Supporting Information

Hu *et al.* 10.1073/pnas.0812209106

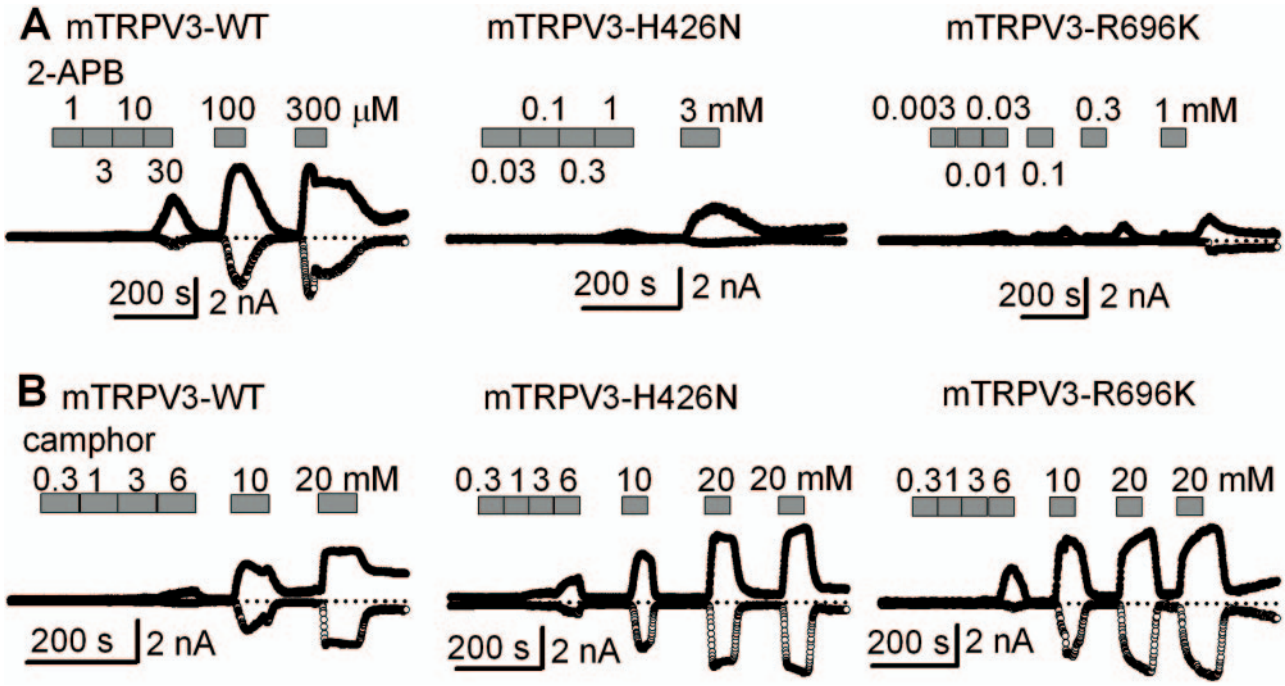

Fig. S1. Whole-cell patch clamp recording revealed decreased 2-APB but not camphor responses in HEK293 cells transfected with mTRPV3-H426N and mTRPV3-R696K mutants. (*A*) Whole-cell current traces of HEK293 cells transiently transfected with mTRPV3, mTRPV3-H426N, and mTRPV3-R696K in response to increasing 2-APB concentrations taken at +100 mV or −100 mV; 2-APB sensitivity is strongly diminished in both H426N and R696K mutants. Note that responses to higher concentrations of 2-APB are shown for mutants compared with WT. (*B*) Camphor responses in TRPV3, TRPV3-H426N, and TRPV3-R696K are similar in equivalent recordings.

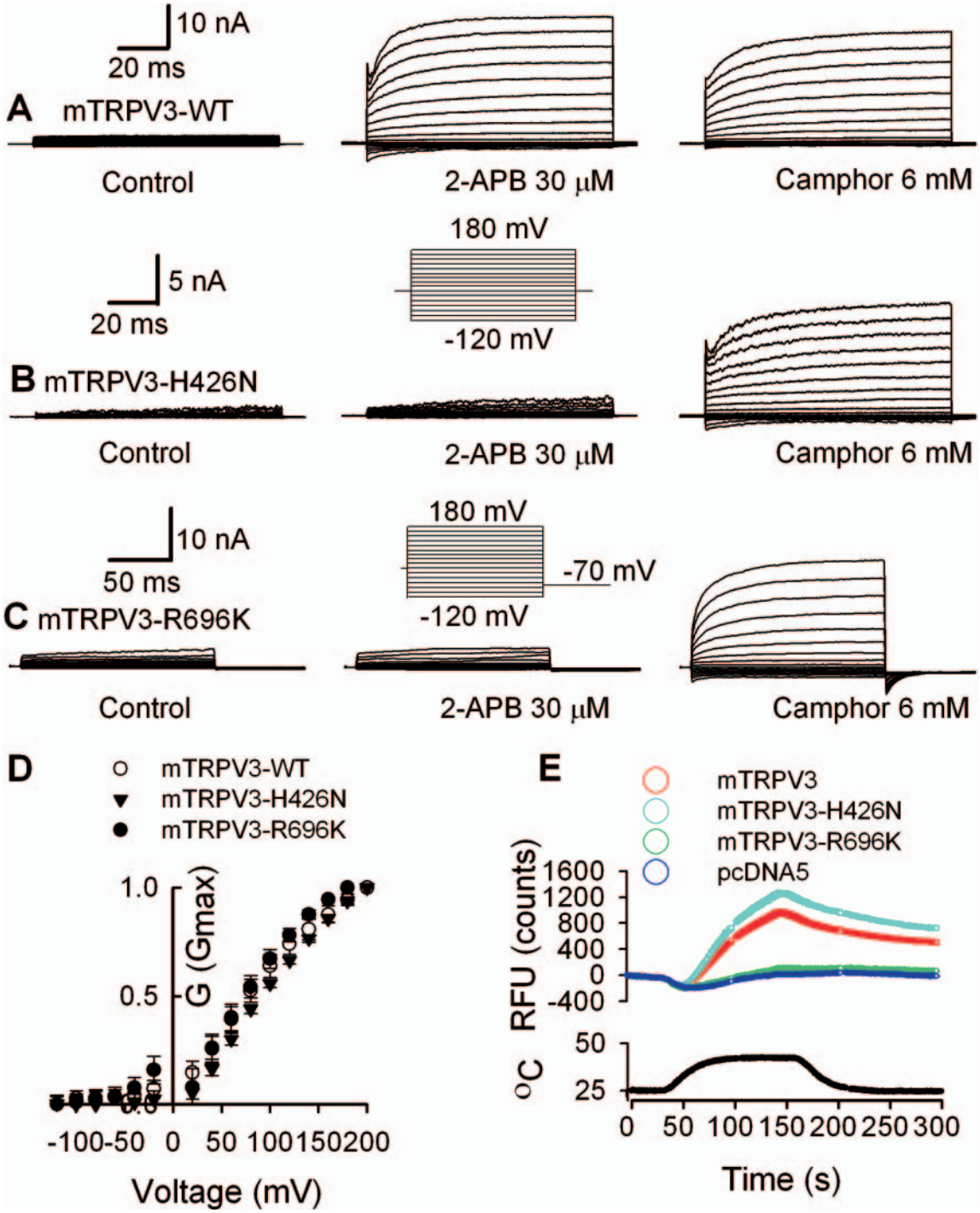

Fig. S2. Voltage-dependence and temperature sensitivity of wild-type and 2-APB-deficient TRPV3 channels. (*A*–*C*) Whole-cell voltage-step patch-clamp current traces from HEK293 cells transfected with wild-type TRPV3 (*A*), TRPV3-H426N (*B*), or TRPV3-R696K (*C*) channels in response to 2-APB or camphor. Due to the desensitizing nature of R696K mutant channel to 2-APB (see Fig. S3), we used a tail current protocol to measure $V_{1/2}$ of 2-APB-activated response in this mutant. However, because the camphor response was not desensitizing in R696K mutant, we used the steady state current to calculate $V_{1/2}$ for consistency with measurements in wild-type TRPV3 and TRPV3-H426 mutants. (*D*) Conductance-voltage (GV) curves of camphor-activated responses in HEK293 cells transfected with wild-type mTRPV3, mTRPV3-H426N, and mTRPV3-R696K mutants. (*E*) Temperature sensitivity in TRPV3-H426N and TRPV3-R696K. Time sequence of calcium responses evoked by a heat step from 25 to 42 to 25 °C at ≈2 °C/s in HEK293 cells transfected with TRPV3, TRPV3-H426N, TRPV3-R696K, or pcDNA5 vector only. Each trace is the time sequence of each clone averaged from 24 wells. Error bars are SEs. Baseline subtraction was applied to all for easy comparison of the net responses (for details of heating device and method, see Grandl J, *et al*. (2008) Pore region of TRPV3 ion channel is specifically required for heat activation. *Nat Neurosci* 1:1007–1013).

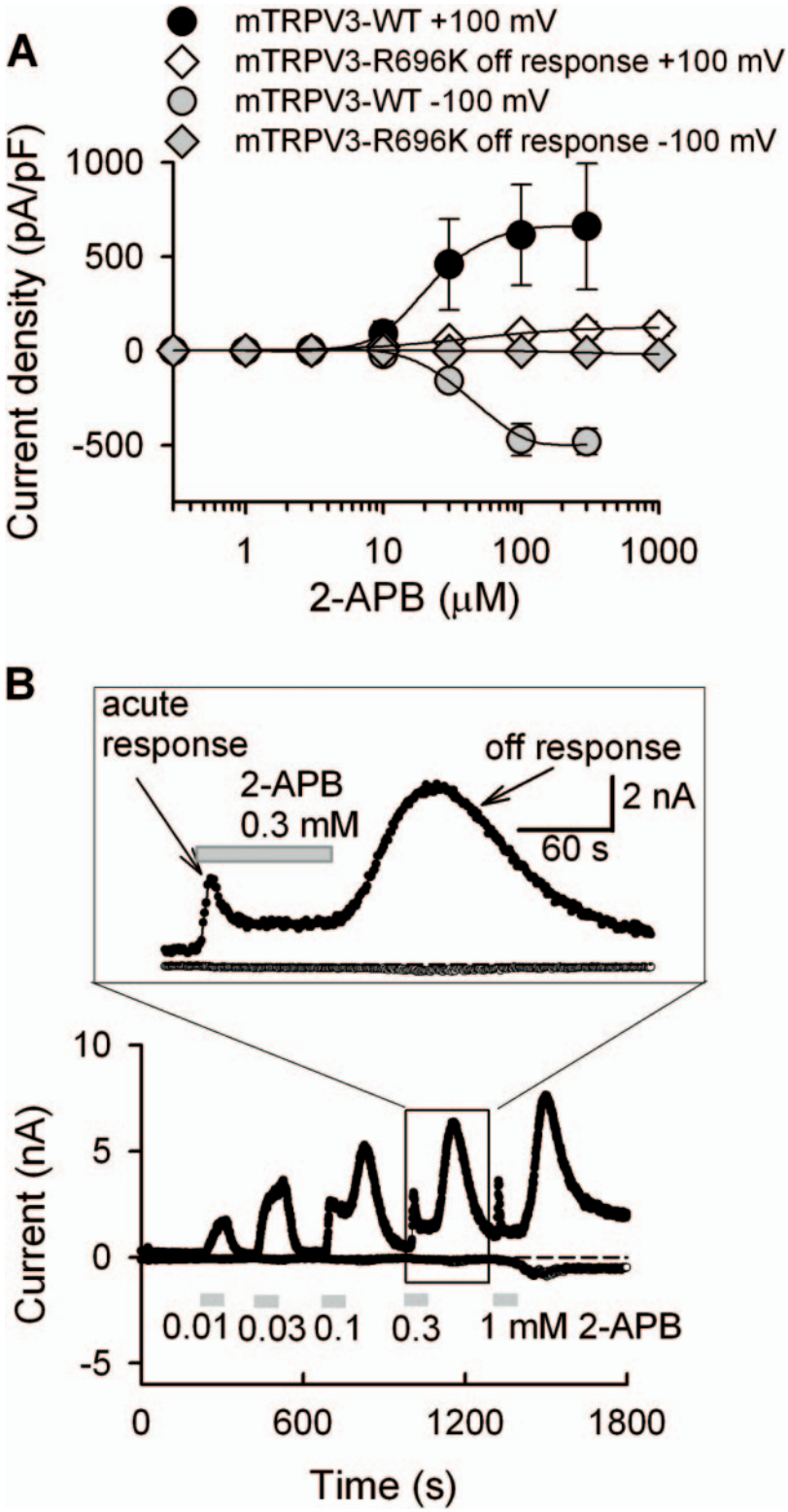

Fig. S3. The 2-APB-evoked ''off response'' in TRPV3-R696. (*A*) Concentration-response curves of 2-APB response in wild-type TRPV3 and 2-APB-evoked off response in TRPV3-R696 mutant. Error bars are SEs, $n = 6$ for each clone. (*B*) Whole cell current traces of 2-APB-activated response in a HEK293 cell transfected with R696K mutant taken at both +100 and −100 mV. Note that 2-APB at high concentrations had a desensitizing response and followed by an off response after washout. Blown-up image on top illustrate the ''acute response'' on application of 100 μM 2-APB that desensitized in the presence of 2-APB. An off response followed the washout of 2-APB.

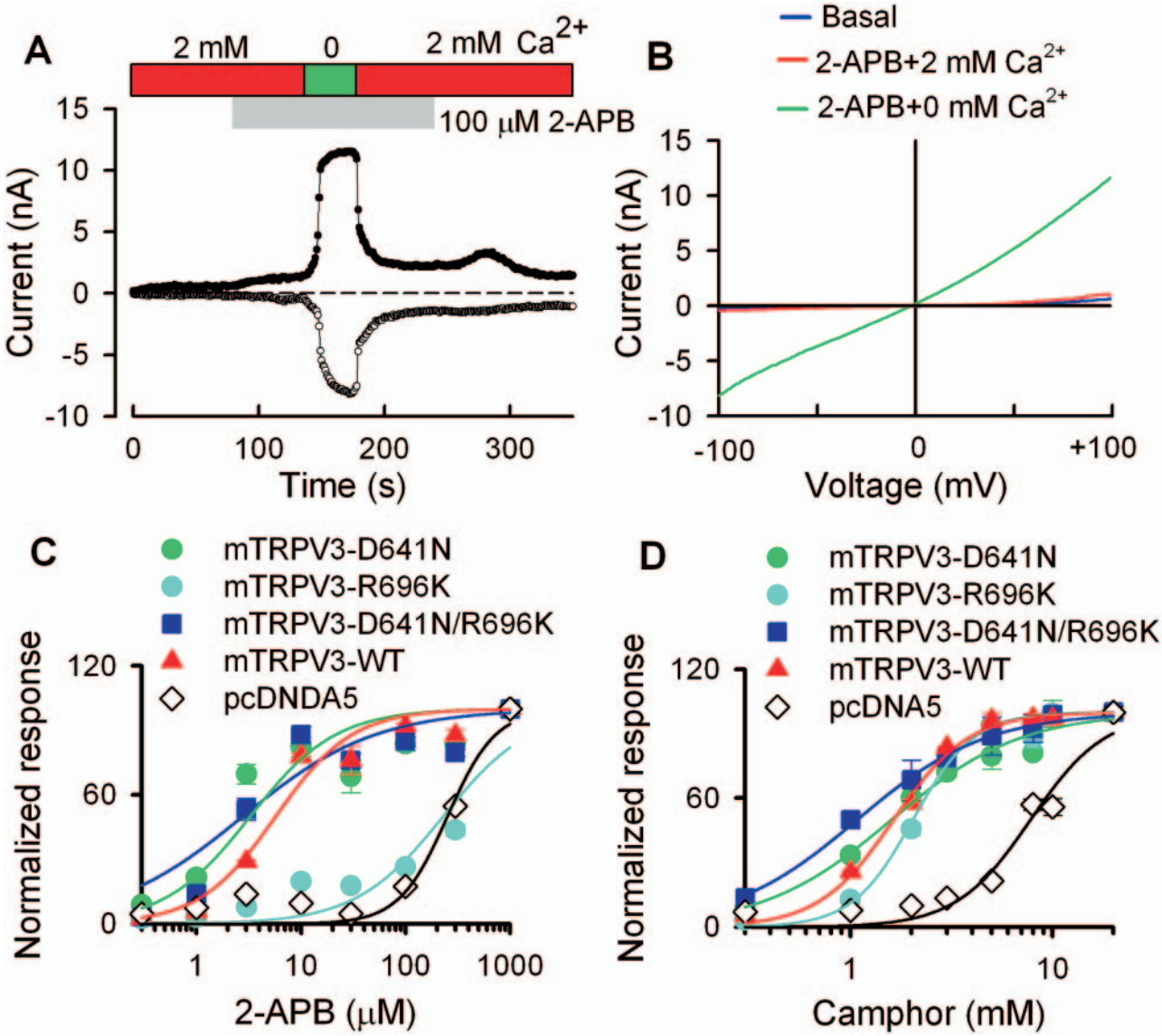

Fig. S4. By removing extracellular calcium inhibition, the 2-APB response in TRPV3-R696K is restored. (*A*) A a small response was evoked by 100 μM 2-APB in a R696K-transfected HEK293 cell in whole cell configuration in the presence of 2 mM extracellular calcium; 2-APB-activated current was dramatically increased when nominal calcium free solution was applied. (*B*) Current-voltage relationship illustrates the effect of removing extracellular calcium on 2-APB response in *A*. (*C*) Neutralization of the acidic residue D641 in the putative selective filter region of the TRPV3 channel pore restores 2-APB response in R696K mutant. FLIPR assay reveals that double mutation of D641N/R696K has similar concentration-response curve as wild-type TRPV3 and D641N, but R696K mutant is severely decreased in 2-APB responses. (*D*) Camphor-evoked responses are similar among wild-type and mutant TRPV3 channels. Error bars are SEs, $n = 4$ for each points.

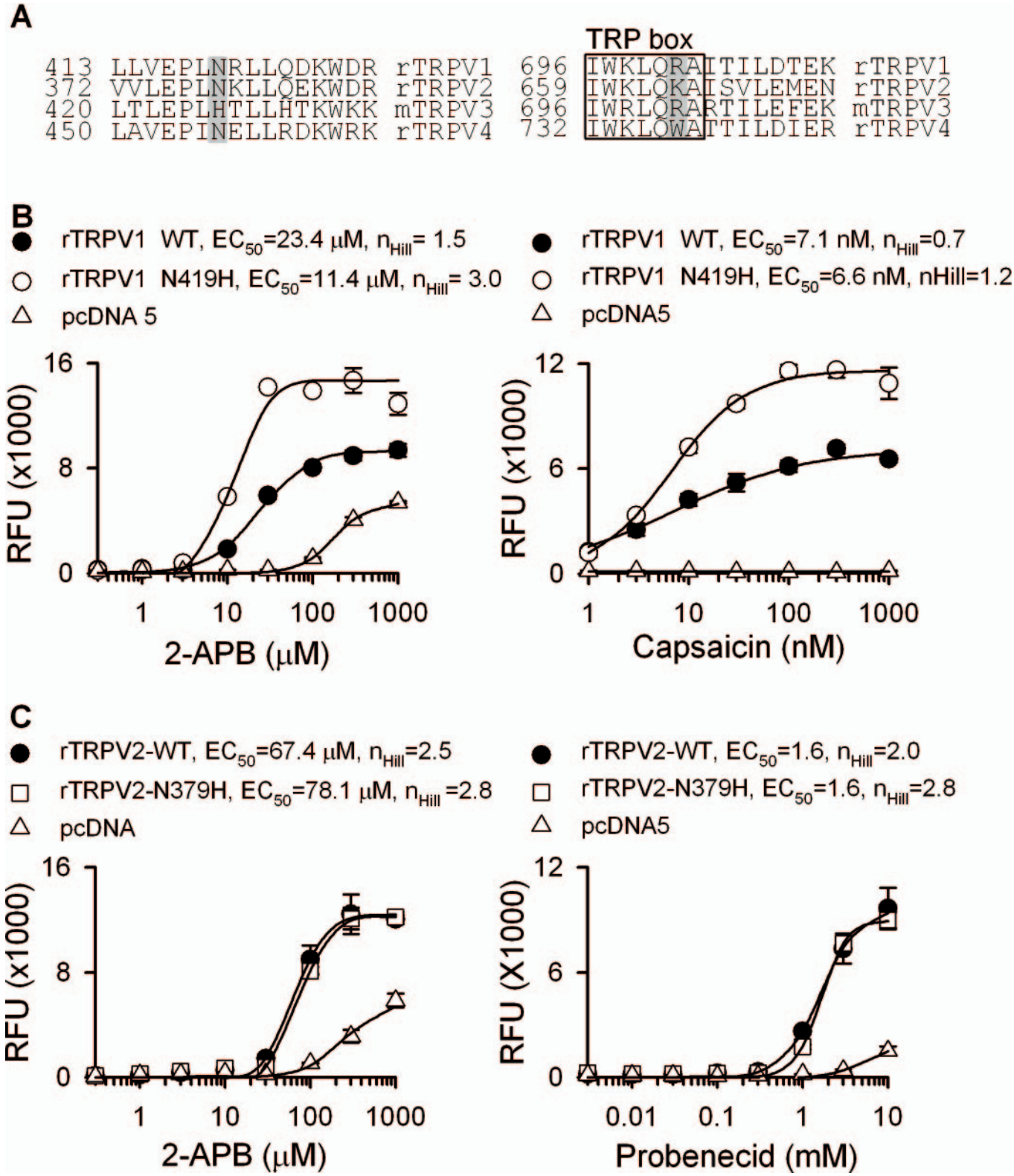

Fig. S5. Effects of mutations of equivalent position to mTRPV3-H426 identified in TRPV1 and TRPV2 on 2-APB response. (*A*) Sequence alignment of the N-terminal and C-terminal TRP box of rat TRPV1, TRPV2, mouse TRPV3, and rat TRPV4. Amino acids specifically involved in 2-APB interaction are indicated in gray. (*B*) Concentration-response curves of 2-APB and capsaicin responses in HEK293 cells transfected with wild-type TRPV1 and N419H mutants. (*C*) Concentration-response curves to 2-APB and probenecid in HEK293 cells transfected with wild-type TRPV2 and N379H mutants. Error bars are SEs, $n = 6$ for each clone.

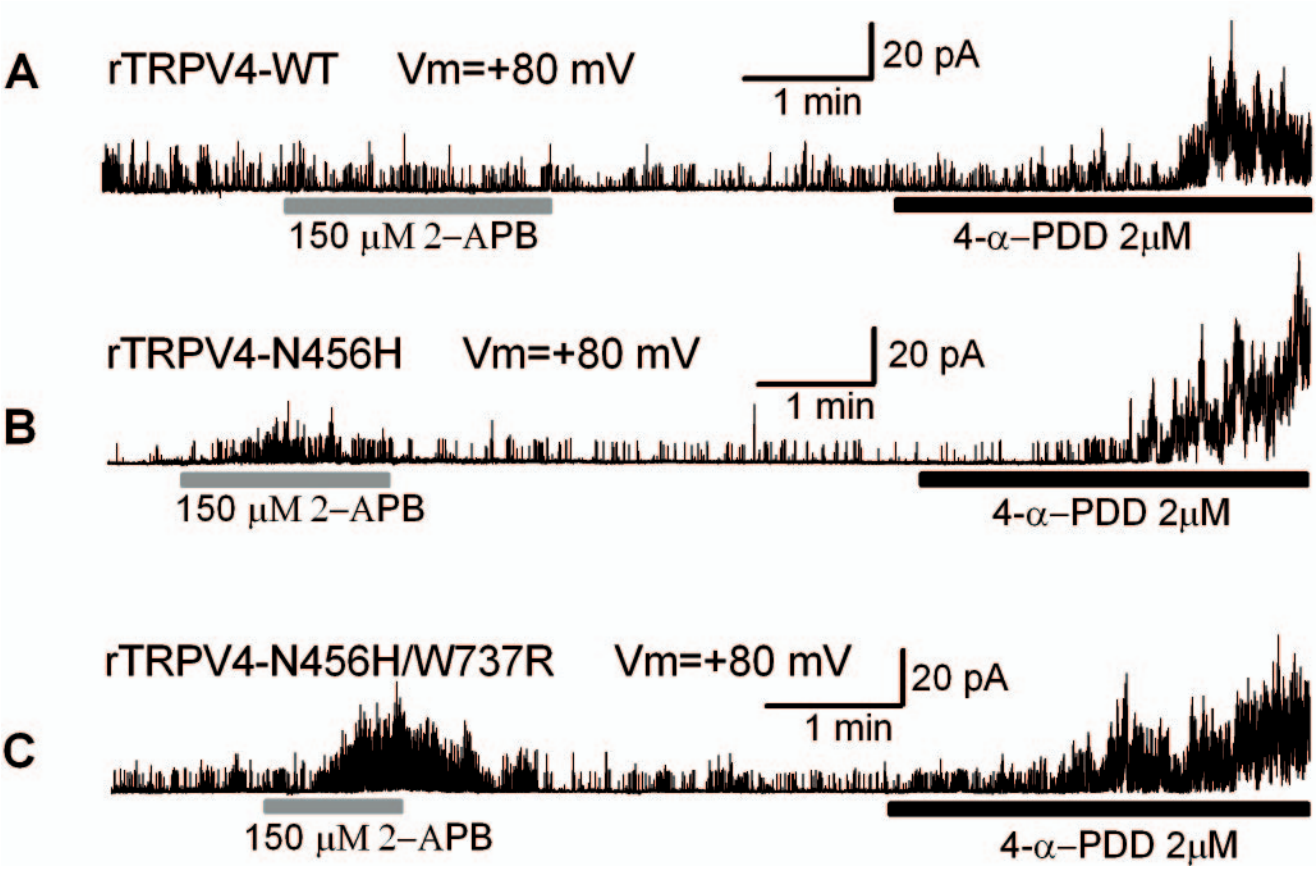

Fig. S6. Activity of 2-APB activity in inside-out patches expressing wild-type TRPV4 and TRPV4 mutants. Single-channel current traces taken from excised inside-out patches expressing wild-type rTRPV4 (*A*) or rTRP4-N456H (*B*) or rTRPV4-N456H/W737R channels. All patches were treated with 150 μM 2-APB and followed by 2 μM 4-α-PDD. All recordings were taken at +80 mV, $n = 5$ for wild-type rTRPV4, $n = 7$ for rTRPV4-N456H mutant, and $n = 5$ for rTRPV4-N456H/W737R mutant.

Table S1. His426 mutants

Clones	2-APB $EC_{50}/\mu M$	2-APB n_{Hill}	Camphor $EC_{50}/\mu M$	Camphor n_{Hill}	Emax, 100% to 20 mM camphor response
WT	11.5 ± 2.0	1.2	1.83 ± 0.09	2.8	100
H426A	332.1 ± 12.4	1.0	1.50 ± 0.09	3.3	99.6 ± 5.0
H426C	197.6 ± 3.4	1.2	1.74 ± 0.15	3.4	104.4 ± 2.6
H426D	392.0 ± 4.2	1.4	1.35 ± 0.26	2.6	91.6 ± 1.0
H426E	355.4 ± 11.3	1.3	1.97 ± 0.50	2.5	106.2 ± 13.2
H426G	125.9 ± 7.9	1.3	1.78 ± 0.22	3.9	91.2 ± 2.1
H426I	419.9 ± 45.7	1.4	2.60 ± 0.14	2.1	98.2 ± 2.4
H426K	148.2 ± 8.8	1.3	1.73 ± 0.13	3.9	102.6 ± 1.2
H426L	266.8 ± 11.5	1.0	2.48 ± 0.15	3.3	95.2 ± 1.4
H426M	271.3 ± 2.3	1.5	2.46 ± 0.32	2.1	104.6 ± 1.4
H426N	268.8 ± 3.4	1.5	1.66 ± 0.07	3.2	99.5 ± 2.4
H426P	373.3 ± 17.5	1.1	3.89 ± 0.16	3.8	94.0 ± 1.7
H426Q	262.1 ± 0.6	1.1	1.50 ± 0.08	2.5	82.3 ± 1.8
H426R	206.3 ± 9.4	1.2	1.73 ± 0.07	3.6	100.6 ± 5.4
H426S	218.8 ± 19.3	1.3	2.06 ± 0.19	1.8	105.1 ± 1.6
H426V	178.4 ± 20.3	1.1	2.25 ± 0.06	2.6	108.2 ± 2.0
H426Y	416.0 ± 2.5	1.7	2.31 ± 0.09	3.9	95.4 ± 2.1

H426F, H426T, and H426W mutants did not respond to either 2-APB or camphor. Data are presented as EC_{50} ± SE and Emax ± SE; $n = 3$ for each data point.

Table S2. Arg696 mutants

Clones	2-APB EC_{50}, μM	2-APB n_{Hill}	Camphor EC_{50}, μM	Camphor n_{Hill}	Emax, 100% to 20 mM camphor response
WT	5.6 ± 0.5	1.6	1.20 ± 0.06	2.6	100
R696C	7.6 ± 3.5	2.0	0.34 ± 0.17	1.9	23.3 ± 1.1
R696E	14.4 ± 10.7	2.4	0.49 ± 0.13	3.2	31.7 ± 0.6
R696F	253.2 ± 54.8	2.1	0.81 ± 0.06	2.1	78.0 ± 0.9
R696H	280.2 ± 106.3	1.0	0.46 ± 0.25	2.7	25.1 ± 1.0
R696I	6.3 ± 7.3	1.6	0.66 ± 0.11	2.3	10.7 ± 0.7
R696K	246.0 ± 66.8	1.2	1.30 ± 0.05	2.9	98.6 ± 3.7
R696L	1.0 ± 0.3	2.3	0.66 ± 0.12	2.3	66.6 ± 4.7
R696M	0.9 ± 0.4	2.4	0.52 ± 0.14	2.2	22.9 ± 0.2
R696Q	71.9 ± 35.7	1.2	0.83 ± 0.23	2.3	59.1 ± 1.7
R696V	1.2 ± 0.8	1.1	0.66 ± 0.24	2.0	44.8 ± 0.4
R696W	ND	ND	0.87 ± 0.34	2.5	55.9 ± 1.3
R696Y	ND	ND	0.59 ± 0.17	1.9	19.6 ± 1.7

R696A, R696D, R696G, R696N, R696P, R696S, and R696T did not respond to either 2-APB or camphor. Data are presented as EC_{50} ± SE and Emax ± SE; $n = 3$ for each data point. ND, not distinguishable from pcDNA-transfected cells.