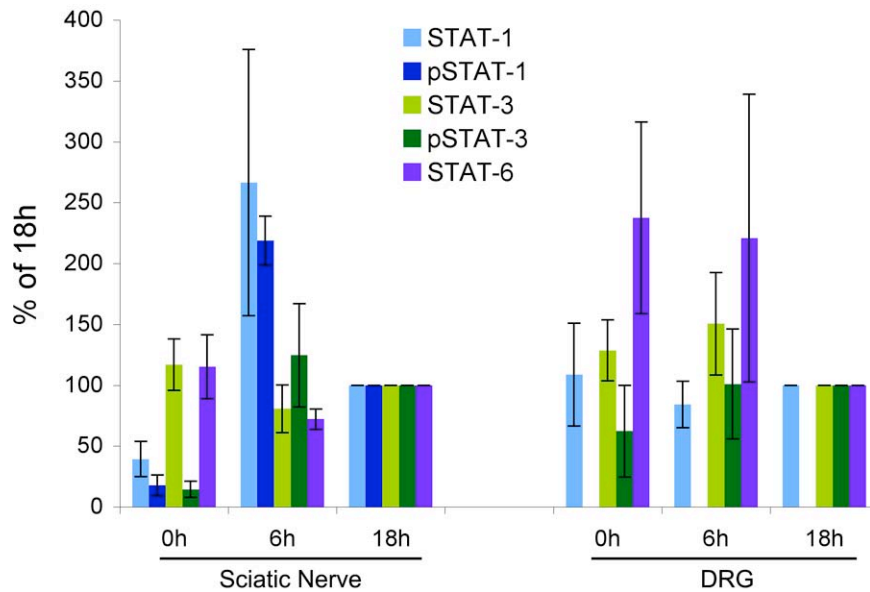


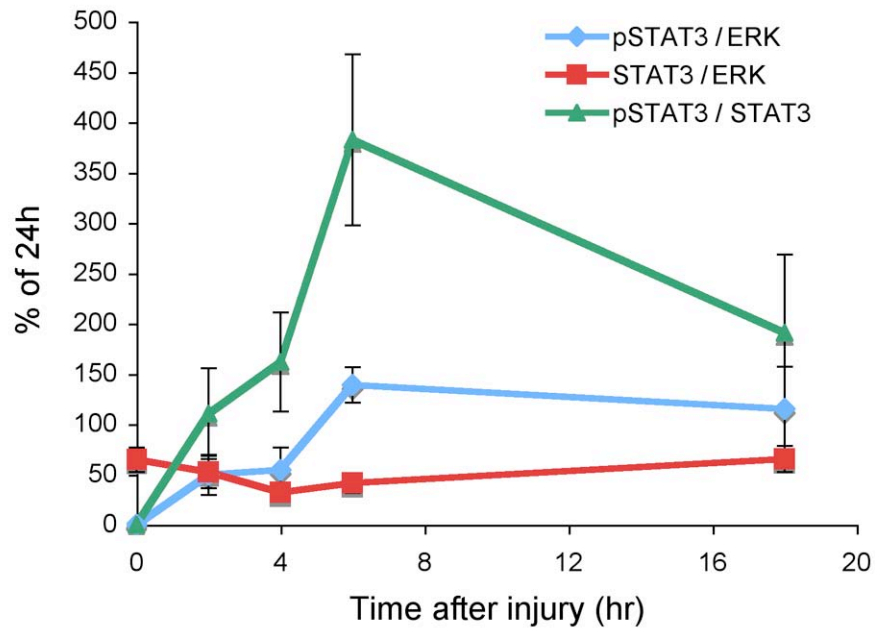
## Supplementary Figures



*Ben-Yaakov et al., Figure S1*

### **Figure S1: Activation of STAT family members in sciatic nerve (SN) and DRG after SN lesion. (Supporting Information for Figure 2A).**

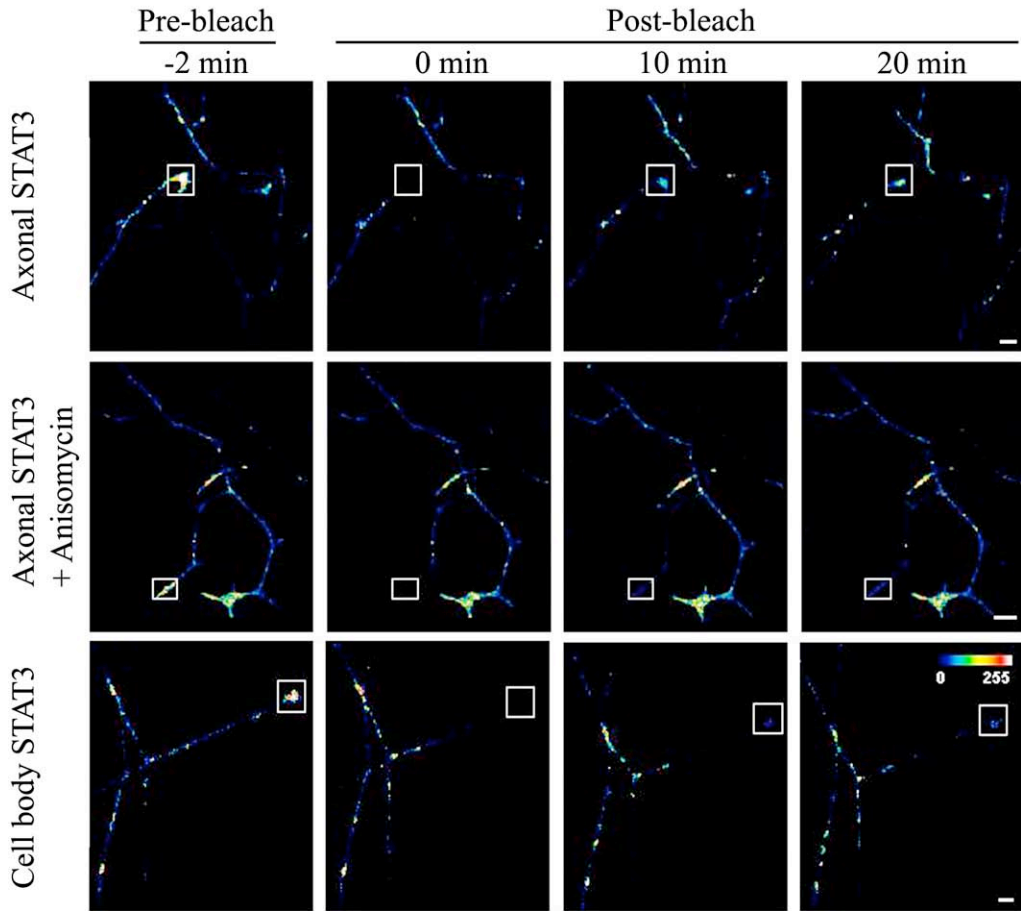
Quantification of the Western blot analyses in Figure 2A, of STAT family members in sciatic nerve axoplasm or DRG extract from control (0h) or injured rat SN (6 or 18 hours after injury).



*Ben-Yaakov et al., Figure S2*

**Figure S2: Activation of STAT3 in sciatic nerve (SN) after lesion.** (*Supporting Information for Figure 2B*).

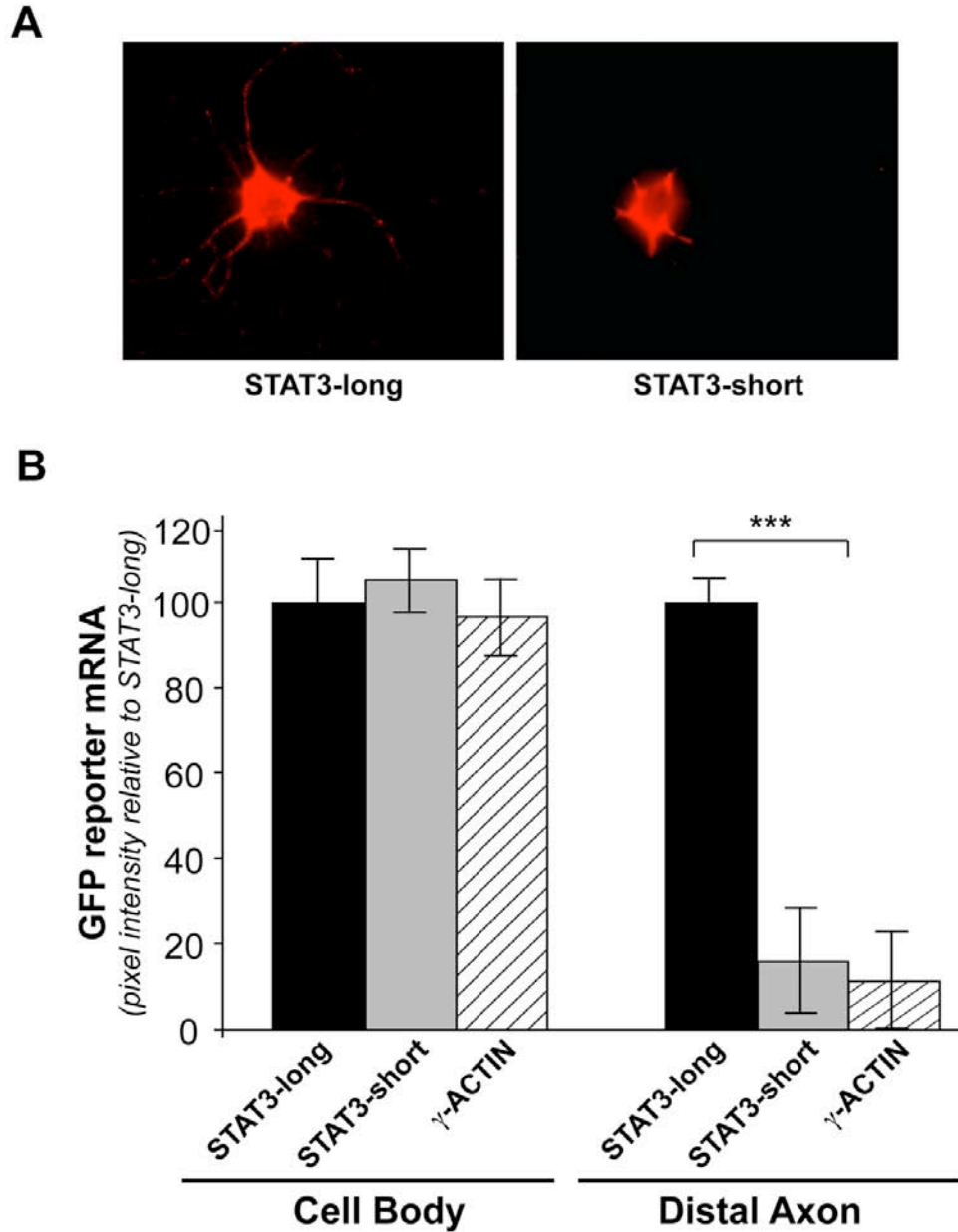
Quantification of the Western blot analyses in Figure 2B, of changes in STAT3 and pSTAT3 levels in sciatic nerve axoplasm after injury. ERK was used as a loading control. Quantification is in percentage at 24 hr post-lesion (average  $\pm$  SEM; n = 5).



*Ben-Yaakov et al., Figure S3*

**Figure S3: Specific 3'UTR variant of STAT3 targets GFP reporter for local translation in axons.** (*Supporting Information for Figure 3E*)

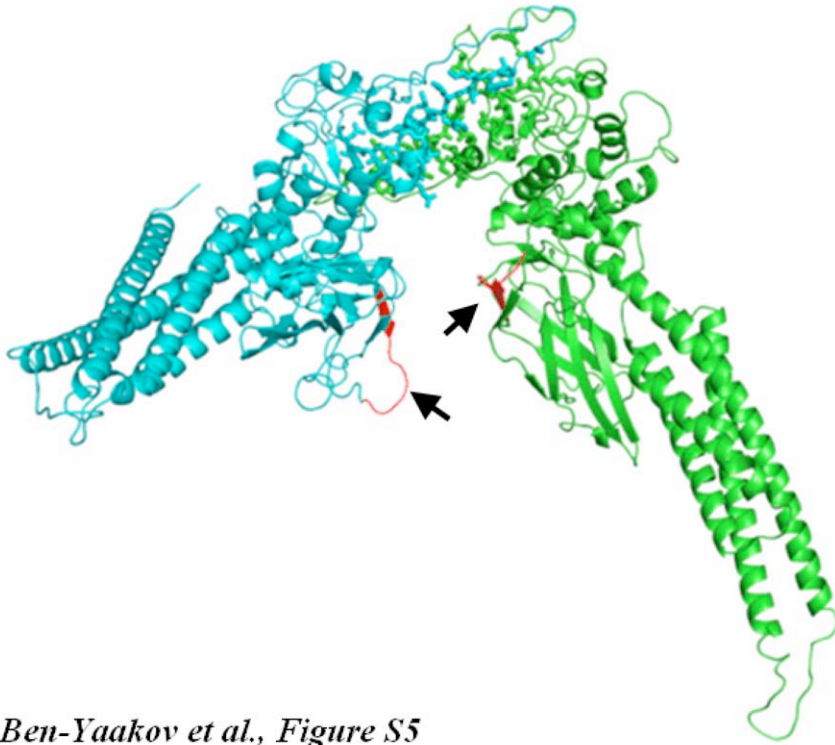
Representative images from time-lapse sequences of photobleach experiments before (2 min) and after photobleaching (0, 10 and 20 min) of adult DRG neurons transfected with the indicated constructs. The boxed regions represent the area subjected to photobleaching (at a distance >800  $\mu\text{m}$  from cell body) with recovery monitored over 20 min. Transfected neurons that were treated with 50  $\mu\text{M}$  anisomycin immediately prior to prebleach imaging are indicated.



*Ben-Yaakov et al., Figure S4*

**Figure S4: FISH analysis on short and long STAT3 3'UTR variants fused to GFP.** (Supporting Information for Figure 3F).

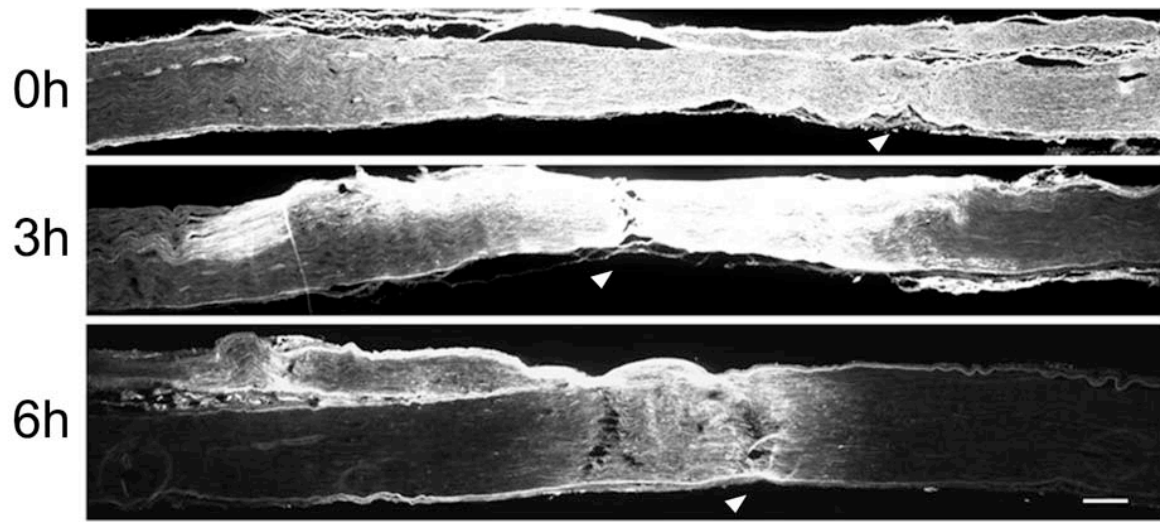
(A) In situ hybridization against GFP RNA sequence reveals mRNA for both short and long STAT3-3'UTR reporter construct in the cell bodies of cultured DRG neurons. (B) Quantitative FISH signal intensity for GFP mRNA in the cell body and distal 200  $\mu\text{m}$  of the axons from DRG neurons transfected with either the STAT3-short or STAT3-long reporter constructs is shown as average pixels/ $\mu\text{m}^2 \pm \text{SEM}$  ( $n \geq 30$  neurons/condition over 3 separate experiments; \*\*\* p-value<0.005).



*Ben-Yaakov et al., Figure S5*

**Figure S5: 3D Model of STAT3 protein and its NLS peptide location.** (*Supporting Information for Figure 5C*).

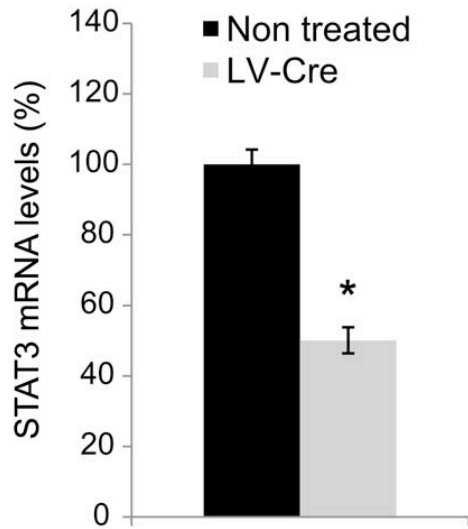
Predicted crystal structure of rat STAT3, modeled using "Swiss model" and aligned over the mouse homodimer STAT3 crystal structure (PDB ID: 1bg1). The region in STAT3 that affects Importin  $\alpha$  association and used to design the STAT3-NLS-peptide is marked in red.



*Ben-Yaakov et al., Figure S6*

**Figure S6: Biotinylated STAT3-NLS peptide is present in sciatic nerve axons up to 6h after injection.** (*Supporting Information for Figure 5 D-F*).

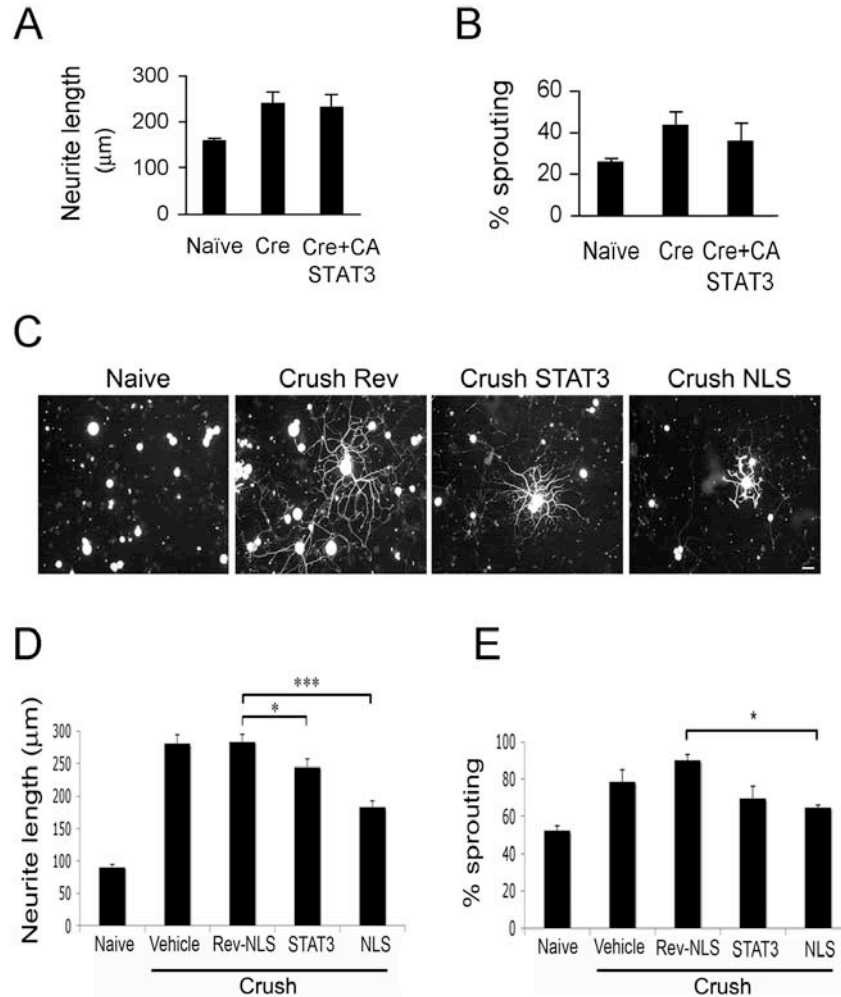
250 $\mu$ g of biotinylated STAT3-NLS peptide were injected to SN concomitantly with a crush injury. At the indicated time points after injury, SN was fixed and sectioned at 10 $\mu$ m width in cryostat, followed by staining with RRX-conjugated streptavidin. The presence of the peptide was visualized by fluorescence microscopy. Distal part of SN is on the right. Crush sites are indicated by arrow heads. Scale bar 100 $\mu$ m.



*Ben-Yaakov et al., Figure S7*

**Figure S7: Activation of Cre by viral transduction of L4/L5 DRG in floxed STAT3 mice reduces STAT3 mRNA levels in DRG.** (*Supporting Information for Figure 6B*).

RNA was extracted from L4/L5 DRGs 7 days after viral transduction, and subjected to reverse transcription and QPCR. Control RNA was extracted from the contralateral DRG. Results are normalized to beta-actin and presented as percentage of control (average  $\pm$  SEM of 3 technical repeats).

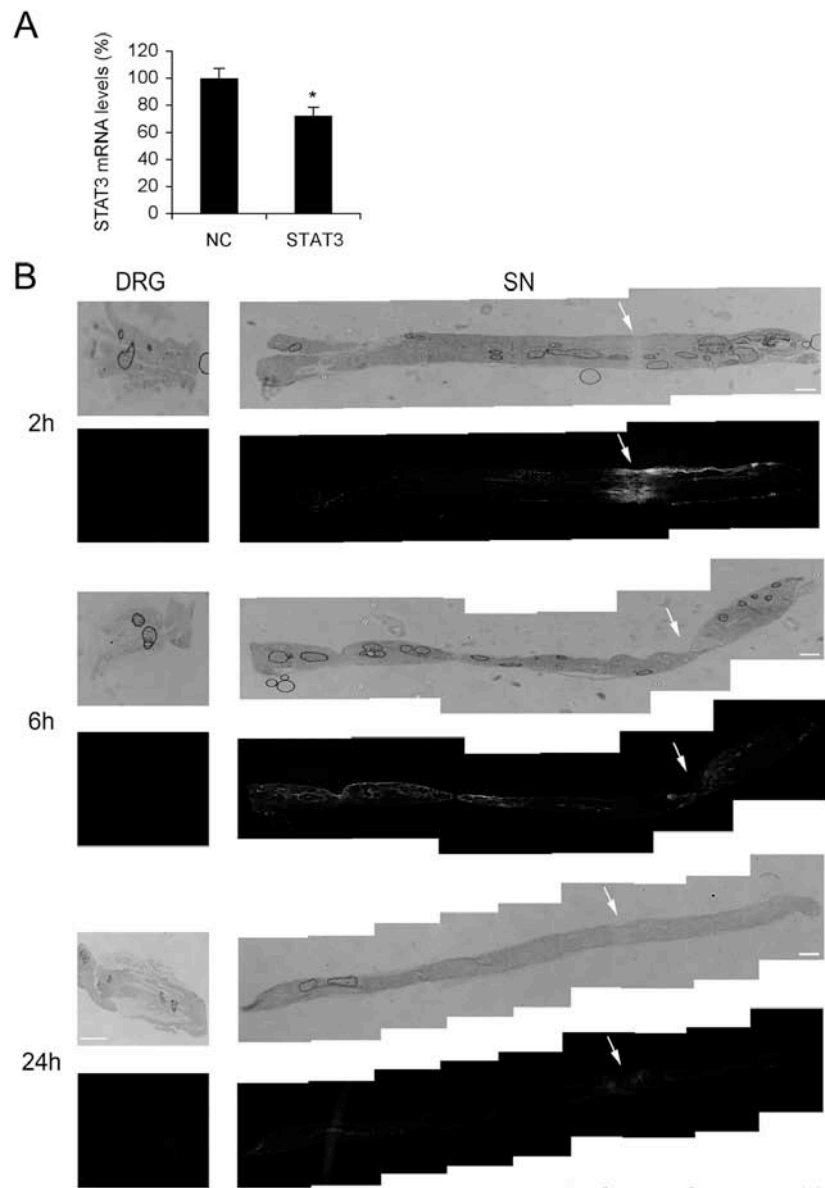


*Ben-Yaakov et al., Figure S8*

**Figure S8: Conditioning lesion responses after interfering with STAT3 signaling.**  
(Supporting Information for Figure 6B,E).

(A, B) Delivery of constitutively active (CA) STAT3 in DRG neurons did not affect the conditioning lesion response in floxed STAT3 mice after Cre activation by viral transduction of L4/L5 DRG. A conditioning crush injury was carried out 7 days after viral transduction, and 3 days later L4/L5 DRG neurons were cultured in-vitro for 18h, fixed, and stained for NFH before measurement of neurite outgrowth. No significant change was obtained with the CA STAT3 compared to Cre alone (average  $\pm$  SEM, n=3). (C-E) Effect of STAT3-NLS peptide on conditioning lesion responses in adult rat DRG neurons. Sciatic nerves were injected with 250mg of NLS, reverse-NLS, or STAT3 peptide, or vehicle alone (PBS), concomitantly with a conditioning crush injury. Three days after the lesion, L4/L5 DRG neurons were cultured in-vitro for 30h, fixed, and stained for NFH before measurement of neurite outgrowth. The neurite length was reduced only slightly by STAT3-NLS peptide, and no significant change was obtained on the percent of neurite-bearing cells, while classical NLS peptide effect was more pronounced on both parameters. Reverse-NLS peptide serve as a negative control (average  $\pm$  SEM, n=4, \*  $p < 0.05$ , \*\*\*  $P < 0.005$ ). Representative pictures are shown in E. Scale bar 50µm.

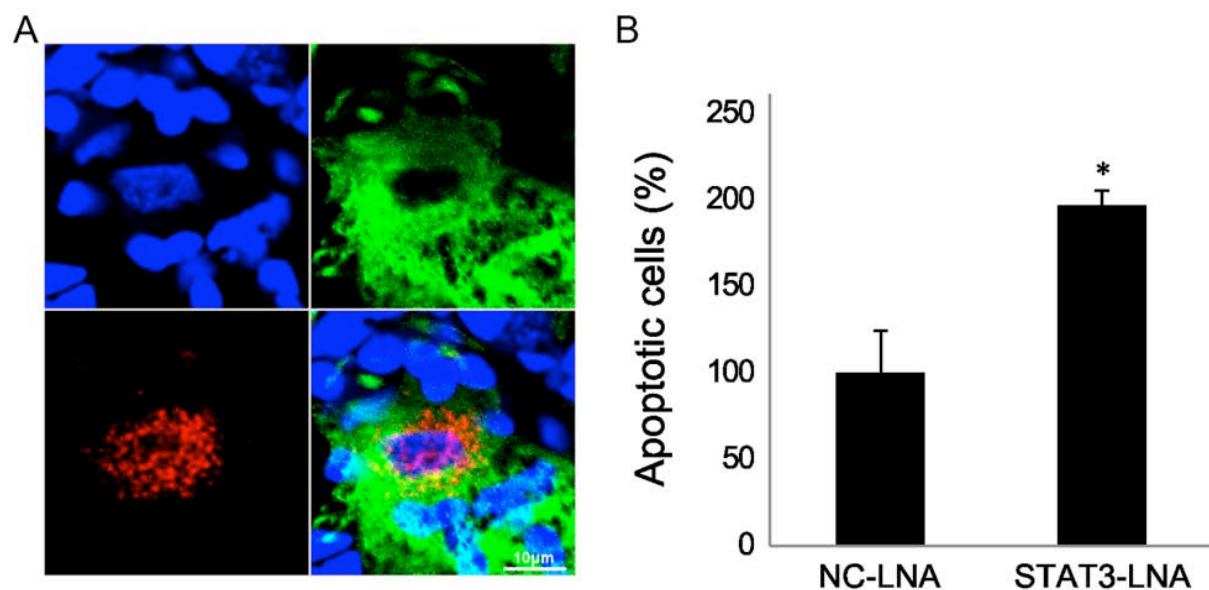




*Ben-Yaakov et al., Figure S9*

**Figure S9: Injection of anti-STAT3 locked nucleic acid (LNA) into sciatic nerve reduces the levels of STAT3 mRNA in axons. (Supporting Information for Figure 6F).**

**(A)** 0.3  $\mu\text{g}$  of anti-STAT3 or negative control (NC) LNA were injected into sciatic nerve two days before and then again concomitantly with lesion. 18h after lesion, RNA was extracted from axoplasm, and subjected to reverse transcription and QPCR. Results are normalized to beta-actin and presented as percentage of negative control (average  $\pm$  SEM,  $n=3$ ,  $*p<0.05$ ). **(B)** Fluorescent nucleic acids injected to SN axons do not reach neuronal cell bodies in DRGs. 0.2nmol (10ul of 20 $\mu\text{M}$ ) of fluorescent RNA duplex (D-001630-02, Dharmacon) were injected to rat sciatic nerve (SN) concomitantly with a crush injury. At the indicated time points after injury, SN and DRG were fixed and sectioned at 10 $\mu\text{m}$  width in cryostat. The presence of the indicator RNA was visualized by fluorescence microscopy. Distal part of SN is on the right. Scale bar 500 $\mu\text{m}$ .



**Figure S10: Injection of anti-STAT3 locked nucleic acid (LNA) into sciatic nerve increases the fraction of apoptotic neurons expressing cleaved caspase 3 in L4/L5 DRG.** (*Supporting Information for Figure 6F*).

**(A)** 0.3 µg of anti-STAT3 or negative control (NC) LNA were injected into sciatic nerve two days before and then again concomitantly with lesion. Seven days later the L4/L5 DRG were excised, fixed, sectioned and stained for NFH (green), cleaved caspase 3 (red) and DAPI. **(B)** Quantification of cleaved caspase 3 positive neurons for the STAT3-LNA treatment as percentage of the control LNA treatment (average ± SEM, n≥3, \* p<0.05).

## Supplementary Table Descriptions

**Table S1.** (*Supporting Information for Figure 1A*).

107 different matrices predicted by Genomica to be the key contributors for gene transcription after injury. Below each matrix are its responsive genes.

**Table S2.** (*Supporting Information for Figure 1B*).

The degree of overlap in regulated genes between pairs of the 107 different matrices predicted by Genomica to be the key contributors for gene transcription after injury.

**Table S3. Dynein-bound Transcription Factors in Axons.** (*Supporting Information for Figure 1D*).

Activation of TFs bound to dynein in axoplasm after nerve injury was tested using Panomics protein/DNA Arrays. Percent TF activity after injury was measured by the spot intensity of each TF, compared to that observed with axoplasm from noninjured nerve. Intensities were normalized to hybridization controls on the array. Only TFs with consistent up or downregulation in at least two independent experiments are shown in the table. In bold are the TFs with more than 15% increase or decrease in dynein-bound active form after injury.

**Table S4. STAT3 responsive genes.** (*Supporting Information for Figure 4G*).

List of STAT3 responsive genes as predicted by Genomica to be differentially expressed. Data presented as log<sub>2</sub> of the expression values in reference to time point of 1 hour post injury.

TFs	Mean percent
AP-1 (2)	32.45±15.71
AP-2 (1)	+
AP-2 (2)	19.71±9.62
AR	-
Bm-3	-
CEBP (1)	-
c-Myb	+
CREB (1)	+
E2F-1	-
EGR (1)	-36.91±14.16
ER	+
Ets (1)	-
Ets-1/Pea-	-
FAST-1	+
GAS/ISRE	-
GATA	26.94±20.72
GR/PR	16.25±10.19
HNF-4 (1)	-
HSE	41.26±9.75
IRF-1	-
MEF-1 (1)	16.78±8.58
MEF-2 (1)	-
MRE	+
Myc/Max	24.92±4.11
NF-1 (1)	-
NFAT-1	30.47±22.65
NF-	18.95±8.90

TFs	Mean percent
NF-E2 (1)	18.12±5.68
NFkB (1)	14.38±2.54
OCT (1)	-
P53 (1)	-
Pax-5 (1)	24.36±18.77
Pbx-1	25.19±16.66
Pit-1 (1)	-
PPAR (1)	29.29±11.59
PRE	-
RAR/DR-	22.97±15.15
RXR/DR-	-
SIE	32.40±9.74
Smad/SBE	35.27±5.86
SMAD-	41.99±5.75
Sp-1	-18.53±3.38
SRE	-
STAT-1	28.10±17.48
STAT-3	17.80±4.34
STAT-4	27.38±5.51
STAT-5	40.17±24.57
STAT-6	46.51±17.17
TFIID	-
TR	-
TR/DR-4	+
USF-1 (1)	32.64±5.87
VDR/DR-	18.80±8.47

**Table S3.** The following elements showed inconsistency during the assay and were excluded: AP-1 (1), CBF and CDP. - indicates below detection levels and + indicates activity detected but didn't change as a result of sciatic nerve injury.

## **Supplementary Experimental Procedures**

### **Nerve Injury, Axoplasm Preparation, and DRG Extraction**

Mouse or rat sciatic nerve crush and axoplasm preparation were as previously described (Hanz et al., 2003; Rishal et al., 2010). DRG extracts were prepared by compression of L4/L5 rat DRGs in lysis buffer (50mM Tris-HCl pH 7.4, 150mM NaCl, 1mM PMSF, 1mM EDTA, 1% Triton X-100, and protease inhibitors as previously described (Perlson et al., 2005).

### **Microinjection of Viruses into the L4 and L5 DRG's**

Floxed-STAT3 mice (20–24 gm) were anesthetized with ketamine – xylazine solution (10mg/ml ketamine and 2mg/ml xylazine, 0.01ml solution/mg body weight). A lateral back incision was performed, starting at the L6 spinal segmental level. The L4/L5 transverse processes were carefully removed after the microsurgical dissection of the spinal column's lumbar region. Finally, L4/L5 DRGs were carefully exposed and prepared for virus microinjection using a microinjector. The microinjection needle was attached to the needle holder of the microinjector system at roughly 45°. The virus was carefully inserted into the capillary. This system was gently moved in small increments under visual control using a microscope to L4/L5 DRGs. The virus injection was performed when the capillary tip was clearly focused and gently inserted in a DRG. The surgery zone was then sutured using a special autoclip wound closing system. All stages of surgery procedures, except the skin incision, were made under a microscope.

### **TFBS Analyses**

Normalized expression values from microarray analyses (Michaevlevski et al., 2010) were uploaded to Genomica (<http://genomica.weizmann.ac.il>) with a gene set of TFs generated as a binary matrix  $M$  of size  $n \times m$ .  $n$  represents the number of genes and  $m$  the number of TFs.  $M_{i,j}=1$  if transcription factor  $j$  is predicted to bind the promoter of gene  $i$ , otherwise  $M_{i,j}=0$ . In order to determine the interaction between TFs and genes the upstream regions (2000 bp) of all rat open reading frames were downloaded and scanned using the SCANACE program (<http://arep.med.harvard.edu/mrnadata/mrnasoft.html>). Positional weight matrices (PWM) representing the binding affinities for all vertebrate TFs were downloaded from the Transfac 7 database (<http://www.gene-regulation.com/pub/databases.html>). For each gene  $i$  and TF  $j$   $M_{i,j}$  was set to one if at least one position in gene's  $i$  upstream sequence had an identity of above 95%.

### **Quantitative PCR (QPCR)**

Total RNA was extracted as described by Michaevlevski et al (2010). QPCR was performed as previously described (Nilsson et al., 2005). Briefly, RNA extracted from L4/L5 DRG's was reverse transcribed (Superscript III, Invitrogen) and QPCR was performed in triplicates on an Applied Biosystems 7300 Real-PCR System using Taqman primer kits for  $\beta$ -Tubulin III or  $\beta$ -actin (normalization control), ATF3, Reg3a, SOCS3 and STAT3.

### **Protein/DNA Array Analysis**

Sciatic nerve axoplasm (500  $\mu$ g) from control or injured nerve (6 hr post-lesion) was subjected to immunoprecipitation with anti-dynein intermediate chain clone 74.1 antibody (Sigma) and protein-A agarose beads. Active TFs bound to dynein in axoplasm were identified using the Panomics Protein/DNA array (#MA1210). Briefly, the dynein immunoprecipitates were incubated with biotin-labeled TF binding probes in the presence of 2 mM EGTA for six hours at 37°C. Complexes were separated and washed, and bound probes were then eluted and hybridized to a membrane containing 56 consensus TFBS, according to manufacturer's instructions.

### **Antibodies, Western Blot and Immunofluorescence**

The following antibodies were from Cell Signaling: mouse anti STAT3 (Cat. #9139), rabbit anti phospho-STAT3-Y705 (Cat. #9131), mouse anti phospho-STAT1-Y701 (Cat. #9171), and cleaved caspase-3 (Cat. #9664). Rabbit polyclonals for STAT1 (ab31369), STAT5 (ab68465), STAT2 (ab53149), and STAT6 (ab44718) were from Abcam. Mouse anti STAT4 was from Invitrogen (#33-2300). Mouse anti phospho-STAT6-Y641 (Cat. #05-590) was from Upstate, and Mouse anti phospho-STAT1-Y701 was from Anaspec. Mouse anti importin- $\beta$ 1 clone 3E9 was from Affinity Bioreagents. Mouse anti-dynein intermediate chain clone 74.1, rabbit anti Erk M5670, and mouse anti-NFH clone N52 were from Sigma. Rabbit anti-NFH AB1989, and chicken anti-NFH were from Chemicon. Chicken anti-neurofilament M, and chicken antineurofilament L were from Aves Laboratories. Rabbit anti-S100 was from Dako. Mouse anti Isl1 (#40.2D6) was from Developmental Studies Hybridoma Bank. Normal mouse IgG (sc-2025) was from Santa-Cruz. DAPI (Sigma D8417) at 5 $\mu$ g/ml was used for nuclear staining. HRP-conjugated secondary antibodies were from BioRad, and fluorescent secondary antibodies were from Jackson ImmunoResearch. Western blots and immunostainings were carried out as described previously (Hanz et al., 2003; Perlson et al., 2005). For Westerns, axoplasm samples were resolved on 10% SDS PAGE, transferred to nitrocellulose, and developed with pico or femto ECL (Pierce) after reaction with the desired antibodies. For immunofluorescence, cultured DRG neurons were fixed with 3% paraformaldehyde, while sciatic nerve segments and DRGs were fixed in 4% paraformaldehyde, frozen with Tissue-Tek, and cross sectioned at 12  $\mu$ m thickness on a Leica cryostat. Neuron cultures and sections of DRG and sciatic nerve were mounted in moviol (Calbiochem) and observed under an Olympus FV500 Confocal laser-scanning microscope. For visualization of DAPI, Cy2, Rhodamine RED-X, and Cy5, we used 350, 489, 543 and 633 nm wavelengths, respectively, in a sequential manner.

### **Immunoprecipitations**

Axoplasm (500  $\mu$ g) from control or injured (6 hr) rat sciatic nerve was precleared for 1hr with Protein A-agarose (Santa Cruz) and 0.5% triton. Following overnight incubation with primary antibody, complexes were incubated on protein-A beads for 2 hr, washed extensively, and eluted by boiling in SDS-PAGE sample buffer before loading on gels for Western blot analysis. For competition assays, 50  $\mu$ g of reverse-NLS (CTPVKRKKKP) or STAT3-NLS peptide

(TLREQRCGNG) were added during incubation with primary antibody.

### **Electron Microscopy**

SN from naïve or injured (6hr) rat were fixed in 4% PFA and 0.1% Glutaraldehyde in 0.1M Cacodylate buffer for 2 hours at room temperature and then at 4°C over night. The nerves were then washed in 0.1M Cacodylate buffer, cut to 1mm fragments and impregnated in 2.3M sucrose in fixation buffer overnight at room temperature. The nerve fragments were then rapidly frozen in liquid nitrogen. Ultrathin sections (70–90 nm) were prepared at –120°C on Leica EM FC6 ultramicrotome with cryo chamber and collected on nickel grids coated with 0.3% Formvar. For immunostaining, grids with sections were reacted with mix of rabbit anti-Dynein HC1 and mouse anti-pSTAT3 antibodies, followed by a mix of secondary anti-rabbit (15nm) and anti-mouse (10nm) IgG gold particles. The grids were then embedded in Methylcellulose with 0.2% uranyl acetate and analyzed under 120 kV on a Tecnai 12 (FEI) Transmission Electron Microscope with the EAGLE (FEI) CCD camera using TIA software. The number of pSTAT3 particles per axon and the percent of pSTAT3 particles adjacent to a dynein particle were quantified.

### **DRG Cultures and Conditioning Lesion**

DRG neuron cultures and *in vivo* conditioning sciatic nerve lesions were as previously described (Hanz et al., 2003; Perlson et al., 2005). For tests of introduced substances, sciatic nerves were injected concomitantly with the crush with 250 µg of NLS, reverse-NLS, or STAT3 peptides. L4/L5 DRG neurons were cultured 3 days after the conditioning lesion. After 30 hr in culture neurons were fixed, stained with NFH, and process lengths were quantified with WIS-Neuromath ([www.wisdom.weizmann.ac.il/~vision/NeuroMath/](http://www.wisdom.weizmann.ac.il/~vision/NeuroMath/)).

### **Metabolic Labeling and Axonal Synthesis**

Mouse or rat sciatic nerve segments were incubated for 2-6 hours in Met/Cys-deficient DMEM medium supplemented with 40µCi/ml of [<sup>35</sup>S]Met/Cys (Amersham), with or without 10 µg/ml of cycloheximide or 100 mM EGTA, or their vehicle. EGTA (100 mM) or Vehicle (PBS) were injected to SN before incubation in the medium. Axoplasm was then immunoprecipitated with anti-STAT3, followed by SDS-PAGE and radioactive detection.

### **Quantification of pSTAT3 Accumulation in DRG Cell Nuclei**

Rat L4/L5 DRGs were dissected at different time points after sciatic nerve injury, fixed in 4% paraformaldehyde, and stained for the neuronal marker NFH, pSTAT3 and DAPI. The percentage of DRG neurons with stronger pSTAT3 signal in their nucleus compared to cytoplasm was calculated against the total number of neurons with DAPI staining. For retrograde transport inhibition, 100µg colchicine (Sigma) was injected one cm proximal to the injury site in sciatic nerve. For competition assay, 250 µg of control reverse-NLS, NLS, or STAT3 peptide were injected to sciatic nerve concomitantly with sciatic nerve crush.

## Fluorescence In-Situ Hybridization and Immunolabelings

cRNA probes for GFP reporter mRNA in transfected neurons were generated and hybridized as previously described (Vuppalanchi et al., 2010). Fluorescent signals were detected by epifluorescence. All image sets from individual experiments were matched for exposure, gain and offset. ImageJ was used to quantify FISH signal (pixels/mm<sup>2</sup>) in the cell body and the distal 200  $\mu$ m of the axon. Antisense oligonucleotide probes for STAT3 were designed using Oligo 6 software and checked for homology and specificity by BLAST. STAT3 probes spanned the following nucleotides of STAT3 mRNA (NM\_012747): 1524-1573 (for STAT3 $\alpha$  and  $\beta$ ), 2329-2378 and 2388-2437 (for STAT3 $\alpha$ ), 2321-2351 and 2401-2430 (for STAT3 $\beta$ ). Probes were synthesized with amino group modifications at four positions each and labeled with digoxigenin (DIG) succinamide ester as per manufacturer's instructions (Roche). Hybridization to DRG neuronal cultures was as previously described (Willis et al., 2007). Hybridization to tissue sections was performed as previously published (Muddashetty et al., 2007), with minor modifications. Briefly, sciatic nerve was fixed for 2 hr in 2% paraformaldehyde. After overnight cryoprotection in 30% sucrose at 4°C, nerves were processed for cryosectioning with 10  $\mu$ m sections laid onto Superfrost<sup>plus</sup> glass slides (Fisher). Cryostat sections were stored at -20°C until used. Sections were warmed to room temperature, rinsed in PBS for 20 min and then soaked in 0.25% acetic anhydride, 0.1 M triethanolamine hydrochloride, and 0.9% NaCl at pH 8.0 for 10 min. Sections were then rinsed in 2X SSC, dehydrated through a graded series of ethanol (70%, 5 min; 95%, 5 min; and 100%, 5 min), delipidated in chloroform for 5 min and rinsed with 100% ethanol. Sections were washed in 1X SSC and then hybridized overnight at 42°C with 1.2 ng/ $\mu$ l digoxigenin labeled probes in 2X SSC plus 40% formamide, 250  $\mu$ g/ml tRNA, 100  $\mu$ g/ml sheared salmon sperm DNA, and 10% dextran sulfate. For immunodetection of digoxigen probes, NF and S100, samples were incubated in primary antibody for 2 hours at room temperature. Also, an additional Cy3-conjugated anti-mouse F'ab fragment antibody was added to increase sensitivity for detecting FISH signals in these tissue sections. Sections were washed in 1X SSC three times for 15 min each at 50°C, PBS for 5 min, and PBS with 0.3% Triton X-100 for another 5 min. For immunodetection, coverslips and sciatic nerve sections used for *in situ* hybridization were equilibrated in IF buffer (1% heat-shock BSA, 1% protease-free BSA (Pandey et al.), 50 mM Tris, and 150 mM NaCl) and then blocked in the same buffer containing 2% heat-shock BSA and 2% fetal bovine serum for 1 hour. After blocking, samples were incubated for 2 hours at room temperature with the following primary antibodies in IF buffer: chicken antineurofilament H (1:1,000; Chemicon), chicken anti-neurofilament M (1:1,000; Aves Laboratories), chicken anti-neurofilament L (1:1,000; Aves Laboratories) and rabbit anti-S100 (1:500; Dako). Samples were washed in IF buffer three times and then incubated for 1 hour in the following secondary antibodies diluted in IF buffer: Cy5-conjugated anti-rabbit, FITCconjugated anti-chicken, and Cy3-conjugated mouse anti-digoxigenin antibodies (1:200 each; Jackson ImmunoRes.). To increase sensitivity of detecting hybridizations in the tissue sections, an additional Cy3-conjugated anti-mouse IgG F(ab') fragments antibody (1:200; Jackson ImmunoRes.) was included with secondary antibody incubation. Coverslips were mounted with gelvatol with 6



mg/ml *n*-propyl gallate. Tissues were imaged using a Leica TCS/SP2 confocal system on an inverted Leica DMIRE2 microscope.

### **Pure Axonal Preps, RT-PCRs, and 3' RACE RT-PCR**

Isolation of DRG axons was carried out as previously described (Zheng et al., 2001). Two hundred nanograms of RNA from cell body and axons were used as template for reverse transcription and PCR. STAT3 primers were as follows: forward 5'-CCCGAGAGCCAGGAGCAC, reverse 5'-AAAGTTGGGAGTTTCAGATGAT.  $\beta$ -actin and  $\gamma$ -actin served as positive and negative controls, as previously described (Zheng et al., 2001). For the 3' RACE experiment we used the STAT3 primer forward:

5'-CCCGAGAGCCAGGAGCAC, and a mixture of four Poly T reverse primers:

5'-GACCACGCGTATCGATGTGCGACTTTTTTTTTTTTTTTTA-3',

5'-GACCACGCGTATCGATGTGCGACTTTTTTTTTTTTTTTTC-3',

5'-GACCACGCGTATCGATGTGCGACTTTTTTTTTTTTTTTT-3',

5'-GACCACGCGTATCGATGTGCGACTTTTTTTTTTTTTTTTG-3'.

The nested PCR was done with primers specific to STAT3-alpha or beta, and primer to the poly linker of the poly T primer, as follows:

STAT3 alpha forward: 5'- AATACCATTGACCTGCCGAT,

STAT3-beta forward: 5'- CATCTGTGTGACACCATTTCATT,

Poly linker reverse: 5'-GACCACGCGTATCGATGTGCGAC.

### **Fluorescence Recovery after Photobleaching (FRAP)**

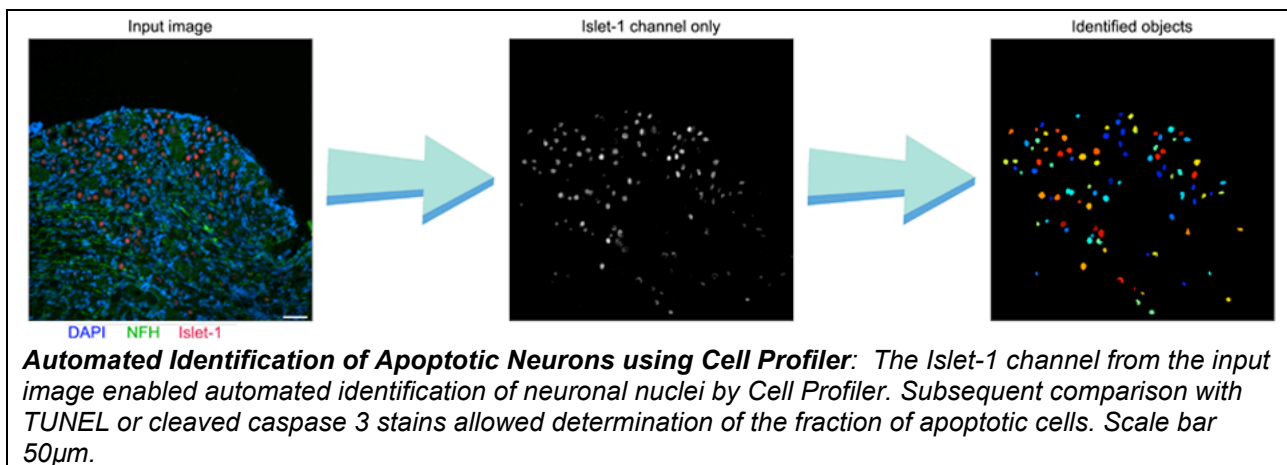
Dissociated DRG cultures were transfected with STAT3 3' UTR axonal and cell body variants using Amaxa Nucleofection system (Lonza, Inc.). Terminal axons were subjected to FRAP sequence at 37°C with 488 nm laser line of Leica TCS/SP2 confocal microscope as described with minor modifications (Yudin et al., 2008). Prior to bleaching, neurons were imaged every 30 sec for 2 min at 15% laser power. For photobleaching, the region of interest (ROI) was exposed to 75% laser power every 1.6 sec for 40 frames. Recovery was monitored every 60 sec over 20 min at 15% laser power. To test for translation dependence, cultures were pretreated with 50  $\mu$ M anisomycin for 30 min before the photobleaching sequence.

### **Quantification of FRAP and Statistical Analysis**

Quantitative data were obtained from at least 2 neurons in each of at least three separate culture preparations for each construct tested ( $n > 6$ ). *ImageJ* was used to calculate average pixels/ $\mu\text{m}^2$  in the ROIs of the raw confocal images. The fluorescence at each time point was normalized to the pre-bleach intensity for each image sequence. Mean  $\pm$  SEM for the normalized percent fluorescent intensity for each time point was calculated. Statistical significance was tested by comparing each post-bleach time point to  $t = 0$  min by one-way ANOVA with Bonferroni post-hoc comparisons using *GraphPad Prism 5* software package.

### TUNEL and cleaved caspase 3 analyses

Rat L4/L5 DRGs were dissected 7 days after SN injury and injection of 250 $\mu$ g NLS, reverse-NLS or STAT3-NLS peptide, 10 $\mu$ g colchicine or its vehicle (PBS), or 0.3 $\mu$ g anti-STAT3 (CACTGAGCCATCCTGCCG) or negative control (GTGTAACACGTCTATACGCCCA) locked nucleic acid (LNA). L4/L5 DRGs from floxed-STAT3 mice were dissected 14 days after injection of lentiviruses to the DRGs and 7 days after SN crush. The DRGs were then fixed in 4% paraformaldehyde, and cross sectioned at 20 $\mu$ m thickness on a Leica cryostat for cell death analyses as previously described (McKay Hart et al., 2002). Briefly, serial sections were stained in alternate order for apoptotic markers (TUNEL or cleaved caspase 3) versus NFH, Islet1 and DAPI. TUNEL staining was with the TMR red In-Situ Cell Death Detection kit (Roche) according to the manufacturer's instructions. Immunostaining for cleaved caspase 3 was performed as previously described (Schoenmann et al., 2010). Images were taken using an Olympus IX71 inverted fluorescence microscope. Islet1 positive cells were counted using the Cell Profiler software (<http://www.cellprofiler.org>) as exemplified graphically below and the mean number of apoptotic cells per number of Islet1 positive cells was determined. At least 1,000 neurons were counted for each experimental replicate.



### References for Supplementary Experimental Procedures

- Alexa, A., Rahnenfuhrer, J., and Lengauer, T. (2006). Improved scoring of functional groups from gene expression data by decorrelating GO graph structure. *Bioinformatics* 22, 1600- 1607.
- Hanz, S., Perlson, E., Willis, D., Zheng, J.Q., Massarwa, R., Huerta, J.J., Koltzenburg, M., Kohler, M., van-Minnen, J., Twiss, J.L., and Fainzilber, M. (2003). Axoplasmic importins enable retrograde injury signaling in lesioned nerve. *Neuron* 40, 1095-1104.
- McKay Hart, A., Brannstrom, T., Wiberg, M., and Terenghi, G. (2002). Primary sensory neurons and satellite cells after peripheral axotomy in the adult rat: timecourse of cell death and elimination. *Exp Brain Res* 142, 308-318.
- Muddashetty, R.S., Kelic, S., Gross, C., Xu, M., and Bassell, G.J. (2007). Dysregulated metabotropic glutamate receptor-dependent translation of AMPA receptor and postsynaptic

density-95 mRNAs at synapses in a mouse model of fragile X syndrome. *J Neurosci* 27, 5338-5348.

Nilsson, A., Moller, K., Dahlin, L., Lundborg, G., and Kanje, M. (2005). Early changes in gene expression in the dorsal root ganglia after transection of the sciatic nerve; effects of amphiregulin and PAI-1 on regeneration. *Brain Res Mol Brain Res* 136, 65-74.

Pandey, A., Blagoev, B., Kratchmarova, I., Fernandez, M., Nielsen, M., Kristiansen, T.Z., Ohara, O., Podtelejnikov, A.V., Roche, S., Lodish, H.F., and Mann, M. (2002). Cloning of a novel phosphotyrosine binding domain containing molecule, Odin, involved in signaling by receptor tyrosine kinases. *Oncogene* 21, 8029-8036.

Perlson, E., Hanz, S., Ben-Yaakov, K., Segal-Ruder, Y., Seger, R., and Fainzilber, M. (2005). Vimentin-dependent spatial translocation of an activated MAP kinase in injured nerve. *Neuron* 45, 715-726.

Rishal, I., Michaelevski, I., Rozenbaum, M., Shinder, V., Medzihradzky, K.F., Burlingame, A.L., and Fainzilber, M. (2010). Axoplasm isolation from peripheral nerve. *Dev Neurobiol* 70, 126-133.

Schoenmann, Z., Assa-Kunik, E., Tiomny, S., Minis, A., Haklai-Topper, L., Arama, E., and Yaron, A. (2010). Axonal degeneration is regulated by the apoptotic machinery or a NAD<sup>+</sup>-sensitive pathway in insects and mammals. *J Neurosci* 30, 6375-6386.

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., and Ideker, T. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 13, 2498-2504.

Willis, D.E., van Niekerk, E.A., Sasaki, Y., Mesngon, M., Merianda, T.T., Williams, G.G., Kendall, M., Smith, D.S., Bassell, G.J., and Twiss, J.L. (2007). Extracellular stimuli specifically regulate localized levels of individual neuronal mRNAs. *J Cell Biol* 178, 965-980.

Yudin, D., Hanz, S., Yoo, S., Iavnilovitch, E., Willis, D., Segal-Ruder, Y., Vuppalachchi, D., Ben-Yaakov, K., Hieda, M., Yoneda, Y., *et al.* (2008). Localized regulation of axonal RanGTPase controls retrograde injury signaling in peripheral nerve. *Neuron* 59, 241-252.

Zheng, J.Q., Kelly, T.K., Chang, B., Ryazantsev, S., Rajasekaran, A.K., Martin, K.C., and Twiss, J.L. (2001). A functional role for intra-axonal protein synthesis during axonal regeneration from adult sensory neurons. *J Neurosci* 21, 9291-9303.