nature cell biology

DNA zip codes control an ancient mechanism for gene targeting to the nuclear periphery

Sara Ahmed[1], Donna G. Brickner[1], William H. Light[1], Ivelisse Cajigas[1], Michele McDonough[2], Alexander B. Froyshteter[1], Tom Volpe[2] and Jason H. Brickner[1,3]

Many genes in *Saccharomyces cerevisiae* are recruited to the nuclear periphery after transcriptional activation. We have identified two gene recruitment sequences (GRS I and II) from the promoter of the *INO1* gene that target the gene to the nuclear periphery. These GRSs function as DNA zip codes and are sufficient to target a nucleoplasmic locus to the nuclear periphery. Targeting requires components of the nuclear pore complex (NPC) and a GRS is sufficient to confer a physical interaction with the NPC. GRS I elements are enriched in promoters of genes that interact with the NPC, and genes that are induced by protein folding stress. Full transcriptional activation of *INO1* and another GRS-containing gene requires GRS-mediated targeting of the promoter to the nuclear periphery. Finally, GRS I also functions as a DNA zip code in *Schizosaccharomyces pombe*, suggesting that this mechanism of targeting to the nuclear periphery has been conserved over approximately one billion years of evolution.

The spatial organization of DNA within the nucleus compartmentalizes the genome into different subnuclear environments that might affect gene expression. Both transcriptionally active and inactive genes localize at the nuclear periphery[1–3]. Localization of individual genes within the nucleus can also be dynamically controlled. For example, in the budding yeast *S. cerevisiae*, many inducible genes rapidly relocalize from the nucleoplasm to the nuclear periphery after activation[4–9].

How genes are targeted from one location to another within the nucleus is unclear. Localization could simply reflect changes in transcriptional status, chromatin structure or the production of nascent RNA. Targeting of certain genes seems to involve nascent RNA transcripts that might mediate recruitment to the nuclear periphery in yeast[6,7,10]. Alternatively, changes in localization could represent gene targeting, controlled by *cis*-acting DNA elements. Consistent with this idea, peripheral targeting of certain genes in budding yeast is independent of transcription[11,12].

We have approached this problem by studying the mechanism by which the yeast *INO1* gene is recruited to the nuclear periphery. This gene is targeted to the nuclear periphery after activation[5]. Localization of *INO1* to the nuclear periphery is controlled by two *cis*-acting DNA elements. These elements function as DNA zip codes that are sufficient to target an ectopic locus to the nuclear periphery. One of these elements is also sufficient to target an ectopic locus to the nuclear periphery in the highly divergent fission yeast *S. pombe*, suggesting that this mechanism of targeting is ancient. Finally, we show that full transcriptional activation of *INO1* requires at least one of these DNA zip codes in the promoter of the gene. This suggests that the genome can encode for its own spatial organization and that this affects gene expression.

RESULTS

Identification of a DNA element required for recruitment of *INO1* to the nuclear periphery

We monitored gene localization with respect to the nuclear periphery by expressing the lac-repressor fused to GFP in a strain that has an array of lac-repressor binding sites integrated at the chromosomal locus of interest (Fig. 1a)[13,14]. We then quantified the fraction of the population in which the GFP spot colocalizes with the nuclear envelope[5]. A nucleoplasmic locus such as *URA3* colocalizes with the nuclear envelope marker in about 27% of cells[5] (Fig. 1b, indicated as a hatched blue line throughout). On activation by inositol starvation, *INO1* colocalized with the nuclear envelope in about 60% of cells in the population (Fig. 1b). To determine whether targeting of *INO1* is dependent on chromosomal context, we integrated the *INO1* gene and the lac-repressor array beside the *URA3* gene (*URA3:INO1*; Fig. 1a). We found that this hybrid locus was targeted to the nuclear periphery on inositol starvation (Fig. 1b). The *INO1* gene, therefore, was sufficient to confer peripheral targeting of the *URA3* locus.

To identify *cis*-acting subnuclear targeting element(s), we deleted several 100-bp segments from the *INO1* promoter sequence (Fig. 1c) and tested their ability to target *URA3* to the nuclear periphery (Fig. 1d). Loss of segment 4 resulted in unregulated peripheral targeting of *URA3:INO1* (Fig. 1d) and unregulated, modest *INO1* transcription (similar to the

[1]Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208, USA. [2]Department of Cell and Molecular Biology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA.
[3]Correspondence should be addressed to J.H.B (e-mail: j-brickner@northwestern.edu)

Received 22 October 2009; accepted 23 December 2009; published online 24 January 2010; corrected online 27 January 2010; DOI: 10.1038/ncb2011

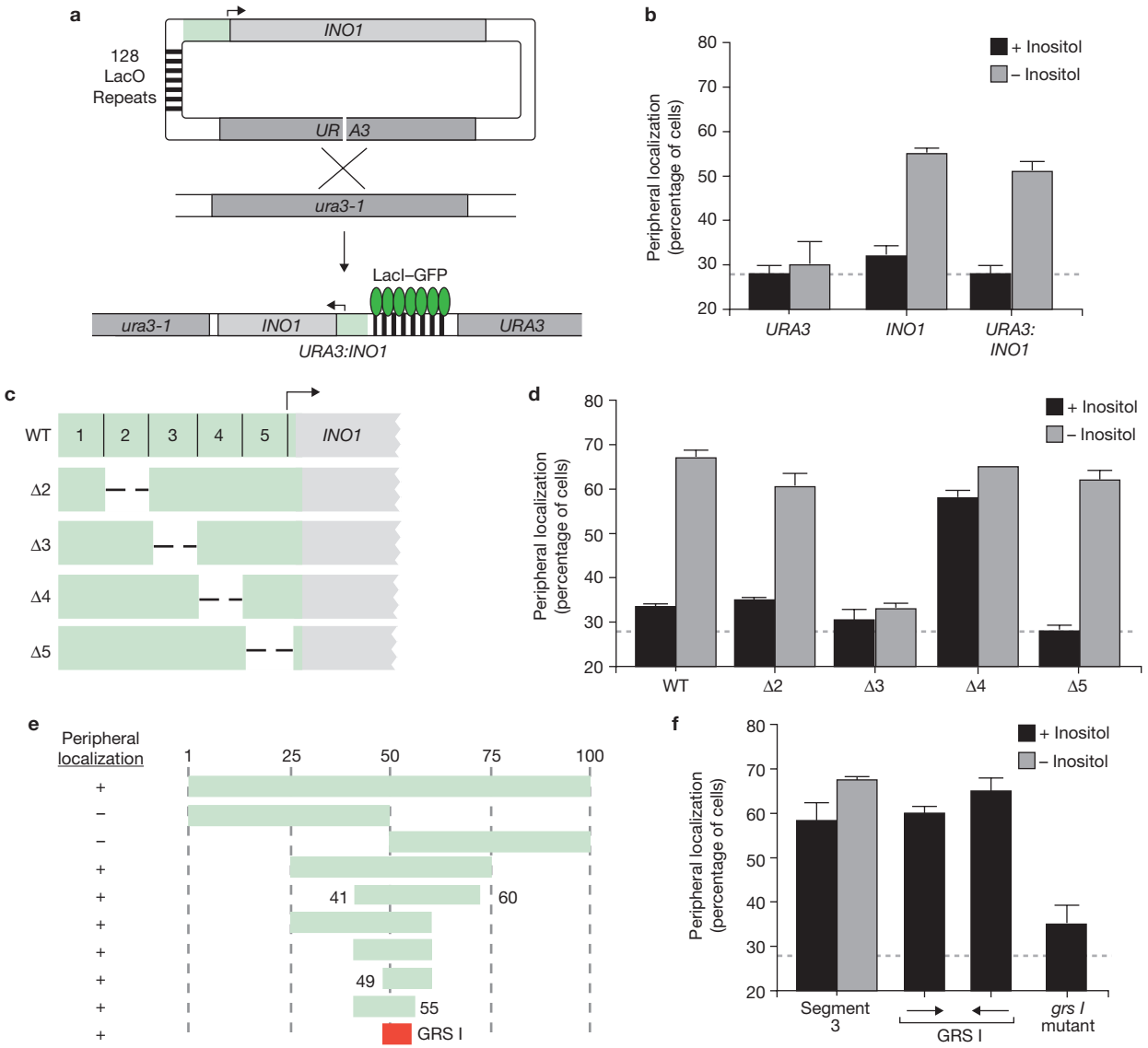

Figure 1 Identification of a gene recruitment sequence (GRS) in the *INO1* promoter. (**a**) Integration scheme for integrating *INO1* and the lac-repressor array at *URA3* by homologous recombination. The *INO1* gene included 504 bp upstream and 685 bp downstream of the coding sequence. (**b**) The fraction of the population in which the GFP spot colocalized with the nuclear envelope marker Sec63–Myc for cells grown in the presence or absence of myoinositol (100 μM). The hatched blue line represents the mean peripheral localization for *URA3*. The maximal peripheral localization observed using this assay is about 80% of cells for a gene that is artificially tethered to the nuclear envelope[5]. Therefore, the dynamic range of this assay is 20–80%. Data represent the mean ± s.e.m from 5 biological replicates (30–50 cells were analysed per replicate). (**c**, **d**) Map (**c**) and peripheral localization (**d**) of 100 bp non-overlapping deletions in the *INO1* promoter integrated at *URA3*. (**e**) Map of fragments within segment 3 that were integrated at *URA3* and their peripheral localization (see Supplementary Information, Fig. S1a for complete data). (**f**) Peripheral localization of segment 3 in the presence and absence of inositol, the 8 bp GRS I in either orientation (indicated by the arrows) or a mutant version of GRS I (*grs 1* mutant) integrated at *URA3*. $n = 5$ (**b**) or 3 (**d** and **f**), 30–50 cells per biological replicate).

INO1-100 mutant[15]; data not shown). Loss of segment 3 blocked targeting of *URA3:INO1* to the nuclear periphery (Fig. 1d), suggesting that segment 3 contains DNA sequences necessary for targeting *URA3:INO1* to the nuclear periphery.

A DNA element required for *INO1* targeting to the nuclear periphery functions as a DNA zip code

We next integrated the 100-bp segment 3 alone beside *URA3* and found that it was sufficient to target *URA3* to the nuclear periphery (Fig. 1e, f). Segment 3, therefore, functioned as a DNA zip-code: a DNA sequence that is sufficient to target an ectopic locus to a particular subnuclear location. When removed from the *INO1* promoter, segment 3-mediated peripheral localization was no longer regulated by inositol (Fig. 1f). This suggests that the peripheral targeting element is ordinarily negatively regulated in the context of the *INO1* promoter.

To identify a minimal gene recruitment sequence (GRS), we integrated a series of smaller fragments from segment 3 at *URA3* and determined their peripheral targeting activity (Fig. 1e; Supplementary Information, Fig. S1). All the DNA fragments that were active for peripheral targeting contained a common 8-bp sequence (Fig. 1e). When this 8-bp fragment

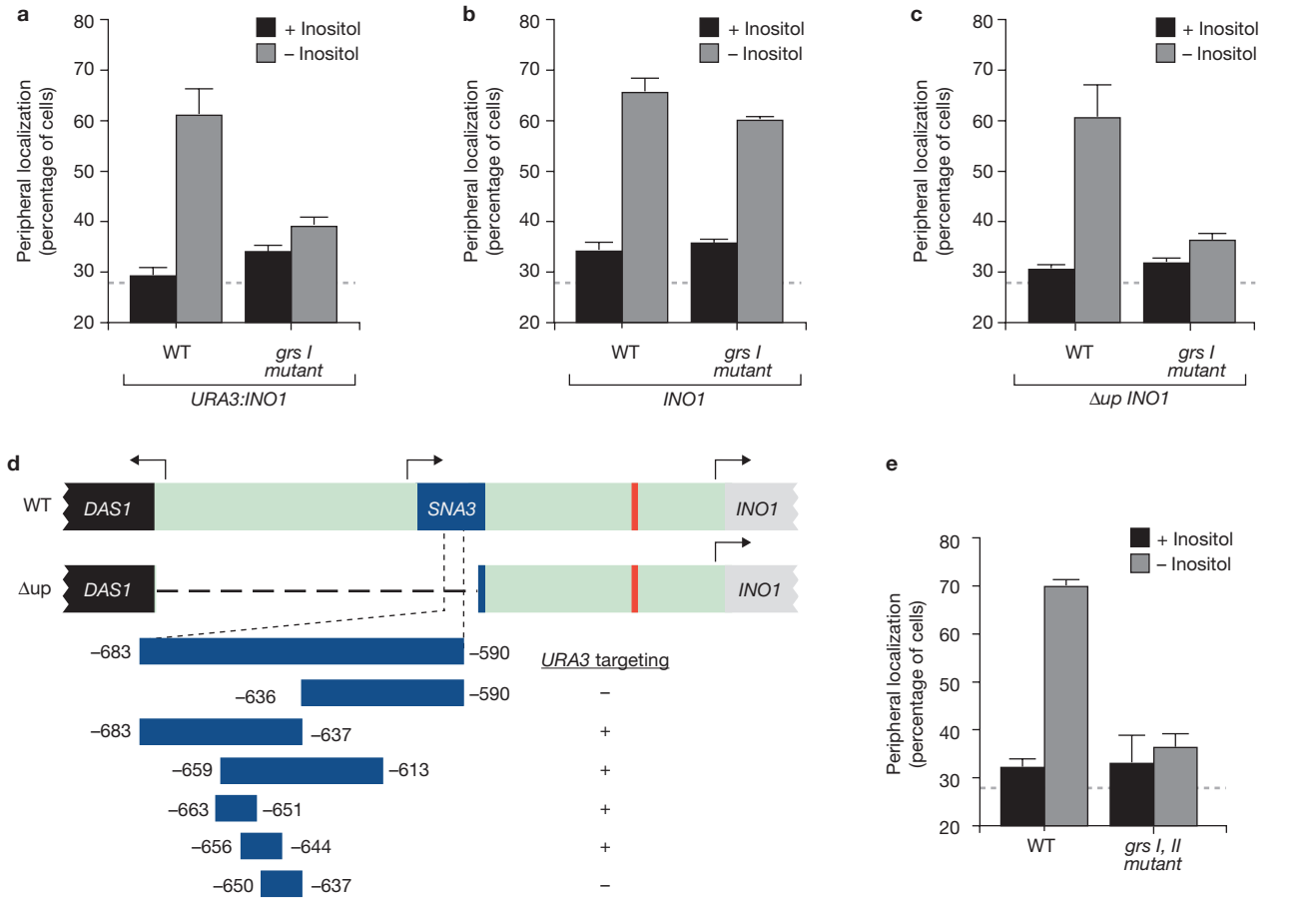

Figure 2 Targeting of the endogenous *INO1* gene is mediated by two redundant DNA zip codes. (**a**, **b**) Localization of wild-type (WT) *INO1* and *grs I* mutant *INO1* integrated either at *URA3* (**a**) or at *INO1* (**b**). (**c**) Localization of wild-type or *grs I* mutant *INO1* at endogenous *INO1* in a strain lacking the 943 bp upstream of GRS I as depicted in panel **d**. Bars in **a**–**c** are means ± s.e.m.; for panels **a**–**d**, $n = 3$, 30–50 cells per replicate. (**d**) Map of fragments used to identify GRS II by integration at *URA3* and their peripheral localization (see Supplementary Information, Fig. S1a for complete data). (**e**) Peripheral localization of a combined mutation in GRS I and GRS II at endogenous *INO1*. Bars are means ± s.e.m.; $n = 3$, 30–50 cells per replicate. The hatched blue line in panels **a**–**c** and **e** represents the mean peripheral localization for *URA3*.

(GRS I; see below) was integrated in either orientation beside *URA3*, it functioned to target *URA3* to the nuclear periphery (Fig. 1f).

To verify that GRS I is responsible for peripheral targeting of full-length *INO1*, we introduced a transition mutation in GRS I (*grs I*) in the *INO1* promoter and tested the effect of this mutation on peripheral targeting of *URA3:INO1*. Mutation of GRS I blocked targeting of *URA3:INO1* to the nuclear periphery, confirming that it was the element responsible for this relocalization (Fig. 2a).

Peripheral targeting of the endogenous *INO1* gene is mediated by two redundant DNA zip codes

We then introduced the *grs I* mutation into the promoter of the endogenous *INO1* gene. This mutation did not block targeting of *INO1* to the nuclear periphery (Fig. 2b). We hypothesized that additional, redundant targeting elements also contribute to peripheral targeting of endogenous *INO1*. We found that deletion of a 943-bp region upstream of *INO1* (*Δup INO1*; Fig. 2d) led to GRS I-dependent targeting of *INO1* (Fig. 2c). This suggests that additional targeting elements exist within this 943-bp region.

We integrated a series of fragments from this 943-bp region at *URA3* and tested their targeting activity (Fig. 2d; Supplementary Information, Fig. S1b, c). We identified a second DNA zip code, GRS II, embedded within the upstream *SNA3* gene. GRS I and GRS II are redundant; mutation of either element alone had no effect on peripheral targeting of *INO1* (Fig. S2a). However, loss of both GRS I and GRS II blocked targeting of endogenous *INO1* to the nuclear periphery (Fig. 2e).

GRS I-containing genes in the *S. cerevisiae* genome

From their sequences, GRS I (5′-GGGTTGGA-3′) and GRS II (5′-GAATGATTGCTGGGAAGAAT-3′) are not obviously related. GRS I does not correspond to any known binding site[16,17]. A sequence within GRS II (5′-TGCTGG-3′) resembles the binding site for the daughter-specific transcription factor Ace2 (ref. 18). However, peripheral targeting does not correlate with Ace2-dependent transcription and we have not observed an interaction of the *INO1* promoter with Ace2 *in vivo* using chromatin immunoprecipitation (ChIP; data not shown). Therefore, these elements probably represent previously uncharacterized DNA binding sites.

Perfect matches of GRS I appear 280 times in the yeast genome as a whole and 97 times in 94 promoters (within 1000 bp 5′ of the transcription initiation site). Among these genes, the most significantly overrepresented gene ontology class was 'cellular response to heat' (corrected hypergeometric $P = 0.007$; refs 19, 20). The *INO1* gene is transcriptionally induced not only by inositol starvation, but also by unfolded protein

Table 1 Co-regulation of GRS I-element genes#

	<90th percentile§		>90th percentile†			
Condition	**Expected**	**Observed**	**Expected**	**Observed**	χ^2	***P***
Tunicamycin[24], 60 min	54	49	6	11	4.630	0.0314*
Dithiothreitol[24], 60 min	54	49	6	11	4.630	0.0314*
Dithiothreitol + Heat shock[24]	58	49	7	16	12.968	0.0003***
opi1Δ‡,[23]	67	63	7	11	2.525	0.1121
Heat shock[22], 5 min	52	47	6	11	4.647	0.031*
Heat shock[22], 10 min	56	49	6	13	9.042	0.0026**
Heat shock[22], 15 min	59	50	7	16	12.944	0.0003***
Heat shock[22], 20 min	53	47	6	12	6.679	0.0098**
Heat shock[22], 30 min	57	51	6	12	6.632	0.0100*
Nitrogen depletion, 8 h	64	61	7	10	1.426	0.2324
Nitrogen depletion, 12 h	64	60	7	11	2.536	0.1113

#Genes with GRS I element within 775 bp of the translational start site
§Expected <90th percentile = 0.9 × [number of GRS I genes in expression data]
†Expected >90th percentile = 0.1 × [number of GRS I genes in expression data]
‡This strain lacks the repressor of *INO1* transcription and overexpresses inositol-repressed genes
***Extremely significant enrichment
**Very significant enrichment
*Significant enrichment

Table 2 GRS I genes interact with the nuclear pore complex

	Non-GRS I§		GRS I†			
ChIP	**Expected**	**Observed**	**Expected**	**Observed**	χ^2	***P***
Cse1	454	443	7	18	17.552	0.0001***
Nup116	528	518	8	18	12.689	0.0004***
Nup2	290	283	4	11	12.419	0.0004***
Mlp1	392	384	6	14	10.830	0.0010***
Xpo1	384	376	6	14	10.833	0.0010***
Nup60	169	164	3	8	8.481	0.0036**
Nup100	1383	1371	21	33	6.961	0.0083**
Mlp2	431	425	7	13	5.226	0.0222*
Nup84	54	52	1	3	4.074	0.0435*
Nup145	216	214	3	5	1.352	0.2450
Kap95	94	93	1	2	1.011	0.3147
Nsp1	114	113	2	3	0.509	0.4757
Nic96	583	582	9	10	0.113	0.7369

§Expected non-GRS I genes = [number of bound targets] – [expected GRS I genes]
†Expected GRS I genes = [number of GRS I genes] x ([number of bound targets]/[number of microarray probes])
***Extremely significant enrichment
**Very significant enrichment
*Significant enrichment

stress in the endoplasmic reticulum (ER)[21], heat shock[22] and nitrogen starvation[22]. We assessed whether genes containing GRS I elements were co-regulated with *INO1* under any of these conditions (Supplementary Information, Table S1). We observed significant enrichment of GRS I-containing genes among the genes most highly induced under heat shock and ER stress conditions[23] but no significant enrichment of GRS I genes among those highly induced by inositol starvation or nitrogen deprivation (Supplementary Information, Table S1). The greatest enrichment of GRS I-containing genes was among the >90th percentile of genes induced under conditions of combined heat shock and ER stress[24]. This enrichment was more significant if we limited our analysis to genes in which the GRS I element is less than 775-bp upstream of the translational start site (Table I). This suggests that GRS I-containing promoters are significantly enriched for genes that are co-regulated by protein folding stress in the ER and the cytoplasm.

One perfect match of GRS I exists in the promoter of the *TSA2* gene, which encodes an inducible thioredoxin peroxidase that is activated by heat shock and oxidative stress[25]. We localized the *TSA2* gene and found that it localized in the nucleoplasm in the absence of stress (peripheral in 37 ± 2% of cells; Fig. 3a). In the presence of oxidative stress, *TSA2* localized to the nuclear periphery in 73 ± 3% of cells (Fig. 3a). When we introduced the *grs I* mutation into the *TSA2* promoter, targeting of *TSA2* to the nuclear periphery was blocked (Fig. 3a). This strongly suggests that GRS-mediated targeting is a general mechanism used by genes in *S. cerevisiae*.

GRS I is functional in *S. pombe*

We next asked whether GRS-mediated peripheral targeting is an evolutionarily conserved mechanism. To address this question, we tested whether the GRS I element was sufficient to direct peripheral targeting in the fission yeast *S. pombe*. This yeast is distantly related to *S. cerevisiae*, having diverged from a common ancestor between four hundred million and one billion years ago[26]. We integrated the Lac operator array plasmid with or without a single copy of GRS I at the *ura4* locus in *S. pombe* (see Methods). We then quantified the fraction of the population in which the lac operator array colocalized with the nuclear pore protein Nup120 (Fig. 3b). In the strain without the GRS I element, the *ura4* locus was nucleoplasmic, colocalizing with the nuclear envelope in 35 ± 1% of the cells in the population (Fig. 3c). However, in the strain with the GRS I element integrated at *ura4*, we observed an increase in the colocalization of the *ura4* locus with the nuclear envelope to 50 ± 3% of the cells in the population (Fig. 3c). Although this level of peripheral localization was not as high as we had observed for *URA3* in budding yeast, it represents a significant change in the localization of *ura4* ($P = 0.002$, one-tailed *t*-test). This suggests that the mechanism of GRS I targeting is ancient and conserved between two highly divergent species.

GRS I targets chromosomal loci to the NPC

Many studies have suggested that genes that are recruited on activation are targeted to the NPC[4,6–8,11,12,27–30]. We monitored peripheral targeting of *INO1* in a collection of 30 viable null mutant yeast strains lacking proteins that make up the NPC or that associate with the nuclear periphery (Fig. 4a; Supplementary Information, Fig. S3). Most of the proteins that make up the core channel[31] of the NPC were dispensable for peripheral targeting of *INO1* (Fig. 4a). By contrast, most of the proteins associated with the nucleoplasmic face of the NPC were required for peripheral localization of *INO1* (Fig. 4a). Nup1, proteins in the SAGA complex and proteins in the TREX2 complex are also required for localization of the *GAL1-10* locus to the nuclear periphery on galactose induction[11,12,28]. However, Mlp1, which is required for recruitment of *GAL1-10* (ref. 27), *GAL2* and *HSP104* (ref. 7) to the nuclear periphery, was not required for recruitment of *INO1* to the nuclear periphery (Fig. 4a). Instead, Mlp2, a homologous protein, was required for *INO1* targeting to the nuclear periphery. This suggests that different genes may use overlapping, but distinct, targeting mechanisms.

The NPC protein Nup2 interacts physically with active genes that localize at the nuclear periphery[4,11]. To test whether the *INO1* promoter associates physically with the NPC under activating conditions, we used ChIP. Nup2–TAP co-immunoprecipitated with the *INO1* promoter when the gene was active (Fig. 4b). We did not observe an interaction of Nup2–TAP with either the repressed *INO1* promoter or with a nearby intergenic region (Fig. 4b). The interaction of *INO1* with the NPC requires the GRS elements; we did not observe an interaction of the *INO1* promoter with Nup2–TAP when both GRS I and GRS II were mutated (Supplementary Information, Fig. S2c, d).

NPC mutants that blocked targeting of *INO1* to the nuclear periphery also blocked targeting of *URA3* to the nuclear periphery by GRS I alone (Fig. 4c). Furthermore, the GRS I element at *URA3* was sufficient to confer an interaction with Nup2–TAP by ChIP (Fig. 4d). This suggests that the GRS elements control targeting of *INO1* to the NPC, and that the interaction of *INO1* with the NPC observed by ChIP is mediated by DNA elements and is not a result of post-transcriptional interaction with nascent mRNA.

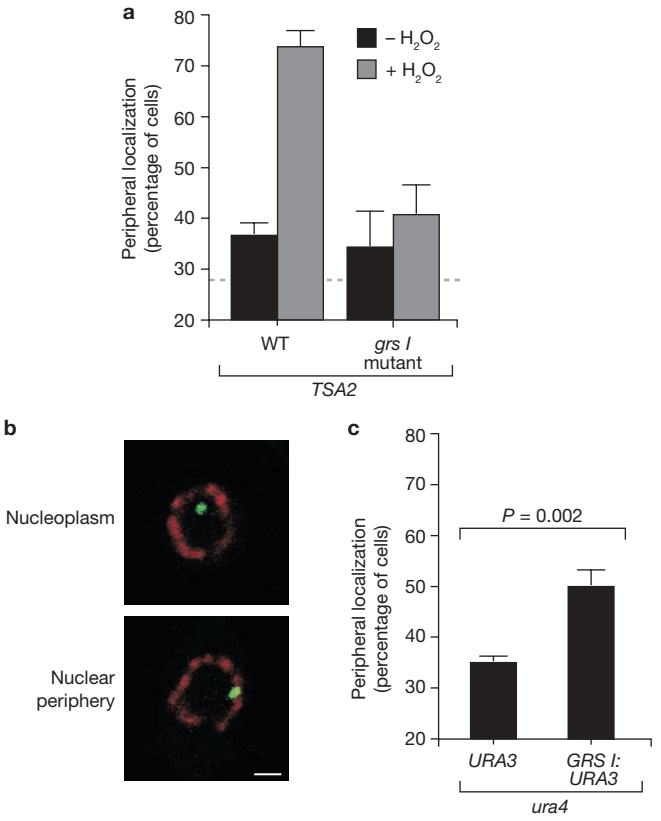

Figure 3 GRS I mediated targeting to the nuclear periphery is general and ancient. (**a**) Peripheral localization of the wild-type (WT) or *grs I* mutant *TSA2* gene was determined for a population of cells for cells grown in yeast peptone dextrose medium (YPD; $-H_2O_2$ repressing conditions) or in YPD + 0.5mM H_2O_2 (activating conditions; $n = 3$, 30–50 cells per replicate). The hatched blue line represents the mean peripheral localization for *URA3*. (**b**) Introduction of the GRS into *S. pombe*. Representative confocal micrographs of immunofluorescence against LacI–GFP (green) and Nup120–Myc (red) in *S. pombe* that were scored as either nucleoplasmic or peripheral. Scale bar, 1 μm. (**c**) The fraction of the population in which *ura4* colocalized with Nup120–Myc in cells with either the lac-repressor array plasmid or the lac-repressor array plasmid with a single copy of GRS I. Bars are means ± s.e.m.; $n = 4$, 60–100 cells per replicate.

We next asked whether GRS I-containing promoters are enriched among genes that have been shown to physically associate with the NPC by ChIP[4]. Using chi square analysis, we found that GRS-containing promoters were significantly enriched among genes that associate with the nuclear pore proteins Nup2, Mlp1, Mlp2, Nup60 and Nup116 and the transport factors *Cse*1 and *Xpo*1 (Table 2). As a control, we performed the same analysis with a reversed GRS I (GRS I_{rev}; 5′-AGGTGGG-3′). We observed no enrichment of GRS I_{rev} containing promoters among genes that interact with NPC proteins (data not shown). Furthermore, we noticed that the GRS I from *INO1* and *TSA2* is related to a sequence motif that was previously found to be overrepresented in promoters of NPC-associated genes[4] (Fig. 5). This suggests that GRS I-like elements control the interaction of many genes with the NPC.

Peripheral targeting enhances transcription of *INO1* and *TSA2*

We next tested the functional significance of peripheral localization for transcriptional activation of *INO1*. Mutation of the GRS I element of *URA3:INO1* resulted in poor accumulation of *INO1* mRNA after

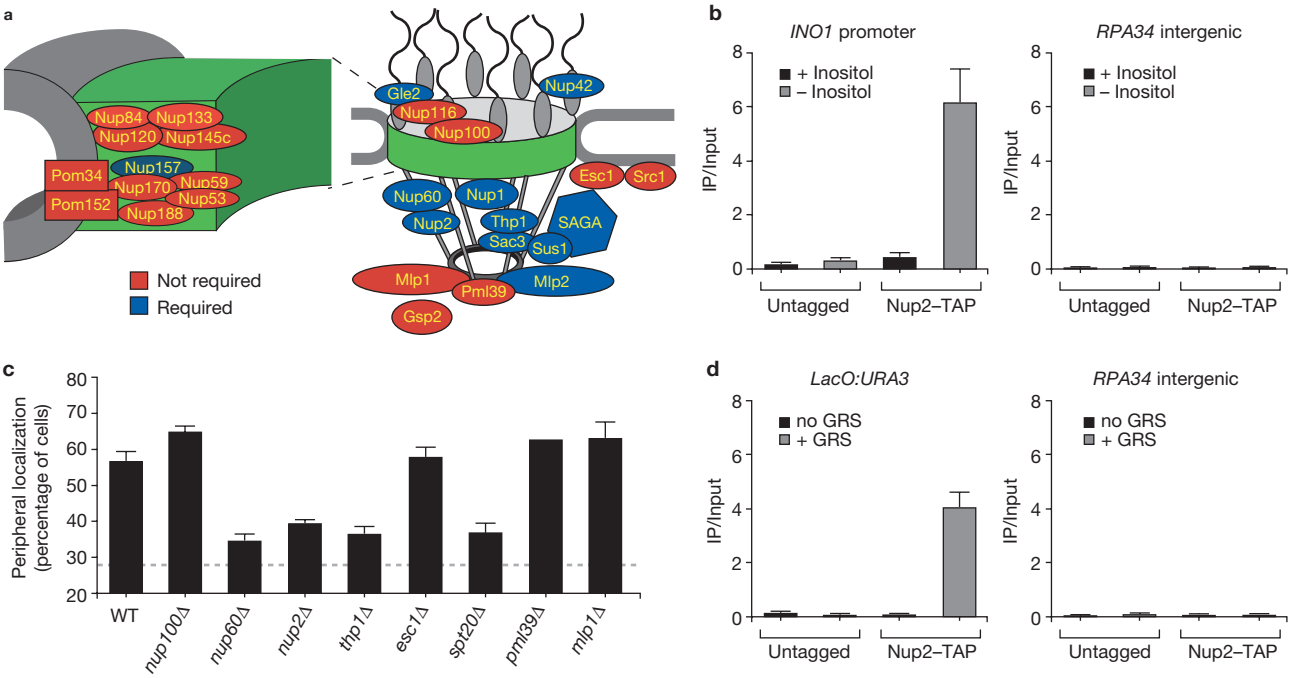

Figure 4 *INO1* recruitment to the nuclear periphery requires components of the nuclear pore complex (NPC) and associated factors. (**a**) Summary of nuclear pore proteins and associated factors required for *INO1* targeting to the nuclear periphery (see Supplementary Information, Fig. S3 for complete data; data for Nup2 has previously been published[12]). Proteins filled in blue were required for *INO1* peripheral targeting. Proteins filled in red were not required for *INO1* peripheral targeting. The positions of proteins within the pore and contacts between them are approximations based on a model of the NPC structure[31]. The left panel shows an expanded view of the core channel of the NPC. (**b**) ChIP of Nup2-TAP under repressing and activating conditions. Recovery of the *INO1* promoter (left panel) or the *RPA34* intergenic region about 5000 bp upstream (right panel) with IgG magnetic beads (Invitrogen) was quantified relative to the input by real-time quantitative PCR. (**c**) Nuclear pore requirements for GRS I mediated targeting of *URA3* ($n = \geq 2$, 30–50 cells per replicate). Cells were grown in the presence of inositol. (**d**) Recovery of *URA3* or *RPA34* with Nup2 (as in panel **b**) from strains with or without GRS I at the *URA3* locus (see Methods). For panels **b** and **d**, bars are mean ± s.e.m.; $n = 3$. The hatched blue line in **c** represents the mean peripheral localization for *URA3*.

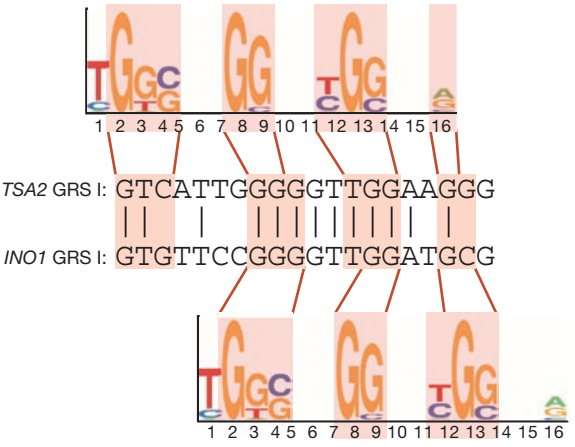

Figure 5 GRS I is enriched among genes that interact with many nuclear pore proteins. Comparison of GRS I from *INO1* and *TSA2* to a motif, identified by *Casolari et al.*[4], that is overrepresented in genes associated with Mlp1, Mlp2 or Nic96. Two alternative alignments are shown.

3 h of induction (Fig. 6a). At steady state, we observed a twofold difference in mRNA levels (Supplementary Information, Fig. S4a). This decrease did not correlate with a difference in the rate of mRNA decay, suggesting that it is due to a difference in transcription (Supplementary Information, Fig. S4b). Furthermore, we also observed a similar defect in the activation of *grs I* mutant *TSA2*, relative to wild-type *TSA2* (Supplementary Information, Fig. S4c).

We have noticed that plasmid-borne *INO1*, integrated either at *URA3* or in place of the endogenous gene, is normally regulated by inositol starvation but is expressed at higher levels than endogenous *INO1* (Supplementary Information, Fig. S4d). For this reason, we also compared the transcription of wild-type with *grs* mutant forms of *INO1* after introducing chromosomal mutations to remove GRS I and II at the endogenous locus. We observed a clear decrease in *INO1* mRNA levels at the endogenous *INO1* locus in strains lacking both GRS elements (Fig. 6c). Peripheral targeting correlated with transcription; expression of endogenous *INO1* was not significantly affected by the *grs I* mutation or *grs II* mutation alone (Supplementary Information, Fig. S2b). The GRS elements, therefore, are redundant for both *INO1* localization and transcription and full activation of *INO1* and *TSA2* requires DNA zip codes that confer peripheral localization.

Transcription of *INO1* is enhanced by promoter targeting to the nuclear periphery

We next asked whether the part of the gene that is targeted to the nuclear periphery is important for transcription. We re-introduced the GRS I element either upstream or downstream of the coding sequence of the *grs I* mutant *URA3:INO1* and quantified the *INO1* mRNA levels on activation. Re-introduction of GRS I about 450 bp upstream (that is, 5′) of *grs I* mutant *INO1* restored regulated targeting to the nuclear periphery

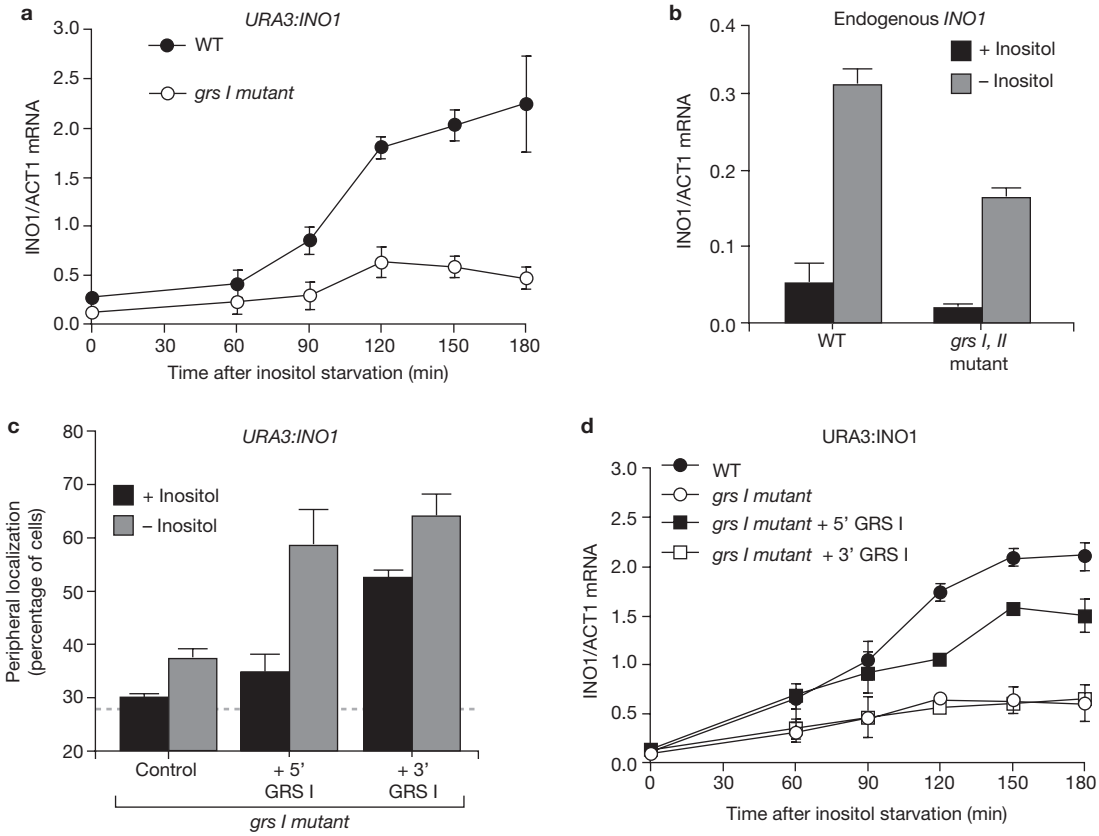

Figure 6 Localization at the nuclear periphery enhances transcription of *INO1*. (**a**) *INO1* mRNA levels were quantified by reverse transcription-qPCR, relative to *ACT1*, following induction by inositol starvation from strains having either plasmid-borne wild-type (WT) *INO1* or *grs I* mutant *INO1* integrated at *URA3*. (**b**) Strains were constructed in which the *grs I, II* mutations were introduced at the chromosomal *INO1* locus. Wild-type and *grs I, II* mutant strains were grown overnight in the presence or absence of inositol and the mRNA levels quantified as in **a**. (**c**, **d**) GRS I was reintroduced either at the 5′ end or the 3′ end of *grs I* mutant *INO1* and these plasmids were integrated at *URA3*. These strains were compared with wild-type and *grs I* mutant *INO1* for localization (**c**) and transcription (**d**). For panels **a**–**d**, data are mean ± s.e.m; $n = 4$. The hatched blue line in **c** represents the mean peripheral localization for *URA3*.

(Fig. 6c) and largely suppressed the defect in transcription (Fig. 6d). By contrast, re-introduction of GRS I at the 3′ end of *grs I* mutant *INO1* caused the gene to localize to the nuclear periphery constitutively, but it did not suppress the defect in transcription (Fig. 6c, d). This suggests that the targeting of the promoter, not the gene *per se,* to the nuclear periphery is associated with full transcriptional activation.

We also tested whether GRS I was sufficient to promote transcriptional activation. We introduced GRS I upstream of a crippled *CYC1* promoter (*CYC1**) driving a β-galactosidase (*LacZ*) reporter gene. The well-established unfolded protein response element (UPRE) functions as an enhancer in this context and was sufficient to promote LacZ expression. However, the GRS I element did not enhance transcription of *CYC1*–LacZ* (Supplementary Information, Fig. S5a). Therefore, GRS I might not simply be an enhancer and its important role might be in controlling promoter interactions with factors at the nuclear envelope or the NPC that promote transcription of certain genes.

DISCUSSION

The GRS elements are small, well-defined DNA elements with the ability to target ectopic chromosomal loci to a particular subnuclear location. The existence of such DNA zip codes suggests that genomes code for their own spatial organization. The DNA zip codes that we have identified are negatively regulated when *INO1* is repressed. Previous work has implicated transcriptional regulators in promoting peripheral targeting of genes[5,11] and this might be why the regulation of GRS-mediated targeting requires that they are located within the promoter. Regulation of peripheral targeting was lost either when expression was constitutive (Fig. 1; Δ4 mutant) or when the element was introduced downstream of *INO1* (Fig. 4c). We also observed unregulated peripheral targeting when GRS I was introduced downstream of *GAL1*, another regulated gene that is recruited to the nuclear periphery (Supplementary Information, Fig. S6). Therefore, transcription factors might regulate both transcription and gene targeting to the nuclear periphery. This suggests that the spatial organization coded by DNA can be dynamic and regulated.

Our previous work has shown that peripheral localization of genes can establish 'transcriptional memory', which promotes the reactivation of genes like *INO1* and *GAL1* after they are repressed[12]. We show here that targeting to the nuclear periphery is also important for full expression of *INO1* and *TSA2*. It is still unclear how localization promotes activation. We found that peripheral targeting of the promoter, but not the 3′ end of the gene, promotes *INO1* transcription. Similarly, introduction of the GRS I element downstream of the *GAL1* gene had no effect on its activation (Supplementary Information, Fig. S6). The GRS elements might promote transcription by recruiting transcription factors that both activate transcription and target genes to the nuclear periphery. However, the GRS I element was not sufficient to promote transcription

from a crippled promoter. Therefore, it also remains possible that the important activity of the GRS elements is to function as DNA zip codes, and that the expression of certain genes is promoted by protein–DNA interactions at the nuclear periphery or with the NPC.

The GRS I element functions as a DNA zip code in an organism that is approximately one billion years diverged from the organism in which it was identified. Perfect matches of GRS I occur 112 times in the *S. pombe* genome, 19 of which are clearly in promoters (Supplementary Information, Table S2). It will be interesting to determine whether these elements control subnuclear localization of these genes. Our work suggests that GRS-mediated targeting to the nuclear periphery is an ancient mechanism that could be shared by many eukaryotes. We conclude that DNA zip codes represent an additional level of genetic information that controls the spatial organization of the genome and affects gene expression. □

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturecellbiology/

Note: Supplementary Information is available on the Nature Cell Biology website.

ACKNOWLEDGEMENTS

We acknowledge Robert Lamb for generously sharing his confocal microscope. Also, we thank Audrey Gasch, Richard Carthew, Rick Gaber, Sandy Westerheide, Jonathan Widom, Susan Wente, Michael Rout and members of the Brickner lab for helpful discussions. This work was supported by the Searle Leadership Fund at Northwestern University, a gift of The Searle Funds at The Chicago Community Trust (J.H.B.), an Institutional Research Grant from the American Cancer Society (J.H.B.) and National Institutes of Health grant GM080484 (J.H.B.).

AUTHOR CONTRIBUTIONS

S.A., D.G.B, T.V. and J.H.B designed the experiments, S.A., D.G.B., W.H.L., M.M., I.C., A.B.F. and J.H.B performed the experiments, S.A. and J.H.B wrote the manuscript.

COMPETING INTERESTS

The authors declare that they have no competing financial interest.

Published online at http://www.nature.com/naturecellbiology/
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions/

1. Akhtar, A. & Gasser, S. M. The nuclear envelope and transcriptional control. *Nature Rev. Genet.* **8,** 507–517 (2007).
2. Ahmed, S. & Brickner, J. H. Regulation and epigenetic control of transcription at the nuclear periphery. *Trends Genet.* **23,** 396–402 (2007).
3. Taddei, A. Active genes at the nuclear pore complex. *Curr. Opin. Cell Biol.* **19,** 305–310 (2007).
4. Casolari, J. M. *et al.* Genome-wide localization of the nuclear transport machinery couples transcriptional status and nuclear organization. *Cell* **117,** 427–439 (2004).
5. Brickner, J. H. & Walter, P. Gene recruitment of the activated *INO1* locus to the nuclear membrane. *PLoS Biol.* **2,** e342 (2004).
6. Casolari, J. M., Brown, C. R., Drubin, D. A., Rando, O. J. & Silver, P. A. Developmentally induced changes in transcriptional program alter spatial organization across chromosomes. *Genes Dev.* **19,** 1188–1198 (2005).
7. Dieppois, G., Iglesias, N. & Stutz, F. Cotranscriptional recruitment to the mRNA export receptor Mex67p contributes to nuclear pore anchoring of activated genes. *Mol. Cell Biol.* **26,** 7858–7870 (2006).
8. Taddei, A. *et al.* Nuclear pore association confers optimal expression levels for an inducible yeast gene. *Nature* **441,** 774–778 (2006).
9. Sarma, N. J. *et al.* Glucose-responsive regulators of gene expression in *Saccharomyces cerevisiae* function at the nuclear periphery via a reverse recruitment mechanism. *Genetics* **175,** 1127–1135 (2007).
10. Abruzzi, K. C., Belostotsky, D. A., Chekanova, J. A., Dower, K. & Rosbash, M. 3′-end formation signals modulate the association of genes with the nuclear periphery as well as mRNP dot formation. *EMBO J.* **25,** 4253–4262 (2006).
11. Schmid, M. *et al.* Nup-PI: the nucleopore-promoter interaction of genes in yeast. *Mol. Cell* **21,** 379–391 (2006).
12. Brickner, D. G. *et al.* H2A.Z-mediated localization of genes at the nuclear periphery confers epigenetic memory of previous transcriptional state. *PLoS Biol.* **5,** e81 (2007).
13. Straight, A. F., Belmont, A. S., Robinett, C. C. & Murray, A. W. GFP tagging of budding yeast chromosomes reveals that protein-protein interactions can mediate sister chromatid cohesion. *Curr. Biol.* **6,** 1599–1608 (1996).
14. Robinett, C. C. *et al.* *In vivo* localization of DNA sequences and visualization of large-scale chromatin organization using lac operator/repressor recognition. *J. Cell Biol.* **135,** 1685–1700 (1996).
15. Swift, S. & McGraw, P. INO1–100: an allele of the *Saccharomyces cerevisiae INO1* gene that is transcribed without the action of the positive factors encoded by the *INO2*, *INO4*, *SWI1*, *SWI2* and *SWI3* genes. *Nucleic Acids Res.* **23,** 1426–1433 (1995).
16. Harbison, C. T. *et al.* Transcriptional regulatory code of a eukaryotic genome. *Nature* **431,** 99–104 (2004).
17. Lee, T. I. *et al.* Transcriptional regulatory networks in *Saccharomyces cerevisiae*. *Science* **298,** 799–804 (2002).
18. Colman-Lerner, A., Chin, T. E. & Brent, R. Yeast Cbk1 and Mob2 activate daughter-specific genetic programs to induce asymmetric cell fates. *Cell* **107,** 739–750 (2001).
19. Carmona-Saez, P., Chagoyen, M., Tirado, F., Carazo, J. M. & Pascual-Montano, A. GENECODIS: a web-based tool for finding significant concurrent annotations in gene lists. *Genome Biol.* **8,** R3 (2007).
20. Nogales-Cadenas, R. *et al.* GeneCodis: interpreting gene lists through enrichment analysis and integration of diverse biological information. *Nucleic Acids Res.* **37,** W317–W322 (2009).
21. Cox, J. S., Chapman, R. E. & Walter, P. The unfolded protein response coordinates the production of endoplasmic reticulum protein and endoplasmic reticulum membrane. *Mol. Biol. Cell* **8,** 1805–1814 (1997).
22. Gasch, A. P. *et al.* Genomic expression programs in the response of yeast cells to environmental changes. *Mol. Biol. Cell* **11,** 4241–4257 (2000).
23. Travers, K. J. *et al.* Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. *Cell* **101,** 249–258 (2000).
24. Leber, J. H., Bernales, S. & Walter, P. IRE1-independent gain control of the unfolded protein response. *PLoS Biol.* **2,** E235 (2004).
25. Wong, C. M., Ching, Y. P., Zhou, Y., Kung, H. F. & Jin, D. Y. Transcriptional regulation of yeast peroxiredoxin gene TSA2 through Hap1p, Rox1p, and Hap2/3/5p. *Free Radic. Biol. Med.* **34,** 585–597 (2003).
26. Heckman, D. S. *et al.* Molecular evidence for the early colonization of land by fungi and plants. *Science* **293,** 1129–1133 (2001).
27. Luthra, R. *et al.* Actively transcribed GAL genes can be physically linked to the nuclear pore by the SAGA chromatin modifying complex. *J. Biol. Chem.* **282,** 3042–3049 (2007).
28. Cabal, G. G. *et al.* SAGA interacting factors confine sub-diffusion of transcribed genes to the nuclear envelope. *Nature* **441,** 770–773 (2006).
29. Menon, B. B. *et al.* Reverse recruitment: the Nup84 nuclear pore subcomplex mediates Rap1/Gcr1/Gcr2 transcriptional activation. *Proc. Natl Acad. Sci. USA* **102,** 5749–5754 (2005).
30. Chekanova, J. A., Abruzzi, K. C., Rosbash, M. & Belostotsky, D. A. Sus1, Sac3, and Thp1 mediate post-transcriptional tethering of active genes to the nuclear rim as well as to non-nascent mRNP. *RNA* **14,** 66–77 (2008).
31. Alber, F. *et al.* The molecular architecture of the nuclear pore complex. *Nature* **450,** 695–701 (2007).

METHODS

Chemicals and reagents. Unless stated otherwise, chemicals were from Sigma Aldrich, DNA oligonucleotides were from Operon and Integrated DNA Technologies, restriction enzymes were from New England Biolabs and yeast media components were from Q-Biogene. Antibodies against GFP, fluorescent secondary antibodies and Human Pan Mouse IgG dynabeads were from Invitrogen and the antibody against myc was from Santa Cruz Biotechnology.

Plasmid construction. Plasmids pRS306-INO1[5], pRS304-Sec63-Myc[12] and pAFS144 (ref. 13) have been described previously. pRS306-INO1′ contains the same *INO1* insert as pRS306-INO1 but in the opposite orientation, with the 5′ end of the gene near the unique *Xho*I site in pRS306[32]. Plasmids pRS306-*grs I*mutINO1 and pRS306 *grs I*mutINO1′ are derived from these two plasmids and were generated by Quick Change site directed mutagenesis to convert the GRS I sequence in the *INO1* promoter from 5′-GGGTTGGA-3′ to 5′-AAACCAAA-3′. To re-introduce GRS I at the 5′ or 3′ end of *INO1*, GRS I (41–60) was cloned into pRS306-*grs I*mutINO1 and pRS306-*grs I*mutINO1′ using *Xma*I and *Not*I. Plasmids were digested with either *Stu*I to integrate at *URA3* or *Bgl*II to integrate at *INO1*. The Δ2 (–351 to –450), Δ3 (–251 to –350), Δ4 (–151 to –250) and Δ5 (–50 to –150) mutants were generated by deletion of non-overlapping regions of the *INO1* promoter in pRS306-INO1′. For DNA localization experiments, a fragment of 128 Lac operator repeats was moved from p6LacO128 (ref. 5) into each of these plasmids using *Hin*dIII and *Xho*I.

GRS I and GRS II mapping plasmids were created in p6LacO128 (ref. 5). Fragments larger than 150 bp were generated by PCR amplification from genomic DNA then cloned into p6LacO128. Smaller fragments were cloned as 5′ phosphorylated oligonucleotides with one of the following combinations of overhangs: 1) *Sac*I and *Spe*I; 2) *Xho*I at both ends; 3) *Xma*I and *Not*I;or 4) *BamH*I and *Not*I. Sequences for all oligonucleotides are described in Supplementary Information, Table S3.

pRS305Nup2–TAP was generated by cloning of Nup2–TAP into pCR2.1 (Invitrogen) using PCR amplification from BY4741 Nup2–TAP[33] genomic DNA. Nup2–Tap was ligated into pRS305 using *Not*I and *Spe*I and the resulting plasmid was digested with *Swa*I and integrated at the *LEU2* locus in yeast strains WLY53 and WLY54.

The UPRE was removed from pJC002 (ref. 34) by digestion with *Xho*I and re-ligation of the cut plasmid to generate pJC002CYC1*LacZ. GRS I (41–60) was cloned into *Xho*I cut pJC002 using 5′ phosphorylated oligonucleotides to generate pJC002GRSCYC1*LacZ.

Yeast strains. Yeast strains used in this study are described in Supplementary Information, Table S4. Except for BY4741 Nup2–TAP[33] all strains were constructed from CRY1 *(ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1* Mat a). Deletions in yeast proteins were made using the PCR-based deletion system[35]. Deletions were confirmed by PCR using genomic DNA.

Yeast strains for GRS mapping were generated by integration of *Stu*I-digested plasmids containing the LacO array and relevant GRS fragments at *URA3*. For integration of smaller GRS fragments ≤10bp the PCR based integration system[35] was adapted to integrate the relevant GRS fragment along with the kanMX6 marker at *URA3* as follows. Plasmid p6LacO128 was first integrated at *URA3*. GRS fragments were included in the primers used to amplify the *kanMX6* gene. The 5′ 45 bp of the primers had homology to the β-lactamase gene in pRS306. As a control, we integrated the *kanMX6* marker alone at *URA3*. This integration had no effect on the localization of *URA3*. This PCR-based integration strategy was also used to introduce GRS I at the 3′ end of *GAL1* using a strain containing p6LacO*GAL1* integrated downstream of *GAL1* (ref. 12).

Chromosomal mutations in GRS I and GRS II at endogenous *INO1* locus were made using homologous recombination. Fragments including the entire *INO1* gene, promoter and 3′-UTR that were mutant for *grs I* (AAACCAAA), *grs II* (deletion of the central TGCTGG sequence) or both, were transformed into a *proΔino1* mutant strain. This strain lacks the 450 bp upstream of the *INO1* transcriptional start site and a part of the coding sequence, which is replaced by the *kanMX6* marker[35]. We selected for strains that had recovered *INO1* by selecting for inositol prototrophy. Ino+ transformants that had lost the kanMX6 marker were confirmed by DNA sequencing. As a control for this approach, wild-type *INO1* was also recreated in this way and was used as the wild-type control.

The *TSA2 grs I* mutant was generated in the chromosome using the *delitto perfetto* strategy[36]. To introduce GRS I into *S. pombe*, we took advantage of the ability of *URA3* from *S. cerevisiae* to complement the *ura4* mutation in the orthologous gene from *S. pombe*[37]. The *Ura4+* gene was replaced with a non-functional fragment of the *URA3* gene from *S. cerevisiae* called *ura3.1*. We amplified the *ura3.1* mutant by two rounds of PCR. First, using *S. pombe* genomic DNA as template, we carried out two reactions using either the ura4up + ura3.1up primer pair or the ura3.1down + ura4down primer pair. These reactions generated two products of 215 bp, each having 25 bp of homology to *URA3* at one end. These products were then used as primers to amplify 835 bp of *URA3* corresponding the coding sequence, but lacking the promoter using pRS306 as template[32]. The PCR product was transformed into strain 972 h- and 5 fluoroorotic acid resistant transformants were isolated to create strain MM160 (ref. 37). Strain MM160 was transformed with *Stu*I-digested p6LacO128 or p6LacO (41–75) to generate strains MM162 and MM163, respectively. MM169 (*NUP120myc, ura4-Δ18, ade6+, his7+::LacI-GFP*) was then crossed to MM162 and MM163 to obtain strains MM170 and MM171, respectively. PCR analyses confirmed expected insertions within all strains.

Reverse transcriptase real-time quantitative PCR. RNA preparation and RT Q-PCR analysis was performed as described previously[12].

Chromatin immunoprecipitation. Chromatin immunoprecipitation was performed as described[12], except that TAP-tagged Nup2 was recovered using Pan Mouse IgG dynabeads (Invitrogen). DNA was quantified by real-time quantitative PCR[5].

Chromatin localization assay. Samples were visualized on a Zeiss LSM510 confocal microscope in the Northwestern University Biological Imaging Facility. Chromatin localization experiments in *S. cerevisiae* were performed as described previously[5,12]. Briefly, methanol fixed, spheroplasted, detergent-extracted cells were probed with anti-Myc monoclonal antibody (1:200 dilution) to detect Sec63–Myc, and rabbit polyclonal anti-GFP antibody (1:1000 dilution) to detect GFP–Lac repressor. Secondary antibodies were diluted 1:200. A single *z* slice through each cell with the brightest and most focused anti-GFP spot was collected. Cells in which this anti-GFP spot colocalized with Sec63-*myc* nuclear membrane staining were scored as peripheral and all other cells were scored as nucleoplasmic. For each biological replicate, the fraction of cells in a population of 30–50 cells that scored as peripheral was determined. Error bars represent the s.e.m. between biological replicates.

For experiments in *S. pombe,* 10 ml of cells were grown to mid-log phase in YEA, transferred to YEA + sorbitol (2.4 M) for 30 min followed by fixation with 3.5% formaldehyde for 1 h. Cells were washed twice with 1 ml PEMS (100 mM PIPES, 1 mM EGTA, 1 mM $MgSO_4$, 1.2 M sorbitol pH, 6.9) and resuspended in 1 ml PEMS containing 0.2% BME and 1 mg ml^{-1} Zymolyase 100T. Spheroplasting was checked under the microscope by mixing 0.5 µl of 20% SDS and 9.5µl of cells. Spheroplasts were spun down, washed three times with 1 ml PEMS, resuspended in PEMS + 1% Triton, resuspended in 1 ml PEMBAL (100 mM PIPES, 1 mM EGTA, 1 mM $MgSO_4$, 1% BSA, 0.1% sodium azide, 100 mM L-lysine hydrochloride) and rotated for 30 min at room temperature. Spheroplasts were spun down, and resuspended in 100 µl of PEMBAL containing Myc (1:100) and GFP (1:500) antibodies for 3 h or overnight. Spheroplasts were washed three times with 1 ml PEMBAL, resuspended in 100 µl of PEMBAL containing a 1:100 dilution of Alexa Fluor 594 goat anti-mouse IgG and Alexa Fluor 488 goat anti-rabbit IgG and incubated for 3 h. Spheroplasts were washed three times with 1ml PEMBAL and once with PBS + 0.1% sodium azide. Cells (10 µl) were spotted onto polylysine-treated slides and sealed with 2 µl of mounting medium.

Statistical methods. Tables I, II and S1 used the chi square test. For Table 1 and Supplementary Information, Table S1, each expression dataset was ranked according to signal (mRNA levels relative to untreated control). The number of GRS I-containing genes that were above and below the top 10% of the range was compared with the predicted number (10% and 90% of the total within each set, respectively) by chi square test. For Table II, the number of non-GRS I or GRS I genes was calculated as described in the footnote of Table II and compared with the observed number of non-GRS I and GRS I genes in each ChIP by chi square test.

β-galactosidase Miller assay. Cells (10 ml) were grown to log phase and an equal number of cells was pelleted and resuspended in 2 ml Z buffer (40 mM NaH_2PO_4, 60 mM Na_2HPO_4). An aliquot of cells (0.5 ml) was used for each reaction. SDS (0.1; %20 µl) and 2 drops of chloroform were added and the reaction incubated at 30° C for 15 min. Ortho-nitrophenyl-β-galactoside (4mg ml^{-1}; 160 µl) was added and the reaction incubated at 30° C until a pale yellow colour developed. The

DOI: 10.1038/ncb2011

development time was noted and the reaction quenched by addition of Na_2CO_3 (1 M; 400 µl). Absorbance values at 420 nm and 550 nm were read. Miller units were calculated using the following formula[38]:

$$\text{Miller units} = 1000 \times [(A_{420}) - (1.75 \times A_{550})]$$
$$\text{time} \times \text{volume of cells (0.5 ml)} \times A_{600}$$

32. Sikorski, R. S. & Hieter, P. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* **122,** 19–27 (1989).
33. Ghaemmaghami, S. *et al*. Global analysis of protein expression in yeast. *Nature* **425,** 737–741 (2003).
34. Cox, J. S. & Walter, P. A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. *Cell* **87,** 391–404 (1996).
35. Longtine, M. S. *et al*. Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. *Yeast* **14,** 953–961 (1998).
36. Storici, F., Durham, C. L., Gordenin, D. A. & Resnick, M. A. Chromosomal site-specific double-strand breaks are efficiently targeted for repair by oligonucleotides in yeast. *Proc. Natl Acad. Sci. USA* **100,** 14994–14999 (2003).
37. Grimm, C., Kohli, J., Murray, J. & Maundrell, K. Genetic engineering of *Schizosaccharomyces pombe*: a system for gene disruption and replacement using the ura4 gene as a selectable marker. *Mol. Gen. Genet.* **215,** 81–86 (1988).
38. Miller, J. H. *Experiments in Molecular Genetics*. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1972).

nature cell biology

DOI: 10.1038/ncb2011

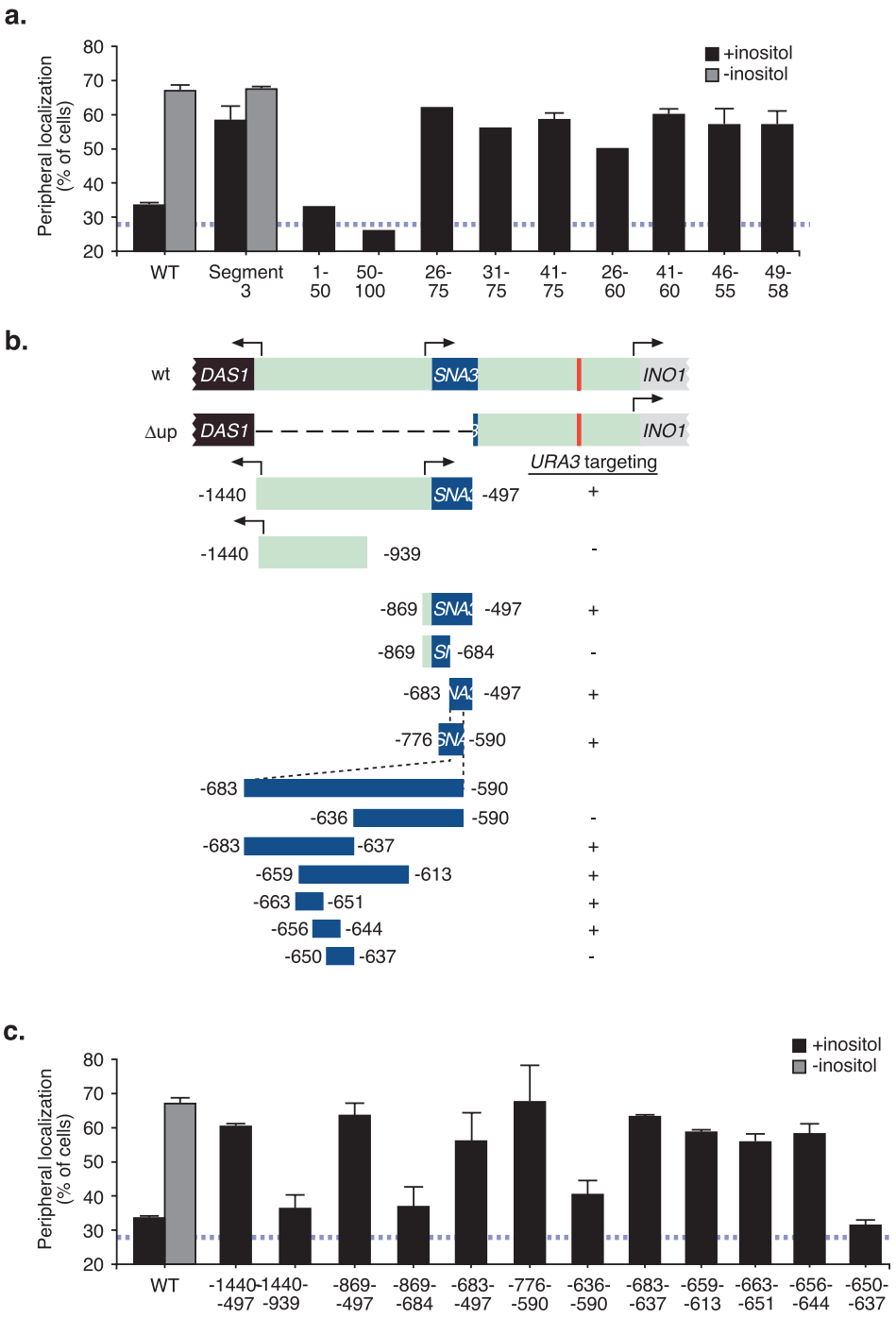

Figure S1 Mapping of the Gene Recruitment Sequences. (**a**) GRS I mapping. Segment 3 (1-100) and fragments of it were integrated along with a lac repressor array at *URA3*. The fraction of cells in which the lac repressor colocalized with the nuclear envelope was determined for each strain. (**b** & **c**) GRS II mapping. (**b**) Fragments upstream of *INO1* were cloned and integrated along with a lac repressor array at *URA3*. Coordinates represent the position of the fragments relative to the *INO1* transcriptional start site. (**c**) The fraction of cells in which the lac repressor co localized with the nuclear envelope was determined for each strain.

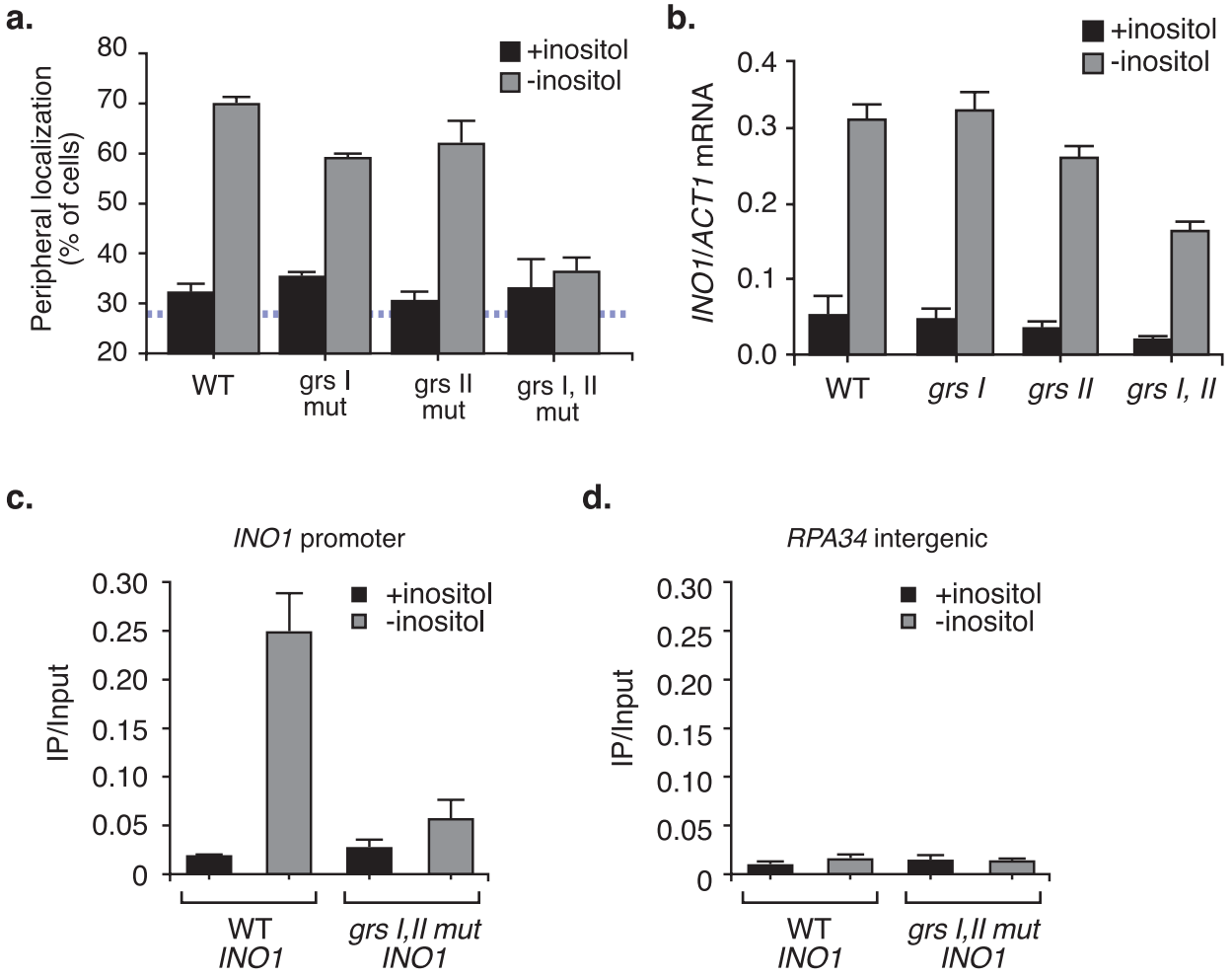

Figure S2 GRS I and GRS II are redundant for peripheral targeting, transcription and Nup2 association at the endogenous *INO1* locus. (**a**) The localization of the indicated forms of *INO1* was determined with respect to the nuclear envelope. (**b**) Measurement of the steady-state *INO1* mRNA levels relative to *ACT1* mRNA in strains having wild type, *grs I* mutant, *grs II* mutant or *grs I,II* mutant *INO1* at the endogenous *INO1* locus, grown overnight in the presence or absence of inositol. (**c** & **d**) Chromatin immunoprecipitation with Nup2-TAP under repressing and activating conditions from strains having either wild type *INO1* or a *grs I,II* mutant *INO1* at the endogenous chromosomal locus. Recovery of the *INO1* promoter (**c**) or the *RPA34* intergenic region ~5000 bp upstream (**d**) with IgG magnetic beads (Invitrogen) was quantified relative to the input by real-time quantitative PCR.

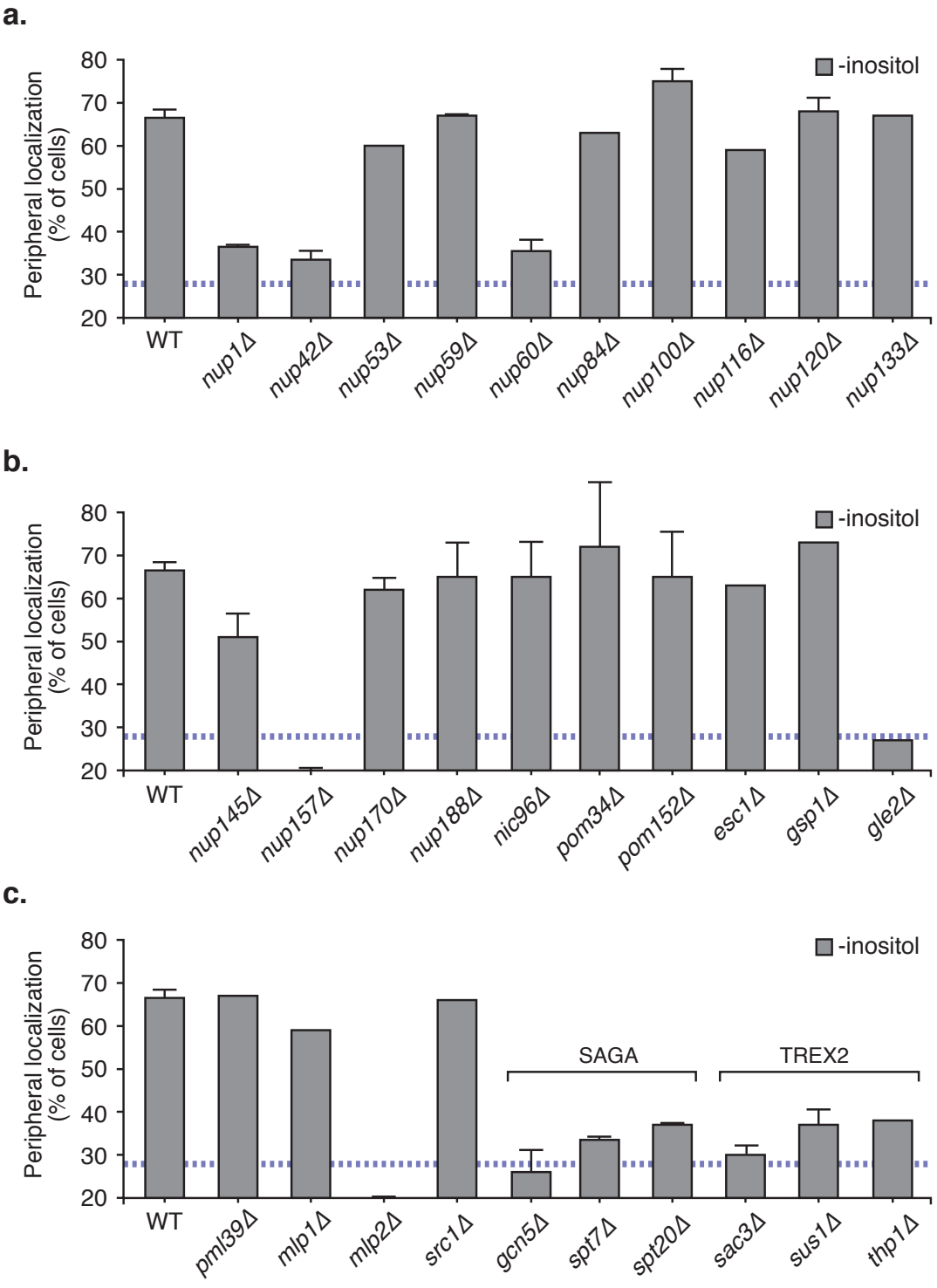

Figure S3 *INO1* targeting to the nuclear periphery requires components of the nuclear pore complex and associated factors. Panels **a, b**, & **c**: null mutants in genes encoding nuclear pore proteins and other peripherally localized proteins were tested for *INO1* targeting to the nuclear periphery upon inositol starvation.

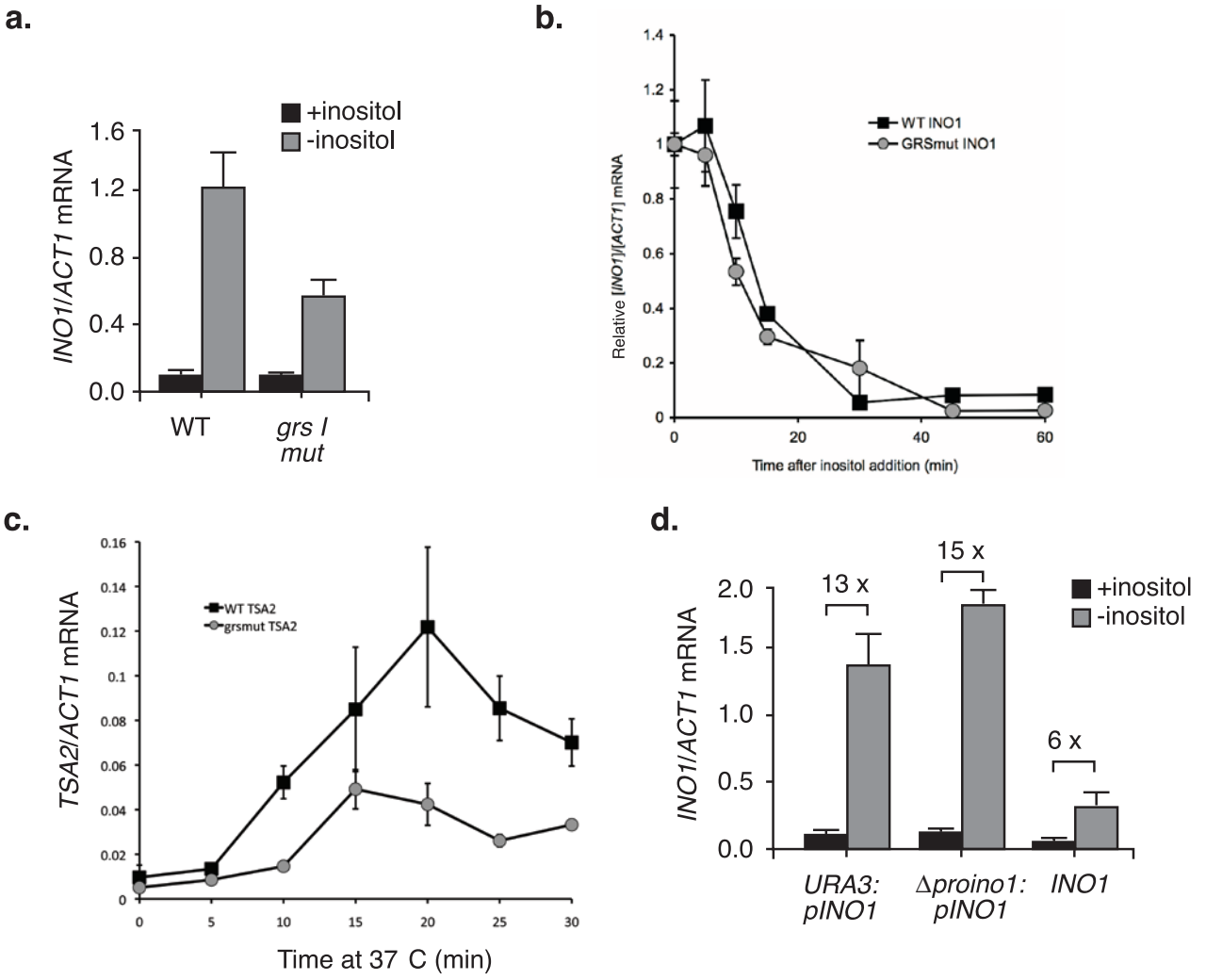

Figure S4 GRS I targeting promotes transcription. (**a**) Measurement of the steady-state *INO1* mRNA levels relative to *ACT1* mRNA in strains having either wild type or *grs I* mutant *INO1* integrated at the *URA3* locus, grown overnight in the presence or absence of inositol. (**b**) *INO1* mRNA decay is unaffected by the GRS I mutation. Cells having either wild type *INO1* or *grs I* mutant *INO1* integrated at *URA3* were grown in the absence of inositol to induce *INO1*. At the beginning of the time course, inositol (100μM) was added. mRNA was prepared from cells collected at the indicated times and the amount of *INO1* mRNA remaining was quantified relative to *ACT1* mRNA using RT-qPCR. Because the amount of *INO1* mRNA produced in these strains is different, the data were normalized to the starting concentration to facilitate comparison. (**c**) Wild type and *grs I* mutant *TSA2* strains were grown in YPD at 25˚C, harvested and resuspended in 37˚C medium. The cultures were maintained at 37˚C and RNA was harvested at the indicated time points. *TSA2* mRNA levels were quantified relative to *ACT1* by RT QPCR. (**d**) An integrating plasmid bearing the *INO1* gene, including the promoter and 3' UTR, was integrated either at *URA3* or at *INO1* in a *proΔino1* strain (lacking the promoter and the 5' half of the gene). Cells were grown overnight in medium with or without inositol and *INO1* and *ACT1* mRNA levels were determined by RT-qPCR. Above each pair of conditions is shown the fold induction. For comparison, we quantified *INO1* transcript levels from an *INO1* strain.

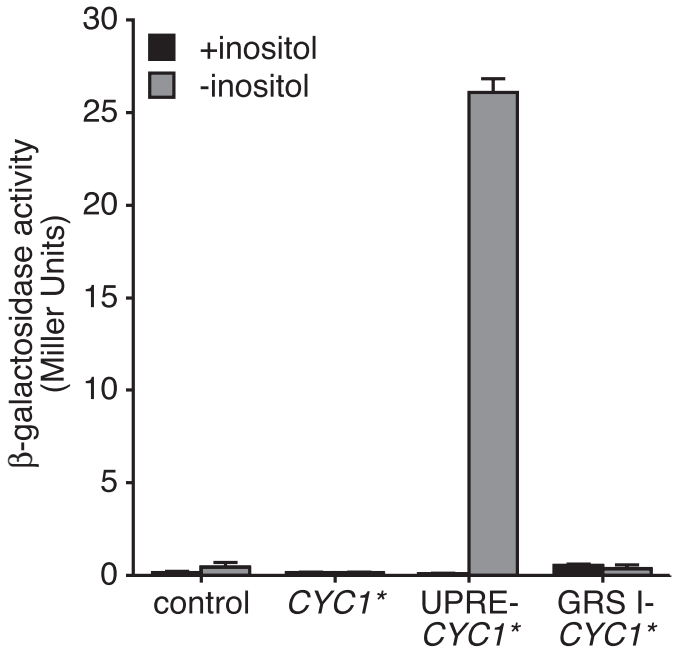

Figure S5 GRS I is not an enhancer element for the *CYC1* promoter. The GRS was inserted upstream of a crippled *CYC1* promoter (*CYC1**) [6] and tested for the ability to activate transcription of a β galactosidase (*LacZ*) reporter gene (Methods). Control: no *LacZ* reporter, *UPRE-CYC1**: unfolded protein response element fusion to *CYC1** used as a positive control and activates transcription upon inositol starvation[7].

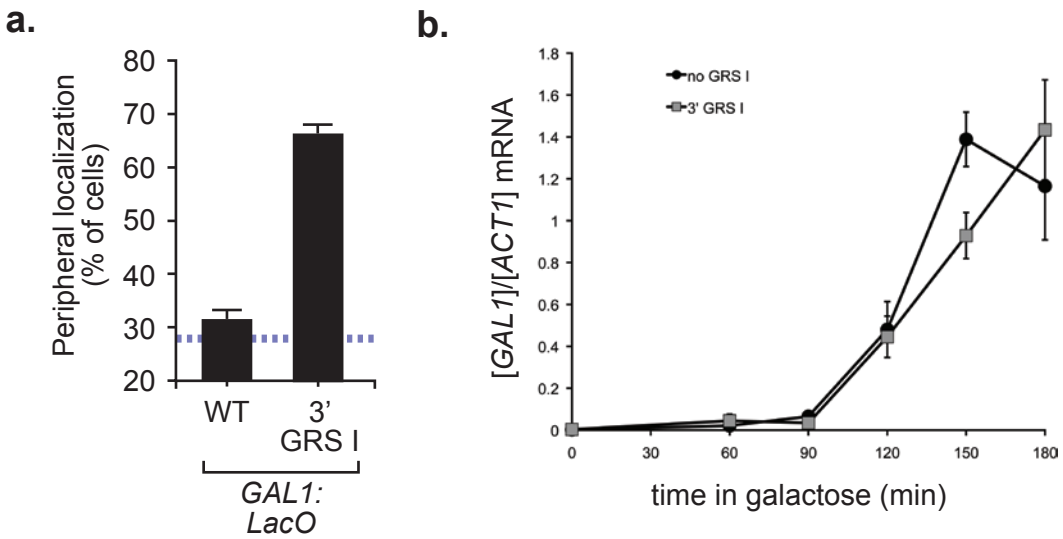

Figure S6 GRS I constitutively targets the *GAL1* gene to the nuclear periphery. The Lac repressor array plasmid with or without GRS I element was integrated at the 3' end of *GAL1*. (**a**) Cells were grown in glucose (repressing conditions) and the percent of cells where the lac repressor colocalized with the nuclear envelope was determined for each strain. (**b**) The strains used in panel **a** were shifted from glucose to galactose and mRNA was recovered after the indicated times. *GAL1* and *ACT1* mRNA levels were determined by RT Q-PCR.

Supplementary Materials

Table S1: Regulation of genes having a GRS I-element within 1000 bp upstream of start site

	<90th percentile		>90th percentile		
Condition	**Exp.**	**Obs.**	**Exp.**	**Obs.**	***P***
Tm$^{\Psi}$, 60min	**74**	**71**	**8**	**11**	**0.2642**
DTT$^{\Sigma}$, 60min	**74**	**70.5**	**8**	**11.5**	**0.1366**
DTT + HS$^{\theta}$	**80**	**72**	**9**	**17**	**0.0049****
***opi1*Δ^{Ω}**	**80**	**85**	**10**	**14**	**0.1797**
HS 5min$^{\varpi}$	**69**	**64**	**8**	**13**	**0.0618**
HS 10min$^{\varpi}$	**73**	**66**	**8**	**15**	**0.0091****
HS 15min$^{\varpi}$	**77**	**68**	**9**	**18**	**0.0015****
HS 20min$^{\varpi}$	**70**	**65**	**8**	**13**	**0.0620**
HS 30min$^{\varpi}$	**74**	**67**	**8**	**15**	**0.0092****
Nitrogen Depl., 8h$^{\varpi}$	**81**	**79**	**9**	**11**	**0.4822**
Nitrogen Depl., 12h$^{\varpi}$	**81**	**76**	**9**	**14**	**0.0789**

$^{\Psi}$ Tunicamycin, averaged from two replicates from Leber et al.[1]
$^{\Sigma}$ Dithiothreitol, from Leber et al.[1]
$^{\theta}$ Dithiothreitol + Heat shock, from Leber et al.[1]
** Statistically significant
$^{\Omega}$ From Travers et al.[2] This strain lacks the repressor of *INO1* transcription and overexpresses inositol-repressed genes.
$^{\varpi}$ From Gasch et al.[3]

Table S2: GRS I matches in the *S. pombe* genome

#	Sequence	Position	Element
Chromosome I			
1	TGTCTTCAAAGGGTTGGAACAATATAT	245067	CDS
2	GGGAACTTTCTTGGGTTGGATTTTCCAGCGG	360702	CDS
3	CTTCGGGTGGGTTGGATAAAAG	415904	CDS
4	ATGGGAGCGGGGTTGGAATTGAAGGAAGGT	452330	CDS
5	ATGCGTCTGTGGGTTGGATGAGACTCGGT	467374	CDS
6	GCAATAACTCAAGGGTTGGAAATGCTTTTCTT	652713	CDS
7	ATAGGGATTCGGGTTGGAGGATTTAAA	677619	CDS
8	CTAATGACTTTAGGGGGTTGGATAATGCAGGT	697388	CDS
9	CACGTATTGTGGGTTGGATAACAATATT	870758	Promoter
10	ACAAATTACCAGGGTTGGAATTTGAAATA	877348	Genic
11	ATTCGGAATTGGGTTGGAAAAAGGTAGG	1211850	CDS
12	GCCAAGTAGGGTTGGAGATGTATACATGGG	1231603	CDS
13	CTTTTTAGGAGGGTTGGAATAAAAACGTAATA	1282410	CDS
14	TTACTGATGGGGTTGGAATGGCATCATTA	1327087	CDS
15	CTATCAACTGGGTTGGAAATCTCATCAT	1395319	CDS
16	TCCAAGTAGAAGGGTTGGAATTTCTGTGGA	1445500	CDS
17	CGTCCTTTTGGGTTGGAATTATTTGAC	1489678	Promoter
18	CCTTTTCACTTAATTGGGTTGGATCACAAACT	1659395	CDS
19	TGGGAAAACGTAAGGGTTGGAAGCGATG	1667590	Promoter
20	TCAACCGCCCCTGGGTTGGATGCCCAATGGG	1781433	CDS
21	CAATAAGATTGGGTTGGAACAGGTTGCCAATT	1846492	CDS
22	AGCTCATCGTTGGGTTGGATTCCTCTTATA	1855845	CDS
23	TGCCTAACAATGGGTTGGAAATCTTGGACTT	1893317	CDS
24	ATTGAGTGCGTGGGTTGGAATGGGATGATTG	1923426	CDS
25	AGCCCATCACCGGGTTGGAGTTTACCAACA	1970690	CDS
26	AGCGCAGACGGGTTGGATTACACCAATCTCC	2145684	CDS
27	CAAATAATTATGGGTTGGATGATGATAG	2316923	CDS
28	CAAGTAAAGTAGGGTTGGATCTATTCATA	2571236	CDS
29	ATCCCAACAGGGTTGGACTTCTGGTATGATCA	2725050	CDS
30	CTTTTATAGCTGGGGTTGGAACAGGAGTCGGT	2846236	Promoter
31	TACCATTTGGTGGGTTGGATTAGGTTT	2991880	CDS
32	TTCCTTTCCAGTGGGTTGGAGAACACTGGCT	3124356	CDS
33	GAATTTTGGGTTGGATTTAATTTAA	3331068	Promoter
34	AATACCCTGAATGGGTTGGAAAGGTCGTGTT	3480254	CDS
35	ATACACTATACGGGTTGGATGTAAGCAGAAAG	3618967	Promoter
36	TAGGATACAATTGAGGGTTGGATATTAATTAA	3680387	Intergenic
37	CTAAAGAAGCTGGGTTGGATCGATGTATTGGT	3841926	CDS
38	CATCGCTTTCAGCGGGTTGGACACGACGAA	3898817	CDS
39	TCGGGGGTTGGGTTGGAGTAGGCTAA	4153139	Promoter
40	TGGTAGTCGGGTTGGACCGCGTGTTT	4226474	Promoter
41	GCATCTACGCGGGTTGGAGTAAGCTGTC	4336303	CDS
42	CATTAATGGGTTGGAAATCAAGACTGG	4424859	Intergenic
43	GCCACTTACTGGGTTGGACATCCTGCA	4475865	CDS

Table S2 (cont'd)

44	GAAAACGGGGTTGGAGGAAGTTAGT	4550932	CDS
45	CGTGTAGCGAGGGTTGGACTTTAAGCGAA	4680276	Promoter
46	GTCCAGAACTTAGGGTTGGATAAACTGATA	4727029	CDS
47	CAATGGAAGCAGATGGGTTGGATGGAGCCTTT	4940977	CDS
48	TCTTCTGATCCTTGGGTTGGATCACGAAGCCT	4976512	CDS
49	GGCGATCAGTTGGGTTGGATTCATCTATGAG	5093320	CDS
Chromosome II			
50	ACCTATTTTATTTGGGTTGGAAGATACCTT	233162	Intergenic
51	TTTTAGTATGGGTTGGAGAAGATTTC	251482	CDS
52	CTAGTCGACACGGGTTGGAAACCAAC	370005	CDS
53	GTTGAGTTTTGGGTTGGATGTCCATACTC	423818	CDS
54	ACCAGACGAAGGGTTGGAAGAGGAAG	622826	CDS
55	AAACTCAGGGGTTGGAACAGCAATCT	869366	CDS
56	GGATATAAAGTTGGGTTGGATTTAAAGAC	944585	CDS
57	GACATACTTAGGGTTGGAGGTACTACCCC	1112818	CDS
58	TAAACAGTGGGGGTTGGATGGCTATCA	1130466	Promoter
59	GTGTGATAAACATTGGGTTGGAATCTCTCAC	1162006	CDS
60	GCTACTCGTGGGTTGGACATTAAAGGC	1201228	CDS
61	AAAGGCTATAGTGGGGTTGGATTTTATGTAA	1287616	CDS
62	TGGAGAGAGCGGGTTGGATAGAGGCG	1295387	CDS
63	CAAAGCTGAGGGTTGGAAAAGAGCTT	1476476	CDS
64	TTCGTCGGGTTGGATGCGGGCATTG	1949756	CDS
65	TCTCGTAAACGGGTTGGAATCATCCGCT	1955784	CDS
66	GGAGCAGGAGGGTTGGAAGTTTCAGC	1975050	CDS
67	GAAGCTGGAAGGGTTGGACTTTGGAAAC	2049851	CDS
68	GCGTCAATTCGGGTTGGACCATGTTGTG	2125233	CDS
69	CACGTCCCTTAGGGTTGGAACGACCAGG	2173047	CDS
70	CTCAAAGATGGGGTTGGAGAACAGCGTT	2444864	CDS
71	TGGTAGCTGGGTTGGAAAAAACTGGA	2449858	CDS
72	GGTATAATTTTTGGGTTGGATGGATCGAGGA	2492147	CDS
73	GGCAAAACGAGGGTTGGAAGTTTGAACAC	2940499	CDS
74	GCTTCCTTTGTTCGGGGTTGGAAAGGTCTGC	3068049	CDS
75	CGATTCTTCTGGGTTGGACTTTATGT	3195519	CDS
76	CCTAATCATAGGGTTGGAAGAATAAATGTCC	3445885	CDS
77	CTGGTTTATGGGTTGGAAAAGAAGGGCCAT	3541890	CDS
78	CCCATGTTGGGGTTGGAATCAGCGGTAT	3564645	CDS
79	GGTGTTGGGGTTGGAATGATTTTA	3604400	CDS
80	TCAGCTCCATGGGTTGGAATGCTTCTTTC	3820174	CDS
81	AAAAGAAGAGGGGTTGGAAGTGACCATGC	3891666	CDS
82	GCTCCAAATGGGTTGGATTCTGAACT	3957915	CDS
83	CAAGCTATGCGGGTTGGATATTTCTG	4205739	CDS
84	GAGAATGTGGGGTTGGAATTCCTCG	4234474	CDS
85	CGGGTACTTGGGTTGGAAAGATATGTA	4337530	CDS
86	AAAATGCAGGGTTGGATCTAGT	4340819	CDS
87	GAACGAAGGGTTGGACGAGTCTTT	4389004	CDS
88	ATCCTATCGGGTTGGAGGATATCGG	4443247	CDS

Table S2 (cont'd)

Chromosome III			
89	GAGCTATAGGGTTGGATCCTATTA	286548	Promoter
90	AAGGAATAAATGGGTTGGAGTGCCAA	305407	Promoter
91	TTTCTCAACGGGTTGGAATGTATTAA	420905	CDS
92	GAATGAATCAGCAGGGTTGGACGTTAATG	480512	CDS
93	AAAATATGGGTTGGAAGTTTCCCTC	494998	CDS
94	CCTCAATCGGGGTTGGATGACCC	557919	CDS
95	ATTTTGTTGAGCTTGGGTTGGAATTATAGC	558979	Promoter
96	CTATTCGTTTGGGTTGGACGAGTTTAGAC	658503	Promoter
97	GAAGAATCCGGGTTGGACAGGAATCAA	659344	CDS
98	GCTGATTTTGGGTTGGAAGACAAAGTAC	663459	CDS
99	CTAGTTTAGGGGTTGGATAATATGA	1211663	Promoter
100	TTTTTTCTGGGTTGGACGTCTTTTCTAA	1246200	CDS
101	ATTATTAAAGATGGGTTGGAGCATTTCC	1332087	CDS
102	CTTTAAAAGGGTTGGATATATA	1373496	Promoter
103	CGTAAGTTGATGGGTTGGACACCAGGT	1455138	CDS
104	GAAGAAAGGGTTGGAGCGCTCCA	1475741	Promoter
105	TGCAGTGACGGGTTGGATAGCCTCA	1512636	CDS
106	TTAGATTTGGGTTGGATGATGC	1586611	CDS
107	GAAGGGGAGGGGTTGGAAAGGAAAAGA	1588235	CDS
118	GGGAATACCATGGGTTGGATGCTTAGTAAC	1604090	CDS
109	CTTGAGTTGGGGTTGGAGGTCGAT	1890253	CDS
110	GTTGATGCTGGGTTGGATGCTAAGC	1965368	Promoter
111	CATAAAACCAGGGTTGGACTGTAGAAG	2188244	Promoter
112	ATCATACTTGGGTTGGAGATGGAC	2425187	CDS

19 promoters
2 convergent intergenic

Table S3: Oligonucleotides used in this study

Oligonucleotide	Sequence
GRSI(1-100) forward	CCCTTTTGTTCTTCACGTCCTTTTTATGAAATACGTGCCGGTGTTC CGGGGTTGGATGCGGAATCGAAAGTGTTGAATGTGAAATATGCGG AGGCCAAGTAA
GRSI(1-100) reverse	CTAGTTACTTGGCCTCCGCATATTTCACATTCAACACTTTCGATTCC GCATCCAACCCCGGAACACCGGCACGTATTTCATAAAAAGGACGT GAAGAACAAAAGGGAGCT
GRSI(1-50) forward	TCGAGCCTTTTGTTCTTCACGTCCTTTTTATGAAATACGTGCCGGT GTTCCGGGGC
GRSI(1-50) reverse	TCGAGCCCCGGAACACCGGCACGTATTTCATAAAAAGGACGTGAA GAACAAAAGGC
GRSI(51-100) forward	TCGAGTTGGATGCGGAATCGAAAGTGTTGAATGTGAAATATGCGG AGGCCAAGTAC
GRSI(51-100) reverse	TCGAGTACTTGGCCTCCGCATATTTCACATTCAACACTTTCGATTC CGCATCCAAC
GRSI(26-75) forward	TCGAGTGAAATACGTGCCGGTGTTCCGGGGTTGGATGCGGAATC GAAAGTGTTGAC
GRSI(26-75) reverse	TCGAGTCAACACTTTCGATTCCGCATCCAACCCCGGAACACCGGC ACGTATTTCAC
GRSI (26-60) forward	TCGAGTGAAATACGTGCCGGTGTTCCGGGGTTGGATGCGGC
GRSI (26-60) reverse	TCGAGCCGCATCCAACCCCGGAACACCGGCACGTATTTCAC
GRSI (41-75) forward	TCGAGTGTTCCGGGGTTGGATGCGGAATCGAAAGTGTTGAC
GRSI (41-75) reverse	TCGAGTCAACACTTTCGATTCCGCATCCAACCCCGGAACAC
GRSI (41-60) forward	CCGGGTGTTCCGGGGTTGGATGCGGGC
GRSI (41-60) reverse	GGCCGCCCGCATCCAACCCCGGAACAC
GRSI(46-55)F1	GCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGCGGG GTTGGACGGATCCCCGGGTTAATTAA
GRSI(49-58)F1	GCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGGGTTG GATGCCGGATCCCCGGGTTAATTAA
GRSI(48-55)F1	AAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCT GGGTTGGATCCCCGGGTTAATTAAG
GRSI8flip forward	CCGGGTGTTCCGTCCAACCCTGCGGGC
GRSI8flip reverse	GGCCGCCCGCAGGGTTGGACGGAACAC
GRSImid8transition-1	TCGAATGTTCCAAAACCAAATGCGGC
GRSImid8transition-2	TCGAGCCGCATTTGGTTTTGGAACAT
GRSII(-1440-497)F	GTCTTCATCATATGCTGGA
GRSII(-1440-497)R	TGATATGAATATAAGCTGA
GRSII(-869-497)F	GGATCCGTCTTCATCATATGCTGGAGGCTG
GRSII(-869-497)R	GCGGCCGCCCCAGCAGAAAGCAAACAACC
GRSII(-869-684)F	GGATCCGTTCAATAGTGTATCCCT
GRSII(-869-684)R	GCGGCCGCCCCAGCAGAAAGCAA
GRSII(-683-497)F	GGATCCGTCTTCATCATATGCTGG
GRSII(-683-497)R	GCGGCCGCTACTTCTCTTCCTACTGTTA
GRSII(-776-590)F	GGATCCGTGAAAGATCGTACGAAC
GRSII(-776-590)R	GCGGCCGCACGACTTGTTGTTAATG
GRSII(-636-590)F	GATCCGTGAAAGATCGTACGAACGTTCACTACTCGTTTCATATACA ACGTAGGC
GRSII(-636-590)R	GGCCGCCTACGTTGTATATGAAACGAGTAGTGAACGTTCGTACGA TCTTTCACG

Table S3 Oligonucleotides (p. 2 of 2)

GRSII(-683-637)F	GATCCCAAGCGTGAATGATTGCTGGGAAGAATAACAGTAGGAAGA GAAGTAAGC
GRSII(-683-637)R	GGCCGCTTACTTCTCTTCCTACTGTTATTCTTCCCAGCAATCATTC ACGCTTGG
GRSII(-659-613)F	GATCCCTACTCGTTTCATATACAACGTAGCAAGCGTGAATGATTG CTGGGAAGC
GRSII(-659-613)R	GGCCGCTTCCCAGCAATCATTCACGCTTGCTACGTTGTATATGAA ACGAGTAGG
GRSII(-650-637)F1	GCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGCAAG CGTGAATGATCGGATCCCCGGGTTAATTAA
GRSII(-651-663)F1	GCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGTGCT GGGAAGAATCGGATCCCCGGGTTAATTAA
GRSII(-656-644)F1	GCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGGAAT GATTGCTGGCGGATCCCCGGGTTAATTAA
pRS306URAR1	CGAAATCAAAAAAAAGAATAAAAAAAAAATGATGAATTGAATTGAG AATTCGAGCTCGTTTAAAC
INO1CDS F	TAGTTACCGACAAGTGCACGTACAA
INO1CDS R	TAGTCTTGAACAGTGGGCGTTACAT
ACT1CDS F	GGTTATTGATAACGGTTCTGGTATG
ACT1CDS R	ATGATACCTTGGTGTCTTGGTCTAC
INO1Chip F	CCTTTTGTTCTTCACGTCCTTTTTA
INO1Chip R	GCCTCCGCATATTTCACATTC
GRSIURA3ChipF	CGTATGTTGTGTGGAATTGTGAGCG
GRSIURA3ChipR	GGTACCCAGCTTTTGTTCCCTTTAG
RPA34 intergenic F	GCGTATGTGCGTATAACTGTGTGTAACATAAG
RPA34 intergenic R	CATTCATCAGTTTCCACCAGCAGAAATGCC
TSA2 upstream	GGTACCTCCACTCGTGTTCAACAAGGA
TSA2 downstream	GGTACCCCGCCAAGAAATTCGAAGAT
TSA2 GRSIcore F	ATCTTAACTATATGCGCCCCTCTAGTTTACAAGTTTTAGTCATTGG GGGTTTCGTACGCTGCAGGTCGAC
TSA2 GRSIcore R	CTCGCCCGCTCCTAAACGACGCCAATTGTAAGGGGGGATCAGCC CTTCCATAGGGA-TAACAGGGTAAT-CCGCGCGTTGGCCGATTCAT
TSA2 GRSItransitionF	ATCTTAACTATATGCGCCCCTCTAGTTTACAAGTTTTAGTCATTGG AAACCAAAAGGGCTGATCCCCCCTTACAATTGGCGTCGTTTAGGA GCGGGCGAG
TSA2 GRSItransitionR	CTCGCCCGCTCCTAAACGACGCCAATTGTAAGGGGGGATCAGCC CTTTTGGTTTCCAATGACTAAAACTTGTAAACTAGAGGGGCGCATA TAGTTAAGAT
ura4up	aggatttcgaccaggatatggt
ura3.1up**	CGTTCCTTATATGTAGCTTTCGACATtaataccctcgcctggcact
ura3.1down**	CTAAACTCACAAATTAGAGCTTCAAggtcgtaatcctgttgtcgaa

*Primer pRS306URA R1 was used in combination with F1 primers to integrate GRS constructs of ≤ 20 bp at *URA3*.

**Capital letters correspond to sequences from *URA3* and lower case letters correspond to sequences from *Ura4+*.

Table S4: Yeast strains used in this study

Strain	Genotype	Reference
DBY37	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 sus1Δ::Kan^r Mat* ***a***	This study
DBY39	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 nup42Δ::Kan^r Mat* ***a***	This study
DBY41	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 nup145ΔC::Kan^r Mat* ***a***	This study
DBY54	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1Δ3:LacO128:URA3 Mat* ***a***	This study
DBY55	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1Δ5:LacO128:URA3 Mat* ***a***	This study
DBY66	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1Δ2:LacO128:URA3 Mat* ***a***	This study
DBY67	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1Δ4:LacO128:URA3 Mat* ***a***	This study
DBY77	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 nup120Δ::Kan^r Mat* ***a***	This study
DBY79	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 nup170Δ::Kan^r Mat* ***a***	This study
DBY83	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 nup188Δ::Kan^r Mat* ***a***	This study
DBY84	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 gsp1Δ::Kan^r Mat* ***a***	This study
DBY87	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 sac3Δ::Kan^r Mat* ***a***	This study
DBY97	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 esc1Δ::Kan^r Mat* ***a***	This study
DBY98	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 pml39Δ::Kan^r Mat* ***a***	This study
DBY99	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 pom34Δ::Kan^r Mat* ***a***	This study
DBY100	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 pom152Δ::Kan^r Mat* ***a***	This study
DBY101	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 src1Δ::Kan^r Mat* ***a***	This study
DBY108	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 thp1Δ::Kan^r Mat* ***a***	This study

Table S4: Yeast strains used in this study (p.2 of 5)

Strain	Genotype	Reference
DBY109	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 nup53Δ::Kan^r Mat* ***a***	This study
DBY112	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 nup100Δ::Kan^r Mat* ***a***	This study
DBY119	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 mlp1Δ::Kan^r Mat* ***a***	This study
DBY128	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 gle2Δ::Kan^r Mat* ***a***	This study
DBY134	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 nup116Δ::Kan^r Mat* ***a***	This study
DBY137	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 nic96Δ::Kan^r Mat* ***a***	This study
DBY141	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 spt7Δ::Kan^r Mat* ***a***	This study
DBY142	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 spt20Δ::Kan^r Mat* ***a***	This study
DBY143	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 gcn5Δ::Kan^r Mat* ***a***	This study
DBY151	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 nup59Δ::Kan^r Mat* ***a***	This study
DBY196	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 nup157Δ::Kan^r Mat* ***a***	This study
JBY397	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc::Kan^r INO1:LacO128:URA3 Mat* ***a***	[4]
JBY463	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 nup60Δ::Kan^r Mat* ***a***	This study
JBY466	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc:TRP1 INO1:LacO128:URA3 mlp2Δ::Kan^r Mat* ***a***	This study
JBY497	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 p6LacO(41-75):URA3 mlp1Δ::Kan^r Mat* ***a***	This study
MM170	*NUP120+::13myc-KAN, HIS7+::LacI-GFP,URA4::URA3.1::URA3+p6LacO128,LEU1+ADE6+*	This study
MM171	*NUP120+::13myc-KAN, HIS7+::LacI-GFP,URA4::URA3.1::URA3+p6LacOGRSI(41-75), LEU1+ADE6+*	This study
Nup2-TAP	*his3Δ1 leu2Δ1 met15Δ0 ura3Δ0 NUP2-TAP:His5+*	[5]
SAY21	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc::Kan^r p6LacOsegment3:URA3 Mat* ***a***	This study

Table S4: Yeast strains used in this study (p.3 of 5)

Strain	Genotype	Reference
SAY49	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc::Kan^r p6LacOGRSI(1-50):URA3 Mat* ***a***	This study
SAY50	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc::Kan^r p6LacOGRSI(50-100):URA3 Mat* ***a***	This study
SAY51	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc::Kan^r p6LacOGRSI(26-75):URA3 Mat* ***a***	This study
SAY56	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc::Kan^r p6LacOGRSI(41-75):URA3 Mat* ***a***	This study
SAY57	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc::Kan^r p6LacOGRSI(31-75):URA3 Mat* ***a***	This study
SAY61	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc::Kan^r p6LacOGRSI(41-60):URA3 Mat* ***a***	This study
SAY96	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc::Kan^r p6LacOGRSI-8bptransitionmutant:URA3 Mat* ***a***	This study
SAY108.1	*ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1, Sec63myc:TRP, LacI-GFP:HIS3 p6LacO128:URA3 kanMX6::URA3 Mat* ***a***	This study
SAY109	*ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1, Sec63myc:TRP, LacI-GFP:HIS3 p6LacO128:URA3 GRSI(41-60):kanMX6:URA3 Mat* ***a***	This study
SAY110	*ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1, Sec63myc:TRP, LacI-GFP:HIS3 p6LacO128:URA3 GRSI(46-55):kanMX6:URA3 Mat* ***a***	This study
SAY112	*ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1, Sec63myc:TRP, LacI-GFP:HIS3 p6LacO128:URA3 GRSI(49-58):kanMX6:URA3 Mat* ***a***	This study
SAY114	*ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1, Sec63myc:TRP, LacI-GFP:HIS3 p6LacO128:URA3 GRSI(48-55) or 8bp:kanMX6:URA3 Mat* ***a***	This study
SAY124	*ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1, Sec63myc:TRP, LacI-GFP:HIS3 LacOGRSI-8bpmutantINO1:INO1:URA3 Mat* ***a***	This study
SAY145	*ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1,ino1prΔ::his5 pRS306INO1:INO1:URA3 Mat* ***a***	This study
SAY146	*ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1,ino1prΔ::his5 pRS306INO1GRSI-8bpmutant:INO1:URA3 Mat* ***a***	This study
SAY161	*ino1Δ::kan^r ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 INO1:URA3 Mat* ***a***	This study
SAY162	*ino1Δ::kan^r ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1GRSI-8bpmutant INO1:URA3 Mat* ***a***	This study
SAY172	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 p6LacOGRSI(41-75):URA3 nup60Δ::Kan^r Mat* ***a***	This study
SAY173	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 p6LacOGRSI(41-75):URA3 esc1Δ::Kan^r Mat* ***a***	This study
SAY175	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 p6LacOGRSI(41-75):URA3 nup100Δ::Kan^r Mat* ***a***	This study
SAY176	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 p6LacOGRSI(41-75):URA3 pml39Δ::Kan^r Mat* ***a***	This study
SAY177	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 Sec63-13myc: TRP1 p6LacOGRSI(41-75):URA3 nup133Δ::Kan^r Mat* ***a***	This study

Table S4: Yeast strains used in this study (p.4 of 5)

Strain	Genotype	Reference
SAY179	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 Sec63-13myc: TRP1 p6LacOGRSI(41-75):URA3 nup84Δ::Kan^r Mat* ***a***	This study
SAY183	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC63-13myc::Kan^r p6LacOGRSI':URA3 Mat* ***a***	This study
SAY184	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 p6LacOGRSI(41-75):URA3 nup2Δ::Kan^r Mat* ***a***	This study
SAY185	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 p6LacOGRSI(41-75):URA3 thp1Δ::Kan^r Mat* ***a***	This study
SAY186	*ino1Δ::kan^r ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 INO1+3'GRSI:URA3 Mat* ***a***	This study
SAY187	*ino1Δ::kan^r ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 GRS8bpmutINO1'+5'GRSI:URA3 Mat* ***a***	This study
SAY188	*ino1Δ::kan^r ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 GRS8bpmutINO1+3'GRSI:URA3 Mat* ***a***	This study
SAY189	*ino1Δ::kan^r ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 GRSI-8bpmutINO1':URA3 Mat* ***a***	This study
SAY191	*ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1,LacI-GFP:HIS3 Sec63-13myc::Kan^r LacOINO1GRSI-8bpmutant + 3' GRSI:URA3 Mat* ***a***	This study
SAY192	*ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1,LacI-GFP:HIS3 Sec63-13myc::Kan^r LacOINO1GRSI-8bpmutant + 5' GRSI:URA3 Mat* ***a***	This study
SAY232	*ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1,LacI-GFP:HIS3 Sec63-13myc::TRP1 ino1das1Δpr::kanMX6 p6LacOGRS1-mutINO1: INO1 Mat* ***a***	This study
SAY234	*ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1,LacI-GFP:HIS3 Sec63-13myc::TRP1 ino1das1Δpr::kanMX6 p6LacOINO1: INO1 Mat* ***a***	This study
SAY236	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 Sec63-13myc::Kan^r LacOGRSII(-939-1440):URA3 Mat* ***a***	This study
SAY239	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 Sec63-13myc::Kan^r LacOGRSII(-869-497):URA3 Mat* ***a***	This study
SAY240	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 Sec63-13myc::Kan^r LacOGRSII(-869-684):URA3 Mat* ***a***	This study
SAY241	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 Sec63-13myc::Kan^r LacOGRSII(-683-497):URA3 Mat* ***a***	This study
SAY242	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 Sec63-13myc::Kan^r LacOGRSII(-659-613):URA3 Mat* ***a***	This study
SAY243	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 Sec63-13myc::Kan^r LacOGRSII(-683-637):URA3 Mat* ***a***	This study
SAY244	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 Sec63-13myc::Kan^r LacOGRSII(-636-590):URA3 Mat* ***a***	This study
SAY254	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 Sec63-13myc::Kan^r LacOGRSII(-656-644):URA3 Mat* ***a***	This study
SAY255	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 Sec63-13myc::Kan^r LacOGRSII(-663-651):URA3 Mat* ***a***	This study
SAY259	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 Sec63-13myc::Kan^r LacOGRSII(-650-637):URA3 Mat* ***a***	This study
SAY262	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 WT INO1 Mat* ***a***	This study

Table S4: Yeast strains used in this study (p.5 of 5)

SAY263	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 GRSImut INO1 at INO1 Mat* ***a***	This study
SAY264	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 GRSIImut INO1 at INO1 Mat* ***a***	This study
SAY265	*ade2-1 can1-100 LacI-GFP:HIS3: his3-11,15 leu2-3,112 trp1-1 ura3-1 GRSI,GRSII mut INO1 at INO1 Mat* ***a***	This study
WLY51	*ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 p6LacOGRSI(41-75):URA3 Mat* ***a***	This study
WLY52	*ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 p6LacO128:URA3 Mat* ***a***	This study
WLY53	*ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 p6LacOGRSI(41-75):URA3 Nup2-TAP:LEU2 Mat* ***a***	This study
WLY54	*ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 p6LacO128:URA3 Nup2-TAP:LEU2 Mat* ***a***	This study

Supplementary references

1. Leber, J.H., Bernales, S. & Walter, P. IRE1-independent gain control of the unfolded protein response. *PLoS Biol* 2, E235 (2004).
2. Travers, K.J. *et al.* Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. *Cell* 101, 249-258 (2000).
3. Gasch, A.P. *et al.* Genomic expression programs in the response of yeast cells to environmental changes. *Mol Biol Cell* 11, 4241-4257 (2000).
4. Brickner, J.H. & Walter, P. Gene recruitment of the activated INO1 locus to the nuclear membrane. *PLoS Biol* 2, e342 (2004).
5. Ghaemmaghami, S. *et al.* Global analysis of protein expression in yeast. *Nature* 425, 737-741 (2003).
6. Guarente, L. & Mason, T. Heme regulates transcription of the CYC1 gene of S. cerevisiae via an upstream activation site. *Cell* 32, 1279-1286 (1983).
7. Cox, J.S. & Walter, P. A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. *Cell* 87, 391-404 (1996).