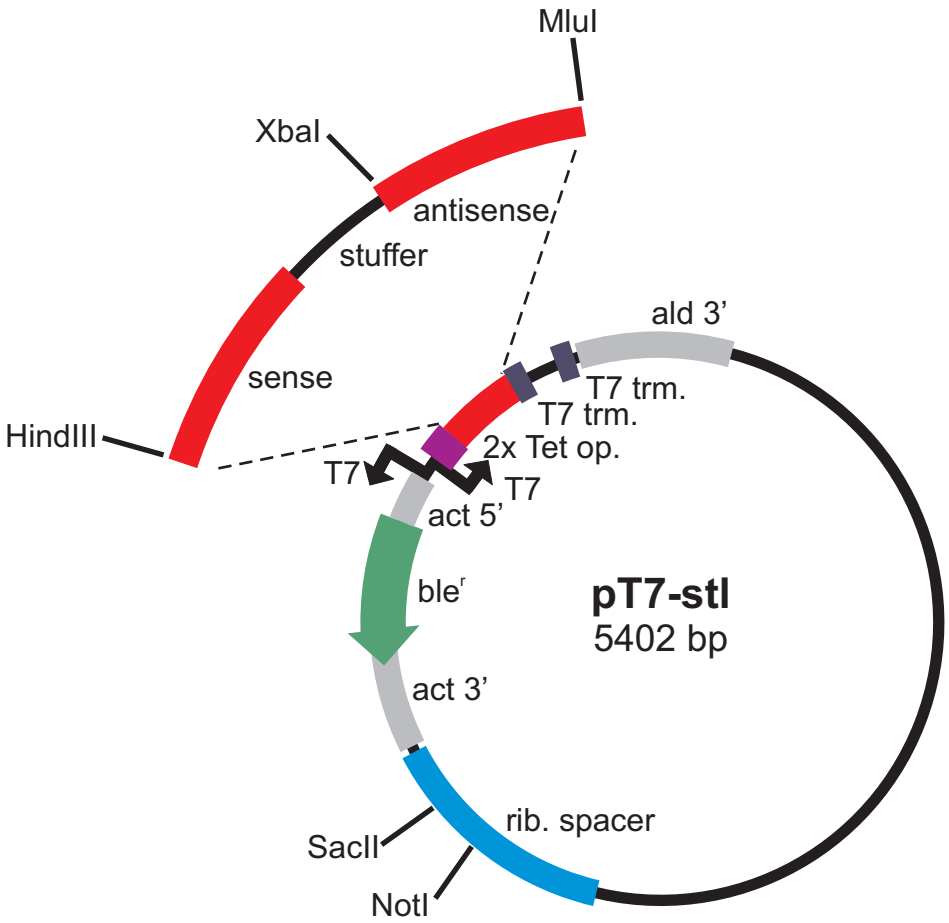

Figure S1: Schematic drawn to scale of the T7-stl vector for conditional dsRNA expression in *T. brucei.* pT7-stl is a derivative of pLEW100 (Wirtz *et al*, 1999) with two gene cassettes arranged head-to head. To the left, a T7 promoter (black arrow) drives the expression of the bleomycin resistance gene (ble^r, green arrow) which is bounded by the actin A gene flanks providing *trans* splicing and polyadenylation signals (act 5' and act 3', minor gray boxes). The downstream ribosomal sequence (rib. spacer, blue) facilitates targeting of the construct into the transcriptionally silent spacer region of an *RRNA* locus. To the right, a T7 promoter under the control of two tetracycline operators (2x Tet op., purple) drives the expression of a stem-loop RNA whose coding region (red) can be introduced into pT7-stl analogously to the established stem-loop cloning strategy (Shi *et al*, 2000). The stem-loop RNA coding region is followed by two T7 transcription terminators (T7 trm.) and an aldolase gene 3' flank (ald 3').

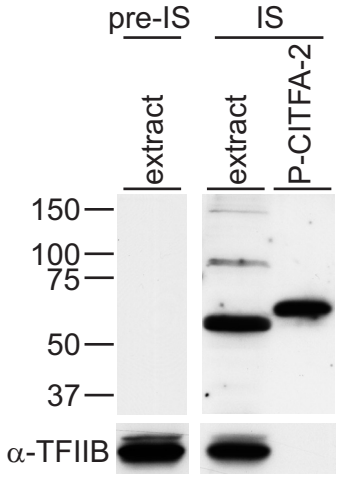

Figure S2: Immunoblots of procylic extract were probed with anti-CITFA-2 pre-immune serum (pre-IS) and immune serum (IS). In the latter, purified CITFA-2 (P-CITFA-2) was co-analyzed. Detection of TFIIB served as a loading control.

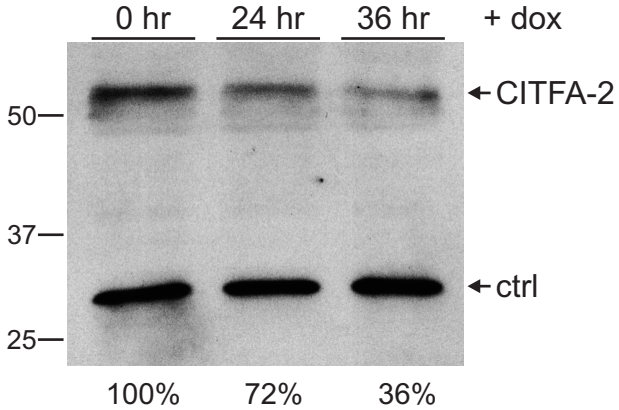

Figure S3: Immunoblot analysis of *CITFA-2* silencing in procyclic trypanosomes. Whole lysates of 10^6 cells harvested before and 24 and 36 hr after induction of *CITFA-2* dsRNA synthesis were separated on a 12% SDS/polyacrylamide gel, immunoblotted and detected with anti-CITFA-2 serum. The non-specific protein band of 30 kDa served as a loading control. The relative CITFA-2 signal strength normalized with the control signal is given below each lane. In contrast to bloodstream RNAi cells, the pZJM-transfected procyclics grew poorly and the silencing of CITFA-2 was less effective.

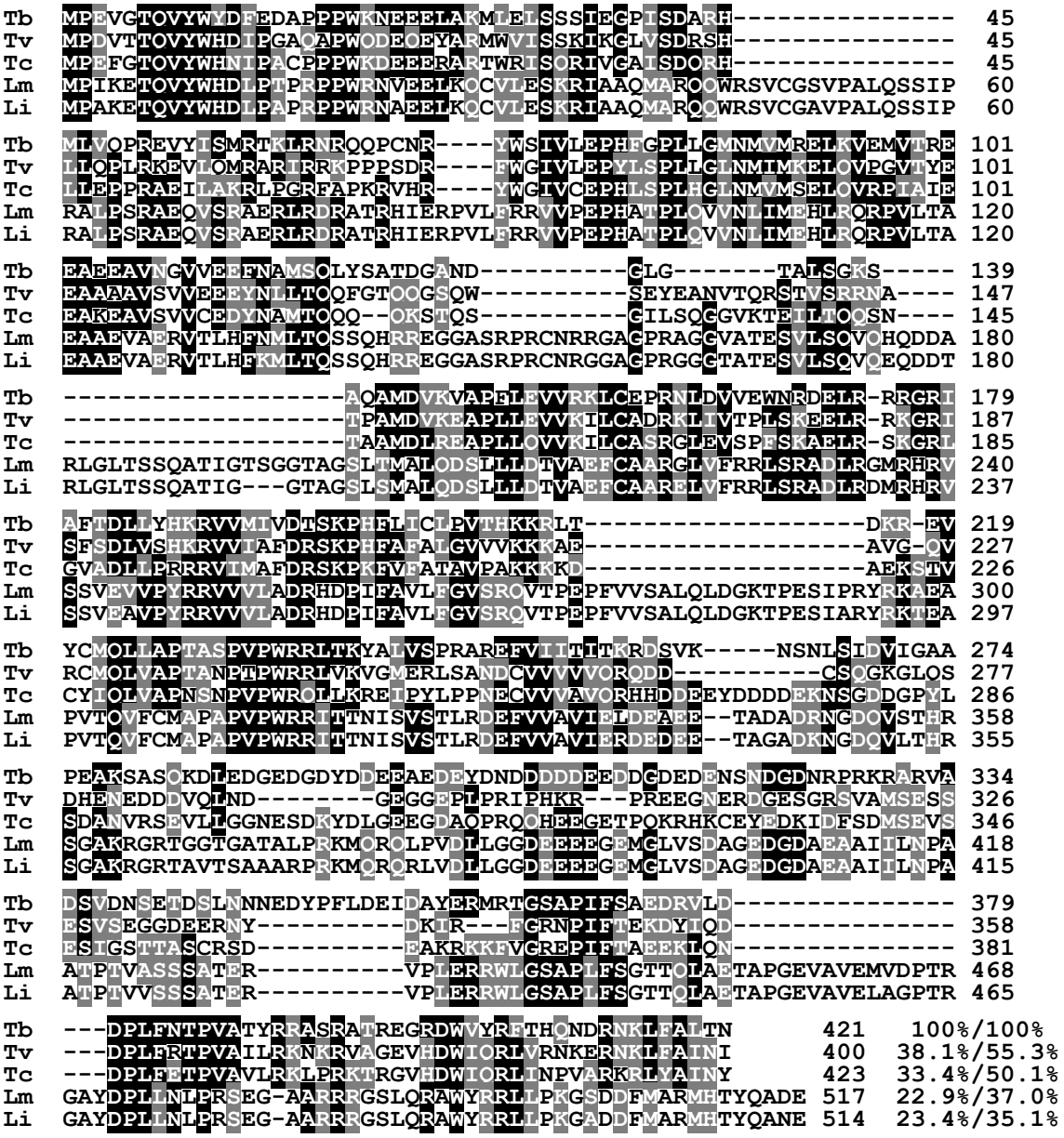

Figure S4. Sequence alignment of trypanosomatid CITFA-2 orthologues. ClustalW alignment of CITFA-2 sequences from *T. brucei* (Tb, GeneDB accession number Tb09.211.3440), *Trypanosoma vivax* (Tv, tviv913g02.p1k_2), *Trypanosoma cruzi* (Tc, Tc00.1047053510741.100), *Leishmania major* (Lm, LmjF35.3150), and *Leishmania infantum* (Li, LinJ35.3250). Identities and similarities are shaded in black and gray, respectively. Only positions with a minimum of three identical or conserved residues are shaded. Identity / similarity values specified at the end of each sequence were determined by pair-wise comparison with the *T. brucei* sequence using the EMBOSS program (http://www.ebi.ac.uk/emboss/align/) at default settings. *T. cruzi* has a nearly identical CITFA-2 paralogue (Tc00.1047053510661.150) which was not included in this analysis.

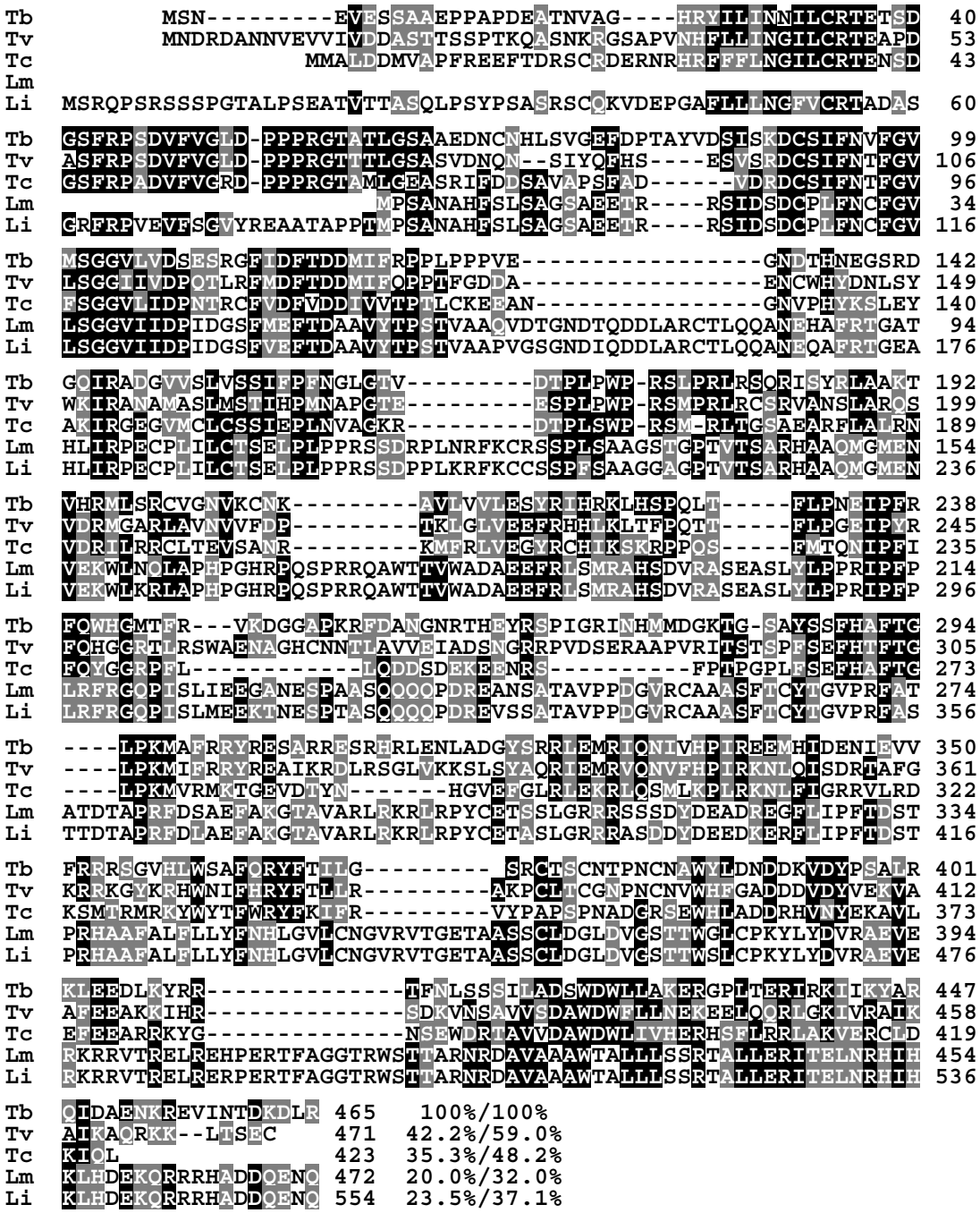

Figure S5. ClustalW sequence alignment of trypanosomatid CITFA-1 orthologues corresponding to Figure S4. GeneDB accession numbers of the sequences are Tb11.47.0010 (Tb), tviv163a09.p1k_0 (Tv), Tc00.1047053508505.30 (Tc), LmjF27.0830 (Lm), and LinJ31.0920 (Li). The *T. cruzi* paralogue (Tc00.1047053503893.160) is nearly identical to the TcCITFA-1 analyzed.

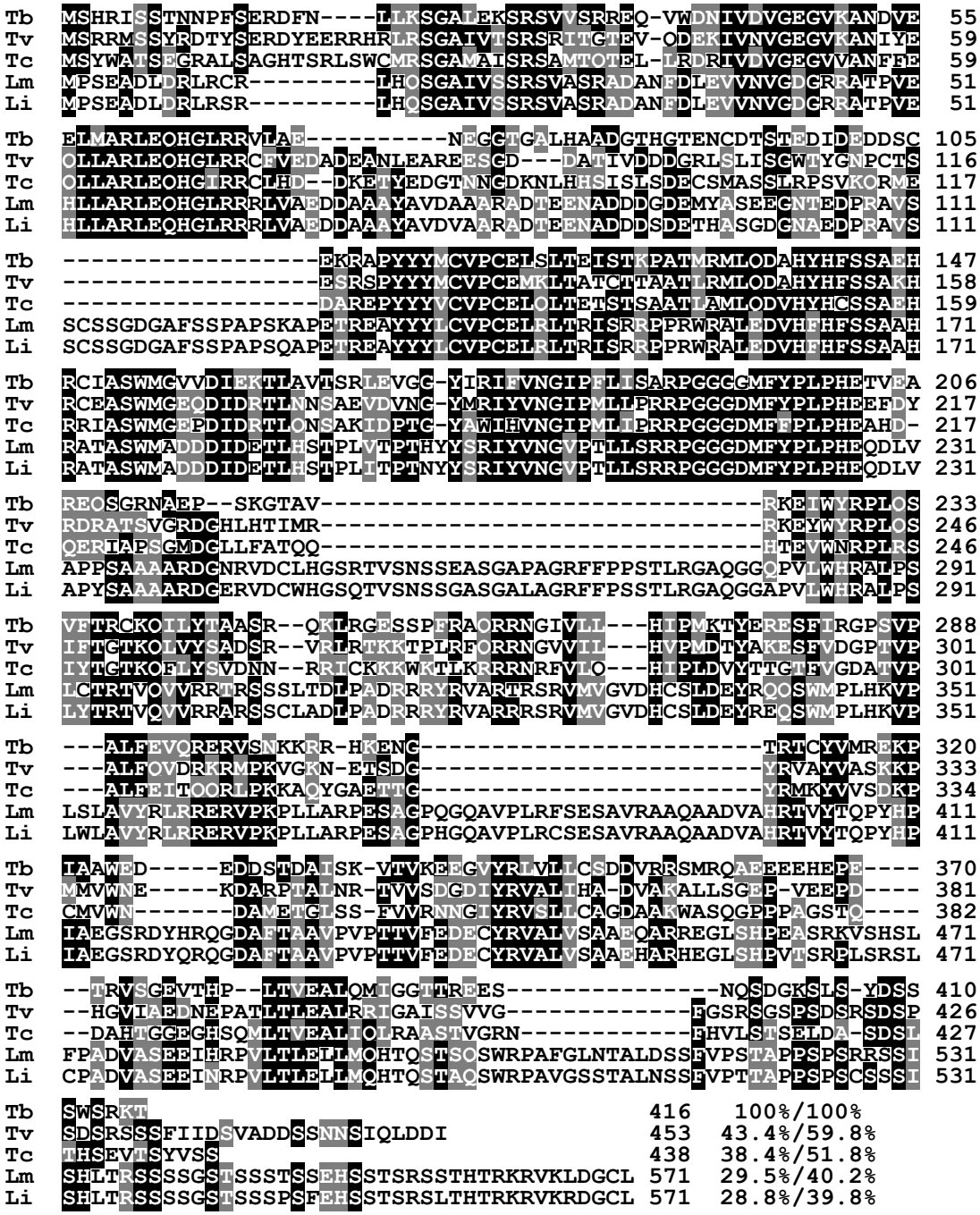

Figure S6. ClustalW sequence alignment of trypanosomatid CITFA-3 orthologues. GeneDB accession numbers of the sequences are Tb11.47.0008 (Tb), tviv163a09.p1k_2 (Tv), Tc00.1047053508505.10 (Tc), LmjF27.0850 (Lm), and LinJ27.0440 (Li). The *T. cruzi* gene Tc00.1047053503893.181 is at the end of a contig and predicted to encode the identical N-terminal 128 amino acid sequence of the TcCITFA-3 analyzed suggesting the existence of a paralogous gene.

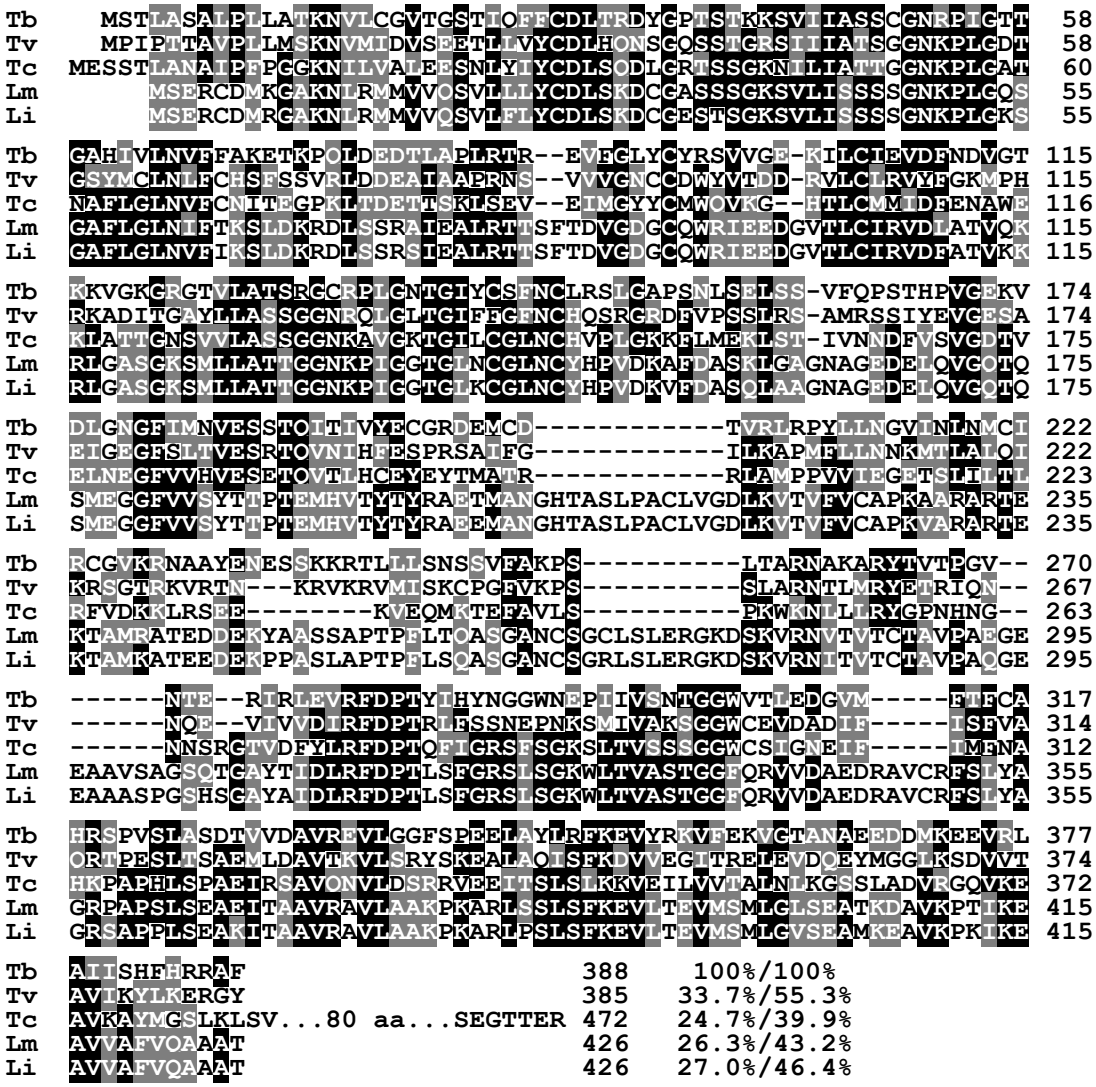

Figure S7. ClustalW sequence alignment of trypanosomatid CITFA-4 orthologues. GeneDB accession numbers of the sequences are Tb11.01.0240 (Tb), tviv140b09.q1k_4 (Tv), Tc00.1047053508819.20 (Tc), LmjF28.0430 (Lm), and LinJ28.0320 (Li). The *T. cruzi* paralogue (Tc00.1047053509595.40) is nearly identical to the TcCITFA-4 analyzed.

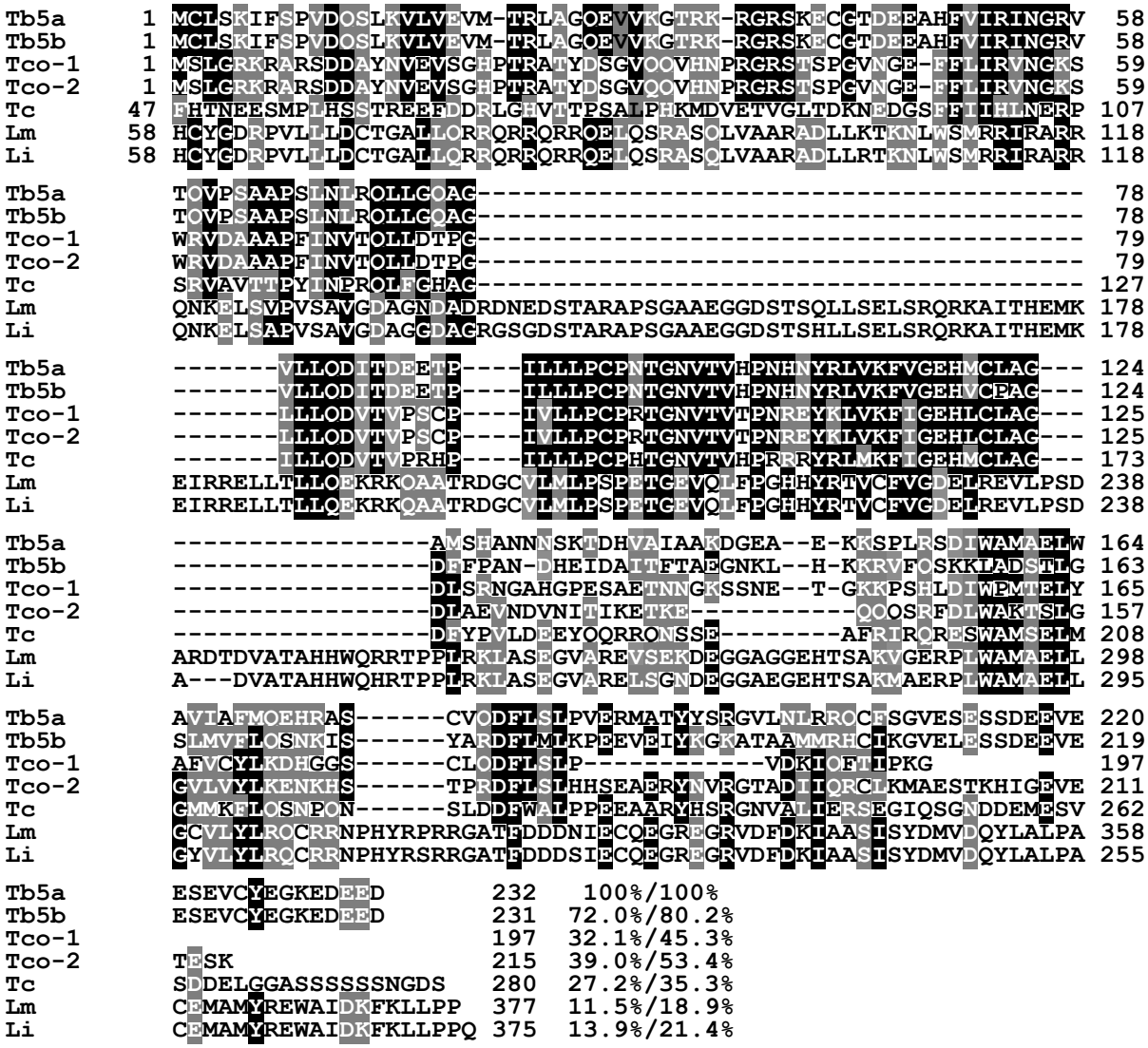

Figure S8. ClustalW sequence alignment of trypanosomatid CITFA-5 homologues. Since the available *T. vivax* CITFA-5 sequences were incomplete, CITFA-5 sequences of *Trypanosoma congolense* (Tco), another African trypanosome, were analyzed instead. GeneDB accession numbers of the sequences are Tb927.8.4030 and Tb927.8.4080 (Tb5a), Tb927.8.4130 (Tb5b), congo400e01.p1k_3 (Tco-1), congo240a09.q1k_19 (Tco-2), Tc00.1047053503897.140 (Tc), LmjF10.0650 (Lm), LinJ10.0990 (Li). In contrast to the CITFA-5 paralogues of African trypanosomes, the *T. cruzi* paralogue (Tc00.1047053509561.50) was nearly identical to its TcCITFA-5 counterpart and therefore not analyzed. Putative N-terminal extensions of the *T. cruzi* and *Leishmania* proteins were not included in this alignment.

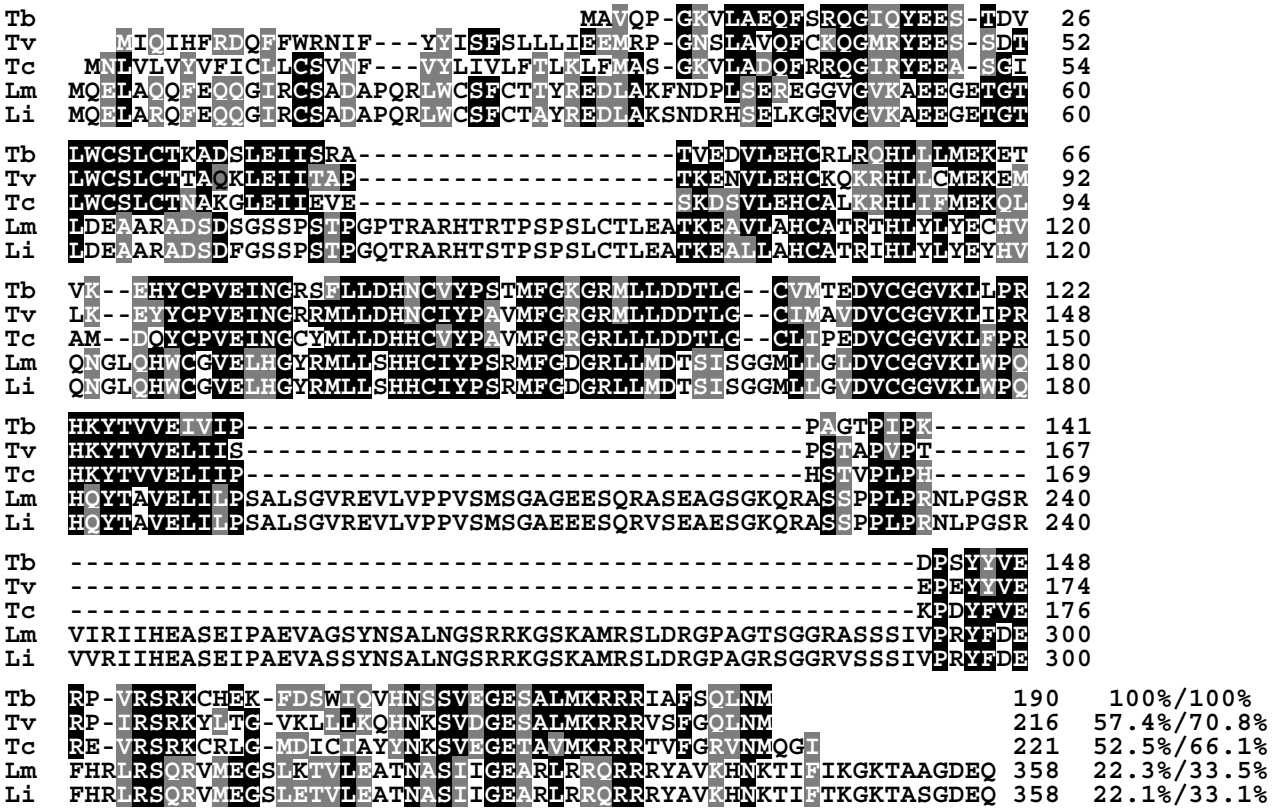

Figure S9. ClustalW sequence alignment of trypanosomatid CITFA-6 orthologues. GeneDB accession numbers of the sequences are Tb927.5.970 (Tb), tviv1195b03.q1k_2 (Tv), Tc00.1047053508303.44 (Tc), LmjF35.1270 (Lm), LinJ35.1360 (Li). The *T. cruzi* paralogue (Tc00.1047053511179.114) is nearly identical to the TcCITFA-6 analyzed.

Table S1: List of peptides identified by LC/MS/MS

CITFA-1: Tb11.47.0010 (19% coverage)

YILINNILCR
EVINTDKDLR
SGVHLWSAFQR
HRLENLADGYSR
TGSAYSSFHAFTGLPK
GTATLGSAAEDNCNHLSVGEFDPTAYVDSISK

CITFA-2: Tb09.211.3440 (30% coverage)

KLCEPR
HMLVQPR
EVYISMR
YALVSPR
ELKVEMVTR
VAPFLEVVR
EFVIITITKR
IAFTDLLYHKR
MLELSSSIEGPISDAR
EVYCMQLLAPTASPVPWR
TGSAPIFSAEDRVLDDPLFNTPVATYR

CITFA-3: Tb11.47.0008 (30% coverage)

TCYVMR
DFNLLK
QILYTAASR
LRGESSPFR
ANDVEELMAR
ISSTNNPFSER
LVLLCSDDVRR
GPSVPALFEVQR
MLQDAHYHFSSAEHR
EQVWDNIVDVGEGVK
VSGEVTHPLTVEALQMIGGTTR

CITFA-4: Tb11.01.0240 (37% coverage)

FKEVYR
GTVLATSR
LAIISHFHR
YTVTPGVNTER
STLASALPLLATK
ETKPQLDEDTLAPLR
SPVSLASDTVVDAVR
EVLGGFSPEELAYLR
VGTANAEEDDMKEEVR
GCRPLGNTGIYCSFNCLR
TLLLSNSSVFAKPSLTAR

CITFA-5a: Tb927.8.4030 / Tb927.8.4080 (33% coverage); * peptides specific for this paralogue

MATYYSR*
LAGQEVVK
ECGTDEEAHFVIR
VTQVPSAAPSLNLR
ASCVQDFLSLPVER*
SKECGTDEEAHFVIR
FVGEHMCLAGAMSHANNNSK*

CITFA-5b: Tb927.8.4130 (22% coverage); * peptides specific for this paralogue

LAGQEVVK
ECGTDEEAHFVIR
DFLMLKPEEVEIYK*
VTQVPSAAPSLNLR
SKECGTDEEAHFVIR

CITFA-6: Tb927.5.970 (80% coverage)

IAFSQLNM
VLAEQFSR
QHLLLMEK

ADSLEIISR
RIAFSQLNM
ATVEDVLEHCR
EHYCPVEINGR
ETVKEHYCPVEINGR
SFLLDHNCVYPSTMFGK
YTVVEIVIPPAGTPIPK
QGIQYEESTDVLWCSLCTK
MLLDDTLGCVMTEDVCGGVK
FDSWIQVHNSSVEGESALMK

DYNLL1: Tb11.50.0007 / Tb11.0845 (18% coverage); ** substilisin digest

NADMPEDMQSDAVEVA**

Initial purification and analysis of the promoter-binding complex **(detailed protocol)**

Thirty liters of procyclic cells were harvested, washed with 200 ml of medium without FBS or hemin, and resuspended in lysis buffer (20 mM PIPES pH 7.5, 15 mM NaCl, 60 mM KCl, 0.5 mM EGTA, 4 mM EDTA, 0.5 mM DTT, and protease inhibitors — 0.5 mM PMSF, 1 μM leupeptin, 2 μg/ml aprotinin, 1 μM pepstatin A, 0.1 mM TLCK) at a density of 4 x 10^9 cells/ml. Cells were disrupted by two cycles of freezing at –20°C and thawing. Nuclei were pelleted by centrifugation for 20 min at 15,000 g and 4°C, resuspended in nuclei buffer (20 mM HEPES pH 7.6, 420 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 25% glycerol, and protease inhibitors) at a concentration equivalent to 2 x 10^{10} cells/ml, and homogenized with 20 strokes of a type B pestle in a Dounce homogenizer. The homogenate was centrifuged for 30 min at 25,000 g and 4°C. The soluble nuclear extract was subjected to chromatographic purification by a protocol that was devised by empirical testing. All steps were carried out at 4°C and purification was monitored by EMSA. A 25–50% saturation ammonium sulfate precipitate was extensively dialyzed against buffer A1 (20 mM HEPES pH 7.6, 20% glycerol, 0.2 mM EDTA, 2mM DTT, and protease inhibitors) and passed through a column of S-Sepharose cation exchanger (Amersham) equilibrated with the buffer A1. The flow-through was adjusted to 2.5 mM $MgCl_2$ and 0.2 M NaCl and applied to a HiTrap Heparin column (Amersham) pre-equilibrated with buffer A1 containing 2.5 mM $MgCl_2$ and 0.2 M NaCl. After eluting with 0.8 M NaCl, precipitating with 50% ammonium sulfate, and dialyzing extensively against buffer A3 (10 mM HEPES pH 7.6, 10% glycerol, 60 mM NaCl, 0,2 mM EDTA, 2 mM DTT), the sample was adjusted to 40 mM DTT, 0.1 mM spermine, 1 mM spermidine and 0.05% Tween20, and loaded onto a Streptavidin Sepharose High Performance column (Amersham) pre-equilibrated with PBS and loaded with an 82-bp *VSG* ES promoter, PCR-amplified from plasmid ESP3 using one biotinylated and one normal primer. After elution with buffer A3 containing 1 M NaCl, and extensive dialysis against buffer B1 (bis-Tris-propane pH 7.0, 10% glycerol, 100 mM NaCl and 2 mM DTT), the extract was loaded to a Resource Q column (Amersham) pre-equilibrated with buffer B1. After a stepwise increase in NaCl concentration to 350 mM, the complex was eluted with a linear gradient 350-500 mM NaCl. The active fractions were loaded onto 4 ml 15–45% (v/v) linear glycerol gradients in buffer A4 (10 mM HEPES, 150 mM NaCl, 0.1 mM EDTA, 1 mM DTT) and centrifuged at 50,000 rev/min in a Beckman SW 60 rotor for 16 hr at 7°C. Fractions of 250 μl were collected from the bottom.

The purified protein complex was reduced (20 mM dithiothreitol) and alkylated (100 mM iodoacetamide) in Laemmli loading buffer, resolved on a 10% SDS-PAGE Tris-glycine gel and silver-stained. Each visible band was excised, destained, dehydrated and treated with 100 ng of modified TPCK-trypsin for 8 hr at 37°C. Peptides were extracted by sonication in an aqueous mixture of 2.5% formic acid and 0.1% TFA for 5 min at 50°C, then transferred to a ZipTip (Millipore) containing 200 μg Poros 20R2 beads (Applied Biosystems). After multiple washes with 0.1% aqueous TFA, peptides were eluted with 20 μl 40% acetonitrile, 0.1% TFA, followed by 20 μl 80% acetonitrile, 0.1% TFA, and these eluates were combined prior to lyophilization. The resulting peptide mixtures were resuspended in 0.01% (v/v) TFA in water/methanol/acetic acid (945:50:5 v/v/v) and subjected to LC-ESI-MS/MS analysis using an HPLC system (Smart System, Amersham) connected directly to an electrospray ion trap mass spectrometer (LCQ DECA, Thermo Fisher Scientific). Peptide mixtures were resolved on a reverse-phase column (Magic C_{18}, 50 mm x 0.2 mm, 100Å; 5 μm; Michrom Bioresources) with a fast-rising methanol gradient (7 min) at a flow rate of 40 μl/min pre-column split to 2.8 μl/min. MS fragmentation analysis was performed on the most intense ion observed at 2-second intervals. Protein searches were performed with SonarMSMS (Genomic Solutions) using locally collated versions of the available *T. brucei* genome data in 2001, with a re-analysis in May 2005.