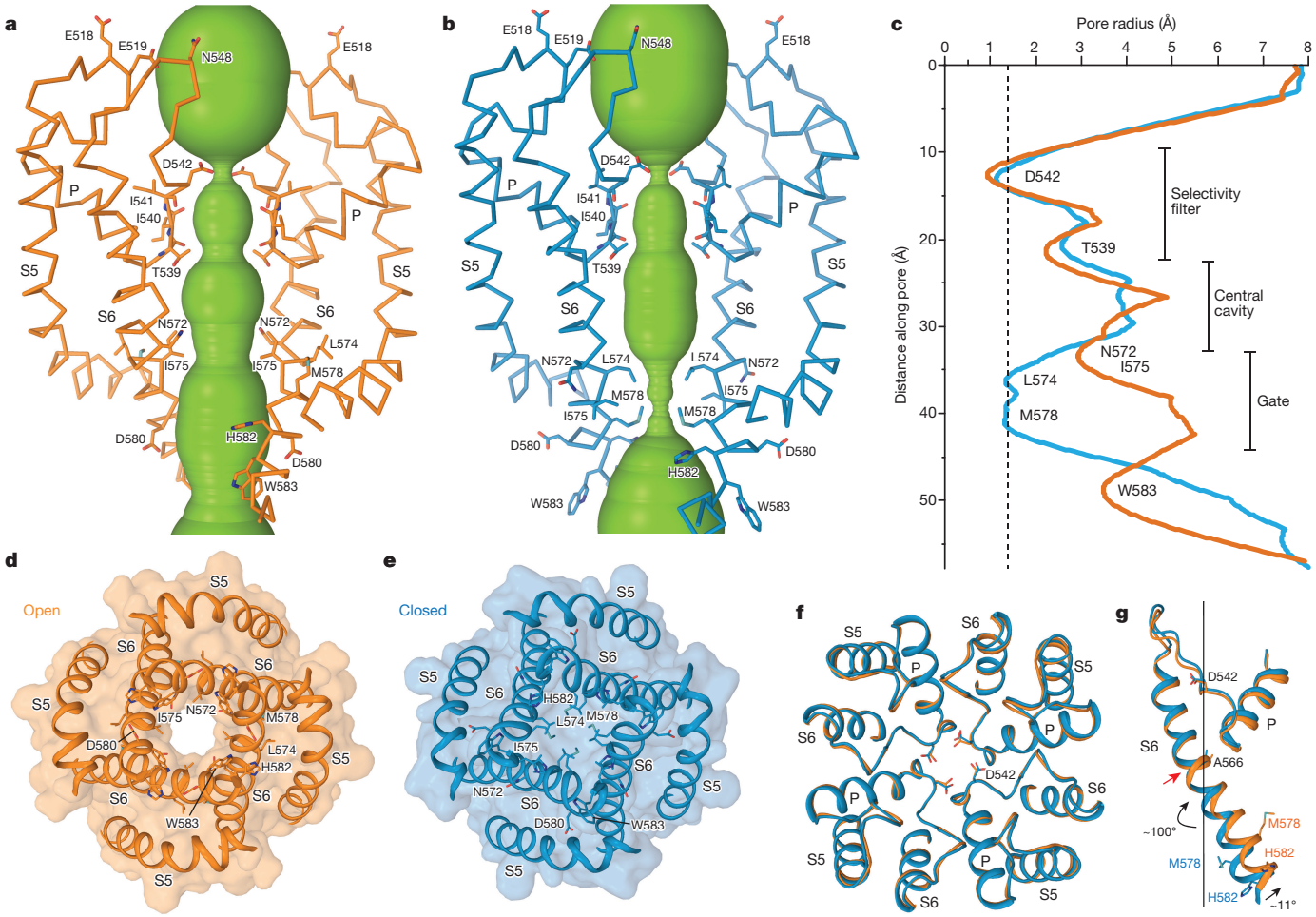

Figure 3 | Open and closed ion channel pore. a, **b**, Ion conduction pathway (green) in open hTRPV6 (**a**) and closed hTRPV6(R470E) (**b**), with residues lining the selectivity filter and around the gate shown as sticks. Only two of four subunits are shown, with the front and back subunits removed for clarity. **c**, Pore radius calculated using HOLE[29] for hTRPV6 (orange) and hTRPV6(R470E) (blue). Dashed line corresponds to 1.4 Å (radius of a water molecule). **d**, **e**, Intracellular view of the S6 bundle crossing in hTRPV6 (**d**) and hTRPV6(R470E) (**e**). **f**, Superposition of the selectivity filter regions in hTRPV6 (orange) and hTRPV6(R470E) (blue), viewed extracellularly. **g**, Superposition of the P loop and S6 in hTRPV6 (orange) and hTRPV6(R470E) (blue), viewed parallel to the membrane. The straight line shows the pore axis, red arrow indicates the position of the gating hinge alanine A566 and black arrows illustrate ~100° rotation and ~11° bending away from the pore axis of the C-terminal part of S6.

mobility of the putative bound lipid. Because rTRPV6 and hTRPV6 were purified in similar conditions, have 89% overall sequence identity, and have identical amino acid compositions of their site 2 lipid-binding pockets, it remains unclear why one channel was closed and the other open. For example, some lipids within the membranes of the protein-expressing HEK 293 cells may be important for opening of hTRPV6 but not rTRPV6. Different conformations of rTRPV6 and hTRPV6 might also reflect the ease with which these constitutively active channels rapidly transition between gating states, and that very subtle changes can push this equilibrium towards one state or the other.

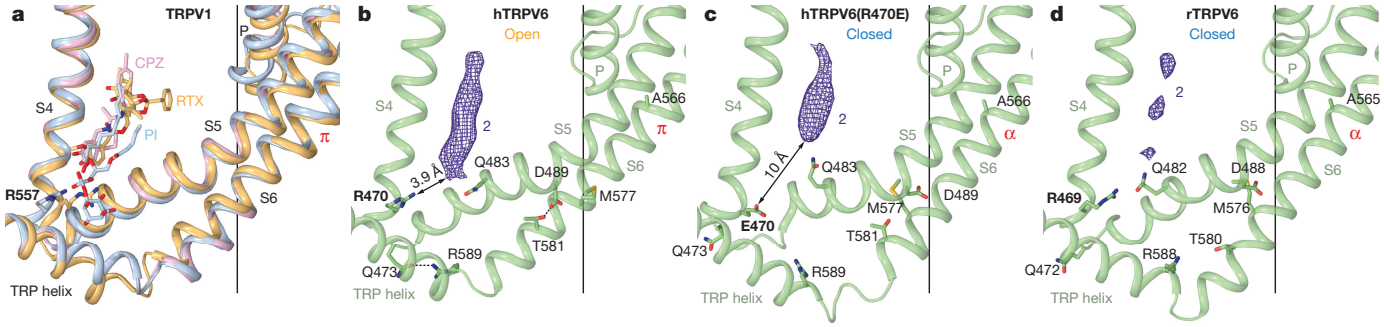

Figure 4 | Activation-related lipid binding pocket. a, Superposition of the agonist binding site in TRPV1 structures in the phosphatidylinositol (PI)-bound closed state (blue, PDB ID: 5IRZ), antagonist CPZ-bound closed state (pink, PDB ID: 5IS0) and agonist RTX-bound open state (orange, PDB ID: 5IRX). **b**–**d**, Putative activating lipid binding site in open hTRPV6 (**b**), closed hTRPV6(R470E) (**c**) and closed rTRPV6 (**d**), with densities filtered to the same resolution (4.24 Å) and shown at 5.3σ as purple mesh. Residues involved in gating are shown as sticks. Dashed lines in **b** indicate bonds between the residues.