**Structure of *C. elegans* TMC-1 complex illuminates auditory mechanosensory transduction**

Hanbin Jeong[1*], Sarah Clark[1*], April Goehring[1,2], Sepehr Dehghani-Ghahnaviyeh[3], Ali Rasouli[3], Emad, Tajkhorshid[3] and Eric Gouaux[1,2]

1. Vollum Institute, Oregon Health & Science University, Portland, Oregon 97239, USA.

2. Howard Hughes Medical Institute, Oregon Health & Science University, Portland, Oregon 97239, USA.

3. Theoretical and Computational Biophysics Group, NIH Center for Macromolecular Modeling and Bioinformatics, Beckman Institute for Advanced Science and Technology, Department of Biochemistry, and Center for Biophysics and Quantitative Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA.

*These authors made equal contributions.

Correspondence to Eric Gouaux: gouauxe@ohsu.edu

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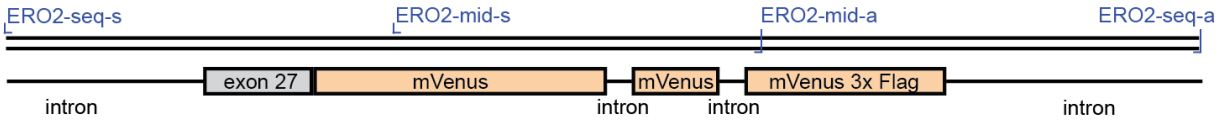
Supplementary Figures 1-3

**a**




**b** syb2173 (*tmc1::mVenus*)

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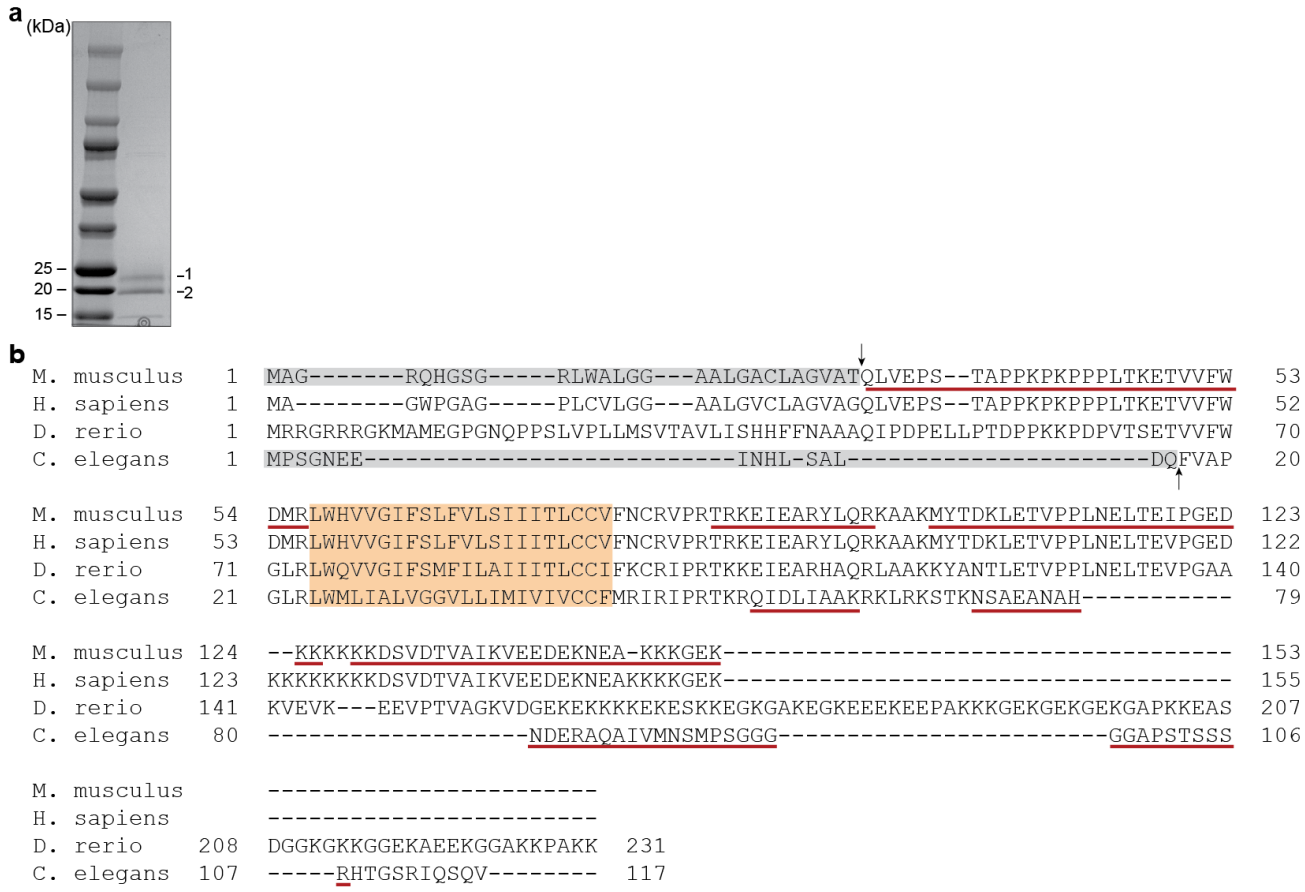
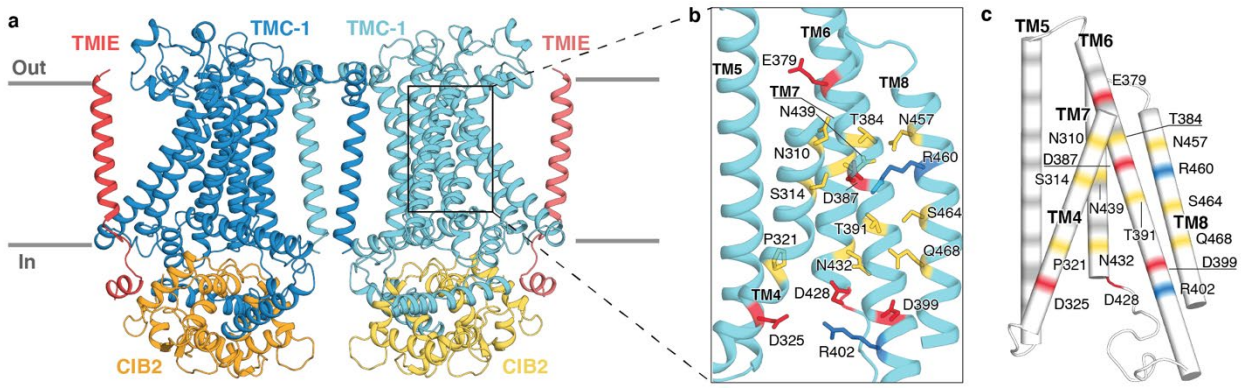
**Supplementary Fig. 1. *tmc-1::mVenus* strain. a**, Schematic diagram of *tmc-1::mVenus* strain. Location of the PCR and sequencing primers relative to the insertion site of 3C precision protease site-mVenus-3xFLAG prior to the stop codon of *tmc-1*in exon 27. **b,** Sequence of the *tmc-1::mVenus* strain. Introns are in lower case and exons in upper case letters. The 3C precision protease site-mVenus-3xFLAG insert is highlighted in orange. Primer annealing sites for PCR amplification and sequencing of insert are labeled with blue lines.




**Supplementary Fig. 2. N-terminal sequence of mouse TMIE. a,** Representative Coomassie staining of sodium dodecyl sulfate-polyacrylamide gel electrophoresis of recombinantly expressed mouse TMIE. Upper and lower bands for mouse TMIE sample submitted for N-terminal sequencing and LC-MS/MS are indicated as 1 and 2, respectively. **b,** Results from N-terminal sequencing of mouse TMIE and mass spectrometry for both mouse and *C. elegans* TMIE. The cleavage site for both mouse TMIE bands was identical and is indicated with an arrow. The identified peptides for both mouse and *C. elegans* TMIE are underlined in red and the signal peptide and transmembrane domain are highlighted in grey and orange, respectively. The peptides that were identified for mouse TMIE were the same in both the upper and lower bands, suggesting the difference in the upper and lower bands is not due to N-terminal or C-terminal cleavage and is most likely due to post translational modification.




**Supplementary Fig. 3. Residue composition of the putative ion conduction pathway of the human TMC-1 complex homology model. a,** Homology model of human TMC-1 complex: TMC-1 (dark blue and light blue), CIB2 (orange and yellow), and TMIE (red and pink). **b,** An expanded view of the putative ion conduction pathway, highlighting pore-lining residues. Polar (yellow), acidic (red), and basic (blue) residues are shown as sticks. **c**, Electrostatic potential of pore-lining residues are depicted in different colors: grey = nonpolar, yellow = polar, red = acid, blue = basic. Acidic, basic, and polar residues are labeled.