

Supplementary Information (Kirkham et al.)

Table S1 Accession numbers of kinetoplastid *CITFA2* genes

FIG S1 Kinetoplastids harbor two distinct, conserved *LC8* genes

FIG S2 Generation of a specific rat anti-*T. brucei* LC8 immune serum

FIG S3 *LC8* silencing results in an increase in both cell size and DNA content

Supplemental References

Table S1 Accession numbers of kinetoplastid *CITFA2* genes

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

A

| | | |
|----------------|---|------|
| | | XXXX |
| <i>TbLC8</i> | MSTDRKAI IKNADMPEDMQSDAVEVALQALEKFNIEKDIAAYIKKEFDKKYQPTWHCIVG 60 | |
| <i>TbLC8DV</i> | MMSDRKT NVKILSDISEEMQNDALLVAARAVKEHQLERDIAAHIKKEFDKRHNPTWQCIAG 60 | |
| | * :****: :* :*: * :** * : ** :*: : :*:*****:*****:*****:*****:*****:* | |
| <i>TbLC8</i> | RNFGSYVT THEHSFLYFYFGQVAILLFKSG 90 | |
| <i>TbLC8DV</i> | RNFGADV VHESK HFIYFYVGQISILLWKTG 90 | |
| | *****: * ***: * :*** * :***:***:* | |

B

| | | |
|-----------------|--|----|
| <i>TcLC8DV</i> | MSDRKPN VKEAD I SEEMONDAM T VATKAIKE H OMEKDIAAHIKKEF | 46 |
| <i>TbLC8DV</i> | MMSDRKT NVKILSDISEEMONDAL I VAARAVKE H OLERDIAAHIKKEF | 47 |
| <i>TvLC8DV</i> | MSDRKT NVKE D ISEEMONDAL I VAARAVKE H OLERDIAAHIKKEF | 46 |
| <i>BsLC8DV</i> | MAERKP NIKEAD I SDDMONDAVE V ATKAIQE H OMEKDIAAHIKREF | 46 |
| <i>LmLC8DV</i> | MSERKP DVKLIAD I SPEMOTDALD I ATKAIKE H HLEKDMAAHIKREF | 46 |
| <i>CFLC8DV</i> | MSERKP NIKVAD I SPEMOSDAVE V ATKAIKE H OMEKDIAAHIKREF | 46 |
| <i>AtLC8</i> | MIGRSSLPEVEASPPACKRAVI KSADM K DDMOK E AIEIAL S AEFEKY S VEKDIAENIKKEF | 60 |
| <i>SpDLC2</i> | MAVI KAVDMSE K MQQEAI H AAVQAMEKF T IEKDIAAEIKREF | 42 |
| <i>MmDYNLL1</i> | MCDRKAVIK LVDMDSE E MOODSVRCAL Q ALEKYSTE K DIAAHIKKEF | 46 |
| <i>CeDLC-1</i> | MVDRKAVIK NADM M DDM00DAIDCAT Q ALEKYNIEKDIAAYIKKEF | 46 |
| <i>XtDYNLL1</i> | MSERKAVIK NADM M EM00DAVDCAT Q ALEKENIEKDIAAYIKKEF | 46 |
| <i>HsDYNLL2</i> | MSDRKAVIK NADM M SEDMD00DAVDCAT Q AMEKYNIEKDIAAYIKKEF | 46 |
| <i>MnDYNLL2</i> | MSDRKAVIK NADM M SEDMD00DAVDCAT Q AMEKYNIEKDIAAYIKKEF | 46 |
| <i>GgDYNLL2</i> | MSDRKAVIK NADM M SEDMD00DAVDCAT Q AMEKYNIEKDIAAYIKKEF | 46 |
| <i>XtDYNLL2</i> | MSDRKAVIK NADM M SEDMD00DAVDCAT Q AMEKYNIEKDIAAYIKKEF | 46 |
| <i>DrDYNLL2</i> | MTDRKAVIK NADM M SEDMD00DAVDCAT Q AMEKYNIEKDIAAYIKKEF | 46 |
| <i>DmLC8</i> | MSDRKAVIK NADM M EM00DAVDCAT Q ALEKYNIEKDIAAYIKKEF | 46 |
| <i>DrDYNLL1</i> | MSDRKAVIK NADM M EM00DAVECAT Q ALEKYNIEKDIAAYIKKEF | 46 |
| <i>HsDYNLL1</i> | MCDRKAVIK NADM M EM00DSVECAT Q ALEKYNIEKDIAAHIKKEF | 46 |
| <i>GgDYNLL1</i> | MSDRKAVIK NADM M EM00DSVECAT Q ALEKYNIEKDIAAHIKKEF | 46 |
| <i>BsLC8</i> | MAADRKA VKNADM A EDMOTDAIEV ST QAMEKENIEKDIAAYIKKEF | 48 |
| <i>LmLC8</i> | MYNNND HKATVKNA D MPEDM0ADAIEV T QAMEKF N IEKDIAAYIKKEF | 48 |
| <i>CFLC8</i> | MYNNND HKATVKNA D MPEDM0ADAIEV T QAMEKF N IEKDIAAYIKKEF | 48 |
| <i>TbLC8</i> | MSDRK AIKNADMPEDMQSDAVE V ALQALEKFNIEKDIAAYIKKEF | 47 |
| <i>TvLC8</i> | MSVDRKAVIK NADM P EDMQSDAI E VALQAMEKF N IEKDIAAYIKKEF | 47 |
| <i>TcLC8</i> | MSADRKA VKNADM P EDM0ADAIEV ALQ AMEKF N IEKDVAAYIKKEF | 47 |

| | | |
|-----------------|---|--|
| <i>TcLC8DV</i> | DKRYNPT WOCIAGRSF AA VVHESKHL I YFYVGQMS ILLWKTG 89 | |
| <i>TbLC8DV</i> | DKRHNP TWOClAGRNF GAD VVHESKHF I YFYVGQI SILLWKTG 90 | |
| <i>TvLC8DV</i> | DKRHNP TWOClIVGRNFG GAD VVHESKHF I YFYVGQI SILLWKTG 89 | |
| <i>BsLC8DV</i> | DKKHSPT WOCIVGRQFG AD VVHESKHF V YFYLGQ I AVILLWKTG 89 | |
| <i>LmLC8DV</i> | DKRYEPT WHCIVGRNFGAD V HEAKNFI I YLYVGQ V SLLLWKT A 89 | |
| <i>CFLC8DV</i> | DKRYEPT WHCIVGRSFGAD V HENKNFI I YFYVGQ L SLLLWKT G 89 | |
| <i>AtLC8</i> | DKKHGAT WHCIVGRNFGSYV T THE N HF V YFYLD DKAVILLFKSG 103 | |
| <i>SpDLC2</i> | DKKESPT WHCIVGRNFGSF V THE SR HF I YFYLG T VAELL FKSG 85 | |
| <i>MmDYNLL1</i> | DKKYNPT WHCIVGRNFGSYV T THE TK HF I YFYLG Q VAILL FKSG 89 | |
| <i>CeDLC-1</i> | DKKYNPT WHCIVGRNFGSYV T THE TK HF I YFYLG Q VAILL FKSG 89 | |
| <i>XtDYNLL1</i> | DKKYNPT WHCIVGRNFGSYV T THE TK HF I YFYLG Q VAILL FKSG 89 | |
| <i>HsDYNLL2</i> | DKKYNPT WHCIVGRNFGSYV T THE TK HF I YFYLG Q VAILL FKSG 89 | |
| <i>MnDYNLL2</i> | DKKYNPT WHCIVGRNFGSYV T THE TK HF I YFYLG Q VAILL FKSG 89 | |
| <i>GgDYNLL2</i> | DKKYNPT WHCIVGRNFGSYV T THE TK HF I YFYLG Q VAILL FKSG 89 | |
| <i>XtDYNLL2</i> | DKKYNPT WHCIVGRNFGSYV T THE TK HF I YFYLG Q VAILL FKSG 89 | |
| <i>DrDYNLL2</i> | DKKYNPT WHCIVGRNFGSYV T THE TK HF I YFYLG Q VAILL FKSG 89 | |
| <i>DmLC8</i> | DKKYNPT WHCIVGRNFGSYV T THE TK HF I YFYLG Q VAILL FKSG 89 | |
| <i>DrDYNLL1</i> | DKKYNPT WHCIVGRNFGSYV T THE TK HF I YFYLG Q VAILL FKSG 89 | |
| <i>HsDYNLL1</i> | DKKYNPT WHCIVGRNFGSYV T THE TK HF I YFYLG Q VAILL FKSG 89 | |
| <i>GgDYNLL1</i> | DKKYNPT WHCIVGRNFGSYV T THE TK HF I YFYLG Q VAILL FKSG 89 | |
| <i>BsLC8</i> | DKKYNPT WHCIVGRNFGSYV T THE TK HF I YFYLG Q VAILL FKSG 91 | |
| <i>LmLC8</i> | DKKYOPT WHCIVGRNFGSF V THE TC FL I YFYLG Q VAILL FKCG 91 | |
| <i>CFLC8</i> | DKKYOPT WHCIVGRNFGSF V THE TC FL I YFYLG Q VAILL FKCG 91 | |
| <i>TbLC8</i> | DKKYOPT WHCIVGRNFGSYV T THE HS FL I YFYLG Q VAILL FKSG 90 | |
| <i>TvLC8</i> | DKKYOPT WHCIVGRNFGSYV T THE HS FL I YFYLG Q VAILL FKSG 90 | |
| <i>TcLC8</i> | DKKYOPT WHCIVGRNFGSYV T THE HS FL I YFYLG Q VAILL FKSG 90 | |

C

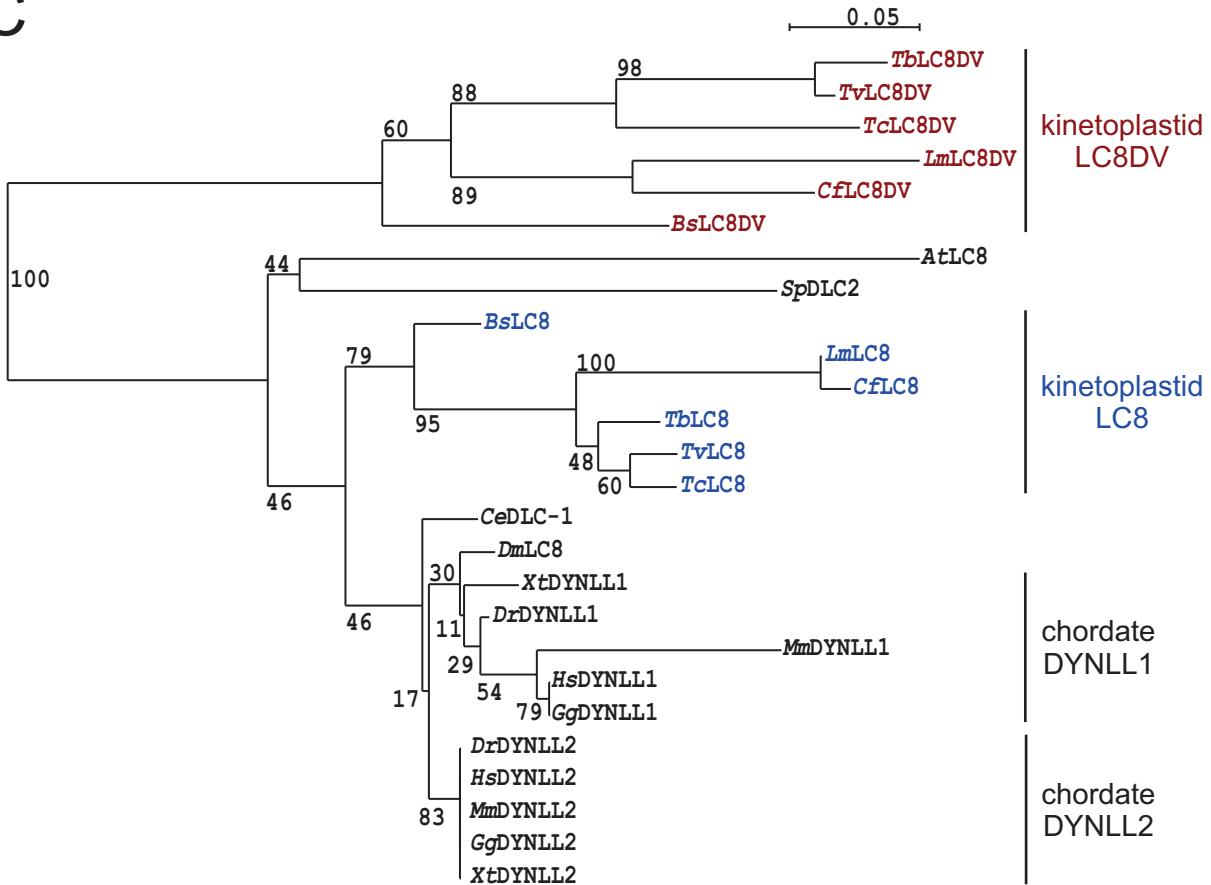


FIG S1 Kinetoplastids harbor two distinct, conserved LC8 genes. (A) Clustal Omega alignment of amino acid sequences (1) deduced from *TbLC8* (accession number Tb927.11.18680) and *TbLC8DV* (Tb927.11.320) coding regions. Identical and similar positions are indicated by asterisks and colons, respectively. 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* (*HsDYNLL1*, accession number NP_001032584), *Mus musculus* (*MmDYNLL1*, NP_001001185), *Gallus gallus* (*GgDYNLL1*, XP_003642263), *Xenopus tropicalis* (*XtDYNLL1*, NP_001005077) and *Danio rerio* (*DrDYNLL1*, NP_998189), of DYNLL2 from the same organisms (*HsDYNLL2*, NP_542408; *MmDYNLL2*, NP_080832; *GgDYNLL2*, XP_004946822; *XtDYNLL2*, NP_001165079; *DrDYNLL2*, NP_956393), LC8 sequences from *Drosophila melanogaster* (*DmLC8*, NP_525075), *Caenorhabditis elegans* (*CeDLC-1*, NP_498422), *Schizosaccharomyces pombe* (*SpDLC2*, NP_594368), *Arabidopsis thaliana* (*AtLC8*, CAB46031) and from the kinetoplastids *T. brucei* (*TbLC8*), *Trypanosoma vivax* (*TvLC8*, TvY486_1100540 & TvY486_1100570), *Trypanosoma cruzi* (*TcLC8*, TCDM_13942), *Leishmania major* (*LmLC8*, LmjF.32.0230), *Cryptosporidium fasciculata* (*CfLC8*, CfaC1_32_0390) and *Bodo saltans* (*BsLC8*, BS21670.1..pep & BS74770.1..pep), and divergent LC8 sequences from the same kinetoplastid organisms (*TbLC8DV*; *TvLC8DV*, TvY486_0034050; *TcLC8DV*, TcCLB.504109.24; *LmLC8DV*, LmjF.25.0260; *CfLC8DV*, CfaC1_28_0460; *BsLC8DV*, 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.

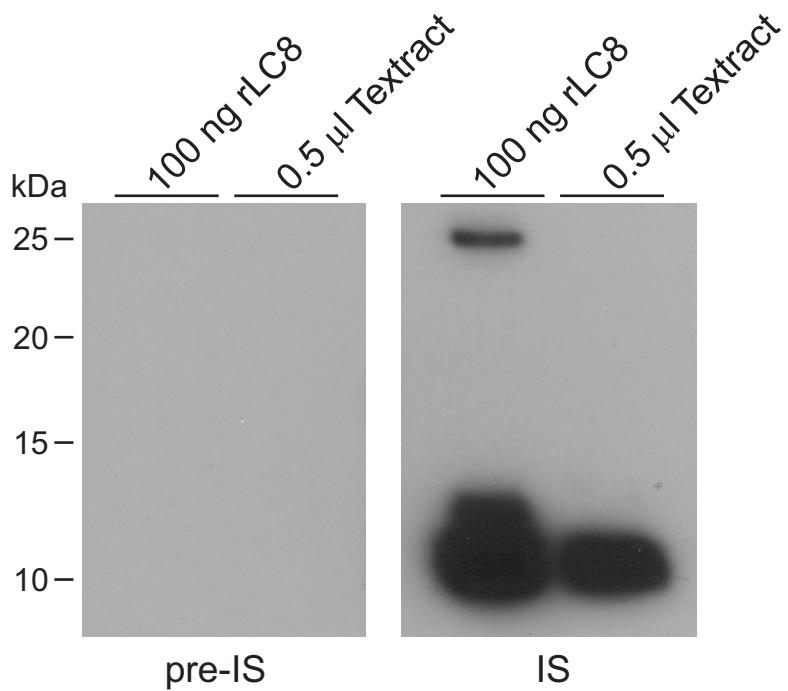


FIG S2 Generation of a specific rat anti-*T. brucei* LC8 immune serum. Immunoblot of 100 ng of trypanosome recombinant LC8 (rLC8) that was expressed in *E. coli* as a GST tag fusion, purified from bacterial extract via glutathione affinity chromatography and subjected to thrombin digest to remove the 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).

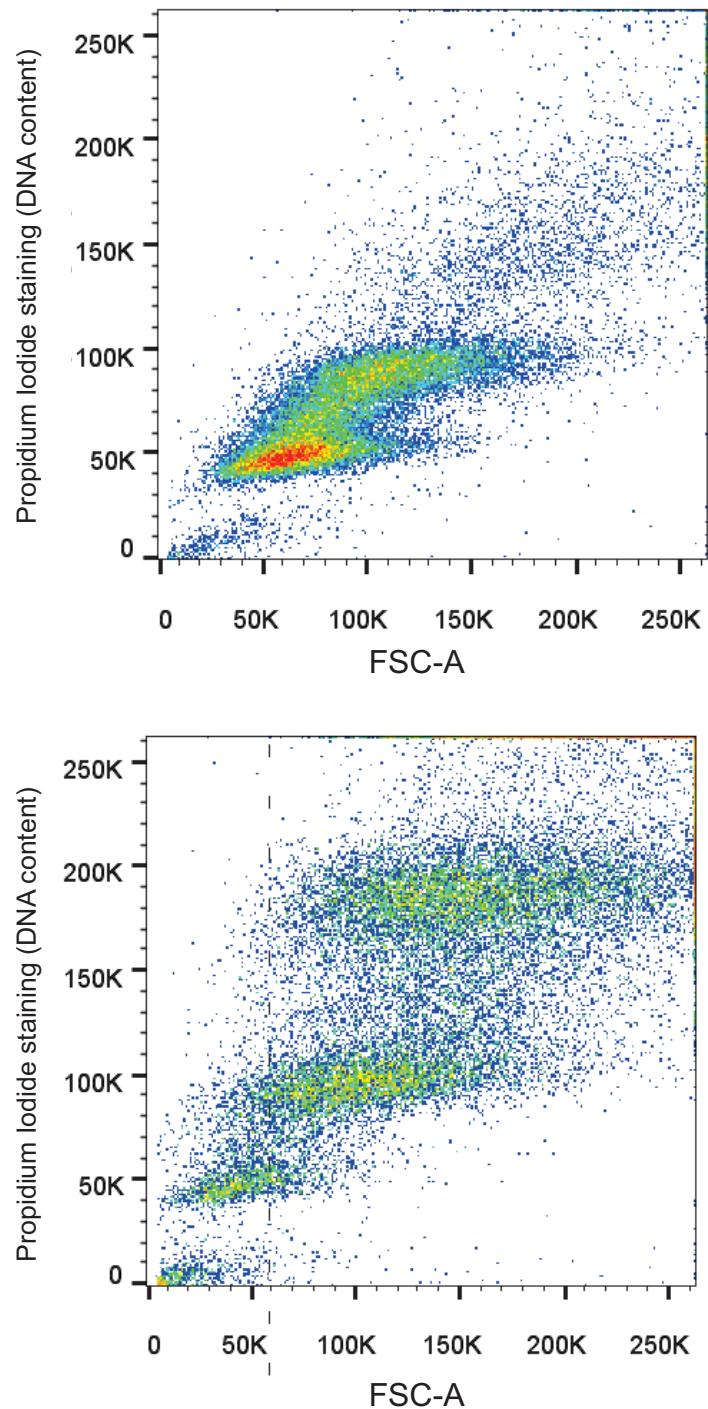


FIG S3 *LC8* silencing results in an increase in both cell size and DNA content. Ungated count data from one of three replicate experiments comparing non-induced cells (top panel) to cells in which *LC8* was silenced for 1 day (bottom panel). The y-axis represents the per-cell DNA content, as measured 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, Schaaaf 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.