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Additional file 1

# Responses of perivascular macrophages to circulating lipopolysaccharides in the subfornical organ with special reference to endotoxin tolerance

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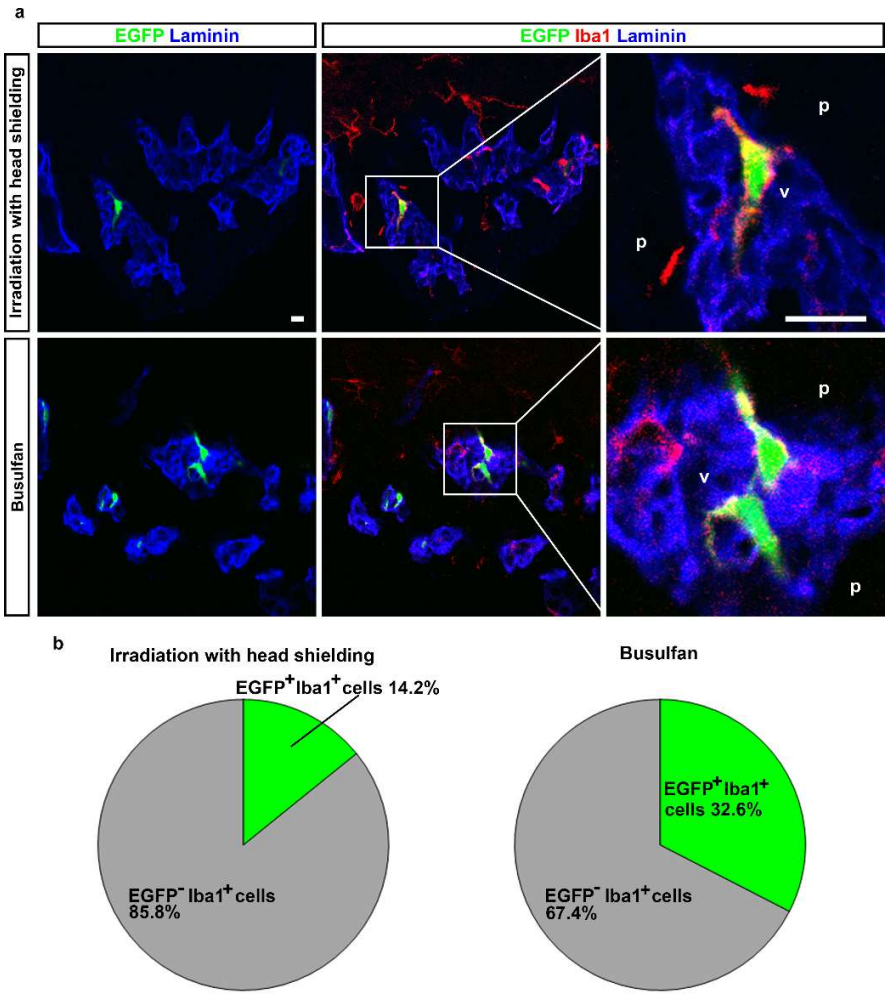
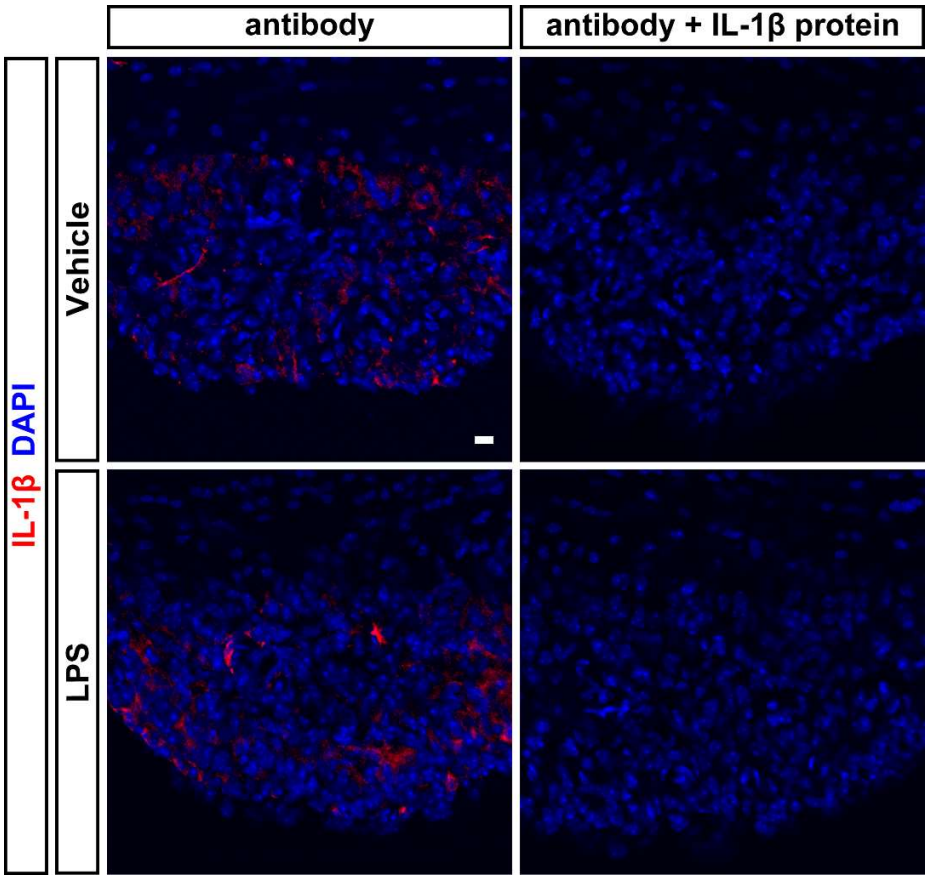
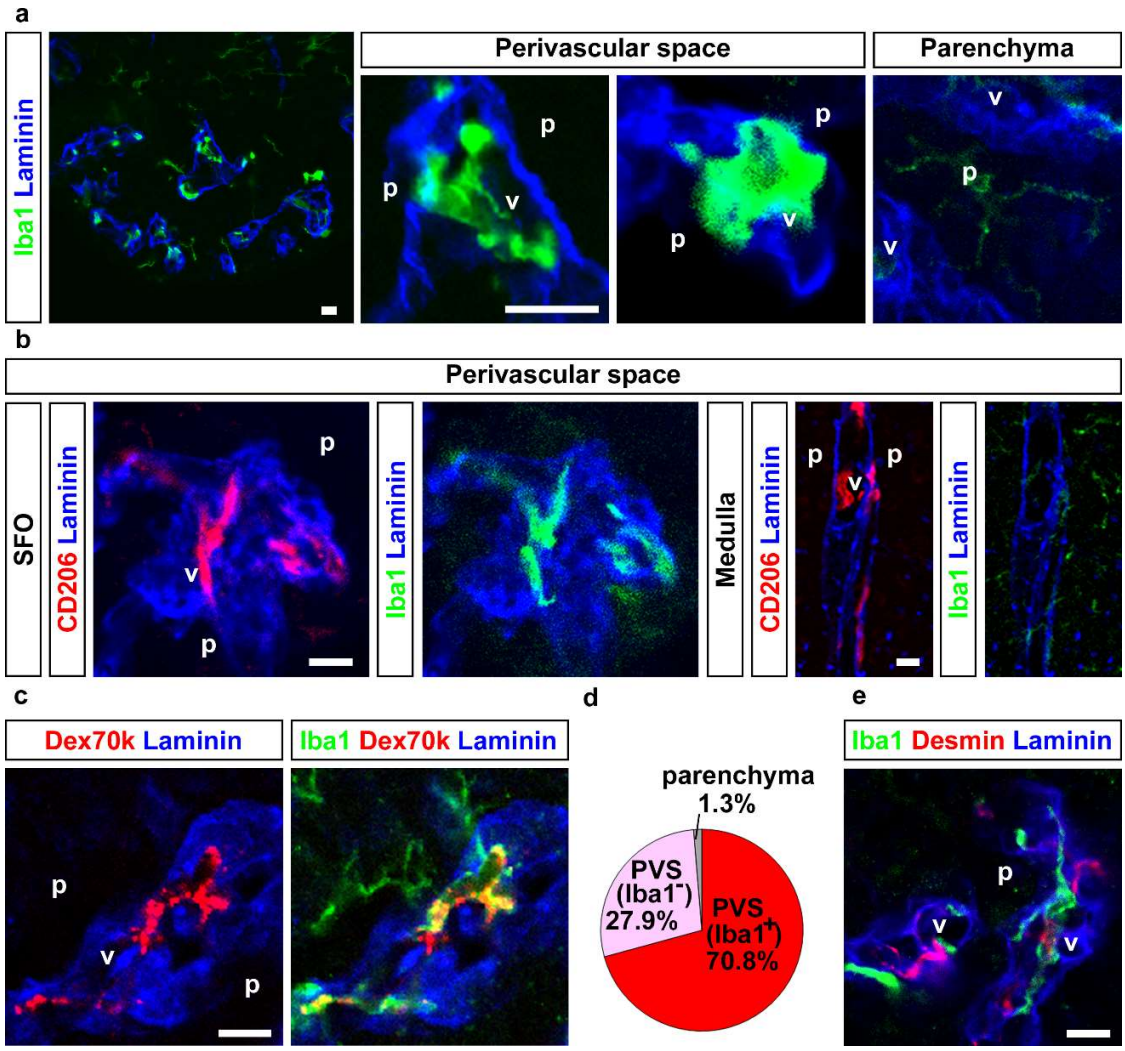



**Supplementary Fig. 1.** Engraftment of bone marrow-derived cells into the SFO. Cryosections were immunostained with antibodies against the myeloid lineage cell marker Iba1 and the outer basement membrane marker laminin-111. (a) Immunohistochemistry shows EGFP$^+$ Iba1$^+$ cells in the SFO of irradiated chimeric mice with head shield 4 weeks after reconstitution (upper) and that of busulfan-treated chimeric mice 4 weeks after reconstitution (lower). (b) Quantification of results from a. Data were obtained from 4-5 animals (37-46 sections) and 289-470 Iba1$^+$ perivascular macrophages were examined. *Laminin* laminin-111, *p* parenchymal area, *v* vasculature. Scale bars are 10 μm.

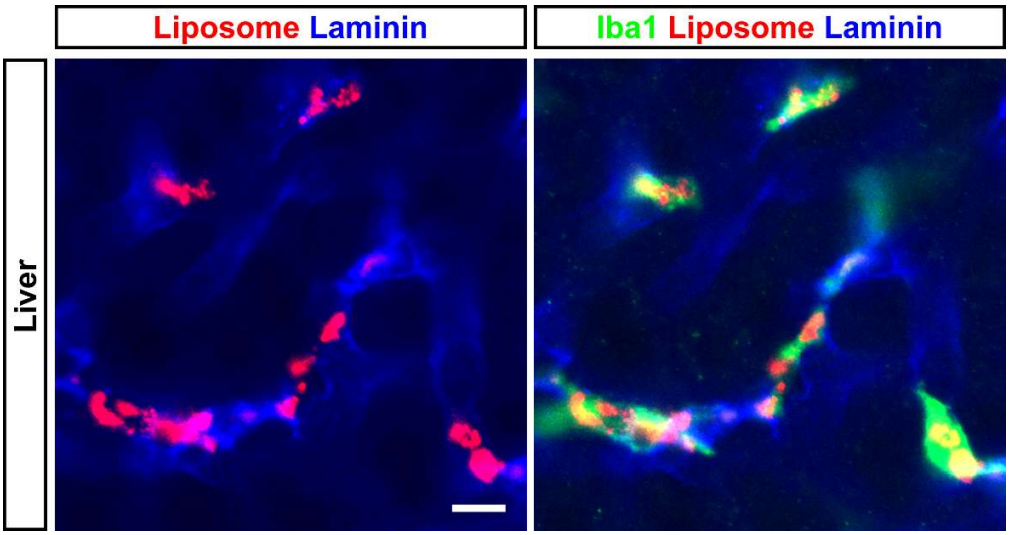



**Supplementary Fig. 2.** Specificity controls for IL-1β antibody. (upper) Immunohistochemistry was performed on mouse brain sections with anti-IL-1β antibody. (b) Anti-IL-1β antibody was pre-adsorbed with IL-1β recombinant protein and used for immunohistochemistry preadsorption control. The IL-1β staining was significantly diminished or abolished when the diluted primary antibody was preincubated with the IL-1β recombinant protein. (lower) The IL-1β staining was also abolished by antibody pre-incubation with the IL-1β recombinant protein in the SFO of LPS-injected mice. Nuclei were counterstained with 4,6-diamidino-2-phenylindole. Scale bar is 10 μm.




**Supplementary Fig. 3.** Characterization of the SFO perivascular Iba1⁺ cells. Cryosections were immunostained with antibodies against the myeloid lineage cell marker Iba1, the outer basement membrane marker laminin-111, the mannose receptor CD206, and the pericyte marker desmin. (a) Immunohistochemistry revealed that the Iba1⁺ cells were widely distributed in the SFO. Perivascular Iba1⁺ cells were seen inside, or sometimes spanning, the laminin-111⁺ outer basement membrane, and were strongly immunoreactive for Iba1 and exhibited elongated or ameboid-like morphology. Microglia were seen outside the laminin-111⁺ outer basement membrane, were immunoreactive for Iba1, and exhibited ramified morphology. (b) Both the SFO perivascular Iba1⁺ cells and typical perivascular macrophages were CD206⁺, but typical perivascular macrophages were weakly

immunoreactive for Iba1. (c) The SFO perivascular Iba1$^+$ cells were able to phagocytose circulating dextran. (d) Quantification of results from c. Data were obtained from 5 animals (48 sections) and 383 Dextran 70,000$^+$ spots were examined. (e) The SFO perivascular Iba1$^+$ cells were negative for the pericyte marker desmin. *Dex70k* Dextran 70,000, *Laminin* laminin-111, *p* parenchymal area, *PVS* perivascular space, *v* vasculature. Scale bars are 10 μm.




**Supplementary Fig. 4.** Localization of peripherally injected control liposomes in the SFO. Cryosections were immunostained with antibodies against the myeloid lineage cell marker Iba1 and the outer basement membrane marker laminin-111. DiI-labeled control liposomes were found in the liver and co-localized with Iba1 immunoreactivity. *Laminin* laminin-111. Scale bar is 10 µm.