Supplemental Information

Supplementary Figure Titles and Legends

Fig. S1: Increased H3K27me3 is a signature of aging (related to Fig. 1)

(A) H3.3/H3 % from LC-MS/MS raw area values in young and old livers. Data are summarized as mean ± S.E.M. * $p<0.05$ in a Welch's two-tailed unpaired t-test with corrections for multiple comparisons at 1% FDR (two-stage step-up Benjamini, Krieger, and Yekutieli). **(B)** Abundance of monomethyl (me1), dimethyl (me2), trimethyl (me3) and acetyl (ac) -ated histones from LC-MS/MS in young and old livers. Data are summarized as mean ± S.E.M. A Welch's two-tailed unpaired t-test did not yield any significant results. **(C)** Volcano plot of single hPTMs from LC-MS/MS data in muscle stem cells [1] and **(D)** human post-mortem brain samples [2]. The significantly increased (green) and decreased (blue) hPTMs are labeled (two-tailed unpaired t-test). **(E)** Representative H3K27me3 IF images (young 12 weeks and old 80 weeks). Scale bar is 10 µm. H3K27me3 intensity is plotted on the right. * $p<0.05$ in a Welch's two-tailed unpaired t-test. **(F)** Human spike-in alignment rate plotted as a % in H3, input and H3K27me3 immunoprecipitated samples. **(G)** PCA plot of H3 subtracted H3K27me3 genome coverage and **(H)** called peaks from liver ChIP-seq data. Note for (G-H), PC1 accounts for the variation in age and PC2 in sex. **(I)** Bar plot of liver H3K27me3 ChIP-seq peak numbers. Data are summarized as mean ± S.E.M. A Welch's two-tailed unpaired t-test did not yield any significant results. **(J)** Violin plots of H3K27me3 peak characteristics in young and old livers. *** indicates $p<0.001$ from a Welch's two-tailed unpaired t-test. **(K)** Representative PhenoImager images for young and old liver stained with antibodies indicated on the right. Scale bar is 100 µm and 50 µm for young and old respectively. **(L)** Cell composition and **(M)** H3K27me3 signal in each cell type in young and old livers. For (K-M), data are summarized as mean ± S.E.M. * $p<0.05$ and ** $p<0.01$ in a Welch's two-tailed unpaired t-test with corrections for multiple comparisons at 10% FDR (two-stage step-up Benjamini, Krieger, and Yekutieli). For (A-B) young 11-12 weeks and old 79-95 weeks, n=3 biological replicates per group. For (E), young 12 weeks and old 80 weeks, n=6 biological replicates per group. For (F-J) young 10-12 weeks and old 79-95 weeks, n=3 biological replicates per group. For (K-M), young 14 weeks and old 95 weeks, n=3 biological replicates per group.

Fig. S2: Quality control of all genome-wide datasets (related to Figs. 1-5 and 7)

(A) Sequencing depth of all mouse ChIP and human CUT&Tag libraries generated in this study. **(B)** The alignment rates of all mouse ChIP and human CUT&Tag libraries, plotted as a percentage of mapped fragments. For (A-B) young 10-12 weeks and old 79-95 weeks, n=3-10 biological replicates per group for mice and 19-26 years and old 71-79 years, n=3 young and 5 old biological replicates for humans. **(C)** Sequencing depth of libraries made from salt fractionated chromatin of young and old mouse livers. **(D)** The alignment rates of the libraries from different salt fractions, plotted as a percentage of mapped fragments. For (C-D) young 11 weeks and old 81 weeks, n=2 replicates per group for mice. For (A-D), the boxes are bounded by the 25th and 75th percentile values with the median represented as the bar in the middle. The whiskers extend to 1.5X the interquartile length. A Welch's two-tailed unpaired t-test did not yield any significant results for young vs old comparisons.

Fig. S3: H3K27me3 patterns in mouse and human livers (related to Fig. 1)

(A) Schematic showing the samples and comparisons made in (B-E). **(B)** Genome browser snapshot of overlayed H3K27me3 ChIP signal (H3 subtracted) over chr5 in sex-matched pairs of young and old mouse livers. **(C)** Genome browser snapshot of H3K27me3 ChIP signal (H3 subtracted) over chr5 in individual young and old mouse livers. **(D)** Genome browser snapshot of overlayed H3K27me3 ChIP signal (H3 subtracted) over chr18 in sex-matched pairs of young and old mouse livers. **(E)** Genome browser snapshot of H3K27me3 ChIP signal (H3 subtracted) over chr18 in individual young and old mouse livers. For (B-E), young 10-12 weeks and

old 79-95 weeks, n=3 biological replicates per group and gray area in the whole chromosome is expanded on the right. **(F)** Volcano plot of diffReps output showing differential H3K27me3 peaks in young and old human livers. Significantly enriched H3K27me3 regions in old (FDR<0.05) are indicated in green and those depleted are in blue. **(G)** Top 20 GO terms for genes near the 1117 young peaks identified as differentially enriched in young (A). Young 19-26 years and old 71-79 years, n=3 young and 5 old biological replicates. *p*-values are from Fisher's exact test.

Fig. S4: Age-domains are heterochromatinized while H3K27me3 peak regions are euchromatinized during aging (related to Fig. 3)

(A) Genome browser snapshot of salt fraction enrichments over chr18 in both young and old samples. Inset 1 is euchromatinized and inset 2 is heterochromatinized with age and expanded on the right. Note the overlap of 350mM and pellet fractions in the old with a H3K27me3 domain in inset 2. Young 11 weeks and old 81 weeks, n=2 replicates per group.

Fig. S5: Transcriptomic signatures of aging and regeneration (related to Figs. 4 and 6)

(A) Schematic of experimental procedure, timeline, and assays. **(B)** Steps of 70% PH. **(C)** Age and sex distribution of mice used. Data are summarized as mean ± S.E.M. with individual data points representing each mouse (n=10 males and 20 females). **(D)** Volcano plot of differentially expressed mRNAs in young and old livers (young 10-11 weeks and old 79-95 weeks, n=3 biological replicates per timepoint). mRNAs significantly upregulated (p<0.05 from Benjamini-Hochberg procedure) in old are in green and those downregulated are in blue. Biological process GO terms are indicated for genes upregulated in the old (right). **(E)** Same as (D) except 120 h post-resection (young 10-16 weeks and old 79-98 weeks, n=3 biological replicates per timepoint). Cell proliferation genes in the GO analysis are in red. **(F)** Same as (D) except 72 h post-resection (young 10-13 weeks and old 79-98 weeks, n=3 biological replicates per timepoint). **(G)** Same as (D) except 240 h post-resection (young 10-12 weeks and old 79-95 weeks, n=3 biological replicates per timepoint). For (F-G) genes were too few for GO analysis. **(H)** Quantification of % positive cells (left) and intensity (right) of Ki67 in an immunohistochemistry assay and **(I)** immunofluorescence assay across age and regeneration. For (H-I), young 10-16 weeks and old 79-98 weeks, n=3 biological replicates per timepoint. ** p<0.01 and *** p<0.001 from a Kolmogorov-Smirnov test with corrections for multiple comparisons at 1% FDR (two-stage step-up Benjamini, Krieger, and Yekutieli). An individual data point indicates one field. **(J)** Heatmap of ImpulseDE2 output showing upregulated mRNAs (red) and downregulated mRNAs (blue) in young (young 10-15 weeks and old 79-83 weeks, n=3 biological replicates per timepoint). p<0.05 from Benjamini-Hochberg procedure. **(K)** GO terms associated with transiently upregulated genes in the young identified in (J). Cell proliferation genes are in red. **(L)** GO terms associated with transiently downregulated genes in the young identified in (J). For all GO analyses, *p*-values are from Fisher's exact test.

Fig. S6: Delayed and reduced liver regeneration in aged mice (related to Fig. 6)

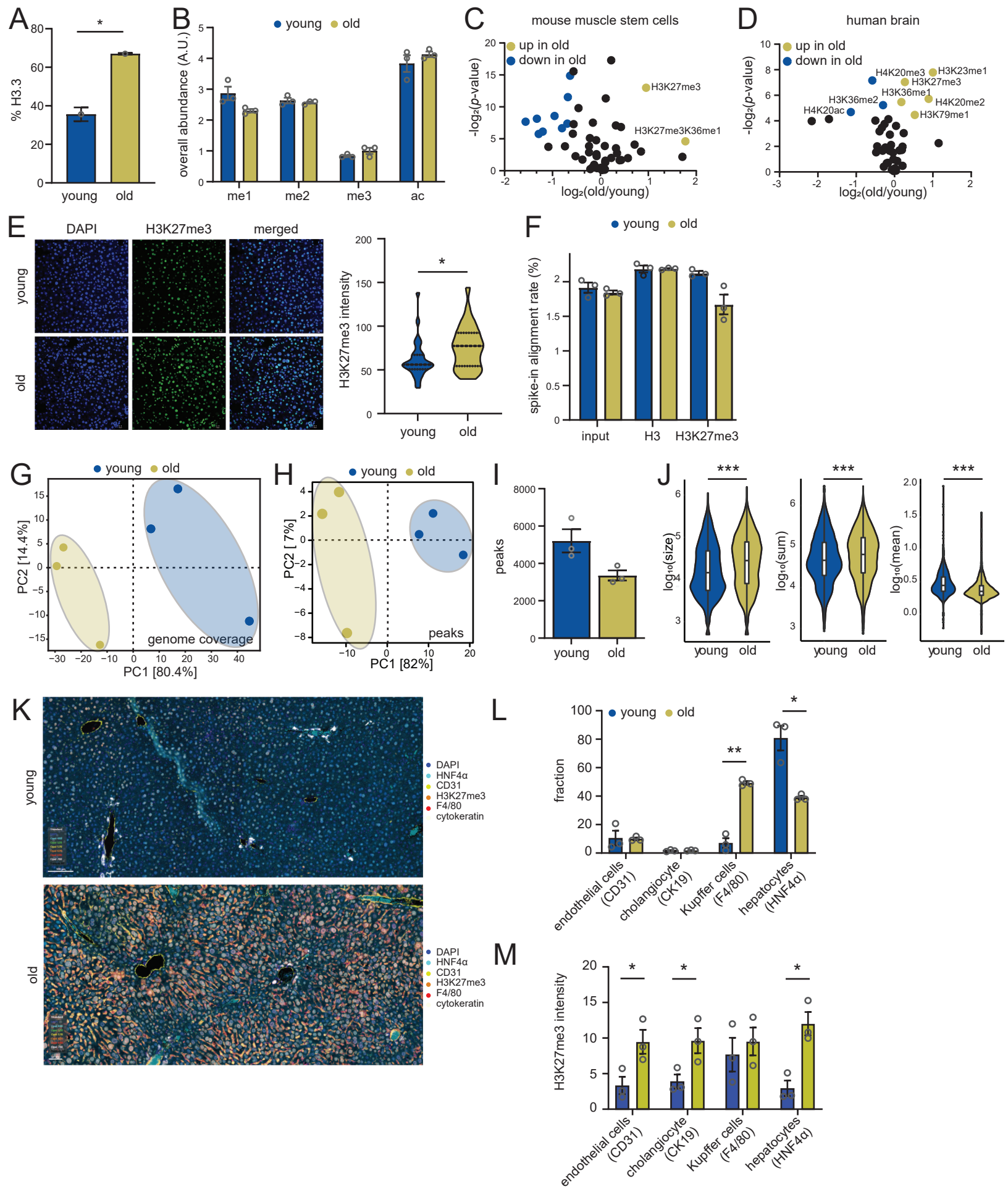
(A) Representative images of Ki67 immunohistochemistry and **(B)** Ki67 immunofluorescence in young and old liver sections across age and regeneration. For both (A-B), young 11-16 weeks and old 83-86 weeks, n=5 biological replicates per group and 1 per timepoint. Scale bar is 50μm.

Fig. S7: Multiple tissues show features of hyper-quiescent chromatin during aging (related to Fig. 1)

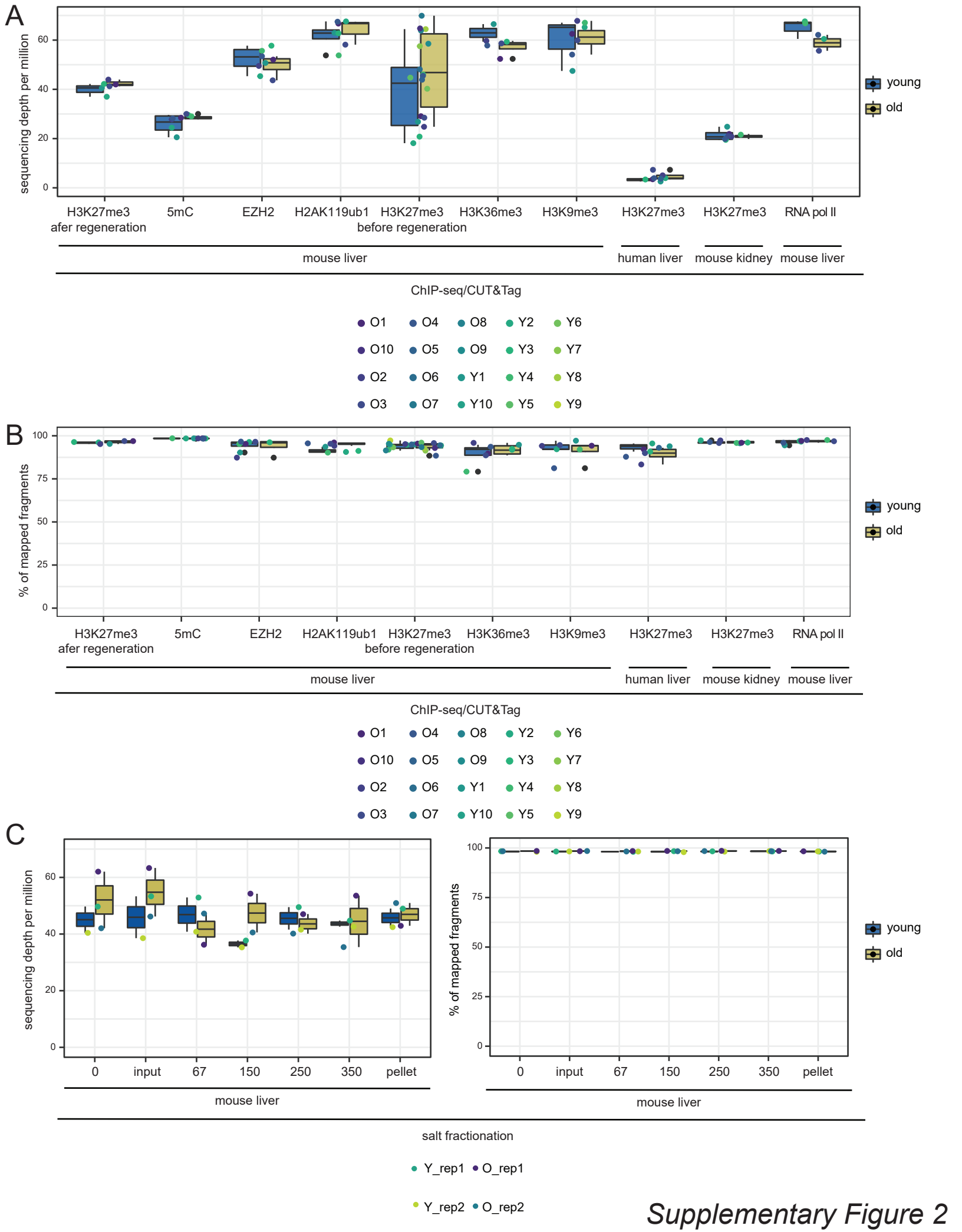
(A) Genome browser snapshot of overlayed H3K27me3 ChIP signal (H3 subtracted) over chr5 and **(B)** chr18 in individual young and old mouse liver, kidneys, muscle, and heart. For kidneys, young 11 weeks and old 80 weeks, n=4 biological replicates per group. For muscle and heart, young 6 months and old 24 months, n=3 biological replicates per group. Muscle and heart datasets were mined from Sleiman et al, 2020 [3] and replicates were merged. Location of age-domains are shown on the top of each browser snapshot.

References

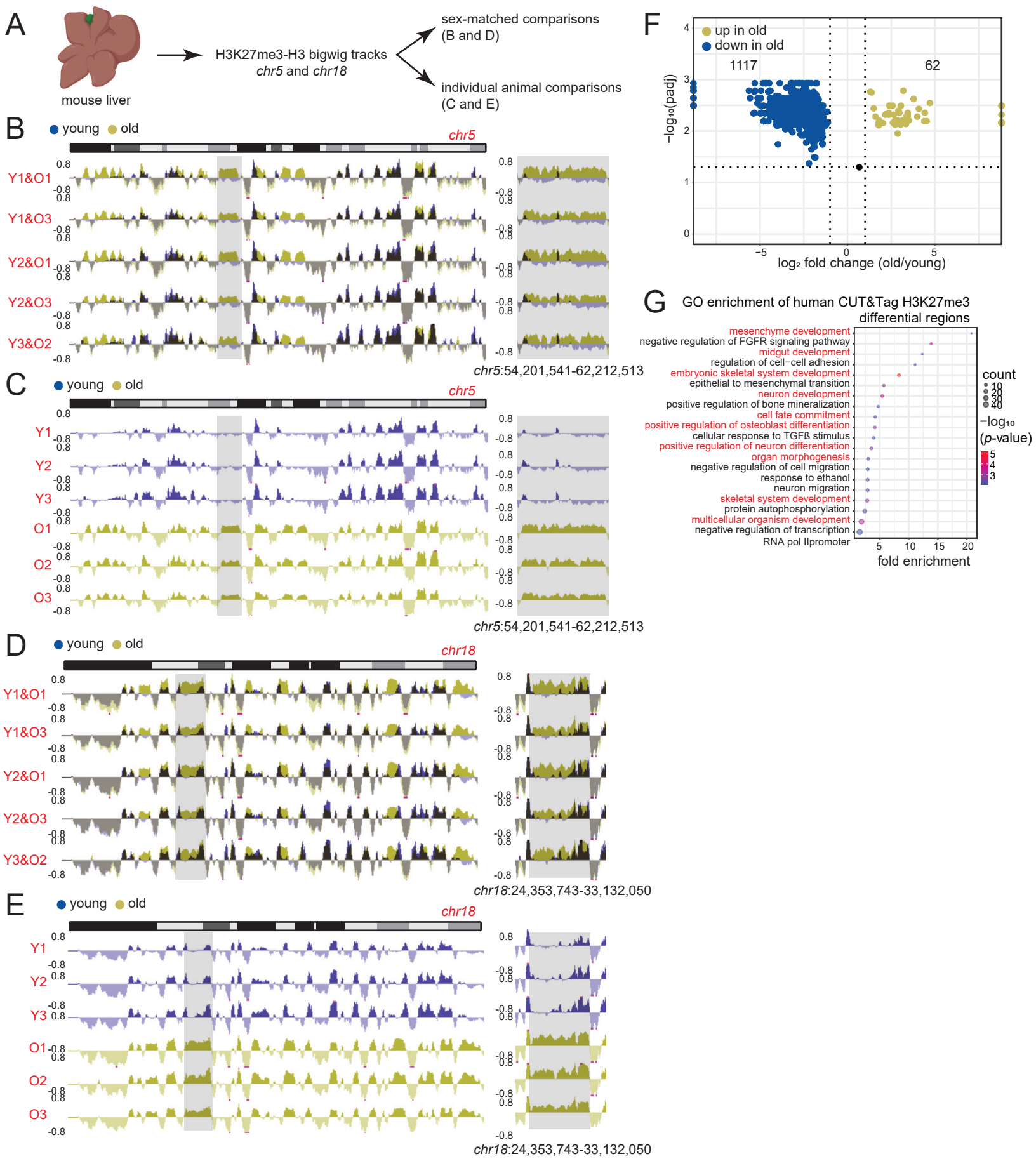
1. Schworer, S., et al., *Epigenetic stress responses induce muscle stem-cell ageing by Hoxa9 developmental signals.* Nature, 2016. **540**(7633): p. 428-432. 10.1038/nature20603.
2. Nativio, R., et al., *An integrated multi-omics approach identifies epigenetic alterations associated with Alzheimer's disease.* Nat Genet, 2020. **52**(10): p. 1024-1035. 10.1038/s41588-020-0696-0.
3. Bou Sleiman, M., et al., *The Gene-Regulatory Footprint of Aging Highlights Conserved Central Regulators.* Cell Rep, 2020. **32**(13): p. 108203. 10.1016/j.celrep.2020.108203.



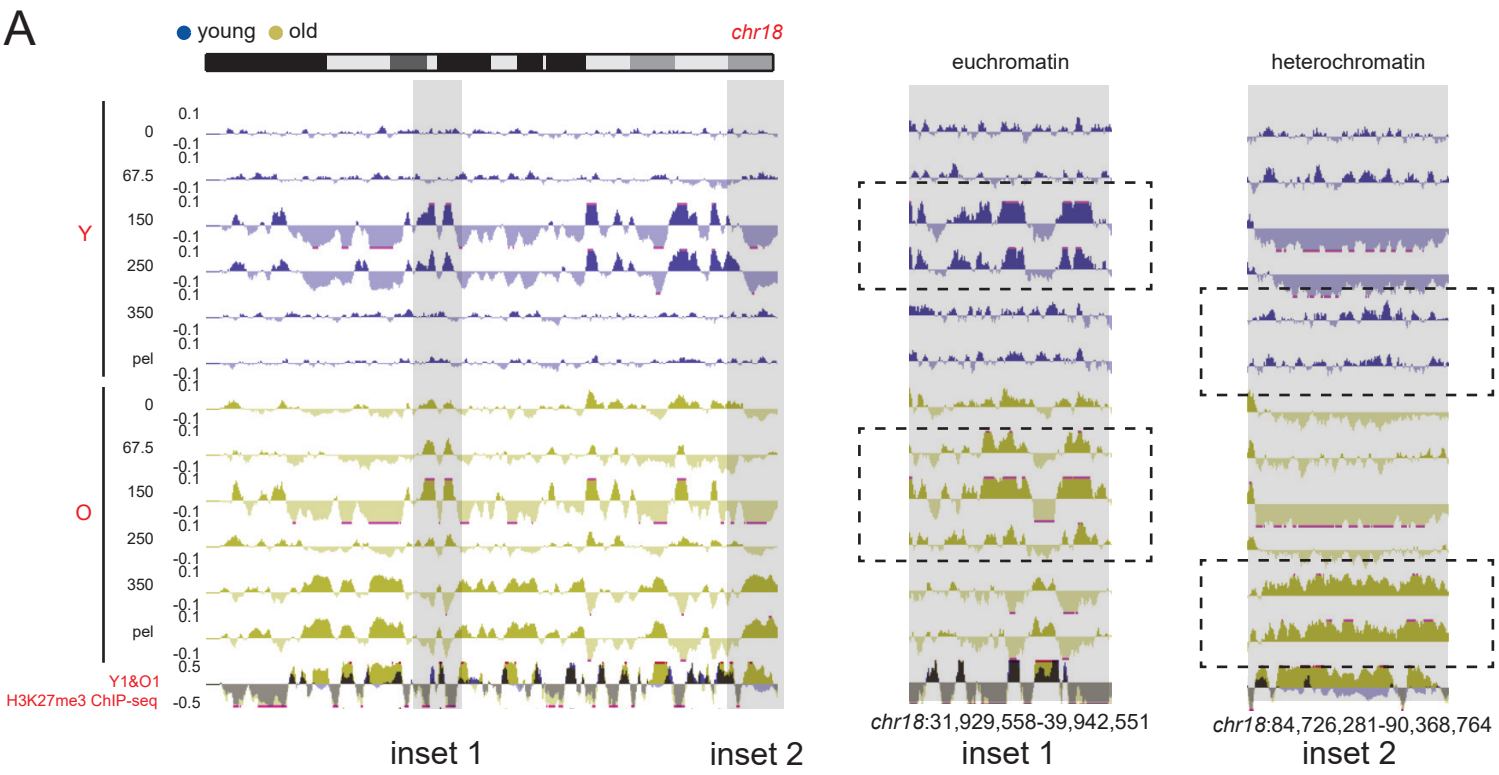
Supplementary Figure 1



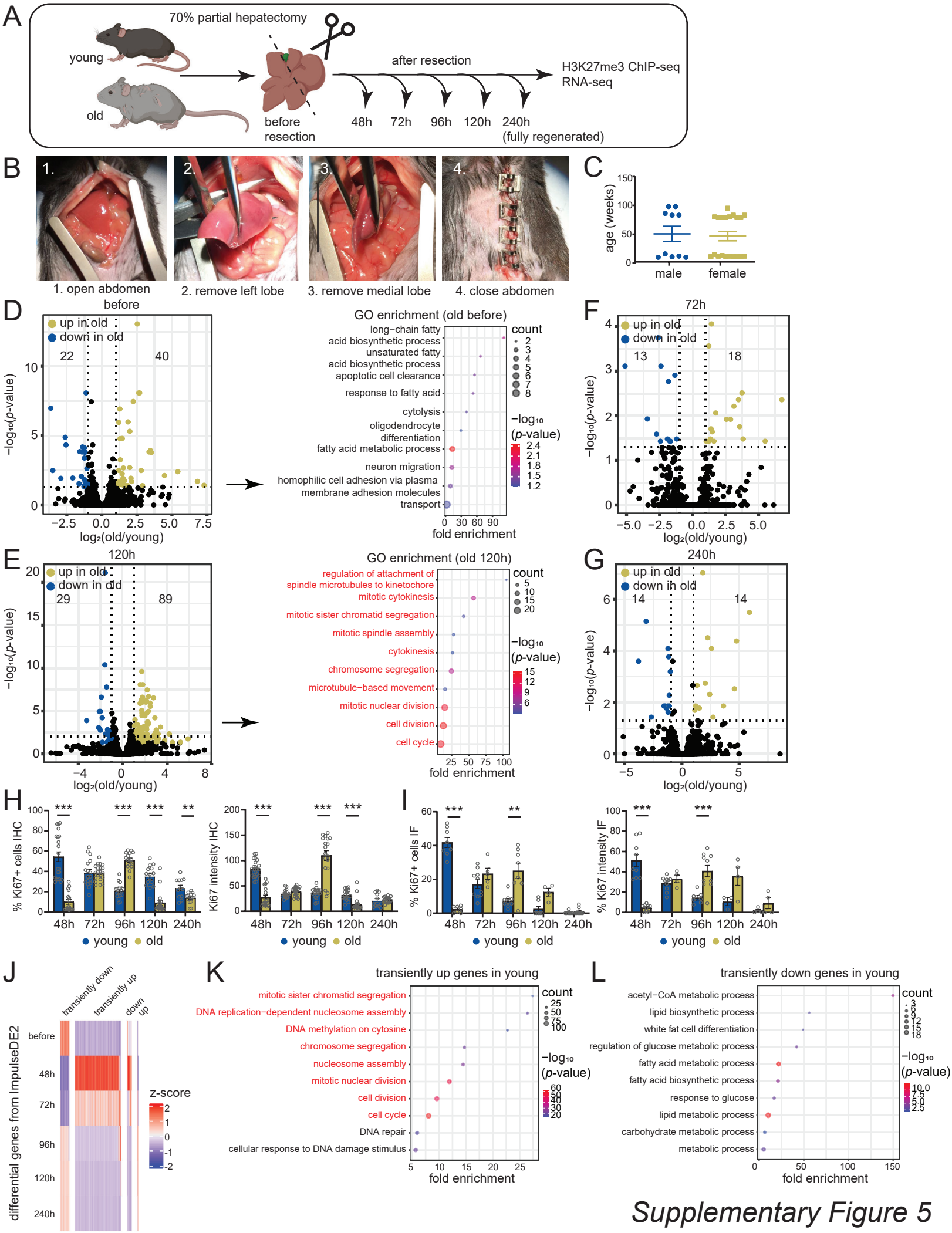
Supplementary Figure 2



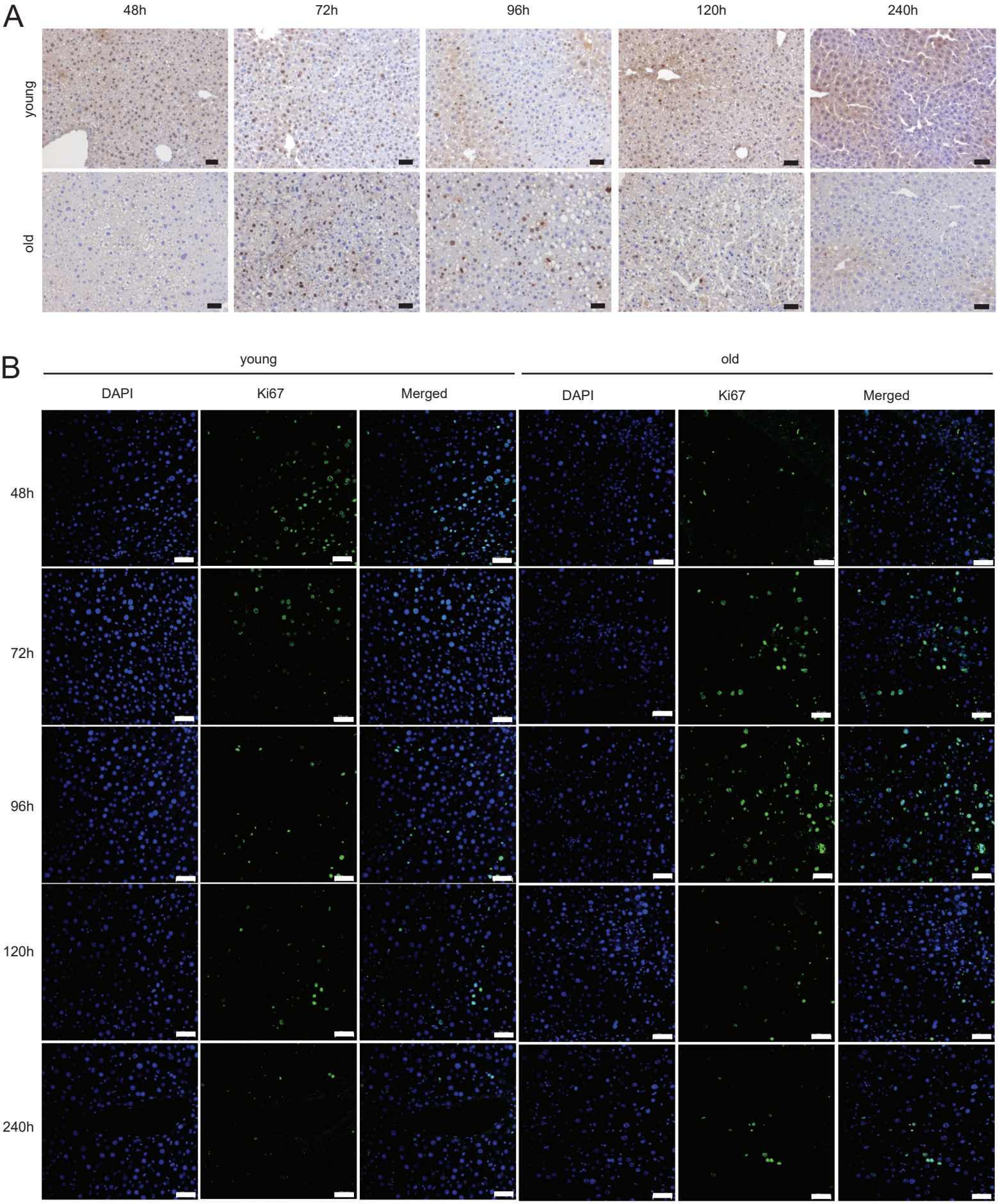
Supplementary Figure 3



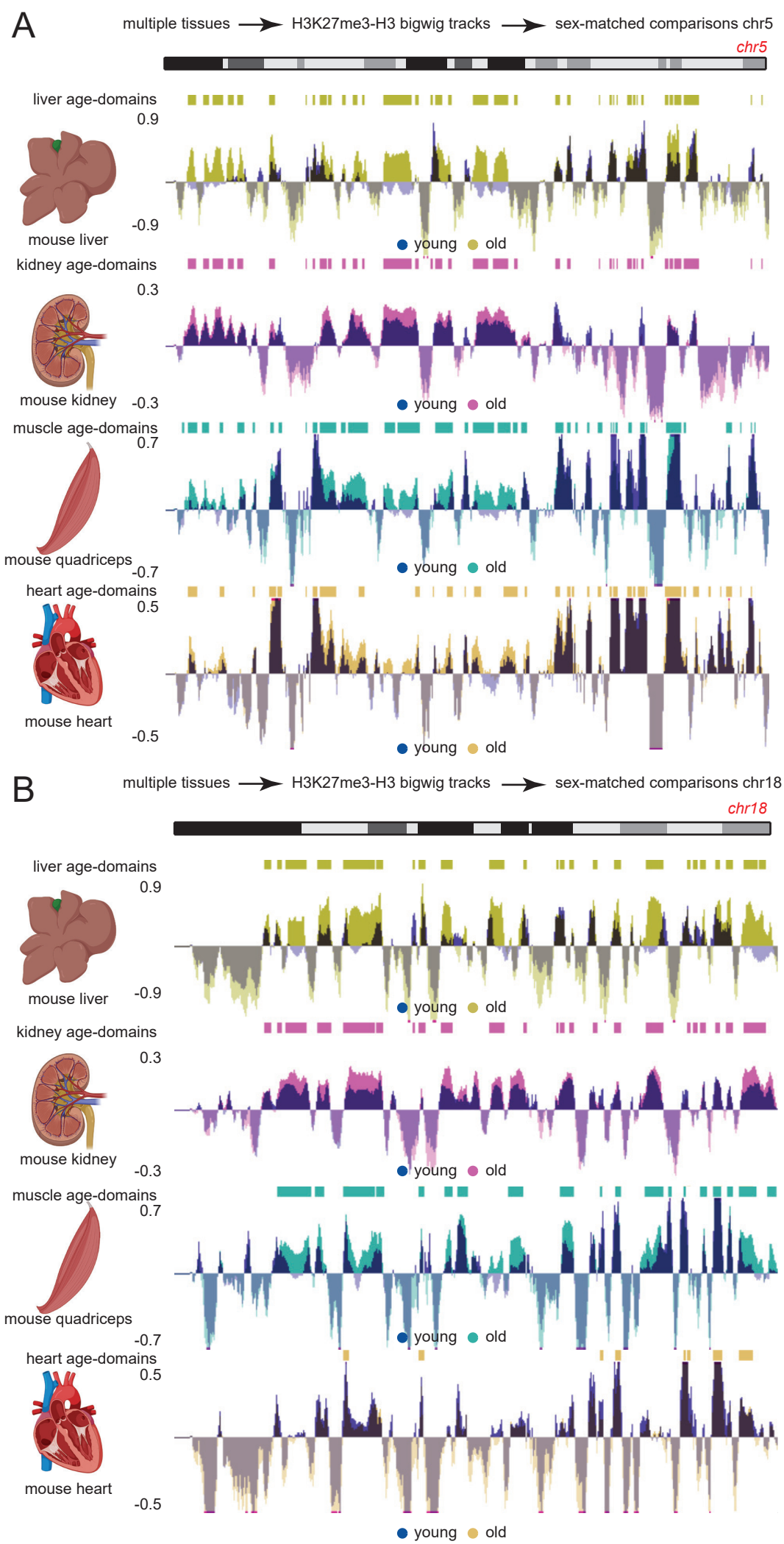
Supplementary Figure 4

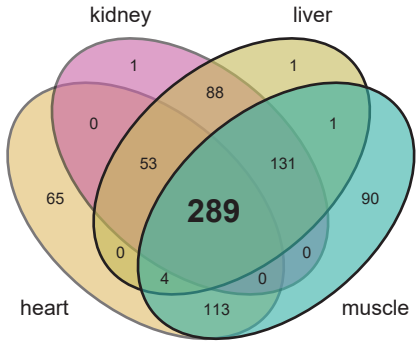


Supplementary Figure 5



Supplementary Figure 6





Supplementary Figure 7