Structural basis of temperature sensation by the TRP channel TRPV3

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We present structures of mouse TRPV3 in temperature-dependent open, closed and intermediate states that suggest two-step activation of TRPV3 by heat. During the strongly temperature-dependent first step, sensitization, the channel pore remains closed while S6 helices undergo α-to-π transitions. During the weakly temperature-dependent second step, channel opening, tight association of the S1–S4 and pore domains is stabilized by changes in the carboxy-terminal and linker domains.

Temperature perception is mediated by temperature-sensitive transient receptor potential (TRP) channels, thermo-TRPs, that exhibit unusually high temperature coefficients (Q10) values compared to non-temperature-sensitive ion channels.1–4 Four members of the vanilloid subfamily TRP channels are thermo-TRPs: TRPV1 and TRPV2 are activated by noxious heat (>43 °C), while TRPV3 and TRPV4 can respond to warm temperatures (<33 °C).5–8 TRPV3 is predominantly expressed in skin and mediates warm and pain sensation.5,8 Temperature-dependent activation of TRPV3 is use-dependent and hysteretic; its initial activation requires higher temperatures (33 °C)5–8. TRPV3 and TRPV4 can respond to warm temperatures (<33 °C), while TRPV3 is activated by noxious heat (>43 °C), and TRPV3 is unsurprising because of the low P0 of the channel (Fig. 1c), which requires higher temperatures for efficient activation.9–11

To determine the structure of TRPV3 in a temperature-activated open state, we looked for constructs with increased temperature sensitivity. We tested TRPV3Y564A, which contains a single residue substitution in binding site 2 (Extended Data Fig. 4)11. Presumably, Y564 is directly involved in putative lipid 2 binding; its substitution in binding site 2, which was identified earlier in TRPV3 WT-Closed-4 °C (Extended Data Fig. 3)11. Because of the limited resolution, we cannot unambiguously identify these densities as lipids, or determine the exact type of these putative lipids. The 4.5-Å resolution sensitized-state structure (TRPV3 WT-Sensitized-42 °C) shows an α-to-π transition in the middle of each S6 helix. This results in an ~100° axial rotation of the carboxy (C)-terminal half of S6, which becomes longer by two helical turns, while the TRP helix becomes shorter by two helical turns. Consequently, a different set of S6 residues faces the channel pore compared with the closed state (Fig. 1g), including the highly conserved N671 (ref. 19). In TRPV3 WT-Sensitized-42 °C, the side chain of each M677 points away from the pore. Nevertheless, the pore remains closed as a result of the I674 hydrophobic seal. Not observing an open conformation of TRPV3 WT at 42 °C is unsurprising because of the low P0 of the channel (Fig. 1c), which requires higher temperatures for efficient activation.10,11

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We purified full-length wild-type (WT) mouse TRPV3 (TRPV3 WT) and tested its function in lipid bilayers (Methods). No TRPV3 WT activity was detected at room temperature, while a weak temperature sensitivity. We tested TRPV3Y564A, which contains a single residue substitution in binding site 2 (Extended Data Fig. 4)11. Presumably, Y564 is directly involved in putative lipid 2 binding; its substitution in binding site 2, which was identified earlier in TRPV3 WT-Closed-4 °C (Extended Data Fig. 3)11. Because of the limited resolution, we cannot unambiguously identify these densities as lipids, or determine the exact type of these putative lipids. The 4.5-Å resolution sensitized-state structure (TRPV3 WT-Sensitized-42 °C) shows an α-to-π transition in the middle of each S6 helix. This results in an ~100° axial rotation of the carboxy (C)-terminal half of S6, which becomes longer by two helical turns, while the TRP helix becomes shorter by two helical turns. Consequently, a different set of S6 residues faces the channel pore compared with the closed state (Fig. 1g), including the highly conserved N671 (ref. 19). In TRPV3 WT-Sensitized-42 °C, the side chain of each M677 points away from the pore. Nevertheless, the pore remains closed as a result of the I674 hydrophobic seal. Not observing an open conformation of TRPV3 WT at 42 °C is unsurprising because of the low P0 of the channel (Fig. 1c), which requires higher temperatures for efficient activation.10,11

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We first determined the TRPV3Y564A structure at 4 °C (Extended Data Figs. 1 and 2) at an overall resolution of 4.1 Å, which is higher than the resolution of the previous structure11. Due to its high resemblance to TRPV3 WT-Sensitized-42 °C, we termed this structure TRPV3Y564A-Sensitized-4 °C. The similarity in structures and single-channel conductances (Figs. 1b and 2b) justifies usage of the Y564A mutant as a reasonable qualitative model of wild type for structural and functional studies. Nonetheless, increased affinity to 2-APB (ref. 12), higher open probability and reduced temperature sensitivity of TRPV3Y564A compared to TRPV3 WT suggest that quantitative comparisons should be made with caution.

At 37 °C, the majority of the TRPV3Y564A particles were classified into low-resolution reconstructions (Extended Data Figs. 1, 2 and 5), likely representing an ensemble of heterogeneous conformations.

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Functional characterization and structures of TRPV3 at 42 °C.

Fig. 1 | Functional characterization and structures of TRPV3 WT at 42 °C. a, Representative single-channel recordings of TRPV3 WT-mediated currents at 22 °C and 42 °C and at +100 mV membrane potential (n = 5; 2,538 were analyzed). b, TRPV3 WT current-voltage (I–V) relationship at 42 °C. The data (mean ± s.e.m.) represent 15 independent experiments and 114,629 events. Fitting the data allows the temperature coefficient to be estimated, Q10 = 26.9 ± 7.4 (n = 19). c, Temperature dependence of P0, recorded at +100 mV (n = 12; 67,673 events were analyzed). Fitting the data allows the temperature coefficient to be estimated, Q10 = 26.9 ± 7.4 (n = 19). d, e, Structure of TRPV3 WT-Closed-42 °C viewed from the side (d) or the bottom (e) with each of the four subunits colored differently and semitransparent, the density for the C-terminal domain colored red and the lipid-like densities colored blue. f, g, TRPV3 WT pore-forming domains in the closed (TRPV3 WT-Closed-42 °C), f and sensitized (TRPV3 WT-Sensitized-42 °C, g) states with the residues lining the pore shown as sticks. Only two of four subunits are shown; the front and back subunits are omitted for clarity. The pore profiles are shown as space-filling models (gray). Residues in the closed-state TRP helix form the C-terminal part of S6 in the sensitized state (red arrows).

However, we determined three distinct structures comprising the minority of particles. The first, 5.18-Å resolution structure shows α-to-π transitions in its S6 helices, along with a pore hydrophobically sealed by I674 (Fig. 2g), similar to TRPV3 WT-Sensitized-42 °C (Fig. 1g) and TRPV3 Y564A-Sensitized-4 °C (Fig. 2i). Therefore, we refer to this structure as TRPV3 Y564A-Sensitized-37 °C.

The second, 4.48-Å resolution structure (Fig. 2d,e) has an apparently open ion channel pore (Fig. 2h). Similar to the 2-APB-bound TRPV3 Y564A structure, the ion conduction pathway is lined by polar or negatively charged side chains or backbone carbonyls, and has an overall negatively charged surface, likely important for TRPV3 cation selectivity. The pore’s narrowest constriction at the intracellular gate region is defined by the side chains of I674 (interatomic distance of 9.7 Å) and is similar to the intracellular gate regions of open TRPV1 (ref. 17) (9.3 Å, I679) and TRPV6 (ref. 18) (9.6 Å, I575). We concluded that this structure (TRPV3 Y564A-Open-37 °C) represents an open state of TRPV3 Y564A that had been activated by heat.

The third, 4.75-Å resolution structure of TRPV3 Y564A at 37 °C exhibits C2 symmetry (Fig. 2j,k), as seen previously in ligand-bound TRPV3 structure. This reconstruction lacks clear density for the pore domain, presumably because of strong conformational heterogeneity. In the intracellular skirt, the C termini of two diagonal subunits, B and D, wrap around their respective three-stranded β-sheets at intersubunit interfaces (Fig. 2l), similar to the closed- and sensitized-state structures. In contrast, the C termini of the A and C subunits point away from the membrane plane (Fig. 2m), similar to the open-state structure. This C2 symmetrical structure (TRPV3 Y564A-Intermediate-37 °C) likely represents an intermediate state of the channel transitioning between the sensitized and open states.

To understand heat-induced conformational changes in TRPV3, we compared the structures of TRPV3 in the closed and sensitized states and TRPV3 Y564A in the sensitized and open states. The three structures in the sensitized state, TRPV3 WT-Sensitized-42 °C, TRPV3 Y564A-Sensitized-4 °C and TRPV3 Y564A-Sensitized-37 °C, superpose well overall (root mean squared deviation, r.m.s.d. = 1.01–1.12 Å) and have similar pore architecture and dimensions (Figs. 1g and 2f,g,i). Therefore, we propose that they represent the same state in the temperature-gating mechanism of TRPV3. Because of low resolution, we exclude the TRPV3 WT-Sensitized-37 °C structure from further detailed comparisons. Although all structures look grossly similar, the open-state structure appears to be ~5 Å shorter compared with the closed- and sensitized-state structures and its intracellular skirt rotates ~8° clockwise, when viewed intracellularly (Fig. 3a,b). The most dramatic local conformational changes between the states occur in the transmembrane, linker and C-terminal domains.

Conformational changes in the transmembrane domain are correlated with the presence of two lipid-like densities in binding sites 1 and 2. The first density is wedged in between the extracellular part of S4 and S6 (site 1), while the second one nests in a pocket formed by the S1–S4 bundle and the C-terminal part of the TRP helix (site 2). The densities in these two sites are prominent in the closed state, become weaker in the sensitized state and disappear completely in the open state (Fig. 3c–f). In the TRPV3 Y564A-Sensitized-4 °C structure, the site 1 density disappears but another density appears in closer proximity to the S4–S5 linker (Fig. 3e). Dissociation of the site 1 density may promote S4 and S6 association (Supplementary Table 2). This transition is accompanied by S6 tilting towards S4, the α-to-π transition in S6 and, consequently, the two helical turn elongation of S6, the two helical turn shortening of the TRP helix and tilting of the TRP helix. Based on our structural results, channel opening, however, appears to require not only complete removal of the densities from sites 1 and 2, but also conformational changes in the linker and C-terminal domains.

Strikingly, the ankyrin repeat domains (ARDs) in all structures are nearly identical (r.m.s.d. = 0.43–1.07 Å, Fig. 3g,h). During the closed-to-sensitized and sensitized-to-open-state transitions, the ARDs move as rigid bodies while the domains connecting them to each other and to the transmembrane domain undergo substantial...
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structural rearrangements. Among these connecting domains, the most drastic changes occur in the ARD finger 3, the AR5 helix connecting loop, the linker domain, which includes helices LH1, LH2 and pre-S1, the three-stranded β-sheet and the C terminus. Indeed, numerous studies have revealed that mutations in these domains affect thermo-TRP temperature-dependent gating.

Fig. 2 | Functional characterization and structures of TRPV3Y564A at 4 °C and 37 °C. a, Representative single-channel recordings of TRPV3Y564A-mediated currents at 22 °C and 42 °C and at +100 mV membrane potential (n = 5; 2,538 events were analyzed). b, TRPV3Y564A current–voltage (I–V) relationship at 22 °C. The data (mean ± s.e.m.) represent 15 independent experiments and 114,629 events. c, Temperature dependence of $P_o$ recorded at +100 mV (n = 12; 67,673 events were analyzed). Fitting the data yields the temperature coefficient, $Q_{10} = 1.21 ± 0.20$ (n = 21). d–f, Structure of TRPV3Y564A-Open-37 °C viewed from the bottom (d) or the side (e) with each of the four subunits colored differently and semitransparent, and the density for the C-terminal domain colored red. f–h, Pore-forming domain of TRPV3Y564A in the sensitized (TRPV3Y564A-Sensitized-4 °C, f; TRPV3Y564A-Sensitized-37 °C, g) and open (TRPV3Y564A-Open-37 °C, h) states with residues lining the pore shown as sticks. Only two of four subunits are shown; the front and back subunits are omitted for clarity. The pore profiles are shown as space-filling models (gray). i, Pore radii calculated using HOLE29 for TRPV3WT-Closed-42 °C (blue), TRPV3WT-Sensitized-42 °C (dark green), TRPV3Y564A-Sensitized-4 °C (green), TRPV3Y564A-Sensitized-37 °C (light green) and TRPV3Y564A-Open-37 °C (orange). The vertical dashed line denotes the radius of a water molecule, 1.4 Å. j, k, TRPV3Y564A-Intermediate-37 °C viewed from the side (j) or the bottom (k). l, m, Expanded views of the intersubunit interfaces between subunits D and A (l) and A and B (m). The C terminus is highlighted in red and thickened for clarity. Conserved residues at the C terminus–ARD interfaces are shown as sticks.
 swapping of C termini not only made temperature sensitivity stron-
ger or weaker, but also reversed temperature sensitivities, making
heat-sensitive channels cold-sensitive and vice versa. The
consensitized-to-open-state transition in TRPV3 is accompanied
by substantial conformational changes in the C terminus
(Extended Data Fig. 6). In the closed and sensitized states, the C terminus wraps
around the three-stranded β-sheet, while in the open state it unwraps
and projects towards the cytoplasm (Figs. 1–3). In the open state,
the dissociated portion of the C terminus is partially replaced by a
12-residue-long polypeptide bound to the intersubunit interface,
which likely represents a portion of the amino (N) terminus that
was unfolded and unobserved in the closed and sensitized states;
although the possibility that the polypeptide density corresponds
to a more distal portion of the C terminus cannot be excluded. The
C terminus appears to function as a latch that structurally supports
the closed and sensitized states and that needs to be released for the
channel to open. This mechanism resembles the regulatory switch previously proposed for TRPV3 (ref. 27). The C terminus interacts
with residues that are conserved in the thermo-TRPs but not in
TRPV5/6 (Supplementary Fig. 1). For example, conserved residues
W739 and W742 interact with hydrophobic residues and R226 in
the ARDs. Mutating any of these residues altered channel activity27,28.

The C terminus has been proposed to play an important role
in thermo-TRP temperature-dependent gating21–26. Deletions or
swapping of C termini not only made temperature sensitivity stronger or weaker, but also reversed temperature sensitivities, making heat-sensitive channels cold-sensitive and vice versa. Consistently, the sensitized-to-open-state transition in TRPV3 is accompanied by substantial conformational changes in the C terminus (Extended Data Fig. 6). In the closed and sensitized states, the C terminus wraps around the three-stranded β-sheet, while in the open state it unwraps and projects towards the cytoplasm (Figs. 1–3). The C terminus is partially replaced by a 12-residue-long polypeptide bound to the intersubunit interface, which likely represents a portion of the amino (N) terminus that was unfolded and unobserved in the closed and sensitized states; although the possibility that the polypeptide density corresponds to a more distal portion of the C terminus cannot be excluded. The C terminus appears to function as a latch that structurally supports the closed and sensitized states and that needs to be released for the channel to open. This mechanism resembles the regulatory switch previously proposed for TRPV3 (ref. 27). The C terminus interacts with residues that are conserved in the thermo-TRPs but not in TRPV5/6 (Supplementary Fig. 1). For example, conserved residues W739 and W742 interact with hydrophobic residues and R226 in the ARDs. Mutating any of these residues altered channel activity27,28. Additionally, the thermo-TRPV linker domains contain an 11-residue insertion that, in our structures, interacts with the C-terminal region in both the closed and sensitized states, but not in the open state, and likely transmits conformational changes between the transmembrane domain and ARD during gating. Changes in the linker domain result in altered TRPV3 temperature-dependent gating21–26.

Superposition of the open-state structures obtained in the presence of the agonist 2-APB (ref. 11) or at high temperature (Extended Data Fig. 7a–c) shows that they are very similar (r.m.s.d. = 1.01 Å) and have similar pore architectures and dimensions. However, in TRPV3WT-Open-37°C, the 2-APB-binding pockets are unoccupied (Extended Data Fig. 7d–f). It appears that 2-APB and heat activate TRPV3 via similar mechanisms; both lead to S4 and S6 interactions and the release of the C-terminal latch. Better understanding of these mechanisms and the role of lipids in temperature sensation by TRPV3 and thermo-TRPs in general await further investigation.

Online content
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Fig. 3 | Conformational changes in response to heat. a, b, Overall superposition of TRPV3WT-Closed-42°C (blue), TRPV3WT-Sensitized-42°C (dark green), TRPV3Y564A-Sensitized-4°C (light green) and TRPV3Y564A-Open-37°C (orange) structures viewed from the side (a) or the bottom (b). Only two of four subunits are shown in a; the front and back subunits are omitted for clarity. c-f, The S1–S5 and TRP helix of one subunit and S6 of the adjacent subunit from TRPV3WT-Closed-42°C (c) and the same region superposed on TRPV3WT-Sensitized-42°C (d), TRPV3Y564A-Sensitized-4°C (e) and TRPV3Y564A-Open-37°C (f). The densities at sites 1 and 2 are shown as a purple mesh. Domain movements relative to the closed state are indicated by red arrows. g, h, Superposition of TRPV3 structures based on the ARD. The C terminus is thickened for clarity. Domain movements relative to the closed state are indicated by red arrows.
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Methods

Construct. The full-length mouse TRPV3 WT (residues 1–825) was cloned into a PET BacMam vector13, with the C-terminal thrombin cleavage site (LYPRG) followed by the streptavidin affinity tag (WSHPQFEK), as was done previously for TRPV3 (ref. 9). The TRPV3Y564A construct was produced using a standard mutagenesis procedure.

Expression and purification. TRPV3 constructs were expressed and purified as was done previously for TRPV3, with minor differences14. Bacmid and baculoviruses were produced as described in the literature15. In short, baculovirus was made in Sf9 cells for ~72 h (Thermo Fisher Scientific, mycoplasma test negative, Gibco, catalog no. 12659017) and was applied to the suspension-adapted HEK293S cells lacking N-acetyl-glucosaminyltransferase I (GnT1; mycoplasma test negative, ATCC, catalog no. CRL-3022) that were maintained in Freestyle 293 media (Gibco-Life Technologies, catalog no. 12338-018) supplemented with 2% FBS in a 90% presence of CO2. The cells were cultured at 37 °C for 1–3 d at 37 °C for ~10 h with a baculovirus, after which 10 mM sodium butyrate was added to the cells with a concurrent reduction in temperature to 30 °C. At 48–60 h post-transduction, the cells were pelleted by centrifugation at 5,471 g for 15 min using a Sorvall Evolution RC Centrifuge (Thermo Scientific), washed in PBS pH 8.0 and pelleted again by centrifugation at 3,202 g for 10 min using an Eppendorf Centrifuge 5810. After resuspension in buffer (50 ml per 800 ml of culture) containing 150 mM NaCl, 20 mM Tris–HCl (pH 8.0), 1 mM βME (β-mercaptoethanol) and protease inhibitors (0.8 μM aprotinin, 2 μg/ml leupeptin, 2 μg/ml pepstatin A and 1 mM phenylmethylsulfonyl fluoride) the cells were subjected to sonication with a Misonix Sonicator (power level set to 0.1) for ~20 min to disrupt the cell membrane and release the cytoplasm. The resulting supernatant was added to a streptavidin-linked resin and incubation of TRPV3 WT grids at 42 °C prior to plunge freezing resulted in vitreous ice and, as a result, precluded particle visualization using cryo-EM. However, although TRPV3Y564A-Intermediate-37 °C, which resulted in a final 3D reconstruction at 4.07-Å resolution. Nevertheless, despite the absolute numbers of particles in the cryo-EM datasets not reaching all of the transmembrane domains, limiting our efforts to determine the structure. To increase the number of particles in the open state, we collected a total of four datasets on a Titan Krios, which resulted in 18,901 micrographs. The total number of particles (~6,000,000) was extracted with a box size of 220 pixels. These extracted particles were subjected to multiple rounds of heterogeneous classification showing the presence of at least two conformations in processed particles, resolving the open and closed states of TRPV3 (Fig. 1A). The reason for this discrepancy is the inherent difference between the conditions of the protein sample in cryo-EM and the lipid bilayer experiments. These include a different physical environment for the transmembrane portion of the protein (detergent micelle versus lipid bilayer), as well as protein interactions with the thin layer of vitreous ice (including surface tension effects) and possibly with the material of the grid (gold mesh). Nevertheless, despite the absolute numbers of particles in the cryo-EM datasets not matching representations of the corresponding states in functional experiments, the relative presence of these states is likely preserved.

The reported resolutions of the final maps were estimated using the Fourier shell correlation (FSC) = 0.143 criterion16 on masking-effect-corrected FSC curves calculated between two independent half maps17. The local resolutions were estimated with unfiltered half maps using ResMap18 and EM density visualization was done in UCSF Chimera19.

Model building. To build the closed, sensitized- and open-state models of TRPV3 in Coot20, we used the previously published cryo-EM structures of TRPV3 as guides21. The models were tested for overfitting (Extended Data Fig. 2) by shifting their coordinates by 0.5 Å (using shake) in PHENIX22, refining each shaken model against the corresponding half map and generating intermediate maps. For each model, we calculated the root-mean-square deviation (RMSD) for the resulting models in Chimera. FSC was calculated between the densities generated in Chimera and both unfiltered half maps and the sum maps, using EMAN22 (ref. 22). The local resolution of the transmembrane domains in all our reconstructions, except TRPV3Y564A-Intermediate-37 °C, was higher compared to the resolution of the soluble domains, as evidenced by local resolution predictions made using ResMap23. High resolution of the transmembrane domains allowed us to unambiguously define the conformation of the pore-lining S6 helices in the closed, sensitized- and open-state structures. Structures were visualized and figures were prepared in Chimera and Pymol24.

Planar lipid bilayer measurements. Planar lipid bilayer measurements were performed as described previously25. Briefly, planar lipid bilayers were formed from a solution of synthetic 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-1'-rac-glycerol (POPG), 1-palmitoyl-2-oleoyl-glycerol-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-glycerol-3-phosphoethanolamine (POPE; Avanti Polar Lipids) at a 3:1:1 ratio in n-decane (Sigma-Aldrich). The solution was used to
paint a bilayer in an aperture of diameter ~150 μm in a Delrin cup (Warner Instruments) between symmetric aqueous bathing solutions of 150 mM KCl, 0.02 mM MgCl₂, 1 mM CaCl₂, and 20 mM HEPES (pH 7.2). Unless specified otherwise, all experiments were performed in the presence of 2.5 μM 1-(1,2R-dioctanoylphosphatidyl)inositol-4,5-bisphosphate trisodium salt (DiC₈-PIP₂, Cayman Chemical) added to both compartments. All reagents (Sigma-Aldrich) were ultrapure (>99%). Bilayer capacitances were in the range of 50−75 pF.

After the bilayers had formed, the micellar solution of TRPV3 protein (0.02 mM µl⁻¹) was added by painting. Unitary currents were recorded using the Axopatch 200B patch-clamp amplifier (Molecular Devices). The trans solution (command voltage side) was connected to the CV 201A head-stage input, while the cis solution was held at a virtual ground via a pair of matched Ag–AgCl electrodes. Currents through the voltage-clamped bilayers (background conductance, <1 pS) were filtered at the amplifier input (low pass, −3 dB at 10 kHz, 8-pole Bessel response). Data were filtered at 100 Hz through an 8-pole Bessel filter (950 TAF; Frequency Devices) and digitized at 1 kHz with an analog-to-digital converter Digidata 1322A controlled by pClamp v.10.3 software (Molecular Devices). Single-channel conductance events, all-points histograms, open probability (Pₒ) and other parameters were identified and analyzed with Clampfit v.10.3 software (Molecular Devices). The experiments were performed in the temperature range 22−42 °C. For the temperature-dependence measurements, the bilayer recording chamber was fitted onto a conductive stage containing a pyroelectric heater/cooler, and other parameters were identified and analyzed with Clampfit v.10.3 software (Molecular Devices). The experiments were performed in the temperature range 22−42 °C. For the temperature-dependence measurements, the bilayer recording chamber was fitted onto a conductive stage containing a pyroelectric heater/cooler, which was controlled by a temperature controller (CL-100; Warner Instruments). Deionized water was circulated through the stage and pumped into the system to remove the generated heat. The temperature of the bath was constantly monitored using a thermocouple and was reliably controlled within ±0.5 °C. The temperature coefficients (Q₁₀) for Pₒ were calculated using equation 1:

\[ Q_{10} = \left( \frac{X_2}{X_1} \right) \left( \frac{T_2}{T_1} \right) \]

where X₁ and X₂ are Pₒ values obtained at T₁ and T₂, temperatures measured in kelvin. Statistical analysis was performed using Origin v.9.0 (Microcal Software). Statistical significance was calculated using one-way analysis of variance followed by Fisher’s least significant difference test. All data are presented as mean ± s.e.m.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

Cryo-EM density maps have been deposited in the Electron Microscopy Data Bank (EMDB) under accession numbers EMD-20492 for TRPV3WT-Closed-42 °C, EMD-20493 for TRPV3WT-Sensitized-42 °C, EMD-20494 for TRPV3WT-Sensitized-4 °C, EMD-20495 for TRPV3WT-Sensitized-37 °C, EMD-20496 for TRPV3WT-Sensitized-37 °C. Open-37 °C and EMD-20497 for TRPV3WT-Intermediate-37 °C. Model coordinates have been deposited in the Protein Data Bank (PDB) under accession numbers 6PVL for TRPV3WT-Closed-42 °C, 6PVN for TRPV3WT-Sensitized-42 °C, 6PVP for TRPV3WT-Sensitized-4 °C, 6PVO for TRPV3WT-Sensitized-37 °C, 6PVP for TRPV3WT-Intermediate-37 °C and 6PVO for TRPV3WT-Sensitized-37 °C. All other data are available from the corresponding author upon request.

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**Author contributions**

A.K.S. and A.I.S. designed the project. A.K.S. and L.L.M. made constructs, prepared protein samples, carried out cryo-EM data collection and processing. A.K.S. and A.I.S. built molecular models. L.D. and M.L. carried out bilayer experiments. A.K.S., L.L.M., E.Z. and A.I.S. wrote the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

Extended data is available for this paper at https://doi.org/10.1038/s41594-019-0318-7.
Supplementary information is available for this paper at https://doi.org/10.1038/s41594-019-0318-7.

**Correspondence and requests for materials** should be addressed to A.I.S.

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Extended Data Fig. 1 | Overview of cryo-EM data collected for TRPV3<sub>WT</sub> and TRPV3<sub>Y564A</sub>. a, Example micrographs from different TRPV3-construct collections with example particles circled in red. b, Reference-free 2D class averages in different orientations. c, Euler angle distribution of particles contributing to the final reconstructions with larger red cylinders representing orientations comprising more particles.
Extended Data Fig. 2 | Resolution of TRPV3<sub>WT</sub> and TRPV3<sub>y564A</sub> cryo-EM reconstructions. a, FSC curves calculated between half maps. b, FSC curves calculated between two unfiltered half-maps and the final map and a model whose coordinates were randomized and refined against only half map 1. c, Local resolution predicted by ResMap<sup>10</sup>.
Extended Data Fig. 3 | Comparison of TRPV3<sub>WT</sub> closed-state structures at 4 °C and 42 °C. a-b, Overall superposition of TRPV3<sub>WT</sub>-closed-4 °C (red) and TRPV3<sub>WT</sub>-closed-42 °C (blue) structures viewed from the side (a) or the bottom (b). c, Pore radii calculated using HOLE<sup>29</sup>. The vertical dashed line denotes the radius of a water molecule, 1.4 Å. d-e, Expanded view of the transmembrane domain of one TRPV3<sub>WT</sub>-Closed-4 °C (d) or TRPV3<sub>WT</sub>-Closed-42 °C (e) subunit with lipid-like densities shown as purple mesh.
Extended Data Fig. 4 | Architecture and domain organization of TRPV3. a–b, Top (a) and side (b) views of the TRPV3 tetramer, with each subunit shown in a different color. c, Domain organization diagram of the TRPV3 subunit. d, Structure of TRPV3 subunit, with domains colored as in c. Alanine substituting tyrosine 564 in TRPV3Y564A is shown in red space-filling representation (a, b and d) or indicated by the red circle (c).
Extended Data Fig. 5 | 3D reconstruction workflow for TRPV3ΔY564A.
Extended Data Fig. 6 | C-terminus unlatching during channel opening. a–b, Expanded view of the cytosolic intersubunit interface in TRPV3_Y564A-Sensitized-4 °C (a) and TRPV3_Y564A-Open-37 °C (b). The C-termini and the putative N-terminus fragment from the TRPV3_Y564A-Open-37 °C adjacent subunit are thickened for clarity. Conserved residues at the C-terminus-ARD interfaces are shown as sticks. Movement of the AR5 loop in the open state relative to the sensitized or closed state is indicated by a red arrow.
Extended Data Fig. 7 | Comparison of heat- and ligand-activated open states.  

**a-c**, Overall superposition of TRPV3_{Y564A}-Open-37°C (orange) and TRPV3_{Y564A}-Open-2-APB (grey) viewed from the top (**a**) and the side (**b**) and an expanded view of the transmembrane domain of one subunit (**c**). 2-APB molecules bound to TRPV3_{Y564A}-Open-2-APB structure are shown as space-filling models.  

**d-f**, Expanded views of binding sites 2 (**d**), 3 (**e**) and 4 (**f**). The 2-APB molecules bound to TRPV3_{Y564A}-Open-2-APB are shown as sticks and the TRPV3_{Y564A}-Open-37°C density is shown as blue mesh. TRPV3_{Y564A}-Open-37°C residues that would clash with the 2-APB molecules are shown in stick representation.
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Software and code

Policy information about availability of computer code

Data collection: Data on Titan Krios TEM equipped with a Gatan K2 Summit DED camera was collected using Leginon

Data analysis: Origin 9.1.0, Leginon, MotionCor2, gCTF, RELION 3.0, UCSF Chimera, ResMap, COOT, PHENIX, EMAN2, Pymol

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size          | Amount of cryo-EM data collected was limited by time allocation at the microscopes. |
|----------------------|-----------------------------------------------------------------------------------|
| Data exclusions      | No data have been excluded.                                                       |
| Replication          | No replication attempts have failed. All cryo-EM data collections were consistent from the beginning to the end. |
| Randomization        | N/A                                                                               |
| Blinding             | N/A                                                                               |

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