The Polycomb protein RING1B enables estrogen-mediated gene expression by promoting enhancer-promoter interaction and R-loop formation

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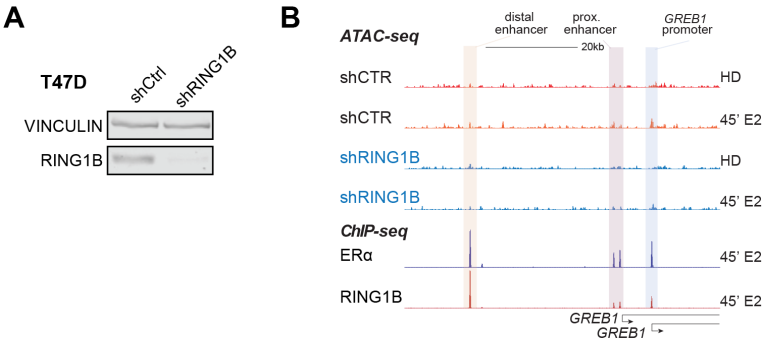
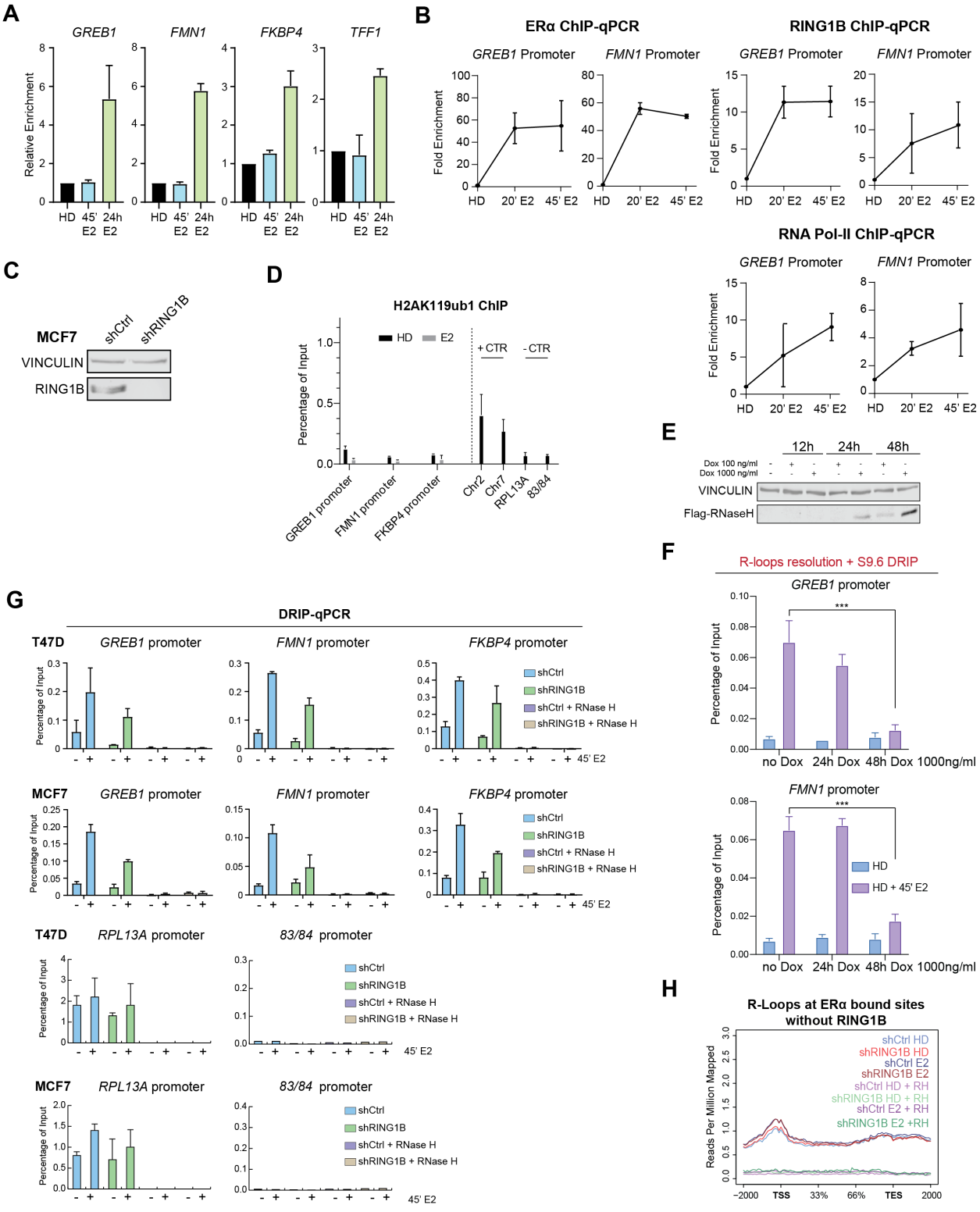
Supplementary Figures

Figure S1



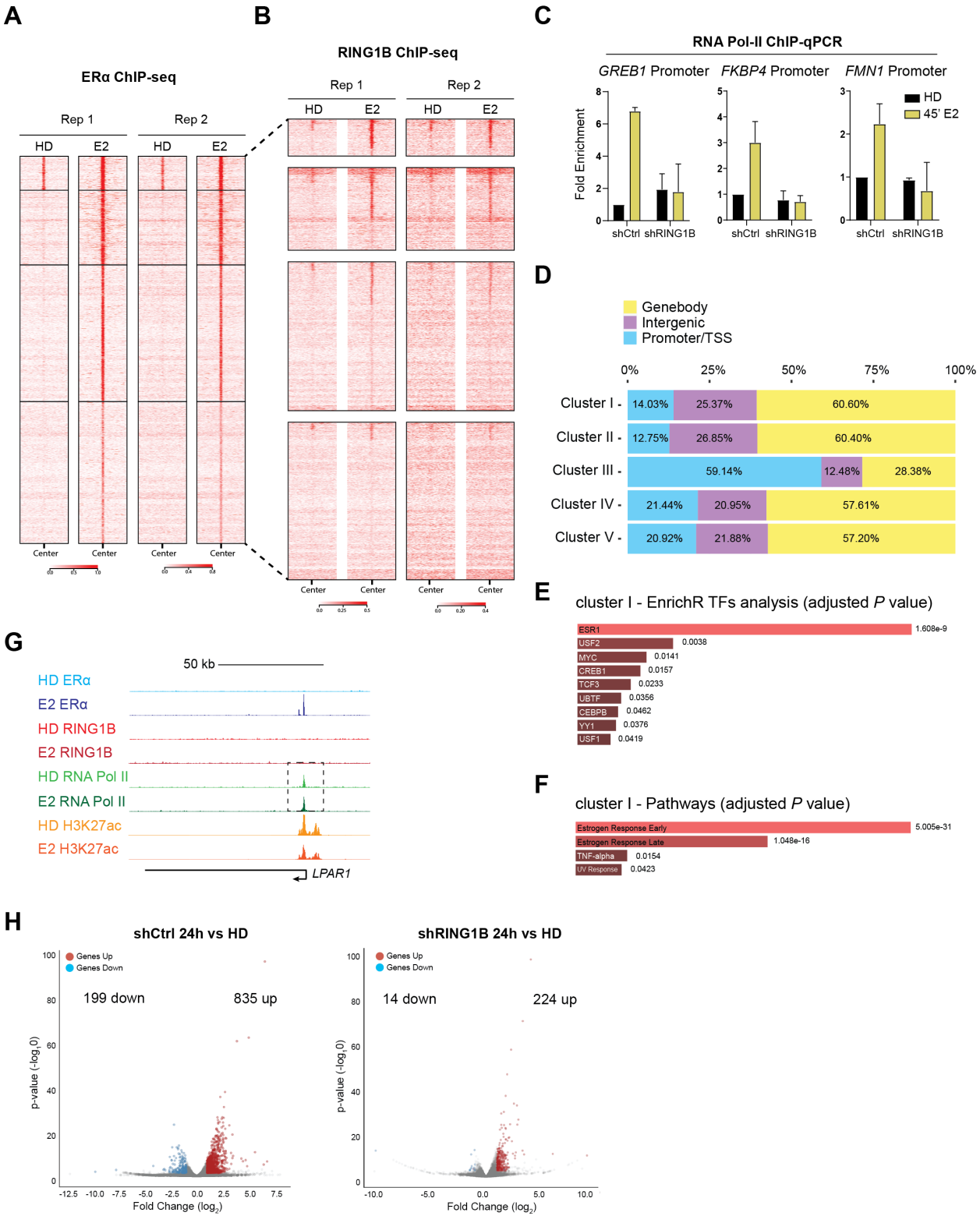
Supplementary Figure S1. **(A)** Western blot of RING1B and VINCULIN (loading control) in control and RING1B-depleted T47D cells. **(B)** ATAC-seq signal in control and RING1B-depleted T47D cells before and after 45min of E2. The three H3K27ac-enriched sites bound by both RING1B and ERα are highlighted and labeled as distal enhancer, proximal enhancer, and *GREB1* promoter.

Figure S2


A
GREB1
FMN1
FKBP4
TFF1
Relative Enrichment
HD
45' E2
24h E2
B
ERα ChIP-qPCR
RING1B ChIP-qPCR
GREB1 Promoter
FMN1 Promoter
Fold Enrichment
HD
20' E2
45' E2
RNA Pol-II ChIP-qPCR
C
MCF7
shCtrl
shRING1B
VINCULIN
RING1B
D
H2AK119ub1 ChIP
HD
E2
+ CTR
- CTR
Percentage of Input
GREB1 promoter
FMN1 promoter
FKBP4 promoter
Chr2
Chr7
RPL13A
83/84
E
12h
24h
48h
Dox 100 ng/ml
Dox 1000 ng/ml
VINCULIN
Flag-RNaseH
F
R-loops resolution + S9.6 DRIP
GREB1 promoter
FMN1 promoter
Percentage of Input
***
no Dox
24h Dox
48h Dox 1000ng/ml
HD
HD + 45' E2
G
DRIP-qPCR
T47D
MCF7
GREB1 promoter
FMN1 promoter
FKBP4 promoter
RPL13A promoter
83/84 promoter
shCtrl
shRING1B
shCtrl + RNase H
shRING1B + RNase H
45' E2
H
R-Loops at ERα bound sites without RING1B
Reads Per Million Mapped
shCtrl HD
shRING1B HD
shCtrl E2
shRING1B E2
shCtrl HD + RH
shRING1B HD + RH
shCtrl E2 + RH
shRING1B E2 +RH
−2000
TSS
33%
66%
TES
2000

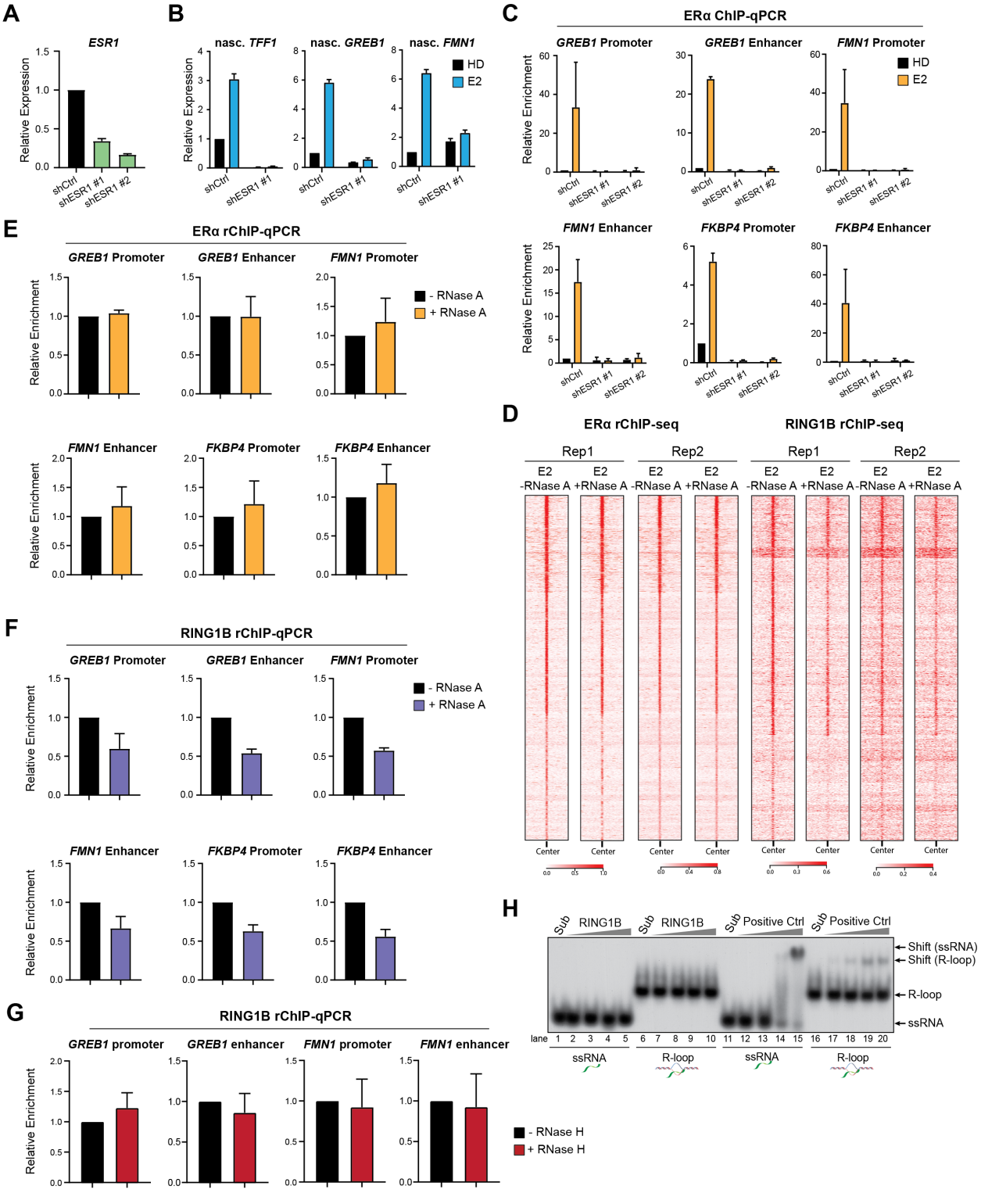
Supplementary Figure S2. **(A)** RT-qPCR of *GREB1*, *FMN1*, *FKBP4*, and *TFF1* in T47D parental cells before and after 45min and 24h of E2 stimulation. Error bars represent the standard deviation of two independent replicates. **(B)** ERα, RING1B, and RNA Pol-II ChIP-qPCR in T47D parental cells before and after 20min and 45min of E2 at RING1B and ERα co-regulated sites within the *GREB1* and *FMN1* promoters. Error bars represent the standard deviation of two independent replicates. **(C)** Western blot of RING1B and VINCULIN (loading control) in control and RING1B-depleted MCF7 cells. **(D)** H2AK119ub1 ChIP-qPCR before and after 45min of E2 administration at promoters co-occupied by RING1B and ERα. Two regions in chromosomes 2 and 7 were used as a positive control (4) and *RPL13A* and *83/84* were used as negative controls. **(E)** Flag WB in dox-inducible Flag-RNase H T47D cells treated with 100ng/ml or 1000ng/ml of dox for 12h, 24h and 48h. VINCULIN was used as a loading control. **(F)** DRIP-qPCR of RING1B/ERα co-targets before and after 45min of E2 induction in the presence of no dox or dox (1000ng/ml) for 24h and 48h. *** p-value < 0.001, ** p-value < 0.005, two-tailed t-test. **(G)** DRIP-qPCR in T47D and MCF7 shCTR and shRING1B cells before and after 45min of E2 addition at ERα and RING1B co-bound sites within the promoters of ERα target genes. No signal was detected in samples treated with RNase H, demonstrating that the detected enrichment is specific for R-loops. *RPL13A* and the *83/84* locus were used as positive and negative controls, respectively. Error bars represent the standard deviation of two biological replicates. **(H)** Average DRIP-seq signal at ERα target genes not occupied by RING1B.

Figure S3


A
ERα ChIP-seq
Rep 1
Rep 2
HD
E2
Center
B
RING1B ChIP-seq
C
RNA Pol-II ChIP-qPCR
GREB1 Promoter
FKBP4 Promoter
FMN1 Promoter
Fold Enrichment
HD
45' E2
shCtrl
shRING1B
D
Genebody
Intergenic
Promoter/TSS
0%
25%
50%
75%
100%
Cluster I - 14.03% 25.37% 60.60%
Cluster II - 12.75% 26.85% 60.40%
Cluster III - 59.14% 12.48% 28.38%
Cluster IV - 21.44% 20.95% 57.61%
Cluster V - 20.92% 21.88% 57.20%
E
cluster I - EnrichR TFs analysis (adjusted P value)
ESR1 1.608e-9
USF2 0.0038
MYC 0.0141
CREB1 0.0157
TCF3 0.0233
UBTF 0.0356
CEBPB 0.0462
YY1 0.0376
USF1 0.0419
F
cluster I - Pathways (adjusted P value)
Estrogen Response Early 5.005e-31
Estrogen Response Late 1.048e-16
TNF-alpha 0.0154
UV Response 0.0423
G
50 kb
HD ERα
E2 ERα
HD RING1B
E2 RING1B
HD RNA Pol II
E2 RNA Pol II
HD H3K27ac
E2 H3K27ac
LPAR1
H
shCtrl 24h vs HD
Genes Up
Genes Down
199 down
835 up
p-value (-log10)
Fold Change (log2)
shRING1B 24h vs HD
14 down
224 up

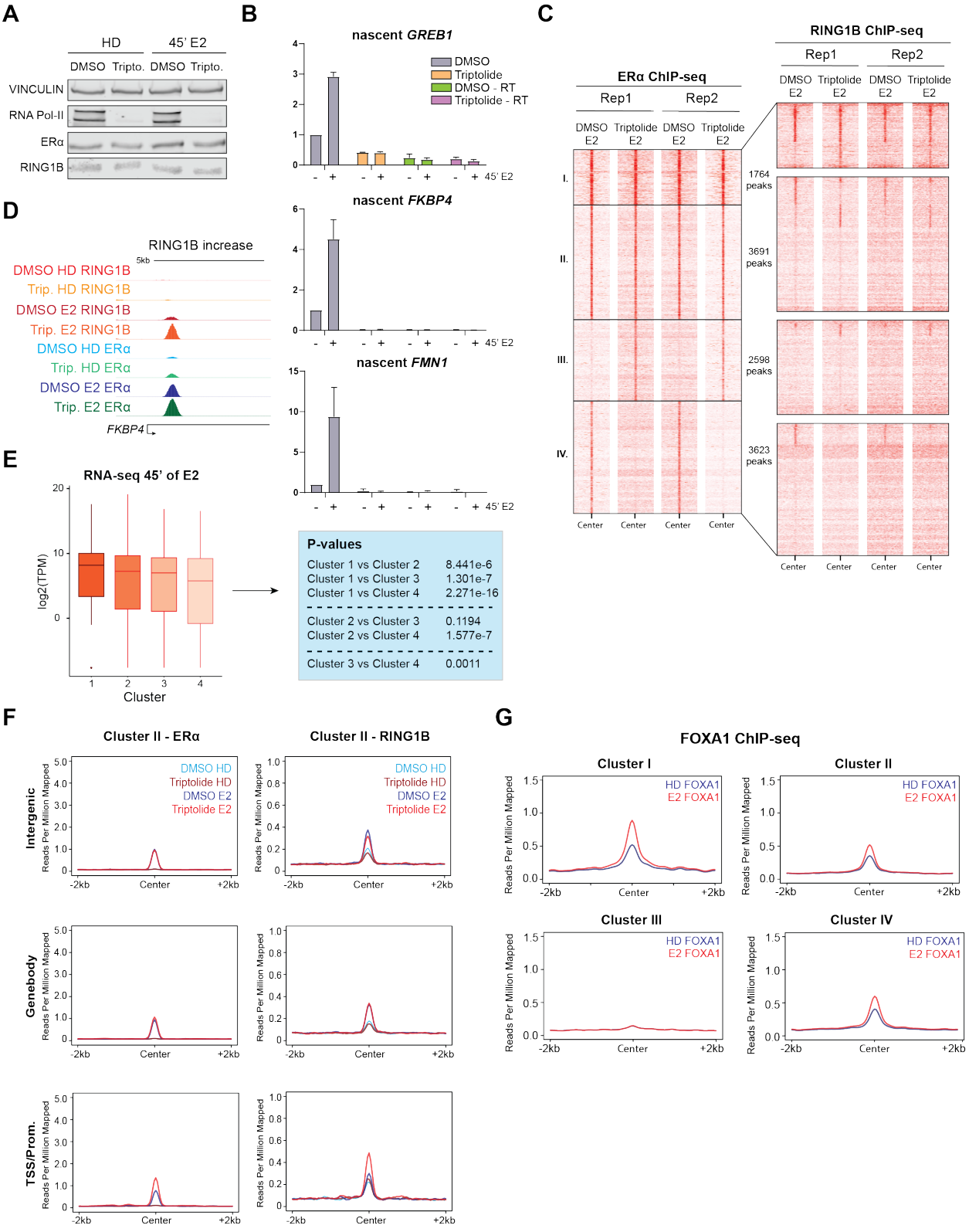
Supplementary Figure S3. **(A-B)** Heatmap of each ERα (A) or RING1B (B) ChIP-seq replicate before and after 45min of E2 organized in order of the 4 clusters from Figure 3A. **(C)** RNA Pol-II ChIP-qPCR in shCTR and shRING1B T47D cells before and after 45min of E2 at RING1B and ERα co-regulated sites. Error bars represent the standard deviation of two independent replicates. **(D)** Genomic annotation of RNA Pol II peaks within each cluster from Figure 3C. **(E-F)** EnrichR TF analysis (E) and EnrichR pathway analysis (F) of genes assigned to cluster I RNA Pol II peaks. **(G)** ERα, RING1B, RNA Pol II, and H3K27ac signal before and after 45min of E2 at *LPAR1*, which is regulated only by ERα. **(H)** Volcano plots (adjusted *P* value) of deregulated genes in T47D-shCTR and shRING1B cells after 24 hours of E2.

Figure S4


A
ESR1
Relative Expression
shCtrl
shESR1 #1
shESR1 #2
B
nasc. TFF1
nasc. GREB1
nasc. FMN1
HD
E2
C
ERα ChIP-qPCR
GREB1 Promoter
GREB1 Enhancer
FMN1 Promoter
FMN1 Enhancer
FKBP4 Promoter
FKBP4 Enhancer
Relative Enrichment
E
ERα rChIP-qPCR
- RNase A
+ RNase A
D
ERα rChIP-seq
RING1B rChIP-seq
Rep1
Rep2
E2
-RNase A
+RNase A
Center
F
RING1B rChIP-qPCR
G
RING1B rChIP-qPCR
GREB1 promoter
GREB1 enhancer
FMN1 promoter
FMN1 enhancer
- RNase H
+ RNase H
H
Sub
RING1B
Positive Ctrl
Shift (ssRNA)
Shift (R-loop)
R-loop
ssRNA
lane
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Supplementary Figure S4. (A) RT-qPCR of *ESR1* in T47D shCTR and two shESR1 cell lines, showing ~66% ERα depletion in shESR1-1 and ~85% depletion in shESR1 #1. Error bars represent the standard deviation of two independent replicates. **(B)** RT-qPCR of nascent *TFF1*, *GREB1*, and *FMN1* transcripts in T47D shCTR and shESR1-1 cells before and after 45min of E2 stimulation. Error bars represent the standard deviation of two independent replicates. **(C)** ERα ChIP-qPCR in control and ERα-depleted T47D cells before and after 45min of E2 stimulation at RING1B and ERα co-targets. Error bars represent the standard deviation of two independent replicates. **(D)** Heatmaps of each rChIP-seq replicate of ERα (left) and RING1B (right) after 45min of E2 stimulation. **(E-F)** rChIP-qPCR of ERα (E) and RING1B (F) after 45min of E2 stimulation with and without RNase A treatment. Error bars represent the standard deviation of two independent replicates. **(G)** rChIP-qPCR of RING1B after 45min of E2 stimulation with and without prior RNase H treatment of chromatin. Error bars represent the standard deviation of two independent replicates. **(H)** Electrophoretic mobility shift assay (EMSA) using ssRNA and R-loops demonstrate a shift in ssRNA and R-loop migration when incubated with a positive control but not when incubated with RING1B.

Figure S5


A
HD
45' E2
DMSO Tripto. DMSO Tripto.
VINCULIN
RNA Pol-II
ERα
RING1B
B
nascent GREB1
DMSO
Triptolide
DMSO - RT
Triptolide - RT
- + - + - + - + 45' E2
nascent FKBP4
nascent FMN1
C
ERα ChIP-seq
Rep1
Rep2
DMSO E2
Triptolide E2
RING1B ChIP-seq
I.
II.
III.
IV.
1764 peaks
3691 peaks
2598 peaks
3623 peaks
Center
D
RING1B increase
5kb
DMSO HD RING1B
Trip. HD RING1B
DMSO E2 RING1B
Trip. E2 RING1B
DMSO HD ERα
Trip. HD ERα
DMSO E2 ERα
Trip. E2 ERα
FKBP4
E
RNA-seq 45' of E2
log2(TPM)
Cluster
P-values
Cluster 1 vs Cluster 2 8.441e-6
Cluster 1 vs Cluster 3 1.301e-7
Cluster 1 vs Cluster 4 2.271e-16
Cluster 2 vs Cluster 3 0.1194
Cluster 2 vs Cluster 4 1.577e-7
Cluster 3 vs Cluster 4 0.0011
F
Cluster II - ERα
Cluster II - RING1B
Intergenic
Genebody
TSS/Prom.
Reads Per Million Mapped
DMSO HD
Triptolide HD
DMSO E2
Triptolide E2
-2kb
Center
+2kb
G
FOXA1 ChIP-seq
Cluster I
Cluster II
Cluster III
Cluster IV
HD FOXA1
E2 FOXA1

Supplementary Figure S5. (A-B) Western blot (A) of RNA Pol II, ERα, RING1B, and VINCULIN (loading control) and RT-qPCR (B) in T47D cells treated with vehicle (DMSO) or triptolide, before and after 45min of E2. Error bars represent the standard deviation from two biological replicates. **(C)** Heatmap of both replicates of ERα (left) and RING1B (right) ChIP-seq in cells treated with vehicle (DMSO) or triptolide upon 45 min of E2 organized in order of the ERα clusters from Figure 4F. **(D)** RING1B and ERα ChIP-seq before and after 45min of E2 in cells treated with DMSO or triptolide demonstrating increased ERα and RING1B recruitment at the *FKBP4* promoter following triptolide treatment. **(E)** Boxplot depicting TPM values of genes assigned to each cluster from Figure 4F (left) with comparisons between the 4 clusters (right). **(F)** Average profiles showing the levels of ERα and RING1B chromatin binding at cluster 2 ERα sites, classified by genomic annotation, before and after 45 min of E2 in cells treated with DMSO or triptolide. **(G)** FOXA1 ChIP-seq signal at each of the four ERα clusters before and after 45min of E2 treatment.