

Supporting Information for

Pathway-dependent cold activation of heat-responsive

TRPV channels

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This Supporting Information includes:

Tables S1, S2, S3, S4, S5, S6, S7 and Figure S1.

Table S1 Tertiary noncovalent interactions along the PI-dependent minimal gating pathway from I387 to K710 in each subunit of closed PI-bound rTRPV1- Δ (604-626) in MSP2N2 at 4 °C (PDB ID, 5IRZ)

Noncovalent interaction	Cut-off distance	Linked residues
Salt bridge	3.2-4 Å	D471-R474, D576-R579
H-bond	<3.9 Å	I387/D388-S394, S402-R409, K425-E709 , Y441-Q519, T449-W549, S512-PI-R557, Q519-N551, R557-PI-E570, Y584-T641 /Y666, S629-S632
π - π interaction	2.65–6.5 Å	F434/F438 /Y441-Y555, Y441-Y444/F488/F516 , Y444-F488 , Y453-Y454 , Y463-Y530 , Y487-F488 , F488-F516 , F489-F490/F517 , F516-Y554 , F522-F543 , Y530-F531 , Y530-Y537 , Y554-Y555 , F591-Y666 , Y627-F649 , F640-Y666 , F649/Y653-F659
cation- π interaction	<6.0 Å	Y401-R499, W426-R701
CH ₃ /CH- π interaction	2.65-3.01 Å	H410-I696, Y444-F448 , M445-W549 , P456-Y463, F489-L524, Y495-E513 , F580-L678, F580-F584, F589-T593 , E636-F649 , E651-Y653
Lone pair- π interaction	3-3.7 Å	Y666-T670

Note: Bold interactions were conserved in both closed and open states of rTRPV1- Δ (604-626).

Table S2 Tertiary noncovalent interactions along the PI-dependent gating pathway from I387 to K710 in each subunit of open PI-free rTRPV1- Δ (604-626) in MSP2N2 at 25 °C (PDB ID, 8U3L)

Noncovalent interaction	Cut-off distance	Linked residues
Salt bridge	3.2-4 Å	R534-E536, E636-K639
H-bond	<3.9 Å	S403-T406, E416-R420, K425-E709 , Y463-Y537, Q498-R499, S502-S505, S512-E513, Y584-T641
π - π interaction	2.65–6.5 Å	F434-W426/Y555 , F438-Y555, Y441-Y444/ F488/F516/Y555 , Y444-F488 , Y453-Y454 , Y463-Y530 , Y487-F488-F516 , F489-F517 , Y495-F496, F516-Y554 , F522-F543 , Y530-F531/Y537 , Y554-Y555 , Y584-Y666, F591-Y666 , Y627-F649 , F640-Y666 , F649/Y653-F659
cation- π interaction	<6.0 Å	W426-R701
CH ₃ /CH- π interaction	2.65-3.01 Å	Y444-F448 , M445-W549 , Y495-E513 , Q560-W697, F589-T593 , E636-F649 , D654-F655
Lone pair- π interaction	3-3.7 Å	

Note: Bold interactions were conserved in both closed and open states of rTRPV1- Δ (604-626).

Table S3. Tertiary noncovalent interactions along the PC-dependent minimal gating pathway from D396 to K705 in each subunit of the closed reduced hTRPV3 channel at 4 °C (PDB ID, 6UW4).

Noncovalent interaction	Cut-off distance	Linked residues
Salt bridge	3.2-4 Å	E405-K705, E418-R690, K500-E501, PC-R567, E689-R693
H-bond	<3.9 Å donor-H-acceptor <60°	D396-K432-E704 , Y409-E702, R416-D519, Y448-Q529-Y451, T456-W559 , E501-H523, T566-S576, D586-T680 , Y594-Y661 , E610-K649 , E631-K634
π - π interaction	2.65–6.5 Å	H426-H430, W433-F441-Y565, F449-F445-Y565 , F447-W493-F489 -Y451, Y448-Y451 , Y448-F526 , Y448-Y565 , F449-W559 , Y451-W493 , Y460-Y461, W521-F522-Y564 , F526-Y564 , Y540-Y547 , F542-Y544, Y564-Y565 , F601-Y661 , Y622-F654
cation- π interaction	<6.0 Å	W433-R696
CH ₃ /CH- π interaction	2.65-3.01 Å	W433-K438 , N452-W559, W521-PC, F527-V531, Q570-W692 , F590-L673 , F597-L664, L632-Y661, F633-I637
Lone pair- π interaction	3-3.7 Å	F656-T660, T665-Y661

Note: Bold interactions were conserved in both closed hTRPV3 and open hTRPV3/K169A.

Table S4. Tertiary noncovalent interactions along the PC-dependent minimal gating pathway from D396 to K705 in each subunit of the open reduced hTRPV3-K169A channel at 4 °C (PDB ID, 6UW6).

Noncovalent interaction	Cut-off distance	Linked residues
Salt bridge	3.2-4 Å	E610-K649. E689-R693 , R698-E702
H-bond	<3.9 Å donor-H-acceptor <60°	D396-K432-E704 , T397-E704, N412-D512, Q529-Y451 , T456-W559 , D519-R567, D586-T680 , Y594-Y661 , Y594-T636-Y661
π - π interaction	2.65–6.5 Å	F441-Y565 , F449-F445-Y565 , F447-W493-F489 , Y448-Y451 , Y448-F526 , Y448-Y565 , F449-W559 , Y451-W493 , W521-F522-Y564 , F526-Y564 , Y540-Y547 , Y564-Y565 , F601-Y661 , Y622-F654 ,
cation- π interaction	<6.0 Å	W433- R696
CH ₃ /CH- π interaction	2.65-3.01 Å	W433-K438 , N452-W559 , Q570-W692 , F590-Y594, F590-L673
Lone pair- π interaction	3-3.7 Å	T665-Y661

Note: Bold interactions were conserved in both closed hTRPV3 and open hTRPV3/K169A.

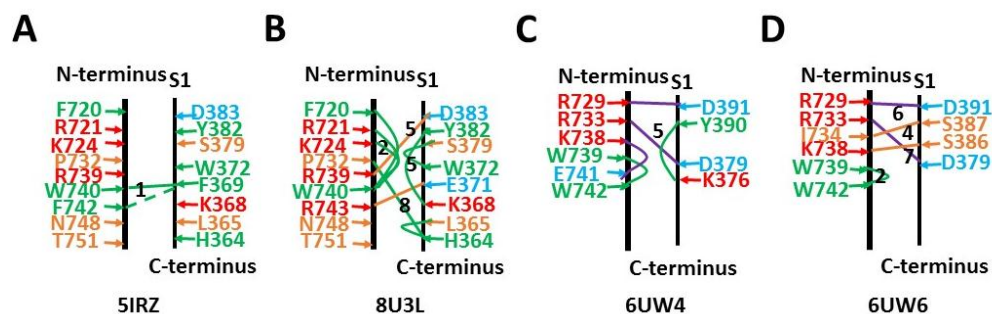


Fig.S1. The grid-like noncovalently interacting mesh network between N- and C-terminal domains of reduced rTRPV1-Δ(604-626) or hTRPV3 beyond the PI/PC-dependent minimal gating pathway. (A) The reduced and closed rTRPV1-Δ(604-626) channel in MSP2N2 at 4 °C (PDB ID, 5IRZ). (B) The reduced and open rTRPV1-Δ(604-626) channel in MSP2N2 at 25 °C (PDB ID, 8U3L). (C) The reduced and closed hTRPV3 channel in MSP2N2 at 4 °C (PDB ID, 6UW4). (D) The reduced and open hTRPV3-K169A channel in MSP2N2 at 4 °C (PDB ID, 6UW6). The N- and C-terminal domains are indicated in black along with S1. Salt bridges, π interactions, and H-bonds between paired amino acid side chains are denoted in purple, green, and orange, respectively. The specific grid sizes necessary to regulate the least-stable noncovalent interactions in the grids are indicated with black numbers.

Table S5. Tertiary noncovalent interactions along the ligand-dependent minimal gating pathway from D425 to R746 in each subunit of the closed reduced hTRPV4 channel with RhoA/GSK279 bound at 4 °C (PDB ID, 8FC7).

Noncovalent interaction	Cut-off distance	Linked residues
Salt bridge	3.2-4 Å	D425-K462, E435-R746, K727-E728, D743-R746
H-bond	<3.9 Å donor-H-acceptor <60°	Y439-R746, K462-E745, Y502-Y567
π - π interaction	2.65–6.5 Å	W737-W463-F471-Y472 , F471- F592-Y478 -F524/Y553, F485-Y556-F524-Y553 , Y490-Y491 , F549/Y553-Y591, Y567-Y574 , Y591-F592, F617-Y621, F624-Y628-Y702 , W733-W737
cation- π interaction	<6.0 Å	
CH ₃ /CH- π interaction	2.65-3.01 Å	M482-W586 , (P498-Y567), F525-I529, K597-W733, Y624-L709, F707-L711
Lone pair- π interaction	3-3.7 Å	

Note: Bold interactions were conserved in both closed and open hTRPV4.

Table S6. Tertiary noncovalent interactions along the ligand-dependent minimal gating pathway from D425 to R746 in each subunit of the open reduced hTRPV4 channel with 4 α -PDD bound at 4 °C (PDB ID, 8FCA).

Noncovalent interaction	Cut-off distance	Linked residues
Salt bridge	3.2-4 Å	
H-bond	<3.9 Å donor-H-acceptor <60°	Y628-T701
π - π interaction	2.65–6.5 Å	H447-H731, W737-W463-F471-Y472 , F592-Y478, F485-Y556 , Y490-Y491 , Y500-Y502, Y556-F524-Y553-F592 , Y567-Y574 , F624-Y628-Y702 , W733-W737
cation- π interaction	<6.0 Å	Y502-R503
CH ₃ /CH- π interaction	2.65-3.01 Å	Y621-L710, Y478- M482-W586
Lone pair- π interaction	3-3.7 Å	

Note: Bold interactions were conserved in both closed and open hTRPV4.

Table S7. Tertiary noncovalent interactions along the PE-dependent minimal gating pathway from D347 to R683 in each subunit of closed hTRPV2 homology model based on close state 1 of rTRPV2 in DMNG/MSP2N2 at pH 8 and 4 °C (PDB ID, 6U84).

Noncovalent interaction	Cut-off distance	Linked residues
Salt bridge	3.2-4 Å	D347/E350-R683, R367-D467, R533-E647
H-bond	<3.9 Å donor-H-acceptor <60°	E356-R457, K383-E672, T406-W507, Q412-H436/Y495, Y469-Q528, D534-R537, Y542-T604/Y629, L553-Y590, N586-Q615, E599-K602, L600-Y629
π - π interaction	2.65–6.5 Å	F360-F460, W384-F391-Y513, F392-F517, F395-Y513, H436-Y495, Y445-Y675/W676/W677, W452-W676, Y453-F454/F460, W455-H458, Y469-F470, F470-Y512, F474-Y513, Y512-Y513, F538-Y542, Y542-F601, F549-Y629, F603-Y629, H617-F618, W676-W677
cation- π interaction	<6.0 Å	W384-K664
CH ₃ /CH- π interaction	2.65-3.01 Å	H368-I659, N398-Y513, M402-W507, F474-N409, C488-Y495, Q518-W660, Y523-I527, F538-M640, F603-V630-Y634, W660-K664
Lone pair- π interaction	3-3.7 Å	E559-H617, W561-E614, Y629-T633