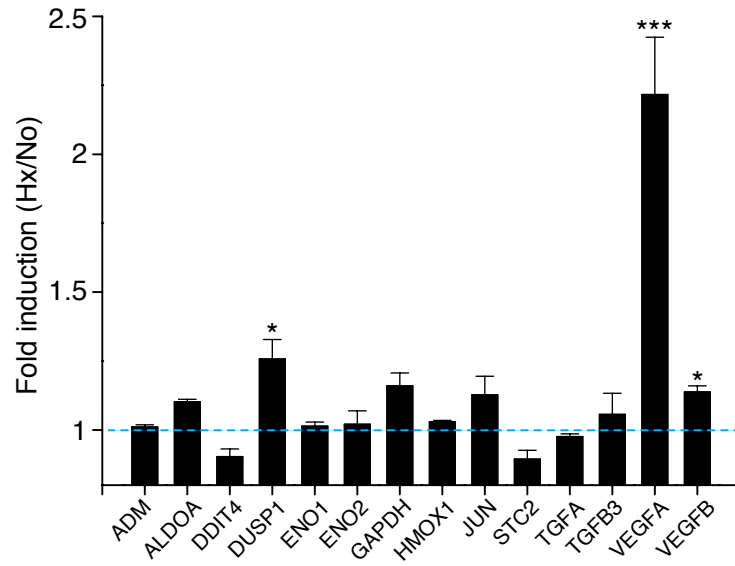
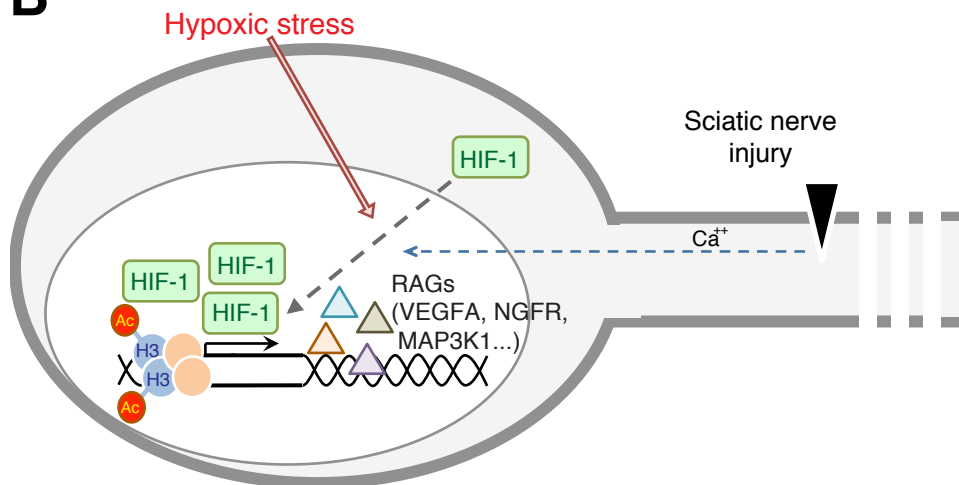


A**B**

Supplemental Figure Legends

Figure S1. qPCR analysis of selected HIF-1-target genes. (Related to figure 1).

Quantitative PCR analysis was done using RNA samples prepared from embryonic DRG cultures infected with control or HIF-1 α -knock down lentivirus. Seven HIF-1-target genes were tested (STC2, VEGFA, ENO2, JUN, TGFB3, ALDOA and GAPDH). Two genes were used as internal controls to confirm HIF-1-dependency (SF3B5 and MRPL10). Knockdown efficiency of HIF1A was confirmed from the each experimental set (** $p < 0.01$, * $p < 0.05$ by *t*-test; mean \pm SEM; ns, not significant)-

Figure S2. Knock down or overexpression of HIF-1 α in mouse embryonic DRG culture. (Related to figure 3).

(A) HIF-1 α was knocked down by two different shRNA lentiviruses (shHIF1A-1 and shHIF1A-2). Human GFP-HIF-1 α was overexpressed by lentivirus. Arrowhead points to endogenous HIF-1 α and arrow points to GFP- HIF-1 α . (B) Normalized intensity calculated from (A) ($n=4$; *** $p < 0.001$ by one-way ANOVA with Tukey test; mean \pm SEM)

Figure S3. Expression levels of HIF-1 α in control or HIF1AcKO DRGs and following sciatic nerve injury. (Related to figure 4).

(A) Representative western blot of HIF-1 α from control or HIF1AcKO mice. L4 and L5 DRGs were dissected from mice that received (+Ax) or not (-Ax) a prior (1 day) sciatic nerve axotomy and analyzed by western blot. (B) HIF-1 α were normalized to tubuli (TUJ1) and quantification of

HIF-1 α ($n=3$; *** $p<0.001$, * $p<0.05$ by one-way ANOVA with Tukey test; mean \pm SEM). (C) Representative HIF-1 α immunostaining from control or HIF1A cKO mice. L4 DRGs were dissected 1 day following sciatic nerve axotomy. Scale bar, 100 μ m and 20 μ m (for magnified images (A) and (B)). (D) Quantification of fluorescence intensity of nuclear HIF-1 α ($n=77$ and 99 from three control and three HIF1A cKO mice; *** $p<0.001$ by t -test; mean \pm SEM). (E) Western blot analysis of mouse L4 and L5 DRG tissues dissected at 24 hours after sciatic nerve injury. Protein samples were prepared from four individual mice. (F) Normalized intensity calculated from (A) ($n=4$; * $p<0.05$ by t -test; mean \pm SEM; -Ax, not injured; +Ax, injured). (G) Expression level of HIF1A mRNA by real time PCR analysis. Mouse L4 and L5 DRG tissues were dissected 24 hours after sciatic nerve injury and prepared for RNA extraction ($n=4$; * $p<0.05$ by t -test, -Ax, not injured; +Ax, injured). (H) Western blot analysis of mouse L4 and L5 DRG tissues dissected 24 hours after sciatic nerve injury in mice that received at the site of injury vehicle or BAPTA-AM treatment. (I) Normalized intensity calculated from (D) ($n=3$; ** $p<0.01$ by t -test; mean \pm SEM; -Ax, not injured; +Ax, injured)

Figure S4. Deletion of DLK in DRG neurons does not alter up-regulation of nuclear HIF-1 α induced by sciatic nerve injury. (Related to figure 5).

(A) Immunohistochemistry images of HIF-1 α -staining with (+Ax) or without (-Ax) sciatic nerve injury from control or DLK cKO mice. Scale bar, 50 μ m. Note that in this genetic background, the HIF-1 α - staining in the absence of axotomy appears diffuse in the cytoplasm, but nuclear accumulation is clearly induced by injury. (B) Quantification of fluorescence intensity of nuclear HIF-1 α ($n=55$, 106, 78 and 89 for control (-Ax), control (+Ax), DLK cKO (-Ax) and DLK cKO (+Ax), respectively. Two mice were used for each condition; *** $p<0.001$ by one-way ANOVA with Tukey test; mean \pm SEM; ns, not significant).

Figure S5. Deletion of HIF1A impairs VEGFA gene expression in mouse DRG tissue. (Related to figure 6).

(A) Quantitative PCR analysis of VEGFA from L4 DRGs in control or HIF1A cKO mice ($n=2$ per group, performed in triplicate ; $**p<0.01$, $*p<0.05$ by t -test; mean \pm SEM).

Figure S6. Live imaging showing the nuclear GFP-HIF-1 α intensity after Doxycyclin induction. (Related to figure 7).

(A) Representative time-lapse images of GFP-HIF-1 α accumulation in the nucleus of cultured DRG neurons. Scale bar, 5 μ m. The number indicates the hour after Doxycyclin treatment. (B) Average fluorescence intensity calculated from (A) ($n=6$; mean \pm SEM)

Figure S7. Specific HIF-1-target genes are activated from L4 and L5 DRG tissues after AIH *in vivo*. (Related to figure 8).

(A) Quantitative PCR analysis of fourteen HIF-1-target genes from L4 and L5 DRG tissues after a 2 h AIH regime ($n=3$; $***p<0.001$, $*p<0.05$ by one-way ANOVA with Tukey test; mean \pm SEM) .

(B). Proposed model for the role HIF-1 α in activating a pro-regenerative program. Axon injury induces nuclear accumulation of HIF-1 α , possibly via the injury-induced elevation of intracellular calcium ion. Nuclear HIF-1 α is required for the injury-induced increase in histone H3 acetylation and for the transcription of injury-responsive genes regulating axon regeneration. Hypoxic stress can be applied after injury, as a post-conditioning, to promote HIF-1 α stabilization and improve axon regeneration.

Symbol	3h	8h	12h	40h	3h	8h	12h	40h
ABCB6	0.17	0.05	0.21	0.36	●	●	●	●
ACVR1B	0.36	1.01	0.05	-0.84	●	●	●	●
AK3L1	-0.14	-0.42	0.54	0.55	●	●	●	●
APLP1	0.27	0.09	0.13	0.35	●	●	●	●
AVPI1	0.10	0.46	0.48	0.63	●	●	●	●
BCKDHA	0.29	0.26	0.39	0.27	●	●	●	●
BCL2L11	0.23	0.13	2.16	1.92	●	●	●	●
BCL6	0.27	-1.04	1.72	0.87	●	●	●	●
BCOR	-0.09	-0.15	0.08	0.34	●	●	●	●
BMPR2	0.17	0.37	-0.38	0.38	●	●	●	●
BRSK2	0.33	0.43	0.10	-0.01	●	●	●	●
CAV1	0.06	-0.65	0.32	-0.31	●	●	●	●
CCNL1	0.89	-0.09	0.56	0.06	●	●	●	●
CDH22	-1.36	0.52	-0.08	0.05	●	●	●	●
DARS	0.28	0.15	-0.03	-0.31	●	●	●	●
DDR1	0.16	-0.42	0.10	0.55	●	●	●	●
DDX4	0.11	0.38	1.06	0.25	●	●	●	●
DTNA	0.44	0.30	0.32	-0.03	●	●	●	●
DYSF	-0.98	-0.04	-0.34	0.72	●	●	●	●
ELF3	0.36	1.38	0.64	-0.18	●	●	●	●
FBXO42	0.34	0.48	0.05	0.70	●	●	●	●
FGF11	0.27	0.16	0.38	0.24	●	●	●	●
FIBCD1	0.35	0.50	0.63	1.28	●	●	●	●
FNIP1	-0.01	-0.33	0.68	0.26	●	●	●	●
GADD45B	0.24	-0.14	1.05	0.58	●	●	●	●
GJA1	-0.73	-0.68	-0.34	0.33	●	●	●	●
GPT2	0.54	-0.30	-0.11	-0.11	●	●	●	●
GYS1	0.63	0.53	0.37	-0.01	●	●	●	●
HIVEP2	0.20	0.18	-0.29	0.29	●	●	●	●
HOMER1	-0.45	0.09	0.70	0.82	●	●	●	●
HOXA4	0.00	0.19	0.26	0.44	●	●	●	●
KLF10	0.70	-1.15	-0.19	0.48	●	●	●	●
KLF4	0.97	1.41	1.26	1.55	●	●	●	●
KLF6	0.02	0.89	-0.20	-0.81	●	●	●	●
MDGA2	-0.03	0.33	0.39	0.27	●	●	●	●
MNT	-0.40	-0.56	-0.11	0.57	●	●	●	●
MTTP	0.29	0.56	0.31	-0.19	●	●	●	●
NXPH1	0.71	-0.10	-0.44	-1.13	●	●	●	●
OXSR1	-0.31	0.28	-0.22	1.33	●	●	●	●
PIM2	0.42	0.87	0.52	-0.07	●	●	●	●
PLXND1	0.22	0.26	0.60	0.93	●	●	●	●
PNRC1	0.18	0.29	0.93	0.63	●	●	●	●
PSD3	-0.03	0.42	0.18	0.07	●	●	●	●
RAP2B	-0.19	0.16	0.45	-0.03	●	●	●	●
RASSF1	0.26	0.07	0.73	0.42	●	●	●	●
RCOR2	-0.01	-0.10	0.24	0.32	●	●	●	●
RORC	0.28	-0.36	-0.40	-4.15	●	●	●	●
SCN1A	-0.14	0.54	0.77	0.07	●	●	●	●
SFRS5	0.23	0.11	0.18	0.39	●	●	●	●

Symbol	3h	8h	12h	40h	3h	8h	12h	40h
SFXN3	0.13	0.13	0.32	-0.11	●	●	●	●
SIAH2	-0.26	-0.54	0.36	-0.18	●	●	●	●
SLC25A36	0.02	-0.08	0.38	0.01	●	●	●	●
ST6GAL1	-0.47	-0.07	0.07	0.29	●	●	●	●
SYT5	0.24	0.17	0.42	0.51	●	●	●	●
TCEAL3	0.17	0.33	0.58	0.22	●	●	●	●
TEX264	0.24	0.25	0.65	0.46	●	●	●	●
TSC22D3	0.73	0.79	0.67	0.38	●	●	●	●
XPNPEP1	0.32	0.25	0.19	-0.26	●	●	●	●
YPEL5	0.35	0.62	1.13	1.02	●	●	●	●
ZFX	-0.35	-0.66	1.06	0.62	●	●	●	●
ZNF653	0.28	0.10	-0.01	0.26	●	●	●	●
AMOTL2	0.18	-0.12	-0.37	-1.16	●	●	●	●
ANKRD12	0.11	0.04	-0.04	0.15	●	●	●	●
CHST8	-0.07	-0.26	-0.39	-0.37	●	●	●	●
CLK3	-0.12	0.08	0.00	0.00	●	●	●	●
EFNA3	-0.21	0.06	0.11	0.12	●	●	●	●
ELOVL4	0.08	0.13	-0.02	0.06	●	●	●	●
EMID2	0.22	0.03	-0.31	-0.53	●	●	●	●
GNAI1	0.09	0.07	0.08	-0.10	●	●	●	●
HUWE1	-1.96	-0.71	-1.25	-1.80	●	●	●	●
IER5L	-0.06	0.16	-0.05	0.09	●	●	●	●
KLF7	0.15	0.13	-0.01	0.08	●	●	●	●
KLHDC3	0.01	-0.10	-0.06	0.13	●	●	●	●
LDB1	0.25	0.16	0.19	0.02	●	●	●	●
MKNK2	-0.18	-0.08	-0.19	-0.07	●	●	●	●
MTCH2	-0.09	-0.17	-0.46	-0.51	●	●	●	●
NEGR1	-0.91	0.15	-0.41	-2.37	●	●	●	●
NRXN2	0.02	0.03	0.04	0.15	●	●	●	●
PCOLCE2	-0.40	-0.46	-0.61	-1.54	●	●	●	●
PCYT1B	-0.46	-0.41	-0.57	-0.21	●	●	●	●
PHF21A	-0.48	-0.06	-0.87	-1.64	●	●	●	●
PLCH2	-0.02	-0.02	-0.13	0.06	●	●	●	●
PPME1	0.09	0.13	0.11	0.06	●	●	●	●
PPP1R3C	-0.04	-0.26	0.02	-0.02	●	●	●	●
PPP1R9A	0.00	0.01	0.00	0.07	●	●	●	●
PTPRR	-0.16	-0.53	-0.54	-1.14	●	●	●	●
RCOR1	-0.17	0.20	-0.72	-0.82	●	●	●	●
RRAGD	0.02	-0.09	0.08	-0.17	●	●	●	●
SERGEF	-0.04	0.02	0.04	0.05	●	●	●	●
SPOCK1	-0.17	-0.03	-0.11	-0.02	●	●	●	●
TPDS2	-0.07	-0.03	-0.27	0.10	●	●	●	●
UGP2	-0.13	-0.29	-0.22	-0.40	●	●	●	●
UVRAG	0.09	-0.06	-0.10	-0.13	●	●	●	●
VAPA	-0.61	-0.61	-0.51	-1.01	●	●	●	●
YEATS2	-0.17	0.00	-0.32	0.00	●	●	●	●
ZC3H6	-0.24	-0.03	-0.35	-0.10	●	●	●	●
ZDHHC9	-0.06	-0.04	-0.23	-0.14	●	●	●	●
ZNF292	-0.94	-0.38	-0.64	-0.75	●	●	●	●

Supplemental Tables

Table S1. mRNA expression fold changes of genes known as HIF-1 α target genes after axotomy *in vitro*. (Related to figure 1A).

HIF-1 α -target genes from the reference (Benita et al., 2009) were selected from the microarray results previously performed (Cho et al., 2013) and listed with expression fold changes at different hours after axotomy. PID indicates the Pubmed ID for the individual reference showing the evidence of HIF-1 α -dependency. The 3h, 8h, 12h and 40h indicate the time after *in vitro* axotomy. Each value indicates log₂-transformed expression fold changes. The red dot indicates the highest up-regulation at the indicated hours after axotomy over 0.263. The gray dot indicates the mild up-regulation at the indicated hours after axotomy over 0 and below 0.263. The black dot indicates the down-regulation at the indicated hours after axotomy

Table S2. mRNA expression fold changes of genes predicted as HIF-1 target genes after axotomy *in vitro*. (Related to figure 1B).

Predicted HIF-1 α -target genes from the reference (Ortiz-Barahona et al., 2010) were selected from the microarray result previously performed (Cho et al., 2013) and listed with expression fold changes at different hours after axotomy. The 3h, 8h, 12h and 40h indicate the time after *in vitro* axotomy. Each value indicates log₂-transformed expression fold changes. The red dot indicates the highest up-regulation at the indicated hours after axotomy over 0.263. The gray dot indicates the mild up-regulation at the indicated hours after axotomy over 0 and below 0.263. The black dot indicates the down-regulation at the indicated hours after axotomy

Table S3. List of genes up-regulated by constitutive overexpression of HIF-1 α . (Related

to figure 2B).

Probe indicates Illumina gene ID. The number indicates \log_2 -transformed average fold change by constitutive overexpression of HIF-1 α (green, $\log_2[\text{average fold}] \leq 1.20$; orange, $1.20 < \log_2[\text{average fold}] < 1.9$; red, $1.9 \leq \log_2[\text{average fold}]$).

**Table S4. List of genes down-regulated by constitutive overexpression of HIF-1 α .
(Related to figure 2B).**

Probe indicates Illumina gene ID. The number indicates \log_2 -transformed average fold change by constitutive overexpression of HIF-1 α (gray, $-2.0 \leq \log_2[\text{average fold}]$; light blue, $-2.0 < \log_2[\text{average fold}] < -2.9$; blue, $\log_2[\text{average fold}] \leq -2.9$).

**Table S5. List of genes screened as HIF-1 α -dependent injury-induced genes (1 to 493)
(Related to figure 2C).**

Probe indicates Illumina gene ID. The number “Cslope” or “KSlope” indicates the calculated slopes of relative fold changes of expression. The number “O/C” indicates the relative expression level of genes in HIF-1 α -overexpressing DRG neurons over control DRG neurons (see Experimental Procedures for details; RNA preparations, qPCR and microarray analyses).

Supplemental Experimental Procedures

Antibodies and lentiviruses

The following antibodies were used in this study: anti-HIF-1 α (Novus, NB100-479; Abcam, ab113642 and ab2185), anti-SCG10 (Novus, NBP1-49461), anti- β III tubulin (Covance, MMS-435P), anti-GFP (Santa Cruz, sc-9996), anti-p-PKC μ (Cell Signaling, #2051), anti-PKC μ (Cell Signaling, #2052), anti-PKC δ (Cell Signaling, #9616), anti-p-PKC (pan) (Cell Signaling, #9371), anti-p-JNK (Cell Signaling, #9912), anti-p-c-Jun (Cell Signaling, #9261), anti-c-Jun (Cell Signaling, #9165) and anti-DLK (Antibodies Incorporated, #75-355). To express HIF-1 α in cultured DRG neurons, human HIF1A cDNA (Addgene, 18949) was sub-cloned into FUGW lentiviral vector for constitutively overexpression. HA-HIF1 α -pcDNA3 was a gift from Dr. William Kaelin (Addgene plasmid #18949 (Kondo et al., 2002)). For inducible expression, human HIF1A cDNA was sub-cloned into tetracycline-responsive lentiviral expression vector LV-TRE-GFP-Ubi-rtTA3 by the Viral Vector Core (Washington University, School of Medicine). Mouse VEGF 164 (493-MV-025/CF) from R&D systems was used in sciatic nerve regeneration assays.

Lentiviral-mediated knock down of HIF-1 α and selected HIF-1 α dependent genes.

To knock down HIF-1 α and selected HIF-1 α dependent genes for *in vitro* regeneration assay, MISSION shRNAs from Sigma were used and lentiviruses were generated as previously described (Cho and Cavalli, 2012). MISSION shRNAs from Sigma were used: HIF-1 α , TRCN0000232222, TRCN0000232223; PDE1B, TRCN0000438413, TRCN0000434573; HMOX1, TRCN0000234077, TRCN0000234075; MAP3K1, TRCN0000361582, TRCN0000361513; ARHGEF3, TRCN0000110045, TRCN0000110048; ARHGAP29, TRCN0000023924, TRCN0000023926; NGFR, TRCN0000065555, TRCN0000065554; VEGFA, TRCN0000066818, TRCN0000066822, TRCN0000315978).

Meta analysis and Microarray analyses

Raw data from our previous microarray (Cho et al., 2013) was used to obtain a list of genes up-regulated at 0, 3, 8, 12 and 40 h after *in vitro* axotomy. Quantile normalized expression levels were used. Genes with *p* value less than 0.01 at each time-point and with a fold expression change at any point >1.2 compared to the 0 h control were included in our list of injury-induced genes. This list of injury-induced genes was compared with lists of known HIF-1 α -known target genes (Benita et al., 2009) or HIF-1 α -predicted target genes (Ortiz-Barahona et al., 2010).

To study HIF-1 α -dependent gene regulation, a microarray analysis was done as previously described (Cho et al., 2013), comparing control DRG neurons to DRG neurons in which HIF1A was knocked down or DRG neurons in which human HIF1A was overexpressed. Total RNA was extracted at 0, 3 and 12 h after axotomy from control cultured DRG neurons, DRG neurons in which, HIF1A was knocked down or DRG neurons in which human HIF1A was overexpressed. To analyze expression profile, MouseRef-8 v2.0 Bead-Chips (Illumina) from Genome Technology Access Center at Washington University was used. To screen for the minimal sets of injury-responsive HIF-1 α -dependent genes, the genes detected with *p* value smaller than 0.1 were selected. Injury-responsive genes were then filtered by expression level at 3 or 12 hours after axotomy. Only genes with expression levels 16% higher at 3 or 12 hours than at 0 hour time point were selected ($[\text{expression level}_{3 \text{ hours}}] / [\text{expression level}_{0 \text{ hour}}] > 1.16$ OR $[\text{expression level}_{12 \text{ hours}}] / [\text{expression level}_{0 \text{ hour}}] > 1.16$). To screen for HIF-1 α -dependent genes, “slope of expression level” was calculated. The slope of expression level was calculated using the gene expression fold change at 0, 3 and 12 hours after axotomy from the control sets (“Cslope”) or HIF-1 α -knock down sets (“Kslope”). Based on the calculated slope, only the genes that have a higher slope in the control sets compared to HIF-1 α -knock down sets either at 3 or 12 hours were selected. ($[\text{expression level of control}_{3 \text{ hours}}] / [\text{expression level of}$

HIF-1 α -knock down_3 hours] > 1 OR [expression level of control_12 hours] / [expression level of HIF-1 α -knock down_12 hours] > 1). To further restrict the HIF-1 α -dependency, only the genes showing higher expression level in overexpressed HIF-1 α compared to the control were selected ([expression level of HIF-1 α -overexpression_0 > [expression level of control_0 hour], O/C).

Surgeries and tissue preparations

Sciatic nerve injury experiments were performed as described (Cho and Cavalli, 2012). For double crush injury experiments, we followed previous protocols (Shin et al., 2012). Briefly, the nerve was crushed with fine forceps for 20 s. The site of the first injury was marked by dipping the tips of forceps in 1 μ m orange fluorescence beads (FluoSpheres; Invitrogen). The second crush was given at 1mm proximal to the first crush site under an SZX12 fluorescence-dissecting microscope (Olympus) to injure all axons.

For mouse L4 dorsal root nerve crush surgery, an ~2cm skin incision was made above the L2 vertebra and the soft tissue below dissected. Mice were then placed in a custom frame with 2 horizontal stainless steel 'arms' that were slid under each L2 transverse process in order to stabilize the mouse. The connective tissue between the L2 and L3 vertebra was then removed and a small laminectomy of the caudal portion of the L2 vertebra was performed to create a small opening exposing the spinal cord and lumbar dorsal roots. Fine forceps were then used to pierce through the dura perpendicular to the spinal cord and crush the right L4 dorsal root ~6mm from the L4 DRG for 5 seconds. The underlying tissue was sutured with 6.0 silk sutures and the skin closed with metal clips.

For western blot analysis, mouse DRG tissues were dissected at the indicated time after nerve injury. Mouse DRG tissues were homogenized in lysis buffer (Cell Signaling) as described (Cho and Cavalli, 2012). Protein concentration was quantified by Bio-Rad protein assay kit (Bio-

Rad). For immunohistochemistry, mouse DRG tissues were prepared as described (Cho and Cavalli, 2012). L4 or L5 DRG were dissected and fixed for 1 hour in 4% paraformaldehyde in PBS, incubated overnight in 30% sucrose, embedded in OCT solution (Tissue-Tek) and frozen in dry ice-cooled methylbutane. 10 μ m cryostat sections were stained with the indicated antibodies using standard methods. To measure the intensity of nuclear HIF-1 α , region-of-interest (ROI) of DAPI-positive area from TUJ-1-positive cells was selected and HIF-1 α -fluorescence intensity was measured using ImageJ.

Embryonic DRG neuron spot culture and *in vitro* regeneration assay

Embryonic DRG neurons were cultured as previously described (Cho and Cavalli, 2012). Briefly, DRG neurons of mouse embryos at E13.5 were dissected, and trypsinized and dissociated. For spot culture, 10,000 DRG neurons were resuspended in 2.5 μ l of medium and plated on PDL/laminin-coated culture dishes and incubated for 20 minutes before adding more medium. To knock down or overexpressing HIF-1 α , or knock down HIF-1 α target genes, lentiviruses were infected at DIV4. *In vitro* regeneration assay was performed as previously described (Cho and Cavalli, 2012). DRG spot cultures were axotomized with a blade (FST, 10035-10) at DIV7 under a dissection scope (Nikon, SMZ645) and immunostained for SCG10 and β III tubulin antibodies. Images were acquired on a Nikon, TE2000E microscope. A regeneration index was calculated from the images acquired 40 h post axotomy by measuring the fluorescence intensity of a square area (2.7x0.1mm) at 0.1 mm distal to the axotomy line and normalizing this intensity to the similar area 0.1 mm proximal to the axotomy line.

***In vivo* regeneration assays**

To test for axon regeneration *in vivo*, sciatic nerves were dissected at the indicated time after a crush injury. Longitudinal sections of fixed sciatic nerves were stained with SCG10 and TUJ1, as described in (Shin et al., 2012). For each animal, SCG10 fluorescence intensity was

measured along the length of the nerve using a line scan macro in ImageJ in five serial sections. A regeneration index was calculated by measuring the average SCG10 intensity at several distances away from the crush site, which is defined by the position along the sciatic nerve length with maximal SCG10 intensity (Shin et al., 2014). To measure reinnervation of the neuromuscular junction after sciatic nerve injury, the tibialis anterior muscle as well as the distal 5 mm of the deep peroneal nerve from thy1-YFP16 mice was removed and the extensor hallucis longus (EHL) muscle was dissected, fixed and stained with Alexa Fluor 647-conjugated α -bungarotoxin (Invitrogen) and mounted for confocal imaging. The number of NMJ endplates re-occupied (O) or non-occupied (N) by YFP signal was counted and normalized to the total number of endplates to calculate the percentage.

Hypoxia treatment

For *in vitro* hypoxia, established protocols were followed (Wu and Yotnda, 2011). Briefly, embryonic DRG cultures were placed in a gas-tight hypoxia chamber containing a Petri dish filled with sterile water to maintain humidity. To obtain hypoxic condition, a tube attached to a gas tank containing 5% CO₂ balanced with nitrogen was connected to the chamber with airtight connector. The air in the chamber was flushed for 10 minutes by opening the gas tank at a flow rate of 20 L/min. The chamber was then placed back in the cell culture incubator for the indicated amount of time. Control cultures underwent the same procedure but the air in the control chamber was flushed with 5% CO₂ balanced with normal air.

For *in vivo* hypoxia, all procedures were approved by Washington University in St. Louis, School of Medicine Animal Studies Committee. To perform whole body-hypoxia treatment, an established protocol (Zhang et al., 2004) was followed with minor modifications. Briefly, conscious mice were exposed to AIH by being placed in a gas-tight chamber (E-Z Anesthesia).

Air was continuously flushed at a rate of 1L/min. Chamber was flushed with oxygen balanced with nitrogen: 10 min episodes of 8% O₂; 10 min intervals with normal air. After 6 hypoxic episodes with equivalent normoxic intervals (120min) the mice were returned to their cages until the next exposure. For sciatic nerve axon regeneration experiments, the 120 min AIH protocol began 2 hours after sciatic nerve injury and was applied once daily for 3 days. For muscle reinnervation experiments, the 120 min AIH protocol was administered daily for 7 days starting 1 hour after sciatic nerve injury and mice were returned to their cages for 5 days. For control groups, mice were placed in the chamber for the same durations but normal air was supplied.