

Supplementary Information

The Janus transcription factor HapX controls fungal adaptation to both iron starvation and iron excess

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Table S1. Biomass production, TAFC production and PpIX accumulation of mutant strains. Liquid cultures were grown under conditions of iron starvation (-Fe), iron sufficiency (+Fe, 0.03 mM FeSO₄) and high iron availability (hFe, 3 mM FeSO₄) at 37°C for 24 h. The data represent the mean ± SD from biological triplicates. Statistically significant differences compared to the wild type and *hapX^R*, respectively, are shown in red (two-tailed, unpaired T-test; p<0.05).

(A) Biomass production of $\Delta cccA$ and $\Delta hapX$. Compared strains are *A. fumigatus* ATCC 46645 derivatives.

Strain	Biomass production (g)		
	-Fe	+Fe	hFe
wt	0.28±0.015	0.65±0.021	0.64±0.030
$\Delta cccA$	0.28±0.016	0.64±0.021	0.42±0.026
$\Delta hapX$	0.13±0.010	0.62±0.028	0.24±0.019

(B) Biomass production, TAFC production and PpIX content of $\Delta hapX$, $\Delta hapX\Delta cccA$ and $hapX^{VENUS}$. Compared strains are *A. fumigatus* AfS77 derivatives.

Strain	Biomass production (g)			TAFC production (Abs _{435nm} /biomass)	PpIX accumulation (pmol/mg protein)
	-Fe	+Fe	hFe	-Fe	-Fe
wt	0.19±0.014	0.59±0.019	0.59±0.026	0.44±0.028	2031.3±223.39
$\Delta hapX$	0.11±0.013	0.57±0.030	0.19±0.020	0.19±0.016	11278.5±1323.96
$\Delta hapX\Delta cccA$	0.12±0.010	0.56±0.019	0.18±0.014	0.16±0.020	10966.1±394.46
Strain	Biomass production (g)				
	-Fe	+Fe	hFe		
wt	0.18±0.025	0.63±0.042	0.45±0.031		
$\Delta hapX$	0.11±0.012	0.62±0.027	0.29±0.018		
$hapX^{VENUS}$	0.19±0.024	0.61±0.015	0.47±0.034		

(C) Biomass and TAFC production of strains harboring cysteine-mutations within the HapX protein. Compared strains are *A. fumigatus* AfS77 derivatives.

Strain	Biomass production (g)			TAFC production (Abs _{435nm} /biomass)
	-Fe	+Fe	hFe	-Fe
$\Delta hapX$	0.09±0.014	0.60±0.031	0.21±0.015	0.13±0.008
$hapX^R$	0.20±0.019	0.61±0.040	0.61±0.042	0.50±0.036
$hapX^{C115A}$	0.09±0.007	0.58±0.007	0.21±0.010	0.10±0.018
$hapX^{C126A}$	0.17±0.019	0.56±0.020	0.61±0.029	0.43±0.040
$hapXA2^{C203A}$	0.20±0.002	0.58±0.030	0.36±0.016	0.54±0.034
$hapXB3^{C286A}$	0.20±0.016	0.59±0.036	0.29±0.013	0.50±0.026
$hapXC3^{C353A}$	0.21±0.014	0.54±0.024	0.57±0.011	0.54±0.036
$hapXD2^{C380A}$	0.20±0.018	0.57±0.028	0.63±0.024	0.55±0.015
$hapX^{C422A}$	0.19±0.013	0.52±0.032	0.62±0.013	0.54±0.045

(D) Biomass production, TAFC production and PpIX content of strains expressing C-terminal truncated HapX protein. Compared strains are *A. fumigatus* AfS77 derivatives.

Strain	Biomass production (g)			TAFC production (Abs _{435nm} /biomass)	PpIX accumulation (pmol/mg protein)
	-Fe	+Fe	hFe	-Fe	-Fe
wt	0.23±0.012	0.65±0.022	0.63±0.011	0.46±0.022	1122.3±120.56
$hapX^{464}$	0.13±0.005	0.65±0.037	0.65±0.011	0.16±0.018	8594.0±669.35
$hapX^{398}$	0.12±0.007	0.62±0.029	0.61±0.010	0.11±0.022	7763.1±424.41
$hapX^{374}$	0.11±0.001	0.66±0.032	0.64±0.013	0.09±0.016	7055.5±267.92
$hapX^{296}$	0.10±0.012	0.63±0.026	0.65±0.021	0.07±0.001	6761.4±228.61
$hapX^{158}$	0.15±0.009	0.59±0.031	0.18±0.002	0.14±0.005	5065.6±632.79
$\Delta hapX$	0.11±0.006	0.62±0.027	0.20±0.005	0.08±0.006	9831.9±735.56

Table S2. Proteases that are transcriptionally repressed by iron (Schrettl et al, 2008; Schrettl et al, 2010).

Gene	Protease
AFUA_2G01250	serine peptidase, family S28, putative
AFUA_3G05450	glutamate carboxypeptidase, putative
AFUA_3G11400	aspartic endopeptidase Pep2
AFUA_5G03990	aspartyl aminopeptidase
AFUA_5G04330	aminopeptidase, putative
AFUA_5G07330	carboxypeptidase S1, putative
AFUA_5G09210	alkaline serine protease Alp2
AFUA_6G13540	carboxypeptidase Y (CpyA), putative
AFUA_5G13300	aspartic endopeptidase Pep1
AFUA_8G00410	methionine aminopeptidase, type II, putative
AFUA_8G04730	oligopeptidase family protein

Table S3. Strains used in this study.

Strain	Genotype	Reference
wt, ATCC 46645, clinical isolate		American Type Culture Collection
$\Delta hapX$	ATCC 46645, $\Delta hapX::ptrA$	(Schrettl et al, 2010)
$\Delta hapXcccA^{oe}$	ATCC 46645, $\Delta hapX::ptrA$, $cccA^{oe}$, hph	This study
$\Delta cccA$	ATCC 46645, $\Delta cccA::ptrA$	(Gsaller et al, 2012)
$\Delta cccAcccA^{oe}$	ATCC 46645, $\Delta cccA::ptrA$, $cccA^{oe}$, hph	(Gsaller et al, 2012)
$\Delta sreA$	ATCC 46645, $\Delta sreA::hph$	(Schrettl et al, 2008)
wt, AfS77	ATCC 46645, $\Delta akuA::loxP$	(Hartmann et al, 2010)
$\Delta hapX$	AfS77, $\Delta hapX::ptrA$	This study
$\Delta hapX\Delta cccA$	AfS77, $\Delta hapX::ptrA$, $\Delta cccA::hph$	This study
Amino acid substitution		
$hapX^R$	AfS77, $\Delta hapX::ptrA$, $hapX^{S16G}$, hph	This study
$hapX^{C115A}$	AfS77, $\Delta hapX::ptrA$, $hapX^{C115A}$, hph	This study
$hapX^{C126A}$	AfS77, $\Delta hapX::ptrA$, $hapX^{C126A}$, hph	This study
$hapXA2^{C203A}$	AfS77, $\Delta hapX::ptrA$, $hapX^{C203A}$, hph	This study
$hapXA2^{C208A}$	AfS77, $\Delta hapX::ptrA$, $hapX^{C208A}$, hph	This study
$hapXBI^{C277A}$	AfS77, $\Delta hapX::ptrA$, $hapX^{C277A}$, hph	This study
$hapXBI^{C286A}$	AfS77, $\Delta hapX::ptrA$, $hapX^{C286A}$, hph	This study
$hapXC2^{C350A}$	AfS77, $\Delta hapX::ptrA$, $hapX^{C350A}$, hph	This study
$hapXC3^{C353A}$	AfS77, $\Delta hapX::ptrA$, $hapX^{C353A}$, hph	This study
$hapXD2^{C380A}$	AfS77, $\Delta hapX::ptrA$, $hapX^{C380A}$, hph	This study
$hapXD3^{C389A}$	AfS77, $\Delta hapX::ptrA$, $hapX^{C389A}$, hph	This study
$hapX^{C422A}$	AfS77, $\Delta hapX::ptrA$, $hapX^{C422A}$, hph	This study
Truncated HapX		
$hapX^{464}$	AfS77, $\Delta hapX::ptrA$, $hapX^{464}$, hph	This study
$hapX^{398}$	AfS77, $\Delta hapX::ptrA$, $hapX^{398}$, hph	This study
$hapX^{374}$	AfS77, $\Delta hapX::ptrA$, $hapX^{374}$, hph	This study
$hapX^{296}$	AfS77, $\Delta hapX::ptrA$, $hapX^{296}$, hph	This study
$hapX^{158}$	AfS77, $\Delta hapX::ptrA$, $hapX^{158}$, hph	This study
Venus tagged HapX		
$hapX^{VENUS}$	AfS77, $\Delta hapX::ptrA$, $hapX^{VENUS}$, hph	
A. nidulans strains		
wt, BPU1	<i>pyrG89; biA; wA3; argB2; pyroA4; pAR5 (argB); ArgB+</i>	(Tanaka et al, 2002)
$\Delta hapX$, BPU Δ X1	<i>pyrG89; biA; wA3; argB2; pyroA4; hapXΔ::argB; ArgB+</i>	(Tanaka et al, 2002)
F. oxysporum strains		
wt, <i>Fusarium oxysporum</i> f sp <i>lycopersici</i> race 2 - 4287		FGSC 9935
$\Delta hapX$, <i>Fusarium oxysporum</i> f sp <i>lycopersici</i> race 2 - 4287	$\Delta hapX::hph$	(Lopez-Berges et al, 2012)

Table S4. Oligonucleotides (5'→3') used in this study.**(A) Disruption of *hapX* and *cccA* coding sequence.*****hapX* disruption**

oAfhapX-1	AGCGACTATAGCCGGATG
oAfhapX-2	CCTTGGGTCTTGAAGCTTGCG
oAfhapX-3	TCATCTAGACTGCCCAAGCTTCATACC
oAfhapX-4	ATCAGAGCTGGAGAGGCA
oAfhapX-5	TGGAGTTCGGATTGGTGC
oAfhapX-6	ATCCCGCTTCTTTCACCC
oAoPtrA1	GAGGACCTGGACAAGTAC
oAoPtrA2	CATCGTGACCAGTGGTAC

***cccA* disruption**

oAfcccA1	CAACTCGGGCTCTGGGTG
oAfcccA5	CGGTGTCCTTGGTATTGC
oAfcccAr4	TGGGGCCTGGTTAGCGTC
oAfcccA3	TTGTAAGCTTGATCGACCATGGTTC
oAfcccAr6	AAGGATGCCGCCAATGTG
oAfcccAr2	GGGAGATCTCGCTGGCAC
ohph14	GAAGATGTTGGCGACCTC
ohph15	GAGAGCCTGACCTATTGC

(B) Generation of *phapX^R*-*hph*.

oAfhapX-S1	CGATACTTTGACCGACGGTTTGCCGACAAAGGAGCTGGTGCAGGCGCTG
oAfhapX-S2	CGCCAGCACATGGACAGCTAATTTATCGCATCTCTGCTTGTAGCATCACTC
oAfhapX-1	AGCGACTATAGCCGGATG
oAfhapX-S3	CGACGGTTTGCCGACAAA
oAfhapX-S4	TTTATCGCATCTCTGCTTGTAG
oAfhapX-7	CGCAAGCTTCAAGACCCAAGG
oAfhapX-8	GGGTGAAAGAAGCGGGAT
ohyg-1	CCATTAACCTAGGTACAG
ohyg-2	CTCCACTCGACCTGCAGG

(C) Site directed mutagenesis.

oAfHapX-C115A.f	TCGCGAGGTGGAGCAGGCGCGCTCCGAGATGACCTGGTG
oAfHapX-C115A.r	ACCAGGTCATCTCGGAGCGCGCTGCCACCTCGCGAG
oAfHapX-C126A.f	ACCTGGTGGCGCATCGTGCATGCGTTGGAGAAGGAG
oAfHapX-C126A.r	TCCTTCTCCAACGCATGCGCAGATCGCGCCACCAGGTC
oAfHapX-C203A.f	GTTAGGCTGCAATGATGCATCCACATCGCATTGCC
oAfHapX-C203A.r	GGCAATGCGATGTGGATGCATCATTGCAGCCTAAC
oAfHapX-C208A.f	ATTGCTCCACATCGCATGCGCAGTGCATTGAGGATGC
oAfHapX-C208A.r	GCATCCTCAATGCACTGCGCATGCGATGTGGAGCAAT
oAfHapX-C277A.f	CCTGCCGTGGATCCC GCCGGCTTCTGTTCCGATGGC
oAfHapX-C277A.r	GCCATCCGAACAGAAGCCGGCGGGATCCACGGCAGG
oAfHapX-C286A.f	TTCGGATGGCACGCCGGCCGTTTGC GCGGAGATG
oAfHapX-C286A.r	CATCTCCGCGCAAACGGCCGCGTGCATCCGAAC
oAfHapX-C350A.f	CAACGGGCCGGGCACAGCTGCGCAGTGTCTTGCAG
oAfHapX-C350A.r	CTGCAAGACACTGCGCAGCTGTGCCCGGCCGTTG
oAfHapX-C353A.f	CCGGGCACATGCGCGCAAGCGCTTGCAGATCCGCGGAGG
oAfHapX-C353A.r	CCTCCGCGGATCTGCAAGCGCTTGC GCGCATGTGCCCGG
oAfHapX-C380A.f	CTGCCCGTCCGGATGCGCAGGAGGTAAGGCGCGG
oAfHapX-C380A.r	CCGCGCCTTTACCTCCTGCGCATCCC GACGGGGCAG
oAfHapX-C389A.f	AAGGCGCGGATGGCGGGGCATGCCAGTCCGCTAACAC
oAfHapX-C389A.r	GTGTTACGCGACTGGCATGCCCCGCCATCCGCGCCTT
oAfHapX-C422A.f	CATCCTTGACTTTATCTGCAGCCGACGCCTACACGAC
oAfHapX-C422A.r	GTCGTGTAGGCGTCCGGCTGCAGATAAAGTCAAGGATG

(D) Generation of truncated *hapX*. (^{aa}, amino acids in length of truncated HapX protein)

hapXtrunc-f	GAATGCACAGGTACACTTG	HapX ^{aa}
hapX464r	TTAATTAATTCGAATCACCCACGATCGGTAAAGGG	HapX ⁴⁶⁴
hapX398r	TTAATTAATTCGAATCACGAAACATTGGTGTACGC	HapX ³⁹⁸
hapX374r	TTAATTAATTCGAATCAAGCGCTAGGGGAGCGGA	HapX ³⁷⁴
hapX296r	TTAATTAATTCGAATCATTCTCTGAGCGGCCAT	HapX ²⁹⁶
hapX158r	TTAATTAATTCGAATCATCTTACAGCCTCCCTGTC	HapX ¹⁵⁸

(E) Generation of Venus tagged HapX. (overlapping complementary sequences are in same color)

5'hapX-f	TGCCTAGTGAATGCTCCG
5'hapXvenus-r	CTCGCCCTTGCTGACCATGGTTACGGATGATGAGACT
5'hapXvenus-f	AGTCTCATCATCCGTAACCATGGTCAGCAAGGGCGAG
venus-r	GTTAATGCAGCTGGCACG
venushapX-f	TGTACAAGGGCGCCAGATCTATGTCTACACCTTCAATAGC
hapX3'-r	ATATCACGAGTCCGTTTGG
5'hapX-f2	ACAACCTCTCCTATGAGTCG
venushapX-r	GTAGACATAGATCTGGCGCCCTGTACAGCTCGTCCATG
hapXtrunc-f	GAATGCACAGGTACACTTG
hapX-r	TTACTTAATTCGAATCATTGTGCGCAAACCGTCCG

(F) Generation of DIG-labeled hybridization probes.

oAfccc1-1.f	GATTCCGACACCCTAGAC	<i>cccA</i>
oAfccc1-1.r	GCGATGATGTTGTCCCTG	
hemA-FG1	ATGACTAGTGGTGCACCTG	<i>hemA</i>
hemA-FG2r	CTAGGGTGATCTAGATGTTC	
oAfLeu1-f	GGTCTCACATACTTCGGC	<i>leuA</i>
oAfLeu1-r	TTCACGCACATCGGCAAG	
oAfhapX2-seq	TAATGTCCATCCAGATCGC	<i>hapX</i>
oHapX-seq2	CGAGTCCGTTTGGGTATC	
oAfmirB1me	AAGCCGAGAAAAAGGGGG	<i>mirB</i>
oAfmirB2me	AACCCAGATGAAGCCAG	
oAfAT1me	ACAATCAAGGCTCAGCCC	<i>sidG</i>
oAfAT2me	ACTTCGAGTCATGCTGGG	
oAfcytC1	CCCTTCTTGACAGTGTCC	<i>cycA</i>
oAfcytC2	CCGCGCATCTGCTTTTAC	

(G) Surface plasmon resonance analysis (CCAAT boxes are underlined).

BcccAp-369-37	Biotin-AAATCC <u>ACCAAT</u> CAACGCCTCCGTGATTGTGAGCAAT
cccAp-369-37	ATTGCTGACAATCACGGAGGCGTTG <u>ATTGGTGGATTT</u>
BcccAp-369-34	Biotin-AAATCC <u>ACCAAT</u> CAACGCCTCCGTGATTGTGAGC
cccAp-369-34	GCTGACAATCACGGAGGCGTTG <u>ATTGGTGGATTT</u>
BcccAp-369-32	Biotin-AAATCC <u>ACCAAT</u> CAACGCCTCCGTGATTGTCA
cccAp-369-32	TGACAATCACGGAGGCGTTG <u>ATTGGTGGATTT</u>
BcccAp-369-28	Biotin-AAATCC <u>ACCAAT</u> CAACGCCTCCGTGATT
cccAp-369-28	AATCACGGAGGCGTTG <u>ATTGGTGGATTT</u>

(H) ChIP analysis of *cccA* and *actA*.

cccA-ChIPf	CCTCTAGACACTTATAAGGATTTACATTGTGT
cccA-ChIPrev	CGGATTATCATAACACAGACCACCAG
actA-ChIPf	TGTTGTGTAGGAGGGAGAGAAGT
actA-ChIPrev	TTTCTTTTCTTGGACCAGTCGC

(I) qRT-PCR analysis of *hapX*, *sreA* and *actA*.

actA-qPCRf	CCACGTCACCACTTTCAACTC	<i>actA</i>
actA-qPCRR	CTGCATACGGTCGGAGATAC	
hapX-qPCRf	GCCTACACGACCTCTCACG	<i>hapX</i>
hapX-qPCRR	GCCCATCACACTCGCAGCC	
sreA-qPCRf	GGCAGTGCAGAAGAAGCGAAC	<i>sreA</i>
sreA-qPCRR	GGGGGAAGGATTGGCAGTTG	

CBC binding domain

AnHapX MA--AQPALAIAPSAAPLAPALVAKPTVSPSPGPGTSGSVTSKEWVIPP RP KPGRK PATD 58
AfuHapX ---MSTPSIAPAPAP--LVPALAAKPAISPSPGPGTSGSITSKEWVIPP RP KPGRK PATD 55
AoryHapX -MATPAPSIAPAPTAPLAPALAAKPTISPSPGPGTSGSVTSKEWVIPP RP KPGRK PATD 59
NfiHapX ---MSTPSIAPTAP--LVPALAAKPAISPSPGPGTSGSITSKEWVIPP RP KPGRK PATD 55
AflaHapX -MATPAPSIAPAPTAPLAPALAAKPTISPSPGPGTSGSVTSKEWVIPP RP KPGRK PATD 59
AnigHapX MATPAAPSLAPAPGPAPLAPALAAKPAISPSPGPGTSGSVTSKEWVIPP RP KPGRK PATD 60
AclaHapX ---MSAPSMAPTAPASLAPALAAKPAVSPSPGPGTSGSITTSKEWVIPP RP KPGRK PATD 57
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b (ZIP) Coiled coil 115

AnHapX TPPTKRKAQNRAAQRAFRRRAARVSELEDQIKCIEDDHEIHVATFKEQIANLSREVEQC 118
AfuHapX TPPTKRKAQNRAAQRAFRRRAARVNELEEQIKKIEDEHEIHIAAFKEQITNLSREVEQC 115
AoryHapX TPPTKRKAQNRAAQRAFRRRAARVNELEDQIKKIEEHDIHVAAFKEQISNLSHEVEQC 119
NfiHapX TPPTKRKAQNRAAQRAFRRRAARVNELEEQIKKIEDEHEIHIAAFKEQITNLSREVEQC 115
AflaHapX TPPTKRKAQNRAAQRAFRRRAARVNELEDQIKKIEEHDIHVAAFKEQISNLSHEVEQC 119
AnigHapX TPPTKRKAQNRAAQRAFRRRAARVNELEDQIKKIEDDHEIHVLQLKEQISNLSHEVEQC 120
AclaHapX TPPTKRKAQNRAAQRAFRRRAARVNELEDQIKKIEDEHDIHIAAFKEQIGNLSCVEVEQC 117
*****:*** **::** :**** ** *****

126 158

AnHapX RTEMGWWRDRCHALEKEVSVERAARETLVKELRSSLPEKNTSGTDAVPLP-----PRSSR 173
AfuHapX RSEMTWWRDRCHALEKEVSVERSAKEAIVKEFRSSLSDRNAVSDKGLAPLTTSTPQARS 175
AoryHapX RNEMTWWWRDRCHALEKEVSVERSAKEALVKEFRSSLSKKNAP---AGRAPLTRVSARNSG 176
NfiHapX RSEMTWWRDRCHALEKEVSVERSAKEAIVKEFRSSLSDRNAASDKGLLPLATST-QNRS 174
AflaHapX RNEMTWWWRDRCHALEKEVSVERSAKEALVKEFRSSLSKKNAP---AGRAPLTRVSARNSG 176
AnigHapX RSEMAWWRDRCHALEKEVSVERSAKESLVKEFRSSLSKKNASRSKAPLTR---IPRDS 177
AclaHapX RSEMTWWRDRCHTLEKEVSVERSAKEALVKEFRSLSDKNAASSDAVPFPRATSS----- 172
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CRR-A

AnHapX SSRMELEKSSPVDRRSELG-----EEVPLGNCNRCSTSHCQCIEDAF-GMPPIEMNRP 225
AfuHapX SDRPDN--GDASNDSGEG-----REEVPLGCNDCSTSHCQCIEDAF-TMPGVVAQEQS 226
AoryHapX SGRATNERSSPSNANSGSN---DDEQEVEPLGCPCSSSTHCQCIEDAF-AMPGVES-LHS 231
NfiHapX SERPDN--GDASNDSGEG-----REEVPLGCNDCSTSHCQCIEDAF-TMPGVVAQEQS 225
AflaHapX SGRATNERSSPSNANSGSN---DDEQEVEPLGCPCSSSTHCQCIEDAF-AMPGVES-LHS 231
AnigHapX AAHAAVENN-----MHEER-----EEVPLGCNCSNVHCQCIEDAFSGMPGIERTSAQ 225
AclaHapX --RPEND-GVPANNASHDDGHSDNREEVPLGCNDCSTSHCQCIEDAF-AMPGVLAQEE 228
: . ***** ** ***** ** :

CRR-B

AnHapX -----EPKIKPEPEEMEIDFTTRFAAPHHEETAASPVASPPVDPCGFCQDGT 273
AfuHapX RR-LDTTKPLGSEPEIKPDPEEMEIDFTSRFAATQ-QDQSPSTSVSSPAVDPCGFCSDGT 284
AoryHapX KR-LSTTGQGRAEPEIKPDPEEMEIDFTTRFAAPQ-PQEDNATAVSSPAVDPCGFCQDGT 289
NfiHapX RS-LDTTKPRSEPEIKPDPEEMEIDFTSRFAAIQ-QDHSPTSVSSPAVDPCGFCSDGT 283
AflaHapX KR-LSTTGQGRAEPEIKPDPEEMEIDFTTRFAAPQ-PQEDNATAVSSPAVDPCGFCQDGT 289
AnigHapX SKRPDSSQSHAPEPEIKPEPEEMEIDFTTRFAAPHVQDDTVTNVSSPAVDPCGFCQDGT 285
AclaHapX RR-PGSTKPRLEPEIKPDPEEMEIDFTARFATMK-PQDHSPTSVSSPAVDPCGFCQDGT 286
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AnHapX PCICAEMAAQEEERRNSTFESNRLAPIQNLISQFTPPPSDSDVRSNVTLPPIQATAAN 333
AfuHapX PCICAEMAAQEQRRPRNSFENNRLAPIQNLISQFTPPPSDGDVRS-DVTLPPISQAT--N 341
AoryHapX PCICAEMAAQEEQ--RRQSFENNRLAPIQNLISQFTPPPSDGDVRS-EVTLPSINQAT--N 344
NfiHapX PCICAEMAAQEQRRPRNSFENNRLAPIQNLISQFTPPPSDGDVRS-EVTLPPISQAT--N 340
AflaHapX PCICAEMAAQEEQ--RRQSFENNRLAPIQNLISQFTPPPSDGDVRS-EVTLPSINQAT--N 344
AnigHapX PCICAEMAAQEEERRNSTFENNRLAPIQNLISQFTPPPSDGDVRS-EVTLPPISQAT--N 342
AclaHapX PCICAEMAAQEEENERRNTFENNRLAPIQNLISQFTPPPSDGDVRS-EMTLPSLSQAT--N 343
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CRR-C 374 CRR-D 398

AnHapX PCANGPGTCAACLSDPRRTLFCCKTLAASRSASGTPSGCCGGKGRDGGCCQSQRSTSAPRR 393
AfuHapX PCANGPGTCAQCLADPRRTLFCCKTLAASRSPSAAPSGCCGGKGRDGGCCQS--RNTNVS 399
AoryHapX PCANGPGTCAQCLADPKSTLFCCKTLAASRSASVASSGCCGGKGRDGGCCQS--RSSNPPR 402
NfiHapX PCANGPGTCAQCLADPRRTLFCCKTLAASRSPSAAPSGCCGGKGRDGGCCQS--RNTNVS 398
AflaHapX PCANGPGTCAQCLADPKSTLFCCKTLAASRSASVASSGCCGGKGRDGGCCQS--RSSNPPR 402
AnigHapX PCANGPGTCAQCLADPRRTLFCCKTLAASRPASAAPSGCCGGKGRDGGCCMS--RNSNPQR 400
AclaHapX PCANGPGTCAQCLADPRSTLFCCKTLAASRSASGTPSGCCGGKGRDGGCCQS--RSSNPSR 401
***** **::* :*****.* :***** .**** * * : *

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                                422
AnHapX  -----SNTDRSATP-LTTLSCADAFTTLSRHPNFSRASDELASWLPKLHTLPNPRDVS 444
AfuHapX  GRSGSNNTSSGSSAAPSLTTLSCADAYTTLSRHPNFSRATDELSTWLPKLHTLPKPRDFP 459
AoryHapX  ---AAAAKSTSGRSTTPSLTTLSCADAFTTLSRHPNFSRASDEISTWLPKLHTLPNPKDVA 459
NfiHapX  GRSGSNNTSSGSSAAPSLTTLSCADAYTTLSRHPNFSRATDELSAWLPKLHTLPKPRDFP 458
AflaHapX  ---AAAAKSTSGRSTTPSLTTLSCADAFTTLSRHPNFSRASDEISTWLPKLHTLPNPKDVA 459
AnigHapX  -----SGSRRSATPSLTTLSCADAFTTLSRHPNFSRASDDISSWLPKLHTLPNPS-QS 451
AclaHapX  SGSTGNANSIPGPSTTPSLTTLSCADAFTTLSRHPSFTRATDDISNWLPKLHTLPNPRDIP 461
          : . *:* * *****:*****.*:*:*::: *****:* .

          464
AnHapX  QT---TPASRAAMEVEAASVMGVLRYFDRRFADK 475
AfuHapX  LTD--RVPRAALEVEAASVMGVLRYFDRRFADK 491
AoryHapX  SPD--RCSRAALEVEAASVMGVLRYFDRRFADK 491
NfiHapX  LND--RGVPRAAMEVEAASVMGVLRYFDRRFADK 490
AflaHapX  SPD--RCSRAALEVEAASVMGVLRYFDRRFADK 491
AnigHapX  QKEVMQPGSRAALEVEAASVMGVLRYFDRRFADK 485
AclaHapX  LSD--RGTBRAAMEVEAASVMGVLRYFDRRFADK 493
          .***:*****

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Figure S1. Alignment of *Aspergilli* HapX orthologs. The three single Cys 115, 126 and 422 as well as CRR-A, -B, -C, -D are conserved in the seven HapX orthologs. An, *A. nidulans*; Afu, *A. fumigatus*; Aory, *Aspergillus oryzae*; Nfi, *Neosartorya fischeri*; Afla, *Aspergillus flavus*; Anig, *Aspergillus niger*; Acla, *Aspergillus clavatus*.


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          CRR-D
AfuHapX SGCCGGKGDGGCCQSR-----NTNVSRRGRSG 403
AnHapX  SGCCGGKGRDGGCCQSQ-----SRTSAPRRSN 395
CaHap43 SSTRQISIRTNKTSIS-----IDSLNPNQSP 542
UmYap1  SPSRPGMSRSGTTGQLLPYPSRGETIPSAWRQIRSHPRFSQWGGDLLAEVVSKRSGN 631
      *      .      .      .

          422
AfuHapX SNNNTSSGS---SAAPSLTTLSCADAYTTLSRHPNFSRATDELSTWLPKLHTLP----KP 455
AnHapX  TDR-----SATP-LTTLSCADAFTTLSRHPNFSRASDELASWLPKLHTLP----NP 440
CaHap43 IPPPLLANN---KTGGSNAPTPTPAPSTPSHSSSVSSNSGIFIPCADAYKTLRHKKFNS 599
UmYap1  VHSPLLSSKRPREASVEIEPSRSNTISATSNDRATAKPLLHTTSDTTVHTVP-QIKTED 690
      .:      : : : : * .      :      .      :*: .      :

AfuHapX RDFPLTDRGVPRAALEVEEAASVMGVLRYFDRRFADK----- 491
AnHapX  RDVSQTTP-ASRAAMEVEEAASVMGVLRYFDRRFADK----- 475
CaHap43 VDFTTLVGKLTTRGMQVEVQSVANVLRRELDRRAYN----- 634
UmYap1  EDDGLDAREQKRRRILIDREAVQEALALLDAGTAAQPRAGASLSTNPGTGKEEQPCPCPW 750
      *      : : : * . * : *

AfuHapX -----
AnHapX  -----
CaHap43 -----
UmYap1  RPGERRTP 758

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Figure S2. Alignment of *A. fumigatus*, *A. nidulans*, *C. albicans* HapX orthologs and *U. maydis* Yap1.

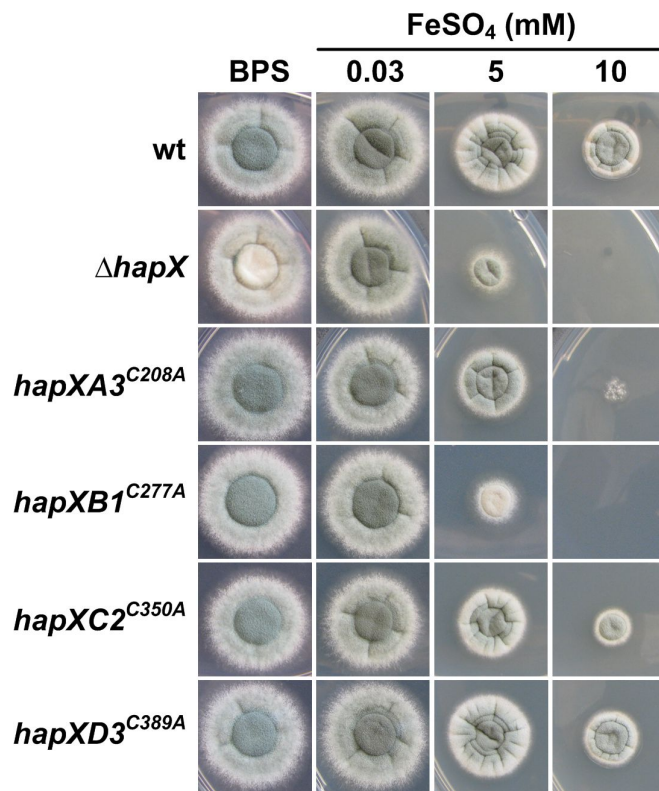


Figure S3. Cysteine-to-alanine mutations within the same CRR cause an identical phenotype (compare to Fig. 4). Strains were grown for 48h at 37°C on agar plates with the given iron concentration. Strains are derivatives of *A. fumigatus* AfS77.

Species	Strand	Start	p-value	Sites
<i>Trichophyton equinum</i>	-	489	2.76e-14	GCAGCCAATAGGCGGCCCTCTTATTGTAAGC
<i>Trichophyton tonsurans</i>	-	491	2.76e-14	GCAGCCAATAGGCGGCCCTCTTATTGTAAGC
<i>Trichophyton rubrum</i>	-	481	2.76e-14	GCAGCCAATAGGGGGCCCGCTTATTGTAAGC
<i>Trichophyton verrucosum</i>	-	580	6.32e-14	GCAGCCAATGGGGGGCCCGCTTATTGTAAGC
<i>Arthroderma benhamiae</i>	-	582	7.40e-14	GCAGCCAATGGGCTGCCCGCTTATTGTAAGC
<i>Paracoccidioides brasiliensis</i>	-	528	6.28e-13	GCAGCCAATCAGCACCGCTGGGAATGTCAGC
<i>Arthroderma otae</i>	-	455	7.14e-13	GCAGCCAATAGGAGGGCAGATGATTGTAAGC
<i>Aspergillus flavus</i>	-	370	1.04e-12	CCGGCCAATCACATCTCCGTTAATAGTCAGC
<i>Aspergillus oryzae</i>	-	369	1.04e-12	CCGGCCAATCACATCTCCGTTAATAGTCAGC
<i>Paracoccidioides lutzii</i>	-	523	3.77e-12	GCAGCCAATCAGCACCGTTGGGAATGTAAGC
<i>Ajellomyces dermatitidis</i>	-	369	3.77e-12	CCAGCCAATCAGCACAGCGATAAATGTAAGT
<i>Aspergillus kawachii</i>	-	317	3.77e-12	CCGGCCAATCAAGCCCGCTAATGTCAGC
<i>Arthroderma gypseum</i>	-	486	1.08e-11	CCAGCCAATAGAGGGTGCGCTGATTGTAATC
<i>Aspergillus niger</i>	-	315	1.08e-11	TGGGCCAATCACAAGCCCGCTAATGTCAGC
<i>Penicillium chrysogenum</i>	+	535	1.45e-11	GCAGCCAATCACATCACAGGCAAAGGTCAGC
<i>Aspergillus terreus</i>	-	307	2.12e-11	CGAGCCAATCAGCATTGCGGTAATGGTCAGC
<i>Talaromyces marneffeii</i>	-	441	3.07e-11	TCAGCCAATGGGAGAAACTGTAATGTAATC
<i>Uncinocarpus reesii</i>	-	434	4.37e-11	GCACCCAATCAAAGCAATGCTGATTGTAAGC
<i>Ajellomyces capsulatus</i>	-	346	7.28e-11	CCGGCCAATCAGCACGGCGGTAATGTAAGG
<i>Neosartorya fischeri</i>	-	532	1.09e-10	TCCACCAATCAACGCATCTGTGATTGTCAGC
<i>Penicillium digitatum</i>	+	609	1.18e-10	GCAGCCAATCGCATCAGCTGCAAAGGTCATC
<i>Aspergillus fumigatus</i>	-	365	1.49e-10	TCCACCAATCAACGCCTCCGTGATTGTCAGC
<i>Coccidioides posadasii</i>	-	415	2.17e-10	CAGGCCAATAGCGTCACAGCAAATAGTAAGC
<i>Coccidioides immitis</i>	-	417	2.17e-10	CAGGCCAATAGCGTCACAGCAAATAGTAAGC
<i>Talaromyces stipitatus</i>	-	467	2.89e-10	TTAGCCAATGAAAACCGTTATAAATGTAAGC
<i>Fusarium oxysporum</i>	-	281	3.11e-10	CCAGCCAATCACGAAGCATGTTTTAGTAATC
<i>Aspergillus clavatus</i>	-	382	3.11e-10	CTGGCCAATCAGCGAGTGCATAAATGTCAGC
<i>Aspergillus nidulans</i>	-	475	6.50e-08	CGAGCCAATCAGAGCGAAGTGATTACCAACC

Figure S4. Identification of an evolutionary conserved motif in the *cccA* promoter regions of 28 fungal species. Sites identified by MEME analysis and their relative positions to the ATG start codon of the *CccA* coding sequences are shown.

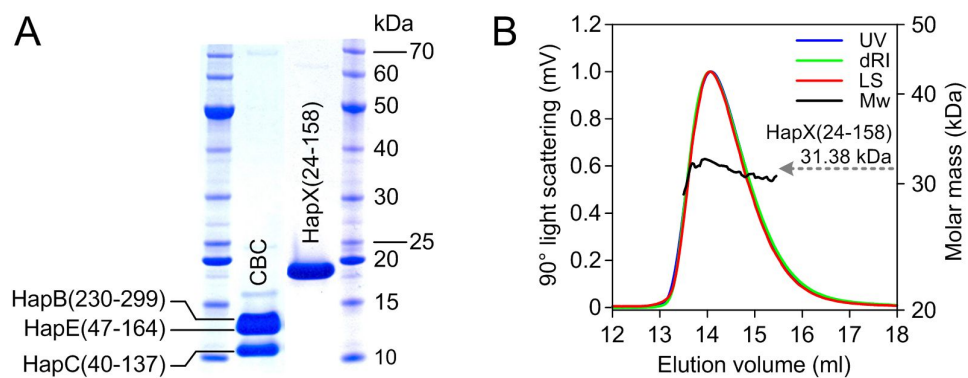


Figure S5. Characterization of purified recombinant proteins used in this study. (A) SDS-PAGE analysis of the CBC and HapX(24-158). (B) Analysis of the solution oligomeric state of HapX(24-158) by size exclusion chromatography and multiangle static light scattering. The light scattering signal (LS) is shown overlaid with the calculated molar mass (Mw) across the elution profile monitored by the absorbance at 280 nm (UV) and changes of the refractive index (dRI).

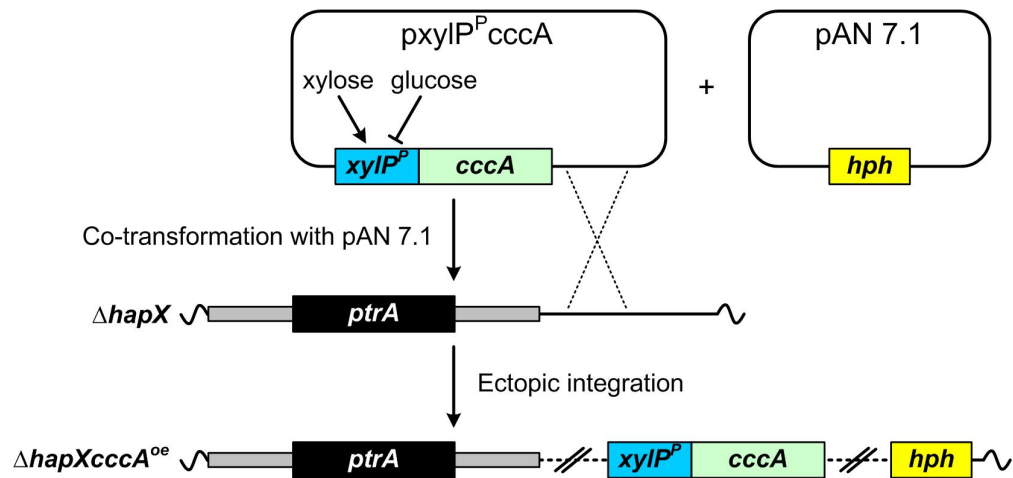


Figure S6. Ectopic integration of inducible *cccA*. $p_{xyIP^P} cccA$ was co-transformed with $pAN 7.1$, a plasmid containing a hygromycin resistance cassette (*hph*) for selection of transformants.

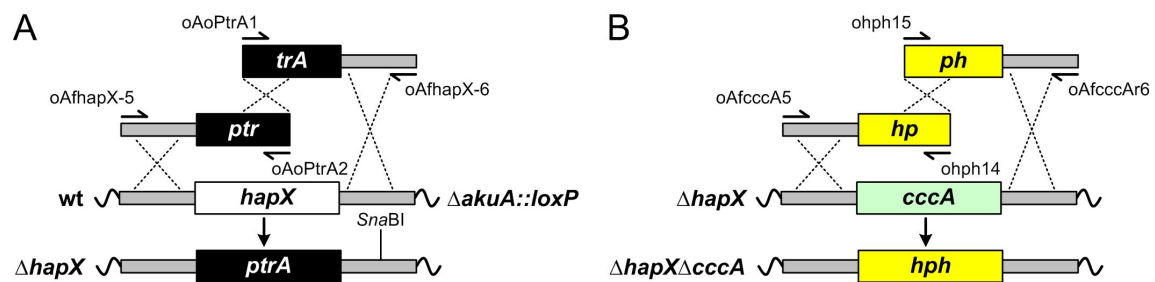


Figure S7. Scheme of *hapX* (A) and *cccA* (B) disruption using the bipartite technique.

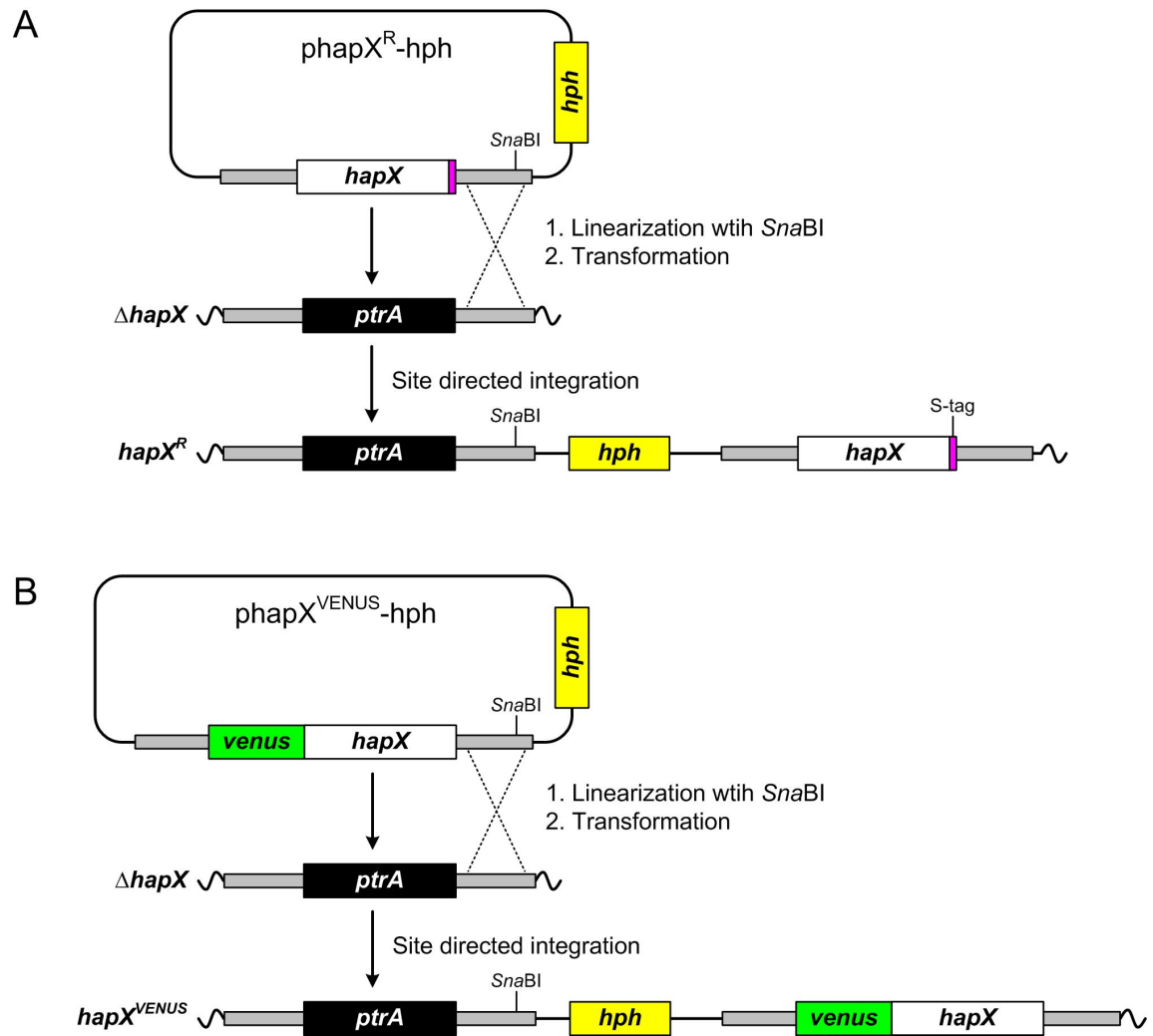


Figure S8. Scheme of site-directed integration of *hapX* plasmids. (A) Site-specific integration is illustrated for plasmid phapX^R-hph, which was transformed into Δ *hapX*. The same transformation strategy was performed with plasmids of modified *hapX* (mutations and truncations). (B) Site-directed integration of N-terminally *venus*-tagged *hapX*.