

Supplemental Figures: Minimizing the *ex vivo* confounds of cell-isolation techniques on transcriptomic profiles of purified microglia

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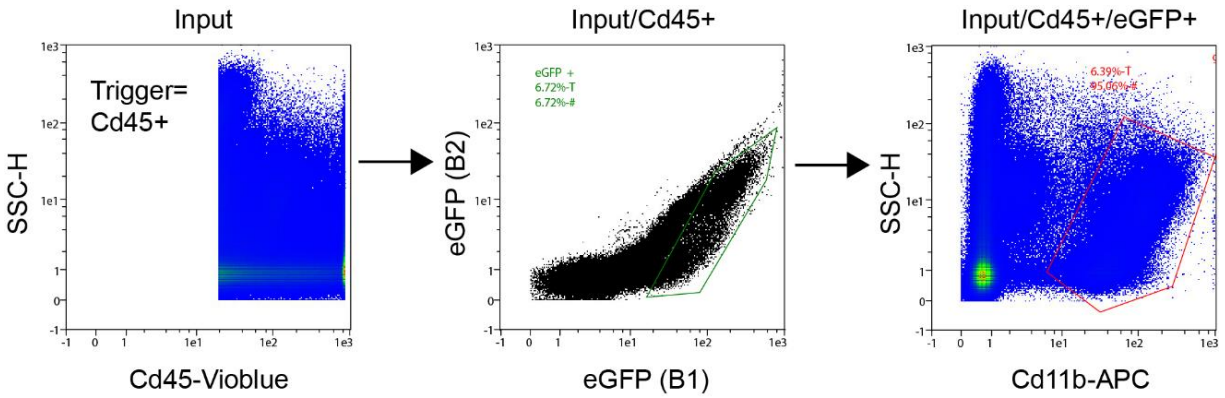


Figure S1. Cardette-based FACS gating strategy for microglial sorting on Miltenyi Biotec MACSQuant Tyto.

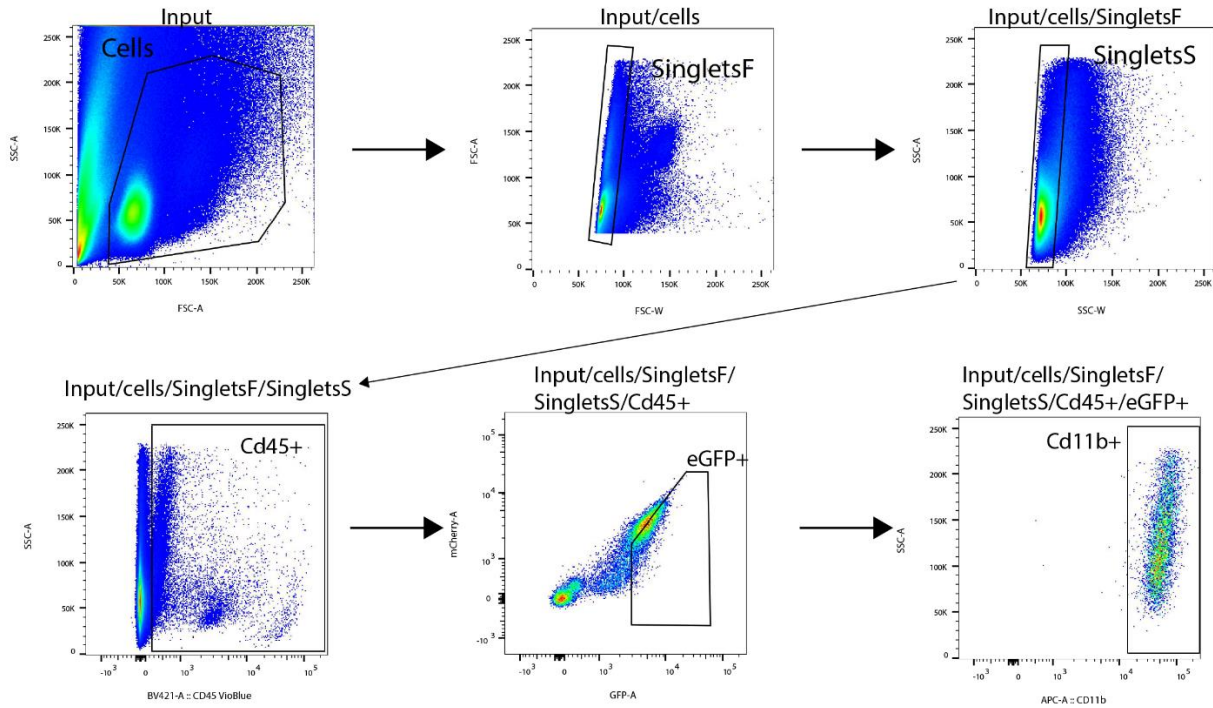


Figure S2. Cytometer-based FACS gating strategy for microglial sorting on FACSria.

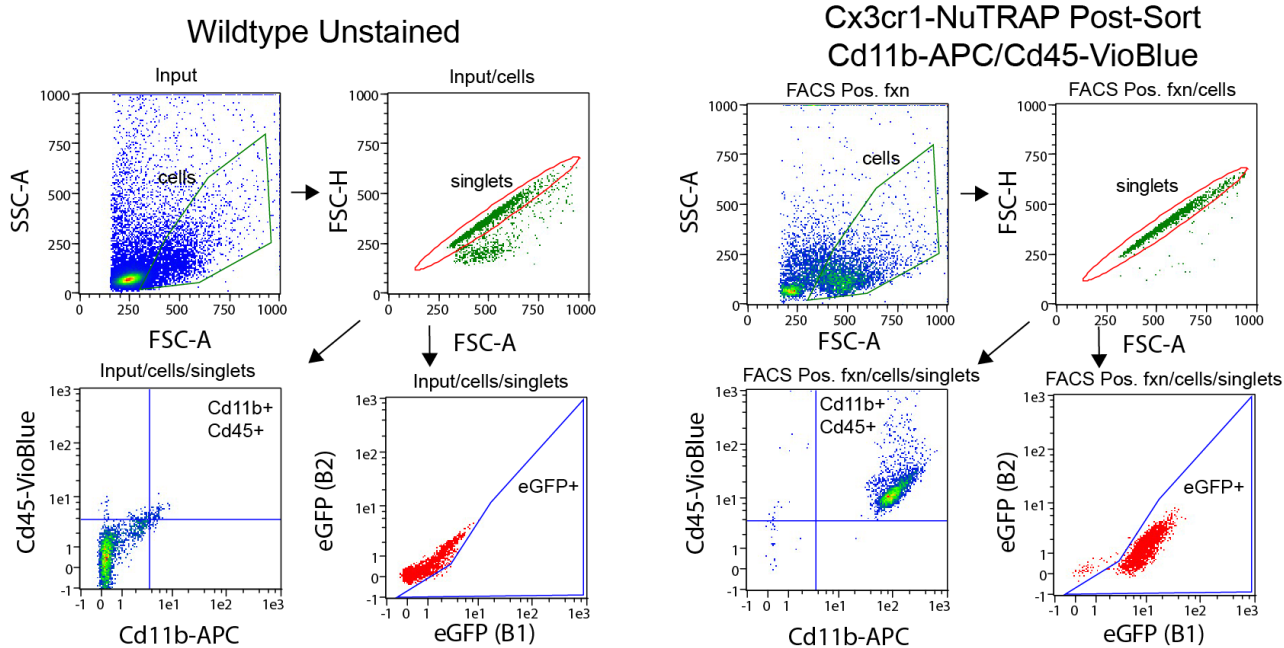


Figure S3. Gating strategy for assessment of microglial purity by different sort methods.

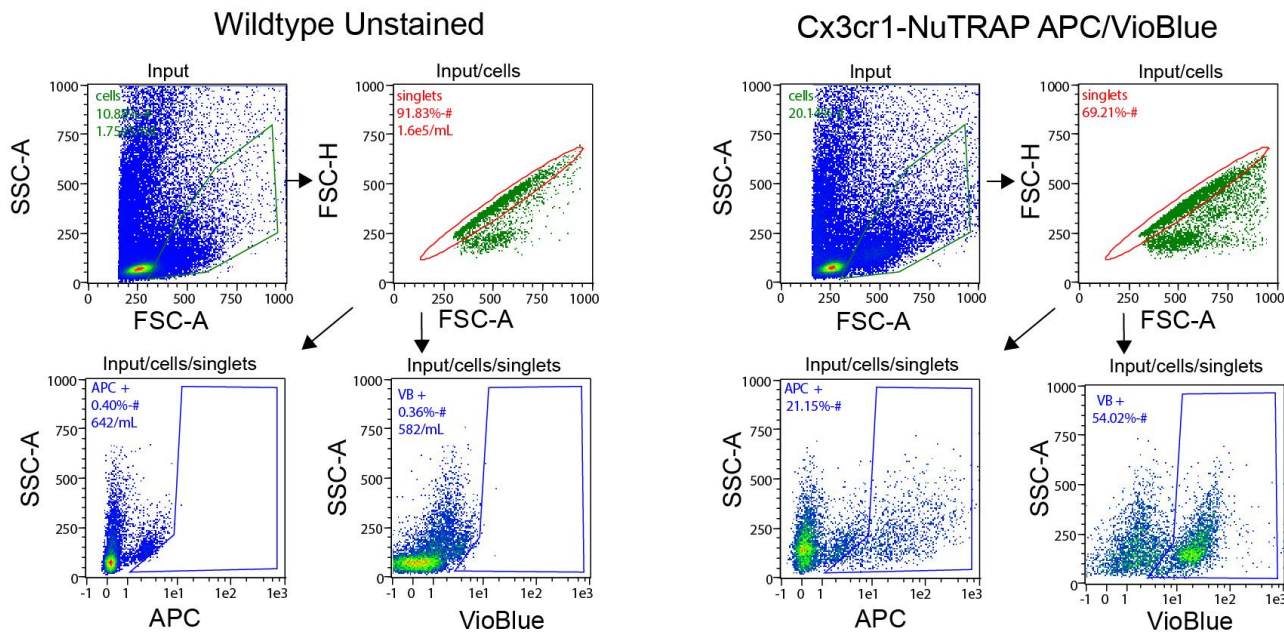


Figure S4. Gating strategy for assessment of cellularity.

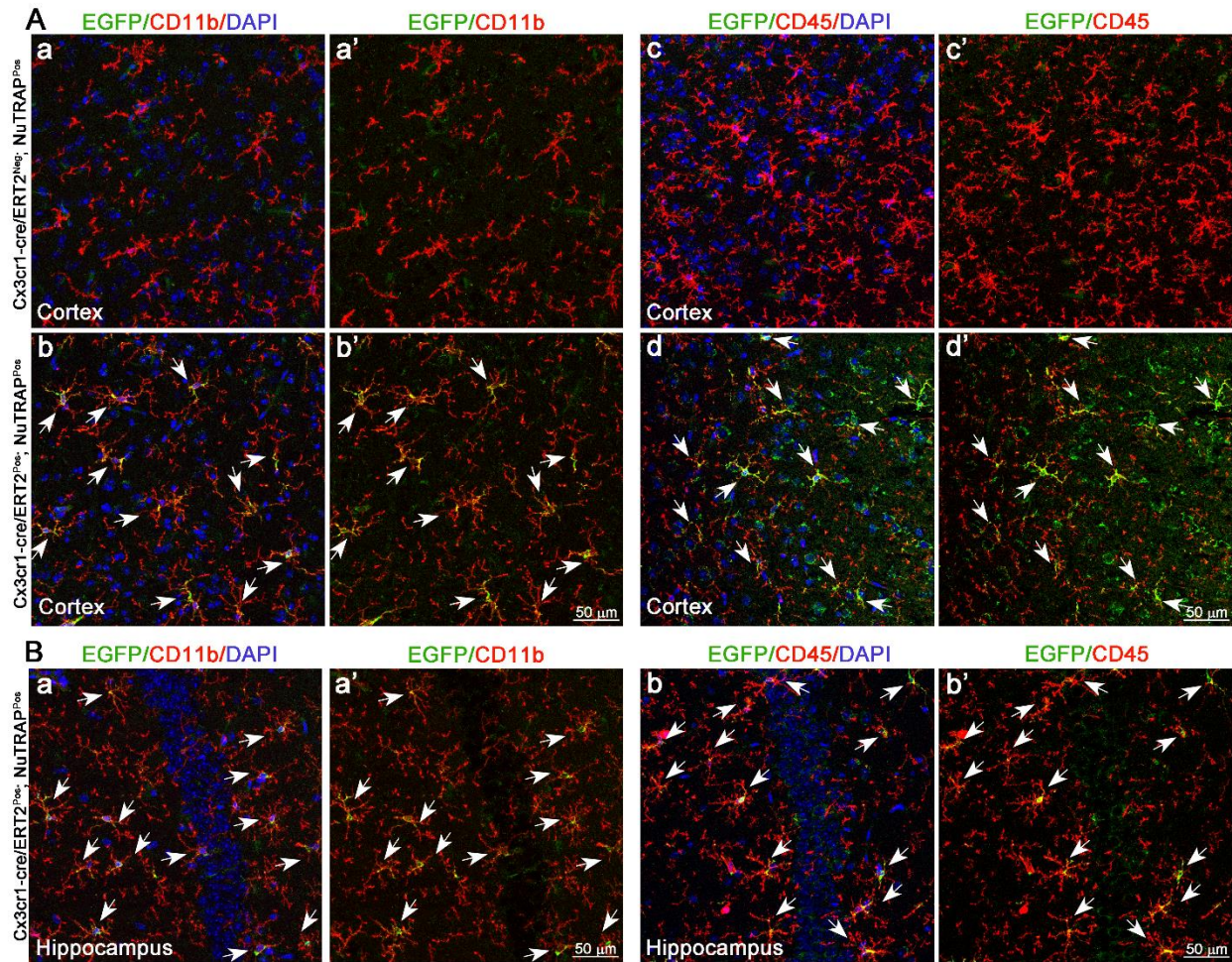


Figure S5. Validation of microglial identity of recombined cells in the Cx3cr1-NuTRAP brain. Two months after Tam treatment, brains were harvested from Cx3cr1-NuTRAP and cre negative NuTRAP⁺ (control) mice for immunohistochemistry (IHC). **A.** Representative confocal fluorescent microscopy images of sagittal brain sections captured in the cortex show EGFP expression (green signal) in cells that co-expressed CD11b (red signal, **a-a'-b-b'**) and CD45 (red signal, **c-c'-d-d'**) in Cx3cr1-NuTRAP brains but not in the cre negative counterparts (n=2/group). **B.** Representative confocal fluorescent microscopy images captured in the hippocampus show EGFP expression (green signal) in cells that co-expressed CD11b (**a-a'**) and CD45 (**b-b'**) in Cx3cr1-NuTRAP brains DAPI: nuclear counterstain. Scale bar: 50 μ m