Developmental Cell

Supplemental Information

Nuclear Pore Basket Proteins Are Tethered to the Nuclear Envelope and Can Regulate Membrane Curvature

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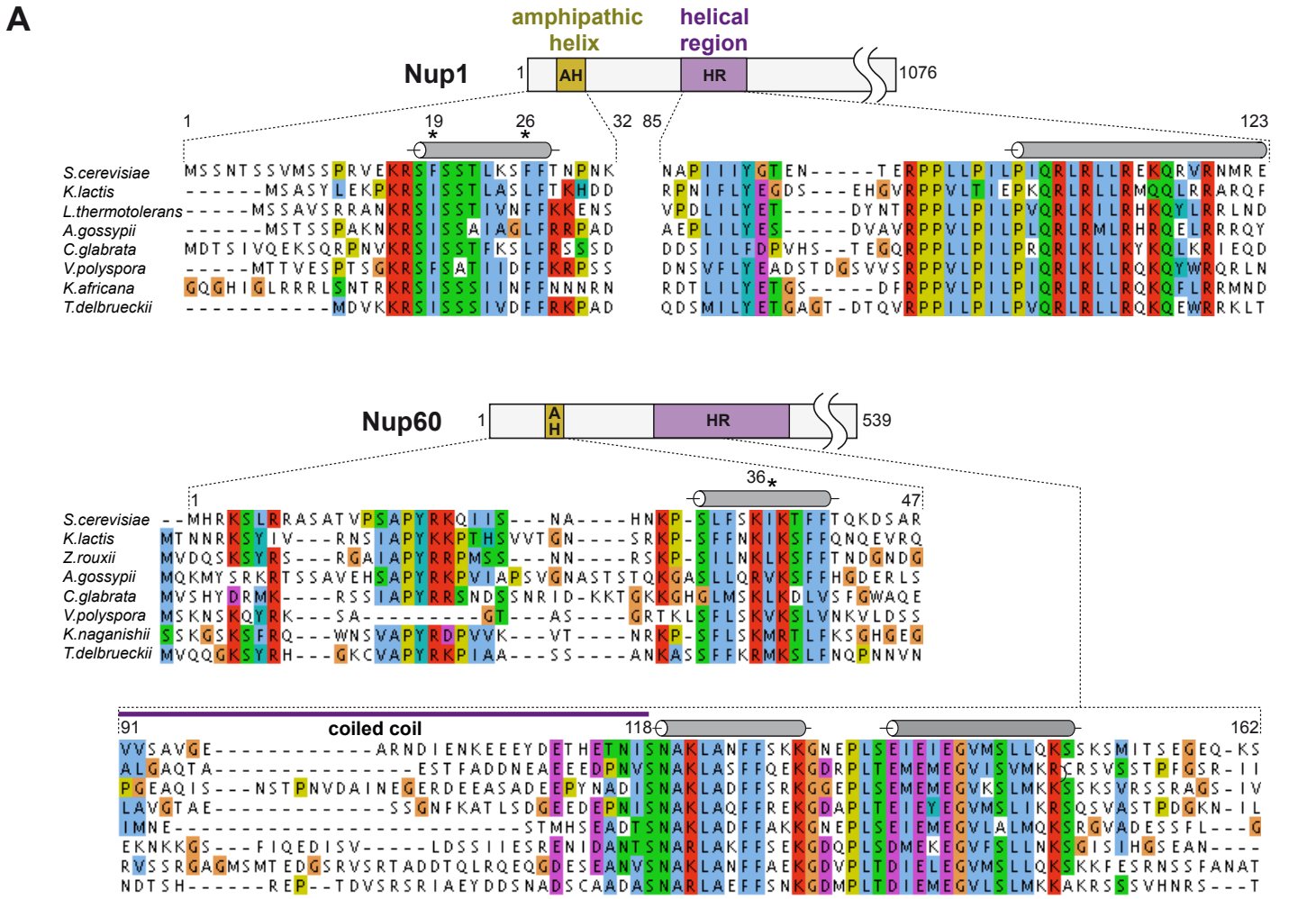
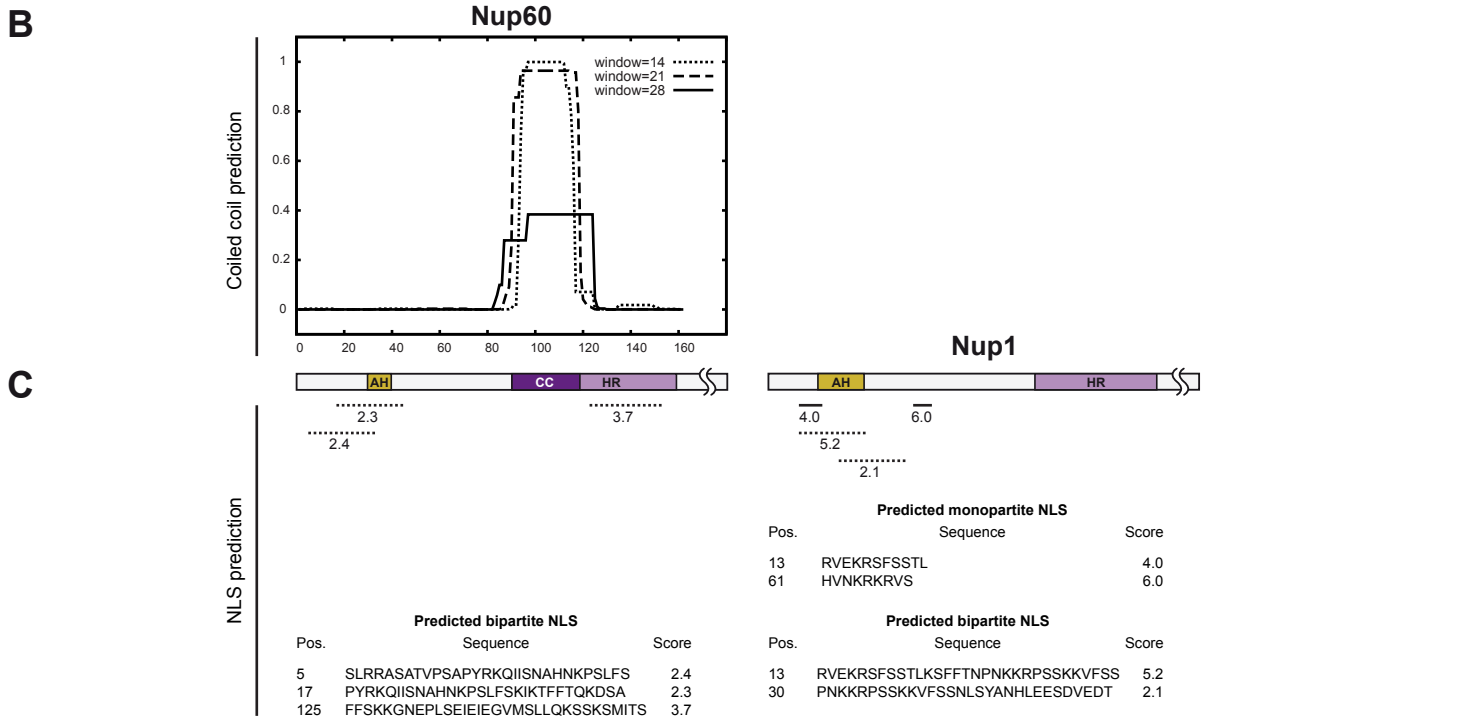

A
amphipathic helix
helical region
Nup1
AH
HR
1076
S.cerevisiae
K.lactis
L.thermotolerans
A.gossypii
C.glabrata
V.polyspora
K.africana
T.delbrueckii
Nup60
539
S.cerevisiae
K.lactis
Z.rouxii
A.gossypii
C.glabrata
V.polyspora
K.naganishii
T.delbrueckii
coiled coil


B
Nup60
Coiled coil prediction
window=14
window=21
window=28
C
Nup1
NLS prediction
Predicted bipartite NLS
Pos. Sequence Score
5 SLRRASATVPSAPYRKQIISNAHNKPSLFS 2.4
17 PYRKQIISNAHNKPSLFSKIKTFFTQKDSA 2.3
125 FFSKKGNEPLSEIEIEGVMSLLQKSSKSMITS 3.7
Predicted monopartite NLS
Pos. Sequence Score
13 RVEKRSFSSTL 4.0
61 HVNKRKRVS 6.0
Predicted bipartite NLS
Pos. Sequence Score
13 RVEKRSFSSTLKSFFTNPNKKRPSSKKVFSS 5.2
30 PNKKRPSSKKVFSSNLSYANHLEESDVEDT 2.1

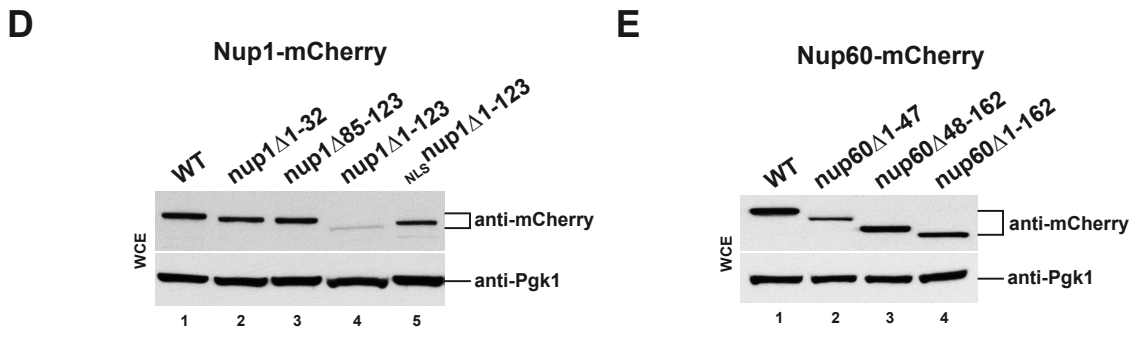
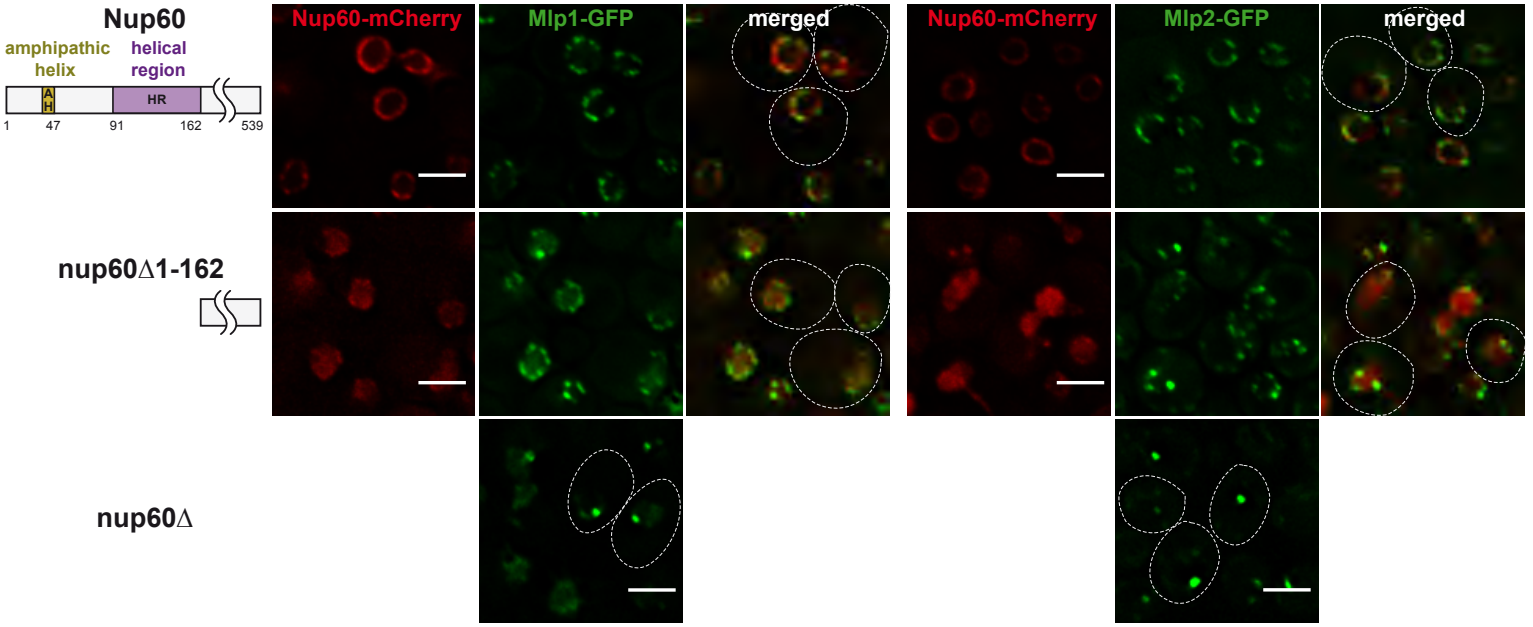

D
Nup1-mCherry
WT
nup1Δ1-32
nup1Δ85-123
nup1Δ1-123
nup1Δ1-123 NLS
WCE
anti-mCherry
anti-Pgk1
E
Nup60-mCherry
WT
nup60Δ1-47
nup60Δ48-162
nup60Δ1-162
WCE
anti-mCherry
anti-Pgk1

Figure S1

A



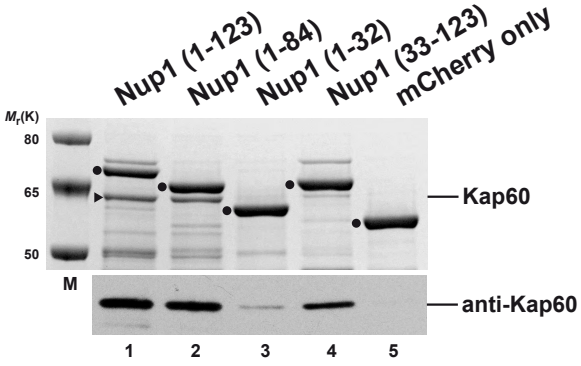
B

Mass Spectrometry Analysis
Nup1 (1-123)-TAP

	Protein	Unique Peptides	Total Peptides
I	**Nup170**	11	23
	Fas1	6	14
II	**Kap121**	36	78
	Nup1*	16	18
	Pfk1	7	14
	Kgd1	6	12
III	**Nup2**	65	162
	Nup1*	15	21
	Eft1	7	13
IV	**Kap95**	78	663
	Pdi1	8	19
	Air2	7	11
	Kre5	6	21
V	**Nup1***	27	48
	Nup85	11	29
	Nop2	6	11
	Pin4	6	14
	Smc5	5	19
VI	Kar2	77	484
	Gal10	11	30
	Spt8	9	17
	Trp5	7	20
	Act1	6	19
VII	Ssa1	80	780
	Ssb2	44	121
	Nup60	10	21
	Myo4	6	25
	Pab1	5	12
VIII	**Kap60**	38	385
	Gcd11	11	48
	Hem4	10	21
	Pdc1	8	19
	Pet111	7	22
	Ddr48	6	29
	Dal4	5	18
IX	**Nup1(1-123)-CBP**	15	39
	Lsm6	5	12

* Nup1 peptides that originate from the endogenous Nup1 protein. These contain additional peptide sequences beyond the last C-terminal residue of the bait.

C



D

Mass Spectrometry Analysis
Nup60 (1-162)-TAP

	Protein	Unique Peptides	Total Peptides
I	**Kap123**	68	805
	Ssa1	6	7
	Def1	5	12
II	Sse1	36	78
	Pin4	9	12
	Ski2	5	15
III	Ssc1	44	155
	Ssa1	41	158
	Ssa3	19	27
	Ssa4	6	18
	Ssa2	8	14
IV	Ssa1	51	503
	Ssa2	10	123
	Ssa4	7	88
	Ssb1	6	27
	Ssa3	5	49
V	**Nup60(1-162)-CBP**	78	663
VI	**Nup60(1-162)-CBP**	89	775
	Nop10	5	12
	Mam33	5	9

E

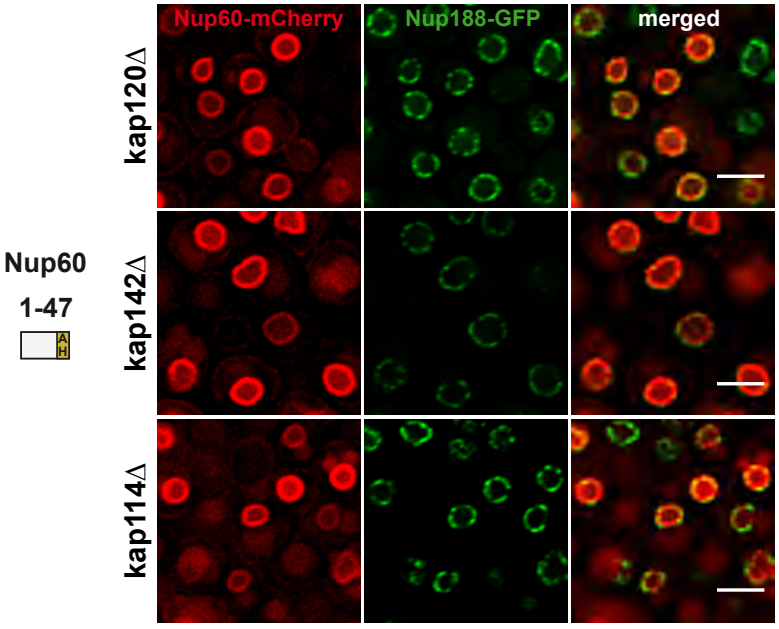
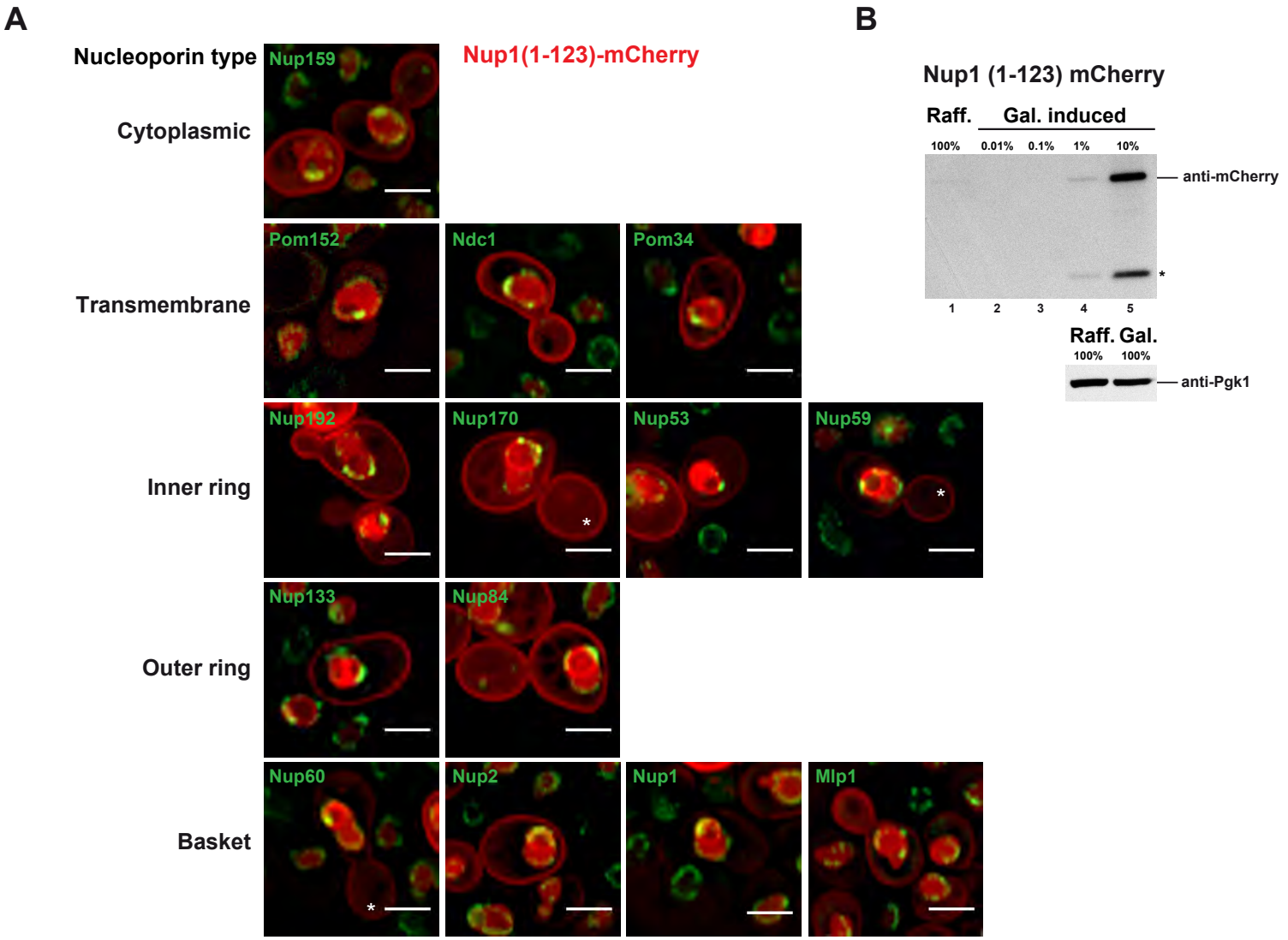
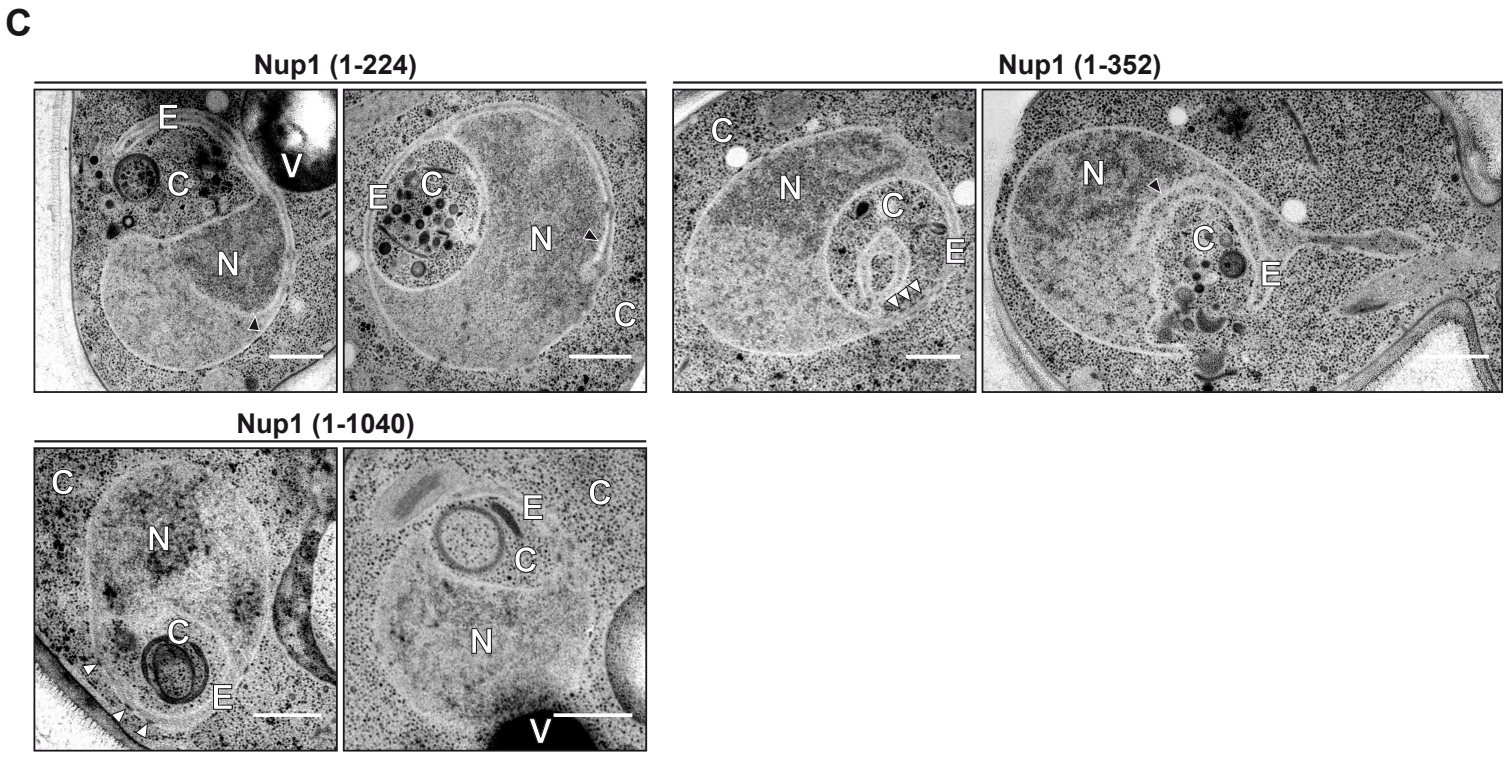


Figure S2


A
Nucleoporin type
Nup1(1-123)-mCherry
Cytoplasmic
Nup159
Transmembrane
Pom152
Ndc1
Pom34
Inner ring
Nup192
Nup170
Nup53
Nup59
Outer ring
Nup133
Nup84
Basket
Nup60
Nup2
Nup1
Mlp1
B
Nup1 (1-123) mCherry
Raff.
Gal. induced
100%
0.01%
0.1%
1%
10%
anti-mCherry
1
2
3
4
5
Raff. Gal.
100%
100%
anti-Pgk1


C
Nup1 (1-224)
Nup1 (1-352)
Nup1 (1-1040)
E
C
V
N

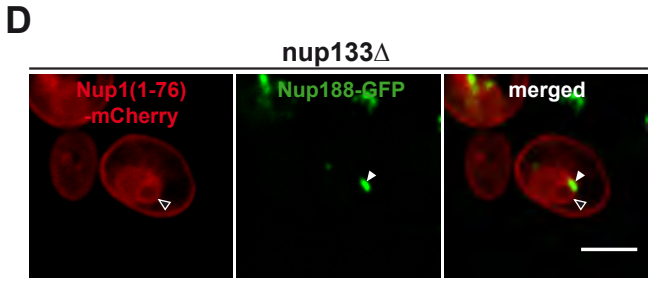

D
nup133Δ
Nup1(1-76)
-mCherry
Nup188-GFP
merged

Figure S3

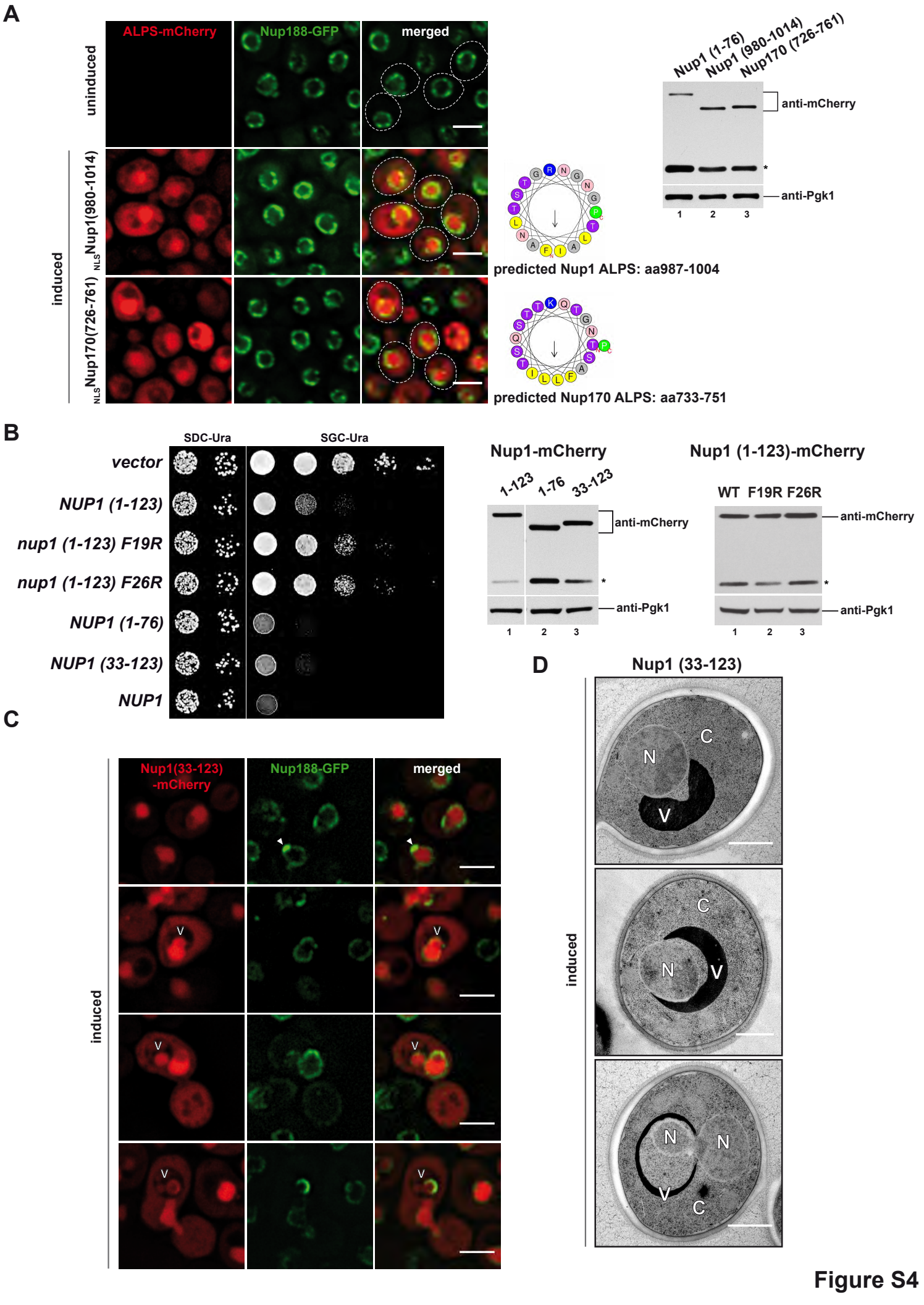

A
ALPS-mCherry
Nup188-GFP
merged
uninduced
induced
NLS Nup1(980-1014)
NLS Nup170(726-761)
predicted Nup1 ALPS: aa987-1004
predicted Nup170 ALPS: aa733-751
Nup1 (1-76)
Nup1 (980-1014)
Nup170 (726-761)
anti-mCherry
anti-Pgk1
B
SDC-Ura
SGC-Ura
vector
NUP1 (1-123)
nup1 (1-123) F19R
nup1 (1-123) F26R
NUP1 (1-76)
NUP1 (33-123)
NUP1
Nup1-mCherry
Nup1 (1-123)-mCherry
1-123
1-76
33-123
WT F19R F26R
C
Nup1(33-123) -mCherry
V
D
Nup1 (33-123)
N
C
V

Figure S4

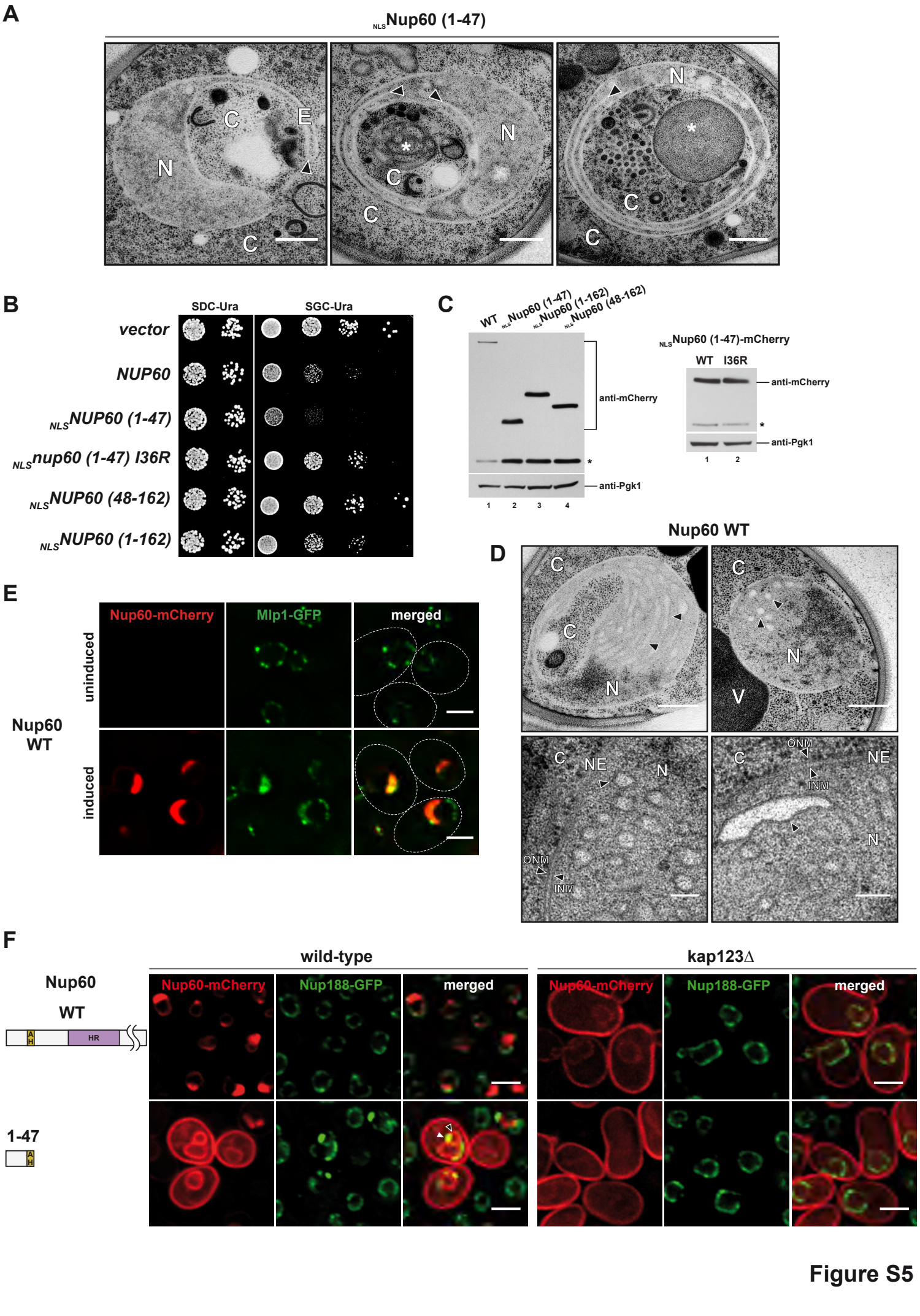
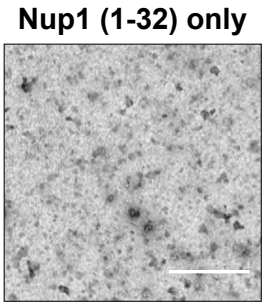
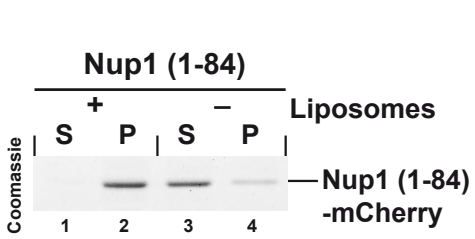

A
NLSNup60 (1-47)
C
E
N
C
N
C
C
N
C
C
B
SDC-Ura
SGC-Ura
vector
NUP60
NLSNUP60 (1-47)
NLSnup60 (1-47) I36R
NLSNUP60 (48-162)
NLSNUP60 (1-162)
C
WT
NLSNup60 (1-47)
NLSNup60 (1-162)
NLSNup60 (48-162)
anti-mCherry
anti-Pgk1
1 2 3 4
NLSNup60 (1-47)-mCherry
WT I36R
anti-mCherry
anti-Pgk1
1 2
D
Nup60 WT
C
C
N
C
N
V
C
NE
N
ONM
INM
C
ONM
NE
INM
N
E
Nup60-mCherry
Mlp1-GFP
merged
uninduced
induced
Nup60
WT
F
wild-type
kap123Δ
Nup60
WT
HR
1-47
Nup60-mCherry
Nup188-GFP
merged
Nup60-mCherry
Nup188-GFP
merged

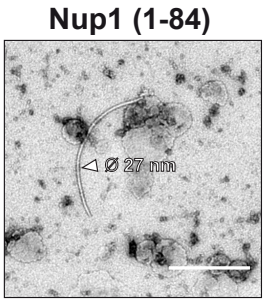
Figure S5

A

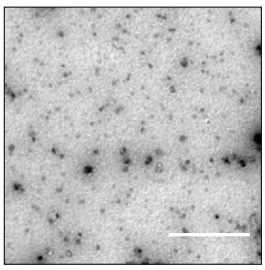


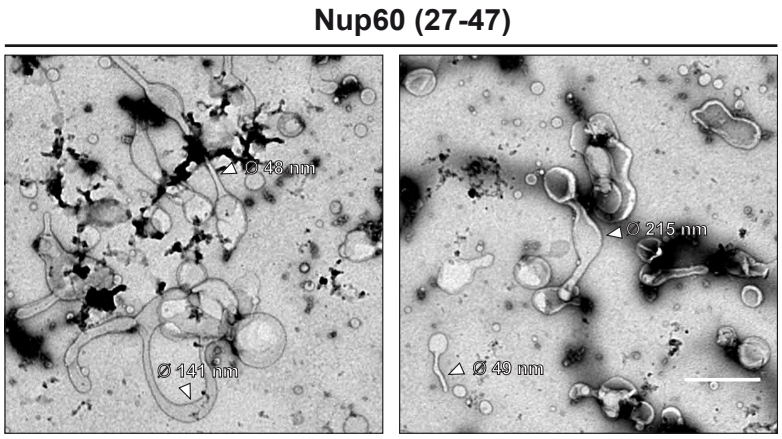
B





C





D

Extrusion filter diameter	Measured diameter (DLS)	% Polydispersity
30 nm	114.8	19.1
50 nm	115.8	12.1
100 nm	129.4	11.9
200 nm	174	14.9
400 nm	217.4	20.7

E

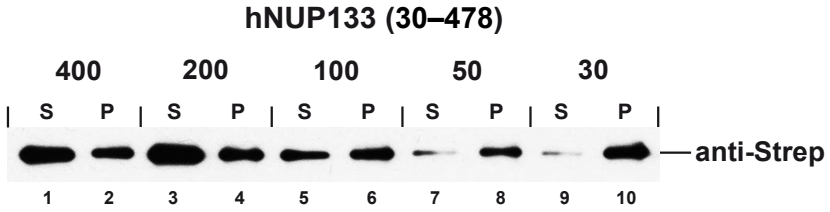
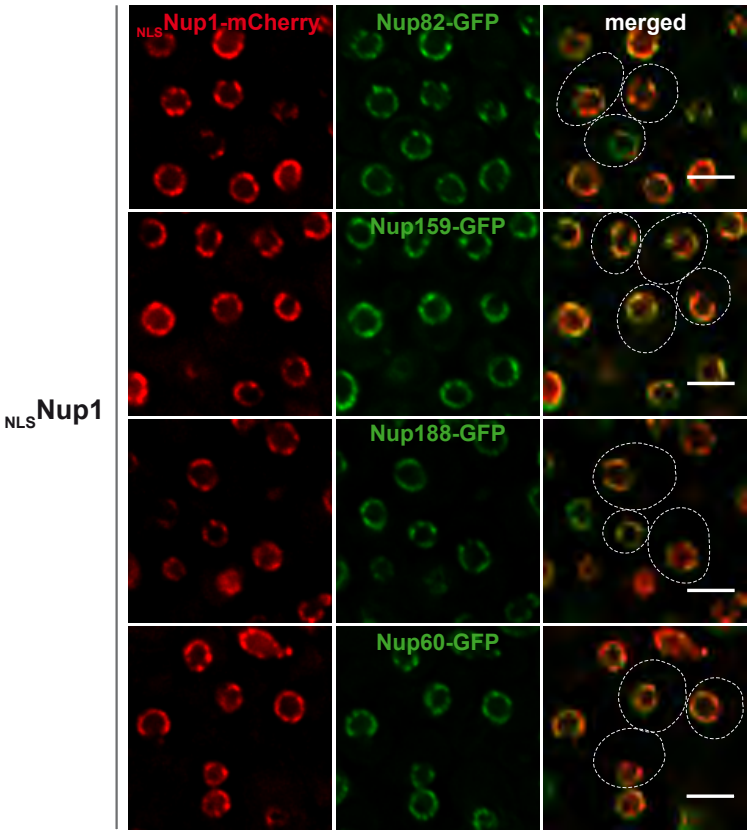


Figure S6

A


NLSNup1-mCherry
Nup82-GFP
merged
Nup159-GFP
Nup188-GFP
Nup60-GFP
NLSNup1

B

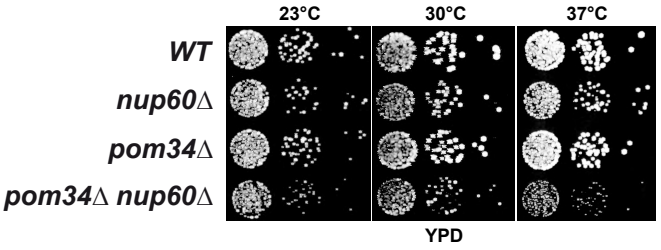

23°C
30°C
37°C
WT
nup60Δ
pom34Δ
pom34Δ nup60Δ
YPD

Figure S7

Supplemental Information

Figure S1-7

Supplemental Figure Legends

Supplemental Experimental Procedures including Yeast strains and Plasmids

Supplemental References

Figure S1, Related to Figure 1

(A) Sequence alignments of Nup1 and Nup60 orthologs across representative fungal species were performed with Clustal Omega and colored by JalView. Predicted α-helices (JPred) are grey, coiled-coil (CC) is dark purple. Numbers denote residue positions. Asterisks indicate mutated residues (**Figure 3C** and **4A**).

(B) Prediction of coiled coil regions for Nup60 N-terminus (aa1-162) was carried out with COILS (http://embnet.vital-it.ch/software/COILS_form.html) using default settings. The predictions using three possible window sizes (14, 21 and 28 residues) are shown.

(C) Prediction of importin α-dependent nuclear localization signals for the N-termini of Nup1 (1-123) and Nup60 (1-162) was performed with cNLS Mapper (http://nls-mapper.iab.keio.ac.jp/cgi-bin/NLS_Mapper_form.cgi). The positions of predicted monopartite (solid line) and bipartite (dashed line) NLSs in the protein sequences are indicated together with scores. Predicted NLS strength ranges from 2 to 10.

(D) and **(E)** Protein levels of the indicated Nup1 and Nup60 mCherry-tagged proteins, which were expressed from plasmids under the control of their endogenous promoter in the respective deletion strain. Denaturing extracts were prepared and immunoblotted with the indicated antibodies. Pgk1 (3-phosphoglycerate kinase) is used as a loading control.

Figure S2, Related to Figure 2

(A) Fluorescence microscopy of *nup60*Δ cells containing wild-type, truncated *NUP60-mCherry* (endogenous promoter) or no plasmid in strains with genomically GFP-tagged Mlp1 or Mlp2. Scale bar: 3 µm.

(B) Details of mass spectrometry analysis of a Nup1 (1-123)-TAP purification. Proteins with fewer than 5 unique peptides are excluded from the list. Roman numerals indicate cut out band from gel.

(C) Kap60 and the indicated GST-Nup1 constructs were co-expressed in *E.coli* and subjected to GST-affinity purifications. Kap60 directly interacts with the Nup1 N-terminus. Kap60 binding was reduced when the Nup1 AH was deleted (lane 4). Conversely, weak Kap60 binding to the AH alone was detected (lane 3). Kap60 binding may be a composite function of several predicted NLS sequences in the Nup1 N-terminus (**Figure S1C**). Proteins were eluted with GSH and analyzed by SDS-PAGE and Coomassie staining. Filled circles indicate bait proteins, arrow indicates Kap60 as identified by mass spectrometry. The same eluates were immunoblotted using an anti-Kap60 antibody.

(D) Details of mass spectrometry analysis of a Nup60 (1-162)-TAP purification as in **(B)**.

(E) Fluorescence microscopy of live *kap120*Δ*, kap142*Δ *and kap114*Δ cells expressing genomically tagged Nup188-GFP and plasmid-borne Nup60 (1-47)-mCherry (*GAL* promoter) grown in raffinose media. Scale bar: 3 µm.

Figure S3, Related to Figure 3

(A) Fluorescent live imaging of cells expressing representative GFP-tagged Nups from different parts of the NPC and plasmid-borne, overproduced Nup1 (1-123)-mCherry (4hrs of galactose induction). Note that cells are enlarged and frequently contain large empty buds (asterisks) suggesting mitotic defects. Scale bar: 2 µm.

(B) Quantitative Western blotting shows expression levels of Nup1 (1-123)-mCherry cells grown in raffinose (uninduced) and an at least 100-fold increase of protein levels upon 4hrs of galactose induction. Asterisk indicates degradation band. Pgk1; loading control.

(C) Gallery of TEM images of cells overproducing the indicated Nup1-mCherry fragments (4hrs of induction). As in Nup1 (1-123) overexpressing cells, distinct expansion membranes, engulfment of cytoplasmic material, intranuclear membranes (black arrows) and NPC clusters (white arrows) were observed. The Nup1 (1-352) expressing cell on the right undergoes cell

division. Scale bars: 500 nm. N, nucleus; C, cytoplasm; E, expansion membrane; V, vacuole.

(D) Overexpression of the Nup1 (1-76)-mCherry fragment (4hrs of induction) in *nup133*Δ *NUP188-GFP* cells. Black arrows indicate expansion membrane, white arrows label NPC clusters. Scale bar: 2 µm.

Figure S4, Related to Figure 3

(A) Fluorescent imaging of cells expressing Nup188-GFP and plasmid-based ALPS-motifs of Nup1 and Nup170 fused to mCherry and tagged with an SV40 NLS (*GAL* promoter). Induced refers to 4hrs of protein overproduction in galactose-containing medium. Helical wheel projections of the ALPS motifs are depicted. Protein levels of the overexpressed ALPS constructs were compared to the overexpressed Nup1 (1-76)-mCherry fragment (4hrs of induction). Denaturing extracts were prepared and immunoblotted with the indicated antibodies. Pgk1; loading control. Scale bar: 3 µm.

(B) The indicated Nup1-mCherry overexpression plasmids were transformed into wild-type yeast cells. Cells were spotted in 10-fold serial dilutions and growth at 30°C was followed on SDC-Ura (repressed; loading control) and on SGC-Ura (galactose induced) plates. Protein levels of the indicated constructs were measured after 4hrs of galactose induction. Whole cell extracts were prepared and immunoblotted with the indicated antibodies (all lanes are derived from the same blot membrane). Pgk1; loading control. Asterisks mark degradation products. Note that Nup1 (1-76) was the most highly expressed (also higher level of degradation product) and toxic AH construct. Overexpressing the HR construct (Nup1 aa33-123) or full-length Nup1 was also highly toxic.

(C) Gallery of cells expressing Nup188-GFP and plasmid-borne Nup1 (33-123)-mCherry (*GAL* promoter) after 4hrs of galactose induction. Note the close proximity of nuclei and vacuoles and encapsulation of nuclear material in vacuoles. This phenotype was most often seen in cells with the highest Nup1 (33-123)-mCherry levels. White arrow points to NPC cluster. V; vacuole. Scale bar: 2 µm.

(D) TEM images of cells overexpressing the toxic Nup1 (33-123)-mCherry protein after 4hrs of galactose induction. Different hypothetical stages of a

“nucleophagy-like” phenotype are shown. Scale bar: 1 µm. N, nucleus; C, cytoplasm; V, vacuole.

Figure S5, Related to Figure 4

(A) TEM images showing different stages of NE deformation/proliferation upon overexpression of the Nup60 AH ($_{NLS}$Nup60 aa1-47-mCherry; *GAL* promoter) captured in a population of exponentially growing cells that was induced with galactose for 4hrs. Left: focal NE expansion engulfing part of the cytoplasm. Black arrow points to the highly curved leading edge of the expansion membrane. Middle and right: entrapment of cytoplasmic material and emergence of additional membrane sheets (black arrows). Asterisks mark cytoplasmic membrane structures that have undergone significant morphology changes after intranuclear engulfment. Scale bar: 500 nm. N, nucleus; C, cytoplasm; E, expansion membrane.

(B) Growth assay showing toxicity of Nup60 overexpression. Wild-type yeast cells were transformed with empty plasmid (vector) or plasmids for inducible expression of Nup60 and its fragments (*GAL* promoter). Cells were grown on SDC-Ura (repressed; loading control) or SGC-Ura (galactose induced) at 30 °C for 2 and 3 days, respectively. The Nup60 AH construct ($_{NLS}$Nup60 aa1-47), which produced a NE expansion membrane, was the most toxic. Intranuclear membrane tubules induced by $_{NLS}$Nup60 (1-162) had a milder phenotype.

(C) Protein levels of the indicated constructs were measured after 4hrs of galactose induction. Denaturing extracts were prepared and immunoblotted with the indicated antibodies. Asterisks mark degradation products. Pgk1; loading control.

(D) TEM images of cells overproducing wild-type Nup60 fused to mCherry (*GAL* promoter; 4hrs of induction). Upper images display longitudinal (left) and cross-sections (right) of intranuclear tubules. Note engulfment of cytoplasmic material. Lower images show higher magnifications of single-layered tubules and more irregular sac-like structures (black arrows). EM protocol was optimized for membrane staining. Scale bars: 500 nm (upper images), 100 nm (lower images). N, nucleus; C, cytoplasm; NE, nuclear envelope; V, vacuole; INM, inner nuclear membrane; ONM, outer nuclear membrane.

(E) Live cell imaging of cells expressing Mlp1-GFP and plasmid-borne wild-type Nup60-mCherry (*GAL* promoter). Cells were grown in raffinose- (uninduced) or galactose-containing medium (induced for 4hrs). Mlp1 and Nup60 co-cluster in crescent-shaped foci, which likely correspond to the membrane tubules shown in (**D**). Scale bar: 2 µm.

(F) Fluorescence microscopy of wild-type or *kap123Δ* cells expressing genomically tagged Nup188-GFP and overproducing the indicated Nup60-mCherry constructs (*GAL* promoter; 4hrs of galactose induction). Kap123 deletion abolishes nuclear import, NE deformation and NPC clustering by the Nup60 (1-47) fragment. Similarly, full-length Nup60 no longer accumulates into crescent-shaped nuclear foci and is mistargeted to the PM instead. Note a residual faint NE labeling in *kap123*Δ cells expressing Nup60-mCherry. This suggests that other karyopherins also contribute to the import of full-length Nup60, while the Nup60 AH strictly depends on Kap123. White arrow points to NPC cluster, black arrow marks expansion membrane. Scale bar: 3 µm.

Figure S6, Related to Figure 5

(A) Electron microscopy image of negatively stained Strep-Nup1 (1-32)-mCherry (recombinant protein only; final concentration: 2.5 µM). Scale bar: 500 nm.

(B) *In vitro* liposome binding and deformation assays for recombinant Strep-Nup1 (1-84)-mCherry. Note that the protein binds tightly to liposomes but also has a tendency to aggregate in the absence of liposomes (lane 4). Protein-liposome mixtures were further analyzed by negative stain EM. The outer tubule diameter is indicated. Scale bar: 500 nm.

(C) Negatively stained His-Nup60 (27-47)-mCherry protein only control or when mixed with liposomes (final protein concentration: 2.5 µM). Images show large-scale liposome deformation that is sometimes observed besides the thin tubules. Diameters of representative structures are indicated. Scale bar: 500 nm.

(D) Average liposome diameters measured by dynamic light scattering. Liposomes were prepared by extrusion through polycarbonate membranes of defined pore size. The heterogeneity of the sample is indicated by the polydispersity value. Consistent with (Nath, Dancourt et al. 2014), the actual

distribution of liposome sizes is narrower than the range of filter pore sizes. Liposomes extruded through larger pores typically contain many smaller liposomes.

(E) Liposome co-sedimentation assay of human NUP133. Recombinant Strep-NUP133 (30-478) was incubated with liposomes of different sizes (diameter: 400, 200, 100, 50, 30 nm), pelleted and analyzed by immunoblotting against the Strep-tag. hNUP133 preferentially binds to liposomes with a smaller diameter (compare lanes 1/2 to 9/10).

Figure S7, Related to Figures 6 and 7

(A) Live cell imaging of *nup1Δ* cells expressing representative GFP-tagged Nups from different parts of the NPC and plasmid-borne $_{NLS}$Nup1-mCherry. Appending an SV40 NLS to full-length Nup1 does not result in comparable Nup82/Nup159 mislocalization phenotypes. Scale bar: 3 µm.

(B) Growth analysis of the indicated strains reveals no major synthetic interaction between *nup60*Δ and *pom34*Δ at 30°C consistent with (Miao et al., 2006). A mild synthetic effect is seen at 37°C. Cell density was normalized, and cells were spotted onto plates in 10-fold serial dilutions. Plates were incubated for 2-3 days at the indicated temperatures.

Supplemental Experimental Procedures

Electron microscopy

Yeast cells were mixed 1:1 with 8% BSA, pelleted, and immediately frozen in a Wohlwend HPF Compact 01 high-pressure freezer. Samples were subsequently transferred into a Leica EM AFS2 freeze substitution unit and substituted for 4 days in a medium of anhydrous acetone containing 2% osmium tetroxide, 0.25% uranyl acetate and alternatively 2% water (for better preservation of membrane structures). The substituted specimens were embedded in Agar 100 resin. Ultra-thin sections were cut at a nominal thickness of 70 nm and post-stained with 2% aqueous uranyl acetate followed by Reynold's lead citrate.

Recombinant protein expression and purification

Strep-tagged yeast Nup1 was affinity-purified with a 5 ml *Strep-Tactin*® Superflow column (buffer: 300 mM NaCl, 50 mM Tris-HCl pH 7.5, 0.5 mM DTT). Proteins were eluted in 2.5 mM desthiobiotin, 150 mM NaCl, 50 mM Tris-HCl pH 7.5, 0.5 mM DTT. His-tagged yeast Nup60 was purified by Ni^{2+}-affinity purification and eluted by imidazole (wash buffer: 300-600 mM NaCl, 50 mM Tris-HCl pH 7.5, 50 mM imidazole). GST-tagged versions of Nup1 were co-expressed with untagged Kap60p and purified by glutathion (GSH) affinity purification (buffer: 200 mM NaCl, 50 mM Tris-HCl pH 7.5, 0.5 mM DTT, 0.15% NP-40).

TAP Purification and Mass Spectrometry

Tandem Affinity Purifications from yeast were performed according to a published procedure (Rigaut et al., 1999). Cells were grown in selective SRC medium and induced with galactose for 4 hrs (see GAL induction). Coomassie-bands were cut individually from the gel and analyzed by microcapillary LC-MS/MS techniques on Orbitrap mass spectrometers (Thermo Scientific).

Yeast strains

Name	Genotype	Reference
BY4741	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*	Euroscarf
nup60Δ	*Mat a; his3Δ1; leu2Δ0; met15k0; ura3Δ0; nup60Δ::kanMX4*	Euroscarf
nup60Δ MLP1-GFP	*Mat a; his3Δ1; leu2Δ0; met15k0; ura3Δ0; nup60Δ::kanMX4 MLP1-GFP::natNT2*	This study
nup60Δ MLP2-GFP	*Mat a; his3Δ1; leu2Δ0; met15k0; ura3Δ0; nup60Δ::kanMX4 MLP2-GFP::natNT2*	This study
nup60Δ NUP188-GFP	*Mat a; his3Δ1; leu2Δ0; met15k0; ura3Δ0; nup60Δ::kanMX4 NUP188-GFP::natNT2*	This study
nup1Δ[#] (created by sporulation and tetrad dissection of BY4743-NUP1/nup1Δ diploid strain)	*Mat α; his3Δ1; leu2Δ0; ura3Δ0; nup1Δ::kanMX4*	This study
nup1Δ[#] NUP60-GFP	*Mat α; his3Δ1; leu2Δ0; ura3Δ0; nup1Δ::kanMX4 NUP60-GFP::natNT2*	This study
nup1Δ[#] NUP159-GFP	*Mat α; his3Δ1; leu2Δ0; ura3Δ0; nup1Δ::kanMX4 NUP159-GFP::natNT2*	This study
nup1Δ[#] NUP188-GFP	*Mat α; his3Δ1; leu2Δ0; ura3Δ0; nup1Δ::kanMX4 NUP188-GFP::natNT2*	This study
nup1Δ[#] NUP82-GFP	*Mat α; his3Δ1; leu2Δ0; ura3Δ0; nup1Δ::kanMX4 NUP82-GFP::natNT2*	This study
nup1Δ[#] nup53Δ	*Mat α; his3Δ1; leu2Δ0; ura3Δ0; nup1Δ::kanMX4 nup53Δ::natNT2*	This study
nup1Δ[#] nup60Δ	*Mat α; his3Δ1; leu2Δ0; ura3Δ0; nup1Δ::kanMX4 nup60Δ::natNT2*	This study
nup1Δ[#] pom34Δ	*Mat α; his3Δ1; leu2Δ0; ura3Δ0; nup1Δ::kanMX4 pom34Δ::natNT2*	This study
nup1Δ[#] pom152Δ	*Mat α; his3Δ1; leu2Δ0; ura3Δ0; nup1Δ::kanMX4 pom152Δ:: natNT2*	This study

nup1Δ[#] mlp1Δ	*Mat α; his3Δ1; leu2Δ0; ura3Δ0; nup1Δ::kanMX4 mlp1Δ:: natNT2*	This study
nup1Δ[#] nup60Δ1-47 pom152Δ	*Mat α; his3Δ1; leu2Δ0; ura3Δ0; nup1Δ::kanMX4 nup60Δ::natNT2 pom152Δ::hphNT1 pRS315-nup60Δ1-47-mCherry*	This study
nup1Δ[#] nup60Δ1-47 pom34Δ	*Mat α; his3Δ1; leu2Δ0; ura3Δ0; nup1Δ::kanMX4 nup60Δ::natNT2 pom34Δ::hphNT1 pRS315-nup60Δ1-47-mCherry*	This study
nup1Δ[#] nup60Δ1-47 pom33Δ	*Mat α; his3Δ1; leu2Δ0; ura3Δ0; nup1Δ::kanMX4 nup60Δ::natNT2 pom33Δ::hphNT1 pRS315-nup60Δ1-47-mCherry*	This study
nup1Δ[#] nup60Δ1-47 nup53Δ	*Mat α; his3Δ1; leu2Δ0; ura3Δ0; nup1Δ::kanMX4 nup60Δ::natNT2 nup53Δ::hphNT1 pRS315-nup60Δ1-47-mCherry*	This study
kap123Δ NUP188-GFP	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; kap123Δ::kanMX4 NUP188-GFP::natNT2*	This study
kap120Δ NUP188-GFP	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; kap120Δ::kanMX4 NUP188-GFP::natNT2*	This study
kap142Δ NUP188-GFP	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; kap142Δ::kanMX4 NUP188-GFP::natNT2*	This study
kap114Δ NUP188-GFP	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; kap114Δ::kanMX4 NUP188-GFP::natNT2*	This study
NUP159-GFP	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; NUP159-GFP::HIS3MX6*	(Huh, Falvo et al. 2003)
POM152-GFP	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; POM152-GFP::HIS3MX6*	(Huh, Falvo et al. 2003)
POM34-GFP	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; POM34-GFP::HIS3MX6*	(Huh, Falvo et al. 2003)

NUP192-GFP	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; NUP192-GFP::HIS3MX6*	(Huh, Falvo et al. 2003)
NUP188-GFP	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; NUP188-GFP::HIS3MX6*	(Huh, Falvo et al. 2003)
NUP170-GFP	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; NUP170-GFP::HIS3MX6*	(Huh, Falvo et al. 2003)
NUP53-GFP	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; NUP53-GFP::HIS3MX6*	(Huh, Falvo et al. 2003)
NUP59-GFP	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; NUP59-GFP::HIS3MX6*	(Huh, Falvo et al. 2003)
NUP133-GFP	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; NUP133-GFP::HIS3MX6*	(Huh, Falvo et al. 2003)
NUP84-GFP	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; NUP84-GFP::HIS3MX6*	(Huh, Falvo et al. 2003)
NUP60-GFP	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; NUP60-GFP::HIS3MX6*	(Huh, Falvo et al. 2003)
NUP2-GFP	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; NUP2-GFP::HIS3MX6*	(Huh, Falvo et al. 2003)
NUP1-GFP	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; NUP1-GFP::HIS3MX6*	(Huh, Falvo et al. 2003)
MLP1-GFP	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; MLP1-GFP::HIS3MX6*	(Huh, Falvo et al. 2003)
SEC63-GFP	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; SEC63-GFP::HIS3MX6*	(Huh, Falvo et al. 2003)
NUP188-GFP nup133Δ	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; NUP188-GFP::HIS3MX6 nup133Δ:: natNT2*	This study
pom34Δ	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; pom34::kanMX4*	Euroscarf
nup60Δ pom34Δ	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; nup60Δ::kanMX4 pom34Δ::natNT2*	This study

[#] strains contain a pRS316-NUP1 cover plasmid

Plasmids

Name	Description	Reference
Yeast plasmids based on the pRS31X series	*ARS/CEN*	(Sikorski and Hieter 1989)
pRS316-NUP1	NUP1 promoter	This study
pRS313-NUP1-mCherry	NUP1 promoter	This study
pRS313-$_{NLS}$NUP1-mCherry	NUP1 promoter SV40 NLS	This study
pRS313-NUP1Δ1-32-mCherry	NUP1 promoter	This study
pRS313-NUP1Δ85-123-mCherry	NUP1 promoter	This study
pRS313-NUP1Δ1-123-mCherry	NUP1 promoter	This study
pRS313-$_{NLS}$NUP1Δ1-123-mCherry	NUP1 promoter SV40 NLS	This study
pRS313-pGAL1-NUP1-mCherry	GAL1 promoter	This study
pRS313-pGAL1-NUP1 (1-32)-mCherry	GAL1 promoter	This study
pRS313-pGAL1-NUP1 (1-76)-mCherry	GAL1 promoter	This study
pRS313-pGAL1-NUP1 (1-123)-mCherry	GAL1 promoter	This study
pRS313-pGAL1-NUP1 (33-123)-mCherry	GAL1 promoter	This study
pRS313-pGAL1-NUP1 (1-224)-mCherry	GAL1 promoter	This study
pRS313-pGAL1-NUP1 (1-352)-mCherry	GAL1 promoter	This study
pRS313-pGAL1-NUP1 (1-1040)-mCherry	GAL1 promoter	This study
pRS316-pGAL1-NUP1 (1-32)-mCherry	GAL1 promoter	This study
pRS316-pGAL1-NUP1 (1-76)-mCherry	GAL1 promoter	This study
pRS316-pGAL1-NUP1 (1-123)-mCherry	GAL1 promoter	This study
pRS316-pGAL1-NUP1 (1-123) F19R-mCherry	GAL1 promoter	This study
pRS316- pGAL1-NUP1 (1-123) F26R-mCherry	GAL1 promoter	This study
pRS316-pGAL1-NUP1 (33-123)-mCherry	GAL1 promoter	This study
pRS316- pGAL1-$_{NLS}$NUP1 (980-1014)-mCherry	GAL1 promoter SV40 NLS	This study
pRS315-pGAL1-NUP1 (1-123)-TAP	GAL1 promoter	This study
pRS316-pGAL1-$_{NLS}$NUP170 (726-761)-mCherry	GAL1 promoter SV40 NLS	This study
pRS313-pGAL1-NUP60-mCherry	GAL1 promoter	This study
pRS313-pGAL1-$_{NLS}$NUP60 (1-47)-mCherry	GAL1 promoter SV40 NLS	This study
pRS313-pGAL1-$_{NLS}$NUP60 (1-162)-mCherry	GAL1 promoter SV40 NLS	This study
pRS313-pGAL1-$_{NLS}$NUP60 (48-162)-mCherry	GAL1 promoter SV40 NLS	This study
pRS316-pGAL1-NUP60-mCherry	GAL1 promoter	This study
pRS316-pGAL1-NUP60 (1-47)-mCherry	GAL1 promoter	This study
pRS316-pGAL1-$_{NLS}$NUP60 (1-47)-mCherry	GAL1 promoter SV40 NLS	This study
pRS316-pGAL1-$_{NLS}$NUP60 (1-47) I36R-mCherry	GAL1 promoter SV40 NLS	This study

pRS316-pGAL1-NUP60 (1-162)-mCherry	GAL1 promoter	This study
pRS316-pGAL1-$_{NLS}$NUP60 (1-162)-mCherry	GAL1 promoter SV40 NLS	This study
pRS316-pGAL1-NUP60 (48-162)-mCherry	GAL1 promoter	This study
pRS316-pGAL1-$_{NLS}$NUP60 (48-162)-mCherry	GAL1 promoter SV40 NLS	This study
pRS315-NUP60-mCherry	NUP60 promoter	This study
pRS315-NUP60Δ1-47-mCherry	NUP60 promoter	This study
pRS315-NUP60Δ1-162-mCherry	NUP60 promoter	This study
pRS315-NUP60Δ48-162-mCherry	NUP60 promoter	This study
pRS315-pGAL1-NUP60 (1-162)-TAP	GAL1 promoter	This study
pET24d-Strep-NUP1 (1-32)-mCherry	kanamycin	This study
pET24d-Strep-NUP1 (1-32) F19R-mCherry	kanamycin	This study
pET24d-Strep-NUP1 (1-32) F26R-mCherry	kanamycin	This study
pET24d-Strep-NUP1 (1-84)-mCherry	kanamycin	This study
pET24d-GST-TEV-NUP1 (1-32)-mCherry-FLAG	kanamycin	This study
pET24d-GST-TEV-NUP1 (1-84)-mCherry-FLAG	kanamycin	This study
pET24d-GST-TEV-NUP1 (1-123)-mCherry-FLAG	kanamycin	This study
pET24d-GST-TEV-NUP1 (33-123)-mCherry-FLAG	kanamycin	This study
pET24d-GST-TEV-mCherry-FLAG	kanamycin	This study
pET24d-Strep-mCherry	kanamycin	This study
pPROEX-Htb-NUP60 (27-47)-mCherry	ampicilin	This study
pPROEX-Htb-NUP60 (27-47) I36R-mCherry	ampicilin	This study
pST50Trc3-KAP60	ampicillin	This study
pET24d-Strep-hNUP133 (30-478)	kanamycin	This study
pFA6a::natNT2		(Janke, Magiera et al. 2004)
pFA6a::hphNT1		(Janke, Magiera et al. 2004)
pFA6a-GFP::natNT2		(Kressler, Roser et al. 2008)

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