Supporting Information

Lippman and Broach 10.1073/pnas.0907027106

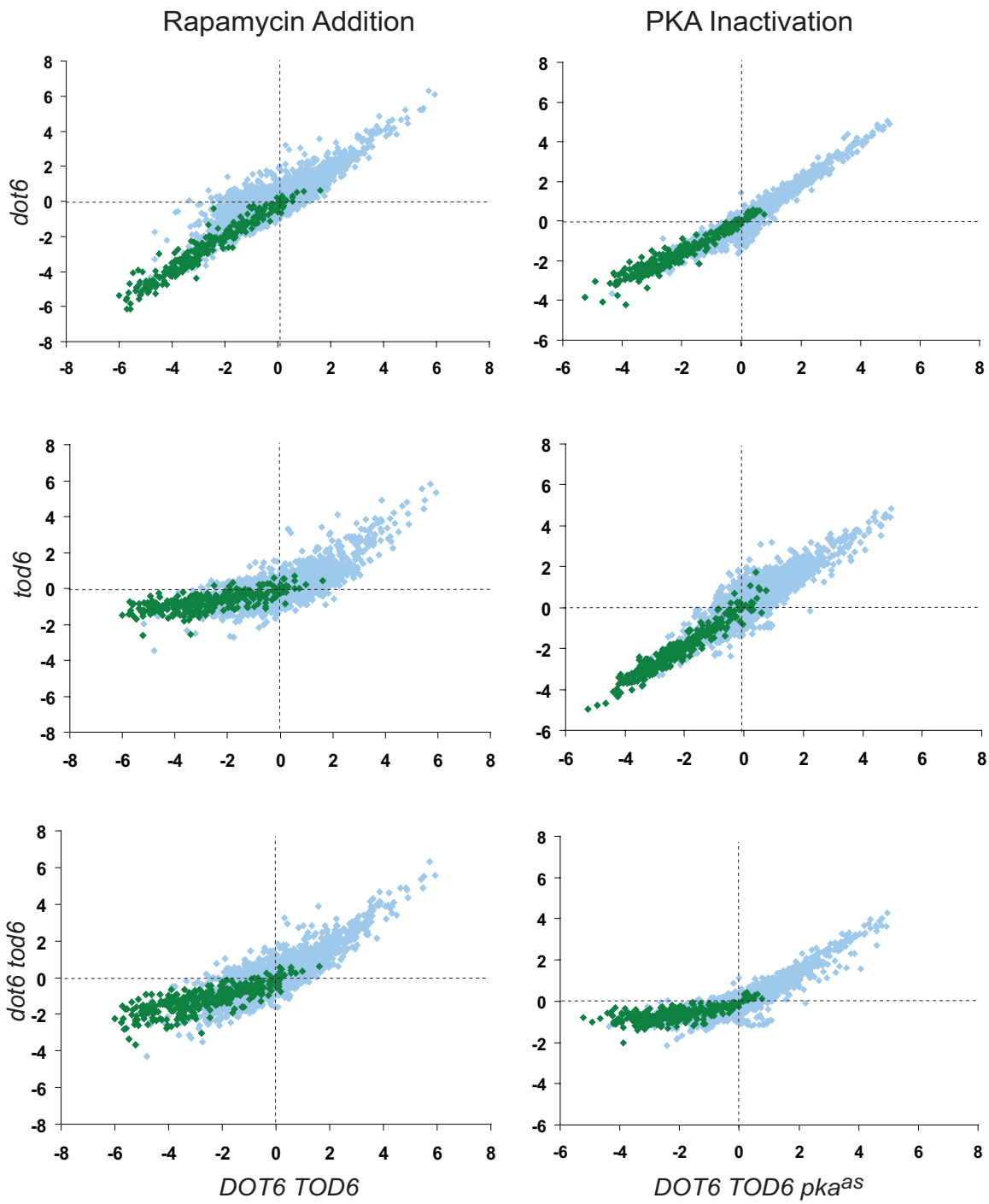

Fig. S1. Dot6 and Tod6 are required for repression of Ribi genes at early times after inactivation of TORC1 or PKA. Data are presented as in Fig. 2 for samples taken 20 min after treatment with rapamycin or 1NM-PP1.

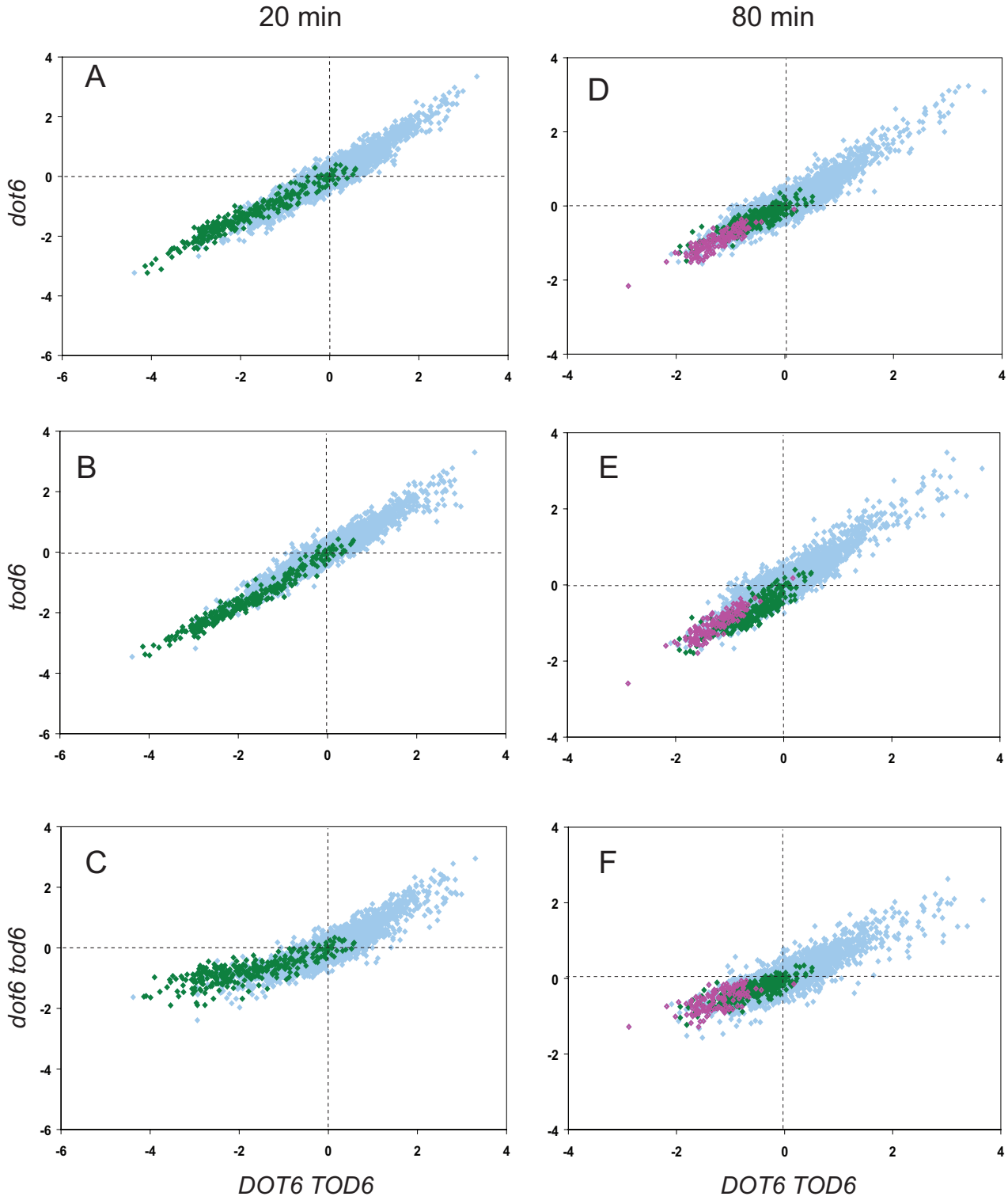

Fig. S2. Dot6 and Tod6 are required for repression of Ribi genes after inactivation of Sch9. Shown are scatter plots of microarray data for ≈5,600 yeast genes obtained from two different strains after inactivation of Sch9 by addition of 100 nM 1NM-PP1 to strains carrying the analog-sensitive allele *sch9*(T492G), presented as described in the legend to Fig. 2. Each point represents the $\log_2$ change in levels of the mRNA for a single gene for strain Y3507 (*sch9*^{as} *DOT6 TOD6*) in the horizontal dimension and the $\log_2$ change in mRNA levels for that gene in a *sch9*^{as} *dot6* (Y3711; *A* and *D*), *sch9*^{as} *tod6* (Y3712; *B* and *E*), or *sch9*^{as} *dot6 tod6* (Y3713; *C* and *F*) at 20 min (*A*–*C*) and 80 min (*D*–*F*) after addition. Points corresponding to genes annotated as Ribi genes are shown in green, and those corresponding to RP genes are shown in pink.

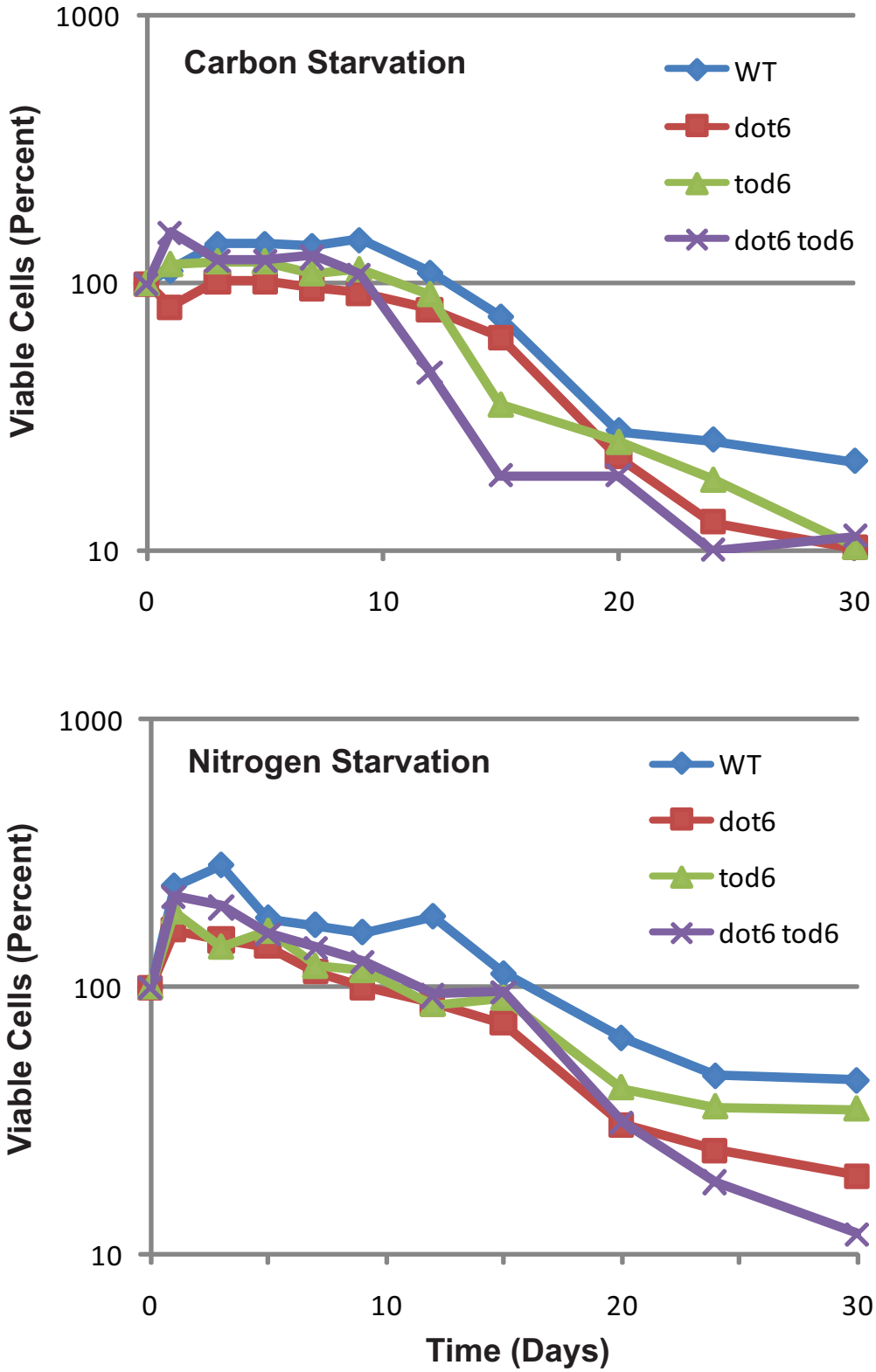

Fig. S3. Dot6 and Tod6 are required for sustained viability after nutrient starvation. WT and deletion prototroph strains were grown overnight in SC medium plus 2% glucose and 0.5% ammonium sulfate, diluted into the same medium but limiting for glucose (0.8%) or ammonium sulfate (0.03%) and grown overnight. Strains were then diluted 1:200 into media entirely lacking either glucose or nitrogen sources and incubated at 30 °C. Samples were taken at the times indicated, and viable cell count was determined by plating appropriate dilutions on YPD agar plates. Data are plotted as a percent of the initial viable cell count for each strain.

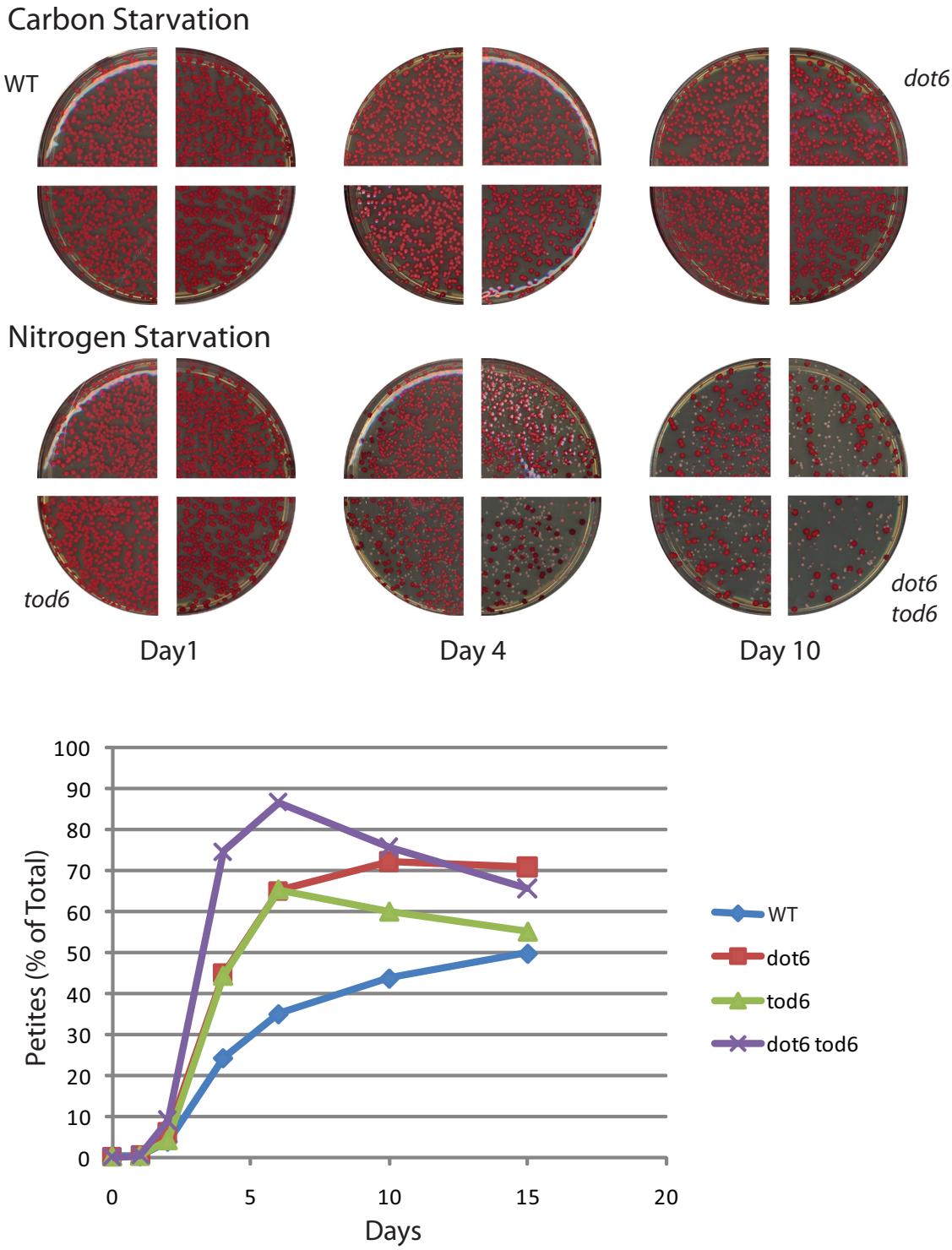

Fig. S4. Nitrogen starvation induces petites. (*Upper*) Quadrants of YEPD plates on which have been plated samples of isogenic WT (upper left quadrant), *dot6* (upper right quadrant), *tod6* (lower left quadrant), and *dot6 tod6* (lower right quadrant) prototrophs starved for either glucose or nitrogen for the indicated period. The same volume equivalent was plated at all time points. Three days after plating the samples, colonies were overlaid with agar containing 0.2% tetrazolium and incubated at room temperature in the dark as described in Ogur et al. [Ogur M, St. John R, Nagai S (1957) *Science* 125:928–929]. Red colonies are respiratory competent, and white colonies are petites. (*Lower*) Petite formation, presented as the percent of total viable cells, is shown as a function of time of nitrogen starvation of the indicated prototrophic strains.

Table S1. Genes affected by DOT6 and TOD6 deletion are enriched for Ribi- and PAC-containing genes

Gene type*	Condition*	No. of affected genes†	No. of Ribi genes affected‡	No. of PAC-containing genes§
dot6Δ	Rapamycin 20 min	49	3 (0.99)	6 (0.99)
dot6Δ	Rapamycin 80 min	0	—	—
tod6Δ	Rapamycin 20 min	224	154 (0)	146 (8.6×10^{-141})
tod6Δ	Rapamycin 80 min	218	149 (0)	125 (2.1×10^{-99})
dot6Δ tod6Δ	Rapamycin 20 min	152	107 (7.4×10^{-278})	108 (7.9×10^{-116})
dot6Δ tod6Δ	Rapamycin 80 min	50	44 (3.3×10^{-144})	47 (2.2×10^{-71})
pka^{as} ***dot6Δ***	1NM-PP1, 20 min	42	39 (1.6×10^{-135})	35 (5×10^{-45})
pka^{as} ***dot6Δ***	1NM-PP1, 80 min	94	70 (1.6×10^{-191})	74 (4.6×10^{-90})
pka^{as} tod6Δ	1NM-PP1, 20 min	6	1 (0.82)	2 (0.69)
pka^{as} tod6Δ	1NM-PP1, 80 min	9	0 (0.97)	1 (1)
pka^{as} dot6Δ tod6Δ	1NM-PP1, 20 min	116	103 (0)	101 (9.5×10^{-142})
pka^{as} dot6Δ tod6Δ	1NM-PP1, 80 min	25	20 (7.3×10^{-58})	21 (6.4×10^{-27})
sch9^{as} ***dot6Δ***	1NM-PP1, 20 min	34	32 (1.6×10^{-112})	31 (8.8×10^{-45})
sch9^{as} ***dot6Δ***	1NM-PP1, 80 min	1	1 (0.0016)	0 (0.98)
sch9^{as} ***tod6Δ***	1NM-PP1, 20 min	2	1 (0.10)	1 (0.58)
sch9^{as} ***tod6Δ***	1NM-PP1, 80 min	4	0 (0.99)	0 (0.97)
sch9^{as} dot6Δ tod6Δ	1NM-PP1, 20 min	35	33 (2.5×10^{-116})	35 (3.8×10^{-57})
sch9^{as} dot6Δ tod6Δ	1NM-PP1, 80 min	0	—	—

Deletion of DOT6 and TOD6 predominantly affects repression of Ribi genes and genes whose promoters contain PAC sites.

*Strains and conditions are as described in legends to Figs. 1 and 2 and Figs. S1 and S2.

†Number of genes whose repression by the indicated treatment is attenuated at least 4-fold in the mutant strain (or at least 2-fold, for the boldface strains) relative to that in an isogenic *DOT6 TOD6* strain.

‡Number of those affected genes that are annotated in SGD among those involved in ribosome biogenesis (303 total). The *P* value for that overlap occurring by chance is provided in parentheses.

§Number of those affected genes whose promoters contain one or more PAC sites (647 genes in total). The *P* value for that overlap occurring by chance is provided in parentheses.

Table S2. Dot6 and Tod6 phosphorylation is affected by glucose upshift

Protein	Site*	Change in phosphorylation†
Dot6	476-LNALSSDADML**S**P**T**H**S**PQK	1.0
	245-SN**S**H**S**FTNSLNQDPIVR	1.0
	310-RRG**S**LANW**S**R	1.8–2.0
	310-RRG**S**LANWSR	1.3
	521-DV**S**PDPIFSPDPADDSSNTSDAGS	0.8
	507-SGSTTDDDKG**S**D	0.8
Tod6	330-RRA**S**LVV**S**PYM**S**P	3.0
	330-RRA**S**LVV**S**PYMSP	>1.0
	330-RRASLVV**S**PYMSP	0.8
	224-SF**S**HSITTNTPNV	1.4–1.8

*Phosphopeptide with phosphorylated S/T residue highlighted in bold. Starting residue of each peptide is indicated, and putative PKA sites are underlined

†Change in phosphopeptide levels 5 min after glucose addition to 2% to a culture of strain Y3615 (*arg4*::kan *lys1*::kan *car1*::HIS3) grown in SC plus 2% glycerol supplemented with ^{12}C-arginine and ^{12}C-lysine relative to that of the same strain grown in SC plus 2% glycerol supplement with ^{13}C-arginine and ^{13}C-lysine. Ratios were determined by total phosphoproteome measurements after trypsin digestion, SCX/IMAC enrichment, and MS/MS analysis of the mixed extracts as described previously [Gruhler A, et al. (2005) *Mol Cell Proteomics* 4:310–327; Villen J, Gygi SP (2008) *Nat Protoc* 3:1630–1638]. Data were provided by N. Petrenko, W. Kim, S. Gygi, and J.R.B.

Table S3. Strains used in this study

Strain	Genotype	Source
Y2092	*ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	(1)
Y3705	*dot6*Δ ::KanMX *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	This study
Y3706	*tod6*Δ ::*HIS3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	This study
Y3707	*tod6*Δ ::*HIS3 dot6*?::KanMX *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	This study
Y3561	*tpk1*(M164G) *tpk2*(M147G) *tpk3*(M165G) *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	(2)
Y3708	*dot6*Δ ::KanMX *tpk1*(M164G) *tpk2*(M147G) *tpk3*(M165G) *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	This study
Y3709	*tod6*Δ ::*TRP1 tpk1*(M164G) *tpk2*(M147G) *tpk3*(M165G) *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	This study
Y3710	*tod6*Δ ::*TRP1 dot6*Δ ::KanMX *tpk1*(M164G) *tpk2*(M147G) *tpk3*(M165G) *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	This study
Y3507	*sch9*(T492G) *gal1*::*HIS3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	(3)
Y3711	*dot6*Δ ::KanMX *sch9*(T492G) *gal1*::*HIS3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	This study
Y3712	*tod6*Δ ::TRP1 *sch9*(T492G) *gal1*::*HIS3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	This study
Y3713	*tod6*Δ ::*TRP1 dot6*Δ ::KanMX *sch9*(T492G) *gal1*::*HIS3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	This study
Y3628	Prototroph	(4)
Y3714	*dot6*Δ ::KanMX	This study
Y3715	*tod6*Δ ::*TRP1 trp1-1*	This study
Y3716	*dot6*Δ ::KanMX *tod6*Δ ::*TRP1 trp1-1*	This study

1. Lee TI, et al. (2002) Transcriptional regulatory networks in Saccharomyces cerevisiae. *Science* 298:799–804.
2. Zaman S, Lippman SI, Schneper L, Slonim N, Broach JR (2009) Glucose regulates transcription in yeast through a network of signaling pathways. *Mol Syst Biol* 5:245.
3. Regenberg B, et al. (2006) Growth-rate regulated genes have profound impact on interpretation of transcriptome profiling in *Saccharomyces cerevisiae*. *Genome Biol* 7:R107.
4. Prototroph derived from W303, kindly provided by Fred Cross.