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Supplementary Information for

Permanent neuroglial remodeling of the retina following infiltration of CSF1R-inhibition resistant peripheral monocytes.

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Fig. S1. Microglia repopulation after PLX5622 treatment occurs primarily by peripheral CX3CR1^{+} cells.

(A-C) Flat mount retinas from busulfan myelodepleted *CX3CR1^{CreERT2}::ROSA26-tdTomato* bone marrow transfer mice stained with Iba1-FITC 2 months after cessation PLX5622 treatment. Cre-Lox recombination was induced with 5 daily tamoxifen injections, 1 week prior to imaging. Two months after cessation of PLX5622 treatment peripheral tdTomat$^{+positive}$ FITC$^{+positive}$ and non-peripheral tdTomat$^{-negative}$ FITC$^{+positive}$ CX3CR1^{+} cells repopulate the retinal. (D) Magnification showing ramified tdTomat$^{-negative}$ FITC$^{+positive}$ microglia and ameboid tdTomat$^{+positive}$ FITC$^{+positive}$ peripheral monocyte repopulating the retina. (E) Representative schematic of flat mount retina as used for confocal imaging; center white circle represents the optic nerve head, red lines the retinal vessels, and green dots the peripheral monocytes. (F) Flow cytometry analysis of the retina 2 months after cessation of PLX5622 treatment confirms that microglia repopulation occurs primarily from peripheral CX3CR1^{+} cells with a smaller contribution (7%) from non-peripheral CX3CR1^{+} microglia progenitors. (G) In the setting of ocular injury, the contribution of non-peripheral CX3CR1^{+} microglia progenitors in retinal repopulation increases to 20%. n=3 mice per group. (A-D) Scale bar: 100µm.

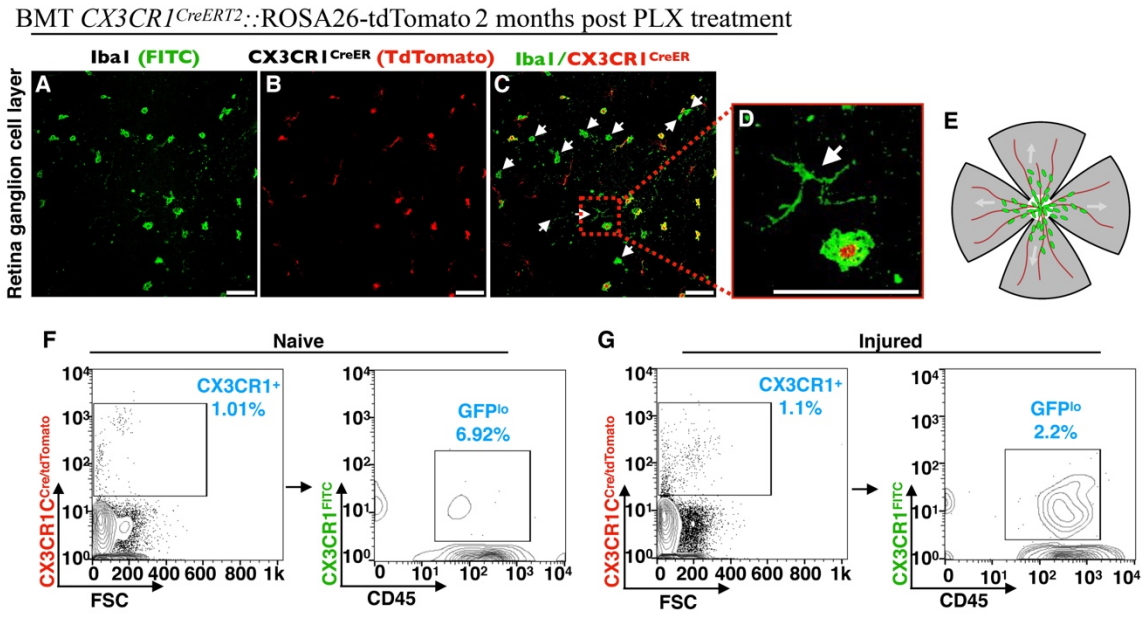


Fig. S2. PLX5622-resistant CX3CR1^{+} cells have low or no expression of CSF1R. (A) Experimental setup to assess CSF1R expression of PLX5622-resistant retinal CX3CR1^{+} cells. (B) Following ocular injury, PLX5622-resistant CX3CR1^{+} cells have low or no expression of CSF1R while MHC-II expression is elevated. n=3 mice per group.

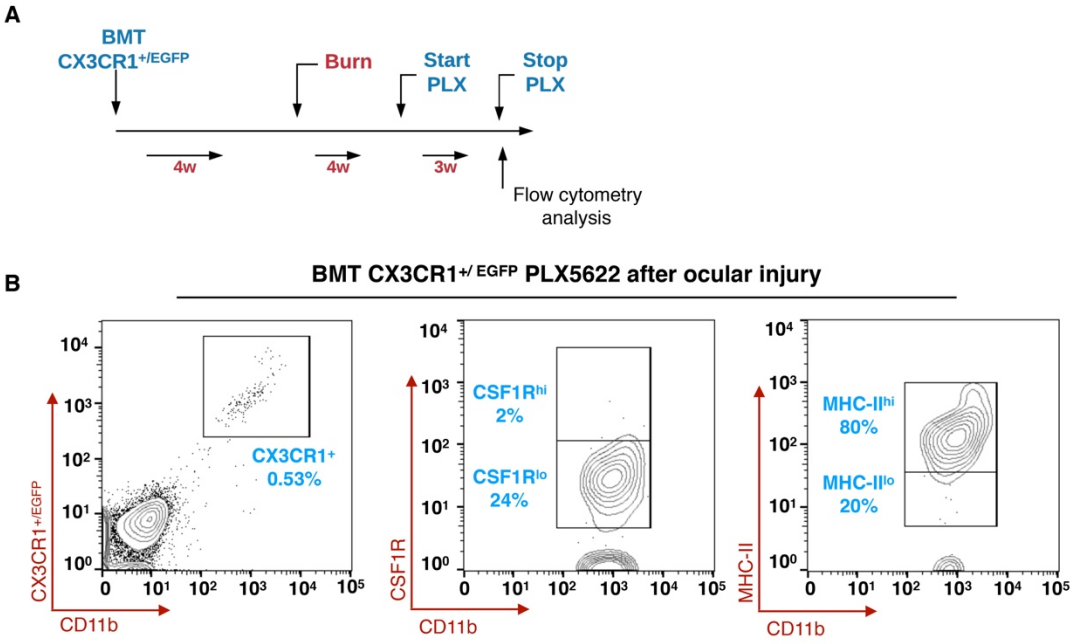


Fig. S3. Progressive neuroretinal degeneration after ocular injury.

(A-D) Retinal flat mounts showing progressive loss of β3-tubulin tissue 2, 4 months, and 2.5 years after ocular injury as compared to naïve eyes. P-Phenylenediamine (PPD) staining of optic nerve sections of (E) naïve mice or of mice (F) 3 months and (G) 2 years after ocular injury showing progressive axonal degeneration and loss of myelin (white arrows). n=3 mice per group. (A-D) Scale bar: 100μm. (E-G) Scale bar: 25μm.

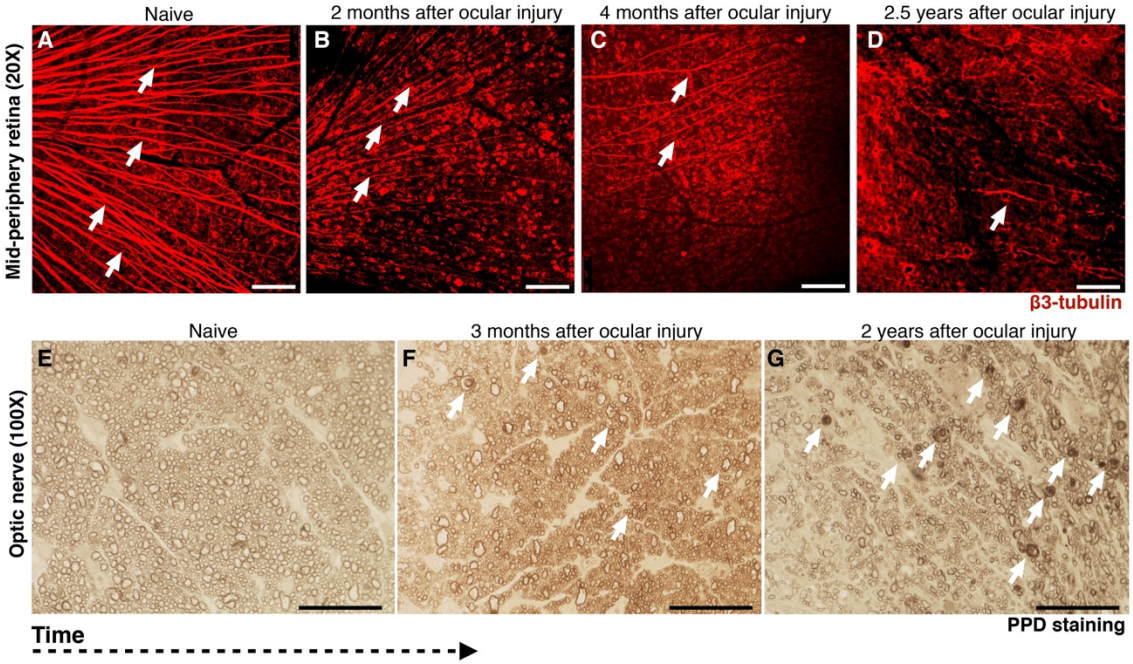
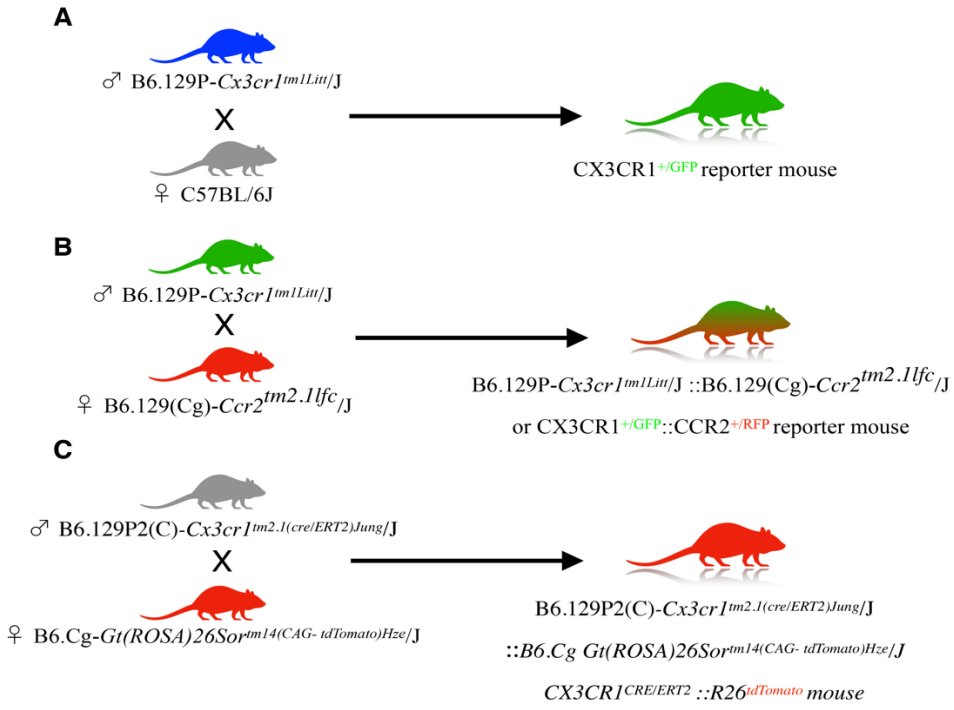


Fig. S4. Mouse breeding schemes

(A) CX3CR1$^{+/EGFP}$ (B) CX3CR1$^{+/EGFP}$::CCR2$^{+/RFP}$, and (C) CX3CR1$^{CRE/ERT2}$::R26tdTomato reporter mice were generated in house using the depicted breeding schemes. (D) Explanation of mouse strain nomenclatures, as provided by Jackson Laboratory.



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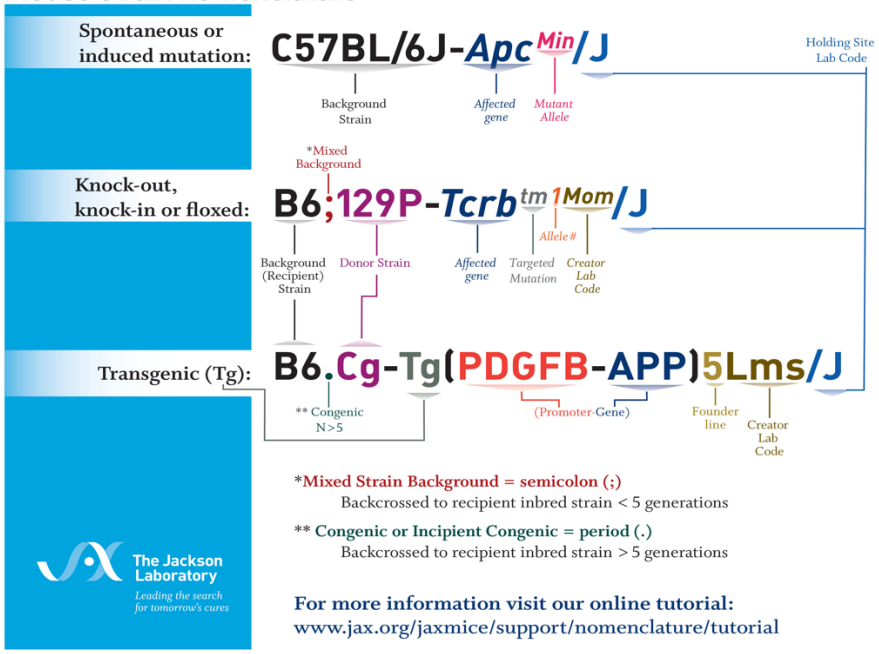

Movie S1. Peripheral CX3CR1^{+} cells engraft permanently in the retina after corneal injury.

CX3CR1$^{+/EGFP}$ bone marrow chimera model 16 months after cell transfer. In the control eye, very few blood-derived CX3CR1$^{+/EGFP}$ cells that are present into the retina are localized around major retinal vessels and the optic nerve head. These cells are primarily positioned in the ganglion cell layer and do not migrate deeper into the tissue (left video panel). In contrast, after acute ocular surface injury significant number of peripheral CX3CR1$^{+/EGFP}$ monocytes infiltrate and permanently engraft into the retina. Sixteen months after the injury, peripherally engrafted CX3CR1$^{+/EGFP}$ cells have occupied the three distinct retinal microglia strata and transformed to ramified cells (right video panel). The morphology and arrangement of these cells resembles that of yolk-sac derived retinal microglia.

Movie S2. Three-dimensional reddening of peripherally engrafted CX3CR1^{+} monocytes engulfing β3-tubulin^{+} neuronal tissue 20 weeks after ocular injury.

There dimensional rendering of confocal scans showing peripherally engrafted CX3CR1^{+} cells (semitransparent green) engulfing β3-tubulin^{+} cells (red) 20 weeks after acute ocular surface injury (right video panel). In contrast, CX3CR1^{+} microglia of naive eye do not engulf nor internalize β3-tubulin^{+} tissue, although they come in contact with β3-tubulin^{+} tissue (left video panel).