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Supplemental Information

The Neuromodulator Adenosine Regulates Oligodendrocyte Migration at Motor Exit Point Transition Zones

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Figure S1

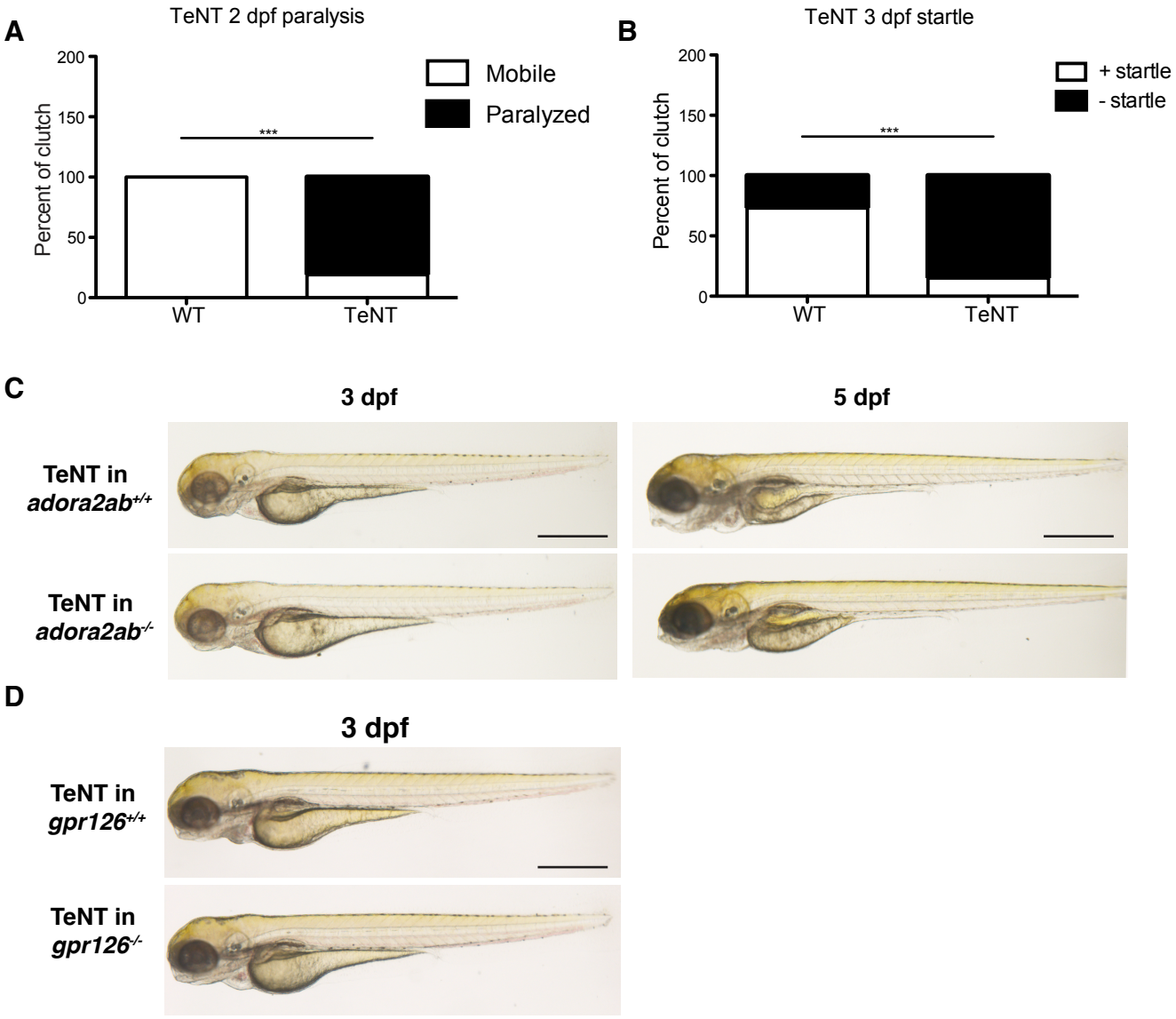

Figure S1 Related to Figures 1&4. Blocking of vesicular release results in larval paralysis.
(A) Percentage of embryos that were mobile or paralyzed during dechorionation with forceps at 2 dpf. Analyzed by Chi-squared test; ***p<0.0001, n = 54 (WT), n = 57 (TeNT). (B) Percentage of fish with positive and negative startle responses at 3 dpf. Analyzed by Fisher's exact test; ***p<0.0001, n = 52 (WT), n = 53 (TeNT). (C) Brightfield images of 3 and 5 dpf WT and *adora2ab*$^{-/-}$ siblings injected with TeNT mRNA at the one-cell stage. Note that in the 5 dpf larvae, neither WT nor *adora2ab* mutants have inflated swim bladders because of their inability to swim and get to the surface of the petri dish to gulp air in order to inflate their swim bladder. (D) Brightfield images of 3 dpf WT and *gpr126*$^{-/-}$ siblings injected with TeNT mRNA at the one-cell stage. Scale bars (C&D), 0.5 mm.

Figure S2

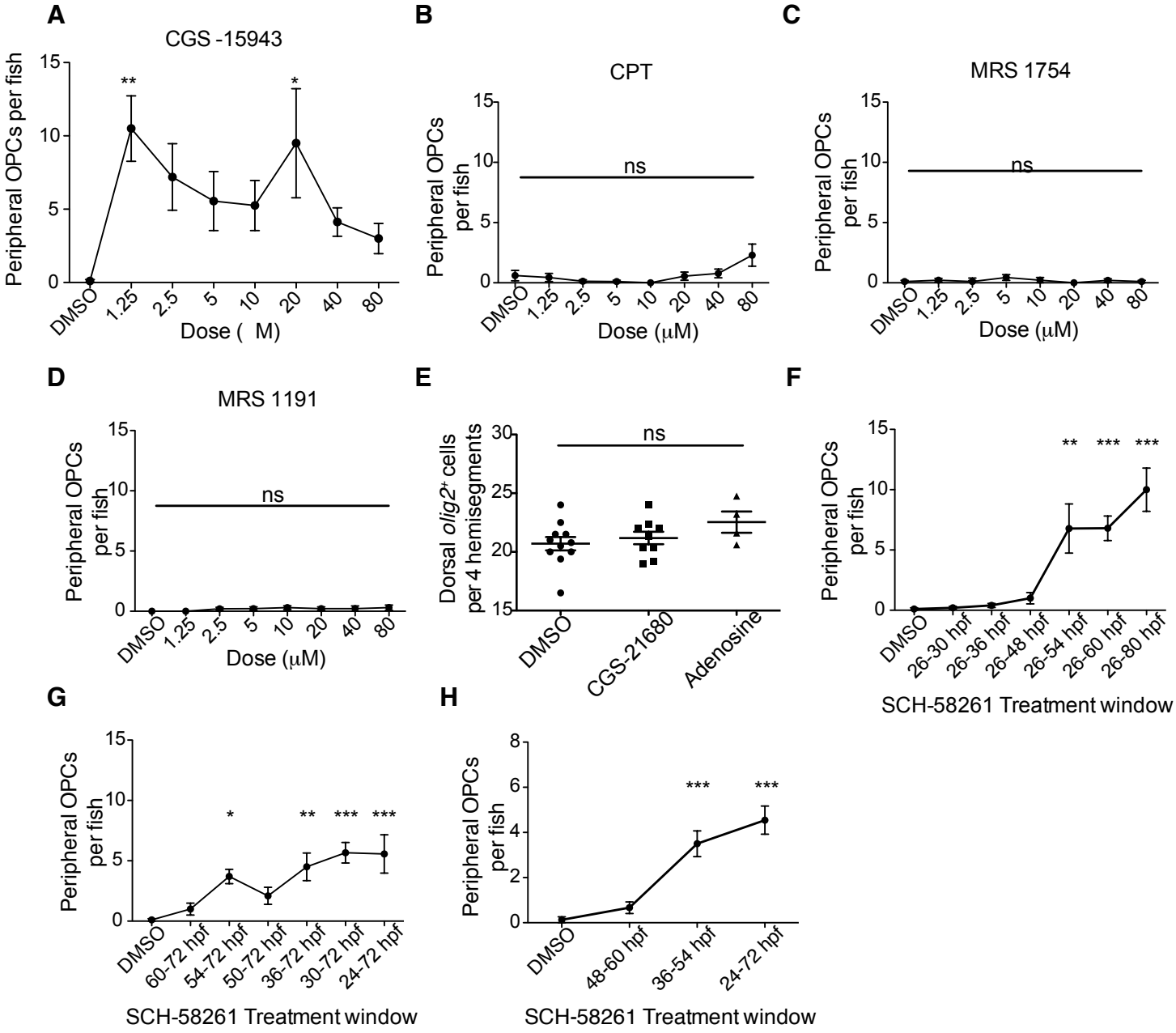

Figure S2 Related to Figure 2. A2a AR specifically mediates OPC migration at the MEP TZ.
(A-D) Dose response curves for the general AR antagonist CGS-15943 and antagonists selective for A1 (CPT), A2b (MRS 1754), and A3 (MRS 1191). (E) Mean ± SEM dorsal *olig2*$^+$ cells at 3 dpf in *olig2:dsred* larvae treated with DMSO (n = 11), CGS-21680 (n = 9), or adenosine (n = 4), p=0.23. (F-H) Numbers of peripheral OPCs in larvae treated with 10 μM SCH-58261 during various developmental stages. All data presented are mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 compared to DMSO, n = 9-12 fish per condition.

Figure S3

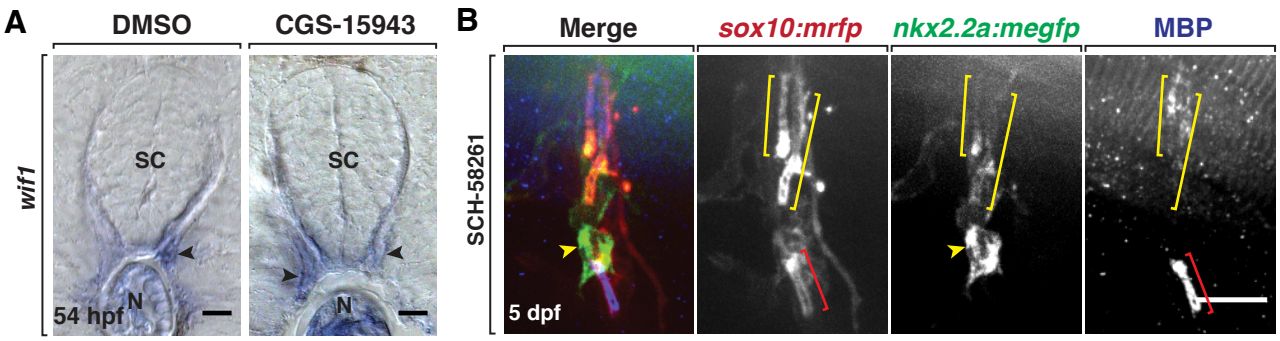

Figure S3 Related to Figure 3. A2a antagonism does not affect spinal motor nerve development.
(A) In situ hybridization for *wif1* in 54 hpf larvae treated 36 to 54 hpf with DMSO or 1.25 µM CGS-15943. Arrowheads denote MEP glia. SC, spinal cord; N, notochord. (B) Image of a 5 dpf larva treated from 30 hpf to 3 dpf with SCH-58261. Arrowhead marks a *nkx*$^{+}$*/sox10*$^{+}$ oligodendrocyte on the nerve, which is myelinating proximal segments of the nerve with *nkx*$^{+}$*/sox10*$^{+}$/MBP^{+} myelin (yellow brackets). A differentiated Schwann cell also myelinates a nerve segment with a *nkx*$^{-}$*/sox10*$^{+}$/MBP^{+} myelin sheath (red bracket). Scale bars, (A) 20 µm, (B) 50 µm.

Figure S4

Figure S4 Related to Figure 4. A2a AR protein sequence homology.
Clustal Omega sequence alignment for human A2a (top), zebrafish A2ab (middle), and zebrafish A2aa (bottom). Conserved sequence is in blue text, transmembrane domains are denoted in yellow highlight and ligand binding domains are bolded, underlined text.

Figure S5

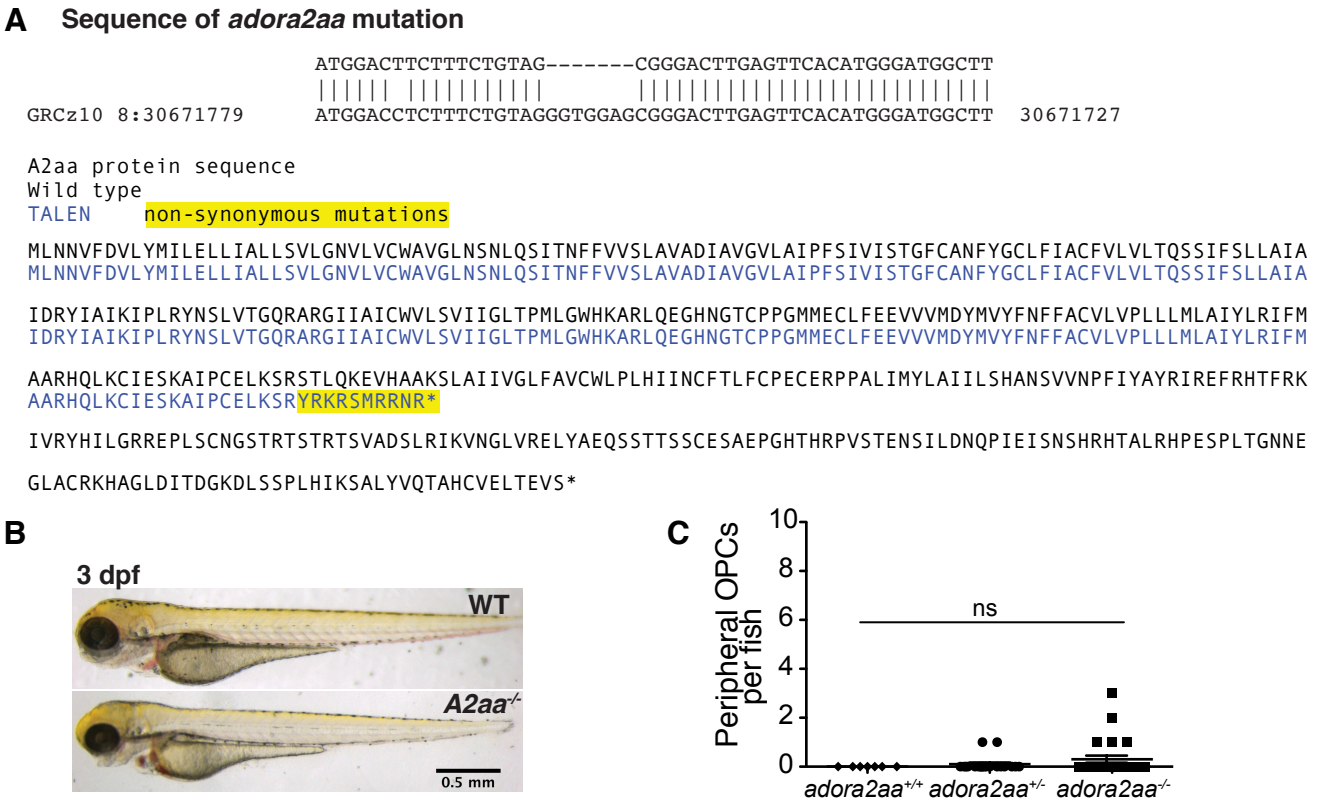

Figure S5 Related to Figure 4. Characterization of adora2aa mutant larvae.
(A) DNA sequence (top) and predicted protein sequence of adora2aa^{ct845} compared to WT.
(B) Brightfield images of 3 dpf WT and *adora2aa*$^{-/-}$ larvae. (C) Mean ± SEM of peripheral OPCs per larvae for *adora2aa*$^{+/-}$ and *adora2aa*$^{-/-}$ larvae at 3 dpf. p=0.24, n = 20 (*adora2aa*$^{+/-}$), n = 26 (*adora2aa*$^{-/-}$). Scale bar, 0.5 mm.

Figure S6

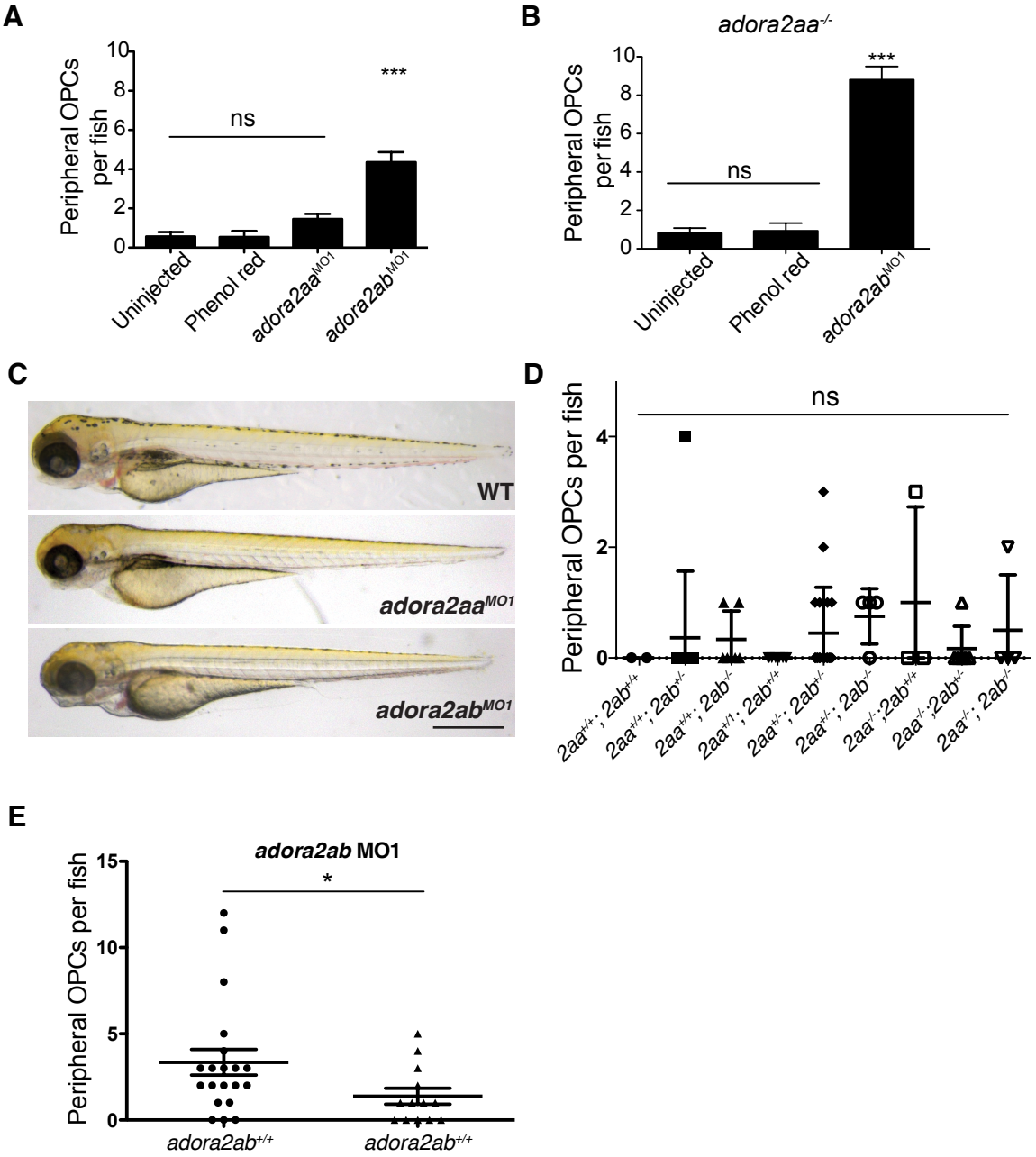

Figure S6 Related to Figure 4. Knockdown of adora2ab, but not adora2aa, results in peripheral OPCs. (A) Mean ± SEM of peripheral OPCs in WT *olig2:dsred* embryos injected with vehicle, 1 ng/nl *adora2aa* MO1 or 1 ng/nl *adora2ab* MO1. n = 42 (WT), n = 11 (phenol red), n = 65 (*adora2aa* MO1) and n = 42 (*adora2ab* MO1). ***p<0.0001 *adora2ab* MO1 compared to uninjected. (B) Mean ± SEM of peripheral OPCs at 3 dpf in *olig2:dsred;adora2aa*$^{-/-}$ larvae injected with *adora2ab* MO1. n = 44 (uninjected), n = 30 (phenol red) and n = 84 (*adora2ab* MO1). ***p<0.0001 compared to uninjected. (C) Brightfield images of 3 dpf WT, *adora2aa* and *adora2ab* morphant larvae. (D) Mean ± SEM of peripheral OPCs at 3 dpf in *olig2:dsred;adora2aa*$^{-/-}$;*adora2ab*$^{-/-}$ larvae. p=0.75, n = 2 (*adora2aa*$^{+/+}$;*adora2ab*$^{+/+}$), n = 11 (*adora2aa*$^{+/+}$; *adora2ab*$^{+/-}$), n = 6 (*adora2aa*$^{+/+}$; *adora2ab*$^{-/-}$), n = 8 (*adora2aa*$^{+/-}$; *adora2ab*$^{+/+}$), n = 20 (*adora2aa*$^{+/-}$; *adora2ab*$^{+/-}$), n = 4 (*adora2aa*$^{+/-}$; *adora2ab*$^{-/-}$), n = 3 (*adora2aa*$^{-/-}$; *adora2ab*+/+), n = 6 (adora2aa-/-; adora2ab+/-) and n = 4 (adora2aa-/-; adora2ab$^{-/-}$). Scale bar, 0.5 mm. (E) Mean ± SEM of peripheral OPCs at 3 dpf in *olig2:dsred;adora2ab*$^{-/-}$ and *adora2ab*$^{+/+}$ larvae injected with 1ng/nl *adora2ab* MO at the one-cell stage. *p = 0.03, n = 20 (*adora2ab*$^{+/+}$); n = 13 (*adora2ab*$^{-/-}$).

Figure S7

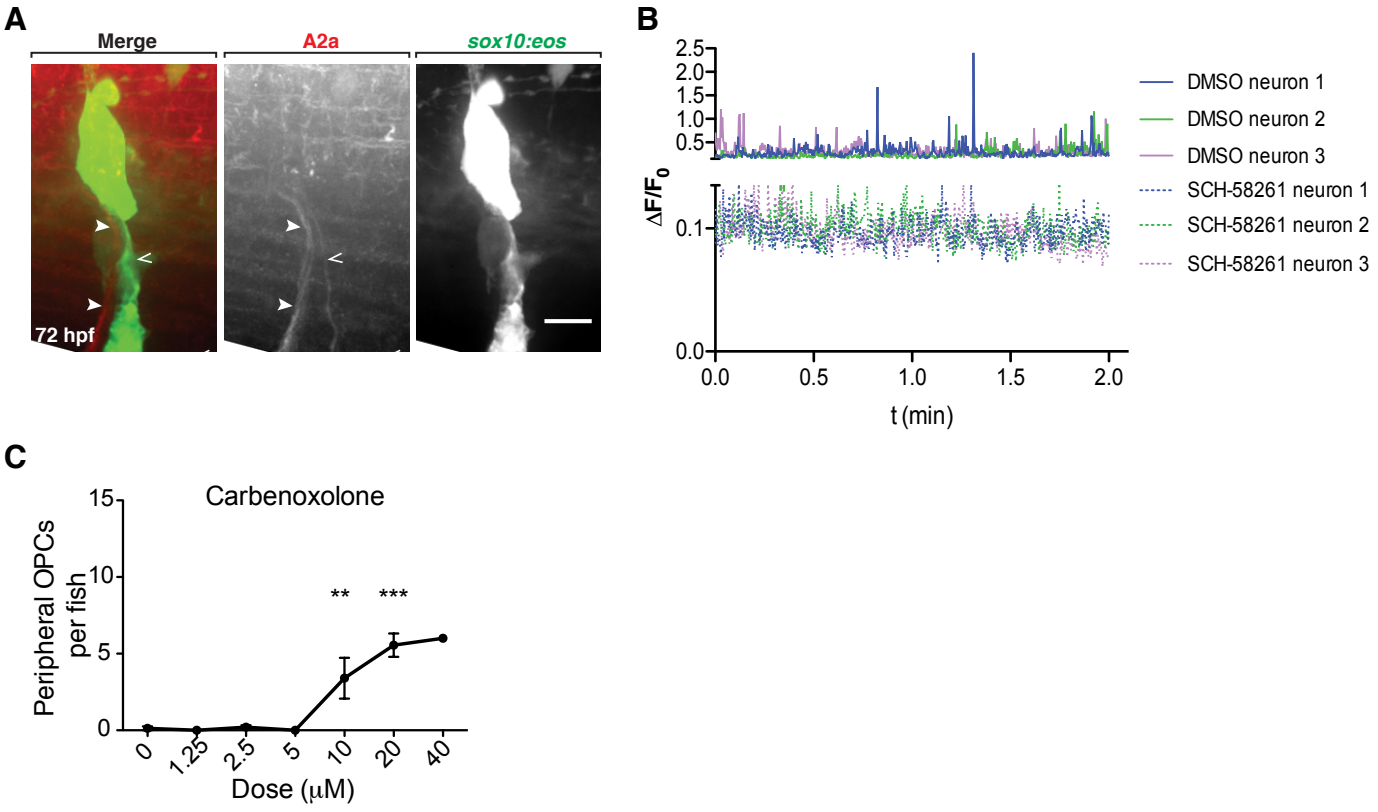

Figure S7 Related to Figure 5. Modulation of neuronal activity affects OPC migration.
(A) A2a antibody expression in motor axons (solid arrowheads) and sensory axons (open arrowheads) in a 72 hpf *sox10:eos* larva. (B) Example traces for calcium responses in individual GCaMP-expressing neurons in larvae treated with DMSO or SCH-58261. (C) Mean ± SEM peripheral OPCs per larvae at 3 dpf after treatment with carbenoxolone from 36 hpf to 3 dpf. n = 8 (0 μM), n = 9 (1.25 μM), n = 10 (2.5μM), n = 9 (5 μM), n = 5 (10 μM), n = 9 (20 μM) and n = 1 (40 μM). 40 μM dose was excluded from statistical analysis. *p<0.05, **p<0.001 compared to 0 μM.

Table S1 Related to Star Methods. Transgenes used in this study, with abbreviations and descriptions of structures labeled.

Transgene name	Abbreviation	Description
Tg(sox10(4.9):eos)^{w9}	*sox10:eos*	Photoconvertible Eos protein expressed by OPCs, Schwann cells, MEP glia and some interneurons
Tg(sox10(4.9):nls-eos)^{w18}	*sox10:nls-eos*	Photoconvertible Eos protein expressed in the nucleus of Sox10-expressing cells
Tg(olig2:egfp)^{vu12}	*olig2:egfp*	GFP expressed by motor neurons and axons, OPCs, MEP glia and some interneurons
Tg(olig2:dsred2)^{vu19}	*olig2:dsred*	DsRed2 expressed by motor neurons and axons, OPCs, MEP glia and some interneurons
Tg(nkx2.2a:megfp)^{vu17}	*nkx2.2a:megfp*	Membrane-tethered GFP expressed by perineurial glia and myelinating oligodendrocytes
Tg(mbp:egfp-CAAX)^{ue2}	*mbp:megfp*	Membrane-tethered GFP expressed by myelinating glia
Tg(neurod:gal4)^{uva22}	*neurod:gal4*	Gal4 expressed by neurons