Supplementary Information

Molecular Basis for Heat Desensitization of TRPV1 Ion Channels

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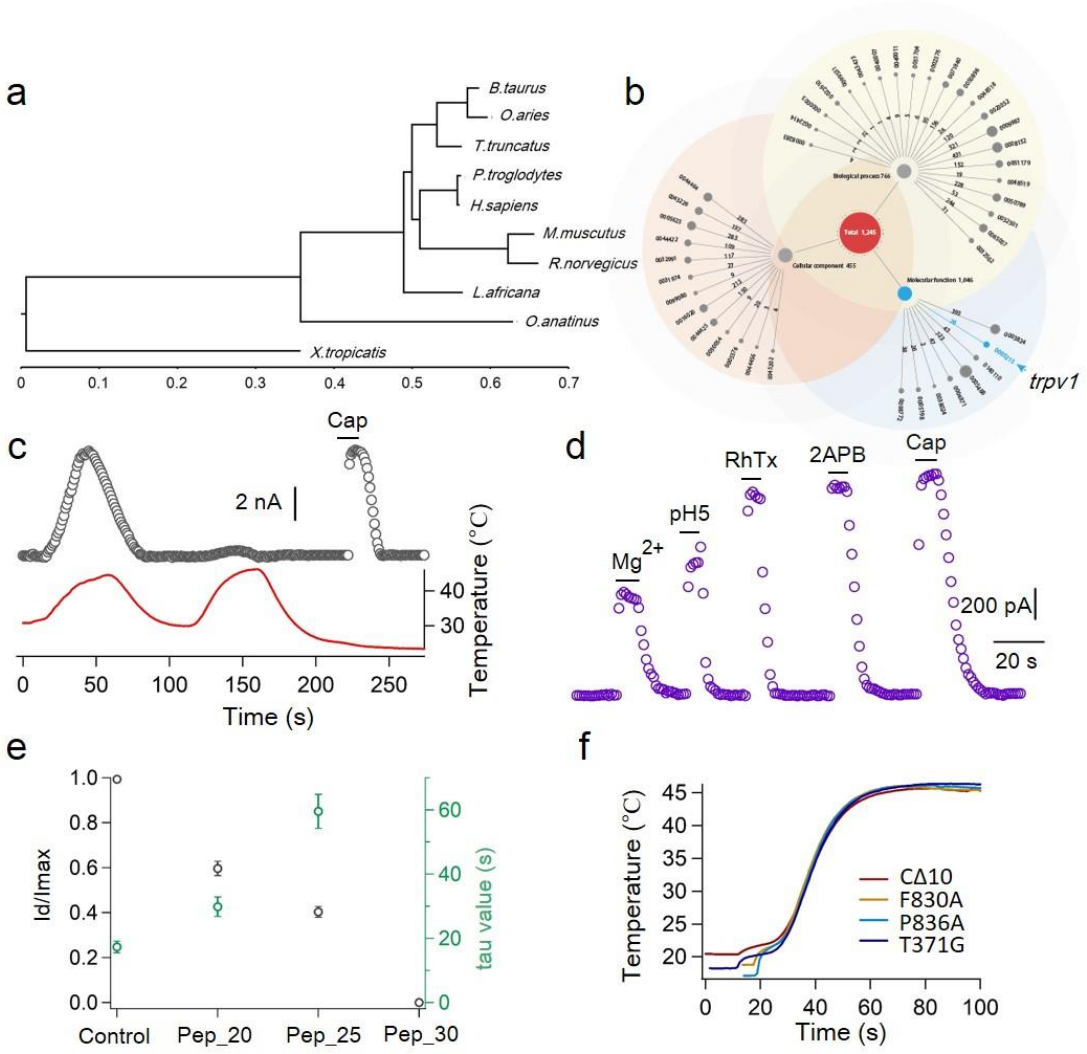
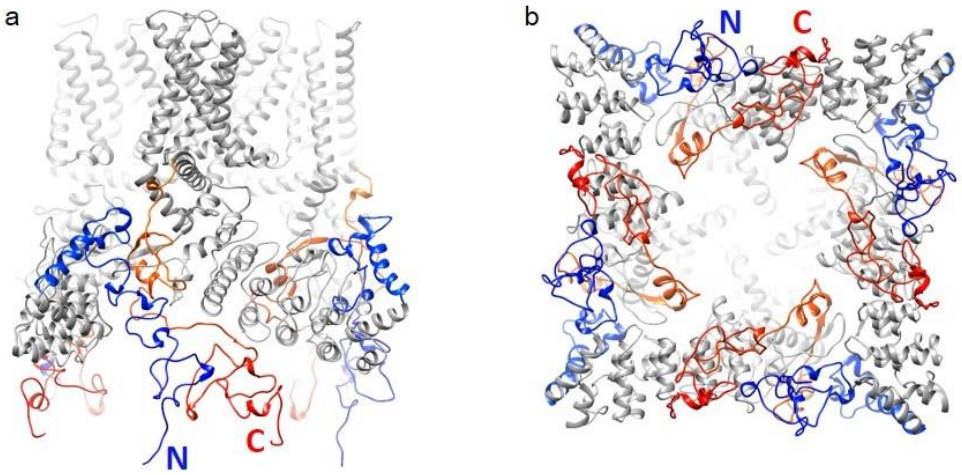
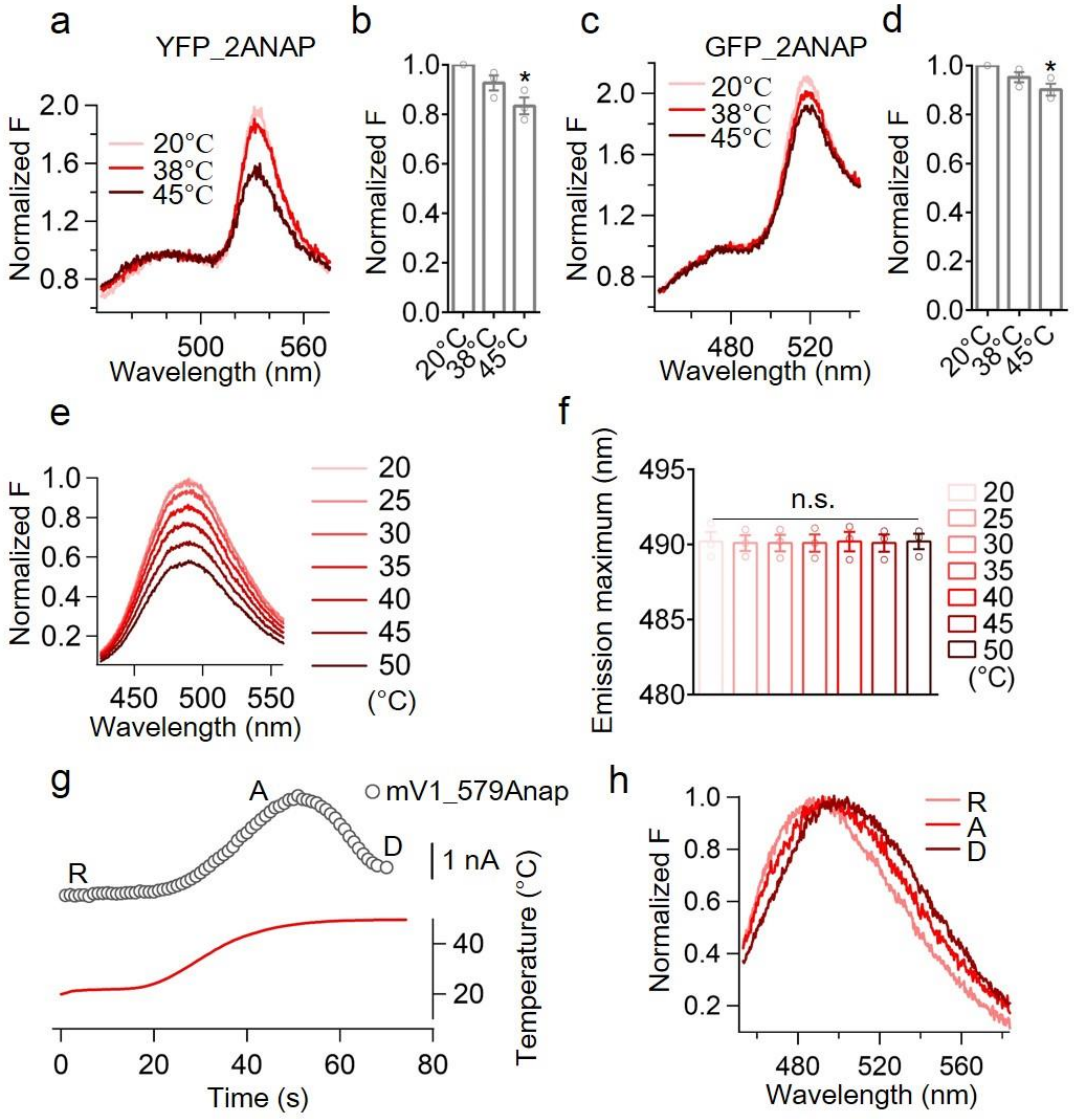


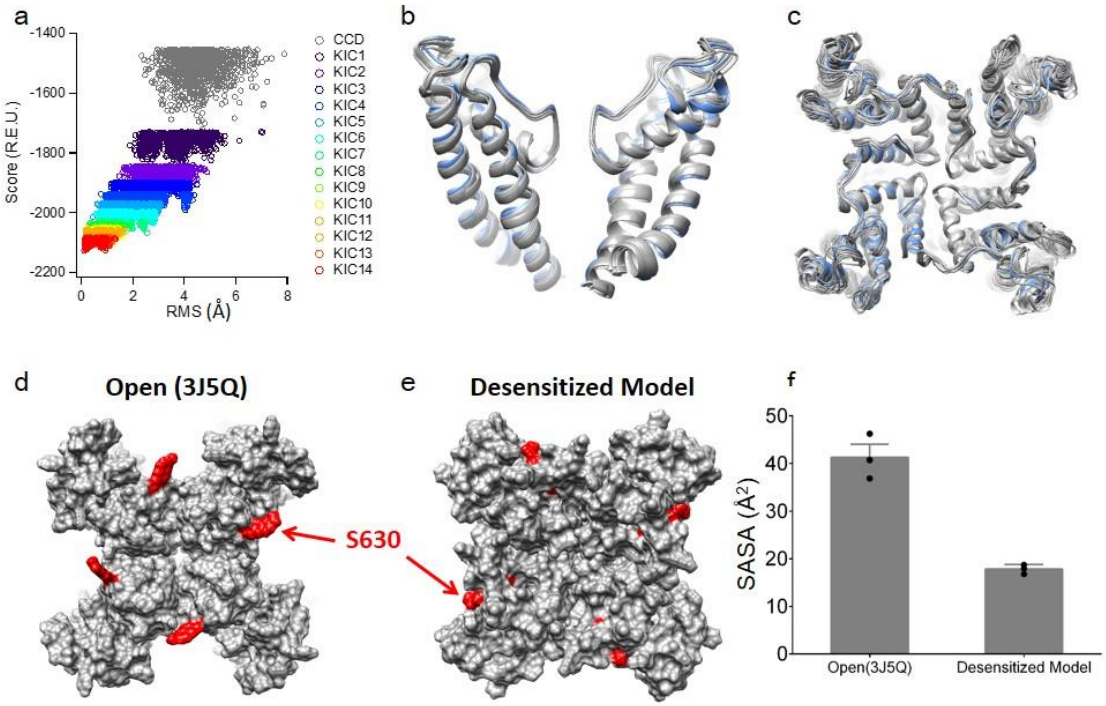
Supplementary Figure 1. Positive selection of platypus *trpv1* and functional conformation of platypus TRPV1 channel (pV1). **a** Consensus phylogenetic tree of species used in this study. The divergence time is labeled on the nodes. **b** Functional annotation of genes with positive selection in platypus based on PANTHER. **c** Representative current traces from HEK293T cells expressing mouse TRPV1 (mV1) evoked by repeated application of heat and supersaturated capsaicin. **d** Whole-cell pV1 currents elicited by application of Mg^{2+} (130 mM), pH 5, RhTx (10 μM), 2-APB (3 mM), capsaicin (10 μM). **e** Normalized heat desensitization (Dh) degree and tau values of mV1 with C-termini peptides of different lengths. **f** The temperature ramps used in electrophysiological recordings from channel mutants, respectively. All statistical data are given as mean ± s.e.m.



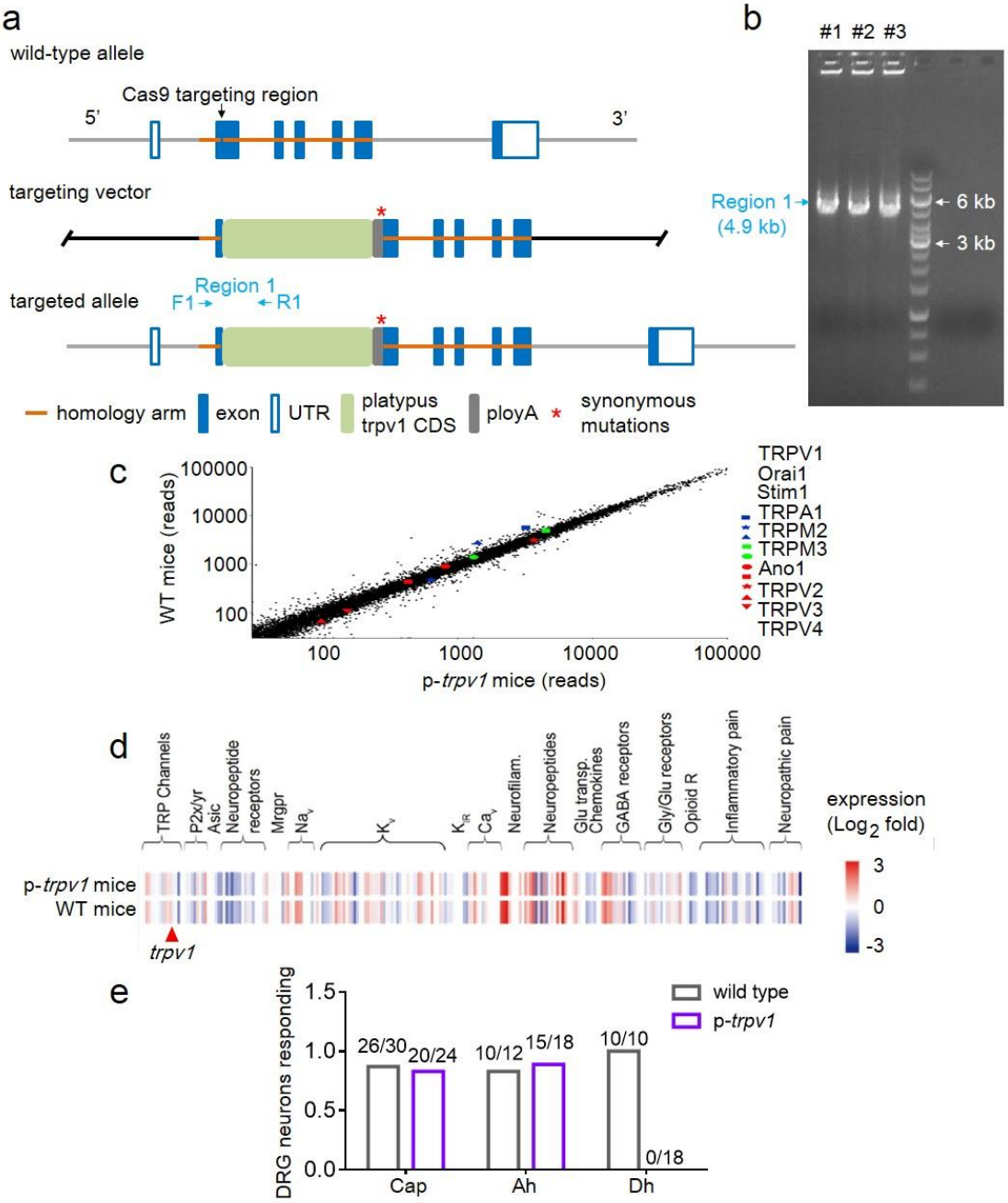
Supplementary Figure 2. *de novo* model of the distal N and C termini based on the cryo-EM structures. Ribbon diagram of mV1 atomic model with each of the four identical subunits color-coded, showing views from side (**a**) and bottom (**b**) focused on N and C termini.



Supplementary Figure 3. Heat-evoked current and emission spectra of ANAP-incorporated mouse TRPV1 (mV1_579ANAP) or YFP/GFP. **a** The spectra of YFP_2ANAP were acquired at 20, 38 and 45 °C. **b** The spectra in panel **a** were normalized to the ANAP intensity (n=3, two-sided t-test: *p<0.01). **c** The spectra of GFP_2ANAP were acquired at 20, 38 and 45 °C. **d** The spectra in panel **c** were normalized to the ANAP intensity (n=3, two-sided t-test: *p<0.01). **e** The spectra of ANAP were acquired at different temperatures. **f** The emission peak of ANAP at different temperatures (n=3, two-sided t-test: n.s., not significant). **g** Representative current induced by a temperature ramp recorded from HEK 293T cells expressing mV1_579ANAP. **h** Representative emission spectra at resting (R), activated (A) and desensitized (D) states of ANAP incorporated mV1 (mV1_579ANAP). Data are mean ± s.e.m.

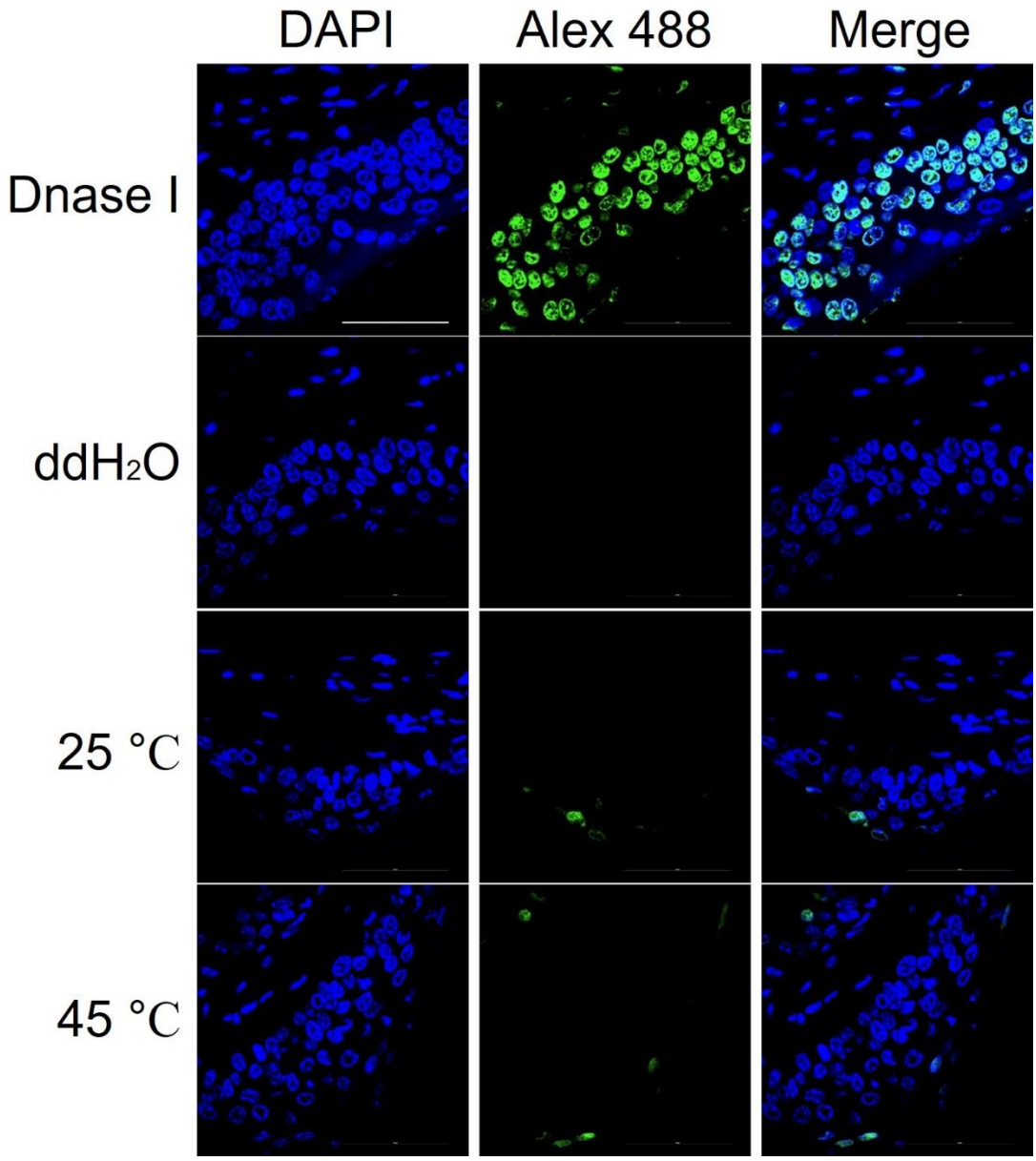


Supplementary Figure 4. Modeling of the TRPV1 channel in the desensitized state. **a** The models after fifteen rounds of KIC loop modeling exhibited a funnel-shaped distribution of total energy calculated by Rosetta (R.E.U., Rosetta energy unit). **b** and **c** Top five models after the fifteen round of loop modeling were well converged. The model with the lowest energy (blue) was chosen as the desensitized-state model. **d** and **e** ANAP was incorporated at the 630 sites (red) in the open (**d**) and desensitized (**e**) states, respectively. **f** SASA of ANAP at mV1_630 site was significantly decreased in the desensitized state (n=3, two-sided t-test: ***p<0.001). Data are mean ± s.e.m.



Supplementary Figure 5. p-*trpv1* transgenic mice construction and functional confirmation. **a** Schematic depiction of p-*trpv1* mice targeting strategy. Genomic region of mouse *trpv1* locus is diagrammed. Solid bars represent open reading frames (ORFs); open bars represent UTRs. **b** Northern blot analysis of p-*trpv1* mRNA expression in DRG from p-*trpv1* $^{+/+}$ mice. The 4.9-kb band identified with the primer pair illustrated in panel a. **c** Transcriptome-wide comparison of mRNA expression between wild-type and p-*trpv1* mice. Several ion channels that have been previously implicated in heat sensing are indicated. **d** Heat map showing the differential expression levels in wild-type and p-*trpv1* mice of a set of about 200 genes implicated in somatosensation. **e** The

percentage of wild-type and p-*trpv1* dorsal root ganglion neurons (DRGs) responding to 10 μM capsaicin, and percentages of heat-induced activation and desensitization of wild-type and p-*trpv1* DRG neurons responding to continues heat.



Supplementary Figure 6. Detection of apoptosis induced by heat using TUNEL assay. Apoptosis was detected by TUNEL staining (green fluorescence) and the nuclei were stained by DAPI (blue fluorescence) in pV1 over-expressing cells. Scale bar, 50 μm.

Supplementary Table 1. Functional comparison of seven mammalian TRPV1 channels.

Species	EC50_Capsaicin (nM)	Threshold of Ah (°C)	Dh transition	τ value_Dh (s)
Mouse	289 ± 17 (n = 3)	39.8 ± 0.6 (n = 5)	Yes	17.3 ± 0.5 (n = 3)
Platypus	307 ± 23 (n = 5)	34.0 ± 0.5 (n = 8)	No	n. d.
Human	269 ± 16 (n = 3)	40.1 ± 0.7 (n = 4)	Yes	17.4 ± 0.5 (n = 3)
Fruit bat	354 ± 25 (n = 6)	40.2 ± 0.7 (n = 3)	Yes	18.2 ± 0.4 (n = 3)
Tree shrew	5260 ± 140 (n = 3)	36.1 ± 0.5 (n = 4)	Yes	19.1 ± 0.5 (n = 3)
Polar bear	313 ± 21 (n = 3)	40.6 ± 0.4 (n = 3)	Yes	17.7 ± 0.6 (n = 3)
Camel	540 ± 37 (n = 5)	41.3 ± 0.9 (n = 6)	Yes	20.3 ± 0.4 (n = 4)

The Latin name of all species was shown in the method. All statistical data are expressed as means ± s.e.m. n.d. indicates no data. EC_{50}, effector concentration for half-maximum response; Ah, heat-induced activation; Dh, heat-induced desensitization; τ_Dh, tau value of the heat-induced desensitization.

Supplementary Table 2. Functional comparison of mV1, pV1 and pV1's chimeras.

Channel name	EC_{50}_Capsaicin (nM)	Threshold of Ah (°C)	Dh transition	τ value_Dh (s)
mV1	299 ± 10 (n = 3)	39.8 ± 0.5 (n = 6)	Yes	17.3 ± 0.5 (n = 5)
pV1	301 ± 12 (n = 5)	34.0 ± 0.4 (n = 8)	No	n. d.
pV1_mC	310 ± 19 (n = 6)	34.2 ± 0.4 (n = 5)	No	n. d.
pV1_mN	313 ± 16 (n = 6)	33.7 ± 0.4 (n = 5)	No	n. d.
pV1_mNC	306 ± 13 (n = 6)	34.0 ± 0.5 (n = 9)	Yes	16.9 ± 0.4 (n = 5)

All statistical data are expressed as means ± s.e.m. n.d. indicates no data. EC_{50}, effector concentration for half-maximum response; Ah, heat-induced activation; Dh, heat-induced desensitization; τ_Dh, tau value of the heat-induced desensitization.

Supplementary Table 3. Functional comparison of mV1 and its truncation mutants.

Channel name	EC_{50}_Capsaicin (nM)	Threshold of Ah (°C)	Dh transition	τ value_Dh (s)
mV1	299 ± 10 (n = 3)	39.8 ± 0.5 (n = 3)	Yes	17.3 ± 0.5 (n = 3)
CΔ30 (Glu^{747}-Phe^{776})	372 ± 43 (n = 5)	37.6 ± 0.6 (n = 6)	Yes	18.8 ± 0.9 (n = 6)
CΔ23 (Ser^{777}-Asp^{799})	398 ± 51 (n = 3)	37.8 ± 0.6 (n = 6)	Yes	20.0 ± 0.7 (n = 6)
CΔ28 (Ala^{800}-Ala^{827})	343 ± 37 (n = 3)	38.2 ± 0.6 (n = 6)	Yes	20.3 ± 7 (n = 6)
CΔ10 (Phe^{830}-Lys^{839})	354 ± 39 (n = 6)	39.0 ± 0.5 (n = 9)	No	n. d.

Supplementary Table 4. Functional comparison of mV1 and its point mutations.

Channel name	EC_{50}_Capsaicin (nM)	Threshold of Ah (°C)	Dh transition	τ value_Dh (s)
mV1	299 ± 10 (n = 3)	39.8 ± 0.5 (n = 3)	Yes	17.3 ± 0.5 (n = 3)
S116A	186 ± 11 (n = 3)	39.5 ± 0.6 (n = 3)	Yes	18.0 ± 0.6 (n = 4)
T371G	322 ± 16 (n = 3)	39.9 ± 0.6 (n = 4)	Yes	34.5 ± 1.9 (n = 6)
F830A	338 ± 27 (n = 3)	40.4 ± 0.5 (n = 3)	Yes	40.0 ± 0.6 (n = 6)
K831A	354 ± 30 (n = 3)	39.9 ± 0.4 (n = 3)	Yes	17.5 ± 0.6 (n = 3)
D832A	361 ± 35 (n = 3)	39.4 ± 0.3 (n = 3)	Yes	17.8 ± 0.4 (n = 3)
S833A	344 ± 26 (n = 3)	39.4 ± 0.5 (n = 3)	Yes	17.1 ± 0.5 (n = 3)
M834A	329 ± 23 (n = 3)	39.9 ± 0.4 (n = 3)	Yes	16.8 ± 0.6 (n = 3)
P836A	315 ± 24 (n = 3)	40.1 ± 0.4 (n = 3)	Yes	39.9 ± 0.7 (n = 6)
E838A	565 ± 54 (n = 3)	39.1 ± 0.4 (n = 3)	Yes	17.4 ± 0.5 (n = 3)
K839A	293 ± 12 (n = 3)	39.5 ± 0.6 (n = 3)	Yes	17.7 ± 0.4 (n = 3)

All statistical data are expressed as means ± s.e.m. n.d. indicates no data. EC_{50}, effector concentration for half-maximum response; Ah, heat-induced activation; Dh, heat-induced desensitization; τ_Dh, tau value of the heat-induced desensitization.

Supplementary Table 5. Functional confirmation of ANAP-incorporated TRPV1 channels.

Labeled position	EC_{50}_Capsaicin (nM)	low pH	2APB	Threshold of Ah (°C)	Dh transition
mV1_241	290 ± 9 (n = 5)	Yes	Yes	37.9 ± 0.4 (n = 3)	Yes
pV1_241	302 ± 11 (n = 6)	Yes	Yes	33.9 ± 0.3 (n = 6)	No
mV1_575	n. d.	n. d.	n. d.	n. d.	n. d.
mV1_579	293 ± 8 (n = 3)	Yes	Yes	37.8 ± 0.4 (n = 3)	Yes
mV1_600	n. d.	n. d.	n. d.	n. d.	n. d.
mV1_603	n. d.	n. d.	n. d.	n. d.	n. d.
mV1_605	n. d.	n. d.	n. d.	n. d.	n. d.
mV1_630	299 ± 10 (n = 4)	Yes	Yes	37.9 ± 0.3 (n = 5)	Yes
pV1_643	306 ± 12 (n = 6)	Yes	Yes	33.8 ± 0.3 (n = 7)	No
mV1_633	n. d.	n. d.	n. d.	n. d.	n. d.
mV1_636	n. d.	n. d.	n. d.	n. d.	n. d.
mV1_640	n. d.	n. d.	n. d.	n. d.	n. d.
mV1_648	296 ± 7 (n = 6)	No	Yes	38.2 ± 0.3 (n = 4)	Yes
mV1_650	n. d.	n. d.	n. d.	n. d.	n. d.
mV1_651	288 ± 8 (n = 6)	Yes	Yes	37.7 ± 0.4 (n = 5)	Yes
mV1_654	304 ± 10 (n = 6)	Yes	Yes	38.1 ± 0.3 (n = 6)	Yes
mV1_656	n. d.	n. d.	n. d.	n. d.	n. d.
mV1_672	n. d.	n. d.	n. d.	n. d.	n. d.
mV1_673	n. d.	n. d.	n. d.	n. d.	n. d.
mV1_676	n. d.	n. d.	n. d.	n. d.	n. d.
mV1_677	n. d.	n. d.	n. d.	n. d.	n. d.
mV1_680	n. d.	n. d.	n. d.	n. d.	n. d.
mV1_684	n. d.	n. d.	n. d.	n. d.	n. d.
mV1_685	295 ± 11 (n = 8)	Yes	Yes	37.8 ± 0.4 (n = 6)	Yes

All statistical data are expressed as means ± s.e.m. n.d. indicates no data. EC_{50}, effector concentration for half-maximum response; Ah, heat-induced activation; Dh, heat-induced desensitization; low pH, pH5; 2APB, 2-aminoethoxydiphenyl borate.

Supplementary Table 6. Primers used in this study to identify the genotype of transgenic mice.

primer	sequence
F1	5’-CAGAGTGCTAGAGATGCTTTCAATC-3’
R1	5’-TGCGTTTAATCCCTTCACTGTTC-3’

Supplementary Table 7. A routine urine test in WT & p-*trpv1* mice.

Project	WT (n=3)	p-*trpv1* (n=3)
PH	5.5 ± 0.0	5.5 ± 0.0
Glu - (mmol/L)	0 ± 0.0	0 ± 0.0
Vc +3 (mmol/L)	5.6 ± 0.13	5.6 ± 0.14
SG	1.030 ± 0.000	1.030 ± 0.007
BLD - (Cell /μL)	0 ± 0.0	0 ± 0.0
PRO +- (g/L)	0.15 ± 0.01	0.15 ± 0.00
BIL - (μmol/L)	0 ± 0.0	0 ± 0.0
URO +1 (μmol/L)	33 ± 0	33 ± 0
KET +1 (mmol/L)	1.5 ± 0.05	1.5 ± 0.05
WBC- (Cell /μL)	0 ± 0.0	0 ± 0.0

Data are expressed as mean ± s.e.m. from 3 mice.

Supplementary Table 8. Routine blood test in WT & p-*trpv1* mice.

Project	WT (n=3)	p-*trpv1* (n=3)
WBC ($\times 10^9$/L)	5.0 ± 1.92	12.3 ± 1.40
Lymph# ($\times 10^9$/L)	2.7 ± 1.12	8.1 ± 2.09
Mon# ($\times 10^9$/L)	0.2 ± 0.05	0.5 ± 0.12
Gran# ($\times 10^9$/L)	2.1 ± 0.28	3.7 ± 0.62
Lymph (%)	54.5 ± 2.49	66.2 ± 7.27
Mon (%)	3.1 ± 0.22	3.7 ± 0.49
Gran (%)	42.4 ± 6.06	30.1 ± 3.35
RBC ($\times 10^{12}$/L)	9.81 ± 1.02	11.13 ± 0.80
HGB (g/L)	148 ± 10.66	165 ± 13.91
HCT (%)	46.5 ± 4.02	54.7 ± 7.30
MCV (fL)	47.5 ± 1.27	49.2 ± 2.19
MCH (pg)	15.0 ± 0.74	14.8 ± 0.37
MCHC (g/L)	318 ± 7.76	301 ± 11.34
RDW (%)	14.5 ± 0.38	14.2 ± 0.45
PLT ($\times 10^9$/L)	464 ± 18.57	435 ± 17.93
MPV (fL)	5.3 ± 0.34	5.7 ± 0.60
PDW	15.9 ± 0.75	16.7 ± 0.54
PCT (%)	0.245 ± 0.004	0.247 ± 0.004

Data are expressed as mean ± s.e.m. from 3 mice.

Supplementary Table 9. Blood bio-chemistry examination in WT & p-*trpv1* mice.

Project	WT (n=3)	p-*trpv1* (n=3)
TP (g/L)	48.3 ± 3.7	55.8 ± 4.4
ALB (g/L)	33.7 ± 1.9	37.5 ± 2.8
GLB (g/L)	14.6 ± 2.1	18.3 ± 1.9
ALB/GLB	2.31 ± 0.20	2.05 ± 0.04
ALT (IU/L)	61.10 ± 4.80	80.00 ± 8.50
AST (IU/L)	254.8 ± 17.1	337.5 ± 33.2
AST/ALT	4.20 ± 0.08	4.20 ± 0.05
TB (μmol/L)	0.8 ± 0.1	1.3 ± 0.2
DB (μmol/L)	0.70 ± 0.05	0.8 ± 0.08
IDBIL (μmol/L)	0.2 ± 0.0	0.5 ± 0.1
TBA (μmol/L)	7.6 ± 0.4	6.7 ± 0.8
ALP (IU/L)	261.0 ± 30.8	231.5 ± 22.1
Urea (mmol/L)	9.21 ± 0.76	11.38 ± 0.99
Cre (μmol/L)	10.20 ± 0.80	13.40 ± 0.67
UA (μmol/L)	64.7 ± 9.9	105.3 ± 15.2
GLU (mmol/L)	7.9 ± 0.4	7.6 ± 0.4
TC (mmol/L)	1.88 ± 0.19	2.57 ± 0.40
F-CHOL (mmol/L)	0.38 ± 0.07	0.55 ± 0.07
TG (mmol/L)	1.05 ± 0.06	1.17 ± 0.13
HDL-C (mmol/L)	1.42 ± 0.20	1.87 ± 0.28
LDL-C (mmol/L)	0.41 ± 0.03	0.55 ± 0.05
apoA1 (g/L)	0.03 ± 0.00	0.03 ± 0.00
apoB (g/L)	0.12 ± 0.03	0.20 ± 0.04
Ca (mmol/L)	2.29 ± 0.15	2.56 ± 0.13

Data are expressed as mean ± s.e.m. from 3 mice.

Supplementary Table 10. Primers used in this study to generate TRPV1 chimeras.

Name	Primer-Forward	Primer-Reverse
pV1_mNC_mV1	ctgatgggggagacagtgaa caagattgcacaagagagc	gaagttgaagtaaaatatgc gcttgacaaatctgtcccac
pV1_mNC_pV1	gacagatttgtcaagcgc atattttacttcaacttcttc	ctcttgtgcaatcttgttca ctgtctcccccatcagggc
pV1_mC_mV1	ctgatgggggagacagtga acaagattgcacaagagagc	ccgggcccgcggtaccgtt ttctcccctggggccatgga
pV1_mC_pV1	atggccccaggggagaaaa cggtaccgcgggcccgggat	ctcttgtgcaatcttgttca ctgtctcccccatcagggc
pV1_mN_mV1	gatctcgagctcaagcttat ggagaaatgggctagctta	gaagttgaagtaaaatatgc gcttgacaaatctgtccca
pV1_mN_pV1	gacagatttgtcaagcgc atattttacttcaacttcttc	gctagcccatttctccataa gcttgagctcgagatctga
mC_mV1	gctagcgtttaaacttaaaa caagattgcacaagagagc	ctggatatctgcagaatttt tctcccctggggccatgga
pC_pV1	gctagcgtttaaacttaaaa caaggtctcgcaagaaagc	ctggatatctgcagaatttt cttctaaaataagtgactc

Supplementary Table 11. Primers used in this study to generate mV1 truncation mutants.

Name	Primer-Forward	Primer-Reverse
CΔ30 (Glu^{747}-Phe^{776})	agggtggattccctgcgg tcaggccgagtttcaggg	ccgcagggaatccaccct gaagcaccaccggaagtc
CΔ23 (Ser^{777}-Asp^{799})	ctgagcttcgcaagcact cgagataggcatagcacc	agtgcttgcgaagctcag ggtgcgcttgacgccctc
CΔ28 (Ala^{800}-Ala^{827})	ctgagggacgaggtcttc aaggattccatggcccca	gaagacctcgtccctcag aaggggaaccagggcaaa
CΔ10 (Phe^{830}-Lys^{839})	gctgaggtctcagggaag ccgaattctgcagtcgac	cttccctgagacctcagc atcctctggcttaaggga

Supplementary Table 12. Primers used in this study to generate mV1 point mutations.

Name	Primer-Forward	Primer-Reverse
S116A	gatcgcagggcaatcttcgacgctgtggctcagagc	gtcgaagattgccctgcgatcatagagccttggggg
T371G	aggaagttcgcagaatgggcctatgggcccgtgcac	ggcccattctgcgaacttcctggacaggtgccggca
F830A	gctgaggtcgcaaaggattccatggccccaggggag	ggaatcctttgcgacctcagcatcctctggcttaag
K831A	gaggtcttcgcagattccatggccccaggggagaaa	catggaatctgcgaagacctcagcatcctctggctt
D832A	gtcttcaaggcatccatggccccaggggagaaatca	ggccatggatgccttgaagacctcagcatcctctgg
S833A	ttcaaggatgcaatggccccaggggagaaatcaggg	tggggccattgcatccttgaagacctcagcatcctc
M834A	aaggattccgcagccccaggggagaaatcagggaag	ccctggggctgcggaatccttgaagacctcagcatc
P836A	tccatggccgcaggggagaaatcagggaagccgaat	tttctcccctgcggccatggaatccttgaagacctc
E838A	gccccaggggcaaaatcagggaagccgaattctgca	ccctgattttgcccctggggccatggaatccttgaa
K839A	ccaggggaggcatcagggaagccgaattctgcagtc	cttccctgatgcctcccctggggccatggaatcctt

Supplementary Table 13. Primers used in this study to generate ANAP-incorporated TRPV1 channels.

Name	Primer-Forward	Primer-Reverse
mV1_241	aagaaaacctaggggaggcctggcttctactttggt	aggcctcccctaggttttcttgaagaagtccccgtt
pV1_241	aagaaaacctagggccggccgggcttttattttggt	cggccggccctaggttttcttaaagaagtcaccatg
mV1_575	aagatgatctagagagacctgtgtcggtttatgttc	caggtctctctagatcatcttctcaatcatgacagc
mV1_579	agagacctgtagcggtttatgttcgtctacctcgtg	cataaaccgctacaggtctctgaggatcatcttctc
mV1_600	gtgacactgtaggaggatgggaagaataactcactg	cccatcctcctacagtgtcactacggctgtggaaaa
mV1_603	atcgaggattagaagaataactcactgcctgtggag	gttattcttctaatcctcgatcagtgtcactacggc
mV1_605	gatgggaagtagaactcactgcctgtggagtcccca	cagtgagttctacttcccatcctcgatcagtgtcac
mV1_630	tcttacaactagctgtattccacatgtctggagctg	ggaatacagctagttgtaagagttacctggcctgca
pV1_643	tcctataactagctgtactctacttgcctggagctc	agagtacagctagttataggaaggggaagaaggctt
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mV1_636	tccacatgttaggagctgttcaagttcaccatcggc	gaacagctcctaacatgtggaatacaggctgttgta
mV1_640	gagctgttctagttcaccatcggcatgggtgacctg	gatggtgaactagaacagctccagacatgtggaata
mV1_648	atgggtgactaggagttcaccgagaactatgacttc	ggtgaactcctagtcacccatgccgatggtgaactt
mV1_650	gacctggagtagaccgagaactatgacttcaaggct	gttctcggtctactccaggtcacccatgccgatggt
mV1_651	ctggagttctaggagaactatgacttcaaggctgtc	atagttctcctagaactccaggtcacccatgccgat
mV1_654	accgagaactaggacttcaaggctgtcttcatcatc	cttgaagtcctagttctcggtgaactccaggtcacc
mV1_656	aactatgactagaaggctgtcttcatcatcctgtta	gacagccttctagtcatagttctcggtgaactccag
mV1_672	attctcacctagatcctcctgctcaacatgctcatt	caggaggatctaggtgagaatcacataggccagtaa
mV1_673	ctcacctactagctcctgctcaacatgctcattgct	gagcaggagctagtaggtgagaatcacataggccag
mV1_676	atcctcctgtagaacatgctcattgctctcatgggc	gagcatgttctacaggaggatgtaggtgagaatcac
mV1_677	ctcctgctctagatgctcattgctctcatgggcgag	aatgagcatctagagcaggaggatgtaggtgagaat
mV1_680	aacatgctctaggctctcatgggcgagactgtcaac	catgagagcctagagcatgttgagcaggaggatgta
mV1_684	gctctcatgtaggagactgtcaacaagattgcacaa	gacagtctcctacatgagagcaatgagcatgttgag
mV1_685	ctcatgggctagactgtcaacaagattgcacaagag	gttgacagtctagcccatgagagcaatgagcatgtt

Supplementary Methods

Commands in Rosetta to perform loop modeling:

```
/home/fanyang/rosetta/main/source/bin/loopmodel.linuxgccrelease \
-in:path:database /home/fanyang/rosetta2015.25/main/database \
-score:weights membrane_highres_Menv_smooth.wts \
-in:file:fullatom \
-membrane:normal_cycles 100 \
-membrane:normal_mag 15 \
-membrane:center_search \
-ignore_unrecognized_res \
-symmetry:symmetry_definition
/home/fanyang/projects/input_files/3J5Q_2018/4D_ABCD_r_4D_after_ccd_relaxed_r.s
ymm \
-symmetry:initialize_rigid_body_dofs \
-in:file:spanfile /home/fanyang/projects/input_files/3J5Q_2018/3J5Q_FL.span \
-in:file:s                    /share/work/fanyang/work/3J5Q_FL_KIC5-3J5Q_2018-
_/top20_score_filtered/_1D/${SLURM_ARRAY_TASK_ID}.pdb \
-loops:loop_file /home/fanyang/projects/input_files/3J5Q_2018/3J5Q_FL_SF.loop \
-loops:remodel perturb_kic \
-loops:refine refine_kic \
-loops:relax no \
-loops:strict_loops \
-loops:build_attempts 20 \
-relax:bb_move false \
-max_inner_cycles 30 \
-nstruct 51 \
-out:prefix msymm-loop-kic- \
-out:file:silent              /share/work/fanyang/work/3J5Q_FL_KIC6-3J5Q_2018-
_/${SLURM_ARRAY_TASK_ID}/msymm-relax-_3J5Q_2018_${SLURM_ARRAY_TASK_ID}.silent \
-out:file:silent_struct_type binary \
-mute all
```

Rosetta scripts to perform SASA calculation and filtering:

```
<ROSETTASCRIPTS>
    <RESIDUE_SELECTORS>
    <Not name="630_unselected">
        <Index resnums=272/>
    </Not>
    </RESIDUE_SELECTORS>
    <TASKOPERATIONS>
    <OperateOnResidueSubset name="630_only" selector="6300_unselected" >
        <PreventRepackingRLT/>
    </OperateOnResidueSubset>
    </TASKOPERATIONS>
    <SCOREFXNS>
    </SCOREFXNS>
 <FILTERS>
    <TotalSasa     name=630_sasa     threshold=10     task_operations=630_only
report_per_residue_sasa=1/>
 </FILTERS>
    <MOVERS>
    </MOVERS>
    <PROTOCOLS>
    <Add filter=630_sasa/>
    </PROTOCOLS>

</ROSETTASCRIPTS>
```

Commands in Rosetta to incorporate ANAP into TRPV1 models

```
-database /home/fan/rosetta/main/database
-in:file:fullatom
-ignore_unrecognized_res
-s /home/fan/Rosetta/Project_ANAP_Parameterization/3J5Q.pdb
-backrub:ntrials 10
-nstruct 1
-mc_kt 0.6
-resfile /home/fan/Rosetta/Project_ANAP_Parameterization/630ANP_3J5Q.resfile
-overwrite
```

630ANP_3J5Q.resfile:

```
NATRO
start
630 A EMPTY NC ANP
630 B EMPTY NC ANP
630 C EMPTY NC ANP
630 D EMPTY NC ANP
```