**Supplementary file 2. Cryo-EM data collection parameters and model refinement statistics.**

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|  | Dynein AAA3 Walker B mutant (E2488Q) bound to Lis1 |
| **Models**PDBEMDB**Data collection and processing** | 7MGM23829 |
| MicroscopeCamera  | Titan KriosGatan K2 Summit |
| Voltage (kV) | 300 |
| Electron exposure (e–/Å2) | 58.3 |
| MagnificationDefocus range (μm) | 2 – 2.7 |
| Pixel size (Å) | 1.31 |
| **Reconstruction**Symmetry imposed | C1 |
| Initial particle images (no.) | 561 397 |
| Final particle images (no.) | 83 975 |
| Micrographs collected (no.)Map resolution (Å) (0.143 FSC threshold) | 23783.1 |
|  |  |
| **Model Refinement** |  |
| Initial model used (PDB code) | 5NUG |
| Map-to-model resolution (0.5 FSC threshold) (Å)Sphericity (3D FSC) | 3.20.972 |
| Map sharpening *B* factor (Å2)Model to Map Fit CC mask CC volume CC peaks | 78.770.870.870.83 |
| *Model composition* Non-hydrogen atoms Protein residues Ligands | 24 08029644 |
| *B* factors (Å2) Protein Ligand | 74.2356.39 |
| *R.m.s. deviations* Bond lengths (Å) Bond angles (°) | 0.0040.481 |
| *Validation* MolProbity score Clashscore Poor rotamers (%)  | 1.405.740.00 |
| *Ramachandran*  Favored (%) Allowed (%) Disallowed (%) | 97.602.400.00 |