

Supplemental Materials

Figure S1. Quality control of snRNA-seq data

- A. The ratio of heart weight to body weight and the ratio of heart weight to lung weight were measured in young and aged cynomolgus monkeys. Data are presented as the mean \pm SEM. $n = 8$ monkeys for each group.
- B. The distribution of quality controls, including median number of genes, sequencing saturation, and mapping rate to the genome, in the snRNA-seq data of each sample.
- C. Scatter plot showing the proportion of mitochondrial genes for each cell. The color key from gray to red indicates less to more percentage of mitochondrial genes. Low quality cells are colored by gray.
- D. Histograms showing the number of genes detected in individual cells.
- E. Histograms showing the number of unique molecular identifiers (UMIs) detected in individual cells.
- F. Boxplot showing the value of ROGUE (A method for accurately quantify the purity of an identified cell clusters) of each cell type. Box shows the median and the quartile range (25-75%) and the length of whiskers represents $1.5 \times$ the IQR.

Figure S2. DEG analysis in multiple cell types and cell-cell communication

- A. Dot plots showing the expression of representative genes for each cell type in monkey LV.
- B. Bar plot showing changes in the proportion of cells between young and old monkey LV.
- C. Bar plots showing aging-related upregulated (left) and downregulated (right) DEG enriched GO terms or pathways in the seven cell types with the highest DEG numbers in the monkey LV.
- D. Network plots showing changes in the number of ligand-receptor interactions detected between each two cell types and/or within a single cell type in the old vs. young groups. The changes in the number of ligand-receptor interactions are indicated by connection lines. The thickness of the lines is positively correlated with the number of ligand-receptor interactions. Red lines indicated an increase in the number of ligand-receptor interactions in the old group, and blue lines indicated a decrease.
- E. The circle plot showing the top 100 highly expressed ligand-receptor interactions in the old vs. young groups. The gene types of ligands and receptor are colored by yellow, green and black, and are annotated on the right. The ligand-receptor interaction events are indicated by connecting lines with arrows. The thickness of the line is positively correlated with the expression level of the ligand, and the size of the arrow is positively correlated with the expression level of the receptor. Red lines indicated upregulated ligand and/or receptor in the old group, blue lines indicated ligand-receptor pairs downregulated in the old group.

Figure S3. Single-nucleus transcriptomic signatures in NHP CM and bulk RNA-seq quality control in primate CM

- A. The heatmap showing the expression levels of aging-related core regulatory transcription factors (TFs) in monkey ventricular cells, with red representing up-regulated and blue representing down-regulated. The size of the dot plot indicates the number of target genes regulated by a certain transcription factor.
- B. Dot plots showing that DEGs overlapped with genes from specific heart disease-associated gene sets. Red parts of nodes, upregulated genes; blue parts of nodes,

- downregulated genes. The node size indicates the frequency of DEGs.
- C. Dot plot showing cardiomyocytes aging-related DEGs overlapped with genes from the heart disease-related gene sets. Red nodes, upregulated genes; blue nodes, downregulated genes.
 - D. Heatmap showing the activity (row z-score) of 18 aging-related regulators in monkey cardiomyocytes. Top panel, age of each cardiomyocyte (green, young group; yellow, old group).
 - E. Heatmap showing the relative expression level (row-scaling) of DEGs in siNC and siFOXP1 hCMs by RNA-sequencing.
 - F. Volcano plot showing the DEGs of siNC and siFOXP1 hCMs by bulk RNA-sequencing.

Supplemental Table Legends

Table S1. Marker genes for each cell type in cynomolgus monkey left ventricle.

Table S2. Aging-related differentially expressed genes (DEGs) for each cell type in the cynomolgus monkey left ventricle.

Table S3. Gene list for the genes annotated in the Aging Atlas database, cardiac fibrosis-related genes, SASP related genes, and heart disease-related gene sets.

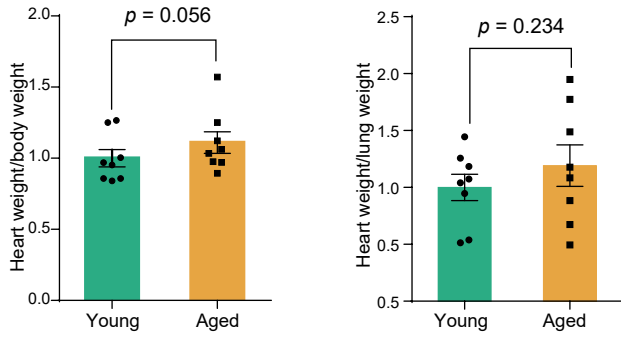
Table S4. Differentially expressed genes (DEGs) in hCMs upon silence of FOXP1.

Table S5. The list of antibodies used in this study.

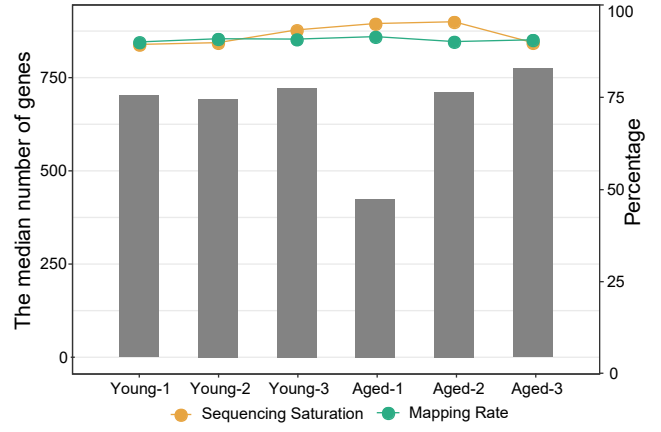
Table S6. Primer list for qRT-PCR and sequence of siFOXP1#1, siFOXP1#2.

Figure S1

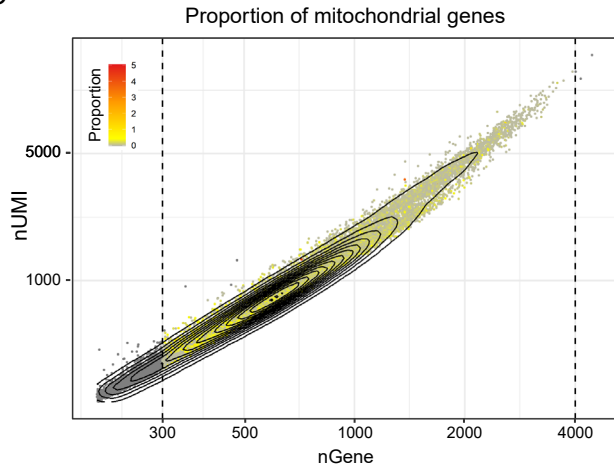
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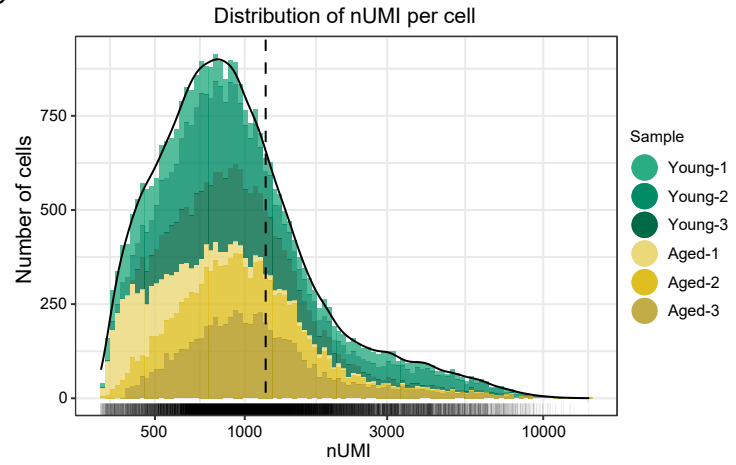
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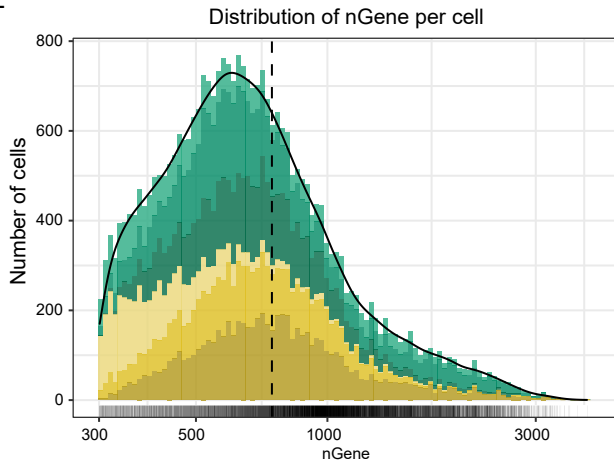
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D



E



F

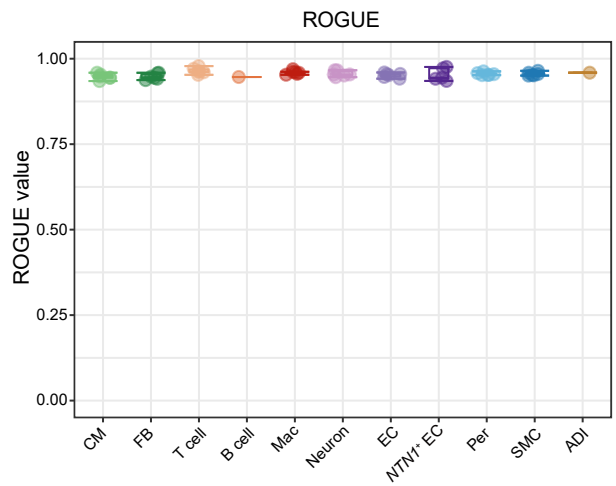


Figure S2

