Supplemental Table 1 – *Aspergillus fumigatus* strains used in this study

Strain	Description	Purpose	Reference
akuBKU80	*akuBKU80::pyrG,* in CEA17	Parental strain	*Da Silva Ferreira *et al.* 2006
AfchoAΔ::HPH	*akuBKU80::pyrG AfchoAΔ::HPH*	Deletion of *AfCHOA*	This study
AfbadAΔ::HPH	*akuBKU80::pyrG AfbadAΔ::HPH*	Deletion of *AfBADA*	This study
AfchoAΔAfbadAΔ::HPH	*akuBKU80::pyrG AfchoAΔAfbadAΔ::HPH*	Double deletion of *AfCHOA* and *AfBADA*	This study
Dal::eGFP::promoter::RFP	*Dal::eGFP::promoter::RFP*	Transcriptional fusion	This study
AfchoAΔAfbadAΔ:: AfCHOA-eGFP + AfBADA-RFP	*AfchoAΔAfbadAΔ::*eGFP*::AfCHOA*::promoter::*AfBADA*::RFP	Revertant strain and translational fusion	This study

***da Silva Ferreira ME, Kress MR, Savoldi M, Goldman MH, Hartl A, Heinekamp T, Brakhage AA, Goldman GH.** 2006. The akuB(KU80) mutant deficient for nonhomologous end joining is a powerful tool for analyzing pathogenicity in *Aspergillus fumigatus*. Eukaryot Cell **5:**207-211.

Supplementary Table 2. Culture media and various stresses tested on the viability of mutants

rich complex media	
Sabouraud medium.	2% glucose 1% peptone 2 % agar pH 5.6
Malt medium	2 % malt extract 2 % agar
YPD medium	1% *yeast* extract 2% peptone 2% dextrose 2% agar
YE medium	1% *yeast* extract 2% agar

synthetic media	
RPMI medium	see RPMI-1640 Media Formulation (sigma-aldrich)
Minimal medium (MM)	1 % glucose 0.92 g/L ammonium tartrate 0.5 g KCl, 0.5 g MgSO4, 7H2O and 2 g KH2PO4 1 mL of trace element solution at pH 6.5 2% agar
MM w/o nitrogen	1 % glucose 0.5 g KCl, 0.5 g MgSO4, 7H2O and 2 g KH2PO4 1 mL of trace element solution at pH 6.5 2% agar
MM w/o carbon	0.92 g/L ammonium tartrate 0.5 g KCl, 0.5 g MgSO4, 7H2O and 2 g KH2PO4 1 mL of trace element solution at pH 6.5 2% agar
MM w/o nitrogen and carbon	0.5 g KCl, 0.5 g MgSO4, 7H2O and 2 g KH2PO4 1 mL of trace element solution at pH 6.5 2% agar

stresses tested on the viability of mutants	
osmotic stresses	solid MM (or solid rich medium) + 1 - 2.5 M NaCl solid MM (or solid rich medium)+ 1.2 M Mannitol solid MM (or solid rich medium)+ 1,2 M Sorbitol
oxidative stresses	solid MM (or solid rich medium) + 1 -5 mM H_2O_2 solid MM (or solid rich medium) + 5 -80 µM ménadione
pH stresses	solid MM at pH 2 (MES buffer) solid MM at pH 9 (Tris buffer)
Thermic stresses	solid MM (or rich medium) at 4°C solid MM (or rich medium) at 50°C
freezing shock	conidial viability after 24h at -20°C and growth on MM
heat shock	conidial viability after 5min at 75°C and growth on MM

Supplemental Table 3. List of oligonucleotides using during this study.

Primers	Sequence (5'-3')	Purpose in this study	PCR
55-*CHOA*	CACAGCTACTCCTGAGTACGACTTCCTCCA	*AfCHOA disruption cassette*	PCR1
53-*CHOA*	CTCGTGAATCTTTTACCAGATCGGAAGCAATTCCAGCACCTCTGAAGATTGGTAAGTGAG	*AfCHOA disruption cassette*	PCR1
5-*CHOA-HPH*	CTCACTTACCAATCTTCAGAGGTGCTGGAATTGCTTCCGATCTGGTAAAAGATTCACGAG	*AfCHOA disruption cassette*	PCR3
3-*CHOA-HPH*	AATAAGCCAGCATCTGTACACAAGAAGCCAATCAGAGCAGATTGTACTGAGAGTGCACCA	*AfCHOA disruption cassette*	PCR3
35-*CHOA*	TGGTGCACTCTCAGTACAATCTGCTCTGATTGGCTTCTTGTGTACAGATGCTGGCTTATT	*AfCHOA disruption cassette*	PCR2
33-*CHOA*	ATACCTTCCAGTAAACCTCTGGGGTTGAGC	*AfCHOA disruption cassette*	PCR2
55-*BADA*	CACAGCTTCATGTACAAGCACTTTTTCTCG	*AfBADA disruption cassette*	
53-*BADA*	TCGTGAATCTTTTACCAGATCGGAAGCAATTGAATGTGTACAACTTATCCGCCAAAGGAG	*AfBADA disruption cassette*	
5-*BADA-HPH*	CTCCTTTGGCGGATAAGTTGTACACATTCAATTGCTTCCGATCTGGTAAAAGATTCACGA	*AfBADA disruption cassette*	
3-*BADA-HPH*	TACCTCTCTAGTACTCTGACCGACCTGCCCATCAGAGCAGATTGTACTGAGAGTGCACCA	*AfBADA disruption cassette*	
35-*BADA*	TGGTGCACTCTCAGTACAATCTGCTCTGATGGGCAGGTCGGTCAGAGTACTAGAGAGGTA	*AfBADA disruption cassette*	
33-*BADA*	ATGGCTAATCCACCGCACCTAAGAGGTAAT	*AfBADA disruption cassette*	
55-*CHOABADA*	CACAGCTTCATGTACAAGCACTTTTTCTCG	*AfCHOA and AfBADA disruption cassette*	
53-*CHOABADA*	TCGTGAATCTTTTACCAGATCGGAAGCAATTGAATGTGTACAACTTATCCGCCAAAGGAG	*AfCHOA and AfBADA disruption cassette*	
5-*CHOABADA-HPH*	CTCCTTTGGCGGATAAGTTGTACACATTCAATTGCTTCCGATCTGGTAAAAGATTCACGA	*AfCHOA and AfBADA disruption cassette*	
3-*CHOABADA-HPH*	TTATGGATTATTTATCATGGGCGAAGACGGATCAGAGCAGATTGTACTGAGAGTGCACCA	*AfCHOA and AfBBADA disruption cassette*	
35-*CHOABADA*	TGGTGCACTCTCAGTACAATCTGCTCTGATCCGTCTTCGCCCATGATAAATAATCCATAA	*AfCHOA and AfBADA disruption cassette*	
33-*CHOABADA*	GGAAGCTCCAAGTCAGTCAAAATGATCACA	*AfCHOA and AfBADA disruption cassette*	
CHOA-KL1-F	GGCTGTGTCATTGCTAGTCG	RT-PCR	
CHOA-KL1-R	AGCCTGCACCCAGTCCTT	RT-PCR	
BADA-KL2-F	CCACTAGCGGAAATCCATGT	RT-PCR	
BADA-KL2-R	TGAAATCTACCGGGAAGCTG	RT-PCR	
EF1a-F	CCATGTGTGTCGAGTCCTTC	RT-PCR	
EF1a-R	GAACGTACAGCAACAGTCTGG	RT-PCR	
NdeI-PCR1-transcript-F	GGGAATTCCATATGTTACTTGTACAGCTCGTCCATGCCGAG	Transcriptional fusion	PCR1
PCR1-transcript-R	AATCATCATAACATTCCATTCTATCACTATCATTCGACATGGTGAGCAAGGGCGAGGAGCT	Transcriptional fusion	PCR1
PCR2-transcript-F	AGCTCCTCGCCCTTGCTCACCATGTCGAATGATAGTGATAGAATGGAATGTTATGATGATT	Transcriptional fusion	PCR2
PCR2-transcript-R	CTTAATCAGCTCTTCGCCCTTAGACACCATTCTCTTTCAGCTTGAAGGGGCCCC	Transcriptional fusion	PCR2
PCR3-transcript-F	GGGGCCCCTTCAAGCTGAAAGAGAATGGTGTCTAAGGGCGAAGAGCTGATTAAG	Transcriptional fusion	PCR3
PCR3-transcript-NdeI-R	GGGAATTCCATATGTTACTTGTACAGCTCGTCCATGCCATTAAGTT	Transcriptional fusion	PCR3
NdeI-PCR1-translational-F	GGGAATTCCATATGTTACTTGTACAGCTCGTCCATGCCGAG	Translational fusion	PCR1

PCR1-translational-R	GGACTCGGGAACAGCCCAAGCTGATGGTGAGCAAGGGCGAGGAGCT	Translational fusion	PCR1
PCR2-translational-F	AGCTCCTCGCCCTTGCTCACCATCAGCTTGGGCTGTTCCCGAGTCC	Translational fusion	PCR2
PCR2-translational-R	CTTAATCAGCTCTTCGCCCTTAGACACCATCAATTTTGCAAAGACAGTAGCAACCCGT	Translational fusion	PCR2
PCR3-translational-F	ACGGGTTGCTACTGTCTTTGCAAAATTGATGGTGTCTAAGGGCGAAGAGCTGATTAAG	Translational fusion	PCR3
PCR3-translational-NdeI-R	GGGAATTCCATATGTTACTTGTACAGCTCGTCCATGCCATTAAGTT	Translational fusion	PCR3
NdeI-ATG-*AfCHOA*	GGGAATTCCATATGGCCACCACAAACGATTTCC	Production of recombinant *Af*ChoAp	
AfCHOA-stop-BamHI	CGCGGATCCCTACAGCTTGGGCTGTTCCCG	Production of recombinant *Af*ChoAp	
NdeI-ATG-*AfBADA*	GGGAATTCCATATGGCGGACCTACCTTTCGCTC	Production of recombinant *Af*BadAp	
AfBADA-stop-BamHI	CGCGGATCCCTACAATTTTGCAAAGACAGTAGCAACCC	Production of recombinant *Af*BadAp	
CHOA forward	GCTATGACGCCGCAACCATC	Expression in vivo	
CHOA reverse	CACGCTTAATCCAATCCTTGAAC	Expression in vivo	
BADA forward	CTTCGGACCCTTGAGTTCTGC	Expression in vivo	
BADA reverse	GCCATAGAGGAGCGTTGCC	Expression in vivo	
GEL4 forward	ATACGCCACCGAGCAGGAC	Expression in vivo	
GEL4 reverse	GGAAGAATCACCGCACCACTC	Expression in vivo	
GAPDH forward	CCACTCACGGCAAATTCAAC	Expression in vivo	
GAPDH reverse	GTAGACTCCACGACATACTCA	Expression in vivo	
TPS1 forward	CACCCAAAACGAGGCGAGAC	Expression of trehalose metabolism enzymes	
TPS1 reverse	GCCACTAAGACCGCTGACC	Expression of trehalose metabolism enzymes	
TPS2 forward	ACCAATGCCTCAAATGCCACTC	Expression of trehalose metabolism enzymes	
TPS2reverse	GGTAGAGCGGTATCGGAGACG	Expression of trehalose metabolism enzymes	
TPS3forward	TGACGCCAGCACCCAATG	Expression of trehalose metabolism enzymes	
TPS3reverse	CAGCCTTTGTGATGTCCGATGTG	Expression of trehalose metabolism enzymes	
TSL1forward	CGCCAGAGACGCCACAATAAG	Expression of trehalose metabolism enzymes	
TSL1reverse	CCGCTGACCGAGAGACATACG	Expression of trehalose metabolism enzymes	
CLOCK9forward	CGGATGTGTTGGACGGGATTAAAC	Expression of trehalose metabolism enzymes	
CLOCK9reverse	TGGCACCACTTATTGAACAGTCG	Expression of trehalose metabolism enzymes	
M1PDHforward	GAAGAGGGCGAGAAAACCAAGAC	Expression of mannitol metabolism enzymes	
M1PDHreverse	CGACGGCACAAGTGACAACG	Expression of mannitol metabolism enzymes	
M2DHforward	GGGTAAACGACTACGCCATTTGC	Expression of mannitol metabolism enzymes	
M2DHreverse	AGGAGTTGATGCTGCCGATGAC	Expression of mannitol metabolism enzymes	

Supplementary figures

Fig S1. (A) Purification of recombinant *Af*CodAp and *Af*BadAp heterologously expressed in *E. coli* Rosetta (DE3)pLysS as analyzed by SDS-PAGE . Lane 1: cell free extract. Lane 2: after 65% ammonium sulfate precipitation and dialysis. Lane 3: after DEAE-Sepharose column chromatography. Lane 4: purified choline oxidase. Lane 5: Molecular mass markers: choline oxidase from *A. globiformis* (60 kDa) and nitronate monooxygenase from *Neurospora crassa* (40 kDa). Lane 6; BADA cell free extract. Lane 7 purified BADA. (B) UV-visible absorbance spectra of choline oxidase in 100 mM imidazole, pH 7.0, containing 10% glycerol. Oxidized enzyme (thick line) reduced enzyme (dotted line) after anaerobic substrate reduction with choline. (C) . Determination of betaine aldehyde produced during catalytic turnover of *Af*ChoAp with choline as substrate. *Af*ChoAp at a concentration of 0.5 µM was incubated with saturating choline (50 mM) and 1.1 mM oxygen in 20 mM Tris-Cl at 25 °C. At the indicated times, aliquots of the reaction mixture were withdrawn and the concentration of betaine aldehyde was determined colorimetrically using 2,4-dinitrophenylhydrazine as dye. (●) Expermiental data; (○) expected values based on the kinetic parameters of the enzyme if all the betaine aldehyde produced under the experimental conditions used accumulates in the reaction mixture without being further oxidized to GB.

Fig. S2. A. Deletion of *AfCHOA* gene by targeted gene replacement using the *HPH* gene. Restriction maps of genomic fragments containing the *AfCHOA* wild-type (A1), the *AfchoA*Δ*::HPH* deletion allele (A2). The black boxes indicate the *AfCHOA* 5'- and 3'-flanking sequences used for homologous recombination. (A3) Southern hybridization of *AfCHOA* wild-type strain (*akuB$_{ku}$*80) and deletion mutant strain (*AfchoA*Δ*::HPH*). Genomic DNA of each strain was digested with *Eco*RI or *Nco*I and hybridized and probed with RBDNA fragments. B. Deletion of *AfBADA* gene by targeted gene replacement using the *HPH* gene. Restriction maps of genomic fragments containing the *AfBADA* wild-type (B1), the *AfbadA*Δ*::HPH* deletion allele (B2). Southern hybridization of *AfBADA* wild-type strain (*akuB$_{ku}$*80) and deletion mutant strain (*AfbadA*Δ*::HPH*). Genomic DNA of each strain was digested with *Nco*I or *Hind*III hybridized and probed with LB DNA fragments (B3). C. Deletion of *AfCHOA* and *AfBADA* gene cluster by targeted gene replacement using the *HPH* gene. Restriction maps of genomic fragments containing the *AfCHOA* and *AfBADA* wild-type (C1), the *AfchoA*Δ*AfbadA*Δ*::HPH* deletion allele (C2). The black boxes indicate the *AfCHOA* and *AfBADA* cluster 5'- and 3'-flanking sequences used for homologous recombination. (C3) Southern hybridization of *AfCHOA* and *AfBADA* wild-type strain (*akuB$_{ku}$*80) and deletion mutant strain (*AfchoA*Δ*AfbadA*Δ*::HPH*). Genomic DNA of each strain was digested with *Nco*I or *Eco*RI hybridized and probed with LB and RB DNA fragments, respectively. (C4) Construction of translational fusion proteins. Translational fusion was performed by fusion of three PCR fragments: the PCR1 fragment encoding for eGFP, the PCR2 fragment containing the *AfCHOA* ORF, the intergenic region (1.650 kb) of the *AfCHOA* and *AfBADA* genes and the *AfBADA* ORF and the PCR3 fragment encoding for RFP-T. This construction was used for the complementation of the double mutant *AfchoA*Δ*AfbadA*Δ*::HPH.* LB (Left border flanking 5' sequences of the target gene), RB (Right border flanking 3' sequences of the target gene) used for homologous recombination.

Fig. S3. A. Use of choline as a carbon source. The *akuB*$_{ku80}$ parental and the *AfchoA*Δ*::HPH*, *AfbadA*Δ::*HPH* and *AfchoA*Δ*AfbadA*Δ::*HPH* mutants were grown on agar plates with MM without carbon source, MM with choline chloride (100 mM) or with GB (100 mM) as sole carbon source. B. Use of choline as both carbon and nitrogen sources.

Fig.S4. ^{1}H, ^{13}C-HSQC (upper spectrum; CH_1 and CH_3 peaks plotted in blue, CH_2 peaks plotted in red) and ^{1}H, ^{13}C-HMBC (lower spectrum) of the intracellular fraction from *A. fumigatus akuB*$_{ku80}$ strain cultured in MM (right panel) and that from *A. fumigatus AfchoA*Δ*::HPH* strain (left panel). GB signals are circled in green. Two signals (δ_H/δ_C=3.219/56.06 for NCH_3 and 3.857/68.83 ppm for CH_2) in the ^{1}H,^{13}C-HSQC spectrum and ^{1}H-^{13}C long range correlations between NCH_3 proton and CH_2 carbon (δ_H/δ_C=3.219/68.83 ppm), CH_2 proton and NCH_3 carbon (δ_H/δ_C=3.857/56.06 ppm), and CH_2 proton and CO carbon (δ_H/δ_C=3.857/171.97 ppm) in the ^{1}H,^{13}C-HMBC spectrum were identified in the intracellular fraction of *A. fumigatus akuB*$_{ku80}$ strain corresponding to those of the GB standard, but not in that of *A. fumigatus AfchoA*Δ*::HPH* strain.

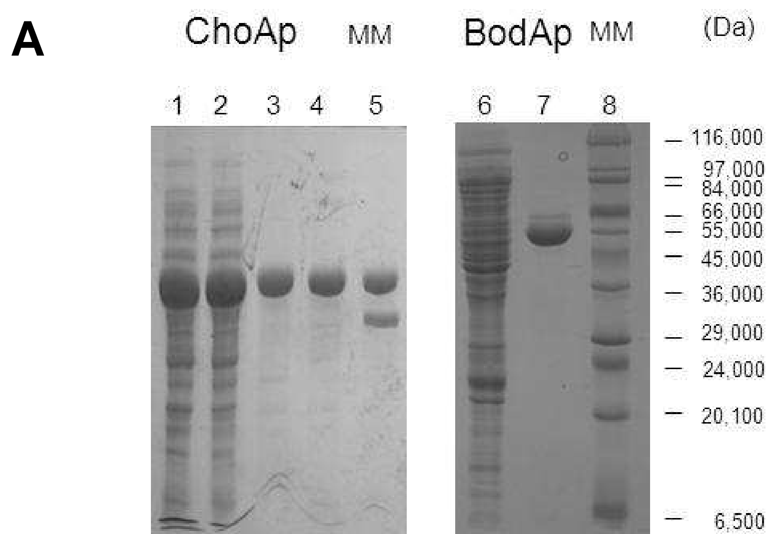
Fig. S5. Analysis of *akuBku80*; *ΔchoA*, *ΔbadA* and *ΔchoA/ΔbadA* strains under an osmotic shock .(A) Note the absence of growth differences between the parental strain and the mutants when grown in MM medium containing 0, 0.6 or 1.2 M of NaCl (B). RT-PCR data showing the lack of difference in the expression of *BADA* and *CHOA* genes in mycelium of *akuBku80*; *ΔbadA*, ΔchoA and *ΔchoA/ΔbadA* strains grown for 16 hrs in Sabouraud medium with 0 or 0.6 M NaCl. Gel4 gene was used as a control of constitutive expression.

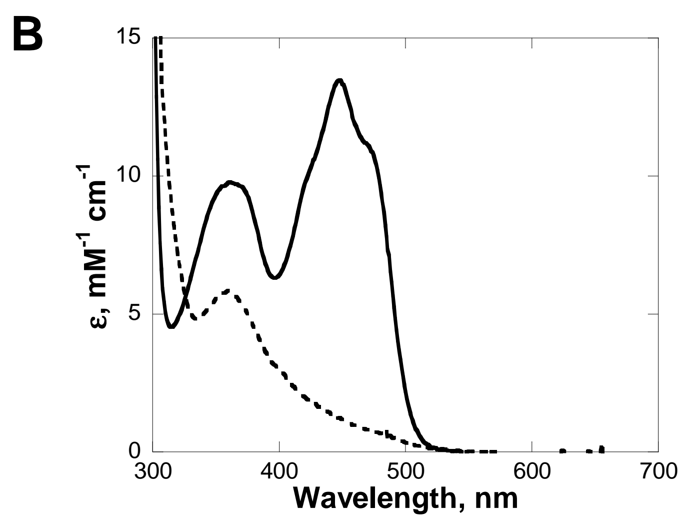
Fig. S6 Kinetics of the concentrations of trehalose and polyols during the conidial germination of *A. fumigatus*. Concentrations are expressed in μg/2.5 10^7 conidia

Fig. S7. Expression of trehalose and mannitol biosynthesis enzymes during conidial germination determined by RT–PCR in *akuB*$_{ku80}$ and *AfchoA*Δ*AfbadA*Δ::*HPH* mutant in resting conidia (0 h) and germinated conidia incubated in YPD broth at 37 °C for 3 h. Specific primer pairs designed to include an intron in the PCR product to verify the lack of genomic DNA amplification (Table S2) were used for cDNA amplification. The sizes of amplified products are indicated. *EF1α* gene (TEF) was constitutively expressed and used as a control of constitutive expression. Genes tested: trehalose phosphate synthase/phosphatase complex (*TPS1*, *TPS2*, *TPS3*, *TSL1*); *CLOCK9* enzyme; Mannitol 1 phosphate 5 dehydrogenase (*M1PDH*) and Mannitol2 dehydrogenase (*M2DH*).

Fig. S8 A. Expression of *CHOA* and *BADA* in the lung of mice infected by *A. fumigatus*. *GEL4* was used as a fungal positive control gene. Lungs from immune-suppressed non infected mice (lanes 5, 6, 7 and 8) were used as a negative control for fungal genes but as a positive control for the mouse gene *GAPDH*. B. Growth of the *AfchoA*Δ*::HPH*, *AfbadA*Δ::*HPH* and *AfchoA*Δ*AfbadA*Δ::*HPH* mutants is not ore affected in the lung of immunocompetent mice than the parental strain. CFUs of conidia in BALs 36 hours after inoculation to mice.

Fig. S1





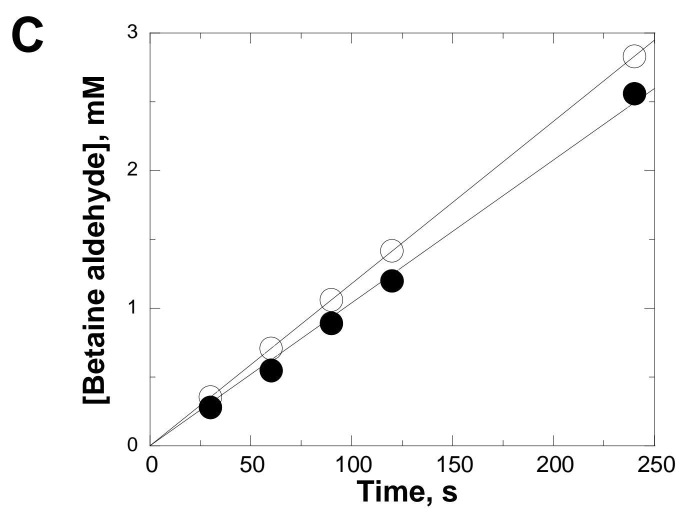


Fig. S2

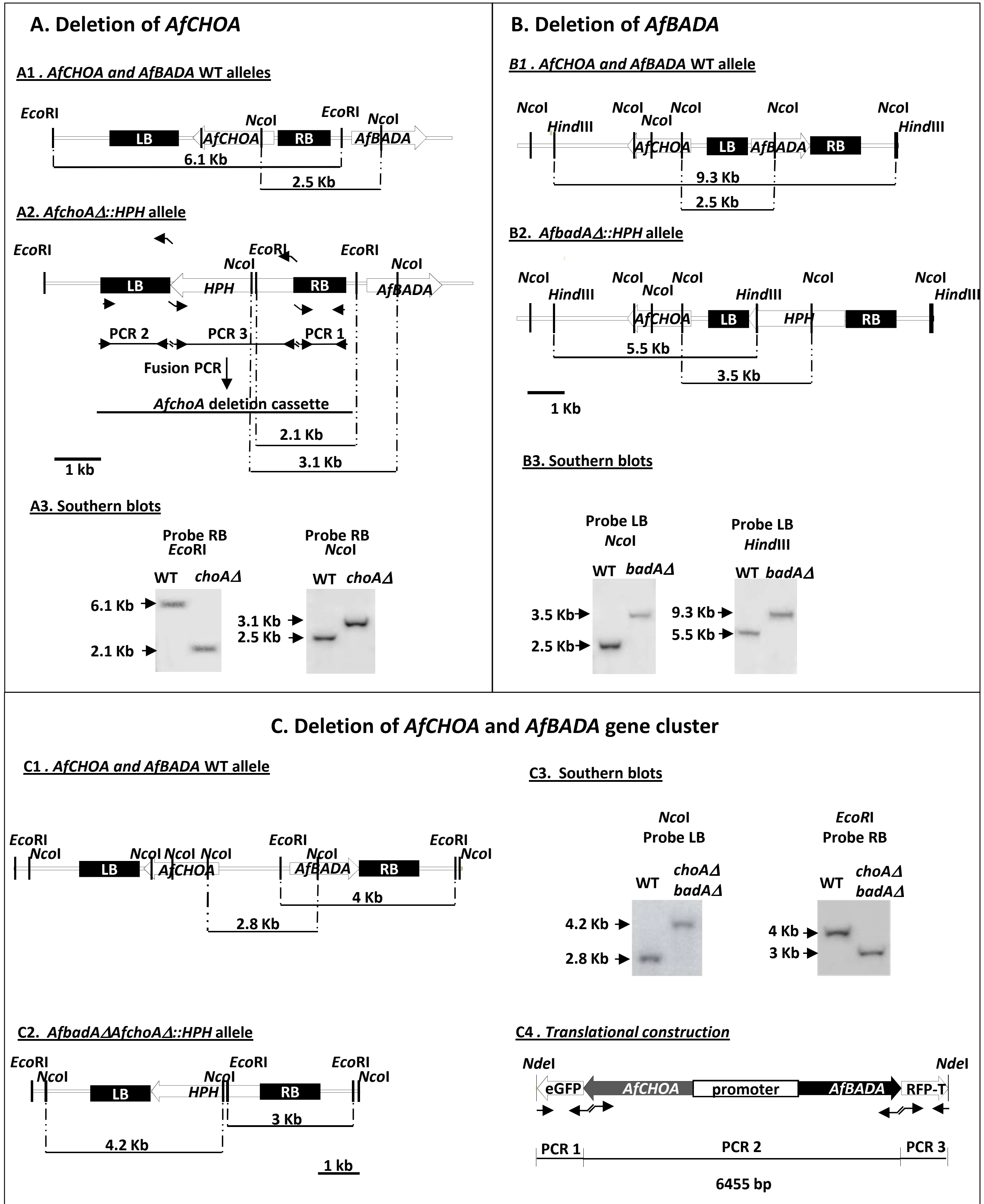
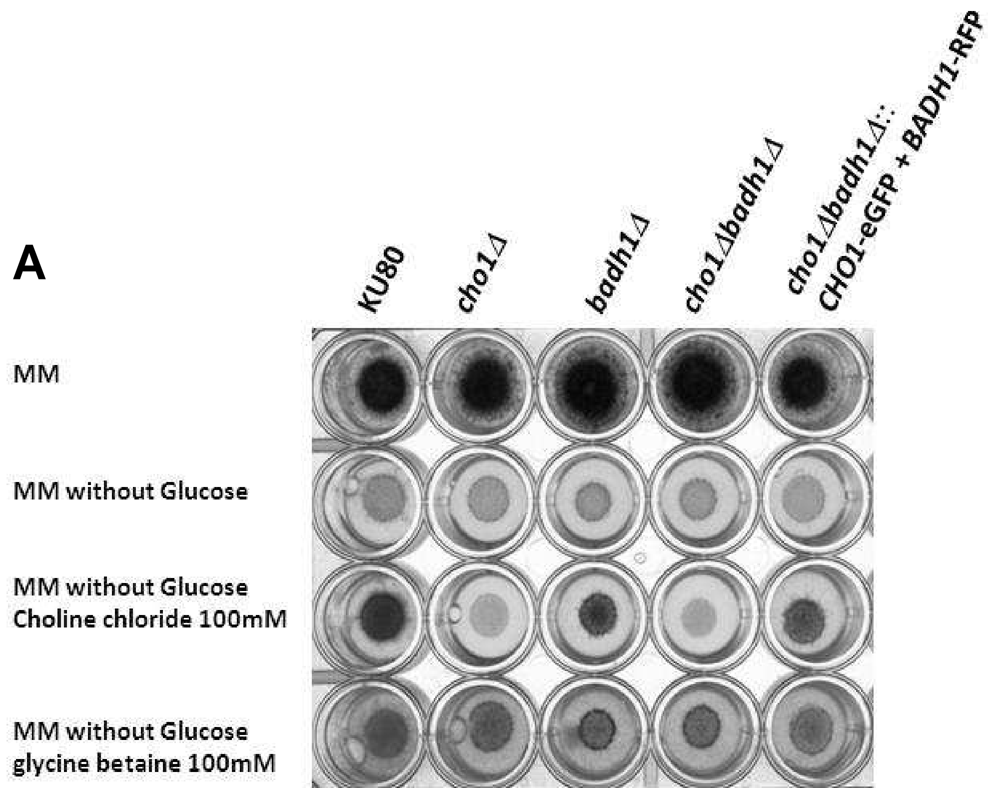


Fig. S3



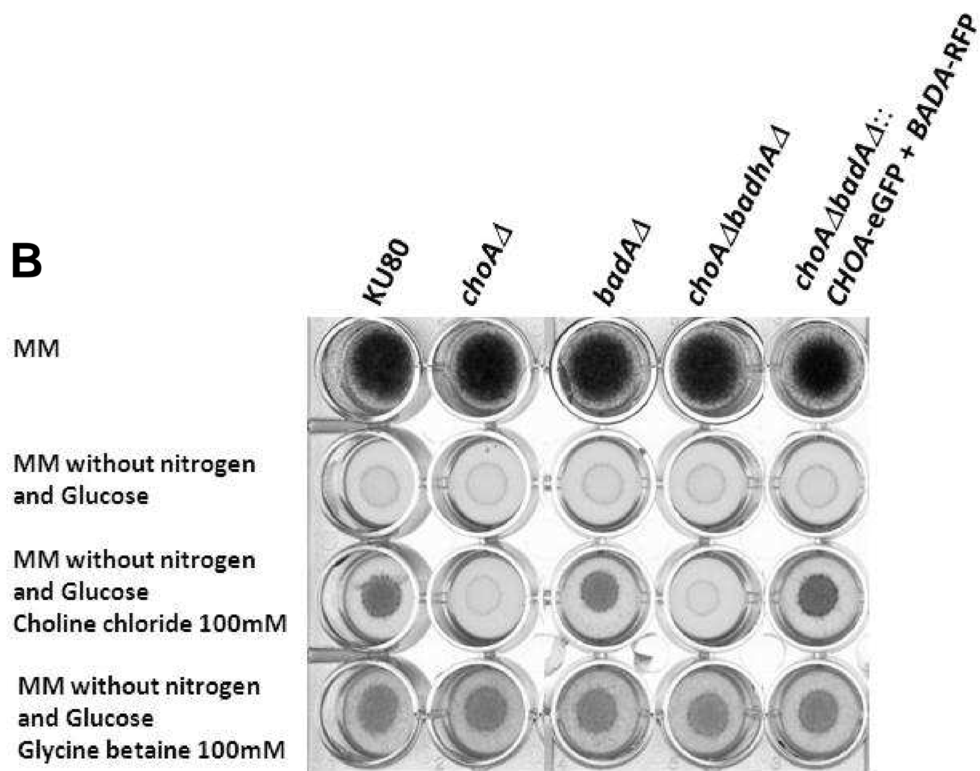


Fig. S4

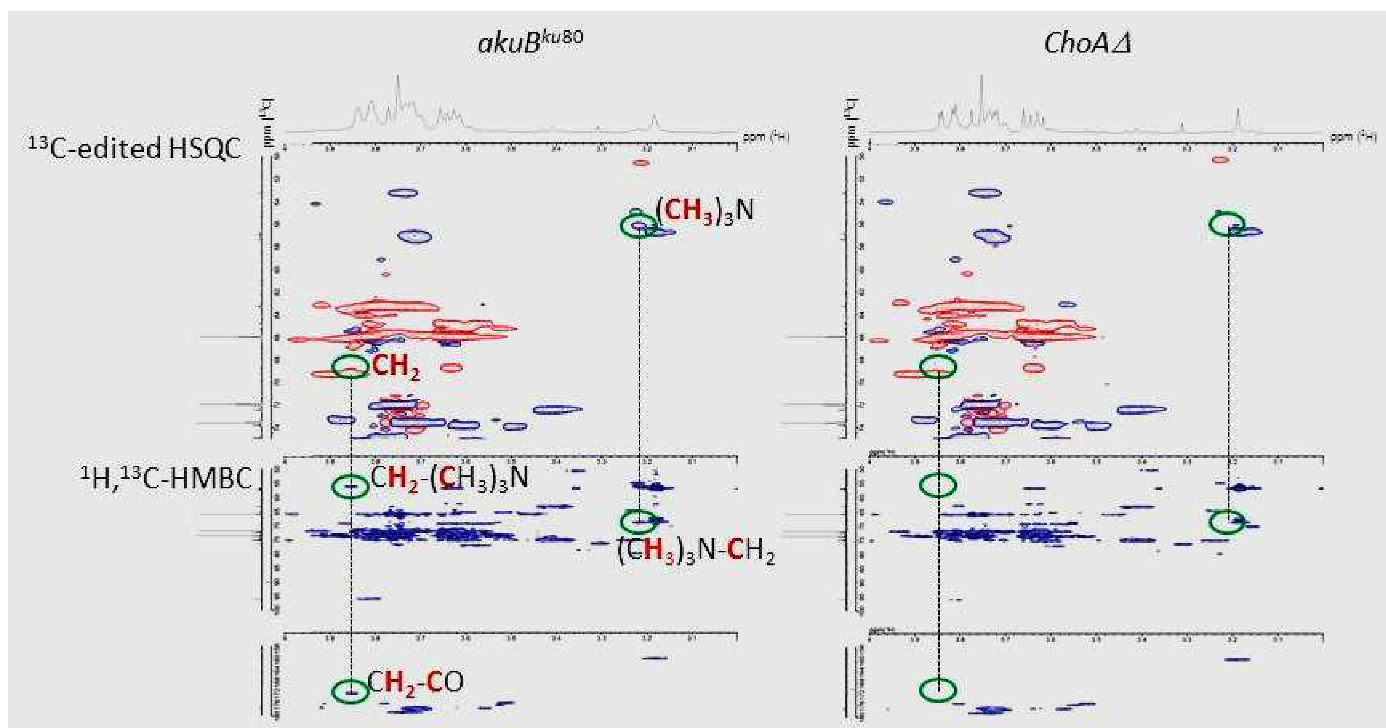
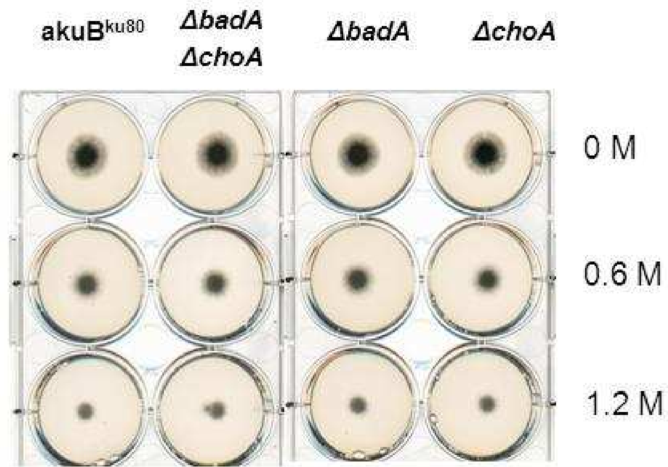


Fig. S5

(A) NaCl



(B) RT-PCR

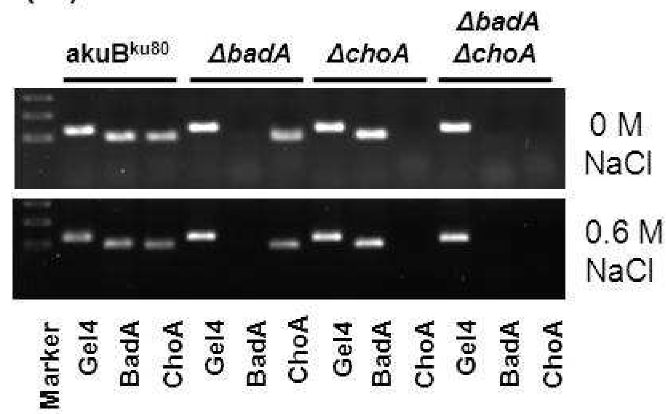
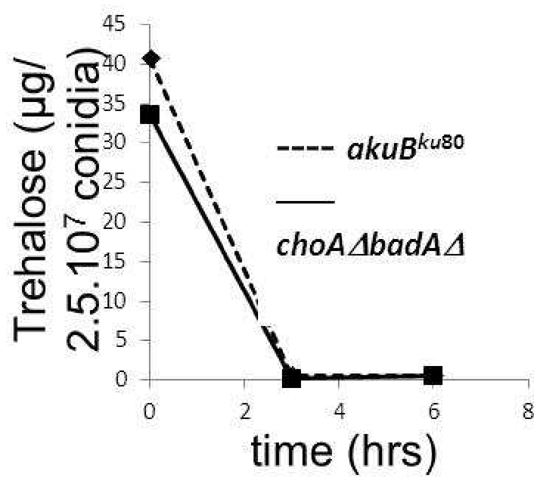
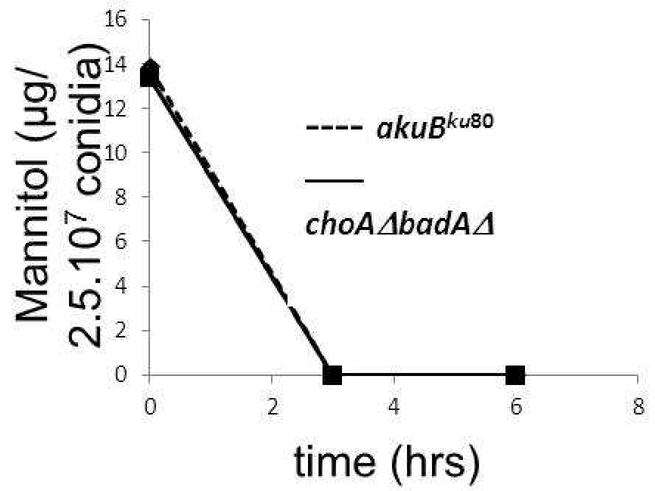
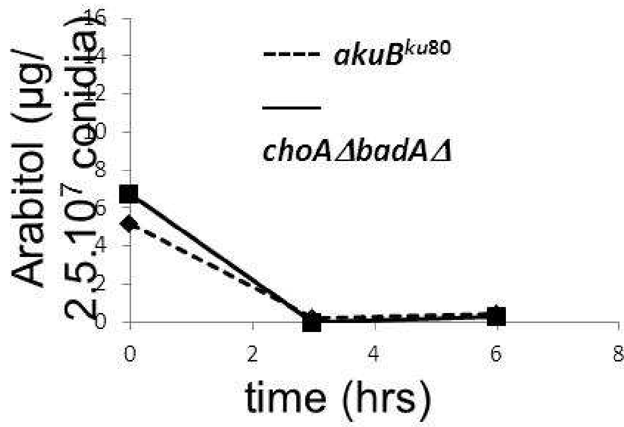


Fig. S6







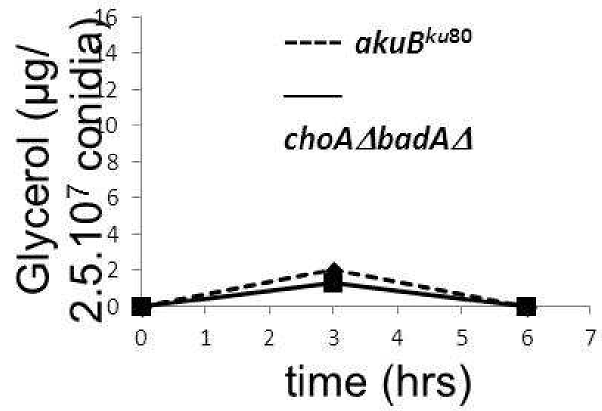


Fig. S7

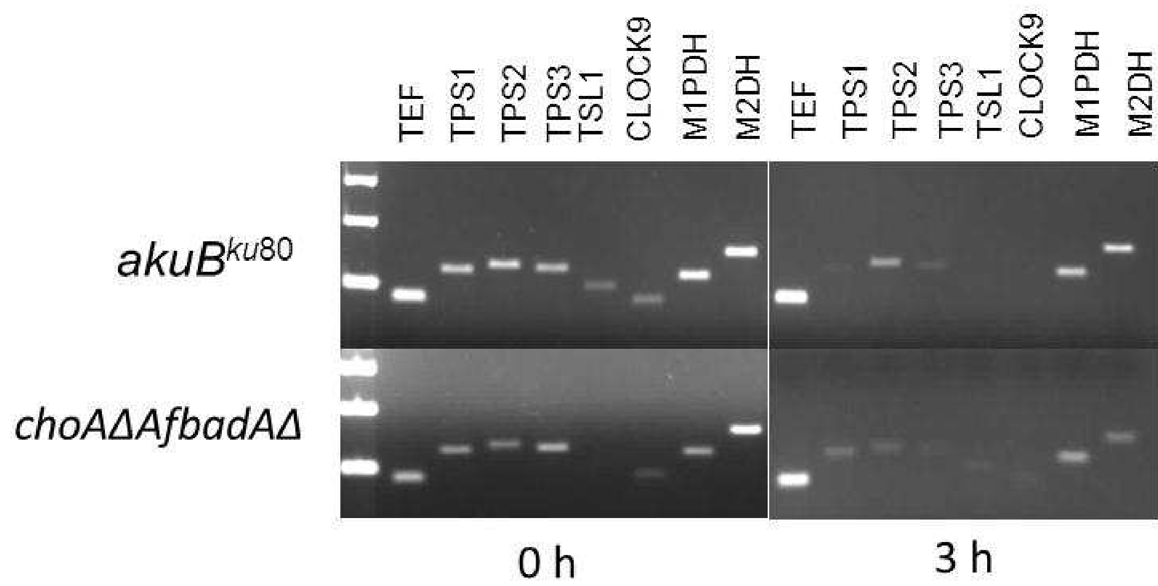


Fig. S8