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Supplementary Materials for

Synchronization of stochastic expressions drives the clustering of functionally related genes

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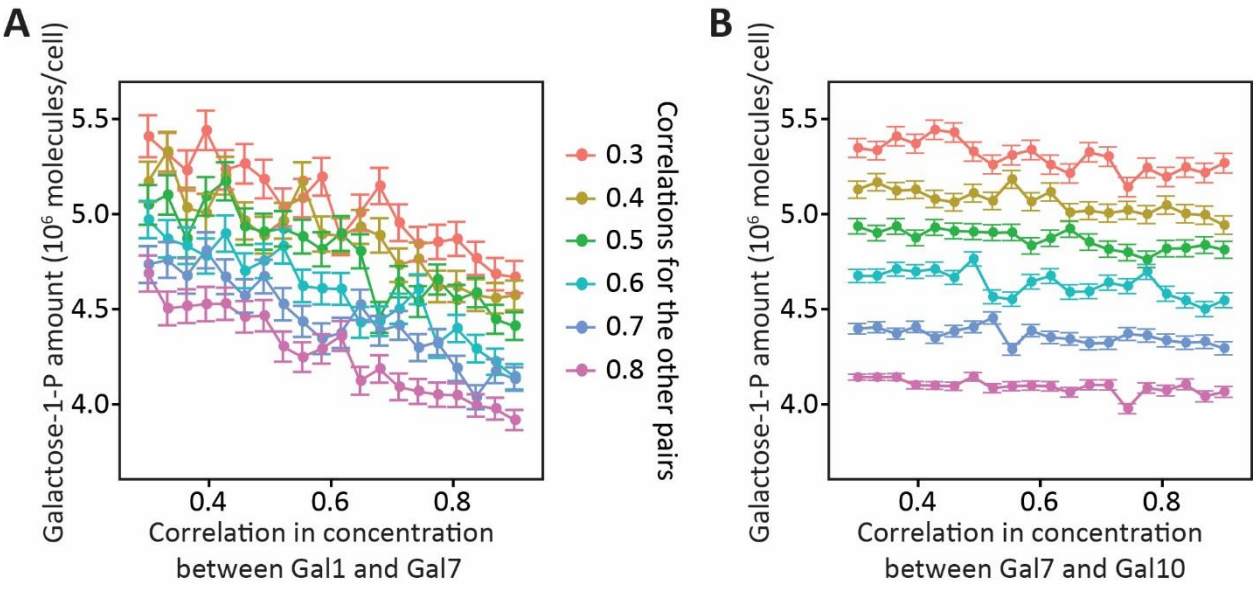

Fig. S1. Simulation of the *GAL* pathway. (**A**) Expected concentrations of galactose-1-P under different levels of correlation between the concentrations of Gal1 and Gal7, determined by non-steady state numerical simulations. The correlation coefficient in concentration between Gal1 and Gal10 and that between Gal7 and Gal10 are fixed at various levels indicated on the right of the figure. Each circle indicates the average from 2,000 simulations. Error bars show SEs. (**B**) Expected concentrations of galactose-1-P under different levels of correlation between the concentrations of Gal7 and Gal10, determined by non-steady state numerical simulations. The correlation coefficient in concentration between Gal1 and Gal10 and that between Gal1 and Gal7 are fixed at various levels indicated on the right of the figure. Each circle indicates the average from 10,000 simulations. Error bars show SEs.

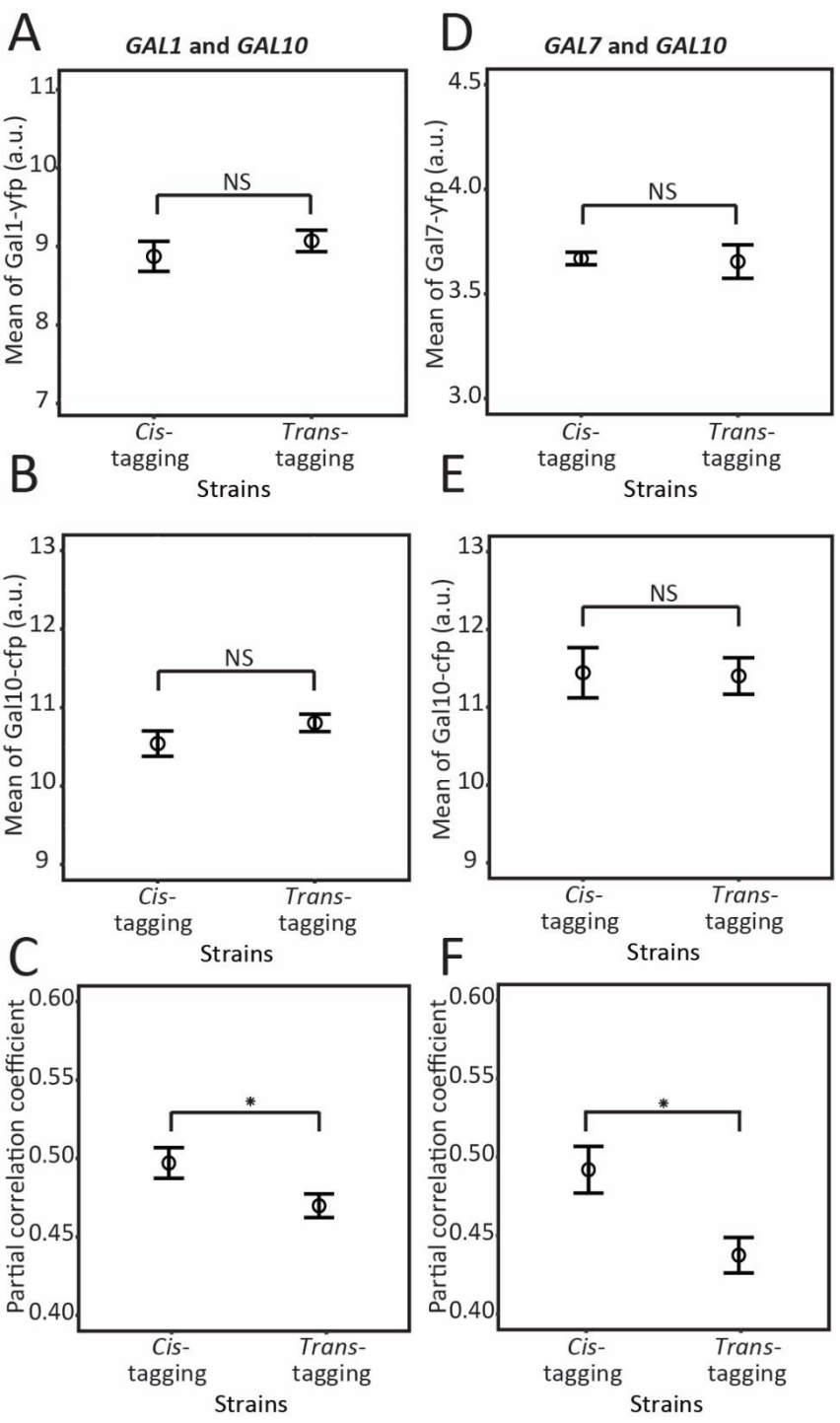
A
GAL1 and GAL10
Mean of Gal1-yfp (a.u.)
11
10
9
8
7
NS
Cis-tagging
Trans-tagging
Strains
D
GAL7 and GAL10
Mean of Gal7-yfp (a.u.)
4.5
4.0
3.5
3.0
NS
Cis-tagging
Trans-tagging
Strains
B
Mean of Gal10-cfp (a.u.)
13
12
11
10
9
NS
Cis-tagging
Trans-tagging
Strains
E
Mean of Gal10-cfp (a.u.)
13
12
11
10
9
NS
Cis-tagging
Trans-tagging
Strains
C
Partial correlation coefficient
0.60
0.55
0.50
0.45
0.40
*
Cis-tagging
Trans-tagging
Strains
F
Partial correlation coefficient
0.60
0.55
0.50
0.45
0.40
*
Cis-tagging
Trans-tagging
Strains

Fig. S2. Comparison of protein expression levels of *GAL* genes between cis- and trans-tagging strains. (**A** to **C**) Study of the pair of *GAL1* and *GAL10* genes. Mean Gal1-yfp (**a**) and mean Gal10-cfp (**B**) protein levels are not significantly different between *cis*- and *trans*-tagging strains, but the partial correlation between Gal1-yfp and Gal10-cfp protein levels among cells upon the control for cell morphology is significantly higher in the *cis*- than *trans*-tagging strains (**C**). (**D** to **F**) Study of the pair of *GAL7* and *GAL10* genes. Mean Gal7-yfp (**D**) and mean Gal10-cfp (**E**) protein levels are not significantly different between the *cis*- and *trans*-tagging strains, but the partial correlation between Gal7-yfp and Gal10-cfp protein levels among cells upon the control for cell morphology is significantly higher in the *cis*- than *trans*-tagging strains (**F**). In all panels, each circle represents the average of eight biological replicates, each with 5000 cells. Error bars show SEs. Significance levels are indicated as follows: NS, $P \geq 0.05$; *, $P < 0.05$.

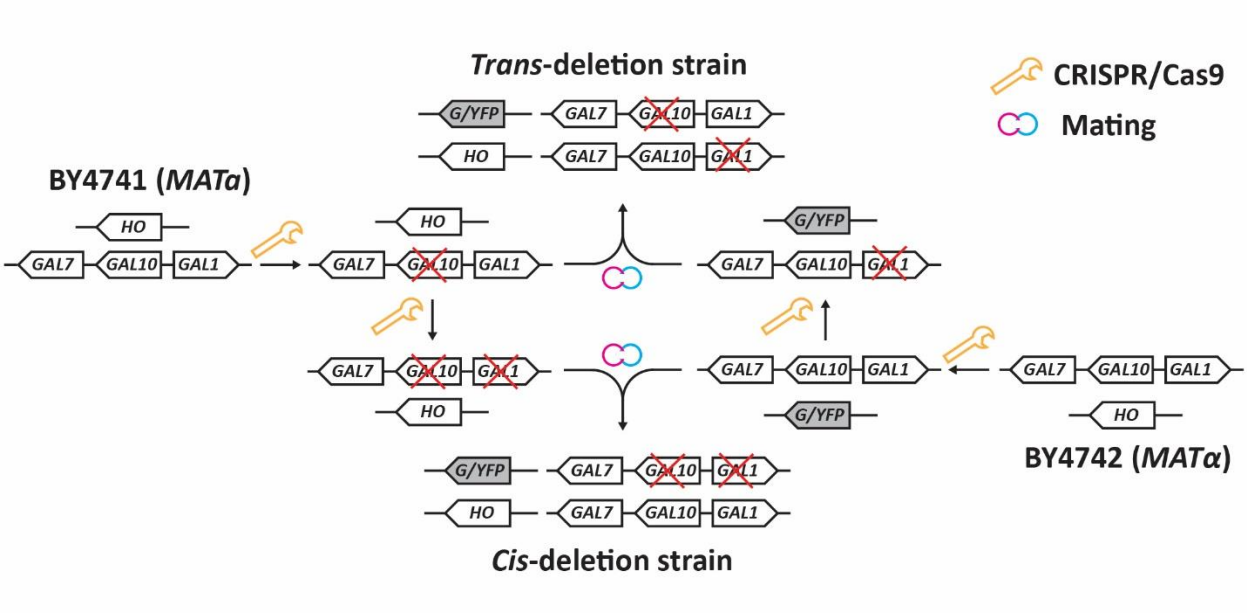

Fig. S3. Flowchart showing the construction of cis- and trans-deletion strains for the pair of *GAL1* and *GAL10* genes.

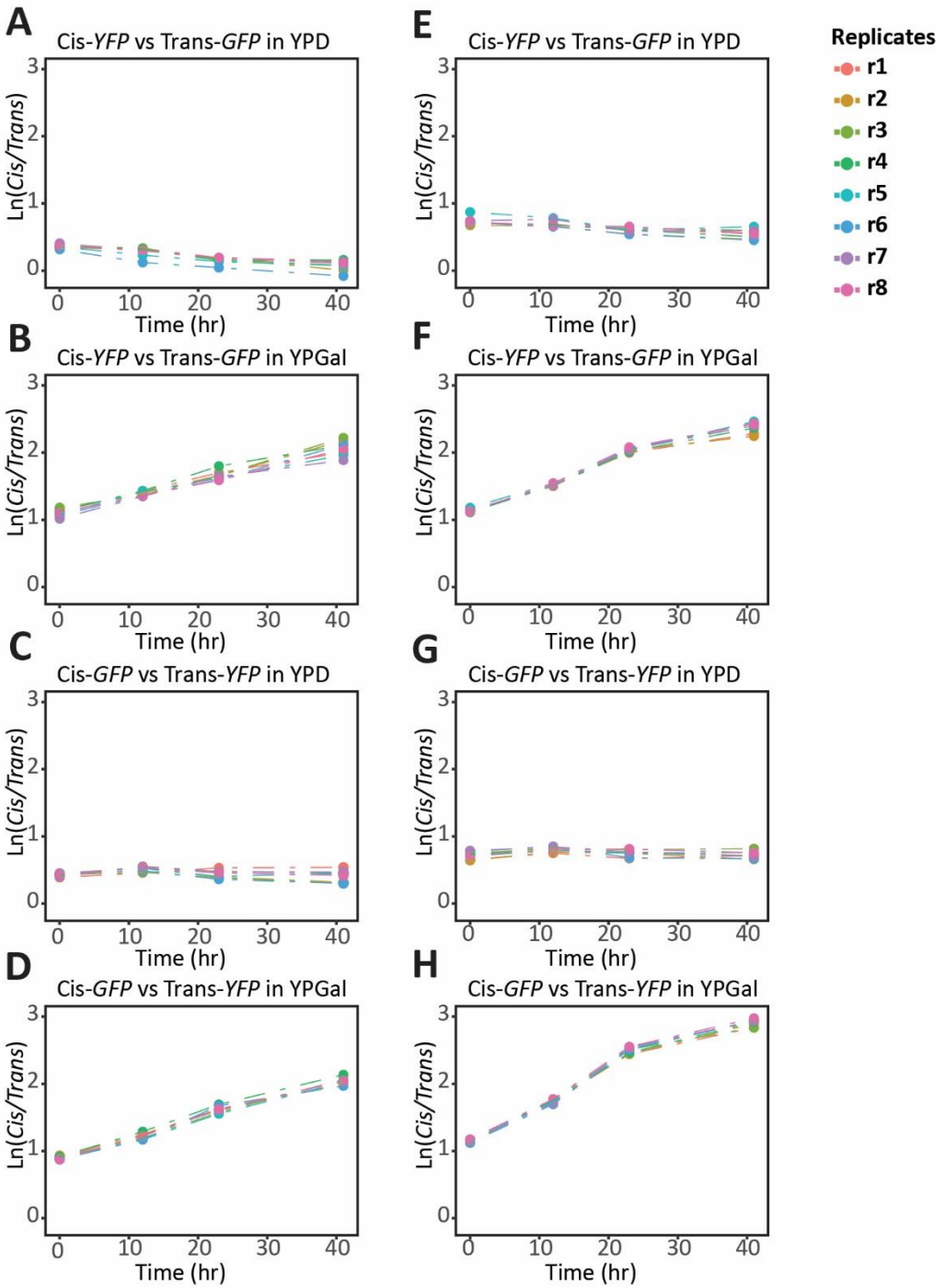

Fig. S4. Ln(cell number ratio between the cis- and trans-deletion strains) as a function of competition time. (**A** to **D**) Competitions between *cis*- and *trans*-deletion strains involving *GAL1* and *GAL10* gene deletions. (**E** to **H**) Competitions between *cis*- and *trans*-deletion strains involving *GAL7* and *GAL10* gene deletions. Note the different competition media used and the variation in the fluorescence marker of each strain.

Table S1. Parameter values used in the simulation of the linear pathway with two reactions.

Parameter	Value	Explanation
$[M_1]$	3.2×10^7 molecule/cell[a]	Based on the intracellular glucose concentration (*46*)
k_{cat1}	702 min^{-1}	From the parameter for Gal1 (*47*)
k_{cat2}	900 min^{-1}	From the simplified parameter for Gal7 (*47*)
K_{m1}	2.3×10^7 molecule/cell[a]	Based on the parameter for Gal1 (*47*)
K_{m2}	8.6×10^7 molecule/cell[a]	Based on the simplified parameter for Gal7 (*47*)

[a] The conversion from mM to number of molecules per cell is done by assuming 1 gram of dry cell has a volume of 2.38 ml (*48*) and a haploid cell weight of 1.5×10^{-11} gram (*49*). This means one haploid cell has a volume of 3.57×10^{-14} L.

Table S2. Parameter values used in the simulation of the *GAL* pathway.

Parameter	Value	Explanation
[Gal]	8×10^6 molecule/cell [a]	Arbitrarily set to enable the simulation to approach equilibrium in a reasonable time
[UGl]	1976024 molecule/cell [a,b]	The intial UDP-glucose concentraion estimated according to a previous study (*50*)
[UGa]	4023976 molecule/cell [a,b]	The intial UDP-galactose concentration estimated according to a previous study (*50*)
k_{cat1}	3350 min^{-1}	k_{cat} of the Gal1 reaction from a previous simulation study (*51*)
k_{cat2}	59200 min^{-1}	k_{cat} of the Gal7 reaction from a previous simulation study (*51*)
k_{cat3}	3890 min^{-1}	k_{cat} of the Gal10 reaction from a previous simulation study (*51*)
K_{m1}	1.29×10^7 molecule/cell [a]	K_m in the Gal1 reaction from a previous simulation study (*51*)
K_{m2}	8.6×10^7 molecule/cell [a]	K_m for G1P in the Gal7 reaction from a previous simulation study (*51*)
K_{m3}	5.6×10^6 molecule/cell [a]	K_m for UGl in the Gal7 reaction from a previous simulation study (*51*)
K_{m4}	4.73×10^6 molecule/cell [a]	K_m for UGa in the Gal10 reaction from a previous simulation study (*51*)
K_{m5}	5.37×10^6 molecule/cell [a]	K_m for UGl in the Gal10 reaction from a previous simulation study (*51*)
K_{eq}	3.5	Equilibrium constant for the reaction in Eq. (5) in Materials and Methods (*51*)

[a] The conversion from mM to number of molecules per cell is done by assuming that 1 gram of dry cell has a volume of 2.38 ml (*48*) and that a haploid cell weights 1.5×10^{-11} gram (*49*). This means one haploid cell has a volume of 3.57×10^{-14} L.

[b] The initial [UGl] and [UGa] are modified so that reactions are at equilibrium when simulation starts

Table S3. Primers for strain construction.

Name	Sequence (5'→3')	Description
xhq3	GATCTGAAAAACGGTGAAACTTACGTTTTAGAGCTAG	For *GAL10* gRNA hybridization
xhq4	CTAGCTCTAAAACGTAAGTTTCACCGTTTTTCA	For *GAL10* gRNA hybridization
xhq5	GATCTATAGACAGCTGCCCAATGCGTTTTAGAGCTAG	For *GAL1* gRNA hybridization
xhq6	CTAGCTCTAAAACGCATTGGGCAGCTGTCTATA	For *GAL1* gRNA hybridization
xhq7	ATAGAAAAAATATGATATGAATGAATATTCCACTTTCTTTTTTTGAGGGA	For *GAL7* Cas9 deletion's donors hybridization
xhq8	TCAACATGATAAAAAAAAACAGTTGAATATTCCCTCAAAAAAAGAAAGTG	For *GAL7* Cas9 deletion's donors hybridization
xhq9	ATAGAGTGCATATTTTCAAGAAGGATAGTAAGCTGGCAAATTATATTGAA	For *GAL10* Cas9 deletion's donors hybridization
xhq10	ATCCAAAAAAAAAAGTAAGAATTTTTGAAAATTCAATATAATTTGCCAGCT	For *GAL10* Cas9 deletion's donors hybridization
xhq11	ATATACCTCTATACTTTAACGTCAAGGAGAAAAAACTATAGTATACTTC	For *GAL1* Cas9 deletion's donors hybridization
xhq12	TGAGAAGTTGTTCTGAACAAAGTAAAAAAAAGAAGTATACTATAGTTTTTTCTCCTTG	For *GAL1* Cas9 deletion's donors hybridization
xhq13	TAGGTGCAGGATTTCCATCG	For the confirmation of Cas9 deletion of *GAL7*
xhq14	TATACTTCGGAGCACTGTTGAG	For the confirmation of Cas9 deletion of *GAL7*
xhq15	TGCTCCGAAGTATAGCTTTCC	For the confirmation of Cas9 deletion of *GAL10*
xhq16	AGGAGAGTCTTCCGTCGGAG	For the confirmation of Cas9 deletion of *GAL10*
xhq17	GTTCCTGAAACGCAGATGTG	For the confirmation of Cas9 deletion of *GAL1*
xhq18	GCAGCGGTTGAAAGCATATC	For the confirmation of Cas9 deletion of *GAL1*

xhq19	TATTAGGTGTGAAACCACGAAAAGT	For the confirmation of replacing *HO* by *YFP* or *GFP*
xhq20	CATGTCTTCTCGTTAAGACTGCAT	For the confirmation of replacing *HO* by *YFP* or *GFP*
xhq21	ACTTTTATTACATACAACTTTTTAAACTAATATACACA TT	For constructing donors for replacing *HO* by *YFP* or *GFP*
xhq22	CTCATAAGCAGCAATCAATTCTATCTATACTTTAAAAA GTGTAAACGCGAAGCTTGCAAA	For constructing donors for replacing *HO* by *YFP* or *GFP*
xhq23	GATCGCCGGCTTGATCGACTCAGAGTTTTAGAGCTAG	For *HO* gRNA of replacing *HO* by *YFP* or *GFP* hybridization
xhq24	CTAGCTCTAAAACTCTGAGTCGATCAAGCCGGC	For *HO* gRNA of replacing *HO* by *YFP* or *GFP* hybridization
xhq27	GATCTTGGTTGGTTTTGAATTGTTGTTTTAGAGCTAG	For *GAL7* gRNA hybridization
xhq28	CTAGCTCTAAAACAACAATTCAAAACCAACCAA	For *GAL7* gRNA hybridization
xhq63	TGTTCCATACTGGGCCATCTGG	For the confirmation of *GAL7-FP* fusion
xhq64	GTCACTCCGTTCAAGTCGACAACC	For the confirmation of *GAL7-FP* fusion
xhq69	CGGTGATTTCTTGTCTGCTGG	For the confirmation of *GAL10-FP* fusion
xhq72	GCCCTTGCCAATGAGTTCTAC	For the confirmation of *GAL1-FP* fusion
xhq90	TAATAGAAAAAATATGATATGAATGAATATTCCACTTT CTTTAGCTTGCCTCGTCCCCGC	For donors of *GAL7-YFP-KanMX* fusion
xhq92	AATGAGAAGTTGTTCTGAACAAAGTAAAAAAAAGAAG TATACAGCTTGCCTCGTCCCCGC	For donors of *GAL1-YFP-KanMX* fusion
xhq107	AGAGTGCATATTTTCAAGAAGGATAGTAAGCTGGCAA A AGCTTGCCTCGTCCCCGC	For donors of *GAL10-CFP-NatMX* fusion with linker
xhq120	TAAACCAGCATTGGGCAGCTGTCTATATGAATTA GGTGACGGTGCTGGTTTAATTAAC TCTAAAGGTGAAGAATTATTCACTGG	For donors of *GAL1-YFP-KanMX* fusion with linker
xhq121	TTTAGATGGTCAGATTCATTATCTACAAAGACTG GGTGACGGTGCTGGTTTAATTAAC TCTAAAGGTGAAGAATTATTCACTGG	For donors of *GAL7 YFP-KanMX* fusion with linker
xhq123	CGGTGAAACTTACG GGTCCAAGATTGTCTACAGATTTTCC GGTCGACGGATCCCCGGG	For donors of *GAL10 CFP-NatMX* fusion with linker

xhq126	GATCGTACGGGGACGACGGCGACCGTTTTAGAGCTAG	For constructing gRNA for knocking out *NatMX* coding gene
xhq127	CTAGCTCTAAAACGGTCGCCGTCGTCCCCGTAC	For constructing gRNA for knocking out *NatMX* coding gene
xhq130	ACTAAAAGATATAGAGTGCATATTTTCAAGAAGGATA GTA AGCTGGCAAACTATTTGTAC	For constructing donors for deleting *NatMX* gene
xhq131	TTGTTACTGCTGCTGGTATTACCCTAGGTATGGATGAA TT GTACAAATAGTTTGCCAGCT	For constructing donors for deleting *NatMX* gene
xhq134	TGACGGTGCTGGTTTAATTAAC	Primer on *YFP* fusion linker
xhq135	GATCCCCGGGTTAATTAACAGT	Primer on *CFP* fusion linker
xhq137	GTACGGGGACGACGGCGACC	For checking guide RNA construction of *NatMX*

Table S4. gRNA target sequences.

Guide RNA target	Target sequence
GAL10	TGAAAAACGGTGAAACTTAC
GAL1	TATAGACAGCTGCCCAATGC
GAL7	GCCGGCTTGATCGACTCAGA
HO	TTGGTTGGTTTTGAATTGTT
NatMX	GTACGGGGACGACGGCGACC

Table S5. Doubling times for various trans-deletion strains.

Strain name	Medium	Doubling time (hr)[1]
4743 Trans *gal10*Δ and *gal1*Δ *HO*Δ*::GFP*	YPD	1.102 ± 0.016
4743 Trans *gal10*Δ and *gal1*Δ *HO*Δ*::GFP*	YPGal	1.458 ± 0.016
4743 Trans *gal10*Δ and *gal1*Δ *HO*Δ*::YFP*	YPD	1.087 ± 0.039
4743 Trans *gal10*Δ and *gal1*Δ *HO*Δ*::YFP*	YPGal	1.461 ± 0.013
4743 Trans *gal10*Δ and *gal7*Δ *HO*Δ*::GFP*	YPD	0.919 ± 0.135
4743 Trans *gal10*Δ and *gal7*Δ *HO*Δ*::GFP*	YPGal	1.616 ± 0.045
4743 Trans *gal10*Δ and *gal7*Δ *HO*Δ*::YFP*	YPD	1.000 ± 0.100
4743 Trans *gal10*Δ and *gal7*Δ *HO*Δ*::YFP*	YPGal	1.607 ± 0.027

[1]Mean ± standard error estimated from five replicates.