Supporting Information

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SI Materials and Methods

Reagents. Lestaurtinib and crizotinib were purchased from LC Laboratories, KW-2449 and foretinib from Selleckchem, and tozasertib from BioVision. Antibodies: Phospho-JNK, Thr183/Tyr185 (4671); JNK (9258); c-Jun (9165); Phospho-MKK7 (4171) and MKK7 (4172) (Cell Signaling Technology); monoclonal anti–alpha-tubulin antibody (T6074) (Sigma); mouse neuronal class βIII tubulin (TUJ1) (Covance); goat polyclonal Brn3 (C-13); and rabbit anti-Cre (Novus).

PLGA Microspheres. Polymer microparticles loaded with tozasertib were prepared using poly(lactic-*co*-glycolic acid) (PLGA) by a single-emulsion solvent evaporation method. Microparticle size was determined using a Multisizer IIe (Beckman-Coulter, Inc.). Drug release rates were determined in vitro by spectrophotometry at 252 nm for tozasertib, following a standard protocol (1).

Retinal Ganglion Cell Purification, Culture, Screening, and Imaging. All animal use was in accordance with the Association for Research in Vision and Opthalmology (ARVO) Statement for the Use of Animals, following animal protocols approved by the Institutional Animal Care and Use Committee at Johns Hopkins University. Retinas were isolated from postnatal 0–5-d mice and dissociated with papain. Microglia were immunodepleted with anti-CD11b conjugated Dynabeads (Life Technologies). The suspension of retinal cells was immunopanned on plates preconjugated with anti-Thy1.2 antibody (Serotec, MCA028) and goat antimouse IgM (Jackson Immunoresearch) at room temperature (RT). After washing, retinal ganglion cellS (RGCs) were released from the plate by a cell lifter, counted, and seeded at a density of 10,000 per well in 96-well plates in the media composed of Neurobasal (Life Technologies), B27, N2 supplement, L-glutamine, and penicillin/streptomycin. After a 72-h culture at 37°C, RGCs were stained with calcein AM (acetomethoxy derivate of calcein), ethidium homodimer, and Hoechst 33342. Images were taken from portions of each well with a Cellomics ArrayScan VTI HCS Reader (Thermo Fisher), and cell survival was quantified using the Cellomics Neuronal Profiling bioapplication. As indicated, RGC viability was alternatively measured by CellTiter-Glo (Promega) luminescence.

For siRNA-based screening, the siRNAs from the Sigma Mission Mouse Kinome library were complexed with NeuroMag (Oz Biosciences) at a final concentration of 20 nM. RGCs were then reverse transfected on a stationary magnet and assayed for survival 72 h later using CellTiter-Glo (Promega). Confirmatory siRNAs were obtained from both Dharmacon and Ambion. Adenoviruses expressing wild-type or kinase-dead dual leucine zipper kinase, GFP, or Cre were added to RGCs at a multiplicity of infection of 100–1000.

Rat Intravitreal Injections. Six-week old male Wistar rats were anesthetized with ketamine/xylazine. A partial peritomy was made to expose the sclera. The injection site was approximately at the ora serrata, and the injection glass pipet was angled toward the optic disk to avoid lens injury. Five microliters (10 μg) of PLGA microspheres were injected with a glass pipet and Hamilton syringe.

Electrophysiology. Recordings were made by using the whole-cell patch-clamp technique in both current- and voltage-clamp modes with an Axopatch 200B (Molecular Devices). Data were low-pass filtered at 1 kHz (Bessel) and sampled at 10 kHz. A liquid junction potential of −2 mV has been corrected, and the resting potential was estimated to be -62 ± 2.2 mV (mean ± SEM; $n = 13$). The recording pipette was filled with the following intracellular solution (in mM): 100 K-gluconate, 50 KCl, 20 Hepes, 10 EGTA, 5 $MgCl_2$, 2 ATP, 0.1 GTP, pH adjusted to 7.33 with KOH. The cells were continuously perfused with (in mM) 140 NaCl, 5 KCl, 1 $MgCl_2$, 2.5 $CaCl_2$, 10 glucose, 10 Hepes, pH 7.4, with NaOH.

Production of Adeno-Associated Virus Vectors. Adeno-associated virus vector was produced by the 2-plasmid, cotransfection method with modifications (2).

1. Tang BC, Fu J, Watkins DN, Hanes J (2010) Enhanced efficacy of local etoposide delivery by poly(ether-anhydride) particles against small cell lung cancer in vivo. *Biomaterials* 31(2):339–344.

2. Zolotukhin S, et al. (2002) Production and purification of serotype 1, 2, and 5 recombinant adeno-associated viral vectors. *Methods* 28(2):158–167.

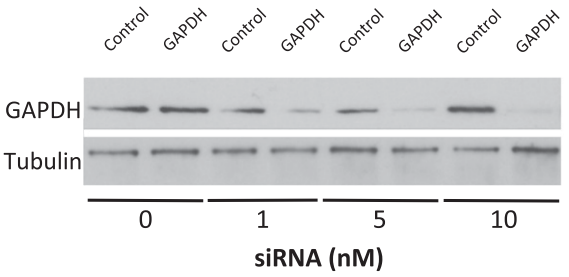

Fig. S1. Efficient delivery to primary RGCs. RGCs were reverse transfected with increasing doses of GAPDH or control small interfering RNA oligonucleotide in the presence of a fixed amount of NeuroMag and immunoblotted for GAPDH protein 24 h later.

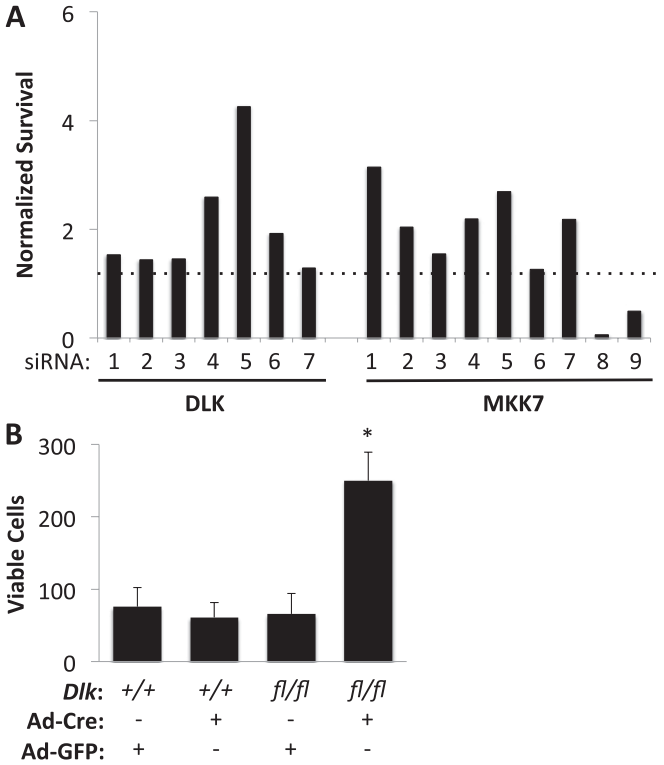

Fig. S2. Secondary screening confirms the neuroprotective activity of dual leucine zipper kinase and MKK7 small interfering RNA oligonucleotides (siRNAs). (*A*) RGCs were immunopanned and transfected with an independent set of siRNAs not used in the initial screen. Candidate genes were considered confirmed if 75% of the secondary screening siRNA increased survival more than three SDs above the control siRNAs (dashed line). (*B*) RGCs were isolated from wild-type or $Dlk^{fl/fl}$ mice and immediately transduced with adeno-GFP or adeno-Cre. After 72 h, RGCs were imaged for viability by calcein-AM staining.

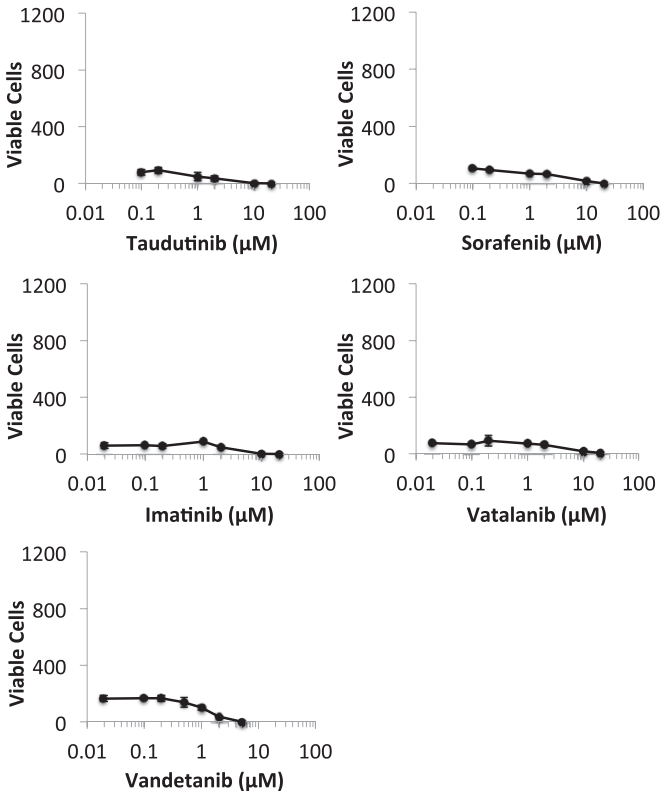

Fig. S3. Kinase inhibitors without dual leucine zipper kinase activity do not affect RGC survival. Survival of immunopanned RGCs, treated with increasing doses of the indicated kinase inhibitors, after 72 h in culture.

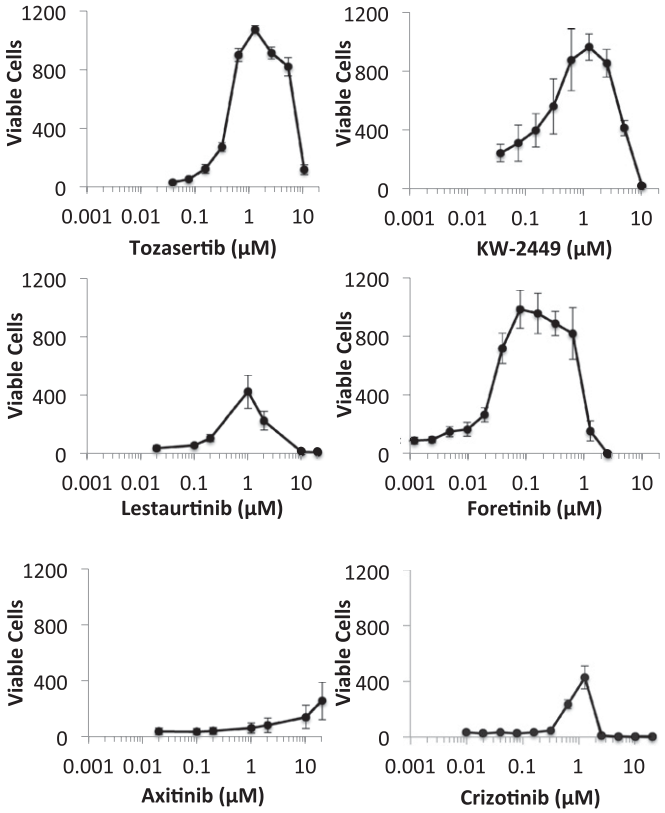

Fig. S4. Kinase inhibitors with dual leucine zipper kinase activity increase RGC survival. Survival of immunopanned RGCs, treated with increasing doses of the indicated dual leucine zipper kinase inhibitors, after 72 h in culture.

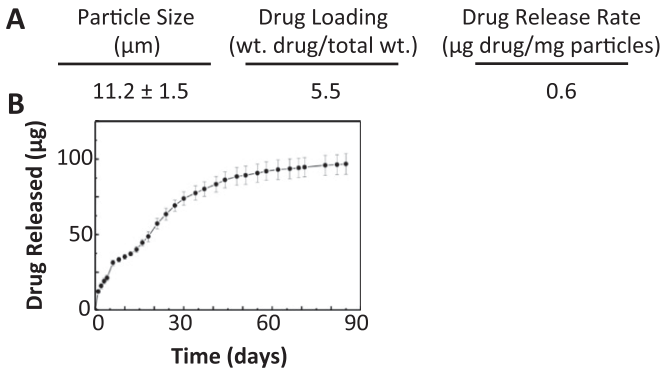

Fig. S5. Characterization of drug-eluting microspheres. (*A*) Kinetics of drug-release from microspheres. (*B*) Elution of tozasertib in vitro.

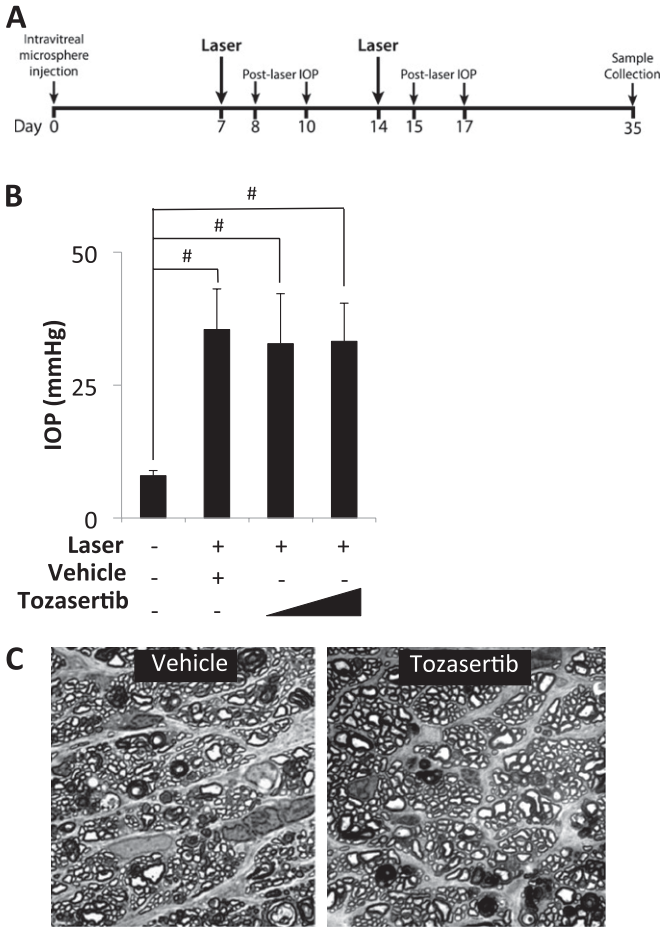

Fig. S6. Laser-induced ocular hypertension. (*A*) Schematic of rat glaucoma experiment. (*B*) Mean intraocular pressure increase 24 h after the first administration of diode laser to the trabecular meshwork. $^{\#}P < 0.005$; error bars, SD.