

# Naturally-aged microglia exhibit phagocytic dysfunction accompanied by gene expression changes reflective of underlying neurologic disease

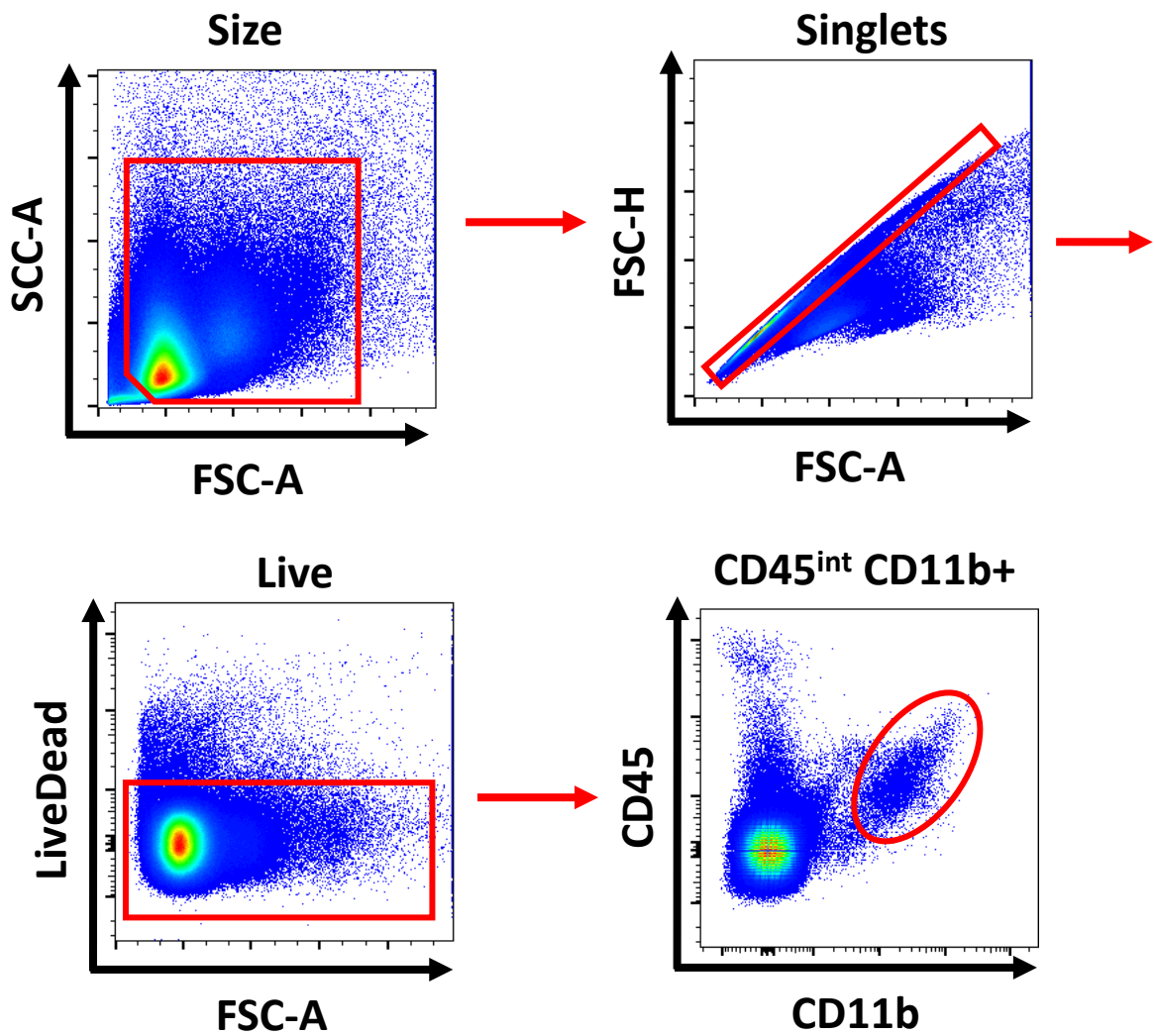
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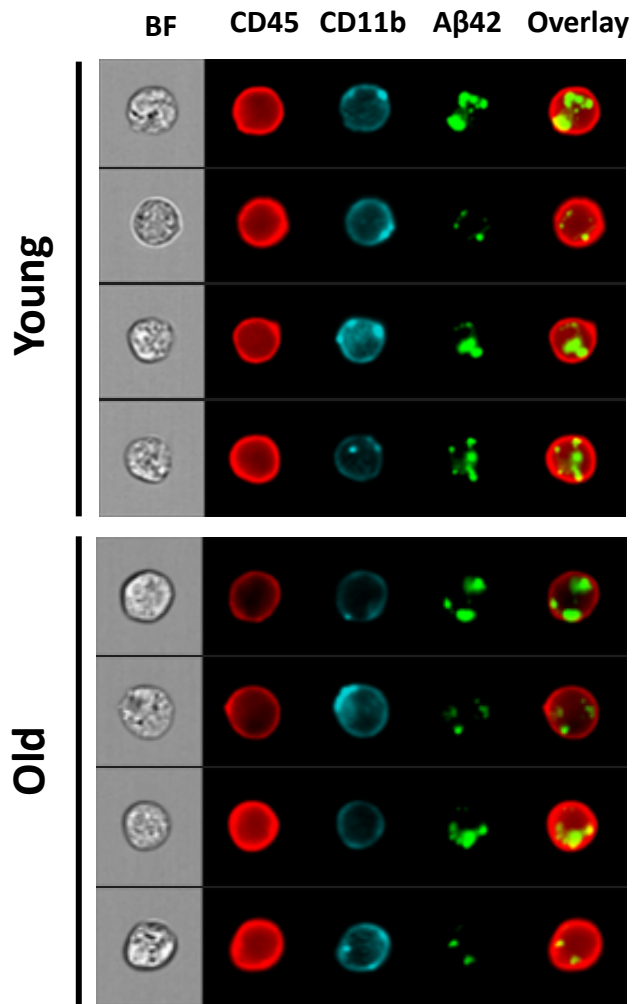
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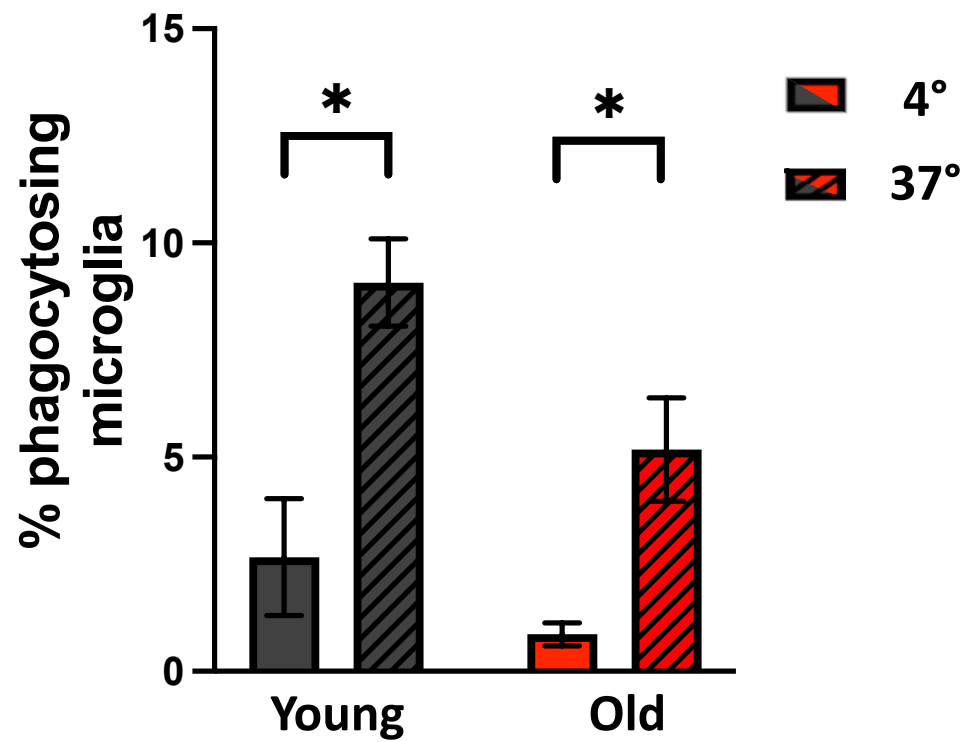
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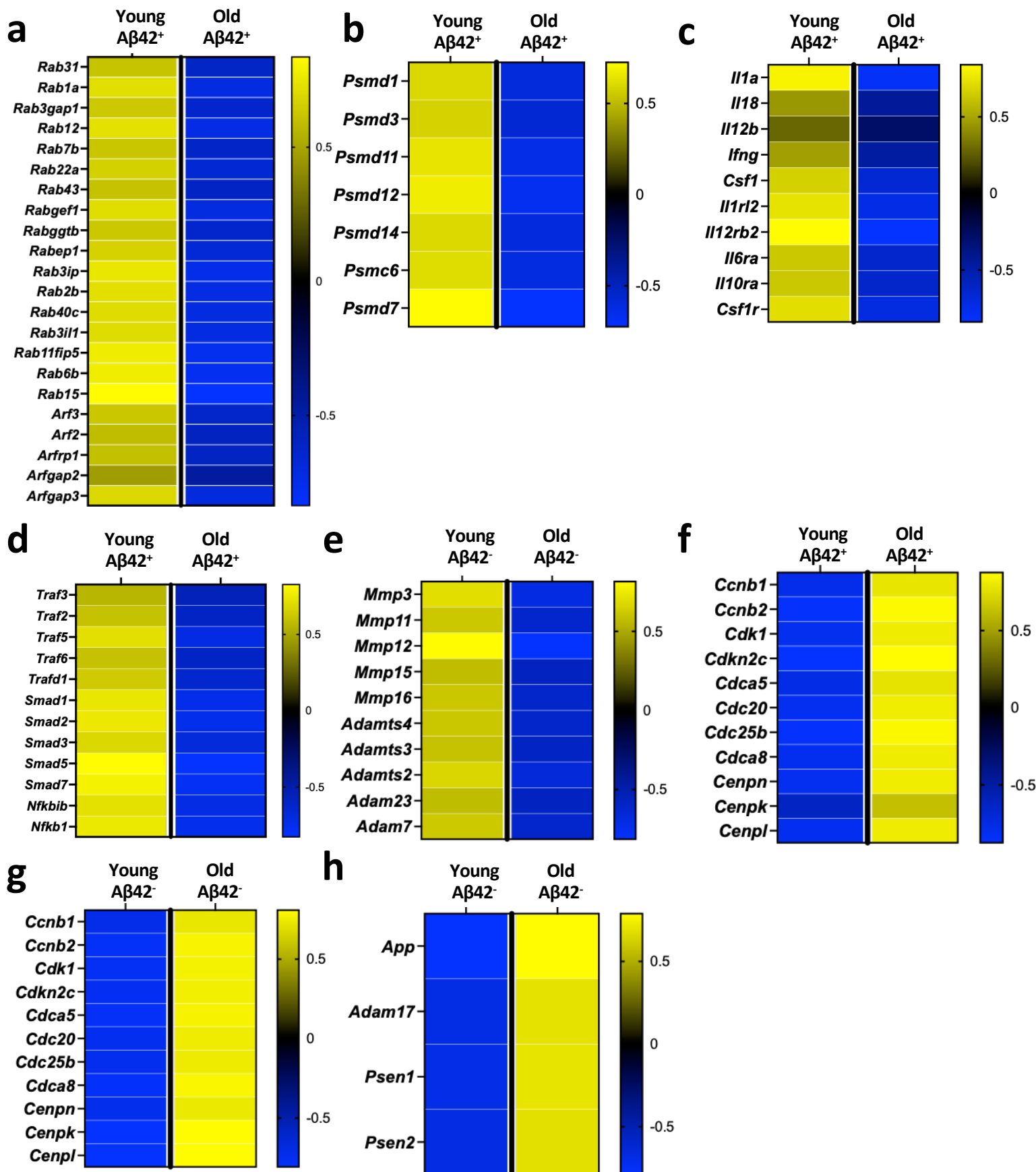
**Supplemental Figure 1: Flow cytometry gating strategy to identify microglia after immune cell isolation of the brain.** Representative flow cytometry gating of single cell suspension of isolated immune cells from the brain. Microglia were identified as live, CD45<sup>int</sup> CD11b<sup>+</sup> cells.



**Supplemental Figure 2: Imaging flow cytometry of A $\beta$ 42 uptake.** Representative imaging flow cytometry images of microglia upon 2hr incubation of A $\beta$ 42. BF, brightfield. Red indicates CD45, blue indicates CD11b, and green indicates A $\beta$ 42. Original magnification 60X.



**Supplemental Figure 3: Microglial phagocytosis at 4° and 37°.** Brains from female young (3 months) and old (22 months) mice (n=3-4) were processed and live CD45 intermediate CD11b<sup>+</sup> cells (microglia) were assessed for phagocytic capacity. Cells were incubated with 0.5μM of fluorescent fibril Aβ42 or medium (control) for 1 hour and phagocytosis was assessed by flow cytometry. Graphs are shown as mean ± s.e.m % of microglia showing phagocytosis of fluorescent Aβ42 are graphed \* $p \leq 0.05$ , Student's *t* Test



**Supplemental Figure 4: Average gene expression of select genes from bulk RNAseq.** Heatmap representations of selected genes from bulk RNAseq. Z score comparisons are shown as average gene expression of 4 mice per group. **a), b), c), d), and f)** Heatmaps compare young Aβ42<sup>+</sup> microglia to old Aβ42<sup>+</sup> microglia. **e), g), and h)** Heatmaps compare young Aβ42<sup>-</sup> microglia to old Aβ42<sup>-</sup> microglia.