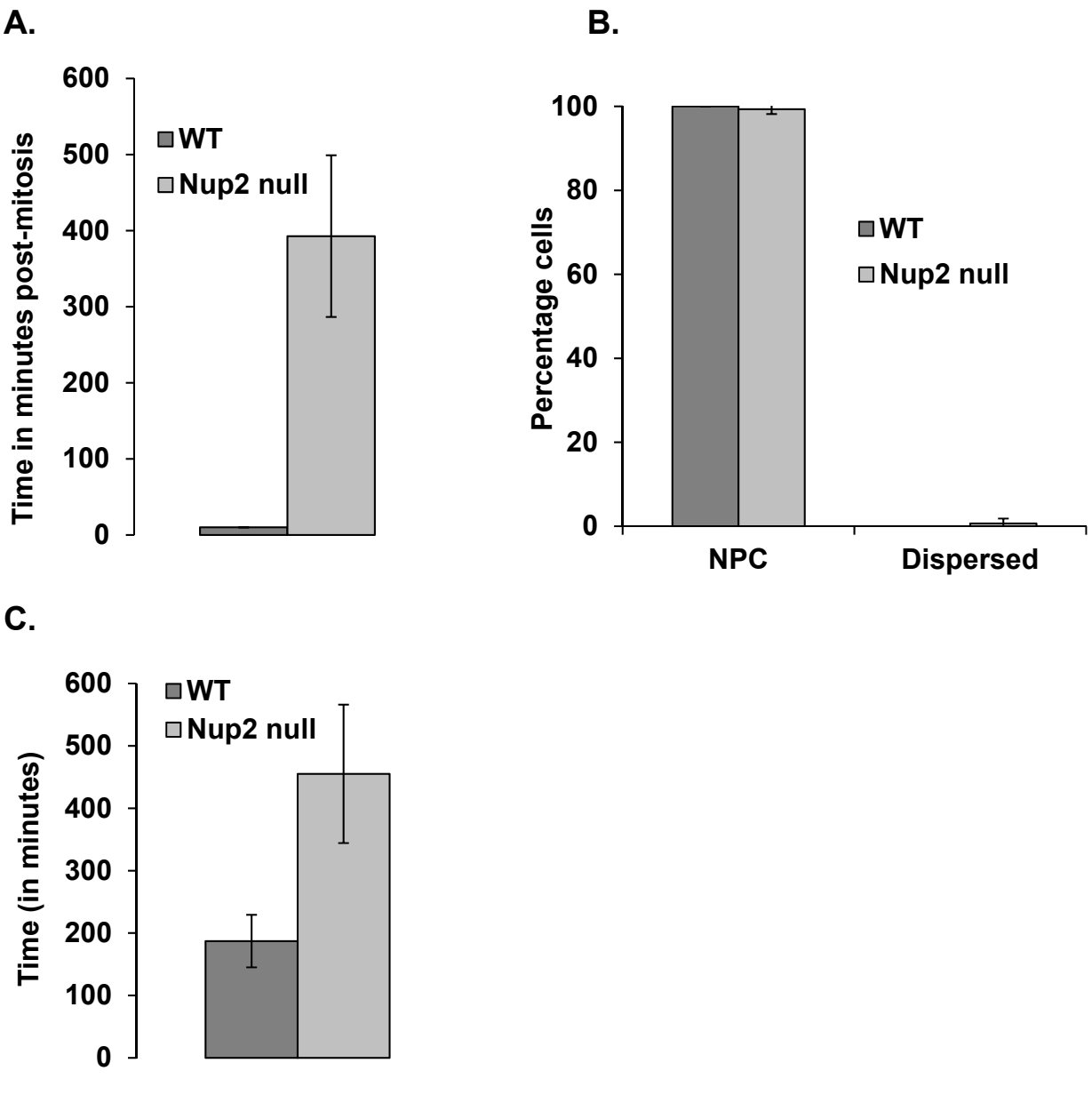
Supplemental Materials

Molecular Biology of the Cell

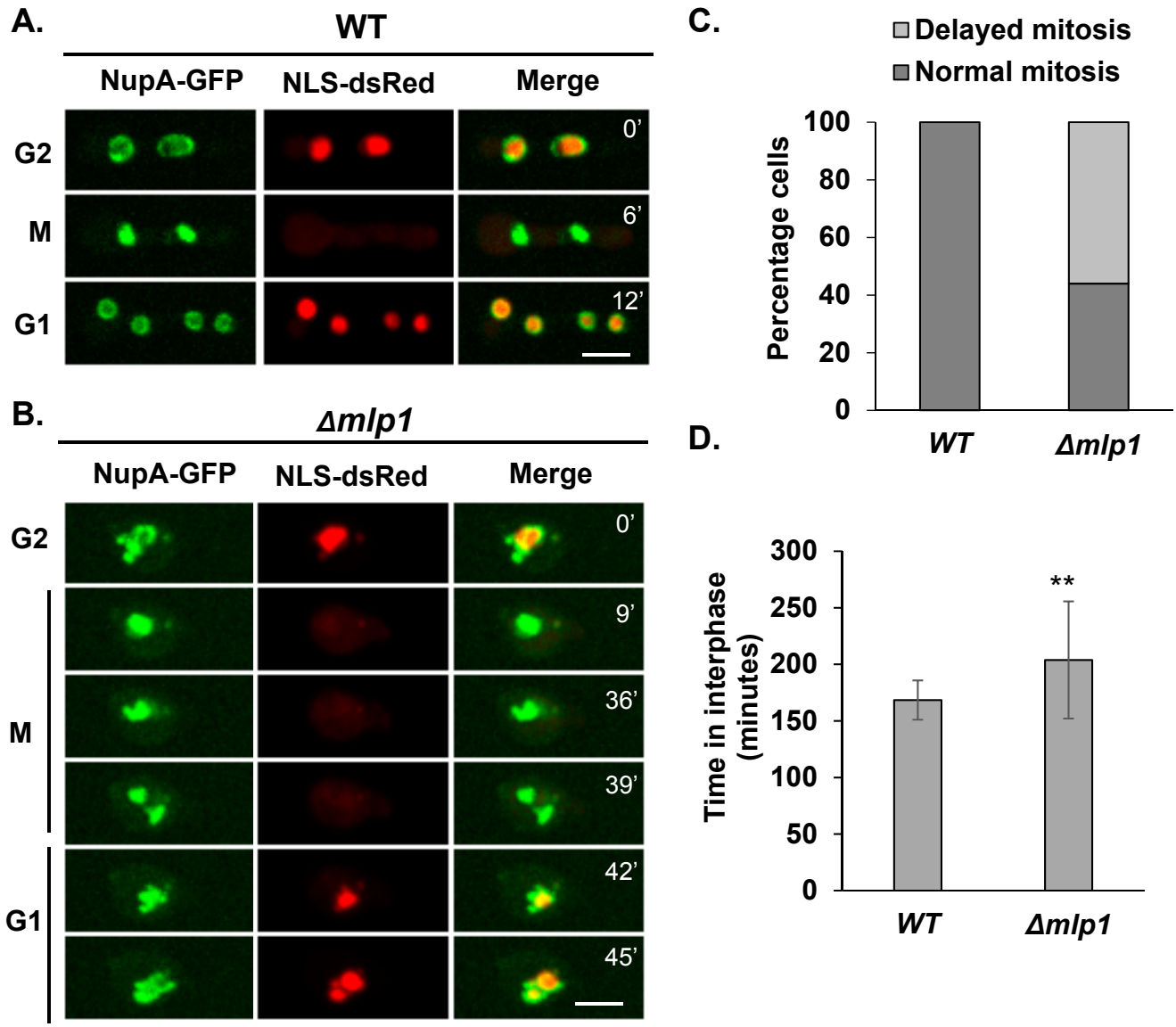
Suresh et al.

Supplementary Figure S1



Supplementary Figure S1. Mlp1 gets incorporated into NPCs after a prolonged interphase in Nup2-deleted cells. (A) Time taken for Mlp1 to be incorporated into NPCs post-mitosis was calculated in wildtype and Nup2-deleted cells (n = 20 mitoses. p-value <0.0001). (B) Percentage cells with Mlp1 either at NPCs or dispersed just before mitosis was calculated in wildtype and Nup2-deleted cells (n = 20 mitosis) indicating that Mlp1 comes back to NPCs in most cases before mitosis. (C) Time spent in interphase was calculated by following NLS-dsRed and recording the time between its mitotic dispersal from nuclei in wildtype and Nup2-deleted cells (n = 50 mitosis. p-value <0.0001).

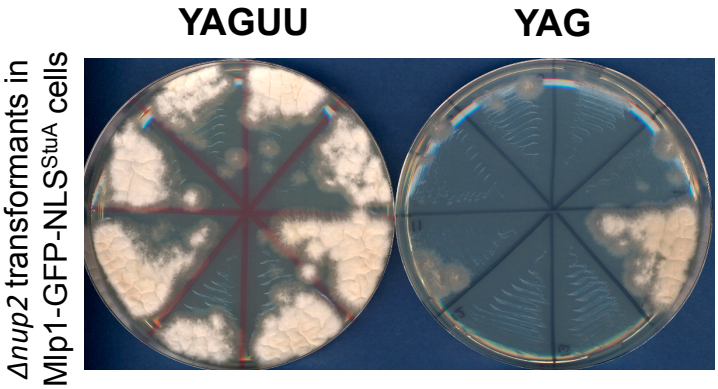
Supplementary Figure S2



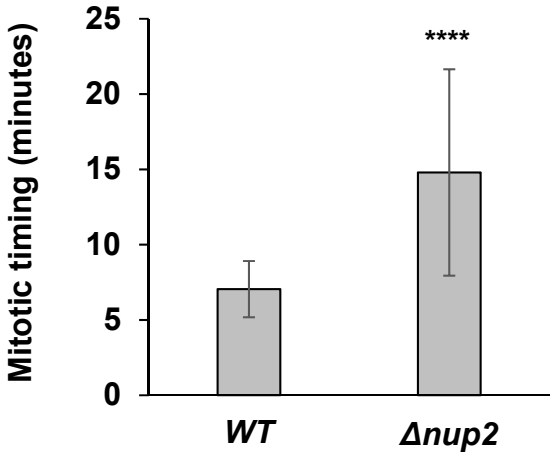
Supplementary Figure S2. Mlp1 deletion causes interphase and mitotic delay. (A) Imaging of NupA-GFP and NLS-dsRed during G2-M-G1 transitions in wildtype (SM86) and (B) *Δmlp1* cells (SGS250-H). (C) Percentage of cells displaying mitotic delay (greater than 10 minutes in mitosis was measured as delayed mitosis) in wildtype and *Δmlp1* cells measured by following NLS-dsRed (n = 70 mitosis). (D) Time spent in interphase in WT versus *Δmlp1* cells by following NLS-dsRed and recording the time between its mitotic dispersal from nuclei (n = 25 mitoses. p-value < 0.01). Scale bar, ~ 5μm.

Supplementary Figure S3

A.

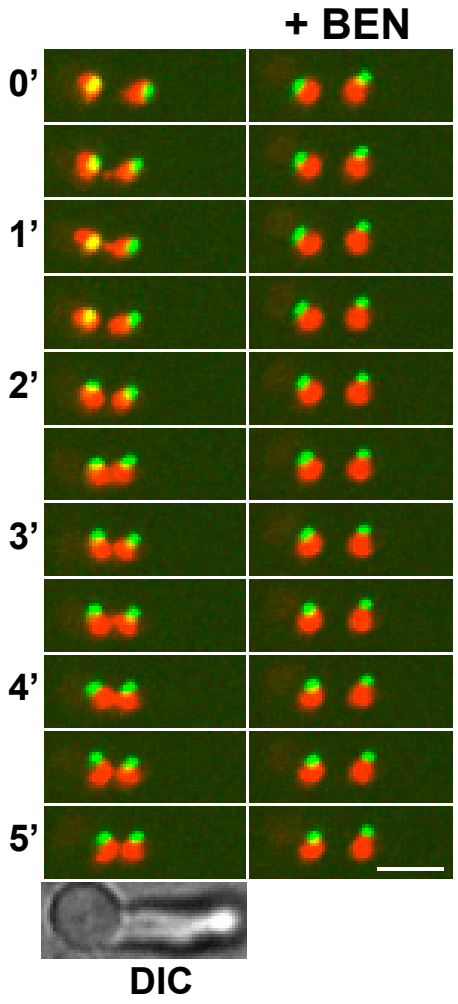


B.



Supplementary Figure S3. Retargeting Mlp1 to interphase NPCs does not rescue mitotic delay or the growth defects in *Δnup2* cells. (A) Using the heterokaryon rescue technique (Osmani, Oakley, et al., 2006), Mlp1-GFP-NLS^{StuA} was found to not complement Nup2 function. The one colony growing on selective media was a diploid. Heterokaryons form spontaneously when essential genes are deleted using a nutritional marker gene. When selection for the nutritional marker is imposed, nuclei carrying the essential gene deletion provide the nutritional marker function while the non-deleted nuclei provide the essential gene function. The heterokaryotic state is broken during the asexual cycle because all asexual spores contain a single nucleus. Growth of the uninucleated spores from heterokaryons on selective media therefore permits the growth of the null mutant to its terminal arrest phenotype but not the nuclei carrying the essential gene. Growth of the same spores on non-selective media allows the parent non-deleted spores to grow and form a colony. (B) Time in mitosis was measured in Mlp1-GFP-NLS^{StuA} cells with or without Nup2 (n = 45 mitoses. p-value < 0.0001).

Supplementary Figure S4



Supplementary Figure S4. Ima1 foci formed in the absence of Nup2 remain after benomyl treatment to depolymerize microtubules. Time-lapse microscopy of Ima1-GFP and NLS-dsRed in a *Δnup2* cell (SGS159-H) before treatment and after 15 minute treatment with 3μg/ml benomyl (+BEN) as indicated. Scale bar, ~5 μm.

Supplementary Table 1. *Aspergillus nidulans* strains used in this study

Strain	Genotype	Source background
SGS90	Mlp1::GFP::pyroA^{Af} (pyroA4); pyrG89; wA3; fwA1; chaA1; Δmus51::argB (argB2); SE15; nirA14	SO451
SGS102	Mlp1-GFP-pyroA^{Af} (pyroA4); ΔyA::NLS-DsRed; wA2?/wA3?; Δmus51::argB (argB2); pyrG89; fwA1?; chaA1?; SE15	SGS91XCDS553
SGS105 (H)	ΔnupA::pyrG^{Af} (pyrG89); Mlp1-GFP-pyroA^{Af} (pyroA4); ΔyA::NLS-DsRed; Δmus51::argB (argB2); wA2?/wA3?; fwA1?; chaA1?; SE15	SGS102
SGS121 (H)	Δnup2::pyrG^{Af} (pyrG89); Mlp1-GFP-pyroA^{Af} (pyroA4); ΔyA::NLS-DsRed; Δmus51::argB (argB2); wA2?/wA3?; fwA1?; chaA1?; SE15	SGS102
SGS159 (H)	Δnup2::pyrG^{Af} (pyrG89); Ima1-GFP::pyroA^{Af} (pyroA4); nimT23; ΔyA::NLS-DsRed ; ΔnkuA::argB (argB2?)	HA449
SGS193	AN2226-GFP-pyroA^{Af} (pyroA4); pyrG89; wA3; fwA1; chaA1; Δmus51::argB (argB2); SE15; nirA14	SO451
SGS197	ΔyA::NLS-DsRed; pyrG89; pyroA4; ∆mus51:: argB (argB2); wA2/wA3; fwA1?; chaA1?; sE15	(Suresh et al., 2017)
SGS206	AN5694-GFP-pyroA^{Af} (pyroA4?); ΔyA::NLS-DsRed; pyrG89; Δmus51::argB (argB2); wA2/wA3; fwA1?; chaA1?; SE15	SGS185XCDS553

SGS212	AN2226-GFP-pyroA^{Af} (pyroA4?); ΔyA::NLS-DsRed; pyrG89; Δmus51::argB (argB2); wA2/wA3; fwA1?; chaA1?; SE15; nirA14	SGS193XCDS553
SGS250 (H)	Δmlp1::pyrG^{Af} (pyrG89); NupA-GFP::pyroA^{Af} (pyroA4); nimT23; ∆mus51::argB; ∆yA::NLS-DsRed-StuA	SM86
SGS263 (H)	Δnup2::pyrG^{Af} (pyrG89); AN2226-GFP-pyroA^{Af} (pyroA4?); ΔyA::NLS-DsRed; Δmus51::argB (argB2); wA2/wA3; fwA1?; chaA1?; SE15; nirA14	SGS212
SGS282	Md2A-GFP::pyroA^{Af} (pyroA4?); ΔyA::NLS-DsRed; pyrG89; ΔnKuA^{Ku70}::argB (argB2); SE15?; nirA14?; wA2/3?; fwA1?; chaA1?	CDS553XCDS634
SGS285 (H)	Δnup2::pyrG^{Af} (pyrG89); Md2A-GFP::pyroA^{Af} (pyroA4?);ΔyA::NLS-DsRed; ΔnKuA^{Ku70}::argB (argB2); SE15?; nirA14?; wA2/3?; fwA1?; chaA1?	SGS282
SGS287 (H)	ΔnupA::pyrG^{Af} (pyrG89); Md2A-GFP::pyroA^{Af} (pyroA4?); ΔyA::NLS-DsRed; ΔnKuA^{Ku70}::argB (argB2); SE15?; nirA14?; wA2/3?; fwA1?; chaA1?	SGS282
SGS290 (H)	Δnup2::pyrG^{Af} (pyrG89); AN5694-GFP-pyroA^{Af} (pyroA4?); ΔyA::NLS-DsRed; Δmus51::argB (argB2); wA2/wA3; fwA1?; chaA1?; SE15	SGS206
SGS306 (H)	Δnup2::pyrG^{Af} (pyrG89); Mlp1-GFP-StuANLS::pyroA^{Af} (pyroA4); ΔyA::NLS-DsRed; ∆mus51:: argB(argB2); wA2/wA3; fwA1?; chaA1?; sE15	SGS311

SGS311	Mlp1-GFP-StuANLS::pyroA^{Af} (pyroA4); pyrG89; ΔyA::NLS-DsRed; ∆mus51:: argB (argB2); wA2/wA3; fwA1?; chaA1?; sE15	SGS197
SGS337	Nup2ΔIABDΔRBD-CR-pyrG^{Af} (pyrG89); Mlp1::GFP::pyroA^{Af} (pyroA4); wA3; fwA1; chaA1; Δmus51::argB (argB2); SE15; nirA14	SGS90
SGS340	Nup2ΔIABDΔRBD-CR-pyrG^{Af} (pyrG89); AN2226-GFP-pyroA^{Af} (pyroA4); wA3; fwA1; chaA1; Δmus51::argB (argB2); SE15; nirA14	SGS193
SM86	pyrG89 ΔyA::StuA-NLS-DsRed; nimT23; pyroA4 Δmus51::argB; nupA-GFP::pyroA^{Af}	(Markossian et al., 2015)
SM106 (H)	ΔnupA::pyrG^{An}; Ima1-GFP::pyroA^{Af}; nimT23; ΔyA::NLS-DsRed-StuA; pyrG89; (pyroA4; argB2?); ΔnkuA^{ku70}::argB	HA449
HA449	Ima1-GFP::pyroA^{Af}; nimT23; ΔyA::NLS-DsRed; pyrG89; (pyroA4; argB2?) ; ΔnkuA::argB	(Liu et al., 2015)
SGS91	Mlp1::GFP::pyroA^{Af} (pyroA4); pyrG89; wA3; fwA1; chaA1; Δmus51::argB (argB2); SE15; nirA14	SO451
CDS553	ΔyA::NLS-DsRed; argB2; wA2	AY02
SO451	pyrG89; pyroA4; wA3; fwA1; chaA1; Δmus51::argB (argB2); SE15; nirA14	(Osmani, Davies, et al., 2006)
SGS185	AN5694-GFP-pyroA^{Af} (pyroA4); pyrG89; wA3; fwA1; chaA1; Δmus51::argB (argB2); SE15; nirA14	SO451

SGS193	AN2226-GFP-pyroA^{Af} (pyroA4); pyrG89; wA3; fwA1; chaA1; Δmus51::argB (argB2); SE15; nirA14	SO451
CDS634	Md2A-GFP::pyroA^{Af} (pyroA4); ΔnKuA^{Ku70}::argB (argB2); pyrG89; sE15; nirA14; wA3; fwA1; chaA1	(De Souza et al., 2009)
AY02	pyrG89; pyroA4; ΔyA:NLS-DsRed; ΔnKuA::argB; (argB2)	(Shen, Osmani, Govindaraghavan, & Osmani, 2014)

Some nutritional and color markers could be covered by, or be recessive to, other markers in the strain and are designated by a question mark.

^{Af} represents genes from *A. fumigatus* used for complementation of the corresponding *A. nidulans* nutritional mutations.

(H) Represents a heterokaryon.

Supplementary Table 2. Primers used in this study

Oligo	Sequence
GS15	CTGTTCCAGCCTCGTCTTCC
GS16	CCAACTCCAACTCCTGCACC
GS28	GGCTCCAGCGCCTGCACCAGCTCCGCCGGCACCACTTCTAGCAA
GS29	CATTTATCGAGACAACAGTACAGCACCCGC
GS30	GCGGGTGCTGTACTGTTGTCTCGATAAATGCGTGCTGGCACACCGTCC
GS31	GGCTCCAGCGCCTGCACCAGCTCCCTTTTGAGCGTCTCCATCGG
GS51	TGGCATGGATGAACTATACAAACGCTACGGCCAAGGCACCGTTG
GS52	GAAGAGCATTGTTTGAGGCGTTAACGACGAGCACTTATCAGACTGCC
GS59	GGAGAGGCTAGAGGCAAGTG
GS60	TGGACTTGGTGCAGGCTCAG
GS61	GGCTCCAGCGCCTGCACCAGCTCCAGCCCCTCGTCGAGCACGCTTGTT
GS62	GCATCAGTGCCTCCTCTCAGACAGTTGCTGTCTTCAGAGATACAC
GS63	CAATCGTCTTGTCCACATCC
GS64	CAGATGCAGTATGCCAGAAG
GS83	GATCTTGTCCTCCTCTCCAC
GS84	TGCCTGTTATCGTGTCGGTG
GS85	GGCTCCAGCGCCTGCACCAGCTCCGCTGACCGCGATGAGCCCTT
GS86	GCATCAGTGCCTCCTCTCAGACAGATCTAGCAGTTAAGTGATGGCATACG
GS87	CGCCTTCTGTTCATTCATCG
GS88	CTTCTCGAAGGTTCGCGGCA

GS95	AGGAAGAGTGCGGCTAGCTG
GS101	TTTGTATAGTTCATCCATGCCATGTGTAATCCC
GS102	GGGATTACACATGGCATGGATGAACTATACAAACGCTACGGCCAAGGCACCGTTG
GS105	CACACAGGCCAAGGTCGACA
GS106	GACACCTAAGCCTTCGCCAG
GS107	CGCCTTGATTATCTACCGTG
GS108	CGGTTGTTGAGCAAGAGATG
GS111	GCAGCACCAGCTTCTGAATC
GS112	GGCCGGTGGATGCATTAGTG
JD143	CAGAAATATGACCGAGTGGACCCAGCTCAG
JD144	AGATCCCCGTGTATGACCACCACCGCGCATC
JD145	GATGCGCGGTGGTGGTCATACACGGGGATCTGGAGCTGGTGCAGGCGCTGGAGCCG
JD146	CACATTCCAATGCAAAATGCAGCTTGCCACCTGTCTGAGAGGAGGCACTGATGC
JD147	GTGGCAAGCTGCATTTTGCATTGGAATGTG
JD148	GCCATATCATGTCCCGTTAGCAGGTTGTTC
JD151	AGGACGTCATGTTGAAGTACCCCATAAGCG
JD152	GTTGAACTTAAATAGATCAGTGTCCTTATCC
JD153	GGATAAGGACACTGATCTATTTAAGTTCAAC-CGCCTCAAACAATGCTCTTC
JD154	GCCGGTGGATGCATTAGTGATGAAGACTGT-CTGTCTGAGAGGAGGCACTGATGC

JD155	ACAGTCTTCATCACTAATGCATCCACCGGC
JD156	TATCAAGGAGATCCGCGCTTTCGCTACTCG
JD163	GTTGACGAAAGTACAGTAGCCAGCACGG
JD164	TGAACTGAGTGAGAAGGTCGTCAAACGC
JD240	AACGCCTGAACATAGCCTTGGTCATTTGCC
JD241	TGCTTTGAAAATTCCAACCTACAGCCAG
JD242	TCCTGGCTGTAGGTTGGAATTTTCAAAGCACGCCTCAAACAATGCTCTTC
JD243	CACATTCCAATGCAAAATGCAGCTTGCCACCTGTCTGAGAGGAGGCACTGATGC
JD147	GTGGCAAGCTGCATTTTGCATTGGAATGTG
JD244	CGCAGCTCTCCGATACTGATAGCTTCATAG
JD336	CGCTCATTCCTACCCCAGTAGATTG
JD337	ACGAACTTTGCCGGTGATGACATGC
SM36	GGTATGTTGTCGCACTTGGA
SM39	CGCTTACAGAACTACCAACC
SM40	GAAGAGCATTGTTTGAGGCGTCTGACTGTATATTTGTGTTTGCGC
SM42	GCATCAGTGCCTCCTCTCAGACAGTAGAGTGTCGTGGTTTTTGTTACAT
LU233	CGCCTCAAACAATGCTCTTC
FN01	CTGTCTGAGAGGAGGCACTGATGC

Supplementary Table 3. Importin α/β interacting proteins. Table showing the chosen importin α/β interacting proteins, their predicted orthologues in yeast, the number of unique peptides identified by mass spectrometry and the % sequence coverage.

Purified protein	**Co-purifying protein**	**Orthologues (*S. cerevisiae* - *S. pombe*)**	**Length (aa) / Size (kDa)**	**Unique peptides in interphase (% sequence coverage)**
Importin α	AN2226 (Tho2)	Tho2-Tho2	2429 / 269.5	23 (13)
Importin α	AN5694 (CutB)	TRF5-Cid14	694 / 77.7	7 (17)
Importin β	AN5694 (CutB)	TRF5-Cid14	694 / 77.7	4 (11)

Supplementary Movie S1. Δ*nupA* cell expressing Mlp1-GFP and NLS-DsRed going through mitosis. Images were collected every 8 minutes.