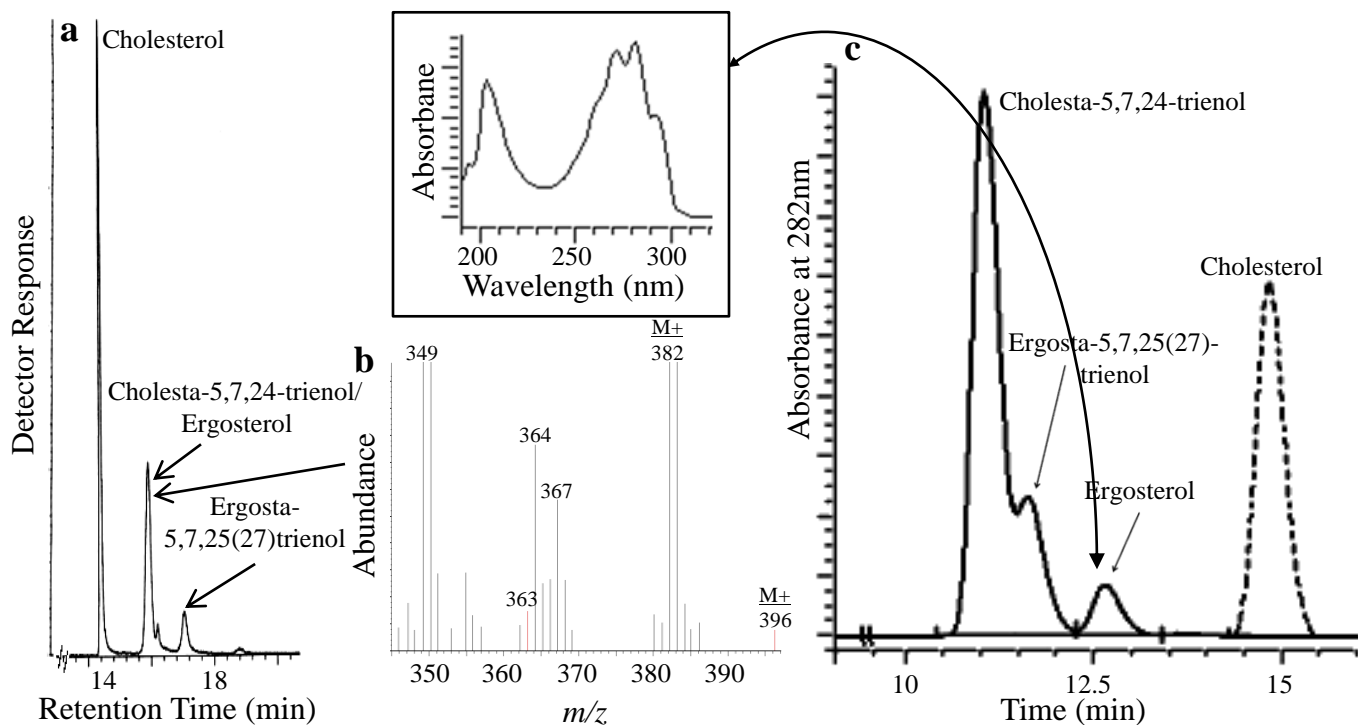


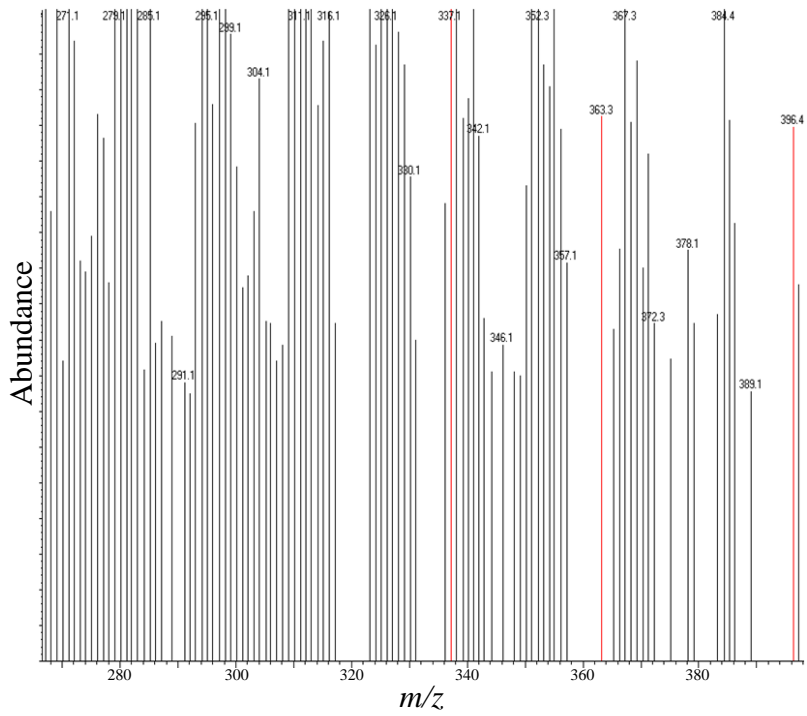
## Supplemental Information

### Discovery of an ergosterol-signaling factor that regulates *Trypanosoma brucei* growth

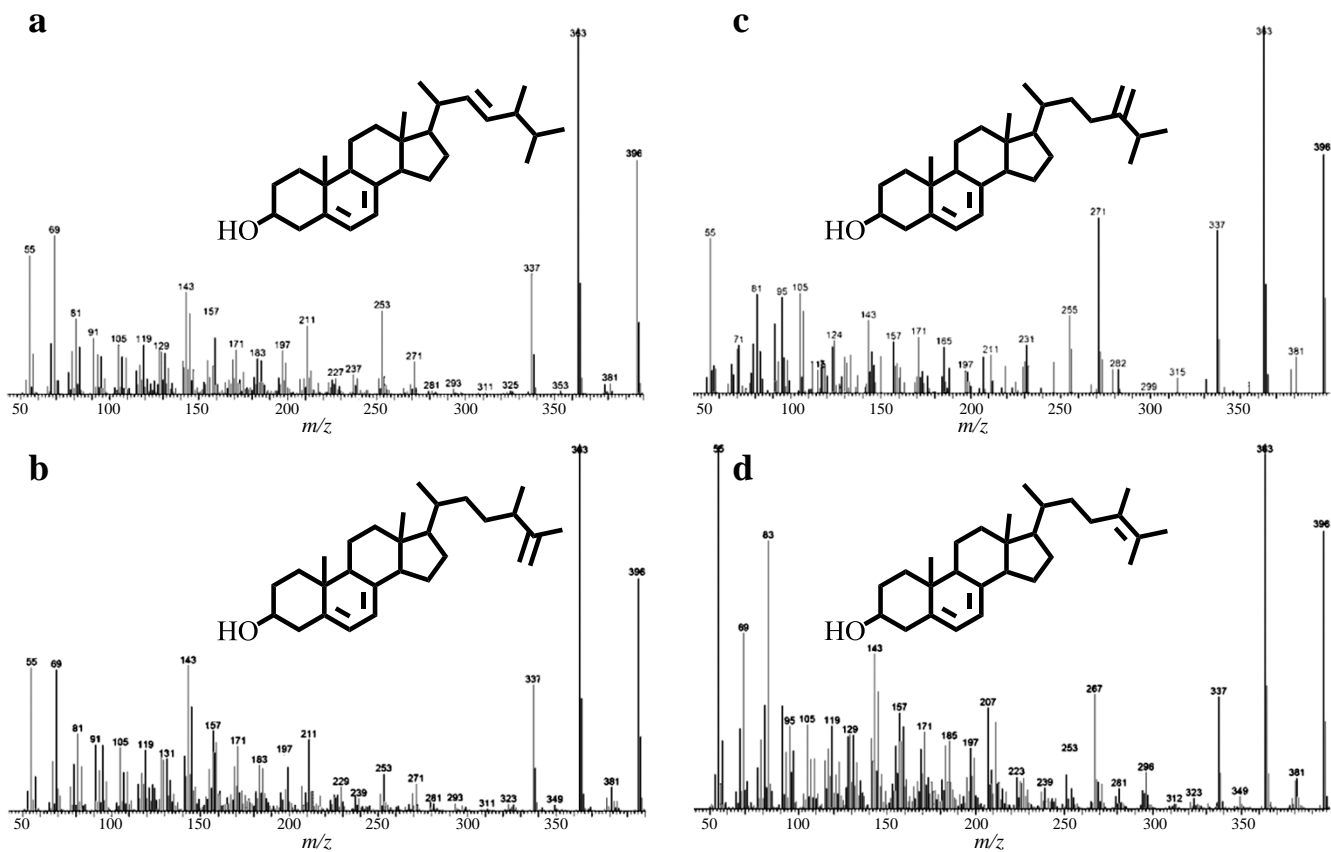
Brad A. Haubrich,<sup>1\*</sup> Ujjal K. Singha,<sup>2\*</sup> Matthew B. Miller,<sup>1‡</sup> Craigen R. Nes,<sup>1‡</sup> Hosanna Anyatonwu,<sup>1‡</sup> Laurence Lecordier,<sup>3</sup> David J. Leaver,<sup>1,4</sup> Presheet Patkar,<sup>1</sup> Fernando Villata,<sup>2</sup> Benoit Vanhollebeke,<sup>3</sup> Minu Chaudhuri<sup>2</sup> and W. David Nes<sup>1¶</sup>



Supplementary Figure 1. SF1. Sterol analysis of procyclic cells cultured in FGM. (A) GC-MS analysis of neutral lipids. (B) High end mass spectrum (enhanced) of GC peak corresponding to cholesta-5,7,24-trienol and ergosterol. (C) HPLC chromatogram of neutral lipids of PCF cultured in FGM (Inset show the UV spectrum of the compound eluting at 12.6 min). See text for details of sterol analysis.

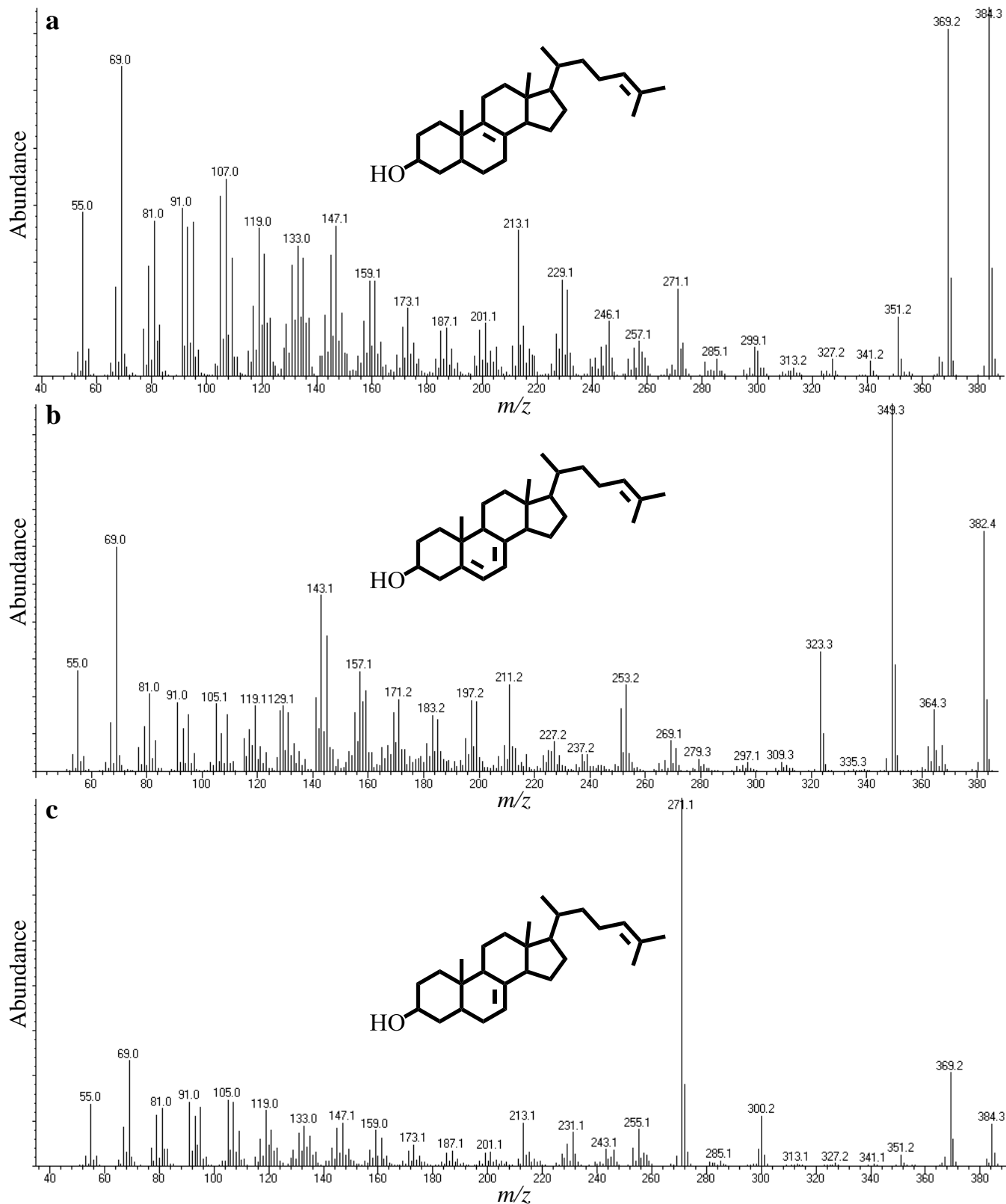


Supplementary Figure 2. SF2. Mass spectrum of ergosterol purified by HPLC from procyclic cells cultured in FGM.

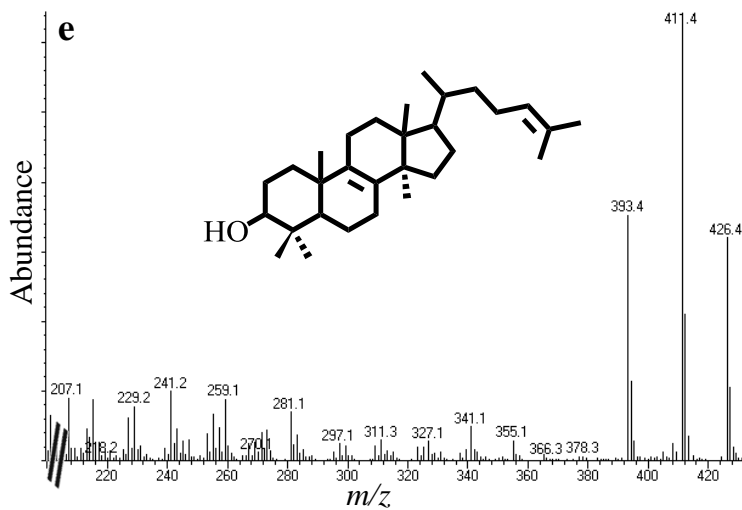
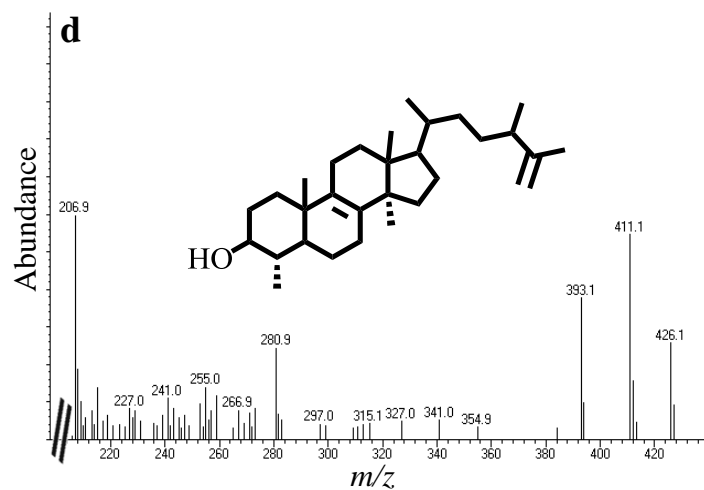
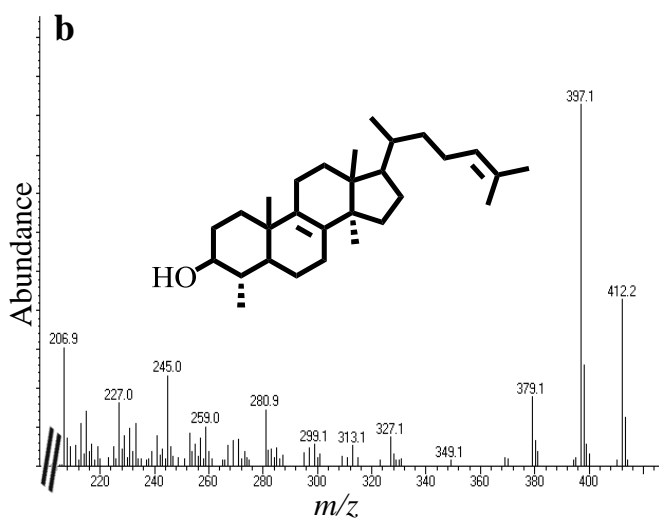
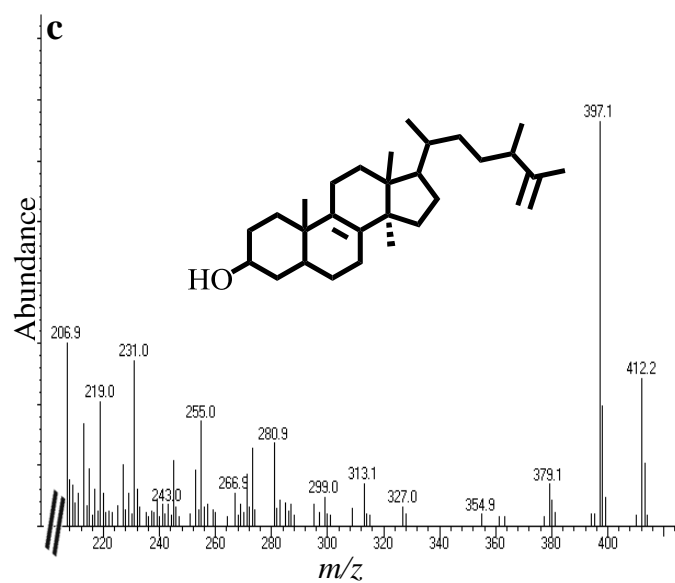
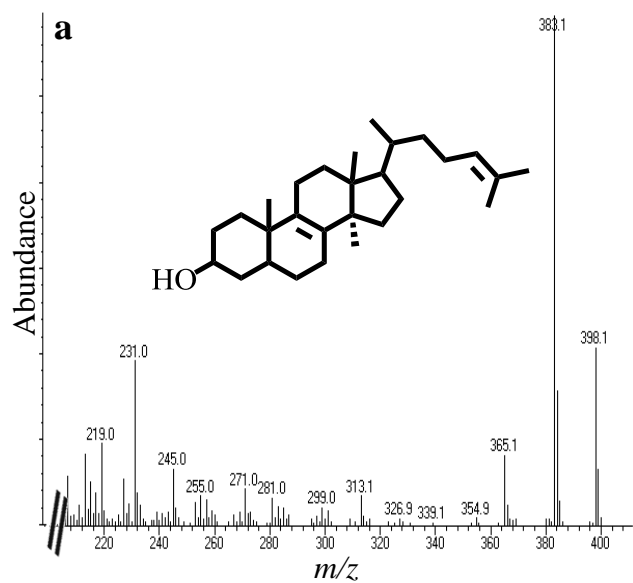


Supplementary Figure 3. SF3. Mass spectra of ergosterol isomers (retention times in GC and HPLC are reported in reference 4 in the text and serve as standards) isolated from procyclic cells cultured in LDM.

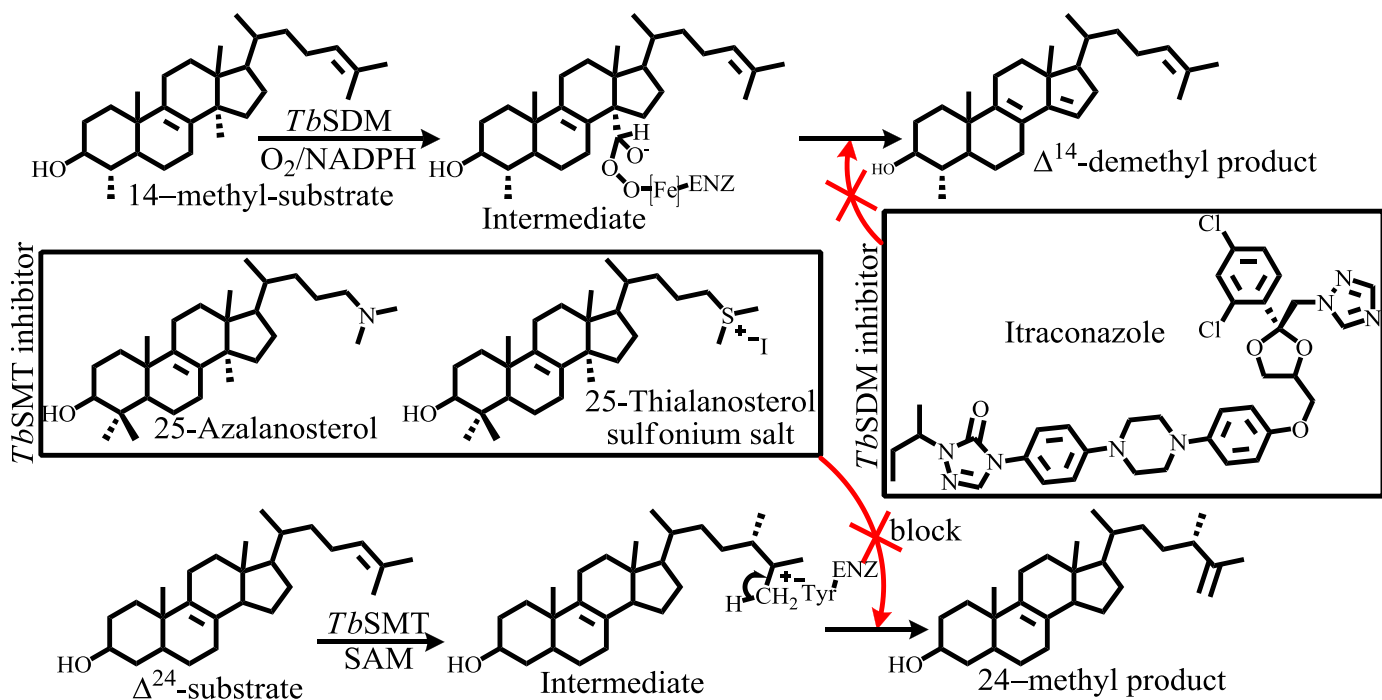
(A) Ergosta-5,7,22-trienol (Ergosterol), RRTc, 1.10; (B) Ergosta-5,7,24(28)-trienol, RRTc, 1.19, (C) Ergosta-5,7,25(27)-trienol (tryptosterol), RRTc, 1.18, and (D) Ergosta-5,7,24(25)-trienol, RRTc, 1.27. Retention times are relative to the retention time of cholesterol.



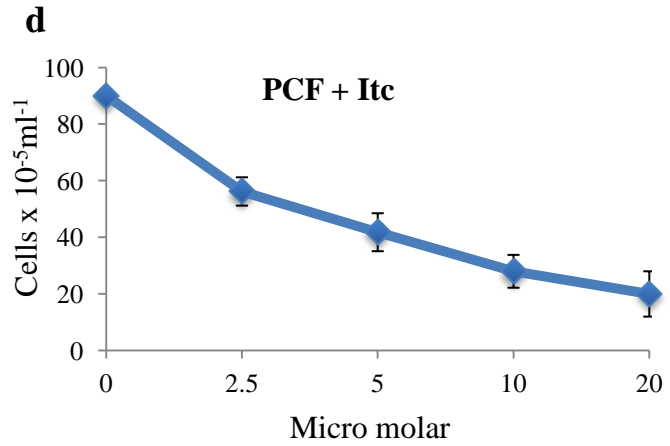
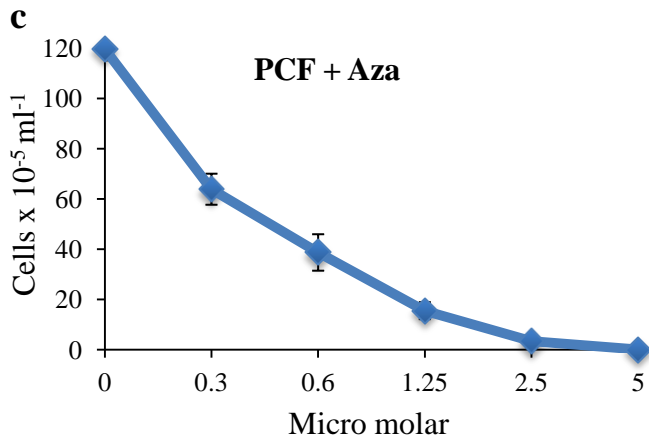
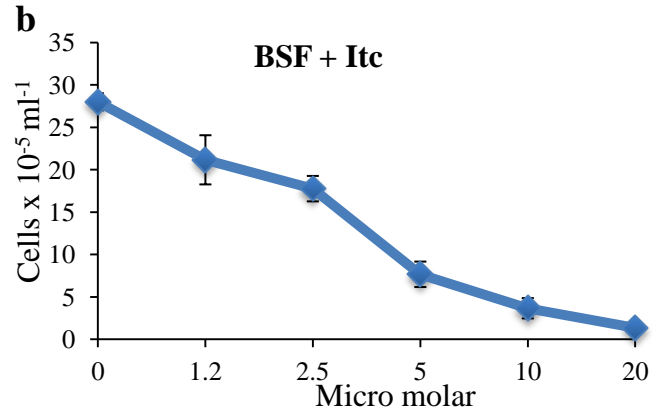
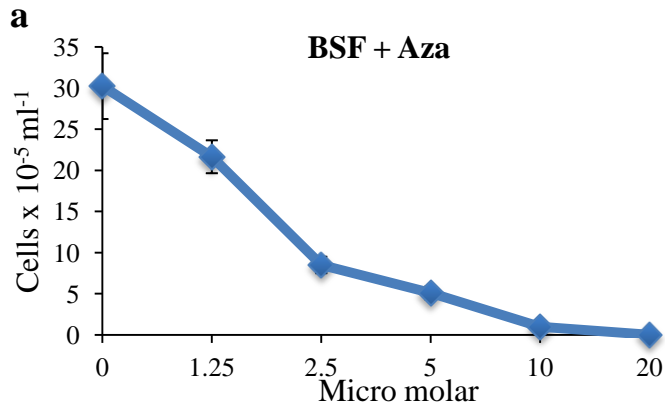
Supplementary Figure 4. SF4. Mass spectra of major sterols isolated from DOX-induced TbSMT RNAi lines or after treatment with 25-azalanosterol or 25-thialanosterol sulfonium salt.



Supplementary Figure 5. SF5. Mass spectra of major sterol detected in *TbSDM* or itraconazole treated cultures

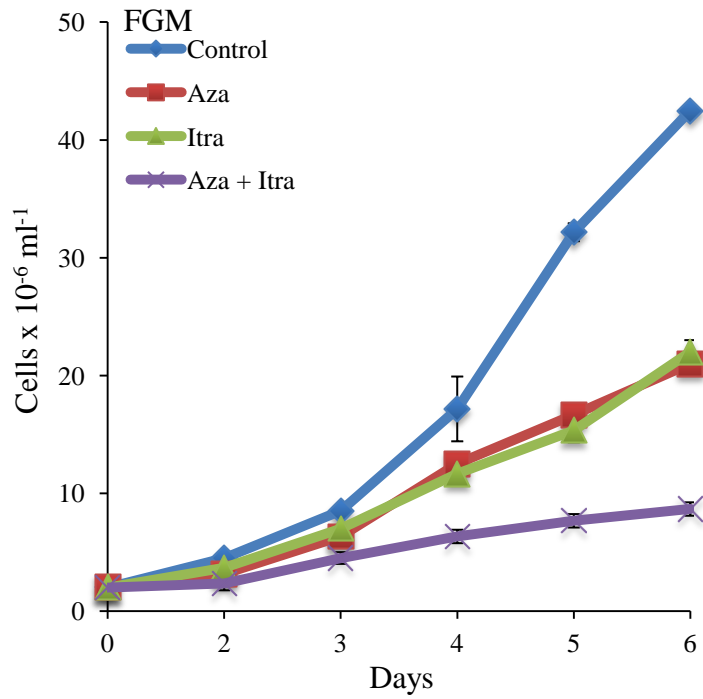


Supplementary Figure 6 SF. Proposed conversion of substrate to product in the biosynthesis of ergosterol.

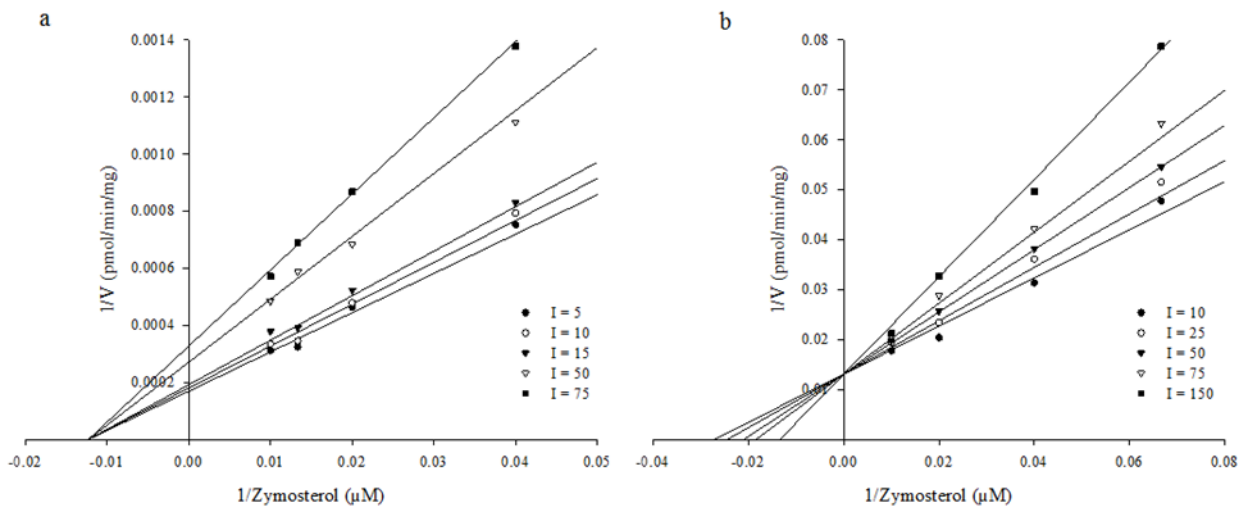


Supplementary Figure 7. SF7. Growth curves of bloodstream form (BSF) and procyclic form (PCF) in FGM treated with 25-azalanosterol (Aza) and itraconazole (Itc).

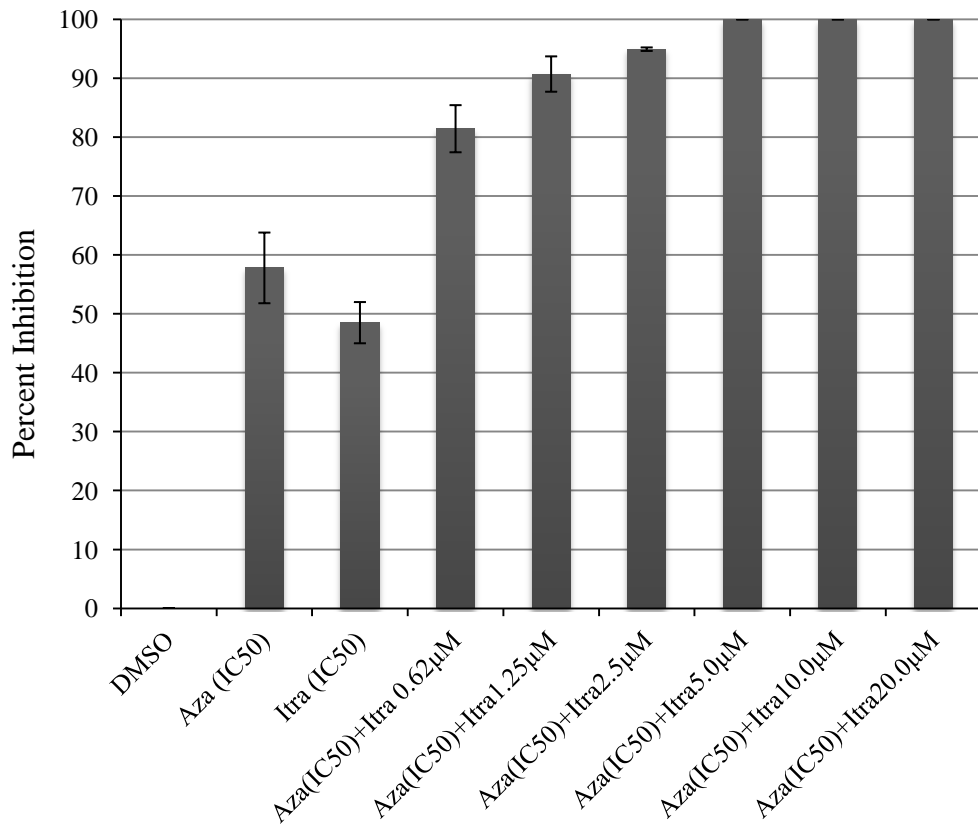




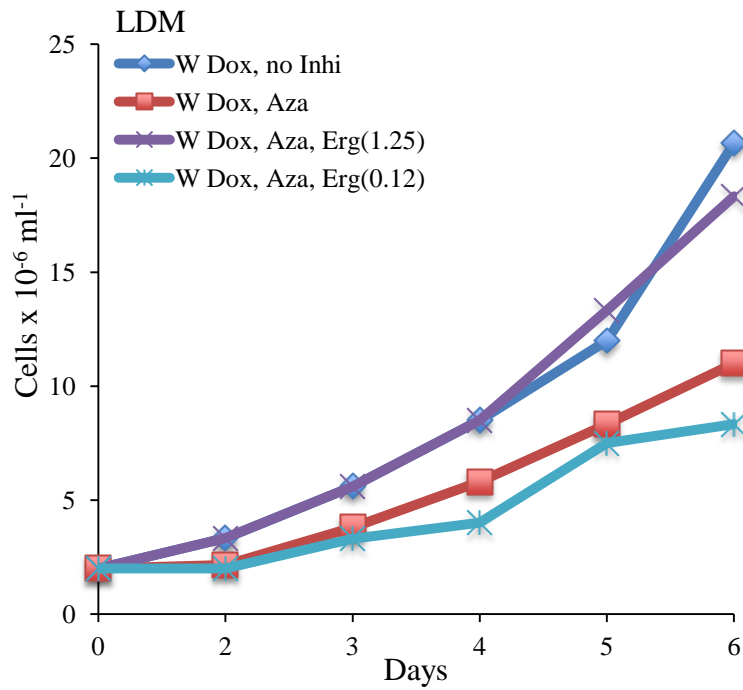
Supplementary Figure 8. SF8. Growth curve of treated wild-type procyclic form cells cultured in full-growth media (FGM). 25-azalanosterol (Aza) is supplied at 1  $\mu$ M and itraconazole (Ira) is supplied at 1  $\mu$ M to the media as shown.



Supplementary Figure 9. SF9. Inhibition kinetics of *TbSMT* against substrate analog inhibitors. In A- inhibitor (I) is 25-thialanosterol and in B- inhibitor is lanosterol. Activity assays were performed for 45 min at 35 °C and pH 7.5; see text for complete details of enzyme assays.



Supplementary Figure 10. SF10. Histogram of bloodstream form showing effect of increasing concentrations of itraconazole (Ira) against fixed concentrations of 25-azalanosterol (Aza).



Supplementary Figure 11. SF11. Growth curve of procyclic form *TbSMT* RNAI cells cultured with doxyxycycline (W, DOX) in full growth media and ergosterol at increasing concentrations of ergosterol (ergosterol at 12  $\mu$ M grew in a manner similar to cultures supplied 1.2  $\mu$ M ergosterol, data not shown).

Supplemental Table 1. Sterol composition of procyclic *Trypanosoma brucei* cells.

Sterol	Percent Total Sterol								
	A	B	C	D	E	F	G	H	I
Cholesterol	20	21	51	43	91	83	87	76	85
Zymosterol	1	tr		20	2				
4 $\alpha$ -Methyl cholesta-8,24-dienol	0.1				tr.				
4 $\alpha$ -Methyl cholesta-8,14,24-trienol	tr.								
Cholesta-5,7,24-trienol	50	52	35	23	4			22	
Ergosterol	0.3	tr.	4	ND	ND			tr	tr
Cholesta-7,24-dienol	8	9	3		tr			1	
Ergosta-8,25(27)-dienol	1								
31-Norlanosterol	0.3								2
Fecosterol	0.1								
14 $\alpha$ -Methylzymosterol				3					11
14 $\alpha$ -Methylergosta-8,25(27)-dienol				2		11	8		
4 $\alpha$ ,14 $\alpha$ -Dimethylergosta-8,25(27)-dienol						1	1		tr.
Ergosta-5,7,25(27)-trienol	14	12	8	8	tr				1
Ergosta-5,7,24(28)-trienol	0.1								
Ergosta-5,7,24-trienol	3	3	tr.						tr.
24-Dimethylergosta-5,7,25(27)-trienol	tr.								
Lanosterol	1	tr		1		5	4		1
Others	1.1	tr	tr	tr	3	tr	tr	1	tr

**A**-Detailed sterol composition of previously determined PCF strain 427 cultured in LDM as reported in reference 4- all remaining sterol analyses are of studies performed herein for strain 427; **B**- PCF cultured in LDM; **C**- PCF cultured in FGM; **D**- PCF treated with 25-azalanosterol at ED50; **E**- PCF treated with 25-azalanosterol at IC90; **F**- PCF treated with itraconazole at ED50; **G**- PCF treated with itraconazole at ED90; **H**- PCF DOX-induced *TbSMT* RNAi cell line; and **I**- PCF DOX-induced *TbSDM* RNAi cell line. ND- not detected within our limits of GC-MS detection; tr, trace amounts of sterol at <1% of total sterol. 25-Thialanosterol sulfonium salt-treated cells generated sterol compositions very similar to 25-azalanosterol-treated cells and therefore are not shown in the table. See text for details on culture conditions.