

A**TcBDF3**

MGSTGRKAVDEVNHWIAYIDCALSHPHPLPKGKHVFRSDLSTVPEVRDIYDCLYKLYAESASASAFREPVNALELGVFNYYEVVTEPMSLRTVLDRIAEGGHYSQASQVLADVEKIWSNCE

KYNGADSALVKEAKKCGI LARLRERLAEEQPAPNAELDKIISAFESADESVLGELAYFRREDPSLIISNGDVDLTALRVKHLKAMKAILERAMNGGGGRG

B

TcBDF1	RDEVHRL	LENSFYRECRSVLNAV	MVKQDENGIFASN-	PAALPEYVLM	SRPIWVKLI	SRRLDRY-EY	GTKMEFLHDMRL	VIDNCYAYNGEVS	VVAALGRRLEVV	MEDLDFVTKL
TcBDF2	KRGREGI	LDKARCLAFVHQLW	DKDKLKFHHPVS--	AAELPDYHKA	NYPVDLSTIR	QGIESG-TYDS	DADVQNAVAQMI	ANALEYNAKGT	FWHHQALSFRN	IYLDVARQCG
TcBDF3	SDLSTV	EVVRDIYDCLYKLY	AESASASAFREPV	NALELGVFNYYE	VVTEPMSLRTV	LDRIAEGGHYSQ	ASQVLADVEKI	WSNCEKYN	GADSLVKEAKK	CGILARLRERLA
TcBDF4	IKRRVLR	RRRRLIRAVDEVWR	AGKDAADFLLPVT--	EREAPDYRRVR	QPVCIASIYCS	VWDA-EVEDY	AGLKAFTLMRS	NCELYNGAGS	PLVAACQGL	VRVGFRAAREAQ
DmBrahma	DKRSKK	MHKIMS	AVIKHNQIGRTLSE	FMKLP	--RQRLPDY	EITKRPV	DKKILQRIEDC	-KYADLNELEK	DFMQLCNAQI	YNEEASLIYLD
HsGCN5	ELKDPD	QLYTTLKNLLA	QIKSHPSA-WF	FMEPVK--KSE	APDYEVIRFP	DLKTMTERL	RSR-YVTRK	LFVADLQ	RVIANCREYN	FPDSEYCRCA
DmGCN5	ESTDPE	KLATSFA	SVLQSVRCHTTA-	WFLR	PVT--AAE	VPDYDHIKYP	MDLKTMG	ERLKKG-Y	YQTRRL	FMA
ScGcn5p	PKRGP--	HDAAIQ	NILTELOHAAA-	WF	LQPVN--KEE	VPDYDFIK	PEMDLST	MEIKLESN-	KYQKME	DFI
DmTAF1a	RTDPVV	LSSILEI	IHNE-LFS	MPDVSE	FLFPVS--	AKKVPDY	RVVTKPMD	LQTMREY	TRQF-RYT	SREMF
DmTAF1b	DDDQV	LSFIFDKL	HSQ-IKQL	PESWFL	PKPVN--	KKQVKD	YTVIKR	PMDLET	IGKNIEAH-	RYHSRAE
HsTAF1a	RTDPMV	LSSILESI	IIND-M	DLPNTY	FHTPVN--	AKVVKD	YKITT	REMDL	QTLREN	VKRRLYPS
HsTAF1b	DDDQV	FSFILD	NIVTQKMM	AVPDSW	FFHHPVN--	KKFV	PDYKVI	VNPM	DLSTIR	KNISKH-
ScBDF1a	IPKHQ	ERHALLA	IAKAVKRL	KIAR---	FLQ	PVDPVKLD	IPFENY	IKR	PMDLSTIER	KNINVG-AYE
ScBDF1b	RLQQAM	FCQSVL	KELMAK	KHASYN-Y	FLE	PVDPVSMN	LPTFY	FDYV	KEPMDL	GTI
ScBDF2a	LPPHQS	YLLSS	IKATKRL	KIAR---	FLK	PVDP	PIALNIP	HFENY	VQTPMD	LSTIET
ScBDF2b	TLQKFF	TCLKIL	KVLM	SKKNSDIN-	FF	LQPV	DPIALN	LPNY	FDVVK	NPM
ScSpt7p	ERIGQE	ELYEACE	KVLELRN	YTEHST	FLNK	YS--KRE	APNYHQI	IKK	SMDLNT	VLKKISF-
AfSpt7	DKIGQE	ELYEAAE	KVLS	SELKAMTE	HSSAFL	TRVN--	KRD	APDY	TIHK	PEMDL
HsSMCA4	NLT	TKMK	KIVDA	VIK	YK	YDSSGR	QLSEVFI	QLPS--	RKEL	PEY
HsSMCA2	-----	---	SQLE	IEGNSS	GR	QLSEVFI	QLPS--	RKEL	PEY	ELIRK
ScSNF2	EKVAKQ	ALDLYH	FALNYE	NAEGR	KLSDI	FLSK	PS--KAL	Y	PDY	YMI

α Z
 α A
 α B
 α C

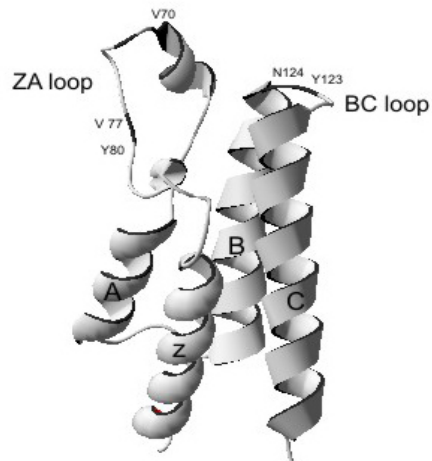
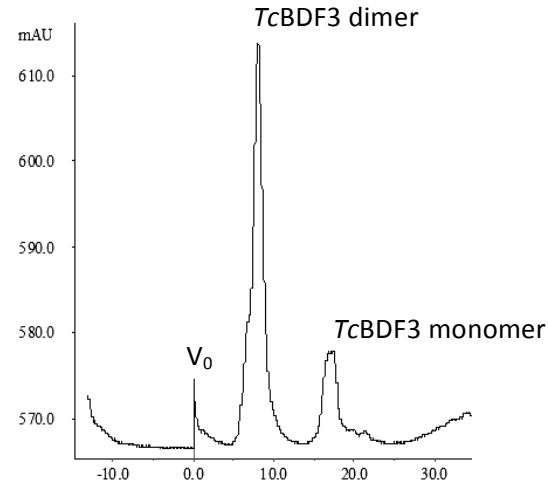
C**D**

Figure S1. Sequence analysis and dimeric state of TcBDF3. (A) Aminoacidic sequence of TcBDF3. 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-helices 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): *DmBrahma* (*Drosophila melanogaster*; P25439), *HsSMCA4* (*Homo sapiens*; P51532), *HsSMCA2* (*H. sapiens*; P51531), *DmGCN5* (*D. melanogaster*; AAC39102.1), *HsGCN5* (*H. sapiens*; AAC39769.1), *ScGcn5p* (*Saccharomyces cerevisiae*; NP_011768.1), *ScBDF1a* and *ScBDF1b* (*S. cerevisiae*; P35817), *ScBDF2a* and *ScBDF2b* (*S. cerevisiae*; YDL070W), *DmTAF1a* (TAFII250) and *DmTAF1b* (TAFII250) (*D. melanogaster*; P51123), *HsTAF1a* (TAFII250) and *HsTAF1b* (TAFII250) (*H. sapiens*; P21675), *ScSpt7* (*S. cerevisiae*; NP_009637.1), *AfSpt7* (*Aspergillus fumigatus*; XP_754519.1), *TcBDF1* (*Trypanosoma cruzi*; TcCLB.506247.80), *TcBDF2* (*T. cruzi*; TcCLB.506553.20), *TcBDF3* (*T. cruzi*; TcCLB.510719.70), *TcBDF4* (*T. cruzi*; TcCLB.504213.70). (C) Secondary structure of TcBDF3 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 helices (α A, α B, α C and α Z) and the ZA and BC loops. (D) Determination of the multimeric state of TcBDF3 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).

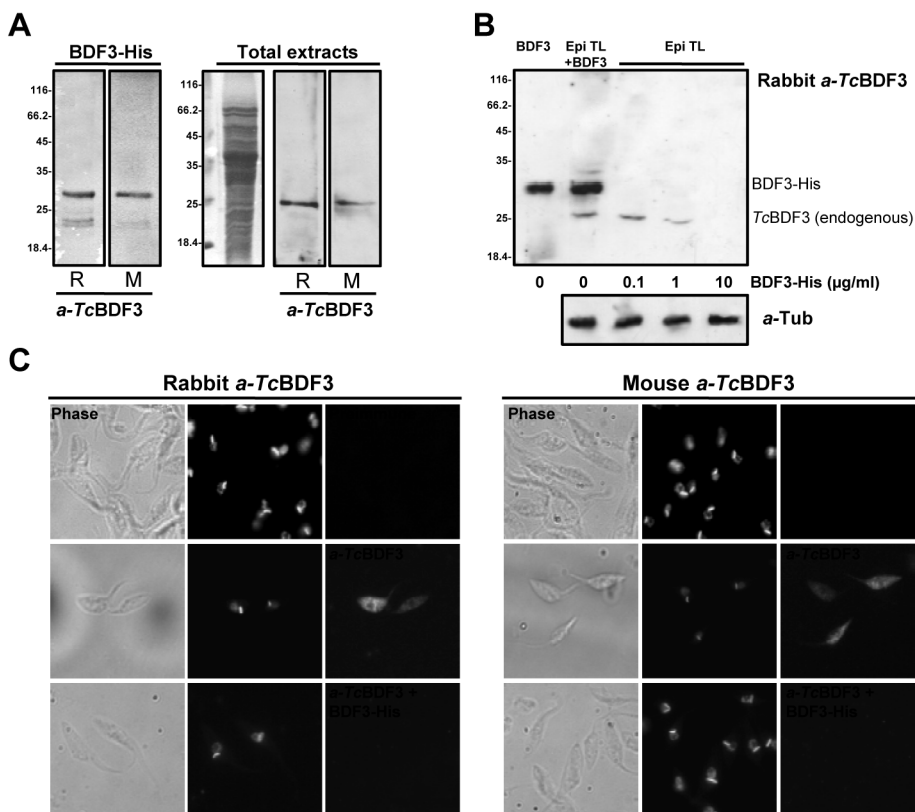


Figure S2. Anti-TcBDF3 antibodies raised in rabbit and mouse are specific and do not cross react with other *T. cruzi* bromodomains. (A) Western blot analysis with rabbit (R) and mouse (M) anti-TcBDF3

antibodies (*a-TcBDF3*). Recombinant *TcBDF3* (BDF3-His) and total protein extracts were tested. (B) Western blot analysis with rabbit anti-*TcBDF3* antibodies competed with different amounts of recombinant *TcBDF3* in total lisates from epimastigotes (Epi TL). Recombinant *TcBDF3* (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) Immunofluorescence assays in epimastigotes using rabbit and mouse pre-immune sera, purified anti-*TcBDF3* antibodies and anti-*TcBDF3* antibodies competed with 1 μ g/ml of recombinant *TcBDF3*. Anti-rabbit IgG conjugated to Fluorescein was used as secondary antibody. Nucleus and kinetoplast were labelled with DAPI.

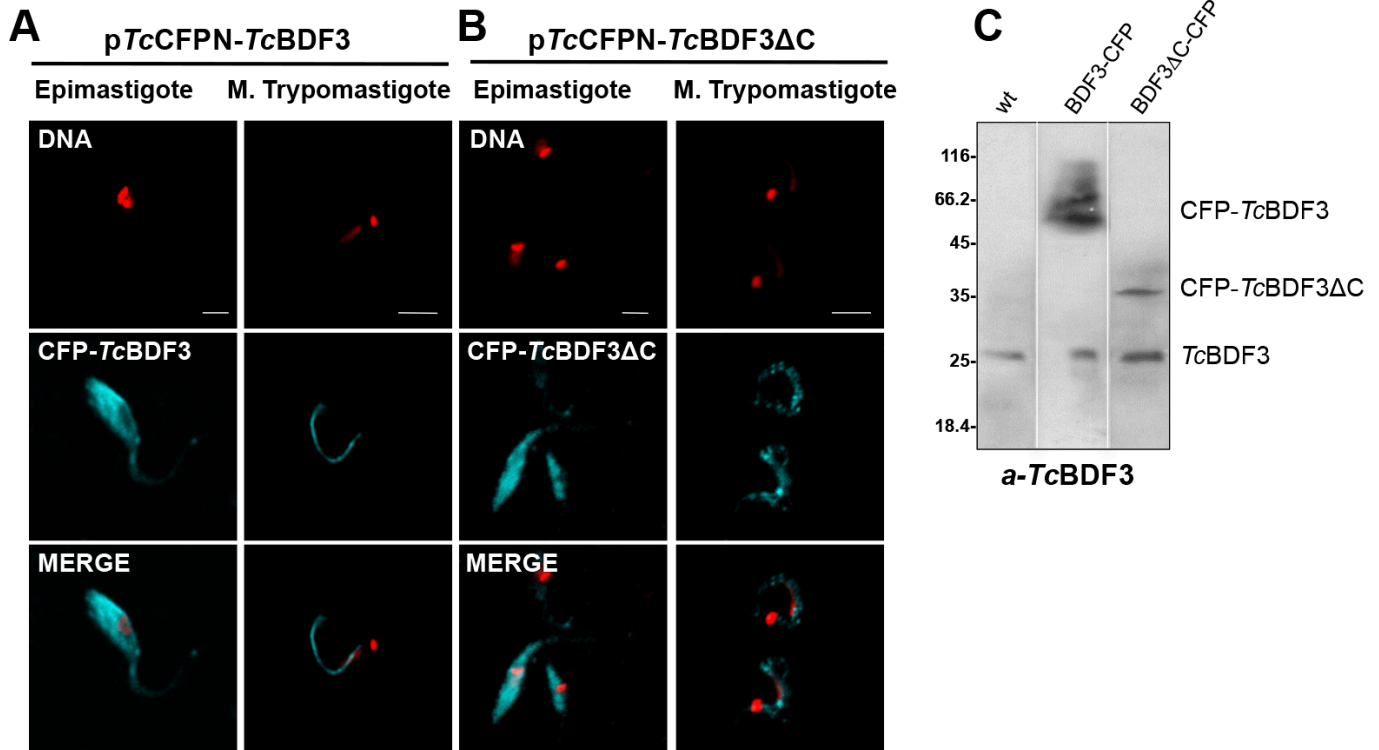


Figure S3: Truncated *TcBDF3* miss-localizes in metacyclic trypomastigotes. CFP-*TcBDF3* (A) and CFP-*TcBDF3*ΔC (B) localization (cyan) in epimastigotes and metacyclic trypomastigotes was determined by confocal microscopy. Nucleus and kinetoplast was stained with propidium iodide (red) (DNA). Bars = 2 μ m. (C) Western blot analysis of epimastigotes total extracts of: Dm28c (wt), Dm28c pTcCFPN-*TcBDF3* (BDF3-CFP) and Dm28c pTcCFPN-*TcBDF3*ΔC (BDF3ΔC-CFP). Endogenous *TcBDF3*, CFPN-*TcBDF3* and CFPN-*TcBDF3*ΔC were detected with anti-*TcBDF3* antibodies.