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Supplemental information

Noncanonical protease-activated receptor 1 regulates lymphatic differentiation in zebrafish

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Supplemental information

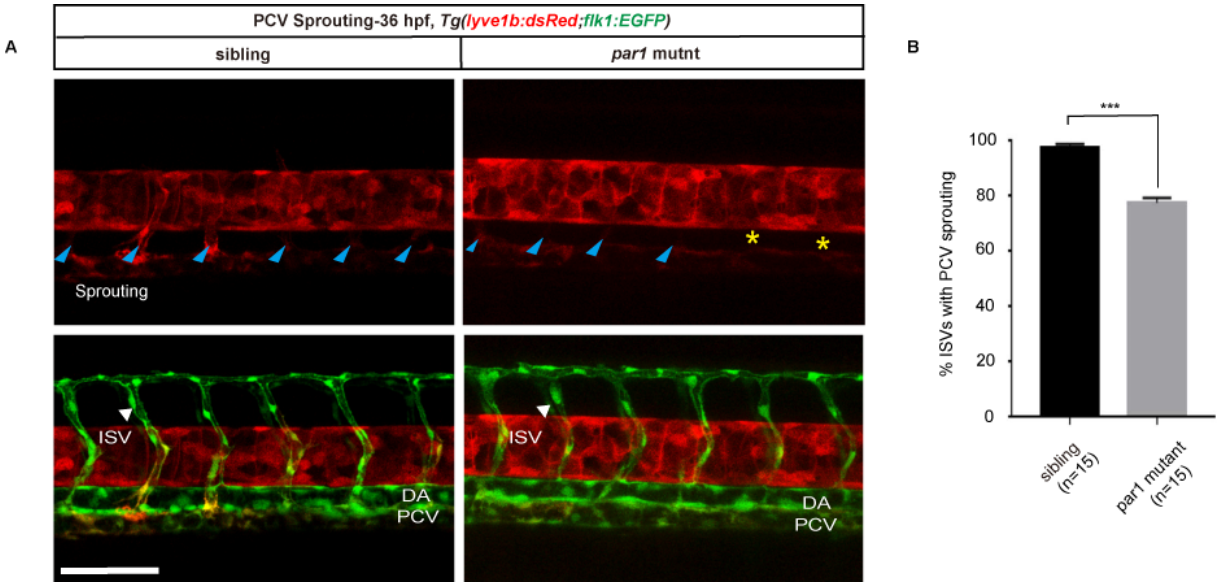

Figure S1. *par1* mutants show defective lympho-venous sprouting in zebrafish embryos, related to Figure 1.

(A) Confocal images showing lympho-venous sprouting in the posterior cardinal vein (PCV) area in sibling and *par1* mutants in *Tg(lyve1b:dsRed;flk1:EGFP)* line at 36 hpf. Blue arrowheads indicate lympho-venous sprouting from the PCV area in each intersegmental vessel (ISV); yellow asterisks represent a lack of lympho-venous sprouting from the PCV area in each ISV; the white arrowhead indicates the ISV, and the dorsal aorta (DA) and PCV areas are also noted. Scale bars: 100 μm. (B) Percentage of lympho-venous sprouting from the PCV in sibling (n = 15 embryos) and *par1* mutants (n = 15 embryos); 8 ISVs/embryo were used for quantification. In (B), values represent means ± SEMs. **P* ≤ 0.001 in the Student's t-test.

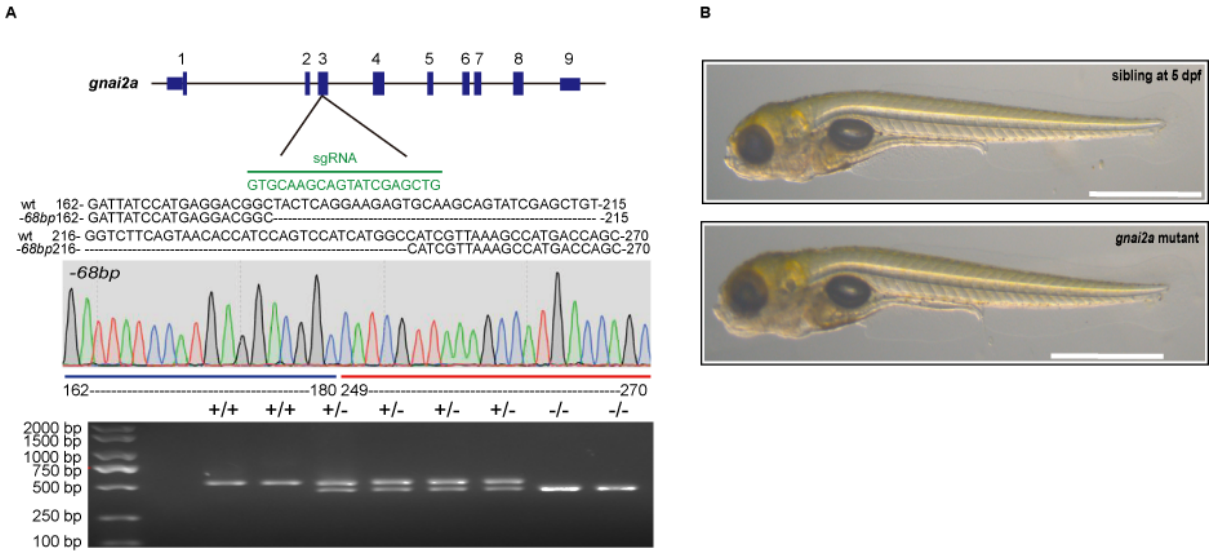

Figure S2. Generation of *gnai2a* zebrafish mutants, related to Figure 4.
(A) Top, schematic representation of the generated *gnai2a* zebrafish mutant (68 bp deletion in its exon 3); middle, results of sequencing for validating *gnai2a* mutants; bottom, DNA gel results for genotyping wildtype (+/+), heterozygous (+/-), and homozygous mutant (-/-) embryos. (B) Brightfield lateral views of siblings and *gnai2a* zebrafish mutants at 5 dpf. Scale bars: 1 mm.

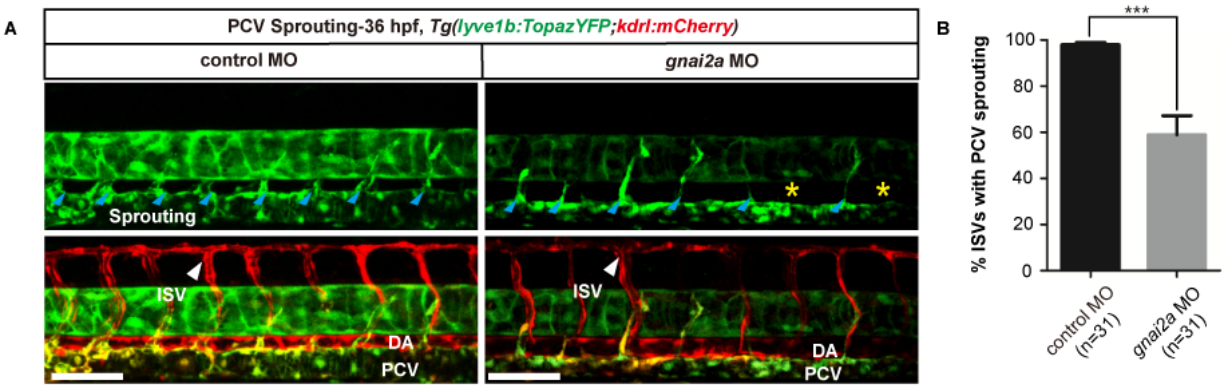

Figure S3. Knockdown of *gnai2a* by *gnai2a* MO impaired lympho-venous sprouting in zebrafish embryos, related to Figure 4.

(A) Confocal images showing lympho-venous sprouting in the PCV area in *Tg(lyve1b:TopazYFP;kdrl:mCherry)* injected with control MO and *gnai2a* MO at 36 hpf. Blue arrowheads indicate lympho-venous sprouting from the PCV area in each ISV; yellow asterisks represent the lack of lympho-venous sprouting from the PCV area in each ISV; the white arrowhead indicates the ISV, and DA and PCV areas are also noted. Scale bars: 100 μm. (B) Percentage of lympho-venous sprouting from the PCV in embryos injected with control MO (n = 31 embryos) or 4 ng *gnai2a* MO (n = 31 embryos); 8 ISVs/embryo were used for quantification. In (B), values represent means ± SEMs. **P* ≤ 0.001 in the Student's t-test.

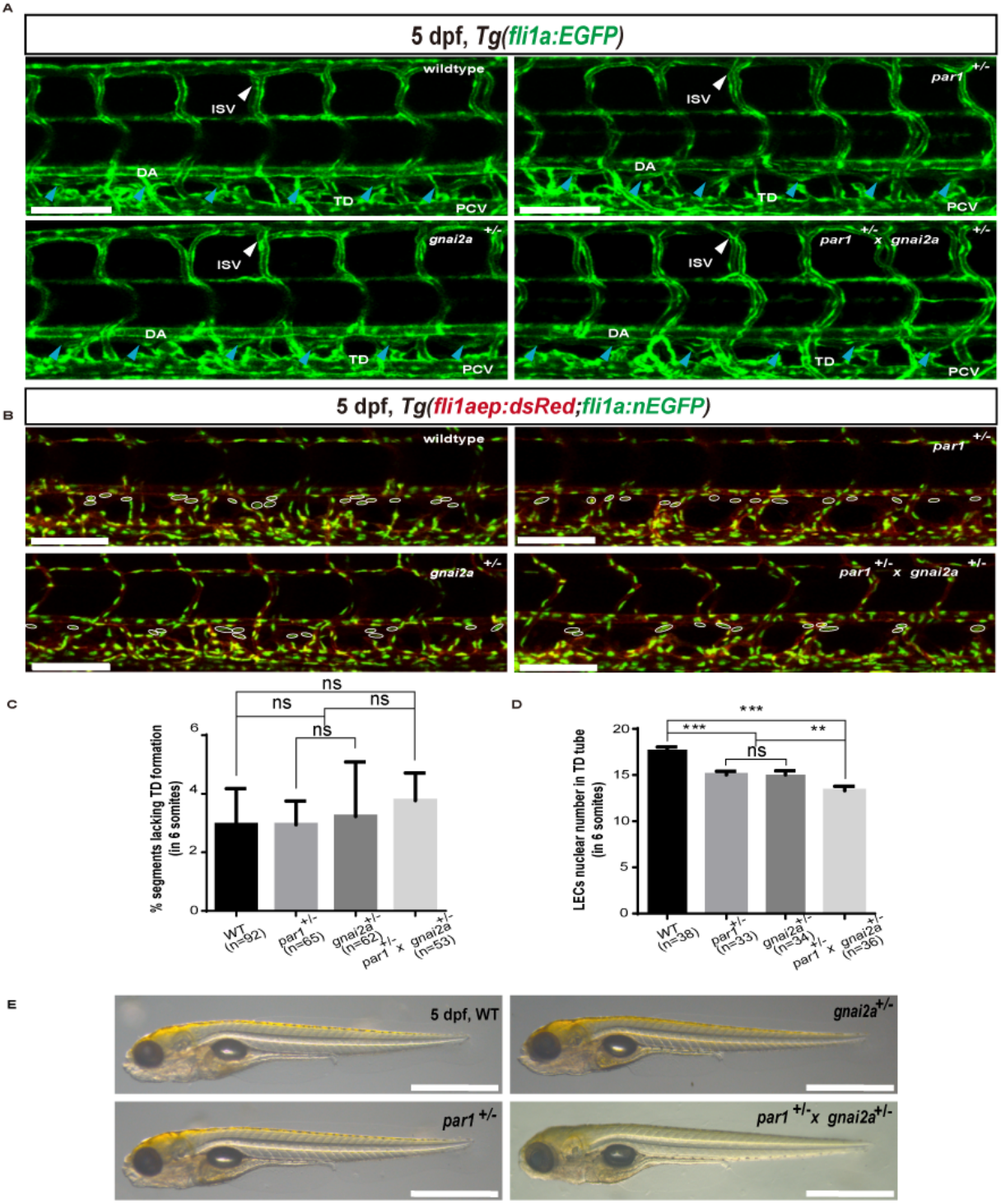

Figure S4. Genetic interaction analyses between *par1*$^{+/-}$ and *gnai2a*$^{+/-}$ zebrafish mutants, related to Figure 4.

(A) Confocal images showing TD formation of wildtype, *par1*$^{+/-}$ heterozygous mutants, *gnai2a*$^{+/-}$ heterozygous mutants, and *par1*$^{+/-}$ and *gnai2a*$^{+/-}$ double heterozygous mutants in the *Tg(fli1a:EGFP)* line at 5 dpf. Scale bars: 100 µm. (B) Confocal images showing LECs nuclear numbers in the TD tube of wildtype, *par1*$^{+/-}$ heterozygous mutants, *gnai2a*$^{+/-}$ heterozygous mutants, and *par1*$^{+/-}$ and *gnai2a*$^{+/-}$ double heterozygous mutants in the *Tg(fli1a:EGFP)* and *Tg(fli1aep:dsRed;fli1a:nEGFP)* lines at 5 dpf. White circles indicate the presence of LECs nuclear numbers in the TD tube. Scale bars: 100 µm. (C) Percentage of somites lacking TD formation in wildtype (n = 92), *par1*$^{+/-}$ heterozygous mutants (n = 65), *gnai2a*$^{+/-}$ heterozygous mutants (n = 62), and *par1*$^{+/-}$ and *gnai2a*$^{+/-}$ double heterozygous mutants (n = 53) in the *Tg(fli1a:EGFP)* line at 5 dpf; 6 somites/embryo were used for quantification. (D) Quantification of LECs nuclear number in the TD tube of wildtype (n = 38), *par1*$^{+/-}$ heterozygous mutants (n = 33),

gnai2a$^{+/-}$ heterozygous mutants (n = 34), and *par1*$^{+/-}$ and *gnai2a*$^{+/-}$ double heterozygous mutants (n = 36) in the *Tg(fli1aep:dsRed;fli1a:nEGFP)* line at 5 dpf; 6 segments/embryo were used for quantification. (E) Brightfield lateral views of morphology of wildtype, *par1*$^{+/-}$ heterozygous mutants, *gnai2a*$^{+/-}$ heterozygous mutants, and *par1*$^{+/-}$ and *gnai2a*$^{+/-}$ double heterozygous mutants at 5 dpf. Scale bars: 1 mm. In (C) and (D), values represent means ± SEMs. **P* ≤ 0.01, ***P* ≤ 0.001, ns, not significant in the Student's t-test.

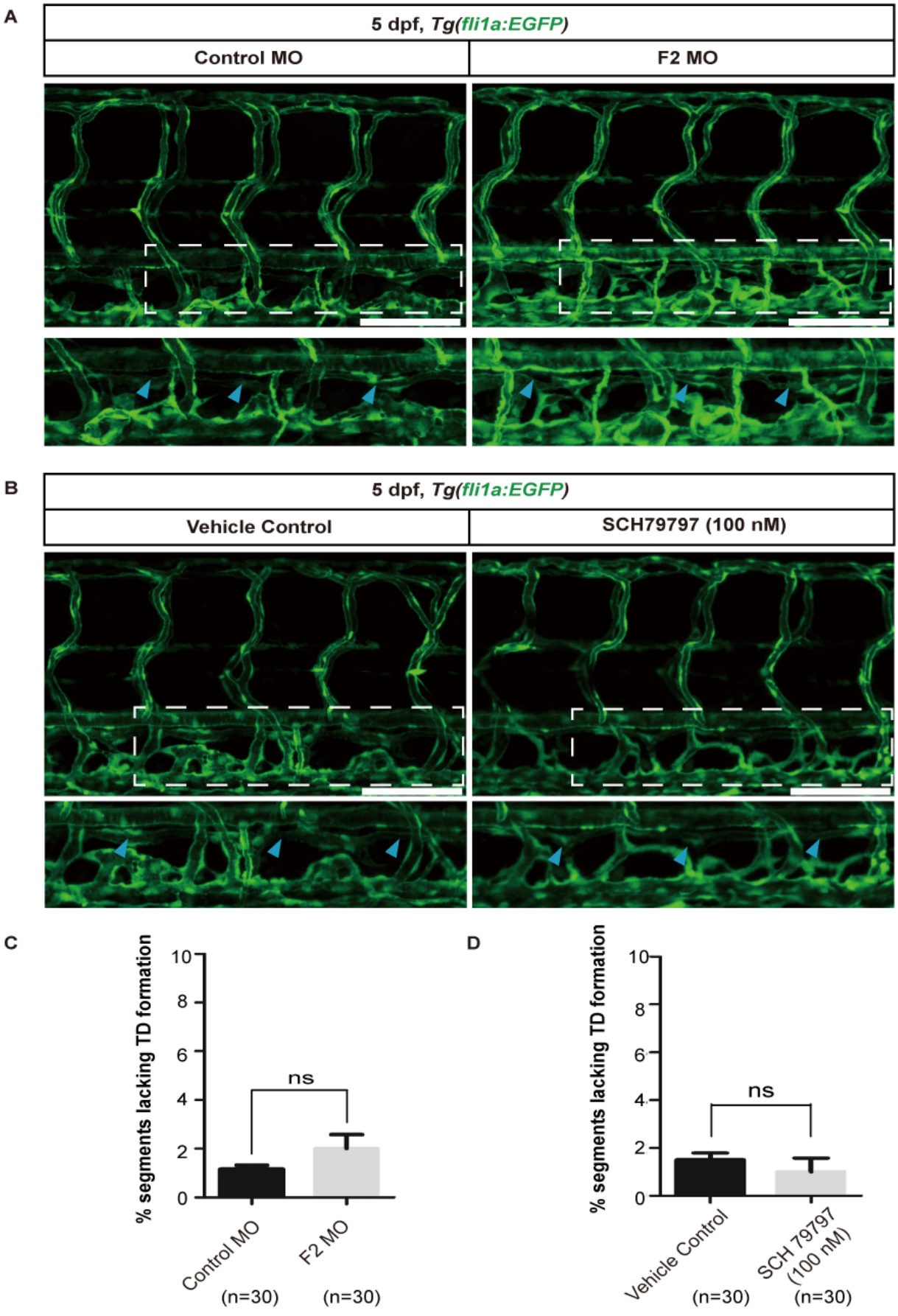

Figure S5. Classic Par1 ligand thrombin (F2) is not required for zebrafish lymphatic

development, related to Figure 5.
(A) Confocal images showing TD formation after injecting *Tg(fli1a:EGFP)* with 4 ng control MO and 4 ng F2 MO at 5 dpf. Blue arrowheads indicate TD formation in each somite. Scale bars: 100 μm. (B) Confocal images showing TD formation of the vehicle control group and SCH79797-treated group at 5 dpf. Blue arrowheads indicate TD formation in each somite. Scale bars: 100 μm. (C) Percentage of somites lacking TD formation in control embryos (n = 30 embryos) or F2 morphants (n = 30 embryos); 6 somites/embryo were used for quantification. (D) Percentage of somites lacking TD formation in embryos treated with vehicle control (n = 30 embryos) or 100 nM SCH79797 (n = 30 embryos); 6 somites/embryo were used for quantification. In (C) and (D), values represent means ± SEMs. ns, not significant in the Student's t-test.

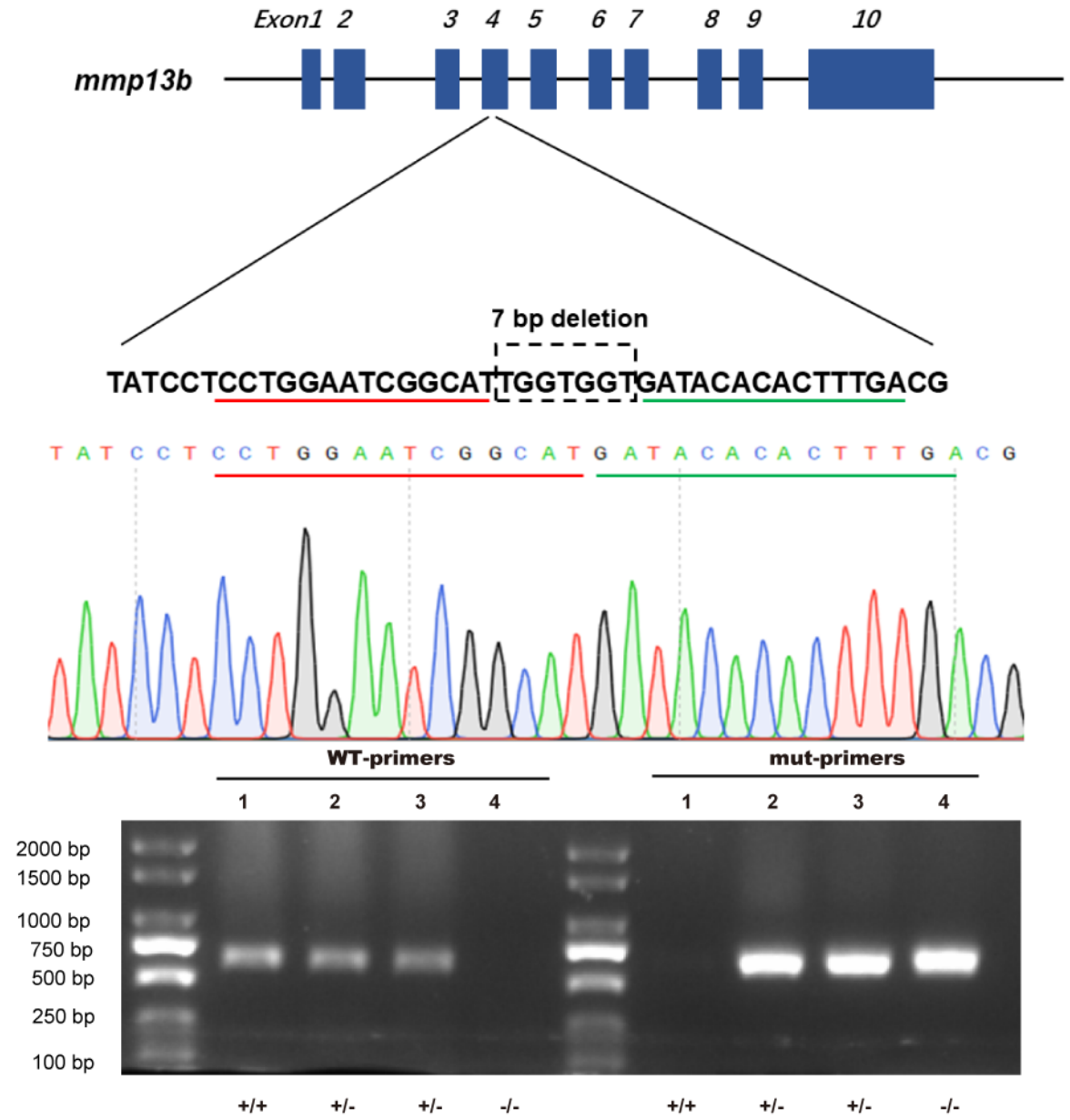

Figure S6. Generation of *mmp13b* zebrafish mutant, related to Figure 5.
Top, schematic representation of the generated *mmp13b* zebrafish mutant (7 bp deletion in its exon 4); middle, results of sequencing for validating *mmp13b* mutants; bottom, DNA gel results for genotyping wildtype (+/+), heterozygous (+/-), and homozygous mutant (-/-) embryos.

Table S1. Sequence information in this study, related to STAR Methods.

Gene	Purpose	Sequence
par1 (zebrafish)	Primers for mutant genotyping	F: 5'-GAGCCGTTTGATTATCTGGACG-3' R: 5'-CGGCTCCGTATATCCAGTTG-3'
	Probe for WISH	F: 5'-TTCTGTGCTGCTGATTATGT-3' R: 5'-CTTGTGCTGGAGGTGAAC-3'
gnai2a (zebrafish)	Primers for mutant genotyping	F: 5'-ATGCACGCCGCTAAATATTTCATA-3' R: 5'-AGGCTGTGGTTTTCCAAATTCAG-3'
	Probe for WISH	F:5'-GTCTTCAGTAACACCATCCA-3' R: 5'-CCTCATCCTCAGCCAGTA-3'
gnai2b (zebrafish)	Primers for WISH	F: 5'-GAGCCGTGGTTTACAGCAAC-3' R: 5'-CCTGCTGGGTGGGAATGTAG-3'
mmp13b (zebrafish)	Primers for mutant genotyping	WT-F: 5'-CGGCATTGGTGGTGATACAC-3' R: 5'-TGTGGTGTATGAGGACATGTGTTA-3' MU-F: 5'-AATCGGCATGATACACACT-3' R: 5'-TGTGGTGTATGAGGACATGTGTTA-3'
	Probe for WISH	F: 5'-CGACATTGAGGGCATCCAGT-3' R: 5'-GAACGACTTTCCTTGCGCTG-3'
flt4 (zebrafish)	Probe for WISH	F: 5'- AGTCAAGTGCGACGGATGAT-3' R: 5'- ACCATCCCACTGTCTGTCTG-3'
PAR1 (human)	Primers for qPCR	F: 5'-CCAGTGAGGACAGATGCAGA-3' R: 5'-GCAGTGGCACCATCCAA-3'
GNA11 (human)	Primers for qPCR	F: 5'-GTCTACCAGAACATCTTCACCG-3' R: 5'-GTACTGATGCTCGAAGGTGG-3'
GNAQ (human)	Primers for qPCR	F: 5'-ATCAGAACATCTTCACGGCC-3' R: 5'-AAAGCAGACACCTTCTCCAC-3'
GNA13 (human)	Primers for qPCR	F: 5'-GTCGAGAATTTCAACTGGGTG-3' R: 5'-CAAAGTCGTATTCATGGATGCC-3'
GNA12 (human)	Primers for qPCR	F: 5'-AAGTCCACGTTCCTCAAGC-3' R: 5'-CCAAGGAATGCCAAGCTTATC-3'
GNAI2 (human)	Primers for qPCR	F: 5'-ATGGACTGGATGGTGTTGCT-3' R: 5'-GGAGGTGAAGTTGCTGCTGT-3'
GNAI1 (human)	Primers for qPCR	F: 5'-TCTGAATAACCAGCTTCATGGAT-3' R: 5'-GGAGCGGAGTAAGATGATCG-3'
GNAI3 (human)	Primers for qPCR	F: 5'-CATCCTCTGAATAGCCATCCTC-3' R: 5'-AAGATGATCGACCGCAACTT-3'
MMP1 (human)	Primers for qPCR	F: 5'-GCACAAATCCCTTCTACCCG-3' R: 5'-TGAACAGCCCAGTACTTATTCC-3'
MMP13 (human)	Primers for qPCR	F: 5'-GATGACGATGTACAAGGGATCC-3' R: 5'-ACTGGTAATGGCATCAAGGG-3'
APC (human)	Primers for qPCR	F: 5'-ACGACAGCTTTTACAGTCCC-3' R: 5'-TTGAACCCTGACCATTACCAG-3'
F2 (human)	Primers for qPCR	F: 5'-ATGTCTGGAAGGTAACTGTGC-3' R: 5'-CAGGATGGGTAGTGGAGTTG-3'
GZMA (human)	Primers for qPCR	F: 5'-AACTCCTATAGATTTCTGGCATCC-3' R: 5'-CCATGTAGGGTCTTGAATGAGG-3'
PLG (human)	Primers for qPCR	F: 5'-GAAGACCCCAGAAAACTACCC-3' R: 5'-TTTCAGGTTGCAGTACTCCC-3'

PROX1 (human)	Primers for qPCR	F: 5’-GGCATTGAAAAACTCCCGTA-3’ R: 5’-ACAGGGCTCTGAACATGCAC-3’