SUPPLEMENTARY DATA

Table S1. List of proteins used in the phylogenetic analysis.

1	jgi|129416|Ceriporiopsis/1-449
2	EGN98663.1|Serpula/1-440
3	EAK83082.1|Ustilago/1-386
4	jgi|121644|Fomitiporia/1-494
5	jgi|78388|Auricularia/1-506
6	jgi|41987|Coniophora/1-490
7	jgi|52833|Rhodotorula/1-357
8	EFJ02265.1|238-698|Schizophyllum/1-461
9	jgi|392714|Heterobasidion/1-477
10	EDR15581.1|Laccaria/1-446
11	jgi|113595|Ganoderma/1-470
12	jgi|148196|Gymnopus/1-515
13	EDP43994.1|Malassezia/1-356
14	jgi|120089|Jaapia/1-464
15	jgi|33049|Sporobolomyces/1-364
16	jgi|123931|Stereum/1-491
17	EFP75931.1|Puccinia/1-370
18	AAW43133.1|Cryptococcus/1-389
19	CBQ72626.1|378-767|Sporisorium/1-390
20	CNAG_01385|Cryptococcus/1-391
21	jgi|117371|Agaricus/1-467
22	jgi|151176|Dichomitus/1-472
23	jgi|233482|Galerina/1-439
24	jgi|26317|Phlebia/1-452
25	jgi|69710|Fomitopsis/1-483
26	jgi|172856|Botryobasidium/1-554
27	jgi|182294|Agaricus/1-476
28	jgi|83334|Phanerochaete/1-450
29	jgi|448758|Hebeloma/1-444
30	jgi|64646|Paxillus/1-467
31	jgi|129444|Punctularia/1-493
32	jgi|61473|Wallemia/1-402
33	ADV24329.1|Cryptococcus/1-392
34	jgi|145354|Bjerkandera/1-447
35	jgi|39553|Tremella/1-392
36	jgi|25742|Trametes/1-456
37	jgi|135302|Gloeophyllum/1-462
38	jgi|135257|Wolfiporia/1-473
40	CCA73538.1|554-950|Piriformospora/1-397
41	jgi|25863|Phlebiopsis/1-449
42	jgi|1111534|Pleurotus/1-461
43	jgi|56170|Plicaturopsis/1-455

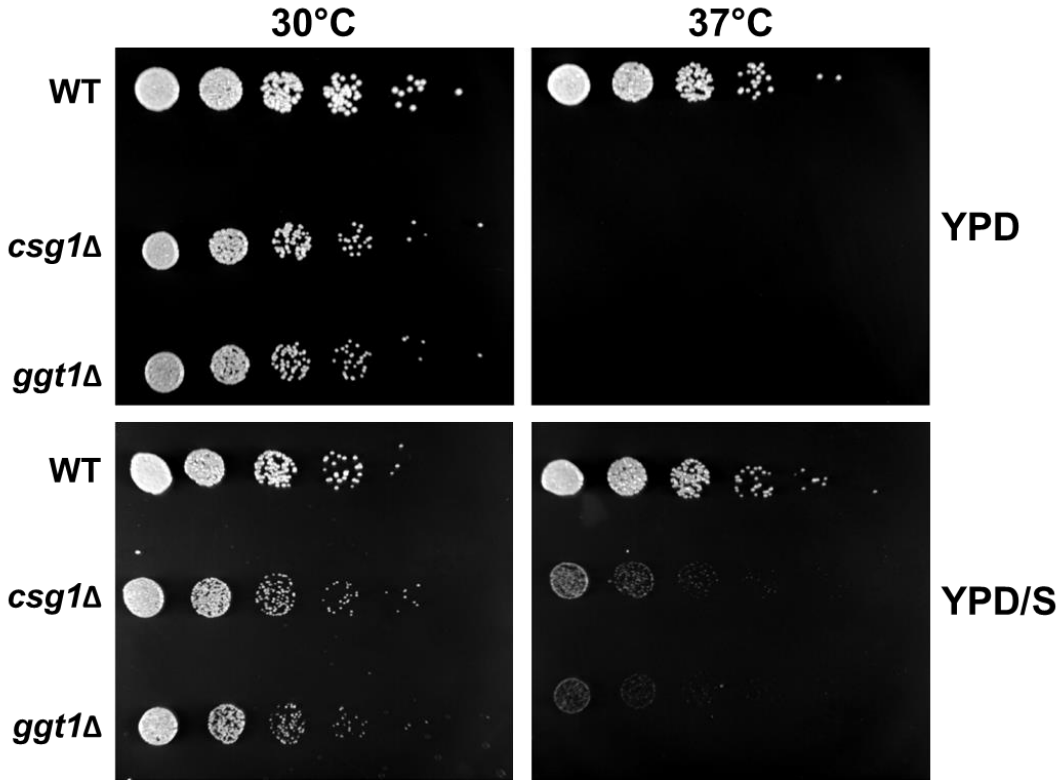
Table S2. *C. neoformans* var. *grubii* strains used in this work.

Strain	Designation	Relevant genotype	References
TDY-451	KN99α	Wild type	(Nielsen, K., Cox, G.M., et al. 2003)
TDY-1212	H99 *ggt1*Δ	*ggt1*Δ*::NAT*	This study
SMA-646	*csg1*Δ	*csg1*Δ*::NAT*	This study
TDY-1267	$GGT1_{empty}$	*ggt1*Δ*::NAT* pIBB103	This study
TDY-1268, 1269	$GGT1_{long}$	*ggt1*Δ*::NAT* pIBB103-$GGT1_{long}$	This study
TDY-1270	$GGT1_{short}$	*ggt1*Δ*::NAT* pIBB103-$GGT1_{short}$	This study

Table S3. Primers used in gene disruption and *CnGGT1* cloning. The sequence indicated in bold is complementary to the M13 primers used to amplify the resistance gene and mediate the overlap PCR used to assemble the disruption cassette. Restriction sites are underlined.

Primer	Sequence (5' to 3')	Comment
Csg1-L1	CCCCGAAGCCAAATAATCCACG	5'-flanking reg.
Csg1-L2	**CTGGCCGTCGTTTTAC**GTGGGGGGATTTGTATCTTGCC	5'-flanking reg.
Csg1-R1	**GTCATAGCTGTTTCCTG**GGGAAACGTTAAGGGATAAGGGTC	3'-flanking reg.
Csg1-R2	GTTACACAATCTAATAAGCCGCACG	3'-flanking reg.
Ggt1-L1	CTTATTGACTCTCGTGCCCGTC	5'-flanking reg.
Ggt1-L2	**CTGGCCGTCGTTTTAC**TGCGCTCTGTACATGCCATTC	5'-flanking reg.
Ggt1-R1	**TCATAGCTGTTTCCTG**GCTTAAGAAGGAAGTCGGCCTG	3'-flanking reg.
Ggt1-R2	GCACCAGCAAGCAATTGGAAC	3'-flanking reg.
M13fwd	GTAAAACGACGGCCAG	
M13rev	CAGGAAACAGCTATGAC	
AM84	CATGCCACTAGTGCTGCGAGGATGTGAGCTGG	$P_{ACT,}$ sense
AM87	CTGTCATGAGCATGGGCCCCTCCTTGTTTGCAATAACACG	$GGT1_{long,}$ antisense
AM92	TAGCTTGGGCCCTACAGTGGCGCGCCGGGCGAGTTTACTAATGG	$P_{ACT1,}$ antisense
AM93	ACTGTAGGCGCGCCATGCCTTTCCACGC	$GGT1_{long,}$ sense
AM94	ACTGTAGGCGCGCCATGCCTCAAGCGGATCTC	$GGT1_{short,}$ sense

A


30°C
37°C
WT
csg1Δ
ggt1Δ
YPD
WT
csg1Δ
ggt1Δ
YPD/S

B

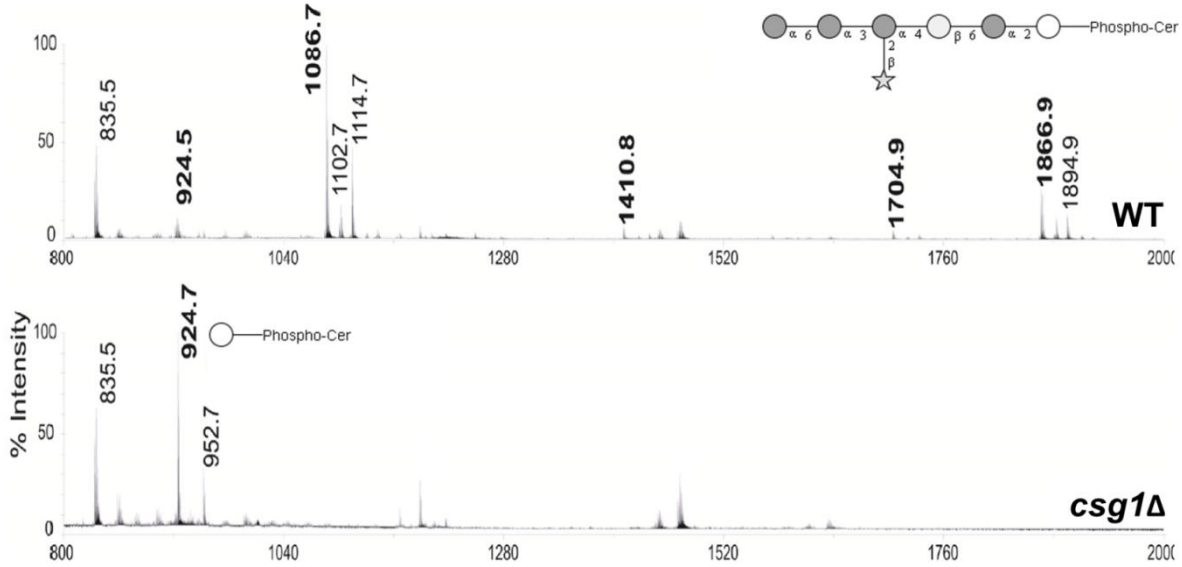

% Intensity
100
50
0
835.5
924.5
1086.7
1102.7
1114.7
1410.8
1704.9
1866.9
1894.9
Phospho-Cer
WT
800
1040
1280
1520
1760
2000
835.5
924.5
952.7
Phospho-Cer
csg1Δ

Fig. S1. Growth of *C. neoformans csg1*Δ and *ggt1*Δ mutants on rich culture medium with and without sorbitol and GIPC structure of *C. neoformans csg1*Δ mutant. **(A)** *C. neoformans* wild type (WT), the *csg1*Δ mutant lacking the first mannosyltransferase, and the *ggt1*Δ mutant deficient in the successive galactosyltransferase were grown in rich liquid culture medium (YPD). A five-fold dilution series starting at OD_{600} of 0.1 was prepared and equal volumes were spotted on YPD agar plates with (YPD/S) and without (YPD) the osmotic stabilizing agent sorbitol (1M). Plates were incubated at either 30°C or 37°C. **(B)** For GIPC analysis by MALDI-MS lipids were extracted from *C. neoformans* WT and *csg1*Δ mutant and analyzed as described in Materials and Methods. The numbers in bold indicate masses (Da) of GIPC. In most cases GIPC species are accompanied by two adjacent peaks corresponding to derivatives with an additional hydroxyl-group (+16) or extended fatty acid moiety (C2:+28), as previously reported (Guan, X.L. and Wenk, M.R. 2006, Heise, N., Gutierrez, A.L., et al. 2002). 924.5: IPC; 1086.7: MIPC; 1866.9: GIPC with hexasaccharide; 1410.8: GIPC with trisaccharide; 1704.9: GIPC with pentasaccharide; 835.6: PI; other masses could not be assigned. White circle: inositol; dark grey circle: mannose; light grey circle: galactose; star: xylose.

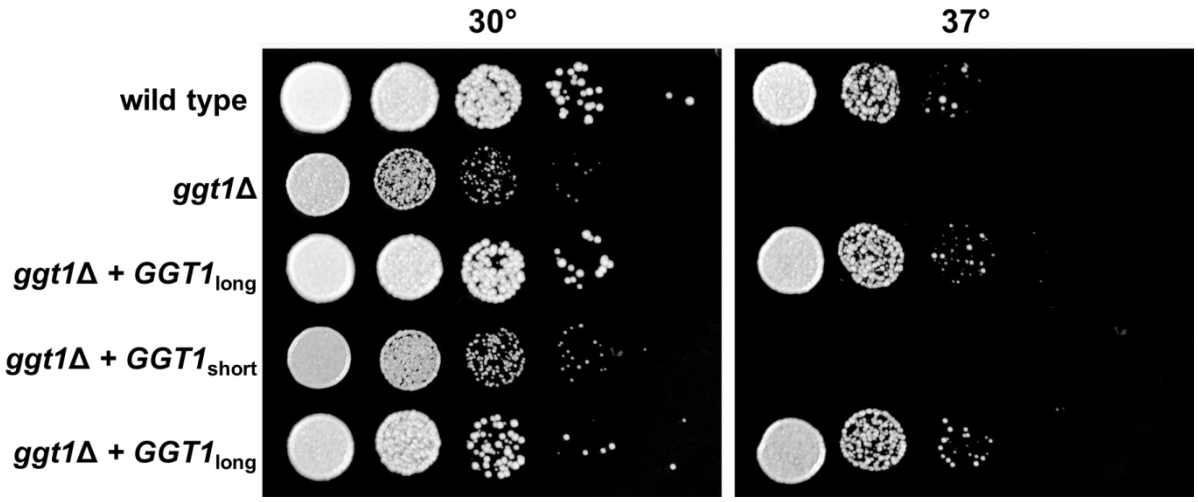
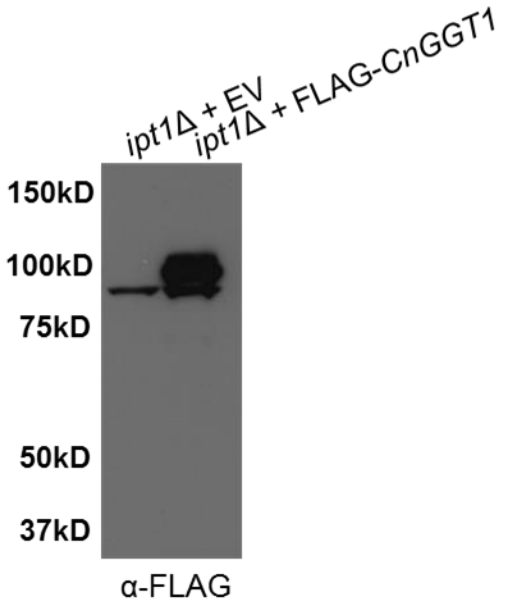
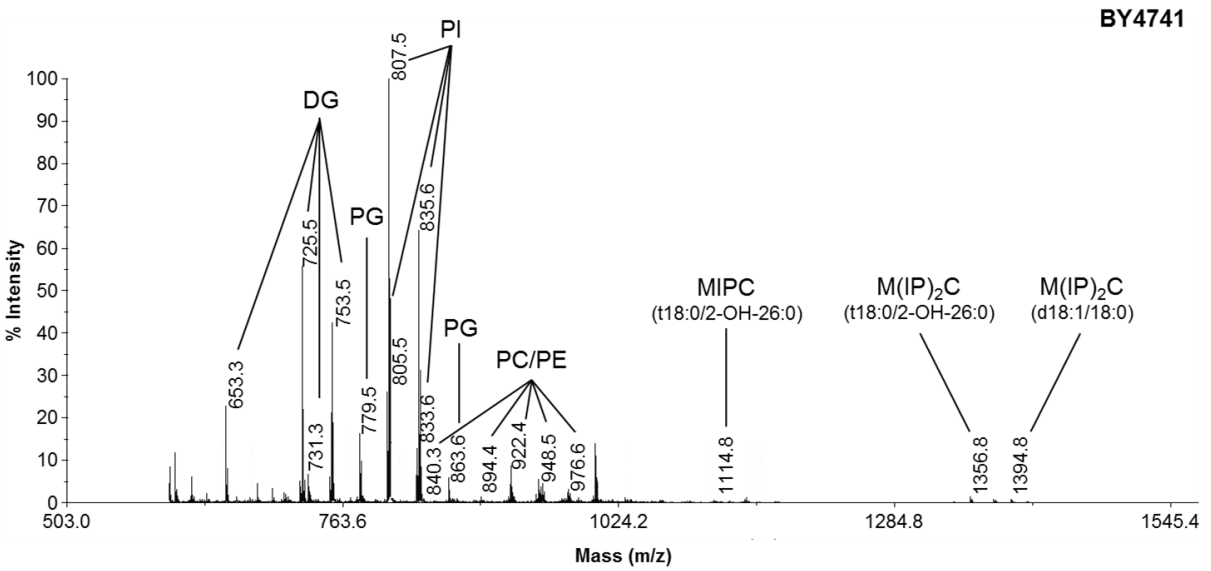


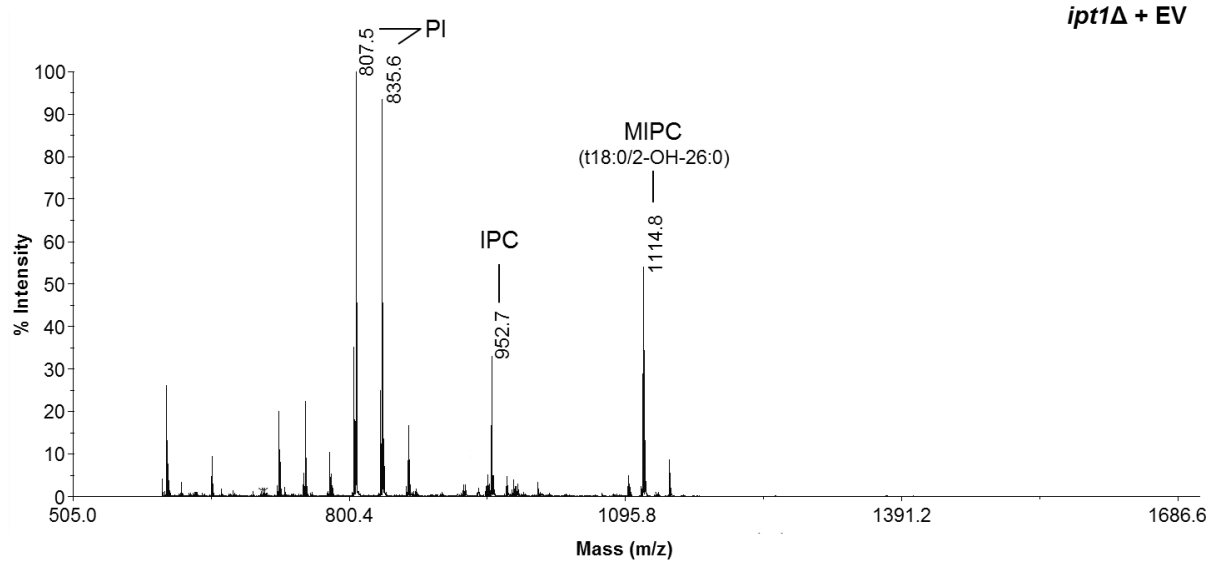
Fig. S2. Episomal expression of *GGT1* in *C. neoformans* on YPD + G418. Serial 10-fold dilutions of the indicated strains were spotted on YPD plates containing G418 and incubated at the indicated temperatures for three days. Two independent transformants are shown for the plasmid containing the long form of the gene.

A



B


BY4741
% Intensity
Mass (m/z)
PI
DG
PG
PG
PC/PE
MIPC
(t18:0/2-OH-26:0)
M(IP)2C
(t18:0/2-OH-26:0)
M(IP)2C
(d18:1/18:0)
807.5
725.5
753.5
835.6
653.3
805.5
779.5
833.6
731.3
840.3
863.6
894.4
922.4
948.5
976.6
1114.8
1356.8
1394.8
503.0
763.6
1024.2
1284.8
1545.4


*ipt1*Δ + EV
% Intensity
Mass (m/z)
PI
807.5
835.6
MIPC
(t18:0/2-OH-26:0)
IPC
952.7
1114.8
505.0
800.4
1095.8
1391.2
1686.6

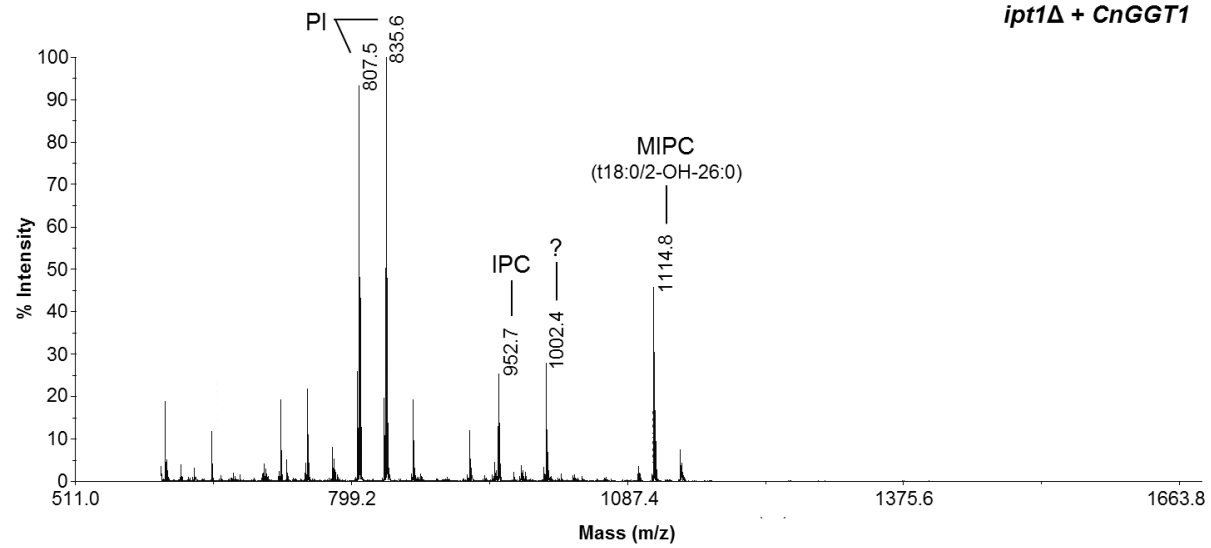

*ipt1*Δ + *CnGGT1*
% Intensity
Mass (m/z)
PI
807.5
835.6
MIPC
(t18:0/2-OH-26:0)
IPC
?
952.7
1002.4
1114.8
511.0
799.2
1087.4
1375.6
1663.8

Figure S3. Heterologous expression of *CnGGT1* in *Saccharomyces cerevisiae*. **(A)** The *CnGGT1* ORF was N-terminally FLAG-tagged and cloned into a low-copy pRS415 vector under the control of the GPD promoter. FLAG-*CnGGT1* was expressed in an *ipt1*Δ *S. cerevisiae* strain (EUROSCARF *BY474 ipt1::kanMX4)* at 30°C overnight. Western blot analysis with an anti-FLAG antibody confirmed expression of CnGgt1 in *S. cerevisiae*. **(B)** GIPCs were extracted from *S. cerevisiae* wild type (BY4741), *ipt1*Δ harboring the empty vector (*ipt1*Δ + EV) and *ipt1*Δ expressing *CnGGT1* (*ipt1*Δ + *CnGGT1*) as described in Materials and Methods. Peaks at m/z of 1114.8 and 1356.8/1394.8 correspond to MIPC and $M(IP)_2C$, respectively. $M(IP)_2C$ was detected in *S. cerevisiae* BY4741 but was absent in the *ipt1*Δ strain as previously described (Dickson, R.C., Nagiec, E.E., et al. 1997). Absence of a peak at 1276.78 m/z in the *ipt1*Δ + *CnGGT1* spectrum indicates that no Gal-MIPC is synthesized in *S. cerevisiae* upon heterologous expression of *CnGGT1*. The same result was obtained for C-terminally FLAG-tagged *CnGGT1* ORF (data not shown).

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