

Proliferation dynamics of organotypic vascular endothelium during aging

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(Supplementary Figures S1-S8)

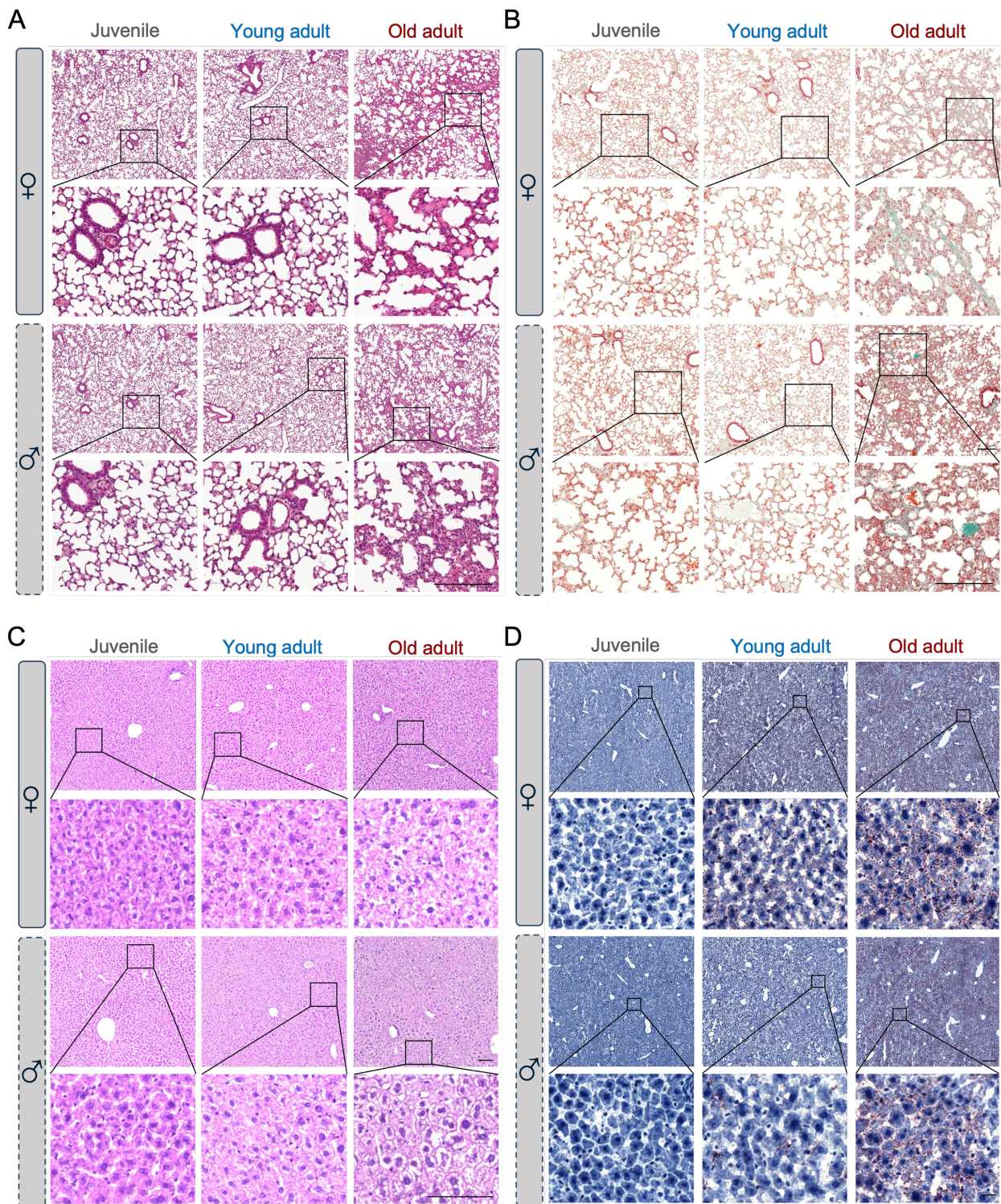


Figure S1: Age-related histological characterization of lung and liver tissues in female and male mice. (A) Representative images of H&E staining on lung tissues. Scale bar: 200 μm . **(B)** Representative images of Masson Goldner staining on lung tissues. Scale bar: 200 μm . **(C)** Representative images of H&E staining on liver tissues. Scale bar: 200 μm . **(D)** Representative images of Oil O Red staining on liver tissues. Scale bar: (overview) 200 μm ; (zoom-in) 20 μm .

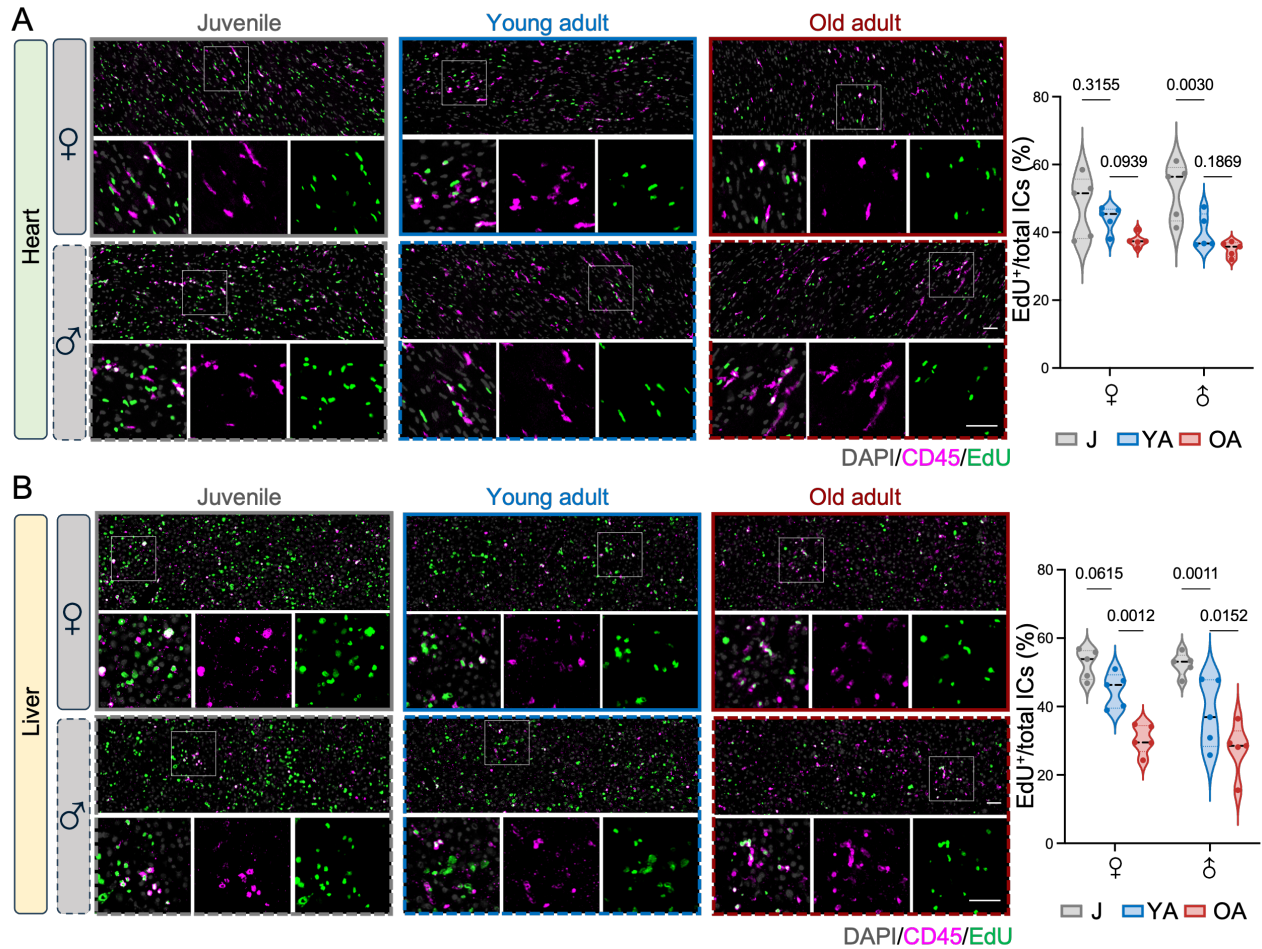


Figure S2. Immune cell proliferation in heart and liver tissues during aging. (A,B) Representative images and quantitative analysis of EdU⁺ immune cells in heart (A) and liver (B) tissues from mice of different ages and sexes ($n_{males} = 5$ and $n_{females} = 5$ per age group). Quantitative data are presented as violin plots showing interquartile range, with each dot representing a value corresponding to a biological replicate. *P*-values are shown as numerical values computed using two-way ANOVA test. Scale bar: 50 μ m.

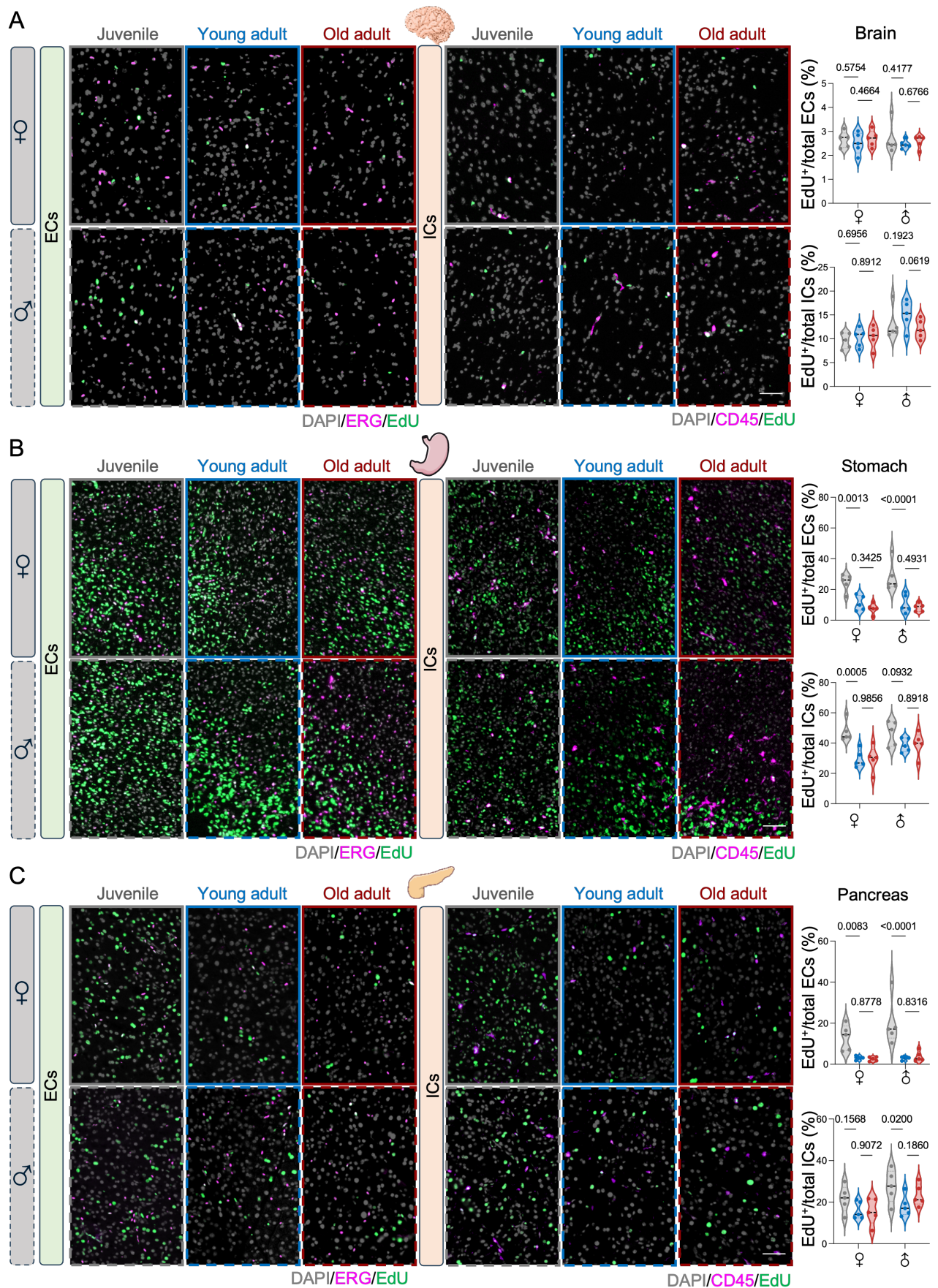


Figure S3. Age-related dynamics of EC and IC proliferation in brain, stomach, and pancreas tissues. (A-C) Representative images and quantitative analysis of EdU+ ECs and ICs in brain (A), stomach (B), and pancreas (C) tissues from mice of different ages and sexes ($n_{males} = 5$ and $n_{females} = 5$ per age group). Quantitative data are presented as violin plots showing interquartile range, with each dot representing a value corresponding to a biological replicate. *P*-values are shown as numerical values computed using two-way ANOVA test. Scale bar: 50 μ m.

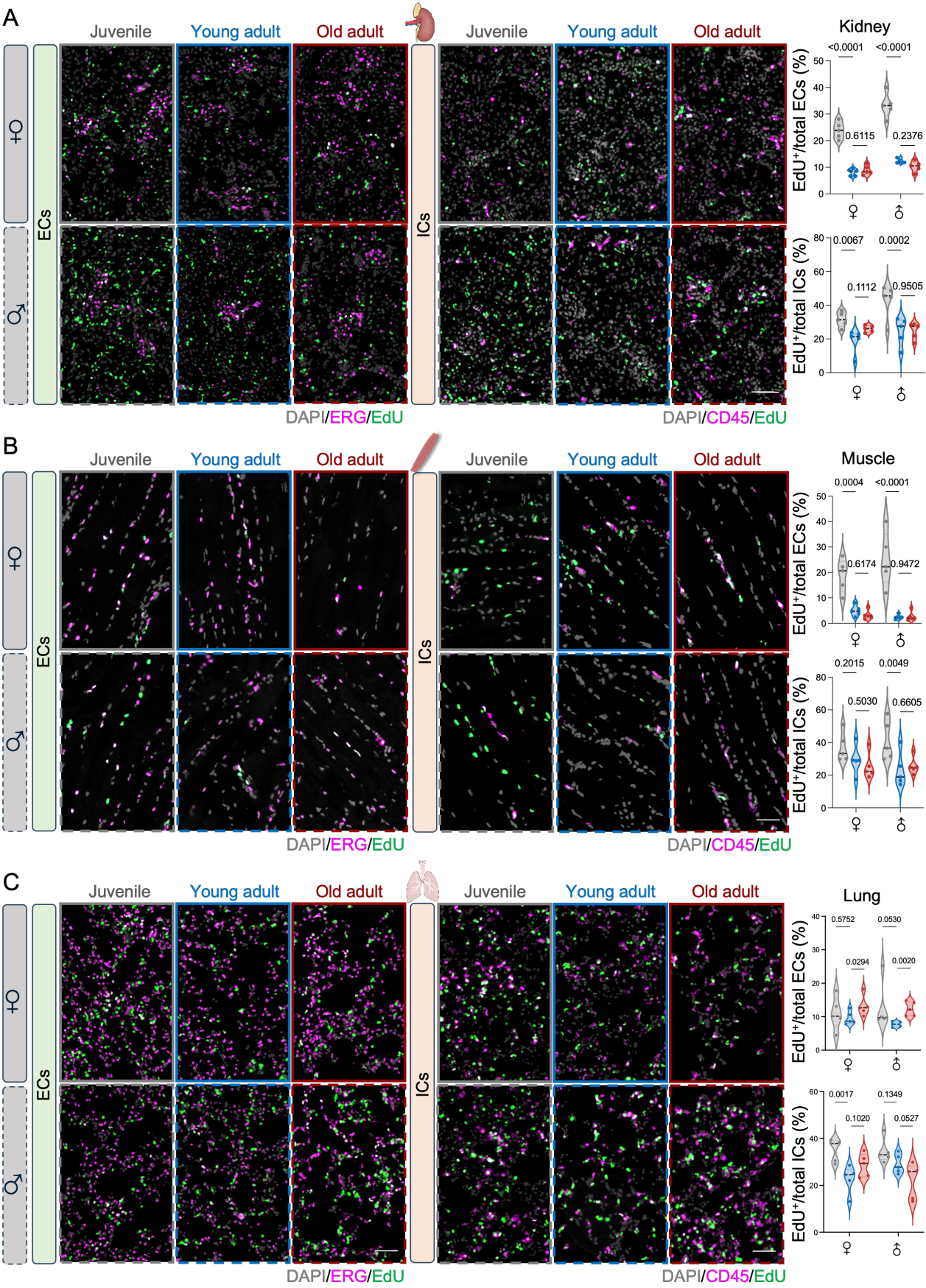


Figure S4. Age-related dynamics of EC and IC proliferation in kidney, muscle, and lung tissues. (A-C) Representative images and quantitative analysis of EdU+ ECs and ICs in kidney (A), muscle (B), and lung (C) tissues from mice of different ages and sexes ($n_{males} = 5$ and $n_{females} = 5$ per age group). Quantitative data are presented as violin plots showing interquartile range, with each dot representing a value corresponding to a biological replicate. *P*-values are shown as numerical values computed using two-way ANOVA test. Scale bar: 50 μ m.

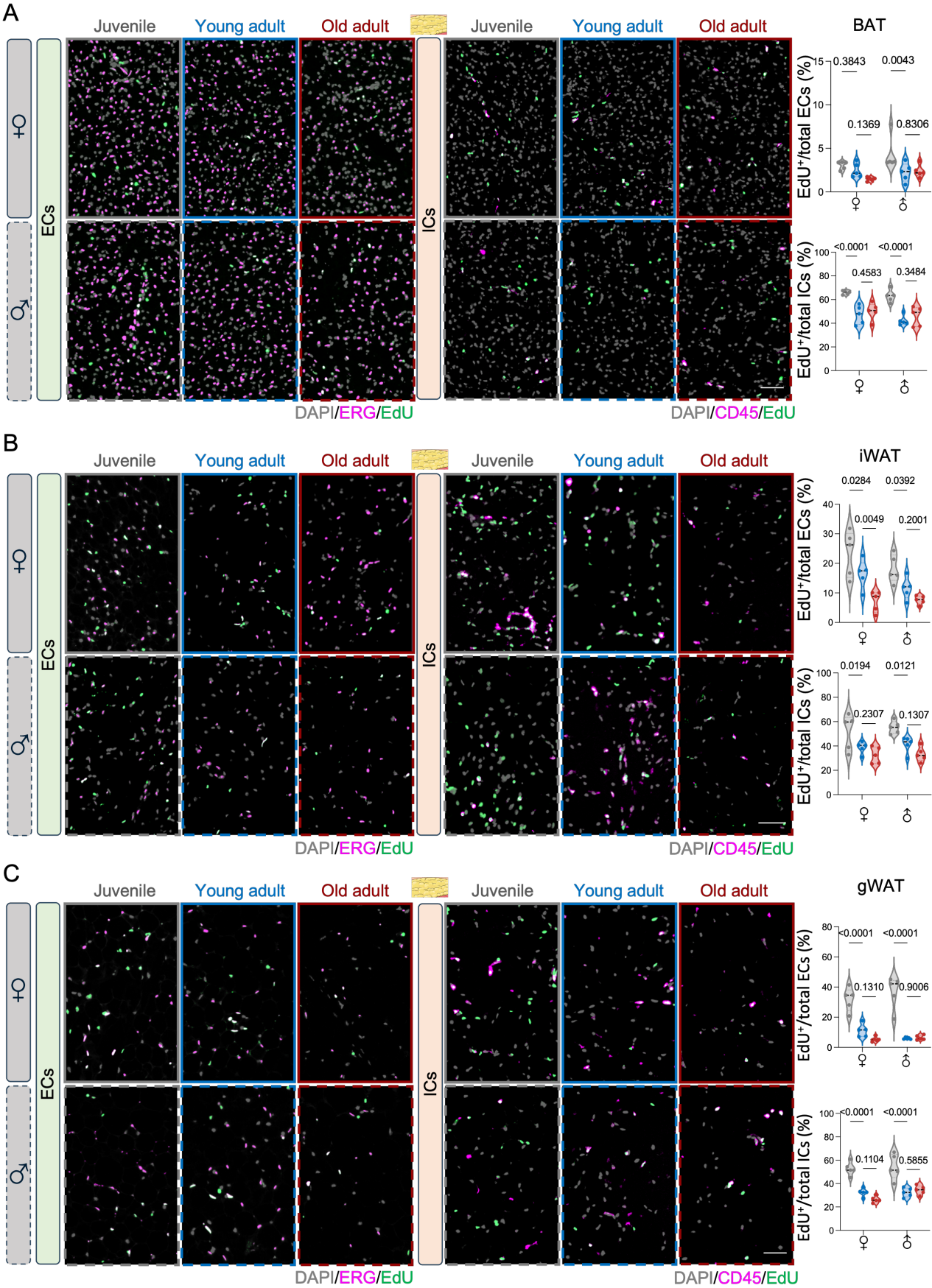


Figure S5. Age-related dynamics of EC and IC proliferation in different adipose depots. (A-C) Representative images and quantitative analysis of EdU+ ECs and ICs in brown (BAT, **A**), inguinal white (iWAT, **B**), and gonadal white (gWAT, **C**) tissues from mice of different ages and sexes ($n_{males} = 5$ and $n_{females} = 5$ per age group). Quantitative data are presented as violin plots showing interquartile range, with each dot representing a value corresponding to a biological replicate. *P*-values are shown as numerical values computed using two-way ANOVA test. Scale bar: 50 μ m.

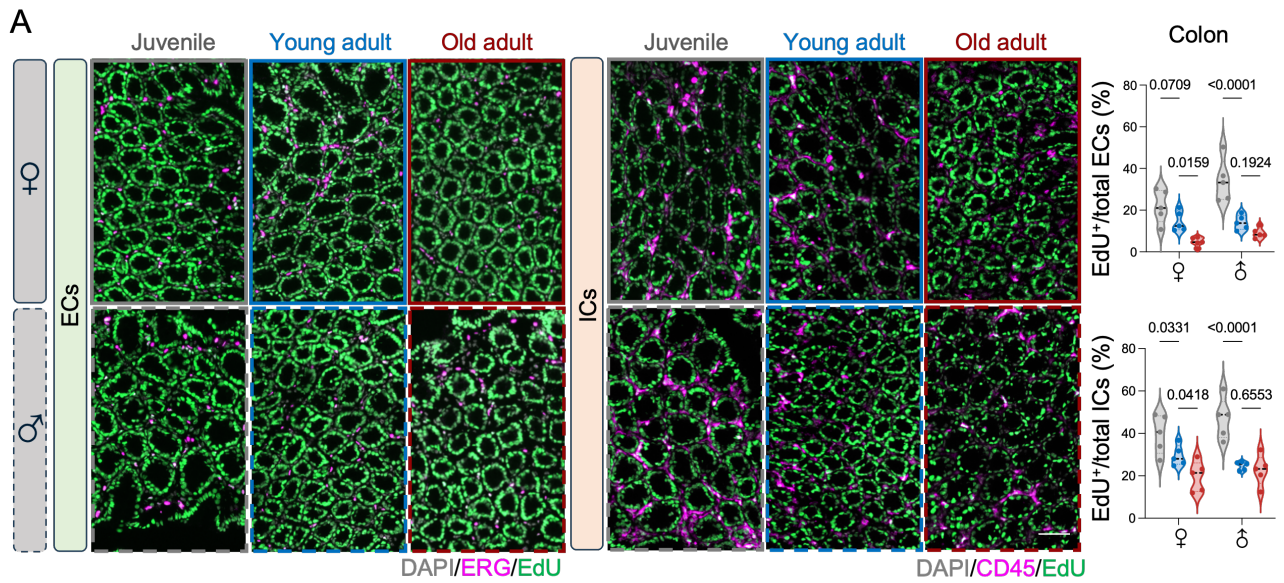


Figure S6. Age-related dynamics of EC and IC proliferation in colon tissues. (A) Representative images and quantitative analysis of EdU⁺ ECs and ICs in colon tissues from mice of different ages and sexes ($n_{males} = 5$ and $n_{females} = 5$ per age group). Quantitative data are presented as violin plots showing interquartile range, with each dot representing a value corresponding to a biological replicate. *P*-values are shown as numerical values computed using two-way ANOVA test. Scale bar: 50 μ m.

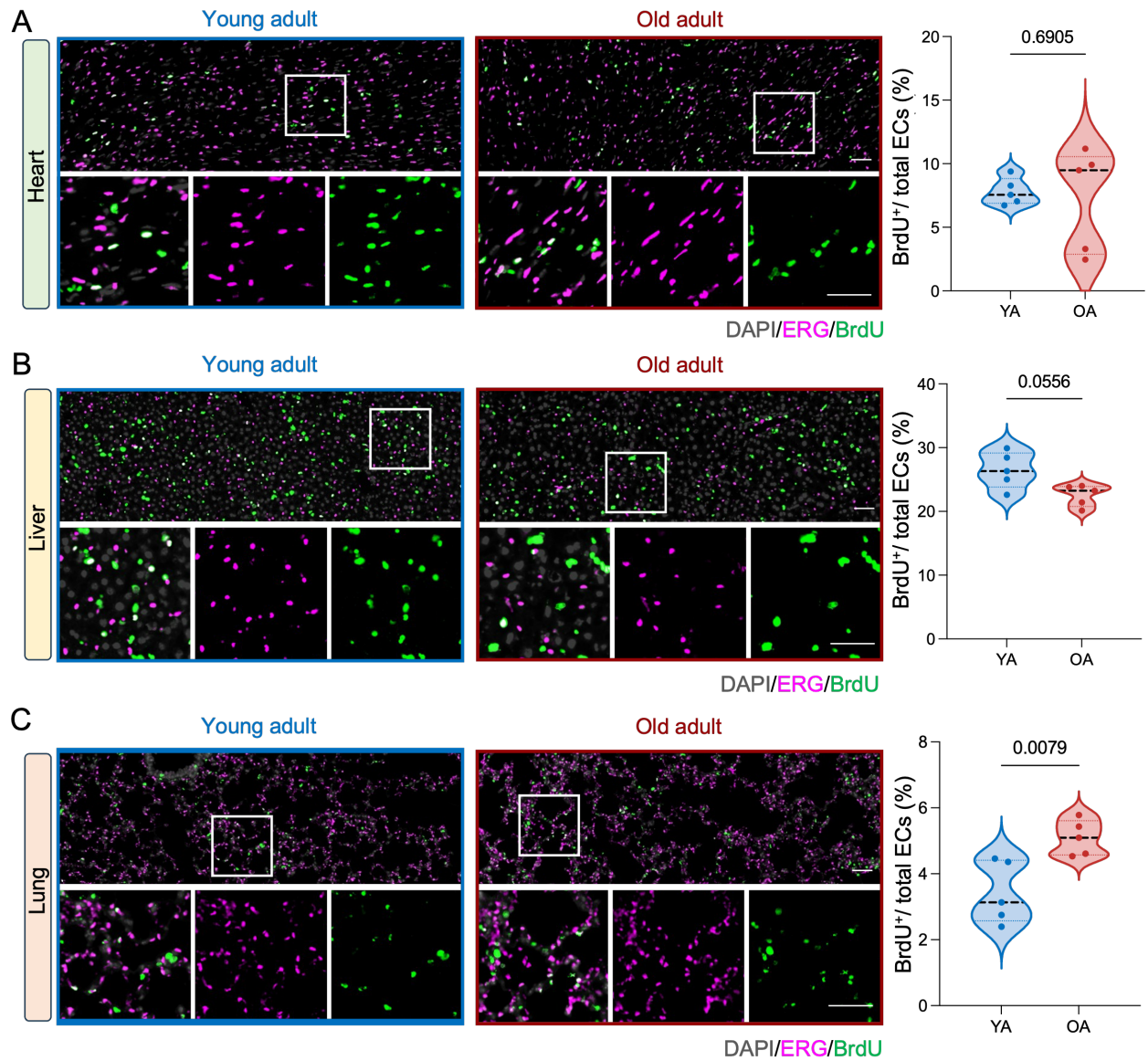


Figure S7. Age-related dynamics of BrdU-based EC proliferation in heart, liver, and lung tissues in female mice. (A-C) Representative images and quantitative analysis of BrdU⁺ ECs in heart (A), liver (B), and lung (C) tissues from female mice of different ages ($n_{females} = 5$ per age group). Quantitative data are presented as violin plots showing interquartile range, with each dot representing a value corresponding to a biological replicate. *P*-values are shown as numerical values computed using Mann-Whitney U test. Scale bar: 50 μ m.

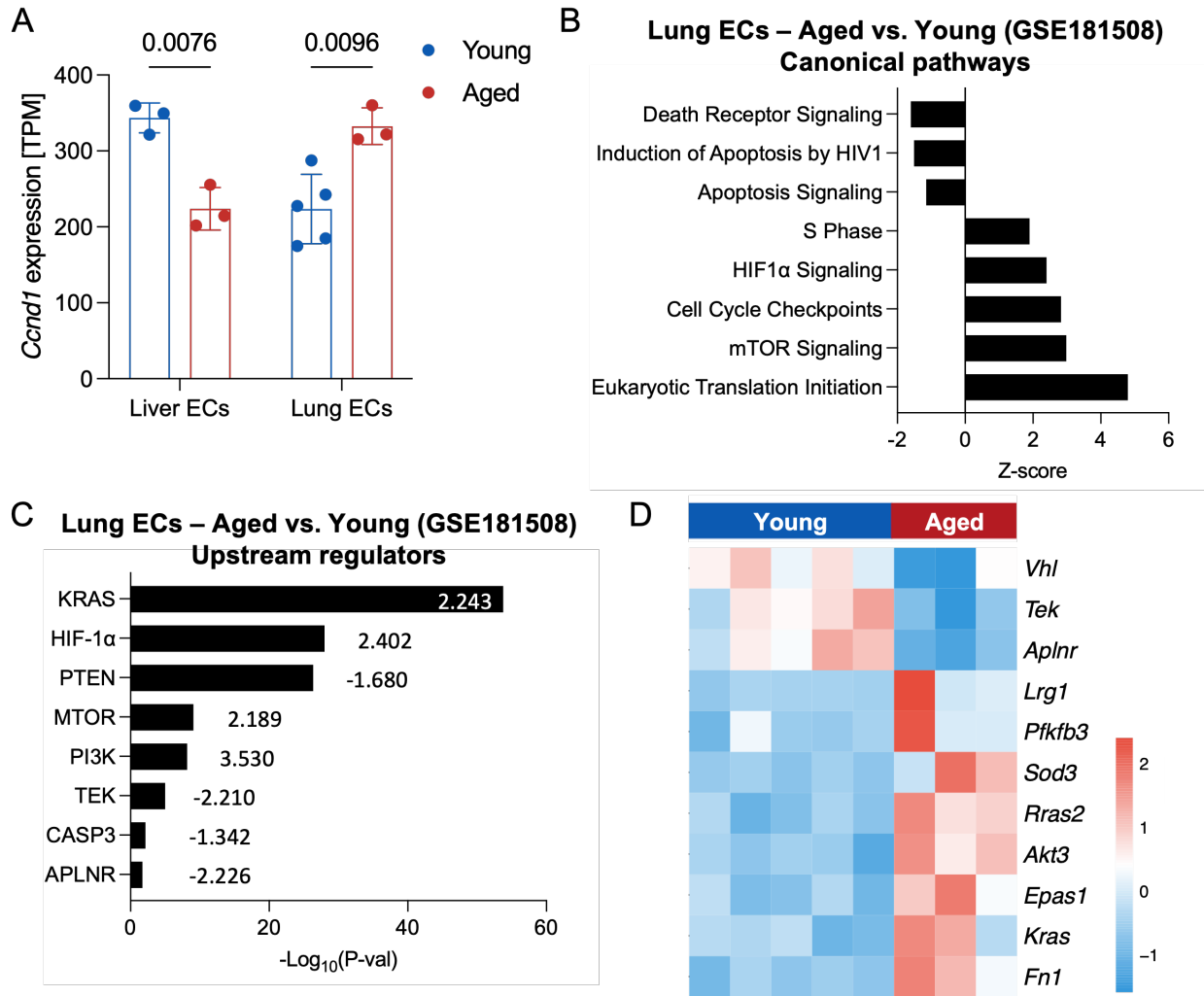


Figure S8. Molecular changes in lung ECs during aging. (A) *Ccnd1* expression in liver and lung ECs isolated from young and aged mice, reanalyzed from publicly available bulk transcriptomic datasets (GSE216592 – liver ECs; GSE181508 – lung ECs). Data are presented as mean $\pm$ SD. *P*-values are shown as numerical values computed using multiple Mann-Whitney tests. TPM: Transcripts Per Million reads. (B,C) Aging-associated DEGs in lung ECs were analyzed with Ingenuity Pathway Analysis. Shown are enriched canonical pathways (B) and upstream regulators (C) in aged vs. young lung ECs in the GSE181508 dataset. In panel C, numerical values reflect the corresponding z-score. (D) Heatmap comparing the expression of selected DEGs between young and aged lung ECs in the GSE181508 dataset.