

SUPPLEMENTARY DATA

Peak	m/z ^a	HR m/z ^b	Lipid ^c	Principal component ^d	Theor m/z ^e
1	520	520.30	18:2	0:0/18:2	520.3398
2	522	522.31	18:1	0:0/18:1	522.3554
3	524	524.33	18:0	0:0/18:0	524.3711
4	544	544.34	20:4	0:0/20:4	544.3398
5	546	546.32	20:3	0:0/20:3	546.3554
6	548	548.37	20:2	0:0/20:2	548.3711
7	568	568.35	22:6	0:0/22:6	568.3398
8	570	570.37	22:5	0:0/22:5	570.3554
9	572	572.37	22:4	0:0/22:4	572.3711
10	646	646.45	26:2	0:0/26:2	646.4443
11	648	648.47	26:1	0:0/26:1	648.4599
12	650	650.49	26:0	0:0/26:0	650.4756
13	660	660.59	a-28:2	a-14:0/14:2	660.5363
14	662	663.55	a-28:1	a-14:0/14:1	662.5119
15	664	664.51	a-28:0	a-14:0/14:0	664.5276
16	674	674.49	28:2	14:0/14:2	674.4756
17	676	676.52	28:1	14:0/14:2	676.4912
18	678	878.53	28:0	14:0/14:0	678.5069
19	675	675.52	SM(14:0)	Cer 18:1/14:0	675.54
20	686	686.32	e-30:2	e-14:1/16:2	686.5220
21	688	686.29	e-30:1, a-30:2	a-14:0/16:2	688.5376
22	690	690.56	e-30:0, a-30:1	a-14:0/16:1	690.5432
23	692	692.53	a-30:0	a-14:0/16:0	692.5589
24	698	698.49	30:4	16:2/14:2	698.4756
25	700	700.52	30:3	16:2/14:1	700.4912
26	702	702.54	30:2	16:0/14:2	702.5069
27	704	703.62	SM(16:0)	Cer 18:1/16:0	703.58
28	704	704.55	30:1	16:0/14:1	704.5225
29	706	706.54	30:0	16:0/14:0	706.5382
30	716	716.57	e-32:1, a-32:2	e-16:1/16:1	716.5589
31	718	718.59	e-32:0, a-32:1	e-16:1/16:0	718.5745
32	720	720.62	a-32:0	a-16:0/16:0	720.5902
33	728	728.54	32:3	16:1/16:2	728.5225
34	730	730.55	32:2	16:0/16:2	730.5382
35	732	732.57	32:1	16:0/16:1	732.5538
36	732	731.64	SM(18:0)	Cer 18:1/18:0	731.61
37	734	734.58	32:0	16:0/16:0	734.5695
38	738	738.58	e-34:4, a-34:5	a-14:0/20:5	738.5433
39	740	740.57	e-34:3, a-34:4	a-14:0/20:4	740.5589
40	742	742.59	e-34:2, a-34:3	a-14:0/20:3	742.5745
41	744	744.63	e-34:1, a-34:2	a-16:0/18:2	744.5902
42	746	746.63	e-34:0, a-34:1	a-16:0/18:1	746.6058
43	748	748.61	a-34:0	a-16:0/16:0	748.6215
44	754	754.55	34:4	16:2/18:2	754.5382
45	756	756.57	34:3	16:1/18:2	756.5538
46	758	758.59	34:2	16:0/18:2	758.5695

47	760	759.62	SM(20:0)	Cer18:1/20:0	759.64
48	760	760.59	34:1	16:0/18:1	760.5851
49	762	762.62	34:0	16:0/18:0	762.6008
50	766	766.55	e-36:4, a-36:5	e-16:1/20:4	766.5745
51	768	768.55	e-36:3, a-36:4	a-16:0/20:4	768.5902
52	770	770.62	e-36:2, a-36:3	e-18:1/18:2	770.6058
53	772	772.65	e-36:1, a-36:2	a-18:0/18:2	772.6215
54	774	774.63	e-36:0, a-36:1	a-18:0/18:1	774.6371
55	776	776.63	a-36:0	a-18:0/18:0	776.6528
56	778	778.55	36:6	16:2/20:4	778.5382
57	780	780.53	36:5	16:1/20:4	780.5538
58	782	782.53	36:4	16:0/20:4	782.5695
59	784	784.56	36:3	18:1/18:2	784.5851
60	786	786.59	36:2	18:0/18:2 & 18:1/18:1	786.6008
61	788	788.57	36:1	18:0/18:1	788.6164
62	790	789.65	SM(22:0)	Cer18:0/22:0	789.68
63	790	790.54	e-38:6	e-16:1/22:6	790.5745
64	790	790.61	36:0	18:0/18:0	790.6321
65	792	792.57	e-38:5, a-36:6	a-16:0/22:6	792.5902
66	794	794.54	e-38:4, a-38:5	e-18:1/20:4	794.5695
67	796	796.84	e-38:3, a-38:4	a-18:0/20:4	796.6215
68	798	798.64	e-38:2, a-38:3	e-18:1/20:2	798.6371
69	800	800.68	e-38:1, a-38:2	a-18:0/20:2	800.6528
70	802	802.68	e-38:0, a-38:1	a-18:0/20:1	802.6684
71	804	804.54	38:7	16:1/22:6	804.5538
72	804	804.69	a-38:0	a-18:0/20:0	804.6841
73	806	806.53	38:6	16:0/22:6	806.5695
74	808	808.53	38:5	16:0/22:5	808.5851
75	810	810.58	38:4	18:0/20:4 & 16:0/22:4	810.6008
76	812	812.60	38:3	18:0/20:3	812.6164
77	814	814.62	38:2	18:0/20:2	814.6321
78	816	816.63	38:1	18:0/20:1	816.6477
79	818	818.57	e-40:6	e-18:1/22:6	818.6058
80	818	818.67	38:0	18:0/20:0	818.6634
81	820	820.61	e-40:5, a-40:6	a-18:0/22:6	820.6215
82	822	822.61	e-40:4, a-40:5	e-18:1/22:4	822.6371
83	824	824.63	e-40:3, a-40:4	a-18:0/22:4	824.6528
84	826	826.61	e-40:2, a-40:3	e-18:1/22:2	826.6321
85	828	828.68	e-40:1, a-40:2	a-18:0/22:2	828.6841
86	830	830.68	e-40:0, a-40:1	e-18:1/22:0	830.6997
87	832	832.70	a-40:0	a-18:0/22:0	832.7154
88	830	830.55	40:8	18:2/22:6	830.5695
89	832	832.57	40:7	18:1/22:6	832.5851
90	834	834.59	40:6	18:0/22:6	834.6008
91	836	836.59	40:5	18:0/22:5	836.6164
92	838	838.62	40:4	18:2/22:4	838.6321
93	840	840.65	40:3	18:1/22:2	840.6477
94	842	842.68	40:2	18:0/22:2	842.6634
95	844	844.72	40:1	18:1/22:0	844.6790
96	846	846.73	40:0	18:0/22:0	846.6947

97	848	848.66	e-42:6	e-18:1/24:6	848.6528
98	850	850.66	e-42:5	e-18:1/24:4	850.6684
99	852	852.68	a-42:4	a-18:0/24:4	852.6841
101	852	852.59	42:11	20:4/22:5	852.5538
102	854	854.72	a-42:3	e-18:1/24:2	854.6997
103	854	854.57	42:10	20:4/22:6	854.5695
104	856	856.73	a-42:2	a-18:0/24:2	856.7154
105	856	856.59	42:9	20:4/22:5	856.5851
106	858	858.75	a-42:1	a-18:1/24:0	858.7310
107	859	858.59	42:8	18:2/24:6	858.6008
108	861	860.57	42:7	18:1/24:6	860.6164
109	860	860.72	a-42:0	a-18:0/24:0	860.7467
110	862	862.61	42:6	18:0/24:6	862.6321
111	864	864.63	42:5	18:0/24:5	864.6477
112	866	866.66	42:4	18:2/24:4	866.6634
113	868	868.69	42:3	18:1/24:2	868.6790
114	870	870.73	42:2	18:0/24:2	870.6947
115	872	872.73	42:1	18:1/24:0	872.7103
116	874	874.75	42:0	18:0/24:0	874.7260
117	878	878.59	44:12	20:6/24:6	878.5695
118	880	852.58	44:11	20:6/24:5	852.5565
119	882	882.59	44:10	20:4/24:6	882.6008
120	886	886.61	44:8	20:4/24:4	886.6321
121	888	888.61	44:7	20:2/24:5	888.6477
122	890	890.62	44:6	20:1/24:6	890.6634
123	892	892.63	44:5	20:0/24:5	892.6790
124	894	894.65	44:4	18:0/26:4	894.6947
125	896	896.68	44:3	20:1/24:2	896.7103
126	898	898.70	44:2	20:0/24:2	898.7260
127	900	900.72	44:1	18:0/26:1	900.7416
128	902	902.73	44:0	18:0/26:0	902.7573

Table S1: Mass spectrometry analysis of PC and sphingomyelin (SM) molecular species in the bloodstream form of *T.brucei*

^a [M+H]⁺ ions over charge.

^b HR [M+H]⁺ ions over charge (high resolution survey scan) $\pm 0.05m/z$

^c Peak identities refer to total number of carbon atoms and double bonds. The peaks containing the most abundant PC/SM species are in bold.

^d Defined here as the [M+H]⁺ ion within the series with the greatest intensity. Precise fatty acyl constituents and their positions are not discernable in positive ion mode; however, where possible, the most likely fatty acid candidates (in parentheses) for the *sn*-1 and *sn*-2 constituents of the molecular species presented were deduced from the available literature.

^e All of the molecular species detected within the area of the peak are contained within the lowest and highest mass range outliers listed, the degree of unsaturation decreases by one from the lowest mass in each series until the highest mass is met. e = plasmenyl (alkenylacyl); a = plasmanylyl (alkylacyl)

SUPPLEMENTARY FIGURE LEGENDS

Figure S1; (A) ClustalW alignment of the predicted amino acid sequences of the *T. brucei* nSMase with nSMases from other eukaryotes: *Leishmania major* (XP 001681949), *Trypanosoma cruzi* (XP 814233), *Homo sapiens* (CAA10995), *Saccharomyces cerevisiae* (NP 010935) and *Apis mellifera* (XP 395633). Identical residues are shown in reverse type with black background, conserved residues are shown in reverse type with grey background. Residues required for catalytic activity, substrate binding, and Mg²⁺ binding, in other homologues are indicated by asterisks. The two putative transmembrane helices are underlined. The Endonuclease/Exonuclease/phosphatase Pfam motif PF03372 covers residues 5 – 364.

(B) Phylogenetic analysis using ClustalW of the nSMase homologues from Figure 1A.

Figure S2; Activity of recombinant *TbnSMase*

Enzyme activity of *TbnSMase* activity in washed *E.coli* membranes expressing either GST-*TbnSMase* (1) or N-terminal-His₆-*TbnSMase* (2) or empty vector (3).

Figure S3; Further examples of ER localisation of *TbnSMase-HA^{Ti}* in bloodstream form *T. brucei*. Fixed *TbnSMase-HA^{Ti}* cells were incubated with rat anti-HA antibody, and rabbit anti-rat FITC conjugated antibody and DNA stained with DAPI.

FIGURE S4; RT-PCR analysis: Total RNA from procyclic cells (Lane 2), bloodstream form cells (Lane 3). The RT-PCR was performed with *TbnSMase* orf primers, as detailed in *Experimental procedures*.

Figure S5: Lipids were extracted from *T. brucei* wild type (A) and *Tb nSMase* conditional knockout without tetracycline for 42h (B) and analysed by ES-MS-MS for choline-phosphate containing phospholipids by collision induced fragmentation in positive ion mode of parents of 184m/z in wild type cells (A) and *Tb nSMase* conditional knockout without tetracycline for 42h (B).

Figure S6: Lipids were extracted from *Tb nSMase* conditional knockout without tetracycline for 42h and analysed by negative ion mode ES-MS (A) and ES-MS-MS by collision induced fragmentation in negative ion mode of parents of 241m/z for PI species (B), parents of 196m/z for PE species (C) and neutral loss of 87m/z for PS species (D) as described in *Experimental procedures*.

Figure S7: ES-MS-MS daughter ion spectrum of the m/z 771 ion (A), m/z 670 ion (B) and collision induced fragmentation in negative ion mode of parents of 241m/z for Ino-phosphate containing species from the lipid extract of the P100 fraction of *Tb nSMase* conditional knockout without tetracycline for 42h as described in *Experimental procedures*.

Figure S8: Further examples of TEM of wild-type bloodstream form *T. brucei* (A-D) and *TbnSMase* cKO (-tet) showing a big-eye phenotype. . FL, flagellum; FP, flagellar pocket; K, kinetoplast; M, mitochondria; G, glycosome; *, enlarged concentric membrane structure, may be part of the endosomal network.

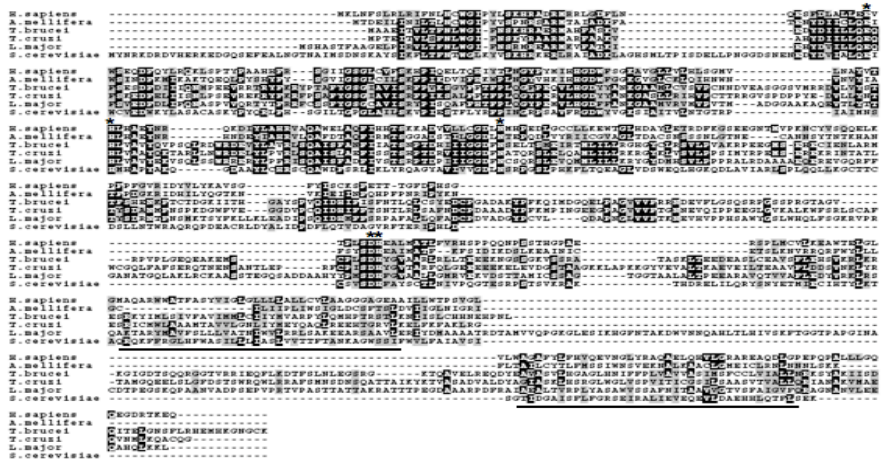
Figure S9: (A) Coomassie stain protein gel of the IP [³⁵S]methionine labelled P67 for Figure 7A, Wild type (lanes 2 and 3) and *TbnSMase* cKO cells grown in the absence of tetracycline for 42 hours (4 and 5). Lanes 6 and 7 show the coomassie blue stain of the total protein [³⁵S]-methionine labelling with the corresponding autoradiograph (lanes 8 and 9). (B) Anti-VSG Western of VSG in total lysate (lanes 1 and 2) and sVSG preparations (lanes 3 and 4) from wild type (lanes 1 and 3) and *TbnSMase* cKO cells grown in the absence of tetracycline for 42 hours (2 and 4).

Figure S10; Further examples of the morphological “Big-Eye” phenotype of the *TbnSMase* conditional knockout grown in the absence of tetracycline for 42 hours are shown as merged fluorescence-phase images of DAPI and anti-trypanopain.

Figure S11; Anti-PDI-2 western of wild type (lane 1) and *TbnSMase* cKO cells grown in the absence of tetracycline for 42 hours (lane 2).

Figure S1

A



B

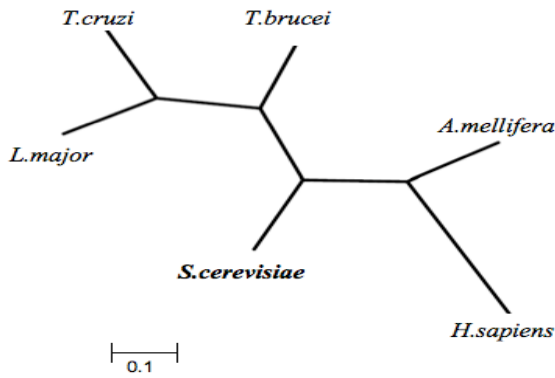


Figure S2

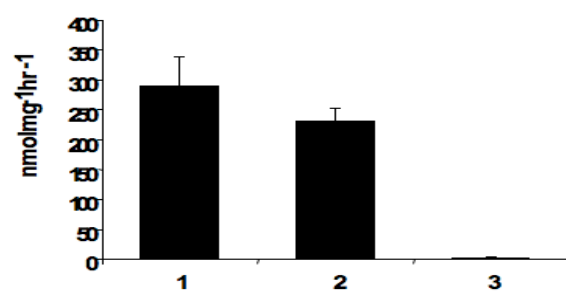


Figure S3

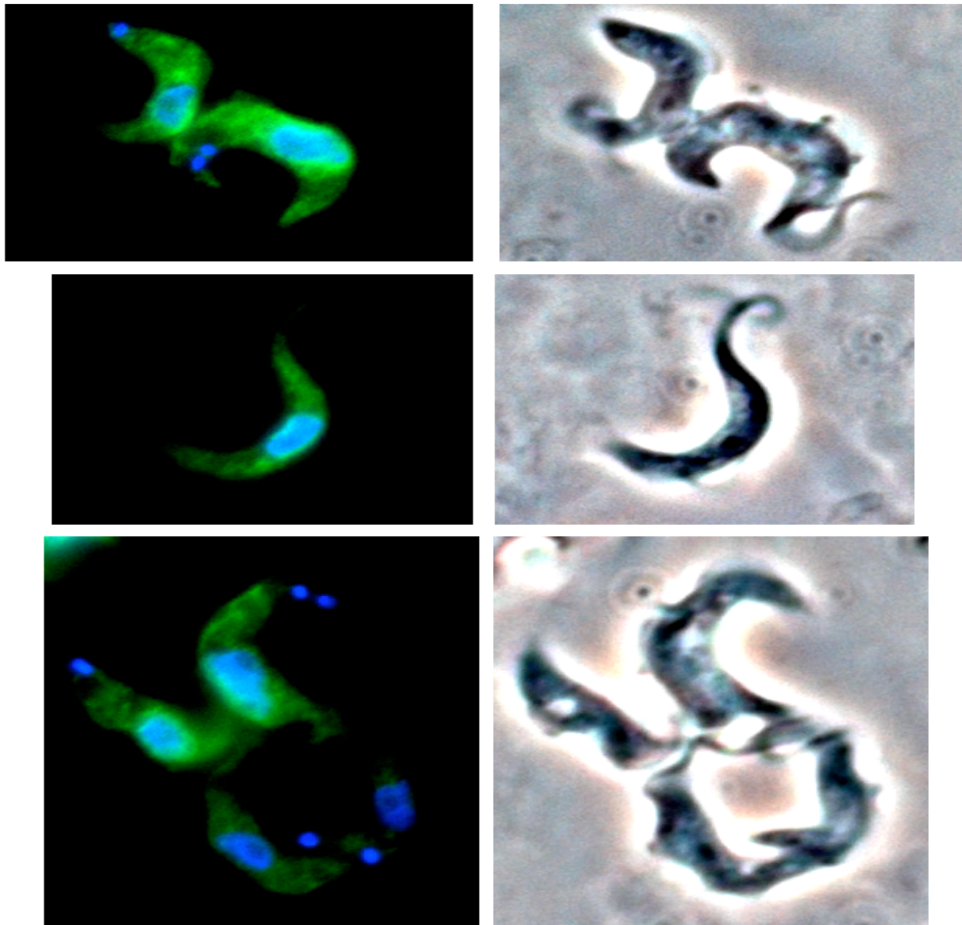


Figure S4

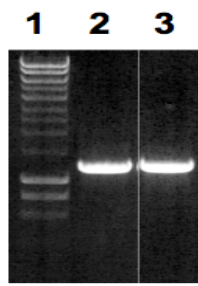


Figure S5

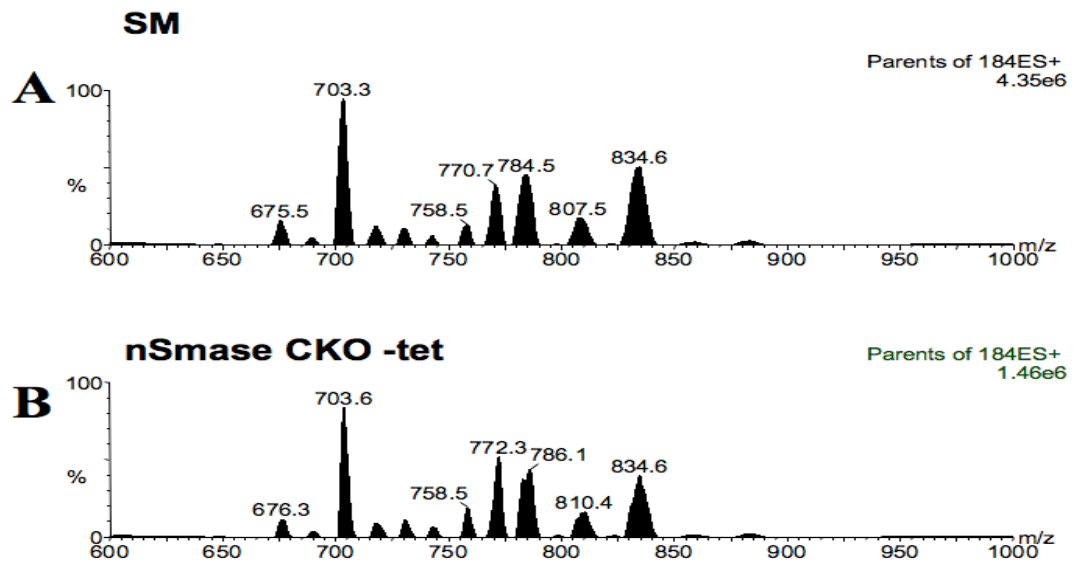


Figure S6

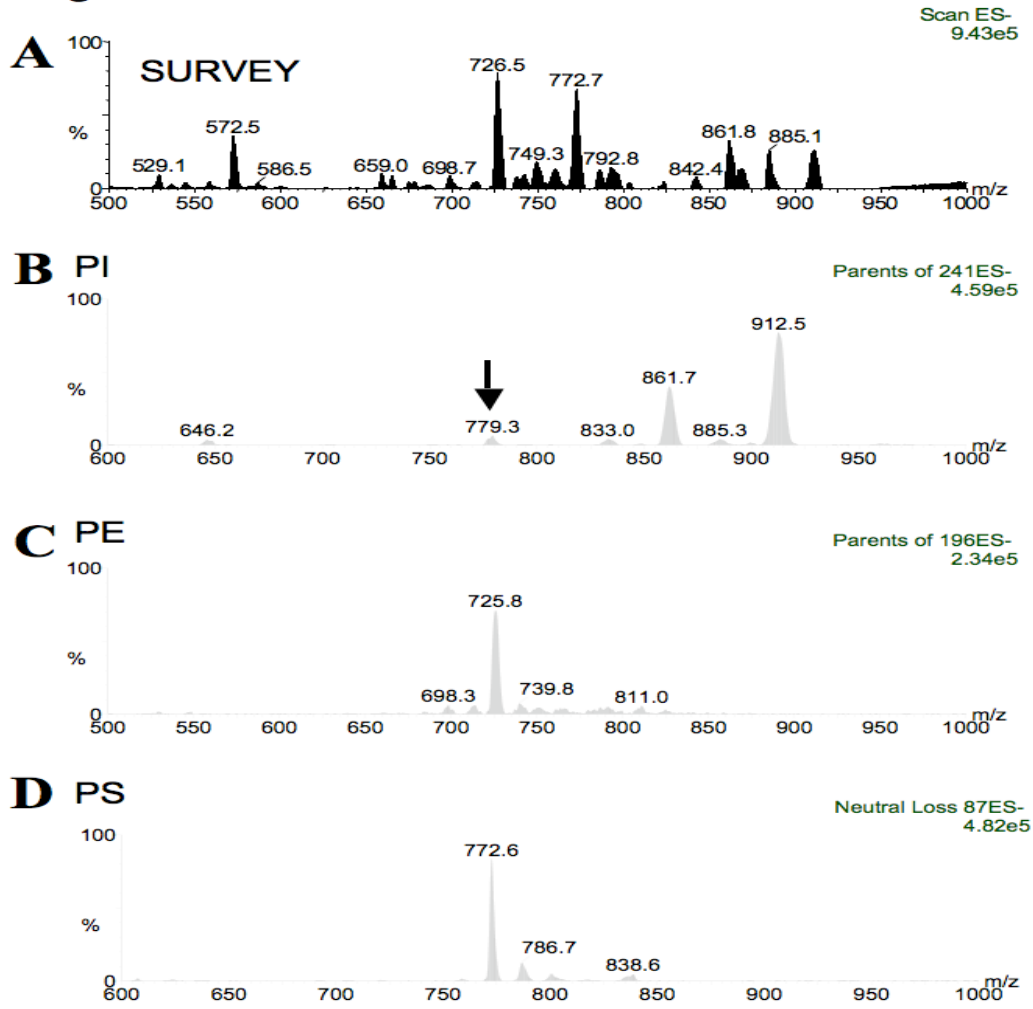


Figure S7

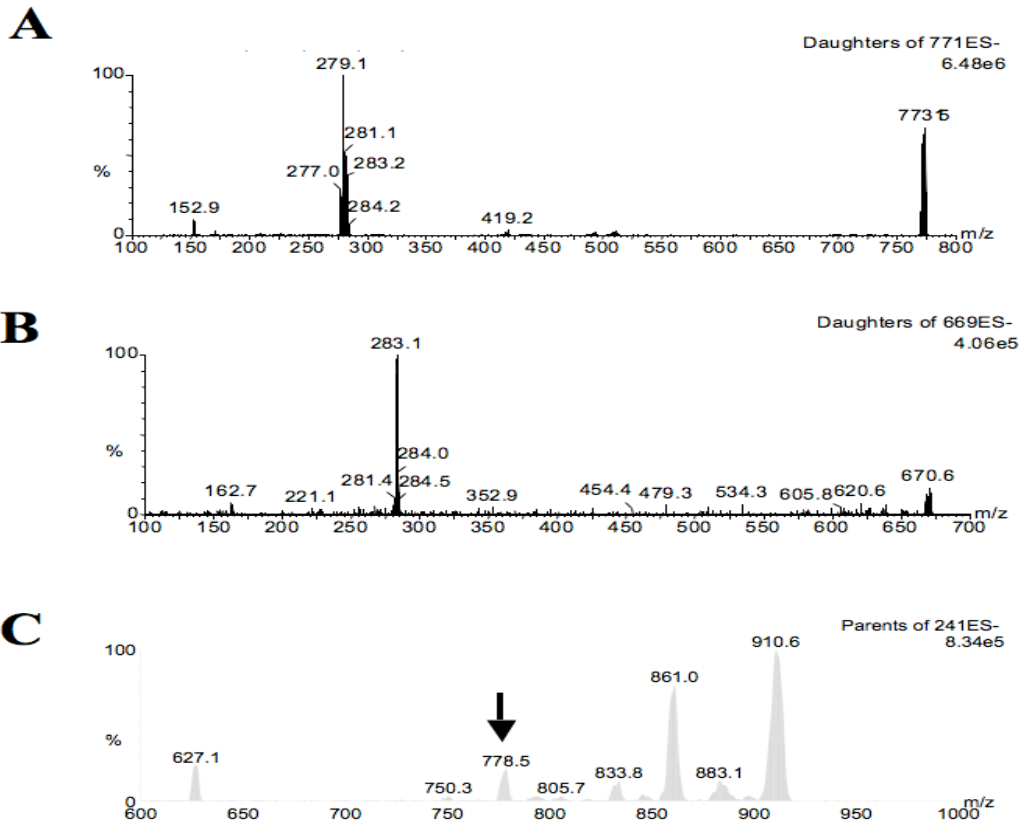


Figure S8

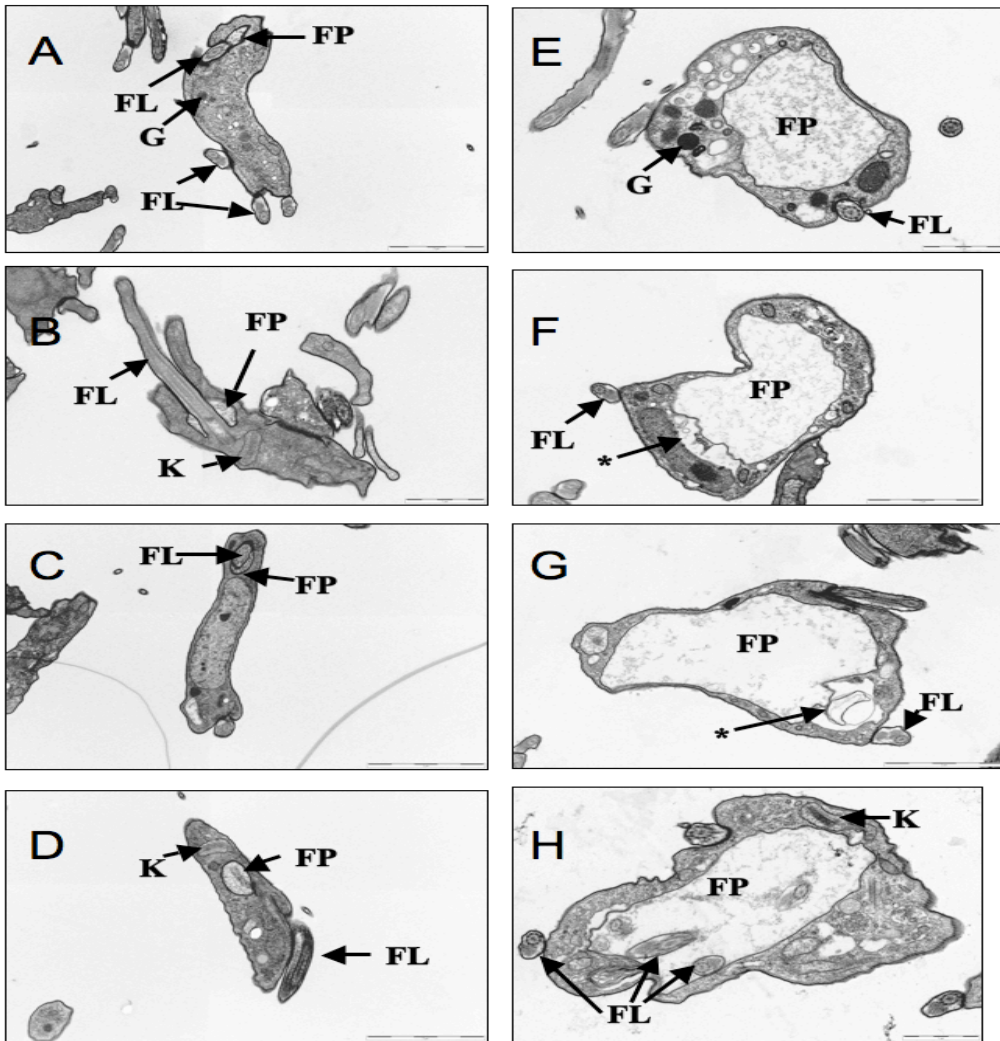
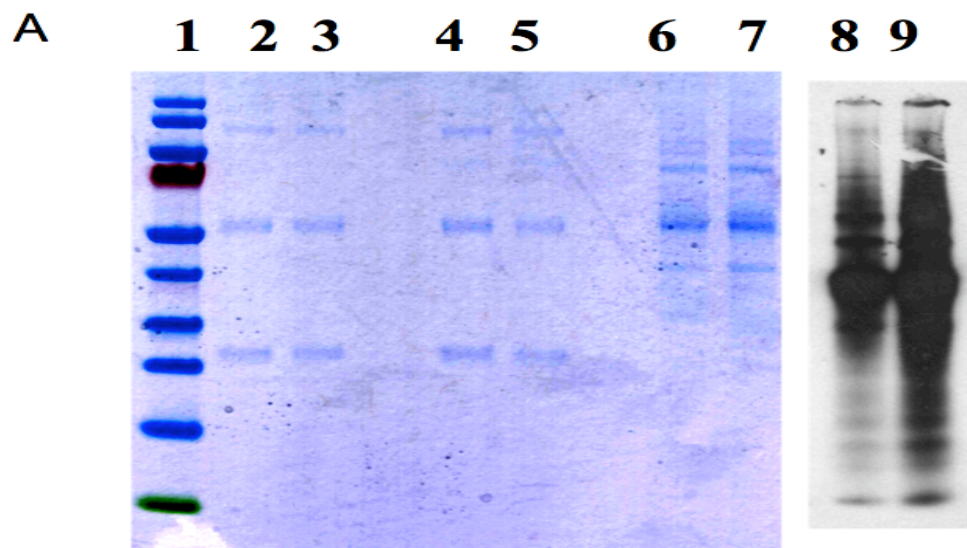


Figure S9



B

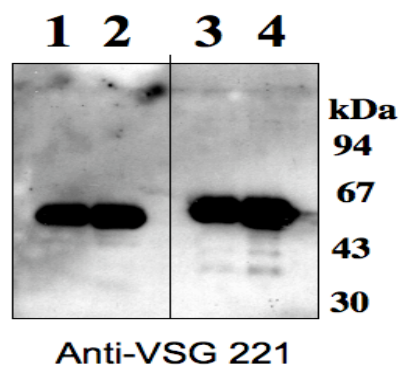


Figure S7

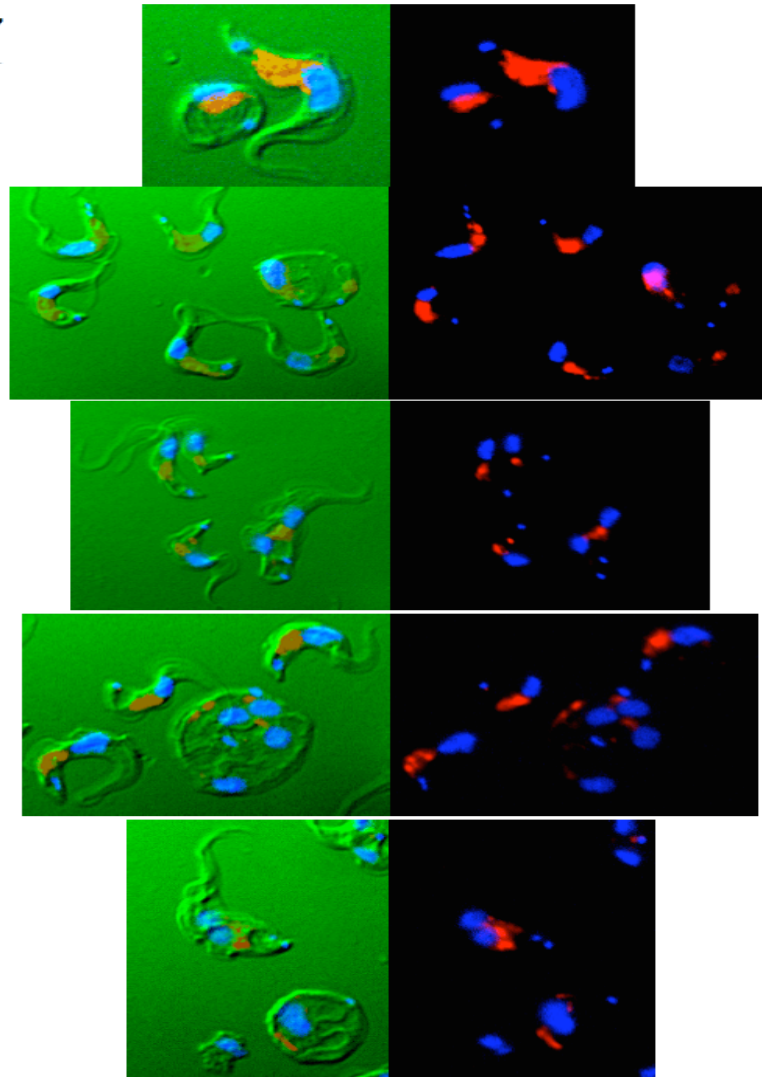


Figure S11

