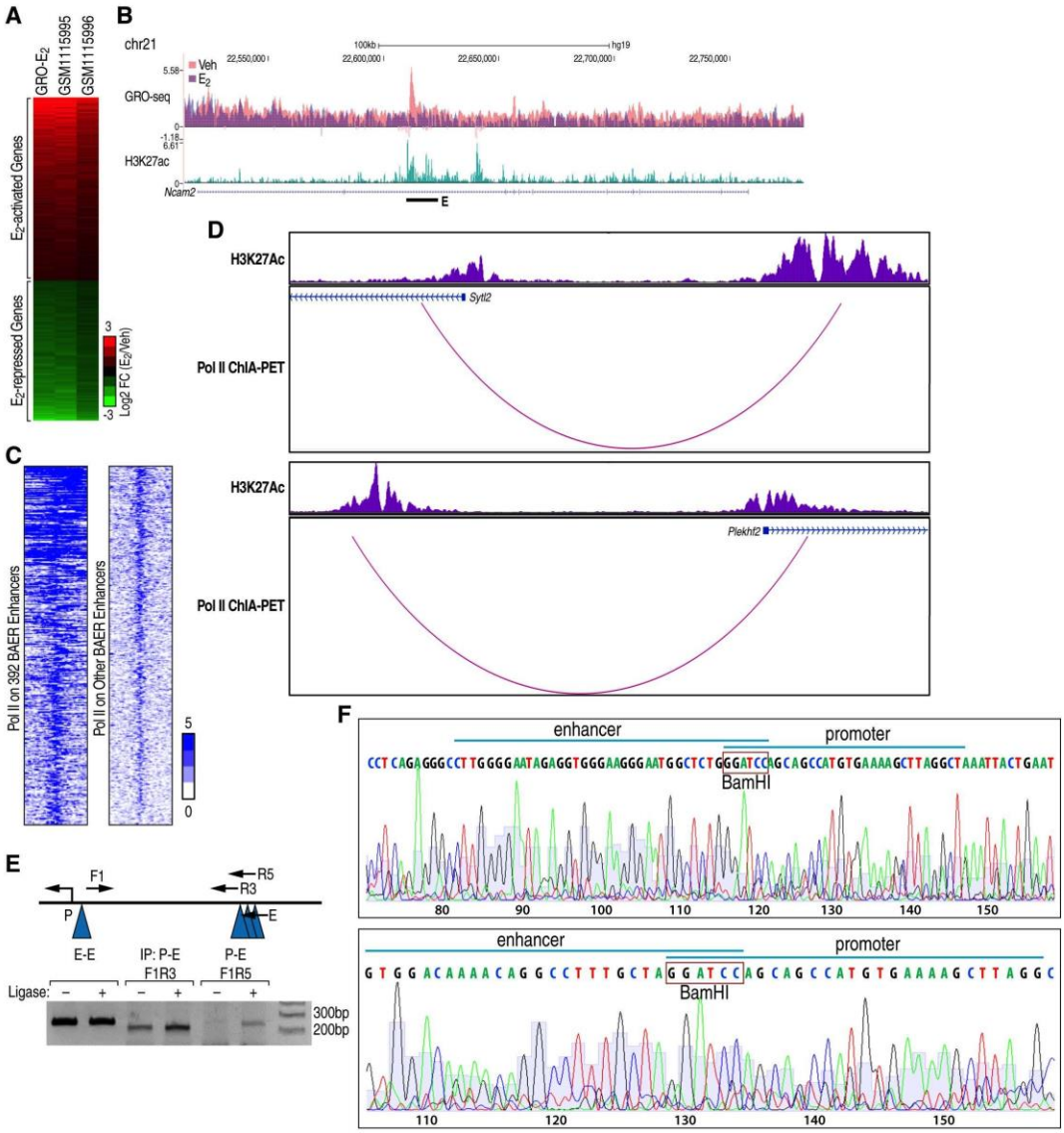
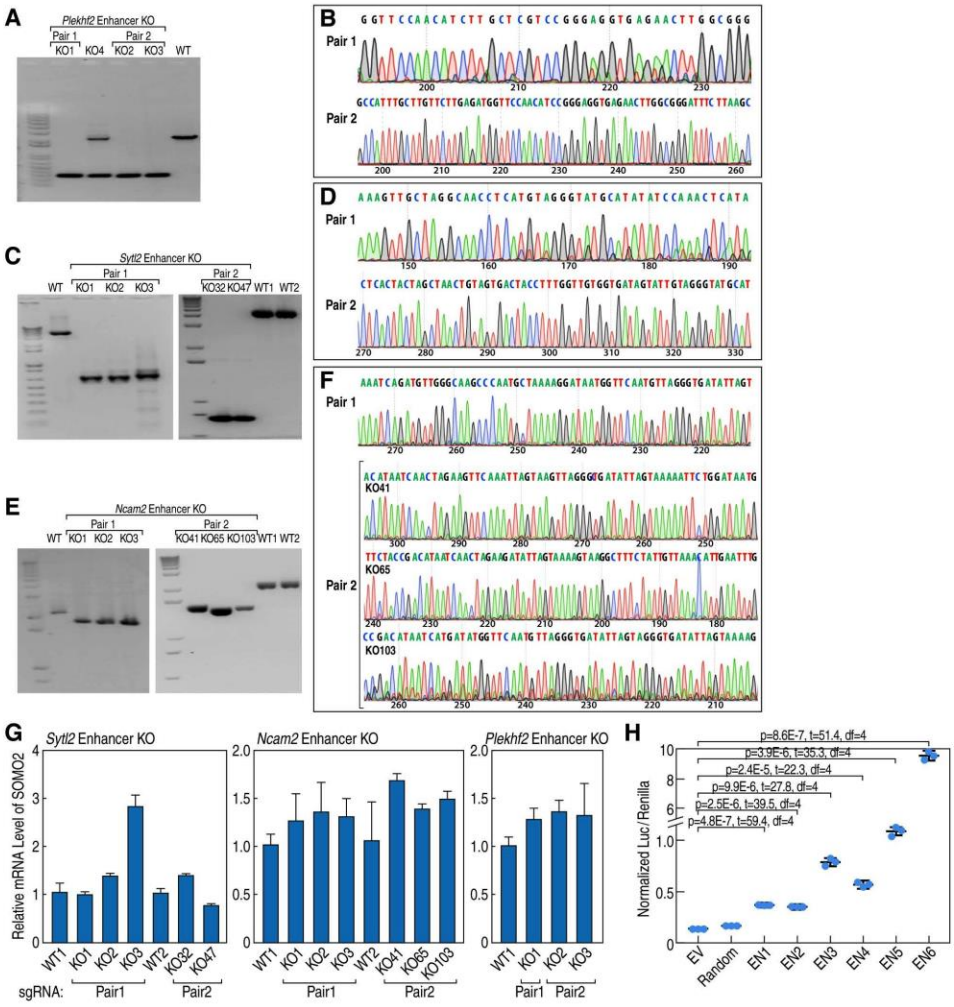


Tan et al. 2018, Sup Fig 1

Supplementary Figure 1 (Related to Figure 1). E_2 induces a large program of transcriptional repression in MCF7 cells.

A, Heatmap shows the expression of ERα regulated genes upon Veh (EtOH) and E_2 treatment as revealed by GRO-seq. ERα regulated genes are calculated assessed by the edgeR with a fold change (FC) > 1.5 and FDR < 0.01. The fold changes of expression

level shown in the heatmap were normalized to the Veh condition in logarithmic scale (Log_2). **B**, GRO-seq tag counts are showed at Veh and E_2 condition for *Ncam2* genomic loci. **C**, The enrichment of Pol II spanning ±3kb region from the center of 392 BAER enhancers, detected in MCF7 cells with normal cell culture medium, and the rest of the other BAER enhancers under Veh condition are represented as heatmaps. **D**, Interaction loop view of Pol II ChIA-PET (GSE33664) in *Plekhf2* and *Sytl2* loci. H3K27Ac is used to indicate both enhancer and promoter regions. **E**, 3C-PCR assays show a specific E (Enhancer): P (Promoter) looping in the *Sytl2* locus upon vehicle or E_2 treatment. **F**, Sanger sequencing of the 3C-PCR products show that the ligated fragment comprises regions from *Sytl2* promoter and enhancer.

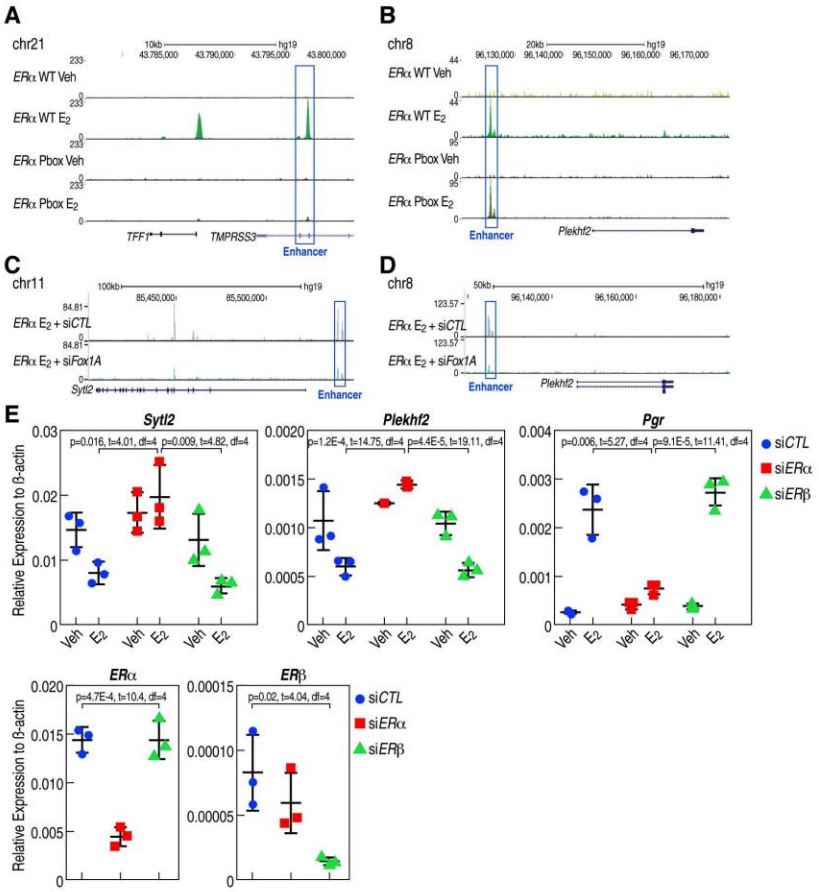


Tan et al. 2018, Sup Fig 2

Supplementary Figure 2 (Related to Figure 2). BAER enhancers regulate the transcription of E_2 repressed genes in MCF7 cells cultured under vehicle condition.

A, Identification of *Plekhf2-enhancer* knockout cells by PCR. KO 1, 2, 3 are homozygous, KO 1 was generated with gRNA pair 1; KO 2 and KO 3 was generated with gRNA pair 2; heterozygous clone (KO4) showed both long and short fragment. **B**, The documentation of DNA sequencing for pair 1 and pair 2 gRNA generated homozygous *Plekhf2*-enhancer

knock out clones. **C**, Identification of *Sytl2-enhancer* knockout cells by PCR, KO 1, 2, 3 (right) were generated with gRNA pair 1, KO 32, 47 were generated with gRNA pair 2 (Left). **D**, The documentation of DNA sequencing for pair 1 and pair 2 gRNA generated homozygous *Sytl2*-enhancer knock out clones. **E**, Identification of *Ncam2-enhancer* knockout cells by PCR, KO 1, 2, 3 (right) were generated with gRNA pair 1, KO 41, 65, 103 were generated with gRNA pair 2 (Left) with double nicking strategy. **F**, The documentation of DNA sequencing for pair 1 and pair 2 gRNA generated homozygous *Ncam2*-enhancer knock out clones. **G**, Analysis of the expression level of *Sumo2* in wild type and BAER enhancer knockout cells by RT-qPCR to exclude the effect of cancer cell variations in these enhancer knockout cell lines. **H**, Luciferase assay shows that BAER enhancers could induce gene expression in HEK-293T cells. 6 different BAER enhancers were cloned into pGL4.23 and transfected into HEK-293T cells, the luciferase value was detected by Dual-Luciferase® Reporter Assay System (Promega).

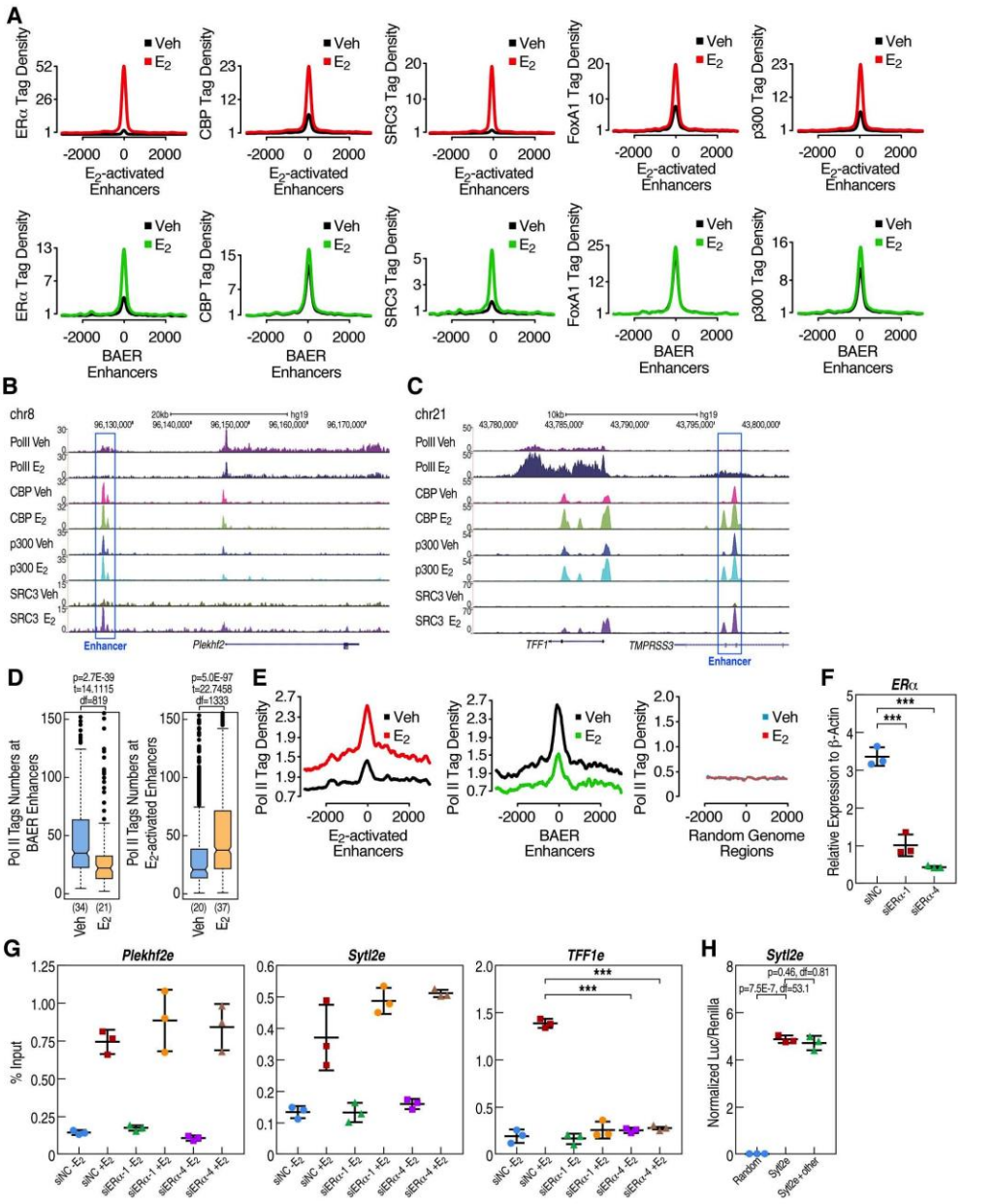


Tan et al. 2018, Sup Fig 3

Supplementary Figure 3 (Related to Figure 3). FOXA1 servers as pioneer for ERα *trans*-recruitment to BAER enhancers.

A, **B**, Genomic loci shows ERα binding at E_2 activated enhancer-*Tff1* enhancer (**A**) and BAER enhancer-*Plekhf2* enhancer (**B**) at both Veh and E_2 condition. **C**, **D**, UCSC genome browser shows ERα ChIP-seq signals upon si*CTL* and si*FoxA1* conditions at *Plekhf2* (**C**) and *Sytl2* (**D**) loci when MCF7 cells were treated with E_2. **E**, RT-qPCR shows gene

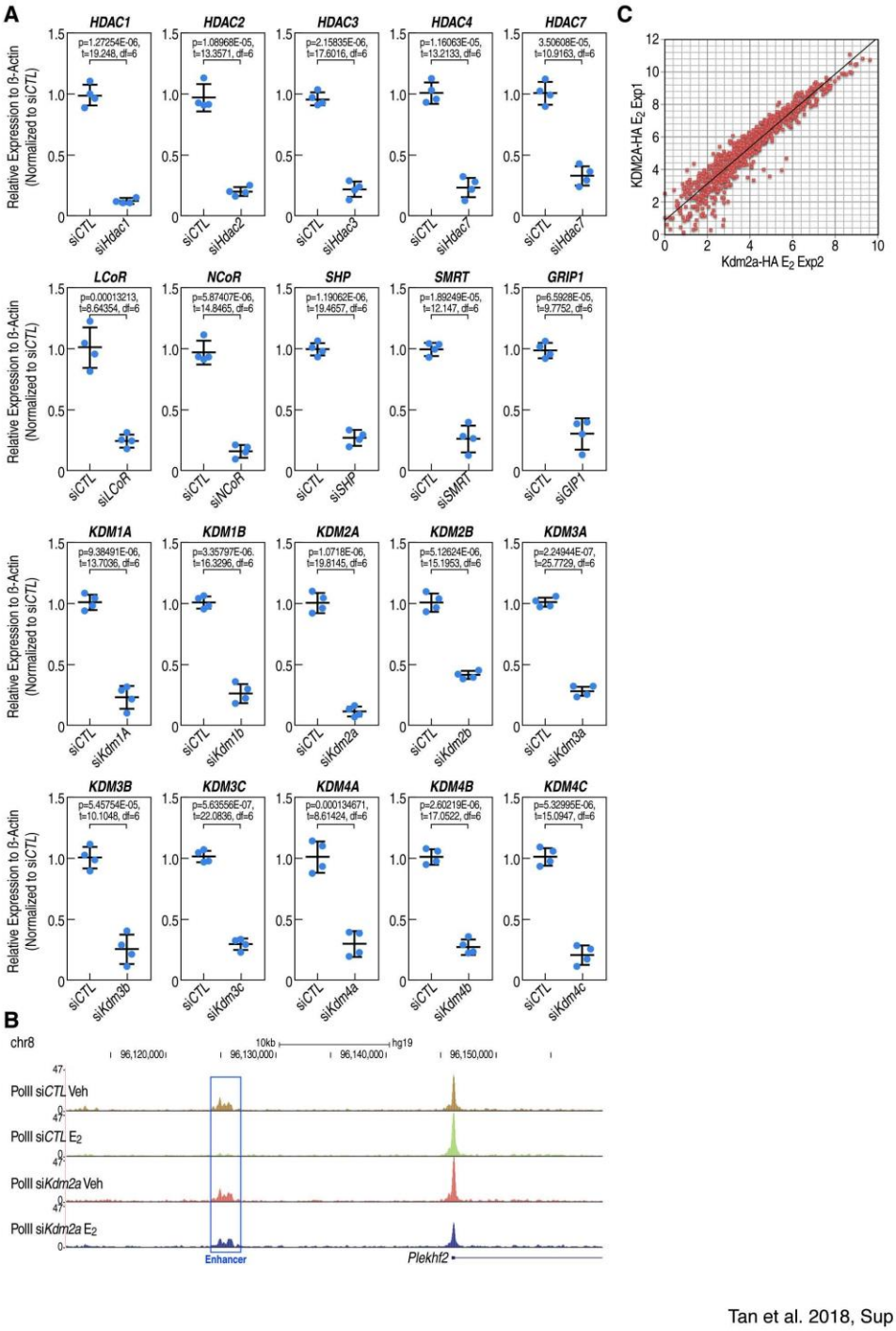
expression levels of E_2 repressed gene-*Sytl2* and *Plekhf2*, and E_2 active gene-*Pgr* in response of E_2 when ERα or ERβ was knockdown by siRNAs. Data are presented as mean ± s.d. P values denote differences between si*CTL* and siERα or siERα and siERβ are shown (N = 3; unpaired *t* test) at E_2 condition.



Tan et al. 2018, Sup Fig 4

Supplementary Figure 4 (Related to Figure 4). Coactivators are recruited to the BAER enhancers upon E_2 treatment.

A, Tag density plot shows the enrichment of ERα (E-TABM-828), FOXA1 (GSM588929/30), CBP (E-TABM-785), P300 (E-TABM-785), SRC13 (E-TABM-785), H3K27Ac and Pol II at E_2 activated and BAER enhancers upon vehicle and E_2 treatment. **B**, **C**, UCSC genome browser shows Pol II, CBP (E-TABM-785), p300 (E-TABM-785), SRC3 (E-TABM-785) ChIP-seq signals at *Plekhf2* (**B**) and *Tff1* (**C**) locus when MCF7 cells were treated with Veh or E_2. **D**, Box-and-whisker plots show Pol II ChIP-seq signals (tag counts) in MCF7 cells before and after E_2 treatment (1 h) at E_2 activated enhancers and BAER enhancers. P values denote statistical differences between treatment conditions. Center lines show the medians, box limits indicate the 25th and 75th percentiles, and whiskers extend 1.5× the interquartile range from the 25th and 75th percentiles. **E**, Tag density plots show the enrichment of Pol II at genome random regions, E_2 activated enhancers, or BAER enhancers upon vehicle and E_2 treatment. **F**, RT-qPCR shows knockdown efficacy of *Esr1* (ERα) by siRNAs targeting its 3'UTR in ERα P-box mutant cells (N = 3; unpaired *t* test). **G**, Knockdown of endogenous ERα in ERα P-box mutant cells does not affect the binding of HA-tagged P-box mutated ERα at *Pelkhf2*-enhancer and *Sytl2*-enhancer, presented by ChIP-qPCR using antibody targeting HA-tag (N = 3; unpaired *t* test). **H**, Insertion of a random sequence of comparable length without Pol II transcription terminators to *Sytl2* enhancers does not decrease downstream target reporter gene (luciferase) expression in HEK-293T cells. The luciferase value was detected by Dual-Luciferase® Reporter Assay System (Promega).

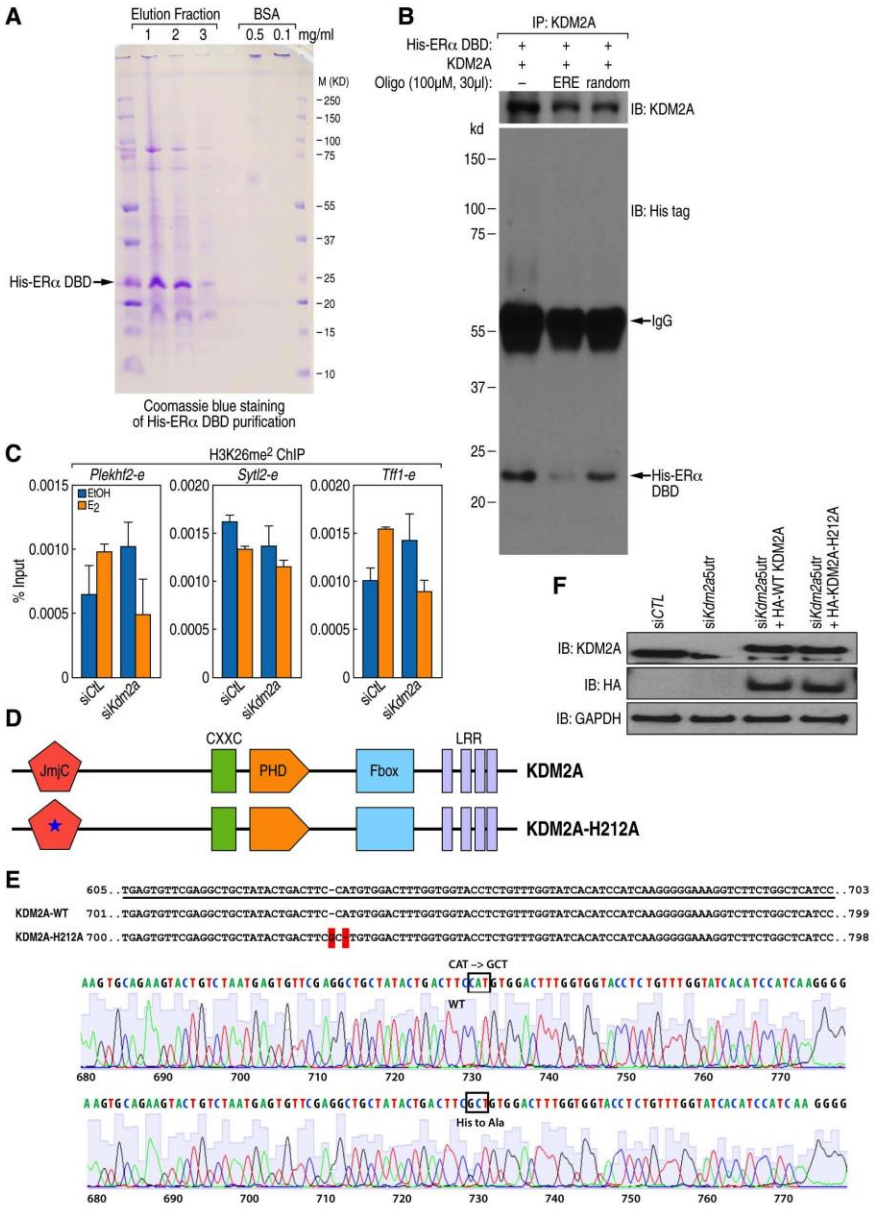


Tan et al. 2018, Sup Fig 5

Supplementary Figure 5 (Related to Figure 5). KDM2A is involved in ERα mediated enhancer decommissioning.

A, RT-qPCR shows knockdown efficacy of corepressors and histone demethylases when they were knockdown by their cognate siRNAs. Data are presented as mean ± s.d. P values denote differences between si*CTL* and target siRNAs are shown (N = 3; unpaired *t* test). **B**, Genomic loci for *Plekhf2* indicates knockdown of *Kdm2a* reverse the reduction of

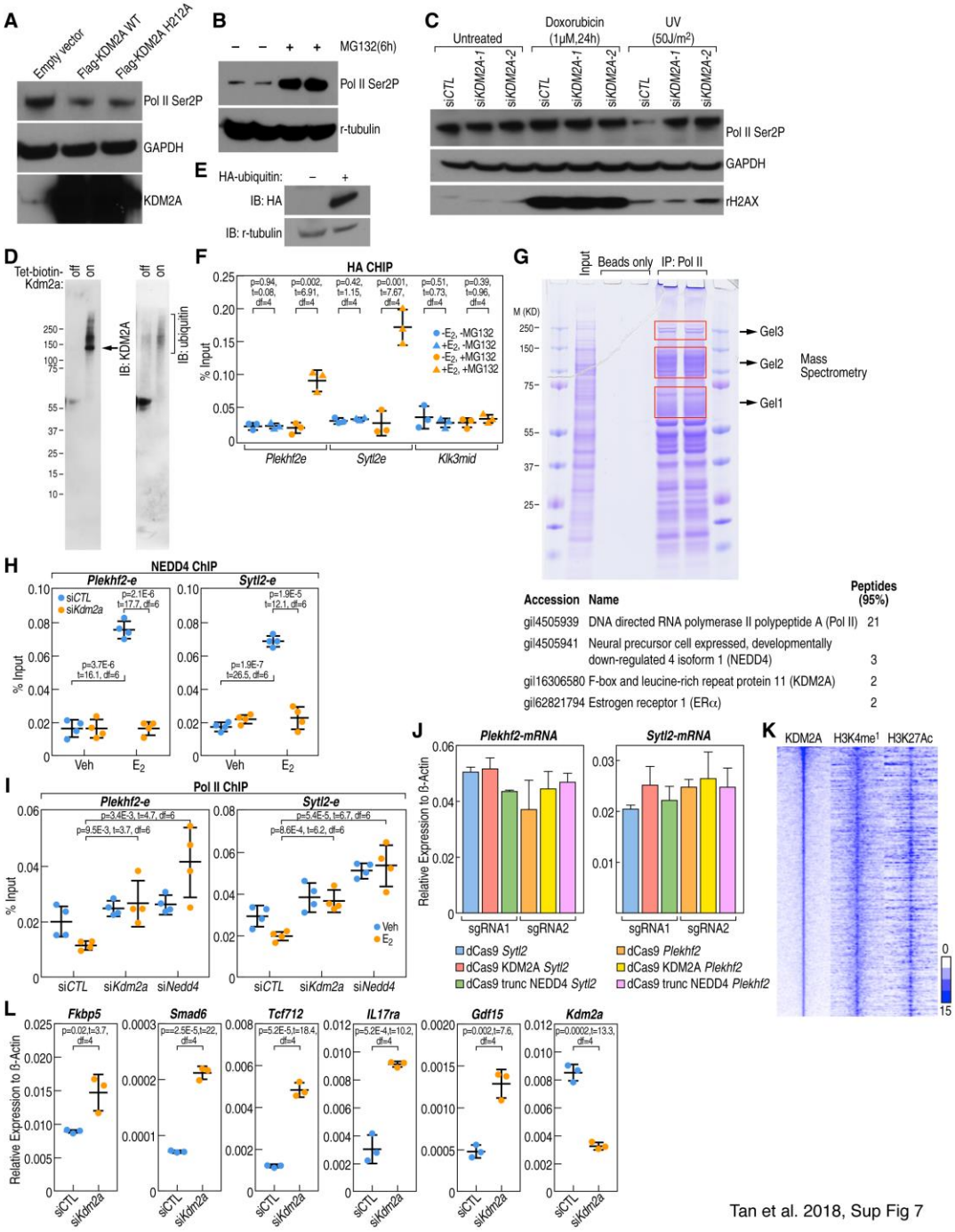
Pol II by E_2 treatment. **C**, Scatterplot shows the Pearson correlation between our two sets of HA-tagged KDM2A ChIP-seq experiments. ChIP-Seq tags counts (Log_2) of HA-KDM2A at enhancers were generated using HOMER (Heinz et al., 2010).



Tan et al. 2018, Sup Fig 6

Supplementary Figure 6 (Related to Figure 6). ***Trans*-bound ERα recruits KDM2A to BAER enhancers.**

A, His-tagged ERα DBD was expressed and purified from bacteria BL21. Coomassie brilliant blue (CBB) staining of purified protein and BSA (concentration control). **B**, *In vitro* immunoprecipitation of KDM2A in presence of ERα DBD protein, with or without 2xERE motif or control ~60mer oligonucleotides (100μM, 30μl) as competitors. **C**, ChIP-qPCR showing the absence of H3K36me2 upon si*CTL* and si*Kdm2a* treatment at *Plekhf2*, *Sytl2* and *Tff1* enhancers. **D**, KDM2A with domain organization and mutations is indicated. **E**, Sanger sequencing of the KDM2A-H212A mutation is shown. **F**, Western blot analysis shows roughly equal protein expression between endogenous KDM2A and HA-tagged KDM2A cell lines. The endogenous KDM2A was knocked down by siRNA target to KDM2A 5'UTR (si*Kdm2a5utr*).



Supplementary Figure 7 (Related to Figure 7). Pol II ubiquitylation is mediated by KDM2A and is the key signature for Pol II dismissal at BAER enhancers.

A, Western blot detects Ser2-phosphorylated RNA polymerase II (PolIISer2P) protein level in HEK293T cells with transfection of empty vector, or KDM2A or KDM2A-H212A overexpressed-plasmid 2 days before harvest cells. **B**, PolIISer2P expression following MG132 treatment (10 μM, 6h) is shown by Western blot. **C**, Western blot detects

PolIISer2P proteins in HEK293T cells treated with Doxorubicin (1 μM, 24h) or 50J/m^2 UV-radiation and allowed to recover for 1h, with si*CTL* or *Kdm2a* knockdown. **D**, KDM2A containing complexes were pulled-down by streptavidin beads from MCF7 cells stable expressing Tet-on biotinylated KDM2A, and then subjected *in vitro* ubiquitylation assay. **E**, Western blot shows that the expression of HA-tagged ubiquitin in MCF7 cells, HA tag was employed to indicate protein expression. **F**, Increased HA-ubiquitin following E_2 treatment at the *Plekhf2* and *Sytl2* enhancers with 10 μM MG132 treatment for 3 h, but not at genome control regions (*Klk3mid*), shown by HA ChIP-qPCR. ChIP signals are presented as percentage of input. Data are presented as mean ± s.d. P values denote differences between Veh and E_2 condition are shown. **G**, Protein complexes that are co-immunoprecipitated by Pol II antibody in MCF7 cells are showing by coomassie blue staining. The red frame indicates gel pieces subjected to mass spectrometry analysis. **H**, NEDD4 was recruited by KDM2A upon E_2 treatment under si*CTL* conditions at the *Plekhf2*-enhancer and *Sytl2*-enhancer; ChIP signals are presented as percentage of input. Data are presented as mean ± s.d. P values denoting differences between Veh and E_2 under si*CTL* conditions and between si*CTL* and si*Kdm2a* under E_2 treatment are shown (N = 4; unpaired *t* test). **I**, Decreasing of Pol II upon E_2 treatment under si*CTL* conditions at the *Plekhf2* and *Sytl2* enhancer was abolished following *Kdm2a* or *Nedd4* knockdown by siRNAs, as showed by Pol II ChIP-qPCR. ChIP signals are presented as percentage of input. Data are presented as mean ± s.d. P values denoting differences between si*CTL* and si*Kdm2a* or si*Nedd4* are shown (N = 4; unpaired *t* test). **J**, *Plekhf2* and *Sytl2* enhancers were repressed by dCas9 fused with KDM2A or truncated NEDD4 (containing C terminal HECT domain) proteins generated using two different groups of sgRNAs. The expression level of *Plekhf2* and *Sytl2* mRNA are measured by RT-qPCR to ascertain the specificity of dCas9-sgRNA experiments. Data are presented as mean ± s.d. **K,** The enrichment of H3K27Ac and H3K4me1 spanning ±3kb region from the center of KDM2A-marked mouse ESCs enhancers are represented as heatmaps. **L**, The expression level of genes targeted by KDM2A-marked enhancers in mESCs under si*CTL* or si*Kdm2a* conditions is measured by RT-qPCR. Data are presented as mean ± s.d. P values denoting differences between control *siRNA* and *siKdm2a*. (N = 3; unpaired *t* test).