Supplementary Material

The structure of a conserved Piezo channel domain reveals a novel beta sandwich fold

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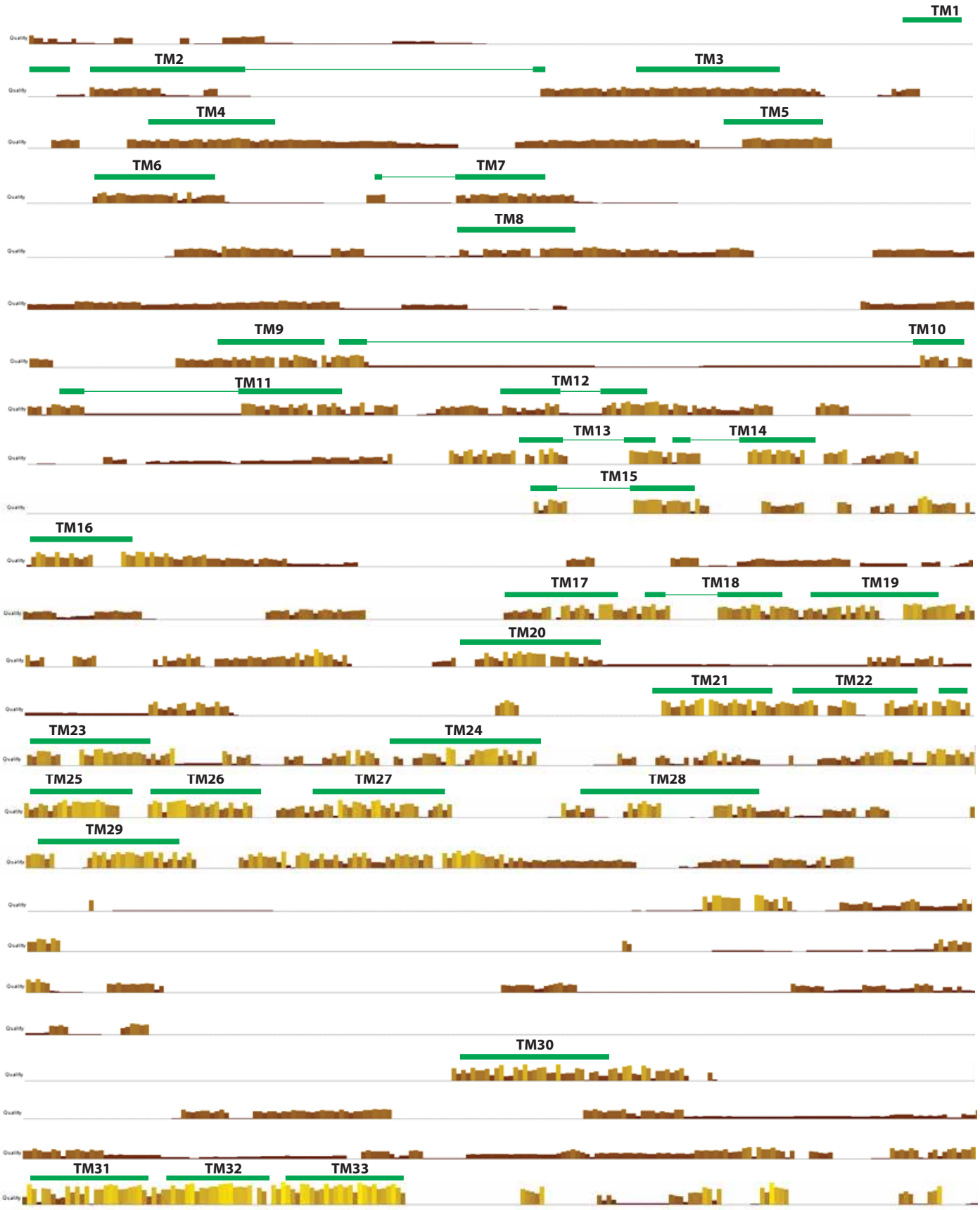
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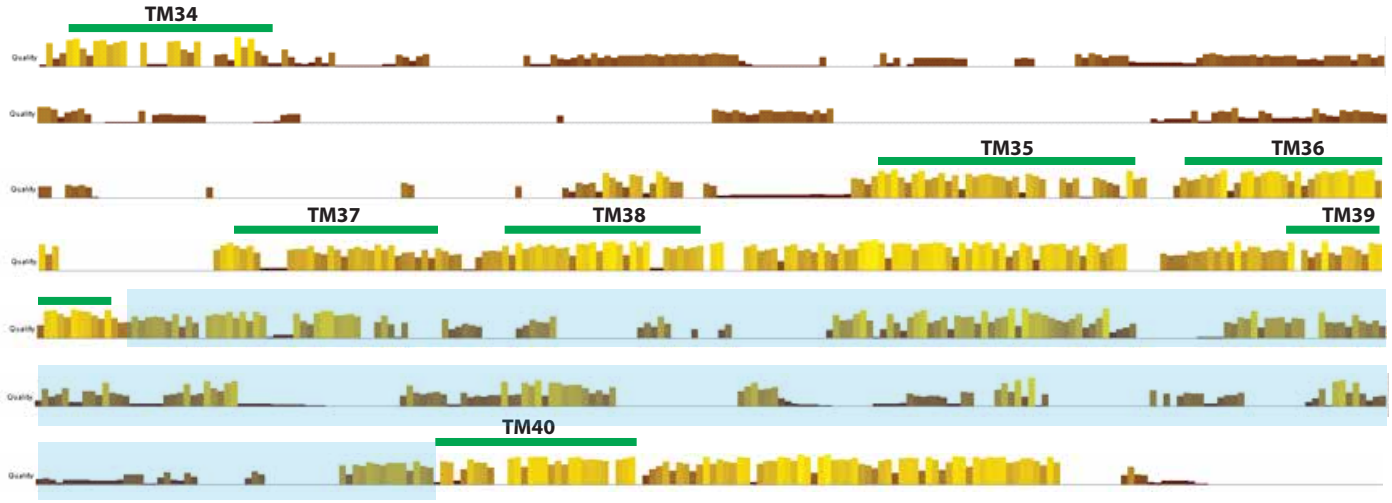


Figure S1, related to Figure 1. Multiple sequence alignment of Piezo homologs.

Multiple sequence alignment was performed using representative Piezo homologs (listed below) from vertebrate Piezo1, vertebrate Piezo2, invertebrate Piezo, plant Piezo, and unicellular eukaryote Piezo. Protein sequence conservation is represented by the shading and height of the bar corresponding to each residue, with the tall yellow bar, short brown bar and no bar representing high levels, low levels and poor sequence conservation, respectively. The green bars highlight the transmembrane (TM) segment for mouse PIEZO1 as predicted by Topcons (Bernsel et al., 2009). CTL2 (shaded in light blue) is the largest conserved Piezo soluble domain. The Piezo sequence conservation is relatively higher in the C-terminal region. The diagram was generated using the MAFFT multiple sequence alignment program (Katoh et al., 2002), available at the EMBL-EBI web page.

List of Piezo homologs used:

H. sapiens 1 (NP_001136336.2), D. rerio 1 (XP_696355.4), R. norvegicus 1 (NP_001070668.2), M. lucifugus 1 (XP_006097426.1), O. garnettii 1 (XP_003800876.1), B. Taurus 1 (XP_001256011.4), S. harrisii 1 (XP_003758542.1), G. gorilla 1 (XP_004058200.1), C. porcellus 1 (XP_003460961.1), E. caballus 1 (XP_005615040.1), P. alecto 1 (XP_006927190.1), M. domestica 1 (XP_007477361.1), L. Chalumnae 1 (XP_006002160.1), T. rubripes 1 (XP_003978351.1), Anolis carolinensis 1 (XP_008120472.1), C. lupus 1 (XP_005620631.1), M. gallopavo 1 (XP_003209947.1), S. partitus 1 (XP_008279724.1), X. tropicalis 1 (XP_002933721.2), C. simum 1 (XP_004437180.1), H. glaber 1 (XP_004843264.1), O. rosmarus 1 (XP_004392217.1), M. musculus 1 (NP_001032375.1), O.orca 1 (XP_004280184.1), M. musculus 2 (NP_001034574.4), G. Gallus 2 (XP_419138.4), H. sapiens 2 (NP_071351.2), B. Taurus 2 (XP_003587835.2), C. porcelius 2 (XP_005001285.1), C. simum 2 (XP_004437180.1), H. glaber 2 (XP_004843264.1), M. lucifugus 2 (XP_006089356.1), C. millii 2 (XP_007887509.1), O. orca 2 (XP_004276120.1), O. aries 2 (XP_004020692.1), G. gorilla 2 (XP_004059237.1), O. rosmarus 2 (XP_004417033.1), M. lucifugus 2 (XP_006089356.1), X. tropicalis 2 (XP_002937522.2), C. lupus 2 (XP_005623199.1), M. gallopavo 2 (XP_003205004.1), A. platyrhynchos 2 (XP_005013163.1), T. guttata 2 (XP_002192627.2), S. harrisii 2 (XP_003760113.1), A. aegyptii (XP_001657818.1), B. impatiens (XP_003494661.1), H. saltator (EFN75267.1), C. elegans (CAA92491.3), C. sinensis (GAA51253.1), D. melanogaster (AFB77909.1), M occidentalis (XP_003747214.1), P. humanus (XP_002428649.1), N. vitripennis (XP_008202351.1), E. histolytica (XP_655549.2), P. tetraurelia (XP_001461126.1), O. trifallax (EJY84567.1), T. cruzi (EKG00857.1), S. lycopersicum (XP_004247483.1), G. max (XP_006605262.1), T. cacao (XP_007030785.1), A. thaliana (NP_182327.6), C rubella (XP_006293550.1), P. persica (XP_007200947.1), O. tauri (XP_003079754.1)

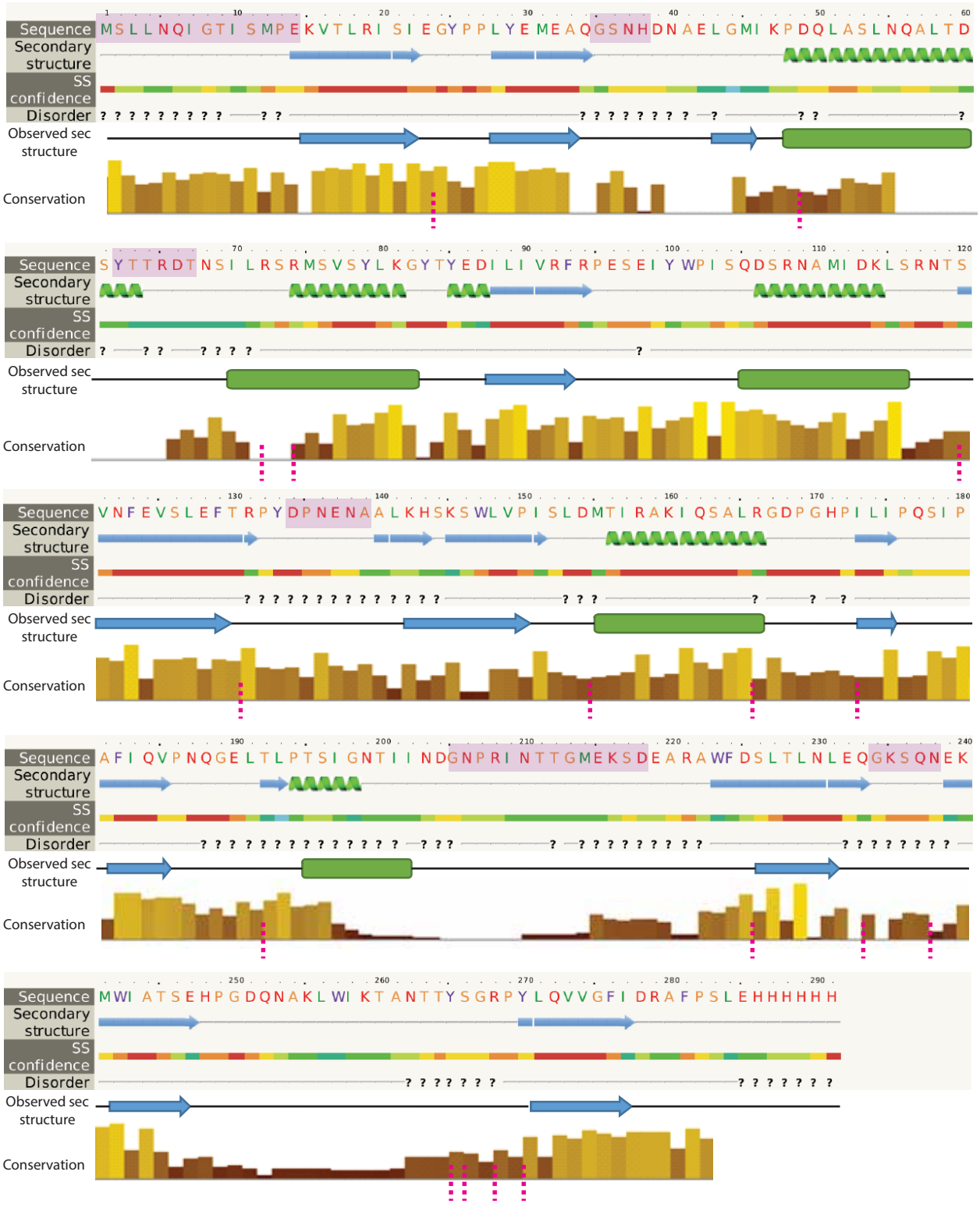
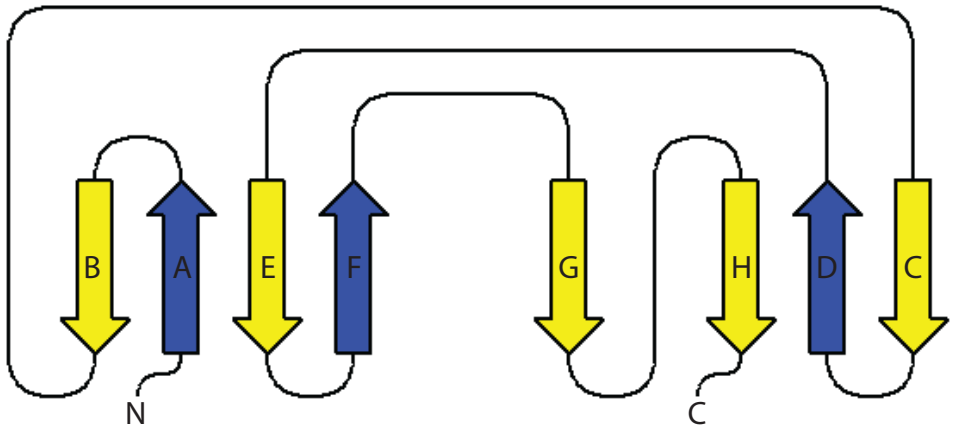


Figure S2, related to Figures 2 and 3. Comparison of the secondary structure of the WT *C. elegans* CTL2 from the crystal structure and from the Phyre2 prediction.

The observed secondary structure in the crystal structure of *C. elegans* CTL2 (drawn below the 'Disorder' row) generally resembles the secondary structure assignments predicted by Phyre2 (Kelley and Sternberg, 2009), (drawn below the 'Sequence' row). Amino acid residues that are not visible in the electron density of either the wild type or M31R mutant structures (shaded in purple) coincide with the predicted disordered region. The sequence conservation representation follows the convention used in Fig S1, with gaps in the multiple sequence alignment indicated by the magenta dotted lines.

A



B

Hit	Z-score	rmsd	PDB	Method	Description	CATH #	Sheet 1	Sheet 2
1	3.6	3.3	3N9D	SSM	Monoclinic LigD (P. aeruginosa)	N/A	ABCD	HGFE
2	2.8	4.1	3QK0	DALI	Phospatidylinositol-4,5-biphosphate 3-kinase	N/A	GHAD	CBEF
3	2.6	4.1	3L9B	DALI - SSM	Otoferlin	N/A	GHAD	CBEF
4	2.6	3.4	3NSJ	DALI	Perforin-1	N/A	GHAD	CBEF
5	2.6	2.5	2QZ5	DALI	Axin interactor, dorsalization associated	N/A	DEAIH	GFBC
6	2.4	3.4	1TFP	DALI - SSM	Transthyretin	2.60.40.180	DAGH	FEBC
7	2.4	3.8	2NQ3	DALI	ITCH-homolog E3 ubiquitin ligase	N/A	GHAD	CBEF
8	2.3	3.7	3JZY	DALI	Intersectin 2	N/A	GHAD	CBEF
9	2.1	3.4	2CJS	DALI	UNC-13 homolog A	N/A	DAHG	FEBC
10	2.1	4.1	1CJY	DALI	Cytosolic phospholipase A2	2.60.40.150	GABD	CEF
11	2.1	4.7	1D5R	DALI	Phosphoinositide phosphatase PTEN	2.60.40.150	GABD	CEF
12	2.0	4.2	1UGK	DALI	Synaptotagmin IV	2.60.40.150	GABD	CEF
13	2.0	3.9	1GMI	DALI	Protein kinase C, epsilon type	2.60.40.150	GABD	CEF
14	2.0	3.1	2H6U	DALI	5-Hydroxyisourate hydrolase	2.60.40.180	DAGH	FEBC

Figure S3, related to Figure 2. Comparison of the beta strand connectivity of the Piezo CTL2 domain to potentially related domains.

Each beta strand in the connectivity diagram is drawn as an arrow. Beta strands that are parallel to the first beta strand are colored blue, while those that are antiparallel are colored yellow (A). Connectivity diagrams for each of the 14 closest matches to the Piezo CTL2 loop identified by DALI (Holm and Rosenstrom, 2010) and SSM (Krissinel and Henrick, 2004) are summarized in table (B). No exact matches in strand orientation are observed between CTL2 and these candidate structural homologs.

Figure S4, related to Figures 3 and 4. Multiple sequence alignment of Piezo homologs around the M31 mutation site.

A methionine residue that is mutated into arginine in DHS patients (colored in red) is conserved across Piezo homologs. The position of this methionine (M31 in our *C. elegans* CTL2 construct) is in close proximity to an arginine residue (colored in blue) that is not conserved. There are three glutamate residues (colored in purple) around M31 that provide a net negative charge at this location.

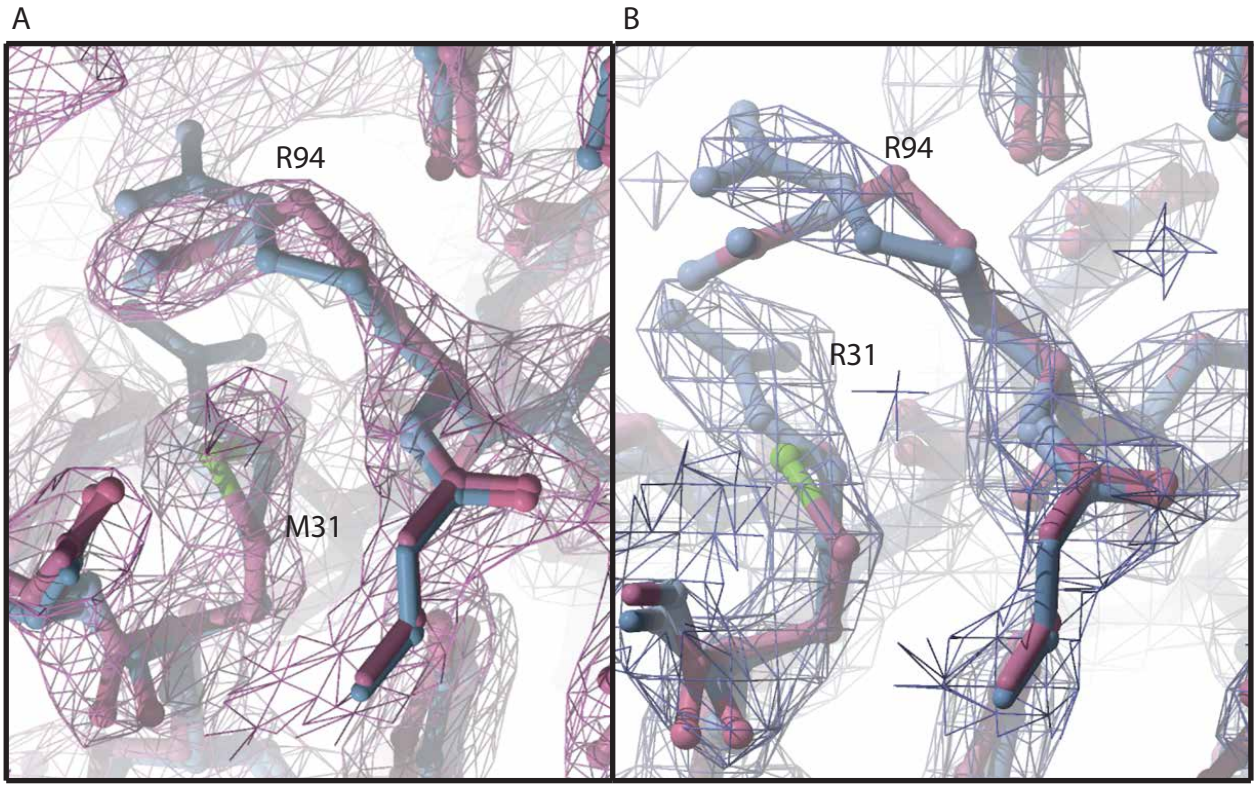


Figure S5, related to Figure 4. Substitution of M31 with Arg leads to repositioning of the R94 sidechain.

The structure of the wild type *C. elegans* PIEZO CTL2 structure is displayed in magenta, while the M31R mutant structure is displayed in blue. A: the electron density of WT Piezo loop is displayed in magenta. B: the electron density of the M31R mutant CTL2 domain is displayed in blue.