

Supplemental Table S1. gRNA targets and verification primers used to amplify the target region.

Primer name	Sequence
<i>vwa1</i> gRNA Target1 F	TAATACGACTCACTATAGGCTCACATGATCTGGGCCCCTTTAGAGCTAGAAATAGC
Target1 verification primer-F	GTTGAATTGCTGTGAAGGCGA
Target1 verification primer-R	CAGTAGGCGGACTCACGAC
<i>vwa1</i> gRNA Target2 F	TAATACGACTCACTATAGGTCGTGAGTCCGCCTACTGGTTTTAGAGCTAGAAATAGC
Target2 verification primer-F	TACGAGTTCTCCCGCATGGT
Target2 verification primer-R	ACCCACCAATGATTGAGTTCC
<i>vwa1</i> gRNA Target3 F	TAATACGACTCACTATAGGGATCTGTCCCGTCTCATGTTTTAGAGCTAGAAATAGC
Target3 verification primer-F	CATCCGCGCTGAGAGACTG
Target3 verification primer-R	TGTGTATGTGCTACTCACCTGT
<i>vwa1</i> gRNA Target4 F	TAATACGACTCACTATAGGCACTCGAAGCGGGCCACTGTTTTAGAGCTAGAAATAGC
Target4 verification primer-F	CAGATCCAGCTGTTGTCCTCTT
Target4 verification primer-R	GTGTATTAATGTGAACTCTCCTCAC
<i>vwa1</i> gRNA R	AAAAAAAGCACCGACTCGGTGCCAC

Supplemental Table S2. Primers used in RT-qPCR of *vwa1*, *fgfr1a*, *fgfr2*, *fgfr3*, *fgfr4*, *fgf8a*, *fgf8b*, and *runx2a*.

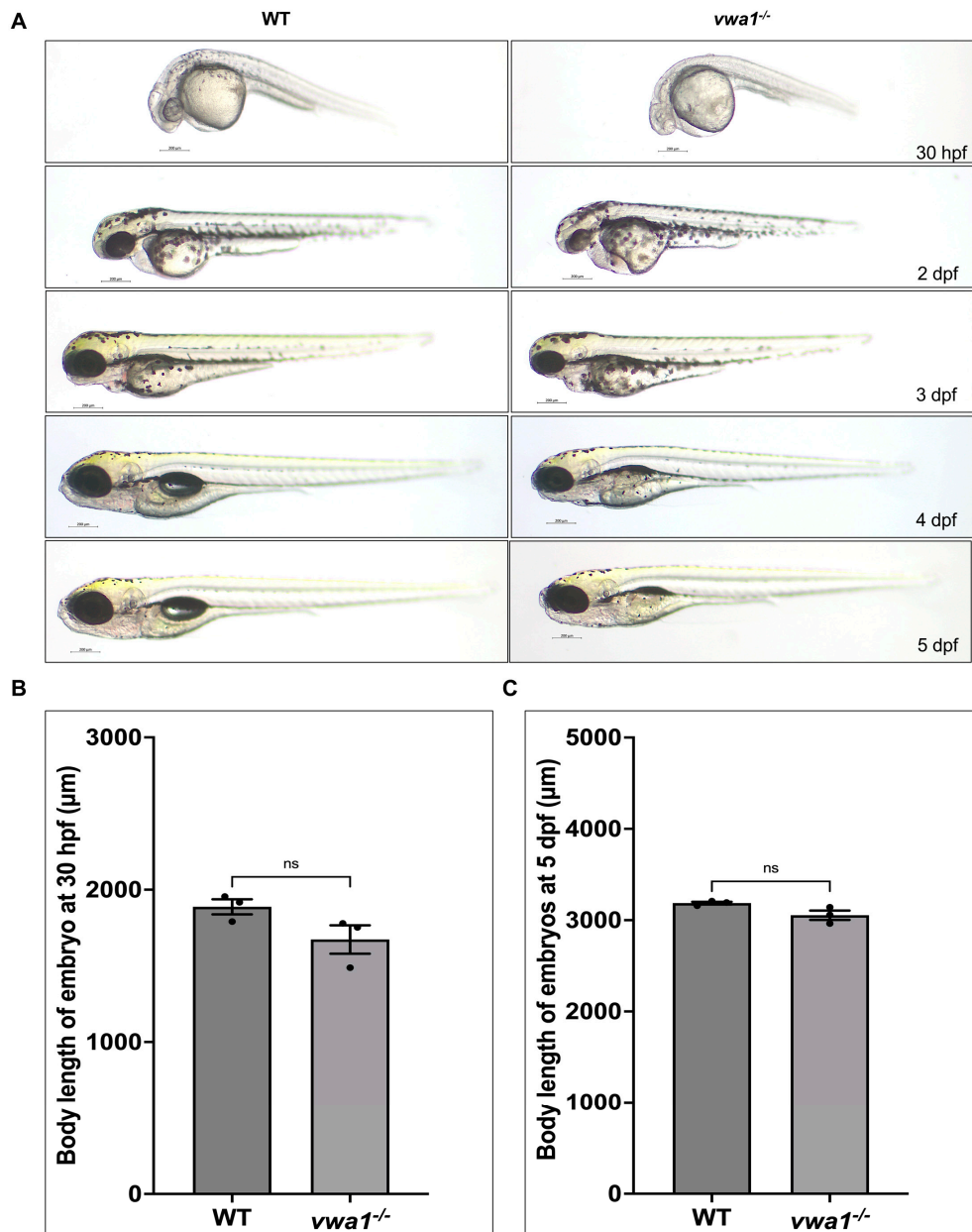
Primer name	Sequence
<i>vwa1</i> qPCR F	CATCATCGGCGAAGACCTGA
<i>vwa1</i> qPCR R	GATCTGTCCCGTCTCATGG
<i>fgfr1a</i> qPCR F	CGGAAACTCTATCGGCCACT
<i>fgfr1a</i> qPCR R	GGAGTCCACAGACACTGTTACC

<i>fgfr2</i> qPCR F	ATAGGTGTGTTTCTGATCGCCT
<i>fgfr2</i> qPCR R	TGACGACACTGTTACCTGGC
<i>fgfr3</i> qPCR F	AGTAGTTCTGTCCTGCACGC
<i>fgfr3</i> qPCR R	CCTGCCTCGTCCTCATCTTC
<i>fgfr4</i> qPCR F	CTCTTGGACGTGTTGGAACG
<i>fgfr4</i> qPCR R	GGAATGCCATCAGGCCCAT
<i>fgf8a</i> qPCR F	GCCGTAGACTAATCCGGACC
<i>fgf8a</i> qPCR R	TGGCTTCACTCTTCAACGCT
<i>fgf8b</i> qPCR F	TGAGGCTGAAATCATCGAGGT
<i>fgf8b</i> qPCR R	GTTTACCGCTGGTTCGGCTA
<i>runx2a</i> qPCR F	CAACTTTCTGTGCTCGGTGC
<i>runx2a</i> qPCR R	GCGGTGGGTTCGTGAATACT
<i>actb1</i> qPCR F	ACCACGGCCGAAAGAGAAAT
<i>actb1</i> qPCR R	ATGTCCACGTCGCACTTCAT

Supplemental Table S3. Primers used in the cloning of *crestin*, *dlx2a*, *tbx1*, *barx1*, *nkx2.3*, *fgf8a*, *fgfr2*, *fgfr3*, *sox9a*, and *col2a1a* for RNA probe synthesis before whole-mount in situ hybridization.

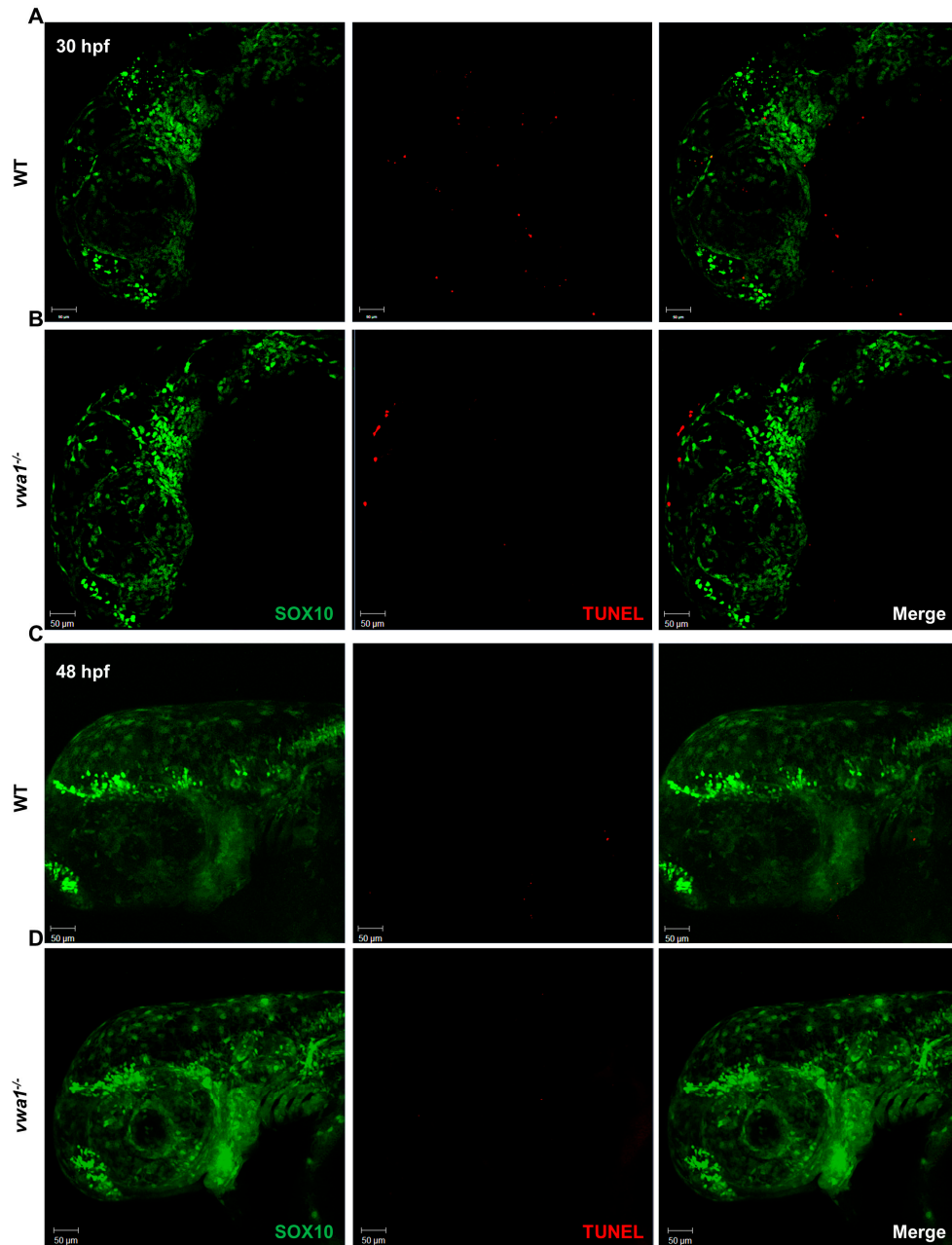
Primer name	Sequence
<i>crestin</i> in situ F	GAGAAGCCCTCATCAGAGAGTTTG
<i>crestin</i> in situ R	GTTGCTTGTCTCAGGCAGAATCAGG
<i>dlx2a</i> in situ F	CACAGTTCTGCTTTGCGTCG
<i>dlx2a</i> in situ R	CCCAAGTCGGCAGAGTCAAA
<i>tbx1</i> in situ F	GCAGCTGTCCATTTTTCG
<i>tbx1</i> in situ R	ACGGCGGTAAATCTGGTCTC
<i>barx1</i> in situ F	CTGGGCGGATCAGACTTCTC
<i>barx1</i> in situ R	GCTTCTCGTGTCTCTCTCTG
<i>nkx2.3</i> in situ F	TCGTGTTTTCTCGGAGGTGG
<i>nkx2.3</i> in situ R	GCGCATTAGTGGACGTGTTC
<i>sox9a</i> in situ F	CCTCGACCCCTACCTGAAGA
<i>sox9a</i> in situ R	GGCGGGAGGTATTGGTCAAA
<i>col2a1a</i> in situ F	TCTGAAGTCCATCAACGGGC
<i>col2a1a</i> in situ R	TTTTCCGTCACGCTAAACGC

<i>fgf8a</i> in situ F	GCCGTAGACTAATCCGGACC
<i>fgf8a</i> in situ R	TGGCTTCACTCTTCAACGCT
<i>fgfr2</i> in situ F	GACCCTGATCATGGAGAGCG
<i>fgfr2</i> in situ R	TGACGACACTGTTACCTGGC
<i>fgfr3</i> in situ F	CGCAAGACTTCCTCCTTCCC
<i>fgfr3</i> in situ R	AGTGTGCTTGATTGTCCCGT



Supplemental Figure S1. Comparison of the body sizes of *vwa1*^{-/-} mutants and wildtype controls from 30 hpf to 5 dpf. **(A)** Light microscopy demonstrated some *vwa1*^{-/-} mutants had shorter body lengths and smaller head circumferences than the wildtype control (WT). **(B, C)** There was no statistical size difference in body length

between mutants and WT at 30 hpf and 5 dpf, although the average body length of the mutants was slightly shorter than WT.



Supplemental Figure S2. Apoptosis did not significantly increase in *vwa1*^{-/-} mutants at 30 and 48 hpf. (A, B) TUNEL assay (red fluorescence) at 30 hpf demonstrated similar amount of apoptosis cells in the cranial region and dorsal tissues of wildtype controls (WT) and mutants. (C, D) TUNEL assay (red fluorescence) at 48 hpf showed similar level of apoptosis in WT and mutants