

Supplementary Materials for
**TRPV3 activation by different agonists accompanied by lipid dissociation
from the vanilloid site**

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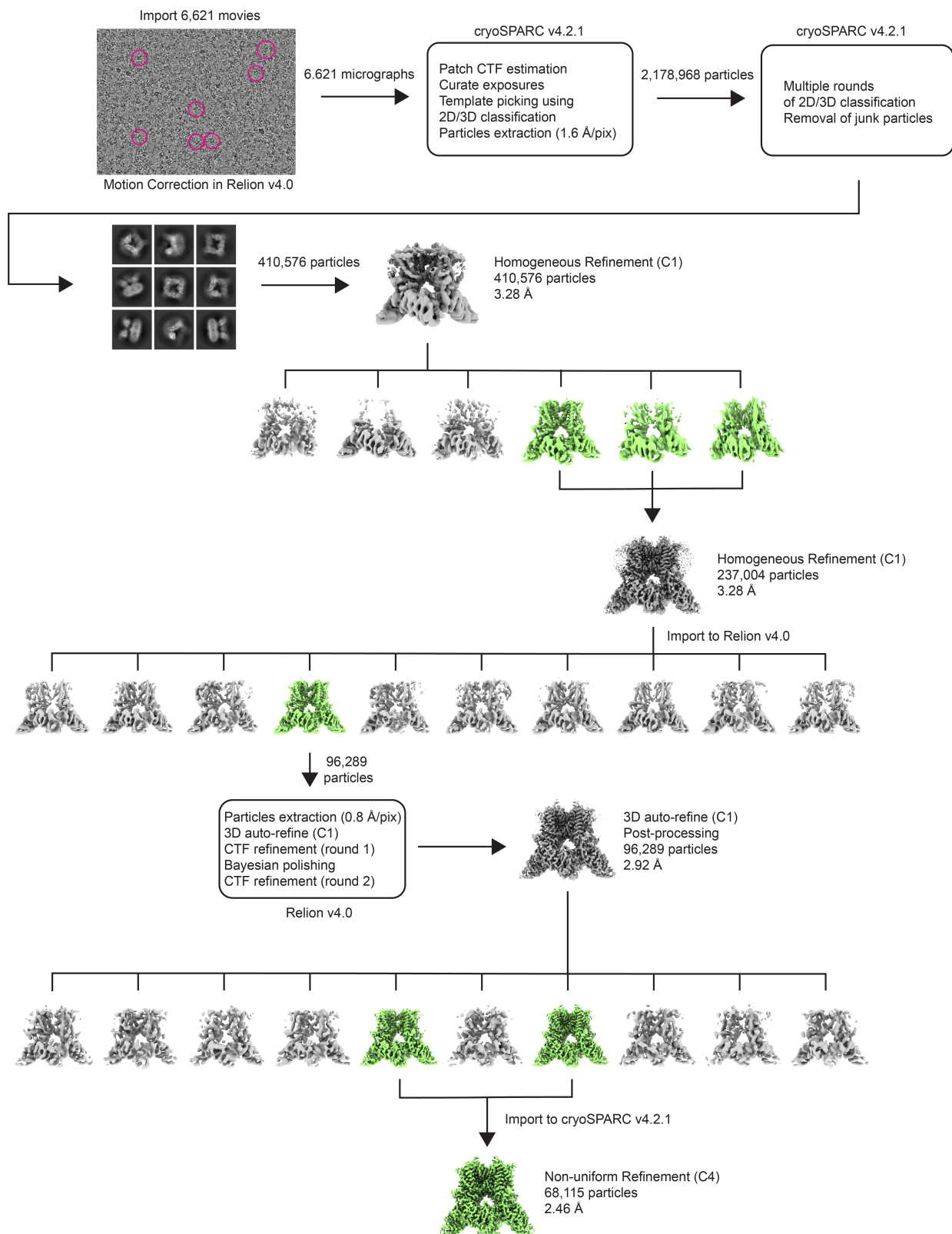


Fig. S1. Cryo-EM data processing workflow for the APO structure

The workflow includes examples of micrographs, 2D class averages and 3D classes.

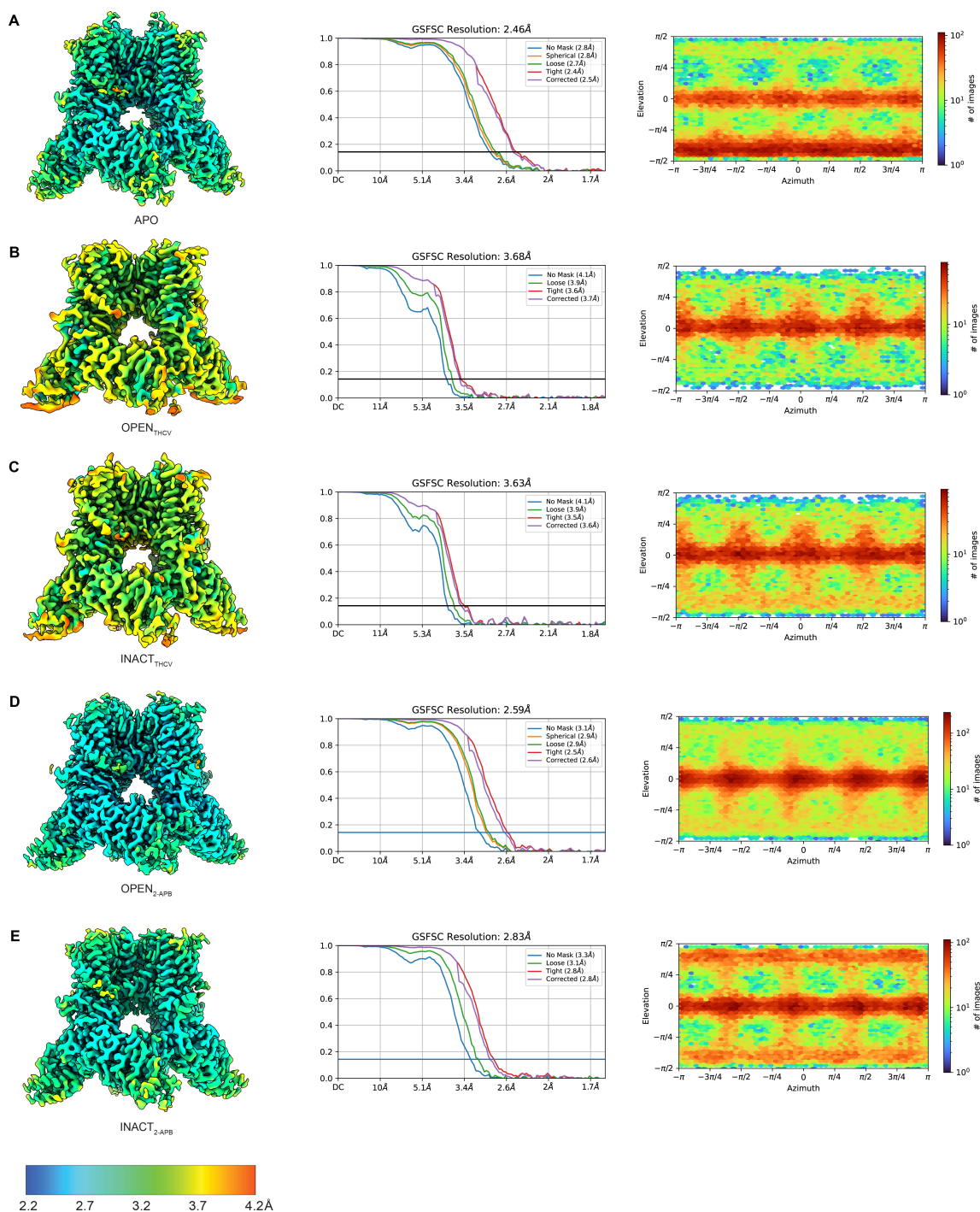


Fig. S2. Characteristics of cryo-EM reconstructions.

(A-E) Cryo-EM maps colored according to the local resolution estimation in cryoSPARC (left), FSC curves calculated between half maps, with the overall resolution estimated using the FSC = 0.143 criterion (middle) and angular distribution of particles calculated using the 3D refinement reconstruction algorithm in cryoSPARC (right) for the APO (A), OPEN_{THCV} (B), INACT_{THCV} (C), OPEN_{2-APB} (D) and INACT_{2-APB} (E) structures.

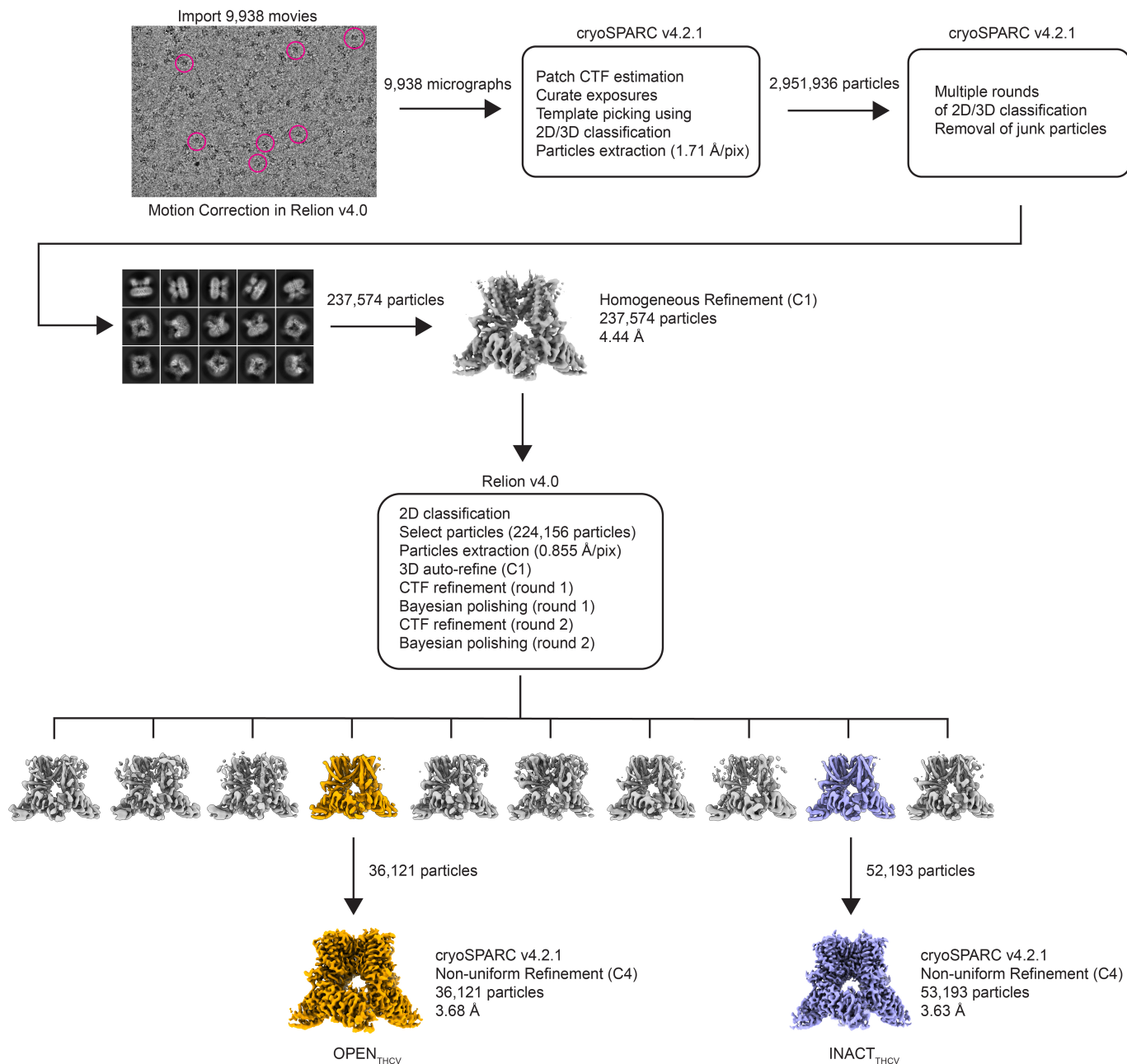


Fig. S3. Cryo-EM data processing workflow for TRPV3 in the presence of THCV.

The workflow includes examples of micrographs, 2D class averages and 3D classes.

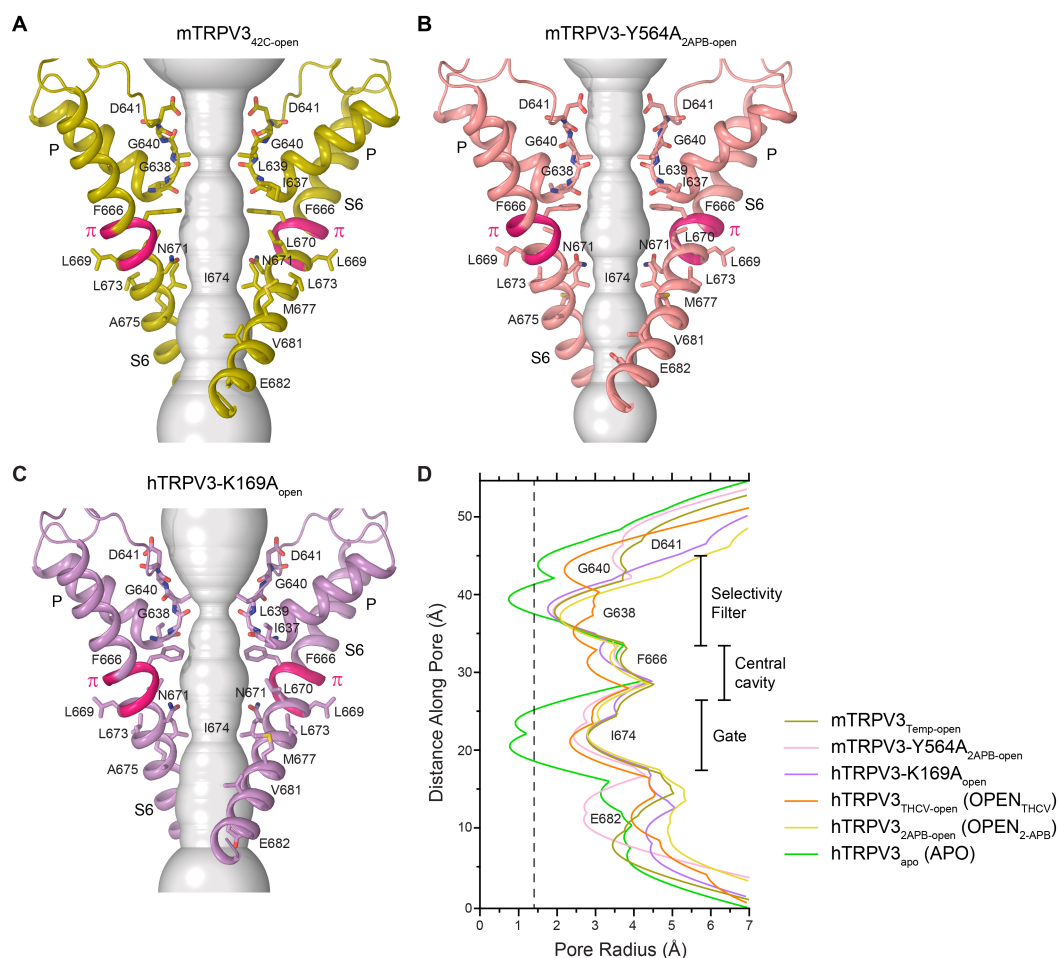


Fig. S4. Comparison of pores in open-state structures of TRPV3.

(A-C) Pore-forming domains in mTRPV3_{Temp-open} (A, PDB ID: 7MIO), mTRPV3-Y564A_{2APB-open} (B, PDB ID: 6DVZ) and hTRPV3-K169A_{open} (C, PDB ID: 6UW6), with residues contributing to pore lining shown as sticks. Only two of four subunits are shown, with the front and back subunits omitted for clarity. The pore profile is shown as a space-filling model (grey). The region that undergoes the α -to- π transition in S6 is highlighted in pink. (D) Pore radius for different TRPV3 structures calculated using HOLE. The vertical dashed line denotes the radius of a water molecule, 1.4 Å.

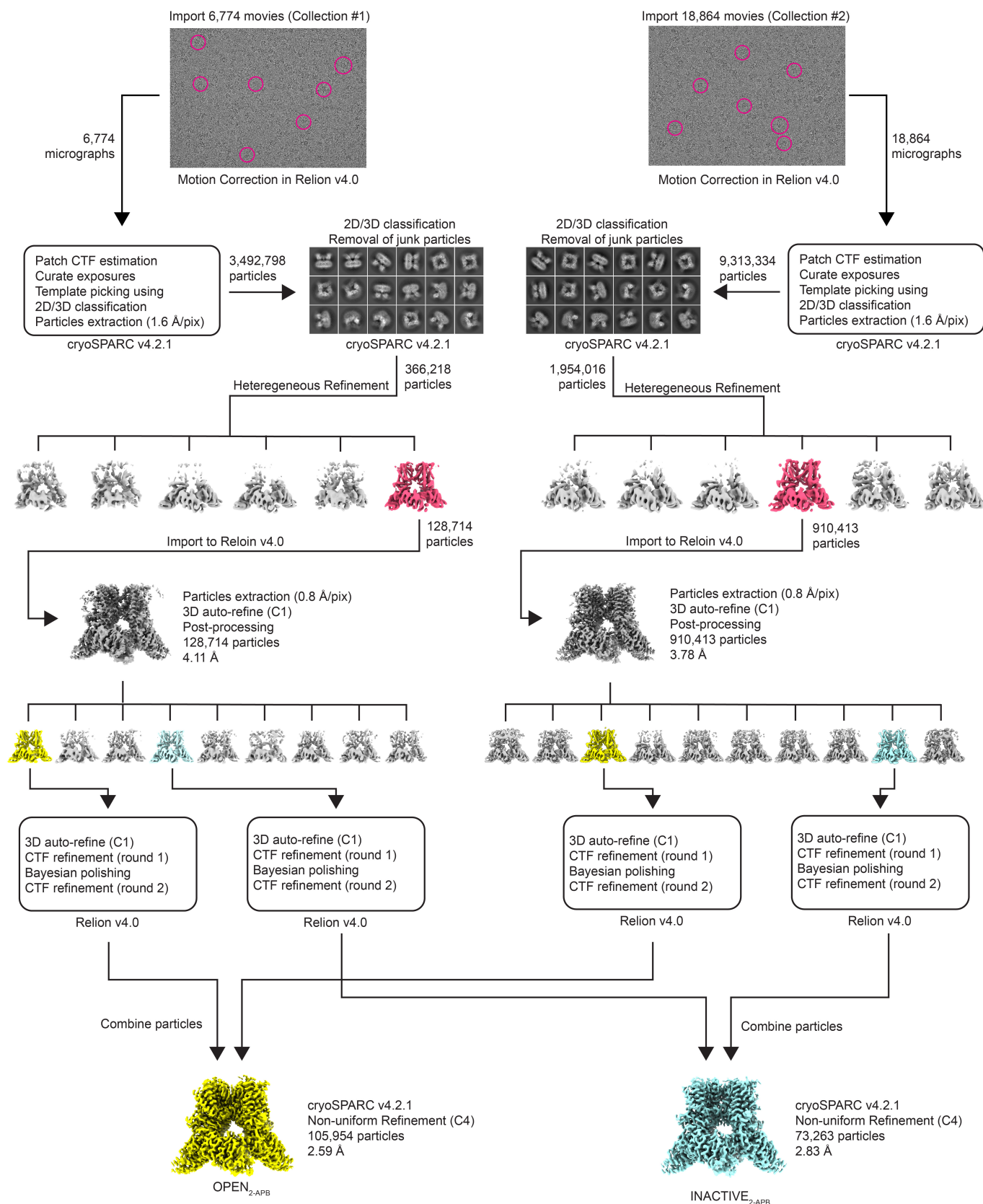


Fig. S5. Cryo-EM data processing workflow for TRPV3 in the presence of 2-APB.

The workflow includes examples of micrographs, 2D class averages and 3D classes.

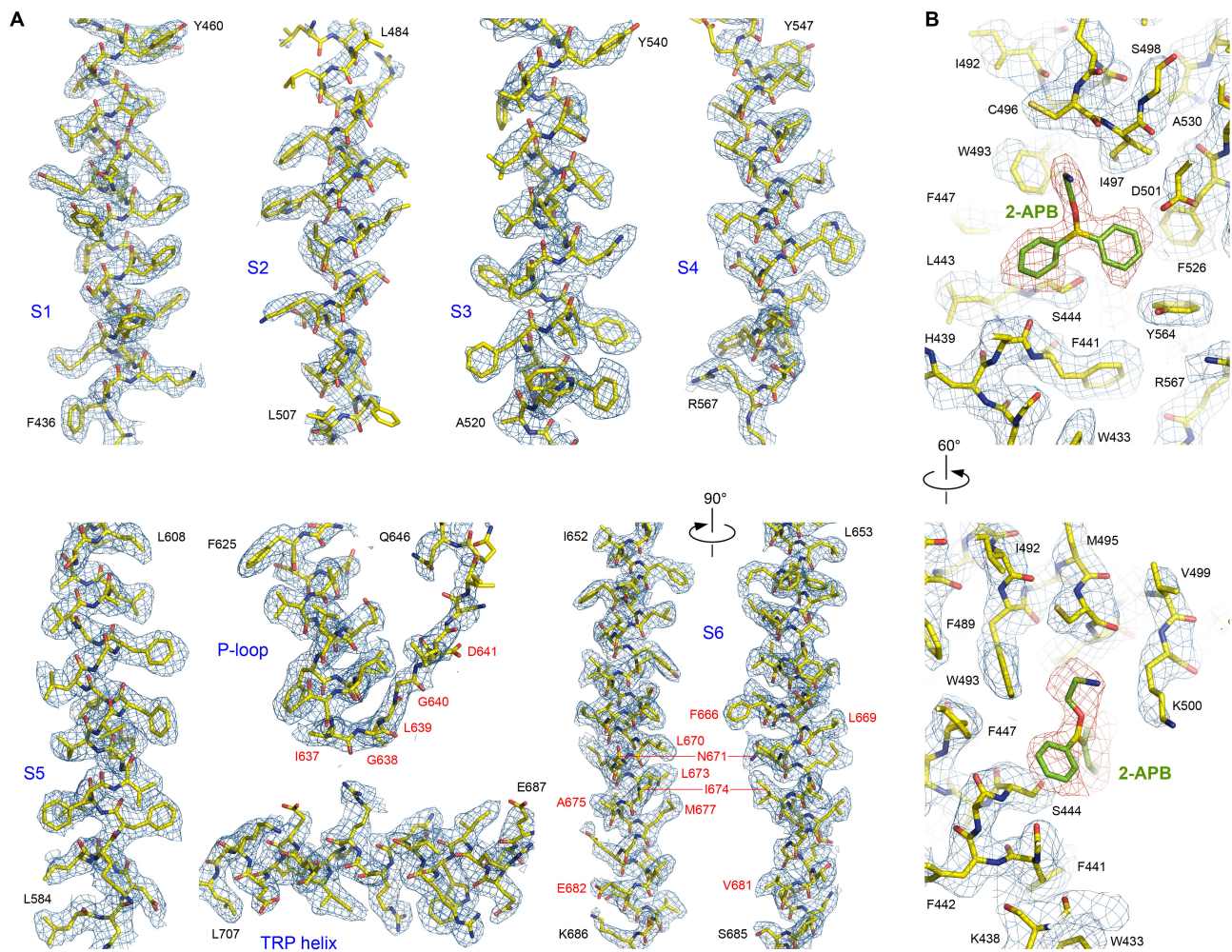


Fig. S6. Cryo-EM density of TRPV3.

(A) Fragments of the OPEN_{2-APB} TMD with the structural model shown as yellow sticks and the corresponding cryo-EM density as a blue mesh. Red labels indicate residues that contribute to pore lining. **(B)** Close-up view of the S1-S4 base site (site 2) with the protein and 2-APB molecule shown as yellow and green sticks and the corresponding density as red and blue mesh.

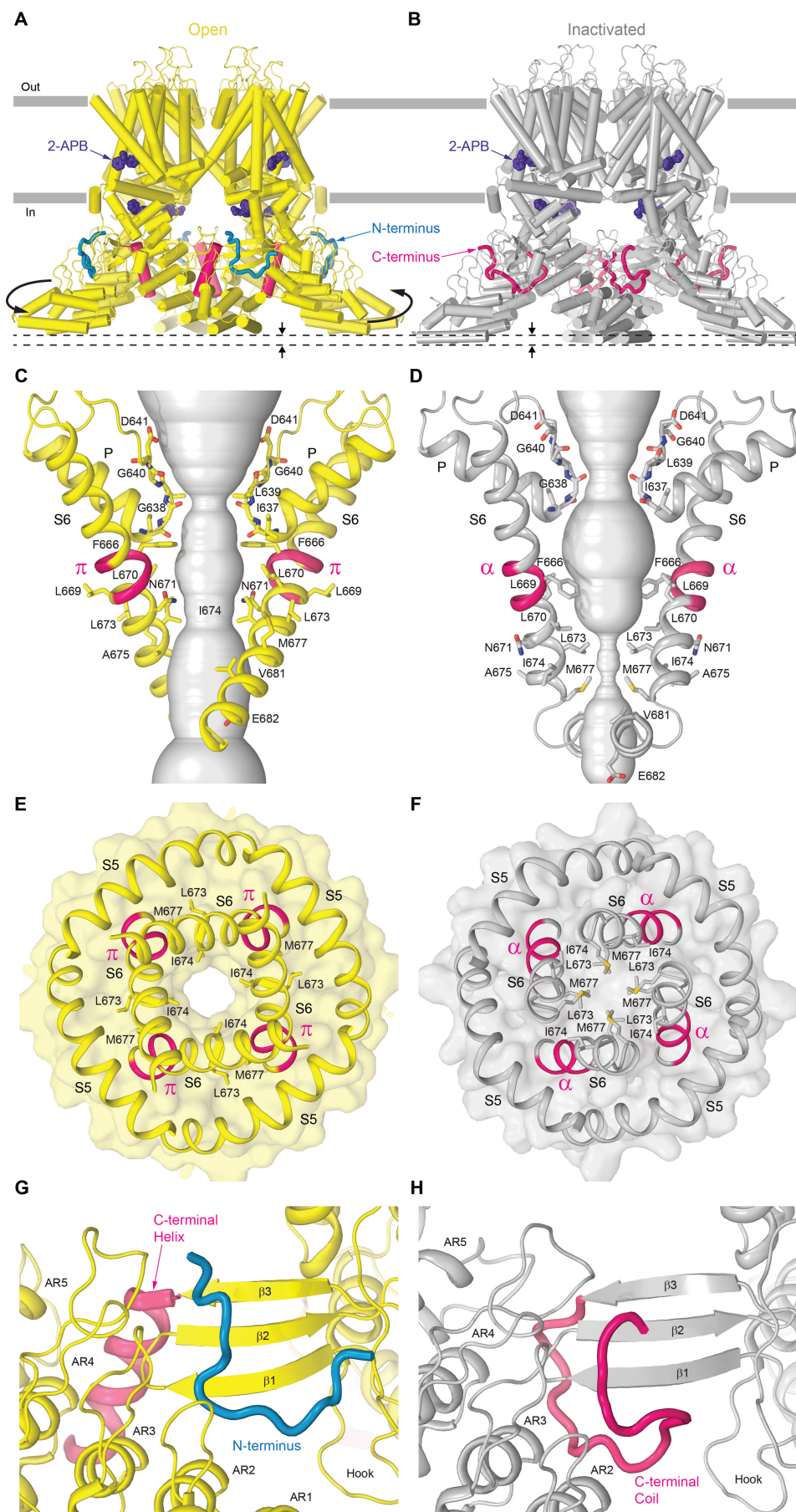


Fig. S7. 2-APB-bound open and inactivated states.

(**A-B**) Structures of OPEN_{2-APB} (**A**) and INACT_{2-APB} (**B**), viewed parallel to the membrane, with the N- and C-termini colored blue and pink, respectively, and the molecules of 2-APB (purple) shown as space-filling models. (**C-D**) Pore-forming domains in OPEN_{2-APB} (**C**) and INACT_{2-APB} (**D**), with residues contributing to pore lining shown as sticks. Only two of four subunits are shown, with the front and back subunits omitted for clarity. The pore profile is shown as a space-filling model (grey). The region that undergoes the α -to- π transition in S6 is highlighted in pink. (**E-F**) Intracellular view of the S6 bundle crossing in OPEN_{2-APB} (**E**) and INACT_{2-APB} (**F**), with the surface shown in the corresponding color. (**G-H**) Interface between the neighboring subunits in OPEN_{2-APB} (**G**) and INACT_{2-APB} (**H**), with the C-terminus (pink) adapting coiled (**H**) or helical (**G**) conformations and N-terminus (blue) appearing in the open state (**G**).

Table S1. Cryo-EM data collection, refinement, and validation statistics.

Structure	APO	OPEN_{THCV}	INACT_{THCV}	OPEN_{2APB}	INACT_{2APB}
Ligand	-	THCV	THCV	2-APB	2-APB
EMDB accession code	EMDB-42994	EMDB-42995	EMDB-42996	EMDB-42997	EMDB-42998
PDB accession code	8V6K	8V6L	8V6M	8V6N	8V6O
Data collection and processing					
Magnification	29,000x	105,000x	105,000x	29,000x	29,000x
Voltage (kV)	300	300	300	300	300
Electron exposure (e ⁻ Å ⁻²)	60	60	60	60	60
Defocus range (μm)	-0.75 to -1.5	-0.5 to -1.75	-0.5 to -1.75	-0.75 to -1.5	-0.75 to -1.5
Reported pixel size (Å)	0.788	0.855	0.855	0.394	0.394
Calibrated pixel size (Å)	0.80	0.855	0.855	0.80	0.80
Exposures (no.)	6621	9938	9938	25,638	25,638
Processing software					
Platform software for particle picking	cryoSPARC v4.2.1	cryoSPARC v4.2.1	cryoSPARC v4.2.1	cryoSPARC v4.2.1	cryoSPARC v4.2.1
Motion correction	MotionCor2	MotionCor2	MotionCor2	MotionCor2	MotionCor2
CTF estimation	Patch CTF	Patch CTF	Patch CTF	Patch CTF	Patch CTF
Software for 2D/3D class. & refinements	Relion v4.0	Relion v4.0	Relion v4.0	cryoSPARC v4.2.1	cryoSPARC v4.2.1
Symmetry imposed	C4	C4	C4	C4	C4
Initial particle images (no.)	2,178,968	2,951,936	2,951,936	12,806,132	12,806,132
Final particle images (no.)	68,115	36,121	52,193	105,954	73,263
Map resolution (Å)	2.46	3.68	3.63	2.59	2.83
FSC 0.143					
Map resolution range (Å)	2.21/3.19/32.95	2.50/5.056/9.62	2.46/5.28/9.51	2.22/3.56/32.78	2.49/4.56/16.37
min/median/max					
Refinement					
Initial models used (PDB code)	6UW6	This study	This study	This study	This study
Model resolution (Å)	2.46	3.68	3.63	2.61	2.83
FSC threshold					
Map sharpening	-76.7	-143.2	-150.3	-95.1	-84.7
B factor (Å ²)					
Model composition					
Non-hydrogen atoms	22,537	22,387	21,547	22,011	22,639
Protein residues	2560	2612	2560	2544	2560
Ligand atoms	2045	1127	711	1449	1847
R.m.s. deviations					
Bond lengths (Å)	0.004	0.002	0.004	0.003	0.003
Bond angles (°)	0.913	0.550	0.966	0.799	0.805
Validation					
CC (across whole map volume)	0.7077	0.7417	0.6148	0.5623	0.5961
CC (only across atoms in the model)	0.7762	0.7559	0.7101	0.7363	0.6883
Clashscore, all atoms	2.18	3.15	2.33	3.67	4.34
Poor rotamers (%)	0.00	5.12	0.00	0.00	0.35
Ramachandran plot					
Favored (%)	92.67	86.59	88.24	94.40	94.51
Allowed (%)	6.54	13.41	10.66	5.60	5.49
Disallowed (%)	0.78	0.00	1.10	0.00	0.00

Table S2. EC_{50} values for TRPV3 activation by THCV and 2-APB. The number of independent measurements, n , equals to 3 where the information is not provided.

Mutation	Site	EC_{50}, 2-APB, μM	n_{HILL}, 2-APB	EC_{50}, THCV, μM	n_{HILL}, THCV
WT		93.03 ± 5.02 $n = 6$	1.61 ± 0.04 $n = 6$	6.1 ± 0.5	1.63 ± 0.13
L557A	site 1	43.46 ± 1.84	1.02 ± 0.05	0.081 ± 0.001	1.03 ± 0.09
A560L	site 1	117.6 ± 6.6	1.52 ± 0.11	3.59 ± 0.19	1.95 ± 0.18
A560M	site 1	98.3 ± 12.7	1.41 ± 0.14	9.35 ± 0.91	2.36 ± 0.22
L563A	site 1	127.6 ± 14.6	1.27 ± 0.13	6.04 ± 0.19	1.88 ± 0.10
L563F	site 1	7572 ± 3639	0.96 ± 0.21	>100	
N561A	site 1	24.4 ± 1.7	1.65 ± 0.17	20.33 ± 1.41	2.95 ± 0.42
S444A	site 2	109.2 ± 8.5	1.67 ± 0.11	2.43 ± 0.14	2.08 ± 0.24
S444L	site 2	10.62 ± 0.75	1.63 ± 0.10	3.89 ± 0.58	1.80 ± 0.39
E501A	site 2	> 10000	N/A	35.48 ± 0.48	2.10 ± 0.05
C496A	site 2	34.58 ± 3.14	1.37 ± 0.13		
H426A	site 3	52.4 ± 10.4 $n = 6$	1.30 ± 0.05	1.41 ± 0.09	3.46 ± 0.44
L420A	site 3	245.3 ± 29.3	1.18 ± 0.04	2.66 ± 0.15	1.84 ± 0.17
H430A	site 3	63.4 ± 8.9	1.34 ± 0.14	3.84 ± 0.18	2.36 ± 0.20
R696K	site 3	118.8 ± 13.2	1.29 ± 0.17	3.88 ± 0.28	2.41 ± 0.31
R693F	site 3	499 ± 28	2.93 ± 0.32	20.1 ± 4.9	1.83 ± 0.58