



Extended Data Figure 6 | Overview of single-particle cryo-EM for hTRPV6(R470E) in amphipols. **a**, Example cryo-EM micrograph for hTRPV6(R470E) in amphipols with example particles circled in red. **b**, Reference-free two-dimensional class averages of hTRPV6(R470E) in amphipols illustrating different particle orientations. **c**, Local resolution mapped on density at 0.017 threshold level (UCSF Chimera) calculated using Resmap and two unfiltered half maps, with the highest resolution

observed for the channel core. **d**, Orientations of particles that contribute to the final 4.2 Å reconstruction. Longer red rods represent orientations that comprise more particles. **e**, FSC curve calculated between half maps. **f**, Cross-validation FSC curves for the refined model versus unfiltered half maps (only half map1 was used for PHENIX refinement) and the unfiltered summed map.