Supplementary figures and legends, Drieu et al.

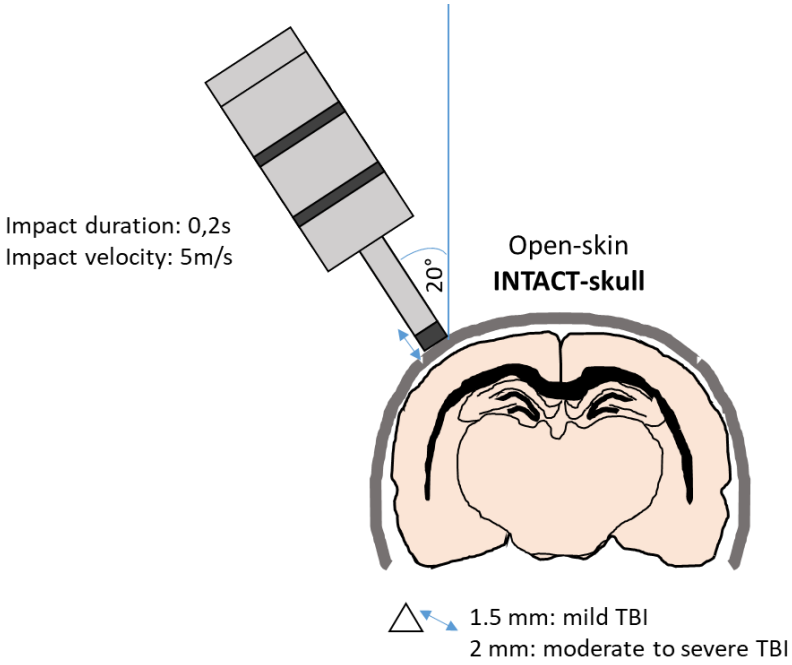


Figure S1. Schematic representation of the model of TBI.

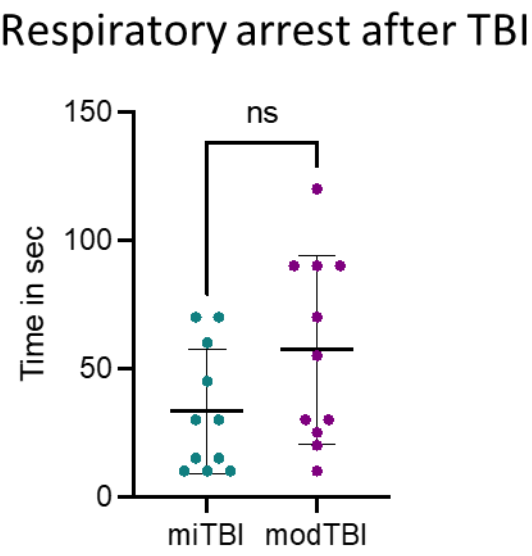
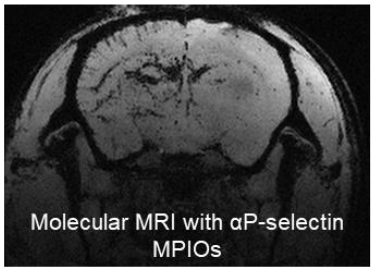


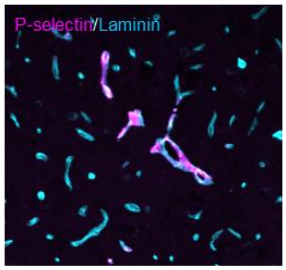
Figure S2. Respiratory arrest after mTBI. Mice were put in a recovery box following mi- or modTBI. The time when mice got their first breath was manually recorded. n=10/11 mice/group, p=0.1024, Mann-Whitney test.

LPS + 24h

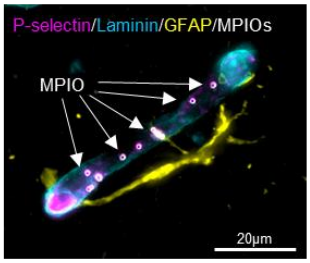
A



B

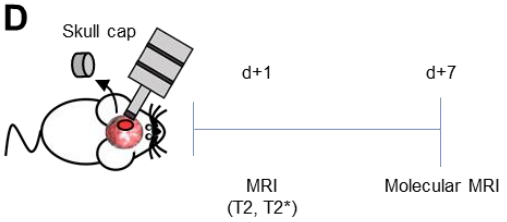


C

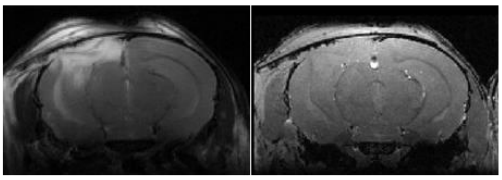


Severe TBI d+7

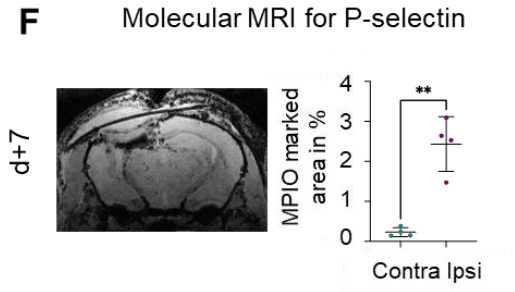
D



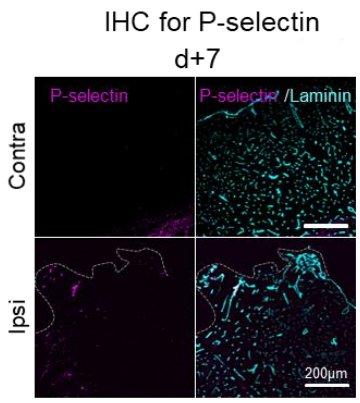
E



F



G



H

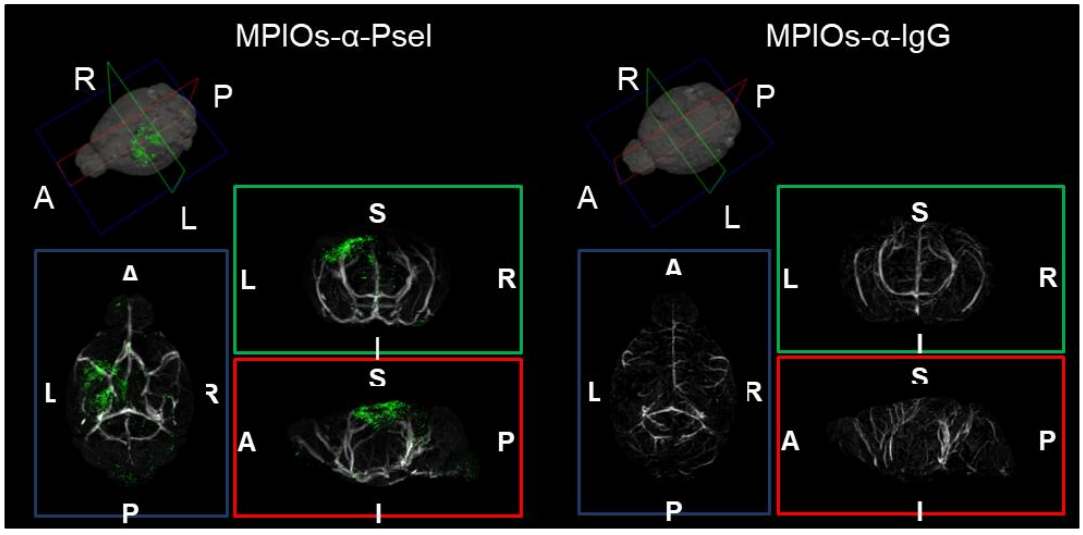


Figure S3. Controls for molecular MRI. (A) Representative T2*-GEFC αP-selectin MPIO acquisitions 24h after intrastriatal injection of LPS. (B) Representative P-selectin immunostaining 24h after intrastriatal injection of LPS. (C) Localization of P-selectin-MPIOs in a brain blood vessel of a mouse 24h after intrastriatal injection of LPS. (D) Schematic representation of the experimental design showing that severe TBI was induced after craniectomy. (E) Representative T2-weighted and T2*-weighted MRI acquisitions in mice 24 hours after severe TBI. (H) Representative projection of signal obtained with MPIOs coupled to either an antibody against P-selectin, or a control immunoglobulin. Signals are mapped on a template and on the corresponding angiography. (F) Representative T2*-GEFC αP-selectin MPIO acquisitions 7 days after severe TBI and its MPIO-marked area quantifications. (G) Representative photomicrographs of P-selectin immunostaining 7 days after sTBI (blood vessels were stained with laminin).

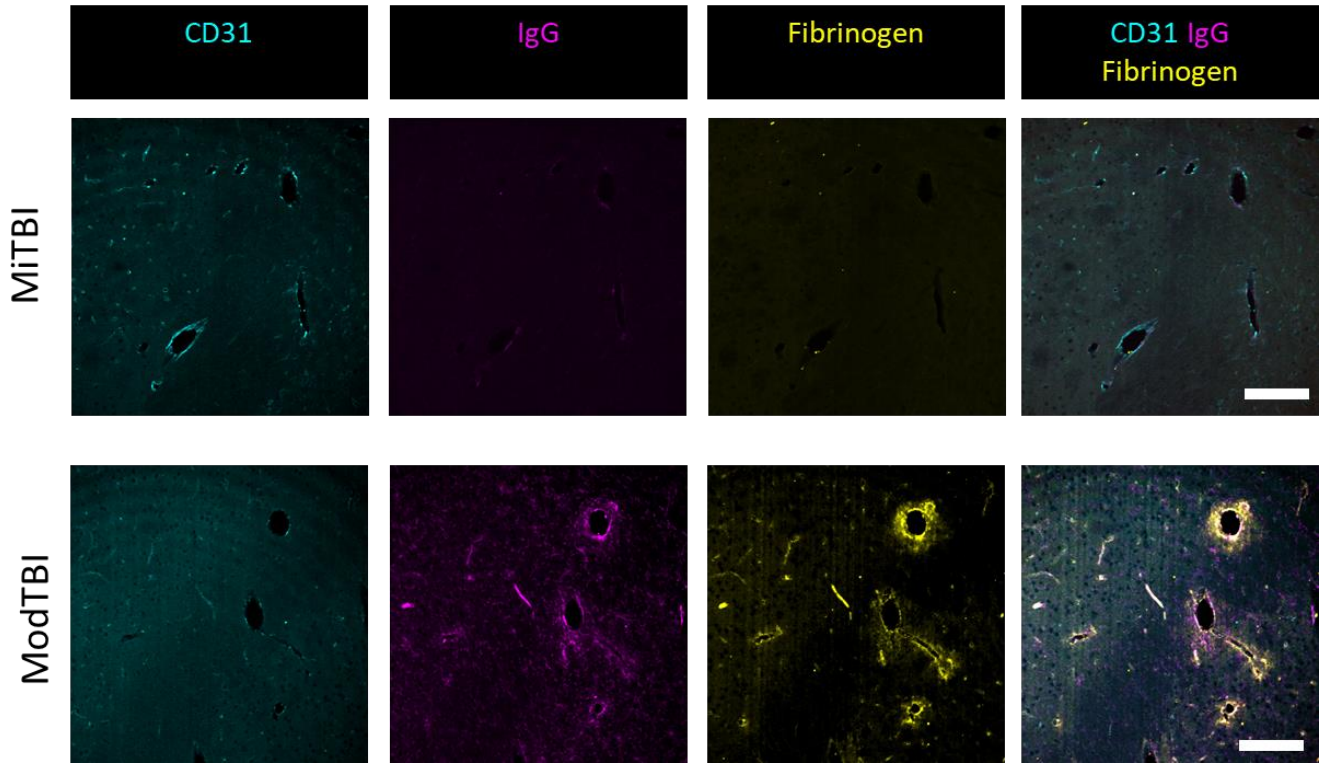


Figure S4. Mild TBI does not induce BBB leakage after 24h in contrast to moderate TBI. Representative immunostainings for CD31, fibrinogen and immunoglobulin extravasation after mild or moderate TBI (n=4 animals per group). Scale bar: 100µm.

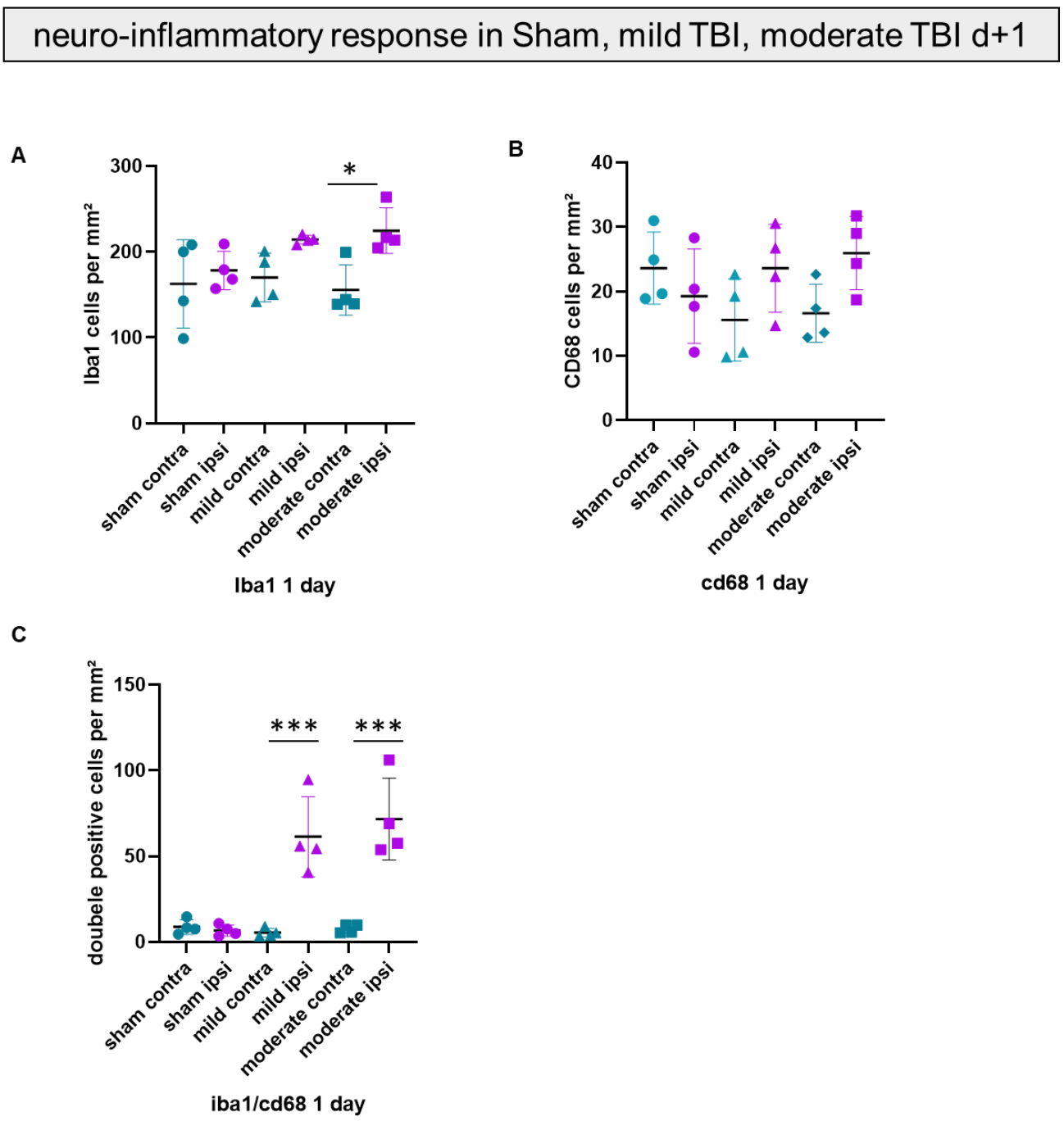


Figure S5. Quantification of microglial cell (A) and macrophages number (B), as well as microglial activation (C) in the cortex of mice subjected to sham conditions, miTBI or modTBI after 24h (n=4 per group).

*p<0.05, ***p<0.001 vs contralateral hemisphere, Ordinary one-way ANOVA with Tukey's multiple comparisons.

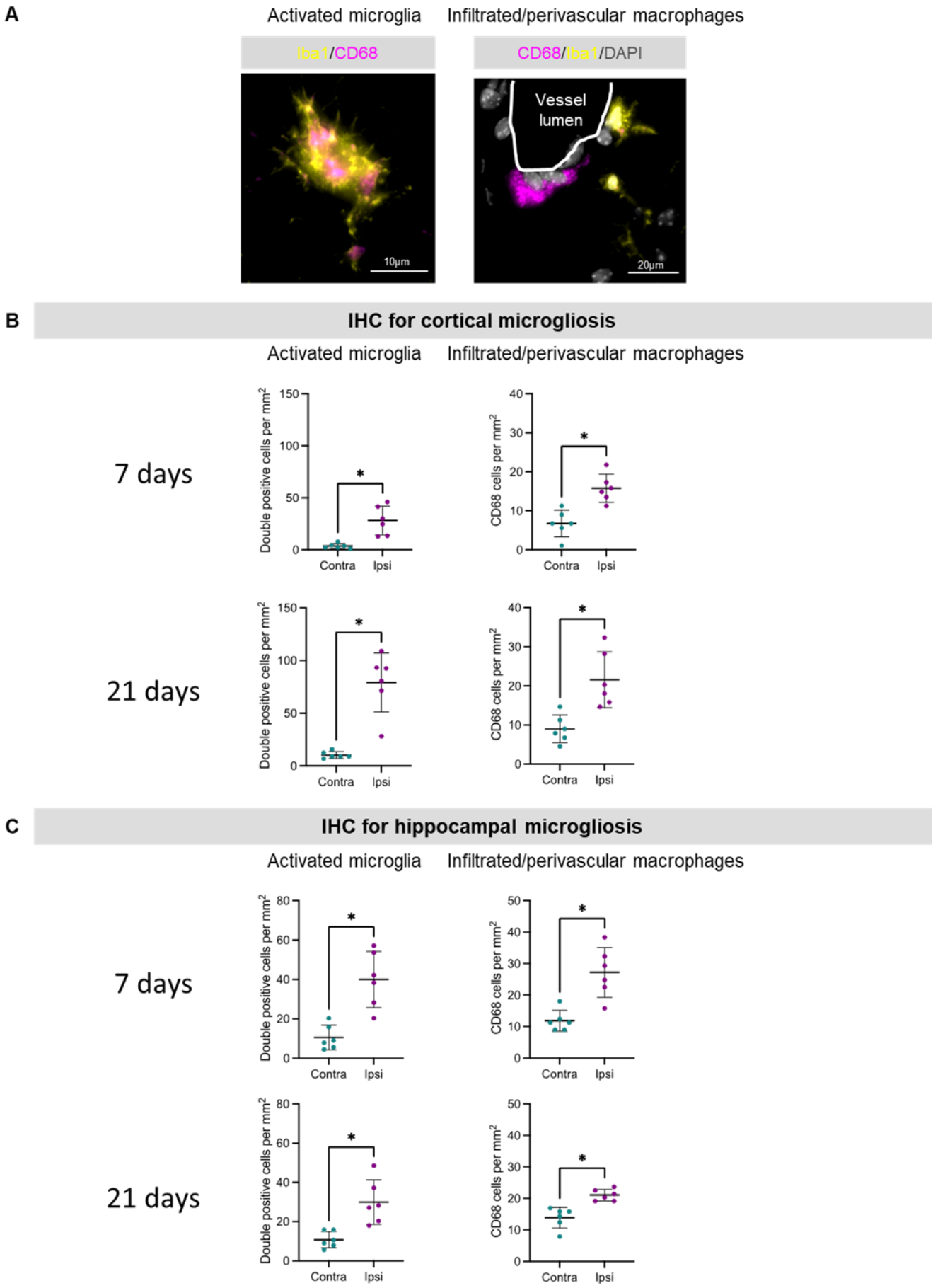

A
Activated microglia
Infiltrated/perivascular macrophages
Iba1/CD68
CD68/Iba1/DAPI
Vessel lumen
10μm
20μm
B
IHC for cortical microgliosis
Activated microglia
Infiltrated/perivascular macrophages
7 days
21 days
Double positive cells per mm2
CD68 cells per mm2
Contra
Ipsi
C
IHC for hippocampal microgliosis
Activated microglia
Infiltrated/perivascular macrophages
7 days
21 days
Double positive cells per mm2
CD68 cells per mm2
Contra
Ipsi

Figure S6. Mild TBI provokes a long-lasting microglial activation and infiltrated/perivascular macrophage recruitment. (A) Representative photomicrographs of an activated microglia (left panel, $Iba1^{+}CD68^{high}$) and an infiltrated/perivascular macrophage sitting next to a blood vessel (dotted line) ($Iba1^{low}CD68^{high}$). (B) Cortical microgliosis. Left: quantification of cortical $Iba1^{+}CD68^{high}$ microglial cell numbers 7 (top) and 21 (bottom) days after miTBI. Right: quantification of cortical $Iba1^{low}CD68^{high}$ macrophages 7 (top) and 21 (bottom) days after miTBI. (C) Hippocampal microgliosis. Left: quantification of hippocampal $Iba1^{+}CD68^{high}$ microglial cell numbers 7 (top) and 21 (bottom) days after miTBI. Right: quantification of $Iba1^{low}CD68^{high}$ macrophages 7 (top) and 21 (bottom) days after miTBI. Right: quantification of hippocampal $Iba1^{low}CD68^{high}$ macrophages 7 (top) and 21 (bottom) days after miTBI. n=6 mice/group; *$p<0.05$, **$p<0.01$ vs contralateral hemisphere, Wilcoxon paired-test.