Supplementary material

Tmc proteins are essential for zebrafish hearing where Tmc1 is not obligatory

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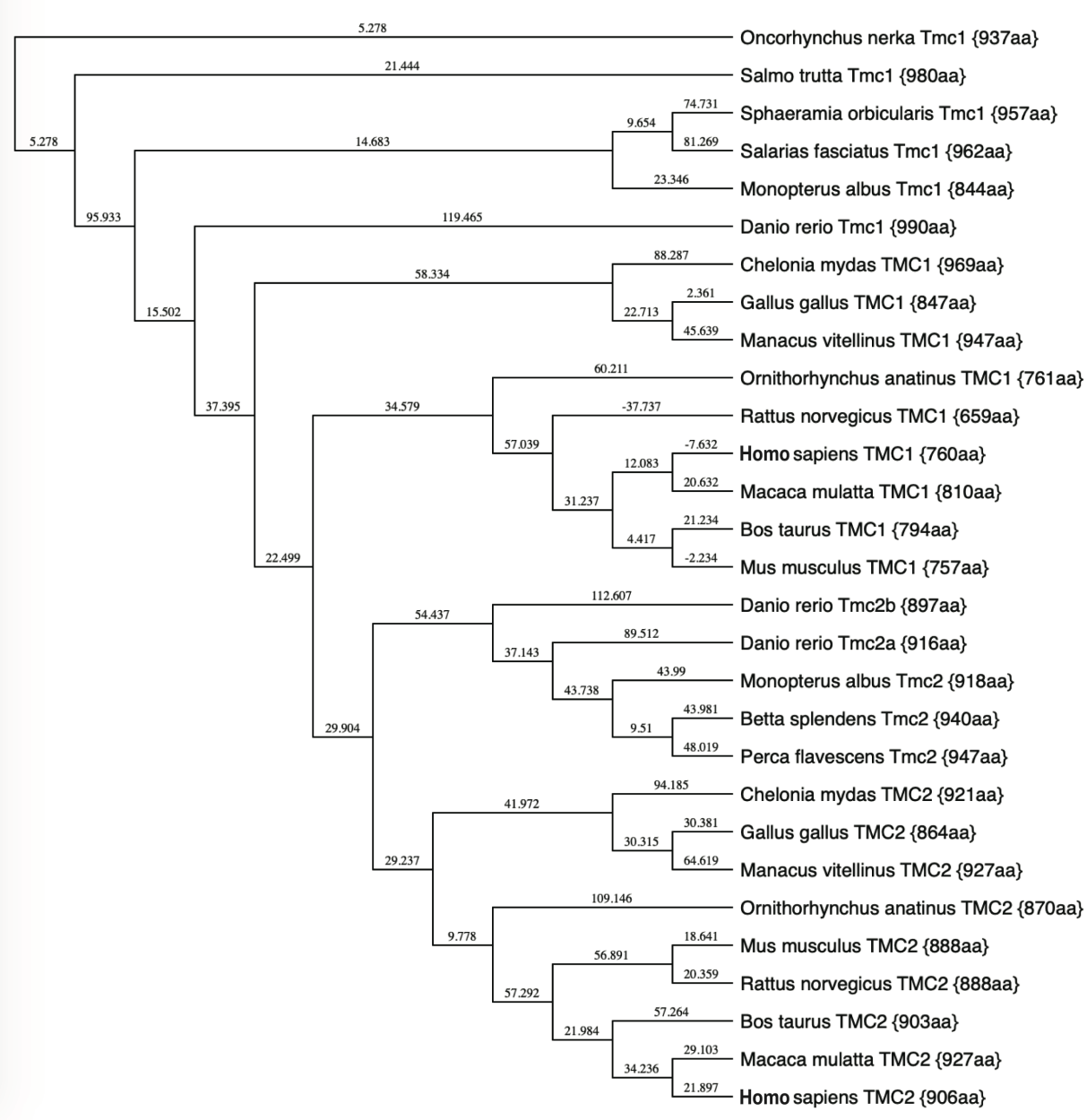

Fig. S1. A phylogenetic tree of zebrafish Tmc1, Tmc2a, Tmc2b, and other relevant vertebrate orthologous proteins using ClustalW (neighbor joining tree with absolute number difference). GenBank accession numbers are as follows: zebrafish (*Danio rerio*: Tmc1, NP_001299610.1; Tmc2a, NP_001289166.1; Tmc2b, NP_001289152.1), mouse (*Mus musculus*: TMC1, NP_083229.1; TMC2, NP_619596.1),

human (*Homo sapiens*: TMC1, NP_619636.2; TMC2, NP_542789.2), cattle (*Bos taurus*: TMC1, XP_024851786.1; TMC2, NP_001107238.1), green sea turtle (*Chelonia mydas*: TMC1, XP_007065675.1; TMC2, XP_007070749.2), chicken (*Gallus gallus*: TMC1, NP_001006580.1; TMC2, NP_001034413.1), rhesus macaque (*Macaca mulatta*: TMC1, XP_014973432.1; TMC2, XP_015004873.2), golden-collared manakin (*Manacus vitellinus*: TMC1, XP_017941330.1; TMC2, XP_017930609.2), swamp eel (*Monopterus albus*: Tmc1, XP_020446228.1; Tmc2, XP_020470803.1), platypus (*Ornithorhynchus anatinus*: TMC1, XP_028911690.1; TMC2, XP_028927221.1), rat (*Rattus norvegicus*: TMC1, NP_001101991.1; TMC2, NP_001099980.1), sockeye salmon (*Oncorhynchus nerka*: Tmc1, XP_029534853.1), brown trout (*Salmo trutta*: Tmc1, XP_029617773.1), blenny (*Salarias fasciatus*: Tmc1, XP_029960010.1), cardinalfish (*Sphaeramia orbicularis*: Tmc1, XP_029999672.1), yellow perch (*Perca flavescens*: Tmc2, XP_028457984.1), and Siamese fighting fish (*Betta splendens*: Tmc2, XP_029025885.1).

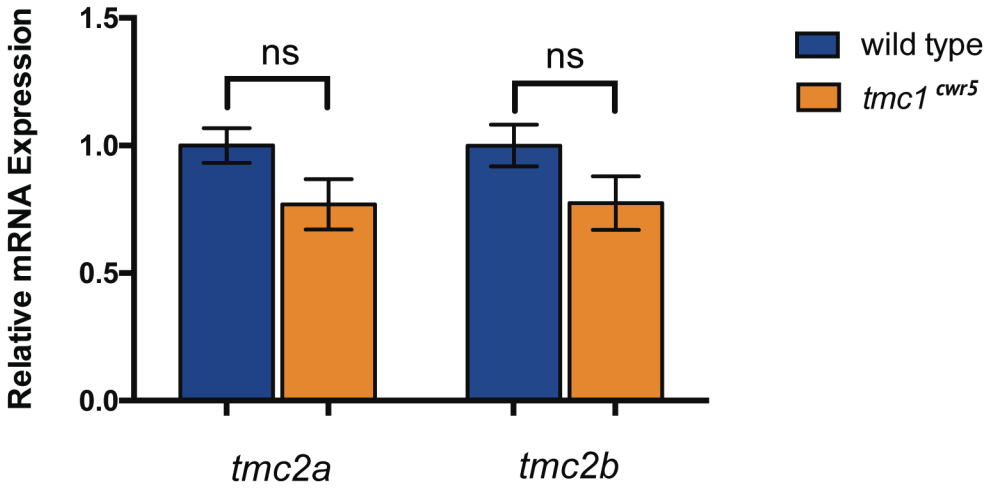

Fig. S2. Relative *tmc2a* and *tmc2b* mRNA levels in the *tmc1* ^{cwr5} mutant. Quantitative RT-PCR analyses for *tmc2a* and *tmc2b* mRNAs from 6-dpf larvae. Unpaired Student's *t*-test, P = 0.096 and 0.13 for *tmc2a* mRNA and *tmc2b* mRNA, respectively (n = 5).

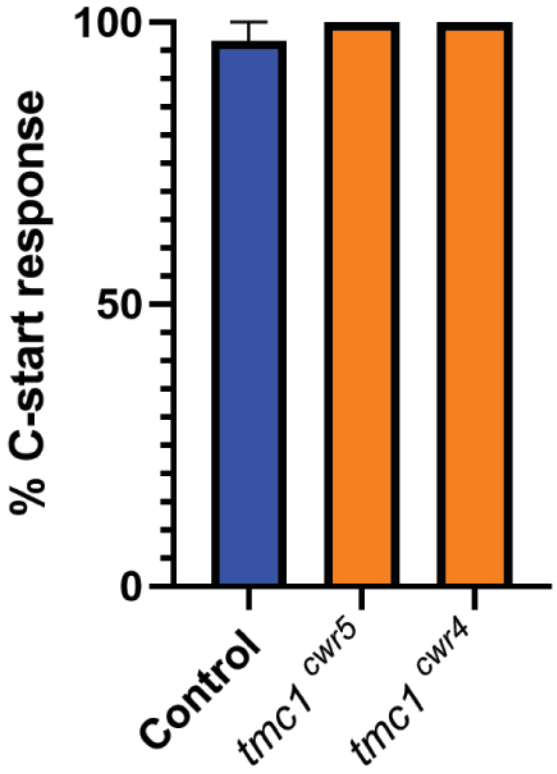

Fig. S3. Graph of the mean percentages of adult zebrafish that exhibit a C-start response when presented with vibrational stimuli ± SEM. Each fish was presented with 10 vibrational stimuli. All mutants responded to all stimuli. $n_{control\ (wild\ type)} = 3$, $n_{tmc1\ ^{cwr5}} = 5$, $n_{tmc1\ ^{cwr4}} = 1$; n = number of fish.

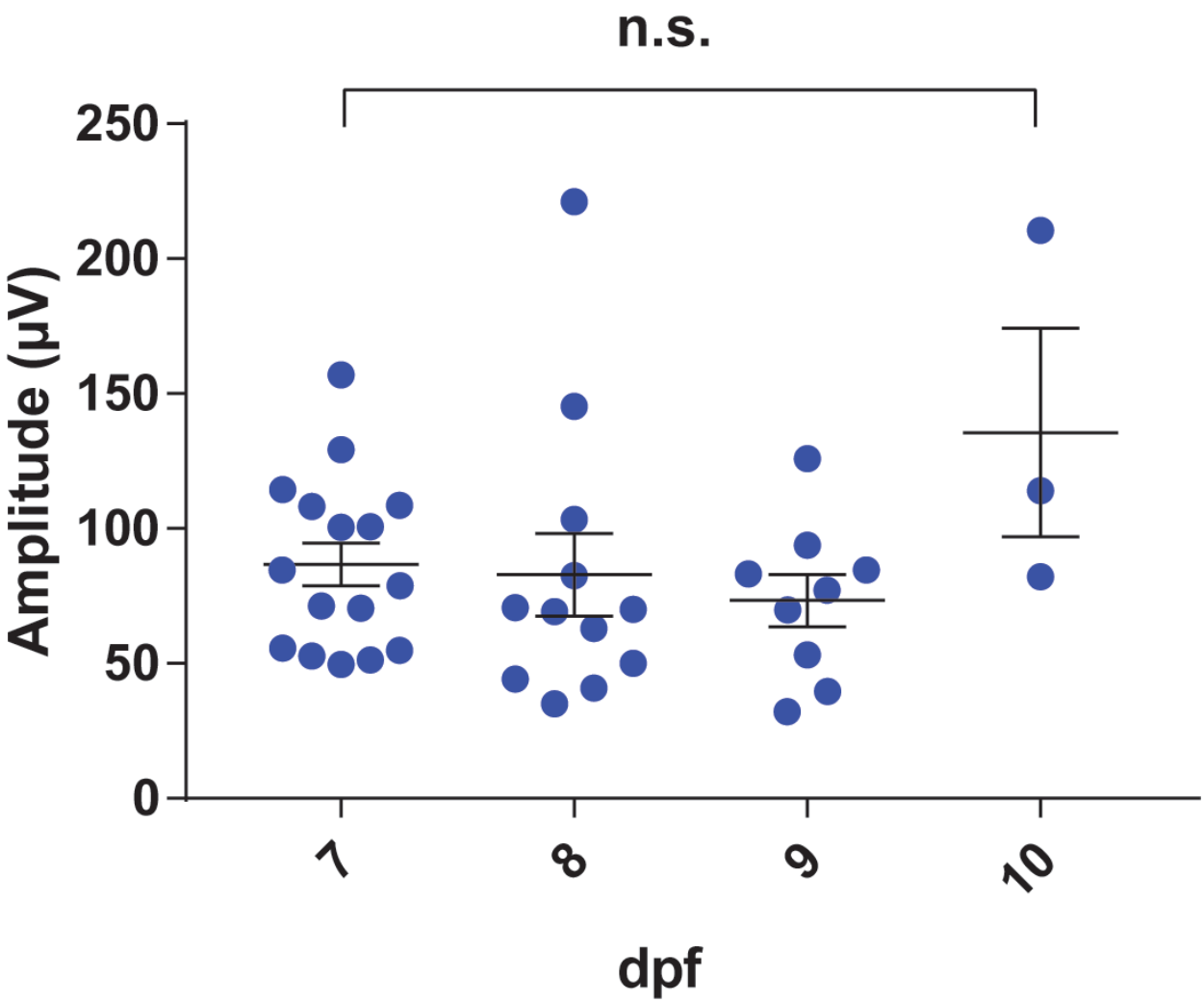

Fig. S4. Mean microphonic potential amplitudes of wild-type fish by age of fish. The mean amplitudes of microphonic potentials ± SEM are 86.8 ± 7.8 μV for fish at 7 dpf (n = 16), 83.0 ± 15.3 μV for fish at 8 dpf (n = 12), 73.3 ± 9.6 μV for fish at 9 dpf (n = 9), and 135 ± 38.6 μV for fish at 10 dpf (n = 3). One-way ANOVA, P = 0.18.

Movie descriptions:

Movie 1. Startle reflex of a *tmc1* ^{cwr5} larva.

Movie 2. Startle reflex of a *tmc1* $^{cwr5/+}$ control larva.

Movie 3. Startle reflex of a *tmc1* ^{cwr4} larva.

Movie 4. Startle reflex of a wild-type control adult.

Movie 5. Startle reflex of a *tmc1* ^{cwr4} adult.

Movie 6. Startle reflex of a *tmc2b* ^{cwr2} *tmc2a* ^{cwr3} double-mutant larva with no response to a stimulus.

Movie 7. A *tmc2b* ^{cwr2} *tmc2a* ^{cwr3} double-mutant larva with a response to a stimulus.

The same fish from **Movie 6** was used in this movie, demonstrating the inconsistency in responses of the double mutant to provided stimuli.

Movie 8. A *tmc2b* ^{cwr8} *tmc1* ^{cwr4} *tmc2a* ^{cwr6} triple-mutant larva lacks a response to a stimulus.