Genome-wide Study of Functional Cross-talks Between ERRα and COUP-TFII, Two Orphan Nuclear Receptors

Xinhao Zhao

Department of Biochemistry
McGill University
Montreal, Quebec, Canada
August 2010

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

ABSTRACT

Nuclear receptors play essential roles in many aspects of cellular regulation such as cell development, differentiation, cell death, energy homeostasis, and metabolism of the organism. ERRα and COUP-TFII are both orphan nuclear receptors known to recognize similar DNA response elements. ERRα often binds to an extended half site TNAAGGTCA in the promoter regions of target genes, whereas COUP-TFII prefers to bind to a number of variably spaced imperfect AGGTCA direct or inverted repeats. To better understand the cross talk between these two proteins, ChIP-on-chip experiments were performed on mouse liver in order to identify the targets of ERRα and COUP-TFII on a genome-wide scale. 299 genes with a large variety of biological processes were found to be common targets of ERRα and COUP-TFII, meaning that these genes recruit the two nuclear receptors to the same binding sites. In these cases, ERRα and COUP-TFII are likely to compete for the common DNA binding site in order to regulate their target genes.

RÉSUMÉ

Les récepteurs nucléaires jouent un rôle critique dans plusieurs aspects de la physiologie cellulaire, incluant le développement, la différentiation, l'apoptose, l'homéostasie et le métabolisme énergétique. ERRα et COUP-TFII sont tous deux des récepteurs nucléaires orphelins connus pour se lier à des éléments de réponse similaires au niveau de l'ADN. ERRα se lie généralement à un demi-site allongé TNAAGGTCA situé dans la région du promoteur de ses gènes cibles. COUP-TFII se lie quant à lui à des motifs AGGTCA, en répétition directe ou en palindrome, séparés par un nombre variable de nucléotides. Afin de mieux comprendre les interactions entre ces deux protéines, une analyse génomique ChIP-sur-chip (pour Chromatin ImmunoPrécipitation sur Chip) a été faite dans des tissus de foie murin. Cette analyse a permis d'établir la liste des gènes cibles d'ERRα et COUP-TFII dans cet organe. 299 gènes communs aux deux récepteurs nucléaires ont été identifiés. Ces gènes, impliqués dans une grande variété de processus biologiques, sont liés au même élément de réponse de l'ADN par les deux récepteurs. Nos résultats suggèrent qu'ERRα et COUP-TFII exercent une liaison compétitive à l'ADN, sur les éléments de réponse commun, pour la régulation de leurs gènes cibles.

PREFACE-CONTRIBUTION OF AUTHORS

The research conducted and presented in this thesis is entirely my own work except for the ChIP-on-chip experiments. The ChIP-on-chip experiments were optimized and performed by Catherine Rosa Dufour. This thesis was written by me and corrected by Dr. Vincent Giguère and Lillian Eichner.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my supervisor, **Dr. Vincent Giguère**, for his encouragement and endless support during my Master's studies and for giving me the opportunity to work in his laboratory and expand my knowledge in research.

I would also like to give special thanks to **Catherine Rosa Dufour**, for all her time, support, suggestions, and crucial contributions to my thesis and precious friendship.

I wish to acknowledge all present and past members of Dr. Giguère's laboratory: **Majid Ghahremani** for managing the lab so well; **Lillian Eichner** and **Brian Wilson** for all the support, advice, technique training, enlightening discussions and laughs; **Geneviève Deblois**, **Marie-Claude Perry**, **Guillaume Sylvain-Drolet** and **Xingxing Liu** for their technical assistance, scientific criticisms and suggestions. **Aymen Shatnawi**, **Carlo Ouellet**, **Cedric Chaveroux**, **Marie-Pier Levasseur, Annie Tremblay** and **Alexis Charest-Marcotte** for all their kind support and help.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

ACSL1	acyl-CoA synthetase long-chain family member 1
ACTR	activator of thyroid and retinoic acid receptors
ADIPOR2	adiponectin receptor 2
AF	activation function
AR	androgen receptor
BAD	BCL2-associated agonist of cell death
BMP2K	BMP2 inducible kinase
ChIP	chromatin immunoprecipitation
CNS	central nervous system
COUP-TF	chicken ovalbumin upstream promoter transcription factor
CtBP	C-terminal binding protein
CTE	carboxy terminal extension
CYP7A	7α-hydeoxylase gene
DBD	DNA-binding domain
EDTA	ethylenediaminetetraacetic acid
ER	estrogen receptor
ERE	estrogen response element
ERR	estrogen-related receptor
ERRE	estrogen-related receptor response element
FXR	farnesoid X receptor
GR	glucocorticoid receptor

GRN	granulin
GRIP1	glucocorticoid receptor interacting protein 1
HDAC	histone deacetylase
HNF	hepatocyte nuclear factor
HRE	hexanucleotide respond element
HSP	heat shock proteins
IFN	interferon
JUN	Jun oncogene
LBD	ligand-binding domain
LBP	ligand-binding pocket
LXR	liver X receptor
MTOR	mechanistic target of rapamycin (serine/threonine kinase)
MR	mineralocorticoid receptor
NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
NLS	nuclear location signal
NR	nuclear receptor
Nr5a2	nuclear receptor subfamily 5, group A, member 2
OXPHOS	oxidative phosphorylation
PGC-1α	peroxisome proliferator-activated receptor γ coactivator-1α
PPAR	peroxisome proliferator-activated receptor
PR	progestin receptor
PRKAG2	protein kinase, AMP-activated, gamma 2 non-catalytic

	subunit
RAR	retinoic acid receptor
RIP140	receptor interacting protein of 140 kDa
RT-PCR	real-time polymerase chain reaction
RXR	9-cis retinoic acid receptor
SOCS5	suppressor of cytokine signaling 5
SHP	small heterodimer partner
SHR	steroid hormone receptor
SRC	steroid receptor coactivator
TR	thyroid hormone
VDR	vitamin D_3 receptor
WAT	white adipose tissue
Zfp524	zinc finger protein 524

CHAPTER I-Literature Review

1. Nuclear Receptor Superfamily

Nuclear receptors represent a large family of transcription factors that play essential roles in many aspects of cellular regulation including cell development, differentiation, cell death, energy homeostasis, and metabolism of the organism (Laudet, Hanni et al. 1992; Gronemeyer, Gustafsson et al. 2004; Bain, Heneghan et al. 2007). To date, over 300 nuclear receptors have been identified in various species (Zhang, Burch et al. 2004). Those activated by ligands are classified as ligand-dependent nuclear receptors, whereas the other NRs without known endogenous ligands are called “orphan” receptors (Giguere 1999).

1.1 Ligand-Dependent Nuclear Receptors

Ligand-dependent nuclear receptors function to mediate specific transcriptional responses upon binding to their ligands. These receptors can be subdivided into two subfamilies. One subfamily includes genes encoding receptors for progestins (PR), estrogens (ER), androgens (AR), glucocorticoids (GR), and mineralocorticoids (MR), which are also referred to as steroid hormone receptors (SHRs). In the absence of ligands, the nuclear location signal (NLS) of the SHRs are masked by the heat shock proteins (HSPs) and the SHRs are therefore sequestered in a non-active

state in the cytosol (McKenna, Lanz et al. 1999). Once bound to their ligands, SHRs will change conformation, disassociate from HSPs and translocate into the nucleus. They usually function as homodimers and activate transcription by binding to two palindromic sequences, AGGACA. One exception in this family is ER, which recognizes AGGTCA, also known as the estrogen response elements (ERE), or as a half site (Beato, Herrlich et al. 1995; Carolyn M. Klinge 1997).

The other subfamily of ligand-dependent receptors consists of those for thyroid hormone (Warnmark, Treuter et al. 2003), 9-cis retinoic acid (RXR), all-trans retinoic acid (Horard and Vanacker 2003), and vitamin D_3 (VDR). The classical non-steroid nuclear receptors are typically not bound to HSPs. Instead, they are found more predominantly in the nucleus rather than in the cytosol. They are able to bind to their cognate promoters in the absence of ligand and often induce a basal level repression (McKenna, Lanz et al. 1999). Upon ligand binding, they can form either homodimers or heterodimers and control target gene expression by occupying two ERE half sites (Chen and Evans 1995; Carolyn M. Klinge 1997).

1.2 Orphan Nuclear Receptors

In contrast to ligand-dependent nuclear receptors, orphan nuclear receptors are defined as gene products that share similar structures with classic nuclear receptors, but which have no known natural ligand (Giguere 1999).

A strategy, named “reverse endocrinology” has been developed and used over the years to search for ligands using the receptors as screen targets. A few ligands have actually been discovered for some members of the orphan receptor family using this method, such as 9-cis retinoic acids for RXRs (retinoid X receptors), fatty acids for PPARs (peroxisome proliferator-activated receptors), farnesol metabolites for FXRs (farnesoid X receptors), and 24(S) hydroxycholesterol for LXRs (liver X receptors) (Gottlicher, Widmark et al. 1992; Heyman, Mangelsdorf et al. 1992; Levin, Sturzenbecker et al. 1992; Forman BM 1995; Janowski, Willy et al. 1996). This leads to the discovery of novel signaling system and helps to develop new therapeutic compounds for various diseases.

1.3 Nuclear Receptor Anatomy

Nuclear receptors are composed of an amino (N) terminal region, a highly conserved DNA-binding domain (DBD), a hinge region and a moderately conserved C-terminal ligand-binding domain (LBD) (Giguere 1999).

The N terminal region, also known as the A/B domain, is the least conserved region among NRs in terms of size and sequence. This region often contains a transcriptional activation domain, the AF-1, which exhibits <15% conservation across the nuclear receptor Superfamily (Bain, Heneghan et al. 2007). Consequently, this could result in the differential regulation of gene promoters by closely related NRs binding to the same

response element. In addition, isoforms of NRs that differ exclusively in the amino terminal region are generated by alternative splicing or using different promoters (Warnmark, Treuter et al. 2003).

The DNA binding domain (DBD) is centrally located and is the most conserved domain of NR proteins, which consists of two zinc fingers and a carboxyl terminal extension (CTE). NRs usually bind to DNA in forms of monomers, heterodimers or homodimers (Giguere 1999). The P-box domain of the DBD mediates the NRs to the hexanucleotide respond elements (HREs) containing one or two consensus core half sites sequences (Umesono and Evans 1989). D- and DR-boxes of DBDs provide the necessary interface for NRs to form dimers while binding to DNA (Zechel, Shen et al. 1994). The CTE also forms interfaces assisting protein-DNA and protein-protein interactions (Rastinejad, Perlmann et al. 1995; Zhao, Khorasanizadeh et al. 1998).

The hinge region of NRs serves to link the DBD and the LBD. The length and sequence of this region is quite variable. The flexibility of this structure allows the DBD to rotate freely so that dimers of NRs are able to bind to direct or inverted HREs (Glass 1994).

The ligand binding domain (LBD) serves many important functions including ligand binding, transcriptional activation, and dimerization. It

contains another transcriptional activation domain, the AF-2. In contrast to the AF-1, the AF-2 domain is moderately conserved. It can synergize with the AF-1 in the amino terminal region and give NRs full transcriptional activity (Warnmark, Treuter et al. 2003).

2. The Estrogen Related Receptor (ERR) Family

There are three members of the estrogen related receptor family, namely ERRα, ERRβ and ERRγ, all of which are recognized as orphan nuclear receptors. ERRα and β were first isolated during the search for genes related to the estrogen receptors in kidney and heart (Giguere, Yang et al. 1988), whereas ERRγ was identified a decade later when looking for the critical gene causing the Usher syndrome, although ultimately ERRγ was shown not to be responsible for this disease (Hong, Yang et al. 1999). ERRs recognize the consensus extended half site, TNAAGGTCA, also known as the ERR-response element (ERRE), and they bind to this element as either homo or heterodimers. Upon binding their target genes, ERRs recruit co-regulators and activate transcription, without requiring ligands (Giguere 2002).

2.1 The Orphan Nature of the ERRs

ERRs and ERs are evolutionarily related to each other. ERRs share great sequence similarity with ERs. The sequence is over 60% identical in the DBD region and ~35% identical sequence in the LBD region in human

(Horard and Vanacker 2003). However, ERRα has been shown not to bind to estrogen, like ER, or any of the other major classes of steroids (Giguere, Yang et al. 1988). The crystal structure of the ERRα LBD has been isolated and the putative ligand-binding pocket (LBP) was found to be completely occupied by the bulky side chains (Kallen, Schlaeppi et al. 2004). In the mean time, the crystal structure of the ERRγ LBD revealed that the LBP is so small that only molecules half the size of estrogen could fit in it. The ERRβ LBP is very similar to that of ERRγ, with only two differing amino acids, suggesting that estrogen cannot bind to ERRβ either (Greschik, Wurtz et al. 2002). Although small molecules have the potential to bind to the LBD, to date, no natural ligand has been identified for the ERRs.

While the search for ligands for ERRs continues, evidence indicates that ERRs are constitutively in a transcriptionally active state (Hong, Yang et al. 1999; Xie, Hong et al. 1999; Chen, Zhou et al. 2001). One group has resolved the crystal structure of ERRγ bound with the co-activator SRC-1. No ligand is found in the LBP. However, the LBD of ERRγ adopts the typical transcriptionally active conformation (Greschik, Wurtz et al. 2002). Mutants made with an enlarged LBP remains transcriptional active with or without ligands. This further indicates that the presence of ligands is neither necessary nor likely for the ERRs .

2.2 ERRα

ERRα has been found to be highly expressed during the development of the embryo and adults. The expression of ERRα is detected as early as 8.5 d.p.c. in the trophoblast, mesoderm cells of the visceral sac, the primitive heart and the neural tube. In adults, ERRα is mostly expressed in tissues with high-energy demand, such as the heart, kidneys, liver, skeletal muscle, and brown fat (Giguere 1999; Giguere 2002). Its expression can be induced by stimuli, such as exposure to cold, exercise and fasting, which require large amounts of energy consumption (Ichida, Nemoto et al. 2002; Schreiber, Knutti et al. 2003). ERRα has also been shown to be important in bone development and remodeling (Bonnelye, Merdad et al. 2001; Bonnelye, Kung et al. 2002).

2.2.1 Physiological Function of ERRα

ERRα knockout mice are viable, fertile and do not present gross abnormalities. Extensive studies have been carried out with this mouse model. One group has shown that there is no significant change in food consumption and energy expenditure between the wild type and KO mice. However, the ERRα null mice have less fat and body weight compared to the controls and they are more resistant to high-fat-induced obesity (Luo, Sladek et al. 2003). Other studies show that ERRα null mice have difficulty maintaining their core body temperature when exposed to cold, develop signs of heart failure under cardiac overload, and are deficient in response

to IFN-γ stimulated oxidative metabolism necessary to clear infection (Huss, Imahashi et al. 2007; Sonoda, Laganiere et al. 2007; Villena, Hock et al. 2007). Altogether, ERRα null mice have defects in handling stressors requiring high-energy production or utilization, furthering supporting the important role of ERRα as a metabolic regulator.

2.2.2 Co-regulators of ERRα

Even though ERRα activates transcription in a ligand-independent manner, the transcriptional activity of ERRα has been shown to be cell context and promoter dependent, indicating the presence and absence of specific co-regulators (Bonnelye, Vanacker et al. 1997; Sladek, Bader et al. 1997; Zhang and Teng 2000). One well-studied ERRα co-activator is peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α). Correlating with the expression of ERRα, PGC-1α is highly expressed in tissues such as the heart, kidney, muscle, and brown adipose tissue. Its expression also is modulated in response to signals such as cold and starvation. One group has shown that the induction of PGC-1α not only increases the expression of ERRα, but also enhances the transcriptional activity of ERRα significantly (Schreiber, Knutti et al. 2003). Together, those two proteins control important processes necessary for energy metabolism. Other co-activators of ERRα have also been reported, such as ACTR, SRC1 and GRIP1, which

promote the transcriptional activity of ERRα in different cell contexts and on different gene promoters (Xie, Hong et al. 1999; Zhang and Teng 2000).

Several co-repressors of ERRα have been identified as well, including small heterodimer partner (SHP) and receptor interacting protein (RIP140). However, they are less characterized than the co-activators (Sanyal, Kim et al. 2002; Castet, Herledan et al. 2006). RIP140, for example, is known to interact with a few NRs, such as ERs, ERRs, RARs, RXRs, and TRs (Lhorset, Dauvois et al. 1996). It is believed that the repressive activity of RIP140 involves histone deacetylase (HDACs), CtBPs and additional inhibitory partners (Castet, Herledan et al. 2006).

2.2.3 ERRα and Cancer

The abnormal expression of ERRα has been noticed in many human cancer types, including breast cancer, ovarian cancer, colorectal cancer, prostate cancer and endometrial cancer (Ariazi, Clark et al. 2002; Cavallini, Notarnicola et al. 2005; Cheung, Yu et al. 2005; Sun, Sehouli et al. 2005; Gao, Sun et al. 2006). Take breast cancer for example. Two independent clinical studies have established a direct link between the expression of ERRα and unfavorable breast cancer clinical outcomes, including the high risk of recurrence and low survival rate. Thus ERRα was considered to be a negative prognostic factors for breast cancer (Ariazi, Clark et al. 2002; Suzuki, Miki et al. 2004). One other group has shown that the knockdown

of ERRα in a xenograft model of ERα-negative breast cancer cells significantly inhibits tumor growth. This result provides evidence that the expression of ERRα is able to affect breast cancer development independently of ERα and suggests ERRα could be a potential therapeutic target for either ERα-positive or ERα-negative breast cancer (Stein, Chang et al. 2008). An ERRα antagonist, *N*-[(2*Z*)-3-(4,5-dihydro-1,3-thiazol-2-yl)-1,3-thiazolidin-2-yl idene]-5H dibenzo[*a,d*][7]annulen-5-amine, has been recently identified and shown to effectively inhibit cell proliferation in both ERα-positive and ERα-negative breast cancer cell lines, further supporting the important role of ERRα in breast cancer (Chisarnore, Wilkinson et al. 2009).

3. The Chicken Ovalbumin Upstream Promoter Transcription Factor (COUP-TFs) Family

COUP-TFs are orphan receptors belonging to the steroid/thyroid hormone receptor superfamily. COUP-TFs were first identified in the study of ovalbumin gene expression. It was found to bind to the COUP element and regulate transcription of the ovalbumin gene (Pastorcic, Wang et al. 1986).

To date, two genes termed COUP-TFI (also known as EAR3 or NR2F1) and COUP-TFII (also known as ARP-1 or NR2F2) have been found in mammals. COUP-TFI and COUP-TFII have identical DBDs across different species, indicating they could bind to similar or even

identical response elements. The putative LBDs of COUP-TFI and COUP-TFII also share great similarity (~99.6%) among vertebrates and between human and *Drosophila* (~90%). Such a high degree of sequence conservation suggests that the LBD domains must possess important functions for the COUP-TFs, even though no ligand has yet been found for the COUP-TFs . The amino-terminal domains, on the other hand, have only 45% identity between COUP-TFI and II. This difference suggests that the two members could have distinct functions (Tsai and Tsai 1997).

3.1 COUP-TF Binding Sites

Biochemical studies indicate that COUP-TFs homodimerize or heterodimerize with retinoid X receptor (RXR) or a few other NRs to a number of variably spaced imperfect AGGTCA direct or inverted repeats (Tsai and Tsai 1997). In general, COUP-TFs bind to direct repeats with higher affinity than to inverted palindromic sequences. In vitro assays show that COUP-TFs bind to direct repeats of the core half sites separated by one nucleotide (DR1) with the highest affinity and prefer to bind to consensus sequences with 0 spacing of the palindromic sequence (Cooney, Tsai et al. 1992). In fact, DR1 is the most commonly found COUP-TF binding site for natural gene promoters. COUP-TFs have been shown to bind to many gene promoters with DR1 consensus-regulatory elements, including rat apolipoprotein CIII (Haddad, Ordovas et al. 1986), human apolipoprotein AI (Ladias, Hadzopoulou-Cladaras et al. 1992), chicken apolipoprotein

VLDLII (Wijnholds, Muller et al. 1991), etc. At the same time, COUP-TFs were found to be able to bind to the other DR elements in nature as well. For example, the DR6 of the rat insulin 2 promoter, and the DR9 of HIV-LTR are all considered to be COUP elements (Hwung, Wang et al. 1988; Cooney, Tsai et al. 1991).

3.2 Molecular Mechanism of COUP-TFs

COUP-TFs were originally found to activate the transcription of the ovalbumin gene. There are three different ways that COUP-TFs can act as activators. They can directly bind to a nuclear receptor elements and activate transcription. For example, it has been shown that COUP-TFII can bind to a DR4 element on the promoter of the rat cholesterol 7α-hydeoxylase gene (CYP7A) and a DR7 element of the promoter of the arrestin gene to activate transcription (Lu, Salbert et al. 1994; Stroup, Crestani et al. 1997). Alternatively, instead of directly affecting transcription, COUP-TFs can serve as accessory factors to facilitate the regulation of gene expression (Hall, Sladek et al. 1995). COUP-TFs have also been reported to activate transcription through protein-protein interaction. For example, COUP-TFs act through HNF-4 to increase the promoter activity of HNF-1, without directly binding to it (Ktistaki and Talianidis 1997).

Even though COUP-TFs were first identified to be activators, their negative regulatory properties are more recognized. Four main mechanisms have been studied. First, COUP-TFs can compete for binding sites of the other NRs. For example, COUP-TFs, vitamin D3 (VDR), thyroid hormone (TR) and retinoic acid receptor (RXR) all bind to AGGTCA repeats. Overexpression of COUP-TFs can compete for the available binding sites and inhibit the hormone-induced transactivation of VDR, TR and RAR (Cooney, Leng et al. 1993). Secondly, COUP-TFs can compete for RXR with other NRs. Homodimers of some NRs including RAR, TR, VDR, and PPAR cannot be formed properly, and they require RXR to form functional heterodimers. COUP-TFs have been shown to readily bind to RXR in coimmunoprecipitation assays. Thus, COUP-TFs are able to occupy RXRs and make less RXRs available to other NRs (Cooney, Leng et al. 1993). Thirdly, COUP-TFs show basal transcriptional repression activities for some genes possessing COUP-TF binding sites. The repression is response element specific since reporter genes without COUP-TF binding sites show no signs of silencing. It has also been shown that the repression domain of COUP-TFs lies within the C-terminus of the ligand-binding domain (LBD) (Leng, Cooney et al. 1996). Finally, COUP-TFs repress transcription by inhibiting the activity of other NRs through transrepression. They can directly bind to the LBD of other NRs such as TR, RXR or RAR and form heterodimers. Since these NRs require their cognate ligands in order to

function, by occupying the LBD, COUP-TFs are able to inactivate the activities of ligand dependent NRs (Leng, Cooney et al. 1996).

3.3 COUP-TFII

The expression of COUP-TFs has been observed in multiple tissues and organs during the development of the embryo including the central nervous system (CNS), lung, testis, prostate, pancreas, etc. The expression patterns of COUP-TFI and II are distinct but also overlaping, indicating they could have redundant functions in certain contexts (Jonk, de Jonge et al. 1994; Lu, Salbert et al. 1994; Pereira, Qiu et al. 1995).

The COUP-TFII null-mice are reported to be embryonic lethal. The homozygous knockout mice (COUP-TFII$^{-/-}$) die around E10. Enlarged blood vessels, defects in the development of the atria and sinus venosus and malformed cardinal veins were observed, suggesting a critical role of COUP-TFII in the developments of the embryonic heart and vasculature (Pereira, Qiu et al. 1999). The female heterozygous knockout mice (COUP-TFII$^{+/-}$) are found to have impaired reproductive function, however, the male mice are fertile and do not present any major anatomical difference from the wild type mice (Takamoto, Kurihara et al. 2005). The COUP-TFII$^{+/-}$ mice have much less WAT mass compared to the wild type mice, and exhibit significantly improved glucose tolerance, resistant to high-fat diet-induced obesity and aged related weight gain. This model provides

global evidence for the regulatory role of COUP-TFII in WAT development and metabolism (Li, Xie et al. 2009).

The COUP-TFII$^{+/-}$ mouse model, however, is not sufficient for a complete understanding of the COUP-TFII molecular and cellular functions. Conditional knockout mice were generated to fulfill this purpose. One group utilized the Cre/loxp system to specifically delete COUP-TFII in the limb bud. They noticed that the limbs of the mutants are obviously shorter than the control littermates due to the slower proliferation rate of the mesenchyme in limbs lacking COUP-TFII (Lee, Li et al. 2004). This indicates that COUP-TFII is required for the maintenance of limb bud outgrowth. Different applications of the conditional knockout mouse model are still needed in order to further study of the functions of COUP-TFII in the development of different tissues.

4. ChIP-on-chip

Standard chromatin immunoprecipitation (ChIP) offers a way to study the interaction of protein and DNA in *vivo* by selectively precipitating the protein of interest together with the DNA sequence associated with it (Aparicio, Geisberg et al. 2004; Collas 2010). One limitation of ChIP is that it is only useful to study protein-DNA interactions at previously defined or predicted sites, as PCR with specific primers is used in the subsequent analysis. On the other hand, by combining ChIP with microarray

technology, ChIP-on-chip allows the identification of protein-bound sites on a genome-wide scale (Ren, Robert et al. 2000). DNA sequences that co-precipitate with the protein of interest can be amplified and labeled with Cy5. They are then applied to a microarray spotted with a large number of oligonucleotide probes covering the regions of interest. Double stranded DNA will be formed whenever labeled DNA hybridizes to a probe on the array, and this will generate a signal when viewed under fluorescent light, indicating a positive direct target gene.

GOAL OF THE STUDY

Both nuclear receptors ERRα and COUP-TFII play important roles as metabolic regulators. They also share similar response elements. For example, the DR0 for COUP-TFII, AGGTCAAGGCAT, contains a perfect binding site for ERRα, which is TCAAGGTCA. Other DRs could also harbor a potential binding site for ERRα depending on the nucleotides separating the two half sites. This raises the question as to whether ERRα and COUP-TFII compete with each other for binding to response elements, and if they serve redundant or opposite functions. Our goal was to identify the common target genes of ERRα and COUP-TFII. Further analysis of these genes could help reveal the cross-talk between ERRα and COUP-TFII in the regulation of different metabolic pathways.

MATERIALS AND METHODS

ChIP, ChIP-on-chip Experiments and Genome-wide Location Analyses

ChIP experiments, ChIP-on-chip experiments, and Genome-wide location analyses were performed as previously described (Dufour, Wilson et al. 2007; Charest-Marcotte, Dufour et al. 2010) with modifications on adult male mouse livers.

In COUP-TFII ChIP-on-chip experiments, chromatin isolated from 4 g of initial liver mass taken from a pool of 10 mouse livers, was diluted in 2.5X ChIP dilution buffer (0.5% Triton X-100, 2 mM EDTA, 100 mM NaCl, 20 mM Tris-HCl, pH 8.1). 600 μl of a 50% slurry of salmon sperm DNA/protein A beads (Upstate) was added to the chromatin and incubated for 2.5 hr at 4°C for pre-clear. 50 μl of anti-hCOUP-TFII (Persesus Proteomics Inc., PP-H7147-00), which is proven to be specific for both mouse and human COUP-TFII proteins, was used for overnight immunoprecipitation at 4°C. The precipitated chromatin was then incubated with 600 μl of a 50% slurry of salmon sperm DNA/protein A beads at 4°C for 3 hr. Beads were then collected and washed.

For the mouse liver ERRα ChIP-on-chip experiment, refer to the work of Charest-Marcotte *et al*. (Charest-Marcotte, Dufour et al. 2010).

Quantitative Real-Time PCR

Quantitative Real-Time PCR (qPCR) was performed to validate the binding of ERRα and COUP-TFII at specific promoters identified by ChIP-on-chip experiments using the same DNA enriched from mouse livers. A mixture of 4.75 μl of H_2O, 6.25 μl of SyBr Green PCR Master Mix (Qiagen), 0.5 μl of each forward and reverse primer (10 μM) and 1 μl of enriched DNA material was used for each PCR reaction performed on a LightCycler II machine (Roche). DNA was amplified for 45 cycles at the desired temperature for each primer sets. Amplified DNA fragments were normalized against the mean enrichment from two amplified control regions located ~4kb upstream of the ERRα start site and ~55 kb upstream of the Prox1 start site. The following formula was used to assess the promoter enrichment for ERRα or COUP-TFII:

$$2^{(\text{Q-PCR cycle of no-antibody control DNA - Q-PCR cycle of enriched DNA})}$$

Table 1-4 shows the primers tested for the purpose of this validation.

siRNA Knockdown Assays and Western Blot Analysis

siRNA knockdown assays were performed to check if the binding of ERRα and COUP-TFII to specific promoters has a real effect on transcription. 0.25 X 10^6 Hepa1-6 cells were seeded in 4 ml DMEM media (Invitrogen, 10569-044) supplemented with 10% fetal bovine serum per 6 cm plate right before the siRNA transfection. 1 ml of DMEM media was mixed with 37.5

μl of Hiperfect transfection reagent (Qiagen) and 5 μl of 100 μM of On-Target smart pool siERRα (Dharmacon, L-040772-00-0005) or 5 μl of 100 μM of On-Target smart pool siCOUP-TFII (Dharmacon, L-063437-01-0005) or 5 μl of 100 μM of On-Target smart pool siControl (Dharmacon). The mixtures were incubated at room temperature for 10 min and added to a 6 cm plate dropwise. The cells were allowed to grow for 72 hr before the experiment was harvested.

Cells were collected in 500 μl of RIPA lysis buffer (50 mM Tris-HCl, pH 7.4, 1% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl, 1 mM EDTA) supplemented with protease inhibitors (Roche) and Phosphatase inhibitors (Roche) per plate. Cells were then lysed open by sonication and centrifuged at 11,000 rpm at 4°C for 10 min to get rid of cell debris. The collected protein was quantified by the BCA method. 75 μg of total cell lysate was loaded on a 10% SDS-PAGE gel to separate. Immunoblot detection was carried out with anti- ERRα (1:10,000), anti-COUP-TFII (1: 400, Persesus Proteomics Inc., PP-H7147-00) and anti-RPLP (1:2000, Proteintech Group, 11290-2-AP).

Quantitative Reverse Transcription PCR

Total RNA was isolated from Hepa1-6 siRNA knockdown samples using the RNeasy mini kit (Qiagen). 1.5 μg of RNA was used to make cDNA, and it was reverse transcribed using Superscript II RNase H Reverse

Transcriptase. Specific primers (Table 5) were used to check the transcript levels of the predicted target genes of ERRα and COUP-TFII using the qPCR method as described above. The expression of target genes was normalized to the expression of RPLP0.

Functional Classification of Target Genes

Functional Analysis was done by Ingenuity Pathways Analysis software (IPA, https://analysis.ingenuity.com) and using NCBI gene descriptions. When two genes shared one promoter region recognized by ChIP-on-chip, both genes were counted in the functional analysis.

Computational Motif Analysis

De novo motif analysis for ERRα and COUP-TFII was performed with Macvector software. Together with all the common target segments (282) of ERRα and COUP-TFII, 100 segments with the smallest p-value (P <10^5) were chosen from the list of COUP-TFII targets only and the list of ERRα targets only, respectively, to look for binding motifs. Sequences +/- 250 bp of the strongest binding probes on each gene promoter region were chosen for Macvector analysis. The subsequence TNAAGGTCA was used to search for promoters containing an ERRE, allowing 1 mismatch; the subsequence AGGTCAAGGTCA was used to search for promoters containing a DR0 and the subsequence AGGTCANAGGTCA was used to search for promoters containing a DR1, allowing 3 mismatches. Promoters

that had, for example, one DR1 and one ERRE at the same position (merged sites), were counted once in each category. Promoters that had, for example, one DR1 and one ERRE at different positions, were arbitrarily assigned just one binding motif by manual checking (*eg*. a site with less mismatches or more centered in the probing region was chosen). The enriched motif logo pictures were generated using Weblogo (http://weblogo.berkeley.edu/logo.cgi).

Results

Genome-wide Identification of Promoters bound by ERRα and COUP-TFII in Mouse Liver Through ChIP-on-chip Experiments

Both of the orphan nuclear receptors ERRα and COUP-TFII have been shown to play important roles in cellular energy metabolism and cancer development (Nakshatri, Mendonca et al. 2000; Ariazi, Clark et al. 2002; Giguere 2002; More, Fellner et al. 2003; Villena, Hock et al. 2007; Villena and Kralli 2008; Li, Xie et al. 2009). However, the cross talk between these two receptors has not been well studied. ChIP-on-chip experiments, which couples standard ChIP with microarray technology, was used to identify the common target genes of ERRα and COUP-TFII. Further data analysis allowed for a characterization of the binding patterns of these two receptors to their common target genes, and revealed the interaction between them in different cellular pathways.

ChIP-on-chip experiments were performed with mouse liver chromatin using a DNA microarray containing regions spanning -5.5 kb upstream and +2.5 kb downstream of the transcriptional start sites of ~ 17,000 of the most well-defined mouse genes. 2373 segments were found to be bound by ERRα, and 1766 segments were found to be bound by COUP-TFII. Parameters used to identify significantly bound regions have been

described previously (Charest-Marcotte, Dufour et al. 2010). Among the identified segments, 709 of them were shared by both nuclear receptors as shown in Figure 1. This accounts for 30% of the total ERRα targets and 40% of the total COUP-TFII targets. The significant overlap of target genes between ERRα and COUP-TFII indicates that they could co-regulate many genes in the same cellular pathways. The target genes identified for COUP-TFII are listed in Table 6, target genes for ERRα refer to the work of Charest-Marcotte *et al*. (Charest-Marcotte, Dufour et al. 2010) and the overlap target genes of ERRα and COUP-TFII are listed in Table 7.

ERRα and COUP-TFII Binding Validation

To gain more confidence about the promoters enriched by ERRα and COUP-TFII identified by the ChIP-on-chip experiments, random genes were chosen from the target gene lists for chromatin immunoprecipitation validation. Specific primers were designed (Tables 1-4) for qPCR using the same ChIP samples as were used for the ChIP-on-chip experiments. As shown in figure 2A, different levels of promoter enrichment were detected for the all the genes randomly picked, suggesting that target genes identified at p-value ≤ 0.01 are most likely all true positives.

Arrays containing probes spanning every 200 bp across promoter regions in ChIP-on-chip experiments were used. Depending on the binding

strength of each probe to the chromatin, binding profiles are generated to indicate at which location on the promoters the ERRα or COUP-TFII proteins tend to bind. Four categories of promoter regions targeted by ERRα and COUP-TFII are identified. For example, in figure 2B, only one peak for the ERRα binding profile is shown on the promoter region of *Grn*, indicating that the promoter of *Grn* contains a binding site for ERRα, but not for COUP-TFII. At the same time, the promoter region of *Zfp524* is only bound by COUP-TFII. On the promoter regions bound by both ERRα and COUP-TFII, the peak for the ERRα binding profile is either separated or superimposed upon the peak for the COUP-TFII binding profile, suggesting that the common target gene promoters can recruit ERRα and COUP-TFII to distinct binding sites or to the same binding site. By further analysis, 410 genes from the common list were found to contain distinct binding sites within the promoter region, whereas 299 genes from the common list were found to have binding at the same probe (Figure 1; Table 8). From here on, only the 299 genes are referred to as the common target genes of ERRα and COUP-TFII in this thesis.

Computational Motif Analysis for ERRα and COUP-TFII

ERRα is known to bind to a consensus extend half site, TNAAGGTCA, whereas COUP-TFII has been shown to bind to direct repeats of the core half site separated by one or more nucleotides (Cooney, Tsai et al. 1992;

Giguere 2002). Our ChIP-on-chip experiments revealed that ERRα and COUP-TFII share the same DNA binding sites for the 299 of their common target genes. This leads to the question of whether there is a conserved sequence that both ERRα and COUP-TFII recognize for their common targets and whether ERRα and COUP-TFII compete for their binding sites.

Macvector software was used to perform the computational motif analysis. Together with all the common target segments (282) of ERRα and COUP-TFII, 100 segments with the smallest p-value ($P < 10^{-5}$) were picked from the list of COUP-TFII targets only and the list of ERRα targets only, respectively, to look for binding motifs. Sequences +/- 250 bp of the most strongly bound probes on each gene promoter region were chosen for Macvector analysis. As shown in Figure 3A, in the COUP-TFII targets only list, DR1 is the most predominant binding site enriched for the COUP-TFII protein. 77% of the segments contain a DR1. ERREs and DR0s are also observed, but much less frequently, supporting the idea that most COUP-TF elements found in nature are DR1s. In contrast, 78% of the segments contain an ERRE and 69% of the segments contain a DR0 in the ERRα targets only list. Since DR0s naturally harbor an ERRE site within it, the results here agree with the literature that the ERRE is the main binding site for ERRα. For ERRα and COUP-TFII common targets, as shown in Figure 3C, all ERRE, DR0, and DR1 sites are approximately equally enriched. However, the pie chart in Figure 3D clearly shows that the merged

DR0/ERRE site is the major site for the binding of ERRα and COUP-TFII. Merged sites DR1/ERRE and DR0/DR1/ERRE also make up great proportions of all the binding sites. Since the ERRE is the main binding site for the ERRα protein, and the DR0 and DR1 are the preferable binding sites for the COUP-TFII protein, the results here suggest that ERRα and COUP-TFII could compete for DNA binding sites on many of their common target genes. The enriched DR1 motif in the COUP-TFII targets only list, the enriched ERRE motif in the ERRα targets only list and the enriched ERRE, DR0, DR1 motifs in the ERRα and COUP-TFII common targets list are shown is Figure 4.

Transcriptional Regulation by ERRα and COUP-TFII of Their Common Target Genes

We have discovered that ERRα and COUP-TFII can bind to the same site on the promoters of 299 genes, and that conserved binding sequences are also enriched for the binding of ERRα and COUP-TFII. We next asked if ERRα and COUP-TFII truly regulate the transcription of their 299 common target genes. To answer this question, ERRα and COUP-TFII, individually, were knocked down in mouse liver Hepa 1-6 cells with siRNA. The mRNA levels were checked by qPCR and normalized to the expression of RPLP0, the transcription of which is not regulated by either ERRα or COUP-TFII. mRNA levels of *Esrra* and *COUP-TFII* were down-regulated by 80% and 90%, respectively, as shown in Figure 5A. Their protein expression after

siRNA knockdown were examined by western blot. No protein of ERRα or COUP-TFII was detected, further confirming the successful knockdown of the two receptors (Figure 5B). 20 genes were randomly picked from the 299 genes to validate their transcriptional regulation by ERRα and COUP-TFII. *Jun*, *Nr5a2* and *Bmp2k* were found to be regulated by both NRs (Figure 5C). Therefore, approximately 15% of the common target genes are truly regulated by ERRα and COUP-TFII in Hepa 1-6 mouse liver cancer cells. Even though there are a lot of false positives in this cell context, the ChIP-on-chip experiments are still helpful in identifying the common target genes for both NRs for a huge number of mouse genes. Since the knockdown of both proteins resulted in the transcription of their target genes changing in the same direction, ERRα and COUP-TFII may serve the same role in regulating transcription.

Biological Process Regulation by ERRα and COUP-TFII

Biological process analysis for ERRα and COUP-TFII was done with Ingenuity Pathways Analysis software (https://analysis.ingenuity.com). ERRα is mainly involved in energy metabolism pathways as previous shown, such as oxidative phosphorylation, mitochondrial dysfunction and citrate cycle (Schreiber, Emter et al. 2004; Dufour, Wilson et al. 2007; Deblois, Hall et al. 2009). COUP-TFII specific target genes were mostly enriched for cell proliferation, tissue development or cancer (Figure 6A). For instance, *BAD*, which is known to inhibit cell proliferation in breast

cancer, is shown as one of the specific targets of COUP-TFII (Fernando, Foster et al. 2007).

Despite the distinct functions of ERRα and COUP-TFII when comparing their specific gene targets against the genome, their common target genes were enriched for a significant number of biological pathways (Figure 5B). Genome-wide expression analysis has shown that in pre-diabetic and diabetic individuals, the expression of mitochondrial oxidative phosphorylation (OXPHOS) genes downstream of PGC-1α were significantly reduced (Mootha, Lindgren et al. 2003; Patti, Butte et al. 2003). Since PGC-1α regulates most of its OXPHOS genes via ERRα, ERRα could be a potential target in type II diabetes (Schreiber, Knutti et al. 2003; Schreiber, Emter et al. 2004). At the same time, COUP-TFII knockout mice show improved insulin resistance and, therefore, could also contribute to the development of type II diabetes (Bardoux, Zhang et al. 2005; Li, Xie et al. 2009). Thus, the Type II Diabetes Mellitus Signaling pathway identified when comparing the ERRα and COUP-TFII common target genes against the genome is particularly noteworthy. *SOCS5, PRKAG2, NFKBIA, MTOR, ACSL1, PPARG,* and *ADIPOR2* were all common targets of ERRα and COUP-TFII, and are all involved in the Type II Diabetes Mellitus Signaling pathway. Other important genes in this pathway are also specific targets of ERRα or COUP-TFII, as shown in Figure 6C.

Discussion

ERRα and COUP-TFII are orphan nuclear receptors involved in multiple biological processes such as adipogenesis and energy metabolism (Dufour, Wilson et al. 2007; Villena and Kralli 2008; Li, Xie et al. 2009). They have also been shown to share similar response elements. ERRα often recognizes the extended half site TNAAGGTCA for DNA binding, whereas COUP-TFII prefers to bind to a number of variably spaced imperfect AGGTCA direct or inverted repeats (Giguere, Yang et al. 1988; Tsai and Tsai 1997). The cross-talk between these two proteins, however, is less studied. The ChIP-on-chip technique is a powerful tool for the identification of potential target genes of NRs on a genome-wide scale. Our ChIP-on-chip experiments on the orphan nuclear receptors ERRα and COUP-TFII have allowed for the identification of common targets of ERRα and COUP-TFII, and have revealed their interplay in different biological processes.

ERRα and COUP-TFII have been both reported to be expressed in liver and serve functions as metabolism regulators (Lou, Tannour et al. 1999; Giguere 2008). Therefore, mouse liver was chosen as a primary source of tissue for ERRα and COUP-TFII ChIP-on-chip experiments. 2373 and 1766 segments were enriched for ERRα and COUP-TFII respectively. Among all of the targets identified, 709 of the segments were bound by both ERRα and COUP-TFII. This significant overlap indicates

that the two NRs might co-regulate many genes. All of the genes randomly chosen from target gene lists are shown to be true positives in the binding validation assays, suggesting that with p-value ≤ 0.01, target genes indentified from the ChIP-on-chip experiments are most likely true positives. However, it is noteworthy that this cut-off p-value may be too stringent and, therefore, a few real targets could have been left out from the lists. Other p-values should be tested if the complete transcriptional regulation capacities of the two NRs are to be identified. For the purpose of this thesis, p-value ≤ 0.01 was kept to ensure strict binding site specificity.

Computational motif search revealed that the merged sites DR0/ERRE, DR1/ERRE and DR0/DR1/ERRE are the main sites that recruit ERRα and COUP-TFII to the promoters of the 299 common target genes. The results support the hypothesis that ERRα and COUP-TFII are likely to compete for binding sites in regulating their common target genes. For the other gene promoters that do not contain merged sites, ERRα and COUP-TFII proteins could bind to the same site with imperfect ERREs or COUP-TFII response elements. Some studies have already shown that the core sequence AGGTCA is sufficient for the binding of ERRα to some genes (Carrier, Deblois et al. 2004; Zhang, Chen et al. 2006). If more mismatches are allowed, merged sites might be detectable for those genes. Moreover, the DNA binding sites of COUP-TFII are not limited to DR0s and DR1s (Cooney, Tsai et al. 1992). Other DR sites are also detected in the promoter

regions of the COUP-TFII target genes (data not shown). Thus, all the other merged sites, DR2/ERRE for example, are not included in this analysis and could be the real binding site for ERRα and COUP-TFII.

The next step to confirm if ERRα and COUP-TFII co-regulate or compete for their common binding sites would be to compare the overlap between the liver transcript profiles for COUP-TFII obtained from ERRα mutant mice and the 299 identified common target genes. If the common target genes are found in the liver transcript profiles obtained from ERRα mutant mice, it suggests that those common target genes are able to recruit COUP-TFII to their promoters independent of ERRα. Therefore, ERRα and COUP-TFII are likely to compete for binding sites for the regulation of those genes. On the other hand, if the common target genes are not found in the liver transcript profiles obtained from ERRα mutant mice, it indicates that those common target genes need ERRα to recruit COUP-TFII to their promoters. Thus, ERRα and COUP-TFII are more likely to co-regulate for the transcription of those genes. This approach will help to further confirm our hypothesis that ERRα and COUP-TFII compete for binding sites in regulating their common target genes.

siRNA knockdown of either ERRα or COUP-TFII in Hepa1-6 cells followed by qPCR shows that the two NRs regulate their common target genes in the same manner. They both either activate the same gene or

repress the same gene, indicating they could serve the same function in transcriptional regulation. It was also found that only 15% of their common target genes are truly regulated by both of them. There could be different reasons for this discrepancy between the predicted and experimental results. First of all, the ChIP-on-chip experiments for ERRα or COUP-TFII were carried out in mouse liver. There is much more complicated signaling pathway regulation and cross talk in this context compared to the liver cancer cell line, Hepa1-6. The targets regulated by ERRα or COUP-TFII in mouse liver are not necessarily controlled by these NRs in Hepa1-6 cells and vice versa. In this case, target gene expression should be checked in knockout mice livers instead of cell lines. The downside of this approach is that the isoforms of COUP-TFs and ERRs may compensate for the lost of COUP-TFII or ERRα. Further more, COUP-TF I and II are similar proteins and are potentially serve the same function (Qiu, Tsai et al. 1994; Tsai and Tsai 1997; Pereira, Tsai et al. 2000). Only knocking down COUP-TFII in the cells might not be enough to see a difference in the transcription of some genes. The same reasoning can also be applied to the three different isoforms of the ERRs (Lu, Kiriyama et al. 2001; Zhang, Ma et al. 2006). Therefore, a double knockdown for the COUP-TFs or the ERRs in Hepa1-6 cells could help to verify more of their co-regulated genes.

A significant number of common biological pathways have been identified involving the regulation of both ERRα and COUP-TFII such as

mitochondrial dysfunction, oxidative phosphorylation, citrate cycle and molecular mechanism of cancer (Figure 6B). These results could be of great assistance for understanding the cross-talk between ERRα and COUP-TFII in *vivo*.

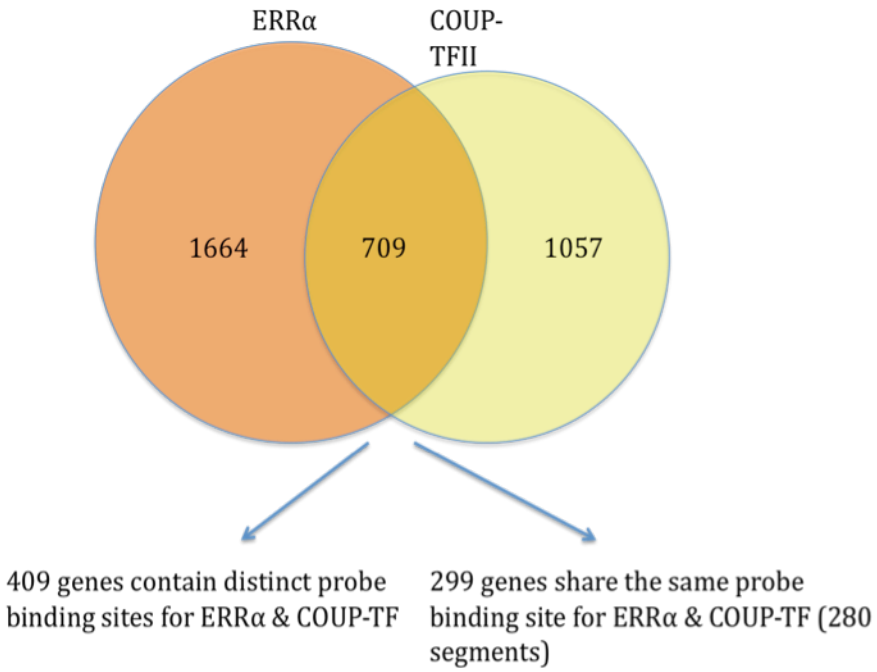
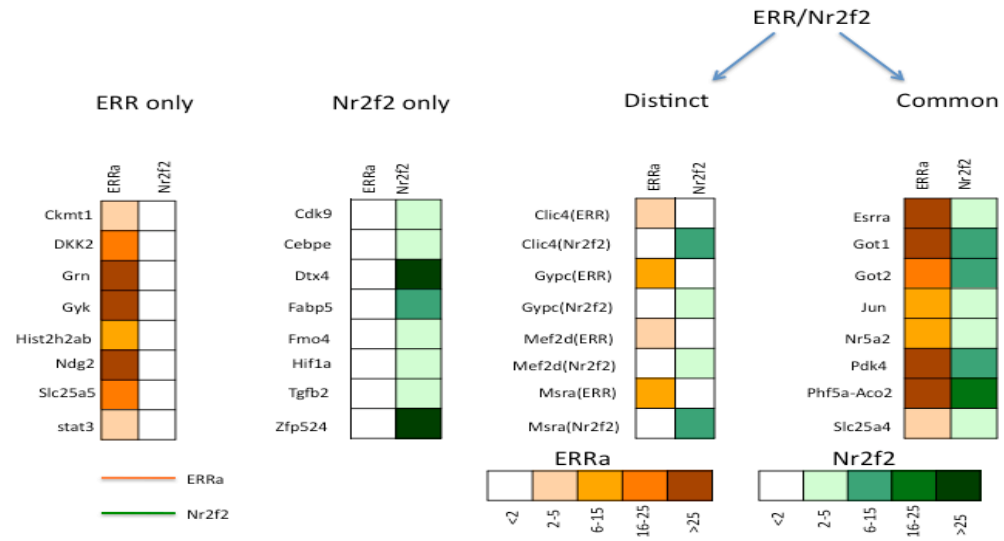


Figure 1. Genome-wide identification of promoters bound by ERRα and COUP-TFII. Venn diagram demonstrating the promoters enriched by ERRα and COUP-TFII in the mouse liver ChIP-on-chip experiments with a cut-off p-value ≤ 0.01. A total number of 2373 segments were identified to be enriched for ERRα and 1766 segments were identified to be enriched for COUP-TFII.

A



B

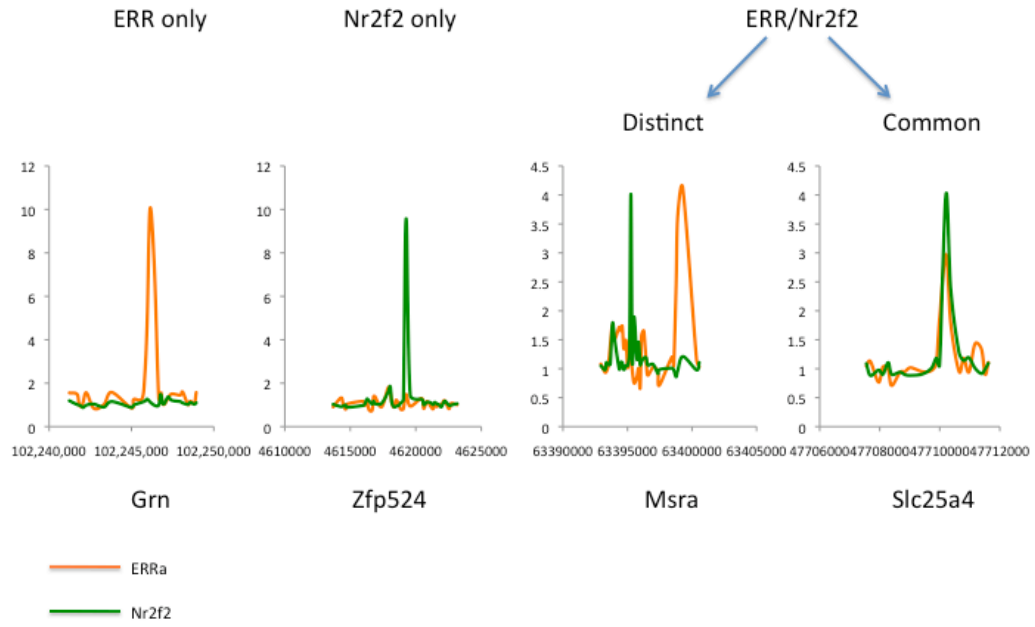


Figure 2. Binding Validation for ERRα and COUP-TFII ChIP-on-chip Experiments. A) Genes were randomly picked for validation from four subsets of target genes including gene groups bound by ERRα only, bound by COUP-TFII only, bound by ERRα and COUP-TFII but at distinct binding sites, bound by ERRα and COUP-TFII at the same binding site. Cut-off p-value is set at ≤ 0.01 (Fold enrichment ≥2); B) Examples of binding profiles for each subset of genes.

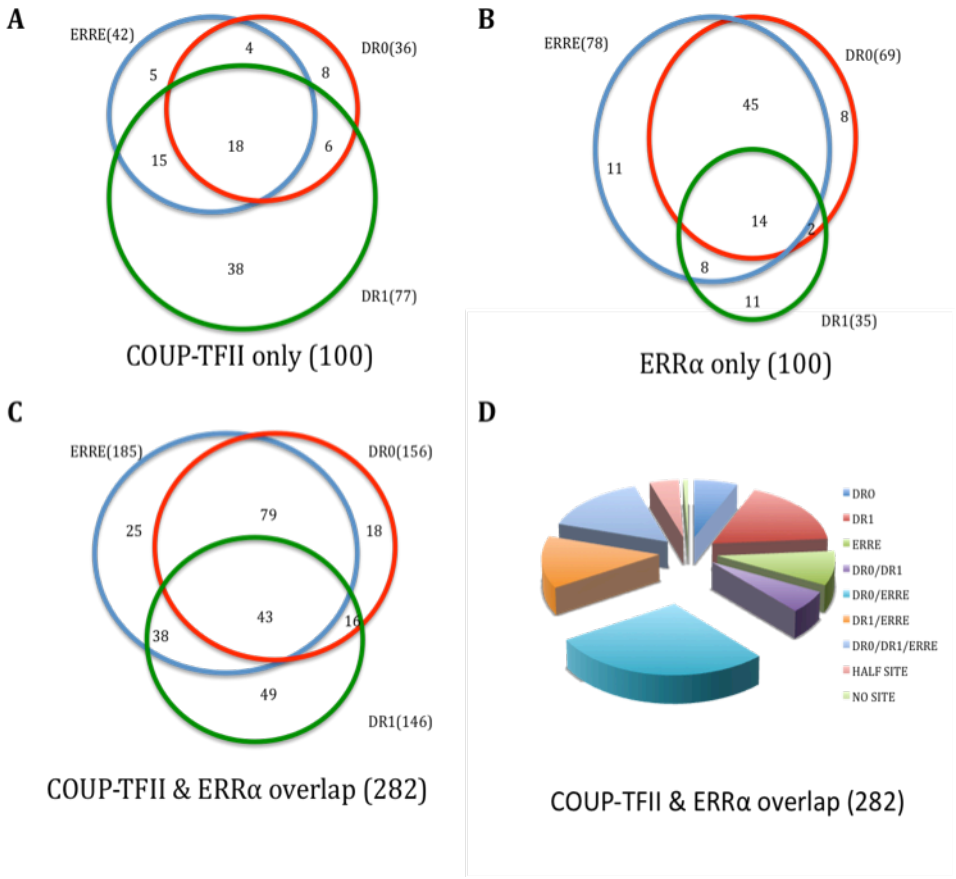


Figure 3. Computational Motif Analysis for ERRα and COUP-TFII. A) 100 segments with the smallest p-value (P <10^5) were picked from the COUP-TFII only list and each assigned with a most likely binding site for COUP-TFII; B) 100 segments with the smallest p-value (P <10^5) were picked from the ERRα only list and each assigned with a most likely binding site for ERRα; C) 282 common target segments of ERRα and COUP-TFII were each assigned with a most likely binding site for ERRα and COUP-TFII; D) Pie chart representation of binding sites for ERRα and COUP-TFII on their common target segments.

A

B

C

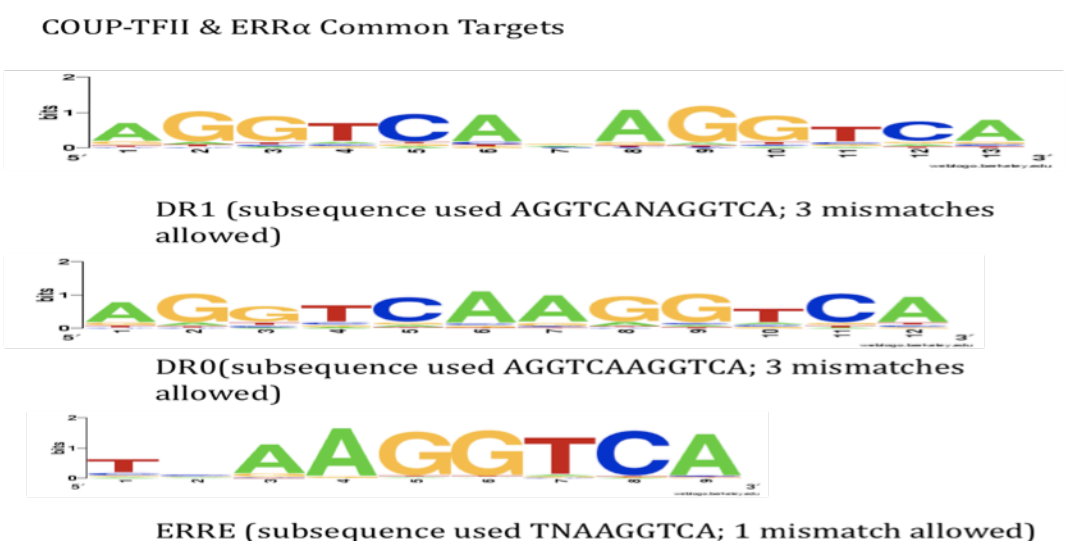


Figure 4. Motifs Enriched for Each Subset of Target Genes for ERRα and COUP-TFII. A) DR1 site enriched for the COUP-TFII only list; B) ERRE site enriched for the ERRα only list; C) DR1, DR0, and ERRE sites enriched for the ERRα and COUP-TFII common targets list.

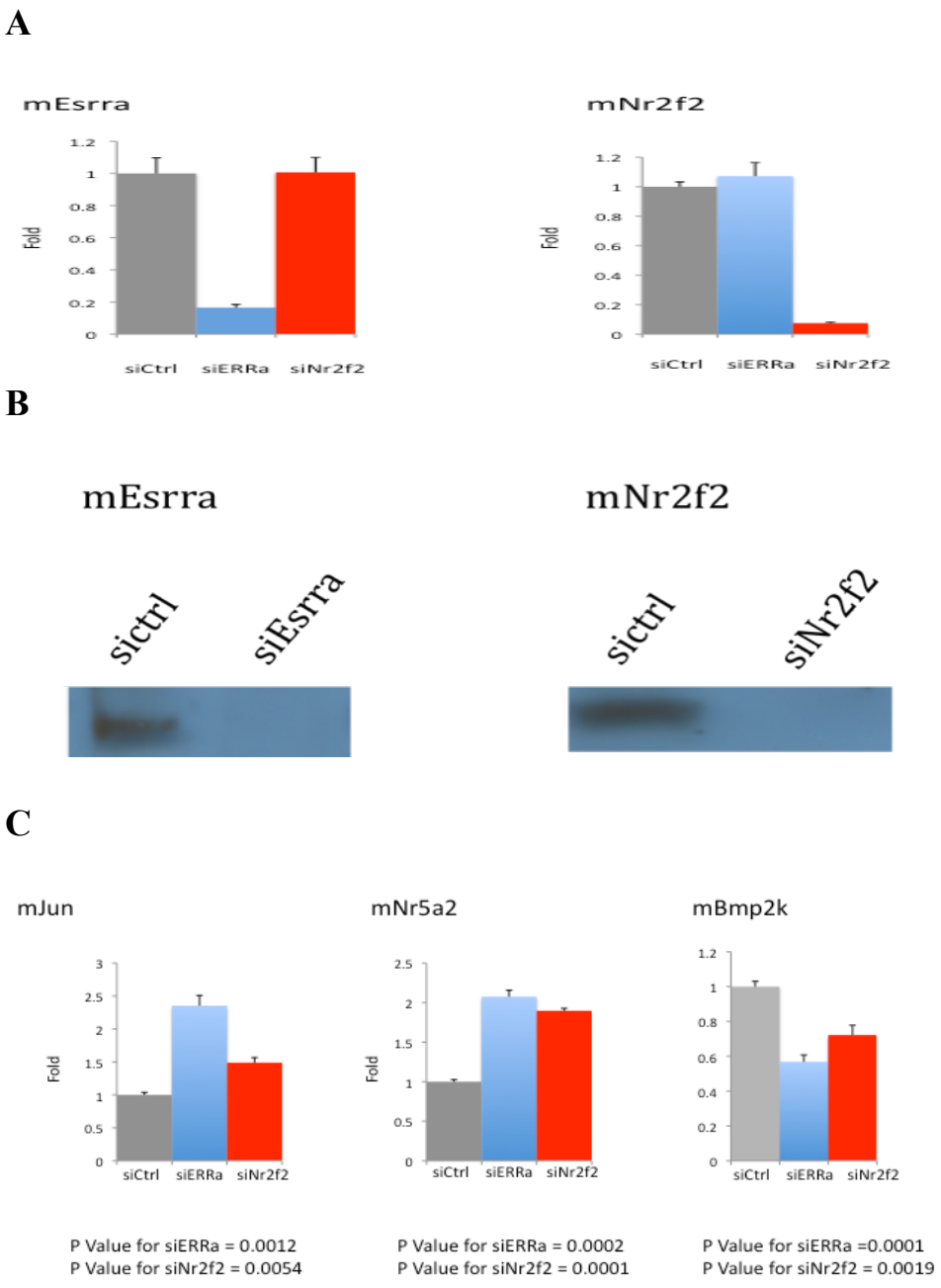
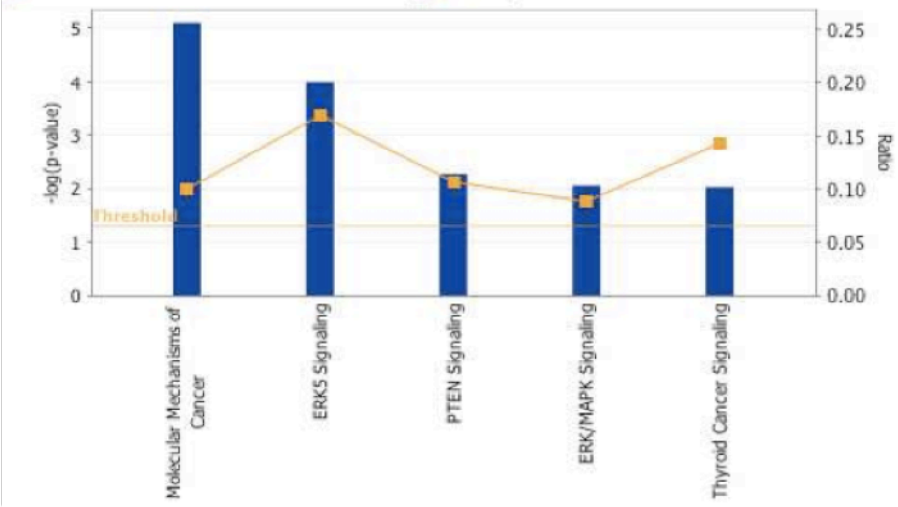


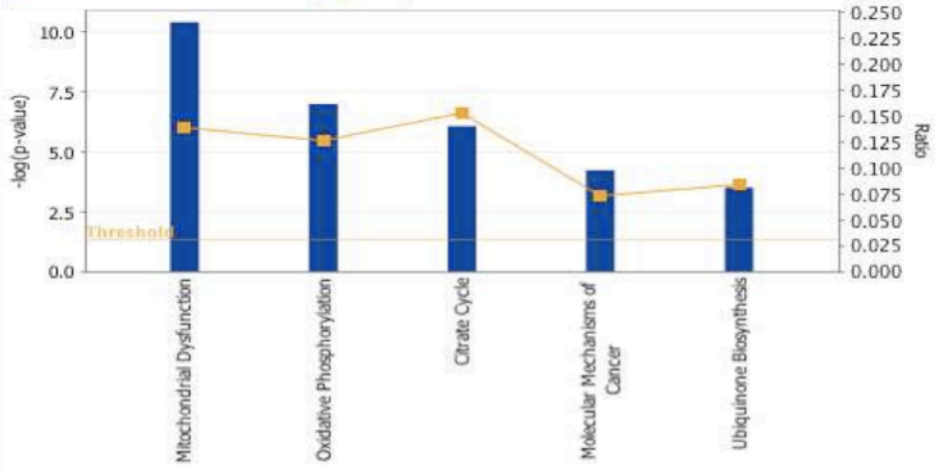
Figure 5. Transcriptional Regulation by ERRα and COUP-TFII of Their Common Target Genes. A) mRNA levels of *Esrra* and *COUP-TFII* after siRNA knockdown in Hepa1-6 cells; B) Protein levels of ERRα and COUP-TFII after siRNA knockdown in Hepa1-6 cells; C) mRNA levels of genes from the ERRα and COUP-TFII common targets list after knockdown of ERRα or COUP-TFII.

A



Nr2f2 Specific Vs Genome

B



Common Target Genes of ERRα and COUP-TFII Vs Genome

C

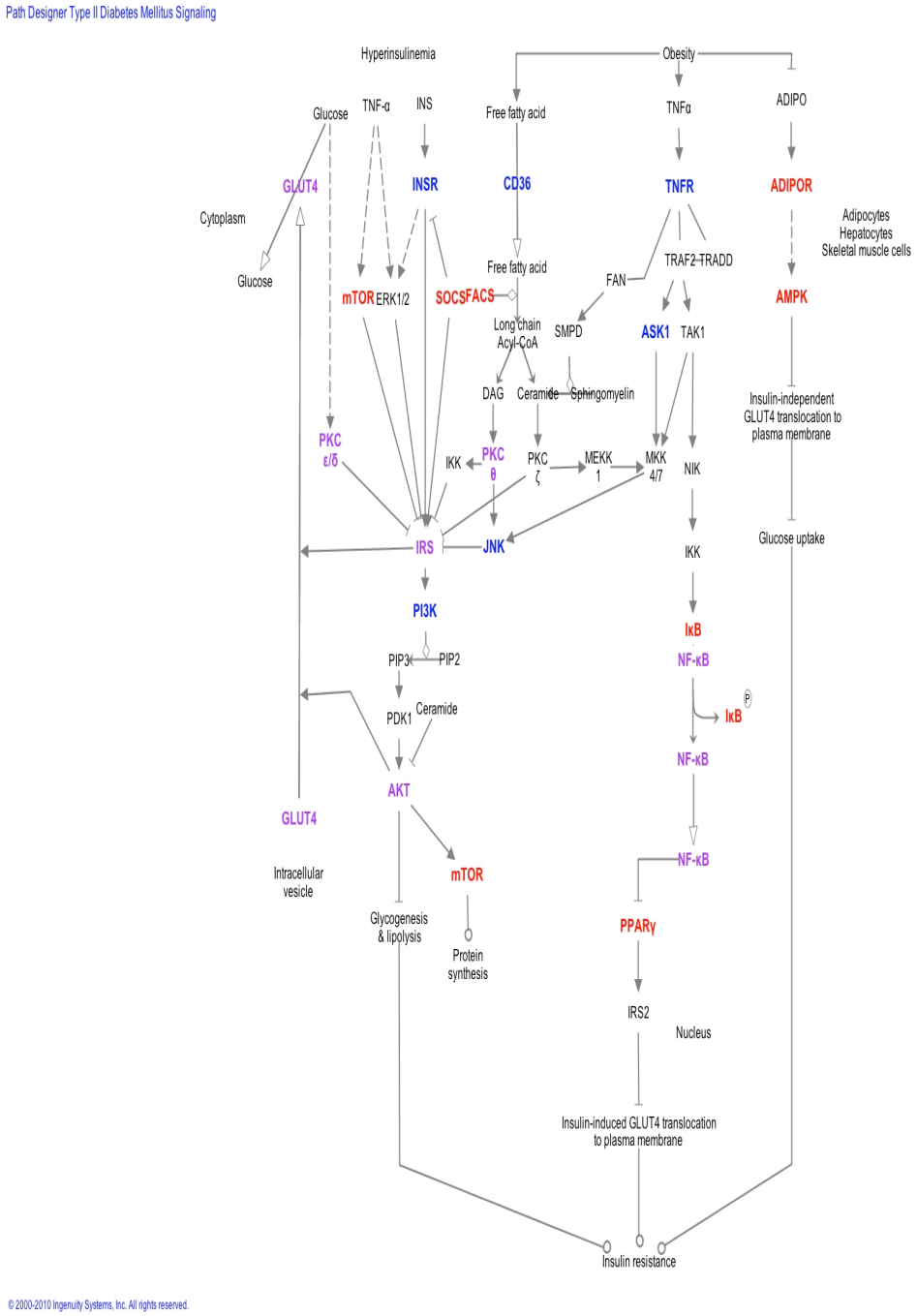


Figure 6. Biological Processes Regulated by ERRα and COUP-TFII. A) Biological pathways involving COUP-TFII specific target genes; B) Biological pathways involving ERRα and COUP-TFII common target genes; C) Type II Diabetes Mellitus Signaling pathway. Genes in red are the common targets of ERRα and COUP-TFII; genes in purple are COUP-TFII specific targets; genes in blue are ERRα specific targets.

Table 1. Mouse Primers to Validate The Enrichment of Promoters by ERRα Only

GENE	PRIMERS	
Ckmt1	Forward	GGCAGTGAGTTTGGGAGACAGAATAGAC
	Reverse	GGAAAGACAGCACAGCCTCAAGGTCACC
DKK2	Forward	GCGTTAAATTAGAGTCCTGGACAGGTTG
	Reverse	CTAGCAGTGTCCTTGAGCCAAAATGGAG
Grn	Forward	TACTCGCCCTTGAACCTTTATTAG
	Reverse	TACATTCCTGTCTCTTCACGCCTG
Gyk	Forward	CGCTCGCTTTATTAACCACTGTGGA
	Reverse	TCTCACTGCGGGTCCAGTCCAAAG
Hist2h2ab	Forward	CCCCAGATCAAACGAGGACATC
	Reverse	GACAGTGGCTCCCCATCTTTGC
Ndg2	Forward	GAGGGAAACGTGGGTGCTCTCC
	Reverse	CCTCTACTCCAGGGTCCAGTGG
Slc25a5	Forward	GGAGCAGGTTCCCAGGGTGC
	Reverse	GAAGGTGGGCTGTTTACTGCTTAAATTC
Stat3	Forward	CCATTATTCTTTTGTCAAAGTTTGG
	Reverse	CTATGGCCTCTCCTATTTGCTAAATG

Table 2. Mouse Primers to Validate The Enrichment of Promoters by COUP-TFII Only

GENE	PRIMERS	
Cdk9	Forward	GGTAAATCCAGGAGGGGACTAGAG
	Reverse	GTTTGGGCAATCCACAGGAAGCAG
Cebpe	Forward	GGGGTCCGAAGGAGAAGCTGGTAG
	Reverse	CACAACCAAACTGGAGAGAGCAGG
Dtx4	Forward	GGTTCCCTGCGTTTACATAGAGAG
	Reverse	CGAGTCTAGCTTGCGTTTGTTCTG
Fabp5	Forward	CGCATAGATAGCTCAGAAACTG
	Reverse	TACTTCCTCCAGCTCTTCCTCC
Fmo4	Forward	CACGGGGGTCTTTCCTTTGGATAG
	Reverse	CGGAAGGCATGTGATTAGCTTCTG
Hif1a	Forward	GCACTTGGCATGTTTTGCTTTCAG
	Reverse	TGACCTTTGACCTAATTGGCAGTG
Tgfb2	Forward	AGTGAGAGACAATAGCAAAGGTTTGG
	Reverse	CATGCAGTGGGGGTGGGGGTGTCCTC
Zfp524	Forward	TCCCCCGACAGAATCTGCTGCTAG
	Reverse	TCCAGGACTTTCCCCAGTGCCCAC

Table 3. Mouse Primers to Validate The Enrichment at Promoters of ERRα and COUP-TFII at Distinct Binding Sites

GENE	ERRα PRIMERS	
Clic4	Forward	GAATTGCCGTCAGTGAATGTCACC
	Reverse	CAACTCACTCATCACCCTAGCCTG
Gypc	Forward	TCAACCAAGGGCAAGGCACCAAAC
	Reverse	TCGGAGCAGAGAGAATTGACTTCC
Mef2d	Forward	GTGTTCTCAGACACCCAGCTTC
	Reverse	CAAGGTTCTCAGCCACTCCCAC
Mrsa	Forward	GTATGTGTCTCCCACCATTCCTTC
	Reverse	GTACAGAGCCACATCTCCCTTCTG
	COUP-TFII PRIMERS	
Clic4	Forward	CTACATCTCCAATCCCTTCCCTAG
	Reverse	ACCGCAAACACAACAATCACACAG
Gypc	Forward	TCCCTTAATTTGTCATCCCACAGG
	Reverse	GGTGAATTTGTTCTTGCCTTCTTG
Mef2d	Forward	CTTAAAAATTCACCACGCCCAGAG
	Reverse	ACCACTACCTCCCTTGCCTCACAG
Mrsa	Forward	AGCGAGCTAGAAGAAAGGCAGACC
	Reverse	GTGGTGTTGAGAATTGTGCTTTGG

Table 4. Mouse Primers to Validate The Enrichment at Promoters of ERRα and COUP-TFII at The Same Binding Site

GENE	PRIMERS	
Esrra	Forward	GTGGCCCCGCCTTTCCCCGTGACCTTCATT
	Reverse	ACCCCTGAGGACCCTCAAGTGGAGAAGCAG
Got1	Forward	CTAGTATGTGCTCATCCCTTACTTTCTGC
	Reverse	CAAGTGTCTGGCACAGGACTCTAGTGGTC
Got2	Forward	GCTTCCAGTTAATCATCCGCTACGC
	Reverse	GGACATGAAAGCATTCTGAACCTTG
Jun	Forward	GGCGACGTGAGAAGGTCCGAG
	Reverse	CGTTCCTCCAGTCCGAGAGCG
Nr5a2	Forward	GCATGGGGAAGGACAGACTCTGCTCTTC
	Reverse	GGCAACTGGTCAAATGGTAAGTAAATACC
Pdk4	Forward	GGATAGATCCCAGGTCGCTAGG
	Reverse	GGCTACTGTAAAAGTCCCGCTCTG
Phf5a-Aco2	Forward	CATGCTTCCGCCAAGTATGTTG
	Reverse	CCTTGTCACCTTTGCCCTTG
Slc25a4	Forward	GGGACGACCTGGGGACAGAGAG
	Reverse	GACAACGGGAAGGGGTGGAAGC

Table 5. Mouse Primers to Validate The Transcriptional Regulation of ERRα and COUP-TFII of Their Common Target Genes

GENE	PRIMERS	
Jun	Forward	CAGTGGGTGCCAACTCATGCTAAC
	Reverse	CCAGTCCATCTTGTGTACCCTTGG
Nr5a2	Forward	AACAACCTCCTGAGTCTCGCACAG
	Reverse	TGCTGGTGGTAGTCTTCGGCATAG
Bmp2k	Forward	TGTATGTCAATAACACACCCGACC
	Reverse	TCCATTAAGATAAGCACTTCCCAC
Got2	Forward	CAAGAGGTGAAAGGCATGGCTGAC
	Reverse	TCATGTAGACCGAGAACTCCTTGG
Phf5a	Forward	CCGATGCCTACTACTGTAAAGAGTG
	Reverse	AGCAGTTTGATGGGGGAGGAAGGAG
Aco2	Forward	CCCTTTACCCCTGACTTGGCTCAC
	Reverse	ATGGTGAACTGGGACTTGCACTTG
Ahcy	Forward	CCCACCCAGATAAATACCCTGTTG
	Reverse	AAGGACAGCCCCAGCACTCAGTAG
DKK4	Forward	GGAAGCCCAGTACGAAGAAATCAC
	Reverse	GCCCCCTCCTGGAGCAGACTTGTC
Fth1	Forward	GCCTCCTACGTCTATCTGTCTATG
	Reverse	CGGTCTGGTTTCTTTATATCCTGC
Fadd	Forward	GACCTGTTCACGGTGCTGCTGGAG
	Reverse	ACACACAATGTCAAATGCCACCTG

GENE	PRIMERS	
Gga1	Forward	GTGATGCCACAGCCAGCTCCATTC
	Reverse	GAAGGTGGTGTCGGGTCACTTAGG
Psmd9	Forward	CACCAGCTCAGACTGATTCCAACC
	Reverse	CCATTCTTCTCCAGATCCTTACAC
Pgk1	Forward	TGCCTGTTGACTTTGTCACTGCTG
	Reverse	CCAAACAATCTGCTTAGCTCGACC
Gata4	Forward	AATGCGGAAGGAGGGGATTCAAAC
	Reverse	CTTCACTGCTGCTGCTGCTGCTAG
Serpina1e	Forward	GTCAACTTTGCAGAGTCAGAGGAG
	Reverse	GCTTGCTTAGTGTTCTCAGGATCG
Dsc3	Forward	ACAGTTTCACTCAACCCCGACTTG
	Reverse	GTCCCTCTTCTTCCTGCTTTTCAC
Acox1	Forward	GAGGGGGAGAACACTGTTATGATG
	Reverse	GGCTGTTAATGTCCACCAGAGTTG
Serpinc1	Forward	CGCACCGAGGATGGCTTCAGTCTG
	Reverse	ACTCGCTGCTGCTTCACTGCCTTC
Rxrb	Forward	CTGACCTACTCGTGTCGTGATAAC
	Reverse	CCATCTCCATCCCCGTCTTTGTCC
Esrra	Forward	TGGAGGAAGCGGAGTAGGAAGCAG
	Reverse	CTGGCTGGACATGGTGCTGGTCAC

Table 6. List of Genes, the Promoters of Which are Bound by COUP-TFII in ChIP-on-chip Experiments with cut-off p-value ≤0.01

0610011F06Rik	Aco2	Atox1	Ccdc33	Cradd
1110007C09Rik	Acot12	Atp10d	Ccdc38	Creld1
1190002N15Rik	Acox1	Atp5a1	Ccdc56	Crip2
1300010F03Rik	Acp2	Atp5b	Ccdc83	Crls1
1600014C10Rik	Acp6	Atp5d	Ccne1	Crp
1700029G01Rik	Acsl1	Atp5e	Ccnt2	Crsp7
1700040L02Rik	Actg1	Atp5g3	Cd1d1	Csk
1700041G16Rik	Acy3	Atxn7	Cd274	Csnk1d
1810011O10Rik	Add3	B3gnt2	Cd302	Ctsa
2010003K11Rik	Adfp	B630019A10Rik	Cdc14a	Ctsb
2200001I15Rik	Adipor2	Banf1	Cdc25a	Cugbp2
2310016E02Rik	Agpat1	BC017612	Cenpa	Cxcl11
2310028O11Rik	Ahcy	BC017643	Cenpq	Cxcl12
2310044H10Rik	Ahsa1	BC043934	Centb2	Cycs
2410015M20Rik	AI132487	BC048546	Cerk	Cyp4f13
2510027J23Rik	AI413782	Bcdo2	Ces6	D17Wsu92e
2610301F02Rik	AI662250	Bckdha	Cfb	D4Wsu53e
2610507B11Rik	Ak3	Bckdk	Cflar	Dact2
3110009E18Rik	Akap8l	Bcl2l1	Chchd3	Dad1
4933403F05Rik	Akr1b7	Bcl3	Chdh	Dbi
4933426M11Rik	Aldh1l1	Bhlhb2	Chic2	Dcakd
5330417C22Rik	Aldh4a1	Bin1	Chmp4b	Ddit3
6330503K22Rik	Alkbh7	Birc3	Chst1	Ddo
6330512M04Rik	Amacr	Bmp2k	Cideb	Decr1
6330578E17Rik	Anapc1	Brap	Cish	Dedd2
9130011J15Rik	Ank3	Brsk1	Clic4	Depdc7
9530058B02Rik	Ankrd27	Btd	Clip1	Dexi
9930012K11Rik	Ap3m2	Bub1b	Cln8	Dhcr24
A230050P20Rik	Apoa2	C130022K22Rik	Clock	Dhps
A930018P22Rik	Apoc1	C1qtnf6	Cml5	Dhx8
Aars2	Aqp5	C3	Cnnm4	Diap1
Aass	Arf4	C430004E15Rik	Cobl	Dido1
Abcb8	Arhgap26	C8b	Cobll1	Disc1
Abhd4	Arhgef12	Cabp1	Copz1	Dkk4
Acaa1a	Armc1	Calr	Coq7	Dnaja3
Acaa2	Armc5	Car8	Coro7	Dnajc19
Acadm	Armet	Carhsp1	Cox10	Dok4
Acads	Arsa	Carkl	Cox17	Dpep1
Acat2	Asgr1	Ccdc106	Cox6c	Dullard
Acbd4	Atf4	Ccdc151	Cox8b	Dusp6

Table 6. Continued.

Dvl1	Fndc4	Hadh	Itih1	Mcts1
E130309D14Rik	Fosl2	Hdac5	Itih2	Mef2d
E2f2	Fth1	Hdgfrp2	Itih3	Men1
E2f3	Fubp3	Hdlbp	Jmjd3	Mertk
Edem1	G0s2	Helb	Jun	Mesdc1
Eepd1	Gaa	Hes6	Junb	Mesdc2
Efcab2	Gabarapl1	Hfe	Kazald1	Mettl7b
Eif1ad	Galt	Hhat	Kcnk5	Mgrn1
Eif2ak3	Gapdh	Hhex	Kifc3	Midn
Eif4ebp1	Gata4	Hist2h4	Klb	Mier1
Eif4ebp2	Gata6	Hnrpll	Klhl24	Mir16
Ela2	Gbe1	Hook2	Kptn	Mkln1
Elk4	Gckr	Hoxd12	Kynu	Mlf2
Emilin1	Gfod1	Hsdl2	Lace1	Mllt6
Eno1	Gga1	Hsp90b1	Lasp1	mmu-let-7c-2
Enpp4	Ggt6	Hyal1	Lenep	mmu-mir-122a
Ephb4	Ghdc	Hyal2	Letm2	mmu-mir-365-2
Ern1	Gm129	Hyou1	Lifr	Mocs2
Errfi1	Gmfg	Icam1	Lims1	Mospd3
Esrra	Gmnn	Id3	Lipc	Mpp1
Etfb	Gna12	Idh1	Lipe	Mprip
Etfdh	Gnpnat1	Idh3b	Lman1	Mrap
F10	Gns	Idh3g	Lrch1	Mrpl12
F7	Golga5	Ier2	Lrmp	Mrpl34
Fadd	Got1	Ifitm1	Lrp1	Mrpl35
Fam100a	Got2	Ifitm2	Lrp6	Mrpl36
Fam102a	Gpatch3	Ifnar2	Lrrc8d	Mrpl4
Fam109a	Gpd1	Ifrg15	Lsm8	Mrps21
Fam167b	Gpr124	Igfbp2	Ltbr	Mrps33
Fam18a	Gpr182	Il13ra1	Lypla1	Mrps5
Fam20a	Gramd3	Il17rb	Macrod1	Msi2
Farsla	Grhpr	Immt	Map1lc3b	Msra
Fbxl19	Grlf1	Ing5	Map3k7ip1	Mtap7d1
Fbxo31	Grwd1	Inmt	Mapk14	Mtmr11
Fdxr	Gsdmdc1	Inppl1	Mapkapk3	Mtmr12
Fgf1	Gsk3a	Insig2	Masp2	Mul1
Fgfr4	Gstk1	Iscu	Mbd6	Mut
Fh1	Gypc	Isg20l1	Mcart1	Mx2
Fkbp5	Gys1	Islr2	Mcfd2	Myst2
Fn1	H1f0	Isoc2b	Mcoln1	Naca

Table 6. Continued.

Nadk	Otud5	Pnpla3	Reep6	Serpinf2
Nags	Oxa1l	Polr1e	Ret	Setd8
Narf	Oxnad1	Ppara	Rhod	Sh3bp5
Ndufa2	P2rx4	Pparg	Rhof	Sin3a
Ndufa4	Pacs1	Ppcs	Rilp	Skap2
Ndufb3	Pag1	Ppm1b	Rmnd1	Slc12a7
Ndufb8	Paics	Ppm1d	Rmnd5a	Slc22a4
Ndufs1	Park7	Ppm1k	Rnase10	Slc25a10
Ndufs2	Pbx2	Ppp1cb	Rnf5	Slc25a13
Ndufs8	Pck1	Ppp1r3b	Rpl10a	Slc25a28
Ndufv1	Pcolce	Prdx2	Rpl36	Slc25a39
Nedd8	Pctk3	Prkaca	Rreb1	Slc25a4
Nek4	Pde8a	Prkag2	Rsad2	Slc25a42
Nfatc3	Pdha1	Prmt1	Rtl1	Slc26a1
Nfe2l1	Pdk2	Psap	Rtn4ip1	Slc26a6
Nfib	Pdk4	Pscd1	Rtp3	Slc26a8
Nfkbia	Pdxp	Psen2	Ruvbl2	Slc29a1
Nkx2-5	Pemt	Psmc4	Rxrb	Slc38a3
Nola2	Pex11c	Psmd9	S100a13	Slc38a7
Nostrin	Pex5	Pspc1	Sacm1l	Slc39a4
Npc1	Pgk1	Ptbp1	Safb2	Slc44a1
Nphp3	Pgm2	Ptdss2	Samd4	Slc45a3
Nqo2	Phb	Ptp4a1	Saps3	Slc47a1
Nr1d1	Phf10	Ptp4a2	Sardh	Slc9a8
Nr1d2	Phf5a	Pvrl1	Sart3	Slmap
Nr1h4	Phpt1	Pvrl2	Sbno2	Slmo2
Nr2f6	Phyhd1	Pxk	Scml4	Smad3
Nr5a2	Pigr	Qdpr	Scn1b	Smap1l
Nrp	Pigv	R3hdm1	Sdha	Smcr7
Nrp1	Pik3ap1	Rab3a	Sdhd	Snrpa
Nsmce1	Pik4cb	Rab9	Sds	Snx13
Nsun4	Pim1	Rai12	Sec14l2	Socs5
Nucb1	Pim3	Rap2c	Sec24c	Sod2
Nwd1	Pipox	Rasgrp2	Sema4g	Sorbs3
Oaf	Pitpnm2	Rasl11b	Sepp1	Sp3
Oaz2	Plcl4	Rassf3	Serf2	Spata17
Ogdh	Plekho1	Rbpms	Serpina1e	Spr
Ogt	Plxnd1	Rcor1	Serpina6	Spred2
Optn	Pnkd	Rdh1	Serpinc1	Spry2
ORF61	Pnpla2	Recc1	Serpine1	Srxn1

Table 6. Continued.

St3gal3	Tmem85	Xdh	2310036O22Rik	Acot7
St6galnac6	Tmprss4	Ypel4	2310051M13Rik	Acsl4
Stambpl1	Tnfrsf9	Ythdf1	2310065K24Rik	Acss3
Stard7	Tom1l2	Zadh1	2610034B18Rik	Actb
Stk11	Tomm40	Zbtb5	2610209A20Rik	Actn4
Strn	Tomm40l	Zcchc7	2810026P18Rik	Adamts1
Strn3	Tor1aip1	Zfand2a	2810410L24Rik	Adamts12
Stub1	Tpcn1	Zfat1	2900010M23Rik	Adamts13
Sult1a1	Tpi1	Zfp101	2900073G15Rik	Adamts2
Supt5h	Trappc3	Zfp106	3110001D03Rik	Adamts4
Supt7l	Trim56	Zfp112	3200002M19Rik	Admr
Susd4	Trim7	Zfp143	4921517D22Rik	Adora2a
Suv420h2	Trip10	Zfp219	4921524J17Rik	Adrbk1
Tada1l	Trp53	Zfp27	4930412F15Rik	Agbl2
Tagap1	Trp53i13	Zfp444	4930455C21Rik	Agtrap
Tbc1d10a	Trpm7	Zfp46	4930483J18Rik	Ahdc1
Tcf19	Tsku	Zfp469	4930546H06Rik	AI182371
Tcfap2e	Ttpal	Zfp661	4931406C07Rik	AI847670
Tect1	Tulp2	Zfp691	5031414D18Rik	AI987944
Tef	Tusc2	Zfp710	5730455P16Rik	Aip
Tenc1	Txndc12	Zfp768	5930416I19Rik	Akap2
Tfb1m	Tyki	Zfp787	6720460F02Rik	Akt1
Thnsl2	Ubc	Zhx3	8430410A17Rik	Aldh2
Thrsp	Ube2i	Zkscan1	9130227C08Rik	Aldh3b1
Tk1	Ubxd1	Zkscan14	9330133O14Rik	Alg14
Tlcd2	Upb1	Zmym2	9430015G10Rik	Alox12e
Tm4sf4	Uqcrb	Zmynd12	9530048O09Rik	Ampd2
Tmco4	Uqcrh	Mtor	A130023I24Rik	Anapc5
Tmed10	Uqcrq	(March9)	A530016L24Rik	Ankrd33b
Tmem106a	Usp2	(Sept11)	A530095I07Rik	Ankrd43
Tmem110	Vamp8	(Sept4)	A630091E08Rik	Anxa2
Tmem120a	Vasp	0610009B22Rik	A830007P12Rik	Anxa3
Tmem147	Vim	1110018G07Rik	Abcb1a	Aof1
Tmem14c	Vkorc1	1110034B05Rik	Abcc9	Ap1m2
Tmem161a	Wdfy3	1110038D17Rik	Abcd2	Ap2a2
Tmem161b	Wdr18	1700012B15Rik	Abhd1	Apbb2
Tmem166	Wdr51a	1700123O20Rik	Abhd15	Apoc2
Tmem171	Wdr79	1810046J19Rik	Abl1	Apom
Tmem50a	Wiz	2310003C23Rik	Acbd5	Arf5
Tmem77	Wsb2	2310035K24Rik	Acin1	Arglu1

Table 6. Continued.

Arhgap17	Bclaf1	Ccdc72	Cldn15	Cygb
Arhgap4	Bcmo1	Ccdc84	Clec14a	Cyr61
Arhgef15	Bcor	Ccdc85b	Clic1	D14Ertd449e
Arhgef2	Bin3	Ccdc97	Cltc	D15Ertd621e
Arhgef7	Bmp6	Ccnb1	Cmtm6	D15Wsu169e
Arid1a	Bnip1	Ccnd1	Cmtm8	D19Wsu162e
Arih2	Brca1	Ccng2	Cnih	D1Ertd622e
Arnt	Bst2	Cct8	Cnr2	D5Wsu178e
Arrb1	Btf3	Cd151	Cntln	D830013H23Rik
Arrdc4	Btg1	Cd2bp2	Col4a1	D830014E11Rik
Arsb	Btrc	Cd52	Copa	Dag1
Art4	C130060K24Rik	Cda	Cotl1	Dcbld1
Asb8	C1galt1	Cdc25b	Cox6a2	Dctd
Ascc2	C1qtnf1	Cdc25c	Cp	Ddah2
Ascc3l1	C1rl	Cdc26	Cpne2	Ddx3x
Asf1b	C2	Cdc34	Cpped1	Ddx42
Asna1	C6	Cdc42ep3	Cpsf7	Dedd
Atg2a	C730034F03Rik	Cdca5	Cpt1c	Dennd4b
Atg4c	C8g	Cdcp1	Creb3l2	Dhrs1
Atoh8	Camk1	Cdipt	Crlf3	Dhrs3
Atp2c1	Camta2	Cdk9	Crtac1	Dhrs7
Atp6v1e2	Cant1	Cdo1	Cryzl1	Dhx32
Atrip	Capn5	Cdx1	Csdc2	Dirc2
Aurkb	Capza1	Cebpa	Csnk1g2	Dll1
Avil	Capzb	Cebpe	Csrnp1	Dmpk
AW209491	Car10	Cebpg	Cst3	Dmwd
Axud1	Car13	Cenpb	Ctbp2	Dnaja1
B230396O12Rik	Car4	Cenpj	Ctbs	Dnajc17
B2m	Card10	Centb1	Ctf1	Dnajc27
B530045E10Rik	Carkd	Ces3	Ctgf	Dnm1
B630005N14Rik	Casc3	Cfl1	Ctnna1	Dock6
Bad	Casp8ap2	Cgref1	Ctnnb1	Dph5
Bahcc1	Cat	Cgrrf1	Ctsl	Dpp4
Barhl2	Cbln3	Chchd5	Cuedc1	Dpysl3
Baz1b	Cbr1	Chmp7	Cxcl10	Dsc3
BC004728	Cc2d2a	Chn1	Cxcl15	Dtx4
BC019943	Ccbp2	Chst2	Cxcl5	Dusp14
BC026585	Ccdc115	Chtf8	Cxcr5	Dysfip1
BC050777	Ccdc47	Churc1	Cyb5	E130203B14Rik
Bcam	Ccdc51	Ciz1	Cyb5r4	Eaf1

Table 6. Continued.

Ebna1bp2	Fam49a	Gabarap	Grb14	Inoc1
Eef1b2	Fam53c	Gas2l1	Grb7	Inpp5a
EG634650	Fam78a	Gats	Gse1	Insl3
Egfl7	Fam83a	Gatsl3	Gstm7	Ipmk
Egr1	Fam92b	Gba	Gsto1	Irf1
Ei24	Fam96b	Gbf1	Gtf2a2	Isca1
Eif1	Fastkd2	Gbl	Gtf2f1	Isgf3g
Eif2a	Fbln1	Gcgr	Gtpbp2	Itga1
Eif2ak1	Fbxl8	Gcn5l2	Gtpbp6	Itgb4bp
Eif2ak4	Fbxo6	Gda	Guf1	Itih4
Eif2s3y	Fbxw10	Gdf9	Hax1	Itm2b
Eif3s7	Fbxw5	Gdpd3	Hcfc1r1	Itpr1
Eif4g2	Fcgrt	Gemin5	Herpud2	Ivns1abp
Eif4g3	Fdps	Gfi1b	Hexim1	Jarid1c
Eif5a2	Fdx1l	Ggnbp2	Hif1a	Jph4
Eml2	Fgd2	Gipc2	Hif1an	Jub
Eno3	Fhdc1	Git2	Hip2	Jundm2
Enpp6	Fibp	Gja4	Hipk2	Katnal1
Epb4.1l4a	Fignl1	Gjb2	Hirip3	Katnb1
Erc1	Fis1	Glis2	Hmha1	Kcnk6
Erh	Fkbp6	Gm11961	Hmmr	Kctd2
Esam1	Fli1	Gm237	Hnrpc	Kifc1
Evi5l	Flot1	Gm5420	Hnrpul1	Kit
Exoc1	Fmo4	Gm5464	Homer3	Klc4
Exosc5	Fndc5	Gm6377	Hoxa5	Klf15
F12	Folr1	Gmfb	Hr	Klhl28
Fabp4	Fosl1	Gmip	Hrg	Kmo
Fabp5	Foxg1	Gmpr2	Hs1bp3	Krt18
Fam105a	Foxo3a	Gnai2	Hs3st3b1	Krt222
Fam108b	Foxq1	Gnaq	Hspbap1	L7Rn6
Fam125a	Frs3	Gnb1	Icam5	Larp2
Fam131a	Fryl	Gnb2l1	iffo1	Lbh
Fam134c	Fscn2	Gnpda1	Ifi47	Lbp
Fam13a	Fsd1	Golga4	Ifitm5	Lepr
Fam149b	Ftl1	Gpiap1	Ifngr2	Lgals3bp
Fam160b1	Fv1	Gpr157	Ift172	Lgals8
Fam168b	Fxn	Gpsn2	Ift80	Lgals9
Fam176b	Fzd7	Gpx3	Il3ra	Lhx8
Fam20b	G3bp	Gpx7	Ilvbl	Lima1
Fam45a	G3bp2	Gramd1b	Imp4	Lincr

Table 6. Continued

Lipt1	Med8	Myo10	Oit3	Pfn1
Llph	Mfsd3	Myoz1	Olig2	Pfn4
Lman1l	Mfsd5	Napa	Omp	Pgm1
Lnx2	Mgat1	Narg1l	Orai1	Phf12
Loxl2	Mical1	Nbr1	Orm3	Phf20
Lpin3	Miip	Ncapd3	Osbpl9	Phgdhl1
Lrch4	Mina	Ncbp1	Oscp1	Phkg2
Lrdd	Mios	Ncl	Oxsr1	Phlda3
Lrp10	Mllt10	Ncln	Pacsin1	Phldb2
Lrp12	mmu-mir-143	Ncor2	Pafah1b2	Phospho2
Lrp4	mmu-mir-145	Ndst1	Paip2	Pi4k2a
Lrp5	mmu-mir-29a	Ndufs6	Pak4	Pif1
Lrrc48	Mpv17l2	Nek2	Pank3	Pink1
Lrrc49	Mpzl1	Nelf	Pank4	Pip5k2a
Lrrc9	Mrpl1	Nfat5	Papln	Pitpnb
Lsm4	Mrpl11	Nfia	Parp3	Pitpnc1
Lsr	Mrpl16	Nfkb2	Pbld	Pkig
Ltc4s	Mrpl2	Nfkbil2	Pcaf	Pkmyt1
Luc7l2	Mrpl22	Nid1	Pcdh7	Pla2g6
Lyn	Mrpl38	Ninj1	Pcgf1	Plec1
Lysmd4	Mrps26	Nit2	Pcnxl3	Plekhg2
Lzts2	Mrps28	Nkx2-3	Pcsk7	Plekhh3
Maf	Mrps30	Nme2	Pctk2	Plekhq1
Mafg	Mrvi1	Nnp1	Pdcd6ip	Plscr3
Mafk	Msh5	Nod1	Pde4dip	Plxdc1
Magoh	Mt2	Nodal	Pdgfc	Plxnb1
Man1a	Mtch1	Nol4	Pdgfrb	Pml
Map3k11	Mthfr	Notch1	Pdia4	Pmpcb
Map3k8	Mtmr4	Npas2	Pdia5	Pnkp
Mapk15	Mtrf1	Npepl1	Pdia6	Pnrc2
Mapln3	Mus81	Nr2f2	Pdlim1	Pold3
Mavs	Mx1	Nrbf2	Pdlim4	Polr3h
Mbd2	Mxra8	Nsun5	Pdlim7	Pomt2
Mbd3	Myc	Nthl1	Pdp2	Popdc2
Mccc1	Mycl1	Ntn1	Pdzd11	Por
Mcph1	Myd116	Nuak1	Pea15	Pou2f3
Mdh1b	Myd88	Nuak2	Pear1	Ppdpf
Mdk	Myh9	Nudcd2	Pebp1	Ppfibp2
Med11	Myl6	Nuf2	Per3	Ppl
Med25	Mylip	Oas1b	Pes1	Ppm1h

Table 6. Continued.

Ppox	Rab13	Rnf20	Sfrs7	Smoc1
Ppp1ca	Rab2	Rod1	Sgk	Snai3
Ppp1r11	Rab33b	Rpa3	Sgpp1	Snd1
Ppp1r12a	Rab37	Rpgrip1	Sh2b2	Sned1
Ppp1r15b	Rab3ip	Rpl24	Sh2d3c	Snip1
Ppp1r1b	Rab4b	Rpl26	Sh3pxd2a	Snrnp27
Ppp2r2a	Rab8b	Rpl3	Sh3tc1	Snx15
Ppp2r3c	Rabgef1	Rpl4	Shank3	Snx30
Ppp2r5b	Rabl4	Rpl6	Shc1	Socs2
Ppp2r5e	Rad54l	Rplp2	Shf	Socs7
Pprc1	Ralb	Rpo1-4	Shisa3	Sox18
Ppt2	Ranbp10	Rqcd1	Shkbp1	Spa17
Prcp	Rap1a	Rras	Shoc2	Spag9
Preb	Rarres2	Rspry1	Shroom4	Sparc
Prkce	Rbbp5	Rsrc2	Sipa1	Spats2l
Prkci	Rbbp6	Rtkn	Skil	Spred1
Prkcsh	Rbks	Rufy1	Skiv2l	Spry1
Prkcz	Rbm4	S100a6	Slc12a9	Srebf2
Prkd2	Rbm4b	Sars2	Slc16a5	Srms
Prnpip1	Rbm9	Sat1	Slc16a7	Ssbp4
Prpf4b	Rbp1	Scaf1	Slc17a7	Ssr2
Prrt1	Rcbtb1	Scnm1	Slc1a5	Sssca1
Pscd2	Rcn1	Scoc	Slc25a21	St3gal4
Pscd3	Rdbp	Sdccag1	Slc25a22	Stag1
Psma3	Rdh11	Selm	Slc25a35	Star
Psma5	Rec8L1	Selp	Slc25a45	Stard6
Psmb4	Rel	Sema3f	Slc2a3	Stat6
Psmd12	Rela	Sema4a	Slc2a4	Steap4
Ptch1	Rfwd3	Senp1	Slc35a2	Stmn1
Pthr1	Rg9mtd2	Sepx1	Slc35d1	Stom
Ptms	Rgl3	Serinc2	Slc37a1	Stoml3
Ptpn12	Rhobtb1	Serinc5	Slc37a3	Strada
Ptpn9	Ric8b	Serpinb6b	Slc39a8	Stx3
Ptprf	Rin3	Serpine2	Slc40a1	Suds3
Ptprm	Rinl	Sertad1	Slc41a1	Sulf1
Pus10	Rnase4	Setd1b	Slc46a1	Sult2b1
Pxmp2	Rnf128	Setd4	Slc9a3r2	Sumo3
Pycrl	Rnf13	Sfrs3	Slco2b1	Suox
Qscn6l1	Rnf144	Sfrs4	Smad6	Syngr1
R74862	Rnf183	Sfrs6	Smarcd2	Synpo2l

Table 6. Continued.

Syt12	Tmem101	Trpc4ap	Wdr67	Zfp95
Syt6	Tmem123	Tsen2	Wdtc1	Zgpat
Taf1d	Tmem125	Tssk3	Wfdc1	Zmiz2
Taf5	Tmem140	Ttc16	Wnk1	Zswim3
Tagln2	Tmem141	Ttc25	Wwtr1	Zswim4
Tanc1	Tmem143	Ttc32	X99384	Zwilch
Tap1	Tmem150	Ttc38	Xab2	
Tbc1d12	Tmem160	Ttf1	Xrcc6bp1	
Tbc1d17	Tmem176b	Txnip	Yif1b	
Tbc1d22a	Tmem183a	Txnrd2	Ypel3	
Tbc1d22b	Tmem20	Tyw3	Ythdf2	
Tbc1d9b	Tmem205	Ube1x	Zan	
Tbcd	Tmem24	Ube2d3	Zbtb40	
Tbx6	Tmem51	Ube2g1	Zbtb7a	
Tbxa2r	Tmem65	Ube2s	Zbtb7b	
Tcam1	Tmem79	Ubiad1	Zc3hav1	
Tcea2	Tmem86b	Ubr1	Zc3hc1	
Tcf3	Tmem87b	Ubxd5	Zdhhc18	
Tcf4	Tmem88	Ulk2	Zfp128	
Tcfcp2	Tmem98	Uncx	Zfp13	
Tcirg1	Tnfrsf14	Ung	Zfp157	
Tcp11l2	Tnfsf13	Urm1	Zfp189	
Tctex1d4	Tnip1	Use1	Zfp207	
Tead3	Tomm70a	Usp10	Zfp273	
Tera	Top3b	Usp39	Zfp313	
Tgfb1	Tpcn2	Usp43	Zfp39	
Tgfb2	Tpd52l2	Usp52	Zfp428	
Tgfbrap1	Tpm4	V1re11	Zfp438	
Tha1	Tpr	Vat1	Zfp446	
Timm13	Traf7	Vcpip1	Zfp513	
Timm17b	Traip	Vdp	Zfp523	
Tinagl	Trf	Vmn2r57	Zfp524	
Tle2	Trhde	Vmo1	Zfp551	
Tle3	Trib2	Vps54	Zfp639	
Tle6	Trim21	Vps72	Zfp667	
Tln1	Trim25	Vwa1	Zfp689	
Tln2	Trim46	Vwa3b	Zfp706	
Tlr12	Trim50	Wbp2	Zfp740	
Tmcc2	Trim8	Wdr23	Zfp809	
Tmeff2	Trp53inp1	Wdr45	Zfp870	

Table 7. List of Genes, the Promoters of Which are Bound by Both ERRα and COUP-TFII in ChIP-on-chip experiments with Cut-off p-value ≤0.01

1110007C09Rik	Apoa2	Ces6	Eif4ebp2	Grlf1
1190002N15Rik	Aqp5	Chdh	Ela2	Grwd1
1300010F03Rik	Arf4	Chic2	Emilin1	Gsk3a
1700040L02Rik	Arhgef12	Chst1	Enpp4	Gys1
1700041G16Rik	Armc1	Cideb	Ephb4	Hdac5
1810011O10Rik	Arsa	Clip1	Ern1	Hdgfrp2
2010003K11Rik	Atox1	Cln8	Etfb	Hdlbp
2310016E02Rik	Atp10d	Clock	Etfdh	Helb
2310044H10Rik	Atp5a1	Cml5	F7	Hes6
2410015M20Rik	Atp5b	Cnnm4	Fadd	Hhat
2510027J23Rik	Atp5d	Cobll1	Fam167b	Hist2h4
4933403F05Rik	Atp5e	Cox10	Fam18a	Hnrpll
6330503K22Rik	Atp5g3	Cox6c	Farsla	Hoxd12
9130011J15Rik	Atxn7	Cox8b	Fbxl19	Icam1
9530058B02Rik	B630019A10Rik	Cradd	Fkbp5	Idh3b
A930018P22Rik	Banf1	Creld1	Fn1	Ifitm1
Aass	BC043934	Crp	Fndc4	Ifitm2
Abcb8	BC048546	Csk	Fosl2	Igfbp2
Abhd4	Bckdk	Ctsa	Fth1	Il13ra1
Acaa1a	Bin1	Cugbp2	Fubp3	Il17rb
Acadm	Btd	Cxcl12	G0s2	Ing5
Acat2	C130022K22Rik	Cyp4f13	Gaa	Inppl1
Acbd4	C1qtnf6	D17Wsu92e	Gabarapl1	Iscu
Aco2	C3	D4Wsu53e	Gapdh	Isg20l1
Acot12	C430004E15Rik	Dad1	Gbe1	Islr2
Acsl1	Calr	Dbi	Gckr	Isoc2b
Actg1	Car8	Dcakd	Gfod1	Itih1
Acy3	Carhsp1	Dedd2	Gga1	Jun
Adfp	Carkl	Depdc7	Ggt6	Kazald1
Ahcy	Ccdc33	Dhx8	Ghdc	Kptn
Ahsa1	Ccdc83	Dkk4	Gm129	Kynu
AI132487	Ccne1	Dnajc19	Gmfg	Lasp1
AI413782	Ccnt2	Dok4	Gmnn	Lenep
AI662250	Cd274	Dpep1	Gna12	Lifr
Akr1b7	Cdc14a	Dullard	Gnpnat1	Lims1
Aldh4a1	Cdc25a	Dvl1	Gns	Lipc
Alkbh7	Cenpa	E2f3	Golga5	Lipe
Amacr	Cenpq	Edem1	Gpatch3	Lrch1
Anapc1	Centb2	Eif1ad	Gpr124	Lrp1
Ap3m2	Cerk	Eif2ak3	Gpr182	Lsm8

Table 7. Continued

Lypla1	Nola2	Ppcs	Serf2	Tmem106a
Macrod1	Nostrin	Ppm1b	Serpinc1	Tmem147
Mapk14	Npc1	Ppm1d	Serpinf2	Tmem14c
Masp2	Nqo2	Ppm1k	Setd8	Tmem161a
Mcart1	Nr1d1	Ppp1cb	Skap2	Tmem171
Mcoln1	Nr1h4	Prdx2	Slc12a7	Tmem50a
Mcts1	Nr5a2	Prkag2	Slc22a4	Tmem77
Men1	Nsmce1	Psap	Slc25a10	Tmem85
Mertk	Nwd1	Pscd1	Slc25a13	Tmprss4
Mettl7b	Oaz2	Psmc4	Slc25a28	Tnfrsf9
Mgrn1	Ogt	Ptdss2	Slc25a4	Tomm40l
Midn	Otud5	Pvrl1	Slc26a1	Tpi1
Mier1	Oxa1l	R3hdm1	Slc26a8	Trappc3
Mir16	Oxnad1	Rai12	Slc47a1	Trim7
Mkln1	Pacs1	Rap2c	Slc9a8	Trp53
Mlf2	Pag1	Rasgrp2	Slmap	Trp53i13
Mllt6	Paics	Rasl11b	Slmo2	Tsku
mmu-let-7c-2	Park7	Rbpms	Smap1l	Ubxd1
mmu-mir-122a	Pck1	Rcor1	Snrpa	Uqcrh
Mospd3	Pcolce	Rdh1	Sod2	Vkorc1
Mpp1	Pctk3	Reep6	Sorbs3	Wdfy3
Mrap	Pdk4	Ret	Sp3	Wdr18
Mrpl4	Pdxp	Rilp	Spata17	Wdr79
Mrps21	Pex11c	Rmnd1	Spr	Wiz
Mrps33	Pex5	Rnase10	Srxn1	Wsb2
Mrps5	Pgk1	Rpl10a	St6galnac6	Ythdf1
Msi2	Phf5a	Rpl36	Strn3	Zadh1
Mtap7d1	Phyhd1	Rtn4ip1	Stub1	Zbtb5
Mtor	Pigr	Ruvbl2	Sult1a1	Zcchc7
Mul1	Pim1	Sacm1l	Supt5h	Zfand2a
Mut	Pim3	Samd4	Supt7l	Zfat1
Mx2	Pipox	Sardh	Tada1l	Zfp101
Myst2	Pitpnm2	Sart3	Tcfap2e	Zfp106
Naca	Plcl4	Sbno2	Tect1	Zfp112
Ndufa2	Plxnd1	Scml4	Tef	Zfp143
Ndufb3	Pnkd	Sdha	Tenc1	Zfp219
Ndufs2	Pnpla2	Sdhd	Tfb1m	Zfp27
Ndufv1	Pnpla3	Sds	Thnsl2	Zfp46
Nek4	Polr1e	Sema4g	Tk1	Zfp469
Nkx2-5	Ppara	Sepp1	Tmed10	Zfp661

Table 7. Continued.

Zfp691	B3gnt2	Ddit3	Gypc	Mef2d
Zfp710	BC017612	Ddo	H1f0	Mesdc1
Zfp768	BC017643	Decr1	Hadh	Mesdc2
Zkscan14	Bcdo2	Dexi	Hfe	mmu-mir-365-2
Zkscan5	Bckdha	Dhcr24	Hhex	Mocs2
Zmynd12	Bcl2l1	Dhps	Hook2	Mprip
0610011F06Rik	Bcl3	Diap1	Hsdl2	Mrpl12
1600014C10Rik	Bhlhb2	Dido1	Hsp90b1	Mrpl34
1700029G01Rik	Birc3	Disc1	Hyal1	Mrpl35
2200001I15Rik	Bmp2k	Dnaja3	Hyal2	Mrpl36
2310028O11Rik	Brap	Dusp6	Hyou1	Msra
2610301F02Rik	Brsk1	E130309D14Rik	Id3	Mtmr11
2610507B11Rik	Bub1b	E2f2	Idh1	Mtmr12
3110009E18Rik	C8b	Eepd1	Idh3g	Nadk
4933426M11Rik	Cabp1	Efcab2	Ier2	Nags
5330417C22Rik	Ccdc106	Eif4ebp1	Ifnar2	Narf
6330512M04Rik	Ccdc151	Elk4	Ifrg15	Ndufa4
6330578E17Rik	Ccdc38	Eno1	Immt	Ndufb8
9930012K11Rik	Ccdc56	Errfi1	Inmt	Ndufs1
A230050P20Rik	Cd1d1	Esrra	Insig2	Ndufs8
Aars2	Cd302	F10	Itih2	Nedd8
Acaa2	Cfb	Fam100a	Itih3	Nfatc3
Acads	Cflar	Fam102a	Jmjd3	Nfe2l1
Acox1	Chchd3	Fam109a	Junb	Nfib
Acp2	Chmp4b	Fam20a	Kcnk5	Nfkbia
Acp6	Cish	Fbxo31	Kifc3	Nphp3
Add3	Clic4	Fdxr	Klb	Nr1d2
Adipor2	Cobl	Fgf1	Klhl24	Nr2f6
Agpat1	Copz1	Fgfr4	Lace1	Nrp
Ak3	Coq7	Fh1	Letm2	Nrp1
Akap8l	Coro7	Galt	Lman1	Nsun4
Aldh1l1	Cox17	Gata4	Lrmp	Nucb1
Ank3	Crip2	Gata6	Lrp6	Oaf
Ankrd27	Crls1	Got1	Lrrc8d	Ogdh
Apoc1	Crsp7	Got2	Ltbr	Optn
Arhgap26	Csnk1d	Gpd1	Map1lc3b	ORF61
Armc5	Ctsb	Gramd3	Map3k7ip1	P2rx4
Armet	Cxcl11	Grhpr	Mapkapk3	Pbx2
Asgr1	Cycs	Gsdmdc1	Mbd6	Pde8a
Atf4	Dact2	Gstk1	Mcfd2	Pdha1

Table 7. Continued.

Pdk2	Sec14l2	Tom1l2		
Pemt	Sec24c	Tomm40		
Pgm2	Serpina1e	Tor1aip1		
Phb	Serpina6	Tpcn1		
Phf10	Serpine1	Trim56		
Phpt1	Sh3bp5	Trip10		
Pigv	Sin3a	Trpm7		
Pik3ap1	Slc25a39	Ttpal		
Pik4cb	Slc25a42	Tulp2		
Plekho1	Slc26a6	Tusc2		
Pparg	Slc29a1	Txndc12		
Ppp1r3b	Slc38a3	Tyki		
Prkaca	Slc38a7	Ubc		
Prmt1	Slc39a4	Ube2i		
Psen2	Slc44a1	Upb1		
Psmd9	Slc45a3	Uqcrb		
Pspc1	Smad3	Uqcrq		
Ptbp1	Smcr7	Usp2		
Ptp4a1	Snx13	Vamp8		
Ptp4a2	Socs5	Vasp		
Pvrl2	Spred2	Vim		
Pxk	Spry2	Wdr51a		
Qdpr	St3gal3	Xdh		
Rab3a	Stambpl1	Ypel4		
Rab9	Stard7	Zfp444		
Rassf3	Stk11	Zfp787		
Recc1	Strn	Zhx3		
Rhod	Susd4	Zkscan1		
Rhof	Suv420h2	Zmym2		
Rmnd5a	Tagap1			
Rnf5	Tbc1d10a			
Rreb1	Tcf19			
Rsad2	Thrsp			
Rtl1	Tlcd2			
Rtp3	Tm4sf4			
Rxrb	Tmco4			
S100a13	Tmem110			
Safb2	Tmem120a			
Saps3	Tmem161b			
Scn1b	Tmem166			

Table 8. List of Genes that Recruit ERRα and COUP-TFII to the Same Binding Site on their Promoter Regions

1110007C09Rik	BC043934	Dok4	Hnrpll	Mospd3
1300010F03Rik	BC048546	Dpep1	Hoxd12	Mprip
1600014C10Rik	Bckdk	Dusp6	Hsdl2	Mrap
1700041G16Rik	Bin1	Dvl1	Icam1	Mrpl4
1810011O10Rik	C1qtnf6	E2f3	Idh3b	Msi2
2010003K11Rik	C3	Edem1	Ifitm1	Mtap7d1
2200001I15Rik	C430004E15Rik	Eif1ad	Ifitm2	Mul1
2310016E02Rik	Calr	Eif2ak3	Ifnar2	Mx2
2510027J23Rik	Ccdc83	Ela2	Il17rb	Naca
2610507B11Rik	Cd274	Emilin1	Immt	Nags
9530058B02Rik	Cd302	Enpp4	Ing5	Ndufa2
A930018P22Rik	Cdc14a	Esrra	Inppl1	Ndufb3
Aass	Cdc25a	Fadd	Iscu	Nkx2-5
Abhd4	Chchd3	Fam167b	Isg20l1	Nola2
Acaa1a	Chdh	Fam18a	Jun	Nostrin
Acaa2	Chst1	Farsla	Kazald1	Nqo2
Acads	Cish	Fbxl19	Kptn	Nr1d1
Acat2	Clip1	Fdxr	Kynu	Nr1h4
Aco2	Cml5	Fkbp5	Lasp1	Nr5a2
Acot12	Cnnm4	Fosl2	Lenep	Nsmce1
Actg1	Cobll1	Fth1	Lifr	Nucb1
Acy3	Coq7	Fubp3	Lims1	Ogdh
Ahcy	Cox10	Gbe1	Lipc	Ogt
Ahsa1	Cox6c	Gfod1	Lrch1	Otud5
AI413782	Cox8b	Gga1	Lsm8	Oxnad1
AI662250	Cradd	Ggt6	Lypla1	Pag1
Akr1b7	Creld1	Gm129	Macrod1	Paics
Alkbh7	Crp	Gmfg	Mapk14	Pck1
Amacr	Ctsa	Golga5	Mbd6	Pcolce
Ankrd27	Cxcl12	Got1	Mcart1	Pctk3
Ap3m2	Cyp4f13	Got2	Mcoln1	Pdha1
Arf4	D17Wsu92e	Gpr124	Mertk	Pdk4
Arsa	Dad1	Gpr182	Mettl7b	Pdxp
Atp10d	Dcakd	Grwd1	Mgrn1	Pex11c
Atp5e	Ddit3	Gsk3a	Midn	Pgk1
Atp5g3	Dedd2	Gys1	Mkln1	Phf10
Atxn7	Depdc7	Hdac5	Mlf2	Phf5a
B630019A10Rik	Dhx8	Hdlbp	Mllt6	Phyhd1
Banf1	Diap1	Helb	mmu-let-7c-2	Pigr
BC017612	Dkk4	Hes6	mmu-mir-122a	Pim1

Table 8. Continued.

Pim3	Rap2c	Serpinf2	Tcfap2e	Wiz
Pipox	Rasgrp2	Setd8	Tef	Wsb2
Plcl4	Rasl11b	Sh3bp5	Tenc1	Ythdf1
Plxnd1	Rbpms	Slc25a10	Tfb1m	Zadh1
Pnpla2	Rcor1	Slc25a13	Tk1	Zbtb5
Pnpla3	Rdh1	Slc25a4	Tlcd2	Zcchc7
Polr1e	Reep6	Slc26a8	Tmed10	Zfp101
Ppara	Rilp	Slc47a1	Tmem106a	Zfp106
Ppcs	Rmnd1	Smap1l	Tmem77	Zfp112
Ppm1b	Rnase10	Snx13	Tmprss4	Zfp219
Ppm1d	Rtn4ip1	Sorbs3	Tpi1	Zfp27
Ppm1k	Ruvbl2	Sp3	Trappc3	Zfp444
Ppp1cb	Rxrb	Spata17	Trim7	Zfp469
Ppp1r3b	Sacm1l	Spr	Trpm7	Zfp691
Prdx2	Sart3	Srxn1	Tsku	Zfp710
Psap	Scml4	Strn3	Tulp2	Zfp787
Psmd9	Sds	Stub1	Upb1	Zkscan14
Pspc1	Serpina1e	Supt7l	Uqcrq	Zkscan5
Pvrl1	Serpinc1	Tada1l	Vkorc1	Zmynd12
R3hdm1	Serpine1	Tcf19	Wdr18	

Conclusion

ChIP-on-chip experiments showed enrichment of 2373 and 1766 segments for ERRα and COUP-TFII, respectively. Of those, 299 genes were identified as common targets of the two NRs, recruiting ERRα and COUP-TFII to the same site in the promoter regions. The main sites for the binding of ERRα and COUP-TFII on their common target genes were found to be the DRs/ERRE merged sites, indicating these two proteins are likely to compete for binding when regulating target gene expression. In the Hepa1-6 cell line, approximately 15% of common target genes of ERRα and COUP-TFII were found to be co-regulated by both NRs. It confirms the usefulness of ChIP-on-chip experiments and gives informative results by revealing the common targets of ERRα and COUP-TFII. Finally, a significant number of biological pathways were found to be potentially modulated by both NRs, a contribution which could assist future studies of cross talk between these two receptors.

Reference:

Aparicio, O., J. V. Geisberg, et al. (2004). "Chromatin immunoprecipitation for determining the association of proteins with specific genomic sequences in vivo." Curr Protoc Cell Biol **Chapter 17**: Unit 17 17.

Ariazi, E. A., G. M. Clark, et al. (2002). "Estrogen-related receptor alpha and estrogen-related receptor gamma associate with unfavorable and favorable biomarkers, respectively, in human breast cancer." Cancer Research **62**(22): 6510-6518.

Bain, D. L., A. F. Heneghan, et al. (2007). "Nuclear receptor structure: Implications for function." Annual Review of Physiology **69**: 201-220.

Bardoux, P., P. Zhang, et al. (2005). "Essential role of chicken ovalbumin upstream promoter-transcription factor II in insulin secretion and insulin sensitivity revealed by conditional gene knockout." Diabetes **54**(5): 1357-1363.

Beato, M., P. Herrlich, et al. (1995). "Steroid-Hormone Receptors - Many Actors in Search of a Plot." cell **83**(6): 851-857.

Bonnelye, E., V. Kung, et al. (2002). "Estrogen receptor-related receptor impinges on the estrogen axis in bone: Potential function in osteoporosis." Endocrinology **143**(9): 3658-3670.

Bonnelye, E., L. Merdad, et al. (2001). "The orphan nuclear estrogen receptor-related receptor alpha (ERR alpha) is expressed throughout osteoblast differentiation and regulates bone formation in vitro." Journal of Cell Biology **153**(5): 971-983.

Bonnelye, E., J. M. Vanacker, et al. (1997). "The ERR-1 orphan receptor is a transcriptional activator expressed during bone development." Molecular Endocrinology **11**(7): 905-916.

Carolyn M. Klinge, D. L. B. (1997). "Binding of type II nuclear receptor and estrogen receptor to full and half-stie estrogen response elements in vitro." Nucleic Acids Research **25**(10): 1903-1912.

Carrier, J. C., G. Deblois, et al. (2004). "Estrogen-related receptor alpha (ERRalpha) is a transcriptional regulator of apolipoprotein A-IV and controls lipid handling in the intestine." J Biol Chem **279**(50): 52052-52058.

Castet, A., A. Herledan, et al. (2006). "Receptor-interacting protein 140 differentially regulates estrogen receptor-related receptor transactivation depending on target genes." Molecular Endocrinology **20**(5): 1035-1047.

Cavallini, A., M. Notarnicola, et al. (2005). "Oestrogen receptor-related receptor alpha (ERR alpha) and oestrogen receptors (ER alpha and ER beta) exhibit different gene expression in human

colorectal tumour progression." European Journal of Cancer **41**(10): 1487-1494.
Charest-Marcotte, A., C. R. Dufour, et al. (2010). "The homeobox protein Prox1 is a negative modulator of ERR alpha/PGC-1 alpha bioenergetic functions." Genes & Development **24**(6): 537-542.
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., D. Zhou, et al. (2001). "Molecular basis for the constitutive activity of estrogen-related receptor alpha-1." J Biol Chem **276**(30): 28465-28470.
Cheung, C. P., S. Yu, et al. (2005). "Expression and functional study of estrogen receptor-related receptors in human prostatic cells and tissues." Journal of Clinical Endocrinology & Metabolism **90**(3): 1830-1844.
Chisarnore, M. J., H. A. Wilkinson, et al. (2009). "Estrogen-related receptor-alpha antagonist inhibits both estrogen receptor-positive and estrogen receptor-negative breast tumor growth in mouse xenografts." Molecular Cancer Therapeutics **8**(3): 672-681.
Collas, P. (2010). "The Current State of Chromatin Immunoprecipitation." Molecular Biotechnology **45**(1): 87-100.
Cooney, A. J., X. H. Leng, et al. (1993). "Multiple Mechanisms of Chicken Ovalbumin Upstream Promoter Transcription Factor-Dependent Repression of Transactivation by the Vitamin-D, Thyroid-Hormone, and Retinoic Acid Receptors." Journal of Biological Chemistry **268**(6): 4152-4160.
Cooney, A. J., S. Y. Tsai, et al. (1991). "Chicken Ovalbumin Upstream Promoter Transcription Factor Binds to a Negative Regulatory Region in the Human-Immunodeficiency-Virus Type-1 Long Terminal Repeat." Journal of Virology **65**(6): 2853-2860.
Cooney, A. J., S. Y. Tsai, et al. (1992). "Chicken Ovalbumin Upstream Promoter Transcription Factor (Coup-Tf) Dimers Bind to Different Ggtca Response Elements, Allowing Coup-Tf to Repress Hormonal Induction of the Vitamin-D(3), Thyroid-Hormone, and Retinoic Acid Receptors." Molecular and Cellular Biology **12**(9): 4153-4163.
Deblois, G., J. A. Hall, et al. (2009). "Genome-Wide Identification of Direct Target Genes Implicates Estrogen-Related Receptor alpha as a Determinant of Breast Cancer Heterogeneity." Cancer Research **69**(15): 6149-6157.
Dufour, C. R., B. J. Wilson, et al. (2007). "Genome-wide orchestration of cardiac functions by the orphan nuclear receptors ERRalpha and gamma." Cell Metabolism **5**(5): 345-356.

Fernando, R., J. S. Foster, et al. (2007). "Breast cancer cell proliferation is inhibited by BAD: regulation of cyclin D1." J Biol Chem **282**(39): 28864-28873.
Forman BM, G. E., Chen J, Oro AE, Bradley DJ, Perlmann T, Noonan DJ, Burka LT, McMorris T, Lamph WW, Evans RM, Weinberger C. (1995). "Identification of a nuclear receptor that is activated by farnesol metabolites." cell **81**(5): 687-693.
Gao, M., P. Sun, et al. (2006). "Expression of estrogen receptor-related receptor isoforms and clinical significance in endometrial adenocarcinoma." International Journal of Gynecological Cancer **16**(2): 827-833.
Giguere, V. (1999). "Orphan nuclear receptors: From gene to function." Endocrine Reviews **20**(5): 689-725.
Giguere, V. (2002). "To ERR in the estrogen pathway." Trends in Endocrinology and Metabolism **13**(5): 220-225.
Giguere, V. (2008). "Transcriptional control of energy homeostasis by the estrogen-related receptors." Endocrine Reviews **29**(6): 677-696.
Giguere, V., N. Yang, et al. (1988). "Identification of a new class of steroid hormone receptors." Nature **331**(6151): 91-94.
Glass, C. K. (1994). "Differential Recognition of Target Genes by Nuclear Receptor Monomers, Dimers, and Heterodimers." Endocrine Reviews **15**(3): 391-407.
Gottlicher, M., E. Widmark, et al. (1992). "Fatty-Acids Activate a Chimera of the Clofibric Acid-Activated Receptor and the Glucocorticoid Receptor." Proceedings of the National Academy of Sciences of the United States of America **89**(10): 4653-4657.
Greschik, H., J. M. Wurtz, et al. (2002). "Structural and functional evidence for ligand-independent transcriptional activation by the estrogen-related receptor 3." Molecular Cell **9**(2): 303-313.
Gronemeyer, H., J. A. Gustafsson, et al. (2004). "Principles for modulation of the nuclear receptor superfamily." Nature Reviews Drug Discovery **3**(11): 950-964.
Haddad, I. A., J. M. Ordovas, et al. (1986). "Linkage, Evolution, and Expression of the Rat Apolipoprotein a-I, C-Iii, and a-Iv Genes." Journal of Biological Chemistry **261**(28): 3268-3277.
Hall, R. K., F. M. Sladek, et al. (1995). "The Orphan Receptors Coup-Tf and Hnf-4 Serve as Accessory Factors Required for Induction of Phosphoenolpyruvate Carboxykinase Gene-Transcription by Glucocorticoids." Proceedings of the National Academy of Sciences of the United States of America **92**(2): 412-416.
Heyman, R. A., D. J. Mangelsdorf, et al. (1992). "9-Cis Retinoic Acid Is a High-Affinity Ligand for the Retinoid-X Receptor." cell **68**(2): 397-406.

Hong, H., L. Yang, et al. (1999). "Hormone-independent transcriptional activation and coactivator binding by novel orphan nuclear receptor ERR3." J Biol Chem **274**(32): 22618-22626.
Horard, B. and J. M. Vanacker (2003). "Estrogen receptor-related receptors: orphan receptors desperately seeking a ligand." J Mol Endocrinol **31**(3): 349-357.
Huss, J. M., K. I. Imahashi, et al. (2007). "The nuclear receptor ERR alpha is required for the bioenergetic and functional adaptation to cardiac pressure overload." Cell Metabolism **6**(1): 25-37.
Hwung, Y. P., L. H. Wang, et al. (1988). "Differential Binding of the Chicken Ovalbumin Upstream Promoter (Coup) Transcription Factor to 2 Different Promoters." Journal of Biological Chemistry **263**(26): 13470-13474.
Ichida, M., S. Nemoto, et al. (2002). "Identification of a specific molecular repressor of the peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 alpha)." Journal of Biological Chemistry **277**(52): 50991-50995.
Janowski, B. A., P. J. Willy, et al. (1996). "An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha." Nature **383**(6602): 728-731.
Jonk, L. J., M. E. de Jonge, et al. (1994). "Cloning and expression during development of three murine members of the COUP family of nuclear orphan receptors." Mech Dev **47**(1): 81-97.
Kallen, J., J. M. Schlaeppi, et al. (2004). "Evidence for ligand-independent transcriptional activation of the human estrogen-related receptor alpha (ERRalpha): crystal structure of ERRalpha ligand binding domain in complex with peroxisome proliferator-activated receptor coactivator-1alpha." J Biol Chem **279**(47): 49330-49337.
Ktistaki, E. and I. Talianidis (1997). "Chicken ovalbumin upstream promoter transcription factors act as auxiliary cofactors for hepatocyte nuclear factor 4 and enhance hepatic gene expression." Molecular and Cellular Biology **17**(5): 2790-2797.
Ladias, J. A., M. Hadzopoulou-Cladaras, et al. (1992). "Transcriptional regulation of human apolipoprotein genes ApoB, ApoCIII, and ApoAII by members of the steroid hormone receptor superfamily HNF-4, ARP-1, EAR-2, and EAR-3." J Biol Chem **267**(22): 15849-15860.
Laudet, V., C. Hanni, et al. (1992). "Evolution of the Nuclear Receptor Gene Superfamily." Embo Journal **11**(3): 1003-1013.
Lee, C. T., L. Li, et al. (2004). "The nuclear orphan receptor COUP-TFII is required for limb and skeletal muscle development." Molecular and Cellular Biology **24**(24): 10835-10843.
Leng, X., A. J. Cooney, et al. (1996). "Molecular mechanisms of COUP-TF-mediated transcriptional repression: Evidence for

transrepression and active repression." Molecular and Cellular Biology **16**(5): 2332-2340.
Levin, A. A., L. J. Sturzenbecker, et al. (1992). "9-Cis Retinoic Acid Stereoisomer Binds and Activates the Nuclear Receptor Rxr-Alpha." Nature **355**(6358): 359-361.
Lhorset, F., S. Dauvois, et al. (1996). "RIP-140 interacts with multiple nuclear receptors by means of two distinct sites." Molecular and Cellular Biology **16**(11): 6029-6036.
Li, L. P., X. Xie, et al. (2009). "The Nuclear Orphan Receptor COUP-TFII Plays an Essential Role in Adipogenesis, Glucose Homeostasis, and Energy Metabolism." Cell Metabolism **9**(1): 77-87.
Lou, D. Q., M. Tannour, et al. (1999). "Chicken ovalbumin upstream promoter-transcription factor II, a new partner of the glucose response element of the L-type pyruvate kinase gene, acts as an inhibitor of the glucose response." J Biol Chem **274**(40): 28385-28394.
Lu, D., Y. Kiriyama, et al. (2001). "Transcriptional regulation of the estrogen-inducible pS2 breast cancer marker gene by the ERR family of orphan nuclear receptors." Cancer Research **61**(18): 6755-6761.
Lu, X. P., G. Salbert, et al. (1994). "An evolutionary conserved COUP-TF binding element in a neural-specific gene and COUP-TF expression patterns support a major role for COUP-TF in neural development." Molecular Endocrinology **8**(12): 1774-1788.
Lu, X. P., G. Salbert, et al. (1994). "An Evolutionary Conserved Coup-Tf Binding-Element in a Neural-Specific Gene and Coup-Tf Expression Patterns Support a Major Role for Coup-Tf in Neural Development." Molecular Endocrinology **8**(12): 1774-1788.
Luo, J. M., R. Sladek, et al. (2003). "Reduced fat mass in mice lacking orphan nuclear receptor estrogen-related receptor alpha." Molecular and Cellular Biology **23**(22): 7947-7956.
McKenna, N. J., R. B. Lanz, et al. (1999). "Nuclear receptor coregulators: Cellular and molecular biology." Endocrine Reviews **20**(3): 321-344.
Mootha, V. K., C. M. Lindgren, et al. (2003). "PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes." Nat Genet **34**(3): 267-273.
More, E., T. Fellner, et al. (2003). "Activation of the MAP kinase pathway induces chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII) expression in human breast cancer cell lines." J Endocrinol **176**(1): 83-94.
Nakshatri, H., M. S. Mendonca, et al. (2000). "The orphan receptor COUP-TFII regulates G2/M progression of breast cancer cells by modulating the expression/activity of p21(WAF1/CIP1), cyclin D1, and cdk2." Biochemical and Biophysical Research Communications **270**(3): 1144-1153.

Pastorcic, M., H. Wang, et al. (1986). "Control of transcription initiation in vitro requires binding of a transcription factor to the distal promoter of the ovalbumin gene." Molecular and Cellular Biology **6**(8): 2784-2791.
Patti, M. E., A. J. Butte, et al. (2003). "Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1." Proc Natl Acad Sci U S A **100**(14): 8466-8471.
Pereira, F. A., Y. H. Qiu, et al. (1995). "Chicken Ovalbumin Upstream Promoter Transcription Factor (Coup-Tf) - Expression during Mouse Embryogenesis." Journal of Steroid Biochemistry and Molecular Biology **53**(1-6): 503-508.
Pereira, F. A., Y. H. Qiu, et al. (1999). "The orphan nuclear receptor COUP-TFII is required for angiogenesis and heart development." Genes & Development **13**(8): 1037-1049.
Pereira, F. A., M. J. Tsai, et al. (2000). "COUP-TF orphan nuclear receptors in development and differentiation." Cell Mol Life Sci **57**(10): 1388-1398.
Qiu, Y., S. Y. Tsai, et al. (1994). "COUP-TF an orphan member of the steroid/thyroid hormone receptor superfamily." Trends Endocrinol Metab **5**(6): 234-239.
Rastinejad, F., T. Perlmann, et al. (1995). "Structural Determinants of Nuclear Receptor Assembly on DNA Direct Repeats." Nature **375**(6528): 203-211.
Ren, B., F. Robert, et al. (2000). "Genome-wide location and function of DNA binding proteins." Science **290**(5500): 2306-2309.
Sanyal, S., J. Y. Kim, et al. (2002). "Differential regulation of the orphan nuclear receptor small heterodimer partner (SHP) gene promoter by orphan nuclear receptor ERR isoforms." Journal of Biological Chemistry **277**(3): 1739-1748.
Schreiber, S. N., R. Emter, et al. (2004). "The estrogen-related receptor alpha (ERRalpha) functions in PPARgamma coactivator 1alpha (PGC-1alpha)-induced mitochondrial biogenesis." Proc Natl Acad Sci U S A **101**(17): 6472-6477.
Schreiber, S. N., D. Knutti, et al. (2003). "The transcriptional coactivator PGC-1 regulates the expression and activity of the orphan nuclear receptor estrogen-related receptor alpha (ERR alpha)." Journal of Biological Chemistry **278**(11): 9013-9018.
Sladek, R., J. A. Bader, et al. (1997). "The orphan nuclear receptor estrogen-related receptor alpha is a transcriptional regulator of the human medium-chain acyl coenzyme A dehydrogenase gene." Molecular and Cellular Biology **17**(9): 5400-5409.
Sonoda, J., J. Laganiere, et al. (2007). "Nuclear receptor ERR alpha and coactivator PGC-1 beta are effectors of IFN-gamma-induced host defense." Genes & Development **21**(15): 1909-1920.

Stein, R. A., C. Y. Chang, et al. (2008). "Estrogen-Related Receptor a Is Critical for the Growth of Estrogen Receptor-Negative Breast Cancer." Cancer Research **68**(21): 8805-8812.

Stroup, D., M. Crestani, et al. (1997). "Orphan receptors chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) and retinoid X receptor (RXR) activate and bind the rat cholesterol 7 alpha-hydroxylase gene (CYP7A)." Journal of Biological Chemistry **272**(15): 9833-9839.

Sun, P. M., J. Sehouli, et al. (2005). "Expression of estrogen receptor-related receptors, a subfamily of orphan nuclear receptors, as new tumor biomarkers in ovarian cancer cells." Journal of Molecular Medicine-Jmm **83**(6): 457-467.

Suzuki, T., Y. Miki, et al. (2004). "Estrogen-related receptor a in human breast carcinoma as a potent prognostic factor." Cancer Research **64**(13): 4670-4676.

Takamoto, N., I. Kurihara, et al. (2005). "Haploinsufficiency of chicken ovalbumin upstream promoter transcription factor II in female reproduction." Molecular Endocrinology **19**(9): 2299-2308.

Tsai, S. Y. and M. J. Tsai (1997). "Chick ovalbumin upstream promoter-transcription factors (COUP-TFs): coming of age." Endocrine Reviews **18**(2): 229-240.

Umesono, K. and R. M. Evans (1989). "Determinants of Target Gene Specificity for Steroid Thyroid-Hormone Receptors." cell **57**(7): 1139-1146.

Villena, J. A., M. B. Hock, et al. (2007). "Orphan nuclear receptor estrogen-related receptor alpha is essential for adaptive thermogenesis." Proceedings of the National Academy of Sciences of the United States of America **104**(4): 1418-1423.

Villena, J. A. and A. Kralli (2008). "ERR alpha: a metabolic function for the oldest orphan." Trends in Endocrinology and Metabolism **19**(8): 269-276.

Warnmark, A., E. Treuter, et al. (2003). "Activation functions 1 and 2 of nuclear receptors: Molecular strategies for transcriptional activation." Molecular Endocrinology **17**(10): 1901-1909.

Wijnholds, J., E. Muller, et al. (1991). "Oestrogen facilitates the binding of ubiquitous and liver-enriched nuclear proteins to the apoVLDL II promoter in vivo." Nucleic Acids Res **19**(1): 33-41.

Xie, W., H. Hong, et al. (1999). "Constitutive activation of transcription and binding of coactivator by estrogen-related receptors 1 and 2." Molecular Endocrinology **13**(12): 2151-2162.

Zechel, C., X. Q. Shen, et al. (1994). "Dimerization Interfaces Formed between the DNA-Binding Domains Determine the Cooperative Binding of Rxr Rar and Rxr Tr Heterodimers to Dr5 and Dr4 Elements." Embo Journal **13**(6): 1414-1424.

Zhang, Y., K. Ma, et al. (2006). "Estrogen-related receptors stimulate pyruvate dehydrogenase kinase isoform 4 gene expression." J Biol Chem **281**(52): 39897-39906.
Zhang, Z., K. Chen, et al. (2006). "Estrogen-related receptors-stimulated monoamine oxidase B promoter activity is down-regulated by estrogen receptors." Molecular Endocrinology **20**(7): 1547-1561.
Zhang, Z. and C. T. Teng (2000). "Estrogen receptor-related receptor alpha 1 interacts with coactivator and constitutively activates the estrogen response elements of the human lactoferrin gene." J Biol Chem **275**(27): 20837-20846.
Zhang, Z. D., P. E. Burch, et al. (2004). "Genomic analysis of the nuclear receptor family: New insights into structure, regulation, and evolution from the rat genome." Genome Research **14**(4): 580-590.
Zhang, Z. P. and C. T. Teng (2000). "Estrogen receptor-related receptor alpha 1 interacts with coactivator and constitutively activates the estrogen response elements of the human lactoferrin gene." Journal of Biological Chemistry **275**(27): 20837-20846.
Zhao, Q., S. Khorasanizadeh, et al. (1998). "Structural elements of an orphan nuclear receptor-DNA complex." Molecular Cell **1**(6): 849-861.