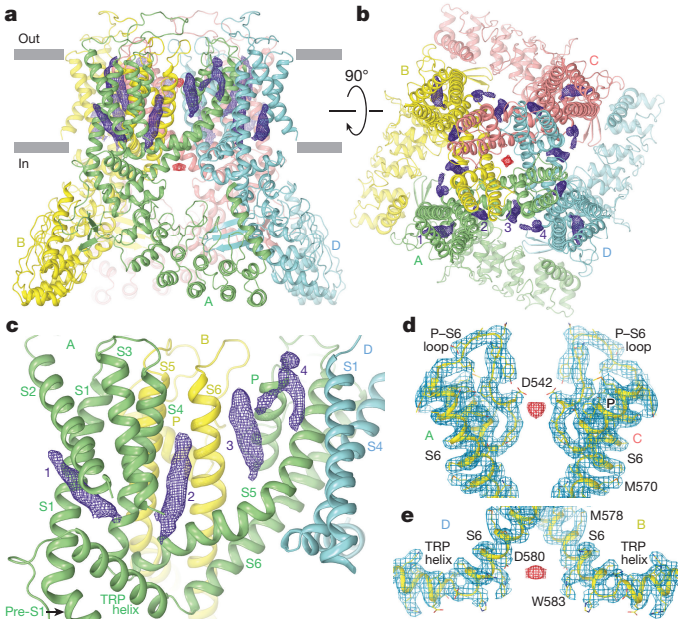

Figure 2 | Structure of hTRPV6. a, **b**, Side (**a**) and top (**b**) views of hTRPV6 tetramer, with each subunit (A–D) shown in a different colour. Putative lipid densities at 3.5σ and ion densities at 4σ are illustrated by purple and red mesh, respectively. **c**, Expanded view of the four (1–4) putative lipid densities per hTRPV6 subunit. **d**, **e**, Expanded views of the putative ion densities at 4σ at the selectivity filter (**d**) and S6 helices bundle crossing (**e**).

head-and-two-tails appearance. Fitting different lipid molecules into density 4 (Extended Data Fig. 5a–c) suggests that the chemical environment around the lipid head group, including the negatively charged aspartate D525 and polar Y349, Y509, Q513 and Y524 residues, supports binding of phosphatidylethanolamine or phosphatidylcholine rather than phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2). Densities 1–3 have sausage-like appearances and might represent a wider variety of lipid-like molecules, including cholesterol or cholesterol hemisuccinate (CHS) (Extended Data Fig. 5d, e). In physiological conditions, some of these sites can bind PtdIns(4,5)P_2[7,9]. For example, the positively charged R470 and K484 and polar T479, Q483 and Q596 residues around density 2 create a permissive chemical environment for the negatively charged head group of PtdIns(4,5)P_2. However, the poor fit of PtdIns(4,5)P_2 into density 2 (Extended Data Fig. 5f) suggests that in our cryo-EM structure, density 2 represents a different molecule.

In the crystal structure of rTRPV6 in the closed state, the M577 side chains form a hydrophobic 'seal' on the cytoplasmic side of the S6 helices[10,11]. By contrast, interatomic distances within the pore of the new structure confirmed that the hTRPV6 channel pore is open (Fig. 3). The pore surface is lined by the side chains of D542, T539, N572, I575, D580 and W583, as well as the backbone-carbonyl oxygens of I540, I541 and G579. The narrowest part of the upper pore, the selectivity filter, is formed by the D542 side chains, one from each subunit, which project towards the centre of the pore (Fig. 3a, c, f). We propose that D542 in hTRPV6, similar to D541 in rTRPV6, plays an important role in Ca^{2+} permeation by directly coordinating dehydrated Ca^{2+} ions[11]. The narrowest part of the hTRPV6 lower pore (9.6 Å interatomic distance) is defined by the side chains of I575 at the S6 bundle crossing (Fig. 3a, c, d). This part of the pore is comparable in size to the pore of open TRPV1 (9.3 Å interatomic distance, measured between side chains of I679)[18] and is wide open for conductance of hydrated Na^+ or Ca^{2+} ions.

Along the axis of the hTRPV6 pore, there is a strong density about 3.9 Å away from the side chains of D542 (Fig. 2d) that is likely to represent a Ca^{2+} ion bound to a site homologous to the main Ca^{2+}-binding site (site 1) at D541 in the pore of rTRPV6[10,11]. An additional strong density along the hTRPV6 pore is observed at the bundle crossing of the S6 helices, about 8.0 Å away from D580 and about 6.6 Å away from W583, suggesting that these residues may play an important role in ion permeation (Fig. 2e). Indeed, W583 is conserved in TRPV6 and TRPV5 channels and is involved in the regulation of calcium uptake, as shown by mutation W583A in TRPV5, which induces cell death due to increased calcium influx[19]. The density at the S6 helices bundle crossing, which was not observed in the pore of the closed-state rTRPV6[10,11], is likely to represent another permeant ion bound in the open pore of hTRPV6.

To determine the structure of hTRPV6 in the closed state, we decided to shift the open–closed state gating equilibrium towards the closed state by interfering with channel activation. Because the open probability of TRPV6 is strongly dependent on membrane lipids[7,9], altering lipid binding by mutagenesis might result in channel closure. TRPV1, for example, is activated through an intramembrane vanilloid-binding site (Fig. 4a), which accommodates agonists, such as resiniferatoxin (RTX) and capsaicin, and antagonists, such as capsazepine (CPZ)[18]. In the absence of ligands, this site is occupied by the lipid phosphatidylinositol, which favours the closed pore conformation[18]. The TRPV1 vanilloid-binding site coincides with hTRPV6 lipid density 2, which may represent the binding site for natural lipid agonists (Fig. 4b).

To test whether this site is critical for channel activation, we mutated R470 to glutamate (R470E). An analogous mutation has previously been shown to eliminate capsaicin-evoked currents in TRPV1[20]. The mutant hTRPV6(R470E) channels were functional (Extended Data Fig. 1b) but their calcium uptake was about ten times slower than that of wild-type channels (Extended Data Fig. 1e, f), consistent with their less frequent openings. In addition, 2-APB, a TRPV6 inhibitor that acts through the membrane, showed increased affinity to and decreased maximum inhibition of hTRPV6(R470E) compared to wild-type channels (Extended Data Fig. 1i–k), consistent with the R470E mutation altering regulation of TRPV6 by lipids.

We solved the hTRPV6(R470E) structure in amphipols by cryo-EM to 4.2 Å resolution (Extended Data Fig. 6). Consistent with the idea that the site 2 density represents an activating lipid, this density was smaller in hTRPV6(R470E) (Fig. 4c) than in hTRPV6 (Fig. 4b). Confirming that the physical occupancy of site 2 differed, the side chain of Q483 in hTRPV6(R470E) has an altered conformation that would cause clashing with the lipid density in wild-type hTRPV6 (Fig. 4b, c). Supporting the role of Q483 in lipid recognition, an hTRPV6(Q483A) mutant, while being functional (Extended Data Fig. 1c), showed an approximately five times slower calcium uptake than wild-type channels (Extended Data Fig. 1e, g). Notably, the ion channel in hTRPV6(R470E) appears to be closed (Fig. 3b, c, e). Indeed, the size of the pore at the S6 bundle crossing becomes comparable to the narrowest point of the selectivity filter. While the latter is formed by the side chains of D542, which directly coordinate calcium ions for selective permeation, the S6 bundle crossing is formed by the side chains of L574 and M578, which create a hydrophobic seal impermeable to ions and water, and therefore represent the channel gate.

The closed-state structure of hTRPV6(R470E) is nearly identical to the closed-state crystal structure of rat TRPV6 (rTRPV6)[10] and their superposition yields a root mean square deviation (r.m.s.d.) of 0.917 Å. To verify that the rTRPV6 crystal structure represents the physiologically relevant conformation, we solved the structure of rTRPV6 by cryo-EM to 3.9 Å using a lipid nanodisc preparation similar to that used for hTRPV6 (Extended Data Fig. 7). Strikingly, the cryo-EM structure of rTRPV6 is nearly identical (r.m.s.d. = 0.781 Å) to the crystal structure of rTRPV6 (Extended Data Fig. 8a–c). As the hTRPV6(R470E) structure is nearly identical to both the rTRPV6 cryo-EM structure (Extended Data Fig. 8d, e, r.m.s.d. = 0.932 Å) and the rTRPV6 crystal structure, we contend that it represents the true closed state of hTRPV6. Consistently, a much weaker density at site 2 in the cryo-EM structure of rTRPV6 (Fig. 4d) suggests either lower occupancy or greater