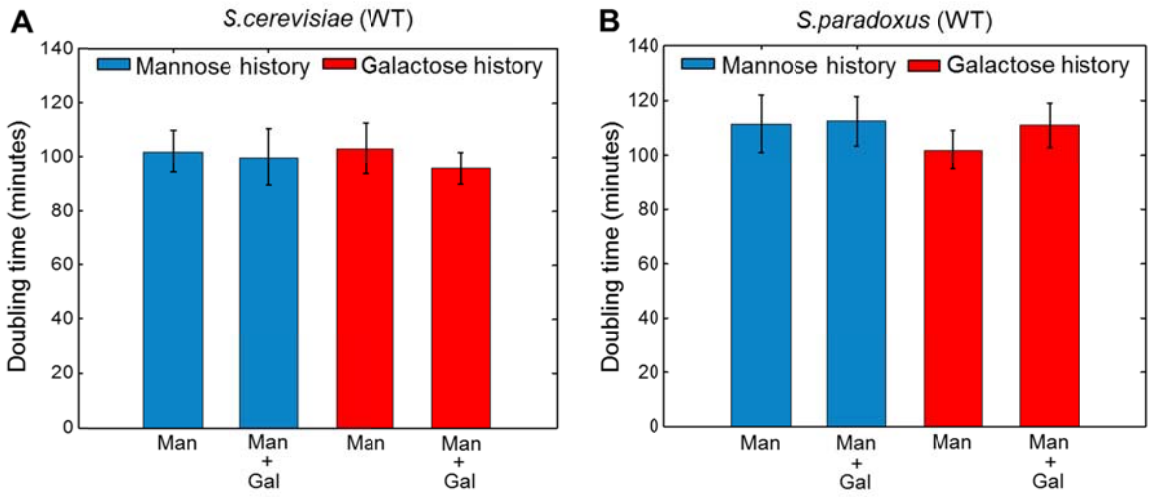
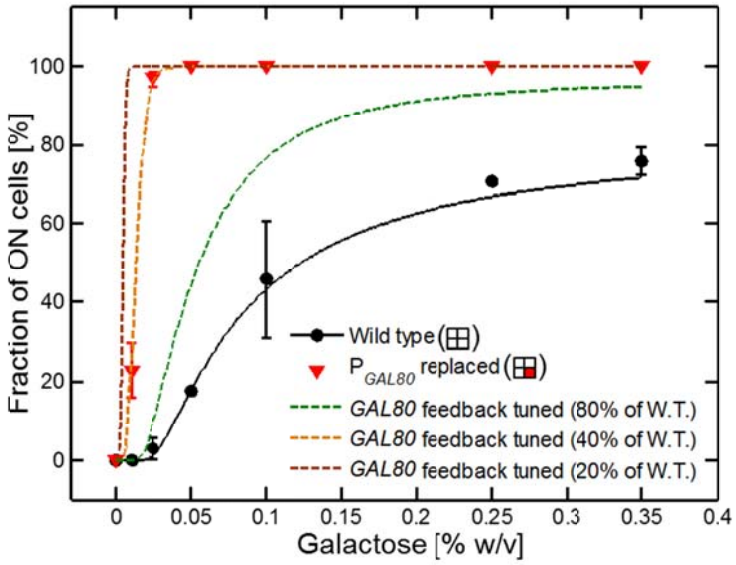
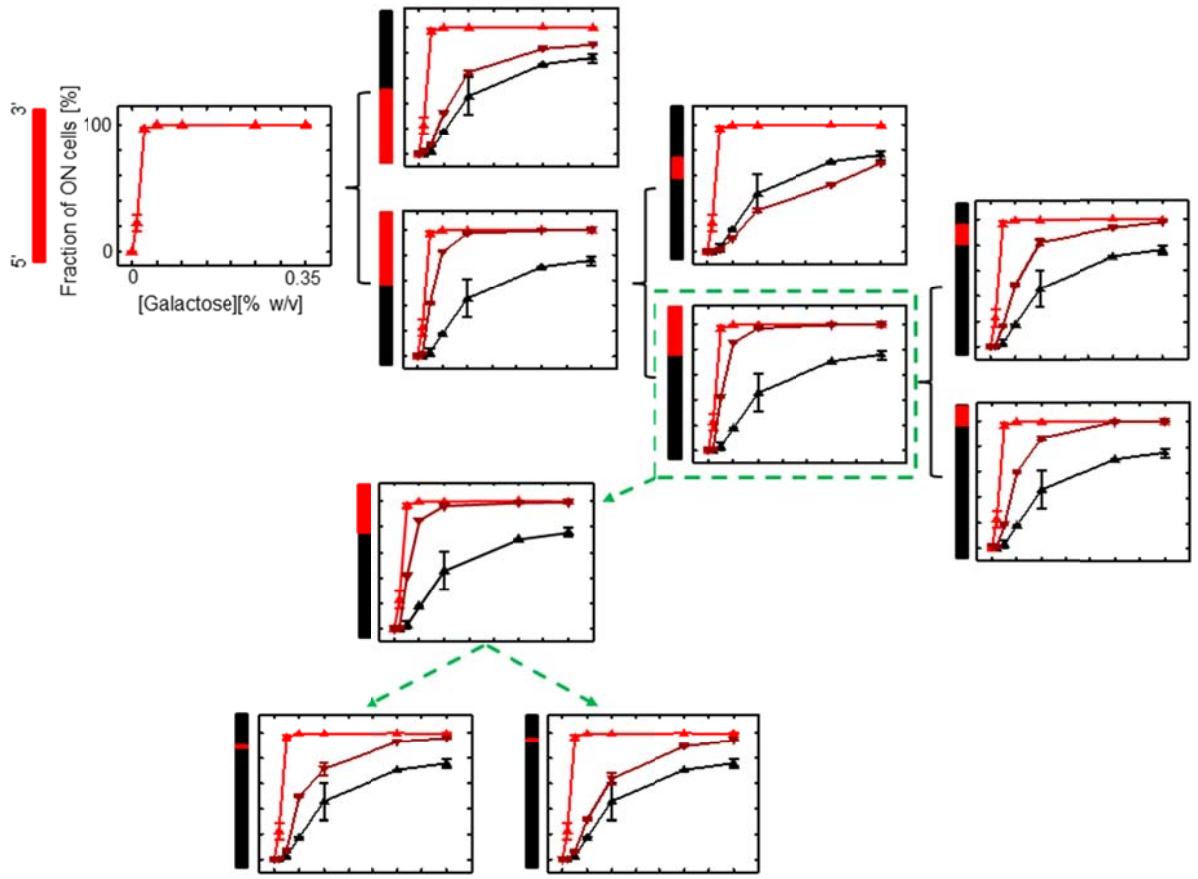
SUPPLEMENTARY FIGURES



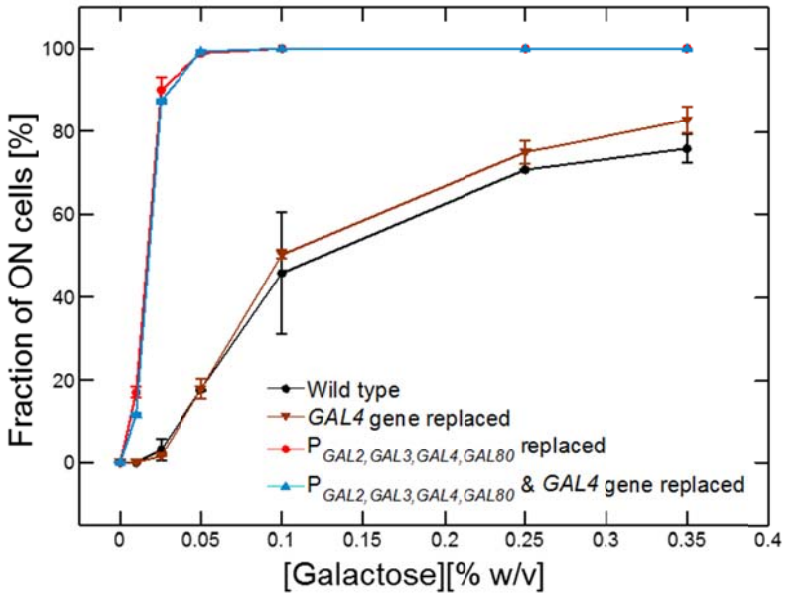
Supplementary Fig. 1. Growth rate measurements of the wild type *S. cerevisiae* (a) and *S. paradoxus* strains (b). Cells grown overnight (22 hours) in 'Mannose History' (minimal media with 0.1% mannose) or 'Galactose History' (minimal media with 0.1% mannose and 0.35% galactose) were induced the next day either in the same media or switched to the opposite media, and growth rates were measured by recording OD_{600} values. The abbreviation 'Man' refers to the presence of 0.1% mannose in the after-overnight growth environment during which growth rates were measured; the abbreviation 'Man+Gal' refers to the presence of both 0.1% mannose and 0.35% galactose in the after-overnight environment. To obtain the growth rates, the OD_{600} recordings were fitted to an exponential function. Growth rates were converted to doubling times by using the following relationship between them: $doubling\ time = ln2\ /\ (growth\ rate)$. In both panels, error bars indicate SEM (N=2).



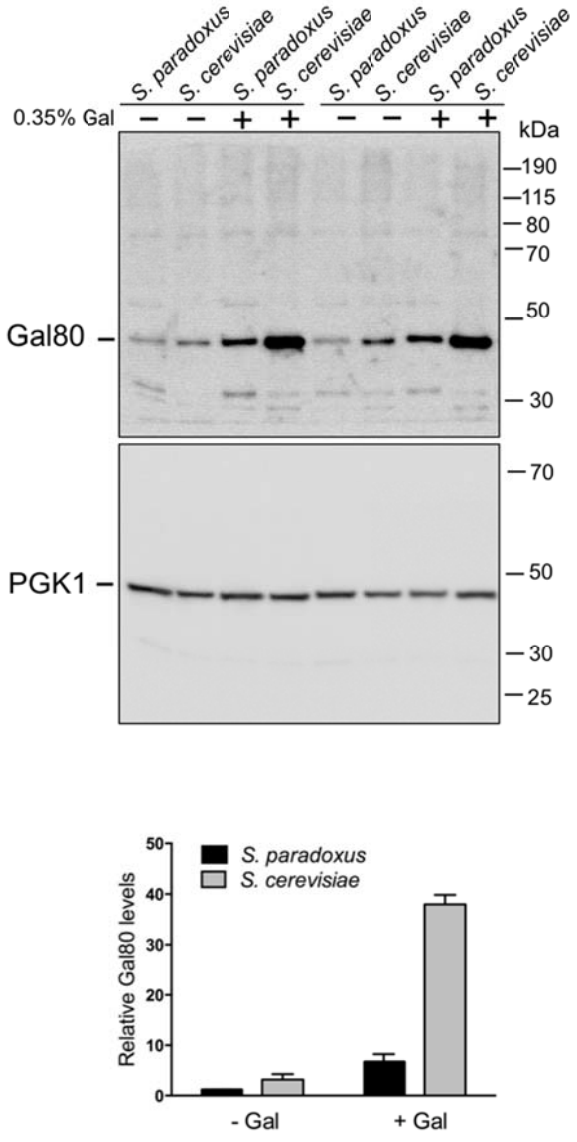
Supplementary Fig. 2. Reducing the *GAL80* feedback strength can reproduce network inducibility levels displayed by the P_{GAL80}-replaced strain. Black and red symbols represent experimental measurements, while dotted lines are model predictions for the GAL network activity in *S. cerevisiae*. Model predictions are obtained by changing the value of only one parameter (θ_{80}, *GAL80* feedback-strength parameter) relative to the value it takes in wild type *S. cerevisiae* GAL network ($\theta_{80} = 1500$ proteins/hr). Error bars indicate SEM (N=2).



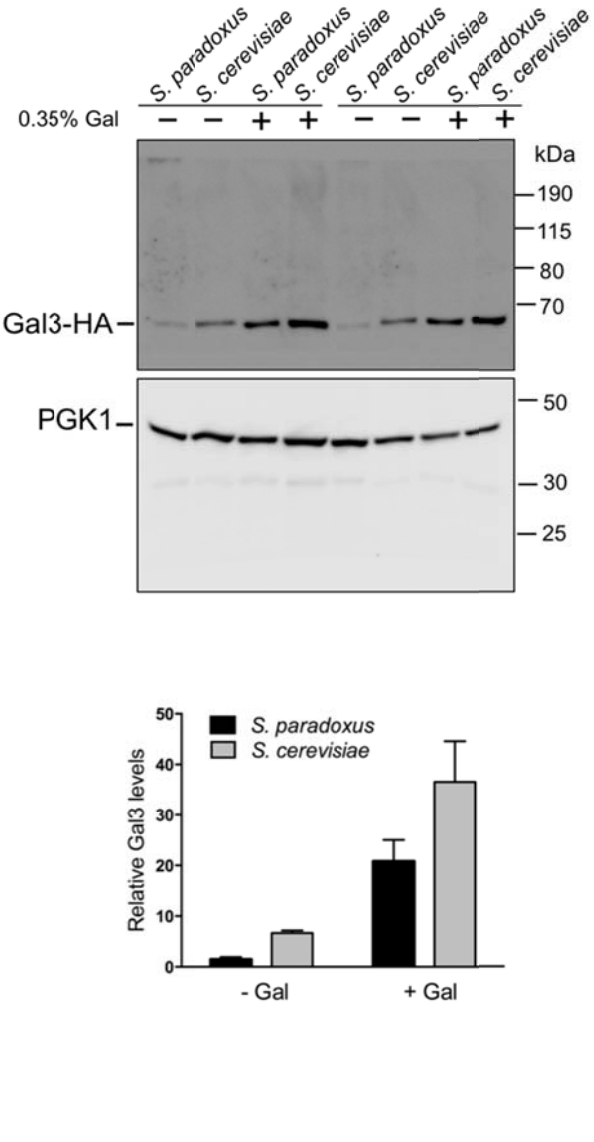
Supplementary Fig. 3. Inducibility profiles of the *S. cerevisiae* strains carrying the hybrid *GAL80* promoters. Red and black data points compose the profiles that are obtained when the whole *GAL80* promoter is from *S. paradoxus* and *S. cerevisiae*, respectively. The brown data points compose the profiles that are obtained when the hybrid *GAL80* promoters replaced the endogenous *GAL80* promoter in *S. cerevisiae*. The vertical bars at the left of the panels represent how the hybrid promoters were contructed, with black color representing the promoter region from *S. cerevisiae* and red representing the region from *S. paradoxus*. When the ~100bp region at the 3' end of a hybrid promoter was from *S. paradoxus* (as in the panel shown in green box), the inducibility profile turned out to be the most similar to the profile obtained from the strain carrying the full/endogenous *S. paradoxus GAL80* promoter (top-left panel). The bottom two panels show the results obtained from strains carrying point mutations (4bp insertion and 1bp mutation) in an otherwise *S. cerevisiae* version of the *GAL80* promoter. Error bars indicate SEM (N=2).



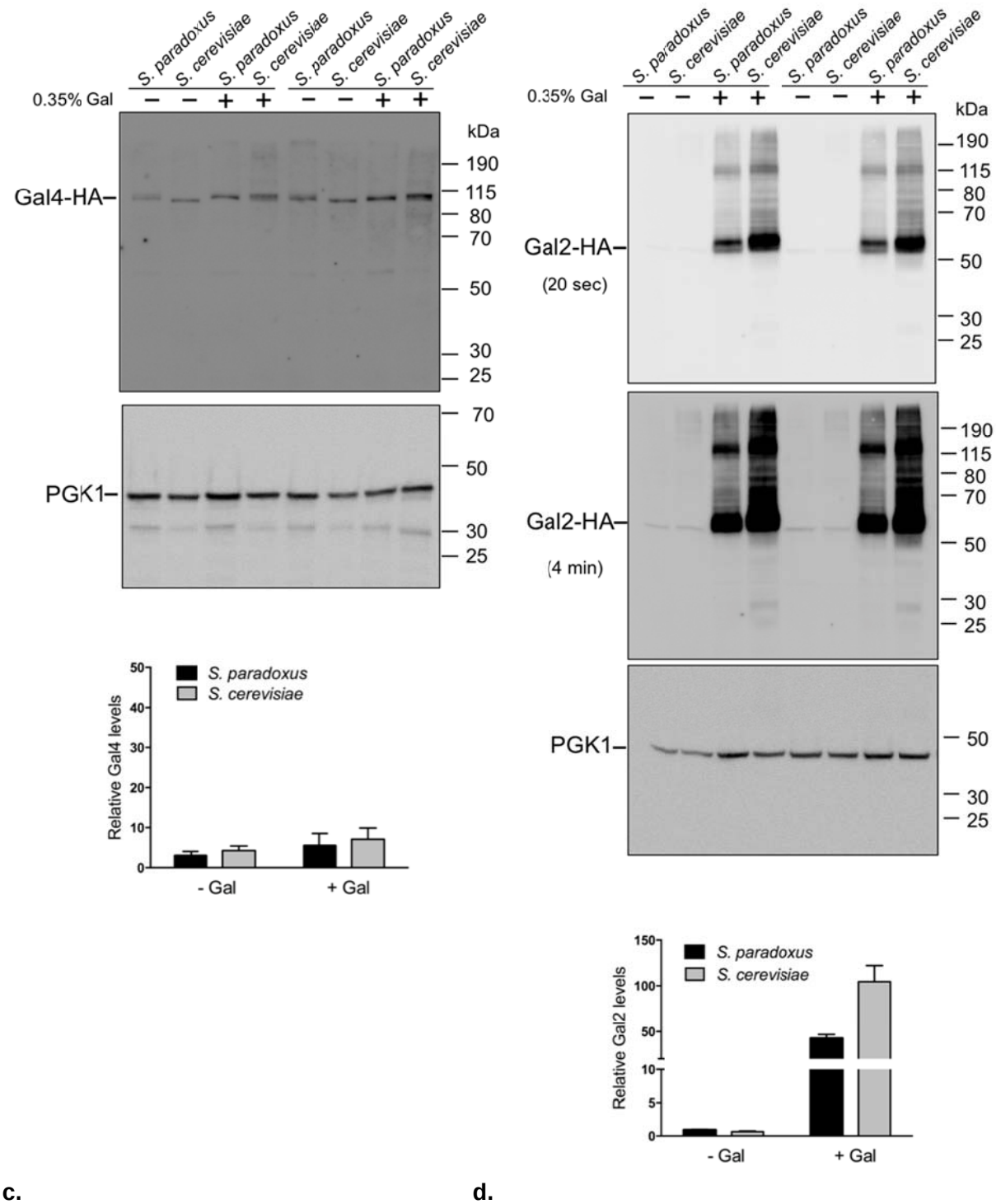
Supplementary Fig. 4. Effect of *GAL4* gene replacement on the inducibility profile of the GAL network in *S. cerevisiae*. All strains used the same P_{GAL1}-YFP reporter. Black: wild type *S. cerevisiae* GAL network profile. Brown: network inducibility profile when the *GAL4* gene was replaced by its counterpart from *S. paradoxus*. Red: network inducibility profile when all four regulatory promoters were replaced by their counterparts from *S. paradoxus*. Blue: network inducibility profile when the *GAL4* gene and all four regulatory promoters were replaced by their counterparts from *S. paradoxus*. Error bars indicate SEM (N=2). The replacement of the Gal4 proteins between the two yeast species does not change the inducibility profile of the network.


S. paradoxus
S. cerevisiae
S. paradoxus
S. cerevisiae
S. paradoxus
S. cerevisiae
S. paradoxus
S. cerevisiae
0.35% Gal
– – + + – – + +
kDa
190
115
80
70
50
30
Gal80
70
50
30
25
PGK1
Relative Gal80 levels
50
40
30
20
10
0
S. paradoxus
S. cerevisiae
- Gal
+ Gal

a.


S. paradoxus
S. cerevisiae
S. paradoxus
S. cerevisiae
S. paradoxus
S. cerevisiae
S. paradoxus
S. cerevisiae
0.35% Gal
– – + + – – + +
kDa
190
115
80
70
Gal3-HA
50
30
25
PGK1
Relative Gal3 levels
50
40
30
20
10
0
S. paradoxus
S. cerevisiae
- Gal
+ Gal

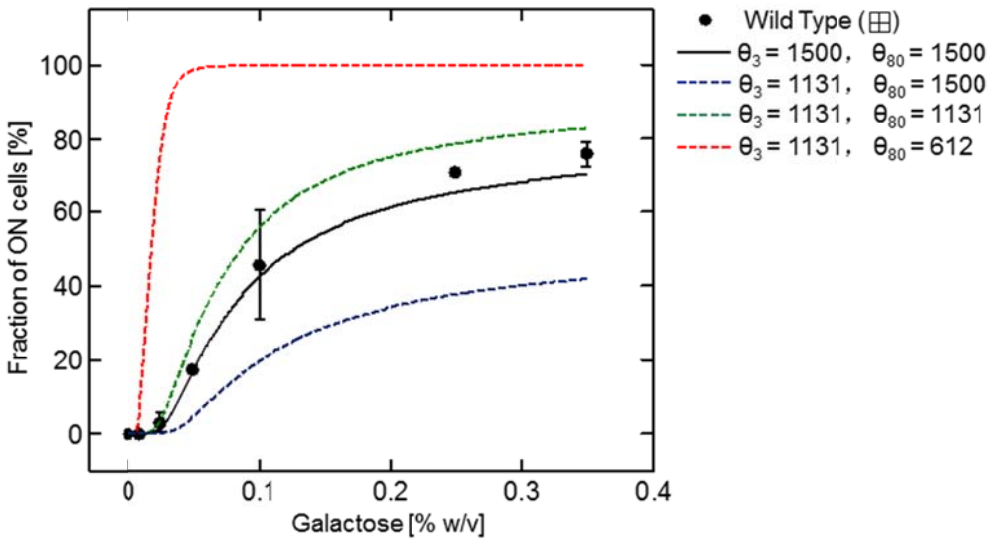
b.



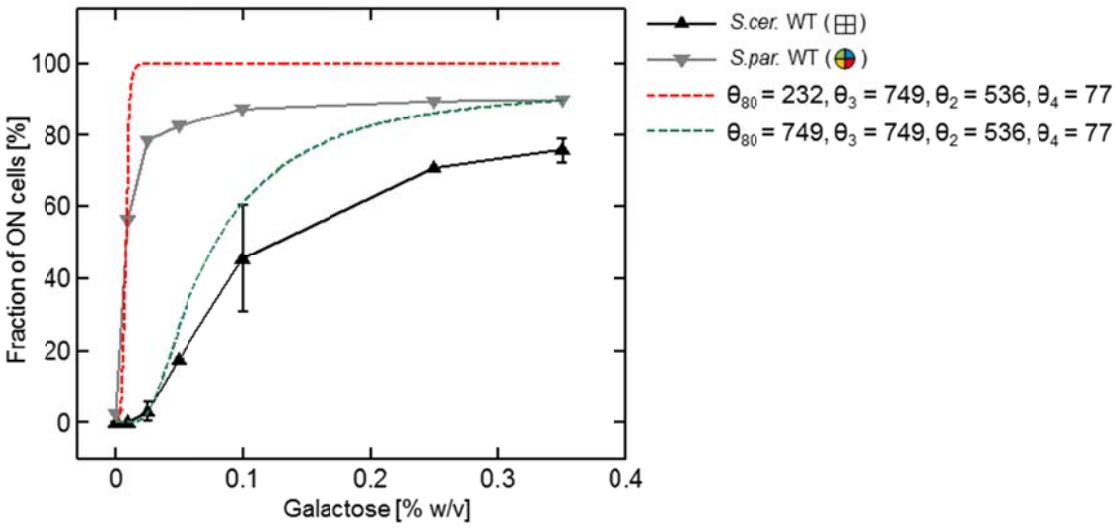
c. **d.**

Supplementary Fig. 5. Western blots comparing **a.** Gal80, **b.** Gal3, **c.** Gal4, **d.** Gal2 protein levels between wild type *S. paradoxus* and wild type *S. cerevisiae*, in two independent experiments. Cells were grown overnight (22 hr) in minimal media containing 0.1% mannose. Then, they were transferred to minimal media containing either 0.1% mannose and 0.35%

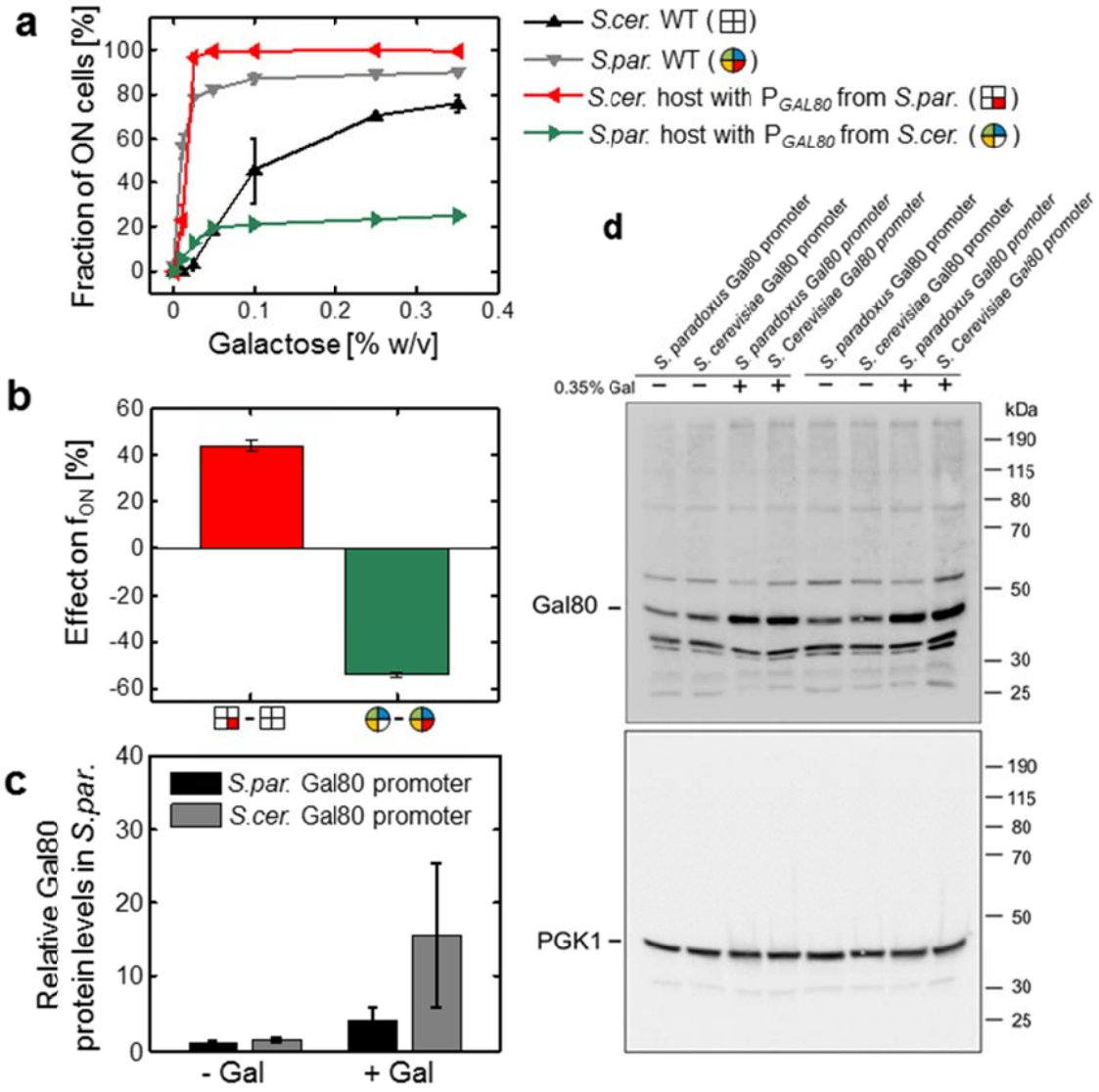
galactose (indicated as ‘+’), or 0.1% mannose (indicated as ‘-’), and grown for another 22 hours. Both low and high exposures of the membrane were shown for Gal2-HA. Relative protein levels were normalized to *PGK1* levels, and then normalized to the first sample of each western blot. Gal4-HA levels were further normalized to Gal3-HA for comparison purposes. In all panels, error bars indicate SEM (N=2).



Supplementary Fig. 6. Relative strength of the *GAL80* promoter with respect to the *GAL3* promoter dictates the network inducibility profile of the GAL network. Black dots represent the experimental inducibility profile of the wild type *S. cerevisiae* GAL network, while the black line is the model prediction for the wild type network activity in *S. cerevisiae* (using the literature-based parameter values $\theta_{80} = \theta_3 =$ 1500 proteins/hr). When we simulated the network inducibility profile using $\theta_{80} = \theta_3 =$ 1131 proteins/hr, the profile did not change in a major way, verifying the compensated nature of the GAL network. However, changing the relative strength of the *GAL80* promoter with respect to the strength of the *GAL3* promoter caused large deviations from the wild type profile, showing that the relative strength difference between the two network promoters (not the absolute values of their strength) dictates the inducibility profile of the GAL network. Error bars indicate SEM (N=2).

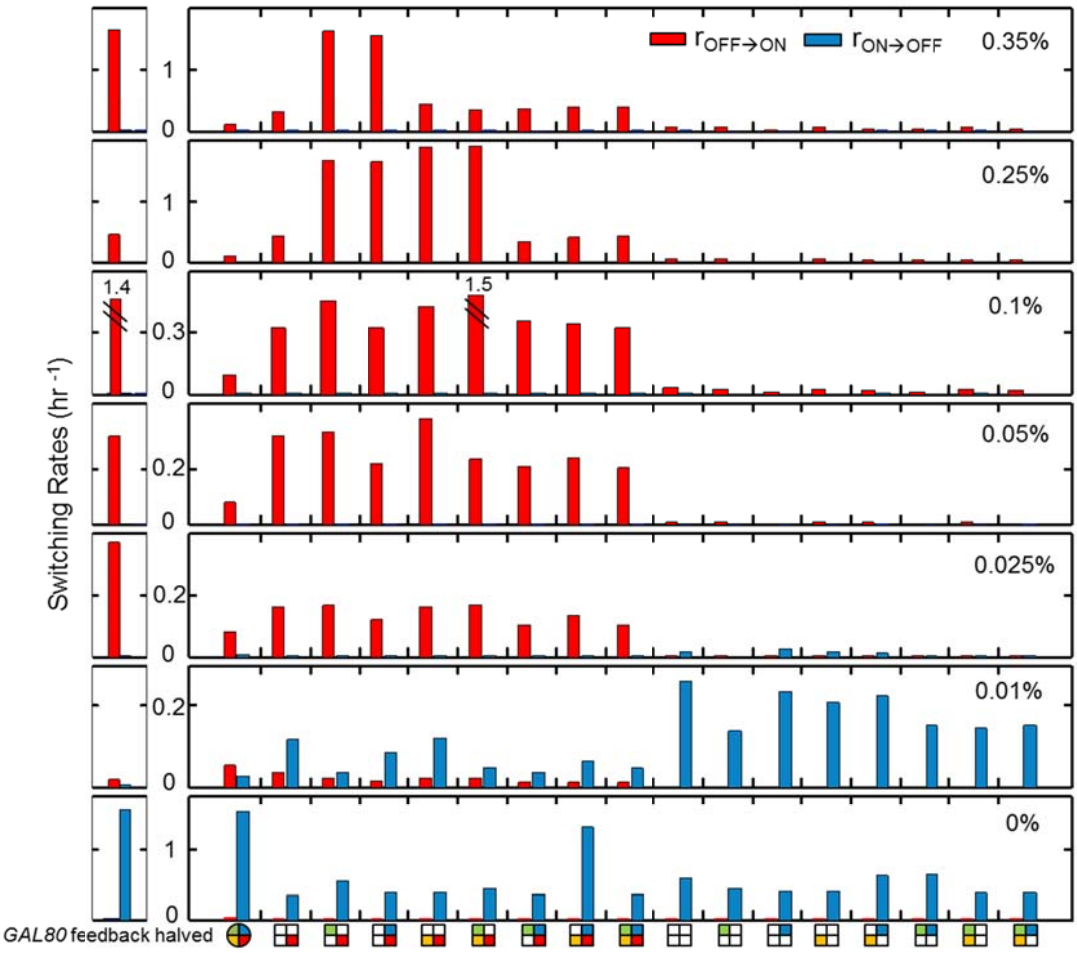


Supplementary Fig. 7. Relatively weak strength of the *S. paradoxus GAL80* promoter is the cause of the high inducibility profile observed in wild type *S. paradoxus*. Black and gray triangles are experimental measurements of the GAL network inducibility in the two wild type species. Using the *S. paradoxus* protein production rates ($\theta_{80} = 232\,\text{proteins/hr}$, $\theta_3 = 749\,\text{proteins/hr}$, $\theta_2 = 536\,\text{proteins/hr}$, $\theta_4 = 77\,\text{proteins/hr}$) estimated from the western blot experiments, we ran a simulation using our model and observed a highly inducible network activity profile (red line), similar to the purely experimental profile (gray triangles). To prove that the strength difference between the *GAL80* and *GAL3* promoters causes this profile, we made the strength of the *GAL80* promoter equal to the strength of the *GAL3* promoter ($749\,\text{proteins/hr}$) and ran a simulation. The resulting inducibility profile (green line) was indeed significantly lower than the original profile. Error bars indicate SEM (N=2).

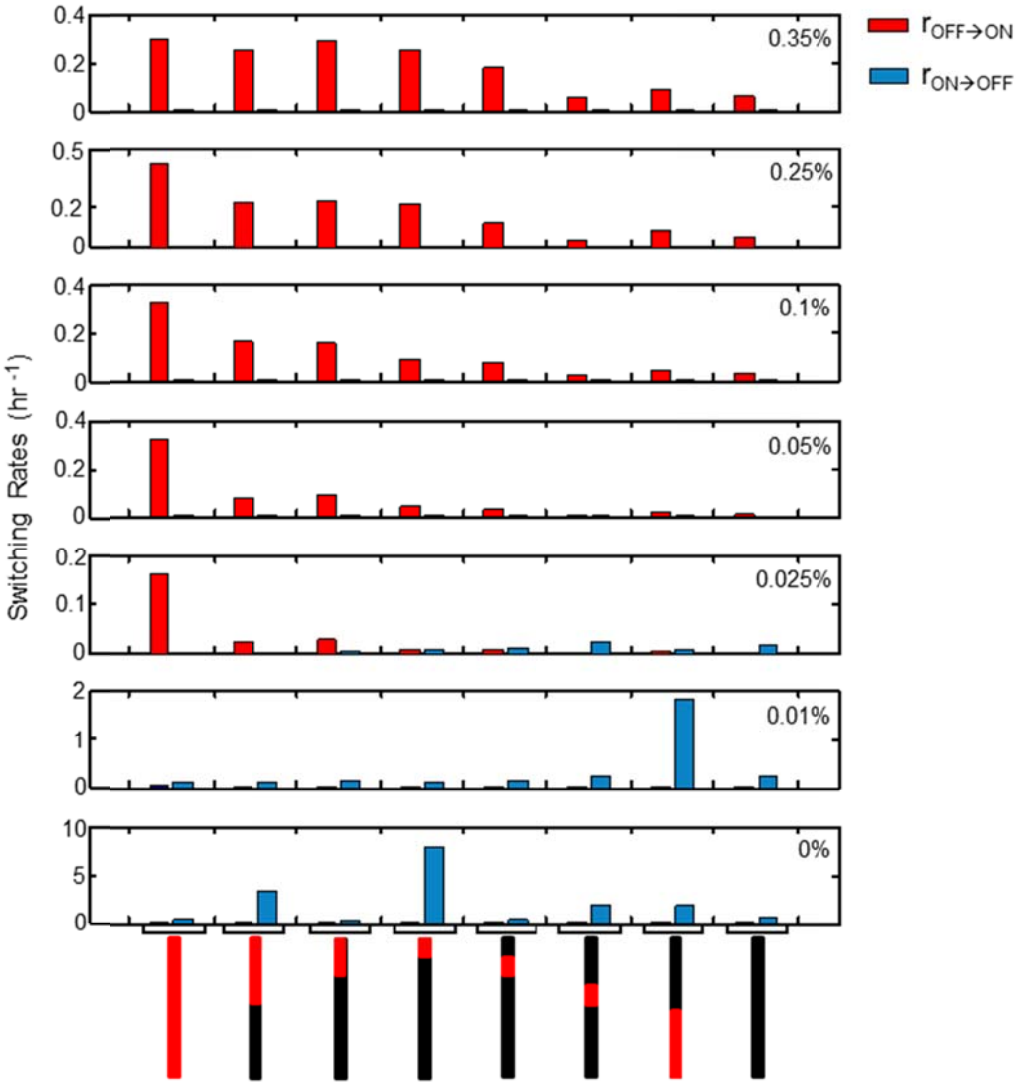


Supplementary Fig. 8. **a.** Replacing the *GAL80* promoter in *S. paradoxus* with its *S. cerevisiae* counterpart lowers the network inducibility profile (green triangles). This indicates that the *S. cerevisiae GAL80* promoter is stronger than its *S. paradoxus* counterpart (gray triangles). **b.** The effect of the *GAL80* promoter replacement on average network inducibility is similar between the two host species. The average differences were obtained by averaging across the seven induction levels. **c-d.** Western blots showing the relative Gal80 protein levels in two *S. paradoxus* strains, one carrying its endogenous *GAL80* promoter (black), and the other carrying the *S. cerevisiae GAL80* promoter (gray). Cells were cultured overnight (for 22 hours) in 0.1% mannose minimal media before being transferred to (and grown for another 22 hours in) minimal media containing either 0.1% mannose and 0.35% galactose (indicated as '+'), or 0.1% mannose only (indicated as '-'). Two independent experiments were performed. Relative protein levels were normalized to *PGK1* levels. Results show that the reduction observed in the network inducibility profile (**a**, green triangles) is due to an increase in the Gal80 protein level. In the '-Gal' environment, the network is in its monomodal OFF state leading to basal expression levels

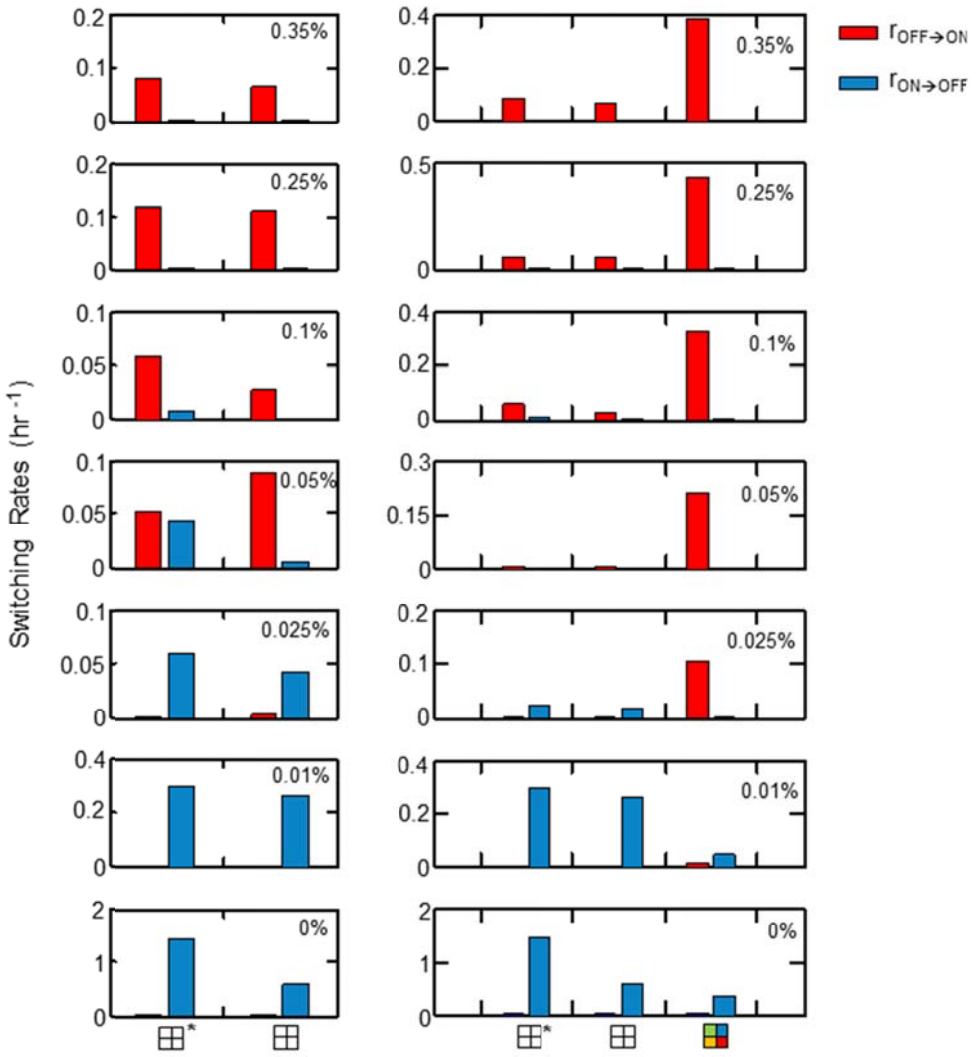
of Gal80 proteins measured by the western blot. In the '+Gal' environment, the network activity in both strains display bimodal activity. Since western blots quantify the Gal80 content from both OFF and ON cells, we used the following equations and plotted (panel **c**) Gal80 protein levels of the ON cells. $Gal80_{total}$ = [$Gal80_{OFF}$ * (1 - f_{ON})] + [$Gal80_{ON}$ * f_{ON}]. From here, we solved for $Gal80_{ON}$ = ($Gal80_{total}$ – [$Gal80_{OFF}$ * (1- f_{ON})]) / f_{ON}. The $Gal80_{total}$ and $Gal80_{OFF}$ values are measured directly by western blots (panel **d**). In all panels, error bars indicate SEM (N=2).



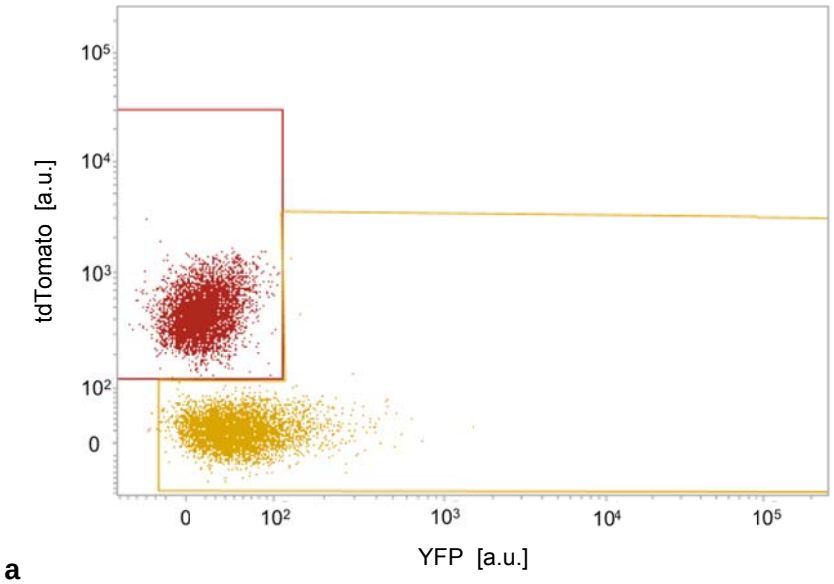
Supplementary Fig. 9. Phenotypic switching rates of the *GAL80* feedback-halved strain (MA0494, far left side) in comparison to the other strains characterized. The numbers (0% to 0.35%) indicate the concentration of galactose used in the induction media. The values shown on the right side of the figure (switching rates of the 17 strains) are identical to the ones shown in the main text; they are shown here for comparison purposes. The numbers (1.4 and 1.5) displayed on two discontinuous bars represent the actual values of the switching rates for those two strains/condition. The genetic background of each haploid *S. cerevisae* strain is specified by the squares below the data panels. The small squares represent the four regulatory promoters replaced (if colored) by their *S. paradoxus* counterparts. The color-coding is: blue for P_{GAL3}, red for P_{GAL80}, green for P_{GAL2}, yellow for P_{GAL4}. The colored circle at left represents the wild type *S. paradoxus* GAL network.



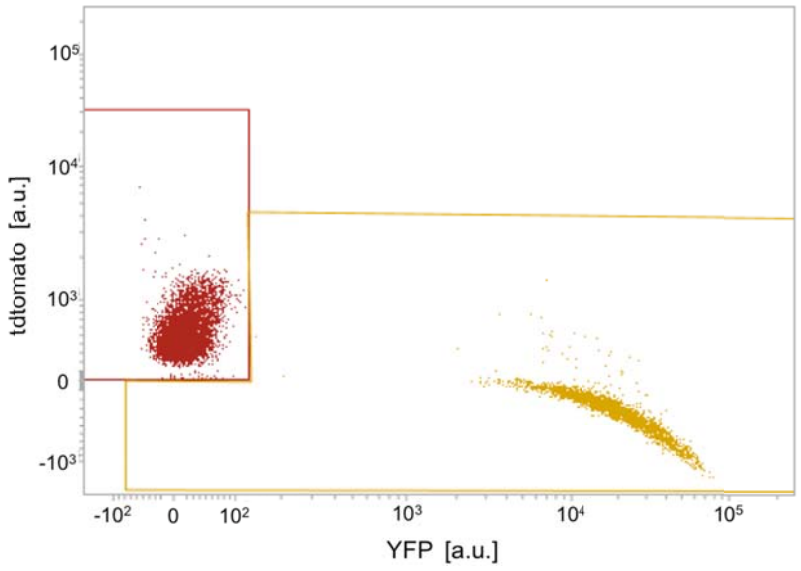
Supplementary Fig. 10. Phenotypic switching rates of the *S. cerevisiae* strains carrying hybrid *GAL80* promoters. The numbers (0% to 0.35%) indicate the concentration of galactose used in the induction media. The vertical bars below the data panels represent how the hybrid promoters were constructed (black color representing promoter regions from *S. cerevisiae* and red color representing the regions from *S. paradoxus)*.



Supplementary Fig. 11. Testing the method used in measuring the switching rates. To see whether or not our method is invariant or robust to different overnight-growth conditions, we used a third set of overnight growth conditions. Instead of growing the cells for 22 hours overnight, we grew them for 15 hours; and instead of using [0.35% galactose + 0.1% mannose], we used [0.1% galactose + 0.1% mannose] for the galactose-containing overnight media (the other overnight media still contained 0.1% mannose only and cells were grown overnight for 15 hours). As expected, this gave rise to a different "after-overnight f_{ON} value" for the galactose-containing case, as well as a different "after-induction f_{ON} value". However, when we calculated the switching rates after the 22-hours induction period, we obtained values (white square marked with ' * ') that were similar to the previously-measured values (unmarked white square). In the left and right panel above, the white squares represent the haploid wild type *S. cerevisiae* strain. The colored square on the right panel above (haploid *S. cerevisiae* strain carrying all four promoter swaps) is shown for comparison purposes: the switching rate differences between a P_{GAL80}-swapped and unswapped strain are still there if a third overnight growth-condition is used.

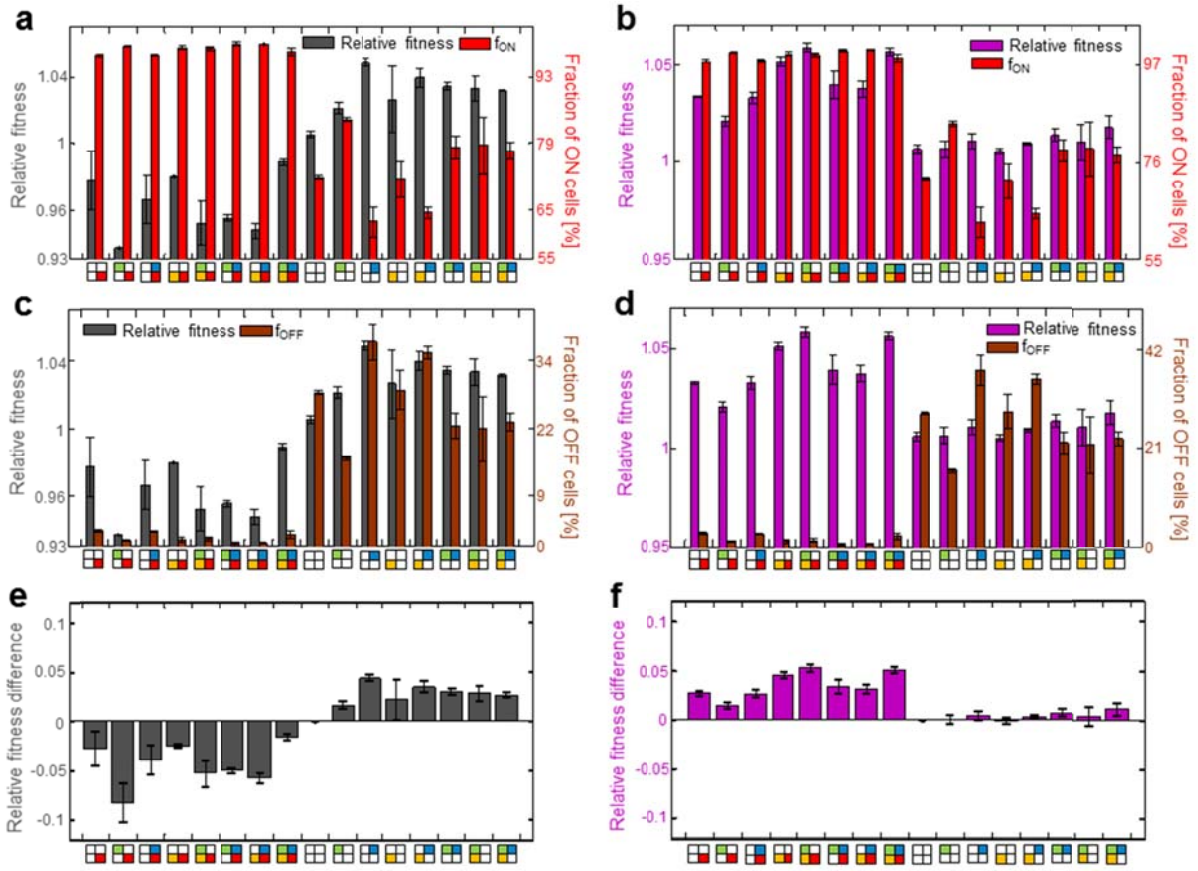


a

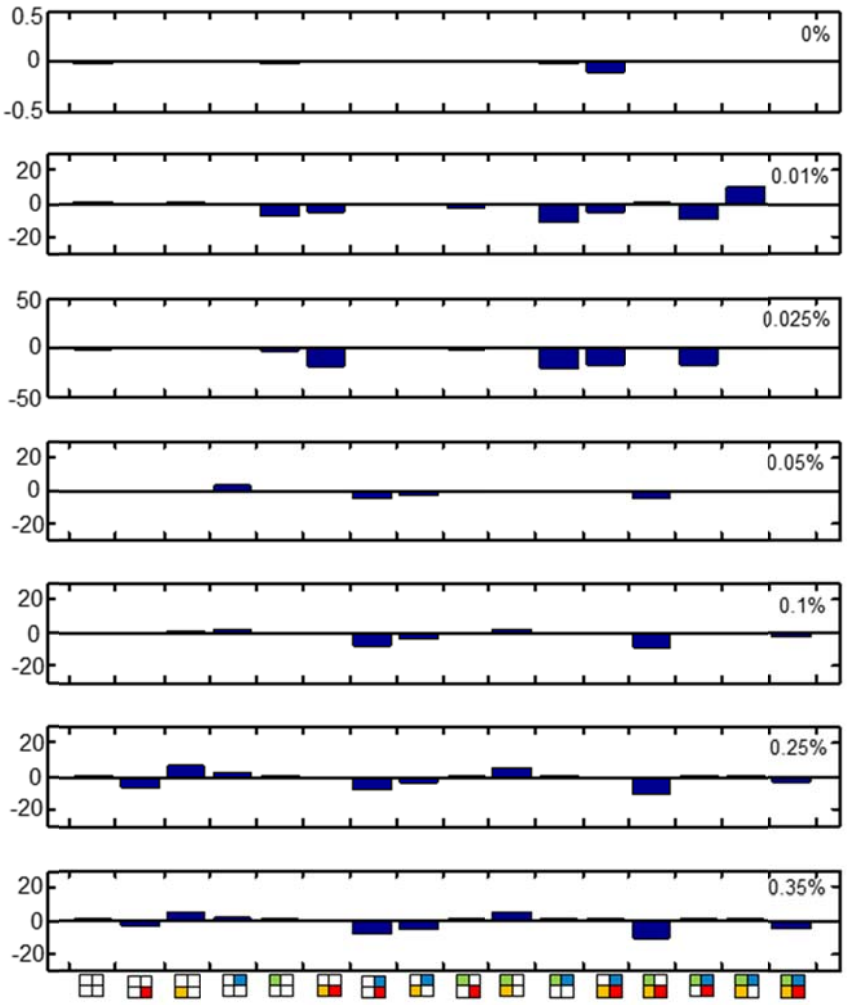


b

Supplementary Fig. 12. Quantifying the relative abundance of YFP- and tdTomato-carrying cells during the competition experiments. The YFP-containing strains were competed against a wild type strain (MA0658) carrying the tdTomato reporter constitutively expressed by the *PGK1* promoter. Using a flow cytometer (BD FACSverse), expression profiles are measured and plotted using bi-exponential axes, and relative fraction of cells carrying each reporter are quantified by gating the plots as shown above. The positions of the gates were slightly changed for some samples in order to improve the separation of the two cell groups. To distinguish the tdTomato-expressing cells from the cells that are in the OFF-state of the YFP distribution, compensation was utilized. **a**, **b**, Example plots showing how the two populations look like, when YFP-containing cells are in the OFF state (**a**), and when they are in the ON state (**b**).



Supplementary Fig. 13. The effect of promoter replacements on cellular fitness profiles. (**a**) Relative fitness of each YFP-containing *S. cerevisiae* strain relative to a wild type *S. cerevisiae* strain carrying the constitutively expressed tdTomato reporter. Strains competed for 48 hours in minimal media containing 0.01% galactose and 0.03% mannose as carbon sources. The dark grey bars indicate the relative fitness level of each strain. The red bars represent the fraction of ON cells (of the YFP-containing strains) measured at 48 hours. At the beginning of the competition, the fraction of ON cells was 0%. (**b**) Relative fitness values of the strains in minimal media environment containing 1% galactose as the sole carbon source. Competing strains of the first environment (a) were transferred to this second environment and competitions lasted for another 44 hours. The fractions of ON cells (of the YFP-containing strains) at the beginning of this environmental exposure are plotted using red color, and the purple color represents the relative fitness levels. All YFP-expressing strains were measured to have 100% ON cells at the end of the 44 hours duration. (**c**, **d**) For the first (**c**) and second (**d**) environment, relative fitness comparisons with respect to the fraction of OFF cells (f_{OFF}). Since $f_{OFF} = 1 - f_{ON}$, information presented in panels **c-d** and **a-b** are complementary to each other. (**e**, **f**) For the first (**e**) and second (**f**) environment, the relative fitness differences between each YFP-containing strain and the wild type strain containing YFP. In all panels, error bars indicate SEM (N=2). In all panels, the genetic background of each haploid *S. cerevisae* strain is specified by the squares below the panels. The small squares represent the regulatory promoters replaced (if colored) by their *S. paradoxus* counterparts (blue for P_{GAL3}, red for P_{GAL80}, green for P_{GAL2}, yellow for P_{GAL4}).



Supplementary Fig. 14. A quantitative perspective on the predictive power of the model by quantifying the residuals. The residuals were quantified in the following way. For each galactose concentration used (from 0% to 0.35%), the experimentally measured fraction of ON cells were subtracted from the values generated by the model. The residual analysis shows the unbiased nature of the model predictions: for most induction conditions used, we observed both positive and negative residual values (y-axes) across different strains (x-axis). The genetic background of each haploid *S. cerevisae* strain is specified by the squares below the panels. The small squares represent the four regulatory promoters replaced (if colored) by their *S. paradoxus* counterparts. The color-coding is: blue for P_{GAL3}, red for P_{GAL80}, green for P_{GAL2}, yellow for P_{GAL4}. The residuals for the predictions of the 11 strains (x-axis, square 6 from left to square 16 from left) were in similar magnitude compared to the residuals of the 5 fitted strains (x-axis, square 1 from left to square 5 from left), validating the predictive ability of the model.

SUPPLEMENTARY TABLES

Parameter	Value
$\theta_2\ (proteins/hr)$	1500
$\theta_3\ (proteins/hr)$	1500
$\theta_4\ (proteins/hr)$	100
$\theta_{80}\ (proteins/hr)$	1500
λ	0.2
α	0.85
β	6.1
η	1.6
μ	0.9
ν	1.3
$K_2\ (proteins)$	600
$K_3\ (proteins)$	4.1
$K_4\ (proteins)$	2
$K_{80}(proteins)$	8.0
$K_g\ (proteins)$	0.052

Supplementary Table 1. Parameter values used in our previous model of GAL network activity[2].

Parameter	Value
α	0.69
β	5.1
η	2.7
μ	0.4
ν	1.1
$K_2\ (proteins)$	1463
$K_3\ (proteins)$	1.8
$K_4\ (proteins)$	3.7
$K_{80}(proteins)$	9.1
$K_g\ (proteins)$	0.027

Supplementary Table 2. Model parameters and values used in the fitting procedure.

Parameter	***S.cer* promoter (value from ref2)**	***S.par* promoter (value from fit)**	***Relative comparison:*** $\boldsymbol{\theta_{S.cer}/\,\theta_{S.par}}$
$\theta_2\ (proteins/hr)$	1500	1087	1.38
$\theta_3\ (proteins/hr)$	1500	1131	1.33
$\theta_4\ (proteins/hr)$	100	109	0.92
$\theta_{80}\ (proteins/hr)$	1500	612	2.45

Supplementary Table 3. Model parameters and values for the protein production rates of GAL network promoters from *S. cerevisiae* and *S. paradoxus*.

Parameter / **Strain**	θ_2	θ_3	θ_4	θ_{80}
Wild type *S. cerevisiae*	1500	1500	100	1500
P_{GAL2} -replaced in *S. cer.*	**1087**	1500	100	1500
P_{GAL3} -replaced in *S. cer.*	1500	**1131**	100	1500
P_{GAL4} -replaced in *S. cer.*	1500	1500	**109**	1500
P_{GAL80} -replaced in *S. cer.*	1500	1500	100	**612**

Supplementary Table 4. The strains used in the fitting procedure. Fitted (**bolded**) and fixed parameter values are shown for the protein production rates from the **replaced** (from *S. paradoxus*) and *S. cerevisiae* GAL network promoters.

Strain	Genotype
MA0048	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-*YFP*
WP0005	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-*YFP*, P_{gal80}::$P_{GAL80\ (S.Par)}$
WP0006	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-*YFP*, P_{gal2}::$P_{GAL2\ (S.Par)}$
WP0007	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-*YFP*, P_{gal3}::$P_{GAL3\ (S.Par)}$
WP0008	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-*YFP*, P_{gal4}::$P_{GAL4\ (S.Par)}$
WP0020	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-*YFP*, P_{gal80}::$P_{GAL80\ (S.Par)}$, P_{gal2}::$P_{GAL2\ (S.Par)}$
WP0021	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-*YFP*, P_{gal3}::$P_{GAL3\ (S.Par)}$, P_{gal80}::$P_{GAL80\ (S.Par)}$
WP0023	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-*YFP*, P_{gal3}::$P_{GAL3\ (S.Par)}$, P_{gal4}::$P_{GAL4\ (S.Par)}$
WP0024	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-*YFP*, P_{gal2}::$P_{GAL2\ (S.Par)}$, P_{gal3}::$P_{GAL3\ (S.Par)}$
WP0056	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-*YFP*, P_{gal80}::$P_{GAL80\ (S.Par)}$, P_{gal4}::$P_{GAL4\ (S.Par)}$
WP0042	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-*YFP*, P_{gal4}::$P_{GAL4\ (S.Par)}$, P_{gal2}::$P_{GAL2\ (S.Par)}$
WP0031	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-*YFP*, P_{gal80}::$P_{GAL80\ (S.Par)}$, P_{gal2}::$P_{GAL2\ (S.Par)}$, P_{gal4}::$P_{GAL4\ (S.Par)}$
WP0045	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-*YFP*, P_{gal3}::$P_{GAL3\ (S.Par)}$, P_{gal80}::$P_{GAL80\ (S.Par)}$, P_{gal2}::$P_{GAL2\ (S.Par)}$
WP0046	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-*YFP*, P_{gal3}::$P_{GAL3\ (S.Par)}$, P_{gal80}::$P_{GAL80\ (S.Par)}$, P_{gal4}::$P_{GAL4\ (S.Par)}$
WP0047	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-*YFP*, P_{gal2}::$P_{GAL2\ (S.Par)}$, P_{gal3}::$P_{GAL3\ (S.Par)}$, P_{gal4}::$P_{GAL4\ (S.Par)}$
WP0048	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-*YFP*, P_{gal80}::$P_{GAL80\ (S.Par)}$, P_{gal2}::$P_{GAL2\ (S.Par)}$, P_{gal4}::$P_{GAL4\ (S.Par)}$, P_{gal3}::$P_{GAL3\ (S.Par)}$
WP0057	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-*YFP*, *gal4*::$GAL4_{(S.Par)}$
WP0063	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-*YFP*, P_{gal80}::$P_{GAL80\ (S.Par)}$, P_{gal2}::$P_{GAL2\ (S.Par)}$, P_{gal4}::$P_{GAL4\ (S.Par)}$, P_{gal3}::$P_{GAL3\ (S.Par)}$, *gal4*::$GAL4_{(S.Par)}$
MA0494	*MAT*a/α, *ade*2::*ADE*2-P_{MYO2}-*rtTA*/*ade*2::*ADE*2-P_{GAL1}-*YFP*, *leu*2/*leu*2::*LEU*2, *his*3::*HIS*3/*his*3, *gal80Δ*::*KanMX*/*GAL80*
MA0653	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-*YFP*, *his4*, *leu2*, *ura3*, *lys1*, *ho*::*KanMX4*
MA0658	*MAT*α, *his3*::*HIS3*-P_{PGK1}-*tdTomato*
WP0094	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-YFP, P_{gal80}::$P_{GAL80\ (hybrid\ 1)}$ where $P_{GAL80\ (hybrid\ 1)}$ = 1-139bp from *S.par*, 140-282bp from *S.cer*
WP0089	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-YFP, *trp1*::*TRP1*-P_{TEF1}-*Cas9*, P_{gal80}::$P_{GAL80\ (hybrid\ 2)}$ where $P_{GAL80\ (hybrid\ 2)}$ = 1-140bp from *S.cer*, 141-288bp from *S.par*
WP0090	*MAT*α, *ade2*::*ADE2*-P_{GAL1}-YFP, *trp1*::*TRP1*-P_{TEF1}-*Cas9*, P_{gal80}::$P_{GAL80\ (hybrid\ 3)}$ where $P_{GAL80\ (hybrid\ 3)}$ = 1-140bp from *S.cer*, 141-180bp from *S.par*, 181-283bp from *S.cer*

WP0099	*MAT*α, *ade2::ADE2*-P_{GAL1}-YFP, P_{gal80}::$P_{GAL80\ (hybrid\ 4)}$ where $P_{GAL80\ (hybrid\ 4)}$ = 1-180bp from *S.cer*, 181-288bp from *S.par*
WP0100	*MAT*α, *ade2::ADE2*-P_{GAL1}-YFP, P_{gal80}::$P_{GAL80\ (hybrid\ 5)}$ where $P_{GAL80\ (hybrid\ 5)}$ = 1-180bp from *S.cer*, 181-220bp from *S.par*, 221-287bp from *S.cer*
WP0095	*MAT*α, *ade2::ADE2*-P_{GAL1}-YFP, P_{gal80}::$P_{GAL80\ (hybrid\ 6)}$ where $P_{GAL80\ (hybrid\ 6)}$ = 1-216bp from *S.cer*, 217-284bp from *S.par*
WP0101	*MAT*α, *ade2::ADE2*-P_{GAL1}-YFP, P_{gal80}::$P_{GAL80\ (4bp\ change)}$ where $P_{GAL80\ (4bp\ change)}$ = 1-186bp from *S.cer*, 187-190bp from *S.par*, 191-287bp from *S.cer*
WP0103	*MAT*α, *ade2::ADE2*-P_{GAL1}-YFP, P_{gal80}::$P_{GAL80\ (1bp\ change)}$ where $P_{GAL80\ (1bp\ change)}$ = 1-212bp from *S.cer*, 213bp from *S.par*, 214-283bp from *S.cer*
YYX67	*MAT*α, *ade2::ADE2*-P_{GAL1}-*YFP*, P_{GAL2}-*GAL2-HA-NatNT2*
YYX56	*MAT*α, *ade2::ADE2*-P_{GAL1}-*YFP*, P_{GAL3}-*GAL3-HA-NatNT2*
YYX61	*MAT*α, *ade2::ADE2*-P_{GAL1}-*YFP*, P_{GAL4}-*GAL4-HA-NatNT2*
YYX66	*MAT*α, *ade2::ADE2*-P_{GAL1}-*YFP, his4, leu2, ura3, lys1, ho::KanMX4*, P_{GAL2}-*GAL2-HA-NatNT2*
YYX55	*MAT*α, *ade2::ADE2*-P_{GAL1}-*YFP, his4, leu2, ura3, lys1, ho::KanMX4*, P_{GAL3}-*GAL3-HA-NatNT2*
YYX58	*MAT*α, *ade2::ADE2*-P_{GAL1}-*YFP, his4, leu2, ura3, lys1, ho::KanMX4*, P_{GAL4}-*GAL4-HA-NatNT2*
YYX70	*MAT*α, *ade2::ADE2*-P_{GAL1}-*YFP, his4, leu2, ura3, lys1, ho::KanMX4*, P_{gal80}::$P_{GAL80\ (S.Cer)}$

Supplementary Table 5. Yeast strains used in this study. The *S. cerevisiae* strains are shown in black and they have the W303 background. *S. paradoxus* strains are shown in red (MA0653 is a wild type *S. paradoxus* strain). The wild type *S. paradoxus* strain (into which P_{GAL1}-YFP was integrated) was obtained from N. Talarek.

Strain	*GAL80*, Ct	*ACT1*, Ct	ΔCt (*GAL80* Ct - *ACT1* Ct)	ΔΔCt (ΔCt *S1* - ΔCt *S2*)	**Strength ratio between *S.par* and *S.cer* P_{GAL80} promoter**
Strain 1 (S1)					
S.cer host with P_{GAL80} from *S.par*	20.89	17.85			
	20.61	18.31			
	21.38	17.59			
	21.02	18.18			
Average	20.97 ± 0.32	17.98 ± 0.28	2.99 ± 0.46	0.25 ± 0.72	**0.68 (68%)**
Strain 2 (S2)					
S.cer WT	21.29	18.41			
	20.89	17.98			
	20.91	17.90			
	20.10	17.94			
Average	20.80 ± 0.50	18.06 ± 0.24	2.74 ± 0.56		

Supplementary Table 6. qPCR measurements for quantifying the cross-species P_{GAL80} promoter strength difference. Two haploid *S. cerevisiae* strains (the wild type and the P_{GAL80}–promoter swapped strain) were grown overnight for 22 hours in minimal media containing 0.1% mannose prior to induction for another 22 hours in minimal media containing 0.1% mannose and 0.35% galactose. The culture volumes during the overnight and induction periods were 10ml and 15ml, respectively, and the OD_{600} values at the end of both periods were ~0.1. At the end of the induction period, cells were harvested for total RNA and cDNA was prepared by using Applied Biosystems' High Capacity RNA-to-cDNA kit (Part No: 4387406). The promoter strength ratio between the *S. paradoxus* and *S. cerevisiae* P_{GAL80} promoters was calculated based on the *GAL80* transcript ratio between the above strains as well as the fraction of ON cells observed at the end of the induction period. The lower and upper bound of the error bar for the 0.68 mean value was 0.41 and 1.12, respectively. The qPCR primers used were the following. qG80-F: ACGGTACCAAGGGAGATTTG. qG80-R: ATACCCCGGGTCTAAAGGAG. ACT1-F: ATCGATTTGGCCGGTAGAG. ACT1-R: AAGTCCAAGGCGACGTAACA. The length amplified in the *S. cerevisiae GAL80* gene was 140bp. The length amplified in the *S. cerevisiae ACT1* gene was 137bp.

SUPPLEMENTARY METHODS

Model description

The activity of the GAL network displays a bimodal profile: the expression of galactose-regulated genes can be either in the ON-state or the OFF-state of the bimodal distribution[1, 2, 3, 4]. The following set of differential equations was used to describe the time evolution of the concentrations of proteins expressed from the GAL network genes.

$$\begin{cases} \frac{dx_2}{dt} = \theta_2\ [\lambda(1-f)+f] - \gamma x_2 \\ \frac{dx_3}{dt} = \theta_3\ [\lambda(1-f)+f] - \gamma x_3 \\ \frac{dx_4}{dt} = \theta_4 - \gamma x_4 \\ \frac{dx_{80}}{dt} = \theta_{80}[\lambda(1-f)+f] - \gamma x_{80} \end{cases} \quad (1)$$

where $f = f(x_2, x_3, x_4, x_{80})$ represents the fraction of ON cells, and $(1-f)$ is the fraction of OFF cells. The variables x_2, x_3, x_4, and x_{80} denote the mean protein expression levels of *GAL2, GAL3, GAL4,* and *GAL80*, respectively. *GAL4* gene is constitutively expressed. Parameter θ_i denotes the promoter strength of the corresponding genes in terms of proteins produced per hour. This parameter represents the coarse-grained processes of transcription, translation, and protein folding. Parameter λ represents the relative transcriptional strength of the OFF-state compared to the ON-state. Parameter γ denotes the effective rate of protein loss, which was measured based on protein dilution due to cell division.

Our model considers that cells stochastically transition between the OFF and ON states of the network and that the total concentration of the different regulatory proteins affects the rate at which the OFF→ON transition takes place.

$$\mathrm{OFF} \underset{h}{\overset{h\,\rho(x_2, x_3, x_4, x_{80})}{\rightleftharpoons}} \mathrm{ON}$$

Here, the parameter h denotes the timescale at which these transitions take place. $\rho(x_2, x_3, x_4, x_{80})$ is a function that quantifies how the total concentrations of different GAL proteins (x_2, x_3, x_4, x_{80}) affect the OFF→ON transition. This description is valid as long as the molecular interactions that shape the regulating function ρ act in a much faster timescale than the one at which protein concentrations change due to the processes of transcription, translation, and protein dilution/degradation, which are generally slower than molecular interactions among the GAL network components.

Following a mean-field approximation approach, the fraction of cells in the ON-state is given by:

$$f(x_2, x_3, x_4, x_{80}) \cong \frac{r_{OFF\to ON}(x_2, x_3, x_4, x_{80})}{r_{OFF\to ON}(x_2, x_3, x_4, x_{80}) + r_{ON\to OFF}} = \frac{1}{1 + \frac{r_{ON\to OFF}}{r_{OFF\to ON}(x_2, x_3, x_4, x_{80})}}$$
$$= \frac{1}{1 + \rho(x_2, x_3, x_4, x_{80})^{-1}} \quad (2)$$

where $r_{OFF\to ON}$ and $r_{ON\to OFF}$ are the rates of phenotypic switching between the OFF and ON state, and $\frac{r_{OFF\to ON}(x_2,x_3,x_4,x_{80})}{r_{ON\to OFF}} = \frac{h\,\rho(x_2,x_3,x_4,x_{80})}{h} = \rho(x_2, x_3, x_4, x_{80})$.

The function ρ is parameterized by taking into account the protein-protein and protein-promoter interactions among the various GAL network components. The detailed interactions are represented schematically in Fig. 1 of the main text, and following is the description of how we parameterized these interactions.

GAL4p is the main transcriptional activator of the network. It is constitutively expressed and the rate of the constitutive production is mathematically represented by θ_4 in equation (1). The interaction of Gal4p with a GAL network promoter is described by the following form:

$$\rho = \left(\frac{x_4^*}{K_4}\right)^{\eta} \quad (3)$$

where K_4 represents the typical concentration scale of the interaction, $\eta > 0$ denotes the effective nonlinearity of the Gal4p-promoter interaction, and x_4^* is the active concentration of Gal4p which is not bound by Gal80p and can therefore freely activate transcription.

Since the amount of free Gal4p (denoted by x_4^*) should be a decreasing function of the concentration of Gal80p and an increasing function of total Gal4p (denoted by x_4), we used the following equation to model the Gal4p-Gal80p interaction:

$$x_4^* = \frac{x_4}{1 + \left(\frac{x_{80}^*}{K_{80}}\right)^{\beta}} \quad (4)$$

where x_{80}^* is the concentration of Gal80p proteins that are not bound by active Gal3p, K_{80} is the scaling parameter, and β is the degree of nonlinearity of the Gal4p-Gal80p interaction.

Since the amount of x_{80}^* should be a decreasing function of active Gal3p proteins (x_3^*), we used the following equation to model the Gal80p-Gal3p interaction:

$$x_{80}^{*} = \frac{x_{80}}{1 + \left(\frac{x_3^{*}}{K_3}\right)^{\alpha}} \quad (5)$$

where x_3^* is the concentration of active Gal3p proteins and the parameter α quantifies the nonlinearity of Gal80p-Gal3p interaction.

The activation of Gal3p proteins by internal galactose (g^*) is described by the following equation:

$$x_3^{*} = \frac{x_3}{1 + \left(\frac{g^{*}}{K_g}\right)^{-v}} \quad (6)$$

Here, the nonlinearity parameter v is a positive number and the amount of active Gal3p is an increasing function of the concentration of internal galactose.

To describe the internal galactose concentration (g^*) as a function of the external galactose concentration (g), we used the following equation:

$$g^{*} = \frac{g}{1 + \left(\frac{x_2}{K_2}\right)^{-\mu}} \quad (7)$$

The nonlinearity parameter μ is a positive number and the concentration of internal galactose increases as a function of increasing Gal2p (galactose transporter) and external galactose concentration.

Equations (3-7) describe a cascade of molecular interactions starting at the external galactose and ending at the binding of Gal4p to its target promoter. They determine how the rate of OFF→ON phenotypic switching is regulated by the concentrations of the GAL network proteins (x_i), concentration of external galactose (g), and the other system parameters. We combined these equations together and obtained a single equation describing ρ as a function of system parameters, and protein and sugar concentrations. Inserting this equation for ρ into equation (2) above let us obtain the functional form for $f(x_2, x_3, x_4, x_{80})$. Finally, using this functional form in equations shown in (1), we numerically solved the set of four differential equations for the values of x_2, x_3, x_4, and x_{80}, and then calculated $f(x_2, x_3, x_4, x_{80})$.

Setting model parameters and the fitting procedure

We set the value of the protein dilution rate γ to $0.42\ h^{-1}$ $(\gamma = ln2\ /doubling\ time)$. This parameter value was obtained by using the experimentally-measured doubling time of yeast in the environments used in this study, which is about 100 minutes (Supplementary Fig. 1).

To understand dosage compensation in networks, our previous work[2] used the above modeling approach to predict the activity of the *S. cerevisiae* GAL network (quantified by the same reporter construct, P_{GAL1}-YFP). Using the parameter values listed in Supplementary Table 1, the model was able to predict the activity of the GAL network in a variety of genetic backgrounds, including the wild type background and the dosage-varied backgrounds (constructed by varying the gene copy numbers of *GAL2, GAL3, GAL4,* and *GAL80)*.

For our current work, for all four *S. cerevisiae* regulatory promoters, we used the protein production rates (θ_i) displayed in Supplementary Table 1. We also set the value of λ to the value estimated in our previous work. Regarding the rest of the model parameters (from α to K_g), we used the above parameter values as initial conditions in the fitting procedure described below.

Using our model described above, we first performed a least-squares fit between the model- and experimentally-obtained inducibility profiles of GAL network activity in 5 *S. cerevisiae* strains shown in Fig. 2a of the main text: wild type strain, P_{GAL2}-replaced strain, P_{GAL3}-replaced strain, P_{GAL4}-replaced strain, and P_{GAL80}-replaced strain. For this, we used the MATLAB function "fminsearch" to minimize the cost function obtained by summing (over 7 different galactose induction levels) the squared differences between the model-obtained fraction of ON cells and the experimentally-obtained ones (from 5 inducibility profiles). The model-obtained fraction of ON cells was produced after numerically solving the coupled differential equations described in equation (1) above at t=22 hours to match the experimental induction duration. Supplementary Table 2 lists the parameter values best-fitting our model to the experimental inducibility profiles of the 5 different *S. cerevisiae* strains.

4 out of 5 *S. cerevisiae* strains (and their inducibility profiles) used in the fitting procedure had one GAL regulatory promoter replaced by its *S. paradoxus* counterpart. The protein production rate (θ) for the replacing *S. paradoxus* regulatory promoter was obtained as a result of the fitting procedure. In other words, we used a fit parameter for the θ of the *S. paradoxus* promoter (Supplementary Tables 3, 4). For all *S. cerevisiae* GAL promoters, we fixed the value of θ based on the estimation we made in our previous work[2].

The fitted curves are shown in Fig. 2a of the main text (columns 1 and 2, showing 5 strains). In addition to these strains, Fig. 2a also depicts (columns 3 to 5) the remaining 11 strains and their experimentally-obtained inducibility profiles across 7 different galactose concentrations. To validate the parameter values used in our model and to test the predictive power of the model, we ran our model to predict the remaining 11 experimental inducibility profiles. We compared the model predictions to our experimental data and saw the agreement between the two (Fig. 2a, columns 3-5). In these predictions, we did not use any free/fit parameters. Supplementary Fig.

14 provides a quantitative perspective on the predictive power of the model by quantifying the residuals.

Measurement of Phenotypic Switching Rates

We used the following equations to determine the cellular switching (transition) rates between the OFF and ON state of the bimodal GAL network. This model consists of two differential equations which describe the dynamics of the numbers of cells in the ON (N_{ON}) and OFF (N_{OFF}) state:

$$\begin{cases} \frac{dN_{ON}}{dt} = \gamma N_{ON} - r_{OFF}N_{ON} + r_{ON}N_{OFF} \\ \frac{dN_{OFF}}{dt} = \gamma N_{OFF} + r_{OFF}N_{ON} - r_{ON}N_{OFF} \end{cases} \quad (8)$$

The parameter γ characterizes the growth rate of the cells, and r_{ON} (r_{OFF}) denotes the OFF→ON (ON→OFF) switching rates. Summing the equations in equation (8) and rearranging, the growth rate γ is obtained as:

$$\gamma = \frac{\left(\frac{dN_{ON}}{dt} + \frac{dN_{OFF}}{dt}\right)}{N_{ON} + N_{OFF}} \quad (9)$$

For a bimodal distribution, the fraction of ON cells (f_{ON}) can be written as the ratio of the number of ON cells to the total number of cells:

$$f_{ON} = \frac{N_{ON}}{N_{ON} + N_{OFF}} \quad (10)$$

Differentiating both sides of this equation with respect to time, we obtain:

$$\begin{aligned} \frac{df_{ON}}{dt} &= \frac{1}{N_{ON} + N_{OFF}}\left(\frac{dN_{ON}}{dt} - \frac{\left(\frac{dN_{ON}}{dt} + \frac{dN_{OFF}}{dt}\right)}{N_{ON} + N_{OFF}}N_{ON}\right) \\ &= \frac{1}{N_{ON} + N_{OFF}}\left(\frac{dN_{ON}}{dt} - \gamma N_{ON}\right) \\ &= \frac{1}{N_{ON} + N_{OFF}}(-r_{OFF}N_{ON} + r_{ON}N_{OFF}) = -(r_{ON} + r_{OFF})f_{ON} + r_{ON} \end{aligned} \quad (11)$$

The analytical solution of equation 11 is given by the following expression:

$$f_{ON}(t) = \frac{r_{ON}}{r_{ON} + r_{OFF}} + \left(f_{ON}(t=0) - \frac{r_{ON}}{r_{ON} + r_{OFF}}\right) e^{-(r_{ON} + r_{OFF})t} \quad (12)$$

To measure the values of r_{ON} and r_{OFF}, we experimentally measured $f_{ON}(t=0)$ and $f_{ON}(t = 22hr)$. For this, we grew each strain overnight (22 hours) separately in minimal media containing [0.1% mannose] or [0.1% mannose and 0.35% galactose]. At the end of the overnight growth, due to the absence or presence of galactose, the FACS-measured expression profiles gave us different $f_{ON}(t=0)$ fractions, where $t=0$ indicates the beginning of the $22hr$ induction period following the overnight growth period. After the overnight growth, for each strain grown in two different overnight conditions, we separately grew them in minimal media for an additional $22hr$ and measured $f_{ON}(t = 22hr)$ fractions by using FACS. This "induction" media contained 0.1% mannose and 7 different concentrations of galactose (from 0% to 0.35%). We used the same type of induction media for cells coming from two different overnight conditions. This way, we had 2 initial measurements (after-overnight f_{ON} values: $[f_{ON}(t=0)]_1$ and $[f_{ON}(t = 0)]_2$ below) and 2 final measurements (after-induction f_{ON} values: $[f_{ON}(22hr)]_1$and $[f_{ON}(22hr)]_2$ below). Each of these four f_{ON} values was the average of two independent measurements performed on different days. We used these experimental values in equation (12) and ended up having the following equations:

$$[f_{ON}(22hr)]_1 = \frac{r_{ON}}{r_{ON} + r_{OFF}} + \left([f_{ON}(t=0)]_1 - \frac{r_{ON}}{r_{ON} + r_{OFF}}\right) e^{-(r_{ON} + r_{OFF})(22hr)} \quad (13)$$

$$[f_{ON}(22hr)]_2 = \frac{r_{ON}}{r_{ON} + r_{OFF}} + \left([f_{ON}(t=0)]_2 - \frac{r_{ON}}{r_{ON} + r_{OFF}}\right) e^{-(r_{ON} + r_{OFF})(22hr)} \quad (14)$$

Since the same galactose induction condition was used for cells grown in two different overnight conditions, equations (13-14) share the same r_{ON} and r_{OFF} for each galactose induction condition (which is 7 in total, from 0% to 0.35%). Using MATLAB, we numerically solved these two nonlinear equations and obtained experimentally-measured values for the switching rates r_{ON} and r_{OFF}. Figure 4 and Supplementary Figure 4 show the results from these analyses. To check the effectiveness of this approach in measuring switching rates, we used a third set of overnight growth conditions (Supplementary Fig. 11). We obtained similar switching rates irrespective of the differences in the after-overnight f_{ON} values.

Quantification of average contribution of promoter replacements to network inducibility and galactose sensitivity

In Fig. 3a and Fig. 3b, we quantified and plotted the average contribution of promoter replacements to network inducibility and galactose sensitivity, respectively. In these quantifications, we used the following method.

Separately for each promoter, all genetic backgrounds in which that promoter was replaced

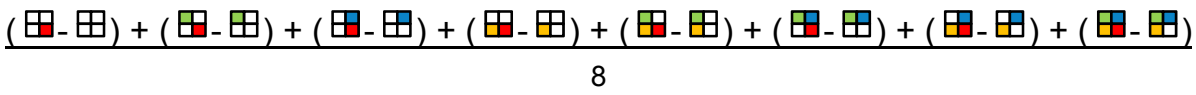
were identified, corresponding to 8 out of 16 strain backgrounds. For example, to quantify the average contribution of P_{GAL80} replacement on network inducibility, we identified the 8 genetic backgrounds in which P_{GAL80} was replaced, which were:

Here, the genetic background of each haploid *S. cerevisae* strain is specified by a square composed of 4 small squares. The small squares represent the four regulatory promoters replaced (if colored) by their *S. paradoxus* counterparts (blue for P_{GAL3}, red for P_{GAL80}, green for P_{GAL2}, yellow for P_{GAL4}). In other words:

 => *S. cerevisiae* wild type

 => *S. cerevisiae* with all four regulatory promoters replaced from *S. paradoxus.*

At all 7 galactose concentrations used, inducibility values of the strains that carry the *S. cerevisae* version of P_{GAL80} promotor were subtracted from the inducibility values of the strains carrying the *S. paradoxus* version of P_{GAL80} in an otherwise identical genetic background and these differences were averaged:



The result of this averaging process (and its SEM as error) was plotted in Fig. 3a of the main text. We used this metric separately for each regulatory promoter and obtained the results depicted in the same figure.

The results depicted in Fig. 3b was also obtained by using this metric. More specifically, the metric used galactose sensitivity values (Fig. 2c) as its entries and quantified the average contribution of different promoter replacements to galactose sensitivity.

Promoter sequence alignments for the GAL network regulatory promoters from *S. cerevisiae* and *S. paradoxus*

EMBOSS Needle Pairwise Sequence Alignment (NUCLEOTIDE) tool was used. The tool is provided online by EMBL-EBI.

Each red box shows the consensus sequence (5'-CGG-N11-CCG-3') for Gal4 protein binding on the promoter.

P_{GAL80} (*S. cerevisiae*) and P_{GAL80} (*S. paradoxus*) promoter sequence alignment:

Similarity: 88.5%

```
PGAL80(S.cer)      1 atggcgcaagttttccgctttgtaatatatatttatacccctttcttctc     50
                     |.||..|||...|.||||||.||||||.||||||||||||.||||||.|||||||
PGAL80(S.par)      1 aggggccaaagctcccgctctgtaaaatatatttatatcccttccttctc     50

PGAL80(S.cer)     51 tcccctgcaatataatagtttaattctaatattaataatatcctatattt    100
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||||
PGAL80(S.par)     51 tcccctgcaatataatagtttaattctaatattaataatatcctatattt    100

PGAL80(S.cer)    101 tcttcatttaccggcgcactctcgcccgaacgacctcaaaatgtctgcta    150
                     ||.|.| ..|||||||||||||||||||||||||||||||..|||||
PGAL80(S.par)    101 tcctta-ccaccggcgcactctcgcccgaacgacctcaaaatgcttgcta    149

PGAL80(S.cer)    151 cattcataataaccaaaagctcataacttttttt----tttgaacctgaa    196
                     ||||||||||||.||||||||.||||||||||||    ||||.|||||||
PGAL80(S.par)    150 cattcataataatcaaaagcttataacttttttttttcctttgtacctgaa    199

PGAL80(S.cer)    197 tatatatacatcacatatcactgctggtccttgccgaccagcgtatacaa    246
                     ||||||||||||.|||.||||||||||||||||||.||||||||||||||
PGAL80(S.par)    200 tatatatacatctcatgtcactgctggtccttgccggccagcgtatacaa    249

PGAL80(S.cer)    247 tctcgatagttgg-tttcccgttctttccactcccgtc    283
                     .||||||||.||| ||||||||||||.|||||||.|||
PGAL80(S.par)    250 cctcgatagctggttttcccgttcttcccactcctgtc    287
```

P_{GAL3} (*S. cerevisiae*) and P_{GAL3} (*S. paradoxus*) promoter sequence alignment:

Similarity: 62.7%

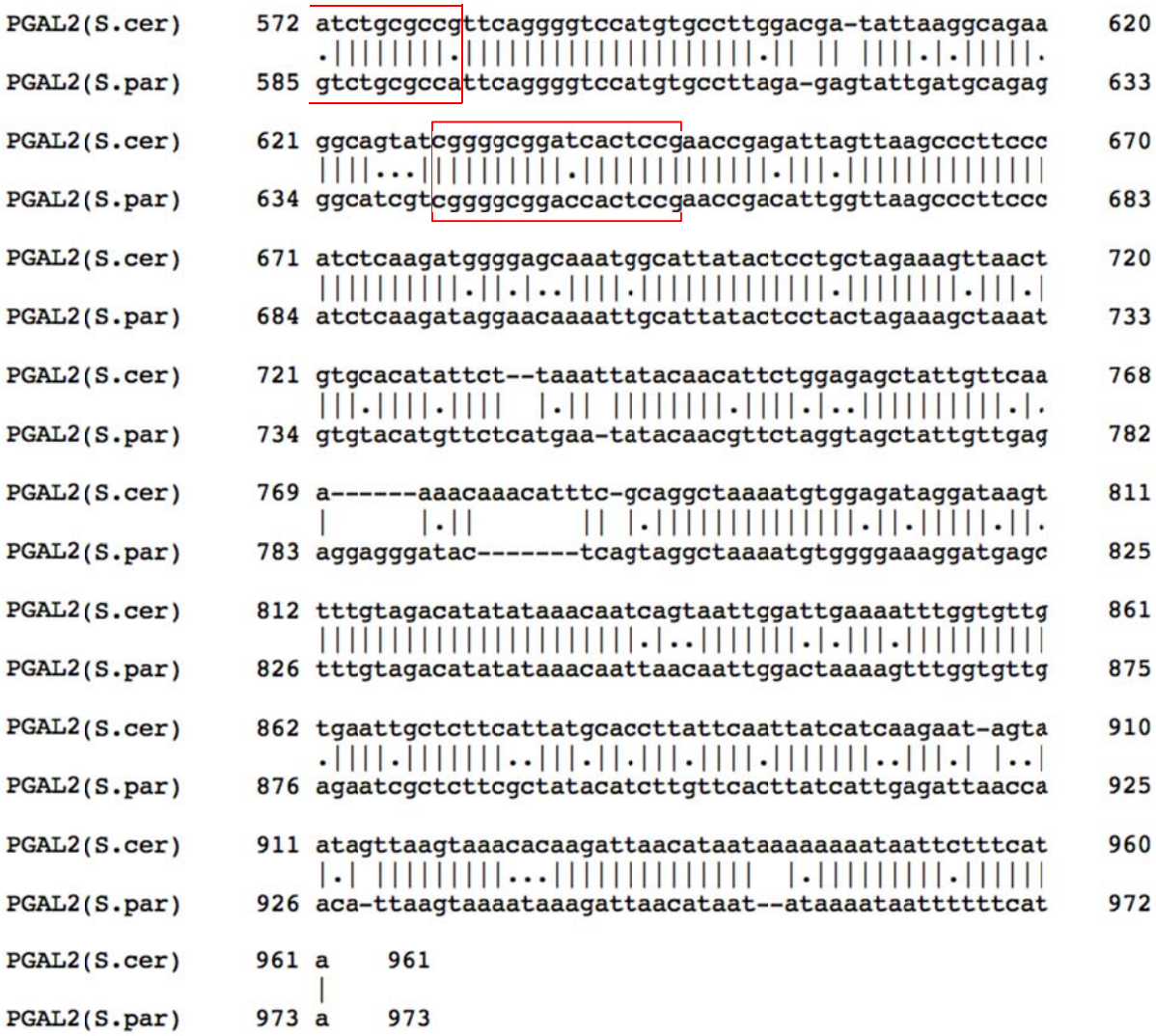
```
PGAL3(S.cer)       1 gttattactgagtagtatttatttaagtattgtttgtgcacttgcctgca     50
                     |||.|..||...||.||||||||||           |..|||||..||||
PGAL3(S.par)       1 gttgtcgctttataatatttattta-----------tctacttgggtgca     39

PGAL3(S.cer)      51 ggccttttgaaaagcaagcataaaagatctaaacataaaa-tctgtaaaa     99
                     |||||||  .|||.||||||...||||..||||.|||||| ||      |
PGAL3(S.par)      40 ggccttt--caaaacaagcagggaagacgtaaatataaaattc------a     81

PGAL3(S.cer)     100 taacaagatgtaaagataatgctaaatcatttggctttttgattgattgt    149
                     |||.||||  .|||.|||||
PGAL3(S.par)      82 taaaaaga--aaaaaataat----------------------------     99

PGAL3(S.cer)     150 acaggaaaatatacatcgcaggggttgacttttaccatttcaccgcaat    199

PGAL3(S.par)     100 --------------------------------------------------     99

PGAL3(S.cer)     200 ggaatcaaacttgttgaagagaatgttcacaggcgcatacgctacaatga    249
                                 ||||.|||..|
PGAL3(S.par)     100 ----------------aagaaaatcct----------------------    110

PGAL3(S.cer)     250 cccgattcttgctagccttttctcggtcttgcaaacaaccgccggcagct    299
                               ||||||.||.|||||.|.|.||||.|||.|.|...|.|.
PGAL3(S.par)     111 -----------ctagccgttcctcggccatacaaataactgtcaataacc    149

PGAL3(S.cer)     300 tagtatataaatacacatgtacatacctctctccgtatcctcgtaatcat    349
                     ||.|.||.|||||.|.||.|.|||||||.|||||  ||.|||..||
PGAL3(S.par)     150 taatgtacaaatataaatattcatacctttctcc--atactccaaa----    193

PGAL3(S.cer)     350 tttcttgtatttatcgtcttttcg-ctgtaaaaactttatcacacttat-    397
                               |||.|||||||| |...||||||.|||.|||   |||
PGAL3(S.par)     194 ------------atcatcttttcgccacaaaaaaccttaccac---tata    228

PGAL3(S.cer)     398 -ctcaaatacacttattaaccgcttttactattatcttctacgctgacag    446
                      |||||||||.||.|.|..||.||||.||||||..|||||||.||..|.|
PGAL3(S.par)     229 cctcaaatacgctcactgcccactttaactattgccttctacactagccg    278

PGAL3(S.cer)     447 taatatcaaacag-----tgacacatattaaacacagtggtttctttgca    491
                     ||||||||||||     |||||||..|||.|..||.|.||||.|||.|.
PGAL3(S.par)     279 taatatcaaacaggatactgacacaagttagatgcaattgtttatttccc    328

PGAL3(S.cer)     492 taa---aca-ccatcagcctcaagtcgtcaagt-aaagatttcgtg-ttc    535
                     |||   .|| ||||   ||||||.||.|||||| |||| ||||||| |||
PGAL3(S.par)     329 taatgtgcagccat---cctcaactcatcaagtgaaag-tttcgtgtttc    374

PGAL3(S.cer)     536 atgcagatagataacaatctatatgttgataattagcgttgcctcatcaa    585
                     ||||||||||.|.|||||.||||.|.||.|||||..||||.|||||||.
PGAL3(S.par)     375 atgcagataggtgacaatatatacgctgctaattcacgttacctcatcag    424
```


PGAL3(S.cer) 586 tgcgagatccgtttaaccggaccctagtgcacttaccccacgttcggtcc 635
PGAL3(S.par) 425 tgctggatccgtttagccggaccctggtgcgcttaccccacgttcggtcc 474
PGAL3(S.cer) 636 actgtgtg-ccgaacatgctccttcactattttaacatgtgga-attctt 683
PGAL3(S.par) 475 acagt-tgaccgaacgtgctccttcaccatttaacatgtggacgttctt 523
PGAL3(S.cer) 684 gaaagaatgaaatcgccatgccaagccatcacacggtcttttatgcaatt 733
PGAL3(S.par) 524 gaaggaatgaaatcgccacattaagcaatcatacaggcttttatgtgatt 573
PGAL3(S.cer) 734 gattgaccgcctgcaacacataggc-agtaaaatttttactgaaacgtat 782
PGAL3(S.par) 574 gattgatcgctagaagggcatatgcaaggaaaattattgctgaatcgtat 623
PGAL3(S.cer) 783 ataatcatcataagcgacaagtgaggcaacacctttgttaccacattgac 832
PGAL3(S.par) 624 ataatcaacataagcgacaagtgaagcgtcgttttgctatcgtattgac 673
PGAL3(S.cer) 833 aaccccaggtattcatacttcctattagcggaatcaggagtgca------ 876
PGAL3(S.par) 674 -atcccagatattcatacttcctattgacagaatcaggagtgcaaggaag 722
PGAL3(S.cer) 877 --aaaagagaaaataaaagtaaaaaggtagggcaacacatagt 917
PGAL3(S.par) 723 ggaaaacagaaagtaaa---aaaaag--agggcacaacatagt 760

P_{GAL2} (*S. cerevisiae*) and P_{GAL2} (*S. paradoxus*) promoter sequence alignment:

Similarity: 77.6% Note: The third Gal4p binding site is partially conserved.

```
PGAL2(S.cer)      1 gctcttgtgtc--tttgtta-tatgggtacaagatataagaaacataact     47
                    .||||||||||  |||||||  |||  ||||||||.|||||||||.||||.|
PGAL2(S.par)      1 actcttgtgtcttttgttattat-ggtacaaggtataagaaatataatt     49

PGAL2(S.cer)     48 attacatac---gaaaat----gtgcatgttatctatatccttctttata     90
                    ||||||.||   .||.||    ||||||  .|||||||.|.|.|||.|.|.
PGAL2(S.par)     50 attacacacatataacatatacgtgcat-atatctattttcgtctgtgtc     98

PGAL2(S.cer)     91 tagatgctgttaacttctttttttttttttgggaaaatcaactgttaaa-     139
                    .||||.||.||||  .||.||||||||.||||       |||.||..||
PGAL2(S.par)     99 cagatacttttaa--actatttttttcttgg-------aacggtataac     139

PGAL2(S.cer)    140 ----cgcgacagtaaaagcagcaaaacattaattttgcttccaagacgac     185
                        |||.||.|||||||||.|||||.||.|||||||||||||||....|
PGAL2(S.par)    140 aaggcgcaacggtaaaagcaacaaaatataaattttgcttccaagtttgc     189

PGAL2(S.cer)    186 agtaatatgtctcctacaataccagtttcgctg--cagaaggcacatcta     233
                    ||||||||.||...||||||||.|||||...||  ||||||||||||.||
PGAL2(S.par)    190 agtaatatatcctgtacaatacaagttttttgtacagaaggcacatgta     239

PGAL2(S.cer)    234 ttacatttactgagcataa---cgggctgtactaatccaaggagg-ttta     279
                    ..|||..||.|||||||||   .||||.||.|||||..||||||| ||||
PGAL2(S.par)    240 acacaactattgagcataaacttgggccgtgctaatttaaggaggtttta     289

PGAL2(S.cer)    280 cggaccaggggaactttccagattcagatcacagcaatataggactagaa     329
                    ||||||||.|||.||||||||||.||||||||.|.||||||||.|.|||.
PGAL2(S.par)    290 cggaccagaggagctttccagatccagatcaccggaatatagggcaagag     339

PGAL2(S.cer)    330 aatatca---ggtagccgcactcaacttgtaactggcaactactttgcat     376
                    ||.||||   .|.||||||||||.|.||.|.|||||||||||||||||||
PGAL2(S.par)    340 aacatcaagcagcagccgcactcgagttatcactggcaactactttgcat     389

PGAL2(S.cer)    377 caaactccaattaaatgcggtagaatcttttc--acaaaaggtactcaac     424
                    ||||||||||||.||||.|.||||||||||  ..||||.|.|||.|.|
PGAL2(S.par)    390 caaactccaattatatgcagcagaatcttttcttttaaaacgcacttagc     439

PGAL2(S.cer)    425 gtcaattcggaaagcttccttccggaatggcttaagtaggttgcaatttc     474
                    |||||.|||||..||||||||||||.|||.||||||..||.||||||||||
PGAL2(S.par)    440 gtcaactcggagggcttccttccgggatgacttaaccagattgcaatttc     489

PGAL2(S.cer)    475 tttttc-tattagtagctaaaaatgggtcacgtgatctatattc--gaaa     521
                    ||||.| |.||| .||.|||||||.||||||||||||||.|.||  ||||
PGAL2(S.par)    490 ttttccatttta-cagttaaaaataggtcacgtgatctaaaatcgagaaa     538

PGAL2(S.cer)    522 ggggcggttgcctcaggaaggcaccggcggtctttcgtccgtgcggagat     571
                    |    ||||||||||.|||||||||||.|||..||.|||||||||||||.
PGAL2(S.par)    539 g----ggttgcctcatgaaggcaccggccgtcactcatccgtgcggagag     584
```


PGAL2(S.cer) 572 atctgcgccgttcaggggtccatgtgccttggacga-tattaaggcagaa 620
PGAL2(S.par) 585 gtctgcgccattcaggggtccatgtgccttaga-gagtattgatgcagag 633
PGAL2(S.cer) 621 ggcagtatcggggcggatcactccgaaccgagattagttaagcccttccc 670
PGAL2(S.par) 634 ggcatcgtcggggcggaccactccgaaccgacattggttaagcccttccc 683
PGAL2(S.cer) 671 atctcaagatggggagcaaatggcattatactcctgctagaaagttaact 720
PGAL2(S.par) 684 atctcaagataggaacaaaattgcattatactcctactagaaagctaaat 733
PGAL2(S.cer) 721 gtgcacatattct--taaattatacaacattctggagagctattgttcaa 768
PGAL2(S.par) 734 gtgtacatgttctcatgaa-tatacaacgttctaggtagctattgttgag 782
PGAL2(S.cer) 769 a------aaacaaacatttc-gcaggctaaaatgtggagataggataagt 811
PGAL2(S.par) 783 aggagggatac-------tcagtaggctaaaatgtggggaaaggatgagc 825
PGAL2(S.cer) 812 tttgtagacatatataaacaatcagtaattggattgaaaatttggtgttg 861
PGAL2(S.par) 826 tttgtagacatatataaacaattaacaattggactaaaagtttggtgttg 875
PGAL2(S.cer) 862 tgaattgctcttcattatgcaccttattcaattatcatcaagaat-agta 910
PGAL2(S.par) 876 agaatcgctcttcgctatacatcttgttcacttatcattgagattaacca 925
PGAL2(S.cer) 911 atagttaagtaaacacaagattaacataataaaaaaaataattctttcat 960
PGAL2(S.par) 926 aca-ttaagtaaaataaagattaacataat--ataaaataatttttcat 972
PGAL2(S.cer) 961 a 961
PGAL2(S.par) 973 a 973

P_{GAL4} (*S. cerevisiae*) and P_{GAL4} (*S. paradoxus*) promoter sequence alignment:

Similarity: 79.9%

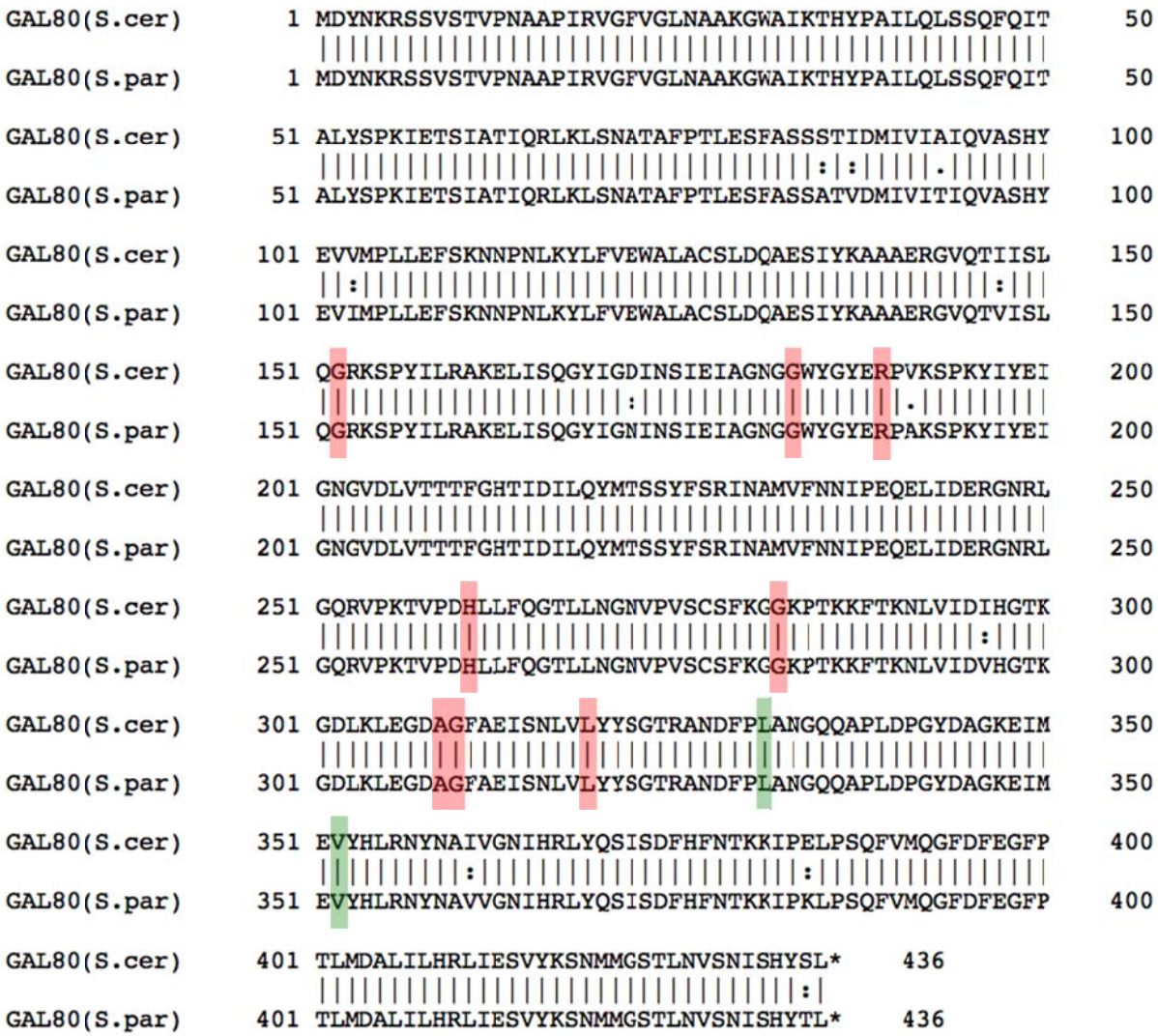
```
PGAL4(sc)          1 Gacagcattcgcccagtattt-tttttattctaca------aaccttcta     43
                     ||||||||||.|   .|.||| |||||.||.||||      |..||||||
PGAL4(sp)          1 gacagcattcac---ttctttctttttttttacatatcttattcttcta     47

PGAL4(sc)         44 taa-tttcaaagtatttacataattctgtatcagtttaatcaccataata     92
                     ||| ||||||.||||||||||.||||||||||||||||||||||||||||
PGAL4(sp)         48 taattttcaacgtatttacatagttctgtatcagtttaatcaccataata     97

PGAL4(sc)         93 tcgttttc-----tttgtttagtgcaattaatttttcctattgttacttc    137
                     |.||||||     ..|..|.|.||||||||..|||||.|||||||.|.||
PGAL4(sp)         98 ttgttttccctcaactaatgaatgcaattagattttcttattgttccctc    147

PGAL4(sc)        138 g-ggcctttttctgttttatgagctatttttttccgtca-tccttccccag    185
                     | |||.|||||.||||||||.|.|||||||||||||||| |.|||||||||
PGAL4(sp)        148 gcggctttttttttgttttataatctatttttttccgtcatttcttccccag    197

PGAL4(sc)        186 attttcagcttcatctccagattgtgtctacgtaatgcacgccatcattt    235
                     ||||.||.|||||||||||||||||||||.||||||||.||.|||||.|
PGAL4(sp)        198 atttccaacttcatctccagattgtgtctatgtaatgcatgctatcatat    247

PGAL4(sc)        236 taagagaggacagagaagcaagcctcctgaaag    268
                     |.|||.|.||.||||||.|||.||||||||||.
PGAL4(sp)        248 tgagaaaagatagagaaacaaccctcctgaaaa    280
```

Amino acid sequence alignments for the GAL network regulatory genes from *S. cerevisiae* and *S. paradoxus*

EMBOSS Needle Pairwise Sequence Alignment (PROTEIN) tool was used. The tool is provided online by EMBL-EBI.

GAL80 gene (*S. cerevisiae*) and GAL80 gene (*S. paradoxus*) amino acid sequence alignment:

Similarity: 99.5%. Red shaded areas show the sites that can prevent *S. cerevisiae* Gal80p-Gal4p binding when mutated[5]. The green shaded areas (amino acids 331 and 352) show the sites that are important for the interaction of Gal80 proteins with Gal3 proteins[5, 6].



GAL3 gene (*S. cerevisiae*) and GAL3 gene (*S. paradoxus*) amino acid sequence alignment:

Similarity: 97.7%

```
GAL3(S.cer)       1 MNTNVPIFSSPVRDLPRSFEQKHLAVVDAFFQTYHVKPDFIARSPGRVNL     50
                    ||||||||||::||.||||||.:||||||:..||||||||||||||||||
GAL3(S.par)       1 MNTNVPIFSSPIKDLSRSFEQKRIAVVDAFYHAYHVKPDFIARSPGRVNL     50

GAL3(S.cer)      51 IGEHIDYCDFSVLPLAIDVDMLCAVKILDEKNPSITLTNADPKFAQRKFD    100
                    ||||||||||||||||||||||||||||||||||||||||.:|||||||
GAL3(S.par)      51 IGEHIDYCDFSVLPLAIDVDMLCAVKILDEKNPSITLTNADARFAQRKFD    100

GAL3(S.cer)     101 LPLDGSYMAIDPSVSEWSNYFKCGLHVAHSYLKKIAPERFNNTPLVGAQI    150
                    ||||||||||||||||||||||||||||||||||||||||||||||||||:
GAL3(S.par)     101 LPLDGSYMAIDPSVSEWSNYFKCGLHVAHSYLKKIAPERFNNTPLVGAQV    150

GAL3(S.cer)     151 FCQSDIPTGGGLSSAFTCAAALATIRANMGKNFDISKKDLTRITAVAEHY    200
                    |||||:||||||||||||.||||||||||||:||||||||||||||||||
GAL3(S.par)     151 FCQSDVPTGGGLSSAFTCAVALATIRANMGKNFNISKKDLTRITAVAEHY    200

GAL3(S.cer)     201 VGVNNGGMDQATSVYGEEDHALYVEFRPKLKATPFKFPQLKNHEISFVIA    250
                    ||||||||||||||||||||||||||||:||||||||||:||.||||||||
GAL3(S.par)     201 VGVNNGGMDQATSVYGEEDHALYVEFRPELKATPFKFPRLKTHEISFVIA    250

GAL3(S.cer)     251 NTLVKSNKFETAPTNYNLRVIEVTVAANALATRYSVALPSHKDNSNSERG    300
                    ||||||||||:||||||||||||||||||||||||||||||||||||||||
GAL3(S.par)     251 NTLVKSNKFESAPTNYNLRVIEVTVAANALATRYSVALPSHKDNSNSERG    300

GAL3(S.cer)     301 NLRDFMDAYYARYENQAQPWNGDIGTGIERLLKMLQLVEESFSRKKSGFT    350
                    ||||||||||||||||||||||||||||||:|||:|||||||.||.|||
GAL3(S.par)     301 NLRDFMDAYYARYENQAQPWNGDIGTGIERLIKMLKLVEESFSGKKIGFT    350

GAL3(S.cer)     351 VHEASTALNCSREEFTRDYLTTFPVRFQVLKLYQRAKHVYSESLRVLKAL    400
                    |.|||:|||||||||||||.||||||||||||||||||||||||:||
GAL3(S.par)     351 VDEASSALNCSREEFTRDYLTIFPVRFQVLKLYQRAKHVYSESLRVLRAL    400

GAL3(S.cer)     401 KMMTSATFHTDEDFFTDFGRLMNESQASCDKLYECSCIETNQICSIALAN    450
                    ||||||||||||||||||||||:||||||||||||||||:||||||||
GAL3(S.par)     401 KMMTSATFHTDEDFFTDFGRLMNDSQASCDKLYECSCIETNKICSIALAN    450

GAL3(S.cer)     451 GSFGSRLTGAGWGGCTIHLVPSGANGNVEQVRKALIEKFYNVRYPDLTDE    500
                    ||||||||||||||:||||||||||||||||||||||||||||||||||
GAL3(S.par)     451 GSFGSRLTGAGWGGCTVHLVPSGANGNVEQVRKALIEKFYNVRYPDLTDE    500

GAL3(S.cer)     501 ELKDAIIVSKPALGTCLYEQ*    521
                    |||||||||:|||||||||.
GAL3(S.par)     501 ELKDAIIVSRPALGTCLYEL*    521
```

GAL2 gene (*S. cerevisiae*) and GAL2 gene (*S. paradoxus*) amino acid sequence alignment:

Similarity: 98.6%

```
GAL2(S.cer)       1 MAVEENNMPVVSQQPQAGEDVISSLSKDSHLSAQSQKYSNDELKAGESGS     50
                    ||||:|||||||||||||.|||.||||||||||||||||||||||||||.
GAL2(S.par)       1 MAVEDNNMPVVSQQPQAAEDVASSLSKDSHLSAQSQKYSNDELKAGESGP     50

GAL2(S.cer)      51 EGSQSVPIEIPKKPMSEYVTVSLLCLCVAFGGFMFGWDTGTISGFVVQTD    100
                    |||:|||||||||.|||||||||||||||||||||||||||||||||||||
GAL2(S.par)      51 EGSRSVPIEIPKKAMSEYVTVSLLCLCVAFGGFMFGWDTGTISGFVVQTD    100

GAL2(S.cer)     101 FLRRFGMKHKDGTHYLSNVRTGLIVAIFNIGCAFGGIILSKGGDMYGRKK    150
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||
GAL2(S.par)     101 FLRRFGMKHKDGTHYLSNVRTGLIVAIFNIGCAFGGIILSKGGDMYGRKK    150

GAL2(S.cer)     151 GLSIVVSVYIVGIIIQIASINKWYQYFIGRIISGLGVGGIAVLCPMLISE    200
                    |||||:.|||||||||||||||||||||||||||||||||||||||||||||
GAL2(S.par)     151 GLSIVIFVYIVGIIIQIASINKWYQYFIGRIISGLGVGGIAVLCPMLISE    200

GAL2(S.cer)     201 IAPKHLRGTLVSCYQLMITAGIFLGYCTNYGTKSYSNSVQWRVPLGLCFA    250
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||
GAL2(S.par)     201 IAPKHLRGTLVSCYQLMITAGIFLGYCTNYGTKSYSNSVQWRVPLGLCFA    250

GAL2(S.cer)     251 WSLFMIGALTLVPESPRYLCEVNKVEDAKRSIAKSNKVSPEDPAVQAELD    300
                    ||||::|||||||||||||||||||||||||||||||||||||||||||||
GAL2(S.par)     251 WSLFLVGALTLVPESPRYLCEVNKVEDAKRSIAKSNKVSPEDPAVQAELD    300

GAL2(S.cer)     301 LIMAGIEAEKLAGNASWGELFSTKTKVFQRLLMGVFVQMFQQLTGNNYFF    350
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||
GAL2(S.par)     301 LIMAGIEAEKLAGNASWGELFSTKTKVFQRLLMGVFVQMFQQLTGNNYFF    350

GAL2(S.cer)     351 YYGTVIFKSVGLDDSFETSIVIGVVNFASTFFSLWTVENLGHRKCLLLGA    400
                    ||||||||||||||||||||||||||||||||||||||||||.|||||||||
GAL2(S.par)     351 YYGTVIFKSVGLDDSFETSIVIGVVNFASTFFSLWTVENLGRRKCLLLGA    400

GAL2(S.cer)     401 ATMMACMVIYASVGVTRLYPHGKSQPSSKGAGNCMIVFTCFYIFCYATTW    450
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||
GAL2(S.par)     401 ATMMACMVIYASVGVTRLYPHGKSQPSSKGAGNCMIVFTCFYIFCYATTW    450

GAL2(S.cer)     451 APVAWVITAESFPLRVKSKCMALASASNWVWGFLIAFFTPFITSAINFYY    500
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||
GAL2(S.par)     451 APVAWVITAESFPLRVKSKCMALASASNWVWGFLIAFFTPFITSAINFYY    500

GAL2(S.cer)     501 GYVFMGCLVAMFFYVFFFVPETKGLSLEEIQELWEEGVLPWKSEGWIPSS    550
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||
GAL2(S.par)     501 GYVFMGCLVAMFFYVFFFVPETKGLSLEEIQELWEEGVLPWKSEGWIPSS    550

GAL2(S.cer)     551 RRGNNYDLEDLQHDDKPWYKAMLE*     575
                    ||||:|||||||||||||||||.
GAL2(S.par)     551 RRGNDYDLEDLQHDDKPWYKAML*-     574
```

GAL4 gene (*S. cerevisiae*) and GAL4 gene (*S. paradoxus*) amino acid sequence alignment:

Similarity: 89.8%. Yellow shaded area (amino acids 1-65) represents the DNA binding domain of the *S. cerevisiae* Gal4 protein[7]. The red shaded area (amino acids 855-870) represents the Gal4 protein's activity domain interacting with the Gal80 proteins[8].

```
GAL4(S.cer)       1 MKLLSSIEQACDICRLKKLKCSKEKPKCAKCLKNNWECRYSPKTKRSPLT    50
                    |||||||||||||||||||||||||||||||||||||||||||||||||||
GAL4(S.par)       1 MKLLSSIEQACDICRLKKLKCSKEKPKCAKCLKNNWECRYSPKTKRSPLT    50

GAL4(S.cer)      51 RAHLTEVESRLERLEQLFLLIFPREDLDMILKMDSLQDIKALLTGLFVQD   100
                    ||||||||.|||||||||||:||.||||||||||.:||.|.|||
GAL4(S.par)      51 RAHLTEVELRLERLEQLFLLIFPRENLDKILKMDSLQDIKVMLTRLLVQD   100

GAL4(S.cer)     101 NVNKDAVTDRLASVETDMPLTLRQHRISATSSSEESSNKGQRQLTVSIDS   150
                    |||||.||..|..||.||||.|.|:.|||.||:||..::.||||||||||
GAL4(S.par)     101 NVNKDTVTVSLVPVENDMPLALGQNGISAKSSAEEDGDEDQRQLTVSIDS   150

GAL4(S.cer)     151 AAHHDNSTIPLDFMPRDALHGFDWSEEDDMSDGLPFLKTDPNNNGFFGDG   200
                    |||||:|||||:||||||||||||||||:.|.|..||.||||||||||
GAL4(S.par)     151 AAHHDDSTIPLNFMPRDALHGFDWSEEDDILDDLSLLKMDPNNNGFFGDG   200

GAL4(S.cer)     201 SLLCILRSIGFKPENYTNSNVNRLPTMITDRYTLASRSTTSRLLQSYLNN   250
                    ||||.||||.:.||||||||:|||||.|||||:|.|||||.|||||||||
GAL4(S.par)     201 SLLCALRSIDYSPENYTNSNINRLPTAITDRYSLTSRSTTFRLLQSYLNN   250

GAL4(S.cer)     251 FHPYCPIVHSPTLMMLYNNQIEIASKDQWQILFNCILAIGAWCIEGESTD   300
                    ||||||||||.||||||||||||||||||||||||||||::|||||
GAL4(S.par)     251 FHPYCPIVHSQTLMMLYNNQIEIASKDQWQILFNCILAIGAWCMDGESTD   300

GAL4(S.cer)     301 IDVFYYQNAKSHLTSKVFESGSIILVTALHLLSRYTQWRQKTNTSYNFHS   350
                    ||:||||||||||||||||||.||.||||||||||||||||||||||||
GAL4(S.par)     301 IDIFYYQNAKSHLTSKVFESGSITLVIALHLLSRYTQWRQKTNTSYNFHS   350

GAL4(S.cer)     351 FSIRMAISLGLNRDLPSSFSDSSILEQRRRIWWSVYSWEIQLSLLYGRSI   400
                    ||||||||||||||||||:||||||||||||||.|||:|:|||||||
GAL4(S.par)     351 FSIRMAISLGLNRDLPSSFNDSSILEQRRRIWWSVYIWEIELALLYGRSI   400

GAL4(S.cer)     401 QLSQNTISFPSSVDDVQRTTTGPTIYHGIIETARLLQVFTKIYELDKTVT   450
                    ||:||||.||||||||||||.||||||.||||||||||:|.|||||.|
GAL4(S.par)     401 QLTQNTIPFPSSVDDVQRTTTCPTIYHGTIETARLLQVFTRICELDKTNT   450

GAL4(S.cer)     451 AEKSPICAKKCLMICNEIEEVSRQAPKFLQMDISTTALTNLLKEHPWLSF   500
                    |||||:.|||||||||||||.||.||||||||||||||||||||||||
GAL4(S.par)     451 AEKSPLSAKKCLMICNEIEEVFRQVPKFLQMDISTTALTNLLKEHPWLSF   500

GAL4(S.cer)     501 TRFELKWKQLSLIIYVLRDFFTNFTQKKSQLEQDQNDHQSYEVKRCSIML   550
                    |||:|||:||||||||||:||.||||:|||| |||||.||||||||||:|
GAL4(S.par)     501 TRFQLKWRQLSLIIYVLREFFKNFTQQKSQL-QDQNDRQSYEVKRCSIIL   549

GAL4(S.cer)     551 SDAAQRTVMSVSSYMDNHNVTPYFAWNCSYYLFNAVLVPIKTLLSNSKSN   600
                    :||||||||||||||||.:|||||||||||||||||||.|:||.||.||.
GAL4(S.par)     550 NDAAQRTVMSVSSYMDNHTITPYFAWNCSYYLFNAVLVHIETLSSNPKSG   599

GAL4(S.cer)     601 AENNETAQLLQQINTVLMLLKKLATFKIQTCEKYIQVLEEVCAPFLLSQC   650
                    |:|::|||.||||:.||||||||||||:||||||||||:||||:|.|.|
GAL4(S.par)     600 ADNDDTAQSLQQISAVLMLLKKLATFKVQTCEKYIQVLEDVCAPYLSSSC   649
```


GAL4(S.cer) 651 AIPLPHISYNNSNGSAIKNIVGSATIAQYPTLPEENVNNISVKYVSPGSV 700
GAL4(S.par) 650 AISSPHISYNHNNGSVIRNIVGPPPVSQYPTLQEDNANNSSVKYVSPGSV 699
GAL4(S.cer) 701 GPSPVPLKSGASFSDLVKLLSNRPPSRNSPVTIPRSTPSHRSVTPFLGQQ 750
GAL4(S.par) 700 GPSPMPLKSGASFSDLVKLLSNRPSSRNSPAAITRSTPPHRSLTPFSGQQ 749
GAL4(S.cer) 751 QQLQSLVPLTPSALFGGANFNQSG-NIADSSLSFTFTNSSNGPNLITTQT 799
GAL4(S.par) 750 QQLQSYVPLTPSALFGGVNSYQSGTNIADSSLSFSFTTNSSGTNLITTPA 799
GAL4(S.cer) 800 NSQALSQPIASSNVHDNFM-NNEITASKIDDGNNSKPLSPGWTDQTAYNA 848
GAL4(S.par) 800 NSQALPQPTATSNLHGNIINNNEITAGRIDD-DNSKPLSPSWTDQTAYNA 848
GAL4(S.cer) 849 FGITTGMFNTTTMDDVYNYLFDDEDTPPNPKKE*---- 882
GAL4(S.par) 849 FGTTTGMFNTTTMDDVYNYLFDDEDTPPNPKKEQNES* 886

Western Blot Experiments

(i) Construction of yeast strains for western blots: *S. cerevisiae* and *S paradoxus* strains carrying Gal2-HA, Gal3-HA, or Gal4-HA were constructed by tagging each gene with 6xHA at C-terminus using one-step PCR transformation as described in literature[9]. P_{GAL80} promoter-swapped *S. paradoxus* strain was constructed by firstly integrating a URA-hphNT1 cassette into wild type *S. paradoxus* strain (MA653) to replace the whole P_{GAL80} promoter, then swapping out this cassette by a PCR product containing the *S. cerevisiae* P_{GAL80} promoter with 200bp flanking sequences on both sides. Colonies were selected on 5-FOA plates and hygromycin plates; colonies that were positive for 5-FOA and negative for hygromycin were selected and verified by DNA sequencing.

(ii) Western Blots: Cells were grown in 10ml minimal media (without adenine) with 0.1% mannose for 22hrs, until OD_{600} reaches 0.08. Cells were then split into two batches (75ml growth volume for each): one batch was cultured in minimal media (without adenine) containing 0.1% mannose and 0.35% galactose; the other batch was cultured in 0.1% mannose minimal media, for a further 22hrs for both batches until OD_{600} reaches 0.08-1. Samples were then harvested for western blotting. Protein samples were extracted using the TCA method. Gal3-HA, Gal4-HA, and Gal80 protein samples were boiled in 2x Laemmli buffer before loading; Gal2-HA protein samples were prepared without boiling due to its high hydrophobicity. Protein lysate were separated on 8.5% Tris Tricine gel. The membrane was imaged using the Fujifilm LAS-4000 imaging system, and protein bands were quantified using Fiji software.

(iii) Antibody: The Gal80 antibody was a kind gift from Dr. Julie Simpson at HHMI's Janelia Research Campus. The epitope is CEQELIDERGNRLGQRV, and it is identical in both *S. paradoxus* and *S. serevisae*. Anti-PGK1 antibody was from Abcam (ab113687), and HA antibody was from Santa Cruz (Y-11). Secondary antibodies were anti-mouse-HRP (ab6728, Abcam) and anti-rabbit-HRP (sc-2004, santa cruz). For the Gal80 and HA antibodies, the dilutions were 1:5,000 and 1:10,000, respectively.

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