Supplementary Information

The two-component response regulator Skn7 belongs to a network of transcription factors regulating morphogenesis in *Candida albicans* and independently limits morphogenesis-induced ROS accumulation

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Supplementary Figure Legends

Figure S1. Effect of ATc on cells after 2 h and 4 h liquid culture. The *SKN7* overexpressing strains CEC4228 (P_{TET}-*SKN7-HA*$_3$) and CEC4848 (P_{TET}-*SKN7*) were grown o/n in rich medium, and diluted at an OD_{600}=0.2 in YPD or YPD supplemented with 3 μg.mL^{-1} ATc. Cultures were grown at 30°C in the dark for 2 h (left panel) or 4 h (right panel).

Figure S2. Expression analysis of selected genes in filament-inducing condition. Expression of the *EED1*, *CPH1, UME6*, *HWP1, IHD1*, *TSA1* and *GPX2* genes was quantified for ΔΔ*skn7* (CEC4220) and ΔΔ*skn7*+*SKN7* (CEC4682) strains grown on solid YPD or Spider media, by RT-qPCR using primers specific for the selected regions (Table S6). *ACT1* was used as a control. The experiments were performed on 3 biological replicates and error bars show the SD. Statistical significance was assigned by performing 2-tailed Student's *t*-tests that compare ΔΔ*skn7* with ΔΔ*skn7*+*SKN7*, followed by false discovery rate according to Benjamini and Hochberg (1995). (** p≤0.01, *** p≤0.001).

Figure S3. Effect of ATc on the colony phenotype of knockout mutants. The WT reference strains SC5314 and the knockout mutants ΔΔ*sfl2* (CEC1535), ΔΔ*sfl1* (CEC2011), ΔΔ*cph1* (CEC2297), ΔΔ*ume6* (CEC2664), ΔΔ*efg1* (CEC3907), ΔΔ*skn7* (CEC4220), ΔΔ*eed1* (CEC4637), ΔΔ*czf1* (CEC4829) and ΔΔ*tec1* (CEC4831) were grown o/n in YPD, and streaked on YPD or YPD supplemented with 3μg.mL^{-1} ATc. Pictures were taken after 2 days incubation in the dark at 30°C, using a Leica M80 stereomicroscope equipped with a DMC2900 colour camera, at a 7.5x magnification.

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Fig. S4. Embedded phenotype of *SKN7* or *CZF1* overexpressing strains. The WT reference strain SC5314 and strains allowing overexpression of either P_{TET}*SKN7* (CEC4963) or P_{TET}*CZF1* (CEC5068) in a WT background were grown in embedded conditions at 25°C for 5 days. For overexpression, 3 μg.mL^{-1} ATc was added to each layer, and plates were incubated in the dark at 25°C for 5 days. Pictures were acquired on a Leica M80 stereomicroscope equipped with a DMC2900 colour camera, at a 16x magnification. Several fields of representative cells might have been merged.

Table S1. Strains used in this study.

Strain ID	Strain name	Genotype	References
SC5314	SC5314		Gillum *et al.*, 1984
CAI4	CAI4	*ura3Δ::λimm434/ura3Δ::λimm434*	Fonzi and Irwin, 1993
SN76	SN76	*arg4Δ/arg4Δ his1Δ/his1Δ ura3Δ::λimm434/ura3Δ::λimm434 iro1Δ::λimm434/iro1Δ::λimm434*	Noble *et al.*, 2005
BWP17	BWP17	*ura3Δ::λimm434/ura3Δ::λimm434 his1Δ::hisG/ his1Δ::hisG arg4Δ::hisG/arg4Δ::hisG*	Wilson *et al.*, 1999
CEC161	BWP17 AH	*ura3Δ::λimm434/ura3Δ::λimm434 his1Δ::hisG/HIS1 arg4Δ::hisG/ARG4*	Firon *et al.*, 2007
CEC377	*ΔΔpga59,pga62*	BWP17 *pga59,pga62Δ::HIS1/pga59,pga62Δ::ARG4 RPS1/RPS1::*CIp10	Moreno-Ruiz *et al.*, 2009
CEC955	BWP17 AH pNIM1	*ura3Δ::λimm434/ura3Δ::λimm434 his1Δ::hisG/HIS1 arg4Δ::hisG/ARG4 ADH1/adh1::P_{ADH1}-cartTA::SAT1::P_{TET}-caGFP*	Chauvel *et al.*, 2012
CEC1084	P_{TET}-*CPH1*	BWP17 AH pNIM1 *RPS1/RPS1::*CIp10-P_{TET}-*CPH1*	This study
CEC1085	P_{TET}-*TEC1*	BWP17 AH pNIM1 *RPS1/RPS1::*CIp10-P_{TET}-*TEC1*	This study
CEC1535	*ΔΔsfl2*	SN76 *sfl2Δ::ARG4/sfl2Δ::HIS1 RPS1/RPS1::*CIp10	Znaidi *et al.*, 2013
CEC1569		SN76 *sfl2Δ::ARG4/sfl2Δ::HIS1 ADH1/adh1::P_{TDH3}-carTA::SAT1*	Znaidi *et al.*, 2013
CEC2001	*ΔΔsfl1*	SN76 *sfl1Δ::ARG4/sfl1Δ::HIS1 RPS1/RPS1::*CIp10	Znaidi *et al.*, 2013
CEC2293	*ΔΔhog1*	BWP17 *hog1::loxP-ARG4-ura3-loxP/hog1::loxP-HIS1-loxP RPS1/RPS1::*CIp10-*gLUC59*	Lab collection
CEC2297	*ΔΔcph1*	CAI4 *cph1Δ::hisG/cph1Δ::hisG efg1Δ::hisG/efg1Δ::hisG RPS1/RPS1::*CIp10-*gLUC59*	Lab collection
CEC2664	*ΔΔume6*	SN76 *ume6Δ::ARG4/ume6Δ::HIS1 RPS1/RPS1::*CIp10	Lab collection
CEC2907	BWP17 AH pNIMX	*ura3Δ::λimm434/ura3Δ::λimm434 his1Δ::hisG/HIS1 arg4Δ::hisG/ARG4 ADH1/adh1::P_{TDH3}-carTA::SAT1*	Chauvel *et al.*, 2012
CEC2994	P_{TET}-*UME6*	BWP17 AH pNIMX *RPS1/RPS1::*CIp10-P_{TET}-*UME6*	This study
CEC3373		SN76 *ume6Δ::ARG4/ume6Δ::HIS1 ADH1/adh1::P_{TDH3}-carTA::SAT1*	This study
CEC3420	*ΔΔefg1+SKN7*	CAI4 *efg1Δ::hisG/efg1Δ::hisG ADH1/adh1::P_{TDH3}-carTA::SAT1 RPS1/RPS1::*CIp10-P_{TET}-*SKN7*	This study
CEC3424	*ΔΔsfl2+SKN7*	SN76 *sfl2Δ::ARG4/sfl2Δ::HIS1 ADH1/adh1::P_{TDH3}-carTA::SAT1 RPS1/RPS1::*CIp10-P_{TET}-*SKN7*	This study
CEC3568		SN76 *tec1Δ::ARG4/tec1Δ::HIS1 ADH1/adh1::P_{TDH3}-carTA::SAT1*	This study
CEC3645		SN76 *cph1Δ::ARG4/cph1Δ::HIS1 ADH1/adh1::P_{TDH3}-carTA::SAT1*	This study
CEC3785	BWP17 AH pNIMX-bcGTW	BWP17 AH pNIMX *RPS1/RPS1::*CIp-P_{TET}-*GtwB*	Cabral *et al.*, 2014
CEC3836	*ΔΔsfl1+SKN7*	SN76 *sfl1Δ::ARG4/sfl1Δ::HIS1 ADH1/adh1::P_{TDH3}-carTA::SAT1 RPS1/RPS1::*CIp10-P_{TET}-*SKN7*	This study
CEC3907	*ΔΔefg1*	CAI4 *efg1Δ::hisG/efg1Δ::hisG RPS1/RPS1::*CaEXP-*URA3*	Lab collection
CEC4219		SN76 *skn7Δ::HIS1/skn7Δ::ARG4*	This study
CEC4220	*ΔΔskn7*	SN76 *skn7Δ::HIS1/skn7Δ::ARG4 RPS1/RPS1::*CIp10	This study
CEC4228	P_{TET}-*SKN7-*HA_3	BWP17 AH pNIMX *RPS1/RPS1::*CIp10-P_{TET}-*SKN7-*HA_3	This study
CEC4272	*skn7*^{D474}	SN76 *skn7Δ::HIS1/skn7Δ::ARG4 ADH1/adh1::P_{TDH3}-carTA::SAT1 RPS1/RPS1::*CIp10-P_{SKN7}-*skn7*$^{D474/A}$-HA_3	This study
CEC4273	*skn7*^{T484}	SN76 *skn7Δ::HIS1/skn7Δ::ARG4 ADH1/adh1::P_{TDH3}-carTA::SAT1 RPS1/RPS1::*CIp10-P_{SKN7}-*skn7*$^{T484/A}$-HA_3	This study
CEC4274	*skn7*^{T496}	SN76 *skn7Δ::HIS1/skn7Δ::ARG4 ADH1/adh1::P_{TDH3}-carTA::SAT1 RPS1/RPS1::*CIp10-P_{SKN7}-*skn7*$^{T496/A}$-HA_3	This study
CEC4277	*skn7*^{F76,L83}	SN76 *skn7Δ::HIS1/skn7Δ::ARG4 ADH1/adh1::P_{TDH3}-carTA::SAT1 RPS1/RPS1::*CIp10-P_{SKN7}-*skn7*$^{F76/A,L83/A}$-HA_3	This study
CEC4393	P_{TET}-*SFL2*	BWP17 AH pNIMX *RPS1/RPS1::*CIp10-P_{TET}-*SFL2-*HA_3	This study

CEC4458	*ΔΔume6+SKN7*	SN76 *ume6Δ::ARG4/ume6Δ::HIS1 ADH1/adh1::*P_{TDH3}*-carTA::SAT1 RPS1/RPS1::*CIp10-P_{TET}*-SKN7*	This study
CEC4462	*ΔΔcph1+SKN7*	SN76 *cph1Δ::ARG4/cph1Δ::HIS1 ADH1/adh1::*P_{TDH3}*-carTA::SAT1 RPS1/RPS1::*CIp10-P_{TET}*-SKN7*	This study
CEC4464	*ΔΔtec1+SKN7*	SN76 *tec1Δ::ARG4/tec1Δ::HIS1 ADH1/adh1::*P_{TDH3}*-carTA::SAT1 RPS1/RPS1::*CIp10-P_{TET}*-SKN7*	This study
CEC4474	*ΔΔskn7+SFL2*	SN76 *skn7Δ::ARG4/skn7Δ::HIS1 ADH1/adh1::*P_{TDH3}*-carTA::SAT1 RPS1/RPS1::*CIp10-P_{TET}*-SFL2*	This study
CEC4617		CAI4 *czf1::hisG/czf1::hisG*	Brown *et al*., 1999
CEC4636		BWP17 *eed1Δ::HIS1/eed1Δ::ARG4*	Martin *et al*., 2011
CEC4637	*ΔΔeed1*	BWP17 *eed1Δ::HIS1/eed1Δ::ARG4 RPS1/RPS1::*CIp10	Martin *et al*., 2011
CEC4649	*ΔΔskn7+TEC1*	SN76 *skn7Δ::ARG4/skn7Δ::HIS1 ADH1/adh1::*P_{TDH3}*-carTA::SAT1 RPS1/RPS1::*CIp10-P_{TET}*-TEC1*	This study
CEC4650	*ΔΔskn7+UME6*	SN76 *skn7Δ::ARG4/skn7Δ::HIS1 ADH1/adh1::*P_{TDH3}*-carTA::SAT1 RPS1/RPS1::*CIp10-P_{TET}*-UME6*	This study
CEC4653	*ΔΔeed1+SKN7*	BWP17 *eed1Δ::HIS1/eed1Δ::ARG4 ADH1/adh1::*P_{TDH3}*-carTA::SAT1 RPS1/RPS1::*CIp10-P_{TET}*-SKN7-*HA_3	This study
CEC4654		BWP17 *eed1Δ::HIS1/eed1Δ::ARG4 ADH1/adh1::*P_{TDH3}*-carTA::SAT1*	This study
CEC4655		SN76 *skn7Δ::ARG4/skn7Δ::HIS1 ADH1/adh1::*P_{TDH3}*-carTA::SAT1*	This study
CEC4656	*ΔΔskn7+CPH1*	SN76 *skn7Δ::ARG4/skn7Δ::HIS1 ADH1/adh1::*P_{TDH3}*-carTA::SAT1 RPS1/RPS1::*CIp10-P_{TET}*-CPH1*	This study
CEC4682	*ΔΔskn7+SKN7*	SN76 *skn7Δ::HIS1/skn7Δ::ARG4 ADH1/adh1::*P_{TDH3}*-carTA::SAT1 RPS1/RPS1::*CIp10-P_{SKN7}*-SKN7-*HA_3	This study
CEC4811		CAI4 *czf1::hisG/czf1::hisG ADH1/adh1::*P_{TDH3}*-carTA::SAT1*	This study
CEC4812	P_{TET}*-EED1*	BWP17 AH pNIMX *RPS1/RPS1::*CIp10-P_{TET}*-EED1*	This study
CEC4813	*ΔΔskn7+EED1*	SN76 *skn7Δ::ARG4/skn7Δ::HIS1 ADH1/adh1::*P_{TDH3}*-carTA::SAT1 RPS1/RPS1::*CIp10-P_{TET}*-EED1*	This study
CEC4815	*ΔΔczf1+SKN7*	CAI4 *czf1::hisG/czf1::hisG ADH1/adh1::*P_{TDH3}*-carTA::SAT1 RPS1/RPS1::*CIp10-P_{TET}*-SKN7*	This study
CEC4829	*ΔΔczf1*	CAI4 *czf1::hisG/czf1::hisG RPS1/RPS1::*CIp10	This study
CEC4831	*ΔΔtec1*	SN76 *tec1Δ::ARG4/tec1Δ::HIS1 RPS1/RPS1::*CIp10	This study
CEC4848	P_{TET}*-SKN7*	BWP17 AH pNIMX *RPS1/RPS1::*CIp10-P_{TET}*-SKN7*	Chauvel *et al*., 2012
CEC5069	*ΔΔskn7+CZF1*	SN76 *skn7Δ::ARG4/skn7Δ::HIS1 ADH1/adh1::*P_{TDH3}*-carTA::SAT1 RPS1/RPS1::*CIp10-P_{TET}*-CZF1ΔattB2*	This study

Table S6. Primers used in this study

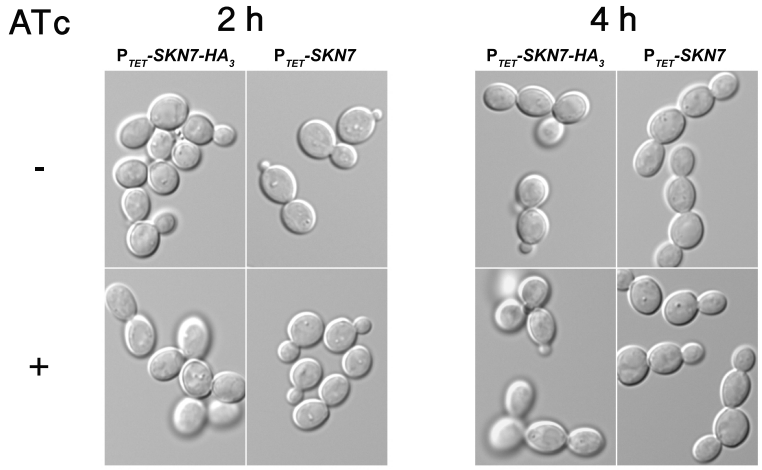
Name	Sequence[1]
ForHindIII	CATACGTCCAATATCGAGTCCT
RevD474	CGAAAGAACGAATCACACTTGTGGCCGTTGCCCCGTCTAGGTTTGGCATAACAATAgCCATTAAAACCAAATCATATTTC
RevT484	ATCGAAAGAACGAATCACACTTGTGGCCGcTGCCCCGTCTAGGTTTGGC
ForT496	CTAGACGGGGCAACGGCCACAAGTGTGATTCGTTCTTTCGATACAAAAgCCCCAATCATTGCCATGACAGG
RevPstI	AACATCAGAGATTTTGAGACACG
ForF76	TCAAACgcTGCCAGTTTTGTACGTCAGgcGAACAAGTATGATTTCCATAAAGTA
RevDraI	CTCTTGATACAAGCTATGTTTATC
ForSacI	ATGTCTTCATTACAACAACCCATAC
RevF76	TTCGTTTGAGATCTTTACTTTATGGAAATCATACTTGTTCgcCTGACGTACAAAACTGGCAgcGTTTGAGTGCTTGAAATGTTT
CipUL	ATTACTATTTACAATCAAAGGTGGTC
CipUR	ATTACTATTTACAATCAAAGGTGGTC
SKN7KO_F	AAAATAAAAGTAAATATTGGAGCATTATCCTATAAATCTGTTCAAGGACAACACTCAATCCATTATTTACCCCCTTGCGTATTCTTGTTCATGTATAATA*gctc ggatccactagtaacg*
SKN7KO_R	CAGTATACTTTGATAAAGTACAGATTTCTCGATATCTACACACCAATAAAAATGACGCATGGGGTACTTCCTTTACCGATTTAGCATACCATTATATTTC*acc agtgtgatggatatctgc*
SB095	GTCGATCCAATGTACGTACTGCGG
SB096	CCTACTTCCAATTTCTAGTTAACCATTTATTATACATGAACAAGAATACGCAAGG
SB204	CAGTTCTTCGAGCTCACCAACTGC
SB205	TTATTAACAGGTATACTTATTTACTTCTGTATTCAACAATACCTC

[1]Uppercase: nucleotides identical to *SKN7* sequence; lowercase: mutated nucleotides; lowercase italics: nucleotides hybridizing on pSN plasmids

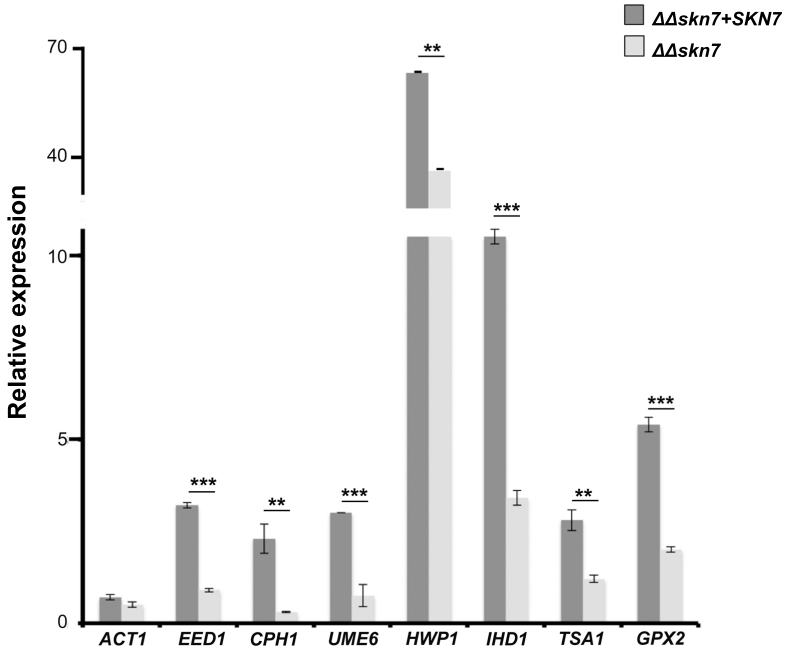
Table S7. List of qPCR primers

Name	Sequence (5’ to 3’)
TEF3F	CAAGAAATGTCCATCTGCTCAATC
TEF3R	TGTTTAGTTTTAACCCCTTCCAAGA
RPL18F	AGAATGGTGGTGAAGCCATT
RPL18R	GTGGACCGAAACCAAAGTGT
ACT1F	TATGAAAGTTAAGATTATTGCTCCACCAGAAA
ACT1R	GGAAAGTAGACAATGAAGCCAAGATAGAAC
TSA1F1-qPCR	GCAGGAATATCGACCCAAAA
TSA1R1-qPCR	CAGCGCAACAGACCATTTTA
RBT4F1-qPCR	GTCACAAAAAGGGGGAACAA
RBT4R1-qPCR	CCTGCCAGGATTTTCAAGTG
DEF1F1-qPCR	AACCCATTTGATACATATACGCTAA
DEF1R1-qPCR	TTCCTGAGAGTAGTTTGTTTGTTTG
UME6F1-qPCR	CAATTAGAAACCAACAGAGGAAAG
UME6R1-qPCR	CAACTCCCGGGAAATTCTATAC
TEC1F1-qPCR	CTACTACTACTACACACTTGACC
TEC1R1-qPCR	CCTATTGTACCTTAAAGGAACAAC
SFL1-qPCR	CAAGAGCTCAAAACCACAAAC
SFL1-qPCR	CTTTATCAATAAAGTGGCGATGG
SFL2-qPCR	GGGAGAATACTTTAAGAAAAATC
SFL2-qPCR	GAATGATGGAATTGAAAATTGTG
SKN7F-qRT	GGTCCAATATCATCAGATACAGCAT
SKN7R-qRT	AGATTCTGTCCAAGTGACTGTTGTT
TSA1F-qRT	ACCAACCACTCCTTGTCCAG
TSA1R-qRT	TTGGAAAGCCTCCAACAATC
UME6F-qRT	TCTACTTCTAATCCAATGGTG
UME6R-qRT	TATCATTACTTGATTTTTTCCGAG
HWP1F-qRT	ATCAGCTCCTGCCACTGAAC
HWP1R-qRT	TGAGTGGAACTGATTCTAATGTAGTTG
DEF1F-qRT	TAGTGGTAATACCCAACGTG
DEF1R-qRT	CTGATATTTGAAATTTTGGAAGCTTTTC
CPH1F-qRT	TATGACGCTTCTGGGTTTCC
CPH1R-qRT	ATCCCATGGCAATTTGTTGT
IHD1F-qRT	GGTACTGCTGCCACCAATAC
IHD1R-qRT	ACCTGTCTTCTTAGCAGCGT
SFL1F-qRT	CCGACACCAGTAAATCATTC
SFL1R-qRT	GCAACAGAACTGTCATTTAG
EFG1F-qRT	TACCAGGTCAACAAGCAGTACCTAT
EFG1R-qRT	ACATGGTAGTTGTTACTCGTGGTCT
UME6F-qRT	TCTACTTCTAATCCAATGGTG
UME6R-qRT	TATCATTACTTGATTTTTTCCGAG
SFL2F-qRT	CAGCATCAGCTTTATCTTCC
SFL2R-qRT	ACGATAGTTGGTTGAATTCA
CZF1F-qRT	GTATTCTGCTGCTGGTA
CZF1R-qRT	TTGTTGCTTGACTTGTTG
BRG1F-qRT	GGTCATATAATAGCAGTGCA
BRG1R-qRT	ATAGTGTAACCCACATTAGG

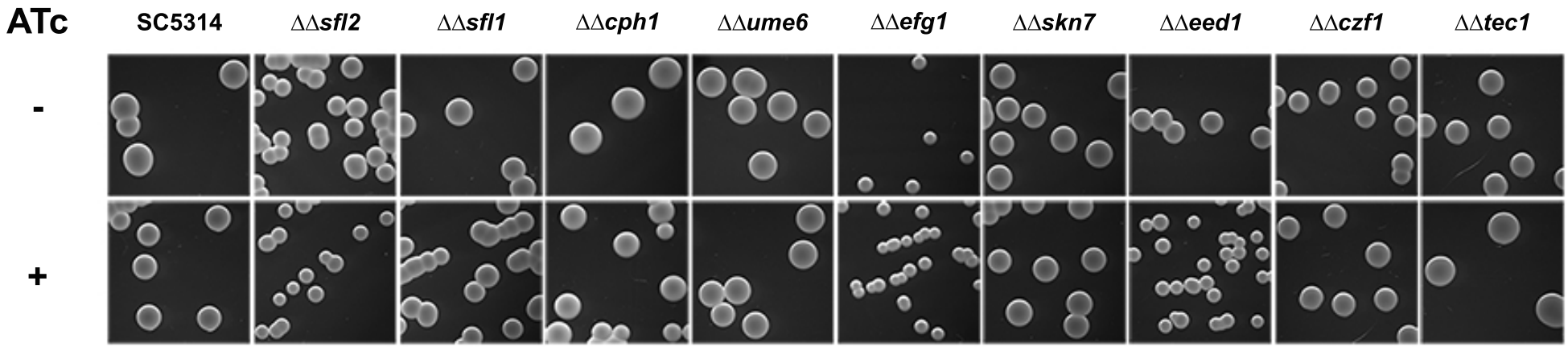
Supplemental Figure 1.



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4