NosA, a transcription factor important in *Aspergillus fumigatus* stress and developmental response, rescues the germination defect of a *laeA* deletion

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Supplementary Information

Table S1: Primers used in this study

Primer Name	**Sequence**	**Purpose**
NosA 5` flank for	CGGCAAGGTCACGAGCAG	*A. fumigatus ΔnosA* and *OE::nosA* constructs
NosA 5` flank Rev pyrG	CATTCCCGTCGTCATCCACCTCCGAGTCG AGGTATCGATAGCTGG	*A. fumigatus ΔnosA*construct
Ap pyrG For	GAGTCGAGGTATCGATAGCTTG	*ΔnosA*construct
Ap pyrG Rev	ATTCGACAATCGGAGAGGCTGC	*ΔnosA*construct
NosA 3` flank for pyrG	TGTGTGTTTCCTTGCGTTGCAGCCTCTCC GATTGTCGAATGA	*A. fumigatus ΔnosA*construct
NosA 3` flank rev	CAGCTTTGAAACATGAACAGCA	*A. fumigatus ΔnosA*construct
NosA 5` flank rev pyrG&gpdA	CATTCCCGTCGTCATCCACGCGTAATACG ACTCACTATAGG	*A. fumigatus OE::nosA* construct
pyrG 3` for pJMP9	CGTAATACGACTCACTATAGGG	*A. fumigatus OE::nosA* construct
gpdA(p) 3`	TACCCCGCTTGAGCAGACATCACA	*A. fumigatus OE::nosA* construct
NosA 3` for gpdA	CGGCCTCGAATGCCGCCAAGAGGGTCGT ACCCCGCTTGAGCAGACATCACA	*A. fumigatus OE::nosA* construct
NosA 3` int	CAAGACCACTGCGGGATTCG	*A. fumigatus OE::nosA* construct
Af nosA 5' flank ext	GCACAACACGATCCTGCTGAAC	*A. fumigatus ΔnosA* mutant screening
Ap pyrG 3' int	CTGGACCAAACACATCGATGC	*A. fumigatus ΔnosA* mutant screening
gpdA 5' int	GCGTCCAAATATCGTGCCTCTCC	*A. fumigatus OE::nosA* mutant screening
Af nosA 3' int 2	GAGGGTCATGCTGAAAGGTGG	*A. fumigatus OE::nosA* mutant screening
Af nosA 5' flank for NotI	NNNGCGGCCGCCGGCAAGGTCACGAGCA G	*A. fumigatus* complementation
Af nosA 3' flank rev NotI	NNNGCGGCCGCGCTGTTCATGTTTCAAA GCTG	*A. fumigatus* complementation

An nosA 5' flank for NotI	NNNGCGGCCGCCGACGAACTCCCCGCACC	*A. nidulans* complementation
An nosA 3' flank rev NotI	NNNGCGGCCGCGTGAAAGAAAGGTATTGTCTGG	*A. nidulans* complementation
An nosA 5' flank rev pyrG	CAAGCTATCGATACCTCGACTCGAGGGCAGGTCCAAAGTCG	*A. nidulans ΔnosA* construct
An nosA 3' flank for pyrG	GCAGCCTCTCCGATTGTCGAATCCTCGATGGGATCTCGTACA	*A. nidulans ΔnosA* construct
An nosA 5' flank for	CGACGAACTCCCCGCACC	*A. nidulans ΔnosA* construct
An nosA 3' flank rev	GTGAAAGAAAGGTATTGTCTGG	*A. nidulans ΔnosA* construct
An nosA 5' flank ext	CTCCAGCTGCACTTCATCACA	*A. nidulans ΔnosA* PCR screening and probe
An nosA 3' int	GTTCTTGATACGTTCCTTGTGC	*A. nidulans ΔnosA* PCR screening and probe
an cpeA 5' for probe	CGTCAGCACAACTCGGTCTC	*A. nidulans* northern probe
an cpeA 3' for probe	GCACACGAGGCGTTCCTCG	*A. nidulans* northern probe
an hxtA 5' for probe	CGTGTCACTGCAATTCAGTTC	*A. nidulans* northern probe
an hxtA 3' for probe	ACGGGACCAGTCCGGTCC	*A. nidulans* northern probe
hphF	CGCTCTAGAACTAGTGGATC	Southern probe
hphR	CACCGGCTGCACATGTCAA	Southern probe
af hxtA 5' for probe F	CAGCAGTATGAGCGGTGCG	*A. fumigatus* northern probe
af hxtA 3' rev probe	CTCGCTGTGAGCAGCGACC	*A. fumigatus* northern probe
af cat2 probe 5'	AGGAGCAGTCGTCGCAGCC	*A. fumigatus* northern probe
af cat2 probe 3'	GGTTCATTACCTTGTTCCACGC	*A. fumigatus* northern probe
af sod1 F	TCATTGCTGTCCTCCGTGGTG	semi-quantitative RT-PCR
af sod1 R 2	GCGCTCGGAGTCCTCAGG	semi-quantitative RT-PCR

af sod2 F 2	GGTAAGATGAACACCGCTTTG	semi-quantitative RT-PCR
af sod2 R	CTTCTCCACAGCCTTCCAGTTG	semi-quantitative RT-PCR
af sod3 F	CTACGATGCCCTCCAACCCTAC	semi-quantitative RT-PCR
af sod3 R 2	AAGAGGGAATGGTTGATGTGC	semi-quantitative RT-PCR
af sod4 F	ACAAGTTCGCCAAGGACATTATCG	semi-quantitative RT-PCR
af sod4 R 2	TGTGGGTGGCCATATCTACC	semi-quantitative RT-PCR
af arpA F 2	CCAAGACGCGATTACCCGC	semi-quantitative RT-PCR
af arpA R	TCTTCGTGCCATTCGTCTG	semi-quantitative RT-PCR
af catA F	CCGATGAAGGCAACTGGGAC	semi-quantitative RT-PCR
af catA R	GGTCAGACATGGCCCACATG	semi-quantitative RT-PCR
af cat1 F 2	CATCAACCAACCTCGCGTTC	semi-quantitative RT-PCR
af cat1 R 2	GGCTGAGACCAGACATCCTC	semi-quantitative RT-PCR
af cat2 F	GCACACGGCCGTCTCCAAC	semi-quantitative RT-PCR
af cat2 R 2	CCCACGAGATCTTGTTGCCG	semi-quantitative RT-PCR
af nosA F	GCAGACGAAGATGAATGAGCG	semi-quantitative RT-PCR
af nosA R	AAGTCTGTCGTTCTGTCTTGAC	semi-quantitative RT-PCR

Figure S1

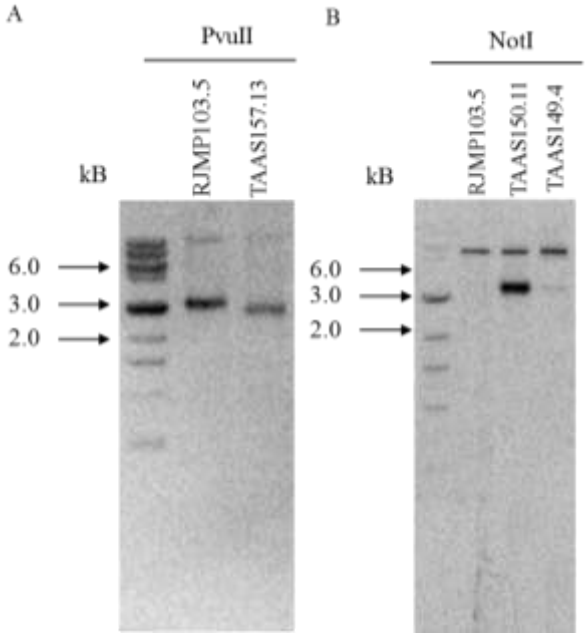

Fig. S1. Southern digests confirm *A. nidulans* transformants. A. Confirmation of TAAS157.13 (*ΔnosA*) using a PvuII digestion. Expected sizes are: RJMP103.5 - 3263 bp; TAAS157.13 – 2708 bp. Probe was created using An nosA 5' flank for and An nosA 5' flank rev pyrG to amplify template. B. Confirmation of transformants for complementation testing using NotI. Expected sizes are: RJMP103.5 – 24945 bp; TAAS150.11 (+*A.n. nosA*) – 4361 bp; TAAS149.4 (+*A.f. nosA*) – 4100 bp. Dual probes were created using An nosA 5' flank for and An nosA 3' flank rev and Af nosA 5' flank for and Af nosA 3' flank rev to amplify templates.

Figure S2

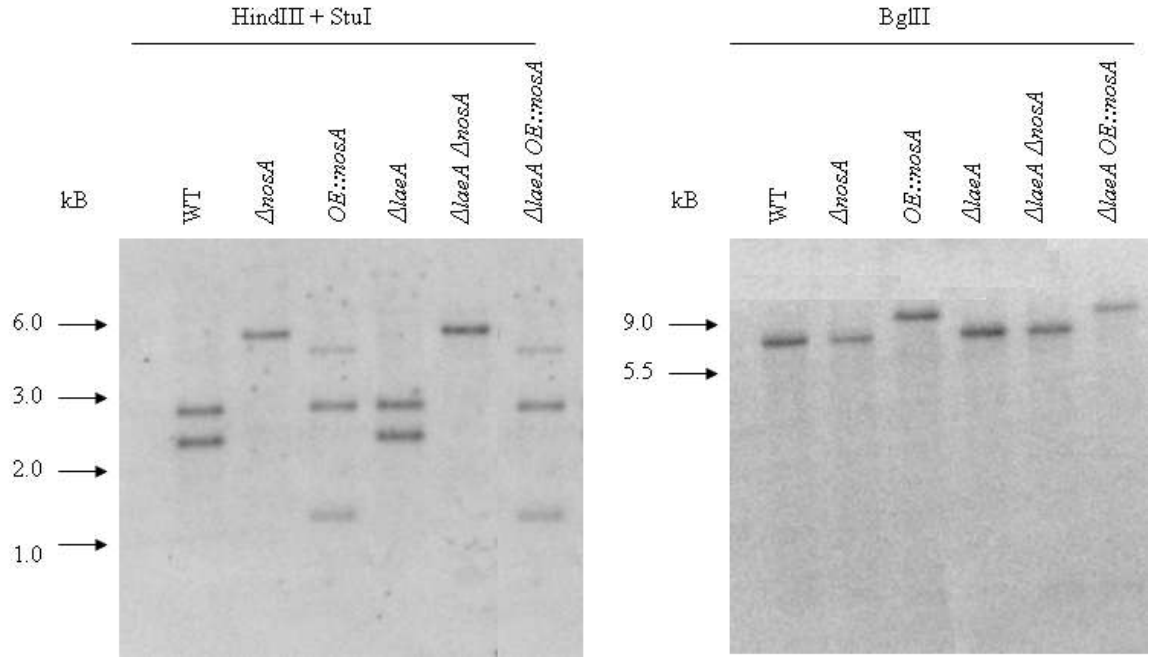

Fig. S2. Southern blot using HindIII + StuI or BglII confirms correct *A. fumigatus* transformants. Predicted fragment sizes in bp for HindIII + StuI are WT: 2955 and 2366, *ΔnosA:* 5494, *OE::nosA*: 4603, 2955, and 1366. Predicted fragment sizes in bp for BglII are wild type: 5567, *ΔnosA*: 5497, *OE::nosA*: 9097. Strains correspond to WT – TFYL20.10, *ΔnosA* - TAAS104, *OE::nosA* – TAAS105, *ΔlaeA* – TFYL19.3, *ΔlaeA ΔnosA* – TAAS107, *ΔlaeA OE::nosA* – TAAS108. Probe was created using Af nosA 5' flank for and Af nosA 3' flank rev to amplify template.

Figure S3

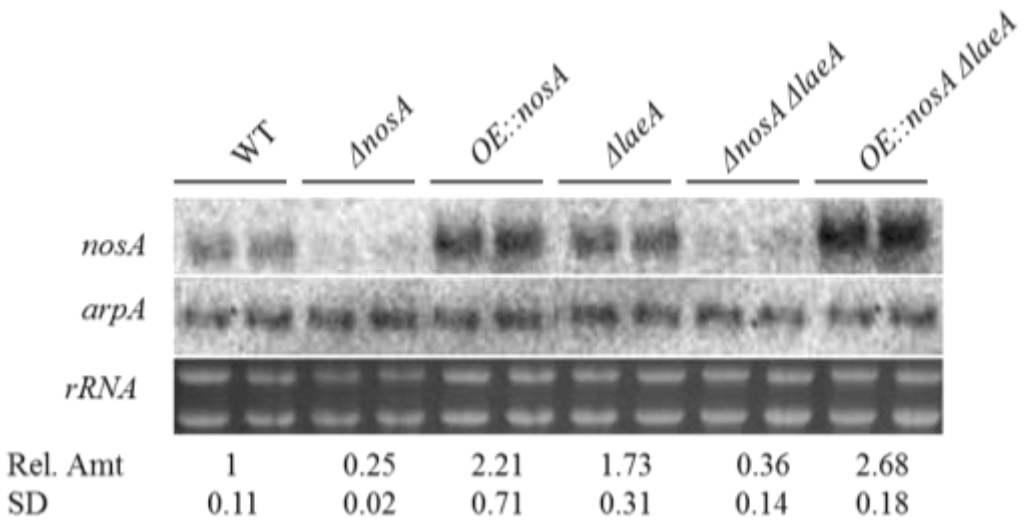

Fig. S3. Northern blots confirm overexpression of *nosA* and deletion of *nosA* in both wild type and *ΔlaeA* backgrounds of *A. fumigatus*. Note that *ΔlaeA* also displays increased *nosA* transcript relative to wild type under these conditions, confirming the results of the previously published microarray (Perrin et al., 2007). Actin related protein A (*arpA*) was used as a loading control. Amounts given are of *nosA* transcript normalized to *arpA* levels, relative to wild type. Strains correspond to WT – TFYL20.10, *ΔnosA* - TAAS104, *OE::nosA* – TAAS105, *ΔlaeA* – TFYL19.3, *ΔlaeA ΔnosA* – TAAS107, *ΔlaeA OE::nosA* – TAAS108.

Figure S4.

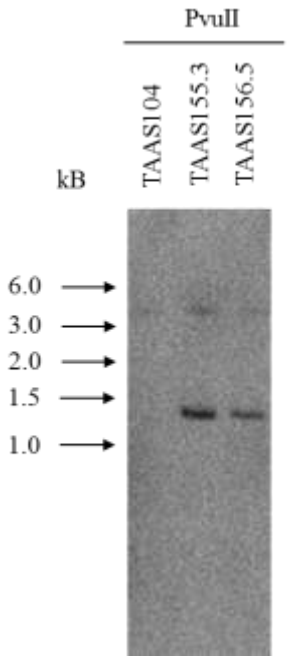

Fig. S4. Southern digest confirming *A. fumigatus* complementation strains using EcoRI. Expected sizes are: TAAS104 – no fragment; TAAS155.3 *(+ A.f. nosA*) and TAAS156.5 (+ *A.n. nosA*) – 1426 bp. hphF and hphR were used to amplify template DNA for the probe.

Figure S5

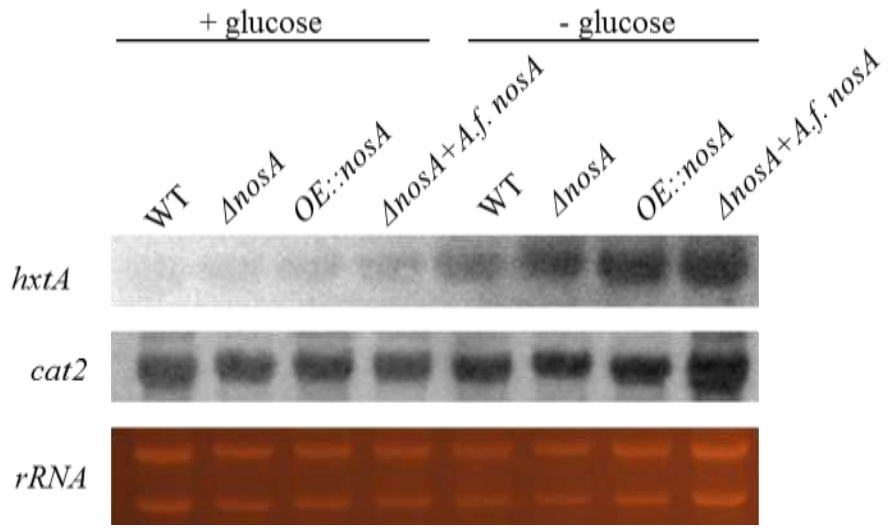

Fig. S5. *A. fumigatus nosA* does not influence expression of *cat2* or *hxtA*. When carbon is absent from the culture, both *cat2* and *hxtA* transcript levels increase in all strains tested, regardless of *nosA* genotype. Strains correspond to WT – TFYL20.10, *ΔnosA* - TAAS104, *OE::nosA* – TAAS105, *ΔnosA* + *A.f. nosA* – TAAS155.3.

Figure S6

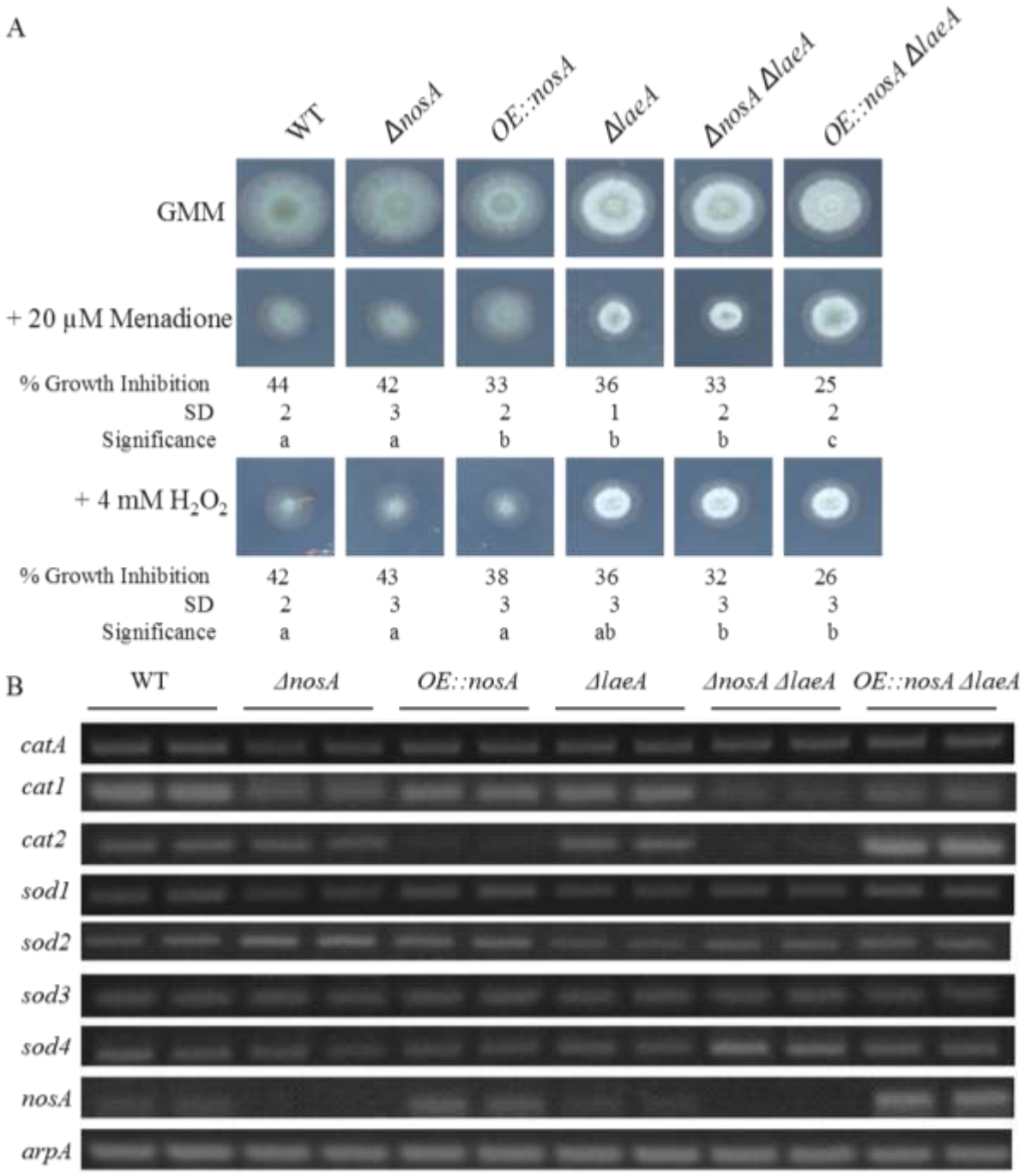

Fig. S6. Resistance to chemicals which generate reactive oxygen species. A. Overexpression of *nosA* results in increased resistance to 20 µM menadione, as evidenced by an increased ratio of

treated/untreated colony diameter. This increase is independent of *laeA*, as increased resistance occurs in both wild type and *ΔlaeA* backgrounds. *ΔlaeA* also exhibits a statistical increase in menadione resistance relative to WT, which is not abrogated by deletion of *nosA*. No significant differences were seen for resistance to 4 mM hydrogen peroxide among *nosA* mutants in their respective control backgrounds (e.g. WT or *ΔlaeA*) although the double *nosA ΔlaeA* mutants were more resistant to hydrogen peroxide than wild type and single *nosA* mutants. Letters represent significant differences at $p < 0.05$. B. Semi-quantitative RT-PCR examining catalase and superoxide dismutase transcript levels in *nosA* mutants. *OE::nosA* strains do not exhibit a significant increase in transcript levels of any catalase or superoxide dismutase tested. *ΔnosA* shows a slight increase in *sod2* transcript, but maintains wild type transcript levels of other genes. Actin related protein A (*arpA*) was used as a loading control. Strains correspond to WT – TFYL20.10, *ΔnosA* - TAAS104, *OE::nosA* – TAAS105, *ΔlaeA* – TFYL19.3, *ΔlaeA ΔnosA* – TAAS107, *ΔlaeA OE::nosA* – TAAS108.

Figure S7

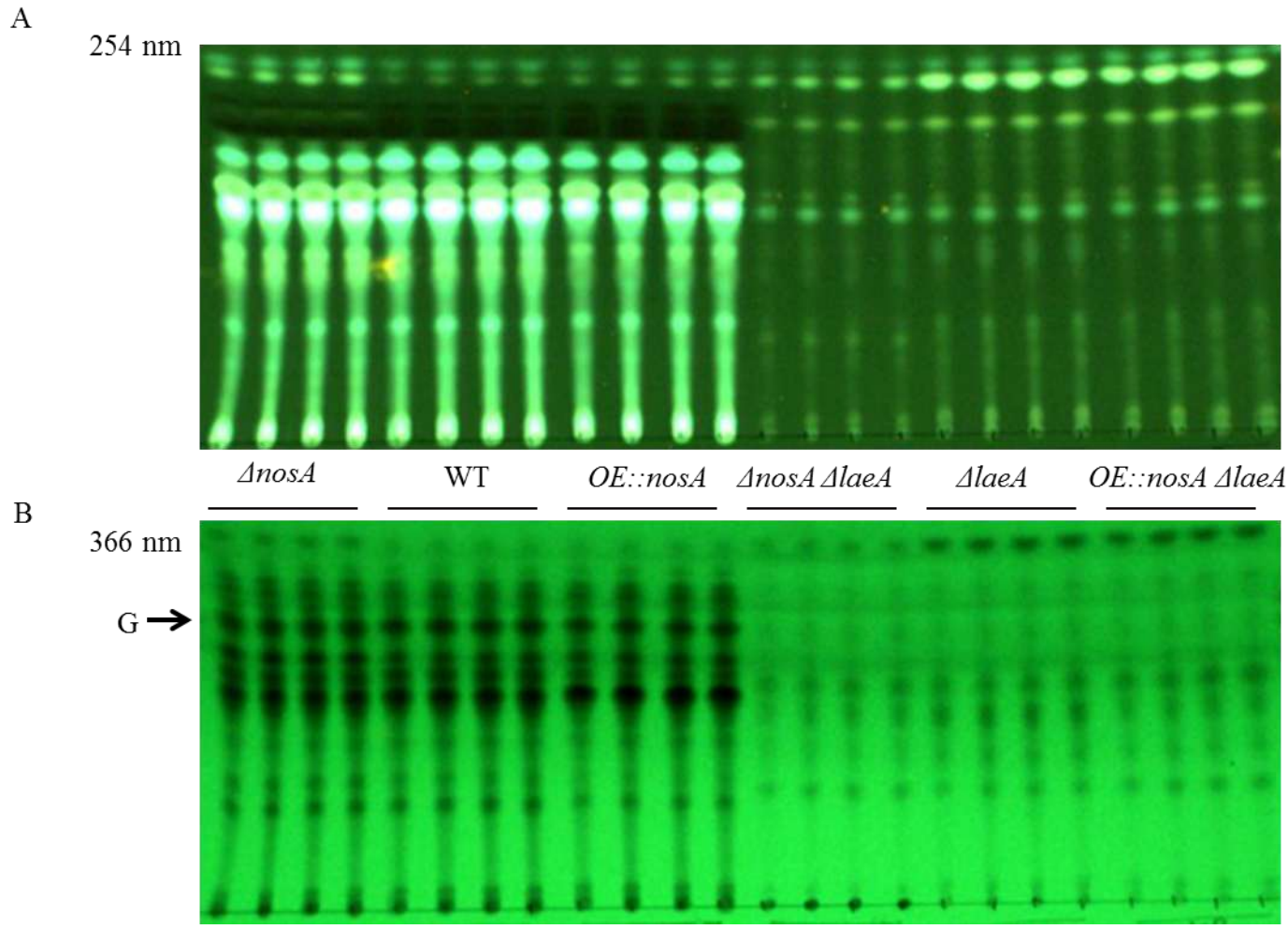

Fig. S7. NosA mutants show only minor differences in SM profiles. A. TLC analysis visualized under 254 nm light. 4 replicates of each strain are shown. B. TLC analysis visualized under 366 nm light. 4 replicates of each strain are shown. The band correlating to gliotoxin is indicated (G). Strains correspond to WT – TFYL20.10, *ΔnosA* - TAAS104, *OE::nosA* – TAAS105, *ΔlaeA* – TFYL19.3, *ΔlaeA ΔnosA* – TAAS107, *ΔlaeA OE::nosA* – TAAS108.

Figure S8

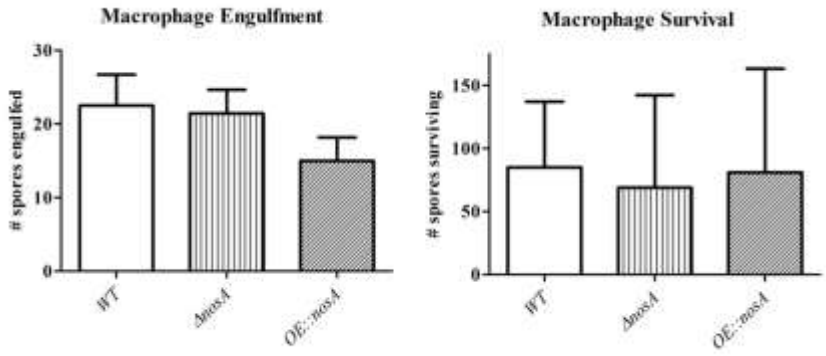

Fig. S8. NosA mutants do not differ in macrophage engulfment or survival. A. Engulfment of condia after a 1 hour incubation with purified human primary macrophages. B. Survival of engulfed conidia. 4 hours post-engulfment, the numbers of surviving conidia were quantified and normalized to engulfed conidia. No differences were seen between the strains for either engulfment or spore survival. Strains correspond to WT – TFYL20.10, *ΔnosA* - TAAS104, *OE::nosA* – TAAS105.