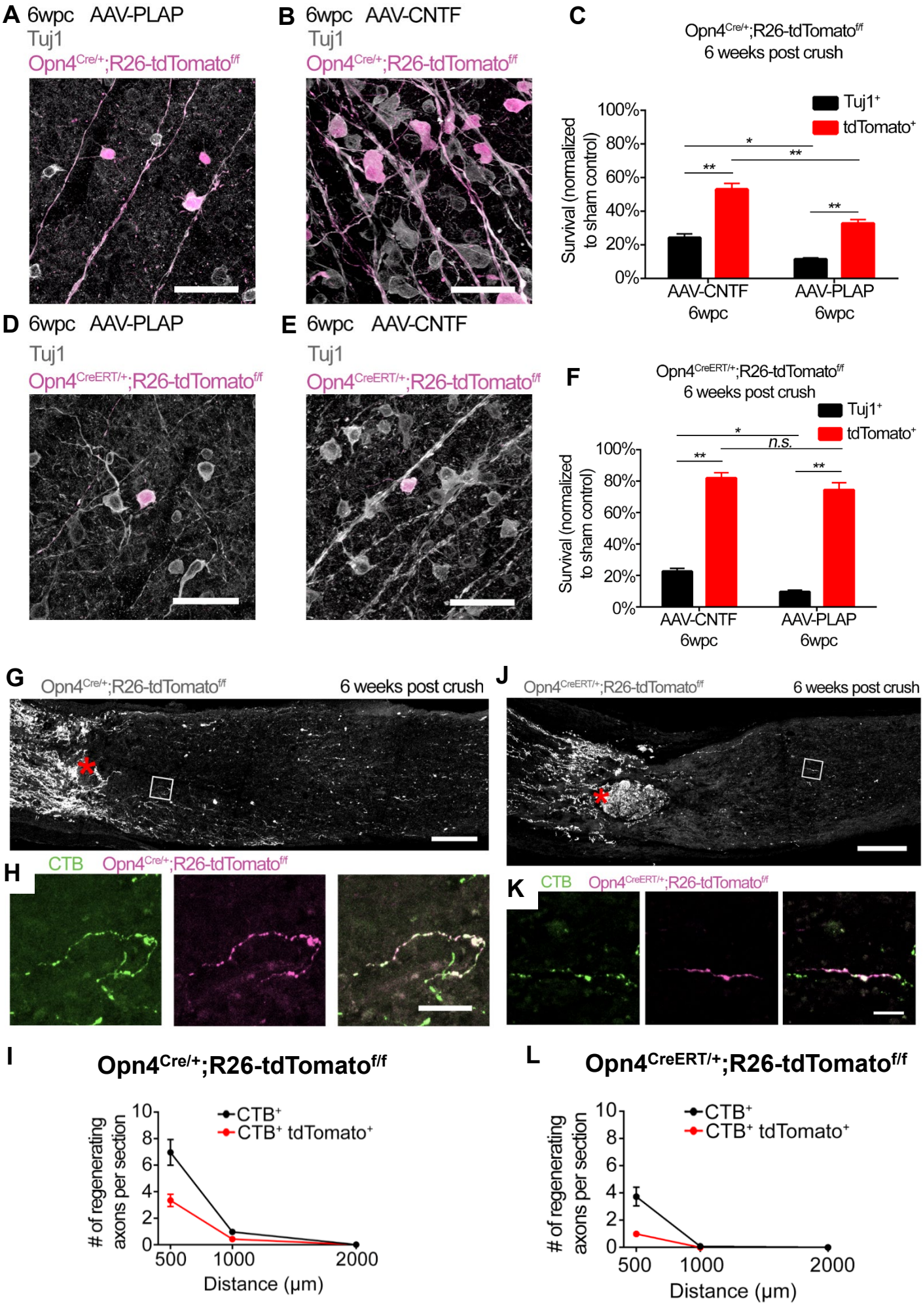
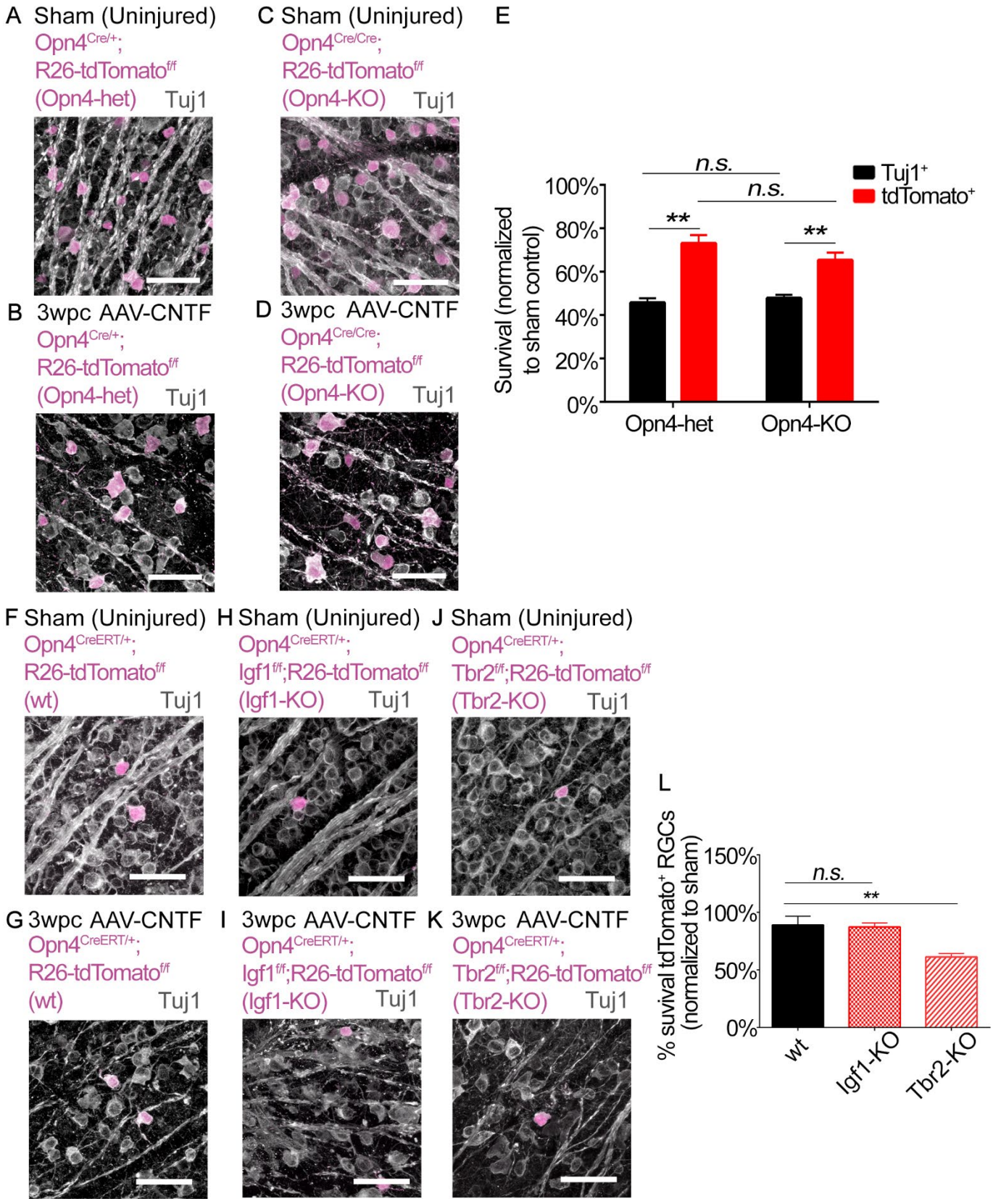
Figure S1


A 6wpc AAV-PLAP
Tuj1
Opn4Cre/+;R26-tdTomatof/f
B 6wpc AAV-CNTF
Tuj1
Opn4Cre/+;R26-tdTomatof/f
C
Opn4Cre/+;R26-tdTomatof/f
6 weeks post crush
Tuj1+
tdTomato+
Survival (normalized to sham control)
100%
80%
60%
40%
20%
0%
*
**
**
**
AAV-CNTF 6wpc
AAV-PLAP 6wpc
D 6wpc AAV-PLAP
Tuj1
Opn4CreERT/+;R26-tdTomatof/f
E 6wpc AAV-CNTF
Tuj1
Opn4CreERT/+;R26-tdTomatof/f
F
Opn4CreERT/+;R26-tdTomatof/f
6 weeks post crush
Tuj1+
tdTomato+
n.s.
G
Opn4Cre/+;R26-tdTomatof/f
6 weeks post crush
J
Opn4CreERT/+;R26-tdTomatof/f
6 weeks post crush
H
CTB
Opn4Cre/+;R26-tdTomatof/f
K
CTB
Opn4CreERT/+;R26-tdTomatof/f
I
Opn4Cre/+;R26-tdTomatof/f
CTB+
CTB+ tdTomato+
# of regenerating axons per section
10
8
6
4
2
0
500
1000
2000
Distance (μm)
L
Opn4CreERT/+;R26-tdTomatof/f
CTB+
CTB+ tdTomato+
# of regenerating axons per section
Distance (μm)

Supplementary Figure 1 (Related to Figure 1): RGCs labelled in Opn4$^{Cre/+}$;R26-tdTomato$^{f/f}$ and Opn4$^{CreERT/+}$;R26-tdTomato$^{f/f}$ mice have high survival following optic nerve crush, and ipRGCs can spontaneously regenerate axons following optic nerve crush. (A, B, D and E) Images of retinal whole mounts showing Tuj1-labeled RGCs (grey) and tdTomato-labeled ipRGCs (magenta) (A-B) Opn4$^{Cre/+}$;R26-tdTomato$^{f/f}$ or (D-E) Opn4$^{CreERT/+}$;R26-tdTomato$^{f/f}$. (A and D) AAV-PLAP injected 6 weeks post crush. (B and E) AAV-CNTF injected 6 weeks post crush. Scale bars, 50 μm. **(C)** Quantification of RGC survival for (A and B). Percentage of Tuj1$^+$ or tdTomato$^+$ RGCs in each line normalized to the Sham uninjured retina. **(F)** Quantification of RGC survival for (D and E). *** $p < 0.01$, * $p < 0.05$, n.s. $p \geq 0.05$*, *ANOVA, Tukey's post-hoc test*. (C) Opn4$^{Cre/+}$;R26-tdTomato$^{f/f}$. *n=8 for AAV-CNTF, 6 for AAV-PLAP;* (F) Opn4$^{Cre/+}$;R26-tdTomato$^{f/f}$ *n=8 for AAV-CNTF, 7 for AAV-PLAP*. Error bars, SEM.

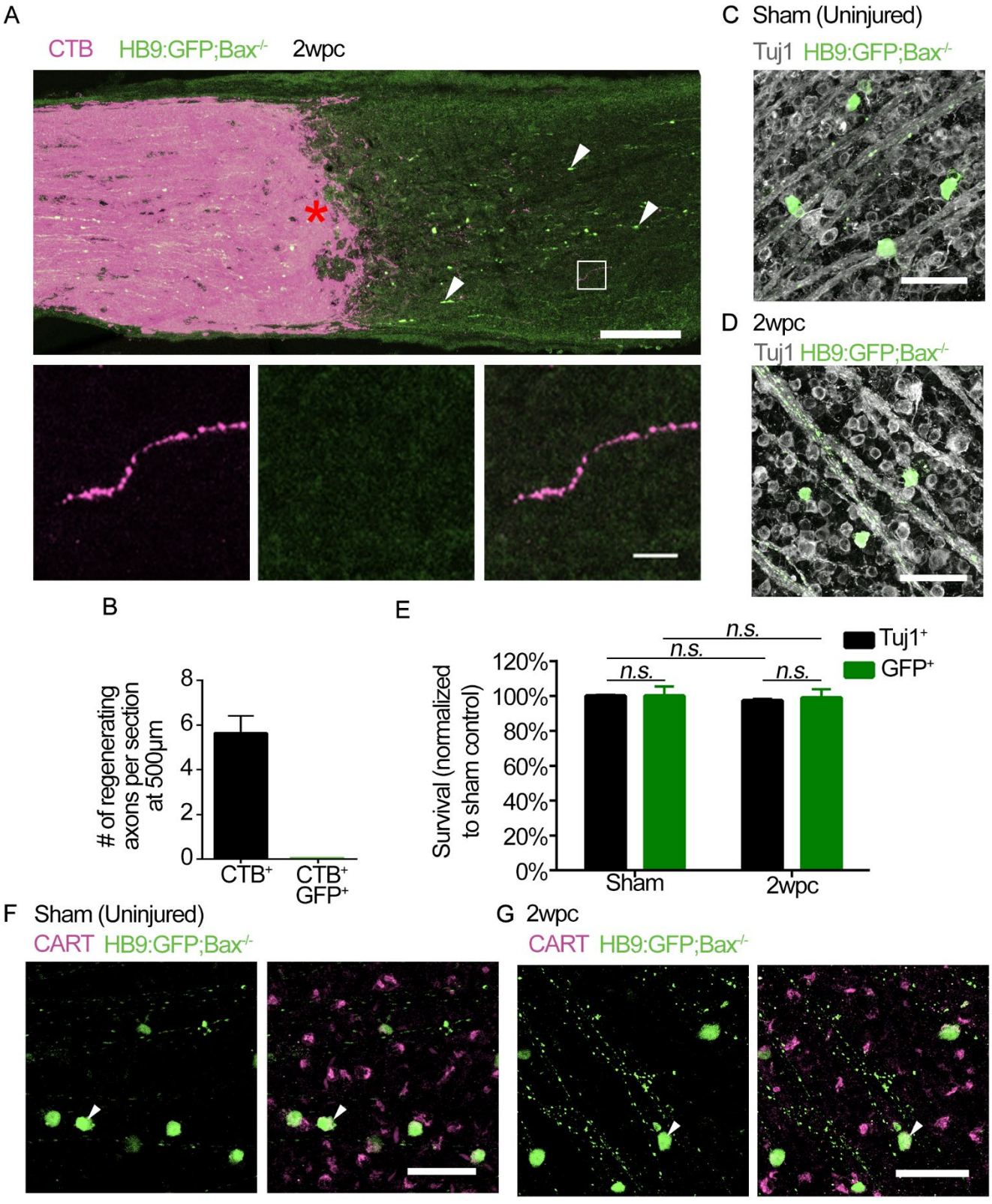
(G) Optic nerve showing tdTomato-labeled axons (grey) in AAV-PLAP injected Opn4$^{Cre/+}$;R26-tdTomato$^{f/f}$ 6 weeks following crush. Scale bar, 100 μm. **(H)** High-magnification image of the boxed areas in (G). CTB (green), tdTomato (magenta) and merge. Scale bar, 10 μm. **(I)** Quantification of axon regeneration for (G) showing the number of CTB$^+$ or CTB$^+$ tdTomato$^+$ axons at each distance. **(J)** Optic nerve showing tdTomato-labeled axons (grey) in AAV-PLAP injected Opn4$^{CreERT/+}$;R26-tdTomato$^{f/f}$ mice 6 weeks following crush. Scale bar, 100 μm. **(K)** High-magnification image of the boxed areas in (J). CTB (green), tdTomato (magenta) and merge. Scale bar, 10 μm. **(L)** Quantification of axon regeneration for (J) showing the number of CTB$^+$ or CTB$^+$ tdTomato$^+$ axons at each distance. *n=6 for Opn4$^{Cre/+}$;R26-tdTomato$^{f/f}$, 7 for Opn4$^{CreERT/+}$;R26-tdTomato$^{f/f}$*. Error bars, SEM.

Figure S2


A Sham (Uninjured)
Opn4Cre/+;
R26-tdTomatof/f
(Opn4-het) Tuj1
C Sham (Uninjured)
Opn4Cre/Cre;
R26-tdTomatof/f
(Opn4-KO) Tuj1
B 3wpc AAV-CNTF
Opn4Cre/+;
R26-tdTomatof/f
(Opn4-het) Tuj1
D 3wpc AAV-CNTF
Opn4Cre/Cre;
R26-tdTomatof/f
(Opn4-KO) Tuj1
E
n.s.
n.s.
**
**
Tuj1+
tdTomato+
Survival (normalized to sham control)
100%
80%
60%
40%
20%
0%
Opn4-het
Opn4-KO
F Sham (Uninjured)
Opn4CreERT/+;
R26-tdTomatof/f
(wt) Tuj1
H Sham (Uninjured)
Opn4CreERT/+;
Igf1f/f;R26-tdTomatof/f
(Igf1-KO) Tuj1
J Sham (Uninjured)
Opn4CreERT/+;
Tbr2f/f;R26-tdTomatof/f
(Tbr2-KO) Tuj1
G 3wpc AAV-CNTF
Opn4CreERT/+;
R26-tdTomatof/f
(wt) Tuj1
I 3wpc AAV-CNTF
Opn4CreERT/+;
Igf1f/f;R26-tdTomatof/f
(Igf1-KO) Tuj1
K 3wpc AAV-CNTF
Opn4CreERT/+;
Tbr2f/f;R26-tdTomatof/f
(Tbr2-KO) Tuj1
L
% suvival tdTomato+ RGCs (normalized to sham)
150%
100%
50%
0%
n.s.
**
wt
Igf1-KO
Tbr2-KO

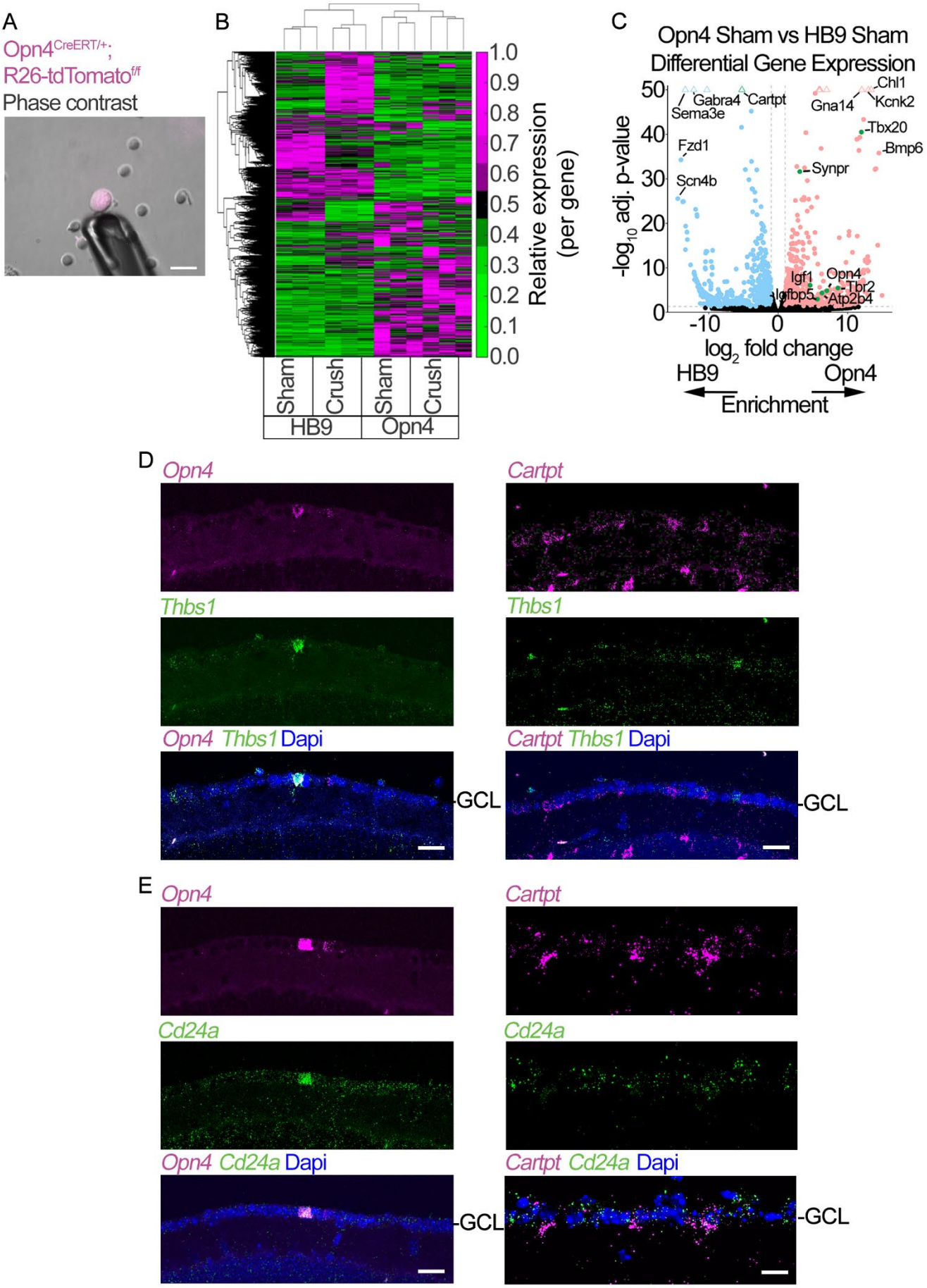
Supplementary Figure 2 (Related to Figure 3): Opn4, Igf1, Tbr2 knock-out retinas and RGC survival. (A-D) Retinal whole mounts showing Tuj1-labeled RGCs (grey) and tdTomato-labeled ipRGCs (magenta) (A and B) $Opn4^{Cre/+}$;R26-$tdTomato^{f/f}$ (Opn4-het) or (C and D) $Opn4^{Cre/Cre}$;R26-$tdTomato^{f/f}$ (Opn4-KO). (A and C) Sham (uninjured). (B and D) AAV-CNTF and 3 weeks post crush. Scale bars, 50 μm. **(E)** Quantification of RGC survival for (A-D). Percentage of $Tuj1^{+}$ and $Tuj1^{+}tdTomato^{+}$ neurons in injured retina normalized to the sham retinas in the Opn4-het and Opn4-KO mice. *ANOVA with Tukey's post-hoc ** $p \leq 0.01$.* **(F-K)** Retinal whole mounts showing Tuj1-labeled RGCs (grey) and tdTomato-labeled ipRGCs (magenta) of (F and G) $Opn4^{CreERT/+}$;R26-$tdTomato^{f/f}$ (wt), (H and I) $Opn4^{CreER/+}$;$Igf1^{f/f}$;R26-$tdTomato^{f/f}$ (Igf1-KO) and (J and K) $Opn4^{CreER/+}$;$Tbr2^{f/f}$;R26-$tdTomato^{f/f}$ (Tbr2-KO). (F, H and J) Sham (uninjured). (G, I and K) AAV-CNTF and 3 weeks post crush. Scale bars, 50 μm. (**L**) Quantification of RGC survival for (F-K). *** $p < 0.01$, n.s. $p \geq 0.05$, ANOVA, Bonferroni's post hoc vs wt. n=8 for $Opn4^{CreERT/+}$;R26-$tdTomato^{f/f}$, 6 for $Opn4^{CreERT/+}$;$Igf1^{f/f}$;R26-$tdTomato^{f/f}$, 7 for $Opn4^{CreERT/+}$;$Tbr2^{f/f}$;R26-$tdTomato^{f/f}$.* Error bars, SEM.

Figure S3


A
CTB HB9:GFP;Bax-/- 2wpc
C Sham (Uninjured)
Tuj1 HB9:GFP;Bax-/-
D 2wpc
Tuj1 HB9:GFP;Bax-/-
B
# of regenerating axons per section at 500μm
8
6
4
2
0
CTB+
CTB+ GFP+
E
Survival (normalized to sham control)
120%
100%
80%
60%
40%
20%
0%
n.s.
n.s.
n.s.
n.s.
Tuj1+
GFP+
Sham
2wpc
F Sham (Uninjured)
CART HB9:GFP;Bax-/-
G 2wpc
CART HB9:GFP;Bax-/-

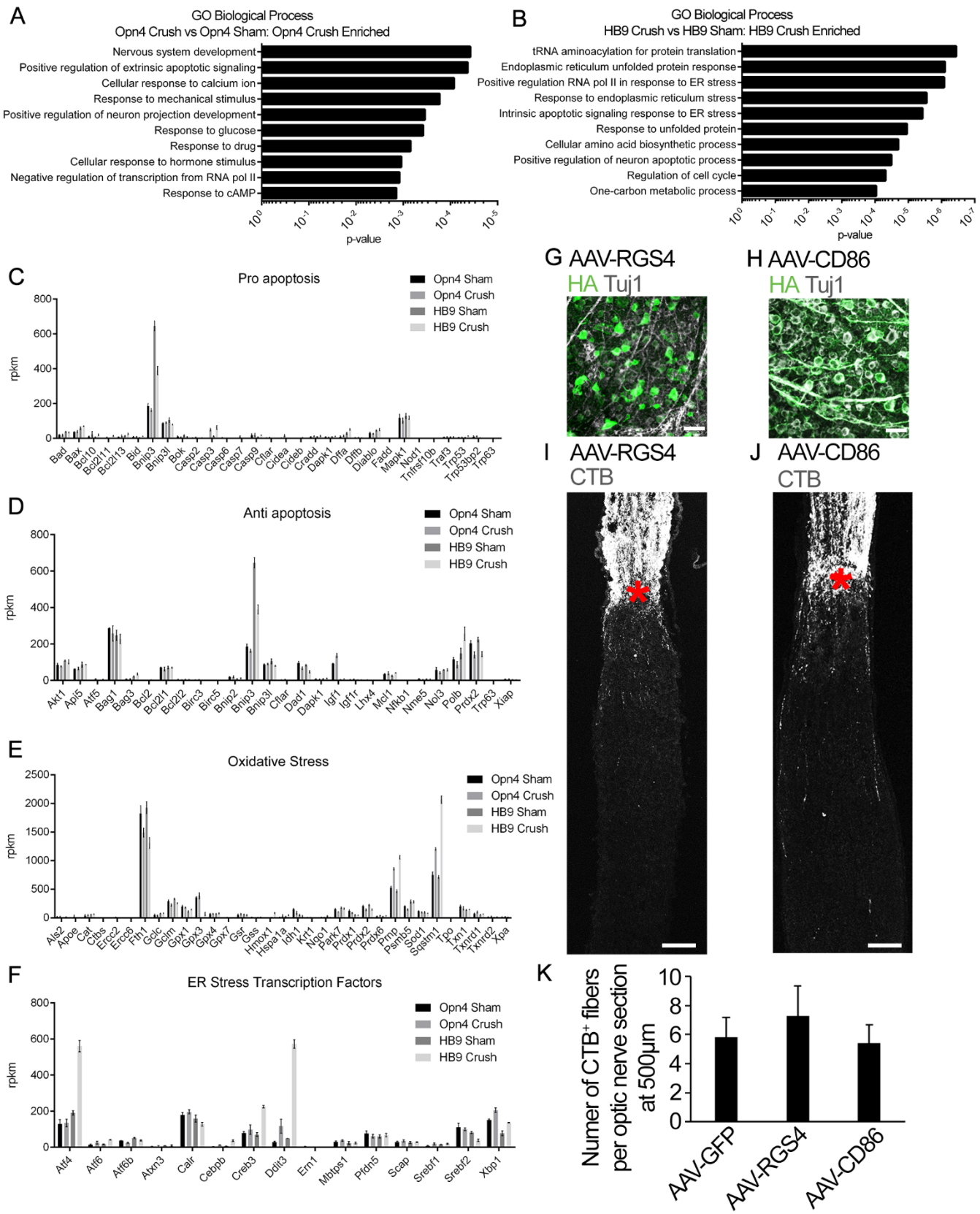
Supplementary Figure 3 (Related to Figure 4): Opn4$^{CreERT/+}$;R26-tdTomato$^{f/f}$ RGCs are, but HB9:GFP RGCs are not capable of spontaneous axon regeneration following optic nerve crush even under Bax KO background. (A) Image of an optic nerve section showing CTB-labeled axons (magenta) and GFP-labeled HB9:GFP;Bax$^{-/-}$ axons (green) 2 weeks post crush. Arrow heads, examples of distal degenerating (CTB-negative) axon fragments. Asterisks, lesion site. Scale bars, 100 μm. Insets below, high-magnification of the boxed area in (A) showing CTB (magenta), GFP (green), and merge. Scale bar, 10 μm. **(B)** Quantification of axon regeneration for (A). The number of CTB^{+} and CTB^{+} GFP^{+} axons at 500 μm distal to the lesion site. Error bars, SEM. **(C and D)** Images of retinal whole mounts showing Tuj1-labeled RGCs (grey) and GFP-labeled HB9:GFP;Bax$^{-/-}$ RGCs (green). (C) Sham (uninjured). (D) 2 weeks post optic nerve crush. Scale bars, 50 μm. **(E)** Quantification of RGC survival for (C and D). Percentage of Tuj1^{+} or GFP^{+} RGCs in HB9:GFP;Bax$^{-/-}$ mice normalized to the sham retinas. *n.s. $p \geq 0.05$*, *ANOVA, Tukey's post-hoc test*. Error bars, SEM. *n=3 per condition*. **(F and G)** Images of retinal whole mounts showing CART-labeled ooDSGCs (magenta) and GFP-labeled HB9:GFP;Bax$^{-/-}$ RGCs (green). Arrow heads show cells with co-localization. (F) Sham (uninjured). (G) 2 weeks post optic nerve crush. Scale bars, 50 μm.

Figure S4


A
Opn4CreERT/+;
R26-tdTomatof/f
Phase contrast
B
Relative expression
(per gene)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Sham
Crush
Sham
Crush
HB9
Opn4
C
Opn4 Sham vs HB9 Sham
Differential Gene Expression
-log10 adj. p-value
log2 fold change
HB9
Opn4
Enrichment
Sema3e
Gabra4
Cartpt
Gna14
Chl1
Kcnk2
Tbx20
Bmp6
Fzd1
Synpr
Scn4b
Igf1
Opn4
Tbr2
Igfbp5
Atp2b4
D
Opn4
Cartpt
Thbs1
Thbs1
Opn4 Thbs1 Dapi
Cartpt Thbs1 Dapi
GCL
GCL
E
Opn4
Cartpt
Cd24a
Cd24a
Opn4 Cd24a Dapi
Cartpt Cd24a Dapi
GCL
GCL

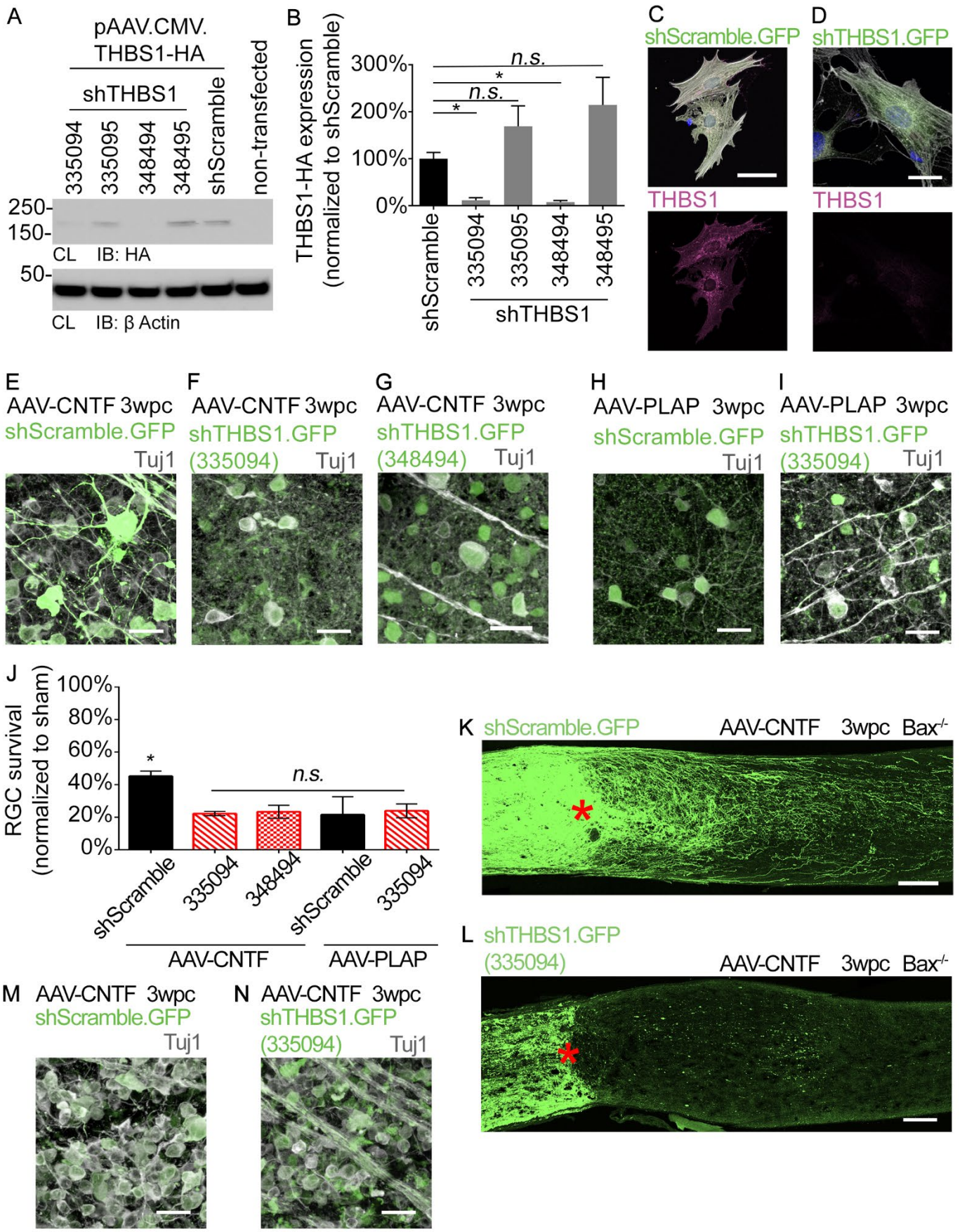
Supplementary Figure 4 (Related to Figure 4): RNA-seq performed in isolated Opn4 and HB9 RGCs, and fluorescent in situ hybridization (FISH) validation. (A) Example of a tdTomato-labeled (magenta) RGC being manually collected. Scale bar, 20 μm. **(B)** Hierarchical clustering of RNA-seq samples, showing normalized expression of each gene. **(C)** Volcano plot showing differential gene expression between uninjured Opn4 and HB9 RGCs. Positive $\log_2$FC indicates higher expression in Opn4 RGCs relative to HB9 RGCs. Genes are considered significantly different if expression ±1 $\log_2$FC and *adjusted (adj) p-value ≤ 0.05,* vertical and horizontal reference lines at respective values. Triangles indicate genes with an *adj p-value* $< 1*10^{-50}$*, these values* were fixed at $1*10^{-50}$. Green points indicate that a gene is a known marker of the RGC subtypes. **(D and E)** FISH validation in vivo. (D) Retina sections 3 days post crush, probed for *Opn4* (magenta) or *Cartpt* (magenta) and *Thbs1* (green). Dapi in blue. (E) Retina sections 3 days post crush, probed for *Opn4* (magenta) or *Cartpt* (magenta) and *cd24a* (green). Dapi in blue. Scale bar, 25 μm.

Figure S5


A
GO Biological Process
Opn4 Crush vs Opn4 Sham: Opn4 Crush Enriched
Nervous system development
Positive regulation of extrinsic apoptotic signaling
Cellular response to calcium ion
Response to mechanical stimulus
Positive regulation of neuron projection development
Response to glucose
Response to drug
Cellular response to hormone stimulus
Negative regulation of transcription from RNA pol II
Response to cAMP
p-value
B
GO Biological Process
HB9 Crush vs HB9 Sham: HB9 Crush Enriched
tRNA aminoacylation for protein translation
Endoplasmic reticulum unfolded protein response
Positive regulation RNA pol II in response to ER stress
Response to endoplasmic reticulum stress
Intrinsic apoptotic signaling response to ER stress
Response to unfolded protein
Cellular amino acid biosynthetic process
Positive regulation of neuron apoptotic process
Regulation of cell cycle
One-carbon metabolic process
p-value
C
Pro apoptosis
Opn4 Sham
Opn4 Crush
HB9 Sham
HB9 Crush
rpkm
Bad Bax Bcl10 Bcl2l11 Bcl2l13 Bid Bnip3 Bnip3l Bok Casp2 Casp3 Casp6 Casp7 Casp9 Cflar Cidea Cideb Cradd Dapk1 Dffa Dffb Diablo Fadd Mapk1 Nod1 Tnfrsf10b Traf3 Trp53 Trp53bp2 Trp63
D
Anti apoptosis
Opn4 Sham
Opn4 Crush
HB9 Sham
HB9 Crush
rpkm
Akt1 Api5 Atf5 Bag1 Bag3 Bcl2 Bcl2l1 Bcl2l2 Birc3 Birc5 Bnip2 Bnip3 Bnip3l Cflar Dad1 Dapk1 Igf1 Igf1r Lhx4 Mcl1 Nfkb1 Nme5 Nol3 Polb Prdx2 Trp63 Xiap
E
Oxidative Stress
Opn4 Sham
Opn4 Crush
HB9 Sham
HB9 Crush
rpkm
Als2 Apoe Cat Cbs Ercc2 Ercc6 Fth1 Gclc Gdm Gpx1 Gpx3 Gpx4 Gpx7 Gsr Gss Hmox1 Hspa1a Idh1 Krt1 Ngo1 Park7 Prdx1 Prdx2 Prdx6 Prnp Psmb5 Sod1 Sqstm1 Tpo Txn1 Txnrd1 Txnrd2 Xpa
F
ER Stress Transcription Factors
Opn4 Sham
Opn4 Crush
HB9 Sham
HB9 Crush
rpkm
Atf4 Atf6 Atf6b Atxn3 Calr Cebpb Creb3 Ddit3 Ern1 Mbtps1 Pfdn5 Scap Srebf1 Srebf2 Xbp1
G AAV-RGS4
HA Tuj1
H AAV-CD86
HA Tuj1
I AAV-RGS4
CTB
J AAV-CD86
CTB
K
Numer of CTB+ fibers per optic nerve section at 500µm
AAV-GFP
AAV-RGS4
AAV-CD86

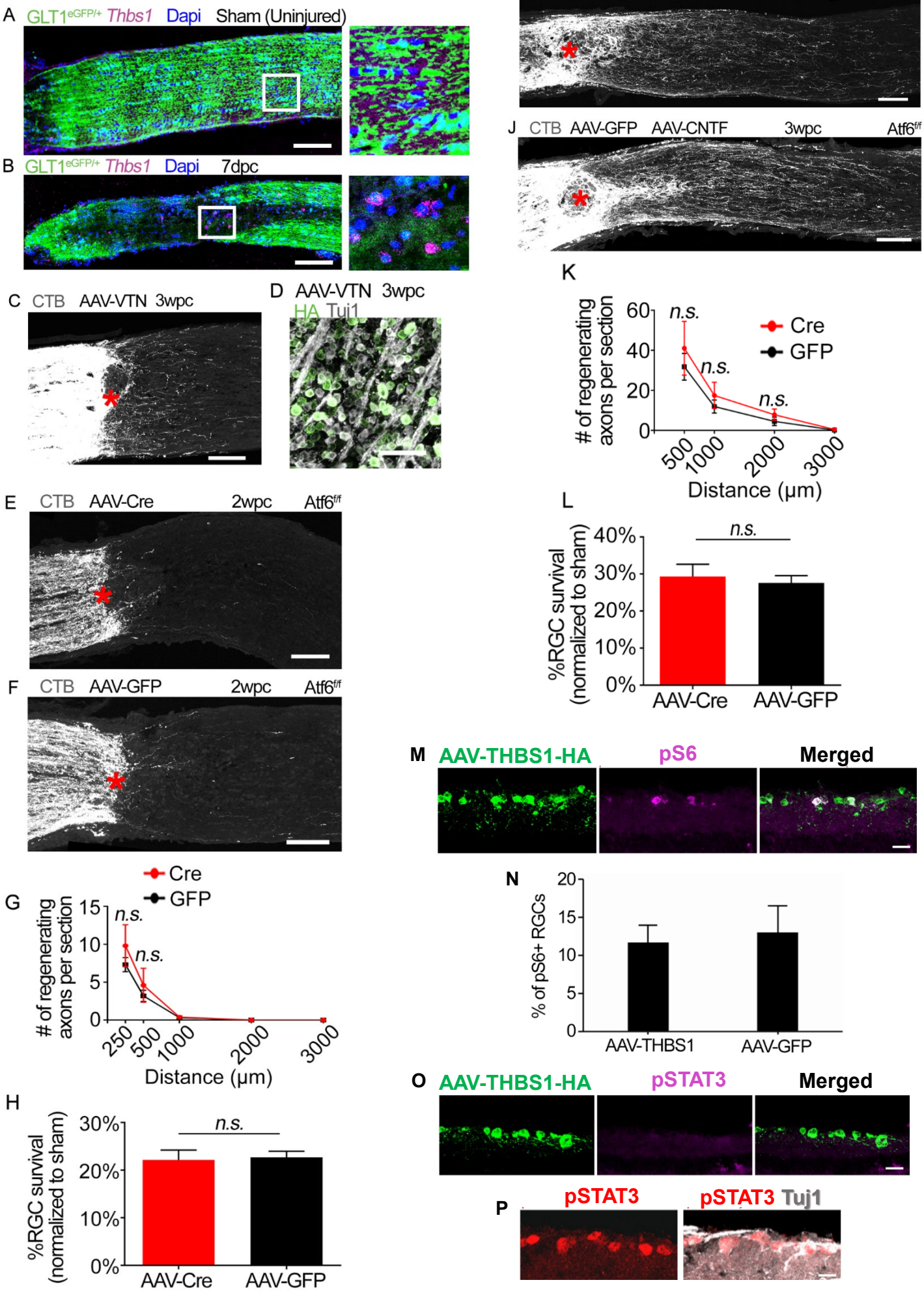
Supplementary Figure 5 (Related to Figure 4): Pathway and gene set analyses, and in vivo examination of effects of overexpressing select candidate genes. (A and B) Top 10 GO biological processes enriched for each comparison as stated in graph title. Enriched genes defined as ±1 $\log_2$FC and adjusted (adj) p-value ≤ 0.05. **(C-F)** Average expression of genes associated with (C) pro-apoptosis, (D) anti-apoptosis, (E) ameliorating oxidative stress and (F) ER stress transcription factors. Error bars, SEM. **(G and H)** Representative retinas of (G) AAV2-RGS4-HA and (H) AAV2-CD86-HA injected C57BL/6J mice after immunostaining using Tuj1 and HA antibodies. Retinas were removed 2 weeks after intravitreal AAV injection. Scale bar, 25 μm. **(I and J)** Representative images of optic nerve sections showing CTB-labeled axons 3 weeks post crush (wpc). C57BL/6J mice received an intravitreal injection of either (I) AAV2-RGS4-HA or (J) AAV2-CD86-HA at 2 weeks prior to crush. Asterisk, lesion site. Scale bar, 100 μm. **(K)** Quantification of axon regeneration for (I and J). The number of CTB^+ axons at 500 μm distal to the lesion site. *n=7 for AAV-RGS4, 5 for AAV-CD86, 5 for AAV-GFP.*

Figure S6


A
pAAV.CMV.
THBS1-HA
shTHBS1
335094
335095
348494
348495
shScramble
non-transfected
250-
150-
CL IB: HA
50-
CL IB: β Actin
B
THBS1-HA expression
(normalized to shScramble)
300%
200%
100%
0%
n.s.
*
n.s.
*
shScramble
335094
335095
348494
348495
shTHBS1
C
shScramble.GFP
THBS1
D
shTHBS1.GFP
THBS1
E
AAV-CNTF 3wpc
shScramble.GFP
Tuj1
F
AAV-CNTF 3wpc
shTHBS1.GFP
(335094)
Tuj1
G
AAV-CNTF 3wpc
shTHBS1.GFP
(348494)
Tuj1
H
AAV-PLAP 3wpc
shScramble.GFP
Tuj1
I
AAV-PLAP 3wpc
shTHBS1.GFP
(335094)
Tuj1
J
RGC survival
(normalized to sham)
100%
80%
60%
40%
20%
0%
*
n.s.
shScramble
335094
348494
shScramble
335094
AAV-CNTF
AAV-PLAP
K
shScramble.GFP
AAV-CNTF 3wpc Bax-/-
*
L
shTHBS1.GFP
(335094)
AAV-CNTF 3wpc Bax-/-
*
M
AAV-CNTF 3wpc
shScramble.GFP
Tuj1
N
AAV-CNTF 3wpc
shTHBS1.GFP
(335094)
Tuj1

Supplementary Figure 6 (Related to Figure 6): Validation of THBS1 knock-down and additional RGC survival and regeneration data from the THBS1-knockdown experiment. **(A)** HEK293T cells were co-transfected with AAV-THBS1-HA and shRNA containing plasmid. Cell lysate (CL) of HEK293T cells immunoblotted (IB) for HA and β-Actin. shTHBS1 identifiers are the last six digits of Broad Institutes TCRN identifier. **(B)** Densitometry quantification of (A). Each sample normalized to β-Actin. Error bars, SEM. *$p < 0.05$, n.s. $p \geq 0.05$, n=4 each condition, ANOVA with Bonferroni post-hoc vs shScramble.* **(C and D)** Mouse embryonic fibroblasts nucleofected with shRNA-GFP plasmids. Immunocytochemistry for GFP (green), Phalloidin (grey), Hoechst (blue) and THBS1 (magenta). Top panels, merged images showing all colors. Bottom panels, images showing THBS1 immunoreactivity only. (C) shScramble-treated cells. (D) shTHBS1 335094-treated cells. Scale bars, 50 μm. **(E-I)** Retinas showing Tuj1 (grey) and AAV-shRNA.GFP (shScramble or shTHBS1) infected GFP-labeled RGCs (green). (E, F and G) AAV-CNTF injected mice 3 weeks post crush (wpc). (H and I) AAV-PLAP injected mice 3wpc. Scale bar, 25 μm. **(J)** Quantification of RGC survival for (E-I). *$p < 0.05$ *AAV-CNTF + shScramble vs all others, n.s. $p \geq 0.05$ all other comparisons*, *ANOVA, Tukey's post-hoc test*. Error bars, SEM. **(K and L)** Optic nerves showing AAV-shRNA.GFP infected GFP-labeled RGC axons (green) in $Bax^{-/-}$ mice 3 weeks after crush and AAV-CNTF. (K) AAV-shScramble.GFP animal, (L) AAV-shTHBS1(335094).GFP animal. Scale bars, 100 μm. (**M and N)** Retinas showing Tuj1 (grey) and AAV-shRNA.GFP infected GFP-labeled RGCs (green) in $Bax^{-/-}$ mice 3 weeks after crush and AAV-CNTF. (M) AAV-shScramble.GFP animal, (N) AAV-shTHBS1(335094).GFP animal. Scale bars, 25 μm.

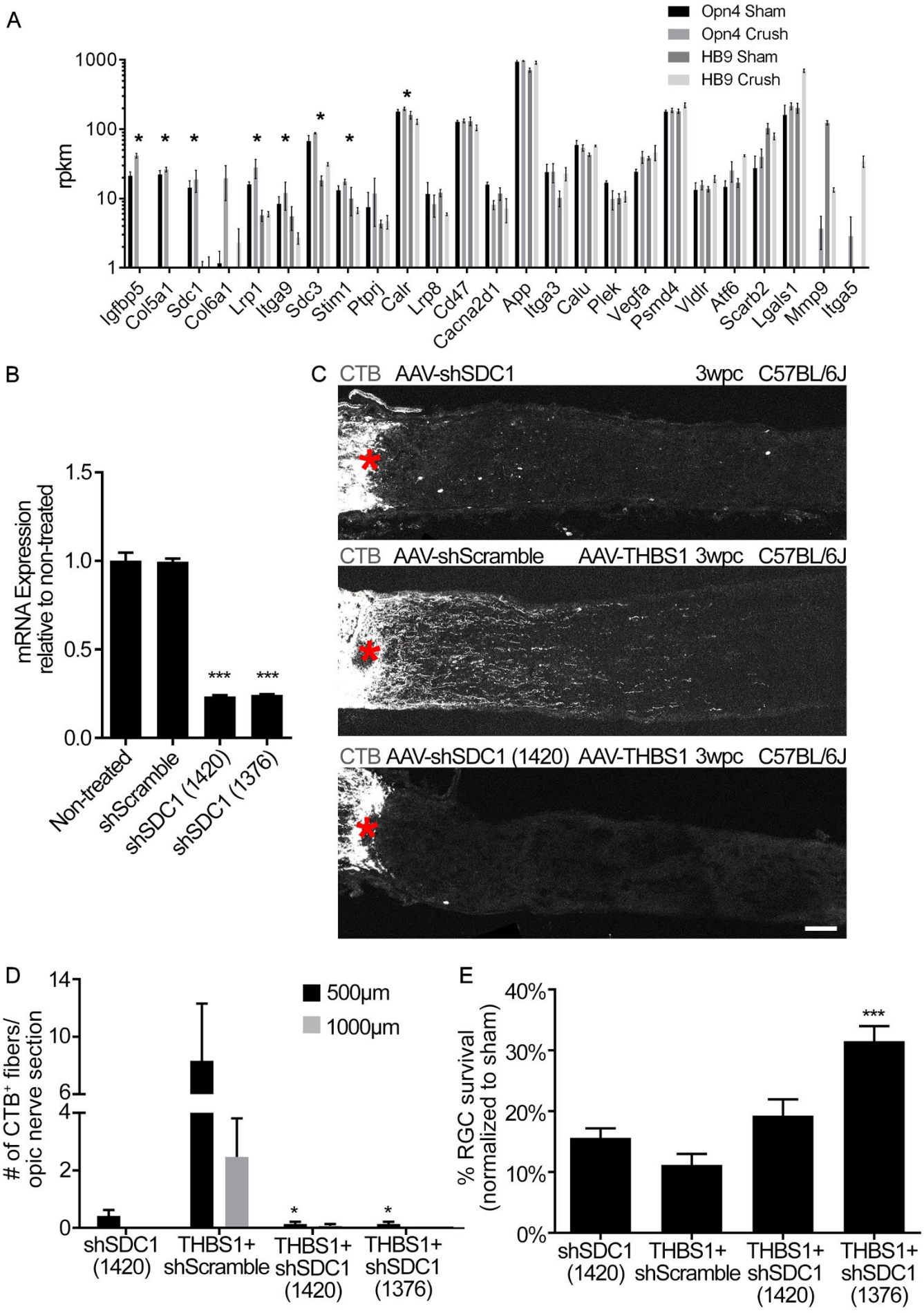
Figure S7


A GLT1eGFP/+ Thbs1 Dapi Sham (Uninjured)
B GLT1eGFP/+ Thbs1 Dapi 7dpc
C CTB AAV-VTN 3wpc
D AAV-VTN 3wpc
HA Tuj1
E CTB AAV-Cre 2wpc Atf6f/f
F CTB AAV-GFP 2wpc Atf6f/f
G
Cre
GFP
# of regenerating axons per section
n.s.
n.s.
250
500
1000
2000
3000
Distance (μm)
H
%RGC survival (normalized to sham)
n.s.
AAV-Cre
AAV-GFP
I CTB AAV-Cre AAV-CNTF 3wpc Atf6f/f
J CTB AAV-GFP AAV-CNTF 3wpc Atf6f/f
K
# of regenerating axons per section
n.s.
n.s.
n.s.
Cre
GFP
500
1000
2000
3000
Distance (μm)
L
%RGC survival (normalized to sham)
n.s.
AAV-Cre
AAV-GFP
M AAV-THBS1-HA pS6 Merged
N
% of pS6+ RGCs
AAV-THBS1
AAV-GFP
O AAV-THBS1-HA pSTAT3 Merged
P pSTAT3 pSTAT3 Tuj1

Supplementary Figure 7 (Related to Figure 7): Examination of THBS1's potential downstream mechanism: ATF6 (a known intracellular effector of THBS1), pS6 and pSTAT3. (A) Uninjured optic nerve of adult *GLT1-eGFP* mouse (a glial cell-reporter mouse) subjected to FISH staining. *Thbs1* in magenta; GFP in green; Dapi in blue. Right image shows higher magnification of the boxed region on the left. **(B)** Crushed optic nerve of adult GLT1-eGFP mouse. The regions that are devoid of GFP^+ cells indicate the lesion site. *Thbs1* in magenta; GFP in green; Dapi in blue. Scale bar, 100 μm. **(C)** Representative optic nerve of an AAV-vitronectin (VTN)-HA injected mouse. AAV-VTN was injected 2 weeks before crush. wpc, weeks post crush. Scale bar, 100 μm. **(D)** Whole-mount retina from an AAV-VTN-HA injected mouse stained with HA and Tuj1 antibodies. Retina was removed 2 weeks after AAV injection to validate VTN-HA expression. Scale bar, 100 μm. **(E and F)** Optic nerves showing CTB-labeled axons (grey) from $Atf6^{f/f}$ mice. (E) AAV-Cre injected animal 2 weeks after crush, (F) AAV-GFP injected animal. **(G)** Quantification of axon regeneration for (E and F). **(H)** Quantification of RGC survival for (E and F). Percentage of $Tuj1^+$ RGCs in each group normalized to the sham uninjured retina. **(I)** AAV-Cre + AAV-CNTF injected $Atf6^{f/f}$ animal 3 weeks post crush, and **(J)** AAV-GFP + AAV-CNTF injected $Atf6^{f/f}$ animal. Asterisks, lesion site. Scale bars, 100 μm. **(K)** Quantification of axon regeneration for (I and J). **(L)** Quantification of RGC survival for (I and J). Percentage of $Tuj1^+$ RGCs in each group normalized to the sham uninjured retina. *n.s.* $p \geq 0.05$*, 2 tailed unpaired t-test at each distance.* Error bars, SEM. *n = 6 for AAV-Cre + 2 week post crush, 5 for AAV-GFP + 2 week post crush, 6 for both AAV-CNTF 3wpc groups.*

(M) Images of retina sections (3 days post crush) from mice injected with AAV-THBS1-HA, co-stained against pS6 (magenta). HA in green. Scale bar, 25 μm. **(N)** Quantification of percentage of $pS6^+$ RGCs per retina in AAV-THBS1 and AAV-GFP (control) animal groups. n= 3 per condition. Error bars, SEM. **(O)** Images of retina sections (3 days post crush) from mice injected with AAV-THBS1-HA, co-stained against pSTAT3 (magenta). Scale bar, 25 μm. **(P)** Validation of the pSTAT3 antibody. To ensure that the lack of $pSTAT3^+$ RGCs seen after AAV-THBS injection is not due to staining issue, we performed the immunostaining using the same protocol on retinal sections from a mouse subjected to intravitreal AAV-CNTF injection, known to activate STAT3 in injured RGCs. pSTAT3 in red Tuj1 in grey.

Figure S8


A
Opn4 Sham
Opn4 Crush
HB9 Sham
HB9 Crush
rpkm
1000
100
10
1
Igfbp5
Col5a1
Sdc1
Col6a1
Lrp1
Itga9
Sdc3
Stim1
Ptprj
Calr
Lrp8
Cd47
Cacna2d1
App
Itga3
Calu
Plek
Vegfa
Psmd4
Vldlr
Atf6
Scarb2
Lgals1
Mmp9
Itga5
B
mRNA Expression relative to non-treated
1.5
1.0
0.5
0.0
***
***
Non-treated
shScramble
shSDC1 (1420)
shSDC1 (1376)
C
CTB AAV-shSDC1 3wpc C57BL/6J
CTB AAV-shScramble AAV-THBS1 3wpc C57BL/6J
CTB AAV-shSDC1 (1420) AAV-THBS1 3wpc C57BL/6J
D
# of CTB+ fibers/ opic nerve section
14
10
6
4
2
0
500µm
1000µm
*
*
shSDC1 (1420)
THBS1+ shScramble
THBS1+ shSDC1 (1420)
THBS1+ shSDC1 (1376)
E
% RGC survival (normalized to sham)
40%
30%
20%
10%
0%
***
shSDC1 (1420)
THBS1+ shScramble
THBS1+ shSDC1 (1420)
THBS1+ shSDC1 (1376)

Supplementary Figure 8 (Related to Figures): Syndecan-1 is highly expressed in Opn4 but not in HB9 RGCs: THBS1 induced- and spontaneous RGC axon regeneration require syndecan-1. (A) Graph showing the expression levels of genes known to bind to THBS1 in Opn4 and HB9 RGCs with (crush) or without (sham) optic nerve crush. Genes were included if geometric mean expression was greater than 10 RPKM in Opn4 Crush or HB9 Crush condition. * *FDR adjusted p-value ≤ 0.05*. **(B)** Validation of two different shRNAs against syndecan-1. shRNA-GFP plasmids encoding control (shScramble) or syndecan-1-targeting shRNAs (shSDC1) were validated in Neuro-2a cells. shSDC1 (1420), shRNA targeting mRNA nucleotide position 1420-1439; shSDC1 (1376), shRNA targeting mRNA nucleotide position 1376-1396. Values are presented relative to the syndecan-1 expression in non-treated cells (n=3; mean ± sd). *** *p < 0.0001, ANOVA, Bonferroni's post hoc vs shScramble.* **(C)** Representative optic nerve sections from animals subjected to either AAV-shSDC1 alone, AAV-THBS1 + AAV-shScramble or AAV-THBS1 + AAV-shSDC1. Animals (C57BL/6J) received co-injection of the AAVs followed by crush 2 weeks later. Regeneration was analyzed about 2 weeks after crush. Asterisks, lesion site. Scale bar, 100 μm. **(D)** Quantification of axon regeneration for (C). The number of CTB^+ fibers at each distance distal to the lesion site. *n=4 for AAV-shSDC1 (1420), 5 for AAV-THBS1 + AAV-shScramble, AAV-THBS1 + AAV-shSDC1 (1420) and AAV-THBS1 + AAV-shSDC1 (1376). ANOVA with Bonferroni post-hoc vs THBS1 + shScramble.* * *p < 0.05;* Error bars, SEM. **(E)** Quantification of RGC survival for (C and D). Percentage of $Tuj1^+$ RGCs in each group normalized to the sham uninjured retinas. *ANOVA with Bonferroni post-hoc vs THBS1 + shScramble.* *** *p < 0.001;* Error bars, SEM.

Table S1. qPCR Primers (Related to Figures 1, 3 and S8)

Gene Symbol	Ref-seq ID (one isoform)	Forward (5' -> 3')	Reverse (5' -> 3')
Gapdh	NM_008084.3	CAAAATGGTGAAGGTCGGTGTG	TGATGTTAGTGGGGTCTCGCTC
Opn4	NM_013887.2	TGGTGATCACACGTCCACTG	GCACGTAGGCACTCCAACC
Tbr2	NM_010136.3	CAGGGTTCTCCGCTCTACGG	CATGCGCCTGCCCTGTTTG
Rho	NM_145383.1	GCCACCACTCAGAAGGCAG	GATGGAAGAGCTCTTAGCAAAG
cJun	NM_010591.2	CCTTCTACGACGATGCCCTC	GGTTCAAGGTCATGCTCTGTTT
Cartpt	NM_013732.7	ACGAGAAGGAGCTGATCGAAG	CAGTCACACAGCTTCCCGAT
Atf3	NM_007498.3	AACTGGCTTCCTGTGCACTT	TGAGGCCAGCTAGGTCATCT
Thbs1	NM_011580.4	AGTGGAAGAGCATCACGCTG	CACCACGTTGTTGTCAAGGG
sdc1	NM_011519.2	ATGAGACGCGCGGCGCTCTG	GCGTAGAACTCCTCCTGCTTGG

Table S2. Cloning Primers (Related to Figures 5, 6, 7 and S7 and S8)

Table 2: Cloning primers and oligonucleotides		
shRNA backbone primers	**Primer (5'-> 3')**	**Introduced Cut Site**
CMV_mutant F	tcgatCAATTGtcgccaccatggtgagcaa	MfeI
CMV_mutant R	tcgatCTCGAGaggcggggaggcg	XhoI
MluI-U6 F	tcgatACGCGTAGAGAGGGCCTATTTCCCATGA	MluI
MluI-U6 R	tcgatACGCGTAGATCTAGAATTCAAAAATGAAACCGA	MluI
shRNA oligonucleotides	**Oligonucleotide**	
shThbs1 (TCRN0000335094) (+)	CCGGGCTGGAAAGATTTCACTGCATCTCGAGATGCAGTGAAATCTTTCCAG CTTTTTG	
shThbs1 (TCRN0000335094) (-)	AATTCAAAAAGCTGGAAAGATTTCACTGCATCTCGAGATGCAGTGAAATCTTT CCAGC	
shThbs1 (TCRN0000335095) (+)	CCGGGCGCCTATTTACTTCCCACTACTCGAGTAGTGGGAAGTAAATAGGCG CTTTTTG	
shThbs1 (TCRN0000335095) (-)	AATTCAAAAAGCGCCTATTTACTTCCCACTACTCGAGTAGTGGGAAGTAAAT AGGCGC	
shThbs1 (TCRN0000348494) (+)	CCGGTGAAACCGATTTCCGACAATTCTCGAGAATTGTCGGAAATCGGTTTCA TTTTTG	
shThbs1 (TCRN0000348494) (-)	AATTCAAAAATGAAACCGATTTCCGACAATTCTCGAGAATTGTCGGAAATCG GTTTCA	
shThbs1 (TCRN0000348495) (+)	CCGGATCATCAGCTGCCAATCATAACTCGAGTTATGATTGGCAGCTGATGAT TTTTTG	
shThbs1 (TCRN0000348495) (-)	AATTCAAAAAATCATCAGCTGCCAATCATAACTCGAGTTATGATTGGCAGCT GATGAT	
shScramble (+)	CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTT GTTTTTG	
shScramble (-)	AATTCAAAAACAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATC TTGTTG	
shSdc1-1420 (+)	CCGGTCCACACCTGTCGTCCACTCTTCAAGAGAGAGTGGACGACAGGTGTG GTTTTTTG	
shSdc1-1420 (-)	AATTCAAAAAACCACACCTGTCGTCCACTCTCTCTTGAAGAGTGGACGACAGGTGTGGA	
shSdc1-1376 (+)	CCGGGCTTGGGTGCAAAGGGTTTCTCGAAAGAAACCCTTTGCACCCAAGCT TTTTG	
shSdc1-1376 (-)	AATTCAAAAAGCTTGGGTGCAAAGGGTTTCTTTCGAGAAACCCTTTGCACCC AAGC	
pscAAV.CMV.SV40.mcs oligonucleotides	**Oligonucleotide**	
Oligo_SV40_mcs (+)	GATCCTCTAGAGTCGACCTGCAGAAGCTTGCCTCGAGCAGCGCTGCTCGAG AGATCTA	
Oligo_SV40_mcs (-)	GGCCTAGATCTCTCGAGCAGCGCTGCTCGAGGCAAGCTTCTGCAGGTCGA CTCTAGAG	
pAAV.CMV.SV40 backbone primers	**Primer (5'-> 3')**	**Introduced Cut Site**
NotI_SV40 F	tcgatGCGGCCGCTCGTGACCTAGGCATATGCCAAG	NotI
NotI_SV40 R	tcgatGCGGCCGCAGTTTAAAAAACCTCCCACACCT	NotI

Table S2. continued

Thbs1	**Primer (5'-> 3')**	**Introduced Cut Site**
Thbs1-TSS F	tcgatAAGCTTatgaccatgGAGCTCCTGCGGGGACTA	HindIII
Thbs1-HA R	gctacCTCGAGTTAagcgtaatctggaacatcgtatgggtaGGAATCTCGACACTCGTATTTCAT	XhoI
pAAV.CMV.C-HA	**Oligonucleotide**	
Oligo_C-HA (+)	agcttacccatacgatgttccagattacgcttag	
Oligo_C-HA (-)	gatcctaagcgtaatctggaacatcgtatgggta	
Thbs1 truncation mutants	**Primer (5'-> 3')**	
Thbs1-T3-8 R	gcactgatgtcaaaattctcaggacagat	
Thbs1-EGFL1-3 R	Gcgcagatgatgccattgcctgca	
Thbs1-ΔTSR1	**Primer (5'-> 3')**	
TSR1	atggagctcctgcggggac	
TSR2	ggaatctcgacactcgtatttcatgtctg	
TSR3	ctctgctgacgatggcattgatggatgcctgtccaatccct	
TSR4	ggacaggcatccatcaatgccatcgtcagcagagtcgc	
Thbs1-ΔCC	**Primer (5'-> 3')**	
CC1	GCTGTGGAACGGAAAGACAACA	
CC2	AACAGGACGACCATGGAGAC	
CC3	GGCCTCTCCTGTCCTCCCCTCTGCTTTCACAATGG	
CC4	CAGAGGGGAGGACAGGAGAGGCCACAGATAGC	
Thbs4	**Primer (5'-> 3')**	
Thbs4-F	GAGTCACCATGCCGGCCCCA	
Thbs4-R	TTATCCAAGCGGTCGAAACTCTGG	
Bcl2	**Primer (5'-> 3')**	
Bcl2-F	GATGGCGCAAGCCGGGAGAA	
Bcl2-R	GCCTTGTGGCCCAGGTATGCACC	
Vitronectin	**Primer (5'-> 3')**	
Vtn-F	ATGGCACCCCTGAGGCCCTTTTTCATACTAGCCCTG	
Vtn-R	TTCTCAGAGGTCGGGCAGCC	
Cd86	**Primer (5'-> 3')**	
Cd86-F	ACCCACGATGGACCCCAGA	
Cd86-R	TCTGCATTTGGTTTTGCTGAAGC	
RGS4	**Primer (5'-> 3')**	
RGS4-F	atgtgcaaaggacttgcaggtctgccgg	
RGS4-R	gcacactgggagaccagggaagtgcagtct	
Syndecan 1	**Primer (5'-> 3')**	
Sdc1-F	ATGAGACGCGCGGCGCTCTG	
Sdc1-R	GCGTAGAACTCCTCCTGCTTGG	
pAAV.GFAP.Thbs1-HA.SV40-polyA	**Primer (5'-> 3')**	**Introduced Cut Site**
GFAP-F	CTGAGCTAGCAACATATCCTGGTGTGGAGT	NheI
GFAP-R	CATATCCGGACCCCGCGAGCAGCGGAGGTGATGCGT	BspEI
pAAV.GFAP.EGFP.SV40-polyA	**Primer (5'-> 3')**	**Introduced Cut Site**
EGFP-F	CATGAAGCTTATGGTGAGCAAGGGCGAGGA	HindIII
EGFP-R	CATGCTCGAGTTACTTGTACAGCTCGTCCATGCCGAGAGTGATCCCG	XhoI
Sanger Sequencing Primers		
Primer Name	**Primer (5' -> 3')**	
U6 F	CGCGTAGAGAGGGCCTATTT	
CMV R	tacacgcctaccgccca	
Thbs1_seq_1 F	ACCAACGTCCTTCTTACCCTTGA	
Thbs1_seq_2 F	TCACCATGGGACATCTGCTCT	
Thbs1_seq_3 F	TCCATTACAACCCAGCCCAGT	
Thbs1_seq_4 F	TGACCCTGGACTTGCTGTAGGT	