Figure S1

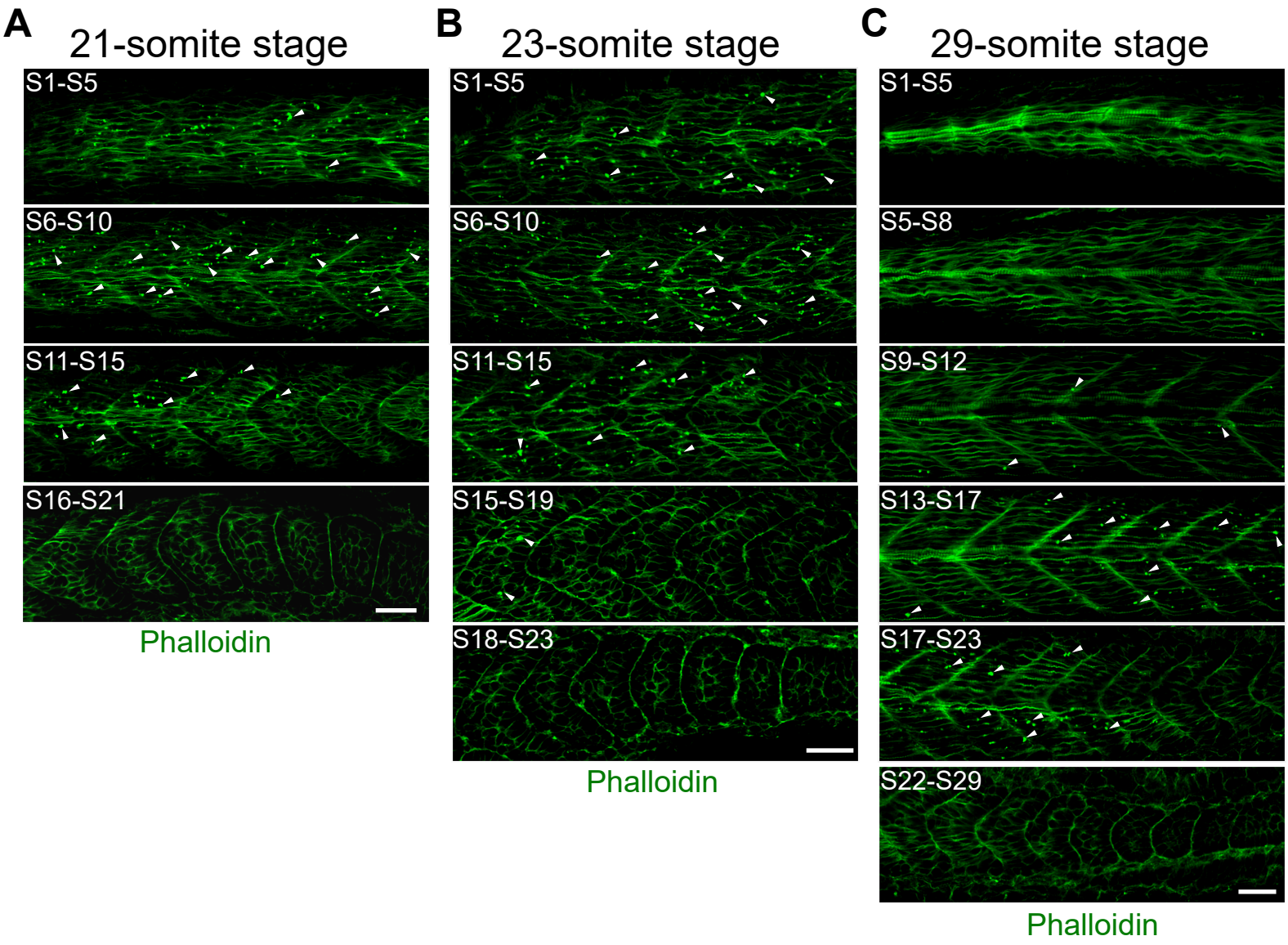

A
21-somite stage
S1-S5
S6-S10
S11-S15
S16-S21
Phalloidin
B
23-somite stage
S1-S5
S6-S10
S11-S15
S15-S19
S18-S23
Phalloidin
C
29-somite stage
S1-S5
S5-S8
S9-S12
S13-S17
S17-S23
S22-S29
Phalloidin

Figure S1. F-actin foci are present in the developing zebrafish myotome. Related to Figure 1.

(A-C) Wild-type embryos were collected at 21-somite stage (A), 23-somite stage (B), and 29-somite stage (C), fixed with 4% PFA, and stained with phalloidin to visualize F-actin. Arrows indicate randomly selected F-actin foci. Scale bars, 25 μm.

Figure S2

A

Jam2a protein sequence

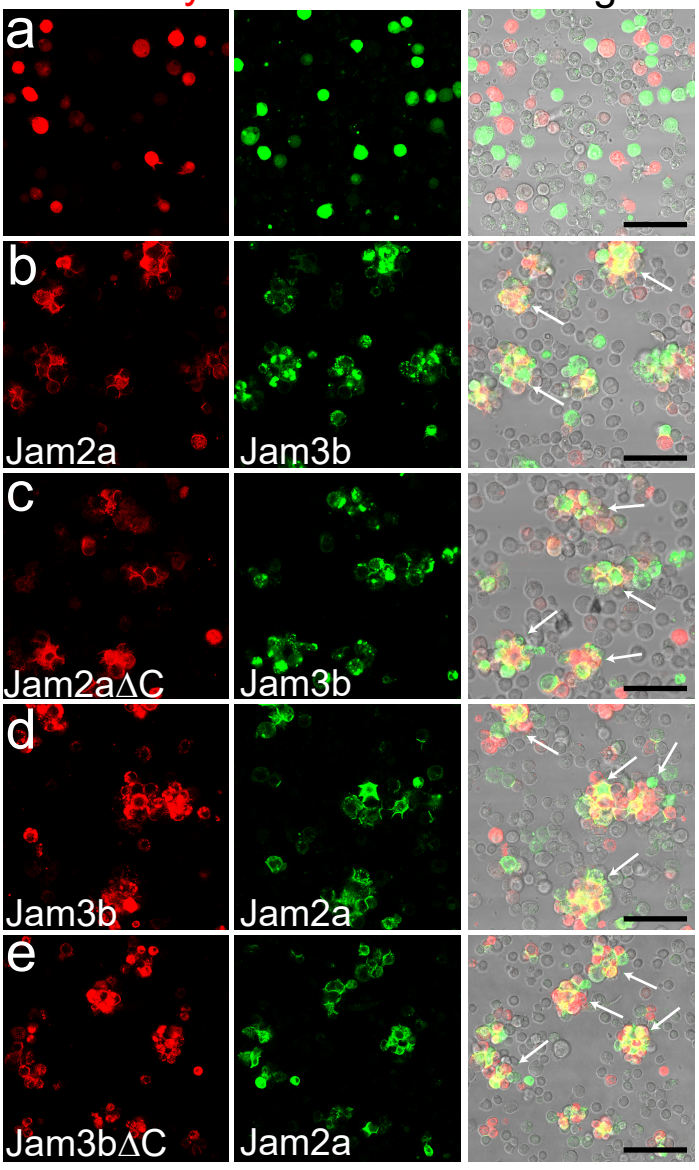
MLVCVSLLILIHSVPVSPVTVSSRNPKVEVHEFSDAELSCEFKTEKDTNPRI
EWKRKDKEKDVSFVYYGERFVGPFQDRADIEGATVRLRRVTQADAGEY
RCEVSAPSDSISLGETNVTLRVLVPPQTPSCDVPSSALTGSQVELRCRDR
HSIPPAVYTWYKDNRALPIRHPNATYTVNEFTGVLMFQTVSRSDAGQYH
CEAKNGVGPPKSCQHTHMQIDDLNVAAVVSAVVLVCVILVLCAFGVCLAH
RQGYFSRHRGRSFWIPHCHGVTHISSQNLNPSEHTQHSGYSHPPKEPQ
DFKHTQSFML

B

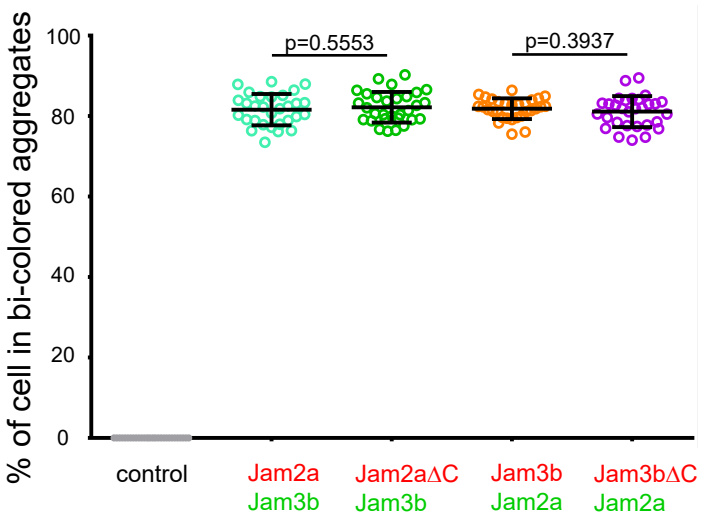
Jam3b protein sequence

MYSQTEHFTDSKMALTPLACVLLLLSMQCYISTLAVLLKSTNSKPWVNEF
GSIELSCMIESITTTKPRIEWKKIKNGDPSYVYFDNQISGDLERRAKIREPA
TLVILNATRSDSADYRCEVTAPNDQKSFDEILISLTVRVKPVVPRCSVPKSI
PVGKPAELHCLEDEGYPKSQYQWFRNKEEIPLDPKSSPKFFNSTYTLDG
EMGTLKFSAVRKEDAGEYYCRAKNEAGISECGPQMMEVYDINIAGIILGV
VVVVMVLLCITVGIFCAYKRGYFTSQKQTGNNYKPPAKGDGVDYVRTED
EGDFRHKSSFVI

C



D



E

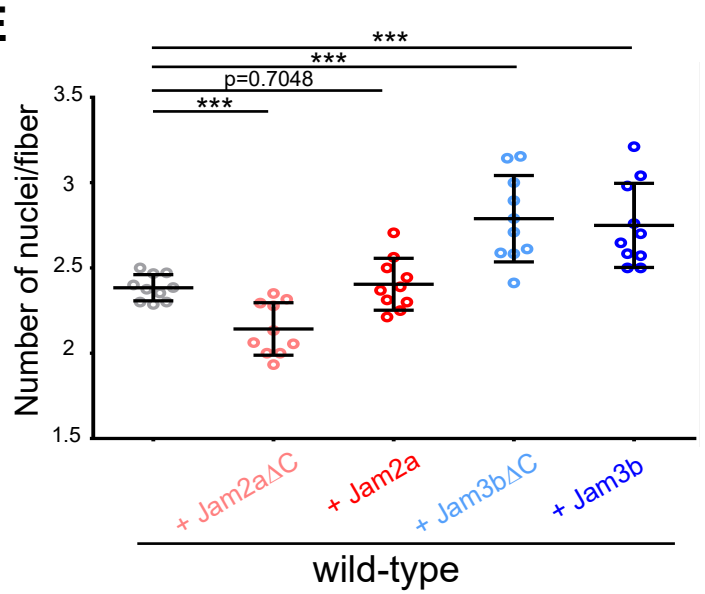


Figure S2. Jam2aΔC and Jam3bΔC exhibited normal cell adhesion properties. Related to Figure 5.

(A and B) Protein sequences of Jam2a (A) and Jam3b (B). Yellow-highlighted sequences, transmembrane (TM) domains; underlined sequences, Jam2aΔC in (A) and Jam3bΔC in (B).

(C) Jam2aΔC and Jam3bΔC exhibit normal cell adhesion properties. Two groups of *Drosophila* S2 cells expressing indicated adhesion molecules were mixed and allowed to adhere for 16 hours. Representative images of each cell mixture are shown in (**b-e**). Arrows indicate bi-colored cell aggregates. The control mixture with mCherry- and mNeonGreen (mNG)-expressing cells (**a**) showed no aggregate. Scale bar, 50 μm.

(D) Quantification of the results in (C). Dot plot showing the percentage of cells in bi-colored aggregates in each cell mixing experiment. Note that Jam2aΔC and Jam3bΔC mediated normal cell adhesion as their full-length counterparts (compare 81.59 ± 3.91% in **b** with 82.18 ± 3.81% in **c,** and 81.83 ± 2.57% in **d** with 81.10 ± 3.85% in **e**). Thirty random 20x microscopic fields were analyzed in three independent experiments.

(E) Overexpressing Jam2a, Jam2aΔC, Jam3b and Jam3bΔC in wild-type embryos led to different effects in myoblast fusion. Dot plot showing the number of nuclei per fiber in somite 16 of uninjected wild-type (2.42 ± 0.09), or wild-type overexpressed with Jam2aΔC-mCherry (2.19 ± 0.18), Jam2a-mCherry (2.42 ± 0.09), Jam3bΔC-mCherry (2.65 ± 0.18) and Jam3b-mCherry (2.77 ± 0.18). 200 myofibers from 10 embryos in each experiment were analyzed. Each data point represents the mean value of an embryo. Mean ± s.d. values are shown in the dot plot, and significance was determined by the unpaired parametric t test. *** indicates $p < 0.001$.

Figure S3

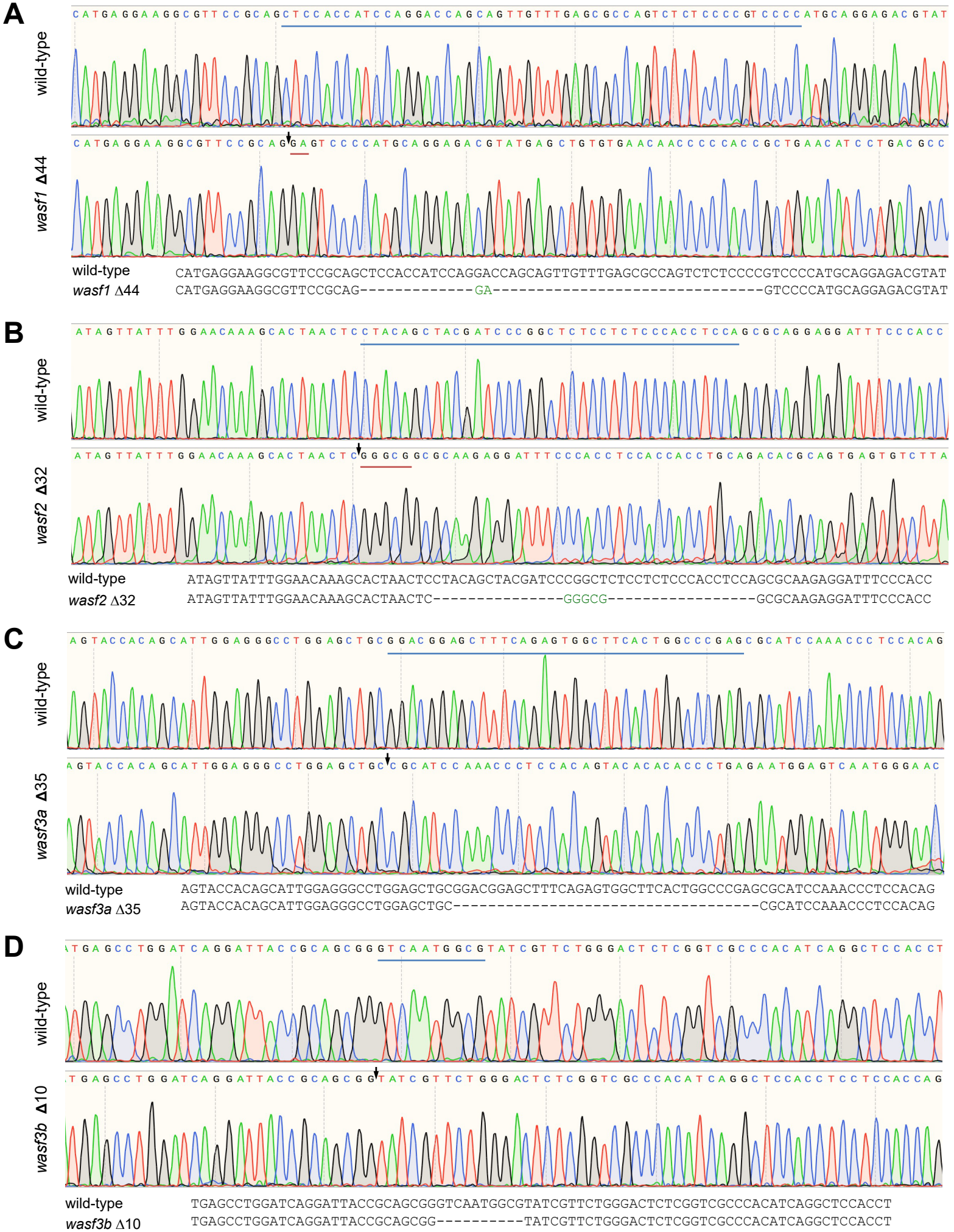

A
wild-type
wasf1 Δ44
wild-type CATGAGGAAGGCGTTCCGCAGCTCCACCATCCAGGACCAGCAGTTGTTTGAGCGCCAGTCTCTCCCCGTCCCCATGCAGGAGACGTAT
wasf1 Δ44 CATGAGGAAGGCGTTCCGCAG----------GA----------GTCCCCATGCAGGAGACGTAT
B
wild-type
wasf2 Δ32
wild-type ATAGTTATTTGGAACAAAGCACTAACTCCTACAGCTACGATCCCGGCTCTCCTCTCCCACCTCCAGCGCAAGAGGATTTCCCACC
wasf2 Δ32 ATAGTTATTTGGAACAAAGCACTAACTC----------GGGCG----------GCGCAAGAGGATTTCCCACC
C
wild-type
wasf3a Δ35
wild-type AGTACCACAGCATTGGAGGGCCTGGAGCTGCGGACGGAGCTTTCAGAGTGGCTTCACTGGCCCGAGCGCATCCAAACCCTCCACAG
wasf3a Δ35 AGTACCACAGCATTGGAGGGCCTGGAGCTGC----------CGCATCCAAACCCTCCACAG
D
wild-type
wasf3b Δ10
wild-type TGAGCCTGGATCAGGATTACCGCAGCGGGTCAATGGCGTATCGTTCTGGGACTCTCGGTCGCCCACATCAGGCTCCACCT
wasf3b Δ10 TGAGCCTGGATCAGGATTACCGCAGCGG----------TATCGTTCTGGGACTCTCGGTCGCCCACATCAGGCTCCACCT

Figure S3. The *wave* mutant alleles. Related to Figure 6.

DNA sequences of the *wasf1* (A), *wasf2* (B), *wasf3a* (C) and *wasf3b* (D) mutants are shown. On the sequencing chromatogram – arrow, mutation start site; blue underline, deleted sequence; and red underline, inserted sequence. In the sequence alignment – dashed line, deleted sequence; and green letters, inserted nucleotides.

Figure S4

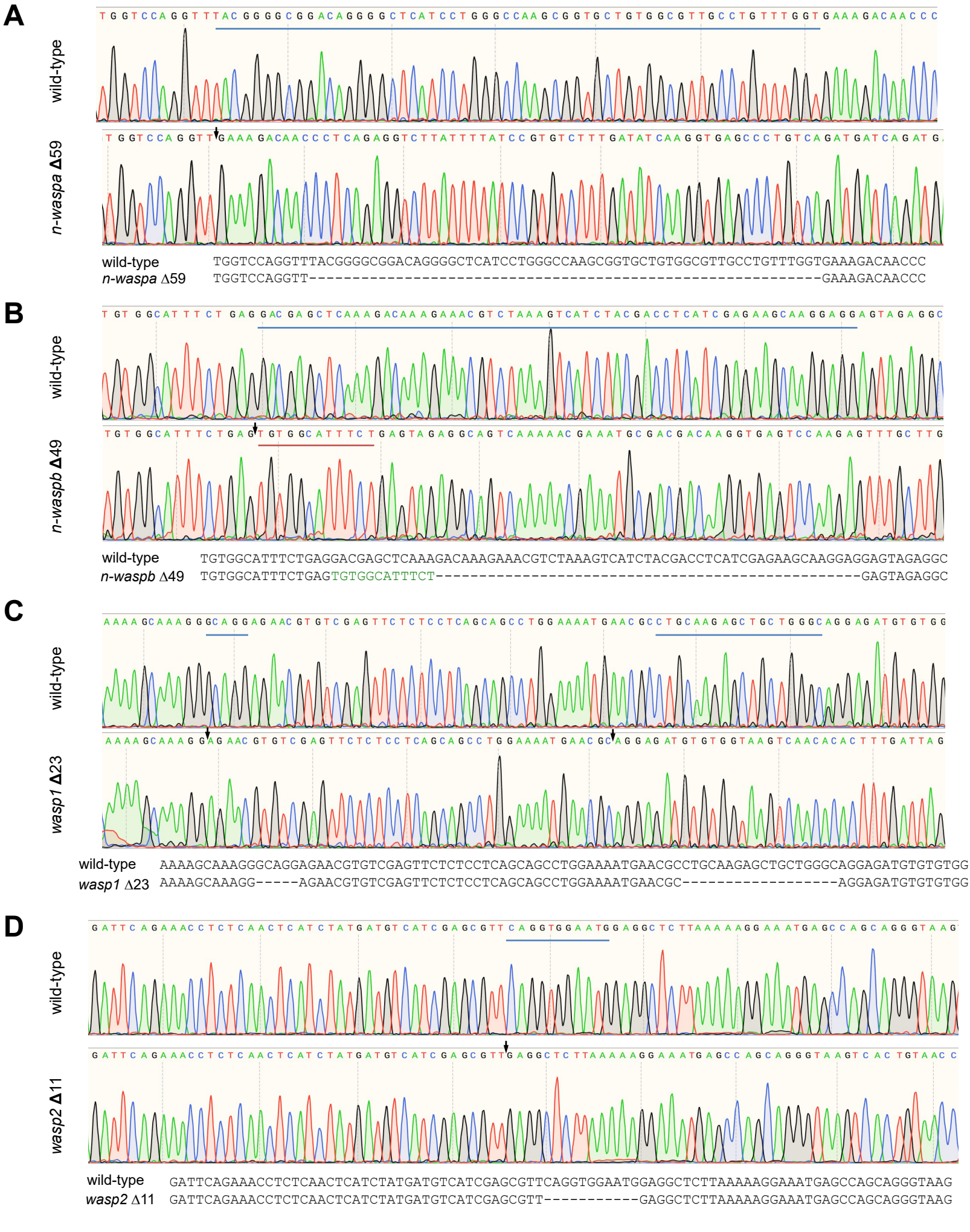

A
wild-type
n-waspa Δ59
wild-type TGGTCCAGGTTTACGGGGCGGACAGGGGCTCATCCTGGGCCAAGCGGTGCTGTGGCGTTGCCTGTTTGGTGAAAGACAACCC
n-waspa Δ59 TGGTCCAGGTT----------GAAAGACAACCC
B
wild-type
n-waspb Δ49
wild-type TGTGGCATTTCTGAGGACGAGCTCAAAGACAAAGAAACGTCTAAAGTCATCTACGACCTCATCGAGAAGCAAGGAGGAGTAGAGGC
n-waspb Δ49 TGTGGCATTTCTGAGTGTGGCATTTCT----------GAGTAGAGGC
C
wild-type
wasp1 Δ23
wild-type AAAAGCAAAGGGCAGGAGAACGTGTCGAGTTCTCTCCTCAGCAGCCTGGAAAATGAACGCCTGCAAGAGCTGCTGGGCAGGAGATGTGTGTGG
wasp1 Δ23 AAAAGCAAAGG-----AGAACGTGTCGAGTTCTCTCCTCAGCAGCCTGGAAAATGAACGC----------AGGAGATGTGTGTGG
D
wild-type
wasp2 Δ11
wild-type GATTCAGAAACCTCTCAACTCATCTATGATGTCATCGAGCGTTCAGGTGGAATGGAGGCTCTTAAAAAGGAAATGAGCCAGCAGGGTAAG
wasp2 Δ11 GATTCAGAAACCTCTCAACTCATCTATGATGTCATCGAGCGTT----------GAGGCTCTTAAAAAGGAAATGAGCCAGCAGGGTAAG

Figure S4. The *wasp* mutant alleles. Related to Figure 6.

DNA sequences of *n-waspa* (A), *n-waspb* (B), *wasp1*(C) and *wasp2* (D) mutants are shown. On the sequencing chromatogram – arrow, mutation start site; blue underline, deleted sequence; and red underline, inserted sequence. In the sequence alignment – dashed line, deleted sequence; and green letters, inserted nucleotides.

Figure S5

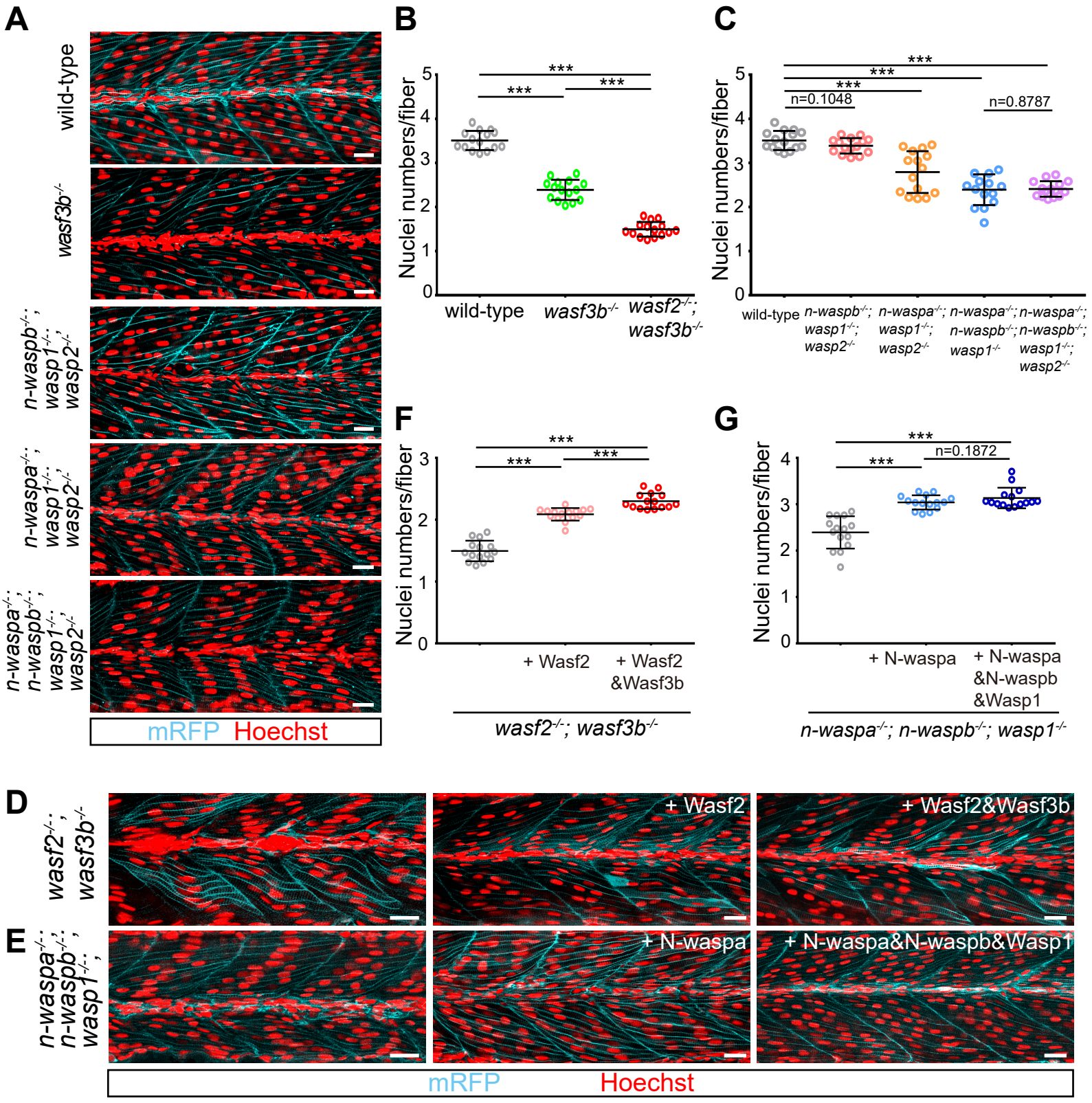

A
wild-type
wasf3b-/-
n-waspb-/-; wasp1-/-; wasp2-/-
n-waspa-/-; wasp1-/-; wasp2-/-
n-waspa-/-; n-waspb-/-; wasp1-/-; wasp2-/-
mRFP Hoechst
B
Nuclei numbers/fiber
***
***
***
wild-type
wasf3b-/-
wasf2-/-; wasf3b-/-
C
Nuclei numbers/fiber
***
***
***
n=0.1048
n=0.8787
wild-type
n-waspb-/-; wasp1-/-; wasp2-/-
n-waspa-/-; wasp1-/-; wasp2-/-
n-waspa-/-; n-waspb-/-; wasp1-/-
n-waspa-/-; n-waspb-/-; wasp1-/-; wasp2-/-
F
Nuclei numbers/fiber
***
***
***
+ Wasf2
+ Wasf2 &Wasf3b
wasf2-/-; wasf3b-/-
G
Nuclei numbers/fiber
***
***
n=0.1872
+ N-waspa
+ N-waspa &N-waspb &Wasp1
n-waspa-/-; n-waspb-/-; wasp1-/-
D
wasf2-/-; wasf3b-/-
+ Wasf2
+ Wasf2&Wasf3b
E
n-waspa-/-; n-waspb-/-; wasp1-/-
+ N-waspa
+ N-waspa&N-waspb&Wasp1
mRFP Hoechst

Figure S5. Zebrafish WAVE and WASP family members have redundant functions in myoblast fusion. Related to Figure 6.

(A) Myoblast fusion phenotype in *wave* and *wasp* mutant embryos. Confocal images of 48 hpf wild-type, *wasf3b*$^{-/-}$ single mutant, *n-waspb*$^{-/-}$*; wasp1*$^{-/-}$*; wasp2*$^{-/-}$ and *n-waspa*$^{-/-}$*; wasp1*$^{-/-}$*; wasp2*$^{-/-}$ triple mutant, and *n-waspa*$^{-/-}$*; n-waspb*$^{-/-}$*; wasp1*$^{-/-}$*; wasp2*$^{-/-}$ quadruple mutant embryos.

(B) Quantification of myoblast fusion defect in *wave* mutant embryos. Dot plot showing the average nuclei number per myofiber per embryo in wild-type (3.51 ± 0.22; 1254 myofibers from 15 embryos), *wasf3b*$^{-/-}$ single mutant (2.39 ± 0.23; 1374 myofibers from 15 embryos) and *wasf2*$^{-/-}$*; wasf3b*$^{-/-}$ double mutant (1.49 ± 0.17; 1359 myofibers from 15 embryos) embryos.

(C) Quantification of myoblast fusion defect in *wasp* mutant embryos. Dot plot showing the average nuclei number per myofiber per embryo in wild-type (3.51 ± 0.22; 1254 myofibers from 15 embryos), *n-waspb*$^{-/-}$*; wasp1*$^{-/-}$*; wasp2*$^{-/-}$ (3.39 ± 0.17; 1299 myofibers from 15 embryos), *n-waspa*$^{-/-}$*; wasp1*$^{-/-}$*; wasp2*$^{-/-}$ (2.79 ± 0.47; 1404 myofibers from 15 embryos), and *n-waspa*$^{-/-}$*; n-waspb*$^{-/-}$*; wasp1*$^{-/-}$ (2.39 ± 0.35; 1374 myofibers from 15 embryos) triple mutant, and *n-waspa*$^{-/-}$*; n-waspb*$^{-/-}$*; wasp1*$^{-/-}$*; wasp2*$^{-/-}$ quadruple mutant (2.41 ± 0.18; 1380 myofibers from 15 embryos) embryos.

(D) The fusion defect in *wasf2*$^{-/-}$*; wasf3b*$^{-/-}$ double mutant embryos was rescued by injecting mRNAs of Wasf2 alone or Wasf2&Wasf3b. Confocal images of 48 hpf un-injected, Wasf2-injected, or Wasf2&Wasf3b-injected *wasf2*$^{-/-}$*; wasf3b*$^{-/-}$ double mutant embryos are shown.

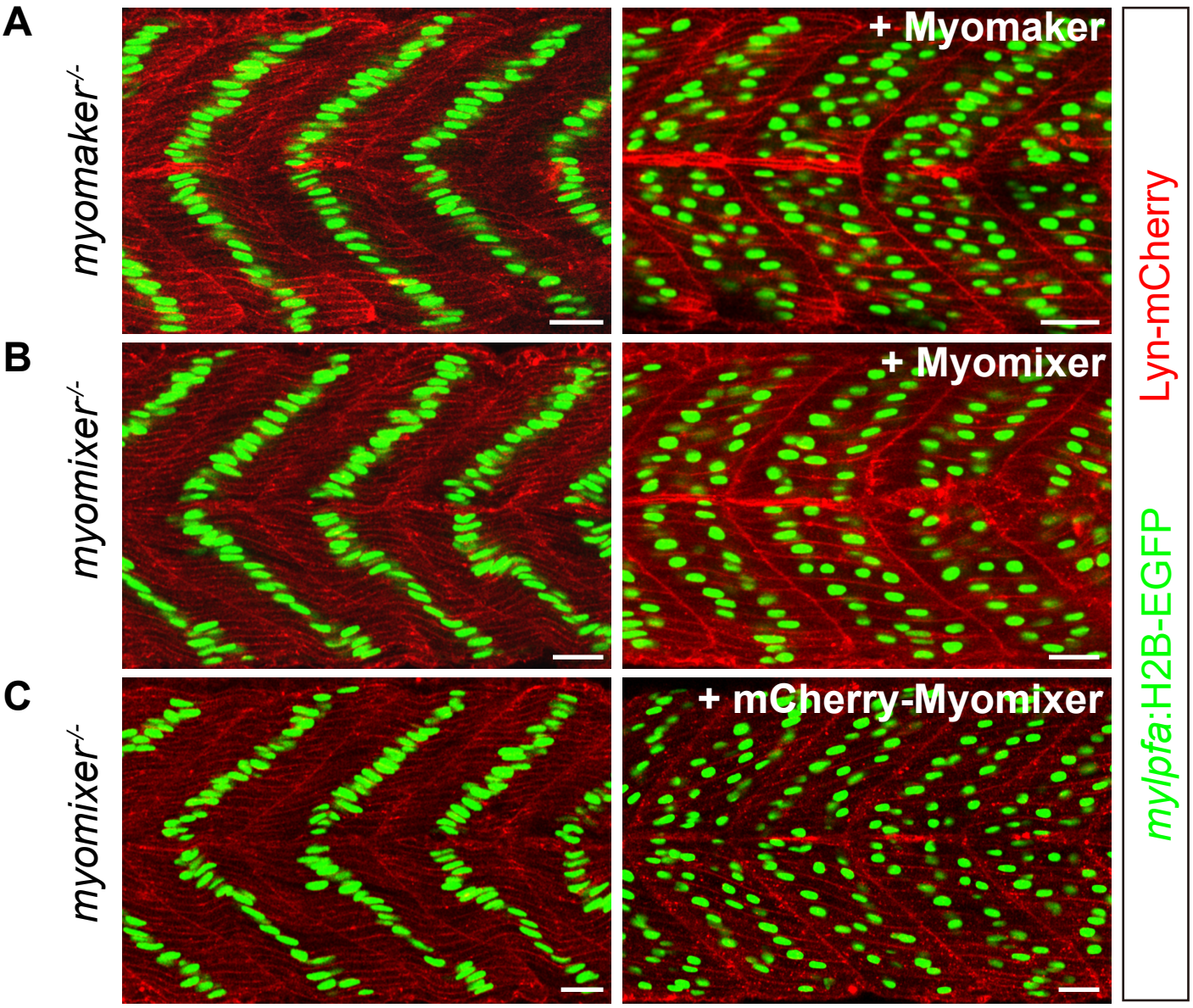
(E) The fusion defect in *n-waspa*$^{-/-}$*; n-waspb*$^{-/-}$*; wasp1*$^{-/-}$ triple mutant embryos was rescued by injecting mRNAs of N-waspa alone or N-waspa&N-waspb&Wasp1. Confocal images of 48 hpf un-injected, N-waspa-injected, or N-waspa&Nwaspb&Wasp1-injected *n-waspa*$^{-/-}$*; n-waspb*$^{-/-}$*; wasp1*$^{-/-}$ triple mutant embryos are shown. In (A), (D) and (E), embryos were stained with Hoechst to visualize the nuclei (red), and a membrane-targeted mRFP (cyan) was expressed to label the cell membrane. Scale bar, 25 μm.

(F) Quantification of the data shown in (D). Dot plot showing the average nuclei number per myofiber per embryo (in somites 10-12) in *wasf2*$^{-/-}$*; wasf3b*$^{-/-}$ double mutant (1.49 ± 0.17; 1359 myofibers from 15 embryos), Wasf2 mRNA-rescued (2.09 ± 0.10; 1839 myofibers from 15 embryos), and Wasf2&Wasf3b mRNA-rescued (2.30 ± 0.13; 1821 myofibers from 15 embryos) embryos.

(G) Quantification of the data shown in (E). Dot plot showing the average nuclei number per myofiber per embryo (in somites 10-12) in *n-waspa*$^{-/-}$*; n-waspb*$^{-/-}$*; wasp1*$^{-/-}$ triple mutant (2.39 ± 0.35; 1374 myofibers from 15 embryos), N-waspa mRNA-rescued (3.04 ± 0.15; 1620 myofibers from 15 embryos), and N-waspa&N-waspb&Wasp1 mRNA-rescued (3.13 ± 0.22; 1599 myofibers from 15 embryos) embryos.

In (B), (C), (F), and (G), each data point represents the mean value of an embryo. Mean ± s.d. values are shown in the dot plot, and significance was determined by the unpaired parametric t test. *** $p < 0.001$.

Figure S6


A
myomaker-/-
+ Myomaker
B
myomixer-/-
+ Myomixer
C
myomixer-/-
+ mCherry-Myomixer
Lyn-mCherry
mylpfa:H2B-EGFP

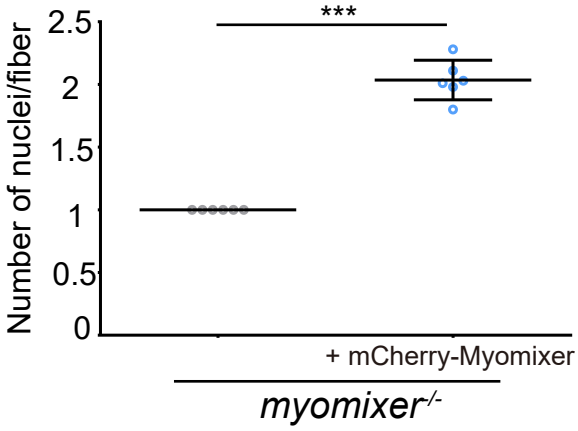

D
Number of nuclei/fiber
2.5
2
1.5
1
0.5
0
***
+ mCherry-Myomixer
myomixer-/-

Figure S6. The myoblast fusion defects in *myomaker*$^{-/-}$ and *myomixer*$^{-/-}$ mutants are rescued by mRNA injection. Related to Figure 7.

(A) The fusion defect in *myomaker*$^{-/-}$ mutant embryos was rescued by Myomaker mRNA injection. Confocal images of *myomaker*$^{-/-}$ mutant embryos uninjected (left) or injected with Myomaker mRNA (right).

(B) The fusion defect in *myomixer*$^{-/-}$ mutant embryos was rescued by Myomixer mRNA injection. Confocal images of *myomixer*$^{-/-}$ mutant embryos uninjected (left) or injected with Myomixer mRNA (right).

(C) The fusion defect in *myomixer*$^{-/-}$ mutant embryos was rescued by mCherry-Myomixer mRNA injection. Confocal images of *myomixer*$^{-/-}$ mutant embryos uninjected (left) or injected with mCherry-Myomixer mRNA (right).

In (A), (B) and (C), 48 hpf embryos are shown. Nuclei were labelled by Tg(*mylpfa*:H2B-EGFP) and cell membranes were labelled by Lyn-mCherry (red). Scale bar, 25 μm.

(D) Quantification of the results in (C). Dot plot showing the average nuclei number per myofiber per embryo (in somites 10-12) in *myomixer*$^{-/-}$ mutant embryos uninjected or injected with mCherry-Myomixer mRNA (2.04 ± 0.16; 485 myofibers from 6 embryos). Each data point represents the mean value of an embryo. Mean ± s.d. values are shown in the dot plot, and significance was determined by the unpaired parametric t test. *** indicates $p < 0.001$.