G3 Genes | Genomes | Genetics

Systematic global analysis of genes encoding protein phosphatases in *Aspergillus fumigatus*

Lizziane K. Winkelströter[1], Stephen K. Dolan[2], Thaila Fernanda dos Reis[1], Vinícius Leite Pedro Bom[1], Patrícia Alves de Castro[1], Daisuke Hagiwara[3], Raneem Alowni[2], Gary W. Jones[2], Sean Doyle[2], Neil Andrew Brown[1] and Gustavo H. Goldman[1,4]

[1]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brazil; [2]Department of Biology, Maynooth University, Maynooth, Co. Kildare, Ireland; [3]Medical Mycology Research Center, Chiba University, Chiba, Japan; [4]National Laboratory of Science and Technology of Bioethanol (CTBE), Campinas, Brazil

Corresponding author: Dr. Gustavo H. Goldman

Departamento de Ciências Farmacêuticas

Faculdade de Ciências Farmacêuticas de Ribeirão

Preto, Universidade de São Paulo

Av. do Café S/N, CEP 14040-903, Ribeirão Preto

São Paulo, Brazil, Phone/Fax: 55-16-36024280/81

e-mail address: ggoldman@usp.br

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Figure S1 Southern blot analysis for all the phosphatase deletion mutants (A to Y), complementation analysis (W), and strategy to fuse *niiA* promoter to the essential genes (Z).

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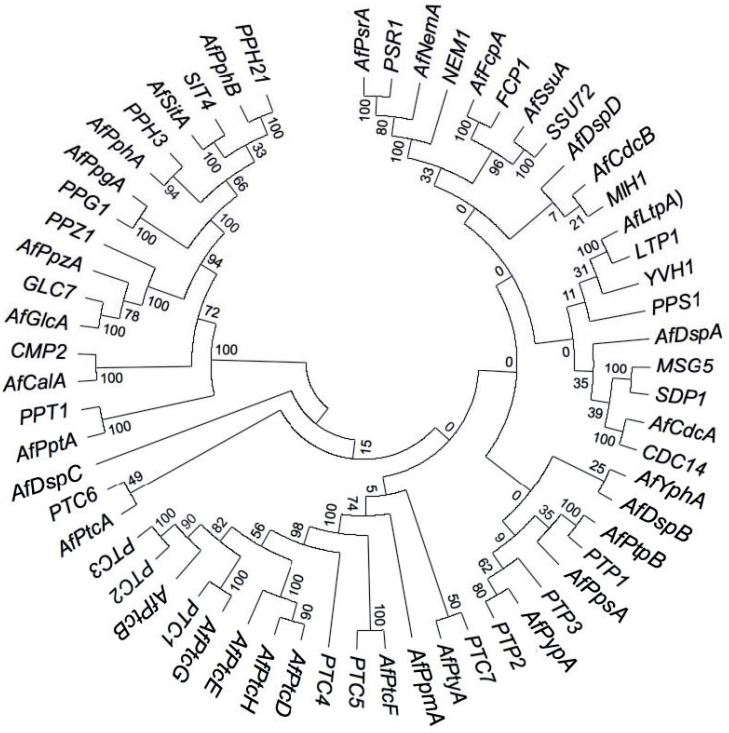

Figure S2 Phylogeny of *A. fumigatus* and *S. cerevisiae* protein phosphatases. The optimal tree for the *A. fumigatus* and *S. cerevisiae* phosphatases is represented. The tree was inferred using the Neighbourg-Joining Method. The bootstrap values calculated on 500 replicates are indicated on the tree branches. When homologues are available, *A. fumigatus* proteins were named according to the *S. cerevisiae* protein names (see http://www.yeastgenome.org) (see Table 1). Sequences were aligned with ClustalW and the tree was constructed by using MEGA6. Sequences are available in File S1.

Figure S3 Phenotypes for selected phosphatase deletion mutants (A to P). The order of the mutants follows the order of the mutants in Table 2.

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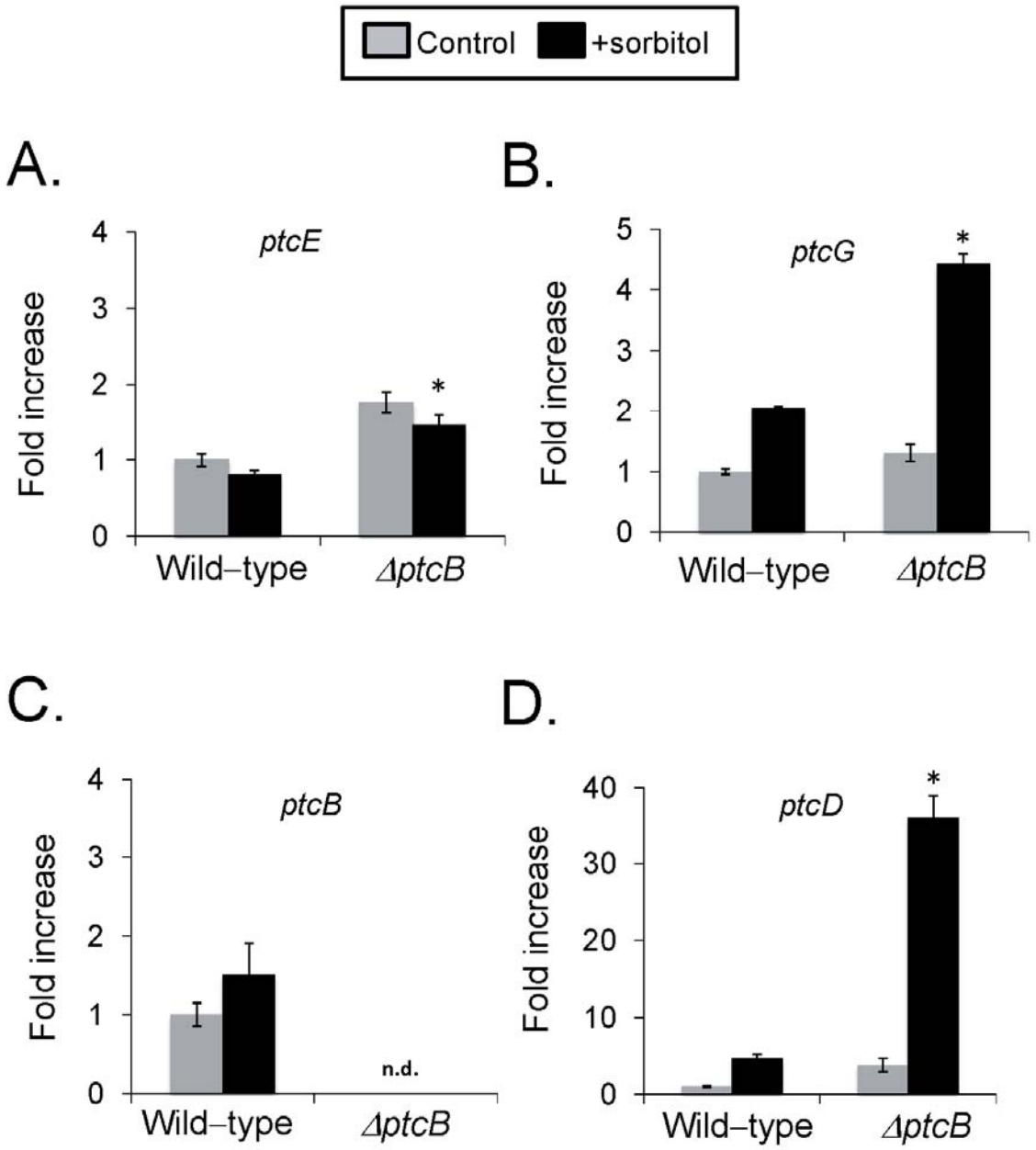

Figure S4 Phosphatase encoding genes were induced by exposure to sorbitol or fluodioxonil in an SskA-dependent manner. The wild-type and the phosphatase null mutants were grown for 18 h at 37°C. Then, sorbitol (1 M final concentration) or fluodioxonil were added for 0 (control) and 10 minutes. The mycelium was harvested at the indicated times, and total RNA was extracted. The absolute quantitation of the selected genes (A to D) and *actA* (Afu6g04740, encoding the actin) was determined by a standard curve (i.e., C_T –values plotted against a logarithm of the DNA copy number). The results are the means (± standard deviation) of four biological replicates (*, $p < 0.001$, comparison of the treatments with the time zero control).

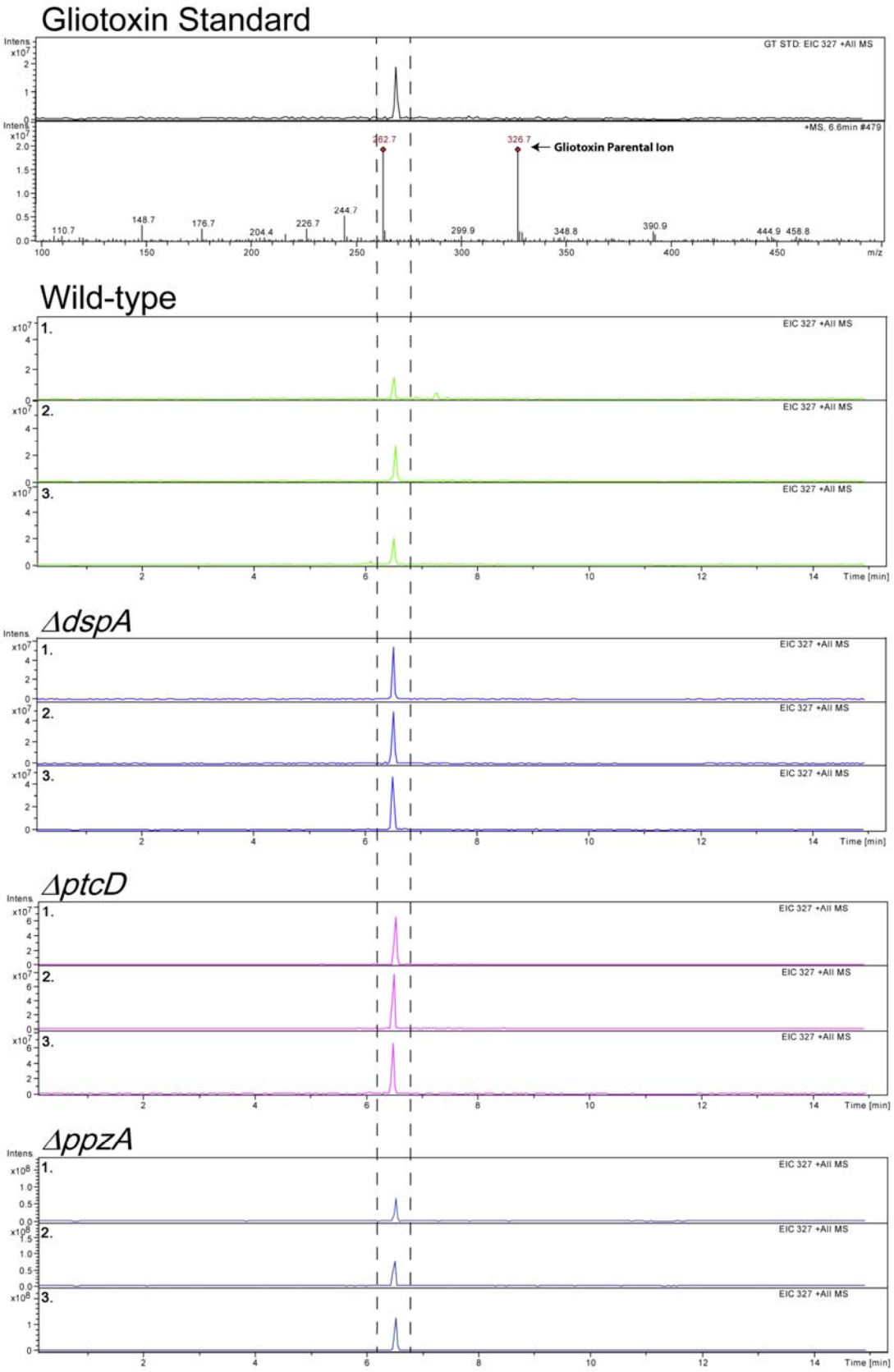

Figure S5 Gliotoxin chromatograms for the wild-type and phosphatase mutants. Dotted lines show the gliotoxin standard graph.

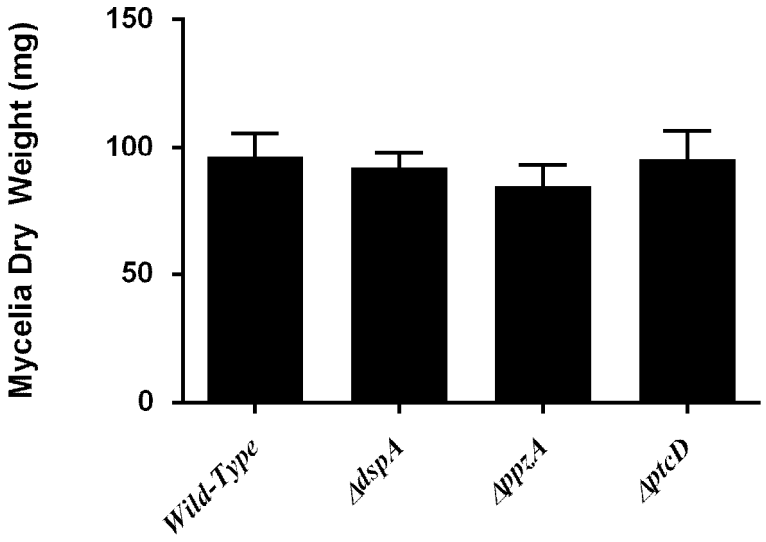

Figure S6 Dry mycelial weight for the wild-type and the phosphatase mutants used for the gliotoxin analysis shown in Figure S5.

File S1

Phosphatase sequences used for the phylogenetic analysis

Available for download as a PDF file at www.g3journal.org/lookup/suppl/doi:10.1534/g3.115.016766/-/DC1

Table S1 Oligonucleotide sequences of the primers used in this study

Afu5g06700 pRS426 5fw	5’-GTAACGCCAGGGTTTTCCCAGTCACGACGGGCCTCCTCCTCCAACGCAC-3’
Afu5g06700 pyrG 5rv	5’-GCACTTAAGGACAAGAACTCTGCAAAGCGTAGTAATCGACCG-3’
Afu5g06700 pyrG 3fw	5´-CACTTGAAGGACAAGAACTCTATGTCTCTCTAGGCTTATGCG-3´
Afu5g06700 pRS426 3rv	5'-GCGGTTAACAATTTCTCTCTGGAAACAGCCGCCAGTTCCTCAATGATAACC-3'
Afu5g06700 ORF fw	5´- ATGGCTGCATCCGATCTCGAGG -3´
Afu5g06700 ORF rv	5´- TCAGCTTTGTAATTTGAGCAGACACG -3´
Afu5g06700 5’ext fw	5’-CTGCAGTCTAACGTAGCGG-3’
Afu5g06700_hygromycin 3rv	5'TCCTGTGTGAAATTGTTATCCGCTGAAATGACGATGGCTCATGAGC-3'
Afu5g13740 pRS426 5fw	5’-GTAACGCCAGGGTTTTCCCAGTCACGACGTTGTTCTCTGATGGACTCGAA-3’
Afu5g13740 pyrG 5rv	5’-GCCTCCTCTCAGACAGAATTTTGCTGTTTGCTCTGAGGAG - 3’
Afu5g13740 pyrG 3fw	5´-GCATTGTTTGAGGCGAATTCTTGGGCTTAGCATGGCGTGGCG- 3´
Afu5g13740 pRS426 3rv	5'-GCGGTTAACAATTTCTCTCTGGAAACAGCTATCGGAACCATGCTGCATTGC-3'
Afu5g013740 ORF fw	5'-CCTCTATCTAGTTCTGCATCGGGCG-3'
Afu5g13740 ORF rv	5'-GTTGCATTATCACTCATCGGGCCC-3'
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Afu5g12010 pyrG 5rv	5’-GCACTTAAGGACAAGAACTCTTCTTCGTAACCCATACGACG-3’
Afu5g12010 pyrG 3fw	5´-CACTTGAAGGACAAGAACTTGCACTTATCTTTCCTTCTAAG-3´
Afu5g12010 pRS426 3rv	5'-GCGGTTAACAATTTCTCTCTGGAAACAGCCTTTCAACCTCAGCTTCATATC-3'
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Afu5g12010 ORF rv	5´-TCACATGGTATCACTGCGCGCA-3´
Afu5g12010 5’ext fw	5´-GACACTCTAGAACACAAAGCTG-3´
Afu5g12010_hygromycin 3rv	5’-TCCTGTGTGAAATTGTTATCCGCTCAGCGATGATGTTCATGTTCATC-3’
Afu4g00720 pRS426 5fw	5’-GTAACGCCAGGGTTTTCCCAGTCACGACGGATTGGACTATATAACTGGAGCTG-3’
Afu4g00720 pyrG 5rv	5’-CAGTGCCTCCTCTCAGACAGAATCTTACTGTCCGTCGAAGACCGC-3’
Afu4g00720 pyrG 3fw	5´-GAGCATTGTTTGAGGCGAATTCCATGCTTGACGTACGGATCATG-3´

Afu4g00720 pRS426 3rv	5'-GCGGTTAACAATTTCTCTCTGGAAACAGCGAGCGCAGATGCAAGTAAGTGG-3´
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Afu6g06650 pyrG 5rv	5'-CAGTGCCTCCTCTCAGACAGAATTTACGACATCGCTTTTGCCGTCC-3'
Afu6g06650 pyrG 3fw	5´-GAGCATTGTTTGAGGCGAATTCGTAATTATGGGCGGATACAATGG-3
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Afu2g03890 pRS426 5fw	5'GTAACGCCAGGGTTTTCCCAGTCACGACGGTTTTCATTTCGCTTGAAGTGACAG-3'
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Afu2g03890 pyrG 3fw	5´-GAGCATTGTTTGAGGCGAATTCAGCGAAGGCAGAGCAGGCCTC- 3´
Afu2g03890 pRS426 3rv	5'-GCGGTTAACAATTTCTCTCTGGAAACAGCAGCGTCAGAGTGTCAATGGTGG-3'
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Afu2g03890 ORF rv	5'-TAGGTGTTTTGACCGTCCAATAGAG-3'
Afu2g03890 5´UTR ext fw	5´-GGAAAGGGAGGTACGGAGTG-3´
Afu2g03890_hygromycin 3rv	5'-TCCTGTGTGAAATTGTTATCCGCTTTGACCGGAGAAAGGCACCTTCTC-3'
Afu2g03950 pRS426 5fw	5'-GTAACGCCAGGGTTTTCCCAGTCACGACGGCCCAGCAAATCCCAGCAACCGG-3'
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Afu2g03950 5'ext fw	5´-GATCAACAACTACTCAGCCC-3´
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Afu1g06860 pRS426 5fw	5'GTAACGCCAGGGTTTTCCCAGTCACGACGCCTGCGACCTGTCCCAGTTTCTTC-3'
Afu1g06860 pyrG 5rv	5'-CAGTGCCTCCTCTCAGACAGAATTGAACTTGCAGTAACGTTCAGG- 3'
Afu1g06860 pyrG 3fw	5´-GAGCATTGTTTGAGGCGAATTCCATCTTCTACATGGTCTTCACC- 3´
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Afu1g09280 pyrG 5rv	5’-GCCTCCTCTCAGACAGAATCACGAATAGGCGGCAACCAG- 3’
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Afu1g09280_hygromycin 3rv	5'-TCCTGTGTGAAATTGTTATCCGCTCCCAGGAAAGCCCCC-3'
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Afu4g07000 5´UTR ext fw	5´-GATGCTGGCGTTGGGCTTGGACG-3´
Afu4g07000_hygromycin 3rv	5´-TCCTGTGTGAAATTGTTATCCGCT-3´
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Afu8g04580 ORF fw	5'-GATCCTGCTGGCCTCCTCCCGT-3'
Afu8g04580 ORF rv	5'-GCAAGATTCATGACCTCGACCCTCTTC-3'
Afu8g04580_hygromycin 3rv	5'-TCCTGTGTGAAATTGTTATCCGCTGCTTCTCTGTGTCTTGGAGTGC-3'
Afu6g11470 pRS426 5fw	5'-GTAACGCCAGGGTTTTCCCAGTCACGACGCGGGATCTTGGTCAATTGTACC-3'
Afu6g11470 pyrG 5rv	5'- GCCTCCTCTCAGACAGAATGGTGCGGGTGCGGACGAACG -3'
Afu6g11470 pyrG 3fw	5´- GCATTGTTTGAGGCGAATTCCCTAGCATGCTTGATGGTCTG -3´
Afu6g11470 pRS426 3rv	5'-GCGGTTAACAATTTCTCTCTGGAAACAGCCCTGATTATTCCATTCCCGC-3'
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Afu6g11470 ORF rv	5'-GAGGTATTCTACAAGAAGTACTCGCTGCGGCCCG-3'
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Afu1g15800 pRS426 5fw	5'-GTAACGCCAGGGTTTTCCCAGTCACGACGGTTGATCTTCCTGCTGCGGC-3'
Afu1g15800 pyrG 5rv	5'-GCACTTAAGGACAAGAACTTTTCCTTGCTGTTTGGATTTACC-3'
Afu1g15800 pyrG 3fw	5´-CACTTGAAGGACAAGAACTGCACGAGTCATTAGTACCCCC-3´
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Afu1g15800 ORF fw	5´- ATGGACTTCCTGGGCCTGCATAC -3´
Afu1g15800 ORF rv	5´- TCATCGCCTGGAACTGCGCGG -3´

Afu1g15800 5’ext fw	5´-ACGTTGCCGGTGTGAACTTC-3´
Afu1g15800_hygromycin 3rv	5'-TCCTGTGTGAAATTGTTATCCGCTCGTACTTATCGCCAACTTTACATA-3'
Afu4g07080 pRS426 5’fw	5’-GTAACGCCAGGGTTTTCCCAGTCACGACGGTCTTCAAGATGCCCAGTCATAC-3’
Afu4g07080 pyrG niiA 5’rv	5’-GGAGCTCGTATTTTTCCCTGCGCTGATGCCACGTTATAGCCGAT-3’
Afu4g07080 niiA orf fw	5’-GGCGTTGAGACTTCGTCACGATGGCTATGAACAAGATCCC-3’
Afu4g 07080 pRS426 orf rv	5’GCGGATAACAATTTCACACAGGAAACAGCTGAATCATCTGGAGCTCTCAT-3’
Afu1g4950 pRS426 5’fw	5’-CAGGGTTTTCCCAGTCACGACGTTCTGGCCTGCATCACGACTC-3’
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Afu1g4950 niiA orf fw	5’-CTGGCGTTGAGACTTCGTCACGATGGCTGATCAAGAAGTGGATC-3’
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Afu6g10830 pRS426 orf rv	5’-CAATTTCACACAGGAAACAGCTCACAGGAAGTAATCAGGTGTACG-3’
Afu3g11410 pRS426 5fw	5’-GTAACGCCAGGGTTTTCCCAGTCACGACGGGCCAAGTAATGTGTCTCTGC-3’
Afu3g11410 pyrG niiA 5’rv	5’- GGAGCTCGTATTTTTCCCTGCTCTTGTATCGGGCAGGCGAGG-3’
Afu3g11410 niiA orf fw	5’-CTGGCGTTGAGACTTCGTCACGATGCTTCTTCGGTTACCGCCC-3’
Afu3g11410 pRS426 orf rv	5’-CAATTTCACACAGGAAACAGCCTACCCGTTCTCAGCTGCAATC-3’
RT-actin-F	5'-CTGTGCAGATTGTCGCCAGGG-3'
RT-actin-R	5'-GTCCAGATTAAGCTGTCGCGC-3'
RT-catA-F	5'-CACCGACGATCCACTTCTCCAAGG-3'
RT-catA-R	5'-GACGGTTGATTGGCAGCTCTTCCC-3'
RT-dprA-F	5'-GTCCAACATGATACATAAGGTG-3'
RT-dprA-R	5'-GTCGGCGGCCCTAGAGTCATGG-3'
RT-dprB-F	5'-GTCTGGACTGATGCACAAGGTC-3'
RT-dprB-R	5'-GTGGTCTGGTGGGTGCCGTGG-3'
RT-2g03890-F	5'-GTTTCGCAATACCATCTTGTCTACC-3'

RT-2g03890-R	5'-CGATCCTCCTGAAAAGGTCGAGCC-3'
RT-3g10970-F	5'-GGCGCATCCCGCCATCAAAATCGATACG-3'
RT-3g10970-R	5'-CGGAACCGGATACCACATCGAAACCACC-3'
RT-5g13340-F	5'-GTTCAGTGGCTCCTCGAGCCCACCC-3'
RT-5g13340-R	5'-CGTCGGTCTTGACGGTCAGGGACGGG-3'
RT-5g13740-F	5'-GGGTCCTCTATCTAGTTCTGCATCGG-3'
RT-5g13740-R	5'-GTTGCGAAGGTCGAGATCCTAGGGCG-3'
RT-1g03540-F	5'-GCACTTCTCATTCGCAATGAGCTCTATC-3'
RT-1g03540-R	5'-CGGACTTCCAGAGCTGTAAAGCAGCG-3'
RT-1g09280-F	5'-CAGCATGGAGGACGCCCATGCTGCGG-3'
RT-1g09280-R	5'-TATCTCCACCATGACCGTCATATACACC-3'
RT-1g09460-F	5'-GAACTCGTTGAATATCCTATCTTCCCG-3'
RT-1g09460-R	5'-CCGATTTCTCCCTGCGAACGCGACC-3'
RT-3g12250-F	5'-GTCTGGATATATGGGCGGACCCGGCC-3'
RT-3g12250-R	5'-GAGCTCCTAGGACATTTCCTGGACAGG-3'
RT-4g04710-F	5'-GTCCACTACGGCTGACCAGAGACAATCACC-3'
RT-4g04710-R	5'-GGTATCTCGCAGTGATAATAAAGACGCTGG-'3
RT-1g06860-F	5'-GCGTCGCCTGGCCCTCCAGACTTTCCG-3'
RT-1g06860-R	5'-CCTAGAGGCTGATGATTATGGGAGAACG-3'
RT-1g13040-F	5'-GACTCCGGCTCGTTGCATTGAGGCTTCG-3'
RT-1g13040-R	5'-ATTGGCAGACGAGTCTTTGCACTTCC-3'
RT-5g11690-F	5'-GGCTACGGTGTTGGTACAGCAAACGC-3'
RT-5g11690-R	5'-GGTAAATGTTTGTTTGGCACCGGCGACGGG-3'
RT-4g07000-F	5'-GGCTAAACCACTTAGTGAGAAGTCTTC-3'
RT-4g07000-R	5'-GCAAAGGACGATAACCAAGGGAAGAAGAC-3'
sakA-U-F	5'-GGTGAAGCTAACGTAGGTTTGAATCAGG-3'
sakA-D-R	5'-GTCGAGCTGTCGTGAACAGGCGATTCC-3'

Table S2 Taq Man probes used in this work

Assay Name	Forward Primer Sequence	Reverse Primer Sequence	Reporter 1 Sequence
βTUB	GTGAGGCTGAAGGCTGTGA	GGTACCACCACCGAGAGAGT	CCTCCAGGGCTTCCAG
SIDA	GTGCGCAACAATTTTCGGATGTA	GAGCTGCTGGGATCAGACTTC	CTTCCCCGTAAGCCAC
SIDC	GGGCAAGGCGATGTTAGC	CCGAGCATAGCCTTCGATCAATC	CTGTTGCGAGTTCTCC
SIDG	CGAGTCTCGCCGAAGTTCTG	AAGGCATAGCGAACCATCTCC	CCTCCTTCGCGTACCC
CCCA	CTTGGATCTCAGCCATCACACT	AAGTAAGGTATGAGCGGGATGAAC	CCTCCCACGAAATAC
SREA	TGGTTCCTGTCCTGGTGGA	TTGTAGGCAGGGCAACCAT	TCCCTCGGCTCCGCC
HAPX	CCAGACGTCTTGATACCACCAA	GGCAAATCGGGAAGTGAAATCAATTT	CCGAGCCTCAGATCAA

Reporter 1 Dye: FAM

Reporter 1 Concentration (µM): 5

Reporter 1 Quencher: NFQ

Forward Primer Concentration (µM): 18

Reverse Primer Concentration (µM): 18