Supplemental Material

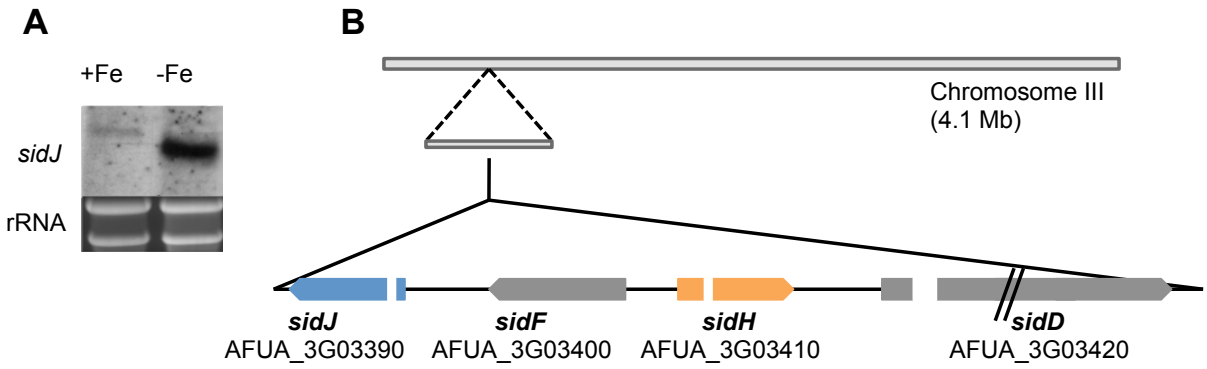

Fig. S1. Iron regulated expression (A) and genomic location of *sidJ* (B). (A) Northern analysis of *sidJ* expression was performed with *A. fumigatus* total RNA, which was extracted from mycelia grown in liquid iron-replete (+Fe, 30 µM $FeSO_4$) and iron-limited (-Fe) minimal medium for 24 hours. The *sidJ* hybridization probe was PCR-amplified using primers oAf538AH1-f and oAf538AH1-r. The primer sequences are listed in Table S1. Ethidium bromide stained rRNA is shown as a control for loading and quality of RNA. (B) Schematic view of the genomic localization of *sidJ* on supercontig 100 (http://www.broad.mit.edu/annotation/genome/aspergillus) and its clustering with the FSC biosynthetic genes *sidF, sidH,* and *sidD* (Schrettl et al., PLoS Pathog. 3:1195-1207, 2007). *SidD* is displayed shortened.

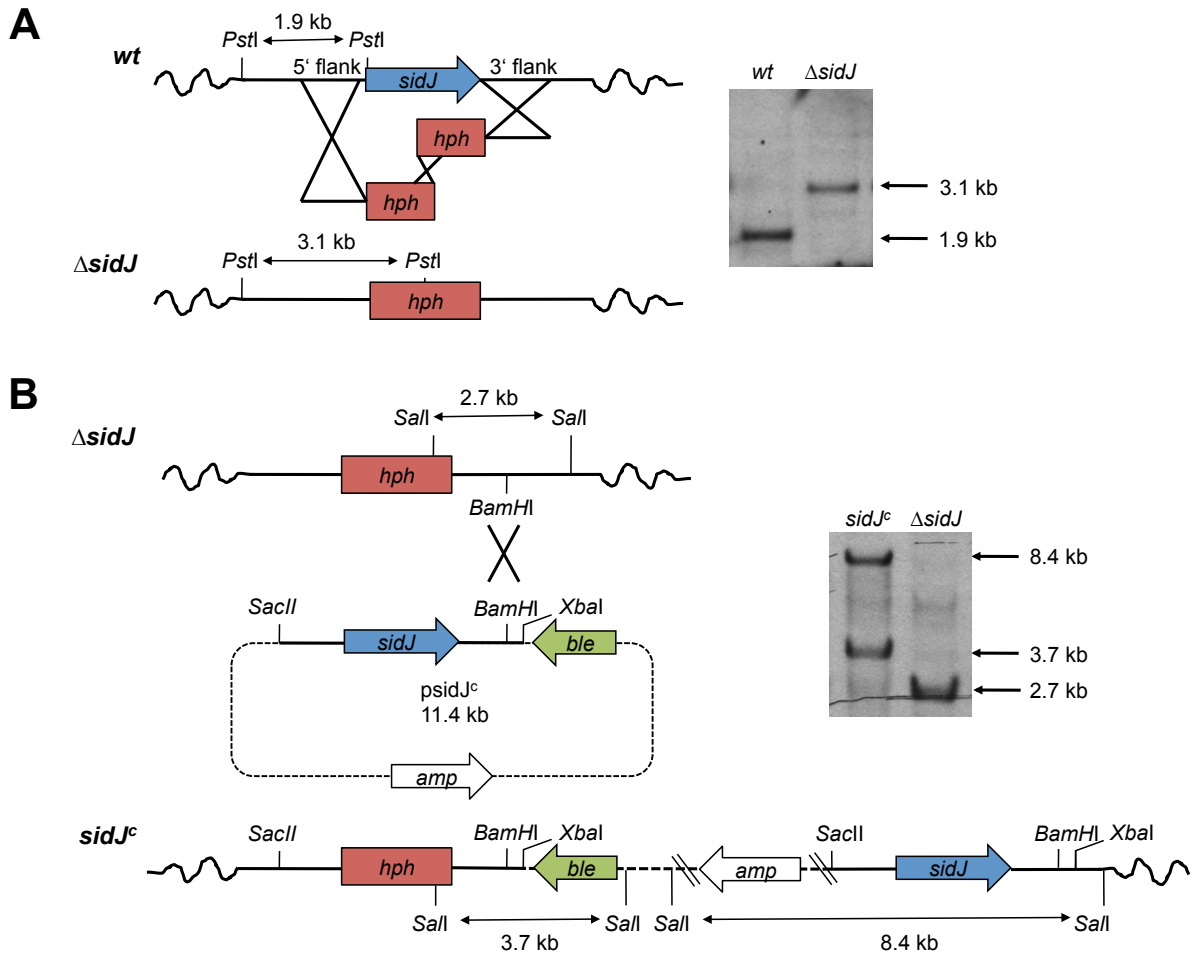

Fig. S2: Generation of *A. fumigatus ΔsidJ* (A) and *sidJ*^{c} (B) strains.

(A) To generate *ΔsidJ*, the 5′ flanking region of *sidJ* (1.2 kb) was amplified using primers oAfAH1-1 and oAfAH1-4 (containing an add-on restriction site for *Not*I), and the 3′ flanking region (1.3 kb) was amplified using oAfAH-2 and oAfAH1-3 (containing an add-on restriction site for *Bam*HI). Subsequently, the *Not*I–*Bam*HI fragment of hygromycin resistance cassette *hph* released from pHY-TK was ligated with the *sidJ* 5′and 3′ flanking regions, respectively. Each ligation product was then used as template for the amplification of the bipartite deletion constructs using primers oAfAH1-5 and ohph-14 for the 5′ flanking region as well as primers oAfAH1-6 and ohph-15 for the 3′ flanking region. Transformation of *A. fumigatus* with these fragments was performed as described earlier (Schrettl et al., PLoS Pathog. 3:1195-1207, 2007). Transformants were selected with 200 µg/ml of hygromycin B and homologous recombination was confirmed by Southern blot analysis. DNA digested with *Pst*I was blotted and probed with a hybridization probe for the 5' prime flanking region of *sidJ* using primers oAfAH1-4 and oAfAH1-5. The 3.1 kb fragment confirms *sidJ* deletion by hph insertion.

(B) For the reconstitution of the Δ*sidJ* strain with a functional *sidJ* copy, a 5.0-kb *Sac*II-*Xba*I fragment released from the cosmid psidD-COS (also designated as pANsCosSidD) was subcloned into plasmid pPhleo carrying the phleomycin resistance marker gene *ble*. The resulting 11.4-kb plasmid psidJc was linearized with *Bam*HI and used to transform *A. fumigatus* Δ*sidJ*. Transformants selected with 50 µg/ml phleomycin carrying a single homologous reconstitution of the *sidJ* gene (*sidJ*^{c}) were screened by Southern blot analysis. DNA digested with *Sal*I was blotted and probed with a hybridization probe for the 3' prime flanking region of *sidJ* using primers oAfAH1-3 and oAfAH1-6. A 3.7 kb fragment confirms the *ΔsidJ* deletion allele, while the 8.4 kb fragment represents the insertion of the *sidJ* containing plasmid. Primers are listed in Table S1.

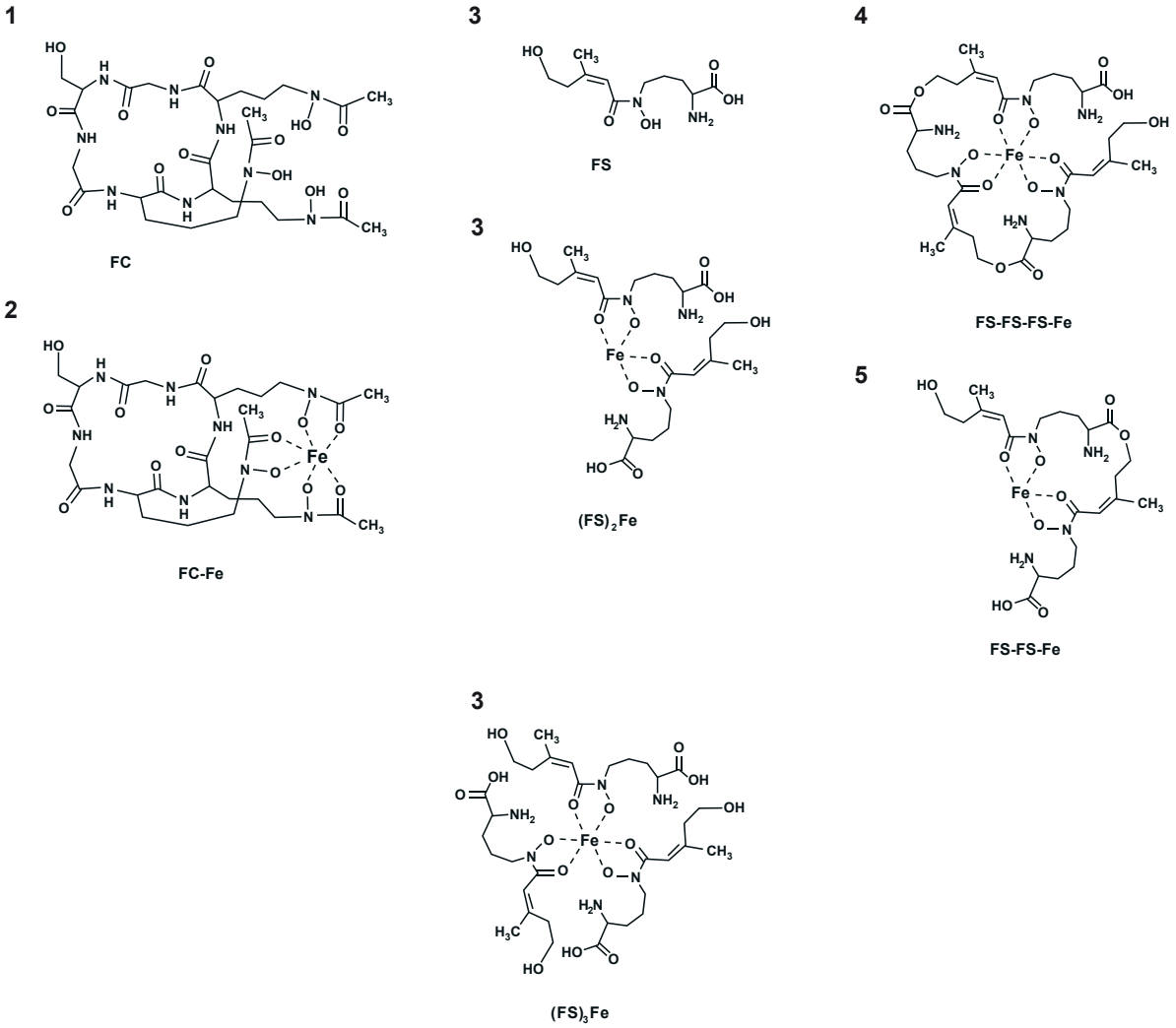

Fig. S3: Chemical structures of the siderophores and their degradation products. The numbers correspond to the peak numbers shown in Fig. 2. (1) and (2) are the intracellular siderophore FC in the desferri- and ferri-form, respectively. Hydrolysis of all three FSC ester bonds leads to FS and FS/iron complexes (3). The ferri-FS dimer (4) derives from hydrolysis of two FSC ester bonds. Hydrolysis of one ester bond leads to ferri-fusarinine B (5).

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A. fumigatus|XP_748659.1|       97 KYIKEYKTEKFGGSA-------SSGKIVLMGHSTGSQCVLHYLSRPNPHT 139
A. oryze|XP_001826766.          97 NYIKEYKTEKFG-----------NGKIVLMGHSTGSQCVVHYLSRPNPHT 135
P. chryogenum|XP_002565941.     96 EYIKSYKGDKFG-----------GGKIALMGHSTGSQCVLHYLSQSNPHT 134
T. reesei|EGR44124.1|           99 RYIKSYKNAKYG-----------YSKLILMGHSTGSQCVLHYLSKPNPHV 137
A. nidulans|XP_663843.1|        97 NYIRDYKSGKFASTNNSGFKGHSNSKVVLMGHSTGSQCVIHYLSKPNPHT 146
G. destructans|ELR06329.1|      97 KYIKDYKTDKFG-----------DGKIVLMGHSSGSQFVMHYLYRPNPHT 135
B. fuckeliana|CCD56338.1|       96 AYFRTIK---------------SGKIILMGHSTGCQDVVEYLTGPG--- 126
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Fig. S4. Conserved putative lipase/esterase domain in fungal SidJ orthologs. Multiple alignment of *A. fumigatus* SidJ (XP_748659.1) with representative fungal orthologs. The pro site domain PS00120 is boxed with serine as active site in black. The overall sequence identity of these proteins is 64-76%.

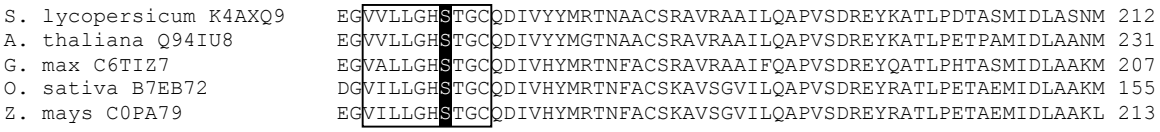

Fig. S5. Conserved putative lipase/esterase domains in plant proteins of DUF1749 family. Representative plant members of the DUF1749 family from the protein database (http://embl-ebi.org/interpro/). The pro site domain PS00120 is boxed with serine as active site in black.

Table S1 Primers used in this study. Introduced restriction sites are underlined.

primer	sequence 5' to 3'
oAfAH1-1	AAA CAG GCC TAA TGA CTG GTC CAG GCT C
oAfAH1-2	TAC TCT AGA CAC AAG ACC TAC CAC TCC
oAfAH1-3	CAT GGA TCC AGT GGC GAA AGA GGA TGC
oAfAH1-4	TCG GCG GCC GCG GTT CAA GCT TGT CTC GG
oAfAH1-5	AGT TAG CCG AGT CCA CAG
oAfAH1-6	TTT GAC TCA CTG CGC TGC
oAf_{538}AH1-f	CCA CAC AGT CTC CTC TTC
oAf_{538}AH1-r	TAC CGC TGT TTT CGT CCC