Structural mechanism underlying capsaicin binding and activation of TRPV1 ion channel

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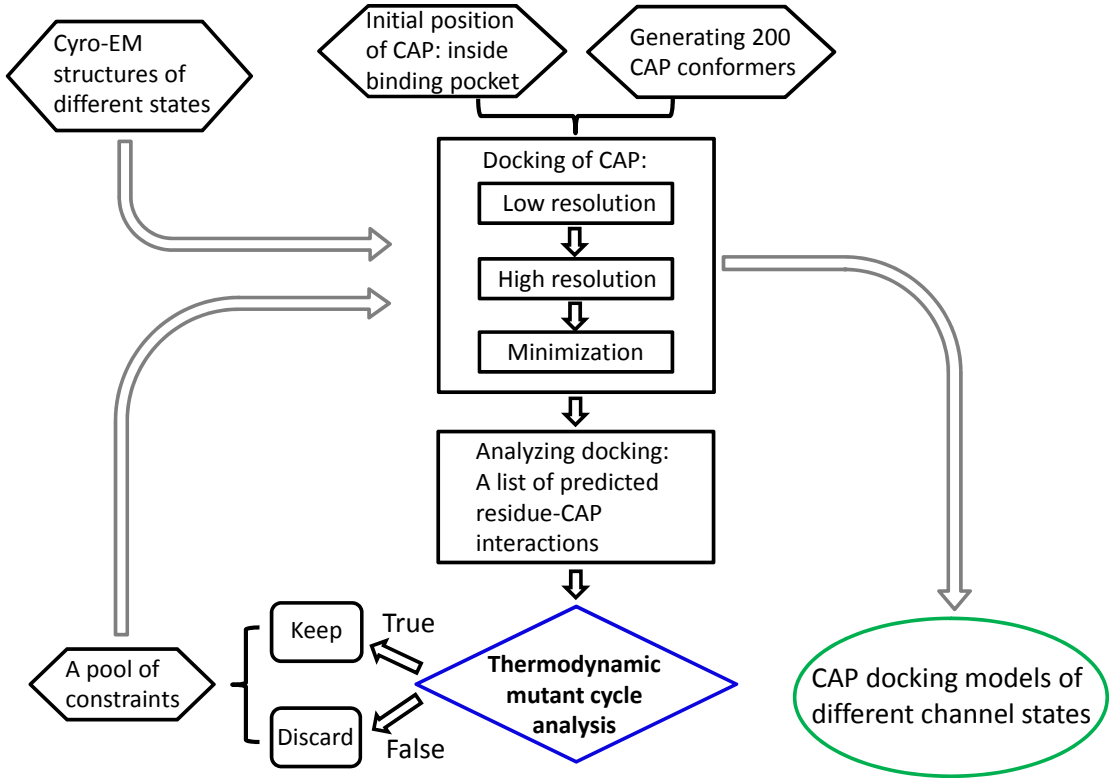
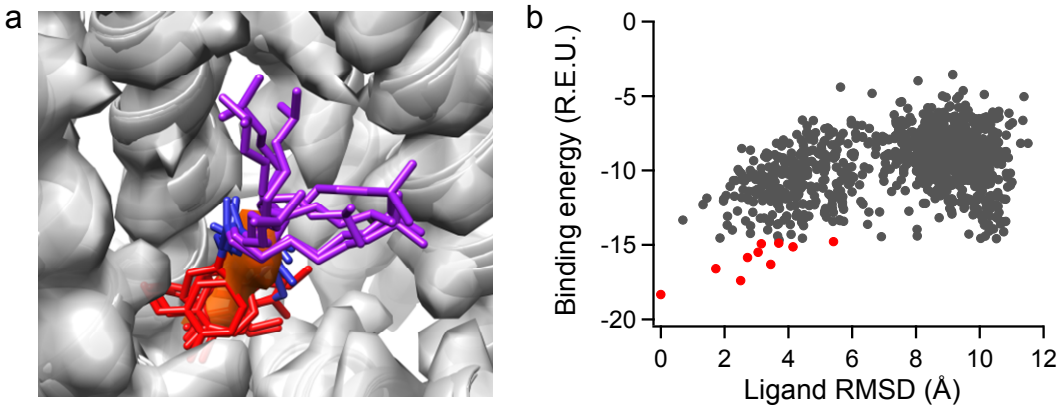
Supplementary Information

Supplementary Results

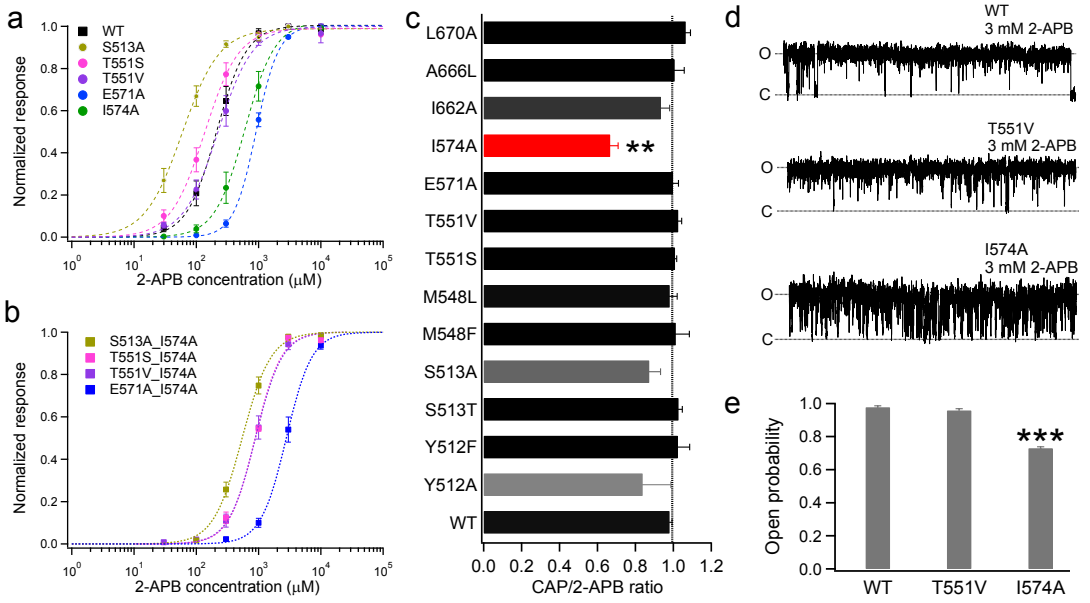
Supplementary Fig. 1. Work flow of iterative computational docking of capsaicin and functional analyses to reveal structural configuration of capsaicin inside its binding pocket and different capsaicin bound states of TRPV1.



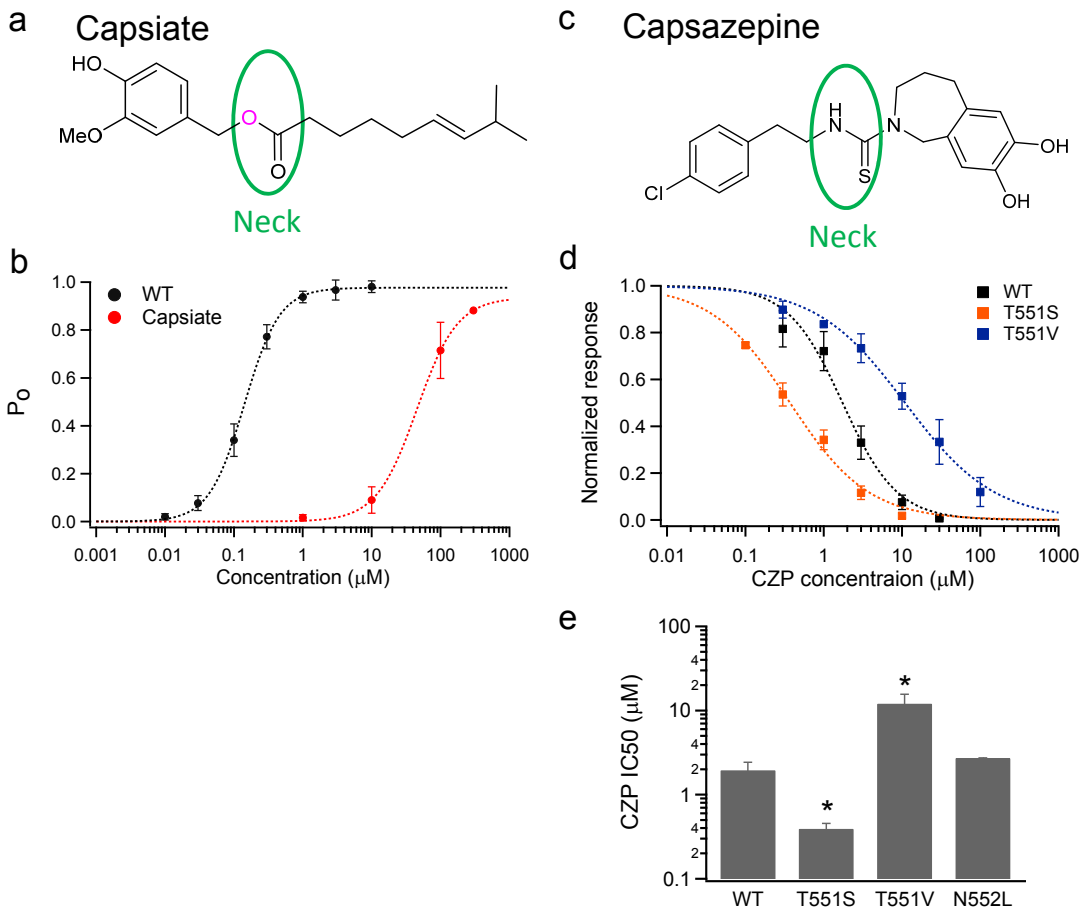
Supplementary Fig. 2. Docking of capsaicin. (**a**) Top 10 models of capsaicin with lowest binding energy from docking. Vanillyl, amide and aliphatic chain groups are colored in red, blue and purple, respectively. The small electron density corresponding to capsaicin is colored in orange. (**b**) Capsaicin binding energy is plotted against its unsuperimposed root-mean-square deviation (RMSD) over the top 1000 models with the lowest total energy score, from which the top 10 binding models (red dots) were selected. The model with lowest binding energy was set as the reference. While the vanillyl and amide groups of the top 10 models converged well, overall unsuperimposed ligand RMSD among these models are 2-to-6 Å, reflecting the large flexibility of the aliphatic chain.



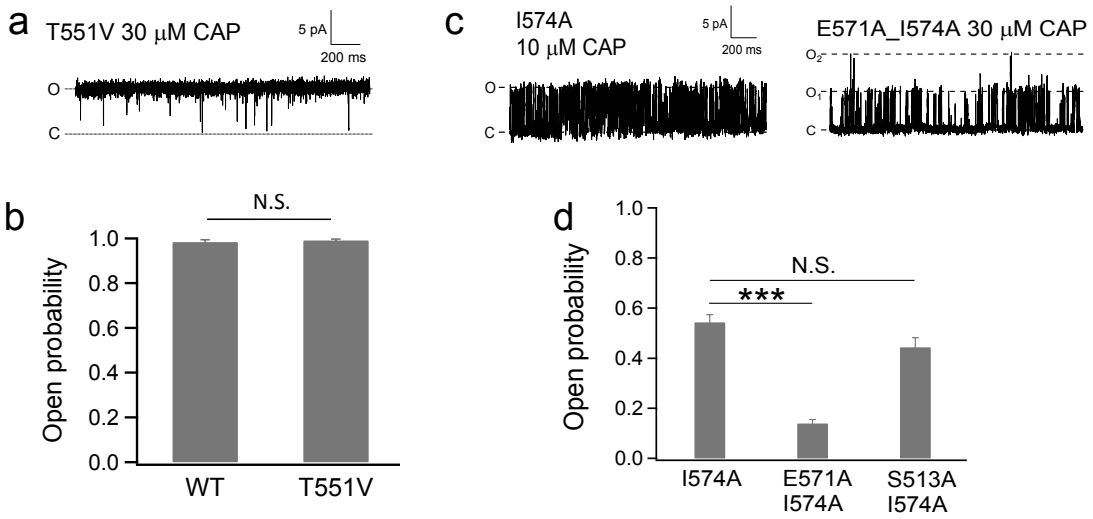
Supplementary Fig. 3. 2-Aminoethoxydiphenyl borate (2-APB) activation of TRPV1 and mutants. (**a**) and (**b**), concentration-response curves of single point mutants and double mutants, respectively (Supplementary Table 2). 3 mM is a saturating concentration for most of the mutants. Note that 2-APB is less sensitive to mutations than capsaicin. (**c**) Ratio of currents elicited by saturating concentration of capsaicin and 3 mM 2-APB. (**d**) representative single-channel recordings of TRPV1 WT and mutants in response to saturating concentration of 2-APB. (**e**) *Po* of the channels activated by 3 mM 2-APB calculated from single-channel recordings. n = 3-to-5.



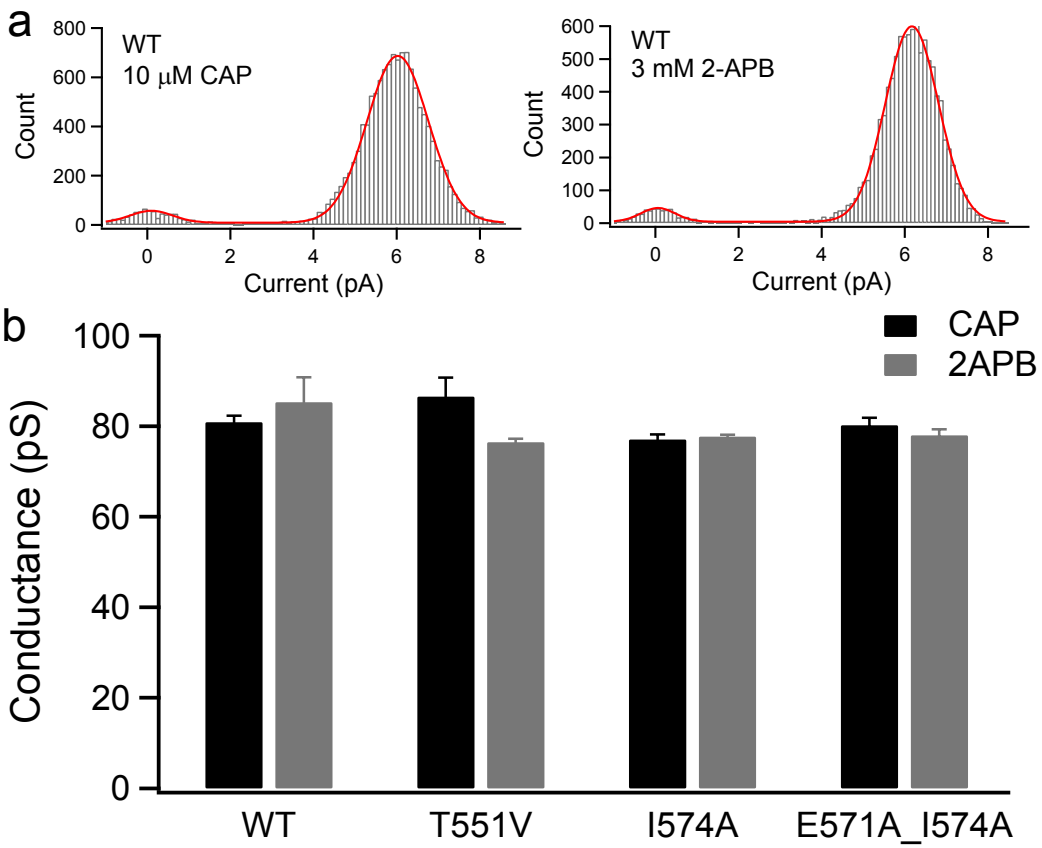
Supplementary Fig. 4. Effects of capsiate and capsazepine on the channel. (**a**) Chemical structure of capsiate. Note that the Neck –NH– of capsaicin is substituted by an oxygen atom (in magenta) in capsiate. (**b**) Capsiate exhibits much weaker binding but similar efficacy as capsaicin. (**c**) Chemical structure of capsazepine, a competitive antagonist. Note that it has a similar “neck” region as in capsaicin. (**d**) Concentration-response curves of channel inhibition by capsazepine. Capsaicin concentration at EC90 of each mutant was used to activate the channels. (**e**), Mutations to T551 affected capsazepine inhibition whereas mutation to the neighboring residue N552 didn’t. n = 4-to-5.



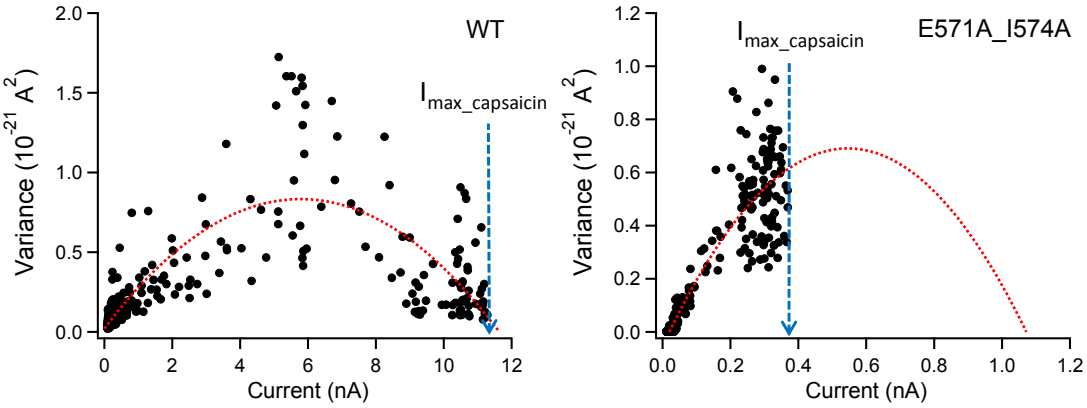
Supplementary Fig. 5. Maximum *Po* of mutant channels at saturating concentration of capsaicin. (**a**) Representative single-channel recordings of T551V at saturating concentration of capsaicin. (**b**) Maximum *Po* by capsaicin calculated from single-channel recordings. (**c**) Representative single-channel traces of I574A and E571A_I574A at saturating concentration of capsaicin. (**d**) Maximum *Po* by capsaicin calculated from single-channel recordings (right panel). n = 3-to-5.



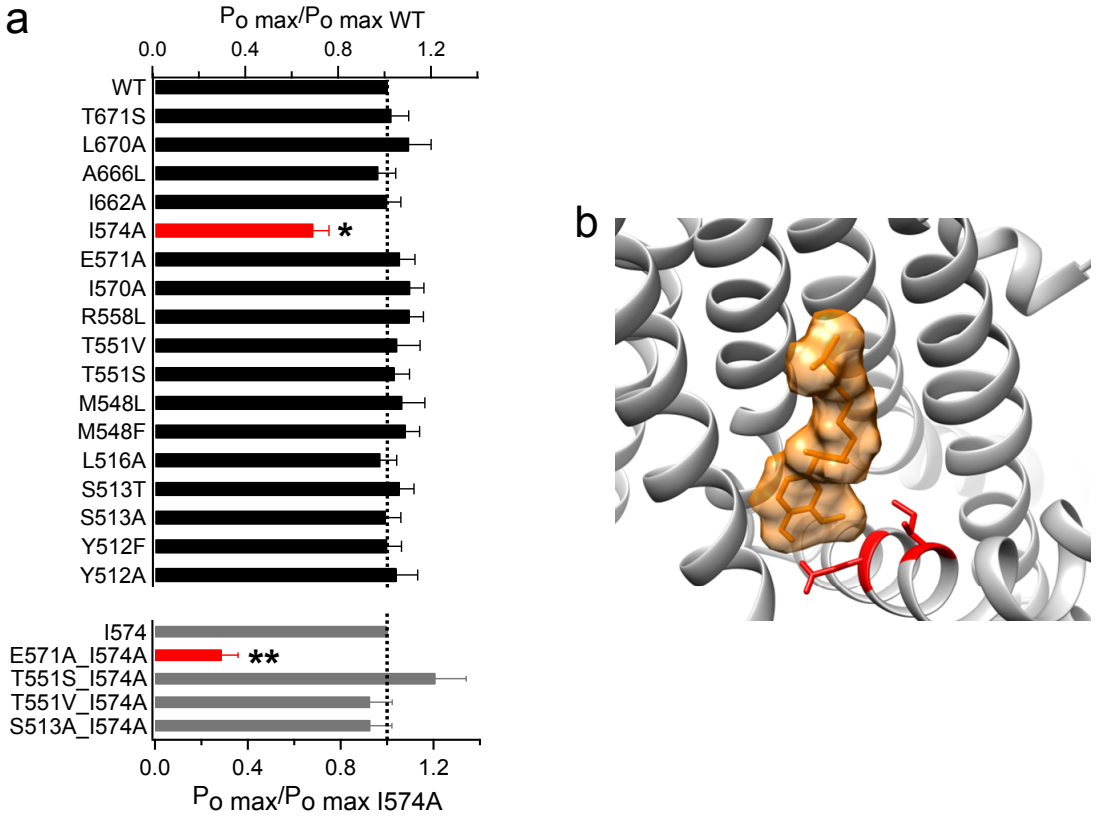
Supplementary Fig. 6. Single-channel conductance is not changed by point mutations or ligand. (**a**) Representative all-point histogram of single-channel recordings of WT channel at +80 mV. To determine current amplitude, such histograms were fitted to a double-Gaussian function (red curves). (**b**) Calculated conductance for WT and mutants. Activated by either saturating capsaicin or 2-APB, all the channels show similar conductance. n = 3-to-6.



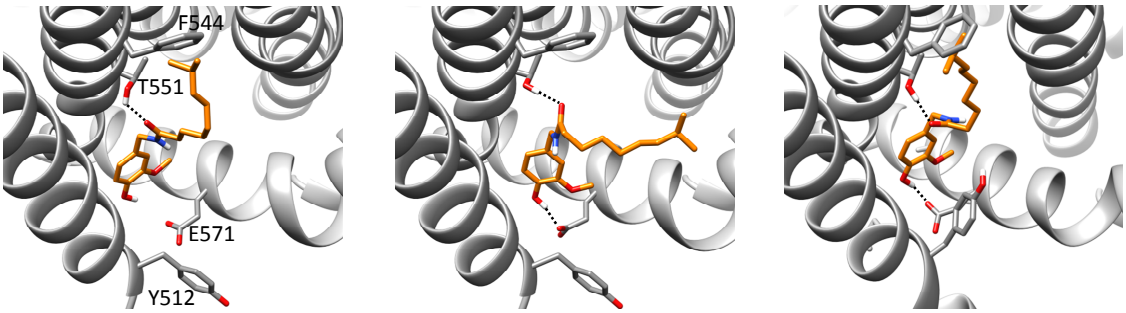
Supplementary Fig. 7. Representative noise analysis for capsaicin activation. For WT channel, current activated by saturating capsaicin ($I_{max\_capsaicin}$) approached the maximum current predicted by noise analysis, indicating the *Po* was very close to unity. For E571A_I574A double mutant, capsaicin activated current was far from reaching the maximum, indicating a reduced *Po_max*.



Supplementary Fig. 8. Comparison of *Po_max* at saturating concentration of capsaicin. (**a**) For single point mutants, their *Po_max* value is normalized to that of TRPV1 WT (top portion of the plot). For mutants in the I574A background, their *Po_max* value is normalized to that of I574A (bottom portion). n = 3-to-6. (**b**) Mapping of the two residues I574A and E571A (in red) with a major gating effect. Capsaicin and its molecular surface are colored in orange.

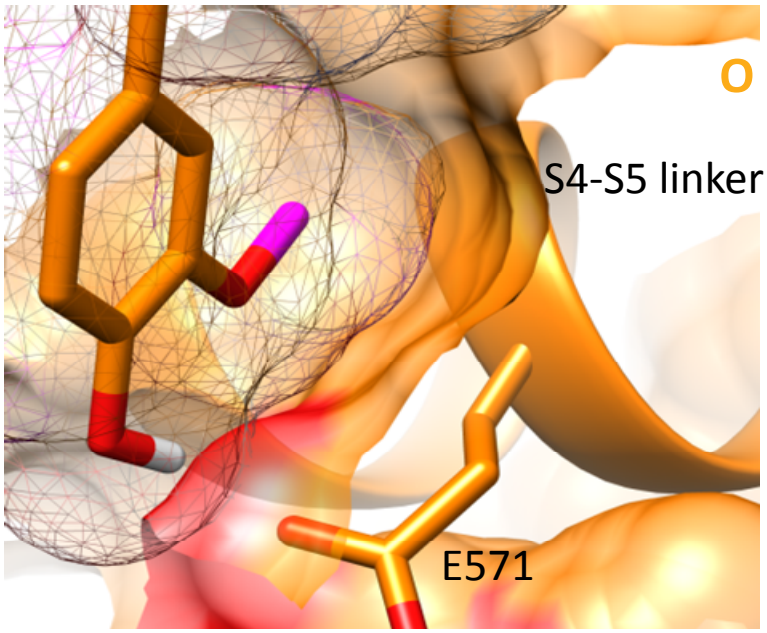


Supplementary Fig. 9. Interactions between capsaicin and its binding pocket in C_0, C_1 and O states. Hydrogen bond is denoted by black dotted line. Note that there is only one hydrogen bond between the Neck and T551 in C_0, but two in C_1 and O.

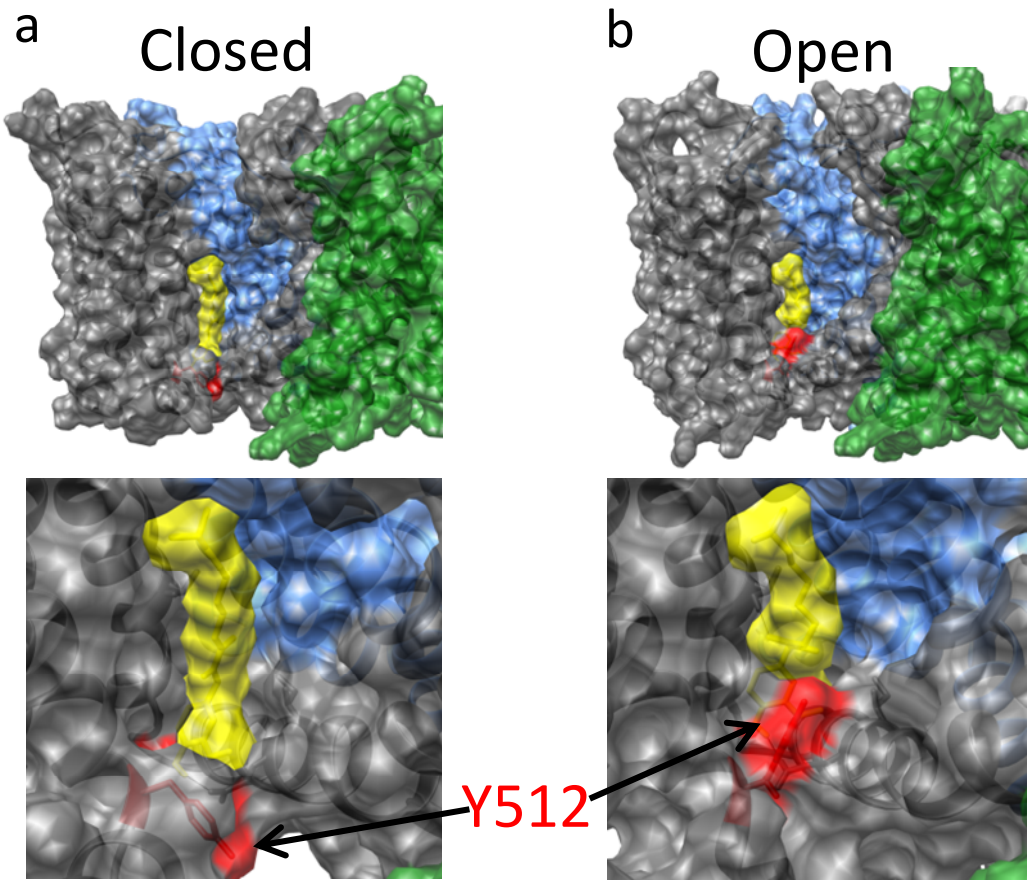


C_0 C_1 O

Supplementary Fig. 10. The Head methyl group (magenta) of capsaicin contacts S4-S5 linker in the open state. Surface of capsaicin and the S4-S5 liner is shown as mesh and solid, respectively.



Supplementary Fig. 11. Capsaicin binding pocket is closed by Y512 (red) in open state. Capsaicin molecule is colored in yellow, while different TRPV1 subunits are colored in grey, blue and green, respectively.



Supplementary Table 1. Capsaicin concentration-response parameters for TRPV1 WT and mutants. (For each channel type, n = 3-to-8)

	EC50 (μM)	Hill Coefficient
WT	0.15 ± 0.02	1.82 ± 0.22
Y512A	12.84 ± 2.83	1.55 ± 0.19
Y512F	1.03 ± 0.14	1.31 ± 0.06
S513T	0.43 ± 0.11	1.56 ± 0.11
S513Y	40.14 ± 7.78	1.44 ± 0.21
S513A	0.48 ± 0.08	1.23 ± 0.16
L516A	0.26 ± 0.05	1.38 ± 0.02
F544Y	0.02 ± 0.01	1.13 ± 0.07
M548F	0.16 ± 0.09	1.53 ± 0.39
M548L	0.11 ± 0.02	1.37 ± 0.22
T551A	0.46 ± 0.02	1.80 ± 0.12
T551S	0.05 ± 0.01	1.76 ± 0.25
T551V	1.56 ± 0.20	1.74 ± 0.13
N552L	9.44 ± 2.58	1.48 ± 0.07
R558L	0.03 ± 0.01	1.19 ± 0.17
I570A	23.21 ± 13.80	1.53 ± 0.07
E571A	1.53 ± 0.10	1.86 ± 0.07
I574A	0.28 ± 0.07	1.49 ± 0.05
I662A	0.16 ± 0.07	0.92 ± 0.01
A666L	0.22 ± 0.07	1.20 ± 0.05
T671V	0.10 ± 0.03	0.98 ± 0.06
T671S	0.10 ± 0.07	1.28 ± 0.18
S513A_I574A	1.16 ± 0.28	1.57 ± 0.09
T551S_I574A	1.17 ± 0.15	1.75 ± 0.10
T551V_I574A	5.92 ± 0.99	1.59 ± 0.13
E571A_I574A	6.86 ± 1.12	2.43 ± 0.27
T551A_E571A	14.09 ± 0.41	2.29 ± 0.12

Supplementary Table 2. 2-APB concentration-response parameters for TRPV1 WT and mutants. (For each channel type, n = 4-to-8)

	EC50 (μM)	Hill Coefficient
WT	212.24 ± 8.82	1.80 ± 0.12
S513A	59.34 ± 3.57	1.46 ± 0.12
T551S	135.87 ± 7.86	1.58 ± 0.13
T551V	223.16 ± 15.10	1.57 ± 0.15
E571A	912.17 ± 4.91	2.41 ± 0.04
I574A	602.47 ± 36.30	1.80 ± 0.15
S513A_I574A	552.71 ± 52.12	1.95 ± 0.14
T551S_I574A	879.36 ± 34.35	2.13 ± 0.19
T551V_I574A	898.14 ± 96.25	2.15 ± 0.17
E571A_I574A	2518.26 ± 277.98	2.70 ± 0.32

Supplementary Table 3. Lipid partition coefficient of capsaicin Tail analogs.

	LogP
Capsaicin	2.35
C_2	0.55
C_3	1.26
C_4	1.54
C_5	2.15
C_6	2.12
C_7	1.86
C_{11}	4.57

Supplementary Table 4. Estimation of number of channels in single-channel recordings.

	Observed Po with saturating capsaicin	Observed current level in recording	Number of opening events(n_0)	Probability of having ***N*** channels under a patch		
				One	**Two**	**Three**
WT	0.98 ± 0.01	One	182	≈ 1	$< 1\times10^{-300}$	$< 1\times10^{-300}$
I574A	0.54 ± 0.03	One	469	≈ 1	5.0×10^{-129}	4.6×10^{-207}
T551V	0.99 ± 0.01	One	134	≈ 1	3.4×10^{-205}	2.2×10^{-244}
E571A_I574A	0.14 ± 0.02	Two	1639	N.A.	≈ 1	5.3×10^{-227}

Supplementary Movie 1. Capsaicin binding and induced conformational changes to activate TRPV1. Upon capsaicin binding, a morph between closed and open states shows the induced conformational changes to activate TRPV1. Hydrogen bond is indicated as black lines. Different subunits are colored distinctly.

The Rosetta scripts to dock capsaicin

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