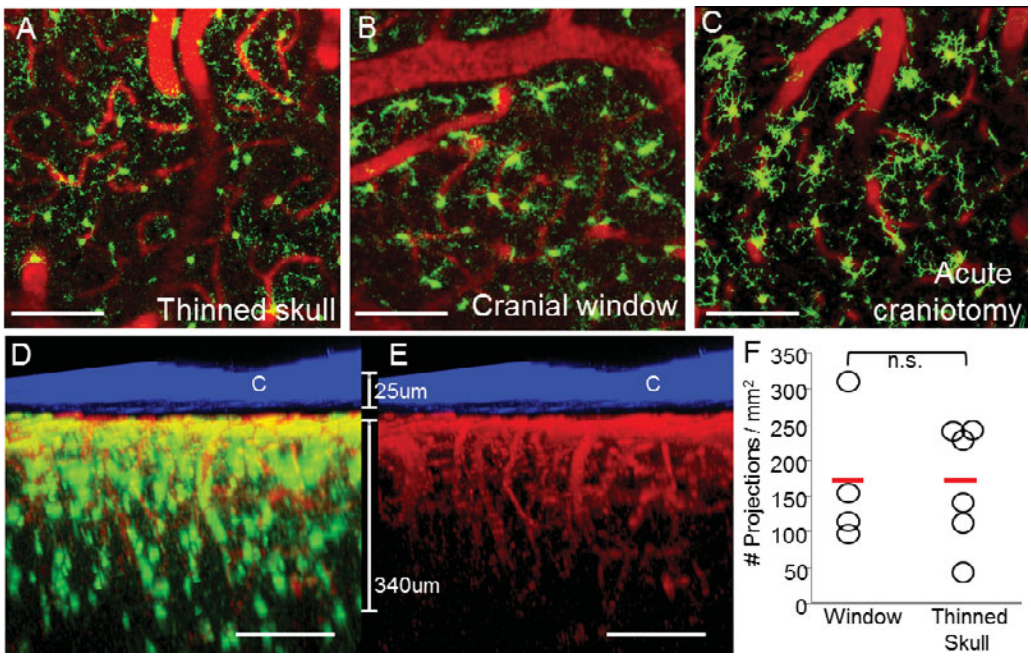
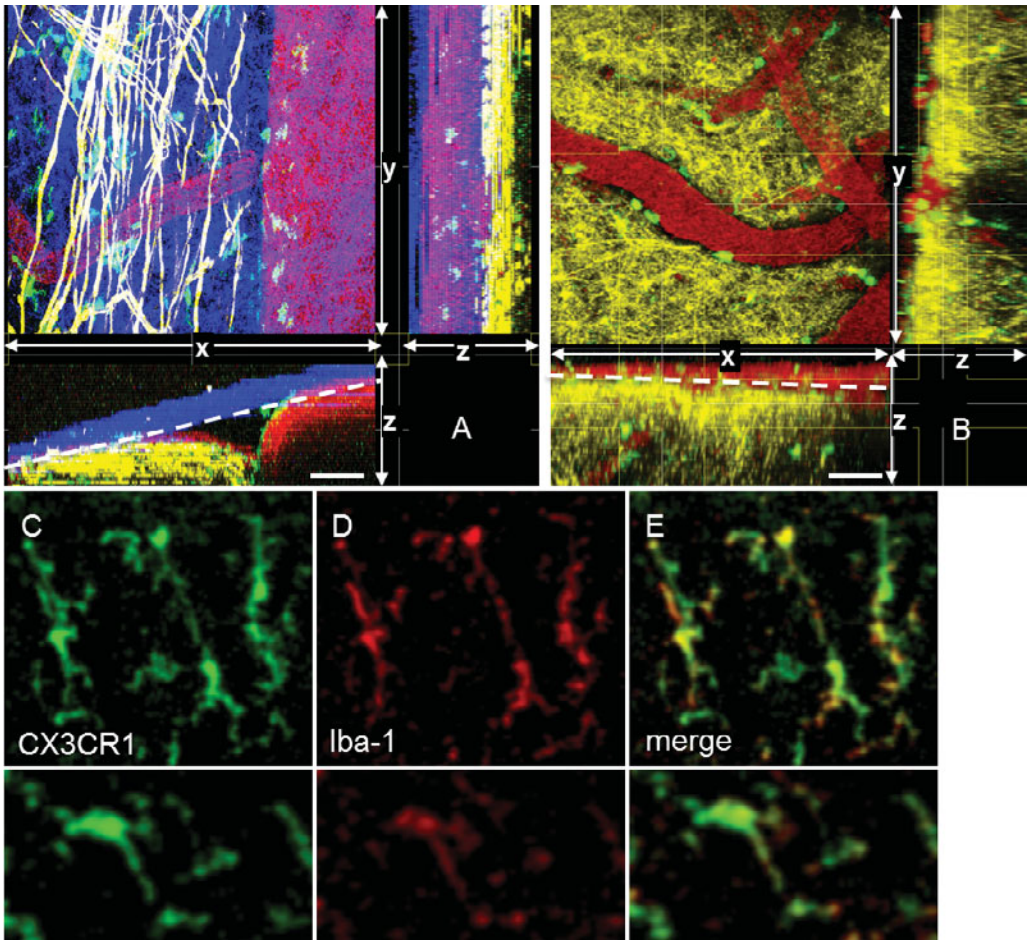
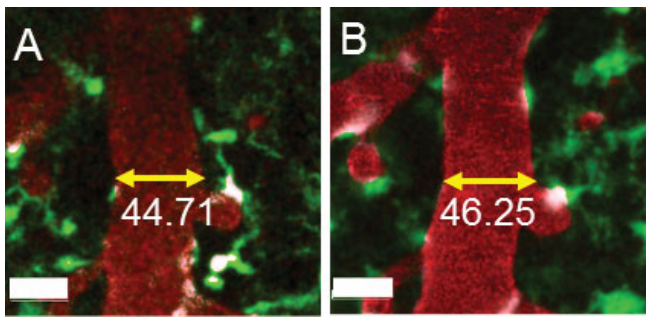


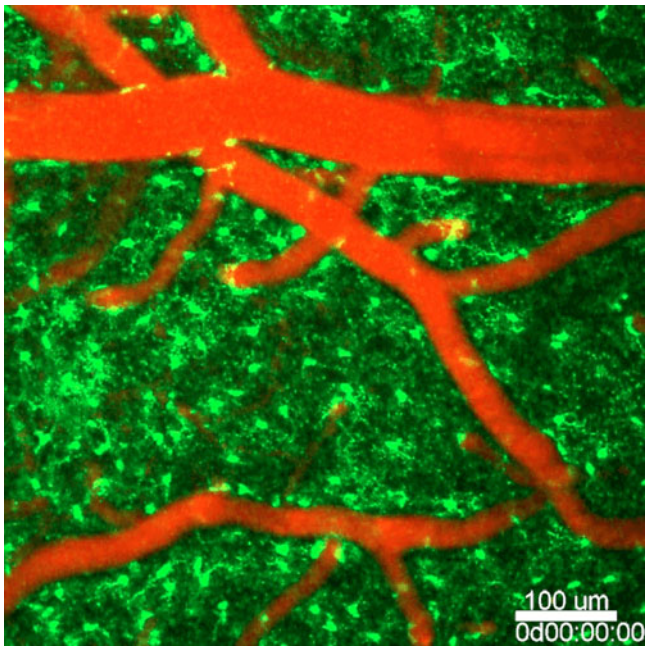
Supplementary Figure 1. Comparison of CX3CR1^{+} cellular morphology and baseline projection frequency using different tissue preparations. The morphology of CX3CR1^{+} cells (green) in control *Cx3cr1*$^{+/GFP}$ mice are shown using: (**A**) thinned-skull approach, where the images were obtained with an intact bone thickness of 25–50 μm (**D**, **E**; c: cranial bone); (**B**) cranial window implanted 4 days prior to imaging; and (**C**) acute open craniotomy through a glass window on the day of imaging. CX3CR1^{+} cellular morphology was similar in all cases, with some differences in the overall imaging properties as imaging depth and structural resolution was greatest with open craniotomy and least with thinned-skull preparation. Parenchymal vessels were labeled with TRITC-dextran (red). Scale bar = 100 μm. (**F**) Baseline number of intravascular projections was quantified from four individual *Cx3cr1*$^{+/GFP}$ mice using 4-day implantation window and six individual *Cx3cr1*$^{+/GFP}$ mice using thinned-skull preparations, showing an average of 172.5 $\pm$ 96.8 projections/mm^2 and 171.5 $\pm$ 82.3 projections/mm^2, respectively. n.s. = not significant. Only CX3CR1^{+} cells in the parenchyma were analyzed (Supplementary Fig. 2).



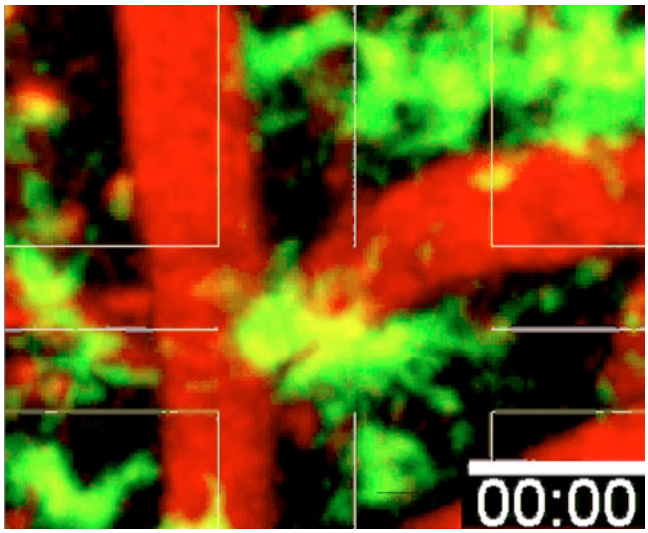
Supplementary Figure 2. Visualization, identification, and analysis of parenchymal CX3CR1^{+} cells with intravital imaging methods. Three-dimensional (*xy*, *xz*, *yz*) display views of the maximum intensity projection images of the spinal cord (**A**) and the brain (**B**) of a control Thy-1-YFP-H $\times$ *Cx3cr1*$^{+/GFP}$ mouse acquired through an open laminectomy (**A**) and a cranial window (**B**), showing the relative positions of individual Thy-1-YFP-H axons (yellow), CX3CR1^{+} cells (green), intact TRITC-dextran labeled blood vessels (red), and dura (blue, second harmonic signals). All of our quantitative analyses took place in the layers where Thy-1-YFP is present below the meninges and pial surface (dotted line). (**C**) Immunofluorescence histology of fixed tissue sections from Figure 1 confirmed that the majority of CX3CR1^{+} GFP^{+} (green) cells in the visualized field also co-stained (**E**) for Iba-1 (**D**, red), further identifying them as belonging to the activated monocytic lineage (Imai & Kohsaka, 2002; Kanazawa et al., 2002).



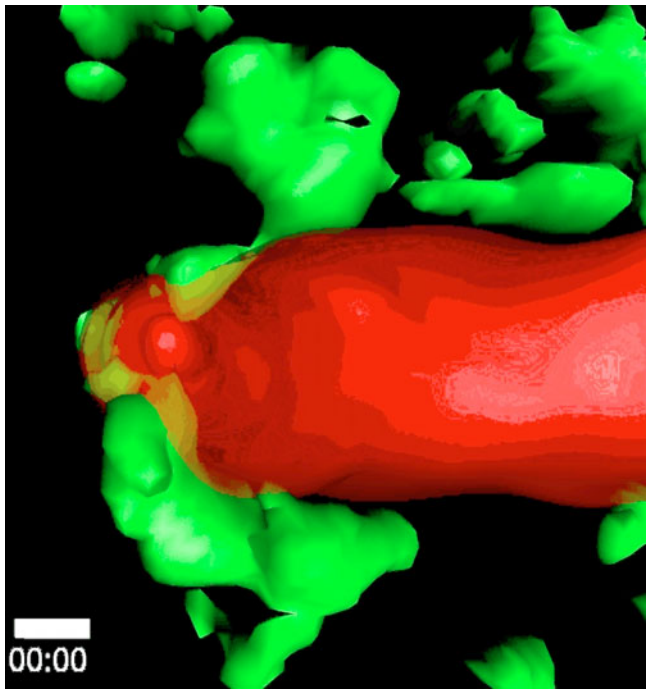
Supplementary Figure 3. Vessel boundary outlined by different *in vivo* labeling techniques. CNS vessel lumen boundary measured similarly during intravital imaging of the same region by sequentially injecting (**A**) TRITC-dextran (700 μg/mouse) and (**B**) tomato-lectin (16 μg/mouse) *i.v.* on two consecutive imaging days. Scale bar = 10 μm.



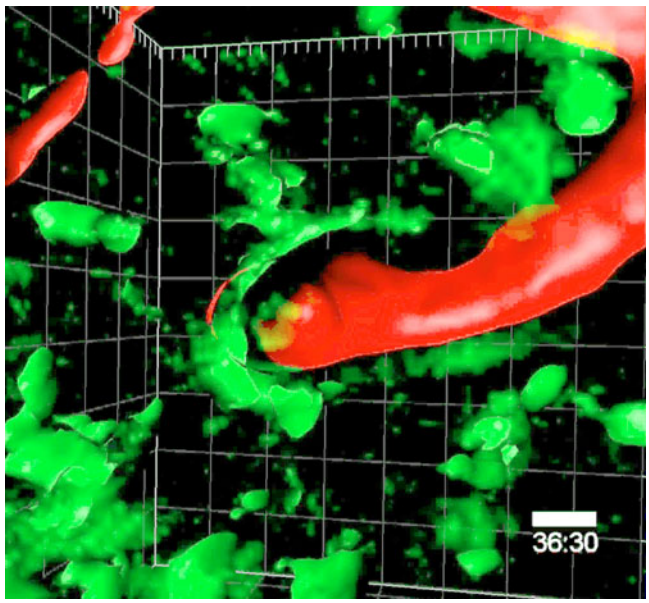
Supplementary Movie 1. Microglia morphology and distribution in noninflamed CNS parenchyma of a *Cx3cr1*$^{+/GFP}$ mouse. Sequential imaging of the parietal lobe of a *Cx3cr1*$^{+/GFP}$ mouse was captured through a cranial window implanted 4 days prior to imaging. CNS vessels are highlighted by TRITC-dextran. Total imaging time: 60 min. Playback speed: 300×.



Supplementary Movie 2. Dynamic dendritic motility of stationary extravascular CX3CR1$^+$ cells. A zoomed-in view of a GFP$^+$ CX3CR1$^+$ cell (green) next to an intact CNS blood vessel (red) illustrates the highly dynamic motility of dendritic extensions probing the extravascular space. Other smaller, spherical CX3CR1$^+$ cells can be seen crawling in the blood vessel lumen, which most likely represents circulating CX3CR1$^+$ NK cells or monocytes (Jung et al., 2000). Total imaging time: 45 min. Playback speed: 300×.



Supplementary Movie 3. Extravascular CX3CR1$^+$ cells project dendrites into CNS vessels. Dendritic projections of extravascular CX3CR1$^+$ cells are vividly visualized within the CNS vessel lumen. The extravascular microglia body projects stably into the vessel lumen for at least 30 min. Dendritic projections from two CX3CR1$^+$ cells can be seen contacting each other within the vessel lumen. Note the absence of TRITC-dextran dye in the surrounding parenchyma at the site of intravascular dendritic insertions. Total time: 45 min. Playback speed = 300×.



Supplementary Movie 4. Three-dimensional view of the intravascular dendritic extension by extravascular CX3CR1$^+$ cells. A snapshot from Supplementary Movie 3 (at time stamp = 36 min 30 s) is shown in a 3D rendering view, demonstrating the relative position of the green dendritic body with respect to intact CNS blood vessel wall. Total time: 22 min. Playback speed: 450×. Scale bar = 15 μm.