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SUPPLEMENTARY INFORMATION

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Direct vascular channels connect skull bone marrow and the brain surface enabling myeloid cell migration

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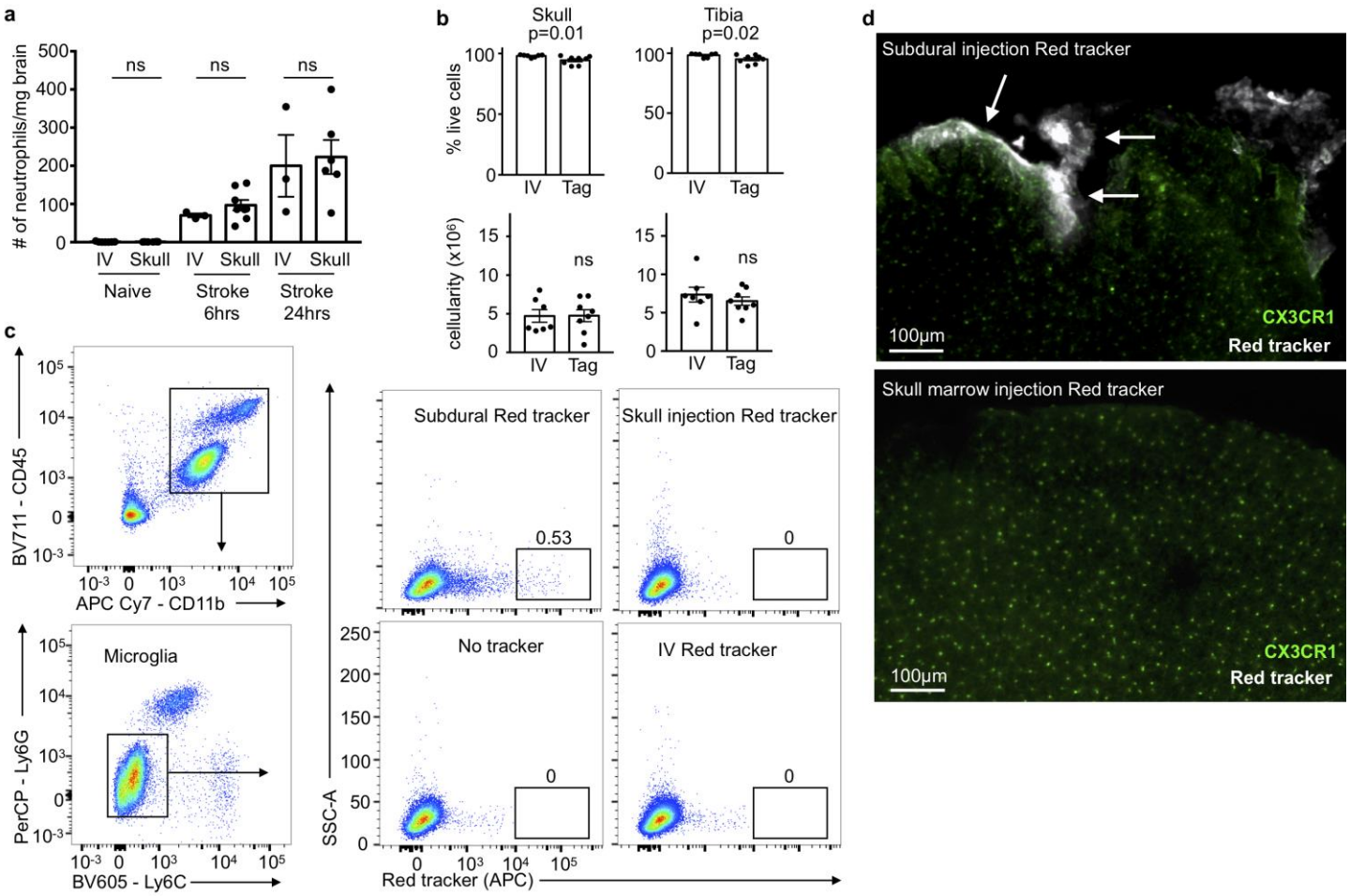
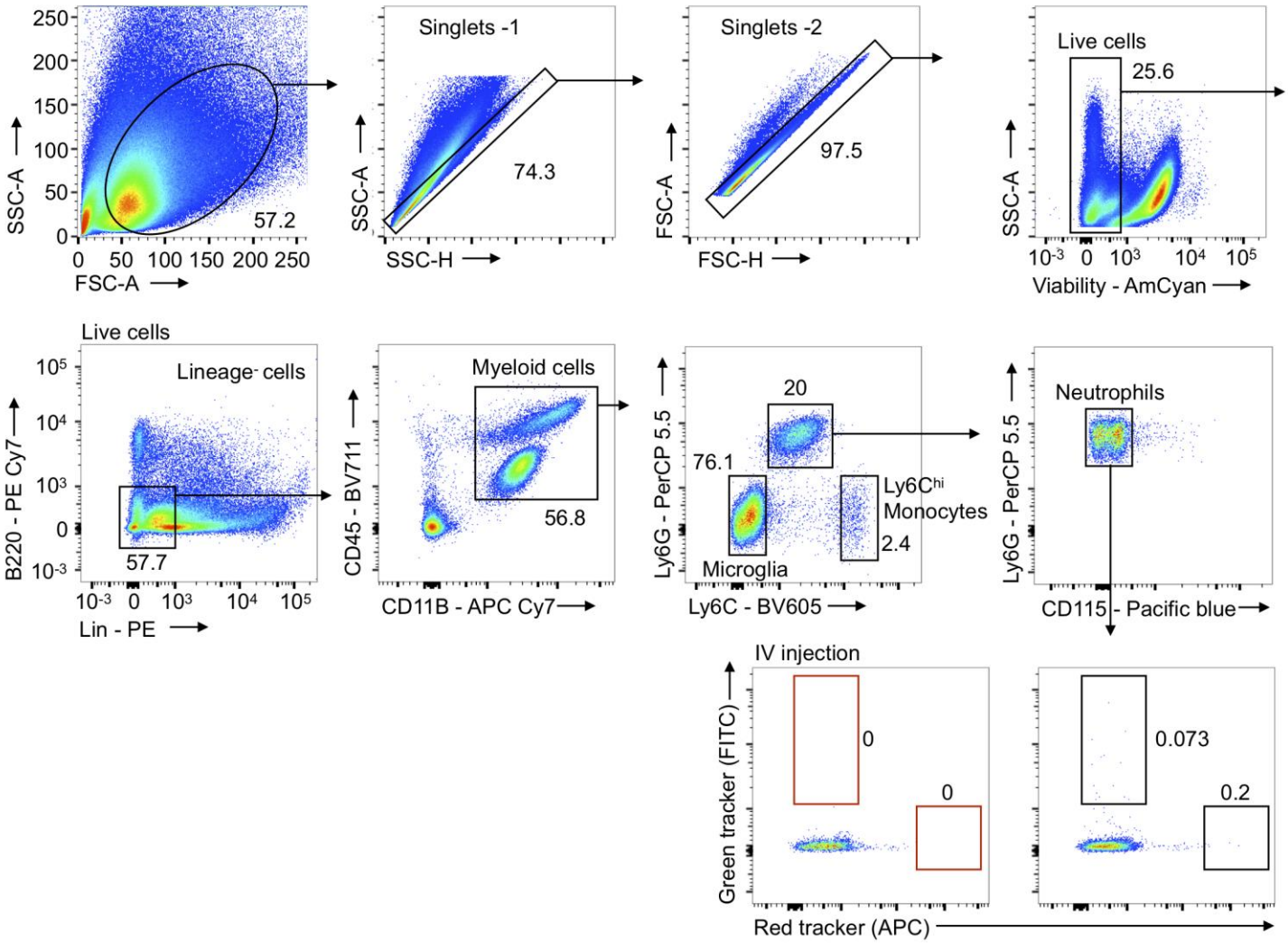


Supplementary Figure 1

Bone marrow cell-tagging effect on cell viability or function.

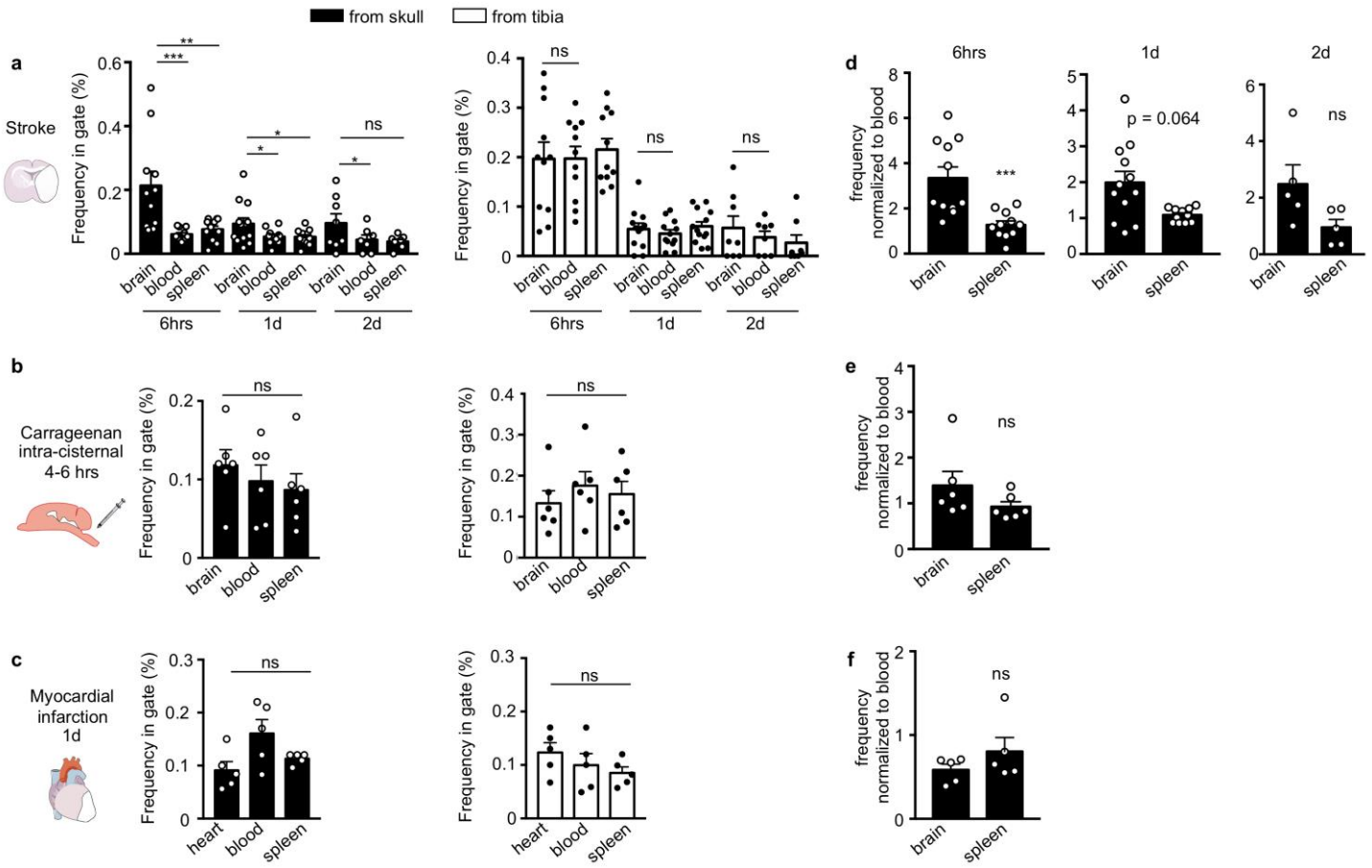
a, Neutrophil recruitment to the brain after intravenous or marrow injection of cell tracker. Two-tailed Mann-Whitney test, naive (n=7 IV, n=6 skull tag, 4 experiments), P= 0.52; stroke 6hrs (n=3 IV, n=8 skull tag, 3 experiments), P=0.28; stroke 24hrs (n=3 IV, n=6 skull tag, 3 experiments), P=0.71; **b**, Viability (upper panel) and cellularity (lower panel) of bone marrow after intravenous or marrow injection of the red (skull) and green (tibia) dyes in naive mice (n=7 IV, n=8 Tag, 6 experiments). Two-tailed Mann Whitney test, viability skull, P= 0.01; viability tibia, P=0.02; cellularity skull P>0.99; cellularity tibia, P=0.61. **c**, Gating for microglia and uptake of red cell tracker 4 hrs after cisternal carrageenan injection (single experiment), after subdural injection of 10 µl (upper left), no tracker (bottom left), 10µl in the calvarium (upper right), IV injection of 10 µl (bottom right). Data are mean ± s.e.m.. Mann-Whitney test, ns indicates not significant. **d,** Brain sections of a naive Cx3cr1^{GFP} mouse, after 10 µl red cell tracker injected in the sub-dural area (upper panel, arrow shows injection site) or locally in the skull marrow (lower panel), single experiment.



Supplementary Figure 2

Flow cytometry gating for brain.

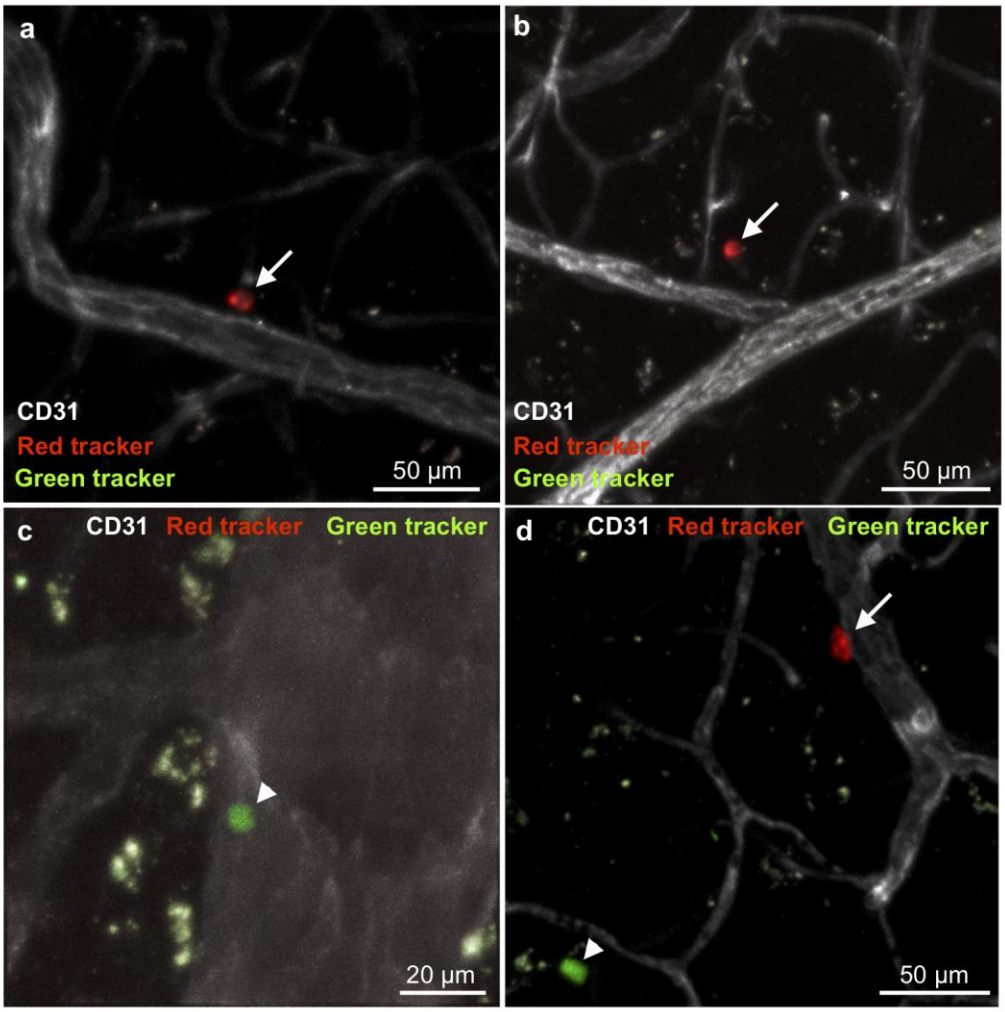
Bottom right panel shows gates for cells originating from tibia (green tracker, FITC channel) and skull (red tracker, APC channel) based on the signal obtained in the circulation after IV injection (bottom left panel).



Supplementary Figure 3

Additional cell-tracking analyses.

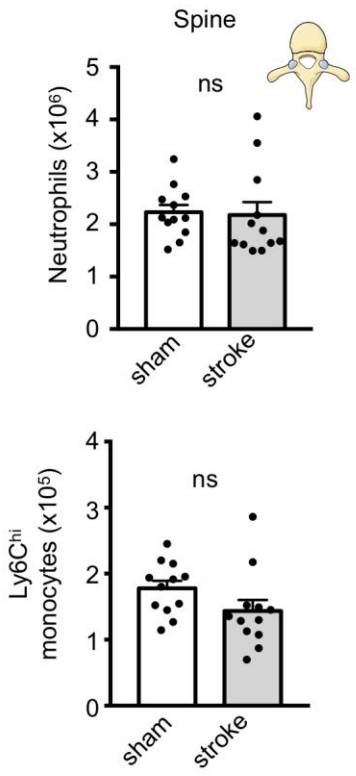
a-c, Frequency of cells tracked from skull and tibia marrow in (**a**) stroke, 6hrs, n=11, 5 experiments; 1 day, n=13 for brain and spleen and n=12 for blood, 5 experiments; 2 days, n=7, 2 experiments; skull ***P=0.001 and **P=0.002 at 6hrs, *P=0.022, *P=0.03 at day 1; *P=0.03, ns P=0.22 at 2 days; tibia ns P=0.46 at 6hrs, ns P=0.69 at day 1, (**b**) carrageenan, n=6, 4 experiments, Kruskall Wallis test, skull P=0.51, tibia ns P=0.65, (**c**) myocardial infarction, n= 5, 1 experiment, Kruskall Wallis test skull ns P=0.11, tibia ns P=0.32. **d-f,** Frequency of tracked cells in respective organs relative to the circulation after (**d**) stroke, at 6 hrs, n=11, 5 experiments, ***P=0.002, 1 day, n=12, 5 experiments, P=0.064 and 2 days, n=5, 2 experiments, ns P=0.13 , (**e**) carrageenan injection, n=6, 4 experiments and (**f**) after myocardial infarction, n= 5, 1 experiment. Data are mean ± s.e.m., ns indicates not significant, two-tailed Wilcoxon test unless otherwise specified.



Supplementary Figure 4

Location of tracked cells from skull and tibia after aseptic meningitis.

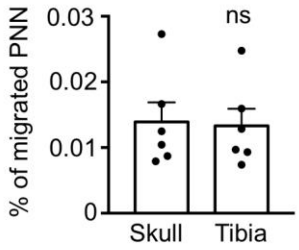
Cells originating from skull (red, arrow) and tibia (green, arrow head) in brain one day after induction of aseptic meningitis (n=2 mice). Cells are outside (**a,b,d**) or inside (**c**) the vasculature. Speckles in c are present in all channels and present autofluorescence. Collapsed Z stacks show cells at a depth of 20-100 µm below the brain tissue surface.



Supplementary Figure 5

The spine marrow during acute stroke.

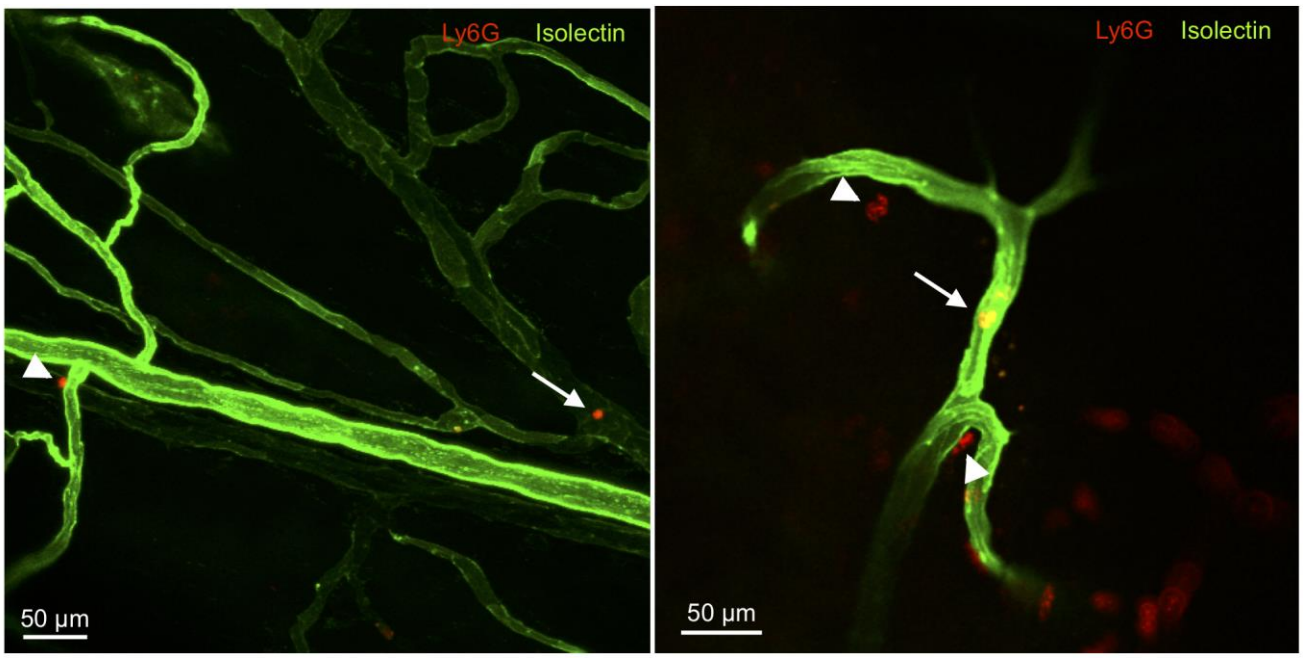
N= 12 per group, 3 experiments. Data are mean ± s.e.m. Two-tailed Mann-Whitney test, neutrophils, P=0.29 and Ly6C^{hi} monocytes, P=0.052.



Supplementary Figure 6

Competitive in vitro transmigration assay through activated endothelium comparing skull and tibial neutrophils.

Cells from 6 mice (6 skull-tibia pairs, red or green fluorescence staining for location-specific tracking) were subjected to migration through a TNFα-activated brain endothelium towards fMLP. Results are migrated cells as % of initial population. Data are mean ± s.e.m.. P=0.69, two-tailed Wilcoxon test.



Supplementary Figure 7

Histology of the dural vasculature 1 d after stroke.

Dural vasculature one day after stroke induced by permanent occlusion (representative of two experiments). Arrows show cells inside vasculature, arrow heads indicate cells outside of vasculature.