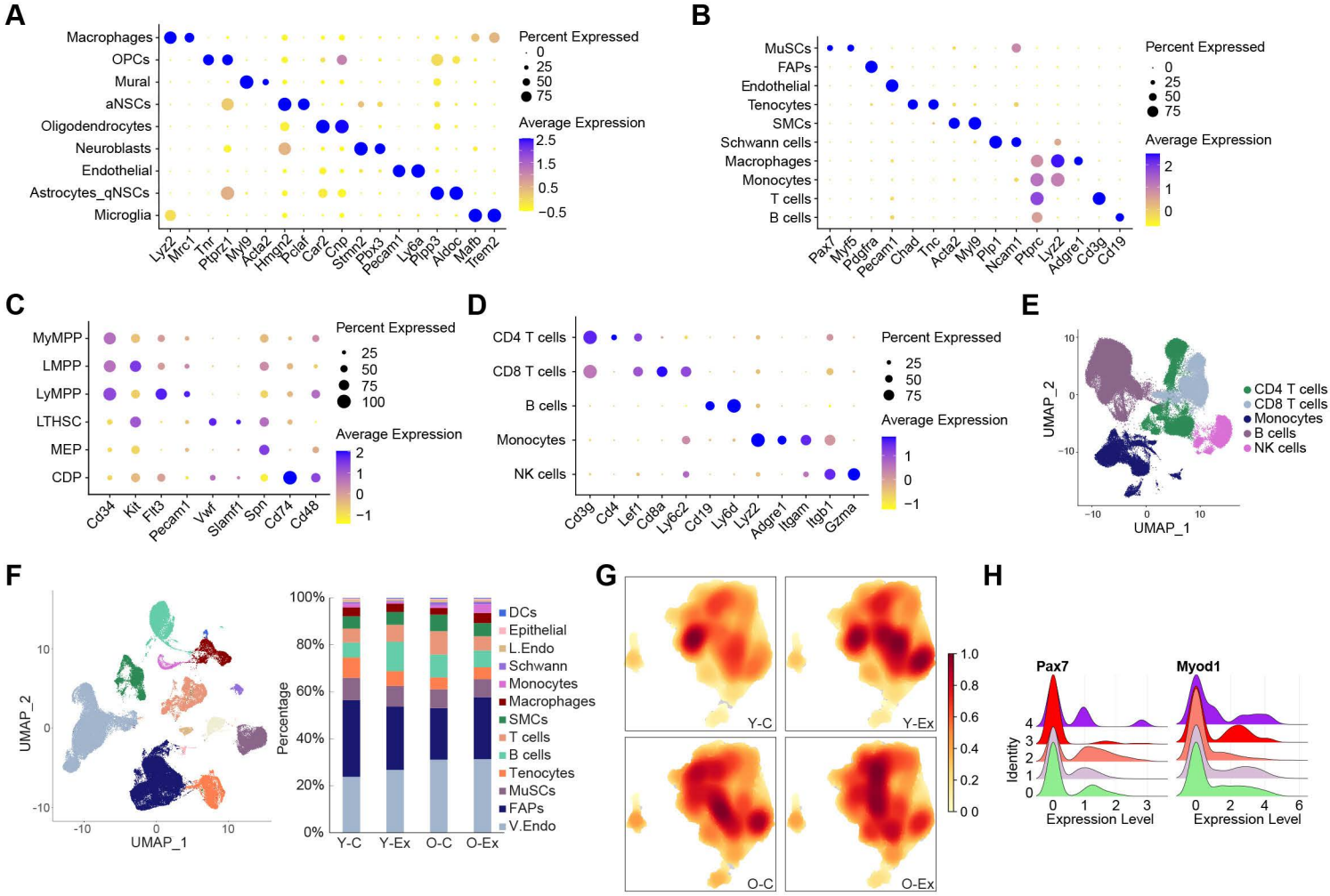

Figure S1: Annotation of cell types in aging and exercise mouse single-cell atlases, related to Figure 1.
(A-D) Dot plots showing markers used for cell type annotation in the SVZ, in skeletal muscle, in the HSC compartment, and in blood immune cells.
(E) UMAP showing major immune cell types in the blood.
(F) UMAP of cell types in skeletal muscle. Fraction of each cell type is shown on the right.
(G) Heatmap of cell density of MuSCs.
(H) Ridge plots of the expression of myogenic markers Pax7and Myod1 in the MuSC clusters illustrated in Fig. 1G.

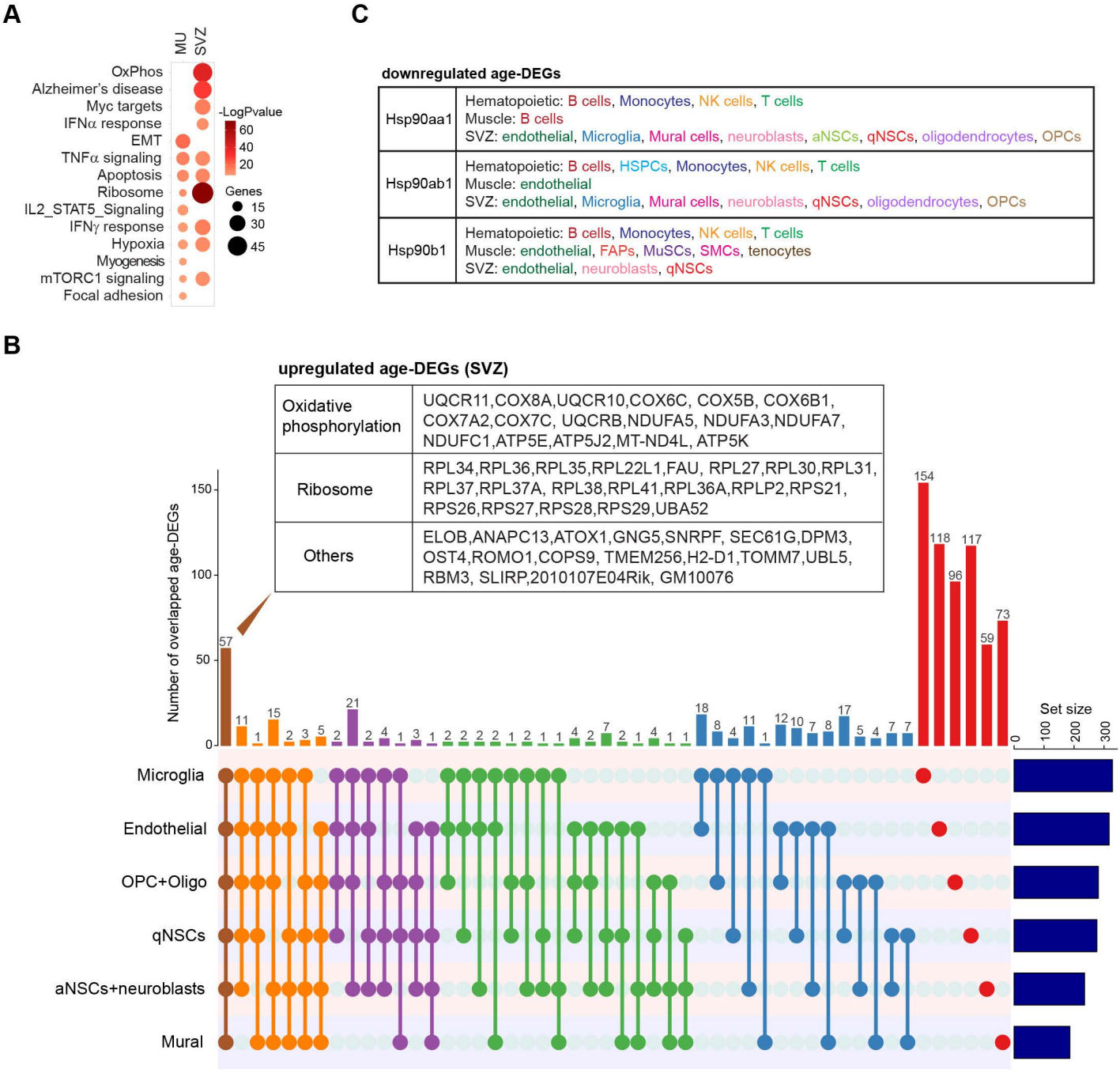

Figure S2: Common patterns of transcriptomic changes in stem cell compartments during aging, related to Figure 2.
(A) Dot plot summarizing the top 10 (ranked by P value) biological pathways enriched among age-DEGs in endothelial cells of the muscle (MU) and the SVZ.
(B) UpSet plot demonstrating common upregulated age-DEGs among major cell types in the SVZ. The age-DEGs are organized by the number of cell types in which they are found in common, with those expressed in all six cell types on the far left and those found in only one cell type on the far right. The common age-DEGs that were upregulated in all 6 types of cells are listed in the table. Oligo: oligodendrocytes.
(C) Table listing cell types in which members of the Hsp90 family were downregulated with age.

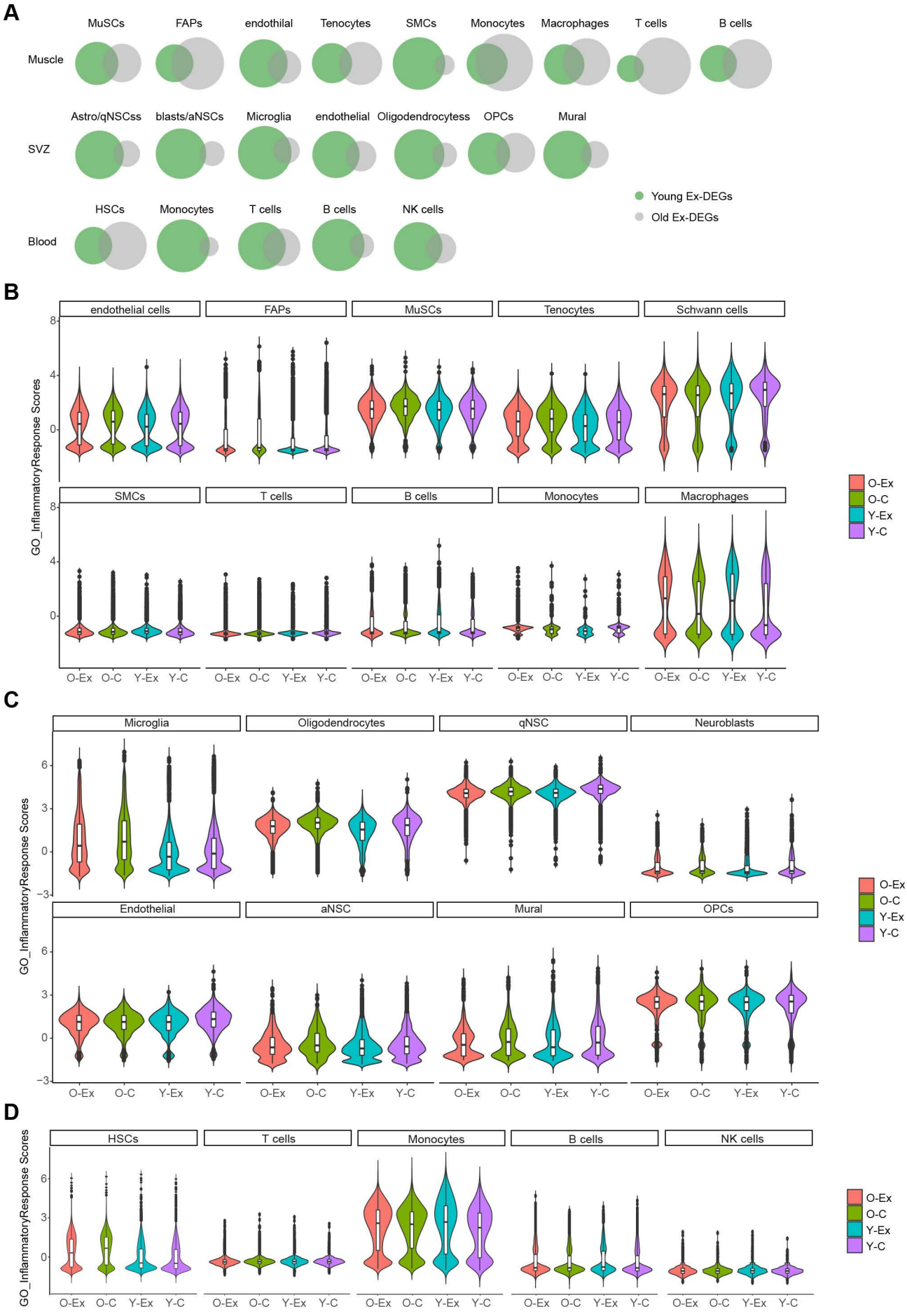

Figure S3: The effect of exercise on gene expression changes in various cell types, related to Figure 3.
(A) Venn diagrams demonstrating the percentage of common Ex-DEGs of each cell type in young and old cells.
(B-D) Violin plots showing the inflammatory scores of cells from muscle, cells from the SVZ, and hematopoietic cells.

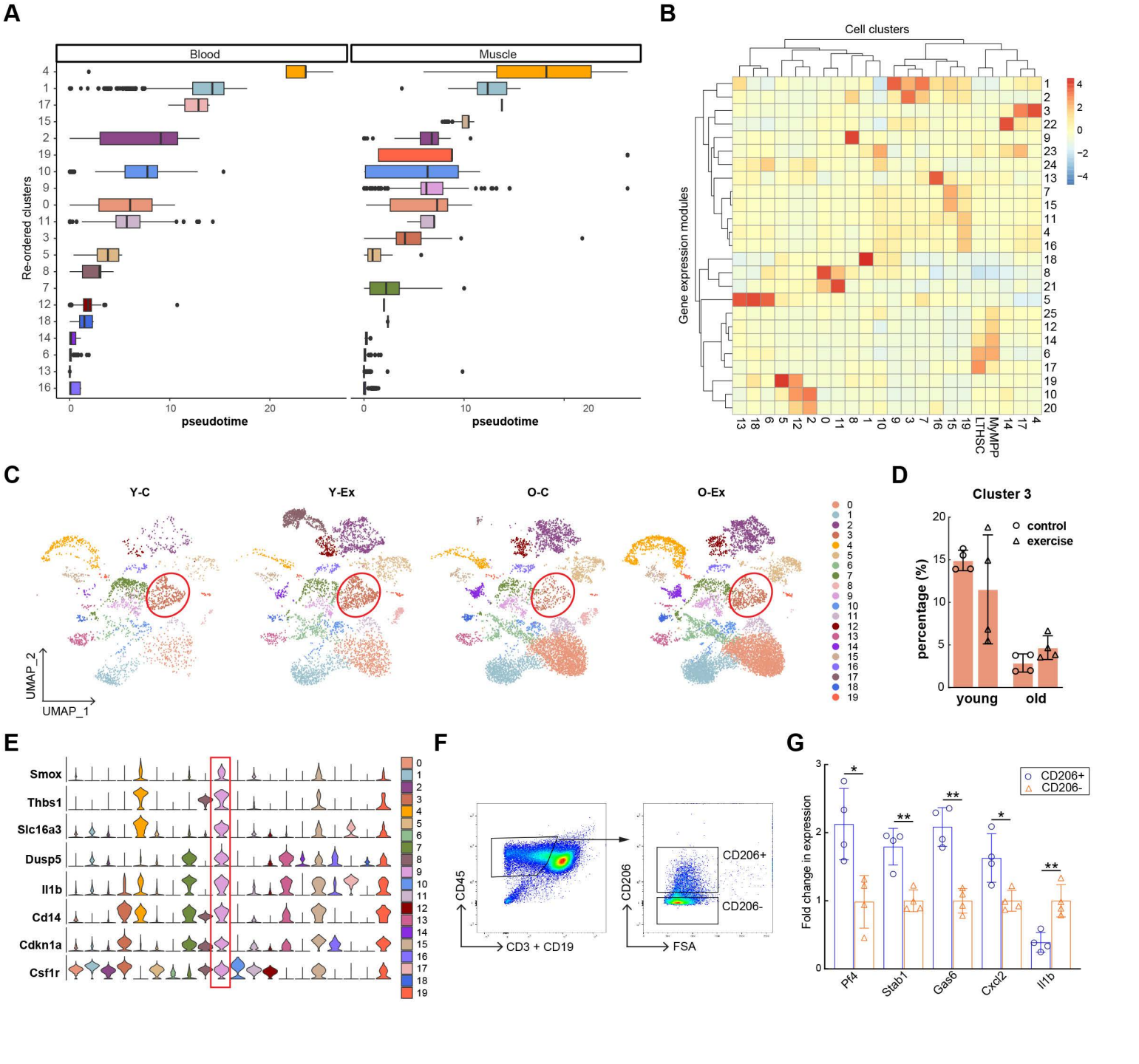

Figure S4: Aging and exercise-induced changes in monocytes/macrophages, related to Figure 4.
(A) Bar graphs representing the position of each clusters in Figure 4A along the pseudotime trajectory.
(B) Clustered heat map demonstrating the gene expression modules in all clusters in Figure 4A.
(C) UMAPs of the 20 monocyte/macrophage clusters in Y-C, Y-Ex, O-C and O-Ex mice. Cluster 3 is marked by the red circle.
(D) Bar graph demonstrating the relative ratio of cluster 3 in all monocytes/macrophages in Y-C, Y-Ex, O-C and O-Ex mice.
(E) Violin plots of selected marker genes of cluster 9 of monocytes/macrophages.
(F) Representative of FACS plots of CD45+/CD3-/CD19-/CD206+ and CD45+/CD3-/CD19-/CD206- cells from skeletal muscle.
(G) Bar graph demonstrating the relative expression Pf4, Stab1, Gas6, Cxcl2, and Il1b in CD206+ and CD206- monocytes/macrophages in skeletal muscle determined by RT-qPCR analysis.

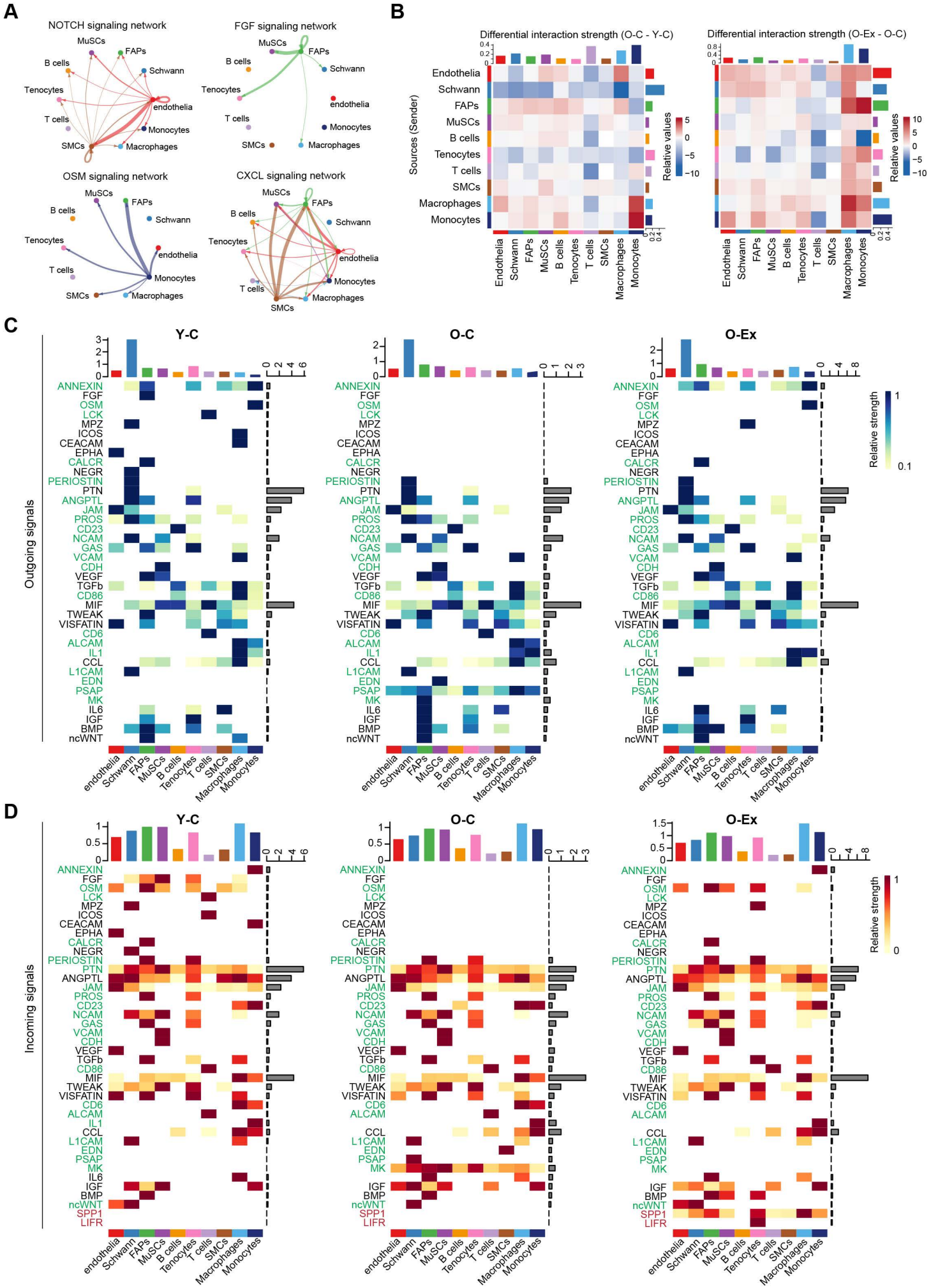
A
NOTCH signaling network
FGF signaling network
OSM signaling network
CXCL signaling network
MuSCs
FAPs
B cells
Schwann
Tenocytes
endothelia
T cells
Monocytes
SMCs
Macrophages
B
Differential interaction strength (O-C - Y-C)
Differential interaction strength (O-Ex - O-C)
Sources (Sender)
Relative values
Endothelia
Schwann
FAPs
MuSCs
B cells
Tenocytes
T cells
SMCs
Macrophages
Monocytes
C
Y-C
O-C
O-Ex
Outgoing signals
Relative strength
ANNEXIN
FGF
OSM
LCK
MPZ
ICOS
CEACAM
EPHA
CALCR
NEGR
PERIOSTIN
PTN
ANGPTL
JAM
PROS
CD23
NCAM
GAS
VCAM
CDH
VEGF
TGFb
CD86
MIF
TWEAK
VISFATIN
CD6
ALCAM
IL1
CCL
L1CAM
EDN
PSAP
MK
IL6
IGF
BMP
ncWNT
D
Incoming signals
SPP1
LIFR

Figure S5: Changes in intercellular communication networks in the MuSC niche during aging and in response to exercise, related to Figure 5.
(A) Circle plots showing the NOTCH, FGF, OSM, and CXCL signaling networks in cells from muscle of young mice. The arrows point to the cell types that express the receptors. The colors of the lines represent the source of the ligands. The thickness of the lines represents signaling strength between the signal sending and receiving cell.
(B) Heatmaps showing the differential overall signaling strength between young and old mice (left) and between old mice without and with exercise (right). The top bars and right bars represent the sum of incoming and outgoing signaling strength of each cell type, respectively. In the left, red and blue represent higher and lower signaling strength in the O-C mice, respectively, in comparison to Y-C mice. In the right, red and blue represent lower and higher signaling strength in the O-C mice, respectively, in comparison to O-Ex mice.
(C) Heatmaps showing the outgoing and incoming signaling pathways in various cell types in the skeletal muscle that change with age in Y-C, O-C and O-Ex mice. The pathways are ranked by the differential overall signaling flow between the Y-C and O-C conditions. The names of the pathways that were restored by exercise in old mice are labeled in green. The names of the pathways that were activated by exercise are labeled in red. The top colored bars represent the overall signaling strength in each cell type. The horizontal gray bars represent the summarized strength of each signaling pathway from all cell types in the muscle. The color scale represents the relative contribution of a cell type to the pathway.

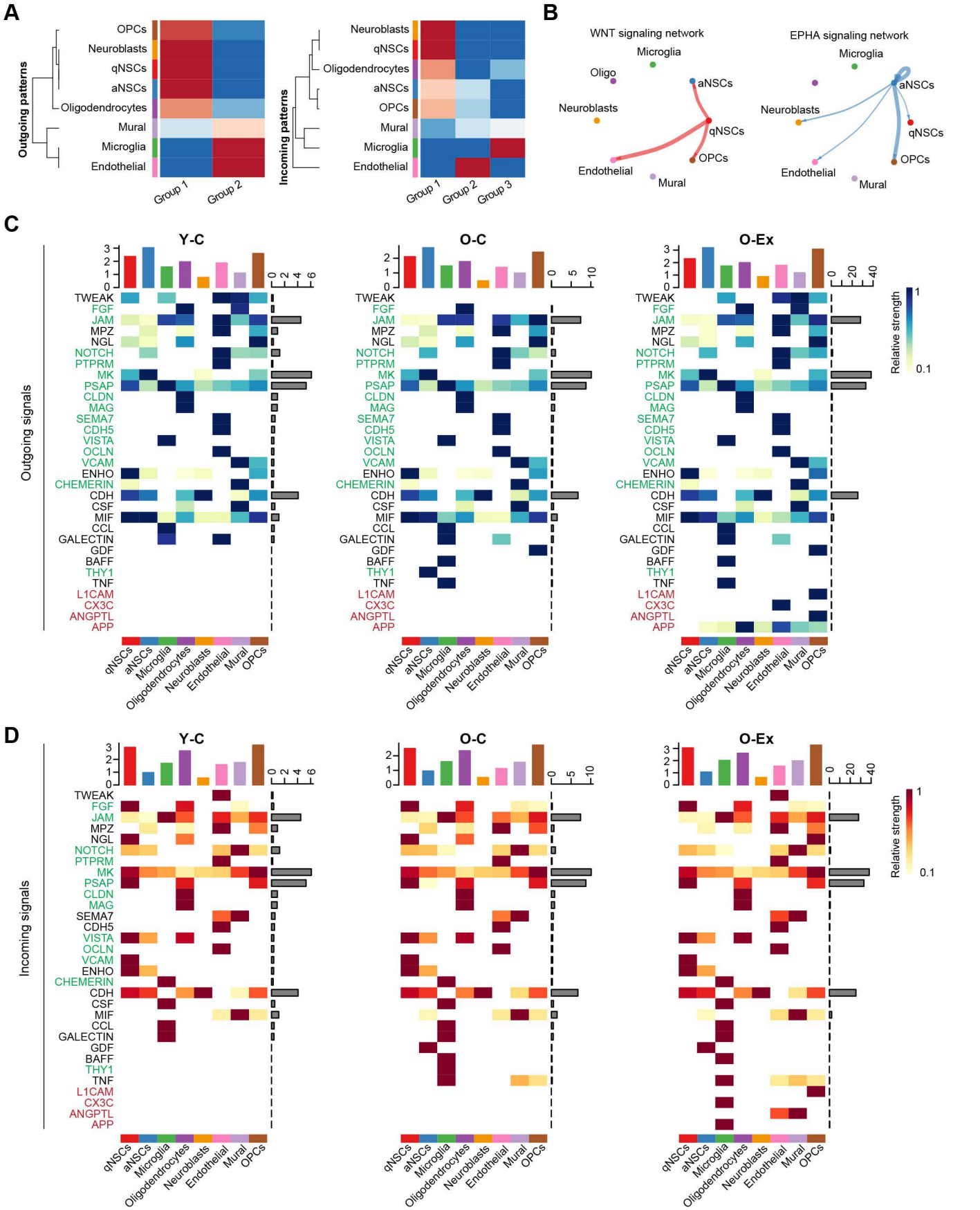

Figure S6: Changes in intercellular communication networks in the NSC niche during aging and in response to exercise, related to Figure 6.

(A) Heatmaps showing the outgoing and incoming signaling patterns mediated by secreted and cell surface molecules in the SVZ.

(B) Circle plot showing the WNT and EPHA signaling networks in the muscle of young mice.

(C) Heatmaps showing the outgoing and incoming signaling pathways in various cell types in the SVZ that change with age in Y-C, O-C and O-Ex mice. The pathways are ranked by the differential overall signaling flow between the Y-C and O-C conditions. The names of the pathways that were restored by exercise in old mice are labeled in green. The names of the pathways that were activated by exercise are labeled in red. The top colored bars represent the overall signaling strength in each cell type. The horizontal gray bars represent the summarized strength of each signaling pathway from all cell types in the muscle.

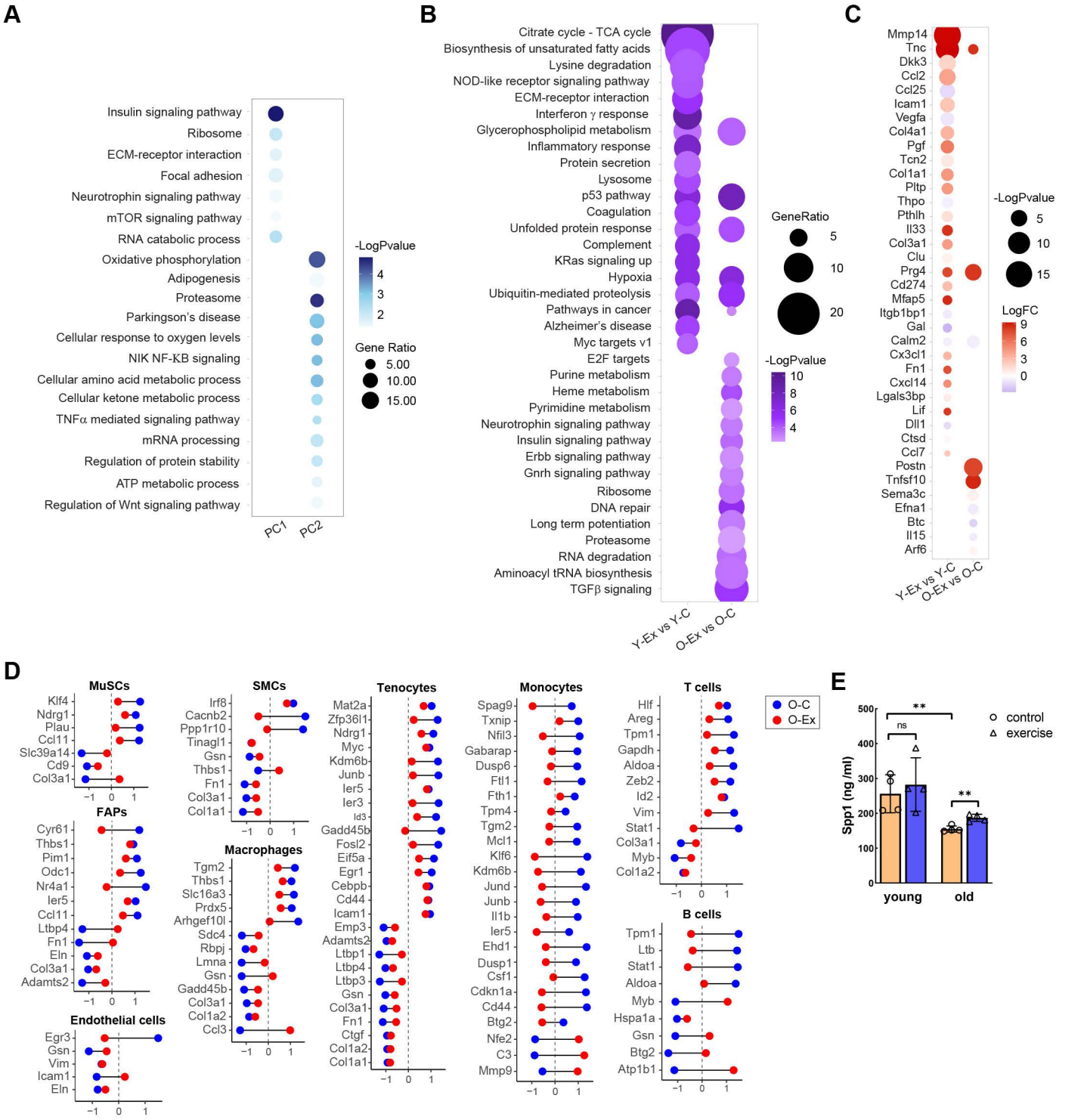

Figure S7: Gene expression changes in myofibers during aging and in response to exercise, related to Figure 7.
(A) Dot plot summarizing biological pathways enriched in the two major principle components from the PCA plot in Figure 7A that distinguish fiber transcriptomes by age and exercise status.
(B) Dot plot summarizing biological pathways enriched in exercised-induced genes in muscle fibers from young (left) and old (right) animals.
(C) Dot plot summarizing genes encoding secreted ligands that were induced by exercise in muscle fibers from young (left) and old (right) animals. Red indicates higher expression in exercised animals.
(D) Dumbbell plots demonstrating SPP1 target genes whose expression was reversed in muscle cell types by exercise in old mice. The x axis represents scaled relative expression to that in Y-C mice.
(E) Bar graph showing the level of Spp1, detected by ELISA, in plasma from control and exercised young and old mice. Data are shown as mean ± SEM. *p < 0.05, **p<0.01, ***p < 0.001 (unpaired t tests).