JEM

Supplemental material

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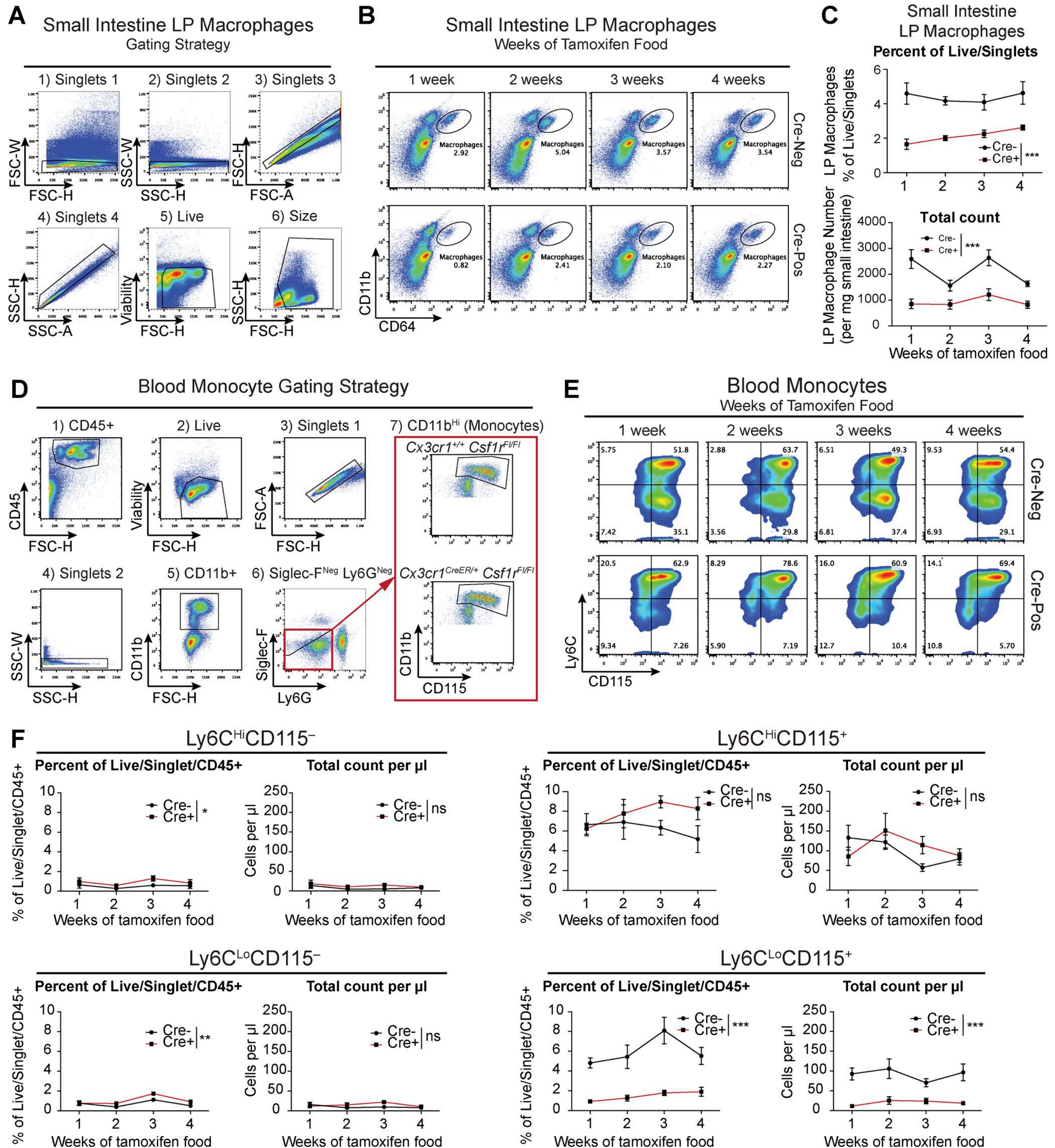

Figure S1. **Cx3cr1-expressing resident myeloid cells in the periphery are depleted in *Cx3cr1^CreER/+^::Csf1r^flox/flox^* mice fed tamoxifen. (A)** Initial gating strategy for live cells in the lamina propria (LP). **(B)** Representative flow cytometry plots of lamina propria macrophages, pregated as depicted in A. **(C)** Counts of lamina propria macrophages (n = 5–6 mice per group; two-way ANOVA; ***, $P < 0.001$; pooled from two independent experiments). **(D)** Initial blood monocyte gating strategy. **(E)** Representative flow cytometry plots of blood monocytes, pregated as depicted in D. **(F)** Counts of blood monocyte populations (n = 5–6 mice per group; two-way ANOVA, ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; pooled from two independent experiments). Error bars represent ±SEM.

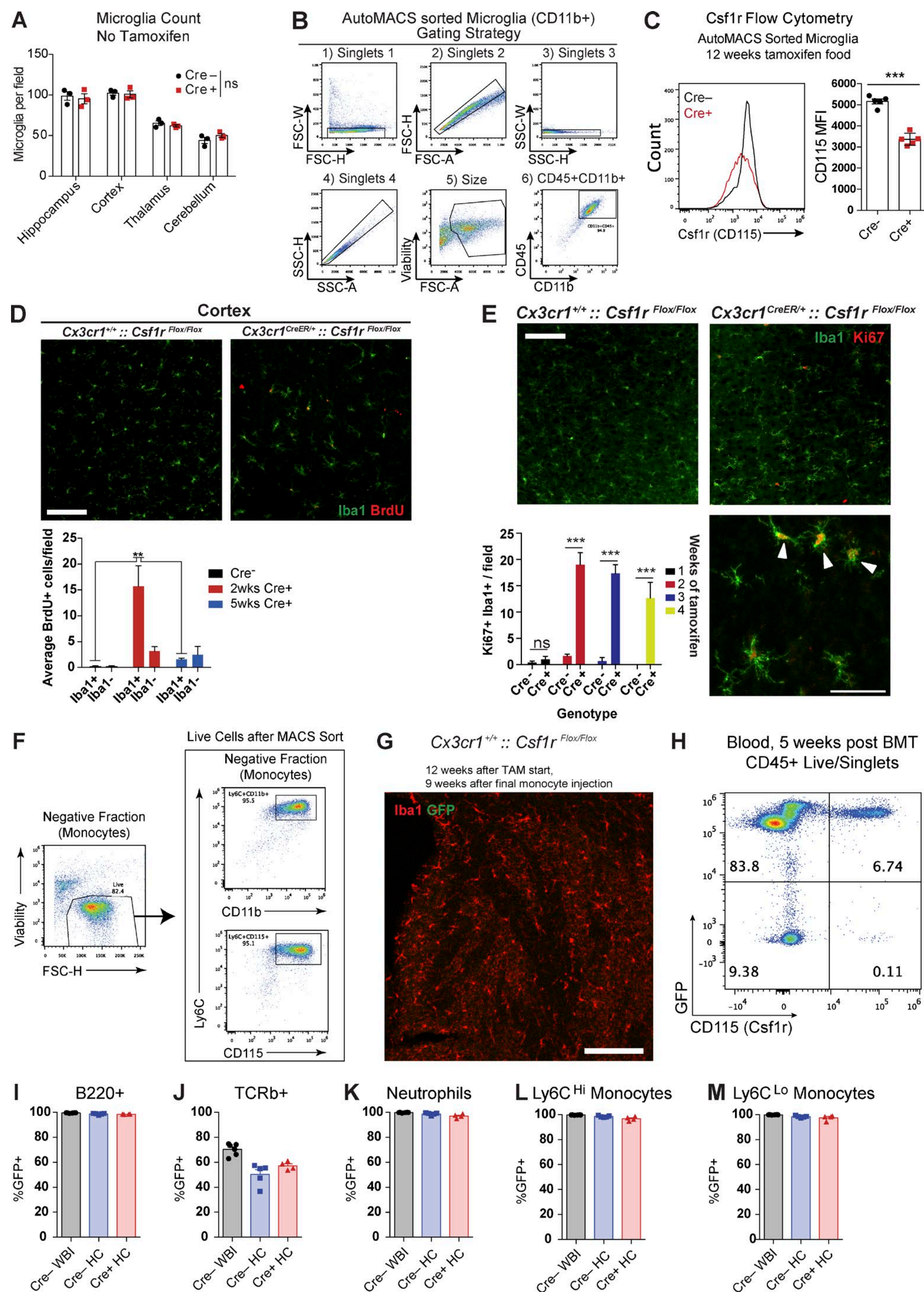

Figure S2. **Supplemental data supporting Fig. 1. (A)** Counts per field of microglia in *Cx3cr1*$^{CreER/+}$ or *Cx3cr1*$^{+/+}$*::Csf1r*$^{Flox/Flox}$ mice not treated with tamoxifen (*n* = 3 mice/group, two-way ANOVA with Tukey's multiple comparisons post-test, not significant [ns]). **(B)** Gating strategy for MACS-sorted microglia. Microglia were gated on singlets/live/size/CD45$^+$/CD11b$^+$ events. Cells were ~95% microglia. **(C)** Histogram and median fluorescence intensity (MFI) of CD115/Csf1r expressed on sorted microglia (*n* = 5 per group; two-sided Student's *t* test; ***, P < 0.001; representative of two independent experiments). **(D)** Representative images of *Cx3cr1*$^{CreER/+}$*::Csf1r*$^{Flox/Flox}$ mice and Cre-negative controls after 2 wk of tamoxifen treatment. Quantification of cells positive for BrdU and Iba1 (*n* = 2–3 mice per group, two-way ANOVA with Tukey's multiple comparisons test, **, P < 0.01; experiment performed once). Bar, 100 µm. **(E)** Representative images of *Cx3cr1*$^{CreER/+}$*::Csf1r*$^{Flox/Flox}$ mice and Cre-negative controls after 2 wk of tamoxifen treatment. Arrowheads point to Ki67$^+$ microglia. Microglia are green (Iba1$^+$) with red (Ki67$^+$) nuclei. Bar, 100 µm. Quantification of cells positive for both Ki67 and Iba1 (*n* = 3–4 mice per group, two-way ANOVA with Tukey's multiple comparisons test; ***, P < 0.001; experiment performed once). **(F)** Representative flow cytometry plots of MACS-sorted monocytes before adoptive transfer demonstrating ~95% pure CD45$^+$Ly6c^{hi}CD11b$^+$CD115$^+$ monocytes among live cells. **(G)** Representative image of *Cx3cr1*$^{+/+}$*::Csf1r*$^{Flox/Flox}$ cortex in mice injected with sorted monocytes 9 wk after the final monocyte injection (12 wk after tamoxifen initiation) per the experimental design outlined in Fig. 1 E, demonstrating no GFP+ peripheral cell engraftment. Bar, 200 µm. **(H)** Representative flow cytometry plot from blood of mice after BMT demonstrating repopulation with UBC-GFP$^+$ bone marrow cells. **(I–M)** Percentage of B cells (I), T cells (J), neutrophils (K), Ly6c^{hi} monocytes (L), and Ly6c^{lo} monocytes (M; *n* = 4–6 mice per group; experiment performed once). HC, head covered; WBI, whole-body irradiation. Error bars represent ±SEM.

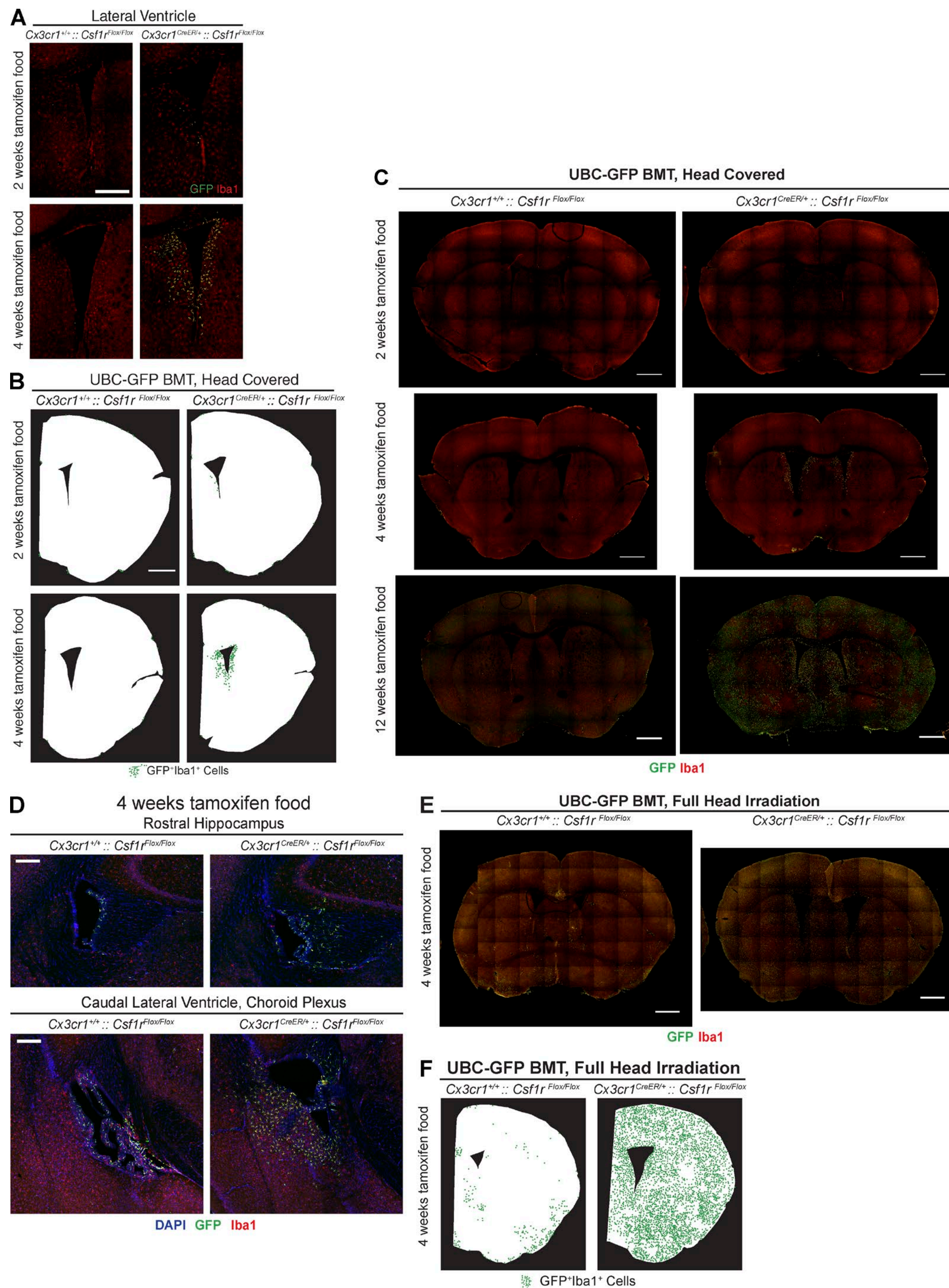

Figure S3. **Supplemental images demonstrating engraftment of beMφs in *Cx3cr1*$^{CreER/+}$*::Csf1r*$^{flox/flox}$ mice with or without head shielding and BMT.** **(A–D)** *Cx3cr1*$^{+/+}$*::Csf1r*$^{flox/flox}$ and *Cx3cr1*$^{CreER/+}$*::Csf1r*$^{flox/flox}$ mice were γ irradiated with head covering, repopulated with UBC-GFP bone marrow cells, and allowed to repopulate their immune systems for 5 wk. Mice were fed tamoxifen chow and euthanized after 2, 4, or 12 wk of tamoxifen chow. (A) Representative images of beMφs after 2 or 4 wk of tamoxifen chow. All macrophages/microglia are stained with Iba1, whereas beMφs are also GFP$^+$. Images are representative of *n* = 3–6 mice per group (pooled from two independent experiments). Bar, 500 µm. **(B)** Illustrations of beMφ locations after 2 or 4 wk of tamoxifen chow. Silhouettes of brain sections were generated on actual brain slices, and beMφ locations were marked with a green dot. Each dot represents a single GFP$^+$Iba1$^+$ beMφ. Images are representative of *n* = 3–6 mice per group, pooled from two independent experiments. Bar, 1,000 µm. **(C)** Representative images of whole brain slices stained for Iba1 and GFP after 2, 4, or 12 wk of tamoxifen chow. These images were used to generate illustrations in Fig. 1 Giii and Fig. S3 B. Bars, 1,000 µm. **(D)** Representative images of periventricular regions from *Cx3cr1*$^{+/+}$*::Csf1r*$^{flox/flox}$ and *Cx3cr1*$^{CreER/+}$*::Csf1r*$^{flox/flox}$ mice γ–irradiated with head covering and repopulated with UBC-GFP bone marrow cells 4 wk after initiation of tamoxifen chow. Images are representative of six mice per group, pooled from two independent experiments. Bars, 250 µm. **(E)** Representative images from *Cx3cr1*$^{+/+}$*::Csf1r*$^{flox/flox}$ and *Cx3cr1*$^{CreER/+}$*::Csf1r*$^{flox/flox}$ mice given whole-body γ–irradiation, repopulated with UBC-GFP bone marrow cells, and fed tamoxifen. 4 wk after tamoxifen initiation, brains were stained for GFP and Iba1. Images are representative of two mice per group (experiment performed once). Bars, 1,000 µm. **(F)** Representative placement of engrafting macrophages. Each green dot represents one GFP$^+$Iba1$^+$ cell.

Table S1 is provided as an Excel file. This table contains the differential gene expression analysis of the RNA-sequencing data generated from the three experimental models utilized in this study.

Table S2 is provided as an Excel file and shows comparatively enriched functions between beMφs and microglia.

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