

Supplemental information

**Microglia contribute to the postnatal development
of cortical somatostatin-positive inhibitory cells
and to whisker-evoked cortical activity**

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Figure S1

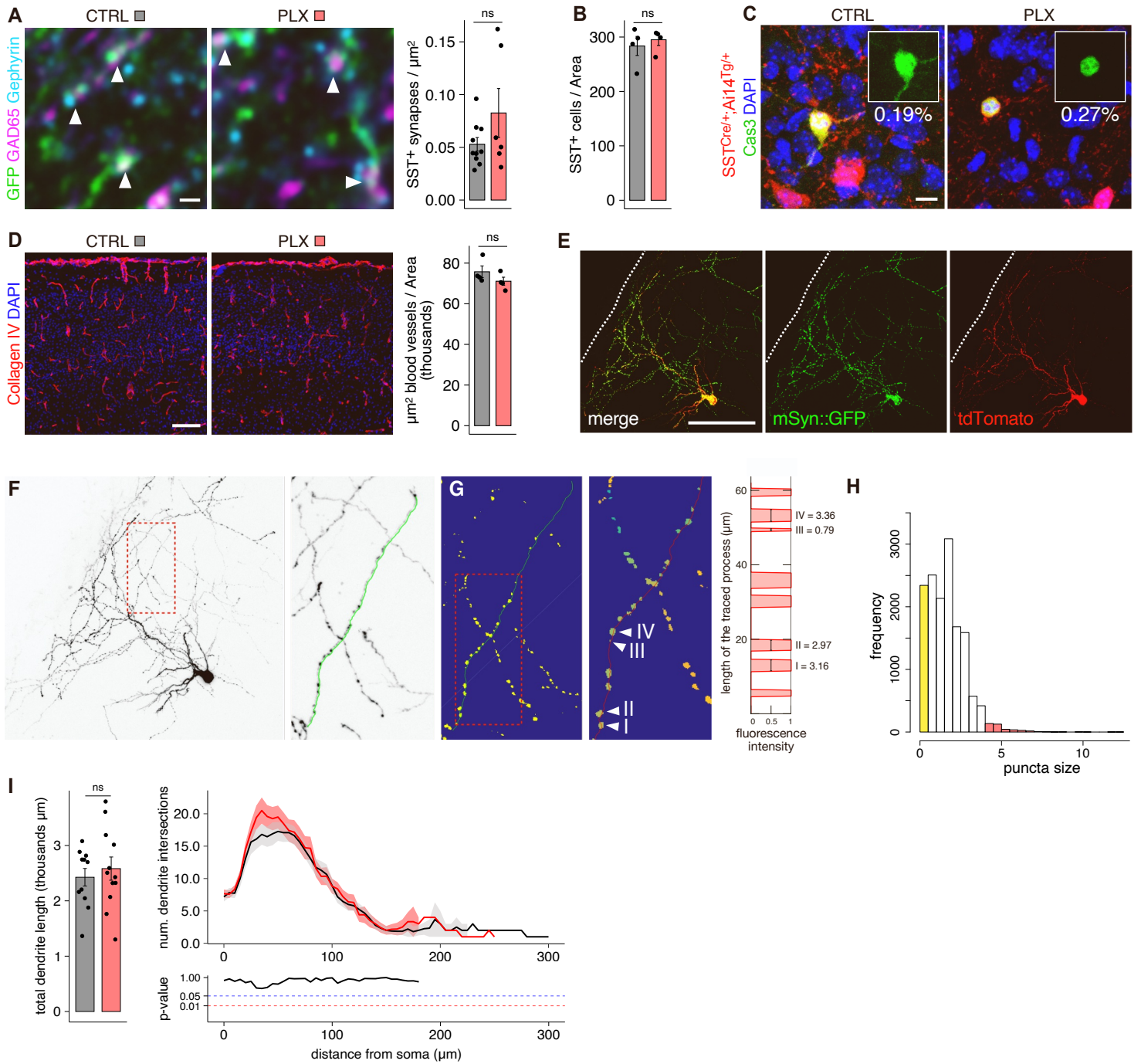


Figure S1, related to Figure 1 in the main text: **A.** Images and quantification of SST⁺ synapses (identified as GAD65⁺ and Gephyrin⁺ puncta encased in GFP⁺ axons labeled using a SST^{Cre/+};RCE mouse) in S1 L1 at P30 (scale bar = 1 μm); arrowheads indicate colocalization (each dot is a brain; CTRL n = 10, mean ± SEM = 0.053 ± 0.006; PLX n = 6, mean ± SEM = 0.082 ± 0.023; T-test p = 0.26). **B.** SST⁺ cells counted per field of view (from L1 to L6) at P10 (each dot is a brain; CTRL n = 4, mean ± SEM = 283.616 ± 17.482; PLX n = 4, mean ± SEM = 295.033 ± 10.552; T-test p = 0.6). **C.** Cleaved Cas3 immunofluorescent staining in L2/3 of a section from an SST^{Cre/+};Ai14^{Tg/+} mouse at P7. Percentages indicate SSTCre-fate mapped cells that are Cas3⁺ (CTRL = 3 brains, mean ± SEM = 0.187 ± 0.005; PLX = 3 brains, mean ± SEM = 0.269 ± 0.043; T-test p = 0.19) (scale bar = 10 μm). **D.** Collagen IV immunofluorescent staining of S1 at P10 (scale bar = 100 μm) and quantification of vasculature area (collagen IV area) measured as μm² per field of view (each dot is a brain; CTRL n = 4, mean ± SEM = 75739.18 ± 2842.839; PLX n = 4, mean ± SEM = 71088.94 ± 1968.866; T-test p = 0.23). **E.** Example of an electroporated cell using a pCAG_loxP-STOP-loxP-tdTomato-T2A-mSynaptophysin::GFP plasmid (scale bar = 100 μm; pia surface is indicated by the dotted line). **F and G.** Sequential steps of the MATLAB program to measure green puncta density (details are reported in the STAR methods). In panel F, an example of a traced axon marked in green is shown. In figure G, the puncta along the traced axon are mapped and segmented. On the right, the output of the MATLAB program shows fluorescent intensity distribution over axonal length. Puncta size is measured at half of the intensity (sizes of puncta I-IV are reported). **H.** The histogram shows distribution of puncta sizes. The yellow column includes puncta smaller than 0.5 μm; red columns include puncta measured as “outliers”. **I.** Total length of traced dendrites (each dot is a cell; CTRL n = 11, mean ± SEM = 2426.203 ± 158.356; PLX n = 12, mean ± SEM = 2582.161 ± 209.96; T-test p = 0.56) and Sholl analysis (the plot shows mean ± SEM; CTRL n = 11 cells; PLX n = 12 cells; T-test p-values are plotted below; two-way ANOVA RM test from 0 to 150 μm p = 0.66; CTRL group from figure 2G, PLX group from figure 2H). (CTRL = control)

Figure S2

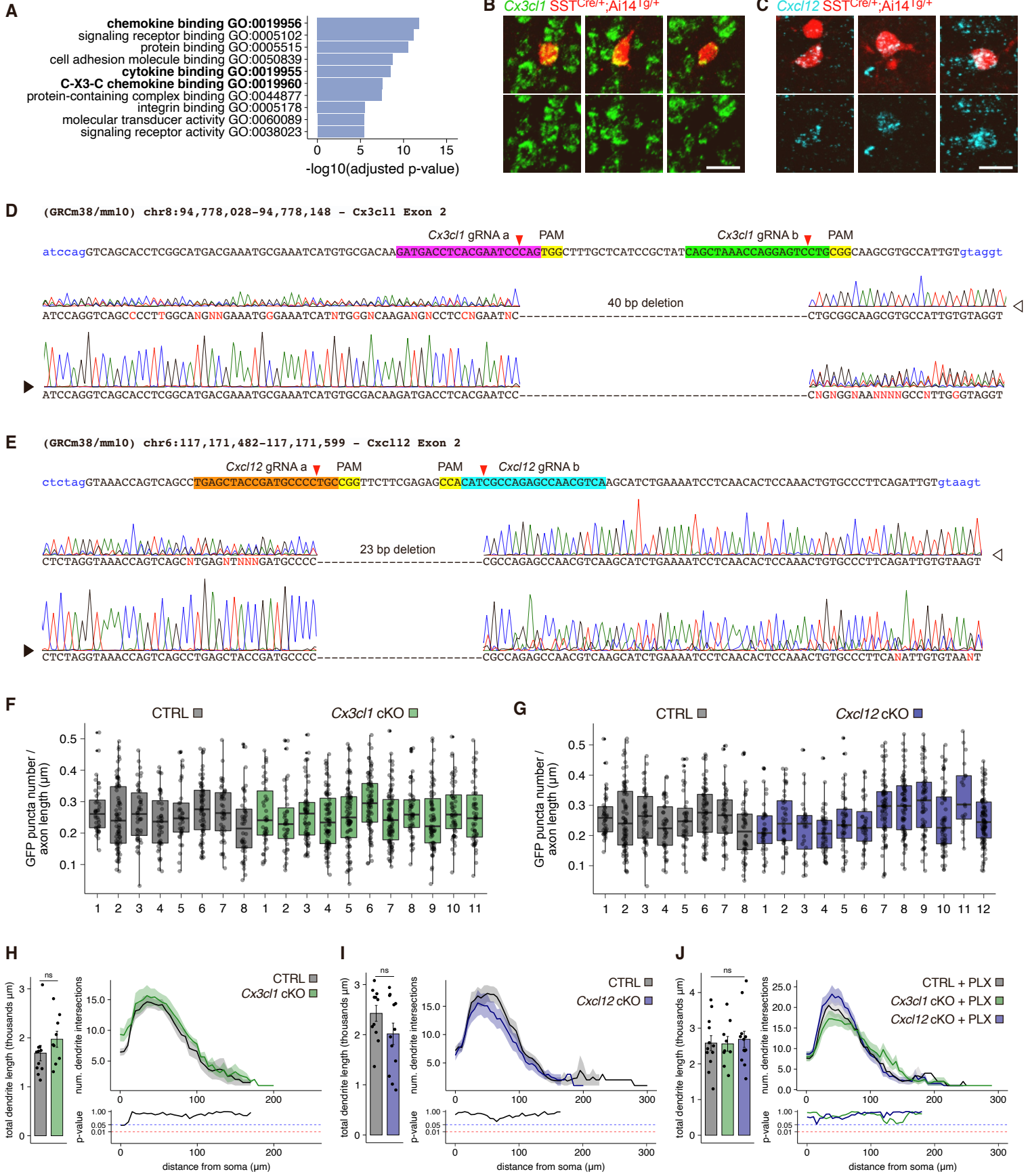


Figure S2, related to Figure 2 in the main text: **A.** Gene ontology (GO) enrichment analysis (molecular function class) performed with gProfiler (Reimand et al., 2016); cytokine gene ontology terms are in bold. **B and C.** *In situ* hybridization showing *Cx3cll* (green) and *Cxcl12* (cyan) mRNA expression in SST^{Cre/+};Ai14^{Tg/+} cells (stained with anti tdTomato; three examples each) at P11 (scalebar = 20 μ m). **D and E.** Sanger sequencing result alignments of *Cx3cll* and *Cxcl12* targeted exon (see STAR methods for details). Sequencing has been done in both directions as indicated by full and empty arrowheads. Red arrowheads indicate the Cas9 cutting sites. **F and G.** Synaptic densities per cell. Each boxplot is a cell. Each dot is the synaptic density of a single traced axonal branch for any given cell. **H.** Total length of traced dendrites (each dot is a cell; CTRL n = 11, mean $\pm$ SEM = 1686.742 $\pm$ 157.014; *Cx3cll* cKO n = 9, mean $\pm$ SEM = 1970.836 $\pm$ 163.926; Wilcoxon test p = 0.11) and Sholl analysis (the plot shows mean $\pm$ SEM; T-test p-values are plotted below; two-way ANOVA RM test from 0 to 125 μ m p = 0.99). **I.** Total length of traced dendrites (each dot is a cell; CTRL n = 11; mean $\pm$ SEM = 2426.203 $\pm$ 158.356; *Cxcl12* cKO n = 12; mean $\pm$ SEM = 2017.468 $\pm$ 214.968; T-test p = 0.14) and Sholl analysis (T-test p-values are plotted below; two-way ANOVA RM test from 0 to 140 μ m p = 0.73). **J.** Total length of traced dendrites (each dot is a cell; CTRL+PLX n = 12, mean $\pm$ SEM = 2582.161 $\pm$ 209.96; *Cx3cll* cKO+PLX n = 9, mean $\pm$ SEM = 2561.333 $\pm$ 206.486; *Cxcl12* cKO+PLX n = 12, mean $\pm$ SEM = 2689.306 $\pm$ 226.907; CTRL+PLX vs *Cx3cll* cKO+PLX T-test p = 0.94; CTRL+PLX vs *Cxcl12* cKO+PLX T-test p = 0.73; one-way ANOVA test p = 0.9) and Sholl analysis (T-test p-values are plotted below; two-way ANOVA RM test from 0 to 167 μ m p = 0.06). (CTRL = control; cKO = conditional knock-out)

Figure S3

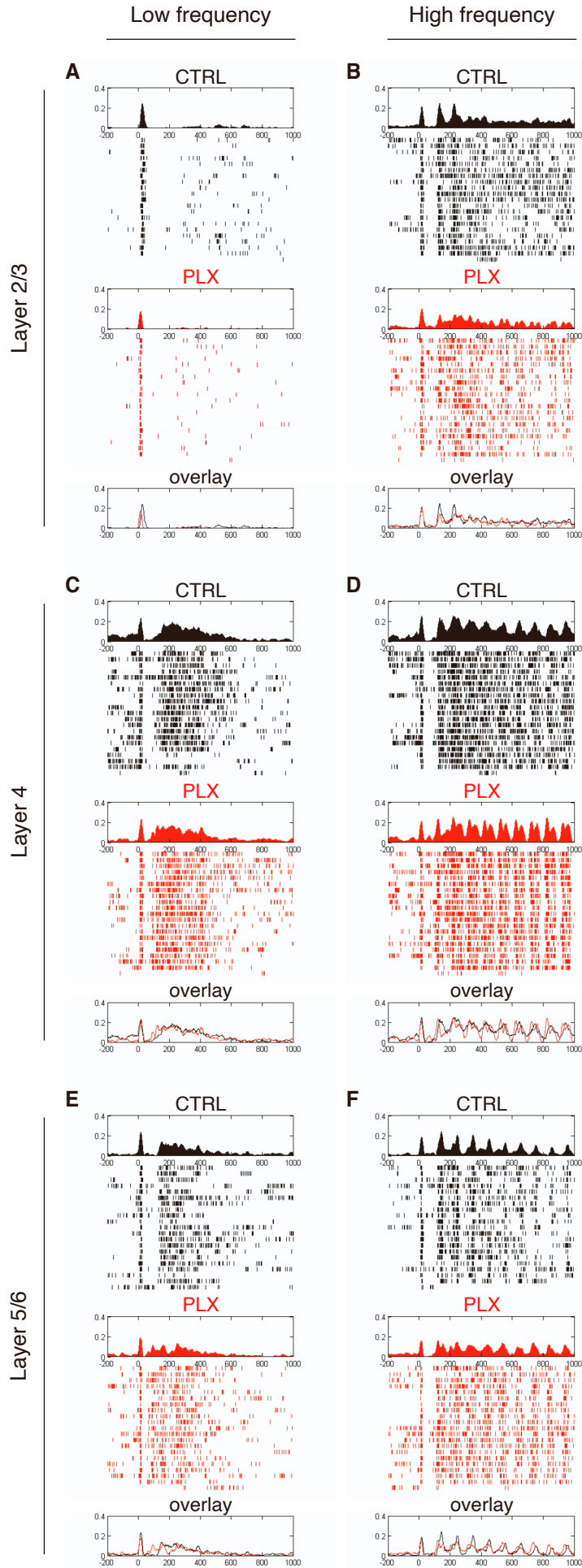


Figure S3, related to Figure 3 in the main text: A-F. Representative examples of raster plots of silicon probe recordings (black is control, red is PLX treated animals-microglia depleted brains). (CTRL = control)

Table S1, related to Figures 1-3 in the main text: The table reports mean $\pm$ SEM exact values.

Figure	n	mean $\pm$ SEM
Figure 1A	P4 n = 3 mice	0.002 $\pm$ 0.001
Figure 1A	P8 n = 6 mice	0.018 $\pm$ 0.004
Figure 1A	P11 n = 3 mice	0.019 $\pm$ 0.002
Figure 1B	P10 n = 6 mice	5.076 $\pm$ 0.678
Figure 1B	P12 n = 3 mice	8.954 $\pm$ 1.285
Figure 1B	P15 n = 6 mice	9.670 $\pm$ 1.211
Figure 1D	P10 CTRL n = 4 mice	0.047 $\pm$ 0.006
Figure 1D	P10 PLX n = 7 mice	0.030 $\pm$ 0.007
Figure 1D	P12 CTRL n = 3 mice	0.047 $\pm$ 0.009
Figure 1D	P12 PLX n = 5 mice	0.041 $\pm$ 0.007
Figure 1D	P15 CTRL n = 6 mice	0.030 $\pm$ 0.003
Figure 1D	P15 PLX n = 4 mice	0.044 $\pm$ 0.004
Figure 1F	P10 CTRL n = 9 cells	0.223 $\pm$ 0.010
Figure 1F	P10 PLX n = 9 cells	0.221 $\pm$ 0.017
Figure 1F	P15 CTRL n = 17 cells	0.162 $\pm$ 0.012
Figure 1F	P15 PLX n = 14 cells	0.212 $\pm$ 0.008
Figure 1H	CTRL n = 11 cells	13330.21 $\pm$ 1559.138
Figure 1H	PLX n = 12 cells	11702.95 $\pm$ 1200.689
Figure 1I	CTRL n = 15 cells	218.733 $\pm$ 27.578
Figure 1I	PLX n = 15 cells	355.533 $\pm$ 49.706
Figure 2E	CTRL n = 8 cells	0.230 $\pm$ 0.009
Figure 2E	<i>Cx3cl1</i> cKO n = 11 cells	0.237 $\pm$ 0.007
Figure 2E	<i>Cxcl12</i> cKO n = 12 cells	0.245 $\pm$ 0.010
Figure 2F	CTRL n = 11 cells	7496.511 $\pm$ 497.372
Figure 2F	<i>Cx3cl1</i> cKO n = 9 cells	9561.762 $\pm$ 615.668
Figure 2G	CTRL n = 11 cells	13330.21 $\pm$ 1559.138
Figure 2G	<i>Cxcl12</i> cKO n = 12 cells	9939.092 $\pm$ 942.275
Figure 2H	CTRL+PLX n = 12 cells	11702.95 $\pm$ 1200.689
Figure 2H	<i>Cx3cl1</i> cKO+PLX n = 9 cells	13214.51 $\pm$ 1458.866
Figure 2H	<i>Cxcl12</i> cKO+PLX n = 12 cells	10199.99 $\pm$ 1137.148
Figure 2M	CTRL n = 6 cells from 4 mice	50.467 $\pm$ 6.020
Figure 2M	<i>Cx3cl1</i> cKO n = 11 cells from 6 mice	47.321 $\pm$ 10.129
Figure 2M	<i>Cxcl12</i> cKO n = 7 cells from 4 mice	38.346 $\pm$ 8.960
Figure 2N	CTRL n = 9 cells	0.315 $\pm$ 0.069
Figure 2N	<i>Cx3cl1</i> cKO n = 8 cells	0.729 $\pm$ 0.089
Figure 2N	<i>Cxcl12</i> cKO n = 7 cells	0.571 $\pm$ 0.174
Figure 3A	CTRL n = 8 mice	12.630 $\pm$ 4.014
Figure 3A	PLX n = 4 mice	15.093 $\pm$ 2.324

Figure 3B	CTRL n = 8 mice	15.100 ± 3.724
Figure 3B	PLX n = 4 mice	21.077 ± 3.616
Figure 3C	CTRL n = 8 mice	8.051 ± 1.807
Figure 3C	PLX n = 4 mice	12.825 ± 2.563
Figure 3D'	CTRL n = 8 mice	27.319 ± 5.030;
Figure 3D'	PLX n = 4 mice	11.908 ± 0.617;
Figure 3D''	CTRL n = 8 mice	15.901 ± 4.824
Figure 3D''	PLX n = 4 mice	5.234 ± 1.048
Figure 3E'	CTRL n = 8 mice	34.468 ± 4.008
Figure 3E'	PLX n = 4 mice	21.870 ± 4.760
Figure 3E''	CTRL n = 8 mice	26.540 ± 4.083
Figure 3E''	PLX n = 4 mice	17.150 ± 3.564
Figure 3F'	CTRL n = 8 mice	28.052 ± 4.483
Figure 3F'	PLX n = 4 mice	14.347 ± 2.832
Figure 3F''	CTRL n = 8 mice	18.803 ± 4.001
Figure 3F''	PLX n = 4 mice	10.998 ± 2.369
Figure 3G'	CTRL n = 8 mice	54.160 ± 6.065
Figure 3G'	PLX n = 4 mice	36.906 ± 8.660
Figure 3G''	CTRL n = 8 mice	41.825 ± 6.140
Figure 3G''	PLX n = 4 mice	22.142 ± 5.054
Figure 3H'	CTRL n = 8 mice	71.721 ± 9.801
Figure 3H'	PLX n = 4 mice	57.181 ± 14.525
Figure 3H''	CTRL n = 8 mice	63.745 ± 10.118
Figure 3H''	PLX n = 4 mice	51.788 ± 13.143
Figure 3I'	CTRL n = 8 mice	55.657 ± 8.533
Figure 3I'	PLX n = 4 mice	39.548 ± 14.963
Figure 3I''	CTRL n = 8 mice	46.063 ± 7.701
Figure 3I''	PLX n = 4 mice	34.626 ± 13.965