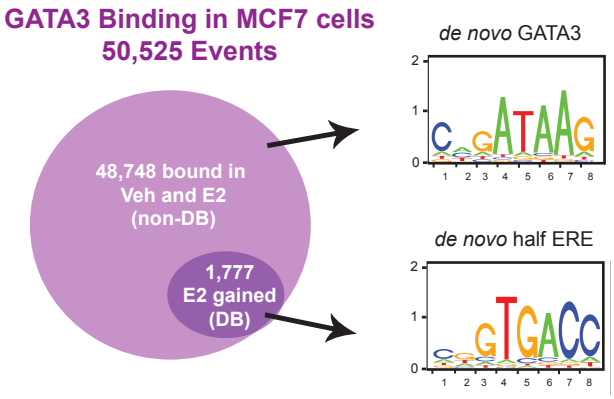
Supplemental Figure 1

(A) GATA3 ChIP-Seq in MCF7 identified 50,525 binding events, most of which are bound in the absence of estrogen. Differential Binding Assay (DBA) identified 1,777 events which are significantly enriched following E2 treatment (DB**). De novo motif analysis revealed a longer GATA3 motif and a half ERE from these two classes of GATA3 binding.
(B) Log concentration of reads from all replicate experiments shows that the non Differentially Bound (non-DB) GATA3 are strong events in Veh conditions (approximate median 3). The Differentially Bound (DB) GATA3 events, which are weak or unbound in Veh (median of 1.4), become strongly bound after E2 treatment (median of 4.2, p<10-16 by Wilcoxon rank sum test).
(C) Known transcription factor motif enrichment analysis. Binding events, centered in the middle of each peak and with the addition of 1kb on either side, were investigated, within 100bp windows, for their enrichment of known motifs from the Jaspar Core Vertebrates. Subsequently, only motifs with a p-value < 0.0005 were used. Enriched DNA motifs in the different sub-classes of ESR1, GATA3 and FOXA1 binding events presented in Fig.1B.
(D) Overlap of the binding sub-classes of ESR1, GATA3 and FOXA1 with ChIA-PET data (Fullwood, 2009) that represent ESR1 contact points involved in chromosomal interactions. The first column shows the percentage of ESR1 ChIA-PETs in the seven sub-classes of binding events. The second column shows the percentage of the seven sub-classes.

A



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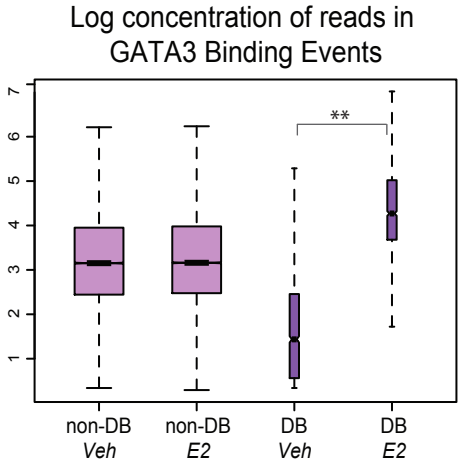

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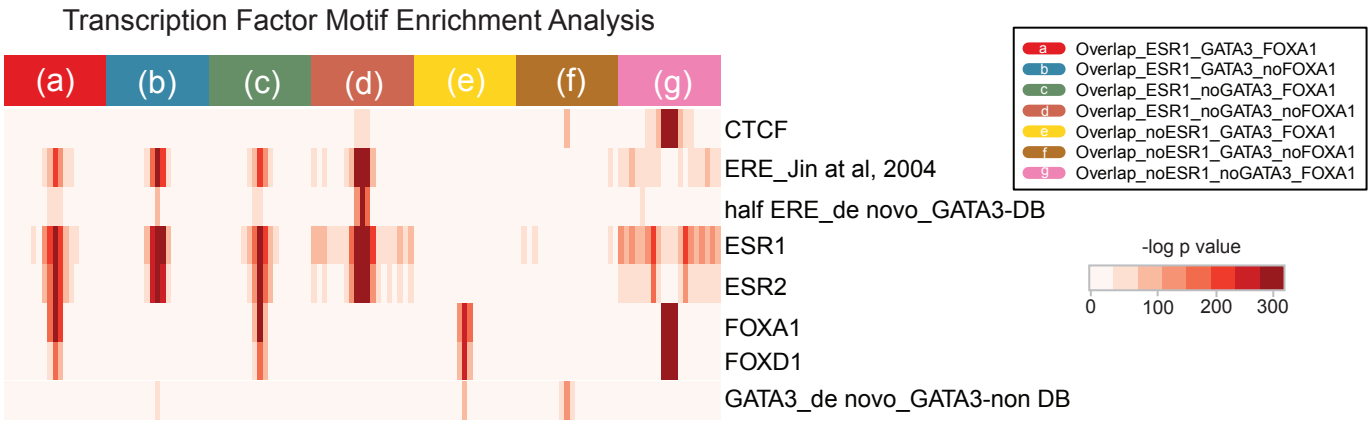


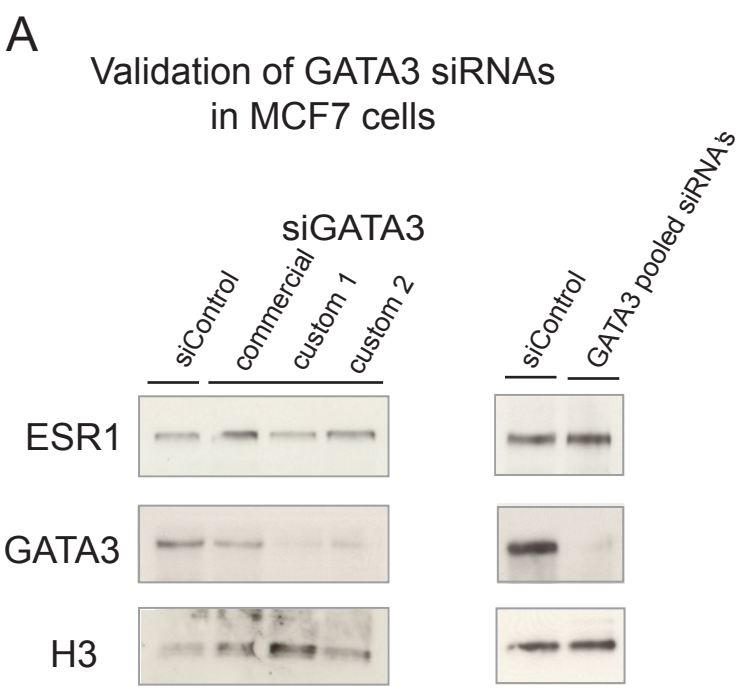
D

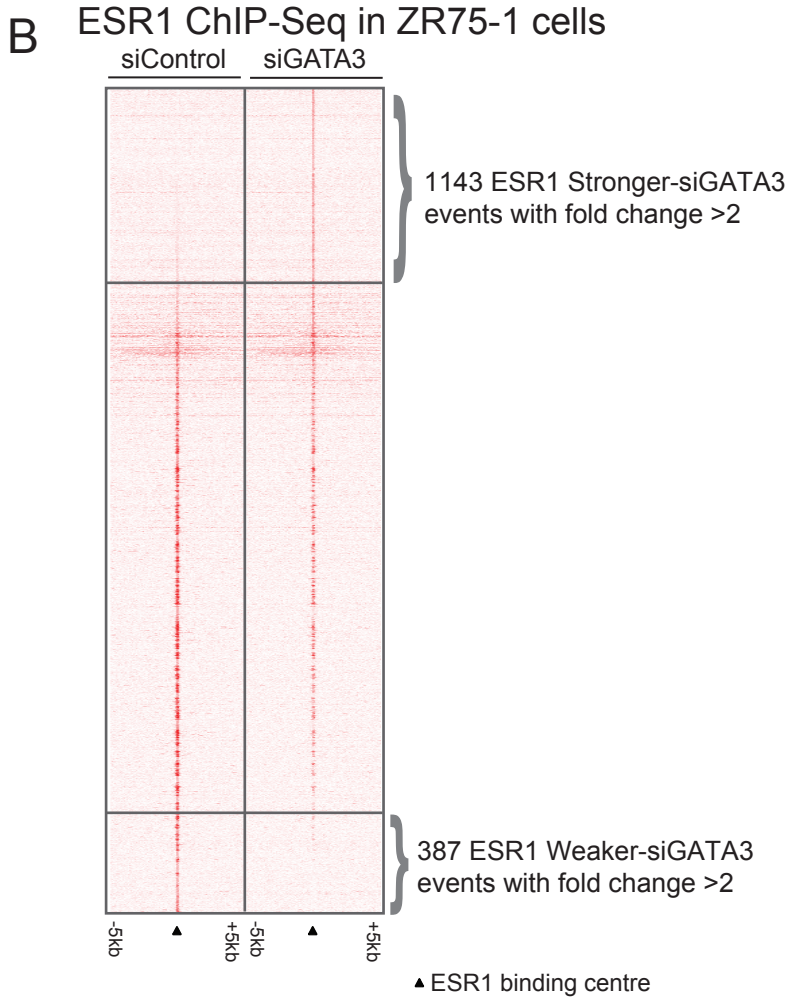
Sub-classes of binding events	Percentage of ESR1 looping events	Percentage of sub-classes binding events
(a) ESR1:GATA3:FOXA1	44	9
(b) ESR1:GATA3	18	7
(c) ESR1:FOXA1	12	4
(d) ESR1	18	2
(e) GATA3:FOXA1	<1	<0.1
(f) GATA3	<1	<0.1
(g) FOXA1	<1	<0.1

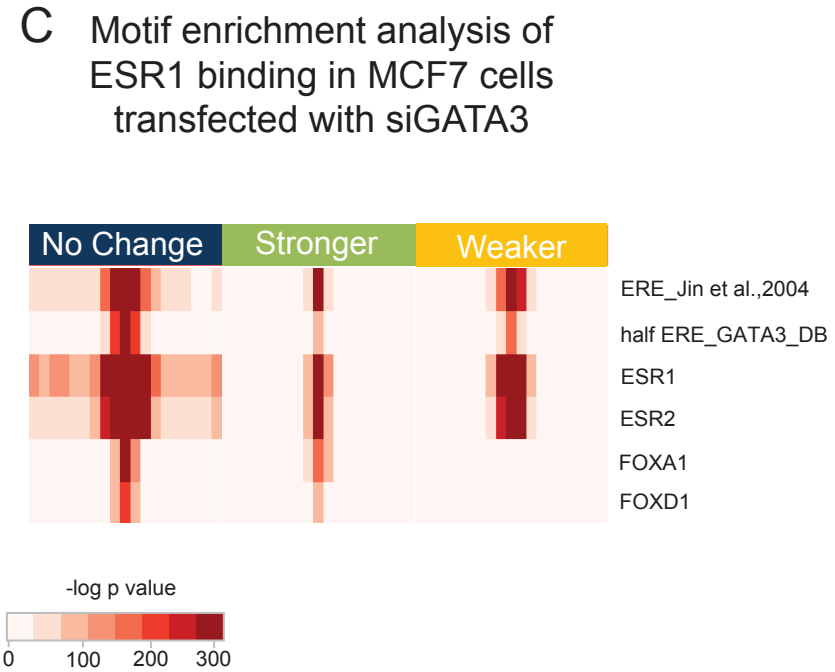
Overlap of ESR1, GATA3 and FOXA1 binding with ESR1 events in chromatin loops

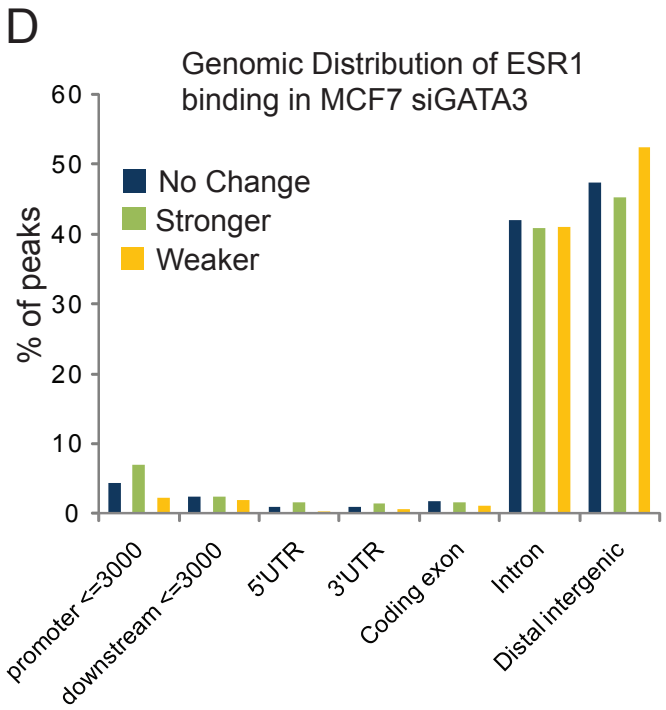
Supplemental Figure 2

(A) Western blots show that efficient silencing of GATA3 does not affect ESR1 protein levels. On the left panel three siRNAs, one commercial and two custom designed, against GATA3 were assessed individually for their knock-down efficiency. On the right, we pooled the custom made siRNAs for our ChIP-Seq experiments.
(B) Silencing GATA3 in another ESR1 positive cell line, ZR75-1, confirms that ESR1 binding is also reprogrammed. Heatmap shows the intensity of the ER binding signal in siControl and siGATA3 conditions.
Based on one replicate; 1143 ESR1 Stronger-siGATA3 binding events show more than 2-fold enrichment in binding signal and 387 ESR1 Weaker-siGATA3 events have less than 2-fold decrease in signal in siGATA3 treated cells.
(C) Enriched motifs in the ESR1 binding events after GATA3 silencing in MCF7 cells.
(D)The genomic distribution of the reprogrammed ESR1 binding events in siGATA3 MCF7 cells have distinct patterns. The ESR1 Stronger events are more enriched at promoters, 5' and 3' UTR elements compared to the whole.



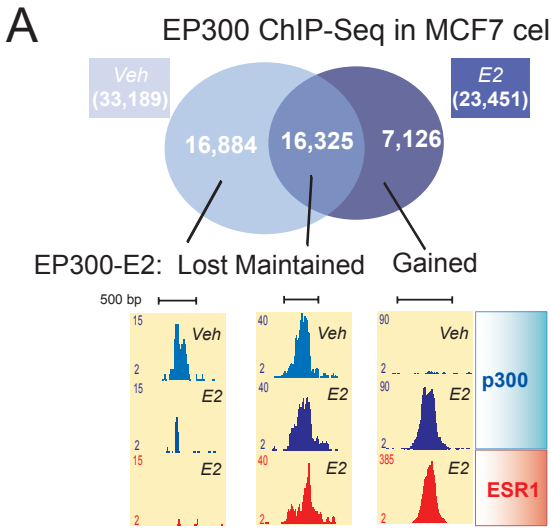


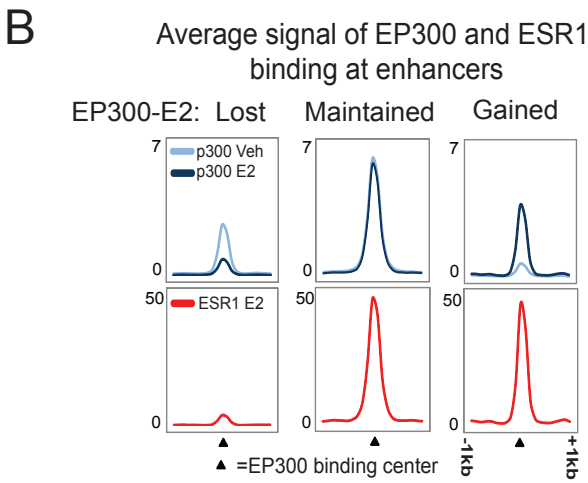


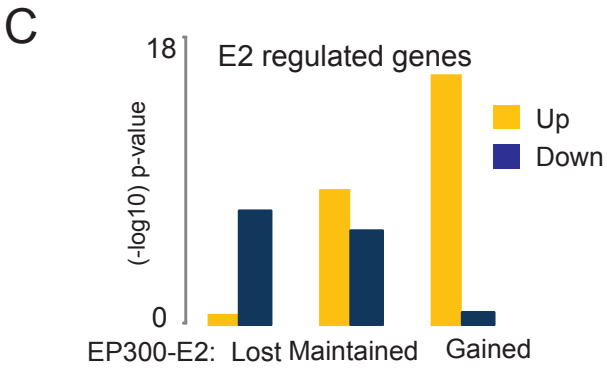


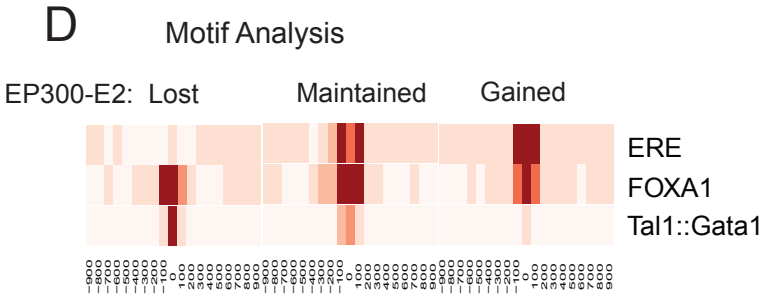
Supplemental Figure 3

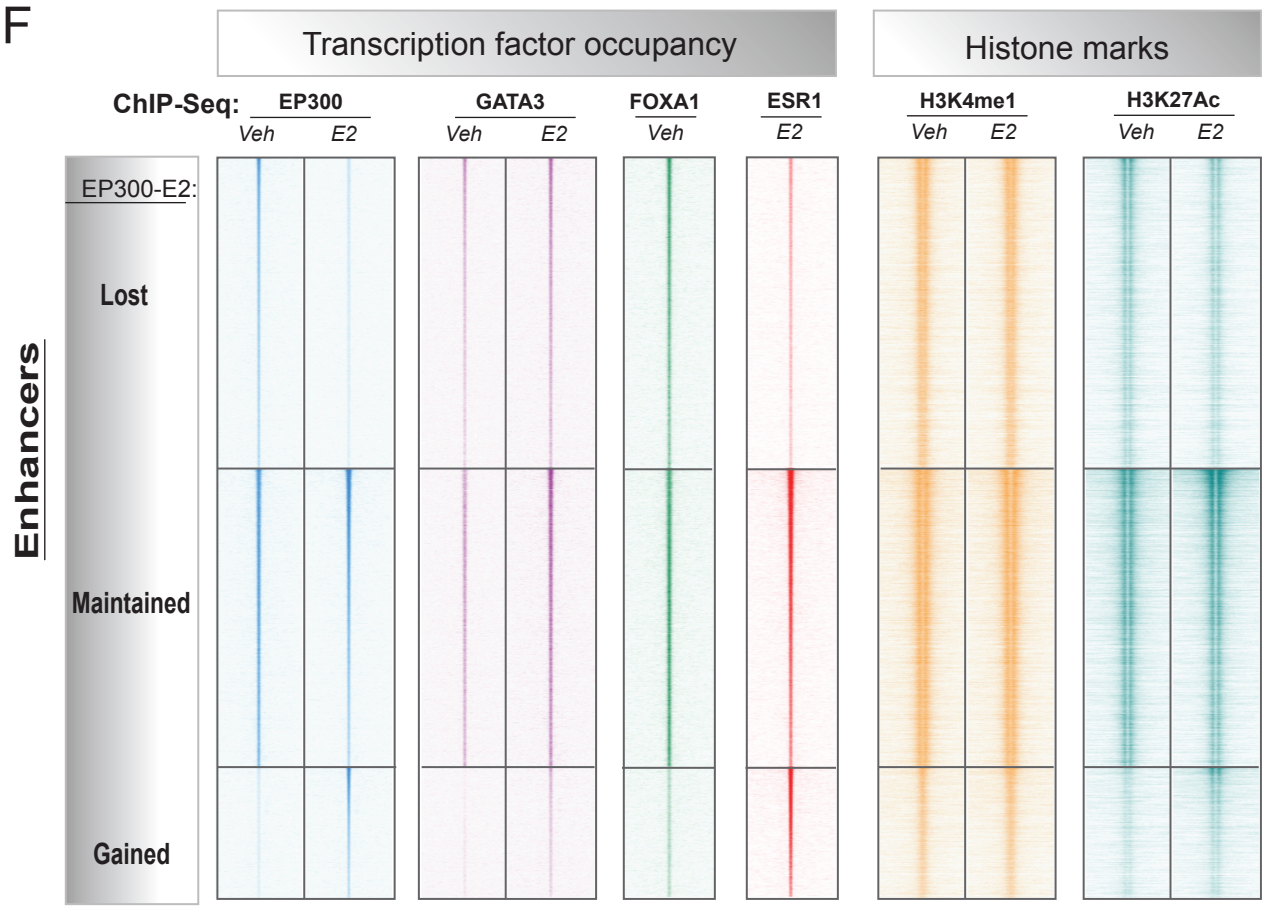
EP300 redistribution is associated with GATA3 and FOXA1 and defines functional enhancer elements involved in the estrogenic response. (A) ChIP-Seq of EP300 in MCF7 cells in estrogen deprived (Veh) and stimulated conditions (E2). (B) Average signal intensity of EP300 and ESR1 binding within the three different EP300 binding categories. (C) EP300 re-destribution in relation to ESR1 transcriptional target genes. E2-gained EP300 binding is enriched near E2 up-regulated genes, whereas the EP300-E2 lost is found in proximity to E2 down-regulated target genes. Y axis shows the –log of p-value from a hypergeometric test, BH corrected. (D) Transcription factor motif enrichment analysis. (E) Heatmap showing ChIP-seq data from different transcription factors (EP300, GATA3, FOXA1 and ESR1), histone modifications (H3K4me1 and H3K27Ac). The heatmap shows signal in a window of -/+5kb and is categorised based on the different EP300 binding categories.





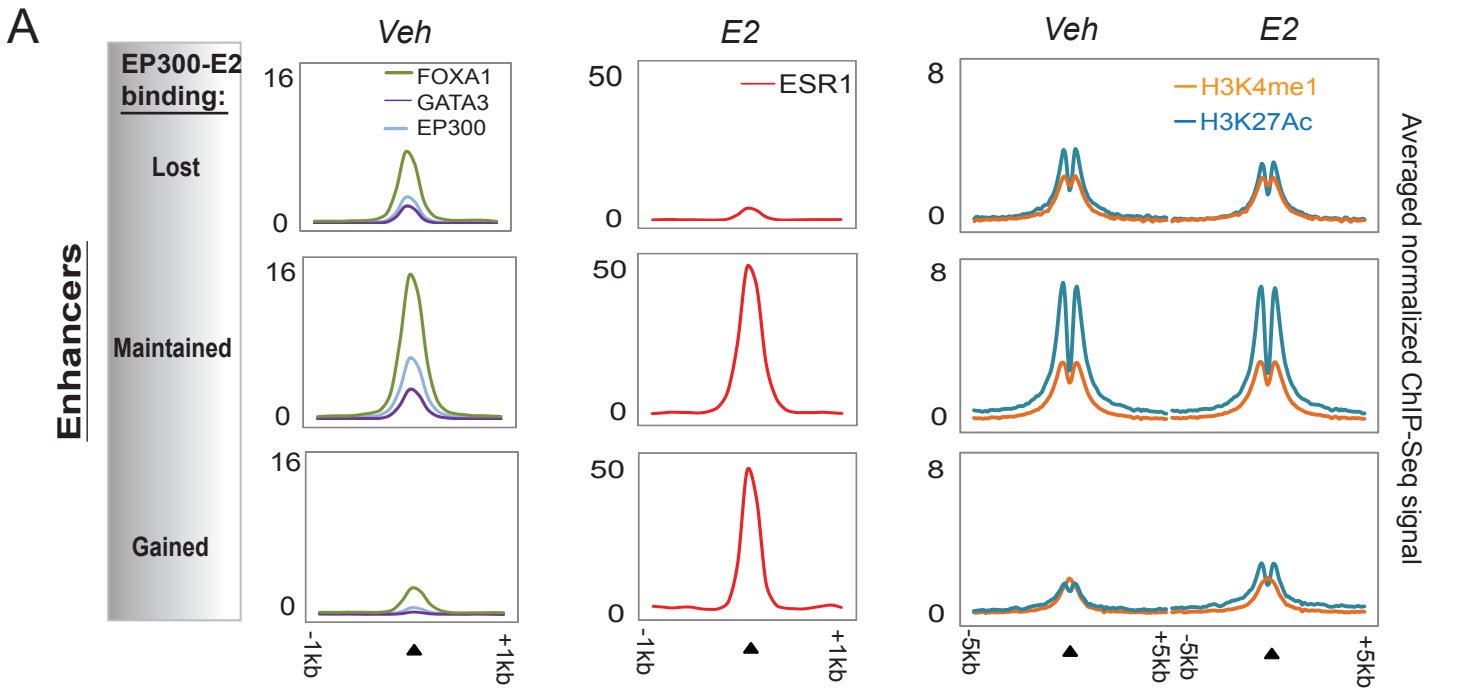


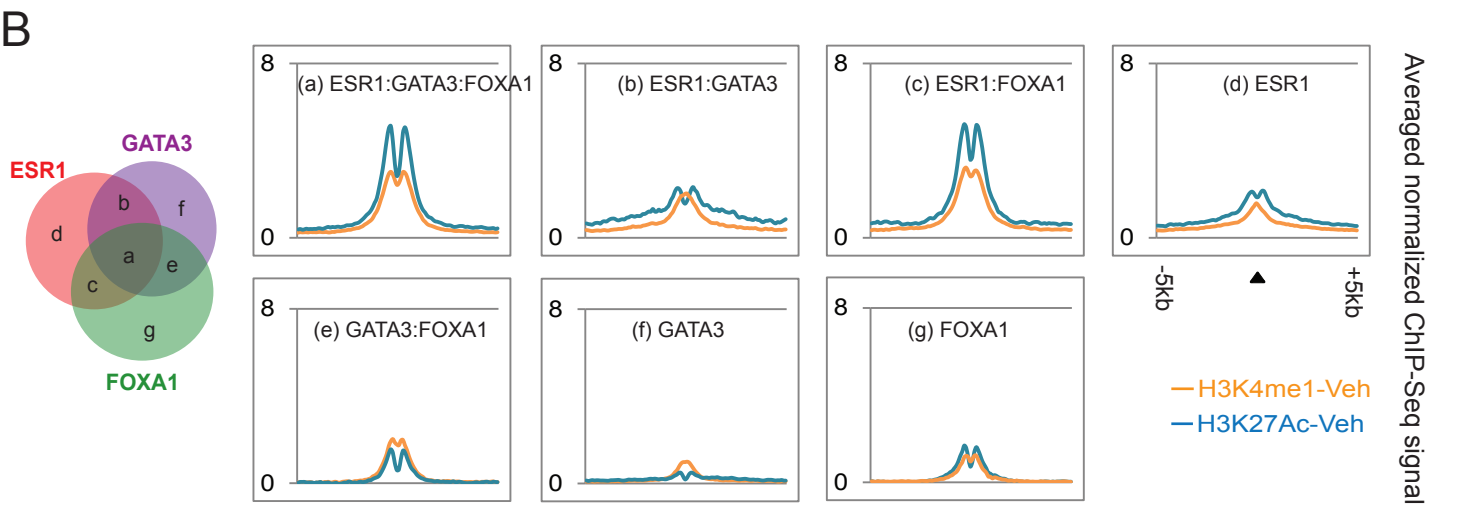


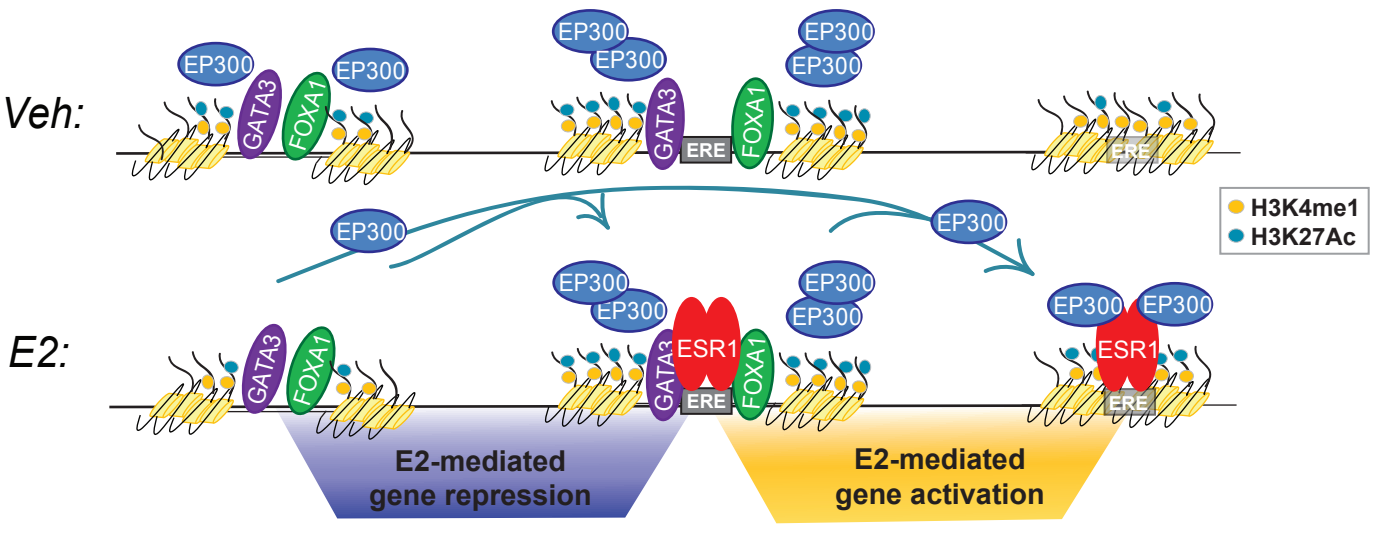


Supplemental Figure 4

(A) Average binding signal intensity of GATA3, FOXA1, EP300 and ESR1 plus the histone modifications, based on the regions where EP300 binding is reprogrammed following estrogen treatment (from Supplemental Fig. 3F). All data is normalized over input.
(B) Averaged signal of H3K4me1 and H3K27Ac histone marks in unstimulated conditions (Veh), centered on the middle of the binding events within the seven sub-classes of ESR1, GATA3 and FOXA1 co-occupied regions.
(C) Model representing the events that occur on the chromatin at enhancer elements prior to E2 treatment and following E2 treatment. Depicted is the redistribution of EP300 following E2 treatment and the properties of these different regions.

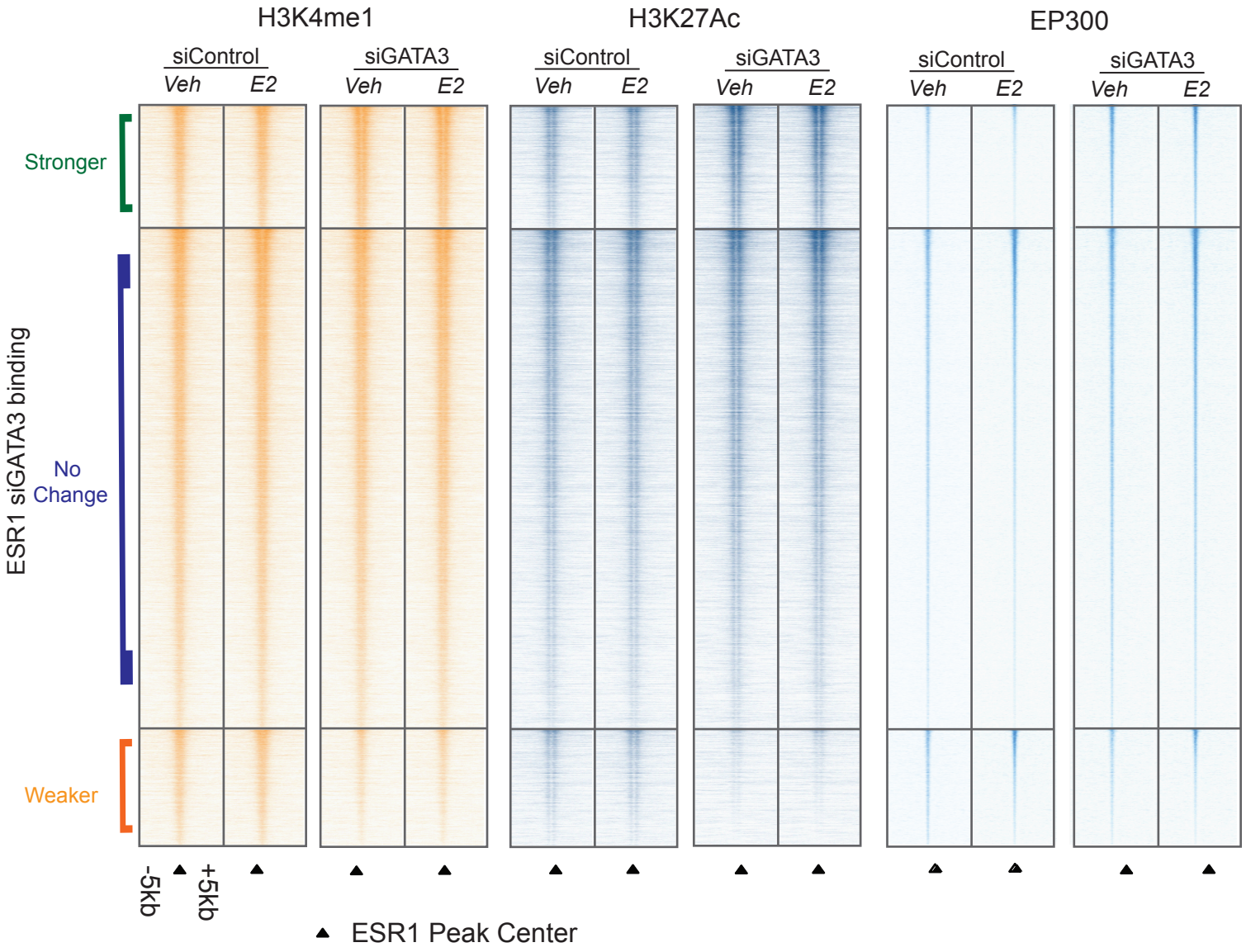






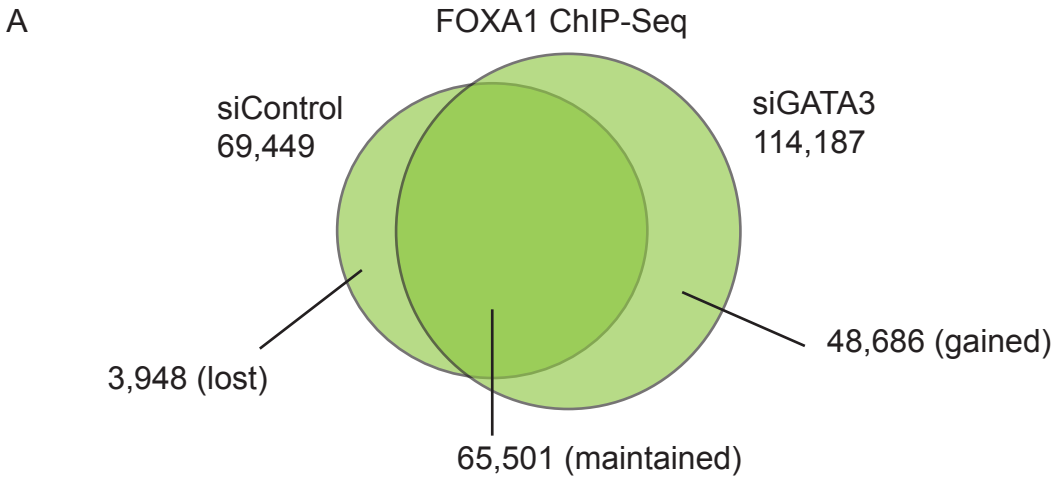
Supplemental Figure 5

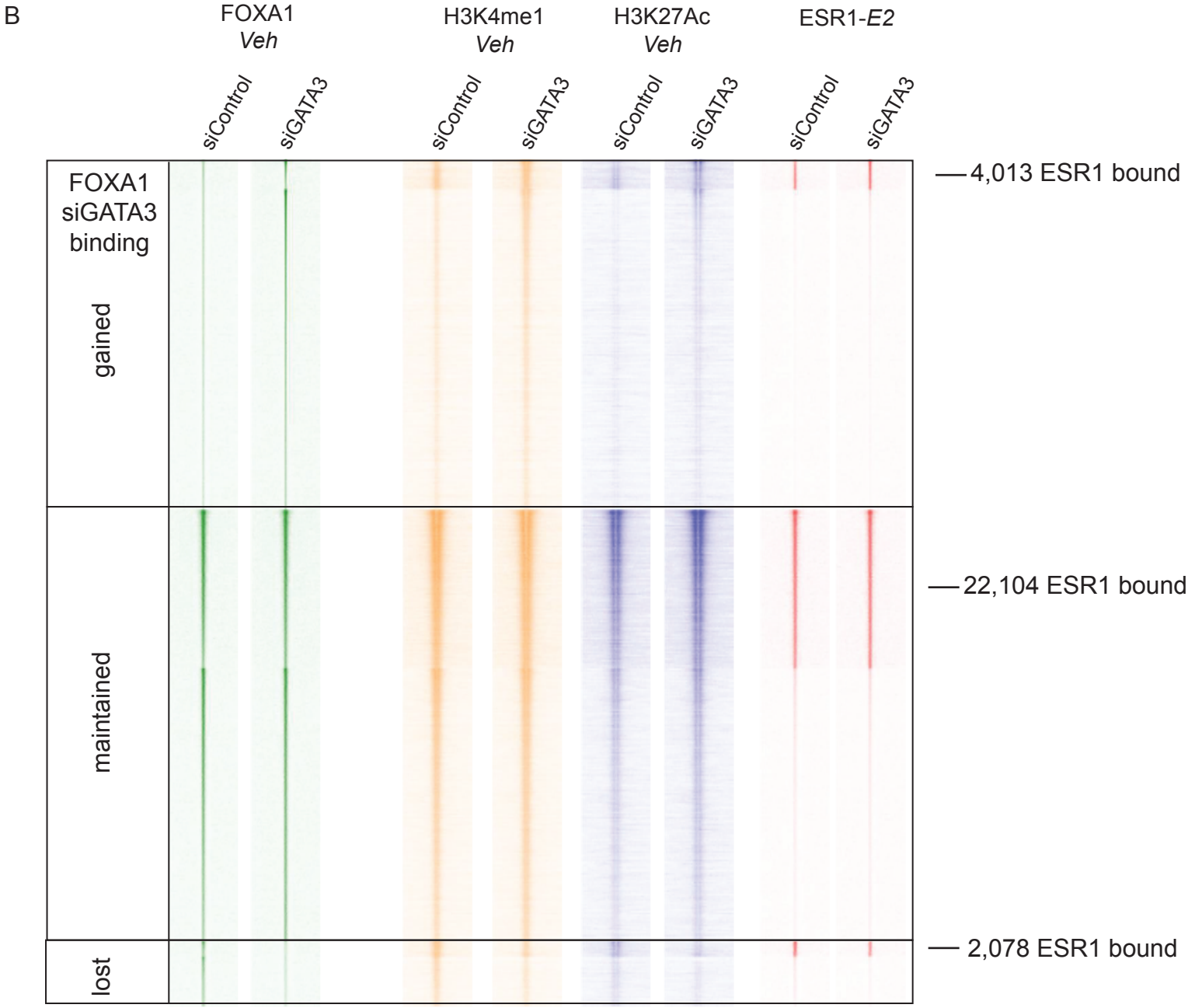
Histone modifications at ESR1 binding events after silencing GATA3.
Heatmaps of H3K4me1, H3K27Ac and EP300 ChIP-Seq data, centered on the ESR1 binding events.
Signal represents enrichment over input and normalized to 10 million reads.



Supplemental Figure 6

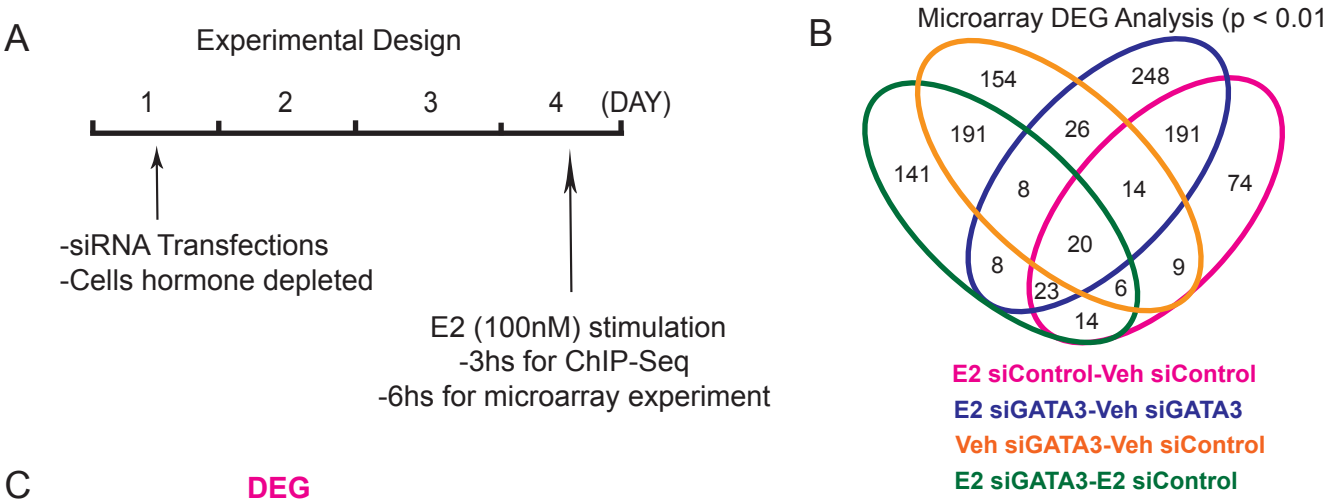
(A) Venn diagram of the FOXA1 binding events in siControl and siGATA3 unstimulated MCF7 cells
(B) Heatmaps of the FOXA1, H3K4me1, H3K27Ac and ESR1 ChIP-Seq signal centered on the FOXA1 binding events presented in (A). The number of sites co-bound by ESR1 is indicated on the right of the heatmaps.

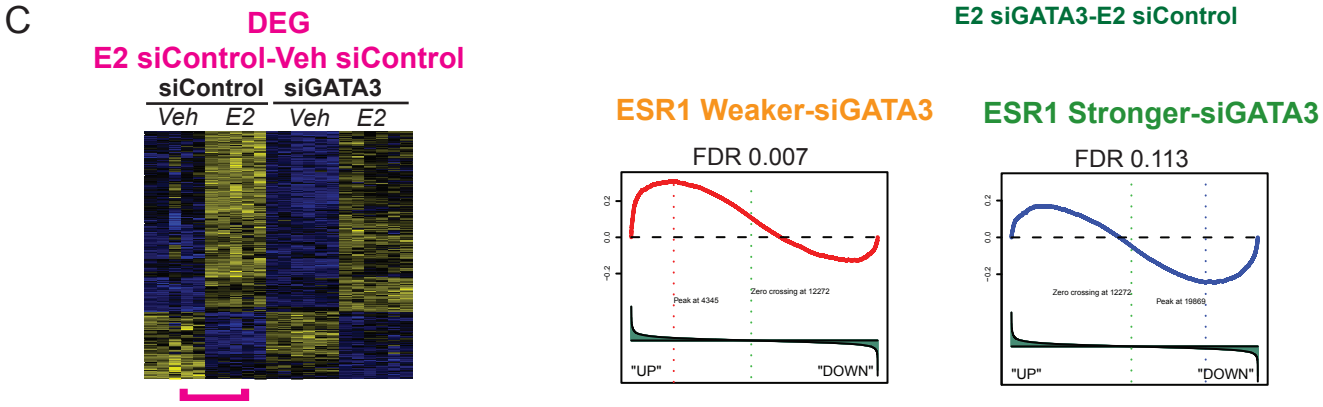


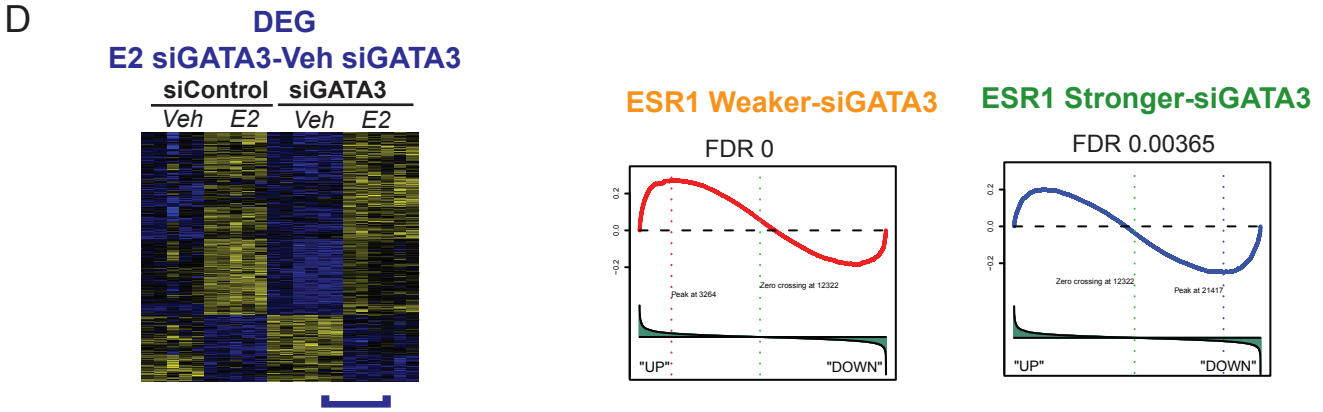


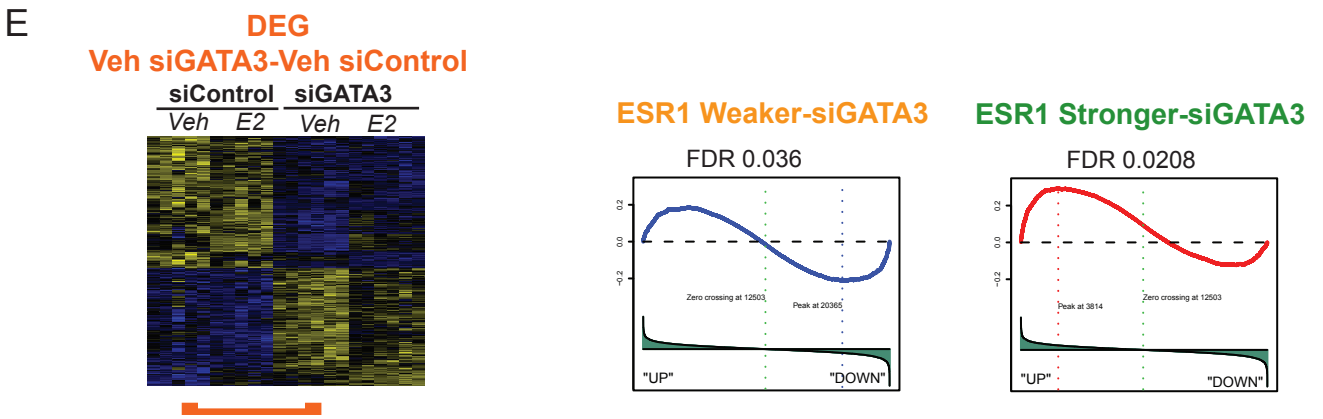
Supplemental Figure 7

(A) Overview of the experimental design used in this study. Cells were transfected with siRNA at final concentration of 50nM and after 6 hours were put in hormone depleted charcoal-dextran treated (CDT) media. The cells were cultured for three days with daily media replacement. On the fourth day, cells were treated with E2 (100nM) for 3 hours for the ChIP-Seq experiments and 6 hours for gene expression.
(B) Venn diagram of the significant differentially expressed genes (DEG at p<0.01, FDR corrected) for four comparisons. DEG genes from all four comparisons are show in the heatmap of Figure 5A.
(C-E) GSEA shows enrichment of the GATA3 dependent changes in ER binding sites within various contrasts tested in the expression microarray experiment: E2 target genes in control conditions (C), E2 target genes in the absence of GATA3 (D) and genes differentially regulated in hormone depleted conditions when GATA3 was silenced (E). ( ]) under heatmap shows the comparison that led to the specific gene list.



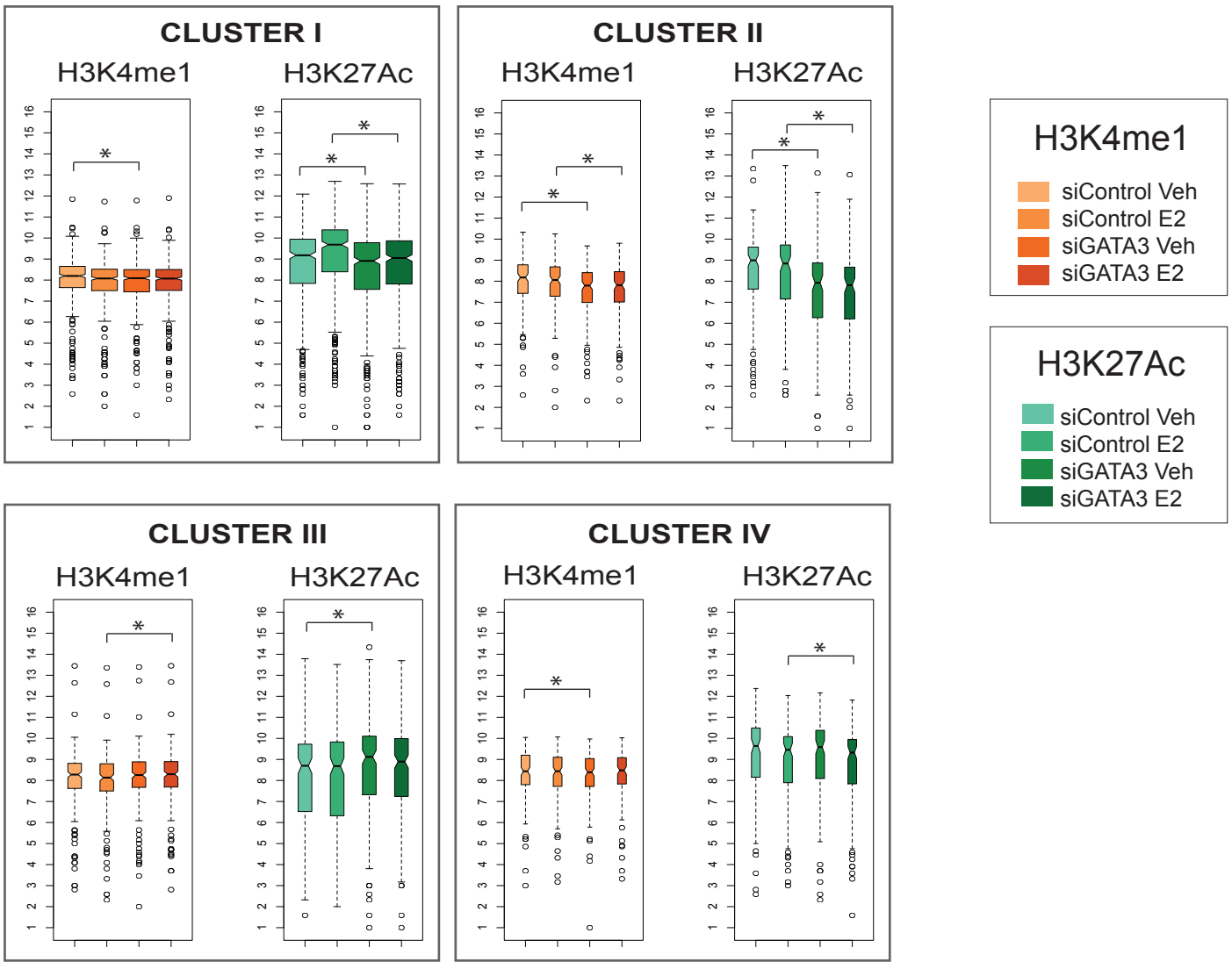






Supplemental Figure 8

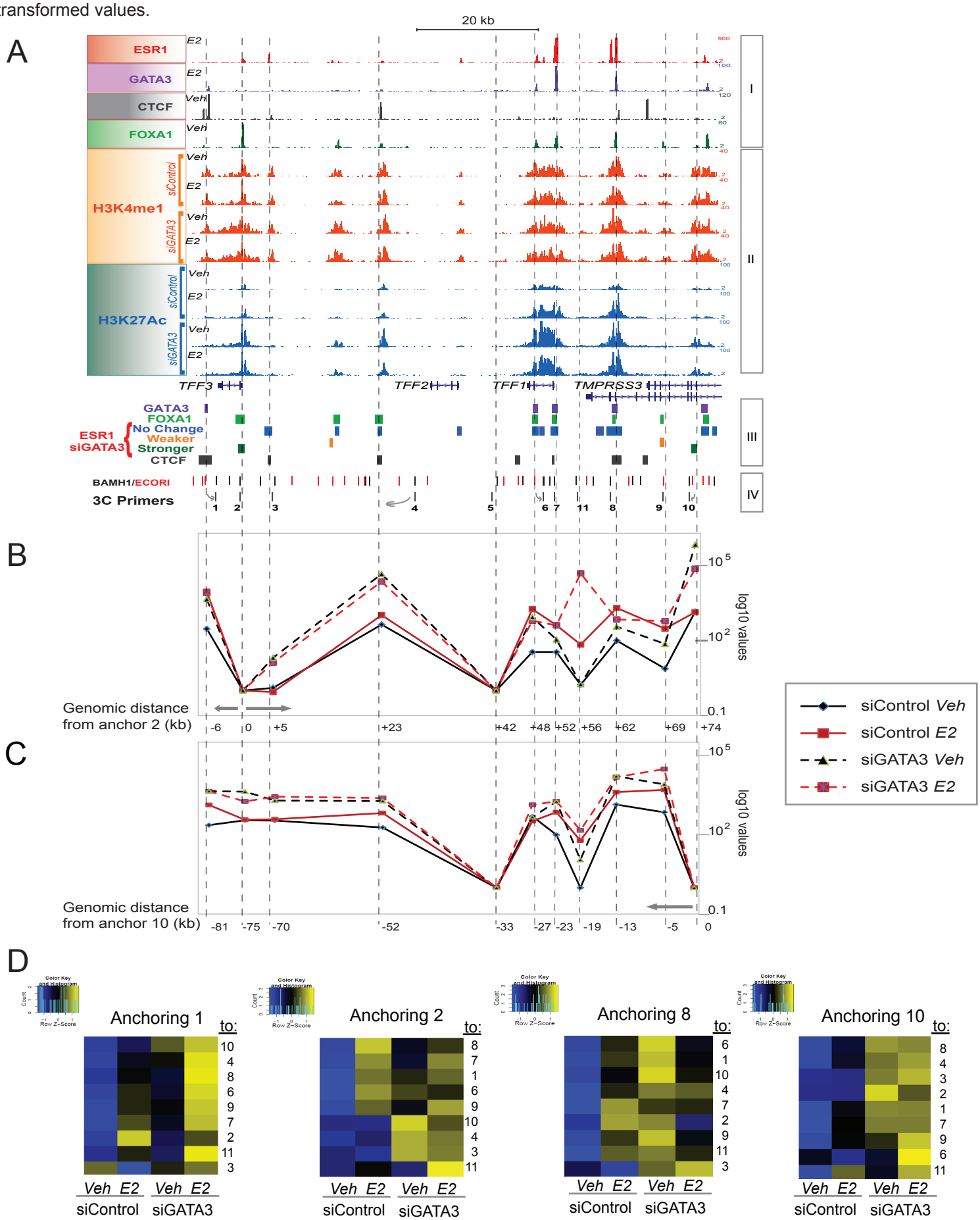
The Differentially Expressed Genes after GATA3 silencing exhibit a remodelled histone landscape. Changes in active histone modifications occur in the same direction as the observed changes in gene expression (Fig. 4A). Y axis shows log concentration of ChIP-Seq reads within a 5kb window from the TSS (1kb up and 4kb downstream) of genes in corresponding gene clusters (I-IV).
Significant changes were identified using Wilcoxon test.



Supplemental Figure S9

Silencing of GATA3 results in increased chromatin loops at the TFF locus

A. Transcription factor binding at the TFF locus, a cluster of E2-upregulated genes that are aberrantly up-regulated in MCF7 cells depleted of GATA3. Also shown are the location of the primers and restriction enzyme sites used for chromosome conformation capture (CCC). We show (I) ChIP-Seq tracks of transcription factor binding, (II) ChIP-Seq tracks of histone marks in siGATA3 and siControl cells (III) Peaks of transcription factor binding and (III) restriction enzymes and primers used for the 3C experiments. B. Normalised CCC signal between anchor region 2, an ESR1-siGATA3 stronger event, and all other regions. The distance between the anchor primer and the other assessed regions is shown. CCC interaction values are shown on a log scale. C. Normalized CCC signal between anchor region 10, an ESR1-siGATA3 stronger event, and all other regions. The distance between the anchor primer and the other assessed regions is shown. CCC interaction values are shown on a log scale. D. Quantitative CCC enrichment between assessed regions, representing the average of five independent replicates. The heatmaps represent z-score transformed values.



Supplemental Figure 10

(A) Aberrant expression of E2 up-regulated genes at the *TFF* gene cluster when GATA3 is silenced. Boxplots represent normalized microarray expression data.
(B) 3C interactions. On the Y axis log10 transformation of the averaged, normalized 3C-qPCR values. X axis shows the interacting genomic regions. Dashed line shows the threshold of positive interactions.
(C) Schematic representation of the interactions at the *TFF* locus in wild-type MCF7 cells.
(D) A model representing the hierarchical order of different transcription factors in the ESR1 pathway.