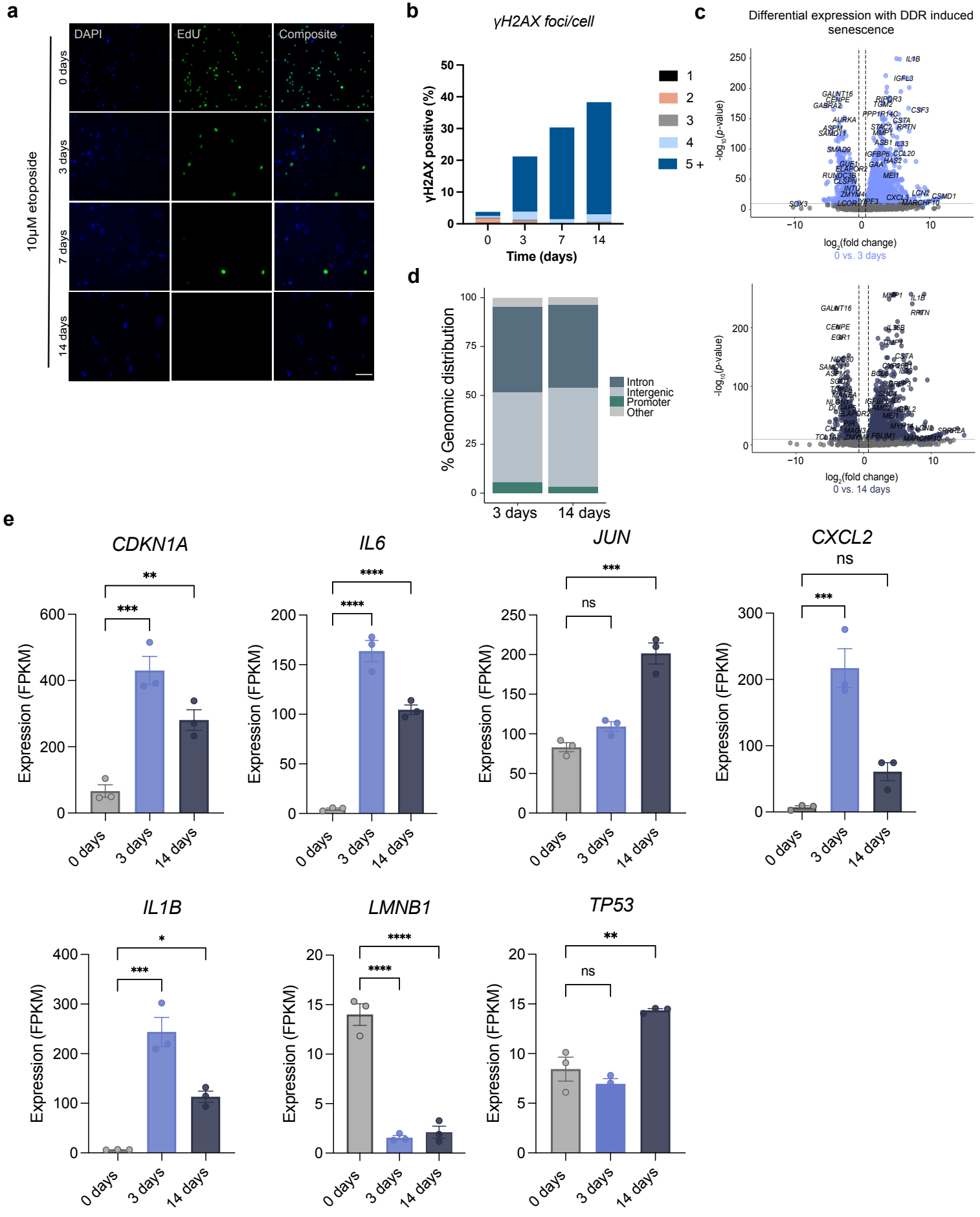
Supplementary Information

FOXO3 pioneers chromatin changes in a model of DNA damage-induced senescence

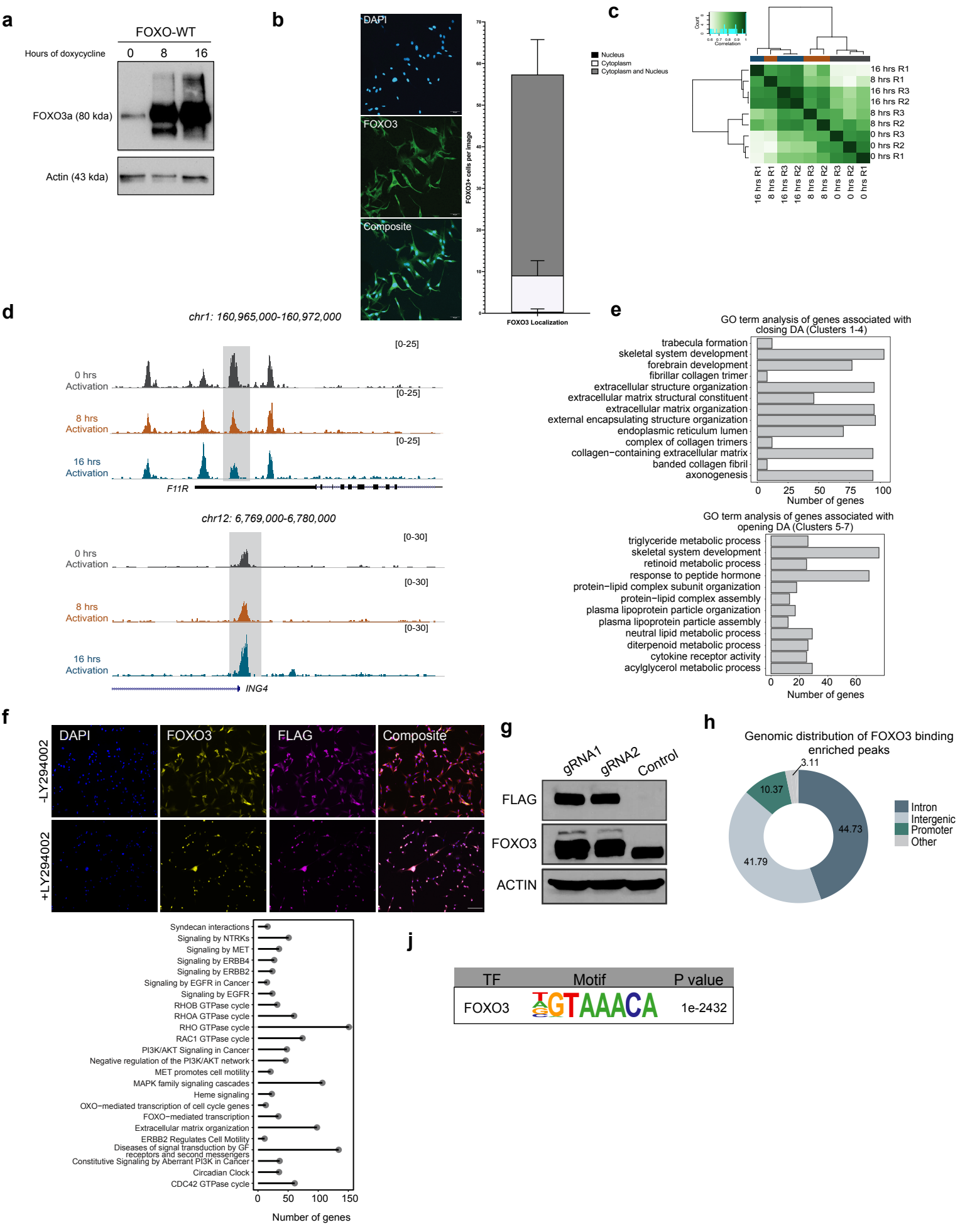
Abigail K. Brown, José A. Arevalo, Angelica Holcom, Adele E. Finch, Sun Y. Maybury-Lewis, Avery D. McNamara, Ashley E. Webb

Supplementary Fig. 1



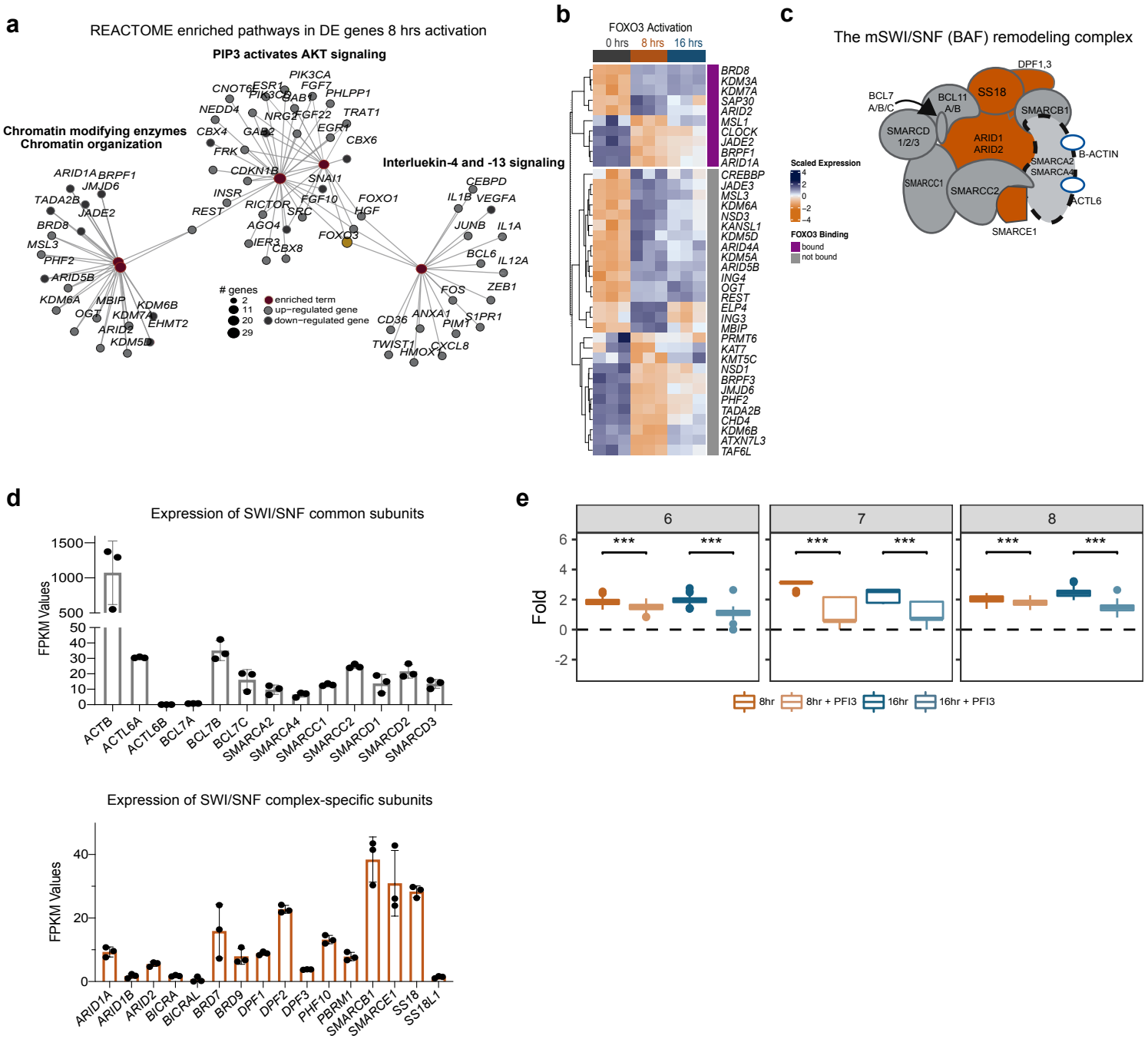
Supplementary Fig. 1 Chemotherapy induced senescence alters chromatin accessibility and the transcriptome. a) Representative images of the EdU incorporation assay after 0, 3, 7 and 14 days of etoposide treatment. Equal number of cells were plated in triplicate and EdU incorporation was performed for 24 hours. Scale bar, 120 microns. **b)** Quantification of the number of cells containing γH2AX positive foci. **c)** Volcano plots depicting differential gene expression with etoposide treatment for 3 (top) and 14 (bottom) days compared with no etoposide treatment. Genes significantly differentially expressed are highlighted in light and dark blue, respectively. **d)** Stacked bar showing the genomic distribution of the DA sites with etoposide treatment for 3 and 14 days compared with 0 days of etoposide. **e)** FPKM expression values of selected senescence associated factors. ****p<0.0001, ***p<0.0005, ***p*<0.005, **p*<0.05 one-way ANOVA, Bonferroni's multiple comparisons test.

Supplementary Fig. 2



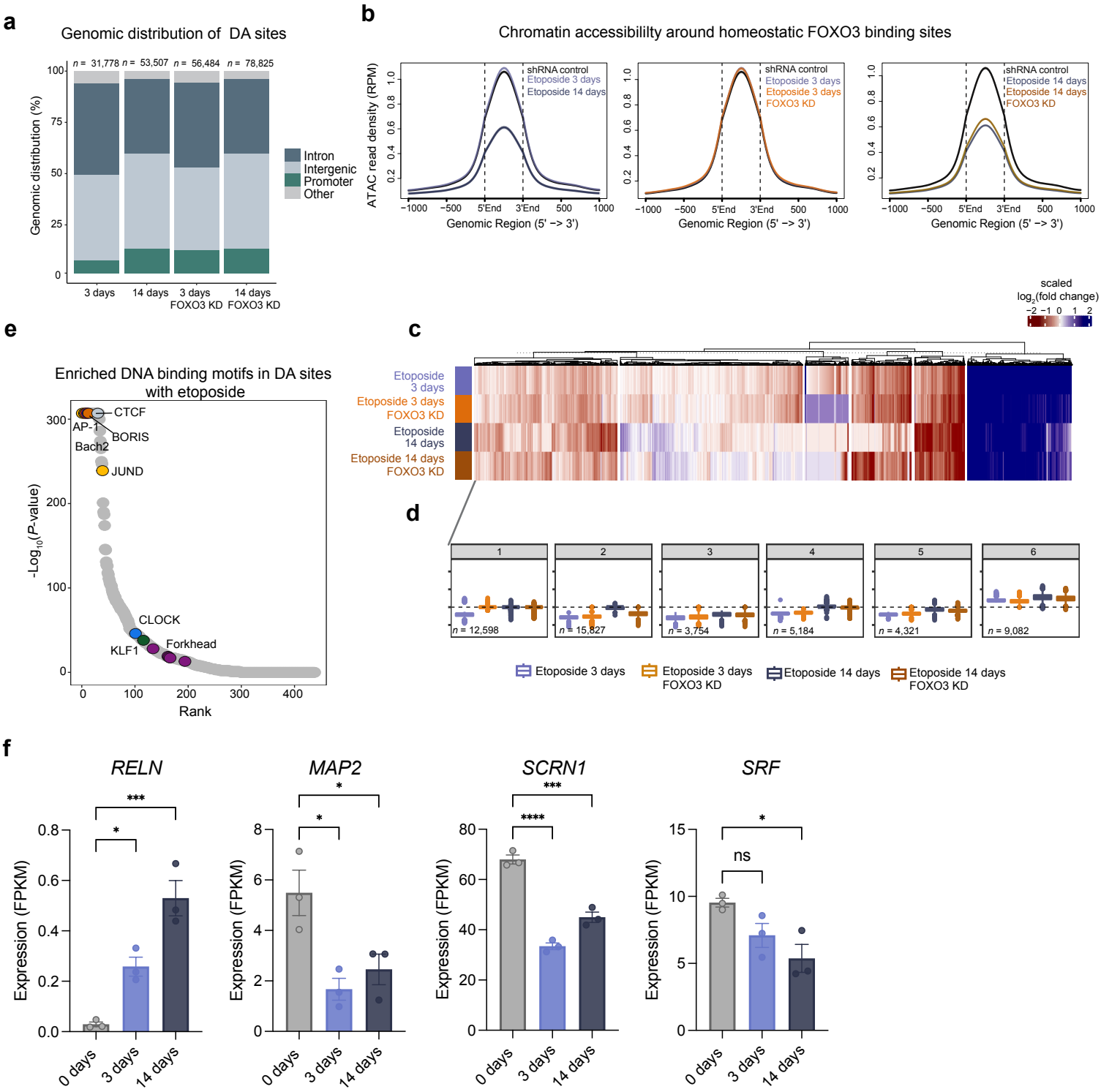
Supplementary Fig. 2 Identification of direct targets of FOXO3. a) Western blot validating overexpression of FOXO3 in U87-MG cells. **b)** quantification of the distribution of endogenous FOXO3 in U87-MG cells. **c)** Heatmap representing the Pearson's correlation of ATAC-seq replicates. **d)** UCSC genome browser view of the changes in ATAC-seq peaks with FOXO3 activation at the *F11R* (top) and *ING4* (bottom) gene loci. **e)** Top 12 enriched (p. adjust < 0.05, Benjamini-Hochberg corrected) gene ontology (GO) term analysis of the genes associated with clusters decreasing (top) and increasing (bottom) in accessibility with FOXO3 activation. **f)** Representative images of U87MG cell lines stably expressing FLAG-FOXO3 stained for endogenous FOXO3 (yellow) or FOXO3-FLAG (magenta). Cells are shown under basal conditions (-LY294002) or PI3K-inhibited conditions (+LY294002). Note the localization of the FOXO3 to the nucleus upon PI3K inhibition. Scale bar, 100 μm. **g)** Western blot showing validation of FLAG-tagged FOXO3 in two replicate cell lines (n = 3, biological independent experiments). **h)** Donut graph shows the genomic distribution of all the FOXO3 binding sites. Promoter regions were defined as -1/+1kb around the TSS. Peaks were annotated based on distance from designated promoter region. **i)** REACTOME pathway analysis of FOXO3 targets. **j)** Homer motif enrichment analysis of the called peaks following CETCh-seq.

Supplementary Fig. 3



Supplementary Fig. 3 FOXO3 recruits SWI/SNF chromatin remodelers to alter chromatin accessibility. a) REACTOME pathways analysis of significantly (FDR < 0.01) differentially expressed genes between FOXO3 activation for 0 and 8 hours. Enrichment was calculated and hierarchical clustering of the terms using pathfindR package. **b)** Heatmap depicting the differential expression of genes enriched in the chromatin organization and chromatin modifying enzymes categories with FOXO3 activation. Rows show scaled expression (log_2fold change), samples are in columns. Annotation bars show whether the genes are direct target genes of FOXO3. **c)** Schematic of the mammalian SWI/SNF (BAF) complex. SMARCA2/SMARCA4 (Brg/Brm) is the catalytic subunit inhibited by PFI-3 and is highlighted by the dashed black outline. **d)** Transcript levels (FPKM values) of chromatin remodeler genes that comprise the mammalian SWI/SNF complex. Expression levels of the common subunits (*top*), complex specific members (*bottom*) are under basal conditions. Error bars represent mean with SD. **e)** Boxplots of accessibility changes per cluster log_2(fold change) in both timepoints. The first and third quartiles are denoted by the lower and upper hinges, and the center is the median. Whisker extends to the largest and smallest values with 1.5X the interquartile range. Data points beyond the 1.5X interquartile range are plotted as individual points. n = 3 biological replicates, one-way t. test comparative analysis with cluster 6 p= 1.979094^{-22}, 1.095722^{-96}, cluster 7 p= 3.557987^{-22}, 5.641509^{-54}, cluster 8 p= 5.079048^{-13}, 1.679138^{-54}, and cluster 9 p= 7.736112^{-12}, 1.509848^{-38}. P values reported represent 8hrs activation vs 8hrs activation + PFI3, and 16hrs activation vs 16hrs activation + PFI3, respectively.

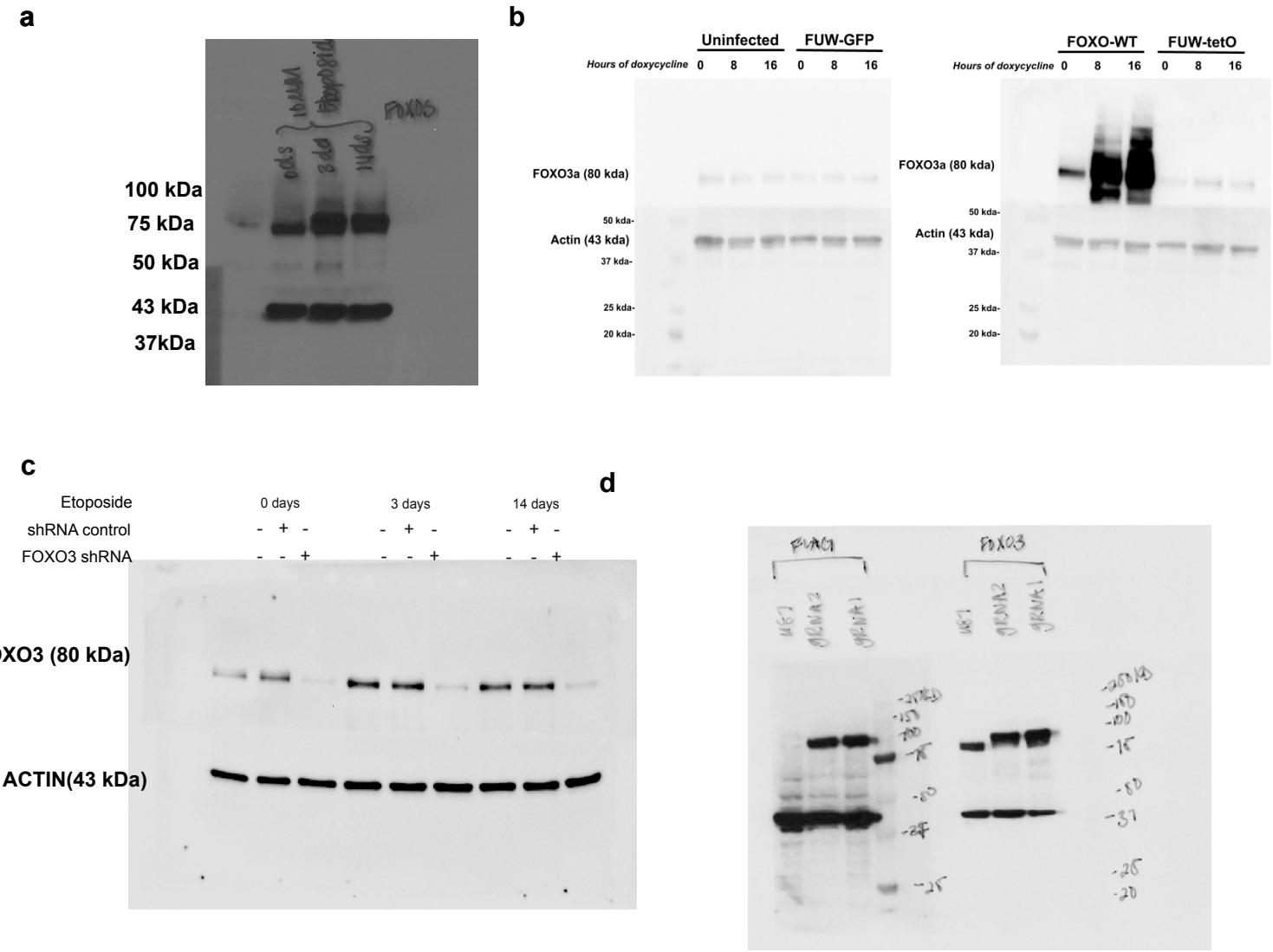
Supplementary Fig. 4



Supplementary Fig. 4 FOXO3 alters defined genomic regions with induction of senescence

a) Stacked bar plot showing the genomic distribution of the DA sites with etoposide treatment for 3 and 14 days with and without FOXO3 when compared with 0 day etoposide and shRNA control. **b)** NGS plot of the consensus ATAC-seq signal in etoposide treated and FOXO3 knockdown samples around the FOXO3 binding sites. **c)** Heatmap depicting scaled log_2(fold change) changes in chromatin accessibility in cells with etoposide treatment for 3 and 14 days, and etoposide treatment for 3 and 14 days in cells with FOXO3 knockdown. **d)** Boxplots of accessibility changes per cluster log_2(fold change) in both timepoints (n = 3 replicates). The first and third quartiles are denoted by the lower and upper hinges, and the center is the median. Whisker extends to the largest and smallest values with 1.5X the interquartile range. Data points beyond the 1.5X interquartile range are plotted as individual points. **e)** Homer motif enrichment analysis of differentially accessible (DA) sites from the heatmap Supplementary Fig. 4c. Forkhead, AP-1, CTCF, and other co-factor motifs are highlighted. **f)** FPKM expression values of select neuronal genes. ****p<0.0001, ***p<0.0005, **p<0.005, *p<0.05 one-way ANOVA, Bonferroni's multiple comparisons test.

Supplementary Fig. 5



Supplementary Fig. 5 FOXO3 protein expression under different conditions

a) Full western blot showing the protein expression when cells are treated with 10μM etoposide for 3 and 14 days. **b)** Full western blot image of FOXO3 protein expression. FOXO3 is activated for 8 and 16 hours. **c)** Full western blot image of FOXO3 protein expression in cells that are transfected with FOXO3 shRNA and treated with 10μM of etoposide for 3 and 14 days. **d)** Full western blot showing FOXO3 protein expression with anti-FOXO3 and anti-FLAG antibodies from the CETCh-seq derived lines.