Supplemental Material

Transcriptomic and Functional Studies Reveal Undermined Chemotactic and Angiostimulatory Properties of Aged Microglia During Stroke Recovery

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Running title: Transcriptome analysis of aged microglia in stroke

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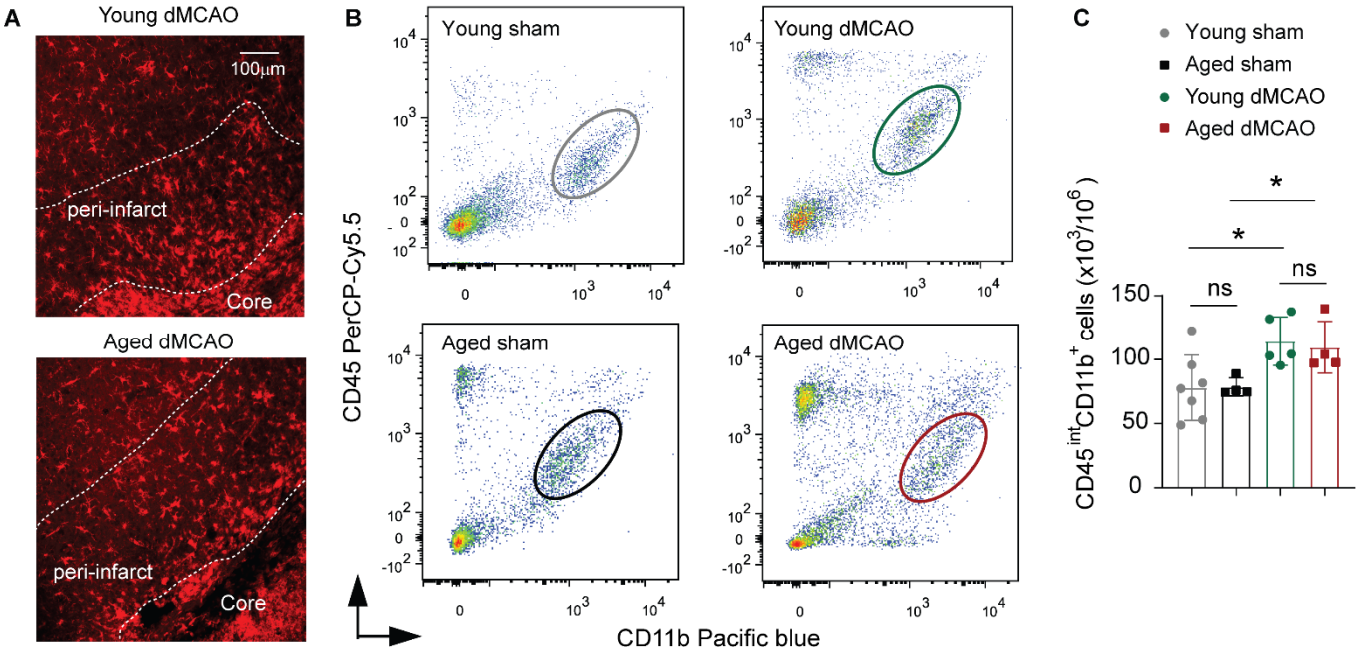

Figure S1. Iba1 staining and flow cytometry analysis. A. Immunostaining showing Iba1^{+} cells in the ischemic core and peri-infarct areas in both young and aged mice 14 days after stroke. **B.** CD45^{int}CD11b^{+} microglia were collected from young and aged brains 14 days dMCAO surgery or with sham operation. **C.** Quantification of CD45^{int}CD11b^{+} microglia in young and aged mice after sham or dMCAO surgery. **p<0.01, one-way ANOVA followed by Bonferroni *post hoc*.

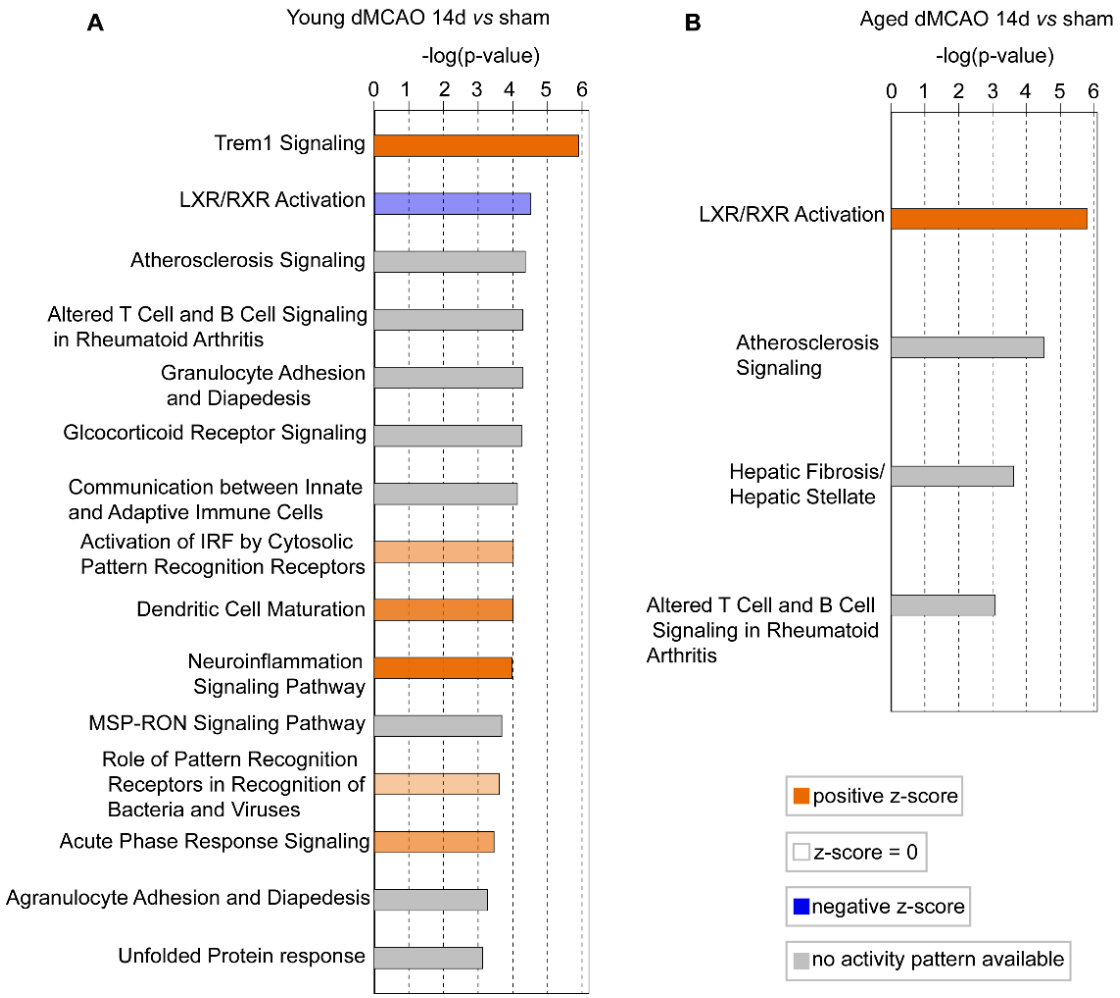

Figure S2. IPA canonical pathway analysis reveals the top pathways changed in young (A) and aged (B) microglia 14 days after dMCAO. Cut off p-value < 0.001. The bar charts represent the $-\log_{10}$(p-value) calculated based on Fisher's exact test.

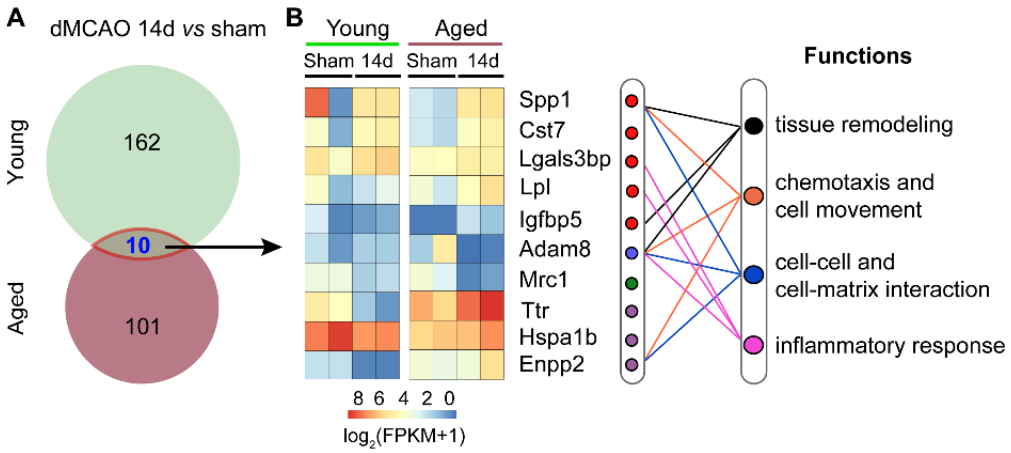

Figure S3. DEGs that are significantly altered in both young and aged microglia at 14 days after stroke. A. Venn diagram shows DEGs in young and aged microglia. There are 172 DEGs in young group and 111 DEGs in aged group, with 10 genes changed in both young and aged microglia. **B.** Heatmap shows the expression [log_2(FPKM+1)] of 10 DEGs in both young and aged microglia. The main functions of these genes are listed.

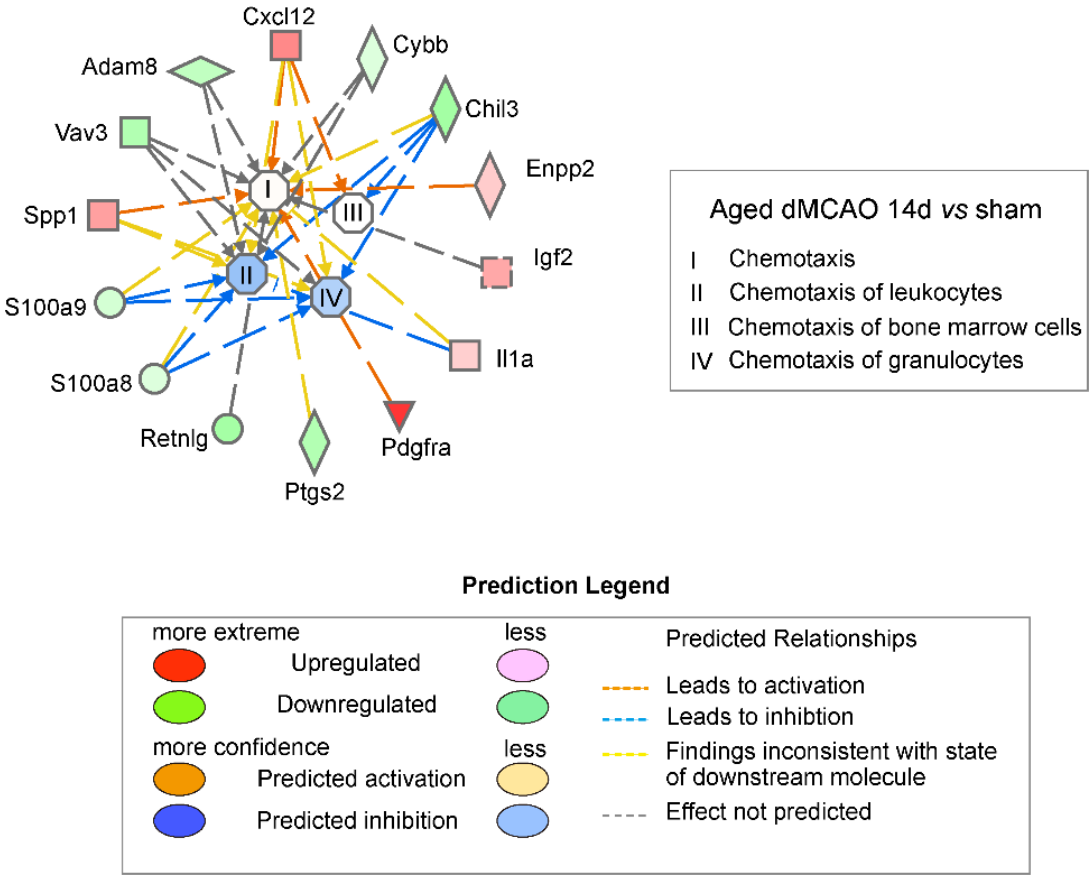

Figure S4. Aged microglia show reduced chemotaxis 14 days after stroke. IPA analysis identified chemotaxis-related functions enriched in aged microglia. The color of outer circle nodes indicates the expression of assigned genes. The color of inner circle nodes indicates the z-scores of enriched functions. The color of segments between gene nodes or function nodes implies gene activation or inhibition.

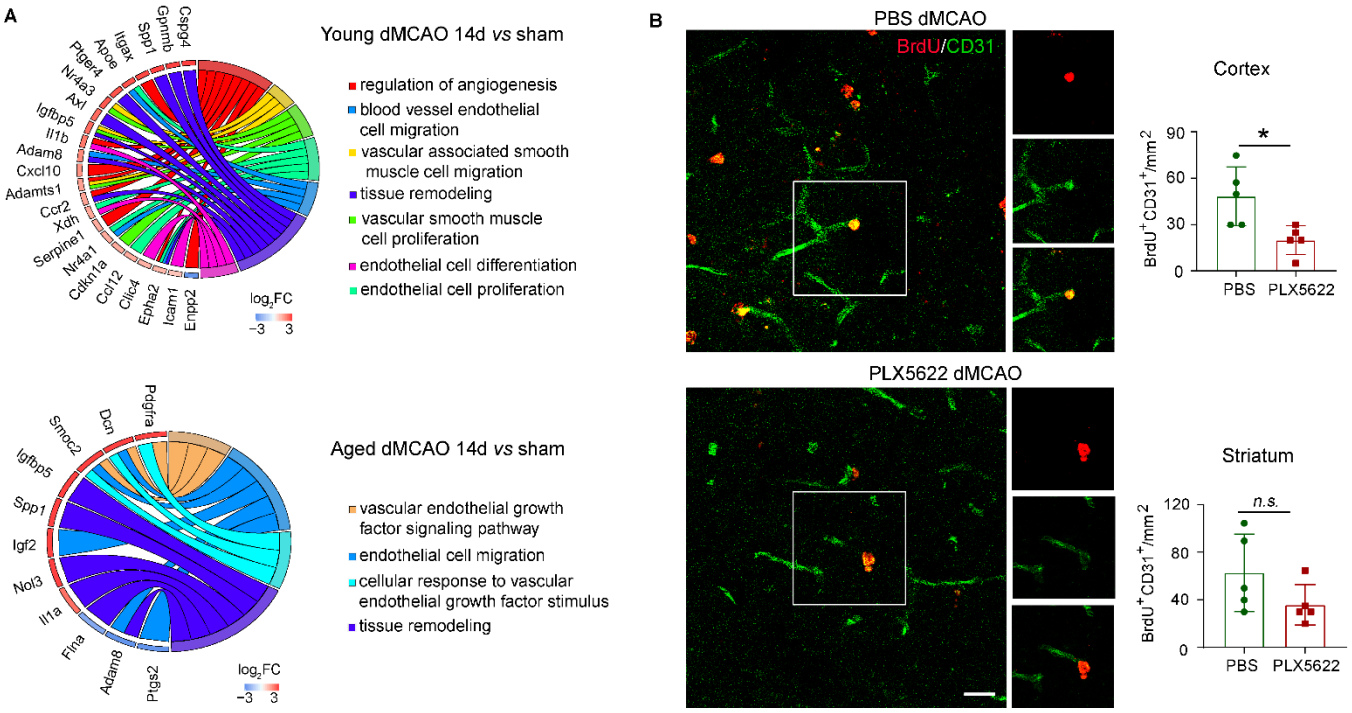

Figure S5. Senescent microglia are associated with impaired angiogenesis after stroke. A. Chord diagrams illustrate the DEGs (left semicircle) and enriched biological processes related to angiogenesis (right semicircle) in young (upper) and aged (lower) groups. The genes (rectangles) were linked to corresponding GO terms *via* colored ribbons. The expressions of genes were presented as $\log_2$(fold change) and listed according to color intensity in a descending order. Upregulated genes were displayed in red and downregulated in blue. **B.** Microglia/macrophages were depleted by dietary intake of PLX5622 7 days before 60 min MCAO. BrdU (red) and CD31 (green) double staining was performed 14 days after MCAO. Dual-labeled cells were quantified in the peri-infarct area in both cortex and striatum.

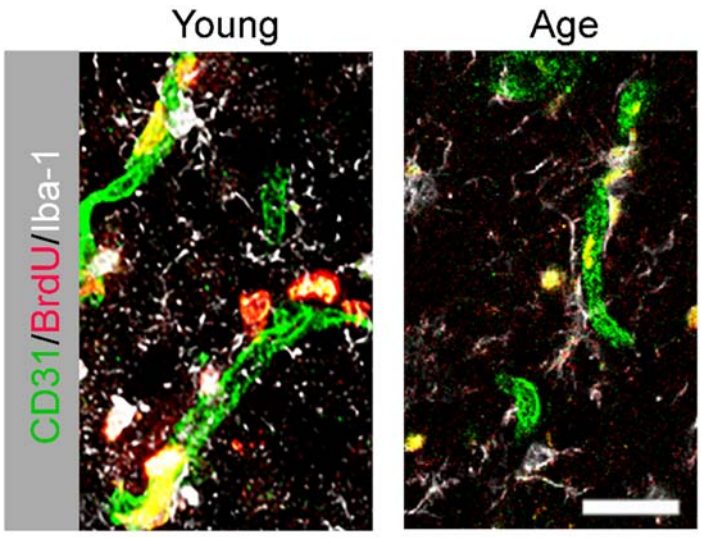

Figure S6. Iba1^{+} microglia/macrophages interact with BrdU^{+}CD31^{+} newly generated vessels in young and aged mice 14d after dMCAO. Scale bar: 40μm.