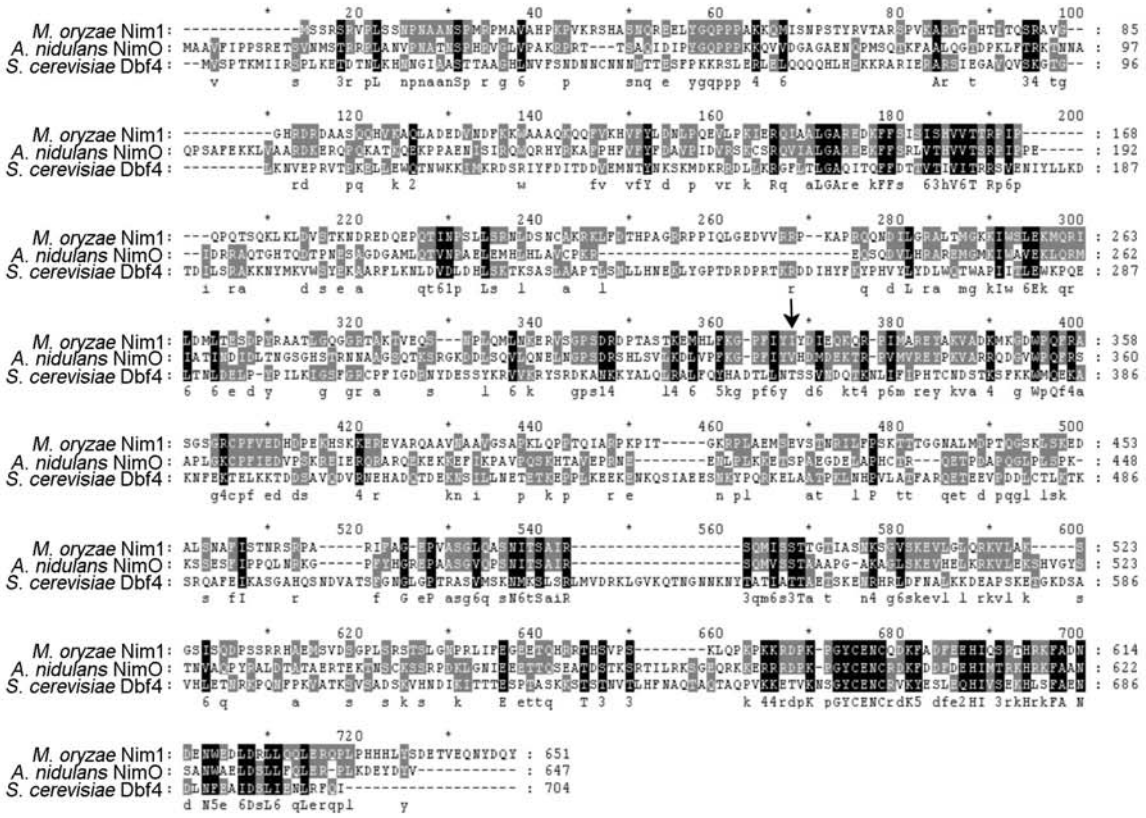
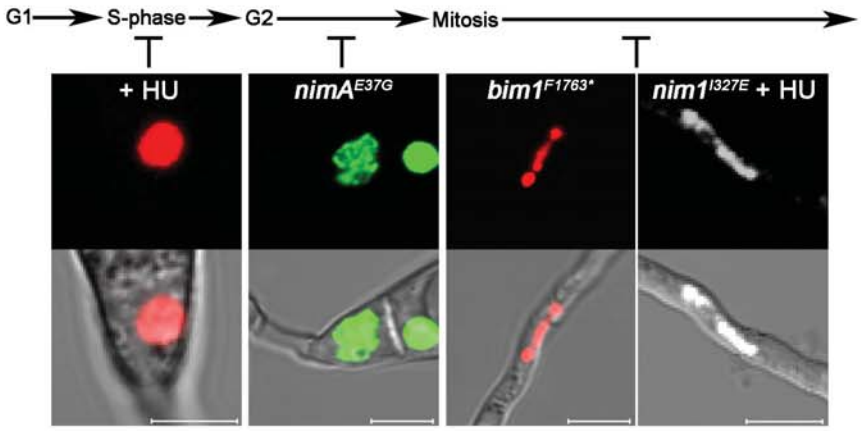


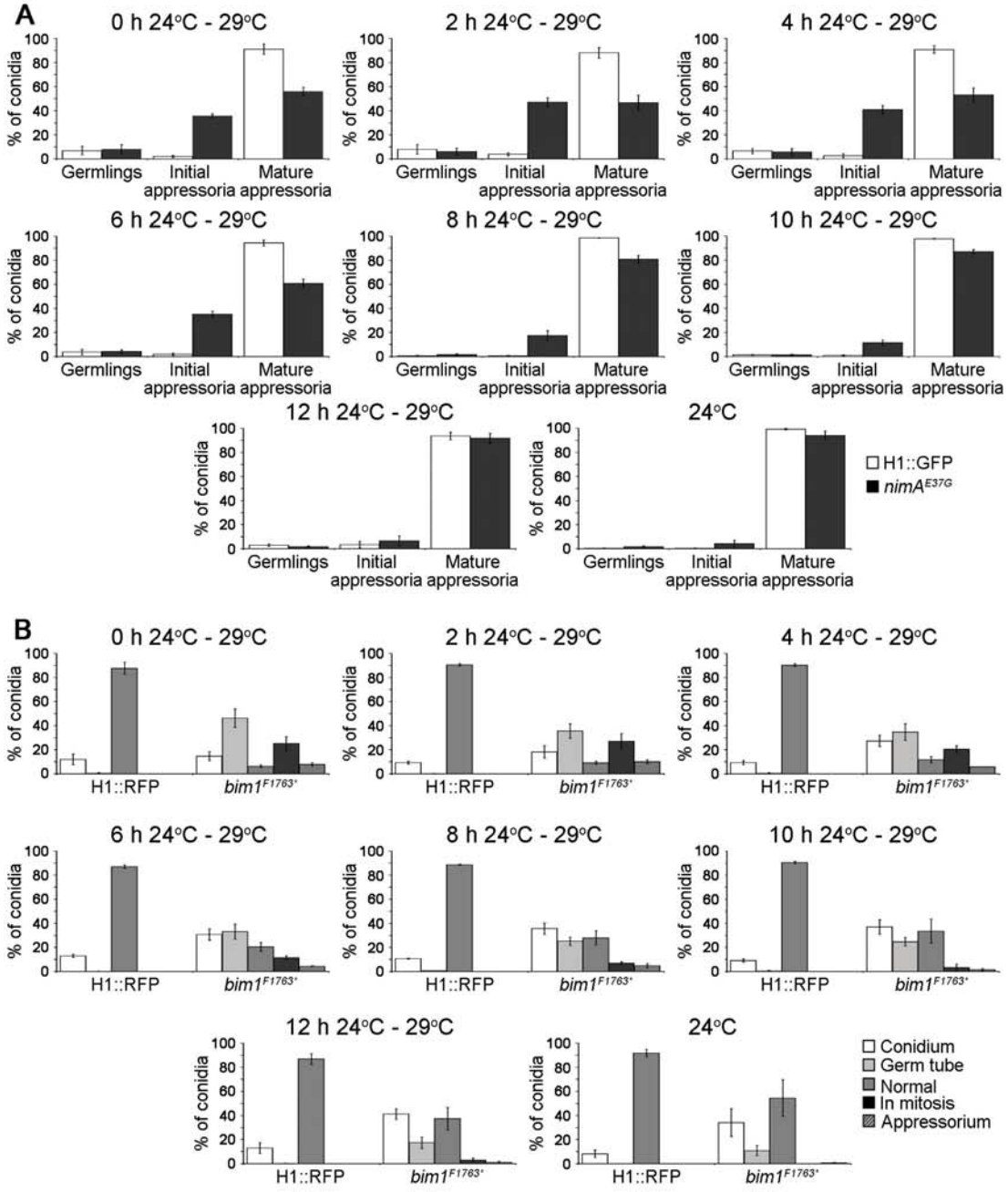
Supplemental Figure 1. Alignment of the predicted M. oryzae Nim1 amino acid sequence with A. nidulans NimO and S. cerevisiae Dbf4.

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 80% amino acid conservation. Arrow indicates position of nucleotide substitution.



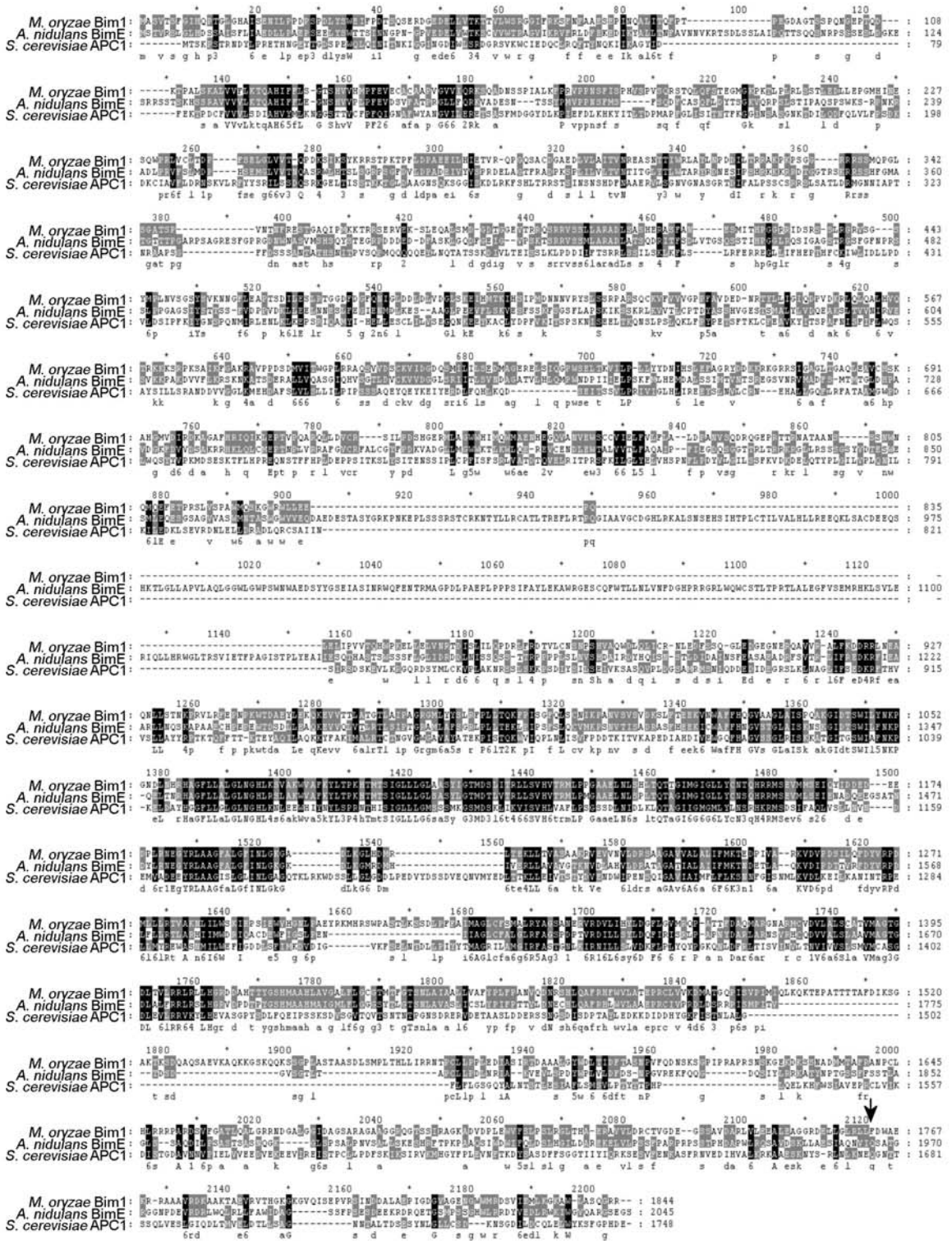
Supplemental Figure 2. High resolution micrographs of chromatin status of M. oryzae arrested at different stages of the cell cycle.

Laser confocal micrographs to show nuclear morphology of M. oryzae H1:RFP strain in the presence of hydroxyurea (HU), the nimAE37G mutant expressing H1:GFP (Veneault-Fourrey et al., 2006), The bim1F1763* mutant expressing H1:RFP, and the nim1I327E mutant expressing H1:RFP. Scale bar = 5 µm.



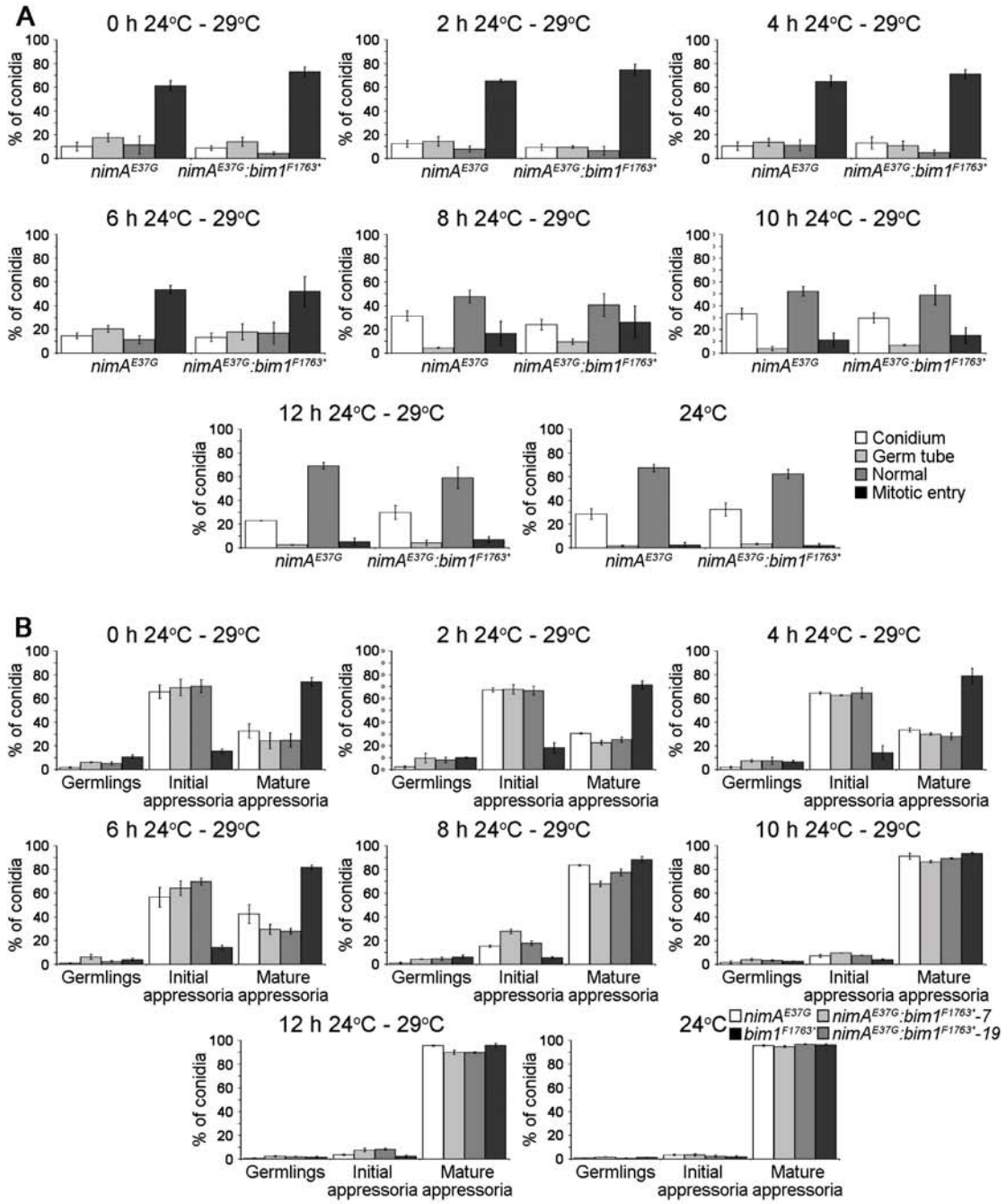
Supplemental Figure 3. Mitotic entry is sufficient for M. oryzae appressorium maturation and bim1F1763* inhibits nuclear division during appressorium morphogenesis.

A Quantitative analysis of appressorium development in the M. oryzae nimAE37G mutant. Conidial suspensions were prepared from the M. oryzae nimAE37G mutant and Guy11 expressing H1:GFP and incubated to allow appressorium development at 24ºC, or transferred to 29ºC 0, 2, 4, 6, 8, 10 or 12 hours post-inoculation (pi). Appressorium development was recorded 24 hours later. B. Bar charts to illustrate nuclear distribution during appressorium development by bim1F1763*. Conidial suspensions were prepared from the bim1F1763* mutant and H1:RFP wild-type strain and incubated at 24ºC, or transferred to 29ºC 0, 2, 4, 6, 8, 10 or 12 hours pi. Nuclear distribution was recorded 24 hours later. Conidium, daughter nucleus returns to conidium; Germ tube, daughter nucleus remains in the germ tube and fails to migrate; Normal, one daughter nucleus migrates to the conidium and is degraded during conidial cell death, the other migrates to the appressorium; Mitotic entry, daughter nuclei fail to separate. h, hours; Error bars = 1 standard error.



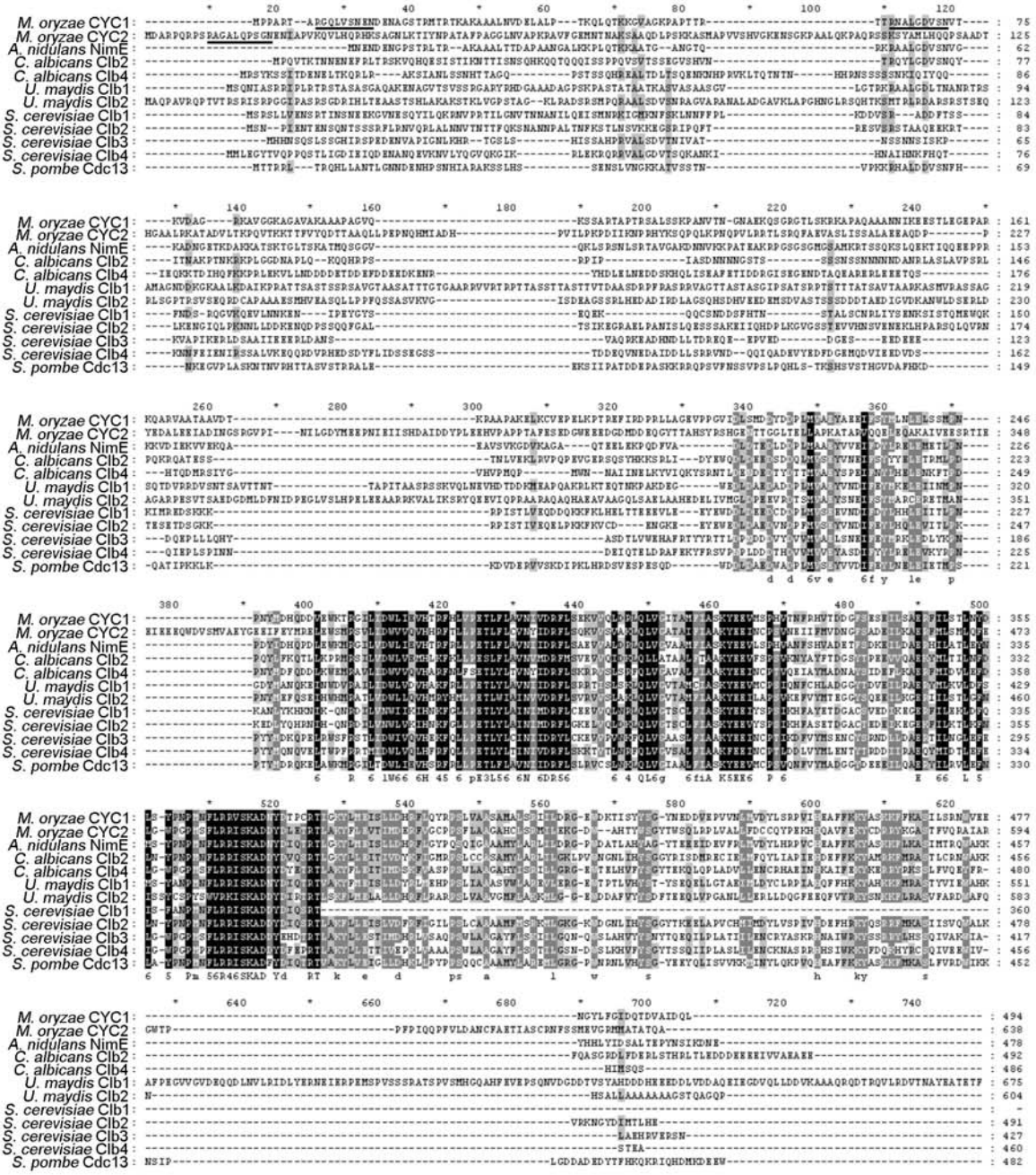
Supplemental Figure 4. Alignment of the predicted M. oryzae Bim1 amino acid sequence with A. nidulans BimE and S. cerevisiae Apc1.

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



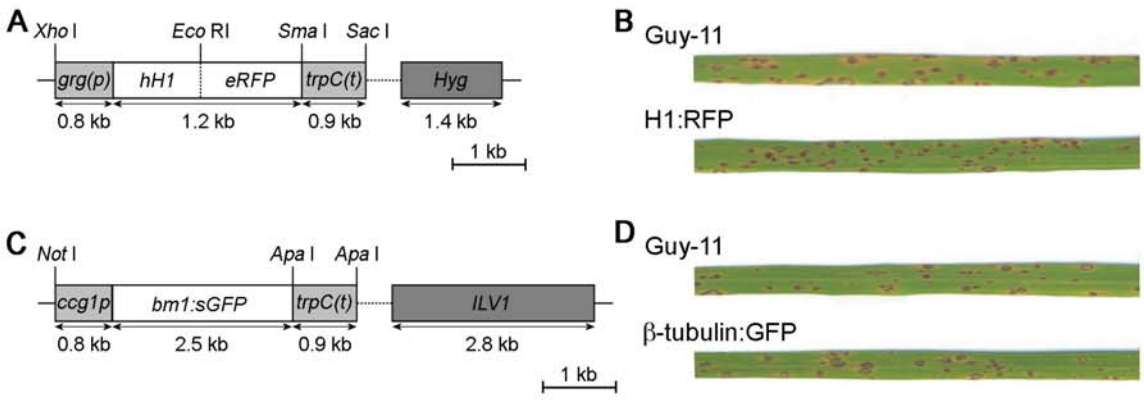
Supplemental Figure 5. The M. oryzae nimAE37G bim1F1763* displays a typical nimAE37G phenotype during appressorium development.

A. Quantitative analysis of nuclear distribution during appressorium development in the M. oryzae nimAE37G bim1F1763* mutant. Conidial suspensions were prepared from nimAE37G::bim1F1763* and nimAE37G mutants and incubated to allow appressorium development at 24ºC, or transferred to 29ºC 0, 2, 4, 6, 8, 10 or 12 hours post-inoculation (pi). Nuclear distribution was recorded 24 hours pi. For key see Supplementary Figure 3. B. Bar charts to illustrate appressorium development by nimAE37G bim1F1763*. Conidial suspensions were prepared from nimAE37G bim1F1763*, nimAE37G and bim1F1763* mutants and incubated to allow appressorium development at 24ºC, or transferred to 29ºC 0, 2, 4, 6, 8, 10 or 12 pi. Appressorium development was recorded 24 hours later. h, hours; Error bars are 1 standard error.



Supplementary Figure 6. Alignment of the predicted M. oryzae B-type cyclins with characterised B-type cyclins from A. nidulans, U. maydis, C. albicans, S. cerevisiae and S. pombe, which act specifically at the G2-mitotic transition.

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 80% amino acid conservation. Underlined regions are predicted destruction box sequences.



Supplemental Figure 7. Construction of the grg(p):H1:RFP and ccg1(p):bm1:sGFP gene fusion vectors and expression in M. oryzae.

A. Scale diagram depicting the grg(p):H1:RFP gene fusion vector. grg1(p), N. crassa grg promoter; hH1, N. crassa Histone H1-encoding gene; eRFP, gene encoding the enhanced red fluorescent protein (RFP), tdTomato; trpC(t), A. nidulans trpC termination sequence; Hyg, E. coli hygromycin phosphotransferase gene under the A. nidulans trpC promoter. B. Leaves from the dwarf Indica rice (Oryza sativa) cultivar, CO-39 following inoculation with 5 x 104 spores mL-1 of the H1:RFP expressing strain and the wild type strain, Guy 11. C. Scale diagram representing the ccg1(p):bm1:sGFP gene fusion vector. ccg1(p), N. crassa ccg1 promoter; Bm1, N. crassa β-tubulin gene; sGFP, gene encoding a green fluorescent protein derivative; ILV1, M. oryzae acetolactase synthase gene conferring sulfonylurea resistance. D. Leaves from the dwarf Indica rice (Oryza sativa) cultivar, CO-39 following inoculation with 5 x 104 spores mL-1 of the bm1:sGFP expressing strain (β-tubulin:GFP) and the wild type strain, Guy 11.

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

Strain background	Genotype	Strain name	Description
Guy-11	*H1:RFP*	H1:RFP	Strain expressing Histone H1-encoding gene (*H1*) from *Neurospora crassa*, fused to *eRFP*.
H1:RFP	*H1:RFP; β-tubulin:GFP*	β-tubulin:GFP	Strain expressing β-tubulin:GFP fusion protein; β-tubulin from *Neurospora crassa.*
Guy11	$nim1^{I327E}$	$nim1^{I327E}$	Strain expressing the n$im1^{I327E}$ thermosensitive allele.
Guy-11	$bim1^{F1763*}$ +/- *H1:RFP*	$bim1^{F1763*}$	Strain expressing the $bim1^{F1763*}$ thermosensitive allele; the H1:RFP fusion protein was introduced post-transformation
Guy-11	*H1:RFP;* $ICL1(p)$*:* $cyc1^{-}_{D1D2}$	$CYC1^{-D1D2}$	H1:RFP strain expressing stabilised *CYC1* under acetate inducible expression
Guy-11	*H1:RFP; ICL1(p)::* $cyc2^{-D1}$	$CYC2^{-D1}$	H1:RFP strain expressing stabilised *CYC2* under acetate inducible expression

Supplemental Table 2. Detailed information of the primers used in this study.

Primer name	Primer sequence[1]	Strand	Annealing temp. (°C)	Template
5grg-H1-XhoI	5'AAc tcg agC GGC CGC TAG AAG GAG CAG TC3'	+	65	pMF280 (Freitag et al., 2004)
3grg-H1-EcoRI	5'AAg aat tcT GCC TTC TCG GCA GCG GGC3'	-	65	pMF280 (Freitag et al., 2004)
5RFP-EcoRI-Link	5'AAg aat tcA TTA ACA TGG TGA GCA AGG GCG AGG3'	+	60	pFA6a-tdtomato-hph (Hagen, unpublished data)
3RFP-SmaI	5'AAc ccg ggT TAC TTG TAC AGC TCG TCC ATG3'	-	60	pFA6a-tdtomato-hph (Hagen, unpublished data)
5TrpCT-SmaI	5'AAc ccg ggC ATG GAT CCA CTT AAC GTT AC3'	+	65	*ICL1(p):sGFP* fusion construct (Wang et al., 2003)
3TrpCT-SacI	5'AAg agc tcA AGC TTG CAT GCC TGC AGG TCG3'	-	65	*ICL1(p):sGFP* fusion construct (Wang et al., 2003)
5TrpCT-ApaI	5'AAg ggc ccC ATG GAT CCA CTT AAC GTT AC3'	+	65	*ICL1(p):sGFP* fusion construct (Wang et al., 2003)
3TrpCT-ApaI	5'AAg ggc ccA AGC TTG CAT GCC TGC AGG TCG3'	-	65	*ICL1(p):sGFP* fusion construct (Wang et al., 2003)
5F1nimO-KpnI	AAg gta ccC TTT GTC TCT AAT TTT G	+	50	*M. oryzae* Guy-11 genomic DNA
3F1nimO-link	CTT CTG CTC GAT ATC GTA TTC GTA GAT GAA CGG GCC CTT G	-	50	*M. oryzae* Guy-11 genomic DNA
5F2nimO-link	CAA GGG CCC GTT CAT CTA CGA ATA CGA TAT CGA GCA GAA G	+	65	*M. oryzae* Guy-11 genomic DNA
3F2nimO-SpeI	AAa cta gtC TGG ACT GGC AGA GTG ACA G	-	65	*M. oryzae* Guy-11 genomic DNA
5SU-SpeI	AAa cta gtG TCG ACG TGC CAA CGC CAC AG	+	62	pCB1532 (Sweigard et al., 1997)
3SU-nimO-link	ATG ATC TAC TCA GGT GGG TGT CGA CGT GAG AGC ATG CAA TTC	-	62	pCB1532 (Sweigard et al., 1997)
5nimO-2kb-link	GAA TTG CAT GCT CTC ACG TCG ACA CCC ACC TGA GTA GAT CAT	+	65	*M. oryzae* Guy-11 genomic DNA
3NnimO-2kb-	AAg cgg ccg cgg tac cCT GCC CAA	-	65	*M. oryzae* Guy-

N-K	TCC CTC CTG CAA G			11 genomic DNA
5BimE-HindIII	5'AAa agc ttA TGG CTT CGG TGA CCT CGT T3'	+	58	*M. oryzae* Guy-11 complementary DNA
3BimE-EcoRI	5'AAg aat tcT TAT CTT CTG CCC TGG CTC G3'	-	58	*M. oryzae* Guy-11 complementary DNA
5ABimE-F1	5'ATG TCT ACG GTG CGG TCT CTC3'	+	60	*A. nidulans* WT and bimE7 genomic DNA
3ABimE-F1	5'ACA CCA CTG CCA CAA CCT TC3'	-	60	*A. nidulans* WT and *bimE7* genomic DNA
5ABimE-F2	5'TGT CAA TTC TGG ACT TTG CTC3'	+	60	*A. nidulans* WT and *bimE7* genomic DNA
3ABimE-F2	5'CTA AGA GCC TTC AGA ACC CC3'	-	60	*A. nidulans* WT and *bimE7* genomic DNA
5MgBimE-SpeI	5'AAa cta gtA AGC ACA CTA TGA CTT CTA TAG3'	+	62	*M. oryzae* Guy-11 genomic DNA
3MgBimE-link	5'CGC CTT GTC CCG TAC CGC GCG GCC GCG CCT CTT TTC GGC CCA GTC **TT**A CAG CAA CCG G3'	-	62	*M. oryzae* Guy-11 genomic DNA
5MgBimE-link	5'T**AA** GAC TGG GCC GAA AAG AGG CGC GGC CGC GCG GTA CGG GAC AAG GCG3'	+	65	*M. oryzae* Guy-11 genomic DNA
3MgBimE-SpeI	5'AAa cta gtT CGG CAC CGT TAC CAG ATC G3'	-	65	*M. oryzae* Guy-11 genomic DNA
5SU-1.2Kb-NotI	5'AAg cgg ccg cGT CGA CGT GCC AAC GCC ACA G3'	+	62	pCB1532 (Sweigard et al., 1997)
3SU-P2	5'CGG CTC TGA GCT AGG CGA GTC GAC GTG AGA GCA TGC AAT TC3'	-	62	pCB1532 (Sweigard et al., 1997)
51.2Kb-P3	5'GAA TTG CAT GCT CTC ACG TCG ACT CGC CTA GCT CAG AGC CGG AC3'	+	65	*M. oryzae* Guy-11 genomic DNA
3BimE1.2-NotI	5'AAg cgg ccg cTC CTC AGC TCT GGG CCC TTG3'	-	65	*M. oryzae* Guy-11 genomic DNA
5Basta-NotI	5'AAg cgg ccg cGT CGA CAG AAG ATG ATA TTG3'	+	65	pCB1530 (Sweigard et al., 1997)
3Basta-1.2BimE	5'GTC CGG CTC TGA GCT AGG CGA GTC GAC CTA AAT CTC GGT G3'	-	65	pCB1530 (Sweigard et al., 1997)
5BimE1.2RF-Basta	5'CAC CGA GAT TTA GGT CGA CTC GCC TAG CTC AGA GCC GGA C3'	+	65	*M. oryzae* Guy-11 genomic DNA
3BimE1.2RF-	5'AAg cgg ccg cGG GCC CTT GAG	-	65	*M. oryzae* Guy-

NotI	GTT GGG GAA3'			11 genomic DNA
5BimE-Seq	TCA AAG ATG ACC GCC GTC TA	+	58	Putative *M. oryzae bim1F1763** and *nimAE37G::bim 1F1763** transformants
3BimE-Seq	TCG GCA CCG TTA CCA GAT C	-	58	Putative *M. oryzae bim1F1763** and *nimAE37G::bim 1F1763** transformants
5NimA	ATG CCG GAG AGC GAT AAT TA	+	58	Putative *M. oryzae bim1F1763** and *nimAE37G::bim 1F1763** transformants
3NimA	TCA ACG ACC AGC GGA GAC	-	58	Putative *M. oryzae bim1F1763** and *nimAE37G::bim 1F1763** transformants
5CyclinB1	5'ATG CCC CCA GCC CGG ACT GC3'	+	62	*M. oryzae* Guy-11 complementary DNA
3CyclinB1	5'CTA CAG CTG GTC GAT AGC TAC G3'	-	62	*M. oryzae* Guy-11 complementary DNA
5TOPO	5'TTA CCG GAT AAG GCG CAG CGG3'	+	63	Various inserts introduced to pCR®2.1-TOPO
3B1-D1-P1	5'CGC AGT CCG GGC TGG GGG C3'	-	63	*CYC1* introduced to pCR®2.1-TOPO
5B1-D1-P2	5'GCC CCC AGC CCG GAC TGC GGA TGA GAA TGC AGG GTC AAC3'	+	63	*CYC1* introduced to pCR®2.1-TOPO
3TOPO	5'ATG CAT GCT CGA GCG GCC GCC3'	-	63	Various inserts introduced to pCR®2.1-TOPO
3B1-D2-PstI	5'AAc tgc agC TGC TTC GTG GGA AGT GCA AG3'	-	65	*CYC1*$^{-D1}$ introduced to pCR®2.1-TOPO
3B1-D2-link	5'CCA GCA ACC CCC TTC TTT GTT TGC AAC TGC TTC GTG GGA AGT GCA AG3'	-	65	*CYC1*$^{-D1D2}$ introduced to pCR®2.1-TOPO
5B1-D2-PstI	5'AAc tgc agA CAA AGA AGG GGG TTG CTG GTA AAC CCG CGC CGA CGA CAA GAA CCA CAG TCA CCA AGG TCG ATG CTG G3'	+	65	*CYC1*$^{-D1}$ introduced to pCR®2.1-TOPO

5B1-D2-link	5'CTT GCA CTT CCC ACG AAG CAG CTG CAG ACA AAG AAG GGG GTT GCT GG3'	+	62	*CYC1*$^{-D1D2}$ introduced to pCR®2.1-TOPO
5CyclinB2	5'ATG GAC GCA AGG CCA CAA CG3'	+	62	*M. oryzae* Guy-11 complementary DNA
3CyclinB2	5'TTA TGC TTG CGT CGC GGT GGC3'	-	62	*M. oryzae* Guy-11 complementary DNA
5B2-D1	5'ATG GAC GCA AGG CCA CAA CGT CCC TCC GAG AAC ATT GCA CCG GTC AAA C3'	+	60	*CYC2* introduced to pCR®2.1-TOPO
3ICL1P-B1	5'CAG TCC GGG CTG GGG GCA TCT CGG GAA TAT GGT TCT TAC G3'	-	60	*ICL1(p):sGFP* fusion construct (Wang et al., 2003)
3ICL1P-B2	5'CGT TGT GGC CTT GCG TCC ATC TCG GGA ATA TGG TTC TTA CG3'	-	60	*ICL1(p):sGFP* fusion construct (Wang et al., 2003)
5B1-ICL1P	5'CGT AAG AAC CAT ATT CCC GAG ATG CCC CCA GCC CGG ACT G3'	+	65	*CYC1*$^{-D1D2}$ introduced to pCR®2.1-TOPO
3B1-D1-D2-SpeI	5'AAa cta gtC TAC AGC TGG TCG ATA GCT AC3'	-	62	*CYC1*$^{-D1D2}$ introduced to pCR®2.1-TOPO
5B2-ICL1P	5'CGT AAG AAC CAT ATT CCC GAG ATG GAC GCA AGG CCA CAA CG3'	+	62	*CYC2*$^{-D1}$ introduced to pCR®2.1-TOPO
3B2-D1-SpeI	5'AAa cta gtT TAT GCT TGC GTC GCG GTG G3'	-	62	*CYC2*$^{-D1}$ introduced to pCR®2.1-TOPO
5TrpCT-SpeI	5'AAa cta gtC ATG GAT CCA CTT AAC GTT AC3'	+	65	pAN52.1 (Mullaney et al., 1985)
3TrpCT-NotI	5'AAg cgg ccg cAA GCT TGC ATG CCT GCA GGT CG3'	-	65	pAN52.1 (Mullaney et al., 1985)

1. Lowercase denotes restriction endonuclease recognition sequences, underscore indicates additional nucleotides encoding amino acid linker regions and bold font signifies nucleotide substitutions.

Supplemental methods

Vector construction for live cell imaging of nuclei and cytoskeletal components of *M. oryzae*

A 2.0 kb amplicon consisting of the *Neurospora crassa grg* promoter and the Histone H1-encoding gene (*H1*) was amplified from pMF280 (Freitag et al., 2004; GenBank; AY598429) (with primers 5grg-H1-Xho1 and 3grgH1-EcoR1) and inserted into pCB1004, which carries the hygromycin resistance selectable marker gene (Carroll et al., 1994). All primers used in this study are shown in Supplemental Table 2 online. The 1.4 kb *tdtomato* (*eRFP*) fragment (Shaner et al., 2004) was amplified and fused in-frame to the C-terminus of the *H1* gene in pCB1004 (primers 5RFP-EcoRI-link and 3RFP-SmaI). The 0.8 kb *Aspergillus nidulans trpC* termination sequence was amplified from pAN52.1 with primers 5TrpCT-SmaI and 3TrpCT-SacI (Mullaney et al., 1985, Genbank; Z32697) and fused to the C-terminus of the *eRFP* gene in pCB1004 to create pDS103 (see Supplemental Figure 7 online).

The *N. crassa* 3.3 kb *ccg1(p):bm1:sGFP* gene fusion was excised from pMF309 (Freitag et al., 2004; GenBank; AY598430) by digestion with *Apa* I and *Not* I, and inserted into pCB1532 (Sweigard et al., 1997). The 0.8 kb *A. nidulans trpC* termination sequence was amplified from pAN52.1 with primers 5TrpCT-ApaI and 3TrpCT-ApaI (Mullaney et al., 1985) and fused to the C-terminus of the *sGFP* gene in pCB1532 to create pDS104 (Supplemental Figure 7 online).

Construction of the *M. oryzae nim1*^{I327E} gene replacement vector

A 4.1 kb fragment of the *M. oryzae NIM1* gene (including 1.5 kb upstream sequence, the 2.3 kb *NIM1* coding sequence and 0.3 kb of the 3'UTR was amplified from genomic DNA (*M. oryzae* genomic locus MGG00597; *A. nidulans* nimO

Genbank AF0142812). The 4.1 kb fragment was amplified initially in two fragments of 2.5 kb and 1.6 kb, either side of the region requiring nucleotide substitution. Substitutions A1055G, T1056A and C1057A were introduced by PCR to change amino acid 327 from isoleucine to glutamic acid (primers 5F1nimO-kpn1, 3F1nimO-link, 3F2nimO-SpeI, 5nimO-2kb-link, 3NnimO-2kbN-K). The 2.5 kb and 4.1 kb fragments were then joined by fusion PCR (Yu et al., 2004). The 2.8 kb modified *ILV1* allele, conferring resistance to sulfonylurea, was amplified from pCB1532 with primers 5SU-SpeI and 3SU-nimO-link (Sweigard et al., 1997; GenBank; AF013601). A 2.0 kb region downstream of the *NIM1* locus was amplified from genomic DNA to provide a region of homology 3' to the *ILV1* gene in the gene replacement construct. The 2.8 kb and 2.0 kb fragments were then joined by fusion PCR (Yu et al., 2004) and the 4.8 kb amplicon introduced into the pCR®2.1-TOPO cloning vector (Invitrogen), to create pDS105. The 4.1 kb $nim1^{I327E}$ gene replacement region was then inserted into pDS105. The full length 8.9 kb gene replacement construct was excised by digestion with *Kpn* I, gel-purified and a 10 µg aliquot of DNA used for fungal transformation of the *M. oryzae* strain, Guy-11.

Construction of the *M. oryzae bim1*^{F1763*} gene replacement vector

The A. nidulans bimE7 allele was amplified and sequenced (primers 5ABimE-F1, 3ABimE-F1, 5ABimE-F1, 3ABimE-F2) to reveal a single point mutation, which altered a glutamine residue to a premature stop codon at position 1966. A 2.9 kb *BIM1* gene fragment including 2.4 kb of the *BIM1* ORF and 0.5 kb of the 3'UTR was amplified from genomic DNA (*M. oryzae* genomic locus MGG03314) using primers 5BimE-HindIII and 3BimE-EcoR1 (Supplemental Table 2). The 2.9 kb region was amplified initially in two fragments, 2.1 kb and 0.8 kb, either side of the region

requiring nucleotide substitution. The primers used to amplify the two fragments introduced nucleotide substitutions T6136A and C6137A, which in turn changed amino acid 1763 from phenylalanine to a stop codon, thereby truncating the corresponding protein(5MgBimESpe1, 3MgBimE-link, 3MgBimE-link, 3MgBimE-SpeI). The 3'-end 0.8 kb gene fragment was amplified and the resulting 2.1 kb and 0.8 kb *BIM1* gene fragments joined by fusion PCR.

The 2.8 kb modified *ILV1* gene, conferring resistance to sulfonylurea, was amplified from pCB1532 (Sweigard et al., 1997; GenBank; AF013601). A 1.2 kb region adjacent and downstream to the *BIM1* locus was amplified using the PCR from genomic DNA in order to provide a region of homology 3' to the *ILV1* gene in the gene replacement construct using primers 5SU-1.2kb-Not1 and 3SU-P2. The 2.8 kb modified *ILV1* gene and the 1.2 kb region adjacent to the *BIM1* locus were joined and the resulting 4.0 kb amplicon gel-purified and ligated to pSC-A vector arms (Stratagene). The resulting pSC-A vector, containing the 4.0 kb *ILV1:BIM1* downstream region, was ligated to the 2.9 kb *Spe* I *BIM1* fragment. The full length 6.9 kb gene replacement construct was excised with *Xba* I and *Apa* I, and introduced into *M. oryzae*. Following validation of potential $bim1^{F1837*}$ transformants, two transformants were selected and transformed with the *grg(p):H1:eRFP* gene fusion construct.

In order to study the epistatic relationship between *BIM1* and *NIMA* in *M. oryzae* the $bim1^{F1837*}$ temperature sensitive allele was introduced into the $nimA^{E37G}$ temperature sensitive strain of *M. oryzae* (Veneault-Fourrey et al., 2006). The $bim1^{F1837*}$ temperature sensitive allele and bialophos selectable marker were amplified using primers 5Basta-not1, 3Basta-1.2BimE, 5BimE1.2RF-Basta, 3BimE1.2RF-Not1 and ligated together to create a new allelic replacement construct,

pDS106, which contains the bialophos resistance selectable marker from pCB1530 (Sweigard et al., 1997).

Putative *M. oryzae bim1*^{F1763*} and *nimA*^{E37G}*:bim1*^{F1763*} transformants were subject to DNA sequence analysis (primers 5BimE-Seq, 3BimE-Seq) to ensure the relevant mutations in *NIMA* and *BIM1* had been incorporated.

Removal of the putative destruction boxes from *M. oryzae CYC1* and *CYC2*

The 1.5 kb *M. oryzae CYC1* coding sequence was used as a template to amplify two fragments either side of the first destruction box, D1, which corresponds to nucleotides 22 to 48 of the coding sequence. The fragments were then joined by fusion PCR and the resulting 2.3 kb amplicon sequenced to confirm the successful removal of the D1 destruction box (primers 3B1-D1-P1, 5B1-D1-P2). The second destruction box, D2, in *M. oryzae CYC1* which corresponds to nucleotides 193 to 219 of the coding sequence was removed in the same way and the two fragments joined (primers 3B1-D2-Pst1, 3B1-D2-link, 5B1-D2-Pst1, 3B1-D2-link, 5B1-D2-link). The resulting 1.4 kb amplicon was gel-purified and subjected to comprehensive DNA sequence analysis on both strands to ensure removal of the destruction boxes and to check for any rearrangements or substitutions that may have resulted from extensive use of the PCR.

The 1.9 kb *M. oryzae CYC2* coding sequence was used as a template for PCR-based removal of the putative destruction box sequence, nucleotides 28 to 57 of the coding sequence (5B2-D1, 3B2-D1-Spe1). The resulting allele was sequenced to confirm removal of the destruction box sequence.

Regulated expression of *CYC1*$^{-D1D2}$ and *CYC2*$^{-D1}$

The 1.5 kb *ICL1* promoter, *ICL1(p)*, which drives expression of the isocitrate lyase-encoding gene in *M. oryzae*, was amplified from a *ICL1(p):sGFP* fusion construct (Wang et al., 2003) and joined to the *CYC1*$^{-D1D2}$ and *CYC2*$^{-D1}$ alleles to create in-frame promoter fusions (primers 5B1-ICL1P, 3ICL1PB1, 5B2-ICL1P, 3B2-ICL1P). The 0.8 kb *trpC* terminator, *trpC(t)*, was ligated into pSC-A containing *CYC1*$^{-D1D2}$ or *CYC2*$^{-D1}$. The resulting *CYC1*$^{-D1D2}$*:trpC(t)* and *CYC2*$^{-D1}$*:trpC(t)* gene fusions were then excised and ligated to the *ICL1(p)* sequence. The 2.2 kb *CYC1*$^{-D1D2}$*:trpC(t)* and 2.7 kb *CYC2*$^{-D1}$*:trpC(t)* amplicons were gel-purified and joined to the 1.5 kb *ICL1(p)* by fusion PCR. The resulting amplicons were ligated into pCB1532 (Sweigard et al., 1997) and introduced into the *M. oryzae H1:RFP* strain.

The addition of sodium acetate has been shown to induce expression of genes placed under the *ICL1* promoter which drives expression of the isocitrate lyase encoding gene in *M. oryzae* (Wang et al., 2003). Hyphal growth on minimal media (MM; Talbot et al., 1993) and appressorium development was assessed in *M. oryzae* strains containing genes under *ICL1(p)*-directed expression, through the presence or absence of 50 mM sodium acetate over time.