

## Supplementary Information for

### Structural insight into TRPV5 channel function and modulation

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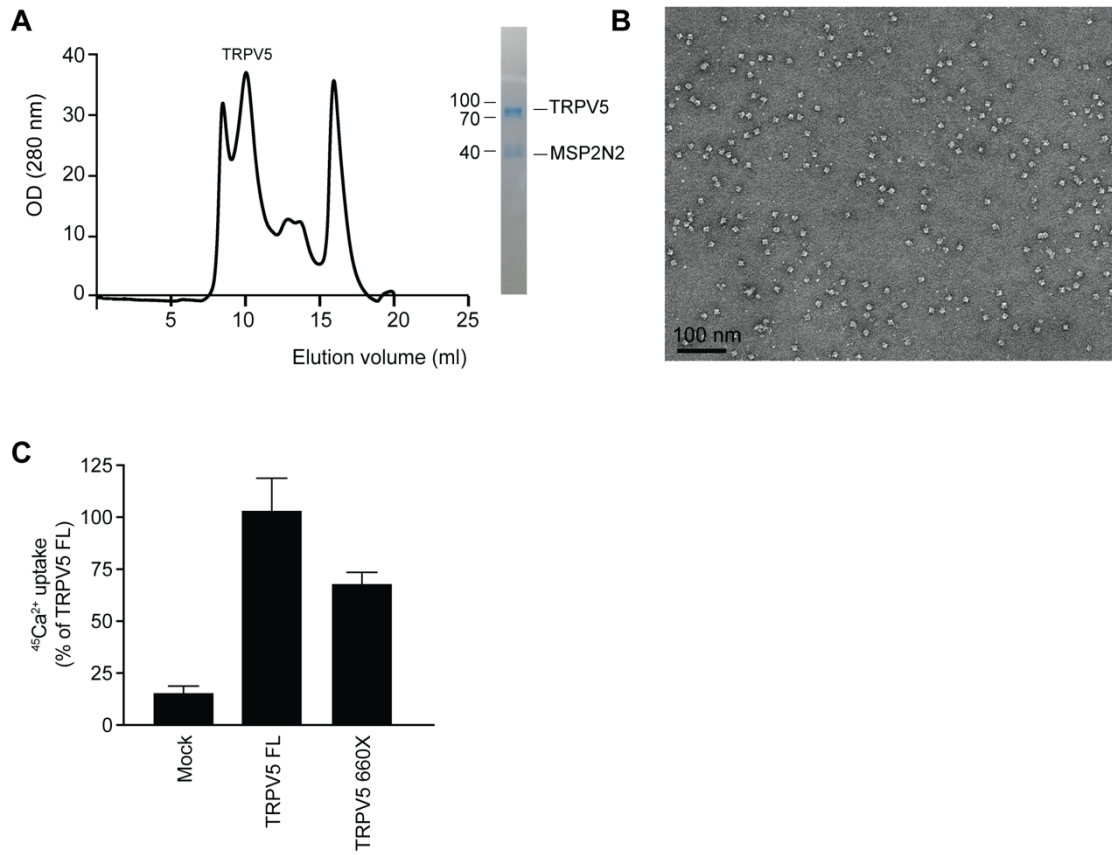
Email: [David.Julius@ucsf.edu](mailto:David.Julius@ucsf.edu)

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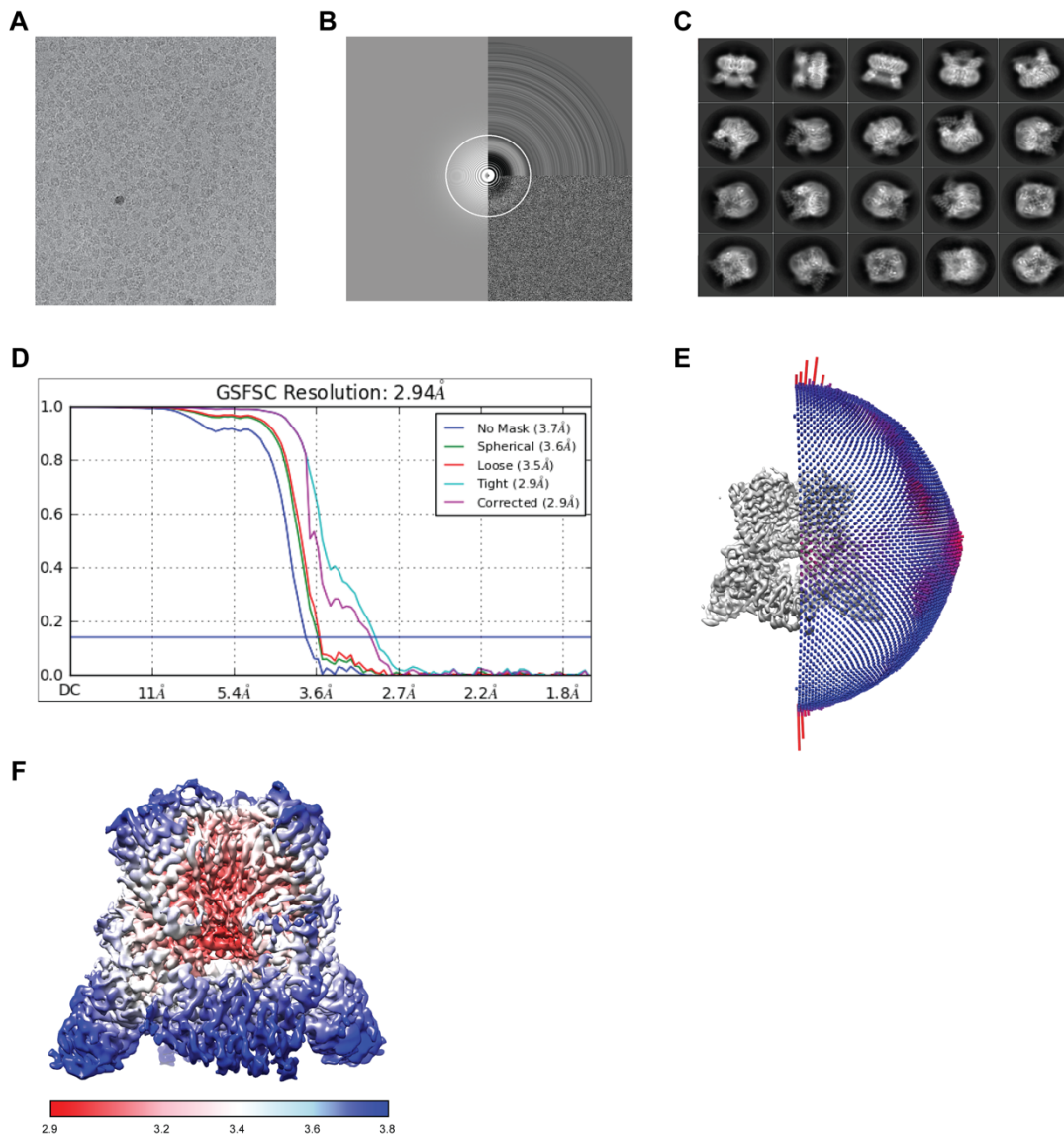
Figs. S1 to S11

Table S1

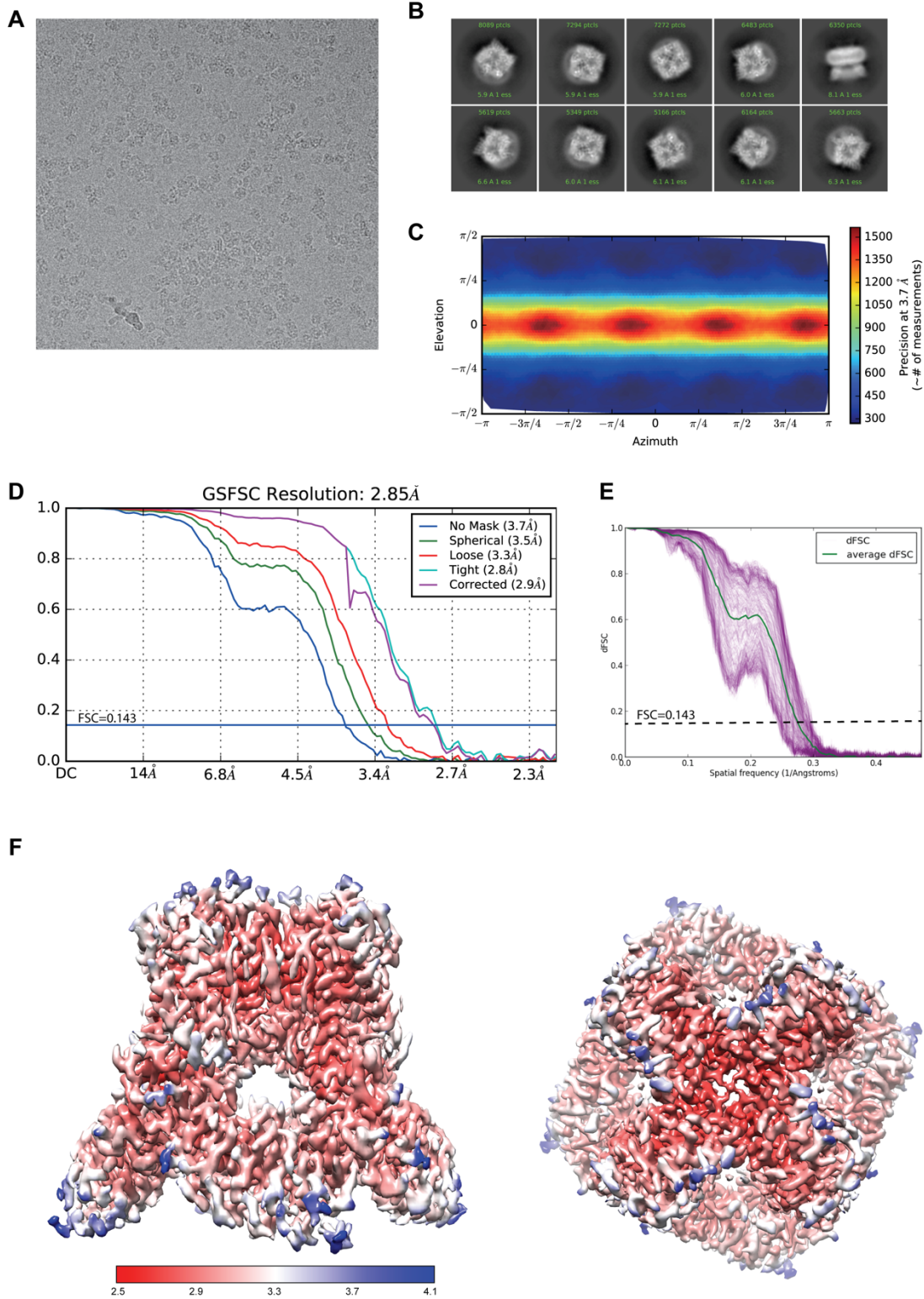
## Supplemental figures



**Fig. S1.** Lipid nanodisc reconstitution and function of TRPV5. (A) Size-exclusion profile of nanodisc-reconstituted TRPV5 1-660 after proteolytic removal of the MBP tag. Insert: Coomassie blue staining of the TRPV5-nanodisc complex peak. (B) Representative negative stain image of purified TRPV5 1-660. (C) Radioactive  $^{45}\text{Ca}$  uptake assay of HEK293 cells transfected with mock, full-length TRPV5, and C-truncated (660X) TRPV5. Uptake is shown as the mean $\pm$ SEM (N=3).

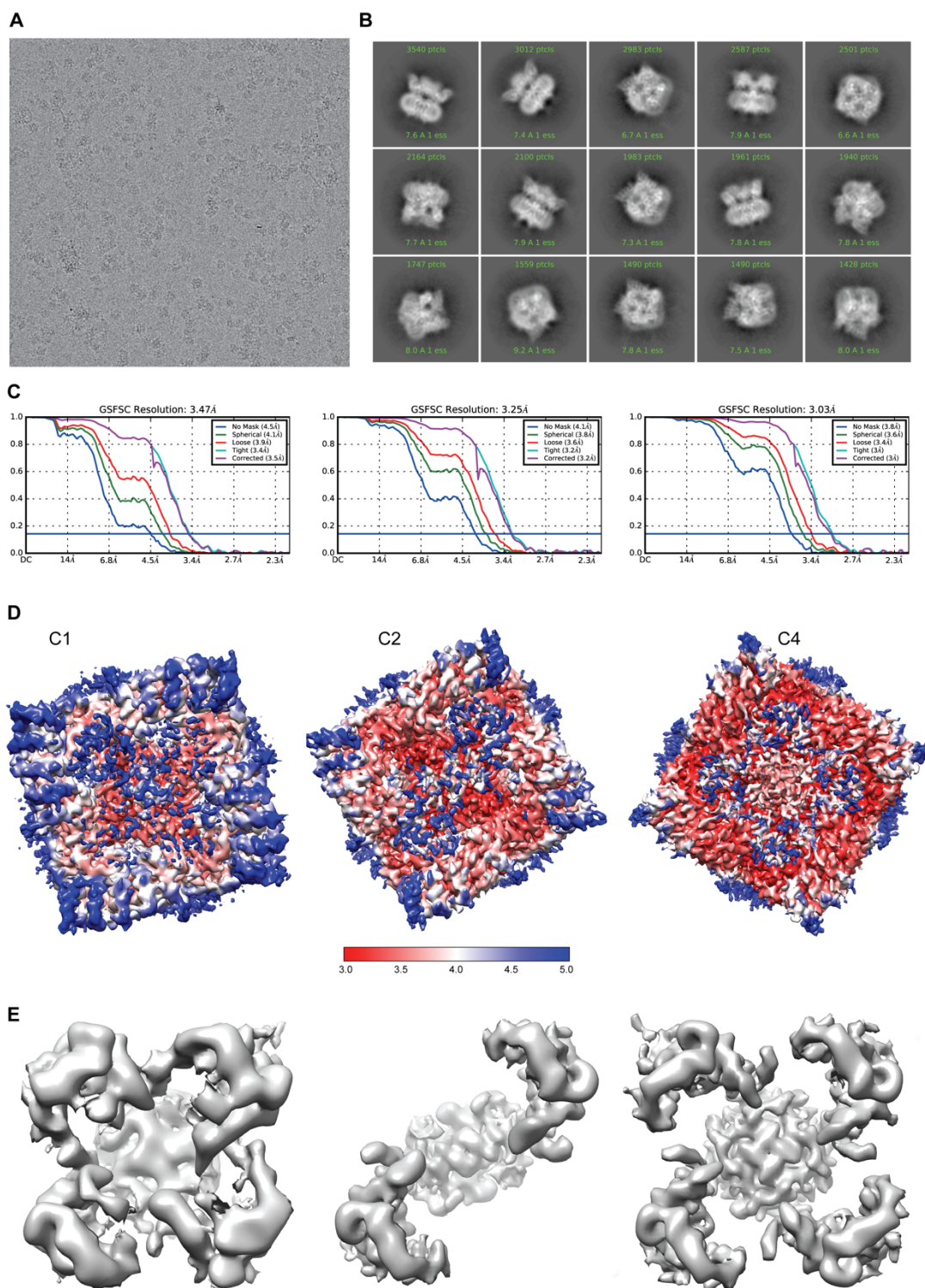


**Fig. S2.** Cryo-EM structure of TRPV5 1-660. (A) An example of an electron micrograph of TRPV5 1-660 in lipid nanodiscs. (B) Fourier power spectrum calculated from the micrograph shown in (A). (C) Representative 2D class averages calculated from selected particles. (D) FSC curves of the 3D reconstructions of the entire molecule as a tetramer with imposed C4 symmetry. (E) Euler angle distributions of all particles used in the final nanodisc-stabilized TRPV5 3D reconstruction. (F) Side view of the density map, colored according to the local resolution estimation made by Resmap.



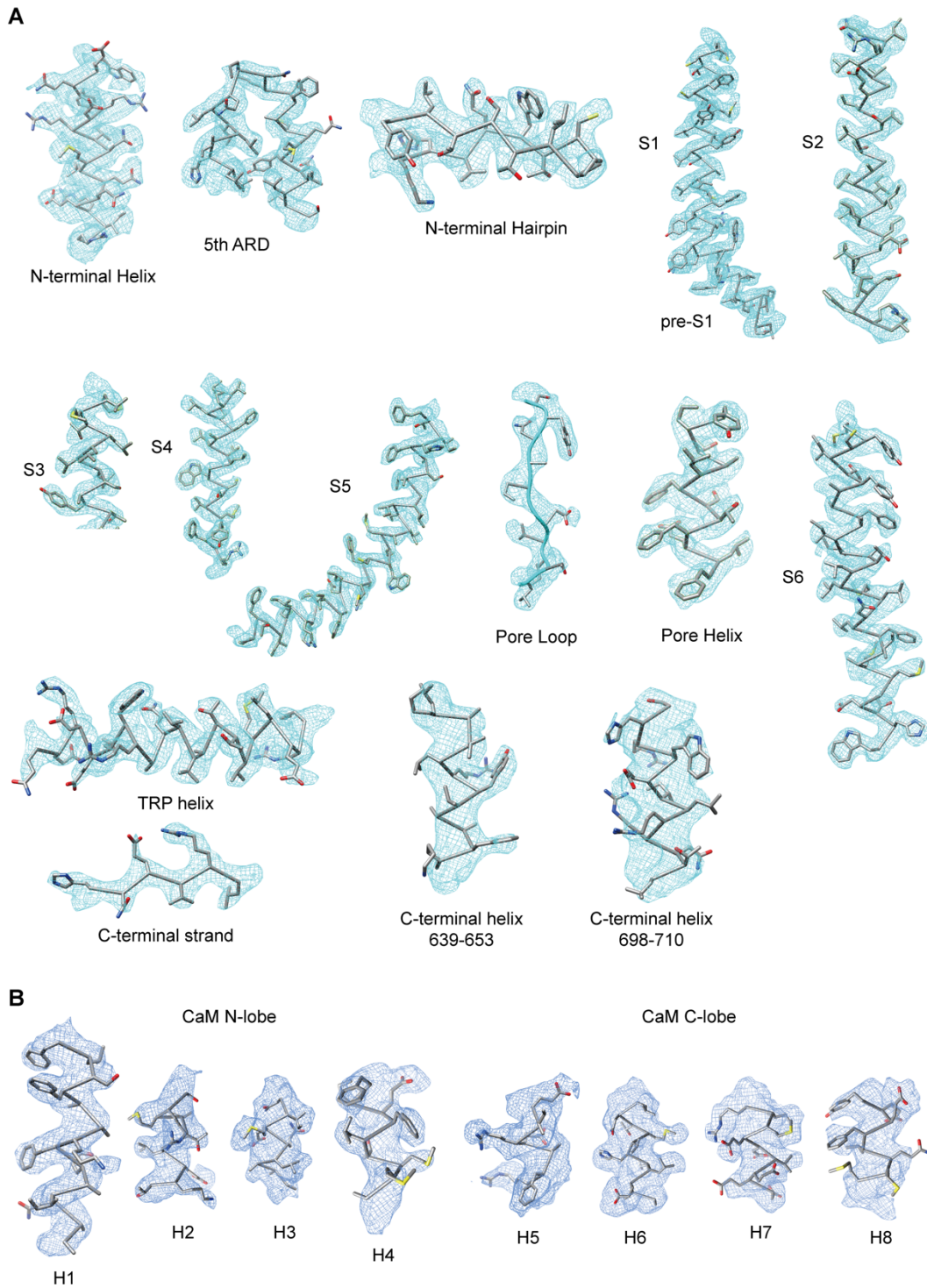
**Fig. S3.** Cryo-EM structure of TRPV5 W583A. (A) An example of an electron micrograph of TRPV5 W583A in lipid nanodiscs. (B) Representative 2D class averages calculated from selected particles. (C) Posterior precision directional distributions of all particles used in the final 3D reconstruction.

(D) FSC curves of the 3D reconstructions of the entire molecule as a tetramer with imposed C4 symmetry. (E) Directional FSC from different Fourier cones. Each curve indicates a different direction. (F) Side view and bottom view of the density map, colored according to the local resolution estimation made by Resmap.



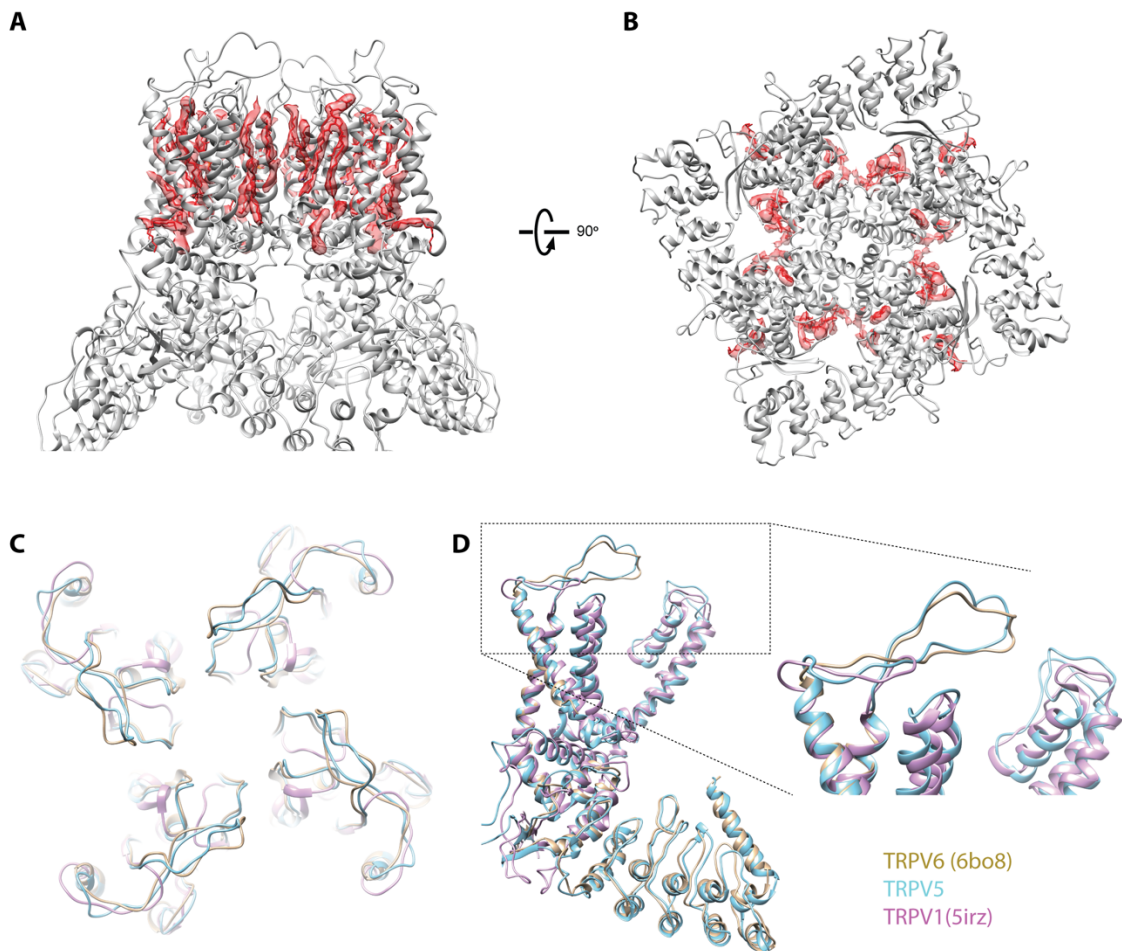
**Fig. S4.** Cryo-EM structure of TRPV5-CaM. (A) An example of an electron micrograph of TRPV5-CaM complex in LMNG. (B) Representative 2D class averages calculated from selected particles. (C) Posterior precision directional distributions of all particles used in the final 3D reconstruction

for C1, C2 and C4 refinement respectively. (D) Bottom view of the density map in order to show CaM density clearly, for C1, C2 and C4 refinement respectively, colored according to the local resolution estimation made by Resmap. (E) Bottom views of the density map with CaM density isolated, with C1, C2 and C4 symmetry applied during the refinement.



**Fig. S5.** Cryo-EM densities of TRPV5-CaM. (A,B) Fragments of the helical domains of TRPV5 (A) and CaM (B). The density is shown as blue mesh and the corresponding structural model in grey with side chains of residues depicted to demonstrate the quality of the map from various regions of the reconstructions.





**Fig. S6.** Lipid densities and S1-S2 linker comparison. (A,B) Side and top view of the TRPV5 tetrameric complex, with lipid densities indicated in red. (C) top view overlay of the S1-S2 linker region of TRPV5 (cyan), TRPV6 (gold), and TRPV1 (purple). (D) comparison of the single monomers of TRPV5 (cyan), TRPV6 (gold), and TRPV1 (purple), with a zoomed insert of the outer pore region to indicate differences in the S1-S2 linker.

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1          1q          2q
rbTRPV5 MGAC.....PPKAKGP.....WA.....QLQKLLISWPVGEQ.....
rbTRPV6 M.....VSLDSEGEEDLPEDTCP.....D.....LLDGD.SNAKPPPAKPHIFS.TA
rbTRPV1 MKRW.....VSLDSEGEEDLPEDTCP.....D.....LLDGD.SNAKPPPAKPHIFS.TA
rbTRPV2 M.....VSLDSEGEEDLPEDTCP.....D.....LLDGD.SNAKPPPAKPHIFS.TA
rbTRPV3 MNVLPKDMVPLMGRRAITAGGNP.....AVLLEKRPAAEVTPTKSAHFLEIEGFESNPVTTKTSPPIFSKPM
rbTRPV4 MADP.....SDSP.....RAGPGEAAEPPGDESATAGEAF.....PLSSLANLFEDEGS.PAPLPTDAGRPAAGPDA

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rbTRPV5 .....
rbTRPV6 .....
rbTRPV1 KRSRSL.....FGKGDSEET.....SPMDCSYEEGELAPCPAI.....TVSSVIVQRSGDGPTCARQLSQ
rbTRPV2 .....YGAG.....P.....PMEBSRFQEDERNFPFQI.....KVN.....N
rbTRPV3 DSNIRP.....CVSGNCDMDSP.....QSPQDDVTETPSNPSF.....
rbTRPV4 RPNLRMKFGAFKKGVPNPIDLESLEYESSVAPAKKAPMDSLFDYGYRHHPSDNKRRRKRVEKQPQ.....S

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3q          4q          5q          6q          7q          8q          9q
rbTRPV5 ...DWEQYRD.RVNMLQOERIRDSPLLQAAKENDLRLKILLNQ.....SCD.....FQORGAVETAAHVAIY..DNLEAAT
rbTRPV6 ...SWAQSRD.EQNLLQQRWESPLLLAAKENVQAPNKIKYD.....SCE.....VHORGALETAATHIAYI..DNLEAAM
rbTRPV1 DSVAAAGAER.PKLY.....DRRRIFEVAVAQNQCDESELCFLQSRKRLTDS.....EFKDPETCKPCLKAMNLSHGQNDTI
rbTRPV2 YRKGAGASQ.PDLNRF.....DRDRLFNVVARGNPELDAGLEYLRLRSTKYLTD.....EYEGSTCKPCLKAMNLSHGQNDTI
rbTRPV3 .SANLAKEEKRRRKH.....LKKRIFTAVSEGCQEDVLELLEQLCRRHRGLDVSDFLMHKLTAASDCKPCLKAMNLSHGQNDTI
rbTRPV4 PKAPAPQPPP.VLKVF.....NRPILFDIVSRGSTADLVGELPFLTHKRLTDE.....EFREPSCKPCLKAMNLSHGQNDTI

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10q         11q         12q         13q         14q         15q         16q         17q
rbTRPV5 .LIME.....AAPELAKEPALCEPFVGTALHIAVMNQNLNLVRAILARASISARATIAARRRSP.HNLIYGEHPLSFAACVGSSEI
rbTRPV6 .LIME.....AAPDLVPEPMTSELYGQTALHIAVMNQNLNLVRAILARASISARATIAARRRSP.HNLIYGEHPLSFAACVGSSEI
rbTRPV1 PLLEIARQDSDSKEFVNASYTDSYKQGTALHIAERRNMAVLTLLVENSADVOAANQDFPKKTKRGRGFPVCEPLSLAACTNQLAI
rbTRPV2 QPHEIIDRDSGNPQPLVNAOCTDEYRCHSALHIAIERRSLQCVKLVENGANVHAKACQHPKNO.DTCFVPGGELSLAACTKQWDV
rbTRPV3 RILITFAEENGILDRFINAEYTEEAYEGQTALHIAIERRDRIAAALIAAGADVNAHAKAVFPNPKYQHEGFPVGGC.....
rbTRPV4 PALLDIAERTGNMREFINAPFRDIYRGTALHIAIERRCKHYVELVAQCADVHAQARERFQPKDEGGYFVCEPLSLAACTNQPHI

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18q         19q         20q         21q         22q         23q         24q         25q         26q
rbTRPV5 VRLIEH...GADIRAQDSLGNTVLHLILIQ....PNKFFACQMYNLLSYDEHSDHLQSLVLPVHQSTGPFKLGVEINTVMFOHLM
rbTRPV6 VRLIEH...GADIRAQDSLGNTVLHLILIQ....SNKFFACQMYNLLSYDGHSDHLQSLVLPVHQSTGPFKLGVEINTVMFOHLM
rbTRPV1 VKFLLNSWQPADISARDSVGNVTLHALVEADNTPDNTKPVSMYNEILGLAKLHPTLKEELIKKCLTPALAKGSKIGVLAAIL
rbTRPV2 VNYLLENPHQPASLQADSLGNTVLHALVMIADDSANSLAVLRMYDGLQAGARLCPNVQLEGIPNLECLTPKLKLAKECKIIFKHIL
rbTRPV3 VNYLLENPHKADMRRQDSRGNTVLHALVAIADNTRENTKPVTKMYDLLLKCARLFPDSNLEAVLNDLSLMLMAKTKIGIQHYII
rbTRPV4

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27q         28q         29q         30q         31q         32q         33q
rbTRPV5 QK.....RKHVQWTCGLTSTLYDITEIDSGEELSFELVUVSS.KKREA...QILEQTEVKEVSVFKKKYGRPYEVCVLASLYI
rbTRPV6 QK.....RKHVQWTCGLTSTLYDITEIDSGEELSFELVUVSS.KKREA...HILDQTEVKEVSVLKKKRYGRPYEVCVLGAIYL
rbTRPV1 QREILEPECRHLSRKPTEWAYGCVVHSSLYDLSCIDTCERNVSEVLEIAYSSSETPNHDMLLVEPLNRLQDKKRRVRIEYFNPFVYC
rbTRPV2 QREIFA.PCQSLSRKPTEWAYGCVVRSLYDLASVDSW.EENSVEIIFAFH.SRSPHHRMVVLEPLNKLQAKWDLRI.PRECENFLCYL
rbTRPV3 QREIKKPLRGLSRKFTDWAYGCVVSSLYDLSTVDT.TONSVEIIVYN.TNIDNHEMLTLEPLTLHMKKKKFAKYMFLSFCFYF
rbTRPV4 RREVTDEDRHLSRKFKDWAYGCVVSSLYDLSSLDTGCEASVEIILVYN.SKIENHEMLAVEPINELWLDKRRKFGAVSEYINVVSYL

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34q         35q         36q         37q         38q         39q         40q         41q         42q
rbTRPV5 LYMICP...TCCIIYR...LKL.RD.DNRTDPRDITILQOKLQOAY.VTHODNIRLV...ELVTVTGAVIILLLEIPDI...VLRGASRVFGQITLGGP
rbTRPV6 LYITCF...MCCIIYR...LKL.RP.SNRTDPRDNTLLQOKLQOAY.VTSRDDIRLV...ELVTVTGAVIILLLEIPDI...VLRGTRFFGHTILGGP
rbTRPV1 LYMIIF...TAAYYR...VDG.LP.PY...SNRTDPRDNTLLQOKLQOAY.VTSRDDIRLV...ELVTVTGAVIILLLEIPDI...VLRGTRFFGHTILGGP
rbTRPV2 VYMLIF...AVAYHQ...ALEKQG.FP...PLR.NLPGDYFRVT...EILSVAGGVYFFFRGIQY...LQRRR.PSMKALFVDSY
rbTRPV3 FYNITL...LVSYR...REE.EALPH...PLATLTHNGWLQLL...RMFVLLWATCISVKEGIAI...MLRPS.DLQSLSDAW
rbTRPV4 CAMVIF...LTAYYR...LEG.TP.PY...RTTYDFLRLA...EITLLTGVLVFLTSTIKLD...MKKCP.GVNSLFDWGS

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43q         44q         45q         46q         47q         48q         49q         50q         51q
rbTRPV5 FHVIIITYASLVLLTMVRLTNMNGEVVPLSFALV...LWCVSVM...FARGFQMLCPPTI...MIFG...LMLR...CWLMAVILL...CFAS...PHITFQT
rbTRPV6 FHVLIITYAFVMLTMMVRLTNDGGEVPMFSFALV...LWCVSVM...FARGFQMLCPPTI...MIFG...LMLR...CWLMAVILL...CFAS...PHITFQT
rbTRPV1 SEMLFFVQALFMLATVVLVYFVSHCKEYVATMVVSLAL...LWCVSVM...FARGFQMLCPPTI...MIFG...LMLR...CWLMAVILL...CFAS...PHITFQT
rbTRPV2 FELFLQALVTLVSVLFLATEWVLLVSSLV...LWCVSVM...FARGFQMLCPPTI...MIFG...LMLR...CWLMAVILL...CFAS...PHITFQT
rbTRPV3 FHFVFFVQALVTLVSVLFLATEWVLLVSSLV...LWCVSVM...FARGFQMLCPPTI...MIFG...LMLR...CWLMAVILL...CFAS...PHITFQT
rbTRPV4 FQLLYFIYSVLVISAALYLAGLAVLAVMVFALV...LWCVSVM...FARGFQMLCPPTI...MIFG...LMLR...CWLMAVILL...CFAS...PHITFQT

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52q         53q         54q         55q         56q         57q         58q
rbTRPV5 EDPNN.....LGEFSDYPTALFSTFELFLTIIDGPANY.SVDLPFMYCITYAFAFIATLMLNLFIAM...GDTHW
rbTRPV6 QDPE.....LGHFYSYPMALFSTFELFLTIIDGPANY.AVDLPFMYCITYAFAFIATLMLNLFIAM...GDTHW
rbTRPV1 KNSSTSAESTSHRWGFGCRS...SDSSYNSLYSTCLLEFKFTIIMGDLEFTE.NYDFKAVFILLLAVVILTYI...LLNMLIAL...GSETVN
rbTRPV2 AQNSRTPAGPNATEVGGPQAGQDEAPPYRSLDASLELFKFTIIMGELAFQE.QLRFVGVVLLLLAYVLLTV...LLNMLIAL...GSETVN
rbTRPV3 VLRRA...SSGYSFGTAVLSSQAQCSGLDGLNIQAEPPPTSPCFCSWLLINYV.ISLR...PTSNHRL...GSETVN
rbTRPV4 CANNKVCDEGQSNTTPTY...P...SCRDSETFFLDLFLKTIIMGDLEMLG.SAKYPVVFIILLVITYIILT...LLNMLIAL...GSETV*

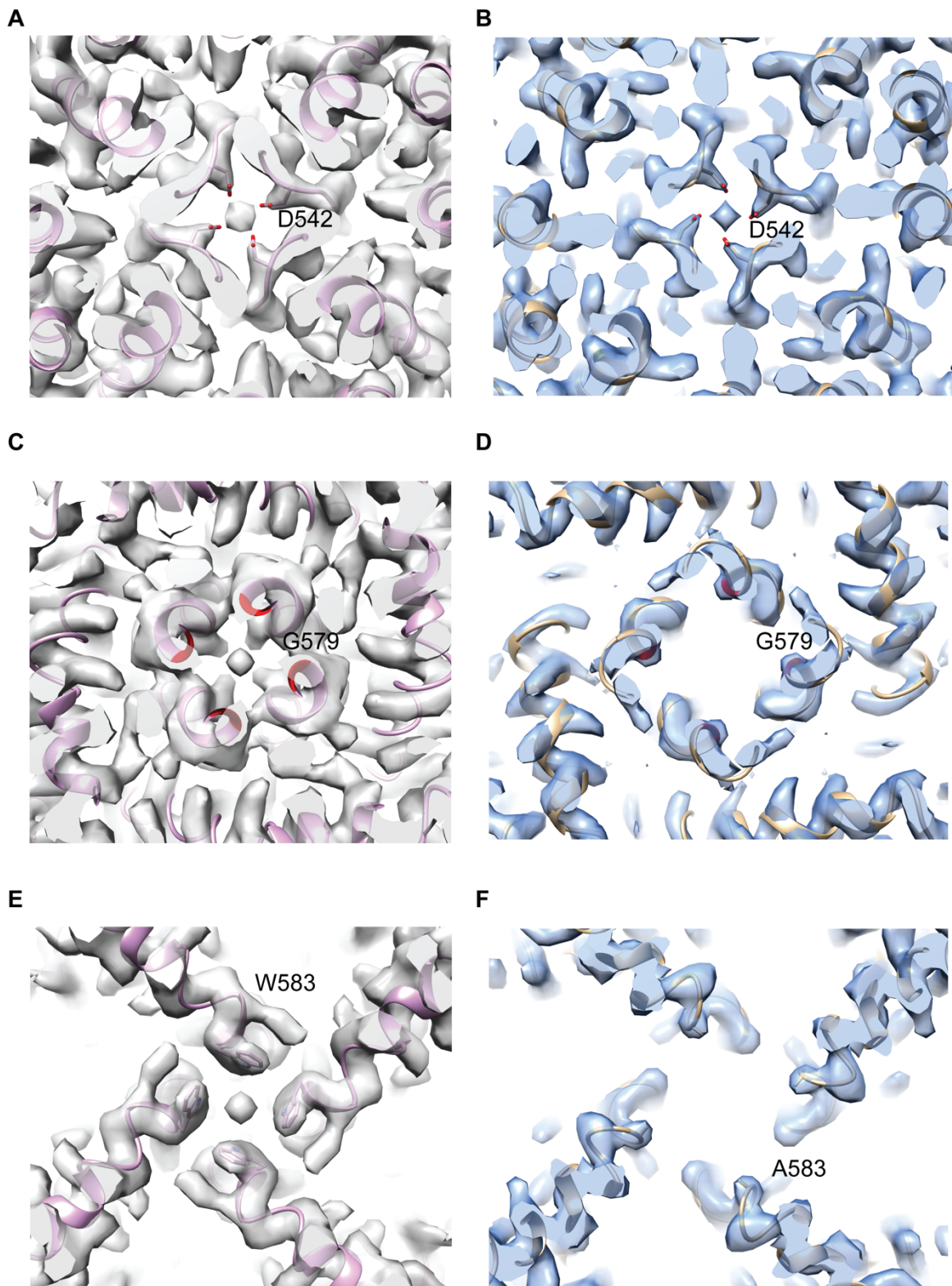
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59q         60q         61q         62q         63q         64q         65q         66q
rbTRPV5 RVAQERDELW...RAOVVATTVMLE...RKMPRLWPRSGIC...YEYGL...GD...WFL...VENHHDQNLPLRVL.R.YVFAFKCSKDEKDGQEQ
rbTRPV6 RVAHERDELW...RAOVVATTVMLE...RKLPRLWPRSGIC...CKRYGL...GD...WFL...VEDRDQDLNRRMR.R.YAQFNLSGSDDLDDK
rbTRPV1 KVAQDSKSIW...KLORAITILD...KGLKCMRKAF.RSCKLLQVGYTPDGKDCR...WCF...RVEVNWTTWNTNVG.II...
rbTRPV2 SIAETSWSIW...KLOKAVISVLE...NGYWCRRKQ.RAGVMLVTVTRPDGSPDR...WCF...RVGEMNWTWQTLPTL...
rbTRPV3 NVSKETKRIKACX...ORARTILEF...KMLPEWLRSRF.RMGELCVKA...EEDF...RCL...RINEVKTWETKTHVS.FL...
rbTRPV4 QVSKSKHIW...KLOWATTILD...ERSFPVRRKAF.RSGEMVTVGKSSGSDGSPDR...WCF...RVEVNWWSHWNQNLG.II...

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67q         68q         69q         70q         71q
rbTRPV5 LSEKRPSTVESGMLSR.ASVAFQTPSLRSTTSQSSNSHRGWELRRLNTL...GHLNLGLD...
rbTRPV6 SEEKL...ELRHPLG.PROPFPMPALSRASRSS...MNWEKLRQAGLR...DL.R.GVINRALE...
rbTRPV1 ...NEDPGNCE...GVKRTLSF...SLRSG...RNVKFNALVPLLRDASTRDRRHPXPPEDVHLRPPVVG...
rbTRPV2 ...CEEPGAA...AP.GVMKNTPPASQGED...SASEEDHPLQLQSR...
rbTRPV3 ...KEDPGPK...RT.Ad.L...NKIQD...SSRS...NS.K.TTLNAFEE...
rbTRPV4 ...NEDPGKNE...TY.QYYGF...SHTVG...RLRR...DRWS...SVVPRVVEL...KNNSNPDVAVVPLAHSVGN

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72q         73q
rbTRPV5 ...LGEGDGEEV.YHF
rbTRPV6 ...DEEGWEYQ...I
rbTRPV1 SLKPGDAELFKDSVAAAEK
rbTRPV2 ...MEEFPETSV...
rbTRPV3 ...PSGPGHQSHPPQVAD...

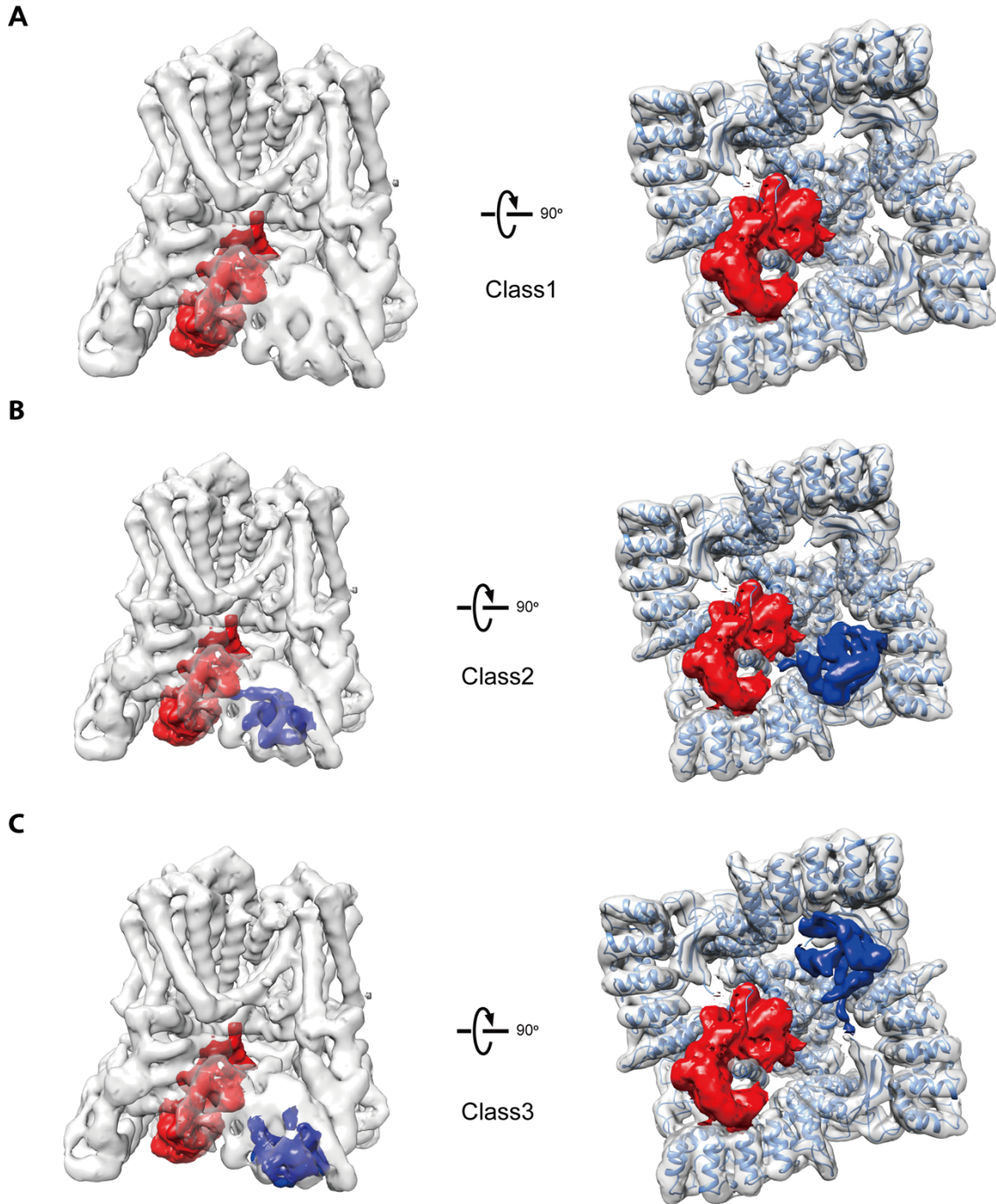
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**Fig. S7.** Sequence alignment of the TRPV family members. Complete sequence alignment of all rabbit members of the TRPV subfamily. Domains and corresponding secondary structures are marked according to rbTRPV5. Full conserved residues are marked by dark grey overlay and regions discussed in the main text are highlighted in light gray. Asterisks denote all residues that are mentioned in the main text.

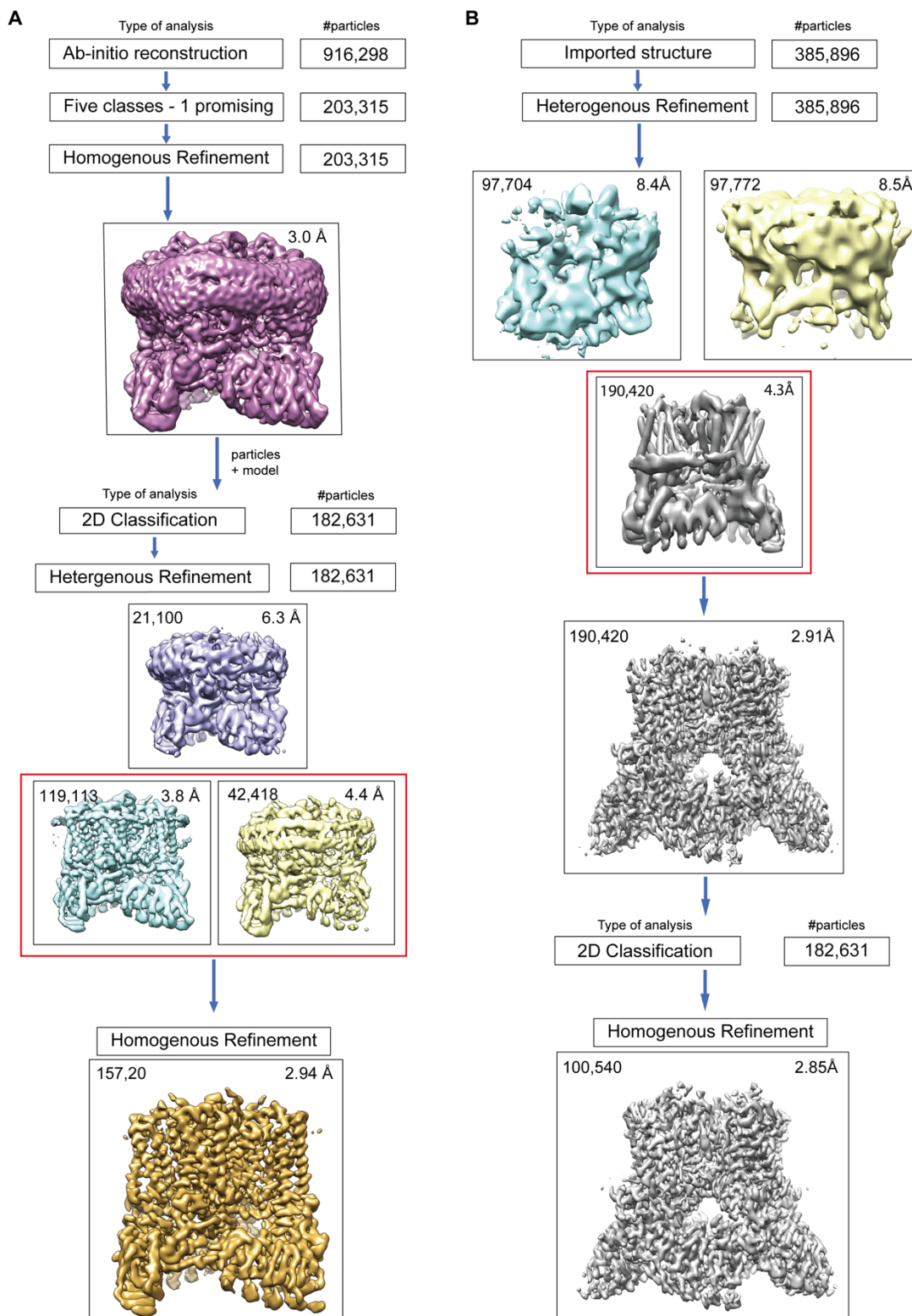


**Fig. S8.** Densities in the pore of TRPV5 1-660 and TRPV5 W583A. (A,B) Top view indicating the presence of the density in the selectivity filter of the nanodisc-reconstituted TRPV5 1-660 (A) and TRPV5 W583A (B). The side chains are visualized for D542. (C,D) Bottom view indicating the presence and absence of the density in nanodisc-reconstituted TRPV5 1-660 (C) and TRPV5

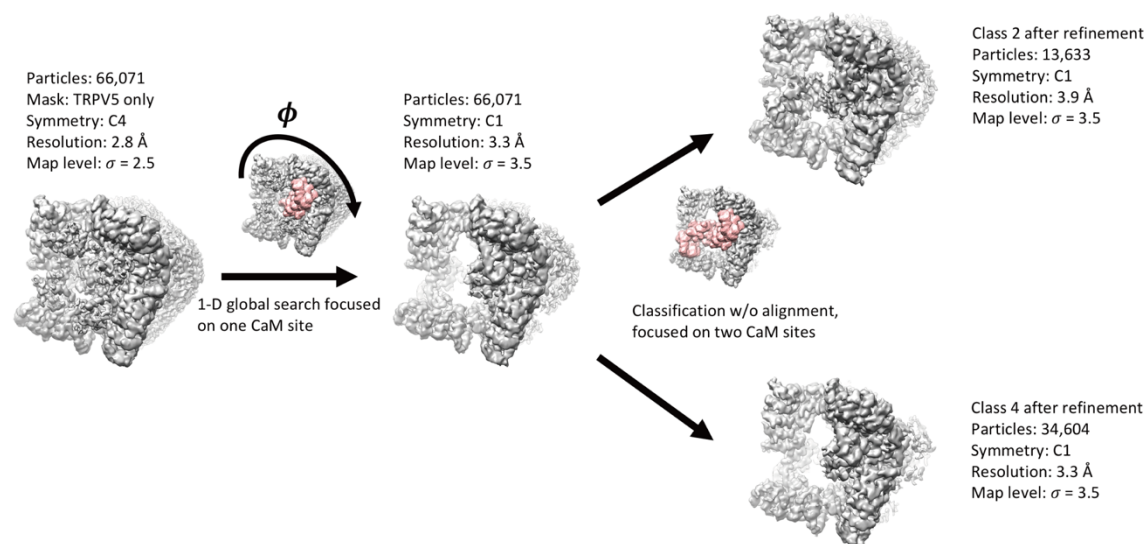
W583A (D), respectively. Residue G579 is projected in red. (E,F) Bottom view indicating the presence and absence of the density in nanodisc-reconstituted TRPV5 1-660 (E) and TRPV5 W583A (F), respectively. The side chains are visualized for the residues at the lower part of the pore, W583 (E) and A583 (F).



**Fig. S9.** Comparison of cryo-EM densities for TRPV5-CaM from different classes. (A-C) Front and bottom views of cryo-EM densities for TRPV5-CaM showing 3 different classes obtained after 3D classification in RELION. Extra densities belonging to CaM are indicated in red (N- and C-lobe) and blue (only N-lobe).



**Fig. S10.** Data processing and model building (A) Data processing workflow with the number of particles and the reconstruction resolutions of the TRPV5 1-660 sample indicated at every step. (B) Data processing workflow with the number of particles and the reconstruction resolutions of the TRPV5 W583A sample indicated at every step.



**Fig. S11.** Data process workflow of further alignment and classification. Bumber of particles, resolution and symmetry applied are indicated at every step.



**Table S1. Summary of Cryo-EM Data Collection and Model Refinement**

	1-660	Full-length in ND	W583A	V5-CaM
<b>Data Collection/Processing</b>				
<b>Voltage (kV)</b>	300	300	300	300
<b>Magnification</b>	130,000	22,500	22,500	22,500
<b>Defocus Range (<math>\mu\text{m}</math>)</b>	0.6 – 2.3	0.6 – 2.5	0.8 – 2.0	0.7 – 2.2
<b>Pixel Size (<math>\text{\AA}</math>)</b>	0.84	1.059	1.059	1.059
<b>Total Electron Dose (<math>\text{e}^-/\text{\AA}^2</math>)</b>	68	64	70	63
<b>Exposure Time (s)</b>	12	10	10	10
<b>Number of Images</b>	2968	930	1146	1135
<b>Number of Frames/Image</b>	60	50	50	50
<b>Initial Particle Number</b>	916,298	408,785	385,896	482,348
<b>Final Particle Number</b>	157,203	87,603	100,540	66,071
<b>Resolution (unmasked, <math>\text{\AA}</math>)</b>	3.7	3.8	3.7	3.8/4.1/4.5*
<b>Resolution (masked, <math>\text{\AA}</math>)</b>	2.9	3.0	2.8	3.0/3.2/3.3*
<b>Refinement</b>				
<b>Number of Atoms</b>	19584	19584	19612	20950
<b>RMS Deviations</b>				
<b>Bond Lengths (<math>\text{\AA}</math>)</b>	0.010	0.011	0.010	0.008
<b>Bond Angles (<math>^\circ</math>)</b>	1.262	1.366	1.076	1.082
<b>Ramachandran</b>				
<b>Favored (%)</b>	95.07	94.09	96.73	93.87
<b>Allowed (%)</b>	4.76	5.91	3.27	6.09
<b>Outlier (%)</b>	0.16	0.0	0.0	0.04
<b>Molprobity Score</b>	1.62	1.87	1.93	1.94
<b>EMRinger Score</b>	2.36	2.87	2.95	2.2

\*Resolution shows refinement with C4/C2/C1 symmetry applied respectively.