

## Supplemental Materials

### Figure S1. Quality control for spatial transcriptome data of NHP ovarian aging.

- (A) H&E staining (top) and spot type annotation of spatial transcriptomic data (bottom) for all samples in this study.
- (B) Spatial visualization showing the number of genes detected in each spot of all samples.
- (C) Bar plot showing the number of genes detected in each spot of all samples.
- (D) Bar plot showing the mean read of each spot for all samples.
- (E) Bar plot showing the spot number for all samples.

### Figure S2. Spatial expression of marker genes for major spot types in monkey ovary.

### Figure S3. CD31 immunofluorescence staining of ovaries from young and old monkeys.

Representative images are shown on the left. Scale bars, 20  $\mu\text{m}$  and 10  $\mu\text{m}$  (zoomed-in images). The numbers of CD31-positive cells and CD31-positive area were quantified as fold changes (Old vs. Young), and presented as mean  $\pm$  SEMs on the right. n = 4 monkeys for each group.

### Figure S4. Age-related changes of a panel of aging hallmarks in NHP ovaries.

- (A) CD163 immunofluorescence staining of ovaries from young and old monkeys. Representative images are shown on the left. Scale bars, 20  $\mu\text{m}$  and 10  $\mu\text{m}$  (zoomed-in images). The numbers of CD163-positive cells were quantified as fold changes (Old vs. Young), and presented as mean  $\pm$  SEMs on the right. n = 4 monkeys for each group.
- (B) Violin plot showing the gene set score of inflammatory response-related genes across different spot types in young and old groups.
- (C) Violin plot showing the gene set score of SASP-related genes across different spot types in young and old groups.
- (D) Violin plot showing the gene set score of fibrosis-related genes across different spot types in young and old groups.
- (E) Ridge plot (left) and violin plot (right) showing the gene set score of TGF- $\beta$  pathway-related genes in young and old groups. The corresponding dashed lines represent the median of each group.
- (F) Violin plot showing the gene set score of lipid storage-related genes across different spot types in young and old groups.
- (G) Ridge plot (left) and violin plot (right) showing the gene set score of ATF6 pathway-related genes in young and old groups. The corresponding dashed lines represent the median of each group.
- (H) Ridge plot (left) and violin plot (right) showing the global distribution density the gene set score of IRE1 pathway-related genes in young and old groups. The corresponding dashed lines represent the median of each group.
- (I) Ridge plot (left) and violin plot (right) showing the gene set score of PERK pathway-related genes in young and old groups. The corresponding dashed lines represent the median of each group.
- (J) Ridge plot (left) and violin plot (right) showing the gene set score of autophagy-related genes in young and old groups. The corresponding dashed lines represent the median of each group.

- (K) Violin plot showing the gene set score of apoptosis-related genes across different spot types in young and old groups.
- (L) Cleaved-caspase 3 immunostaining of ovarian tissues from young and aged monkeys. Representative images are shown on the left. Scale bar, 20  $\mu\text{m}$  and 10  $\mu\text{m}$  (zoomed-in images). Cleaved-caspase 3-positive cells in the tissues were quantified as fold changes (Old vs. Young), and shown as mean  $\pm$  SEMs on the right. n = 4 monkeys for each group.

**Figure S5. Analysis of the canonical aging hallmark-related genes set in NHP ovaries.**

- (A) Violin plot showing the gene set score of ROS-related genes across different spot types in young and old groups.
- (B) Violin plot showing the gene set score of DNA repair-related genes across different spot types in young and old groups.
- (C) Violin plot showing the gene set score of senescence-related genes across different spot types in young and old groups.
- (D) SA- $\beta$ -Gal staining of ovaries from young and old monkeys. Scale bars, 200  $\mu\text{m}$  and 50  $\mu\text{m}$  (zoomed-in images). SA- $\beta$ -Gal-positive areas were quantified as fold changes (Old vs. Young), and presented as mean  $\pm$  SEMs on the right. n = 4 monkeys for each group.

**Figure S6. Correlation analysis between OSAGs score and multiple major aging-related pathways.**

- (A) The scatter plot showing the correlation coefficients between OSAG-upregulated or downregulated gene set scores and gene set scores of 11 age-related pathways.
- (B) Correlation analysis between OSAG-upregulated set score and gene set scores of 11 age-related pathways across different spot types in monkey ovary.
- (C) The spatial distribution and colocalization presentation of OSAG-upregulated gene set score and scores of fibrosis, senescence, and inflammatory response-related gene sets.

**Figure S7. The integrative analysis of APOE-positive spots and spots with a high PCOA score.**

- (A) The spatial distribution and colocalization presentation of spots highly expressing *APOE* and those with a high PCOA score, including SASP-related gene set score, fibrosis-related gene set score, senescence-related gene set score and inflammatory response-related gene set score.
- (B) The scatter plot showing the correlation coefficient between *MT2* expression and *APOE* expression.
- (C) The spatial distribution and colocalization presentation of *MT2* expression and *APOE* expression in cynomolgus monkey ovary.

**Supplementary Tables**

**Table S1.** Marker genes for each spot type in non-human primate ovaries.

**Table S2.** Aging -related differentially expressed genes (DEGs) for each spot type.

**Table S3.** Gene list for genes used in this study.

Figure S1

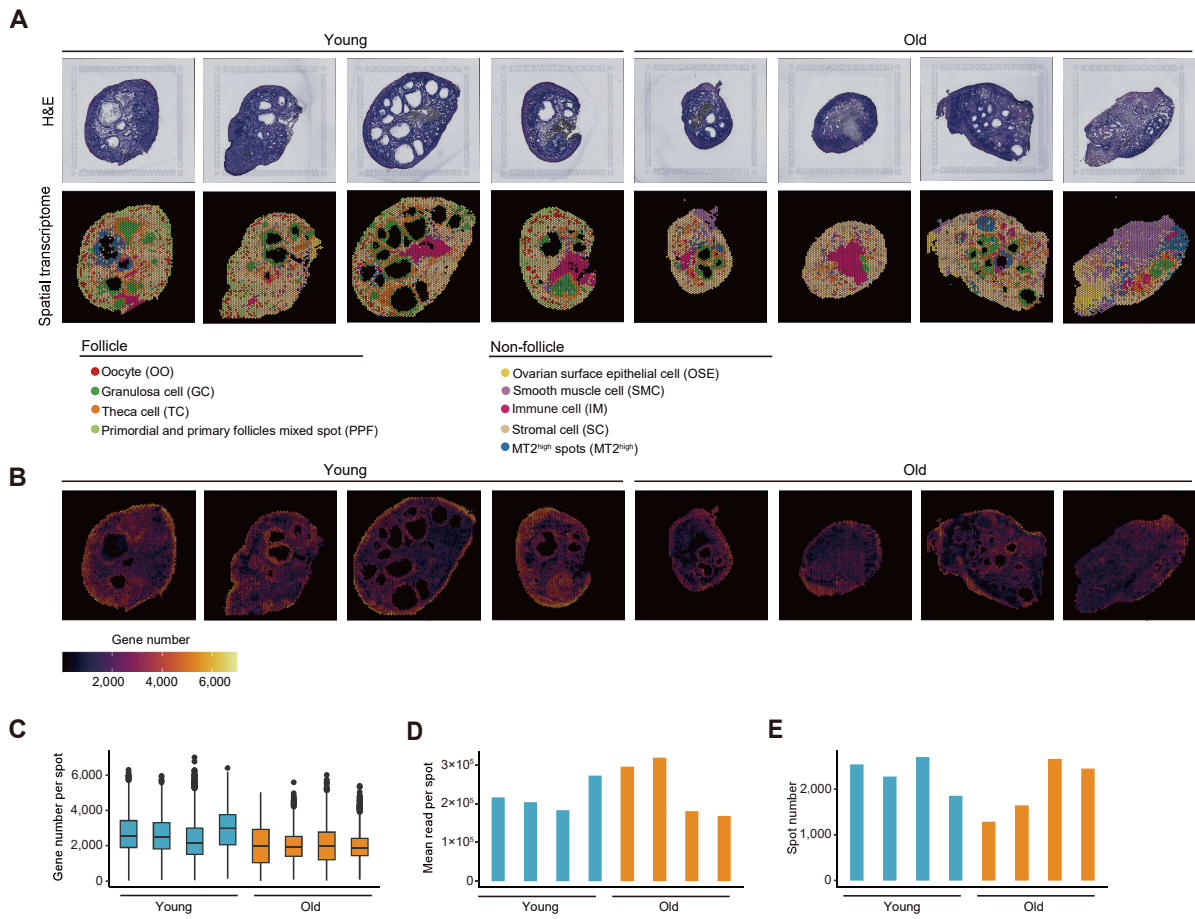


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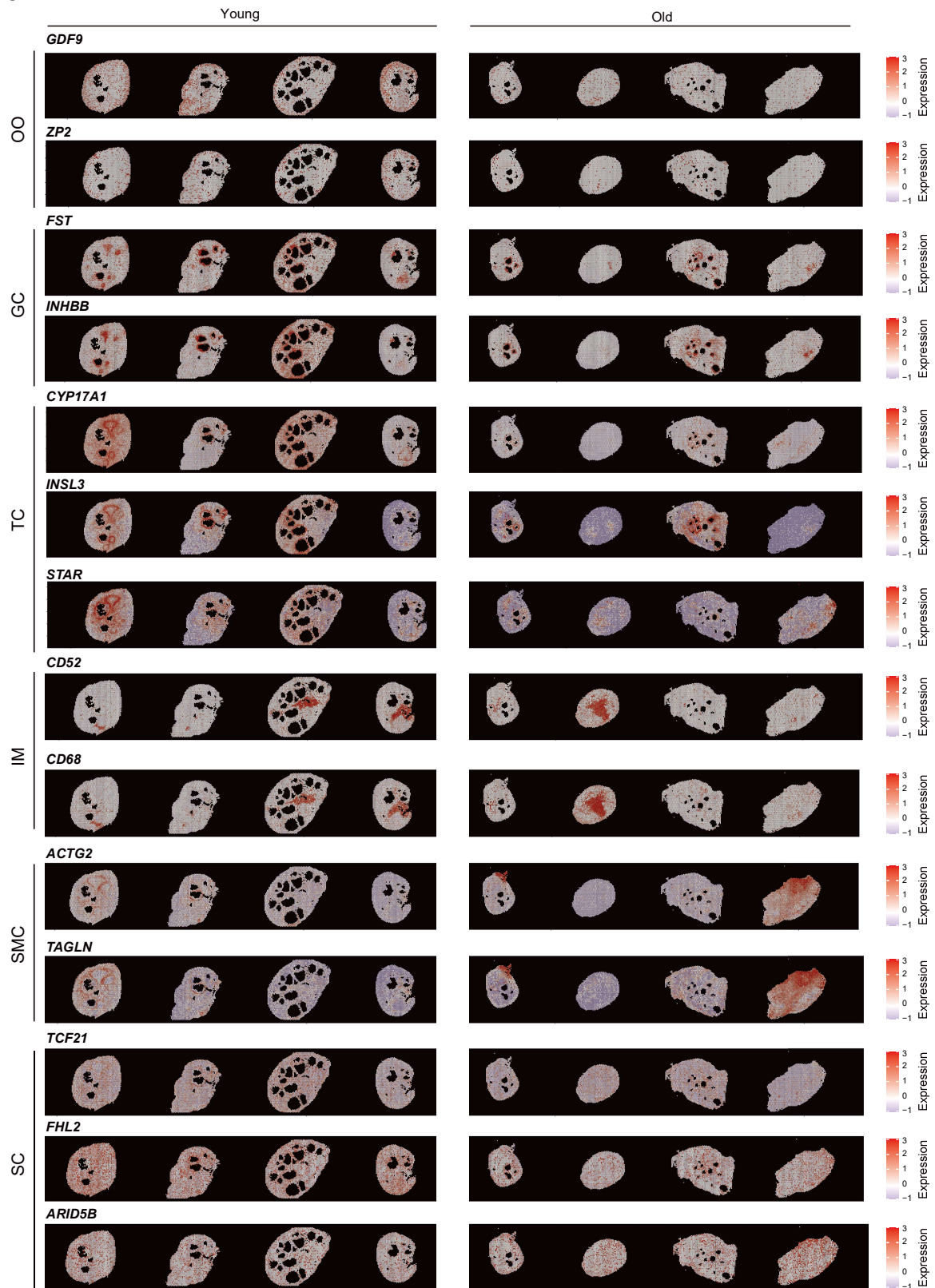


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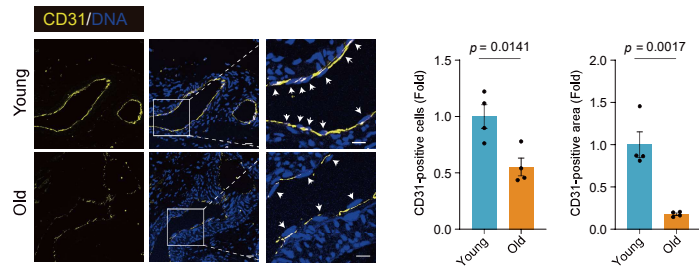
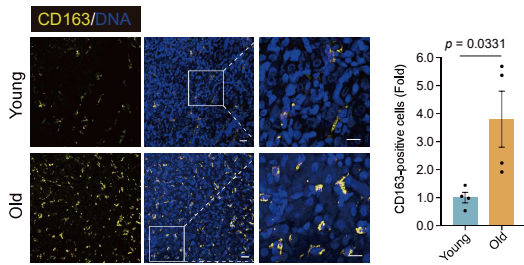
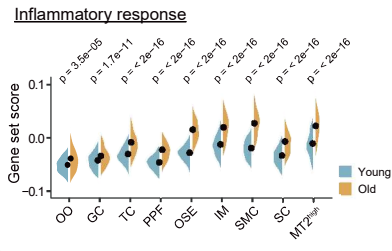


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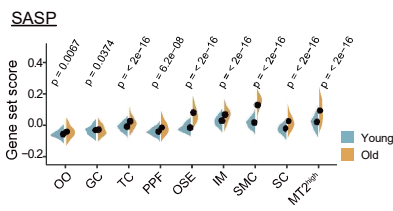
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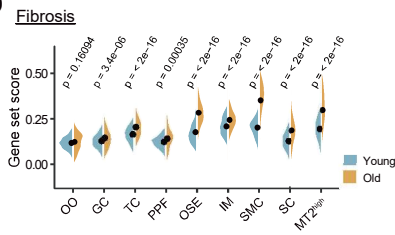
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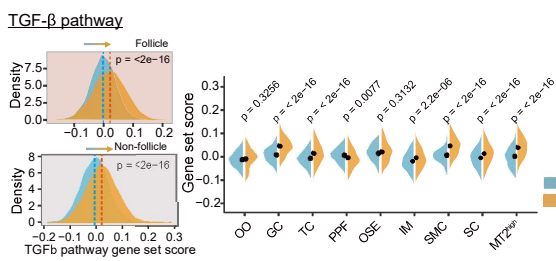
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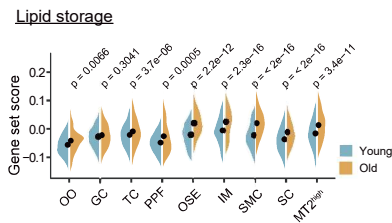
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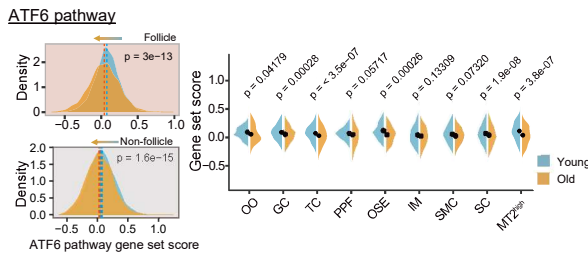
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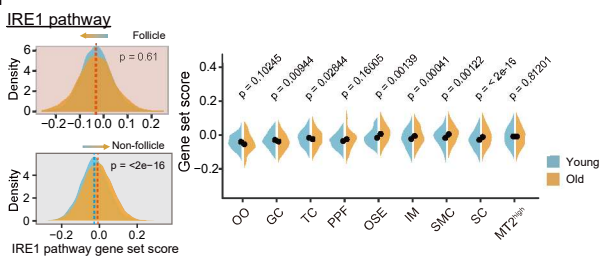
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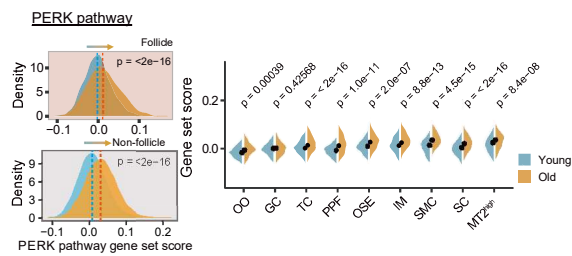
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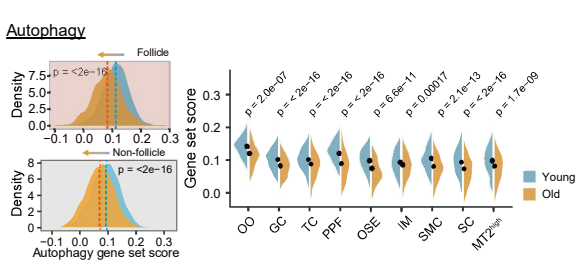
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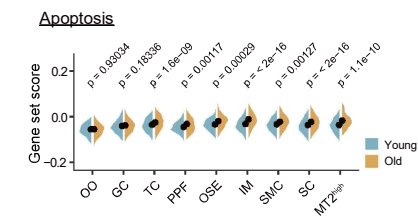
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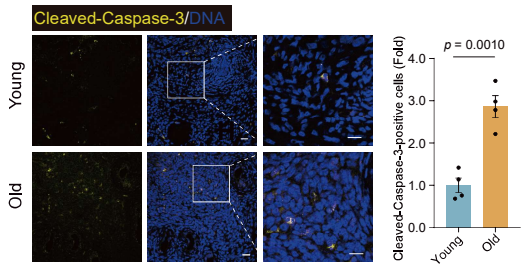


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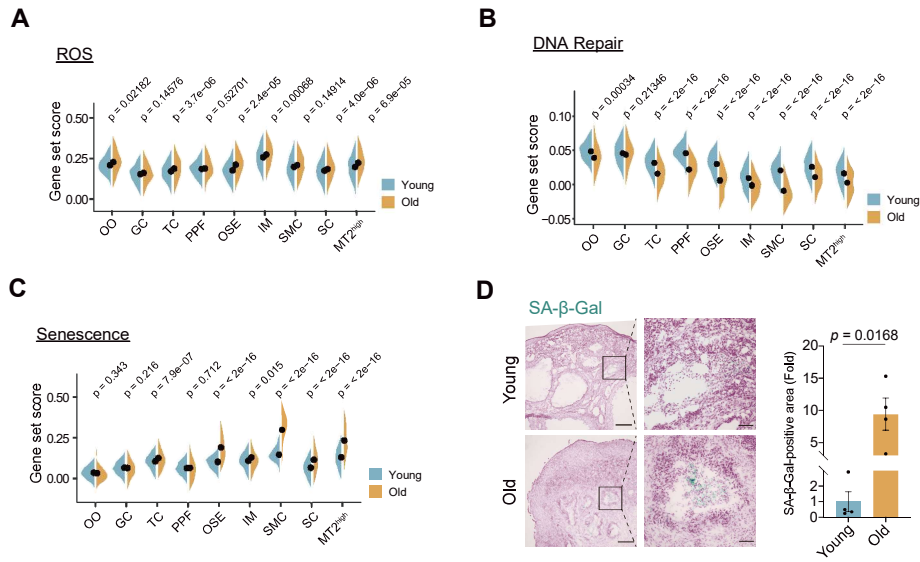
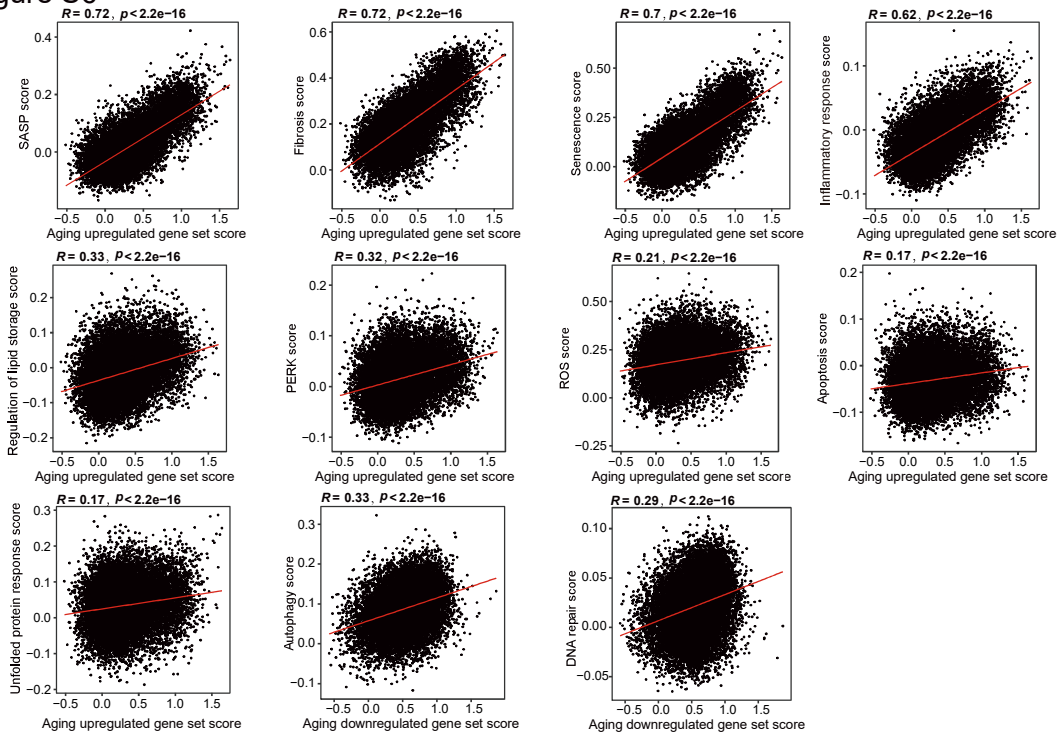
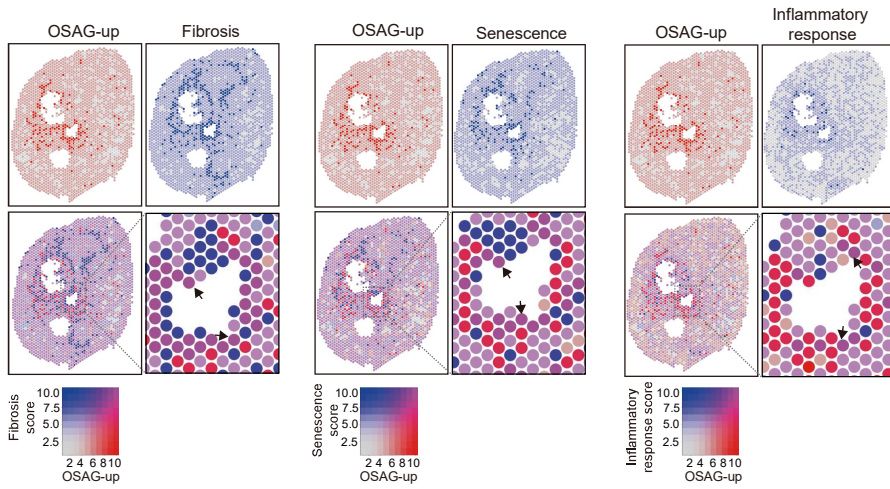


Figure S6

A



B



C

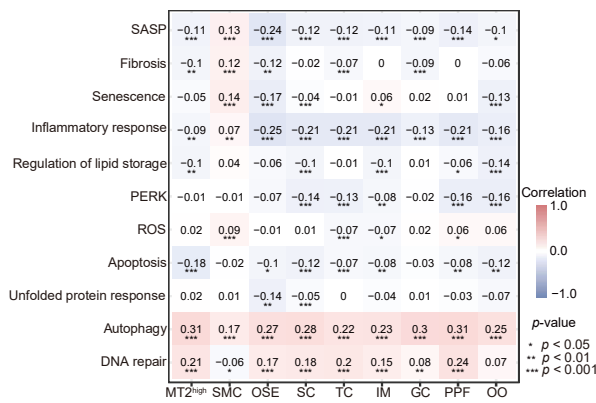
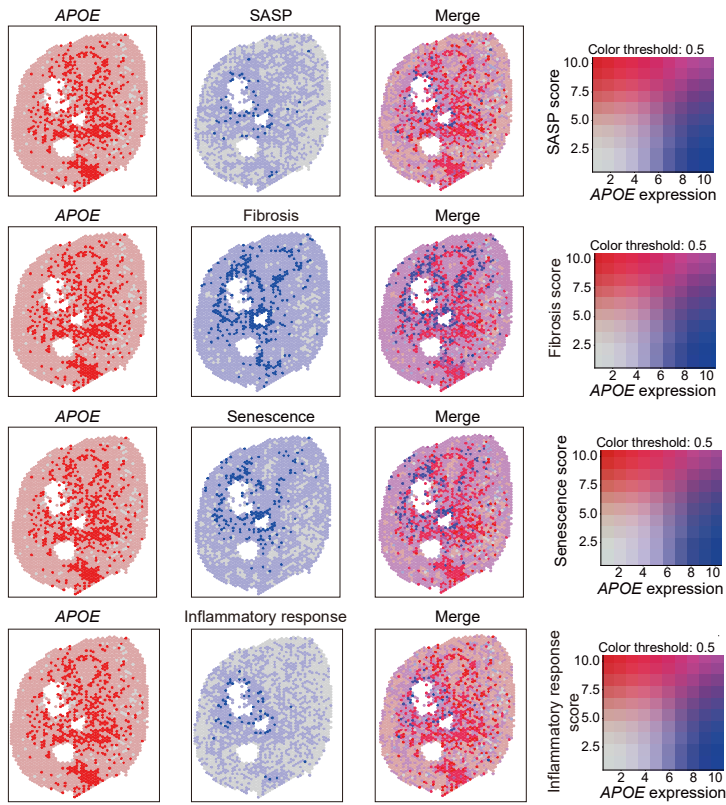
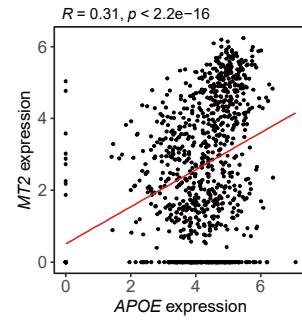


Figure S7

**A**



**B**



**C**

