Supplementary Information

Functional characterization of the *Aspergillus nidulans* glucosylceramide pathway reveals that LCB Δ8-desaturation and C9-methylation are relevant to filamentous growth, lipid raft localization and *Ps*d1 defensin activity.

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Supplemetary Table S1 – Sexual crosses of Δ*sdeA*, Δ*smtA*, Δ*smtB* and Δ*gcsA* mutants with GR5 strain (wild-type).

| | Number of segregants | | | |
|---|---|---|---|---|
| Cross | *pyrG+sdeA+* | *pyrG+sdeA-* | *pyrG-sdeA+* | *pyrG-sdeA-* |
| Δ*sdeA* x wild-type | 0 | 34 | 62 | 0 |
| Cross | *pyrG+smtA+* | *pyrG+smtA-* | *pyrG-smtA+* | *pyrG-smtA-* |
| Δ*smtA* x wild-type | 0 | 44 | 56 | 0 |
| Cross | *pyrG+smtB+* | *pyrG+smtB-* | *pyrG-smtB+* | *pyrG-smtB-* |
| Δ*smtB* x wild-type | 0 | 43 | 40 | 0 |
| Cross | *pyrG+gcsA+* | *pyrG+gcsA-* | *pyrG-gcsA+* | *pyrG-gcsA-* |
| Δ*gcsA* x wild-type | 0 | 51 | 49 | 0 |

* Δ*sdeA* and Δ*gcsA* segregants were selected based on their radial diameter and reduced conidiation in MM while

Δ*smtA* and Δ*smtB* segregants were selected based on Congo Red resistance.

Supplementary Information

Supplementary Table S2 - List of primers used for *sdeA*, *smtA*, *smtB* and *gcsA* deletion cassettes construction and amplification. P1-P2, P9-P10, P11-P12 were used for FGSC deletion cassette amplification; P3 to P8 were used for *smtB* deletion cassette construction; P1, P3, P8-P9, P11, P13-P14 were used to check cassette integration and gene disruption by PCR; P15 to P22 primers were used to amplify 5' UTR regions that served as probes in Southern Blot analysis.

| | Primer | Sequence |
|---|---|---|
| P1 | *sdeA* 5' UTR F | GTAACGCCAGGGTTTTCCCAGTCACGACGCTTACATGACTCGCTGTGAC |
| P2 | *sdeA* 3' UTR R | GCGGATAACAATTTCACACAGGAAACAGCCTGGTCTAACAGACGAAGC |
| P3 | *smtA* 5' UTR tag pRS426 F | GTAACGCCAGGGTTTTCCCAGTCACGACGGACAACCTCGTAGCTATTCA |
| P4 | *smtA* 5' UTR tag *pyrG* R | CGCATCAGTGCCTCCTCTCAGACAGAATCGTCGATTGATTTTTGACCTC |
| P5 | smtA 3' UTR tag *pyrG* F | GGTGAAGAGCATTGTTTGAGGCGAATTCGCTGCTGTTGGTGAATCTGTT |
| P6 | *smtA* 3' UTR tag pRS426 R | GCGGATAACAATTTCACACAGGAAACAGCGTCAGTGGTACTTGGCATTG |
| P7 | *pyrG* F | ATTCTGTCTGAGAGGAGGCACTGATGCG |
| P8 | *pyrG* R | GAATTCGCCTCAAACAATGCTCTTCACC |
| P9 | *smtB* 5' UTR F | GTAACGCCAGGGTTTTCCCAGTCACGACGGGAGACCTATACTCAGCCAT |
| P10 | *smtB* 3' UTR R | GCGGATAACAATTTCACACAGGAAACAGCGCCGACTAGATGAAAGCTAC |
| P11 | *gcsA* 5' UTR F | GTAACGCCAGGGTTTTCCCAGTCACGACGGAGATACCCGCTACAACTCT |
| P12 | *gcsA* 3' UTR R | GCGGATAACAATTTCACACAGGAAACAGCGCTGTCTCCTACATTACCCT |
| P13 | *pyrG* FGSC RI | GACAGAAGATGATATTGAAGGAGC |
| P14 | *pyrG* FGSC RII | AGGAGTCTGGTATTGCTGTC |
| P15 | *sdeA* 5' UTR F | TTACATGACTCGCTGTGAC |

| P16 | *sdeA* 5' UTR R | GGCTGTCAAGCAATAACC |
|---|---|---|
| P17 | *smtA* 5' UTR F | GCAACGAGCGGACTATTC |
| P18 | *smtA* 5' UTR R | ACGAATTGACCAAGACGACC |
| P19 | *smtB* 5' UTR F | GTAATAAGCCGTCTCTCGT |
| P20 | *smtB* 5' UTR R | TGAGGACAACAAGCTGGTTG |
| P21 | *gcsA* 5' UTR F | GACGATAAATCAGCAGGCG |
| P22 | *gcsA* 5' UTR R | ACTGGAATCCGCCATAGC |

Supplementary Information

Supplementary Table S3 - List of primers used for *niiA::smtB* cassette construction, amplification and analysis of *smtB* and *tubC* expression. P23-P30 were used for *niiA::smtB* cassette construction; P23 and P29 primers were used for *niiA::smtB* cassette amplification, P23 and P26 were used to check cassette integration and *smtB* promoter replacement by PCR; P31-P34 were used to quantify *smtB* and *tubC* mRNA abundance by qPCR.

|  | Primer | Sequence |
|---|---|---|
| P23 | *smtB* 5' UTR tag pRS426 F | GTAACGCCAGGGTTTTCCCAGTCACGACGCCATGCTTCTCATACAGCCCGCG |
| P24 | *smtB* 5' UTR tag pyro R | GACCCAACAACCATGATACCAAGGGATGGAGGAGAGTTGAGGAC |
| P25 | *pyro* F | TGGTATCATGGTTGTTGGGTC |
| P26 | *pyro* R | AGCATCCACATGATCGACAG |
| P27 | *niiA* tag pyro F | CTGTCGATCATGTGGATGCTCATTGCTCAGAGTACTACAGG |
| P28 | *smtB* tag niiA R | CTGGCGTTGAGATTCGTCACGTCAGGCTTCACTCTCAATTGAAC |
| P29 | *smtB* tag pRS426 R | GCGGTTAACAATTTCTCTCTGGAAACAGCATATTTTTGTTCATAATCGTGTCG |
| P30 | *smtB* 5' ext F | TGACCAAAGTGAGAAAACAGC |
| P31 | *smtB* sybr F | TTGCTAAGCGTCCTTCCTGT |
| P32 | *smtB* sybr R | GTCTGCATGGGGATCTTGTT |
| P33 | *tubC* sybr F | AGCTGGCGGTAACAAATACG |
| P34 | *tubC* sybr R | ACCTGATCCACCAATTCTGC |
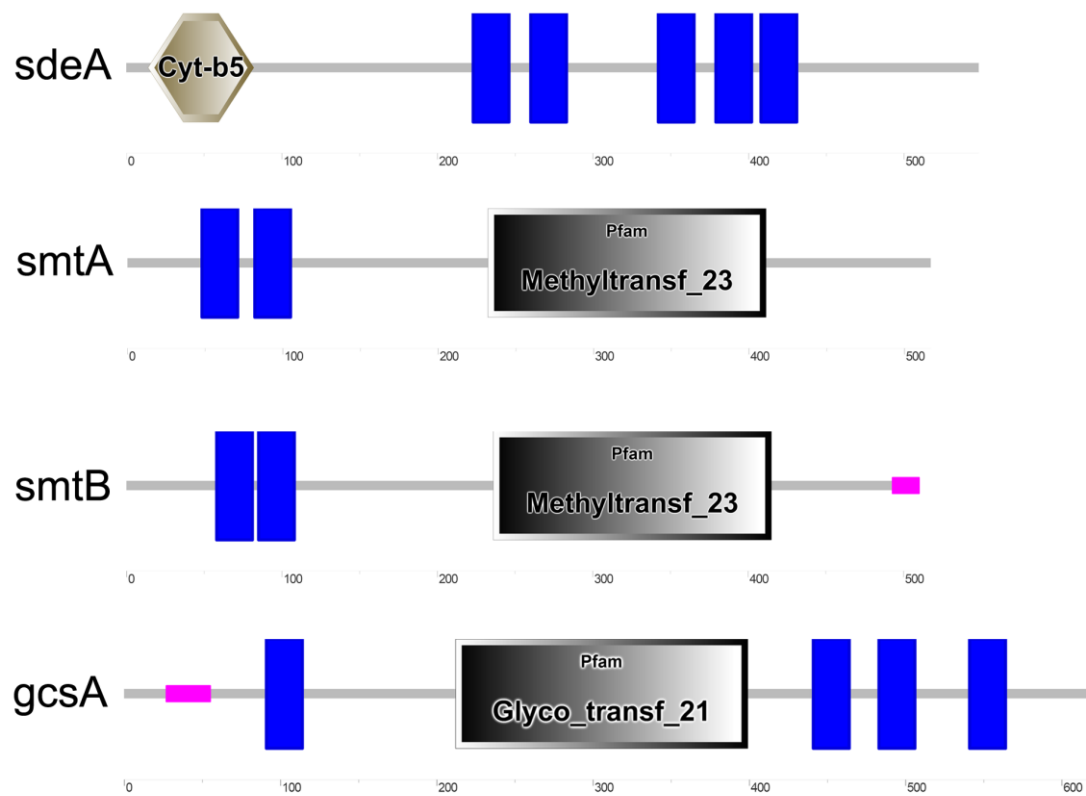
Supplementary Figure S1 - Predicted domains of *A. nidulans* SdeA, SmtA, SmtB and GcsA enzymes. SMART diagrams illustrate putative motifs and their location in amino acid sequences (shown by the numeric scales). Transmembrane and low complexity regions are indicated by blue and pink bars, respectively. Cytochrome b5-like heme/sterol binding domain is illustrated by the brown hexagon. Pfam domains and their predicted functions are represented by grey boxes.
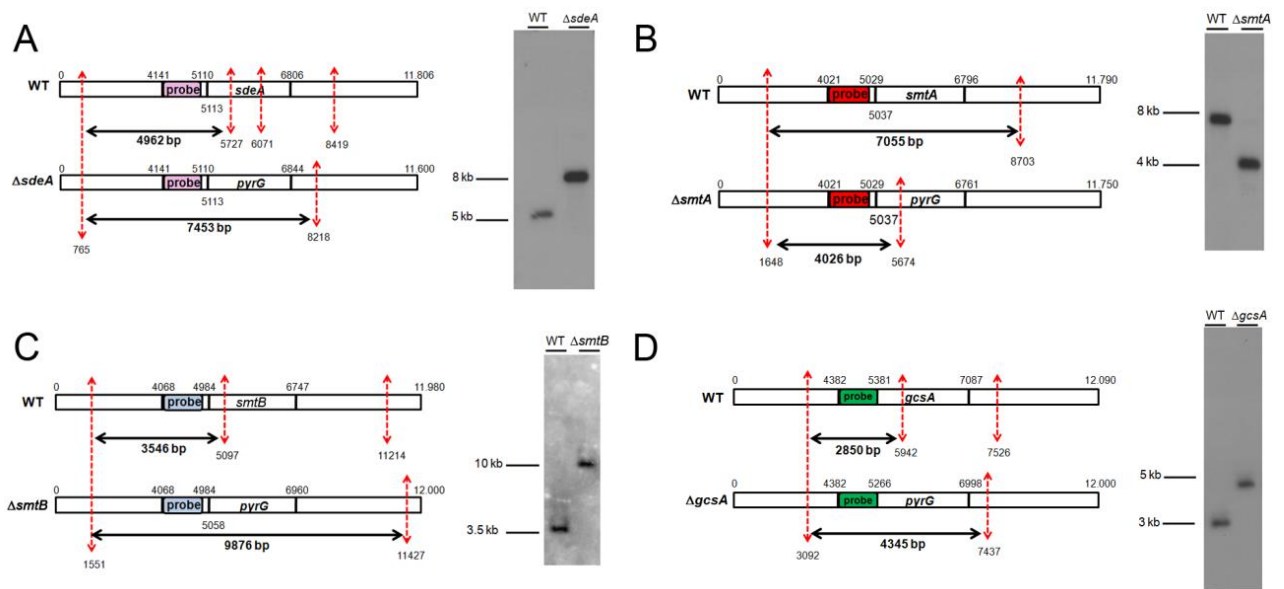
Supplementary Figure S2 - Schematic illustration of gene deletion strategy and Southern Blot analysis. Genomic DNA from ∆sdeA, ∆smtA, ∆smtB and ∆gcsA candidates and parental strain was isolated and digested with EcoRV (1A, 1B and 1D) or BamHI (1C) enzymes, whose restriction sites are shown by dotted red lines. 1 kb of each 5' UTR region was used as amplification probe, leading to the recognition of7453 (A), 9876 (B), 4026 (C) and 4345 (D) bp bands present in the respective mutant strains but not in wild type, confirming gene disruption.
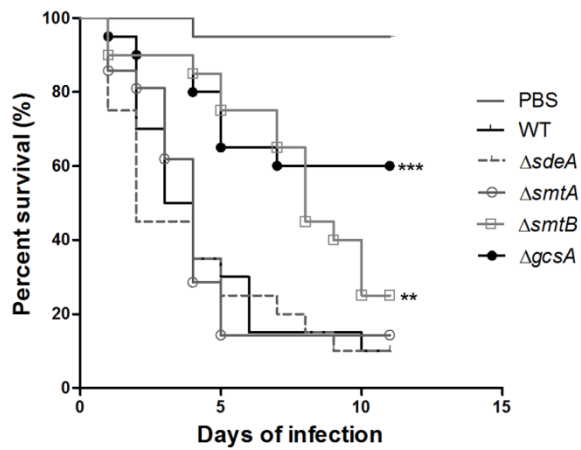
Supplementary Figure S3 - Survival of *G. mellonella* larvae infected with alternative Δ*sdeA*, Δ*smtA*, Δ*smtB* and Δ*gcsA* transformants. $1.10^6$ conidia or an equal volume of saline solution were injected in 20 caterpillars/group and viability was monitored for 10 days after inoculation. *sdeA*, *smtA* and *smtB* null mutants caused approximately 80 % lethality, comparable to wild type strain, while *gcsA* disruption impaired fungal virulence; **p< 0.01, ***p< 0.001.

A
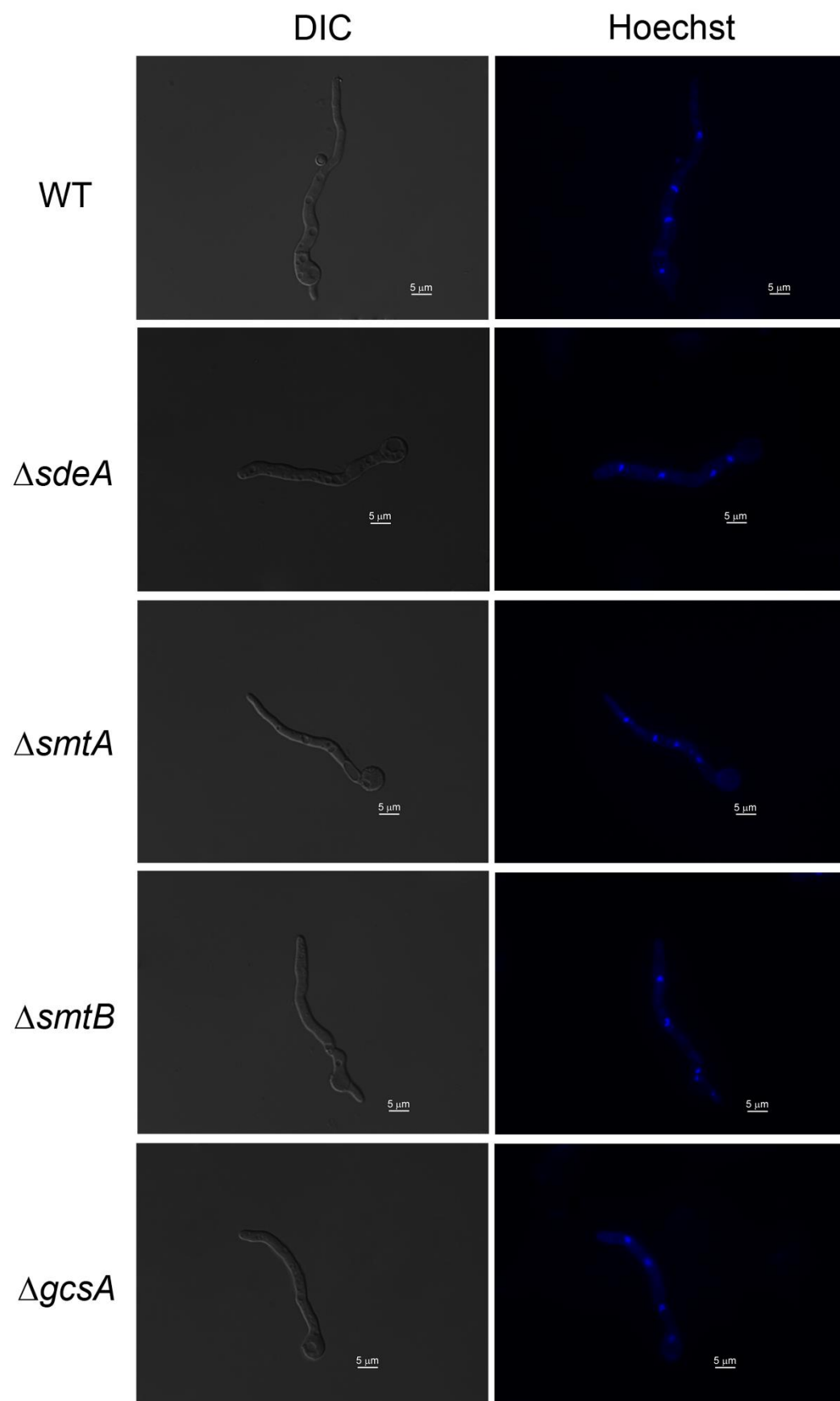
B
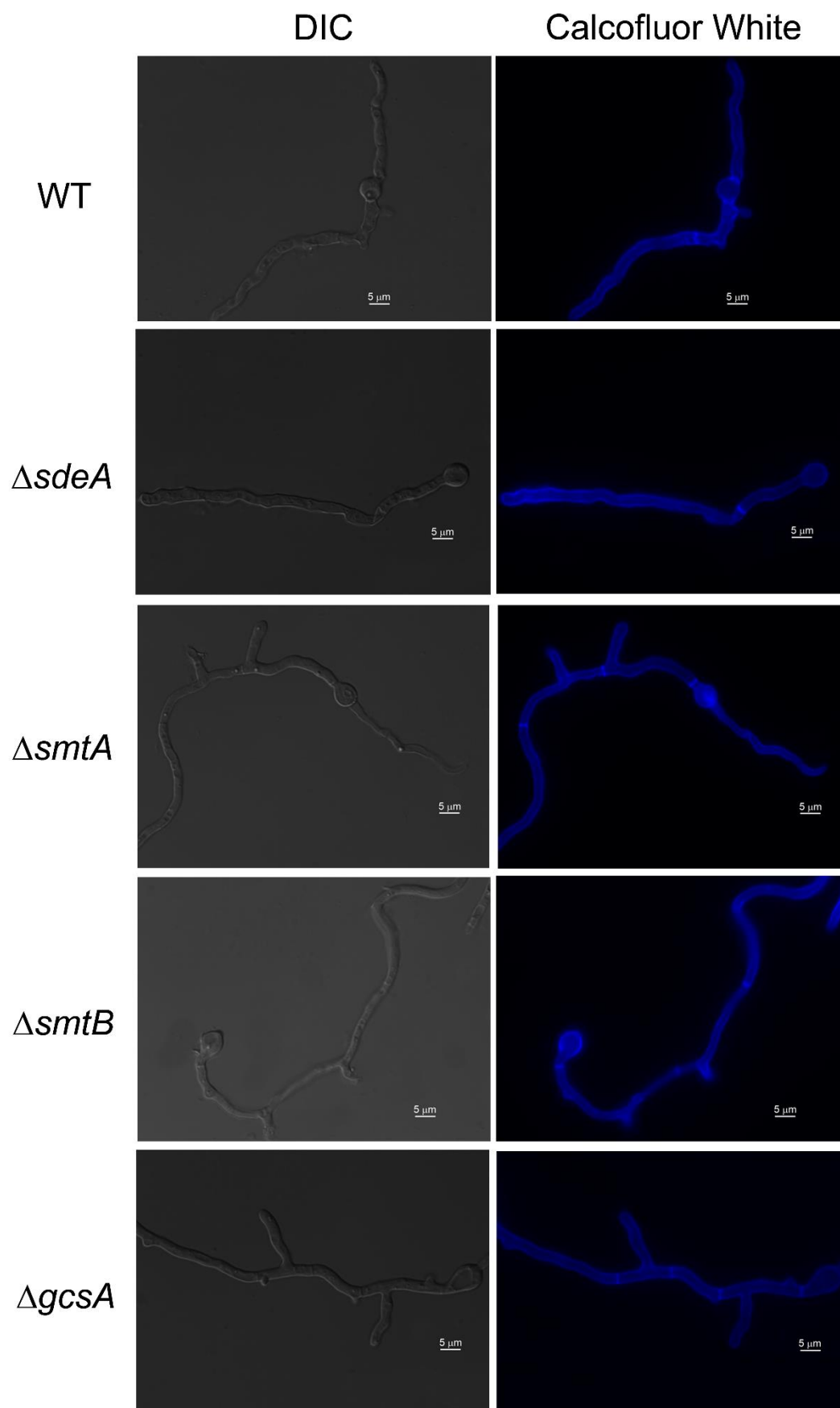
Supplementary Information

Supplementary Figure S4 - The strains deficient in GlcCer synthesis show regular nuclei distribution (A) and chitin staining (B). Wild-type, Δ*sdeA*, Δ*smtA*, Δ*smtB* and Δ*gcsA* conidia were grown in MM + UU + pyro media for 16 h at 25 °C and then stained with 10 μg.ml$^{-1}$ Hoechst (A) or 2 μg.ml$^{-1}$ Calcofluor White (B). The bars represent 5 μm.