

Supplementary Information

Table S1. Fungal strains and transformants.

<i>Aspergillus parasiticus</i> SU1
<i>Aspergillus parasiticus</i> BN9
<i>Aspergillus parasiticus</i> RHΔaflJ, ptrA ⁻
<i>Aspergillus parasiticus</i> RHΔaflJ-gpd-A. <i>flavusaflJ</i>
<i>Aspergillus parasiticus</i> RHΔaflJ-gpd-D. <i>septosporumafJ</i>
<i>Aspergillus parasiticus</i> RHΔaflJ-gpd-A. <i>nidulans</i> ANid7819aflJ
<i>Aspergillus parasiticus</i> RHΔaflJ-gpd-A. <i>nidulans</i> ANid10021aflJ
<i>Aspergillus parasiticus</i> RHΔaflJ ptrA ⁺ gpdA-aflJ::GFP
<i>Aspergillus parasiticus</i> BN9 ptrA ⁺ gpdA-aflR::GFP
<i>Aspergillus flavus</i> CA14 Δku70 ΔpyrG-ptrA ⁻
<i>Aspergillus flavus</i> CA14 Δku70 ΔpyrG-ptrA ⁺ -gpdA-c-myc::aflJ
<i>Aspergillus flavus</i> CA14 Δku70 ΔpyrG-gpdA- ptrA ⁺ -c-myc::aflR
<i>Aspergillus flavus</i> CA14 Δku70 ΔpyrG-amyB-Ct-eYFP::aflJ
<i>Aspergillus flavus</i> CA14 Δku70 amyB-Nt-eYFP::aflR
<i>Aspergillus flavus</i> CA14 Δku70 ptrA ⁺ -amyB-Nt-eYFP::aflR; amyB- Ct-eYFP::aflJ

Table S2. Oligonucleotides used for QPCR ^a.

Quantitative PCR in chromatin immunoprecipitation assays		
Name	Location	Sequence
<i>pksA-F</i>	15865	TTCGTCGTTGACCGATGAGCTGAGT
<i>pksA-R</i>	16007	CGATGGCCACATGGTGCAATAA
<i>nor-1-F</i>	17347	AACTCGGCCAGCGACCAACACA
<i>nor-1-R</i>	17484	GCCTCTCTTGATCGTGCTGGCTAA
<i>fasB-F</i>	25282	ATCGGTTCAATGCTCGAACACCTAA
<i>fasB-R</i>	25413	CAGCCTGGCTGCCATTCTTGA
<i>aflJ-F</i>	34810	GGGACGTTTCAGTAGCTCTCCTTGCA
<i>aflJ-R</i>	34938	GGCGCAGGTTTCTAGGTCAGTCA
<i>ver-1-F</i>	41839	CGCCGCCCGATGAGCTACTGGT
<i>ver-1-R</i>	42100	GACGGCGATGGCAGCACCGA

^a Location is in *A. parasiticus* aflatoxin cluster sequence; GenBank Accession number AY371490. Oligonucleotide sequences are written from the 5' to 3' direction. Oligos designed using designed using Primer Express3, Applied Biosystems.

Table S3. PCRs to prepare DNAs used for aflJ gene complementation.

Name	Sequence
AF-aflJ-Not-F	aatagcgccgcATGACCTTGACTGACCTAGAAACC
AF-aj-Rsr-R2	aggctcggaccgGGCGTGATAGAGTCTTCCGGCTA
Nid-7819-NF	actagagcgccgccaccATGACCGGTGCTAACAAAGTAA
Nid-7819-CR	aggctcggaccgTTAGTGCTTTCTAGCAGACGGTTC
Nid-10021-NF	actgtagcgccgccACCATGTCTAGTCTATCCGA
Nid-10021-CR	gtcgacgggtccgatTCACCTGTTATAGGCCTGGT
Doth-aj-FNot	actgtagcgccgcCACCATGTGCGGATCTCT
Doth-aj-RKpn	aggcatgtgaccTCATCCTAAGAGCTGCGGCTG

Plasmids were created in pTRI-gpdA-trpC [4] by cloning into NotI and RsrII or SmaI sites.

Table S4. Oligos to check for expression of *A. parasiticus* aflatoxin cluster genes.

Name	Sequence	DNA product size	RT-PCR size
pksA-F	ATGGCTCAATCAAGGCAACT	756	625
pksA-R	CTTAGAGATGCAGCTGATCCAC	-	-
fasA-F2	CAAGAATGCCATCTTTGTTGAG	331	281
fasA-R2	CGCCATAGCTGATGCTCA	-	-
omtA-F	ACAGGATATCATTGTGGACGG	465	387
omtA-R	ATACCTAGATCAAAGCGGCG	-	-
Ver-1 F2	GGATCCTGAGGCAACTGC	331	281
Ver-1 R2	CCTTGGACCCGGAGTATACA	-	-
Doth-F	AGAAGTGTTGCTGGCGGAA	949	854
Doth-R	GTCGCAGAATGTAGACGGCT	-	-

fasA, *pksA*, *ver-1*, *omtA* in *Aspergillus parasiticus* SRRC2043- Δ *aflJ* complemented with *gpd-A. flavus aflJ*; *gpd-D. septosporum aflJ*.

Table S5. Oligos for preparation of constructs used for Y2H studies.

Name	Sequence
AflR-F (<i>NdeI</i>)	gagcatatgGTTGACCATATCTCC
AflR-R (<i>BamHI</i>)	attggatccCATTCTCGATGCAGGTAAT
AflJ-F (<i>NdeI</i>)	cagcatatgACCTTGACTGACCTAGA
AflJ-R (<i>BamHI</i>)	attggatccTTATATCGGTTGTCAT

Table S6. Oligos for cloning into pTRI-*gpd-trpC* for expression of AflJ with myc tag.

Name	Sequence
pgb-cmyc-F (<i>NotI</i>)	gagcatatgGTTGACCATATCTCC
aflJ-R (<i>RsrII</i>)	gcattcggtccgTTATATCGGTTGTCATTG
aflJ-R (<i>SmallI</i>)	TCATTCTCGATGCAGGTAAT

Table S7. Oligos for cloning into puc18-*gpdA-eGFP-nmt1* for expression of AflR and AflJ with the GFP tag.

Name	Sequence
AflJ-F (<i>NcoI</i>) (A.p.)	CGAGCCATGGCCTTGACTGACCTAGA
AflJ-R (<i>NcoI</i>) (A.p.)	GCATTCCATGGcATATCGGTTGTCAT
aflR-InFusion*-pUC-F	GCAGACATCACCATGGATGGTTGACCATATC
aflR-InFusion*-pUC-R	CCCTTGCTCACCATGGccttcgatgcagga
aflR-aflJ-InFusion*-F	GCATGATGGCCGTCATGCATTTCATTCTCGATGCAGGTA
aflR-aflJ-InFusion*-R	CCCTTGCTCACCATGGCATATCGGTTGTCATC

* InFusion is a non-restriction enzyme method for cloning developed by Clontech.

Figure S1. Plasmids used for the fungal transformations. (A). pTRI-*gpdA-trpC* was derived from pPTRI a cloning plasmid available from Takara with the *A. oryzae* *ptrA* (pyrithiamine resistance gene) for selection on pyrithiamine containing media. This plasmid was used for construction of the expression plasmids for the *aflJ* homologs; (B) This pUC-based plasmid was used for construction of the expression plasmids for the *aflJ* homologs and for preparation of the *aflJ::GFP* and *aflR::GFP* plasmids Introduction into *Aspergillus* was by co-transformation with Pptri; (C) pTRI-*gpdA-trpC* was used to create the N-terminal split YFP vector, where the *gpdA* promoter was replaced by *amyB* promoter and Nt-eYFP-*aflR* was introduced by overlap PCR from a plasmid first created in pMCBapx; (D) pMCB17apx-*amyB* was used as the plasmid for construction of the C-terminal split eYFP vector. The *gpdA* promoter of the pMCB17apx vector was replaced with the *amyB* promoter as a *EcoRI*-*KpnI* fragment.

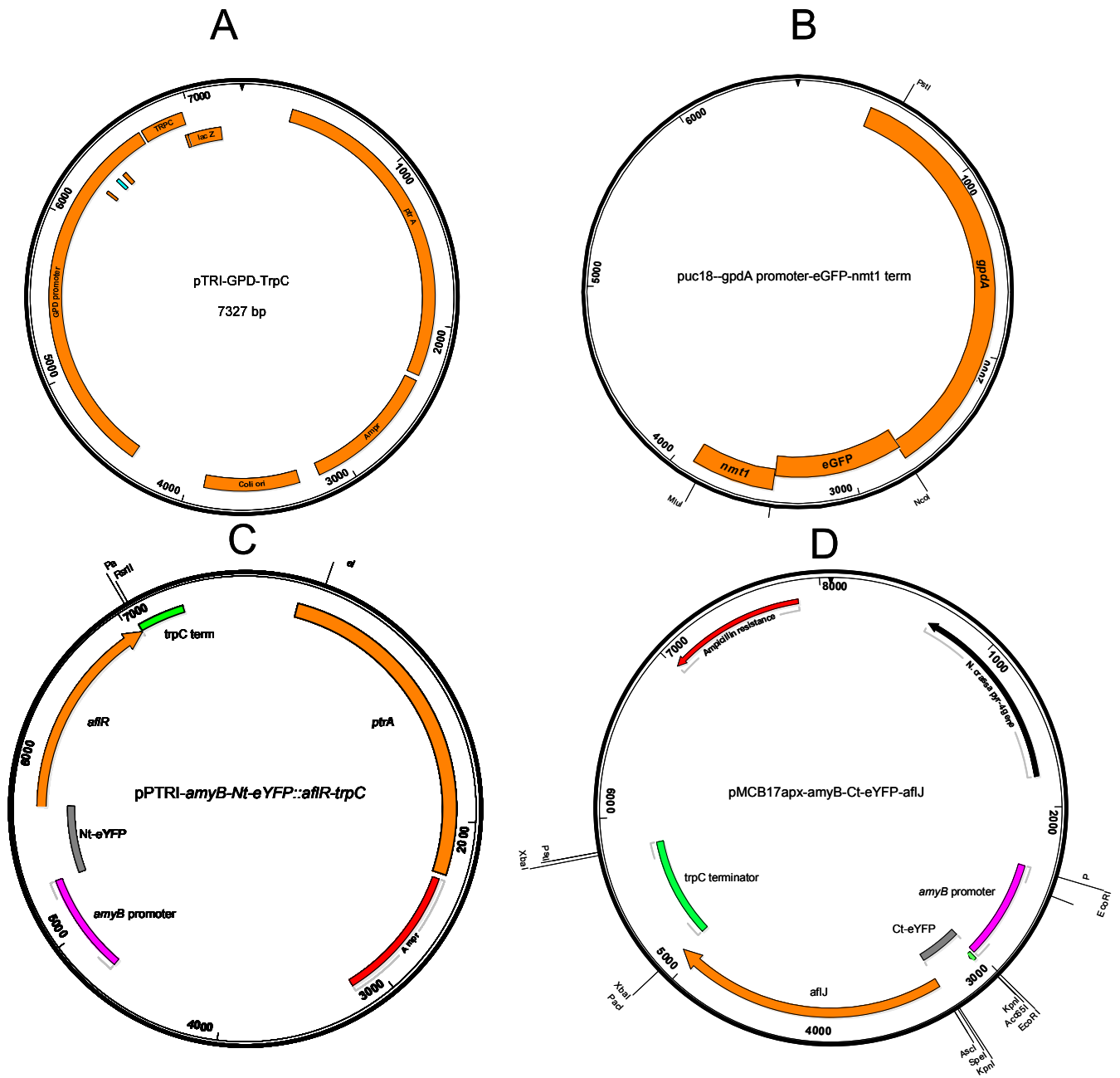
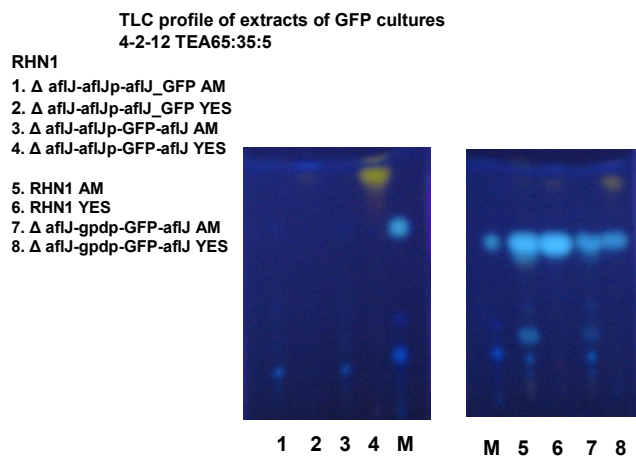
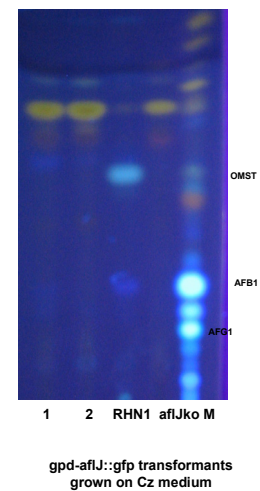


Figure S2. Thin layer chromatography results (various transformants TLC of extracts of mycelia from *A.parasiticus* SRRC2043 Δ *aflJ*-*aflJ*::*GFP* transformants (accumulates OMST). (A) Δ *aflJ* produces no OMST whereas the *gpd*::*aflJ*::*GFP* complemented mutant produces OMST in quantities that approach those of the parental strain (RHN1 = AP2043 Δ *niaD*) in the Figure; (B) When *aflJ* is the promoter the yield of OMST is barely visible when grown in two media conducive for OMST expression. On Czapek's medium, *gpd*-*aflJ*::*GFP* does not complement the deletion as expected; (C) Δ *aflJ* complemented with wild-type *aflJ* and *aflJ* fused to different fluorescent tags.

A



B



C

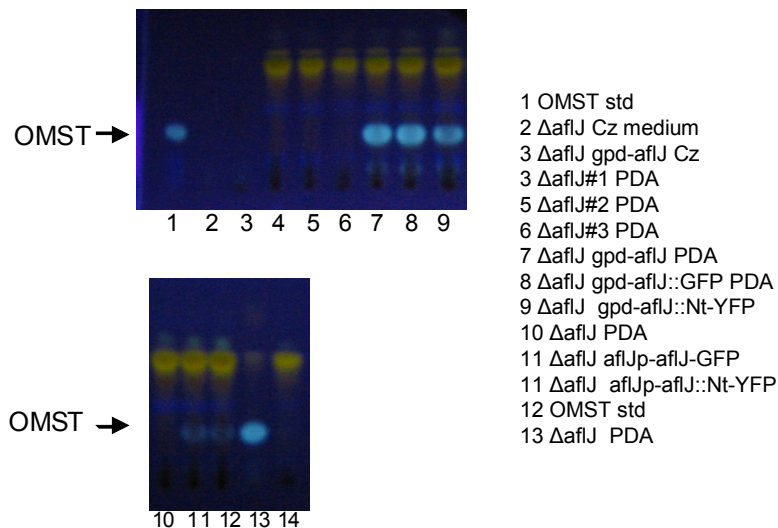


Figure S3. Sequence of *Dothistroma septosporum aflJ* (Provided by Dr Rosie Bradshaw).

DNA sequence

>aflJdothistroma-genomic(1-1482)

ATGTCGCGGATCTCTCGGCTGGGTGCATGCGCGGAGGAGCTTGCCATCGCTGCCACGACC
 ATTGCGGCATTCTGCAAACACCATAGCAATTCTGGCCTACCTGGCGATAGCATACCGCCG
 GACGCTCCCCAGAAGGTGCTGCAGGCCAAGCAATCCGTCATCACCAACTCACAGAAATTA
 GAAGTGTGCTGGCGGAACCGGCGGACTTTATACAACGTCTTGCGCGAGAGGTTGCTCA
 CAAACCCAATATGTCCATTTGCCGTCCATACTGATTGTGCTGTTCAGAACCAACTGCTGG
 CTTGCTTGCAATGGCTCGGCGAGTTCCAAGTGCTTGCTGCATTCTATCGTGGATTCCG
 TACTACTACAGCGACGTGGCCGACCTTGCTTGCCTACCGGTCGATCAGCTGCGACGAATTG
 CTCGCATGACAATCACGGCAGGCTTCTCCAAGAGCCGAAGCCAGGGTATGTCGCTCACA
 GCGGACTGTGCGCGCCGTTCTGTAACAGCCCGTGCTGCTAGATGCAGCGATGTTCTTGT
 CCGAGACCCTCGCGCCGTCTGCTCTTCACATGTCACTAGCGACGAAGCGCCATGGTCGAA
 CTCACCAGACTGACCAATGCGCGTTCAATACCGCATTCAATACCAAGGCCAGTTTCGCGG
 ATTCACTCGGACGAAGGCCTAGGCTGCAACGTCAATGGCCATCATTCTCAAGATACGCCA
 TCGCCGATGACGAGGCTGGCGTCGAAGATGTTATGACTCGCTTGGACTGGCTTAGCCTAG
 GCGAAGCTACAGTGGTCGATGTAGGTGCTGTGCCGAACAGTCATTAGGTCTGAGTACCCC
 ATTGATCACGATCTACAGGTCTGTGCGAAGACAGCATCTCTCGCGACGGCACTGACGAGC
 AAATATCCGTGCTGCGGTTTCGTCGTTCAAAGTGAAGAACAGTGCCAGAACCATACTTGG
 TCGCGATCATTGTGACGACAAAGCTGCACAATGGTTTGTGACACCTCCCGAATCCGAT
 ACCGGACCCGCTGCACGGGCTGCCAAGGCAAGTGAACGTCTTGAGCTGCAACAACGAGCG
 TTGGGCTCGCCGCAGAATGTCACCAATGCAGCCGTCTACATTCTGCGACTCGGTACAGCC
 TCGCCTTTCACGTCCTGGCACAAGCTCAGAGCGCAGGCTACAGCAGAGCTCAGCGCTCAT
 GCTGACATCTTGCGCAAAGAGCATGGATCAAGACTCATCCTGGTGACCCGCACTTTGCCG
 AAGCCTGGCGAGGTAGAGACCACTGTGCGAGGCCATGGCACGATTCGAGACCTCACCTG
 ATGCAGCTGGCCAACGTAAGGGAAGTGGAACTTCCGAAGTCGTGGAATTATTGAACAGC
 GTCCACATCGAGGGCGGATGCCTCGTGCTCACGAACGAGCTGAGAACCAGAAACAGCGGT
 ATGATCGCATTGAGGCGGACTTATCAGCCGCAGCTCTTAGGA

Protein sequence

MSRISRLGACAEELAIAATTIAAFCKHHSNSGLPGDSIPPDAPQKVLQAKQSVITNSQKLEVLLAEPADF
 IQLARENQLLACLQWLGEFQVLACIPIVDSVHYSDVADLACVPVDQLRRIARMTITAGFLQEPKPGYVA
 HSGLSAPFVKQPVLLDAAMFLSETLAPSALHMSLATKRHGRTHQTDQCAFNTAFNTKASFADSLGRRPRL
 QRQWPSFSRYAIADDEAGVEDVMTRLDWLSLGEATVVDVCAKTASLATALTSKYPSLRFVQSEEQCQNHTWS
 RSLSATKLHNGLSTPPESDTGPAARAAKASERLELQQRALGSPQNVTNAAVYILRLGTASPFTSWHK
 LRAQATAELSAHADILRKEHGSRLILVTRTLPKPGEVETTVEAMARFRDLTLMQLANVRELETSEVVELL
 NSVHIEGGCLVLTNELRTRNSGMIAFEATYQPQLLG*

Figure S4. Sequences of *A. nidulans aflJ* homologs.

A. nidulans AN_10021

Genomic sequence

>ANID_10021

ATGTCTAGTCTATCCGACCTTGAAACCCACGCCAGTGAGCTCACAAGCGCTGTCAAGACG
ATCATCTCGCAATGCCCTCGCCAAAATGCCGCCTCTCGCAGCAGAACTCAACCCCTCATC
ACCTCTAGCGCTTCCAAGGAAGCGCATCGAGCCCAACAATCGATCTTATCAACCATTCT
GGCCTCCAGAAGCTCCTCACCAGCCCAACCGACTTCCTCCACCACCTCGCCGTTCAGAAC
CAGCTGCTTGCCTGCCTACAATGGCTCGGAGAGTTCCAAGTCCTCGCTTGCATTCCCCTC
ACCGGCACCGTTCCCATAAAAGATGTCGCTGAGCTGGCCGGTGTCCCAGAGACTCATCTC
TCACGTATTATCCGGATGACAGCCACCGCTGGCTTCCTGGATGAGCCAGACCCCGGTCAA
GTCGCTCACAGCGCGCTCTCCGCTCCTTTCGTCACCAAACCGTCTTATCTTGACGCTGTG
ATGTTTTTGGCTGGCACCATTGCCCCCTTCTGCTTTGCAGATGCCTACTGCAACGCAGCGA
TTTGGCGGAGTTTGCCTCCGAACGAGACCGCGTACAACCTAGCTTTAAATAACCCAGCG
ACATTCGCCAGTACGTCTGAGCAACGGCCAAAGCTTCAACGCCAGTGGCCTGCTTTTCTT
CAGTATGGGACCAGTGATACCGACGATCGAGTGACGGATCTGTTGTCGAGGCTGGACCAT
TTTCGAAGAGGAAGTATATCTGTCGTTGAGGTAATTCATCCAATCCATCTTCATTTTGA
ATCATCCATACGATTGTCTAACCAATTGCCCAAATTATAGGTCAGCGCCCGCTCCCTCGA
CCGCGCAACAACCCCTTGCAAACCTCTACCCATCCATCAACATCACAGTCCAAATCGCATC
CCCAGCAGGCCCAACTGCCTGGTCACCAGCACACCCCAATCCCATCCGCCCCCAACTCC
CGGCGGTAGCCACAAACACGACGACCTTCGCGCACTCACTGCAAGCACGGCCAGTACAAC
ACCGGCCTCTAGCCACAACCACACCCATACGCATACCACCAATAGCATACCCAGGCCTC
CAACATAACGATCCAACACCGGCTTCCAACAGCACCGCAACCCATTACCTCAGCAAATCT
CTACATCCTACACCTCCCCTCTCCCTCACCAACAGTTCCTTTGCCTCCCTTGCAACGCA
CATCCTCGCAGAACTCCGCTCACATCTCGACATCCTCCGCTCAAACCCATCGGCGACCT
GATTCTCACCCCGCGGCCCTTGCTGAACCTCAGCTGTGCATAGCGAGGTGCAAGCAAG
CGCGCGACTGCGCGACTTGACGCTGATGCAGTTGGCAAATGAGCGTGAGATTGAGCTGGC
GGAGTGGATTAATCTGCTGAGCAATGTCAGTGATAGTATGGGCCGGTTGGTGGTGGTAA
TAAGATTCAGTCCAGAGAAAGCACGGTAGTTTTTGTGGAGATTCGGTACCAGGCCTATAA
CAGGTGA

AN_10021 protein

MSSLSDLETHASELTSVKTIIQCPRQNAASRSRTQPLITSSASKEAHRAQQSILSTIS
GLQKLLTSPDHLHHLAVQNQLLACLQWLGEFQVLACIPLTGTVPKDVAEAGVPETHL
SRIIRMTATAGFLDEPDGQVAHSALSAPFVTKPSYLDVDMFLAGTIAPSALQMPTATQR
FGASLRPNETAYNLALNNPATFASTSEQRPKLQRQWPAFLQYGTSDTDDRVTDLRLDH
FRRGSISVVEVSARSLDRATTLANLYPSINITVQIASPAGPTAWSPAHPNPIRPPTPGGS
HKHDDLRLTASTSTTPASSHNHTHTHTNSIPQASNITIQHRLPTAPQPITSANLYIL
HLPSPTVPFASLATHILAELRSHLDILRSNPATLILTPRPLPEPSAVHSEVEASARL
RDLTLMQLANEREIELAEWINLLSNVSDSMGRLVVVNKIQSRESTVVLLEIRYQAYNR

Figure S5. CMEIAS: Definitions of measurement features.

(CMEIAS© Ver 1.28 [7] and <http://cme.msu.edu/cmeias>)

Major Axis Length: The maximum distance between points on the object's boundary.

Area: Area of the object, measured as the number of pixels (scaled to the user-defined unit for image calibration) in the polygonal approximation of the cell. This measurement of size tends to slightly overestimate the object's true area because the borders of the pixels may extend beyond the true perimeter of the cell.

Perimeter: Length of the outside contour of the object represented as a polygon in the digital image.

Roundness (also called "circularity" or "shape factor"): Computed as $(4\pi \text{ Area}/\text{Perimeter}^2)$. This shape feature measures the degree of object roundness. Values lie between 0 and 1. The greater the value, the rounder is the object.

Elongation: The ratio of the length of the major axis to the length of its minor axis. The result is a value ≥ 1 . If the elongation is 1, the object is roughly circular or square. The ratio increases from 1 as the object becomes more elongated.

Compactness: Computed as:

$$\frac{\sqrt{4\text{Area}/\pi}}{\text{Major Axis Length}}$$

The feature measures the object's circularity, representing the ratio of the Feret diameter (defined below) to the object's major axis length, and ranges between 0 and 1. Objects with a compactness value of 1 are roughly circular.

Feret Diameter: Diameter of a circle having the same area as the object, computed as $(\sqrt{4\text{Area}/\pi})$.

ABR: ratio of the object's area to the area of the smallest bounding box.

Figure S6. Additional eYFP fluorescence assays. Positive and negative controls for split eYFP studies. As a positive control a fusion protein was made with transformants expressing Nt-eYFP-veA + Ct-eYFP-laeA. In this control eYFP fluorescence was only detected in nuclei and not in organelles. The negative control used in the experiment was Nt-YFP-afIR + Ct-YFP-veA and in this case no fluorescence could be detected when grown on maltose-containing (inducing) medium.

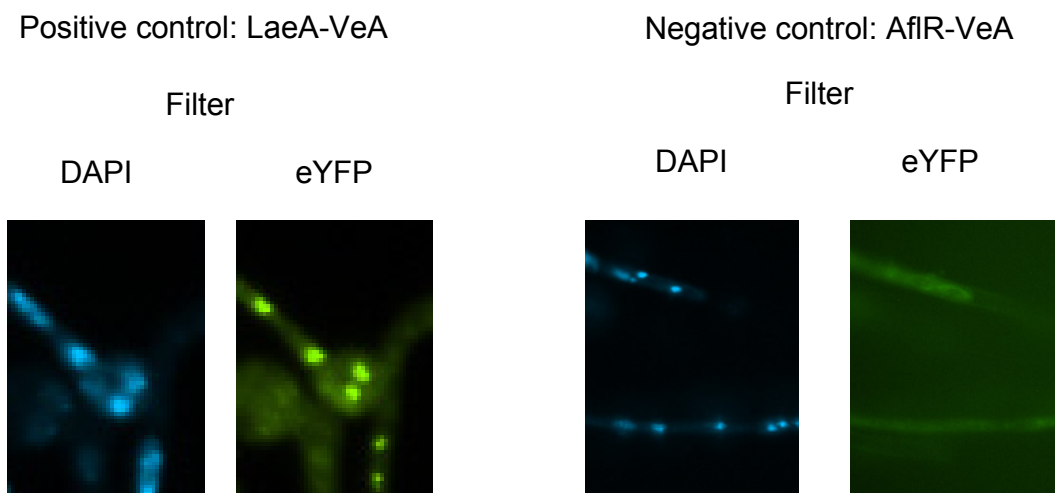
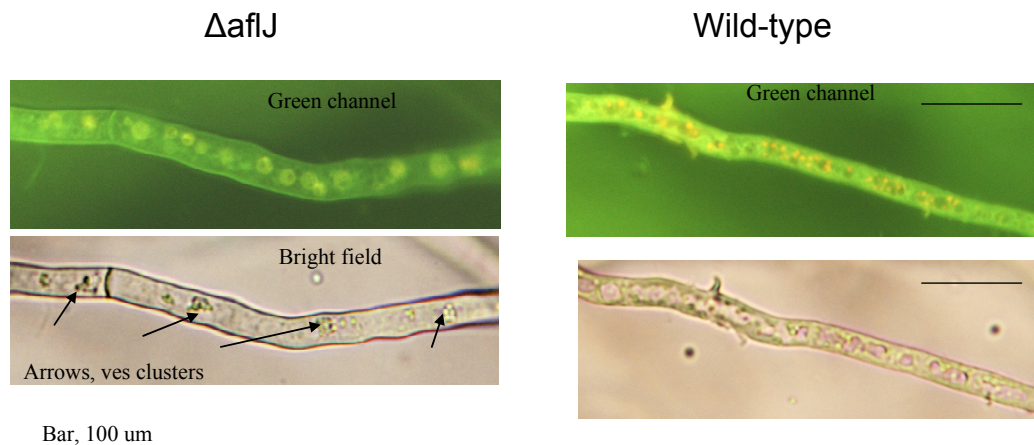


Figure S7. MDY staining of vacuoles in *A. parasiticus* $\Delta aflJ$ and wild-type. Cultures were grown on YES liquid medium for 68 h with shaking. Morphology was evaluated at indicated time points using bright field microscopy and fluorescent microscopy (Nikon). Mycelia were rinsed with sterile PBS, stained with MDY-64 (0.5 μ L per slide). MDY-64 (Molecular Probes) is a green fluorescent dye that preferentially labels vacuolar membranes. $\Delta aflJ$ and wild-type both contained numerous medium size vacuoles (stained with MDY-64 and much smaller vesicles (not stained). In some cells vesicles that appeared to stain with MDY-64 had small vacuoles underlying the vesicles.



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