Cell Reports

Supplemental Information

[*SWI*$^{+}$] abolishes yeast multicellular growth by triggering conformational changes of multiple regulators required for flocculin gene expression

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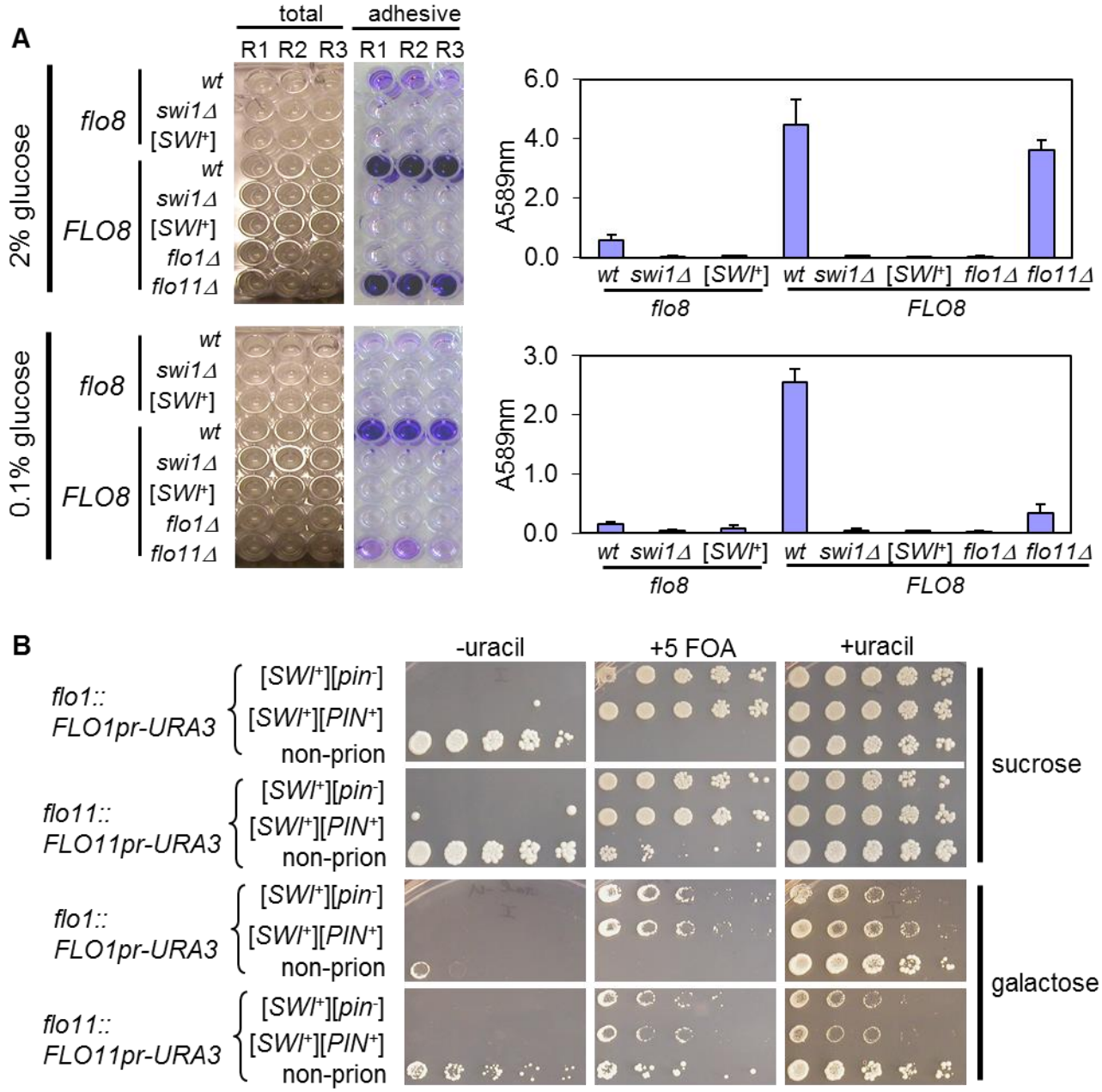

Figure S1. Related to Figure 1 and Figure 2D. Plastic surface adhesion and *FLO* gene expression are impaired similarly in *swi1*Δ or [*SWI*$^+$] cells. (A) Related to Figure 1. The indicated strains were grown in YPD medium containing either 2% or 0.1% of glucose for 24 h in micro-titer plates before washing and staining with crystal blue as described in the Supplemental Experimental Procedures. The left panels are representative images from independent culture (R1-R3),and the right are a summative data (Error bars represent standard deviation). **(B)** Related to Figure 2D. Transcriptional defect of *FLO genes* is independent of carbon source and [*PIN*$^+$]. The indicated strains were spotted onto SC plates with (+uracil) or without (-uracil) uracil, or on SC plates supplemented with 5 FOA (+5FOA), with 2% sucrose or galactose as sole carbon source. Shown are representative results of multiple times of repeated assays.

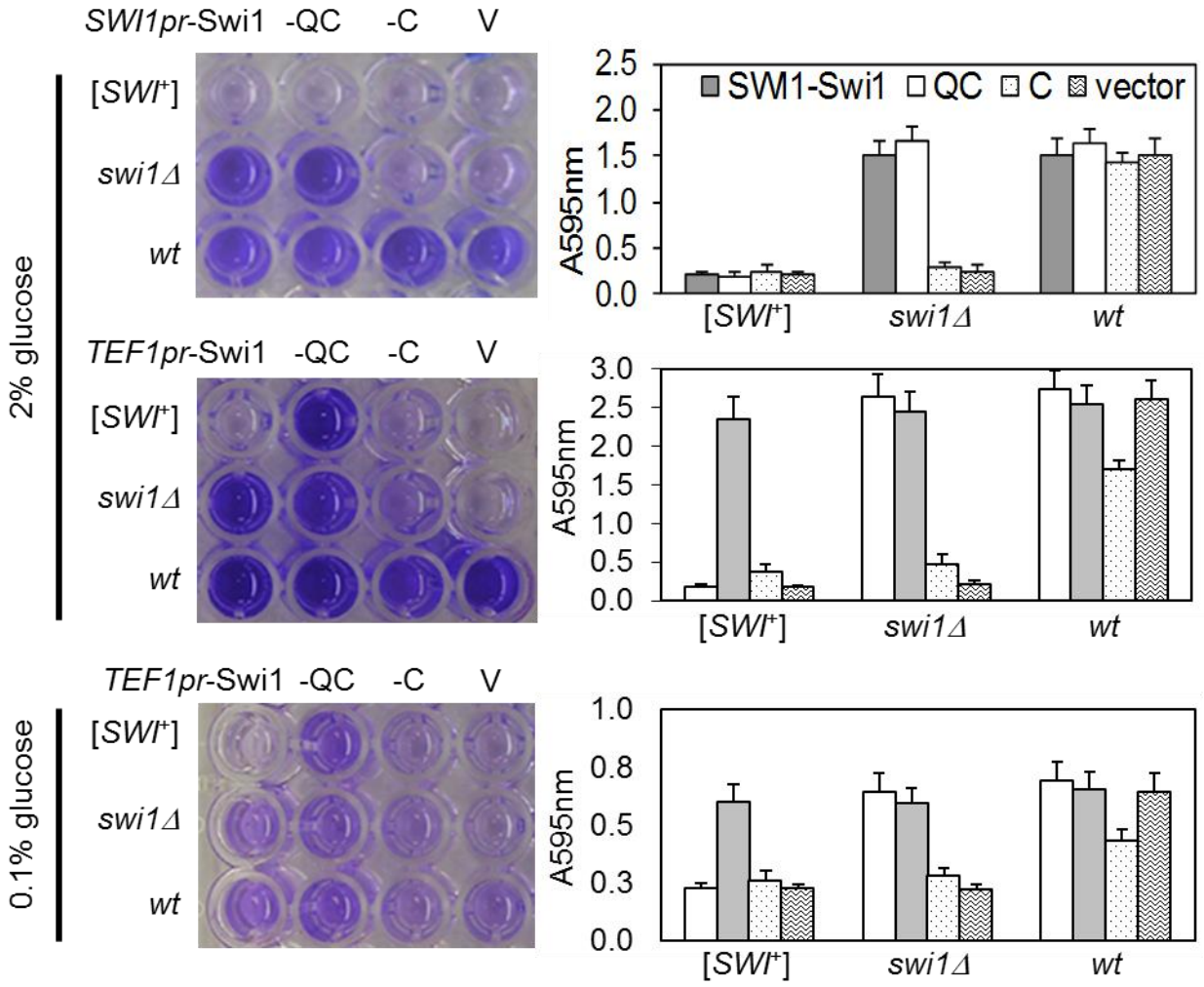

Figure S2. Related to Figure 3C. Effect of Swi1 and its functional domains on plastic surface adhesion of [*SWI*$^+$] cells. Strains with the indicated genetic backgrounds were repaired for *FLO8*, and transformed with a plasmid carrying either a *SWI1* promoter (*SWI1pr*)- or *TEF1* promoter (*TEF1pr*)-driven ORF encoding the full-length Swi1 (Swi1), or a truncated Swi1 functional domain with (QC) or without (C) the Q-rich region. V, vector control. Overnight cultures were properly diluted and grown in selective SC medium containing either 2% or 0.1% of glucose for 24 h in micro-titer plates before washing and staining with crystal blue as described in Supplemental Experimental Procedures. The left panels are representative images of three independent experiments, and the right panels are a summary of quantitative assays of the three experiments. The error bars represent standard deviation. Notably, even though similar patterns were observed, adhesion on plastic surface favors a higher glucose concentration.

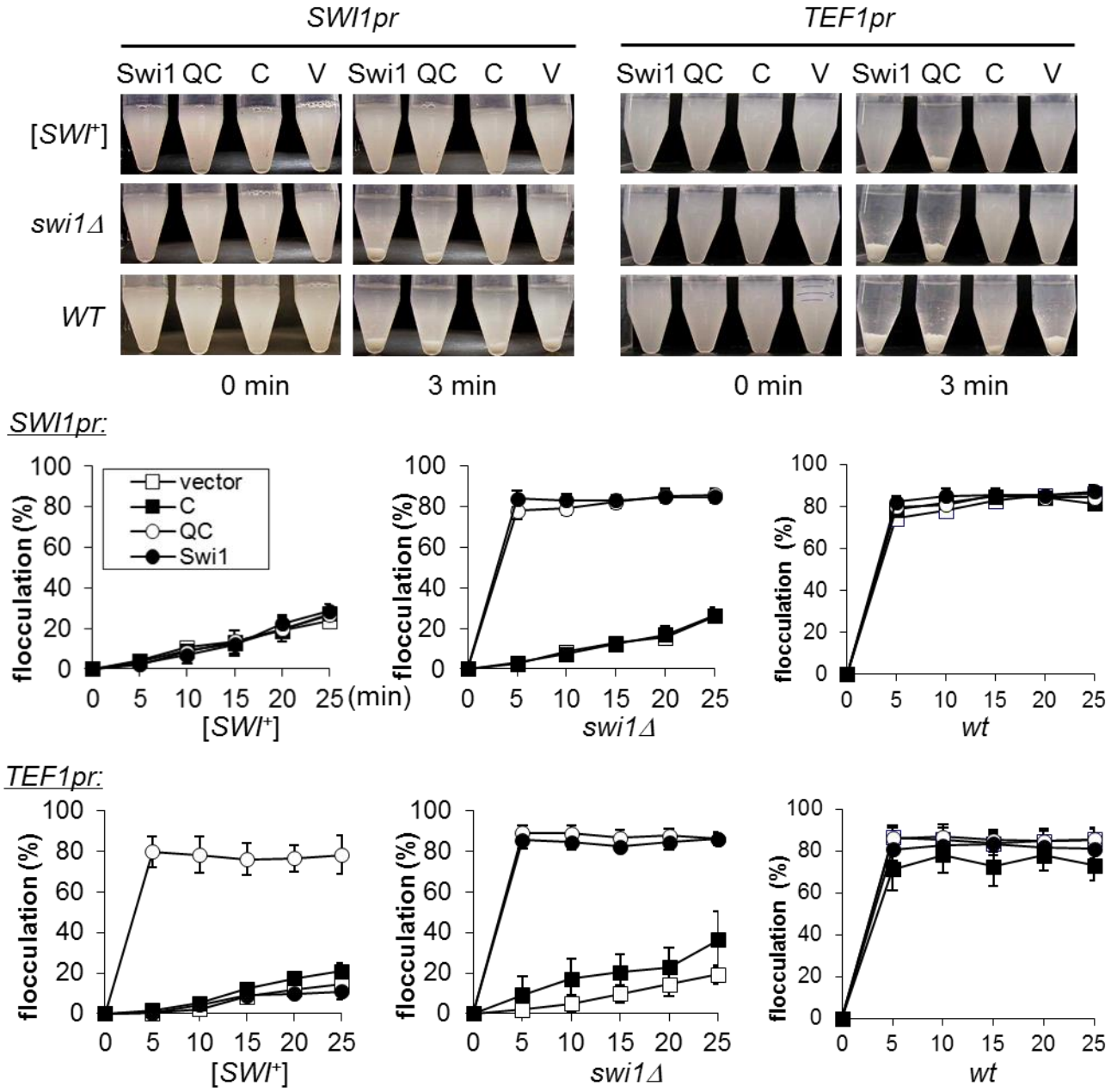

Figure S3. Related to Figure 3D. Effect of Swi1 and its functional domains on flocculation of [*SWI*$^+$] cells. Strains with the indicated genetic backgrounds were repaired for *FLO8* and transformed with a plasmid carrying either a *SWI1* promoter (*SWI1pr*)- or *TEF1* promoter *(TEF1pr*)-driven ORF encoding the full-length Swi1 (Swi1), or truncated Swi1 functional domain with (QC) or without (C) the Q-rich domain. V, vector control. After growth in selective SC medium for 48 h, flocculation assay was done as described in the Supplemental Experimental Procedures. The upper panels show representative flocculation phenotype. The lower panels represent summative data from three quantitative assays. The error bars represent standard deviation. The “min” herein stands for “minute” that the cultures stayed still post vortex.

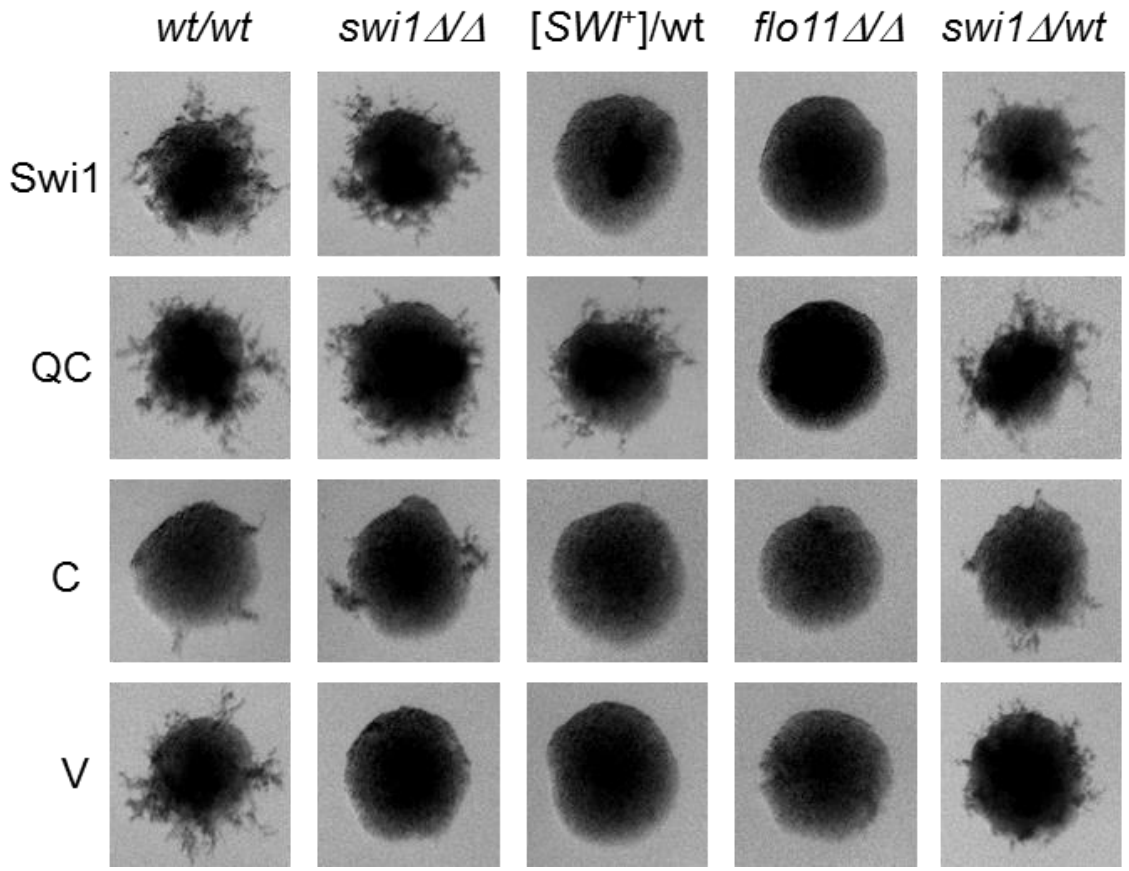

Figure S4. Related to Figure 3E. Effect of Swi1 and its functional domains on pseudohyphal formation of [*SWI*$^+$] diploid cells. The *FLO8* gene was repaired for a *MATa*-type strain that was used to mate with a *MATα* strain to construct the indicated diploids with an indicated genetic background. These diploids were transformed with a plasmid carrying a *TEF1* promoter *(TEF1pr*)-driven ORF encoding full-length Swi1 (Swi1), Swi1 functional domain with (QC) or without (C) the Q-rich domain, or transformed with an empty vector (V). Transformants were analyzed for pseudohyphal formation using a method described in the Supplemental Experimental Procedures. Show are representative results of at least three independent experiments.

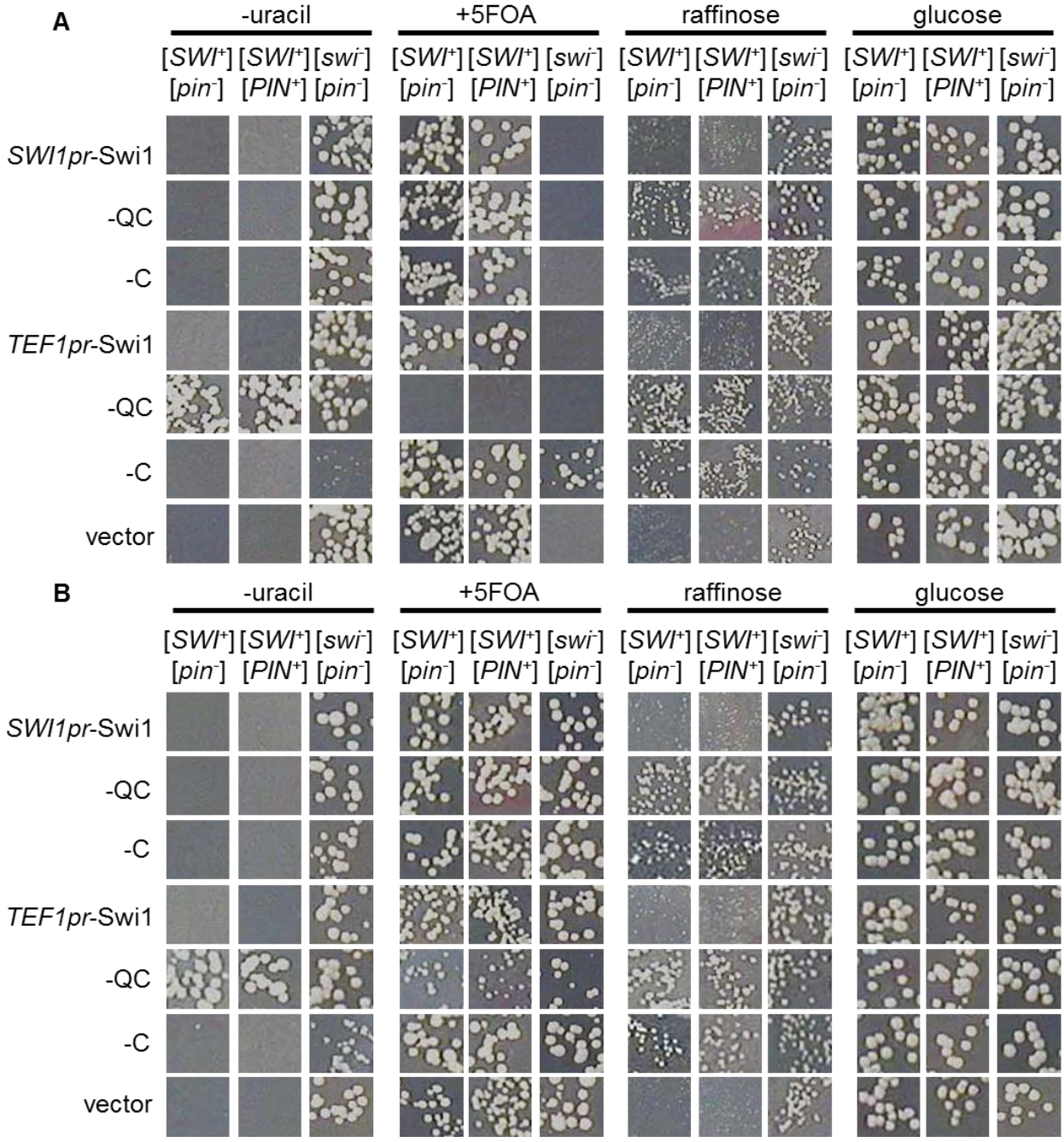

Figure S5. Related to Figure 3F. The effect of Swi1 and its functional domains on Raf$^{\pm}$ and *FLO* gene transcription in [*SWI*$^+$] cells is not attributable to [*PIN*$^+$]. The *FLO8::HIS3 flo1::FLO1pr-URA3* **(A)** and *FLO8::HIS3 flo11::FLO11pr-URA3* **(B)** strains with the indicated prion backgrounds were transformed with a plasmid carrying either a *SWI1* promoter (*SWI1pr*)- or *TEF1* promoter (*TEF1pr*)-driven ORF encoding full-length Swi1 (Swi1), Swi1 functional domain with (QC) or without (C) the Q-rich domain, and spread onto the indicated selective SC plates. Images were taken after 3 days of incubation. –uracil, SC plated without uracil; +5FOA, SC plates with 5 FOA; raffinose, SC raffinose plates; glucose, SC plates with glucose.

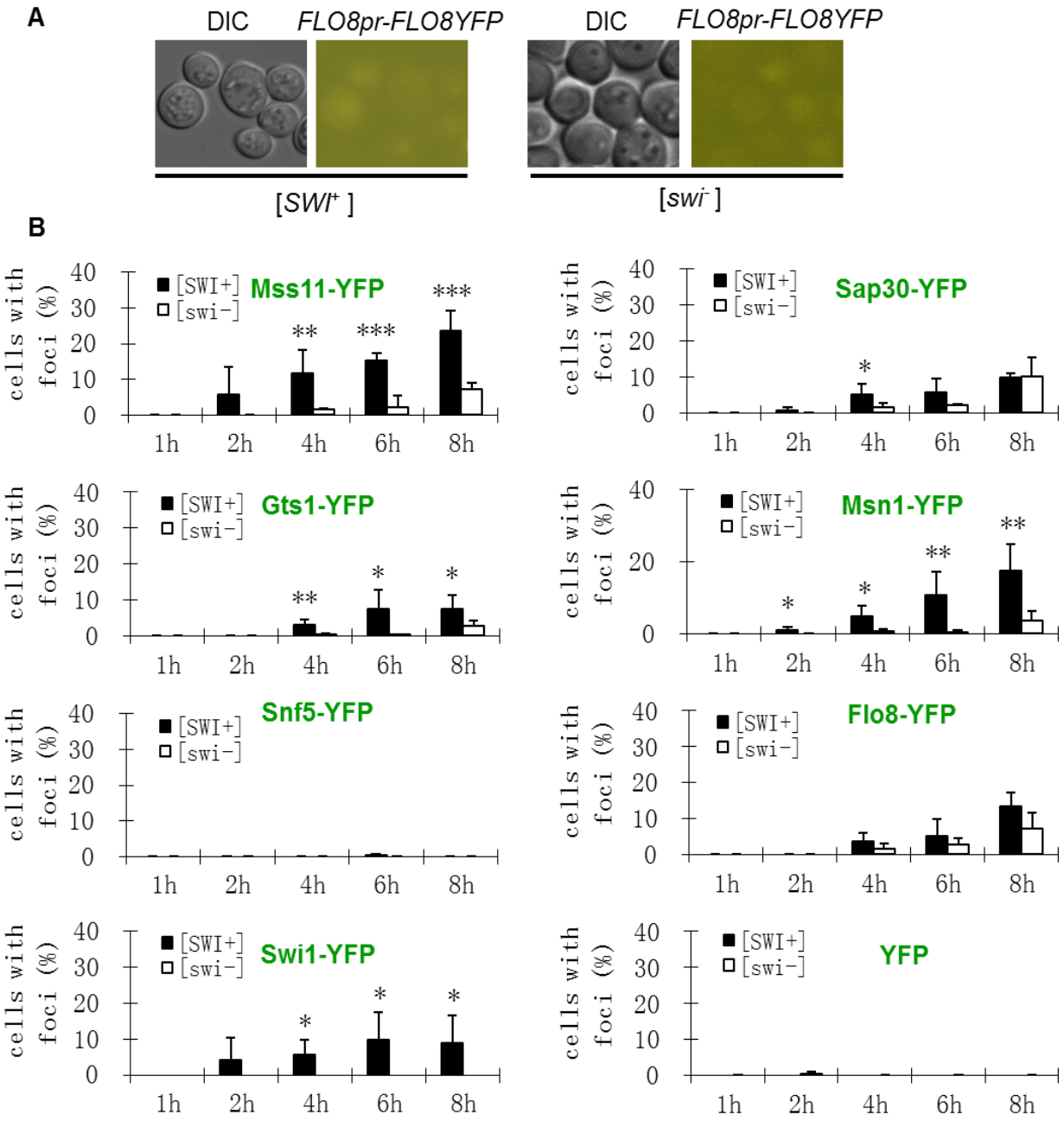

Figure S6. Related to Figure 5. Multiple *FLO* gene up-regulators have higher aggregation frequency in [*SWI*$^+$] than in [*swi*$^-$] cells. (A) The defect of [*SWI*$^+$] cells in *FLO* gene expression and multicellularity is not caused by repression of *FLO8* gene expression. Flo8-YFP was expressed in the indicated strains driven by its own promoter and assayed by fluorescence microscopy. **(B)** Each YFP fusion was expressed under *GAL1* promoter from a 2μ-plasmid in [*SWI*$^+$] and [*swi*$^-$] cells. SC + 2% sucrose log-phase cultures were induced by 0.5% galactose, and aggregation frequency was assayed in a time course. Shown is the result of five independent experiments. Error bars are standard deviations and significance of the difference between [*SWI*$^+$] and [*swi*$^-$] pairs for each protein was performed by t-test (***, $P<0.001$; **, $P<0.01$; *, $P < 0.05$, single tail test).

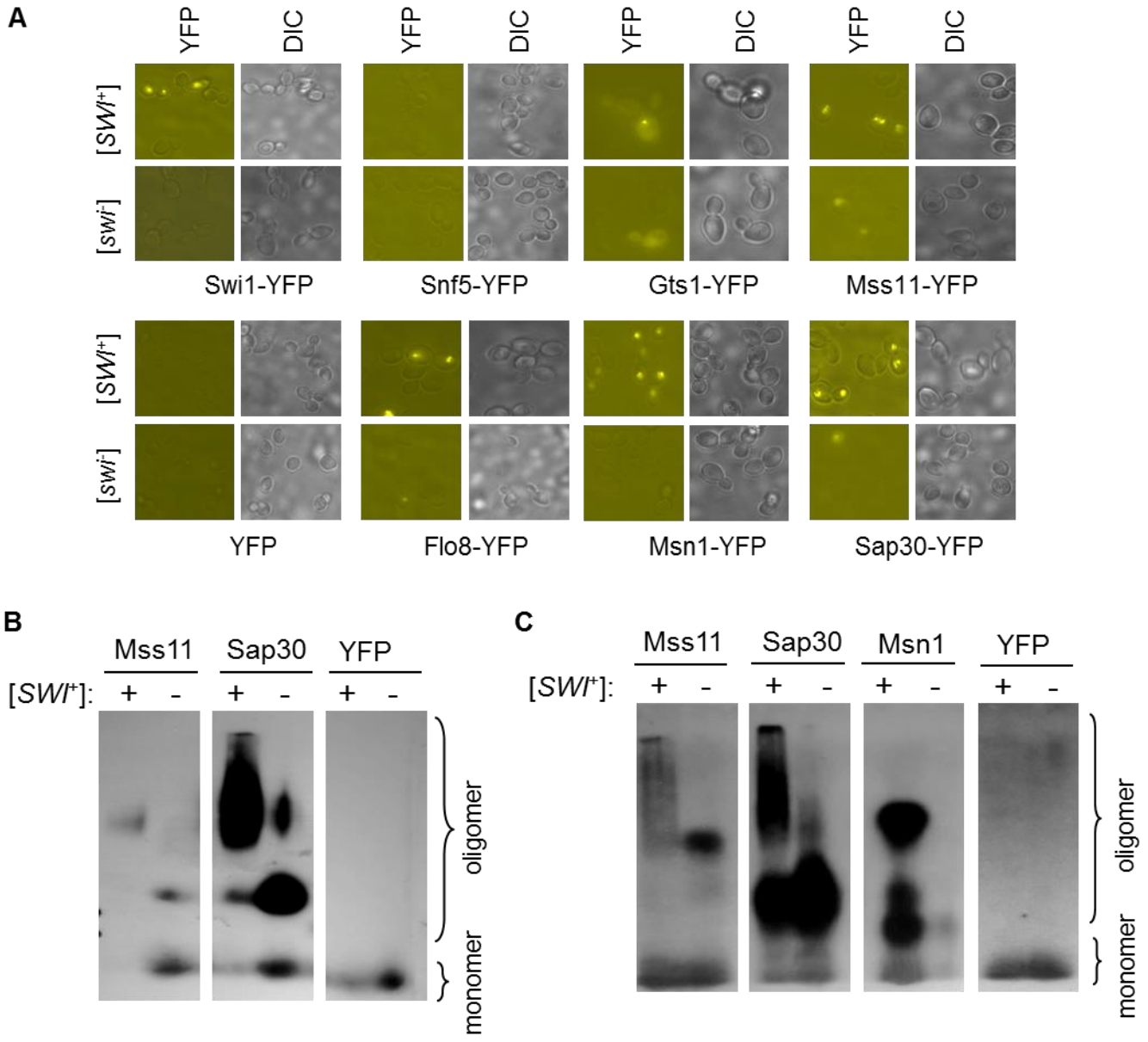

Figure S7. Related to Figure 5. Conformational changes of *FLO* gene up-regulators in [*SWI*$^+$] cells. (A) Representative images showing the aggregation of *FLO* gene activators in [*SWI*$^+$] and [*swi*$^-$] cells (from experiments shown in Figure S6B). **(B)** [*SWI*$^+$] and [*swi*$^-$] strains transformed with a *cen*-plasmid expressing the indicated YFP fusions driven by *TEF1* promoter were cultured to late-log-phase and used for SDD-AGE assay. Pellets of lysates were re-suspended in lysis buffer after centrifugation at 4000 g for 5 min, and treated with 2% SDS for 30 min at 25^{o}C before loading. Eight proteins were assayed and only those showing differences between [*SWI*$^+$] and [*swi*$^-$] are shown. As a note, Msn1 also exhibited SDS tolerant aggregate species frequently in [*SWI*$^+$] cells. **(C)** The experiments were done similarly as in (B) except that the strains carried a 2µ-plasmid containing a *GAL1*-driven YFP fusion. In a sucrose containing medium, 0.5% galactose was used as an inducer and cells were lysed after 12 h of induction.

Table S1. Related to Experimental Procedures. Oligoes used in this study.

Name	Sequence (5'-3')	Description
For plasmid construction (used in PCR):		
FLO8F	gcg-tctaga (*Xba*I)-ATGAGTTATAAAGTGAATAGT(nt 1 of *FLO8*)	forward, create *FLO8-YFP*
FLO8R	ccc-cccggg (*Xma*I)-A-GCCTTCCCAATTAATAAAAT(nt 2397 of *FLO8*)	reverse, create *FLO8-YFP*
Gts1ORF-F	ggcg-actagt (*Spe*I)-ATGAGGTTTAGGAGTTCTTC (nt 1 of *GTS1*)	forward, create *GTS1-YFP*
GtsR	ccc-cccggg (*Xma*I)-a-TTGTGTGTAGAAATAACCTTG (nt 1188 of *GTS1*)	reverse, create *GTS1*- and *GTS1 PrD*-like region-*YFP*
GtsF	gcg-actagt (*Spe*I)-atg-AGTTTTCGATGGTACCCTTC (nt 870 of *GTS1*)	forward, create *GTS1 PrD*-like-*YFP* fusion
M1-F	gcg-actagt (*Spe*I)-ATGGATAACACGACCAATATTAATAC (nt 1 of *MSS11*)	forward, create *MSS11-YFP*
M2274-R	ccc-cccggg (*Xma*I)-a-GCTATCCATTAGATCAGGAGAAAAG (nt 2275 of *MSS11*)	reverse, create *MSS11-YFP*
Msn1F	gcg-actagt (*Spe*I)-ATGAATAATGATCAAACTGC (nt 382 of *MSN1*)	forward, create *MSN1 PrD*-like-*YFP* fusion
Msn1R	ccc-cccggg (*Xma*I)-a-CTGTTCCTCCTCAAATGTCG (nt 780 of *MSN1*)	reverse, create *MSN1 PrD*-like-*YFP* fusion
Msn1ORF-F	ggcg-actagt (*Spe*I)-ATGGCAAGTAACCAGCACATAG (nt 1 of *MSN1*)	forward, create *MSN1-YFP*
Msn1ORF-R	gccc-cccggg (*Xma*I)-c-CTTCAAAGTCTCTGGAATATG (nt 1146 of *MSN1*)	reverse, create *MSN1-YFP*
Mss11F1	gcg-actagt (*Spe*I)-atg-GCACCTGATACTGCTATAAA (nt 724 of *MSS11*)	forward, create *MSS11 PrD*-like region *I or I/II-YFP*
Mss11R1	ccc-cccggg (*Xma*I)-t-TGTTTTCATCTTATTGAAGC (nt 1413 of *MSS11*)	reverse, create *MSS11 PrD*-like region *I-YFP*
Mss11F2	gcg-actagt (*Spe*I)-atg-AATGACAATAACGCAAATGG (nt 1342 of *MSS11*)	forward, create *MSS11 PrD*-like region *II-YFP*
Mss11R2	ccc-cccggg (*Xma*I)-a-TTCCTTGGTCTTTGTTTTAC (nt 2040 of *MSS11*)	reverse, create *MSS11 PrD*-like region *II or I/II-YFP*
Sap1-F	gcg-actagt (*Spe*I)-ATGGCTAGGCCAGTTAATACAAACG (nt 1 of *SAP30*)	forward, create *SAP30* and *SAP30 PrD*-like region-*YFP*
SAP604-R	ccc-cccggg (*Xma*I)-a-ACCCCGAAATTCCATCTTGAATTTC (nt 604 of *SAP30*)	reverse, create *SAP30* and *SAP30 PrD*-like region-*YFP*
Sap30F	gcg-actagt (*Spe*I)-ATGGCTAGGCCAGTTAATACA (nt 1 of *SAP30*)	forward, create *SAP30 PrD*-like region-*YFP*
Sap30R	ccc-cccggg (*Xma*I)-c-GGTGATATGTGTCTCTATGA (nt 267 of *SAP30*)	reverse, create *SAP30 PrD*-like region-YFP
M-741F	ccgc-gagctc (*Sac*I)-ATCGATAAAGAGTTCAAAAAGCTCTC (nt -741 of *MSS11*)	forward, amplify *MSS11* promoter
M-1R	ggcg-actagt (*Spe*I)-ATCTTTATCATGCACCTTTTTCTTATTTC (at -1 of *MSS11*)	reverse, amplify *MSS11* promoter
Sap-611F	tGAAGAGCTC (*Sac*I)-TTGACGTATCACTC (nt -614 of *SAP30*)	forward, amplify *SAP30* promoter
Sap-1R	ggcg-actagt (*Spe*I)-TACTACTCAGTATATACCTATCCTTC (nt -1 of *SAP30*)	reverse, amplify *SAP30* promoter
9-10F	gggg-ccgcgg (*Sac*II)-ATTCTCATCGAGAGCCGAGC (nt -2000 of *FLO11*)	forward, dissect *FLO11* promoter
1-3R	atat-cccggg (*Xma*I)-AGTGTGCGTATATGGATTTT (nt -1 of *FLO11*)	reverse, dissect *FLO11* promoter
URA3N-F	gtta-cccggg (*Xma*I)-atgcat (*Nsi*I)-ATGTCGAAAGCTACATATAAG (nt 1 of *URA3*)	forward, amplify 963-bp *URA3* ORF+terminator
URA3XN-R	gggg-*ctcgag* (*Xho*I)-TTTATCGTTGGATACTTGAAAA TTGTGCCCAATGGTAAAT CAACTTGCAG -(nt 2729 of *LYS2*)–GCGGCCGC(*Not*I)-TAATAACTGATATAATTAAATTG (nt 879 of *URA3*)	reverse, amplify 963-bp *URA3* ORF+terminator

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SL-F	gggg-gagctc (*Sac*I)-*gtcgac* (*Sal*I)-GTTACGTCTATATTCATTGAAACTGATT ATTCGATTTTCTTCTTGCTGAC (nt -350 of *LYS2*)-CCGCGG(*Sac*II)-GCAACAACCTATAATTGAGTTAAG (nt -901 of *SUC2*)	forward, amplify 749-bp *SUC2-LEU2* promoter
SL-R	gggg-ctcgag (*Xho*I)-atgcat (*Nsi*I)-cccggg (*Xma*I)-TAGAATGGTATATCCTTGAAATAT (nt -1 of *LEU2*)	reverse, amplify 749-bp *SUC2-LEU2* promoter
For yeast strain engineering:		
Flo11ex-F	GAAAGCTGTGCGGGAAAAC (nt -1650 of *FLO11*)	forward, to replace *FLO11* with *URA3*
Flo11ura3-R	GTATTTTCGTTGTAACCGTATAGTTGGACGGTACCTTTTGGACCAGTG AC (nt 350 of *FLO11*)-TAATAACTGATATAATTAAATTG (nt 879 of *URA3*)	reverse, to replace *FLO11* with *URA3*
Flo11ex-R	GTATTTTCGTTGTAACCGTAT (nt 350 of *FLO11*)	reverse, PCR for *FLO11* replacement and detection
flo1ura-F	TCCGGGTTCTTATTTTTAATTCTTGTCACCAGTAAACAGAACATCCAAA A (nt -50 of *FLO1*)-ATGTCGAAAGCTACATATAAG (nt 1 of *URA3*)	forward, PCR to replace *FLO1* ORF with *URA3* ORF
flo1ura-R	TGATCGGCGGTTGCTGTTGAGCACAACAGTTGAACGCGGTTGCACCA CCT (nt 550 of *FLO1*)-TTAGTTTTGCTGGCCGCATC (nt 804, the stop code of *URA3*)	reverse, PCR to replace the *FLO1* ORF with *URA3* ORF
Flo1-F	TCCGGGTTCTTATTTTTAATTC (nt -50 of *FLO1*)	forward, to replace *FLO1* with *URA3*
flo1-R	TGATCGGCGGTTGCTGTTG (nt 550 of *FLO1*)	forward, to replace *FLO1* with *URA3*
flo1t-F	GTCACCAGTAAACAGAACATC (nt -26 of *FLO1*)	forward, detection of *FLO1* replacement by *URA3*
MET15-F	ATGCCATCTCATTTCGATACTGTTC (nt 1 of *MET15*)	forward, amplify *MET15*
MET15-R	TCATGGTTTTTGGCCAGCGAAAAC (nt 1335 of *MET15*)	reverse, amplify *MET15*
HtoM-F	CTTCGAAGAATATACTAAAAAATGAGCAGGCAAGATAAACGAAGGCAA AG (nt -50 of *HIS3*)-ATGCCATCTCATTTCGATACTGTTC (nt 1 of *MET15*)	forward, to replace *HIS3* with *MET15*
HtoM-R	TATACACATGTATATATATCGTATGCTGCAGCTTTAAATAATCGGTGTC A (nt 713 of *HIS3*)-TCATGGTTTTTGGCCAGCGAAAAC (nt 1335 of *MET15*)	reverse, to replace *HIS3* with *MET15*
For DNA sequencing and RT-PCR:		
FLO1-F	CTGCGAATGAAGAGTCTG	forward, RT-PCR of *FLO1*
FLO1-R	AGGACGCAATGAAGACAC	reverse, RT-PCR of *FLO1*
GPDpro-F	TAATTCTGTAAATCTATTTC (nt -115 of *TDH3*)	forward, for DNA sequencing
TEFup	AGTTTCATTTTTCTTGTTCTATTAC (nt -91 of *TEF1*)	forward, for DNA sequencing
CYCdn	CATAACTATAAAAAAATAAATAGGG (nt 474 of *CYC1*)	reverse, for DNA sequencing
Mss11-1968R	GCTGGAGCTAGAAGTACA (nt 1968 of *MSS11*)	reverse, sequencing *Mss11*
Mss11-759F	GCAGCAGTACGCAACTAT (nt 759 of *MSS11*)	forward, sequencing *Mss11*
msn1-243R	TTCAGCGTGGCGTTGTTG (nt 243 of *MSN1*)	reverse, sequencing *MSN1*

Note: Lower-case bases are inserted nucleotide (nt). Restriction sites are underlined and denoted. The starting position of a homologous sequence is indicated (from 5', an upstream position is "-" and downstream is "+" taking the first base of a start code as 1).

Table S2. Related to Experimental Procedures. Plasmids used in this study.

Name	Marker	Replicon	Promoter	Protein to express	Source
p315CUP1-RNQ1GFP	*LEU2*	*CEN6/ARSH4*	*CUP1*	Rnq1-GFP	(Sondheimer and Lindquist, 2000),
p413TEF	*HIS3*	*CEN6/ARSH4*	*TEF1*		ATCC
p415ADH	*LEU2*	*CEN6/ARSH4*	*ADH1*		ATCC
p415GAL1	*LEU2*	*CEN6/ARSH4*	*GAL1*		ATCC
p415TEF	*LEU2*	*CEN6/ARSH4*	*TEF1*		ATCC
p415TEF-NQYFP	*LEU2*	*CEN6/ARSH4*	*TEF1*	Swi1 NQ region (aa 1-536)-YFP	(Du et al., 2010)
p416ADH	*URA3*	*CEN6/ARSH4*	*ADH1*		ATCC
p416GPD-SNF5YFP	*URA3*	*CEN6/ARSH4*	*GPD*	Snf5-YFP	Lindquist lab
p416SWI1-CYFP	*URA3*	*CEN6/ARSH4*	*SWI1*	Swi1 C region (aa 537-1314)-YFP	(Du et al., 2010)
p416SWI1-NQYFP	*URA3*	*CEN6/ARSH4*	*SWI1*	Swi1 NQ region (aa 1-536)-YFP	(Du et al., 2010)
p416TEF-C	*URA3*	*CEN6/ARSH4*	*TEF1*	Swi1 C region (aa 537-1314)	(Du et al., 2010)
p416TEF-CYFP	*URA3*	*CEN6/ARSH4*	*TEF1*	Swi1 C region (aa 537-1314)-YFP	(Du et al., 2010)
p416TEF-NQYFP	*URA3*	*CEN6/ARSH4*	*TEF1*	Swi1 NQ region (aa 1-536)-YFP	(Du et al., 2010)
p416TEF-NYFP	*URA3*	*CEN6/ARSH4*	*TEF1*	Swi1 N region (aa 1-327)-YFP	(Du et al., 2010)
p416TEF-QC	*URA3*	*CEN6/ARSH4*	*TEF1*	Swi1 QC region (aa 332-1314)	(Du et al., 2010)
p416TEF-SWI1	*URA3*	*CEN6/ARSH4*	*TEF1*	Swi1	(Du et al., 2010)
p416TEF-SWI1mCherry	*URA3*	*CEN6/ARSH4*	*TEF1*	Swi1-mCherry	(Crow et al., 2011)
p416TEF-SWI1YFP	*URA3*	*CEN6/ARSH4*	*TEF1*	Swi1-YFP	(Du et al., 2008)
p416TEF-YFP	*URA3*	*CEN6/ARSH4*	*TEF1*	YFP	Lindquist lab
p425GAL1	*LEU2*	*2 micron*	*GAL1*		ATCC
p426GAL1	*URA3*	*2 micron*	*GAL1*		ATCC
pCUP1-RNQ1GFP	*URA3*	*CEN6/ARSH4*	*CUP1*	Rnq1-GFP	(Sondheimer and Lindquist, 2000),
pHL1	*URA3*	*CEN6/ARSH4*	*FLO8*	Flo8	(Liu et al., 1996)
pHL11	*URA3*		*FLO8*	repair and expression of Flo8	(Liu et al., 1996)
pLS7	*TRP1*	*CEN6/ARSH4*	*SUC2-LEU2*	LacZ	(Sarokin and Carlson, 1985)
pRS303	*HIS3*				Lindquist lab
pRS313	*HIS3*	*CEN6/ARSH4*			Lindquist lab
p413SWI1-QC	*HIS3*	*CEN6/ARSH4*	*SWI1*	Swi1 QC region (aa 332-1314)	this study
p413TEF-QC	*HIS3*	*CEN6/ARSH4*	*TEF1*	Swi1 QC region (aa 332-1314)	this study
p413TEF-SWI1mCherry	*HIS3*	*CEN6/ARSH4*	*TEF1*	Swi1-mCherry	this study
p415ADH-FLO8YFP	*LEU2*	*CEN6/ARSH4*	*ADH1*	Flo8-YFP	this study
p415ADH-GTSfullYFP	*LEU2*	*CEN6/ARSH4*	*ADH1*	Gts1-YFP	this study
p415ADH-MfullYFP	*LEU2*	*CEN6/ARSH4*	*ADH1*	Mss11-YFP	this study
p415ADH-MSNfullYFP	*LEU2*	*CEN6/ARSH4*	*ADH1*	Msn1-YFP	this study
p415ADH-Mss11aYFP	*LEU2*	*CEN6/ARSH4*	*ADH1*	Mss11 Q-rich fusion (aa 242-471)	this study
p415ADH-NQYFP	*LEU2*	*CEN6/ARSH4*	*ADH1*	Swi1 NQ fusion (aa 1-536)	this study
p415ADH-SfullYFP	*LEU2*	*CEN6/ARSH4*	*ADH1*	Sap30-YFP	this study
p415F19-URA3	*LEU2*	*CEN6/ARSH4*	*FLO11*	Ura3	this study
p415GAL1-MfullYFP	*LEU2*	*CEN6/ARSH4*	*GAL1*	Mss11-YFP	this study
p415GAL1-MsnfullYFP	*LEU2*	*CEN6/ARSH4*	*GAL1*	Msn1-YFP	this study
p415GAL1-NQYFP	*LEU2*	*CEN6/ARSH4*	*GAL1*	Swi1 NQ fusion (aa 1-536)	this study

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p415GAL1-SfullYFP	*LEU2*	*CEN6/ARSH4*	*GAL1*	Sap30-YFP	this study
p415GAL1-Swi1YFP	*LEU2*	*CEN6/ARSH4*	*GAL1*	Swi1-YFP	this study
p415MSS11-MfullYFP	*LEU2*	*CEN6/ARSH4*	*MSS11*	Mss11-YFP	this study
p415SAP30-SfullYFP	*LEU2*	*CEN6/ARSH4*	*SAP30*	Sap30-YFP	this study
p415SL	*LEU2*	*CEN6/ARSH6*	*SUC2-LEU2*		this study
p415SL-URA3	*LEU2*	*CEN6/ARSH5*	*SUC2-LEU2*	Ura3	this study
p415SWI1-C	*LEU2*	*CEN6/ARSH4*	*SWI1*	Swi1 C region (aa 537-1314)	this study
p415SWI1-Mss11YFP	*LEU2*	*CEN6/ARSH4*	*SWI1*	Mss11 Q/N (aa 242-680)	this study
p415SWI1-NQYFP	*LEU2*	*CEN6/ARSH4*	*SWI1*	Swi1 NQ region (aa 1-536)-YFP	this study
p415SWI1-QC	*LEU2*	*CEN6/ARSH4*	*SWI1*	Swi1 QC region (aa 332-1314)	this study
p415SWI1-SWI1	*LEU2*	*CEN6/ARSH4*	*SWI1*	Swi1	this study
p415TEF-C	*LEU2*	*CEN6/ARSH4*	*TEF1*	Swi1 C region (aa 537-1314)	this study
p415TEF-FLO8YFP	*LEU2*	*CEN6/ARSH4*	*TEF1*	Flo8-YFP	this study
p415TEF-Gts1YFP	*LEU2*	*CEN6/ARSH4*	*TEF1*	Gts1 Q/N-rich fusion (aa 290-396)	this study
p415TEF-GTSfullYFP	*LEU2*	*CEN6/ARSH4*	*TEF1*	Gts1-YFP	this study
p415TEF-MfullYFP	*LEU2*	*CEN6/ARSH4*	*TEF1*	Mss11-YFP	this study
p415TEF-Msn1YFP	*LEU2*	*CEN6/ARSH4*	*TEF1*	Msn1 Q/N-rich fusion (aa 128-260)	this study
p415TEF-MSNfullYFP	*LEU2*	*CEN6/ARSH4*	*TEF1*	Msn1-YFP	this study
p415TEF-Mss11aYFP	*LEU2*	*CEN6/ARSH4*	*TEF1*	Mss11 Q-rich fusion (aa 242-471)	this study
p415TEF-Mss11bYFP	*LEU2*	*CEN6/ARSH4*	*TEF1*	Mss11 N-rich fusion (aa 448-680)	this study
p415TEF-Mss11YFP	*LEU2*	*CEN6/ARSH4*	*TEF1*	Mss11 Q/N (aa 242-680)	this study
p415TEF-NYFP	*LEU2*	*CEN6/ARSH4*	*TEF1*	Swi1 N region (1-327)-YFP	this study
p415TEF-QC	*LEU2*	*CEN6/ARSH4*	*TEF1*	Swi1 QC region (aa 332-1314)	this study
p415TEF-Sap30YFP	*LEU2*	*CEN6/ARSH4*	*TEF1*	Sap30 Q/N-rich fusion (aa 1-89)	this study
p415TEF-SfullYFP	*LEU2*	*CEN6/ARSH4*	*TEF1*	Sap30-YFP	this study
p415TEF-SNF5YFP	*LEU2*	*CEN6/ARSH4*	*TEF1*	Snf5-YFP	this study
p415TEF-SWI1	*LEU2*	*CEN6/ARSH4*	*TEF1*	Swi1	this study
p415TEF-YFP	*LEU2*	*CEN6/ARSH4*	*TEF1*	YFP	this study
p425GAL1-FLO8YFP	*LEU2*	*2 micron*	*GAL1*	Flo8-YFP	this study
p425GAL1-GTSfullYFP	*LEU2*	*2 micron*	*GAL1*	Gts1-YFP	this study
p425GAL1-MfullYFP	*LEU2*	*2 micron*	*GAL1*	Mss11-YFP	this study
p425GAL1-MSNfullYFP	*LEU2*	*2 micron*	*GAL1*	Msn1-YFP	this study
p425GAL1-Sap30YFP	*LEU2*	*2 micron*	*GAL1*	Sap30 Q/N-rich fusion (aa 1-89)	this study
p425GAL1-SfullYFP	*LEU2*	*2 micron*	*GAL1*	Sap30-YFP	this study
p425GAL1-SNF5YFP	*LEU2*	*2 micron*	*GAL1*	Snf5-YFP	this study
p425GAL1-SWI1YFP	*LEU2*	*2 micron*	*GAL1*	Swi1-YFP	this study
p425GAL1-YFP	*LEU2*	*2 micron*	*GAL1*	YFP	this study
p426GAL1-NQYFP	*URA3*	*2 micron*	*GAL1*	Swi1 NQ region (aa 1-536)-YFP	this study
p426GPD-SNF5YFP	*URA3*	*2 micron*	*GPD*	Snf5-YFP	this study
pRS303-FLO8	*HIS3*		*FLO8*	repair and expression of Flo8	this study
pRS313-FLO8	*HIS3*	*CEN6/ARSH4*	*FLO8*	Flo8	this study

Note: All these plasmids harbor an amplicilin resistance gene (ampR) for the growth selection in *E. coli*. aa, amino acid.

Table S3. Related to Experimental Procedures. Yeast strains used in this study.

Name	Backbone	Genotype Description	Source
LY422	74D-694	*MATa ade1-14 trp1-289his3-200 ura3-52 leu2-3, 112*	(Chernoff et al., 1995)
DY902	74D-694	*MATa ade1-14 trp1-289::TRP1::TEF1^{promoter}-RNQ1CFP his3-200 ura3-52 leu2-3, 112 [SWI^{+}]*	(Crow et al., 2011)
LY720	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8 swi1Δ::KanMX4*	(Du et al., 2008)
LY742	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8 [SWI^{+}]*	(Du et al., 2008)
LY750	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8 mss11Δ::KanMX4*	Invitrogen
LY751	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8 sap30Δ::KanMX4*	Invitrogen
LY768	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8 gts1Δ::KanMX4*	Invitrogen
LY769	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8 msn1Δ::KanMX4*	Invitrogen
LY549	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8 snf5Δ::KanMX4*	Invitrogen
LY746	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8 [PIN^{+}]*	ATCC
LY732	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8 flo1Δ::KanMX4*	(Liu et al., 1996)
LY733	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8 flo11Δ::KanMX4*	(Liu et al., 1996)
LY734	BY4742	*MATalpha his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 flo8 flo11Δ::KanMX4*	(Liu et al., 1996)
LY747	BY4743	*MATa/MATalpha his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 lys2Δ0/+ met15Δ0/+ ura3Δ0/ura3Δ0 flo8/flo8ΔSWI1::KanMX4/+*	ATCC
LY742	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8 [SWI^{+}]*	(Du et al., 2008)
LY129	wild	*D585a*	Lindquist lab
LY130	wild	*D84alpha*	Lindquist lab
DY760	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8::FLO8::HIS3*	this study
DY761	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8::FLO8::HIS3 swi1Δ::KanMX4*	this study
LY741	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8::FLO8::HIS3 [SWI^{+}]*	this study
DY762	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8::FLO8::HIS3 flo1Δ::KanMX4*	this study
DY763	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8::FLO8::HIS3 flo11Δ::KanMX4*	this study
DY755	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8::FLO8::HIS3 flo1Δ::FLO1^{promoter}-URA3*	this study
LY735	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8::FLO8::HIS3 flo1Δ::FLO1^{promoter}-URA3 [SWI^{+}]*	this study
LY736	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8::FLO8::HIS3 flo1Δ::FLO1^{promoter}-URA3 [SWI^{+}][PIN^{+}]*	this study
DY758	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8::FLO8::HIS3 flo11Δ::FLO11^{promoter}-URA3*	this study
LY737	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8::FLO8::HIS3 flo11Δ::FLO11^{promoter}-URA3 [SWI^{+}]*	this study
LY738	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8::FLO8::HIS3 flo11Δ::FLO11^{promoter}-URA3 [SWI^{+}][PIN^{+}]*	this study
LY770	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8::FLO8::MET15 flo1Δ::FLO1^{promoter}-URA3 [SWI^{+}]*	this study
LY773	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8::FLO8::MET15 flo1Δ::FLO1^{promoter}-URA3*	this study
LY756	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8 swi1Δ::KanMX4 p416TEF-SWI1 [SWI^{+}]*	this study
LY762	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8::FLO8::HIS3 swi1Δ::KanMX4 flo1Δ::FLO1^{promoter}-URA3*	this study
LY763	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8::FLO8::HIS3 swi1Δ::KanMX4 flo1Δ::FLO11^{promoter}-URA3*	this study

Continued from proceeding page (Table S3)

DY894	BY4742	*MATalpha his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8::FLO8::HIS3*	this study
DY895	BY4742	*MATalpha his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8::FLO8::HIS3 swi1Δ::KanMX4*	this study
DY896	BY4742	*MATalpha his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8::FLO8::HIS3 flo11Δ::KanMX4*	this study
DY771	BY4742	*MATalpha his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8 pCUP1-RNQ1GFP*	this study
DY770	BY4742	*MATalpha his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 flo8 swi1Δ::KanMX4 [PIN$^+$] p315CUP1-RNQ1GFP*	this study
DY441	BY4742	*MATa his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 flo8 swi1Δ::KanMX4*	this study
LY787	BY4742	*MATalpha his3Δ1 leu2Δ0 ura3Δ0 flo8::FLO8*	this study
DY777	BY4742	*MATalpha his3Δ1 leu2Δ0 ura3Δ0 flo8 [PIN$^+$] pCUP1-RNQ1GFP*	this study
DY897	BY4743	*MATa/MTAalpha his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 met15Δ0/met15Δ0 ura3Δ0/ura3Δ0 flo8::FLO8::HIS3/flo8::FLO8::HIS3*	this study
DY898	BY4743	*MATa/MTAalpha his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 met15Δ0/met15Δ0 ura3Δ0/ura3Δ0 flo8::FLO8::HIS3/flo8::FLO8::HIS3 swi1Δ::KanMX4/swi1Δ::KanMX4*	this study
DY899	BY4743	*MATa/MTAalpha his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 met15Δ0/met15Δ0 ura3Δ0/ura3Δ0 flo8::FLO8::HIS3/flo8::FLO8::HIS3 [SWI$^+$]*	this study
DY900	BY4743	*MATa/MTAalpha his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 met15Δ0/met15Δ0 ura3Δ0/ura3Δ0 flo8::FLO8::HIS3/flo8::FLO8::HIS3 flo11Δ::KanMX4/flo11Δ::KanMX4*	this study
DY901	BY4743	*MATa/MTAalpha his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 met15Δ0/met15Δ0 ura3Δ0/ura3Δ0 flo8::FLO8::HIS3/flo8::FLO8::HIS3 swi1Δ::KanMX4/+*	this study

Note: +, wild-type; *MATalpha*, mating type alpha. In general, BY4741, BY4742 and BY4743-based strains are derivatives of S288c that are *MATa, MATalpha* or diploid, respectively, in which *FLO8* gene is not functioning.

Supplemental Experimental Procedures.

Plasmid construction.

Oligonucleotides for plasmid construction, yeast engineering and DNA sequencing are shown in Table S1.

There are 82 plasmids used in this study and 55 are newly constructed (Table S2). By inserting a 4.2-kb fragment (including *FLO8* gene and its promoter) from *pHL1* into *pRS303* and *pRS313* via *Xba*I/*Xho*I sites, *pRS303-FLO8 and pRS313-FLO8* were created, respectively. *SWI1 QC* and *SWI1mCherry* were subcloned from *p416TEF-QC* and *p416TEF-SWI1mCherry* to *p413TEF* through *Spe*I/*Xho*I sites, resulting *p413TEF-QC and p413TEF-SWI1mCherry*, respectively. To make *p413TEF-C, p415TEF-QC, p415TEF-NYFP, P415TEF-SWI1, p415TEF-SWI1YFP* and *p415TEF-YFP*, corresponding *Spe*I/*Xho*I fragments derived from *p416TEF-C, p416TEF-QC, p416TEF-NYFP, p416TEF-SWI1, p416TEF-SWI1YFP* and *p416TEF-YFP* were cloned into the same sites of *p415TEF*. Also, through *Spe*I/*Xho*I, *SWI1YFP* and *SWI1-NQYFP* from *p416TEF-SWI1YFP* and *p416TEF-NQYFP* were cloned into *p525GAL1* and p*426GAL1*, resulting plasmid *p425GAL1-SWI1YFP* and *p426GAL1-NQYFP*, respectively. Plasmid *p415SWI1-NQYFP* was created by cloning *SWI1-NQYFP* from *p416SWI1-NQYFP* to *p415TEF* through sites of *Sac*I/*Xho*I. Two Snf5-YFP expression plasmids, *p25GAL1-SNF5YFP and p426GPD-SNF5YFP* were constructed by sub-cloning a 3.49-kb *Bam*HI/*Xho*I fragment from *p416GPD-SNF5YFP* into the same sites of *p425GAL1* and *p426GPD*, respectively. To make p*415TEF-FLO8YFP, FLO8* gene was PCR-amplified from *pHL1* with primer pair of FLO8F and FLO8R, and used to replace the NQ fragment of *p415TEF-NQYFP* after *Xba*I/*Xma*I digestion. To make prion-like domain (PrLD)-YFP fusions, PCR products amplified from chromosomal DNA of yeast 74D-694 (#LY422) using primer pairs of GtsF/GtsR, Msn1F/Msn1R, Sap30F/Sap30R, Mss11F1/Mss11R1, Mss11F2/Mss11R2, and Mss11F1/Mss11R2 were used to replace the NQ fragment of *p415TEF-NQYFP* through *Spe*I/*Xma*I, generating *p415TEF-Gts1YFP, p415TEF-Msn1YFP, P415TEF-Sap30YFP, p415TEF-Mss11aYFP, p415TEF-Mss11bYFP*, and *P415TEF-Mss11YFP*, respectively.

The 1.5-kb *ADH1* promoter was cleaved from *p415ADH*, and used to replace the corresponding *SWI1* or *TEF1* promoter harbored by plasmid *p415SWI1-NQYFP, p415TEF-FLO8YFP, p415TEF-Mss11aYFP* through *Sac*I/*Spe*I sites, resulting *p415ADH-NQYFP*, *p415ADH-FLO8YFP*, and *p415ADH-Mss11aYFP*, respectively. Replacement of *TEF1* promoter in plasmid *p413TEF-QC* and *p415TEF-Mss11YFP* with the 0.7-kb *SWI1pr* from *p416SWI1-CYFP* and *p416SWI1-NQYFP* through *Sac*I/*Spe*I sites generated *p413SWI1-QC* and *p415SWI1-Mss11YFP*, respectively. 1.2-kb *GTS1* gene and 1.1-kb *MSN1* gene were PCR amplified with primer pairs of GtsORF-F/GtsR and Msn1ORF-F/Msn1ORF-R, and used to replace the *SWI-N* fragment of *p415TEF-NYFP* after *Spe*I/*Xma*I cleavage, resulting *p415TEF-GTSfullYFP* and *p415TEF-MSNfullYFP*, respectively. To obtain plasmid *p415TEF-SNF5YFP*, the 3.5-kb *SNF5YFP* from *p426GPD-SNF5YFP* was subcloned into *p415TEF* through sites of *Spe*I/*Xho*I. Also, by *Spe*I/*Xho*I digestion, *SAP30 PrD -YFP* from *p415TEF-Sap30YFP* and *YFP* from

p415TEF-YFP were cloned into *p425GAL1* to obtain *p425GAL1-Sap30YFP* and *p25GAL1-YFP*, respectively.

Replacement of the *MSS11 Q-rich PrLD* in plasmid *p415ADH-Mss11aYFP* through *Xma*I/*Spe*I sites with a 604-bp PCR product amplified with a primer pair of SAP1-F/SAP604-R from chromosomal DNA of 74D-694 (#LY422) created *p415ADH-SfullYFP*. To create *p415ADH-GTSfullYFP, p415ADH-MSNfullYFP*, and *p415ADH-MfullYFP*, MSS11a fragment in *p415ADH-Mss11aYFP* was replaced by *GTS1* from *p415TEF-GTSfullYFP, MSN1* from *p415TEF-MSNfullYFP*, and a 2.3-kb PCR product of *MSS11* amplified with primers of M1-F/M2274-R from chromosomal DNA or 74D-694 (#LY422), through *Spe*I/*Xma*I sites, respectively. The Q/N-rich region of *MSS11* in *p415SWI1-Mss11YFP* was replaced by *SWI1-C* from *p416TEF-CYFP, SWI1-QC* from *p416TEF-QC*, and *SWI1* from *p416TEF-SWI1* upon *Spe*I/*Xho*I digestion, resulting plasmids of *p415SWI1-C, p415SWI1-QC* and *p415SWI1-SWI1*, respectively. The *GAL1-NQYFP* fragment from *p426GAL1-NQYFP* was used to replace the *ADH-NQYFP* fragment in *p415ADH-NQYFP* through SacI/XhoI sites, resulting *p415GAL1-NQYFP*. Vector *p425GAL1* was partially digested by *Xba*I and fully by *Xho*I, and the resulting 7.4-Kb vector fragment was used to ligate with a 3.2-kb *FLO8* ORF-YFP fragment from *p415ADH-FLO8YFP* after cutting by *Xba*I/*Xho*I, creating *p425GAL1-FLO8YFP*.

Replacing the 1.5-kb *ADH1* promoter of *p415ADH-MfullYFP* and *p415ADH-SfullYFP* with a 741-bp *MSS11* promoter PCR product using primers of M-741F/M-1R, and with a 611-bp *SAP30* promoter PCR product using primers of SAP-611F/SAP-1R through sites of *Sac*I/*Spe*I, generated *p415MSS11-MfullYFP* and *p415SAP30-SfullYFP*, respectively. To construct *p415GAL1-MfullYFP, p415GAL1-MsnfullYFP, p415GAL1-SfullYFP*, and *p415GAL1-SWI1YFP*, corresponding *Spe*I/*Xma*I fragments were used to replace *SWI1 NQ* in *p415GAL1-NQYFP*. Full-length *MSN1-YFP, SAP30-YFP*, and *GTS1-YFP* from *p415ADH-MSNfullYFP, p415ADH-SfullYFP*, and *p415ADH-GTSfullYFP* were sub-cloned into *p425GAL1* through *Spe*I/*Xho*I sites to created *p425GAL1-MSNfullYFP, p425GAL1-SfullYFP*, and *p425GAL1-GTSfullYFP*, respectively. *MSS11* ORF from *p415ADH-MfullYFP* was used to replace *SAP30-PrLD* in *p425GAL1-Sap30YFP* after *Spe*I/*Xma*I digestion, resulting *p425GAL1-MfullYFP*.

The *MSS11* promoter in *p415MSS11-MfullYFP* and the *SAP30* promoter in *p415SAP30-SfullYFP* were replaced by *TEF1* promoter from *p415TEF-NYFP* through *Sac*I/*Spe*I sites to create *p415TEF-MfullYFP* and *p415TEF-SfullYFP*, respectively. To make *p415SL*, the *SUC2-LEU2* chimeric promoter was PCR-amplified from *pLS7* with primer pair of SL-F/SL-R, and used to replace the *TEF1* promoter in *p415TEF* through digestion with *Sac*I/*Xho*I. Subsequently, *URA3* ORF with its terminator and a linker sequence was PCR-amplified with primer pair of URA3N-F/URA3XN-R using *p416TEF* as template, and cloned into *p415SL* after digestion with *Xma*I/*Xho*I, resulting *p415SL-URA3*. Finally, *SUC2-LEU2* promoter in *p415SL-URA3* was replaced with *FLO11* promoter that was PCR-amplified with primer 9-10F and 1-3R from chromosomal DNA of BY4741 (#LY746), through *Xma*I/*Sac*II sites, created *p415F19-URA3*. All the newly created plasmids were confirmed by restriction digestion and DNA sequencing. PrimeSTAR HS DNA Polymerase (TaKaRa), a novel enzyme with high efficiency was used in PCR to amplify DNA for cloning purpose. To prepare chromosomal DNA templates for PCR,

yeast cells were simply boiled for 10 min in 20 mM NaOH solution and briefly cleared at 600 g for 2 min. The crude supernatants were used as PCR templates.

Yeast strains and engineering.

Eighty-three *S. cerevisiae* strains were used and 54 were created in this study, which are all S288C-derivatives with the exception of LY422 and DY902 that are derivatives of 74D-694 (Table S3). All yeast cultures were grown at 30^{o}C.

By sporulation and tetrad dissection, *MATa* (LY220) and *MATα* (DY441) *swi1Δ* haploid strains were obtained from a diploid strain (LY747). To generate *swi1Δ* and wild-type S288C, a [*PIN*$^{+}$] wild-type BY4741 strain (LY746) was transformed with *p315CUP1-RNQ1GFP*, mated with DY441 carrying plasmid *p416TEF-SWI1*. Diploids were selected by growth on synthetic complete (SC) medium without histidine and uracil. The acquired diploids were further sporulated and assayed for their genotypes, leading to LY756, DY771, DY770 and DY777. These strains including other [*PIN*$^{+}$] strains were further grown on 5 mM guanidine hydrochloride (GdnHCl)-containing media to get rid of the [*PIN*$^{+}$] prion, which was confirmed by re-introduction of *pCUP1-RNQ1GFP* or *p315CUP1-RNQ1GFP* and fluorescence microscopy assays for Rnq1GFP status. The RNQ1-GFP plasmids were then removed from these strains before further experiments.

To repair the *FLO8* point mutation of S288C-derived strains, either *pHL11* or *pRS303-FLO8* was linearized by *Bgl*II or *Sal*I, and used to transform various yeast strains, which confer Ura^{+} and His^{+} phenotype, respectively. For wild-type strains, gain of adhesive growth (Adh^{+}) was an indication of a successful repair of *FLO8*. DY760 and DY894 were derived from LY746 and DY771 after restoration of the *FLO8* gene with linearized *pRS303-FLO8*, respectively. DY777 without containing prions and plasmids was transformed with linearized *pHL11,* and the resulting Ura^{+} and isolates were subsequently counter-selected by growing on 5 FOA-containing medium to loop out the chromosomal *URA3* gene. The resulting strain of LY787 was Ura^{-} but still Adh^{+}, an indication of *FLO8* maintenance. LY741, a *FLO8::HIS3* [*SWI*$^{+}$] strain, was generated from LY742 upon repairing *FLO8* with plasmid *pRS303-FLO8*. Selection of the right clones was assisted by the fact that real *FLO8*-repaired strain would turned from His^{+} Raf^{-} Adh^{-} to His^{-} Raf^{+} Adh^{+} upon GdHCl treatment. To repair *FLO8* of two *swi1Δ* strains, LY720 (*MATa*) and DY770 (*MATα*), they were transformed with *p416TEF-C* to provide Swi1 function before introducing the linearized *pRS303-FLO8*. A successful repair was suggested by a phenotypic switch from His^{+} Raf^{+} Adh^{+} to His^{+} Raf^{-} Adh^{-} after removing *p416TEF-C* by counter-selection with 5 FOA, resulting DY761 and DY895, respectively. Similarly, DY762, DY763 and DY896 were derived from LY732, LY733 and LY734 after repairing the *FLO8* gene with *pRS303-FLO8* that was linearized with *Sal*I.

To replace *FLO1* ORF with *URA3* ORF, *URA3* ORF was firstly PCR-amplified with primer pair of flo1ura-F and flo1ura-R from template of *p416TEF*, and further amplified from the 1st PCR products with primer pair of Flo1-F and Flo1-R to keep integrity of the *FLO1* upstream and downstream extensions for efficient homologous recombination. The acquired PCR products were then used to transform DY760 that was selected on SC-his-ura plates to get

DY755. The right isolates were verified by PCR detection with primer pair of Flo1-F/Flo1-R and by loss of Adh^+ phenotype. Similar procedure was followed to replace the *FLO1* gene of a *swi1Δ* strain (DY761) to generate LY762, except that *p415TEF-C* was pre-introduced into the strain to provide to assist the screen. To replace *FLO11* ORF with *URA3* ORF, *URA3* ORF was firstly PCR-amplified with primers of Flo11ex-F and Flo11URA3-R with *p415F19-URA3* as template, and secondary PCR was conducted with primer pair of Flo11ex-F/Flo11ex-R to keep integrity of its upstream and downstream extensions. The PCR products were then used to transform DY760 that was selected on SC-his-ura plates, and subsequently screened for isolates that Adh^+ phenotype, and then verified by PCR with primer pair of Flo11ex-F/Flo11ex-R, resulting DY758. Similarly, starting from DY761 (*FLO8::HIS3 swi1Δ*) containing *p415TEF-C, FLO11* was replaced, resulting LY763. To generate isogenic [SWI^+] and [SWI^+][PIN^+] strains with *FLO1* or *FLO11* ORF replaced by the ORF of *URA3*, [SWI^+] was introduced in to DY755 and DY758 by crude protein extract transformation using DY902 as a donor, with co-introduction of plasmid *p415TEF-NQYFP*, resulting LY735, LY736, LY737, and LY738, respectively. Similar extract transformation was carried to introduce [SWI^+] into LY787 to create LY786, also using DY902 as a donor. The resulting strains were selected and confirmed based on their phenotypic switch from His^+ Raf^- Adh^- to His^+ Raf^+ Adh^+ upon GdnHCl treatment, and by checking their *MET15* and *LYS2* markers. The *swi1Δ* [SWI^+] strain LY756 was generated independently in the study with a method similar to what published earlier (Crow et al., 2011). In brief, crude cell extract prepared from DY902 were introduced in LY720 that had been transformed with *p416TEF-SWI1*, and *p415TEF-NQYFP* was also co-introduced to facilitate the prion detection.

To remove the auxotrophic marker *HIS3* from strain of *FLO8::HIS3 flo1Δ::URA3* [SWI^+] (DY735), *HIS3* ORF was replaced with *MET15* ORF further from DY735, resulting LY770. To do so, *MET15* ORF was firstly PCR-amplified from the chromosomal DNA of LY422 using primer pair of MET15-F/MET15-R, and the secondary PCR was conducted with primer pair of HtoM-F/HtoM-R using the 1^{st} round of PCR products as templates. Upon transforming DY735 with the PCR products, transformants were selected for His^- Met^+ and curable [SWI^+] phenotypes. Isogenic *FLO8::MET15 flo1Δ::URA3* [swi^-] strain (LY773) was obtained after treatment of LY770 with GdnHCl.

To test the effect of Flo8, Flo1, Flo11, swi1, and [SWI^+] on pseudohyphae formation, diploids without, with one copy of, or with two copies of the *FLO8* gene were created by mating. As shown in Table S3, only those with two copies of *FLO8::HIS3* were listed. To assist the mating experiment, all the used *MATα* strains were transformed with *p415TEF* that is Leu^+, and all the *MATa* strains contained *p416TEF* that is Ura^+. After mating, the resulting diploids were selected by auxotrophic markers and confirmed by their morphology, genotypes and phenotypes. Subsequently, plasmids in these diploids were removed either by counter-selection with 5 FOA (for *p416TEF*), or by spontaneous loss (for *p415TEF*). These plasmid-free diploids were then transformed with *p416TEF-NQYFP* and *pCUP1-RNQ1* to check the Swi1 and Rnq1 status by fluorescence microscopy before experiments. Eventually, diploid strains of DY897, DY898, DY899, DY900, and DY901 were from mating of pairs of haploids, DY760/DY894, DY761/DY895, LY741/DY894, DY763/DY896, DY760/DY895, respectively.

Media, chemicals, and buffers.

General yeast cultivation and culture manipulations were performed as described previously (Fan et al., 2007; Park et al., 2006). LB, Yeast extract-peptone-dextrose (YPD) and synthetic complete (SC) with dropout of certain amino acids were the most used media in this study. SC+5 FOA is a regular SC medium with 0.1% 5 FOA, pH4.0. Raffnose media were described earlier (Du et al., 2008). In a glactose induction experiment, 2% sucrose was used to substitute glucose as carbon source. Semi-solid YPD was the same as YPD except it contains only 0.3% bacto agar and 0.2% glucose, which was used for biofilm formation assay (Reynolds and Fink, 2001). SLAD (2% bacto agar, 2% glucose, 0.67% bacto yeast nitrogen base without amino acids or ammonium sulfate, 50 μM ammonium sulfate, and essential amino acid supplements) was used in pseudohyphae formation assay (Gimeno et al., 1992). In this study, 4% glucose was used instead of 2% for SLAD and the agar was washed twice with water before autoclave and cells were incubated for 6 days. Sterile ammonium sulfate and amino acid stocks were made by filtration through a 0.22 μm filter. Pre-sporulation plate (0.8% yeast extract, 0.3% bacto peptone, 10% glucose, 1.5% bacto agar, and 100 mg/L adenine sulfate that just for 74D-694) and Sporulation plate (0.98% potassium acetate, 0.05% glucose, 0.1% yeast extract, 1.5% bacto agar, 100 mg/L adenine sulfate for strains of 74D-694 background, and 1X concentrations of other auxotrophies depending on the strain backgrounds) were used for sporulation. Mating-type test of haloid yeasts was done with –AA medium (6.75 g/L bacto yeast nitrogen base without amino acids, 20 g/L glucose and 19 g/L bacto agar). The bottom agar, top agar, ST buffer, STC buffer, PTC buffer, and SOS buffer and other reagents used in the crude cell extract transformation experiments to transfer [*SWI*$^+$] from a donor to recipients were described previously (Du et al., 2010). Antimycin A (Sigma) was supplemented to a final concentration of 1 μg/ml in the raffinose medium. The G418 concentration was 300 μg/ml for yeast cultures. To express a *CUP1* promoter-controlled protein, 50 μM final $CuSO_4$ was supplemented in a specific SC medium. For *GAL1* promoter, either 2% or 0.5% galactose, the inducer, was added as final concentration to a log-phase selective SC culture with sucrose as sole carbon source. To cure prions, cells were grown in a liquid medium or solid agar plate supplemented with 5 mM GdnHCl. In LB media, 100 μg/ml ampicillin and/or 30 mg/ml kanamycin could be supplemented for *Escherichia coli* culture to maintain the plasmids the strain harbors. Crystal violet with a final concentration of 0.1% was used to stain yeast cells adhesive onto polystyrene surfaces.

Mating, mating-type test, and [*SWI*$^+$] transfer.

Plasmid-assistant mating between *MATa* and *MATα* haploid strains was realized by introducing proper plasmids into the mating partners to assist selection prior to mating. Mating was conducted by mixing equal amount of the log-phase cultures of mating partners and fresh medium, and by incubating the mixture at 30 ^{o}C for ~6 h on a wheel rotator at a low speed (~50 RPM). After a slow speed of spin at 100-200 g for 1 min, cell pellets were re-suspected in fresh medium, vigorously vortexed, and spread onto selective SC media. Mating-type test was performed using D585a and D84α as testers. A protein transformation-based transfer of [*SWI*$^+$]

was described previously (Du et al., 2010), which was a modified protocol published earlier (Tanaka and Weissman, 2006). In such an experiment, a 74D-694 *TRP1::TEF::RNQ1CFP* [SWI^+] (Crow et al., 2011), DY902, was used as [SWI^+] donor to transfer the prion into a set of S288C-derivatives as recipients. The advantage of DY902 being a [SWI^+] donor is that it's convenient to monitor the Rnq1 prion ([PIN^+]) status. In these experiments, the donor strain was ensured to be [pin^-] right before preparing cell lysates.

Adhesive growth, flocculation, cell mobility and psedohyphal formation assays.

Adhesive growth on YPD and SC plates were performed as described previously (Braus et al., 2003; Fichtner et al., 2007; Roberts and Fink, 1994) with minor changes. In brief, cells from fresh cultures or agar plates were washed with TE pH7.5 buffer, properly diluted, and spotted onto YPD agars. Plates were incubated for 3 d at 30^{o}C and shifted to room temperature for extra 3 d before washing assays. A gentle wash was performed in a water bowl in a time course, and a vigorous wash was done using tap water with or without rubbing. Similar procedure was followed for SC plates with the exception that either 2% or 0.2% glucose was supplemented as carbon source. Images were taken prior to and after each wash steps. To see the possible morphological changes upon starvation, cells at each washing step were collected and observed with microscope.

Adhesion to plastic surfaces was conducted with flat-bottom 96-well micro-titer polystyrene plates (Reynolds and Fink, 2001), with modifications. In brief, overnight cultures were prepared from regular YPD and SC selective media, washed twice with sterile water before diluting cells into 150 µl regular YPD, YPD with 0.1% glucose, regular SC, or SC with 0.1% glucose. After growing overnight, plates were rinsed twice with PBS buffer before being stained with 0.1% crystal violet for 10 min. The stained cells were then extracted with 200 µl ethanol and measured for optical density at 595 nm. Images were taken for total growth and after staining and elusion.

Flocculation assays were carried our similarly to previous reports (Kobayashi et al., 1996). Basically, after two days of growth in YPD or SC media, flocculating ability of cells was assessed on the presence of visible cell aggregates. To quantify, cells were washed twice with 250 mM EDTA, and once with 250 mM NaCl pH 2.0, and re-suspended in 3 ml 250 mM NaCl pH4.5. A 20 µl aliquot was taken to 180 µl 250 mM NaCl pH2.0 as control sample (ck). Upon adding 4 mM pH4.5 $CaCl_2$, cell suspensions were mixed well by inverting the tubes for 18 times and stay in RT. At each time of the stay, a 20 µl aliquot was taken to mix with 180 µl 250 mM NaCl pH2.0 as sample. Flocculation was judged with the absoebance at 620 nm, with an equation of % of setlled cells=$100*(A^{ck}-A^{sample})/A^{ck}$.

Yeast's mobility associated with biofilm formation was assayed on semi-soft YPD plates with 0.3% and 0.2% glucose according to an earlier study (Reynolds and Fink, 2001). The regular YPD plate was used as control. Plates were photographed in a time course up to 13 days of incubation at 25°C. The diameter of the cell lawn was used as the judgment of the cell motility. Pseudohyphal growth of diploids was tested on SLAD plates (Gimeno and Fink, 1992). 2% or 4%

glucose was supplemented as carbon source and the pseudohyphae formation was observed under microscope in a time course up to 6 days of incubation.

Fluorescence microscopy.

In this study, GFP, YFP, and mCherry served as tags for variety of fluorescent fusion proteins. These fusions were produced either ectopically from a plasmid or endogenously from chromosome. Methods for fluorescence microscopy were described previously (Du and Li, 2014). For constitutive promoter-driven expression, either log-phase cultures or fresh colonies were used for the assay. For an inducible promoter such as the *CUP1* or *GAL1* promoter, $CuSO_4$ or galactose was added at log-phase of a culture to induce the expression before being observed by fluorescence microscope in a time course.

Semi-denaturing detergent agarose gel electrophoresis (SDD-AGE).

SDD-AGE assay was carried out based on the published protocol (Halfmann and Lindquist, 2008). Briefly, endogenous GFP-fusion proteins, or ectopically expressed YFP fusion proteins were used in the assay. The ectopic YFP fusions were either expressed from a *CEN-TEF-*, *CEN-GAL1-*, or *2μ-GAL1*-based plasmid. For the *GAL1* promoter set, 0.5% galactose was added in log-phase cultures developed from various media with sucrose as the sole carbon source. In a time course, expression of these YFP fusions was monitored by fluorescence microscopy. Cells with visible YFP signals whose intensities were comparable between an [SWI^+] strain and its isogenic [swi^-] partner were collected for SDD-AGE assays. For *CEN-TEF*-based expression, log-phase cultures were harvested for the assay. Spheroplasting was done with Lyticase from *Arthrobacter luteus* (Sigma). Generally, a pre-clearing step with a centrifugation speed of 4000 g X 2 min was used as described (Halfmann and Lindquist, 2008). In this condition, both the supernatant and pellet fractions were run for SDD-AGE. For *CEN-GAL1*-based expression, the clearance was also conducted at 500 g X 5 min followed by DNase I treatment (10U/ml lysate) for 10 min at room temperature to reduce the viscosity of the lysates. Samples were treated at 25 ^oC or 100 ^oC for 30 min in 2% SDS before loading to a 1.5% agarose gel. After capillary transfer, PDVF membranes were probed with the monoclonal anti-GFP antibody as well.

RT-PCR.

Total mRNA was extracted and purified with RNeasy Mini Kit (GIAGEN, cat# 74104), and cDNA was synthesized with SuperScript First-strand Synthesis SuperMix (Invitrogen, cat# 18080-400) with oligo(dT) according to the manufacturer's instructions. PCR was performed with primer pair of FLO1-F and FLO1-R to detect the *FLO1* gene transcription, using 50 ng cDNA as template in a 50 μL system. Regular Taq polymerase was used for PCR, and the reaction was terminated after 25 cycles.

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