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Gic1 is a novel heterochromatin boundary protein in vivo

Risa Mitsumori[1], Kaori Shinmyozu[2], Jun-ichi Nakayama[3],

Hiroyuki Uchida[1] and Masaya Oki[1, 4, 5]*

[1]Department of Applied Chemistry & Biotechnology, Graduate School of Engineering, University of Fukui, Fukui 910-8507, Japan. [2]Proteomics Support Unit Center for Developmental Biology, RIKEN, Kobe 650-0047, Japan. [3]Graduate School of Natural Sciences, Nagoya City University, Nagoya 467-8501, Japan. [4]Research and Education Program for Life Science, University of Fukui, Fukui 910-8507, Japan. [5]PRESTO, Japan Science and Technology Agency (JST), Kawaguchi 332-0012, Japan.

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* **Corresponding author:** Masaya Oki

Department of Applied Chemistry & Biotechnology, Graduate School of Engineering, University of Fukui, 3-9-1 Bunkyo, Fukui 910-8507, Japan

Tel: 81-776-27-8640. Fax: 81-776-27-8640. Email: ma4sa6ya@u-fukui.ac.jp.

ABSTRACT

In *Saccharomyces cerevisiae*, *HMR/HML,* telomeres and ribosomal DNA are heterochromatin-like regions in which gene transcription is prevented by the silent information regulator (Sir) complex. The Sir complex (Sir2, Sir3 and Sir4) can spread through chromatin from the silencer. Boundaries prevent Sir complex spreading, and we previously identified 55 boundary genes among all ~6,000 yeast genes. These boundary proteins can be distinguished into two types: those that activate transcription to prevent spreading of silencing, and those that prevent gene silencing by forming a boundary. We selected 44 transcription-independent boundary proteins from the 55 boundary genes by performing a one-hybrid assay and focused on *GIC1* (GTPase interaction component 1). Gic1 is an effector of Cdc42, which belongs to the Rho family of small GTPases, and has not been reported to function in heterochromatin boundaries in vivo. We detected a novel boundary-forming activity of Gic1 at *HMR*-left and telomeric regions by conducting a chromatin immunoprecipitation assay with an anti-Sir3 antibody. We also found that Gic1 bound weakly to histones in two-hybrid analysis. Moreover, we performed domain analysis to identify domain(s) of Gic1 that are important for its boundary activity, and identified two minimum domains, which are located outside its Cdc42-binding domain.

INTRODUCTION

HMR/HML, telomeres, and ribosomal DNA (rDNA) are heterochromatic regions in *Saccharomyces cerevisiae*. Polymerization of the silent information regulator (Sir) proteins Sir2, Sir3, and Sir4 is required to achieve and maintain transcriptional silencing (Loo and Rine, 1995; Sun et al., 2011). Sir2 is a NAD+-dependent histone deacetylase and deacetylates the N-terminal of histone H3/4 (Tanny et al., 1999; Imai et al., 2000; Ito et al., 2000; Landry et al., 2000). The Sir complex (Sir2/3/4) spreads from the silencer (*HML*-E, *HML*-R, *HMR*-E, and *HMR*-R) at *HMR/HML* and inhibits transcriptional activity (Triolo and Sternglanz, 1996; Moazed, 2001), while at telomeres the complex spreads from *TG1–3* repeats. Silencing at rDNA does not require Sir3 or Sir4, in contrast to that at telomeres and *HMR/HML*. However, Sir2 is required for silencing at rDNA regions (Rusche et al., 2003; Moazed et al., 2004).

However, spreading of the silencing region must be stopped to allow the transcription of essential genes. Thus, a boundary must exist to prevent spreading of gene silencing and protect gene activity. Two types of boundary models have been reported (Oki and Kamakaka, 2002). One boundary type is specific to the DNA sequence and protects against spreading of gene silencing. For example, tDNA near to the *HMR*-left site and the *STAR* sequence at telomeres are specific DNA sequences for

boundaries. tDNA is a well-known boundary in *S. cerevisiae* (Donze et al., 1999; Donze and Kamakaka, 2001; Oki and Kamakaka, 2005). tDNA binds to *TFIIIC*, a component of DNA polymerase III, and can prevent spreading of silencing (Simms et al., 2008; Valenzuela et al., 2009). In telomeres, the *STAR* sequence, which consists of the *STR* sequence in the X-region and Y`-*STAR* in the *3`* terminal region of the *Y`* element, acts as a specific DNA sequence at boundaries (Fourel et al., 1999). Tbf1 and Reb1 can bind to the *STAR* sequence and prevent spreading of silencing. The other model is the DNA sequence-independent model, in which proteins protect against gene silencing. Sas2 is one such well-known protein (Kimura et al., 2002; Suka et al., 2002). Sas2 is a histone acetyltransferase (HAT) and competes with Sir2, a NAD+-dependent histone deacetylase. They form a boundary and change the chromatin structure from heterochromatin to euchromatin and vice versa.

To identify other boundary proteins, our group previously developed a genome-wide screening system (Oki et al., 2004). This system detected 55 boundary proteins among the total of ~6000 *S. cerevisiae* genes. These 55 genes are classified into eight groups (Histone modifications, *SWI/SNF*, *TFIID*, Mediator, Transcription, Cell cycle, Other, and Unknown). We previously investigated the function of some of these 55 proteins in boundary formation. For example, Sgf29 belongs to the Histone modifications group and is a component of the Spt-Ada-Gcn5-acetyltransferase (SAGA)

complex (Kamata et al., 2014). It is conserved from human to yeast and is important to maintain transcription. Domain analysis revealed that Sgf29 has two boundary elements, and a tag fusion system revealed that two small elements form a complex with Gcn5, Ada1, and Ada3. We also analyzed another boundary protein, Ycr076c/Fub1, which belongs to the Unknown category and interacts with multiple subunits of the 20S proteasome core particle. We also found that the proteasome and Fub1 work together to establish a boundary using a proteasome factor-deficient strain (Hatanaka et al., 2011).

In this study, we investigated a new boundary element to demonstrate DNA sequence-independent boundary formation and to identify a new form of boundary organization. We isolated proteins with DNA sequence-independent boundary function among the 55 boundary proteins using a one-hybrid assay and investigated the boundary function of Gic1 (GTPase interaction component 1). Gic1 did not activate transcription and we sought to understand its relationship with the silencing region. Gic1 is an effector of Cdc42, a Rho family small GTPase (Johnson, 1999), and is involved in cytokinesis and bud growth, localization, and appearance in yeast (Brown et al., 1997; Chen et al., 1997; Hofken and Schiebel, 2004). Recent studies reported that Gic1 is involved in septin recruitment, ring formation, and dissociation (Iwase et al., 2006; Lindsey et al., 2010; Sadian et al., 2013). Gic1 has a homologous

protein, Gic2 (GTPase interaction component 2), which is also an effector of Cdc42 (Jaquenoud and Peter, 2000). Gic1 and Gic2 colocalize with Cdc42 as cell polarity is established during the cell cycle and during mating in response to pheromones. The double mutant of *gic1/gic2Δ* has a temperature-sensitive defect in polarity establishment, with perturbed actin cytoskeleton organization (Brown et al., 1997; Chen et al., 1997). Gic1 and Gic2 have a CRIB (Cdc42/Rac interactive binding) motif, which assists transport to cell membranes and binding to Cdc42, and is conserved from human to yeast (Burbelo et al., 1995). Another study reported a new Gic1 domain, the BR (basic-rich) domain, which is located at the N-terminal of the CRIB domain and is basic and hydrophobic (Takahashi and Pryciak, 2007). A fluorescence assay of BR-EGFP revealed that BR localizes both in the nucleus and cytoplasm, and is important for Gic1 activity at actin and in septin association. Interestingly, the nuclear localization of Gic1 depends on the basic residues of the BR domain.

In this study, we report the novel function of Gic1 in boundary formation at heterochromatic regions.

MATERIALS AND METHODS

Strains The yeast strains used in this study are listed in Supplementary Table S1. Target genes were replaced by the *KanMAX* gene (Knockout Strain Collection, Open Biosystems). PCR products were

amplified from genomic DNA and transformed into yeast cells.

Plasmids The plasmids used in this study are listed in Supplementary Table S2. All domains were obtained by PCR amplification and cloned into the pFOM44 plasmid. In domain *gic1Δ* mutant, pFOM781–784 was generated using pFOM56 by self-PCR and checked by sequencing. The primers are listed in Supplementary Table S3.

One- and two-hybrid assays To determine the transcriptional activity and cooperation of Gic1, histones, and Sir proteins, a β-galactosidase activity assay was performed as described previously (Reynolds et al., 2001). Yeast cells were transformed with *GBD*, *GBD*-fusion genes, or *GBD*-fusion genes and *GAD*-fusion genes and then grown in synthetic dextrose (SD) medium containing adenine, leucine, lysine, uracil, and histidine (but lacking tryptophan), or adenine, lysine, uracil, and histidine (but lacking leucine and tryptophan) for 2 or 3 days at 30°C. Inoculated overnight cultures grew to mid-log phase (OD_{600}=1.5–2.0). Cells collected from 1 ml of each medium were re-suspended in 1 ml of Z buffer (60 mM $Na_2HPO_4{\cdot}7H_2O$, 40 mM $NaH_2PO_4{\cdot}H_2O$, 10 mM KCl, 1 mM $MgSO_4{\cdot}7H_2O$, and 50 mM 2-mercaptoethanol) and placed on ice. Thereafter, 0.2 ml of 4 mg/ml ONPG was added and samples were placed in a water bath at 28°C. When a medium-yellow color developed, the reaction was stopped by adding 0.5 ml of 1 M Na_2CO_3, the time was noted, and OD_{420} was measured. Units

were calculated using the following equation: $U=1000\times(OD_{420})-(1.75\times OD_{600})/T\times V\times OD_{600}$. This was calculated three times independently and normalized by the value of the gene with the highest activity.

Chromatin immunoprecipitation (ChIP) assay To determine the boundary activity of Gic1 in vivo, a ChIP assay was performed as described previously (Hatanaka et al., 2011). Cells were harvested from 200 ml of YPD medium at log phase (OD_{600}=2.0), washed once with phosphate-buffered saline, and crosslinked with 1% formaldehyde for 30 min at room temperature. Immunoprecipitation (IP) was performed using a rabbit polyclonal anti-Sir3p antibody (a gift from Dr. Kamakaka). The amount of DNA immunoprecipitated was analyzed using the Applied Biosystems SYBR Green RT-PCR system. Each immunoprecipitation was normalized by the *MAT* control. The primers are listed in Supplementary Table S4.

Mating assay To identify the minimum domain(s) of Gic1 required for its boundary function, a mating assay was performed as described previously (Oki et al., 2004). Yeast were transformed with *GBD* and *GBD*-fusion genes and grown in SD medium lacking tryptophan at 30°C for 3 days. Single colonies were streaked onto SD plates containing mating tester lawns with an OD_{600} of 2.0. Plates were incubated at 30°C for 3 days.

RESULTS

Selection of Gic1 in the screening of boundary proteins By performing a mating assay, we previously identified 55 genes related to boundary formation among 6000 genes in *S. cerevisiae* (Oki et al., 2004). In this method, the *Gal4*-binding sequence was inserted between E silencer and the reporter gene *a1* in the silencing region of *HMR*, and it was determined whether each GBD-fusion protein had boundary function. However, some of the 55 boundary proteins were predicted to have transcriptional activity. First, to exclude proteins that had a transcription-associated boundary (TAB) among the 55 proteins identified, we performed a one-hybrid assay, to identify proteins with low transcriptional activity that had a transcription-independent boundary (TIB) (Fig. 1). This method detected β-galactosidase activity and calculated transcriptional activity. We used a threshold level of β-galactosidase activity of around 0.2 to permit visual appraisal of the change in color, and we identified 44 genes with a transcription-independent boundary (TIB) function.

We selected *GIC1*, which belongs to categories other than those of histone modification, transcription, cell cycle, or Mediator, for boundary screening. *GIC1* is known to bind to the bud of yeast (Hofken and Schiebel, 2004). However, to our knowledge, *GIC1* has not been reported to be

associated with boundary formation; therefore, we expected to identify a new boundary formation system by studying *GIC1*.

Gic1 has boundary activity at *HMR* and *Tel-VR* in vivo Because we used an artificial *GBD* system to isolate boundary proteins among all *S. cerevisiae* genes, we next investigated where Gic1 has boundary function in vivo by performing ChIP analysis with an anti-Sir3 antibody. If Gic1 functions at boundaries in some regions, occupancy of Sir3 around these regions would be increased in the *gic1Δ* strain. In *S. cerevisiae*, *HMR*, *HML*, telomeric regions of chromosome ends, and rDNA are well-known heterochromatic regions, and boundary regions might be located close to them (Fourel et al., 1999; Donze and Kamakaka, 2001; Bi, 2002; Oki and Kamakaka, 2005). We analyzed *HMR*, telomeric, and rDNA regions (Fig. 2). First, we focused on the *HMR* region and compared Sir3 occupancy between wild type (WT) and *gic1Δ* strains. Sir3 occupancy was increased at boundary regions (R7 and R10), located either side of the *HMR*, in the *gic1Δ* (Fig. 2, a and b). These data suggest that Gic1 has a boundary function in the *HMR* region. In addition, its level at the *HMR* core region (R9) was also increased. The reason for this effect is unclear. Gic1 might have a function in stabilizing the chromatin association of Sir3, in addition to its role in boundary formation.

Next, we assessed telomeric and rDNA regions (Fig. 2c). *YER187W*, *YER188W*, and *YER186C* are

located close to the right telomere of chromosome V, and *ARR3* is located close to the right telomere of chromosome XVI. Sir3 occupancy at *YER188W* was higher in the *gic1Δ* strain, but the other regions were not appreciably different. These data suggest that Gic1 also has a boundary function close to *YER188W* in the right arm of telomere V, similar to its boundary function in the *HMR* region.

Gic1 binds to histones and functions at boundaries independent of other boundary proteins We detected boundary activity of Gic1 at *HMR* and telomeric regions using the ChIP assay. We next investigated whether Gic1 can bind to nucleosomes. Gic1 reportedly localizes to both the nucleus and cytoplasm (Chen et al., 1997; Hofken and Schiebel, 2004; Takahashi and Pryciak, 2007). However, it has not been reported whether Gic1 can bind to nucleosomes. To investigate this, we performed two-hybrid analysis with histone H2A, histone H2B, histone H3, histone H4, and Sir3. In this assay, FUY49 (for one or two-hybrid assay strains, see Supplementary Table S1) was transformed with GAD- and GBD-fusion plasmids (Supplementary Table S2). Gic1 could bind to all histones, but not to Sir3 (Fig. 3). These data suggest that Gic1 binds to histones directly and has boundary function.

Next, we investigated whether Gic1 works together with other boundary proteins to form a boundary. We selected 11 boundary proteins (Ada1, Ada2, Sas2, Sas5, Dot1, Snf6, Taf47, Med2, Clb1, Ycr076c, and Gds1), which were in each of the categories in our screening. Each of these proteins still had

boundary activity in the *gic1Δ* strain, meaning that the boundary activity of Gic1 is independent of other boundary proteins (Supplementary Fig. S1).

Gic1 has two small boundary domains We performed deletion analysis of Gic1 to identify the minimum region(s) necessary for its boundary function by using the patch mating system used for boundary screening (Oki et al., 2004)(Fig. 4a). A low level or absence of mating indicated boundary activity (Fig. 4b). First, we divided Gic1 into two regions, its N-terminal domain (1–151 amino acids (aa)), which includes the CRIB motif, and its C-terminal domain (167–315 aa). The CRIB motif in the N-terminal domain did not appear to have any role in Gic1 boundary function. A series of Gic1 deletion mutants of the C-terminal domain were constructed and tested using patch mating. These data indicate that 228–241 aa and 283–302 aa of Gic1 had boundary activity. To confirm this, we generated strains in which these regions were deleted (Δ228–241 aa and Δ283–302 aa) and performed patch mating. These strains did not have boundary activity. If endogenous Gic1 forms a homodimer with these two minimum regions, this would explain these data and mean their function is not related to boundaries. When we performed the same experiment with the *gic1Δ* strain, both domains still had boundary function (Fig. 5). Next, we investigated why Gic1 has two domains important for its boundary function and whether the same proteins can bind to both domains. We performed mass

spectrometry to identify binding proteins of the boundary domains (228–241 aa and 283–302 aa) by constructing strains expressing the minimum domains tagged with G196 (gift from Dr. Urano) (Kamata et al., 2013). However, we could not identify a protein that specifically binds to these domains (data not shown). The level of Gic1 was low in cells; therefore, it might not be detectable under the conditions used for affinity purification.

DISCUSSION

Gic1 has a novel boundary function in vivo In this report, we investigated the novel boundary protein Gic1. Gic1 has boundary activity according to a mating assay; however, its boundary function in vivo is unknown (Oki et al., 2004). First, we attempted to identify transcription-independent boundary (TIB) genes among 55 genes by performing a one-hybrid assay (Reynolds et al., 2001). This showed that 11 genes had a transcription-associated boundary (TAB) function and 44 genes had a transcription-independent boundary (TIB) function. Some of these 44 genes were previously reported to function at boundaries. For example, Sas2, the most well-known boundary protein, prevents the deacetylase activity of Sir2 (Kimura et al., 2002; Suka et al., 2002). Sgf29 and Sgf73 are components

of the SAGA, SAGA-like, ADA, and HAT-2 complexes (Kamata et al., 2013, 2014). *GIC1* does not belong to the Histone modification, Transcription, or Cell cycle groups. Our data suggest that Gic1 has novel boundary function by working together with another protein. Therefore, we performed liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis to identify a novel binding protein; however, we could not detect a novel binding partner of Gic1 (data not shown). We speculate that the interactions between Gic1 and such proteins are very weak or very transient, or that the concentration of such complexes is low.

Gic1 has boundary function in vivo Next, we showed that Gic1 has native boundary activity at *HMR* and telomere sites. Gic1 has many basic residues (BR domain, 29–33 aa). The BR domain is located close to the CRIB domain and is involved in protein-protein and protein-membrane interactions and localization of nuclear-targeting activity (Takahashi and Pryciak, 2007). Therefore, we predicted that the nuclear localization of Gic1 prevents heterochromatin in vivo. ChIP analysis using an anti-Sir3 antibody revealed the boundary function of Gic1 at *HMR* and telomeric regions. Elongation of heterochromatin from *HMR-left* to R7–R6, from *HMR*-right to R10-R11 and from the telomere to *YER188W* was not prevented in the *gic1Δ* strain (Fig. 2). However, the function of Gic1 in histone modifications or transcription regulation is unknown. We confirmed the novel heterochromatin

boundary function of Gic1. We next investigated whether Gic1 can bind directly to these boundary regions. We performed ChIP with a strain expressing Flag-Gic1; however, we could not detect Gic1 at these boundary regions (data not shown), although in our previous boundary screening study Gic1 had boundary activity. Given that Gic1 has many functions in living cells, the level of Gic1 at boundary regions might be low.

Gic1 interacts with histones Gic1 exhibits a genetic interaction with Cdc42, Rsr1, Bem1, Cdc24, and Bem2 (Chen et al., 1997; Johnson, 1999; Kawasaki et al., 2003; Gandhi et al., 2006). These proteins are associated with bud emergence, actin and septin localization, and mitosis. However, no reports have shown an interaction between Gic1 and histones. Such interactions are very important to form a heterochromatin boundary; therefore, we performed two-hybrid analysis (Fig. 3). Our results suggest that Gic1 weakly interacts with histones and that this is related to heterochromatin boundaries.

Gic1 has two minimum boundary domains Some boundary proteins have several functional boundary domains and interact with other proteins (Hatanaka et al., 2011; Kamata et al., 2014). We hypothesized that Gic1 also has boundary domains. We performed domain analysis using a mating assay to identify the boundary domain(s) of Gic1 and found two minimum boundary domains (Fig. 4b). These two domains do not contain the CRIB or BR domains, which assist budding at the neck and

nuclear localization of Gic1. It is suggested that Gic1 has a novel function to prevent elongation of heterochromatin in relation to histone modifications. Moreover, we checked the ability of the two minimum boundary domains to protect *a1* gene transcription from heterochromatin elongation using the *gic1Δ* strain. If Gic1 forms a homodimer, these two minimum domains might bind to and recruit intact Gic1. Both domains were independently capable of preventing heterochromatin elongation in the *gic1Δ* strain (Fig. 5). Next, we investigated how the two minimum domains function in the boundary activity of full-length Gic1 by generating *gic1* mutants in which each minimum boundary domain was disrupted. Interestingly, the mutant in which both domains were disrupted did not have boundary activity, whereas those in which only one domain was disrupted had weak boundary activity. These data suggest that these two minimum boundary domains work together to assemble a protein complex required for boundary formation. Therefore, we performed LC-MS/MS analysis with strains expressing Flag-tag fusion proteins to identify proteins that bind to these minimum boundary domains. We identified some weak binding proteins, one of which was Spa2, which is a component of the polarisome and can bind to Sir2 (Liu et al., 2010). The Gic1-Spa2-Sir2 complex might be important for boundary activity; however, further studies are required to prove this.

Gic1 associates with Zds1, which affects cell polarity and transcriptional silencing (Bi and Pringle,

1996; Zanelli and Valentini, 2005). In the *zds1* mutant, HM region and rDNA silencing is up-regulated, whereas telomere silencing is down-regulated (Roy and Runge, 2000). Moreover, rDNA recombination and life span are affected in the *zds1* mutant. We hypothesized that Gic1 might work together with Zds1 for boundary activity. Therefore, we performed the boundary assay with the minimum Gic1 boundary domain in the *zds1Δ* strain. However, boundary activity was retained, indicating that Gic1 does not work together with Zds1 to form a boundary (Supplementary Fig. S2).

Gic1 interacts with many proteins and has many functions in vivo. More experiments need to be performed in order to elucidate the native boundary mechanism in which Gic1 functions.

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FIGURE LEGENDS

Fig. 1. Measurement of the transcriptional activity of boundary elements.

The transcriptional activity of 55 boundary elements was assessed by a one-hybrid assay. FUY49 was transformed with plasmids harboring each boundary element fused to *GBD* (see Supplementary Table S2). The X-axis shows the 55 boundary genes, and the Y-axis shows β-galactosidase activity. Transcriptional activity was normalized against that of *ACE1*, which was highest. Error bars show standard deviation of three independent experiments.

Fig. 2. Gic1 affects heterochromatin boundaries at *HMR* and telomeric regions.

We compared the distribution of Sir3 between WT (FUY31) and *gic1Δ* (FUY53) strains at *HMR* and telomeric regions by performing a ChIP assay. (a) Schematic diagram of the *HMR* region. R4–R13 were the primer sets used for the ChIP assay. (b) Results of the ChIP assay for the *HMR* region. (c) Schematic diagram of telomeres. Black points denote the primer sets used for the ChIP assay. (d) Results of the ChIP assay with WT (white bars) and *gic1Δ* (black bars) strains at telomeres. The X-axis shows the primer set used. The Y-axis shows Sir3 distribution. The positive control is tel0.5 and the negative control is tel7.5. Data are normalized against that of *MAT*. The error bars show

standard deviation of three independent experiments.

Fig. 3. Gic1 is associated with histones.

GIC1 and *GAL11* were fused to GBD. Histones and *SIR3* were fused to GAD (see Supplementary Table S2). FUY49 was transformed with these plasmids. The X-axis shows each combination. The Y-axis shows β-galactosidase activity. Data were normalized against those obtained using a combination of GBD-*GAL11* and GAD.

Fig. 4. Identification of the minimum boundary domains of Gic1.

(a) Cartoon for our boundary screening system. If X protein does not have boundary activity, the yeast can grow on the selection plate (OFF). If X protein has boundary activity, the yeast cannot grow on the selection plate (ON). For details, see our previous paper (Oki et al., 2004).

(b)Plasmids were constructed harboring various domains of Gic1 and a mating assay was performed. The plasmids are listed in Supplementary Table S2. The numbers on the left denote the aa of Gic1. Black boxes denote domains, boxes containing slanted lines denote the CRIB domain, and boxes containing dots denote the BR domain. The right panel shows the mating results with four independent

colonies.

Fig. 5. The two minimum boundary domains of Gic1 form a boundary by themselves.

ROY2042 (Gal4bd+), FUY163 (*gic1Δ,* Gal4bd-), and FUY164 (*gic1Δ,* Gal4bd+) were transformed with a plasmid harboring one of the minimum boundary domains, and the mating assay was performed.

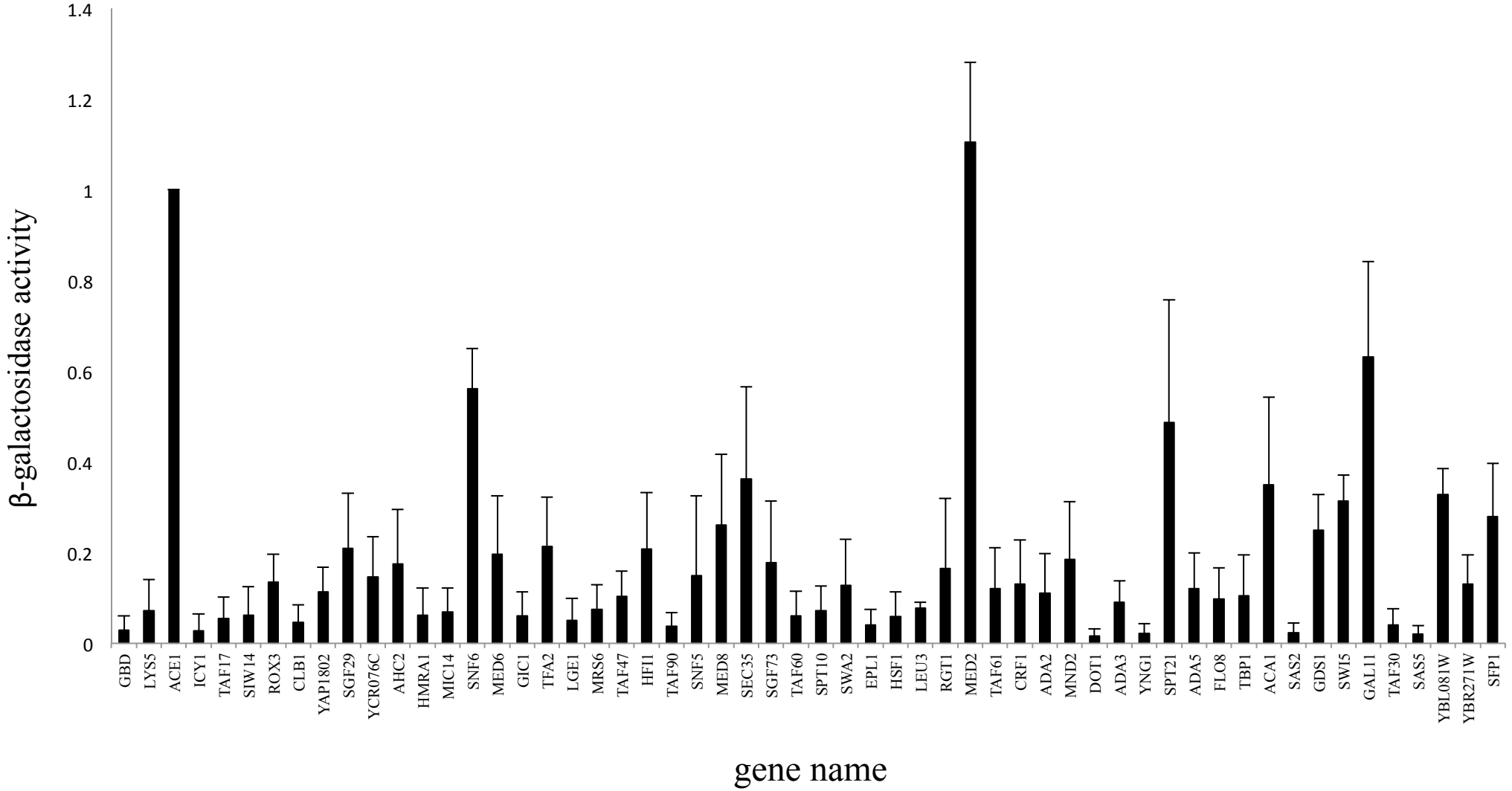
Supplementary Fig. S1. Gic1 has boundary activity independent of other boundary proteins.

ROY2042 (Gal4bd+), FUY163 (*gic1Δ,* Gal4bd-), and FUY164 (*gic1Δ*, Gal4bd+) were transformed with GBD-fusion plasmids, and the mating assay was performed with four independent colonies for boundary analysis.

Supplementary Fig. S2. Gic1 has boundary activity in the *zds1Δ* strain.

The mating assay for boundary screening was performed as shown in Fig. 4. FUY1509 was transformed with GBD-fusion plasmids, and the mating assay was performed with four independent colonies for boundary analysis.

Figure 1


β-galactosidase activity
1.4
1.2
1
0.8
0.6
0.4
0.2
0
GBD
LYS5
ACE1
ICY1
TAF17
SIW14
ROX3
CLB1
YAP1802
SGF29
YCR076C
AHC2
HMRA1
MIC14
SNF6
MED6
GIC1
TFA2
LGE1
MRS6
TAF47
HFI1
TAF90
SNF5
MED8
SEC35
SGF73
TAF60
SPT10
SWA2
EPL1
HSF1
LEU3
RGT1
MED2
TAF61
CRF1
ADA2
MND2
DOT1
ADA3
YNG1
SPT21
ADA5
FLO8
TBP1
ACA1
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GDS1
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GAL11
TAF30
SAS5
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YBR271W
SFP1
gene name

Figure 2

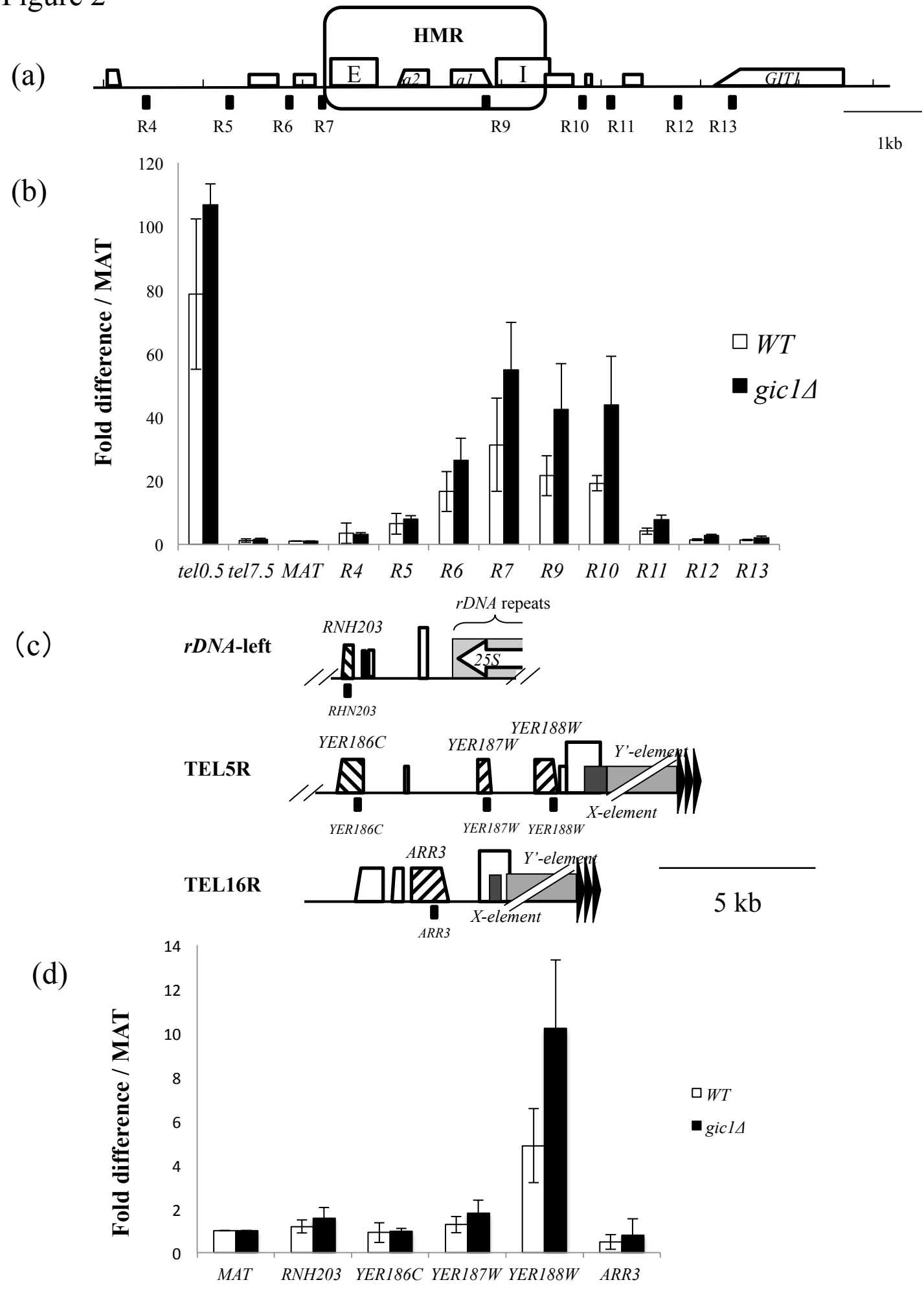
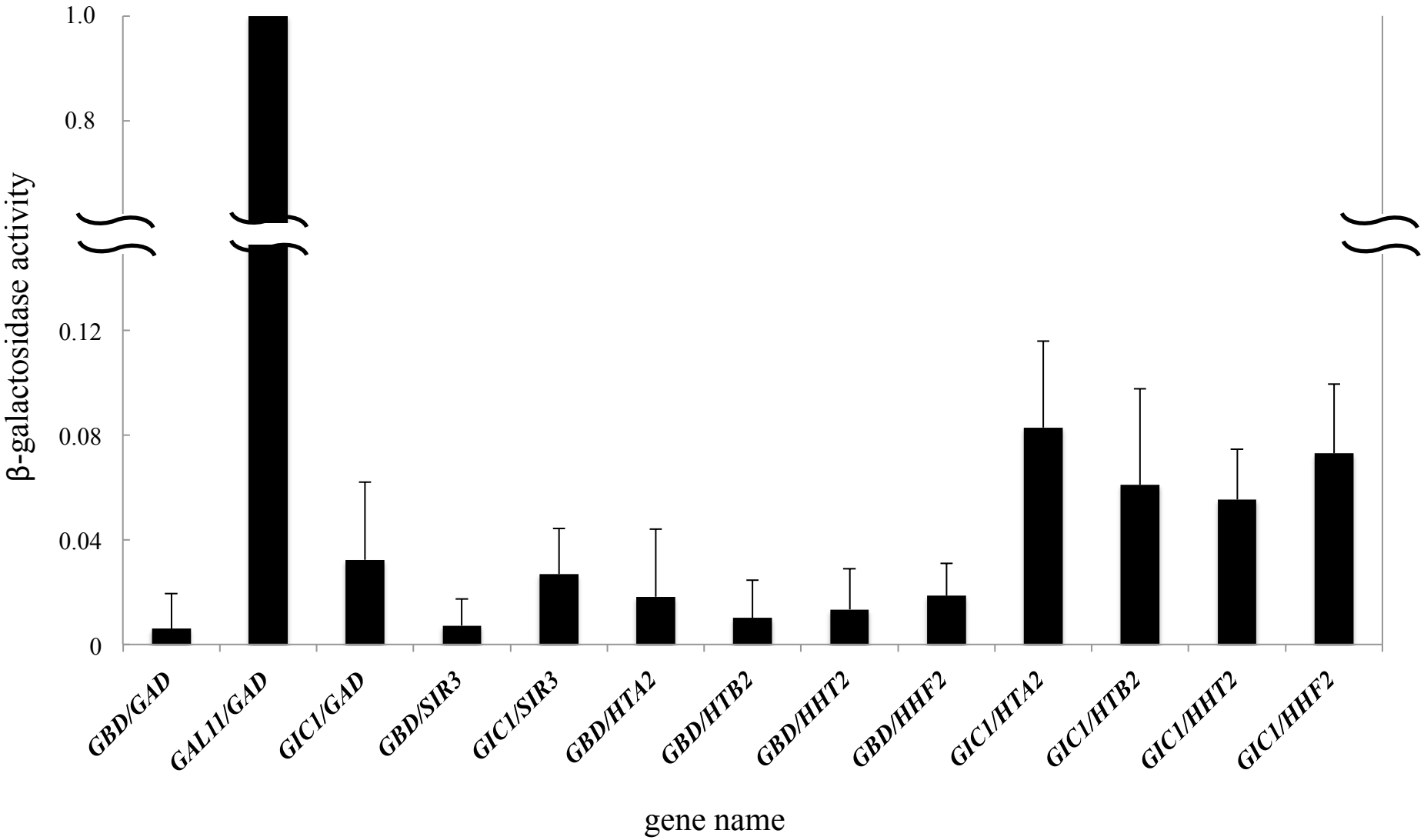

(a)
HMR
E
a2
a1
I
GIT1
R4
R5
R6
R7
R9
R10
R11
R12
R13
1kb
(b)
Fold difference / MAT
120
100
80
60
40
20
0
WT
gic1Δ
tel0.5
tel7.5
MAT
R4
R5
R6
R7
R9
R10
R11
R12
R13
(c)
rDNA repeats
rDNA-left
RNH203
25S
RHN203
TEL5R
YER186C
YER187W
YER188W
Y'-element
X-element
TEL16R
ARR3
5 kb
(d)
Fold difference / MAT
14
12
10
8
6
4
2
0
WT
gic1Δ
MAT
RNH203
YER186C
YER187W
YER188W
ARR3

Figure 3


1.0
0.8
0.12
0.08
0.04
0
β-galactosidase activity
GBD/GAD
GAL11/GAD
GIC1/GAD
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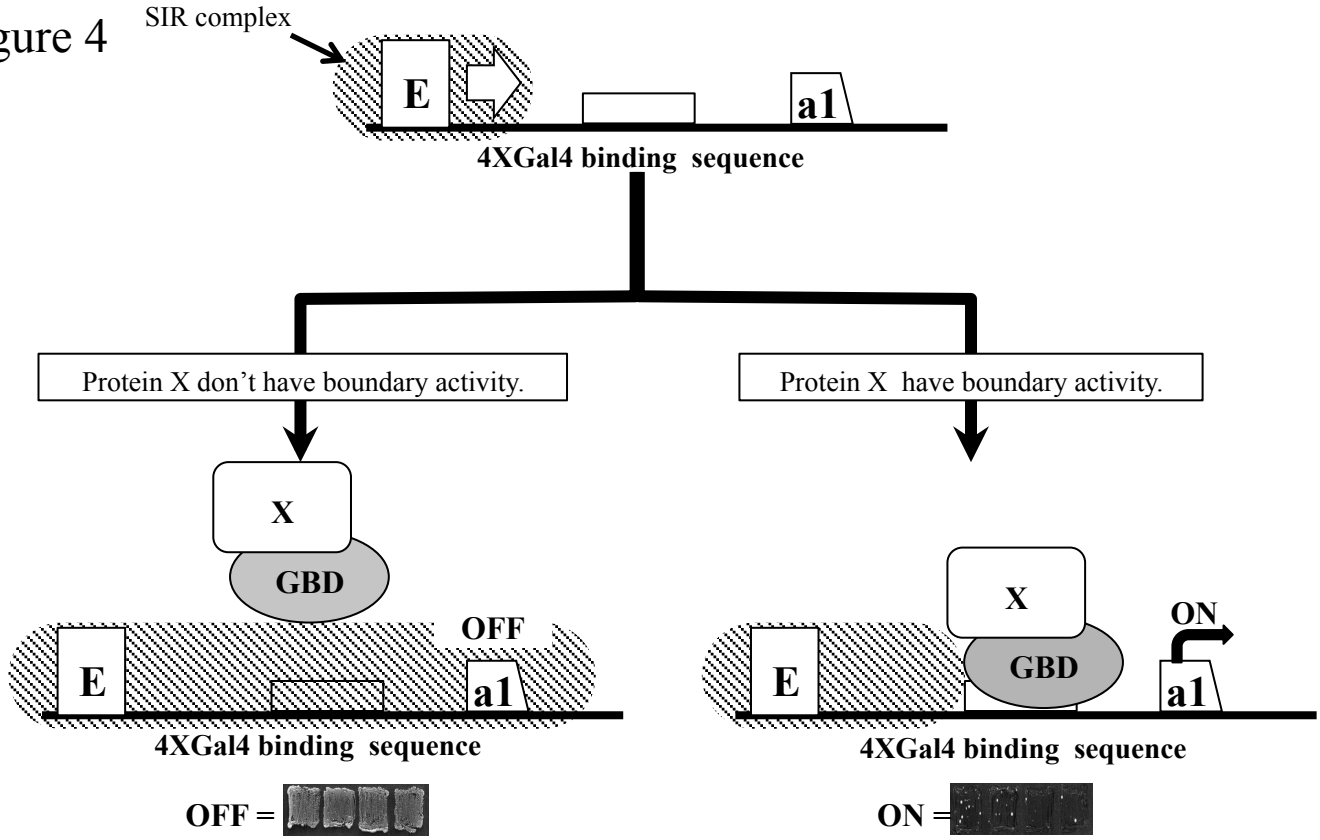
GIC1/SIR3
GBD/HTA2
GBD/HTB2
GBD/HHT2
GBD/HHF2
GIC1/HTA2
GIC1/HTB2
GIC1/HHT2
GIC1/HHF2
gene name

Figure 4

(a)


SIR complex
E
a1
4XGal4 binding sequence
Protein X don't have boundary activity.
Protein X have boundary activity.
X
GBD
OFF
E
a1
4XGal4 binding sequence
X
ON
GBD
E
a1
4XGal4 binding sequence
OFF =
ON =

(b)

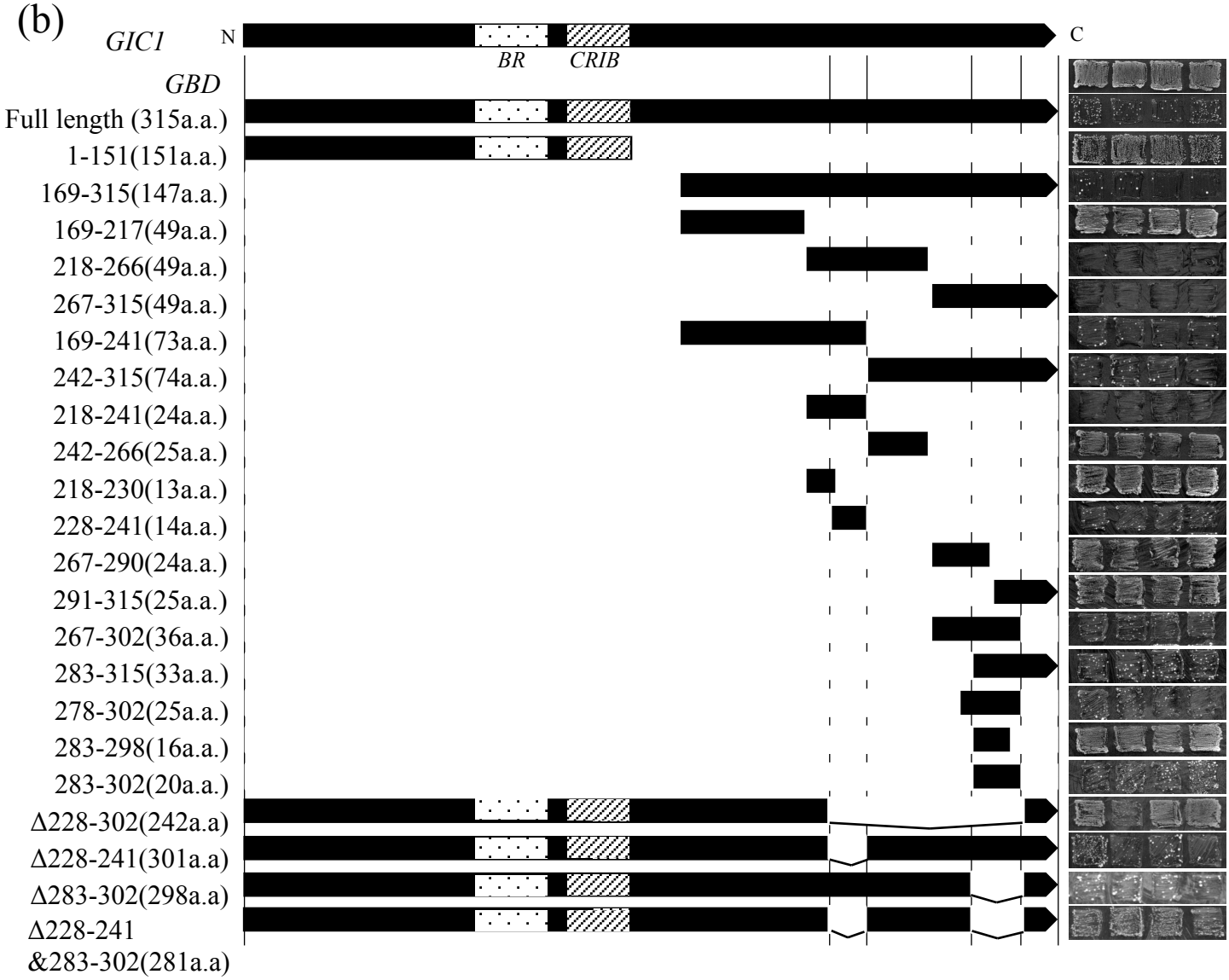
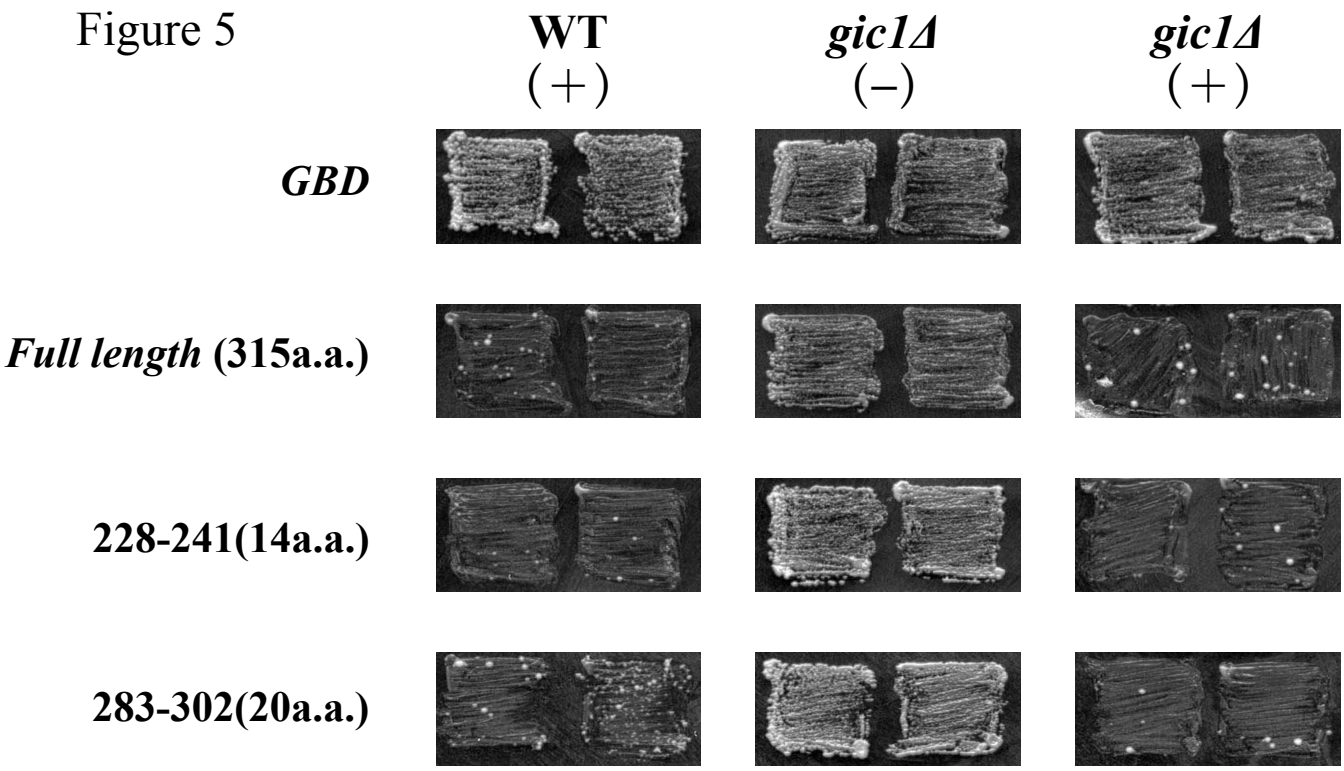

GIC1
N
C
BR
CRIB
GBD
Full length (315a.a.)
1-151(151a.a.)
169-315(147a.a.)
169-217(49a.a.)
218-266(49a.a.)
267-315(49a.a.)
169-241(73a.a.)
242-315(74a.a.)
218-241(24a.a.)
242-266(25a.a.)
218-230(13a.a.)
228-241(14a.a.)
267-290(24a.a.)
291-315(25a.a.)
267-302(36a.a.)
283-315(33a.a.)
278-302(25a.a.)
283-298(16a.a.)
283-302(20a.a.)
Δ228-302(242a.a)
Δ228-241(301a.a)
Δ283-302(298a.a)
Δ228-241
&283-302(281a.a)

Figure 5


WT
(+)
gic1Δ
(–)
gic1Δ
(+)
GBD
Full length (315a.a.)
228-241(14a.a.)
283-302(20a.a.)

Supplementary Table S1. Yeast strains

Strain	MAT	Genotype	Source
JRY19a	*a*	*ADE2 LYS2 his4-519 les2-3,112 trp1 ura3-52*	Rine J
ROY2041	@	*ADE2 lys2 trp1 his3 hmrΔ::bgl-bclΔ ura3-11::HMRΔI::URA3*	Oki M et al. 2004
ROY2042	@	*ADE2 lys2 trp1 his3 hmrΔ::bgl-bclΔ ura3-11::HMRΔ ＋4×Gal4bs at mat a2(EcoNI)::URA3*	Oki M et al. 2004
FUY31	*a*	*ADE2 lys2Δ his3-11 leu2-5,112 trp1-1 ura3-1*	W303 wild type
FUY49	*a*	*trp1-901 leu2-3,112 ura3-52 his3-200gal4(deleted) gal80(deleted) LYS::GAL11-HIS3 GAL-ADE2 met::GAL7-lacZ*	PJ69-4A
FUY53	*a*	*ADE2 lys2 his3-11 leu2-5,112 trp1-1 ura3-1 gic1Δ::KamMX*	this study
FUY163	@	*ADE2 lys2 trp1 his3 hmrΔ::bgl-bclΔ ura3-11::HMRΔI::URA3 gic1Δ::KamMX*	this study
FUY164	@	*ADE2 lys2 trp1 his3 hmrΔ::bgl-bclΔ ura3-11::HMRΔ ＋4×Gal4bs at mat a2(EcoNI)::URA3 gic1Δ::KamMX*	this study
FUY1509	@	*ADE2 lys2 trp1 his3 hmrΔ::bgl-bclΔ ura3-11::HMRΔ+4×Gal4bs at mat a2(EcoNI)::URA3 zds1 Δ ::KanMX*	this study

Supplementary Table S2. plasmid list

Plasmid		Marker	Comment
pACTII	GAD	*LEU2,Amp*	
pGBK-RC	GBD	*TRP1* , Kan^R	Ito, T et al. 2000
pRO586	GBD-Snf6p	*TRP1* , Kan^R	Oki M et al. 2004
pRO587	GBD-Taf47p	*TRP1* , Kan^R	Oki M et al. 2004
pRO589	GBD-Med2p	*TRP1* , Kan^R	Oki M et al. 2004
pRO590	GBD-Sas2p	*TRP1* , Kan^R	Oki M et al. 2004
pRO591	GBD-Sas5p	*TRP1* , Kan^R	Oki M et al. 2004
pRO592	GBD-Ada2p	*TRP1* , Kan^R	Oki M et al. 2004
pRO594	GBD-Clb1p	*TRP1* , Kan^R	Oki M et al. 2004
pRO596	GBD-Gds1p	*TRP1* , Kan^R	Oki M et al. 2004
pRO637	GBD-Dot1p	*TRP1* , Kan^R	Oki M et al. 2004
pFOM18	GBD-Hmra1p	*TRP1* , Kan^R	this study
pFOM48	GBD-Hfi1p	*TRP1* , Kan^R	this study
pFOM50	GBD-Ada3p	*TRP1* , Kan^R	this study
pFOM56	GBD-Gic1p	*TRP1* , Kan^R	this study
pFOM57	GBD-Ycr076cp	*TRP1* , Kan^R	this study
pFOM58	GBD-Sfp1p	*TRP1* , Kan^R	this study
pFOM59	GBD-Epl1p	*TRP1* , Kan^R	this study
pFOM60	GBD-Yng1p	*TRP1* , Kan^R	this study
pFOM61	GBD-Ada5p	*TRP1* , Kan^R	this study
pFOM62	GBD-Sgf29p	*TRP1* , Kan^R	this study
pFOM62	GBD-Sgf29p	*TRP1* , Kan^R	this study
pFOM63	GBD-Lge1p	*TRP1* , Kan^R	this study
pFOM83	GBD-Spt10p	*TRP1* , Kan^R	this study
pFOM84	GBD-Sgf73p	*TRP1* , Kan^R	this study
pFOM85	GBD-Tbp1p	*TRP1* , Kan^R	this study
pFOM86	GBD-Taf17p	*TRP1* , Kan^R	this study
pFOM87	GBD-Taf90p	*TRP1* , Kan^R	this study
pFOM88	GBD-Taf60p	*TRP1* , Kan^R	this study
pFOM89	GBD-Taf61p	*TRP1* , Kan^R	this study
pFOM90	GBD-Taf30p	*TRP1* , Kan^R	this study
pFOM91	GBD-Snf5p	*TRP1* , Kan^R	this study
pFOM92	GBD-Gal11p	*TRP1* , Kan^R	this study
pFOM93	GBD-Rox3p	*TRP1* , Kan^R	this study
pFOM94	GBD-Med6p	*TRP1* , Kan^R	this study
pFOM95	GBD-Med8p	*TRP1* , Kan^R	this study
pFOM96	GBD-Ace1p	*TRP1* , Kan^R	this study
pFOM97	GBD-Hsf1p	*TRP1* , Kan^R	this study
pFOM98	GBD-Leu3p	*TRP1* , Kan^R	this study
pFOM99	GBD-Rgt1p	*TRP1* , Kan^R	this study
pFOM100	GBD-Flo8p	*TRP1* , Kan^R	this study

pFOM101	GBD-Aca1p	*TRP1* , Kan^R	this study
pFOM102	GBD-Swi5p	*TRP1* , Kan^R	this study
pFOM104	GBD-Spt21p	*TRP1* , Kan^R	this study
pFOM105	GBD-Tfa2p	*TRP1* , Kan^R	this study
pFOM106	GBD-Siw14p	*TRP1* , Kan^R	this study
pFOM107	GBD-Mnd2p	*TRP1* , Kan^R	this study
pFOM108	GBD-Lys5p	*TRP1* , Kan^R	this study
pFOM109	GBD-Icy1p	*TRP1* , Kan^R	this study
pFOM111	GBD-Sec35p	*TRP1* , Kan^R	this study
pFOM112	GBD-Swa2p	*TRP1* , Kan^R	this study
pFOM113	GBD-Mrs6p	*TRP1* , Kan^R	this study
pFOM114	GBD-Ybl081wp	*TRP1* , Kan^R	this study
pFOM115	GBD-Ybr271wp	*TRP1* , Kan^R	this study
pFOM117	GBD-Mic14p	*TRP1* , Kan^R	this study
pFOM118	GBD-Crf1p	*TRP1* , Kan^R	this study
pFOM138	GBD-Gic1p(1-151)	*TRP1*, Kan^R	this study
pFOM139	GBD-Gic1p(169-315)	*TRP1*, Kan^R	this study
pFOM247	GAD-Sir3p	*LEU2,Amp*	this study
pFOM248	GAD-H2Ap(HTA2)	*LEU2,Amp*	this study
pFOM249	GAD-H2Bp(HTB2)	*LEU2,Amp*	this study
pFOM250	GAD-H3p(HHT2)	*LEU2,Amp*	this study
pFOM251	GAD-H4p(HHF2)	*LEU2,Amp*	this study
pFOM270	GBD-Gic1p(169-217)	*TRP1* , Kan^R	this study
pFOM271	GBD-Gic1p(218-266)	*TRP1* , Kan^R	this study
pFOM272	GBD-Gic1p(267-315)	*TRP1* , Kan^R	this study
pFOM273	GBD-Gic1p(169-241)	*TRP1* , Kan^R	this study
pFOM274	GBD-Gic1p(242-315)	*TRP1* , Kan^R	this study
pFOM275	GBD-Gic1p(218-241)	*TRP1* , Kan^R	this study
pFOM276	GBD-Gic1p(242-266)	*TRP1* , Kan^R	this study
pFOM277	GBD-Gic1p(169-242)	*TRP1* , Kan^R	this study
pFOM278	GBD-Gic1p(242-316)	*TRP1* , Kan^R	this study
pFOM280	GBD-Gic1p(218-242)	*TRP1* , Kan^R	this study
pFOM281	GBD-Gic1p(242-267)	*TRP1* , Kan^R	this study
pFOM282	GBD-Gic1p(169-243)	*TRP1* , Kan^R	this study
pFOM283	GBD-Gic1p(242-317)	*TRP1* , Kan^R	this study
pFOM671	GBD-Gic1p(278-302)	*TRP1* , Kan^R	this study
pFOM672	GBD-Gic1p(283-298)	*TRP1* , Kan^R	this study
pFOM673	GBD-Gic1p(283-302)	*TRP1* , Kan^R	this study
pFOM781	GBD-Gic1p(228-302)	*TRP1* , Kan^R	this study
pFOM782	GBD-Gic1p(Δ228-241)	*TRP1* , Kan^R	this study
pFOM783	GBD-Gic1p(Δ283-302)	*TRP1* , Kan^R	this study
pFOM784	GBD-Gic1p(Δ228-241&283-302)	*TRP1* , Kan^R	this study

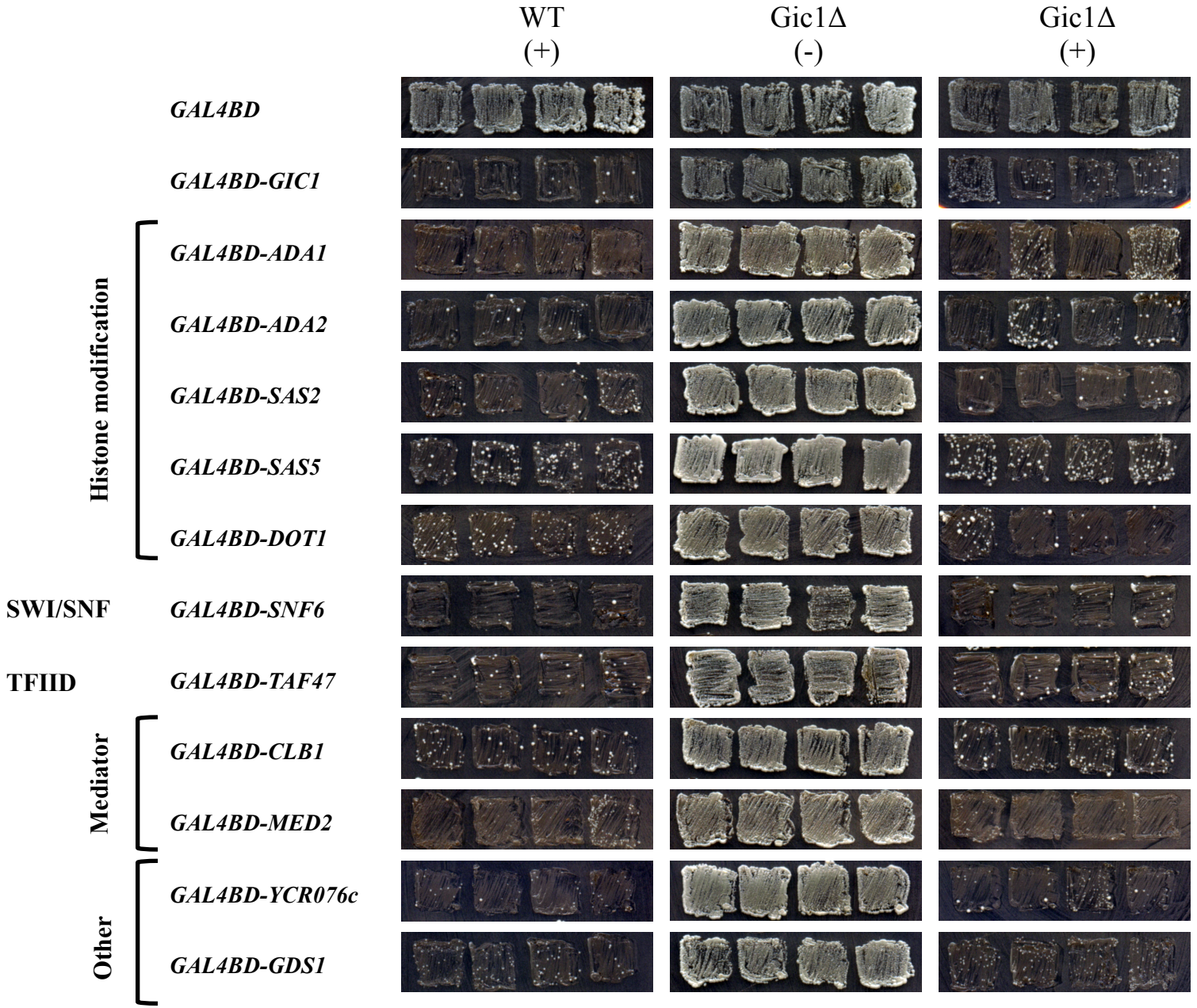
Supplementary Table S3. Primer for make plasmid or yeast

Name	leght	Sequence	Comment
79-KanMX-up600SalI	35	TTAGTCGACCTATGTCAGTCCAATACCTGTTGGTT	to make *gic1Δ* mutation
80-KanMX-down600SalI	35	TTGGTCGACGCTCAATACTTGATGCTAAAGTAGTC	to make *gic1Δ* mutation
900-ZDS1Ts-1480-C	25	ATGGGAATTCGAGAAAGAAGCCGAA	to make pFOM1509
901-ZDS1Ts-Rv-1370-C	31	ACGATCTCGAGCTATTCCCATTTCTTGTTCC	to make pFOM1509
168-GIC1-m40-N	20	ATCGCCGGAATTGGGATCCG	to make pFOM138,270,273
169-GIC1-440-C	31	GATATCTGCAGCTTCGTGCGACTCAAGAGGG	to make pFOM138,
170-GIC1-500-N	32	GTATAGAATTCCCCCGACCAGATTCAAACGTC	to make pFOM139,
171-GIC1-u33-C	21	CCGGAATTAGCTTGGCTGCAG	to make pFOM139,272,274,281,283
958-GIC1-C-49-N	35	ATCGGAATTCCAATTAGACTCGCCTACAGATTTGG	to make pFOM271,275,277
959-GIC1-C-49-C	35	AGCTCTGCAGTTACTTATTGCCATTTGGGGTATTG	to make pFOM170
960-GIC1-C-73-N	31	ATGCGAATTCGGAGATAGCGTCAGCGAAAAG	to make pFOM274,276
961-GIC1-C-73-C	32	CTGACCTGCAGTTAAAGAACAGAAGGAAATGT	to make pFOM273,275,278
962-GIC1-C-98-N	33	CTGAAGAATTCGAGTTGAGTGCGCTACATACGC	to make pFOM272,280,282
963-GIC1-C-98-C	33	GCGCCTGCAGTTATCTTGGCTTGAATTTGCCAG	to make pFOM271,276
980-GIC1-C-59-N	34	ATTTGGAATTCACCTTGGAAGACTTGAGAAATTA	to make pFOM278
981-GIC1-C-62-C	35	CGAATCTGCAGTTATTCCAAGGTCATTTCCAAATC	to make pFOM277
999-GIC1-C-122-C	34	ATCAGCTGCAGTCAACCAGGGGAATTTAGCGACT	to make pFOM280
1000-GIC1-C-122-N	33	ATCGAGAATTCAACAGAATATCTGTGGATGACG	to make pFOM281
1002-GIC1-109-N	35	ACTGCGAATTCAATTGTTTCAATGTAGATCAGTCG	to make pFOM671
1004-GIC1-C-114-N	29	ATCGAGAATTCGATCAGTCGCTAAATTCC	to make pFOM283,672,673
1005-GIC1-C-130-Rv	35	CAGATCTGCAGTTACACGTCATCCACAGATATTCT	to make pFOM672
1006-GIC1-C-134-Rv	35	TCGATCTGCAGTTAGTAGAATTTTAGCACGTCATC	to make pFOM282,671,673
2590-invGIC1-9-Rv	31	CATTTCCAAATCTGTAGGCGAGTCTAATTGC	to make pFOM781,782,784
2591-invGIC1-9-F	27	GGAGATAGCGTCAGCGAAAAGACCAAT	to make pFOM782,784
2592-invGIC1-28-Rv	35	TACATTGAAACAATTTCCTAATTCGGGCGTATGTA	to make pFOM783,784
2593-invGIC1-28-F	35	TATCAATGTAGTGAAACTAGTACTCCTCGAAATAC	to make pFOM781,783,784

Supplementary Table S4. primer set for real time PCR

region	Fr sequence	Rv sequence	Psition
tel0.5	CCTTTTTTGATATAACTGTCGGAGAGT	TCCGAACGCTATTCCAGAAAGT	Chr6R tel
tel7.5	TGTAGACTTCCCACTGTATTTGAATGA	CGTGAAAGTTCAGCGCAACA	Chr6R tel
MAT	AAGTTCACCCTGTTTCCATTGG	AACGAACGCAAATGATGAATTG	Chr3
R4	CCCTCCACTACGGCCATT	GACGTTAACATGCAACTCAACCA	Chr3 HMR
R5	CATTTTAAAGCAACATCCACATTGA	GCGAGAAAAACGCCCTGAA	Chr3 HMR
R6	CAACGCGTCATGAAAAAGAGTT	GGATGTCGCGAAGCATTACA	Chr3 HMR
R7	CGTATCTTGTACCCTTTTTATTGCATAT	TGGACGAAAAGAAATGCGATT	Chr3 HMR
R9	GCAAAGCCTTAATTCCAAGGAA	CCCAAACTCTTACTTGAAGTGGAGTA	Chr3 HMR
R10	TAGAATTGTCAAGCGCAAATCC	GAATAAACCTGGTCTCAAATAAAATTGG	Chr3 HMR
R11	TCGGAGATCTCTTACGGCTTATG	TGACGCACTGAATGTCATCAAA	Chr3 HMR
R12	GCATATGATATCGCCACTGCAT	CCTTAGCTATTTTCGGTGTCTTAATGA	Chr3 HMR
R13	CAGCAATAATAAAAGTCCATCTTGCA	AGTAGGCGTCGAATGTTTCCA	Chr3 HMR
RNH203	CCATCCTCTCGGGAAACACA	TGCCCTCTCTTTCGTAATTATTCA	Chr12
RNH203	CCATCCTCTCGGGAAACACA	TGCCCTCTCTTTCGTAATTATTCA	Chr12
YER186C	GGAGAGGGCTTGCCGTTATT	CCACATAATCTCGCCTAACAAAAA	Chr5
YER187W	TGCTGAGGACACGAAAAACG	TGCACCGTCACCAGGTATGT	Chr5
YER188W	CGACGAACAAACTGCAGCAA	TGACCGACTTGGCGTACTGA	Chr5
ARR3	TTGCCATGGTGCTAATTTGG	CAAGCACGACGCAGAGATCA	Chr16

Supplementary Fig. S1.



Supplementary Fig. S2.