild, K. Tsafou, C. Stolte, S. I. O'Donoghue, R. Schneider and L. J. Jensen (2014). "COMPARTMENTS: unification and visualization of protein subcellular localization evidence." Database (Oxford) **2014**: bau012.

Bladen, C. L., D. Udayakumar, Y. Takeda and W. S. Dynan (2005). "Identification of the polypyrimidine tract binding protein-associated splicing factor.p54(nrb) complex as a candidate DNA double-strand break rejoining factor." J Biol Chem **280**(7): 5205-5210.

Bonte, E. and P. B. Becker (1999). "Preparation of chromatin assembly extracts from preblastoderm Drosophila embryos." Methods in molecular biology **119**: 187-194.

Boonyaratanakornkit, V., V. Melvin, P. Prendergast, M. Altmann, L. Ronfani, M. E. Bianchi, L. Taraseviciene, S. K. Nordeen, E. A. Allegretto and D. P. Edwards (1998). "High-mobility group chromatin proteins 1 and 2 functionally interact with steroid hormone receptors to enhance their DNA binding in vitro and transcriptional activity in mammalian cells." Mol Cell Biol **18**(8): 4471-4487.

Boonyaratanakornkit, V., M. P. Scott, V. Ribon, L. Sherman, S. M. Anderson, J. L. Maller, W. T. Miller and D. P. Edwards (2001). "Progesterone receptor contains a proline-rich motif that directly interacts with SH3 domains and activates c-Src family tyrosine kinases." Mol Cell **8**(2): 269-280.

Botta, A., C. Tandoi, G. Fini, G. Calabrese, B. Dallapiccola and G. Novelli (2001). "Cloning and characterization of the gene encoding human NPL4, a protein interacting with the ubiquitin fusion-degradation protein (UFD1L)." Gene **275**(1): 39-46.

Bottardi, S., L. Mavoungou, H. Pak, S. Daou, V. Bourgoin, Y. A. Lakehal, B. Affar el and E. Milot (2014). "The IKAROS interaction with a complex including chromatin remodeling and transcription elongation activities is required for hematopoiesis." PLoS Genet **10**(12): e1004827.

Brackertz, M., J. Boeke, R. Zhang and R. Renkawitz (2002). "Two highly related p66 proteins comprise a new family of potent transcriptional repressors interacting with MBD2 and MBD3." J Biol Chem **277**(43): 40958-40966.

Braganca, J., J. J. Eloranta, S. D. Bamforth, J. C. Ibbitt, H. C. Hurst and S. Bhattacharya (2003). "Physical and functional interactions among AP-2 transcription factors, p300/CREB-binding protein, and CITED2." J Biol Chem **278**(18): 16021-16029.

Broers, J. L., F. C. Ramaekers, G. Bonne, R. B. Yaou and C. J. Hutchison (2006). "Nuclear lamins: laminopathies and their role in premature ageing." Physiol Rev **86**(3): 967-1008.

Bruggemeier, U., M. Kalff, S. Franke, C. Scheidereit and M. Beato (1991). "Ubiquitous transcription factor OTF-1 mediates induction of the MMTV promoter through synergistic interaction with hormone receptors." Cell **64**(3): 565-572.

Brunelle, M., A. Nordell Markovits, S. Rodrigue, M. Lupien, P. E. Jacques and N. Gevry (2015). "The histone variant H2A.Z is an important regulator of enhancer activity." Nucleic Acids Res **43**(20): 9742-9756.

Bulger, M., T. Ito, R. T. Kamakaka and J. T. Kadonaga (1995). "Assembly of regularly spaced nucleosome arrays by Drosophila chromatin assembly factor 1 and a 56-kDa histone-binding protein." Proc Natl Acad Sci U S A **92**: 11726-11730.

Bunch, H., X. Zheng, A. Burkholder, S. T. Dillon, S. Motola, G. Birrane, C. C. Ebmeier, S. Levine, D. Fargo, G. Hu, D. J. Taatjes and S. K. Calderwood (2014). "TRIM28 regulates RNA polymerase II promoter-proximal pausing and pause release." Nat Struct Mol Biol **21**(10): 876-883.

Byrne, J. L., H. F. Paterson and C. J. Marshall (1996). "p21Ras activation by the guanine nucleotide exchange factor Sos, requires the Sos/Grb2 interaction and a second ligand-dependent signal involving the Sos N-terminus." Oncogene **13**(10): 2055-2065.

Cahill, M. A., J. A. Jazayeri, S. M. Catalano, S. Toyokuni, Z. Kovacevic and D. R. Richardson (2016). "The emerging role of progesterone receptor membrane component 1 (PGRMC1) in cancer biology." Biochim Biophys Acta.

Calvo, V. and M. Beato (2011). "BRCA1 counteracts progesterone action by ubiquitination leading to progesterone receptor degradation and epigenetic silencing of target promoters." Cancer Res **71**(9): 3422-3431.

Carney, J. P., R. S. Maser, H. Olivares, E. M. Davis, M. Le Beau, J. R. Yates, 3rd, L. Hays, W. F. Morgan and J. H. Petrini (1998). "The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response." Cell **93**(3): 477-486.

Chaouki, A. S. and H. K. Salz (2006). "Drosophila SPF45: a bifunctional protein with roles in both splicing and DNA repair." PLoS Genet **2**(12): e178.

Chauchereau, A., L. Amazit, M. Quesne, A. Guiochon-Mantel and E. Milgrom (2003). "Sumoylation of the progesterone receptor and of the steroid receptor coactivator SRC-1." J Biol Chem **278**(14): 12335-12343.

Chawla, A., J. J. Repa, R. M. Evans and D. J. Mangelsdorf (2001). "Nuclear receptors and lipid physiology: opening the X-files." Science **294**(5548): 1866-1870.

Chen, E. Y., C. M. Tan, Y. Kou, Q. Duan, Z. Wang, G. V. Meirelles, N. R. Clark and A. Ma'ayan (2013). "Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool." BMC Bioinformatics **14**: 128.

Chen, H., R. J. Lin, R. L. Schiltz, D. Chakravarti, A. Nash, L. Nagy, M. L. Privalsky, Y. Nakatani and R. M. Evans (1997). "Nuclear receptor coactivator ACTR is a novel

histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300." Cell **90**(3): 569-580.

Chen, H. C., W. C. Lin, Y. G. Tsay, S. C. Lee and C. J. Chang (2002). "An RNA helicase, DDX1, interacting with poly(A) RNA and heterogeneous nuclear ribonucleoprotein K." J Biol Chem **277**(43): 40403-40409.

Chen, J. D. and R. M. Evans (1995). "A transcriptional co-repressor that interacts with nuclear hormone receptors." Nature **377**(6548): 454-457.

Chen, S., L. R. Bohrer, A. N. Rai, Y. Pan, L. Gan, X. Zhou, A. Bagchi, J. A. Simon and H. Huang (2010). "Cyclin-dependent kinases regulate epigenetic gene silencing through phosphorylation of EZH2." Nat Cell Biol **12**(11): 1108-1114.

Childs, K. S. and S. Goodbourn (2003). "Identification of novel co-repressor molecules for Interferon Regulatory Factor-2." Nucleic Acids Res **31**(12): 3016-3026.

Cho, Y. W., T. Hong, S. Hong, H. Guo, H. Yu, D. Kim, T. Guszczynski, G. R. Dressler, T. D. Copeland, M. Kalkum and K. Ge (2007). "PTIP associates with MLL3- and MLL4-containing histone H3 lysine 4 methyltransferase complex." J Biol Chem **282**(28): 20395-20406.

Choi, H., B. Larsen, Z. Y. Lin, A. Breitkreutz, D. Mellacheruvu, D. Fermin, Z. S. Qin, M. Tyers, A. C. Gingras and A. I. Nesvizhskii (2011). "SAINT: probabilistic scoring of affinity purification-mass spectrometry data." Nat Methods **8**(1): 70-73.

Choi, H. J., J. H. Park, M. Park, H. Y. Won, H. S. Joo, C. H. Lee, J. Y. Lee and G. Kong (2015). "UTX inhibits EMT-induced breast CSC properties by epigenetic repression of EMT genes in cooperation with LSD1 and HDAC1." EMBO Rep **16**(10): 1288-1298.

Chung, H. H., S. K. Sze, A. S. Tay and V. C. Lin (2014). "Acetylation at lysine 183 of progesterone receptor by p300 accelerates DNA binding kinetics and transactivation of direct target genes." J Biol Chem **289**(4): 2180-2194.

Chymkowitch, P., V. Eldholm, S. Lorenz, C. Zimmermann, J. M. Lindvall, M. Bjoras, L. A. Meza-Zepeda and J. M. Enserink (2012). "Cdc28 kinase activity regulates the basal transcription machinery at a subset of genes." Proc Natl Acad Sci U S A **109**(26): 10450-10455.

Cirillo, L. A. and K. S. Zaret (2007). "Specific interactions of the wing domains of FOXA1 transcription factor with DNA." J Mol Biol **366**(3): 720-724.

Coelho, M. B., J. Attig, J. Ule and C. W. Smith (2016). "Matrin3: connecting gene expression with the nuclear matrix." Wiley Interdiscip Rev RNA **7**(3): 303-315.

Condon, J. C., D. B. Hardy, K. Kovaric and C. R. Mendelson (2006). "Up-regulation of the progesterone receptor (PR)-C isoform in laboring myometrium by activation of nuclear factor-kappaB may contribute to the onset of labor through inhibition of PR function." Mol Endocrinol **20**(4): 764-775.

Cong, L., S. B. Pakala, K. Ohshiro, D. Q. Li and R. Kumar (2011). "SUMOylation and SUMO-interacting motif (SIM) of metastasis tumor antigen 1 (MTA1) synergistically regulate its transcriptional repressor function." J Biol Chem **286**(51): 43793-43808.

Corona, D. F. and J. W. Tamkun (2004). "Multiple roles for ISWI in transcription, chromosome organization and DNA replication." Biochim Biophys Acta **1677**(1-3): 113-119.

Cui, Y., A. Niu, R. Pestell, R. Kumar, E. M. Curran, Y. Liu and S. A. Fuqua (2006). "Metastasis-associated protein 2 is a repressor of estrogen receptor alpha whose overexpression leads to estrogen-independent growth of human breast cancer cells." Mol Endocrinol **20**(9): 2020-2035.

D'Arcy, S., K. W. Martin, T. Panchenko, X. Chen, S. Bergeron, L. A. Stargell, B. E. Black and K. Luger (2013). "Chaperone Nap1 shields histone surfaces used in a nucleosome and can put H2A-H2B in an unconventional tetrameric form." Mol Cell **51**(5): 662-677.

Daniel, A. R., E. J. Faivre and C. A. Lange (2007). "Phosphorylation-dependent antagonism of sumoylation derepresses progesterone receptor action in breast cancer cells." Mol Endocrinol **21**(12): 2890-2906.

Daniel, A. R., A. L. Gaviglio, L. M. Czaplicki, C. J. Hillard, D. Housa and C. A. Lange (2010). "The progesterone receptor hinge region regulates the kinetics of transcriptional responses through acetylation, phosphorylation, and nuclear retention." Mol Endocrinol **24**(11): 2126-2138.

Daniel, A. R. and C. A. Lange (2009). "Protein kinases mediate ligand-independent derepression of sumoylated progesterone receptors in breast cancer cells." Proc Natl Acad Sci U S A **106**(34): 14287-14292.

Danielsen, J. M., K. B. Sylvestersen, S. Bekker-Jensen, D. Szklarczyk, J. W. Poulsen, H. Horn, L. J. Jensen, N. Mailand and M. L. Nielsen (2011). "Mass spectrometric analysis of lysine ubiquitylation reveals promiscuity at site level." Mol Cell Proteomics **10**(3): M110 003590.

Davis-Smyth, T., R. C. Duncan, T. Zheng, G. Michelotti and D. Levens (1996). "The far upstream element-binding proteins comprise an ancient family of single-strand DNA-binding transactivators." J Biol Chem **271**(49): 31679-31687.

Dawson, M. A., A. J. Bannister, B. Gottgens, S. D. Foster, T. Bartke, A. R. Green and T. Kouzarides (2009). "JAK2 phosphorylates histone H3Y41 and excludes HP1alpha from chromatin." Nature **461**(7265): 819-822.

Dayal, S., A. Sparks, J. Jacob, N. Allende-Vega, D. P. Lane and M. K. Saville (2009). "Suppression of the deubiquitinating enzyme USP5 causes the accumulation of unanchored polyubiquitin and the activation of p53." J Biol Chem **284**(8): 5030-5041.

De Amicis, F., S. Zupo, M. L. Panno, R. Malivindi, F. Giordano, I. Barone, L. Mauro, S. A. Fuqua and S. Ando (2009). "Progesterone receptor B recruits a repressor complex to a half-PRE site of the estrogen receptor alpha gene promoter." Mol Endocrinol **23**(4): 454-465.

Dean, D. A., G. Urban, I. V. Aragon, M. Swingle, B. Miller, S. Rusconi, M. Bueno, N. M. Dean and R. E. Honkanen (2001). "Serine/threonine protein phosphatase 5 (PP5) participates in the regulation of glucocorticoid receptor nucleocytoplasmic shuttling." BMC Cell Biol **2**: 6.

Deplus, R., B. Delatte, M. K. Schwinn, M. Defrance, J. Mendez, N. Murphy, M. A. Dawson, M. Volkmar, P. Putmans, E. Calonne, A. H. Shih, R. L. Levine, O. Bernard, T. Mercher, E. Solary, M. Urh, D. L. Daniels and F. Fuks (2013). "TET2 and TET3 regulate GlcNAcylation and H3K4 methylation through OGT and SET1/COMPASS." EMBO J **32**(5): 645-655.

Diaz Flaque, M. C., N. M. Galigniana, W. Beguelin, R. Vicario, C. J. Proietti, R. Russo, M. A. Rivas, M. Tkach, P. Guzman, J. C. Roa, E. Maronna, V. Pineda, S. Munoz, M. Mercogliano, E. H. Charreau, P. Yankilevich, R. Schillaci and P. V. Elizalde (2013). "Progesterone receptor assembly of a transcriptional complex along with activator protein 1, signal transducer and activator of transcription 3 and ErbB-2 governs breast cancer growth and predicts response to endocrine therapy." Breast Cancer Res **15**(6): R118.

Diep, C. H., A. R. Daniel, L. J. Mauro, T. P. Knutson and C. A. Lange (2015). "Progesterone action in breast, uterine, and ovarian cancers." J Mol Endocrinol **54**(2): R31-53.

Dong, X., J. Sweet, J. R. Challis, T. Brown and S. J. Lye (2007). "Transcriptional activity of androgen receptor is modulated by two RNA splicing factors, PSF and p54nrb." Mol Cell Biol **27**(13): 4863-4875.

Dong, X., C. Yu, O. Shynlova, J. R. Challis, P. S. Rennie and S. J. Lye (2009). "p54nrb is a transcriptional corepressor of the progesterone receptor that modulates transcription of the labor-associated gene, connexin 43 (Gja1)." Mol Endocrinol **23**(8): 1147-1160.

Dowhan, D. H., E. P. Hong, D. Auboeuf, A. P. Dennis, M. M. Wilson, S. M. Berget and B. W. O'Malley (2005). "Steroid hormone receptor coactivation and alternative RNA splicing by U2AF65-related proteins CAPERalpha and CAPERbeta." Mol Cell **17**(3): 429-439.

Dressing, G. E., T. P. Knutson, M. J. Schiewer, A. R. Daniel, C. R. Hagan, C. H. Diep, K. E. Knudsen and C. A. Lange (2014). "Progesterone receptor-cyclin D1 complexes induce cell cycle-dependent transcriptional programs in breast cancer cells." Mol Endocrinol **28**(4): 442-457.

Dufu, K., M. J. Livingstone, J. Seebacher, S. P. Gygi, S. A. Wilson and R. Reed (2010). "ATP is required for interactions between UAP56 and two conserved mRNA export proteins, Aly and CIP29, to assemble the TREX complex." Genes Dev **24**(18): 2043-2053.

Duncan, R., L. Bazar, G. Michelotti, T. Tomonaga, H. Krutzsch, M. Avigan and D. Levens (1994). "A sequence-specific, single-strand binding protein activates the far upstream element of c-myc and defines a new DNA-binding motif." Genes Dev **8**(4): 465-480.

Edwards, D. P., S. E. Wardell and V. Boonyaratanakornkit (2002). "Progesterone receptor interacting coregulatory proteins and cross talk with cell signaling pathways." J Steroid Biochem Mol Biol **83**(1-5): 173-186.

Elbi, C., D. A. Walker, G. Romero, W. P. Sullivan, D. O. Toft, G. L. Hager and D. B. DeFranco (2004). "Molecular chaperones function as steroid receptor nuclear mobility factors." Proc Natl Acad Sci U S A **101**(9): 2876-2881.

Eldridge, A. G., Y. Li, P. A. Sharp and B. J. Blencowe (1999). "The SRm160/300 splicing coactivator is required for exon-enhancer function." Proc Natl Acad Sci U S A **96**(11): 6125-6130.

Enrich, C., C. Rentero, S. V. de Muga, M. Reverter, V. Mulay, P. Wood, M. Koese and T. Grewal (2011). "Annexin A6-Linking Ca(2+) signaling with cholesterol transport." Biochim Biophys Acta **1813**(5): 935-947.

Evans, R. M. (1988). "The steroid and thyroid hormone receptor superfamily." Science **240**(4854): 889-895.

Faivre, E. J., A. R. Daniel, C. J. Hillard and C. A. Lange (2008). "Progesterone receptor rapid signaling mediates serine 345 phosphorylation and tethering to specificity protein 1 transcription factors." Mol Endocrinol **22**(4): 823-837.

Fan, Z., P. J. Beresford, D. Zhang and J. Lieberman (2002). "HMG2 interacts with the nucleosome assembly protein SET and is a target of the cytotoxic T-lymphocyte protease granzyme A." Mol Cell Biol **22**(8): 2810-2820.

Fasulo, B., R. Deuring, M. Murawska, M. Gause, K. M. Dorighi, C. A. Schaaf, D. Dorsett, A. Brehm and J. W. Tamkun (2012). "The Drosophila MI-2 chromatin-remodeling factor regulates higher-order chromatin structure and cohesin dynamics in vivo." PLoS Genet **8**(8): e1002878.

Felsenfeld, G. and M. Groudine (2003). "Controlling the double helix." Nature **421**(6921): 448-453.

Feng, Q., R. Cao, L. Xia, H. Erdjument-Bromage, P. Tempst and Y. Zhang (2002). "Identification and functional characterization of the p66/p68 components of the MeCP1 complex." Mol Cell Biol **22**(2): 536-546.

Feng, Q. and Y. Zhang (2001). "The MeCP1 complex represses transcription through preferential binding, remodeling, and deacetylating methylated nucleosomes." Genes Dev **15**(7): 827-832.

Fernandes, I., Y. Bastien, T. Wai, K. Nygard, R. Lin, O. Cormier, H. S. Lee, F. Eng, N. R. Bertos, N. Pelletier, S. Mader, V. K. Han, X. J. Yang and J. H. White (2003). "Ligand-dependent nuclear receptor corepressor LCoR functions by histone deacetylase-dependent and -independent mechanisms." Mol Cell **11**(1): 139-150.

Fischle, W., B. S. Tseng, H. L. Dormann, B. M. Ueberheide, B. A. Garcia, J. Shabanowitz, D. F. Hunt, H. Funabiki and C. D. Allis (2005). "Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation." Nature **438**(7071): 1116-1122.

Fisher, R. A. (1925). "Statistical Methods for Research Workers." Oliver and Boyd (Edinburgh).

Fondell, J. D., H. Ge and R. G. Roeder (1996). "Ligand induction of a transcriptionally active thyroid hormone receptor coactivator complex." Proc Natl Acad Sci U S A **93**(16): 8329-8333.

Freedman, L. P., B. F. Luisi, Z. R. Korszun, R. Basavappa, P. B. Sigler and K. R. Yamamoto (1988). "The function and structure of the metal coordination sites within the glucocorticoid receptor DNA binding domain." Nature **334**(6182): 543-546.

Freeman, B. C. and K. R. Yamamoto (2002). "Disassembly of transcriptional regulatory complexes by molecular chaperones." Science **296**(5576): 2232-2235.

Fritz, J., A. Strehblow, A. Taschner, S. Schopoff, P. Pasierbek and M. F. Jantsch (2009). "RNA-regulated interaction of transportin-1 and exportin-5 with the double-stranded RNA-binding domain regulates nucleocytoplasmic shuttling of ADAR1." Mol Cell Biol **29**(6): 1487-1497.

Fudenberg, G., M. Imakaev, C. Lu, A. Goloborodko, N. Abdennur and L. A. Mirny (2016). "Formation of Chromosomal Domains by Loop Extrusion." Cell Rep **15**(9): 2038-2049.

Fujiki, R., W. Hashiba, H. Sekine, A. Yokoyama, T. Chikanishi, S. Ito, Y. Imai, J. Kim, H. H. He, K. Igarashi, J. Kanno, F. Ohtake, H. Kitagawa, R. G. Roeder, M. Brown and S. Kato (2011). "GlcNAcylation of histone H2B facilitates its monoubiquitination." Nature **480**(7378): 557-560.

Furukawa, K. (1999). "LAP2 binding protein 1 (L2BP1/BAF) is a candidate mediator of LAP2-chromatin interaction." J Cell Sci **112 (Pt 15)**: 2485-2492.

Gaume, X., K. Monier, F. Argoul, F. Mongelard and P. Bouvet (2011). "In vivo Study of the Histone Chaperone Activity of Nucleolin by FRAP." Biochem Res Int **2011**: 187624.

Giangrande, P. H., E. A. Kimbrel, D. P. Edwards and D. P. McDonnell (2000). "The opposing transcriptional activities of the two isoforms of the human progesterone receptor are due to differential cofactor binding." Mol Cell Biol **20**(9): 3102-3115.

Gill, G. (2004). "SUMO and ubiquitin in the nucleus: different functions, similar mechanisms?" Genes Dev **18**(17): 2046-2059.

Ginisty, H., F. Amalric and P. Bouvet (1998). "Nucleolin functions in the first step of ribosomal RNA processing." EMBO J **17**(5): 1476-1486.

Gnatovskiy, L., P. Mita and D. E. Levy (2013). "The human RVB complex is required for efficient transcription of type I interferon-stimulated genes." Mol Cell Biol **33**(19): 3817-3825.

Gonzalez-Sandoval, A. and S. M. Gasser (2016). "On TADs and LADs: Spatial Control Over Gene Expression." Trends Genet **32**(8): 485-495.

Gorla-Bajszczak, A., C. Juge-Aubry, A. Pernin, A. G. Burger and C. A. Meier (1999). "Conserved amino acids in the ligand-binding and tau(i) domains of the peroxisome proliferator-activated receptor alpha are necessary for heterodimerization with RXR." Mol Cell Endocrinol **147**(1-2): 37-47.

Gregory, R. I., K. P. Yan, G. Amuthan, T. Chendrimada, B. Doratotaj, N. Cooch and R. Shiekhattar (2004). "The Microprocessor complex mediates the genesis of microRNAs." Nature **432**(7014): 235-240.

Gromak, N., M. Dienstbier, S. Macias, M. Plass, E. Eyras, J. F. Caceres and N. J. Proudfoot (2013). "Drosha regulates gene expression independently of RNA cleavage function." Cell Rep **5**(6): 1499-1510.

Guo, Z., L. Qian, R. Liu, H. Dai, M. Zhou, L. Zheng and B. Shen (2008). "Nucleolar localization and dynamic roles of flap endonuclease 1 in ribosomal DNA replication and damage repair." Mol Cell Biol **28**(13): 4310-4319.

Gururaj, A. E., R. R. Singh, S. K. Rayala, C. Holm, P. den Hollander, H. Zhang, S. Balasenthil, A. H. Talukder, G. Landberg and R. Kumar (2006). "MTA1, a transcriptional activator of breast cancer amplified sequence 3." Proc Natl Acad Sci U S A **103**(17): 6670-6675.

Hagan, C. R. and C. A. Lange (2014). "Molecular determinants of context-dependent progesterone receptor action in breast cancer." BMC Med **12**: 32.

Hagan, C. R., T. M. Regan, G. E. Dressing and C. A. Lange (2011). "ck2-dependent phosphorylation of progesterone receptors (PR) on Ser81 regulates PR-B isoform-specific target gene expression in breast cancer cells." Mol Cell Biol **31**(12): 2439-2452.

Hakimi, M. A., D. A. Bochar, J. A. Schmiesing, Y. Dong, O. G. Barak, D. W. Speicher, K. Yokomori and R. Shiekhattar (2002). "A chromatin remodelling complex that loads cohesin onto human chromosomes." Nature **418**(6901): 994-998.

Hakimi, M. A., Y. Dong, W. S. Lane, D. W. Speicher and R. Shiekhattar (2003). "A candidate X-linked mental retardation gene is a component of a new family of histone deacetylase-containing complexes." J Biol Chem **278**(9): 7234-7239.

Han, H. J., J. Russo, Y. Kohwi and T. Kohwi-Shigematsu (2008). "SATB1 reprogrammes gene expression to promote breast tumour growth and metastasis." Nature **452**(7184): 187-193.

Hanakahi, L. A., L. A. Dempsey, M. J. Li and N. Maizels (1997). "Nucleolin is one component of the B cell-specific transcription factor and switch region binding protein, LR1." Proc Natl Acad Sci U S A **94**(8): 3605-3610.

Hassig, C. A., J. K. Tong, T. C. Fleischer, T. Owa, P. G. Grable, D. E. Ayer and S. L. Schreiber (1998). "A role for histone deacetylase activity in HDAC1-mediated transcriptional repression." Proc Natl Acad Sci U S A **95**(7): 3519-3524.

Hayes, J. J., D. J. Clark and A. P. Wolffe (1991). "Histone contributions to the structure of DNA in the nucleosome." Proc Natl Acad Sci U S A **88**(15): 6829-6833.

He, Z., L. A. Henricksen, M. S. Wold and C. J. Ingles (1995). "RPA involvement in the damage-recognition and incision steps of nucleotide excision repair." Nature **374**(6522): 566-569.

Heberle, H., G. V. Meirelles, F. R. da Silva, G. P. Telles and R. Minghim (2015). "InteractiVenn: a web-based tool for the analysis of sets through Venn diagrams." BMC Bioinformatics **16**: 169.

Helsen, C. and F. Claessens (2014). "Looking at nuclear receptors from a new angle." Mol Cell Endocrinol **382**(1): 97-106.

Hernandez, J., E. Bechara, D. Schlesinger, J. Delgado, L. Serrano and J. Valcarcel (2016). "Tumor suppressor properties of the splicing regulatory factor RBM10." RNA Biol **13**(4): 466-472.

Heyd, F. and K. W. Lynch (2010). "Phosphorylation-dependent regulation of PSF by GSK3 controls CD45 alternative splicing." Mol Cell **40**(1): 126-137.

Hilgers, V. (2015). "Alternative polyadenylation coupled to transcription initiation: Insights from ELAV-mediated 3' UTR extension." RNA Biol **12**(9): 918-921.

Holliday, D. L. and V. Speirs (2011). "Choosing the right cell line for breast cancer research." Breast Cancer Res **13**(4): 215.

Hong, W., R. J. Resnick, C. Rakowski, D. Shalloway, S. J. Taylor and G. A. Blobel (2002). "Physical and functional interaction between the transcriptional cofactor CBP and the KH domain protein Sam68." Mol Cancer Res **1**(1): 48-55.

Hori, T., F. Osaka, T. Chiba, C. Miyamoto, K. Okabayashi, N. Shimbara, S. Kato and K. Tanaka (1999). "Covalent modification of all members of human cullin family proteins by NEDD8." Oncogene **18**(48): 6829-6834.

Horlein, A. J., A. M. Naar, T. Heinzel, J. Torchia, B. Gloss, R. Kurokawa, A. Ryan, Y. Kamei, M. Soderstrom, C. K. Glass and et al. (1995). "Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor." Nature **377**(6548): 397-404.

Hsiao, P. W., D. L. Lin, R. Nakao and C. Chang (1999). "The linkage of Kennedy's neuron disease to ARA24, the first identified androgen receptor polyglutamine region-associated coactivator." J Biol Chem **274**(29): 20229-20234.

Hu, S. B., J. F. Xiang, X. Li, Y. Xu, W. Xue, M. Huang, C. C. Wong, C. A. Sagum, M. T. Bedford, L. Yang, D. Cheng and L. L. Chen (2015). "Protein arginine methyltransferase CARM1 attenuates the paraspeckle-mediated nuclear retention of mRNAs containing IRAlus." Genes Dev **29**(6): 630-645.

Hua, G., K. P. Ganti and P. Chambon (2016). "Glucocorticoid-induced tethered transrepression requires SUMOylation of GR and formation of a SUMO-SMRT/NCoR1-HDAC3 repressing complex." Proc Natl Acad Sci U S A **113**(5): E635-643.

Huang, J. and W. S. Dynan (2002). "Reconstitution of the mammalian DNA double-strand break end-joining reaction reveals a requirement for an Mre11/Rad50/NBS1-containing fraction." Nucleic Acids Res **30**(3): 667-674.

Huang, L., H. Fu, C. M. Lin, A. L. Conner, Y. Zhang and M. I. Aladjem (2011). "Prevention of transcriptional silencing by a replicator-binding complex consisting of SWI/SNF, MeCP1, and hnRNP C1/C2." Mol Cell Biol **31**(16): 3472-3484.

Humphrey, G. W., Y. Wang, V. R. Russanova, T. Hirai, J. Qin, Y. Nakatani and B. H. Howard (2001). "Stable histone deacetylase complexes distinguished by the presence of SANT domain proteins CoREST/kiaa0071 and Mta-L1." J Biol Chem **276**(9): 6817-6824.

Hurtado, A., K. A. Holmes, C. S. Ross-Innes, D. Schmidt and J. S. Carroll (2011). "FOXA1 is a key determinant of estrogen receptor function and endocrine response." Nat Genet **43**(1): 27-33.

Huttlin, E. L., L. Ting, R. J. Bruckner, F. Gebreab, M. P. Gygi, J. Szpyt, S. Tam, G. Zarraga, G. Colby, K. Baltier, R. Dong, V. Guarani, L. P. Vaites, A. Ordureau, R. Rad, B. K. Erickson, M. Wuhr, J. Chick, B. Zhai, D. Kolippakkam, J. Mintseris, R. A. Obar, T. Harris, S. Artavanis-Tsakonas, M. E. Sowa, P. De Camilli, J. A. Paulo, J. W. Harper and S. P. Gygi (2015). "The BioPlex Network: A Systematic Exploration of the Human Interactome." Cell **162**(2): 425-440.

Iannone, C., A. Pohl, P. Papasaikas, D. Soronellas, G. P. Vicent, M. Beato and J. ValcaRcel (2015). "Relationship between nucleosome positioning and progesterone-induced alternative splicing in breast cancer cells." RNA **21**(3): 360-374.

Inoue, A., N. Yamamoto, M. Kimura, K. Nishio, H. Yamane and K. Nakajima (2014). "RBM10 regulates alternative splicing." FEBS Lett **588**(6): 942-947.

Ishimi, Y., S. Ichinose, A. Omori, K. Sato and H. Kimura (1996). "Binding of human minichromosome maintenance proteins with histone H3." J Biol Chem **271**(39): 24115-24122.

Iwasaki, T., W. W. Chin and L. Ko (2001). "Identification and characterization of RRM-containing coactivator activator (CoAA) as TRBP-interacting protein, and its splice variant as a coactivator modulator (CoAM)." J Biol Chem **276**(36): 33375-33383.

Iyengar, S. and P. J. Farnham (2011). "KAP1 protein: an enigmatic master regulator of the genome." J Biol Chem **286**(30): 26267-26276.

Izquierdo, J. M. (2008). "Hu antigen R (HuR) functions as an alternative pre-mRNA splicing regulator of Fas apoptosis-promoting receptor on exon definition." J Biol Chem **283**(27): 19077-19084.

Jacob, A. G., R. K. Singh, F. Mohammad, T. W. Bebee and D. S. Chandler (2014). "The splicing factor FUBP1 is required for the efficient splicing of oncogene MDM2 pre-mRNA." J Biol Chem **289**(25): 17350-17364.

Jacobsen, B. M. and K. B. Horwitz (2012). "Progesterone receptors, their isoforms and progesterone regulated transcription." Mol Cell Endocrinol **357**(1-2): 18-29.

Jamin, A. and M. S. Wiebe (2015). "Barrier to Autointegration Factor (BANF1): interwoven roles in nuclear structure, genome integrity, innate immunity, stress responses and progeria." Curr Opin Cell Biol **34**: 61-68.

Jang, M. K., K. Mochizuki, M. Zhou, H. S. Jeong, J. N. Brady and K. Ozato (2005). "The bromodomain protein Brd4 is a positive regulatory component of P-TEFb and stimulates RNA polymerase II-dependent transcription." Mol Cell **19**(4): 523-534.

Jangal, M., J. P. Couture, S. Bianco, L. Magnani, H. Mohammed and N. Gevry (2014). "The transcriptional co-repressor TLE3 suppresses basal signaling on a subset of estrogen receptor alpha target genes." Nucleic Acids Res **42**(18): 11339-11348.

Jenuwein, T. (2006). "The epigenetic magic of histone lysine methylation." FEBS J **273**(14): 3121-3135.

Jeyabalan, J., M. A. Nesbit, J. Galvanovskis, R. Callaghan, P. Rorsman and R. V. Thakker (2010). "SEDLIN forms homodimers: characterisation of SEDLIN mutations and their interactions with transcription factors MBP1, PITX1 and SF1." PLoS One **5**(5): e10646.

Jindal, H. K. and J. K. Vishwanatha (1990). "Functional identity of a primer recognition protein as phosphoglycerate kinase." J Biol Chem **265**(12): 6540-6543.

Jobert, L., M. Argentini and L. Tora (2009). "PRMT1 mediated methylation of TAF15 is required for its positive gene regulatory function." Exp Cell Res **315**(7): 1273-1286.

Johnson, J. L., T. G. Beito, C. J. Krco and D. O. Toft (1994). "Characterization of a novel 23-kilodalton protein of unactive progesterone receptor complexes." Mol Cell Biol **14**(3): 1956-1963.

Jozwik, K. M. and J. S. Carroll (2012). "Pioneer factors in hormone-dependent cancers." Nat Rev Cancer **12**(6): 381-385.

Ju, B. G., D. Solum, E. J. Song, K. J. Lee, D. W. Rose, C. K. Glass and M. G. Rosenfeld (2004). "Activating the PARP-1 sensor component of the groucho/ TLE1 corepressor complex mediates a CaMKinase IIdelta-dependent neurogenic gene activation pathway." Cell **119**(6): 815-829.

Jurica, M. S. and M. J. Moore (2003). "Pre-mRNA splicing: awash in a sea of proteins." Mol Cell **12**(1): 5-14.

Kahyo, T., T. Nishida and H. Yasuda (2001). "Involvement of PIAS1 in the sumoylation of tumor suppressor p53." Mol Cell **8**(3): 713-718.

Kaiser, F. J., H. J. Ludecke and S. Weger (2007). "SUMOylation modulates transcriptional repression by TRPS1." Biol Chem **388**(4): 381-390.

Kalff, M., B. Gross and M. Beato (1990). "Progesterone receptor stimulates transcription of mouse mammary tumour virus in a cell-free system." Nature **344**(6264): 360-362.

Kalousi, A., A. S. Hoffbeck, P. N. Selemenakis, J. Pinder, K. I. Savage, K. K. Khanna, L. Brino, G. Dellaire, V. G. Gorgoulis and E. Soutoglou (2015). "The nuclear oncogene SET controls DNA repair by KAP1 and HP1 retention to chromatin." Cell Rep **11**(1): 149-163.

Kaneko, S., O. Rozenblatt-Rosen, M. Meyerson and J. L. Manley (2007). "The multifunctional protein p54nrb/PSF recruits the exonuclease XRN2 to facilitate pre-mRNA 3' processing and transcription termination." Genes Dev **21**(14): 1779-1789.

Kang, H. J., M. H. Lee, H. L. Kang, S. H. Kim, J. R. Ahn, H. Na, T. Y. Na, Y. N. Kim, J. K. Seong and M. O. Lee (2014). "Differential regulation of estrogen receptor alpha expression in breast cancer cells by metastasis-associated protein 1." Cancer Res **74**(5): 1484-1494.

Kang, X., W. Chen, R. H. Kim, M. K. Kang and N. H. Park (2009). "Regulation of the hTERT promoter activity by MSH2, the hnRNPs K and D, and GRHL2 in human oral squamous cell carcinoma cells." Oncogene **28**(4): 565-574.

Kar, A., K. Fushimi, X. Zhou, P. Ray, C. Shi, X. Chen, Z. Liu, S. Chen and J. Y. Wu (2011). "RNA helicase p68 (DDX5) regulates tau exon 10 splicing by modulating a stem-loop structure at the 5' splice site." Mol Cell Biol **31**(9): 1812-1821.

Kasembeli, M., W. C. Lau, S. H. Roh, T. K. Eckols, J. Frydman, W. Chiu and D. J. Tweardy (2014). "Modulation of STAT3 folding and function by TRiC/CCT chaperonin." PLoS Biol **12**(4): e1001844.

Kastner, P., A. Krust, B. Turcotte, U. Stropp, L. Tora, H. Gronemeyer and P. Chambon (1990). "Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B." EMBO J **9**(5): 1603-1614.

Kerrien, S., B. Aranda, L. Breuza, A. Bridge, F. Broackes-Carter, C. Chen, M. Duesbury, M. Dumousseau, M. Feuermann, U. Hinz, C. Jandrasits, R. C. Jimenez, J. Khadake, U. Mahadevan, P. Masson, I. Pedruzzi, E. Pfeiffenberger, P. Porras, A. Raghunath, B. Roechert, S. Orchard and H. Hermjakob (2012). "The IntAct molecular interaction database in 2012." Nucleic Acids Res **40**(Database issue): D841-846.

Kester, H. A., B. M. van der Leede, P. T. van der Saag and B. van der Burg (1997). "Novel progesterone target genes identified by an improved differential display technique suggest that progestin-induced growth inhibition of breast cancer cells coincides with enhancement of differentiation." J Biol Chem **272**(26): 16637-16643.

Keydar, I., L. Chen, S. Karby, F. R. Weiss, J. Delarea, M. Radu, S. Chaitcik and H. J. Brenner (1979). "Establishment and Characterization of a Cell Line of Human Breast Carcinoma Origin." Eur J Cancer **15**(5): 659-670.

Khorasanizadeh, S. (2004). "The nucleosome: from genomic organization to genomic regulation." Cell **116**(2): 259-272.

Khorasanizadeh, S. and F. Rastinejad (2001). "Nuclear-receptor interactions on DNA-response elements." Trends Biochem Sci **26**(6): 384-390.

Khurana, S., S. Chakraborty, M. Lam, Y. Liu, Y. T. Su, X. Zhao, M. A. Saleem, P. W. Mathieson, L. A. Bruggeman and H. Y. Kao (2012). "Familial focal segmental glomerulosclerosis (FSGS)-linked alpha-actinin 4 (ACTN4) protein mutants lose ability to activate transcription by nuclear hormone receptors." J Biol Chem **287**(15): 12027-12035.

Kilanczyk, E., K. Gwozdzinski, E. Wilczek and A. Filipek (2014). "Up-regulation of CacyBP/SIP during rat breast cancer development." Breast Cancer **21**(3): 350-357.

Kim, J., M. Guermah and R. G. Roeder (2010). "The human PAF1 complex acts in chromatin transcription elongation both independently and cooperatively with SII/TFIIS." Cell **140**(4): 491-503.

Kim, J. H., A. Sharma, S. S. Dhar, S. H. Lee, B. Gu, C. H. Chan, H. K. Lin and M. G. Lee (2014). "UTX and MLL4 coordinately regulate transcriptional programs for cell proliferation and invasiveness in breast cancer cells." Cancer Res **74**(6): 1705-1717.

Kimura, H., N. Takizawa, E. Allemand, T. Hori, F. J. Iborra, N. Nozaki, M. Muraki, M. Hagiwara, A. R. Krainer, T. Fukagawa and K. Okawa (2006). "A novel histone exchange factor, protein phosphatase 2Cgamma, mediates the exchange and dephosphorylation of H2A-H2B." J Cell Biol **175**(3): 389-400.

Klijn, J. G., B. Setyono-Han and J. A. Foekens (2000). "Progesterone antagonists and progesterone receptor modulators in the treatment of breast cancer." Steroids **65**(10-11): 825-830.

Knee, K. M., O. A. Sergeeva and J. A. King (2013). "Human TRiC complex purified from HeLa cells contains all eight CCT subunits and is active in vitro." Cell Stress Chaperones **18**(2): 137-144.

Knight, J. D., G. Liu, J. P. Zhang, A. Pasculescu, H. Choi and A. C. Gingras (2015). "A web-tool for visualizing quantitative protein-protein interaction data." Proteomics **15**(8): 1432-1436.

Knott, G. J., C. S. Bond and A. H. Fox (2016). "The DBHS proteins SFPQ, NONO and PSPC1: a multipurpose molecular scaffold." Nucleic Acids Res **44**(9): 3989-4004.

Knotts, T. A., R. S. Orkiszewski, R. G. Cook, D. P. Edwards and N. L. Weigel (2001). "Identification of a phosphorylation site in the hinge region of the human progesterone receptor and additional amino-terminal phosphorylation sites." J Biol Chem **276**(11): 8475-8483.

Knutson, T. P., A. R. Daniel, D. Fan, K. A. Silverstein, K. R. Covington, S. A. Fuqua and C. A. Lange (2012). "Phosphorylated and sumoylation-deficient progesterone receptors drive proliferative gene signatures during breast cancer progression." Breast Cancer Res **14**(3): R95.

Ko, L. and W. W. Chin (2003). "Nuclear receptor coactivator thyroid hormone receptor-binding protein (TRBP) interacts with and stimulates its associated DNA-dependent protein kinase." J Biol Chem **278**(13): 11471-11479.

Ko, Y. J. and S. P. Balk (2004). "Targeting steroid hormone receptor pathways in the treatment of hormone dependent cancers." Curr Pharm Biotechnol **5**(5): 459-470.

Koike, A., H. Nishikawa, W. Wu, Y. Okada, A. R. Venkitaraman and T. Ohta (2010). "Recruitment of phosphorylated NPM1 to sites of DNA damage through RNF8-dependent ubiquitin conjugates." Cancer Res **70**(17): 6746-6756.

Koop, R., L. Di Croce and M. Beato (2003). "Histone H1 enhances synergistic activation of the MMTV promoter in chromatin." EMBO J **22**(3): 588-599.

Kotaja, N., S. Aittomaki, O. Silvennoinen, J. J. Palvimo and O. A. Janne (2000). "ARIP3 (androgen receptor-interacting protein 3) and other PIAS (protein inhibitor of activated STAT) proteins differ in their ability to modulate steroid receptor-dependent transcriptional activation." Mol Endocrinol **14**(12): 1986-2000.

Kouzarides, T. (2007). "Chromatin modifications and their function." Cell **128**(4): 693-705.

Kraus, W. L. (2015). "PARPs and ADP-Ribosylation: 50 Years ... and Counting." Mol Cell **58**(6): 902-910.

Kretzschmar, M., K. Kaiser, F. Lottspeich and M. Meisterernst (1994). "A novel mediator of class II gene transcription with homology to viral immediate-early transcriptional regulators." Cell **78**(3): 525-534.

Krietsch, J., M. C. Caron, J. P. Gagne, C. Ethier, J. Vignard, M. Vincent, M. Rouleau, M. J. Hendzel, G. G. Poirier and J. Y. Masson (2012). "PARP activation regulates the RNA-binding protein NONO in the DNA damage response to DNA double-strand breaks." Nucleic Acids Res **40**(20): 10287-10301.

Kumar, L. and E. F. M (2007). "Mfuzz: a software package for soft clustering of microarray data." Bioinformation **2**(1): 5-7.

Kumar, R. and E. B. Thompson (2005). "Gene regulation by the glucocorticoid receptor: structure:function relationship." J Steroid Biochem Mol Biol **94**(5): 383-394.

Kumar, R. and R. A. Wang (2016). "Structure, expression and functions of MTA genes." Gene **582**(2): 112-121.

Kumar, V., J. E. Carlson, K. A. Ohgi, T. A. Edwards, D. W. Rose, C. R. Escalante, M. G. Rosenfeld and A. K. Aggarwal (2002). "Transcription corepressor CtBP is an NAD(+)-regulated dehydrogenase." Mol Cell **10**(4): 857-869.

Kumar, V. and P. Chambon (1988). "The estrogen receptor binds tightly to its responsive element as a ligand-induced homodimer." Cell **55**(1): 145-156.

Kuzmichev, A. and D. Reinberg (2001). "Role of histone deacetylase complexes in the regulation of chromatin metabolism." Curr Top Microbiol Immunol **254**: 35-58.

Lange, C. A. (2004). "Making sense of cross-talk between steroid hormone receptors and intracellular signaling pathways: who will have the last word?" Mol Endocrinol **18**(2): 269-278.

Lange, C. A., T. Shen and K. B. Horwitz (2000). "Phosphorylation of human progesterone receptors at serine-294 by mitogen-activated protein kinase signals their degradation by the 26S proteasome." Proc Natl Acad Sci U S A **97**(3): 1032-1037.

Lange, C. A., T. Shen and K. B. Horwitz (2000). "Phosphorylation of human progesterone receptors at serine-294 by mitogen-activated protein kinase signals their degradation by the 26S proteasome." Proc Natl Acad Sci U S A **97**(3): 1032-1037.

Le Dily, F., D. Bau, A. Pohl, G. P. Vicent, F. Serra, D. Soronellas, G. Castellano, R. H. Wright, C. Ballare, G. Filion, M. A. Marti-Renom and M. Beato (2014). "Distinct structural transitions of chromatin topological domains correlate with coordinated hormone-induced gene regulation." Genes Dev **28**(19): 2151-2162.

Le Romancer, M., I. Treilleux, N. Leconte, Y. Robin-Lespinasse, S. Sentis, K. Bouchekioua-Bouzaghou, S. Goddard, S. Gobert-Gosse and L. Corbo (2008). "Regulation of estrogen rapid signaling through arginine methylation by PRMT1." Mol Cell **31**(2): 212-221.

Lee, J. H. and D. G. Skalnik (2008). "Wdr82 is a C-terminal domain-binding protein that recruits the Setd1A Histone H3-Lys4 methyltransferase complex to transcription start sites of transcribed human genes." Mol Cell Biol **28**(2): 609-618.

Lee, J. H., J. You, E. Dobrota and D. G. Skalnik (2010). "Identification and characterization of a novel human PP1 phosphatase complex." J Biol Chem **285**(32): 24466-24476.

Lee, M. G., R. Villa, P. Trojer, J. Norman, K. P. Yan, D. Reinberg, L. Di Croce and R. Shiekhattar (2007). "Demethylation of H3K27 regulates polycomb recruitment and H2A ubiquitination." Science **318**(5849): 447-450.

Lee, M. G., C. Wynder, N. Cooch and R. Shiekhattar (2005). "An essential role for CoREST in nucleosomal histone 3 lysine 4 demethylation." Nature **437**(7057): 432-435.

Lee, W. S., J. A. Harder, M. Yoshizumi, M. E. Lee and E. Haber (1997). "Progesterone inhibits arterial smooth muscle cell proliferation." Nat Med **3**(9): 1005-1008.

Lei, M. (2005). "The MCM complex: its role in DNA replication and implications for cancer therapy." Curr Cancer Drug Targets **5**(5): 365-380.

Lemon, B., C. Inouye, D. S. King and R. Tjian (2001). "Selectivity of chromatin-remodelling cofactors for ligand-activated transcription." Nature **414**(6866): 924-928.

Leonhardt, S. A., V. Boonyaratanakornkit and D. P. Edwards (2003). "Progesterone receptor transcription and non-transcription signaling mechanisms." Steroids **68**(10-13): 761-770.

Leonhardt, S. A. and A. Edwards (2002). "Mechanism of action of progesterone

antagonists." Exp. Biol. Med. (Maywood) **227**: 969-980.

Li, D. Q., S. B. Pakala, S. D. Reddy, K. Ohshiro, S. H. Peng, Y. Lian, S. W. Fu and R. Kumar (2010). "Revelation of p53-independent function of MTA1 in DNA damage response via modulation of the p21 WAF1-proliferating cell nuclear antigen pathway." J Biol Chem **285**(13): 10044-10052.

Li, M., A. Makkinje and Z. Damuni (1996). "The myeloid leukemia-associated protein SET is a potent inhibitor of protein phosphatase 2A." J Biol Chem **271**(19): 11059-11062.

Li, X. R., M. Liu, Y. J. Zhang, J. D. Wang, Y. Q. Zheng, J. Li, B. Ma and X. Song (2011). "ER, PgR, HER-2, Ki-67, topoisomerase IIalpha, and nm23-H1 proteins expression as predictors of pathological complete response to neoadjuvant chemotherapy for locally advanced breast cancer." Med Oncol **28 Suppl 1**: S48-54.

Liger, D., L. Mora, N. Lazar, S. Figaro, J. Henri, N. Scrima, R. H. Buckingham, H. van Tilbeurgh, V. Heurgue-Hamard and M. Graille (2011). "Mechanism of activation of

methyltransferases involved in translation by the Trm112 'hub' protein." Nucleic Acids Res **39**(14): 6249-6259.

Lin, Y. L., M. K. Shivji, C. Chen, R. Kolodner, R. D. Wood and A. Dutta (1998). "The evolutionarily conserved zinc finger motif in the largest subunit of human replication protein A is required for DNA replication and mismatch repair but not for nucleotide excision repair." J Biol Chem **273**(3): 1453-1461.

Listerman, I., A. K. Sapra and K. M. Neugebauer (2006). "Cotranscriptional coupling of splicing factor recruitment and precursor messenger RNA splicing in mammalian cells." Nat Struct Mol Biol **13**(9): 815-822.

Liu, L., N. Xie, P. Rennie, J. R. Challis, M. Gleave, S. J. Lye and X. Dong (2011). "Consensus PP1 binding motifs regulate transcriptional corepression and alternative RNA splicing activities of the steroid receptor coregulators, p54nrb and PSF." Mol Endocrinol **25**(7): 1197-1210.

Liu, X., C. Y. Lin, M. Lei, S. Yan, T. Zhou and R. L. Erikson (2005). "CCT chaperonin complex is required for the biogenesis of functional Plk1." Mol Cell Biol **25**(12): 4993-5010.

Liu, X., L. Wang, K. Zhao, P. R. Thompson, Y. Hwang, R. Marmorstein and P. A. Cole (2008). "The structural basis of protein acetylation by the p300/CBP transcriptional coactivator." Nature **451**(7180): 846-850.

Liu, Z., N. Lam and C. J. Thiele (2015). "Zinc finger transcription factor CASZ1 interacts with histones, DNA repair proteins and recruits NuRD complex to regulate gene transcription." Oncotarget **6**(29): 27628-27640.

Liu, Z., J. Wong, S. Y. Tsai, M. J. Tsai and B. W. O'Malley (1999). "Steroid receptor coactivator-1 (SRC-1) enhances ligand-dependent and receptor-dependent cell-free transcription of chromatin." Proc Natl Acad Sci U S A **96**(17): 9485-9490.

Long, J., G. Wang, I. Matsuura, D. He and F. Liu (2004). "Activation of Smad transcriptional activity by protein inhibitor of activated STAT3 (PIAS3)." Proc Natl Acad Sci U S A **101**(1): 99-104.

Loy, C. J., K. S. Sim and E. L. Yong (2003). "Filamin-A fragment localizes to the nucleus to regulate androgen receptor and coactivator functions." Proc Natl Acad Sci U S A **100**(8): 4562-4567.

Luco, R. F., M. Allo, I. E. Schor, A. R. Kornblihtt and T. Misteli (2011). "Epigenetics in alternative pre-mRNA splicing." Cell **144**(1): 16-26.

Luger, K., A. W. Mader, R. K. Richmond, D. F. Sargent and T. J. Richmond (1997). "Crystal structure of the nucleosome core particle at 2.8 A resolution." Nature **389**(6648): 251-260.

Lukong, K. E., D. Larocque, A. L. Tyner and S. Richard (2005). "Tyrosine phosphorylation of sam68 by breast tumor kinase regulates intranuclear localization and cell cycle progression." J Biol Chem **280**(46): 38639-38647.

Macias, S., R. A. Cordiner, P. Gautier, M. Plass and J. F. Caceres (2015). "DGCR8 Acts as an Adaptor for the Exosome Complex to Degrade Double-Stranded Structured RNAs." Mol Cell **60**(6): 873-885.

Magni, M., V. Ruscica, G. Buscemi, J. E. Kim, B. T. Nachimuthu, E. Fontanella, D. Delia and L. Zannini (2014). "Chk2 and REGgamma-dependent DBC1 regulation in DNA damage induced apoptosis." Nucleic Acids Res **42**(21): 13150-13160.

Mahajan, M. C., G. J. Narlikar, G. Boyapaty, R. E. Kingston and S. M. Weissman (2005). "Heterogeneous nuclear ribonucleoprotein C1/C2, MeCP1, and SWI/SNF form a chromatin remodeling complex at the beta-globin locus control region." Proc Natl Acad Sci U S A **102**(42): 15012-15017.

Mahajan, S. S. and A. C. Wilson (2000). "Mutations in host cell factor 1 separate its role in cell proliferation from recruitment of VP16 and LZIP." Mol Cell Biol **20**(3): 919-928.

Makarov, A. (2000). "Electrostatic axially harmonic orbital trapping: a high-performance technique of mass analysis." Anal Chem **72**(6): 1156-1162.

Man, J. H., H. Y. Li, P. J. Zhang, T. Zhou, K. He, X. Pan, B. Liang, A. L. Li, J. Zhao, W. L. Gong, B. F. Jin, Q. Xia, M. Yu, B. F. Shen and X. M. Zhang (2006). "PIAS3 induction of PRB sumoylation represses PRB transactivation by destabilizing its retention in the nucleus." Nucleic Acids Res **34**(19): 5552-5566.

Mangelsdorf, D. J., C. Thummel, M. Beato, P. Herrlich, G. Schutz, K. Umesono, B. Blumberg, P. Kastner, M. Mark, P. Chambon and R. M. Evans (1995). "The nuclear receptor superfamily: the second decade." Cell **83**(6): 835-839.

Marechal, A., J. M. Li, X. Y. Ji, C. S. Wu, S. A. Yazinski, H. D. Nguyen, S. Liu, A. E. Jimenez, J. Jin and L. Zou (2014). "PRP19 transforms into a sensor of RPA-ssDNA after DNA damage and drives ATR activation via a ubiquitin-mediated circuitry." Mol Cell **53**(2): 235-246.

Marfil, V., M. Moya, C. E. Pierreux, J. V. Castell, F. P. Lemaigre, F. X. Real and R. Bort (2010). "Interaction between Hhex and SOX13 modulates Wnt/TCF activity." J Biol Chem **285**(8): 5726-5737.

Marino, N., J. C. Marshall, J. W. Collins, M. Zhou, Y. Qian, T. Veenstra and P. S. Steeg (2013). "Nm23-h1 binds to gelsolin and inactivates its actin-severing capacity to promote tumor cell motility and metastasis." Cancer Res **73**(19): 5949-5962.

Martinez-Rucobo, F. W., R. Kohler, M. van de Waterbeemd, A. J. Heck, M. Hemann, F. Herzog, H. Stark and P. Cramer (2015). "Molecular Basis of Transcription-Coupled Pre-mRNA Capping." Mol Cell **58**(6): 1079-1089.

Maruvada, P., C. T. Baumann, G. L. Hager and P. M. Yen (2003). "Dynamic shuttling and intranuclear mobility of nuclear hormone receptors." J Biol Chem **278**(14): 12425-12432.

Matsuda, K., M. Nishi, H. Takaya, N. Kaku and M. Kawata (2008). "Intranuclear mobility of estrogen receptor alpha and progesterone receptors in association with nuclear matrix dynamics." J Cell Biochem **103**(1): 136-148.

Matveeva, E., J. Maiorano, Q. Zhang, A. M. Eteleeb, P. Convertini, J. Chen, V. Infantino, S. Stamm, J. Wang, E. C. Rouchka and Y. N. Fondufe-Mittendorf (2016). "Involvement of PARP1 in the regulation of alternative splicing." Cell Discov **2**: 15046.

Mazumdar, A., R. A. Wang, S. K. Mishra, L. Adam, R. Bagheri-Yarmand, M. Mandal, R. K. Vadlamudi and R. Kumar (2001). "Transcriptional repression of oestrogen receptor by metastasis-associated protein 1 corepressor." Nat Cell Biol **3**(1): 30-37.

Mendez, L. M., J. M. Polo, J. J. Yu, M. Krupski, B. B. Ding, A. Melnick and B. H. Ye (2008). "CtBP is an essential corepressor for BCL6 autoregulation." Mol Cell Biol **28**(7): 2175-2186.

Metzger, E., M. Wissmann, N. Yin, J. M. Muller, R. Schneider, A. H. Peters, T. Gunther, R. Buettner and R. Schule (2005). "LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription." Nature **437**(7057): 436-439.

Migliaccio, A., G. Castoria, M. Di Domenico, A. de Falco, A. Bilancio, M. Lombardi, M. V. Barone, D. Ametrano, M. S. Zannini, C. Abbondanza and F. Auricchio (2000). "Steroid-induced androgen receptor-oestradiol receptor beta-Src complex triggers prostate cancer cell proliferation." EMBO J **19**(20): 5406-5417.

Migliaccio, A., D. Piccolo, G. Castoria, M. Di Domenico, A. Bilancio, M. Lombardi, W. Gong, M. Beato and F. Auricchio (1998). "Activation of the Src/p21ras/Erk pathway by progesterone receptor via cross-talk with estrogen receptor." EMBO J **17**(7): 2008-2018.

Min, H., C. W. Turck, J. M. Nikolic and D. L. Black (1997). "A new regulatory protein, KSRP, mediates exon inclusion through an intronic splicing enhancer." Genes Dev **11**(8): 1023-1036.

Mizuguchi, G., X. Shen, J. Landry, W. H. Wu, S. Sen and C. Wu (2004). "ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex." Science **303**(5656): 343-348.

Mizuta, S., T. Minami, H. Fujita, C. Kaminaga, K. Matsui, R. Ishino, A. Fujita, K. Oda, A. Kawai, N. Hasegawa, N. Urahama, R. G. Roeder and M. Ito (2014). "CCAR1/CoCoA pair-mediated recruitment of the Mediator defines a novel pathway for GATA1 function." Genes Cells **19**(1): 28-51.

Mo, R., S. M. Rao and Y. J. Zhu (2006). "Identification of the MLL2 complex as a coactivator for estrogen receptor alpha." J Biol Chem **281**(23): 15714-15720.

Mohammed, H., C. D'Santos, A. A. Serandour, H. R. Ali, G. D. Brown, A. Atkins, O. M. Rueda, K. A. Holmes, V. Theodorou, J. L. Robinson, W. Zwart, A. Saadi, C. S. Ross-Innes, S. F. Chin, S. Menon, J. Stingl, C. Palmieri, C. Caldas and J. S. Carroll (2013). "Endogenous purification reveals GREB1 as a key estrogen receptor regulatory factor." Cell Rep **3**(2): 342-349.

Mohammed, H., I. A. Russell, R. Stark, O. M. Rueda, T. E. Hickey, G. A. Tarulli, A. A. Serandour, S. N. Birrell, A. Bruna, A. Saadi, S. Menon, J. Hadfield, M. Pugh, G. V. Raj, G. D. Brown, C. D'Santos, J. L. Robinson, G. Silva, R. Launchbury, C. M. Perou, J. Stingl, C. Caldas, W. D. Tilley and J. S. Carroll (2015). "Progesterone receptor modulates ERalpha action in breast cancer." Nature **523**(7560): 313-317.

Mohammed, H., C. Taylor, G. D. Brown, E. K. Papachristou, J. S. Carroll and C. S. D'Santos (2016). "Rapid immunoprecipitation mass spectrometry of endogenous proteins (RIME) for analysis of chromatin complexes." Nat Protoc **11**(2): 316-326.

Mohrmann, L. and C. P. Verrijzer (2005). "Composition and functional specificity of SWI2/SNF2 class chromatin remodeling complexes." Biochim Biophys Acta **1681**(2-3): 59-73.

Molli, P. R., R. R. Singh, S. W. Lee and R. Kumar (2008). "MTA1-mediated transcriptional repression of BRCA1 tumor suppressor gene." Oncogene **27**(14): 1971-1980.

Morris, J. H., L. Apeltsin, A. M. Newman, J. Baumbach, T. Wittkop, G. Su, G. D. Bader and T. E. Ferrin (2011). "clusterMaker: a multi-algorithm clustering plugin for Cytoscape." BMC Bioinformatics **12**: 436.

Morris, S. A., S. Baek, M. H. Sung, S. John, M. Wiench, T. A. Johnson, R. L. Schiltz and G. L. Hager (2014). "Overlapping chromatin-remodeling systems collaborate genome wide at dynamic chromatin transitions." Nat Struct Mol Biol **21**(1): 73-81.

Moudry, P., C. Lukas, L. Macurek, H. Hanzlikova, Z. Hodny, J. Lukas and J. Bartek (2012). "Ubiquitin-activating enzyme UBA1 is required for cellular response to DNA damage." Cell Cycle **11**(8): 1573-1582.

Nacht, A. S., A. Pohl, R. Zaurin, D. Soronellas, J. Quilez, P. Sharma, R. H. Wright, M. Beato and G. P. Vicent (2016). "Hormone-induced repression of genes requires BRG1-mediated H1.2 deposition at target promoters." EMBO J.

Narayanan, R., A. A. Adigun, D. P. Edwards and N. L. Weigel (2005). "Cyclin-dependent kinase activity is required for progesterone receptor function: novel role for cyclin A/Cdk2 as a progesterone receptor coactivator." Mol Cell Biol **25**(1): 264-277.

Nardulli, A. M., G. L. Greene, B. W. O'Malley and B. S. Katzenellenbogen (1988). "Regulation of progesterone receptor messenger ribonucleic acid and protein levels in MCF-7 cells by estradiol: analysis of estrogen's effect on progesterone receptor synthesis and degradation." Endocrinology **122**(3): 935-944.

Newman, M. E. and M. Girvan (2004). "Finding and evaluating community structure in networks." Phys Rev E Stat Nonlin Soft Matter Phys **69**(2 Pt 2): 026113.

Nibu, Y., H. Zhang and M. Levine (1998). "Interaction of short-range repressors with Drosophila CtBP in the embryo." Science **280**(5360): 101-104.

Nichol, J. N., M. D. Galbraith, C. L. Kleinman, J. M. Espinosa and W. H. Miller, Jr. (2016). "NPM and BRG1 Mediate Transcriptional Resistance to Retinoic Acid in Acute Promyelocytic Leukemia." Cell Rep **14**(12): 2938-2949.

Noguchi, E., N. Hayashi, Y. Azuma, T. Seki, M. Nakamura, N. Nakashima, M. Yanagida, X. He, U. Mueller, S. Sazer and T. Nishimoto (1996). "Dis3, implicated in mitotic control, binds directly to Ran and enhances the GEF activity of RCC1." EMBO J **15**(20): 5595-5605.

Nora, E. P., B. R. Lajoie, E. G. Schulz, L. Giorgetti, I. Okamoto, N. Servant, T. Piolot, N. L. van Berkum, J. Meisig, J. Sedat, J. Gribnau, E. Barillot, N. Bluthgen, J. Dekker and E. Heard (2012). "Spatial partitioning of the regulatory landscape of the X-inactivation centre." Nature **485**(7398): 381-385.

Obri, A., K. Ouararhni, C. Papin, M. L. Diebold, K. Padmanabhan, M. Marek, I. Stoll, L. Roy, P. T. Reilly, T. W. Mak, S. Dimitrov, C. Romier and A. Hamiche (2014). "ANP32E is a histone chaperone that removes H2A.Z from chromatin." Nature **505**(7485): 648-653.

Onate, S. A., S. Y. Tsai, M. J. Tsai and B. W. O'Malley (1995). "Sequence and characterization of a coactivator for the steroid hormone receptor superfamily." Science **270**(5240): 1354-1357.

Ouyang, J., Y. Shi, A. Valin, Y. Xuan and G. Gill (2009). "Direct binding of CoREST1 to SUMO-2/3 contributes to gene-specific repression by the LSD1/CoREST1/HDAC complex." Mol Cell **34**(2): 145-154.

Palijan, A., I. Fernandes, M. Verway, M. Kourelis, Y. Bastien, L. E. Tavera-Mendoza, A. Sacheli, V. Bourdeau, S. Mader and J. H. White (2009). "Ligand-dependent corepressor LCoR is an attenuator of progesterone-regulated gene expression." J Biol Chem **284**(44): 30275-30287.

Pan, M. R., H. J. Hsieh, H. Dai, W. C. Hung, K. Li, G. Peng and S. Y. Lin (2012). "Chromodomain helicase DNA-binding protein 4 (CHD4) regulates homologous recombination DNA repair, and its deficiency sensitizes cells to poly(ADP-ribose) polymerase (PARP) inhibitor treatment." J Biol Chem **287**(9): 6764-6772.

Pandit, S., W. Geissler, G. Harris and A. Sitlani (2002). "Allosteric effects of dexamethasone and RU486 on glucocorticoid receptor-DNA interactions." J Biol Chem **277**(2): 1538-1543.

Pankotai, T., C. Bonhomme, D. Chen and E. Soutoglou (2012). "DNAPKcs-dependent arrest of RNA polymerase II transcription in the presence of DNA breaks." Nat Struct Mol Biol **19**(3): 276-282.

Pardo, M. and J. S. Choudhary (2012). "Assignment of protein interactions from affinity purification/mass spectrometry data." J Proteome Res **11**(3): 1462-1474.

Park, J. H., S. W. Lee, S. W. Yang, H. M. Yoo, J. M. Park, M. W. Seong, S. H. Ka, K. H. Oh, Y. J. Jeon and C. H. Chung (2014). "Modification of DBC1 by SUMO2/3 is crucial for p53-mediated apoptosis in response to DNA damage." Nat Commun **5**: 5483.

Paronetto, M. P., M. Cappellari, R. Busa, S. Pedrotti, R. Vitali, C. Comstock, T. Hyslop, K. E. Knudsen and C. Sette (2010). "Alternative splicing of the cyclin D1 proto-oncogene is regulated by the RNA-binding protein Sam68." Cancer Res **70**(1): 229-239.

Paul, A., Y. A. Garcia, B. Zierer, C. Patwardhan, O. Gutierrez, Z. Hildenbrand, D. C. Harris, H. A. Balsiger, J. C. Sivils, J. L. Johnson, J. Buchner, A. Chadli and M. B. Cox (2014). "The cochaperone SGTA (small glutamine-rich tetratricopeptide repeat-containing protein alpha) demonstrates regulatory specificity for the androgen, glucocorticoid, and progesterone receptors." J Biol Chem **289**(22): 15297-15308.

Pavri, R., B. Zhu, G. Li, P. Trojer, S. Mandal, A. Shilatifard and D. Reinberg (2006). "Histone H2B monoubiquitination functions cooperatively with FACT to regulate elongation by RNA polymerase II." Cell **125**(4): 703-717.

Peattie, D. A., M. W. Harding, M. A. Fleming, M. T. DeCenzo, J. A. Lippke, D. J. Livingston and M. Benasutti (1992). "Expression and characterization of human FKBP52, an immunophilin that associates with the 90-kDa heat shock protein and is a component of steroid receptor complexes." Proc Natl Acad Sci U S A **89**(22): 10974-10978.

Pedram, A., M. Razandi, R. C. Sainson, J. K. Kim, C. C. Hughes and E. R. Levin (2007). "A conserved mechanism for steroid receptor translocation to the plasma membrane." J Biol Chem **282**(31): 22278-22288.

Perissi, V., A. Aggarwal, C. K. Glass, D. W. Rose and M. G. Rosenfeld (2004). "A corepressor/coactivator exchange complex required for transcriptional activation by nuclear receptors and other regulated transcription factors." Cell **116**(4): 511-526.

Pierce, N. W., J. E. Lee, X. Liu, M. J. Sweredoski, R. L. Graham, E. A. Larimore, M. Rome, N. Zheng, B. E. Clurman, S. Hess, S. O. Shan and R. J. Deshaies (2013). "Cand1 promotes assembly of new SCF complexes through dynamic exchange of F box proteins." Cell **153**(1): 206-215.

Pierson-Mullany, L. K. and C. A. Lange (2004). "Phosphorylation of progesterone receptor serine 400 mediates ligand-independent transcriptional activity in response to activation of cyclin-dependent protein kinase 2." Mol Cell Biol **24**(24): 10542-10557.

Pina, B., U. Bruggemeier and M. Beato (1990). "Nucleosome positioning modulates accessibility of regulatory proteins to the mouse mammary tumor virus promoter." Cell **60**(5): 719-731.

Poorey, K., R. Viswanathan, M. N. Carver, T. S. Karpova, S. M. Cirimotich, J. G. McNally, S. Bekiranov and D. T. Auble (2013). "Measuring chromatin interaction dynamics on the second time scale at single-copy genes." Science **342**(6156): 369-372.

Popow, J., M. Englert, S. Weitzer, A. Schleiffer, B. Mierzwa, K. Mechtler, S. Trowitzsch, C. L. Will, R. Luhrmann, D. Soll and J. Martinez (2011). "HSPC117 is the essential subunit of a human tRNA splicing ligase complex." Science **331**(6018): 760-764.

Popow, J., J. Jurkin, A. Schleiffer and J. Martinez (2014). "Analysis of orthologous groups reveals archease and DDX1 as tRNA splicing factors." Nature **511**(7507): 104-107.

Postel, E. H., S. J. Berberich, S. J. Flint and C. A. Ferrone (1993). "Human c-myc transcription factor PuF identified as nm23-H2 nucleoside diphosphate kinase, a candidate suppressor of tumor metastasis." Science **261**(5120): 478-480.

Pratt, W. B. (1993). "The role of heat shock proteins in regulating the function, folding, and trafficking of the glucocorticoid receptor." J Biol Chem **268**(29): 21455-21458.

Pratt, W. B. and D. O. Toft (1997). "Steroid receptor interactions with heat shock protein and immunophilin chaperones." Endocr Rev **18**(3): 306-360.

Proietti, C. J., W. Beguelin, M. C. Flaque, F. Cayrol, M. A. Rivas, M. Tkach, E. H. Charreau, R. Schillaci and P. V. Elizalde (2011). "Novel role of signal transducer and activator of transcription 3 as a progesterone receptor coactivator in breast cancer." Steroids **76**(4): 381-392.

Proteau, A., S. Blier, A. L. Albert, S. B. Lavoie, A. M. Traish and M. Vincent (2005). "The multifunctional nuclear protein p54nrb is multiphosphorylated in mitosis and interacts with the mitotic regulator Pin1." J Mol Biol **346**(4): 1163-1172.

Purbey, P. K., S. Singh, D. Notani, P. P. Kumar, A. S. Limaye and S. Galande (2009). "Acetylation-dependent interaction of SATB1 and CtBP1 mediates transcriptional repression by SATB1." Mol Cell Biol **29**(5): 1321-1337.

Qiu, Y., Y. Zhao, M. Becker, S. John, B. S. Parekh, S. Huang, A. Hendarwanto, E. D. Martinez, Y. Chen, H. Lu, N. L. Adkins, D. A. Stavreva, M. Wiench, P. T. Georgel, R. L. Schiltz and G. L. Hager (2006). "HDAC1 acetylation is linked to progressive modulation of steroid receptor-induced gene transcription." Mol Cell **22**(5): 669-679.

Rajbhandari, P., M. S. Ozers, N. M. Solodin, C. L. Warren and E. T. Alarid (2015). "Peptidylprolyl Isomerase Pin1 Directly Enhances the DNA Binding Functions of Estrogen Receptor alpha." J Biol Chem **290**(22): 13749-13762.

Ramsey, C. S., F. Yeung, P. B. Stoddard, D. Li, C. E. Creutz and M. W. Mayo (2008). "Copine-I represses NF-kappaB transcription by endoproteolysis of p65." Oncogene **27**(25): 3516-3526.

Rath, N., Z. Wang, M. M. Lu and E. E. Morrisey (2005). "LMCD1/Dyxin is a novel transcriptional cofactor that restricts GATA6 function by inhibiting DNA binding." Mol Cell Biol **25**(20): 8864-8873.

Razandi, M., G. Alton, A. Pedram, S. Ghonshani, P. Webb and E. R. Levin (2003). "Identification of a structural determinant necessary for the localization and function of estrogen receptor alpha at the plasma membrane." Mol Cell Biol **23**(5): 1633-1646.

Richard-Foy, H. and G. L. Hager (1987). "Sequence-specific positioning of nucleosomes over the steroid-inducible MMTV promoter." EMBO J **6**(8): 2321-2328.

Roberts, S. A., N. Strande, M. D. Burkhalter, C. Strom, J. M. Havener, P. Hasty and D. A. Ramsden (2010). "Ku is a 5'-dRP/AP lyase that excises nucleotide damage near broken ends." Nature **464**(7292): 1214-1217.

Robinson, J. L., T. E. Hickey, A. Y. Warren, S. L. Vowler, T. Carroll, A. D. Lamb, N. Papoutsoglou, D. E. Neal, W. D. Tilley and J. S. Carroll (2014). "Elevated levels of FOXA1 facilitate androgen receptor chromatin binding resulting in a CRPC-like phenotype." Oncogene **33**(50): 5666-5674.

Rocha-Viegas, L., R. Villa, A. Gutierrez, O. Iriondo, R. Shiekhattar and L. Di Croce (2014). "Role of UTX in retinoic acid receptor-mediated gene regulation in leukemia." Mol Cell Biol **34**(19): 3765-3775.

Roelofs, J., S. Park, W. Haas, G. Tian, F. E. McAllister, Y. Huo, B. H. Lee, F. Zhang, Y. Shi, S. P. Gygi and D. Finley (2009). "Chaperone-mediated pathway of proteasome regulatory particle assembly." Nature **459**(7248): 861-865.

Roepstorff, P. and J. Fohlman (1984). "Proposal for a common nomenclature for sequence ions in mass spectra of peptides." Biomed Mass Spectrom **11**(11): 601.

Rogers, P. A., F. Lederman, J. Kooy, N. H. Taylor and D. L. Healy (1996). "Endometrial vascular smooth muscle oestrogen and progesterone receptor distribution in women with and without menorrhagia." Hum Reprod **11**(9): 2003-2008.

Rossow, K. L. and R. Janknecht (2003). "Synergism between p68 RNA helicase and the transcriptional coactivators CBP and p300." Oncogene **22**(1): 151-156.

Roux, K. J., D. I. Kim, M. Raida and B. Burke (2012). "A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells." J Cell Biol **196**(6): 801-810.

Ruepp, A., B. Brauner, I. Dunger-Kaltenbach, G. Frishman, C. Montrone, M. Stransky, B. Waegele, T. Schmidt, O. N. Doudieu, V. Stumpflen and H. W. Mewes (2008). "CORUM: the comprehensive resource of mammalian protein complexes." Nucleic Acids Res **36**(Database issue): D646-650.

Saito, M. and F. Ishikawa (2002). "The mCpG-binding domain of human MBD3 does not bind to mCpG but interacts with NuRD/Mi2 components HDAC1 and MTA2." J Biol Chem **277**(38): 35434-35439.

Sakurikar, N. and A. Eastman (2016). "Critical reanalysis of the methods that discriminate the activity of CDK2 from CDK1." Cell Cycle **15**(9): 1184-1188.

Salton, M., Y. Lerenthal, S. Y. Wang, D. J. Chen and Y. Shiloh (2010). "Involvement of Matrin 3 and SFPQ/NONO in the DNA damage response." Cell Cycle **9**(8): 1568-1576.

Samaan, S., L. C. Tranchevent, E. Dardenne, M. Polay Espinoza, E. Zonta, S. Germann, L. Gratadou, M. Dutertre and D. Auboeuf (2014). "The Ddx5 and Ddx17 RNA helicases are cornerstones in the complex regulatory array of steroid hormone-signaling pathways." Nucleic Acids Res **42**(4): 2197-2207.

Santelli, E., M. Leone, C. Li, T. Fukushima, N. E. Preece, A. J. Olson, K. R. Ely, J. C. Reed, M. Pellecchia, R. C. Liddington and S. Matsuzawa (2005). "Structural analysis of Siah1-Siah-interacting protein interactions and insights into the assembly of an E3 ligase multiprotein complex." J Biol Chem **280**(40): 34278-34287.

Sartorius, C. A., M. Y. Melville, A. R. Hovland, L. Tung, G. S. Takimoto and K. B. Horwitz (1994). "A third transactivation function (AF3) of human progesterone receptors located in the unique N-terminal segment of the B-isoform." Mol Endocrinol **8**(10): 1347-1360.

Sartorius, C. A., G. S. Takimoto, J. K. Richer, L. Tung and K. B. Horwitz (2000). "Association of the Ku autoantigen/DNA-dependent protein kinase holoenzyme and poly(ADP-ribose) polymerase with the DNA binding domain of progesterone receptors." J Mol Endocrinol **24**(2): 165-182.

Sasaki, T., A. Onodera, H. Hosokawa, Y. Watanabe, S. Horiuchi, J. Yamashita, H. Tanaka, Y. Ogawa, Y. Suzuki and T. Nakayama (2013). "Genome-Wide Gene Expression Profiling Revealed a Critical Role for GATA3 in the Maintenance of the Th2 Cell Identity." PLoS One **8**(6): e66468.

Sato, N., M. Maeda, M. Sugiyama, S. Ito, T. Hyodo, A. Masuda, N. Tsunoda, T. Kokuryo, M. Hamaguchi, M. Nagino and T. Senga (2015). "Inhibition of SNW1 association with spliceosomal proteins promotes apoptosis in breast cancer cells." Cancer Med **4**(2): 268-277.

Scheidereit, C., S. Geisse, H. M. Westphal and M. Beato (1983). "The glucocorticoid receptor binds to defined nucleotide sequences near the promoter of mouse mammary tumour virus." Nature **304**(5928): 749-752.

Schiewer, M. J. and K. E. Knudsen (2016). "Linking DNA Damage and Hormone Signaling Pathways in Cancer." Trends Endocrinol Metab **27**(4): 216-225.

Schulke, J. P., G. M. Wochnik, I. Lang-Rollin, N. C. Gassen, R. T. Knapp, B. Berning, A. Yassouridis and T. Rein (2010). "Differential impact of tetratricopeptide repeat proteins on the steroid hormone receptors." PLoS One **5**(7): e11717.

Schultz-Norton, J. R., Y. S. Ziegler, V. S. Likhite, J. R. Yates and A. M. Nardulli (2008). "Isolation of novel coregulatory protein networks associated with DNA-bound estrogen receptor alpha." BMC Mol Biol **9**: 97.

Seo, S. B., P. McNamara, S. Heo, A. Turner, W. S. Lane and D. Chakravarti (2001). "Regulation of histone acetylation and transcription by INHAT, a human cellular complex containing the set oncoprotein." Cell **104**(1): 119-130.

Seo, W. Y., B. C. Jeong, E. J. Yu, H. J. Kim, S. H. Kim, J. E. Lim, G. Y. Kwon, H. M. Lee and J. H. Kim (2013). "CCAR1 promotes chromatin loading of androgen receptor (AR) transcription complex by stabilizing the association between AR and GATA2." Nucleic Acids Res **41**(18): 8526-8536.

Shamanna, R. A., M. Hoque, A. Lewis-Antes, E. I. Azzam, D. Lagunoff, T. Pe'ery and M. B. Mathews (2011). "The NF90/NF45 complex participates in DNA break repair via nonhomologous end joining." Mol Cell Biol **31**(23): 4832-4843.

Shannon, P., A. Markiel, O. Ozier, N. S. Baliga, J. T. Wang, D. Ramage, N. Amin, B. Schwikowski and T. Ideker (2003). "Cytoscape: a software environment for integrated models of biomolecular interaction networks." Genome Res **13**(11): 2498-2504.

Shao, J. and M. I. Diamond (2012). "Protein phosphatase 1 dephosphorylates profilin-1 at Ser-137." PLoS One **7**(3): e32802.

Shao, J., W. J. Welch, N. A. Diprospero and M. I. Diamond (2008). "Phosphorylation of profilin by ROCK1 regulates polyglutamine aggregation." Mol Cell Biol **28**(17): 5196-5208.

Shen, T., K. B. Horwitz and C. A. Lange (2001). "Transcriptional hyperactivity of human progesterone receptors is coupled to their ligand-dependent down-regulation by mitogen-activated protein kinase-dependent phosphorylation of serine 294." Mol Cell Biol **21**(18): 6122-6131.

Sheridan, P. L., M. Schorpp, M. L. Voz and K. A. Jones (1995). "Cloning of an SNF2/SWI2-related protein that binds specifically to the SPH motifs of the SV40 enhancer and to the HIV-1 promoter." J Biol Chem **270**(9): 4575-4587.

Shi, X., T. Hong, K. L. Walter, M. Ewalt, E. Michishita, T. Hung, D. Carney, P. Pena, F. Lan, M. R. Kaadige, N. Lacoste, C. Cayrou, F. Davrazou, A. Saha, B. R. Cairns, D. E. Ayer, T. G. Kutateladze, Y. Shi, J. Cote, K. F. Chua and O. Gozani (2006). "ING2 PHD domain links histone H3 lysine 4 methylation to active gene repression." Nature **442**(7098): 96-99.

Shi, Y., J. Sawada, G. Sui, B. Affar el, J. R. Whetstine, F. Lan, H. Ogawa, M. P. Luke, Y. Nakatani and Y. Shi (2003). "Coordinated histone modifications mediated by a CtBP co-repressor complex." Nature **422**(6933): 735-738.

Shimbo, T., Y. Du, S. A. Grimm, A. Dhasarathy, D. Mav, R. R. Shah, H. Shi and P. A. Wade (2013). "MBD3 localizes at promoters, gene bodies and enhancers of active genes." PLoS Genet **9**(12): e1004028.

Shiohama, A., T. Sasaki, S. Noda, S. Minoshima and N. Shimizu (2007). "Nucleolar localization of DGCR8 and identification of eleven DGCR8-associated proteins." Exp Cell Res **313**(20): 4196-4207.

Shumaker, D. K., K. K. Lee, Y. C. Tanhehco, R. Craigie and K. L. Wilson (2001). "LAP2 binds to BAF.DNA complexes: requirement for the LEM domain and modulation by variable regions." EMBO J **20**(7): 1754-1764.

Silva, A. P., D. P. Ryan, Y. Galanty, J. K. Low, M. Vandevenne, S. P. Jackson and J. P. Mackay (2016). "The N-terminal Region of Chromodomain Helicase DNA-binding Protein 4 (CHD4) Is Essential for Activity and Contains a High Mobility Group (HMG) Box-like-domain That Can Bind Poly(ADP-ribose)." J Biol Chem **291**(2): 924-938.

Sinars, C. R., J. Cheung-Flynn, R. A. Rimerman, J. G. Scammell, D. F. Smith and J. Clardy (2003). "Structure of the large FK506-binding protein FKBP51, an Hsp90-binding protein and a component of steroid receptor complexes." Proc Natl Acad Sci U S A **100**(3): 868-873.

Skarra, D. V., M. Goudreault, H. Choi, M. Mullin, A. I. Nesvizhskii, A. C. Gingras and R. E. Honkanen (2011). "Label-free quantitative proteomics and SAINT analysis enable interactome mapping for the human Ser/Thr protein phosphatase 5." Proteomics **11**(8): 1508-1516.

Skowronska-Krawczyk, D., Q. Ma, M. Schwartz, K. Scully, W. Li, Z. Liu, H. Taylor, J. Tollkuhn, K. A. Ohgi, D. Notani, Y. Kohwi, T. Kohwi-Shigematsu and M. G. Rosenfeld (2014). "Required enhancer-matrin-3 network interactions for a homeodomain transcription program." Nature **514**(7521): 257-261.

Smith, D. F. (1993). "Dynamics of heat shock protein 90-progesterone receptor binding and the disactivation loop model for steroid receptor complexes." Mol Endocrinol **7**(11): 1418-1429.

Smith, D. F. (2000). "Chaperones in progesterone receptor complexes." Semin Cell Dev Biol **11**(1): 45-52.

Smith, D. F., M. W. Albers, S. L. Schreiber, K. L. Leach and M. R. Deibel, Jr. (1993). "FKBP54, a novel FK506-binding protein in avian progesterone receptor complexes and HeLa extracts." J Biol Chem **268**(32): 24270-24273.

Smith, D. F., B. A. Baggenstoss, T. N. Marion and R. A. Rimerman (1993). "Two FKBP-related ProteinAs re Associatedwith Progesterone Receptor Complexes." J Biol Chem **268**(24): 18365-18371.

Smith, D. F., B. A. Stensgard, W. J. Welch and D. O. Toft (1992). "Assembly of Progesterone Receptor with Heat Shock Proteins and Receptor Activation Are ATP Mediated Events." J Biol Chem **267**(2): 1350-1356.

Snijders, A. P., G. M. Hautbergue, A. Bloom, J. C. Williamson, T. C. Minshull, H. L. Phillips, S. R. Mihaylov, D. T. Gjerde, D. P. Hornby, S. A. Wilson, P. J. Hurd and M. J. Dickman (2015). "Arginine methylation and citrullination of splicing factor proline- and glutamine-rich (SFPQ/PSF) regulates its association with mRNA." RNA **21**(3): 347-359.

Soares, L. M. and S. Buratowski (2013). "Histone Crosstalk: H2Bub and H3K4 Methylation." Mol Cell **49**(6): 1019-1020.

Song, E. J., S. L. Werner, J. Neubauer, F. Stegmeier, J. Aspden, D. Rio, J. W. Harper, S. J. Elledge, M. W. Kirschner and M. Rape (2010). "The Prp19 complex and the Usp4Sart3 deubiquitinating enzyme control reversible ubiquitination at the spliceosome." Genes Dev **24**(13): 1434-1447.

Soubeyrand, S., L. Pope, B. Pakuts and R. J. Hache (2003). "Threonines 2638/2647 in DNA-PK are essential for cellular resistance to ionizing radiation." Cancer Res **63**(6): 1198-1201.

Spencer, T. E., G. Jenster, M. M. Burcin, C. D. Allis, J. Zhou, C. A. Mizzen, N. J. McKenna, S. A. Onate, S. Y. Tsai, M. J. Tsai and B. W. O'Malley (1997). "Steroid receptor coactivator-1 is a histone acetyltransferase." Nature **389**(6647): 194-198.

Sterner, D. E. and S. L. Berger (2000). "Acetylation of histones and transcription-related factors." Microbiol Mol Biol Rev **64**(2): 435-459.

Strahl, B. D. and C. D. Allis (2000). "The language of covalent histone modifications." Nature **403**(6765): 41-45.

Strahl, B. D., S. D. Briggs, C. J. Brame, J. A. Caldwell, S. S. Koh, H. Ma, R. G. Cook, J. Shabanowitz, D. F. Hunt, M. R. Stallcup and C. D. Allis (2001). "Methylation of histone H4 at arginine 3 occurs in vivo and is mediated by the nuclear receptor coactivator PRMT1." Curr Biol **11**(12): 996-1000.

Su, G., A. Kuchinsky, J. H. Morris, D. J. States and F. Meng (2010). "GLay: community structure analysis of biological networks." Bioinformatics **26**(24): 3135-3137.

Suzuki, C., R. G. Garces, K. A. Edmonds, S. Hiller, S. G. Hyberts, A. Marintchev and G. Wagner (2008). "PDCD4 inhibits translation initiation by binding to eIF4A using both its MA3 domains." Proc Natl Acad Sci U S A **105**(9): 3274-3279.

Swaminathan, V., A. H. Kishore, K. K. Febitha and T. K. Kundu (2005). "Human histone chaperone nucleophosmin enhances acetylation-dependent chromatin transcription." Mol Cell Biol **25**(17): 7534-7545.

Takimoto, G. S., L. Tung, H. Abdel-Hafiz, M. G. Abel, C. A. Sartorius, J. K. Richer, B. M. Jacobsen, D. L. Bain and K. B. Horwitz (2003). "Functional properties of the N-terminal region of progesterone receptors and their mechanistic relationship to structure." J Steroid Biochem Mol Biol **85**(2-5): 209-219.

Tan, J., S. H. Hall, K. G. Hamil, G. Grossman, P. Petrusz, J. Liao, K. Shuai and F. S. French (2000). "Protein inhibitor of activated STAT-1 (signal transducer and activator of transcription-1) is a nuclear receptor coregulator expressed in human testis." Mol Endocrinol **14**(1): 14-26.

Tan, J. A., J. Song, Y. Chen and L. K. Durrin (2010). "Phosphorylation-dependent interaction of SATB1 and PIAS1 directs SUMO-regulated caspase cleavage of SATB1." Mol Cell Biol **30**(11): 2823-2836.

Tanenbaum, D. M., Y. Wang, S. P. Williams and P. B. Sigler (1998). "Crystallographic comparison of the estrogen and progesterone receptor's ligand binding domains." Proc Natl Acad Sci U S A **95**(11): 5998-6003.

Terret, M. E., R. Sherwood, S. Rahman, J. Qin and P. V. Jallepalli (2009). "Cohesin acetylation speeds the replication fork." Nature **462**(7270): 231-234.

Theodorou, V., R. Stark, S. Menon and J. S. Carroll (2013). "GATA3 acts upstream of FOXA1 in mediating ESR1 binding by shaping enhancer accessibility." Genome Res **23**(1): 12-22.

Thompson, P. J., V. Dulberg, K. M. Moon, L. J. Foster, C. Chen, M. M. Karimi and M. C. Lorincz (2015). "hnRNP K coordinates transcriptional silencing by SETDB1 in embryonic stem cells." PLoS Genet **11**(1): e1004933.

Ting, N. S., P. N. Kao, D. W. Chan, L. G. Lintott and S. P. Lees-Miller (1998). "DNA-dependent protein kinase interacts with antigen receptor response element binding proteins NF90 and NF45." J Biol Chem **273**(4): 2136-2145.

Todd, J. L., K. G. Tanner and J. M. Denu (1999). "Extracellular regulated kinases (ERK) 1 and ERK2 are authentic substrates for the dual-specificity protein-tyrosine phosphatase VHR. A novel role in down-regulating the ERK pathway." J Biol Chem **274**(19): 13271-13280.

Tomsig, J. L., H. Sohma and C. E. Creutz (2004). "Calcium-dependent regulation of tumour necrosis factor-alpha receptor signalling by copine." Biochem J **378**(Pt 3): 1089-1094.

Tong, J. K., C. A. Hassig, G. R. Schnitzler, R. E. Kingston and S. L. Schreiber (1998). "Chromatin deacetylation by an ATP-dependent nucleosome remodelling complex." Nature **395**(6705): 917-921.

Toughiri, R., X. Li, Q. Du and C. J. Bieberich (2013). "Phosphorylation of NuMA by Aurora-A kinase in PC-3 prostate cancer cells affects proliferation, survival, and interphase NuMA localization." J Cell Biochem **114**(4): 823-830.

Trevino, L. S., M. J. Bolt, S. L. Grimm, D. P. Edwards, M. A. Mancini and N. L. Weigel (2016). "Differential Regulation of Progesterone Receptor-Mediated Transcription by CDK2 and DNA-PK." Mol Endocrinol **30**(2): 158-172.

Truss, M., J. Bartsch, A. Schelbert, R. J. Hache and M. Beato (1995). "Hormone induces binding of receptors and transcription factors to a rearranged nucleosome on the MMTV promoter in vivo." EMBO J **14**(8): 1737-1751.

Tsai, M. J. and B. W. O'Malley (1994). "Molecular mechanisms of action of steroid/thyroid receptor superfamily members." Annu Rev Biochem **63**: 451-486.

Turner, B., S. Razick, A. L. Turinsky, J. Vlasblom, E. K. Crowdy, E. Cho, K. Morrison, I. M. Donaldson and S. J. Wodak (2010). "iRefWeb: interactive analysis of consolidated protein interaction data and their supporting evidence." Database (Oxford) **2010**: baq023.

Tuteja, N., R. Tuteja, A. Ochem, P. Taneja, N. W. Huang, A. Simoncsits, S. Susic, K. Rahman, L. Marusic, J. Chen and et al. (1994). "Human DNA helicase II: a novel DNA unwinding enzyme identified as the Ku autoantigen." EMBO J **13**(20): 4991-5001.

Unk, I., I. Hajdu, K. Fatyol, J. Hurwitz, J. H. Yoon, L. Prakash, S. Prakash and L. Haracska (2008). "Human HLTF functions as a ubiquitin ligase for proliferating cell nuclear antigen polyubiquitination." Proc Natl Acad Sci U S A **105**(10): 3768-3773.

Utley, R. T., N. Lacoste, O. Jobin-Robitaille, S. Allard and J. Cote (2005). "Regulation of NuA4 histone acetyltransferase activity in transcription and DNA repair by phosphorylation of histone H4." Mol Cell Biol **25**(18): 8179-8190.

van der Horst, A., A. M. de Vries-Smits, A. B. Brenkman, M. H. van Triest, N. van den Broek, F. Colland, M. M. Maurice and B. M. Burgering (2006). "FOXO4 transcriptional activity is regulated by monoubiquitination and USP7/HAUSP." Nat Cell Biol **8**(10): 1064-1073.

van Domselaar, R., R. Quadir, A. M. van der Made, R. Broekhuizen and N. Bovenschen (2012). "All human granzymes target hnRNP K that is essential for tumor cell viability." J Biol Chem **287**(27): 22854-22864.

Vicent, G. P., C. Ballare, A. S. Nacht, J. Clausell, A. Subtil-Rodriguez, I. Quiles, A. Jordan and M. Beato (2006). "Induction of progesterone target genes requires activation of Erk and Msk kinases and phosphorylation of histone H3." Mol Cell **24**(3): 367-381.

Vicent, G. P., A. S. Nacht, J. Font-Mateu, G. Castellano, L. Gaveglia, C. Ballare and M. Beato (2011). "Four enzymes cooperate to displace histone H1 during the first minute of hormonal gene activation." Genes Dev **25**(8): 845-862.

Vicent, G. P., A. S. Nacht, C. L. Smith, C. L. Peterson, S. Dimitrov and M. Beato (2004). "DNA instructed displacement of histones H2A and H2B at an inducible promoter." Mol Cell **16**(3): 439-452.

Vicent, G. P., A. S. Nacht, R. Zaurin, J. Font-Mateu, D. Soronellas, F. Le Dily, D. Reyes and M. Beato (2013). "Unliganded progesterone receptor-mediated targeting of an RNA-containing repressive complex silences a subset of hormone-inducible genes." Genes Dev **27**(10): 1179-1197.

Vicent, G. P., R. Zaurin, A. S. Nacht, J. Font-Mateu, F. Le Dily and M. Beato (2010). "Nuclear factor 1 synergizes with progesterone receptor on the mouse mammary tumor virus promoter wrapped around a histone H3/H4 tetramer by facilitating access to the central hormone-responsive elements." J Biol Chem **285**(4): 2622-2631.

Vicent, G. P., R. Zaurin, A. S. Nacht, A. Li, J. Font-Mateu, F. Le Dily, M. Vermeulen, M. Mann and M. Beato (2009). "Two chromatin remodeling activities cooperate during activation of hormone responsive promoters." PLoS Genet **5**(7): e1000567.

Vidi, P. A., J. Liu, D. Salles, S. Jayaraman, G. Dorfman, M. Gray, P. Abad, P. V. Moghe, J. M. Irudayaraj, L. Wiesmuller and S. A. Lelievre (2014). "NuMA promotes homologous recombination repair by regulating the accumulation of the ISWI ATPase SNF2h at DNA breaks." Nucleic Acids Res **42**(10): 6365-6379.

Vitari, A. C., K. G. Leong, K. Newton, C. Yee, K. O'Rourke, J. Liu, L. Phu, R. Vij, R. Ferrando, S. S. Couto, S. Mohan, A. Pandita, J. A. Hongo, D. Arnott, I. E. Wertz, W. Q. Gao, D. M. French and V. M. Dixit (2011). "COP1 is a tumour suppressor that causes degradation of ETS transcription factors." Nature **474**(7351): 403-406.

Vogel, J. L. and T. M. Kristie (2000). "The novel coactivator C1 (HCF) coordinates multiprotein enhancer formation and mediates transcription activation by GABP." EMBO J **19**(4): 683-690.

Vomastek, T., M. P. Iwanicki, W. R. Burack, D. Tiwari, D. Kumar, J. T. Parsons, M. J. Weber and V. K. Nandicoori (2008). "Extracellular signal-regulated kinase 2 (ERK2) phosphorylation sites and docking domain on the nuclear pore complex protein Tpr cooperatively regulate ERK2-Tpr interaction." Mol Cell Biol **28**(22): 6954-6966.

von der Ahe, D., J. M. Renoir, T. Buchou, E. E. Baulieu and M. Beato (1986). "Receptors for glucocorticosteroid and progesterone recognize distinct features of a DNA regulatory element." Proc Natl Acad Sci U S A **83**(9): 2817-2821.

Wagner, S., S. Weber, M. A. Kleinschmidt, K. Nagata and U. M. Bauer (2006). "SET-mediated promoter hypoacetylation is a prerequisite for coactivation of the estrogen-responsive pS2 gene by PRMT1." J Biol Chem **281**(37): 27242-27250.

Wang, H., Z. Q. Huang, L. Xia, Q. Feng, H. Erdjument-Bromage, B. D. Strahl, S. D. Briggs, C. D. Allis, J. Wong, P. Tempst and Y. Zhang (2001). "Methylation of histone H4 at arginine 3 facilitating transcriptional activation by nuclear hormone receptor." Science **293**(5531): 853-857.

Wang, H., L. Zhai, J. Xu, H. Y. Joo, S. Jackson, H. Erdjument-Bromage, P. Tempst, Y. Xiong and Y. Zhang (2006). "Histone H3 and H4 ubiquitylation by the CUL4-DDB-ROC1 ubiquitin ligase facilitates cellular response to DNA damage." Mol Cell **22**(3): 383-394.

Wang, L., D. M. Lonard and B. W. O'Malley (2016). "The Role of Steroid Receptor Coactivators in Hormone Dependent Cancers and Their Potential as Therapeutic Targets." Horm Cancer **7**(4): 229-235.

Wang, W., J. Cote, Y. Xue, S. Zhou, P. A. Khavari, S. R. Biggar, C. Muchardt, G. V. Kalpana, S. P. Goff, M. Yaniv, J. L. Workman and G. R. Crabtree (1996). "Purification

and biochemical heterogeneity of the mammalian SWI-SNF complex." EMBO J **15**(19): 5370-5382.

Weaver, A. J., W. P. Sullivan, S. J. Felts, B. A. Owen and D. O. Toft (2000). "Crystal structure and activity of human p23, a heat shock protein 90 co-chaperone." J Biol Chem **275**(30): 23045-23052.

Weigel, N. L. (1993). "Overview and Background: Mechanism of Action of Antiprogestins." NATIONAL ACADEMY PRESS.

Werner, S., S. Frey, S. Riethdorf, C. Schulze, M. Alawi, L. Kling, V. Vafaizadeh, G. Sauter, L. Terracciano, U. Schumacher, K. Pantel and V. Assmann (2013). "Dual roles of the transcription factor grainyhead-like 2 (GRHL2) in breast cancer." J Biol Chem **288**(32): 22993-23008.

West, S., N. Gromak and N. J. Proudfoot (2004). "Human 5' --> 3' exonuclease Xrn2 promotes transcription termination at co-transcriptional cleavage sites." Nature **432**(7016): 522-525.

White, C. L., R. K. Suto and K. Luger (2001). "Structure of the yeast nucleosome core particle reveals fundamental changes in internucleosome interactions." EMBO J **20**(18): 5207-5218.

Wierer, M., G. Verde, P. Pisano, H. Molina, J. Font-Mateu, L. Di Croce and M. Beato (2013). "PLK1 signaling in breast cancer cells cooperates with estrogen receptor-dependent gene transcription." Cell Rep **3**(6): 2021-2032.

Williams, S. P. and P. B. Sigler (1998). "Atomic structure of progesterone complexed with its receptor." Nature **393**(6683): 392-396.

Willmann, T. and M. Beato (1986). "Steroid-free glucocorticoid receptor binds specifically to mouse mammary tumour virus DNA." Nature **324**(6098): 688-691.

Wilson, B. J., G. J. Bates, S. M. Nicol, D. J. Gregory, N. D. Perkins and F. V. Fuller-Pace (2004). "The p68 and p72 DEAD box RNA helicases interact with HDAC1 and repress transcription in a promoter-specific manner." BMC Mol Biol **5**: 11.

Winter, S., W. Fischle and C. Seiser (2008). "Modulation of 14-3-3 interaction with phosphorylated histone H3 by combinatorial modification patterns." Cell Cycle **7**(10): 1336-1342.

Wittmann, B. M., K. Fujinaga, H. Deng, N. Ogba and M. M. Montano (2005). "The breast cell growth inhibitor, estrogen down regulated gene 1, modulates a novel functional interaction between estrogen receptor alpha and transcriptional elongation factor cyclin T1." Oncogene **24**(36): 5576-5588.

Wittmann, B. M., N. Wang and M. M. Montano (2003). "Identification of a novel inhibitor of breast cell growth that is down-regulated by estrogens and decreased in breast tumors." Cancer Res **63**(16): 5151-5158.

Workman, J. L. and R. E. Kingston (1998). "Alteration of nucleosome structure as a mechanism of transcriptional regulation." Annu Rev Biochem **67**: 545-579.

Wortham, N. C., E. Ahamed, S. M. Nicol, R. S. Thomas, M. Periyasamy, J. Jiang, A. M. Ochocka, S. Shousha, L. Huson, S. E. Bray, R. C. Coombes, S. Ali and F. V. Fuller-Pace (2009). "The DEAD-box protein p72 regulates ERalpha-/oestrogen-dependent transcription and cell growth, and is associated with improved survival in ERalpha-positive breast cancer." Oncogene **28**(46): 4053-4064.

Wright, R. H., G. Castellano, J. Bonet, F. Le Dily, J. Font-Mateu, C. Ballare, A. S. Nacht, D. Soronellas, B. Oliva and M. Beato (2012). "CDK2-dependent activation of PARP-1 is required for hormonal gene regulation in breast cancer cells." Genes Dev **26**(17): 1972-1983.

Wright, R. H., A. Lioutas, F. Le Dily, D. Soronellas, A. Pohl, J. Bonet, A. S. Nacht, S. Samino, J. Font-Mateu, G. P. Vicent, M. Wierer, M. A. Trabado, C. Schelhorn, C. Carolis, M. J. Macias, O. Yanes, B. Oliva and M. Beato (2016). "ADP-ribose-derived nuclear ATP synthesis by NUDIX5 is required for chromatin remodeling." Science **352**(6290): 1221-1225.

Wysocka, J., M. P. Myers, C. D. Laherty, R. N. Eisenman and W. Herr (2003). "Human Sin3 deacetylase and trithorax-related Set1/Ash2 histone H3-K4 methyltransferase are tethered together selectively by the cell-proliferation factor HCF-1." Genes Dev **17**(7): 896-911.

Xi, Q., Z. Wang, A. I. Zaromytidou, X. H. Zhang, L. F. Chow-Tsang, J. X. Liu, H. Kim, A. Barlas, K. Manova-Todorova, V. Kaartinen, L. Studer, W. Mark, D. J. Patel and J. Massague (2011). "A poised chromatin platform for TGF-beta access to master regulators." Cell **147**(7): 1511-1524.

Xu, J. and Q. Li (2003). "Review of the in vivo functions of the p160 steroid receptor coactivator family." Mol Endocrinol **17**(9): 1681-1692.

Xu, J., Y. Qiu, F. J. DeMayo, S. Y. Tsai, M. J. Tsai and B. W. O'Malley (1998). "Partial hormone resistance in mice with disruption of the steroid receptor coactivator-1 (SRC-1) gene." Science **279**(5358): 1922-1925.

Xu, J., R. C. Wu and B. W. O'Malley (2009). "Normal and cancer-related functions of the p160 steroid receptor co-activator (SRC) family." Nat Rev Cancer **9**(9): 615-630.

Yaffe, M. B., G. W. Farr, D. Miklos, A. L. Horwich, M. L. Sternlicht and H. Sternlicht (1992). "TCP1 complex is a molecular chaperone in tubulin biogenesis." Nature **358**(6383): 245-248.

Yamaguchi, Y., T. Takagi, T. Wada, K. Yano, A. Furuya, S. Sugimoto, J. Hasegawa and H. Handa (1999). "NELF, a multisubunit complex containing RD, cooperates with DSIF to repress RNA polymerase II elongation." Cell **97**(1): 41-51.

Yamashita, N., N. Shimazaki, S. Ibe, R. Kaneko, A. Tanabe, T. Toyomoto, K. Fujita, T. Hasegawa, S. Toji, K. Tamai, H. Yamamoto and O. Koiwai (2001). "Terminal deoxynucleotidyltransferase directly interacts with a novel nuclear protein that is homologous to p65." Genes Cells **6**(7): 641-652.

Yan, Q., W. Sun, P. Kujala, Y. Lotfi, T. A. Vida and A. J. Bean (2005). "CART: an Hrs/actinin-4/BERP/myosin V protein complex required for efficient receptor recycling." Mol Biol Cell **16**(5): 2470-2482.

Yang, W., Q. Wang, K. L. Howell, J. T. Lee, D. S. Cho, J. M. Murray and K. Nishikura (2005). "ADAR1 RNA deaminase limits short interfering RNA efficacy in mammalian cells." J Biol Chem **280**(5): 3946-3953.

Yang, X., F. Zhang and J. E. Kudlow (2002). "Recruitment of O-GlcNAc transferase to promoters by corepressor mSin3A: coupling protein O-GlcNAcylation to transcriptional repression." Cell **110**(1): 69-80.

Yang, Z., J. H. Yik, R. Chen, N. He, M. K. Jang, K. Ozato and Q. Zhou (2005). "Recruitment of P-TEFb for stimulation of transcriptional elongation by the bromodomain protein Brd4." Mol Cell **19**(4): 535-545.

Yasui, D., M. Miyano, S. Cai, P. Varga-Weisz and T. Kohwi-Shigematsu (2002). "SATB1 targets chromatin remodelling to regulate genes over long distances." Nature **419**(6907): 641-645.

Yavuzer, U., G. C. Smith, T. Bliss, D. Werner and S. P. Jackson (1998). "DNA end-independent activation of DNA-PK mediated via association with the DNA-binding protein C1D." Genes Dev **12**(14): 2188-2199.

Ye, Q., I. Callebaut, A. Pezhman, J. C. Courvalin and H. J. Worman (1997). "Domain-specific interactions of human HP1-type chromodomain proteins and inner nuclear membrane protein LBR." J Biol Chem **272**(23): 14983-14989.

Yik, J. H., R. Chen, R. Nishimura, J. L. Jennings, A. J. Link and Q. Zhou (2003). "Inhibition of P-TEFb (CDK9/Cyclin T) kinase and RNA polymerase II transcription by the coordinated actions of HEXIM1 and 7SK snRNA." Mol Cell **12**(4): 971-982.

Yildirim, O., R. Li, J. H. Hung, P. B. Chen, X. Dong, L. S. Ee, Z. Weng, O. J. Rando and T. G. Fazzio (2011). "Mbd3/NURD complex regulates expression of 5-hydroxymethylcytosine marked genes in embryonic stem cells." Cell **147**(7): 1498-1510.

York, B. and B. W. O'Malley (2010). "Steroid receptor coactivator (SRC) family: masters of systems biology." J Biol Chem **285**(50): 38743-38750.

Youn, B., H. D. Kim and J. Kim (2008). "Nm23-H1/nucleoside diphosphate kinase as a key molecule in breast tumor angiogenesis." Expert Opin Ther Targets **12**(11): 1419-1430.

Zaret, K. S. and J. S. Carroll (2011). "Pioneer transcription factors: establishing competence for gene expression." Genes Dev **25**(21): 2227-2241.

Zhang, C., D. R. Dowd, A. Staal, C. Gu, J. B. Lian, A. J. van Wijnen, G. S. Stein and P. N. MacDonald (2003). "Nuclear coactivator-62 kDa/Ski-interacting protein is a nuclear matrix-associated coactivator that may couple vitamin D receptor-mediated transcription and RNA splicing." J Biol Chem **278**(37): 35325-35336.

Zhang, Q., N. Vo and R. H. Goodman (2000). "Histone binding protein RbAp48 interacts with a complex of CREB binding protein and phosphorylated CREB." Mol Cell Biol **20**(14): 4970-4978.

Zhang, T., J. Ma and X. Cao (2003). "Grb2 regulates Stat3 activation negatively in epidermal growth factor signalling." Biochem J **376**(Pt 2): 457-464.

Zhang, X., I. H. Diab and Z. E. Zehner (2003). "ZBP-89 represses vimentin gene transcription by interacting with the transcriptional activator, Sp1." Nucleic Acids Res **31**(11): 2900-2914.

Zhang, X., A. Krutchinsky, A. Fukuda, W. Chen, S. Yamamura, B. T. Chait and R. G. Roeder (2005). "MED1/TRAP220 exists predominantly in a TRAP/ Mediator subpopulation enriched in RNA polymerase II and is required for ER-mediated transcription." Mol Cell **19**(1): 89-100.

Zhang, Y., C. A. Beck, A. Poletti, D. P. Edwards and N. L. Weigel (1995). "Identification of a group of Ser-Pro motif hormone-inducible phosphorylation sites in the human progesterone receptor." Mol Endocrinol **9**(8): 1029-1040.

Zhang, Y., G. LeRoy, H. P. Seelig, W. S. Lane and D. Reinberg (1998). "The dermatomyositis-specific autoantigen Mi2 is a component of a complex containing histone deacetylase and nucleosome remodeling activities." Cell **95**(2): 279-289.

Zhang, Y., F. Yuan, S. R. Presnell, K. Tian, Y. Gao, A. E. Tomkinson, L. Gu and G. M. Li (2005). "Reconstitution of 5'-directed human mismatch repair in a purified system." Cell **122**(5): 693-705.

Zhang, Z. and G. G. Carmichael (2001). "The fate of dsRNA in the nucleus: a p54(nrb)-containing complex mediates the nuclear retention of promiscuously A-to-I edited RNAs." Cell **106**(4): 465-475.

Zhong, Q., C. F. Chen, S. Li, Y. Chen, C. C. Wang, J. Xiao, P. L. Chen, Z. D. Sharp and W. H. Lee (1999). "Association of BRCA1 with the hRad50-hMre11-p95 complex and the DNA damage response." Science **285**(5428): 747-750.

Zhu, B., Y. Zheng, A. D. Pham, S. S. Mandal, H. Erdjument-Bromage, P. Tempst and D. Reinberg (2005). "Monoubiquitination of human histone H2B: the factors involved and their roles in HOX gene regulation." Mol Cell **20**(4): 601-611.

Zhu, M. L., C. M. Horbinski, M. Garzotto, D. Z. Qian, T. M. Beer and N. Kyprianou (2010). "Tubulin-targeting chemotherapy impairs androgen receptor activity in prostate cancer." Cancer Res **70**(20): 7992-8002.

8. Appendices

Table 1: High confidence interactors.

			IgG FC					
Uniprot ID	Gene name	Description	T00	T01	T05	T15	T30	T60
O00148	DX39A	ATP-dependent RNA helicase DDX39A (EC 3.6.4.13)	33.3	38	50	46	53.3	50
O00712	NFIB	Nuclear factor 1 B-type	3.33	14	26	14	23.3	20
O14686	KMT2D	Histone-lysine N-methyltransferase 2D (MLL2)	0	18	48	50	63.3	66
O14929	HAT1	Histone acetyltransferase type B catalytic subunit	20	34	30	36	28.3	36
O14979	HNRDL	Heterogeneous nuclear ribonucleoprotein D-like	18.7	23.5	31.5	26.7	31.1	28.8
O14980	XPO1	Exportin-1 (Chromosome region maintenance 1 protein homolog)	25.3	38.4	48	32	32	25.6
O15042	SR140	U2 snRNP-associated SURP motif-containing protein	21.7	18	40	52	35	38
O15294	OGT1	UDP-N-acetylglucosamine	13.3	28	50	46	48.3	24
O15355	PPM1G	Protein phosphatase 1G (PP2C-gamma)	20	18	26	24	11.7	22
O15550	KDM6A	Lysine-specific demethylase 6A (UTX)	0	2	28	22	31.7	24
O43143	DHX15	Putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15	64	72	81.6	88	89.3	81.6
O43390	HNRPR	Heterogeneous nuclear ribonucleoprotein R	15.6	18.1	22.9	21.3	20	16.5
O43396	TXNL1	Thioredoxin-like protein 1	24	22.4	22.4	20.8	17.3	22.4
O43684	BUB3	Mitotic checkpoint protein BUB3	46.7	44	66	54	75	58
O43707	ACTN4	Alpha-actinin-4	5.07	7.36	5.44	4.16	3.2	4.8
O43809	CPSF5	Cleavage and polyadenylation specificity factor subunit 5 (Nudix motif 21)	17.3	20.8	20.8	20.8	29.3	24
O60244	MED14	Mediator of RNA polymerase II transcription subunit 14 (Trap170)	0	16	38	44	18.3	16
O60264	SMCA5	SMARCA-5 (hSNF2H)	25	20	32	34	26.7	40
O60341	KDM1A	Lysine-specific histone demethylase 1A (LSD1)	11.7	28	64	58	61.7	78
O60506	HNRPQ	Heterogeneous nuclear ribonucleoprotein Q	5.67	5.6	6.8	7.2	5.33	6.4
O60563	CCNT1	Cyclin-T1	18.3	20	24	24	35	36
O60884	DNJA2	DnaJ homolog subfamily A member 2 (Dnj3)	31.7	20	20	16	18.3	20
O75150	BRE1B	E3 ubiquitin-protein ligase BRE1B	38.3	46	80	82	76.7	92
O75367	H2AY	Core histone macro-H2A.1	18	15.2	23.2	23.2	15.3	22.4
O75369	FLNB	Filamin-B	5.7	6.4	5.38	4.22	1.82	3.64
O75376	NCOR1	Nuclear receptor corepressor 1	5	26	30	24	38.3	44
O75448	MED24	Mediator of RNA polymerase II transcription subunit 24	3.33	20	46	48	51.7	38
O75531	BAF	Barrier-to-autointegration factor	18.7	24	30.4	14.4	18.7	25.6
O75533	SF3B1	Splicing factor 3B subunit 1	25	26	46	46	48.3	48

Table 1 (continued):

			IgG FC					
Uniprot ID	Gene name	Description	T00	T01	T05	T15	T30	T60
O75643	U520	U5 small nuclear ribonucleoprotein 200 kDa helicase (EC 3.6.4.13)	30	38	48	60	60	70
O75925	PIAS1	E3 SUMO-protein ligase PIAS1 (Protein inhibitor of activated STAT protein 1)	0	6	26	20	11.7	10
O94776	MTA2	Metastasis-associated protein MTA2	12.4	14.9	21.3	16.5	24.4	23.5
O94992	HEXI1	Protein HEXIM1	20	12	26	22	21.7	30
O95340	PAPS2	Bifunctional 3'-phosphoadenosine 5'-phosphosulfate synthase 2	23.3	26	24	36	30	24
O95396	MOCS3	Adenylyltransferase and sulfurtransferase MOCS3	10	16	22	16	16.7	16
O95758	PTBP3	Polypyrimidine tract-binding protein 3	17.3	22.4	30.4	25.6	26.7	27.2
O95833	CLIC3	Chloride intracellular channel protein 3	28	33.6	37.6	27.2	31.3	34.4
O95983	MBD3	Methyl-CpG-binding domain protein 3	6.67	24	40	24	26.7	44
P00558	PGK1	Phosphoglycerate kinase 1 (EC 2.7.2.3)	5.52	4.73	5.16	4.15	4.85	5.75
P04350	TBB4A	Tubulin beta-4A chain (Tubulin 5 beta)	4.33	4.57	4.29	2.97	2.9	3.54
P05198	IF2A	Eukaryotic translation initiation factor 2 subunit 1	10	8.8	9.6	7.2	8	7.2
P05549	AP2A	Transcription factor AP-2-alpha	1.67	16	42	36	36.7	14
P06396	GELS	Gelsolin (AGEL) (Actin-depolymerizing factor)	11.1	8.91	9.14	8.91	9.14	9.6
P06401	PRGR	Progesterone receptor (Nuclear receptor subfamily 3 group C member 3)	49.2	51.5	56.2	49	49.2	46.7
P06493	CDK1	Cyclin-dependent kinase 1 (CDK1) (EC 2.7.11.22)	6.93	6.72	8.64	8.64	6.4	8
P06733	ENOA	Alpha-enolase (EC 4.2.1.11)	3.51	3.9	3.72	3.41	3.16	4.56
P06748	NPM	Nucleophosmin (Numatrin)	3.56	3.25	3.52	3.63	3.6	5.17
P07437	TBB5	Tubulin beta chain (Tubulin beta-5 chain)	3.92	4.4	3.97	3.02	2.85	3.38
P07737	PROF1	Profilin-1	4.59	4.27	5.33	5.51	4.44	5.69
P07814	SYEP	Bifunctional glutamate/proline--tRNA ligase	13.3	11.2	6	9.6	6	7.6
P07900	HS90A	Heat shock protein HSP 90-alpha	12	10.2	6.43	5.14	5.54	6.18
P07910	HNRPC	Heterogeneous nuclear ribonucleoproteins C1/C2	8	7.64	8.18	9.78	10.4	10.3
P08107	HSP71	Heat shock 70 kDa protein 1A/1B	3.68	3.42	3.16	2.55	2.63	3.63
P08133	ANXA6	Annexin A6 (67 kDa calelectrin)	6.67	10.8	9.6	8	3.67	6.8
P08238	HS90B	Heat shock protein HSP 90-beta	9.44	7.82	4.77	3.72	4.07	4.49
P08621	RU17	U1 small nuclear ribonucleoprotein 70 kDa	21.7	24	28	32	40	32
P08651	NFIC	Nuclear factor 1 C-type	5	8	22	28	13.3	16
P09429	HMGB1	High mobility group protein B1	11.4	11.7	14	11.2	12.9	14.4
P09651	ROA1	Heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1)	6.79	8.87	11.5	10.5	10.9	11.6
P09874	PARP1	Poly [ADP-ribose] polymerase 1	27.8	24.4	28.8	27	28.2	33.8
P10768	ESTD	S-formylglutathione hydrolase (FGH)	23.3	28	22	24	23.3	24
P11142	HSP7C	Heat shock cognate 71 kDa protein	3.08	2.9	2.67	2.45	2.59	3.22

Table 1 (continued):

			IgG FC					
Uniprot ID	Gene name	Description	T00	T01	T05	T15	T30	T60
P11586	C1TC	C-1-tetrahydrofolate synthase, cytoplasmic (C1-THF synthase)	7.33	7.6	5.6	5.4	4.83	4.2
P11940	PABP1	Polyadenylate-binding protein 1	7.47	12.2	8	5.44	3.2	5.44
P12270	TPR	Nucleoprotein TPR (Megator)	24	21.9	20.3	23.5	17.8	25.1
P12956	XRCC6	X-ray repair cross-complementing protein 6 (Ku70)	37.6	42.9	42.9	37.4	42.4	43.8
P13010	XRCC5	X-ray repair cross-complementing protein 5 (Ku80) (Ku86)	18.4	19.1	18.7	18.7	18.3	21.7
P13639	EF2	Elongation factor 2	5.56	5.2	4.07	3.6	2.72	4.13
P14866	HNRPL	Heterogeneous nuclear ribonucleoprotein L	24	23.4	26.6	25.9	31.2	31
P17844	DDX5	Probable ATP-dependent RNA helicase DDX5	8.76	9.6	11.4	13.3	13.9	11.9
P17931	LEG3	Galectin-3	10.7	12.3	17.1	10.7	13.8	13.3
P17987	TCPA	T-complex protein 1 subunit alpha	10.7	11.2	7.47	11.7	4.89	13.3
P18124	RL7	60S ribosomal protein L7	16	18.4	18.4	24	14.7	17.6
P18615	NELFE	Negative elongation factor E (NELF-E)	36.7	34	50	46	53.3	52
P19338	NUCL	Nucleolin (Protein C23)	4.41	4.8	5.78	7.14	6.15	9.35
P19793	RXRA	Retinoic acid receptor RXR-alpha	1.67	20	36	28	21.7	10
P21333	FLNA	Filamin-A	23.3	26	18	8	6.67	8
P22314	UBA1	Ubiquitin-like modifier-activating enzyme 1	31.3	26.4	32.8	39.2	38.7	59.2
P22392	NDKB	Nucleoside diphosphate kinase B	4.76	4.11	3.43	4.11	4.19	5.71
P22626	ROA2	Heterogeneous nuclear ribonucleoproteins A2/B1 (hnRNP A2/B1)	4.59	5.6	7.02	6.44	7.19	7.47
P23193	TCEA1	Transcription elongation factor A protein 1	31.7	24	32	38	31.7	44
P23246	SFPQ	Splicing factor, proline- and glutamine-rich	6.04	7.58	8.84	8.34	10.6	9.94
P23528	COF1	Cofilin-1 (18 kDa phosphoprotein)	3.14	3.89	3.43	3.09	3.9	4
P23771	GATA3	Trans-acting T-cell-specific transcription factor GATA-3	8.33	36	66	58	68.3	62
P24666	PPAC	Low molecular weight phosphotyrosine protein phosphatase	18.7	19.2	19.2	14.4	24	14.4
P25205	MCM3	DNA replication licensing factor MCM3 (EC 3.6.4.12)	30	34	38	44	33.3	52
P26368	U2AF2	Splicing factor U2AF 65 kDa subunit	23.3	30	42	36	35	46
P26583	HMGB2	High mobility group protein B2	13.3	10.4	10	12.4	15.3	15.6
P26599	PTBP1	Polypyrimidine tract-binding protein 1 (hnRNP I)	34.2	38.4	45.3	40.5	43.1	48.5
P27635	RL10	60S ribosomal protein L10 (Tumor suppressor QM)	16	19.2	22.4	27.2	10.7	20.8
P27694	RFA1	Replication protein A 70 kDa DNA-binding subunit	33.3	28	50	40	45	48
P29373	RABP2	Cellular retinoic acid-binding protein 2	4.57	4.57	5.94	4.69	4.76	5.49
P29401	TKT	Transketolase (TK) (EC 2.2.1.1)	8.53	6.72	5.76	7.68	7.47	8.96

Table 1 (continued):

			IgG FC					
Uniprot ID	Gene name	Description	T00	T01	T05	T15	T30	T60
P30086	PEBP1	Phosphatidylethanolamine-binding protein 1	17.3	16	17.6	19.2	24	22.4
P31942	HNRH3	Heterogeneous nuclear ribonucleoprotein H3	40	54	70	70	53.3	70
P31943	HNRH1	Heterogeneous nuclear ribonucleoprotein H	3.26	3.08	3.79	3.67	4	4.15
P31948	STIP1	Stress-induced-phosphoprotein 1	88.3	74	48	54	35	60
P32119	PRDX2	Peroxiredoxin-2	6	4.53	4.27	5.07	4.22	6.13
P33991	MCM4	DNA replication licensing factor MCM4 (EC 3.6.4.12) (CDC21 homolog)	28.3	24	38	20	28.3	30
P33993	MCM7	DNA replication licensing factor MCM7 (EC 3.6.4.12) (CDC47 homolog)	73.3	56	94	82	98.3	72
P35637	FUS	RNA-binding protein FUS (Oncogene FUS)	15.7	14.8	19.6	19.2	19.7	18.8
P36578	RL4	60S ribosomal protein L4	6.17	4.6	4.2	5.2	6	4
P38159	RBMX	RNA-binding motif protein, X chromosome (hnRNP G)	5.19	6.22	6.58	6.58	7.56	6.93
P38919	IF4A3	Eukaryotic initiation factor 4A-III	9.6	12.8	17.6	16	13.9	16.6
P39748	FEN1	Flap endonuclease 1	50.7	33.6	48	44.8	44	49.6
P40227	TCPZ	T-complex protein 1 subunit zeta	5.63	5.51	5.69	6.04	4.3	6.22
P40763	STAT3	Signal transducer and activator of transcription 3	9.33	12.3	18.1	13.3	17.3	14.4
P42166	LAP2A	Lamina-associated polypeptide 2, isoform alpha	6.46	5.78	6.89	6.89	6.36	6.15
P43243	MATR3	Matrin-3	32	48.8	55.2	82.4	76	68.8
P45973	CBX5	Chromobox protein homolog 5 (HP1 alpha)	32	27.2	30.4	25.6	32	38.4
P45974	UBP5	Ubiquitin carboxyl-terminal hydrolase 5 (EC 3.4.19.12)	35	38	24	34	33.3	40
P46777	RL5	60S ribosomal protein L5	10.2	10.7	8	8	12	10.7
P46781	RS9	40S ribosomal protein S9	20	32	19.2	30.4	20	22.4
P49368	TCPG	T-complex protein 1 subunit gamma	17.3	26.4	26.4	18.4	20	20
P49585	PCY1A	Choline-phosphate cytidylyltransferase A (EC 2.7.7.15) (CCT-alpha)	49.3	43.2	51.2	48	49.3	68.8
P49756	RBM25	RNA-binding protein 2	25	16	12	28	25	34
P49915	GUAA	GMP synthase [glutamine-hydrolyzing] (EC 6.3.5.2)	86.7	84	96	72	103	110
P49916	DNLI3	DNA ligase 3 (EC 6.5.1.1)	33.3	26	28	36	20	44
P50395	GDIB	Rab GDP dissociation inhibitor beta	3	5.6	7.2	5.6	3	6.4
P50990	TCPQ	T-complex protein 1 subunit theta	4.67	4	3.2	3.6	2.89	4.4
P51452	DUS3	Dual specificity protein phosphatase 3 (EC 3.1.3.16)	25.3	27.2	33.6	24	33.3	25.6
P51610	HCFC1	Host cell factor 1 (VP16 accessory protein)	63.3	40	46	52	50	66
P51991	ROA3	Heterogeneous nuclear ribonucleoprotein A3 (hnRNP A3)	8.15	10.3	14.8	14	15.7	14.9
P52209	6PGD	6-phosphogluconate dehydrogenase, decarboxylating (EC 1.1.1.44)	12.5	13.4	13.8	10.2	8.53	10.6

Table 1 (continued):

			IgG FC					
Uniprot ID	Gene name	Description	T00	T01	T05	T15	T30	T60
P52272	HNRPM	Heterogeneous nuclear ribonucleoprotein M	10.6	11.2	15.6	14.6	16.2	16.5
P52597	HNRPF	Heterogeneous nuclear ribonucleoprotein F	4.58	4.87	5.7	5.84	5.57	5.43
P53396	ACLY	ATP-citrate synthase (EC 2.3.3.8)	10.5	7.36	10.2	7.36	7.33	9.44
P53999	TCP4	Activated RNA polymerase II transcriptional coactivator p15	10.7	7.2	11.2	11.2	12	13.6
P55060	XPO2	Exportin-2 (Chromosome segregation 1-like protein)	9	9.6	10.2	10.2	8.33	7.8
P55072	TERA	Transitional endoplasmic reticulum ATPase (VCP)	21.3	20	24	22.4	16	33.6
P55265	DSRAD	Double-stranded RNA-specific adenosine deaminase	15	32	34	34	28.3	36
P55317	FOXA1	Hepatocyte nuclear factor 3-alpha (Forkhead box protein A1)	18.3	26	40	34	46.7	26
P55795	HNRH2	Heterogeneous nuclear ribonucleoprotein H2	3.7	3.82	4.27	4.53	4.81	4.27
P56545	CTBP2	C-terminal-binding protein 2	25	30	46	48	53.3	60
P60981	DEST	Destrin (Actin-depolymerizing factor)	15	32	30	22	21.7	26
P61289	PSME3	Proteasome activator complex subunit 3	28.3	22	24	28	20	30
P61956	SUMO2	Small ubiquitin-related modifier 2	2.17	3.6	4.4	3.4	3.17	3.6
P61978	HNRPK	Heterogeneous nuclear ribonucleoprotein K	5.81	6.27	6.91	6.91	7.84	6.85
P62081	RS7	40S ribosomal protein S7	5.6	7.36	6.72	6.4	5.33	8.64
P62136	PP1A	Serine/threonine-protein phosphatase PP1-alpha catalytic subunit	11.7	16	22	18	18.3	26
P62158	CALM	Calmodulin (CaM)	3.85	4.09	4.98	3.02	3.56	4.8
P62249	RS16	40S ribosomal protein S16	3.33	3.84	4.48	3.84	4	3.52
P62826	RAN	GTP-binding nuclear protein Ran (Androgen receptor-associated protein 24)	3.82	3.95	4.69	4.16	4.36	5.33
P62917	RL8	60S ribosomal protein L8	4.5	4.4	3.8	3.6	4.33	4.2
P62979	RS27A	Ubiquitin-40S ribosomal protein S27a	3.7	4.62	4.09	3.56	3.26	4.27
P62993	GRB2	Growth factor receptor-bound protein 2	16.7	20	50	34	45	38
P62995	TRA2B	Transformer-2 protein homolog beta	11.7	24	26	28	15	20
P63165	SUMO1	Small ubiquitin-related modifier 1	2.67	8	20.8	12.8	16	19.2
P63241	IF5A1	Eukaryotic translation initiation factor 5A-1	16.7	18.4	23.2	16	17.3	19.2
P68371	TBB4B	Tubulin beta-4B chain (Tubulin beta-2 chain)	3.88	4.12	3.79	2.79	2.69	3.23
P78347	GTF2I	General transcription factor II-I	55	42	82	86	83.3	62
P78371	TCPB	T-complex protein 1 subunit beta	5.78	4.53	3.07	3.6	3.89	4.67
P78527	PRKDC	DNA-dependent protein kinase catalytic subunit	42.1	38.8	40.4	36.6	33.3	32.2
P82979	SARNP	SAP domain-containing ribonucleoprotein	24	17.6	24	33.6	18.7	36.8

Table 1 (continued):

			IgG FC					
Uniprot ID	Gene name	Description	T00	T01	T05	T15	T30	T60
P83916	CBX1	Chromobox protein homolog 1 (HP1 beta)	8.67	11.2	16	16	12.7	16.8
P84090	ERH	Enhancer of rudimentary homolog	21.7	22	24	24	16.7	26
P98175	RBM10	RNA-binding protein 10	1.67	12	20	24	28.3	32
Q00796	DHSO	Sorbitol dehydrogenase (EC 1.1.1.14)	28.3	36	38	22	23.3	26
Q00839	HNRPU	Heterogeneous nuclear ribonucleoprotein U	8.38	8.76	9.52	9.14	9.59	10.7
Q01105	SET	Protein SET	28.3	30	46	46	26.7	52
Q01130	SRSF2	Serine/arginine-rich splicing factor 2	12.3	10	12.8	14.4	13	16.4
Q01826	SATB1	DNA-binding protein SATB1	30	66	100	96	86.7	104
Q01844	EWS	RNA-binding protein EWS	10	5.6	9.6	10.4	18.7	10.4
Q02790	FKBP4	Peptidyl-prolyl cis-trans isomerase FKBP4	129	106	52.8	36	34	60
Q04726	TLE3	Transducin-like enhancer protein 3	16.7	34	50	48	46.7	54
Q05048	CSTF1	Cleavage stimulation factor subunit 1	6.67	18	44	30	38.3	34
Q07666	KHDR1	KH domain-containing, RNA-binding, signal transduction-associated protein 1 (Sam68)	46.7	64	75.2	64	73.3	60.8
Q07955	SRSF1	Serine/arginine-rich splicing factor 1	28.3	28	34	36	36.7	46
Q08211	DHX9	ATP-dependent RNA helicase A (RHA)	37.8	39.7	44.8	40.3	45.1	42.1
Q08J23	NSUN2	tRNA (cytosine(34)-C(5))-methyltransferase (EC 2.1.1.203)	25	28	14	22	16.7	16
Q09028	RBBP4	Histone-binding protein RBBP4	8.89	12.8	13.3	13.3	15.1	17.6
Q09472	EP300	Histone acetyltransferase p300	1.67	28	106	84	112	70
Q12857	NFIA	Nuclear factor 1 A-type (CTF-I)	10	12	24	22	13.3	18
Q12905	ILF2	Interleukin enhancer-binding factor 2	23.3	32	32	52	28.3	50
Q12906	ILF3	Interleukin enhancer-binding factor 3	13.8	9.07	16	19.2	16.4	14.9
Q12996	CSTF3	Cleavage stimulation factor subunit 3	11.7	14	36	44	26.7	32
Q13057	COASY	Bifunctional coenzyme A synthase	14.7	14.4	12.8	8	10.7	5.6
Q13148	TADBP	TAR DNA-binding protein 43 (TDP-43)	4.15	4.62	5.69	5.69	6.52	6.58
Q13151	ROA0	Heterogeneous nuclear ribonucleoprotein A0 (hnRNP A0)	33.3	30	48	38	48.3	58
Q13185	CBX3	Chromobox protein homolog 3 (HP1 gamma) (Modifier 2 protein)	9.33	10.6	11.2	11.2	12.3	12.5
Q13242	SRSF9	Serine/arginine-rich splicing factor 9	25	30	40	36	35	30
Q13247	SRSF6	Serine/arginine-rich splicing factor 6	6.67	8	7.47	7.47	8.44	9.6
Q13263	TIF1B	Transcription intermediary factor 1-beta (E3 SUMO-protein ligase TRIM28)	28	24.6	31	29	36.2	32.8
Q13330	MTA1	Metastasis-associated protein MTA1	10.7	6.4	20.8	11.2	21.3	16
Q13363	CTBP1	C-terminal-binding protein 1	43.3	62	74	68	63.3	68
Q13451	FKBP5	Peptidyl-prolyl cis-trans isomerase FKBP5	327	222	88	72	80	96
Q13526	PIN1	Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1	6.67	18	28	34	28.3	32
Q13547	HDAC1	Histone deacetylase 1 (HD1) (EC 3.5.1.98)	1.87	5.12	10.6	8.32	9.33	11.5
Q13573	SNW1	SNW domain-containing protein 1 (SkiP) (NCoA-62)	16.7	20	44	40	36.7	34

Table 1 (continued):

			IgG FC					
Uniprot ID	Gene name	Description	T00	T01	T05	T15	T30	T60
Q13838	DX39B	Spliceosome RNA helicase DDX39B (EC 3.6.4.13)	33.3	42	46	54	53.3	56
Q14103	HNRPD	Heterogeneous nuclear ribonucleoprotein D0	9.33	10.9	13.6	11.5	11.6	13.6
Q14498	RBM39	RNA-binding protein 39 (CAPER alpha)	16.7	22	28	26	28.3	38
Q14566	MCM6	DNA replication licensing factor MCM6	20	20.8	17.6	17.6	22.7	28.8
Q14683	SMC1A	Structural maintenance of chromosomes protein 1A	13.3	24	36	48	30	48
Q14687	GSE1	Genetic suppressor element 1	15	28	80	78	91.7	66
Q14839	CHD4	Chromodomain-helicase-DNA-binding protein 4 (Mi2-beta)	61.7	80	92	110	81.7	96
Q14980	NUMA1	Nuclear mitotic apparatus protein 1	94.7	69.6	80	92.8	103	134
Q15020	SART3	Squamous cell carcinoma antigen recognized by T-cells 3	76.7	56	94	90	103	106
Q15029	U5S1	116 kDa U5 small nuclear ribonucleoprotein component	9	8.4	13.2	14.8	10.3	19.6
Q15056	IF4H	Eukaryotic translation initiation factor 4H	5.17	4.8	5.4	4.6	5.67	4
Q15233	NONO	Non-POU domain-containing octamer-binding protein (p54nrb)	17.6	21.3	30.7	28	30.2	33.3
Q15365	PCBP1	Poly(rC)-binding protein 1 (hnRNP E1)	5.16	4.8	5.33	5.23	7.29	4.48
Q15366	PCBP2	Poly(rC)-binding protein 2 (hnRNP E2)	4.3	4.09	4.8	4.44	5.93	4.89
Q15393	SF3B3	Splicing factor 3B subunit 3	23.3	28	34	46	26.7	36
Q15417	CNN3	Calponin-3	12	16	11.2	12	6	9.6
Q15459	SF3A1	Splicing factor 3A subunit 1	26.7	18	18	30	16.7	44
Q15596	NCOA2	Nuclear receptor coactivator 2 (SRC-2)	0	50	104	88	105	66
Q15637	SF01	Splicing factor 1	23.3	28	56	64	63.3	64
Q15648	MED1	Mediator of RNA polymerase II transcription subunit 1 (Trap220)	1.67	12	32	38	30	26
Q15717	ELAV1	ELAV-like protein 1 (Hu-antigen R)	6.22	6.93	8.53	13.9	10.7	12.8
Q15788	NCOA1	Nuclear receptor coactivator 1 (SRC-1)	0	10	44	12	28.3	22
Q15843	NEDD8	NEDD8 (Neddylin) (Ubiquitin-like protein Nedd8)	8.33	26	20	16	16.7	28
Q16401	PSMD5	26S proteasome non-ATPase regulatory subunit 5	53.3	86	104	102	93.3	90
Q16531	DDB1	DNA damage-binding protein 1	23.3	24	24	36	30	28
Q16576	RBBP7	Histone-binding protein RBBP7	8.15	7.82	9.6	8.89	8.59	11.7
Q16629	SRSF7	Serine/arginine-rich splicing factor 7	20	30	32	32	28.3	22
Q29RF7	PDS5A	Sister chromatid cohesion protein PDS5 homolog A	23.3	20	24	40	26.7	40
Q52LJ0	FA98B	Protein FAM98B	31.7	26	22	22	31.7	24
Q53EL6	PDCD4	Programmed cell death protein 4	15	12	20	32	23.3	38
Q58FF6	H90B4	Putative heat shock protein HSP 90-beta 4	6.22	5.69	2.49	2.31	2.52	2.31
Q5VTR2	BRE1A	E3 ubiquitin-protein ligase BRE1A	46.7	58	80	78	70	88
Q69YN2	C19L1	CWF19-like protein 1 (C19L1)	18.3	20	32	30	20	20
Q6ISB3	GRHL2	Grainyhead-like protein 2 homolog	3.33	48	108	88	68.3	68
Q6NXG1	ESRP1	Epithelial splicing regulatory protein 1	4	9.6	16.8	12.8	12	8.8

Table 1 (continued):

			IgG FC					
Uniprot ID	Gene name	Description	T00	T01	T05	T15	T30	T60
Q6P1J9	CDC73	Parafibromin (Cell division cycle protein 73 homolog)	16.7	34	24	38	16.7	38
Q6P2Q9	PRP8	Pre-mRNA-processing-splicing factor 8	20	26	26	36	26.7	34
Q6PJG2	EMSA1	ELM2 and SANT domain-containing protein 1	3.33	20	42	34	61.7	38
Q6PJT7	ZC3HE	Zinc finger CCCH domain-containing protein 14	10	12	18	28	23.3	28
Q6UXN9	WDR82	WD repeat-containing protein 82 (Protein TMEM113)	11.7	18	28	22	18.3	20
Q7L014	DDX46	Probable ATP-dependent RNA helicase DDX46	38.7	43.2	48	73.6	37.3	65.6
Q7L775	EPMIP	EPM2A-interacting protein 1	16.7	28	28	26	38.3	26
Q7Z5L9	I2BP2	Interferon regulatory factor 2-binding protein 2	31.7	40	52	54	73.3	74
Q86V15	CASZ1	Zinc finger protein castor homolog 1	1.67	8	28	32	26.7	26
Q86VP6	CAND1	Cullin-associated NEDD8-dissociated protein 1	7.56	11.7	16.5	10.1	6.22	11.7
Q86YP4	P66A	Transcriptional repressor p66-alpha (Gatad2a)	4	14.4	14.4	27.2	20	22.4
Q8IU81	I2BP1	Interferon regulatory factor 2-binding protein 1(Probable E3 ubiquitin-protein ligase IRF2BP1)	10	26	50	38	65	60
Q8IX12	CCAR1	Cell division cycle and apoptosis regulator protein 1	11.7	12	18	26	18.3	26
Q8N163	CCAR2	Cell cycle and apoptosis regulator protein 2 (DBC-1)	45	38	54	52	60	66
Q8TAT6	NPL4	Nuclear protein localization protein 4 homolog	33.3	32	44	36	48.3	36
Q8TE68	ES8L1	Epidermal growth factor receptor kinase substrate 8-like protein 1	5	4	8	6	4	6
Q8WX92	NELFB	Negative elongation factor B (NELF-B) (Cofactor of BRCA1)	25	26	42	28	23.3	36
Q8WXF1	PSPC1	Paraspeckle component 1	23.3	38	42	38	48.3	58
Q8WXI9	P66B	Transcriptional repressor p66-beta (Gatad2b)	7.47	9.92	16	15.7	14.7	15
Q92499	DDX1	ATP-dependent RNA helicase DDX1	68	65.6	75.2	75.2	60	76.8
Q92769	HDAC2	Histone deacetylase 2 (HD2) (EC 3.5.1.98)	2.33	7.2	12.8	8.8	9.33	12
Q92793	CBP	CREB-binding protein (EC 2.3.1.48)	0	28	96	86	107	72
Q92804	RBP56	TATA-binding protein-associated factor 2N	26.7	27.2	44.8	43.2	29.3	36.8
Q92841	DDX17	Probable ATP-dependent RNA helicase DDX17 (EC 3.6.4.13)	7.43	11.2	12.3	11.7	11.1	13.7
Q92878	RAD50	DNA repair protein RAD50 (hRAD50) (EC 3.6.-.-)	110	106	102	122	102	172
Q92882	OSTF1	Osteoclast-stimulating factor 1	22.7	28.8	33.6	27.2	28	25.6
Q92890	UFD1	Ubiquitin fusion degradation protein 1 homolog	14.7	16	20.8	16	17.3	17.6

Table 1 (continued):

			IgG FC					
Uniprot ID	Gene name	Description	T00	T01	T05	T15	T30	T60
Q92925	SMRD2	SMARCD2 (BAF60B)	0	16	56	30	33.3	40
Q92945	FUBP2	Far upstream element-binding protein 2	9.14	9.83	11.3	10.5	12.7	11.8
Q93009	UBP7	Ubiquitin carboxyl-terminal hydrolase 7 (EC 3.4.19.12)	56.7	90	100	78	66.7	68
Q93074	MED12	Mediator of RNA polymerase II transcription subunit 12 (Trap230)	5	10	30	20	28.3	22
Q969G3	SMCE1	SMARCE1 (BAF57)	3.33	12	24	20	31.7	36
Q96AE4	FUBP1	Far upstream element-binding protein 1	19.6	19.2	32.5	25.1	27.6	30.4
Q96I24	FUBP3	Far upstream element-binding protein 3	30.7	62.4	76.8	67.2	69.3	54.4
Q96I25	SPF45	Splicing factor 45	13.3	10	22	22	25	32
Q96IU4	ABHEB	Alpha/beta hydrolase domain-containing protein 14B	33.3	44	44	30	41.7	52
Q96KR1	ZFR	Zinc finger RNA-binding protein	8.33	6	12	24	18.3	18
Q96PK6	RBM14	RNA-binding protein 14	33.3	54	80	76	86.7	72
Q96RN5	MED15	Mediator of RNA polymerase II transcription subunit 15	10	16	30	18	25	24
Q99459	CDC5L	Cell division cycle 5-like protein	26.7	20	16	30	33.3	54
Q99729	ROAA	Heterogeneous nuclear ribonucleoprotein A/B (hnRNP A/B)	23.3	24	50	44	38.3	42
Q99829	CPNE1	Copine-1	5	6.4	7.6	6	7	5.6
Q99873	ANM1	Protein arginine N-methyltransferase 1 (EC 2.1.1.-)	21.7	24	32	24	23.3	24
Q9BQ04	RBM4B	RNA-binding protein 4B	20	24	48	46	46.7	44
Q9BRA2	TXD17	Thioredoxin domain-containing protein 17	16	17.6	17.6	12.8	21.3	20.8
Q9BTT0	AN32E	Acidic leucine-rich nuclear phosphoprotein 32 family member E	25	22	32	20	25	30
Q9BUF5	TBB6	Tubulin beta-6 chain (Tubulin beta class V)	4.5	6.6	6	3.6	3.33	4.8
Q9BUJ2	HNRL1	Heterogeneous nuclear ribonucleoprotein U-like protein 1	30	32	46	52	51.7	30
Q9BWF3	RBM4	RNA-binding protein 4	6.4	8	11.8	10.9	12.3	10.9
Q9BXP5	SRRT	Serrate RNA effector molecule homolog	33.3	22	24	32	36.7	32
Q9BY42	RTF2	Protein RTF2 homolog	20	16	20	16	11.7	16
Q9BZK7	TBL1R	F-box-like/WD repeat-containing protein TBL1XR1	35	54	90	78	93.3	70
Q9BZZ5	API5	Apoptosis inhibitor 5	20	32	28	38	21.7	40
Q9H0D6	XRN2	5'-3' exoribonuclease 2	8.33	8	14	20	25	24
Q9H147	TDIF1	Deoxynucleotidyltransferase terminal-interacting protein 1	0	4	28	22	28.3	22
Q9H1K0	RBNS5	Rabenosyn-5	25	16	24	32	25	22
Q9H2P0	ADNP	Activity-dependent neuroprotector homeobox protein	33.3	20	36	48	43.3	38
Q9H444	CHM4B	Charged multivesicular body protein 4b	20	24	22	24	20	34
Q9H6T0	ESRP2	Epithelial splicing regulatory protein 2	4.67	6.4	8	13.6	12.7	9.6

Table 1 (continued):

			IgG FC					
Uniprot ID	Gene name	Description	T00	T01	T05	T15	T30	T60
Q9HAH7	FBRS	Probable fibrosin-1	3.33	10	20	18	23.3	14
Q9HAV4	XPO5	Exportin-5 (Ran-binding protein 21)	11.7	24	30	32	25	22
Q9HB71	CYBP	Calcyclin-binding protein	13.3	12.3	8.53	7.47	5.78	10.1
Q9NR45	SIAS	Sialic acid synthase	36	40	43.2	44.8	46.7	51.2
Q9NVS9	PNPO	Pyridoxine-5'-phosphate oxidase	76.7	76	90	44	48.3	80
Q9NXG2	THUM1	THUMP domain-containing protein 1	20	16	16	18	11.7	24
Q9NZU5	LMCD1	LIM and cysteine-rich domains protein 1 (Dyxin)	33.3	24	44	34	36.7	34
Q9P0W2	HM20B	SMARCE1-related protein	5	16	44	38	45	38
Q9P258	RCC2	Protein RCC2	10.7	11.2	16	12.8	20	19.2
Q9P2J5	SYLC	Leucine--tRNA ligase, cytoplasmic	9.33	12	10.4	8.8	5.33	5.6
Q9P2N5	RBM27	RNA-binding protein 27	5	16	14	32	36.7	34
Q9UHD8	SEPT9	Septin-9	6.4	6.4	6.72	4.8	4	4.48
Q9UHF7	TRPS1	Zinc finger transcription factor Trps1	22	28.3	46.1	39.7	48.9	47.2
Q9UI30	TR112	Multifunctional methyltransferase subunit TRM112-like protein	13.3	14	18	12	6.67	22
Q9UKM9	RALY	RNA-binding protein Raly (HNRNPC2)	26.7	34	28	36	41.7	40
Q9UKN8	TF3C4	General transcription factor 3C polypeptide 4	23.3	20	36	44	41.7	36
Q9UMS4	PRP19	Pre-mRNA-processing factor 19 (EC 6.3.2.-)	61.7	42	68	56	81.7	94
Q9UN79	SOX13	Transcription factor SOX-13 (SRY)	10	24	28	22	35	24
Q9UPN9	TRI33	E3 ubiquitin-protein ligase TRIM33	5	18	36	26	30	20
Q9UPT8	ZC3H4	Zinc finger CCCH domain-containing protein 4	16.7	24	20	24	16.7	28
Q9UQE7	SMC3	Structural maintenance of chromosomes protein 3	11.7	10	22	24	18.3	28
Q9Y224	CN166	UPF0568 protein C14orf166 (CLE7 homolog)	28.3	32	60	40	40	72
Q9Y230	RUVB2	RuvB-like 2 (INO80 complex subunit J)	12.3	14.8	15.6	17.2	15.7	19.6
Q9Y265	RUVB1	RuvB-like 1 (INO80 complex subunit H)	20.7	20	24	20	19.3	20.8
Q9Y2L1	RRP44	Exosome complex exonuclease RRP44	26.7	34	34	24	23.3	26
Q9Y2W1	TR150	Thyroid hormone receptor-associated protein 3 (Trap150)	11.3	10.4	16	10.4	21.3	23.2
Q9Y2X9	ZN281	Zinc finger protein 281	6.67	22	68	48	58.3	52
Q9Y383	LC7L2	Putative RNA-binding protein Luc7-like 2	9.33	8.53	10.7	10.7	11.6	13.9
Q9Y3I0	RTCB	tRNA-splicing ligase RtcB homolog (EC 6.5.1.3)	21.8	21.3	22.9	16	16.4	19.2
Q9Y618	NCOR2	Nuclear receptor corepressor 2	31.7	74	130	124	152	132
Q9Y6Q9	NCOA3	Nuclear receptor coactivator 3(SRC-3)	0	58	176	152	155	86
Q9Y6X2	PIAS3	E3 SUMO-protein ligase PIAS3 (Protein inhibitor of activated STAT protein 3)	0	14	32	24	30	14

Table 2, moderate confidence interactors with the IgG FC for every time point:

			IgG FC					
Uniprot ID	Gene name	Description	T00	T01	T05	T15	T30	T60
O00170	AIP	AH receptor-interacting protein	20	8	8	6	6.67	8
O00193	SMAP	Small acidic protein	16.7	6	6	8	16.7	14
O00267	SPT5H	Transcription elongation factor SPT5	8.33	4	14	12	13.3	20
O00299	CLIC1	Chloride intracellular channel protein 1	21.7	10	16	18	11.7	12
O00571	DDX3X	ATP-dependent RNA helicase DDX3X	4.67	6	6.8	6	3.33	6.8
O14745	NHRF1	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	4	2.49	1.96	2.31	1.93	4.27
O14776	TCRG1	Transcription elongation regulator 1	11.7	8	12	8	6.67	18
O15417	TNC18	Trinucleotide repeat-containing gene 18 protein	1.67	6	16	18	18.3	16
O43175	SERA	D-3-phosphoglycerate dehydrogenase	4	3.48	2.82	2.26	2.12	2.45
O43395	PRPF3	U4/U6 small nuclear ribonucleoprotein Prp3	6.67	12	14	12	13.3	20
O60306	AQR	Intron-binding protein aquarius	10	20	28	36	18.3	24
O60869	EDF1	Endothelial differentiation-related factor 1	13.3	14.4	9.6	11.2	10.7	16
O60934	NBN	Nibrin (Cell cycle regulatory protein p95)	16.7	12	12	20	13.3	16
O75340	PDCD6	Programmed cell death protein 6	2	7.2	9.6	5.6	10.7	4.8
O75937	DNJC8	DnaJ homolog subfamily C member 8	6.67	16	12	12	10	16
O95232	LC7L3	Luc7-like protein 3	16.7	20	28	30	16.7	26
P00338	LDHA	L-lactate dehydrogenase A chain	4	8	5.2	3.2	5.67	6
P04075	ALDOA	Fructose-bisphosphate aldolase A	3.45	3.42	3.03	2.76	2.71	2.7
P04406	G3P	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	2.29	2.43	2.69	1.79	2.67	2.75
P05388	RLA0	60S acidic ribosomal protein P0	4.44	7.47	7.47	6.4	5.33	5.87
P05455	LA	Lupus La protein	11.7	12	12	20	11.7	20
P07305	H10	Histone H1.0	6.22	2.67	3.73	6.4	5.33	4.8
P07355	ANXA2	Annexin A2	3	3.2	2.6	2.49	2.64	2.83
P09661	RU2A	U2 small nuclear ribonucleoprotein A' (U2 snRNP A')	13.3	10	12	10	15	18
P09960	LKHA4	Leukotriene A-4 hydrolase (LTA-4 hydrolase) (EC 3.3.2.6)	29.3	32	17.6	22.4	13.3	24
P0C0S5	H2AZ	Histone H2A.Z (H2A/z)	1.6	1.71	1.81	1.92	1.69	2.24
P0CW22	RS17L	40S ribosomal protein S17-like	4	3.84	3.52	5.12	2.4	4.16
P11388	TOP2A	DNA topoisomerase 2-alpha	15	20	22	18	16.7	22
P12268	IMDH2	Inosine-5'-monophosphate dehydrogenase 2	0.72	1.6	1.85	2.58	0.72	2.71
P13489	RINI	Ribonuclease inhibitor	2.1	3.43	3.66	2.74	2.48	2.97
P13984	T2FB	General transcription factor IIF subunit 2	8.33	12	18	10	11.7	18
P14174	MIF	Macrophage migration inhibitory factor	4.33	4	4	3.6	4.33	3.6
P14625	ENPL	Endoplasmin (HSP90B1)	3.33	2.93	1.87	1.33	2	2.13

Table 2 (continued):

			IgG FC					
Uniprot ID	Gene name	Description	T00	T01	T05	T15	T30	T60
P14678	RSMB	Small nuclear ribonucleoprotein-associated proteins B and B'	8.33	10	28	18	18.3	12
P15531	NDKA	Nucleoside diphosphate kinase A (NDK A)	3.67	3.4	3.2	1.6	2.83	4.2
P15880	RS2	40S ribosomal protein S2	3.81	2.97	3.89	3.89	2.67	3.43
P17480	UBF1	Nucleolar transcription factor 1	5	10	12	20	21.7	14
P18621	RL17	60S ribosomal protein L17	18.3	12	12	30	11.7	18
P18887	XRCC1	DNA repair protein XRCC1	16.7	8	8	18	11.7	20
P21266	GSTM3	Glutathione S-transferase Mu 3	2.93	4.8	4.8	3.52	2.4	3.52
P21291	CSRP1	Cysteine and glycine-rich protein 1	12	9.6	14.4	11.2	9.33	6.4
P23396	RS3	40S ribosomal protein S3	2.59	2.73	2.54	3.01	1.8	3.29
P23526	SAHH	Adenosylhomocysteinase	3.54	3.62	4.03	3.48	2.9	3.27
P24941	CDK2	Cyclin-dependent kinase 2	2.67	4.27	4.27	3.2	3.11	2.67
P25787	PSA2	Proteasome subunit alpha type-2	6	7.2	7.2	8	2.67	9.6
P26373	RL13	60S ribosomal protein L13	2.12	2.35	2.07	2.64	2.51	3.29
P26640	SYVC	Valine--tRNA ligase	11.3	7.2	3.2	2.4	4	4
P26641	EF1G	Elongation factor 1-gamma	2.38	2.86	2.51	2.4	2.1	2.29
P27348	1433T	14-3-3 protein theta	2.67	4	2.8	3.2	3.67	4
P28370	SMCA1	Probable global transcription activator SNF2L1	15	14	26	22	21.7	22
P29590	PML	Promyelocytic leukemia protein	13.3	14	14	14	18.3	20
P30043	BLVRB	Flavin reductase (NADPH)	12	17.6	28.8	14.4	13.3	17.6
P30050	RL12	60S ribosomal protein L12	4	2.88	3.52	2.24	4.8	3.84
P31689	DNJA1	DnaJ homolog subfamily A member 1	23.3	20	26	14	8.33	16
P32969	RL9	60S ribosomal protein L9	13.3	16	11.2	14.4	8	11.2
P33240	CSTF2	Cleavage stimulation factor subunit 2	3.33	4	12	10	15	20
P33992	MCM5	DNA replication licensing factor MCM5	15	16	12	22	18.3	10
P35268	RL22	60S ribosomal protein L22	2.13	2.56	2.88	1.92	1.07	2.88
P35579	MYH9	Myosin-9	3.03	3.05	2.91	2.65	1.82	3.03
P35659	DEK	Protein DEK	3.33	16	24	22	8.33	18
P37108	SRP14	Signal recognition particle 14 kDa protein	16.7	16	14	14	16.7	14
P39023	RL3	60S ribosomal protein L3	4.19	2.97	3.66	4.57	4.38	3.43
P40429	RL13A	60S ribosomal protein L13a	5.78	4.27	5.87	5.33	4.89	6.4
P41182	BCL6	B-cell lymphoma 6 protein	0	4	20	20	16.7	16
P42167	LAP2B	Lamina-associated polypeptide 2, isoforms beta/gamma	3.29	2.88	3.2	3.41	3.2	3.52
P42285	SK2L2	Superkiller viralicidic activity 2-like 2	8.33	10	10	18	13.3	16
P43246	MSH2	DNA mismatch repair protein Msh2	20	16	24	20	10	16
P46776	RL27A	60S ribosomal protein L27a	1.63	1.78	1.96	2.31	1.63	1.96
P46778	RL21	60S ribosomal protein L21	4.27	4.16	3.52	3.84	4	4.48
P46940	IQGA1	Ras GTPase-activating-like protein	0.97	2.04	1.16	0.73	0.61	1.16
P47897	SYQ	Glutamine--tRNA ligase	6	6.4	5.6	3.2	2.67	4
P48643	TCPE	T-complex protein 1 subunit epsilon	15	36	26	18	10	32
P49588	SYAC	Alanine--tRNA ligase, cytoplasmic	5.07	4.16	0.64	1.92	1.33	0.96
P49736	MCM2	DNA replication licensing factor MCM2	20	14	18	32	21.7	34

Table 2 (continued):

			IgG FC					
Uniprot ID	Gene name	Description	T00	T01	T05	T15	T30	T60
P49792	RBP2	E3 SUMO-protein ligase RanBP2	2	9.6	3.2	3.2	0.67	0
P49959	MRE11	Double-strand break repair protein MRE11A	26.7	26	30	36	15	36
P50914	RL14	60S ribosomal protein L14	3.11	2.13	3.2	4.27	4.89	4.8
P50991	TCPD	T-complex protein 1 subunit delta	5.67	6.8	7.2	6.4	2	4.4
P51114	FXR1	Fragile X mental retardation syndrome-related protein 1	4.67	11.2	4.8	7.2	5.33	6.4
P51532	SMCA4	Transcription activator BRG1 (BAF190A) SMARCA4	5	4	10	20	15	20
P51570	GALK1	Galactokinase (EC 2.7.1.6)	11.7	22	16	12	16.7	20
P51858	HDGF	Hepatoma-derived growth factor (HMG-1L2)	13.3	18	16	20	11.7	30
P52943	CRIP2	Cysteine-rich protein 2	2.52	1.24	2.84	3.02	3.11	1.6
P53621	COPA	Coatomer subunit alpha	7.47	5.12	7.36	3.84	3.47	2.88
P54136	SYRC	Arginine--tRNA ligase	3.33	3.2	2	3.2	2.67	0.4
P55209	NP1L1	Nucleosome assembly protein 1-like 1	20	20	10	8	3.33	12
P58546	MTPN	Myotrophin (Protein V-1)	20	20	22	14	13.3	14
P60842	IF4A1	Eukaryotic initiation factor 4A-I	2.76	3.73	3.09	2.67	3.02	2.67
P60903	S10AA	Protein S100-A10	23.3	20	32	14	13.3	24
P61204	ARF3	ADP-ribosylation factor 3	6.22	6.93	8	6.4	5.33	6.4
P61247	RS3A	40S ribosomal protein S3a	2.93	3.52	2.56	6.08	4	6.08
P61254	RL26	60S ribosomal protein L26	4	4.48	3.52	4.16	3.73	5.44
P61313	RL15	60S ribosomal protein L15	2.67	2.8	2.8	3.6	3.33	1.6
P61353	RL27	60S ribosomal protein L27	1.87	1.28	2.88	2.56	2.13	2.56
P61962	DCAF7	DDB1- and CUL4-associated factor 7	0	10	24	24	16.7	12
P62191	PRS4	26S protease regulatory subunit 4	16.7	10	4	12	5	8
P62241	RS8	40S ribosomal protein S8	2.06	1.6	2.33	2.33	2.3	2.04
P62244	RS15A	40S ribosomal protein S15a	3.73	3.52	5.12	3.52	3.47	3.2
P62258	1433E	14-3-3 protein epsilon	2.46	2.83	2.71	1.97	2.26	2.58
P62263	RS14	40S ribosomal protein S14	2.22	3.02	3.02	2.67	2.07	2.31
P62266	RS23	40S ribosomal protein S23	10.7	9.6	11.2	9.6	13.3	12.8
P62273	RS29	40S ribosomal protein S29	5.33	4	4	4	6	4.8
P62277	RS13	40S ribosomal protein S13	4.67	9.6	6.4	9.6	8.67	5.6
P62280	RS11	40S ribosomal protein S11	12	11.2	6.4	12.8	12	9.6
P62304	RUXE	Small nuclear ribonucleoprotein E (snRNP-E)	10	14	20	10	20	16
P62318	SMD3	Small nuclear ribonucleoprotein Sm D3	4.44	6.4	5.33	4.8	4.89	4.8
P62328	TYB4	Thymosin beta-4	4	4.8	7.2	1.6	3.33	7.2
P62424	RL7A	60S ribosomal protein L7a	3.14	2.97	3.09	3.77	2.1	3.54
P62701	RS4X	40S ribosomal protein S4, X isoform	2.89	3.2	3.33	3.47	3	2.8
P62750	RL23A	60S ribosomal protein L23a	1.73	1.12	1.44	2.4	1.6	2.88
P62753	RS6	40S ribosomal protein S6	3.43	2.97	1.6	3.89	2.29	2.97
P62805	H4	Histone H4	1.72	2.22	2.06	2.03	2.05	2.28
P62877	RBX1	E3 ubiquitin-protein ligase RBX1	20	8	14	14	13.3	12
P62906	RL10A	60S ribosomal protein L10a (NEDD-6)	2	2.13	1.87	3.73	1.33	3.47

Table 2 (continued):

			IgG FC					
Uniprot ID	Gene name	Description	T00	T01	T05	T15	T30	T60
P62910	RL32	60S ribosomal protein L32	1.9	0.91	1.37	1.14	1.71	2.51
P62913	RL11	60S ribosomal protein L11	3.5	2	2	3.4	2.83	2
P62937	PPIA	Peptidyl-prolyl cis-trans isomerase A	2.71	2.68	2.87	2.93	3.05	2.96
P63104	1433Z	14-3-3 protein zeta/delta	2.51	2.64	2.92	2.45	2.51	2.82
P63167	DYL1	Dynein light chain 1, cytoplasmic	10	8	12	10	18.3	6
P63173	RL38	60S ribosomal protein L38	13.3	12	12	14	11.7	16
P63220	RS21	40S ribosomal protein S21	5.78	5.87	4.8	5.87	3.11	6.4
P63244	GBLP	Guanine nucleotide-binding protein subunit beta-2-like 1	4.89	6.4	5.87	4.8	6.22	3.73
P67809	YBOX1	Nuclease-sensitive element-binding protein 1	1.33	1.4	1.8	2	1.83	2
P68366	TBA4A	Tubulin alpha-4A chain	2.83	3.07	2.24	2.11	1.92	2.5
P68431	H31	Histone H3.1	2.56	3.07	2.8	3.07	2.11	2.67
P78337	PITX1	Pituitary homeobox 1	0	4	16	16	16.7	12
P83731	RL24	60S ribosomal protein L24	3.33	3.2	1.2	3.2	3	2.8
P84098	RL19	60S ribosomal protein L19	6.67	3.2	5.6	4.8	4.67	11.2
P84103	SRSF3	Serine/arginine-rich splicing factor 3	13.3	16	19.2	19.2	12	16
P98179	RBM3	RNA-binding protein 3	10	6	16	26	26.7	24
Q00341	VIGLN	Vigilin (High density lipoprotein-binding protein)	9.33	19.2	9.6	14.4	5.33	3.2
Q01081	U2AF1	Splicing factor U2AF 35 kDa subunit	6.67	2	14	16	25	6
Q02878	RL6	60S ribosomal protein L6	3.89	3.47	3.2	3.47	3.11	4.13
Q04724	TLE1	Transducin-like enhancer protein 1	8.33	12	20	18	18.3	18
Q04760	LGUL	Lactoylglutathione lyase	16	14.4	17.6	12.8	9.33	12.8
Q06587	RING1	E3 ubiquitin-protein ligase RING1	13.3	8	14	22	18.3	20
Q06830	PRDX1	Peroxiredoxin-1	3.33	3.52	2.8	2.48	2.93	2.32
Q07020	RL18	60S ribosomal protein L18	2	3.2	2.4	4	2	3.2
Q08170	SRSF4	Serine/arginine-rich splicing factor 4	2.67	4.27	3.2	2.13	4	3.73
Q09666	AHNK	Neuroblast differentiation-associated protein	2.83	4.1	2.4	3.07	1.47	3.4
Q10570	CPSF1	Cleavage and polyadenylation specificity factor subunit 1	5	8	8	16	11.7	8
Q12873	CHD3	Chromodomain-helicase-DNA-binding protein 3	16.7	18	30	26	21.7	22
Q12874	SF3A3	Splicing factor 3A subunit	10.7	6.4	6.4	20.8	16	12.8
Q12888	TP53B	Tumor suppressor p53-binding protein 1	20	14	2	22	10	18
Q13200	PSMD2	26S proteasome non-ATPase regulatory subunit 2	6.67	8	5.2	4	3	3.6
Q13310	PABP4	Polyadenylate-binding protein 4	5.33	9.07	6.4	5.87	2.67	4.27
Q13435	SF3B2	Splicing factor 3B subunit 2	23.3	8	14	30	23.3	54
Q13619	CUL4A	Cullin-4A	11.7	14	14	10	10	16
Q13620	CUL4B	Cullin-4B	13.3	12	14	10	5	8
Q14011	CIRBP	Cold-inducible RNA-binding protein (A18 hnRNP)	8	12.8	16	9.6	18.7	16
Q14204	DYHC1	Cytoplasmic dynein 1 heavy chain 1	3.56	4.62	1.78	1.24	1.78	2.49

Table 2 (continued):

			IgG FC					
Uniprot ID	Gene name	Description	T00	T01	T05	T15	T30	T60
Q14240	IF4A2	Eukaryotic initiation factor 4A-II	2.91	3.49	3.05	2.76	3.15	2.47
Q14686	NCOA6	Nuclear receptor coactivator 6	0	8	20	14	16.7	14
Q14847	LASP1	LIM and SH3 domain protein 1	2.93	2	2.32	2.32	2.47	2.64
Q14974	IMB1	Importin subunit beta-1	2.89	4	4	4.8	3.78	4.27
Q15046	SYK	Lysine--tRNA ligase	2.33	2.8	2.8	3.2	2	2.4
Q15287	RNPS1	RNA-binding protein with serine-rich domain 1	6.67	4	16	12	10	12
Q16181	SEPT7	Septin-7 (CDC10 protein homolog)	13.3	30	10	10	1.67	10
Q16543	CDC37	Hsp90 co-chaperone Cdc37	30	20	10	16	13.3	12
Q16630	CPSF6	Cleavage and polyadenylation specificity factor subunit 6	8.33	12	16	10	20	14
Q2TAY7	SMU1	WD40 repeat-containing protein SMU1	10	14	14	18	6.67	26
Q5JVF3	PCID2	PCI domain-containing protein 2	5	6	12	20	11.7	8
Q5TGY3	AHDC1	AT-hook DNA-binding motif-containing protein 1	0	6	20	14	18.3	8
Q68E01	INT3	Integrator complex subunit 3	16.7	14	22	16	13.3	28
Q6ZW49	PAXI1	PAX-interacting protein 1	0	6	22	16	20	22
Q71U36	TBA1A	Tubulin alpha-1A chain	2.6	2.69	2.11	2.19	1.79	2.23
Q7KZF4	SND1	Staphylococcal nuclease domain-containing protein 1	28.3	12	16	12	5	12
Q86U86	PB1	Protein polybromo-1 (BAF180)	5	6	20	18	13.3	14
Q86V81	THOC4	THO complex subunit 4	4.53	3.84	3.84	4.16	4.27	5.44
Q86XP3	DDX42	ATP-dependent RNA helicase DDX42	23.3	24	32	34	33.3	42
Q8IUE6	H2A2B	Histone H2A type 2-B	2.67	2.91	2.47	2.76	3.03	2.76
Q8N684	CPSF7	Cleavage and polyadenylation specificity factor subunit 7	6.67	2	6	18	16.7	8
Q8TAQ2	SMRC2	SWI/SNF complex subunit SMARCC2 (BAF170)	10	22	34	38	33.3	58
Q8WUM4	PDC6I	Programmed cell death 6-interacting protein	5.11	4	4	3.73	2	1.87
Q92616	GCN1L	Translational activator GCN1	5.6	5.12	2.24	1.92	1.87	0.32
Q92754	AP2C	Transcription factor AP-2 gamma	0	10	24	26	15	2
Q92973	TNPO1	Transportin-1	18.3	26	20	14	8.33	20
Q96EP5	DAZP1	DAZ-associated protein 1	10	18	6	16	20	12
Q96FQ6	S10AG	Protein S100-A16	10	18	22	12	13.3	16
Q96FW1	OTUB1	Ubiquitin thioesterase OTUB1	18.3	18	32	14	13.3	18
Q96FZ2	HMCES	Embryonic stem cell-specific 5-hydroxymethylcytosine-binding protein	5	4	20	24	20	22
Q96GM5	SMRD1	SMARCD1 (BAF60A)	0	6	20	6	6.67	12
Q96L91	EP400	E1A-binding protein p400	0	0	12	16	16.7	22
Q9BTC8	MTA3	Metastasis-associated protein MTA3	10.7	6.4	16	11.2	16	14.4
Q9BUQ8	DDX23	Probable ATP-dependent RNA helicase DDX23	15	16	16	24	11.7	16
Q9BY44	EIF2A	Eukaryotic translation initiation factor 2A	12	4.8	8	6.4	4	4.8

Table 2 (continued):

			IgG FC					
Uniprot ID	Gene name	Description	T00	T01	T05	T15	T30	T60
Q9H0L4	CSTFT	Cleavage stimulation factor subunit 2 tau variant	6.67	14	16	16	18.3	20
Q9H1B7	I2BPL	Interferon regulatory factor 2-binding protein-like	3.33	18	26	26	20	38
Q9H3P2	NELFA	Negative elongation factor A (NELF-A)	3.33	12	14	16	8.33	22
Q9H910	HN1L	Hematological and neurological expressed 1-like protein	4.33	3.6	3.2	4.8	3.33	3.6
Q9HCS7	SYF1	Pre-mRNA-splicing factor SYF1	15	12	20	26	18.3	26
Q9NP66	HM20A	High mobility group protein 20A	0	2	20	4	10	8
Q9NPJ6	MED4	Mediator of RNA polymerase II transcription subunit 4	0	12	18	16	16.7	26
Q9NR56	MBNL1	Muscleblind-like protein 1	5	6	18	30	16.7	16
Q9NTI5	PDS5B	Sister chromatid cohesion protein PDS5 homolog B	3.33	4	12	6	5	20
Q9NW64	RBM22	Pre-mRNA-splicing factor RBM22	5	4	2	12	20	14
Q9NYB0	TE2IP	Telomeric repeat-binding factor 2-interacting protein 1	15	14	14	18	15	16
Q9NYF8	BCLF1	Bcl-2-associated transcription factor 1	16.7	10	26	18	23.3	26
Q9P000	COMD9	COMM domain-containing protein 9	8.33	16	22	10	11.7	10
Q9P2K5	MYEF2	Myelin expression factor 2	8.33	12	12	18	13.3	14
Q9UHX1	PUF60	Poly(U)-binding-splicing factor PUF60	21.7	18	12	18	23.3	28
Q9UIG0	BAZ1B	Tyrosine-protein kinase BAZ1B	3.33	2	12	10	6.67	12
Q9UKL0	RCOR1	REST corepressor 1 (Protein CoREST)	0.89	3.2	6.4	4.8	4.89	4.27
Q9ULJ3	ZBT21	Zinc finger and BTB domain-containing protein 21	8.33	12	16	22	18.3	20
Q9UNS2	CSN3	COP9 signalosome complex subunit 3	13.3	10	10	2	5	4
Q9UQ35	SRRM2	Serine/arginine repetitive matrix protein 2	1.33	2	2.4	2.67	2	2.8
Q9UQ80	PA2G4	Proliferation-associated protein 2G4	24	8	9.6	20.8	2.67	4.8
Q9Y314	NOSIP	Nitric oxide synthase-interacting protein	15	14	20	22	18.3	16
Q9Y3A5	SBDS	Ribosome maturation protein SBDS	16.7	20	18	16	15	14
Q9Y3F4	STRAP	Serine-threonine kinase receptor-associated protein	11.7	6	12	10	8.33	6
Q9Y3U8	RL36	60S ribosomal protein L36	13.3	12.8	11.2	11.2	10.7	6.4
Q9Y5S9	RBM8A	RNA-binding protein 8A	5.33	4	5.6	3.2	6	9.6

Table 3: Clusters membership for high confidence proteins, not including basal cluster. Shadowed is highlighted the highest cluster membership of each protein.

		M-fuzz membership		
Uniprot ID	Gene name	cluster 1	cluster 2	cluster 3
O00148	DX39A	0,023	0,818	0,160
O00712	NFIB	0,054	0,763	0,183
O14686	KMT2D	0,029	0,615	0,357
O14929	HAT1	0,152	0,362	0,486
O14979	HNRDL	0,022	0,872	0,107
O14980	XPO1	0,355	0,406	0,239
O15042	SR140	0,075	0,475	0,450
O15294	OGT1	0,088	0,712	0,200
O15355	PPM1G	0,391	0,261	0,349
O15550	KDM6A	0,023	0,821	0,156
O43684	BUB3	0,082	0,657	0,261
O43707	ACTN4	0,832	0,078	0,089
O43809	CPSF5	0,118	0,513	0,369
O60244	MED14	0,131	0,560	0,309
O60264	SMCA5	0,060	0,193	0,747
O60341	KDM1A	0,026	0,546	0,428
O60563	CCNT1	0,078	0,376	0,546
O60884	DNJA2	0,688	0,137	0,176
O75150	BRE1B	0,026	0,460	0,514
O75367	H2AY	0,174	0,316	0,510
O75369	FLNB	0,885	0,053	0,062
O75376	NCOR1	0,073	0,485	0,442
O75448	MED24	0,022	0,856	0,122
O75531	BAF	0,412	0,299	0,289
O75533	SF3B1	0,025	0,697	0,278
O75643	U520	0,036	0,283	0,681
O75925	PIAS1	0,096	0,656	0,248
O94776	MTA2	0,060	0,597	0,343
O94992	HEXI1	0,092	0,292	0,617
O95340	PAPS2	0,201	0,415	0,384
O95396	MOCS3	0,113	0,659	0,228
O95758	PTBP3	0,029	0,828	0,142
O95983	MBD3	0,083	0,467	0,450
P04350	TBB4A	0,884	0,055	0,061
P05549	AP2A	0,081	0,727	0,192
P06748	NPM	0,133	0,198	0,670
P07437	TBB5	0,887	0,054	0,060
P07814	SYEP	0,745	0,109	0,146
P07900	HS90A	0,836	0,074	0,090
P08133	ANXA6	0,692	0,149	0,159
P08238	HS90B	0,832	0,076	0,092
P08621	RU17	0,075	0,568	0,358
P08651	NFIC	0,096	0,522	0,382
P09651	ROA1	0,023	0,770	0,206
P11586	C1TC	0,827	0,083	0,090
P11940	PABP1	0,847	0,073	0,080
P13639	EF2	0,895	0,046	0,060
P17844	DDX5	0,056	0,632	0,313
P17931	LEG3	0,193	0,525	0,282

Table 3 (continued):

		M-fuzz membership		
Uniprot ID	Gene name	cluster 1	cluster 2	cluster 3
P17987	TCPA	0,494	0,173	0,333
P18124	RL7	0,298	0,316	0,386
P18615	NELFE	0,042	0,603	0,355
P19338	NUCL	0,042	0,142	0,816
P19793	RXRA	0,157	0,602	0,241
P21333	FLNA	0,889	0,053	0,058
P22314	UBA1	0,075	0,176	0,749
P22392	NDKB	0,310	0,213	0,478
P22626	ROA2	0,028	0,693	0,279
P23193	TCEA1	0,085	0,190	0,725
P23246	SFPQ	0,053	0,616	0,332
P23771	GATA3	0,015	0,884	0,101
P24666	PPAC	0,374	0,359	0,267
P25205	MCM3	0,073	0,177	0,749
P26368	U2AF2	0,049	0,410	0,540
P26583	HMGB2	0,189	0,290	0,521
P27635	RL10	0,321	0,287	0,392
P27694	RFA1	0,061	0,552	0,387
P29401	TKT	0,317	0,225	0,458
P31942	HNRH3	0,076	0,451	0,473
P31948	STIP1	0,823	0,074	0,103
P33991	MCM4	0,325	0,361	0,314
P33993	MCM7	0,126	0,580	0,294
P36578	RL4	0,418	0,284	0,299
P38919	IF4A3	0,054	0,608	0,338
P39748	FEN1	0,252	0,294	0,455
P40763	STAT3	0,052	0,789	0,159
P43243	MATR3	0,065	0,514	0,421
P45974	UBP5	0,409	0,215	0,376
P46781	RS9	0,457	0,247	0,296
P49368	TCPG	0,469	0,302	0,229
P49585	PCY1A	0,135	0,213	0,652
P49756	RBM25	0,192	0,239	0,568
P49915	GUAA	0,199	0,357	0,444
P49916	DNLI3	0,288	0,202	0,511
P50395	GDIB	0,262	0,347	0,391
P50990	TCPQ	0,665	0,124	0,211
P51610	HCFC1	0,295	0,229	0,476
P51991	ROA3	0,016	0,849	0,135
P52209	6PGD	0,839	0,078	0,083
P52272	HNRPM	0,027	0,658	0,315
P53999	TCP4	0,094	0,293	0,613
P55072	TERA	0,226	0,211	0,563
P55265	DSRAD	0,102	0,486	0,412
P55317	FOXA1	0,085	0,711	0,204
P56545	CTBP2	0,031	0,397	0,571
P60981	DEST	0,341	0,362	0,297
P61956	SUMO2	0,158	0,544	0,298
P62081	RS7	0,303	0,235	0,461
P62136	PP1A	0,062	0,341	0,597
P62158	CALM	0,406	0,273	0,321

Table 3 (continued):

		M-fuzz membership		
Uniprot ID	Gene name	cluster 1	cluster 2	cluster 3
P62993	GRB2	0,032	0,823	0,144
P62995	TRA2B	0,254	0,402	0,344
P63165	SUMO1	0,035	0,730	0,235
P68371	TBB4B	0,894	0,050	0,056
P78347	GTF2I	0,089	0,615	0,296
P78371	TCPB	0,631	0,148	0,221
P82979	SARNP	0,143	0,208	0,649
P83916	CBX1	0,049	0,425	0,527
P84090	ERH	0,370	0,220	0,411
P98175	RBM10	0,037	0,469	0,494
Q00796	DHSO	0,696	0,159	0,145
Q01105	SET	0,120	0,268	0,612
Q01130	SRSF2	0,069	0,189	0,742
Q01826	SATB1	0,032	0,673	0,295
Q01844	EWS	0,160	0,462	0,378
Q02790	FKBP4	0,851	0,066	0,084
Q04726	TLE3	0,028	0,682	0,290
Q05048	CSTF1	0,018	0,891	0,091
Q07666	KHDR1	0,087	0,724	0,189
Q07955	SRSF1	0,039	0,167	0,795
Q08J23	NSUN2	0,711	0,130	0,159
Q09028	RBBP4	0,064	0,361	0,575
Q09472	EP300	0,022	0,878	0,100
Q12857	NFIA	0,112	0,518	0,370
Q12905	ILF2	0,114	0,246	0,639
Q12906	ILF3	0,115	0,448	0,437
Q12996	CSTF3	0,065	0,529	0,406
Q13057	COASY	0,730	0,140	0,130
Q13148	TADBP	0,038	0,526	0,436
Q13151	ROA0	0,067	0,351	0,582
Q13242	SRSF9	0,083	0,726	0,192
Q13330	MTA1	0,103	0,592	0,306
Q13363	CTBP1	0,064	0,681	0,255
Q13451	FKBP5	0,798	0,090	0,112
Q13526	PIN1	0,035	0,602	0,363
Q13547	HDAC1	0,028	0,667	0,304
Q13573	SNW1	0,030	0,811	0,159
Q13838	DX39B	0,043	0,429	0,528
Q14498	RBM39	0,046	0,261	0,693
Q14566	MCM6	0,228	0,253	0,519
Q14683	SMC1A	0,050	0,294	0,656
Q14687	GSE1	0,023	0,845	0,132
Q14839	CHD4	0,087	0,430	0,483
Q14980	NUMA1	0,132	0,227	0,640
Q15020	SART3	0,066	0,407	0,527
Q15029	U5S1	0,057	0,160	0,784
Q15233	NONO	0,026	0,623	0,351
Q15365	PCBP1	0,220	0,468	0,312
Q15393	SF3B3	0,136	0,357	0,507
Q15417	CNN3	0,807	0,088	0,105
Q15459	SF3A1	0,212	0,198	0,590

Table 3 (continued):

		M-fuzz membership		
Uniprot ID	Gene name	cluster 1	cluster 2	cluster 3
Q15596	NCOA2	0,032	0,851	0,117
Q15637	SF01	0,029	0,611	0,360
Q15648	MED1	0,035	0,753	0,212
Q15717	ELAV1	0,061	0,290	0,649
Q15788	NCOA1	0,091	0,685	0,224
Q15843	NEDD8	0,250	0,325	0,426
Q16401	PSMD5	0,059	0,729	0,212
Q16531	DDB1	0,134	0,391	0,475
Q16629	SRSF7	0,226	0,508	0,267
Q29RF7	PDS5A	0,088	0,212	0,700
Q53EL6	PDCD4	0,047	0,164	0,789
Q58FF6	H90B4	0,818	0,083	0,099
Q5VTR2	BRE1A	0,035	0,402	0,564
Q69YN2	C19L1	0,204	0,483	0,313
Q6ISB3	GRHL2	0,052	0,758	0,190
Q6NXG1	ESRP1	0,095	0,703	0,201
Q6P1J9	CDC73	0,263	0,263	0,474
Q6P2Q9	PRP8	0,087	0,289	0,624
Q6PJG2	EMSA1	0,047	0,752	0,201
Q6PJT7	ZC3HE	0,043	0,295	0,662
Q6UXN9	WDR82	0,108	0,630	0,262
Q7L014	DDX46	0,146	0,260	0,594
Q7L775	EPMIP	0,125	0,596	0,279
Q7Z5L9	I2BP2	0,055	0,438	0,507
Q86V15	CASZ1	0,027	0,750	0,223
Q86VP6	CAND1	0,377	0,321	0,302
Q86YP4	P66A	0,075	0,421	0,504
Q8IU81	I2BP1	0,042	0,659	0,298
Q8IX12	CCAR1	0,046	0,222	0,732
Q8N163	CCAR2	0,057	0,318	0,625
Q8TAT6	NPL4	0,116	0,624	0,259
Q8TE68	ES8L1	0,238	0,377	0,385
Q8WX92	NELFB	0,230	0,372	0,398
Q8WXF1	PSPC1	0,070	0,383	0,548
Q8WXI9	P66B	0,018	0,829	0,153
Q92769	HDAC2	0,049	0,669	0,282
Q92793	CBP	0,018	0,884	0,098
Q92804	RBP56	0,125	0,474	0,401
Q92841	DDX17	0,075	0,438	0,487
Q92878	RAD50	0,168	0,193	0,640
Q92925	SMRD2	0,046	0,749	0,205
Q93009	UBP7	0,361	0,382	0,257
Q93074	MED12	0,023	0,869	0,108
Q969G3	SMCE1	0,044	0,484	0,472
Q96AE4	FUBP1	0,053	0,617	0,329
Q96I24	FUBP3	0,095	0,699	0,207
Q96I25	SPF45	0,040	0,257	0,703
Q96IU4	ABHEB	0,260	0,326	0,413
Q96KR1	ZFR	0,079	0,384	0,538
Q96PK6	RBM14	0,019	0,881	0,100
Q96RN5	MED15	0,053	0,752	0,195

Table 3 (continued):

		M-fuzz membership		
Uniprot ID	Gene name	cluster 1	cluster 2	cluster 3
Q99459	CDC5L	0,143	0,220	0,638
Q99729	ROAA	0,043	0,691	0,266
Q99829	CPNE1	0,171	0,601	0,228
Q9BQ04	RBM4B	0,017	0,845	0,137
Q9BRA2	TXD17	0,239	0,367	0,394
Q9BTT0	AN32E	0,262	0,353	0,385
Q9BUF5	TBB6	0,741	0,127	0,133
Q9BUJ2	HNRL1	0,128	0,599	0,273
Q9BWF3	RBM4	0,014	0,905	0,082
Q9BXP5	SRRT	0,216	0,326	0,459
Q9BY42	RTF2	0,701	0,135	0,164
Q9BZK7	TBL1R	0,026	0,871	0,104
Q9BZZ5	API5	0,185	0,261	0,554
Q9H0D6	XRN2	0,056	0,398	0,546
Q9H147	TDIF1	0,017	0,879	0,104
Q9H1K0	RBNS5	0,199	0,379	0,422
Q9H2P0	ADNP	0,110	0,423	0,467
Q9H444	CHM4B	0,181	0,210	0,609
Q9H6T0	ESRP2	0,081	0,524	0,395
Q9HAH7	FBRS	0,036	0,828	0,136
Q9HAV4	XPO5	0,105	0,629	0,266
Q9HB71	CYBP	0,864	0,057	0,079
Q9NVS9	PNPO	0,664	0,152	0,184
Q9NXG2	THUM1	0,403	0,185	0,412
Q9NZU5	LMCD1	0,158	0,504	0,338
Q9P0W2	HM20B	0,011	0,913	0,076
Q9P258	RCC2	0,075	0,500	0,425
Q9P2J5	SYLC	0,784	0,109	0,108
Q9P2N5	RBM27	0,075	0,406	0,519
Q9UHD8	SEPT9	0,834	0,082	0,084
Q9UHF7	TRPS1	0,022	0,796	0,182
Q9UI30	TR112	0,387	0,218	0,395
Q9UKM9	RALY	0,125	0,377	0,498
Q9UKN8	TF3C4	0,056	0,580	0,364
Q9UMS4	PRP19	0,112	0,317	0,570
Q9UN79	SOX13	0,090	0,675	0,235
Q9UPN9	TRI33	0,051	0,806	0,143
Q9UPT8	ZC3H4	0,245	0,243	0,512
Q9UQE7	SMC3	0,035	0,220	0,744
Q9Y224	CN166	0,090	0,305	0,605
Q9Y230	RUVB2	0,047	0,202	0,751
Q9Y2W1	TR150	0,114	0,381	0,506
Q9Y2X9	ZN281	0,015	0,904	0,082
Q9Y383	LC7L2	0,054	0,213	0,734
Q9Y618	NCOR2	0,019	0,843	0,138
Q9Y6Q9	NCOA3	0,042	0,816	0,142
Q9Y6X2	PIAS3	0,059	0,784	0,157

Table 4, RU-486 induced interactors, with IgGFC for T05 and T30. It is also included the results for the T-test comparing RU486 and R5020 MS data.

Uniprot ID	Gene name	Description	T05 FC	T30 FC	T-test for T05	T-test for T30
O00148	DDX39A	ATP-dependent RNA helicase DDX39A	30	23.33	0.5293	0.0195
O14929	HAT1	Histone acetyltransferase type B catalytic subunit	20	30	0.0220	0.1411
O14979	HNRNPDL	Heterogeneous nuclear ribonucleoprotein D-like	31.78	40.33	0.9960	0.1935
O43143	DHX15	Pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15	60	103.33	0.3366	0.0383
O43175	PHGDH	D-3-phosphoglycerate dehydrogenase	5.5	5.17	0.0225	0.0044
O43390	HNRNPR	Heterogeneous nuclear ribonucleoprotein R	24.75	28.42	0.0412	0.0004
O43395	PRPF3	U4/U6 small nuclear ribonucleoprotein Prp3	23.33	26.67	0.0001	0.0171
O43396	TXNL1	Thioredoxin-like protein 1	20	30	0.0380	0.0123
O43684	BUB3	Mitotic checkpoint protein BUB3	33.33	63.33	0.1850	0.0038
O60341	KDM1A	Lysine-specific histone demethylase 1A	53.33	100	0.2819	0.0332
O60506	SYNCRIP	Heterogeneous nuclear ribonucleoprotein Q	11	12.47	0.0206	0.0001
O60884	DNAJA2	DnaJ homolog subfamily A member 2	30	23.33	0.5420	0.0127
O75150	RNF40	E3 ubiquitin-protein ligase BRE1B	63.33	90	0.0227	0.0008
O75376	NCOR1	Nuclear receptor corepressor 1	56.67	143.33	0.0756	0.0028
O75643	SNRNP200	U5 small nuclear ribonucleoprotein 200 kDa helicase	63.33	66.67	0.1283	0.2793
O75934	BCAS2	Pre-mRNA-splicing factor SPF27	33.33	46.67	0.1014	0.0002
O94776	MTA2	Metastasis-associated protein MTA2	30.56	39.11	0.0038	0.0122
O95833	CLIC3	Chloride intracellular channel protein 3	47.67	40.33	0.5260	0.1784
P00338	LDHA	L-lactate dehydrogenase A chain	6.72	7.33	0.3448	0.0356
P00558	PGK1	Phosphoglycerate kinase 1	4.99	6.01	0.8927	0.2289
P01859	IGHG2	Ig gamma-2 chain C region	4.4	4.4	#N/A	#N/A
P04908	HIST1H2AB	Histone H2A type 1-B/E	4.4	3.91	#N/A	#N/A
P05455	SSB	Lupus La protein	36.67	40	0.0006	0.0002

Table 4 (continued):

Uniprot ID	Gene name	Description	T05 FC	T30 FC	T-test for T05	T-test for T30
P05549	TFAP2A	Transcription factor AP-2-alpha	30	46.67	0.0918	0.0002
P06396	GSN	Gelsolin	13.62	16.76	0.0096	0.0000
P06401	PGR	Progesterone receptor	75.33	69.67	0.8437	0.0052
P06493	CDK1	Cyclin-dependent kinase 1	7.33	7.33	0.4333	0.1838
P06733	ENO1	Alpha-enolase	3.74	5.13	0.6316	0.0390
P06748	NPM1	Nucleophosmin	4.58	4.48	0.0084	0.0003
P07437	TUBB	Tubulin beta chain	3.41	2.78	0.8745	0.1432
P07737	PFN1	Profilin-1	5.33	6.33	0.4461	0.0170
P07900	HSP90AA1	Heat shock protein HSP 90-alpha	7.14	7.78	0.7383	0.0262
P07910	HNRNPC	Heterogeneous nuclear ribonucleoproteins C1/C2	7.74	8.96	0.0002	0.0068
P08238	HSP90AB1	Heat shock protein HSP 90-beta	5.53	5.83	0.1128	0.0058
P08397	HMBS	Porphobilinogen deaminase	26.67	20	0.1128	0.0008
P09429	HMGB1	High mobility group protein B1	12.53	13.44	0.0013	0.0608
P09651	HNRNPA1	Heterogeneous nuclear ribonucleoprotein A1	11	14	0.3513	0.1008
P09874	PARP1	Poly [ADP-ribose] polymerase 1	29.79	31.62	0.2236	0.0182
P0DMV8	HSPA1A	Heat shock 70 kDa protein 1A	42.78	56.22	#N/A	#N/A
P10809	HSPD1	60 kDa heat shock protein, mitochondrial	2.36	4.45	0.6528	0.0130
P11586	MTHFD1	C-1-tetrahydrofolate synthase, cytoplasmic	7.33	4.58	0.1477	0.6893
P12268	IMPDH2	Inosine-5'-monophosphate dehydrogenase 2	2.54	2.54	0.0211	0.0000
P12270	TPR	Nucleoprotein TPR	22	23.22	0.1532	0.0031
P12956	XRCC6	X-ray repair cross-complementing protein 6	55	67.22	0.0489	0.0004
P13010	XRCC5	X-ray repair cross-complementing protein 5	19.9	26.19	0.0130	0.0157
P13639	EEF2	Elongation factor 2	3.79	3.29	0.5465	0.0851
P14866	HNRNPL	Heterogeneous nuclear ribonucleoprotein L	31.53	42.53	0.1660	0.7119
P14923	JUP	Junction plakoglobin	2.54	2.82	0.4605	0.0080
P17844	DDX5	Probable ATP-dependent RNA helicase DDX5	11	13.62	0.7396	0.8400
P17931	LGALS3	Galectin-3	25.67	29.33	0.0162	0.0001
P17987	TCP1	T-complex protein 1 subunit alpha	9.78	14.67	0.1957	0.0124
P18124	RPL7	60S ribosomal protein L7	16.5	20.17	0.4673	0.9941

Table 4 (continued):

Uniprot ID	Gene name	Description	T05 FC	T30 FC	T-test for T05	T-test for T30
P18615	NELFE	Negative elongation factor E	36.67	43.33	0.0258	0.1823
P19338	NCL	Nucleolin	6.98	7.86	0.1326	0.0006
P22314	UBA1	Ubiquitin-like modifier-activating enzyme 1	34.83	55	0.8865	0.0549
P22626	HNRNPA2B1	Heterogeneous nuclear ribonucleoproteins A2/B1	7.18	8.1	0.0032	0.0521
P23246	SFPQ	Splicing factor, proline- and glutamine-rich	9.39	8.8	0.0146	0.0281
P23526	AHCY	Adenosylhomocysteinase	3.67	4.05	0.0168	0.0169
P23528	CFL1	Cofilin-1	4.81	4.58	0.0680	0.0326
P23771	GATA3	Trans-acting T-cell-specific transcription factor GATA-3	63.33	66.67	0.2786	0.3643
P24468	NR2F2	COUP transcription factor 2	23.33	36.67	0.0149	0.0245
P25205	MCM3	DNA replication licensing factor MCM3	46.67	73.33	0.9176	0.0063
P25398	RPS12	40S ribosomal protein S12	2.52	2.52	0.0910	0.0001
P25787	PSMA2	Proteasome subunit alpha type-2	8.56	7.33	0.1104	0.0006
P26368	U2AF2	Splicing factor U2AF 65 kDa subunit	33.33	60	0.6861	0.0002
P26583	HMGB2	High mobility group protein B2	18.33	20.17	0.0005	0.2377
P26599	PTBP1	Polypyrimidine tract-binding protein 1	45.22	63.56	0.2612	0.0923
P27348	YWHAQ	14-3-3 protein theta	5.5	7.33	0.4460	0.5821
P27694	RPA1	Replication protein A 70 kDa DNA-binding subunit	43.33	43.33	0.5568	0.2930
P29373	CRABP2	Cellular retinoic acid-binding protein 2	9.95	10.21	0.1039	0.0009
P29401	TKT	Transketolase	9.53	12.47	0.2316	0.0096
P30043	BLVRB	Flavin reductase (NADPH)	33.33	23.33	0.0302	0.2243
P30086	PEBP1	Phosphatidylethanolamine-binding protein 1	36.67	46.67	0.9385	0.1760
P30626	SRI	Sorcin	20	26.67	0.0560	0.0043
P31942	HNRNPH3	Heterogeneous nuclear ribonucleoprotein H3	106.67	113.33	0.0429	0.0032
P31943	HNRNPH1	Heterogeneous nuclear ribonucleoprotein H	4.01	4.58	0.0289	0.0014
P31948	STIP1	Stress-induced-phosphoprotein 1	43.33	53.33	0.1895	0.0745
P31949	S100A11	Protein S100-A11	3.91	3.42	0.2158	0.1110
P33993	MCM7	DNA replication licensing factor MCM7	40	66.67	0.9576	0.0399
P35637	FUS	RNA-binding protein FUS	20.17	23.83	0.0144	0.0266
P36578	RPL4	60S ribosomal protein L4	3.26	3.26	0.8662	0.1441
P38159	RBMX	RNA-binding motif protein, X chromosome	6.93	6.93	0.6252	0.1233

Table 4 (continued):

Uniprot ID	Gene name	Description	T05 FC	T30 FC	T-test for T05	T-test for T30
P38919	EIF4A3	Eukaryotic initiation factor 4A-III	24.44	24.44	0.0775	0.0259
P39023	RPL3	60S ribosomal protein L3	5.3	6.11	0.0087	0.0150
P39748	FEN1	Flap endonuclease 1	36.67	43.33	0.4103	0.0030
P40227	CCT6A	T-complex protein 1 subunit zeta	5.87	9.53	0.5708	0.0005
P40763	STAT3	Signal transducer and activator of transcription 3	22	31.78	0.1382	0.0568
P42166	TMPO	Lamina-associated polypeptide 2, isoform alpha	7.33	7.33	0.0383	0.0418
P43243	MATR3	Matrin-3	80.67	106.33	0.9277	0.0952
P43246	MSH2	DNA mismatch repair protein Msh2	30	40	0.6267	0.0246
P45973	CBX5	Chromobox protein homolog 5	46.67	53.33	0.0574	0.0029
P45974	USP5	Ubiquitin carboxyl-terminal hydrolase 5	26.67	30	0.5541	0.5980
P46777	RPL5	60S ribosomal protein L5	14.67	15.89	0.0094	0.0019
P46778	RPL21	60S ribosomal protein L21	4.71	5.24	0.0362	0.0024
P48643	CCT5	T-complex protein 1 subunit epsilon	46.67	56.67	0.0044	0.0036
P49368	CCT3	T-complex protein 1 subunit gamma	23.83	27.5	0.8495	0.0053
P49585	PCYT1A	Choline-phosphate cytidylyltransferase A	36.67	53.17	0.0024	0.0079
P49736	MCM2	DNA replication licensing factor MCM2	23.33	30	0.1172	0.0717
P49756	RBM25	RNA-binding protein 25	43.33	40	0.3342	0.0137
P49915	GMPS	GMP synthase [glutamine-hydrolyzing]	113.33	143.33	0.0554	0.0014
P50990	CCT8	T-complex protein 1 subunit theta	3.67	4.58	0.4056	0.0005
P51452	DUSP3	Dual specificity protein phosphatase 3	26.67	33.33	0.7119	0.1652
P51610	HCFC1	Host cell factor 1	60	90	0.0146	0.0029
P51991	HNRNPA3	Heterogeneous nuclear ribonucleoprotein A3	8.56	8.15	0.1143	0.0388
P52209	PGD	6-phosphogluconate dehydrogenase, decarboxylating	9.17	13.44	0.0753	0.0417
P52272	HNRNPM	Heterogeneous nuclear ribonucleoprotein M	11.54	13.58	0.0593	0.0333
P52597	HNRNPF	Heterogeneous nuclear ribonucleoprotein F	5.09	4.85	0.0060	0.0001
P52943	CRIP2	Cysteine-rich protein 2	2.44	2.85	0.7267	0.2320
P53396	ACLY	ATP-citrate synthase	5.5	6.97	0.4651	0.0307
P54920	NAPA	Alpha-soluble NSF attachment protein	20	20	0.8449	0.3294

Table 4 (continued):

Uniprot ID	Gene name	Description	T05 FC	T30 FC	T-test for T05	T-test for T30
P55060	CSE1L	Exportin-2	9.17	11.37	0.3959	0.0009
P55072	VCP	Transitional endoplasmic reticulum ATPase	25.67	42.17	0.1987	0.2121
P55795	HNRNPH2	Heterogeneous nuclear ribonucleoprotein H2	4.58	5.68	0.0045	0.0008
P55854	SUMO3	Small ubiquitin-related modifier 3	2.2	2.2	0.0105	0.0003
P60842	EIF4A1	Eukaryotic initiation factor 4A-I	3.44	3.21	0.8846	0.0112
P61956	SUMO2	Small ubiquitin-related modifier 2	2.2	2.57	0.0011	0.0000
P61978	HNRNPK	Heterogeneous nuclear ribonucleoprotein K	8.59	9.28	0.3681	0.0189
P61981	YWHAG	14-3-3 protein gamma	3.95	4.23	0.1618	0.2111
P62081	RPS7	40S ribosomal protein S7	6.6	8.8	0.6831	0.0021
P62158	CALM1	Calmodulin	3.97	8.25	0.9455	0.0247
P62249	RPS16	40S ribosomal protein S16	4.71	4.71	0.1897	0.0305
P62258	YWHAE	14-3-3 protein epsilon	4.79	5.08	0.7060	0.0680
P62424	RPL7A	60S ribosomal protein L7a	2.93	2.93	0.0255	0.0469
P62701	RPS4X	40S ribosomal protein S4, X isoform	2.36	2.1	0.0621	0.0236
P62753	RPS6	40S ribosomal protein S6	4.19	4.71	0.0567	0.0171
P62826	RAN	GTP-binding nuclear protein Ran	3.42	4.64	0.1751	0.3098
P62877	RBX1	E3 ubiquitin-protein ligase RBX1	33.33	36.67	0.3939	0.1081
P62937	PPIA	Peptidyl-prolyl cis-trans isomerase A	3.05	3.36	0.6964	0.0003
P62995	TRA2B	Transformer-2 protein homolog beta	23.33	43.33	0.2774	0.0462
P63165	SUMO1	Small ubiquitin-related modifier 1	40	43.33	0.0076	0.0002
P63241	EIF5A	Eukaryotic translation initiation factor 5A-1	25.67	20.17	0.9151	0.4531
P63261	ACTG1	Actin, cytoplasmic 2	5.78	6.91	#N/A	#N/A
P78347	GTF2I	General transcription factor II-I	66.67	123.33	0.0125	0.0117
P78371	CCT2	T-complex protein 1 subunit beta	5.19	9.17	0.0122	0.0001
P78527	PRKDC	DNA-dependent protein kinase catalytic subunit	53.78	66.41	0.0150	0.0335
P82979	SARNP	SAP domain-containing ribonucleoprotein	36.67	33.33	0.0000	0.0055
Q00839	HNRNPU	Heterogeneous nuclear ribonucleoprotein U	10.65	10.48	0.9427	0.0580
Q01130	SRSF2	Serine/arginine-rich splicing factor 2	7.33	8.56	0.0201	0.0032

Table 4 (continued):

Uniprot ID	Gene name	Description	T05 FC	T30 FC	T-test for T05	T-test for T30
Q01826	SATB1	DNA-binding protein SATB1	100	93.33	0.0995	0.0017
Q01844	EWSR1	RNA-binding protein EWS	22	23.83	0.2155	0.8367
Q02790	FKBP4	Peptidyl-prolyl cis-trans isomerase FKBP4	41.56	47.67	0.0034	0.0540
Q02878	RPL6	60S ribosomal protein L6	3.67	5.02	0.0420	0.0185
Q04726	TLE3	Transducin-like enhancer protein 3	33.33	33.33	0.2078	0.0455
Q06830	PRDX1	Peroxiredoxin-1	2.6	3.36	0.3033	0.3701
Q07666	KHDRBS1	KH domain-containing, RNA-binding, signal transduction-associated protein 1	63.33	46.67	0.0012	0.0307
Q08211	DHX9	ATP-dependent RNA helicase A	55.61	56.22	0.7839	0.1253
Q09028	RBBP4	Histone-binding protein RBBP4	23.22	26.89	0.6928	0.6472
Q09472	EP300	Histone acetyltransferase p300	26.67	36.67	0.5309	0.8152
Q12888	TP53BP1	Tumor suppressor p53-binding protein 1	23.33	26.67	0.0002	0.0002
Q12905	ILF2	Interleukin enhancer-binding factor 2	46.67	60	0.3697	0.0019
Q12906	ILF3	Interleukin enhancer-binding factor 3	13.44	24.44	0.1374	0.0713
Q12996	CSTF3	Cleavage stimulation factor subunit 3	43.33	40	0.9100	0.1203
Q13148	TARDBP	TAR DNA-binding protein 43	8.15	6.93	0.2915	0.0547
Q13151	HNRNPA0	Heterogeneous nuclear ribonucleoprotein A0	30	30	0.2957	0.0804
Q13185	CBX3	Chromobox protein homolog 3	22	18.33	0.0917	0.0002
Q13247	SRSF6	Serine/arginine-rich splicing factor 6	11	15.89	0.0004	0.0007
Q13263	TRIM28	Transcription intermediary factor 1-beta	43.54	54.54	0.9809	0.6646
Q13363	CTBP1	C-terminal-binding protein 1	63.33	63.33	0.0730	0.0056
Q13451	FKBP5	Peptidyl-prolyl cis-trans isomerase FKBP5	66.67	93.33	0.2877	0.0022
Q13547	HDAC1	Histone deacetylase 1	11.73	13.2	0.5250	0.0824
Q13573	SNW1	SNW domain-containing protein 1	23.33	46.67	0.1646	0.0061
Q14011	CIRBP	Cold-inducible RNA-binding protein	26.67	26.67	0.0002	0.0000
Q14103	HNRNPD	Heterogeneous nuclear ribonucleoprotein D0	12.83	15.89	0.6266	0.9559

Table 4 (continued):

Uniprot ID	Gene name	Description	T05 FC	T30 FC	T-test for T05	T-test for T30
Q14566	MCM6	DNA replication licensing factor MCM6	23.33	30	0.0245	0.0160
Q14683	SMC1A	Structural maintenance of chromosomes protein 1A	30	50	0.1314	0.0199
Q14687	GSE1	Genetic suppressor element 1	26.67	43.33	0.6038	0.2736
Q14839	CHD4	Chromodomain-helicase-DNA-binding protein 4	63.33	106.67	0.0481	0.0006
Q14974	KPNB1	Importin subunit beta-1	9.78	7.94	0.0328	0.0119
Q14980	NUMA1	Nuclear mitotic apparatus protein 1	172.33	183.33	0.0691	0.5134
Q15020	SART3	Squamous cell carcinoma antigen recognized by T-cells 3	63.33	76.67	0.0767	0.2794
Q15029	EFTUD2	116 kDa U5 small nuclear ribonucleoprotein component	14.67	20.17	0.9452	0.0173
Q15056	EIF4H	Eukaryotic translation initiation factor 4H	8.25	11.92	0.0185	0.0390
Q15233	NONO	Non-POU domain-containing octamer-binding protein	26.89	33	0.0081	0.0294
Q15365	PCBP1	Poly(rC)-binding protein 1	8.11	8.49	0.4996	0.0623
Q15366	PCBP2	Poly(rC)-binding protein 2	6.86	8.13	0.3112	0.0033
Q15393	SF3B3	Splicing factor 3B subunit 3	20	26.67	0.1729	0.5532
Q15459	SF3A1	Splicing factor 3A subunit 1	23.33	26.67	0.0001	0.1683
Q15637	SF1	Splicing factor 1	90	96.67	0.0991	0.0074
Q15717	ELAVL1	ELAV-like protein 1	9.78	11	0.1661	0.0426
Q15843	NEDD8	NEDD8	30	36.67	0.0037	0.0010
Q16401	PSMD5	26S proteasome non-ATPase regulatory subunit 5	96.67	100	0.7880	0.0652
Q16531	DDB1	DNA damage-binding protein 1	50	66.67	0.0069	0.0011
Q16576	RBBP7	Histone-binding protein RBBP7	14.26	17.52	0.0866	0.0069
Q16629	SRSF7	Serine/arginine-rich splicing factor 7	12.83	16.5	0.7053	0.0754
Q53EL6	PDCD4	Programmed cell death protein 4	23.33	40	0.5939	0.0004
Q5VTR2	RNF20	E3 ubiquitin-protein ligase BRE1A	56.67	66.67	0.1830	0.0570
Q68E01	INTS3	Integrator complex subunit 3	33.33	40	0.0000	0.0061
Q6ISB3	GRHL2	Grainyhead-like protein 2 homolog	33.33	53.33	0.0364	0.0173
Q6P1J9	CDC73	Parafibromin	43.33	56.67	0.0411	0.0023
Q6P2Q9	PRPF8	Pre-mRNA-processing-splicing factor 8	30	60	0.0119	0.0948

Table 4 (continued):

Uniprot ID	Gene name	Description	T05 FC	T30 FC	T-test for T05	T-test for T30
Q6UXN9	WDR82	WD repeat-containing protein 82	23.33	30	0.3041	0.0427
Q7L014	DDX46	Probable ATP-dependent RNA helicase DDX46	43.33	63.33	0.0154	0.0175
Q7Z5L9	IRF2BP2	Interferon regulatory factor 2-binding protein 2	40	46.67	0.0305	0.0009
Q86V81	ALYREF	THO complex subunit 4	2.75	3.67	0.0075	0.0040
Q86VP6	CAND1	Cullin-associated NEDD8-dissociated protein 1	20.78	40.33	0.2326	0.1658
Q8IU81	IRF2BP1	Interferon regulatory factor 2-binding protein 1	53.33	66.67	0.5357	0.4952
Q8N163	CCAR2	Cell cycle and apoptosis regulator protein 2	60	103.33	0.0353	0.0191
Q8TAQ2	SMARCC2	SWI/SNF complex subunit SMARCC2	36.67	76.67	0.1254	0.0895
Q8TAT6	NPLOC4	Nuclear protein localization protein 4 homolog	70	56.67	0.1536	0.0662
Q8WX92	NELFB	Negative elongation factor B	36.67	36.67	0.0953	0.0028
Q8WXI9	GATAD2B	Transcriptional repressor p66-beta	12.47	17.6	0.0557	0.0149
Q92499	DDX1	ATP-dependent RNA helicase DDX1	29.33	33	0.8507	0.0753
Q92769	HDAC2	Histone deacetylase 2	8.25	11	0.4067	0.1871
Q92841	DDX17	Probable ATP-dependent RNA helicase DDX17	10.48	9.95	0.3925	0.0844
Q92878	RAD50	DNA repair protein RAD50	116.67	126.67	0.0003	0.0103
Q92882	OSTF1	Osteoclast-stimulating factor 1	30	33.33	0.6481	0.0119
Q92925	SMARCD2	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 2	20	30	0.4082	0.1429
Q92945	KHSRP	Far upstream element-binding protein 2	15.98	16.24	0.0388	0.0871
Q93009	USP7	Ubiquitin carboxyl-terminal hydrolase 7	50	90	0.8103	0.0039
Q96AE4	FUBP1	Far upstream element-binding protein 1	44	50.11	0.0847	0.0111
Q96FW1	OTUB1	Ubiquitin thioesterase OTUB1	23.33	20	0.1507	0.0012
Q96I24	FUBP3	Far upstream element-binding protein 3	110	86.67	0.0154	0.1571
Q96KK5	HIST1H2AH	Histone H2A type 1-H	4.4	4.4	#N/A	#N/A
Q96PK6	RBM14	RNA-binding protein 14	83.33	66.67	0.0735	0.0001
Q96QV6	HIST1H2AA	Histone H2A type 1-A	7.33	7	#N/A	#N/A
Q99729	HNRNPAB	Heterogeneous nuclear ribonucleoprotein A/B	40	46.67	0.6771	0.2003
Q99829	CPNE1	Copine-1	7.33	9.17	0.4393	0.9043

Table 4 (continued):

Uniprot ID	Gene name	Description	T05 FC	T30 FC	T-test for T05	T-test for T30
Q99832	CCT7	T-complex protein 1 subunit eta	27.5	20.17	0.0015	0.0024
Q9BUJ2	HNRNPUL1	Heterogeneous nuclear ribonucleoprotein U-like protein 1	36.67	53.33	0.0001	0.0379
Q9BWF3	RBM4	RNA-binding protein 4	14.67	13.93	0.1604	0.1371
Q9BY42	RTFDC1	Protein RTF2 homolog	26.67	30	0.0014	0.0320
Q9BZK7	TBL1XR1	F-box-like/WD repeat-containing protein TBL1XR1	116.67	136.67	0.0643	0.1822
Q9BZZ5	API5	Apoptosis inhibitor 5	23.33	30	0.3511	0.5851
Q9H0A0	NAT10	N-acetyltransferase 10	20	26.67	0.0014	0.0005
Q9H1K0	RBSN	Rabenosyn-5	36.67	33.33	0.3701	0.2043
Q9H2P0	ADNP	Activity-dependent neuroprotector homeobox protein	36.67	60	0.7545	0.0148
Q9H6T0	ESRP2	Epithelial splicing regulatory protein 2	12.83	20.17	0.3349	0.2893
Q9NR45	NANS	Sialic acid synthase	26.67	63.33	0.0467	0.0150
Q9NVS9	PNPO	Pyridoxine-5'-phosphate oxidase	93.33	113.33	0.0047	0.0001
Q9NZU5	LMCD1	LIM and cysteine-rich domains protein 1	46.67	43.33	0.0071	0.0163
Q9UHF7	TRPS1	Zinc finger transcription factor Trps1	59.28	72.11	0.1285	0.0062
Q9UHX1	PUF60	Poly(U)-binding-splicing factor PUF60	43.33	33.33	0.8353	0.0598
Q9UKM9	RALY	RNA-binding protein Raly	36.67	43.33	0.0728	0.0740
Q9UKN8	GTF3C4	General transcription factor 3C polypeptide 4	26.67	30	0.4566	0.0926
Q9UMS4	PRPF19	Pre-mRNA-processing factor 19	86.67	90	0.0039	0.1074
Q9UPT8	ZC3H4	Zinc finger CCCH domain-containing protein 4	40	46.67	0.7187	0.0107
Q9UQE7	SMC3	Structural maintenance of chromosomes protein 3	30	36.67	0.7205	0.0588
Q9Y224	C14orf166	UPF0568 protein C14orf166	40	73.33	0.3806	0.0037
Q9Y230	RUVBL2	RuvB-like 2	11	18.33	0.0245	0.0248
Q9Y265	RUVBL1	RuvB-like 1	22	23.83	0.7548	0.0294
Q9Y2W1	THRAP3	Thyroid hormone receptor-associated protein 3	14.67	16.5	0.2694	0.1294
Q9Y2X9	ZNF281	Zinc finger protein 281	50	83.33	0.0642	0.0046
Q9Y3I0	RTCB	tRNA-splicing ligase RtcB homolog	15.89	18.33	0.7271	0.2282
Q9Y5Q9	GTF3C3	General transcription factor 3C polypeptide 3	20	20	0.2985	0.0041

Table 4 (continued):

Uniprot ID	Gene name	Description	T05 FC	T30 FC	T-test for T05	T-test for T30
Q9Y5S9	RBM8A	RNA-binding protein 8A	14.67	14.67	0.0001	0.2921
Q9Y618	NCOR2	Nuclear receptor corepressor 2	113.33	170	0.2243	0.0203
Q9Y6Q9	NCOA3	Nuclear receptor coactivator 3	43.33	66.67	0.3210	0.3639
Q9Y6X2	PIAS3	E3 SUMO-protein ligase PIAS3	50	53.33	0.0023	0.0015
O00170	AIP	AH receptor-interacting protein	16.67	23.33	0.2397	0.1107
O00712	NFIB	Nuclear factor 1 B-type	16.67	23.33	0.3365	0.2604
O14980	XPO1	Exportin-1	36.67	40	0.7009	0.0004
O15042	U2SURP	U2 snRNP-associated SURP motif-containing protein	26.67	63.33	0.9261	0.3875
O15294	OGT	UDP-N-acetylglucosamine--peptide N-acetylglucosaminyltransferase 110 kDa subunit	30	50	0.9301	0.7904
O15355	PPM1G	Protein phosphatase 1G	10	33.33	0.5808	0.0119
O43447	PPIH	Peptidyl-prolyl cis-trans isomerase H	26.67	16.67	0.0035	0.0011
O43670	ZNF207	BUB3-interacting and GLEBS motif-containing protein ZNF207	20	13.33	0.0116	0.0001
O60264	SMARCA5	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 5	16.67	26.67	0.2550	0.0931
O60306	AQR	Intron-binding protein aquarius	13.33	40	0.4821	0.0545
O60934	NBN	Nibrin	16.67	30	0.0003	0.0068
O60936	NOL3	Nucleolar protein 3	16.67	23.33	0.5367	0.0032
O75131	CPNE3	Copine-3	9.17	12.83	0.9182	0.4984
O75367	H2AFY	Core histone macro-H2A.1	14.67	20.17	0.4657	0.4852
O75531	BANF1	Barrier-to-autointegration factor	10	23.33	0.3700	0.0150
O95071	UBR5	E3 ubiquitin-protein ligase UBR5	10	20	0.0520	0.0000
O95340	PAPSS2	Bifunctional 3'-phosphoadenosine 5'-phosphosulfate synthase 2	16.67	26.67	0.1432	0.0005
O95400	CD2BP2	CD2 antigen cytoplasmic tail-binding protein 2	16.67	23.33	0.1987	0.3670
O95758	PTBP3	Polypyrimidine tract-binding protein 3	23.33	30	0.5272	0.0006
O95861	BPNT1	3'(2'),5'-bisphosphate nucleotidase 1	20	13.33	0.5998	0.7879
O95983	MBD3	Methyl-CpG-binding domain protein 3	23.33	46.67	0.0270	0.0003

Table 4 (continued):

Uniprot ID	Gene name	Description	T05 FC	T30 FC	T-test for T05	T-test for T30
P04406	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	2.33	3.56	0.2485	0.0548
P05198	EIF2S1	Eukaryotic translation initiation factor 2 subunit 1	9.17	14.67	0.0011	0.0082
P08708	RPS17	40S ribosomal protein S17	30	33.33	#N/A	#N/A
P08865	RPSA	40S ribosomal protein SA	0.49	2.2	0.3972	0.1652
P09661	SNRPA1	U2 small nuclear ribonucleoprotein A'	16.67	23.33	0.0139	0.0025
P09960	LTA4H	Leukotriene A-4 hydrolase	20	23.33	0.0108	0.0028
P0C0S5	H2AFZ	Histone H2A.Z	1.74	2.32	0.6226	0.7869
P0CG48	UBC	Polyubiquitin-C	11	7.33	#N/A	#N/A
P11142	HSPA8	Heat shock cognate 71 kDa protein	2.77	3.22	0.4554	0.0465
P14174	MIF	Macrophage migration inhibitory factor	2.75	5.5	0.8478	0.0047
P14618	PKM	Pyruvate kinase PKM	2.41	2.96	0.3796	0.1574
P18754	RCC1	Regulator of chromosome condensation	16.67	20	0.0081	0.0266
P18887	XRCC1	DNA repair protein XRCC1	13.33	26.67	0.2068	0.6595
P23193	TCEA1	Transcription elongation factor A protein 1	20	26.67	0.2167	0.1524
P26373	RPL13	60S ribosomal protein L13	1.28	2.93	0.0060	0.0024
P26641	EEF1G	Elongation factor 1-gamma	2	2.33	0.0175	0.7251
P28066	PSMA5	Proteasome subunit alpha type-5	1.47	2.2	0.0310	0.0002
P30050	RPL12	60S ribosomal protein L12	1.22	5.5	0.0711	0.5010
P32119	PRDX2	Peroxiredoxin-2	6.11	5.5	0.5118	0.3807
P33240	CSTF2	Cleavage stimulation factor subunit 2	6.67	36.67	0.3558	0.6755
P33992	MCM5	DNA replication licensing factor MCM5	16.67	36.67	0.6403	0.6543
P40429	RPL13A	60S ribosomal protein L13a	4.89	8.56	0.0116	0.2779
P42285	SKIV2L2	Superkiller viralicidic activity 2-like 2	23.33	23.33	0.2860	0.0916
P42766	RPL35	60S ribosomal protein L35	9.17	11	0.0001	0.0019
P46100	ATRX	Transcriptional regulator ATRX	6.67	20	0.0350	0.0011
P49773	HINT1	Histidine triad nucleotide-binding protein 1	20	23.33	0.6739	0.0417
P49959	MRE11A	Double-strand break repair protein MRE11A	13.33	50	0.0551	0.0488
P50395	GDI2	Rab GDP dissociation inhibitor beta	4.58	5.5	0.2542	0.0033
P50991	CCT4	T-complex protein 1 subunit delta	4.58	9.17	0.8448	0.0054
P53999	SUB1	Activated RNA polymerase II transcriptional coactivator p15	7.33	18.33	0.1771	0.0041

Table 4 (continued):

Uniprot ID	Gene name	Description	T05 FC	T30 FC	T-test for T05	T-test for T30
P55317	FOXA1	Hepatocyte nuclear factor 3-alpha	26.67	43.33	0.4257	0.5343
P56545	CTBP2	C-terminal-binding protein 2	26.67	33.33	0.3680	0.2916
P60866	RPS20	40S ribosomal protein S20	1.63	4.07	0.3057	0.0108
P60981	DSTN	Destrin	16.67	33.33	0.2652	0.8497
P61289	PSME3	Proteasome activator complex subunit 3	13.33	36.67	0.0191	0.0213
P62241	RPS8	40S ribosomal protein S8	2	2.33	0.8092	0.0247
P62244	RPS15A	40S ribosomal protein S15a	1.47	5.13	0.5869	0.0149
P62318	SNRPD3	Small nuclear ribonucleoprotein Sm D3	6.11	8.56	0.0031	0.0036
P62333	PSMC6	26S protease regulatory subunit 10B	3.33	23.33	0.5968	0.0157
P62851	RPS25	40S ribosomal protein S25	1.47	2.2	0.1291	0.0134
P62913	RPL11	60S ribosomal protein L11	3.21	2.75	0.6555	0.3933
P62917	RPL8	60S ribosomal protein L8	2.75	5.04	0.2104	0.8650
P62993	GRB2	Growth factor receptor-bound protein 2	23.33	23.33	0.5149	0.0859
P63244	GNB2L1	Guanine nucleotide-binding protein subunit beta-2-like 1	3.67	8.56	0.3461	0.0820
P68371	TUBB4B	Tubulin beta-4B chain	3.03	2.68	0.7740	0.1209
P68431	HIST1H3A	Histone H3.1	2.12	1.54	0.0674	0.0002
P83916	CBX1	Chromobox protein homolog 1	9.17	20.17	0.0232	0.0372
P84090	ERH	Enhancer of rudimentary homolog	16.67	26.67	0.0522	0.3053
P98179	RBM3	RNA-binding protein 3	20	20	0.6301	0.0725
Q04760	GLO1	Lactoylglutathione lyase	10	23.33	0.9331	0.0008
Q05048	CSTF1	Cleavage stimulation factor subunit 1	3.33	20	0.0655	0.0237
Q06587	RING1	E3 ubiquitin-protein ligase RING1	20	23.33	0.6410	0.0004
Q07955	SRSF1	Serine/arginine-rich splicing factor 1	20	40	0.0803	0.0265
Q12857	NFIA	Nuclear factor 1 A-type	20	23.33	0.6258	0.0040
Q12873	CHD3	Chromodomain-helicase-DNA-binding protein 3	20	43.33	0.8697	0.0881
Q13057	COASY	Bifunctional coenzyme A synthase	16.5	9.17	0.1179	0.0002
Q13242	SRSF9	Serine/arginine-rich splicing factor 9	20	26.67	0.6675	0.1566
Q13435	SF3B2	Splicing factor 3B subunit 2	13.33	40	0.9767	0.1449
Q13765	NACA	Nascent polypeptide-associated complex subunit alpha	3.06	3.67	0.5452	0.0851
Q14166	TTLL12	Tubulin--tyrosine ligase-like protein 12	6.67	20	0.6940	0.0002
Q14498	RBM39	RNA-binding protein 39	23.33	43.33	0.9535	0.1582

Table 4 (continued):

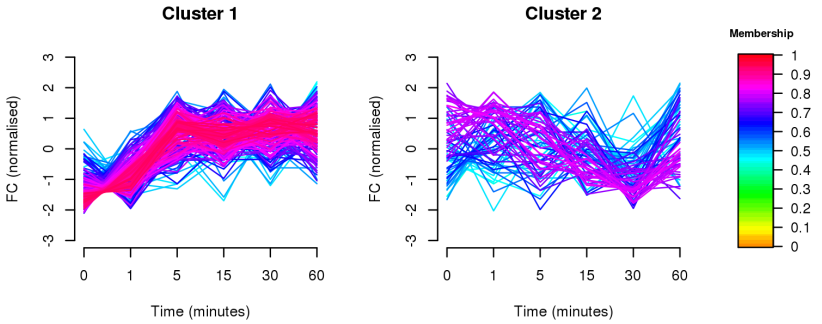
Uniprot ID	Gene name	Description	T05 FC	T30 FC	T-test for T05	T-test for T30
Q14527	HLTF	Helicase-like transcription factor	10	23.33	0.1278	0.0017
Q15102	PAFAH1B3	Platelet-activating factor acetylhydrolase IB subunit gamma	20	13.33	0.0001	0.0195
Q15427	SF3B4	Splicing factor 3B subunit 4	20	33.33	0.0007	0.1355
Q15437	SEC23B	Protein transport protein Sec23B	3.67	12.83	0.8191	0.3652
Q29RF7	PDS5A	Sister chromatid cohesion protein PDS5 homolog A	23.33	50	0.2542	0.4419
Q52LJ0	FAM98B	Protein FAM98B	30	53.33	0.1837	0.0001
Q5VYK3	ECM29	Proteasome-associated protein ECM29 homolog	13.33	33.33	0.4575	0.1550
Q6KC79	NIPBL	Nipped-B-like protein	20	23.33	0.6330	0.7779
Q6NXG1	ESRP1	Epithelial splicing regulatory protein 1	12.83	20.17	0.7426	0.1654
Q6PJG2	ELMSAN1	ELM2 and SANT domain-containing protein 1	16.67	33.33	0.5404	0.6285
Q71U36	TUBA1A	Tubulin alpha-1A chain	2.56	2.47	0.8123	0.0190
Q86XP3	DDX42	ATP-dependent RNA helicase DDX42	20	33.33	0.3175	0.4348
Q8IX12	CCAR1	Cell division cycle and apoptosis regulator protein 1	13.33	26.67	0.0069	0.0008
Q8N684	CPSF7	Cleavage and polyadenylation specificity factor subunit 7	16.67	23.33	0.6434	0.0464
Q8TE68	EPS8L1	Epidermal growth factor receptor kinase substrate 8-like protein 1	0.92	6.42	0.0138	0.2501
Q8WWY3	PRPF31	U4/U6 small nuclear ribonucleoprotein Prp31	16.67	23.33	0.0022	0.0582
Q8WXF1	PSPC1	Paraspeckle component 1	50	20	0.9660	0.1608
Q92804	TAF15	TATA-binding protein-associated factor 2N	36.67	30	0.0610	0.0282
Q969G3	SMARCE1	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1	20	23.33	0.0233	0.0117
Q96I25	RBM17	Splicing factor 45	23.33	26.67	0.0688	0.0836
Q96JP5	ZFP91	E3 ubiquitin-protein ligase ZFP91	13.33	23.33	0.3642	0.0210
Q96QC0	PPP1R10	Serine/threonine-protein phosphatase 1 regulatory subunit 10	20	23.33	0.0502	0.0002

Table 4 (continued):

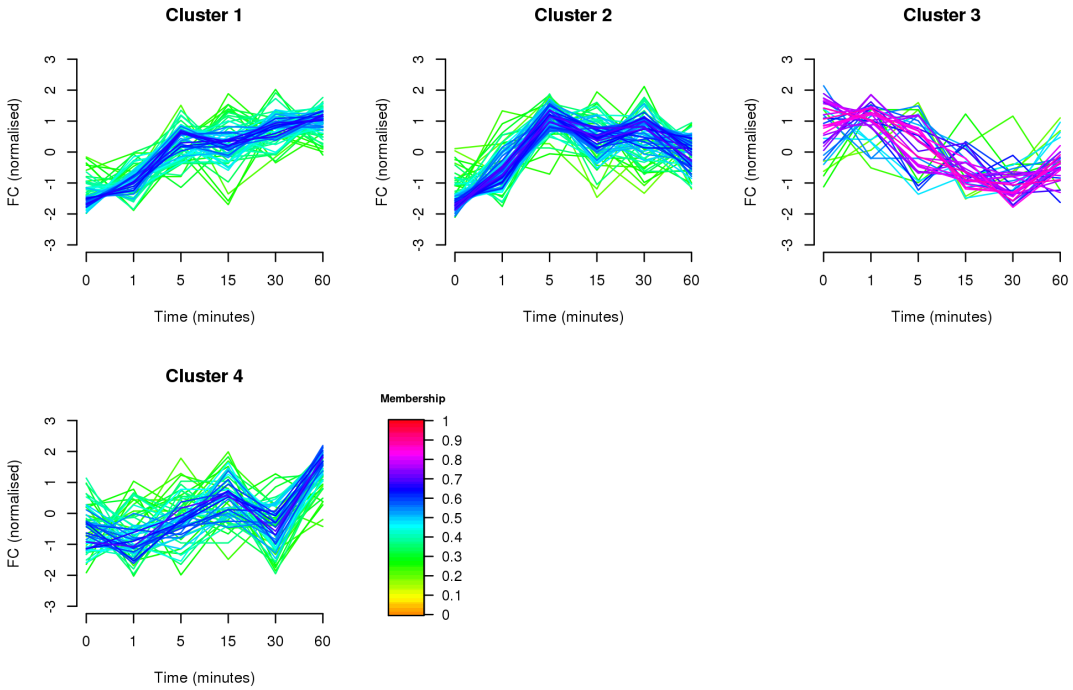
Uniprot ID	Gene name	Description	T05 FC	T30 FC	T-test for T05	T-test for T30
Q99436	PSMB7	Proteasome subunit beta type-7	20	16.67	0.0000	0.0001
Q99459	CDC5L	Cell division cycle 5-like protein	6.67	26.67	0.3266	0.0040
Q99615	DNAJC7	DnaJ homolog subfamily C member 7	6.67	30	0.2779	0.3630
Q9BT78	COPS4	COP9 signalosome complex subunit 4	10	30	0.3757	0.0000
Q9BTC8	MTA3	Metastasis-associated protein MTA3	20	20	0.0003	0.0108
Q9BTT0	ANP32E	Acidic leucine-rich nuclear phosphoprotein 32 family member E	30	16.67	0.1051	0.0077
Q9GZT3	SLIRP	SRA stem-loop-interacting RNA-binding protein, mitochondrial	3.67	5.5	0.6609	0.0009
Q9H0D6	XRN2	5'-3' exoribonuclease 2	16.67	23.33	0.5812	0.1693
Q9H0L4	CSTF2T	Cleavage stimulation factor subunit 2 tau variant	20	60	0.3964	0.0538
Q9H1B7	IRF2BPL	Interferon regulatory factor 2-binding protein-like	13.33	26.67	0.7032	0.0448
Q9HAV4	XPO5	Exportin-5	26.67	26.67	0.1464	0.0109
Q9NR56	MBNL1	Muscleblind-like protein 1	13.33	23.33	0.0509	0.0003
Q9NRX4	PHPT1	14 kDa phosphohistidine phosphatase	16.67	20	0.0000	0.0000
Q9NVH2	INTS7	Integrator complex subunit 7	20	20	0.0086	0.0001
Q9NYB0	TERF2IP	Telomeric repeat-binding factor 2-interacting protein 1	16.67	23.33	0.0421	0.0866
Q9NYF8	BCLAF1	Bcl-2-associated transcription factor 1	26.67	36.67	0.3435	0.0203
Q9P0W2	HMG20B	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1-related	20	40	0.8491	0.0158
Q9P107	GMIP	GEM-interacting protein	13.33	20	0.1215	0.0000
Q9P258	RCC2	Protein RCC2	16.67	26.67	0.0000	0.0249
Q9P2K5	MYEF2	Myelin expression factor 2	16.67	20	0.5050	0.1391
Q9P2N5	RBM27	RNA-binding protein 27	20	23.33	0.1537	0.0347
Q9UN79	SOX13	Transcription factor SOX-13	20	53.33	0.0163	0.0013
Q9UPN9	TRIM33	E3 ubiquitin-protein ligase TRIM33	13.33	23.33	0.6658	0.0106
Q9UQ35	SRRM2	Serine/arginine repetitive matrix protein 2	2.44	2.75	0.4720	0.0208
Q9Y2L1	DIS3	Exosome complex exonuclease RRP44	16.67	26.67	0.3224	0.0411
Q9Y383	LUC7L2	Putative RNA-binding protein Luc7-like 2	14.67	6.11	0.3718	0.7970
Q9Y4B4	RAD54L2	Helicase ARIP4	20	20	0.0001	0.0000

Appendice 1:

a). M-fuzz clustering with high confidence interactors for 2 clusters.



b). M-fuzz clustering with high confidence interactors for 4 clusters.



c). M-fuzz clustering with high confidence interactors for 5 clusters.