## Supplementary Information (Kirkham et al.)

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Tb427tmp.211.3440
Tcon, TcIL3000_9_5170
TvY486_0905960)
TcCLB.510741.100
Tgr.1145.1010
TRSC58_05089
LtaP35.3170)
LmxM.34.3150
LmjF.35.3150
LinJ.35.3200
LdBPK_353200.1

## Α

		XXXX	
	TbLC8	MSTD <mark>RK</mark> AII <mark>K</mark> NADMPEDMQSDAVEVALQALE <mark>K</mark> FNIEKDIAAYI <mark>KK</mark> EFD <mark>KK</mark> YQPTWHCIVG	60
	Tblc8dv	MMSDRKTNVKLSDISEEMONDALLVAARAVKEHOLERDIAAHIKKEFDKRHNPTWOCIAG	60
		* :***: :* :*: *:** **: ** :*::: ::*:****:******	
	Thice		
	TOLC8DV	RNFGADVVHES <mark>K</mark> HFIYFYVGQISILLWKTG 90	
		****: * **:: *:*** **::***:*:*	
D			
D			
	TcLC8DV	MSDRKPNVKFADISEEMONDAMTVATKAIKEHOMEKDIAAHIKKEF	46
	Tblc8dv	M <mark>MSDRKTNVK</mark> LSDISEEMÕNDAL <mark>LV</mark> AARAVKE <mark>H</mark> ÕLERDIAAHIKKEF	47
	TvLC8DV	MS <u>DRKTN</u> VKESDISEEMQNDAL <mark>TV</mark> AARAVKE <mark>H</mark> QLERDIAAHIKKEF	46
	BsLC8DV	MAERKPNIKFADISDDMONDAVEVATKAIOEHOMEKDIAAHIKREF	46
	LmLC8DV		46
		MTCDSSIDFVFASDDACK AVVISADMKDMOKAATTTATSAFFZSOVERDTAFFILKEP	40
	SpDLC2	MAVINGERMON ATTAAVOMEKETTEKDIAAFIKEE	42
	MmDYNLL1	MCDRKAVTKTVDMSEEMÕODSVRCATÕALEKYSTEKDTAAHTKKEF	46
	CeDLC-1	M <sup>V</sup> DRKAVIKNADMSDDMÕÕDAIDCATÕALEKYNIEKDIAAYIKKEF	46
	XtDYNLL1	MSERKAVIKNADMSEEMÕODAVD <mark>C</mark> ATÕALEKFNIEKDIAAGIKKEF	46
	HSDYNLL2	MSDRKAVIKNADMSEDMQQDAVD CATQAMEKYNIEKDIAAYIKKEF	46
	MmDYNLL2	MSDRKAVIKNADMSEDNOODAVDCATOAMEKYNIEKDIAAYIKKEF	46
	GGDINLLZ	MSDRAVIINADMSEDMODAVDCATQAMEAINIEKDIAAIIKEE MSDRAVIINADMSEDMOODAVDCATQAMEAINIEKDIAAIIKEE	40
	DrDYNLL2	MTDRKAVT KNADMSEDMODAVD CATOAMEKYNT EKDTAAYT KKEF	46
	DmLC8	MSDRKAVTKNADMSEEMÕÕDAVDCATÕALEKVNTEKDTAAYTKKEF	46
	DrDYNLL1	MSDRKAVIKNADMSEEMÕÕDAVE <mark>C</mark> ATÕALEKYNIEKDIAAYIKKEF	46
	HSDYNLL1	MCDRKAVIKNADMSEEMQQDSVECATQALEKYNIEKDIAAHIKKEF	46
	GGDYNLL1	MSDRKAVTKNADMSEEMOODSVE CATOALEKYNTEKDTAAHTKKEF	46
	BSLC8		48
		MINDHAAIVANADHPEDMQADATEVILQANEANNISKULAATINKEF	40
	TbLC8	MSTDRKATTKNADMPEDMOSDAVEVALOALEKENTEKDTAAYTKKEF	47
	TvLC8	MSVDRKAVIKNADMPEDMÕSDAIEVALÕAMEKFNIEKDIAAYIKKEF	47
	TcLC8	MS <mark>ADRKAVIKNADM</mark> PEDMÕ <mark>A</mark> DAIEVAHÕAMEKENIEKDVAAYIKKEF	47
	TCLC8DV	DKRYNPHWOCHAERSFAADWYHFISKHI LYFYVEOMSLINWKTE 89	
	TbLC8DV	DKRHNPTWÖCTAGRNFGADVVHESKHFTYFYVGÖISTLLWKTG 90	
	TvLC8DV	DKRHNPTWQCIVGRNFGADVVHESKHFIYFYVGQISILLWKTG 89	
	BsLC8DV	DKKHSPTWQCIVGRQFGADVVHESKHFVYFYLGQIAVLLWKTG 89	
	LmLC8DV	DKRYFPHWHCHVGRNEGADVEHEAKNEHVEVYCOVSVIT WKITA 89	
		DKKYLETWICLVGKSFGADVVIIENKNELYEYVGOLSVIIEWKIG 89 DKKYCAWNYCTVCDNIZCSVIIIEWNYLEWYDOKAVI DKASC 102	
	SpDLC2	DKKESPIWHCIVGRNEGSIVTHDSRHETVEVICTWARDERSC 85	
	MmDYNLL1	DKKYNPTWICIVGRNEGSYVTMETKHEVYEYIGOVATILLEKSG 89	
	CeDLC-1	DKKYNPTWHCIVGRNFGSYVTHETKHFIYFYLGOVAILLFKSG 89	

AtLC8	DKKH <mark>GA</mark> TWHCIVGRNFGSYVTHETNHFVYFYLD <u>OK</u> AVLLFKSG	103
SpDLC2	DKK <mark>FS</mark> PTWHCIVGRNFGSFVTHESRHFIYFYLG <mark>H</mark> VA <mark>F</mark> LLFKSG	85
MmDYNLL1	DKKYNPTWHCIVGRNFGSYVTYETKHFVYFYLGQVAILLFKSG	89
CeDLC-1	DKKYNPTWHCIVGRNFGSYVTHETKHFIYFYLGQVAILLFKSG	89
XtDYNLL1	DKKYNPTWHCIVGRNFGSYVTHETKHFIYFYLGQVAILLFKSG	89
<i>Hs</i> DYNLL2	DKKYNPTWHCIVGRNFGSYVTHETKHFIYFYLGQVAILLFKSG	89
MmDYNLL2	DKKYNPTWHCIVGRNFGSYVTHETKHFIYFYLGQVAILLFKSG	89
GgDYNLL2	DKKYNPTWHCIVGRNFGSYVTHETKHFIYFYLGQVAILLFKSG	89
XtDYNLL2	DKKYNPTWHCIVGRNFGSYVTHETKHFIYFYLGQVAILLFKSG	89
DrDYNLL2	DKKYNPTWHCIVGRNFGSYVTHETKHFIYFYLGQVAILLFKSG	89
DmLC8	DKKYNPTWHCIVGRNFGSYVTHETRHFIYFYLGQVAILLFKSG	89
DrDYNLL1	DKKYNPTWHCIVGRNFGSYVTHETKHFIYFYLGQVAILLFKSG	89
<i>Hs</i> DYNLL1	DKKYNPTWHCIVGRNFGSYVTHETKHFIYFYLGQVAILLFKSG	89
GgDYNLL1	DKKYNPTWHCIVGRNFGSYVTHETKHFIYFYLGQVAILLFKSG	89
BsLC8	DKKYNPTWHCIVGRNFGS <u>YVTHETKH</u> FIYFYLGQVAILLFK <u>S</u> G	91
LmLC8	DKKYQPTWHCIVGRNFGSFVTHDTH <mark>C</mark> FLYFYLGQVAILLFK <mark>C</mark> G	91
CfLC8	DKKYQPTWHCIVGRNFGSFVTHDTH <mark>C</mark> FLYFY <u>L</u> GQVAVLLFK <mark>C</mark> G	91
TbLC8	DKKYQPTWHCIVGRNFGSYVTHETH <mark>S</mark> FLYFY <mark>F</mark> GQVAILLFKSG	90
TvLC8	DRKYQPTWHCIVGRNFGSYVTHETH <mark>S</mark> FLYFY <mark>F</mark> GQVAILLFKSG	90
TcLC8	DKKYQPTWHCIVGRNFGSYVTHETH <mark>SFLYFY</mark> FGQVAILLFKSG	90



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. NP 001032584), (MmDYNLL1. accession number Mus musculus 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; GqDYNLL2, 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, LmiF.32.0230), Crithidia 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 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**

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