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Supplementary information

Restoring metabolism of myeloid cells reverses cognitive decline in ageing

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Restoring metabolism of myeloid cells reverses cognitive decline in ageing

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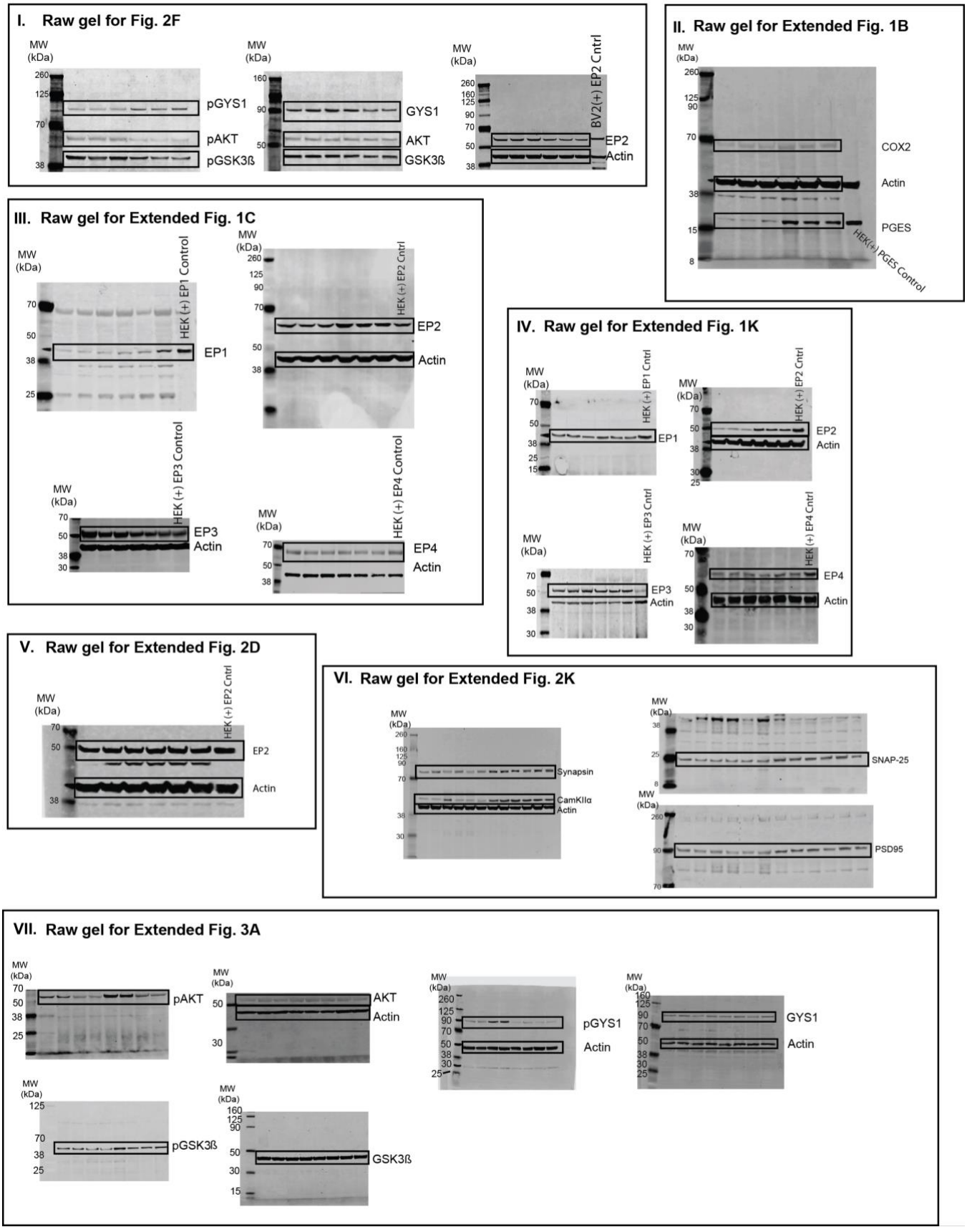
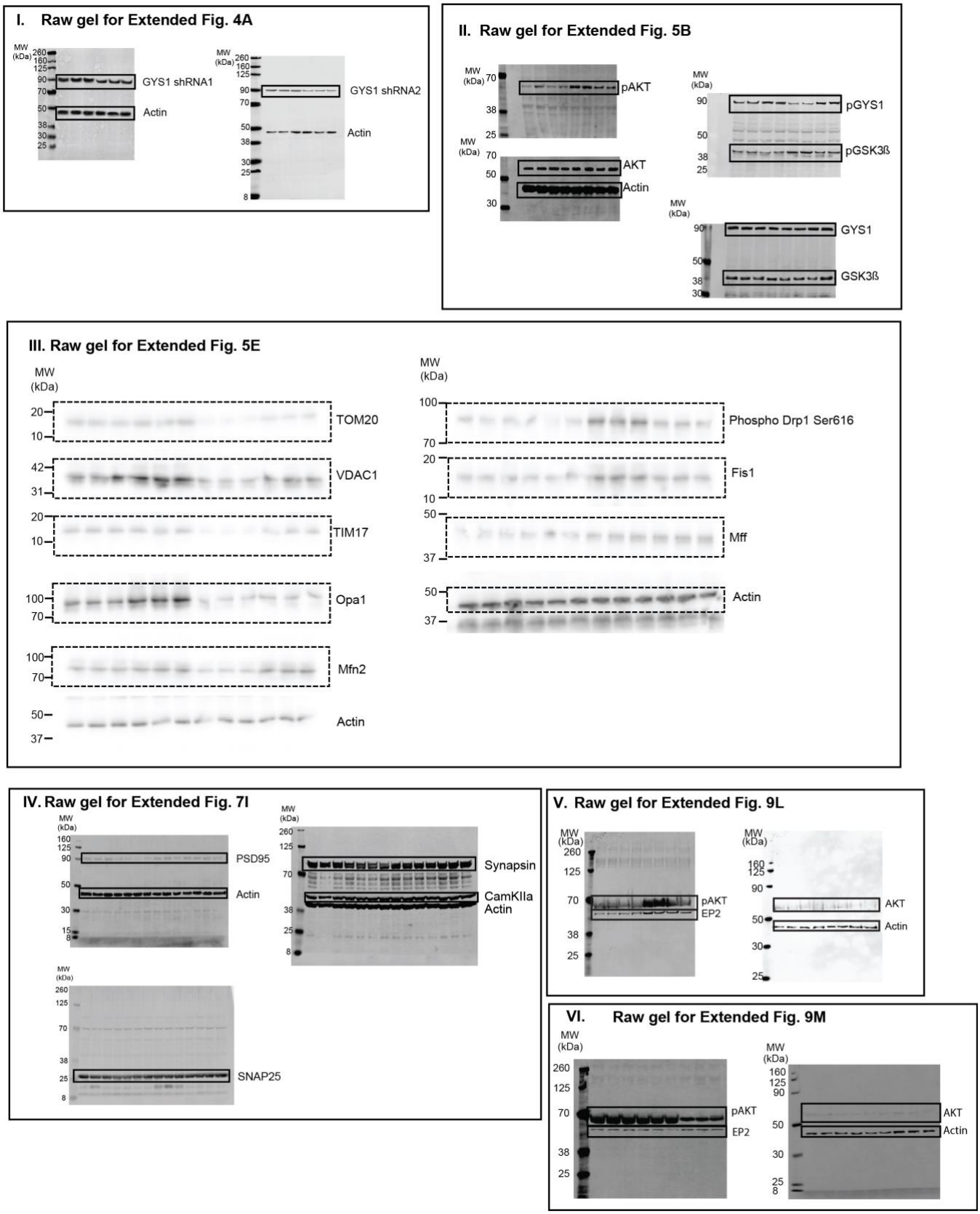
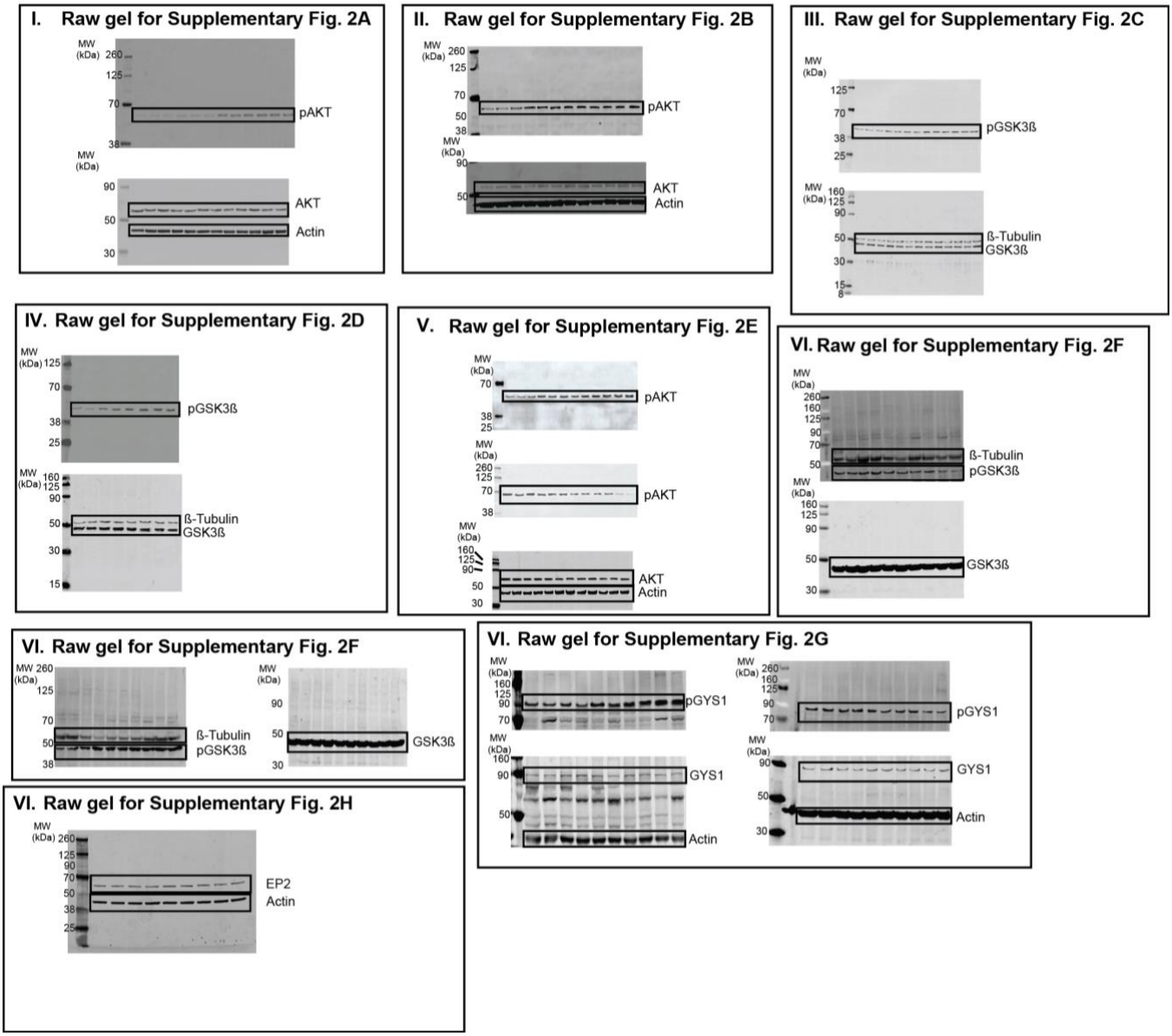
Supplementary Materials Guide:



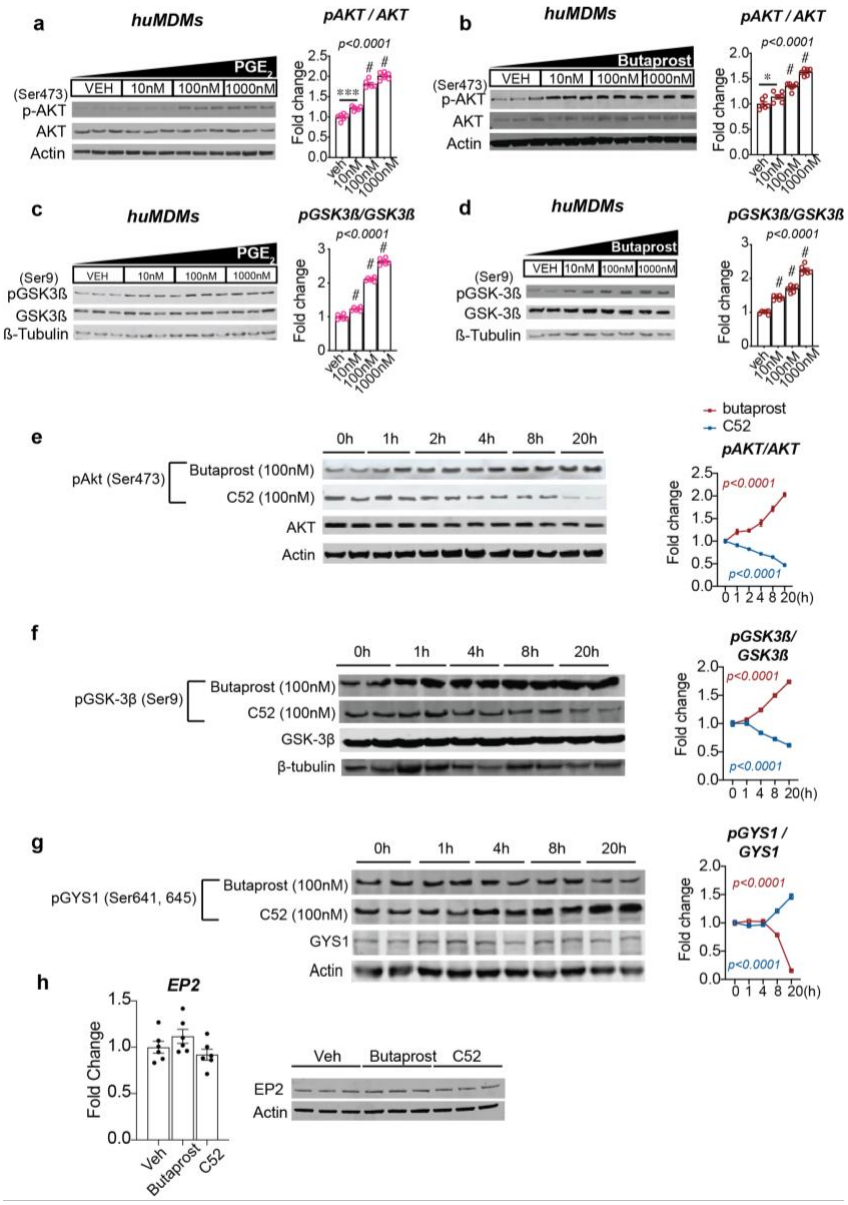
Supplementary Figure 1a



Supplementary Figure 1b



Supplementary Figure 1c



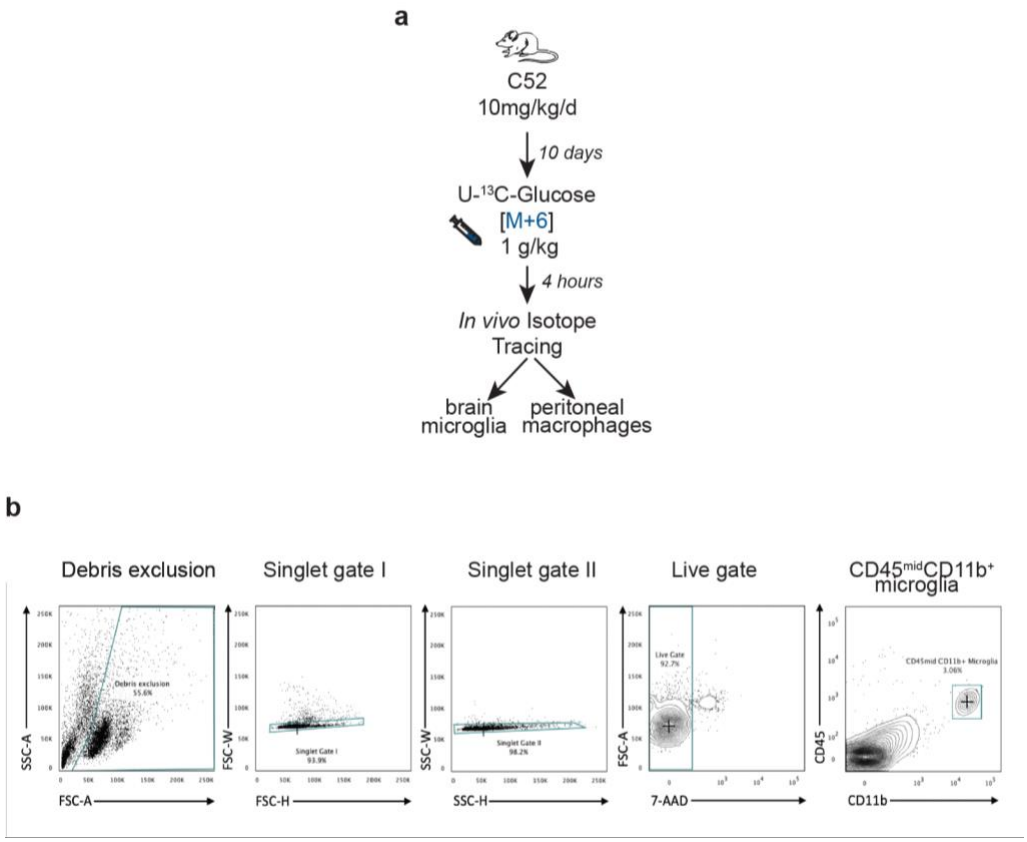
Supplementary Figure 2. **Effects of PGE_2, the EP2 agonist butaprost, and EP2 inhibitor C52 in human MDMs.** Human MDMs are from n = 6 donors (age mean ± SE: 43 ± 8.344 years)**.**

a-b. Representative immunoblots and quantification of two independent experiments measuring effects of ascending doses of PGE_2 (**a**) and the EP2 agonist butaprost (**b**) at 20 h on Ser473 pAKT /total AKT in human MDMs. $P < 0.0001$ by one-way ANOVA; Tukey's post-hoc test $^{*}P = 0.0340$, $^{***}P = 0.006$, $^{\#}P < 0.0001$.

c-d. Representative immunoblots and quantification of two independent experiments measuring the effect of ascending doses of PGE_2 (**c**) and the EP2 agonist butaprost (**d**) at 20 h on Ser9 pGSK3ß / total GSK3ß in human MDMs. $P < 0.0001$ by one-way ANOVA; Tukey's post-hoc test $^{\#}P < 0.0001$.

e-g. Representative immunoblots and quantification of time course of pAKT (Ser473) /total AKT (**e**), pGSK3ß (Ser9) / total GSK3ß (**f**), and pGYS1 (Ser641, 645, 649) /total GYS1 (**g**) from six independent experiments in huMDMs treated with butaprost (100 nM, red) or C52 (100 nM, blue) from 0h to 20 h. $P < 0.0001$ by one-way ANOVA.

h. Representative immunoblot and quantification of two independent experiments measuring effect of butaprost (20h, 100nM) and the C52 (20h, 100nM) on EP2 levels in human MDMs.



Supplementary Figure 3. **Experimental approach for microglial isotope labeling and gating strategy.**

a. Young and aged mice were administered C52 for 10 days at 10 mg/kg/day by oral gavage. On day 10, U-$_{13}$C-Glucose (1g/kg by gavage) was administered for *in vivo* isotope tracing of brain microglia and peritoneal macrophages harvested 4 hours later.

b. Gating strategy for isolation of CD45^{mid}Cd11b^{+} microglia from young (3-4 mo) and aged (22-24 mo) mice.