Supporting Information

Figure legends

Figure S1: Production of glucosylceramides in *G. lamblia*. Chromatograms generated by selection of the ion at m/z 700.5727, corresponding to N-hexadecanoyl-1O-glucosylsphingosine (C16 GlcCer) in: A, a base methanolysed lipid extract of *G. lamblia* trophozoites and B, a standard sample of C16 GlcCer. The exact mass cluster of C16 GlcCer (M+1) calculated and found in B are given in C and D, respectively. Assignment details are given in the table.

Figure S2: Biosynthesis of sphingolipids in mammalian cells. Homologues enzymes annotated in the *Giardia* genome database are labeled in red. The sites of action of the sphingolipid inhibitors used in this study are indicated.

Figure S3: Incorporation of serine in *G. lamblia*. Isolated parasites were labeled with [^{3}H]palmitic acid (palm) or [^{3}H]serine (ser) for 3 h. After extensive washing, cell aliquots were solubilized with 0.1N NaOH and associated radioactivity was measured by liquid scintillation and normalized by protein content. Data are average ± SE (n=3) of a representative from three experiments done in triplicate.

Figure S4: Lipid synthesis in presence of tunicamycin. Isolated parasites were labeled with [^{3}H]palmitic acid for 3 hrs in presence of 60 μM tunicamycin (TM) or solvent (cntl). Lipid aliquots corresponding to equal protein amount were separated by 2D-HPTLC using the solvent systems A. Note the increased amount of GlcCer (spot "A") upon TM treatment.

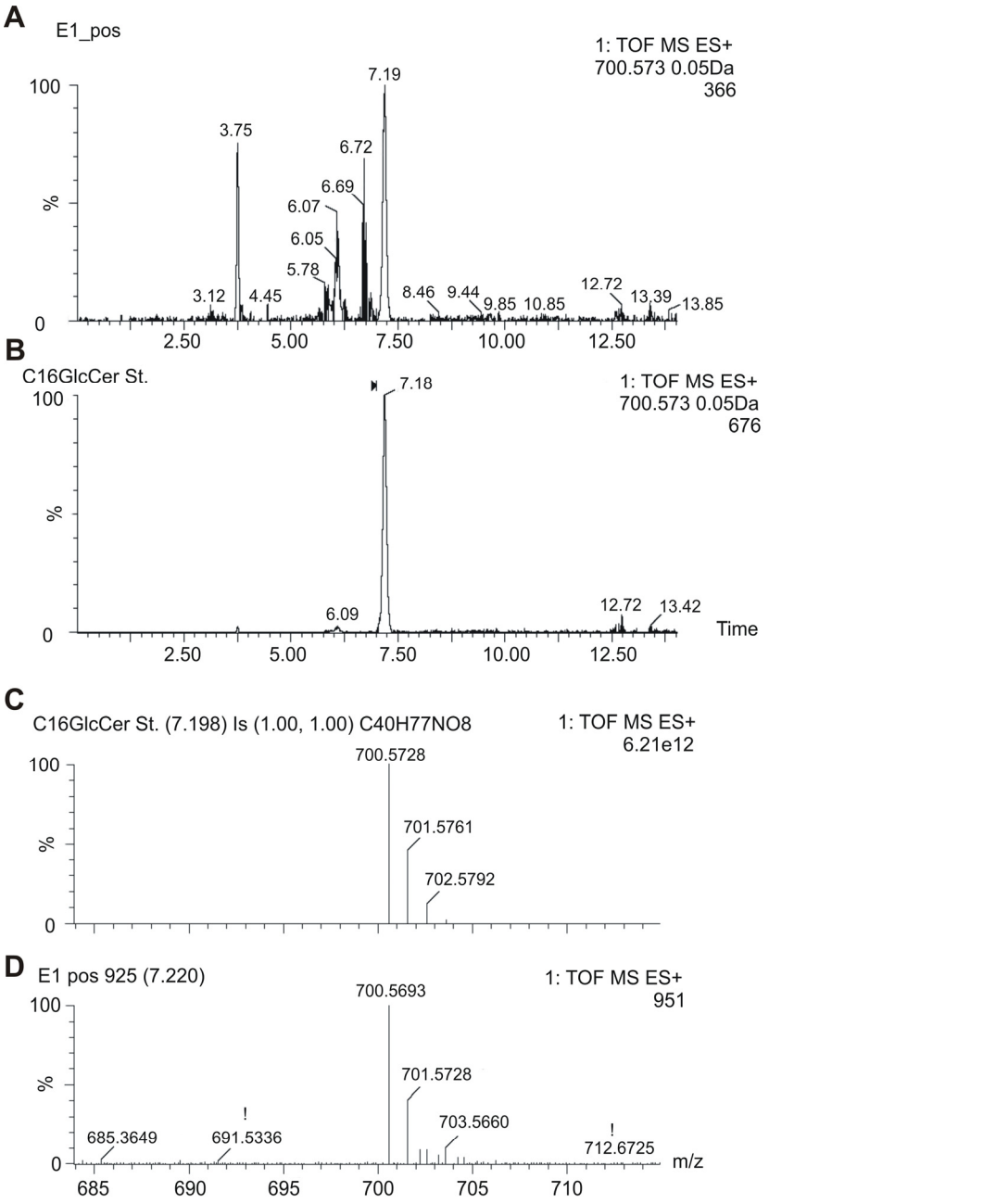
Figure S5: Ultrastructural abnormalities in presence of PPMP. Electron micrographs of *G. lamblia* treated with 10 μM PPMP for 16 h showing coiled multilamellar structures (arrows). N, nucleus. Scale bar: 3 μm.

Figure S6: Nocodazole treatment inhibits cell division and CWP1 expression. Parasites were encysted for 16 h in presence of 10 μM PPMP, 6 μM nocodazole or solvent (cntl). Quantification of parasite number and parasites expressing CWP1 was performed by manual counting. Data are percentage of untreated (cntl) parasites ± SE (n = 9) of three experiments done in triplicate.

Figure S7: Protein sequence comparison of giardial GCS with orthologues retrieved by BLAST data base search. Alignment analysis revealed the highest homology of parasite GCS to plant enzymes in terms of amino acid identity and protein length.

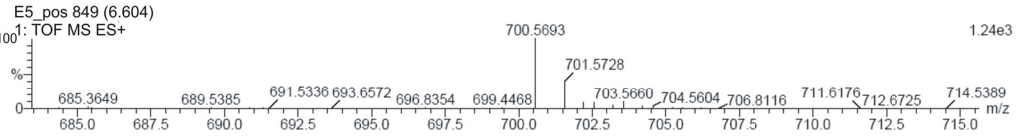
Figure

S1



Elemental composition report
Single mass analysis
Tolerance=5.0 mDa/DBE: min=-1.5, max=50.0
Selected filters: none

Monoisotopic mass, even electron ions
33 formula(e) evaluated with 1 result within limits
(up to 50 best isotopic matches for each mass)
Elements used: C: 0-40 H: 0-80 N: 0-2 O: 0-9



MS-based assignments of C16-GlcCer presents in *G. lamblia* trophozoite extracts

Measured m/z	Theoretical m/z	error ppm	DBE	i-FIT	Molecular formula
700.5727	700.5692	-4.9	2.5	9.3	C40H78NO8

Figure S2

SPT, serine palmitoyl transferase
KSR, 3-ketosphinganine reductase
CerS, ceramide synthase
CerD, ceramide desaturase
GCS, glucosylceramide synthase
GalCS, galactosylceramide synthase
Cerase, ceramidase
SphK, sphingosine kinase
SphPP, sphingosine-phosphate phosphatase
SMS, sphingomyelin synthase
Smase, sphingomyelinase
CerK, ceramide kinase
CerPP, ceramide-phosphate phosphatase

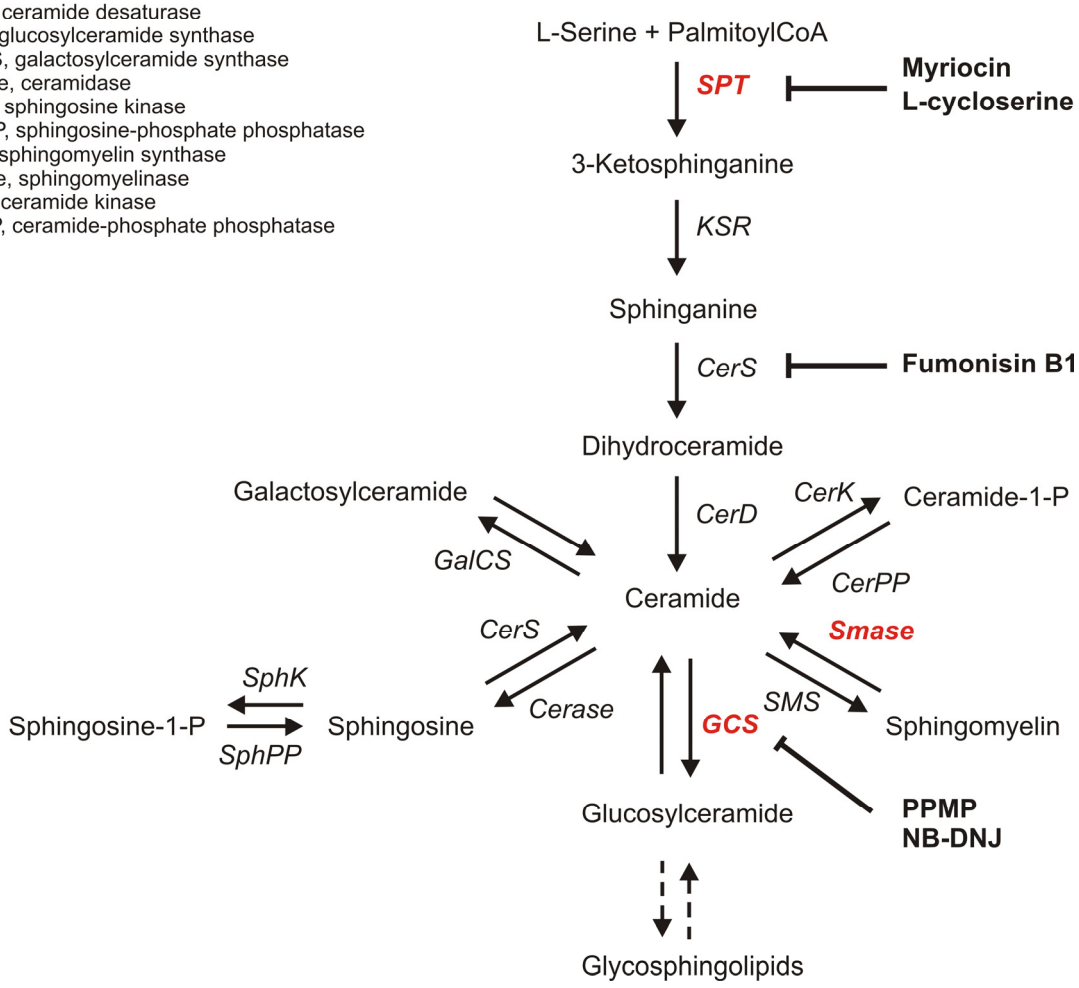


Figure S3

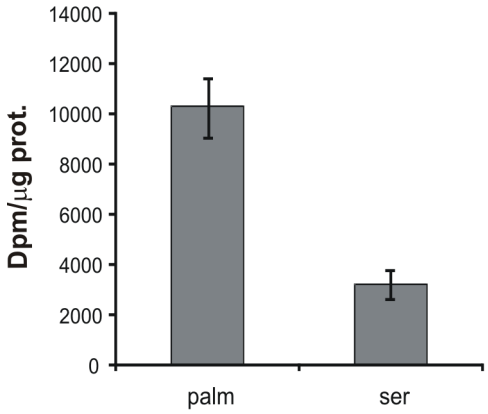


Figure S4

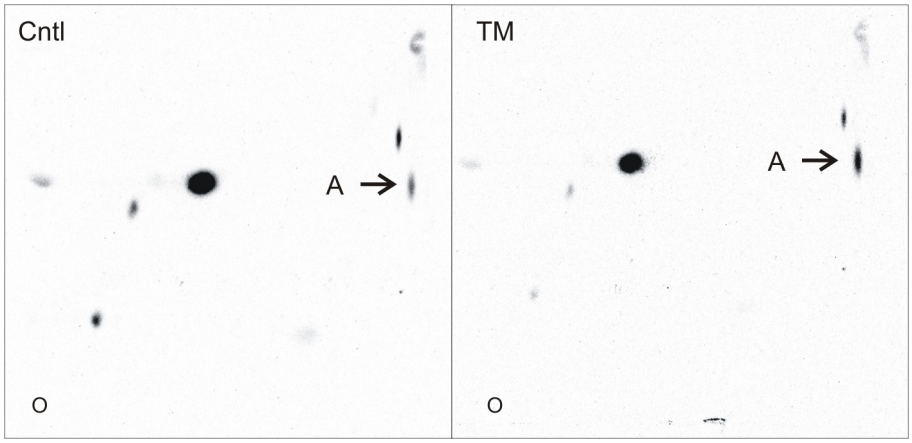


Figure S5

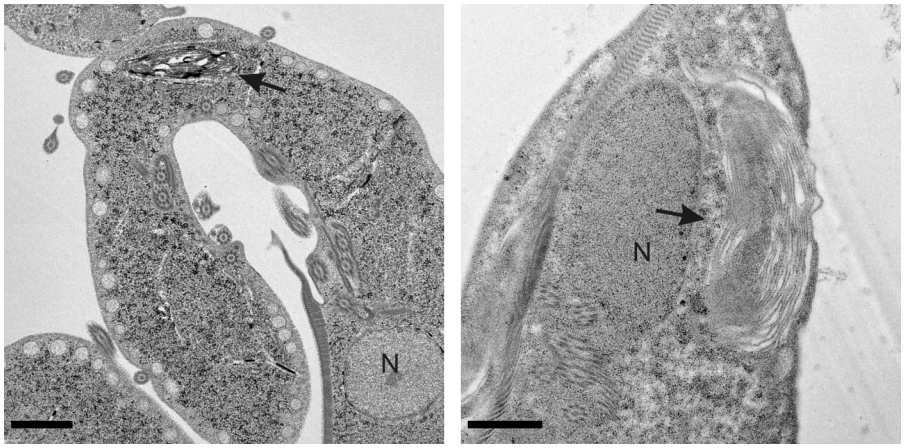


Figure S6

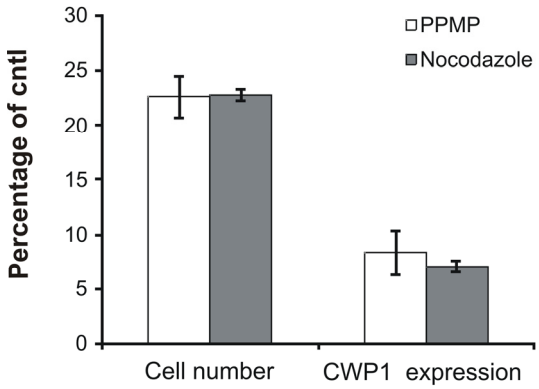

30
25
20
15
10
5
0
Percentage of cntl
PPMP
Nocodazole
Cell number
CWP1 expression

Figure S7

Organism	Identity (%)	Similarity (%)	Length (aa)
G. lamblia	100		537
A. thaliana	21.17	31.49	519
G. arboreum	20.6	32.68	520
M. musculus	12.45	26.76	394
H. sapiens	13.17	25.79	394
C. elegans	11.87	33.02	443
D. melanogaster	15.32	25.18	440