



Extended Data Figure 3 | Overview of single-particle cryo-EM for hTRPV6 in amphipols and comparison to the reconstruction in nanodiscs. **a**, Example cryo-EM micrograph for hTRPV6 in amphipols with example particles circled in red. **b**, Reference-free 2D class averages of hTRPV6 in amphipols illustrating different particle orientations. **c**, Local resolution mapped on density at 0.01 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 4.0 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 map1 was used for PHENIX refinement) and the unfiltered summed map. **g**, **h**, Comparison of putative lipid densities for hTRPV6 in amphipols (**g**) and nanodiscs (**h**), filtered to the same (4.0 Å) resolution and shown at 3.5σ as purple mesh.