Supplemental Information

Microglia Mediate Postoperative Hippocampal Inflammation and Cognitive Decline in Mice

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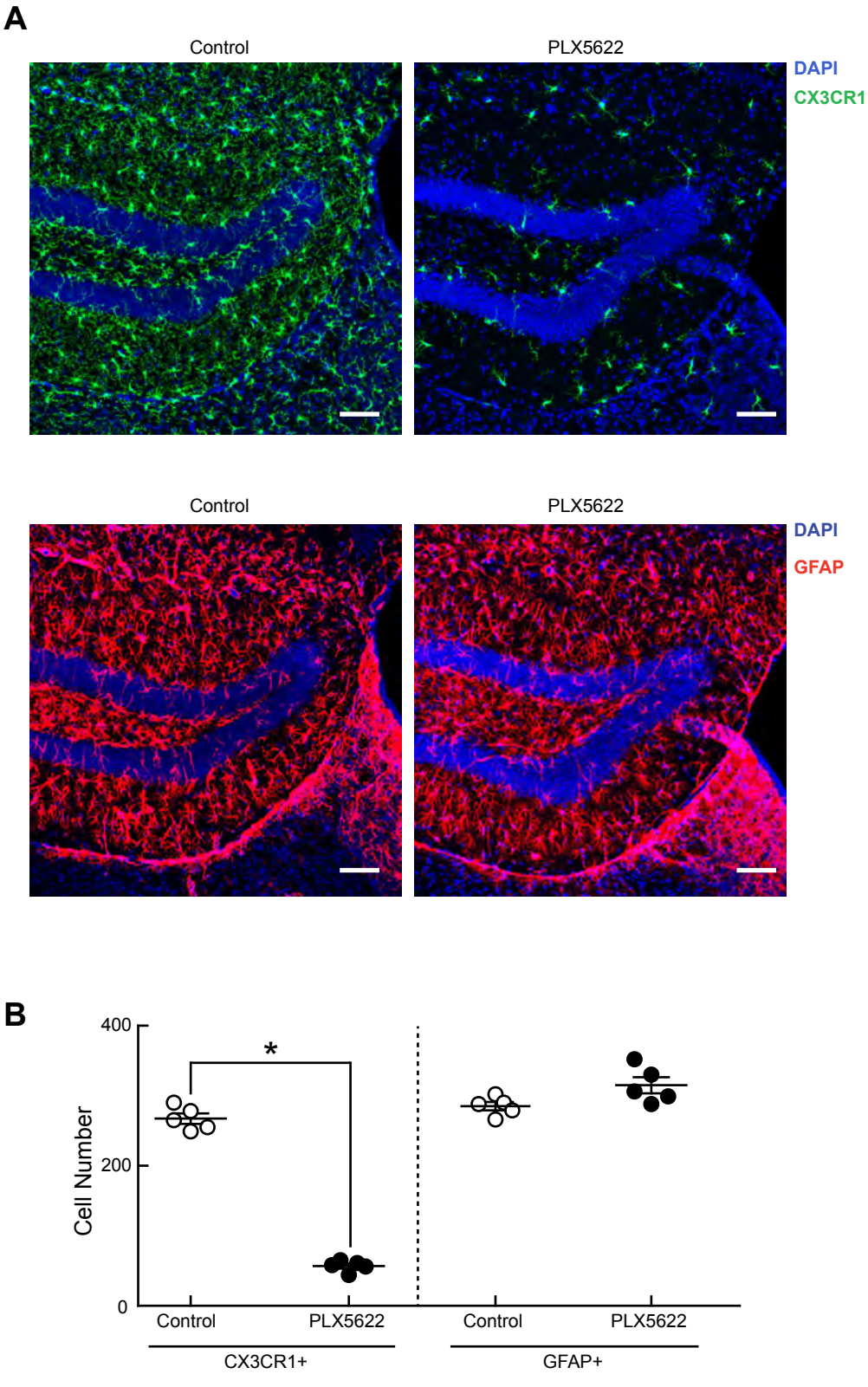
Supplemental Methods

Additional flow cytometry protocols

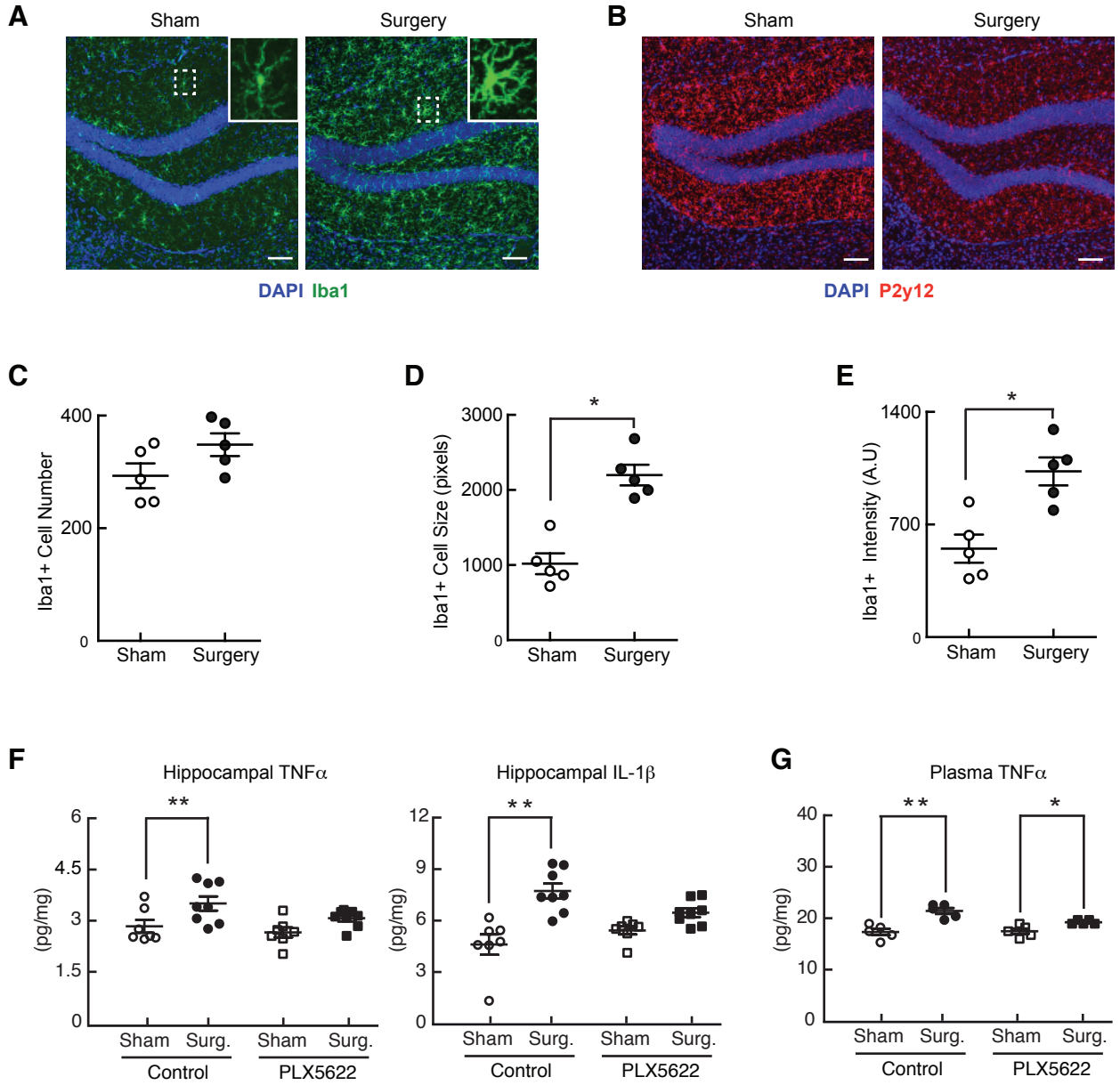
To determine the numbers of neutrophils and monocytes from specific subsets, respectively, among circulating leukocytes in control and PLX5622-treated mice, venous blood samples from live mice were collected into EDTA-containing tubes and treated with ammonium-chloride-potassium (ACK) buffer to lyse the red blood cells. The samples were then gently spun, and the resulting cell pellets were resuspended in FACS buffer (BD bioscience). Total cell numbers were counted using a TC20 automated cell counter (Bio-Rad). The cellular suspensions were blocked (FcBlock; BD Biosciences) for 30 minutes, and then stained for 30 minutes with specific fluorochrome-conjugated antibodies, including conCD11b-BV421 (Biolegend, clone M1/70), CD45-PE-Cyanine7 (Affymetrix, clone 30-F11), Ly6C-PerCP/Cy5.5 (Biolegend, clone HK1.4) and Ly6G-APC (eBioscience, clone 1A8-Ly6g). Fluorescence data indicating the number of neutrophils and monocytes from specific subsets in each sample were acquired as above. “Fluorescence minus one” controls were used to set up gates and analyzed with FlowJo v10 software (Tree Star Inc.).

Additional antibodies

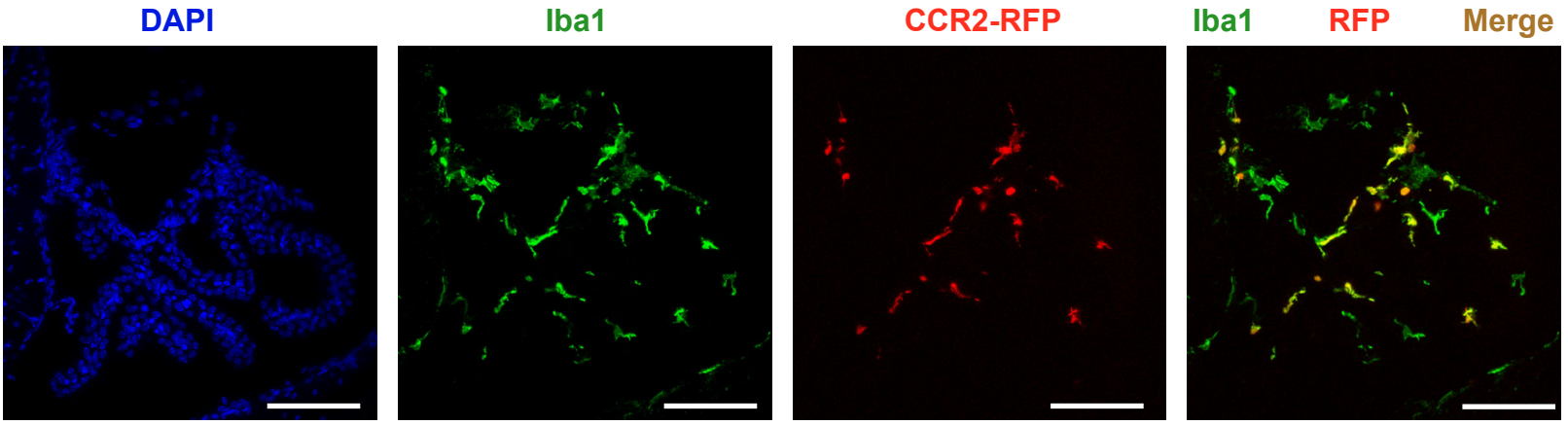
Additional antibodies, including anti-Iba1 (Wako, Cat. No. 019-19741), anti-GFAP (Sigma, G9269), anti-RFP (Rockland, 600-401-379), and anti-P2y12 (kindly provided by Dr. David Julius, University of California San Francisco), were used for immunofluorescence histochemistry experiments.



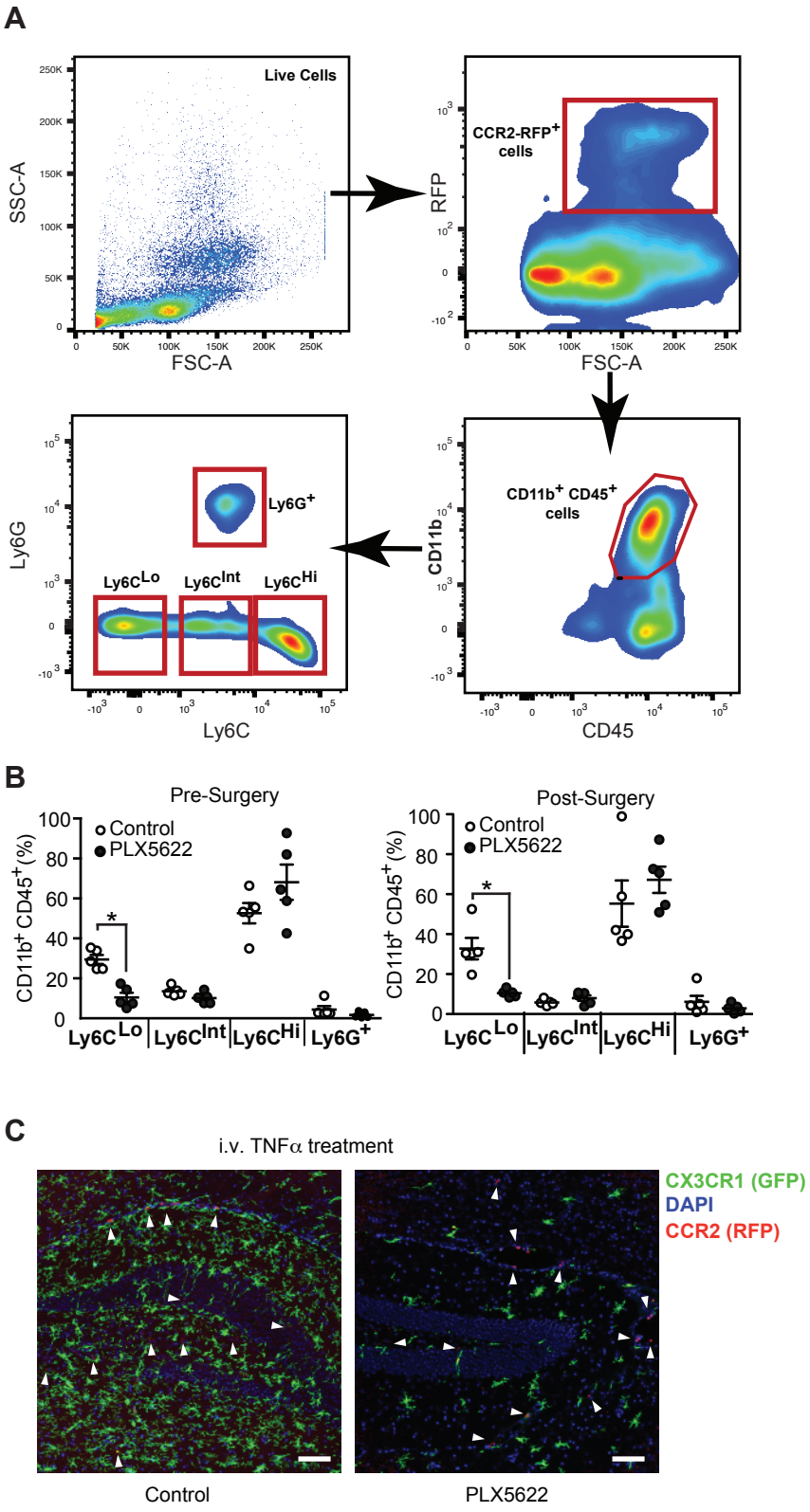
Supplemental Figure 1. Perioperative PLX5622 treatment depletes microglia, but not hippocampal astrocytes. A. Representative immunofluorescence images taken from CX3CR1$^{GFP/+}$ mice 24 hours after surgery (n=5/group), showing that perioperative PLX5622 treatment profoundly reduces the number of microglia (green) that otherwise accumulate within the hippocampus, but does not impact the number of astrocytes (red-stained GFAP^{+} cells) by comparison. Scale bars=50 μm. **B.** Quantification of the comparisons made in A (n = 5/group; *p<0.001.



Supplemental Figure 2. Additional markers indicating that perioperative microglial depletion abrogates surgically induced hippocampal inflammation. A. Representative immunofluorescence image (n=6-7) taken from mice 24 hours following surgery, showing that microglia accumulating in the hippocampus have increased Iba1 expression (green intensity) and have transitioned from a quiescent morphology to one indicative of inflammatory activation. **B.** Representative image from the same time point, showing a subjective reduction in P2Y12 expression (red intensity) among hippocampal microglia in response to surgery. Scale bars=50 μm. **C-E.** Quantification of the number (C), size (D), and fluorescence intensity (E) of Iba1-expressing cells in anatomically matched hippocampal sections from the same groups of mice represented in A (n = 6-7/group). **F.** Tissue ELISA, showing that perioperative PLX5622 treatment of the mice described in Figure 2 prevents the surgically induced rise in hippocampal IL-1b and TNF levels. Data are only shown at 6 hours following surgery, as the induction of each of these cytokines had already returned to sham levels within 24 hours of surgery. Data were analyzed by 2-way ANOVA (n=7-8/group). **G.** Plasma ELISA for the same mice as in Figure 2, showing that both control and PLX5622-treated mice have a similar modest surgically induced rise in circulating TNFα levels. Again, data are only shown at 6 hours following surgery, as levels had fallen to sham levels within 24 hours of surgery. For C-E, **p<0.001 and *p<0.05.



Supplemental Figure 3. CCR2-expressing cells infiltrating the hippocampus in response to surgery are likely monocyte-derived myeloid cells. Representative immunofluorescence images (n=3) taken from CCR2$^{RFP/+}$ mice 24 hours after surgery, showing that the CCR2-expressing (RFP^{+}) cells infiltrating the choroid plexus surrounding the hippocampus have either a round, leukocyte-like morphology, or an elongated morphology of ongoing vascular extravasation. However all of these RFP^{+}cells also express Iba1, indicative of myeloid cells and suggesting that the round cells are monocyte-derived in nature. Note also the presence of rare cells expressing Iba1 but not RFP (green only) with a more stellate morphology, reflective of either resident microglia other myeloid populations (e.g. perivascular or subdural macrophages) that do not express CCR2 highly. Scale bars=50 μm.



Supplemental Figure 4. PLX5622 treatment does not deplete circulating pro-inflammatory monocytes or impair their capacity to infiltrate the hippocampus. A. Multiparameter flow cytometry plots, revealing the stepwise gating strategy to count the number of Ly6CLo, Ly6CInt, and Ly6CHi monocytes, as well as neutrophils (Ly6CInt/Ly6G+), from the pool of CD11b+/CD45+/CCR2-expressing cells in the circulation. **B.** Quantification of data from A (n = 5/group), showing that PLX5622 treatment for 7 days depletes Ly6CLo monocytes from the circulation (*p<0.05; analyzed by Student's t-test), but does not affect the number of Ly6Hi or Ly6CInt monocytes, or the number of neutrophils. C. Representative immunofluorescence (n=3/group) images from CX3CR1GFP/+ : CCR2RFP/+ mice 16 hours following an i.v. injection of 0.15 mg/kg TNFα, showing that whereas PLX5622 treatment depletes microglia (GFP+ cells), CCR2-expressing (RFP+) cells are still able to infiltrate the hippocampus (white arrowheads). Scale bars=50 μm.