

1 **Supplemental Materials**

2 **Figure S1. Information of cynomolgus monkeys and quality control of single-nucleus**  
3 **RNA-seq of NHP skeletal muscle.**

- 4 (A) Information of the cynomolgus monkeys used in this study.  
5 (B) Left, PI and Hoechst double-positive nuclei were enriched by FACS. Right, representative  
6 images of nuclei after sorting. Scale bars, 50  $\mu\text{m}$  and 10  $\mu\text{m}$  (zoomed-in image).  
7 (C) Left, bar plot showing nucleus number for each sample. Right, plot showing the  $\text{Log}_{10}$  ratio  
8 of reads per nucleus and sequencing saturation in each sample.  
9 (D) Left, violin plots showing the unique molecular identifiers (UMIs). Right, number of genes  
10 detected in each nucleus.  
11 (E) UMAP plot showing the distribution of clusters across all samples.  
12 (F) Stack bar plot showing the cell fraction of each sample in 14 cell types.  
13 (G) Bar plot showing the number of nuclei captured across cell types.

14 **Figure S2. Single-nucleus transcriptome profiling of young and old cynomolgus monkey**  
15 **skeletal muscles.**

- 16 (A) Box plots showing the cell proportion of each sample across different cell types. The  $P$   
17 values between old and young groups were calculated using one-sided Wilcoxon Rank Sum  
18 test.  
19 (B) Immunofluorescence staining of VWF and SMA in the skeletal muscle from young and old  
20 monkeys. Representative images are shown on the left; Scale bars, 50  $\mu\text{m}$  and 20  $\mu\text{m}$   
21 (zoomed-in image). The numbers of VWF-positive and SMA-negative cells were quantified  
22 as fold changes in old skeletal muscles vs. in young counterparts and are presented as mean  
23  $\pm$  SEM on the right.  $n = 8$  monkeys for each group.  
24 (C) Heatmap showing the expression profiles of genes differentially expressed in at least five  
25 cell types in the monkey skeletal muscle. Only genes with same direction of differential  
26 expression among different cell types are included.  
27 (D) Heatmap showing the expression profiles of top 5 differentially expressed genes (DEGs)  
28 for each cell type.  
29 (E) Heatmap showing the scaled expression patterns of hotspot genes for muscle diseases-  
30 related genes in different cell types.  
31 (F) Network visualization of upregulated core regulatory TFs in different cell types between  
32 old and young groups. Outer nodes display different cell types and the node size positively  
33 correlates with the number of target genes differentially expressed in corresponding cell  
34 types.  
35 (G) Representative images of negative control of *FOXO3* pre-mRNA labeling in monkey  
36 skeletal muscle by RNA-FISH. Scale bars, 20  $\mu\text{m}$ .

37 **Figure S3. Phenotypic and transcriptomic analyses of human myotubes upon silence or**  
38 **genetic activation of FOXO3.**

- 1 (A) MyHC immunofluorescence staining showing the differentiation efficiency of FOXO3<sup>+/+</sup>  
2 and FOXO3<sup>-/-</sup> hMyotubes. Representative images are shown on the left; Scale bar, 100 μm.  
3 Data are presented as mean ± SEM, n = 3 biological replicates for each group.  
4 (B) Schematic showing the siRNA-mediated knockdown of *FOXO3* in WT hMyotubes.  
5 (C) FOXO3 immunofluorescence staining in FOXO3<sup>+/+</sup> and FOXO3<sup>2SA/2SA</sup> hMyotubes.  
6 Representative images are shown on the left; Scale bars, 50 μm. The numbers of FOXO3-  
7 positive nuclei were quantified as fold changes (FOXO3<sup>2SA/2SA</sup> vs. FOXO3<sup>+/+</sup>) and are  
8 presented as mean ± SEM. n = 3 biological replicates for each group.  
9 (D) Heatmap showing Euclidean distance of gene expression profiles between FOXO3<sup>-/-</sup> and  
10 FOXO3<sup>+/+</sup> hMyotubes.  
11 (E) Heatmap showing the transcript levels of indicated genes in FOXO3<sup>+/+</sup> and FOXO3<sup>-/-</sup>  
12 hMyotubes by RT-qPCR. n = 3 biological replicates for each group.

### 13 **Supplementary Tables**

14 **Table S1.** Differentially expressed genes of bulk RNA-seq and snRNA-seq datasets.

15 **Table S2.** The marker genes of each cell type in monkey skeletal muscle.

16 **Table S3.** Gene sets used in this study.

17 **Table S4.** Core regulatory transcription factors of differentially expressed genes in monkey  
18 skeletal muscle during aging.

19 **Table S5.** Antibodies used in this study.

20 **Table S6.** The sequences for primers used in this study.

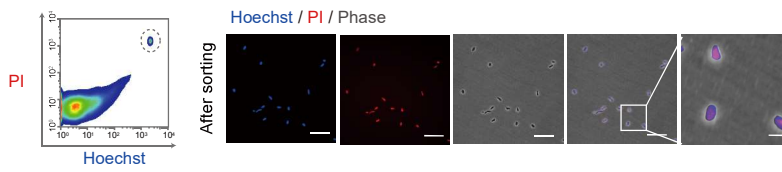
21

**Figure S1**

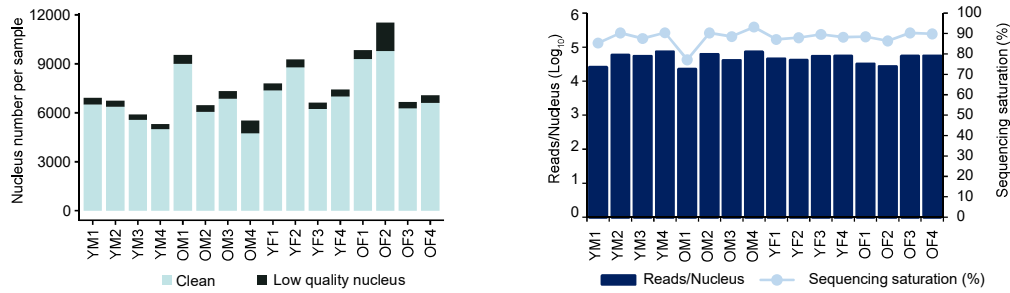
**A**

No.	Sex	Animal Symbol	Age (years)	No.	Sex	Animal Symbol	Age (years)
1	Female	YF1	5	5	Male	YM1	5
2	Female	YF2	5	6	Male	YM2	5
3	Female	YF3	5	7	Male	YM3	6
4	Female	YF4	4	8	Male	YM4	6
9	Female	OF1	18	13	Male	OM1	18
10	Female	OF2	19	14	Male	OM2	19
11	Female	OF3	19	15	Male	OM3	20
12	Female	OF4	20	16	Male	OM4	21

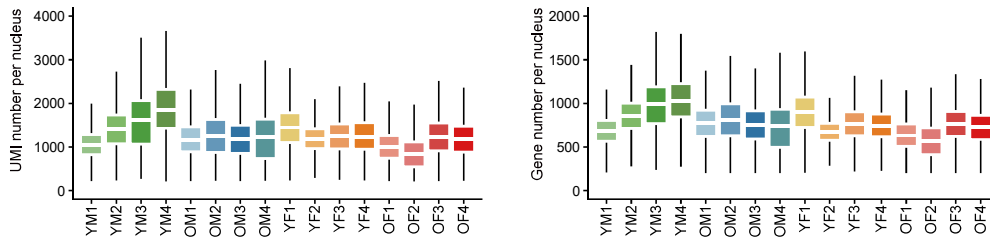
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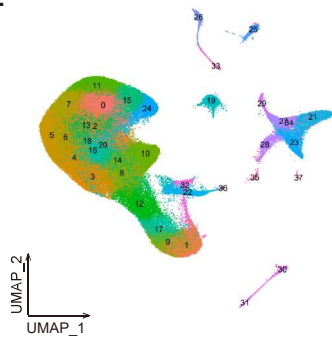
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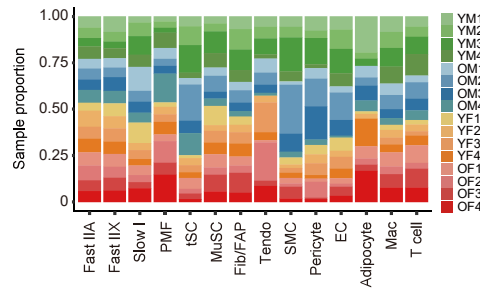
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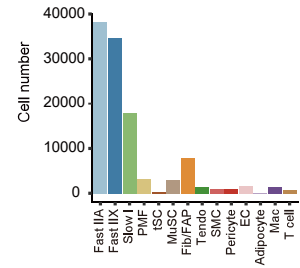
**E**



**F**



**G**





**Figure S3**

