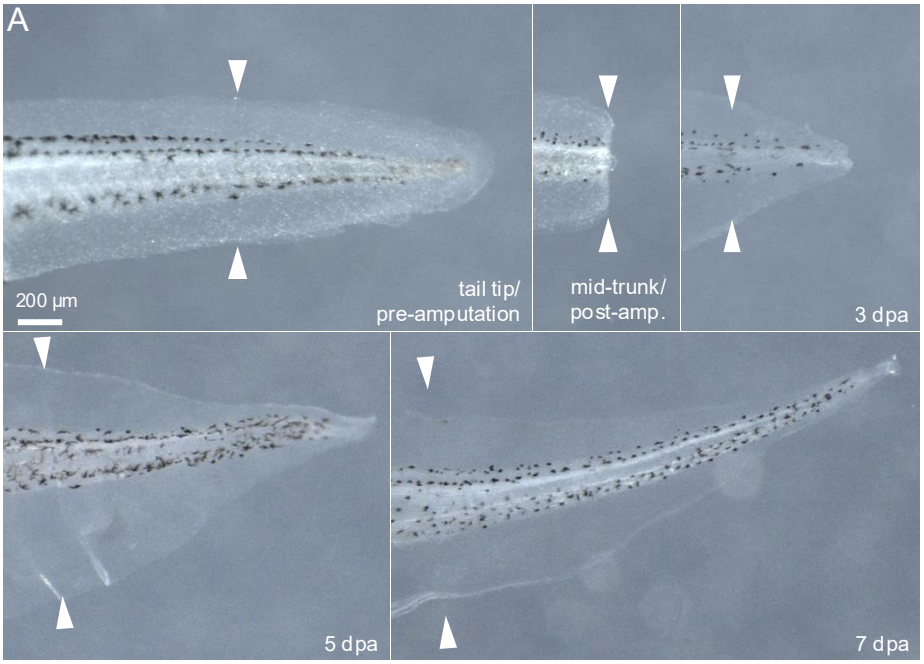
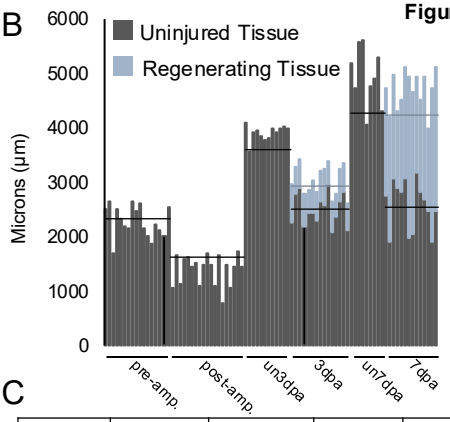
Cardinal Classification	Neurotransmitter	Zebrafish	likely *Xenopus* counterpart	Birth Date	Marker Genes	Potential Subcluster Notes
RBN	Glutamatergic	Rohon Beard Neurons - Tuttle et al., 2024,	Rohon Beard Neurons	Primary	*prdm14, isl2, isl1, tlx3*	NA
dI6	Glycinergic	Commissural Local (CoLo) *- Satou et al. 2009, Kelly et al., 2023*	commissural Interneurons (cIN)	Secondary *-Satou et al., 2009*	*lhx1, dmrt3, lbx1*	NA
V0v	Glutamatergic	Multipolar Commissural Descending (MCoD) and Unipolar Commissural Descending (UCoD) *- Satou et al., 2012, Bernhardt et al., 1990, Kelly et al., 2023*	Dorsolateral commissural (dlc) and/or excitatory commissural Interneurons (eclNs)	Secondary *-Satou et al., 2012*	***evx1***, *lhx1, nrn1,* *developmental ***dbx1***	*cbln2+* subcluster
V1	Glycinergic/ GABAergic	Circumferential Ascending (CiA) *- Higashijima et al., 2004, Kelly et al., 2023*	ascending Interneurons (aINs) *- Li et al., 2004*	Secondary	***en1,*** *lhx1, pnoc, pax2, sp9, foxd3, gbx2, otp*	NA
V2a	Glutamatergic/ Cholinergic	Circumferential Descending (CiD) *- Kimura et al., 2006, Kelly et al., 2023, Kelly et al., 2023*	descending Interneurons (dINs) *- Soffe et al., 2009*	Secondary *- Bernhardt et al., 1990*	***vsx2***, *nrn1, nkx6-2, lhx3*	*chat- sox21+, prdm8+, sox14+* subcluster
V2b	GABAergic	Ventral Longitudinal Descending (VeLD) *- Kumura et al., 2006, Kelly et al., 2023*	descending Interneurons (dINs) *- Soffe et al., 2009, Li et al., 2004*	Primary *- Bernhardt et al., 1990*	*gata2, gata3, lhx1, sox1, tal1*	*slc6a5+* subcluster
KA"	GABAergic	Dorsal Kolmer-Agduhr (KA") *- Yang et al., 2020*	Kolmer-Agduhr Interneuron *- Roberts and Clarke 1982, Kale et al., 1987, Binor and Heathcote, 2001*	Secondary	*pkd1l2, kctd8, sox1, tal1*	NA
KA'	GABAergic/ Dopaminergic	Ventral Kolmer-Agduhr (KA') *- Yang et al., 2020*	Kolmer-Agduhr Interneuron *- Roberts and Clarke 1982, Kale et al., 1987, Binor and Heathcote, 2001*	Secondary	*pkd1l2, sox1, nkx6-2, foxa2, tal1, ptchd*	*chgb, cfap210, sall2, daw1, cdx4*
?	Glycinergic	NA	NA	NA	*megf11+, lhx1*	NA
?	Glycinergic	NA	NA	NA	*foxp2+, pax2+*	NA
		Reviewed in Lewis and Eisen 2003, Sengupta and Bagnall, 2023, Wilson and Sweeney, 2023, and Cucun et al., 2024. Reviewed in mouse in Delile et al., 2019	*Reviewed in Roberts, 2011*	*Saint-Amant and Drapeau 2001*		

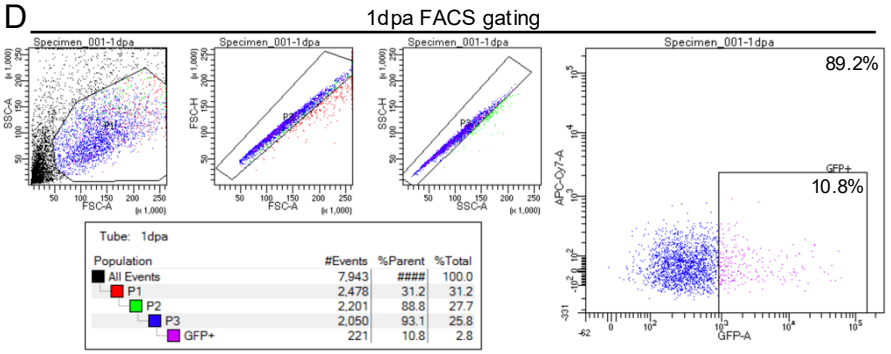
Table S1. Conserved markers from zebrafish can be used to identify *Xenopus* neuron counterparts based on literature analysis. Cardinal neurons have been identified in zebrafish literature by their neurotransmitter use, morphological classification, and birth date. These identities can be tied to their likely *Xenopus* counterpart in our scRNA-seq dataset based on cluster Marker Genes. Bold genes indicate marker genes that have been previously identified in *Xenopus.* For some neurons, additional subclusters could be identified for future studies. Two clusters of glycinergic neurons remain unidentified, and V3 and dI1-5 INs could not be found in our dataset.





C

Condition	Threshold Count	Processed Events Count	GFP+	%GFP+
tail tip	1305845	1102941	67489	5.17%
mid-trunk	1255768	1120835	68640	6.12%
1dpa	679414	668059	17254	2.80%
un1dpa	734238	418012	42498	10.17%
3dpa	495511	491849	30445	6.19%
un3dpa	712636	709516	14681	2.07%
7dpa	218989	208332	3427	1.64%
un7dpa	353498	204674	3310	1.62%



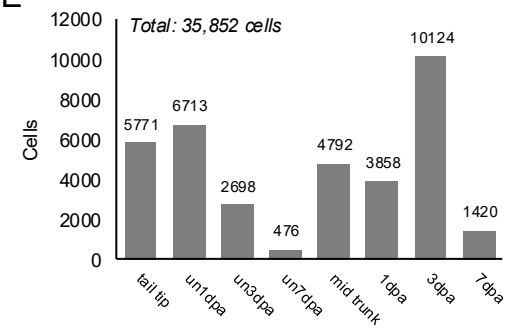


Figure S1. Experimental 7 day time course of regenerating stage 41 tadpoles. A) Brightfield images of tadpoles at tail tip/pre-amputation (pre-amp.) stage 41, mid-trunk/post-amputation (post-amp.), 3 dpa, 5 dpa, and 7 dpa. B) Bar chart of vent-to-tip tail length per tadpole in microns of uninjured (un-) or regenerating tails. Black denotes uninjured tissue, blue-grey denotes regenerated tissue. Black line denotes average uninjured length, blue-grey line denotes average regenerated length. Pre-amp n = 16, post-amp n = 19, un3dpa n = 12, 3dpa n = 14, un7dpa n = 9, 7dpa n = 14 tadpoles. C) Fluorescent Activated Cell Sorting (FACS) metrics for each Sample Condition. D) Example of 1dpa FACS gating strategy, used for all Sample Conditions. E) Bar chart of cells collected by scRNA-seq, post quality control analysis; numbers above bars show cell counts per timepoint, total dataset is 35,852 cells. Source Data are provided as a Source Data file.

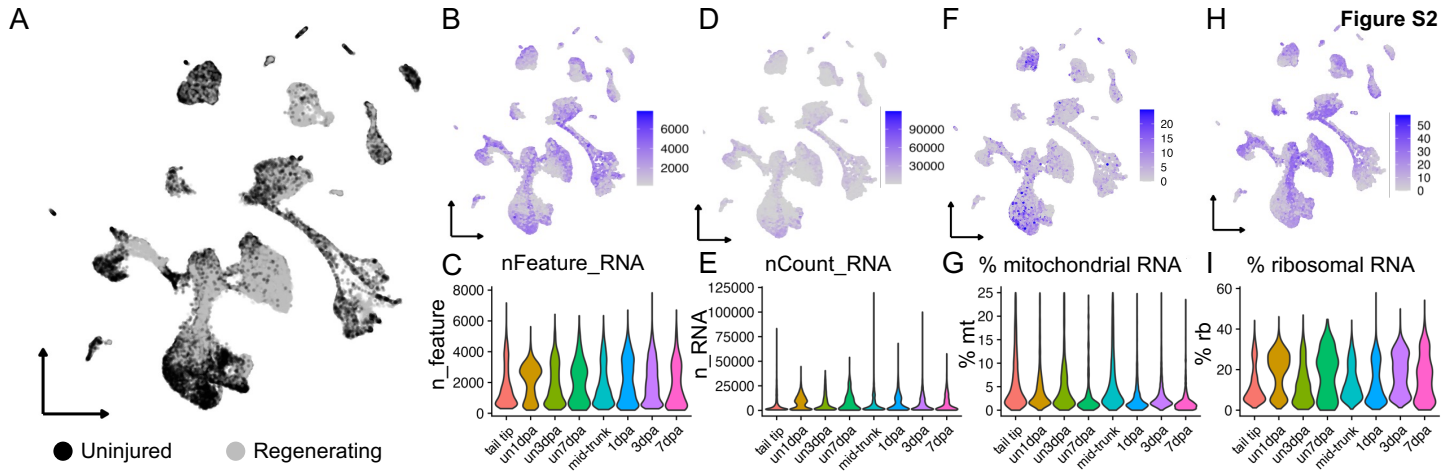


Figure S2. Quality metrics for single-cell RNA sequencing processing. A) UMAP of all cells colored by injury condition. Uninjured cells (tail tip, mid-trunk, un1dpa, un3dpa, and un7dpa) are in black, Regenerating cells (1dpa, 3dpa, and 7dpa) are in grey. B) UMAP of nFeature_RNA, where darker purple indicates a higher number of RNA features (or genes) detected per cell. C) Violin plot of nFeature_RNA by Condition. D) UMAP of nCount_RNA, where darker purple represents a higher number of RNA reads detected per cell. E) Violin plot of nCount_RNA by condition. F) UMAP of % mitochondrial RNA, where darker purple represents a higher percentage of mitochondrial RNA per cell. G) Violin plot of % mitochondrial RNA by Condition. H) UMAP of % ribosomal RNA, where darker purple represents a higher percentage of ribosomal RNA per cell. I) Violin plot of % ribosomal RNA by Condition.

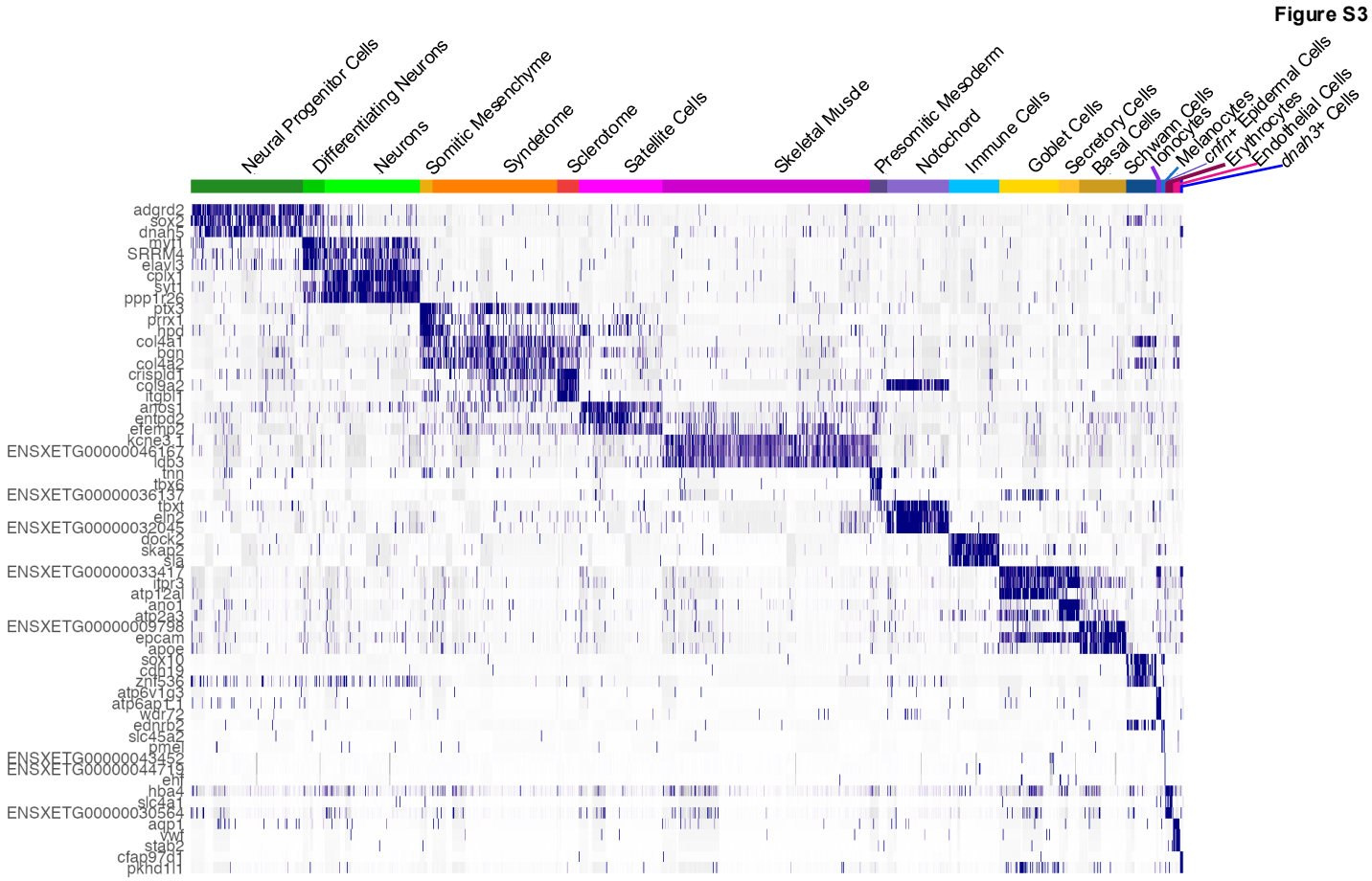
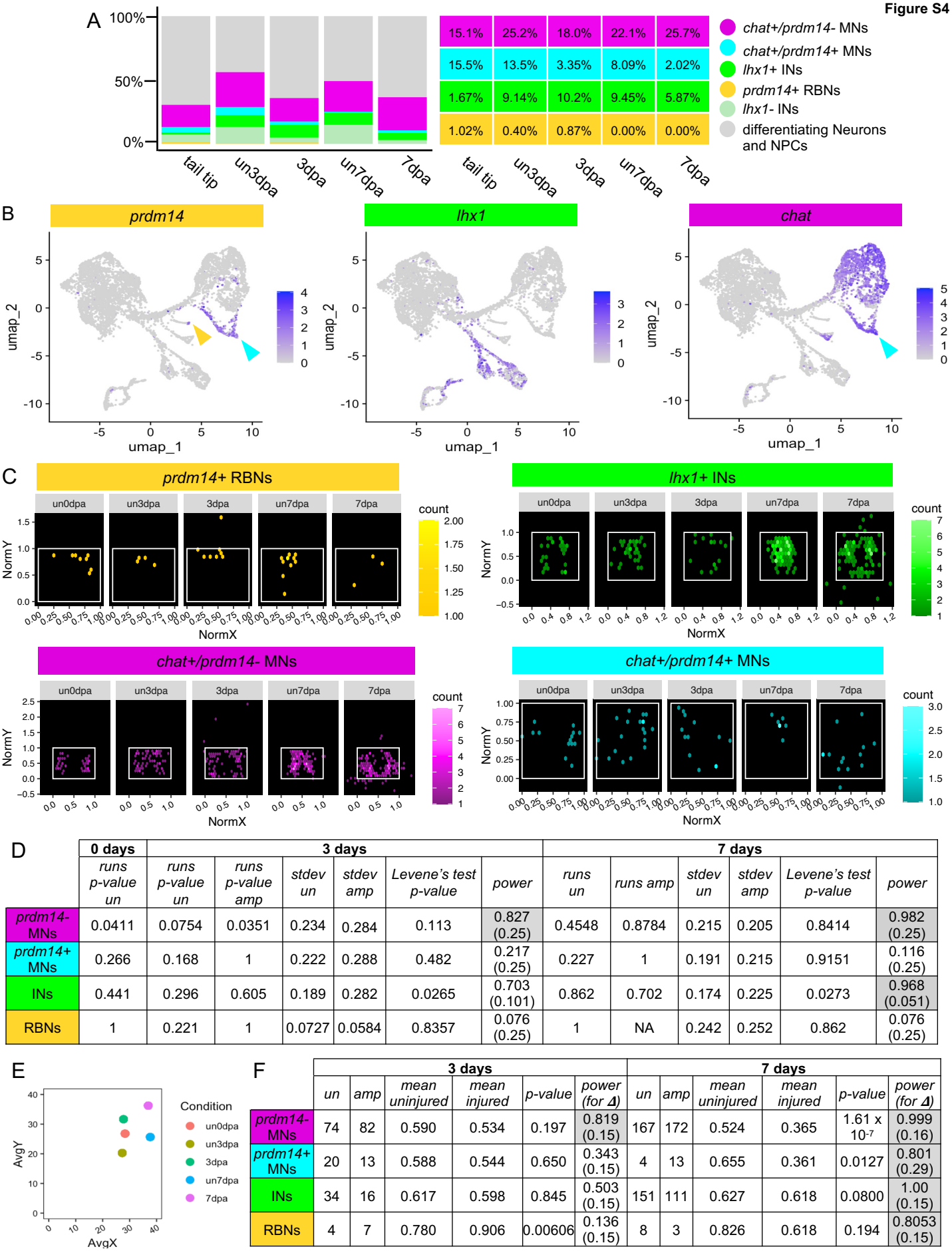


Figure S3. Gene expression patterns identify tail cell types. Heatmap of top three differentially expressed genes for each identified cell type. Purple indicates high expression, white indicates low expression.

Figure S4



D

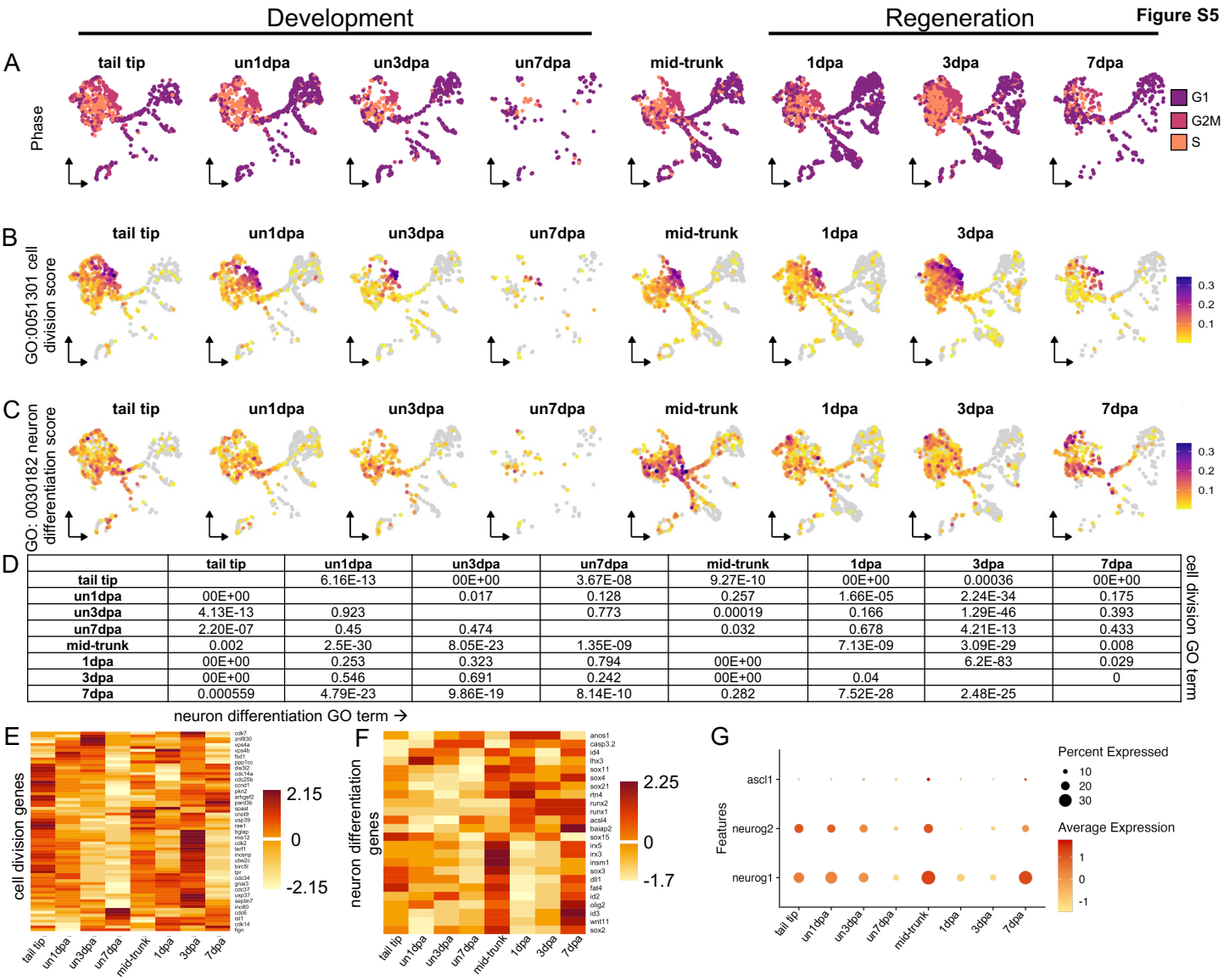
	0 days	3 days						7 days					
	runs p-value un	*runs p-value un*	*runs p-value amp*	*stdev un*	*stdev amp*	*Levene's test p-value*	*power*	*runs un*	*runs amp*	*stdev un*	*stdev amp*	*Levene's test p-value*	*power*
prdm14- MNs	0.0411	0.0754	0.0351	0.234	0.284	0.113	0.827 (0.25)	0.4548	0.8784	0.215	0.205	0.8414	0.982 (0.25)
prdm14+ MNs	0.266	0.168	1	0.222	0.288	0.482	0.217 (0.25)	0.227	1	0.191	0.215	0.9151	0.116 (0.25)
INs	0.441	0.296	0.605	0.189	0.282	0.0265	0.703 (0.101)	0.862	0.702	0.174	0.225	0.0273	0.968 (0.051)
RBNs	1	0.221	1	0.0727	0.0584	0.8357	0.076 (0.25)	1	NA	0.242	0.252	0.862	0.076 (0.25)

E

F

	3 days						7 days					
	un	*amp*	*mean uninjured*	*mean injured*	*p-value*	*power (for Δ)*	*un*	*amp*	*mean uninjured*	*mean injured*	*p-value*	*power (for Δ)*
prdm14- MNs	74	82	0.590	0.534	0.197	0.819 (0.15)	167	172	0.524	0.365	1.61×10^{-7}	0.999 (0.16)
prdm14+ MNs	20	13	0.588	0.544	0.650	0.343 (0.15)	4	13	0.655	0.361	0.0127	0.801 (0.29)
INs	34	16	0.617	0.598	0.845	0.503 (0.15)	151	111	0.627	0.618	0.0800	1.00 (0.15)
RBNs	4	7	0.780	0.906	0.00606	0.136 (0.15)	8	3	0.826	0.618	0.194	0.8053 (0.15)

Figure S4. HCR of neuron types within regenerating tissue. A) Stacked box plots of neuron types over specific timepoints, with the percentage of neurons within total neural population. B) Expression patterns of *prdm14*, *lhx1*, and *chat* in the neural population. Yellow arrow indicates *prdm14*+ RBNs, cyan arrow indicates *prdm14+/chat*+ MNs. C) Hexplots of neurons in the spinal cord over time. Each cell is normalized by spinal cord width and length. White rectangle indicates normalized spinal cord boundary. D) Standard deviation (stdev) of uninjured (un) or amputated (amp) cells by type in Figure 3E. p-value per Levene's test for equality of variances, with Monte Carlo power analysis with the assumption of either 25% change in variance or the found difference in variance, if the p-value is significant. Cells in grey represent values with adequate (>80%) power. E) Scatterplot of average spinal cord width and height by Condition. F) Power analysis of Wilcox test of means of Dorsal/Ventral position of the HCR-identified cells shown in Figure 3E. Cells in grey represent values with adequate (>80%) power. St. 41/uninj n = 98 cells, 4 tadpoles; un3dpa n = 131 cells, 4 tadpoles; 3dpa n = 123 cells, 6 tadpoles; un7dpa n = 330 cells, 3 tadpoles; 7dpa n = 330 cells, 3 tadpoles. Source Data are provided as a Source Data file.



	tail tip	un1dpa	un3dpa	un7dpa	mid-trunk	1dpa	3dpa	7dpa
tail tip		6.16E-13	00E+00	3.67E-08	9.27E-10	00E+00	0.00036	00E+00
un1dpa	00E+00		0.017	0.128	0.257	1.66E-05	2.24E-34	0.175
un3dpa	4.13E-13	0.923		0.773	0.00019	0.166	1.29E-46	0.393
un7dpa	2.20E-07	0.45	0.474		0.032	0.678	4.21E-13	0.433
mid-trunk	0.002	2.5E-30	8.05E-23	1.35E-09		7.13E-09	3.09E-29	0.008
1dpa	00E+00	0.253	0.323	0.794	00E+00		6.2E-83	0.029
3dpa	00E+00	0.546	0.691	0.242	00E+00	0.04		0
7dpa	0.000559	4.79E-23	9.86E-19	8.14E-10	0.282	7.52E-28	2.48E-25	

Figure S5. Faceted UMAPs of cell processes throughout development and regeneration. A) Cell cycle phase (G1, G2M, or S) over scRNA-seq timepoints. B) GO:0051301 cell division module score over scRNA-seq timepoints. C) Neuron Differentiation Module Score over scRNA-seq timepoints. D) Comparative p-values for neuron differentiation GO term (bottom left) and cell division GO term (top right) over developmental and regenerative time via two-sided paired Wilcox Test. E) Heatmap of differentially expressed genes associated with GO:0051301 cell division score. F) Heatmap of differentially expressed genes associated with GO:0030182 neuron differentiation score. G) Plot of neuron differentiation genes *ascl1, neurog2*, *neurog1* over regeneration. Circle radius represents percent of cells expressing gene, color indicates average expression.

Figure S6

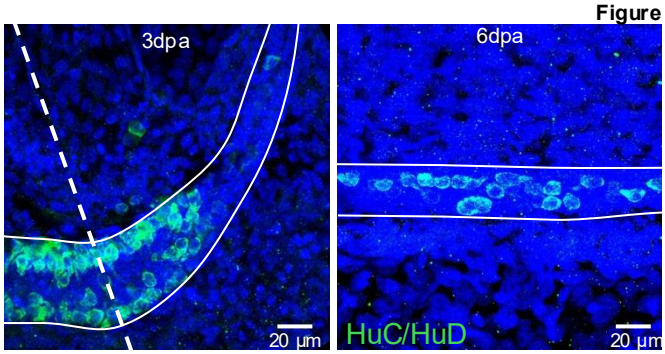


Figure S6. HuC/HuD+ neurons are found in the regenerating spinal cord. Immunohistochemistry of the quantified HuC/HuD neurons (green) in the spinal cord shown in Figure 5E, compared to DAPI (blue). Dashed solid line is amputation plane, solid white line is spinal cord border. 6dpa sample shows tissue posterior to the amputation plane. 3dpa n = 10, 6dpa n = 10.

Figure S7

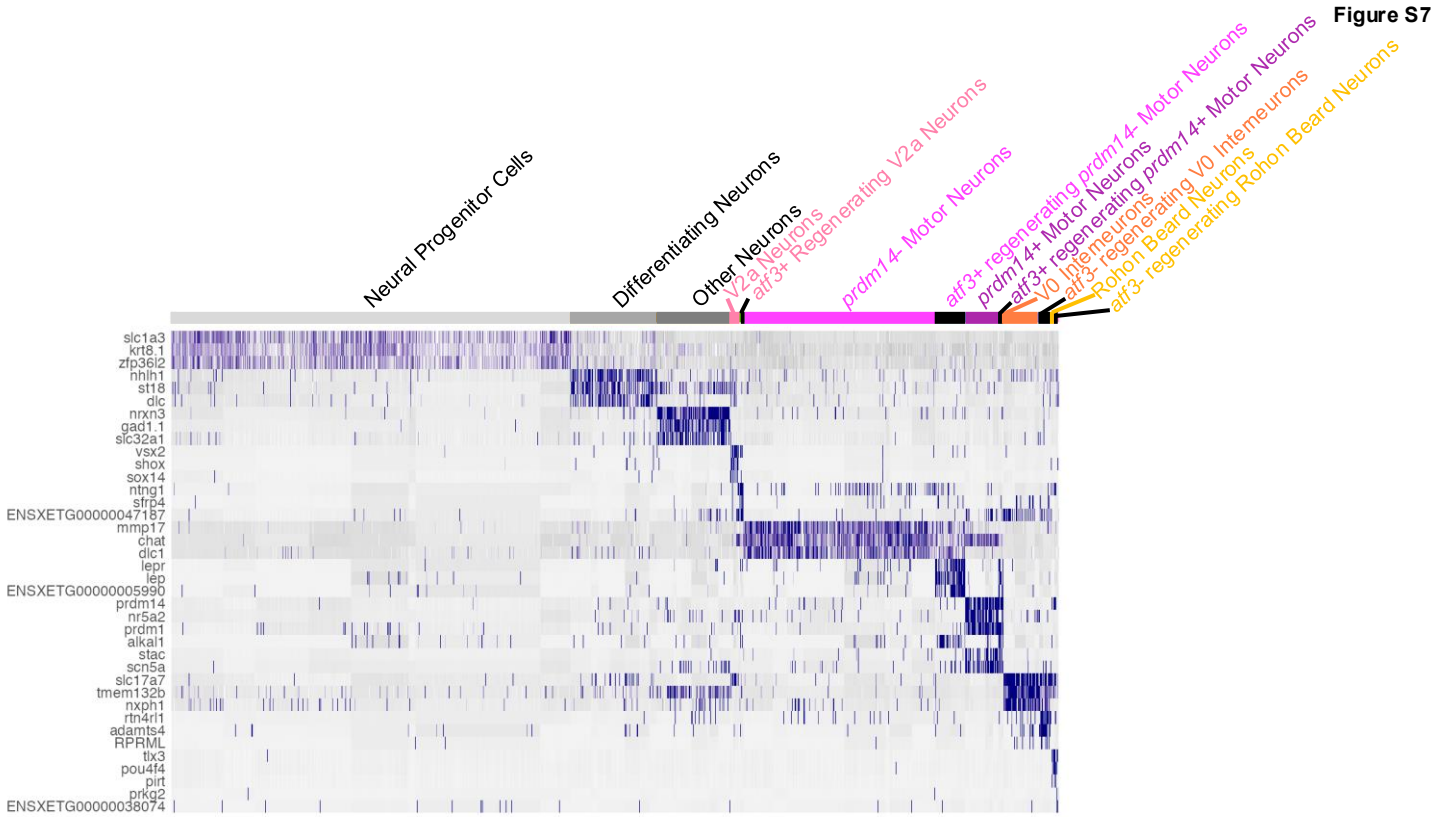


Figure S7. Gene expression patterns differentiate regenerating cell types. Heatmap of top three differentially expressed genes for each regenerating cell type. Other Neurons include V1, V2b, and KA cell types. Purple indicates high expression, white indicates low expression.

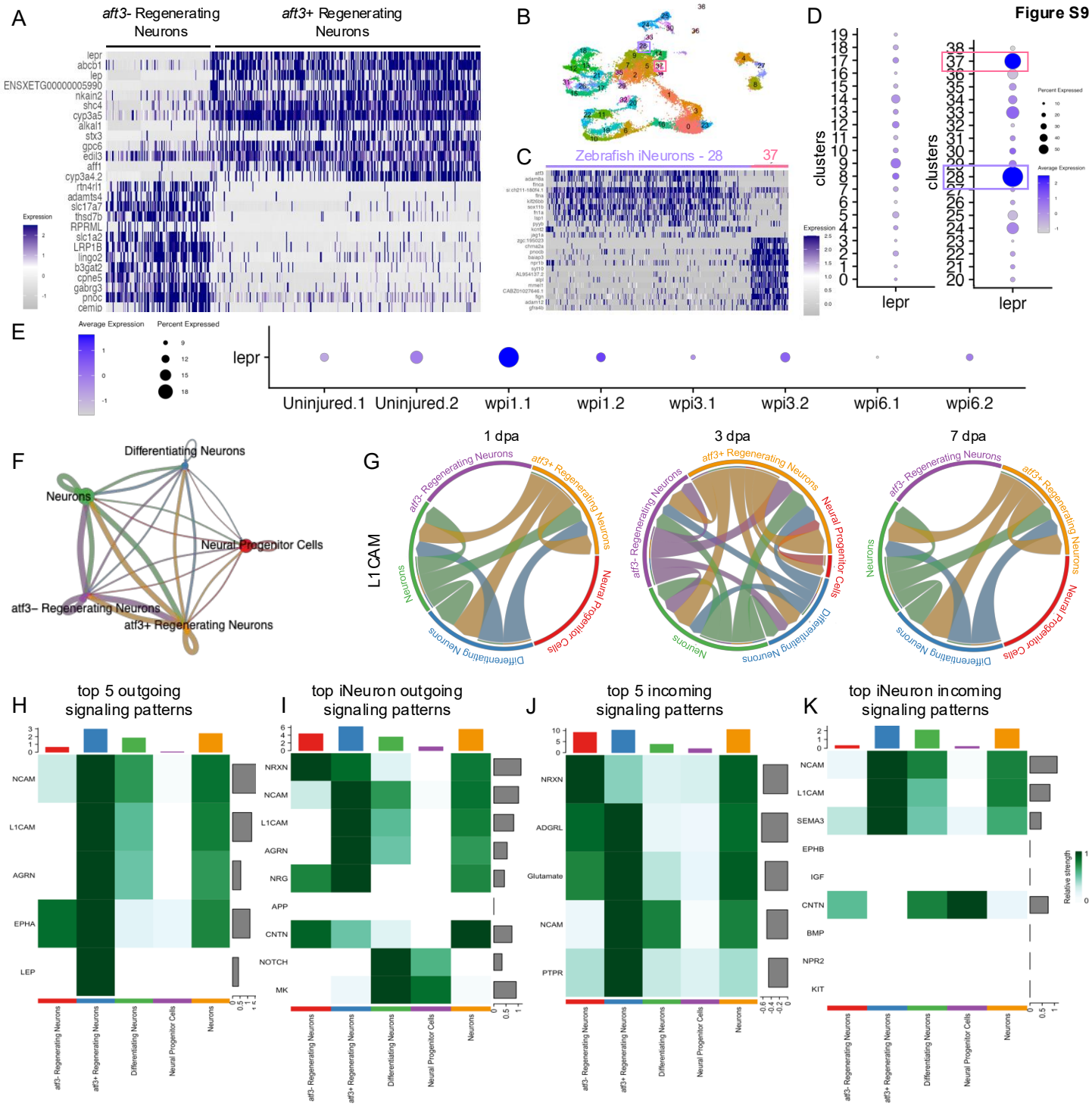


Figure S8. *atf3*+ Regenerating Neurons do not represent a major signaling hub but do show similar top signaling pathways to zebrafish iNeurons. A) Top 12 gene markers for *atf3*- and *atf3*+ Regenerating Neurons. B) UMAP of reprojected zebrafish cells from Saraswathy et al., 2024, with clusters 28 and 37 outlined. C) Top gene markers for zebrafish clusters 28 and 37, showing cluster 28 as *atf3*+ zebrafish iNeurons. D) Expression of *lepr* in zebrafish clusters. E) *lepr* expression in clusters 28 and 37 over regenerating time. F) Comparative signaling strength of regenerating neurons. G) L1CAM signaling during regeneration. H) Top 5 outgoing signaling patterns at 1 dpa I) Visualization of the top outgoing signaling patterns identified zebrafish iNeurons during regeneration in *Xenopus* regenerating neurons. J) Top 5 incoming signaling patterns at 1dpa K) Visualization of the top incoming signaling patterns identified zebrafish iNeurons during regeneration in *Xenopus* regenerating neurons.

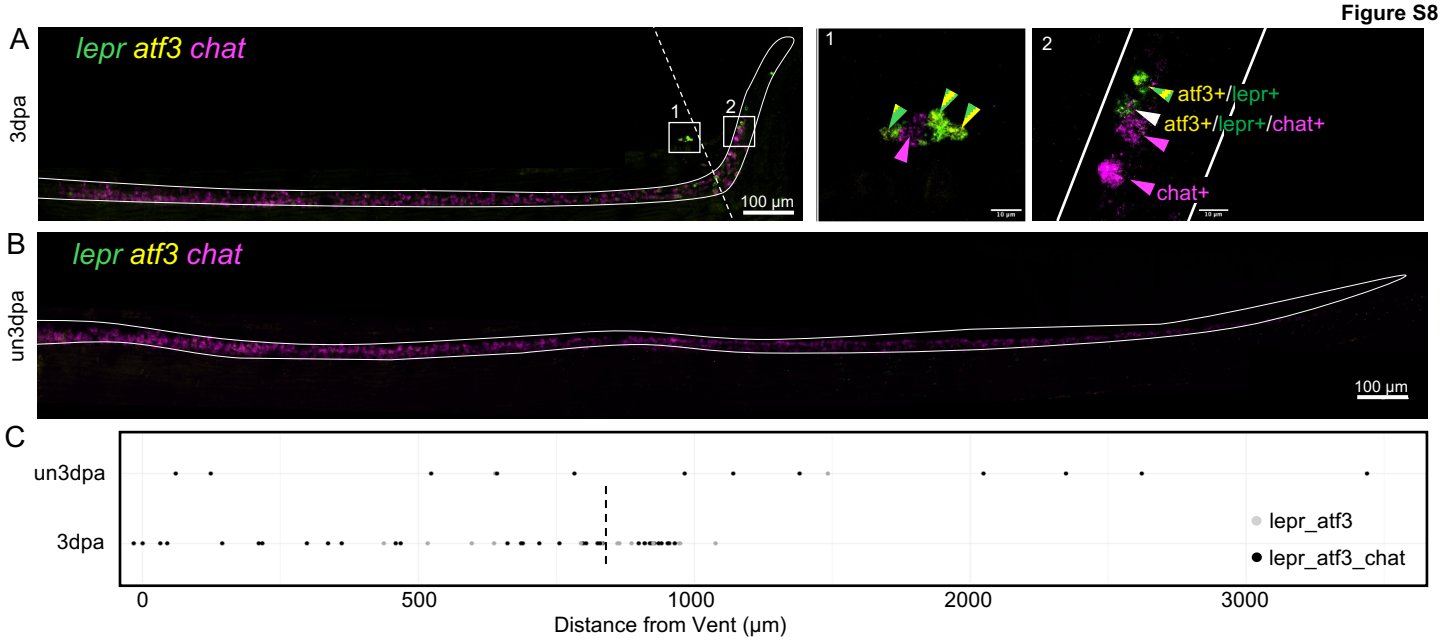


Figure S9. *atf3+/lepr+* cells are located both anterior and posterior to the amputation plane at 3dpa. A-B) HCR for *lepr*, *atf3*, and Motor Neuron marker *chat* of the uninjured (A) and injured 3dpa (B). Inset 1 shows sporadic non-spinal cord localized cells, as previously described in Figure 3. Inset 2 shows *atf3+/lepr+/chat+* (white arrowhead), *atf3+/lepr+* (yellow and green arrowhead), and *chat+* (magenta arrowhead) cells in the regenerated spinal cord. White dashed line represents amputation plane. White solid line represents spinal cord border. Inset scale bar = 10 µm, total scale bar = 100 µm. C) Quantification of cell position from (A-B). Each dot represents a cell along the x axis, calculated as distance from tadpole vent. Black dashed line represents amputation plane. n = 2 animals. Source Data are provided as a Source Data file.

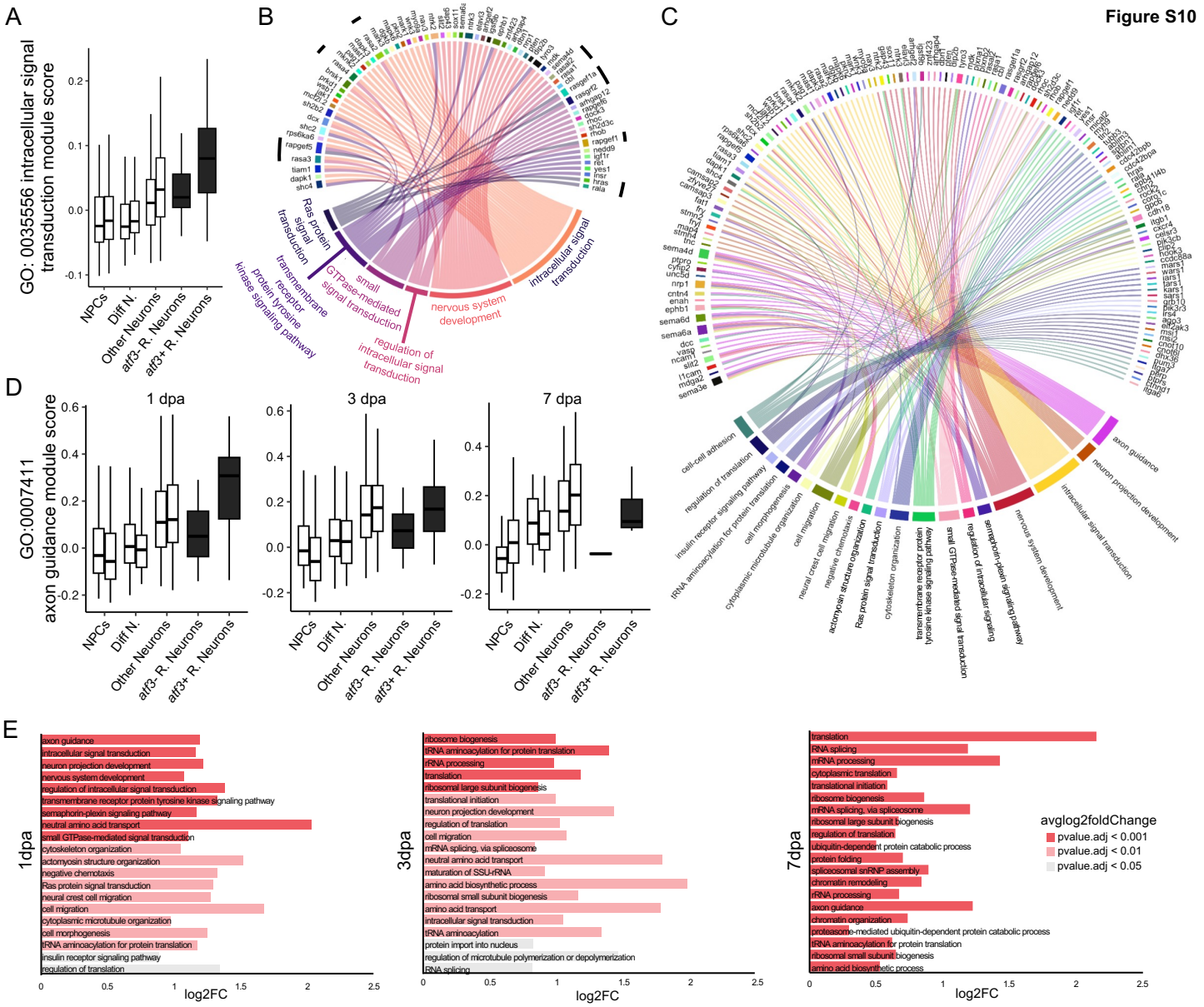


Figure S10. Top 20 GO terms and associated genes in *atf3*+ Regenerating Neurons at 1 dpa compared to 3 and 7 dpa. A) Plot of GO:0035556 intracellular transduction score at 1 dpa in different cell clusters in uninjured (left bar) or regenerating (right bar) timepoints. Black denotes regeneration-specific subclusters. B) Plot of GO:0035556 intracellular transduction score at 1dpaand associated modules at 1 dpa (bottom), compared to upregulated genes representing scores (top). Black bars indicate Ras associated proteins, which represent one of the top enriched gene families. C) Plot of all top 20 GO terms and associated genes at 1dpa. D) Plot of GO:0035556 intracellular transduction score in different cell clusters in uninjured (left bar) or regenerating (right bar) timepoints at 1 dpa (left), 3 dpa (middle), and 7 dpa (right). Black denotes regeneration-specific subclusters. E) Top 20 GO terms upregulated in *atf3*+ regenerating neurons compared to other Neurons at 1 dpa (left), 3 dpa (middle), and 7 dpa (right). For all plots, data is shown as median +/- Q1/Q3 with minimum and maximum values.

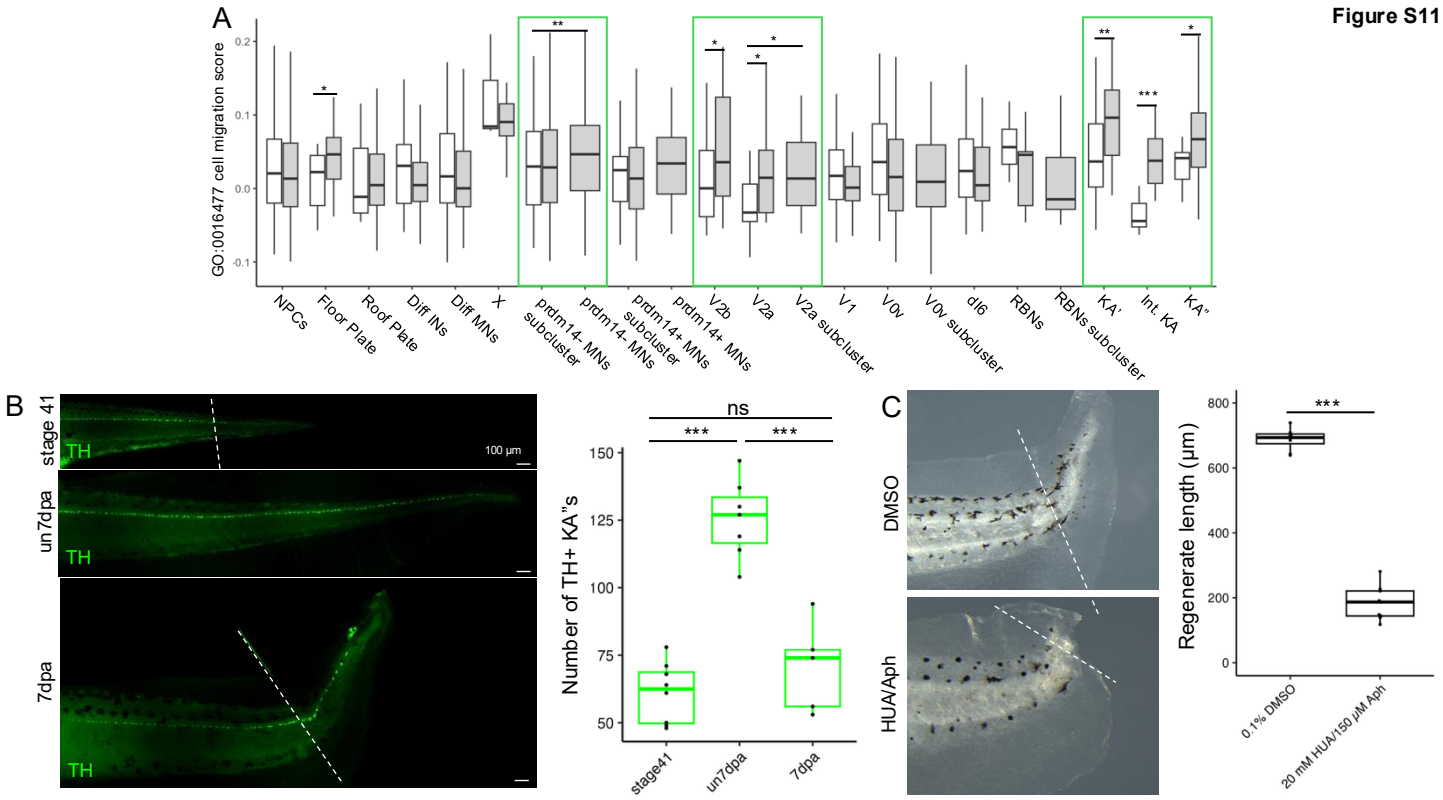
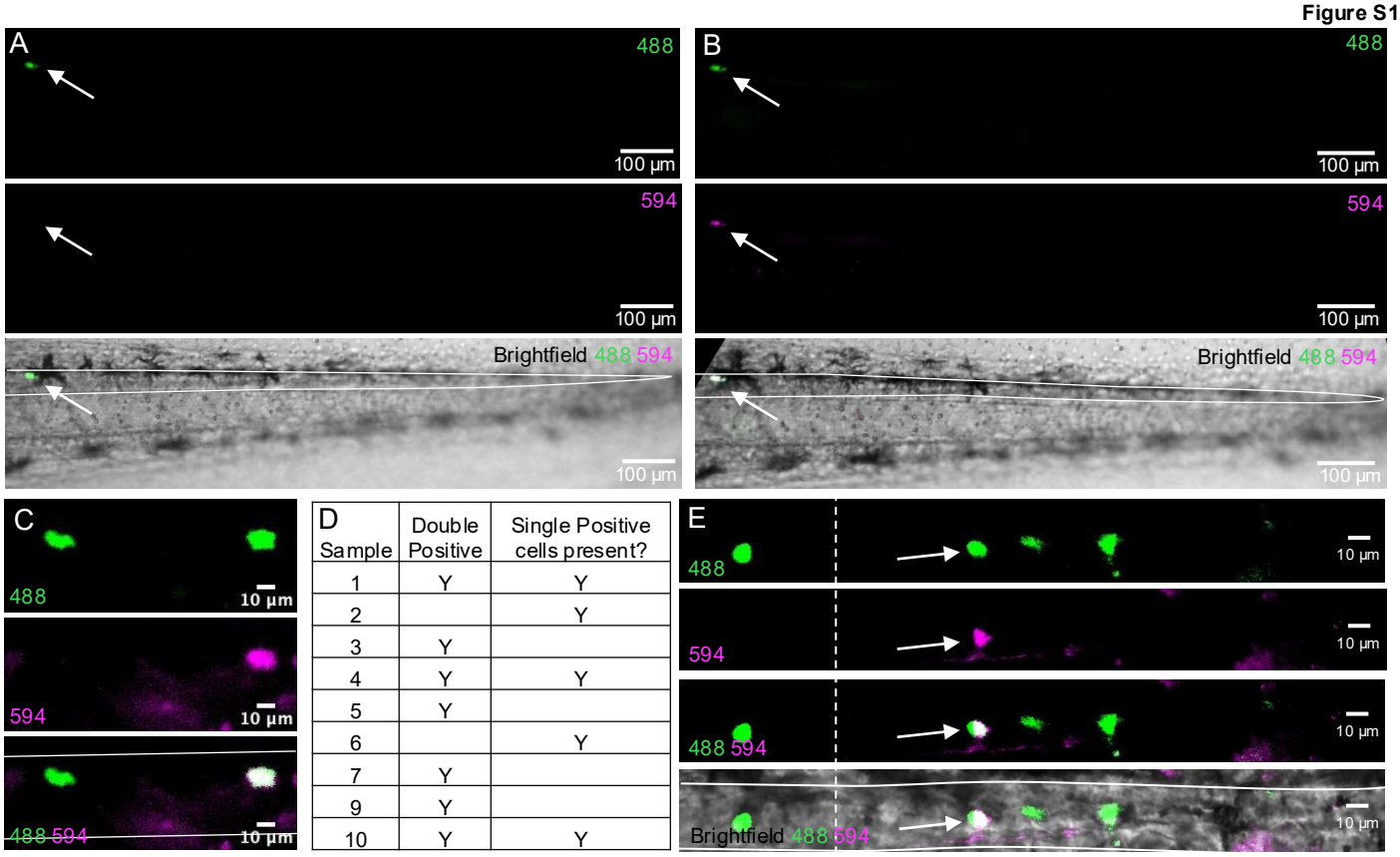


Figure S11. Cell migration may underlie mature neuron displacement. A) GO:0016477 cell migration scores are enriched in regenerating stages in regeneration-specific *prdm14*- MNs, V2bs, V2as, and KA INs (green rectangles). *p-value < 0.05; **p-value < 0.01; ***p-value < 0.001 by Wilcoxon test. B) Images and quantification of TH+ KA" cells at uninjured stage 41, un7dpa, and 7dpa. ***p-value < 0.001, ns = not significant via two-sided t-test (stage41-un7dpa = 0.000000985; stage41-7dpa = 0.235; 7dpa-un7dpa p-value = 0.000123). stage 41 = 8, un7dpa n = 7, 7dpa n = 5 tadpoles. C) Brightfield images and quantification of regenerating tails treated with DMSO or HUA/Aph from 0-3 dpa. ***p-value = 3.41×10^{-13} by two-sided t-test. DMSO n = 8, HUA/Aph n = 9 tadpoles. Source Data are provided as a Source Data file. For all plots, data is shown as median +/- Q1/Q3 with minimum and maximum values.

Figure S12



D Sample	Double Positive	Single Positive cells present?
1	Y	Y
2		Y
3	Y	
4	Y	Y
5	Y	
6		Y
7	Y	
9	Y	
10	Y	Y

Figure S12. Cell migration may underlie mature neuron displacement. A) Dendra2+ tadpole neuron before photoconversion. B) Dendra2+ tadpole neuron after 5 minute photoconversion from 594 to 488 at 405 nm. C) Single positive Dendra2 neuron compared to double positive (photoconverted) neuron at 3dpa. D) Quantification of Dendra2 cells found at 3dpa. n = 10. E) Double positive (photoconverted) neuron cell body next to two single positive (not photoconverted) neuron cell bodies posterior to the amputation plane (dashed white line). In all panels, spinal cord border is represented by solid white line.