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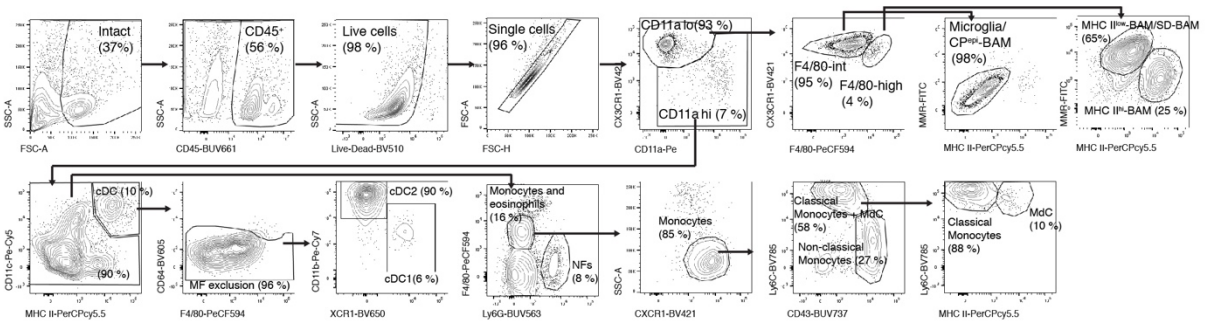
## Supplementary information

# Single-cell RNA and protein profiling of immune cells from the mouse brain and its border tissues

In the format provided by the authors and unedited




**Supplementary Figure 1. Gating strategy for brain myeloid populations, using antibody mix 2.** Single cell suspensions from whole brains of 9-week-old male C57BL/6 mice were stained with antibody mix 2 in BOX1. Brain immune cells are gated as CD45$^{+}$, intact, live, single cells. Microglia/CP$^{epi}$-BAM (in choroid plexus sample) are gated as CX3CR1$^{hi}$ CD11a$^{lo}$ F4/80$^{int}$ MMR$^{lo}$ MHCII$^{lo}$, BAMs are gated as CX3CR1$^{hi}$ CD11a$^{lo}$ F4/80$^{hi}$ and subdivided into MMR$^{hi}$ MHCII$^{lo}$ BAMs (SD-BAM in the subdural meninges sample) and MMR$^{lo}$ MHCII$^{hi}$ BAMs. Conventional dendritic cells (cDCs) are gated as CD11a$^{hi}$ CD11c$^{hi}$ MHCII$^{hi}$ CD64$^{lo}$ F4/80$^{lo}$ and subdivided in CD11b$^{hi}$ cDC2 and XCR1$^{hi}$ cDC1. Monocytes are gated as CD11a$^{hi}$ CD11c$^{lo}$ MHCII$^{lo}$ F4/80$^{int}$ Ly6G$^{lo}$ SSC-A$^{lo}$ CX3CR1$^{+}$ and subdivided in Ly6C$^{hi}$ CD43$^{lo}$ classical monocytes and Ly6C$^{lo\text{-}int}$ CD43$^{hi}$ non-classical monocytes. Neutrophils are gated as CD11a$^{hi}$ CD11c$^{lo}$ MHCII$^{lo}$ F4/80$^{lo}$ Ly6G$^{hi}$. Animal experiments were approved by the Ethische Commissie Dierproeven at the Vrije Universiteit Brussel.