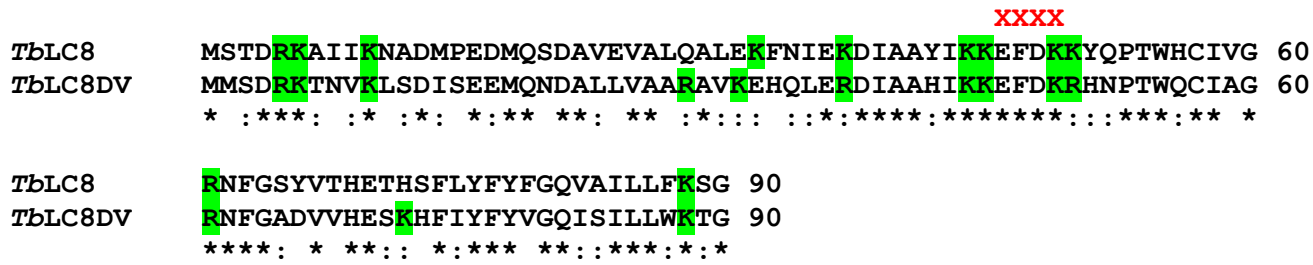
Supplementary Information (Kirkham *et al.*)

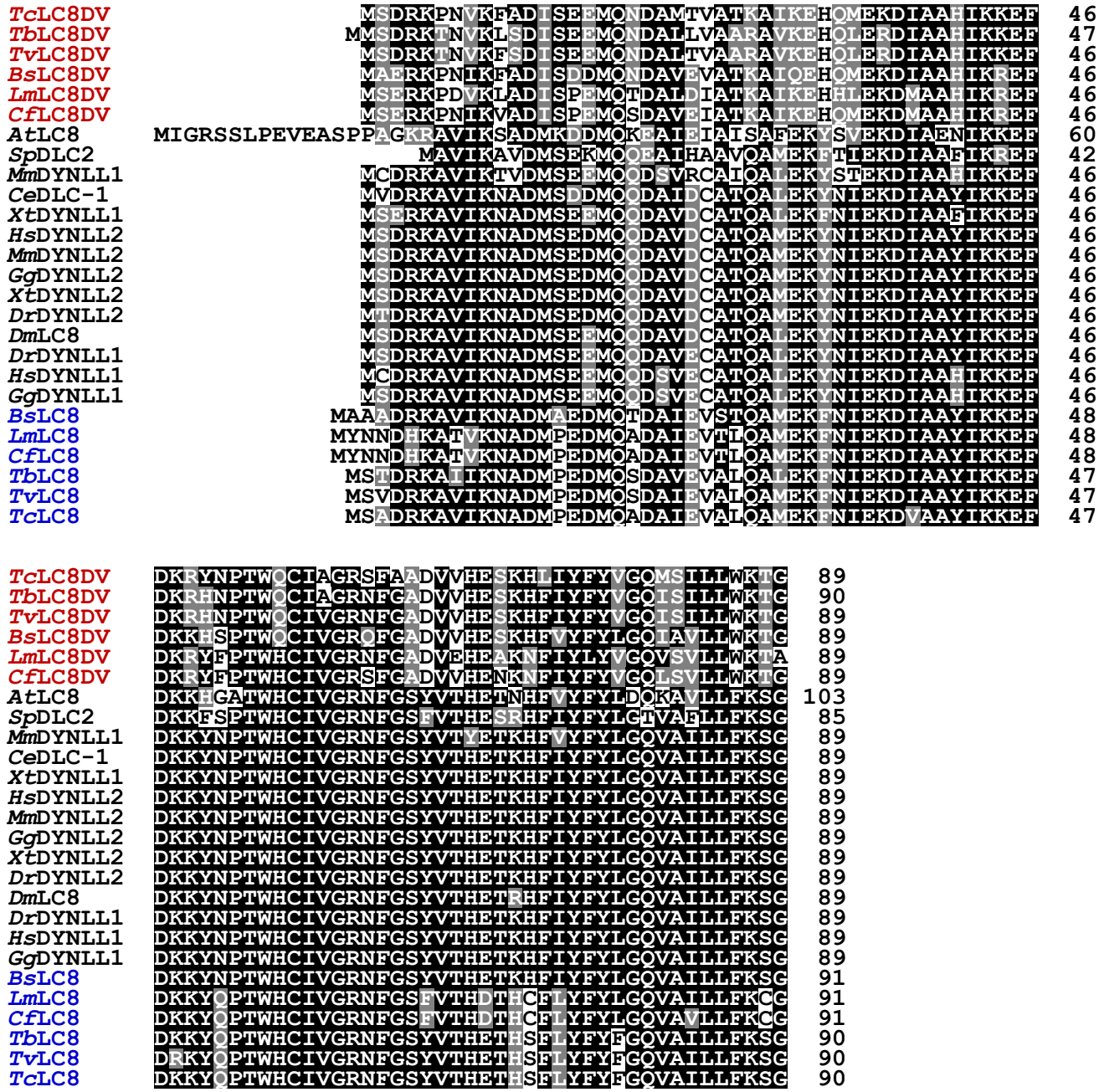
Table S1 Accession numbers of kinetoplastid *CITFA2* genes

Trypanosoma brucei brucei strain 427	Tb427tmp.211.3440
Trypanosoma congolense	Tcon, TcIL3000_9_5170
Trypanosoma vivax	TvY486_0905960)
Trypanosoma cruzi	TcCLB.510741.100
Trypanosoma grayi	Tgr.1145.1010
Trypanosoma rangeli	TRSC58_05089
Leishmania tarentolae	LtaP35.3170)
Leishmania mexicana	LmxM.34.3150
Leishmania major	LmjF.35.3150
Leishmania infantum	LinJ.35.3200
Leishmania donovani	LdBPK_353200.1

A


XXXX
TbLC8 MSTDRKAIIKNADMPEDMQSDAVEVALQALEKFNIEKDIAAYIKKEFDKKYQPTWHCIVG 60
TbLC8DV MMSDRKTNVKLSDISEEMQNDALLVAARAVKEHQLERDIAAHIKKEFDKRHNPTWQCIAG 60
RNFGSYVTHETHSFLYFYFGQVAILLFKSG 90
RNFGADVVHESKHFIYFYVGQISILLWKTG 90

B


TcLC8DV 46
TbLC8DV 47
TvLC8DV 46
BsLC8DV 46
LmLC8DV 46
CfLC8DV 46
AtLC8 60
SpDLC2 42
MmDYNLL1 46
CeDLC-1 46
XtDYNLL1 46
HsDYNLL2 46
MmDYNLL2 46
GgDYNLL2 46
XtDYNLL2 46
DrDYNLL2 46
DmLC8 46
DrDYNLL1 46
HsDYNLL1 46
GgDYNLL1 46
BsLC8 48
LmLC8 48
CfLC8 48
TbLC8 47
TvLC8 47
TcLC8 47
TcLC8DV 89
TbLC8DV 90
TvLC8DV 89
BsLC8DV 89
LmLC8DV 89
CfLC8DV 89
AtLC8 103
SpDLC2 85
MmDYNLL1 89
CeDLC-1 89
XtDYNLL1 89
HsDYNLL2 89
MmDYNLL2 89
GgDYNLL2 89
XtDYNLL2 89
DrDYNLL2 89
DmLC8 89
DrDYNLL1 89
HsDYNLL1 89
GgDYNLL1 89
BsLC8 91
LmLC8 91
CfLC8 91
TbLC8 90
TvLC8 90
TcLC8 90

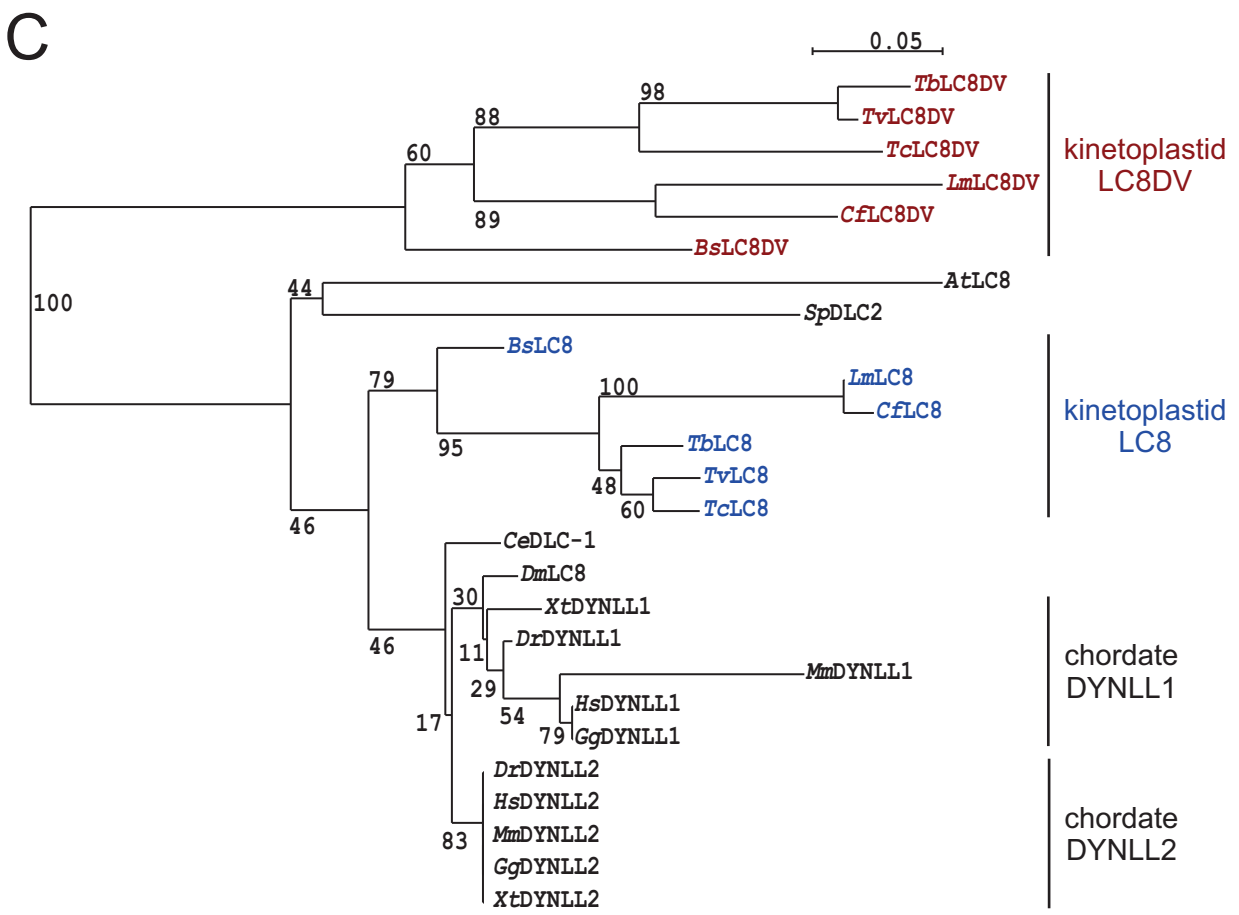


FIG S1 Kinetoplastids harbor two distinct, conserved *LC8* genes. (A) Clustal Omega alignment of amino acid sequences (1) deduced from Tb*LC8* (accession number Tb927.11.18680) and Tb*LC8DV* (Tb927.11.320) coding regions. Identical and similar positions are indicated by asterisks and colons, respectively. Arginines and lysines, marking trypsin cleavage sites, are highlighted in green. The short common trypsin-derived peptide is marked by red Xs. (B) Multiple sequence alignment, carried out with the Clustal Omega server of the European Bioinformatics Institute (http://www.ebi.ac.uk/Tools/services/web/toolform.ebi?tool=clustalo) at default parameters, comprising DYNLL1 amino acid sequences from *Homo sapiens* (*Hs*DYNLL1, accession number NP_001032584), *Mus musculus* (*Mm*DYNLL1, NP_001001185), *Gallus gallus* (*Gg*DYNLL1, XP_003642263), *Xenopus tropicalis* (*Xt*DYNLL1, NP_001005077) and *Danio rerio* (*Dr*DYNLL1, NP_998189), of DYNLL2 from the same organisms (*Hs*DYNLL2, NP_542408; *Mm*DYNLL2, NP_080832; *Gg*DYNLL2, XP_004946822; *Xt*DYNLL2, NP_001165079; *Dr*DYNLL2, NP_956393), LC8 sequences from *Drosophila melanogaster* (*Dm*LC8, NP_525075), *Caenorhabditis elegans* (*Ce*DLC-1, NP_498422), *Schizosaccharomyces pombe* (*Sp*DLC2, NP_594368), *Arabidopsis thaliana (At*LC8, CAB46031) and from the kinetoplastids *T. brucei* (*Tb*LC8), *Trypanosoma vivax* (*Tv*LC8, TvY486_1100540 & TvY486_1100570), *Trypanosoma cruzi* (*Tc*LC8, TCDM_13942), *Leishmania major* (*Lm*LC8, LmjF.32.0230), *Crithidia fasciculata* (*Cf*LC8, CfaC1_32_0390) and *Bodo saltans* (*Bs*LC8, BS21670.1..pep & BS74770.1..pep), and divergent LC8 sequences from the same kinetoplastid organisms (*Tb*LC8DV; *Tv*LC8DV, TvY486_0034050; *Tc*LC8DV, TcCLB.504109.24; *Lm*LC8DV, LmjF.25.0260; *Cf*LC8DV, CfaC1_28_0460; *Bs*LC8DV, BS22550.1..pep). Positions with more than 50% identity or similarity are highlighted in black or gray, respectively. (C) Phylogenetic Tree of the shown sequence alignment using the BIONJ neighbor-joining algorithm (2) with the Seaview version 4 software package (3). Bootstrapping was performed with 1000 replicates with values representing percentages.

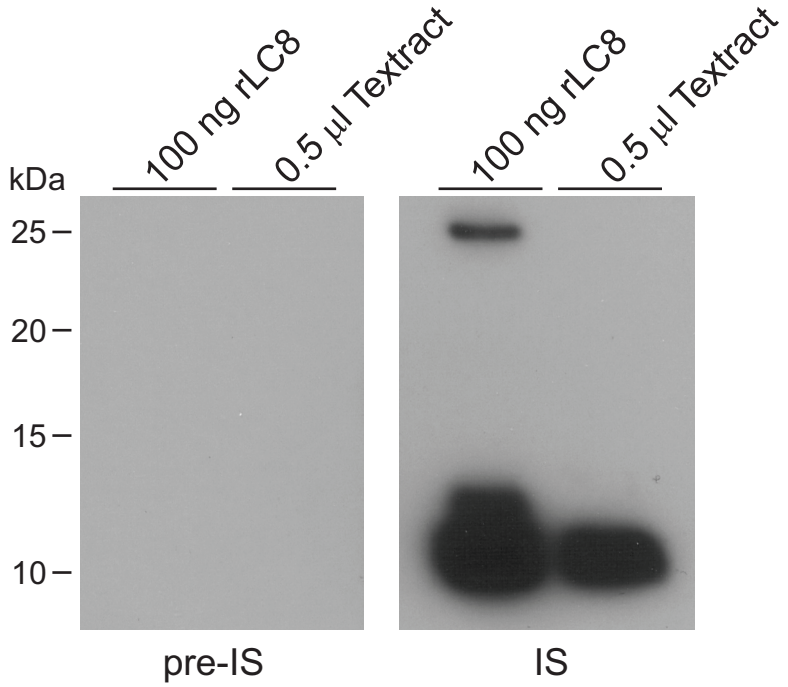


FIG S2 Generation of a specific rat anti-*T. brucei* LC8 immune serum. Immunoblot of 100 ng of trypanosome recombinant LC8 (rLC8) that was expressed in *E. coli* as a GST tag fusion, purified from bacterial extract via gluthathione affinity chromatography and subjected to thrombin digest to remove the tag as well as of transcription extract (Textract) of procyclic form *T. brucei* that was prepared as published (4, 5). LC8 was detected with pre-immune serum (pre-IS) and with immune serum (IS) from one rat that was immunized with purified GST-LC8 according to a standard protocol (6). The sera were diluted 1:1,000 and probed with a 1:5,000 dilution of a goat-derived anti-rat IgG antibody (Southern Biotech).

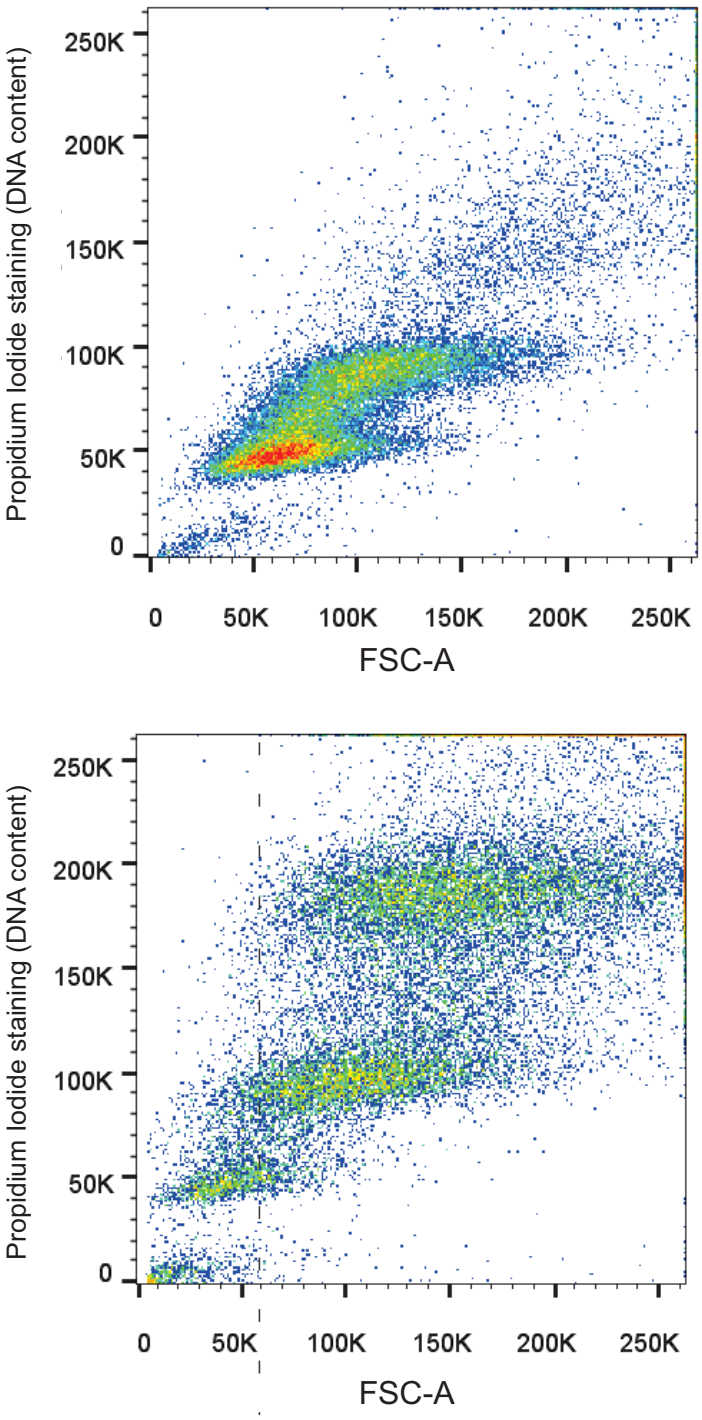


FIG S3 *LC8* silencing results in an increase in both cell size and DNA content. Ungated count data from one of three replicate experiments comparing non-induced cells (top panel) to cells in which *LC8* was silenced for 1 day (bottom panel). The y-axis represents the per-cell DNA content, as measured by propidium iodide staining, while the x-axis represents the forward scatter area (FSC-A), or size, of the cells. Note the appearance of a third population of cells in the induced culture which exhibits an increase in both size and DNA content. Blue represents areas of low count density, while green, yellow, and red represent increasing count densities.

Supplemental References

1. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, et al. 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol. Syst. Biol. **7**:539.

2. Gascuel O 1997. BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. Mol. Biol. Evol. **14**:685-695.

3. Gouy M, Guindon S, Gascuel O 2010. SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol. Biol. Evol. **27**:221-224.

4. Laufer G, Günzl A 2001. *In-vitro* competition analysis of procyclin gene and variant surface glycoprotein gene expression site transcription in *Trypanosoma brucei*. Mol. Biochem. Parasitol. **113**:55-65.

5. Laufer G, Schaaf G, Bollgönn S, Günzl A 1999. *In vitro* analysis of alpha-amanitin-resistant transcription from the rRNA, procyclic acidic repetitive protein, and variant surface glycoprotein gene promoters in *Trypanosoma brucei*. Mol. Cell. Biol. **19**:5466-5473.

6. Schimanski B, Brandenburg J, Nguyen TN, Caimano MJ, Günzl A 2006. A TFIIB-like protein is indispensable for spliced leader RNA gene transcription in *Trypanosoma brucei*. Nucleic Acids Res. 34:1676-1684.