

Figure S1. Two groups of ER α -bound enhancers that respond differentially to E2 were confirmed in separate data sets. Related to Figure 1.

- (A). Workflow of identifying ER α -bound enhancers that produce eRNAs differentially expressed upon E2 treatment.
 (B). ER α binding intensities at E2-downregulated (Down) and E2-upregulated (Up) enhancer regions in the presence of vehicle (Veh.) or E2 using an independent ChIP-Seq data (GSE24166) (Tsai et al., 2010).
 (C). Scatter plot showing the log₂[fold change] (log₂FC) of expression of the defined eRNAs under E2 and vehicle treatment conditions (E2/Veh.) in an independent GRO-Seq data (GSE59532) (Franco et al., 2015).

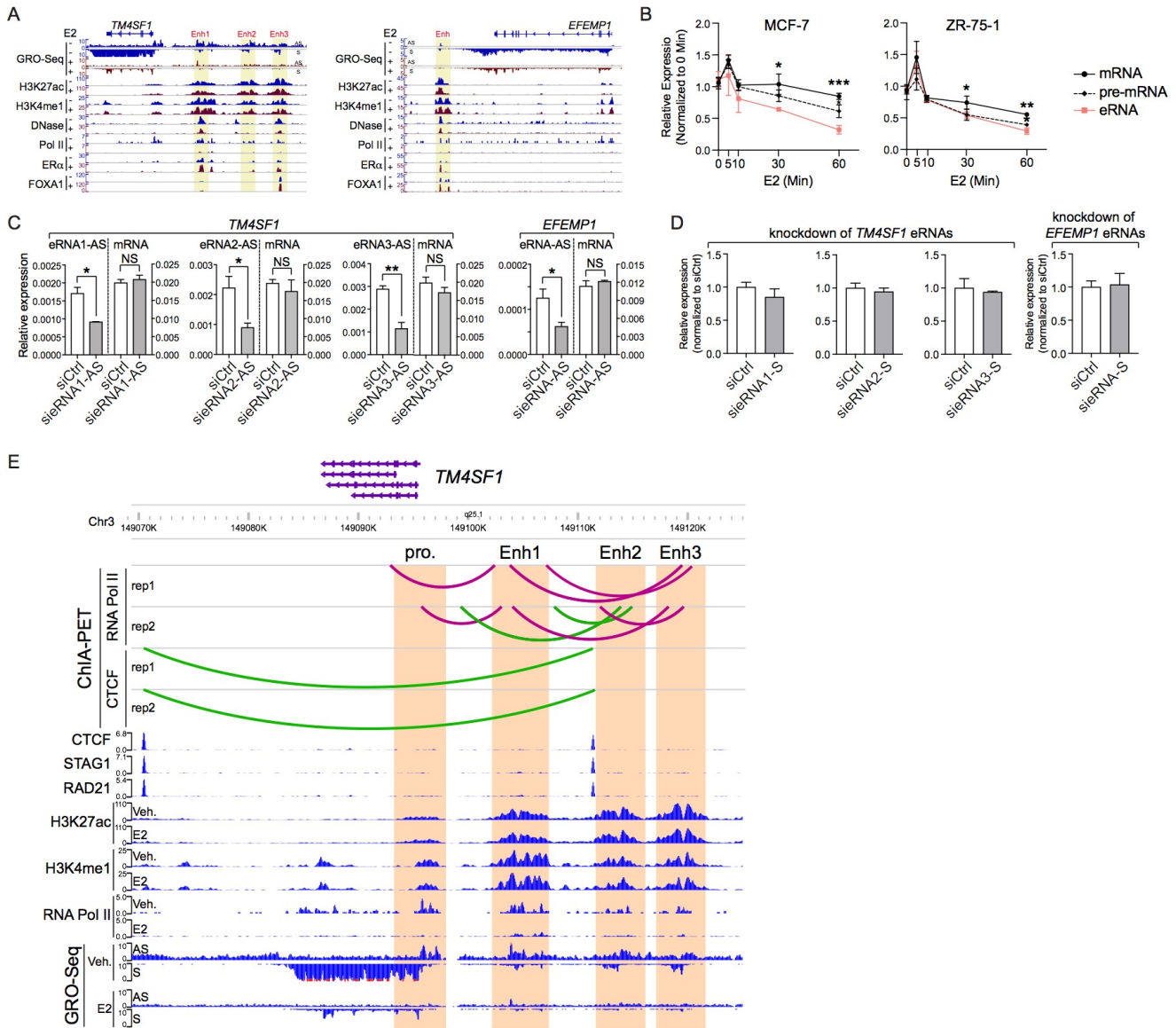


Figure S2. E2-repressed eRNAs control the expression of cognate mRNAs in a specific manner. Related to Figure 2.

(A). The Integrative Genomics Viewer (IGV) of GRO-Seq, ChIP-Seq and DNase-Seq (DNase) at genomic loci of *TM4SF1* and *EFEMP1*. Sense (S)- and antisense (AS)-strand information was indicated. Enhancers of *TM4SF1* (Enh1-3) and *EFEMP1* (Enh) were highlighted. The genome-wide sequencing data sets included in this figure are: GSE45822 (GRO-Seq) (Li et al., 2013), GSE45822 (H3K27ac) (Li et al., 2013), GSE40129 (H3K4me1) (Theodorou et al., 2013), GSE33216 (DNase) (He et al., 2012), GSE45822 (Pol II) (Li et al., 2013), E-TABM-828 (ER α) (Schmidt et al., 2010) and GSE26831 (FOXA1) (Kong et al., 2011).

(B). Expression of mRNA, pre-mRNA and eRNA of *EFEMP1* in MCF-7 and ZR-75-1 cells at different time points upon 100 nM E2 treatment. The forward primer for detecting *EFEMP1* mRNA is aligned across the junction between exon 1 and 2, and the reverse primer targets sequence in exon 3. Both primers for detecting pre-mRNA *EFEMP1* are located in intron 2.

(C). Expression of antisense (AS)-strand eRNA and mRNA of *TM4SF1* and *EFEMP1* in MCF-7 cells after knocking down the specified antisense (AS)-strand eRNAs. Data were normalized to mRNA levels of *GAPDH*.

(D). Expression of *COMMD2* mRNA upon knockdown of sense (S)-strand eRNAs of *TM4SF1* and *EFEMP1*. Data were normalized to conditions with control siRNA (siCtrl) transfection.

Data in (B)-(D) are presented as mean \pm SD with three biological and technical replicates. *P*-values in (B) and (C) were calculated by Student's *t*-test. *, *P* < 0.05; **, *P* < 0.001; ***, *P* < 0.005; NS, not significant.

(E). The chromatin structure around *TM4SF1* gene is specially organized so that the enhancers have distinct functions.

The Integrative Genomics Viewer (IGV) of ChIA-PET, ChIP-Seq and GRO-Seq at genomic locus of *TM4SF1* gene. Sense (S)- and antisense (AS)-strand information was indicated. Data that were generated under vehicle (Veh.) or estradiol (E2) treatment condition were also specified. Promoter (pro.) and enhancers of *TM4SF1* (Enh1-3) were highlighted. Pol II or CTCF-mediated chromatin interactions that involve *TM4SF1* Enh2 were marked in green, otherwise in red. The genome-wide sequencing data sets included in this figure are: GSE33664 (Pol II ChIA-PET) (Li et al., 2012), GSE39495 (CTCF ChIA-PET) (Consortium, 2012), E-TABM-828 (CTCF, STAG1 and RAD21 ChIP-Seq) (Schmidt et al., 2010), GSE45822 (H3K27ac) (Li et al., 2013), GSE40129 (H3K4me1) (Theodorou et al., 2013), GSE45822 (Pol II) (Li et al., 2013), GSE45822 (GRO-Seq) (Li et al., 2013).

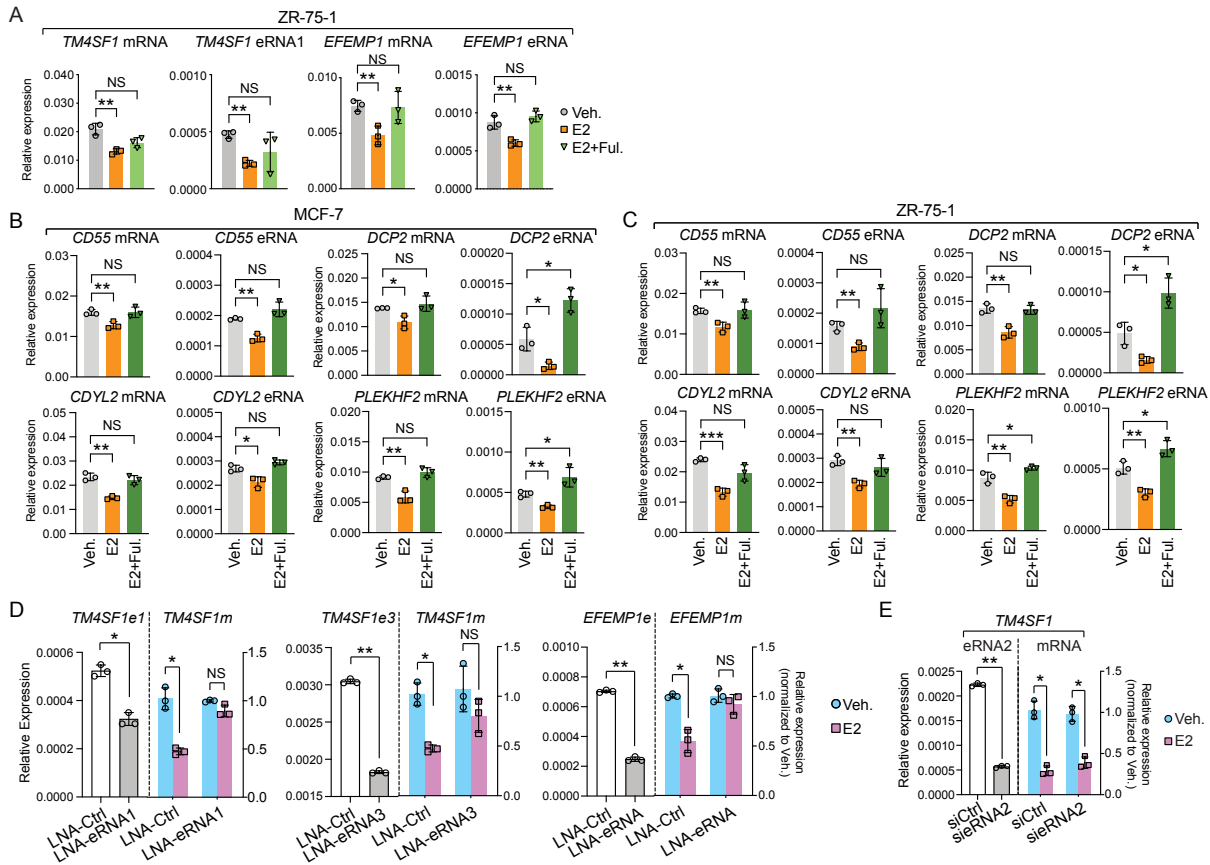


Figure S3. ER α signaling is essential for E2-induced transcriptional silencing of target eRNAs and associated mRNAs. Related to Figure 2.

(A). Expression of mRNA and eRNA of *TM4SF1* and *EFEMP1* in ZR-75-1 cells upon treatment with ethanol (Veh., gray bars), 1 nM estradiol (E2, orange bars) for 3 hrs, or 1 nM estradiol and 1 μ M fulvestrant for 3 hrs (E2+Ful., green bars). Data were normalized to mRNA levels of *GAPDH*.

(B and C). Expression of eRNA and mRNA of four extra E2-repressed genes upon the treatment as described in (A) in MCF-7 (B) and ZR-75-1 (C) cells. Data were normalized to mRNA levels of *GAPDH*.

*, $P < 0.05$; **, $P < 0.001$; NS, not significant.

(D and E). E2-repressed eRNAs are required for E2-induced transcriptional silencing. Expression of eRNA (indicated by the white and gray bars) and mRNA (represented by the blue and light pink bars) of *TM4SF1* and *EFEMP1* upon knockdown of specified eRNAs using locked nucleic acid (LNA) probes (D) or siRNA (E) in the absence (Veh.) or presence (E2) of 100 nM estradiol for 3 hrs. Data were normalized to mRNA levels of *GAPDH*. *, $P < 0.01$; **, $P < 0.001$; NS, not significant.

Data are presented as mean \pm SD with three biological and technical replicates. Statistical significance was calculated by Student's t-test.

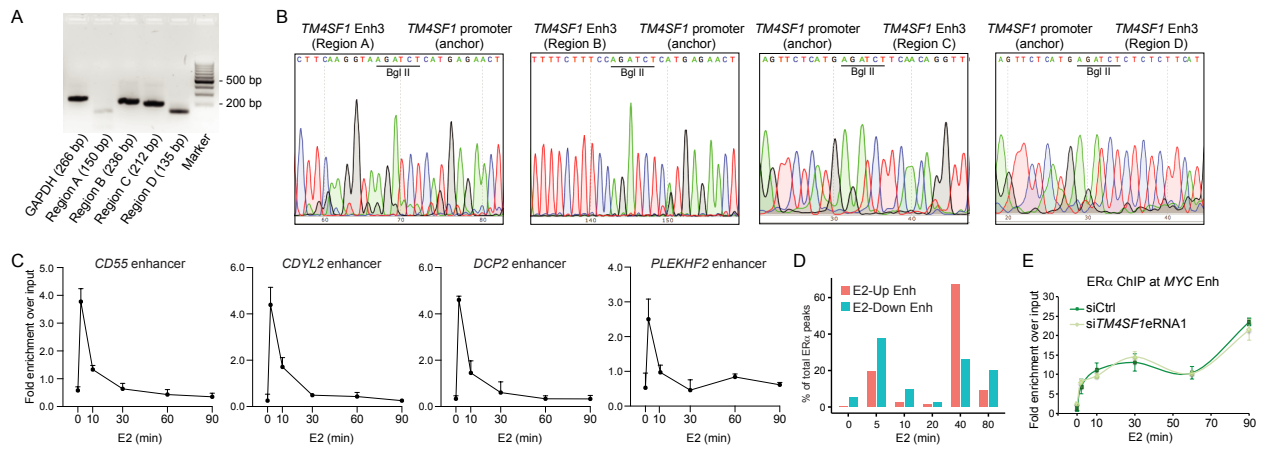


Figure S4. E2-repressed eRNAs play important roles in maintaining functional chromatin environment. Related to Figure 3.

- (A). Agarose gel image showing PCR products of 3C ligation assays.
- (B). Sanger sequencing results of 3C ligation products between *TM4SF1* promoter and different regions around *TM4SF1* Enh3.
- (C). ER α binding at the enhancer regions of indicated genes in MCF-7 cells treated with 100 nM E2 for indicated lengths of time.
- (D). Bar plot showing percentages of E2-upregulated (E2-Up Enh) and E2-downregulated enhancers (E2-Down Enh) that show detectable ER α binding intensities at different time points after E2 treatment in a separate ER α ChIP-Seq data (GSE62789) (Honkela et al., 2015).
- (E). ER α -targeted ChIP at *MYC* enhancer (*MYC* Enh) after knocking down *TM4SF1* eRNA1 at different time points upon 100 nM E2 treatment in MCF-7 cells.
- Data in (C) and (E) are presented as mean \pm SD with three biological and technical replicates.

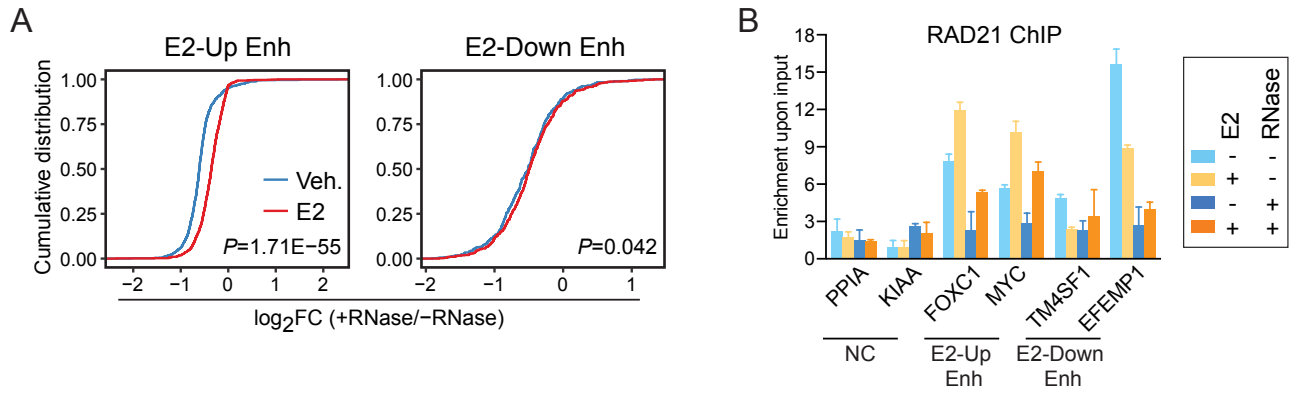


Figure S5. E2-induced ER α binding at E2-downregulated enhancers is particularly dependent on RNA integrity. Related to Figure 4.

(A). Cumulative distribution of ER α binding intensity at E2-upregulated (E2-Up Enh) and E2-downregulated enhancers (E2-Down Enh) before (-) and after (+) RNase treatment in the absence (Veh.) or presence of E2. The fold change of ER α peak intensities was log₂-transformed (log₂FC).

(B). RAD21-targeted ChIP at E2-upregulated (E2-Up Enh) or E2-downregulated enhancers (E2-Down Enh) in MCF-7 cells. Cells were treated with (+) or without (-) 100 nM E2 for 30 min in the presence or absence of RNase. NC, negative controls using promoters of *PPIA* and *KIAA0066* as binding sites. Data are presented as mean + SD with three biological and technical replicates.

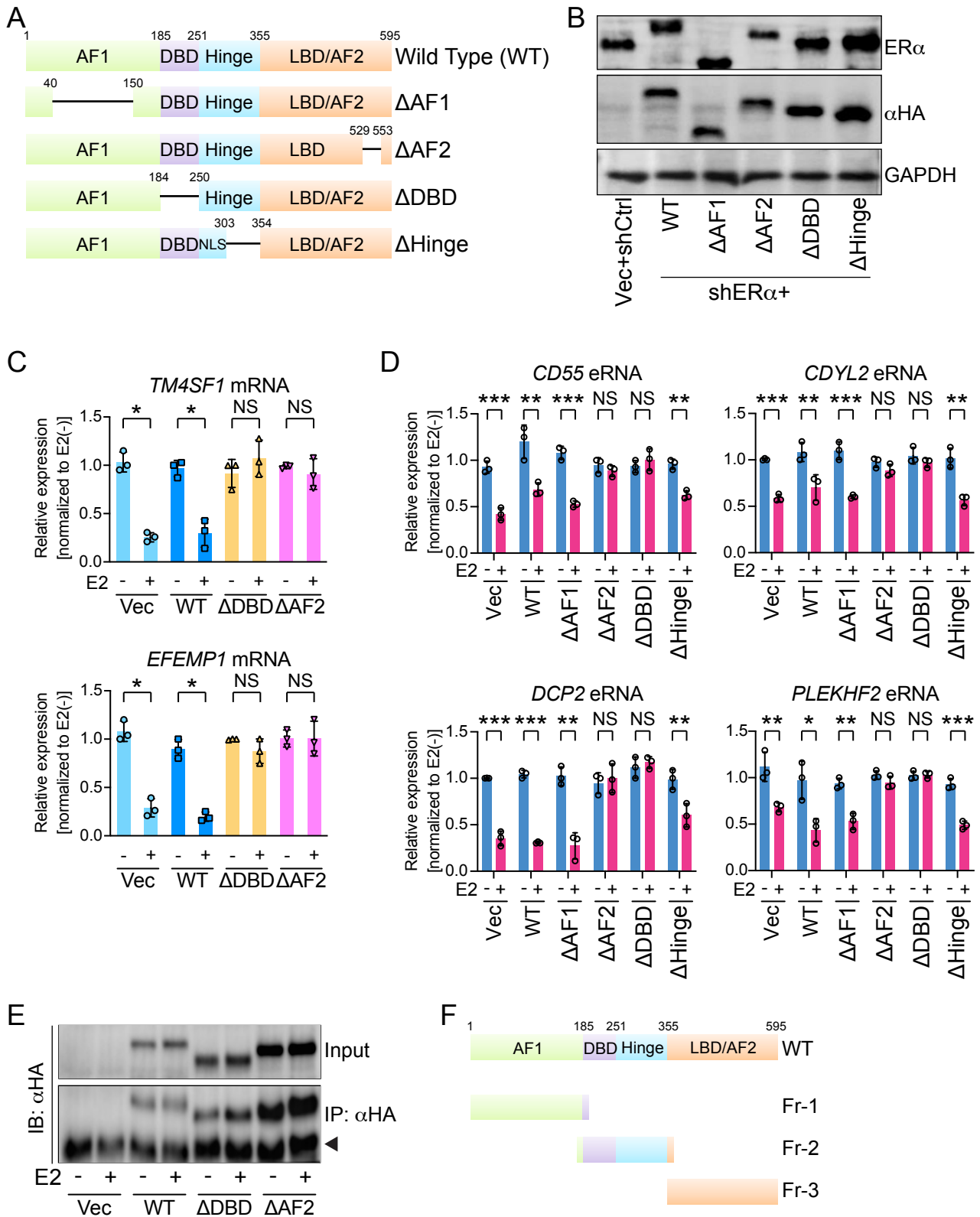


Figure S6. DNA-binding domain (DBD) of ER α plays an essential role in binding with E2-repressed eRNAs. Related to Figure 5.

(A and B). Schematic diagram illustrating the protein structures of wild-type ER α and various truncations (A) and their protein levels after replacing the endogenous ER α in MCF-7 cells (B). Δ AF1, deletion of activation function 1; Δ AF2, deletion of activation function 2; Δ DBD, deletion of DNA-binding domain; Δ Hinge, deletion of hinge region; LBD, ligand-binding domain; NLS, nuclear localization signal. The corresponding residues of each domain or deleted parts are numbered.

(C and D). Expression of *TM4SF1* and *EFEMP1* mRNAs (C) or levels of mRNAs of four additional genes (D) in MCF-7 cells expressing empty vector (Vec), wild-type ER α (WT) or its truncated mutants in the absence (-) or presence (+) of 100 nM E2 for 3 hrs. Data were normalized to corresponding vehicle (-E2) condition. p values denoted differences between Veh and E2 (n=3; Student's t-test).

(E). Immunoprecipitation (IP) efficiency of RIP assay using anti-HA beads in MCF-7 cells. The replacement system was the same as illustrated in (A and B), and cells were treated with 100 nM E2 treatment for 5 min. Arrowhead, IgG heavy chain.

(F). Schematic diagram of full-length ER α (WT) and various GST-tagged fragments (Fr-1, -2 and -3). The amino acid numbers of each protein domain were shown.

Data are presented as mean \pm SD with three biological and technical replicates. P-values were calculated by Student's t-test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant.

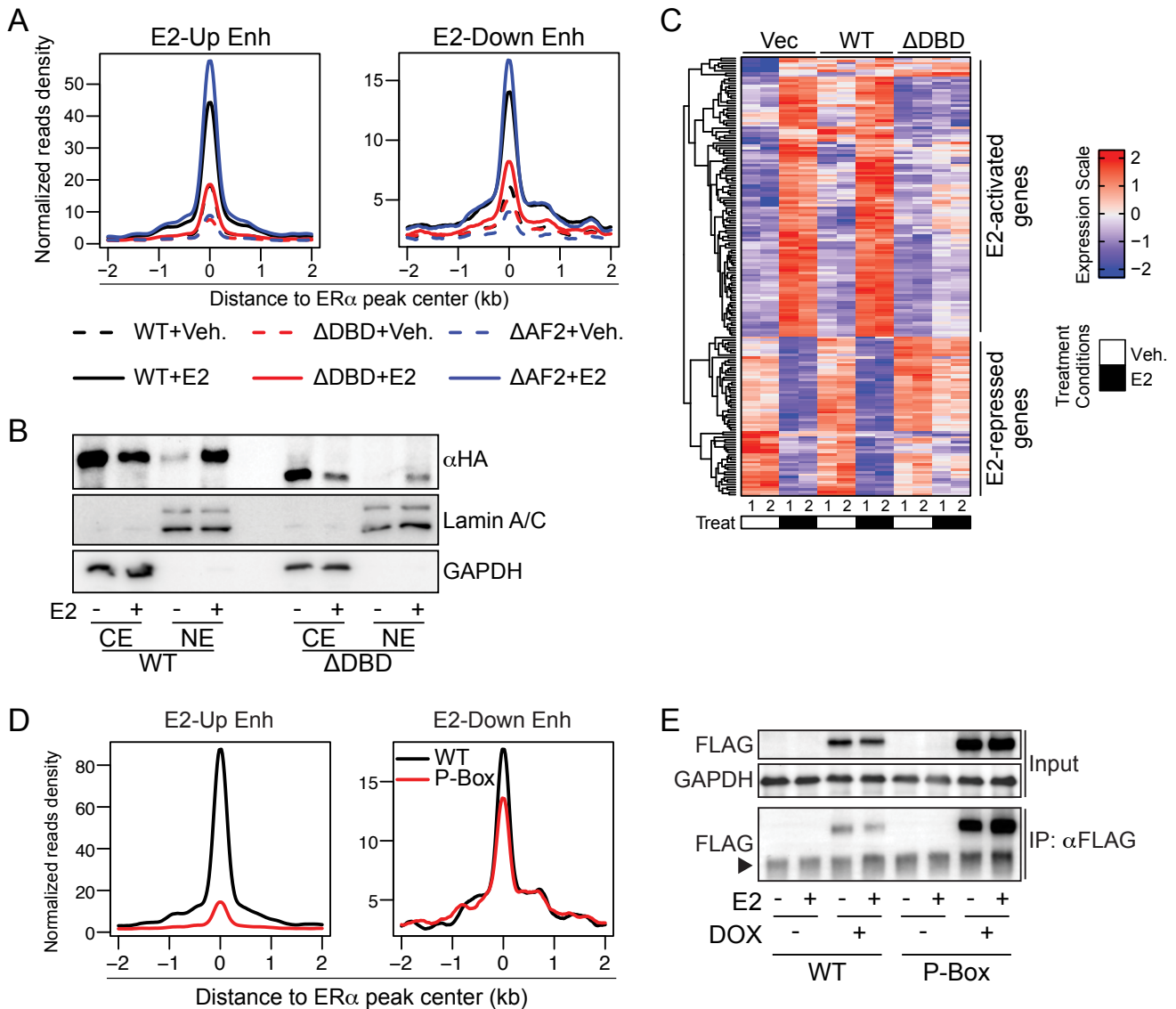


Figure S7. DNA-binding domain (DBD) of ER α is required for indirect chromatin binding of ER α at E2-downregulated enhancers, which is mediated by E2-repressed eRNAs. Related to Figure 5.

(A). Aggregate plots of ER α peak signals at E2-upregulated (E2-Up Enh) or E2-downregulated enhancers (E2-Down Enh) in anti-HA-targeted ChIP-Seq in MCF-7 cells treated with 100 nM E2 for 30 min. WT, wild type; Δ DBD, DNA-binding domain deletion; Δ AF2, activation function 2 deletion.

(B). Cytoplasmic extracts (CE) and nuclear extracts (NE) of MCF-7 cells expressing wild-type ER α (WT) or DBD-deletion mutant (Δ DBD) in the absence (-) or presence (+) of 100 nM E2 for 30 min.

(C). Heat map of E2-dependent expression of E2-activated and E2-repressed coding genes in MCF-7 cells expressing control vector (Vec), wild-type ER α (WT) or DBD-deletion mutant (Δ DBD). Cells were treated with 100 nM E2 for 6 hrs.

(D). Aggregate plot of binding signals of wild-type ER α (WT) or P-Box mutant (P-Box) at E2-upregulated (E2-Up Enh) and E2-downregulated enhancers (E2-Down Enh) in MCF-7 cells that were kept in regular culturing medium.

(E). Immunoprecipitation (IP) efficiency of RIP assay using anti-FLAG antibody in MCF-7 cells expressing inducible wild-type ER α (WT) or P-Box mutant (P-Box). Cells were treated with 100 nM E2 for 5 min and proteins were induced by 2 μ g/ml doxycycline (DOX) overnight. Arrowhead, IgG band.

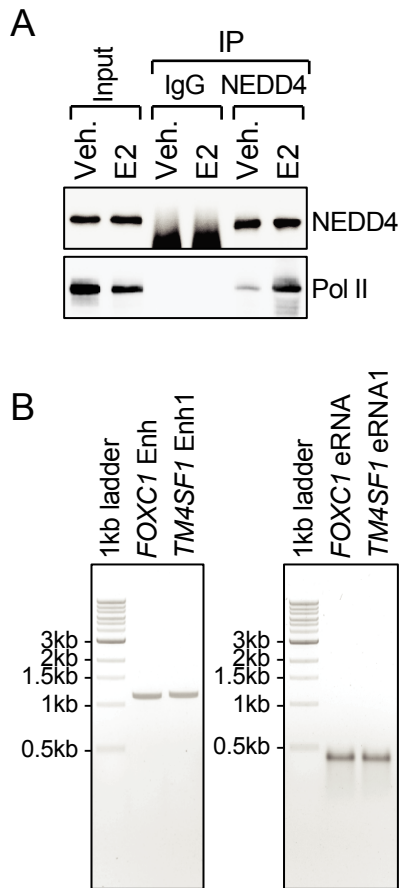


Figure S8. E2-repressed eRNAs assist ER α in its interaction with KDM2A, which leads to association between RNA Pol II and NEDD4. Related to Figure 6.

(A). Immunoprecipitation (IP) of NEDD4 in MCF-7 cells treated with vehicle (Veh.) or 100 nM E2 for 3 hrs.

(B). Agarose gel image showing PCR products that were amplified to contain enhancer regions of *FOXC1* (*FOXC1* Enh) and *TM4SF1* (*TM4SF1* Enh1) (left panel) or *in vitro* transcribed eRNAs of *FOXC1* (*FOXC1* eRNA) and *TM4SF1* (*TM4SF1* eRNA1) (right panel). 1 kb DNA ladder was marked.

Supplemental Tables

Table S1. siRNA and LNA sequences. Related to Figure 2, Figure S2, and Figure S3.

Names	5'-3' Sequences
siTM4SF1 eRNA1-AS_F	CAGAAAGGGAGUUGAGAUUUU
siTM4SF1 eRNA1-AS_R	AAUCUCAACUCCCUUUCUGUU
siTM4SF1 eRNA1-S_F	UGAGAGUGCUGAAGUCAUAUU
siTM4SF1 eRNA1-S_R	UAUGACUUCAGCACUCUCAUU
siTM4SF1 eRNA2-AS_F	GGAGGGGCAUGGAGGUUAAUU
siTM4SF1 eRNA2-AS_R	UUAACCUCCAUGCCCCUCCUU
siTM4SF1 eRNA2-S_F	UCCCAGAGUCCGCCGUAAAUU
siTM4SF1 eRNA2-S_R	UUUACGGCGGACUCUGGGAUU
siTM4SF1 eRNA3-AS_F	UGGUAGAAGUCAUGAGAUUUU
siTM4SF1 eRNA3-AS_R	AAUCUCAUGACUUCUACCAUU
siTM4SF1 eRNA3-S_F	GGUAGGAACUGCAGACUUUUU
siTM4SF1 eRNA3-S_R	AAAGUCUGCAGUCCUACCUU
siEFEMP1 eRNA-AS_F	GGGCUAAAUGCCUCCAAAAUU
siEFEMP1 eRNA-AS_R	UUUUGGAGGCAUUAAGCCCUU
siEFEMP1 eRNA-S_F	GGACAGAGUAGAGGAGAUUUU
siEFEMP1 eRNA-S_R	UAUCUCCUCUACUCUGUCCUU
LNA-TM4SF1-eRNA1	AATTAAAAAGTGTCGT
LNA-TM4SF1-eRNA3	CTGACTAGGTAATGCT
LNA-EFEMP1-eRNA	TTAGTGGTGTGCGAGTG
LNA-negative control	AACACGTCTATACGC

Table S2. Primers for RT-qPCR and RIP-qPCR. Related to Figure 2, Figure 3, Figure 4, Figure 5, Figure 6, Figure S2, Figure S3, and Figure S6.

Gene Names	5'-3' Sequences
GAPDH_F	TGCACCACCAACTGCTTAGC
GAPDH_R	GGCATGGACTGTGGTCATGAG
TM4SF1_F	CACAATGTGCTTGGGTTTCAG
TM4SF1_R	TATGTCTTGATTCCCTCGGC
EFEMP1_F	GCCGCACAGGTATTTTTGCT
EFEMP1_R	TGTCCTGTGACTTGACCAGC
COMMD2_F	TGCTGGTTTAATCTGTTGCCT
COMMD2_R	CGATTCTGAGTGAATTGGCA
TM4SF1-premRNA_F	TACACCTTTGCCAGCACTGA
TM4SF1premRNA_R	AAACACTGGCTAGGTGGGTG
EFEMP1-premRNA_F	CCTGGACTCCTACCACATGC
EFEMP1-premRNA_R	AGGCCACTTACTTGAGAATGC
TM4SF1_eRNA1-S_F	TCTATGACTTCAGCACTCTCAAAGA
TM4SF1_eRNA1-S_R	CTTGGAGAATGCTCACACTGC
TM4SF1_eRNA1-AS_F	AGTTGTTCACTATGGGAACCC
TM4SF1_eRNA1-AS_R	ACAGTCATTTGGCCAGGCTA
TM4SF1_eRNA2-S_F	TCTCACTCAACAACAACTGGC
TM4SF1_eRNA2-S_R	ATTTTTACGGCGGACTCTGG
TM4SF1_eRNA2-AS_F	CTTAACGCCAAGCTCACAGG
TM4SF1_eRNA2-AS_R	CAGACCAGAGATGCACCTCA
TM4SF1_eRNA3-S_F	GTCAGGTAGGAACTGCAGACT
TM4SF1_eRNA3-S_R	ACCAAACTATAGCCTCATCCA
TM4SF1_eRNA3-AS_F	AGCAGTAGCCAGTTTCAGCA
TM4SF1_eRNA3-AS_R	CCTGTTCGGTGTA AAAAGGCC
EFEMP1_eRNA-S_F	AGGAATTTCCAGCAGCTTCA
EFEMP1_eRNA-S_R	CCACCCAGAATCCTGCAGTA
EFEMP1_eRNA-AS_F	TGGAGGACTGGGCTTAATGC
EFEMP1_eRNA-AS_R	GAGGGAGATGTGGTCACCAA
PGLYRP2_eRNA_F	GCATACCGATCACCGAATTGA
PGLYRP2_eRNA_R	TCCTGGCAATGATCGTCAAC
ZDHHC22_eRNA_F	TGCAGCTCCTTGCATCTT
ZDHHC22_eRNA_R	AAGCAGCTTCTGCCTTGA
SEMA3G_eRNA_F	CACTCAGGGTGACCTTCTTTC
SEMA3G_eRNA_R	CTGTGTATTGTACTAAATGGACTGATG
KCNC1_eRNA_F	CAGAGTGAGAGTAGCCTGTAGA
KCNC1_eRNA_R	GTAGTGTCCCGTTGGCATAG
SYT8_eRNA_F	GAGGATGATCTATGCAGGATGTG
SYT8_eRNA_R	CTTGGTGTTCAGGGAGGATTT

PGR_eRNA_F	GCAATTCAGGTCAAGATGTCTAAAT
PGR_eRNA_R	TGGTCTCTACAGTCTACAAGGT
FOXN1_eRNA_F	AGGGAGTGCTCTGGGAA
FOXN1_eRNA_R	AGAGCAGCACTGACATCAC
KCNK5_eRNA_F	GTCCTTTGTATCTTTGGGTCTTC
KCNK5_eRNA_R	CTGCCTTTCTCCCTCTGAATA
ENPP2_eRNA_F	CTGGACTCTCTGGCTTGTTTAG
ENPP2_eRNA_R	ATTCCACAGCAACCCTATG
CT62_eRNA_F	CTGGATCAGACCAACGTGAA
CT62_eRNA_R	AGGATCTGTTTGCTCTACTAAGAAT
SMAD7_eRNA_F	TGGTTCAGTTCAAGTACCTGTT
SMAD7_eRNA_R	TTTCCACAGGTGAGCAGAAA
MYCB_eRNA_F	GTCAGCACAGCACACTAGAT
MYCB_eRNA_R	CTGCTGTTCGTGTTGTCATTT
TTC9_eRNA_F	CAGAAAGCCAAGTCAACAGAAG
TTC9_eRNA_R	GTAGCATCAGCTTATCAGAAGGA
SYDE2_eRNA_F	TTGGGTTTCGAGTGTTACATTC
SYDE2_eRNA_R	TCTTCCACAATCCACCTTCTAC
SPRY1_eRNA_F	GTCAGAAGCCTCAGGGAAATAG
SPRY1_eRNA_R	GGCAGGGCAAGGGTAATAAA
CCNG2_eRNA_F	GTCTGGGCAGCTAATCTATTCC
CCNG2_eRNA_R	TTCTCTCCATTAGGCTCTGA
DSCR8_eRNA_F	CAAGGACATGATTTGGGAGTAAGA
DSCR8_eRNA_R	GACAGCCTAAGCTACAGAAACC
BMF_eRNA_F	CTAGGGACCCATCTCACTCTAT
BMF_eRNA_R	TCTGGAACCTTAAGCTGGCTAAC
KRT7_eRNA_F	CAGTCAGAGCCAATGTGTGA
KRT7_eRNA_R	ACCCTGGCACACCTGAG
GLCCI1_eRNA_F	GAAGGAGGCCTTCAGTCTATTT
GLCCI1_eRNA_R	CCAGGCTTCTTGTAGCCATATTA
PAK2_eRNA_F	GTAAGGAGCCTCTTTCACAATAC
PAK2_eRNA_R	CCTCCAAGACCCAATAAA
WEE1_eRNA_F	AGTAATCTGAACGAAGCCAGTT
WEE1_eRNA_R	GGGTTAGTGACATACACAGGAAA
ARMC9_eRNA_F	GCAATGGAGTCCTAGTGACAAATA
ARMC9_eRNA_R	AGGCTACTTTGCTGCGTAATA
ZNF217_eRNA_F	GGAAGCAGTAACTCATTTCGAAG
ZNF217_eRNA_R	GCAAGAGACAGAGAGAGAGAGA
SALL4_eRNA_F	CTGTAACAGAGAGCCACAGAC
SALL4_eRNA_R	ATCTACGAGCCAAGGAGAGA

PRLR_eRNA_F	CCCACGTTGCACATTCTCTA
PRLR_eRNA_R	AGCAAACCTTCTCCATGTCTGT
TNFRSF11B_eRNA_F	ACAGACAGACCAAGCCCATT
TNFRSF11B_eRNA_R	TTGGAACCTTGGAAGGAAGCT
ARID5B_eRNA_F	CCCATGGAGTTGGCTATCCT
ARID5B_eRNA_R	TGCCTGTTCTACTCTGGTGG
LYPD3_eRNA_F	AGCCCTGAAAAAGAGGCTGA
LYPD3_eRNA_R	GAGTGAGAGGGTTTAGCCGT
TMEM116_eRNA_F	AAGGGTTGCATTGCATTCCC
TMEM116_eRNA_R	CAACAGTGCGTGATGTGCTT
CDYL2_F	GCCCGTCGGTTGAGAAACT
CDYL2_R	GCTTCCGTTTATGGGAGGTCC
CDYL2_eRNA_F	GTCTAGAGACACAGGGAGGC
CDYL2_eRNA_R	TTCCTGGTTCTGCTCTCTGG
CD55_F	AGGCCGTACAAGTTTTCCCG
CD55_R	CCTTCTCGCCAGGAATTTTCAC
CD55_eRNA_F	GCTCCTCAAGTCTCACACCT
CD55_eRNA_R	AGAGATGTGACTGCAGGCAA
DCP2_F	AGAACACACCAGGATTACCTCA
DCP2_R	AGCAAAAACGGACAATGACTGA
DCP2_eRNA_F	TGGAACCAACACCTTCGAAT
DCP2_eRNA_R	ACAGACTTGGTGCACAGCTA
PLEKHF2_F	CCGTCGGGGCTATTAGTGAAA
PLEKHF2_R	TGACCAGCTGCTCCAAAACA
PLEKHF2_eRNA_F	ACGTGGTAGGCAAACAGGAT
PLEKHF2_eRNA_R	GACAATCTGTTCTGGCAGCC

Table S3. Primers for 3C-qPCR. Related to Figure 3.

Names	5'-3' Sequences
Anchor	CTGAATCATGGCAGCAGTTTCCCCCATGC
Region A	TGACAGGACACAGAGAGTTAGGTCAGGGCA
Region B	AGAGAGTTCAGATGACTTGCCCAGAATCCAG
Region C	CACGCTGAGGCTCCACTGACAAGTGCAAG
Region D	GCGAGATGTTCTTTAGGTAGGCAGCCAGGGA
GAPDH_3C_F	ACAGTCCATGCCATCACTGCC (Hagege et al., 2007)
GAPDH_3C_R	GCCTGCTTCACCACCTTCTTG (Hagege et al., 2007)

Table S4. Primers for ChIP-qPCR. Related to Figure 3, Figure 5, Figure 6, Figure S4, and Figure S5.

Names	5'-3' Sequences
TM4SF1_Enh1_F	CACCTAGACCTCATGGCCTC
TM4SF1_Enh1_R	TGCCATGTTGGACTTTGACA
TM4SF1_Enh2_F	TGTGGCTTCTGAAGAGCTGA
TM4SF1_Enh2_R	CATGCCCTCCTTTGTGAAC
TM4SF1_Enh3_F	GTCCAAACATACTGCTGCCA
TM4SF1_Enh3_R	CCAGTGTGTGTTAGTCAGCC
TM4SF1_pro_F	AACTGCCAAGTGCCGAGAT
TM4SF1_pro_R	AGCTCTCAGATTGGAAGCTGT
EFEMP1_Enh_F	TGTTCTAAATGCCAGGGCCT
EFEMP1_Enh_R	CACAGCTGCAGGGATAGGAA
GAPDH_pro_F	TACTAGCGGTTTTACGGGCG (Xu et al., 2012)
GAPDH_pro_R	TCGAACAGGAGGAGCAGAGAGCGA (Xu et al., 2012)
KIAA0066_F	CTAGGAGGGTGGAGGTAGGG (Varambally et al., 2008)
KIAA0066_R	GCCCCAAACAGGAGTAATGA (Varambally et al., 2008)
PPIA_F	GCCAGGCTCCTGTTTTAATG
PPIA_R	GCAGTCTCCGTTTTGAGAG
MYC_Enh_F	CCAGCTTTCACCAACCACTC
MYC_Enh_R	GCAGGTGTCCTAGAGCATGA
FOXC1_Enh_F	GCGTCTCAGAATGGACAGGA
FOXC1_Enh_R	TAAAGATCGCAGTGGCCCTT
CD55_Enh_F	TGAGGCATAGATAGGCACATTAC
CD55_Enh_R	CAGGCAACATTCCCTCCAGAA
CDYL2_Enh_F	GGAGCTAGAATTGGCCACTATG
CDYL2_Enh_R	GGACACCACCTACCATCTACT
DCP2_Enh_F	TGCCAAGCAGATGGTTAAGT
DCP2_Enh_R	CACAGGGAAAGCCTGAAAGA
PLEKHF2_Enh_F	CCTACCACGTTGCAGACAAA
PLEKHF2_Enh_R	ATGGGTCATCAAAGCACTGG