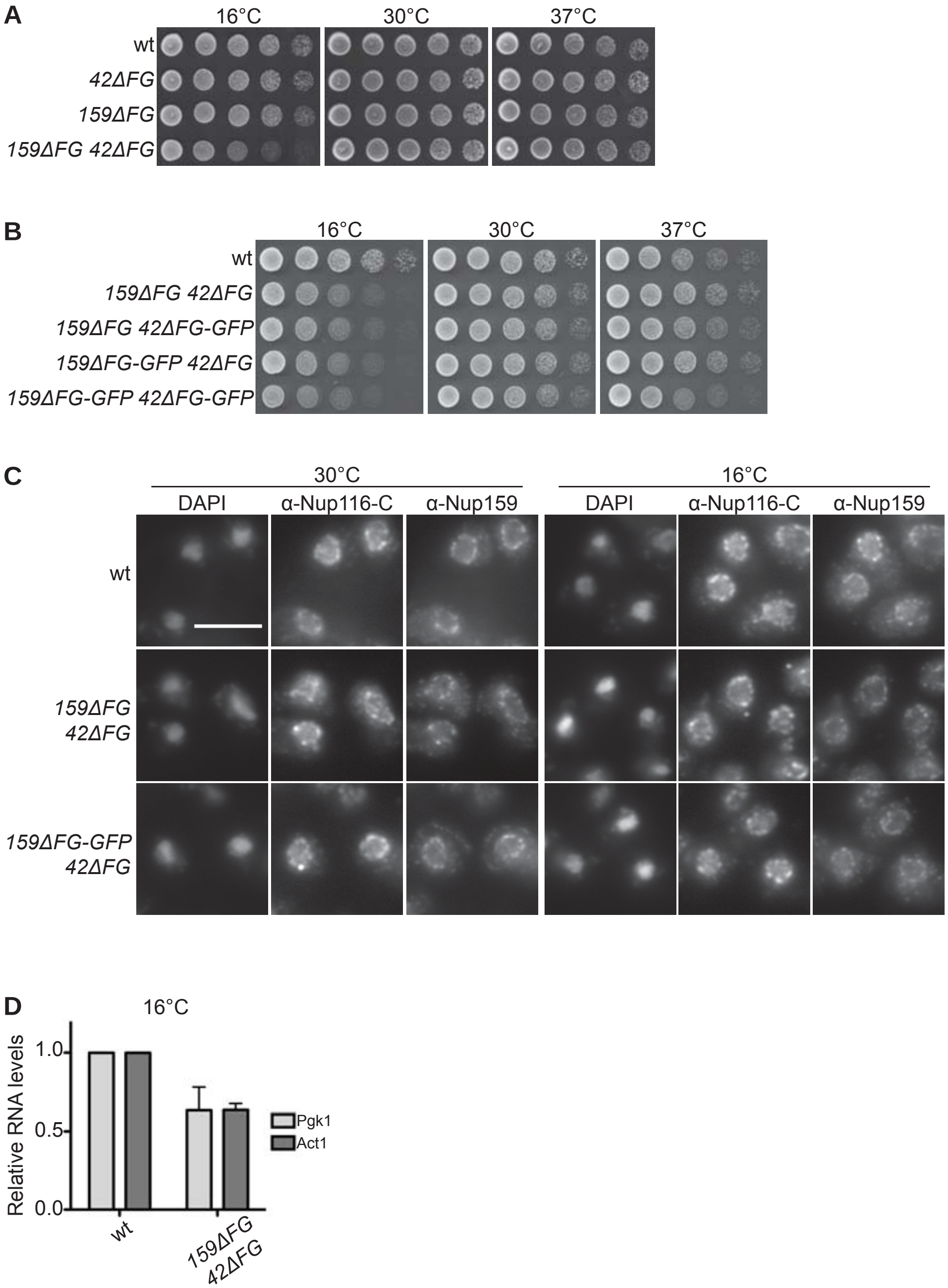

Figure S1 The cold-sensitive mRNA export defect of *nup42ΔFG nup159ΔFG* is not due to mislocalized or non-functional nup42 and nup159 proteins or altered poly(A)+ RNA levels. (A) Deletion of both Nup42 and Nup159 FG domains results in a growth defect at cold temperatures. Yeast strains were grown at 30° and five-fold serially diluted on YPD plates for growth at the indicated temperature. (B) GFP fusions of nup42ΔFG and nup159ΔFG do not result in enhanced growth defects. Yeast strains were grown at 30° and five-fold serially diluted on YPD plates for growth at the indicated temperature. (C) nup159ΔFG and nup159ΔFG-GFP localize to the nuclear envelope at the permissive and restrictive temperatures. Indicated strains were grown at 30°, shifted to 16° or 30° overnight, and processed for immunofluoresence using the indicated antibodies. DAPI staining marks the nucleus. Scale bar, 5μm. (D) Steady-state levels of poly-adenylated transcripts are decreased in *nup42ΔFG nup159ΔFG*. Indicated strains were grown at 30°, shifted to 16° or 30° overnight, and total RNA was isolated. Q-PCR analysis of the resulting cDNA was performed for *Pgk1*, and *Act1*, and normalized to the non-poly-adenylated *Scr1* RNA. Wt levels were set to 1.0, and error bars indicate SEM of triplicate biological replicates. Levels are likely decreased due to feedback mechanisms that reduce transcription in mRNA export mutants.

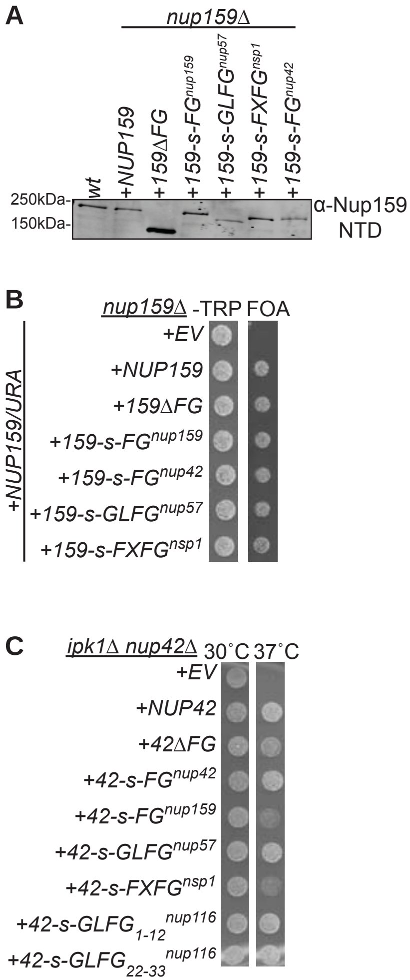

Figure S2 FG swap constructs are expressed and functional. (A) *nup159-s-FG* constructs are expressed. Lysates from a wt strain or *nup159Δ* mutants expressing *nup159-s-FG* vectors were separated by SDS-PAGE and immunoblotted using an α-Nup159-NTD antibody. (B) *nup159-s-FG* constructs are functional. *nup159Δ* strains containing empty vector (EV) or *nup159-s-FG/TRP* vectors were spotted onto -TRP synthetic media or 5-FOA at 25°. Growth on 5-FOA indicates functional complementation. (C) *nup42-s-FG* constructs are functional. *nup42Δ ipk1Δ* mutants containing empty vector (EV) or *nup42-s-FG/TRP* vectors were spotted onto -TRP synthetic media at the indicated temperature. Growth at 37° indicates functional complementation.

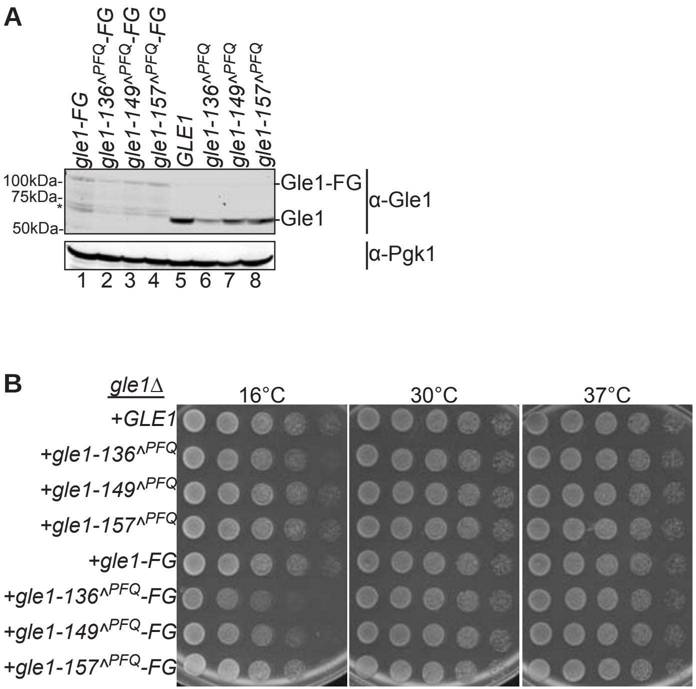

Figure S3 ***gle1-FG* constructs are expressed and show minimal growth defects.** (A) *gle1-FG* fusions are expressed. Lysates from *gle1Δ* strains covered by the indicated vectors were separated by SDS-PAGE and immunoblotted using an α-Gle1 antibody. Pgk1 was used as a loading control. (*) Degradation products from the Nup42 FG domain. (B) *gle1^PFQ-FG* constructs display minimal growth defects. *gle1Δ* strains covered by the indicated vectors were grown at 30° and five-fold serially diluted on YPD plates for growth at the indicated temperatures.

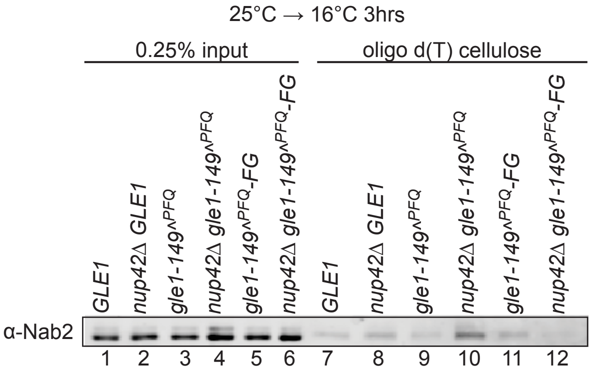

Figure S4 Representative immunoblot from Figure 5E: Fusion of the Nup42 FG domain to the carboxy-terminus of Gle1 rescues the mRNP remodeling defect of *nup42Δ gle1-149^PFQ*. The association of Nab2 protein with poly(A)+ RNA was assessed by shifting strains to 16° for 3hrs, UV crosslinking, isolation of RNA by antisense chromatography, and immunoblotting after treatment with RNAse.

Table S1 Strain Table

Strain	Genotype	Source
SWY2283	*MATa ade2-1::ADE2 ura3-1 his3-11,15 leu2-3,112 lys2 can1-100*	(STRAWN *et al.* 2004)
SWY5703	*MATα ade2-1 ura3-1 his3-11,15 leu2-3 lys2 can1-100*	This Study
SWY2832	*MATα ade2-1::ADE2 ura3-1 his3-11,15 leu2-3,112 lys2 can1-100 HA-LoxP-nup42ΔFG*	(STRAWN *et al.* 2004)
SWY2808	*MATa ade2-1::ADE2 ura3-1 his3-11,15 leu2-3,112 lys2 can1-100 myc-LoxP-nup159ΔFG*	(STRAWN *et al.* 2004)
SWY2846	*MATa ade2-1::ADE2 ura3-1 his3-11,15 leu2-3,112 lys2 can1-100 myc-LoxP-nup159ΔFG HA-LoxP-nup42ΔFG*	(STRAWN *et al.* 2004)
SWY5701	*MATa ade2-1 ura3-1 leu2-3,112 his3-11,15 can1-100 myc-LoxP-nup159ΔFG HA-LoxP-nup42ΔFG*	This Study
SWY5825	*MATa ade2-1::ADE2 ura3-1 his3-11,15 leu2-3,112 lys2 can1-100 myc-LoxP-nup159ΔFG HA-LoxP-nup42ΔFG-GFP:HIS3*	This Study
SWY5826	*MATa ade2-1::ADE2 ura3-1 his3-11,15 leu2-3,112 lys2 can1-100 myc-LoxP-nup159ΔFG-GFP:HIS3 nup42ΔFG*	This Study
SWY5334	*MATa ura3 leu2 his3 rat8-2 (dbp5) +pCA5005*	This Study
SWY4301	*MATa ura3 leu2 his3 trp1 myc-LoxP-nup159ΔFG rat8-2 (dbp5) +pCA5005*	This Study
SWY5542	*MATα ura3 leu2 his3 HA-LoxP-nup42ΔFG rat8-2 (dbp5) +pCA5005*	This Study
SWY4320	*MATα ura3 leu2 his3 trp1 nup42ΔFG myc-LoxP-nup159ΔFG rat8-2 (dbp5) +pCA5005*	This Study
SWY5209	*MATα ura3-1 leu2-3,112 his3-11 trp1-1 gle1-4 +pSW410*	This Study
SWY5208	*MATα ura3-1 leu2-3,112 his3-11 trp1-1 lys2 myc-LoxP-nup159ΔFG gle1-4 +pSW410*	This Study
SWY5206	*MATα ura3-1 leu2-3,112 his3-11 trp1-1 lys2 HA-LoxP-nup42ΔFG gle1-4 +pSW410*	This Study
SWY5207	*MATα ura3-1 leu2-3,112 his3-11 trp1-1 lys2 HA-LoxP-nup42ΔFG myc-LoxP-nup159ΔFG gle1-4 +pSW410*	This Study
Mex67 shuffle	*MATa ade2 his3 leu2 trp1 ura3 mex67::HIS3 +pRS316-MEX67*	(SEGREF *et al.* 1997)
SWY5204	*MATα ade2 his3 leu2 trp1 ura3 HA-LoxP-nup42ΔFG myc-LoxP-nup159ΔFG mex67::HIS3 +pRS316-MEX67*	This Study
SWY5697	*MATα ade2-1 ura3-1 leu2-3,112 his3-11,15 trp1-1 can1-100 gle1::HIS +pSW3345*	This Study
SWY5698	*MATα ade2-1 ura3-1 leu2-3,112 his3-11,15 trp1-1 can1-100 myc-LoxP-nup159ΔFG HA-LoxP-nup42ΔFG gle1::HIS +pSW3345*	This Study
SWY5236	*MATa ura3-1 his3-11,15 leu2-3,112 trp1-1 nab2::HIS3 +pAC717*	This Study
SWY5237	*MATa ura3-1 his3-11,15 leu2-3,112 trp1-1 nab2::HIS3 +pSW3298*	This Study
SWY5238	*MATa ura3-1 his3-11,15 leu2-3,112 trp1-1 HA-LoxP-nup42ΔFG myc-LoxP-nup159ΔFG nab2::HIS3 +pAC717*	This Study
SWY5239	*MATa ura3-1 his3-11,15 leu2-3,112 trp1-1 HA-LoxP-nup42ΔFG myc-LoxP-nup159ΔFG nab2::HIS3 + pSW3298*	This Study
SWY3826	*MATα ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 gle1::HIS3 +pSW399*	(ALCAZAR-ROMAN *et al.* 2010)
SWY4908	*MATα ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 gle1::HIS3 +pSW3743*	(FOLKMANN *et al.* 2013)
SWY4909	*MATα ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 gle1::HIS3 +pSW3742*	(FOLKMANN *et al.* 2013)
SWY4961	*MATα ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 gle1::HIS3 +pSW3760*	(FOLKMANN *et al.* 2013)
SWY5878	*MATα ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 gle1::HIS3 +pSW3936*	This Study
SWY5879	*MATα ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 gle1::HIS3*	This Study

	+pSW3981	
SWY5880	*MATα ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 gle1::HIS3 +pSW3982*	This Study
SWY5881	*MATα ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 gle1::HIS3 +pSW3983*	This Study
SWY5875	*MATα ura3-1 his3-11,15 leu2-3,112 nup42::KAN*^{R} *gle1::HIS3 +pSW410*	This Study
SWY5882	*MATα ura3-1 his3-11,15 leu2-3,112 nup42::KAN*^{R} *gle1::HIS3 +pSW399*	This Study
SWY5883	*MATα ura3-1 his3-11,15 leu2-3,112 nup42::KAN*^{R} *gle1::HIS3 +pSW3742*	This Study
SWY5885	*MATα ura3-1 his3-11,15 leu2-3,112 nup42::KAN*^{R} *gle1::HIS3 +pSW3936*	This Study
SWY5887	*MATα ura3-1 his3-11,15 leu2-3,112 nup42::KAN*^{R} *gle1::HIS3 +pSW3982*	This Study
nup42Δ	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ1 nup42::KAN*^{R}	(Winzeler *et al.* 1999)
SWY2114	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 ipk1::KAN*^{R} *nup42::HIS3*	(Miller *et al.* 2004)
SWY4303	*MATa ura3-1 his3-11,15 trp1-1 leu2-3,112 nup159::KAN*^{R} *+pLG4*	This Study

Table S2 Vector Table

Plasmid	Description	Source
pSW3801	*NUP42/CEN/LEU2*	This study
pSW3802	*NUP42/CEN/TRP1*	This study
pSW3645	*nup42ΔFG/CEN/LEU2*	This study
pSW3657	*nup42ΔFG/CEN/TRP1*	This study
pSW3662	*nsp42-s-FG*^{nup42}*/CEN/TRP1*	This study
pSW3658	*nsp42-s-GLFG*^{nup57}*/CEN/TRP1*	This study
pSW3659	*nsp42-s-FxFG*^{nsp1}*/CEN/TRP1*	This study
pSW3660	*nsp42-s-GLFG*$_{1\text{-}12}^{nup116}$*/CEN/TRP1*	This study
pSW3661	*nsp42-s-GLFG*$_{22\text{-}33}^{nup116}$*/CEN/TRP1*	This study
pSW3841	*nsp42-s-FG*^{nup159}*/CEN/TRP1*	This study
pLG4	*NUP159/URA*	(Gorsch *et al.* 1995)
pSW3647	*NUP159/CEN/TRP1*	This study
pSW3648	*nup159ΔFG/CEN/TRP1*	This study
pSW3692	*nsp159-s-FG*^{nup159}*/CEN/TRP1*	This study
pSW3693	*nsp159-s-GLFG*^{nup57}*/CEN/TRP1*	This study
pSW3695	*nsp159-s-FxFG*^{nsp1}*/CEN/TRP1*	This study
pSW3694	*nsp159-s-FG*^{nup42}*/CEN/TRP1*	This study
pCA5005	*DBP5/CEN/URA3*	(Tseng *et al.* 1998)
pSW410	*GLE1/CEN/URA3*	(Murphy and Wente 1996)
pSW399	*GLE1/CEN/LEU2*	(Murphy and Wente 1996)
pSW3345	*gle1*$^{K377Q/K378Q}$*/CEN/LEU2*	(Alcazar-Roman *et al.* 2010)
pSW3743	*gle1-136*$^{\wedge PFQ}$*/CEN/LEU2*	(Folkmann *et al.* 2013)
pSW3742	*gle1-149*$^{\wedge PFQ}$*/CEN/LEU2*	(Folkmann *et al.* 2013)
pSW3760	*gle1-157*$^{\wedge PFQ}$*/CEN/LEU2*	(Folkmann *et al.* 2013)
pSW3936	*gle1-FG*^{nup42}	This study
pSW3981	*gle1-FG*^{nup42}*-136*$^{\wedge PFQ}$*/CEN/LEU2*	This study
pSW3982	*gle1-FG*^{nup42}*-149*$^{\wedge PFQ}$*/CEN/LEU2*	This study
pSW3983	*gle1-FG*^{nup42}*-157*$^{\wedge PFQ}$*/CEN/LEU2*	This study
pRS316-MEX67	*MEX67/CEN/URA3*	(Segref *et al.* 1997)
pRS314-MEX67	*MEX67/CEN/TRP1*	(Segref *et al.* 1997)
pRS314-mex67-5	*Mex67-5/CEN/TRP1*	(Segref *et al.* 1997)
pAC717	*NAB2/CEN/LEU2*	(Marfatia *et al.* 2003)
pSW3298	*nab2-C437S/CEN/LEU2*	(Tran *et al.* 2007)

SUPPLEMENTAL LITERATURE CITED

ALCAZAR-ROMAN, A. R., T. A. BOLGER and S. R. WENTE, 2010 Control of mRNA export and translation termination by inositol hexakisphosphate requires specific interaction with Gle1. J Biol Chem **285:** 16683-16692.

FOLKMANN, A. W., S. E. COLLIER, X. ZHAN, ADITI, M. D. OHI *et al.*, 2013 Gle1 functions during mRNA export in an oligomeric complex that is altered in human disease. Cell **155:** 582-593.

GORSCH, L. C., T. C. DOCKENDORFF and C. N. COLE, 1995 A conditional allele of the novel repeat-containing yeast nucleoporin RAT7/NUP159 causes both rapid cessation of mRNA export and reversible clustering of nuclear pore complexes. J Cell Biol **129:** 939-955.

MARFATIA, K. A., E. B. CRAFTON, D. M. GREEN and A. H. CORBETT, 2003 Domain analysis of the Saccharomyces cerevisiae heterogeneous nuclear ribonucleoprotein, Nab2p. Dissecting the requirements for Nab2p-facilitated poly(A) RNA export. J Biol Chem **278:** 6731-6740.

MILLER, A. L., M. SUNTHARALINGAM, S. L. JOHNSON, A. AUDHYA, S. D. EMR *et al.*, 2004 Cytoplasmic inositol hexakisphosphate production is sufficient for mediating the Gle1-mRNA export pathway. J Biol Chem **279:** 51022-51032.

MURPHY, R., and S. R. WENTE, 1996 An RNA-export mediator with an essential nuclear export signal. Nature **383:** 357-360.

SEGREF, A., K. SHARMA, V. DOYE, A. HELLWIG, J. HUBER *et al.*, 1997 Mex67p, a novel factor for nuclear mRNA export, binds to both poly(A)+ RNA and nuclear pores. EMBO J **16:** 3256-3271.

STRAWN, L. A., T. SHEN, N. SHULGA, D. S. GOLDFARB and S. R. WENTE, 2004 Minimal nuclear pore complexes define FG repeat domains essential for transport. Nat Cell Biol **6:** 197-206.

TRAN, E. J., Y. ZHOU, A. H. CORBETT and S. R. WENTE, 2007 The DEAD-box protein Dbp5 controls mRNA export by triggering specific RNA:protein remodeling events. Mol Cell **28:** 850-859.

TSENG, S. S., P. L. WEAVER, Y. LIU, M. HITOMI, A. M. TARTAKOFF *et al.*, 1998 Dbp5p, a cytosolic RNA helicase, is required for poly(A)+ RNA export. EMBO J **17:** 2651-2662.

WINZELER, E. A., D. D. SHOEMAKER, A. ASTROMOFF, H. LIANG, K. ANDERSON *et al.*, 1999 Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. Science **285:** 901-906.