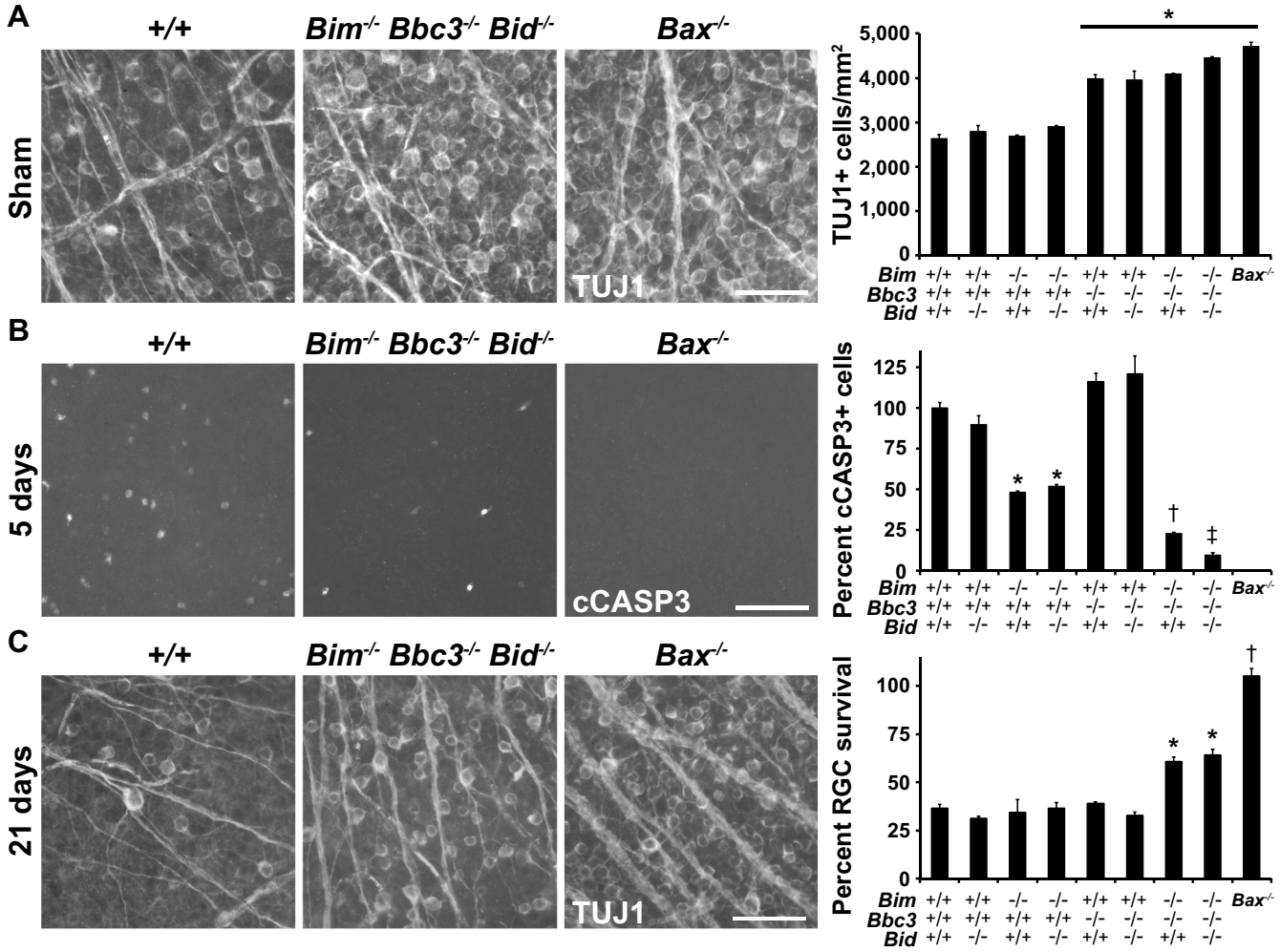
A
+/+
Bim-/- Bbc3-/- Bid-/-
Bax-/-
Sham
TUJ1
TUJ1+ cells/mm2
5,000
4,000
3,000
2,000
1,000
0
*
Bim +/+ +/+ -/- -/- +/+ +/+ -/- -/- Bax-/-
Bbc3 +/+ +/+ +/+ +/+ -/- -/- -/- -/-
Bid +/+ -/- +/+ -/- +/+ -/- +/+ -/-
B
+/+
Bim-/- Bbc3-/- Bid-/-
Bax-/-
5 days
cCASP3
Percent cCASP3+ cells
125
100
75
50
25
0
*
*
†
‡
Bim +/+ +/+ -/- -/- +/+ +/+ -/- -/- Bax-/-
Bbc3 +/+ +/+ +/+ +/+ -/- -/- -/- -/-
Bid +/+ -/- +/+ -/- +/+ -/- +/+ -/-
C
+/+
Bim-/- Bbc3-/- Bid-/-
Bax-/-
21 days
TUJ1
Percent RGC survival
100
75
50
25
0
*
*
†
Bim +/+ +/+ -/- -/- +/+ +/+ -/- -/- Bax-/-
Bbc3 +/+ +/+ +/+ +/+ -/- -/- -/- -/-
Bid +/+ -/- +/+ -/- +/+ -/- +/+ -/-

Figure S1. BIM, BID, and BBC3 are not required for RGC death after axonal injury.

A. To determine the relative number of RGCs in each knockout mouse, the RGC specific marker TUJ1 was used count RGCs in flat mounted retinas. Deleting *Bbc3* significantly increases the number of RGCs in adult mice compared to wild type (*, $P<0.001$), which is consistent with previous reports. The absence of *Bim* and *Bid* did not significantly affect the number of RGCs in the adult retina either alone or in combination with the other genes. Note there was a slight increase in the number of RGCs in adult $Bax^{-/-}$ mice compared to $Bbc3^{-/-}$ mice. However, the $Bax^{-/-}$ mice were on a different genetic background (C57BL/6J) than the $Bbc3^{-/-}$ mice (segregating DBA/2J, C57BL/6J background). Previously, we showed that on the C57BL/6J genetic background, *Bax* deficiency and *Bbc3* deficiency resulted in a similar number of RGCs. Thus, it is likely that the slight increase in RGCs in the $Bax^{-/-}$ retinas compared to $Bbc3^{-/-}$ retinas is caused by genetic background. For TUJ1+ cell counts, cells immunolabeled by anti-TUJ1 (βIII-tubulin; Covance, 1:1000) were counted in the RGC layer in two 40X fields from each quadrant of the retina (a total of 8 fields) approximately 220 μm from the peripheral edge. Error bars denote SEM; N≥3 for single mutants and N≥4 for double and triple mutants; Scale bar, 50μm.

B. The peak of cell death occurs approximately 5 days after axonal injury (controlled optic nerve crush, CONC). CONC was performed in 45-60 day old mice. Following anesthetization, the optic nerve was exposed and clamped where it exits the eye for 3-4 seconds using self-closing forceps (Roboz RS-5027). Sham and unmanipulated eyes were used as controls. 5 days after CONC, cleaved caspase 3+ cells (cCASP3+; R&D Systems, 1:1000) were counted in the RGC layer of retinal flat mounts. Where appropriate, the cCASP3+ cell counts were normalized to the relative number of TUJ1+ RGCs observed in $Bbc3^{-/-}$ mice compared to wild type mice. There are significantly fewer cCASP3+ cells in $Bim^{-/-}$, $Bim^{-/-}$ $Bid^{-/-}$, $Bim^{-/-}$ $Bbc3^{-/-}$, $Bim^{-/-}$ $Bbc3^{-/-}$ $Bid^{-/-}$, and $Bax^{-/-}$ mice compared to wild type mice ($P<0.001$). The contributions of BIM, BBC3, and BID in axonally injured RGCs appear to be additive and hierarchical. Of the single mutants only loss of BIM significantly decreases death at 5 days (*, $P<0.001$). $Bbc3^{-/-}$ mice have been shown to have a decrease in dying RGCs at 3 days and there is no effect at either time point in $Bid^{-/-}$ mice (3 days data not shown). Consistent with these disparities, when combined with the loss of BIM, loss of BBC3 further decreases CASP3 activation (†, $P<0.001$), whereas loss of BID does not ($P=0.613$). However, in the *Bim Bbc3 Bid* triple knockout there are even fewer CASP3+ cells than in the *Bim Bbc3* double knockout uncovering a role for all three of these BH3-only proteins in RGC death after axonal insult (‡, $P<0.001$). cCASP3+ cell counts were performed similarly to TUJ1+ cell counts, except 8 20X fields were counted. Error bars denote SEM; N≥3 for single mutants and N≥4 for double and triple mutants for each experiment and time point; Scale bar, 100μm.

C. Assessment of RGC survival after axonal injury. 21 days after axonal injury RGCs were counted in the RGC layer of retinal flat mounts using the RGC specific marker TUJ1. RGC survival increased in *Bim Bbc3* double knockout and *Bim Bbc3 Bid* triple knockout mice compared to wild type mice. (*, $P<0.001$). Significantly greater protection was observed in *Bax* knockout mice compared to *Bim Bbc3 Bid* triple knockout mice (†, $P<0.001$), as no significant loss of RGCs occurs in *Bax* knockout mice after axonal injury. These results indicate that loss of RGCs did occur when *Bim*, *Bbc3*, and *Bid* were deleted alone or in combination. Error bars denote SEM; N≥3 for single mutants and N≥4 for double and triple mutants; Scale bar, 50μm. Note, all experiments were conducted in accordance with the Association for Research in Vision and Ophthalmology's statement on the use of animals in ophthalmic research and were approved by the University of Rochester's University Committee on Animal Resources. Null alleles of $Bbc3^{tm1Gpz}$ (a generous gift from Gerard Zambetti), $Bim^{tm1.1Ast}$ (The Jackson Laboratory stock # 004524), Bid^{tm1Sjk} (a generous gift from Xiao-Ming Yin), and *Bax* (The Jackson Laboratory stock # 002994) were used.