Wang et al., https://doi.org/10.1083/jcb.201609093

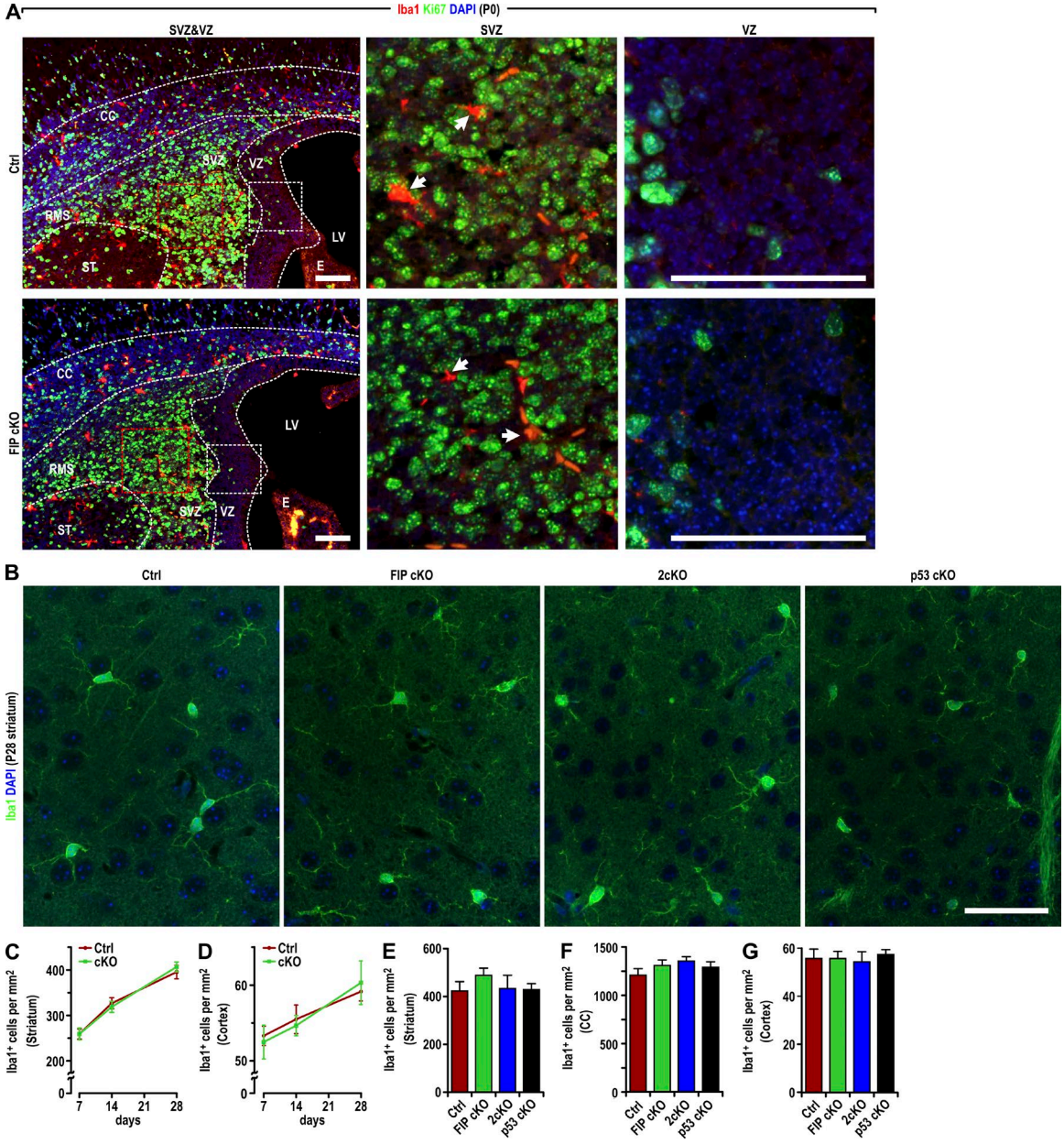

Figure S1. **Infiltration of microglia into SVZ/VZ of P0 mice and brain regions other than SVZ of P28 mice.** (A) Immunofluorescence of Ki67, Iba1, and DAPI in SVZ and ventricular zone (three mice each) of Ctrl and FIP cKO mice at P0. Arrows indicate Iba1+ cells. Dotted lines indicate the boundaries of the SVZ, ventricular zone and CC. Boxed areas in red lines and white lines are shown in detail for staining in SVZ (middle) and ventricular zone (right). (B) Immunofluorescence of Iba1 and DAPI in striatum (three mice each) in Ctrl, FIP cKO, 2cKO, and p53 cKO mice at P28. (C and D) Number of Iba1+ cells per square millimeter in striatum (C) and cerebral cortex (D) sections from Ctrl and FIP cKO mice at P0, P7, P14, and P28 (mean ± SEM; six mice each). (E–G) Number of Iba1+ cells per square millimeter in striatum (E), corpus callosum (F), and cerebral cortex (G) sections from Ctrl, FIP cKO, 2cKO, and p53 cKO mice at P28 (mean ± SEM; six mice each). CC, corpus callosum; E, ependymal layer; LV, lateral ventricle; RMS, rostral migratory stream; ST, striatum; SVZ, subventricular zone; VZ, ventricular zone. Bars: (A) 50 µm; (B) 20 µm.

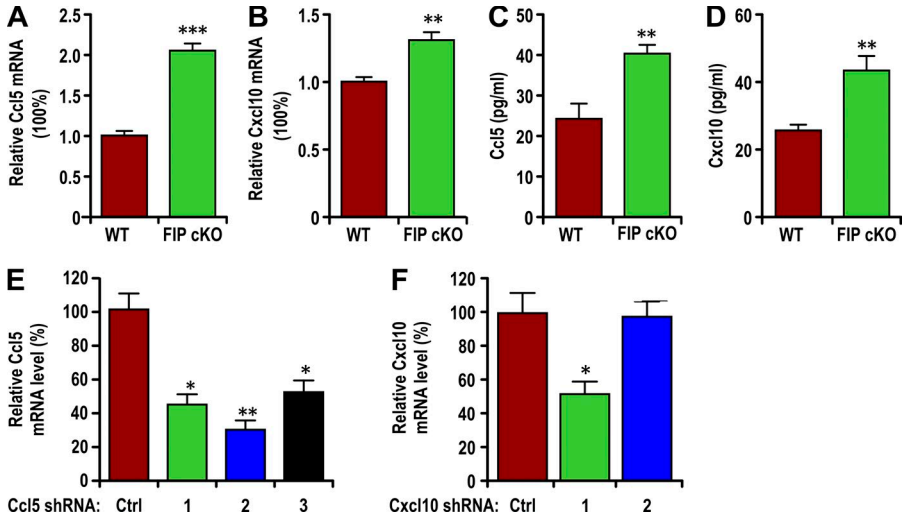

Figure S2. **Increased Ccl5 and Cxcl10 expression in FIP200-deficient astrocytes.** (A and B) mRNA levels of Ccl5 (A) and Cxcl10 (B) in primary astrocytes from Ctrl and FIP cKO mice (mean ± SEM; six mice each). (C and D) Concentration of Ccl5 (C) and Cxcl10 (D) in conditioned medium of astrocytes (normalized to 1 mg total protein lysate of astrocytes) from Ctrl and FIP cKO (mean ± SEM; six mice each). (E and F) Relative mRNA levels of Ccl5 (E) and Cxcl10 (F) in neurospheres infected with recombinant lentiviruses encoding Ctrl shRNA, Ccl5 shRNAs (E), and Cxcl10 shRNAs (F; mean ± SEM; three mice each). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

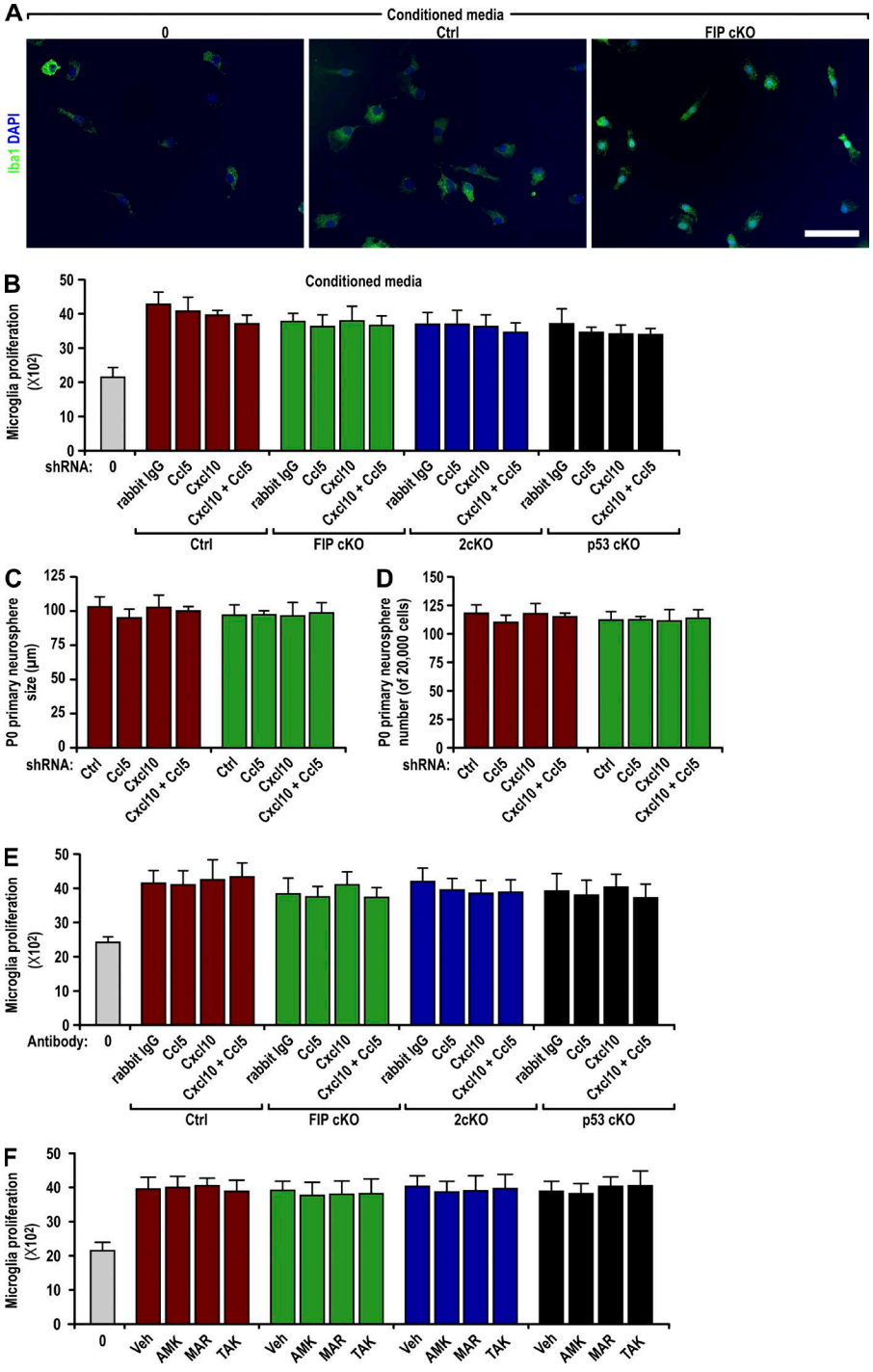

Figure S3. **The effect of Ccl5- and Cxcl10-depleting antibodies, shRNAs, and their receptor antagonists on microglia proliferation and NSCs maintenance in vitro.** (A) Immunofluorescence of Iba1 with DAPI in microglia cultured with naive media (0) or conditioned media from neurospheres of Ctrl and FIP cKO mice. Bar, 20 μm. (B) Number of microglia cultured with naive media (0) or conditioned media from neurospheres of Ctrl, FIP cKO, 2cKO, and p53 cKO mice infected with recombinant lentivirus encoding Ctrl shRNA, Cxcl10 shRNA, Ccl5 shRNA, or a combination of Ccl5 and Cxcl10 shRNAs (mean ± SEM; six mice each). (C and D) Size (C) and number (D) of neurospheres from P0 Ctrl and FIP cKO mice infected with recombinant lentivirus encoding Ctrl shRNA, Ccl5 shRNA, Cxcl10 shRNA, or combination of Ccl5 and Cxcl10 shRNAs (mean ± SEM; three mice each). (E) Number of microglia cultured with naive media or conditioned media from neurospheres of Ctrl, FIP cKO, 2cKO, and p53 cKO mice treated with irrelevant antibody, Cxcl10 antibody, Ccl5 antibody, or a combination of Cxcl10 and Ccl5 antibodies (mean ± SEM; six mice each). (F) Number of microglia cultured with naive media or conditioned media from neurospheres of Ctrl, FIP cKO, 2cKO, and p53 cKO mice treated with vehicle (Veh), AMG-487 (AMG), maraviroc (MAR), or TAK-779 (TAK; mean ± SEM; three mice each).

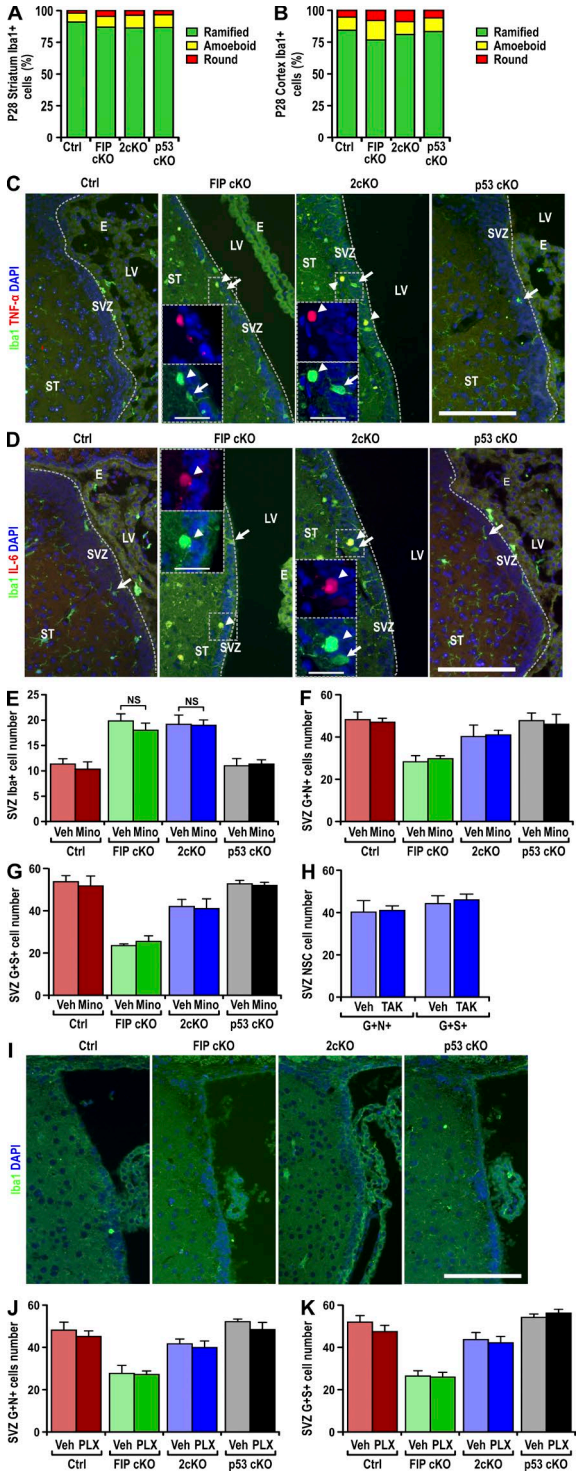

Figure S4. **Minocycline and PLX3397 did not affect NSCs maintenance in vitro.** (A and B) Percentage of ramified, round, and amoeboid microglia in striatum (A) and cortex (B) of Ctrl, FIP cKO, 2cKO and p53 cKO mice at P28 are shown (>300 cells from three mice for each). (C and D) Immunofluorescence of TNF, Iba1 with DAPI (C), and IL-6, Iba1 with DAPI (D) in the SVZ of Ctrl, FIP cKO, 2cKO, and p53 cKO mice at P28. Arrowheads indicate TNF-positive (C) or IL-6–positive (D) microglia, whereas arrows indicate Iba1 single-positive cells. Boxed areas in white lines are shown in detail for staining in SVZ. TNF-positive or IL-6–positive microglia were round or amoeboid in FIP cKO and 2cKO samples. Dotted lines indicate the boundary of the SVZ with the lateral ventricle. (E) Number of Iba1$^+$ microglia per SVZ section of Ctrl, FIP cKO, 2cKO, and p53 cKO mice treated with PBS vehicle (Veh) or minocycline (Mino; mean ± SEM; three mice each). (F and G) Number of GFAP$^+$ and Nestin$^+$ cells (F) or GFAP$^+$ and Sox2$^+$ cells (G) per SVZ section of P28 Ctrl, FIP cKO, 2cKO, and p53 cKO mice treated with vehicle (Veh) or minocycline (Mino; mean ± SEM; three mice each). (H) Number of GFAP$^+$ and Nestin$^+$ cells (left two columns) or GFAP$^+$ and Sox2$^+$ cells (right two columns) per SVZ section of 2cKO mice treated with vehicle (Veh) or TAK-779 (TAK; mean ± SEM; three mice each). (I) Immunofluorescence of Iba1 and DAPI in SVZ (four mice each) of Ctrl, FIP cKO, 2cKO, and p53 cKO mice treated with vehicle (Veh) and PLX3397 (PLX) at P28. (J and K) Number of GFAP$^+$ and Nestin$^+$ cells (J) or GFAP$^+$ and Sox2$^+$ cells (K) per SVZ section of P28 Ctrl, FIP cKO, 2cKO, and p53 cKO mice treated with vehicle (Veh) or PLX3397 (PLX; mean ± SEM; four mice each). E, ependymal layer; LV, lateral ventricle; NS, not significant; ST, striatum; SVZ, subventricular zone. Bars: 50 µm; (C and D, insets) 20 µm.

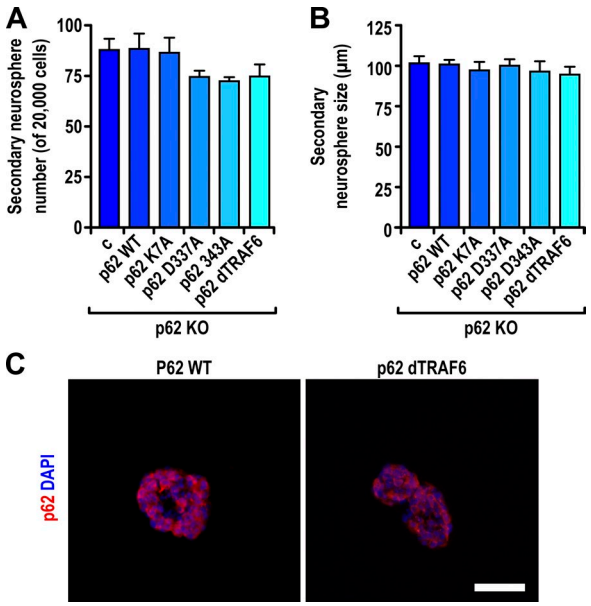

Figure S5. **Effect of GFP-p62-wt and various p62 mutations on NSCs from p62 KO mice.** (A and B) Number (A) and size (B) of secondary p62 KO retrovirus-infected neurospheres encoding GFP-p62-wt and various p62 mutations as indicated (mean ± SEM; five mice each). (C) Immunofluorescence of p62 and DAPI in FIP/p62 2cKO retrovirus-infected neurospheres encoding p62 wild type or p62-dTRAF6 (three mice each). Bar, 20 µm.