Supporting Material

Supplemental Figures and Legends

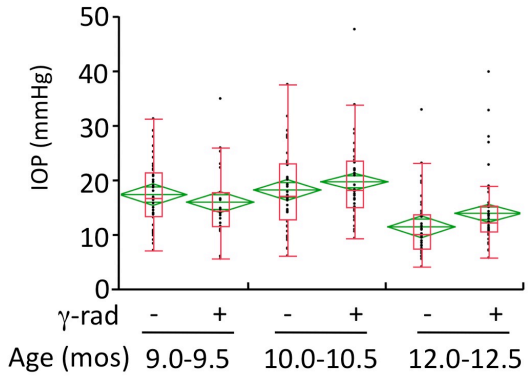

Figure S1. Sublethal irradiation does not affect IOP. γ-radiation treatment has no effect on IOP elevation. 9.0-9.5 mo, P=0.32; 10.0-10.5 mo, P=0.24, 12.0-12.5, P=0.06. n>40 each group.

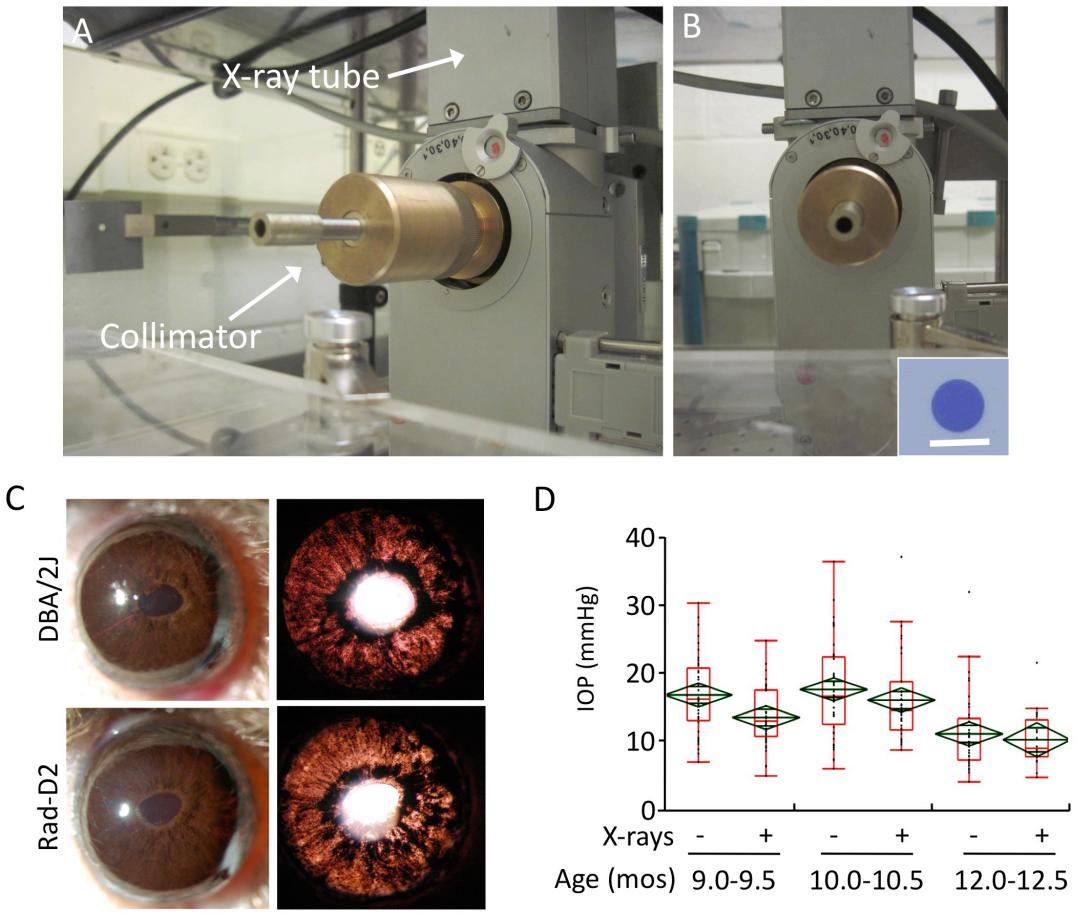

Figure S2: X-ray radiation protects from glaucoma. (**A-B**) X-ray radiation was administered using a specially designed X-ray machine. X-rays are generated in the X-ray tube and a collimator focuses the beam to 3 mm. The machine was calibrated to provide a defined dose to the back of the eye (approximately the location of the optic nerve head and retina, see Methods). Prior to any treatment, radiation-sensitive film was exposed to the X-ray beam to check uniformity and size of beam (inset, B). **(C)** The iris disease, which induces IOP elevation, was no different between irradiation treated and untreated DBA/2J mice (shown at 12mo). Broad beam slit lamp images (left panels) and transillumination images are shown (right panels). (**D**) Despite a slight difference in IOP

profiles at 9.0-9.5 mo in radiation-treated eyes (P=0.008), IOP profiles were not significantly different between treated and untreated eyes at 10.0-10.5 mo (P=0.97) and 12.0-12.5 mo (P=0.57). The degree of difference was not adequate to prevent glaucoma in previous studies [1,2]. n>40 each group. Bar = 3 mm.

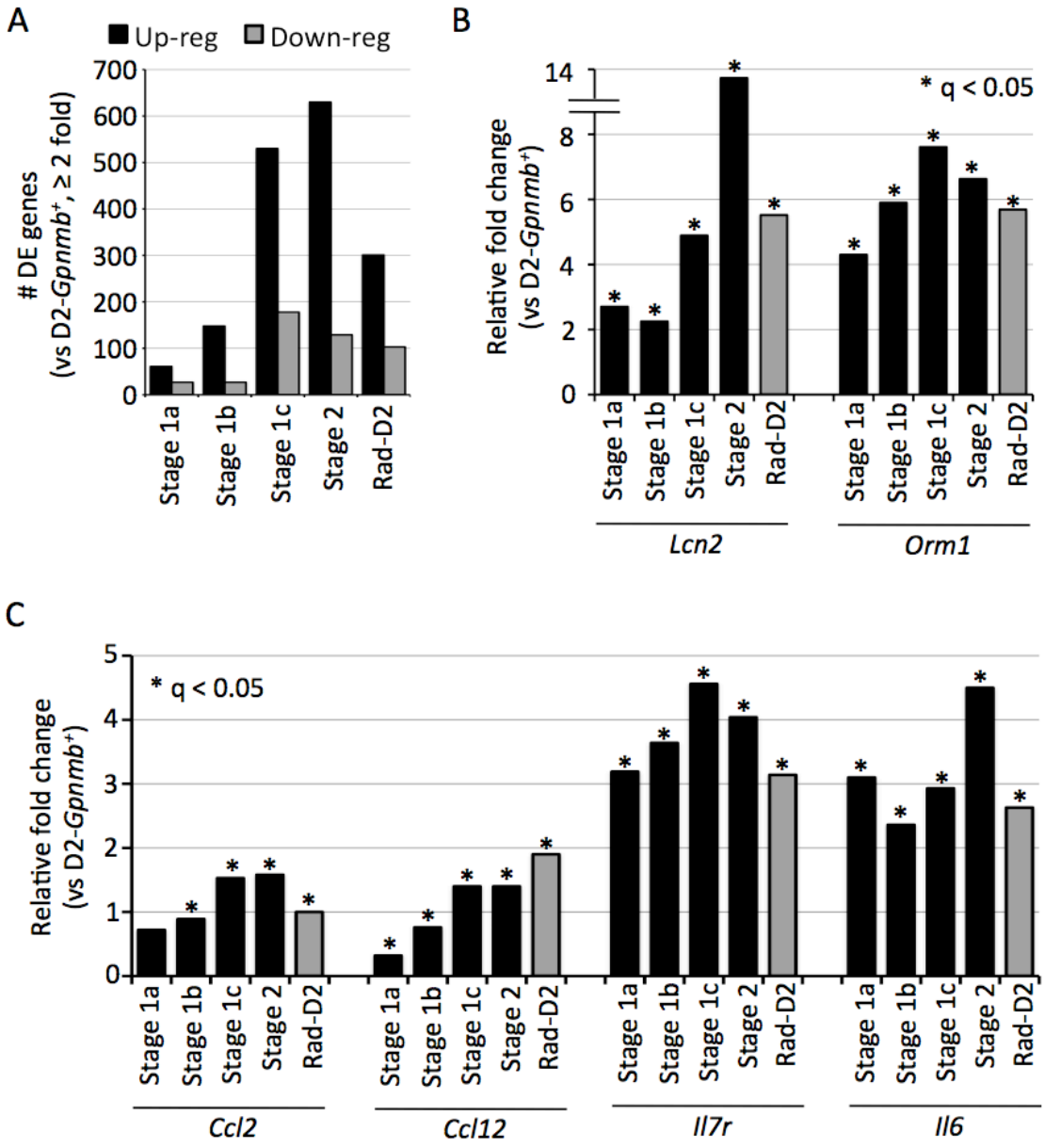

Figure S3. Inflammatory signaling occurs very early in glaucoma and persists in radiation-treated eyes. (**A**) Summary of differentially expressed (DE) genes in glaucoma and radiation-treated eyes. The number of DE genes that are up-regulated or down-regulated (> 2 fold) compared to the D2-*Gpnmb*$^{+}$ control group. (**B**) Two early immune response genes *Lcn2* and *Orm1* are differentially expressed (DE) in all stages and in the radiation-treated group (Rad-D2). (**C**) Various chemokine, cytokine and

cytokine receptor genes are DE in all glaucoma stages and in radiation-treated eyes. This shows that glaucomatous stresses induce inflammatory signaling and that these stresses and signaling persist in radiation-treated eyes. Early inflammatory signaling may be a protective response that subsequently becomes damaging [3,4]. Full details of DE genes are provided in supplemental tables S1-S5.

Figure S4:

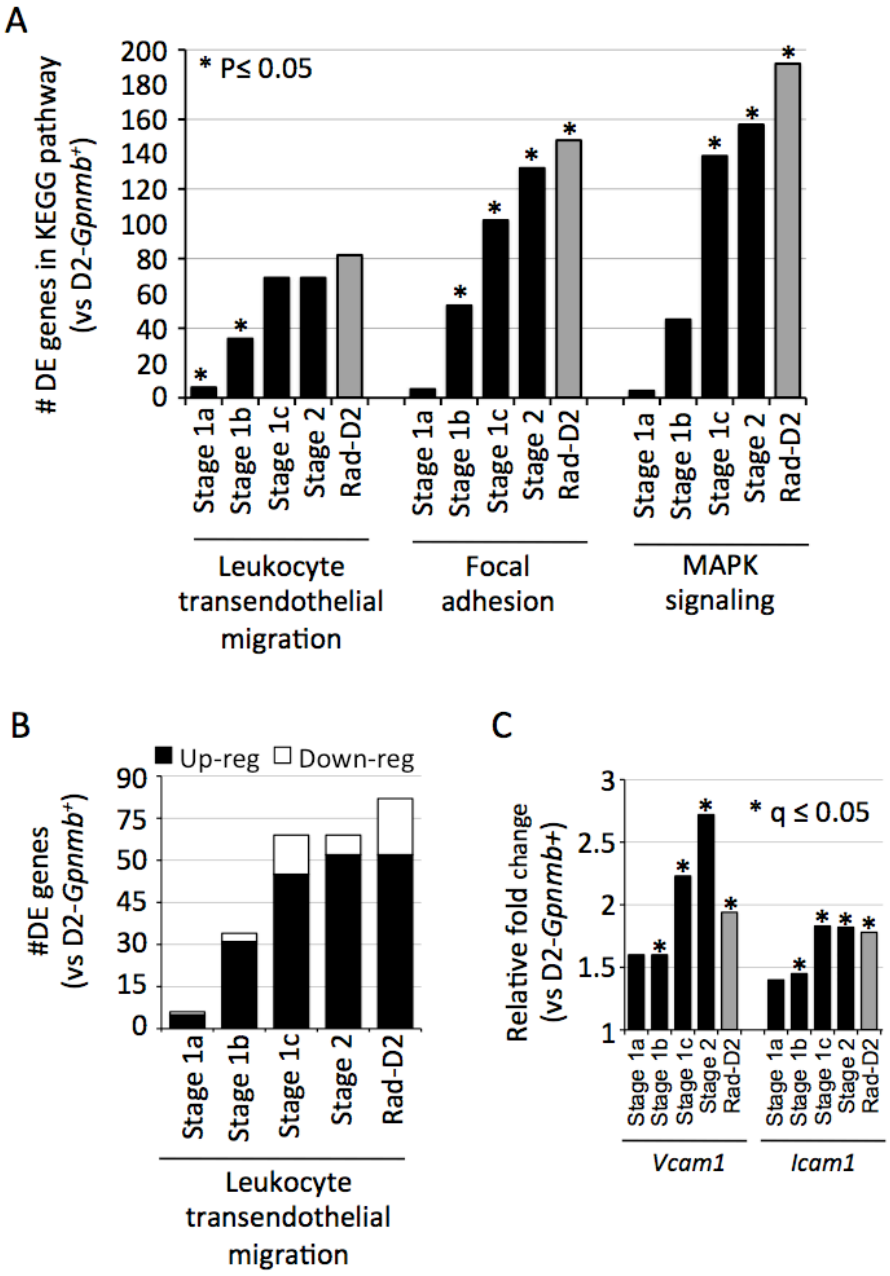

Figure S4. Leukocyte transendothelial migration is the first pathway to significantly change in glaucoma. (**A**) Pathway analysis of differentially expressed DE genes (see Methods) suggested that leukocyte transendothelial migration (LTM), focal adhesion and

MAPK signaling are significantly up-regulated very early in glaucoma (asterisks pathway $P<0.05$). Significance is based on the number of genes DE in the pathway in relation the total number of DE genes for the comparison. The LTM pathway is significantly up-regulated in stage 1a, the earliest glaucoma stage. More LTM genes are significantly DE in stages 1b, 1c and 2, but the pathway does not reach significance in the later stages as the total number of DE genes for all processes is much greater at these stages. Many of these genes are also DE in the radiation-treated group (Rad-D2). Focal adhesion and MAPK signaling pathways are significantly up-regulated by stage 1b and stage 1c respectively. These pathways are also overrepresented in the radiation-treated group. (**B**) The majority of the DE genes in stages 1a, 1b, 1c and 2 that are in the LTM pathway are up-regulated (black bars). Although many of the DE genes are also up-regulated in the radiation-treated group, a larger number of genes are down-regulated (white bars) compared to the glaucoma stages (see also Figure S5). (**C**) The expression levels of the major cell adhesion molecules vascular cell adhesion molecule (*Vcam1*) and intercellular cell adhesion molecule (*Icam1*) are generally lower in the radiation-treated group compared to glaucoma stages.

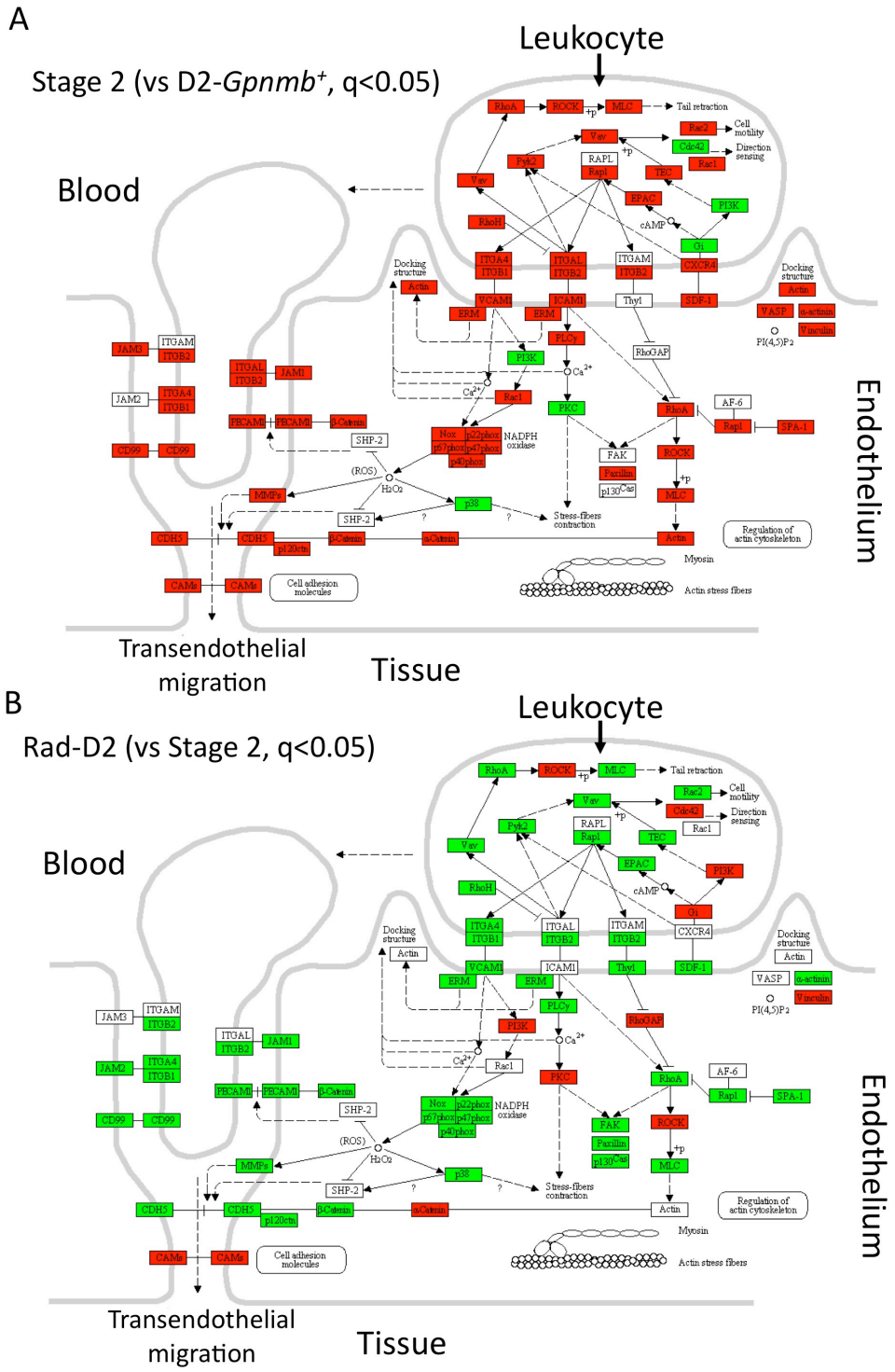

Figure S5. Genes in the leukocyte transendothelial migration (LTM) pathway that are DE in untreated and radiation-treated eyes. (**A**) The majority of genes in the LTM pathway are up-regulated (red) in glaucoma stage 2 compared to D2-*Gpnmb*$^{+}$ group. (**B**)

However, the majority of these genes have a lower expression (green) in the radiation-treated group (Rad-D2) compared to stage 2 (reproduced from KEGG).

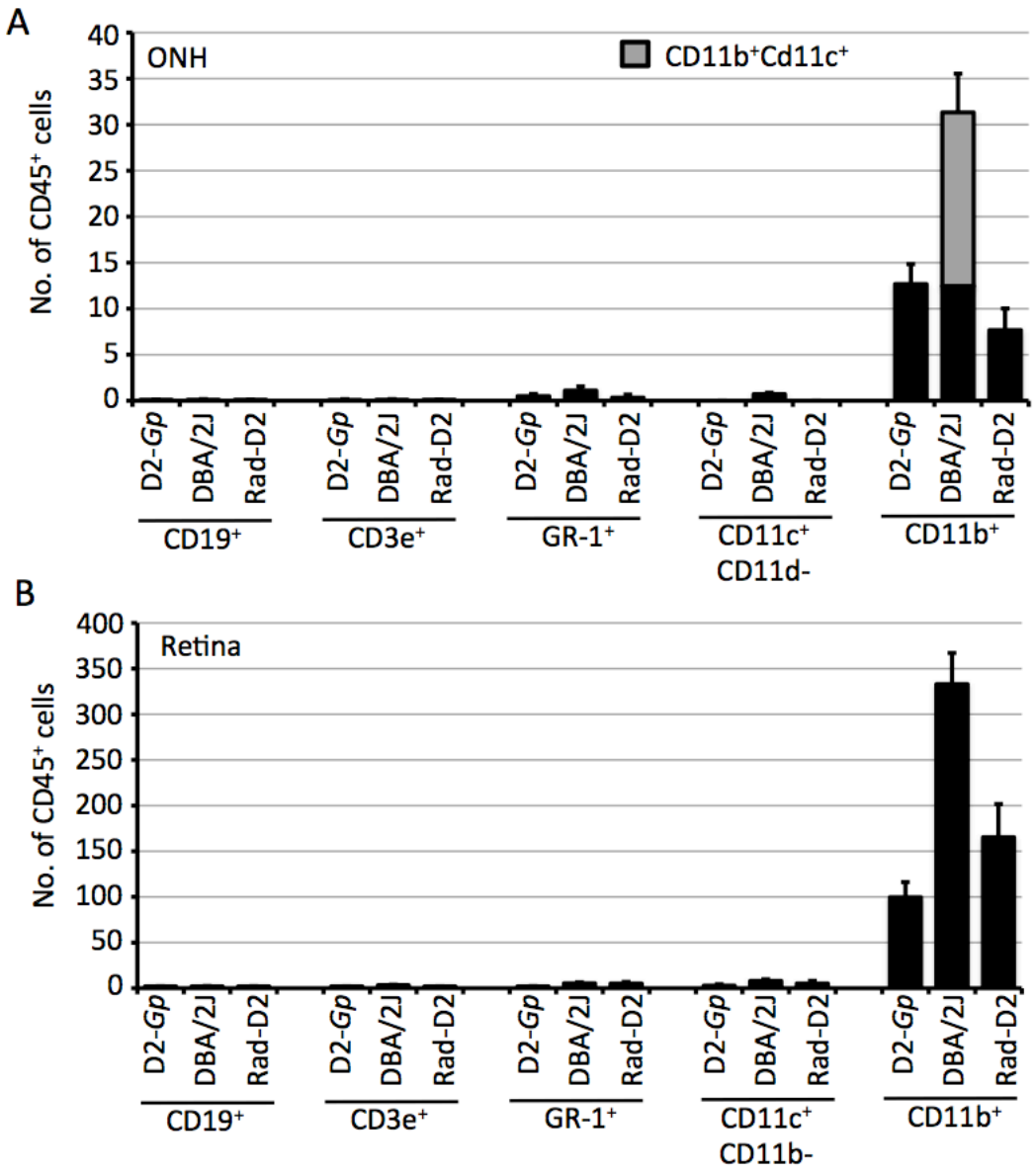

Figure S6. CD11b^{+} monocytes are the major hematopoietic cells to enter the ONH and retina. Cell types were assessed in the ONH and retinas from 10.5 mos D2-*Gpnmb*$^{+}$, DBA/2J and radiation-treated DBA/2J eyes using flow cytometry. The increase in cell numbers in glaucomatous eyes compared to controls is due to CD45^{hi} bone marrow derived cells. Cell type markers were: B cells – CD19; T cells – CD3e; granulocytes –

Gr-1; lymphoid dendritic cells – CD11c alone; monocytes and monocyte-derived cells – CD11b (see Methods). (**A**) In the ONH, the major detected cell type was $CD11b^+$. In untreated DBA/2J eyes, a new $CD11b^+$ $Cd11c^+$ monocyte-derived cell type was present. These cells were completely absent in radiation-treated DBA/2J eyes (Rad-D2) or control (D2-Gp) eyes. (**B**) In the retina, $CD11b^+$ cells were the only major cell type observed in D2-Gp control eyes. $CD11b^+$ cells increased significantly in glaucomatous DBA/2J eyes, but were not present in radiation-treated eyes.

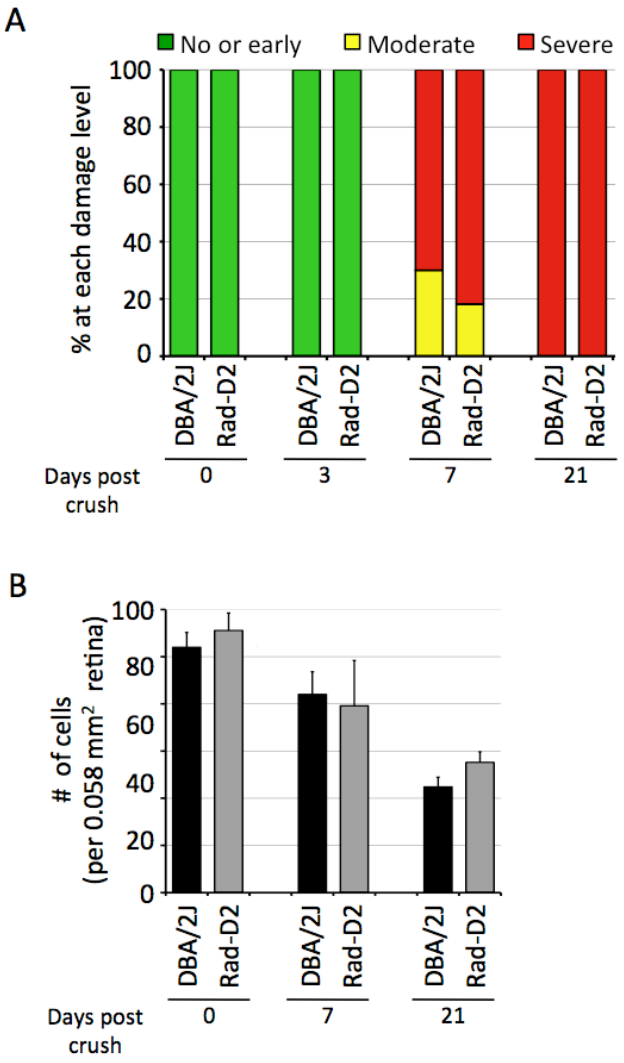

Figure S7. Radiation does not protect RGCs from a direct optic nerve crush insult.

(A-B) Optic nerve crush was performed on untreated and radiation-treated DBA/2J mice. Optic nerve damage and ganglion cell layer (GCL) soma numbers were assessed 3, 7 and 21 days later. The radiation treatment had no protective effect against this direct nerve injury. n>5 for each group.