

Figure S1. Transcription of iron-regulated genes.

Quantitative real-time reverse transcription RT-qPCR. Samples were obtained from the wild-type strain grown at 37°C in liquid Aspergillus Minimal Medium (AMM) -Fe (20 h in iron depletion), sFe (20 h in iron depletion and then supplemented with 10 µM FeSO₄ for 1 h) and +Fe (20 h in 30 µM FeSO₄) (**A**); or grown at 37°C in AMM -Fe and sFe for 15, 30 and 60 min (**B**). Transcript levels of the indicated genes, normalized to *actA*, are expressed relative to those obtained in -Fe. Bars represent standard deviations from two independent biological experiments with two technical replicates each.

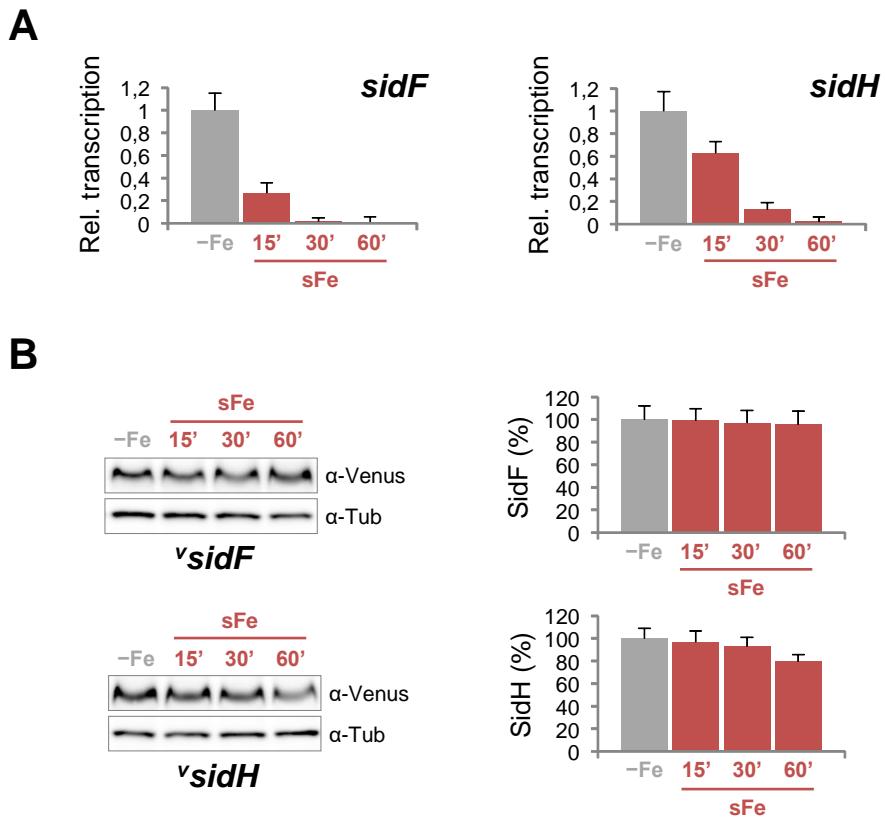


Figure S2. SidF and SidH are stable during sFe conditions.

A. Quantitative real-time reverse transcription RT-qPCR. Samples were obtained from the wild-type strain grown at 37°C in iron-depleted liquid AMM for 20 h (-Fe) and then supplemented with 10 µM FeSO₄ for 15, 30 and 60 min (sFe). Transcript levels of the indicated genes, normalized to *actA*, are expressed relative to those obtained in -Fe. **B.** Protein quantification by Western blot analysis. Samples were obtained from the indicated strains grown as in (A). Left panels: Representative Western blot analysis showing SidF and SidH protein levels. α-Tubulin was used as loading control. Right panels: Densitometric protein quantification. Protein levels were normalized to Tubulin and expressed relative to those in -Fe. Bars represent standard deviations from two independent biological experiments with two technical replicates each.

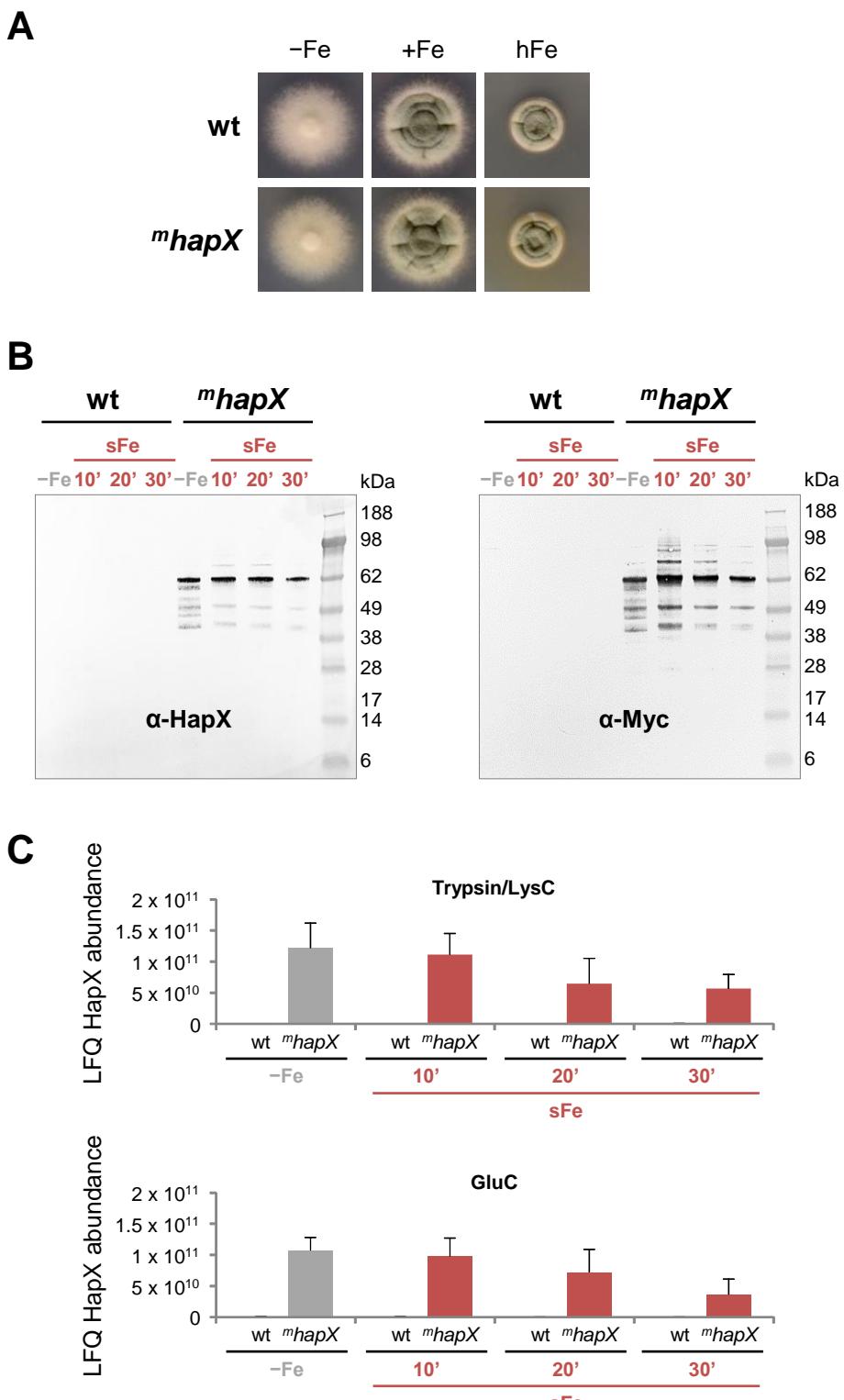
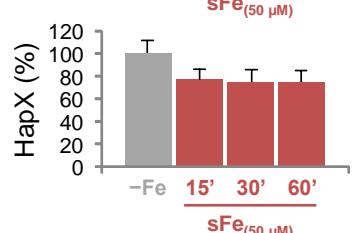
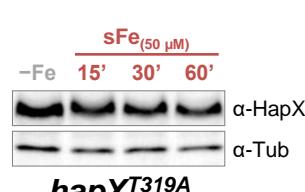
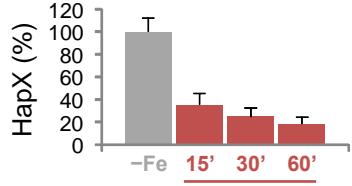
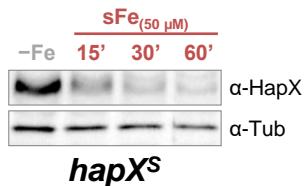
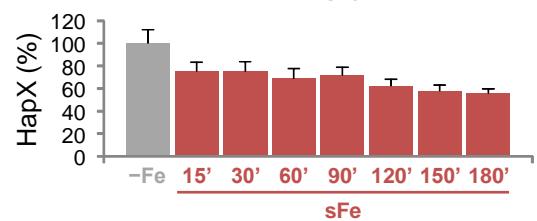
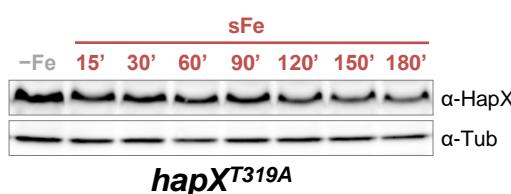
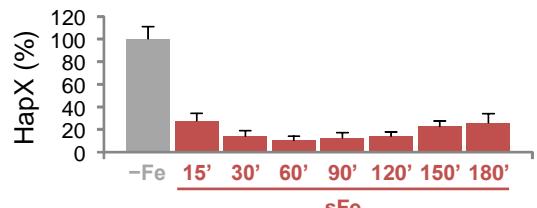
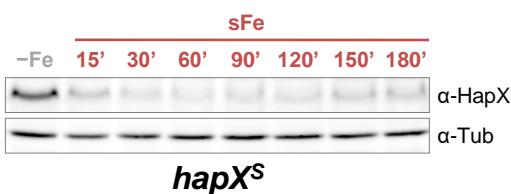


Figure S3. Effective enrichment of *M*HapX validated by Western blot and nLC-MS/MS analysis.

A. Growth of the indicated strains on solid AMM –Fe (iron depletion), +Fe (30 μ M FeSO₄), and hFe (10 mM FeSO₄). Plates were incubated for 2 d at 37°C **B-C.** Samples were obtained from the wild-type and *mhapX* strains grown at 37°C in AMM –Fe and then supplemented with 10 μ M FeSO₄ for 10, 20 and 30 min (sFe) and crude extracts were subjected to Myc-Trap affinity purification. Eluates were subjected to Western blot analysis showing HapX levels with α -HapX and α -Myc antibodies (**B**), or digested with Trypsin/LysC or GluC proteases for Label-Free Quantification (LFQ) abundances of HapX (**C**). Bars represent standard deviations from three independent biological experiments.

A**B****Figure S4. Quantification of HapX in *hapX^S* and *hapX^{T319A}* under different sFe conditions.**

A-B. HapX quantification by Western blot analysis. Samples were obtained from the indicated strains grown at 37°C in liquid AMM sFe (20 h in iron depletion and then supplemented with 50 μM FeSO₄ for 15, 30 and 60 min) (**A**); or grown at 37°C in AMM sFe (20 h in iron depletion and then supplemented with 10 μM FeSO₄ for the indicated time periods) (**B**). Left panels: Representative Western blot analysis showing HapX protein levels. α-Tubulin was used as loading control. Right panels: Densitometric protein quantification. HapX protein levels were normalized to Tubulin and expressed relative to those in -Fe. Bars represent standard deviations from two independent biological experiments with two technical replicates each.

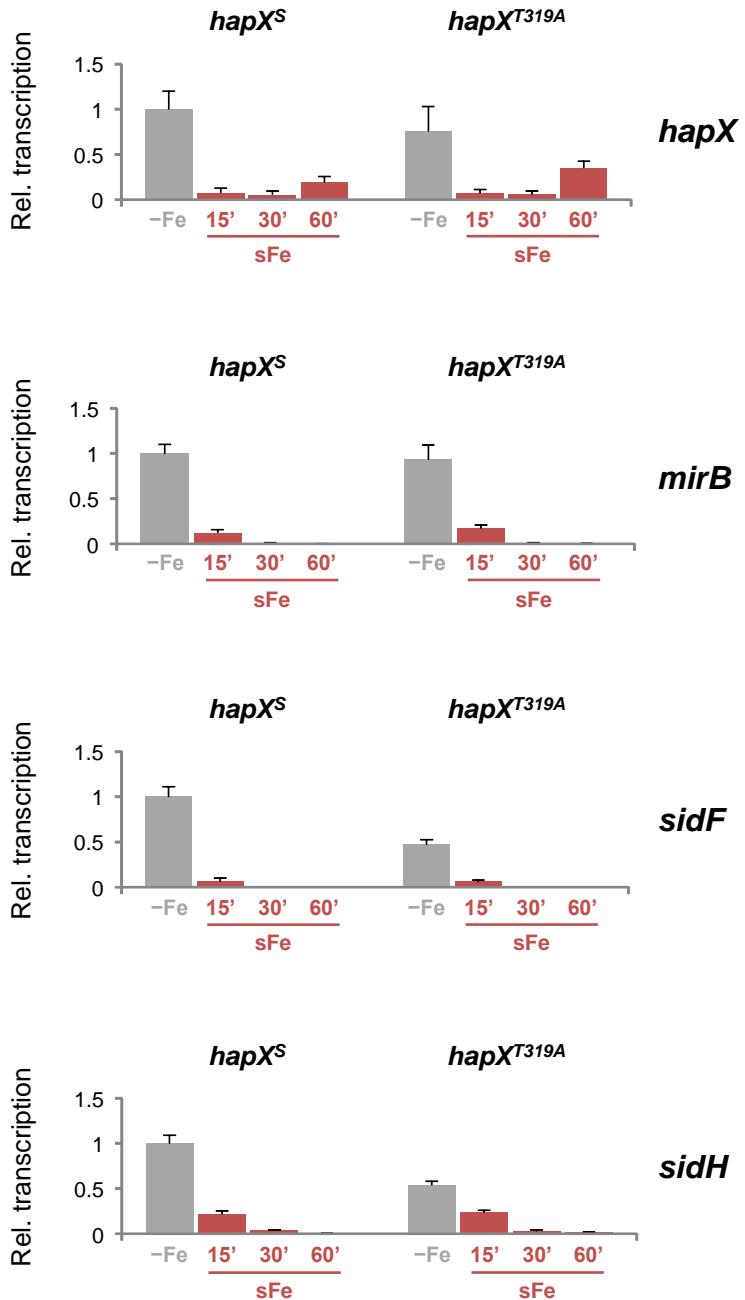


Figure S5. Transcription of iron-repressed genes.

Quantitative real-time reverse transcription RT-qPCR. Samples were obtained from the indicated strains grown as in Figure 2B. Transcript levels of the indicated genes, normalized to *actA*, are expressed relative to those obtained in -Fe. Bars represent standard deviations from two independent biological experiments with two technical replicates each.

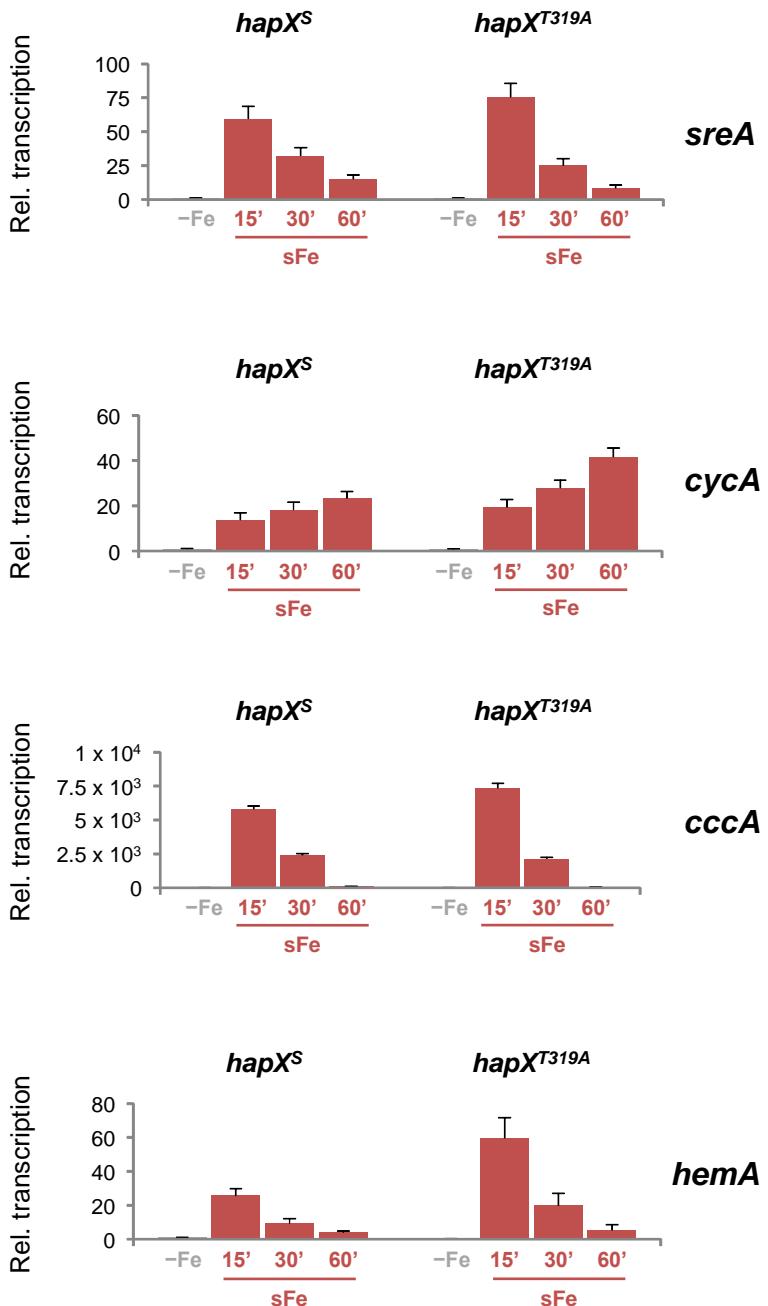


Figure S6. Transcription of iron-induced genes.

Quantitative real-time reverse transcription RT-qPCR. Samples were obtained from the indicated strains grown under sFe conditions as in Figure 2B. Transcript levels of the indicated genes, normalized to *actA*, are expressed relative to those obtained in -Fe. Bars represent standard deviations from two independent biological experiments with two technical replicates each.

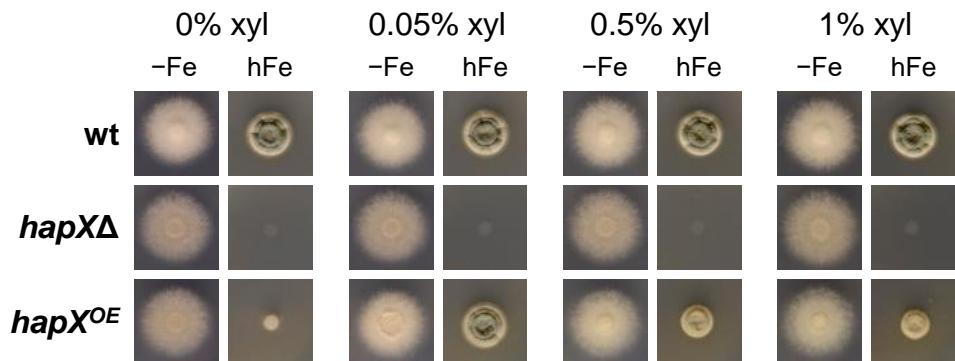


Figure S7. Effect of *hapX* overexpression on -Fe and hFe colony growth.

Growth of the indicated strains on solid AMM -Fe and hFe (10 mM FeSO₄) with the indicated concentration of xylose (%). Plates were incubated for 2 d at 37°C.

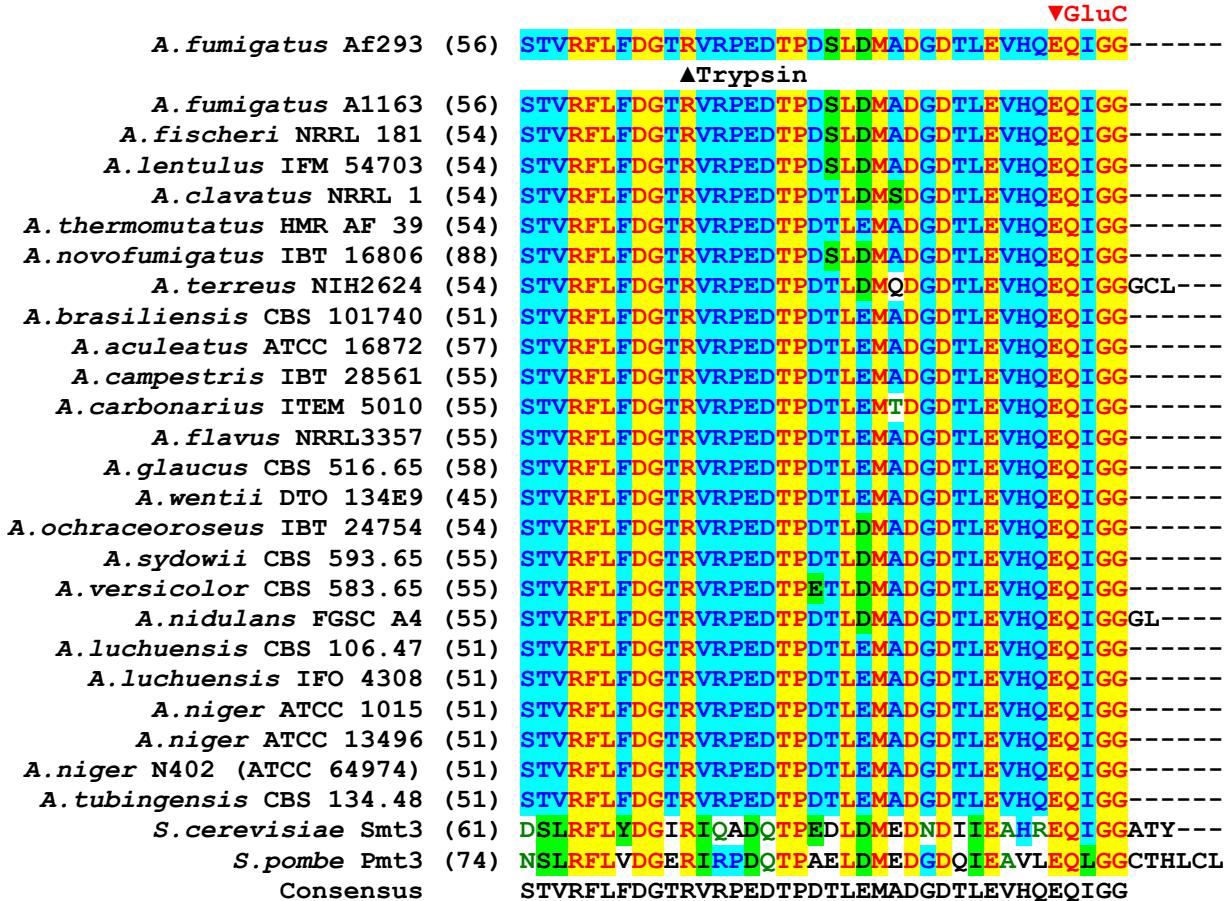


Figure S8. Amino acid sequence alignment of the C-terminal region of SumO orthologs from various *Aspergillus* species.

Sequences included represent all *A. fumigatus* SumO orthologs that were deposited in the publicly accessible database FungiDB (<https://fungidb.org>). Each sequence is preceded by its source organism and the strain ID. For comparison, SumO sequences from *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* are given. Identical residues are marked in yellow, residues conserved in 50% of the sequences are shaded in light blue and blocks of similar residues are marked in green. Red and black triangles mark the closest C-terminal GluC and trypsin P1 cleavage positions in *A. fumigatus* SumO, which after GluC digestion leaves a small residue of 4 amino acids (QIGG) on the modified lysine residue, while a large 27 amino acid residue signature (VRPEDTPDSDLMDADGDTLLEVHQEQQIGG) remains after trypsin digestion (not detectable in LC-MS/MS analysis). Alignments were performed with AlignX (Vector NTI Advance 11).

Table S1. Strains used in this study

Name	Genotype	Reference
wt (AfS77)	akuAΔ	[55]
^v sidF	Afs77; venus:sidF::hyg	This work
^v sidH	Afs77; venus:sidH::hyg	This work
^m hapX	Afs77; myc:hapX::hyg	This work
hapXΔ	Afs77; hapXΔ::ptrA	[16]
fbx22Δ	Afs77; fbx22Δ::hyg	This work
sumOΔ	Afs77; sumOΔ::hyg	This work
hapX ^s	Afs77; hapX:Stag::hyg	[16]
hapX ^{K161R}	Afs77; hapX ^{K161R} :Stag::hyg	This work
hapX ^{K242R}	Afs77; hapX ^{K242R} :Stag::hyg	This work
hapX ^{T319A}	Afs77; hapX ^{T319A} :Stag::hyg	This work
hapX ^{K161R-K242R}	Afs77; hapX ^{K161R-K242R} :Stag::hyg	This work
hapX ^{K161R-T319}	Afs77; hapX ^{K161R-T319A} :Stag::hyg	This work
hapX ^{K242R-T319A}	Afs77; hapX ^{K242R-T319A} :Stag::hyg	This work
hapX ^{OE}	Afs77; PxylP:hapX:Stag::hyg	This work

55. Hartmann, T.; Dumig, M.; Jaber, B. M.; Szewczyk, E.; Olbermann, P.; Morschhauser, J.; Krappmann, S., Validation of a self-excising marker in the human pathogen *Aspergillus fumigatus* by employing the beta-rec/six site-specific recombination system. *Appl Environ Microbiol* **2010**, 76, (18), 6313-7.

16. Gsaller, F.; Hortschansky, P.; Beattie, S. R.; Klammer, V.; Tuppatsch, K.; Lechner, B. E.; Rietzschel, N.; Werner, E. R.; Vogan, A. A.; Chung, D.; Muhlenhoff, U.; Kato, M.; Cramer, R. A.; Brakhage, A. A.; Haas, H., The Janus transcription factor HapX controls fungal adaptation to both iron starvation and iron excess. *The EMBO journal* **2014**, 33, (19), 2261-76.