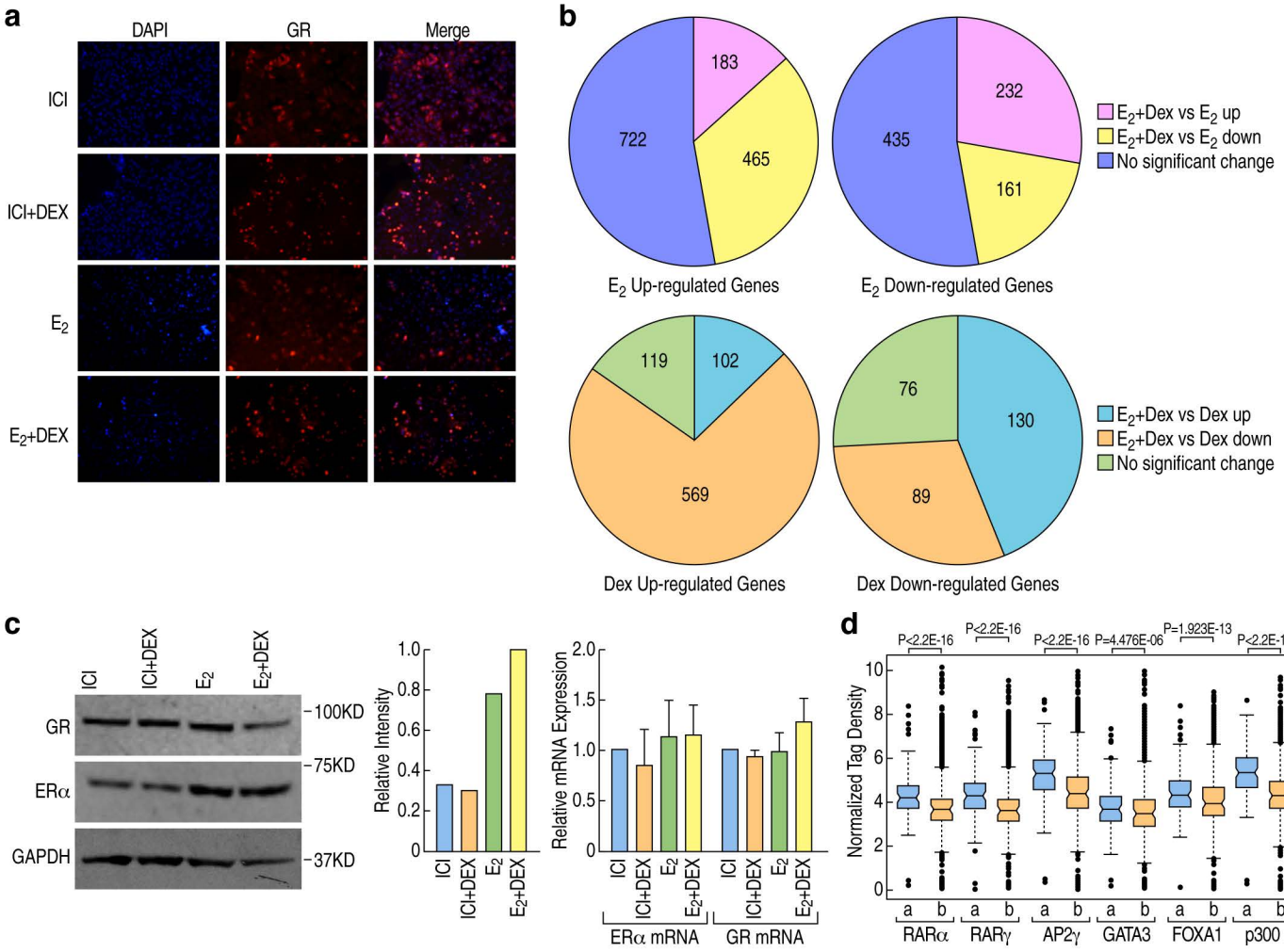
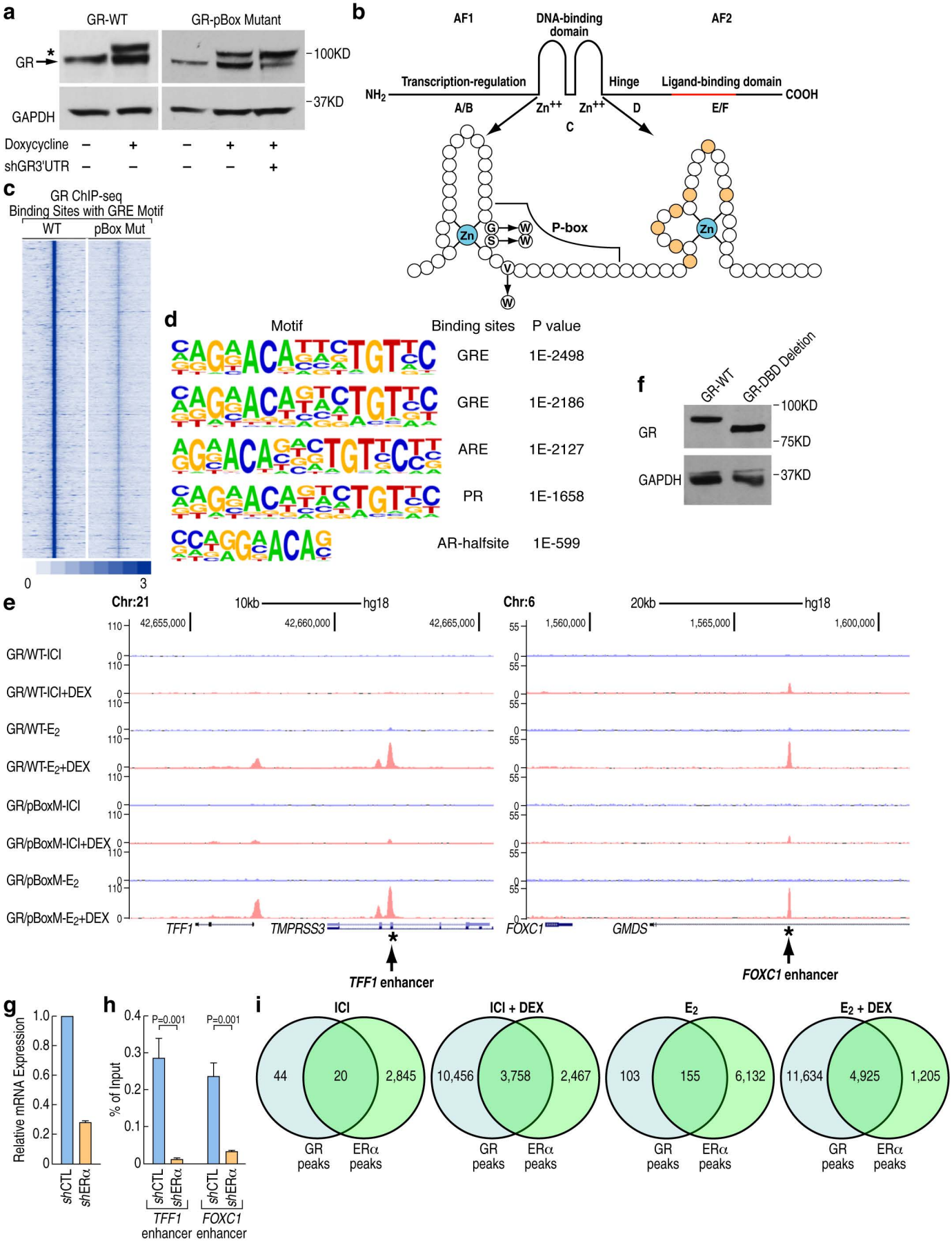
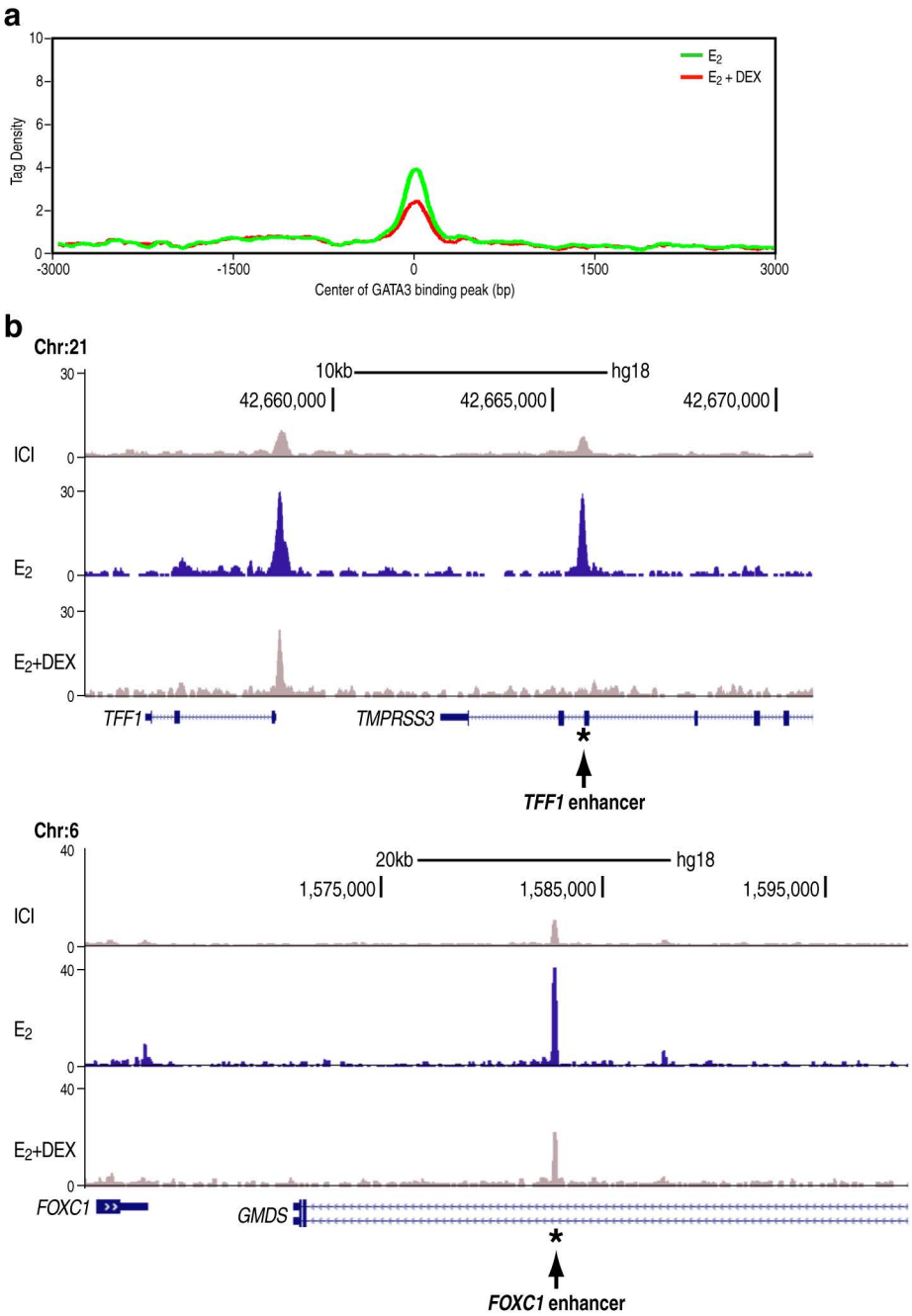


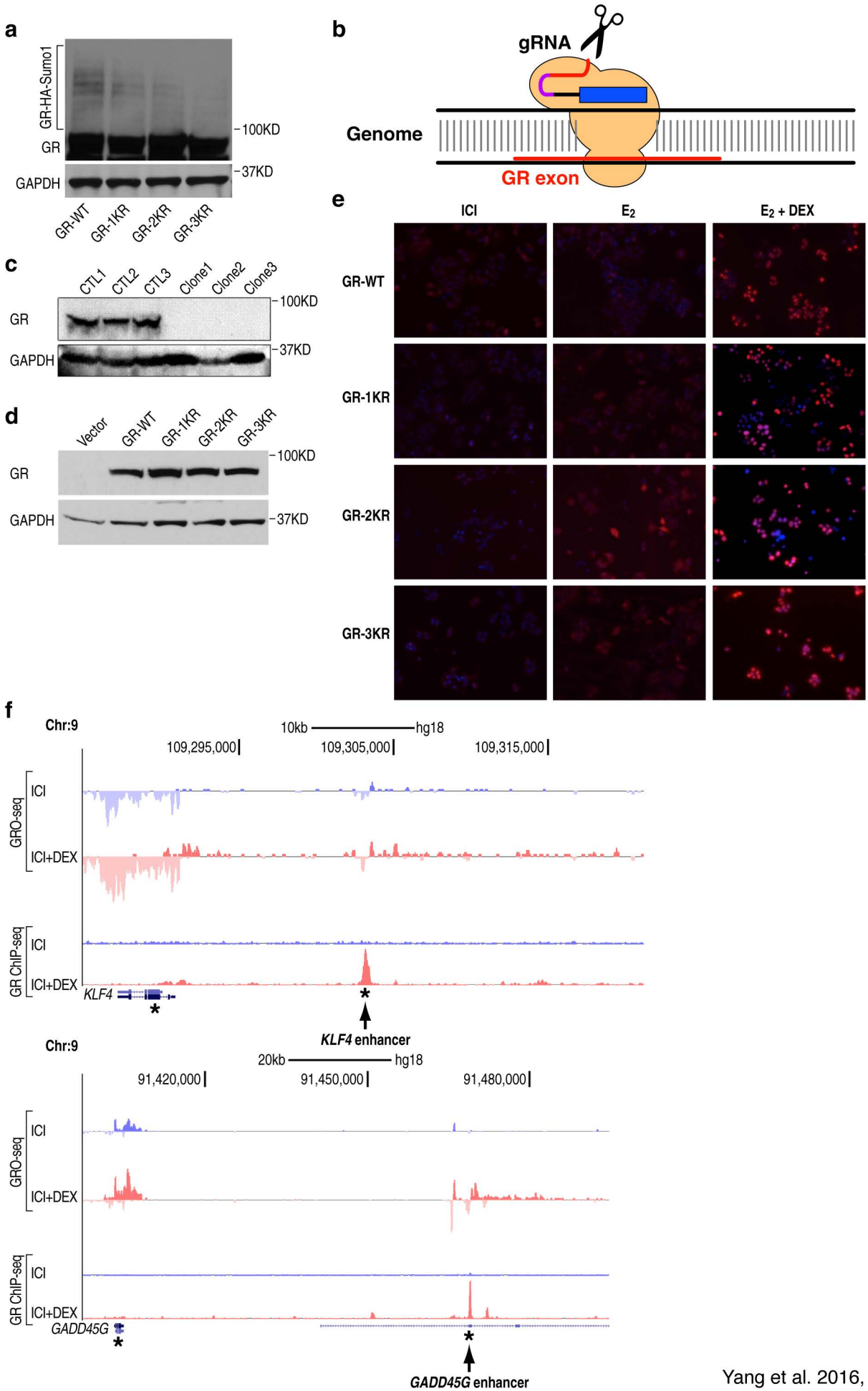
Yang et al. 2016, SupFig 1



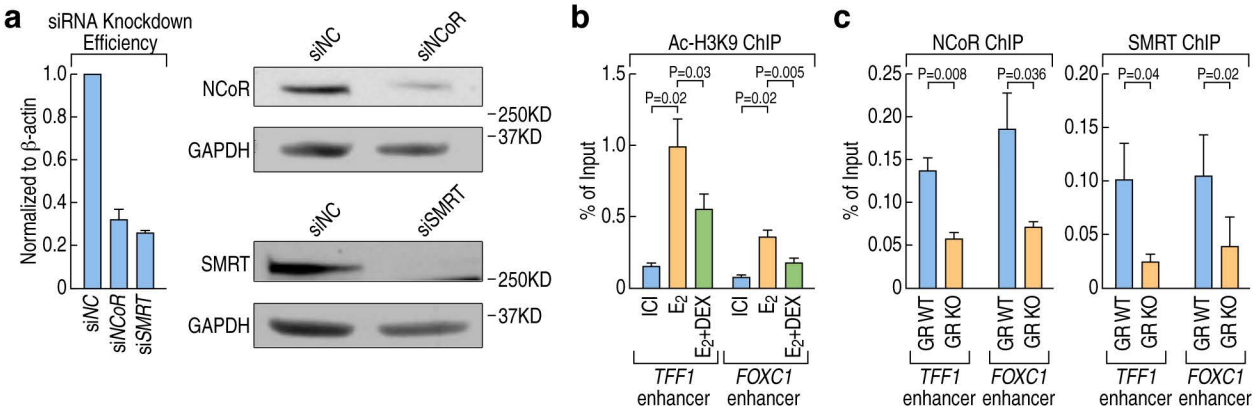
Yang et al. 2016, SupFig 2



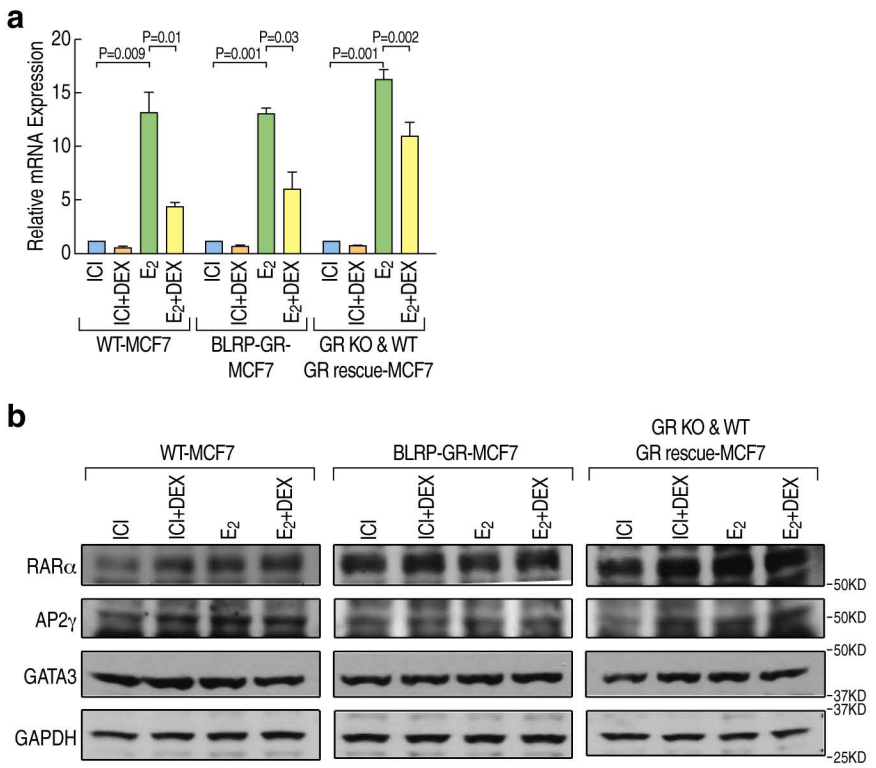
Yang et al. 2016, SupFig 3



Yang et al. 2016, SupFig 4



Yang et al. 2016, SupFig 5



Yang et al. 2016, SupFig 6

Figure S1 related Figure 1

(A) Subcellular localization of GR examined by immunostaining in MCF7 cells when treated with different ligands (ICI, E_2, Dex, E_2+Dex). The cells were immunostained for GR (red) and costained with DAPI for DNA (blue).

(B) Area-proportional Venn diagram showing the number of differentially expressed genes when treated with different ligands based on GRO-seq data analysis.

(C) GR and ERα mRNA and protein levels did not show a significant change in MCF7 cells treated with different ligands (ICI, ICI+Dex, E_2, E_2+Dex), but the ICI treatment significantly reduced ERα expression level.

(D) Boxplot of normalized ChIP-seq tags density showing that MegaTrans complex (RARα/β, AP2γ, GATA3, FOXA1) exhibits a strong binding on 423 E_2+Dex down-regulated enhancers. (a represents 423 significantly down-regulated enhancers, b represents not significantly down-regulated enhancers upon treatment with E_2+Dex compared to E_2 alone, P value is calculated by Wilcoxon rank sum test)

(E) Kaplan-Meier survival analyses for different breast cancer types using the 465 E_2+Dex down-regulated genes (left panels) and 465 randomly selected E_2-upregulated genes (right panels) as input. The breast cancer outcome-linked gene expression data were obtained interrogating the Gene Expression-Based Outcome for Breast Cancer Online (GOBO) tool. The high expression levels of the 465 genes are more significantly predictive of poor outcomes in ER positive lymph node negative breast cancer and HU Luminal A breast cancer patients than the randomly selected group. OS: overall survival

(F) Representative phase-contrast photomicrographs (10× magnification) depicting colony formation in soft agar for MCF7 cells treated with different ligands (ICI, ICI+Dex, E_2, E_2+Dex). The scale bar was indicated.

Figure S2 related Figure 2

(A) Western blots showing doxycycline-induced expression of BLRP-tagged GR wild type and pBox mutant (marked by asterisk) compared to the endogenous protein levels (marked by arrow) in MCF7 transduced with an shRNA targeting GR 3'UTR to inhibit the expression of endogenous GR.

(B) Diagram of GR DNA-binding domain mutation. Arrows point out the mutation sites.

(C) Heatmaps showing ChIP-seq signals for GR wild type and pBox mutant binding sites in MCF7 cells treated with E_2+Dex. Heatmaps are centered on GRE-containing GR binding sites with scales indicated.

(D) De novo motif analyses of GRE-containing GR binding sites by ChIP-seq.

(E) UCSC genome browser (hg18) snapshot showing the ChIP-seq tracks for GR wild type and pBox mutant at the *TFF1* and *FOXC1* loci under different conditions (ICI, ICI+Dex, E_2, E_2+Dex). "*" indicates the position of primers used for qPCR detection.

(F) Western blot showing a similar expression level of GR protein in MCF7 cells stably expressing GR wild type and GR DBD deletion mutant.

(G) shRNAs knockdown efficiency validation for ERα by RT-qPCR in MCF7 cells.

(H) ChIP-qPCR showing GR binding on ERα-activated enhancers (*TFF1* and *FOXC1* enhancers) following shRNA-mediated knock down of ERα in MCF7 cells treated with E_2+Dex. Data are represented as mean ± SD. N⩾3, two-tailed Student's t test.

(I) Area-proportional Venn diagram showing the overlap of GR and ERα binding peaks on enhancers based on ChIP-seq data analysis.

Figure S3 related Figure 3

(A) ChIP-seq tag density profile of GATA3 on the E_2-induced Dex-insensitive enhancers in MCF7 cells when treated with different ligands (E_2, E_2+Dex).

(B) A UCSC genome browser (hg18) snapshot showing the ChIP-seq tracks for GATA3 at the *TFF1* and *FOXC1* loci under different treatments (ICI, E_2, E_2+Dex). "*" indicates the position of primers used for qPCR detection.

Figure S4 related Figure 4

(A) Western blot analysis showing immunoprecipitated GR wild type or SUMOylation mutants in HEK293T cells upon treatment with E_2+Dex. (GR-WT: GR wild type; GR-

1KR: GR C-terminal K703R mutation; GR-2KR: GR N-terminal K277R and K293R two sites mutation; GR-3KR: GR K277R, K293R and K703R all three sites mutation)

(B) A diagram showing the CRISPR-Cas9-mediated knock-out of GR gene in MCF7 cells.

(C) Validation by western blot assay of the GR knock-out stable cell lines. CTL: Cas9 without gRNA transfection into MCF7 cells as a negative control.

(D) Western blot analysis of GR wild type and SUMOylation sites mutants in MCF7 knock-out cells following the overexpression of different constructs. (GR-WT:GR wild type; GR-1KR: GR C-terminal K703R mutation; GR-2KR: GR N-terminal K277R and K293R two sites mutation; GR-3KR: GR K277R, K293R and K703R all three sites mutation)

(E) The sub-cellular localization of GR wild type and SUMOylation sites mutants were examined by immunostaining in MCF7 cells treated with different ligands (ICI, E_2, E_2+Dex). The cells were immunostained for GR (red) and co-stained with DAPI for DNA(blue).

(F) Genome browser image showing normalized GRO-seq tag counts and GR ChIP-seq signals in MCF7 cells under different conditions (ICI, ICI+DEX). KLF4 and GADD45G loci are shown. "*" indicates the position of primers used for qPCR detection.

Figure S5 related Figure 5

(A) Validation by RT-qPCR and western blot of the siRNAs knockdown efficiency for NCoR, SMRT in MCF7 cells.

(B) ChIP-qPCR showing levels of acetyl-H3K9 on ERα-activated enhancers in MCF7 cells treated with ICI, E_2, E_2+DEX. Data are presented as mean ± SD. N$\geqslant$3, two-tailed Student's t test.

(C) ChIP-qPCR showing NCoR/SMRT complex binding on ERα-activated enhancers in GR wild type and knock-out MCF7 cells treated with E_2+Dex. ChIP signals are presented

as percentage of input. Data are represented as mean ± SD. N⩾3, two-tailed Student's t test. GR WT: GR wild type; GR KO: GR knock out.

Figure S6 related Figure 6

(A) qPCR results showing a similar response to E_2 or Dex treatment of different engineered MCF7 cell lines. Data are represented as mean ± SD. N⩾3, two-tailed Student's t test.

(B) Western blot showing similar protein expression levels of MegaTrans components (i.e. GATA3, RARα or AP2γ) for each individual MCF7 cell line when treated with different ligands

Table S1. All qPCR Primers Used in this Study, Related to Experimental Procedures

Name	Sequences 5' to 3'
	Gene primers
GR-qPCR-F	AGCAACATTTGAAGGGCCAG
GR-qPCR-R	AATGAGAGGCTTGCAGTCCT
βactin-qPCR-F	GGA CTT CGA GCA AGA GAT GG
βactin-qPCR-R	AGC ACT GTG TTG GCG TAC AG
Tff1-qPCR-F	CACCATGGAGAACAAGGTGA
Tff1-qPCR-R	TGACACCAGGAAAACCACAA
FoxC1-qPCR-F	AGTCAGCTTGCTTTGAGGCTA
FoxC1-qPCR-R	AGGCATCACCGTGGTAAGAC
KLF4-qPCR-F	AGTTCCCATCTCAAGGCACA
KLF4-qPCR-R	GCCTCTTCATGTGTAAGGCG
GADD45G-qPCR-F	TGCTGGTTGATCGCACTATG
GADD45G-qPCR-R	CAAGACTTTGGCTGACTCGT
NCoR-qPCR-F	GCGTTATGATCAGCTCATGG
NCoR-qPCR-R	AATGGTGGCTGAAAGACCAG
SMRT-qPCR-F	GATGACGAGGACTGGCTTTT
SMRT-qPCR-R	GACAGGTGGGAGGCATAGTC
ERα-qPCR-F	GACAGGGAGCTGGTTCACAT
ERα -qPCR-R	AGGATCTCTAGCCAGGCACA
	ChIP primers
Tff1e-ChIP-F	AGGGGATGTGTGTGAGAAGG
Tff1e-ChIP-R	GCTTCGAGACAGTGGGAGTC
FoxC1e-ChIP-F	CTGAGGAACACAAGACTAGCC
FoxC1e-ChIP-R	ACTGGACTCATTTTGGGACATC
KLF4e-ChIP-F	CCCTCCCTTGGTCAACTTGA
KLF4e-ChIP-R	ACTTTGCTGCAGGGTGATTG
GADD45Ge-ChIP-F	CGTTGAACCTGGCTTGTGAA
GADD45Ge-ChIP-R	GGAGGAAGCGAGGTGTTACT

Table S2. siRNAs and shRNAs Used in this Study, Related to Experimental Procedures

siRNAs			
Gene	Company	Catalog number	Note
siRNA control	Sigma		
N-CoR	Sigma	SASI_Hs01_00238946	
N-CoR	Sigma	SASI_Hs02_00341331	
SMRT	Sigma	SASI_Hs02_00312314	
SMRT	Sigma	SASI_Hs02_00312316	
shRNAs			
Gene	Company	Catalog number	Note
pLKO.1 Control	Sigma	SHC002	TRC1 control shRNA
pLKO.5 Control	Sigma	SHC202	TRC2 control shRNA
GR	Sigma	TRCN0000245007	TRC2 target to 3'UTR
GR	Sigma	TRCN0000245003	TRC2
GR	Sigma	TRCN0000245004	TRC2
GR	Sigma	TRCN0000245005	TRC2
ERα	Sigma	TRCN0000010774	TRC1

Table S3. Oligos Used in the GRO-Seq Protocol, Related to Experimental Procedures

GRO-seq	
Name	Sequences 5’ to 3’
oNTI223	pGATCGTCGGACTGTAGAACTCT;CAAGCAGAAGACGGCATACGATT TTTTTTTTTTTTTTTTTVN
	p = 5’ phosphorylation
	";" = abasic dSpacer furan
	VN = degenerate nucleotides
oNTI200	CAAGCAGAAGACGGCATA
oNTI201	AATGATACGGCGACCACCGACAGGTTCAGAGTTCTACAGTCCGACG
Illumina small RNAseq primer	CGACAGGTTCAGAGTTCTACAGTCCGACGATC

Table S4. Quality Metrics for ChIP-seq and GRO-seq data, Related to Experimental Procedures

Sample name	Cell line	Treatment	Total tags	IP efficiency	Total peaks	Peaks on enhancers	NSC	RSC
GRα-WT ChIP-seq	MCF7	ICI	27597732	0.01%	143	64	1.021081	0.1468148
GRα-WT ChIP-seq	MCF7	ICI+DEX	18398153	6.65%	35003	14214	1.062842	0.1153435
GRα-WT ChIP-seq	MCF7	E2	16426032	0.04%	427	258	1.027229	0.1089213
GRα-WT ChIP-seq	MCF7	E2+DEX	21043422	7.76%	43133	16559	1.057018	0.1143527
GRα-PboxM-ChIP-seq	MCF7	ICI	20039163	0.02%	233	62	1.017882	0.4169963
GRα-PboxM-ChIP-seq	MCF7	ICI+DEX	22918006	2.84%	25684	14037	1.077109	0.9452748
GRα-PboxM-ChIP-seq	MCF7	E2	13452807	0.01%	170	46	1.028031	0.4408001
GRα-PboxM-ChIP-seq	MCF7	E2+DEX	16955432	2.99%	20273	11665	1.122758	0.9716123
ERα ChIP-seq	MCF7	ICI	2403400	2.2%	4141	2865	1.386406	0.3826087
ERα ChIP-seq	MCF7	ICI+DEX	2967430	5.33%	10136	6225	1.075378	0.2477626
ERα ChIP-seq	MCF7	E2	2840623	9.4%	11574	6287	1.075378	0.2477626
ERα ChIP-seq	MCF7	E2+DEX	2024047	11.3%	11196	6130	3.658976	0.6666908
GATA3 ChIP-seq	MCF7	ICI	26825786	0.51%	5652	3157	1.026871	0.2622139
GATA3 ChIP-seq	MCF7	E2	16633206	3.2%	25123	10461	1.107312	1.028149
GATA3 ChIP-seq	MCF7	E2+DEX	13424844	2.54%	19600	7791	1.095174	0.8943474
GRO-seq	MCF7	ICI	21084600					
GRO-seq	MCF7	ICI+DEX	18048409					
GRO-seq	MCF7	E2	22856779					
GRO-seq	MCF7	E2+DEX	12451176					