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Supplemental Information

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

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Figure S1

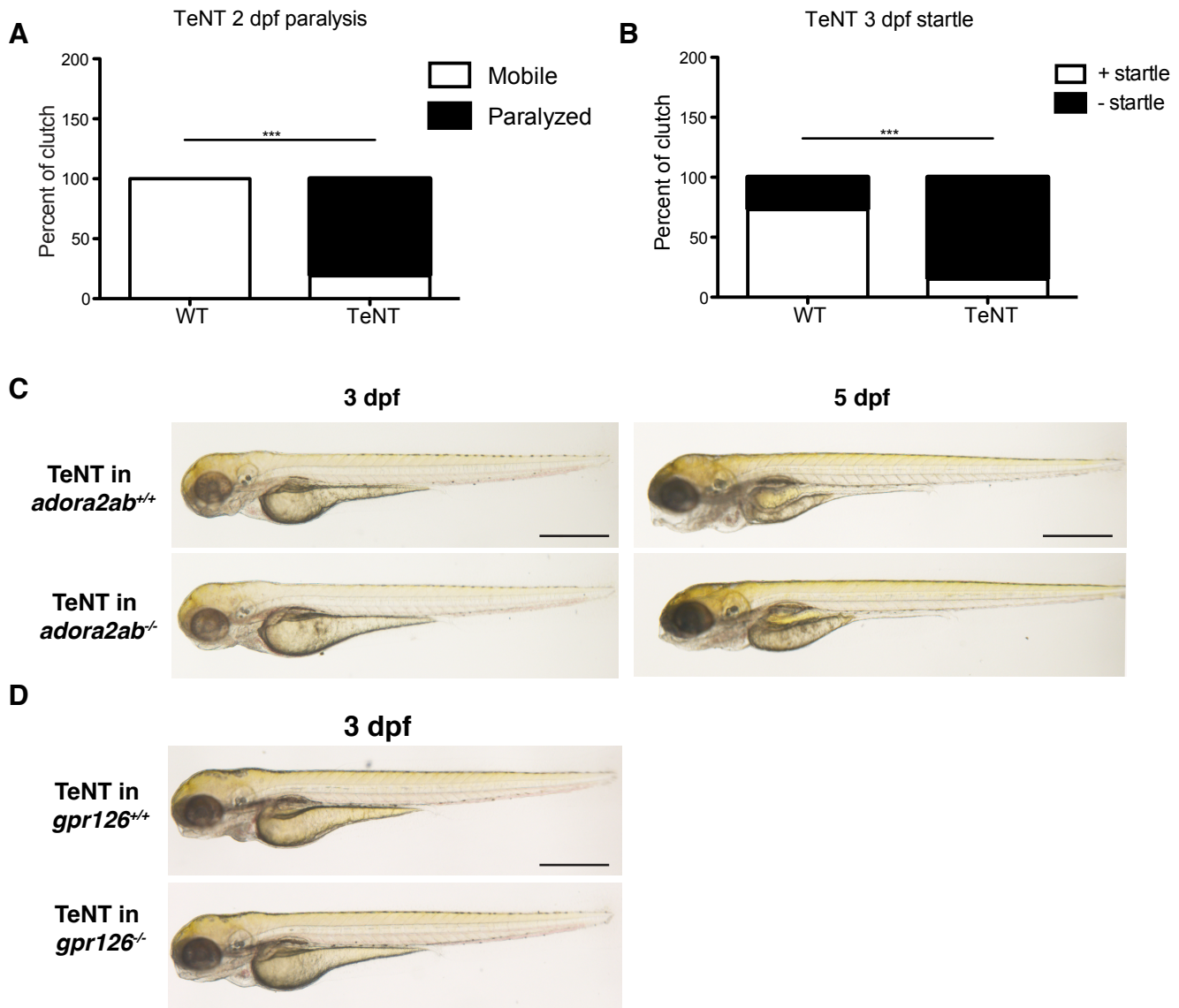


Figure S1 Related to Figures 1&4. Blocking of vesicular release results in larval paralysis.

(A) Percentage of embryos that were mobile or paralyzed during dechoriation 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

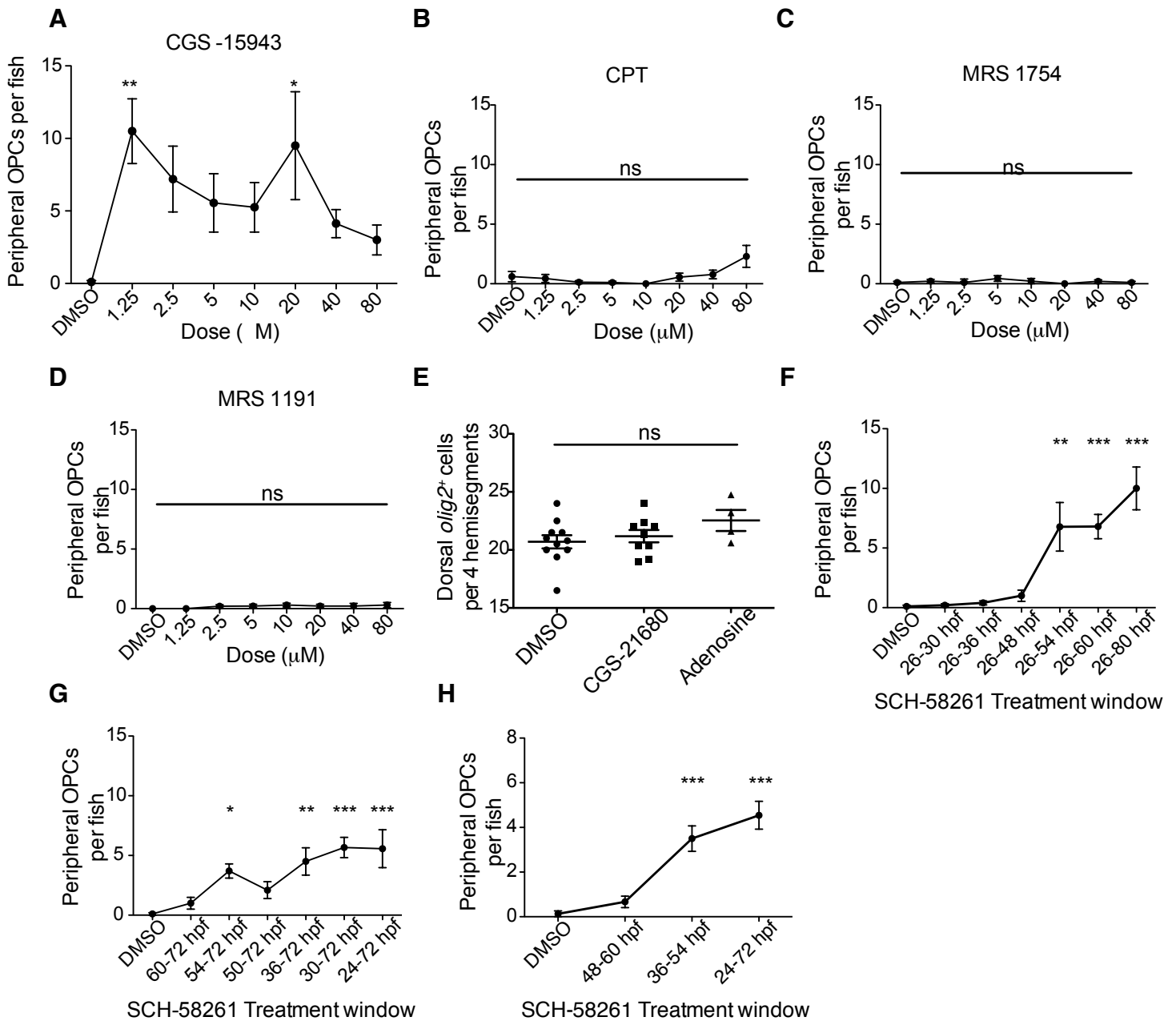


Figure S2 Related to Figure 2. A2a AR specifically mediates OPC migration at the MEP TZ.

(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

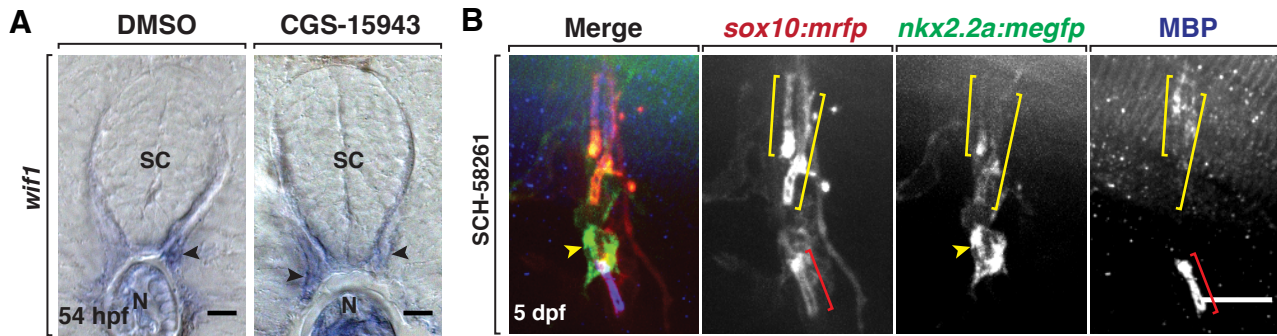


Figure S3 Related to Figure 3. A2a antagonism does not affect spinal motor nerve development. (A) In situ hybridization for *wif1* in 54 hpf larvae treated 36 to 54 hpf with DMSO or 1.25 μ M CGS-15943. Arrowheads denote MEP glia. SC, spinal cord; N, notochord. (B) Image of a 5 dpf larva treated from 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 μ m, (B) 50 μ m.

Figure S4

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CLUSTAL O(1.2.3) multiple sequence alignment
SP|P29274|AA2AR_HUMAN  -MPIMGSSVYITVELAIAVLAAILGNVLVCWAVWLNNSNLQNVTNFFVVSLAAADIAVGVLA 59
TR|Q29ST5|Q29ST5_DANRE  ----MSSLVYIVLELVIAVLAVAGNVLVCWAVCLNSNLQSITNFFVVSLAVADIAVGVLA 56
TR|Q29ST6|Q29ST6_DANRE  MLNNVFDVLYMILELLIALLSVLGNVLVCWAVGLNSNLQSITNFFVVSLAVADIAVGVLA 60

SP|P29274|AA2AR_HUMAN  IPFAITISTGFCAAACHGCLFIACFVLVLTQSSIFSLLAIAIDRYIAIRIPLRYNGLVTGT 119
TR|Q29ST5|Q29ST5_DANRE  IPFAVTISIGFCSNFHGCLFIACFVLVLTQSSVFSLLAIAVDRYIAIKIPLRYNSLVTGR 116
TR|Q29ST6|Q29ST6_DANRE  IPFSIVISTGFCANFYGCLFIACFVLVLTQSSIFSLLAIAIDRYIAIKIPLRYNSLVTGQ 120

SP|P29274|AA2AR_HUMAN  RAKGIIAICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQCGEGQVACLFEDVVPMNYMVY 179
TR|Q29ST5|Q29ST5_DANRE  RAKGIIAVCWILSVVIGLTPMFGWNTSI----DAGTNSSCPQGMTECLFEKVVTMGYMVY 172
TR|Q29ST6|Q29ST6_DANRE  RARGIIAICWVLSVIIGLTPMLGWHKARL---QEGHNGTCCPPGMMECLFEEVVMDYMVY 177

SP|P29274|AA2AR_HUMAN  FNFFACVLVPLLLMLGVYLRIFLAARRQLKQMESQPL-----PGE-RARSTLQKEVHA 231
TR|Q29ST5|Q29ST5_DANRE  FNFFGCILIPLFAMLAITYTWIFTAARRQLRQMEQKLAHLQGHAKGSSSRSTLQKEVHA 232
TR|Q29ST6|Q29ST6_DANRE  FNFFACVLVPLLLMLAIYLRIFMAARHQLKCIESKAI-----PCELKSRSTLQKEVHA 230

SP|P29274|AA2AR_HUMAN  AKSLAIIVGLFALCWLPLHIINCFTFFCPDCSHAPLWLMYLAIVLSHTNSVVNPFYAYR 291
TR|Q29ST5|Q29ST5_DANRE  AKSLAIIVGLFAVCWLPLHIINCFTLFCCPQCDRPQDWVMYLAIILSHANSVVNPFYAYR 292
TR|Q29ST6|Q29ST6_DANRE  AKSLAIIVGLFAVCWLPLHIINCFTLFCCEPECRPPALIMYLAIILSHANSVVNPFYAYR 290

SP|P29274|AA2AR_HUMAN  IREFRQTFRKIIRSHVLROQEPFKAAGTSARVLAAHGS--DGEQVSLRLNGH-----P 342
TR|Q29ST5|Q29ST5_DANRE  IRDFRQTFRRIIRRHFLWHESRLAIGNSNGGMTASSAAVSVIETSCTMSNGYVMDAANPI 352
TR|Q29ST6|Q29ST6_DANRE  IREFRHTFRKIVRYHILGRREPLSCNGSTRTSTRT--S--VADSLRIKVNGL-----V 339

SP|P29274|AA2AR_HUMAN  PGVWANGSAPH-----PERRPNGYALGLVSGGSAQE--SQG--NTGLPDVELLSHE 389
TR|Q29ST5|Q29ST5_DANRE  PGMISCDNFTKELPAKIKPQEEFQDLGYSL----NGSLDH--SF--NANSTPIFSSHSRE 404
TR|Q29ST6|Q29ST6_DANRE  RELYAEQSSTTSSCESAEPGHTHRPVSTENSILDNQPIEISNSHRHTALRHPESPLTGNN 399

SP|P29274|AA2AR_HUMAN  LKGVCPEPPGLDDPLAQDGAGVS----- 412
TR|Q29ST5|Q29ST5_DANRE  EVSSIRDH--VEITTTKDCSDF---THVQDRCLMPVRTSNSSGLAEVS 447
TR|Q29ST6|Q29ST6_DANRE  EGLACRKHAGLDIT---DGKDLSSPLHIKS--ALYVQTAHCVELTEVS 442

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Conserved sequence

Transmembrane domain

Q29ST5 = A2ab

Ligand binding domain

Q29ST6 = A2aa

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 S6

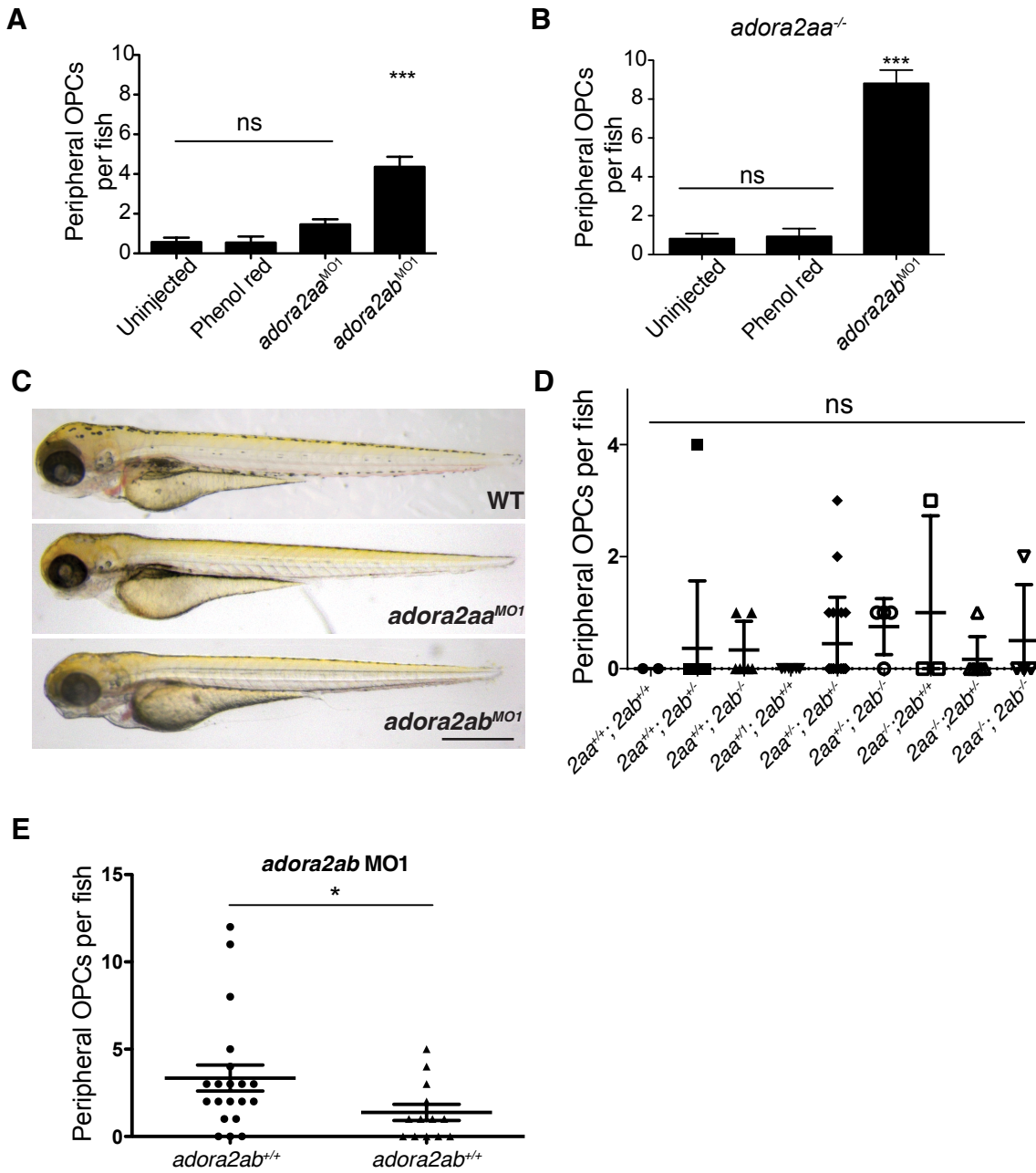


Figure S6 Related to Figure 4. Knockdown of *adora2ab*, but not *adora2aa*, results in peripheral OPCs. (A) Mean ± SEM of peripheral OPCs in WT *olig2:dsred* embryos injected with vehicle, 1 ng/nl *adora2aa* MO1 or 1 ng/nl *adora2ab* MO1. n = 42 (WT), n = 11 (phenol red), n = 65 (*adora2aa* MO1) and 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

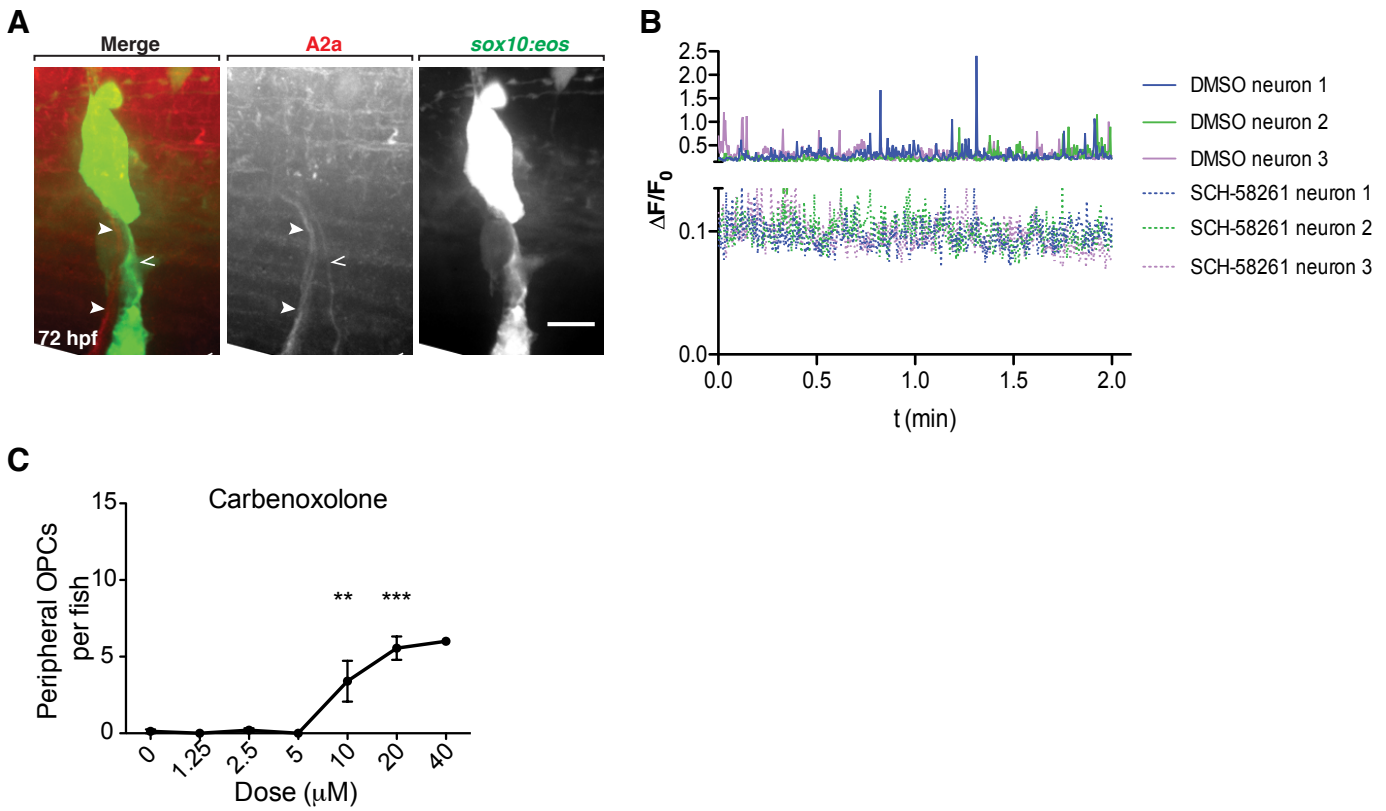


Figure S7 Related to Figure 5. Modulation of neuronal activity affects OPC migration.

(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 \pm 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 (2.5 μM), 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
<i>Tg(sox10(4.9):eos)^{w9}</i>	<i>sox10:eos</i>	Photoconvertible Eos protein expressed by OPCs, Schwann cells, MEP glia and some interneurons
<i>Tg(sox10(4.9):nls-eos)^{w18}</i>	<i>sox10:nls-eos</i>	Photoconvertible Eos protein expressed in the nucleus of Sox10-expressing cells
<i>Tg(olig2:egfp)^{vu12}</i>	<i>olig2:egfp</i>	GFP expressed by motor neurons and axons, OPCs, MEP glia and some interneurons
<i>Tg(olig2:dsred2)^{vu19}</i>	<i>olig2:dsred</i>	DsRed2 expressed by motor neurons and axons, OPCs, MEP glia and some interneurons
<i>Tg(nkx2.2a:megfp)^{vu17}</i>	<i>nkx2.2a:megfp</i>	Membrane-tethered GFP expressed by perineurial glia and myelinating oligodendrocytes
<i>Tg(mbp:egfp-CAAX)^{ue2}</i>	<i>mbp:megfp</i>	Membrane-tethered GFP expressed by myelinating glia
<i>Tg(neurod:gal4)^{uva22}</i>	<i>neurod:gal4</i>	Gal4 expressed by neurons