

Fig. S1. Cleaved caspase-3 expression in the ganglion cell layer after optic nerve transection.

(A) Cleaved caspase-3 (magenta) expression in *isl2b*:GFP<sup>+</sup> (green) flat-mount retinae at 7dpi. DAPI (blue) counterstain for nuclei. (B) Quantification of the percentage of cleaved caspase-3<sup>+</sup> RGCs in the ganglion cell layer after ONT(n=6/condition). Shown are mean±SEM; \*\*p<0.01; Mann-Whitney test. Data derived from 3 experiments. Scale bars = 50µm and 150µm on zoomed images.

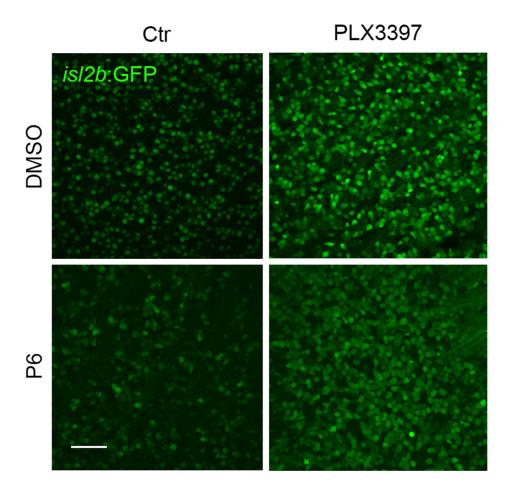


Fig. S2. RGC survival after Jak inhibition and macrophage/microglia depletion.

Flat-mount images of isl2b:GFP retinae after intravitreal injection of P6 or DMSO +/-PLX3397 treatment at 7dpi. Data derived from 3 experiments. Scale bars =  $50\mu$ m.

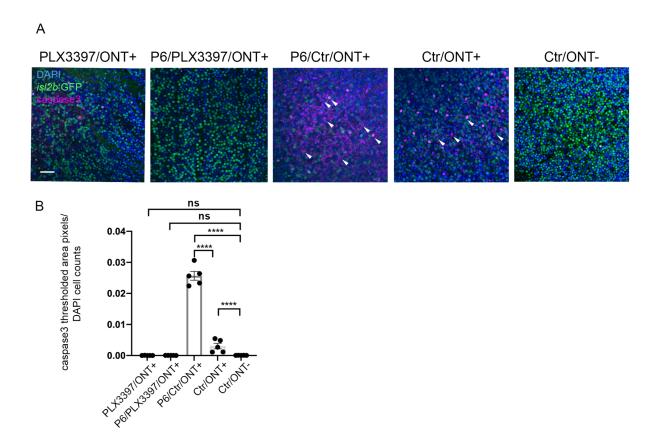


Fig. S3. Cleaved caspase-3 staining after JAK inhibition and macrophage/microglia depletion.

(A) Cleaved caspase-3 (magenta) expression in *isl2b*:GFP<sup>+</sup> (green) flat-mount retinae at 7dpi. DAPI (blue) counterstain for nuclei. Scale bar=50 µm. (B) Quantification of the ratio of the thresholded area pixels of cleaved caspase-3<sup>+</sup> staining to counts of DAPI cells in the ganglion cell layer (n=5/condition). Shown are mean±SD; \*\*\*\*p<0.0001; Kruskal Wallis ANOVA with Dunn's multiple comparisons. Data derived from 2 experiments.

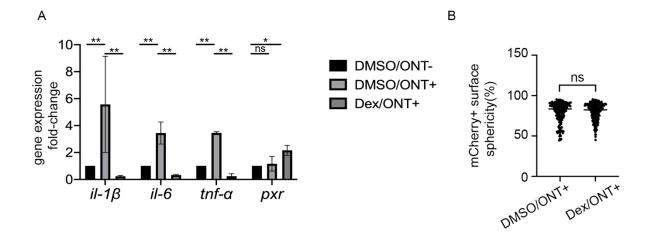


Fig. S4. Quantification of sphericity of macrophages/microglia and qRT-PCR after dexamethasone application.

(A) qRT-PCR for *il1-\beta*, *il6*, *tnf-\alpha* and a downstream transcriptional target of dexamethasone *pxr* (Pascussi et al. 2000) expression in retinae treated with 10µM dexamethasone or 0.05%DMSO at 7dpi,expression fold change of above genes in DMSO/ONT- retinae were normalized to 1 as the control. *gapdh* were used as the housekeeping gene. Shown are mean±SD; \*\*p<0.01; \*p<0.05. Kruskal Wallis ANOVA with Dunn's multiple comparisons. Data derived from 3 experiments. (B) Quantification of the sphericity of mCherry+ surfaces after dexamethasone application at 7dpi. Violin plot showing no significant differences in sphericity of mCherry+ macrophages/microglia in Dex/ONT+ retinae compared to DMSO/ONT+ controls (n=180 in Dex/ONT+ and n=198 in DMSO/ONT+); p=0.4235, unpaired t-test with Welch's correction.

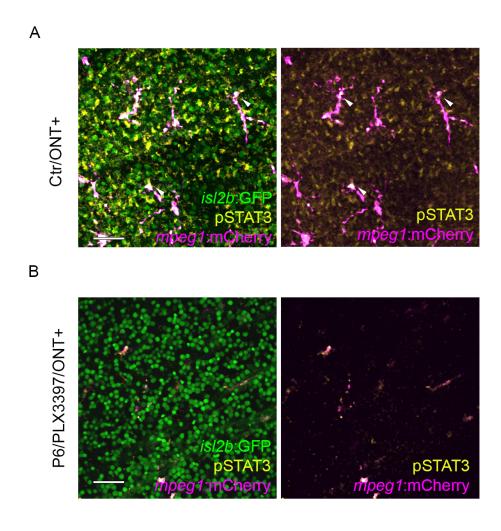


Fig. S5. JAK/STAT pathway activity in macrophages/microglia and after P6/PLX3397 treatment.

(A) Flat-mount images showing pSTAT3 staining (yellow) on *isl2b*:GFP;*mpeg1*:mCherry (*isl2b*:GFP – green; *mpeg1*:mCherry - magenta) retinae at 7dpi. Image to right has *isl2b*:GFP channel removed. (B) Flat-mount images showing pSTAT3 staining on *isl2b*:GFP;*mpeg1*:mCherry (*isl2b*:GFP – green; *mpeg1*:mCherry - magenta) retinae after P6/PLX3397 treatment or housed in system water and injected with 0.05% DMSO (Ctr) at 7dpi. Image to right has *isl2b*:GFP channel removed. Scale bar=50µm.

**Table S1.** Sequences of q-PCR primers

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**Table S2.** 56 up-regulated genes at 24hpi (filtering parameters used: the maximum of the average group RPKM value >1.5, fold change >2, false discovery rate (FDR) p-value <0.05. Genes with TPM=0 in one or more replicates were excluded.

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**Table S3.** 252 down-regulated genes at 24hpi (filtering parameters used: the maximum of the average group RPKM value >1.5, fold change >2, false discovery rate (FDR) p-value <0.05. Genes with TPM=0 in one or more replicates were excluded.

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**Table S4.** 5 up-regulated genes and 3 down-regulated genes at 12hpi (filtering parameters used: the maximum of the average group RPKM value >1.5, absolute fold change >2, false discovery rate (FDR) p-value <0.05. Genes with TPM=0 in one or more replicates were excluded.)

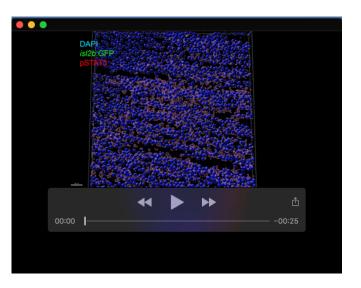
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**Table S5.** 49 up-regulated genes at 12hpi (filtering parameters used: the maximum of the average group RPKM value >1.5, fold change >2, p-value <0.05. Genes with TPM=0 in one or more replicates were excluded.

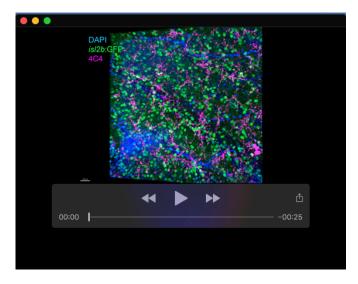
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**Table S6.** 31 down-regulated genes at 12hpi (filtering parameters used: the maximum of the average group RPKM value >1.5, fold change < -2, p-value <0.05. Genes with TPM=0 in one or more replicates were excluded.

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**Movie 1.** Surfaces of pStat3 staining generated in Imaris. Scale bar = 20µm.



**Movie 2.** 3D image of 4C4 staining generated in Imaris. Scale bar =  $20\mu m$ .