**Supplementary File 1: Cryo-EM data information and model validation.**

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| **Models** | **Human Dynein - Lis1** | **Human Dynein - (Lis1)2** |
| PDBEMDBEMPIAR | 8DYVEMD-27783EMPIAR-11373 | 8DYUEMD-27782EMPIAR-11373 |
| **Data collection and processing** |  |
| MicroscopeCameraMagnfication  |  | Talos ArcticaGatan K2 Summit36 000 |
| Voltage (kV) |  | 200 |
| Electron exposure (e–/Å2) |  | 58 |
| Defocus range (μm) |  | 0.3 – 3.7  |
| Pixel size (Å)Number of datasets (no.) |  | 1.163 |
| **Reconstruction** |  |  |
| Symmetry imposed | C1 | C1 |
| Initial particles(no.) | 996 929 | 996 929 |
| Final particles (no.) | 24 217 | 37 288 |
| Micrographs collected (no.)Micrographs final (no.)Map resolution (Å) (0.143 FSC threshold) | 3763 28924.0 | 376328924.1 |
|  |  |  |
| **Model Refinement and Validation** |  |  |
| Initial model used | PDB 5NUG; AlphaFold P43034 | Human Dynein-Lis1 |
| Map-to-model resolution (0.5 FSC threshold) (Å) | 4.3 | 4.4 |
| Map sharpening *B* factor (Å2)Model to Map Fit CC mask | 50.30.70 | 65.40.68 |
| *Model composition* Non-hydrogen atoms Protein residues Ligands | 23 0532 8544 | 2461031744 |
| *B* factors (Å2) Protein Ligand | 11189 | 27649 |
| *R.m.s. deviations* Bond lengths (Å) Bond angles (°) | 0.0061.200 | 0.0050.868 |
| *Validation* MolProbity score Clashscore Poor rotamers (%)  | 1.565.740.20 | 1.394.120.23 |
| *Ramachandran*  Favored (%) Allowed (%) Disallowed (%) | 96.333.630.04 | 96.833.140.03 |