A

***Tc*BDF3**

MGSTGRKAVDEVNHWIAYIDCALSHPHPLPKGKHVFRSDLSTVPEVRDIYDCLYKLYAEESASASFREPVNALELGVFNYYEVVTEPMSLRTVLDRIAEGGHYSQASQVLADVEKIWSNCE

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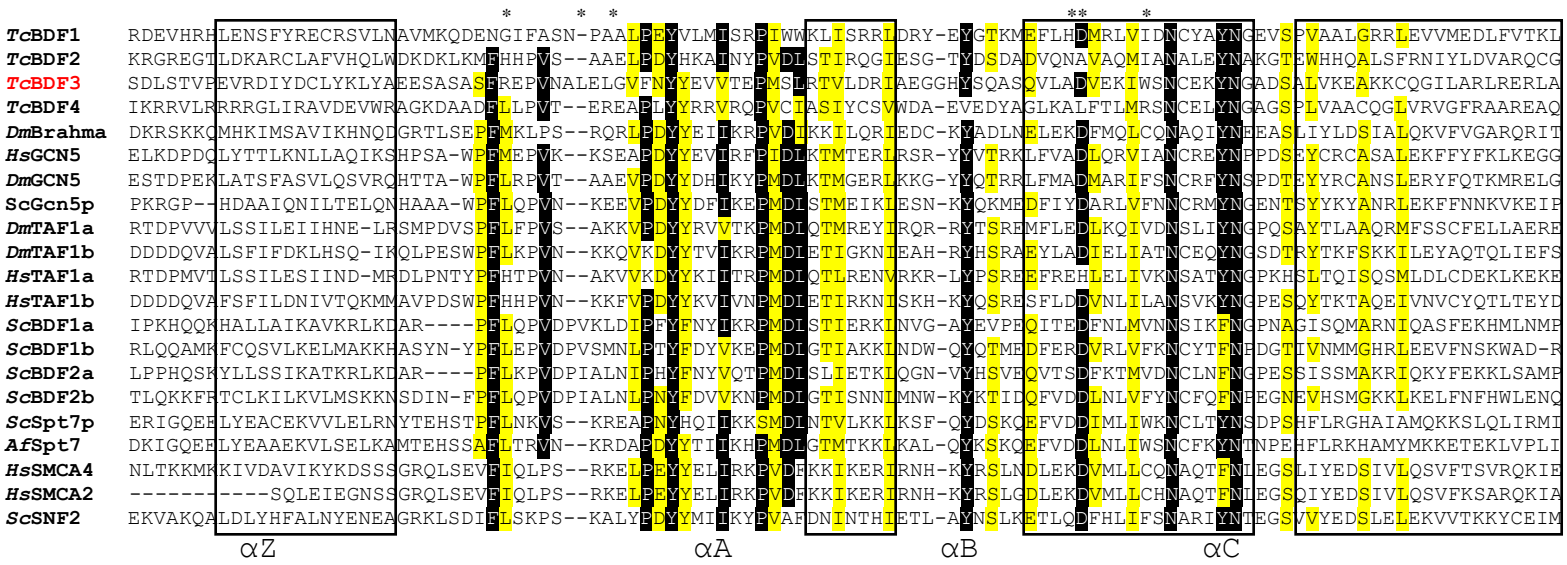
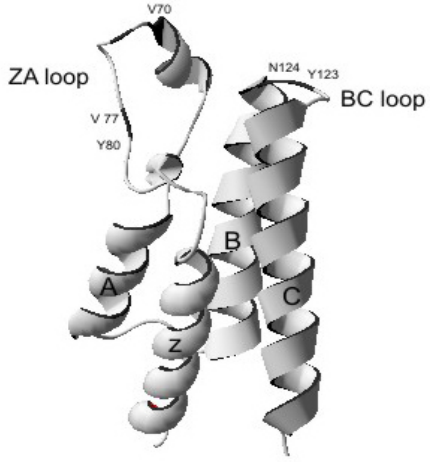
B



C



D

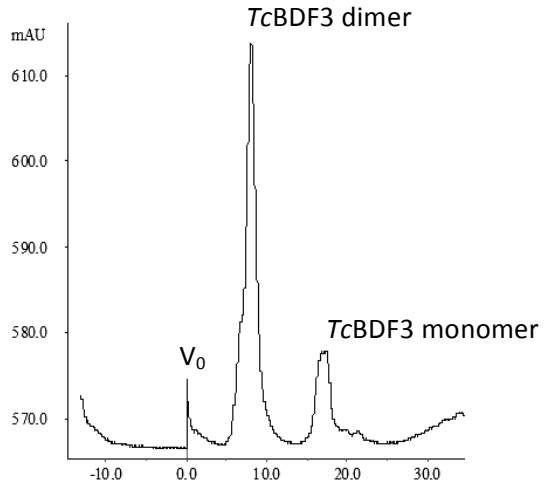


Figure S1. Sequence analysis and dimeric state of *Tc*BDF3. (A) Aminoacidic sequence of *Tc*BDF3. The bromodomain predicted by Pfam is on black background, the acidic region is on light blue background and the basic region is on green background. (B) Sequence alignment of known bromodomains. The sequences were aligned using ClustalW, identical residues are white on black background and conservative changes are black on yellow background. The bromodomain alpha-helixes are marked with boxes. Asterisks (*) indicate residues that are important for the interaction with acetylated lysine residues. Several bromodomain-containing proteins have two bromodomain modules in tandem. In those cases, both were included in the alignment and were differentiated as ‘‘a” –N-terminal domain– and ‘‘b” –C-terminal domain–. The sequences are (species name; GenBank accession number or TriTrypDB accession number): *Dm*Brahma (*Drosophila melanogaster*; P25439), *Hs*SMCA4 (*Homo sapiens*; P51532), *Hs*SMCA2 (*H. sapiens*; P51531), *Dm*GCN5 (*D. melanogaster*; AAC39102.1), *Hs*GCN5 (*H. sapiens*; AAC39769.1), *Sc*Gcn5p (*Saccharomyces cerevisiae*; NP_011768.1), *Sc*BDF1a and *Sc*BDF1b (*S. cerevisiae*; P35817), *Sc*BDF2a and *Sc*BDF2b (*S. cerevisiae*; YDL070W), *Dm*TAF1a (TAFII250) and *Dm*TAF1b (TAFII250) (*D.melanogaster*; P51123), *Hs*TAF1a (TAFII250) and *Hs*TAF1b (TAFII250) (*H. sapiens*; P21675), *Sc*Spt7 (S. cerevisiae; NP_009637.1), *Af*Spt7 (*Aspergillus fumigatus*; XP_754519.1), *Tc*BDF1 (*Trypanosoma cruzi*; TcCLB.506247.80), *Tc*BDF2 (*T. cruzi*; TcCLB.506553.20), *Tc*BDF3 (*T. cruzi*; TcCLB.510719.70), *Tc*BDF4 (*T. cruzi*; TcCLB.504213.70). (C) Secondary structure of *Tc*BDF3 modelled by the Phyre server. The conserved residues that are important for the interaction with acetylated residues in other known bromodomains are indicated, as well as the four alpha helixes (αA, αB, αC and αZ) and the ZA and BC loops. (D) Determination of the multimeric state of *Tc*BDF3 by size exclusion chromatography. V_0, void volume, determined using dextran blue. The standard curve was constructed using: Lisozime (14,7 kDa), GST (27 kDa for the monomeric form and 54 kDa for the dimeric form) and Bovine Serum Albumin (67 kDa) (not shown).

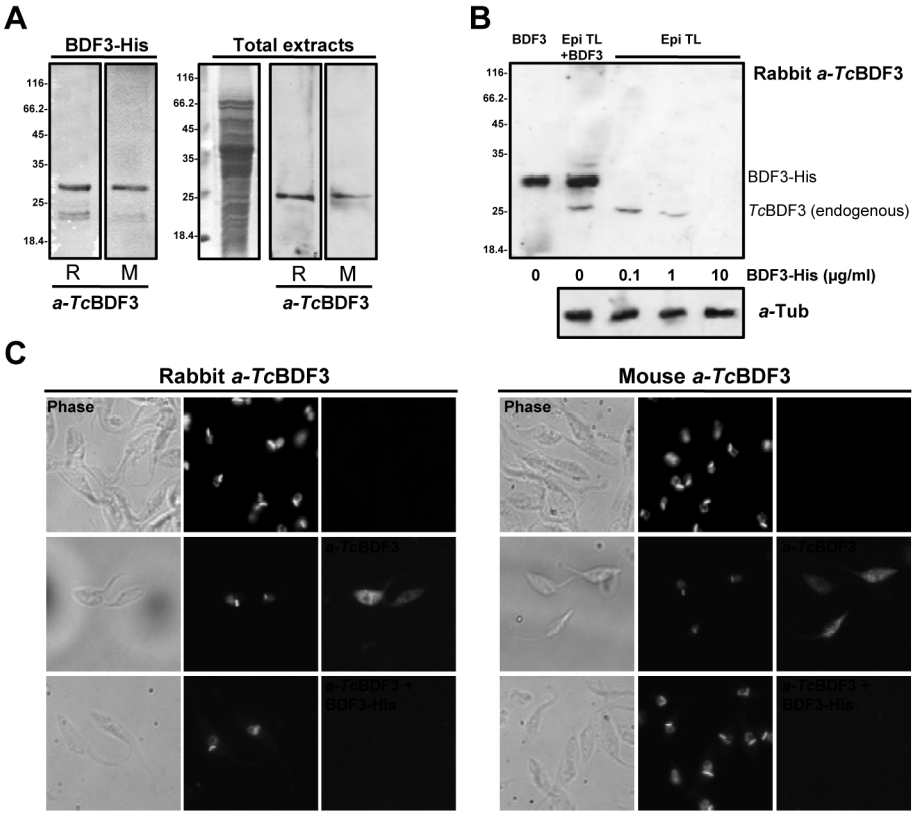


Figure S2. Anti-*Tc*BDF3 antibodies raised in rabbit and mouse are specific and do not cross react with other *T. cruzi* bromodomains. (A) Western blot analysis with rabbit (R) and mouse (M) anti-*Tc*BDF3

antibodies (*a-Tc*BDF3). Recombinant *Tc*BDF3 (BDF3-His) and total protein extracts were tested. (B) Western blot analysis with rabbit anti-*Tc*BDF3 antibodies competed with different amounts of recombinant *Tc*BDF3 in total lisates from epimastigotes (Epi TL). Recombinant *Tc*BDF3 (BDF3) and total lisates from epimastigotes supplemented with BDF3-His were also blotted (Epi TL+BDF3). Mouse anti-α tubulin (*a*-Tub) was used as a loading control. (C) Immunoflurescence assays in epimastigotes using rabbit and mouse pre-immune sera, purified anti-*Tc*BDF3 antibodies and anti-*Tc*BDF3 antibodies competed with 1 µg/ml of recombinant *Tc*BDF3. Anti-rabbit IgG conjugated to Fluorescein was used as secondary antibody. Nucleus and kinetoplast were labelled with DAPI.

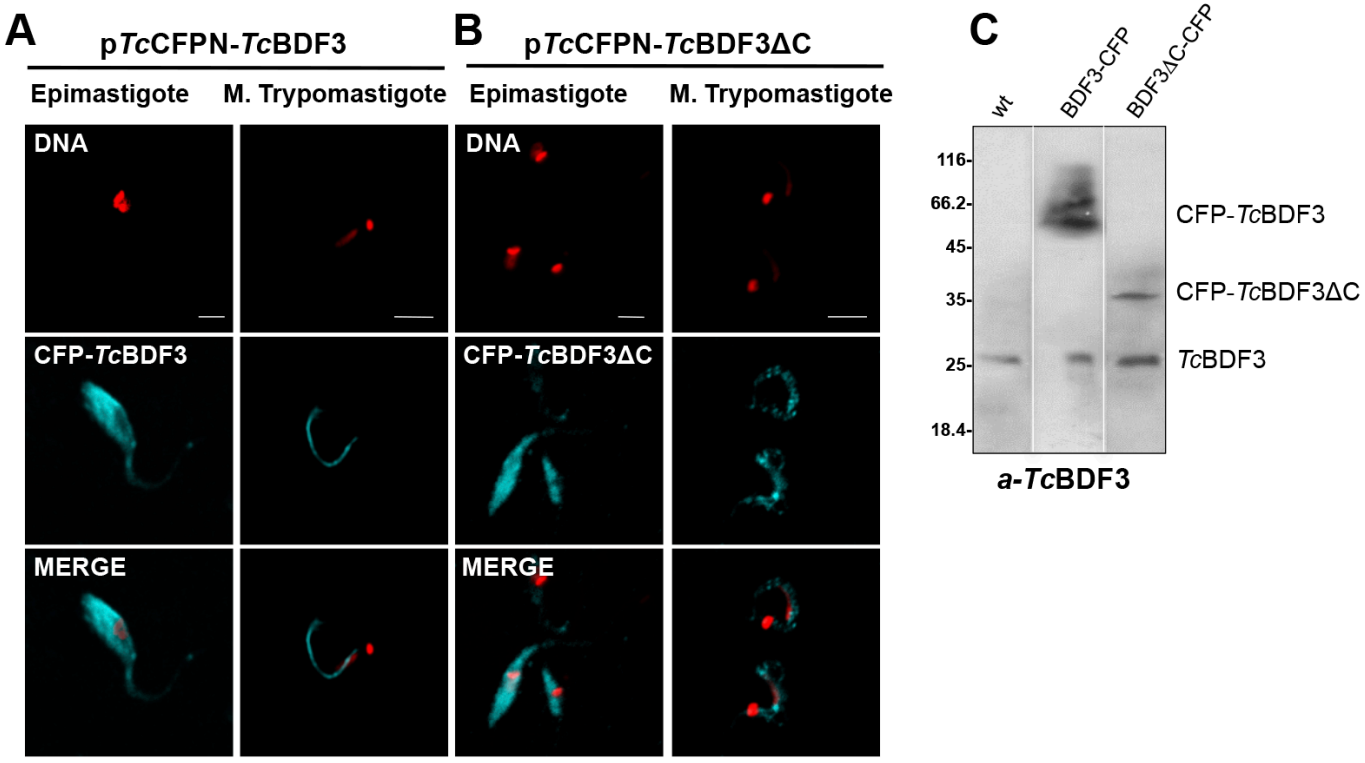


Figure S3: Truncated *Tc*BDF3 miss-localizes in metacyclic trypomastigotes. CFP-*Tc*BDF3 (A) and CFP-*Tc*BDF3ΔC (B) localization (cyan) in epimastigotes and metacyclic trypomastigotes was determined by confocal mycroscopy. Nucleus and kinetoplast was stained with propidium iodide (red) (DNA). Bars = 2 µm. (C) Western blot analysis of epimastigotes total extracts of: Dm28c (wt), Dm28c p*Tc*CFPN-*Tc*BDF3 (BDF3-CFP) and Dm28c p*Tc*CFPN-*Tc*BDF3ΔC (BDF3ΔC-CFP). Endogenous *Tc*BDF3, CFPN-*Tc*BDF3 and CFPN-*Tc*BDF3ΔC were detected with anti-*Tc*BDF3 antibodies.