Table S1. Yeast strains used in this study.

Strain #	Genotype	Source
KWY681	*MATa nup1Δ::HIS3*	(Zeitler and Weis, 2004)
KWY1695	*MATα ada2Δ::KANMX*	this study
KWY1622	*MATα ybr022w::LacO::LEU2 his3::LacI-GFP::HIS3 trp1::dsRED-HDEL::TRP1*	this study
KWY1758	*MATα ybr022w::LacO::LEU2 his3::LacI-GFP::HIS3 trp1::dsRED-HDEL::TRP1 ada2Δ::KANMX*	this study
KWY1823	*MATα ybr022w::LacO::LEU2 his3::LacI-GFP::HIS3 trp1::dsRED-HDEL::TRP1 nup1Δ::NATMX*	this study
KWY2348	*MATa gal1::GAL1-GFP::KANMX*	this study
KWY2645	*MATα ada2Δ::NATMX gal1::GAL1-GFP::KANMX*	this study
KWY2003	*MATa nup2::Nup2-LacI::KANMX*	this study
KWY2082	*MATa ybr022w::LacO::LEU2 his3::LacI-GFP::HIS3 trp1::dsRED-HDEL::TRP1 nup2::Nup2-LacI::KANMX*	this study
KWY2079	*MATa ybr022w::LacO::LEU2 trp1::dsRED-HDEL::TRP1 nup2::Nup2-LacI::KANMX*	this study
KWY2117	*MATa ybr022w::LacO::LEU2 trp1::dsRED-HDEL::TRP1 nup2::Nup2-LacI::KANMX nup1Δ::HIS3*	this study
KWY3272	*MATa ybr022w::LacO::LEU2 his3::LacI-GFP::HIS3 trp1::dsRED-HDEL::TRP1 nup2::Nup2-LacI::KANMX nup1Δ::NATMX*	this study
KWY1302	*MATa rpb1-1*	this study; (Morrissey et al., 1999)
KWY2551	*MATa rpb1-1 nup1Δ::HIS3*	this study
KWY2552	*MATa rpb1-1 ada2Δ::KANMX*	this study
KWY2619	*MATa rpb1-1 ybr022w::LacO::LEU2 his3::LacI-GFP::HIS3 trp1::dsRED-HDEL::TRP1*	this study

All KWY strains listed are derived from W303 [KWY165] (*ura3-1 leu2-3 his3-11,15 trp1-1 ade2-1*).

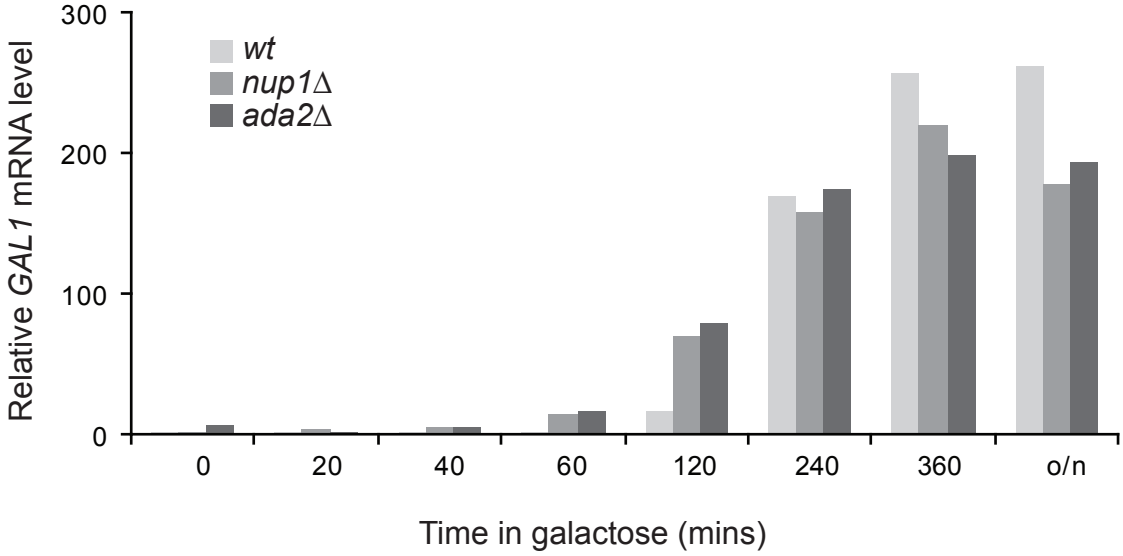
Morrissey, J. P., Deardorff, J. A., Hebron, C., and Sachs, A. B. (1999). Decapping of stabilized, polyadenylated mRNA in yeast pab1 mutants. Yeast *15*, 687-702.

Zeitler, B., and Weis, K. (2004). The FG-repeat asymmetry of the nuclear pore complex is dispensable for bulk nucleocytoplasmic transport in vivo. J Cell Biol *167*, 583-590.

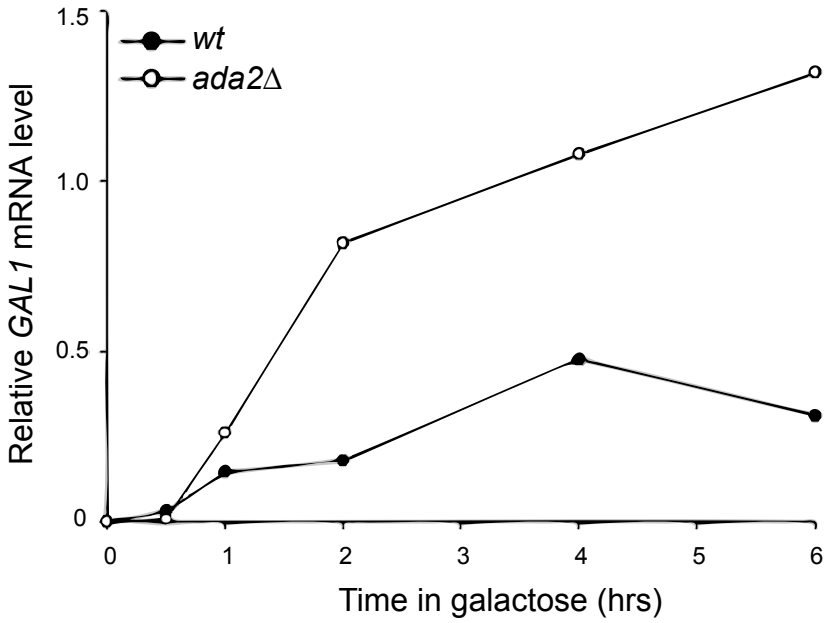
Table S2. Primers and probes used for qRT-PCR.

Primer #	Primer Name	Sequence
UC2955	Act1F	TCCATTGCTTTGTCAAATGG
UC2956	Act1R	CCTGGAACCAAGTGAACAGT
	FAM-Act1-BHQ	AGCCAGCTCCGGTCAAACGG
UC2997	Gal1F	TGGATTCCGGTGATGGTGTT
UC2998	Gal1R	TCAAAATGGCGTGAGGTAGAGA
	FAM-Gal1-BHQ	CTCACGTCGTTCCAATTTACGCTGGTTT
UC3304	Fur4F	GCATATCTATGTGGGGTGGCT
UC3305	Fur4R	ATCGACATTGGCCCATCTTT
UC3306	Kap104F	TCCAACTGGTCTTCAAATGCT
UC3307	Kap104R	GGTCGTTTTCGTCGTTTTGT
UC3219	Gal1probeF	CCGTTTAAATTTCCGCAATTAAAAAAC
UC3220	Gal1probeR	CTAGCATCTTTGTTAACCGTTCGAT
UC3187	Scr1probe	CCTCGCAGAGAGACGGATTCCTCACGCCTCCTGCCAACG
UC3796	Gal7F	TTCTTGGCAAGCATTGACTG
UC3797	Gal7R	CCCATGGCTGTACCTTTGTT
UC3798	Gal10F	AAGTTACGGGCAGAAGAGCA
UC3799	Gal10R	CCTTGCAGGAGTCTTCAACC

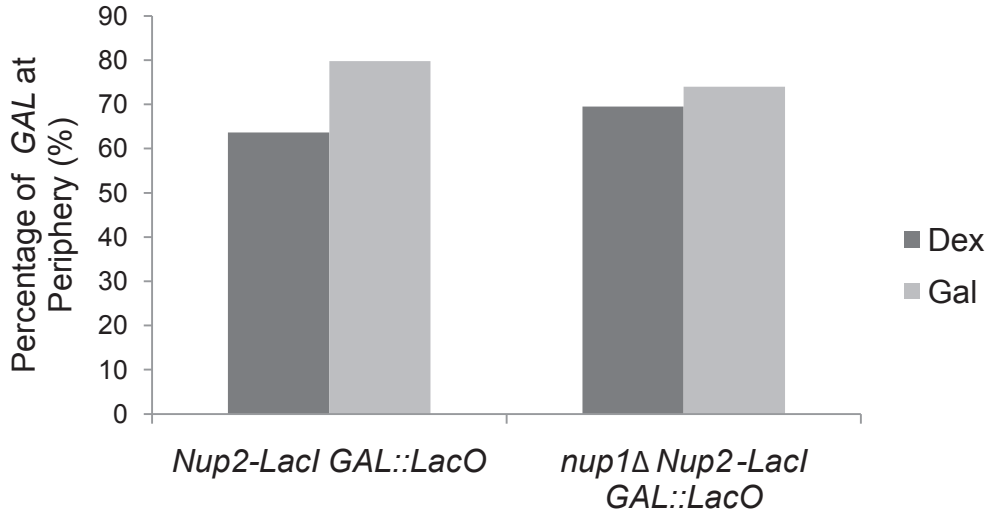
Supplemental Figure 1



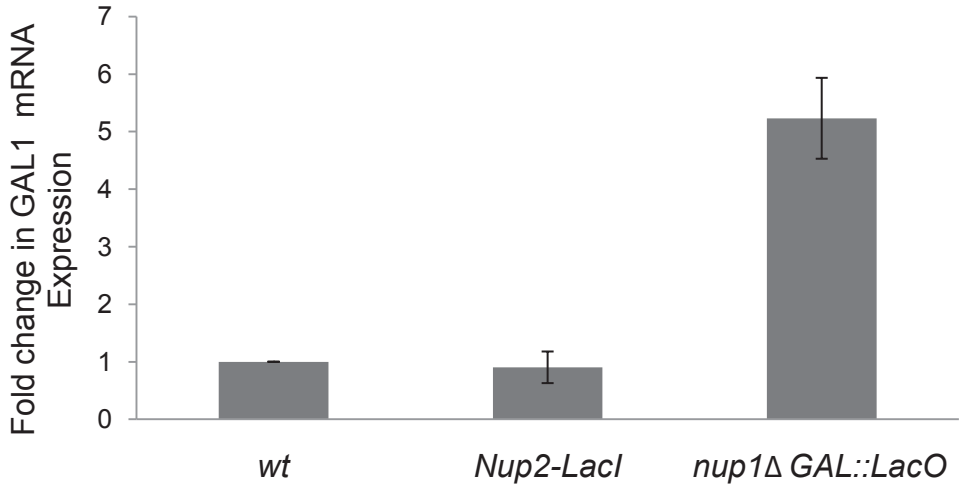
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental figure legends:

Figure S1. Long-term *GAL1* expression in wt and mutant cells.

Wildtype, *nup1Δ* and *ada2Δ* cells were grown as described in **Figure 2A** and *GAL1* mRNA levels were measured at the indicated times in galactose by qRT-PCR and normalized to *ACT1*. (o/n, overnight.)

Figure S2. *GAL1* mRNA expression levels following growth in raffinose.

Wildtype and *ada2*Δ cells were grown in SRaf medium to mid-log phase and then shifted to SGal medium. *GAL1* mRNA levels were measured by Northern blot, normalized to *SCR1* and plotted as a function of time in galactose.

Figure S3. *GAL* nuclear position in the presence of the Nup2-LacI gene tether.

A Nup2-LacI fusion protein which binds the LacO repeats integrated near the *GAL* locus was expressed in both wildtype and *nup1*Δ mutant strains. Cells were grown and *GAL* locus peripheral localization was analyzed as described for **Figure 1A**. Approximately 50-100 cells were scored for each condition.

Figure S4. *GAL1* mRNA levels with the Nup2-LacI gene tether. *GAL1* mRNA levels were measured by qRT-PCR as described in **Figure 3**, after 1 hour induction with galactose.