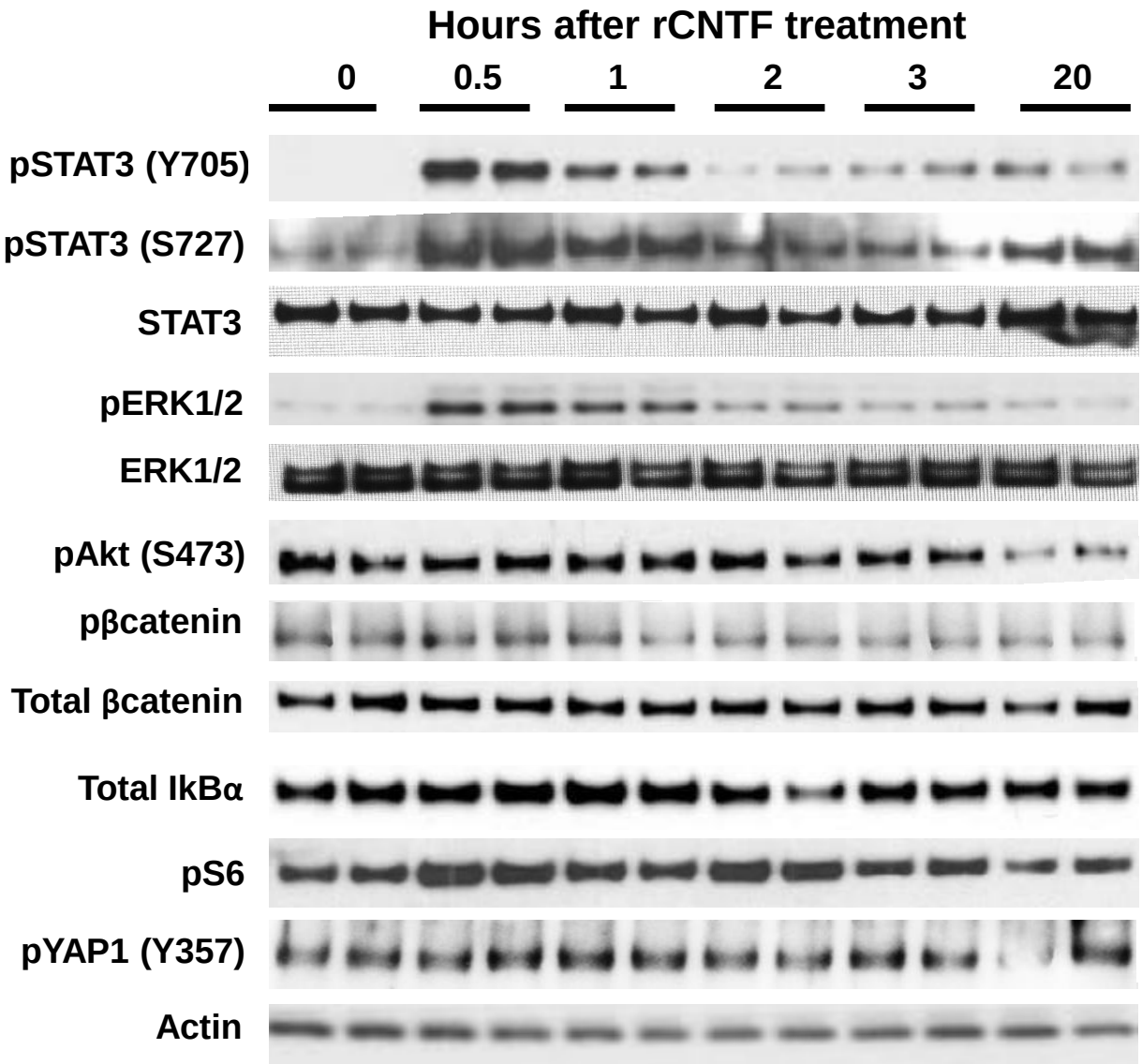
Hours after rCNTF treatment
0
0.5
1
2
3
20
pSTAT3 (Y705)
pSTAT3 (S727)
STAT3
pERK1/2
ERK1/2
pAkt (S473)
pβcatenin
Total βcatenin
Total IkBα
pS6
pYAP1 (Y357)
Actin

Figure S1

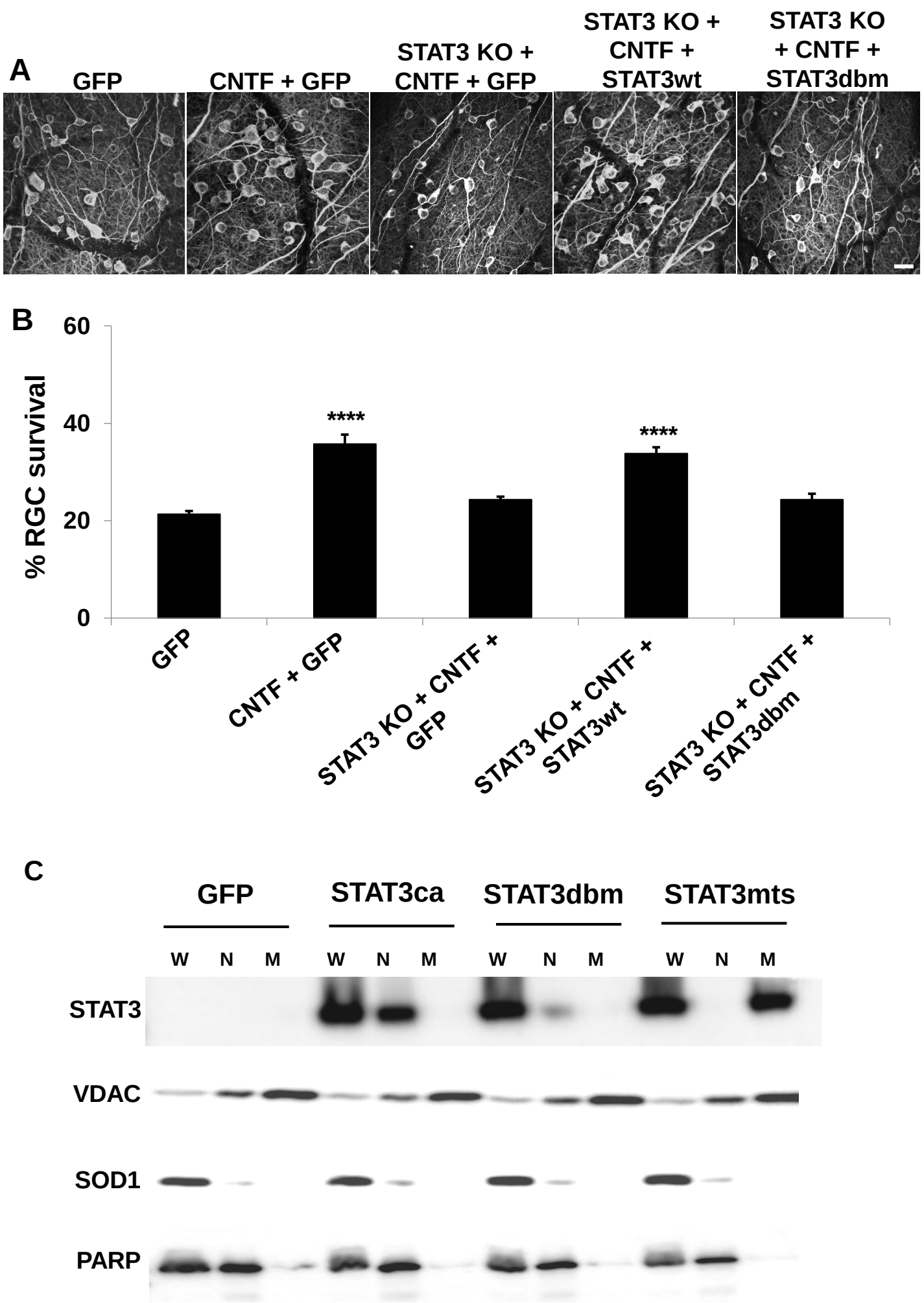
A
GFP
CNTF + GFP
STAT3 KO + CNTF + GFP
STAT3 KO + CNTF + STAT3wt
STAT3 KO + CNTF + STAT3dbm
B
% RGC survival
60
40
20
0
****
****
GFP
CNTF + GFP
STAT3 KO + CNTF + GFP
STAT3 KO + CNTF + STAT3wt
STAT3 KO + CNTF + STAT3dbm
C
GFP
STAT3ca
STAT3dbm
STAT3mts
W N M W N M W N M W N M
STAT3
VDAC
SOD1
PARP

Figure S2

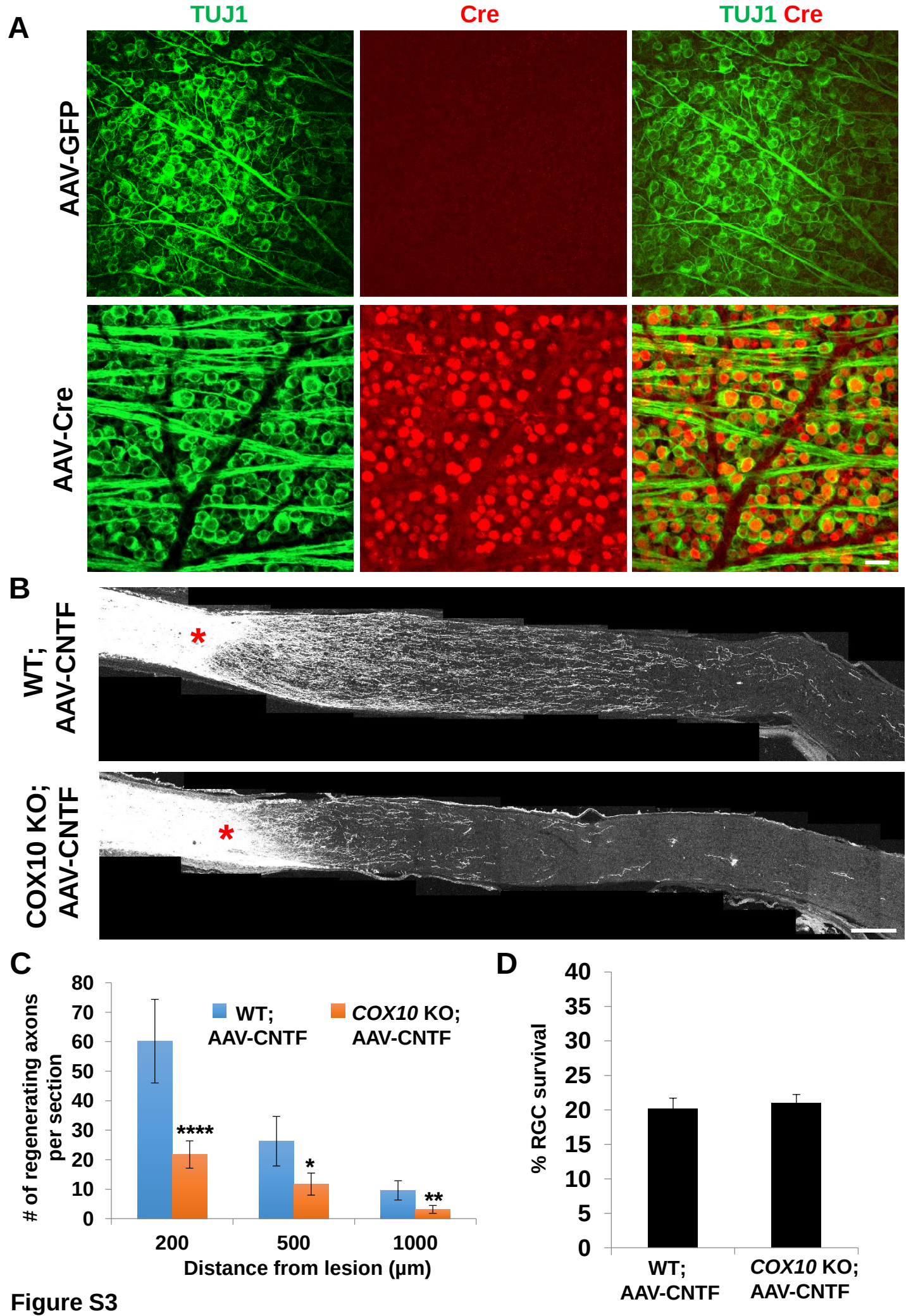
A
TUJ1
Cre
TUJ1 Cre
AAV-GFP
AAV-Cre
B
WT; AAV-CNTF
COX10 KO; AAV-CNTF
C
# of regenerating axons per section
WT; AAV-CNTF
COX10 KO; AAV-CNTF
****
*
**
200
500
1000
Distance from lesion (µm)
D
% RGC survival
WT; AAV-CNTF
COX10 KO; AAV-CNTF

Figure S3

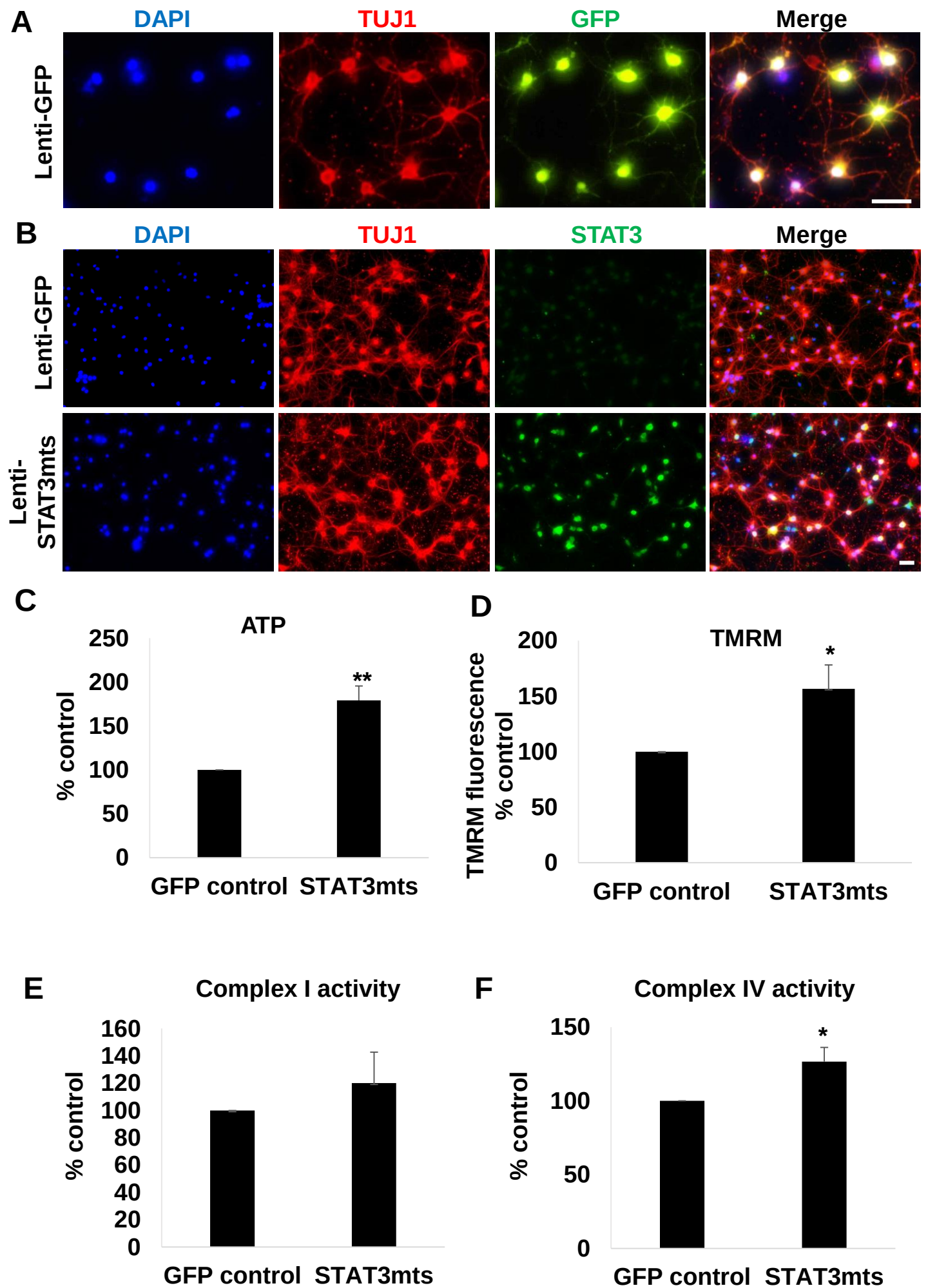
A
DAPI
TUJ1
GFP
Merge
Lenti-GFP
B
DAPI
TUJ1
STAT3
Merge
Lenti-GFP
Lenti-STAT3mts
C
ATP
% control
250
200
150
100
50
0
**
GFP control
STAT3mts
D
TMRM
TMRM fluorescence
% control
200
150
100
50
0
*
GFP control
STAT3mts
E
Complex I activity
% control
160
140
120
100
80
60
40
20
0
GFP control
STAT3mts
F
Complex IV activity
% control
150
100
50
0
*
GFP control
STAT3mts

Figure S4

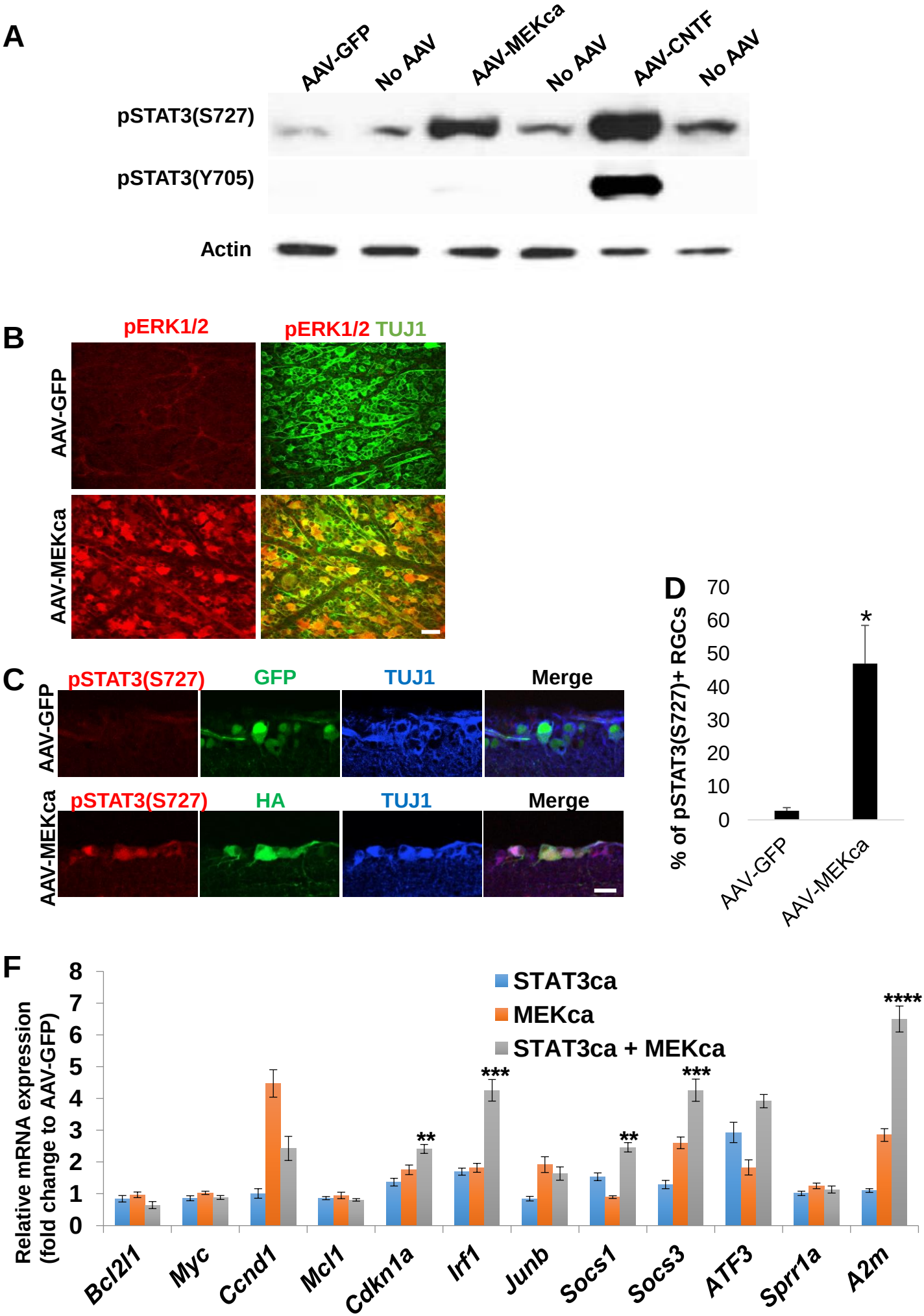
A
AAV-GFP
No AAV
AAV-MEKca
No AAV
AAV-CNTF
No AAV
pSTAT3(S727)
pSTAT3(Y705)
Actin
B
pERK1/2
pERK1/2 TUJ1
AAV-GFP
AAV-MEKca
C
pSTAT3(S727)
GFP
TUJ1
Merge
AAV-GFP
pSTAT3(S727)
HA
TUJ1
Merge
AAV-MEKca
D
% of pSTAT3(S727)+ RGCs
70
60
50
40
30
20
10
0
*
AAV-GFP
AAV-MEKca
F
Relative mRNA expression
(fold change to AAV-GFP)
8
7
6
5
4
3
2
1
0
STAT3ca
MEKca
STAT3ca + MEKca
**
***
**
***
****
Bcl2l1
Myc
Ccnd1
Mcl1
Cdkn1a
Irf1
Junb
Socs1
Socs3
ATF3
Sprr1a
A2m

Figure S5

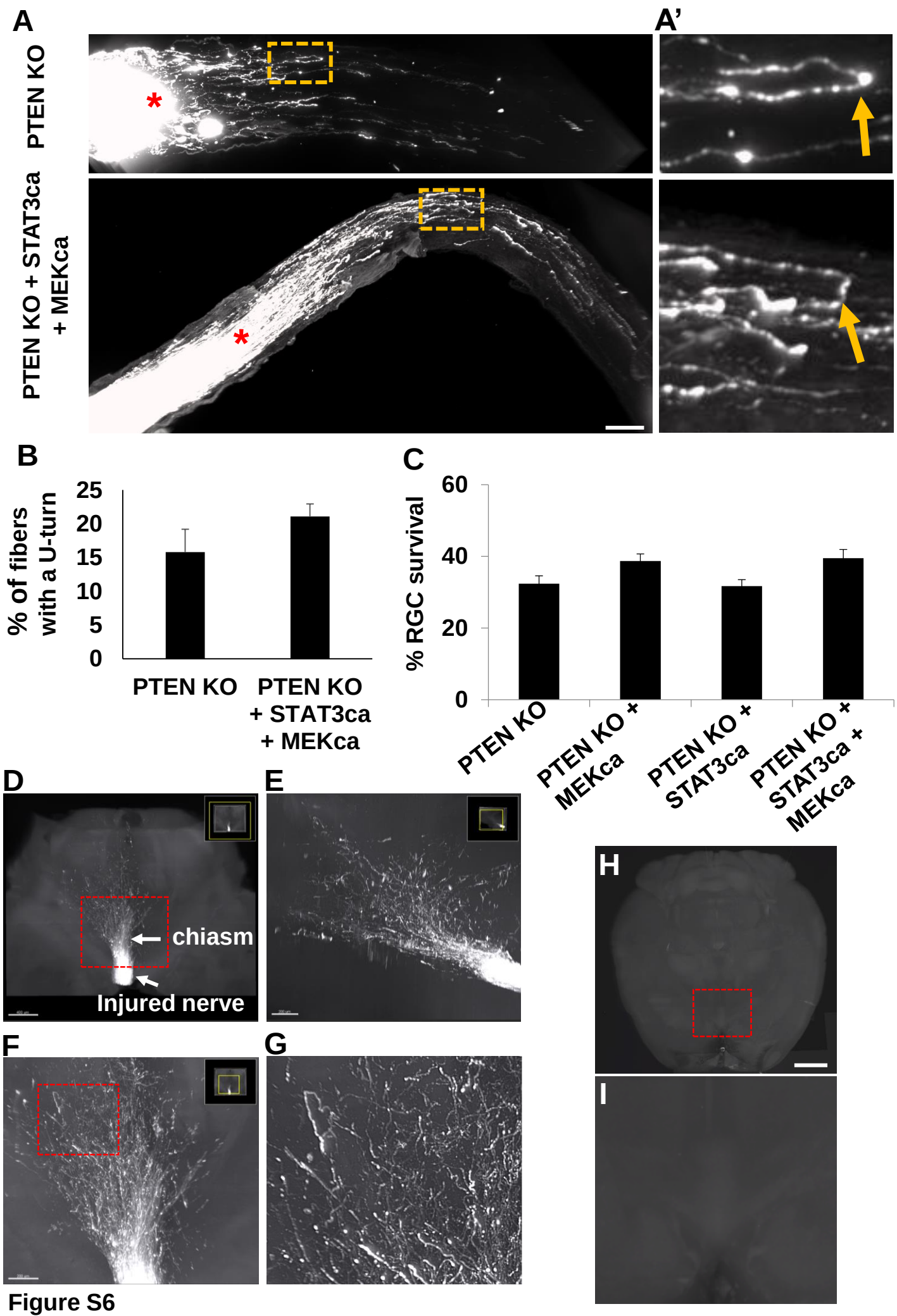
A
A'
PTEN KO
PTEN KO + STAT3ca + MEKca
B
% of fibers with a U-turn
25
20
15
10
5
0
PTEN KO
PTEN KO + STAT3ca + MEKca
C
% RGC survival
60
40
20
0
PTEN KO
PTEN KO + MEKca
PTEN KO + STAT3ca
PTEN KO + STAT3ca + MEKca
D
chiasm
Injured nerve
E
F
G
H
I

Figure S6

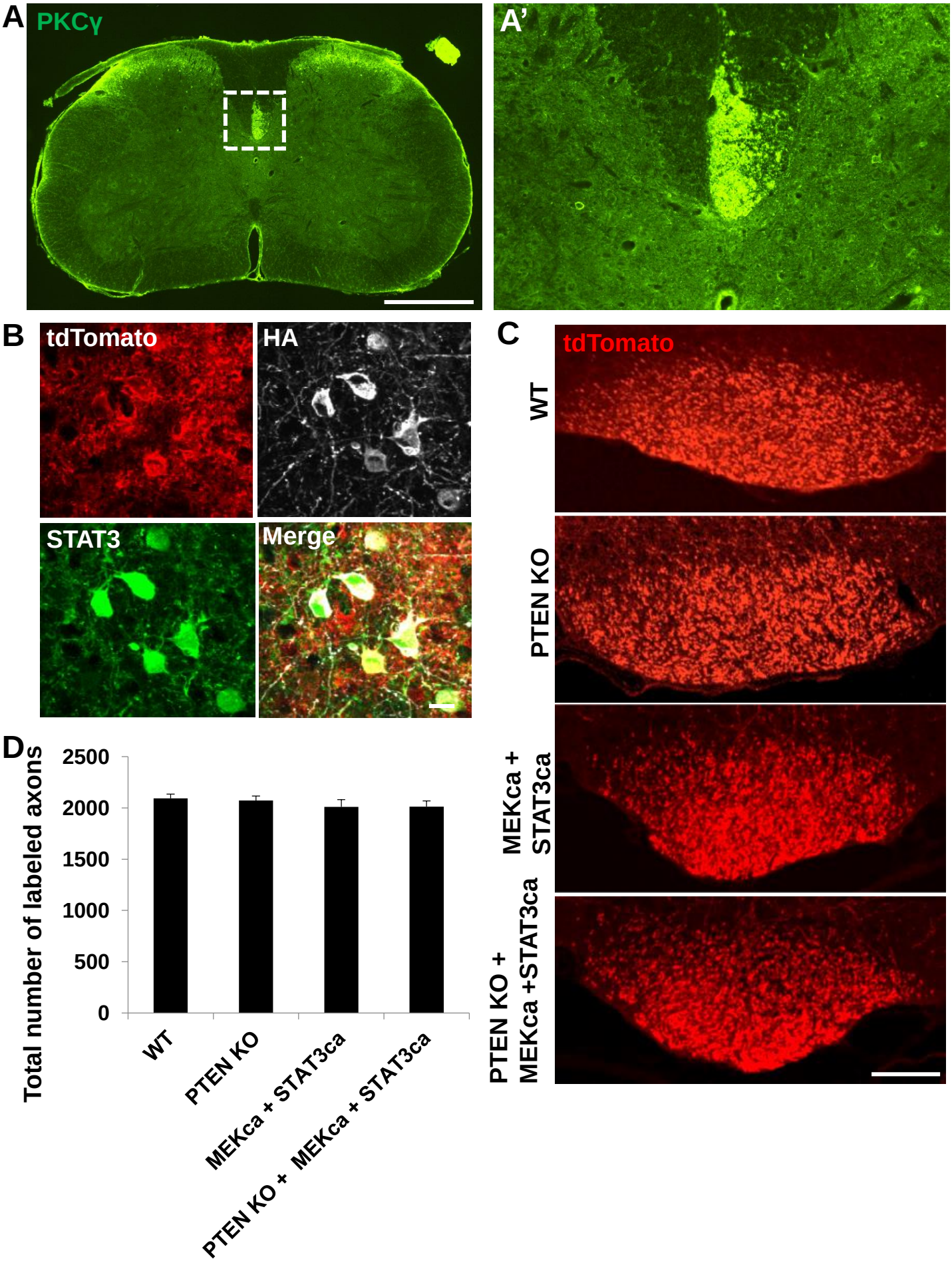
A
PKCγ
A'
B
tdTomato
HA
STAT3
Merge
C
tdTomato
WT
PTEN KO
MEKca + STAT3ca
PTEN KO + MEKca +STAT3ca
D
Total number of labeled axons
2500
2000
1500
1000
500
0
WT
PTEN KO
MEKca + STAT3ca
PTEN KO + MEKca + STAT3ca

Figure S7

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Expression of select cell growth regulatory molecules in P3 rat cortical neurons following CNTF treatment, Related to Figure 1 Western blots of P3 rat cortical neurons at multiple time points following treatment with recombinant CNTF (50 ng/mL). Protein extractions were probed with the indicated antibodies. Among the proteins examined, only STAT3 and ERK1/2 are rapidly phosphorylated in response to CNTF. The experiment was repeated with similar results.

Figure S2. RGC survival at 19 days post-injury in the *Stat3$^{f/f}$;R26-tdTomato* and *R26-tdTomato* animal groups, and overexpressed STAT3 localization, Related to Figures 2 and 3 (A) Representative retinal whole-mount images after TUJ1 immunostaining at 19 days post-injury in the various AAV-Cre injected animal groups; "GFP", AAV-Cre/AAV-GFP co- injected *R26-tdTomato* mice. "CNTF + GFP", AAV-Cre/AAV-GFP co-injected *R26-tdTomato* mice with AAV-CNTF injection at the time of injury. "STAT3 KO + CNTF + GFP", AAV-Cre/AAV-GFP co-injected *Stat3$^{f/f}$;R26-tdTomato* mice with AAV-CNTF injection at the time of injury. "STAT3 KO + CNTF + STAT3wt", AAV-Cre and AAV-STAT3wt co-injected *Stat3$^{f/f}$;R26-tdTomato* mice with AAV-CNTF injection at the time of injury. "STAT3 KO + CNTF + STAT3dbm", AAV-Cre and AAV-STAT3dbm co-injected *Stat3$^{f/f}$;R26-tdTomato* mice with AAV-CNTF injection at the time of injury. (B) Quantification of RGC survival. Values are represented as the percentage of the contra-lateral uninjured retina. STAT3wt but not STAT3dbm rescues CNTF's RGC survival effect in the STAT3 KO mice. n=5/ group. ****P<0.0001 compared to the "GFP" control group using Dunnett's post-test. Scale bar, 20 µm. (C) Representative western blots of fractionated lysates from HEK293 cells transfected with either control plasmid (GFP) or various STAT3 mutants (Table 1). Proteins were extracted 11 hours after transfection. The experiment was repeated twice with similar results. Antibodies against VDAC, SOD1 and PARP were used to validate the level of fractionation purity (mitochondrial, cytoplasmic and nuclear fraction, respectively). W, whole cell lysate; N, nuclear fraction; M, mitochondrial fraction.

Figure S3. Axon regeneration and RGC survival in the *Cox10 KO* mice, Related to Figure 3 (A) Whole-mount retinal staining showing AAV transduction 3 months after injection of AAV-Cre in the *Cox10$^{f/f}$* mice as shown by Cre immunoreactivity (red) in TUJ1$^+$ RGCs (green). No obvious RGC death is seen following *Cox10* deletion alone without optic nerve crush. (B) Representative optic nerve sections showing the degree of axon regeneration 2 weeks after injury in WT or *Cox10* KO animals. Animals received either AAV-GFP ("WT; AAV-CNTF" group) or AAV-Cre ("COX10 KO; AAV-CNTF group") followed by optic nerve crush/AAV-CNTF 8 weeks later. Axons are labeled anterogradely by CTB injection. Red asterisk, lesion site. (C) Quantification of CTB-labeled regenerating axons in the WT control and *Cox10* KO group. n=5/group. *P<0.05, **P<0.01, ****P<0.0001 by unpaired Student's t-test. (D) Quantification of RGC survival. Values are represented as the percentage of the contra-lateral uninjured retina. n=5 animals/group. Scale bars, 20 µm in (A), 100 µm in (B).

Figure S4. The levels of ATP, membrane potential, ETC activity in STAT3mts-expressing cortical neurons, Related to Figure 3 (A) Immunocytochemistry in cortical cell cultures shows 70-90% transduction efficacy of lentivirus expressing GFP ("Lenti-GFP") as evident by GFP$^+$/TUJ1$^+$ neurons. Cells were immunostained with antibodies against GFP and βIII tubulin. (B) Immunocytochemistry in cortical cell cultures shows 70-90% transduction efficacy of lentivirus expressing STAT3mts ("Lenti-STAT3mts") as evident by STAT3$^+$/TUJ1$^+$ neurons.

Cells were immunostained with antibodies against total STAT3 and βIII tubulin. (C) ATP levels were measured using a firefly luciferase/luciferin-based assay. The levels of luminescence were measured and the amount of ATP in each sample calculated with a standard curve. Value of the control group (lenti-GFP treated) is represented as 100%. Values represent means derived from 4 biological replicates. (D) Mitochondrial membrane potential (i.e. indicator of active mitochondria) measured using a TMRM assay kit. Fluorescence intensity was obtained with a fluorescent plate reader for each sample and the value of the control is represented as 100%. Values represent means derived from 4 biological replicates. (E) Complex I activity is measured using a complex I (NADH dehydrogenase) assay kit. The activity is expressed as the change in absorbance per minute per amount of sample. These data are then expressed as rate (mOD_{450}/minute) per μg of cell lysate (see Methods). Value of the control is represented as 100%. Values represent means obtained from 4 biological replicates. (F) Complex IV activity is measured using an enzymatic assay kit. The activity is calculated following the similar protocol to complex I. Value of the control is represented as 100%. Values represent means obtained from 4 biological replicates. $^{*}P<0.05$ and $^{**}P<0.01$ by unpaired Student's t-test. Scale bars, 20 μm.

Figure S5. MEK induces phosphorylation of STAT3(S727) and enhances the expression of STAT3-target genes, Related to Figure 4 (A) Representative western blots in whole retinas. Intravitreal injection of AAV-MEKca results in drastic upregulation of pSTAT3(S727). "GFP", AAV-GFP; "MEKca", AAV-MEKca; "CNTF", AAV-CNTF. The experiment was repeated twice with similar results. (B) Whole-mount retinal staining showing pERK1/2 (red) and TUJ1 (green) immunoreactivity 2 weeks after AAV-GFP or AAV-MEKca injection (n=3 animals/group). (C) Cross sections of mouse retinas 2 weeks after intravitreal injection of AAV-GFP or AAV-MEKca. pSTAT3(S727) is drastically elevated in RGCs compared to AAV-GFP injected retina. n=3 animals/group. (D) Percentage of $TUJ1^{+}$RGCs with pSTAT3(S727) signal. n=3 animals/group; 3-4 retinal sections/animal. $^{*}P < 0.01$ by unpaired Student's t-test. (F) mRNA expression of select genes in cortical neurons treated with either AAV-GFP, AAV-STAT3ca, AAV-MEKca or AAV-STAT3ca/AAV-MEKca. Values are represented as fold change to AAV-GFP-treated group and are means obtained from 4 biological replicates. $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$ compared to the "STAT3ca"group using one way ANOVA followed by Bonferroni post-test. Scale bars, 20 μm.

Figure S6. Characterization of axon regeneration, axon turning and RGC survival in triple AAV-treated mice, Related to Figure 5 A) Cleared, whole optic nerves of AAV-Cre alone treated (PTEN KO) and triple AAV-treated ("PTEN KO + STAT3ca + MEKca") group at 2 weeks post-injury. (A') Higher magnification of the boxed areas in (A) for each group. Some axons (indicated with arrows) make U-turns in the optic nerve as shown in (A'). (B) Percentage of the counted CTB-labeled axons within 1-2 mm from the lesion site that make a U-turn. A total of 15-32 axons were counted for each optic nerves (n=4 animals/group). Red asterisk, lesion site. (C) Quantification of RGC survival at 4 weeks post-injury in $Pten^{f/f}$ animals subjected to AAV-Cre alone (i.e. "PTEN KO"), or AAV-Cre/AAV-MEKca, or AAV-Cre/AAV-STAT3, or AAV-Cre/AAV-STAT3ca/AAV-MEKca (i.e. triple AAV treatment). Values are represented as the percentage of the contra-lateral uninjured retina. n=5 animals/group. (D) Ventral view of a cleared brain of a triple AAV-treated mouse at 4 weeks after injury. (E) Higher magnification lateral view of the red boxed area in (D). (F) Higher magnification ventral view of the red boxed area in (D). (G) Higher magnification of the red boxed area in (F). (H) Ventral view of a cleared

brain of a control injured AAV-GFP mouse. (I) Higher magnification of the boxed area in (H). No CTB^{+} axons are found in the brains of the control animals (n=8). Scale bars , 200 μm in (A);1 mm in (H).

Figure S7. Validation of pyramidotomy model; completeness of the lesion, total labeled axons in the medulla and cellular specificity of intracortical AAV transduction, Related to Figure 7 (A) Representative coronal section of cervical spinal cord showing absence of PKC-γ immunoreactivity in the transected CST tract. Tissues were analyzed 4 weeks after pyramidotomy. (A'), Higher magnification of the boxed area in (A). (B) Immunohistochemistry showing tdTomato, HA and STAT3-labeled cells in the *Pten$^{f/f;}$R26-tdTomato* motor cortex at 5 weeks after triple AAV injection (i.e. AAV-Cre/AAV-STAT3ca/AAV-MEKca). (C) Representative coronal sections of the medullary pyramid showing tdTomato labeled-CST axons 5 weeks after AAV injection. (D) Quantification of total tdTomato labeled-CST axons at the medullary pyramid in each animal groups. n=5-6 animals/group. Scale bars, 500 μm in (A) and c, 20 μm in (B).

Table S1. STAT3 and MEK plasmids, Related to Figures 2, 3 and 4.

Plasmid	**Mutation sites/modification**	**Reference**
Constitutively active STAT3 (STAT3ca)	A661C and N663C	Bromberg et al., 1999, Cell, v98: p295–303
STAT3 DNA binding mutant (STAT3dbm)	VVV461–463AAA and EE434–435AA	Horvath et al., 1995, Genes & Development, v9:984-994; Bromberg et al., 1998, Mol. Cell. Biol. v18: p2553–2558
STAT3 DNA binding mutant and mitochondrial targeting sequence (STAT3mts)	Contains mitochondria-localizing sequence taken from human cytochrome c oxidase subunit VIII gene. Also contains VVV461–463AAA and EE434–435AA.	Szczepanek et al., 2011, J. Biol. Chem. v286: 29610-29620
Constitutively active MEK (MEKca)	S218D and S222D	*Boehm et al. 2007, Cell, v129:1065-1079*

Video 1. 3D visualization of CTB-labeled RGC axonal projections in uninjured adult mouse, Related Figures 5 and 6. Cleared adult mouse optic nerves and brain imaged with the LSFM to show normal RGC axonal projection without optic nerve crush. This video shows 3D visualization of the entire RGC axonal projection from the two eyes labeled with the anterograde tracer CTB. This and other animations were created using Imaris (Bitplane).

Video 2. Optical slices of CTB-labeled RGC axonal projections in injured triple AAV mouse 10 weeks after injury, Related to Figures 5 and 6. A ventrodorsal scan of the horizontal optical slices showing regenerating RGC axons at 10 weeks after injury in cleared adult mouse optic nerves and brain Animals received CTB injection 5 days prior to euthanasia.

Video 3. 3D visualization of the axon regeneration in the triple AAV mouse brain 10 weeks after injury, Related to Figures 5 and 6. Individual images as seen in the Video 2 were compiled to give 3D representation of the entire mouse brain.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Dilution of AAV for multi-viral injection: For intravitreal injection, animals received a total volume of 2 µL of AAV. For injections that involved two or three AAVs in a mixture, equal volumes of AAV was added to make up the mixture (e.g. for the double AAV treatment testing the combined effects of AAV-STAT3ca and AAV-STAT3mts, 1 ul of each AAV was mixed to give a total volume of 2 µL before the injection). Accordingly, to insure fair comparisons among the different AAV groups, the single AAV animal groups (e.g. AAV-STAT3ca alone group) that were being compared to the double AAV groups received AAV diluted at 1:1 in volume with PBS before the injection Similarly, the double AAV groups that were being compared to the triple AAV group (AAV-Cre, AAV-STAT3 and AAV-MEKca) were diluted at 1:1:1 in volume with AAVs and PBS.

RGC survival and RGC axon regeneration quantification: To quantify regenerating RGC axons, the numbers of CTB^+ or $tdTomato^+$ axons that projected various distances from the lesion were counted. At least 4 sections were counted for each animal. To estimate the number of viable RGCs, retinal whole-mount was immunostained with the TUJ1 antibody. $TUJ1^+$ RGCs in the injured and contralateral uninjured retinas were manually counted. For each flat-mounted retina, 10-12 fields were imaged in the peripheral and intermediate and central regions of the retina. Each sample field was 0.263 × 0.263 mm. "The percentage of RGC survival" is the ratio of the mean RGC number injured retina/mean RGC number in uninjured contralateral retina multiplied by 100.

Intracortical AAV injection: $Pten^{f/f}$;R26-tdTomato or *R26-tdTomato* mice (5-6 weeks old) received 2 µl in total volume of AAV into the left sensorimotor cortex at the following six sites: 0.5 mm and 0.1 mm anterior, and 0.3 mm posterior; 1.2 mm and 2.2 mm lateral (in reference to Bregma).

Unilateral pyramidotomy: One week after AAV injection, mice underwent craniotomy of the occipital bone using laminectomy forceps to expose the underlying pyramidal tract of mice. A micro feather scalpel was then used to puncture the dura and transect the entire right pyramidal tract(Lee et al., 2014; Liu et al., 2010).

CST axon counting: For quantifying total labeled CST axons, axons were manually counted at the level of medulla oblongata proximal to the pyramidal decussation. Axons were counted in four rectangular areas randomly placed in the pyramidal tract and this axon density was multiplied by the total area of the tract to obtain the total number of labeled axons. This was done for two sections placed 160um apart and then the two counts were averaged to obtain the final number for each animal. To count the sprouted axons, three vertical lines, adjacent (<50 µm), 250 and 500 µm lateral to the central canal were drawn, and fibers crossing each line were manually counted in each section(Lee et al., 2014). The results were represented after normalization with the number of counted CST fibers at the medulla level: sprouting axon number index is represented as the ratio of the total number of sprouted axons in the denervated spinal cord over the number of labeled axons at the level of medulla. At least three sections were counted for each animal and averaged together.

Immunohistochemistry: Tissue processing and immunohistochemical procedures were performed as described previously (Sun et al., 2011). Mice were euthanized and transcardially perfused with

4% paraformaldehyde (PFA). Tissues were isolated and post-fixed in the same fixative overnight at 4 ℃. Tissues were cryoprotected in 30% sucrose and serial sections (retina, 16 μm; optic nerve, 10 μm; spinal cord, 25 μm; brain, 25 μm) were collected. Tissue sections were immunostained overnight at 4℃ with primary antibody diluted in PBS containing normal goat or donkey serum and 0.1% triton-X 100. Secondary antibodies were applied on tissue sections for 1 hour at room temperature. Sections were washed in PBS between the antibody incubations.

Quantification of pSTAT3(S727)/ATPsynβ immunoreactivity co-localization: Two weeks after intravitreal AAV injection in C57BL6/J mice, retinas were removed, sectioned and stained with antibodies against pSTAT3(S727) (catalogue #, 9134; Cell Signaling Technology (CST), Goettingen, Germany), ATPsynβ (catalogue #, ab14730, Abcam, Cambridge, MA, USA) and βIII tubulin (ab107216, Abcam). The samples were imaged by confocal microscopy and single plane images analyzed with Imaris v8.1.2 (Bitplane, Zurich, Switzerland). ATPsynβ^+ area in RGCs was selected as a Region of Interest (ROI), and pSTAT3(S727) signal was measured within the ROI. Negative controls without primary antibody were used to set thresholds and define ATPsynβ and pSTAT3$^+$ areas. The imaging exposure settings were maintained the same for both control and treated samples. For each retina, 15-20 individual RGCs were measured, and 3 retinas were analyzed for each group.

Quantification of percentage of pSTAT3(S727)$^+$ RGCs: After immunohistochemistry, the numbers of pSTAT3$^+$/TUJ1$^+$ and TUJ1$^+$ RGCs in the retinas of AAV-GFP and AAV-MEKca injected animals were counted in 3-4 non-consecutive 20μm thick retina cross sections, n=3 animals/group. Retina sections without the primary antibody incubation was used as negative controls to set the signal threshold.

Cortical cell culture: Cells from P3 rat pups were dissociated by sequential digestion in papain (20 U/mL; Worthington, Lakewood, NJ, USA) and trypsin (2.5%, Invitrogen), then cultured in supplemented PNGM™ primary neuron growth medium (Lonza, Basel, Switzerland) in 24-well plates (#3524; Corning, Corning, NJ, USA) on a substrate of poly-d-lysine (500 μg/mL, Sigma-Aldrich, Saint-Louis, MO, USA) at a density of 150,000 cells per well. Cultures were maintained at 37° C in a humidified CO^2 incubator. AAV2 ($4x10^8$ total particles) or lentivirus were applied for 2 to 3 days, respectively before lysing or fixing the cells with 4% PFA for subsequent analysis.

Western blot: The protein concentration of the supernatant was determined using the Bio-Rad (Hercules, CA) protein assay reagent. Approximately 20 μg of protein was loaded and separated in a 10% acrylamide–Bis (Bio-Rad, Hercules, CA, USA) gel. The protein was transferred onto Hybond-C Super membrane (Amersham Biosciences, Little Chalfont, UK) and blocked with 5% skim milk in 0.1% Tween 20 in PBS (TPBS). The membranes were incubated with primary antibodies in 5% BSA in TPBS overnight at 4℃. The membranes were incubated in biotinylated secondary antibody at a 1:5,000 dilution for 1 hour at room temperature. The membranes were incubated with HRP-conjugated antibody (Jackson ImmunoResearch, West Grove, PA, USA) before the labeled proteins were detected using the ECL agent (Pierce, Rockford, IL, USA), following the supplier's manual. The intensity of each band was quantified using Image J. The relative level of STAT3 expression was expressed as the ratio to VDAC. Quantification was the mean obtained from at least 3 biological replicates.

Subcellular fractionation: To obtain the cytosolic, nuclear and mitochondrial-enriched fractions, cortical cells were washed twice with PBS and scrapped at 4℃ using a sucrose buffer (buffer 1) (pH7.4) composed of (in mM) 250 sucrose, 50 HEPES, 5 $MgCl_2$, and 0.1 PMSF (phenylmethylsulfonyl fluoride) solution (Sigma-Aldrich), supplemented with 1% Phosphatase Inhibitor Cocktail 3 DMSO solution (Sigma-Aldrich) and Complete Mini, EDTA-free Protease Inhibitor Cocktail tablet (1 tablet per 10 mL of buffer) (Roche, Basel, Switzerland). Then, the extracted cells were homogenized by using a Potter-Elvehjem Pestle (20 strokes) and maintained on ice during 30 min. The homogenates were then centrifuged at 800g for 15 min at 4℃. The resulting supernatant (S1) was collected into new tubes and the pellet (P1) was re-suspended in buffer. The S1 fractions were centrifuged at 800g for 15 min at 4℃, and the P1 fractions were centrifuged at 500g for 15 min at 4℃. After centrifugation, the supernatant (S2) from the S1 fractions was collected into new tubes and the pellet was discarded, while the supernatant from the P1 fractions was discarded and the pellet (P2) was re-suspended in buffer. Both the S2 and P2 fractions were then centrifuged at 11,000g and at 1,000g for 15 min respectively. The resulting supernatant from the P2 fractions was discarded and the pellet (P3) was re-suspended in a buffer (buffer 2) (pH7.9) composed of (in mM) 20 HEPES, 1.5 MgCl2, 500 NaCl, 0.2 EDTA, and 0.1 PMSF supplemented with 10% glycerol, 0.5% Triton-X100, 1% Phosphatase Inhibitor Cocktail 3 DMSO solution and Complete Mini, EDTA-free Protease Inhibitor Cocktail tablet (1 tablet per 10 mL of buffer) and maintained on ice. The resulting supernatant (S3) from the S2 fractions was collected into new tubes while the resulting pellet (P4) was re-suspended in buffer 1. The S3 fractions were mixed with 10% Trichloroacetic acid solution (Sigma-Aldrich) and maintained in ice during 1h in order to precipitate the cytosolic proteins. The P4 fractions were centrifuged at 11,000g for 15 min at 4℃, and the resulting pellet (P5) was re-suspended in a buffer (buffer 3) (pH 6.8) composed of (in mM) 50 Tris-HCl, 1 EDTA, 0.5% Triton-X100, and 0.1 PMSF, supplemented with 1% Phosphatase Inhibitor Cocktail 3 DMSO solution and Complete Mini, EDTA-free Protease Inhibitor Cocktail tablet (1 tablet per 10 mL of buffer), and maintained on ice. The precipitated S3 fractions were centrifuged, and the resulting pellet (P6) was re-suspended with 100% acetone in order to wash off the excess of acid. The P6 fractions were then centrifuged at maximum speed during 20 min at 4℃. The supernatant was discarded and the pellet (P7) was re-suspended in a mix of buffers 1, 2 and 3. The re-suspended P3 (nuclear fraction), P5 (mitochondrial fraction), and P7 (cytosolic fraction) fractions were sonicated on ice, and the P3 fractions were further centrifuged at 9,000g during 30 min at 4℃, and the resulting supernatant (S4) was collected into new tubes (final nuclear fraction). All the samples were stored at -80℃ until use for protein quantification and western blot.

ATP assay: ATP levels were determined using ATP Determination Kit (A22066; Life Technologies, Grand Island, NY, USA). Briefly, 10 µL of cortical cells sample extracts were placed in a clear bottom, white sided 96-well plate (Greiner CELLSTAR 96 well plates white polystyrene wells flat bottom (with micro-clear bottom). Then, 90 µL of a standard reaction solution composed by 1X reaction buffer, 1 mM dithiothreitol (DTT), 0.5 mM D-luciferin, and 1.25 µg/mL firefly luciferase, was mixed with the cell samples, and the luminescence readings were taken after 15 min incubation in a filter-based multi-mode microplate reader (FLUOstar Omega). A standard curve was also generated by replacing the cell samples with different amounts of diluted ATP in order to determine the ATP concentration present in each sample. All the data was normalized to mg of protein of each sample and expressed as the percentage of the control (Lenti-GFP). Quantification was the mean obtained from at 4 biological replicates.

Complex 1, IV activity assay: Complex I and IV activities were measured using Complex I Enzyme Activity Microplate Assay Kit (ab109721; Abcam) and Complex IV Rodent Enzyme Activity Microplate Assay Kit (ab109911; Abcam), respectively. A total of 50 µg protein from cortical cells were incubated with the respective monoclonal antibody for complex I or IV for 3 hours at room temperature in order to capture the sample enzyme complexes in their fully-intact, functionally-active states. After incubation, the wells were washed twice with buffer, and incubated with 200 µL/well of complex I or IV assay solution. For complex I, the activity was determined by following the rate of the oxidation of NADH to NAD^+ and the simultaneous reduction of a dye which leads to increased absorbance at 450 nm. For complex IV, the activity was determined by following the oxidation of reduced cytochrome c which leads to increased absorbance at 550 nm. The activity was determined as the change in absorbance per minute per mg of sample loaded into the well, and expressed as the percentage of the control (Lenti-GFP). Quantification was the mean obtained from 4 biological replicates.

Analysis of mitochondrial membrane potential: Mitochondrial membrane potential ($\Delta\Psi m$) was determined using the cationic fluorescent probe $TMRM^+$ which accumulates inside the negatively charged active mitochondria. Briefly, cortical cells were cultured in a clear bottom, dark sided 96-well plate (Greiner CELLSTAR 96 well plates black polystyrene wells flat bottom (with micro-clear bottom) previously coated overnight with 0.5mg/mL poly-D-lysine (Millipore), for 48h, at 37°C. Then, after changing the cell culture media, in some wells cortical cells were exposed to 50 µM FCCP (carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone)), a H^+ ionophore which uncouples oxidative phosphorylation, disrupting ATP synthesis and promotes mitochondrial membrane depolarization, during 20 min at 37°C. Then, all the wells with or without FCCP were incubated with 300 nM $TMRM^+$ during 30 min at 37°C. After incubation, cortical cells were washed twice in PBS and maintained in PBS for fluorescence readings (540nm excitation and 590 emission) taken in a filter-based multi-mode microplate reader (FLUOstar Omega). The difference between the fluorescence values with and without FCCP treatment for each condition determines the percentage of functional mitochondria that had the capacity to retain $TMRM^+$. The data was normalized to the mg of protein for each condition and expressed as the percentage of the control. Quantification was the mean obtained from 4 biological replicates.

Antibodies and pharmacological inhibitors: Antibodies used were; VDAC (#4866; CST, 1:1,000 dilution), SOD (11407; Santa Cruz, Dallas, TX, USA,1:2,000), PARP (#9542; CST, 1:2,000), nucleolin (NCL; #14574; CST, 1:1,000), vinculin (VCL; #13901; CST, 1:1,000), red fluorescent protein (RFP; 600-401-379; Rockland, Limerick, PA, USA, 1:1000), pS6 (#2211; CST, 1:1,000 for western blot, 1:200 for IHC), pYAP1(Y357) (ab62751; Abcam, 1:1,000), STAT3 (#9139; CST, , 1:1,000), pSTAT3(S727) (#9134; CST, 1:1,000), pSTAT3(Y705) (#9145; CST; 1:1,000 for western blot, 1:100 for IHC), β-catenin (#610153; BD, 1:1,000), pERK1/2 (#4370; CST, 1:1,000 for western blot, 1:100 for IHC), pAKT (#4058; CST, 1:1,000), βIII tubulin (ab107216; Abcam, 1:1,000), HA (11-867-423-001; Roche, 1:1,000), GFP (ab13970; Abcam, 1:1,000), actin (A2228; Sigma-Aldrich, 1:5,000), Cre (PRB-106P; Covance, Princeton, NJ, USA, 1:1,000), ATPsynβ (ab14730; Abcam, 1:2,000).

Quantitative RT-PCR: Three days after adding the AAV2 in cortical cultures, total RNA was extracted from cortical neurons with Trizol reagent (Invitrogen). 1 ug of RNA was used as template for reverse transcription (639506; Clontech, Mountain View, CA, USA) to make

cDNA, which was further analyzed semi-quantitatively by qPCR (4309155; Life Technologies). ΔΔCt method was used to obtain the fold change of mRNA expression level. Primers used were:

A2m-F: cccacagagactaggcgaag, *A2m*-R: attggaccacaggagacagg; *Bcl2l1*-F: accggagagcattcagtgat, *Bcl2l1-R*: tgcaatccgactcaccaata; *Ccnd1*-F: gcgtaccctgacaccaatct, *Ccnd1-R*: ggctccagagacaagaaacg; *Cdkn1a*-F: agcaaagtatgccgtcgtct, *Cdkn1*a-R: acacgctcccagacgtagtt; *Irf1*-F: tggaggggacatcgagatag, *Irf1*-R: atggtgcacaaggaatagcc; *Junb*-F: cagcctttctatcacgacga, *Junb*-R: cctgacccgaaaagtagctg; *Mcl1*-F: agctgcatcgaacctttagc, *Mcl1*-R: aaagccagcagcacatttct; *Myc*-F: gaccagatccctgagttgga, *Myc*-R: ctcgccgtttcctcagtaag; *Socs1*-F: tccgctcccactctgattac, *Socs1*-R: gaagccatcttcacgctgag; *Socs3*-F: ttctttaccaccgacggaac, *Socs3*-R: cgttgacagtcttccgacaa; *ATF3*-F: ccctcctagggaagatggag, *ATF3*-R: ctgatgaaactcccggaaaa; *Sprr1A* -F: aagcagccttgcactgtacc, *Sprr1A* -R: gctctggcaccttaggttga; *GAPDH*-F:gacatgccgcctggagaaac, *GAPDH*-R:agcccaggatgccctttagt.

Tetrahydrofuran (THF)-based tissue clearing: Mice were perfused transcardially with PBS followed by 4% PFA in PBS at 5 ml/min. Optic nerves and brain were dissected and post-fixed with 4% PFA in PBS overnight. Samples were rinsed with PBS and stored at 4℃ until needed. Samples underwent dehydration by incubation in increasing concentration of THF (Sigma-Aldrich) solutions under constant rocking(Luo et al., 2014). Optic nerves were incubated in 50% THF (diluted in water v/v), 80% THF (v/v) and 100% THF for 15 minutes each. Dehydrated optic nerve was rendered clear by incubating in BABB (a mixture of benzyl alcohol and benzyl benzoate (Sigma-Aldrich) at a ratio of 1:2) for 20 minutes. Adult mouse brain was incubated in 50% THF for 12 hours, 80% THF for 12 hours, 100% THF for 3 x 12 hours, and BABB for 12 hours before imaging.

Light Sheet Fluorescent Microscopy (LSFM): Light sheet fluorescent microscope (LaVision, Goettingen, Germany) illuminates specimen with a thin sheet of laser light formed by two lenses, allowing imaging of large tissues, yet with cellular resolution. LSFM was performed as previously described(Luo et al., 2014). Between 100 and 500 optical slices were imaged. The scan speed was 0.5–1.5 s per section, which was about 2-3 minutes for the optic nerve and 5-10 minutes for the brain for a complete scan of the tissue. Images were collected at 2 to 5 µm increment in Z axis.

APEX and Electron Microscopy (EM): Expression constructs were incorporated into a mammalian expression backbone containing the ubiquitin C (uBC) promoter. APEX cDNA (Addgene 42607) was PCR amplified and ligated in frame with STAT3wt or hemagglutinin (HA). APEX constructs were transfected into P3 rat cortical neurons and processed 48 hours later. One hour prior to fixation, rCNTF (50 ng/ml) or PBS was added to the culture media. APEX staining was carried out using a modified protocol(Martell et al., 2012). Cells were rinsed with cold APEX buffer (100 mM sodium cacodylate, 2 mM $CaCl_2$, pH 7.4) and fixed with 2% glutaraldehyde in APEX buffer, for 30 minutes on ice. Following extensive washing and quenching of unreacted glutaraldehyde with 20 mM glycine in APEX buffer, cells underwent diaminobenzene (DAB) staining. DAB staining was performed using a commercially available kit (Vector Labs, Burlingame, CA) at a 0.7 mM H_2O_2 concentration in APEX buffer. Staining was monitored via brightfield microscopy and the reaction was terminated with 5 washes in APEX buffer. Cultures were post-fixed in 2% OsO_4 in 0.1M PO_4 buffer for 1 hour, washed, and dehydrated through an ethanol gradient. Cultures were infused with Embed 812 (Electron Microscopy Sciences) and placed at 64℃ overnight. 50 nm sections were collected and stained

with 0.5% uranyl acetate in ddH_2O for 5 minutes. Sections were imaged on a Phillips CM10 electron microscope.

Quantification of axon turning in cleared whole optic nerves: THF-treated, cleared optic nerves were imaged with LSFM and analyzed using Imaris. To estimate the percentage of axons that make a U-turn, the number of CTB^+ axons within 1-2 mm from the lesion site with at least one U-turn was counted. A total of 15-32 axons were counted for each optic nerve (n=5 animals/group).