Supplemental Data

Generation of A. fumigatus mutant strains.

Generation of the ΔglfB mutant. The 5' and 3' flanking regions (1.5 kb each) of *A. fumigatus glfB* coding sequence were amplified from genomic DNA by PCR with primers JE15/JE14 and JE20/JE17 respectively and linked by fusion PCR to a *ble/tk* cassette conferring resistance to phleomycin (47) amplified by JE06/JE07. From this construct, a second PCR (JE18/JE19) yielded the final deletion construct. Sequence integrity was verified after cloning into pCR2.1-TOPO (Invitrogen). Polyethylene glycol mediated fusion of protoplasts was carried out as described in (34). Transformants were grown on AMM plates containing 1.2 M sorbitol as osmotic stabilizer under appropriate selection conditions and singled out twice before further analysis. Accurate gene deletion and reconstitution were confirmed by southern hybridization. Southern probes were amplified from genomic DNA using primer pairs JE21/JE22, JE25/JE24 and PS20/PS21. All primer sequences are provided in Suppl Table 1.

*Transient complementation of the Δ*glfB *mutant.* A truncated *A. nidulans gpdA* promoter (24) and the *trpC* terminator were amplified from pSK342 (S. Krappmann, unpublished) with primers JE37/38 and JE39/40, respectively. The products were fused by PCR and cloned via XmaI in the pPTRII vector (Takara). Insertion of the *glfB* coding sequence (JE 46/47) via SfiI yielded the non-integrative *A. fumigatus* expression plasmid pPTRII-*glfB* conferring resistance to pyrithiamine. Transformation of the Δ*glfB* mutant strain with pPTRII-*glfB* was carried out as described above. Transformants were grown on AMM plates containing 30 μg/ml phleomycin and 0,3 μg/ml pyrithiamine. For Western Blot analysis, transformant mycelium was picked from the plates and processed as described in Material and Methods.

Generation of A. fumigatus *FLAG*-glfB *mutant.* To restore the *A. fumigatus* wild type from the Δ*glfB* mutant strain, the full-length *gpdA* promoter and the *trpC* terminator were amplified from pME2891 (47) with primers JE103/104 and JE109/110 respectively, and N-terminally FLAG-tagged *glfB* cDNA with primers JE105/106 from pYEScupFLAG-*glfB*. Fragments were fused by overlap PCR and the Δ*glfB* mutant was transformed as described above. Transformants were grown on AMM plates containing 100 μM FUDR, and singled out twice. A 7.4 kb DNA fragment covering the whole *glfB* locus was amplified from genomic DNA of the FLAG-*glfB* strain with primers JE15/17 and sequenced to verify the integrity of the FLAG-*glfB* genomic locus.

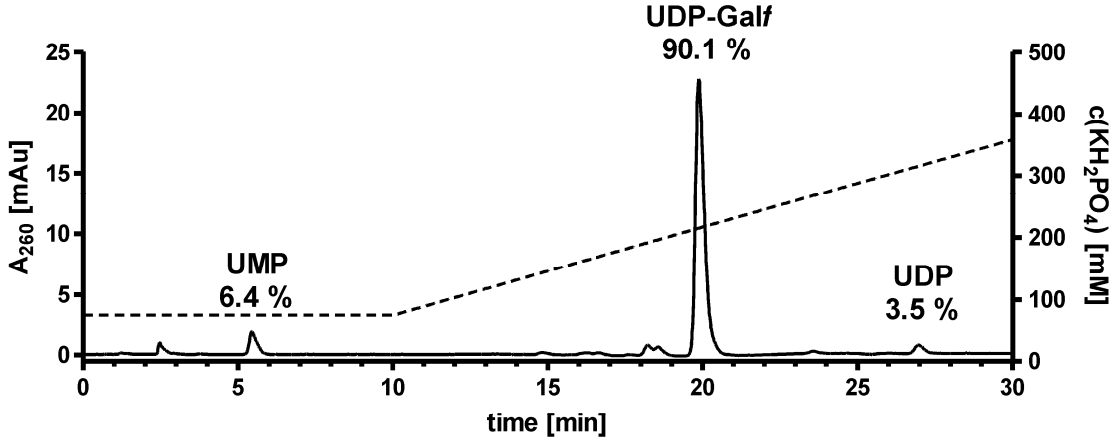
Suppl Table 1 DNA primer sequences

Primer	Sequence (5' -3'), restriction sites underlined	Description (restriction site)
JE06	TCGAGATCTTTCGACACTG	forward primer *ble/tk* cassette
JE07	CTAGAAAGAAGGATTACCTC	reverse primer *ble/tk* cassette
JE14	TCGACGTATTTCAGTGTCGAAAGATCTCGATGTGAAGAGTTGACTCTGGT	reverse primer 5' flanking region +30 nt *ble/tk* cassette overlap
JE15	GATGGAGAGTACGTATAACG	forward primer 5' flanking region
JE17	CGAAACTGTTGCGTTCTGTCC	reverse primer 3' flanking region
JE18	CAAGCTAGAGTAACCTGTCGAT	forward primer nested PCR
JE19	TATCCTTCGACGCTTCACTG	reverse primer nested PCR
JE20	CACTTGTTTAGAGGTAATCCTTCTTTCTAGGCTGGGAATGCGTAAAGAAAATG	forward primer 3' flanking region +30 nt *ble/tk* cassette overlap
JE21	CTCCGTCCGGTAGATAGGCACTGG	forward primer Southern Blot probe 1
JE22	GCCCGAAAATCAGACAAGCAGAGA	reverse primer Southern Blot probe 1
JE24	CCGGTGCGAGGATTGATAAGTGTC	reverse primer Southern Blot probe 2
JE25	CGGATTCACCCTCTTAACCACCTTC	forward primer Southern Blot probe 2
JE26	GCAAGGATCCAGTAACGAAGGAGAAAAAGCC	forward primer *glfB* cDNA (BamHI)
JE28	CGAGTCTAGATTACGCATTCCCAGCAGT	reverse primer *glfB* cDNA (XbaI)
JE37	GCAACCCGGGTTTGCCCGGTGTATGAAACC	forward primer truncated gpdA promotor (XmaI)
JE38	GGTGATGTCTGCTCAAGCGG	reverse primer truncated gpdA promotor
JE39	CCGCTTGAGCAGACATCACCGGCCACGTTGGCCTGACAGGCCGATCCACTTAACGTTACTGAAATC	forward primer trpC terminator (2 SfiI-sites) +20 nt gpdA overlap
JE40	GCAACCCGGGAAAGAAGGATTACCTCTAAACAAG	reverse primer trpC terminator (XmaI)
JE46	GCAAGGCCACGTTGGCCATGAGTAACGAAGGAGAAAAAGCC	forward primer *glfB* cDNA (SfiI)
JE47	GCAAGGCCTGTCAGGCCTCAGTGGTGGTGGTGGTGGTGCTCGAGCGCATTCCCAGCAGTTGC	reverse primer *glfB* cDNA + His-tag (SfiI, XhoI)
JE103	CTAGAAGCTTTCGAGATCTTTCGACACTG	forward primer full-length gpdA promotor (HindIII)
JE104	CGTCATCGTCCTTGTAGTCCATGGTGATGTCTGCTCAAGCGG	reverse primer full-length gpdA Promotor +22 nt FLAG-tag overlap
JE105	ATGGACTACAAGGACGATGACGATAAGGTACCAGGATCCAGTAACGAAGGAGAAAAAGCC	forward primer for *glfB* cDNA +FLAG-tag sequence
JE106	CTAGGGCCTGTCAGGCCTTACGCATTCCCAGCAGT	reverse primer for *glfB* cDNA (SfiI)
JE109	AAGGCCTGACAGGCCCTAGGATCCACTTAACGTTACTGAAATC	forward primer trpC +20 nt JE106 overlap
JE110	GAAAGAAGGATTACCTCTAAACAAG	reverse primer trpC terminator
PS20	AAGGTCGTTGCGTCAGTCCA	forward primer Southern Blot probe 3
PS21	TCGATGTGTCTGTCCTCC	reverse primer Southern Blot probe 3

Suppl Table 2 *Aspergillus fumigatus* (Af293) nucleotide sugar transporter candidates. The number of transmembrane domains (TMDs) predicted by ConPredII (http://bioinfo.si.hirosaki-u.ac.jp/~ConPred2/) is given, together with family classification according to the TC system (http://www.tcdb.org) and putative substrates.

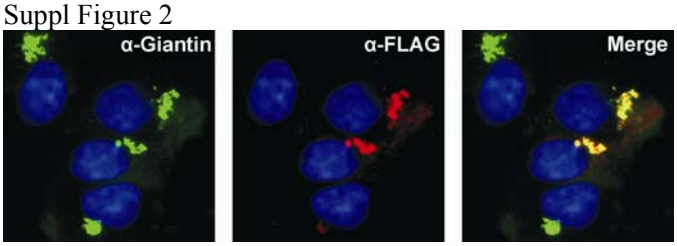
Acc. No.	Description in the RefSeq Database	Locus Tag	TMDs	TC #	TC Subfamily	Possible Substrate
XP_750355	nucleotide-sugar transporter	AFUA_1G06050	8	2.A.7	No subfamily yet	
XP_752298	DUF6 domain protein	AFUA_1G09310	9	2.A.7	No subfamily yet	
XP_756111	integral membrane protein	AFUA_2G17760	9	2.A.7	No subfamily yet	
XP_754351	DUF250 domain membrane protein (GlfB)	AFUA_3G12700	11	2.A.7	No subfamily yet	UDP-Gal*f*
XP_750700	nucleotide-sugar transporter	AFUA_6G08100	10	2.A.7	No subfamily yet	
XP_747139	solute transporter	AFUA_8G00660	8	2.A.7	No subfamily yet	
XP_747138	integral membrane protein	AFUA_8G00670	9	2.A.7	No subfamily yet	
XP_753855	DUF914 domain membrane protein	AFUA_5G07810	10	2.A.7	No subfamily yet	
XP_747907	ER to Golgi transport protein (Sly41)	AFUA_5G04360	8	2.A.7.9	Triose-phosphate Transporter (TPT) Family	
XP_746924	UPD-GlcNAc transporter (Mnn2-2)	AFUA_8G02830	9	2.A.7.10	UDP-N-Acetylglucosamine:UMP Antiporter (UAA) Family	UDP-GlcNAc
XP_750293	UDP-Glc/Gal ER nucleotide sugar transporter	AFUA_1G05440	10	2.A.7.11	UDP-Galactose:UMP Antiporter (UGA) Family	UDP-Gal, PAPS
XP_751184	UDP-galactose transporter	AFUA_6G13070	7	2.A.7.12	CMP-Sialate:CMP Antiporter (CSA) Family	UDP-Gal
XP_746997	nucleotide-sugar transporter	AFUA_8G02090	5	2.A.7.12	CMP-Sialate:CMP Antiporter (CSA) Family	UDP-Gal
XP_754058	Golgi GDP-mannose transporter	AFUA_5G05740	10	2.A.7.13	GDP-Mannose:GMP Antiporter (GMA) Family	GDP-Man
XP_752704	DUF6 domain protein	AFUA_1G13340	10	2.A.7.24	Thiamine Pyrophosphate Transporter (TPPT) Family	Thiamine derivatives
XP_753437	integral membrane protein	AFUA_5G12140	9	2.A.7.24	Thiamine Pyrophosphate Transporter (TPPT) Family	Thiamine derivatives

Suppl Figure 1



Suppl Fig 1 UDP-Gal*f* HPLC profile. 10 µl of a 2 mM UDP-Gal*f* solution were loaded on a CarboPac PA-100 column (Dionex) and eluted with a linear gradient of 75 to 500 mM KH_2PO_4 (dashed line, flow rate 1 ml/min) (45). UMP and UDP were identified from retention times of genuine species, and UDP-Gal*f* retention time was derived from (45). Peak area comparison (assuming identical extinction coefficients) yielded a UMP content of about 6-7 % in the UDP-Gal*f* preparation used in our study.

Suppl Figure 2



Suppl Fig 2 Transient *glfB* expression in HEK293 cells. A HindIII/XbaI fragment was subcloned from pYEScup-FLAG-glfB in pcDNA4 (Invitrogen) to yield pcDNA4-FLAG-glfB. HEK293 cells transfected with pcDNA4-FLAG-glfB were grown on glass coverslips for 48 h without selection. For immunofluorescence stainings, cells were fixed in 4 % paraformaldehyde/PBS and permeabilized with 0.1 % of a *Quillaja saponaria* saponin mixture (Sigma) in PBS containing 0.1 % BSA. Cells were incubated with mouse anti-Flag M5 mAb (Sigma, F4042) and rabbit anti-giantin serum (Golgi marker; Covance, PRB-114C) for 90 min at room temperature. After three washings (0.1 % BSA and 0.1 % Tween-20 in PBS), cells were incubated with secondary antibody conjugates anti–mouse Ig-Cy3 (Sigma, C2181) and anti–rabbit IgG-Alexa Fluor 488 (Molecular Probes, # A21206) for 1 h at room temperature. Cells were stained with the nuclear dye (Hoechst 33258; Hoechst Pharmaceuticals), and after washing with water, slides were mounted (Dako) and analyzed under a microscope (Axiovert 200M; Carl Zeiss, Inc.) using a Plan Apochromat 63×/1.40 oil differential interference contrast objective (M27; Carl Zeiss, Inc.) at room temperature. Images were taken using a camera (AxioCam MRm; Carl Zeiss, Inc.) with filter sets for Hoechst 33258, Alexa Fluor 488, and Cy3 which were converted in blue, green, and red, respectively by Axiovision 4.4 software (Carl Zeiss, Inc.).