**Supplementary File 2. Cryo-EM data collection, refinement and validation statistics**

|  |  |  |
| --- | --- | --- |
|  | HOPS upper(EMDB-xxxx: consensus upper,EMDB-xxxx: SNARE-binding,EMDB-xxxx: backbone)(PDB xxxx) | HOPS lower(EMDB-xxxx: consensus lower,EMDB-xxxx: bottom-Vps18,EMDB-xxxx: bottom-Vps39)(PDB xxxx) |
| **Data collection and processing** |  |  |
| Magnification  | 130,000 | 130,000 |
| Voltage (kV) | 200 | 200 |
| Electron exposure (e–/Å2) | 50 | 50 |
| Defocus range (μm) | -0.8 to -2.8 | -0.8 to -2.8 |
| Pixel size (Å) | 0.924 | 0.924 |
| Symmetry imposed | C1 | C1 |
| Initial particle images (no.) | 2,565,533 (after duplicates removal) | 2,565,533 (after duplicates removal) |
| Final particle images (no.) | 129,388 (129,389 for consensus upper) | 115,273 (115,272 for bottom-Vps39) |
| Map resolution (Å) FSC threshold | 4.2 (consensus upper), 3.6 (SNARE-binding), 4.0 (backbone)0.143 | 4.4 (consensus lower), 4.4 (bottom-Vps18), 5.0 (bottom-Vps39)0.143 |
|  |  |  |
| **Refinement** |  |  |
| Initial model used  | AlphaFold | AlphaFold |
| Model resolution range (Å) FSC threshold | 3.6-5.00.143 | 3.6-5.00.143 |
| Map sharpening *B* factor (Å2) | -90.6 (consensus upper)-76.3 (SNARE-binding)-78.7 (backbone) | -111.4 (consensus lower)-116.1 (bottom-Vps18)-198.2 (bottom-Vps39) |
| Model composition Non-hydrogen atoms Protein residues | 258013581 | 258013581 |
| R.m.s. deviations Bond lengths (Å) Bond angles (°) | 0.0050.979 | 0.0050.979 |
|  Validation MolProbity score Clashscore Poor rotamers (%)  | 2.0811.780 | 2.0811.780 |
|  Ramachandran plot Favored (%) Outliers (%) | 91.870 | 91.870 |
|  |  |  |