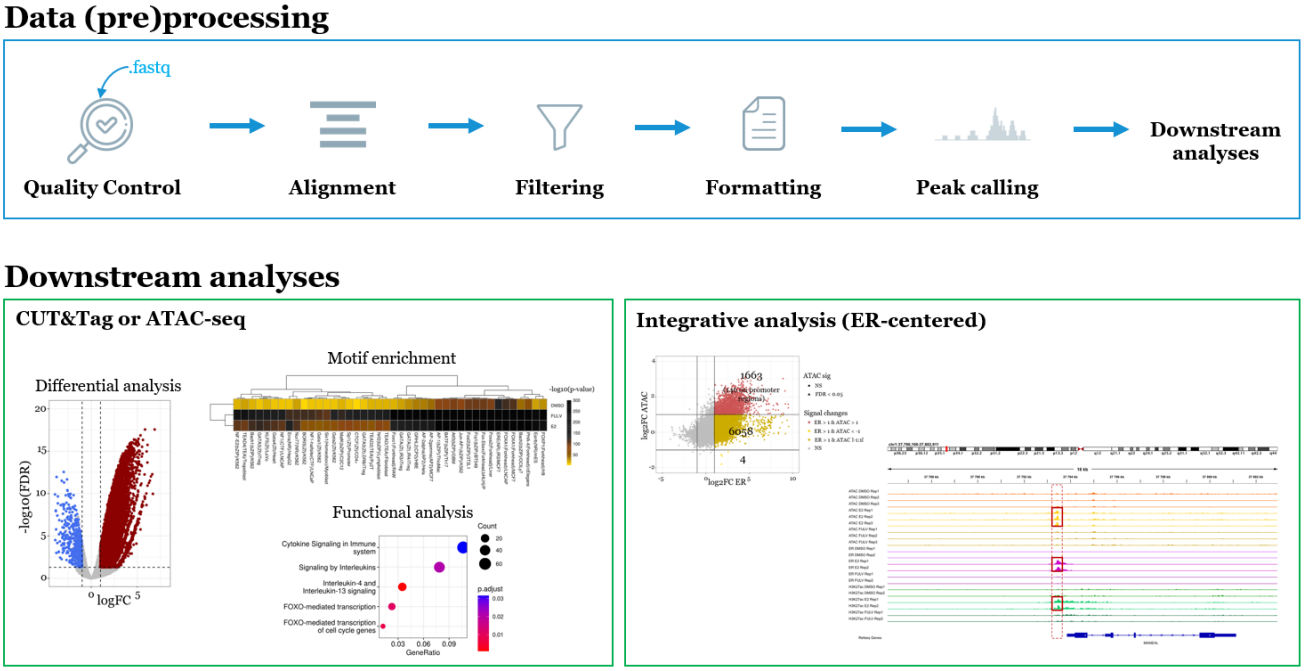

Figure S1. Overview of data processing workflow. Fastq files are first pre-processed for peak calling before downstream analyses. Briefly, a quality control is performed to ensure samples meet quality criteria for further analyses and low-quality reads are filtered out if required. Then, reads are mapped to the human genome hg38 and low-quality mapping reads are removed. In addition, duplicates and blacklisted regions are removed before the peak calling step. Finally, peaks are called using MACS3 and tracks files are generated to visualize signals. The list of peaks is then used to generate a count matrix which is used for downstream analyses. In that regard, similar downstream analyses were performed for CUT&Tag (ER, H3K27ac) and ATAC-seq datasets including a differential analysis comparing treatments and control, a functional analysis based on differential sites and a DNA motif enrichment. Data were then integrated to identify ER binding sites with dysregulation of chromatin accessibility and/or H3K27ac signals.

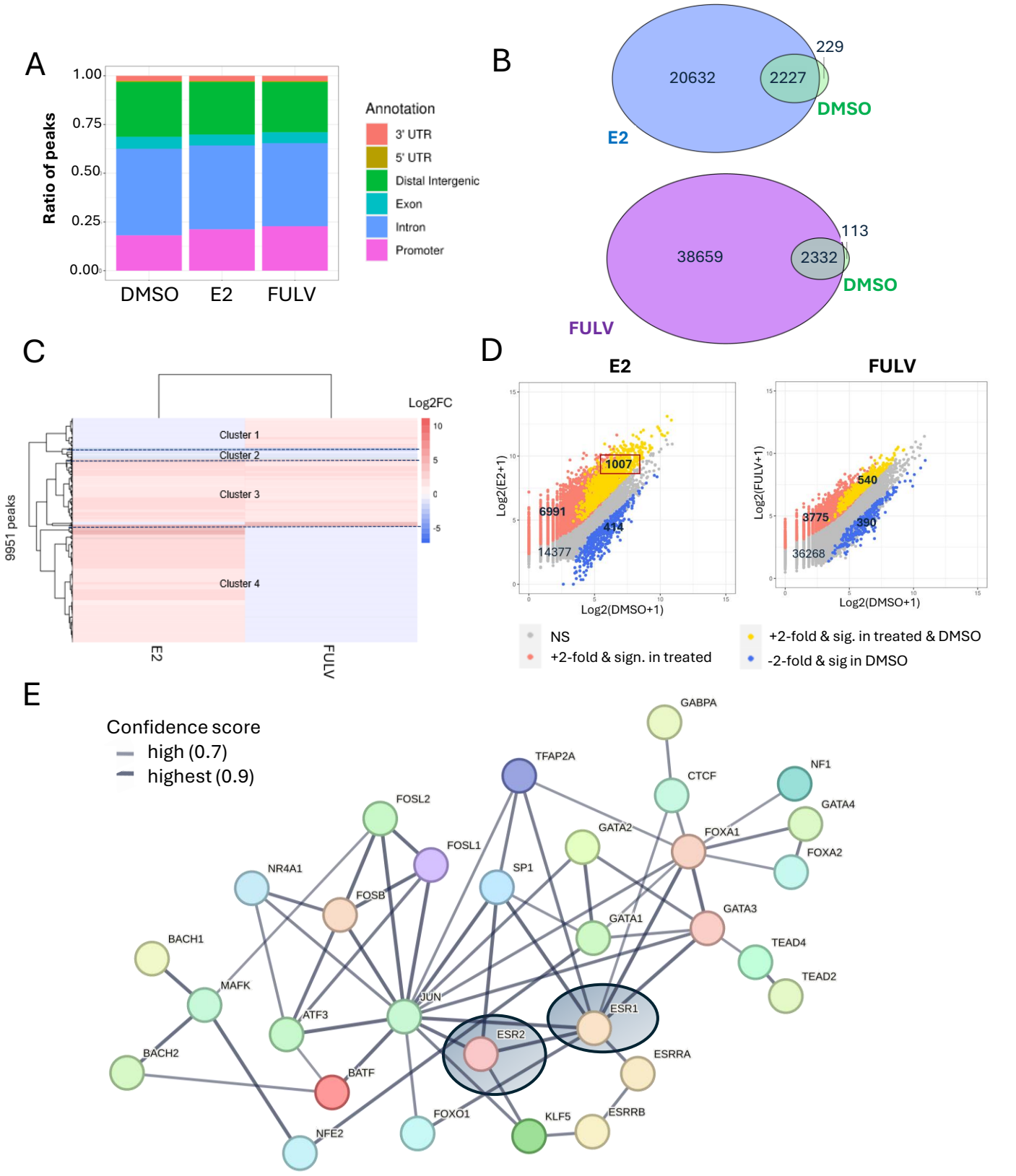

Figure S2. Characterization of ER binding sites identified upon E2 and FULV treatments. (A) Bar plots show the genomic distribution of ER binding sites across conditions. Less than 25% ER peaks fall inside promoter regions (B) Venn diagrams highlight the dramatic increase of ER binding sites upon treatment and shows the large overlap with pre-existing binding sites in the control DMSO condition. Peaks with at least 1bp in common were considered as overlapping (C) Heatmap shows differential ER Cut&Tag signals at 9951 ER binding sites found to be significantly differentially enriched in at least one treatment condition. Positive log2FC in red for increasing signals and negative log2FC in blue for decreasing signals. Four clusters are identified: cluster 1 contains ER sites specifically enriched upon FULV treatment, cluster 2 contains ER sites depleted in both treatment conditions, cluster 3 contains ER sites enriched in both treatment conditions and cluster 4 contains ER sites specifically enriched upon E2 treatment (D) Scatterplots shows the normalized ER Cut&Tag signals in the DMSO control and the annotated treatment condition at ER peaks shown in B. In yellow: peaks were called in both the treated and control conditions, and a minimum 2-fold signal increase is observed upon treatment; in red: peaks were only called upon treatment, and a minimum 2-fold signal increase is observed upon treatment; in blue: peaks were called in the control and/or the treated condition, and a minimum 2-fold signal increase is observed in the control; in grey: no significant changes upon treatment (E) Protein-protein interaction network of transcription factors inferred from DNA motifs enriched with at least p < 1e-100 in DMSO and/or E2 and/or FULV displayed in Figure 2D. The ERE motif directly linked to ER resulted in ESR1 and ESR2 transcription factors highlighted in the network.

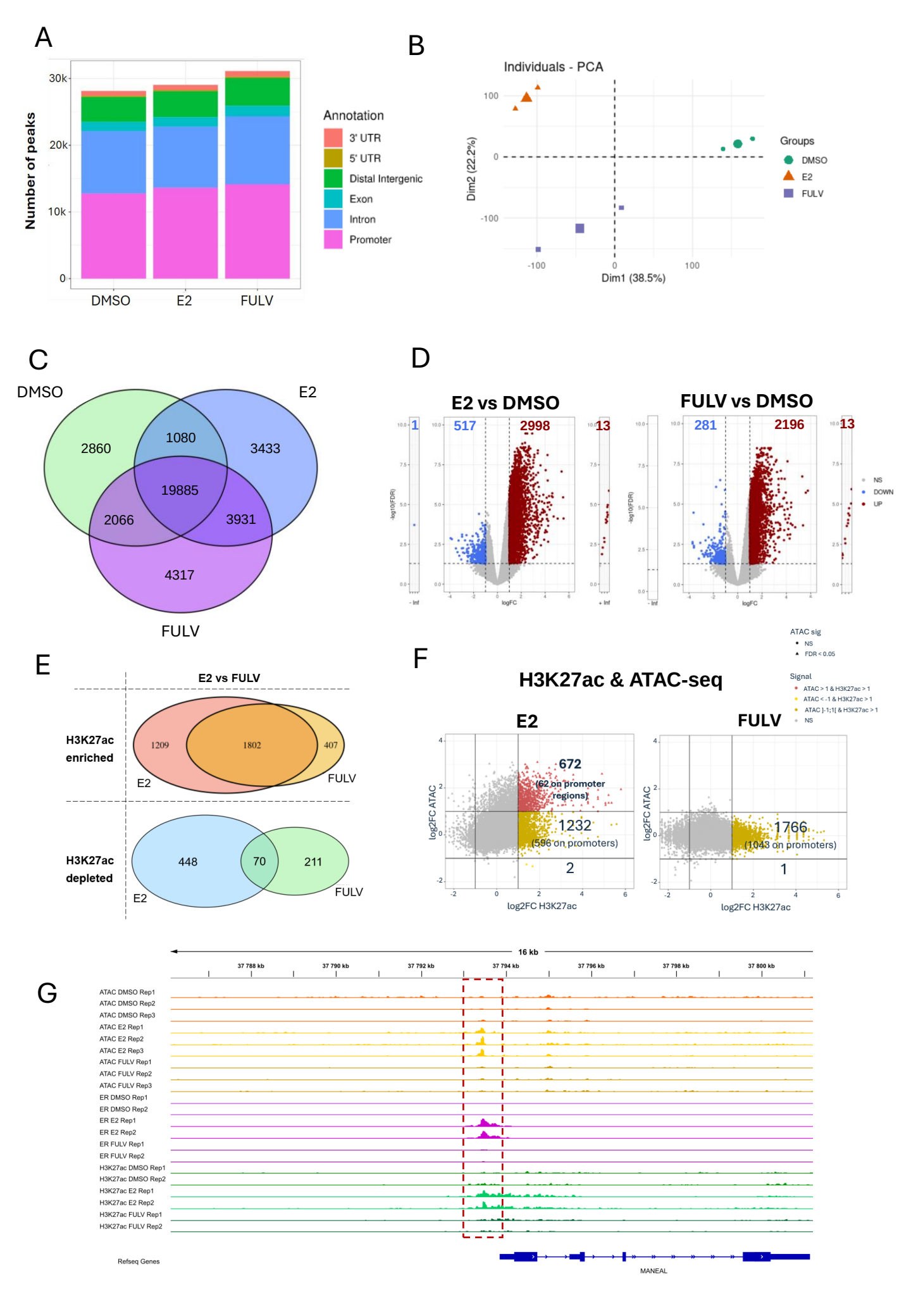
A
Number of peaks
30k
20k
10k
0
DMSO
E2
FULV
Annotation
3' UTR
5' UTR
Distal Intergenic
Exon
Intron
Promoter
B
Individuals - PCA
Dim2 (22.2%)
Dim1 (38.5%)
Groups
DMSO
E2
FULV
C
DMSO
E2
FULV
2860
1080
3433
19885
2066
3931
4317
D
E2 vs DMSO
FULV vs DMSO
1
517
2998
13
281
2196
13
-log10(FDR)
logFC
NS
DOWN
UP
E
E2 vs FULV
H3K27ac enriched
1209
1802
407
E2
FULV
H3K27ac depleted
448
70
211
E2
FULV
F
H3K27ac & ATAC-seq
ATAC sig
NS
FDR < 0.05
Signal
ATAC > 1 & H3K27ac > 1
ATAC < -1 & H3K27ac > 1
ATAC]-1;1[& H3K27ac > 1
NS
E2
FULV
672
(62 on promoter regions)
1232
(596 on promoters)
2
1766
(1043 on promoters)
1
log2FC ATAC
log2FC H3K27ac
G
16 kb
37 788 kb
37 790 kb
37 792 kb
37 794 kb
37 796 kb
37 798 kb
37 800 kb
ATAC DMSO Rep1
ATAC DMSO Rep2
ATAC DMSO Rep3
ATAC E2 Rep1
ATAC E2 Rep2
ATAC E2 Rep3
ATAC FULV Rep1
ATAC FULV Rep2
ATAC FULV Rep3
ER DMSO Rep1
ER DMSO Rep2
ER E2 Rep1
ER E2 Rep2
ER FULV Rep1
ER FULV Rep2
H3K27ac DMSO Rep1
H3K27ac DMSO Rep2
H3K27ac E2 Rep1
H3K27ac E2 Rep2
H3K27ac FULV Rep1
H3K27ac FULV Rep2
Refseq Genes
MANEAL

Figure S3. Genome-wide H3K27ac signal changes upon FULV and E2 treatments. (A) Bar plots show the number and genomic distribution of H3K27ac binding sites across conditions (B) Principal Component Analysis plot based on the fragments counted in H3K27ac peaks called across conditions. Experimental conditions are well separated from each other and replicates remain grouped together based on genome-wide H3K27ac signals A larger symbol represents the centroid of the cluster for each condition (C) Venn diagram shows the overlap of H3K27ac peaks coordinates across experimental condition. Despite a large overlap with pre-existing sites in the control DMSO condition, a large number of sites are specific for each condition (D) Volcano plots show differential H3K27ac signals compared to the DMSO control condition. Both treatments induce robust H3K27ac changes genome-wide. In blue, H3K27ac signal is significantly decreased at least 2-fold upon treatment (FDR < 0.05) and in red, H3K27ac signal is significantly increased at least 2-fold upon treatment (FDR < 0.05). Non significant changes are displayed in grey. –Inf and +Inf panels contain peaks for which no counts were found in one of the condition: in –inf no count was found in the treated condition for the specific peak and, in +inf no count was found in the control condition. (E) Venn diagrams show the overlap between H3K27ac enriched or depleted regions detected upon E2 and FULV treatments. Most H3K27ac enriched regions are common to both E2 and FULV conditions whereas depleted H3K27ac regions appear treatment specific (F) Scatterplots show H3K27ac Cut&Tag and ATAC-seq signal changes at ER binding sites upon E2 or FULV treatment. In red, a minimum 2-fold increase in both ATAC-seq and H3K27ac Cut&Tag signals is observed upon treatment; in light yellow, a minimum 2-fold increase in H3K27ac Cut&Tag signal concomitant with a minimum 2-fold decrease in ATAC-seq signal; in dark gold, a minimum 2-fold increase in H3K27ac Cut&Tag signal concomitant with mild to no change in ATAC-seq signal; The shape of the dots reflects the significance of ATAC-seq signal changes with a triangle showing significant changes (FDR < 0.05) (G) Representative example near the *MANEAL* locus showing increased ER Cut&Tag signals upon E2 treatment in the MCF7 breast cancer cell line with concomitant increases in ATAC-seq and H3K27ac Cut&Tag signals. No changes were detected upon FULV treatment in this region.