

Supplementary Information for

Structural mechanism of the agonist binding on human TRPC3 channel

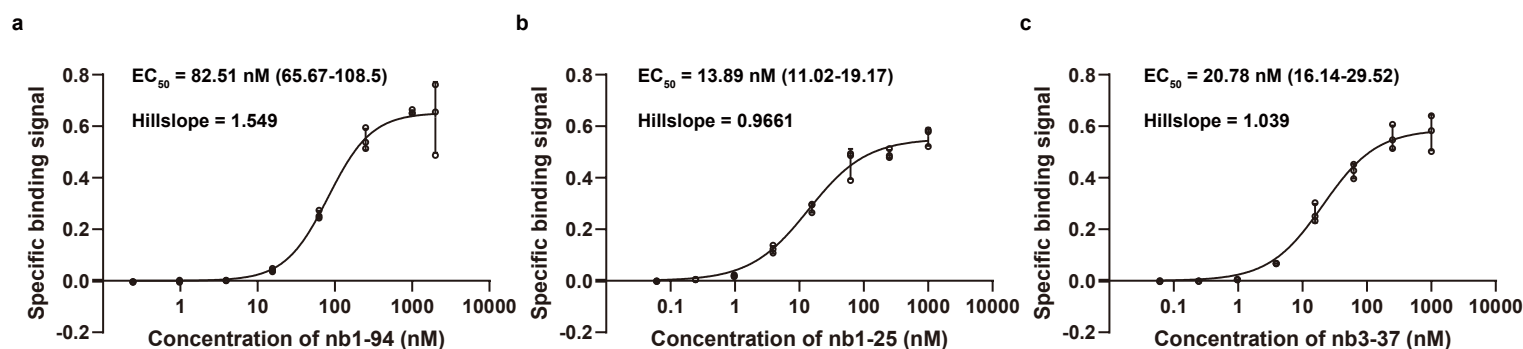
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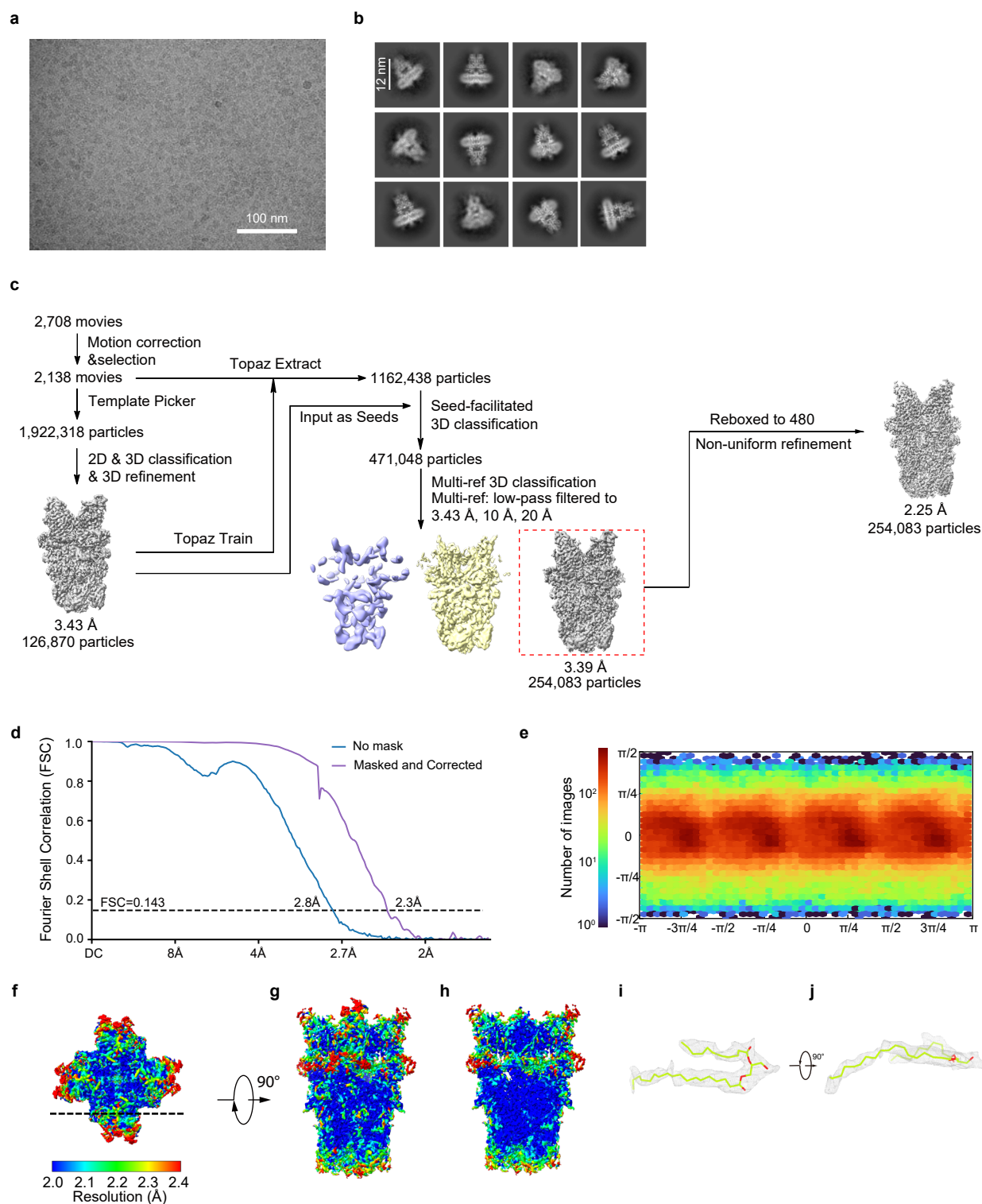


Supplementary Figure 1. Estimation of the affinity between hTRPC3 and nanobodies.

a, Specific ELISA signal of nanobody 1-94 on immobilized hTRPC3 was fitted into a four-parameter variable slope (Hill coefficient) dose-response curve. Estimated affinity (EC_{50}) and 95% confidence intervals (in bracket) are denoted. Each data point is shown as an open circle. Data are mean $\pm$ s.d.; $n = 3$ technical replicates. EC_{50} , median effect concentration. Source data are provided as a Source Data file. The experiments were repeated with two times with similar results.

b, The fitting curve of nanobody 1-25 and hTRPC3 interaction. Data are mean $\pm$ s.d.; $n = 3$ technical replicates. EC_{50} , median effect concentration. Source data are provided as a Source Data file. The experiments were repeated with two times with similar results.

c, The fitting curve of nanobody 3-37 and hTRPC3 interaction. Data are mean $\pm$ s.d.; $n = 3$ technical replicates. EC_{50} , median effect concentration. Source data are provided as a Source Data file. The experiments were repeated with two times with similar results.



Supplementary Figure 2. Cryo-EM image analysis of hTRPC3-Nb1-94 complex.

a, Representative raw micrograph of hTRPC3-Nb1-94 complex.

b, Representative 2D class average of hTRPC3-Nb1-94 complex.

c, Flowchart of hTRPC3-Nb1-94 complex cryo-EM data processing.

d, FSC curves of final refinement for hTRPC3-Nb1-94 complex in high-calcium state (C4 symmetry) after correction for masking effects. Resolution estimations were based on the criterion of the FSC 0.143 cutoff. Source data are provided as a Source Data file.

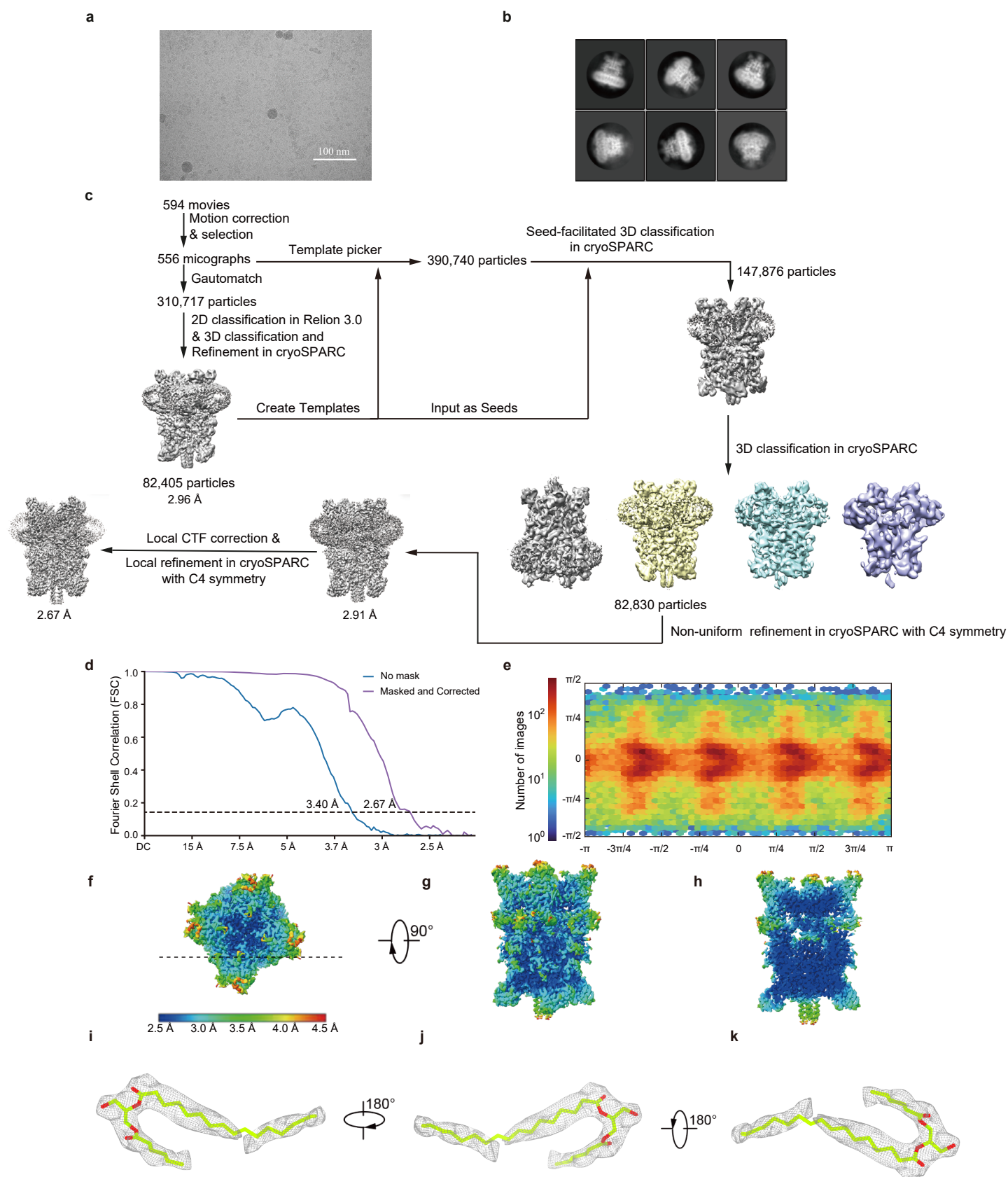
e, Angular distribution of final refinement for hTRPC3-Nb1-94 complex. This is a standard output from cryoSPARC.

f, Local resolution estimation of hTRPC3-Nb1-94 complex in top view.

g, Local resolution estimation of hTRPC3-Nb1-94 complex in side view.

h, The cross-section of local resolution estimation of the hTRPC3-Nb1-94 complex. The position of the cross-section was indicated as a dashed line in **f**.

i-j, Cryo-EM density map of DAG (contoured at 3.20σ).



Supplementary Figure 3. Cryo-EM image analysis of hTRPC3-Nb1-25 complex.

a, Representative raw micrograph of hTRPC3-Nb1-25 complex.

b, Representative 2D class average of hTRPC3-Nb1-25 complex.

c, Flowchart of hTRPC3-Nb1-25 complex cryo-EM data processing.

d, FSC curves of final refinement for hTRPC3-Nb1-25 complex in high-calcium state (C4 symmetry) after correction for masking effects. Resolution estimations were based on the criterion of the FSC 0.143 cutoff. Source data are provided as a Source Data file.

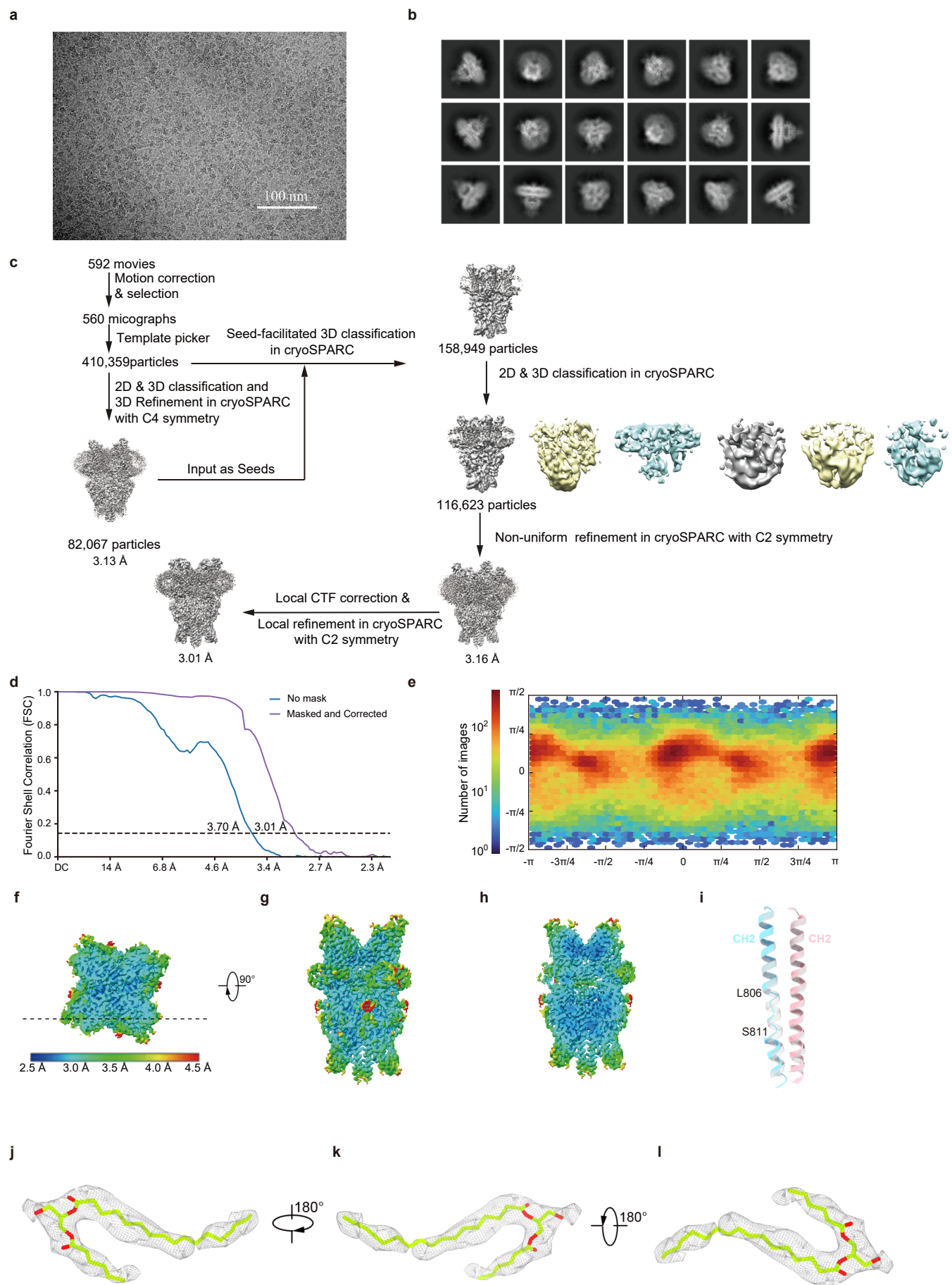
e, Angular distribution of final refinement for hTRPC3-Nb1-25 complex. This is a standard output from cryoSPARC.

f, Local resolution estimation of hTRPC3-Nb1-25 complex in top view.

g, Local resolution estimation of hTRPC3-Nb1-25 complex in side view.

h, The cross-section of local resolution estimation of hTRPC3-Nb1-25 complex. The position of the cross-section was indicated as the dashed line in **f**.

i-k, Cryo-EM density map of DAG (contoured at 3.48σ).



Supplementary Figure 4. Cryo-EM image analysis of hTRPC3-Nb3-37 complex.

a, Representative raw micrograph of hTRPC3-Nb3-37 complex.

b, Representative 2D class average of hTRPC3-Nb3-37 complex.

c, Flowchart of hTRPC3-Nb3-37 complex cryo-EM data processing.

d, FSC curves of final refinement for hTRPC3-Nb3-37 complex in high-calcium state (C2 symmetry) after correction for masking effects. Resolution estimations were based on the criterion of the FSC 0.143 cutoff. Source data are provided as a Source Data file.

e, Angular distribution of final refinement for hTRPC3-Nb3-37 complex. This is a standard output from cryoSPARC.

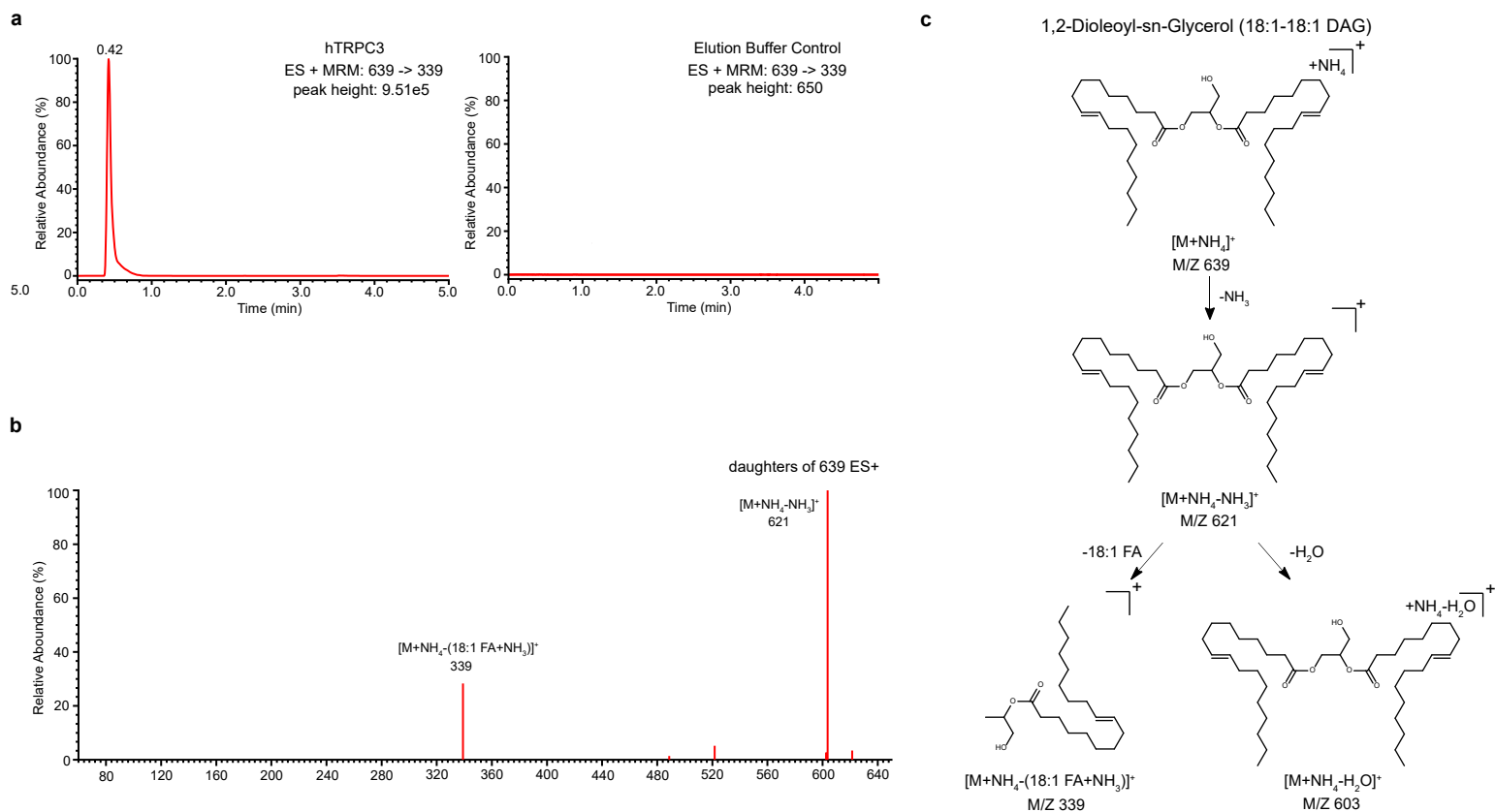
f, Local resolution estimation of hTRPC3-Nb3-37 complex in top view.

g, Local resolution estimation of hTRPC3-Nb3-37 complex in side view.

h, The cross-section of local resolution estimation of the hTRPC3-Nb3-37 complex. The position of the cross-section was indicated as a dashed line in **f**.

i, The Comparison of CH2 between hTRPC3-Nb1-25 complex and hTRPC3-Nb3-37 complex. The CH2 helices belonging to the hTRPC3-Nb3-37 complex are colored the same as in (Fig. 1F), and the CH2 helices belonging to the hTRPC3-Nb1-25 complex are colored in transparent gray.

j-l, Cryo-EM density map of DAG (contoured at 3.35σ).

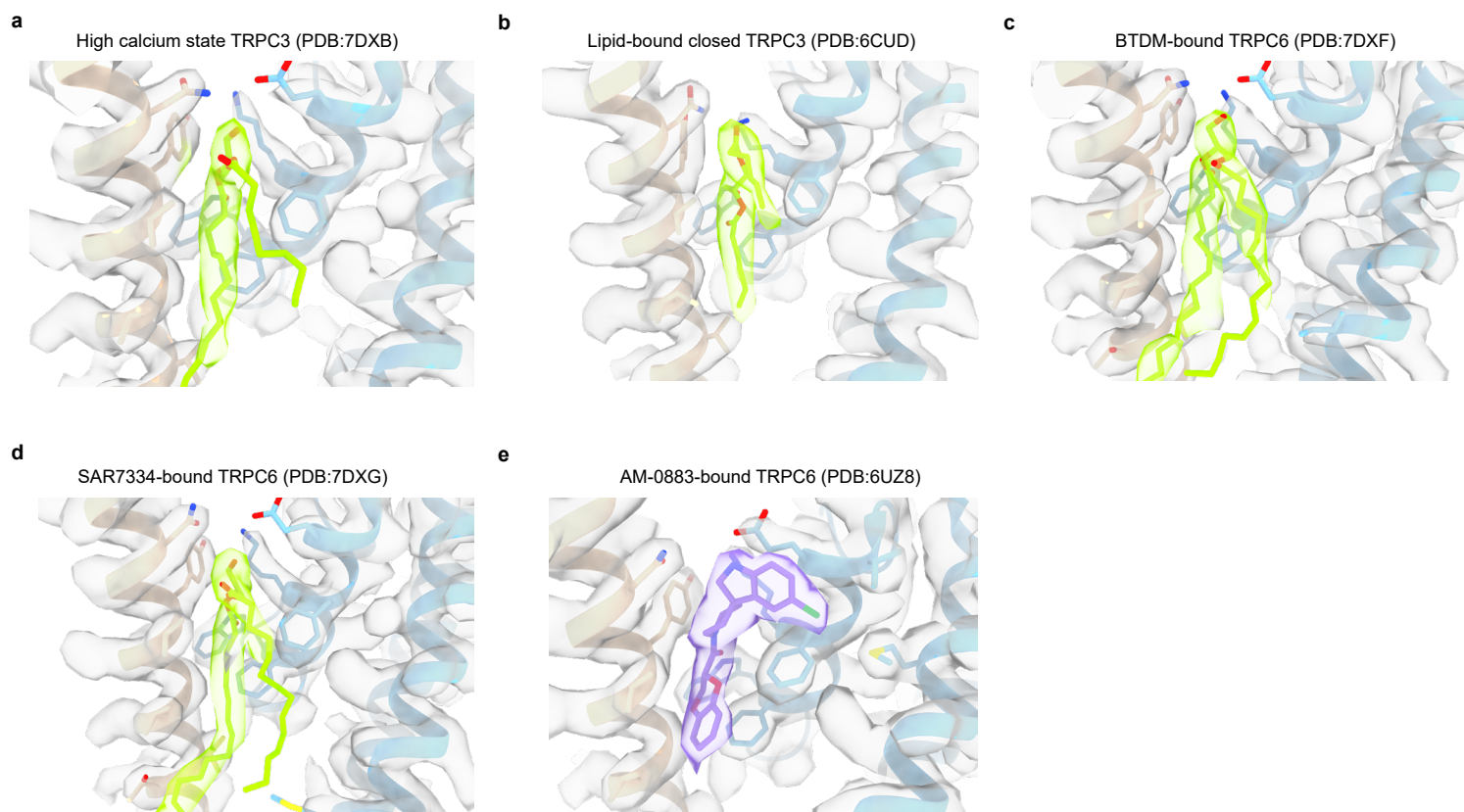


Supplementary Figure 5. The identification of diacylglycerol (DAG) molecule in hTRPC3 protein sample.

a, The HPLC chromatography of C18:1–C18:1 DAG under the MRM transition 639 -> 339 from the hTRPC3 sample (left), and buffer (right). Source data are provided as a Source Data file.

b, MS/MS spectrum of lipid extractants from hTRPC3 sample. Source data are provided as a Source Data file.

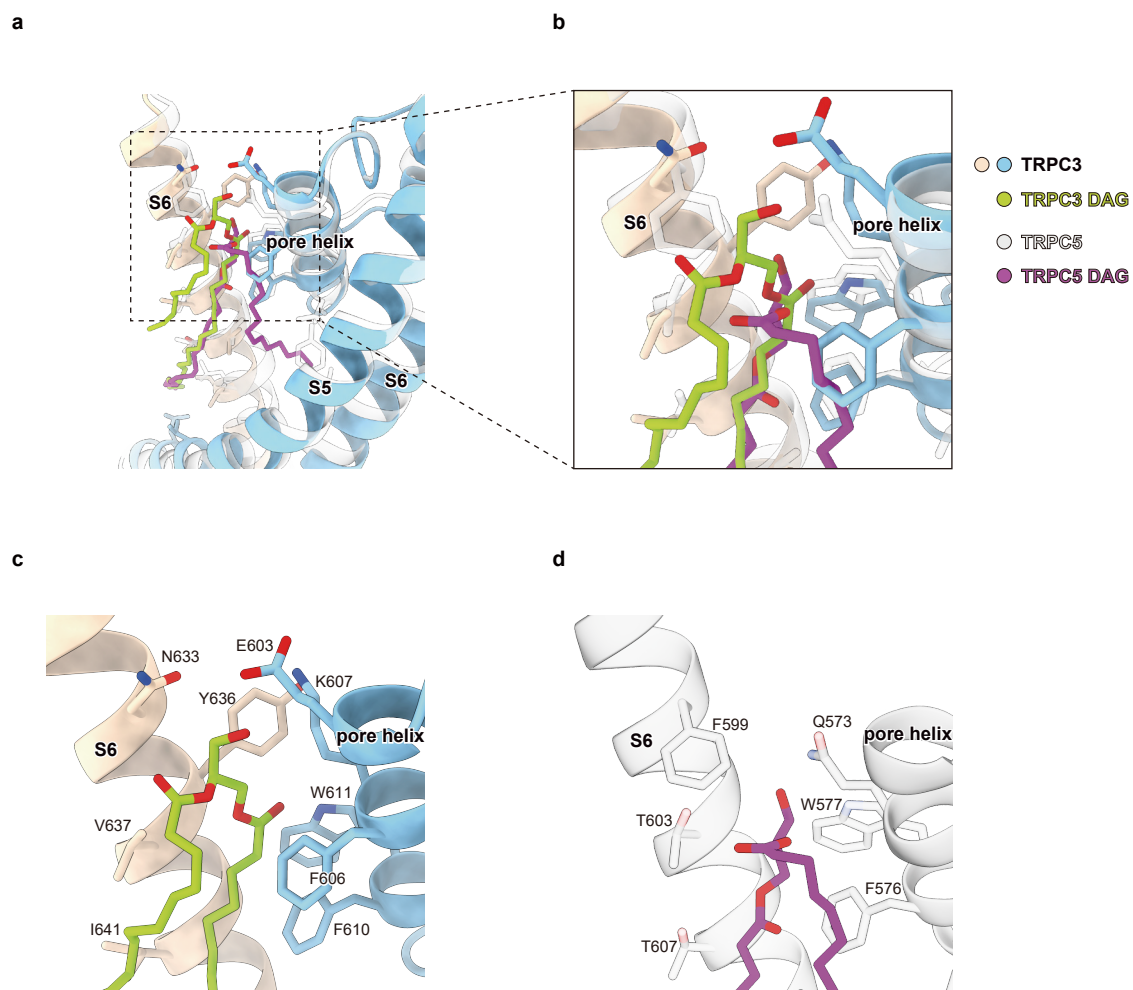
c, Potential MS/MS fragmentation pattern of DAG, using C18:1–C18:1 DAG as an example.



Supplementary Figure 6. Electron densities of DAG and AM-0883 at L2 site in published hTRPC3 and hTRPC6 models.

a-d, Densities of DAG and nearby residues shown in side view. Densities are shown as grey surfaces. The DAG is shown as sticks, and hTRPC3 is shown as cartoon. Both are colored the same as in Fig.1a.

e, Densities of AM-0883 and nearby residues shown in side view. Densities are shown as grey surfaces. The AM-0883 is shown as sticks, and hTRPC3 is shown as cartoons. The AM-0883 is colored purple, and hTRPC3 is colored the same as in Fig.1a



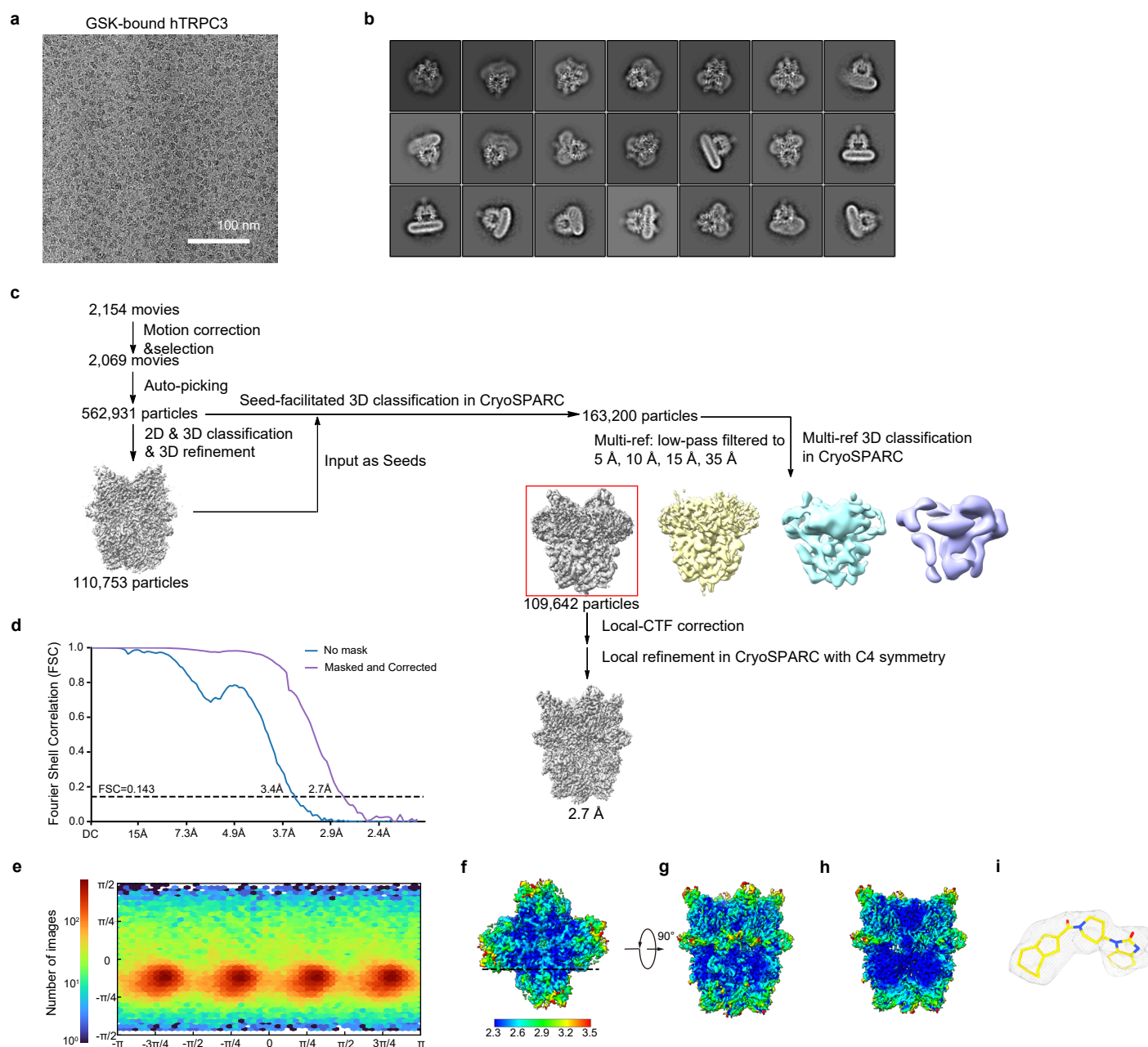
Supplementary Figure 7. Comparison of DAG binding modes between hTRPC3 and hTRPC5.

a, Comparison of DAG binding modes. The color of hTRPC3 structure is the same as that of Fig. 2c, and the DAG bound to TRPC3 is colored in green. The hTRPC5 structure (PDB ID: 7D4P) is displayed in transparent gray, while the DAG bound to hTRPC5 is colored in magenta.

b, The hydrophilic head of DAG and its interacting amino acid residues (shown as sticks).

c, The DAG binding mode of hTRPC3.

d, The DAG binding mode of hTRPC5.



Supplementary Figure 8. Cryo-EM image analysis of GSK1702934A-bound hTRPC3.

a, Representative raw micrograph of GSK1702934A-bound hTRPC3.

b, Representative 2D class average of GSK1702934A-bound hTRPC3.

c, Flowchart of GSK1702934A-bound hTRPC3 cryo-EM data processing.

d, FSC curves of final refinement for GSK1702934A-bound hTRPC3 in the low-calcium state (C4 symmetry) after correction for masking effects. Resolution estimations were based on the criterion of the FSC 0.143 cutoff. Source data are provided as a Source Data file.

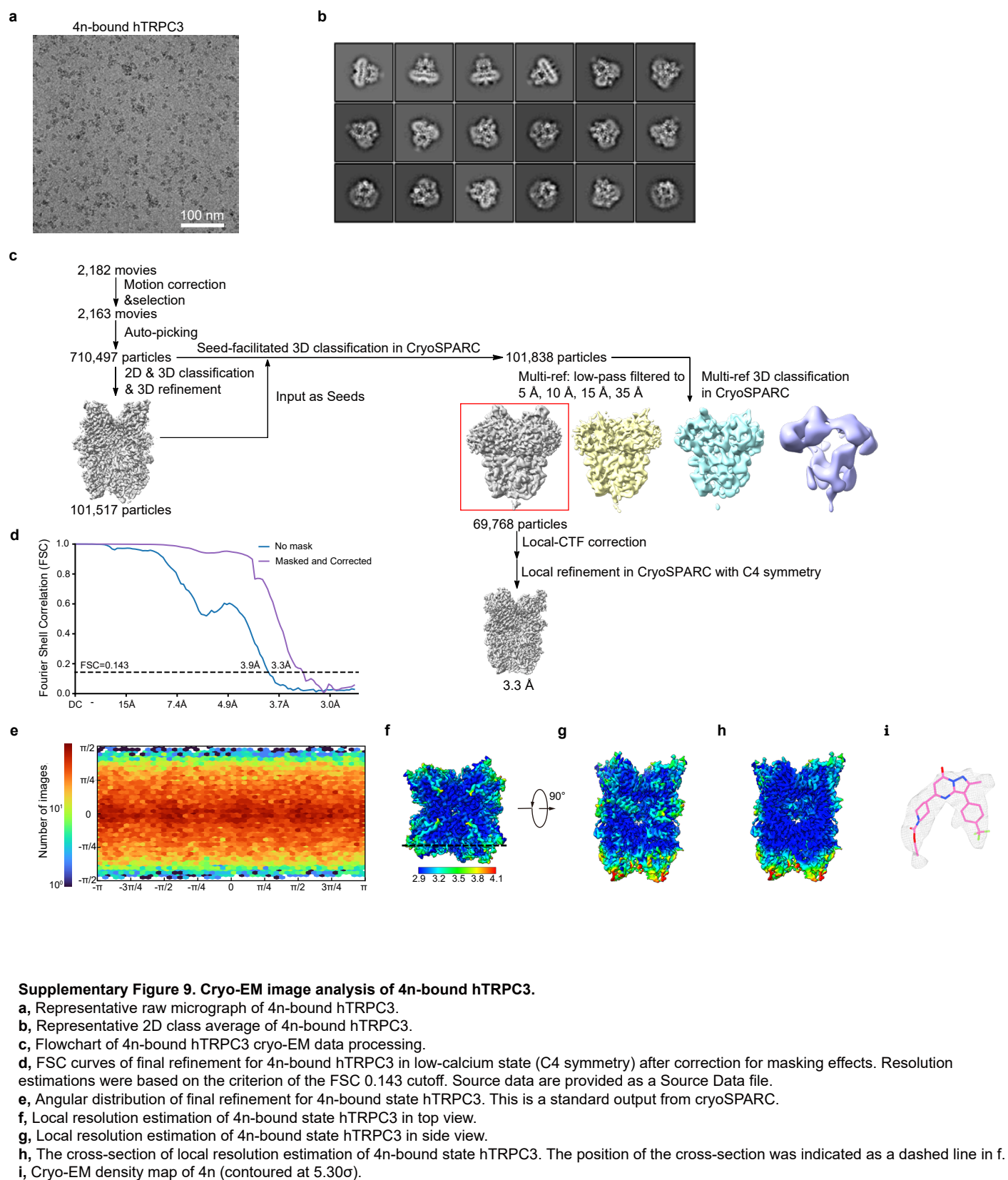
e, Angular distribution of final refinement for GSK1702934A-bound state hTRPC3. This is a standard output from cryoSPARC.

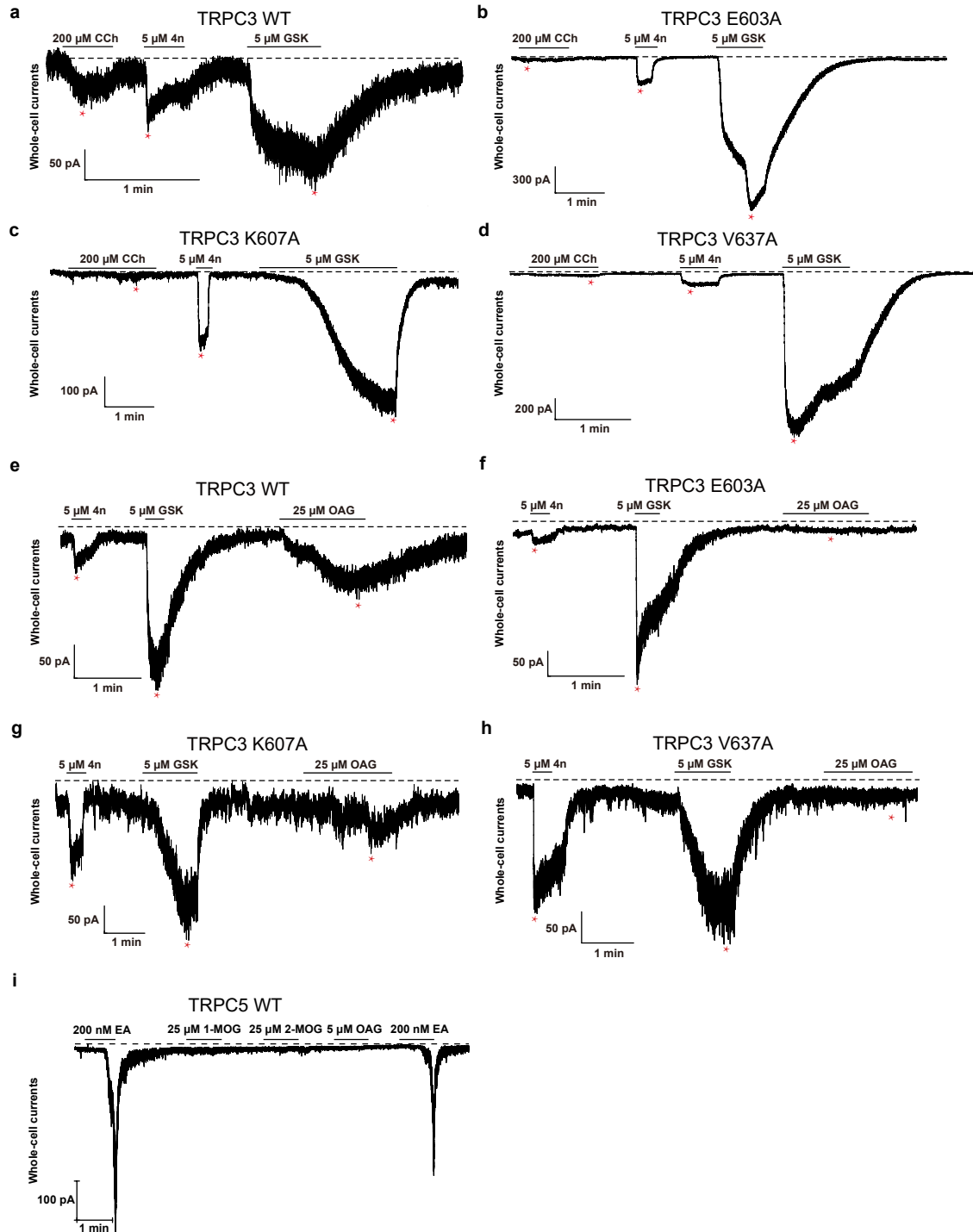
f, Local resolution estimation of GSK1702934A-bound state hTRPC3 in top view.

g, Local resolution estimation of GSK1702934A-bound state hTRPC3 in side view.

h, The cross-section of local resolution estimation of GSK1702934A-bound state hTRPC3. The position of the cross-section was indicated as a dashed line in **f**.

i, Cryo-EM density map of GSK1702934A (contoured at 3.59σ).

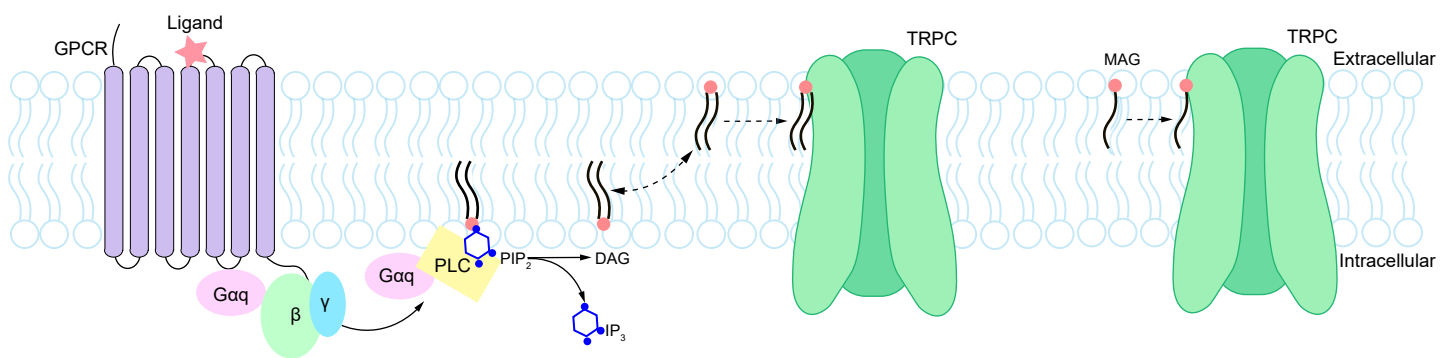




Supplementary Figure 10. The activation effect of different ligands on hTRPC3 and hTRPC5.

a-h, Represented macroscopic currents of wild-type (**a**, **e**), E603A (**b**, **f**), K607A (**c**, **g**), and V637A (**d**, **h**) hTRPC3 recorded in the whole cell mode. The duration of ligand application is indicated by a solid line above. The peak currents used for quantification in Fig.5 are labeled by asterisks. Zero current is indicated by a dashed line. Source data are provided as a Source Data file.

i, Representative macroscopic currents of wild-type hTRPC5 recorded in the whole cell mode. The duration of EA, OAG, 1-MOG, and 2-MOG applications is indicated by a solid line above. Zero current is indicated by a dashed line. Source data are provided as a Source Data file.



Supplementary Figure 11. The cartoon model of GPCR-mediated activation of TRPC3/6/7 channels.

Gαq/11-coupled-receptor activation leads to PLC-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂) to produce inositol 1,4,5-trisphosphate (IP₃) and diacylglycerols (DAG). DAG subsequently flips from the inner leaflet to the outer leaflet to activate TRPC3/6/7 channels. MAGs can also activate TRPC3/6/7 channels by binding to the same binding-site of DAG.

Supplementary Table 1
Cryo-EM data collection, refinement and validation statistics

	nanobody1-94 TRPC3	nanobody1-25 TRPC3	nanobody3-37 TRPC3	GSK1702934A -bound TRPC3	4n-bound TRPC3
PDB ID	9U5C	9KDB	9KDC	9KDD	9KDE
EMDB ID	EMD-63868	EMD-62268	EMD-62269	EMD-62270	EMD-62271
Data collection and processing					
Magnification	60,000 ×	47,000 ×	47,000 ×	48,000 ×	37,800 ×
Voltage (kV)	300	300	300	300	300
Electron exposure (e ⁻ /Å ²)	50	70	70	48	50
Defocus range (μm)	-1.5 to -1.8	-1.5 to -1.8	-1.5 to -1.8	-1.5 to -2.0	-1.0 to -3.0
Pixel size (Å)	0.833	1.067	1.067	1.045	1.324
Symmetry imposed	<i>C4</i>	<i>C4</i>	<i>C2</i>	<i>C4</i>	<i>C4</i>
Initial particle images (no.)	1,922,318	184,521	269,328	562,931	710,497
Final particle images (no.)	254,083	82,830	116,623	109,642	64,003
Map resolution (Å)	2.25	2.67	3.01	2.75	3.34
FSC threshold	0.143	0.143	0.143	0.143	0.143
Map resolution range (Å)	250-2.25	250-2.67	250-3.01	250-2.75	250-3.34
Refinement					
Initial model used (PDB code)	7DXB	7DXB	7DXB	7DXC	7DXC
Model resolution (Å)	2.9	2.9	2.9	3.06	3.06
FSC threshold	0.5	0.5	0.5	0.143	0.143
Model resolution range (Å)	250-2.9	250-2.9	250-2.9	250-3.06	250-3.06
Map sharpening <i>B</i> factor (Å ²)	-103.4	-103.4	-127.6	-122.1	-144.8
Model composition					
Non-hydrogen atoms	27,616	27,676	25,143	21,624	21,968
Protein residues	3,468	3,452	3,136	2,744	2,752
Ligands	16	20	18	8	8
<i>B</i> factors (Å²)					
Protein	29.61	67.50	68.36	46.97	57.23
Ligand	22.66	25.80	24.93	16.80	31.14
R.m.s. deviations					
Bond lengths (Å)	0.002	0.006	0.005	0.003	0.002
Bond angles (°)	0.456	0.980	0.969	0.586	0.542
Validation					
MolProbity score	1.76	1.64	1.89	1.52	1.52
Clashscore	5.93	6.12	7.35	5.65	6.34
Poor rotamers (%)	2.99	1.21	2.25	0.18	0.00
Ramachandran plot					
Favored (%)	97.65	96.37	96.64	96.61	97.05
Allowed (%)	2.35	3.63	3.36	3.39	2.95
Disallowed (%)	0.00	0.00	0.00	0.00	0.00

Supplementary Table 2
Top ten lipids identified by mass spectrometry

Compound	RT (min)	Peak Area	MRM
C18:1–C18:1 DAG	0.43	12585.51	638.60 > 339.30
C16:0–C18:1 DAG	0.43	3735.18	612.60 > 313.30
C18:0–C18:1 DAG	0.42	2108.05	640.60 > 313.30
C16:1–C18:1 DAG	0.43	1958.49	610.60 > 311.30
C16:1–C18:0 DAG	0.42	1619.13	614.60 > 313.30
C18:0–C20:1 DAG	0.43	1074.30	668.60 > 341.30
C16:0–C16:1 DAG	0.43	840.51	584.50 > 311.30
C18:0–C20:0 DAG	0.42	746.91	670.60 > 341.30
C16:0–C20:0 DAG	0.43	394.38	640.60 > 313.30
C16:0–C18:2 DAG	0.43	371.61	610.50 > 313.30