Supplemental Materials

Molecular Biology of the Cell

Merlini et al.

SUPPLEMENTAL FIGURE LEGENDS

Figures S1: Rho1 hyperactivation suppresses lethality and cytokinesis defects of *dma1Δ dma2Δ cla4Δ* cells and the temperature-sensitivity of *cdc5* mutants. A,C-E: Serial dilutions of strains with the indicated genotypes were spotted on YEPD plates and incubated at the indicated temperatures for 2 days. **B:** Wild type and *dma1Δ dma2Δ cla4Δ RHO1-D72N* cells were arrested in G1 by α-factor and released into fresh medium at 25°C. At the indicated times cells were collected for FACS analysis of DNA contents (histograms) and kinetics of budding, nuclear division and septin ring formation by immunofluorescence with anti-Cdc11 antibodies (graphs).

Figure S2. The *RHO1-D72N* allele, but not *CDC42-D65N,* suppresses the temperature-sensitivity of mutants defective in septin organization. A-D: Serial dilutions of stationary phase cultures of strains with the indicated genotypes were spotted on YEPD plates and incubated for 2 days at the indicated temperatures. Centromeric plasmids are indicated in brackets. Several independent transformants or meiotic segregants were analysed for each mutant. **E:** Microcolonies of cells with the indicated genotypes were photographed after 24 hours of incubation at 25°C. **F:** Representative images of cells with the indicated genotypes growing at 25°C.

Figure S3. The *RHO1-D72N* allele does not accelerate actomyosin ring contraction. Live cell imaging of wild type and *RHO1-D72N* cells expressing Myo1-GFP and Shs1-mCherry. The time for AMR contraction has been calculated from the beginning of contraction to disappearance of the AMR.

Figure S4. Hyperactivation of the MAP kinase pathway does not suppress the temperature-sensitivity of *dma1Δ dma2Δ cla4-75* cells. A: Schematic representation of the cell wall integrity (CWI) pathway. **B:** Serial dilutions of stationary phase cultures of strains with the indicated genotypes were spotted on YEPD plates and incubated for two days at the indicated temperatures. Episomal plasmids carrying the tested mutant alleles are in brackets. For each plasmid three independent transformants were analysed. **C:** Log phase cultures of cells with the indicated genotypes were grown at 25°C and shifted at time 0 to 37°C for 30' or 3 hours. Total protein extracts were probed by western blot with phospho-specific and polyclonal anti-Slt2 antibodies.

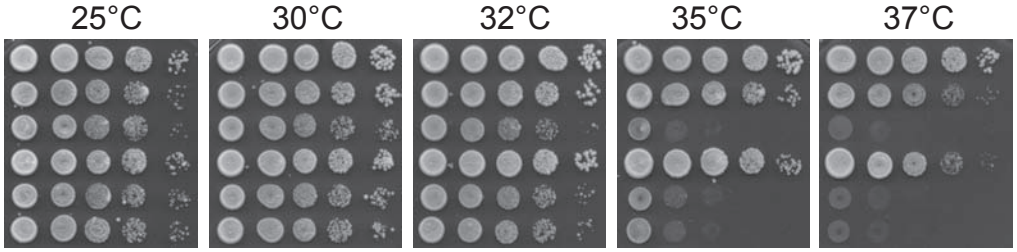
Figure S5. *SYP1* deletion suppresses the temperature-sensitivity of *cdc12-1* and *dma1Δ dma2Δ cla4-75* cells and deletion of *PKC1* or *DMA1* and *DMA2* do not affect the timing of Syp1 localization at the bud neck. A: Serial dilutions of stationary phase cultures of strains with the indicated genotypes were spotted on YEPD plates and incubated for two days at the indicated temperatures. **B:** Wild type and *pkc1Δ* cells were grown in sorbitol-containing YEPD medium at 30°C, arrested in G1 by α-factor and released in YEPD containing sorbitol at 30°C. **C:** Wild type and *dma1Δ dma2Δ* cells were grown in YEPD at 25°C, arrested in G1 by α-factor and released in YEPD at 25°C. At the indicated time points cell samples were collected for FACS analysis of DNA contents (not shown) and to score budding index, nuclear division and Syp1-HA3 localization at the bud neck by indirect immunofluorescence with anti-HA antibodies (B-C).

Table S1. Mass spectrometry analysis of Syp1 phosphorylation sites induced *in vitro* by Pkc1.

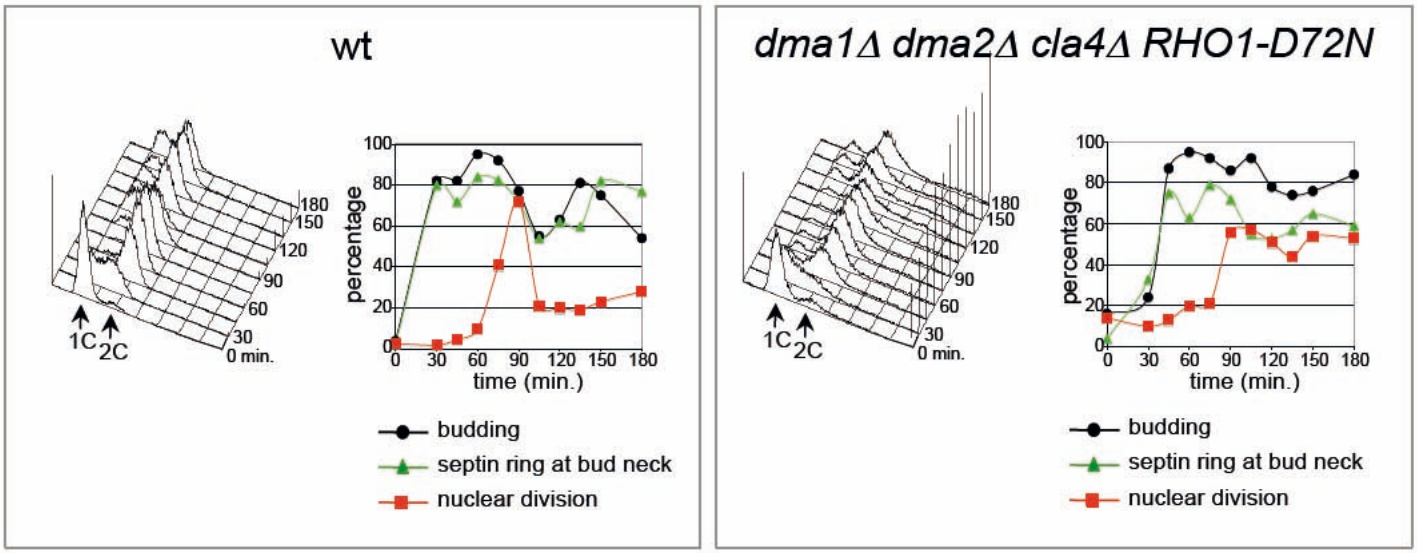
Table S2. Protein coverage of Syp1, Sla1, Ede1 and Fks1 after immunoprecipitation of Syp1-Flag3 and MS/MS analysis.

Table S3. List of strains used in this study.

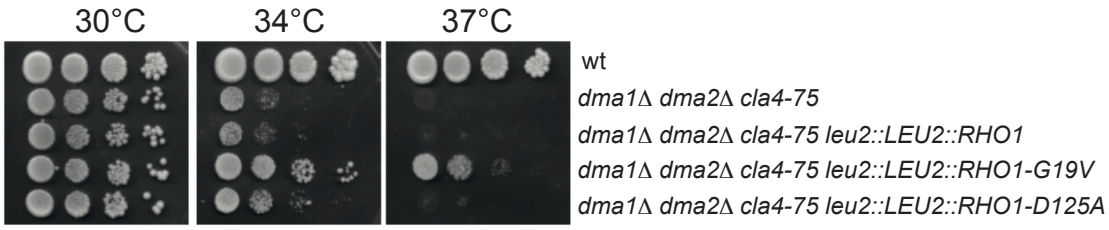
A


25°C
30°C
32°C
35°C
37°C
bud4
dma1Δ dma2Δ bud4
dma1Δ dma2Δ cla4-75 bud4
cla4-75 bud4
dma1Δ dma2Δ cla4-75 bud4
dma1Δ dma2Δ cla4-75 BUD4

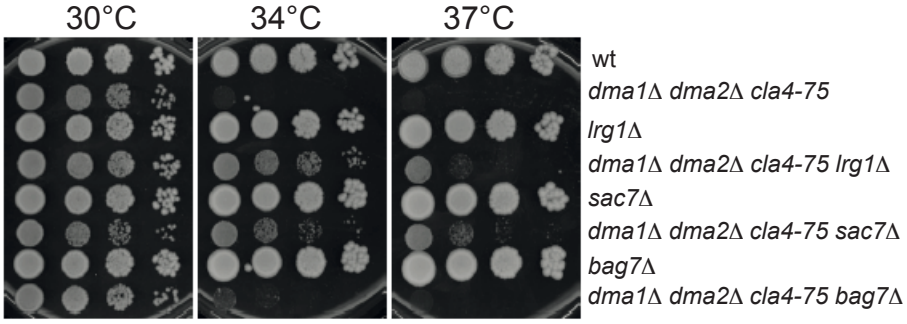
B


wt
dma1Δ dma2Δ cla4Δ RHO1-D72N
1C
2C
180
150
120
90
60
30
0 min.
percentage
time (min.)
budding
septin ring at bud neck
nuclear division

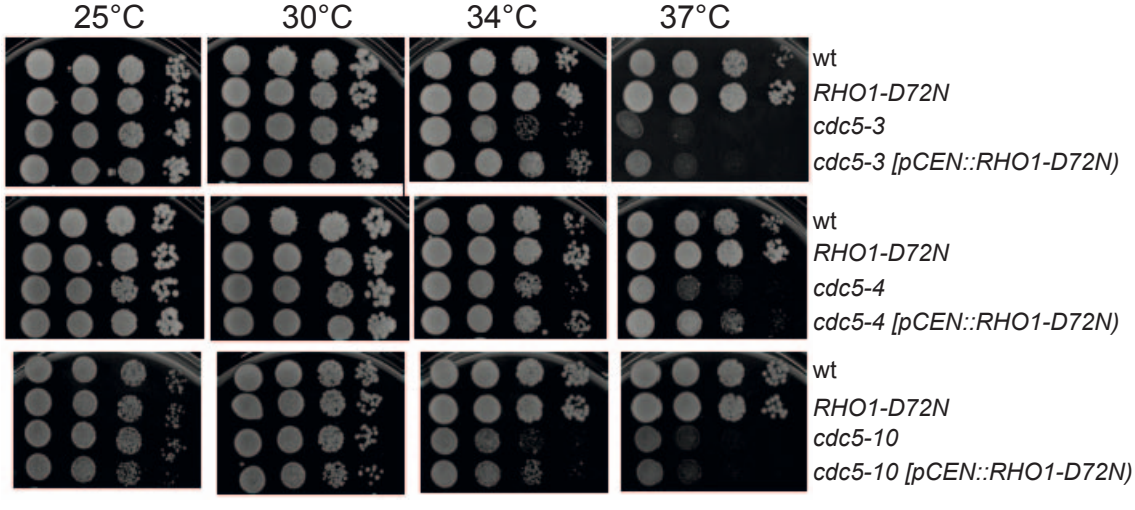
C

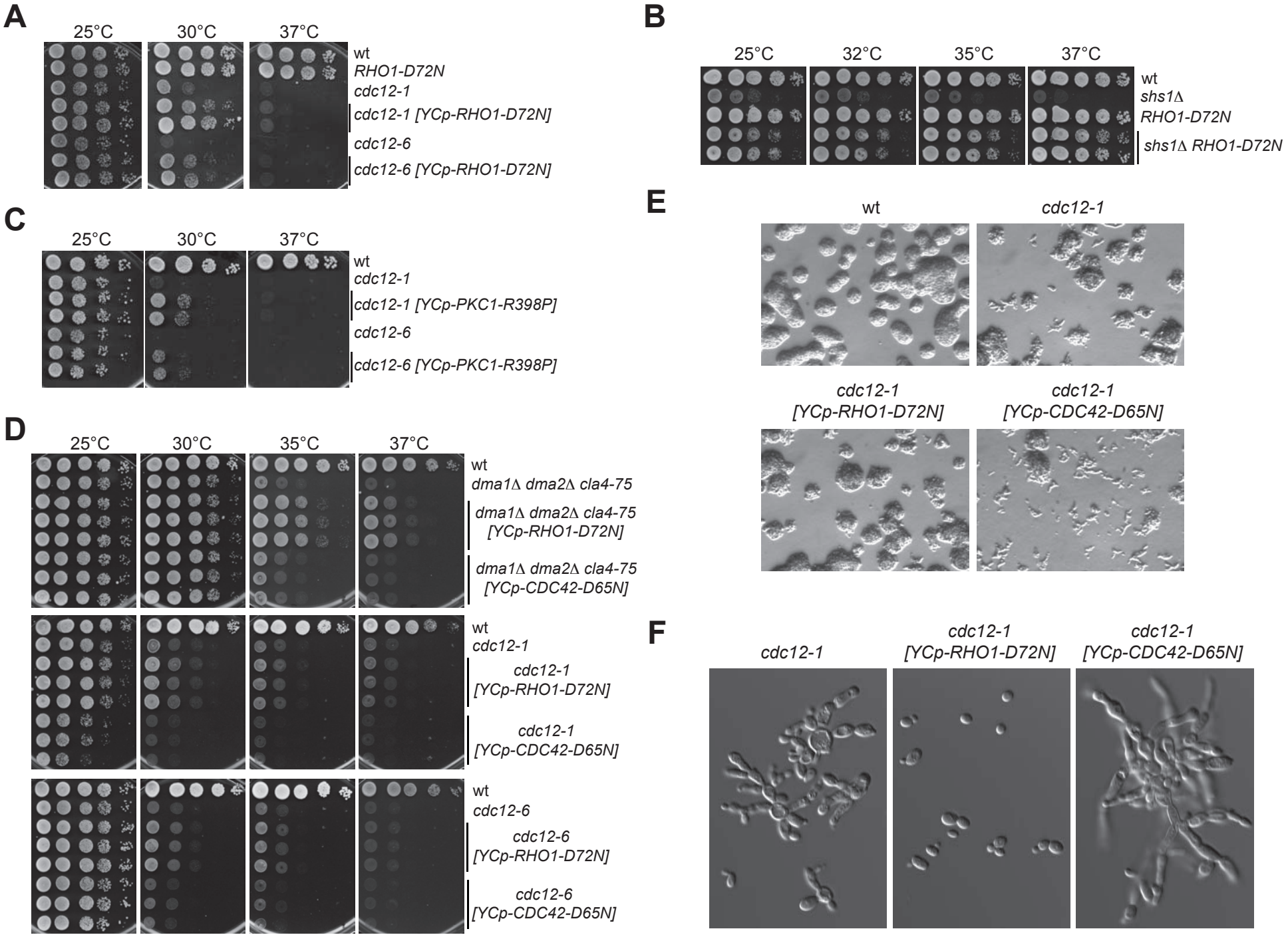

30°C
34°C
37°C
wt
dma1Δ dma2Δ cla4-75
dma1Δ dma2Δ cla4-75 leu2::LEU2::RHO1
dma1Δ dma2Δ cla4-75 leu2::LEU2::RHO1-G19V
dma1Δ dma2Δ cla4-75 leu2::LEU2::RHO1-D125A

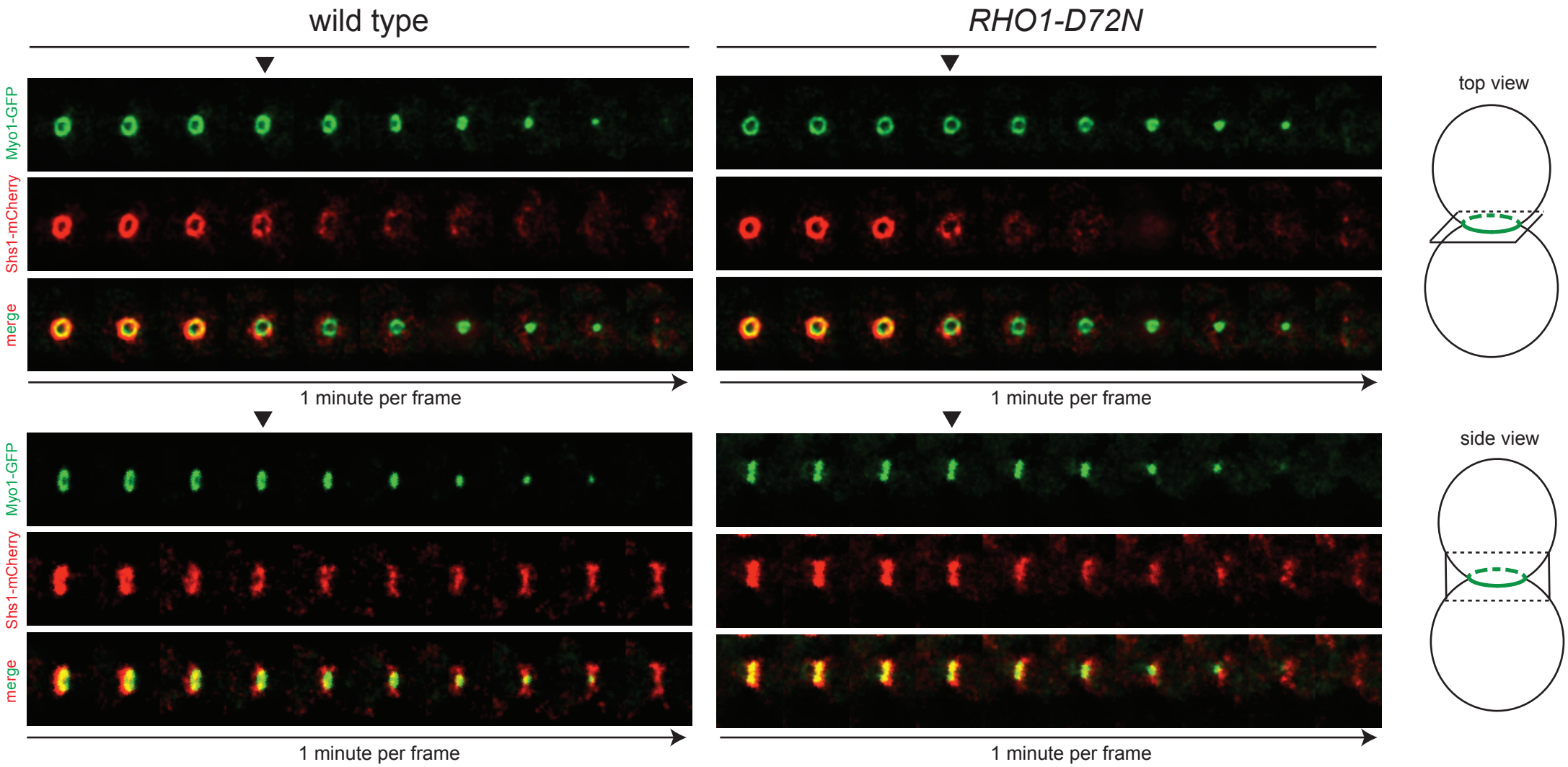
D


30°C
34°C
37°C
wt
dma1Δ dma2Δ cla4-75
lrg1Δ
dma1Δ dma2Δ cla4-75 lrg1Δ
sac7Δ
dma1Δ dma2Δ cla4-75 sac7Δ
bag7Δ
dma1Δ dma2Δ cla4-75 bag7Δ

E

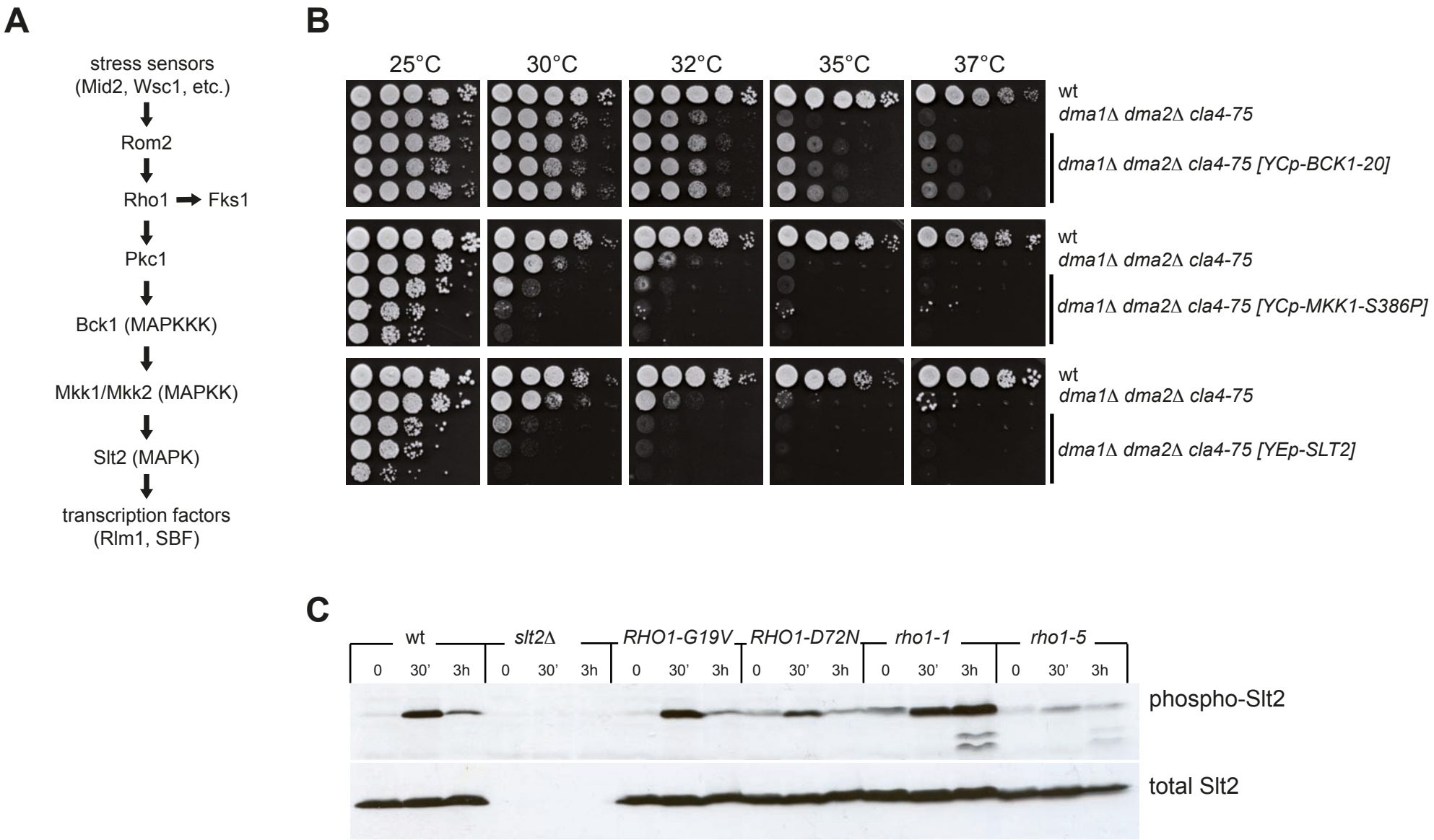

25°C
30°C
34°C
37°C
wt
RHO1-D72N
cdc5-3
cdc5-3 [pCEN::RHO1-D72N)
wt
RHO1-D72N
cdc5-4
cdc5-4 [pCEN::RHO1-D72N)
wt
RHO1-D72N
cdc5-10
cdc5-10 [pCEN::RHO1-D72N)


A
25°C
30°C
37°C
wt
RHO1-D72N
cdc12-1
cdc12-1 [YCp-RHO1-D72N]
cdc12-6
cdc12-6 [YCp-RHO1-D72N]
B
25°C
32°C
35°C
37°C
wt
shs1Δ
RHO1-D72N
shs1Δ RHO1-D72N
C
25°C
30°C
37°C
wt
cdc12-1
cdc12-1 [YCp-PKC1-R398P]
cdc12-6
cdc12-6 [YCp-PKC1-R398P]
D
25°C
30°C
35°C
37°C
wt
dma1Δ dma2Δ cla4-75
dma1Δ dma2Δ cla4-75 [YCp-RHO1-D72N]
dma1Δ dma2Δ cla4-75 [YCp-CDC42-D65N]
wt
cdc12-1
cdc12-1 [YCp-RHO1-D72N]
cdc12-1 [YCp-CDC42-D65N]
wt
cdc12-6
cdc12-6 [YCp-RHO1-D72N]
cdc12-6 [YCp-CDC42-D65N]
E
wt
cdc12-1
cdc12-1 [YCp-RHO1-D72N]
cdc12-1 [YCp-CDC42-D65N]
F
cdc12-1
cdc12-1 [YCp-RHO1-D72N]
cdc12-1 [YCp-CDC42-D65N]



AMR contraction

wild type	*RHO1-D72N*
6.29 ± 0.64 min (n=35)	6.83 ± 0.68 min (n=27)


A
stress sensors
(Mid2, Wsc1, etc.)
Rom2
Rho1 → Fks1
Pkc1
Bck1 (MAPKKK)
Mkk1/Mkk2 (MAPKK)
Slt2 (MAPK)
transcription factors
(Rlm1, SBF)
B
25°C
30°C
32°C
35°C
37°C
wt
dma1Δ dma2Δ cla4-75
dma1Δ dma2Δ cla4-75 [YCp-BCK1-20]
wt
dma1Δ dma2Δ cla4-75
dma1Δ dma2Δ cla4-75 [YCp-MKK1-S386P]
wt
dma1Δ dma2Δ cla4-75
dma1Δ dma2Δ cla4-75 [YEp-SLT2]
C
wt
slt2Δ
RHO1-G19V
RHO1-D72N
rho1-1
rho1-5
0
30'
3h
phospho-Slt2
total Slt2

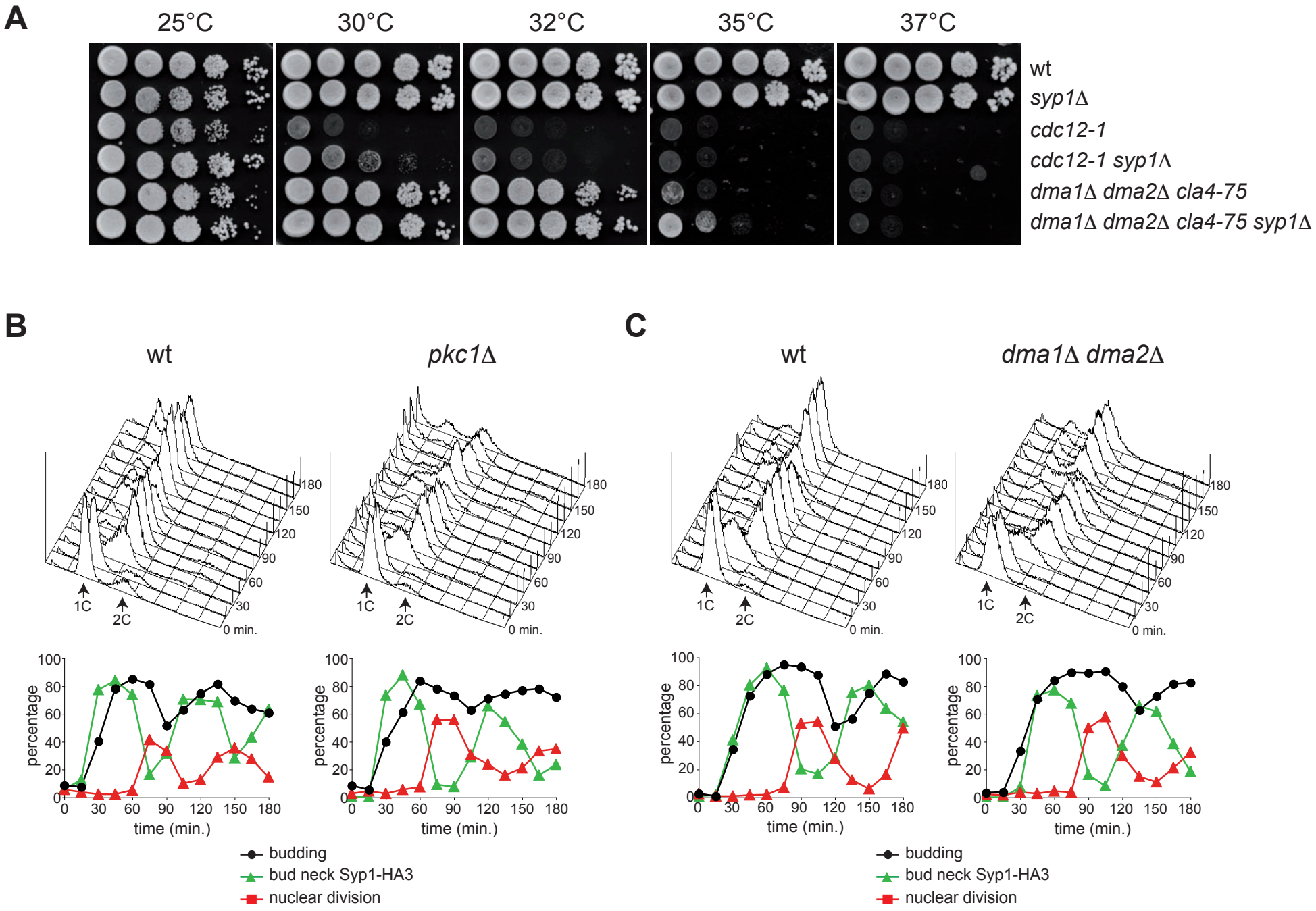

A
25°C
30°C
32°C
35°C
37°C
wt
syp1Δ
cdc12-1
cdc12-1 syp1Δ
dma1Δ dma2Δ cla4-75
dma1Δ dma2Δ cla4-75 syp1Δ
B
wt
pkc1Δ
1C
2C
0 min.
30
60
90
120
150
180
percentage
time (min.)
budding
bud neck Syp1-HA3
nuclear division
C
wt
dma1Δ dma2Δ

Table S3. Lists of *Saccharomyces cerevisiae* strains and plasmids used in this study

Yeast strains (Plasmids are indicated in brackets)

Name	**Relevant genotype**
ySP294	*MATa, cdc12-1*
ySP5183	*MATa, cdc12-6*
ySP5247	*MATa, dma1::K.l.TRP1, dma2::HPHMX, cla4::KanMX4, [CEN::URA3::cla4-75]*
ySP5264	*MATα, dma1::K.l.TRP1, dma2::HPHMX, cla4::KanMX4, [CEN::URA3::cla4-75]*
ySP7342	*MATa, shs1::NatN2*
ySP7343	*MATα, dma1::K.l.TRP1, dma2::HPHMX, cla4::KanMX4, [CEN::URA3::cla4-75], RHO1-D72N*
ySP7863	*MATα, dma1::K.l.TRP1, dma2::HPHMX, cla4::KanMX4, [CEN::URA3::cla4-75], leu2::LEU2::3HA-RHO1-G19V*
ySP7864	*MATα, dma1::K.l.TRP1, dma2::HPHMX, cla4::KanMX4, [CEN::URA3::cla4-75], leu2::LEU2::3HA-RHO1-D125A*
ySP7887	*MATα, dma1::K.l.TRP1, dma2::HPHMX, cla4::KanMX4, RHO1-D72N::K.l.LEU2*
ySP7933	*MATa, RHO1-D72N::K.l.LEU2*
ySP7985	*MATα, cdc12-6, RHO1-D72N::K.l.LEU2*
ySP7986	*MATa, cdc12-6, RHO1-D72N::K.l.LEU2*
ySP7987	*MATa, cdc12-1, RHO1-D72N::K.l.LEU2*
ySP7988	*MATα, cdc12-1, RHO1-D72N::K.l.LEU2*
ySP8034	*MATα, shs1::NatN2, RHO1-D72N::K.l.LEU2*
ySP8035	*MATa, shs1::NatN2, RHO1-D72N::K.l.LEU2*
ySP8107	*MATα, sac7::NatN2*
ySP8123	*MATa, dma1::K.l.TRP1, dma2::HPHMX, cla4::KanMX4, [CEN::URA3::cla4-75], sac7::NatN2*
ySP8150	*MATa, rho1::HIS3, ade3::rho1-1::LU2*
ySP8153	*MATa, rho1::HIS3, ade3::rho1-5::LU2*
ySP8176	*MATa, ura3::URA3::GFP-CDC12*
ySP8177	*MATa, RHO1-D72N::K.l.LEU2, ura3::URA3::GFP-CDC12*
ySP8259	*MATα, lrg1::K.l.LEU2*
ySP8310	*MATa, dma1::K.l.TRP1, dma2::HPHMX, cla4::KanMX4, [CEN::URA3::cla4-75], lrg1::K.l.LEU2*
ySP8321	*MATα, rho1::HIS3, ade3::rho1-5::LEU2, ura3::URA3::GFP-CDC12*
ySP8323	*MATα, rho1::HIS3, ade3::rho1-1::LEU2, ura3::URA3::GFP-CDC12*

ySP8334	*MATa, bag7::NatN2*
ySP8400	*MATa, [CEN::URA3::PKC1-R398P]*
ySP8436	*MATa, dma1::K.l.TRP1, dma2::HPHMX, cla4::KanMX4, [CEN::URA3::cla4-75], bag7::NatN2*
ySP8440	*MATa, cdc5::KanMX4, trp1::TRP1::cdc5-4-3HA, [CEN::LEU2::RHO1-D72N]*
ySP8441	*MATa, cdc5::KanMX4, trp1::TRP1::cdc5-3-3HA, [CEN::LEU2::RHO1-D72N]*
ySP8463	*MATa, slt2::URA3*
ySP8476	*MATa, pkc1::KanMX4*
ySP8501	*MATa, cdc5::KanMX4, trp1::TRP1::cdc5-10-3HA, [CEN::LEU2::RHO1-D72N]*
ySP8594	*MATα, pkc1::KanMX4, ura3::URA3::GFP-CDC12*
ySP8719	*MATa, SYP1-eGFP::KanMX4*
ySP8723	*MATa, syp1::NatNT2*
ySP8725	*MATa, SYP1-3HA::K.l.TRP1*
ySP8741	*MATa, dma1::K.l.TRP1, dma2::HPHMX, cla4::KanMX4, [CEN::URA3::cla4-75], syp1::NatNT2*
ySP8754	*MATa, SYP1-eGFP::KanMX4, pkc1::KanMX4*
ySP8799	*MATa, SYP1-3HA::K.l.TRP1, pkc1::KanMX4*
ySP8784	*MATa, SYP1-3HA::K.l.TRP1, dma1::K.l.TRP1, dma2::K.l.LEU2*
ySP8803	*MATa, syp1::NatNT2, ura3::URA3::GFP-CDC12*
ySP8868	*MATa, syp1::NatNT2, cdc12-1*
ySP8910	*MATα, cdc12-1, [CEN::URA3::PKC1-R398P]*
ySP8912	*MATα, cdc12-6, [CEN::URA3::PKC1-R398P]*
ySP9547	*MATa, MYO1-GFP::KanMX4, SHS1-mCherry::HphMX*
ySP9554	*MATa, MYO1-GFP::KanMX4, SHS1-mCherry::HphMX, RHO1-D72N::K.l.LEU2*
ySP9816	*MATa, SYP1-6Gly-3Flag::KanMX4*
ySP9818	*MATa, SYP1-6Gly-3Flag::KanMX4, pkc1::KanMX4*
ySP10091	*MATa, SYP1-S347A,S389A,S390A::LEU2::syp1::NatNT2, ura3::URA3::GFP-CDC12*
ySP10092	*MATa, SYP1-S347D,S389D,S390D::LEU2::syp1::NatNT2, ura3::URA3::GFP-CDC12*
ySP10121	*MATa, SYP1-S347A,S389A,S390A::LEU2::syp1::NatNT2*
ySP10122	*MATa, SYP1-S347D,S389D,S390D::LEU2::syp1::NatNT2*
ySP10150	*MATa, SYP1-S347A,S389A,S390A-eGFP::KanMX4::LEU2::syp1::NatNT2, ura3::URA3::GFP-CDC12*
ySP10151	*MATa, SYP1-S347D,S389D,S390D-eGFP::KanMX4::LEU2::syp1::NatNT2, ura3::URA3::GFP-CDC12*
ySP10228	*MATa, SYP1-S347A-3HA::K.l.TRP1::LEU2::syp1::NatNT2, ura3::URA3::GFP-CDC12*
ySP10229	*MATa, SYP1-S347A,S389A,S390A-3HA::K.l.TRP1::LEU2::syp1::NatNT2*

ySP10361	*MATa, SYP1-eGFP::KanMX4, SHS1-mCherry::HphMX*
ySP10819	*MATa, SHS1-GFP::LEU2*
ySP10820	*MATα, dma1::K.l.TRP1, dma2::HPHMX, cla4::KanMX4, [CEN::URA3::cla4-75], SHS1-GFP::LEU2*
ySP10821	*MATα, dma1::K.l.TRP1, dma2::HPHMX, cla4::KanMX4, [CEN::URA3::cla4-75], RHO1-D72N, SHS1-GFP::LEU2*
ySP10823	*MATa, dma1::K.l.TRP1, dma2::HPHMX, cla4::KanMX4, [CEN::URA3::cla4-75], PKC1-103, SHS1-GFP::LEU*
ySP11335	*MATa, SHS1-GFP::LEU2, ura3::URA3::GIC2-PBD-(W23A)RFP*
ySP11369	*MATa, bud4::LEU2::BUD4, ura3::URA3::GFP-CDC12*
ySP11416	*MATα, SHS1-GFP::LEU2, ura3::URA3::GIC2-PBD-(W23A)RFP, RHO1-D72N::K.l.LEU2*
ySP11419	*MATa, bud4::URA3::BUD4, dma1::K.l.LEU2, dma2::HPHMX, cla4::NatNT2, [CEN::TRP1::cla4-75],*
ySP11482	*MATa, bud4::LEU2::BUD4, ura3::URA3::GFP-CDC12, pkc1::KanMX4*
ySP11558	*MATa, SHS1-GFP::LEU2, ura3::URA3::GIC2-PBD-(W23A)RFP, PKC1-R398P*
ySP11650	*MATα, bud4::LEU2::BUD4, cdc12-6, GFP-CDC3::URA3, RHO1-D72N::K.l.LEU2*
ySP11651	*MATα, bud4::LEU2::BUD4, cdc12-6, GFP-CDC3::URA3*
ySP11672	*MATα, bud4::LEU2::BUD4, cdc12-6, GFP-CDC3::URA3, PKC1-R398P*

Plasmids

Name	**Description**	**Source/Reference**
pSP340	*cla4-75* cloned in EcoRI/HindIII of Ycplac33 (*CEN, URA3*)	This study
pSP671	*3HA-RHO1-G19V* cloned in pRS305 (*LEU2*)	Tiedje et al., 2008
pSP672	*3HA-RHO1-D125A* cloned in pRS305 (*LEU2*)	Tiedje et al., 2008
pSP675	*RHO1-D72N* cloned in YCplac111 (*CEN, LEU2*)	This study
pSP677	*PKC1-R398P* cloned in YCp50	Gift from M. Hall
pSP789	*SYP1* (-480 to +2770) cloned in YIplac128 (*LEU2*)	This study
pSP794	*SYP1(S347A)* (-480 to +2770) cloned in YIplac128 (*LEU2*)	This study
pSP908	*MKK1-S386P* cloned in YCplac33 (*CEN, URA3*)	Gift from M. Hall
pSP909	*SLT2* cloned in YEp352 (*2μ, URA3*)	Gift from M. Hall
pSP961	*syp1* (aa1-264) cloned in pET28a C/U	Reider et al., 2009
pSP962	*syp1* (aa265-565) cloned in pET28a C/U	Reider et al., 2009
pSP966	*syp1* (aa566-870) cloned in pET28a C/U	Reider et al., 2009
pSP1009	PKC1(HR1-C2)-GFP, URA3, CEN	Kono et al., 2012

pSP1010	PKC1(HR1-C2)(L54S)-GFP, URA3, CEN	Kono et al., 2012
pSP1011	*2HA-BNI1-13myc, TRP, CEN*	Kono et al., 2012
pSP1012	*2HA-BNI1(V360D)-13myc, TRP, CEN*	Kono et al., 2012
pSP1026	*SYP1(S347A, S389A, S390A)* (-480 to +2770) cloned in YIplac128 (*LEU2*)	This study
pSP1027	*SYP1(S347D, S389D, S390D)* (-480 to +2770) cloned in YIplac128 (*LEU2*)	This study
pSP1065	*CDC42-D65N* cloned in pSR315 (*CEN, LEU2*)	Mosch et al., 2001
pSP1089	*BCK1-20* cloned in pRS314 (*CEN, TRP1*)	Gift from G. De Bettignies