Supplementary material

CWH43 is required for the introduction of ceramides into GPI anchors in Saccharomyces cerevisiae

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Figure S1. The profile of sphingolipids made by *cwh43*Δ cells is normal.

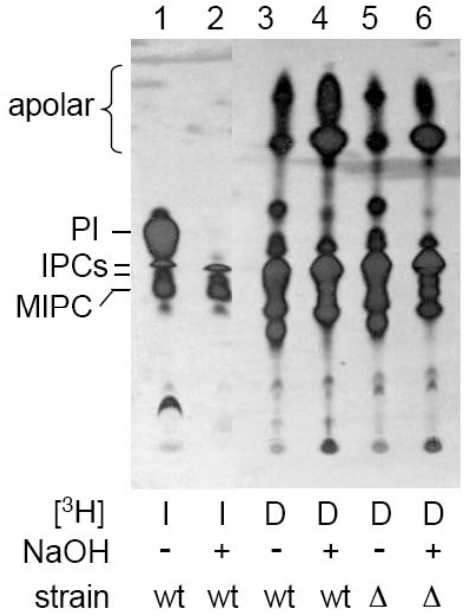

Figure legend S1: In addition to the normal profile of sphingolipids observed in the extract of [^{3}H]inositol labeled *cwh43*Δ cells (Fig. 2C), we also analyzed by TLC the lipid extracts of cells labeled with [^{3}H]inositol (I) or [^{3}H]DHS (D) obtained in the experiment shown in Fig. 2A. As can be seen in the above Figure, the profiles of ceramides and IPCs, whether treated (+) with mild base (NaOH) or not (-) are the same in wt and *cwh43*Δ (Δ) cells. [^{3}H]inositol-labeled lipids of wt cells are included in order to allow the localization of IPCs and mannosyl-IPC (MIPC). It has to be noted that cells can degrade [^{3}H]DHS to [^{3}H]palmitate and that this label then is incorporated into a multitude of glycerophospholipids. The lowest band in the region of apolar lipids represents free fatty acids, and this band strongly increases upon mild base treatment. The region of apolar lipids also contains ceramides. Data argues that the metabolism and the incorporation of [^{3}H]DHS in *cwh43*Δ cells is normal.

Figure S2. ***Cwh43*Δ are not calcofluor sensitive.**

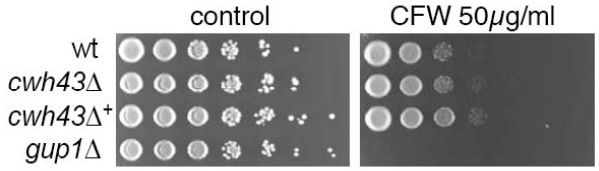

Figure legend S2: Ten fold dilutions of BY4742, *cwh43*Δ, *cwh43*Δ$^+$ (*cwh43*Δ harboring pCWH43) and *gup1*Δ cells were plated onto YPG without (control) or with calcofluor white (CFW) and incubated for 3 days at 30 °C. As reported before, *gup1*Δ cells are highly sensitive to CFW (Bosson et al., 2006) but *cwh43*Δ cells are not.

SUPPLEMENTARY TABLES

Table S1A. Primers for hphNT1 deletion cassettes

Name	Sequence (5' to 3')
Cwh43 hphNT1 F	ctcgaggaataagtaaccaggaatacagaaggtatccaccgccagttatgCGTACGCTGCAGGTCGAC
Cwh43 hphNT1 R	tacacacaatgtgattacactgatttataaaaccaccttacggcctcttaATCGATGAATTCGAGCTCG
Per1 hphNT1 F	tgtgaaaccataccttcgggagaaaagaaacagaagtgtggcaagaaatatgCGTACGCTGCAGGTCGAC
Per1 hphNT1 R	aatatcttaattcacctgtttactatcatgaataatagttatatatgtattATCGATGAATTCGAGCTCG

Nucleotides in lowercase correspond to sequences immediately upstream and downstream of the open reading frame to be deleted.

Table S1B. Primers for cloning *CWH43* into an expression vector

Name	Sequence (5' to 3')
Cwh43 rec1	GAATTCGATATCAAGCTTATCGATACCGTCGACActgatcatcaatggga agatcatccctatagc
Cwh43 rec2	GCGTGACATAACTAATTACATGACTCGAGGTCGACttataagtaataacg tggctcatcaaaaacatgg

Nucleotides in lowercase correspond to the two ends of the open reading frame, nucleotides in uppercase being homologous to the target vector pGREG536 (Jansen et al., 2005).

Table S1C. Primers for point mutations in *CWH43*

Name	Sequence (5' to 3')
D713A F	GATATGGAACTAgctGTGGTAGGTCTAC
D713A R	GTAGACCTACCACagcTAGTTCCATATC
H802A F	CGTCTTTGTATTCgctAGTGGACAAGAAG
H802A R	CTTCTTGTCCACTagcGAATACAAAGACG

Nucleotides in lowercase correspond to the amino acid to be mutated.