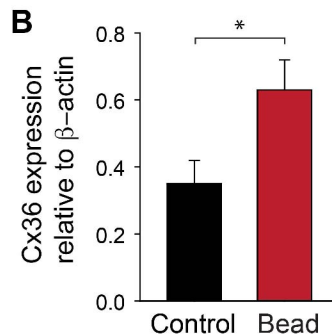
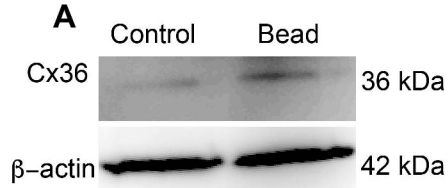
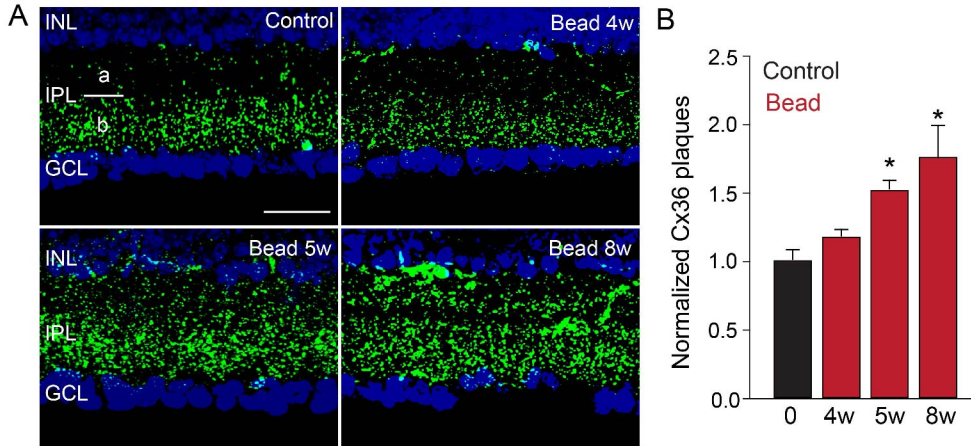


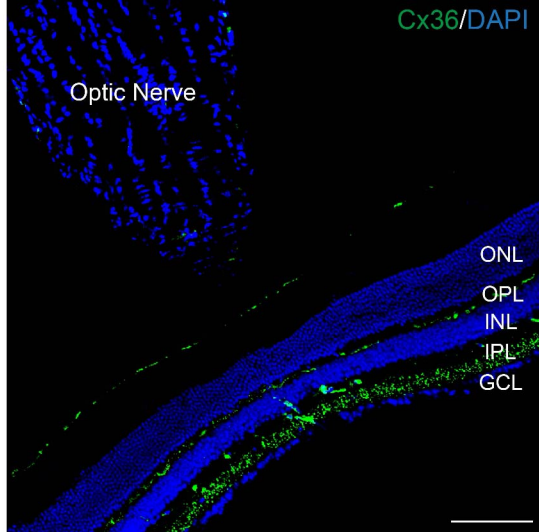
Supplemental Figure 1. Application of MFA is not injurious to the retina. (A-D) Application of MFA to control C57BL/6 mice via osmotic minipumps produced no change in the number of DAPI-labeled cells (total) and Brn3a-labeled RGCs in the GCL. (E, F) Whole mount view of GFAP expression in astrocytes in proximal retina in control and MFA-treated retinas. (G) Vertical section of an MFA-treated retina shows GFAP expression confined to astrocytes in proximal retina. (H) Histogram showing no change in GFAP expression in MFA-treated retinas as compared to levels in control retinas. (I). Application of MFA to control mice produced no change in the thickness of individual retinal layers as compared to measures in untreated retinas. Scale bars = 50 μm in all panels. All data are presented as mean \pm SEM. $P > 0.1$ for all comparisons, Student's t-test ($n = 3-5$ mice for each comparison).



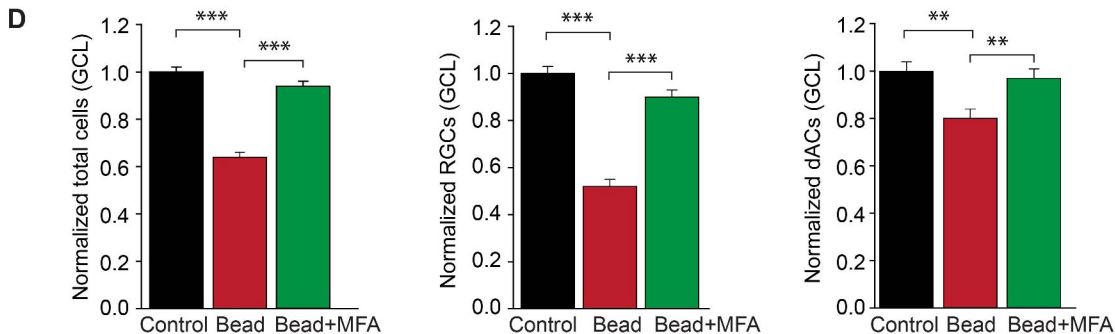
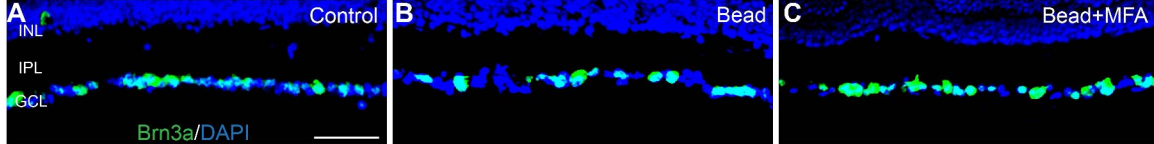
Supplemental Figure 2. Increased expression of Cx36 protein in experimental glaucoma. (A) Representative blots of Cx36 and β -actin in control and glaucomatous eyes. β -actin was probed as a loading control. (B) Histogram quantifying increased level of Cx36 protein in glaucomatous retinas compared to control. Bars represent mean grey intensity \pm SEM from control and microbead-injected eyes ($n = 6$, 2 biological samples for triplicate experiments per condition). * $P < 0.05$, Student's t-test.



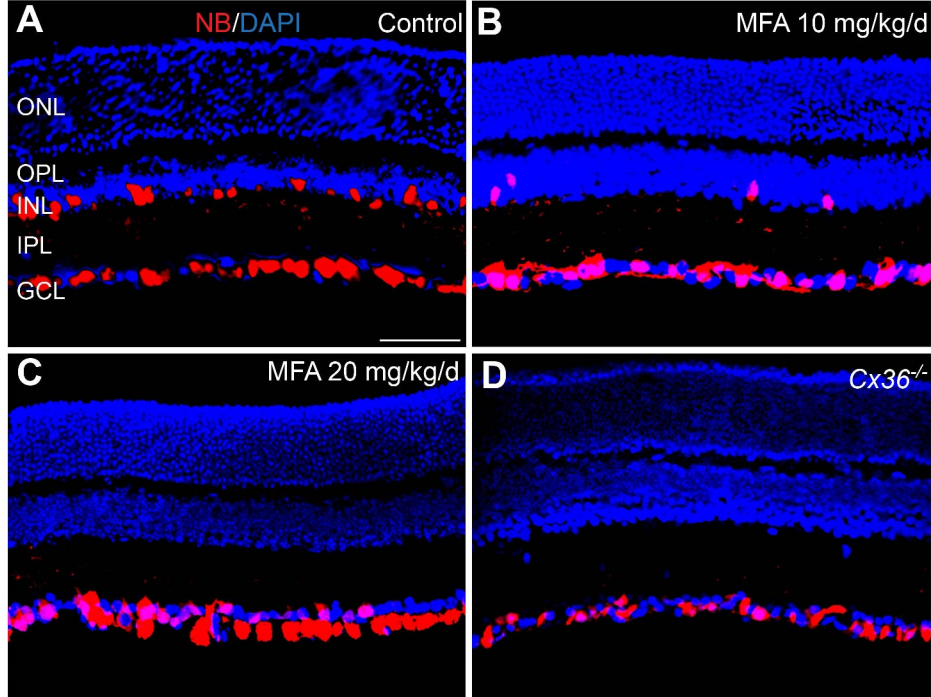
Supplemental Figure 3. The time course of the Cx36 upregulation following microbead injection. (A) Vertical retinal sections immunostained for Cx36 under control condition and at 4, 5 and 8 weeks following microbead injection. Note significant increase in Cx36 immunolabeling in the a- (Off)-sublamina of the IPL after 8 weeks of microbead injection as compared to control. Scale bar=25 μ m for all panels. (B) Quantification of Cx36 plaques in the IPL of control retinas and at different time points after microbead injection. * P <0.05, Student's t-test.



Supplemental Figure 4. Neuronal Cx36 is not expressed in the mouse optic nerve. Vertical retinal section with attached optic nerve was immunostained for Cx36 and counterstained with DAPI. Characteristic punctate labeling of Cx36 is evident in the IPL and OPL whereas no significant staining is seen in the optic nerve. Scale bar = 100 μm .



Supplemental Figure 5. Intravitreal injection of MFA protects inner retinal neurons. (A-C) Representative immunofluorescence confocal images of vertical retinal sections immunostained for RGC marker Brn3a and counterstained with DAPI under control conditions and 8 weeks after initial microbead injection with and without intravitreal MFA application. MFA was injected one day prior to microbead injection and then weekly for 7 weeks. (D) Quantification of changes in total cell count (DAPI-positive), RGC count (Brn3a-positive), and dAC count (DAPI-Brn3a values), in the GCL of glaucomatous retinas from C57BL/6 mice showing the protective effects of an intravitreal application of MFA. Scale bar = 50 μ m for all panels (n = 4 retinas per group). Data are presented as mean \pm SEM. ***P < 0.001, one-way ANOVA followed by Tukey's multiple comparison test.



Supplemental Figure 6. Blockade of gap junctional coupling between AC and RGCs by different doses of MFA delivered through subcutaneous osmotic minipumps. (A) Representative confocal image of a vertical retinal section in which RGCs are retrogradely labeled with Neurobiotin (NB). There are also NB-positive ACs seen in the INL, which presumably represent the movement of NB through GJs made between the ACs and RGCs. Scale bar = 50 μ m for all panels. (B) Systemic delivery of MFA beginning at a dose of 10 mg/kg body weight/day results in a reduction in NB-labeled cells in the INL indicating a partial blockade of GJs between ACs and RGCs. (C) Application of MFA at a dose of 20 mg/kg/day eliminates all NB-positive ACs in the INL by complete blockade of the GJs between ACs and RGCs. (D) No NB-positive ACs seen in the INL of Cx36^{-/-} mice after retrograde labeling of RGCs with NB because the GJs between ACs and RGCs are ablated.