

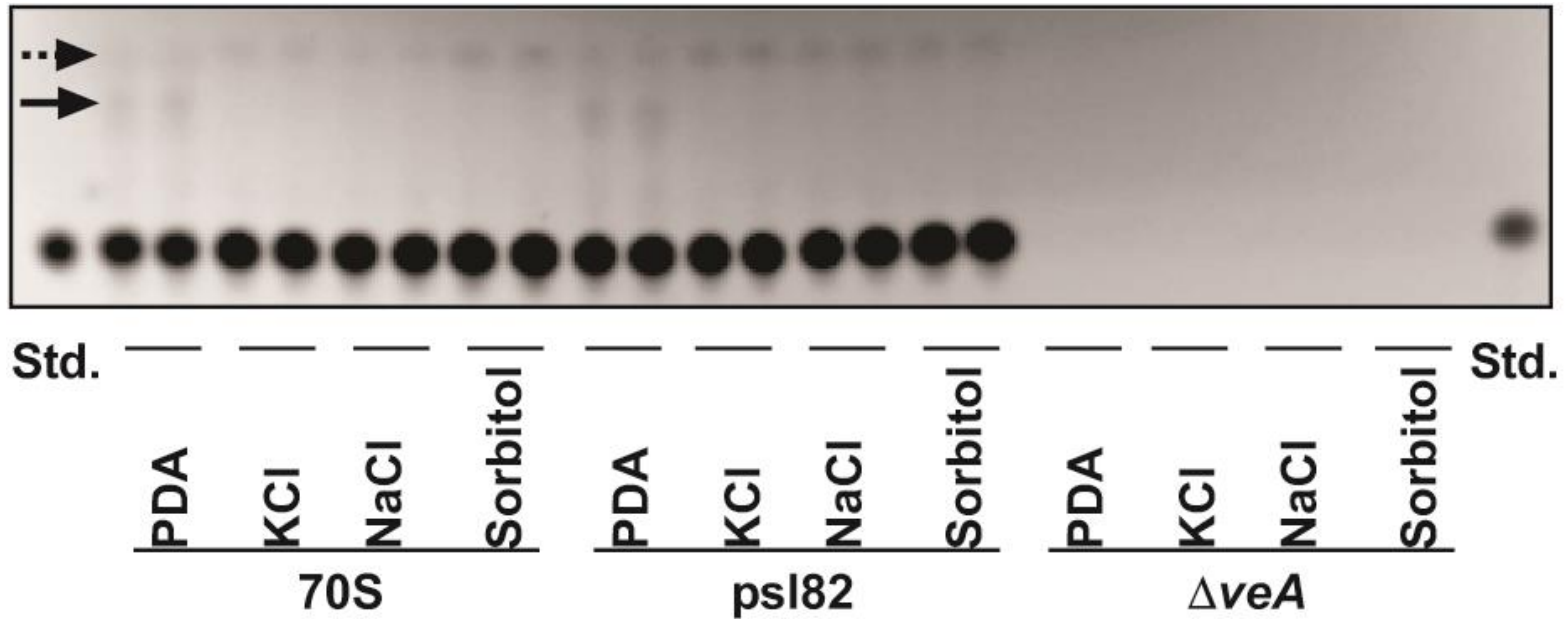
Supplemental Table 1. PCR and qRT-PCR primers used in this study

Primer designation	Oligonucleotide sequence (5'-3')
<i>PCR primers</i>	
5' SphI <i>hogA</i>	<u>GCATGCGAAGACTGATTCAGATAATTAGTTC</u>
5' SalI <i>hogA</i>	<u>GTCGACCTGATGATCTCCACTGTCTGAAC</u>
3' BamHI <i>hogA</i>	<u>GGATCCTATTCCCCGACGACAAGAAGATTTGC</u>
3' EcoRI <i>hogA</i>	<u>GAATTCCTCCGTTGAAAGGCAGTCAGG</u>
<i>hogA</i> Up	CGAGACCTTGTCACATTGGCGTCAACTC
<i>hogA</i> Down	GATGCCTCCTGTCAAGATTGCAATGAAC
<i>qRT-PCR primers</i>	
<i>atfA</i> -F	CACAGAACACGAACATGGATATG
<i>atfA</i> -R	CTGATTGGAAACAGCGAATTG
<i>atfB</i> -F	CAGCGAAGTGCTCAACCTCAAGAACG
<i>atfB</i> -R	GATCGCATGGGAGAAACCAGATCG
<i>catB</i> -F	GGTGTTCAAATCATGGATGAGGAAG
<i>catB</i> -R	CTGCTCAGTCTCGGCAAAGTAGTTG
<i>trxB</i> -F	GATGGTATCGGTGGTACAGAGCTG
<i>trxB</i> -R	CTCATTGTCAGGACCATCGTTCCA
<i>msnA</i> -F	ACACAAGGTTTCGTCGGTGACTACTATAG
<i>msnA</i> -R	CGTTGATGAAGACAGGAGTCATGG
<i>srrA</i> -F	CCACAGCCTTAGTATATCCGAAGATGAGC
<i>srrA</i> -R	GAGTCCGTCGAAAGCAGTATCAATGA
<i>trxA</i> -F	CAGGAGAAGGTTATCGAATCCAAG
<i>trxA</i> -R	GTCGACATCGATCTTGTAGAATTTGG
<i>ypdA</i> -F	CTACGCTTGACGACATGAAAGAACAC
<i>ypdA</i> -R	GCTTTTCCATCTTCTTAAAGGTGC
18S RNA-F	CCACCCGTGTTTACTGTACCTTAG
18S RNA-R	GGAACCAAGAGATCCATTGTTGAAAG

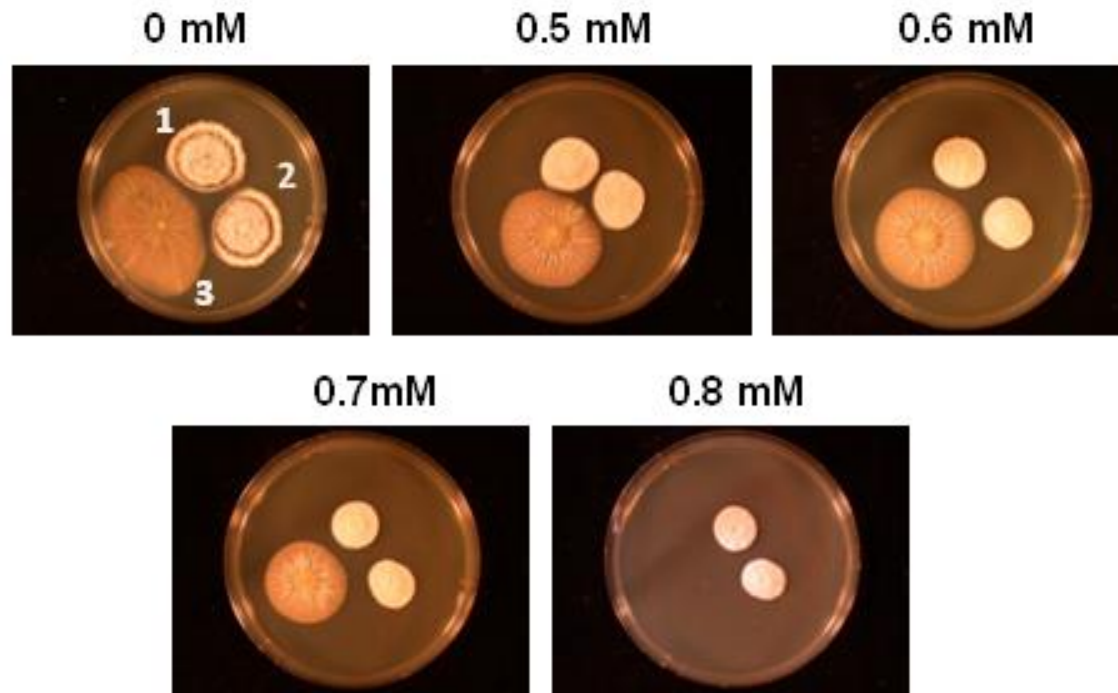
Supplemental Table 2. Primer sequences used to generate double-stranded DNA fragment probes for EMSA

DNA Fragment	Sequence ^a	PCR product (bp)
<i>cat1</i>	F 5' GCGCATCGTGTGCTAACT 3'	518
	R 5' ATTCGCCAAGAATGGATCTG 3'	
<i>trxB</i>	F 5' TACCTGGTCGGAGAGTGAAAG 3'	521
	R 5' AACGACTTTGCTGTGCACC 3'	
cat1-1	F 5' ATCCCAAGACAGGAAGGTAC 3'	259
	R 5' ATTCGCCAAGAATGGATCTG 3'	
cat1-2	F 5' GCGCATCGTGTGCTAACT 3'	259
	R 5' TGTCTTGGGATGCGAATG 3	
trxB-1	F 5' ATAATTAAAGCCGCCTATTTCC 3'	263
	R 5' AACGACTTTGCTGTGCACC 3'	
trxB-2	F 5' TACCTGGTCGGAGAGTGAAAG 3'	258
	R 5' GGCTTTAATTATGGCGTTCAG 3'	

^a F represents forward primers and R represents reverse primers.



Supplemental Figure 1. Osmotic stress does not affect AF production in *A. flavus*. Thin layer chromatography (TLC) analysis of *A. flavus* 70S, psl82, and ΔveA top-agar inoculated cultures after 5 days of incubation. The medium was supplemented with 0.6M NaCl, 0.7M KCl, or 1.0M sorbitol to generate osmotic stress. The arrows indicate two unknown *veA*-dependent compounds. Standard is shown on each side of the TLC silica plate. The experiment was carried out in duplicates.



Supplemental Figure 2. *veA* is necessary for proper response to oxidative stress. Photographs of point-inoculated *A. flavus* 70S, psl82, and ΔveA cultures on YGT supplemented with increasing amounts of menadione. 1= 70S, 2= psl82, and 3= ΔveA . Cultures were incubated at 30 °C for 5 days.

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S.cerevisiae 1 M-----EPIR--TQIPGTTFEINRYNDLNPVGGAFGLVCSATDILTSGOPVAIKKMKKPF
A.clavatus 1 MA---EPIR--AQIPGTTFEINRYNDLNPVGGAFGLVCSARDQLTGQPVAIKKMKKPF
A.fischerianus 1 MA---EPIR--AQIPGTTFEINRYNDLNPVGGAFGLVCSARDQLTGQPVAIKKMKKPF
A.fumigatus 1 MA---EPIR--AQIPGTTFEINRYNDLNPVGGAFGLVCSARDQLTGQPVAIKKMKKPF
A.terreus 1 MA---EPIR--AQIPGTTFEINRYNDLNPVGGAFGLVCSARDQLTGQPVAIKKMKKPF
A.niger 1 MA---EPIR--AQIPGTTFEINRYNDLNPVGGAFGLVCSARDQLTGQPVAIKKMKKPF
A.nidulans 1 MA---EPIR--SDIPLGTTFEINRYNDLNPVGGAFGLVCSARDQLTGQPVAIKKMKKPF
A.oryzae 1 MA---EPIR--SEVIGSVFDTNRYVNVRFVGGAFGLVCSAYDLVRRGQAVAIKLLNPPA
A.flavus 1 MA---EPIR--SEVIGSVFDTNRYVNVRFVGGAFGLVCSAYDLVRRGQAVAIKLLNPPA

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S.cerevisiae 60 TAVLAKRTYRELLKLLKHLRHNELICLDIFLISPLEDIYFVTELLGDTLHRLLLQTRPLEKQ
A.clavatus 57 TPVLSKRTYRELLKLLKHLRHNELICLSDFIISPLEDIYFVTELLGDTLHRLLLTSRPLEKQ
A.fischerianus 57 TPVLSKRTYRELLKLLKHLRHNELICLSDFIISPLEDIYFVTELLGDTLHRLLLTSRPLEKQ
A.fumigatus 57 TPVLSKRTYRELLKLLKHLRHNELICLSDFIISPLEDIYFVTELLGDTLHRLLLTSRPLEKQ
A.terreus 29 -----FVQEN--LPLSDIFISPLEDIYFVTELLGDTLHRLLLTSRPLEKQ
A.niger 57 NATLAKRTYRELLKLLKHLRHNELICLSDFIISPLEDIYFVTELLGDTLHRLLLTSRPLEKQ
A.nidulans 57 STVSAKRTYRELLKLLKHLRHNELICLSDFIISPLEDIYFVTELLGDTLHRLLLTSRPLEKQ
A.oryzae 57 TTANAKQTYRELLKLLKHLRHNELICLDVFIISPRDQVYLVTELLSTDLARLLEAGPLEPQ
A.flavus 57 TTANAKQTYRELLKLLKHLRHNELICLDVFIISPRDQVYLVTELLSTDLARLLEAGPLEPQ

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S.cerevisiae 120 FVQYFLYQIIL-----RGLKYVHSAGVHHRDLKPSNINENCDLKKIC
A.clavatus 117 FVQYFLYQIIL-----RGLKYVHSAGVHHRDLKPSNINENCDLKKIC
A.fischerianus 117 FVQYFLYQIIL-----RGLKYVHSAGVHHRDLKPSNINENCDLKKIC
A.fumigatus 117 FVQYFLYQIIL-----RGLKYVHSAGVHHRDLKPSNINENCDLKKIC
A.terreus 71 FVQYFLYQIIL-----RGLKYVHSAGVHHRDLKPSNINENCDLKKIC
A.niger 117 FVQYFLYQIIL-----RGLKYVHSAGVHHRDLKPSNINENCDLKKIC
A.nidulans 117 FVQYFLYQIIL-----RGLKYVHSAGVHHRDLKPSNINENCDLKKIC
A.oryzae 117 FVQYFLYQIIL-----RGLKYVHSAGVHHRDLKPSNINENCDLKKIC
A.flavus 117 FVQYFLYQIILVRRVASAPYIDSHDSDEPQGLKYVHSAGVHHRDLKPSNINENCDLKKIC

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S.cerevisiae 162 DFGLARIQDPQMTGYVSTRYYRAPEIMLTWQYDVEVDIWSAGCIFAEMLEGKPLFPFGKD
A.clavatus 159 DFGLARIQDPQMTGYVSTRYYRAPEIMLTWQYDVEVDIWSAGCIFAEMLEGKPLFPFGKD
A.fischerianus 159 DFGLARIQDPQMTGYVSTRYYRAPEIMLTWQYDVEVDIWSAGCIFAEMLEGKPLFPFGKD
A.fumigatus 159 DFGLARIQDPQMTGYVSTRYYRAPEIMLTWQYDVEVDIWSAGCIFAEMLEGKPLFPFGKD
A.terreus 113 DFGLARIQDPQMTGYVSTRYYRAPEIMLTWQYDVEVDIWSAGCIFAEMLEGKPLFPFGKD
A.niger 159 DFGLARIQDPQMTGYVSTRYYRAPEIMLTWQYDVEVDIWSAGCIFAEMLEGKPLFPFGKD
A.nidulans 159 DFGLARIQDPQMTGYVSTRYYRAPEIMLTWQYDVEVDIWSAGCIFAEMLEGKPLFPFGKD
A.oryzae 159 DFGLSRPQDHRMTGYVSTRYYRAPEIMLTWQYDVEVDIWSAGCIFAEMLEGKPLFPFGKD
A.flavus 177 DFGLSRPQDHRMTGYVSTRYYRAPEIMLTWQYDVEVDIWSAGCIFAEMLEGKPLFPFGKD

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S.cerevisiae 222 HVNPFISIITDLLGPPDDVITICSENTLRFVKSLPKRERQPLANKFKNADPE---AWD
A.clavatus 219 HVNPFISIITDLLGPPDDVITICSENTLRFVKSLPKRERQPLANKFKNADPE---AWD
A.fischerianus 219 HVNPFISIITDLLGPPDDVITICSENTLRFVKSLPKRERQPLANKFKNADPE---AWD
A.fumigatus 219 HVNPFISIITDLLGPPDDVITICSENTLRFVKSLPKRERQPLANKFKNADPE---AWD
A.terreus 173 HVNPFISIITDLLGPPDDVITICSENTLRFVKSLPKRERQPLANKFKNADPE---AWD
A.niger 219 HVNPFISIITDLLGPPDDVITICSENTLRFVKSLPKRERQPLANKFKNADPE---AWD
A.nidulans 219 HVNPFISIITDLLGPPDDVITICSENTLRFVKSLPKRERQPLANKFKNADPE---AWD
A.oryzae 219 HVNPFISIITDLLGPPDDVITICSENTLRFVKSLPKRERQPLANKFKNADPE---AWD
A.flavus 237 HVNPFISIITDLLGPPDDVITICSENTLRFVKSLPKRERQPLANKFKNADPE---AWD

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S.cerevisiae 278 LLERMLVFPDPKRIIRAGALAHAYEYLAHYHDPTDEPAERKFDWFSFNADLPVDTWKIMMY
A.clavatus 275 LLERMLVFPDPKRIIRAGALAHAYEYLAHYHDPTDEPAERKFDWFSFNADLPVDTWKIMMY
A.fischerianus 275 LLERMLVFPDPKRIIRAGALAHAYEYLAHYHDPTDEPAERKFDWFSFNADLPVDTWKIMMY
A.fumigatus 275 LLERMLVFPDPKRIIRAGALAHAYEYLAHYHDPTDEPAERKFDWFSFNADLPVDTWKIMMY
A.terreus 229 LLERMLVFPDPKRIIRAGALAHAYEYLAHYHDPTDEPAERKFDWFSFNADLPVDTWKIMMY
A.niger 275 LLERMLVFPDPKRIIRAGALAHAYEYLAHYHDPTDEPAERKFDWFSFNADLPVDTWKIMMY
A.nidulans 279 LLERMLVFPDPKRIIRAGALAHAYEYLAHYHDPTDEPAERKFDWFSFNADLPVDTWKIMMY
A.oryzae 275 LLERMLVFPDPKRIIRAGALAHAYEYLAHYHDPTDEPAERKFDWFSFNADLPVDTWKIMMY
A.flavus 293 LLERMLVFPDPKRIIRAGALAHAYEYLAHYHDPTDEPAERKFDWFSFNADLPVDTWKIMMY

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S.cerevisiae 338 -----SEILDPHK--HG-GSD--GQIDISATPDDQVAAATAAAQAQAQAQAQVQLNMPA
A.clavatus 335 -----SEILDPHN--HDQGNDAQALAE-----GLGQQQNPFA
A.fischerianus 335 -----SEILDPHN--HDQGNDAQVLMEG-----GVAQAQNYFA
A.fumigatus 335 -----SEILDPHN--HDQGNDAQVLMEG-----GVAQAQNYFA
A.terreus 289 -----SEILDPHN--HQALNE-GEVLVE-----GAGTAPQGPFA
A.niger
A.nidulans 339 V-HHCSDDVVSFTL-----PFNOQNDLL-----Q
A.oryzae 334 --PAYNLASCYED--PFNOQNDLL-----Q
A.flavus 353 MIAQIKLGLALYFQCSSLFPDDKKICVILHPTPMHAGSQPATE-----

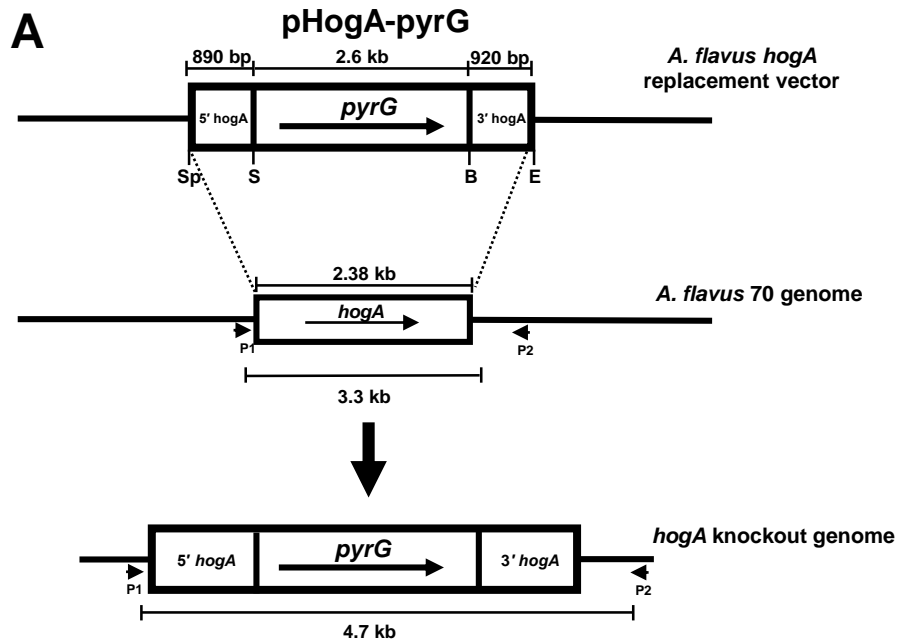
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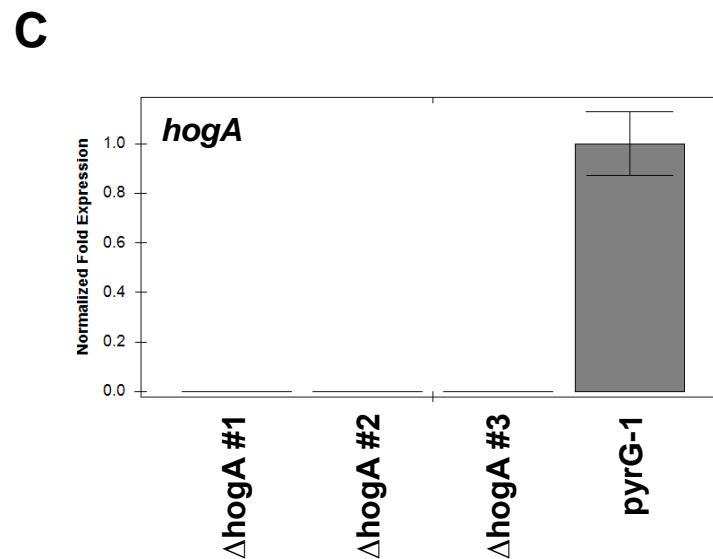
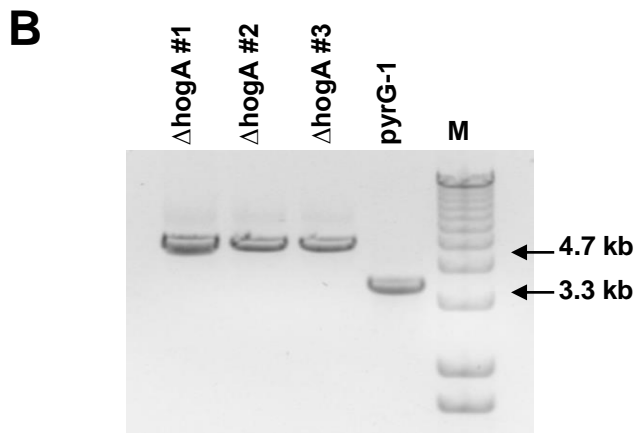
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A.clavatus
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A.nidulans
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A.flavus

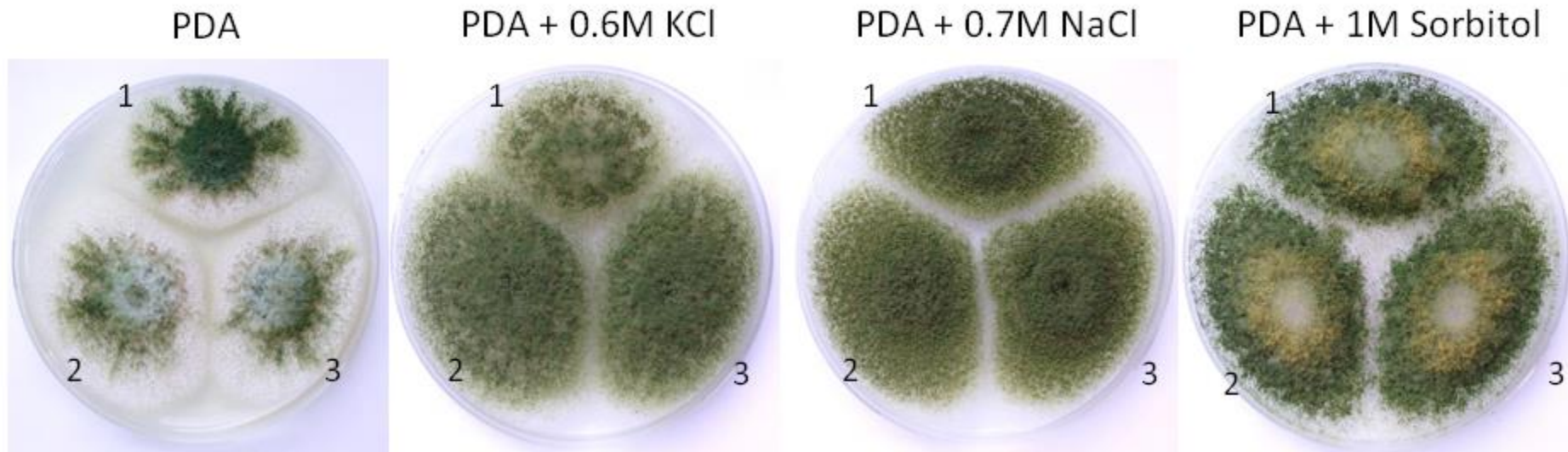
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Supplemental Figure 3. Comparison of putative amino acid sequences of *A. flavus* HogA with those of homologs from other fungal species. Comparisons were done using sequences from Broad Institute (http://www.broadinstitute.org/annotation/genome/aspergillus_group/MultiHome.html) and EMBOSS NEEDLE- Pairwise Sequence Alignment tool from EMBI-EBI (http://www.ebi.ac.uk/Tools/psa/emboss_needle/).

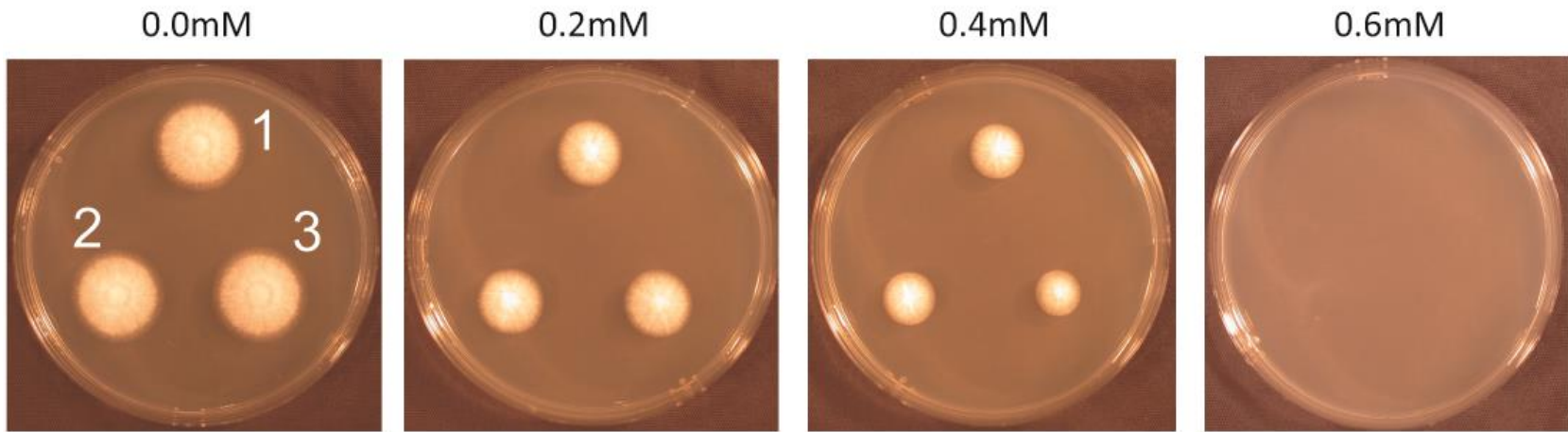


Supplemental Figure 4. Analysis of *A. flavus* deletion mutants by PCR and qRT-PCR. (A) Schematic depicting recombinational inactivation of the *A. flavus* 70S *hogA* gene with the pHogA-*pyrG* disruption vector that utilizes a *pyrG* selectable marker. (B) Successful inactivation of *hogA* was observed in three transformants as indicated by the presence of a 4.7 kb PCR product following amplification of genomic DNA with primers *hogA* Up (P1) and *hogA* Down (P2) (Suppl. Table 1). Amplification of the Af70 *pyrG*-1 control DNA including a *hogA* locus produced a PCR product with the expected size of 3.3 kb. (C) qRT-PCR analysis of *hogA* expression in putative Δ *hogA* mutants and *pyrG*-1 isogenic control.

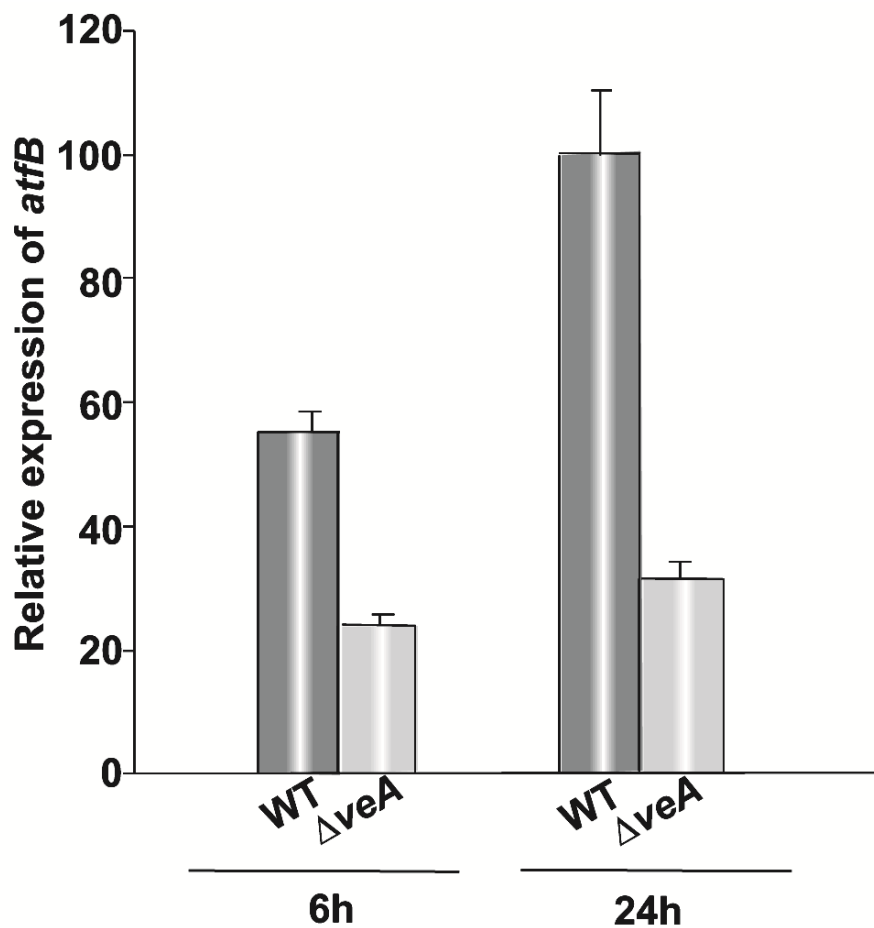




Supplemental Figure 5. *hogA* is dispensable for *A. flavus* response to osmotic stress. *Aspergillus.flavus* wild type (1), and two $\Delta hogA$ mutants (2) and (3) were point-inoculated on PDA supplemented with 0.6M NaCl, 0.7M KCl, or 1.0M sorbitol to induce osmotic stress. Cultures were incubated for 5 days at 30°C.



Supplemental Figure 6. *hogA* is not critical for *A. flavus* response to oxidative stress. *Aspergillus flavus* wild type (1), and two $\Delta hogA$ mutants (2) and (3) were point-inoculated on YGT supplemented with increasing concentrations of menadione. Cultures were incubated for 3 days at 30 °C.



Supplemental Figure 7. *veA* is required for wild-type expression levels of *A. flavus atfB*. Relative expression levels of *atfB* in oxidative stress response 6 hr and 24 hr after addition of 15 mM hydrogen peroxide. *psl82* was used as wild-type control. The relative expression levels were calculated using the method described by Kenneth and Schmittgen (26), and all values were normalized to the expression of the *A. flavus* 18S rRNA gene and to the greatest expression considered as 100.