Supplemental Figures

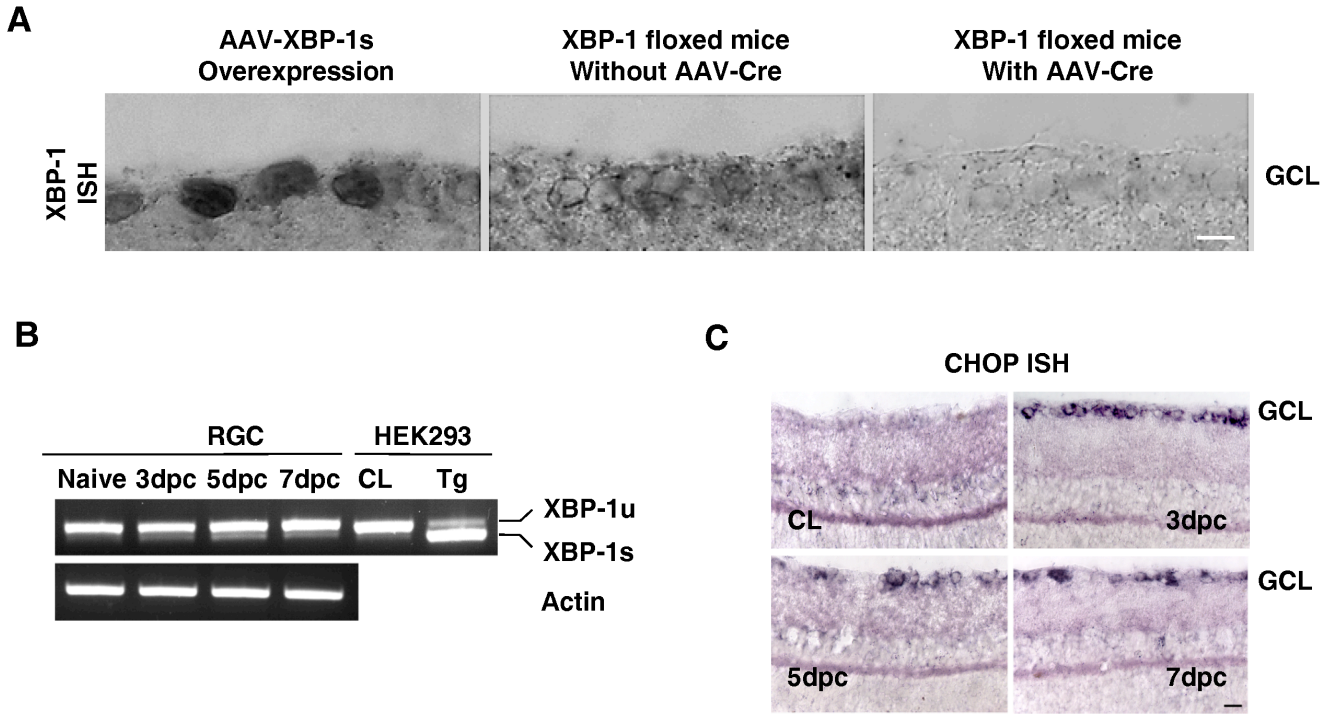

Figure S1. AAV-Cre Mediated XBP-1 KO in RGCs and Dynamic Levels of XBP-1s and CHOP (related to Figure 2)

(A) In situ hybridization images showing the expression of XBP-1 in the ganglion cell layer (GCL) of WT mice injected with AAV-XBP-1s, and XBP-$1^{flox/flox}$ mice with or without intravitreal injection of AAV-Cre. Scale bar: 10 μm. **(B)** Detection of un-spliced and spliced XBP-1 mRNA (XBP-1u or XBP-1s) by RT-PCR. The mRNAs were prepared from retrograde-labeled and FACS-purified adult RGCs from retinas 3, 5 or 7 days post-crush, and contralateral naïve retinas. The mRNAs from HEK 293 cells treated with ER stress inducer thapsigargin (Tg) were used as controls. **(C)** In situ hybridization (ISH) images showing the expression of CHOP in the ganglion cell layer (GCL) at different time points post crush. Scale bar: 20 μm.

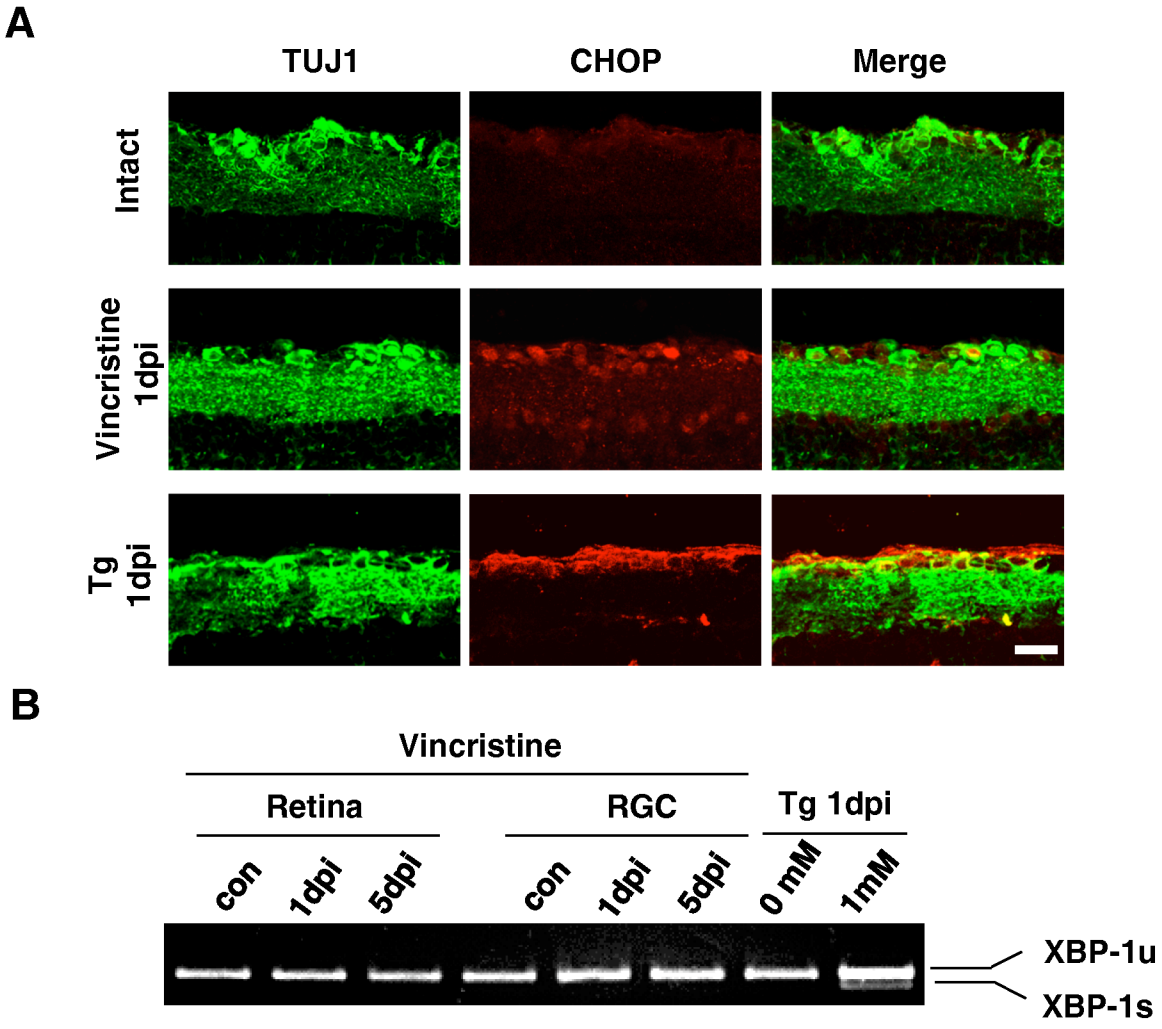

Figure S2. Vincristine Induce CHOP Expression But Not XBP-1 Splicing (related to Figure 1)

(A) Immunohistochemical analysis for CHOP in retinal sections from intact control eyes, vincristine treated eyes and thapsigargin (Tg) treated eyes. Scale bar: 20 μm. 1dpi: 1 day post injection. **(B)** Detection of un-spliced and spliced XBP-1 mRNA (XBP-1u or XBP-1s) by RT-PCR. The mRNAs were prepared from whole retina treated with vincristine for 0, 1 and 5days, FACS-purified adult RGCs 0, 1 and 5 days after vincristine injection, and whole retina mRNAs from thapsigargin (Tg) treated eyes 1 day after injection.

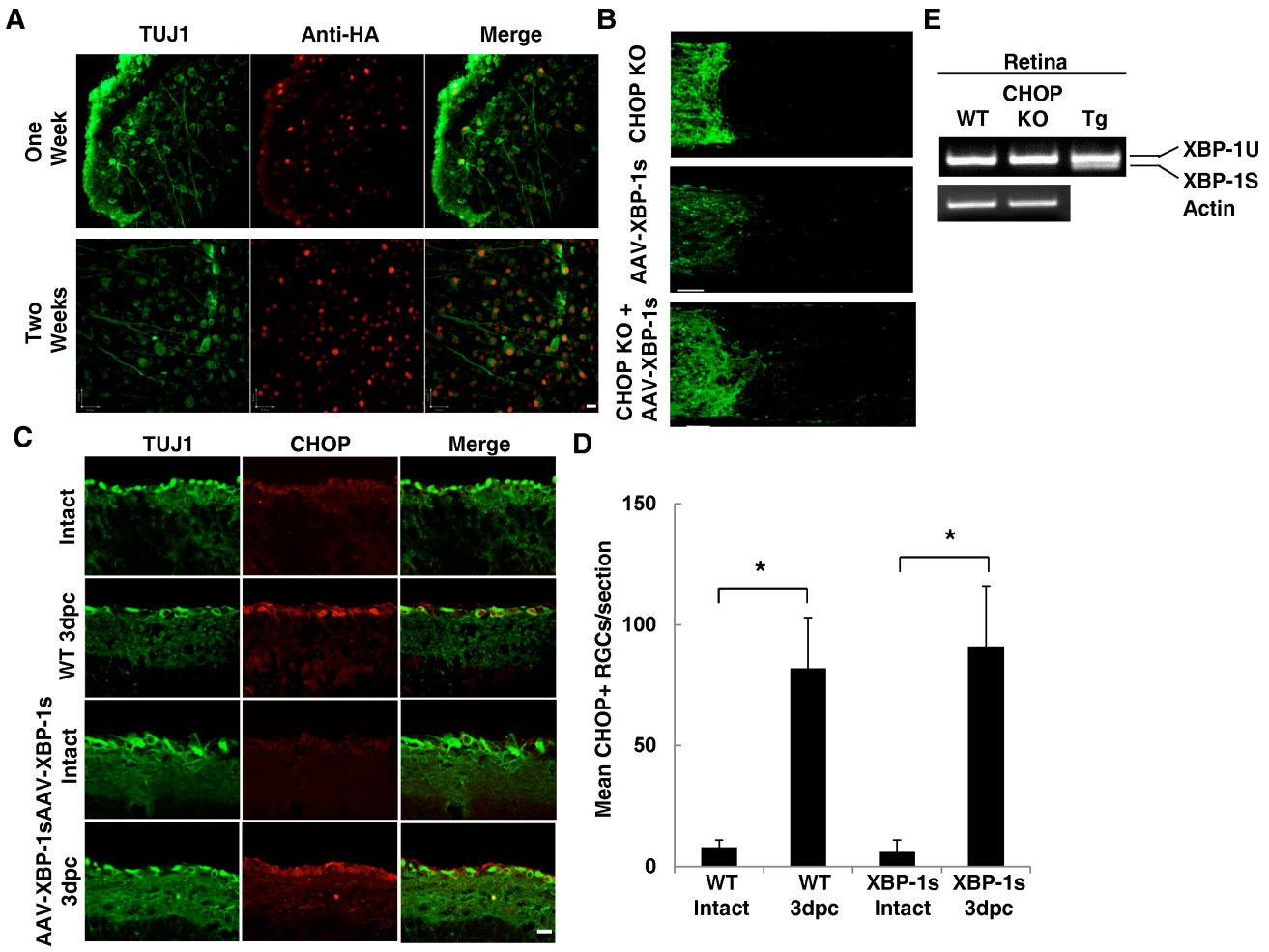

Figure S3. AAV-Mediated Over-expression of XBP-1s in Adult RGCs and Lack of Optic Nerve Regeneration (related to Figure 3)

(A) Retinal flat-mounts staining revealed that XBP-1s expression (as indicated by anti-HA staining, red) are found in 50% of RGCs (TUJ1$^+$) at one week and 80% of RGCs at two weeks after intravitreous AAV-XBP-1s-HA injection. Scale bar: 20 μm. **(B)** Confocal images of optic nerves showing CTB-labeled optic nerve axons around the lesion sites at 14 days after injury from CHOP KO mice, WT mice injected with AAV-XBP-1s or CHOP KO mice injected with AAV-XBP-1s. Scale bar: 50 μm. **(C)** Immunohistochemical analysis for CHOP expression in retinal sections. 3dpc: 3 days post-crush. **(D)** Quantification of CHOP$^+$ RGC cells. * $p<0.01$, student's t test. **(E)** Detection of un-spliced and spliced XBP-1 mRNA (XBP-1u or XBP-1s) by RT-PCR. The mRNAs were prepared from retinas of CHOP KO mice, WT mice or thapsigargin (Tg) treated mice.

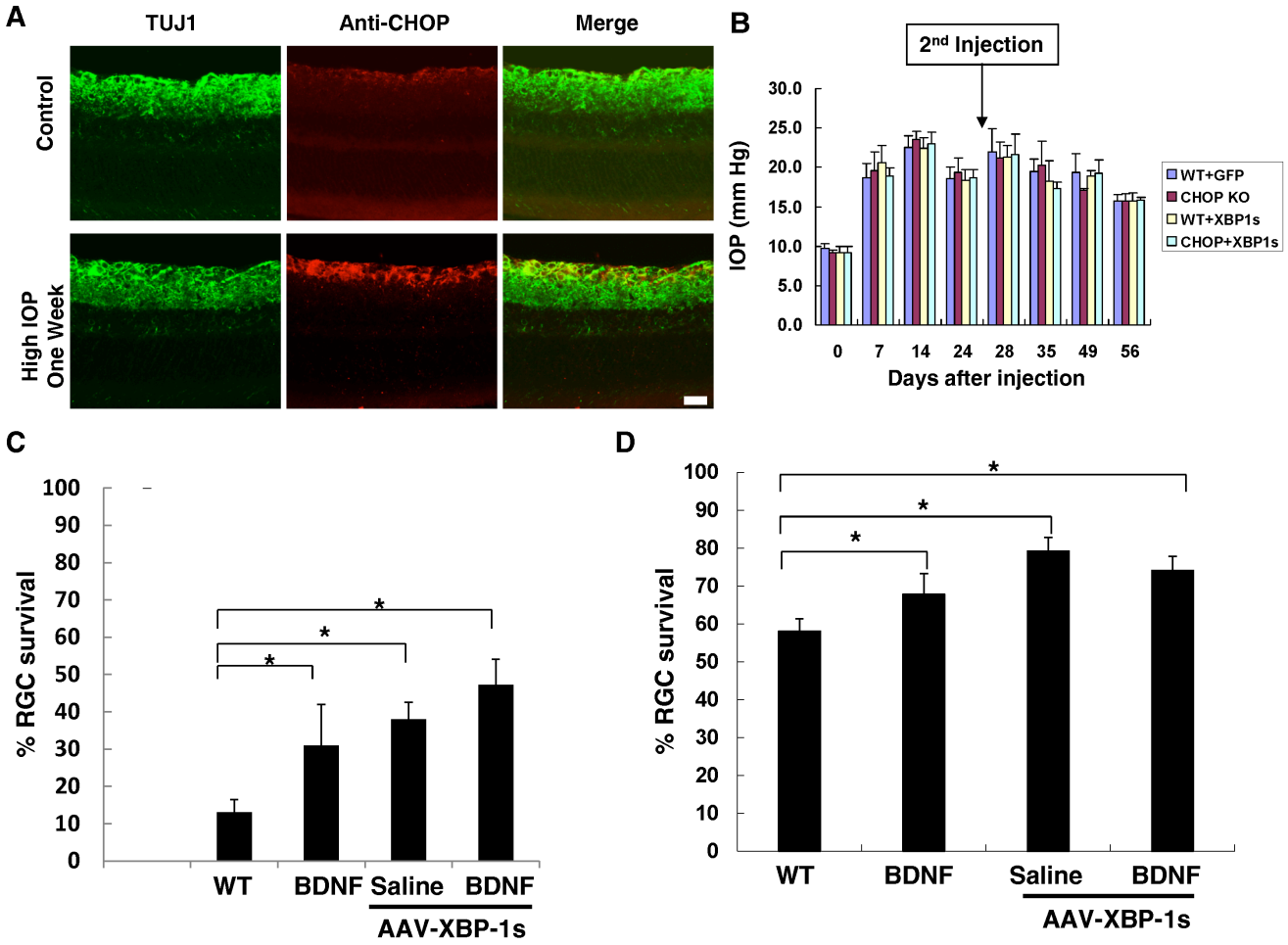

Figure S4. IOP Elevation Induces CHOP Expression in RGCs and No Synergistic Effect of BDNF and XBP-1s on RGC Survival in Both Optic Nerve Crush Model and the Glaucoma Model (related to Figure 4)

(A) Confocal images of retinal sections from high IOP eyes and contralateral controls that were stained with TUJ1 antibody (green) or anti-CHOP antibody (red). Note that many RGCs from high IOP animals showed CHOP immunoreactivity. Scale bar: 20 μm. **(B)** Elevation of IOP was observed in all groups that were subjected to microbeads injection. The magnitude and duration of IOP elevation in WT mice, CHOP KO mice, WT mice injected with AAV-XBP-1s and CHOP KO mice injected with XBP-1s were similar. (**C**) Quantification of TUJ1$^+$ RGCs in injured retina 4 weeks after optic nerve crush, expressed as a percentage of the total number of TUJ1$^+$ RGCs in the contralateral uninjured retina. The counting was performed in the peripheral regions of flat-mounted retinas for all animals. Data are presented as means ± s.e.m and n=6. * $p<0.01$, One way ANOVA and Tukey's Multiple Comparison Test. **(D)** Quantification of TUJ1$^+$ RGCs in injured retina 8 weeks after IOP elevation, expressed as a percentage

of the total number of TUJ1^{+} RGCs in the contralateral uninjured retina. The counting was performed in the peripheral regions of flat-mounted retinas for all animals. Data are presented as means ± s.e.m and n=8. * $p<0.01$, One way ANOVA and Tukey's Multiple Comparison Test.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Mice. CHOP KO and C57BL/6 mice and Sprague-Dawley rats were purchased from the Jackson Laboratories (Bar Harbor, Maine). XBP-1$^{flox/flox}$ mice were described before (Hetz et al., 2008). All experimental procedures were performed in compliance with animal protocols approved by the IACUC at Children's Hospital, Boston.

Intravitreal injection and optic nerve crush. For each intravitreal injection, the micropipette was inserted in peripheral retina just behind the ora serrata, and was deliberately angled to avoid damage to the lens. At two weeks following AAV viral injection, the left optic nerve was exposed intraorbitally and crushed with jeweler's forceps (Dumont #5; Roboz) for 5 seconds approximately 1 mm behind the optic disc, as described previously (Park et al., 2008). To preserve the retinal blood supply, care was taken not to damage the underlying ophthalmic artery. After the surgical procedure, mice received a subcutaneous injection of buprenorphine (0.05 mg/kg, Bedford Lab) as post-operative analgesic. Eye ointment containing atropine sulphate was applied preoperatively to protect the cornea during surgery. BDNF (2 μl, Prospec) was injected intravitreally once a week at the concentration of 0.5μg/μl for 4 weeks. Vincristine (2 μl, VWR) was injected intravitreally at the concentration of 0.1μg/μl.

RGC purification. For retrograde labeling of RGCs for FACS sorting, the superior colliculi of adult Sprague-Dawley rats were injected with DiI (2% in DMF). Briefly, rats were anesthetized and placed in a stereotaxic holder. A hole was made by dentist drill on the skull and approximately 2 μL DiI was then injected directly into the superficial SC (6 mm caudal to Bregma, 1.5 mm lateral to sagittal suture and 4 mm deep to brain surface) via a pulled-glass micropipette attached to an automated nano-injector. 5-7 days after surgery, eyes were removed and retinas were prepared for the cell dissociation procedures. The dissected retinas were incubated in digestion solution (20 U/ml papain, Worthington; 1 mM L-cysteine HCL; 0.004% DNase; 0.5 mM EDTA in Neurobasal) for 25-30 min at 37°C, with gentle shaking every 5 min. Digestion was stopped by adding Ovomucoid/BSA (1 mg/ml) solution before trituration with trituration buffer (0.5% B27; 0.004% DNase; 0.5 mM EDTA in Opti-MEM). Dissociated retinal cells were filtered through 40 μm cell strainer before FACS sorting (FACSVantage SE cell sorter, Becton-Dickinson). The large cell population was gated based on forward and side scatter

to select the RGC neurons. Unlabeled retinal cells were used as control to set up the threshold for DiI positive cells in FL2 channel.

RT-PCR. Total RNA was extract from purified RGCs by Trizol (Invitrogen, Carlsbad, CA). polyA mRNA was reverse-transcribed using M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA) and cDNA was amplified by PCR using primers: rat XBP-1, 5'-TTACGAGAGAAAACTCATGGG-3' AND 5'GGGTCCAACTTGTCCAGAATGC-3'; murine xbp-1, 5'-TTACGGGAGAAAACTCACGGC-3' AND 5'-GGGTCCAACTTGTCCAGAATGC-3'. The 289bp band generated from unspliced form XBP-1 (XBP-1u) and the 263bp band generated from spliced form XBP-1 (XBP-1s) were separated by 2.5% agarose/0.5x TAE gel.

q-PCR. Total RNA (50-100 ng) was reverse transcribed and amplified with TagMan pre-designed real-time PCR assays (Applied Biosystems). Real-time PCR was performed on an ABI 7900HT. Each sample was run in quadruplicate in each assay. GADPH was used as the endogenous control.

Immunohistochemistry of retina. Immunostaining was performed following standard protocols. All antibodies were diluted in a solution consisting of 10% normal goat serum (NGS) and 2% Triton X-100 in PBS. Antibodies used were: mouse neuronal class ß-III tubulin (clone TUJ1, 1:400 dilution; Covance), mouse CHOP (1:500 dilution, ABR), rat HA (clone 3F10, 1:100 dilution, Roche), TUNEL (Promega, Madison, WI), rabbit caspase 3 antibody (Asp175, 1:200 dilution, Cell signaling).. Sections of retinas were incubated with primary antibodies overnight at 4°C and washed three times for 15 minutes each with PBS. Secondary antibodies (Cy2, Cy3 or Cy5-conjugated) were then applied (1:200-400; Jackson) and incubated for 1 hour at room temperature. Sections were again washed three times for 15 minutes each with PBS before a cover slip was attached with Fluoromount-G. For RGC counting, whole-mount were immunostained with the TUJ1 antibody and 6-9 fields were randomly sampled from peripheral region per retina to estimate RGC survival. The people who counted the cells were blinded with the treatment of the samples.

RGC axon anterograde labeling. To identify RGC axons in the optic nerve by anterograde labeling, 1 μl of cholera toxin β subunit (CTB) (2 μg/μl, Invitrogen) was injected into the vitreous with a Hamilton syringe (Hamilton). Animals were fixed by perfusion with 4% paraformaldehyde in PBS 2 days after

CTB injection in the eye. Eyes with the nerve segment still attached were dissected out and post-fixed in the same fixative overnight at 4°C. Tissues were cryoprotected through increasing concentrations of sucrose and optimal cutting temperature compound (Tissue Tek). Then tissues were snap-frozen in dry ice and serial longitudinally cross-sections (8 μm) were cut and stored at –80°C until processed.

In situ hybridization. Mouse cDNA sequences for CHOP, BiP, GADD45a and XBP-1 from Allen Brain Atlas were subcloned into pBluescript II SK+ (Stratagene). In vitro transcription was performed from linearized plasmids using T7 and T3 RNA polymerase (Promega) with digoxigenin-UTP (Roche) for synthesis of sense and antisense probes, respectively. In situ hybridization was performed following the standard protocols. After hybridization, mRNA localization was detected with AP-conjugated anti-digoxigenin Fab fragments followed by visualization with NBT/BCIP.

AAV production. For making AAV2-XBP-1s, the cDNA of XBP-1s-3HA was inserted downstream of the CMV promoter/β-globin intron enhancer in the vector pAAVsc CB6.RBG and viral preparation was made by UMass Gene Therapy Center. The titer determined by silver staining is 1.85×10^{12}.

Induction of chronic IOP elevation in mice. The experimental procedures and the use of animals were conformed to the standards of the Association for Research in Vision and Ophthalmology. The procedure in detail has been described recently (Chen et al., 2010; Sappington et al., 2010). Briefly, in anesthetized mice, elevation of IOP was induced unilaterally in adult mice by anterior chamber injection of fluorescent polystyrene microspheres with a uniform diameter of 10 μm (Invitrogen, Oregon, USA) prior to re-suspending in saline at a final concentration of 7.2×10^6 beads/ml. For experimental animals, the left cornea was gently punctured near the limbus without lesioning lens and iris using a 30 gauge needle. Following this entry wound, a 2 μl microbead solution were injected into the anterior chamber using a glass micropipette. The control group received 2 μl saline to the anterior chamber. In some experimental groups, mice received a second injection of microbeads at 4 week after the first injection. The mice with corneal opacity or signs of inflammation in the anterior chamber (e.g. cloudy anterior chamber) were excluded from further analysis.

IOP measurement. IOP was measured every other day in both eyes using a TonoLab tonometer (Colonial Medical Supply, Espoo, Finland) following product instruction. Briefly, the measurement of

IOP was conducted consistently at the same time in the morning. Measurement was initiated within 2-3 min after animals lost consciousness. The Tonolab tonometer takes six measurements by internal software which generates an average, after elimination of high and low readings. We considered this machine-generated average as one reading. Six machine-generated measurements were obtained from each eye, and the mean was calculated to determine the IOP.

Statistical analyses. Data are presented as means ± s.e.m. We used Student's t-test for two group comparison and One way ANOVA and Tukey's Multiple Comparison Test for multiple comparisons.