Supplemental Figure and Table Legends

Figure S1. Affinity measurements for the binding of different dynein constructs to Lis1 and kymographs for those constructs with and without Lis1, Related to Figure 1. (A) Determination of the binding affinity of Lis1 to $Dyn^{wt\text{-}M}$ (grey), $Dyn^{WA\text{-}M}$ (light green), and $Dyn^{WB\text{-}M}$ (dark green) in the presence of 1 mM ATP-Vi. (n=3 technical replicates per data point). (B) Sample kymographs for Dyn^{wt}, Dyn^{WA} and Dyn^{WB} in the absence, or presence of 300 nM Lis1.

Figure S2. Cryo-EM structure and model validation of $Dyn^{wt\text{-}M}$:Lis1, Related to Figure 2. (A) Representative drift-corrected cryo-EM micrograph. (B) Representative 2D class averages. (C) 3D alignment and classification strategy (see Methods for details). (D) Fourier Shell Correlation (FSC) for 3D reconstruction (Gold-Standard FSC) and atomic model against cryo-EM map (Model vs. map). (E) Local resolution analysis of cryo-EM structure calculated by Bsoft. (F) Euler angle distribution. (G) Example cryo-EM densities with the final Rosetta model docked in. (H) RMSD among the top five Rosetta models. Orange box indicates region shown in (I). (I) Zoomed-in view of the cryo-EM density corresponding to the large domain of AAA5, with the top five Rosetta models docked in. This region has the highest RMSD among the Rosetta models. (J) The $Dyn^{wt\text{-}M}$:Lis1 map was filtered to 20Å to reveal more of the N-terminal density of the linker domain, which is indicated by the purple arrow. The linker was segmented and is colored in purple.

Figure S3. Cryo-EM structure and model validation of Dyn^{WB-M}:Lis1, Related to Figure 3. (A) Representative drift-corrected cryo-EM micrograph. (B) Representative 2D class averages. (C) 3D alignment and classification strategy (see Methods for details). (D) Fourier Shell Correlation (FSC) for 3D reconstruction (Gold-Standard FSC) and atomic model against cryo-EM map (Model vs. map). (E) Local resolution analysis of cryo-EM structure calculated by Bsoft. (F) Euler angle distribution. (G) Example cryo-EM densities with the final Rosetta model docked in. (H) RMSD among the top five Rosetta models. (I) The Dyn^{WB-M}:Lis1 map was filtered to 20Å to reveal more of the N-terminal density of the linker domain, which is indicated by the purple arrow. The linker was segmented and is colored in purple.

Figure S4. Stoichiometry analysis of Dyn^{WB-M}:Lis1 complexes, Related to Figure 3. Chromatogram of Dyn^{WB-M} (solid green line) and Dyn^{WB-M}:Lis1 complexes (dashed green line) purified by size-exclusion chromatography. SDS-PAGE gel of the chromatography fractions was visualized by staining with SYPRO Red. Band intensities were quantified to determine the ratio of Lis1 to Dyn^{WB-M} in the peak fraction. See methods for description of stoichiometry calculations.

Figure S5. Binding affinity and velocity comparisons for Dyn^{wt-M} and Dyn^{EQN-M} with and without Lis1, Related to Figure 5. (A) Sample kymographs for Dyn^{EQN} in the absence, or presence of 300 nM Lis1. (B) Determination of the binding affinity of Lis1 to Dyn^{wt-M} (black – data previously shown in Figure S1A) and Dyn^{EQN-M} (yellow) in the presence of 1 mM ATP-Vi (n=3 per each data point). (C) Sample kymographs for

$Dyn^{WB/EQN}$ in the absence, or presence of 300 nM Lis1. (D) Average velocities for Dyn^{WB} and $Dyn^{WB/EQN}$ in the absence (solid bars) and presence (hatched bars) of 300 nM Lis1 (n>185 events per data point). (E) Normalized binding densities of Dyn^{WB} and $Dyn^{WB/EQN}$ in the absence (solid bars) or presence (hatched bars) of 300 nM Lis1, in the presence of ATP (left) or ATP-Vi (right) (n=12 fields of view per data point). Binding densities were normalized by setting those in the absence of Lis1 to 100%.

Figure S6. Velocity measurements for Dyn^{wt} and Dyn^{EQN} and binding density measurements for $Dyn^{weak/wt}$ and $Dyn^{weak/EQN}$ in plus-end recruitment assay, Related to Figure 6. (A) Minus-end and plus-end velocities of Dyn^{wt} (grey) and Dyn^{EQN} (yellow) on dynamic MTs in the presence of Lis1, Kip2, Bik1 and Bim1 (n>50 events per data point). Statistical significance was calculated using unpaired t-test with Welch's correction. P-values: ns, not significant; **, <0.01. Data are shown as mean and standard error of mean. (B) MT binding densities of $Dyn^{weak/wt}$ (light grey) and $Dyn^{weak/EQN}$ (light yellow) in the presence of Lis1, Kip2, Bik1 and Bim1 (left) or in the presence of Kip2, Bik1 and Bim1 (right) (n=8 fields of view per data point).

Table S1. Summary of velocity and binding data, Related to Figures 1 and 5. Velocity measurements for different dynein constructs with and without Lis1, apparent K_d measurements for the binding of Lis1 to different dynein constructs, and binding densities of different dynein constructs to MTs in different nucleotide conditions.

Table S2. Summary of cryo-EM data, Related to Figures 2 and 3. Overview of data collection and cryo-EM structure determination of $Dyn^{wt\text{-}M}$:Lis1 and $Dyn^{WB\text{-}M}$:Lis1.

Table S3. *S. cerevisiae* strains used in this study, Related to STAR Methods. Overview of yeast strains used in this study with their respective genotypes. P_{GAL1} indicates the galactose-induced promoter. TEV denotes a TEV protease cleavage site. DHA and SNAP denote Halo-tag (Promega) and SNAP-tag (NEB), respectively. Amino acid spacers are indicated by g (glycine), ga (glycine-alanine), and gs (glycine-serine).

Movie S1. Morph between AAA ring conformations of $Dyn^{wt\text{-}M}$:Lis1 and $Dyn^{WB\text{-}M}$:Lis1, Related to Figure 4. The movie begins with the full atomic model for $Dyn^{wt\text{-}M}$:Lis1. We then remove Lis1 and dynein's linker domain to focus on the AAA ring. The movie morphs three times between the two ring conformations and ends with the full atomic model of $Dyn^{WB\text{-}M}$:Lis1.

Supplemental data S1, related to Figure 2, Figure 3, and Figure 4. C distance coordinate files. Compressed (.zip) directory containing all distance comparisons: $Dyn^{wt\text{-}M}$:Lis1 vs. 4RH7 relative to AAA4L (Figure 2H), $Dyn^{wt\text{-}M}$:Lis1 vs. 4AKI relative to AAA4L (Figure 2H), $Dyn^{WB\text{-}M}$:Lis1 vs. 4RH7 relative to AAA4L (Figure 3F), and $Dyn^{wt\text{-}M}$:Lis1 vs. $Dyn^{WB\text{-}M}$:Lis1 relative to AAA3L (Figure 4A). Files must be displayed using UCSF Chimera.