Supplementary Material for

AP-2γ regulates estrogen receptor-mediated long-range chromatin interaction and gene transcription

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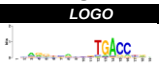
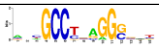
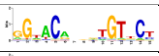
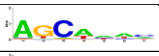
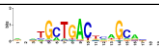
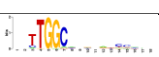
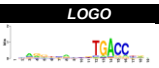
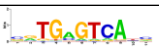
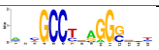
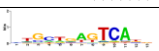
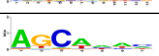
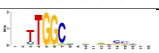
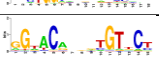
A

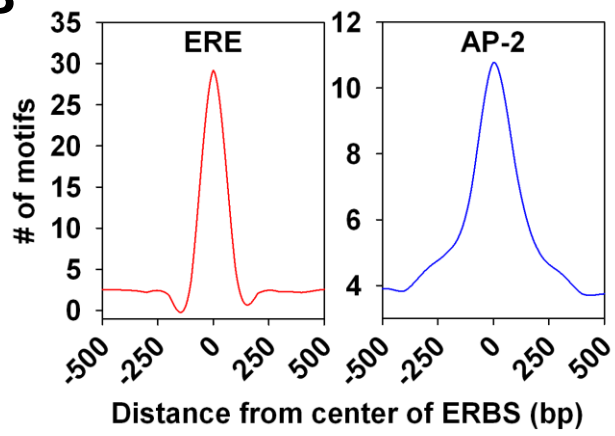
High confidence ERα bindings sites

RANK	FAMILY	TRANSFAC	LOGO	SCORE
1	ERE	V_ER_Q6		683.281538384 out of 2513(27.19%)
2	AP1	V_AP1_Q6		215.796608226 out of 2513(8.59%)
3	BACH	V_BACH2_01		192.807718113 out of 2513(7.67%)
4	AP2	V_AP2ALPHA_02		140.882702135 out of 2513(5.61%)
5	FOX	V_FREAC4_01		120.180841788 out of 2513(4.78%)
6	NRF	V_NRF2_Q4		110.812005739 out of 2513(4.41%)
7	AR	V_AR_01		73.8158806303 out of 2513(2.94%)
8	DBP	V_DBP_Q6		67.4607621247 out of 2513(2.68%)
9	VMAF	V_VMAF_01		65.7244926845 out of 2513(2.62%)
10	NF1	V_NF1_Q6		63.9413676792 out of 2513(2.54%)

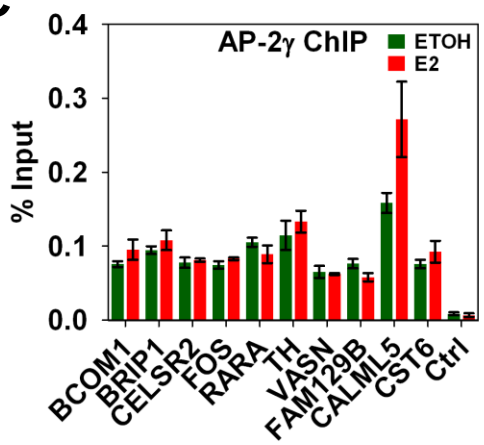
All ERα bindings sites

RANK	FAMILY	TRANSFAC	LOGO	SCORE
1	ERE	V_ER_Q6		2429.04364249 out of 14468(16.79%)
2	AP1	V_AP1_Q6		1049.1138788 out of 14468(7.25%)
3	BACH	V_BACH2_01		1043.75787405 out of 14468(7.21%)
4	AP2	V_AP2ALPHA_02		864.185031754 out of 14468(5.97%)
5	FOX	V_FREAC4_01		755.368713687 out of 14468(5.22%)
6	NRF	V_NRF2_Q4		578.578215857 out of 14468(4%)
7	DBP	V_DBP_Q6		481.803466757 out of 14468(3.33%)
8	NF1	V_NF1_Q6		452.032132608 out of 14468(3.12%)
9	AR	V_AR_01		385.591288433 out of 14468(2.67%)
10	GATA	V_GATA1_05		362.409632178 out of 14468(2.5%)

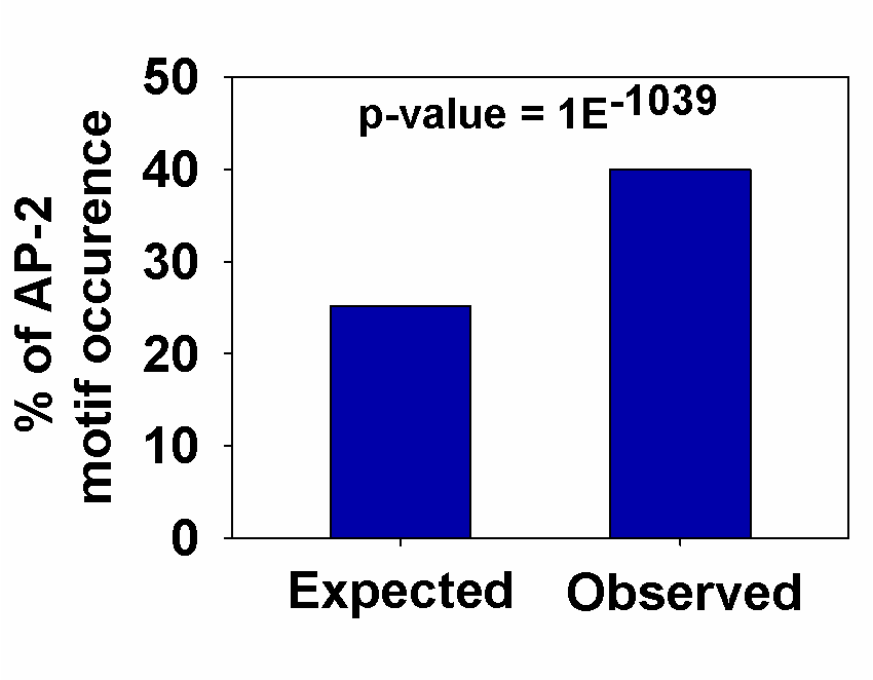
B



C

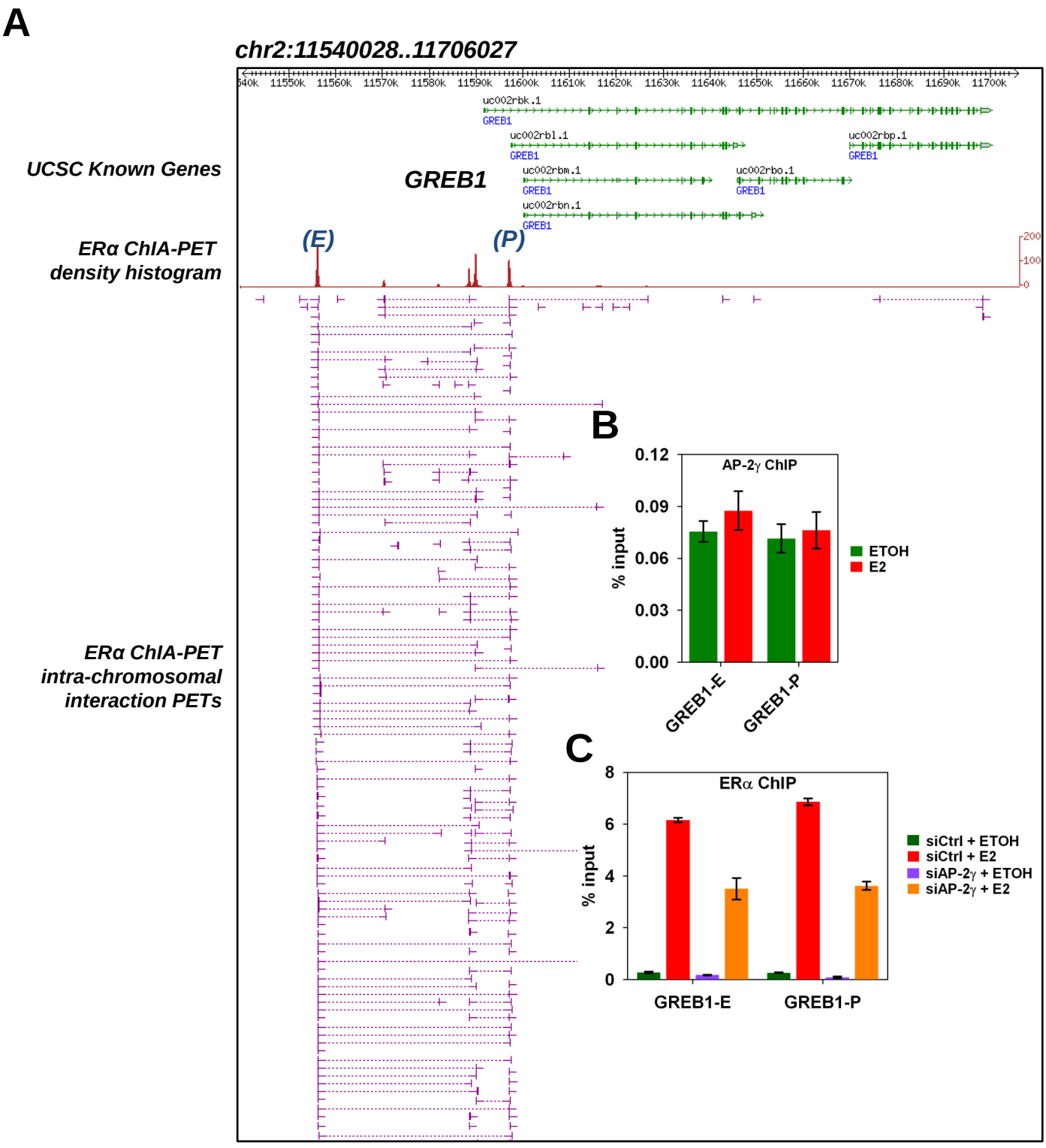


Supplementary Figure 1. AP-2γ is co-occupied at ERBS. (A) Motif screen of high confidence and all ERBS identified from the ChIA-PET of ERα. Genomic regions containing ±250 bp encompassing the peaks of ERBS were scanned for enriched motifs from the TRANSFAC database. The top 10 results from the scan are shown. (B) The frequency of predicted ERE (left panel) and AP-2 (right panel) motif occurrence was analyzed with respect to the center of ERBS. (C) ChIP using anti-AP-2γ was performed in MCF-7 cells treated with or without E2 for 45 min. Binding of AP-2γ was assessed by real-time PCR at ten ERBS with predicted AP-2 motif. The result represents the average of three independent experiments ±S.E.M.

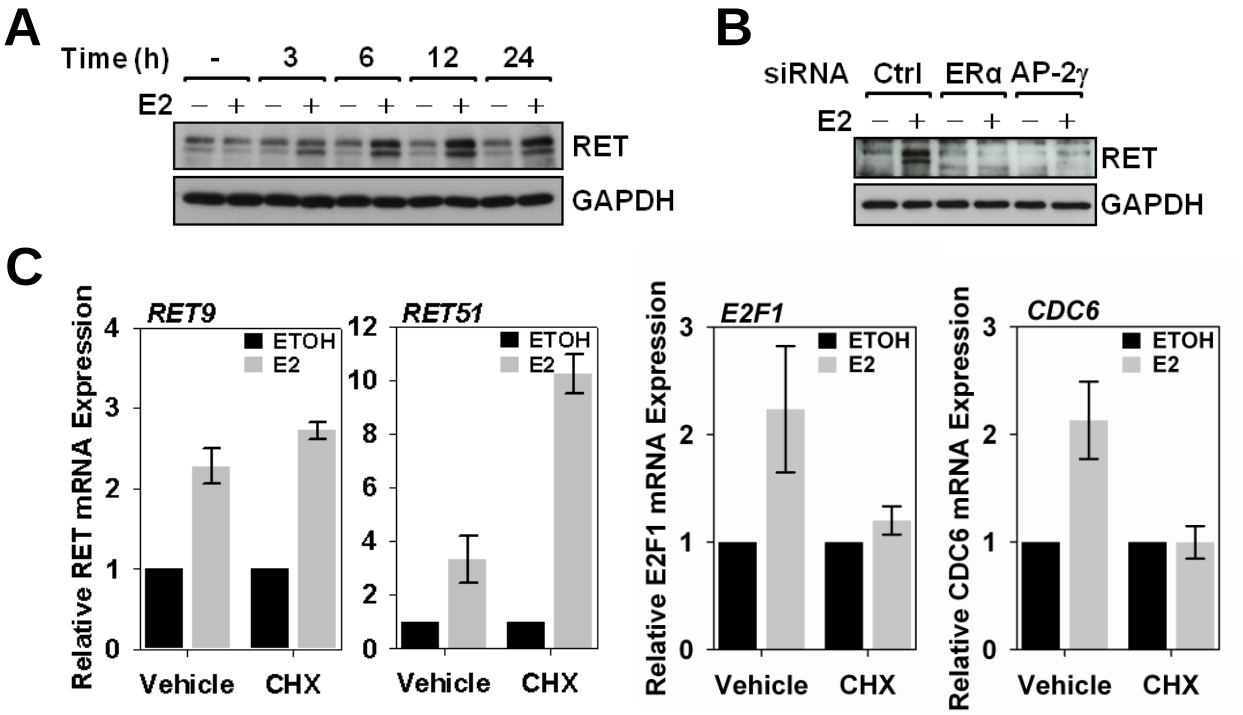


Supplementary Figure 2. AP-2 motif occurrence in ChIA-PET ERBS. Whole genome was sampled every 2 kb (500 bp each), binned according to the GC content and 50,000 sites with varying GC content were selected. Using these 50,000 sites, conditional probability of a 500 bp sequence containing AP-2 motif for a given GC content was tabulated. GC content distribution of ChIA-PET ERBS was determined and 14,468 sequences (500 bp each) with the similar GC content distribution were modeled using the above conditional probability and scored for the expected AP-2 motif occurrence based on the hypothesis that the occurrence is purely due to GC content. *P*-value of the significant difference between the expected motif occurrence and observed motif occurrence in the ChIA-PET ERBS was obtained by approximating the model with normal distribution.

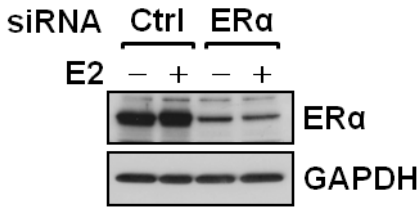
Tan_Suppl. Fig3



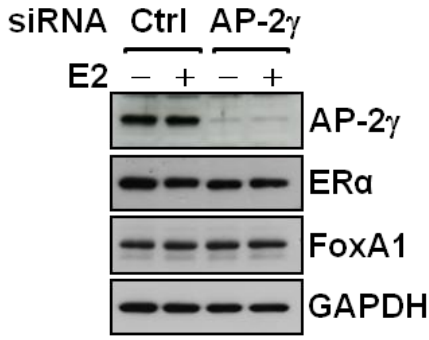
Supplementary Figure 3. Recruitment of AP-2γ to GREB1-associated ERBS. (A) Screenshot of ERα ChIA-PET analysis showing ERα binding and long-range chromatin interactions at the GREB1 gene locus. GREB1-associated ERBS defined as enhancer and promoter are denoted as E and P, respectively. (B) AP-2γ binding was assessed by real-time RT-PCR at GREB1-E and –P after 45 min of E2 or vehicle treatment. (C) ERα binding was assessed at GREB1-E and -P in MCF-7 cells transfected with control or AP-2γ siRNA. All results represent the average of 3 independent experiments ±S.E.M.



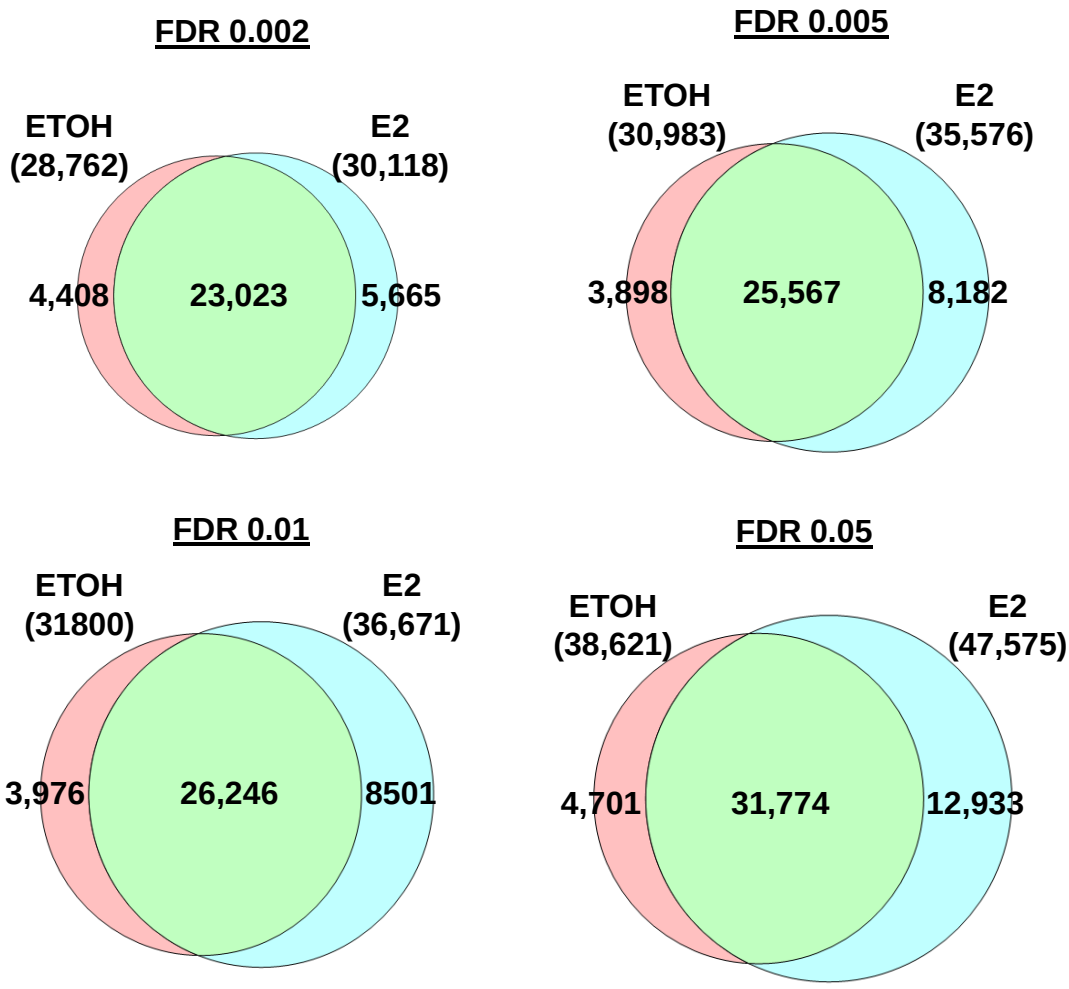
Supplementary Figure 4. AP-2γ is required for the expression of the ERα direct target gene, RET. (A) MCF-7 cells were stimulated with or without E2 for 0, 3, 6, 12 and 24 hours and then analyzed by western blot with antibodies directed to RET and GAPDH. (B) MCF-7 cells were transfected with control, ERα or AP-2γ siRNA, stimulated with or without E2 for 12 hours and then analyzed for RET and GAPDH protein expression. (C) MCF-7 cells were treated with or without cycloheximide (10 μg/ml) for 1 hour prior to 12 hours of E2 or vehicle treatment and then analyzed for RET9 and RET51 mRNA levels. E2F1 and CDC6 which were reported previously as secondary estrogen target genes (Bourdeau et al, 2008) were included as positive controls. All results represent the average of 3 independent experiments ±S.E.M.



Supplementary Figure 5. Knockdown of ERα in MCF-7 cells. Protein level of ERα was assessed in MCF-7 cells transfected with control or ERα siRNA, in the presence and absence of E2 stimulation for 45 min. GAPDH was used as a loading control.



Supplementary Figure 6. Knockdown of AP-2γ in MCF-7 cells. Protein level of AP-2γ, ERα and FoxA1 were assessed in MCF-7 cells transfected with control or AP-2γ siRNA, in the presence and absence of E2 stimulation for 45 min. GAPDH was used as a loading control.

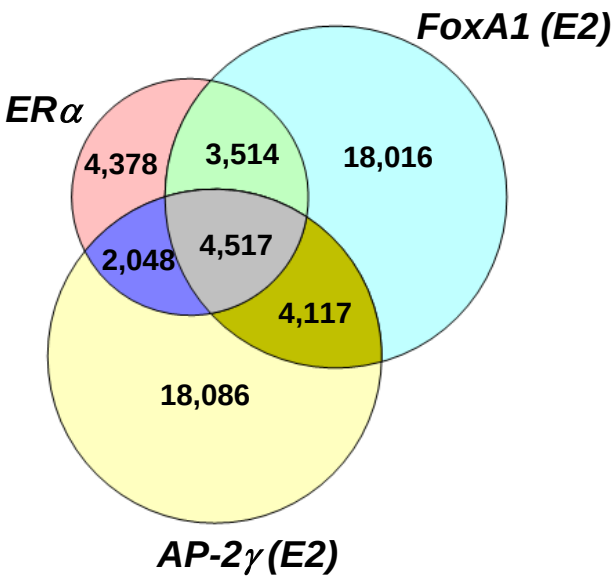


Supplementary Figure 7. Comparison of AP2GBS in MCF-7 cells at different thresholds shows that the majority of AP2GBS are present before and after E2 stimulation. AP2GBS in ETOH and E2 stimulated MCF-7 cells were compared using AP-2γ ChIP-seq peaks called at various false discovery rates (FDRs) ranging from 0.002-0.05. The proportion of overlap between AP2GBS under ETOH and E2 conditions does not change significantly at different FDR cut-offs.

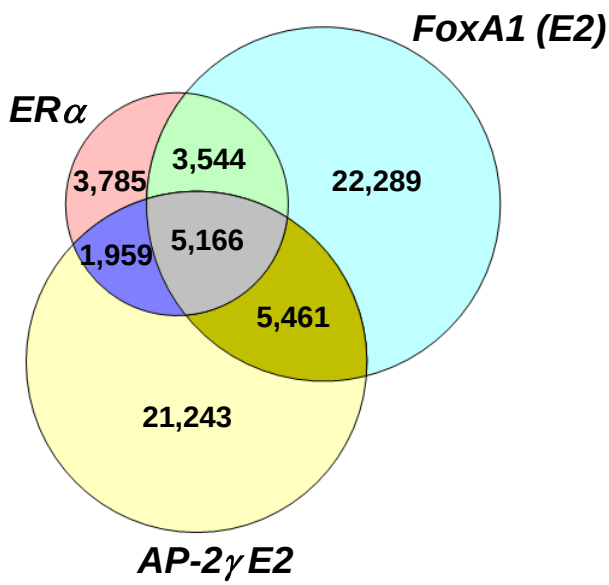
Supplementary Figure 8. Comparison of FoxA1BS in MCF-7 cells at different thresholds shows that the majority of FoxA1BS are present before and after E2 stimulation. FoxA1BS in ETOH and E2 stimulated MCF-7 cells were compared using FoxA1 ChIP-seq peaks called at various false discovery rates (FDRs) ranging from 0.002-0.05. The proportion of overlap between FoxA1BS under ETOH and E2 conditions does not change significantly at different FDR cut-offs.

Tan_Suppl. Fig9

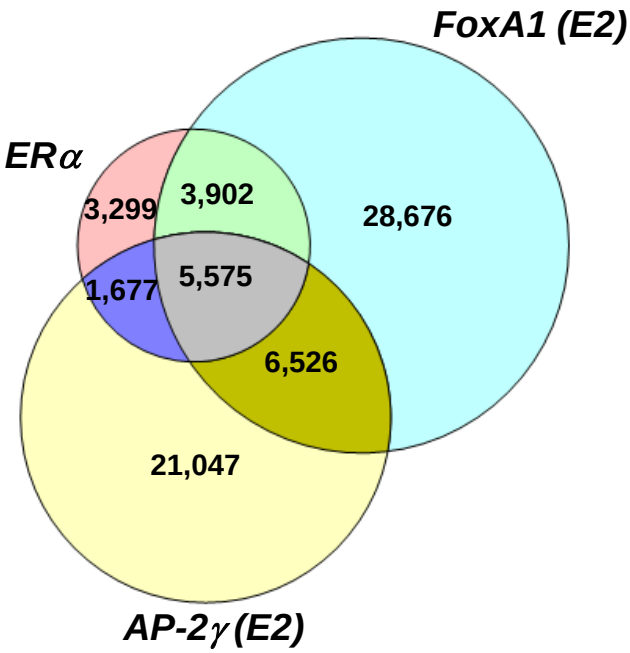
FDR 0.002



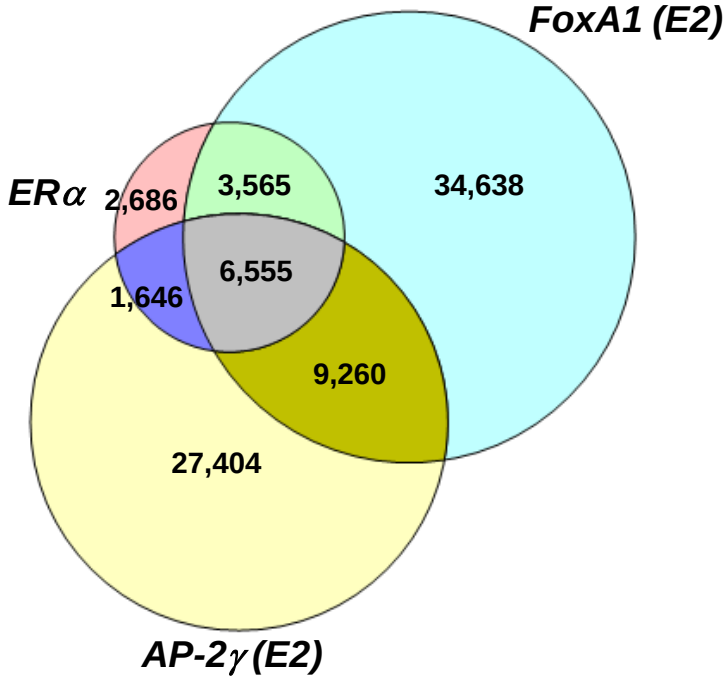
FDR 0.005



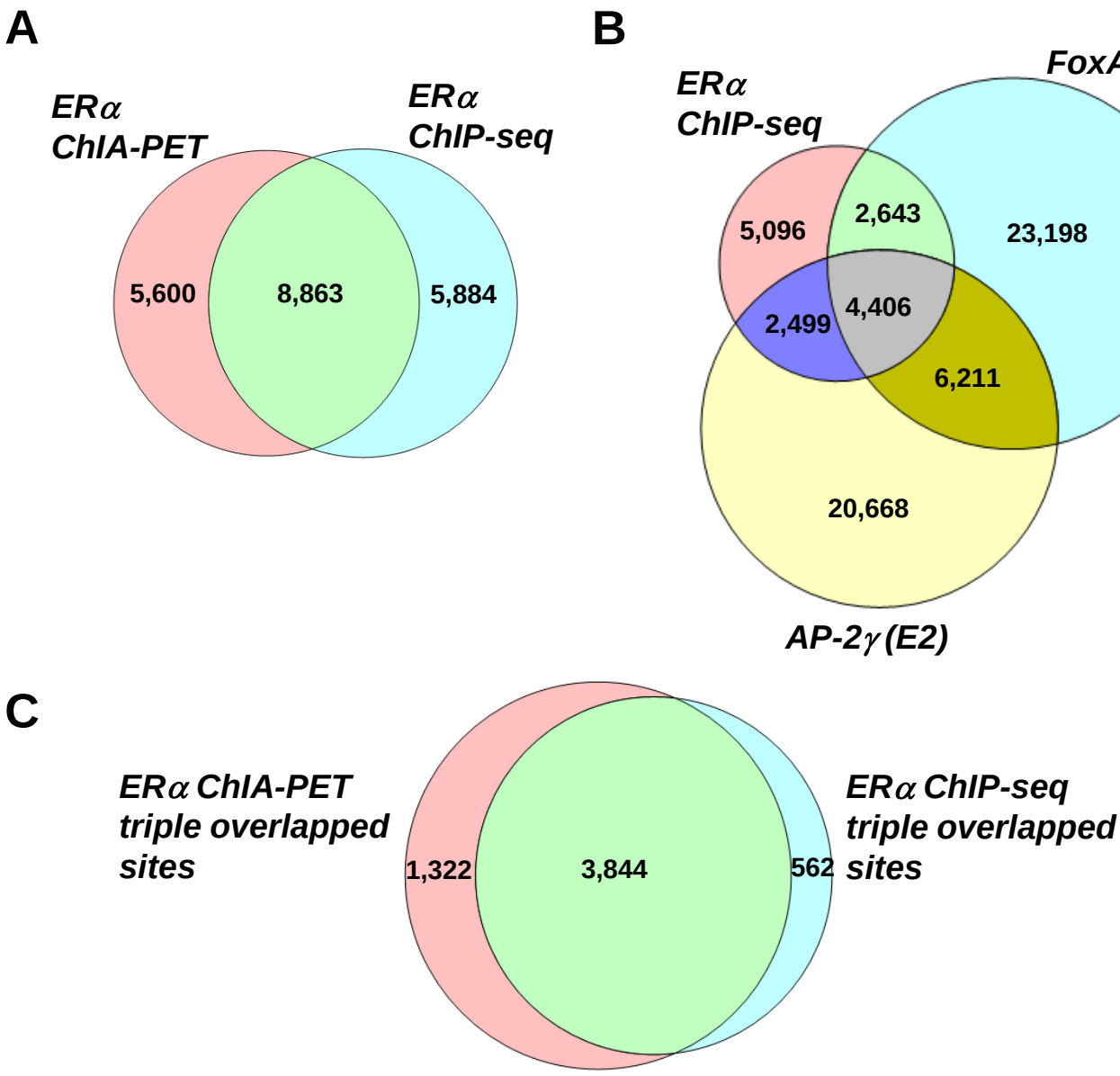
FDR 0.01



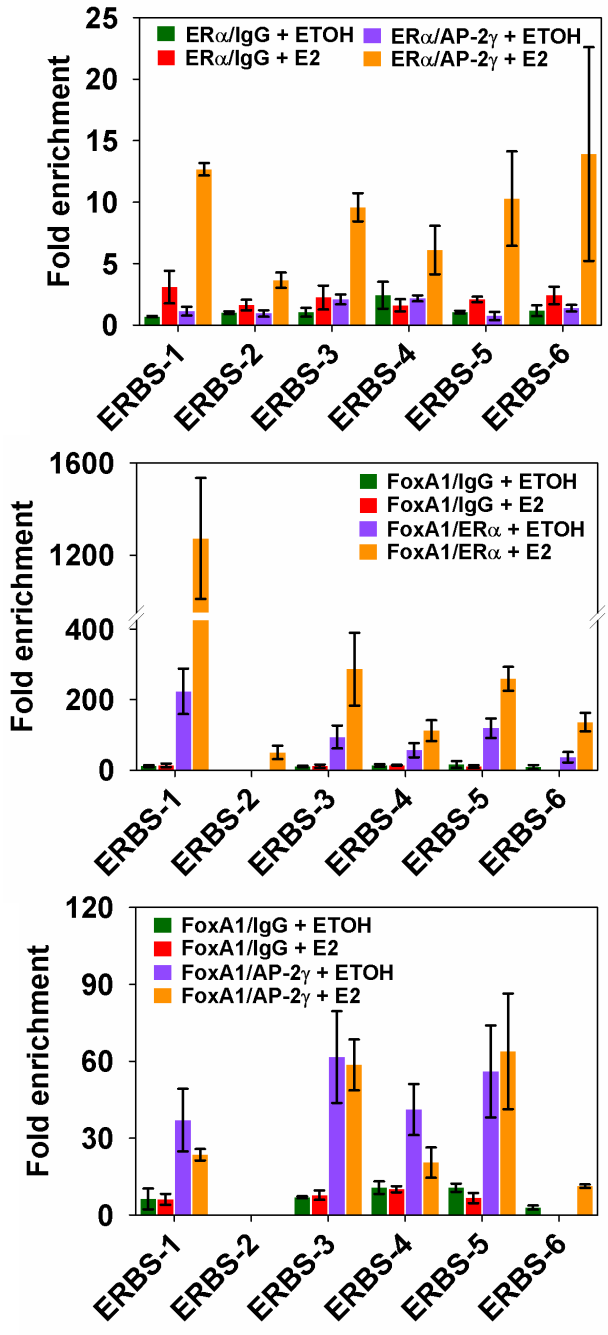
FDR 0.05



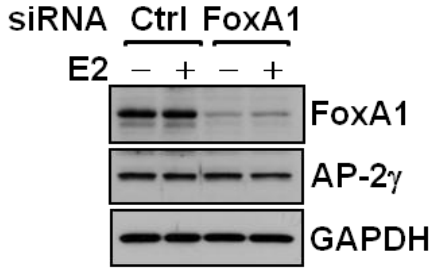
Supplementary Figure 9. Comparison of ChIA-PET ERBS with AP2GBS and FoxA1BS in MCF-7 cells at different thresholds shows that a large co-localization of the three factors. ChIA-PET ERBS were compared using AP-2γ and FoxA1 ChIP-seq peaks called at various false discovery rates (FDRs) ranging from 0.002-0.05. Although the number of AP2GBS and FoxA1BS varies at different FDR cut-off, there is only a slight difference in the triple overlap of ERBS, AP2GBS and FoxA1BS.



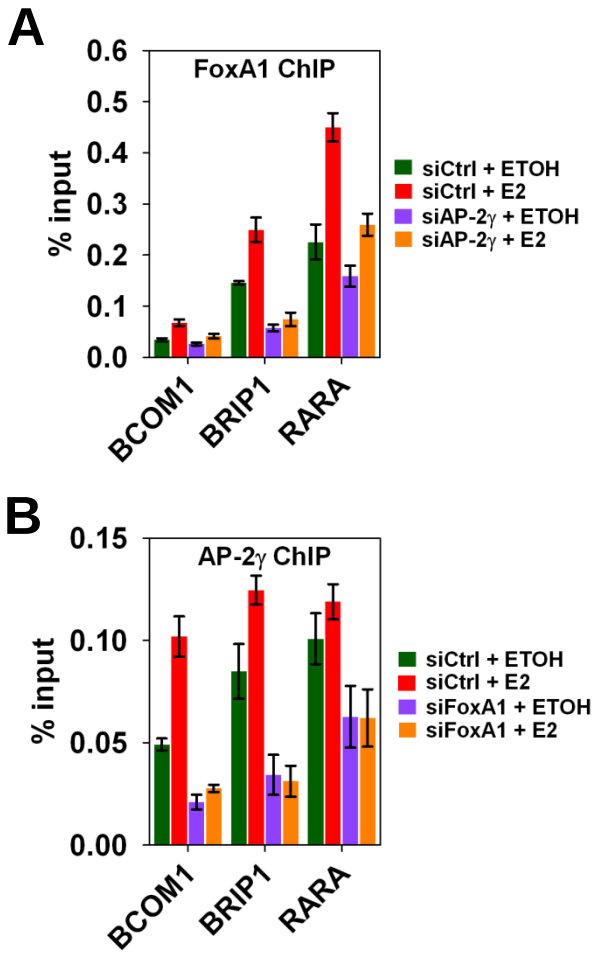
Supplementary Figure 10. ERBS identified from ChIA-PET and ChIP-seq approach are highly similar in their co-localization with AP-2γ and FoxA1. (A) Comparison of ChIA-PET ERBS and ChIP-seq ERBS. (B) ERα ChIP-seq peaks overlapped with AP-2γ and FoxA1 ChIP-seq peaks. (C) Analysis of triple overlapped sites (ERBS/AP2GBS/FoxA1BS) using ERBS identified from ChIA-PET and ChIP-seq.



Supplementary Figure 11. Co-localization of ERα and FoxA1 at ERBS associated with RET. MCF-7 cells were treated with or without E2 for 45 min and sequential ChIP was performed with either ERα or FoxA1 antibodies followed by a second antibody or IgG as a negative control. The binding was expressed as fold enrichment relative to a genomic control site.



Supplementary Figure 12. Knockdown of FoxA1 in MCF-7 cells. Protein level of FoxA1 and AP-2γ were assessed in MCF-7 cells transfected with control or FoxA1 siRNA, in the presence and absence of E2 stimulation for 45 min. GAPDH was used as a loading control.



Supplementary Figure 13. AP-2γ and FoxA1 are mutually dependent for their recruitment at ERBS. (A) ChIP for FoxA1 was performed on MCF-7 cells transfected with control or AP-2γ siRNA and treated with or without E2 for 45 min (B) ChIP for AP-2γ was performed on MCF-7 cells transfected with control or FoxA1 siRNA. Binding of FoxA1 and AP-2γ were examined at ERBS with AP-2γ and FoxA1 co-localization.

Table S1: Summary of ChIP-seq analysis

Library ID	Description	Mapping tags	False discovery rate (FDR)	Number of peaks called
CHM038	AP-2γ EtOH	13468527	0.005	30,983
CHM039	AP-2γ E2	13520837	0.005	35,576
CHM046	FoxA1 EtOH	14341323**	0.005	38,141
CHM047	FoxA1 E2	14341323	0.005	37,478

** Mappable tags from CHM046 library were randomly resampled from 17060651 to 14341323 for more accurate comparison of FoxA1 binding intensities under vehicle and E2 conditons.

Table S2: Cloning and mutagenesis primer sequences

Cloning primers	
Primer name	Sequence (5'-3')
ERBS-1_F	ATCCACACATCCCTTCTGCT
ERBS-1_R	GGAAAGGGAGAGGAGCGAGAT
ERBS-2_F	CCCCAACTAATTCCCTTGGT
ERBS-2_R	GTCAGAGTGTGGATGCTTGGA
ERBS-3_F	GCAGAGCAGTGAGGCACAG
ERBS-3_R	GGAGGGAGCCCTCATCTGAA
ERBS-4_F	CTAGGAGGGAAGGGGAGTTG
ERBS-4_R	GAATGTCTGCCAGGAGAATGC
ERBS-5_F	GGATTGGCGCTGAGACAATG
ERBS-5_R	CTGTAGGGCCACAGGTTCTC
ERBS-6_F	CTCGCCATCTGTGGAACTTT
ERBS-6_R	GCCTGTAATGGCCTGAGGGTA
Mutagenesis primers	
Primer name	Sequence (5'-3')
ERBS-1_EREmt	CAAGGTGCGCGGAGCCCAGAGGGTGATTCAGCTTGCTGACGAG
ERBS-6_EREmt	GAACCTCGAGGCCCTGAATTGCCTTGATATCCAGCTCCCAGGAAC
ERBS-1_AP2mt	TCCGGGACAACGCGAACAGGGGCTCTGGAC
ERBS-6_AP2mt	GTTGAGTCAGGGCCTGAATGGAACTTTTCCTGCCACC
ERBS-1_FOXA1mt	TCACCACGGTAATGCTGTATTGGGGCCTGGCACCATCACC

Mutated nucleotides are underlined

Table S3: Real-time PCR primer sequences

ChIP real-time PCR primers	
Primer name	Sequence (5'-3')
ERBS-1_F	CCCTGAGGGCGCAGAGA
ERBS-1_R	GGGATGGCAAGGTTAGAAGCT
ERBS-2_F	GGAACAGACACCAGCATATCCA
ERBS-2_R	CCTCGGTTTCCCTTTCTTTGA
ERBS-3_F	GGCATAAGCTCTGTGCAAACAT
ERBS-3_R	CATTTCCATGGTGTTTTATTAAAGGA
ERBS-4_F	TGTTCTCTCCCTGCGAGTTGT
ERBS-4_R	GAAGGAGCGACGCAACCA
ERBS-5_F	AAGGAGTGGCTCCACAAAGTGT
ERBS-5_R	TGCAGCGGTGACCTTTCTG
ERBS-6_F	GCTGTCTCCAGGCCCAGTT
ERBS-6_R	TGAGGGTAGAGATTCCCACACA
BCMO1_F	TGTGTGAGGCCCGAGGTT
BCMO1_R	CCAGGTCCACAGTGCTTCCT
BRIP1_F	CAGGCATGCTTTCCAAACAA
BRIP1_R	TGCCTTGGGCCTTTTGC
CELSR2_F	GCCGAGCAGTTGGGAACA
CELSR2_R	GATGCGGAGGCAACTACCA
FOS_F	CCCGAACCACCCAAACCT
FOS_R	CCAAGGAGGCAAAGAGAGACA
RARA_F	GTACCCCGCAGGCAGTGT
RARA_R	GGATAAAGCCACTCCAAGGTAGGT
TH_F	GCTGGCTGTGGCACAAGAT
TH_R	CGCAGGCAAGGTCAGGAT
VASN_F	TGACAGGTCAGGCCAGTCTTC
VASN_R	TGCCACGGCAGACAGGAT
FAM129B_F	CCCCTCAACCACAGTTAATGC
FAM129B_R	GACAGCGGAGAGCAGTCTCAGT
CALML5_F	GCACTGGCCAGGCTCTGA
CALML5_R	GGTCACTCATTGCGACCTGTT
CST6_F	TGGCCGGCATCAAGTACTTC
CST6_R	TGGTCTTGCGGCAGTCTGT
Greb1 Enh_F	GCTGGGTGCCCGTTTTG

Greb1 Enh_R		TCAGTCAGCAGTTTCGGTGAGT
Greb1 Pro_F		CACTGTGACCCAGCAAAACAC
Greb1 Pro_R		GGCAAATGCCACCGTTTC
Control_F		CCTGGAGGGCTTGGAGATG
Control_R		GATCCTACGGCTGGCTGTGA
3C real-time PCR primers		
Primer pairs		Sequence (5'-3')
AB	A	CATGGGAGAAAGATGTAGTCTGGGAGAC
	B	AACCCCGTGTGTCCTTCAG
BC	B	AACCCCGTGTGTCCTTCAG
	C	ATCAAACTGGAGGGAGCAGA
BD	B	AACCCCGTGTGTCCTTCAG
	D	GAAAGGACAGAGAAGGTGCCAGTTG
BE	B	AACCCCGTGTGTCCTTCAG
	E	GCCAGTGGAAGTGTAAGTTGG
BF	B	AACCCCGTGTGTCCTTCAG
	F	CATGTTGTCAGTGGGGGTTA
BG	B	AACCCCGTGTGTCCTTCAG
	G	GGATGTAGTGGCACCAGCCATAGG
BH	B	AACCCCGTGTGTCCTTCAG
	H	AGGGTGGGAAGAGGTGAC
BI	B	AACCCCGTGTGTCCTTCAG
	I	ACCGTCACTTTCCCTGTGTT
BJ	B	AACCCCGTGTGTCCTTCAG
	J	CTAGGGGTGGTCACCTCAGC
BK	B	CCGCGTCCTCTGTACTTGA
	K	CATTCAGTGGCATCAACGTC
IJ	I	AACCCCGTGTGTCCTTCAG
	J	GGCCCTGATGACCTGTCCTTATTC
mRNA real-time RT-PCR primers		
Primer name		Sequence (5'-3')
ERα_F		GAATCTGCCAAGGAGACTCGC
ERα_R		ACTGGTTGGTGGCTGGACAC
AP2γ_F		ACTGTCCCCACCTGAATGCT
AP2γ_R		CGATTTGGCTCTTCTGAGAACA
RET9_F		CCGCTGGTGGACTGTAATAATG
RET9_R		GTAAATGCATGGGAAATTCTACCAT
RET51_F		GAGCCCTCCCTTCCACATG
RET51_R		GGACTCTCTCCAGGCCAGTTC
GREB1_F		CAGGCTTTTGCACCGAATCT
GREB1_R		CAAAGCGTGTCGTCTTCAGCT
E2F1_F		AGATCCCAGCCAGTCTCTACTCA

E2F1_R	TGCCCATCCGGGACAA
CDC6_F	ACGTCTGGGCGATGACAAC
CDC6_R	TTGGTGGAGAACAAGGAGGTAAA
GAPDH_F	GGCCTCCAAGGAGTAAGACC
GAPDH_R	AGGGGAGATTCAGTGTGGTG