Supplemental Materials

Molecular Biology of the Cell

Anderson *et al*.

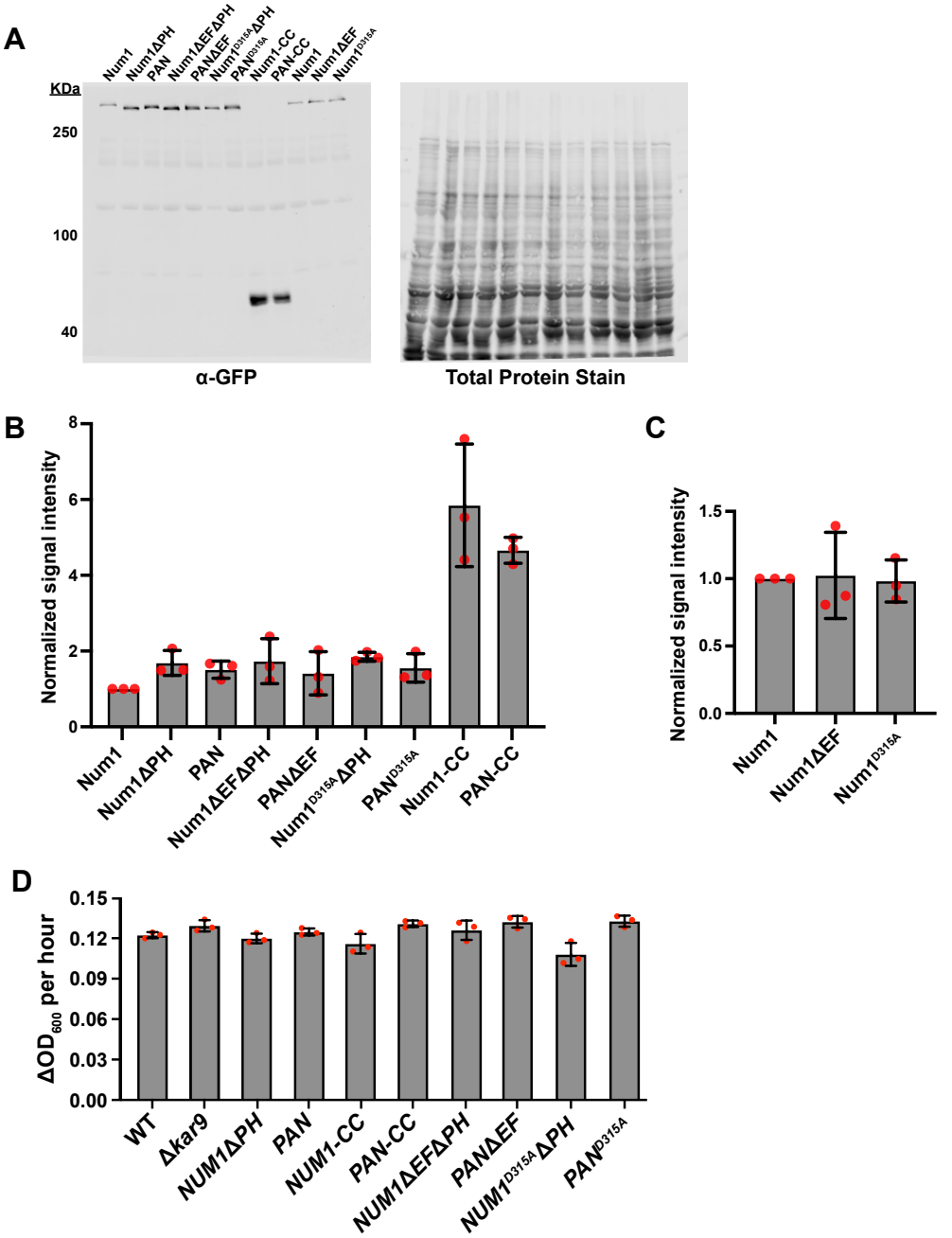

Figure S1. Characterization of synthetically clustered Num1 truncations

(A) Whole cell extracts were analyzed by SDS-PAGE and Western blot for the indicated strains using anti-GFP (left). Total protein stain of the blot (right) was used as the loading control.

(B) Quantified relative protein levels for Num1, Num1ΔPH, PAN, Num1ΔEF, PANΔEF, Num1^{D315A}ΔPH, PAN^{D315A}, Num1-CC, and PAN-CC are shown as the mean ± SD. Each dot represents a biological replicate, n=3 replicates.

(C) Quantified relative protein levels for Num1, Num1ΔEF, and Num1^{D315A} are shown as the mean ± SD. Each dot represents a biological replicate, n=3 replicates.

(D) The change in OD_{600} per hour for the linear portion of growth curves of wildtype W303

(replicated from Fig. 2C), Δ*kar9* (replicated from Fig. 2C), *NUM1*Δ*PH, PAN*, *NUM1-CC*, *PAN-CC* *NUM1*Δ*EF*, *PAN*Δ*EF*, $NUM1^{D315A}$Δ*PH*, and PAN^{D315A}. Three individual biological replicates are shown, each represented by a dot. Each biological replicate is the average of 3-4 technical replicates, mean ± SD is shown.

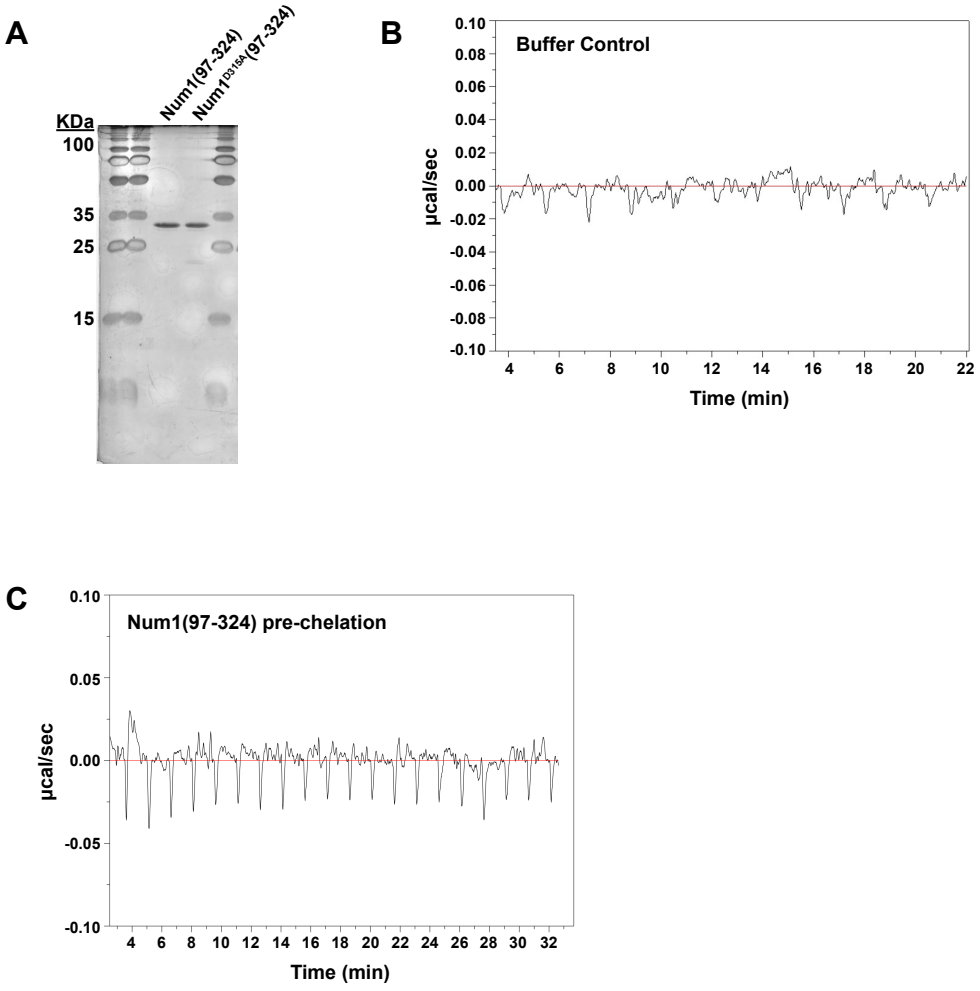

Figure S2. ITC buffer and Num1(97-324) non-EGTA treated controls
(A) Non-cropped silver stained 15% SDS-PAGE gel showing purified Num1(97-324) and Num1^{D315A}(97-324) from Fig. 3A, highlighting the absence of impurities and protein breakdown.
(B) ITC experiment buffer control. Calorimetric titration using only dialysis buffer under the same parameters as in Fig. 3B.
(C) Calorimetric titration of 50 µM Num1(97-324) that was not treated with EGTA using parameters as in Fig. 3B.

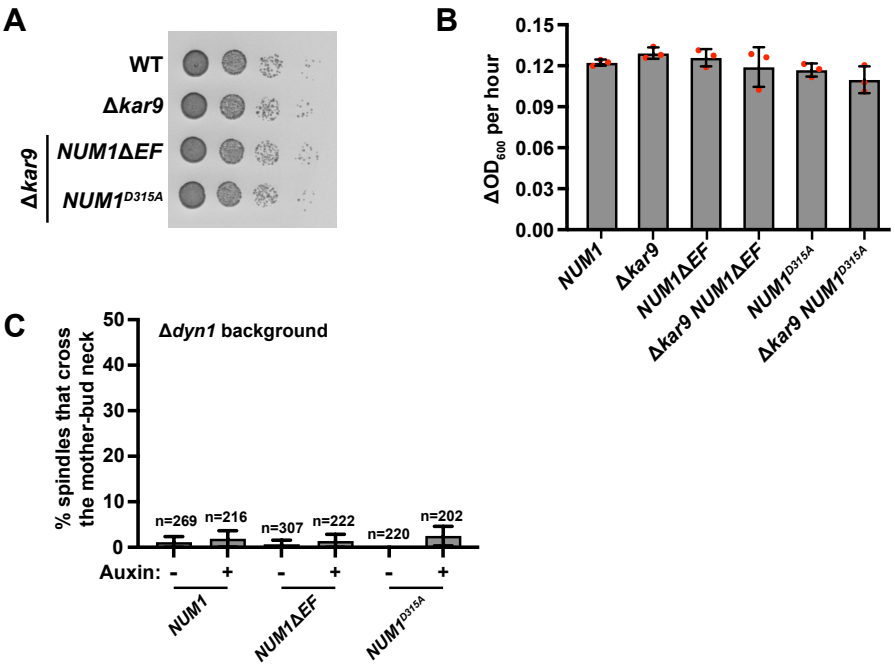

Figure S3. Characterization of dynein activity for full length Num1 EFLM mutants
(A) Tenfold serial dilutions of wildtype W303, Δ*kar9*, Δ*kar9 NUM1*Δ*EF*, and Δ*kar9 NUM1*^{D315A} were plated on YPD medium and grown at 30°C for 24 h. This is a representative example of four replicates.
(B) The changes in OD_{600} per hour for the linear portion of growth curves of WT W303 (replicated from Fig. 2C), Δ*kar9* (replicated from Fig. 2C), *NUM1*Δ*EF*, Δ*kar9 NUM1*Δ*EF*, *NUM1*^{D315A}, and Δ*kar9 NUM1*^{D315A}. Three individual biological replicates are shown, each represented by a dot. Each biological replicate is the average of 3-4 technical replicates, mean ± SD is shown.
(C) Quantification of the percentage of hydroxyurea arrested spindles that crossed the mother-bud neck in the absence and presence of auxin, as indicated under the graph, during a 10 min time lapse movie for *NUM1*, *NUM1*Δ*EF*, and *NUM1*^{D315A} *mito*^{AID} cells harboring mRuby-*TUB1* in a Δ*dyn1* background. n ≥ 202 spindles. Standard error of the proportion with a 95% confidence interval is shown.

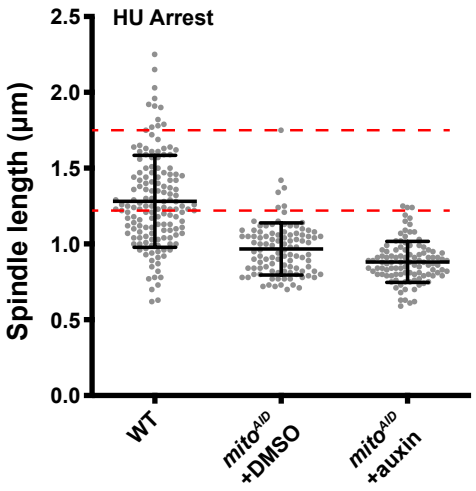

Figure S4. Quantification of pre-anaphase spindle lengths
Quantification of pre-anaphase hydroxyurea arrested spindle lengths for WT, *mito*^{AID} -Auxin, and *mito*^{AID} +Auxin. Red dashed lines indicate 1.25 µm and 1.75 µm. n ≥ 102 spindles.

Supplemental Table 1. Primer List

LL Primer#	Sequence
112	CGCGGATCCATGAACTTTACTTTGGAACTAAGCGAGAATTTGTTG
115	GCAGCTCGAGCTAGTCAATTTCGATCACTATATCAAAAATGTCTTCTTCAC
177	GACATAGAGTACCACAAAGCCGATCATTTGGCAATTTACGAGGTGACGGTG CTGGTTTA
178	GATTATTATTGTTCTTAATTTACTTAGAGTTATTTAGTTTTTTTAATCGATGAA TTCGAGCTCG
226	GTGAAGGTGAAGGTGATGCTAC
259	CTGCCAGTAGGACGGCATCTTTACAAACTTTAGCATCATTGGGTGACGGTG CTGGTTTA
433	TTGGAACTAAGCGAGAATTTGTTGGTTGAGTGTAGGAAATTGCAA
434	TTGCAATTTCCTACACTCAACCAACAAATTCTCGCTTAGTTCCAA
437	GCTCACGTAGTCTTCTTTTTTGAGTAATAAATAATCTG
456	TCCGATACTACAGTAACATCTATTTTTGATATAGTGATCGAA
457	TTCGATCACTATATCAAAAATAGATGTTACTGTAGTATCGGA
458	GGGACCACTAGTGAAGAAGCCATTTTTGATATAGTGATCGAA
459	TTCGATCACTATATCAAAAATGGCTTCTTCACTAGTGGTCCC
513	CGAGTAAAGACGCAACGGTCAAGGCTTTCCACGAGACGTTCGAATATGTCC CACAACAACAGGCATAAAAAGAATAAC
513	TCGTAAATTGCCAAATGATCGGCTTTGTGGTACTCTATGTC
514	GCAGCTCGAGCTAGTCAATTTCGATCACTATATCAAAAATGGCTTCTTCAC
933	CAAAAGAAGTATTATCAAAAACAGCATACTTCCGATACTACAGTAACATCTG GTGACGGTGCTGGTTTA
940	TAAACCAGCACCGTCACCCAATGATGCTAAAGTTTGTAAAGATGCCGTCCTA CTGGCAG

Supplemental Table 2. Strain list

Note the following abbreviations:
NUM1ΔPH = *NUM1(*aa1-2562)
NUM1-CC = *NUM1(*aa1-303)
NUM1ΔEFΔPH = *NUM1*(aa1-2562 Δaa304-315)
NUM1^{D315A}ΔPH = *NUM1(*aa1-2562 with point mutation D315A)
NUM1ΔEF = *NUM1*(Δaa304-315)
NUM1^{D315A} = *NUM1(*point mutation D315A)
PIL1-LaG16 = *PIL1-αGFP*
PIL1-LaG16 NUM1ΔPH-yEGFP = *PAN*
PIL1-LaG16 NUM1-CC-yEGFP = *PAN-CC*
PIL1-LaG16 NUM1ΔEFΔPH-yEGFP = *PANΔEF*
PIL1-LaG16 NUM1^{D315A}ΔPH-yEGFP = *PAN^{D315A}*
MMR1-AID-6*FLAG*::*HYG Δypt11*::*NAT TIR1*::*URA* = *mito^{AID}*

LLY	Genotype	Source
92/93	W303 (ade2–1;leu2–3;his3–11,15;trp1–1;ura3–1;can1–100)	
27/28	W303 Δ*num1*::*HIS*	Ping et al., 2016
123	W303 *NUM1-yEGFP*::*HIS* / pYX142-mito-RED::LEU	Lackner et al., 2013
124	W303 *NUM1ΔPH-yEGFP*::HIS / pYX142-mito-RED::LEU	Lackner et al., 2013
153	W303 *NUM1-yEGFP*::*KAN*	Lackner et al., 2013
330	W303 Δ*kar9*::*NAT*	Schmit et al., 2018
647/648	W303 Δ*num1*::*KAN*	Kraft and Lackner, 2017
1103	W303 *NUM1^{D315A}-yEGFP*::*HIS*	This study
1133	W303 *NUM1^{D315A}-yEGFP*::*KAN* / pYX142-mito-RED::LEU	This study
1184	W303 *PIL1-LaG16*::*CaURA3*	Schmit et al., 2018
1187	W303 *PIL1-LaG16*::*CaURA3 NUM1ΔPH-yEGFP*::*HIS*	Schmit et al., 2018
1223	W303 *NUM1ΔEF-yEGFP*::*HIS*	This study
1224	W303 *NUM1ΔEF-yEGFP*::*HIS* / pYX142-mito-RED::LEU	This study
1366	W303 *MMR1-AID*-6*FLAG*::*HYG Δypt11*::*NAT TIR1*::*URA*	Kraft and Lackner, 2017
1720	W303 *NUM1-yEGFP*::*HIS PHO88-mCherry*::*LEU*	This study
1824	W303 *PIL1-LaG16*::CaURA3 *NUM1ΔPH-yEGFP*::*HIS* pHIS3p:mRuby2-*TUB1*::*HYG*	This study

1906	W303 Δ*kar9*::*NAT NUM1ΔPH-yEGFP*::*HIS*	Schmit et al., 2018
1908	W303 Δ*kar9*::*NAT PIL1-LaG16*::*CaURA3 NUM1ΔPH-yEGFP*::HIS	Schmit et al., 2018
1977	W303 *NUM1ΔPH-yEGFP*::*HIS* pHIS3p:mRuby2-*TUB1*::*HYG*	Schmit et al., 2018
2033	W303 *NUM1ΔPH-yEGFP*::*HIS*	Schmit et al., 2018
2076	W303 *PIL1-LaG16*::*CaURA3 NUM1ΔPH-yEGFP*::*HIS* /pYX142-mito-RED::LEU	Schmit et al., 2018
2085	W303 Δ*lnp1*::*KAN*	This study
2139	W303 pHIS3p:mRuby2-*TUB1*::*HYG*	Schmit et al., 2018
2141	W303 Δ*num1*::HIS pHIS3p:mRuby2-*TUB1*::*HYG*	Schmit et al., 2018
2275	W303 *NUM1-CC-yEGFP*::*HIS*	Ping et al., 2016
2278	W303 *PIL1-LaG16*::*CaURA3 NUM1-CC-yEGFP*::*HIS*	This study
2335	W303 Δ*kar9*::*NAT NUM1-CC-yEGFP*::*HIS*	This study
2337	W303 Δ*kar9*::*NAT PIL1-LaG16*::CaURA3 *NUM1-CC-yEGFP*::*HIS*	This study
2339	W303 *NUM1-CC-yEGFP*::*HIS* / pYX142-mito-RED::LEU	Ping et al., 2016
2341	W303 *PIL1-LaG16*::*CaURA3 NUM1-CC-yEGFP*::*HIS* / pYX142-mito-RED::LEU	This study
2343	W303 *NUM1-CC-yEGFP*::*HIS* pHIS3p:mRuby2-*TUB1*::*HYG*	This study
2345	W303 *PIL1-LaG16*::*CaURA3 NUM1-CC-yEGFP*::*HIS* pHIS3p:mRuby2-*TUB1*::*HYG*	This study
2582	W303 *PIL1-LaG16*::*CaURA3 NUM1ΔEFΔPH-yEGFP*::*HIS*	This study
2649	W303 *NUM1ΔEFΔPH-yEGFP*::*HIS*	This study
3085	W303 *PIL1-LaG16*::*CaURA3 NUM1ΔEFΔPH-yEGFP*::*HIS* pHIS3p:mRuby2-*TUB1*::*HYG*	This study
3144	W303 Δ*kar9*::*NAT NUM1ΔEFΔPH-yEGFP*::*HIS*	This study
3145	W303 Δ*kar9*::*NAT PIL1-LaG16*::*CaURA3 NUM1ΔEFΔPH-yEGFP*::*HIS*	This study
3499	W303 *PIL1-LaG16*::*CaURA3 NUM1ΔPH-yEGFP*::*HIS DYN1-mKate*::*HIS* TEF::mito-TagBFP::TRP	This study
3500	W303 *PIL1-LaG16*::*CaURA3 NUM1-CC-yEGFP*::*HIS DYN1-*	This study

	mKate::*HIS* TEF::mito-TagBFP::TRP	
3501	W303 *PIL1-LaG16*::*CaURA3 NUM1*∆*EF*∆*PH-yEGFP*::*HIS DYN1-mKate*::*HIS* TEF::mito-TagBFP::TRP	This study
3564	W303 *NUM1*^{D315A}∆*PH-yEGFP*::*KAN*	This study
3575	W303 *NUM1-yEGFP*::*KAN DYN1-mKate*::*HIS* TEF::mito-TagBFP::TRP	This study
3576	W303 *NUM1*∆*EF-yEGFP*::*HIS MMR1-AID*-6*FLAG*::*HYG* ∆*ypt11*::*NAT TIR1*::*URA* pHIS3p:mRuby2-*TUB1*::*LEU* TEF::mito-TagBFP::TRP	This study
3577	W303 *PIL1-LaG16*::*CaURA3 NUM1*∆*EF*∆*PH-yEGFP*::*HIS MMR1-AID*-6*FLAG*::*HYG* ∆*ypt11*::*NAT TIR1*::*URA* pHIS3p:mRuby2-*TUB1*::*LEU* TEF::mito-TagBFP::TRP	This study
3597	W303 *NUM1*∆*EF*∆*PH-yEGFP*::*HIS* pHIS3p:mRuby2-*TUB1*::*HYG*	This study
3603	W303 *PIL1-LaG16*::CaURA3 *NUM1*^{D315A}∆*PH-yEGFP*::*KAN*	This study
3607	W303 *PIL1-LaG16*::*CaURA3 NUM1*^{D315A}∆*PH-yEGFP*::*KAN DYN1-mKate*::*HIS* TEF::Mito-TagBFP::TRP	This study
3647	W303 *NUM1*∆*EF-yEGFP*::*HIS* pHIS3p:mRuby2-*TUB1*::*LEU*	This study
3649	W303 *NUM1*∆*EF-yEGFP*::*HIS DYN1-mKate*::*HIS* TEF::MitoTag-BFP::TRP	This study
3713	W303 *PIL1-LaG16*::*CaURA3 NUM1*^{D315A}∆*PH-yEGFP*::*KAN* pHIS3p:mRuby2-*TUB1*::*HYG*	This study
3739	W303 *PIL1-LaG16*::*CaURA3 NUM1*^{D315A}∆*PH-yEGFP*::*HIS MMR1-AID*-6*FLAG*::*HYG* ∆*ypt11*::*NAT TIR1*::*URA* pHIS3p:mRuby2-*TUB1*::*LEU* TEF::mito-TagBFP::TRP	This study
3740	W303 *PIL1-LaG16*::*CaURA3 NUM1*∆*EF*∆*PH-yEGFP*::*HIS* / pYX142-mito-RED::LEU	This study
3742	W303 *PIL1-LaG16*::*CaURA3 NUM1*^{D315A}∆*PH-yEGFP*::*HIS* / pYX142-mito-RED::LEU	This study
3766	W303 ∆*kar9*::*NAT NUM1*^{D315A}∆*PH-yEGFP*::*KAN*	This study
3767	W303 ∆*kar9*::*NAT PIL1-LaG16*::*CaURA3 NUM1*^{D315A}∆*PH-yEGFP*::*KAN*	This study
3809	W303 *PIL1-LaG16*::*CaURA3 NUM1*∆*PH-yEGFP*::*HIS MMR1-AID*-6*FLAG*::*HYG* ∆*ypt11*::*NAT TIR1*::*URA* pHIS3p:mRuby2-*TUB1*::*LEU* TEF::Mito-TagBFP::TRP	This study

3905	W303 Δ*kar9*::*NAT NUM1*Δ*EF-yEGFP*::*HIS*	This study
3906	W303 Δ*kar9*::*NAT NUM1*^{D315A}*-yEGFP*::*HIS*	This study
3915	W303 *NUM1-yEGFP*::*HIS MMR1-AID*-6*FLAG*::*HYG* Δ*ypt11*::*NAT TIR1*::*URA* mRuby-Tub1::LEU TEF::MitoTagBFP::TRP	This study
4103	W303 *MMR1-AID*-6*FLAG*::*HYG* Δ*ypt11*::*NAT TIR1*::*URA* Δ*dyn1*::*NAT NUM1-yEGFP*::*HIS* pHIS3p:mRuby2-*TUB1*::*LEU* TEF::Mito-TagBFP::TRP	This study
4166	W303 *NUM1*^{D315A}*-yEGFP*::*HIS* pHIS3p:mRuby2-*TUB1*::*LEU*	This study
4167	W303 *NUM1*^{D315A}*-yEGFP*::*HIS DYN1-mKate*::*HIS* TEF::Mito-TagBFP::TRP	This study
4168	W303 *MMR1-AID*-6*FLAG*::*HYG* Δ*ypt11*::*NAT TIR1*::*URA* *NUM1*^{D315A}*-yEGFP*::*HIS* pHIS3p:mRuby2-*TUB1*::*LEU* TEF::Mito-TagBFP::TRP	This study
4175	W303 Δdyn1::NAT *MMR1-AID*-6*FLAG*::*HYG* Δ*ypt11*::*NAT TIR1*::*URA NUM1*Δ*EF-yEGFP*::*HIS* pHIS3p:mRuby2-*TUB1*::*LEU* TEF::Mito-TagBFP::TRP	This study
4269	W303 Δdyn1::NAT *MMR1-AID*-6*FLAG*::*HYG* Δ*ypt11*::*NAT TIR1*::*URA NUM1*^{D315A}*-yEGFP*::*HIS* pHIS3p:mRuby2-*TUB1*::*LEU* TEF::Mito-TagBFP::TRP	This study
4422	W303 *NUM1*^{D315A}Δ*PH-yEGFP*::*HIS* / pYX142-mito-RED::LEU	This study
4423	W303 *NUM1*Δ*EF*Δ*PH-yEGFP*::*HIS* / pYX142-mito-RED::LEU	This study
4475	W303 Δ*dyn1*::*NAT* pHIS3p:mRuby2-*TUB1*::*HYG*	This study
4476	W303 *NUM1*^{D315A}Δ*PH-yEGFP*::*HIS* pHIS3p:mRuby2-*TUB1*::*HYG*	This study
4702	W303 Δ*lnp1*::*KAN NUM1-yEGFP*::*HIS PHO88-mCherry*::*LEU*	This study
4703	W303 *NUM1*Δ*EF-yEGFP*::*HIS PHO88-mCherry*::*LEU*	This study
4704	W303 Δ*lnp1*::*KAN NUM1*Δ*EF-yEGFP*::*HIS PHO88-mCherry*::*LEU*	This study
4705	W303 *NUM1*^{D315A}*-yEGFP*::*HIS PHO88-mCherry*::*LEU*	This study
4706	W303 Δ*lnp1*::*KAN NUM1*^{D315A}*-yEGFP*::*HIS PHO88-mCherry*::*LEU*	This study

4979	W303 *MMR1-AID-FLAG*::*HYG* ∆*ypt11*::*NAT TIR1*::*URA NUM1-yEGFP*::*KAN DYN1-yoHalo*::*HIS* TEF::Mito-TagBFP::TRP	This study
4980	W303 *MMR1-AID-FLAG*::*HYG* ∆*ypt11*::*NAT TIR1*::*URA NUM1*∆*EF-yEGFP*::*HIS DYN1-yoHalo*::*KAN* TEF::Mito-TagBFP::TRP	This study
4981	W303 *MMR1-AID-FLAG*::*HYG* ∆*ypt11*::*NAT TIR1*::*URA NUM1^{D315A}-yEGFP*::*HIS DYN1-yoHalo*::*KAN* TEF::Mito-TagBFP::TRP	This study