

**Molecular Cell, Volume 73**

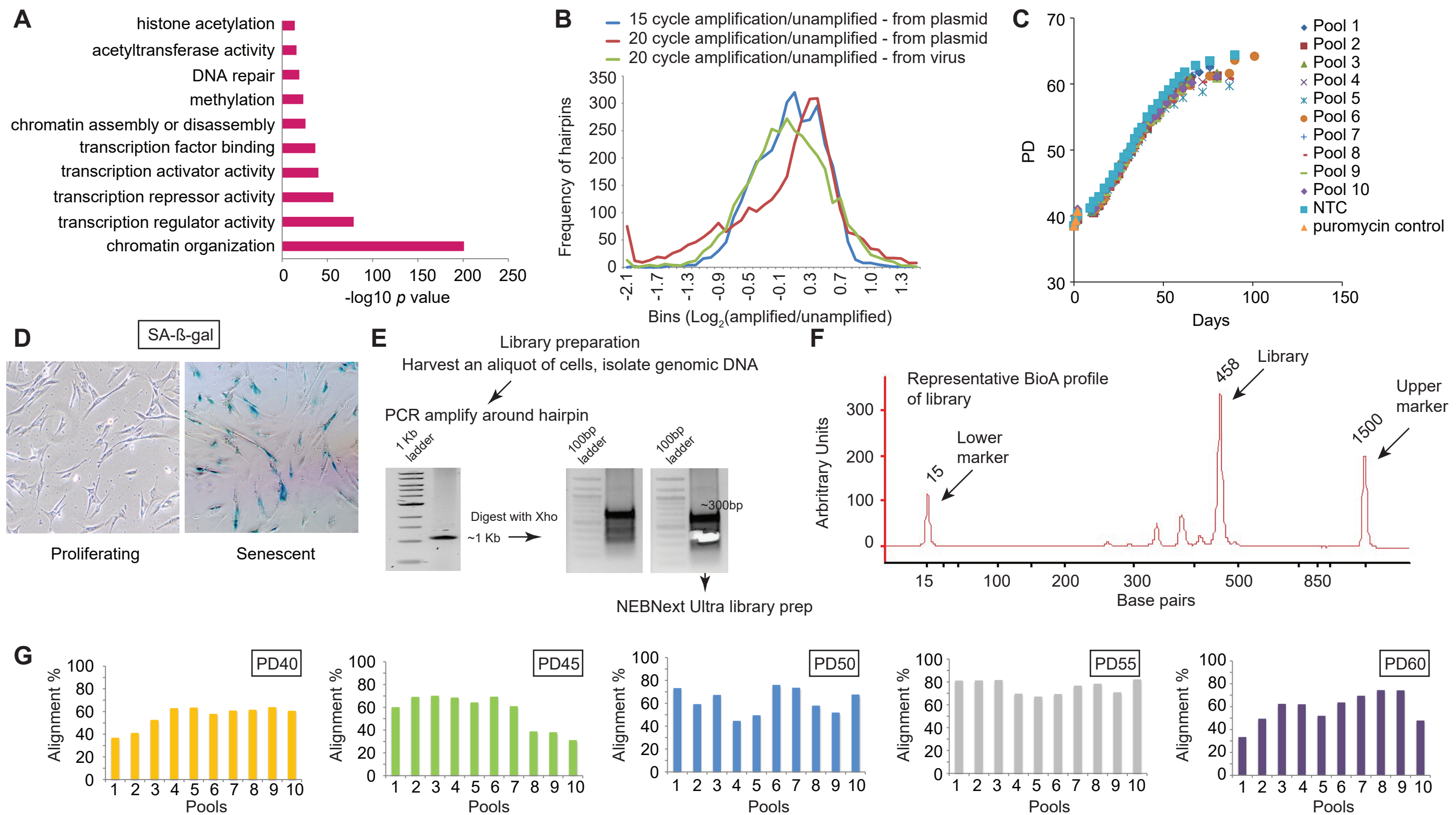
**Supplemental Information**

**Histone Acetyltransferase p300**

**Induces *De Novo* Super-Enhancers**

**to Drive Cellular Senescence**

**Payel Sen, Yemin Lan, Catherine Y. Li, Simone Sidoli, Greg Donahue, Zhixun Dou, Brian Frederick, Qijun Chen, Lacey J. Luense, Benjamin A. Garcia, Weiwei Dang, F. Bradley Johnson, Peter D. Adams, David C. Schultz, and Shelley L. Berger**



**Figure S1**

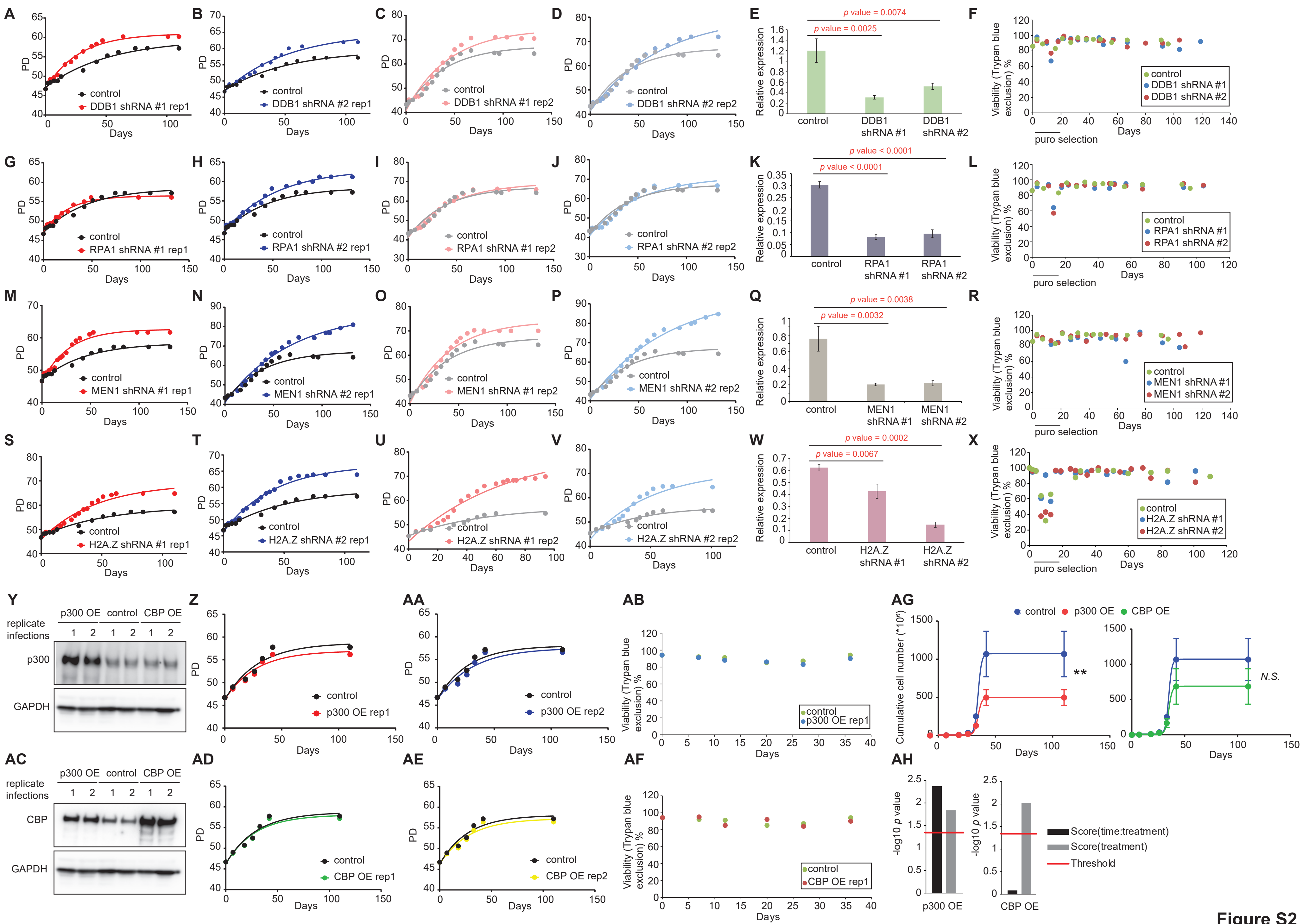
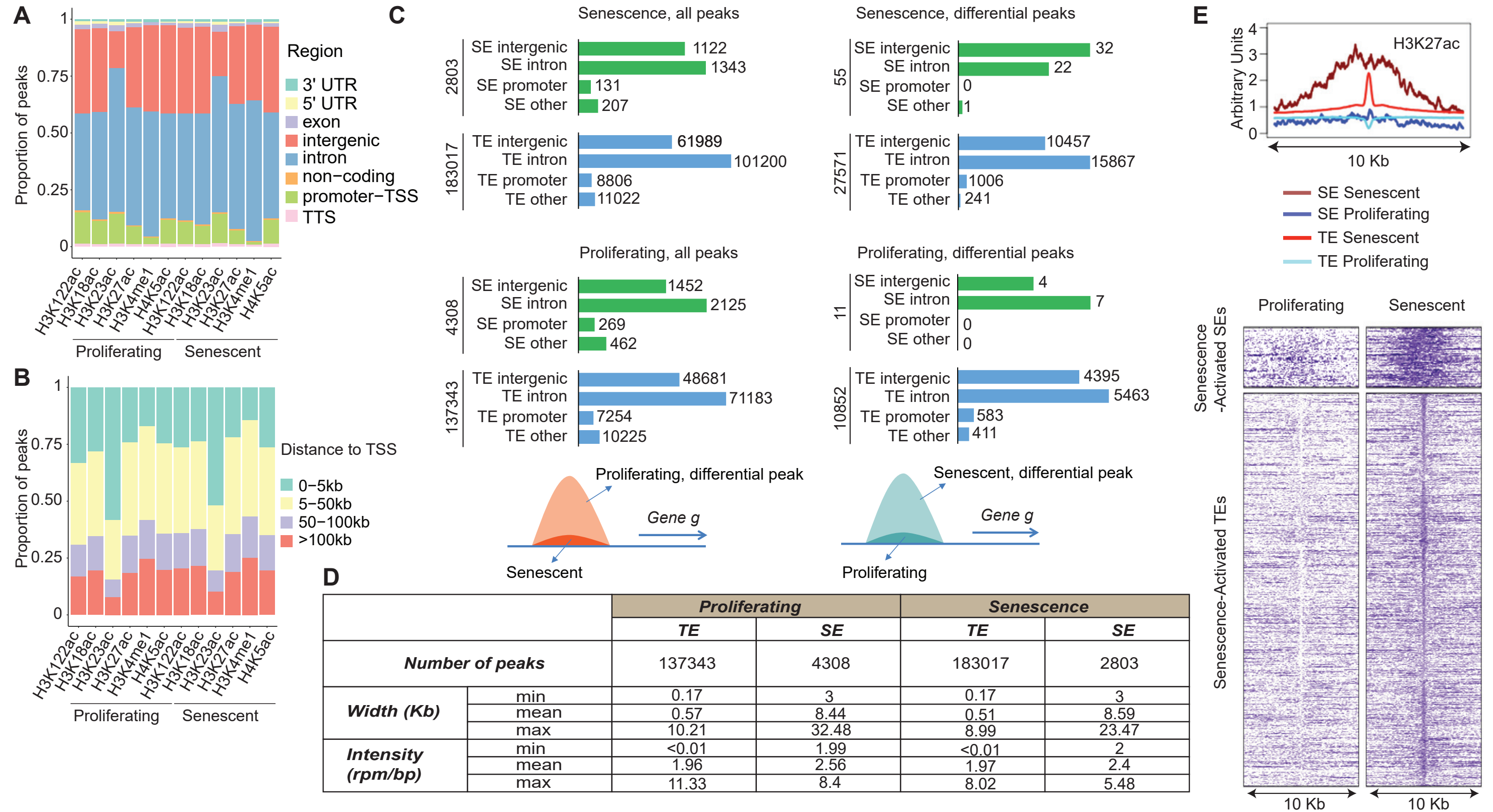


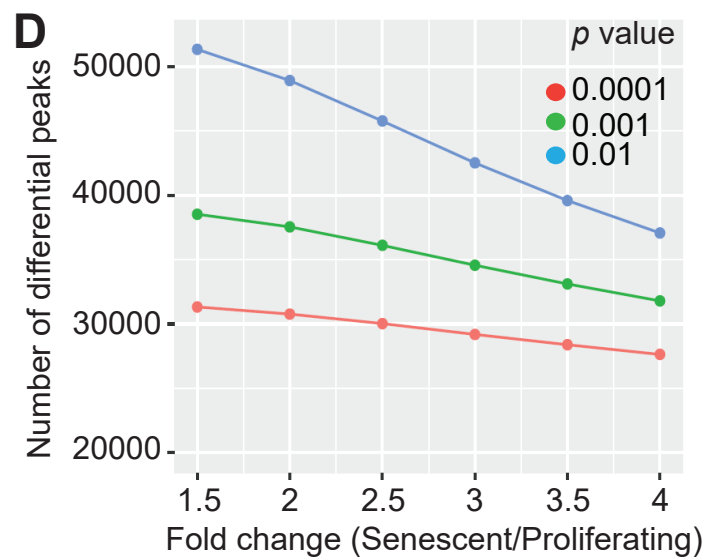
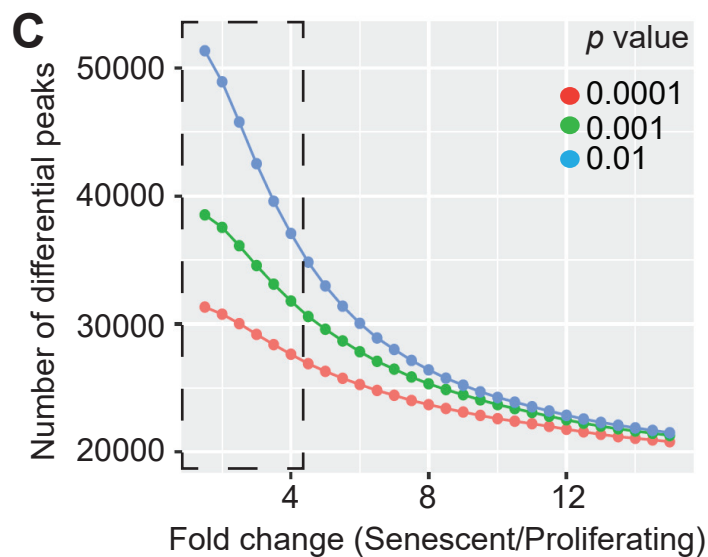
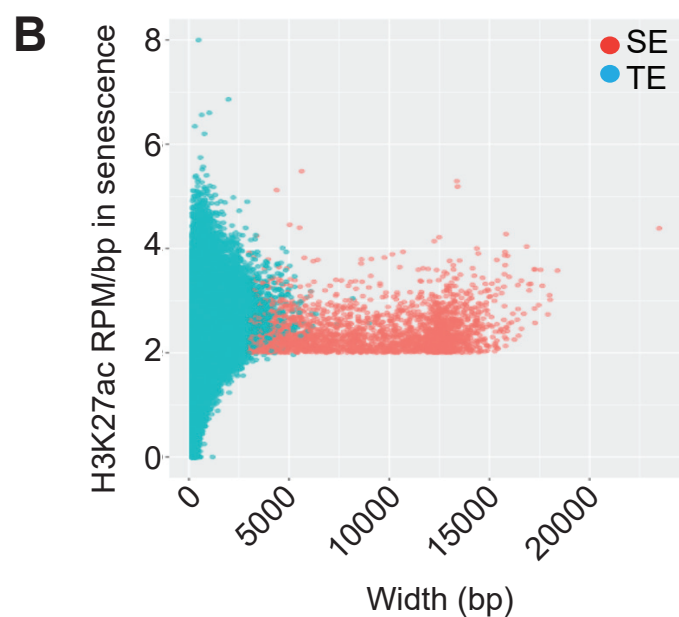
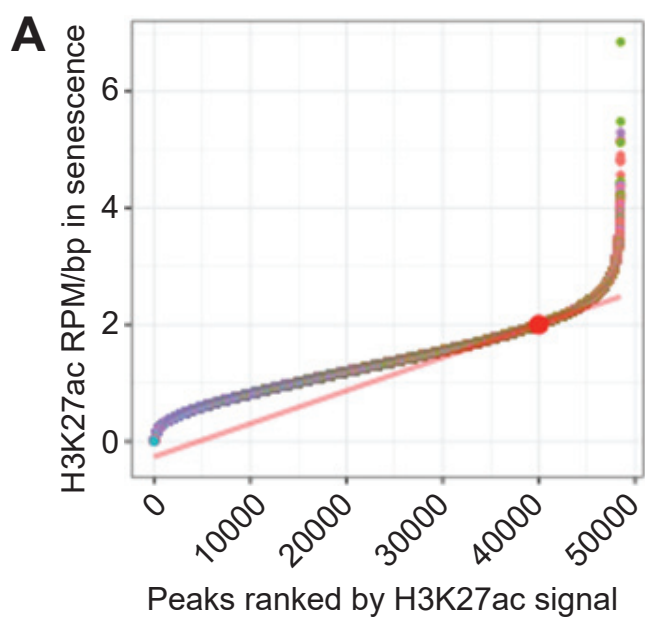
Figure S2





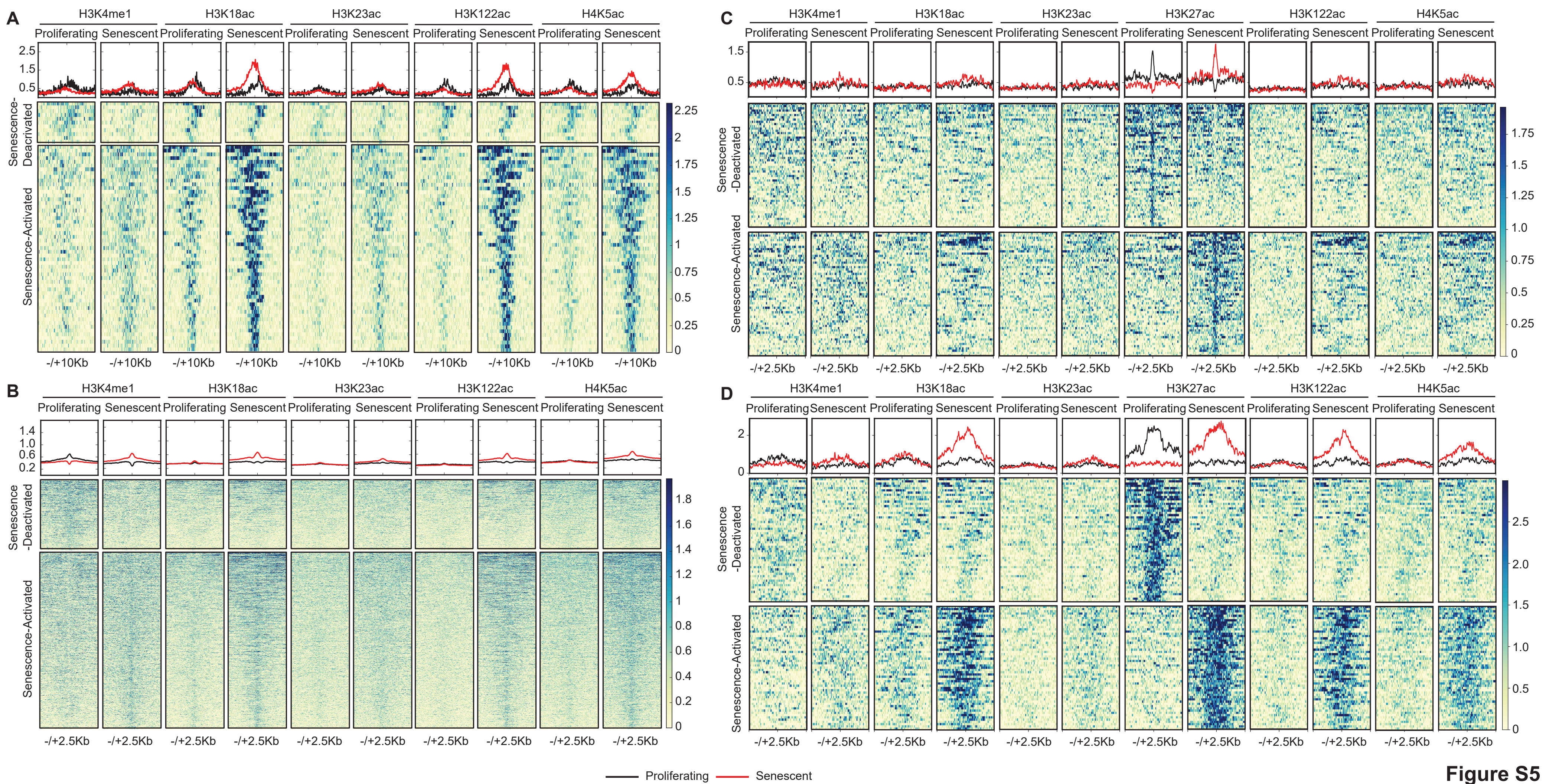
**Figure S3**





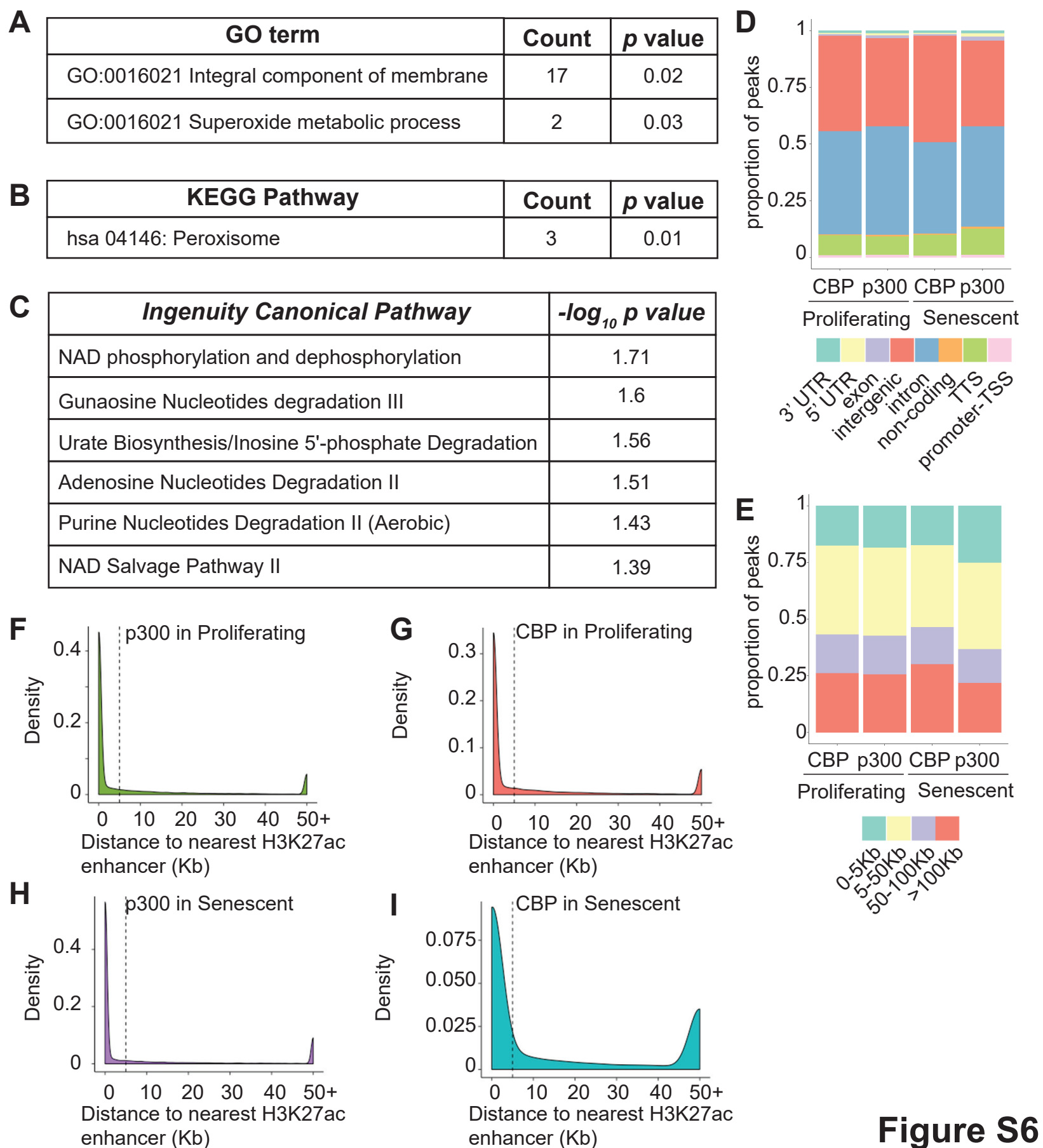
**Figure S4**





**Figure S5**



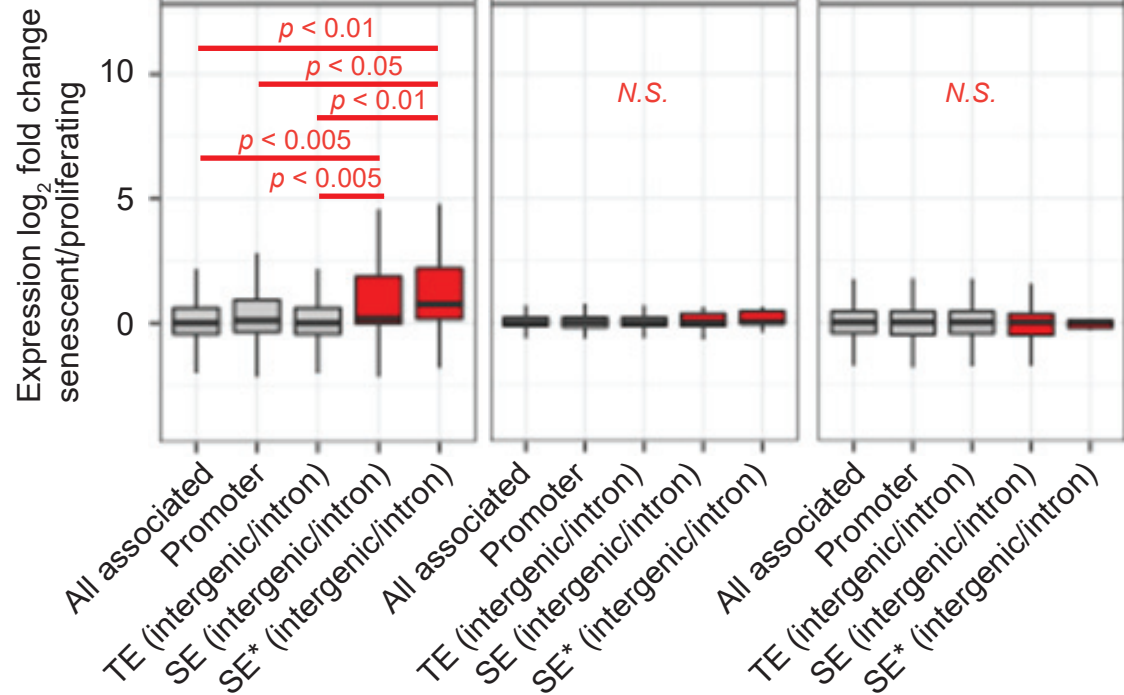


**Figure S6**

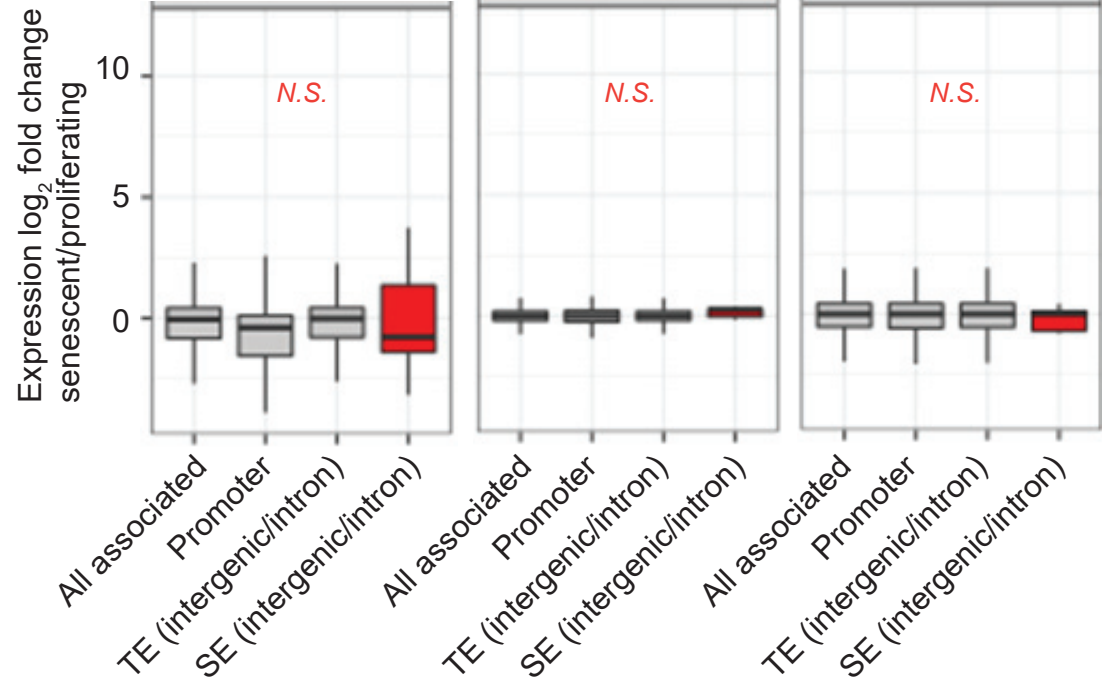


**A**

RNA-seq (nearest gene)

**B**

RNA-seq (nearest gene)

**Figure S7**

## Supplemental Figure legends

### Figure S1 (related to Figure 1): Composition, quality control and execution of the RNAi screen

(A) A Gene Ontology analysis of proteins included in the shRNA pool used for screening. (B) Histogram showing the distribution of amplified and unamplified hairpins before and after packaging into lentiviruses. (C) Replicative lifespan curves of each pool in the RNAi screen including a control infected with a NTC. (D) Representative SA- $\beta$ -gal staining of one of the experimental pools after senescence establishment. (E) Schematic showing the generation of sequencing libraries during quality control of shRNA pools used in the RNAi screen. (F) Representative BioAnalyzer profile of sequencing library generated in the screen. (G) Alignment percentages for each pool at every time-point from the screen.

### Figure S2 (related to Figure 2): Validation studies with potential candidates from the RNAi screen

(A-X) Replicative lifespan curves for cells harboring hairpins against candidates from the RNAi screen along with knockdown efficiency by qPCR and viability of cells throughout a representative lifespan assay is shown for DDB1 (A-F), RPA1 (G-L), MEN1 (M-R) and H2A.Z (S-X). (Y) Western blot showing overexpression of p300 in cells infected with an overexpression vector or an empty vector. (Z-AA) Replicative lifespan curves of cells harboring p300 overexpression constructs in two independent experiments fitted to a one-phase association model. (AB) Representative viability plot during a replicative senescence assay with cells harboring p300 overexpression construct using trypan blue exclusion. (AC) Western blot showing overexpression of CBP in cells infected with an overexpression vector or an empty vector. (AD-AE) Replicative lifespan curves of cells harboring CBP overexpression constructs in two independent experiments fitted to a one-phase association model. (AF) Representative viability plot during a replicative senescence assay with cells harboring CBP overexpression construct using trypan blue exclusion. (AG) Same data as (Z-AA) and (AD-AE) except plotted in cumulative cells numbers instead of PD. Asterisk indicates a  $p$  value  $< 0.006$  in a two way ANOVA test. (AH) Plot showing  $p$  values of lifespan experiments in (Z-AA) and (AD-AE) using a repeat measure analysis. The red line indicates the threshold for significance ( $p=0.05$ ).

### Figure S3 (related to Figure 4): Compartment analysis of histone-acetyl peaks and properties of H3K27ac enhancers in senescence

(A) Bar plot showing the proportion of all called peaks in different compartments in the genome. (B) Bar plot showing the proportion of all called peaks at a given distance from annotated TSSs. (C) Bar plot of all (left) and differential (right) H3K27ac peaks. The schematic of differential peaks is shown below. (D) Table showing the numbers and parameters of all Senescence-Activated and Senescence-Deactivated TEs and SEs. (E) Metaplot (top) and heatmap (bottom) of all Senescence-Activated SEs and TEs.

### Figure S4 (related to Figure 4): Enhancer categorization in senescence based on H3K27ac signal

(A) Distribution of H3K27ac ChIP-seq signal (RPM/bp) across all called enhancers in senescence. The enhancers on the x-axis are ranked. (B) Scatter plot showing the two classes of enhancers (SEs and TEs in senescence) after categorizing them based on width ( $\geq 3$  Kb) and

mean tag density ( $\geq 2$ RPM/bp). **(C)** Graph showing the number of differential H3K27ac peaks at different fold-change cutoffs and at  $p$  values of 0.01, 0.001 and 0.0001 (obtained from an Exact Poisson Test to examine whether tag density in the senescent sample is greater than tag density in the proliferating sample). **(D)** Inset in (C; dashed rectangle) is expanded.

#### **Figure S5 (related to Figure 4): Super-enhancer and typical enhancer profiles in senescence**

**(A)** Metaplot (top) and heatmap (bottom) of all tested histone acetylation signals at Senescence-Activated and Senescence-Deactivated SEs. **(B)** Metaplot (top) and heatmap (bottom) of all tested histone acetylation signals at Senescence-Activated and Senescence-Deactivated TEs. **(C)** Metaplot (top) and heatmap (bottom) of all tested histone acetylation signals at 55 random Senescence-Activated and Senescence-Deactivated TEs. **(D)** Metaplot (top) and heatmap (bottom) of all tested histone acetylation signals at the top 55 Senescence-Activated and Senescence-Deactivated TEs based on H3K27ac signal.

#### **Figure S6 (related to Figure 5-7): Gene Ontology and Pathway Analysis of target genes proximal to Senescence-Activated super-enhancers and compartment analysis of p300 and CBP peaks**

**(A)** Significant gene ontology (GO) terms from DAVID, **(B)** KEGG pathway and **(C)** canonical pathways from IPA for the 55 Senescence-Activated SEs. **(D)** Bar plot showing the proportion of all p300 and CBP peaks in different compartments in the genome. **(E)** Bar plot showing the proportion of all p300 and CBP peaks at a given distance from annotated TSSs. **(F-I)** Distance distribution of p300 and CBP peaks in proliferating and senescent cells.

#### **Figure S7 (related to Figure 5 and 7): Knockdown of CBP in senescent cells does not alter senescence-related gene expression**

**(A)** Box plot of fold change of RNA-seq signal (senescence vs proliferating or control vs CBP knockdown) across different genomic elements called in senescence. **(B)** Box plot of fold change of RNA-seq signal (senescence vs proliferating) across different genomic elements called in proliferating cells. Two-tailed Mann-Whitney-Wilcoxon Test was used to compare bins within and between boxplots. Senescent cells were at PD 75.