Stelter et al., http://www.jcb.org/cgi/content/full/jcb.201105042/DC1

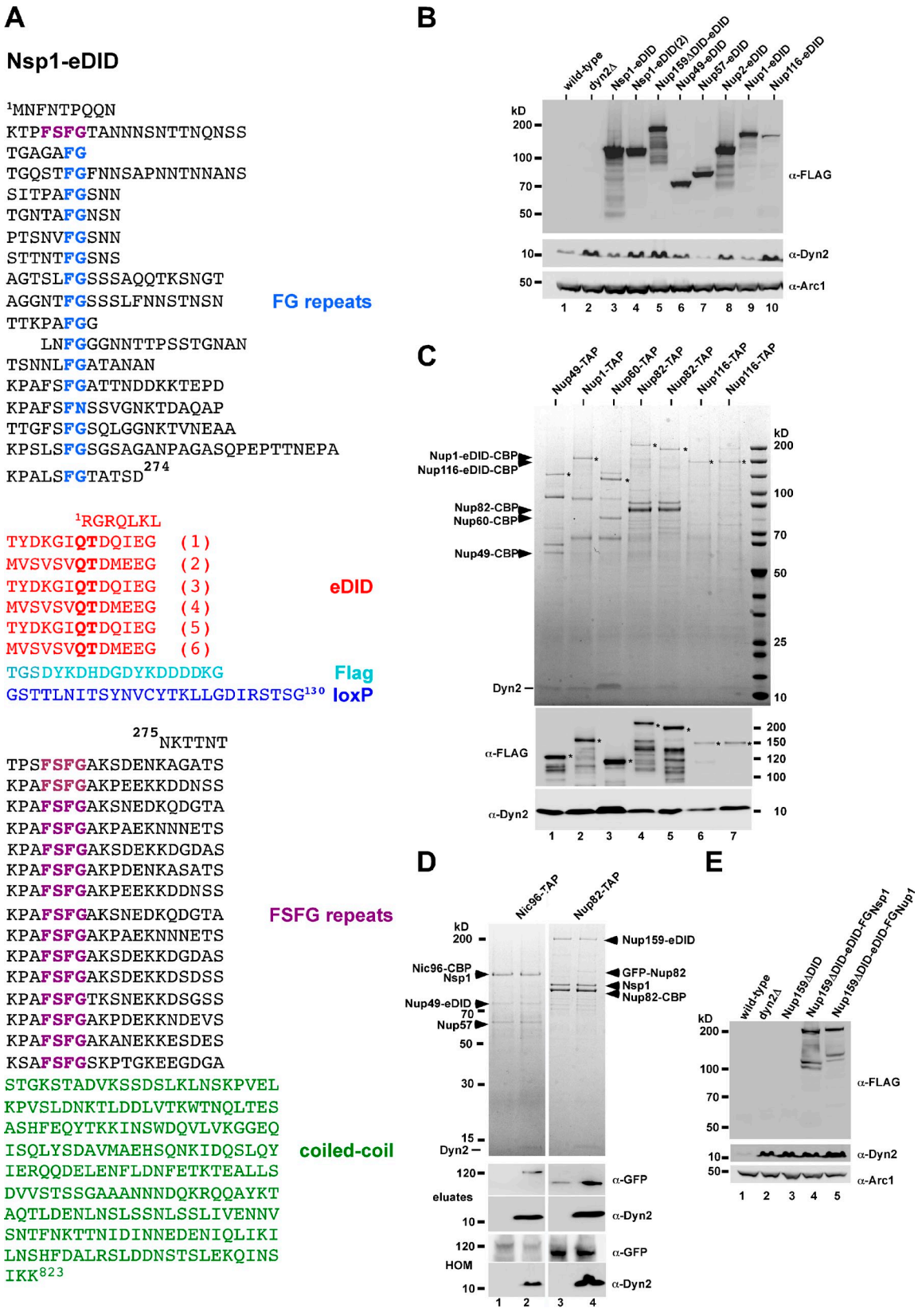

Figure S1. **Dyn2 recruitment to eDID-labeled FG domain Nups.** (A) Schematic drawing of a Nsp1-eDID construct. Insertion of engineered dynein light chain–interacting domain (eDID) followed by the Flag epitope (tag) and a LoxP site (for recombination) into the Nsp1 FG repeat nucleoporin. eDID is composed of six dynein light chain QT binding motifs in a row derived from the dynein intermediate chain Pac11(previously called DID1; Flemming et al., 2010). The amino acid position within the 823-residue-long Nsp1 is indicated, at which eDID, Flag, and loxP were inserted. The different domains of Nsp1 are further indicated. (B) Expression of the Nup-eDID constructs and Dyn2 (*pGAL-DYN2*) after 3-h galactose induction. Whole-cell lysates were separated by SDS-PAGE, blotted onto nitrocellulose, and incubated with anti-Flag, anti-Dyn2, and anti-Arc1 (loading control) antibodies. Wild-type cells revealing endogenous Dyn2 expression (lane 1), *dyn2Δ* cells + *pGAL-DYN2* (lane 2), *NSP1-eDID* + *pGAL-DYN2* (lane 3), *NSP1-eDID*(2) + *pGAL-DYN2* (lane 4), *NUP159ΔDID-eDID* + *pGAL-DYN2* (lane 5), *NUP49-eDID* + *pGAL-DYN2* (lane 6), *NUP57-eDID* + *pGAL-DYN2* (lane 7), *NUP2-eDID*+ *pGAL-DYN2* (lane 8), *NUP1-eDID* + *pGAL-DYN2* (lane 9), and *NUP116-eDID* + *pGAL-DYN2* (lane 10). (C) Coenrichment of Dyn2 with the indicated Nup-eDID constructs. *NUP-eDID dyn2Δ* strains expressing *pGAL-DYN2* were lysed after 3-h galactose induction before the indicated TAP constructs were affinity purified. *NUP49*-TAP expressing *NSP1-eDID* (lane 1) and *NUP1-eDID*-TAP (lane 2), *NUP60*-TAP expressing *NUP2-eDID* (lane 3), *NUP82*-TAP expressing *NUP159ΔDID-eDID*-FG_{Nsp1} (lane 4), *NUP82*-TAP expressing *NUP159ΔDID-eDID* (lane 5), *NUP116-eDID*-TAP *NUP159ΔDID* (lane 6), and *NUP116-eDID*-TAP (lane 7). (D) Dyn2-dependent dimerization of modified eDID Nups. Affinity purification from strain lysates *NIC96*-TAP *NUP49-eDID* (lanes 1 and 2) and *NUP82*-TAP *NUP159-eDID* (lanes 3 and 4) in the presence (lanes 2 and 4) or absence of Dyn2 (lanes 1 and 3), which coexpress GFP-Nup82 and GFP-Nic96, respectively. Eluates and homogenates (HOM) were analyzed by SDS-PAGE and Coomassie blue staining or Western blotting (E) Expression levels of *NUP159ΔDID-eDID*-FG_{Nsp1} and *NUP159ΔDID-eDID*-FG_{Nup1} + *pGAL::DYN2* after 3-h galactose induction. Wild-type cells showing endogenous Dyn2 expression (lane 1), *dyn2Δ* cells + *pGAL::DYN2* (lane 2), *dyn2Δ NUP159ΔDID* cells + *pGAL::DYN2* (lane 3), *dyn2Δ NUP159ΔDID-eDID*-FG_{Nsp1} + *pGAL::DYN2* (lane 4), and *dyn2Δ NUP159ΔDID-eDID*-FG_{Nup1} + *pGAL::DYN2* (lane 5). Whole-cell lysates were boiled in SDS sample buffer and analyzed by SDS-PAGE and Western blotting using anti-Dyn2, anti-Flag, and anti-Arc1 (equal loading) antibodies. CBP, calmodulin-binding peptide

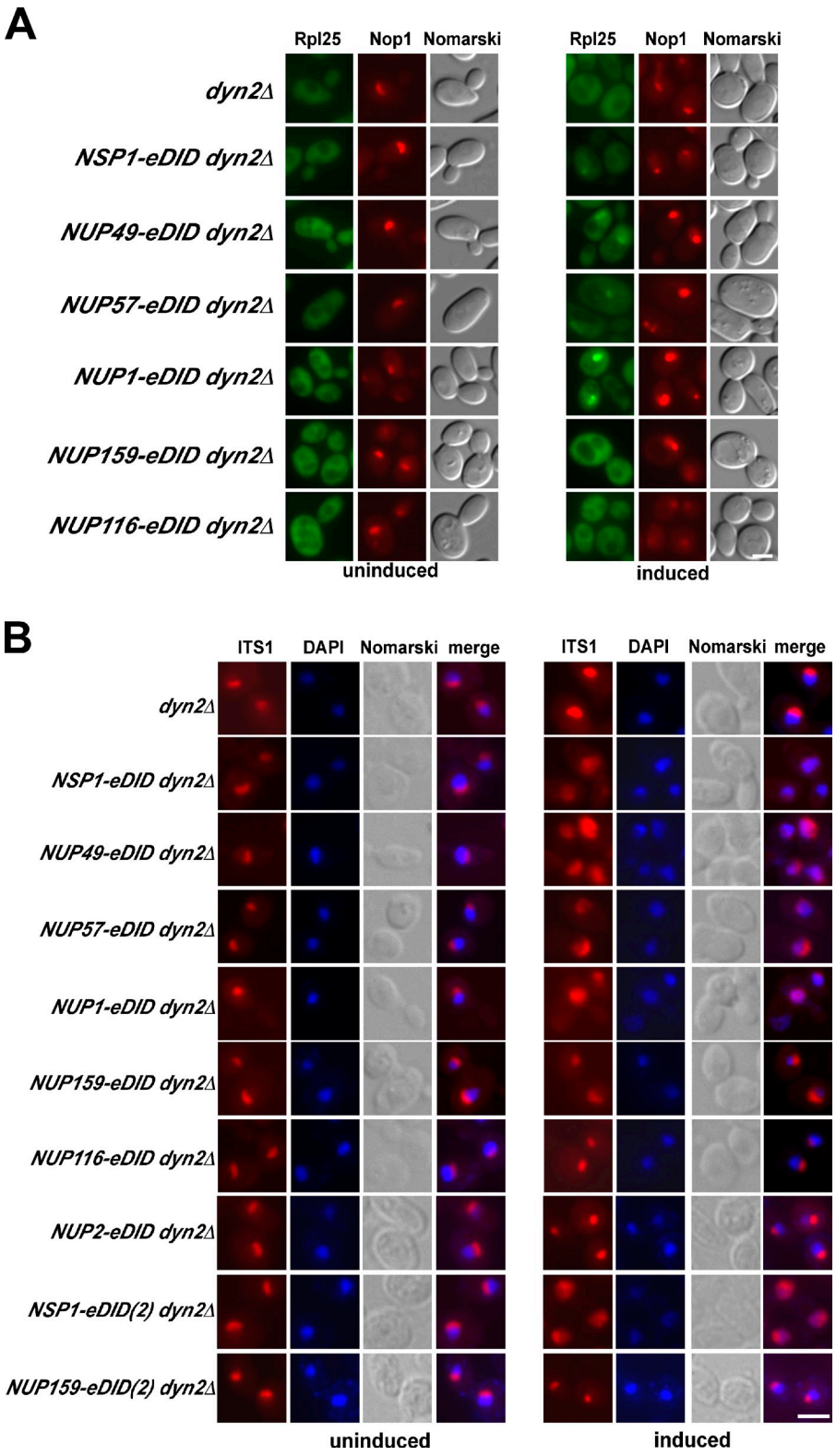

Figure S2. **Analysis of 60S and 40S ribosome export in eDID-labeled FG Nups.** (A) Nuclear export of the 60S ribosomal subunit. Indicated strains were transferred to YPR medium for 4 h, and Dyn2 expression was induced by the addition of galactose (t_0; uninduced) and further incubated for 4 h (induced). Localization of Rpl25-GFP and mRFP-Nop1 (nucleolar marker) was analyzed under the fluorescence microscope. (B) Localization of 20S preRNA and larger precursors. Indicated strains were transferred to YPR medium for 4 h (uninduced), and Dyn2 expression was induced for additional 2 h by addition of galactose (induced). In situ hybridization using a Cy3 fluorescently labeled oligonucleotide probe complementary to the ITS1 region was performed. DNA was stained with DAPI. Bars, 2 µm.

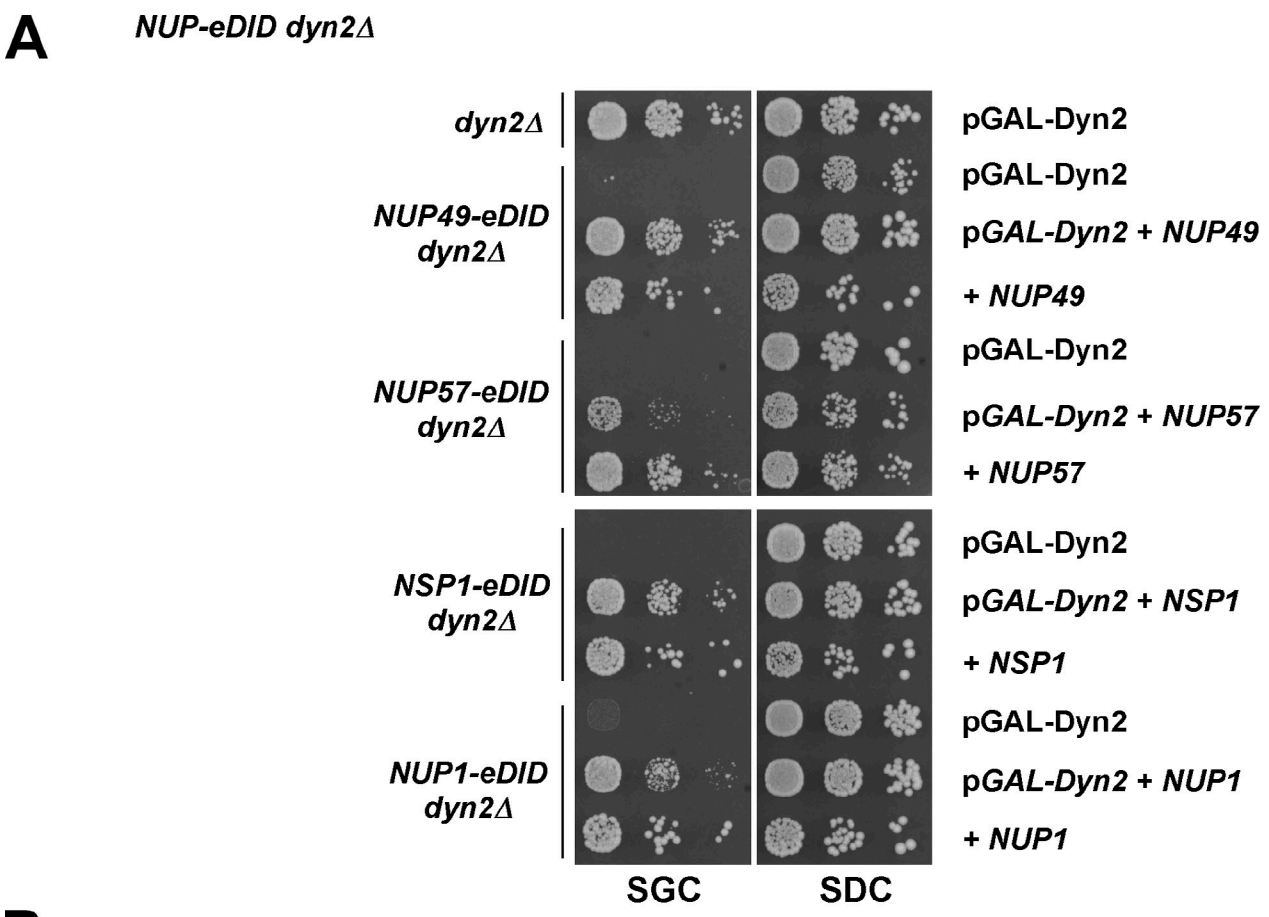

Figure S3. **Analysis of growth and nucleocytoplasmic transport of *NUP-eDID* + pGAL-DYN2 strains transformed with the respective wild-type Nups.** (A) *NUP49-eDID*, *NUP57-eDID*, *NSP1-eDID*, and *NUP1-eDID* were either transformed with *pGAL-Dyn2* or *pGAL-Dyn2 + NUP* wild type or *NUP* wild type alone and spotted on SGC-Leu/-Ura and SDC-Leu/-Ura plates. (B) Poly(A)$^+$ RNA was analyzed by in situ hybridization with a Cy3-labeled oligo d(T) probe, and DNA was stained with DAPI. Bar, 5 µm.

Table S1. **Yeast strains used in this study**

Strains	Genotype	References
RS453[a]	*MAT*a or α *ade2 his3-11 15 leu2-3 112 trp1 ura3-52*	Grandi et al., 1995
Ds1-2b[c]	*MAT*α *leu2-Δ1 trp1-Δ63 his3-Δ200 ura3-52*	Baßler et al., 2001
dyn2Δ[b]	*MAT*α *leu2-Δ1 trp1-Δ63 his3-Δ200 ura3-52 dyn2::LoxP*	This study
Nsp1-eDID dyn2Δ[b]	eDID-LoxP at position 275 aa *dyn2*::LoxP	This study
Nsp1-eDID(2) dyn2Δ[b]	eDID-LoxP at position 2 aa *dyn2*::LoxP	This study
Nup1-eDID dyn2Δ[b]	eDID-LoxP at position 717 aa *dyn2*::LoxP	This study
Nup2-eDID dyn2Δ[b]	eDID-LoxP at position 407 aa *dyn2*::LoxP	This study
Nup57-eDID dyn2Δ[b]	eDID-LoxP at position 71 aa *dyn2*::LoxP	This study
Nup49-eDID dyn2Δ[b]	eDID-LoxP at position 81 aa *dyn2*::LoxP	This study
Nup159ΔDID-eDID dyn2Δ[a]	eDID-LoxP at position 624 aa *nup159*::kanMX4 *dyn2*::kanMX4 pRS414-*NUP159ΔDID-eDID*	This study
Nup159ΔDID-eDID(2) dyn2Δ[a]	eDID-LoxP at position 904 aa *nup159*::kanMX4 *dyn2*::kanMX4 pRS414-*NUP159ΔDID*^{2}*-eDID*	This study
Nup116-eDID dyn2Δ[b]	eDID-LoxP at position 197 aa *dyn2*::LoxP	This study
Nup159ΔDID Nsp1-eDID dyn2Δ[a]	*nup159*::kanMX4 *dyn2*::kanMX4 pRS414-*NUP159ΔDID*, DID-LoxP in *NSP1* at position 275	This study
Nsp1-eDID dyn2Δ Nup159ΔDID[b]	eDID-LoxP at position 275 aa *dyn2*::LoxP *nup159*::HISMX6 pRS414-*NUP159ΔDID*	This study
Nup1-eDID dyn2Δ Nup159ΔDID[b]	eDID-LoxP at position 717 aa *dyn2*::LoxP *nup159*::HISMX6 pRS414-*NUP159ΔDID*	This study
Nup2-eDID dyn2Δ Nup159ΔDID[b]	eDID-LoxP at position 407 aa *dyn2*::LoxP *nup159*::HISMX6 pRS414-*NUP159ΔDID*	This study
Nup49-eDID dyn2Δ Nup159ΔDID[b]	eDID-LoxP at position 81 aa *dyn2*::LoxP *nup159*::HISMX6 pRS414-*NUP159ΔDID*	This study
Nup116-eDID dyn2Δ Nup159ΔDID[b]	eDID-LoxP at position 197 aa *dyn2*::LoxP *nup159*::HISMX6 pRS414-*NUP159ΔDID*	This study
Nup116-eDID-TAP dyn2Δ[b]	eDID-LoxP at position 197 aa *dyn2*::LoxP *NUP116*-TAP::*URA3*	This study
Nup116-eDID-TAP dyn2Δ Nup159ΔDID[b]	eDID-LoxP at position 197 aa *dyn2*::LoxP *NUP116*-TAP::*URA3 nup159*::HISMX6 pRS414-*NUP159ΔDID*	This study
Nup1-eDID-TAP dyn2Δ[b]	eDID-LoxP at position 717 aa *dyn2*::LoxP *NUP1*-TAP::*URA3*	This study
Nup2-eDID Nup60-TAP dyn2Δ[b]	eDID-LoxP at position 407 aa *dyn2*::LoxP *NUP60*-TAP::*URA3*	This study
Nsp1-eDID dyn2Δ Nup49-TAP[b]	eDID-LoxP at position 275 aa *dyn2*::LoxP *NUP49*-TAP::*URA3*	This study
Nup159ΔDID-eDID Nup82-TAP dyn2Δ[a]	eDID-LoxP at position 624 aa *nup159*::kanMX4 *dyn2*::kanMX4 pRS414-*NUP159ΔDID-eDID NUP82*-TAP::*URA3*	This study
Nup49-eDID Nic96-TAP dyn2Δ[b]	eDID-LoxP at position 81 aa *dyn2*::LoxP *NIC96*-TAP::*URA3*	This study
Nup159ΔDID dyn2Δ[a]	*nup159*::kanMX4 *dyn2*::kanMX4 pRS414-*NUP159ΔDID*	Stelter et al., 2007
Nup159 dyn2Δ[a]	*nup159*::kanMX4 *dyn2*::kanMX4 pRS414-*NUP159*	Stelter et al., 2007
dyn2Δ GAL::Dyn2[b]	*GAL-DYN2*::*HISMX6 dyn2*::LoxP	This study
Nsp1-eDID dyn2Δ GAL::DYN2[b]	*GAL-DYN2*:: *HISMX6 dyn2*::LoxP	This study
dyn2Δ Pho4-GFP GAL::DYN2[b]	*PHO4*-GFP::natNT2 *GAL-DYN2*::*HISMX6 dyn2*::LoxP	This study
Nsp1-eDID dyn2Δ Pho4-GFP[b]	*PHO4*-GFP::natNT2 *dyn2*::LoxP	This study
Nsp1-eDID dyn2Δ Pho4-GFP GAL::DYN2[b]	*PHO4*-GFP::natNT2 *GAL-DYN2*::*HISMX6 dyn2*::LoxP	This study
Nup57-TAP GAL::DYN2[c]	*NUP57*-TAP::*TRP1 GAL-DYN2*::*HISMX6*	This study
Nup57-TAP Nsp1-eDID dyn2Δ[b]	*NUP57*-TAP::*TRP1 dyn2*::LoxP	This study
Nup57-TAP Nsp1-eDID dyn2Δ GAL::DYN2[b]	*NUP57*-TAP::*TRP1 GAL-DYN2*::*HISMX6 dyn2*::LoxP	This study
nup159Δ/dyn2Δ shuffle[a]	*nup159*::kanMX4 *dyn2*::kanMX4 pLG4	Stelter et al., 2007
nup159Δ/dyn2Δ GAL::DYN2 shuffle[a]	*nup159*::kanMX4 *dyn2*::kanMX4 *GAL-DYN2*::*HISMX6* pLG4	This study
nsp1Δ/nup57Δ dyn2Δ shuffle[a]	*nsp1::His3 nup57::His3 dyn2*::kanMX4 pCH1122-*NSP1* pRS314-GFP-*NUP57*	This study
Nup159ΔDID-FG_{Nsp1} dyn2Δ[a]	*nup159*::kanMX4 *dyn2*::kanMX4 pRS414-*NUP159ΔDID-eDID-*FG_{NSP1}	This study
Nup159ΔDID-FG_{Nup1} dyn2Δ[a]	*nup159*::kanMX4 *dyn2*::kanMX4 pRS414-*NUP159ΔDID-eDID-*FG_{NUP1}	This study
Nup159 dyn2Δ[a]	*nup159*::kanMX4 *dyn2*::kanMX4 pRS414-*NUP159Δ*	This study
Nup159ΔDID dyn2Δ[a]	*nup159*::kanMX4 *dyn2*::kanMX4 pRS414-*NUP159ΔDID*	This study
Nup159ΔDID-FG_{Nsp1} dyn2Δ[a]	*nup159*::kanMX4 *dyn2*::kanMX4 pRS414-*NUP159ΔDID-*FG_{NSP1}	This study
Nup159ΔDID-eDID-FG_{Nsp1} dyn2Δ[a]	*nup159*::kanMX4 *dyn2*::kanMX4 pRS414-*NUP159ΔDID-eDID-*FG_{Nsp1}	This study
Nup82-TAP Nup159ΔDID dyn2Δ[a]	*NUP82*-TAP::*URA3 nup159*::kanMX4 dyn2::kanMX4 pRS414-*NUP159ΔDID*	This study

Table S1. **Yeast strains used in this study** *(Continued)*

Strains	Genotype	References
Nup82-TAP Nup159ΔDID-eDID-FG_{Nsp1} dyn2Δ[a]	*NUP82*-TAP::*URA3 nup159*::kanMX4 dyn2::kanMX4 pRS414-*NUP159ΔDID-eDID-FG_{Nsp1}*	This study
Nup82-TAP Nup159ΔDID-FG_{Nsp1} dyn2Δ[a]	*NUP82*-TAP::*URA3 nup159*::kanMX4 dyn2::kanMX4 pRS414-*NUP159ΔDID-FG_{Nsp1}*	This study
Nup82-TAP Nup159ΔDID Nsp1-eDID dyn2Δ[a]	*NUP82*-TAP::*URA3 nup159*::kanMX4 dyn2: kanMX4 pRS414-*Nup159ΔDID*	This study
Nup82-TAP Nup159ΔDID-eDID-FG_{Nsp1} dyn2Δ[a]	*NUP82*-TAP::*URA3 nup159*::kanMX4 dyn2::kanMX4 pRS414-*NUP159ΔDID-eDID-FG_{Nsp1}*	This study
GAL::DYN2 dyn2Δ[a]	*GAL-DYN2*::*HISMX6 dyn2*::LoxP	This study
Nup*57*-FG_{Nsp1} Nsp1ΔFG dyn2Δ[a]	*nsp1::His3 nup57::His3 dyn2*::kanMX4 pSB32-P_{ADH1}-*NSP1ΔFG* pRS314-P_{NOP1}-*NUP57-FG_{NSP1}*	This study
Nup*57*-eDID-FG_{Nsp1} Nsp1ΔFG dyn2Δ[a]	*nsp1::His3 nup57::His3 dyn2*::kanMX4 pSB32-P_{ADH1}-*NSP1ΔFG* pRS314-P_{NOP1}-*NUP57-eDID-FG_{NSP1}*	This study

[a]RS453 genetic background.
[b]Ds1-2b genetic background.
[c]dyn2Δ genetic background.

Table S2. **Plasmids used in this study**

Plasmids	Comments	References
pRS414-*NUP159*		Stelter et al., 2007
pRS414-*NUP159ΔDID*		Stelter et al., 2007
pRS414-*NUP159-eDID*	pRS414-*Nup159ΔDID*-eDID[623] eDID-Flag PCR amplified from pfa6a-loxP-eDID-Flag-loxP-HISMX4 and cloned as Bgl2 in the BamH1 cut pRS414-*Nup159ΔDID*	This study
pRS414-*NUP159ΔDID*-FG_{NSP1}	FG_{NSP1}(1–591 aa) was PCR amplified from genomic DNA from a wild-type strain and cloned as Pst1–Apa1 in pRS414-*Nup159ΔDID*	This study
pRS414-*NUP159ΔDID*-eDID-FG_{NSP1}	eDID-FG_{NSP1}(1–591 aa + eDID-Flag-loxP) was PCR amplified from genomic DNA from a *NSP1*-eDID dyn2Δ and cloned as Pst1–Apa1 in pRS414-*Nup159ΔDID*	This study
pRS414-*NUP159ΔDID*-eDID-FG_{NUP1}	eDID-FG_{NUP1}(382–1,076 aa + eDID-Flag-loxP) was PCR amplified from genomic DNA from a *NUP1*-eDID dyn2Δ and cloned as Pst1–Apa1 in pRS414-*Nup159ΔDID*	This study
pRS314-P_{NOP1}	NOP1 Promoter was cloned as BamH1–EcoR1 in pRS314 and a Nde1 restriction site was created before the EcoR1	This study
pRS314-P_{NOP1}-*NUP57ΔFG*	Nup57ΔFG(224–541 aa) was PCR amplified from genomic DNA and cloned as EcoR1–Xho1 in pRS314-P_{NOP1}	This study
pRS314-P_{NOP1}-*NUP57-FG_{NSP1}*	FG_{NSP1}(1–591 aa) was PCR amplified from genomic DNA from a wild-type strain and cloned as Nde1 in pRS314-P_{NOP1}-Nup*57*ΔFG	This study
pRS314-P_{NOP1}-*NUP57-eDID-FG_{NSP1}*	eDID-FG_{NSP1}(1–591 aa + eDID-Flag-loxP) was PCR amplified from genomic DNA from a *NSP1*-eDID dyn2Δ and cloned as Nde1 in pRS314-P_{NOP1}-Nup*57*ΔFG	This study
p*URA3* mRFP-*NOP1 RPL25*-EGFP		Ulbrich et al., 2009
YEp351-*GAL-DYN2*	DYN2 PCR amplified and cloned as BamH1–Hind3	This study
Yep352-*GAL-DYN2*	DYN2 PCR amplified and cloned as BamH1–Hind3	This study
Yep352-*GAL-DYN2*-GFP	DYN2-GFP was a PCR amplified from DYN2-GFP (Stelter et al., 2007) strain and cloned as BamH1–Hind3 in Yep352-*GAL-DYN2* plasmid	This study
pRS303-*GAL::DYN2*	pRS303-GAL::DYN2::*HIS3*	This study
pfa6a-eDID-Flag-loxP-HISMX4-loxP	HindIII-eDID-BamH1-Flag-Sal1-loxP-HISMX4-loxP	This study
pUN100-*NUP49*	Subcloned from genomic library plasmid (3.5 kb)	This study
pRS315-*NUP57*	Subcloned from genomic library plasmid	This study
pSB32-*NSP1*	Subcloned from genomic library plasmid	This study
pUN100-*NUP1*	Subcloned from genomic library plasmid	This study

References

Baßler, J., P. Grandi, O. Gadal, T. Lessmann, E. Petfalski, D. Tollervey, J. Lechner, and E. Hurt. 2001. Identification of a 60S preribosomal particle that is closely linked to nuclear export. *Mol. Cell*. 8:517–529. http://dx.doi.org/10.1016/S1097-2765(01)00342-2

Flemming, D., K. Thierbach, P. Stelter, B. Böttcher, and E. Hurt. 2010. Precise mapping of subunits in multiprotein complexes by a versatile electron microscopy label. *Nat. Struct. Mol. Biol.* 17:775–778. http://dx.doi.org/10.1038/nsmb.1811

Grandi, P., N. Schlaich, H. Tekotte, and E.C. Hurt. 1995. Functional interaction of Nic96p with a core nucleoporin complex consisting of Nsp1p, Nup49p and a novel protein Nup57p. *EMBO J.* 14:76–87.

Stelter, P., R. Kunze, D. Flemming, D. Höpfner, M. Diepholz, P. Philippsen, B. Böttcher, and E. Hurt. 2007. Molecular basis for the functional interaction of dynein light chain with the nuclear-pore complex. *Nat. Cell Biol.* 9:788–796. http://dx.doi.org/10.1038/ncb1604

Ulbrich, C., M. Diepholz, J. Bassler, D. Kressler, B. Pertschy, K. Galani, B. Böttcher, and E. Hurt. 2009. Mechanochemical removal of ribosome biogenesis factors from nascent 60S ribosomal subunits. *Cell*. 138:911–922. http://dx.doi.org/10.1016/j.cell.2009.06.045