Supporting information for "Tracking autophagy during proliferation and differentiation of *Trypanosoma brucei*." by William R. Proto[1], Nathaniel G. Jones[1], Graham H. Coombs[2], and Jeremy C. Mottram[1*]

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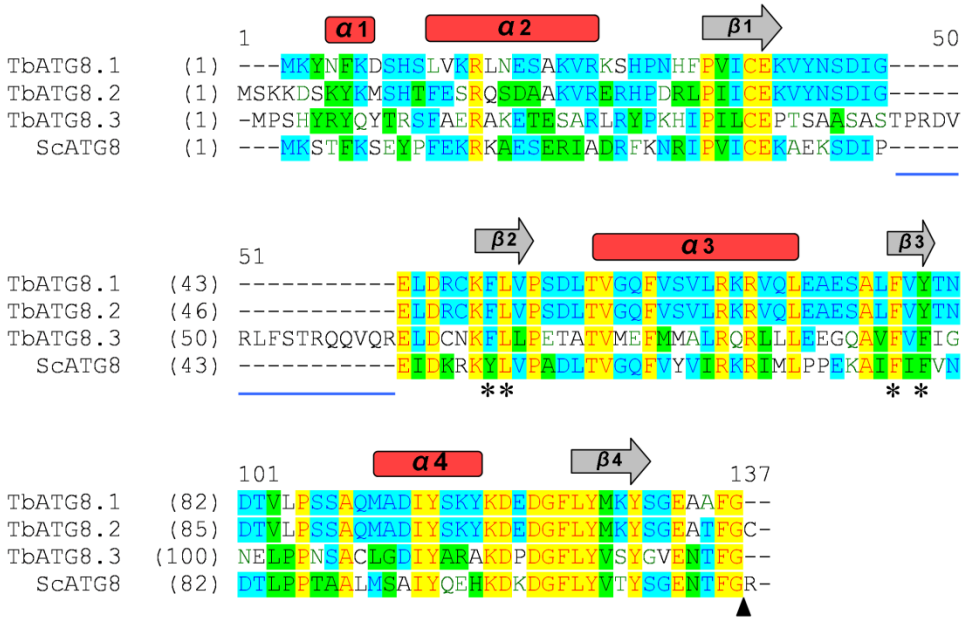
Table S1: Oligonucleotides used in this study

Name	Oligonucleotide
OL2621	CCCAAGCTTCCGCCACCATGAAGTATAACTTCAAG
OL2622	CGGGATCCAGAACCTTATCCAAATGCCGCCTCAC
OL2623	CCCAAGCTTCCGCCACCATGAGTAAAAAAGATAGC
OL2624	CGGGATCCAGAACCTTAGCATCCAAATGTCGC
OL2625	CCCAAGCTTCCGCCACCATGCCTTCACACTACC
OL2626	CGGGATCCAGAACCTCAACCAAATGTATTTTC
OL3668	GCAAGCTTGGAGATGAACTGCTGCAC
OL3669	GCGGATCCGACACGGATGAATGCTGATGC
OL3670	GCAAGCTTTTCTTGTTGACTGACAGCCG
OL3671	GCGGATCCGACCCCTCGTCCTTGTATGAG
TLO411	GGGGACAAGTTTGTACAAAAAAGCAGGCTTGACGACGACGAGGATGCGG
TLO412	GGGGACCACTTTGTACAAGAAAGCTGGGTCATATTCGATGCTCGGCACT
TLO413	GGGGACAAGTTTGTACAAAAAAGCAGGCTTTTCCTTCCAGATACGGTGG
TLO414	GGGGACCACTTTGTACAAGAAAGCTGGGTGAATTTATTCGCCAAAGGCA
OL3810	GGGGACAAGTTTGTACAAAAAAGCAGGCTGAAACTGCAGAAGAGACCCG
OL3811	GGGGACCACTTTGTACAAGAAAGCTGGGTAGGTTAAGTTCAGGCAGCGC
OL3332	GATCGGGCCCGGTACCAAAGGCTCTCGAGAAGAGG
OL3333	GATCAAGCTTCTTAAGAGCGCTGGTAACACCTGAGT
OL2775	AGCTAGCATGAAGTATAACTTCAAGGATTC
OL2776	GCTCGAGTTATCCAAATGCCGCCTCACCTG
TLO291	GGGGACAAGTTTGTACAAAAAAGCAGGCTGGCAGTGCAACTGATGGAGA
TLO292	GGGGACCACTTTGTACAAGAAAGCTGGGTTTGGGAATGTGCAACACACA
TLO293	GGGGACAAGTTTGTACAAAAAAGCAGGCTCAATGGGCTTAATCATTCCC
TLO294	GGGGACCACTTTGTACAAGAAAGCTGGGTACCAAAGTCAATGTGGACGA
TLO295	GGGGACAAGTTTGTACAAAAAAGCAGGCTCTGCTTTGAAGTTGCGATGA
TLO295	GGGGACCACTTTGTACAAGAAAGCTGGGTTCGGTACTTTTGGTGTCGCC
TLO297	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGCAAGCCATTCTCTACAGC
TLO298	GGGGACCACTTTGTACAAGAAAGCTGGGTGGCACAACCAATGACAAGTT

Fig. S1. Protein sequence alignment for *T. brucei* ATG8.1, ATG8.2 and ATG8.3 and the *S. cerevisiae* ATG8.

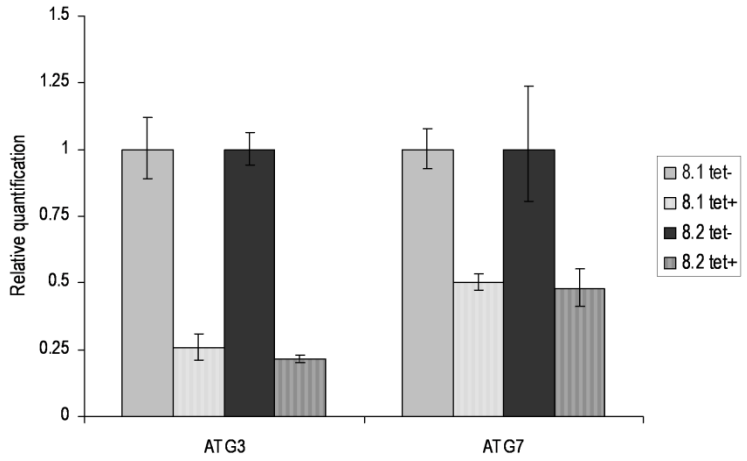


Aligned using Clustal W algorithm of the Align X program. Identical (yellow), well conserved (blue) and weakly conserved amino acids (green) are highlighted. TritrypDB identifiers and accession numbers are: TbATG8.1 (Tb927.7.5900), TbATG8.2 (Tb927.7.5910), TbATG8.3 (Tb927.7.3320) and ScATG8 (YBL078C). The site of ATG4 cleavage, which occurs after the conserved scissile glycine residue is indicated by the black arrowhead. TbATG8.3 insert indicated by blue line. Conserved alpha helices (α red boxes) and beta folds (β grey arrows) of the ubiquitin-like fold, identified from crystal structure (Koopman *et al*., 2009) are indicated. The residues required for ATG4 interaction and lipidation in yeast are marked with star (∗) (Amar *et al*., 2006).

Koopmann R, Muhammad K, Perbandt M, Betzel C, Duszenko M (**2009**) *Trypanosoma brucei* ATG8: Structural insights into autophagic-like mechanisms in protozoa. **Autophagy** 5: 1085-1091. doi:10.4161/auto.5.8.9611

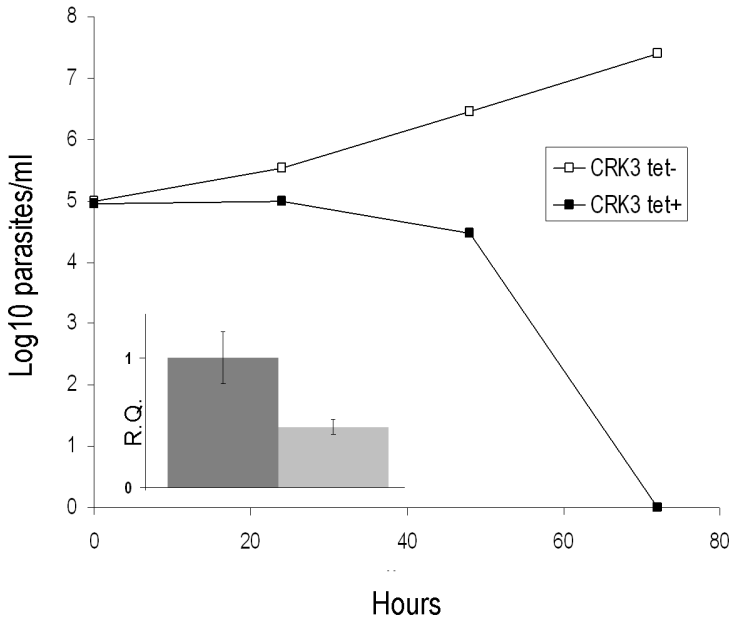
Amar N, Lustig G, Ichimura Y, Ohsumi Y, Elazar Z (**2006**) Two newly identified sites in the ubiquitin-like protein Atg8 are essential for autophagy. **EMBO Rep** 7: 635-642. doi:10.1038/sj.embor.7400698.

Fig. S2. Procyclic form *ATG3* and *ATG7* RNAi real time PCR.



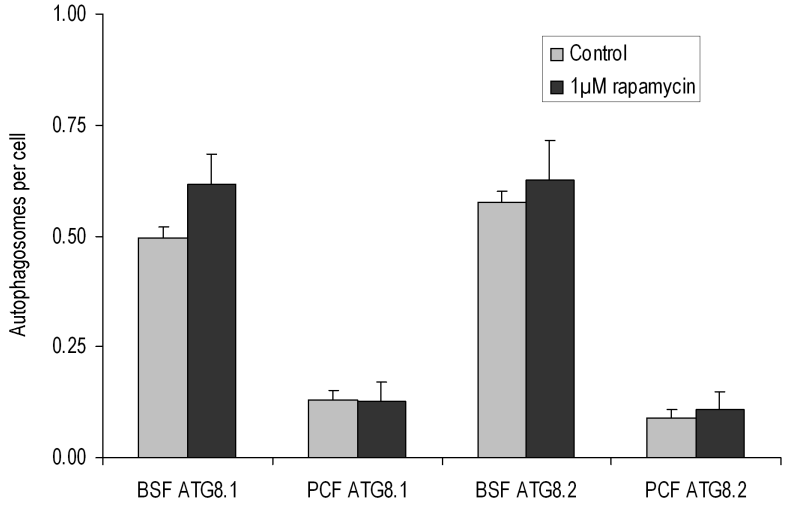
Real time PCR showing downregulation of *ATG3* and *ATG7* transcripts 72 h post tetracycline induction (tet+). Error bars represent one standard deviation derived from three replicates.

Fig. S3. Growth analysis of BSF *CRK3* RNAi mutants *in vitro*.



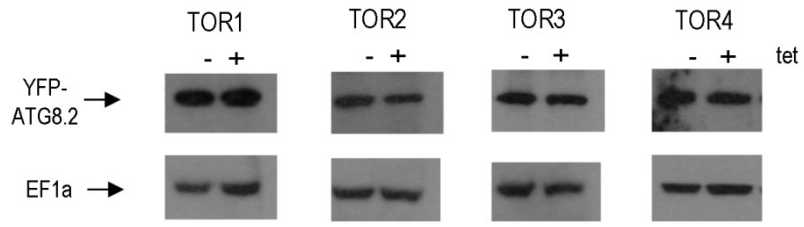
RNAi induced with tetracycline (filled symbols) and growth compared to controls (empty symbols) for 72 h. Cells were reseeded to 1 x 10^5 ml^{-1} at 24 h as required, with cumulative values shown. Inset: confirmation of RNAi specificity by qPCR of *CRK3* RNAi cells 24 h after tetracycline induction (light grey) or control (dark grey) in BSF 2T1. Error bars represent one standard deviation derived from three replicates.

Fig. S4. Effect of rapamycin on *T. brucei* autophagosome formation.



BSF and PCF were engineered to conditionally express YFP-ATG8.1 or YFP-ATG8.2. Fusion protein expression was induced by growing BSF and PCF cells for 24 h in media containing tetracycline. Cultures were then supplemented with rapamycin and maintained for a further 24 h. Fluorescent microscopy was used to monitor the presence of autophagosomes and the mean number of autophagosomes per cell was determined by counting >200 cells with data displayed as a mean of three replicate experiments. Error bars represent standard deviation. No significant difference between treated and control was observed.

Fig. S5. YFP-ATG8 fusion protein expression in BSF 2T1 cells post *TbTOR1-4* RNAi.



To validate the RNAi time points selected for autophagy analysis following *TbTOR1-4* RNAi the expression of YFP-ATG8.1 and YFP-ATG8.2 was confirmed by western blot using anti-TbATG8.1. Cells were grown with or without tetracycline (tet) for: TOR1, 40 h; TOR2, 24 h; TOR3, 72 h; TOR4, 24 h. To demonstrate equal loading the membrane was stripped and reprobed with anti-EF1α.