Supplemental Figures and Legends

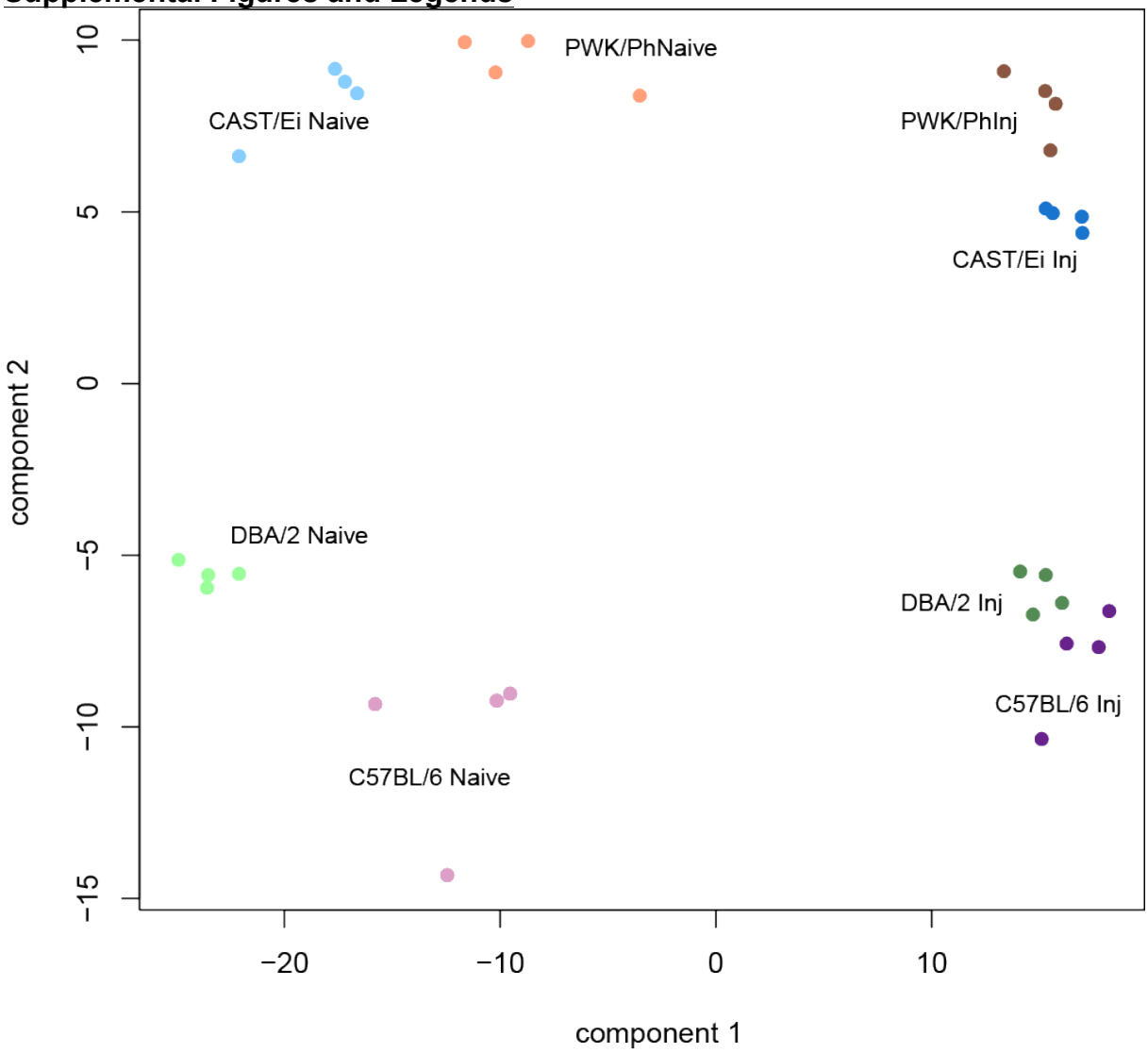

Fig. S1. **Principal component analysis of the transcriptional profile of the four strains under study,** Related to Figure 1. The first component separates the samples according to the treatment (naive vs injured). The second component separates the two *M. m. domesticus* (C57/BL 6 and DBA/2) stains from the two other subspecies (CAST/Ei and PWK/Ph).

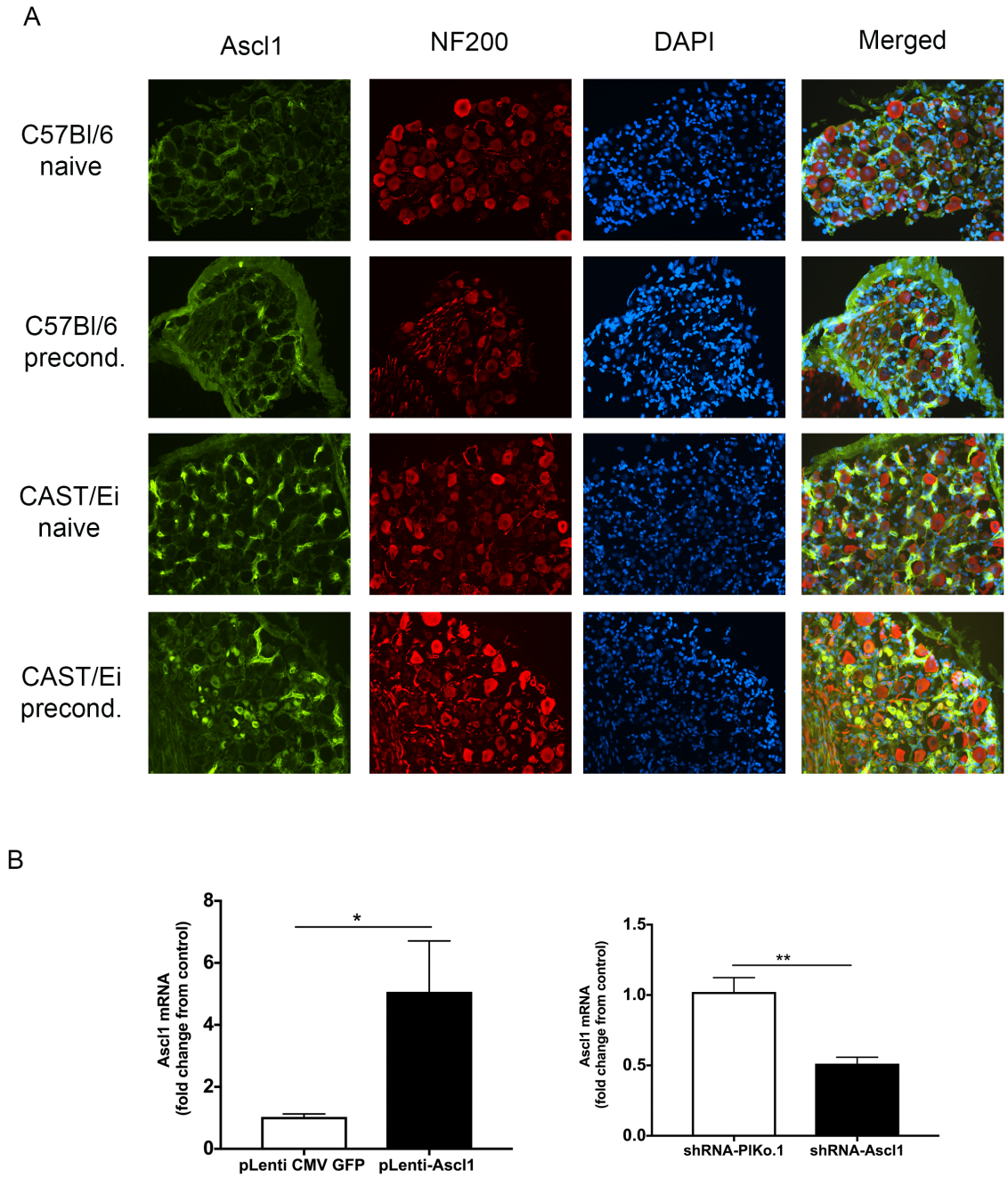

Fig. S2. **Ascl1 expression**, Related to Figure 1. (A) Immunohistochemistry showing Ascl1 expression is increased in a proportion of neurons after injury. (B) Ascl1 mRNA is increased after Ascl1 overexpression and decreased after Ascl1 shRNA.

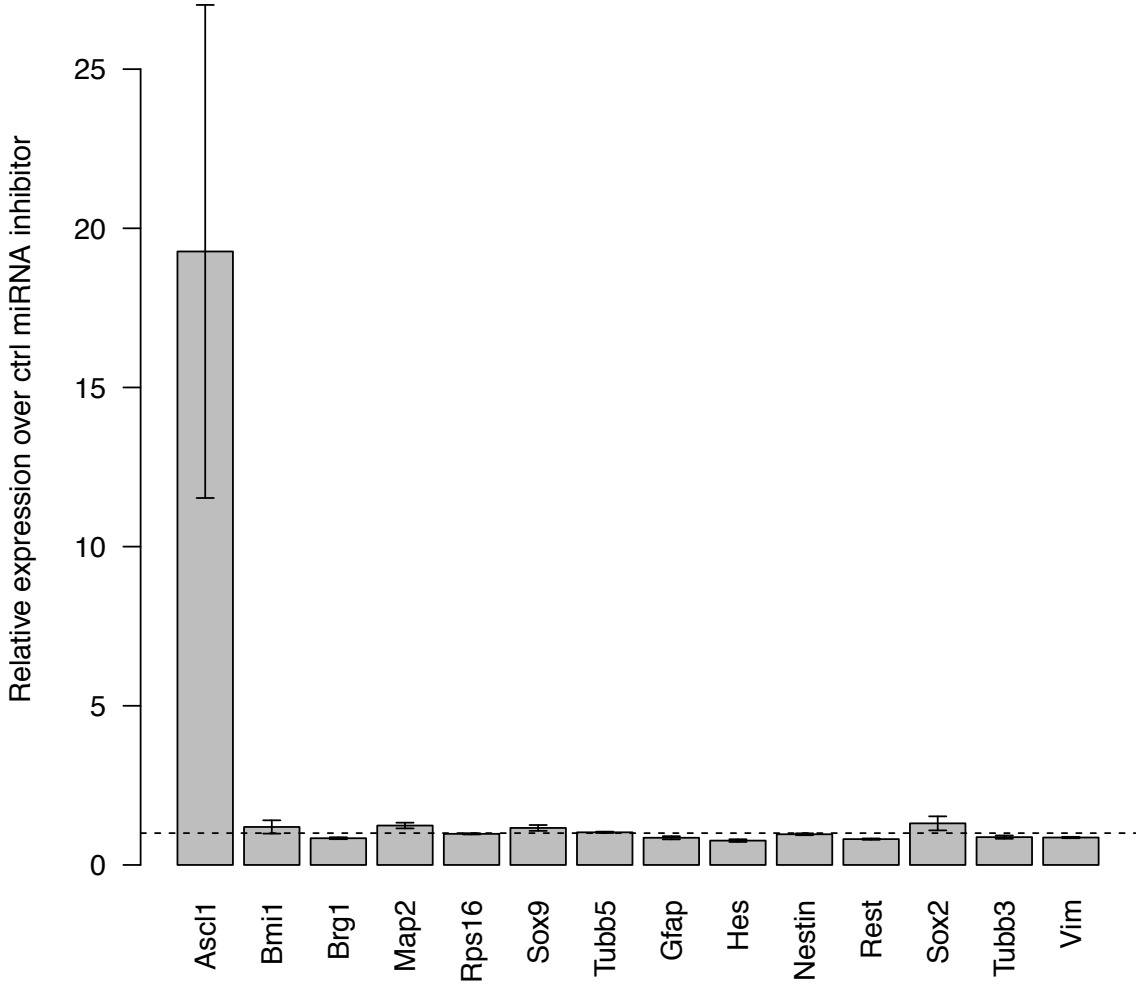

Fig. S3. **miR-7048-3p inhibits ASCL1 at the mRNA level**, Related to Figure 3. Ascl1 mRNA levels are decreased after transfection of C17.2 cells with miR-7048-3p inhibitor. This effect is not observed on the expression of a panel of other genes.

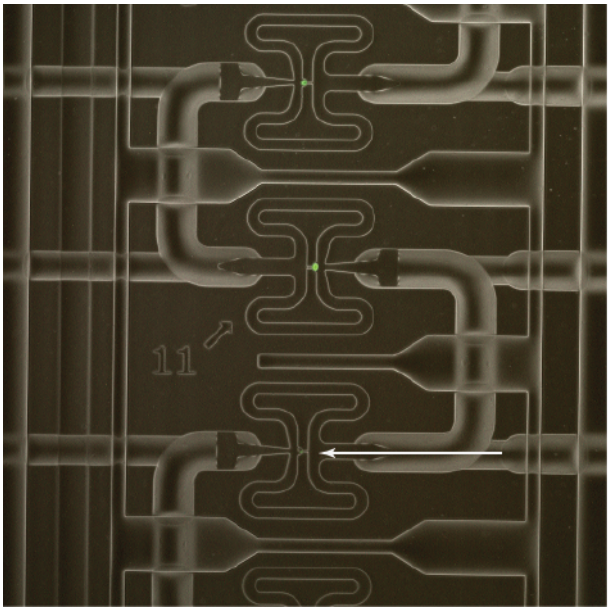

Fig. S4 **C1 harvest site**, Related to Figure 5**.** Representative example of two C1 harvest sites containing a live neuron and one harvest site containing a glia cell. The later one is discarded.

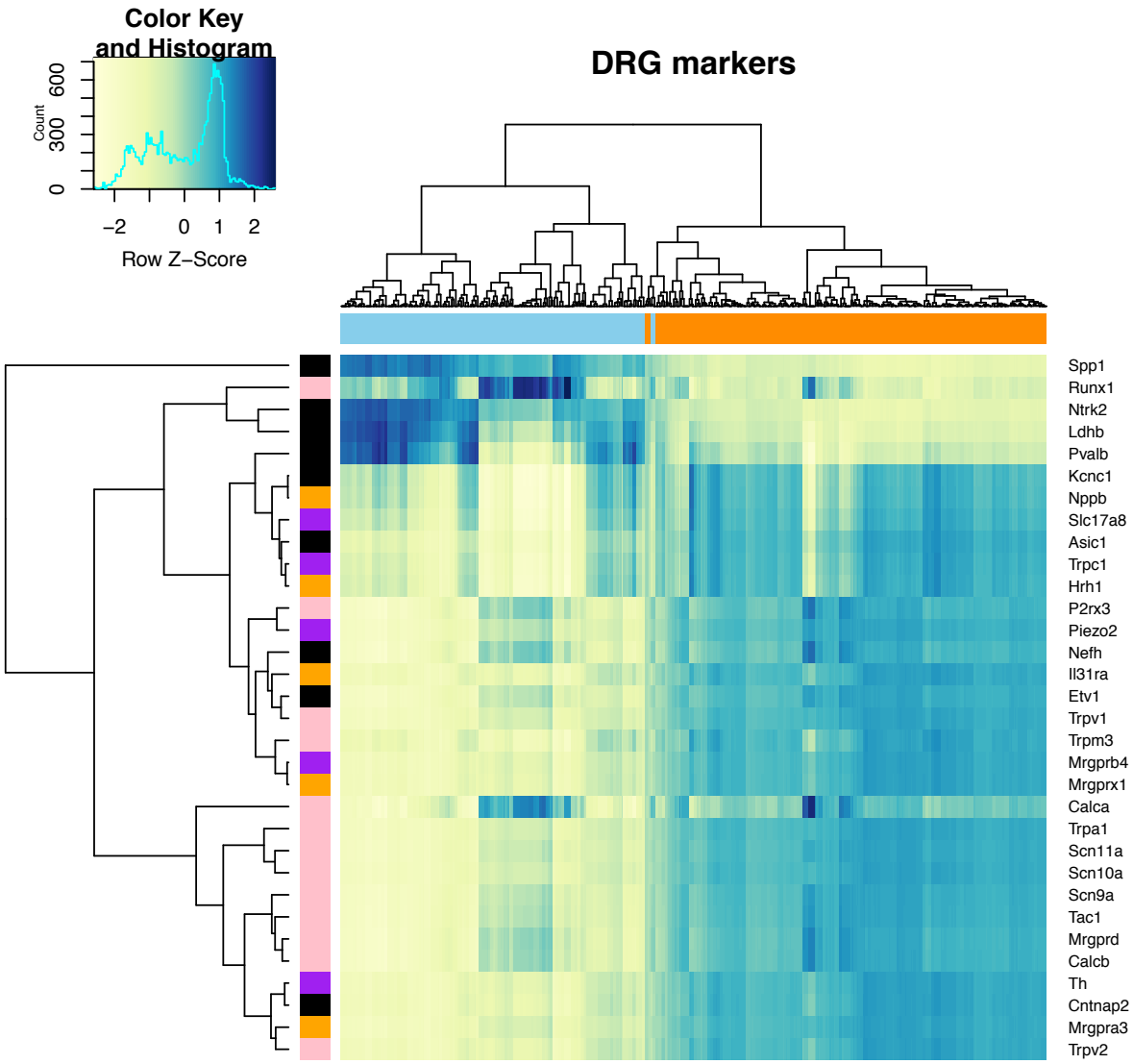

Fig. S5. **DRG neuron markers do not classify scSeq samples**, Related to Figure 5. Known markers of 4 types of DRG neurons were used to cluster single cell sequencing samples. Rows color coded in black are genes associated with proprioception, those color coded in pink are associated with thermoception, those in orange are associated with pruriception whereas the rows color coded in purple are markers of tactile function neurons. Markers for specific functions do not cluster together and therefore cannot be used to assign identity to each of the neurons sequenced.

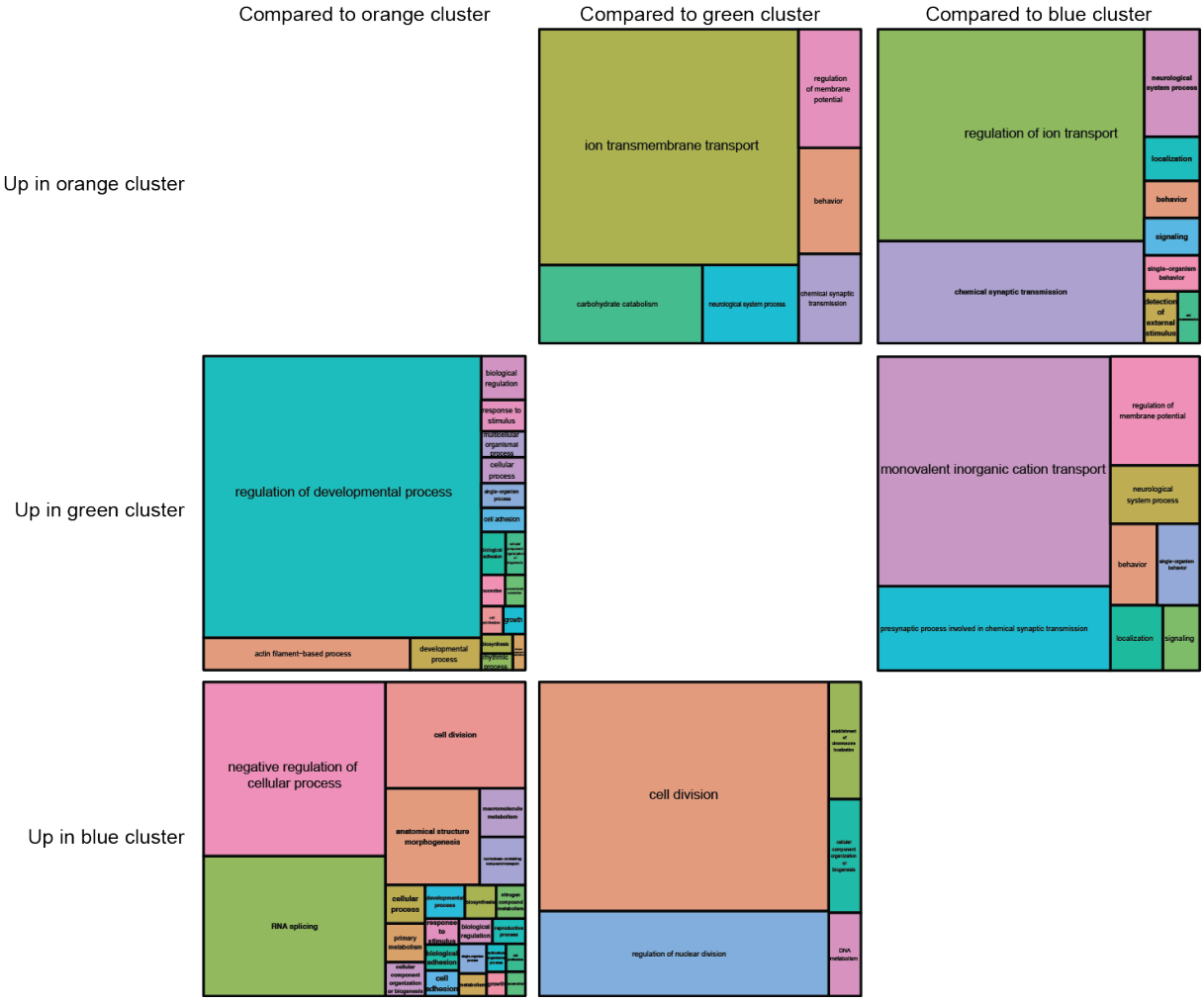

Fig S6. **Comparison of the enriched GO terms associated with the up regulated genes of each cluster of single cell**, Related to Figure 5. The first row are the GO terms enriched in the up regulated genes in the first (orange) cluster compared to the second (green) or third (blue) cluster. The second row presents the enriched GO terms associated with the up regulated genes in the second cluster compared to the first cluster or the third (blue) cluster. The last row showd the GO terms enriched in the upregulated genes of the third cluster compared to the first or second cluster. Comparing the green cells to the blue cells yield similar result to comparing the orange cells to the blue cells whereas comparing the green cells to the orange cells yield similar results to comparing the blue cells to the orange cells.

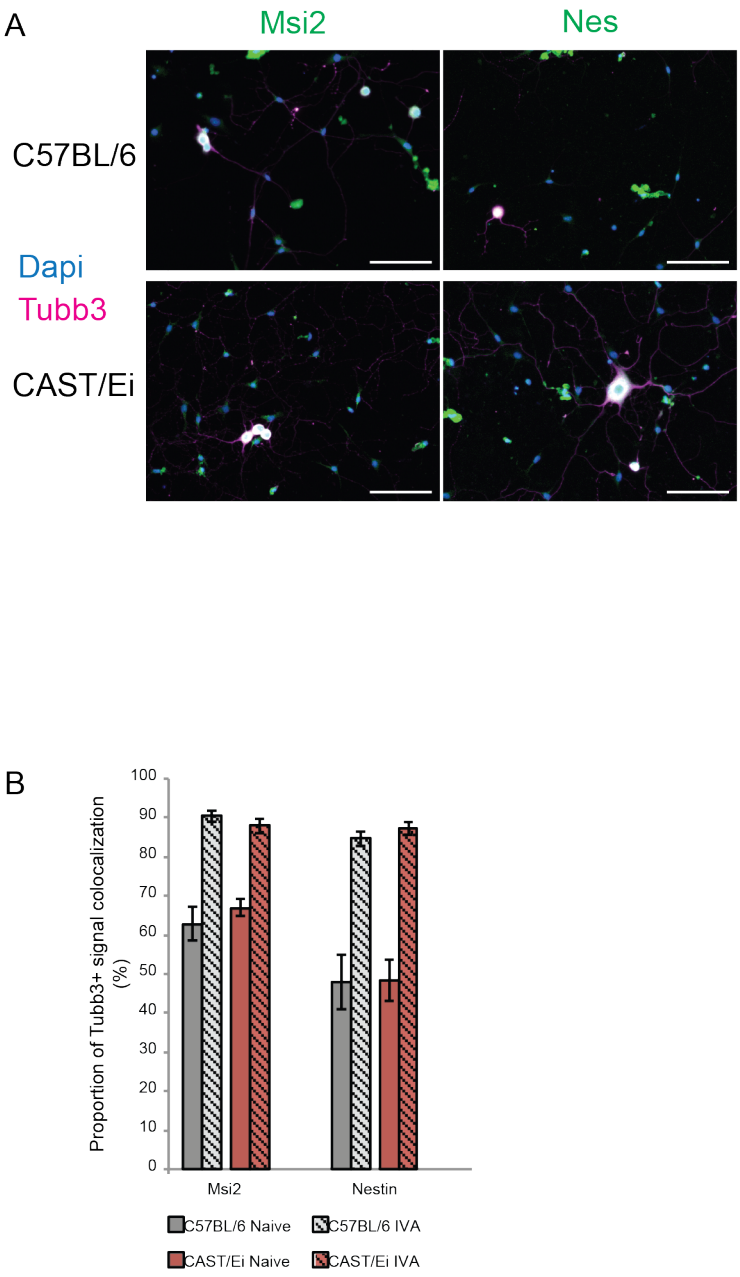

Fig. S7. **Expression of Nestin and Msi2 after IVA**, Related to Figure 5. In both C57BL/6 and CAST/Ei strains, IVA increases the expression of Nestin and Msi2.

Supplemental Tables and Legends

Table SI. Neuronal GO terms associated with miR-7048-3p putative targets, Related to Figure 4

GO Term	Description	P-value	FDR q-value	Enrichment
GO:0050877	neurological system process	3.38E-05	4.40E-02	2.24
GO:0050890	cognition	4.19E-05	4.96E-02	3.11
GO:0007611	learning or memory	4.79E-05	4.79E-02	3.31
GO:0007610	behavior	4.87E-05	4.53E-02	2.4
GO:0050804	modulation of synaptic transmission	1.37E-04	6.83E-02	2.13
GO:0007399	nervous system development	1.42E-04	6.82E-02	2
GO:0097090	presynaptic membrane organization	1.47E-04	6.81E-02	19.06
GO:0051963	regulation of synapse assembly	1.93E-04	7.84E-02	5.13
GO:0007411	axon guidance	2.13E-04	8.14E-02	2.42
GO:0030534	adult behavior	2.28E-04	8.01E-02	3.78
GO:0097485	neuron projection guidance	2.89E-04	8.54E-02	2.38
GO:0050770	regulation of axonogenesis	3.02E-04	8.53E-02	2.2
GO:0050962	detection of light stimulus involved in sensory perception	4.81E-04	1.14E-01	13
GO:0050908	detection of light stimulus involved in visual perception	4.81E-04	1.12E-01	13
GO:0045664	regulation of neuron differentiation	7.33E-04	1.34E-01	1.54
GO:0060291	long-term synaptic potentiation	7.38E-04	1.30E-01	6.27
GO:1900272	negative regulation of long-term synaptic potentiation	7.75E-04	1.33E-01	46.61
GO:0009584	detection of visible light	8.44E-04	1.39E-01	11.56

Supplemental Experimental Procedures

Cell culture

293T, NIH/3T3, Neuro2A cells were obtained from ATCC. 293T and NIH/3T3 cells were grown in DMEM supplemented with 10% fetal bovine serum. Neuro2A cells were grown in EMEM supplemented with 10% fetal bovine serum. All cells were cultured at 37˚C with 5% CO_2.

Ascl1 infection and neurite outgrowth assay

mCherry and Ascl1 were cloned into pLenti CMV GFP using Gibson cloning (New England Biolabs). The Ascl1 sequence was codon optimized (Integrated DNA Technology) and synthesized using GeneArt Strings (Life Tech). DNA overhangs were included in the primers, to generate an optimal Kozac sequence. The DNA sequence was gel-purified, amplified, and inserted into linearized pLenti CMV GFP via a Gibson cloning reaction. Ascl1 and mcherry clones were tested in HEK 293T cells for expression efficiency by evaluating GFP expression 48 h after transfection. Viral particles were produced by viral core at FM Kirby Neurobiology Center, Boston Children's Hospital as previously described (Chandran et al., 2016). Dissociated sensory neurons were plated on PDL-laminin coated 12 well plates in Neurobasal medium (Invitrogen) supplemented with B27 supplement, penicillin, streptomycin, 1 mM L-glutamine, 50 ng/ml NGF, 2 ng/ml GDNF, and 10 uM Cytosine-arabinoside

(AraC, Sigma). Next day, neurons were transduced with virus in presence of Protamine sulfate for 16 h. Media was replaced every 48 h and cultures were replated on 96 wells pre-PDL (Falcon) and laminin (5 mg/well, Invitrogen) coated plates and fixed with 8% paraformaldehyde 24 h after replating. Fixed neurons were stained with anti-mouse beta-tubulin (1:800, Sigma) and visualized using goat anti-mouse 488 (1:500, invitrogen) secondary antibody. Images were captured with the high throughput screening platform ImageXpress Micro Systems, and neurite outgrowth was automatically quantified using the MetaXpress software. In another set of cultures, primary mouse DRG sensory neurons transduced with either pLenti CMV GFP or pLenti Ascl1 overexpressing lentivirus were harvested in Buffer RLT (Qiagen) after 6 days. RNA was isolated using Qiagen RNAmicro isolation kit and cDNA was synthesized using Vilo kit (Invitrogen). mRNA levels were quantified using qRT-PCR for GAPDH and Ascl1. The primers used were, Ascl1: F, CCCCCATTCCAGCGATCT and R, GTCCGAGAACTGACGTTGCTT; GAPDH: F, CATGGCCTTCCGTGTTCCTA, R, GCGGCACGTCAGATCCA

Knockdown of Ascl1

Mission control plasmid containing shRNA sequences for Ascl1 or shRNA control vector, containing a non-specific shRNA, were purchased from Sigma-Aldrich. Viral particles were produced at FM Kirby Neurobiology Center at Boston Children's Hospital viral core as previously described (Chandran et al., 2016). Transfection, replating and analysis were same as described above. In a separate experiment for knockdown efficiency, viral particles containing either control shRNA (pLKO.1) or Ascl1shRNA plasmid were transduced in DRG neurons, RNA was harvested and mRNA levels were quantified using qRT-PCR after 6 days.

Luciferase reporter assays

Ascl1 promoter. The 5kb genomic region upstream of the *Ascl1* start site was amplified from genomic DNA of either C57BL/6 or CAST/EI using the following primers: Fw CTAGCCCGGGCTCGATCCAAGTCTCCTGCTCCATATTATT Rv CAGTACCGGAATGCCATCTTTCTAATTGCTCTCTCGTTCCC. The DNA was gel purified and used for Gibson Assembly into the pGL3-promoter vector (Promega) digested with BglII and HindIII using the NEB Gibson Assembly master mix following the manufacturer's instruction. 20 000 NIH/3T3 cells were plated per well of a 24 well plate 24 hours before transfection and transfected using 3.2ug of PEI, 0.6ug of pGL3-Ascl1 vector and 0.2ug of phRL-TK vector (Promega) containing the Renilla luciferase. 72 hours after transfection, cells were lysed and luciferase activity was assessed using the Dual-Glo luciferase assay system (Promega) according to the manufacturer's instruction. The ratio of firefly luciferase to renilla luciferase was computed. 3 biological replicates and 2 technical replicates were performed.
Ascl1 3'UTR. The 3'UTR of *Ascl1* was cloned downstream of hLuc in the pEZX-MT06 vector (Genecopoeia) which also contains a renilla luciferase. 175 000 Neuro2A cells were plated per well of a 24 well plate and transfected with 100ng of pEZX-MT06-Ascl1 vector and a final concentration of 25nM of the miRNA inhibitor (Exiqon) using 2ul of Lipofectamine 2000. 48 hours after transfection, cells were lysed and luciferase activity assessed using the Luc-Pair Duo-Luciferase Assay kit (Genecopoeia) according to manufacturer's instructions. The ratio of firefly luciferase activity over renilla luciferase activity was computed.

Immunohistochemistry

L3-5 DRGs from naïve and preconditioned c57 and CAST mouse DRGs were harvested 5 days after the sciatic nerve crush. Mice were perfused with 4% paraformaldehyde followed by DRG isolation. DRGs were embedded in OCT and 10 micron cryosections were made. Slides were stained with mouse Ascl1 (1:100, BD Pharmingen) and chicken NF-200 (Cell signaling) and labeled with anti-mouse 488 (1:300) and anti-chicken 568 (1:500). Slides were counterstained with DAPi for nuclear stain. Images were taken at the same exposure using Nikon fluorescence microscope.

RNA sequencing

Bulk samples mRNASeq. RNA sequencing was performed as described (Omura et al., 2015). Reads were mapped to the mm10 mouse genome using TopHat and summarized to gene annotation using the subReads R package. Differential expression analysis were performed using edgeR (Robinson et al., 2010).

Bulk samples smallRNASeq. RNA was extracted using the miRVana kit according to manufacturer's instruction. Libraries were prepared using the Ion Total RNA-Seq kit (Life technologies) according to manufacturer's instruction and sequenced on an Ion Proton. Reads were mapped to the mm10 mouse genome or to the mature miRNA from miRBase release 20 using Bowtie2 (Langmead and Salzberg, 2012) and summarized using the summarizeOverlaps R package. Differential expression analysis were performed using edgeR (Robinson et al., 2010).

Single cells mRNASeq. After harvesting as described above, the naïve or IVA DRG neurons were filtered through a 35um mesh and resuspended in 2ml of complete media before being layered on a Percoll solution (density of 1.040) as described in (Delree et al., 1989) to remove myelin debris. The lower 5ml fraction was washed in PBS and the pellet was resuspended in C1 cell wash buffer. Cells were counted and resuspended at a concentration of 310 cells per ul, stained for viability on ice for 30 minutes and then captured by the C_1 from Fluidigm using the large chip. Cells were imaged at 4X magnification. Cell lysis, reverse transcription and pre-amplification were performed on the C_1 as described by the manufacturer. After preamplification, cDNA was harvested and quality and concentration analyzed on the fragment analyzer from Advanced Analytical using the NGS high sensitivity kit according to manufacturers instructions. Wells with no cell, more than one cell or contaminating debris were discarded. cDNA was diluted and library prepared for Illumina sequencing using the Nextera kit and according to the Fluidigm protocol. Libraries were sequenced on a NextSeq 500 using a high-output 150 cycles chip. Up to 192 cells were sequenced at once and cells from all conditions were combined on each sequencing run.
Reads were mapped to the mm10 mouse genome using Bowtie2 (Langmead and Salzberg, 2012), duplicate reads removed using samtools (Li et al., 2009) and the reads summarized on a gene annotation using RsubReads (Gentleman et al., 2004; Liao et al., 2013). The resulting counts per million (cpm) matrix was denoised using singular value decomposition (SVD). The first dimension of the SVD contained mostly technical noise and therefore was discarded. The second and third dimensions were conserved and the subsequent ones discarded. Gene contribution to the transcriptional profile was computed by multiplying the singular values of the transcriptional matrix by the left singular vectors of that same transcriptionnal matrix for all the non-zero singular values. The top 2000 genes were used for subsequent

analysis. Differential expression analysis of single cell sequencing data and trajectory computation were performed using monocle 2 (Trapnell et al., 2014).

Synthetic-bulk samples. To generate synthetic-bulk samples from single cell RNA seq samples, we randomly split the cells of each strain/treatment combination into 4 groups of 50 cells each. We then summed the denoised counts for each gene across samples grouped together and used this as a synthetic-bulk sample.

Immunocytochemistry and Western blotting

Cells were fixed by addition of an equal volume of 8% paraformaldehyde pre-warmed to 37˚C and incubated for 20 minutes at 37˚C and permeabilized with 0.25% Triton-X100. Non specific staining was blocked using 10% fetal bovine serum in PBS for one hour at room temperature Cells were stained using the following antibodies: TUBB3 (sigma), ASCL1 (BD Pharmigen) at 4˚C overnight followed by secondary antibody staining for 1 hour at room temperature. .

Injured L4 and L5 DRGs were collected in RIPA lysis buffer (Fisher Scientific) containing protease and phosphatase inhibitors (Roche Diagnostics) and protein concentration was estimated. Twenty micrograms of total protein was electrophoresed on SDS-PAGE and then transferred on PVDF membrane and incubated overnight with a polyclonal antibody to Ascl1 (BD Pharmingen) followed by anti mouse IgG HRP secondary antibody (Santa Cruz). Signal was detected using enhanced ECL chemiluminescent reagents (Amersham) and exposing on Hyperfilm (GE Healthcare).

Quantitative microscopy

In order to quantify the levels of Ascl1 in DRG neurons following injury, primary DRG neurons were cultured, fixed, and stained against TUBB3, ASCL1 and DAPI. Z-stacks were obtained using an Olympus 1000 Spectral Confocal microscope (Olympus) with an oil 40x objective. The resulting images were imported and analyzed using the Imaris 8.1 image processing and analysis software (Bitplane). In order to measure ASCL1 intensity in neurons only, the images were processed as follows: nuclei were identified using DAPI signal, the TUBB3 signal was substracted from the nuclei region to remove background glial signal, the remaining Tubb3 signal (cytoplasmic and dendritic localization) was used to identify cells of interest. The mean intensity value of the ASCL1 signal was obtained only from cells of interest. Raw intensity values for ASCL1 were recorded and plotted using R (r-project.org).

miR-7048-3p inhibition in DRG neurons

Adult male mice (8-10 weeks) were terminally anesthetized and DRGs were removed aseptically and digested in 0.2% collagenase/Dispase at 37°C for 90 mins, followed by trituration with fire-polished pipettes in 1 ml DMEM. 96-well plates were prepared for DRG neuron culture by overnight incubation with PDL followed by 3 times rinsing with H_2O. Plates were then incubated 2 hours at 37°C with laminin. Dissociated DRG neurons were layered over 6 ml of a 10% BSA solution for gradient separation removal of Schwann cells, myelin and other debris. The pellet was washed with DMEM and resuspended in Neurobasal media (Invitrogen) supplemented with B27, penicillin, streptomycin, 1 mM L-glutamine, 50 ng/ml NGF, 2 ng/ml GDNF, and 10 uM Cytosine-arabinoside (AraC, Sigma). Purified DRG neurons were transfected with 100 nM miRCURY LNA microRNA inhibitor (mmu-miR-7048-3p, 5nmol, 5`-

fluorescein labeled; cat # 4106101-011, Exiqon) or inhibitor control (miRCURY LNA™ microRNA inhibitor control, 5nmol, 5`-fluorescein labeled; cat #199006-011) by electroporation in a Nucleofector II interfaced with a Lonza nucleocuvette strip. Briefly, 2.5 x 10^5 neurons were placed in each strip-well with 20ul P3 buffer (Lonza), mixed with 2 ul miR-7048-3p inhibitor or inhibitor control, and current was delivered by program CA-133. Following transfection, 80 µl of neuronal culture media (Neurobasal + B27) was immediately added to strip-well. DRG neurons were cultured in the prepared 96-well plates at different densities of DRG neurons per well (1000, 2000, 4000 cells/well). After 24 hours, cells were fixed in 4% PFA for 15 min, rinsed in PBS and blocked with a blocking buffer (Roche) at RT for 1h. The plates were then incubated overnight at 4C with neuronal specific βIII tubulin mouse monoclonal antibody (1:800, Sigma), followed by secondary Goat-anti-Rabbit AlexaFlour 568 conjugated antibody (1:500, Invitrogen) for 1h at RT. Images were captured at 10X with High-Content System (HTS) ImageXpress Micro Systems, and automatically quantified using the MetaXpress software.

BrdU incorporation

Dissociated neurons were grown for four days on PDL-laminin coated coverslips in the presence of 10uM of BrdU (BD biosciences). Cells were fixed with 4% PFA for 20 minutes at 37°C, permeabilized with permeabilization buffer (0.1% Triton® X-100 in PBS) for 20 minutes at room temperature, then acid-washed with first with 1N HCl for 10 minutes at room temperature, then with 2N HCl for 10 minutes at room temperature following which the cells were washed with phosphate/citric acid buffer pH 7.4 and washed three times with permeabilization buffer. Cells were incubated overnight at room temperature in stain solution (PBS, 0.1% Triton, 5% serum) with antibodies against BrdU (BD biosciences) and TUBB3 (Sigma). Cells were washed again with permeabilization buffer, incubated with fluorescently labeled secondary antibodies for one hour at room temperature and washed with permeabilization buffer before being mounted on coverglass using ProLong Gold antifade mountant (Lifetechnologies). Cells were imaged on an Olympus BX-51 microscope with a 10X air objective.

Supplemental References

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