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Supplemental Information

Lis1 Has Two Opposing Modes of Regulating Cytoplasmic Dynein

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Table S1. Summary of velocity and binding data, Related to Figures 1 and 5.

	Average velocity +/- SE (nm/s)
Dyn^{wt}	89.16 +/- 4.54
Dyn^{wt} + 300 nM Lis1	39.55 +/- 2.11
Dyn^{WA}	18.50 +/- 0.52
Dyn^{WA} + 300 nM Lis1	0.22 +/- 0.03
Dyn^{WB}	4.77 +/- 0.19
Dyn^{WB} + 300 nM Lis1	8.73 +/- 0.42
Dyn^{EQN}	81.40 +/- 4.52
Dyn^{EQN} + 300 nM Lis1	42.31 +/- 1.10
$Dyn^{WB/EQN}$	4.96 +/- 0.20
$Dyn^{WB/EQN}$ + 300 nM Lis1	3.28 +/- 0.13
	Apparent K_d +/- SE (nM) for Lis1 in the presence of 1mM ATP-Vi
Dyn^{wt-M}	49.78 +/- 6.1
Dyn^{WA-M}	40.95 +/- 5.1
Dyn^{WB-M}	55.25 +/- 7.5
Dyn^{EQN-M}	196.30 +/- 30.8
	Microtubule binding density +/- SE (pM^{-1} μm^{-1}) in the presence of 1mM ATP
Dyn^{wt}	0.033 +/- 0.002
Dyn^{WA}	0.50 +/- 0.06
Dyn^{WB}	0.50 +/- 0.03
Dyn^{EQN}	0.035 +/- 0.003
$Dyn^{WB/EQN}$	0.48 +/- 0.03
	Microtubule binding density +/- SE (pM^{-1} μm^{-1}) in the presence of 1mM ATP-Vi
Dyn^{wt}	0.037 +/- 0.004
Dyn^{WA}	0.24 +/- 0.03
Dyn^{WB}	0.36 +/- 0.01
Dyn^{EQN}	0.040 +/- 0.002
$Dyn^{WB/EQN}$	0.34 +/- 0.01
	Microtubule binding density +/- SE (pM^{-1} μm^{-1}) in the presence of 1mM ADP
Dyn^{wt}	0.53 +/- 0.03
Dyn^{WA}	1.8 +/- 0.2
Dyn^{WB}	1.6 +/- 0.1
	Microtubule binding density +/- SE (pM^{-1} μm^{-1}) in the absence of nucleotides (Apo)
Dyn^{wt}	1.48 +/- 0.03
Dyn^{WA}	1.8 +/- 0.1
Dyn^{WB}	2.9 +/- 0.1

Table S2: Summary of cryo-EM data, related to Figures 2 and 3

Data collection	**Dyn$^{wt\text{-}M}$:Lis1**	**Dyn$^{WB\text{-}M}$:Lis1**
Microscope	FEI Talos Arctica	FEI Talos Arctica
Voltage (kV)	200	200
Detector	Gatan K2 Summit	Gatan K2 Summit
Pixel size (Å)	0.60	0.60
Defocus range (μm)	1.5-4.0	2.5-5.5
Movies	5614	4826
Frames/movie	53	25
Dose rate (electrons/pixel/s)	10.285	10.00
Total dose (electrons/Å^2)	82	50
Number of particles	25,520	27,807
Map-sharpening *B* factor (Å^2)	-50	-800
Final overall resolution (Å)	7.7	10.2

Table S3. *S. cerevisiae* strains used in this study.

Strain	Genotype
RPY1	W303a (*MATa*, *his3-11,15, ura3-1, leu2-3,112, ade2-1, trp1-1*)
RPY799	W303a *pep4Δ::HIS5, prb1Δ, P_{GAL1}-8HIS-ZZ-SNAP-gs-PAC1, dyn1Δ::cgLEU2*
RPY816	W303a *pep4Δ::HIS5, prb1Δ, P_{GAL1}-8HIS-ZZ-Tev-PAC1, dyn1Δ::cgLEU2, ndl1Δ::Hygro^R*
RPY1099	W303a *pep4Δ::HIS5, prb1Δ, P_{GAL1}-8HIS-ZZ-Tev-KIP2-g- FLAG-ga-SNAP−Kan^R*
RPY1167	W303a *pep4Δ::HIS5, prb1Δ, P_{GAL1}-ZZ-TEV-GFP-3XHA-GST-DYN1(331kDa)-gsDHA-KanR, pac1Δ, ndl1Δ::cgLEU2*
RPY1302	W303a *pep4Δ::HIS5, prb1Δ, PAC11-13xMYC-TRP1, P_{GAL1}-ZZ-Tev-DYN1(331kDa), pac1Δ:: Hygro^R*
RPY1385	*MATa lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63 DYN1-3XGFP::TRP1, ura3-52::CFP-TUB1::URA3, SPC110-tdTomato::SpHIS5, ura3Δ::KanMX*
RPY1536	W303a *pep4Δ::HIS5, prb1Δ, P_{GAL1}-ZZ-TEV-GFP-3XHA-GST-DYN1(331kDa)$^{K3116A,\ K3117A,\ E3122A,\ R3124A}$-gsDHA-KanR, pac1Δ, ndl1Δ::cgLEU2*
RPY1547	W303a *pep4Δ::HIS5, prb1Δ, P_{GAL1}-8HIS-ZZ-Tev-PAC1^{R275A,R301A,R378A,W419A,K437A}, dyn1Δ::cgLEU2, ndl1Δ::Hygro^R*
RPY1630	W303a *pep4Δ::HIS5, prb1Δ, P_{GAL1}-ZZ-TEV-GFP-3XHA-GST-DYN1(331kDa)^{K2424A}-gsDHA-KanR, pac1Δ, ndl1Δ::cgLEU2*
RPY1635	W303a *pep4Δ::HIS5, prb1Δ, PAC11-13xMYC-TRP1, P_{GAL1}-ZZ-Tev-DYN1(331kDa)^{K2424A}, pac1Δ:: Hygro^R*
RPY1653	W303a *pep4Δ::HIS5, prb1Δ, P_{GAL1}-ZZ-TEV-GFP-3XHA-GST-DYN1(331kDa)^{E2488Q}-gsDHA-KanR, pac1Δ, ndl1Δ::cgLEU2*
RPY1654	W303a *pep4Δ::HIS5, prb1Δ, PAC11-13xMYC-TRP1, P_{GAL1}-ZZ-Tev-DYN1(331kDa)^{E2488Q}, pac1Δ:: Hygro^R*
RPY1705	W303a *pep4Δ::HIS5, prb1Δ, P_{GAL1}-ZZ-TEV-GFP-3XHA-GST-DYN1(331kDa)^{E3012A,Q3014A,N3018A}-gsDHA-KanR, pac1Δ, ndl1Δ::cgLEU2*
RPY1707	W303a *pep4Δ::HIS5, prb1Δ, P_{GAL1}-ZZ-TEV-GFP-3XHA-GST-DYN1(331kDa)$^{E2488Q,\ E3012A,Q3014A,N3018A}$-gsDHA-KanR, pac1Δ, ndl1Δ::cgLEU2*
RPY1708	*MATa lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63 DYN1^{E3012A,Q3014A,N3018A}-3XGFP::TRP1, ura3-52::CFP-TUB1::URA3, SPC110-tdTomato::SpHIS5, ura3Δ::KanMX*

RPY1713	W303a *pep4Δ::HIS5, prb1Δ, PAC11-13xMYC-TRP1, P_{GAL1}-ZZ-Tev-DYN1(331kDa)^{E3012A,Q3014A,N3018A}, pac1Δ:: Hygro^{R}*
RPY1717	*MATa lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63 DYN1-3XGFP::TRP1, ura3-52::CFP-TUB1::URA3, SPC110-tdTomato::SpHIS5, ura3Δ::KanMX, pac1Δ::klURA3*
RPY1725	W303a *pep4Δ::HIS5, prb1Δ, P_{GAL1}-ZZ-TEV-GFP-3XHA-GST-DYN1(331kDa)$^{E3012A,Q3014A,N3018A, K3116A, K3117A, E3122A, R3124A}$_gsDHA-KanR, pac1Δ, ndl1Δ::cgLEU2*