SUPPLEMENTARY INFORMATION

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Supplementary Table 1. Diffraction data collection statistics*

Data set	Native	Cs†
Space group	R3	R3
Wavelength (Å)	1.072	1.378
Cell (a x b x c) (Å)	131.8 x 131.8 x 119.1	132.5 x 132.5 x 118.6
Cell (α x β x γ) (°)	90 x 90 x 120	90 x 90 x 120
Resolution (Å)	80-3.0 (3.11-3.0)	80-3.50 (3.56-3.50)
Completeness (%)	99.7 (100)	93.5 (95.7)
Redundancy	3.8 (3.8)	4.0 (3.4)
Rmerge (%)	10.8 (56.8)	9.5 (84.0)
I/σI	12.8 (2.1)	21.4 (1.1)

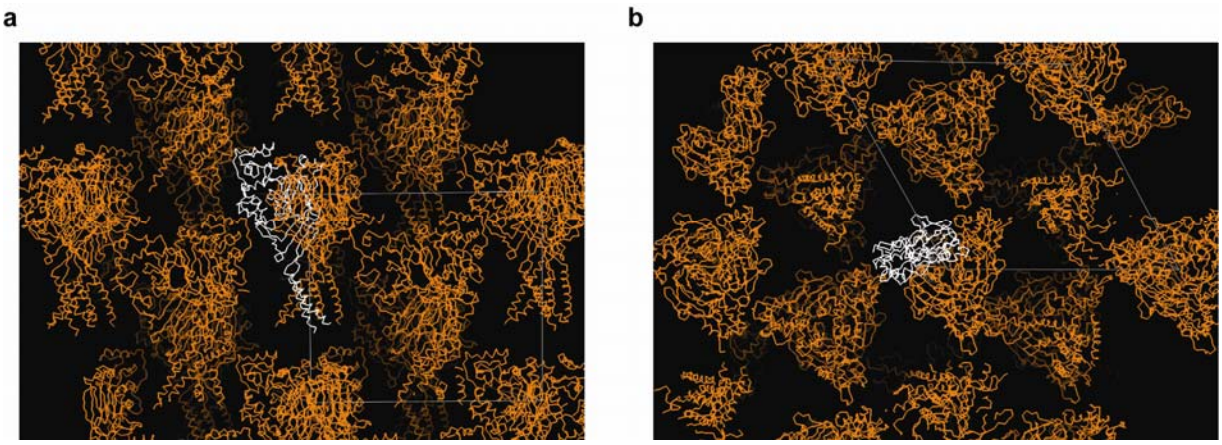
* Statistics for the highest resolution shell are shown in parenthesis.

† Diffraction data for the crystal soaked in 250 mM CsCl.

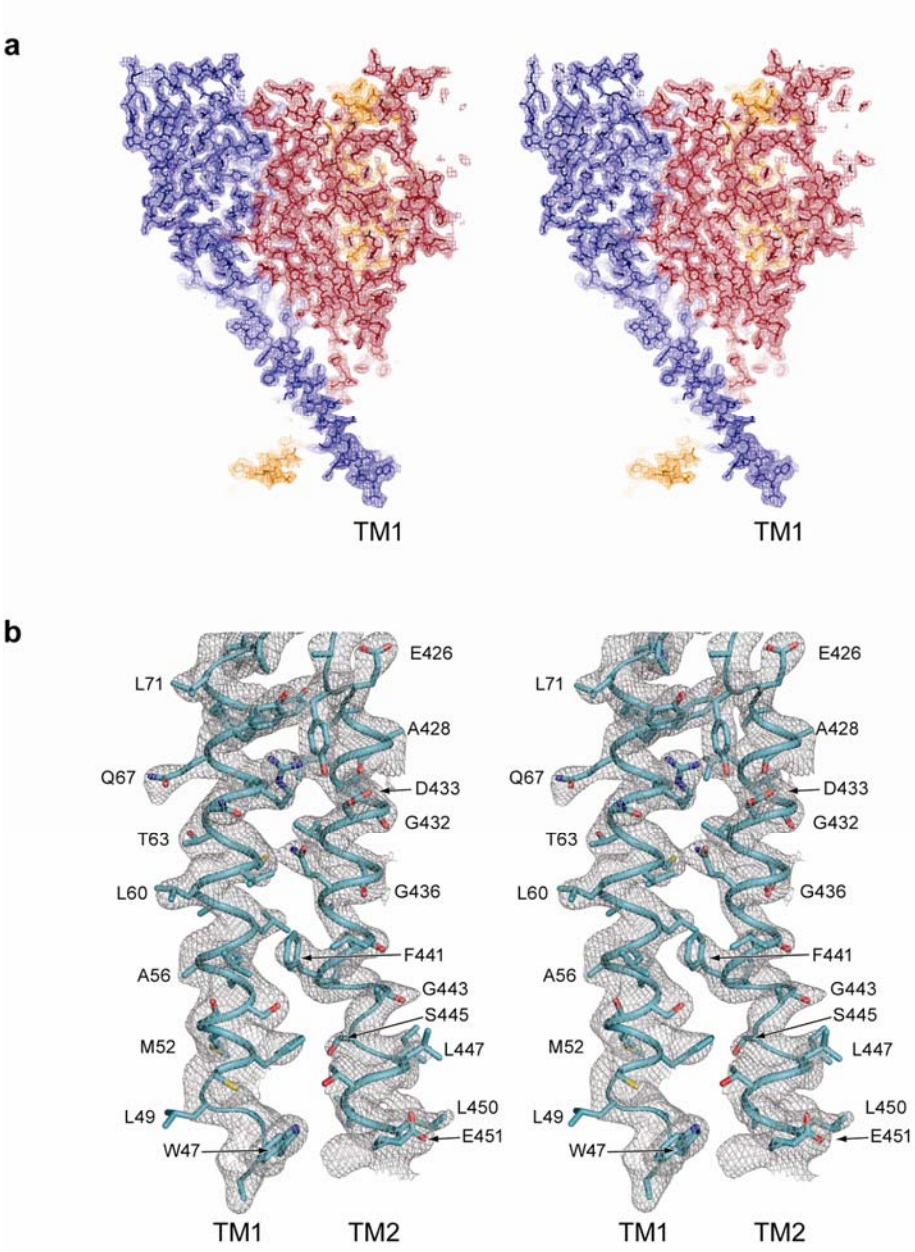
Supplementary Table 2. Crystallographic refinement statistics

Data set	Native
Resolution range (Å)	27.5-3.00
R_{work}/R_{free} †	0.225/0.274
Number of reflections	14,636
Completeness (%)	99.97
Number of atoms	
Protein	3245
Ion	1
Water	54
RMSD bond (Å)	0.007
RMSD angle (°)	1.145
Average B-factors(Å^2)	
Protein	52.0
Ion	77.1
Water	76.1
Ramachandran plot	
Favoured (%)	87.9
Allowed (%)	10.4
Generously allowed (%)	1.7
Disallowed (%)	0

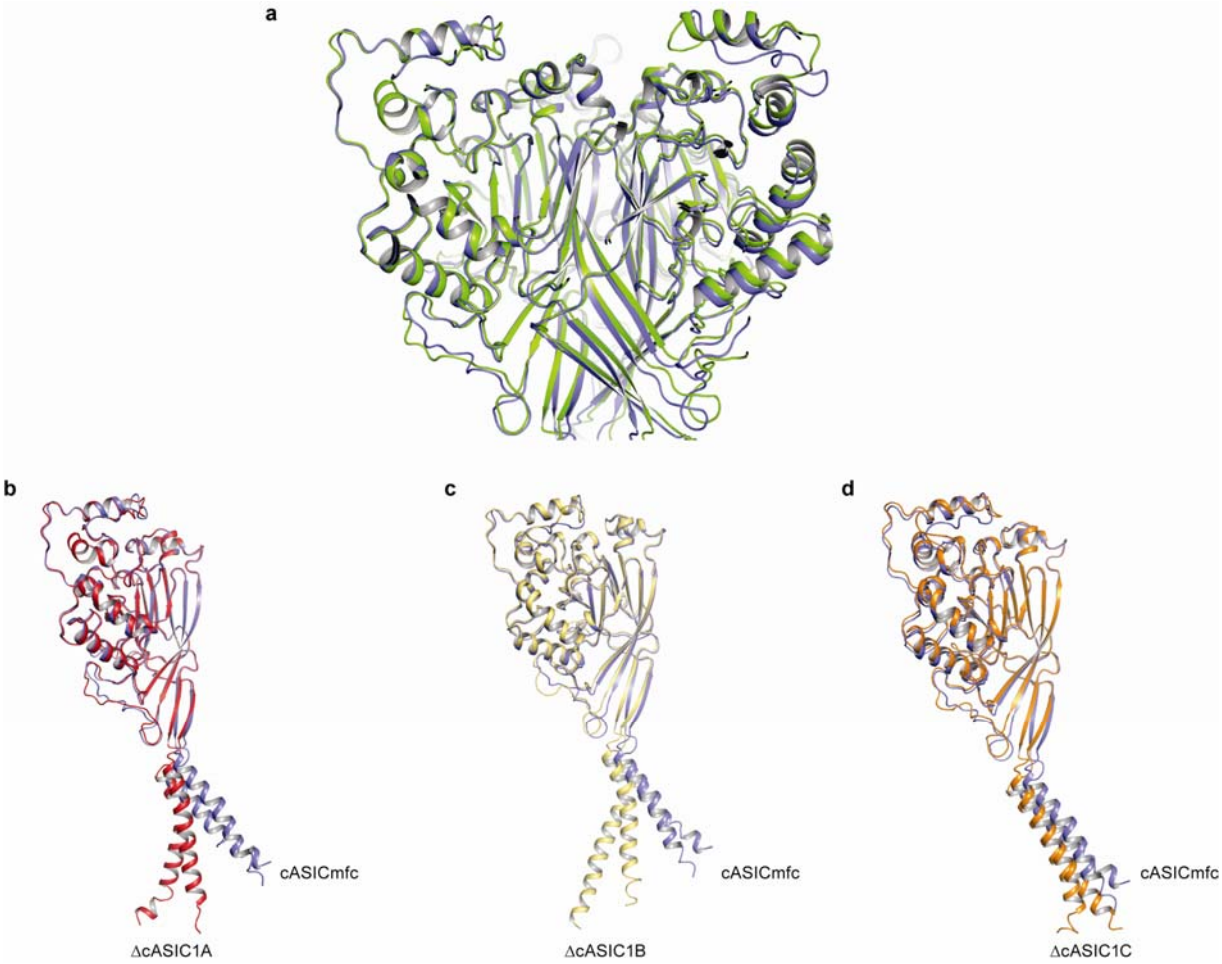
† $R = \Sigma(||F_{obs}|-|F_{calc}||)/\Sigma|F_{obs}|$, with 5.1% of the reflections set aside for the R_{free} calculation



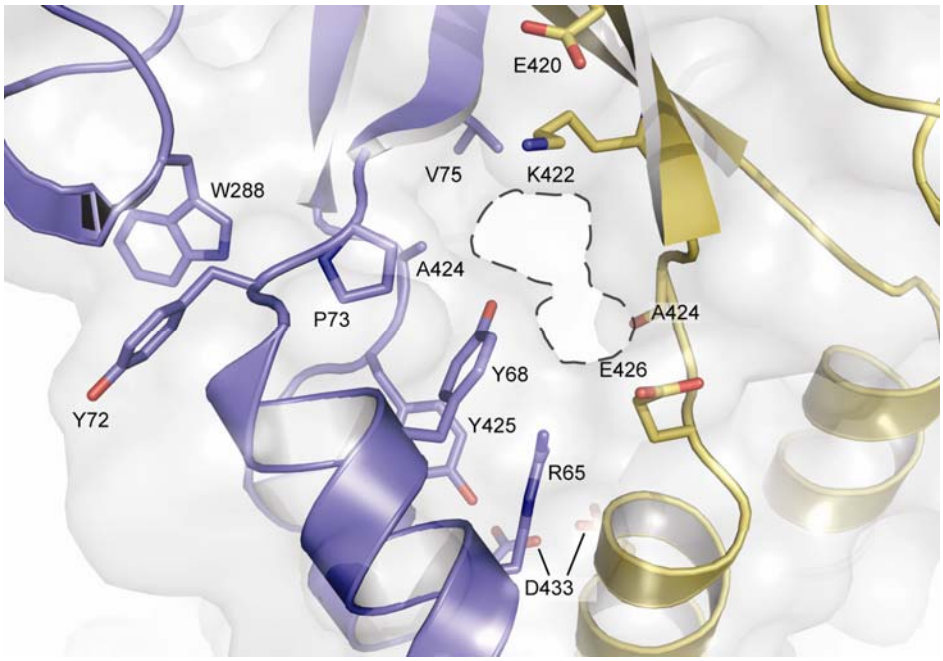
Supplementary Figure 1. cASIC1mfc packing in the R3 crystal form. **a**, Crystal packing viewed perpendicular to the b-c plane. Symmetry-related subunits and molecules are presented as Cα traces in orange. A single subunit and the unit cell axes are shown in white. **b**, Crystal packing viewed perpendicular to the a-b plane.



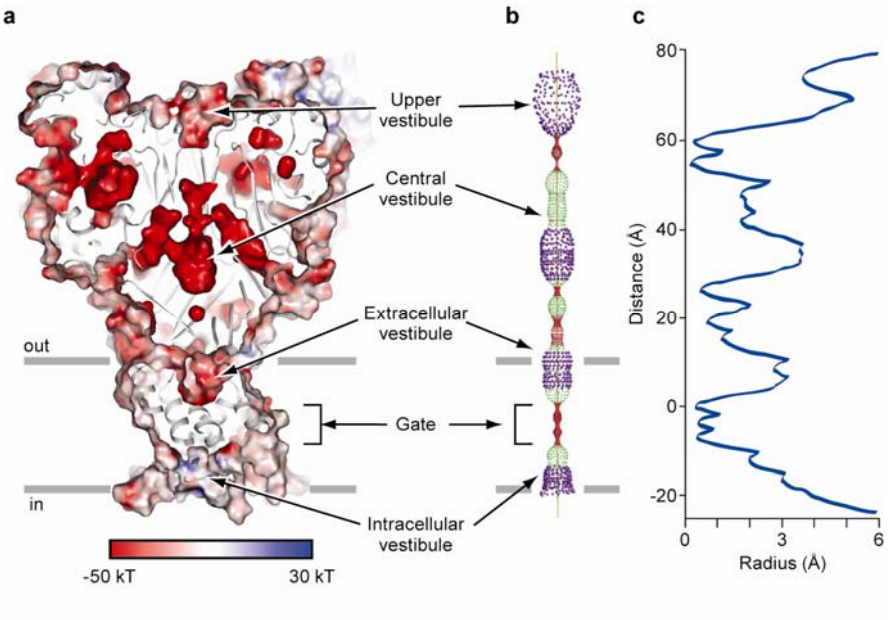
Supplementary Figure 2. Electron density maps of cASIC1mfc. **a**, Stereoview of 2Fo-Fc electron density maps. Individual subunits (blue, red, and orange) are colored. **b**, Stereoview of 2Fo-Fc electron density maps of the TM1 and TM2 domains. Side chain moieties are shown for non glycine residues and carbonyl groups are depicted for G432, G436, and G443. Selected residues within each domain are labeled. Maps are contoured at 1σ.



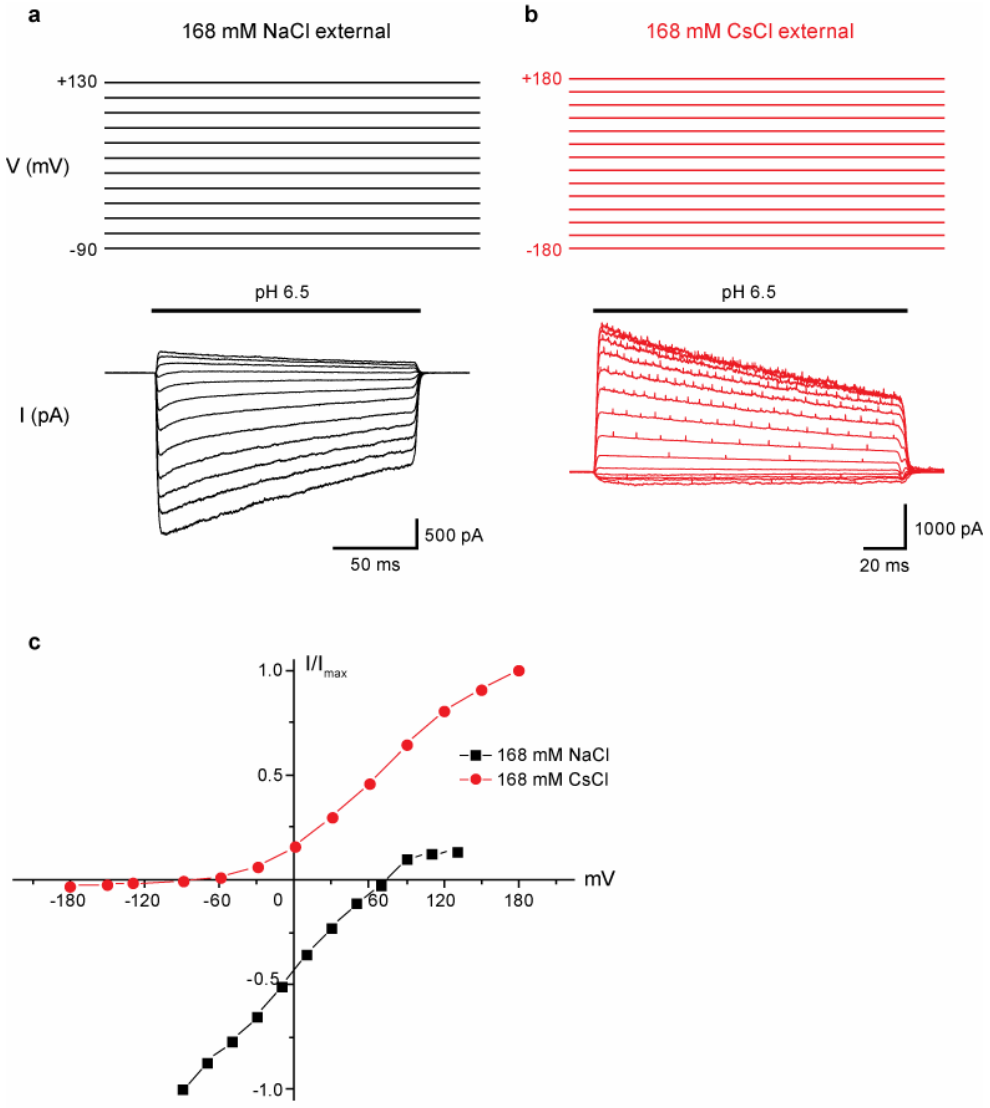
Supplementary Figure 3. cASIC1mfc and ΔcASIC1 comparison. **a**, Overlay of cASIC1mfc (blue) and ΔcASIC1 (green) extracellular domains using residues 77-427 with a root mean square deviation of 1.2 Å. A comparison of the ΔcASIC1 subunit A (ΔcASIC1A; red), subunit B (**b**, ΔcASIC1B; yellow) and subunit C (**c**, ΔcASIC1C; orange), **d**) with the cASIC1mfc subunit (blue). There are pronounced 'kinks' in the transmembrane domains of ΔcASIC1 subunits: ΔcASIC1A at TM2 residues 432-434, ΔcASIC1B at TM1 residues 60-63 and TM2 residues 432-434, and ΔcASIC1C at the C-terminal end of TM2 at residues 443-445.



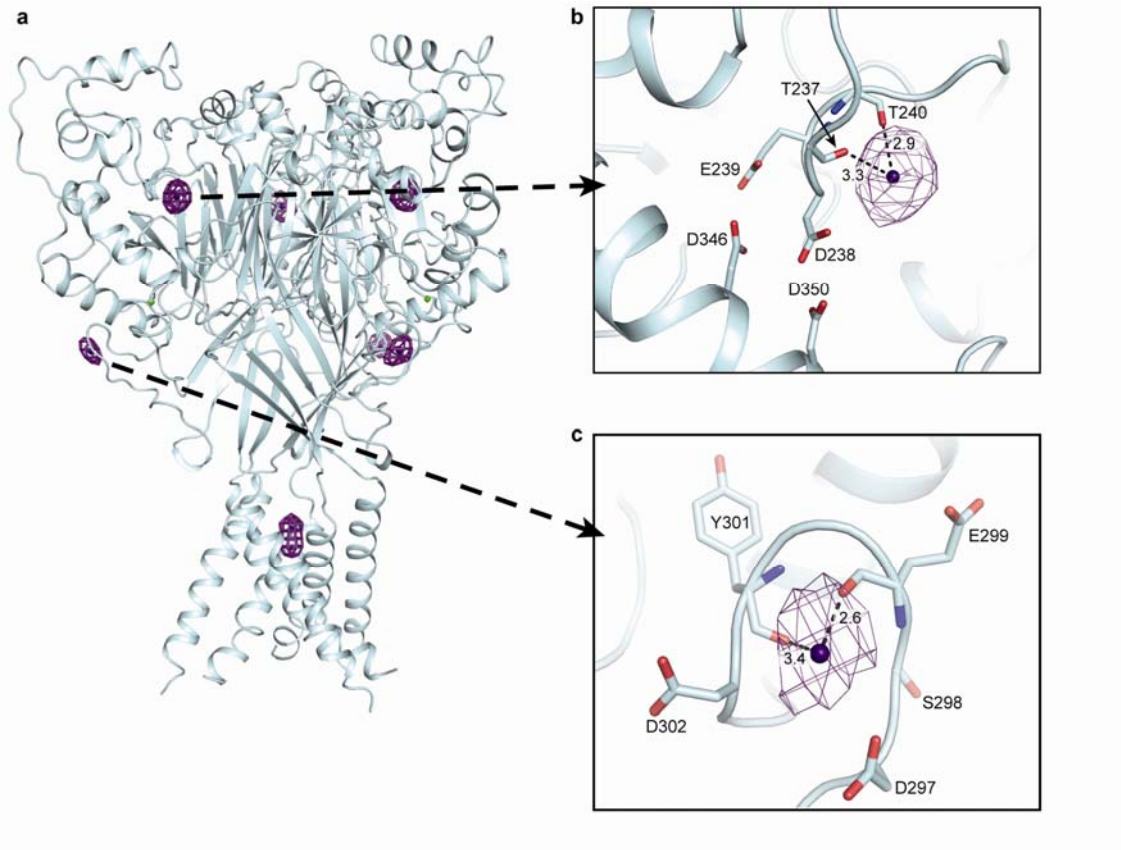
Supplementary Figure 4. Residues lining fenestrations between two subunits. Solvent exposed surface area (grey) and the residues (R65, Y68, P73, V75, E420, K422, A424, and E426) lining the fenestration (dashed line) are in stick representation. Interacting residues between thumb (W288) and transmembrane domain 1 (Y72) are shown. The D433 residues of the second transmembrane domain at the base of the extracellular vestibule are shown.



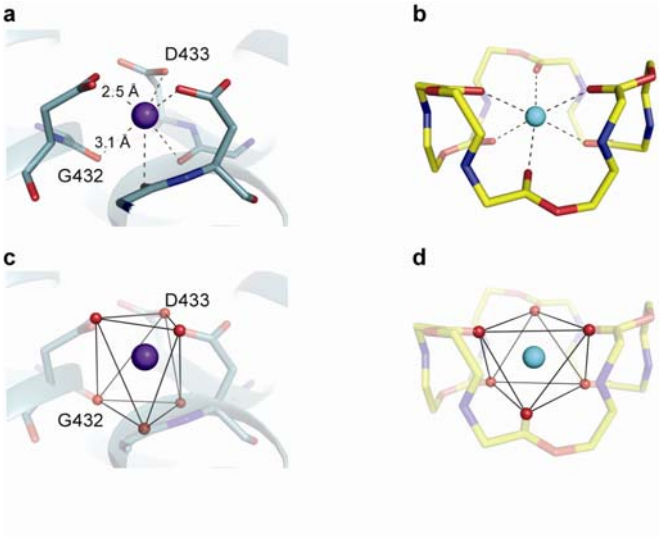
Supplementary Figure 5. Illustration of cavities, vestibules and possible ion permeation pathways in cASIC1mfc. a, An electrostatic potential surface and cartoon representations of cASIC1mfc sliced along the molecular 3-fold axis of symmetry. The surface is coloured based on electrostatic potential, contoured from -50 kT (red) to +30 kT (blue). White is 0 kT. Panels **b** and **c** illustrate the radius of possible pathways on the 3-fold axis, generated using HOLE (red < 1.4 Å< green < 2.3 Å < purple).



Supplementary Figure 6. Current-voltage relationship of monovalent cations in cASIC1mfc. **a**, Representative outside-out patch recording at stepped holding potentials (Vh) of cASIC1mfc in 168 mM NaCl. **b**, Representative outside-out patch recording at stepped holding potentials (Vh) in 168 mM CsCl. **c**, I-V relationship of cASIC1mfc recordings in external solutions containing sodium (black, data shown in panel **a**) or cesium (red, data shown in panel **b**). Currents normalized to maximal response.



Supplementary Figure 7. Cs^+ binding sites in cASIC1mfc. **a**, Anomalous difference Fourier maps showing Cs^+ binding sites (contoured at 3.5σ, purple mesh). **b**, Cs^+ binding site and neighboring residues near putative proton binding sites (D238-D350, E239-D346). The ion is coordinated by carbonyl oxygens of T237 and T240. **c**, Cs^+ binding site at the periphery of the thumb domain. The ion is coordinated by main chain carbonyl oxygens of E299 and Y301. Interactions within 3.5 Å are indicated by dashes and distances are in Å. Cs^+ ions in (**b)** and (**c**) were placed manually.



Supplementary Figure 8. Trigonal antiprism coordination of Cs^+ ions by cASIC1mfc and comparison with potassium coordination with valinomycin. **a**, Stick representation of cASIC1mfc interaction with Cs^+ at site 2. **b**, Structure of valinomycin coordinating a potassium ion. Side chain atoms were removed for clarity. **c**, Trigonal antiprism coordination of a Cs^+ ion by the Gly432 carbonyl and Asp433 carboxyl oxygens. Oxygens (red spheres) form the vertices, while solid lines represent the sides of each of the two staggered triangles of the antiprism. **d**, Valinomycin-K^+ trigonal antiprism coordination[S1].

References

S1 Neupert-Laves, K. & Dobler, M. The crystal structure of a K^+ complex of valinomycin. *Helv Chim Acta* 58, 432-442, (1975).