Supplementary Materials

Figure legends

Figure S1. *GAL* memory lasts for 14hours of growth in glucose. Gal1-mCherry levels, normalized to the constitutively expressed CFP (P_{TDH}-*CFP*), during activation and reactivation after growth in glucose for 12h, 14h and 18h. Error bars represent SEM for ≥ 3 biological replicates of at least 5,000 cells.

Figure S2. Memory Recruitment Sequence (MRS$_{GAL1}$) regulated *GAL1* peripheral localization during memory is sensitive to the fluorescent marker for nuclear envelope. A. Schematic of *GAL1* promoter fragments inserted next to the *URA3:LacO*. The + and - signs indicate fragments that did or did not lead to statistically significant peripheral localization under memory conditions (galactose → glucose, 12h). The MRS$_{GAL1}$ (-336 to -398 within the *GAL1* promoter) is sufficient to target *URA3* to nuclear periphery during memory. Colored boxes indicate the relative positions of the annotated *cis*-regulatory elements (BRICKNER *et al.* 2016). B. The red bars in the schematic represent the segments of the MRS$_{GAL1}$ in which transversion mutations were introduced at every alternate base. Below: localization of wild-type and transversion mutants of MRS$_{GAL1}$ inserted at *URA3:LacO* scored for peripheral localization under memory conditions. *GAL1* peripheral localization either in fixed cells using immunofluorescence (C) or in live cells (D) grown under repressing (glucose), activating (galactose) and memory (galactose → glucose, 12h) conditions with and without overexpressed red fluorescent protein directed to either ER membrane (Heh2-L-mCherry; (MEINEMA *et al.* 2011; EGECIOGLU *et al.* 2014) or ER lumen (dsRed-HDEL; (GREEN *et al.* 2012). D. Left: Representative images of cells having LacO array integrated downstream of *GAL1* gene, expressing GFP-LacI (green) and Pho88-mCherry (red) and scored as localized to nucleoplasm or periphery. The hatched line represents the level of co-localization with the nuclear envelope predicted by chance and error bars represent SEM from at least 3 independent replicates of 30-50 cells. Scale bar = 1 µm. * $p \leq 0.05$ (Student's t-test) relative to repressing condition.

Figure S3. Nup100-dependent *GAL1* peripheral localization during transcriptional memory. The experiments were done under repressing (glucose), activating (galactose) and memory (galactose → glucose, 12h) conditions. A. ChIP of TAP-tagged Nup2 and Nup100. The enrichment for *GAL1* promoter and *RPA34* (negative control) in the IP was quantified relative to the input fraction by qPCR. B. Confocal images of cells with Nup2-FRB-GFP or Nup100-FRB-GFP before and after 1 h rapamycin treatment. Left: GFP fluorescence in live cells, imaged with identical settings. Right: immunofluorescence against Nsp1 shows that NPC number or structural integrity is not altered by anchor away of Nup2 or Nup100. Scale bar = 5 µm. C. Peripheral localization of *GAL1* in live cells depleted of Nup2 and Nup100 by Anchor Away (HARUKI *et al.* 2008). The hatched line represents the level of co-localization with the nuclear envelope predicted by chance. Error bars represent SEM from at least 3 independent replicates of 30-50 cells. * $p \leq 0.05$ (Student's t-test) relative to the repressing condition.

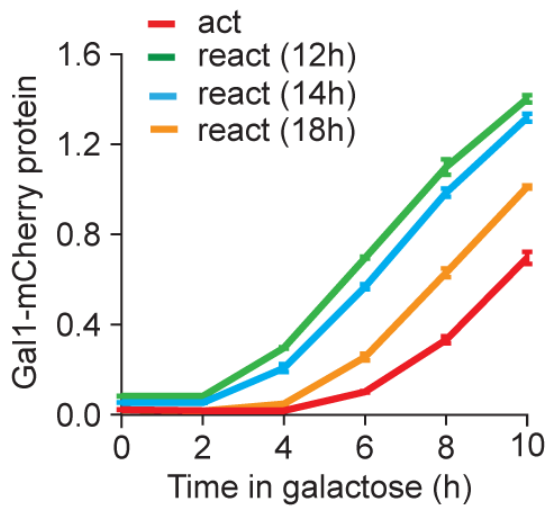
Figure S4. Gal1 expression levels upon shift from galactose to different sugars. Gal1-mCherry levels, normalized to P_{TDH}-*CFP*, in cells that were transferred from galactose to galactose (gal.), glucose (glc.) or raffinose (raff.) for 4h, measured using flow cytometry. Error bars represent SEM from at least 3 independent replicates.

Figure S5. Tup1 is not required for short term *GAL1* memory. Gal1-mCherry levels, normalized to the constitutively expressed CFP (P_{TDH}-*CFP*) upon reactivation during short-term memory in wild-type and *tup1*Δ cells, measured using flow cytometry. To induce short-term *GAL* memory, cells were shifted from glucose to galactose for 2h, back to glucose for 1h and then to galactose for reactivation. Error bars represent SEM from at least 3 independent replicates.

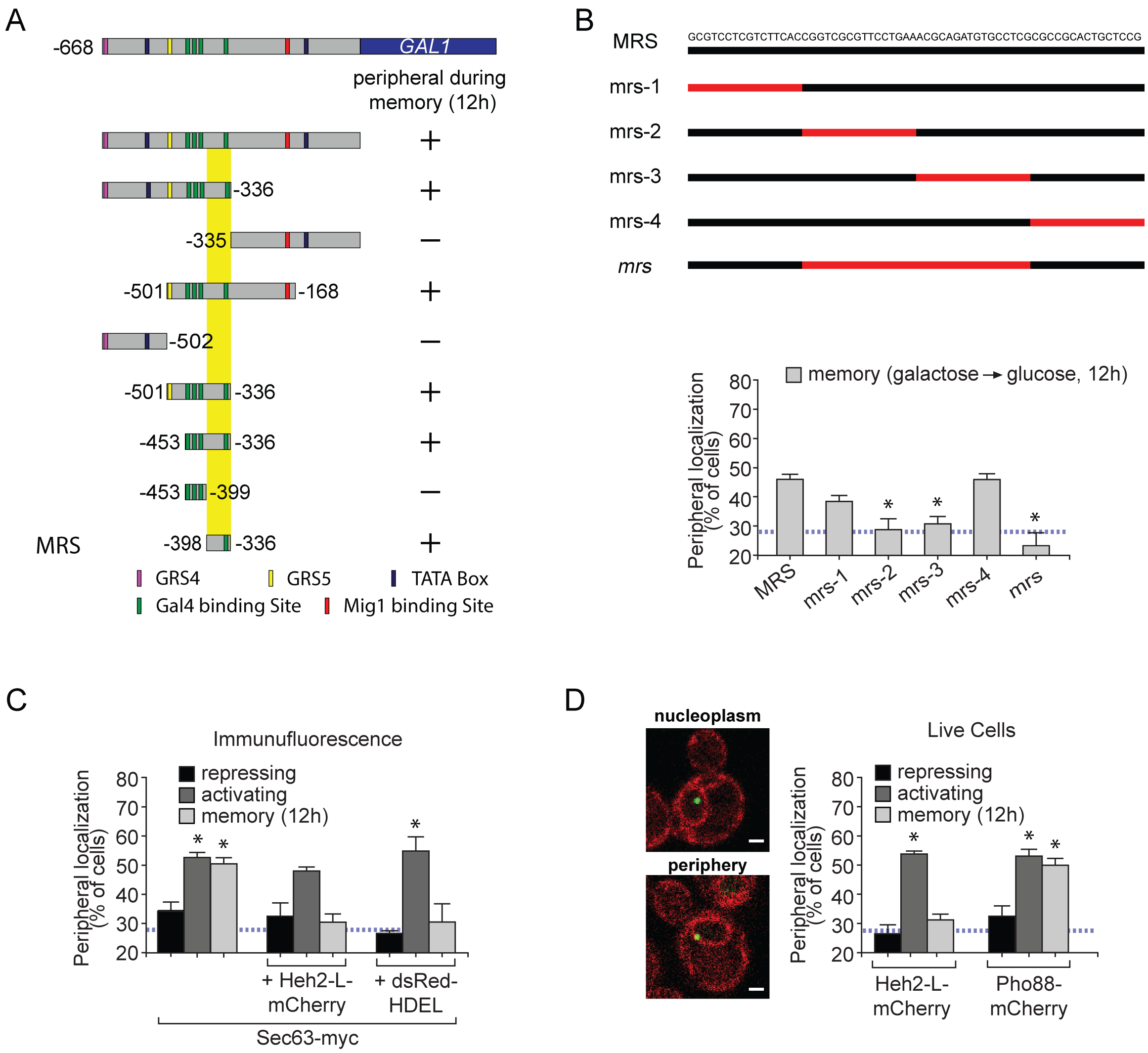
Figure S6. *GAL* transcriptional memory is independent of Sko1. Gal1-mCherry levels, normalized to the constitutively expressed CFP (P_{TDH}-*CFP*), were assayed in wild-type and *sko1*Δ cells at different times during activation (act) or during reactivation (react) after 12h of growth in glucose. Error bars represent SEM from at least 3 independent replicates.

Figure S7. Loss of Mig1 promotes faster/stronger expression of *GAL1* under all conditions. A & B. Time course of RT-qPCR for *GAL1* expression relative to *ACT1* during activation (glucose → galactose, A) and reactivation (galactose → glucose, 12 h → galactose, B) in wild-type and *mig1Δ* cells. Error bars represent SEM from at least 3 independent replicates.

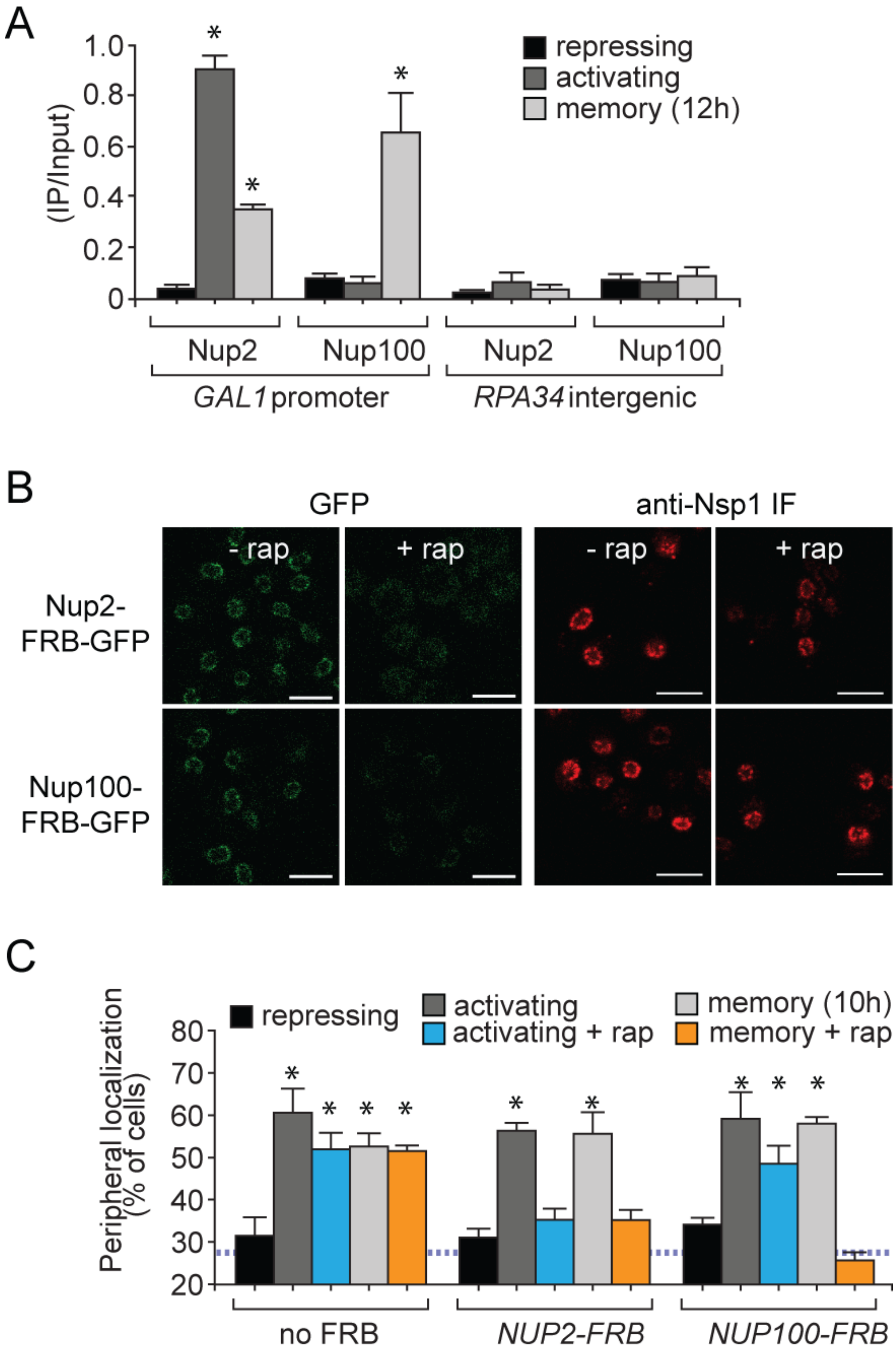
Supplementary Figures



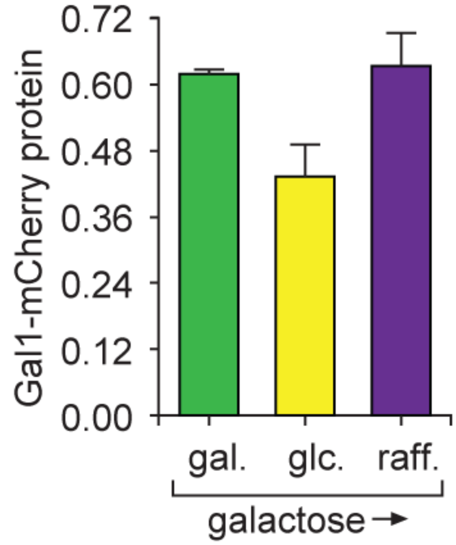
Sood *et al.*, Figure S1



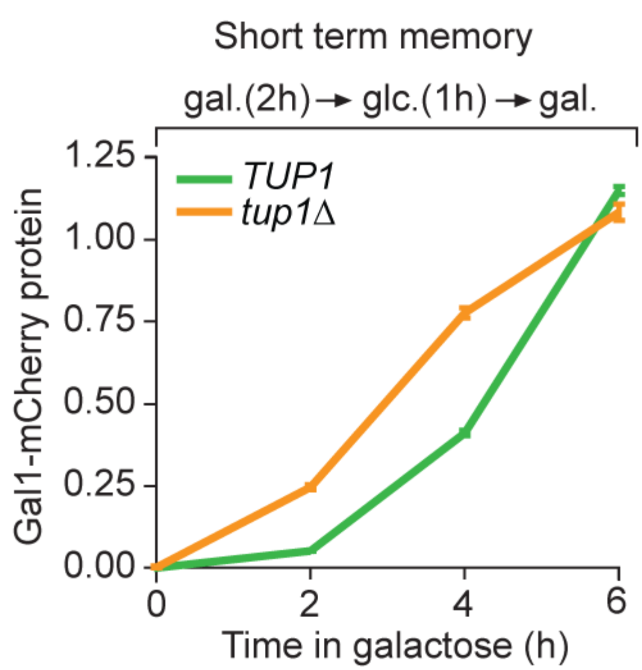
Sood *et al*., Figure S2



Sood *et al.*, Figure S3



Sood *et al.*, Figure S4



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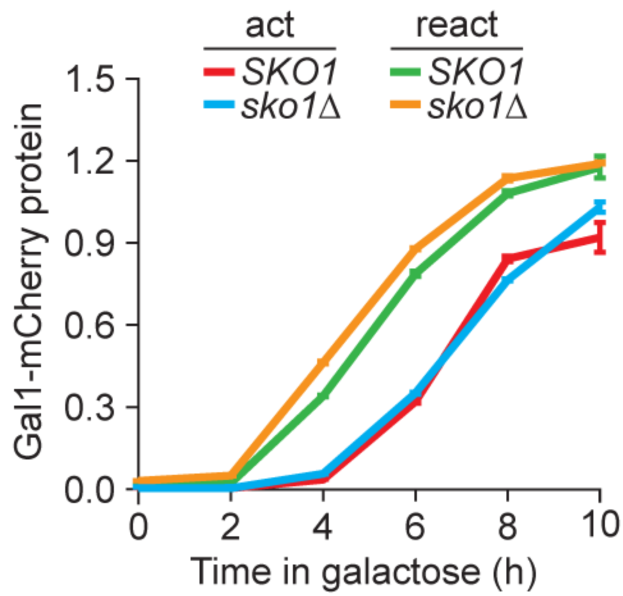
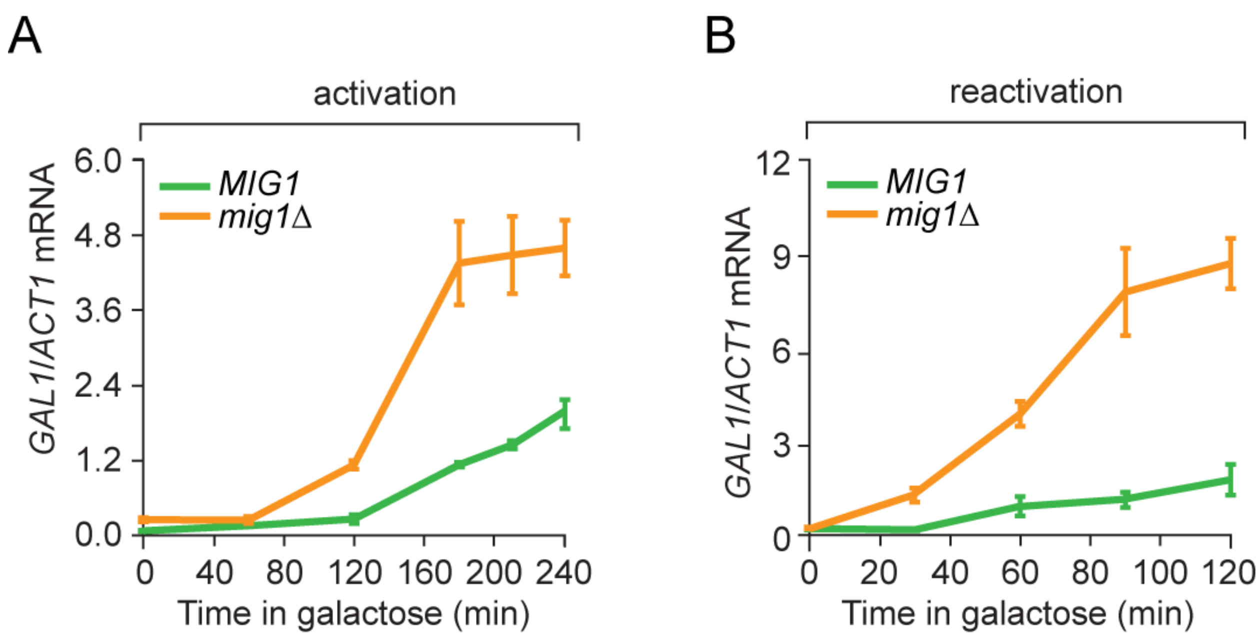


Sood *et al.*, Figure S6



Sood *et al.*, Figure S7

Supplementary Tables

Table 1. Oligonucleotides used in this study

Name	Sequence 5' to 3'
ACT1 CDS F	GGTTATTGATAACGGTTCTGGTATG
ACT1 CDS R	ATGATACCTTGGTGTCTTGGTCTAC
GAL1 CDS F	GTTCGATTTGCCGTTGGACGG
GAL1 CDS R	GGCAAACCTTTCCGGTGCAAG
GAL1 prom F	CCCCACAAACCTTCAAATTAACG
GAL1 prom R	CGCTTCGCTGATTAATTACCCC
GAL2 CDS F	TTGTTTCACAGCAACCCCAA
GAL2 CDS R	CAAAGCAAGGAAACGGTAACA
GAL2 prom F	GATCACTCCGAACCGAGATTAG
GAL2 prom R	GCACAGTTAACTTTCTAGCAGG
MRS-1	TCT TAC TCT TAT GCA TCG GTC GCG TTC CTG AAA CGC AGA TGT GCC TCG CGC CGC ACT GCT CCG AT
MRS-2	GCG TCC TCG TCT TCA CAG TTA GAG GTA CTT ACA CGC AGA TGT GCC TCG CGC CGC ACT GCT CCG AT
MRS-3	GCG TCC TCG TCT TCA CCG GTC GCG TTC CTG AAA GGA ATA GGT TCA TAG CGC CGC ACT GCT CCG AT
MRS-4	GCG TCC TCG TCT TCA CCG GTC GCG TTC CTG AAA CGC AGA TGT GCC TCG AGA CTC AAG GAT ACT AT
mrs	AGT TCG CGT GCA TTA CAC GCA TAG GGG AAT CGC GCC GCA CTG CTC CGA ACA ATA AAG ATT CTA C
GAL2 3' F	ACGTGGATCCTTGGAAATCTGAAGGCTGGA
GAL2 3' R	ACGTGCGGCCGCCGTTCGAACATTCTCACTCCA
BUD3 prom F	CATTCTACTGCTGCTACCT
BUD3 prom R	TTTCAGAGTAAAGAGACGAC

Prm1 CDS F	TTA GTC TTT GGG TCA ATG TTC TCT G
Prm1 CDS R	ATC AGC AGT GCT TTC AAA CAT GGA A
GAL7 CDS F	TTCTAGCCATTCCCATAGACG
GAL7 CDS R	TCCTGTTGACCTAACCAAGGT
RPA34 F	GCGTATGTGCGTATAACTGTGTGTAACATAAG
RPA34 R	CATTCATCAGTTTCCACCAGCAGAAATGCC

Table 2. Yeast strains used in this study

Strain Name	Genotype	Figures
CRY1	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	
CRY2	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	
VSY057	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 HO::P-TDH-CFP-NatMX GAL1-mCHERRY:KanMX6 URA3:ADH1pro-gal1-∆SA*	Figure 1
VSY106	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 GAL1-GFP:KanMx*	Figure 1
ICY083	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 LEU2:LacI-GFP GAL1:URA3p6LacO128 TRP1:ADH1pro-GAL1*	Figure 1
ICY075	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 LEU2:LacI-GFP GAL2:URA3p6LacO128*	Figure 1
ICY150	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 His5::gal1∆ LEU2:LacI-GFP GAL2:URA3p6LacO128*	Figure 1
ICY167	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 LEU2:LacI-GFP GAL2:URA3p6LacO128 TRP1:ADH1pro-GAL1*	Figure 1
VSY034	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 HO::*P_{TDH}*-CFP-NatMX GAL1-mCHERRY:KanMX6*	Figure , S4, S5,
VSY057	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 HO::*P_{TDH}*-CFP-NatMX GAL1-mCHERRY:KanMX6 URA3:ADH1pro-GAL1*	Figure 1,
DBY032	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:Kan^r HIS3:LacI-GFP GAL1:URA3p6LacO128*	Figure 2
VSY039	*MAT a ade2-1 can1-100, his3-11,15 ura3-1 mrsGAL1*	Figure 2
VSY003	*MAT a ade2-1 can1-100, his3-11,15 ura3-1 nup100∆:KanMX6*	Figure 2,
VSY069	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 Pho88-mCherry:HIS5 LEU2:LacI-GFP GAL1:URA3p6LacO128*	Figure2
VSY089	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 Pho88-mCherry:HIS5 LEU2:LacI-GFP GAL1:URA3p6LacO128 nup100∆::KanMX6*	Figure2
ICY185	*MATa ade2-1 can1-100, his3-11,15 ura3-1,112 trp1-1 ura3-1Sec63-myc::TRP1 LEU2:LacI-GFP URA3:p6LacO128-KanMX6*	Figure 2
KVY001	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:Kan^r HIS3:LacI-GFP URA3:GAL1prom-p6LacO128*	Figure 2,
ICY195	*MATa ade2-1 can1-100, his3-11,15 ura3-1 Sec63-myc::TRP1 LEU2:LacI-GFP URA3:p6LacO128Amp∆::Ab2.2-KanMX6*	Figure 2,
VSY096	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 Pho88-mCherry:HIS5 LEU2:LacI-GFP URA3:p6LacO128Ab2.2*	Figure 2
VSY097	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 Pho88-mCherry:HIS5 LEU2:LacI-GFP URA3:p6LacO128Ab2.2 nup100∆::KanMX*	Figure 2
VSY099	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 Pho88-mCherry:HIS5 LEU2:LacI-GFP URA3:p6LacO128Ab2.2 TRP1:ADHprom-GAL1*	Figure 2

VSY042	*MATa ade2-1 can1-100, his3-11,15 ura3-1 TRP1:Sec63-13XMyc LEU2:LacI-GFP URA3:p6LacO128AmpΔ::GAL1prom-KanMX6*	Figure 2
VSY043	*MATa ade2-1 can1-100, his3-11,15 ura3-1 TRP1:Sec63-13XMyc LEU2:LacI-GFP URA3:p6LacO128AmpΔ::mrsGAL1prom-KanMX6*	Figure 2,
VSY047	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 LEU2:LacI-GFP GAL1:URA3p6LacO128 TRP1:Sec63-13XMyc SEC63-13XMyc:KanMX6*	Figure 2
VSY048	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 LEU2:LacI-GFP mrsGAL1:URA3p6LacO128 TRP1:Sec63-13XMyc SEC63-13XMyc:KanMX6*	Figure 2
VSY100	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 Pho88-mCherry:HIS5 LEU2:LacI-GFP GAL1:URA3p6LacO128*	Figure 2
AFY28	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1,HIS3: LacIGFP SEC63-13myc:TRP1 nup100Δ::KANMX GAL1:URA3p6LacO128*	Figure 2
ICY176	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 mig1Δ::His5+ LEU2:LacI-GFP GAL1:URA3p6LacO128*	Figure 3
ICY63	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 tup1Δ::His5 LEU2:LacI-GFP GAL1:URA3p6LacO128*	Figure 3
VSY098	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 Pho88-mCherry:HIS5 LEU2:LacI-GFP URA3:p6LacO128Ab2.2 tup1Δ::KanMX*	Figure 3
ICY29	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 tup1::His5*	Figure 4,
VSY102	*MATa ade2-1 can1-100, his3-11,15 HO::TDH1prm-CFP-NatMX GAL1-mCHERRY:KanMX6 tup1Δ::HIS5*	Figure 4,
VSY103	*MATa ade2-1 can1-100, his3-11,15 HO::TDH1prm-CFP-NatMX GAL1-mCHERRY:KanMX6 tup1Δ::HIS6 URA3:ADH1prom-GAL1*	Figure 4,
DBY051	*MATalpha ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 htz1Δ::HIS5 SEC63-13myc::KANMX HIS3:LacI-GFP GAL1:URA3p6LacO128*	Figure 5
ICY39	*MATalpha ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 htz1Δ::HIS5*	Figure 5
ICY165	*MATalpha ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 htz1Δ::HIS5 URA3:ADH1pro-GAL1*	Figure 5
VSY094	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 GAL1:URA3p6LacO128 SEC63-13myc:KanMX HIS3:LacI-GFP TRP1:Heh2-L-mCHERRY*	Figure S2
VSY095	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 GAL1:URA3p6LacO128 SEC63-13myc:KanMX HIS3:LacI-GFP TRP1:dsRed-HDEL*	Figure S2
ICY186	*MAT A ade2-1 can1-100, his3-11,15 ura3-1 Sec63-myc::TRP1 LEU2:LacI-GFP URA3:p6LacO128AmpΔ::GALproIA-KanMX6*	Figure S2
ICY187	*MAT A ade2-1 can1-100, his3-11,15 ura3-1 Sec63-myc::TRP1 LEU2:LacI-GFP URA3:p6LacO128AmpΔ::GALproIB-KanMX6*	Figure S2
ICY188	*MAT A ade2-1 can1-100, his3-11,15 ura3-1 Sec63-myc::TRP1 LEU2:LacI-GFP URA3:p6LacO128AmpΔ::GALproAb-KanMX6*	Figure S2
ICY189	*MAT A ade2-1 can1-100, his3-11,15 ura3-1 Sec63-myc::TRP1 LEU2:LacI-GFP URA3:p6LacO128AmpΔ::GALproAa-KanMX6*	Figure S2
ICY190	*MAT A ade2-1 can1-100, his3-11,15 ura3-1 Sec63-myc::TRP1 LEU2:LacI-GFP URA3:p6LacO128AmpΔ::GALproAa1-KanMX6*	Figure S2
ICY191	*MAT A ade2-1 can1-100, his3-11,15 ura3-1 Sec63-myc::TRP1 LEU2:LacI-GFP URA3:p6LacO128AmpΔ::GALproAa2-KanMX6*	Figure S2
ICY192	*MAT A ade2-1 can1-100, his3-11,15 ura3-1 Sec63-myc::TRP1 LEU2:LacI-GFP URA3:p6LacO128AmpΔ::GALproUAS1,2,4mut-KanMX6*	Figure S2
ICY193	*MAT A ade2-1 can1-100, his3-11,15 ura3-1 Sec63-myc::TRP1 LEU2:LacI-GFP URA3:p6LacO128 SUP4-o*	Figure S2
ICY194	*MAT A ade2-1 can1-100, his3-11,15 ura3-1 Sec63-myc::TRP1 LEU2:LacI-GFP URA3:p6LacO128AmpΔ::Ab2.1-KanMX6*	Figure S2
VSY036	*MAT a ade2-1 can1-100, his3-11,15 ura3-1 TRP1:Sec63-13XMyc LEU2:LacI-GFP URA3:p6LacO128AmpΔ::mrs1-KanMX6*	Figure S2
VSY037	*MAT a ade2-1 can1-100, his3-11,15 ura3-1 TRP1:Sec63-13XMyc LEU2:LacI-GFP URA3:p6LacO128AmpΔ::mrs2-KanMX6*	Figure S2
VSY038	*MAT a ade2-1 can1-100, his3-11,15 ura3-1 TRP1:Sec63-13XMyc LEU2:LacI-GFP URA3:p6LacO128AmpΔ::mrs3-KanMX6*	Figure S2

VSY039	*MAT a ade2-1 can1-100, his3-11,15 ura3-1 TRP1:Sec63-13XMyc LEU2:LacI-GFP URA3:p6LacO128Amp∆::mrs4-KanMX6*	Figure S2
VSY040	*MAT a ade2-1 can1-100, his3-11,15 ura3-1 TRP1:Sec63-13XMyc LEU2:LacI-GFP URA3:p6LacO128Amp∆::mrs-KanMX6*	Figure S2
Nup100-TAP	*MATa his3∆1 leu2∆0 met15∆1 ura3∆0 Nup100-TAP::His5+*	Figure S3
Nup2-TAP	*MATa his3∆1 leu2∆0 met15∆1 ura3∆0 Nup12-TAP::His5+*	Figure S3
VSY090	*MATalpha tor1-1 fpr1::NAT RPL13A-2×FKBP12::TRP1 LEU2:LacI-GFP Pho88-mCherry:HIS5 GAL1:URA3p6LacO128*	Figure S3
VSY091	*MATalpha tor1-1 fpr1::NAT RPL13A-2×FKBP12::TRP1 LEU2:LacI-GFP Pho88-mCherry:HIS5 GAL1:URA3p6LacO128 NUP2-FRB:KanMX6*	Figure S3
VSY092	*MATalpha tor1-1 fpr1::NAT RPL13A-2×FKBP12::TRP1 LEU2:LacI-GFP Pho88-mCherry:HIS5 GAL1:URA3p6LacO128 NUP100-FRB:KanMX6*	Figure S3
ADY046	*MATalpha tor1-1 fpr1::NAT RPL13A-2×FKBP12::TRP1 NUP100-FRB-GFP:HIS5*	Figure S3
CEY346	*MATalpha tor1-1 fpr1::NAT RPL13A-2×FKBP12::TRP1 NUP2-FRB-GFP:HIS5*	Figure S3
VSY149	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 HO::P_{TDH}-CFP-NatMX GAL1-mCHERRY:KanMX6 sko1Δ::HIS5*	Figure S6
VSY107	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 mig1∆::His5*	Figure S7

Table 3. List of GFP tagged genes used for standard curve between GFP fluorescence and abundance

YKL060C
YLL024C
YCR012W
YLR249W
YAL005C
YPL061W
YLR109W
YHL033C
YBR196C
YAL038W
YHR183W
YNL067W
YDR385W
YDR382W
YER091C
YPR035W
YPR035W
YDR447C
YGR254W
YDL185W

Supplement References

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