

1 **Transcriptional coregulator RIP140: An essential regulator of physiology**

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21 **Abstract**

22 Transcriptional coregulators drive gene regulatory decisions in the transcriptional space. While
23 transcription factors including all nuclear receptors provide a docking platform for coregulators to
24 bind, these proteins bring enzymatic capabilities to the gene regulatory sites. RIP140 is a
25 transcriptional coregulator essential for several physiological processes and aberrations in its function
26 may lead to diseased states. Unlike several other coregulators which are either known for their
27 coactivating or corepressing roles, in gene regulation, RIP140 is capable of acting both as a
28 coactivator and a corepressor. The role of RIP140 in female reproductive axis and recent findings of
29 its role in carcinogenesis and adipose biology have been summarized.

30 **Introduction**

31 We have come a long way in the understanding of transcriptional coregulators from when they were
32 first identified as transcription factor binding proteins (Glass, et al. 1990) (Bastian and Nordeen 1991;
33 Chatterjee, et al. 1991; Wieland, et al. 1991) (Cavailles, et al. 1994; Halachmi, et al. 1994; Onate, et
34 al. 1995) to now when they are recognised as key players in influencing all physiological processes
35 (Dasgupta, et al. 2014; Lonard and O'Malley B 2007; Nautiyal, et al. 2013a; O'Malley, et al. 2008;
36 Stashi, et al. 2014; York and O'Malley 2010). These proteins are essential for gene regulation, by
37 regulating functions of transcription factors including all members of the nuclear receptor superfamily
38 in the genome. Coregulators (a class of molecules which include both transcriptional coactivators and
39 corepressors) function by bringing together multi partner transcriptional machinery by acting as
40 scaffolds and in several cases also possess enzymatic activities to activate (as coactivators) or inhibit
41 (as corepressors) gene expression. In fact, recent findings suggest that these proteins perform
42 functions beyond nucleus in the cytoplasm to regulate several biological functions. Coregulators are
43 secondary transcription factors which are brought to the site of transcription by transcription factors
44 but do not bind to the DNA directly. However, these are the factors which control gene expression by
45 bringing enzymatic potentials to the gene regulatory sites. Numerous studies using transgenic
46 technologies in mice and cell based assays have revealed the powerful potential these proteins possess

47 in regulating normal physiology and driving diseased states. Today 350+ proteins have are known as
 48 coregulators and the number keeps increasing (Millard, et al. 2013; Stashi et al. 2014). This article
 49 elaborates on the biological functions of RIP140, a coregulator that performs several important
 50 physiological functions. RIP140 functions in female reproductive tissues, adipose and current
 51 knowledge on its role in carcinogenesis has been discussed.

52 **Receptor Interacting Protein140 (RIP140)- A switch coregulator**

53 Coregulators are either coactivators (examples, members of Steroid Receptor Coactivator
 54 (SRC) and peroxisome proliferator-activated receptors gamma (PPAR γ) coactivator (PGC-1)
 55 families) or corepressors (examples, Nuclear Receptor Corepressors-1 (NCoR1) and silencing
 56 mediator for retinoid and thyroid hormone receptors (SMRT)). RIP140 however is a uniquely
 57 reported coregulator that functions both as a coactivator and a corepressor (*Figure 1*). It was first
 58 identified in human breast cancer cells in a screen to identify interacting partners of the hormone
 59 binding domain of Oestrogen receptor α (ER) (Cavailles et al. 1994; Cavailles, et al. 1995). At this
 60 stage only the basal transcriptional machinery composed of the TATA box binding protein (TBP) and
 61 TBP-associated factor (TAF), hTAFII30 was known to bind ER (Brou, et al. 1993; Jacq, et al. 1994)
 62 and this binding was not affected by treatment of the cells with oestrogen or anti-oestrogens. Thus,
 63 there was a gap in the understanding of binding partners of ER whose binding was selectively
 64 regulated or inhibited by the ligand oestrogen (E2) and antagonists (Tamoxifen) to effect ER mediated
 65 transcription (Cavailles et al. 1995). As we know it today, RIP140 is a coregulator to most nuclear
 66 receptors and several other transcription factors (Nautiyal et al. 2013a). This elaborate interactome is
 67 due 9 LXXLL motifs and a 10th LXXML motif mapped to the C terminus on RIP140 protein that is
 68 required for the interaction of RAR with RXR (Christian, et al. 2006) (*Figure 1*). Coregulator
 69 interactions with nuclear receptors and transcription factors is brought about by *LXXLL motifs*. This
 70 signature sequence (also called NR box), first identified by Heery et al, is a short alpha helical
 71 sequence motif (where L is leucine and X is any amino acid) (Heery, et al. 1997; Torchia, et al.
 72 1997). LXXLL motifs are common to several transcriptional coregulators. However, there is variation
 73 in the number of LXXLL sequences on different coregulators, which determines their respective

74 interactomes. For example SRC family of proteins have 3 LXXLL motifs while RIP140 has 9
75 LXXLL motifs (Christian et al. 2006; Dasgupta and O'Malley 2014; Leo and Chen 2000).

76 Initial studies indicated a coactivator role for RIP140 (Ikonen, et al. 1997; Joyeux, et al.
77 1997). However soon, duality in RIP140 function in switching from a corepressor to a coactivator was
78 revealed on the rat prolactin promoter. This was driven by changes in the interaction dynamics of
79 pituitary specific transcription factor (Pit1) with ER and thyroid hormone receptors (Chuang, et al.
80 1997) as a result of a truncation in Pit-1. Thus RIP140 could act both as a coactivator and corepressor
81 based on interaction dynamics with other factors. Several studies in physiological models and cells
82 have testified to this remarkable ability of RIP140 to act as a coactivator (Nautiyal et al. 2013a;
83 Nautiyal, et al. 2013b; Rosell, et al. 2011; Zschiedrich, et al. 2008) in some situations and as a
84 corepressor in others (Christian, et al. 2005; Debevec, et al. 2007; Powelka, et al. 2006; Seth, et al.
85 2007) . While mechanisms describing this dual role are not well understood the best hypothesis so far
86 is that, this switch is determined by the cellular environment and is promoter sequence specific.

87 Wei *et al* presented the first biochemical evidence that RIP140 pocesses a Trichostatin A
88 sensitive trans- repressive activity and could directly recruit Histone Deacetylases (HDAC) 1 and 3
89 both in vivo and in vitro. The HDAC-interacting domain on RIP140 was mapped to its N terminus
90 between amino acids 78 and 303 (Wei, et al. 2000). HDACs are enzymes responsible for the removal
91 of the acetyl group on the lysines and restoration of the DNA-histone attractive forces, leading to a
92 closed chromatin confirmation. 4 classes of HDACs, representing about 18 enzymes are known. Of
93 these, HDACs belonging to classes I, II and IV share a related catalytic mechanism involving a zinc
94 metal ion and do not require any cofactor. Members of class III HDACs which include sirtuins 1-7,
95 employ a NAD⁺ dependent mechanism (Dawson and Kouzarides 2012). HDACs are frequently
96 recruited to nuclear receptors by direct binding or brought to the site of transcription by other primary
97 coregulators such as RIP140 (Christian et al. 2006; Heinzl, et al. 1997; Kato, et al. 2011; Nagy, et al.
98 1997).

99 Using deletion mutants Christian *et al* identified 4 distinct autonomous repression domains
 100 (RD) RD1-RD4 in RIP140 (Christian, et al. 2004). These RDs provide platforms for different
 101 corepressor complexes. Both RD1 and RD2 act by recruiting HDACs (Wei et al. 2000). Recruitment
 102 of HDACs to RD2 is mediated by corepressor, C-terminal binding protein (CtBP). As repression is
 103 only relieved partially by HDAC inhibitor Trichostatin A, it is believed that CtBP acts by HDAC
 104 dependent as well as independent mechanisms. Four CtBP binding motifs have been identified in
 105 RIP140 protein. Castet *et al* identified the PIDLS and PINLS motifs which are CtBP binding motifs
 106 on RIP140 and demonstrated that RIP140 also interacts with HDAC 5. This interaction was mapped
 107 to the N terminus between residues 27-199 on RIP140 (Castet, et al. 2004). Since CtBP is a functional
 108 dehydrogenase that is sensitive to NADH:NAD redox ratio, an increase in this ratio may promote
 109 repression mediated by RIP140 (Christian et al. 2006). Thus repression brought about by RIP140 is
 110 likely to be influenced by the redox status of the cells. Interactions between RIP140 and CtBP are
 111 regulated by post translational modifications on RIP140. For example, acetylation through histone
 112 acetyl transferases (HATs), E1A binding protein p300 (p300)/ cAMP response element-binding
 113 protein (CBP) of a N terminal lysine residue on RIP140 led to the disruption of the RIP140-CtBP
 114 complex and thus a loss of repressive potential of RIP140 (Vo, et al. 2001).

115 Gupta *et al*, identified 11 phosphorylation sites on RIP140 using mass spectrophotometry. By
 116 studying constitutively phosphorylated and dephosphorylated mutants of RIP140 at different sites,
 117 this study reported that the strength of the repressive potential of RIP140 through HDACs is
 118 determined by its phosphorylation status. For example, a constitutive dephosphorylation at Thr202
 119 and Thr207 on RIP140, significantly impaired its repressive activity and HDAC recruitment while the
 120 opposite effects were seen with a constitutive phosphorylation of these residues (Gupta, et al. 2005).
 121 Further threonine phosphorylation of RIP140 by Erk2 which promoted p300 recruitment for its lysine
 122 acetylation, enhanced the repressive activity of RIP140 which was shown to influence fat
 123 accumulation in differentiated adipocytes (Ho, et al. 2008). These studies indicate how post
 124 translational modifications on RIP140 regulate its potential to act as a corepressor. Apart from the
 125 interactions with HDACs several studies also suggested that the repressive mechanism of action of

RIP140 is through competition with coactivators like SRC1 (Treuter, et al. 1998) and p300/CBP associated protein (PCAF) (Chen, et al. 2004) (Gupta, et al. 2007). Corepressors NCOR1 and SMRT-1 also act by recruiting HDAC proteins and have specialised domains called the SANT domains through which they modulate the activities of HDACs and help in recognising histones in context with other proteins. The binding sites of several nuclear receptors and transcription factors on NCOR1 and SMRT have been mapped extensively (Wong, et al. 2014).

With the background knowledge of corepressive functions of RIP140 the first mechanistic explanation to its coactivating function came from Stefan Herzig's laboratory. While an intrinsic activation function within RIP140 has not been found so far, this study reported that RIP140 activates expression of several inflammatory cytokines in macrophages by coactivating the NFkB subunit RelA. This is achieved as a result of interaction of RIP140 with the C-terminus, of CBP through its RD1 domain (Zschiedrich et al. 2008). RIP140 was further shown to coactivate CREB mediated transcription on the Amphiregulin (*Areg*) promoter in the ovarian granulosa cells and recent mapping of the RIP140 binding sites in breast cancer cells have shown that CBP and p300 co-occupy several RIP140 binding sites (Nautiyal, et al. 2010; Rosell, et al. 2014). Two distinct C terminal, activation domains (AD1/2), are present in coactivators of the SRC family. AD1 recruits CBP/p300 and pCAF and AD2 recruits arginine methyl transferase (CARM1) and protein arginine N methyl transferase (PRMT1) (Rollins, et al. 2015).

Biological functions of RIP140

Knowledge of the biological functions of RIP140 came from studies on genetically modified mouse models of RIP140, i.e the RIP140 knockout (RIPKO) (White, et al. 2000) and constitutively overexpressing RIP140 transgenic (RIPTg) mice (Fritah, et al. 2010). Two significant phenotypes came to light in RIPKO mice (i) Female RIPKO mice were infertile because of an inability to ovulate (White et al. 2000) and (ii) both RIPKO male and female mice were significantly leaner than their WT counterparts (Leonardsson, et al. 2004). Further studies helped in unravelling the regulatory role of RIP140 and gene targets in ovary (Nautiyal et al. 2010; Tullet, et al. 2005), uterus

(Leonardsson, et al. 2002), adipose tissue (Christian et al. 2005; Hallberg, et al. 2008; Park, et al. 2009; Persaud, et al. 2011; Powelka et al. 2006), muscle (Fritah, et al. 2012; Seth et al. 2007), liver (Berriel Diaz, et al. 2008; Herzog, et al. 2007), heart (Chen, et al. 2012; Fritah, et al. 2011), inflammatory cells (Ho, et al. 2012; Zschiedrich et al. 2008), mammary gland (Nautiyal et al. 2013b), neural functions (Duclot, et al. 2012; Feng, et al. 2014; Feng, et al. 2015; Tsai, et al. 2009) and more recently in the pathophysiology of ageing (Yuan, et al. 2012) and cancer (Aziz, et al. 2015; Docquier, et al. 2013; Gibson, et al. 2016; Lapierre, et al. 2014; Lapierre, et al. 2015a; Rosell et al. 2014). The following sections describe RIP140 biology in hormone responsive female reproductive tissues, recent findings of its role in carcinogenesis and metabolic functions in adipose tissue.

Female fertility and mammary gland development:

RIPKO female mice show a delayed onset of sexual maturation which is linked with ageing and to reduced levels of circulating Insulin-like growth factor-1 (IGF1) (Yuan et al. 2012). While these mice have a normal oestrus cycle (Nautiyal et al. 2013b) they are infertile (White et al. 2000). The infertility phenotype led to examination of the hypothalamic-pituitary-gonadal axis (Leonardsson et al. 2002; Nautiyal et al. 2013b) and studies to examine the ovaries (Leonardsson et al. 2002; Nautiyal et al. 2010; Tullet et al. 2005; White et al. 2000), uterus (Leonardsson et al. 2002) and mammary gland (Nautiyal et al. 2013b). Cross sections through ovaries of mature RIPKO mice revealed follicles at all developmental stages from primordial to the pre-ovulatory stage, indicating normal folliculogenesis. Differentiated corpora lutea were also observed in these mice pointing towards normal luteinisation. However it was remarkable that the corpora lutea retained oocytes thus explaining the anovulatory phenotype (White et al. 2000). Further studies demonstrated defects in the transcriptional regulation and expression in RIPKO mice of the EGF-like growth factor family which includes amphiregulin (AREG), epiregulin (EREG) and betacellulin (BTC) (Nautiyal et al. 2010; Tullet et al. 2005). In WT mice the expression of these growth factors in the pre-ovulatory follicle is one of the earliest events following the luteinising hormone (LH) surge (Park, et al. 2004). Expression of these factors, initiates a cascade of events called cumulus expansion. Cumulus expansion is a biochemical sequence of processes after the LH surge, in the preovulatory follicle

179 where a series of downstream enzymes and factors from the cumulus granulosa cells get expressed
 180 and their products constitute a secreted matrix on which the initially compactly packed cumulus
 181 granulosa cells loosen up and thus expand (*Figure2*). This process is essential for ovulation and
 182 several mouse models have been reported with defects in cumulus expansion and thus are
 183 anovulatory (Richards 2007). Pre-ovulatory RIPKO follicles failed to show expression of EGF
 184 family members and thus a defective cumulus expansion (Tullet et al. 2005). Exposure of the RIPKO
 185 cumulus oocyte complexes (COCs) to recombinant AREG both *in vitro* and *in vivo*, rescued cumulus
 186 expansion and downstream expression of genes involved in the synthesis of the cumulus matrix.
 187 Following the LH surge the EGF-like growth factor family members are known to act in autocrine-
 188 paracrine manner and regulate their own expression as well as expression of several downstream
 189 enzymes that promote cumulus expansion (Richards 2007; Richards and Pangas 2010; Shimada, et al.
 190 2006). It was noteworthy however that exposure of RIPKO COCs to recombinant AREG *in vitro*
 191 rescued cumulus expansion and expression of several enzymes involved in the process but did not
 192 rescue *Areg* expression or that of *Ereg* and *Btc* indicating that RIP140 is essential for the expression
 193 of these genes of the EGF-like growth factor family (Nautiyal et al. 2010). Analysis of *Areg* promoter
 194 in granulosa cell lines demonstrated that RIP140 regulates gene expression of this growth factor
 195 through coactivating CREB and cJun transcription factors (Nautiyal et al. 2010). Ovarian and embryo
 196 transplant experiments in RIPKO mice resulted in only partial restoration of fertility (Leonardsson et
 197 al. 2002). About 50% of the embryos that were transplanted, were resorbed at post coitum day 13.5,
 198 indicating an inadequate uterine environment to support developing embryos and most of the pups
 199 were still born or died soon thereafter (Leonardsson et al. 2002), suggesting inability to survive in
 200 absence of maternal feeding. This indicated that while fertility could be partially restored by ovarian
 201 correction, the uterus and mammary gland in these mice required closer examination.

202 RIPKO mice show clear defects in the mammary ductal elongation at puberty with adult mice
 203 developing a scanty epithelial network. Conversely, RIPTg mice show precocious growth with
 204 extensive cell division and side branching and develop ductal hyperplasia in the mammary gland
 205 (Nautiyal et al. 2013b). RIPTg mice have been reported to develop rapid, progressive postnatal

cardiomyopathy. Examination of the heart tissue in these mice demonstrated mitochondrial degeneration and reduced expression of genes involved in mitochondrial activity, fatty acid metabolism and transport, and impaired signalling of nuclear receptors, PPAR α , PPAR δ and oestrogen related receptors ERR α and ERR γ (Fritah et al. 2010). As a result of cardiac defects, RIPTg mice die early and thus could not be used to study mammary tumorigenesis. Paracrine signalling and crosstalk between epithelium and stromal cells is essential for normal development of the mammary gland (Abubakari, et al. 2009). Tissue recombinant studies at pre-pubertal stages in the RIPKO and RIPTg mouse models helped in highlighting that RIP140 expression is essential in mammary epithelium as well as stroma for mammary gland development (Nautiyal et al. 2013b). Determining gene targets of RIP140 in isolated stromal and epithelial cell populations is warranted to obtain further mechanistic insights in stromal-epithelial crosstalk in mammary gland development. Global ChIP-seq analysis of ER binding sites in the mouse mammary tissue helped in finding several important regulators of mammary gland development that were ER targets and transcriptionally coactivated by RIP140. These genes included *Areg*, progesterone receptor (*Pgr*), cyclin D1 (*ccnd1*), *c-Myc* (*Myc*), and Signal Transducer and Activator of Transcription (*Stat5a*) (Nautiyal et al. 2013b). Analysis of mammary cell populations from RIPTg mice demonstrated that RIP140 overexpression lead to an increase in the basal mammary cell population. This is interesting as the basal cell compartment of the mammary gland is considered home for multi-potent stem cells. Within the luminal cell population there was an increase in the progenitor cells over the differentiated ones indicating that RIP140 promotes proliferation and/or blocks differentiation (Nautiyal et al. 2013b). Thus, RIP140 levels determine cell fate in the mammary gland. Although work here was mainly done to look at ER target genes coregulated by RIP140, it is worth mentioning that its coregulatory potential with other transcription factors in the mammary gland remains to be explored.

Carcinogenesis:

Insights obtained about the role of RIP140 in mammary gland development as a coactivator for ER warranted further mechanistic understanding of its involvement in ER mediated gene transcription. Initial reports had implicated involvement of RIP140 in breast cancer (Docquier, et al.

2012; Hannafon, et al. ; Lee, et al. 2007; Oh, et al. 2006). ChIP-seq analysis was performed to map RIP140 binding with ER in MCF7 breast cancer cell line. 82% of the RIP140 binding sites were co-occupied by ER and its interaction partners FOXA1, GATA3 and other coactivators p300, CBP and p160 family at H3K4me1 demarcated regions in the genome, H3K4me1 being an enhancer specific epigenetic mark of ER binding (Joseph, et al. 2010). Very similar to ER, only 5% of the RIP140 binding was seen at the promoters while the rest was enriched in introns and distal intergenic regions (Fullwood, et al. 2009; Rosell et al. 2014). siRNA knockdown experiments revealed that RIP140 is required for ER mediated transcriptional complex formation and gene regulation which stimulated breast cancer cell proliferation. Genes that changed as a result of RIP140 knockdown in the MCF7 cells were used to generate a gene expression classifier that could be successfully used to stratify patients with breast cancer, who received adjuvant tamoxifen treatment. Patients in the cluster representing poor outcome correlated with active RIP140 gene expression signature, while the cluster representing good outcome represented cases with less active RIP140-responsive gene signature (Rosell et al. 2014). Aziz *et al* demonstrated that RIP140 deficient mice were less susceptible to 7,12 Dimethylbenz[a] anthracene (DMBA) induced carcinogenesis compared to their WT counterparts. While WT mice developed tumours in skin, mammary gland, liver and intestinal tract following DMBA exposure for 6 weeks, RIPKO mice did not develop any tumours, with the exception of smaller skin tumours. Analysis of several breast cancer cellular models showed that RIP140 knockdown induced apoptosis and inhibited cell growth (Aziz et al. 2015).

While the role of RIP140 as a coactivator for ER is appreciated, Docquier *et al* highlighted its role as a corepressor of ER β and in antagonising E2 mediated cellular proliferation in ER positive ovarian cancer cells, stably integrated with an Oestrogen Response Element (ERE) luciferase reporter. With ER β overexpression, there was increased recruitment of RIP140 on the ERE and siRNA knockdown of RIP140 abolished the repressive effect exerted by ER β in the regulation of E2 mediated activation of the ERE (Docquier et al. 2013). It has also been demonstrated in a study using ovarian cancer cells that RIP140 is involved in sensitisation of these cells to chemotherapeutic agents (Lapierre, et al. 2015b). Recently, a tumour suppressor role for RIP140 has been suggested in colon

260 cancer. Examination of RIPKO and RIPTg mice demonstrated that RIP140 inhibits the renewal of
 261 intestinal epithelium by regulating cell proliferation and apoptosis and thus influencing intestinal
 262 homeostasis. When mice were subjected to whole body irradiation, RIPKO mice demonstrated
 263 improved regenerative capacity in the intestine, while the overexpressing RIPTg mice had reduced
 264 renewal of the intestinal villi compared to WT counterparts (Lapierre et al. 2014). This was further
 265 confirmed in cellular models in mouse xenografts in which enhanced RIP140 expression reduced
 266 colon cancer cell proliferation. Clinically RIP140 mRNA and protein levels were lower in human
 267 colon cancer compared to normal tissue and low RIP140 expression correlated with poor prognosis.
 268 Mechanistically, RIP140 stimulates transcription of multiprotein β catenin degradation complex
 269 subunit, Adenomatosis polyposis coli (APC) which inhibits β -Catenin activation in the intestinal
 270 epithelium (Lapierre et al. 2014). Recently RIP140 has emerged as a prognostic marker in
 271 hematologic malignancies such as chronic lymphocytic leukaemia (CLL) (Herold, et al. 2011; van't
 272 Veer, et al. 2006). High expression of RIP140 is a favourable prognostic marker in CLL. The exact
 273 mechanisms and pathways regulated by RIP140 in CLL are not known so far. It is speculated that it
 274 may well be through regulation of nuclear receptors, Wnt and NFkB signalling pathways (Lapierre et
 275 al. 2015a).

276 Two studies to identify mutations and drivers of endometrial cancer (EC) progression, have
 277 recently identified several mutations in RIP140, suggesting its role in impaired ER signalling
 278 (Ferreira, et al. 2014; Gibson et al. 2016). Microsatellite instability (MSI) which is a phenotype
 279 caused by mutations in the DNA mismatch repair (MMR) genes, is a common feature of EC (Cancer
 280 Genome Atlas Research, et al. 2013). In a genome-wide search to identify genes effected by MSI ,
 281 RIP140 was the highest mutated gene (34% of EC) (Ferreira et al. 2014). Exome-sequencing of EC
 282 tumours in another study identified RIP140 mutation in a significant number of tumours (12% of EC).
 283 While several missense mutations were identified, indel (single base pair insertion and deletion)
 284 mutations were highly recurrent on Asn516 and Lys728 on RIP140 (Gibson et al. 2016). Future
 285 studies should focus on the functional effects of RIP140 alterations in driving the development of EC.

Based on examination of patient cohorts and work in mouse and cancer cellular models in the above mentioned studies it is clear that RIP140 plays a role in tumorigenesis. Just like in physiological conditions, the ability of RIP140 to act both as a coactivator and corepressor based on the cellular milieu and in a transcription factor specific context is reflected in cancer as well. For example, RIP140 promotes cancer cell proliferation in breast cancer (Rosell et al. 2014) while it inhibits proliferation in colon cancer (Lapierre et al. 2014). Further functional and molecular studies in individual cancers will determine its use as a biomarker for patient stratification and help in determining individualised approaches for treatment.

Metabolic functions in Adipose Tissue:

Adipose tissue is an endocrine organ that produces hormones such as E2, leptin, resistin, adiponectin and inflammatory cytokines and is a regulator of energy homeostasis (Stern, et al. 2016). Through regulating adipose composition in the body RIP140 maintains the balance between energy storage and expenditure by mechanisms that are coming to light now (Hu, et al. 2015; Kiskinis, et al. 2014; Liu, et al. 2015b). There are mainly two types of adipose tissues. 1) The fat storing white adipose tissue (WAT) which expands under obese conditions and becomes inflammatory, leading to insulin resistance and 2) the fat burning brown adipose tissue (BAT) which is rich in mitochondria and thus expends energy (*Figure2*). Originally BAT was thought to exist only in neonates but it has recently been shown to exist in adult humans (Cypess, et al. 2009).

RIPKO mice are lean, with a 70% reduction in body fat and show resistance to high fat diet induced obesity. While BAT tissue in these mice is histologically normal, the WAT tissue in RIPKO mice shows a significant reduction in the size of the adipocytes (Leonardsson et al. 2004). The WAT in RIPKO mice express genes which are more characteristic of brown fat i.e involved in increasing energy expenditure. These genes include uncoupling protein 1 (UCP1), cell death inducing DNA fragmentation factor (CIDEA), a lipid droplet protein and carnitine palmitoyl transferase (CPT1), a mitochondrial fatty acid transporter (Leonardsson et al. 2004) (Christian et al. 2005). Studies in genetically modified adipose cellular models demonstrated that RIP140 acts as a repressor of

312 catabolic processes such as fatty acid oxidation, oxidative phosphorylation, glycolysis and
 313 tricarboxylic acid cycle (Powelka et al. 2006) and thus ablation of RIP140 leads to upregulation of
 314 gene networks in these pathway and thus expenditure of energy. PGC1 α a transcriptional coactivator
 315 first identified and cloned in BAT by the Spiegelman group (Puigserver, et al. 1998), modulates a
 316 number of genes involved in these metabolic pathways and shares several gene targets with RIP140,
 317 the two coregulators antagonising each other's actions (Hallberg et al. 2008) (Fernandez-Marcos and
 318 Auwerx 2011).

319 The existence of a third type of fat namely 'the beige fat' or BRITE has been recently
 320 appreciated in WAT. This fat has the potential to thrive within the WAT depots on exposure to cold
 321 and β 2 adrenergic agonist treatment. This 'browning of fat' is considered beneficial as beige fat, like
 322 brown fat, is involved in thermogenesis although it is worth mentioning that the brown and beige
 323 adipocytes show distinct gene expression signatures (Walden, et al. 2012) (*Figure2*). Coregulators
 324 PGC1 α and PR domain containing 16 (PRDM16) influence the expression of brown adipocyte genes
 325 within white adipocytes. Further investigation of the role of RIP140 in adipose cell lines indicated
 326 that RIP140 has little role in the regulation of brown fat gene expression. The way RIP140 acts is
 327 through preventing the development of BRITE depots within the white fat. This could be either
 328 through antagonising the co activator potential of PGC1 α on common genes or directly regulating the
 329 expression of PGC1 α (Kiskinis et al. 2014)

330 Obesity, insulin resistance and metabolic dysregulation are inflammatory conditions and the
 331 role of macrophages is appreciated in this. Apart from being crucial effectors of the innate immune
 332 responses, macrophages play vital homeostatic roles which are separate from their function as
 333 immune cells. For example these phagocytic cells clear cellular debris generated during tissue
 334 remodelling and potentially harmful apoptotic products with precision (Mosser and Edwards 2008).
 335 Macrophages are known to have remarkable plasticity and depending on the metabolic cues are
 336 capable of making phenotypic switches to different states. Broadly macrophages can exist in two
 337 states, namely M1 and M2. These two states represent different gene expression and transcriptional

profiles (Gautier, et al. 2012) and their number and activation state can vary, based on the metabolic signals in the WAT tissue (Lumeng, et al. 2007). For example, M2 maintains homeostasis and insulin sensitivity while the M1 represents a state of chronic inflammation. In a high fat diet induced obesity model the number of macrophages increase from 10-15% of the total stromal cells –mostly in the M2 state to 45-60% due to increased inflammation and recruitment of the M1 type. (Odegaard and Chawla 2013). This leads to remodelling of the WAT tissue which enlarges to meet the increased demands of energy storage and also secretes further harmful substances which further aggravate the state of inflammation leading on to insulin resistance (Chawla, et al. 2011; Liu, et al. 2014; Wynn, et al. 2013).

RIP140 has been reported to tip the balance of M1 versus M2 polarization of the macrophage population (Liu et al. 2015b). High fat diet increases RIP140 expression in macrophages. RIP140 knock down specifically in macrophages in mice, led to adipose tissue remodelling as white adipose tissue underwent browning and improved insulin sensitivity was observed in high fat diet fed conditions. In the absence of RIP140 the macrophage profile in these mice altered under high fat diet conditions with increase in macrophages in M2 state and a decrease in M1 state, which improved insulin resistance. This study demonstrated that altering RIP140 expression in macrophages could be used as a new therapeutic strategy in inflammatory disorders and high fat diet induced insulin resistance (Liu et al. 2014). Exposure of preadipocytes to media obtained from cultures of macrophages which are deficient in RIP140 led to differentiation into beige cells. Likewise injecting obese mice with M2 macrophages obtained from WAT of mice which were deficient for RIP140 expression in macrophages, led to improvement of insulin resistance and browning of the white adipose tissue depots providing a proof of concept regarding the therapeutic potential in injecting engineered macrophages for RIP140 expression and correcting metabolic dysregulation (Liu, et al. 2015a).

Concluding Remarks

RIP140 is a coregulator that drives crucial physiological processes and plays a role in the development and progression of cancer, metabolic abnormalities and potentially even fertility related pathologies. Reports of its coactivating and corepressing potential in different physiological or pathological settings are tantalising and more mechanistic insights are needed to clarify this dual role of RIP140. The biomarker and therapeutic potential of RIP140 will be explored in future studies.

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References

- Abubakari AR, Lauder W, Jones MC, Kirk A, Agyemang C & Bhopal RS 2009 Prevalence and time trends in diabetes and physical inactivity among adult West African populations: the epidemic has arrived. *Public Health* **123** 602-614.
- Aziz MH, Chen X, Zhang Q, DeFrain C, Osland J, Luo Y, Shi X & Yuan R 2015 Suppressing NRIP1 inhibits growth of breast cancer cells in vitro and in vivo. *Oncotarget* **6** 39714-39724.
- Bastian LS & Nordeen SK 1991 Concerted stimulation of transcription by glucocorticoid receptors and basal transcription factors: limited transcriptional synergism suggests mediation by coactivators/adaptors. *Mol Endocrinol* **5** 619-627.
- Berriel Diaz M, Krones-Herzig A, Metzger D, Ziegler A, Vegiopoulos A, Klingenspor M, Muller-Decker K & Herzig S 2008 Nuclear receptor cofactor receptor interacting protein 140 controls hepatic triglyceride metabolism during wasting in mice. *Hepatology* **48** 782-791.
- Brou C, Chaudhary S, Davidson I, Lutz Y, Wu J, Egly JM, Tora L & Chambon P 1993 Distinct TFIID complexes mediate the effect of different transcriptional activators. *EMBO J* **12** 489-499.
- Cancer Genome Atlas Research N, Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, Shen H, Robertson AG, Pashtan I, Shen R, et al. 2013 Integrated genomic characterization of endometrial carcinoma. *Nature* **497** 67-73.
- Castet A, Boulahtouf A, Versini G, Bonnet S, Augereau P, Vignon F, Khochbin S, Jalaguier S & Cavailles V 2004 Multiple domains of the Receptor-Interacting Protein 140 contribute to transcription inhibition. *Nucleic Acids Res* **32** 1957-1966.
- Cavailles V, Dauvois S, Danielian PS & Parker MG 1994 Interaction of proteins with transcriptionally active estrogen receptors. *Proc Natl Acad Sci U S A* **91** 10009-10013.
- Cavailles V, Dauvois S, L'Horset F, Lopez G, Hoare S, Kushner PJ & Parker MG 1995 Nuclear factor RIP140 modulates transcriptional activation by the estrogen receptor. *EMBO J* **14** 3741-3751.

399 Chatterjee VK, Madison LD, Mayo S & Jameson JL 1991 Repression of the human glycoprotein
400 hormone alpha-subunit gene by glucocorticoids: evidence for receptor interactions with limiting
401 transcriptional activators. *Mol Endocrinol* **5** 100-110.

402 Chawla A, Nguyen KD & Goh YP 2011 Macrophage-mediated inflammation in metabolic disease. *Nat*
403 *Rev Immunol* **11** 738-749.

404 Chen Y, Hu X & Wei LN 2004 Molecular interaction of retinoic acid receptors with coregulators PCAF
405 and RIP140. *Mol Cell Endocrinol* **226** 43-50.

406 Chen Y, Wang Y, Chen J, Chen X, Cao W, Chen S, Xu S, Huang H & Liu P 2012 Roles of transcriptional
407 corepressor RIP140 and coactivator PGC-1alpha in energy state of chronically infarcted rat hearts
408 and mitochondrial function of cardiomyocytes. *Mol Cell Endocrinol* **362** 11-18.

409 Christian M, Kiskinis E, Debevec D, Leonardsson G, White R & Parker MG 2005 RIP140-targeted
410 repression of gene expression in adipocytes. *Mol Cell Biol* **25** 9383-9391.

411 Christian M, Tullet JM & Parker MG 2004 Characterization of four autonomous repression domains
412 in the corepressor receptor interacting protein 140. *J Biol Chem* **279** 15645-15651.

413 Christian M, White R & Parker MG 2006 Metabolic regulation by the nuclear receptor corepressor
414 RIP140. *Trends Endocrinol Metab* **17** 243-250.

415 Chuang FM, West BL, Baxter JD & Schaufele F 1997 Activities in Pit-1 determine whether receptor
416 interacting protein 140 activates or inhibits Pit-1/nuclear receptor transcriptional synergy. *Mol*
417 *Endocrinol* **11** 1332-1341.

418 Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng YH, Doria
419 A, et al. 2009 Identification and importance of brown adipose tissue in adult humans. *N Engl J Med*
420 **360** 1509-1517.

421 Dasgupta S, Lonard DM & O'Malley BW 2014 Nuclear receptor coactivators: master regulators of
422 human health and disease. *Annu Rev Med* **65** 279-292.

423 Dasgupta S & O'Malley BW 2014 Transcriptional coregulators: emerging roles of SRC family of
424 coactivators in disease pathology. *J Mol Endocrinol* **53** R47-59.

425 Dawson MA & Kouzarides T 2012 Cancer epigenetics: from mechanism to therapy. *Cell* **150** 12-27.

426 Debevec D, Christian M, Morganstein D, Seth A, Herzog B, Parker M & White R 2007 Receptor
427 interacting protein 140 regulates expression of uncoupling protein 1 in adipocytes through specific
428 peroxisome proliferator activated receptor isoforms and estrogen-related receptor alpha. *Mol*
429 *Endocrinol* **21** 1581-1592.

430 Docquier A, Augereau P, Lapierre M, Harmand PO, Badia E, Annicotte JS, Fajas L & Cavailles V 2012
431 The RIP140 gene is a transcriptional target of E2F1. *PLoS One* **7** e35839.

432 Docquier A, Garcia A, Savatier J, Boulahtouf A, Bonnet S, Bellet V, Busson M, Margeat E, Jalaguier S,
433 Royer C, et al. 2013 Negative regulation of estrogen signaling by ERbeta and RIP140 in ovarian
434 cancer cells. *Mol Endocrinol* **27** 1429-1441.

435 Duclot F, Lapierre M, Fritsch S, White R, Parker MG, Maurice T & Cavailles V 2012 Cognitive
436 impairments in adult mice with constitutive inactivation of RIP140 gene expression. *Genes Brain*
437 *Behav* **11** 69-78.

438 Feng X, Krogh KA, Wu CY, Lin YW, Tsai HC, Thayer SA & Wei LN 2014 Receptor-interacting protein
439 140 attenuates endoplasmic reticulum stress in neurons and protects against cell death. *Nat*
440 *Commun* **5** 4487.

441 Feng X, Lin YL & Wei LN 2015 Behavioral stress reduces RIP140 expression in astrocyte and increases
442 brain lipid accumulation. *Brain Behav Immun* **46** 270-279.

443 Fernandez-Marcos PJ & Auwerx J 2011 Regulation of PGC-1alpha, a nodal regulator of mitochondrial
444 biogenesis. *Am J Clin Nutr* **93** 884S-890.

445 Ferreira AM, Tuominen I, Sousa S, Gerbens F, van Dijk-Bos K, Osinga J, Kooi KA, Sanjabi B, Esendam C,
446 Oliveira C, et al. 2014 New target genes in endometrial tumors show a role for the estrogen-receptor
447 pathway in microsatellite-unstable cancers. *Hum Mutat* **35** 1514-1523.

448 Fritah A, Steel JH, Nichol D, Parker N, Williams S, Price A, Strauss L, Ryder TA, Mobberley MA,
 449 Poutanen M, et al. 2010 Elevated expression of the metabolic regulator receptor-interacting protein
 450 140 results in cardiac hypertrophy and impaired cardiac function. *Cardiovasc Res* **86** 443-451.
 451 Fritah A, Steel JH, Nichol D, Parker N, Williams S, Price A, Strauss L, Ryder TA, Mobberley MA,
 452 Poutanen M, et al. 2011 Elevated expression of the metabolic regulator receptor-interacting protein
 453 140 results in cardiac hypertrophy and impaired cardiac function. *Cardiovasc Res* **86** 443-451.
 454 Fritah A, Steel JH, Parker N, Nikolopoulou E, Christian M, Carling D & Parker MG 2012 Absence of
 455 RIP140 reveals a pathway regulating glut4-dependent glucose uptake in oxidative skeletal muscle
 456 through UCP1-mediated activation of AMPK. *PLoS One* **7** e32520.
 457 Fullwood MJ, Liu MH, Pan YF, Liu J, Xu H, Mohamed YB, Orlov YL, Velkov S, Ho A, Mei PH, et al. 2009
 458 An oestrogen-receptor-alpha-bound human chromatin interactome. *Nature* **462** 58-64.
 459 Gautier EL, Shay T, Miller J, Greter M, Jakubzick C, Ivanov S, Helft J, Chow A, Elpek KG, Gordonov S, et
 460 al. 2012 Gene-expression profiles and transcriptional regulatory pathways that underlie the identity
 461 and diversity of mouse tissue macrophages. *Nat Immunol* **13** 1118-1128.
 462 Gibson WJ, Hoivik EA, Halle MK, Taylor-Weiner A, Cherniack AD, Berg A, Holst F, Zack TI, Werner HM,
 463 Staby KM, et al. 2016 The genomic landscape and evolution of endometrial carcinoma progression
 464 and abdominopelvic metastasis. *Nat Genet*.
 465 Glass CK, Devary OV & Rosenfeld MG 1990 Multiple cell type-specific proteins differentially regulate
 466 target sequence recognition by the alpha retinoic acid receptor. *Cell* **63** 729-738.
 467 Gupta P, Huq MD, Khan SA, Tsai NP & Wei LN 2005 Regulation of co-repressive activity of and HDAC
 468 recruitment to RIP140 by site-specific phosphorylation. *Mol Cell Proteomics* **4** 1776-1784.
 469 Gupta P, Park SW, Farooqui M & Wei LN 2007 Orphan nuclear receptor TR2, a mediator of
 470 preadipocyte proliferation, is differentially regulated by RA through exchange of coactivator PCAF
 471 with corepressor RIP140 on a platform molecule GRIP1. *Nucleic Acids Res* **35** 2269-2282.
 472 Halachmi S, Marden E, Martin G, MacKay H, Abbondanza C & Brown M 1994 Estrogen receptor-
 473 associated proteins: possible mediators of hormone-induced transcription. *Science* **264** 1455-1458.
 474 Hallberg M, Morganstein DL, Kiskinis E, Shah K, Kralli A, Dilworth SM, White R, Parker MG & Christian
 475 M 2008 A functional interaction between RIP140 and PGC-1alpha regulates the expression of the
 476 lipid droplet protein CIDEA. *Mol Cell Biol* **28** 6785-6795.
 477 Hannafon BN, Sebastiani P, de Las Morenas A, Lu J & Rosenberg CL Expression of microRNA and their
 478 gene targets are dysregulated in preinvasive breast cancer. *Breast Cancer Res* **13** R24.
 479 Heery DM, Kalkhoven E, Hoare S & Parker MG 1997 A signature motif in transcriptional co-activators
 480 mediates binding to nuclear receptors. *Nature* **387** 733-736.
 481 Heinzel T, Lavinsky RM, Mullen TM, Soderstrom M, Laherty CD, Torchia J, Yang WM, Brard G, Ngo
 482 SD, Davie JR, et al. 1997 A complex containing N-CoR, mSin3 and histone deacetylase mediates
 483 transcriptional repression. *Nature* **387** 43-48.
 484 Herold T, Jurinovic V, Metzeler KH, Boulesteix AL, Bergmann M, Seiler T, Mulaw M, Thoene S, Dufour
 485 A, Pasalic Z, et al. 2011 An eight-gene expression signature for the prediction of survival and time to
 486 treatment in chronic lymphocytic leukemia. *Leukemia* **25** 1639-1645.
 487 Herzog B, Hallberg M, Seth A, Woods A, White R & Parker MG 2007 The nuclear receptor cofactor,
 488 receptor-interacting protein 140, is required for the regulation of hepatic lipid and glucose
 489 metabolism by liver X receptor. *Mol Endocrinol* **21** 2687-2697.
 490 Ho PC, Gupta P, Tsui YC, Ha SG, Huq M & Wei LN 2008 Modulation of lysine acetylation-stimulated
 491 repressive activity by Erk2-mediated phosphorylation of RIP140 in adipocyte differentiation. *Cell*
 492 *Signal* **20** 1911-1919.
 493 Ho PC, Tsui YC, Feng X, Greaves DR & Wei LN 2012 NF-kappaB-mediated degradation of the
 494 coactivator RIP140 regulates inflammatory responses and contributes to endotoxin tolerance. *Nat*
 495 *Immunol* **13** 379-386.
 496 Hu F, Wang M, Xiao T, Yin B, He L, Meng W, Dong M & Liu F 2015 miR-30 promotes thermogenesis
 497 and the development of beige fat by targeting RIP140. *Diabetes* **64** 2056-2068.

Ikonen T, Palvimo JJ & Janne OA 1997 Interaction between the amino- and carboxyl-terminal regions of the rat androgen receptor modulates transcriptional activity and is influenced by nuclear receptor coactivators. *J Biol Chem* **272** 29821-29828.

Jacq X, Brou C, Lutz Y, Davidson I, Chambon P & Tora L 1994 Human TAFII30 is present in a distinct TFIID complex and is required for transcriptional activation by the estrogen receptor. *Cell* **79** 107-117.

Joseph R, Orlov YL, Huss M, Sun W, Kong SL, Ukil L, Pan YF, Li G, Lim M, Thomsen JS, et al. 2010 Integrative model of genomic factors for determining binding site selection by estrogen receptor-alpha. *Mol Syst Biol* **6** 456.

Joyeux A, Cavailles V, Balaguer P & Nicolas JC 1997 RIP 140 enhances nuclear receptor-dependent transcription in vivo in yeast. *Mol Endocrinol* **11** 193-202.

Kato S, Yokoyama A & Fujiki R 2011 Nuclear receptor coregulators merge transcriptional coregulation with epigenetic regulation. *Trends Biochem Sci* **36** 272-281.

Kiskinis E, Chatzeli L, Curry E, Kaforou M, Frontini A, Cinti S, Montana G, Parker MG & Christian M 2014 RIP140 represses the "brown-in-white" adipocyte program including a futile cycle of triacylglycerol breakdown and synthesis. *Mol Endocrinol* **28** 344-356.

Lapierre M, Bonnet S, Bascoul-Mollevi C, Ait-Arsa I, Jalaguier S, Del Rio M, Plateroti M, Roepman P, Ychou M, Pannequin J, et al. 2014 RIP140 increases APC expression and controls intestinal homeostasis and tumorigenesis. *J Clin Invest* **124** 1899-1913.

Lapierre M, Castet-Nicolas A, Gitenay D, Jalaguier S, Teyssier C, Bret C, Cartron G, Moreaux J & Cavailles V 2015a Expression and role of RIP140/NRIP1 in chronic lymphocytic leukemia. *J Hematol Oncol* **8** 20.

Lapierre M, Docquier A, Castet-Nicolas A, Gitenay D, Jalaguier S, Teyssier C & Cavailles V 2015b The emerging role of the transcriptional coregulator RIP140 in solid tumors. *Biochim Biophys Acta* **1856** 144-150.

Lee S, Medina D, Tsimelzon A, Mohsin SK, Mao S, Wu Y & Allred DC 2007 Alterations of gene expression in the development of early hyperplastic precursors of breast cancer. *Am J Pathol* **171** 252-262.

Leo C & Chen JD 2000 The SRC family of nuclear receptor coactivators. *Gene* **245** 1-11.

Leonardsson G, Jacobs MA, White R, Jeffery R, Poulsom R, Milligan S & Parker M 2002 Embryo transfer experiments and ovarian transplantation identify the ovary as the only site in which nuclear receptor interacting protein 1/RIP140 action is crucial for female fertility. *Endocrinology* **143** 700-707.

Leonardsson G, Steel JH, Christian M, Pocock V, Milligan S, Bell J, So PW, Medina-Gomez G, Vidal-Puig A, White R, et al. 2004 Nuclear receptor corepressor RIP140 regulates fat accumulation. *Proc Natl Acad Sci U S A* **101** 8437-8442.

Liu PS, Lin YW, Burton FH & Wei LN 2015a Injecting engineered anti-inflammatory macrophages therapeutically induces white adipose tissue browning and improves diet-induced insulin resistance. *Adipocyte* **4** 123-128.

Liu PS, Lin YW, Burton FH & Wei LN 2015b M1-M2 balancing act in white adipose tissue browning - a new role for RIP140. *Adipocyte* **4** 146-148.

Liu PS, Lin YW, Lee B, McCrady-Spitzer SK, Levine JA & Wei LN 2014 Reducing RIP140 expression in macrophage alters ATM infiltration, facilitates white adipose tissue browning, and prevents high-fat diet-induced insulin resistance. *Diabetes* **63** 4021-4031.

Lonard DM & O'Malley B W 2007 Nuclear receptor coregulators: judges, juries, and executioners of cellular regulation. *Mol Cell* **27** 691-700.

Lumeng CN, Bodzin JL & Saltiel AR 2007 Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest* **117** 175-184.

Millard CJ, Watson PJ, Fairall L & Schwabe JW 2013 An evolving understanding of nuclear receptor coregulator proteins. *J Mol Endocrinol* **51** T23-36.

- 548 Mosser DM & Edwards JP 2008 Exploring the full spectrum of macrophage activation. *Nat Rev*
 549 *Immunol* **8** 958-969.
- 550 Nagy L, Kao HY, Chakravarti D, Lin RJ, Hassig CA, Ayer DE, Schreiber SL & Evans RM 1997 Nuclear
 551 receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. *Cell*
 552 **89** 373-380.
- 553 Nautiyal J, Christian M & Parker MG 2013a Distinct functions for RIP140 in development,
 554 inflammation, and metabolism. *Trends Endocrinol Metab* **24** 451-459.
- 555 Nautiyal J, Steel JH, Mane MR, Oduwale O, Poliandri A, Alexi X, Wood N, Poutanen M, Zwart W,
 556 Stingl J, et al. 2013b The transcriptional co-factor RIP140 regulates mammary gland development by
 557 promoting the generation of key mitogenic signals. *Development* **140** 1079-1089.
- 558 Nautiyal J, Steel JH, Rosell MM, Nikolopoulou E, Lee K, Demayo FJ, White R, Richards JS & Parker MG
 559 2010 The nuclear receptor cofactor receptor-interacting protein 140 is a positive regulator of
 560 amphiregulin expression and cumulus cell-oocyte complex expansion in the mouse ovary.
 561 *Endocrinology* **151** 2923-2932.
- 562 O'Malley BW, Qin J & Lanz RB 2008 Cracking the coregulator codes. *Curr Opin Cell Biol* **20** 310-315.
- 563 Ochsner SA, Day AJ, Rugg MS, Breyer RM, Gomer RH & Richards JS 2003a Disrupted function of
 564 tumor necrosis factor-alpha-stimulated gene 6 blocks cumulus cell-oocyte complex expansion.
 565 *Endocrinology* **144** 4376-4384.
- 566 Ochsner SA, Russell DL, Day AJ, Breyer RM & Richards JS 2003b Decreased expression of tumor
 567 necrosis factor-alpha-stimulated gene 6 in cumulus cells of the cyclooxygenase-2 and EP2 null mice.
 568 *Endocrinology* **144** 1008-1019.
- 569 Odegaard JI & Chawla A 2013 Pleiotropic actions of insulin resistance and inflammation in metabolic
 570 homeostasis. *Science* **339** 172-177.
- 571 Oh DS, Troester MA, Usary J, Hu Z, He X, Fan C, Wu J, Carey LA & Perou CM 2006 Estrogen-regulated
 572 genes predict survival in hormone receptor-positive breast cancers. *J Clin Oncol* **24** 1656-1664.
- 573 Onate SA, Tsai SY, Tsai MJ & O'Malley BW 1995 Sequence and characterization of a coactivator for
 574 the steroid hormone receptor superfamily. *Science* **270** 1354-1357.
- 575 Park JY, Su YQ, Ariga M, Law E, Jin SL & Conti M 2004 EGF-like growth factors as mediators of LH
 576 action in the ovulatory follicle. *Science* **303** 682-684.
- 577 Park SW, Huang WH, Persaud SD & Wei LN 2009 RIP140 in thyroid hormone-repression and
 578 chromatin remodeling of Crabp1 gene during adipocyte differentiation. *Nucleic Acids Res* **37** 7085-
 579 7094.
- 580 Persaud SD, Huang WH, Park SW & Wei LN 2011 Gene repressive activity of RIP140 through direct
 581 interaction with CDK8. *Mol Endocrinol* **25** 1689-1698.
- 582 Powelka AM, Seth A, Virbasius JV, Kiskinis E, Nicoloso SM, Guilherme A, Tang X, Straubhaar J,
 583 Cherniack AD, Parker MG, et al. 2006 Suppression of oxidative metabolism and mitochondrial
 584 biogenesis by the transcriptional corepressor RIP140 in mouse adipocytes. *J Clin Invest* **116** 125-136.
- 585 Puigserver P, Wu Z, Park CW, Graves R, Wright M & Spiegelman BM 1998 A cold-inducible
 586 coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* **92** 829-839.
- 587 Richards JS 2007 Genetics of ovulation. *Semin Reprod Med* **25** 235-242.
- 588 Richards JS & Pangas SA 2010 The ovary: basic biology and clinical implications. *J Clin Invest* **120** 963-
 589 972.
- 590 Rollins DA, Coppo M & Rogatsky I 2015 Minireview: nuclear receptor coregulators of the p160
 591 family: insights into inflammation and metabolism. *Mol Endocrinol* **29** 502-517.
- 592 Rosell M, Jones MC & Parker MG 2011 Role of nuclear receptor corepressor RIP140 in metabolic
 593 syndrome. *Biochim Biophys Acta* **1812** 919-928.
- 594 Rosell M, Nevedomskaya E, Stelloo S, Nautiyal J, Poliandri A, Steel JH, Wessels LF, Carroll JS, Parker
 595 MG & Zwart W 2014 Complex Formation and Function of Estrogen Receptor alpha in Transcription
 596 Requires RIP140. *Cancer Res*.

Seth A, Steel JH, Nichol D, Pocock V, Kumaran MK, Fritah A, Mobberley M, Ryder TA, Rowlerson A, Scott J, et al. 2007 The transcriptional corepressor RIP140 regulates oxidative metabolism in skeletal muscle. *Cell Metab* **6** 236-245.

Shimada M, Hernandez-Gonzalez I, Gonzalez-Robayna I & Richards JS 2006 Paracrine and autocrine regulation of epidermal growth factor-like factors in cumulus oocyte complexes and granulosa cells: key roles for prostaglandin synthase 2 and progesterone receptor. *Mol Endocrinol* **20** 1352-1365.

Stashi E, York B & O'Malley BW 2014 Steroid receptor coactivators: servants and masters for control of systems metabolism. *Trends Endocrinol Metab* **25** 337-347.

Stern JH, Rutkowski JM & Scherer PE 2016 Adiponectin, Leptin, and Fatty Acids in the Maintenance of Metabolic Homeostasis through Adipose Tissue Crosstalk. *Cell Metab* **23** 770-784.

Torchia J, Rose DW, Inostroza J, Kamei Y, Westin S, Glass CK & Rosenfeld MG 1997 The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. *Nature* **387** 677-684.

Treuter E, Albrechtsen T, Johansson L, Leers J & Gustafsson JA 1998 A regulatory role for RIP140 in nuclear receptor activation. *Mol Endocrinol* **12** 864-881.

Tsai NP, Lin YL & Wei LN 2009 MicroRNA mir-346 targets the 5'-untranslated region of receptor-interacting protein 140 (RIP140) mRNA and up-regulates its protein expression. *Biochem J* **424** 411-418.

Tullet JM, Pocock V, Steel JH, White R, Milligan S & Parker MG 2005 Multiple signaling defects in the absence of RIP140 impair both cumulus expansion and follicle rupture. *Endocrinology* **146** 4127-4137.

van't Veer MB, Brooijmans AM, Langerak AW, Verhaaf B, Goudswaard CS, Graveland WJ, van Lom K & Valk PJ 2006 The predictive value of lipoprotein lipase for survival in chronic lymphocytic leukemia. *Haematologica* **91** 56-63.

Vo N, Fjeld C & Goodman RH 2001 Acetylation of nuclear hormone receptor-interacting protein RIP140 regulates binding of the transcriptional corepressor CtBP. *Mol Cell Biol* **21** 6181-6188.

Walden TB, Hansen IR, Timmons JA, Cannon B & Nedergaard J 2012 Recruited vs. nonrecruited molecular signatures of brown, "brite," and white adipose tissues. *Am J Physiol Endocrinol Metab* **302** E19-31.

Wei LN, Hu X, Chandra D, Seto E & Farooqui M 2000 Receptor-interacting protein 140 directly recruits histone deacetylases for gene silencing. *J Biol Chem* **275** 40782-40787.

White R, Leonardsson G, Rosewell I, Ann Jacobs M, Milligan S & Parker M 2000 The nuclear receptor co-repressor nr1p (RIP140) is essential for female fertility. *Nat Med* **6** 1368-1374.

Wieland S, Dobbeling U & Rusconi S 1991 Interference and synergism of glucocorticoid receptor and octamer factors. *EMBO J* **10** 2513-2521.

Wong MM, Guo C & Zhang J 2014 Nuclear receptor corepressor complexes in cancer: mechanism, function and regulation. *Am J Clin Exp Urol* **2** 169-187.

Wynn TA, Chawla A & Pollard JW 2013 Macrophage biology in development, homeostasis and disease. *Nature* **496** 445-455.

York B & O'Malley BW 2010 Steroid receptor coactivator (SRC) family: masters of systems biology. *J Biol Chem* **285** 38743-38750.

Yuan R, Meng Q, Nautiyal J, Flurkey K, Tsaih SW, Krier R, Parker MG, Harrison DE & Paigen B 2012 Genetic coregulation of age of female sexual maturation and lifespan through circulating IGF1 among inbred mouse strains. *Proc Natl Acad Sci U S A* **109** 8224-8229.

Zschiedrich I, Hardeland U, Krones-Herzig A, Berriel Diaz M, Vegiopoulos A, Muggenburg J, Sombroek D, Hofmann TG, Zawatzky R, Yu X, et al. 2008 Coactivator function of RIP140 for NFkappaB/RelA-dependent cytokine gene expression. *Blood* **112** 264-276.

Figure Legends

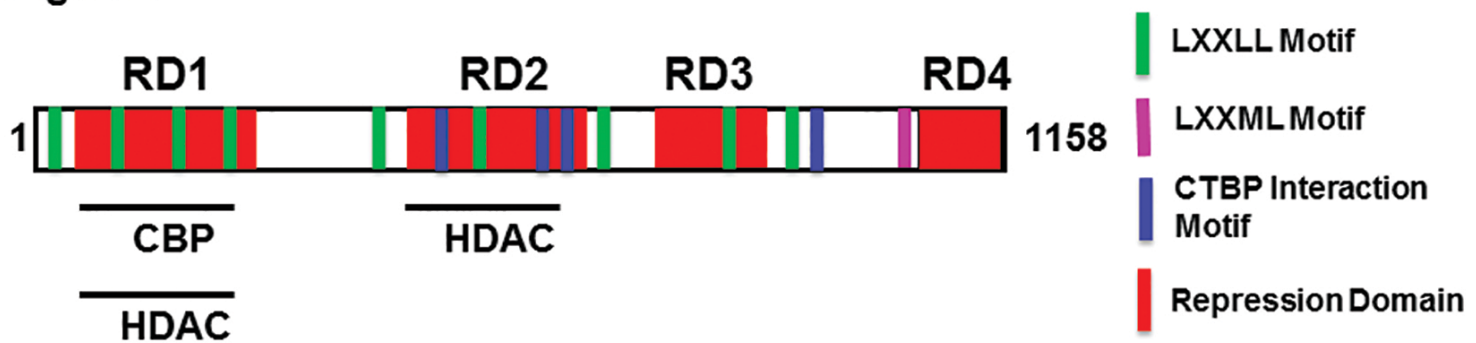
RIP140 a switch coregulator (Figure 1): Cartoon of RIP140 protein showing different known domains (RD1-4) and interaction motifs (LXXLL, LXXML) (Christian et al. 2006) with transcription factors and enzymes which modify histones ie Histone deacetylase (HDAC) and CREB-Binding Protein (CBP). RIP140 can function both as a coactivator (ON Switch) as well as a corepressor (OFF Switch). Key processes regulated by RIP140 are highlighted in green (activation) and red (repression) boxes respectively.

Biological Processes regulated by RIP140 (Figure 2): *Ovary:* Pre-ovulatory follicles with granulosa cells (pink) lining the follicle. The cumulus oocyte complex, which is the oocyte surrounded by cumulus granulosa cells (darker blue) is shown in an unexpanded state on left. Following the LH surge RIP140 activates Amphiregulin (*Areg*) expression which further activates downstream enzymes that synthesise the cumulus matrix. This results in cumulus expansion i.e cumulus granulosa cells spread on a self-synthesised matrix (lighter blue cells), shown on right (Nautiyal et al. 2010; Ochsner, et al. 2003a; Ochsner, et al. 2003b)

Mammary Gland: Shown on the left is a mammary fat pad with rudimentary epithelium at the base representing a pre-pubertal mouse mammary gland. RIP140 activates mitogens and factors which are essential for mammary gland development, side branching and differentiation (Right). These factors include *Areg*, *PGR*, *STAT5a*, *Ccnd1* which are all ER target genes which are coactivated by RIP140 in the mammary gland (Nautiyal et al. 2013a; Nautiyal et al. 2013b).

Adipose Tissue: (Extreme left) Fat storing WAT which is composed of adipocytes and M2 macrophages. WAT adipocytes expand in size on exposure to high fat diet to meet the increasing demands of fat storage and also have an increased infiltration of macrophages which are of the M1 (i.e inflammatory type). Loss of RIP140 promotes formation of BAT. RIP140 KO animals are lean and show a BAT adipose phenotype. BAT (extreme right) has smaller fat droplets and abundant in mitochondria and generate heat. Loss of RIP140 from macrophages promotes the formation of Beige fat depots which are adipocytes within the WAT which are intermediary between WAT and BAT. This is called ‘browning of fat’ which is promoted by RIP140 loss (Liu et al. 2015b).

Figure 1



Corepressor

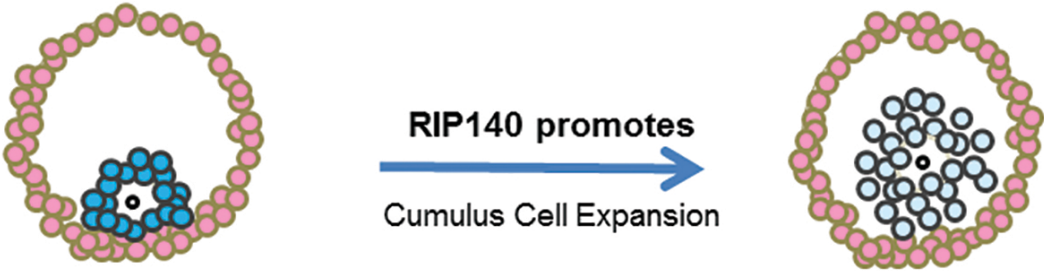
- Formation of Beige/BRITE depots
- Cell division (intestine)
- Respiratory uncoupling
- Lipogenesis
- Gluconeogenesis
- Lipid droplet formation
- Adipocyte differentiation



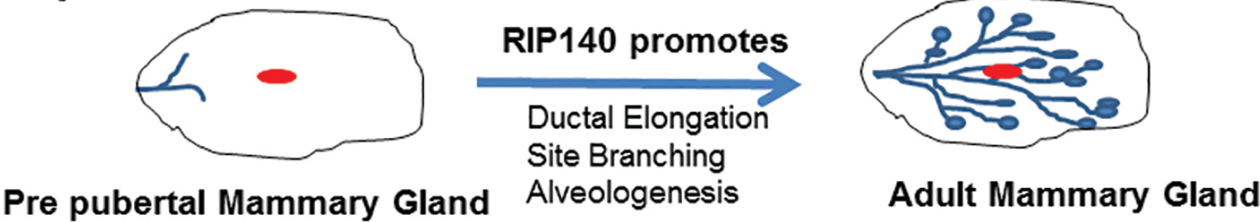
Coactivator

- Inflammation
- Cell division (Mammary gland)
- Sidebranching and Alveologenesis (Mammary Gland)
- Circadian rhythms
- Ovulation (Cumulus Expansion)

Figure 2
Ovary



Mammary Gland



Adipose

