

This is the Supplementary Information for ‘Identification of dosage-sensitive genes in *Saccharomyces cerevisiae* using the genetic tug-of-war method’ by Koji Makanae, Reiko Kintaka, Takashi Makino, Hiroaki Kitano, Moriya Hisao

Table of contents

Supplementary Figures	3
Figure S1. Structure of the pTOWug2-836 plasmid used in gTOW experiments in this study.....	3
Figure S2. Cloning of the target gene into pTOWug2-836.	4
Figure S3. Reproducibility of the gTOW6000 data.....	4
Figure S4. Relationship between CNL and max growth rate.	5
Figure S5. Relationship between CNL and frequency of no-growth.	5
Figure S6. Protein complex components tend to be highly expressed.	6
Figure S7. An example of genes that were not affected by the frameshift mutation.	7
Figure S8. Unknown DNA elements are the determinants of the low CNLs within the locus of <i>DIE2</i> and <i>IRC8</i>	8
Figure S9. Yeast DSGs tend to be highly expressed.	9
Figure S10. Correlation between the copy number limit and protein abundance.	10
Figure S11. Results of 2D-gTOW experiments (plate assay).....	11
Figure S12. Results of 2D-gTOW experiments (copy number determination).....	20
Figure S13. <i>TUB1</i> and <i>TUB3</i> are weak dosage suppressors (partners) of <i>TUB2</i> . .	25
Figure S14. Cloning site of pRS423ks.	26
Figure S15. Construction of a frameshift mutant.	26
Figure S16. Construction of the segmented genes.....	27
Figure S17. Construction of GFP- and GFPdeg-replaced plasmids.	29
Table S2. Statistical data of the 230 empty vector experiments.	30
Table S3. Correlations between max growth rates and copy numbers obtained in the gTOW6000 analysis.	30
Table S7. Partner-seeking experiment results.....	31
Supplementary References	34

Additional files provided as separate files.

Supplementary tables below provides as one Excel file with multiple worksheets.

- **Table S1:** Original gTOW6000 data
- **Table S4:** Copy number data for frameshift analysis
- **Table S5:** yeast DSGs
- **Table S6:** DSGs in Jones clones
- **Table S8:** Primers to construct gTOW6000
- **Table S9:** Additional primers to construct gTOW6000

Supplementary Figures

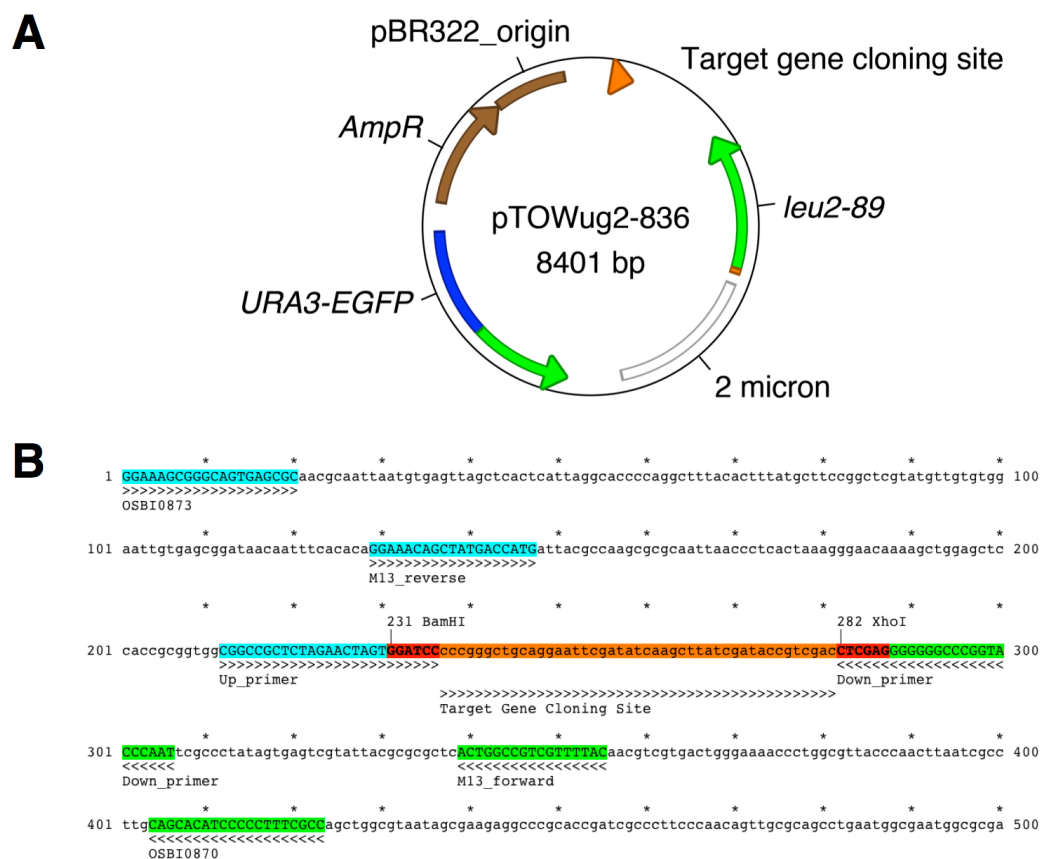


Figure S1. Structure of the pTOWug2-836 plasmid used in gTOW experiments in this study.

(A) A map of pTOWug2-836. (B) The nucleotide sequence of the target-gene cloning site of pTOWug2-836 (5' to 3' direction). The target gene cloning site, the *Bam*HI and *Xho*I sites used to prepare the host vector and the locations of primers used to check the inserts are indicated.

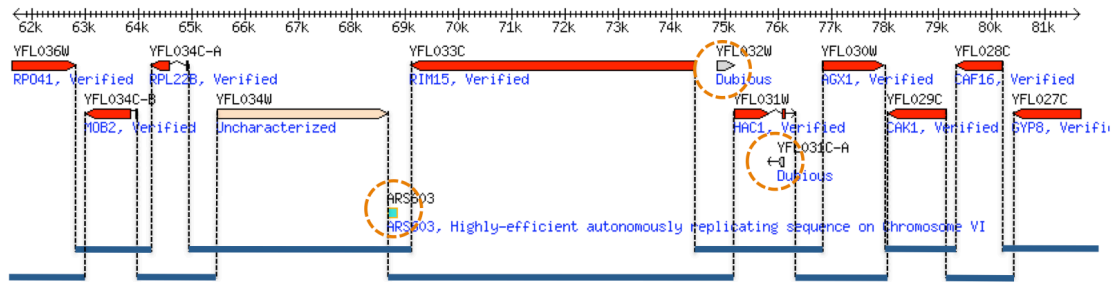
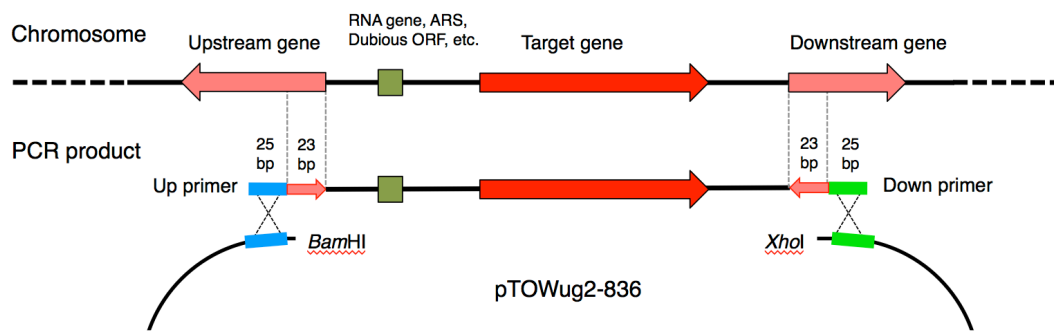
A**B**

Figure S2. Cloning of the target gene into pTOWug2-836.

A part of chromosome VI is shown as an example. The details of this figure are explained in the Method section of the main text.

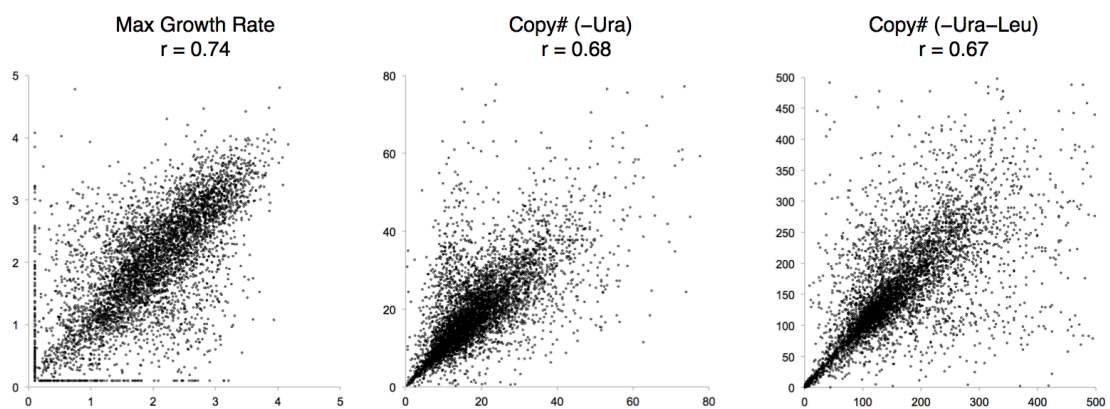


Figure S3. Reproducibility of the gTOW6000 data.

The data from two independent experiments were compared. Pearson's correlation coefficients (r) are shown.

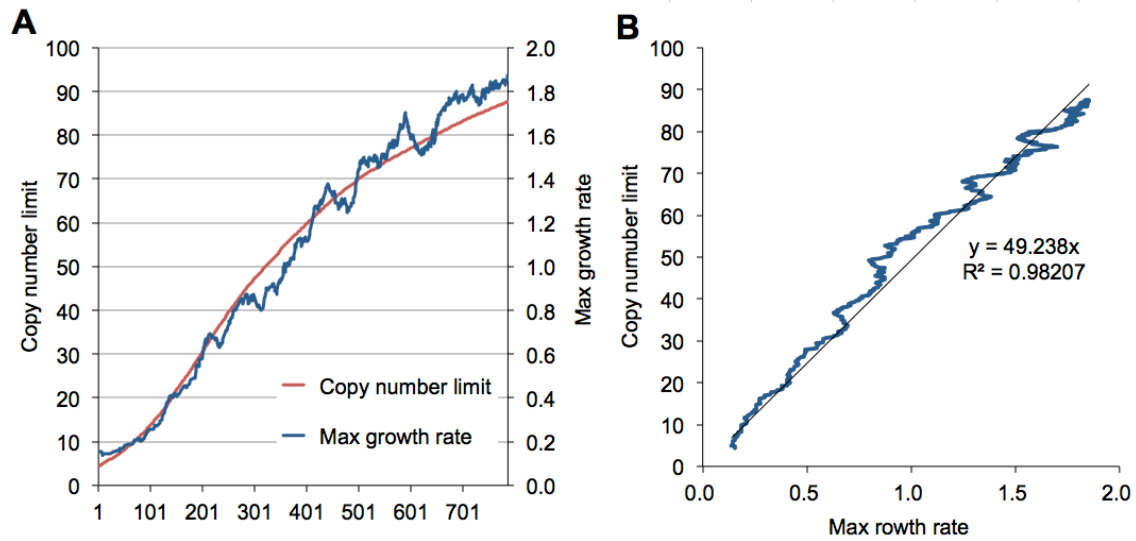


Figure S4. Relationship between CNL and max growth rate.

(A) Moving average of the CNL and the max growth rate of each 100 genes. X-axis shows the number of bin ordered by the CNL. (B) Relationship between the CNL and the max growth rate shown in A. Approximated curve is shown in black line.

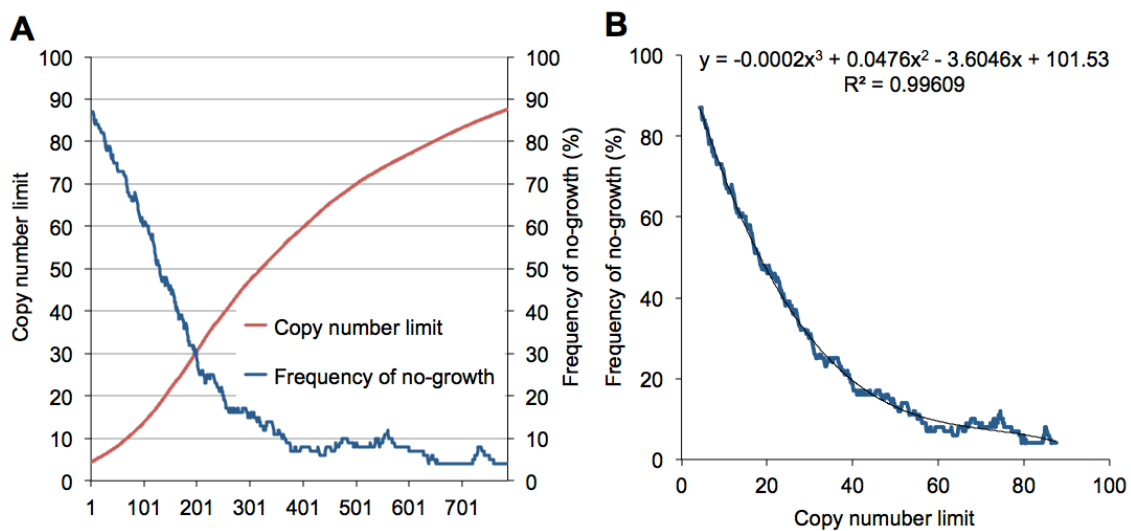


Figure S5. Relationship between CNL and frequency of no-growth.

(A) Moving average of the CNL and the frequency of no-growth of each 100 genes. X-axis shows the number of bin ordered by CNL. (B) Relationship between CNL and frequency of no-growth shown in A. Approximated curve is shown in black line.

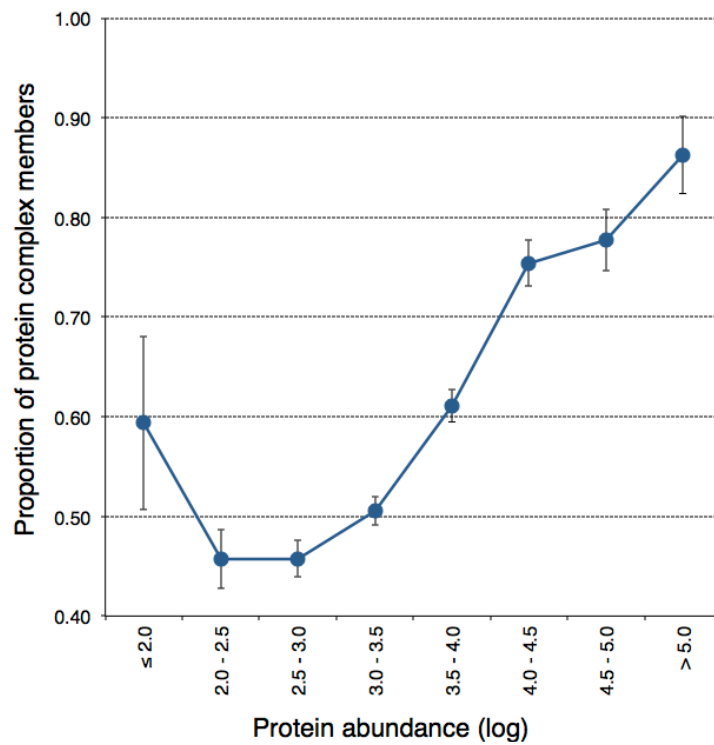


Figure S6. Protein complex components tend to be highly expressed.

The protein abundance data were obtained from Ghaemmaghami *et al.* (2003), and the data for protein complex members were obtained from MIPs (mips; ftp://ftpmips.gsf.de/yeast/catalogues/complexcat/complexcat_data_18052006).

The protein abundance unit is molecules per cell. The error bars indicate the standard error of the mean (SEM).

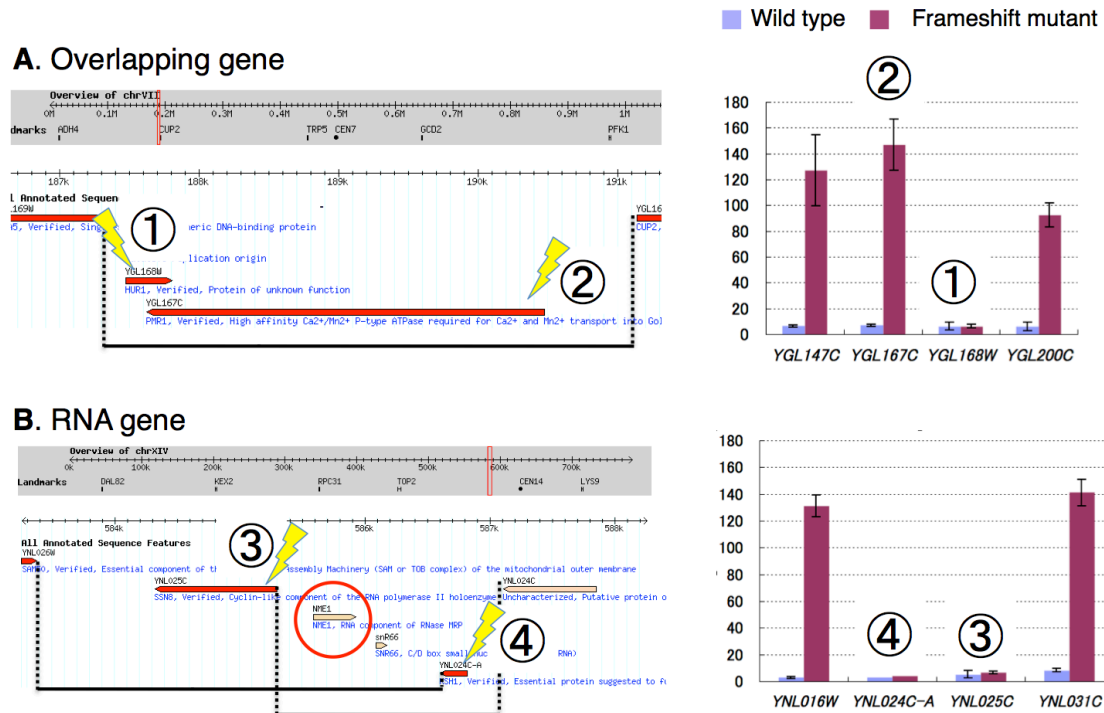


Figure S7. An example of genes that were not affected by the frameshift mutation.

The charts on the left show the chromosomal regions containing the target genes. The sites of frameshift mutations are shown in yellow arcs. A red circle indicates an RNA gene (*NME1*). The graphs on the right show the CNLs of wild-type genes and the frameshift mutants of the target genes. The average results for two independent experiments are shown with the standard deviations, excluding *YNL024C-A/KSH1*, for which only one experiment each was performed for the wild-type and frameshift mutant. The case of overlapping genes (*YGL167C/PMR1* and *YGL168W/HUR1*) (A), and the case of an RNA gene (*NME1*) (B) are shown.

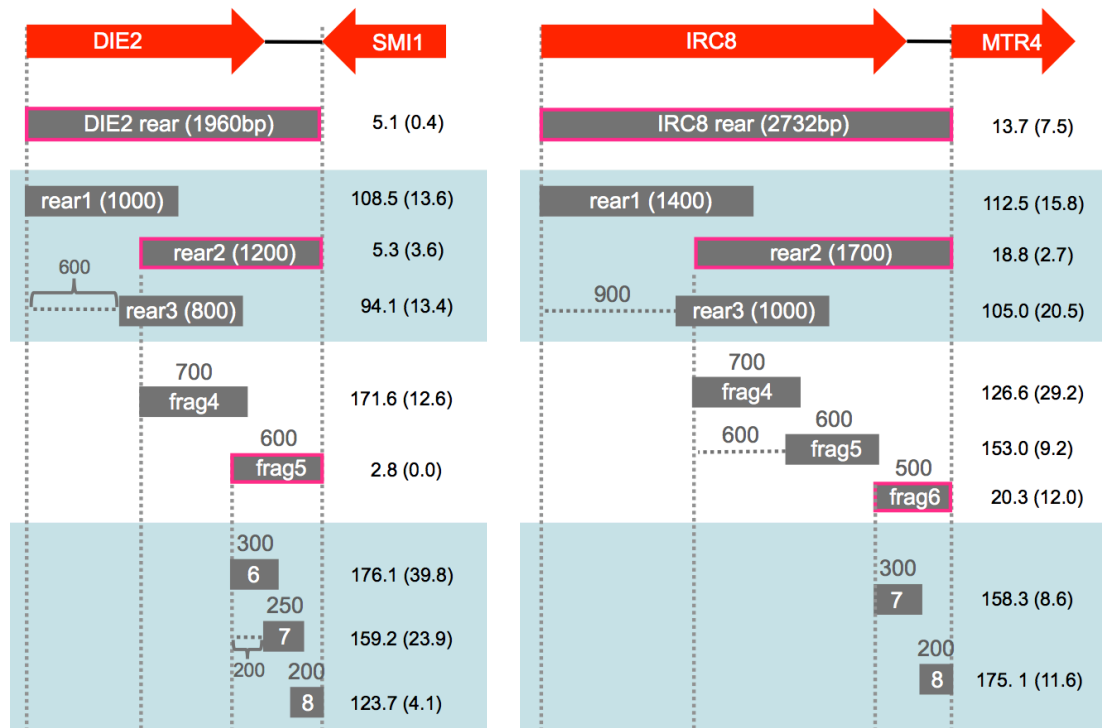


Figure S8. Unknown DNA elements are the determinants of the low CNLs within the locus of *DIE2* and *IRC8*.

The fragments that we analysed are shown in grey boxes with their names, and the size of each fragment is shown above the box. The number on the right side of the box indicates the copy number limit of the individual fragment. Fragments causing the low limit are enclosed with red boxes. Each fragment was amplified by PCR and cloned into pTOWug2-836. The average copy number and the standard deviation (within parentheses) of more than two independent experiments are shown.

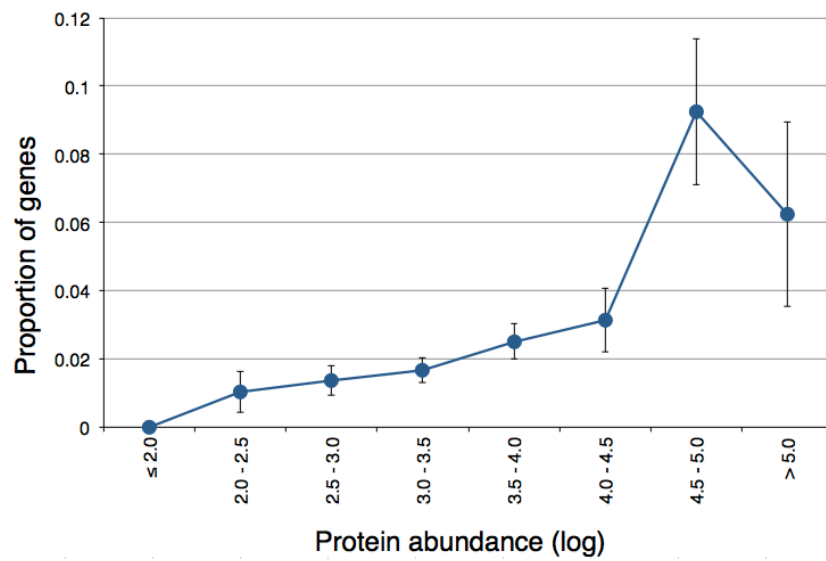


Figure S9. Yeast DSGs tend to be highly expressed.

The distribution of 115 DGSs ordered by their native protein levels. Each bin contains genes ordered by their native protein level (Ghaemmaghami *et al.*, 2003). The protein abundance unit is molecules per cell. The error bars indicate the standard error of the mean (SEM).

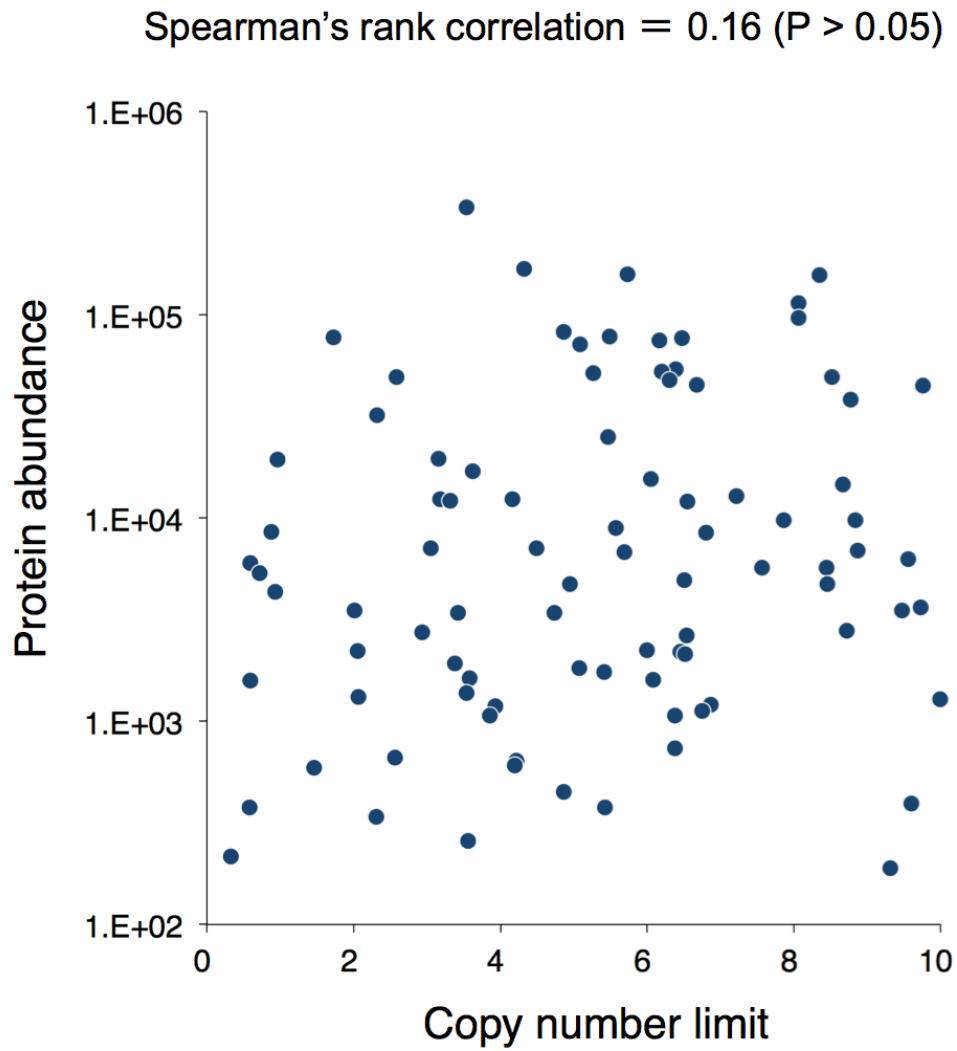


Figure S10. Correlation between the copy number limit and protein abundance. Genes with upper copy number limits ≤ 10 . The protein abundance data were obtained from Ghaemmaghami *et al.* (2003). The protein abundance unit is molecule per cell.

Figure S11. Results of 2D-gTOW experiments (plate assay).

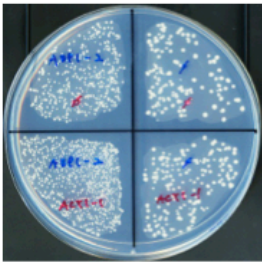
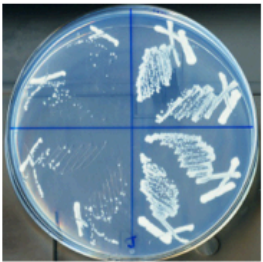
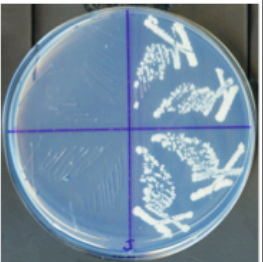
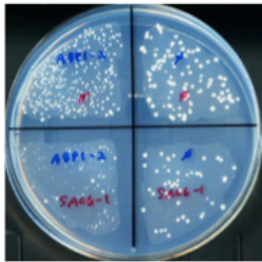
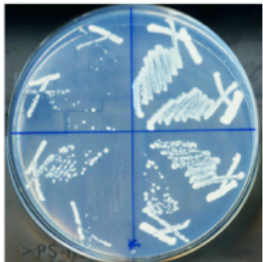
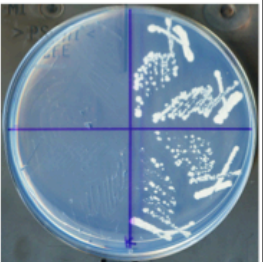
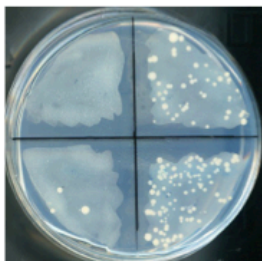
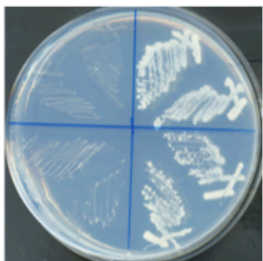
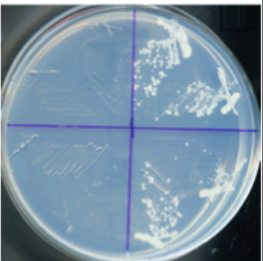
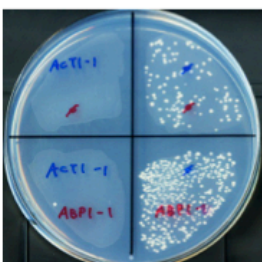
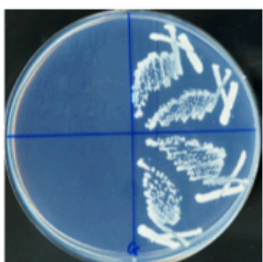
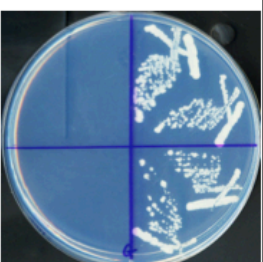
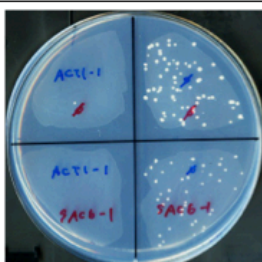
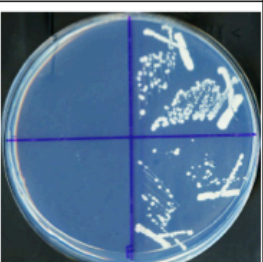
DSG (pTOW)	Partnere candidate (pRS423)	Co-transform	Streaked (-His -Ura)	Streaked (-His -Leu -Ura)
ABP1	ACT1			
ABP1	SAC6			
ACT1	COF1			
ACT1	ABP1			
ACT1	SAC6			

Figure S11 continued

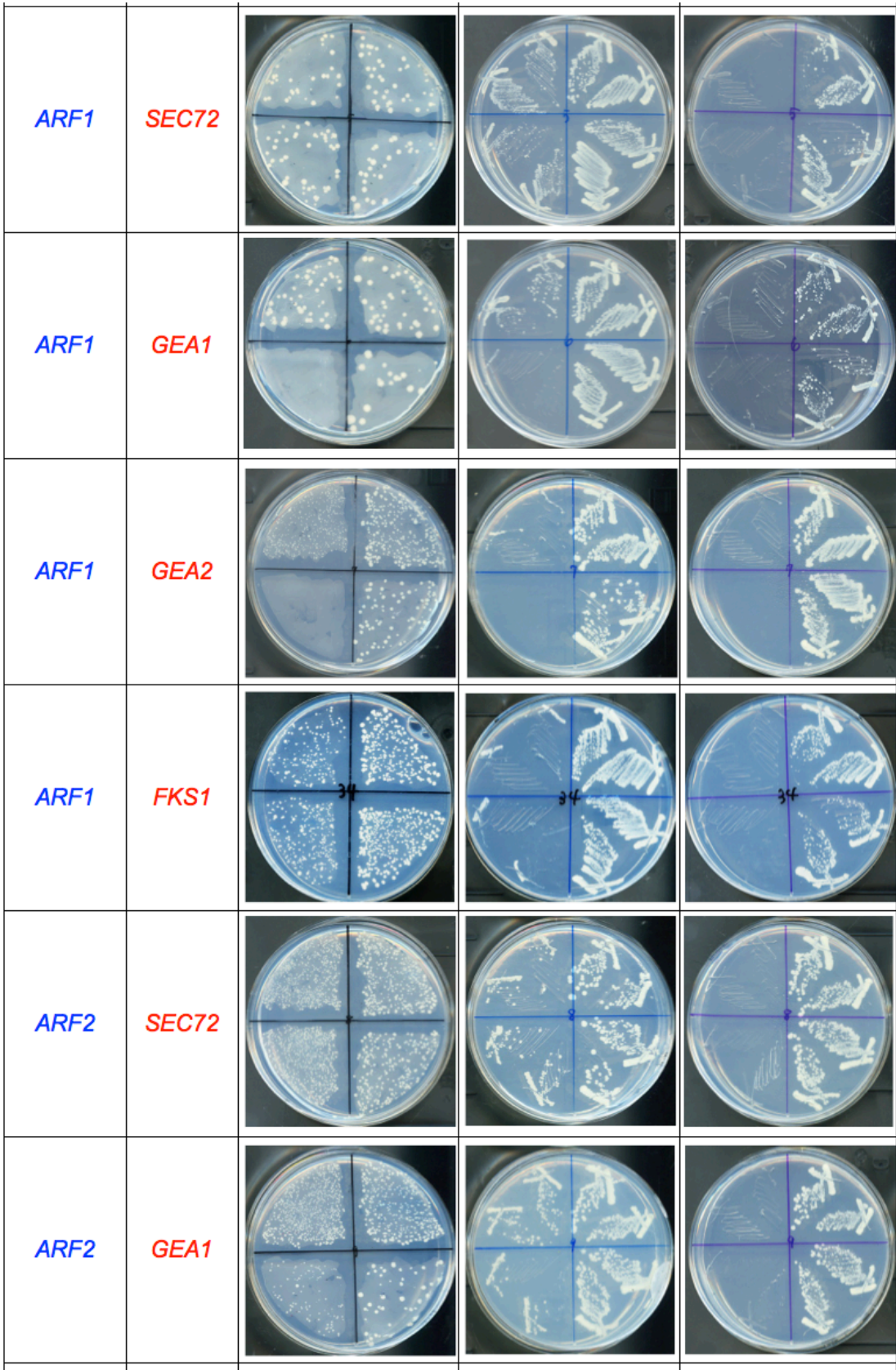


Figure S11 continued

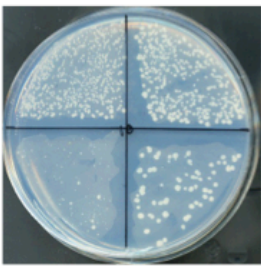
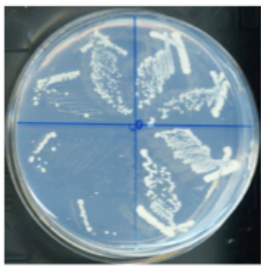
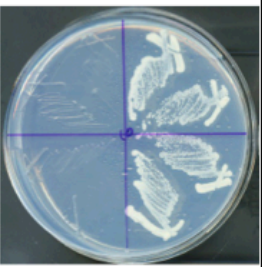
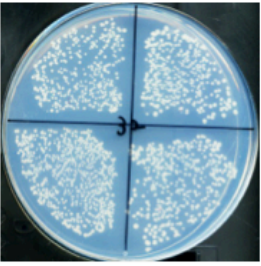
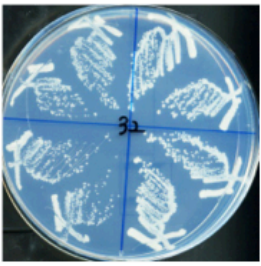
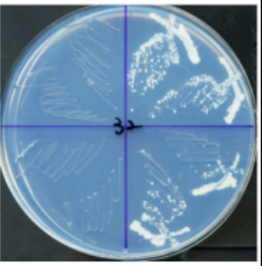
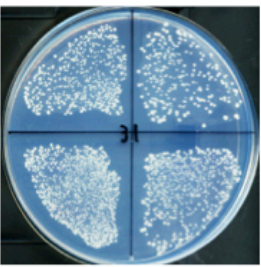
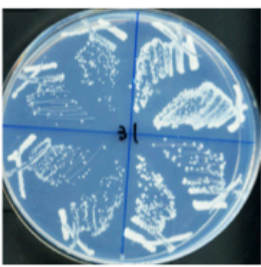
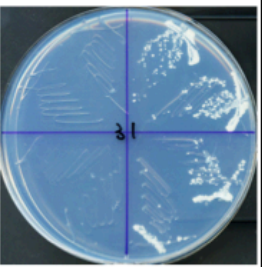
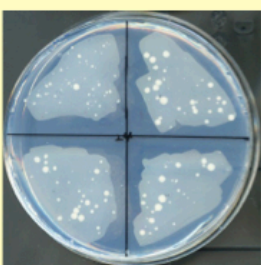
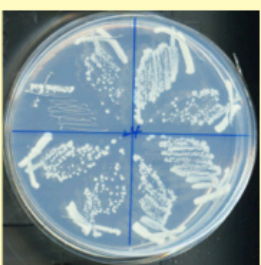
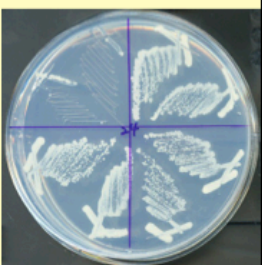
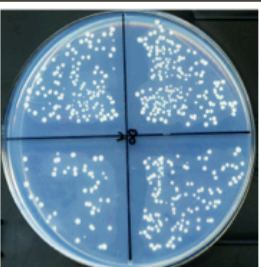
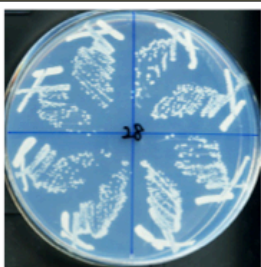
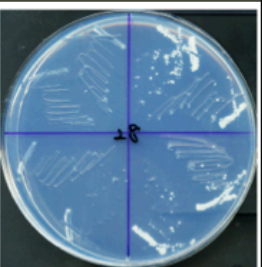
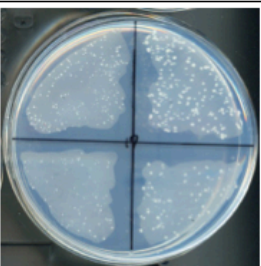
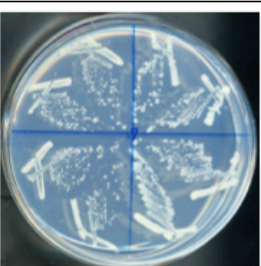
<i>ARF2</i>	<i>GEA2</i>			
<i>ARF2</i>	<i>FKS1</i>			
<i>ARF2</i>	<i>GLO3</i>			
<i>BFA1</i>	<i>TEM1</i>			
<i>BGL2</i>	<i>GSC2</i>			
<i>BGL2</i>	<i>GAS1</i>			

Figure S11 continued

<i>COF1</i>	<i>ACT1</i>			
<i>GAS1</i>	<i>BGL2</i>			
<i>GLN3</i>	<i>URE2</i>			
<i>GSC2</i>	<i>BGL2</i>			
<i>GSP1</i>	<i>RNA1</i>			
<i>MYO1</i>	<i>MLC1</i>			

Figure S11 continued

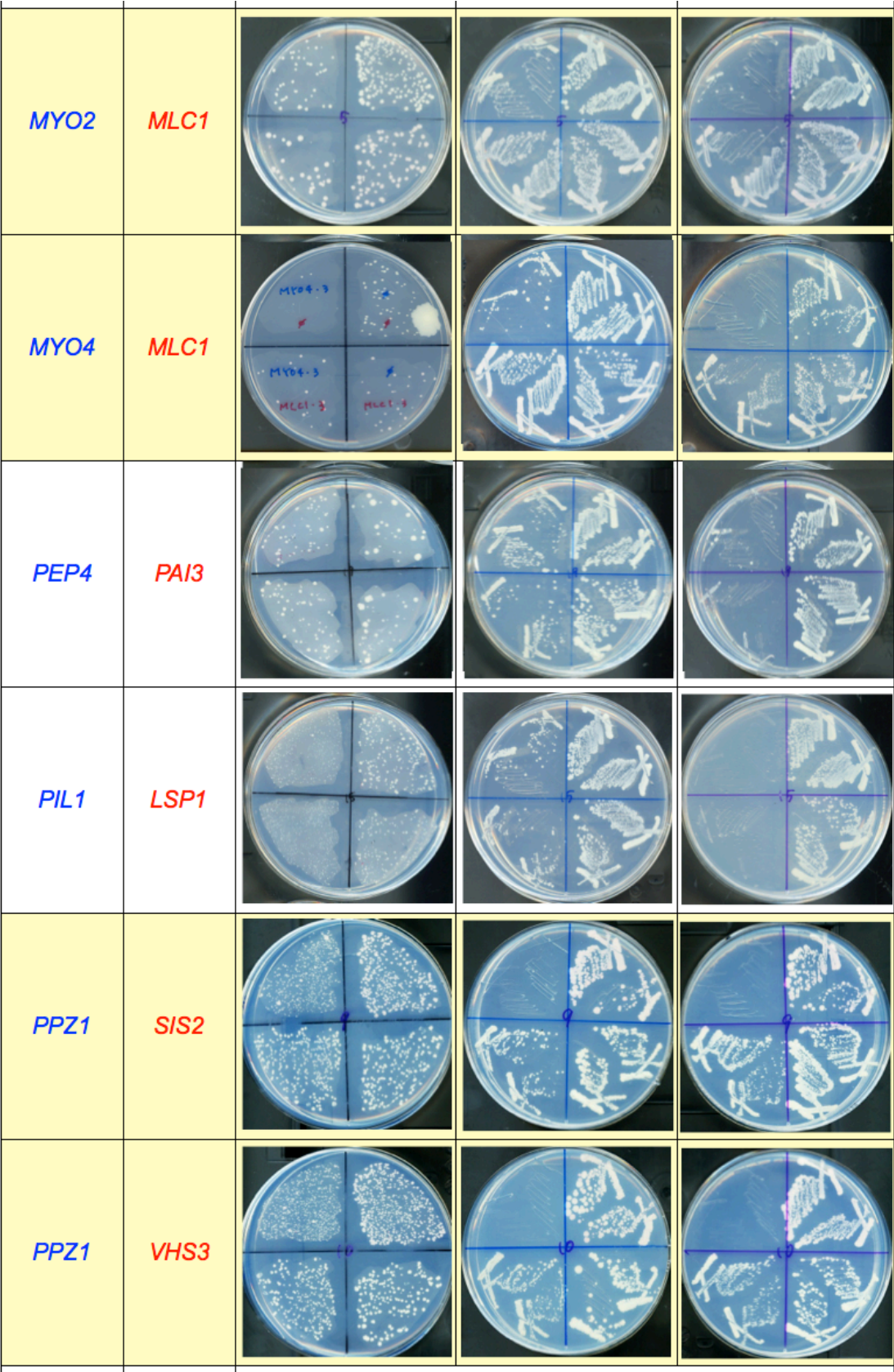


Figure S11 continued

PPZ1	SIS1	Data not shown		
PPZ2	SIS2			
PPZ2	VHS3			
RTN1	YOP1			
SAC6	ACT1			
SAC6	ABP1			

Figure S11 continued

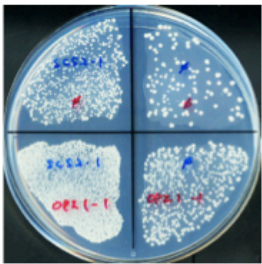
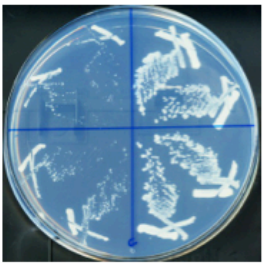
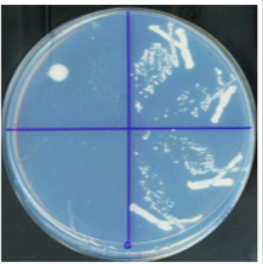
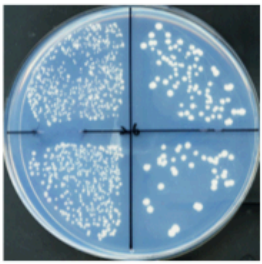
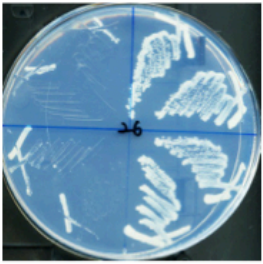
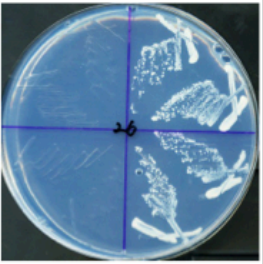
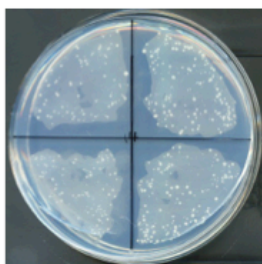
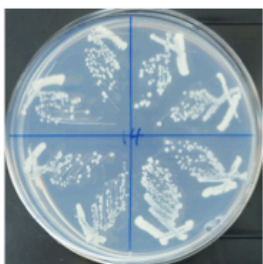
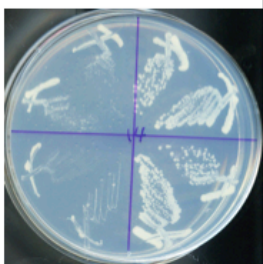
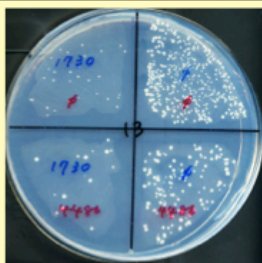
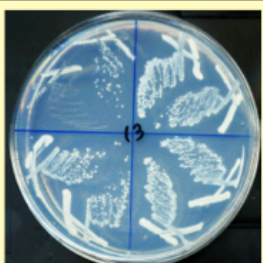
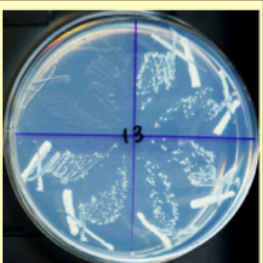
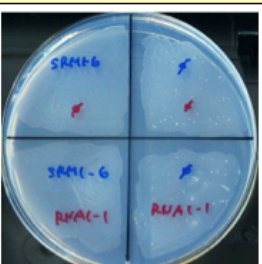
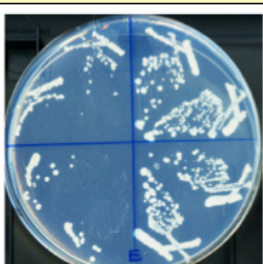
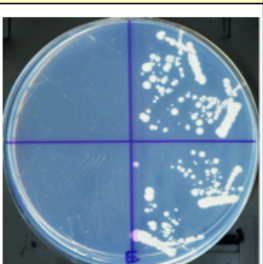
<i>SCS2</i>	<i>OPI1</i>			
<i>SEC23</i>	<i>SAR1</i>			
<i>SEC31</i>	<i>SEC13</i>			
<i>SEC4</i>	<i>SEC2</i>			
<i>SRM1</i>	<i>RNA1</i>			
<i>TEF1</i>	<i>EFB1</i>	Data not shown		

Figure S11 continued

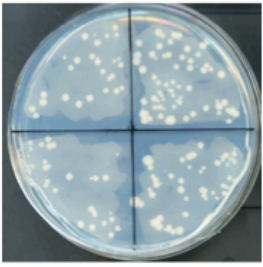
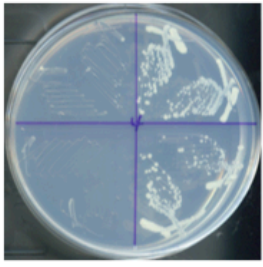
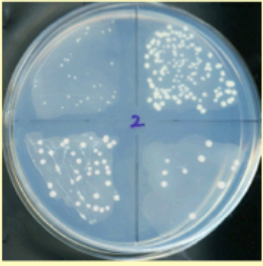
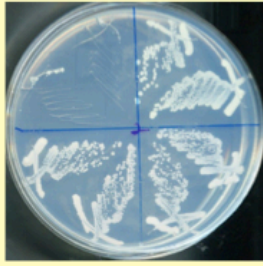
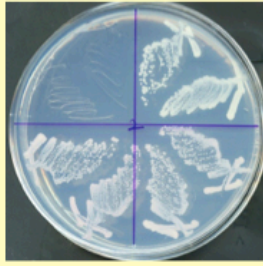
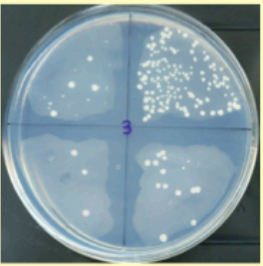
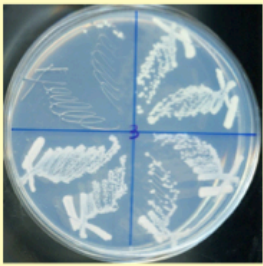
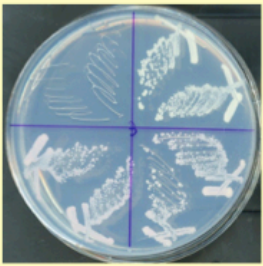
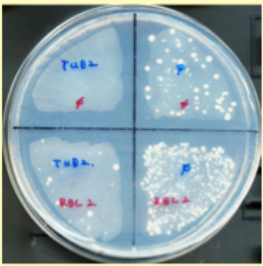
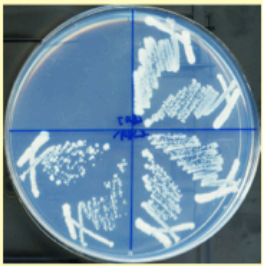
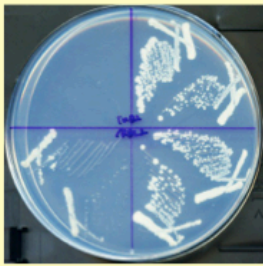
<i>TEF2</i>	<i>EFB1</i>			
<i>TPK1</i>	<i>BCY1</i>	Data not shown		
<i>TPK2</i>	<i>BCY1</i>			
<i>TPK3</i>	<i>BCY1</i>			
<i>TUB2</i>	<i>RBL2</i>			
<i>TUB2</i>	<i>TUB1</i>	See Figure S18		

Figure S11 continued

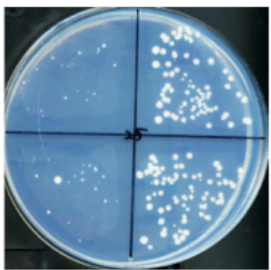
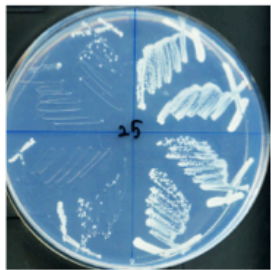
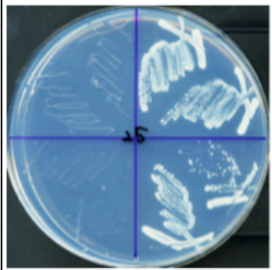
<i>TUB2</i>	<i>TUB3</i>	See Figure S18		
<i>WWM1</i>	<i>MCA1</i>			

Figure S11. Results of 2D-gTOW experiments (plate assay).
In each experiment, yeast strains transformed by the indicated pTOW-DSG and pRS423ks-partner (candidate) were spread onto -His-Ura plates (left plates) and then streaked onto -His-Ura (center plates) and -His-Leu-Ura plates (right plates).

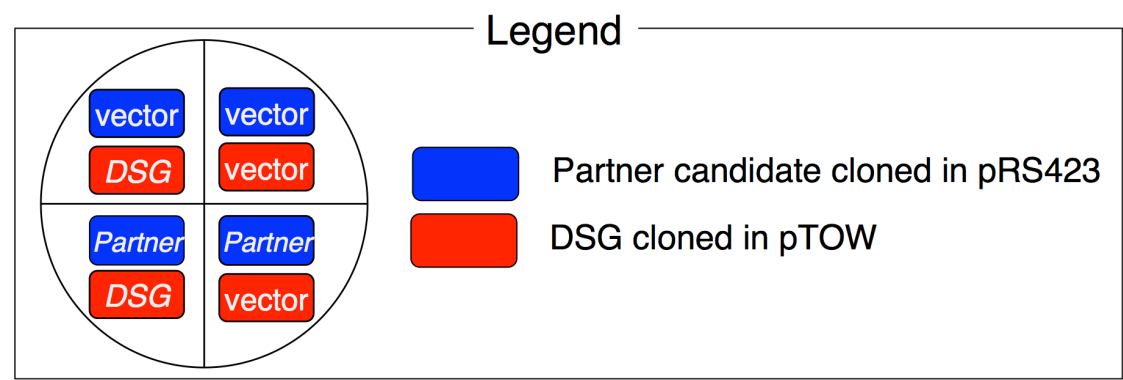


Figure S12. Results of 2D-gTOW experiments (copy number determination)

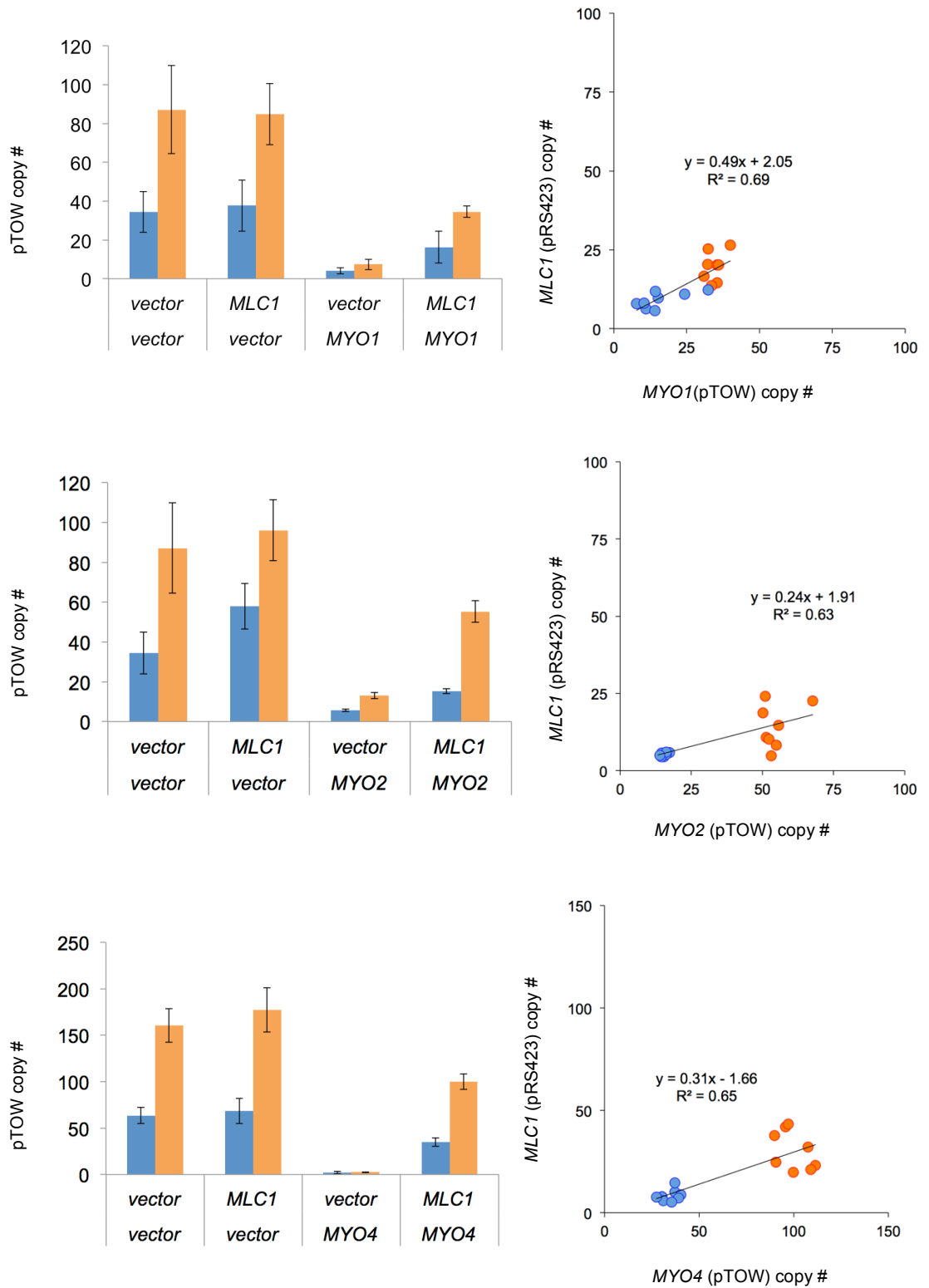


Figure S12 continued

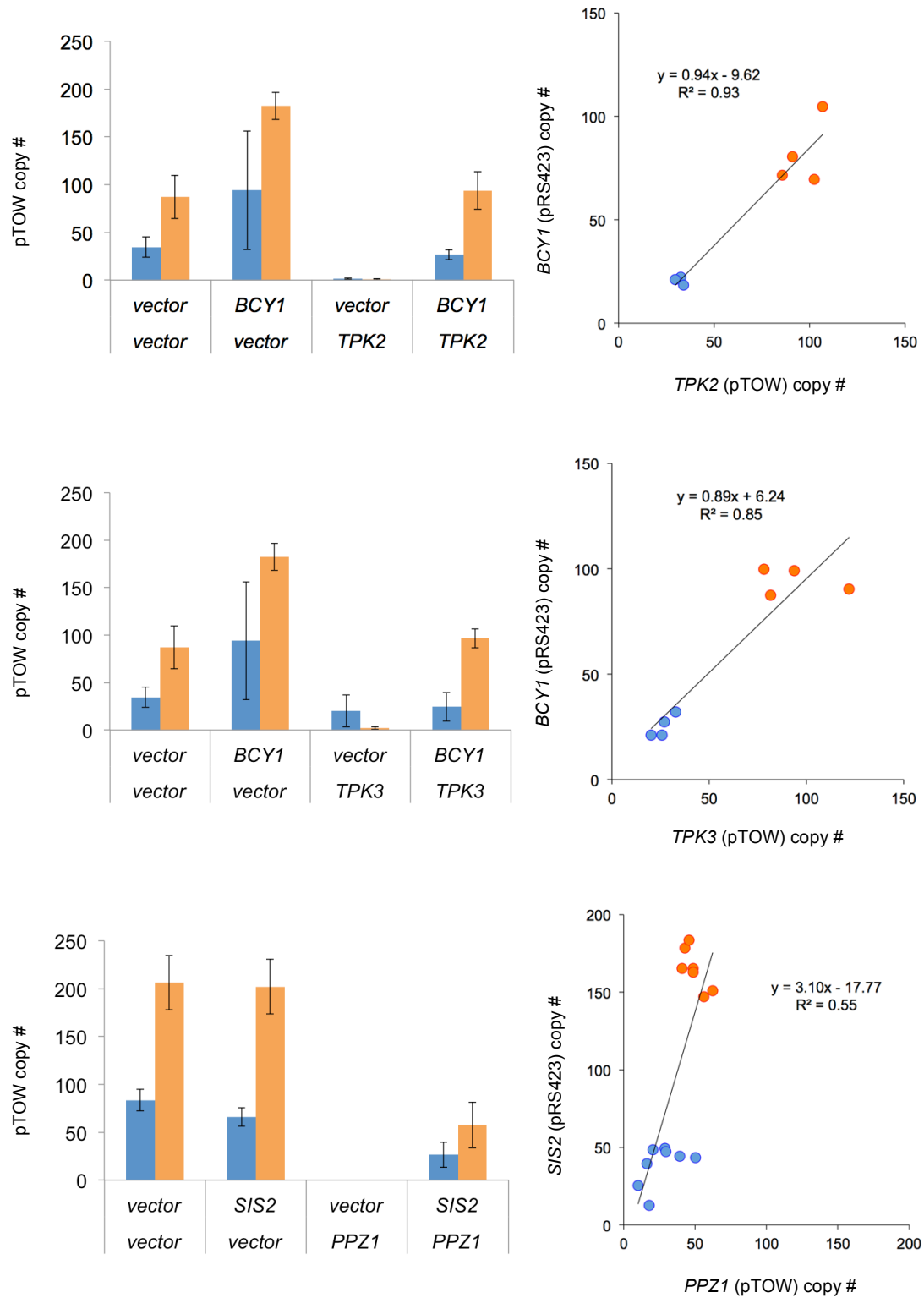


Figure S12 continued

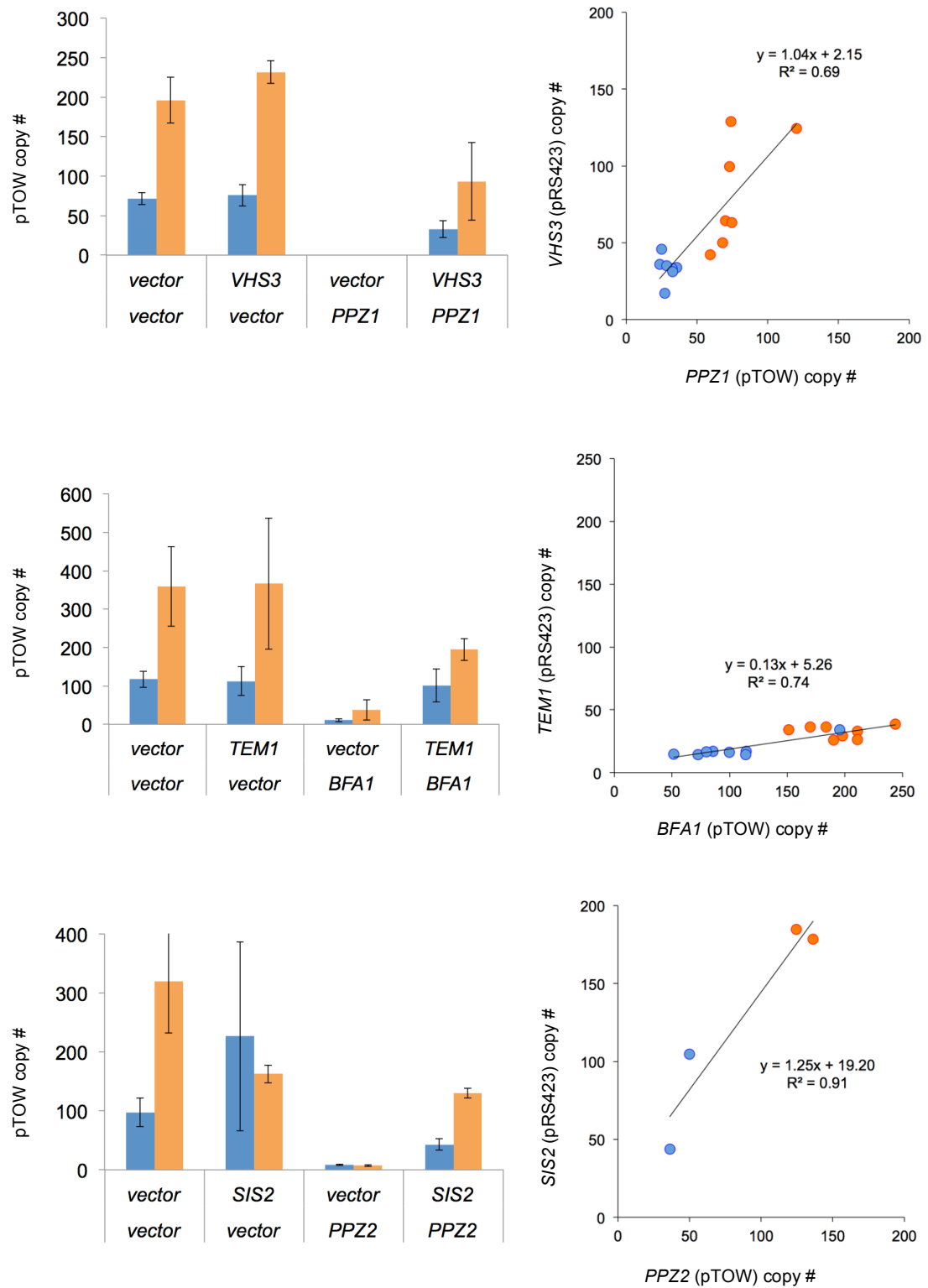


Figure S12 continued

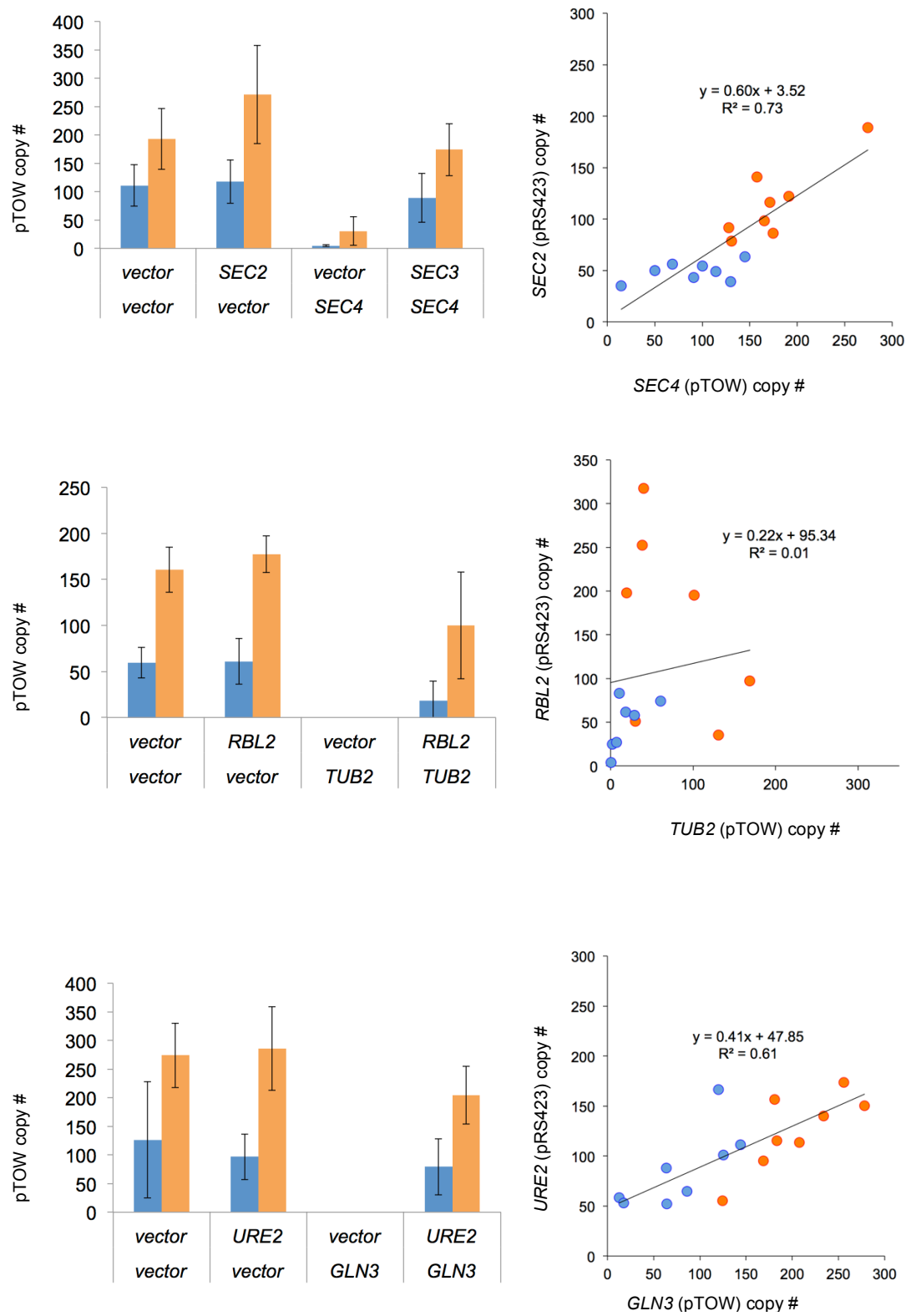


Figure S12 continued

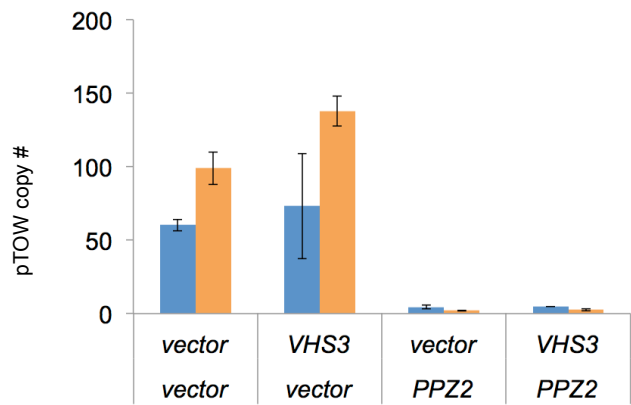


Figure S12. Results of the 2D-gTOW experiments for DSGs and their candidate partners.

The legend for the graph is shown below. The copy numbers of pTOW in the low-copy (–His–Ura) and high-copy (–His–Leu–Ura) conditions are shown in the left panel, and the correlation between the copy numbers of pTOW (DSG) and pRS423ks (candidate partner) are shown in the right panel. The result of the *VHS3* vs. *PPZ2* experiment is shown as a negative example.

pRS423-	Vector	Partner	Vector	Partner
pTOW-	Vector	Vector	DSG	DSG

■ ● –His–Ura ■ ● –His–Leu–Ura

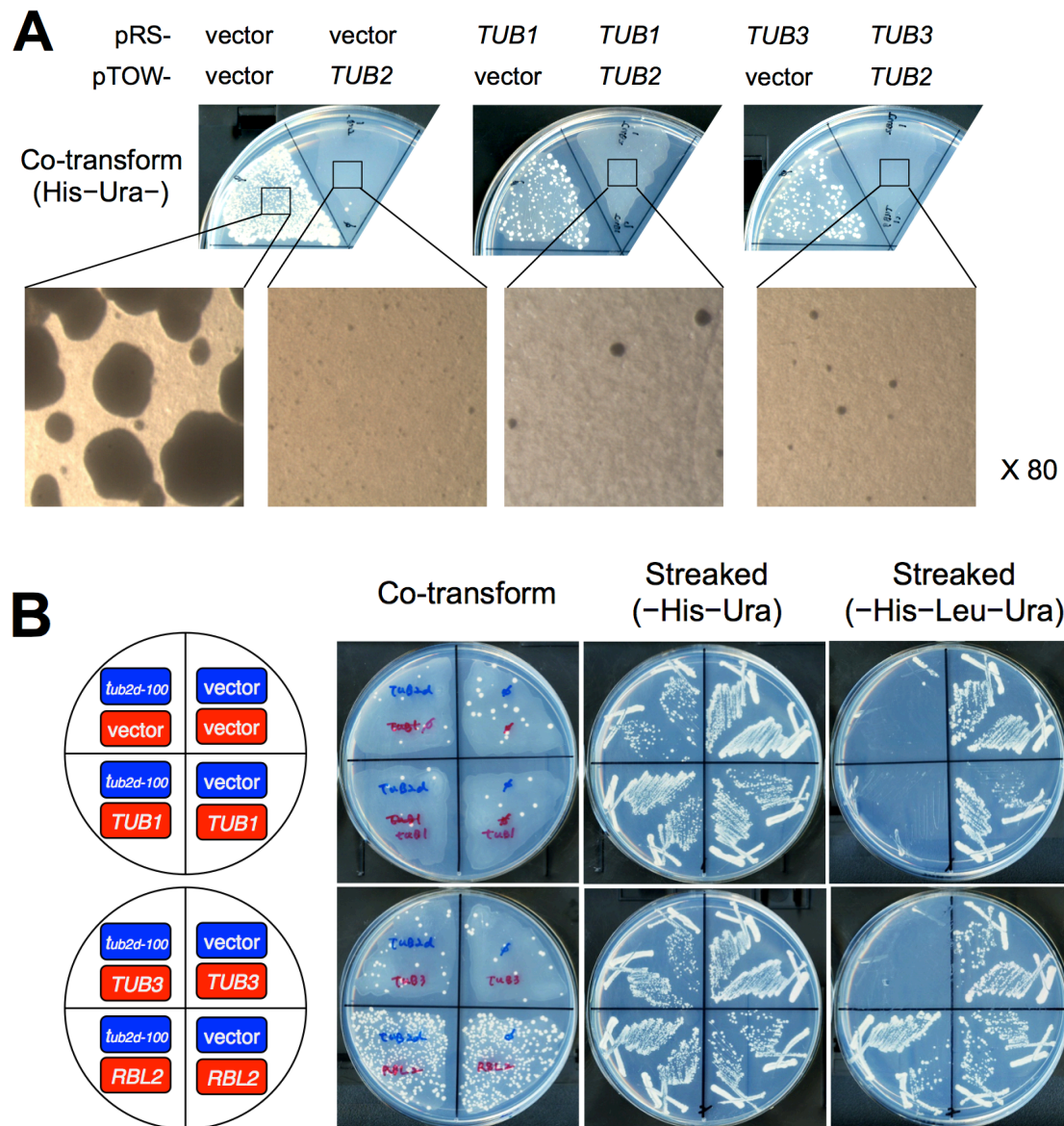


Figure S13. *TUB1* and *TUB3* are weak dosage suppressors (partners) of *TUB2*. *TUB1* and *TUB3* encode α -tubulin; they were suggested to be the partners of *TUB2* (encoding β -tubulin) (Weinstein & Solomon, 1990). However, in our 2D-gTOW experiments, *TUB1* and *TUB3* displayed little suppressive activity (**A** and **B**). By contrast, *RBL2* (encoding a chaperone for a Tub2 monomer) exhibited sufficient suppressive activity (**B**). In **B**, a weakly expressing allele of *TUB2* (*tub2d-100*, see above) was used for 2D-gTOW experiments because *TUB2* is too toxic to obtain any transformant by itself (**A**).

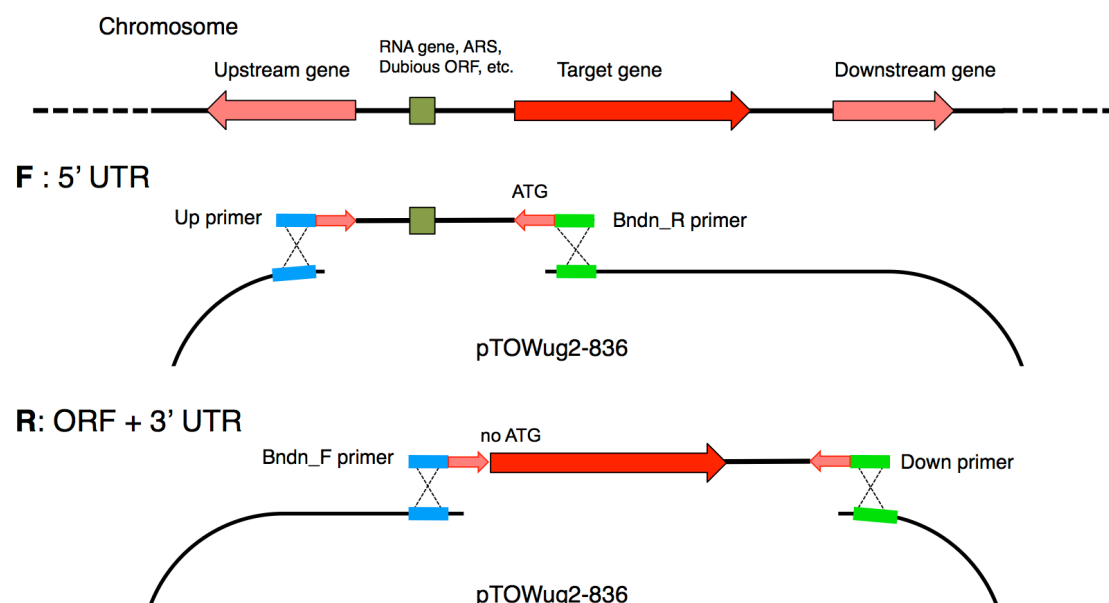
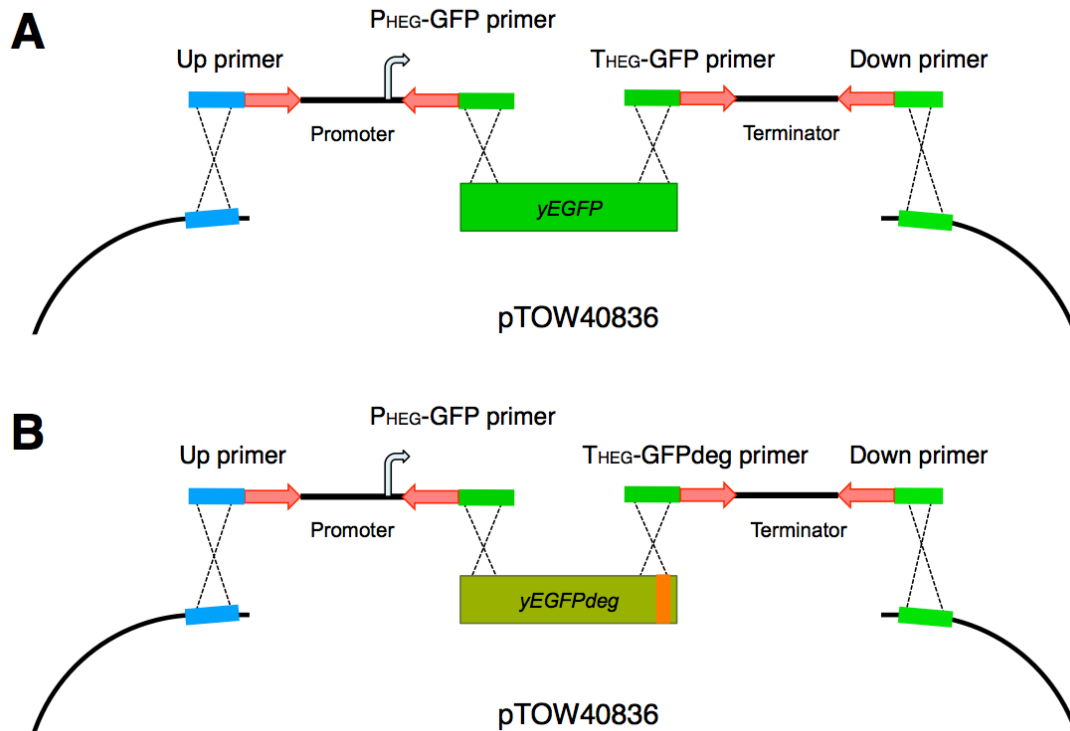


Figure S16. Construction of the segmented genes.

For each target gene, Bdn_R primer and Bdn_F primer were synthesized in order to construct “F” construct that contains 5’ region of the target gene, and “R” construct that contains the ORF (without start codon) and 3’ UTR. PCR-amplified fragments were joined and cloned into pTOWug2-836 by the gap-repair method. The information about the start codon of each gene is obtained from the annotation of *Saccharomyces* Genome Database (released on 28 July 2007).

Figure S17



C: *yEGFP*

```

ATGTCTAAAGGTGAAGAATTATTCAC TGGTGTGCCAATTTGGTTGAATTAGATGGTGATGTTAATGGTCAC
M S K G E E L F T G V V P I L V E L D G D V N G H

AAATTTCTGTCTCCGGTGAAGGTGAAGGTGATGCTACTTACGGTAAATTGACCTTAAAATTTATTTGTACTACT
K F S V S G E G E G D A T Y G K L T L K F I C T T

GGTAAATTGCCAGTTCATGGCCAACCTTAGTCACTACTTTAACTTATGGTGTTCATGTTTTCTAGATACCCA
G K L P V P W P T L V T T L T Y G V Q C F S R Y P

GATCATATGAAACAACATGACTTTTTCAAGTCTGCCATGCCAGAAGTTATGTTCAAGAAAGAACTATTTTTTTC
D H M K Q H D F F K S A M P E G Y V Q E R T I F F

AAAGATGACGGTAACTACAAGACCAGAGCTGAAGTCAAGTTTGAAGGTGATACCTTAGTTAATAGAATCGAATTA
K D D G N Y K T R A E V K F E G D T L V N R I E L

AAAGGTATTGATTTTAAAGAAGATGGTAACATTTTAGGTCACAAATTGGAATACAACATACTCTCACAATGTT
K G I D F K E D G N I L G H K L E Y N Y N S H N V

TACATCATGGCTGACAAACAAAAGATGGTATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGTTCT
Y I M A D K Q K N G I K V N F K I R H N I E D G S

GTTCAATTAGCTGACCATTATCAACAAAATACTCCAATTGGTGATGGTCCAGTCTTGTTACCAGACAACCATTAC
V Q L A D H Y Q Q N T P I G D G P V L L P D N H Y

TTATCCACTCAATCTGCCTTATCCAAAGATCCAAACGAAAAGAGAGACCACATGGTCTTGTTAGAATTTGTTACT
L S T Q S A L S K D P N E K R D H M V L L E F V T

GCTGCTGGTATTACCCATGGTATGGATGAATTGTACAAATAA
A A G I T H G M D E L Y K *

```


D: *yEGFPdeg*

```

ATGTCTAAAGGTGAAGAATTATTCACCTGGTGTGTCCCAATTTTGGTTGAATTAGATGGTGATGTTAATGGTCAC
M S K G E E L F T G V V P I L V E L D G D V N G H

AAATTTTCTGTCTCCGGTGAAGGTGAAGGTGATGCTACTTACGGTAAATTGACCTTAAAATTTATTTGTACTACT
K F S V S G E G E G D A T Y G K L T L K F I C T T

GGTAAATTGCCAGTTCATGGCCAACCTTAGTCACTACTTTAACTTATGGTGTTCATGTTTTTCTAGATACCCA
G K L P V P W P T L V T T L T Y G V Q C F S R Y P

GATCATATGAAACAACATGACTTTTTCAAGTCTGCCATGCCAGAAGGTTATGTTCAAGAAAGAACTATTTTTTTC
D H M K Q H D F F K S A M P E G Y V Q E R T I F F

AAAGATGACGGTAACTACAAGACCAGAGCTGAAGTCAAGTTTGAAGGTGATACCTTAGTTAATAGAATCGAATTA
K D D G N Y K T R A E V K F E G D T L V N R I E L

AAAGGTATTGATTTTAAAGAAGATGGTAACATTTTAGGTCACAAATTGGAATACAACATACTCTCACAAATGTT
K G I D F K E D G N I L G H K L E Y N Y N S H N V

TACATCATGGCTGACAAACAAAAGAATGGTATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGTTCT
Y I M A D K Q K N G I K V N F K I R H N I E D G S

GTTCAATTAGCTGACCATTATCAACAAAATACTCCAATTGGTGATGGTCCAGTCTTGTTACCAGACAACCATTAC
V Q L A D H Y Q Q N T P I G D G P V L L P D N H Y

TTATCCACTCAATCTGCCTTATCCAAAGATCCAAACGAAAAGAGAGACCACATGGTCTTGTTAGAATTTGTTACT
L S T Q S A L S K D P N E K R D H M V L L E F V T

GCTGCTGGTATTACCCATGGTATGGATGAATTGTACAAACTGCCCATGTCTTGTGCCCAGGAGtctatcacaagt
A A G I T H G M D E L Y K L P M S C A Q E S I T S

ttgtacaaaaaagctggttctTAA
L Y K K A G S *

```

Figure S17. Construction of GFP and GFPdeg replaced plasmids.

(A) Constructions of GFP replaced plasmid. (B) Construction of GFPdeg replaced plasmid. The promoter region and the terminator region of each target highly expressed gene and *yEGFP* (or *yEGFPdeg*) were amplified by PCR using indicated primers, and joined and cloned into pTOW40836. (C) The nucleotide and amino acid sequences of *yEGFP* used in A. (D) The nucleotide and amino acid sequences of *yEGFPdeg* used in B. The degron sequence from mouse ornithine decarboxylase gene (cODC1) (Jungbluth *et al.*, 2010) is colored with yellow.

Table S2. Statistical data of the 230 empty vector experiments.

	Max growth rate	Copy# (-Ura)	Copy# (-Leu-Ura)
Average	2.2	22.9	205.2
SD ^{*1}	0.6	12.5	86.6
CV ^{*2}	29.2	54.9	42.2

*1 Standard deviation

*2 Coefficient of variation

Table S3. Correlations between max growth rates and copy numbers obtained in the gTOW6000 analysis.

	Max growth rate vs. U [*]	Max growth rate vs. L [*]	U vs. LU [*]
ALL	0.18	0.35	0.16
Low-limit top 786	0.32	0.54	0.56

* U and LU indicate the copy number in the -Ura and -Leu-Ura conditions, respectively.

Table S7. Partner-seeking experiment results

DSG	Upper limit	Candidate partner	Reference*	Type of interaction	Verified? (Figures S11 and S12)
<i>ABP1</i>	4.2	<i>ACT1</i>	BioGRID	Physical interaction	No
<i>ABP1</i>	4.2	<i>SAC6</i>	BioGRID	Physical interaction	No
<i>ACT1</i>	1.2	<i>COF1</i>	BioGRID	Physical interaction	No
<i>ACT1</i>	1.2	<i>ABP1</i>	BioGRID	Physical interaction	No
<i>ACT1</i>	1.2	<i>SAC6</i>	Sandrock <i>et al.</i> , 1999	Synthetic rescue	No
<i>ARF1</i>	1.0	<i>SEC72</i>	-	-	No
<i>ARF1</i>	1.0	<i>GEA1</i>	-	-	No
<i>ARF1</i>	1.0	<i>GEA2</i>	BioGRID	Physical interaction	No
<i>ARF1</i>	1.0	<i>FKS1</i>	-	-	No
<i>ARF2</i>	5.5	<i>SEC72</i>	-	-	No
<i>ARF2</i>	5.5	<i>GEA1</i>	BioGRID	Physical interaction	No
<i>ARF2</i>	5.5	<i>GEA2</i>	BioGRID	Physical interaction	No
<i>ARF2</i>	5.5	<i>FKS1</i>	-	-	No
<i>ARF2</i>	5.5	<i>GLO3</i>	-	-	No
<i>BFA1</i>	3.5	<i>TEM1</i>	Park <i>et al.</i> , 2004	Synthetic rescue	Yes
<i>BGL2</i>	9.8	<i>GSC2</i>	-	-	No
<i>BGL2</i>	9.8	<i>GAS1</i>	BioGRID	Negative genetic	No

				interaction	
<i>COF1</i>	3.2	<i>ACT1</i>	BioGRID	Physical interaction	No
<i>GAS1</i>	4.2	<i>BGL2</i>	-	-	No
<i>GLN3</i>	1.5	<i>URE2</i>	Palmer <i>et al.</i> , 2009	Synthetic rescue	Yes
<i>GSC2</i>	5.1	<i>BGL2</i>	-	-	No
<i>GSP1</i>	3.5	<i>RNA1</i>	BioGRID	Physical interaction	No
<i>MYO1</i>	6.5	<i>MLC1</i>	-	-	Yes
<i>MYO2</i>	12.1	<i>MLC1</i>	Stevens & Davis, 1998	Dosage rescue	Yes
<i>MYO4</i>	6.5	<i>MLC1</i>	-	-	Yes
<i>PEP4</i>	0.8	<i>PAI3</i>	BioGRID	Physical interaction	No
<i>PIL1</i>	8.1	<i>LSP1</i>	Deng <i>et al.</i> , 2009	Phenotypic enhancement	No
<i>PPZ1</i>	0.3	<i>SIS2</i>	Clotet <i>et al.</i> , 1999	Dosage rescue	Yes
<i>PPZ1</i>	0.3	<i>VHS3</i>	de Nadal <i>et al.</i> , 1998	Synthetic rescue	Yes
<i>PPZ1</i>	0.3	<i>SIS1</i>	-	-	No
<i>PPZ2</i>	9.3	<i>SIS2</i>	BioGRID	Physical interaction	Yes
<i>PPZ2</i>	9.3	<i>VHS3</i>	BioGRID	Physical interaction	No
<i>RTN1</i>	15.1	<i>YOP1</i>	-	-	No
<i>SAC6</i>	2.0	<i>ACT1</i>	Sandrock <i>et al.</i> , 1999	Synthetic rescue	No
<i>SAC6</i>	2.0	<i>ABP1</i>	BioGRID	Physical interaction	No

<i>SCS2</i>	9.5	<i>OPI1</i>	Wilson <i>et al.</i> , 2011	Physical interaction	No
<i>SEC23</i>	4.4	<i>SAR1</i>	Oka & Nakano, 1994	Dosage rescue	No
<i>SEC31</i>	5.1	<i>SEC13</i>	BioGRID	Physical interaction	No Additive
<i>SEC4</i>	5.2	<i>SEC2</i>	Ortiz <i>et al.</i> , 2002	Dosage rescue	Yes
<i>SRM1</i>	6.5	<i>RNA1</i>	-	-	No
<i>TEF1</i>	0.6	<i>EFB1</i>	BioGRID	Physical interaction	No
<i>TEF2</i>	4.9	<i>EFB1</i>	Kinzy & Woolford, 1995	Dosage rescue	No
<i>TPK1</i>	0.9	<i>BCY1</i>	BioGRID	Physical interaction	Yes
<i>TPK2</i>	2.1	<i>BCY1</i>	Nehlin <i>et al.</i> , 1992	Dosage rescue	Yes
<i>TPK3</i>	0.6	<i>BCY1</i>	Mazón <i>et al.</i> , 1993	Phenotypic enhancement	Yes
<i>TUB2</i> [#]	2.7	<i>RBL2</i>	Abruzzi <i>et al.</i> , 2002	Phenotypic suppression	Yes (see also Figure S13)
<i>TUB2</i> [#]	2.7	<i>TUB1</i>	Weinstein & Solomon, 1990	Dosage rescue	Weak (Figure S13)
<i>TUB2</i> [#]	2.7	<i>TUB3</i>	-	-	Weak (Figure S13)
<i>WWM1</i>	0.6	<i>MCA1</i>	Szallies <i>et al.</i> , 2002	Synthetic rescue	No

* BioGRID: <http://thebiogrid.org/>

[#] *tub2-d100* was used in the experiment.

Supplementary References

- Abruzzi, K. C., Smith, A., Chen, W. & Solomon, F. (2002). Protection from free beta-tubulin by the beta-tubulin binding protein Rbl2p. *Mol Cell Biol* **22**, 138-47.
- Clotet, J., Gari, E., Aldea, M. & Ariño, J. (1999). The yeast ser/thr phosphatases sit4 and ppz1 play opposite roles in regulation of the cell cycle. *Mol Cell Biol* **19**, 2408-15.
- de Nadal, E., Clotet, J., Posas, F., Serrano, R., Gomez, N. & Ariño, J. (1998). The yeast halotolerance determinant Hal3p is an inhibitory subunit of the Ppz1p Ser/Thr protein phosphatase. *Proc Natl Acad Sci U S A* **95**, 7357-62.
- Deng, C., Xiong, X. & Krutchinsky, A. N. (2009). Unifying fluorescence microscopy and mass spectrometry for studying protein complexes in cells. *Mol Cell Proteomics* **8**, 1413-23.
- Ghaemmaghami, S., Huh, W. K., Bower, K., Howson, R. W., Belle, A., Dephoure, N., *et al.* (2003). Global analysis of protein expression in yeast. *Nature* **425**, 737-41.
- Jungbluth, M., Renicke, C. & Taxis, C. (2010). Targeted protein depletion in *Saccharomyces cerevisiae* by activation of a bidirectional degron. *BMC Syst Biol* **4**, 176.
- Kinzy, T. G. Woolford, J. L. (1995). Increased expression of *Saccharomyces cerevisiae* translation elongation factor 1 alpha bypasses the lethality of a TEF5 null allele encoding elongation factor 1 beta. *Genetics* **141**, 481-9.
- Mazón, M. J., Behrens, M. M., Morgado, E. & Portillo, F. (1993). Low activity of the yeast cAMP-dependent protein kinase catalytic subunit Tpk3 is due to the poor expression of the TPK3 gene. *Eur J Biochem* **213**, 501-6.
- Nehlin, J. O., Carlberg, M. & Ronne, H. (1992). Yeast SKO1 gene encodes a bZIP protein that binds to the CRE motif and acts as a repressor of transcription. *Nucleic Acids Res* **20**, 5271-8.
- Oka, T. Nakano, A. (1994). Inhibition of GTP hydrolysis by Sar1p causes accumulation of vesicles that are a functional intermediate of the ER-to-Golgi transport in yeast. *J Cell Biol* **124**, 425-34.
- Ortiz, D., Medkova, M., Walch-Solimena, C. & Novick, P. (2002). Ypt32 recruits the Sec4p guanine nucleotide exchange factor, Sec2p, to secretory vesicles;

- evidence for a Rab cascade in yeast. *J Cell Biol* **157**, 1005-15.
- Palmer, L. K., Baptiste, B. A., Fester, J. C., Perkins, J. C. & Keil, R. L. (2009). RRD1, a component of the TORC1 signalling pathway, affects anaesthetic response in *Saccharomyces cerevisiae*. *Yeast* **26**, 655-61.
- Park, J. E., Park, C. J., Sakchaisri, K., Karpova, T., Asano, S., McNally, J., *et al.* (2004). Novel functional dissection of the localization-specific roles of budding yeast polo kinase Cdc5p. *Mol Cell Biol* **24**, 9873-86.
- Sandrock, T. M., Brower, S. M., Toenjes, K. A. & Adams, A. E. (1999). Suppressor analysis of fimbrin (Sac6p) overexpression in yeast. *Genetics* **151**, 1287-97.
- Stevens, R. C. Davis, T. N. (1998). Mlc1p is a light chain for the unconventional myosin Myo2p in *Saccharomyces cerevisiae*. *J Cell Biol* **142**, 711-22.
- Szallies, A., Kubata, B. K. & Duszenko, M. (2002). A metacaspase of *Trypanosoma brucei* causes loss of respiration competence and clonal death in the yeast *Saccharomyces cerevisiae*. *FEBS Lett* **517**, 144-50.
- Weinstein, B. Solomon, F. (1990). Phenotypic consequences of tubulin overproduction in *Saccharomyces cerevisiae*: differences between alpha-tubulin and beta-tubulin. *Mol Cell Biol* **10**, 5295-304.
- Wilson, J. D., Thompson, S. L. & Barlowe, C. (2011). Yet1p-Yet3p interacts with Scs2p-Opi1p to regulate ER localization of the Opi1p repressor. *Mol Biol Cell* **22**, 1430-9.