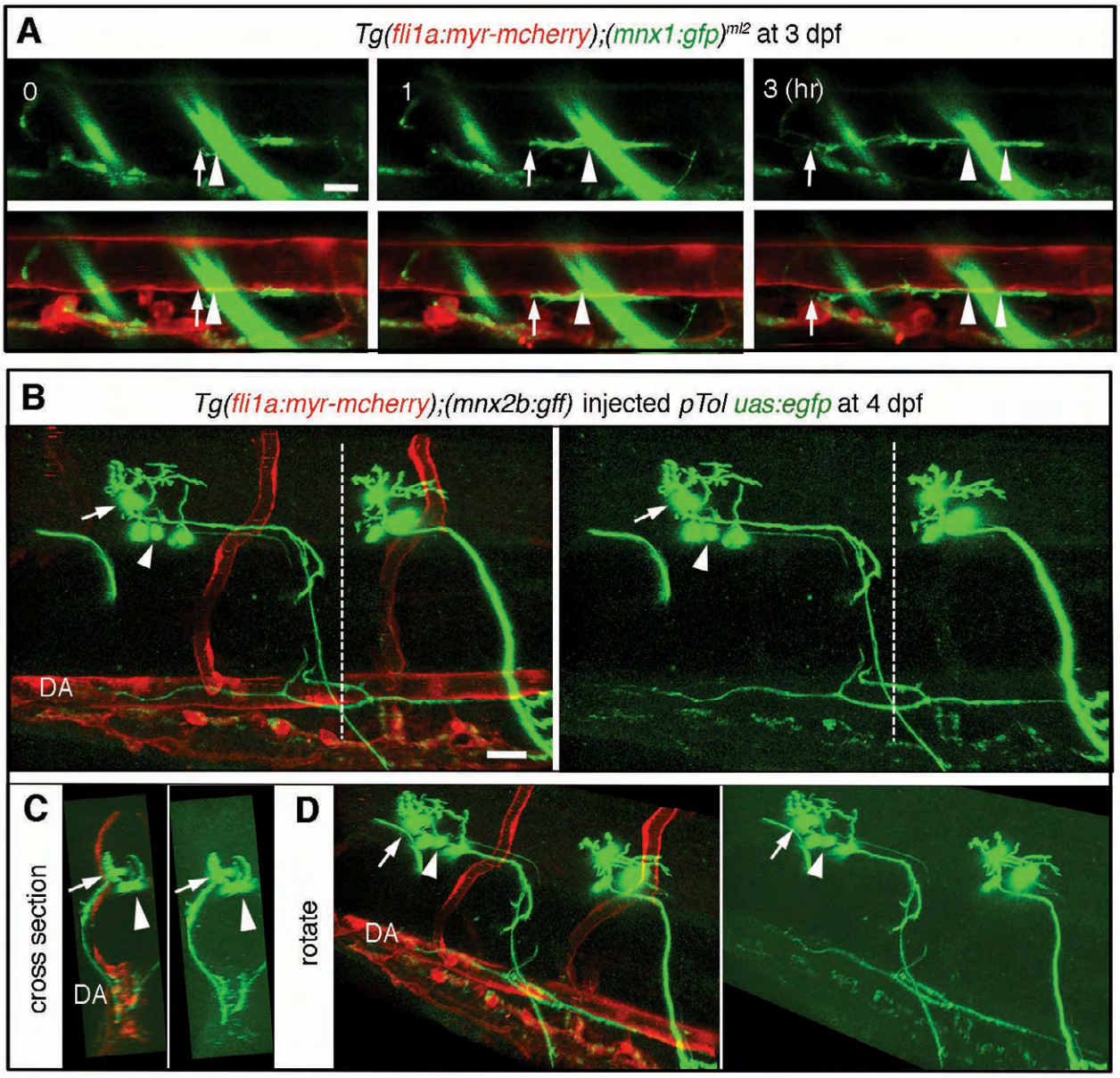


Fig. S1. Motoneurons extend axons rostrally and caudally along the dorsal aorta. (**A**) Time-sequential confocal section images (lateral view) of an embryo expressing *Tg(fli1a:myr mcherry);(mnx1:gfp)*^{ml2}. Elapsed time (hours) from the start point of time-lapse imaging (3 dpf) is shown in the upper panels. Upper panels, GFP images; lower panels, merged images of GFP and mCherry. Arrows indicate the tip of extending neuronal axon. Arrowheads denote the location of the tip when starting time-lapse imaging. Note that axons grow rostrally along the dorsal aorta (DA). (**B**,**C**) 3D volume-rendered confocal stack images of the embryo indicated at the top in which a single cell was labeled. Left, the merged image of mCherry and GFP. Right, GFP image. Arrows and arrowheads indicate the different motoneuronal cell bodies. The 3D volume-rendered confocal stack images anterior to the dashed lines in B are shown in C. (**D**) Oblique views of B. Scale bars: 25 μm.

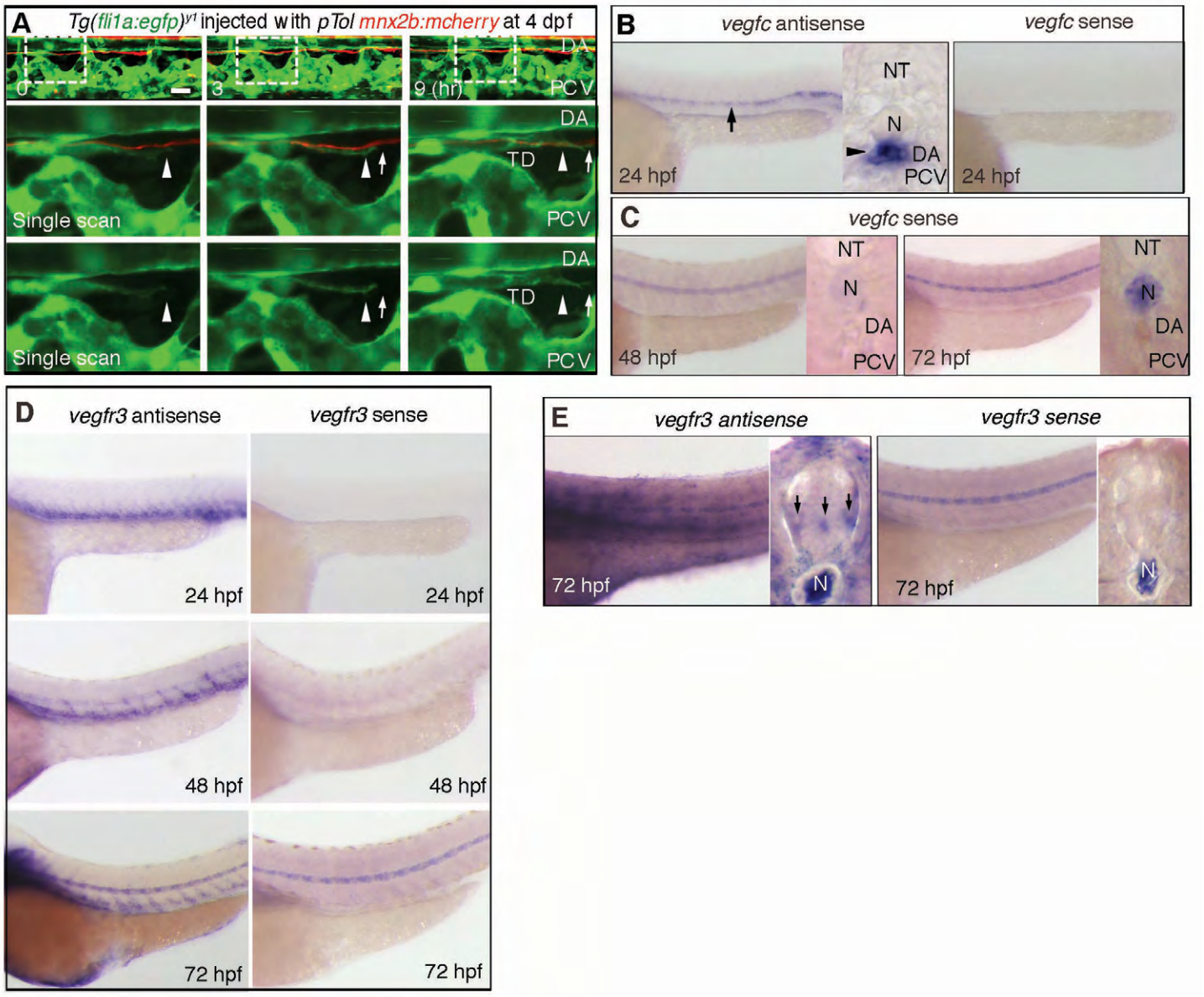


Fig. S2. Development of the thoracic duct along the axons of motoneurons. (**A**) Time-sequential confocal images (lateral view) of a *Tg(fli1a:egfp)*^{y1} embryo injected with *pTol mnx2b:mcherry* plasmid and *transposase* mRNA for Tol2 transposon-mediated gene transfer. Elapsed time (hours) from the start point (4 dpf) of time-lapse imaging is shown in the top panels. Top panels, merged images of GFP and mCherry; middle panels, enlarged single scan images of boxed regions in top panels; bottom panels, enlarged single scan images of GFP images of boxed regions in the same column. Arrows indicate the tip of developing thoracic duct. Arrowheads denote the location of the tip when starting time-lapse imaging. Scale bar: 25 μm. (**B**,**C**) Expression analyses of *vegfc* mRNA by *in situ* hybridization at 24 hpf (B) and negative control of those at 48 and 72 hpf (C). Cross-sectioned images are on the right side of each panel. Arrow and arrowhead indicate the expression of *vegfc* in the DA. (**D**) Expression analyses of *vegfr3* mRNA by *in situ* hybridization at 24 hpf (top), 48 hpf (middle) and 72 hpf (bottom). (**E**) The result of longer reaction of detection of (D, bottom) with the transverse section images. Arrows denote the expression of *vegfr3* in neural tube. DA, dorsal aorta; N, notochord; NT, neural tube; PCV, posterior cardinal vein.

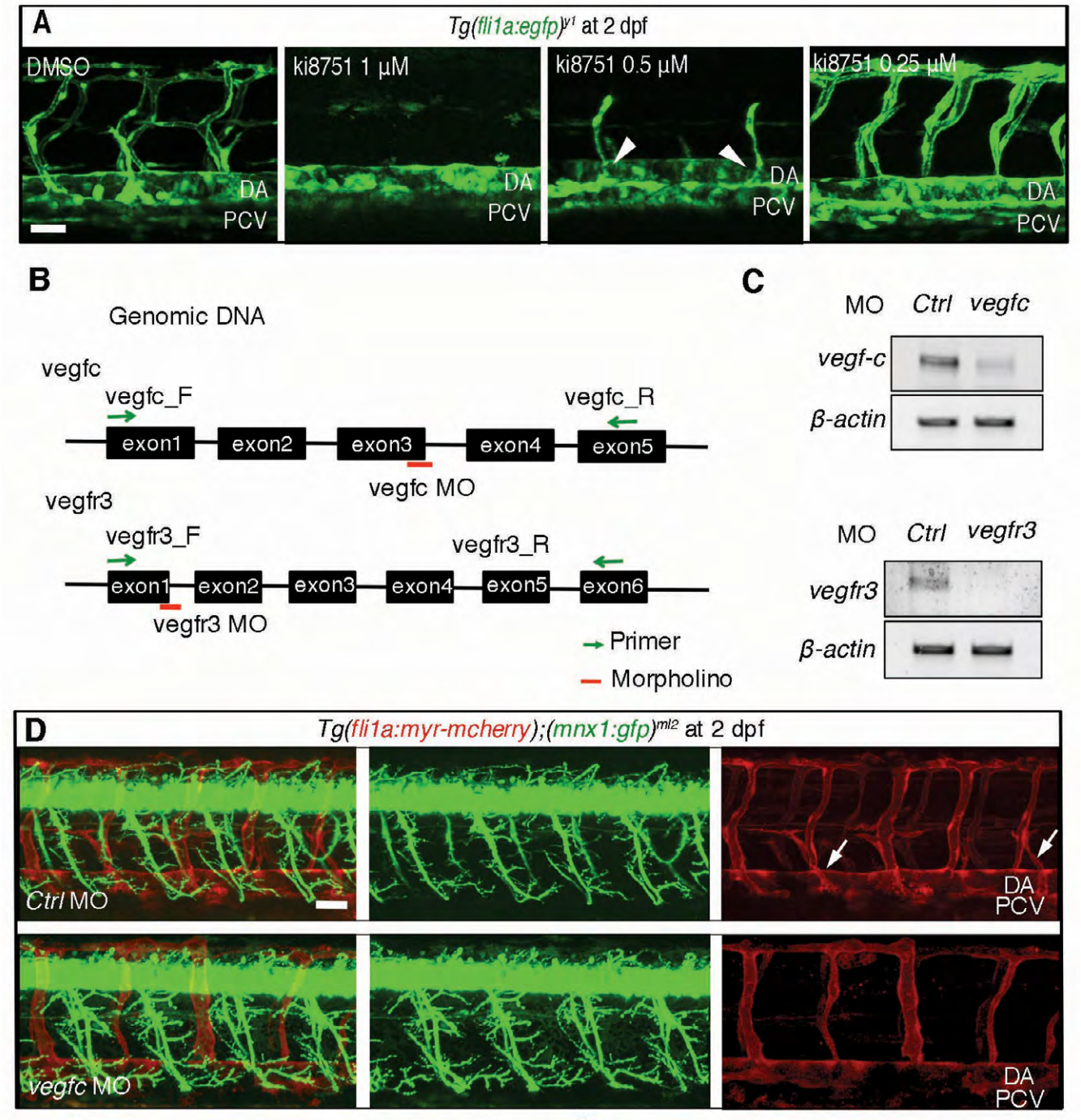


Fig. S3. Schematic illustration of the *vegfc* and *vegfr3* genes and the primer sets for verifying the expression of *vegfc and vegfr3* mRNAs. (**A**) 3D-rendered confocal stack images (lateral view) of a *Tg (fli1a:egfp)*^{y1} embryo treated with DMSO (control) and ki8751 (1 μM, 0.5 μM and 0.25 μM) at 2 dpf. Arrowheads indicate the secondary sprouting from the PCV. (**B**) *vegfc and vegfr3* genes with the primers for examining the expression of *vegfc* and *vegfr3* mRNAs and with the morpholinos (MOs) blocking splicing. (**C**) RT-PCR analyses using the primers indicated at the left and RNAs prepared from the embryos injected with control (Ctrl) MO or target MOs as indicated at the top. (**D**) 3D-rendered confocal stack of fluorescence images (lateral view) of *Tg(fli1a:myr-mcherry);(mnx:gfp)*^{ml2} embryos treated with either control MO (Ctrl, top panels) or *vegfc* MO (bottom panels) at 2 dpf. Left column, merged images; center column, GFP images; right column, mCherry images. Arrows indicate the secondary sprouts from the PCV. Anterior is to the left. DA, dorsal aorta; PCV, posterior cardinal vein. Scale bars: 25 μm.

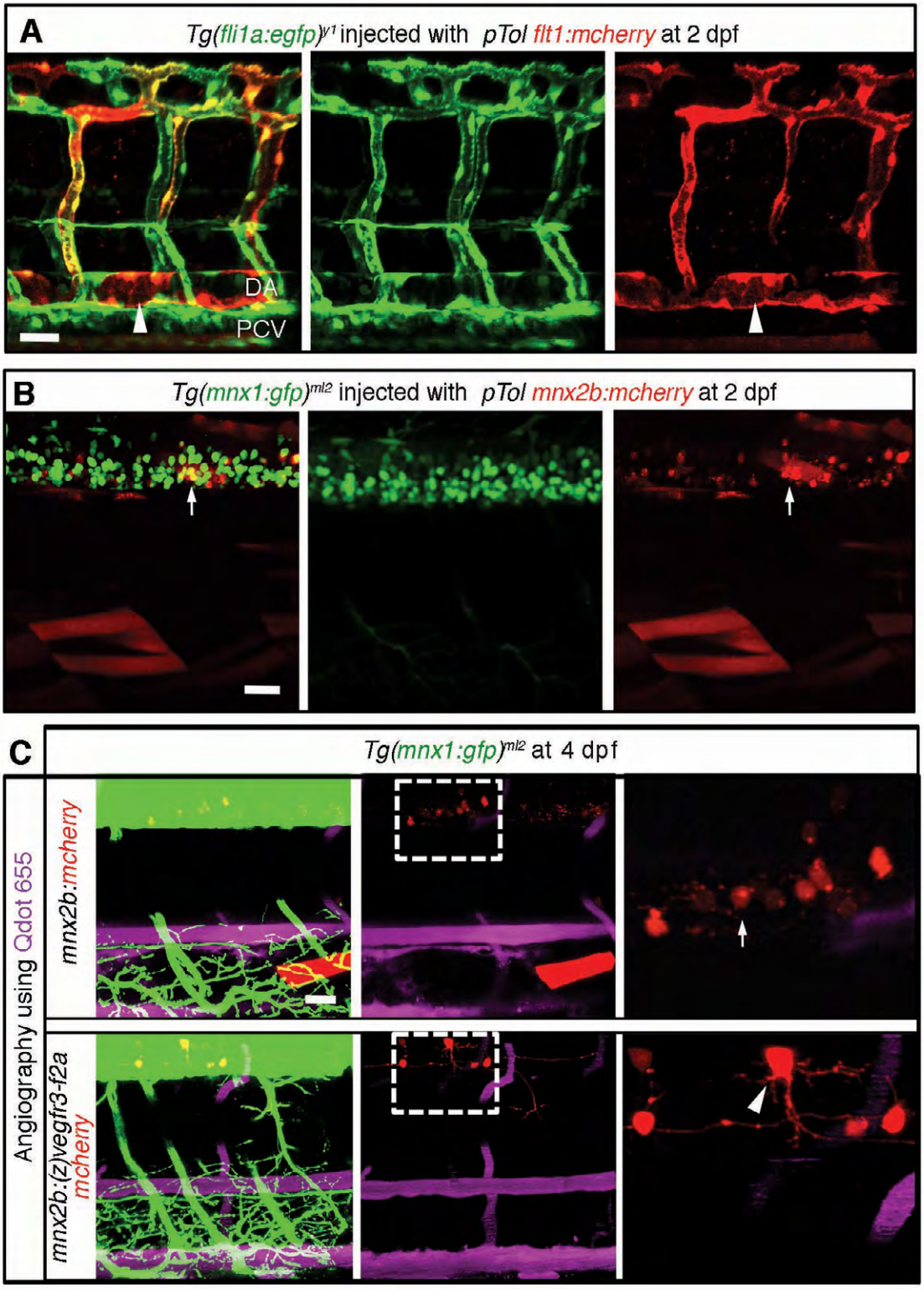


Fig. S4. Tissue-specific expression of genes driven by the *flt1* and *the mnx2b* promoter used for Tol2-mediated gene transfer. (**A**,**B**) 3D-rendered confocal stack of fluorescence images (lateral view) of a *Tg(fli1a:egfp)*^{y1} embryo (2 dpf) injected with *pTol flt1:mcherry* plasmid (A) and of a *Tg(mnx1:gfp)*^{ml2} embryo (2 dpf) injected with *pTol mnx2b:mcherry* plasmid (B). Arrowheads indicate the expression of mCherry in the DA (A). Arrows indicate the expression of mCherry in motoneurons (B). DA, dorsal aorta; PCV, posterior cardinal vein. Note that mCherry is observed in the DA and arterial intersegmental vessels but not in the PCV in A. (**C**) 3D-rendered confocal stack images (lateral view) of *Tg(mnx1:gfp)*^{ml2} embryos transiently expressing the molecules indicated at the left. The embryos were injected with Quantum (Q) dot 655 into the blood vessels at 4 dpf. Left panels, merged images of GFP, mCherry and Qdot 655 images; center panels, merged images of mCherry and Qdot 655 images; right panels, enlarged image of boxed region of the center panels. Arrow and arrowhead indicate expression of mCherry in the motoneurons. Scale bars: 25 μm.

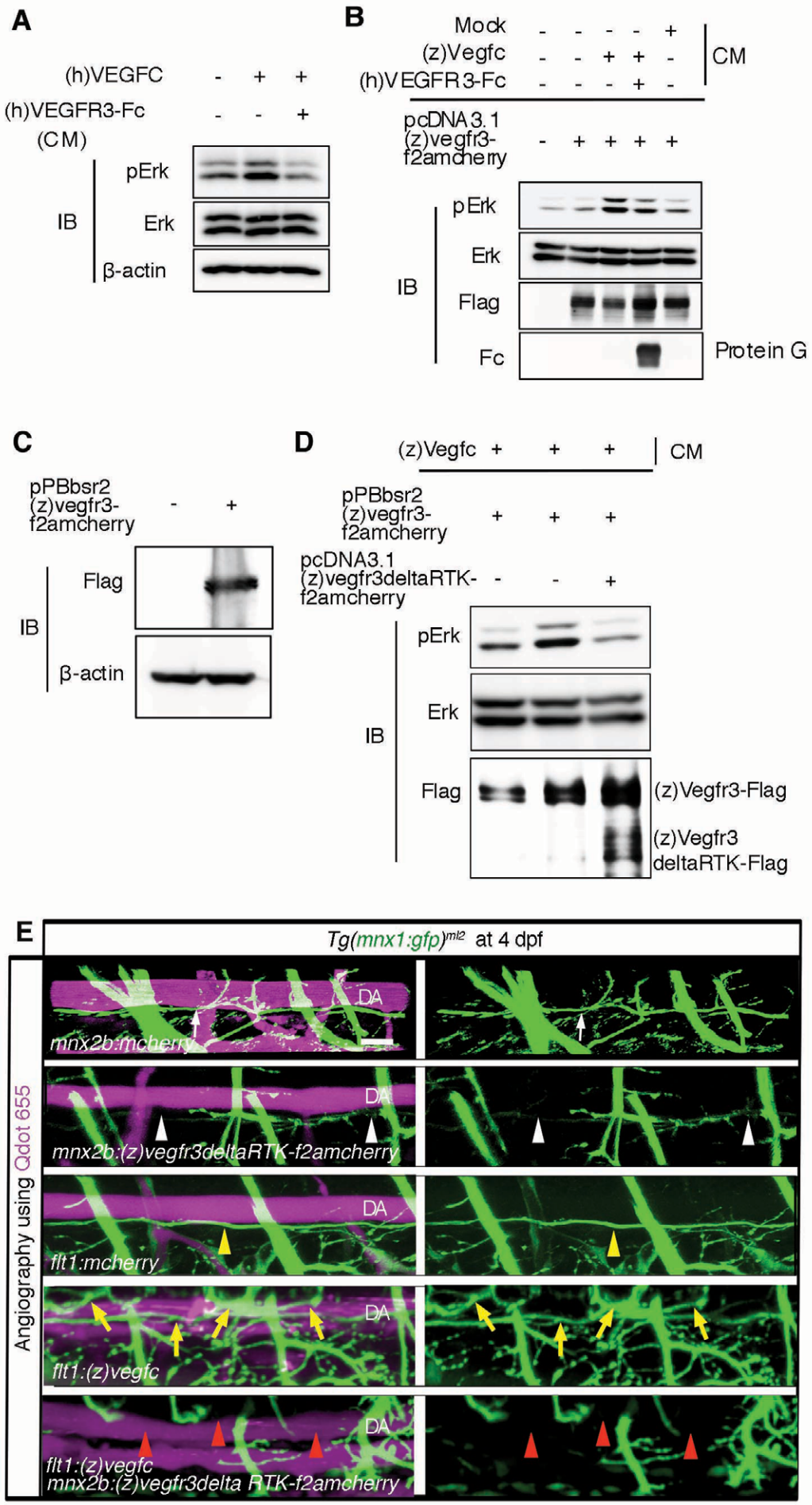
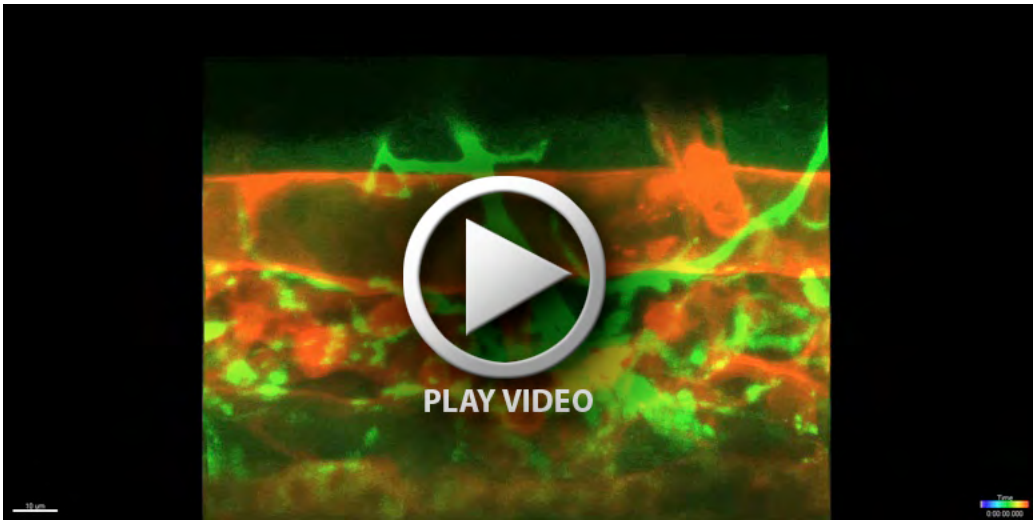

A
(h)VEGFC - + +
(h)VEGFR3-Fc - - +
(CM)
IB
pErk
Erk
β-actin
B
Mock - - - - +
(z)Vegfc - - + + -
(h)VEGFR3-Fc - - - + -
CM
pcDNA3.1
(z)vegfr3-
f2amcherry - + + + +
IB
pErk
Erk
Flag
Fc
Protein G
C
pPBbsr2
(z)vegfr3-
f2amcherry - +
IB
Flag
β-actin
D
(z)Vegfc + + + CM
pPBbsr2
(z)vegfr3-
f2amcherry + + +
pcDNA3.1
(z)vegfr3deltaRTK-
f2amcherry - - +
IB
pErk
Erk
Flag
(z)Vegfr3-Flag
(z)Vegfr3
deltaRTK-Flag
E
Tg(mnx1:gfp)ml2 at 4 dpf
Angiography using Qdot 655
DA
mnx2b:mcherry
mnx2b:(z)vegfr3deltaRTK-f2amcherry
flt1:mcherry
flt1:(z)vegfc
flt1:(z)vegfc
mnx2b:(z)vegfr3delta RTK-f2amcherry

Fig. S5. Impaired parallel growth of motoneurons after inhibition of *vegfr3* in motoneurons. (**A**) Immunoblot analyses with the antibodies indicated at the left using cell lysates of HUVECs treated with recombinant human (h)VEGFC together with or without a truncated mutant of (h)VEGFR3 tagged with human IgG Fc, (h)VEGFR3-Fc, prepared from the conditioned medium (CM) of 293T cells transfected with the plasmids expressing (h)VEGFR3-Fc. (**B**) Immunoblot analyses with the antibodies indicated at the left using cell lysates of 293T cells transfected with or without the plasmid expressing zebrafish (z)Vegfr3 tagged with Flag followed by 2A peptide and mCherry (pcDNA3.1(z)vegfr3-f2amcherry) and treated with the CM indicated at the top. Bottom panel, precipitates on protein G and subjected to immunoblot with anti-human immunoglobulin. (**C**) Immunoblot analyses with the antibodies indicated at the left using the cell lysates of the parental and HEK293 cells stably transfected with pPBbsr2(z)vegfr3-f2amcherry plasmids using *piggyBac* transposon system. (**D**) Immunoblot analyses with the antibodies indicated at the left using the lysates of the cells described in C transfected with the plasmid expressing zebrafish (z)Vegfr3 lacking the cytoplasmic domain tagged with Flag followed by 2A peptide and mCherry (pcDNA3.1(z)vegfr3deltaRTK-f2amcherry) and treated with the conditioned medium (CM) as indicated at the top. (**E**) 3D-rendered confocal stack images (lateral view) of *Tg(mnx1:gfp)*^{ml2} embryos injected with the plasmids indicated at the bottom of each panel at 4 dpf. Blood vessels were visualized by injecting Quantum (Q) dot 655 into the blood vessels. Left panels, merged images of GFP and Qdot 655 images; right panels, GFP images. White arrows and yellow arrowheads indicate parallel growth (PG) of motoneuron axons beneath the DA. White arrowheads and red arrowheads denote the impairment of PG of motoneuron axons and DA. Yellow arrows indicate the increased branches of motoneuron axons. Scale bar: 25 μm. DA, dorsal aorta.



Movie 1. Neuronal axon grows rostrally beneath the dorsal aorta. Time sequential confocal images of a *Tg(fli1a:myr-mcherry);(huc:gfp)* embryo at 3 dpf for 8 hours.

Movie 2. Motoneuron axons grow rostrally and caudally beneath the dorsal aorta. Time sequential confocal images of a *Tg(fli1a:myr-mcherry);(mnx1:gfp)*^{ml2} embryo at 3 dpf for 8 hours.

Movie 3. Motoneurons extend axons outside of the neural tube bilaterally. 3D volume-rendered confocal images of a *Tg(fli1a:myr-mcherry);(mnx2b:gff)* embryo injected with pTol uas:egfp plasmid at 4 dpf.

Table S1. The sequences of primers and morpholinos (MOs)

Category	Name	Sequences
Primer	*vegfc* probe fw	5′-ATGCACTTATTTGGATTTTCTGTCTTCT-3′
	vegfc probe rev	5′-GTCCAGTCTTCCCCAGTATG-3′
	vegfr3 probe fw	5′-CACCAGTATGCCACATTTTT-3′
	vegfr3 probe rev	5′-TTAGAATTCCTTGTCATCGTCATCC-3′
	vegfr3 fw1	5′- ATGATAGAAGCAGGTCAGGCG -3′
	vegfr3 rev1	5′-GGCGAGTCTTCAGGAAACAG-3′
	vegfr3 fw2	5’-ATGAAGAGAGATTTTACGTTTTTCTGTC-3’
	vegfr3 rev2	5’-TTGCCTTTGCGCACATAGTC-3’
	mnx1 fw	5′-ACTTCTGGCTTGCACACCTT-3′
	mnx1 rev	5′-GCCCACCTCACAAACAGATT-3′
	tie1 fw	5′-CATGGAGATCGCTGTCGTAA-3′
	tie1 rev	5′-TGCATTTGCCTTTGTTCTTG-3′
	tie2 fw	5′-AGCACACTCTCCTCACAGCA-3′
	tie2 rev	5′-TTCGCCACAAAGTTCTCTCC-3′
	fli1a fw	5′-CTGCTGCTCCTTTACCCAAG-3′
	fli1a rev	5′-GGAATGGGGTTGATTTTGTG-3′
	ef1α fw	5′-CTGGAGGCCAGCTCAAACAT-3′
	ef1α rev	5′-ATCAAGAAGAGTAGTACCGCTAGCATTAC-3′
	vegfc fw	5’-ATGCACTTATTTGGATTTTCTGTCTTCT-3’
	vegfc rev	5’-GCATAAGTGATTACTCCAGCTG-3’
	β-actin fw	5’-GATCTTCACTCCCCTTGTTCAC-3’
	β-actin rev	5’-CACAGCTTCTCCTTGATGTCAC-3’
	expando seq1	5′-AGCTCTTGATTTGGCTTTAG-3′
	expando seq2	5′- GGAAAGTATCCTTGCTCTGC-3′
Morpholino	*vegfc* MO	5′- ACTTTGACTCACCGTCTTGCTGATG -3′
	vegfr3 MO	5′- TTAGGAAAATGCGTTCTCACCTGAG -3′