Supporting Information

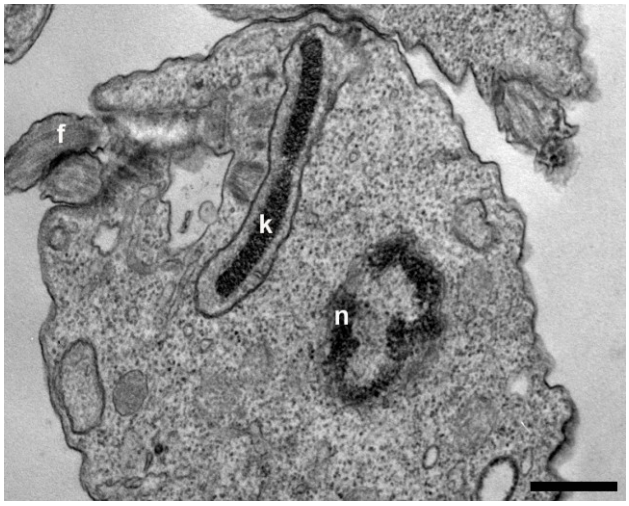

FIG S1: Transmission electron microscopy image of untreated *T. cruzi* epimastigote with normal ultrastructure. Kinetoplast (k), nucleus (n), and flagellum (f) are indicated.

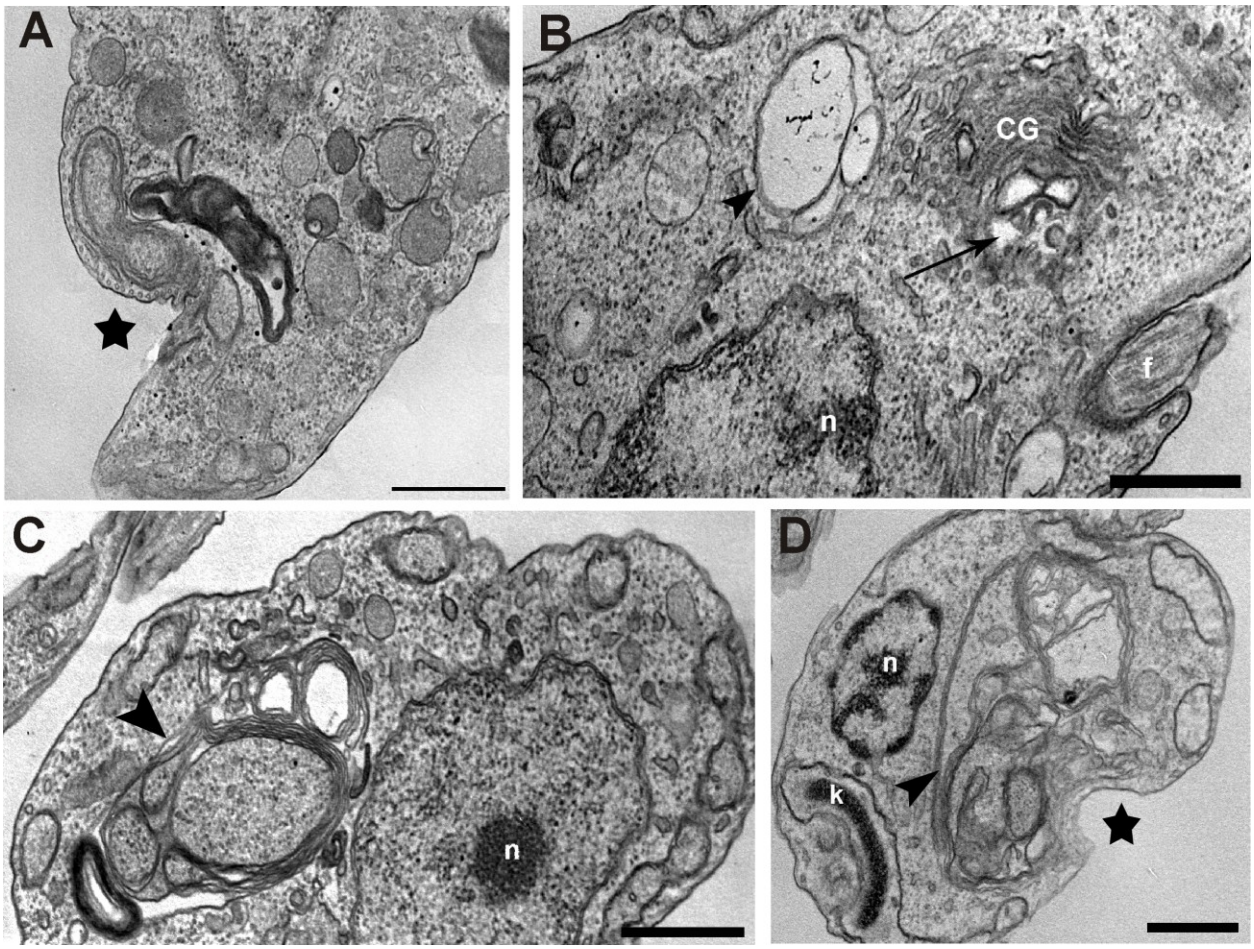

FIG S2: Transmission electron microscopy images of *T. cruzi* epimastigotes treated with 4.6 µM SQ109 for 24 hours (A, B) and 48 hours (C,D) showing Golgi complex alterations (arrow), vacuole and autophagosome formation (arrowhead) and plasma membrane depression (stars). Bars: 0.5 µm.

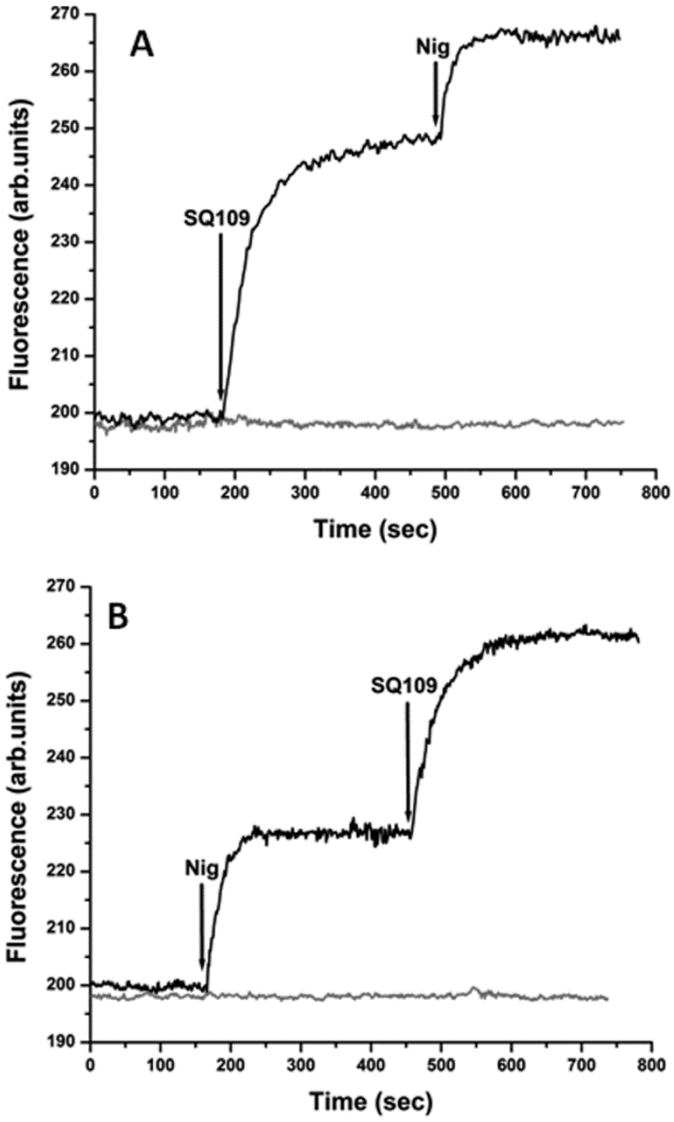

FIG S3: Effects of SQ109 and nigericin onacridine orange fluorescence (increasing fluorescence correlates with alkalinization). A, SQ109 (10 μM) followed by nigericin (10 μM). B, Nigericin (10 μM) followed by SQ109 (10 μM).

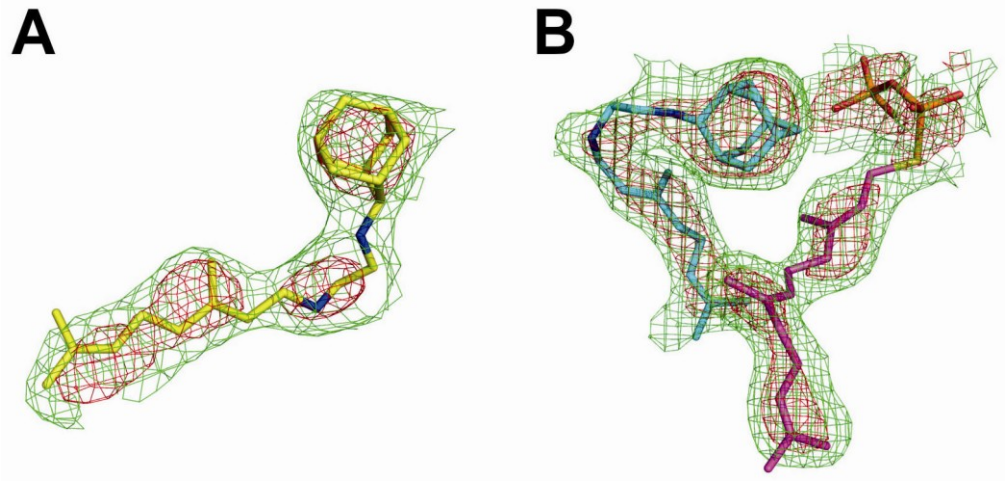

FIG S4: Fo-Fc omit electron density maps contoured at 1□ (green) and 3□ (red).**A**, SQ109 in HsSQS. **B**, FSPP+SQ109 in TcSQS.

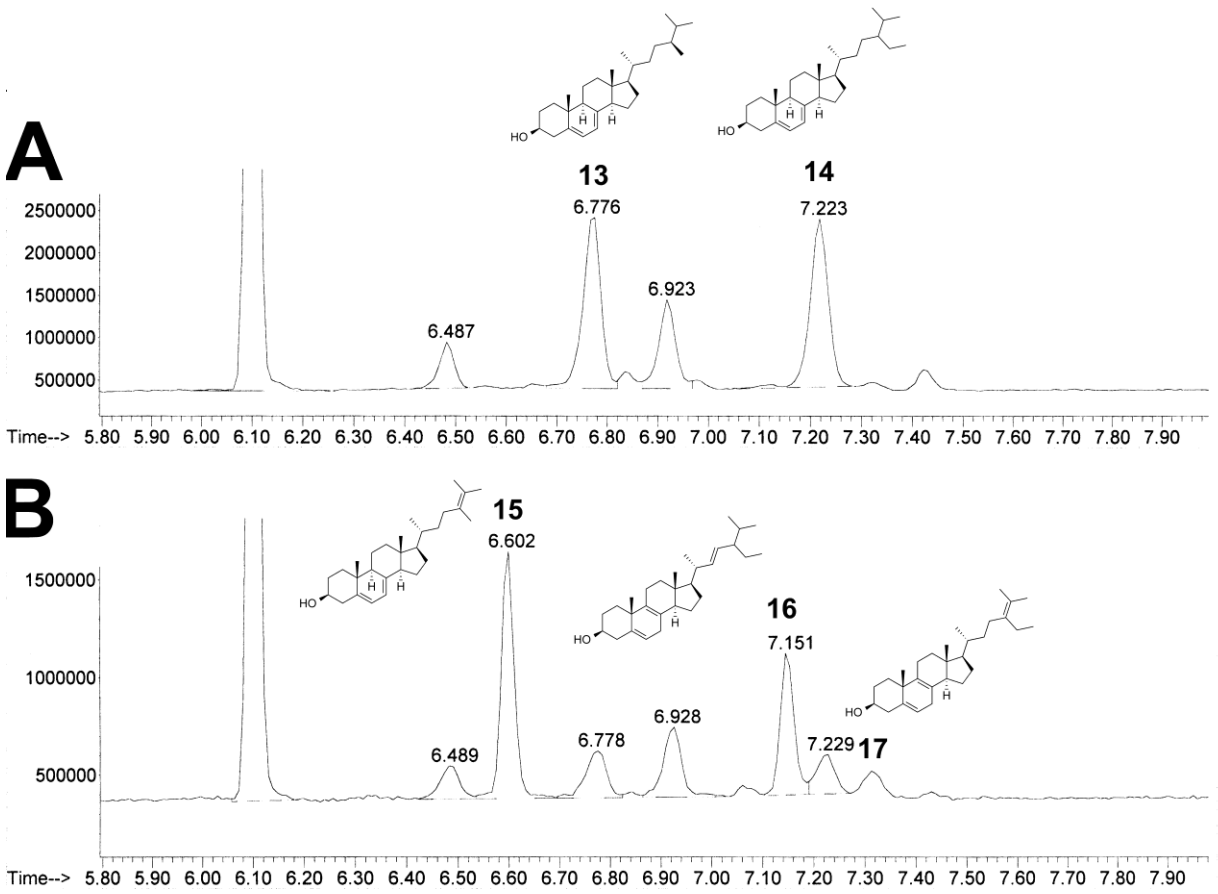

FIG S5: GCMS results for *T. cruzi* epimastigote sterols (as the trimethylsilyl ethers) without and with SQ109 treatment. A, Without SQ109. B, With SQ109 (6 μM). The major sterols in the untreated cells have saturated side-chains (**13,14**). New species (**15,16,17**) appear in the presence of SQ109 and have unsaturated side-chains.

Table S1. Summary of data processing and refinement statistics of TcSQS complex crystals.

Name	TcSQS-SQ109	HsSQS-SQ109
PDB code	3WSB	3WSA
Data collection		
Resolution (Å)	25-2.40 (2.49-2.40)	25-2.90 (3.00-2.90)
Space group	$P2_12_12_1$	$P2_1$
Unit-cell		
a / b / c (Å)	79.36 / 130.57 / 144.63	85.75 / 154.01 / 90.62
β (°)	90.00	91.38
No. of reflections		
Measured	281502 (25911)	190318 (16426)
Unique	57874 (5288)	48463 (4212)
Completeness (%)	97.1 (90.0)	93.5 (81.1)
R_{merge} (%)[a]	5.7 (44.4)	11.1 (41.4)
Mean $I/\sigma(I)$	26.1 (2.3)	10.9 (1.8)
Multiplicity	4.9 (3.8)	3.9 (2.9)
Refinement		
R_{work}(%)	21.6 (29.6)	20.6 (31.3)
R_{free}(%)	27.7 (34.1)	26.1 (33.0)
Geometry deviations		
Bond lengths (Å)	0.007	0.004
Bond angles (°)	1.16	0.77
No. of atoms / Mean B-values (Å^2)		
Protein atoms	11005 (45.7)	15965 (44.0)
Water molecules	433 (57.1)	221 (30.3)
SQ109 atoms	96 (70.3)	96 (65.3)
FSPP atoms	96 (67.3)	
Ramachandran plot (%)		
Most favored	94.0	94.5
Additionally allowed	5.8	5.2
Disallowed	0.2	0.4

Values in parentheses are for the highest resolution shell.

[a] $R_{merge} = \sum_{hkl}\sum_i|I_i(hkl)-\langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$.