Supplemental Data

Quantitative proteomic analysis of replicative and non-replicative forms reveals important insights into chromatin biology of *Trypanosoma cruzi*

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Running title: Chromatin-associated factors of *T. cruzi* life forms

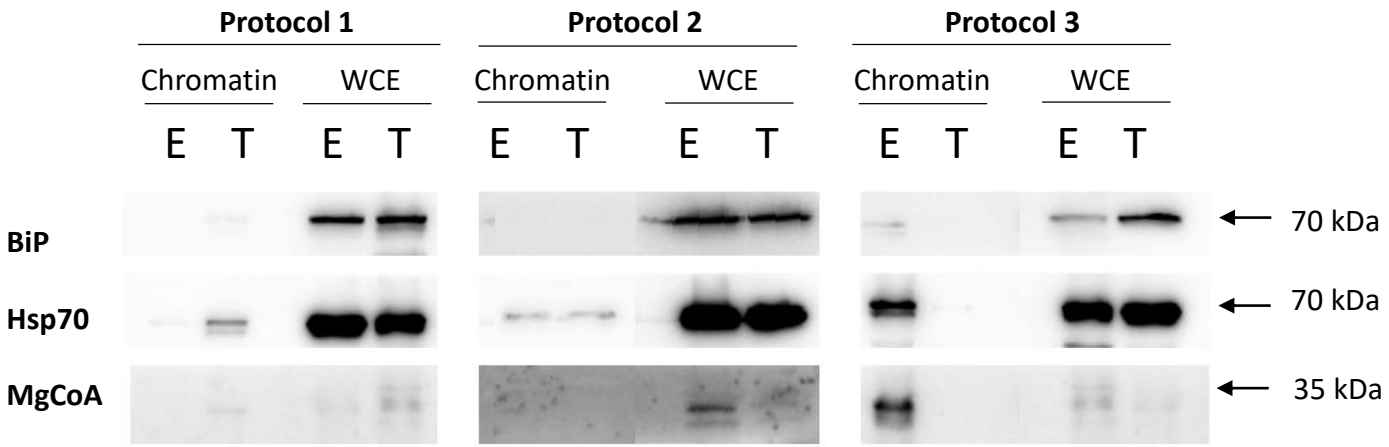


Figure S1. Western blotting using antibodies against BiP (endoplasmic reticulum marker), hsp70 (cytoplasmic marker) and MgCoA (mitochondrial marker) using whole cell extracts (WCE) or chromatin extracts of epimastigotes (E) and trypomastigotes (T) obtained from protocols 1, 2 and 3.

Figure S2. Venn diagram showing the number of proteins found in each non- chromatin enrichment protocol.

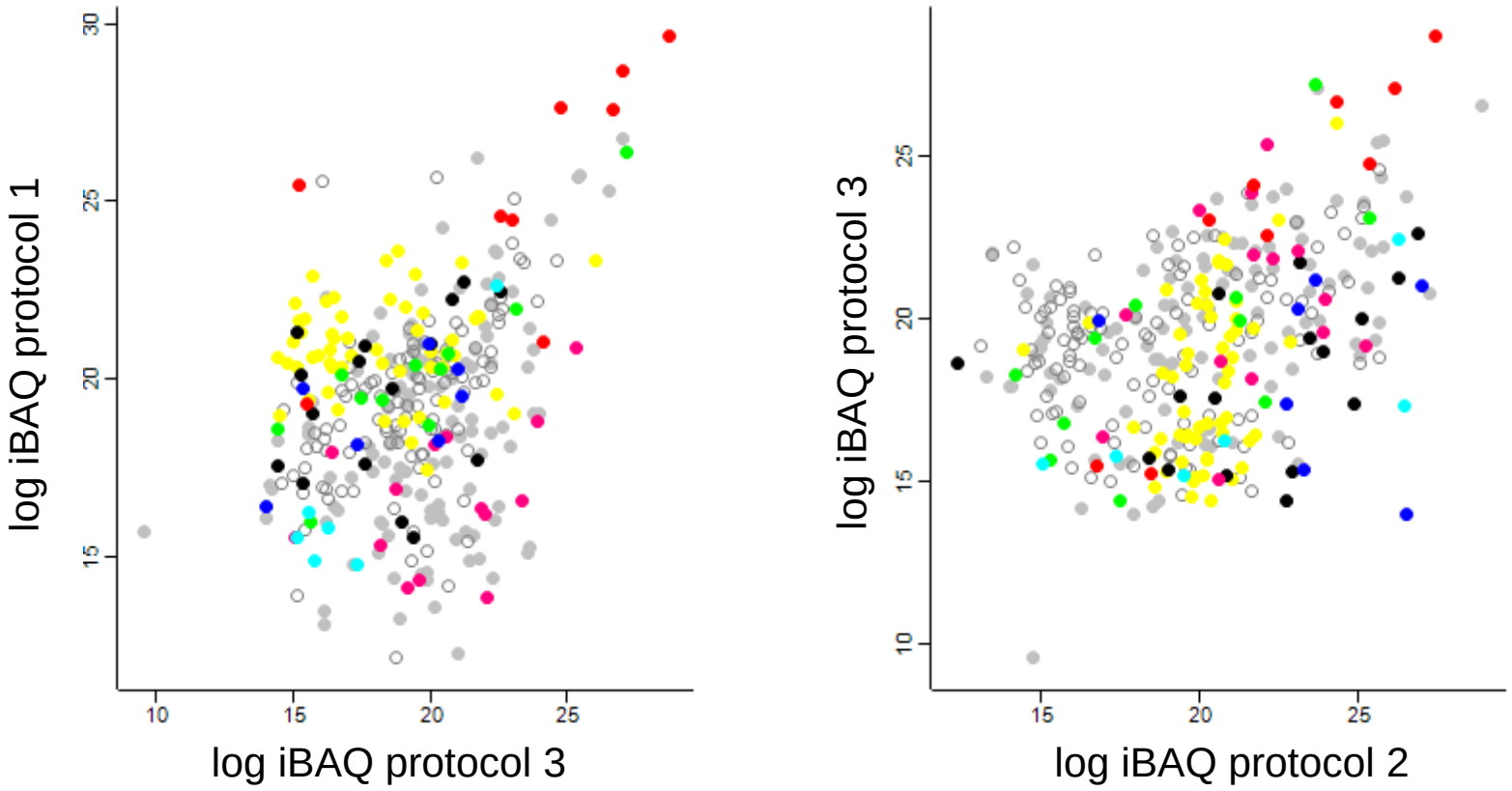
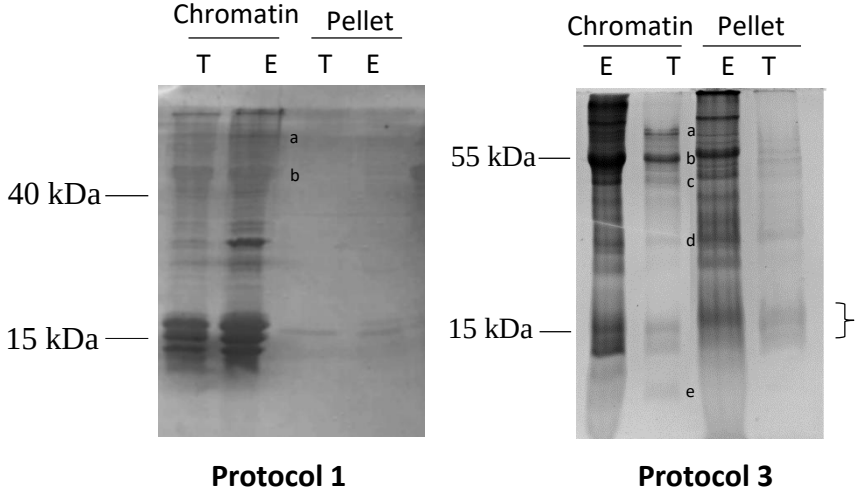


Figure S3. Scatter plot of iBAQ values of chromatin-associated proteins from protocol 1, 2 and 3. Categories from GO predicted function were highlighted: translation (yellow), nucleosome assembly (red), microtubule-based moviment (green), DNA and RNA binding/regulation of replication/transcription/mitosis (black), oxidation-reduction process (light blue), protein folding (dark blue), metabolic process/carbohydrate (pink), hypotheticals (open circle).

A.



B.

	Product Description	ID	Mascot SCORE	MW (Da)	coverage
a	paraflagellar rod	TcCLB.509617.20	3152	69076	65%
b	tubulin beta	TcCLB.506563.40	3638	50352	46%
	tubulin alpha	TcCLB.411235.9	2957	50395	51%
c	dehydrogenase	TcCLB.506943.50	958	39236	47%
	hypothetical protein	TcCLB.503971.50	613	43072	49%
	fructose-biphosphate aldolase	TcCLB.504163.40	568	41207	36%
d	hypothetical protein	TcCLB.506485.100	294	23413	47%
	leucine-richh protein	TcCLB.422189.20	211	26926	26%
	adenylate kinase	TcCLB.503479.30	210	29437	28%
	enoyl-CoA hydratase	TcCLB.508185.10	176	29027	32%
e	kinetoplast-membrane protein	TcCLB.510755.89	177	10876	41%

C.

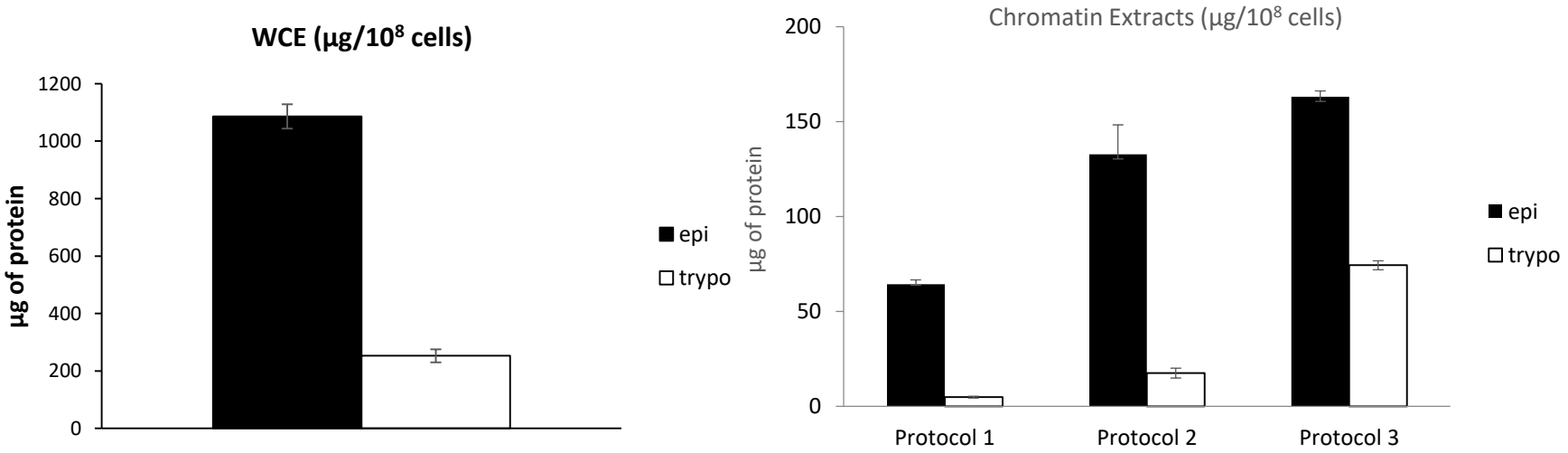


Figure S4. A. SDS-PAGE analysis of chromatin-extracts (protocols 1 and 3) from $1x10^7$ epimastigotes (E) and trypomastigotes (T). At right of each gel, the remaining pellets from each extraction that were solubilized with Coomassie sample buffer. Small letters indicate bands that were excised and analysed by LC-MS/MS. B. MASCOT identification from excised bands on A. C. Comparison of protein amount (in µg) obtained after RIPA (WCE) or chromatin extraction (according to protocols 1, 2 and 3) from 10^8 epimastigotes or trypomastigotes.

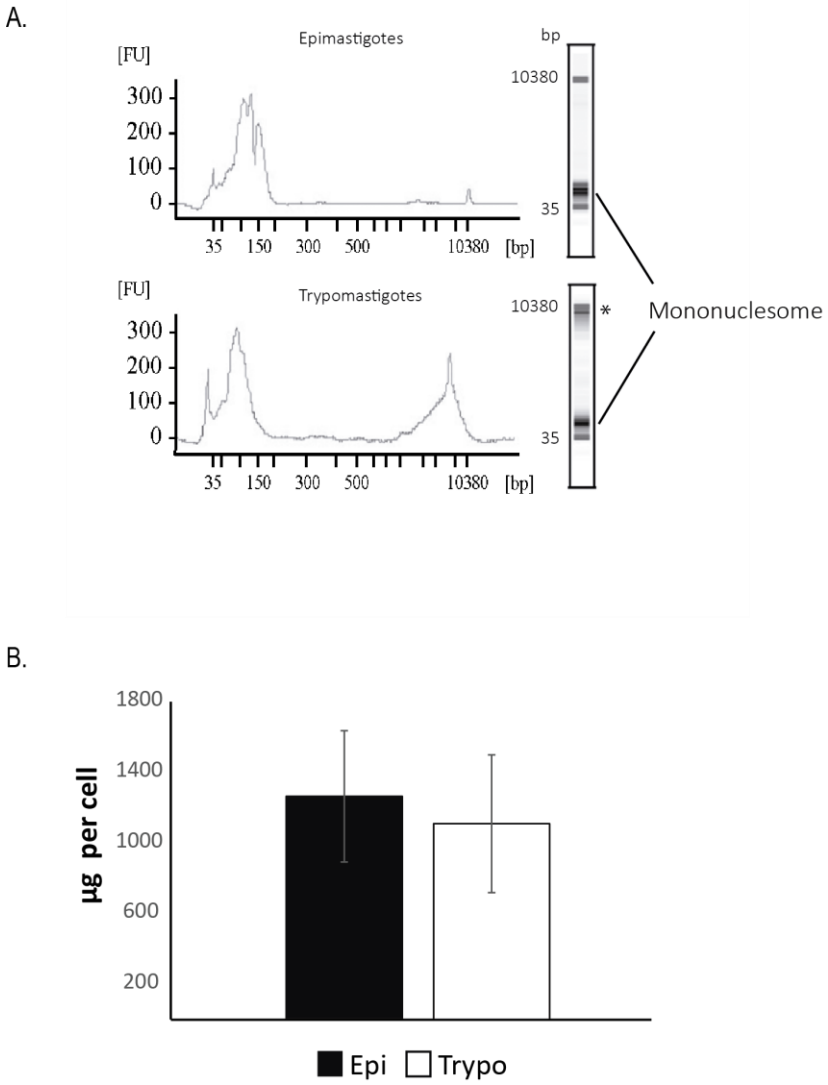


Figure S5. **(A)** Electropherograms of nucleosomal DNA content of epimastigotes and trypomastigotes after MNAse digestion. The data shown are representative of three experiments. Asterisks indicate undigested nucleossomal DNA. **(B)** DNA after MNAse digestion were quantified and normalized by cell number.

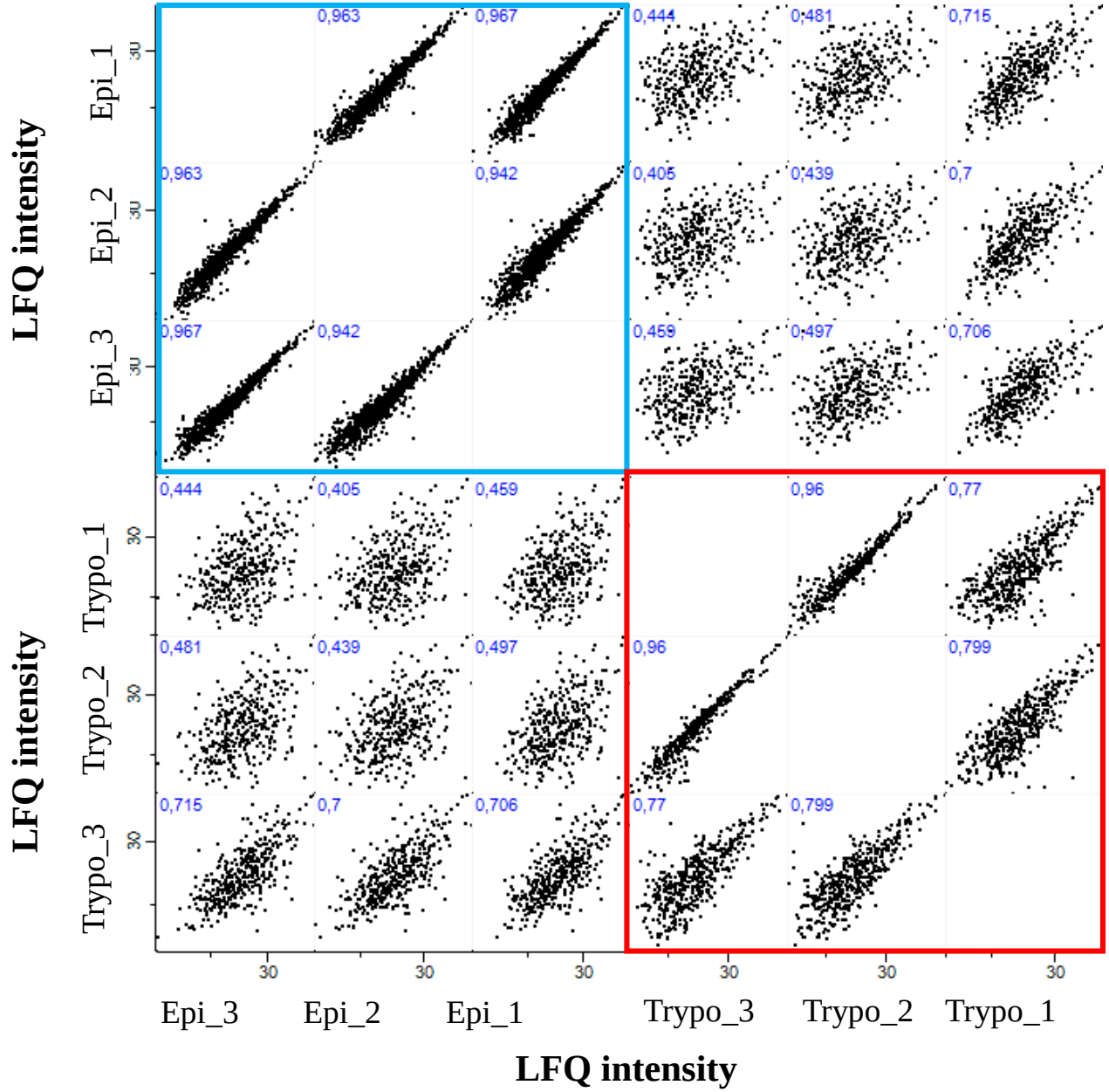


Figure S6. Scatter plots of LFQ intensities (in log) of all identified proteins in chromatin-enriched extracts from epimastigotes (epi) and trypomastigotes (trypo) using three biological replicates obtained by protocol 2. The Pearson coefficient (r) is indicated above each comparison. The graphics outlined in blue represent replicates of epimastigotes and graphics outlined in red represent replicates of trypomastigotes. Pearson correlation between biological replicates of the same parasite life form indicates high reproducibility.

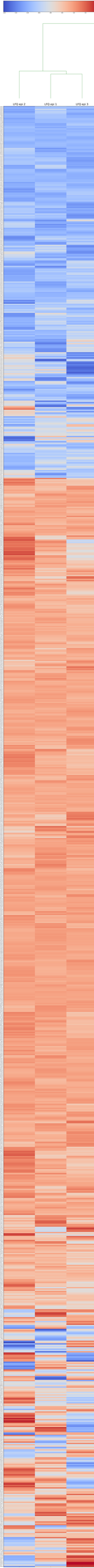

LFQ epi 2
LFQ epi 1
LFQ epi 3
LFQ trypo 3
LFQ trypo 1
LFQ trypo 2

Figure S7. Hierarchical clustering comparing LFQ values from chromatin-associated proteins of epimastigotes and trypomastigotes.

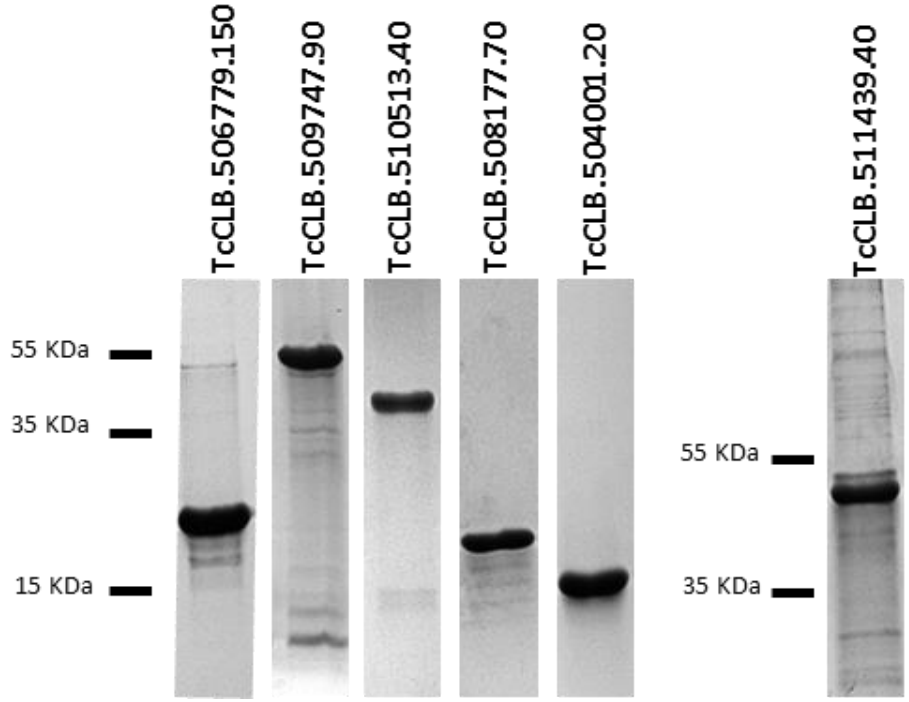


Figure S8. **Recombinant proteins after affinity purification.** The specific band of each protein was cut from the gel for polyclonal antibody production. Molecular markers are indicated. TcCLB.506779.150 (15.6 kDa), TcCLB.509747.90 (47 kDa), TcCLB.510513.40 (34 kDa), TcCLB.508177.70 (16.7 kDa), TcCLB.504001.20 (13 kDa) and TcCLB.511439.40 (52 kDa). Proteins were loaded on a 15%SDS-PAGE or on a 10%SDS-PAGE for TcCLB.511439.40.

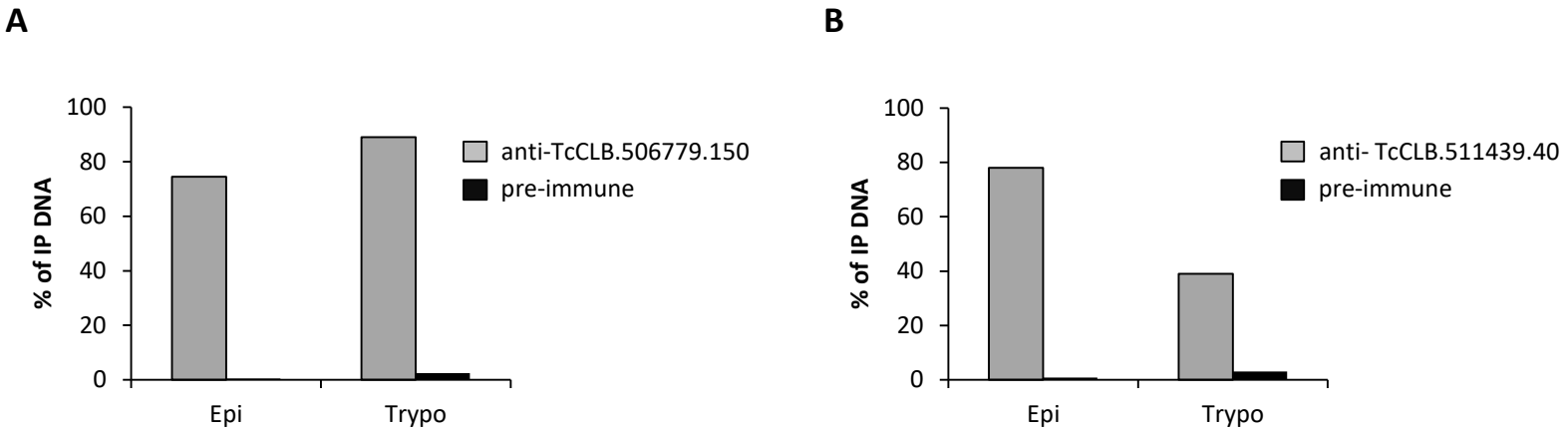


Figure S9. *T. cruzi* epimastigote or trypomastigote cells were submitted to a chromatin immunoprecipitation assay using antibodies or pre-immune serum as indicated. After immunoprecipitation, cross-links were reversed, and DNA was extracted and quantified. Graphs on **A** and **B** show the percentage of immunoprecipitated DNA related to input.