Spatial uncoupling of mitosis and cytokinesis during appressorium-mediated plant infection by the rice blast fungus *Magnaporthe oryzae*

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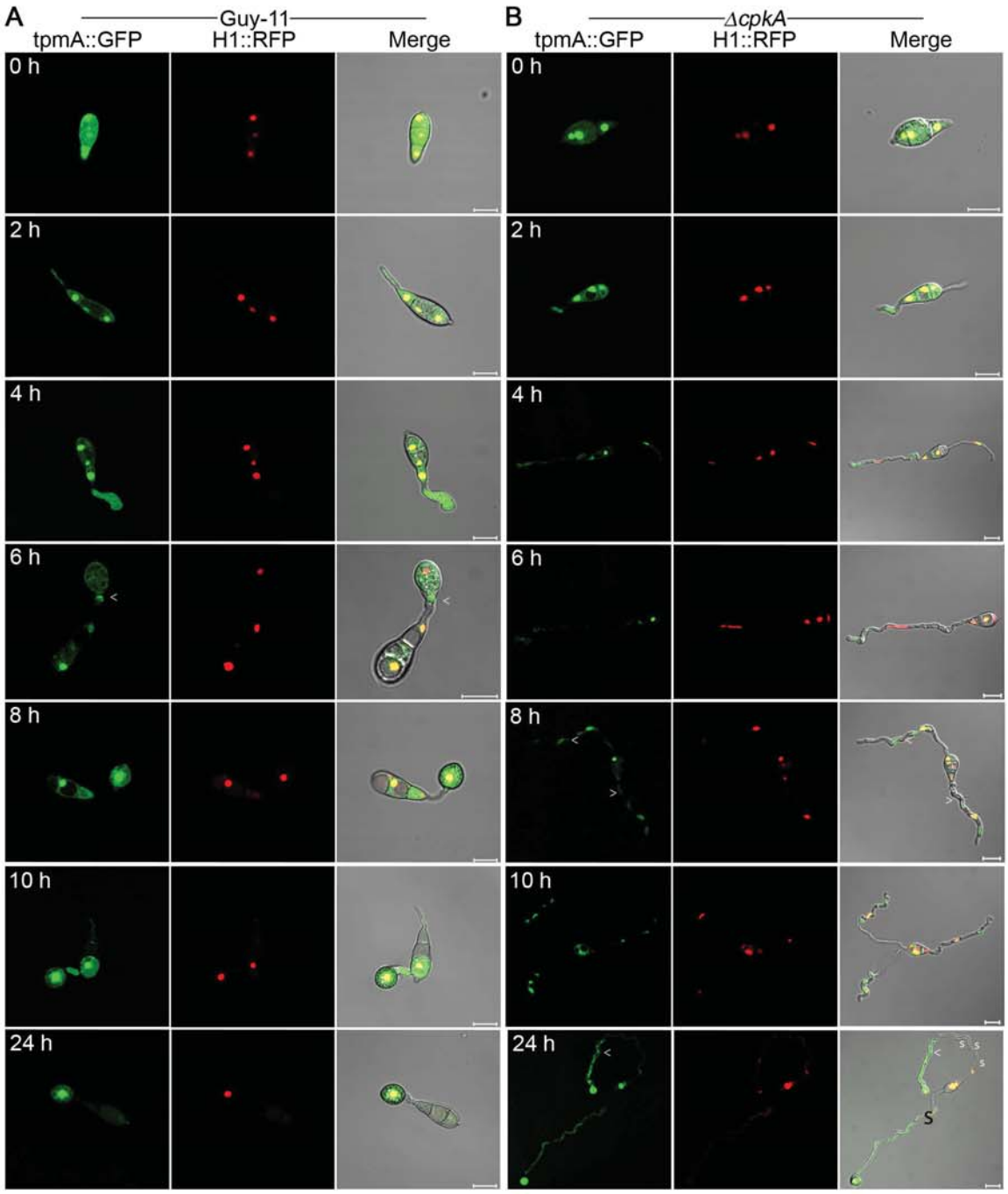
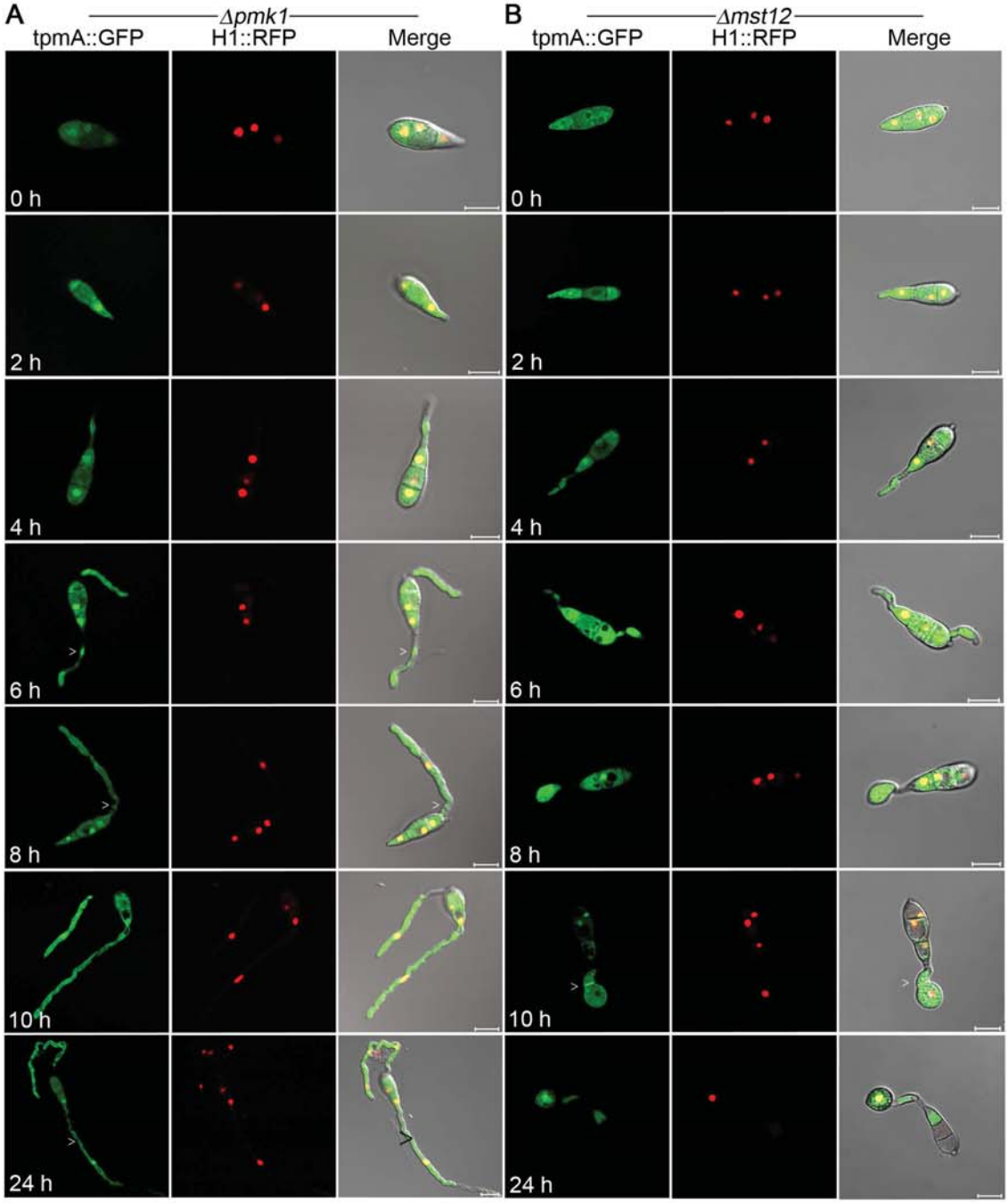
Supplemental Data



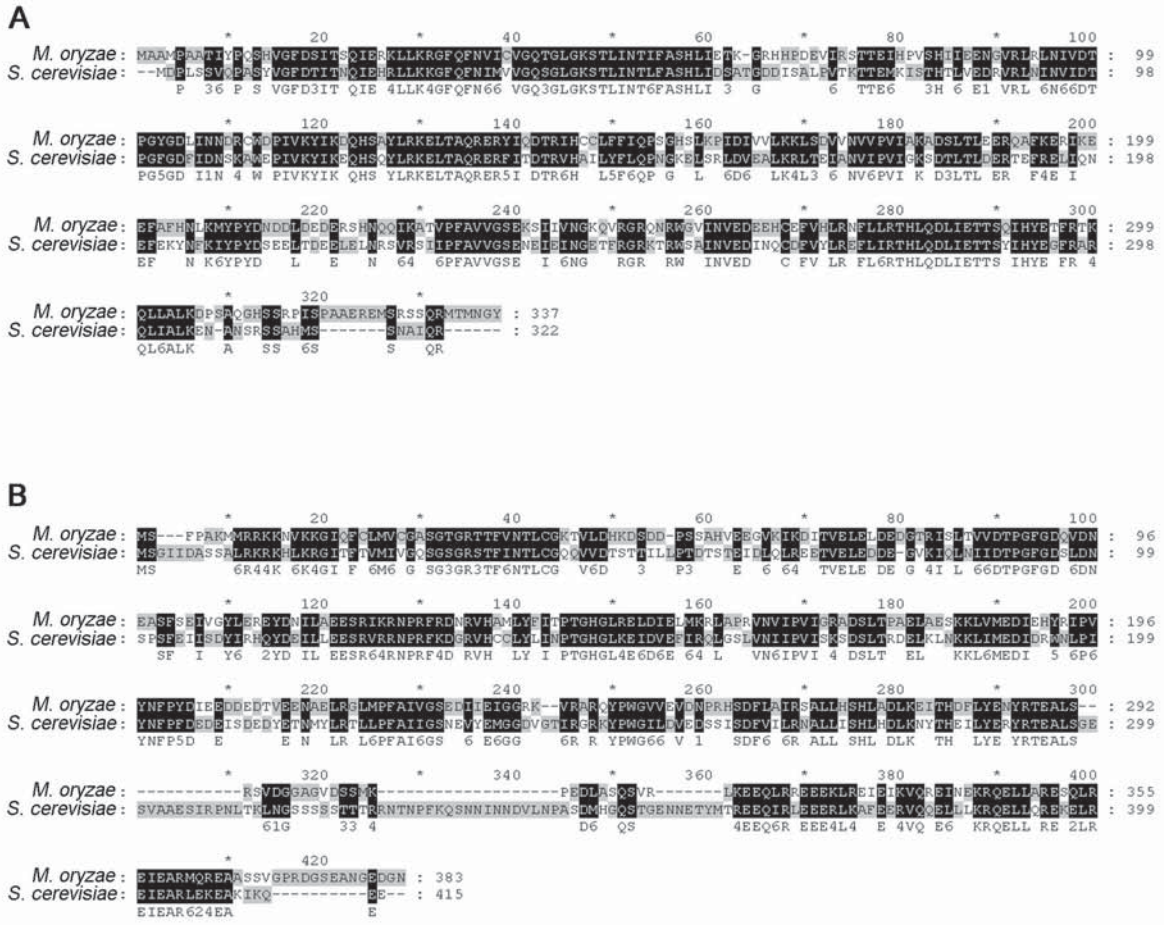
Supplemental Figure 1. Live cell imaging of nuclear dynamics and actomyosin ring formation in the M. oryzae strains Guy-11 and ΔcpkA.

Time series of micrographs to show nuclear division and cytokinesis during appressorium formation. Conidial suspensions were prepared from the M. oryzae Guy-11 (A) and a ΔcpkA mutant (B), expressing H1:eRFP and tpmA:GFP. The suspensions were incubated in conditions to allow appressorium formation and representative images recorded at seven time points during germination and appressorium formation. Septa are highlighted by arrowheads. Scale bars represent 10 µm.



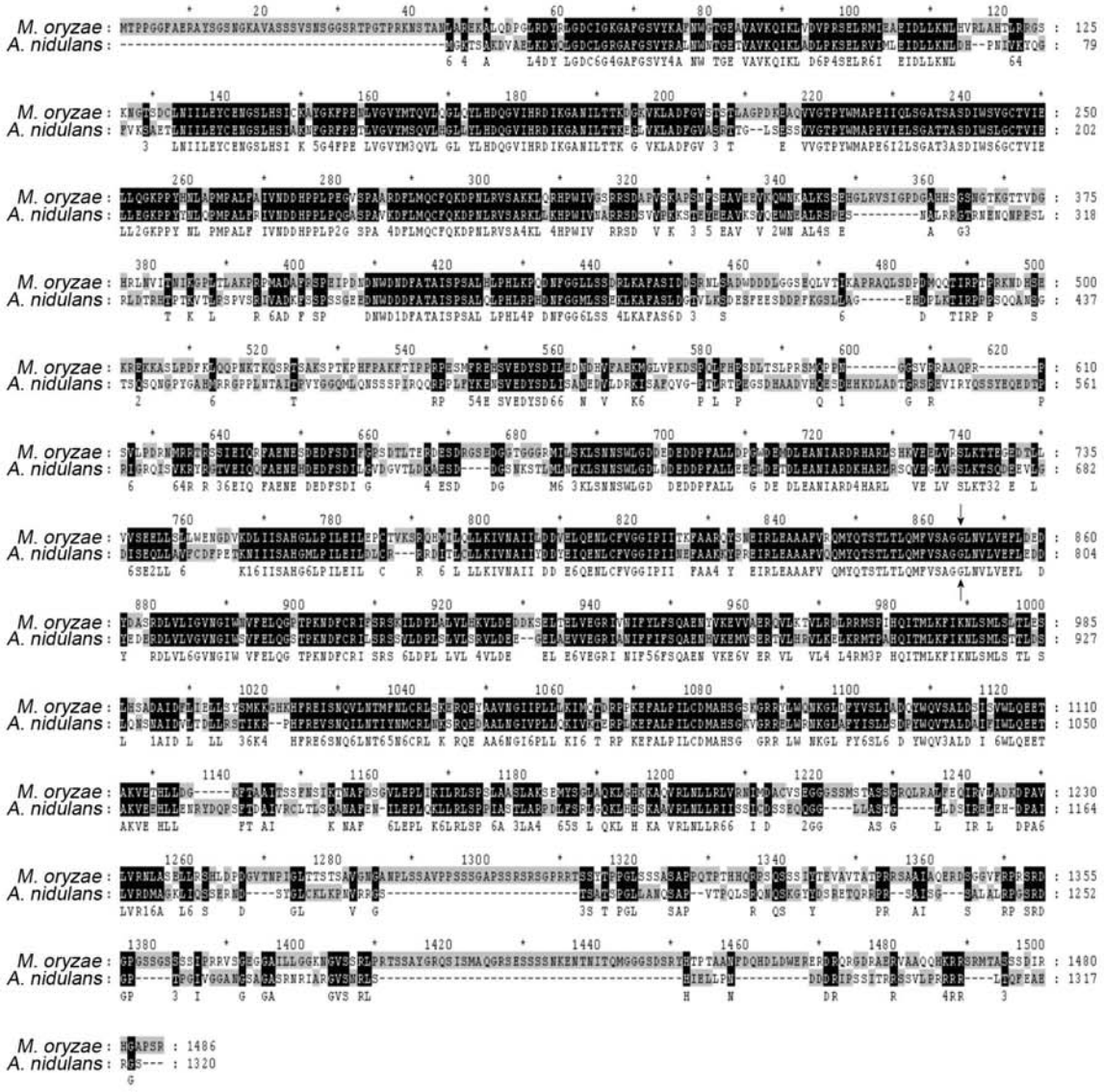
Supplemental Figure 2. Live cell imaging of nuclear dynamics and actomyosin ring formation in the M. oryzae strains Δpmk1 and Δmst12.

Time series of micrographs to show nuclear division and cytokinesis during appressorium formation. Conidial suspensions were prepared from the M. oryzae Δpmk1 (A) and Δmst12 (B) mutants, expressing H1:eRFP and tpmA:GFP. The suspensions were incubated to allow appressorium formation and representative images recorded at seven time points during germination and appressorium formation. Septa are highlighted with arrowheads. Scale bars represent 10 µm.



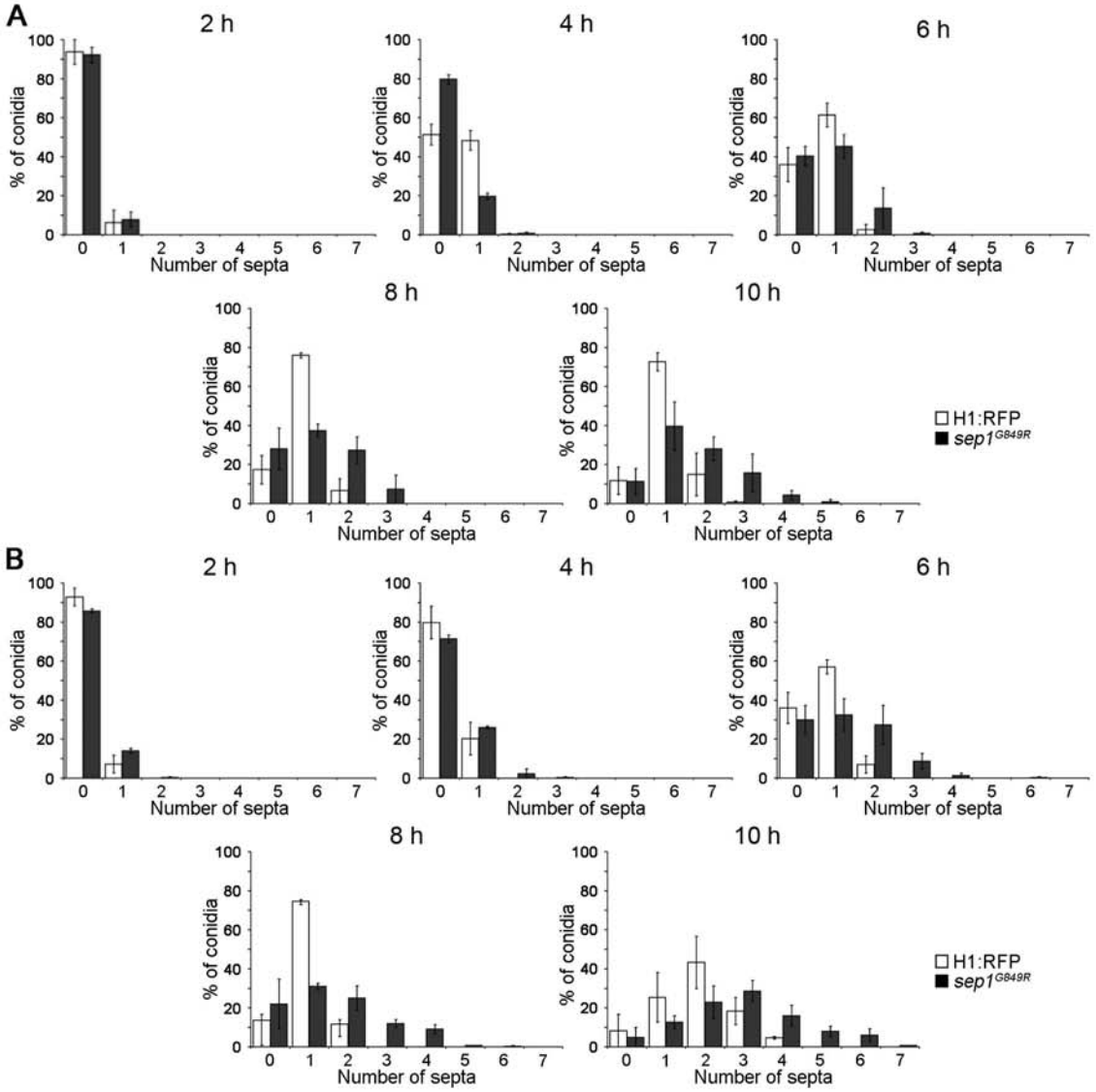
Supplemental Figure 3. Alignment of the predicted M. oryzae Sep4 and Sep5 amino acid sequences with Cdc10 and Cdc11 from S. cerevisiae.

The alignment was generated using ClustalW and shaded using Boxshade v 2.01. Numbers on the right indicate amino acid residue positions. Residues within a black background, dark grey background and light grey background represent 100%, 80% and 60% amino acid conservation.



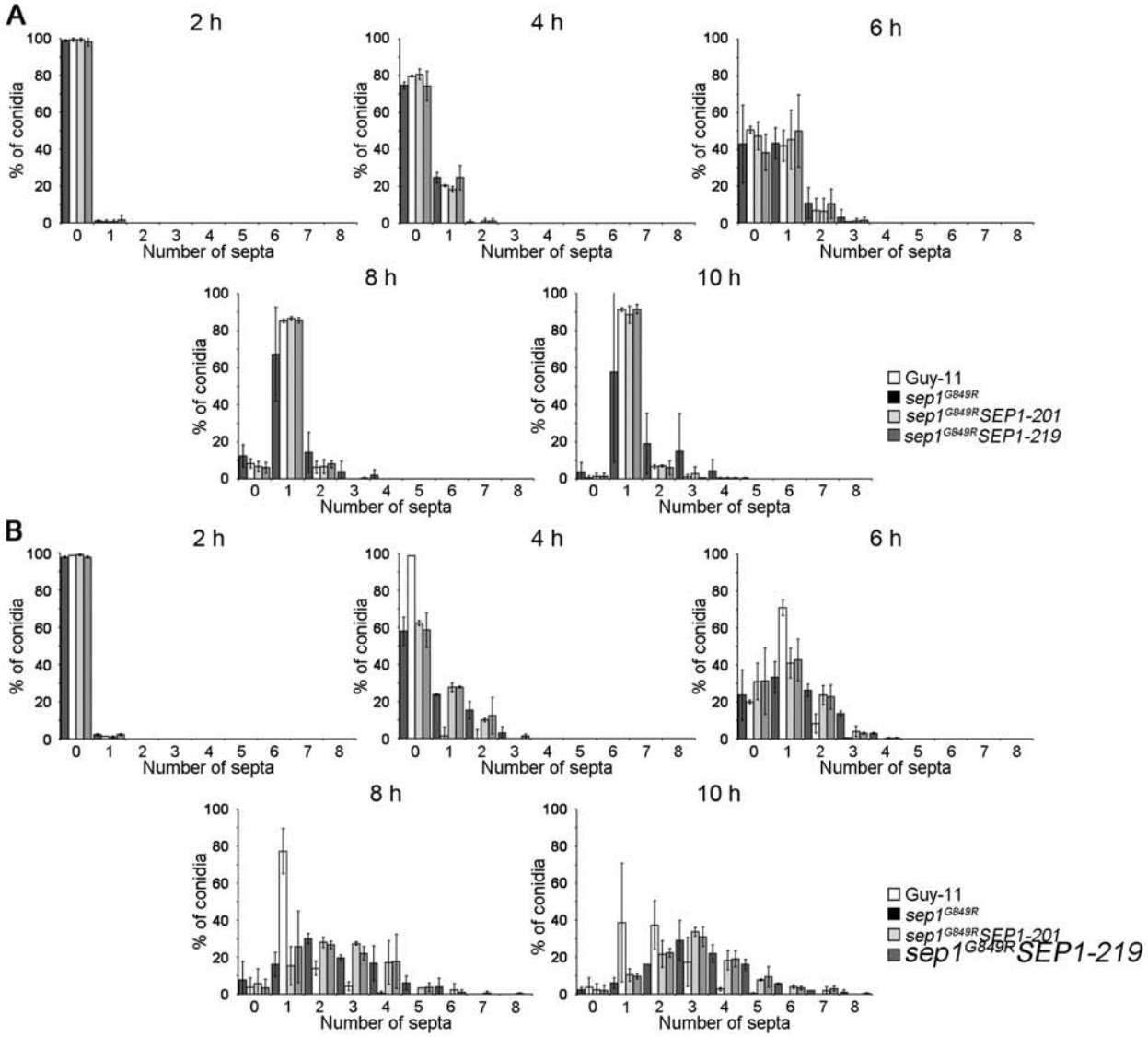
Supplemental Figure 4. Alignment of the predicted M. oryzae Sep1 amino acid sequence with A. nidulans SepH.

The alignment was generated using ClustalW and shaded using Boxshade v 2.01. Numbers on the right indicate amino acid residue positions. Residues within a black background, dark grey background and light grey background represent 100%, 80% and 60% amino acid conservation. Arrow indicates position of nucleotide substitution.



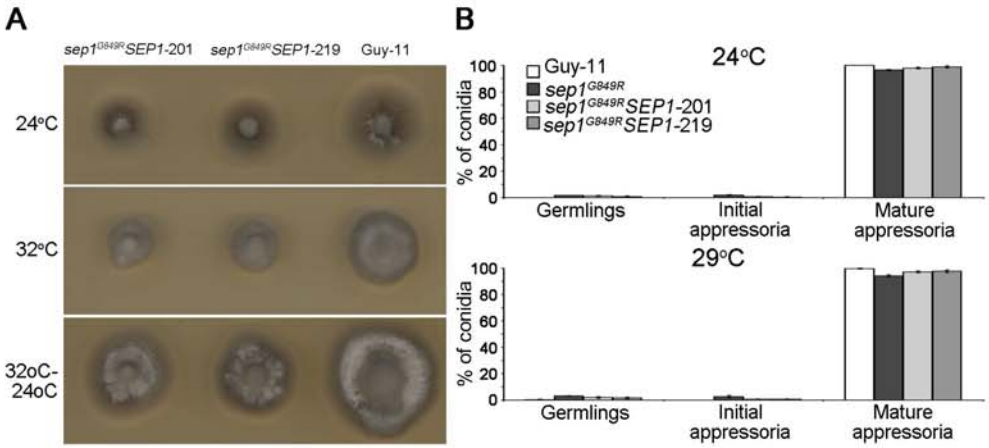
Supplemental Figure 5. A temperature-sensitive mutation in M. oryzae SEP1 increases septation frequency during appressorium formation.

Quantitative analysis of septum formation during appressorium formation in sep1G849R. Conidial suspensions were prepared from the M. oryzae sep1G849R and H1:eRFP strains and incubated in conditions to allow appressorium development at a permissive temperature of 24ºC (A) or after an initial incubation for 1 hour at 24ºC germinating conidia were transferred to the semi-restrictive temperature of 29ºC (B). The number of septa within the germ tube was determined by calcofluor white staining and epifluorescence microscopy. Error bars are 1 standard error.



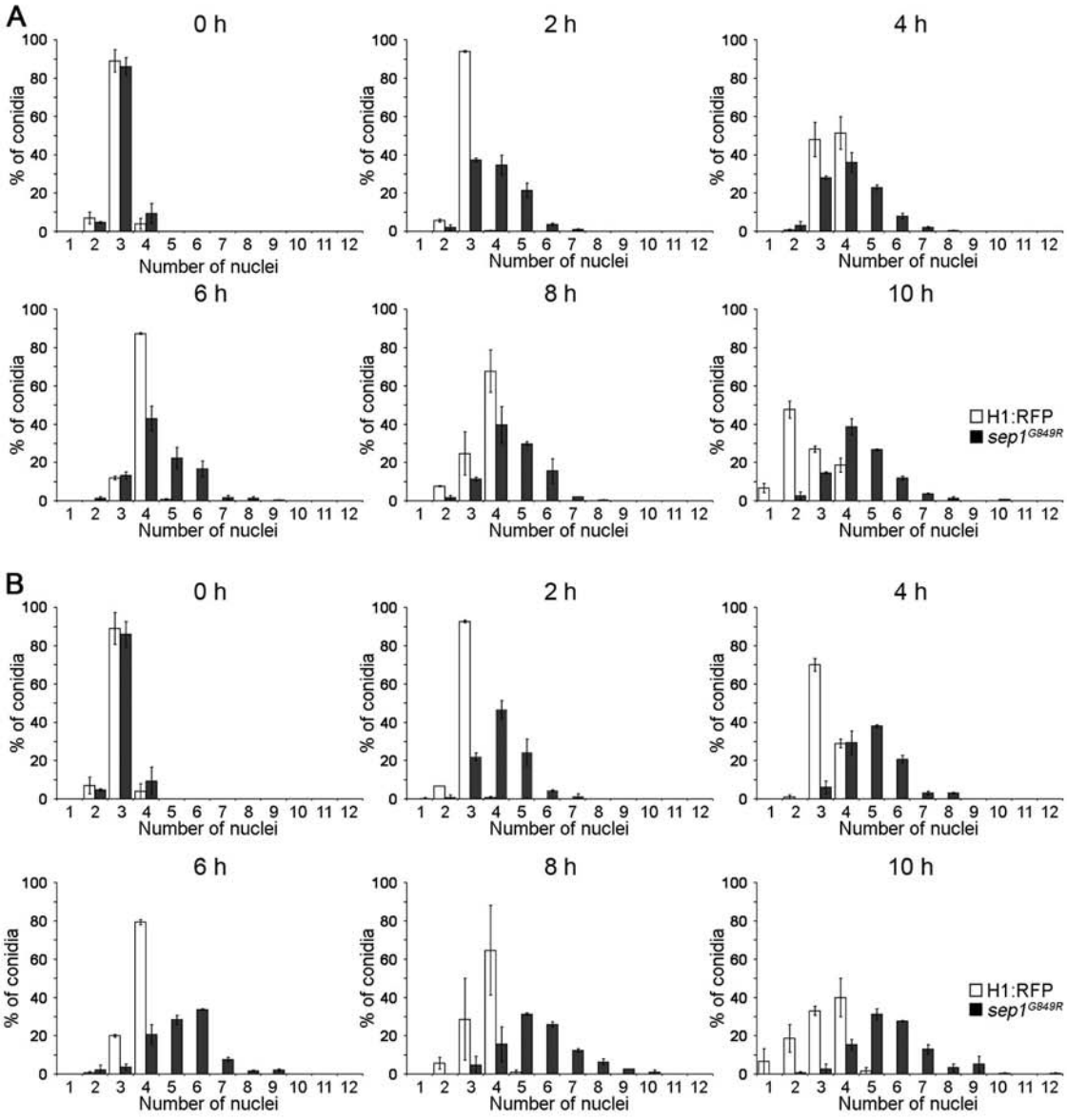
Supplemental Figure 6. The sep1G849R allele increases the frequency of septation during appressorium development even in the presence of a wild type copy of SEP1.

Bar charts to show number of septa present during appressorium development of M. oryzae. Conidial suspensions were prepared from two transformants carrying both a wild type copy of SEP1 and the temperature-sensitive sep1G849R allele -strains sep1G849R SEP1-201, sep1;G849RSEP1-219, as well as a sep1G849R mutant and the wild type H1:eRFP strain of Guy11. Each M. oryzae strain was incubated on hydrophobic borosilicate glass surfaces inductive for appressorium development. The conidial suspensions were incubated at a permissive temperature of 24ºC (A) or after an initial incubation for 1 hour at 24ºC germinating conidia were transferred to the semi-restrictive temperature of 29ºC (B). The number of septa within the germ tube was determined through calcofluor white staining and epifluorescence microscopy. Error bars are 1 standard error.



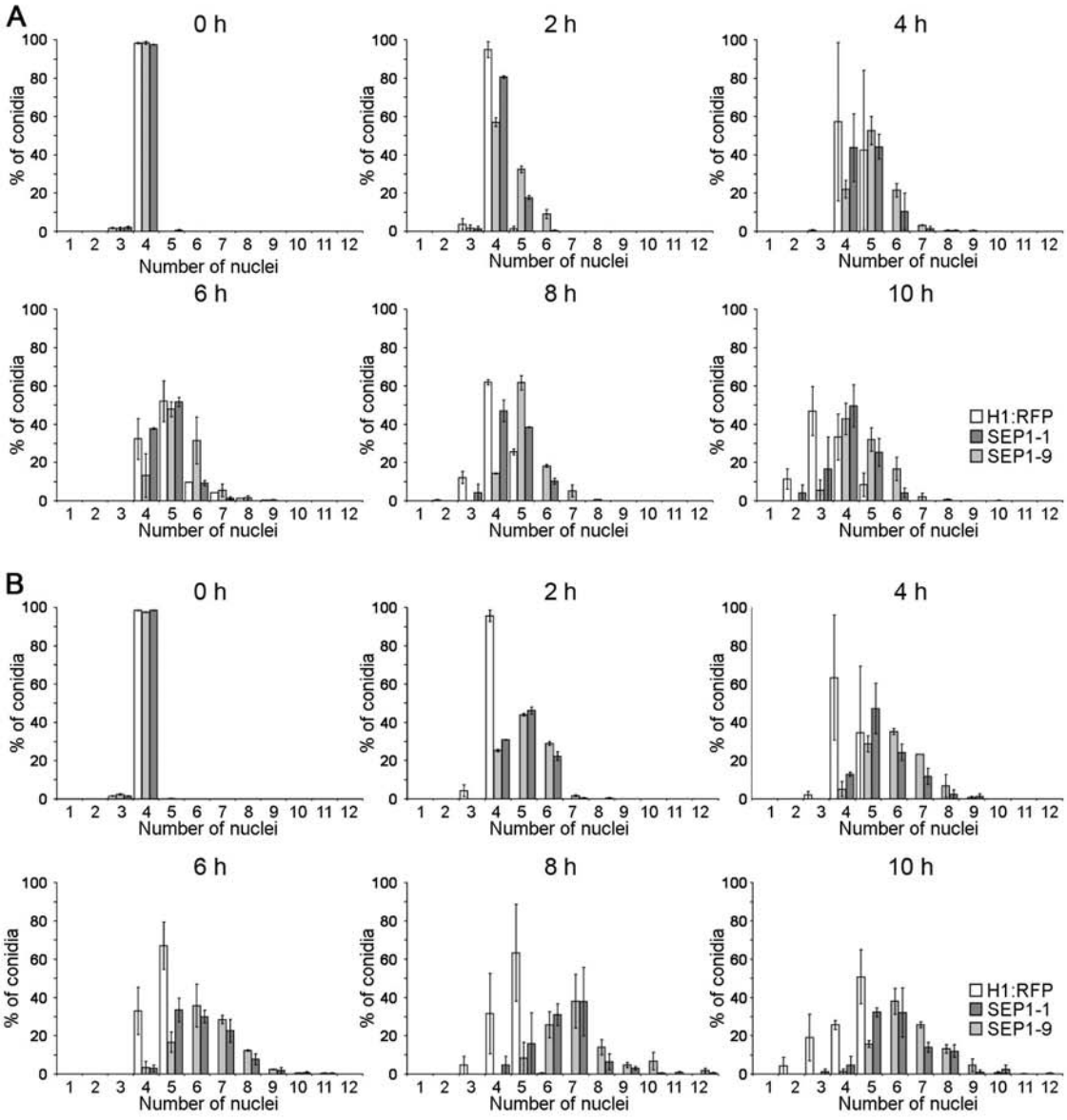
Supplemental Figure 7. The sep1G849R allele reduces hyphal growth but has no effect on appressorium development even in the presence of a wild type copy of SEP1.

A. Plugs of mycelium (5 mm diameter) from sep1G849R SEP1-201, sep1;G849RSEP1-219, alongside Guy-11, were used to inoculate complete medium agar plates incubated at a permissive temperature of 24ºC or a semi-restrictive temperature of 32ºC for 4 days. Reversion of any hyphal growth defects was then assessed by transferring plates to the permissive temperature, of 24ºC, followed by incubation for a further 3 days. B. Bar charts to show frequency of appressorium development. Conidial suspensions of the two independent M. oryzae sep1;G849RSEP1 transformants, alongside Guy-11 and sep1G849R, were prepared and incubated on hydrophobic borosilicate glass slides inductive for appressorium development at either 24ºC or 29ºC. Appressorium development was recorded 24 hours post-inoculation (pi).



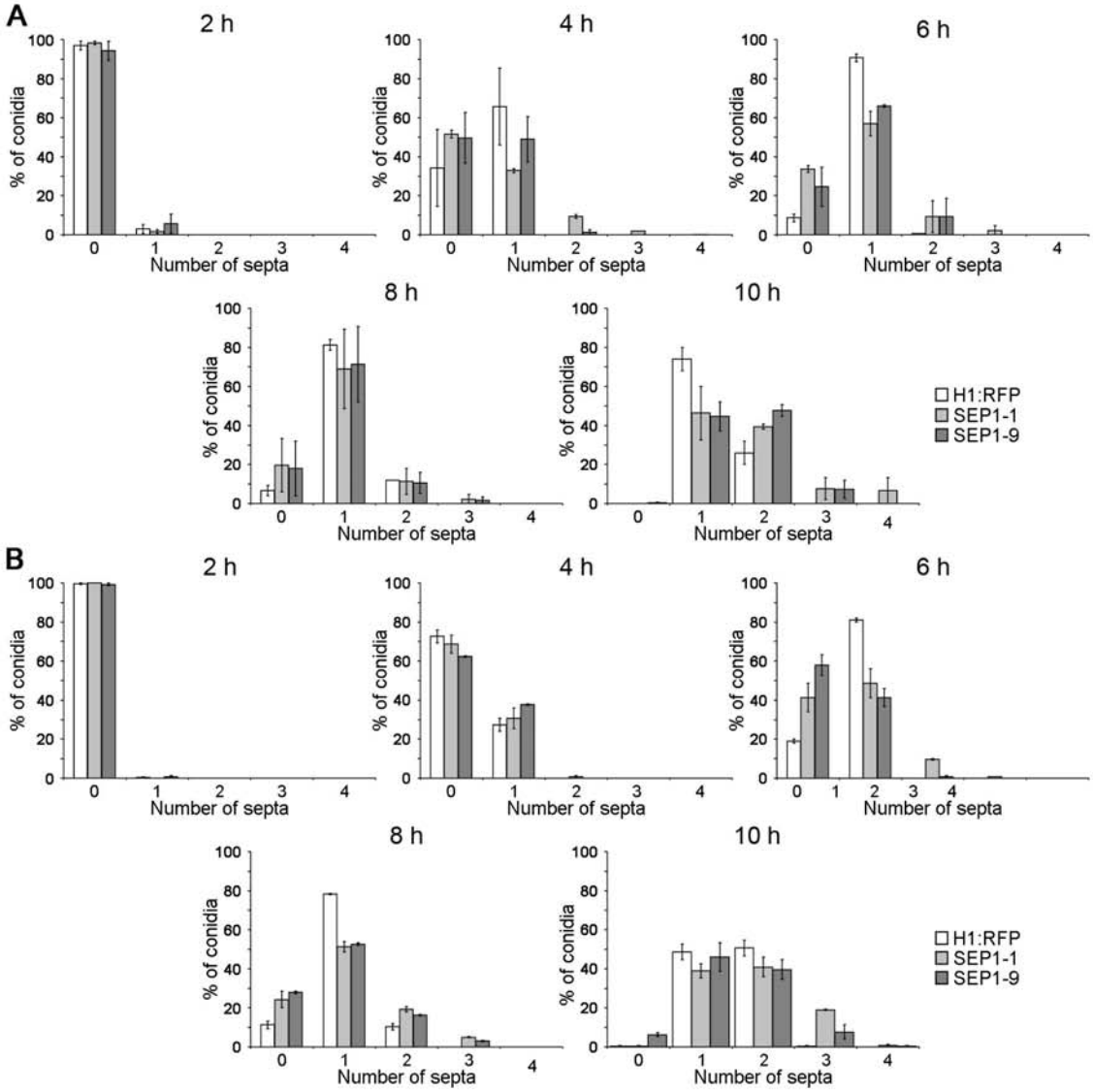
Supplemental Figure 8. A temperature-sensitive mutation in M. oryzae SEP1 alleviates the cell cycle arrest phenotype of non-germinating conidial cells during appressorium development.

Bar charts to show number of nuclei present during a time course of appressorium development in sep1G849R and H1:eRFP. Conidial suspensions were prepared from sep1G849R and H1:eRFP strains and incubated to allow appressorium development. The conidial suspensions were incubated at a permissive temperature of 24ºC (A) or after an initial incubation for 1 hour at 24ºC germinating conidia were transferred to the semi-restrictive temperature of 29ºC (B). The number of nuclei was determined through epifluorescence microscopy. Error bars are 1 standard error.



Supplemental Figure 9. Inducible over-expression of M. oryzae SEP1 alleviates the cell cycle arrest phenotype of non-germinating conidial cells during appressorium development.

Bar charts to show the number of nuclei present during a time course of appressorium development in the presence or absence of acetate. Conidial suspensions were prepared from the M. oryzae SEP1-1, SEP1-9 and H1:RFP strains and incubated in conditions inductive for appressorium development. The conidial suspensions were incubated in the absence (A) or presence (B) of sodium acetate and the number of nuclei was determined through epifluorescence microscopy at various time points during germination and appressorium morphogenesis. Error bars are 1 standard error.



Supplemental Figure 10. Inducible over-expression of M. oryzae SEP1 has no effect on septation frequency during appressorium development.

Quantitative analysis of septum formation during a time course of appressorium development. Conidial suspensions were prepared from the M. oryzae SEP1-1, SEP1-9 and H1:RFP strains and incubated in conditions inductive for appressorium development in the absence (A) or presence (B) of sodium acetate. The number of septa that formed in the germ tube was determined through calcofluor white staining and epifluorescence microscopy at various time points during germination and appressorium morphogenesis. Error bars are 1 standard error.

Supplemental Table 1 *Magnaporthe oryzae* strains used in this study.

Strain	Genotype	Reference
H1:eRFP	*grg(p):H1:eRFP*	Saunders et al., 2010
Guy-11		Leung et al., 1988
DF51	*ΔcpkA*	Xu et al., 1997
MK23	*Δmst12*	Park et al., 2002
nn95	*Δpmk1*	Xu and Hamer, 1996

Supplemental Table 2 *Magnaporthe oryzae* strains generated in this study.

Strain background	Generated strains	Description
H1:eRFP	*tpmA:eGFP*	H1:RFP strain expressing *tpmA:eGFP* fusion protein; *tpmA* from *Aspergillus nidulans* (strain denoted tpmA:GFP)
H1:eRFP	*SEP4:eGFP*	H1:RFP strain expressing SEP4:eGFP fusion protein (strain denoted SEP4:GFP).
H1:eRFP	*SEP5:eGFP*	H1:RFP strain expressing SEP5:eGFP fusion protein (strain denoted SEP5:GFP).
DF51	*ΔcpkA; H1:RFP; tpmA:eGFP*	*ΔcpkA* strain expressing H1:RFP and tpmA:eGFP fusion proteins; *H1* from *N. crassa* and *tpmA* from *A. nidulans*
MK23	*Δmst12; H1:RFP; tpmA:eGFP*	*Δmst12 s*train expressing H1:RFP and tpmA:eGFP fusion proteins; *H1* from *N. crassa* and *tpmA* from *A. nidulans*
Δpmk1	*Δpmk1; H1:RFP; tpmA:eGFP*	*Δpmk1 s*train expressing H1:RFP and tpmA:eGFP fusion proteins; *H1* from *N. crassa* and *tpmA* from *A. nidulans*
Guy-11	$sep1^{G849R}$ +/- *H1:RFP*	Strain expressing the $Mosep1^{G849R}$ temperature sensitive allele; the H1:RFP fusion protein was added post-transformation
H1:eRFP	*H1:RFP; ICL1(p): sep1*	H1:RFP strain expressing *SEP1* under acetate inducible expression (strain denoted SEP1)

Supplemental Table 3. Detailed information of the primers used in this study.

Primer name	Primer sequence[1]	Strand	Annealing Temp. (ºC)	Template
5SU	5'T CGA CGT GCC AAC GCC ACA G3'	+	62	pCB1532 (Sweigard et al., 1997)
3SU	5'T CGA CGT GAG AGC ATG CAA TTC3'	-	62	pCB1532
SEP4-F	5'GATTATTGCACGGGAATTGCATGCTC TCACCTAGTTACTGGACTAGCCTAGAC G3'	+	62	Guy-11 genomic DNA
SEP4-R	5'GGTGAACAGCTCCTCGCCCTTGCTC ACCATGTAGCCATTCATAGTCATCCTT TG3'	-	62	Guy-11 genomic DNA
SEP5-F	5'GATTATTGCACGGGAATTGCATGCTC TCACCGGTCTGCGCACCAGCGTTACC AG3'	+	62	Guy-11 genomic DNA
SEP5-R	5'GGTGAACAGCTCCTCGCCCTTGCTC ACCATGTTGCCGTCTTCCCCGTTTGCC TC3'	-	62	Guy-11 genomic DNA
5SepH-ts1-KpnI	5'AAg gta ccA TGA CAC CCC CGG GAG GCT TTG3'	+	65	Guy-11 genomic DNA
3SepH-KpnI	5'AAg gta ccG TGG AGT CGA AAG GAT TGC C3'	-	65	Guy-11 genomic DNA
3SepH-ts1-NotI	5'AAg cgg ccg cGT GGA GTC GAA AGG ATT GCC3'	-	65	Guy-11 genomic DNA
5SU-ts-NotI	5'AAg cgg ccg cGT CGA CGT GCC AAC GCC ACA G3'	+	65	pCB1532
3SU-ts-NotI	5'AAg cgg ccg cGT CGA CGT GAG AGC ATG CAA TTC3'	-	65	pCB1532
5SepH-ts2-NotI	5'AAg cgg ccg cTT TAG CGG GAA ATA GCT CCG3'	+	65	Guy-11 genomic DNA
3SepH-ts2-XbaI	5'AAt cta gaC GGA CAT GGA GAG CGT CCA GG3'	-	65	Guy-11 genomic DNA
5SepH-ts3-NdeI	5'AAc ata tgA TCT TGC AGC TGC TC3'	+	65	Guy-11 genomic DNA
5SepH-mutant	5'GTT CGT CAG CGC TGG T**C**G **A**CT GAA CGT CCT TG3'	+	-	Guy-11 genomic DNA
5ICL1P-NotI	5'AAg cgg ccg cGA ATT CGT CCA GTA ATC AAA G3'	+	65	*ICL1(p):sGFP* gene fusion (Wang et al., 2003)
3ICL1P-SepH	5'CAA AGC CTC CCG GGG GTG TCA TCT CGG GAA TAT GGT TCT TAC G3'	-	65	*ICL1(p):sGFP*
5SepH-ICL1P	5'CGT AAG AAC CAT ATT CCC GAG ATG ACA CCC CCG GGA GGC TTT G3'	+	62	Guy-11 genomic DNA
3SepH-NotI	5'AAg cgg ccg cTC CTC ATC TAC CCC AGA ATT C3'	-	62	Guy-11 genomic DNA

[1]Lowercase denotes restriction endonuclease recognition sequences and bold font signifies nucleotide substitutions.