Supplementary Information Text

Additional CHARMm parameters for O-palmitoleylserine to facilitate preparation in CHARMM-GUI (not optimised for simulation).

```
* Force Field Parameter File.
*

BOND
OSL CT2  301.50   1.439

ANGLE
CT1 CT2 OSL   67.780     108.420
OSL CT2 HA2   50.840     108.820
CL  OSL CT2   63.630     115.140

DIHEDRAL
X CT2 OSL X      0.383         3       0.0
CT1 CT2 OSL CL   0.383         3       0.0
CT1 CT2 OSL CL   0.800         1     180.0
```

CHARMm topology for *O*-palmitoleylserine to facilitate preparation in CHARMM-GUI (not optimised for simulation).

```
* Lipid-modified protein residues
*
41 1

DECL -CA
DECL -C
DECL -O
DECL +N
DECL +HN
DECL +CA
!DEFA FIRS NTER LAST CTER
AUTO ANGLES DIHE PATCH

RESI SERP         0.00 ! O-palmitoylserine
GROUP                  !
ATOM N    NH1    -0.47 !
ATOM HN   H       0.31 !
ATOM CA   CT1     0.07 !
ATOM HA   HB1     0.09 !      |
GROUP                  !   HN-N
ATOM C    C       0.51 !      |   HB1
ATOM O    O      -0.51 !      |   |
GROUP                  !   HA-CA--CB--OG
ATOM CB   CT2    -0.05 !      |   |    |
ATOM HB1  HA2     0.09 !      |   HB2  |
ATOM HB2  HA2     0.09 !    O=C        |
ATOM OG   OSL    -0.34 !      |        |
ATOM C1   CL      0.63 !           O1=C1
ATOM O1   OBL    -0.52 !              |
ATOM C2   CTL2   -0.08 !          H2A-C2-H2B
ATOM H2A  HAL2    0.09 !              |
ATOM H2B  HAL2    0.09 !              |
```

```
GROUP                   !              |
ATOM C3   CTL2   -0.18 !          H3A-C3-H3B
ATOM H3A  HAL2    0.09 !               |
ATOM H3B  HAL2    0.09 !               |
GROUP                   !              |
ATOM C4   CTL2   -0.18 !          H4A-C4-H4B
ATOM H4A  HAL2    0.09 !               |
ATOM H4B  HAL2    0.09 !               |
GROUP                   !              |
ATOM C5   CTL2   -0.18 !          H5A-C5-H5B
ATOM H5A  HAL2    0.09 !               |
ATOM H5B  HAL2    0.09 !               |
GROUP                   !              |
ATOM C6   CTL2   -0.18 !          H6A-C6-H6B
ATOM H6A  HAL2    0.09 !               |
ATOM H6B  HAL2    0.09 !               |
GROUP                   !              |
ATOM C7   CTL2   -0.18 !          H7A-C7-H7B
ATOM H7A  HAL2    0.09 !               |
ATOM H7B  HAL2    0.09 !               |
GROUP                   !              |
ATOM C8   CTL2   -0.18 !          H8A-C8-H8B
ATOM H8A  HAL2    0.09 !               |
ATOM H8B  HAL2    0.09 !               |
GROUP                   !              |
ATOM C9   CEL1   -0.15 !          H9A-C9-H9B
ATOM H9A  HEL1    0.15 !               |
GROUP
ATOM C10  CEL1   -0.15 !         H10A-C10-H10B
ATOM H10A HEL1    0.15 !               |
GROUP                   !              |
ATOM C11  CTL2   -0.18 !         H11A-C11-H11B
ATOM H11A HAL2    0.09 !               |
ATOM H11B HAL2    0.09 !               |
GROUP                   !              |
ATOM C12  CTL2   -0.18 !         H12A-C12-H12B
ATOM H12A HAL2    0.09 !               |
ATOM H12B HAL2    0.09 !               |
GROUP                   !              |
ATOM C13  CTL2   -0.18 !         H13A-C13-H13B
ATOM H13A HAL2    0.09 !               |
ATOM H13B HAL2    0.09 !               |
GROUP                   !              |
ATOM C14  CTL2   -0.18 !         H14A-C14-H14B
ATOM H14A HAL2    0.09 !               |
ATOM H14B HAL2    0.09 !               |
GROUP                   !              |
ATOM C15  CTL2   -0.18 !         H15A-C15-H15B
ATOM H15A HAL2    0.09 !               |
ATOM H15B HAL2    0.09 !               |
GROUP                   !              |
ATOM C16  CTL3   -0.27 !         H16A-C16-H16B
ATOM H16A HAL3    0.09 !               |
ATOM H16B HAL3    0.09 !             H16C
ATOM H16C HAL3    0.09 !

BOND CB CA   OG CB   N HN  N  CA
```

```
BOND C  CA   C +N  CA HA  CB HB1
BOND CB HB2  OG C1
DOUBLE O  C

DOUBLE  C1  O1
BOND  C1  C2  C2  H2A  C2  H2B
BOND  C2  C3  C3  H3A  C3  H3B
BOND  C3  C4  C4  H4A  C4  H4B
BOND  C4  C5  C5  H5A  C5  H5B
BOND  C5  C6  C6  H6A  C6  H6B
BOND  C6  C7  C7  H7A  C7  H7B
BOND  C7  C8  C8  H8A  C8  H8B
BOND  C8  C9  C9  H9A
BOND  C9  C10 C10 H10A
BOND  C10 C11 C11 H11A C11 H11B
BOND  C11 C12 C12 H12A C12 H12B
BOND  C12 C13 C13 H13A C13 H13B
BOND  C13 C14 C14 H14A C14 H14B
BOND  C14 C15 C15 H15A C15 H15B
BOND  C15 C16 C16 H16A C16 H16B C16 H16C
IMPR C1 O1 OG C2
IMPR N -C CA HN  C CA +N O

DONOR HN N
ACCEPTOR O C
ACCEPTOR O1 C1

IC -C   CA   *N   HN    1.3479 123.93  180.00 114.77  0.9982
IC -C   N    CA   C     1.3479 123.93  180.00 105.89  1.5202
IC N    CA   C    +N    1.4533 105.89  180.00 118.30  1.3498
IC +N   CA   *C   O     1.3498 118.30  180.00 120.59  1.2306
IC CA   C    +N   +CA   1.5202 118.30  180.00 124.50  1.4548
IC HN   N    CA   CB    0.9674 117.70  -36.00 110.95  1.5003
IC CB   N    *CA  C     1.5003 110.95  128.76 106.38  1.5291
IC CB   N    *CA  HA    1.5003 110.95 -121.31 107.62  1.1060
IC N    CA   CB   OG    1.3945 110.95 -174.62 111.79  1.8692
IC OG   CA   *CB  HB1   1.8692 111.79  114.87 107.79  1.1406
IC OG   CA   *CB  HB2   1.8692 111.79 -128.20 109.45  1.0949
IC N    CA   C    O     1.3945 106.38    5.30 121.98  1.2956
IC CA   CB   OG   C1    1.5003 111.79 -178.37 107.64  1.8012
IC CB   OG   C1   C2    1.8692 107.64  158.80 115.77  1.5434
IC C2   OG   *C1  O1    1.5434 115.77 -174.68 119.62  1.2281
IC OG   C1   C2   C3    1.8012 115.77  152.55 111.56  1.5614
IC C3   C1   *C2  H2A   1.5614 111.56 -126.88 116.60  1.0567
IC H2A  C1   *C2  H2B   1.0567 116.60 -120.46 107.56  1.0927
IC C1   C2   C3   C4    1.5434 111.56  178.17 112.38  1.5543
IC C4   C2   *C3  H3A   1.5543 112.38 -115.58 110.36  1.0691
IC H3A  C2   *C3  H3B   1.0691 110.36 -116.74 109.97  1.1344
IC C2   C3   C4   C5    1.5614 112.38  174.26 114.93  1.5290
IC C5   C3   *C4  H4A   1.5290 114.93 -117.82 107.19  1.0636
IC H4A  C3   *C4  H4B   1.0636 107.19 -118.11 109.41  1.1180
IC C3   C4   C5   C6    1.5543 114.93  139.45 116.26  1.5437
IC C6   C4   *C5  H5A   1.5437 116.26 -115.47 116.05  1.1088
IC H5A  C4   *C5  H5B   1.1088 116.05 -122.13 108.08  1.1320
IC C4   C5   C6   C7    1.5290 116.26  159.28 113.90  1.5449
IC C7   C5   *C6  H6A   1.5449 113.90 -120.21 112.43  1.0817
IC H6A  C5   *C6  H6B   1.0817 112.43 -112.42 110.24  1.0503
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IC C5   C6   C7   C8    1.5437 113.90  167.68 115.30  1.5615
IC C8   C6   *C7  H7A   1.5615 115.30 -116.36 108.50  1.1480
IC H7A  C6   *C7  H7B   1.1480 108.50 -115.94 110.56  1.1231
IC C6   C7   C8   C9    1.5449 115.30  165.37 111.83  1.5514
IC C9   C7   *C8  H8A   1.5514 111.83 -113.43 111.91  1.1377
IC H8A  C7   *C8  H8B   1.1377 111.91 -118.34 108.18  1.0480
IC C7   C8   C9   C10   1.5615 111.83  176.59 110.57  1.5526
IC C10  C8   *C9  H9A   1.5526 110.57 -129.97 113.53  1.1310
IC C8   C9   C10  C11   1.5514 110.57 -177.26 112.26  1.5783
IC C11  C9   *C10 H10A  1.5783 112.26 -123.45 106.63  1.1533
IC C9   C10  C11  C12   1.5526 112.26 -163.15 112.58  1.5528
IC C12  C10  *C11 H11A  1.5528 112.58 -121.90 102.65  1.0764
IC H11A C10  *C11 H11B  1.0764 102.65 -122.46 109.95  1.1248
IC C10  C11  C12  C13   1.5783 112.58 -179.16 115.34  1.5375
IC C13  C11  *C12 H12A  1.5375 115.34 -129.55 111.80  1.0856
IC H12A C11  *C12 H12B  1.0856 111.80 -109.80 105.66  1.0741
IC C11  C12  C13  C14   1.5528 115.34 -178.59 114.71  1.5546
IC C14  C12  *C13 H13A  1.5546 114.71 -121.77 106.42  1.0758
IC H13A C12  *C13 H13B  1.0758 106.42 -114.53 102.53  1.0909
IC C12  C13  C14  C15   1.5375 114.71  179.82 117.78  1.5331
IC C15  C13  *C14 H14A  1.5331 117.78 -126.75 106.30  1.1072
IC H14A C13  *C14 H14B  1.1072 106.30 -108.90 106.38  1.0834
IC C13  C14  C15  C16   1.5546 117.78  169.53 109.01  1.4928
IC C16  C14  *C15 H15A  1.4928 109.01 -121.10 100.35  1.1439
IC H15A C14  *C15 H15B  1.1439 100.35 -117.57 115.28  1.1080
IC C14  C15  C16  H16A  1.5331 109.01  -61.78 119.36  1.0834
IC H16A C15  *C16 H16B  1.0834 119.36  138.24 116.61  1.0932
IC H16A C15  *C16 H16C  1.0834 119.36 -107.69 109.08  1.1555
```

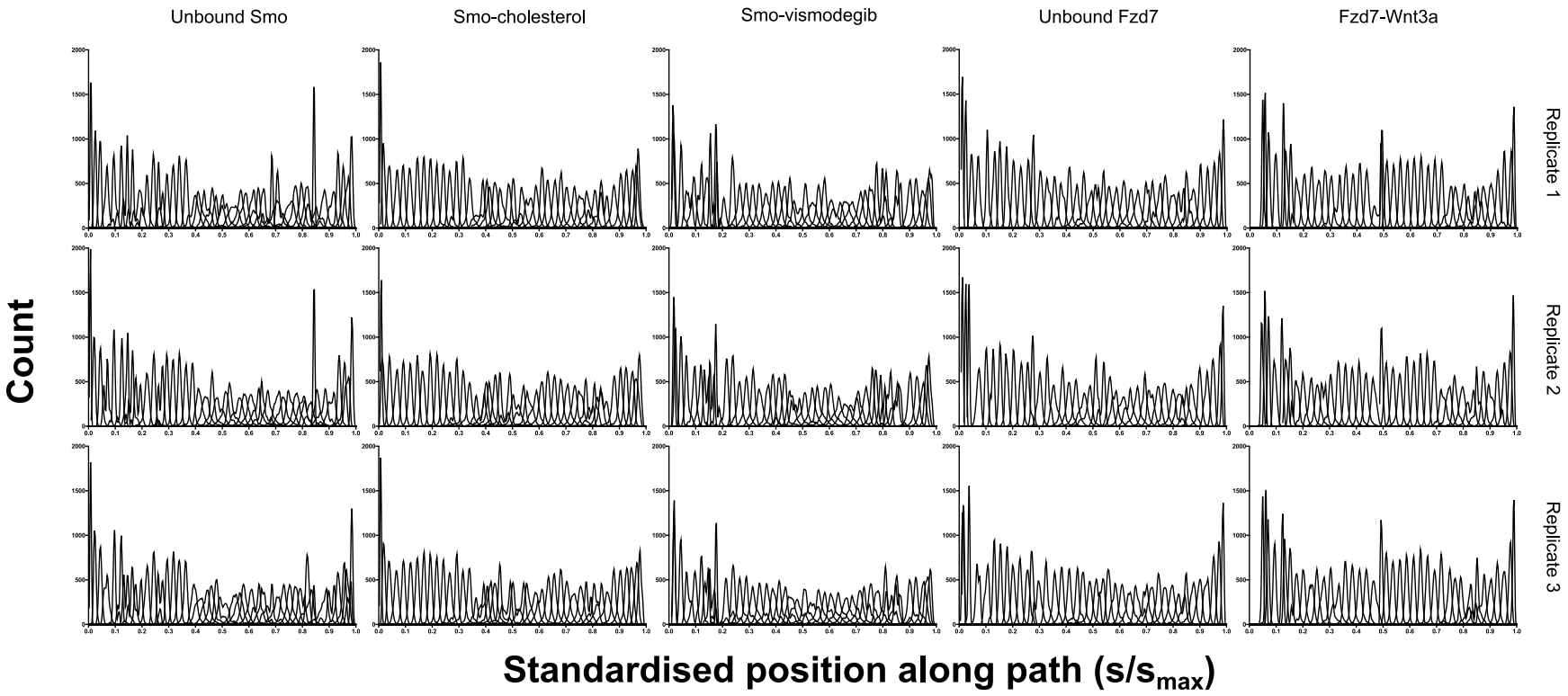

Fig. S1. Histograms for the umbrella sampling simulations. Histograms were prepared with bin width of 0.0025, and smoothed without integration or differentiation to a second order polynomial, with two neighbours on each size.

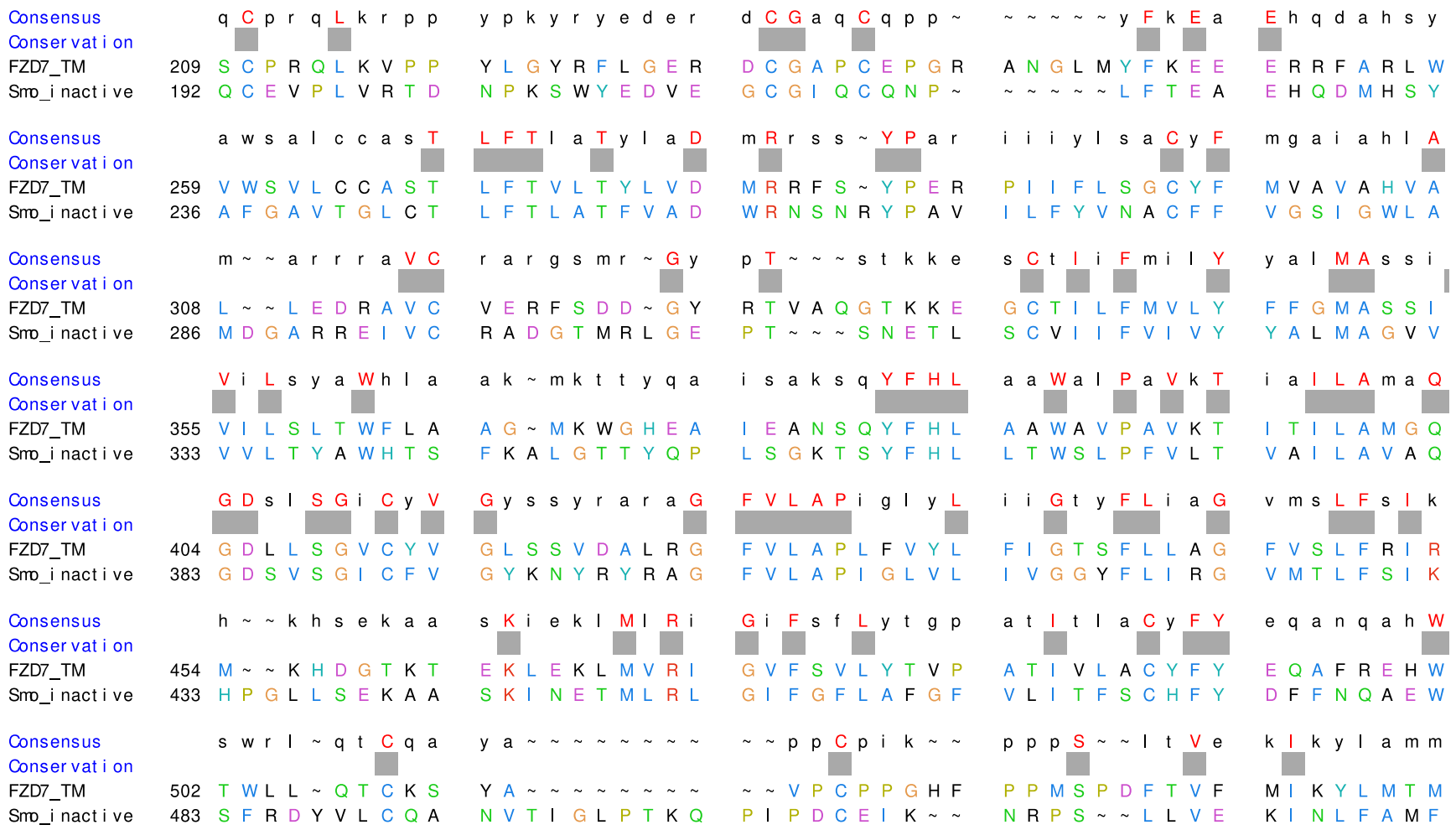

Fig. S2. Sequence alignments between TM regions of Fzd7 and Smo, used to prepare Fzd7 structures.

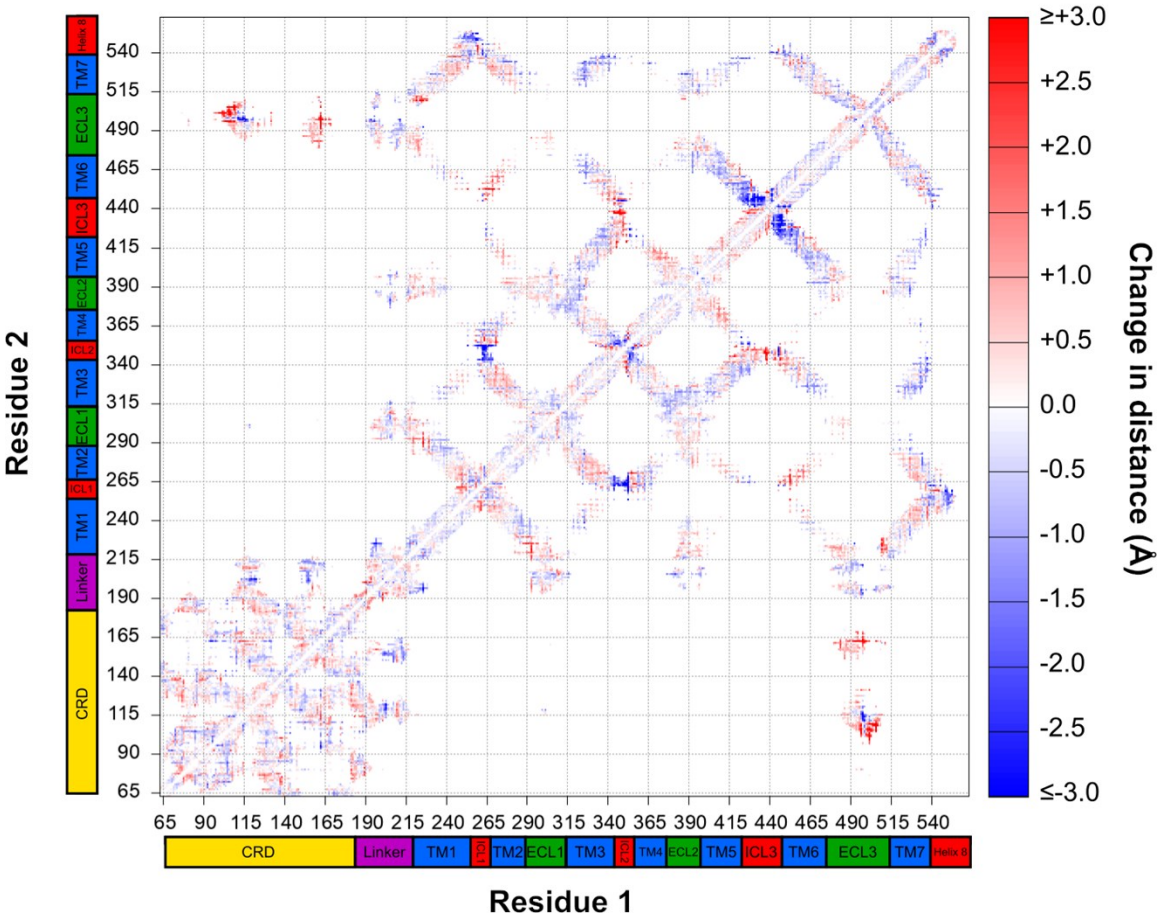

Fig. S3. Difference in distance contact maps between states before and after Barrier (i). Positive changes in distance indicate residue pairs moving apart upon crossing the barrier; negative changes in distance indicate residue pairs closer together upon crossing the barrier.

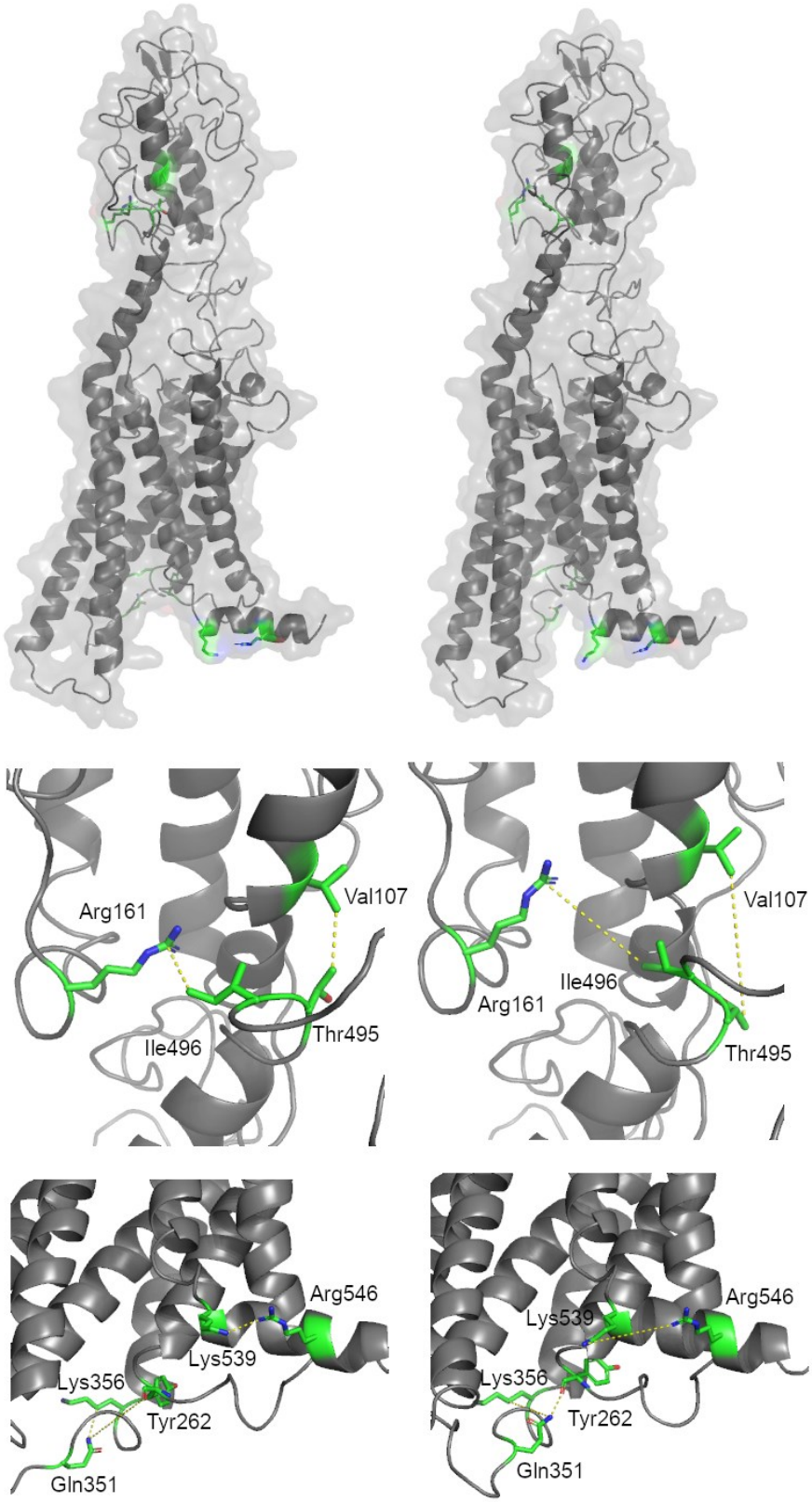

Fig. S4. Larger depiction of states before (first column) and after (second column) Barrier (i) (Figs 4A, 4B, 4G, 4H, 4M, and 4N).

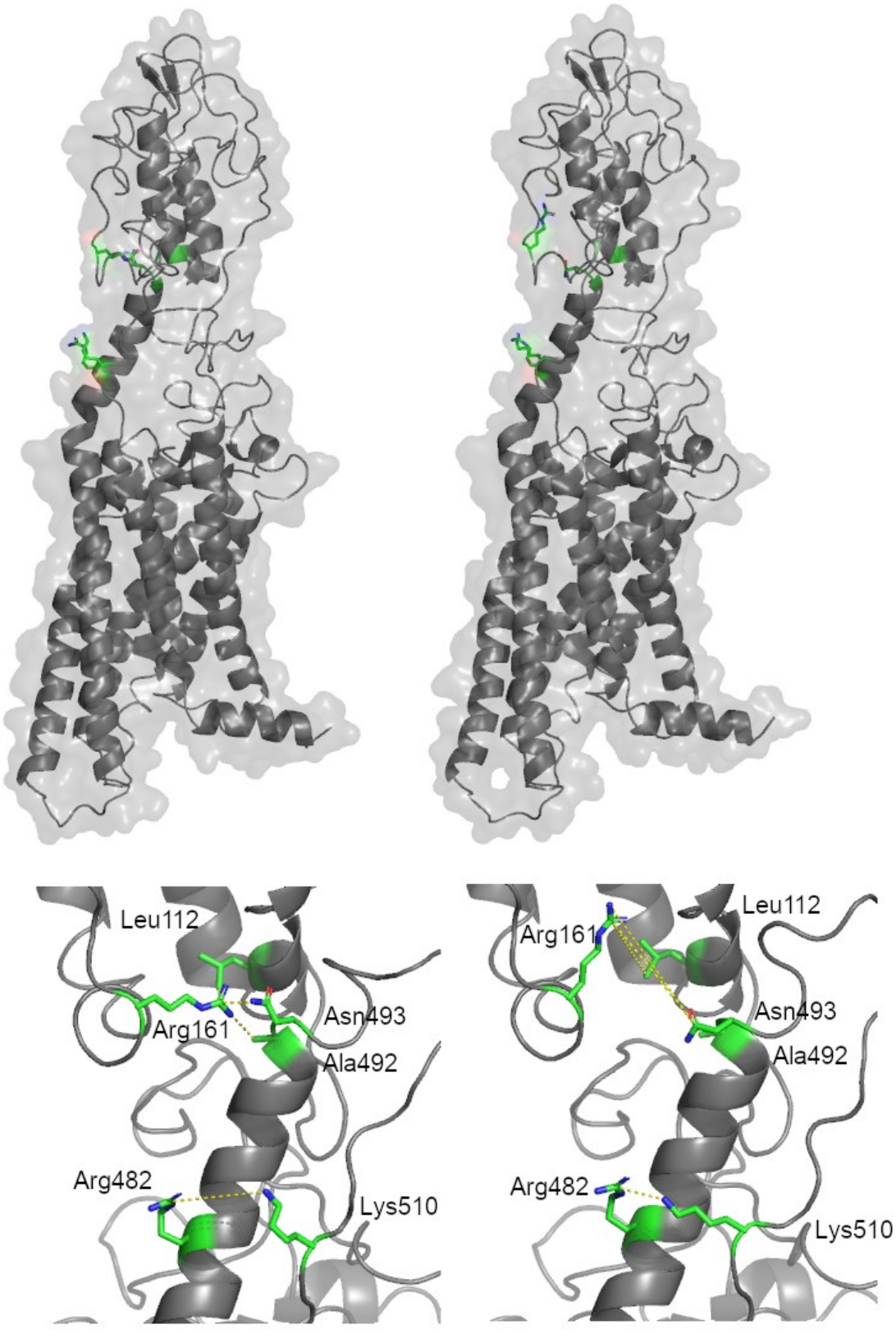

Fig. S5. Larger depiction of states before (first column) and after (second column) Barrier (ii) (Figs 4C, 4D, 4I, and 4J).

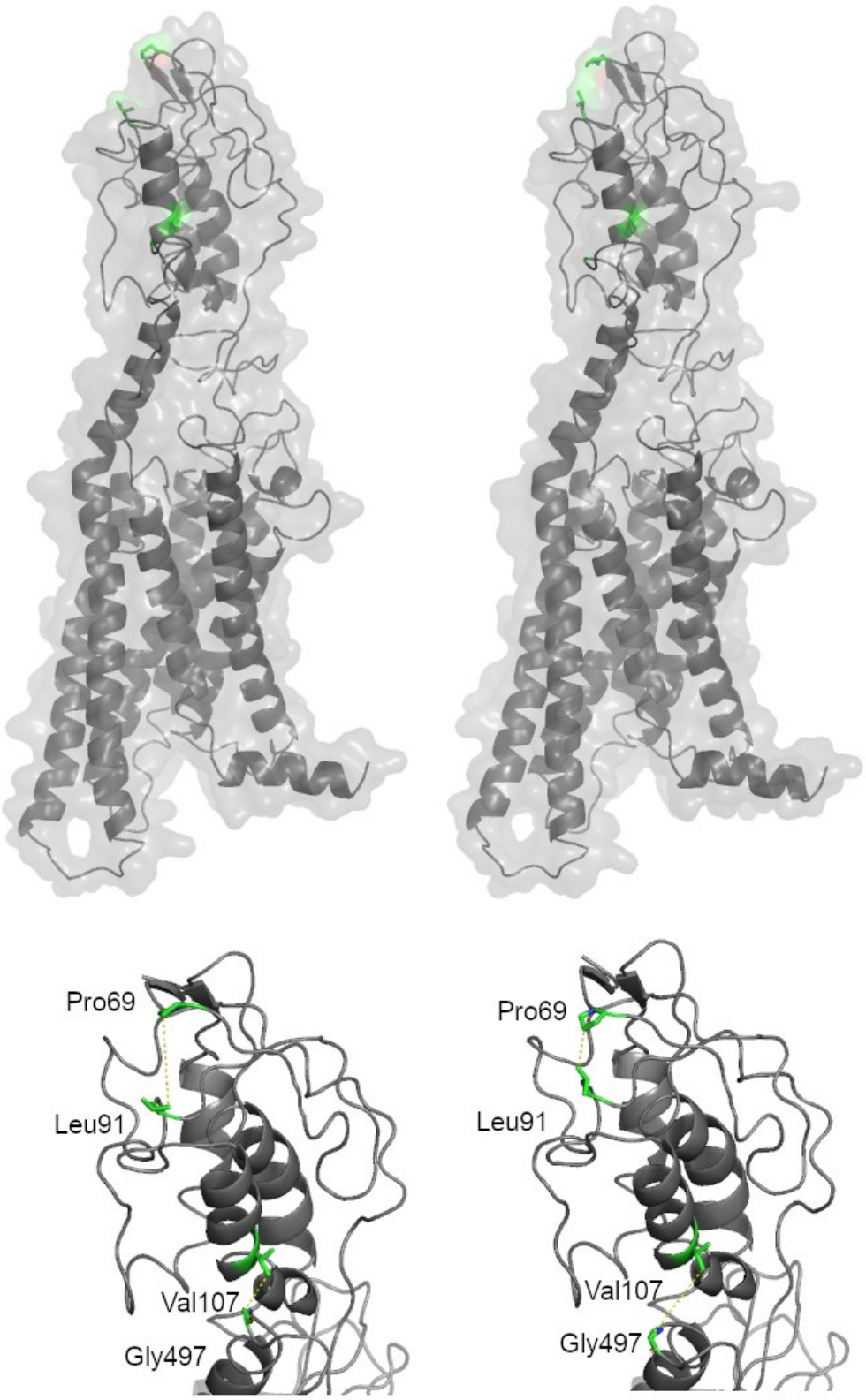

Fig. S6. Larger depiction of states before (first column) and after (second column) Barrier (iii) (Figs 4E, 4F, 4K, and 4L).

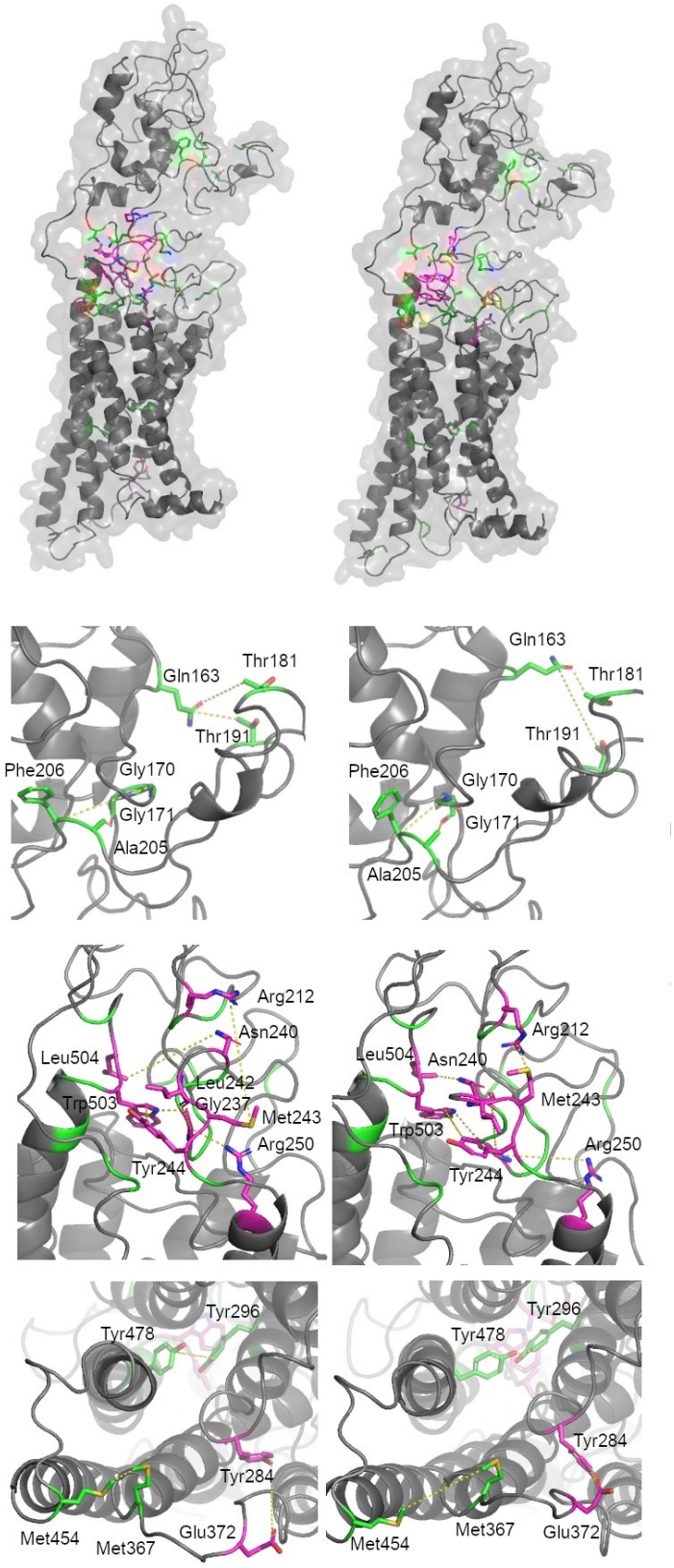

Fig. S7. Larger depiction of states before (first column) and after (second column) Barrier (iv) (Figs 5A, 5B, 5G, 5H, 5Q, and 5R).

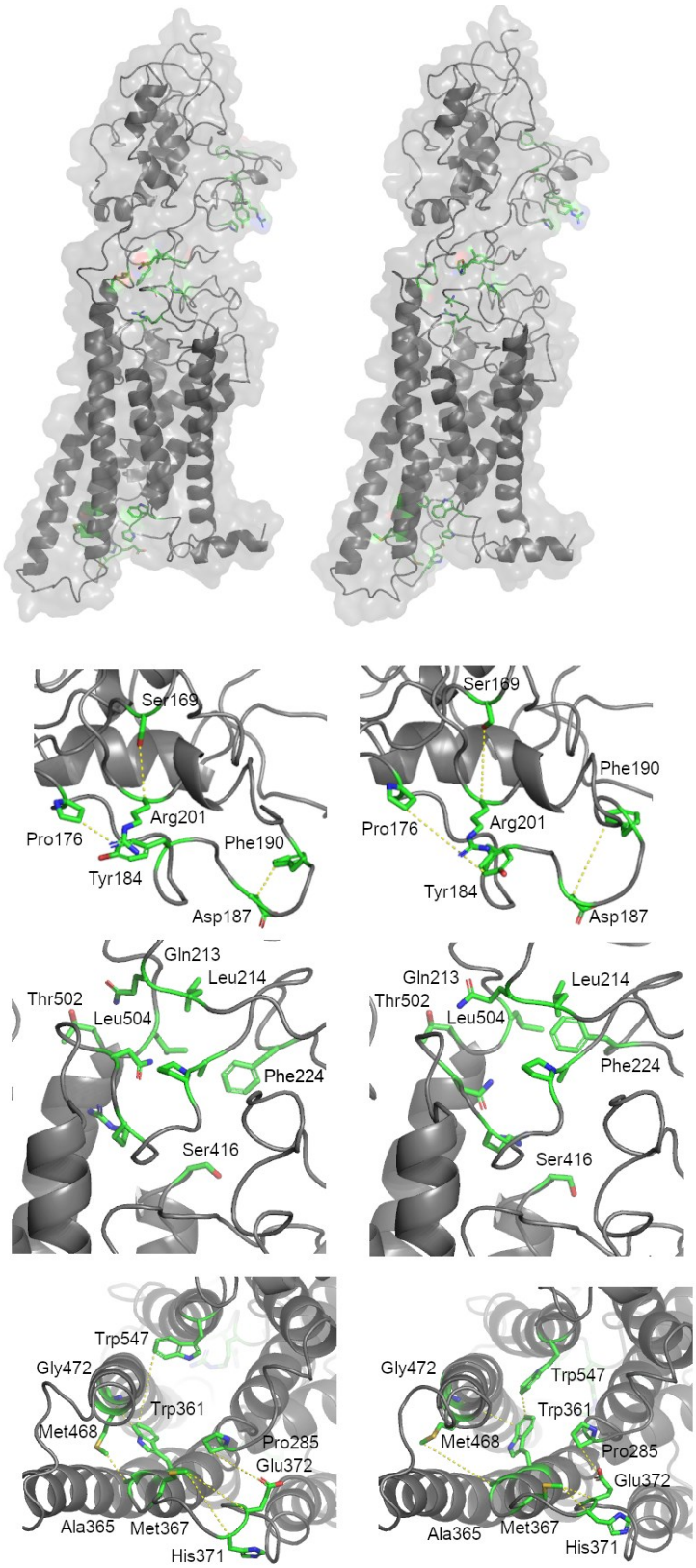

Fig. S8. Larger depiction of states before (first column) and after (second column) Barrier (v) (Figs 5C, 5D, 5I, 5J, 5S, and 5T).

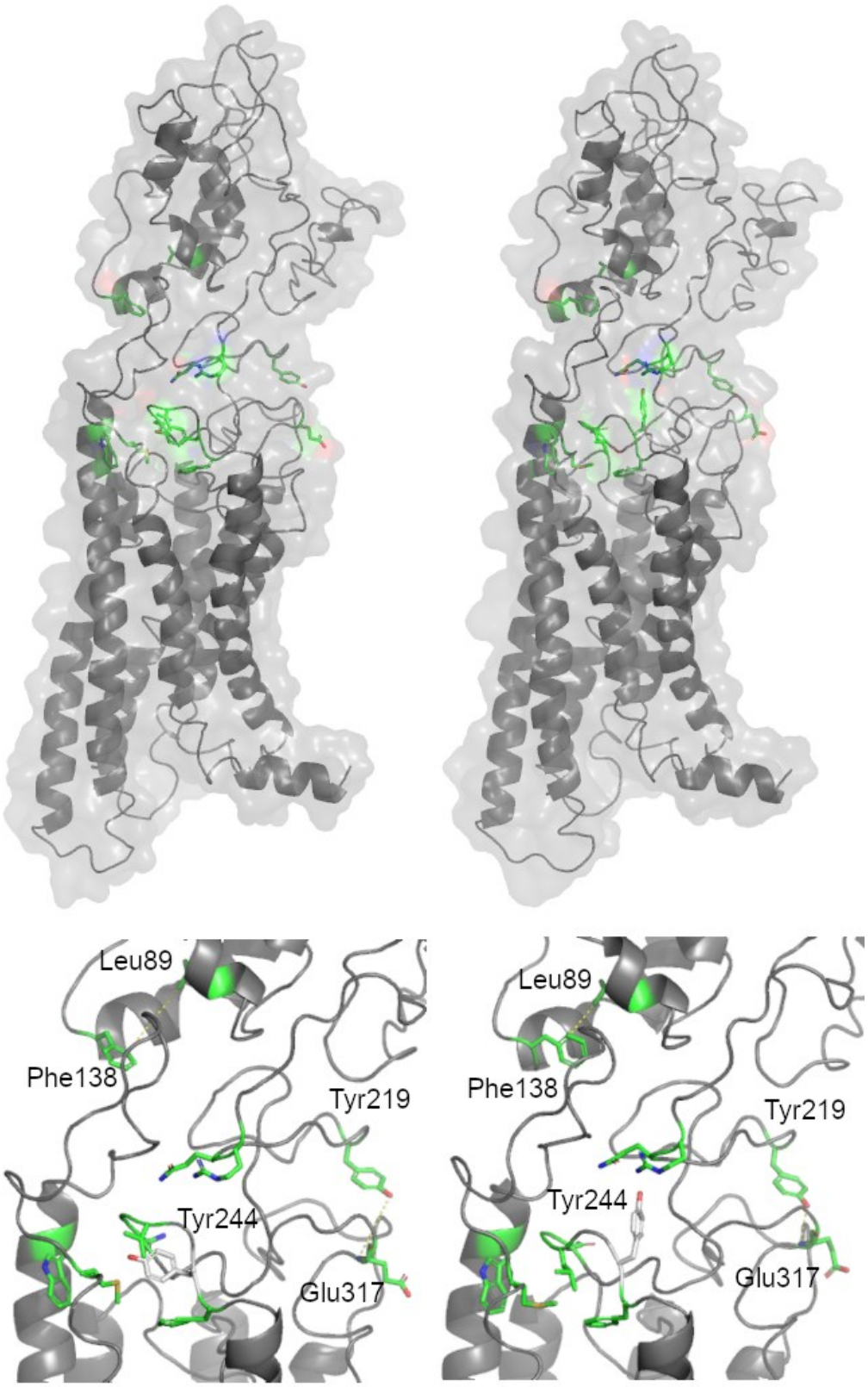

Fig. S9. Larger depiction of states before (first column) and after (second column) Barrier (vi) (Figs 5E, 5F, 5K, and 5L).

Table S1. Quality statistics for initially built and refined models.

System	State	MolProbity Score	TM-Score[a]	Cα deviation (Å)[a]
Unbound Smo	Inactive	1.79	0.98	0.85
	Active	2.23	0.98	0.04
Smo-cholesterol	Inactive	1.16	0.97	0.87
	Active[b]	N/A	N/A	N/A
Smo-vismodegib	Inactive	1.91	0.92	2.26
	Active[b]	N/A	N/A	N/A
Unbound Fzd7	Inactive	2.24	0.81	2.83
	Active	2.34	0.84	2.31
Fzd7-Wnt3a	Inactive[b]	N/A	N/A	N/A
	Active (Wnt3a only)[c]	2.22	0.95	0.62

[a]Calculated for Smo or Fzd7 with respect to PDB 5L7D for inactive structures and PDB 6O3C for active structures (with the exception of Wnt3a in the Wnt3a-Fzd7 complex; see note c). Calculated using TM-Align server (https://zhanglab.ccmb.med.umich.edu/TM-align/). [b]These states were achieved via steered MD starting from the state of the system for which quality statistics are reported; thus, quality statistics are not reported for these states. [c]TM-Score and Cα deviation in this instance are reported with respect to xWnt8a in PDB 4F0A. MolProbity Score indicated is for the entire complex.

Table S2. Variations identified in Smo in cancer patients in the MSK-IMPACT Clinical Sequencing Cohort, PanCancer Studies at residues identified as mediating energetic barriers to activation by the distance contact analysis.

Mutation	Cancer
L112I	Uterine endometrioid carcinoma
R161Q	Rectal adenocarcinoma
R161W	Breast invasive cancer
A492G	Breast invasive cancer
I496V	Non-small cell lung cancer

Table S3. Variations identified in Fzd7 in cancer patients in the MSK-IMPACT Clinical Sequencing Cohort, PanCancer Studies at residues identified as mediating energetic barriers to activation by the distance contact analysis.

Mutation	Cancer
G237A	Mucinous adenocarcinoma of the colon and rectum
M468T	Colon adenocarcinoma

Movie S1 (separate file). Representative ABMD simulation for Smo activation.

Movie S2 (separate file). Representative ABMD simulation for Fzd7 activation.

Movie S3 (separate file). SMD simulation for activation of Smo-cholesterol complex.

Movie S4 (separate file). SMD simulation for activation of Smo-vismodegib complex.

Movie S5 (separate file). SMD simulation for deactivation of Fzd7-Wnt3a complex.

Legends for all movies: brown transparent volume – lipid head groups; grey transparent volume – lipid tails; yellow cartoon – cysteine-rich domain; purple cartoon – linker; blue cartoon – transmembrane regions; red cartoon – intracellular regions; green cartoon – intracellular regions; spheres (grey carbons, CPK-coloured heteroatoms) – ligand (cholesterol [CRD-binding] or vismodegib [TM region-binding]) or post-translational modification (*O*-palmitoleylserine [CRD-binding]).