Supplemental Information

Proteomic Analysis of Coregulators Bound to ERα on DNA and Nucleosomes Reveals Coregulator Dynamics

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1) Supplemental Figures

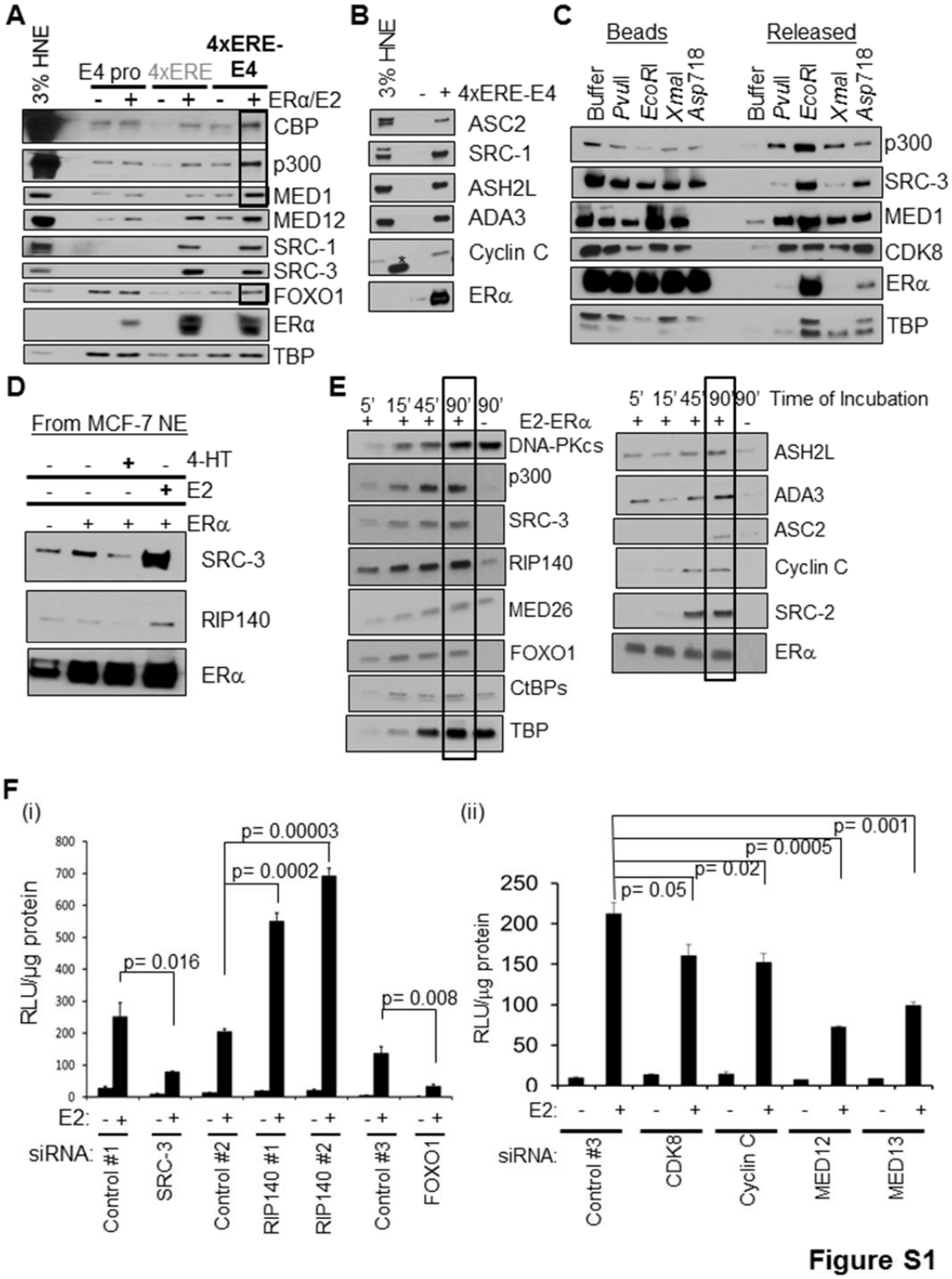

Figure S1

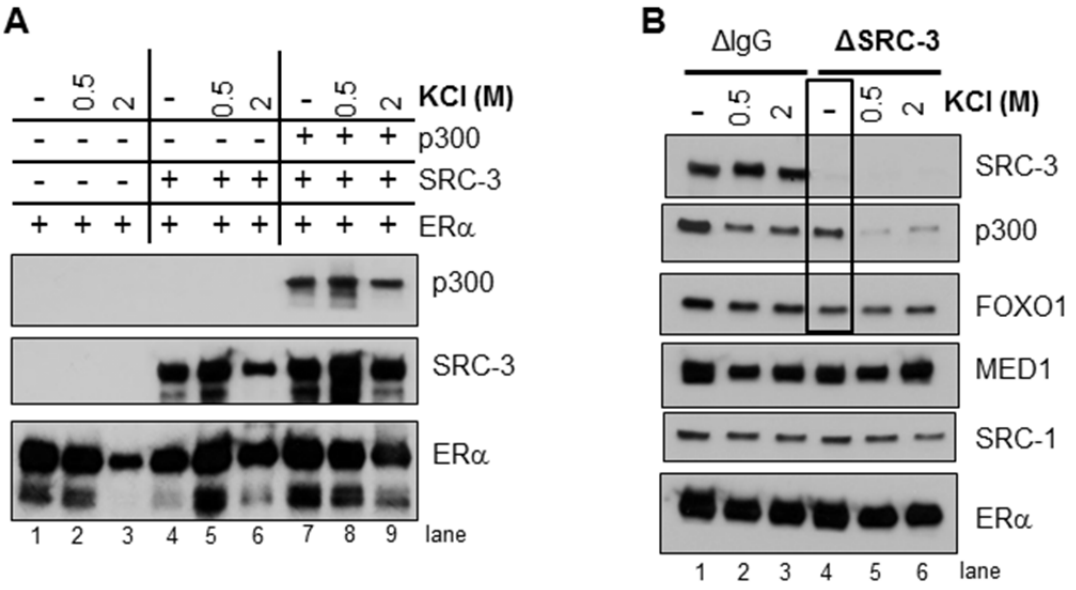
A
- 0.5 2 - 0.5 2 - 0.5 2 KCl (M)
- - - - - - + + + p300
- - - + + + + + + SRC-3
+ + + + + + + + + ERα
p300
SRC-3
ERα
1 2 3 4 5 6 7 8 9 lane
B
ΔIgG
ΔSRC-3
- 0.5 2 - 0.5 2 KCl (M)
SRC-3
p300
FOXO1
MED1
SRC-1
ERα
1 2 3 4 5 6 lane

Figure S2

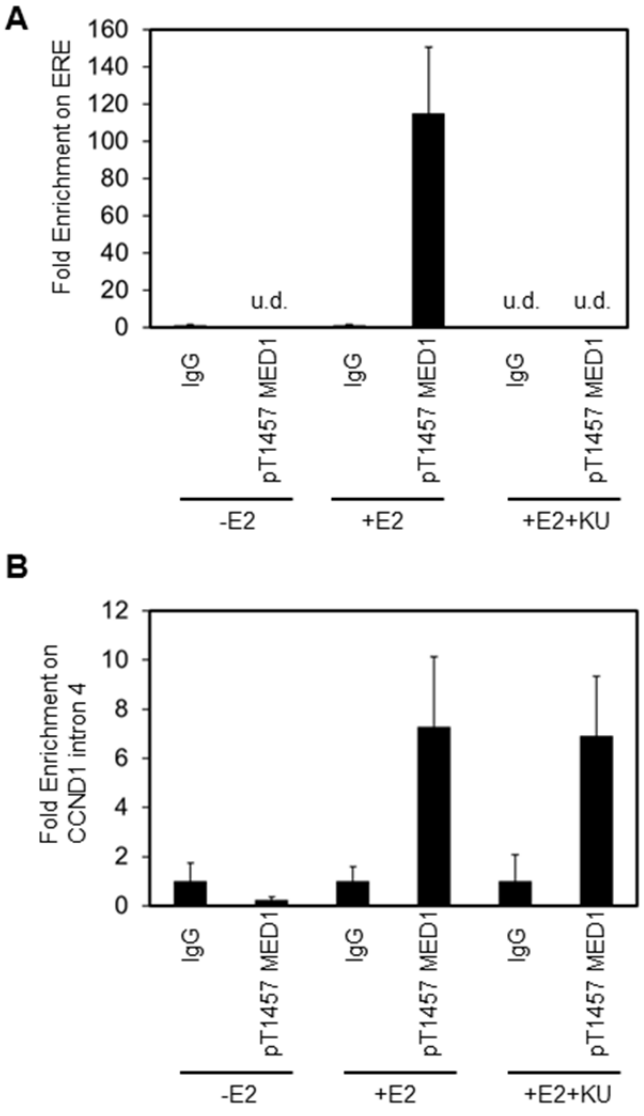
A
Fold Enrichment on ERE
160
140
120
100
80
60
40
20
0
u.d.
u.d.
u.d.
IgG
pT1457 MED1
IgG
pT1457 MED1
IgG
pT1457 MED1
-E2
+E2
+E2+KU
B
Fold Enrichment on CCND1 intron 4
12
10
8
6
4
2
0
IgG
pT1457 MED1
IgG
pT1457 MED1
IgG
pT1457 MED1
-E2
+E2
+E2+KU

Figure S3

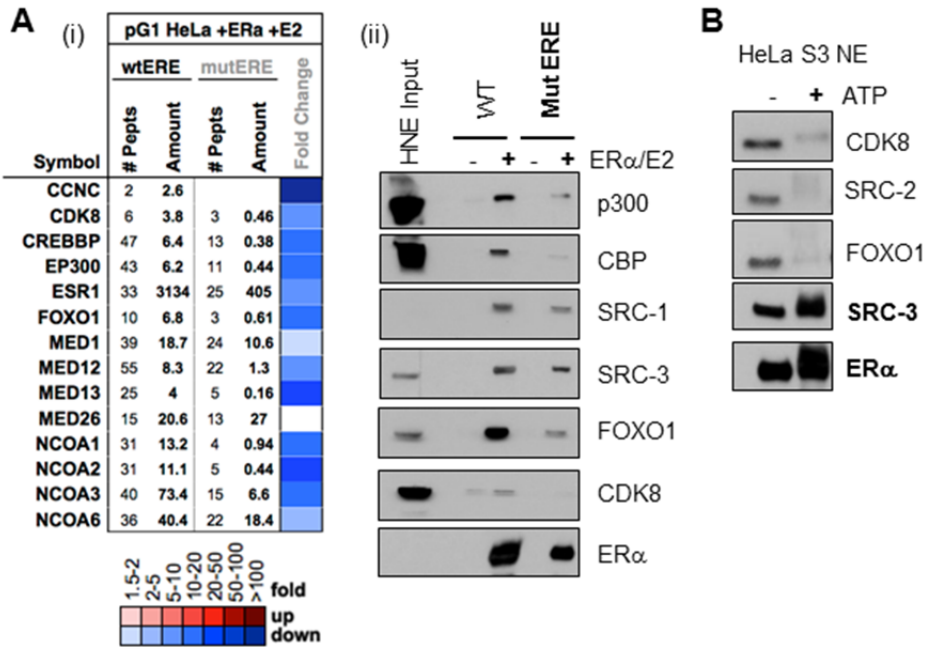
A
(i)
pG1 HeLa +ERa +E2
wtERE
mutERE
Symbol
Pepts
Amount
Pepts
Amount
Fold Change
CCNC 2 2.6
CDK8 6 3.8 3 0.46
CREBBP 47 6.4 13 0.38
EP300 43 6.2 11 0.44
ESR1 33 3134 25 405
FOXO1 10 6.8 3 0.61
MED1 39 18.7 24 10.6
MED12 55 8.3 22 1.3
MED13 25 4 5 0.16
MED26 15 20.6 13 27
NCOA1 31 13.2 4 0.94
NCOA2 31 11.1 5 0.44
NCOA3 40 73.4 15 6.6
NCOA6 36 40.4 22 18.4
1.5-2
2-5
5-10
10-20
20-50
50-100
>100
fold
up
down
(ii)
HNE Input
WT
Mut ERE
- + - +
ERα/E2
p300
CBP
SRC-1
SRC-3
FOXO1
CDK8
ERα
B
HeLa S3 NE
- + ATP
CDK8
SRC-2
FOXO1
SRC-3
ERα

Figure S4

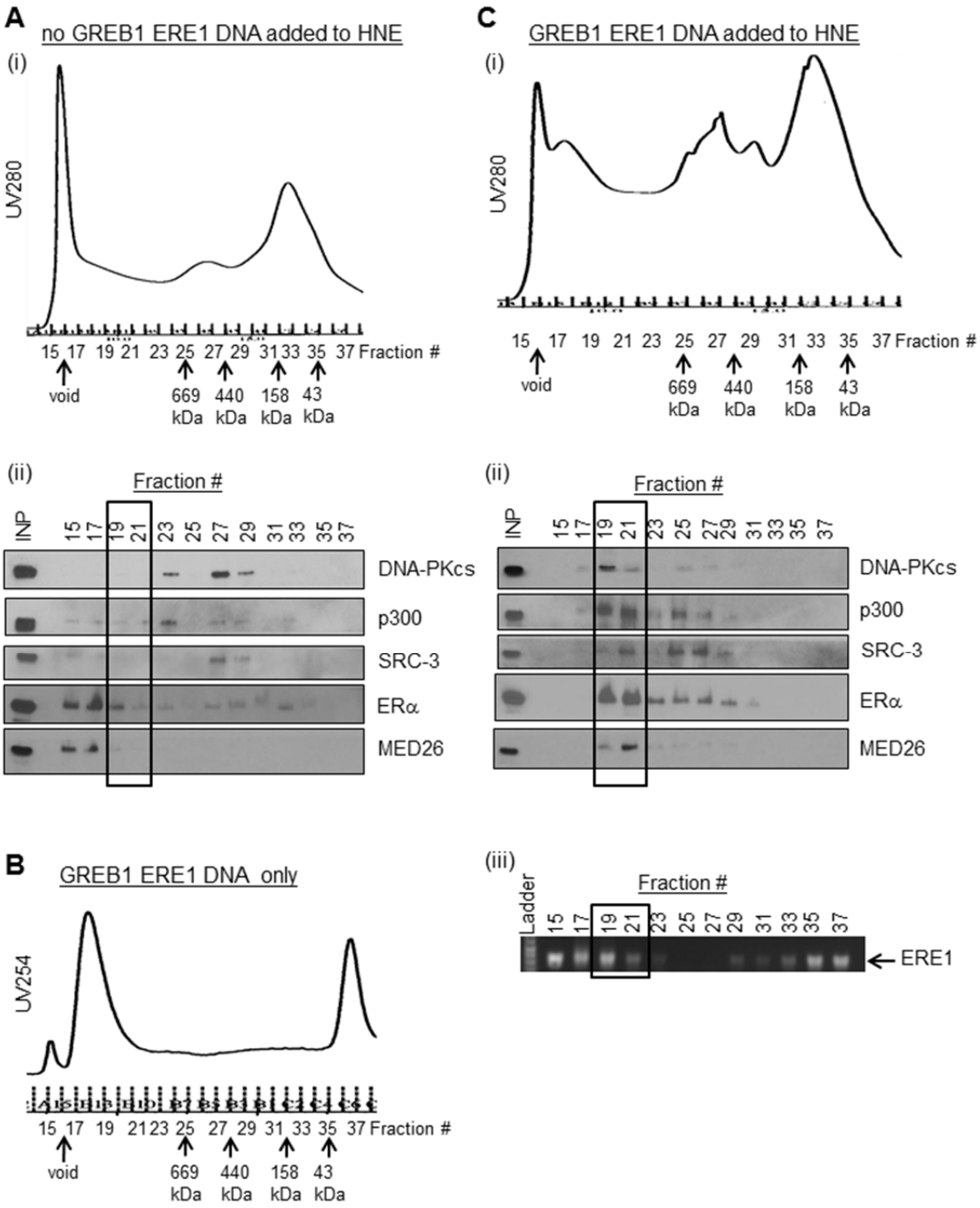
A
no GREB1 ERE1 DNA added to HNE
(i)
UV280
15 17 19 21 23 25 27 29 31 33 35 37 Fraction #
void
669 kDa
440 kDa
158 kDa
43 kDa
C
GREB1 ERE1 DNA added to HNE
(i)
UV280
15 17 19 21 23 25 27 29 31 33 35 37 Fraction #
void
669 kDa
440 kDa
158 kDa
43 kDa
(ii)
Fraction #
INP
15 17 19 21 23 25 27 29 31 33 35 37
DNA-PKcs
p300
SRC-3
ERα
MED26
(ii)
Fraction #
INP
15 17 19 21 23 25 27 29 31 33 35 37
DNA-PKcs
p300
SRC-3
ERα
MED26
B
GREB1 ERE1 DNA only
UV254
15 17 19 21 23 25 27 29 31 33 35 37 Fraction #
void
669 kDa
440 kDa
158 kDa
43 kDa
(iii)
Fraction #
Ladder
15 17 19 21 23 25 27 29 31 33 35 37
ERE1

Figure S5

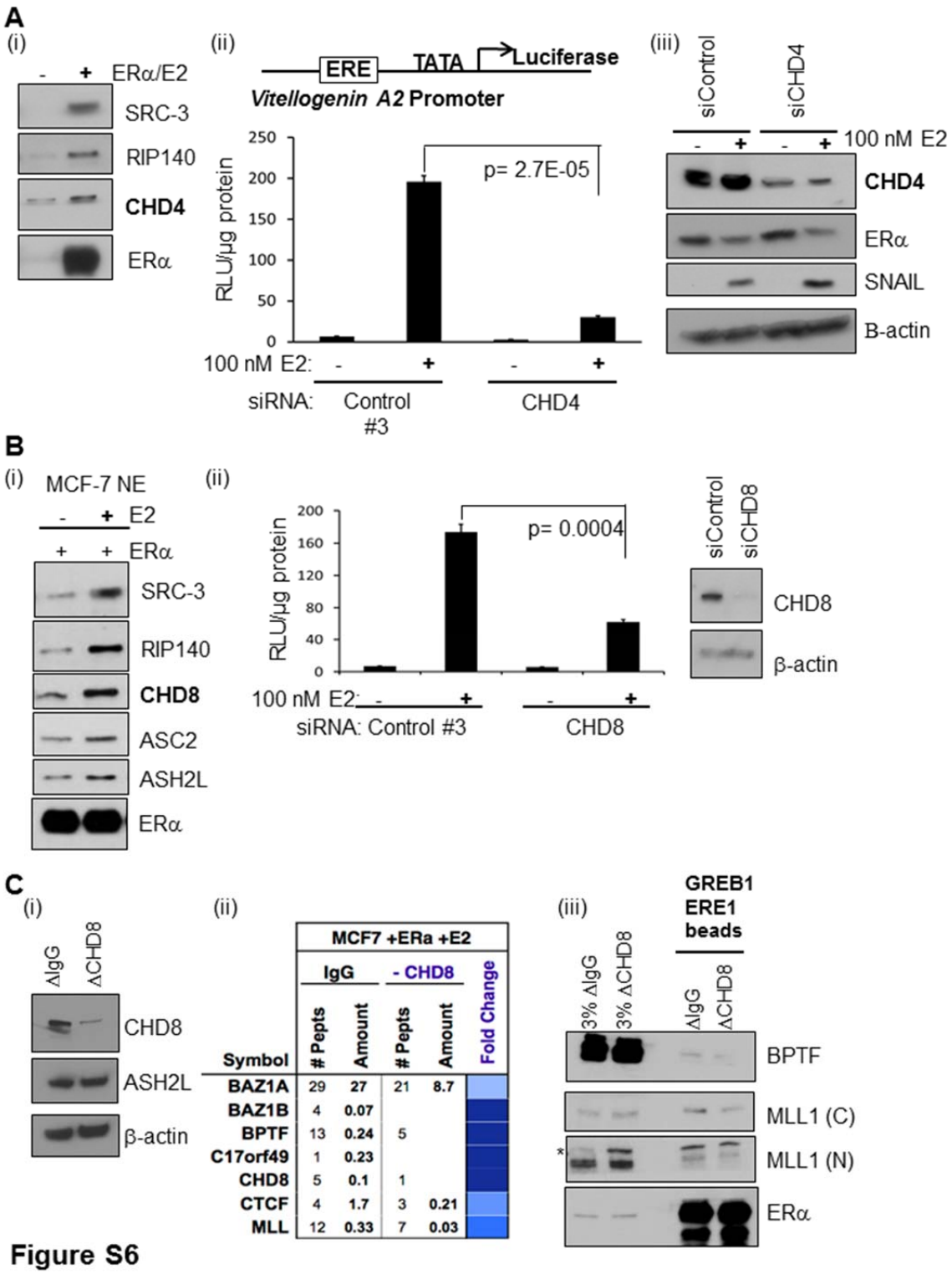
A
(i)
- + ERα/E2
SRC-3
RIP140
CHD4
ERα
(ii)
ERE
TATA
Luciferase
Vitellogenin A2 Promoter
RLU/µg protein
250
200
150
100
50
0
p= 2.7E-05
100 nM E2: - + - +
siRNA: Control #3 CHD4
(iii)
siControl
siCHD4
- + - + 100 nM E2
CHD4
ERα
SNAIL
B-actin
B
(i)
MCF-7 NE
- + E2
+ + ERα
SRC-3
RIP140
CHD8
ASC2
ASH2L
ERα
(ii)
RLU/µg protein
200
160
120
80
40
0
p= 0.0004
100 nM E2: - + - +
siRNA: Control #3 CHD8
siControl
siCHD8
CHD8
β-actin
C
(i)
ΔIgG
ΔCHD8
CHD8
ASH2L
β-actin
(ii)
MCF7 +ERa +E2
IgG
- CHD8
Fold Change
Symbol
Pepts
Amount
Pepts
Amount
BAZ1A 29 27 21 8.7
BAZ1B 4 0.07
BPTF 13 0.24 5
C17orf49 1 0.23
CHD8 5 0.1 1
CTCF 4 1.7 3 0.21
MLL 12 0.33 7 0.03
(iii)
GREB1 ERE1 beads
3% ΔIgG
3% ΔCHD8
ΔIgG
ΔCHD8
BPTF
MLL1 (C)
*
MLL1 (N)
ERα

Figure S6

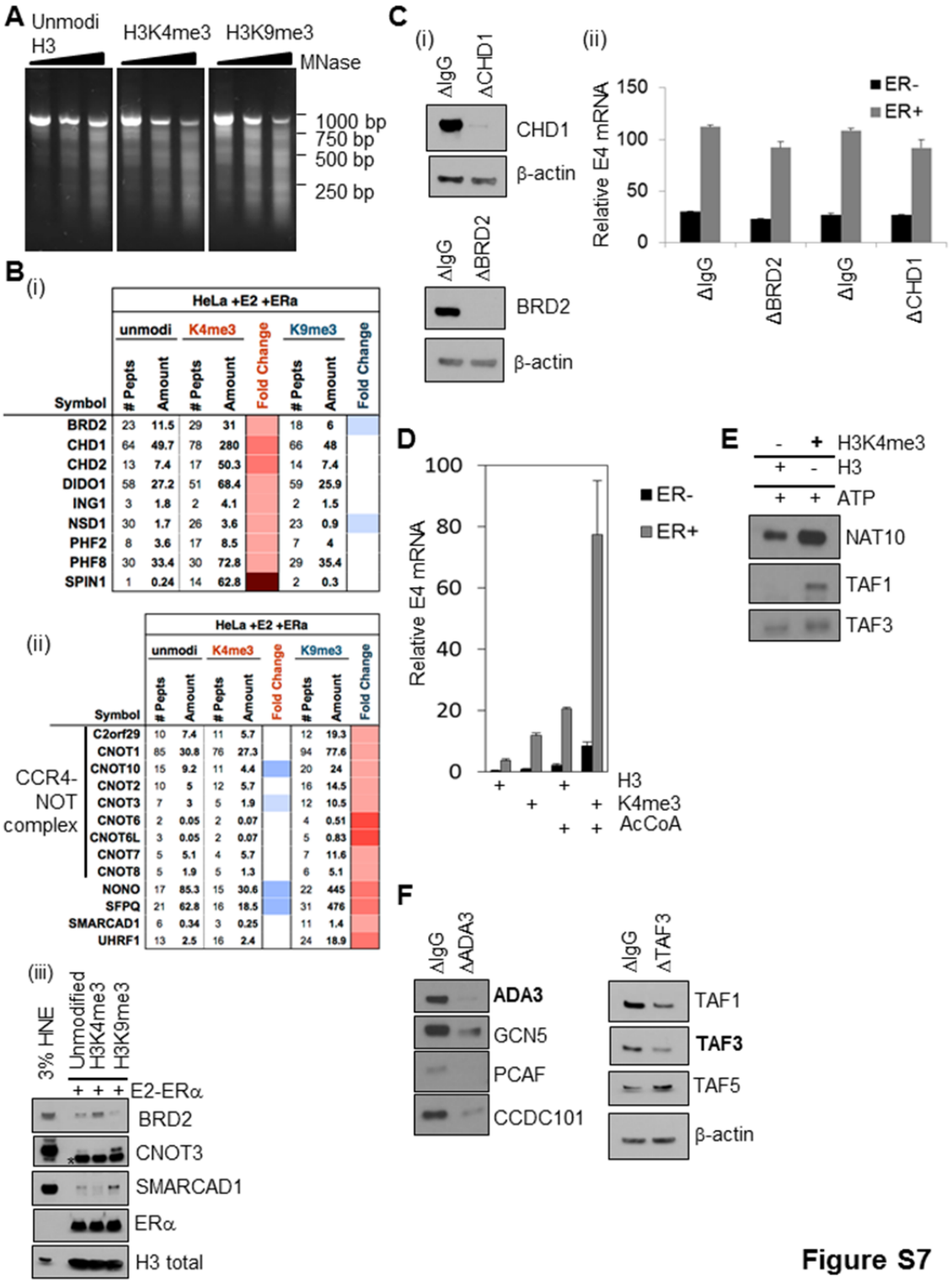
A
Unmodi H3
H3K4me3
H3K9me3
MNase
1000 bp
750 bp
500 bp
250 bp
B
(i)
HeLa +E2 +ERa
unmodi
K4me3
K9me3
Symbol
Pepts
Amount
Fold Change
BRD2 23 11.5 29 31 18 6
CHD1 64 49.7 78 280 66 48
CHD2 13 7.4 17 50.3 14 7.4
DIDO1 58 27.2 51 68.4 59 25.9
ING1 3 1.8 2 4.1 2 1.5
NSD1 30 1.7 26 3.6 23 0.9
PHF2 8 3.6 17 8.5 7 4
PHF8 30 33.4 30 72.8 29 35.4
SPIN1 1 0.24 14 62.8 2 0.3
(ii)
CCR4-NOT complex
C2orf29 10 7.4 11 5.7 12 19.3
CNOT1 85 30.8 76 27.3 94 77.6
CNOT10 15 9.2 11 4.4 20 24
CNOT2 10 5 12 5.7 16 14.5
CNOT3 7 3 5 1.9 12 10.5
CNOT6 2 0.05 2 0.07 4 0.51
CNOT6L 3 0.05 2 0.07 5 0.83
CNOT7 5 5.1 4 5.7 7 11.6
CNOT8 5 1.9 5 1.3 6 5.1
NONO 17 85.3 15 30.6 22 445
SFPQ 21 62.8 16 18.5 31 476
SMARCAD1 6 0.34 3 0.25 11 1.4
UHRF1 13 2.5 16 2.4 24 18.9
(iii)
3% HNE
Unmodified
H3K4me3
H3K9me3
+ + + E2-ERα
BRD2
CNOT3
SMARCAD1
ERα
H3 total
C
(i)
ΔIgG
ΔCHD1
CHD1
β-actin
ΔIgG
ΔBRD2
BRD2
β-actin
(ii)
Relative E4 mRNA
150
100
50
0
ER-
ER+
ΔIgG
ΔBRD2
ΔIgG
ΔCHD1
D
Relative E4 mRNA
100
80
60
40
20
0
ER-
ER+
+ + + H3
+ + + K4me3
+ + AcCoA
E
- + H3K4me3
+ - H3
+ + ATP
NAT10
TAF1
TAF3
F
ΔIgG
ΔADA3
ADA3
GCN5
PCAF
CCDC101
ΔIgG
ΔTAF3
TAF1
TAF3
TAF5
β-actin

Figure S7

2) Supplemental Figure Legends

Figure S1. Optimization and Validation Controls for the Cell-Free ERE DNA Pulldown Assay and Functional Consequence of Loss of Some Coregulators (CoRs), Related to Figure 1

(A) Testing if having a functional RNA Pol II promoter (Adenovirus *E4*) fused to 4xEREs is needed for optimal CoR complex formation from HNE. In this experiment, biotinylated DNA containing only the *E4* promoter (*E4* pro, 773 bp), only 4xEREs (433 bp), or 4xEREs fused to the *E4* promoter (4xERE-E4, 921 bp) was used for the DNA pulldown with recombinant human ERα. CBP, p300, MED1, and FOXO1 have clearly stronger binding when the *E4* promoter is present, as compared to only 4xEREs.

(B) Testing protein binding to Dynabeads alone versus beads with immobilized 4xERE-E4. By immunoblotting, ASC2, SRC-1, ASH2L, ADA3, Cyclin C, and ERα are clearly bound to DNA, not the Dynabeads. *, cross-reaction with protein size standard.

(C) Testing if CoRs and transcription factors bound to 4xERE-E4 DNA can be released from the Dynabeads upon digestion with different restriction enzymes (*Pvu*II, *EcoR*I, *Xma*I, and *Asp*718). In this experiment, only the 3' end of the DNA was biotinylated. 40% of proteins remaining bound to the beads or released in the supernatant were assayed by immunoblotting. As compared to the buffer control, the various enzymes released CoRs, ERα, and TBP to various extents, showing that these proteins were indeed DNA-bound.

(D) Effect of E2 and 4-HT on SRC-3 or RIP140 recruitment from MCF-7 NE to recombinant ERα bound to 4xERE-E4. 100 nM of each ligand was present in reactions,

and 1% ethanol served as the vehicle control. E2 induced CoR binding and 4-HT impaired these recruitments. This data complements Figure 1D data using HeLa NE.

(E) Testing the kinetics of CoR binding to EREs. After various time-points (5, 15, 45, or 90 min) after the addition of ERα to HeLa NE, complexes were isolated, washed, and assayed by immunoblotting. In this experiment, a subset of CoRs are recruited to ERα together (left panel), while others display different kinetics of binding (right panel). The boxes indicate the 90 min data with ERα, and this time-point was chosen for subsequent pulldown-mass spectrometric experiments.

(F) Testing the effect of select CoR knockdown on ERα-driven transcription of a luciferase reporter gene in MCF-7 MAR-ERE-LUC cells. (i) Depletion of SRC-3 or FOXO1 by siRNA (50 nM pools for 3 days) reduces endogenous E2-liganded ERα transcriptional activity, whereas depletion of RIP140 by two different siRNAs (20 nM Stealth siRNA) enhances ERα transcriptional activity in these cells. (ii) Depletion of each of the four proteins in the CDK8-module of the Mediator complex by siRNA (50 nM pools for 3 days) reduces endogenous E2-liganded ERα transcriptional activity. E2: 100 nM; siControl #1: ON-TARGETplus Non-targeting Pool; siControl #2: Stealth Negative Control Med GC siRNA; siControl #3: siGENOME Non-Targeting #3 siRNA. Statistical significance was determined by the Student's t test using data from three independent replicates, with mean +/- SEM shown.

Figure S2. Additional Control Experiments for Salt Stability of ERE-ERα-CoR Complexes, Related to Figure 2

(A) Testing the effect of increasing salt (KCl) concentrations on recombinant purified ERα, SRC-3, and/or p300. High salt (2M KCl) disrupts ERα DNA binding (lane 3), but addition of SRC-3 to the ERα-ERE binding reaction enhances ERα DNA binding (compare lanes 6 and 3). Likewise, p300 addition further stabilizes SRC-3 in the ERα-ERE complex (compare lanes 9 and 6).

(B) Testing the effect of increasing salt (KCl) concentrations on CoR-ERα-ERE complexes when SRC-3 is depleted from HeLa NE (HNE). With increasing salt, p300 binding is reduced in control IgG-treated HNE, but lack of SRC-3 leads to substantial further loss of p300 from the complex (compare lanes 5 and 6 versus 2 and 3). Interestingly, FOXO1, like p300, is affected in recruitment to the ERE complexes when SRC-3 is depleted (indicated by box), yet its salt stability is similar without SRC-3, unlike p300.

Figure S3. ChIP Assays for Phosphorylated MED1 Occupancy at the ERE of the Luciferase Reporter as a Function of E2 and DNA-PK Inhibition, Related to Figure 4

(A) As a test of whether any of the DNA-PK mediated phosphorylations identified in the cell-free ERE DNA pulldown assays might occur at an ERE inside cells, ChIP assays were performed for pT1457 MED1 occupancy at the ERE of the luciferase reporter, as indicated in the Supplemental Experimental Procedures. No pT1457 MED1 was detected (u.d.) in ERE-MAR-LUC cells estrogen-deprived for 2 days, but with 100 nM E2 treatment for 45 min, pT1457 MED1 was readily observed. Importantly, when 5 μM KU60648, a DNA-PK selective inhibitor, was given to cells 3.5 hours before E2 addition,

pT1457 MED1 occupancy was abolished. ChIP-qPCR data was analyzed using the ΔΔCt method, with fold enrichment expressed relative to the IgG control as described (Chakrabarti et al., 2002) and error bars representing the SEM.

(B) To control for the selectivity of the pT1457 MED1 ChIP signal at the ERE, another genomic region (*Cyclin D1* or *CCND1*, intron 4) was assayed by qPCR. While pT1457 MED1 did occupy this region with E2, its binding was not affected with pre-treatment with the DNA-PK inhibitor, thereby suggesting the phosphorylation of MED1 at the ERE of the reporter gene was selectively enhanced by DNA-PK. ChIP-qPCR data was analyzed using the ΔΔCt method, with fold enrichment expressed relative to the IgG control as described (Chakrabarti et al., 2002) and error bars representing the SEM.

Figure S4. Effect of Mutating the ERE1 Sequence in the 364 bp *GREB1* Fragment on CoR Complex Formation Using HeLa NE (HNE) and Effect of Phosphorylation on CoRs Bound to *GREB1*, Related to Figure 5A

(A) Effect of mutations in the ERE1 sequence within the 364 bp *GREB1* fragment on CoR complex formation using HNE as the source of CoRs. This figure complements data shown in Figure 5A using MCF-7 NE. (i) Mutating the ERE1 sequence in the *GREB1* fragment impairs formation of stable CoR-ERE complexes, as seen by reduced peptide detection of CoRs from HNE and ERα. MS data was presented in a heatmap format (with color scale defined below) showing fold change of CoRs binding the mutated ERE (mutERE) over the wild-type ERE (wtERE). (ii) Immunoblotting confirms that when the ERE1 is mutated in the *GREB1* fragment less ERα and six CoRs from HNE are recruited in the presence of 100 nM E2.

(B) Phosphorylation activates CoR complexes formed on the 364 bp *GREB1* ERE1 fragment, as seen by enhanced gel mobility shifts of ERα and SRC-3 (bolded), after 0.5 mM ATP addition to complexes. Also note that CDK8, SRC-2, and FOXO1 were dismissed similar as that observed with 4xERE-E4 in Figure 3A. This figure complements data shown in Figure 5B using MCF-7 NE.

Figure S5. Assaying E2-liganded ERα and Select HeLa Cell CoRs Fractionation on Superose 6 Gel Filtration Columns as a Function of Added *GREB1* ERE1 DNA, Related to Figure 5A

(A) Fractionation profile of HeLa NE and E2-liganded ERα without *GREB1* ERE1 DNA addition. See Supplemental Experimental Procedures for more detail. (i) UV280 (protein) absorbance as a function of fraction number. The void fraction is 16 on our column as estimated from the location of blue dextran. The location of where different size standards fractionated is additionally listed (669 kDa, thyroglobulin; 440 kDa, ferritin; 158 kDa, aldolase; and 43 kDa, ovalbumin). (ii) Immunoblotting of select CoRs (DNA-PKcs, p300, SRC-3, and MED26) and ERα shows differential fractionation (*e.g.*, ERα and MED26 are mainly in the void). INP, 1% HeLa NE/ERα reaction input.

(B) Fractionation profile of *GREB1* ERE1 DNA without added protein on the Superose 6 column. UV254 (DNA) absorbance is shown as a function of fraction number. Void and size standards are defined above.

(C) Fractionation profile of HeLa NE and E2-liganded ERα with *GREB1* ERE1 DNA addition. (i) UV280 (protein) absorbance as a function of fraction number. Void and size standards are defined above. Note the new peak occurring right after the void volume. (ii)

Immunoblotting of select CoRs (DNA-PKcs, p300, SRC-3, and MED26) and ERα shows that they co-fractionate together (*e.g.*, see boxed fractions 19 and 21). INP, 1% HeLa NE/ERα reaction input. (iii) PCR analysis of *GREB1* ERE1 DNA fractionation in this experiment. Consistent with Figure S5B, *GREB1* DNA fractionates in both high and low molecular weight fractions, including fractions 19 and 21 that show CoR and ERα co-fractionation. Ladder, 1 Kb Plus DNA ladder (Invitrogen). This figure complements bead-based data for *GREB1* ERE1 shown in Figures 5A and S4 and supports the concept that a very large CoR complex eluting after the void volume is formed by multiple CoRs binding DNA containing a single ERE.

Figure S6. Identification and Validation of Two Chromodomain-Helicase-DNA-Binding Proteins as ERα Transcriptional Coregulators (CoRs), Related to Figure 5D

(A) Validation of CHD4 as a component of the *GREB1* ERE1 complex and coactivator of ERα's transcriptional activity. (i) Immunoblotting confirms that CHD4 is enriched with E2-liganded ERα on the *GREB1* fragment, in addition to SRC-3 and RIP140. HeLa NE was employed in this experiment. This data complements MS data in Figure 5D. (ii) Knockdown of CHD4 by siRNA reduces ERα transcriptional activity on a stably integrated luciferase reporter gene in MCF-7 MAR-ERE-LUC cells. Cells were transfected with 50 nM siRNAs (siGENOME, Dharmacon) for 3 days. Statistical significance was determined by the Student's t test using data from three independent replicates, with mean +/- SEM shown. (iii) Knockdown of CHD4 increases SNAIL protein level, but does not affect ERα, thereby ruling out decreased ERα protein levels as

the cause for reduced ERE-luciferase expression. SNAIL protein is increased with 100 nM E2 overnight treatment and knockdown of CHD4 protein further increases the SNAIL protein level. ERα is reduced upon overnight E2 treatment, but importantly, loss of CHD4 does not affect that level. β-actin serves as a loading control.

(B) Identification of CHD8 as a component of the *GREB1* ERE1 complex and coactivator of ERα's transcriptional activity. (i) By immunoblotting, CHD8 from MCF-7 NE displays E2-enhanced recruitment to ERα bound on the *GREB1* fragment. In addition, SRC-3, RIP140, ASC-2, and ASH2L showed E2-dependent recruitment to the *GREB1* fragment, thereby validating some MS data shown in Figure 5D. (ii) Knockdown of CHD8 by siRNA reduces E2-liganded ERα transcriptional activity in MCF-7 MAR-ERE-LUC cells. CHD8 protein knockdown was confirmed by immunoblotting, using β-actin as a loading control (right panel). All siRNAs (siGENOME, Dharmacon) were transfected at 50 nM for 3 days. Statistical significance was determined by the Student's t test using data from three independent replicates with the mean +/- SEM shown.

(C) Identification of CHD8-dependent CoRs recruited to the *GREB1* ERE1 complex from MCF-7 NE. (i) Immunoblotting shows that CHD8 was substantially depleted from MCF-7 NE. ASH2L was not affected, and β-actin serves as a loading control. (ii) MS identification of CoRs affected by the loss of CHD8 in terms of recruitment to the *GREB1* ERE1. MS data was presented in a heatmap format (with color scale defined as in Figure S4A) showing fold change of CoRs binding the *GREB1* ERE1 as a function of CHD8 depletion. (iii) Immunoblotting confirms that when CHD8 is depleted less BPTF and MLL1 from MCF-7 NE are recruited to the *GREB1* ERE1, while ERα bound levels

were similar. Two different MLL1 antibodies were tested- one to the N-terminus (N), the other to the C-terminus (C). *, likely cross-reaction in the MLL1 (N) blot.

Figure S7. Validation of 'Reader' Binding from HeLa NE to Recombinant Histone-Assembled 4xERE-E4 and Functional Tests by *In Vitro* Transcription after Immunodepletions, Related to Figure 7

(A) Micrococcal nuclease (MNase) digests of unmodified H3.2 (labeled as unmodi), H3K4me3-containing, or H3K9me3-containing recombinant nucleosomes on 4xERE-E4 reveals similar laddering patterns. These experiments were done as in Figure 6Aii, except that the 921 bp fragment was used instead of the pERE plasmid.

(B) Epigenomic 'readers' selectively enriched on H3K4me3 or H3K9me3 in the presence of E2-liganded ERα were identified by MS. (i) Published binding effectors of H3K4me3 that were observed with ERα on EREs include SPIN1, PHF8, CHD1, DIDO1, ING1, and PHF2. (ii) Published binding effectors of H3K9me3 that were observed with ERα on EREs include SFPQ, NONO, and UHRF1. The color scale for the heatmaps shown for fold change of enrichment with H3K4me3 or H3K9me3 is shown in Figure S4A. Note that CoRs in this figure represent a selected list of the total proteins enriched for either mark (see full datasets at epicome.org). (iii) BRD2, CNOT3, and SMARCAD1 from HeLa NE are H3K4me3 and H3K9me3 selective binding proteins in the presence of E2-liganded ERα. By immunoblotting, BRD2 displayed increased binding to H3K4me3-nucleosomes and decreased binding to H3K9me3-nucleosomes, while CNOT3, a subunit of the CCR4-NOT complex, and SMARCAD1 had the opposite recruitment pattern. Like the MS experiment above, histone H3.2 was the source of unmodified H3. Total H3

levels are shown to demonstrate similar nucleosome presence on the ERE-containing beads. *, likely cross-reaction.

(C) Testing the effect of immunodepleting two CoRs enriched on H3K4me3 (CHD1, BRD2) on *in vitro* transcription from H3K4me3-containing recombinant nucleosomes assembled on 4xERE-E4. (i) Efficient removal of CHD1 or BRD2 from HeLa NE was detected by immunoblotting; β-actin serves as a loading control. (ii) *In vitro* transcription of recombinant nucleosomes bearing H3K4me3 was monitored by RT-qPCR assay for E4 mRNA, as detailed in the Supplemental Experimental Procedures. Loss of CHD1 or BRD2 did not significantly affect ERα-driven transcription promoted by the H4K4me3 mark. Data are represented as mean +/- SEM.

(D) Testing the effect of acetyl CoA (AcCoA) on unmodified H3 or H3K4me3-containing recombinant nucleosomes assembled on 4xERE-E4. *In vitro* transcription assays were performed as above, either with or without 9 μM AcCoA. AcCoA addition increased both unmodified and H3K4me3-driven transcription. Data are represented as mean +/- SEM.

(E) Validation of NAT10 and two TAFs (TAF1, TAF3) enrichment on H3K4me3-containing nucleosomal 4xERE-E4 after 0.5 mM ATP treatment. This immunoblotting data complements the MS data shown in Figure 7E.

(F) Confirmation of efficient ADA3 and TAF3 depletion from HeLa NE. Antibodies to ADA3 (left panel) or TAF3 (right panel) successfully reduced the level of these proteins. As expected, ADA3 depletion reduced GCN5, PCAF, and CCDC101 levels. Interestingly, TAF3 depletion reduced the TAF1, but not TAF5, level. This data complements data shown in Figures 7F and 7G.

3) Supplemental Tables

Table S1. List of mass spectrometry experiments, Related to all Figures

EXP no	Cell	Genotype	Fraction	Treat-ment	Extract Amount	Affinity Name	Affinity Amount	Affinity Adjust ment	MS Instrument	Bands
5778	MCF7	WT (+ESR1)	Nuclear Extract	none	0.2 ml	4xERE	15 pmol		BPRC-Orbitrap-Velos-1	9
5779	MCF7	WT (+ESR1)	Nuclear Extract	E2 (100 nM)	0.2 ml	4xERE	15 pmol		BPRC-Orbitrap-Velos-1	12
5905	HeLa S3	WT (+ESR1)	Nuclear Extract	vehicle EtOH	0.2 ml	4xERE	15 pmol		BCM-Orbitrap-Velos-1	3
5907	HeLa S3	WT (+ESR1)	Nuclear Extract	E2 (100 nM)	0.2 ml	4xERE	15 pmol		BCM-Orbitrap-Velos-1	3
6155	MCF7	WT (+ESR1)	Nuclear Extract	none	0.2 ml	ep-GREB1	15 pmol		BCM-Orbitrap-Velos-1	5
6156	MCF7	WT (+ESR1)	Nuclear Extract	E2 (100 nM)	0.2 ml	ep-GREB1	15 pmol		BCM-Orbitrap-Velos-1	5
6257	MCF7	WT	Nuclear Extract	E2 (100 nM)	2 mg	4xERE	15 pmol		BCM-QExactive-1	12
6258	MCF7	WT (+ESR1)	Nuclear Extract	E2 (100 nM)	2 mg	4xERE	15 pmol		BCM-QExactive-1	12
6259	MCF7	WT	Nuclear Extract	E2 (100nM)	2 mg	4xERE	15 pmol	template with HeLa core histones	BCM-QExactive-1	12
6260	MCF7	WT (+ESR1)	Nuclear Extract	E2 (100 nM)	2 mg	4xERE	15 pmol	template with HeLa core histones	BCM-QExactive-1	12
6312	HeLa S3	WT (+ESR1)	Nuclear Extract	E2 (100 nM)	0.2 ml	ep-GREB1	15 pmol		BCM-Orbitrap-Velos-1	6
6313	HeLa S3	WT (+ESR1)	Nuclear Extract	E2 (100 nM)	0.2 ml	ep-GREB1/ mut ERE	15 pmol		BCM-Orbitrap-Velos-1	6
6314	MCF7	WT (+ESR1)	Nuclear Extract	E2 (100nM)	0.2 ml	ep-GREB1	15 pmol		BCM-Orbitrap-Velos-1	6
6315	MCF7	WT (+ESR1)	Nuclear Extract	E2 (100nM)	0.2 ml	ep-GREB1/ mut ERE	15 pmol		BCM-Orbitrap-Velos-1	6
6420	HeLa S3	WT	Nuclear Extract	E2 (100 nM)	3 mg	4xERE	15 pmol	unmodified H2A, H2B, H3, H4	BCM-Orbitrap-Velos-1	6
6421	HeLa S3	WT (+ESR1)	Nuclear Extract	E2 (100 nM)	3 mg	4xERE	15 pmol	unmodified H2A, H2B, H3, H4	BCM-Orbitrap-Velos-1	6

6422	HeLa S3	WT	Nuclear Extract	E2 (100 nM)	3 mg	4xERE	15 pmol	H3K4me3, unmodified H2A, H2B, H4	BCM-Orbitrap-Velos-1	6
6423	HeLa S3	WT (+ESR1)	Nuclear Extract	E2 (100 nM)	3 mg	4xERE	15 pmol	H3K4me3, unmodified H2A, H2B, H4	BCM-Orbitrap-Velos-1	6
6424	HeLa S3	WT	Nuclear Extract	E2 (100 nM)	3 mg	4xERE	15 pmol	H3K9me3, unmodified H2A, H2B, H4	BCM-Orbitrap-Velos-1	6
6425	HeLa S3	WT (+ESR1)	Nuclear Extract	E2 (100 nM)	3 mg	4xERE	15 pmol	H3K9me3, unmodified H2A, H2B, H4	BCM-Orbitrap-Velos-1	6
6640	HeLa S3	WT (+ESR1)	Nuclear Extract	E2 (100 nM)	3 mg	4xERE	15 pmol	unmodified H2A, H2B, H3, H4; treated with 0.5 mM ATP and 18 μM acetyl CoA for 1 hr	BCM-Orbitrap-Velos-1	5
6642	HeLa S3	WT (+ESR1)	Nuclear Extract	E2 (100 nM)	3 mg	4xERE	15 pmol	H3K4me3, unmodified H2A, H2B, H4; treated with 0.5 mM ATP and 18 μM acetyl CoA for 1 hr	BCM-Orbitrap-Velos-1	5
6761	MCF7	WT (+ESR1)	Nuclear Extract	E2 (100 nM)	2 mg	ep-GREB1	16.7 pmol	control IgG depleted NE	BCM-Elite-1	6
6762	MCF7	WT (+ESR1)	Nuclear Extract	E2 (100 nM)	2 mg	ep-GREB1	16.7 pmol	anti-CHD8 depleted NE	BCM-Elite-1	6

Table S2. List of 20 kinases screened for presence in ERE-ERα-CoR complexes, Related to Figures 3 and 4

Kinase Tested by Immunoblotting	**Bound to 4xERE DNA?**	**Enriched with E2-liganded ERα?**
PKA (C-α)	No	No

p38 MAPK	No	No
JNK (SAPK)	No	No
PKCζ	No	No
CDK2	No	No
CK2α	No	No
GSK3β	No	No
IKKα	No	No
ERK1/2	No	No
ERK3	No	No
AMPK2α	No	No
RSK1	No	No
AKT2	No	No
TAF1	Yes	No
TSC2	Yes	No
PLK3	Yes	No
PAK1	Yes	No
CDK7	Yes	No
DNA-PKcs	Yes	No

4) Supplemental Experimental Procedures

Nuclear Extract (NE) Preparation

NE was made from HeLa S3 cells following the standard protocol (Dignam et al., 1983), while extraction of MCF-7 was done according to our published method (Lanz et al., 2010). Protein concentrations were determined by Bradford assays (Bio-Rad), and aliquots were snap-frozen in liquid N2 and stored at -80°C until usage.

Immunodepletions of FOXO1, SRCs, DNA-PKcs, CBP/p300, BRD2, CHD1, ADA3, TAF3, NAT10, and CHD8 from NE

Appropriate antibody (Ab) was pre-bound to 45 µl protein G Sepharose beads (Invitrogen) that were blocked with BSA (Sigma, Fraction V, 1 mg/ml) in Dulbecco's PBS (D-PBS, Invitrogen) for 4 hr to overnight. Beads were washed in D-PBS once, then added to 2-4 mg of NE for 2 hr to overnight incubation at 4°C. Beads were gently

pelleted, and supernatants were incubated with fresh Ab-protein G beads for another round or two of depletion. Immunoblotting confirmed loss of antigen from the NE. Specifics for each antigen are below:

For FOXO1: 20 μg rabbit polyclonal Ab against FOXO1 (Bethyl, A300-297A) or 20 μg rabbit IgG (Bethyl, P120-101) were incubated with 4 mg HNE. Only two rounds of depletion were necessary.

For SRC-1, -2, and -3: 20 μg rabbit polyclonal Ab against SRC-1 (Bethyl, A300-343A), 20 μg rabbit polyclonal Ab against SRC-2 (Bethyl, A300-346A), 20 μg purified rabbit IgG (Bethyl, P120-101), 20 μg goat polyclonal Ab against SRC-3 (Santa Cruz Biotechnology, sc-7216), or 20 μg normal goat IgG (Santa Cruz, sc-2028) were incubated with 4 mg HNE. Only two rounds of depletion were necessary.

For DNA-PKcs: 16 μg mouse anti-DNA-PKcs three mAb mix (Thermo Scientific, MA5-13404) or 16 μg mouse normal IgG (Santa Cruz, sc-2025) were incubated with 2 mg HNE. Three rounds of depletion were necessary due to the abundance of DNA-PKcs in HNE.

For CBP/p300: 10 μg rabbit anti-CBP (sc-369) and 10 μg rabbit anti-p300 (sc-584) or 20 μg normal rabbit IgG (Santa Cruz, sc-2027) were incubated with 3 mg HNE. Two to three rounds of depletion were performed.

For BRD2: 15 μg rabbit anti-BRD2 (Bethyl, A302-582A) or 15 μg rabbit IgG (Bethyl, P120-101) were incubated with 3 mg HNE. Only two rounds of depletion were necessary.

For CHD1: 20 μg mouse anti-CHD1 (Santa Cruz, sc-271626) or 20 μg normal mouse IgG (Santa Cruz, sc-2025) were incubated with 3 mg HNE. Only two rounds of depletion were necessary.

For ADA3: 20 μg rabbit anti-ADA3 (Santa Cruz, sc-9882) or 20 μg normal rabbit IgG (Santa Cruz, sc-2027) were incubated with 3 mg HNE. Only two rounds of depletion were necessary.

For TAF3: 20 μg rabbit anti-TAF3 (Pierce, PA5-26645) or 20 μg normal rabbit IgG (Santa Cruz, sc-2027) were incubated with 3 mg HNE. Three rounds of depletion were necessary.

For NAT10: 20 μg mouse anti-NAT10 (Santa Cruz, sc-271770) or 20 μg normal mouse IgG (Santa Cruz, sc-2025) were incubated with 3 mg HNE. Only two rounds of depletion were necessary.

For CHD8: 20 μg rabbit anti-CHD8 (Bethyl, A301-224A) or 20 μg rabbit IgG (Bethyl, P120-101) were incubated with 4 mg MCF-7 NE. Three rounds of depletion were necessary.

Hormones, Chemicals, and Pharmacological Inhibitors

Water-soluble 17β-estradiol (E2), ethanol-soluble E2, and ethanol-soluble 4-hydroxy-tamoxifen (4-HT) were obtained from Sigma. AMP-PNP was purchased from Roche. NU7441 was purchased from Tocris Bioscience and dissolved in DMSO. KU60648 was obtained from AXON Medchem.

Biotinylated ERE fragments

A 921 bp 4xERE-E4 doubly biotinylated fragment was made by PCR using *Taq* DNA polymerase (Invitrogen), E4BioF/E4BioR primers biotinylated at their 5' ends (see below) and pERE, gift of W. Lee Kraus, as a template. A 364 bp doubly biotinylated fragment from the human *GREB1* gene was made by PCR using pCR4-Topo-GREB or pCR4-Topo-GREB ERE mut as a template. pCR4-Topo-GREB was constructed by cloning a 364 bp PCR fragment made with high-fidelity *Taq* DNA polymerase (Invitrogen) and MCF-7 genomic DNA (ATCC). Primers used for this PCR (without 5'-biotin moieties) and for creating ERE1 mutations are listed below. ERE1 mutations were made using Quik-Change mutagenesis kit (Stratagene) and confirmed by DNA sequencing. To remove any free biotinylated primers after PCR, reactions were passed through PCR Kleen Spin Columns (Bio-Rad).

ERE-DNA Pulldown Assays

60 µl Dynabeads® M280 streptavidin (Invitrogen) are washed once with 500 µl 1 M NaCl, 0.02%Triton X-100, 1 mM EDTA, 10 mM Tris-Cl, pH 7.5, followed by one wash with 500 µl D-PBS. Beads were pelleted with standard magnetic racks (Millipore). We typically bind 4 µg biotinylated 921 bp 4xERE DNA (7.5 pmol) to the Dynabeads in 150 ul D-PBS by rotation for ~1 hr at 4°C. Bead-immobilized DNAs are then washed as above, with the final PBS removed. If nucleosomal templates are employed (see below), only the D-PBS wash is performed after bead immobilization. NE is thawed on ice and clarified by centrifugation (max speed in a microfuge) at 4°C for 10 min. We add NE (1 mg) to resuspend beads, then add 0.5 µg recombinant purified ERα (Invitrogen or EMD) or its storage buffer, appropriate ligand (100 nM E2 or 1 µM 4-HT), and a 1mM

EDTA/EGTA mix. Reactions are incubated with rotation at 4°C for 1.5 hr. Beads are washed using ice-cold buffers (two washes in NETN- 20 mM Tris-Cl, pH7.5, 150 mM NaCl, 1 mM EDTA, 0.5% lgepal CA-630), followed by one wash in D-PBS). PBS is removed and beads are resuspended in 20-30 µl 2x SDS sample buffer (Fermentas or Pierce). After boiling, protein samples are loaded on 4–15% Mini-PROTEAN® TGX™ Precast gels (Bio-Rad) for immunoblotting (see below for the source of Abs) or on NuPage gels (8% Bis-Tris in MOPS buffer, Invitrogen) for preparative separation-gel slice excision for MS.

Additional Information for Peptide Extraction, Data Acquistion, and Peptide Abundance Estimation by LC-MS/MS

After staining proteins in SDS-PAGE gels with Coomassie blue, gel lanes were sliced into different bands (see Table S1) and in-gel digested overnight at 37°C with trypsin. After digestion, peptides were extracted twice in 200 µl of acetonitrile with re-suspension in 20 µl of 2% formic acid prior to second extraction, dried in a Savant SpeedVac, and dissolved in a 5% methanol/0.1% formic acid solution. The samples were loaded through a 2 cm C18 trap followed by 1 hour 0-30% acetonitrile gradients on a 10 cm C18 column (packed in-house with Reprosil-Pur Basic C18 3 µm beads; Dr. Maisch GmbH, Germany) and measured online with the Thermo Orbitrap Velos or QExactive instruments (Thermo Scientific, Germany). The raw data were searched with Proteome Discoverer 1.3 Mascot engine against a human RefSeq database (downloaded on 2011-07-04) using lenient 1%/5% stricted/relaxed PSM (peptide specific matches) FDR (false discovery rate), and the data was further grouped into gene products that were assigned

homology and identification quality groups using an in-house developed algorithm. All protein gene products that were chosen for follow-up in this study were required to have at least one identification where a spectral match passing <1% FDR and >20 ion score or <5% FDR and >30 ion score thresholds was present. The amounts of each gene product was estimated with a label-free intensity-based absolute quantification (iBAQ) approach (as the sum of peptide areas normalized to the theoretical tryptic peptide potential (Schwanhausser et al., 2011)) and reported as a fraction of total protein iBAQ amount per experiment (in 10^{-5} units for visual comprehension).

In Vitro Phosphorylation of CoR-ERα-ERE Complexes

Kinase reactions consisted of 1x kinase buffer (#9802, Cell Signaling Technology) and 0.5 mM ATP (#9804, Cell Signaling Technology)). Phospho-selective antibodies (Abs) employed were as follows: SRC-3 pT24, pS543, and pS857 have been previously described by our laboratory and SRC-3 pT24 was also from Cell Signaling Technology (#2979S); MED1 pT1032 was a gift from Xiaoting Zhang (University of Cincinnati), while pT1457 was purchased from Abcam (ab60950); and ERα pS118 was obtained from Cell Signaling Technology (#2511) and Millipore (05-793). Recombinant SRC-3 was purified from baculoviral-infected *Sf*9 cells as previously described by our laboratory.

Immunoblotting

Protein samples are transferred from SDS-PAGE gels to PVDF (Bio-Rad) in 1x Tris-glycine, 15% methanol for 1 hr at 250-300 mA at 4°C. Ponceau S (Sigma) staining is

performed to access transfer efficiency before blots are cut into appropriate strips of different size ranges. Strips are blocked at RT for 30 min in 5% nonfat milk/1x PBS, followed by primary antibody addition and incubation at RT for several hours or overnight at 4°C. After PBS washes, appropriate secondary antibody-HRP conjugates were added (donkey anti-rabbit, sheep anti-mouse from GE Healthcare; rabbit anti-goat from Santa Cruz) in blocking buffer for 1 hr at RT, followed by additional PBS washes, and signal development (ECL Plus, Pierce) on X-ray film.

Antibodies (Abs) Used For Immunoblotting

CoR and TF Abs: Monoclonal 6D7 against RIP140 was a gift of Malcolm Parker (Herzog et al., 2007). CDK8 (sc-1521), CBP (sc-369), p300 (sc-584), SRC-1 (sc-32789), MED26 (sc-81237), ADA3 (sc-98821), CtBPs (sc-17805), Cyclin C (sc-1061), DNA-PKcs (sc-1552), TBP (sc-273), GCN5 (sc-20698), PCAF (sc-13124), TAF5 (sc-374644), and ERα (sc-543) were purchased from Santa Cruz Biotechnology. FOXO1 (A300-297A), MED1 (A300-793A), MED12 (A300-774A), MED13 (A301-278A), ASC2 (A300-411A), ASH2L (A300-489A), HDAC1 (A300-712A), CHD4 (A301-082A), BPTF (A300-973A), MLL1 (N) (A300-086A), MLL1 (C) (A300-374A) were purchased from Bethyl Laboratories. SRC-2 (610985), SRC-3 (611104), and SMRT (611386) were purchased from BD Biosciences. CHD8 (7656) was obtained from Cell Signaling Technology. SNAIL (61368) was bought from Active Motif.

Histone 'mark' selective Abs: H3K4me3 (39159), H3K9me3 (39285), H3K9Ac (39917), H3K14Ac (39697), H3K18Ac (39755), and H3K27Ac (39133) were purchased from

Active Motif, whereas total H3 was from Novus (NB500-171). Importantly, the H3K9Ac Ab did not cross-react with the H3K4me3 protein (data not shown).

Kinase Abs (Table S2): PKA (C-α) (Cell Signaling, 4782), p38 MAPK (Cell Signaling, 9212), JNK/SAPK (Cell Signaling, 9258), PKCζ (Millipore, 07-264), CDK2 (Cell Signaling, 2546), CK2α (Cell Signaling, 2656), TAF1 (Santa Cruz, sc-735), GSK3β (Bethyl, A302–048A), IKKα (Bethyl, A301-933A), ERK1/2 (Cell Signaling, 9102), ERK3 (Cell Signaling, 4067), CDK7 (Bethyl, A300-405A), AMPK2α (Bethyl, A300-508A-2), PAK1 (Abcam, 40795), RSK1 (Bethyl, A302-459A), AKT2 (Bethyl, A302-209A), TSC2 (Bethyl, A300-463A), and PLK3 (Cell Signaling, 9785).

H3K4me3 or H3K9me3 binding protein Abs: BRD2 (A302-582A), CNOT3 (A302-156A), and SMARCAD1 (A301-593A) were purchased from Bethyl Laboratories. CCDC101 (also called Sgf29, ab103879) was acquired from Abcam. PHF2 (3497) was obtained from Cell Signaling Technology. UHRF1 antibody was purchased from Abcam (ab57083).

Additional Plasmids Used to Make Biotinylated Templates

pIE-0, a gift of W. Lee Kraus, only contains the minimal Adenovirus *E4* promoter and coding sequence (Pazin et al., 1994).

Additional Biotinylated DNA Fragments Used in Pulldown Assays

a) 4xERE: 433 bp dsDNA made by PCR using *Taq* DNA polymerase, pERE, E4BioF and XbaBioR biotinylated primers

b) E4 pro: 773 bp dsDNA made by PCR using *Taq* DNA polymerase, pIE-O, and biotinylated E4BioF and E4BioR primers.

Restriction Enzyme Digestion of ERE Complexes

After ERα-ERE-CoR complexes were formed from HNE, they were washed as indicated above, then resupended in 20 μl reactions, containing 1x restriction digestion buffer and 3 μl of each enzyme (all four enzymes shown in Figure S1C were from New England Biolabs), and digestion occurred at 37°C for 1 hour. Afterwards, 40% of supernatants (*i.e.*, the released proteins) and final beads were assayed by immunoblotting.

Recombinant ERα-SRC-3-p300-ERE Complexes

Binding reactions were performed as listed above for HNE with the same 4xERE-E4 amount. However, no HNE was added, and where indicated in Figure S2A, 0.476 μg ERα (7.2 pmol), 100 nM E2, 0.575 μg SRC-3 (3.6 pmol, purified as described in Experimental Procedures), or 0.6 μg p300 (1.8 pmol, purchased from ProteinOne, P2004-01) were added together in HNE dialysis buffer for a total volume of 65 μl. After 1.5 hr incubation, washes were performed as indicated above, and complexes were challenged by addition of 30 μl of different KCl concentrations at room temperature for 20 min. After supernatant removal, beads were assayed by immunoblotting.

siRNA Transient Transfections and ERE-Luciferase Reporter Analysis

MCF-7 MAR-ERE-LUC cells (described in (Jiang et al., 2006)) were plated in 6-well dishes at ~$3x10^5$ in stripped media (high-glucose, phenol-red free DMEM, 5% charcoal-

stripped FCS, 1% penicillin/streptomycin) and transfected in triplicate with targeting siRNA or a non-targeting siRNA at a final concentration listed in figure legends using Trans-IT-TKO (Mirus Corp.) for roughly 3 days. E2 (100 nM) was added to cells after the second day.

Sources of siRNA are as follows: siGENOME Non-Targeting siRNA #3 (Dharmacon, D-001210-03-05), siGENOME SMARTpool PRKDC (Dharmacon, M-005030-01-0005), and DNA-PKcs siRNA pool (h) (Santa Cruz, sc-35200). ON-TARGETplus Non-targeting Pool (Dharmacon, D-001810-10-05), ON-TARGETplus SMARTpool NCOA3 (Dharmacon, L-003759-00-0005), Stealth Negative Control Med GC (Invitrogen, 12935-300), Stealth NRIP1 #1 (Invitrogen, HSS112045), Stealth NRIP1 #2 (Invitrogen, HSS112046), siGENOME SMARTpool FOXO1 (Dharmacon, M-003006-03-0005), siGENOME SMARTpool CDK8 (Dharmacon, M-003242-02-0005), Cyclin C siRNA pool (h) (Santa Cruz, sc-35132), siGENOME SMARTpool MED12 (Dharmacon, M-009092-01-0005), siGENOME SMARTpool MED13 (Dharmacon, M-019908-01-0005), siGENOME SMARTpool CHD4 (Dharmacon, M-009774-01-0005), and siGENOME SMARTpool CHD8 (Dharmacon, M-030271-02-0005).

Checking CoR knockdown: To confirm efficiency of siRNA-mediated knockdown, 100 mm dishes of cells were transfected in parallel in scaled-up reactions, and after 3 days, protein was extracted with RIPA buffer (Sigma) containing 1x complete mini protease inhibitors (Roche) and subjected to immunoblotting, with β-actin (Sigma, A5441) serving as the loading control.

Luciferase assays: Cells were harvested 3 days post-transfection in 200 µl CCLR lysis buffer (Promega), and luciferase activity was determined relative to protein level

(assayed by Bradford assays) using a Luciferase Reporter Assay (Promega) and a Berthold 96 well plate reader.

Chromatin Immunoprecipitation (ChIP) of the ERE of the LUC Reporter in MCF-7 Cells

ChIP assays were performed according to an EZ ChIP kit (Millipore, 17-371). Briefly, cells in 15 cm dishes were estrogen-deprived for 2 days by incubation in stripped media, followed 3.5 hour pre-treatment with or without 5 μM KU60648, a DNA-PK selective inhibitor, and with/without 100 nM E2 treatment for 45 min before crosslinking in 1% formaldehyde (Sigma, F8775) in D-PBS for ten minutes and quenching with 125 mM glycine. After chromatin was sheared by sonication using a Branson Sonifier 250, it was precleared with control IgG and protein G agarose beads (Millipore), and then equal volume aliquots were taken out for overnight IP with either normal rabbit IgG (Santa Cruz, sc-2027) or rabbit anti-pT1457 MED1 (Abcam), followed by low salt, high salt, LiCl, and TE washes. DNA fragments were eluted from beads in 1% SDS-100 mM $NaHCO_3$ followed by crosslink reversal and spin-column cleanup. Two μl of purified DNAs were used in qPCR reactions using SYBR green (Applied Biosystems, 4309155) and 200 nM primers on a StepOnePlus machine (Applied Biosystems) with melt curves confirming single species amplicons (data not shown). The sequences of the primers used to amplify 92 bp containing the ERE upstream of the luciferase reporter were as follows: For, 5'- ttttctgcatagcctctttga- 3'; Rev, 5'- gctttgtccaggttgaggtt- 3'. The *CCND1* intron 4 amplicon has been described (Eeckhoute et al., 2006).

ChIP of the ERE1 of *GREB1* in MCF-7 Cells

Cells were either untreated, treated with 100 nM E2 for 45 min, or pre-treated with 1 μM KU60648 for 3.5 hours prior to E2 treatment, followed by crosslinking per the protocol of Active Motif's ChIP-IT® High Sensitivity kit. Chromatin was sheared by sonication using UCD300 Bioruptor (Diagenode), and equal amounts were used for overnight IPs with pS118ERα, pT1457 MED1, CtBPs, and respective control IgGs. The precipitated DNAs were reverse-crosslinked and purified with spin columns (Qiagen), and 3 μl of purified DNA was added to a 20 μl qPCR reaction using the SensiFast SYBR kit (BioLine). The qPCR primers flanking the *GREB1* ERE1 were as follows: For, 5'-GTGGCAACTGGGTCATTCTGA- 3'; Rev, 5'- CGACCCACAGAAATGAAAAGG-3'.

Superose 6 Chromatography

HNE was spun at 100,000xg after thawing for better clarification before application to the Superose 6 column. To 3.2 mg of clarified HNE, 5.8 μg doubly biotinylated 364 bp *GREB1* ERE1 DNA (24 pmol ERE) or water was added, followed by 2 μg recombinant ERα (30 pmol or 60 nM), 100 nM E2, and 1 mM EDTA/EGTA for 3 hours at 4°C with rotation in a final volume of 500 μl. These reactions were then applied to a Superose 6 size exclusion column (10/300 GL, GE Healthcare) that had been equilibrated with buffer D (20 mM HEPES, pH 7.9, 10% glycerol, 0.1 M KCl, 0.2 mM EDTA, 0.5 mM PMSF, and 0.5 mM DTT) using an ÄKTA FPLC (GE Healthcare). Buffer D was chosen to run the column as ERα-CoR complexes from MCF-7 NE were nicely fractionated under this buffer condition (Shi et al., 2011). The column was calibrated with the protein standards listed in Figure S5

legend. The column eluted at a flow rate of 0.5 ml/min, fractions were collected, and every other one was analyzed by immunoblotting (as shown in Figure S5) after TCA precipitation of 90% of each fraction using a kit from Sigma (PROT-PR) and 5 μg chicken ovalbumin as a carrier (Sigma, A2512). One μl from each fraction was added to a 20 μl PCR reaction using *Taq* DNA polymerase and BioGrF/BioGrR primers (listed below) to detect the 364 bp *GREB1* ERE1 DNA. Half of each PCR reaction was run on a 1% agarose gel stained with ethidium bromide.

Chromatin Assembly, Histones, and Micrococcal Nuclease (MNase) Analysis

A recombinant *Drosophila* ACF1/ISWI/NAP-1 assembly system (Ito et al., 1999) was used for chromatin assembly onto EREs. FLAG-tagged ACF1/ISWI and His-tagged NAP-1 recombinant proteins were purified from baculovirus-infected *Sf*9 cells as described (Ito et al., 1999). HeLa core histones were purchased from Active Motif. Recombinant histones were purchased from either Active Motif (*Xenopus* H2A, H2B, H3 (C110A), H3K4me3, H3K9me3, and H4) or from New England Biolabs (human H2A, H2B, H3.2, and H4) as indicated in the figure legends. Recombinant *Xenopus* H2A, H2B, H3 (C110A), H3K4me3, H3K9me3, and H4 were resuspended in 10 mM Hepes (pH 7.6), 10 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM EGTA, 10% glycerol, 10 mM β-glycerol phosphate, 1 mM DTT, and 0.2 mM PMSF. Active Motif generates methylated histones using a patented chemical alkylation reaction to introduce a methyl-lysine analog (MLA) at the desired lysine location for precise control over the site and degree of methylation (see text for citation). Nucleosome assembly reactions (100 μl final volume) were setup in siliconized tubes and contained 1 μg ERE DNA, 3 μg NAP-1, 2.7 μg HeLa core

histones or 0.675 μg each recombinant H2A, H2B, H3, and H4, and 1.6 μg ACF1/ISWI in 30 mM KCl, 1x ATP regeneration system (30 mM phosphocreatine, 3 mM ATP, 3 mM $MgCl_2$, 1 mM DTT, 0.5 μg creatine phosphokinase), 6.1 mM Hepes (pH 7.6), 6.1 mM KCl, 0.9 mM $MgCl_2$, 0.3 mM EGTA, 6.1% glycerol, 6.1 mM β-glycerol phosphate, 0.6 mM DTT, and 0.12 mM PMSF. NAP-1 and histones were first pre-incubated together on ice for 15 min, followed by addition of ACF1/ISWI, 10x ATP regeneration system, and DNA, which were further incubated at 27°C for 4 hours and then stored at 4°C overnight. After assembly, quality of nucleosome assembly was checked by MNase partial digestion using the Chromatin Assembly Kit from Active Motif, followed by agarose gel electrophoresis and ethidium bromide staining.

In Vitro Transcription Reactions and RT-qPCR

To first allow pre-initiation RNA pol II complexes to form, the following were mixed together- 1x reaction buffer (15 mM Hepes, pH8.0, 0.15 mM EDTA, 60 mM KCl, 4 mM $MgCl_2$, 2.5% (w/v) polyvinyl alcohol (M_r 10000, Sigma), 1 mM ATP, 4 units RNase inhibitor (Invitrogen), nucleosomes (10 μl, 0.1 μg of DNA), 60 ng ERα, 100 nM E2, 9 μM acetyl-CoA, and 6 μl HeLa NE and incubated at room temperature for 25 min. After this period, rNTP mix (0.625 mM each) was added to initiate transcription. Final reaction volumes were typically 45 μl, and reactions were incubated at 30°C for 50 min before the resultant E4 RNA was purified the using the Tri-reagent (Molecular Reagent Center, Inc.) following the manufacturer's instructions. Turbo-DNA free reagent (Invitrogen) was used to digest residual DNA in the purified RNA samples. E4 mRNA was quantified by RT-qPCR as follows. 8% of purified RNA (3 μl) was used for RT-qPCR analysis, using the

following primers directed to the E4 cDNA (For, 5'-CCGTGTCGAGTGGTGTTTT-3'; Rev, 5'-AACAACATACAGCGCTTCCA-3', producing a 91 bp amplicon), and SensiFast One-step SYBR Master Mix (Bioline) was used for qPCR reactions. For RT-qPCR analysis, we used the sample predicted to give the highest level of E4 mRNA (i.e., reaction with E2, ERα, and AcCoA) to generate a standard curve, and the relative quantification of E4 mRNA from all IVT reactions was determined by the same standard curve. Each experiment was performed at least two times. GraphPad InStat software was used for the statistical analysis.

Primers Used for Cloning, PCR, and Mutagenesis

Primer	**Sequence (5'->3')**	**Use**
XbaBioR	biotin- GGACTGGGGATCCTCTAGA	Making 433 bp biotinylated 4xERE without E4 promoter by PCR
E4BioF	biotin-GATGACCCTGCTGATTGGTT	Making 433 bp 4xERE with XbaBioR or 921 bp biotinylated 4xERE-E4 by PCR with E4BioR
E4BioR	biotin-AACCGTATTACCGCCTTTGA	Making 921 bp 4xERE-E4
BioGrF	biotin-CTTGATCAGGGCTTGCTTCT	Making 364 bp biotinylated *GREB1* fragments by PCR
BioGrR	biotin-CTAGTGGGGACAAGCACACA	same
ERE1mutF	GTGAAAAAAAGTGTGGCAACTGGcaCATTCTGtgCTAGAAGCAACCAAAATACTTCTG	Mutating ERE1 in *GREB1*, with mutations in small caps
ERE1mutR	CAGAAGTATTTTGGTTGCTTCTAGcaCAGAATGtgCCAGTTGCCACACTTTTTTTCAC	same

5) Supplemental References

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