SUPPLEMENTAL FIGURES

Characterization of *Trypanosoma cruzi* sirtuins as possible drug targets for Chagas Disease

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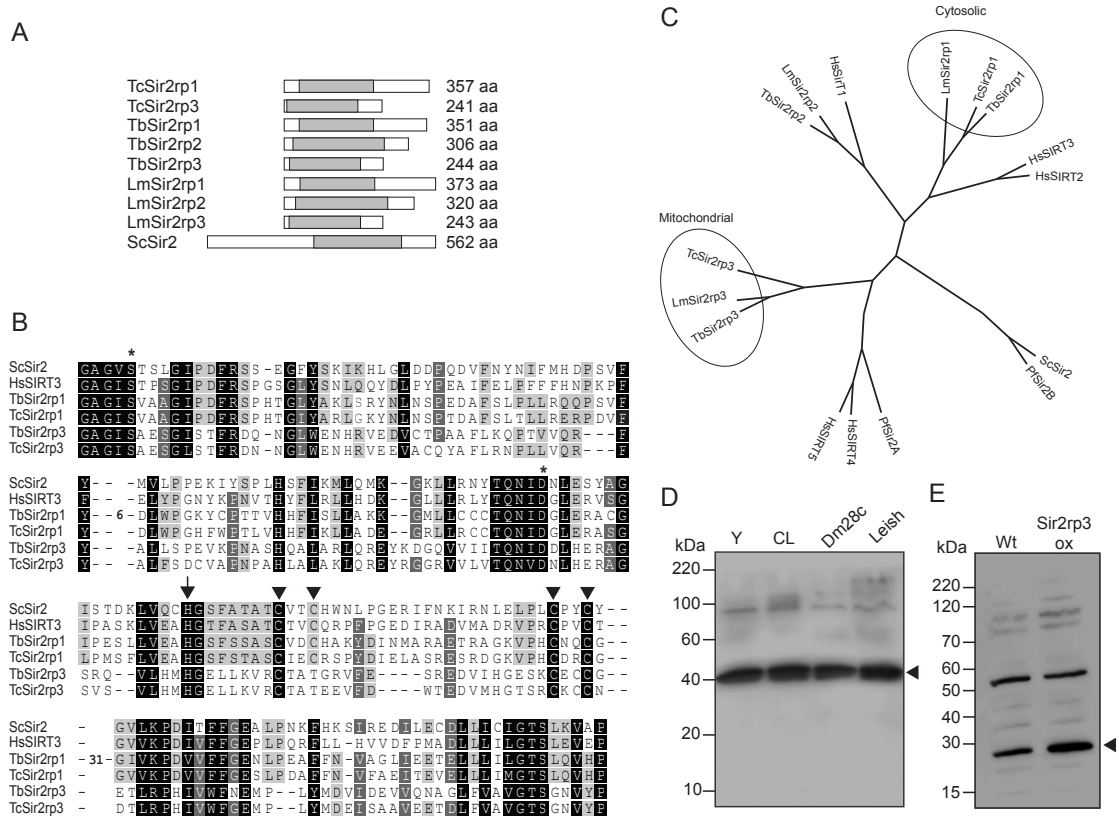

FIG S1. *T. cruzi* sirtuins amino acid sequence analysis. (A) Schematic representation of *T. cruzi* sirtuin domain (showed in grey) compared with *L. major* (LmSir2rp1-3), *T. brucei* (TbSir2rp1-3) and *S. cerevisiae* (ScSir2). **(B)** ClustalW amino acid sequence alignment between the core deacetylase domain of *T. cruzi* sirtuins and sirtuins of *S. cerevisiae* (ScSir2, NP_010242), *H. sapiens* (HsSIRT3, NP_036371.1), and *T. brucei* TbSir2rp1, AAX70528.1 and TbSir2rp3, AAX79070.1). Arrowheads indicate a putative Zn^{2+}-binding motif, arrow indicates critical catalytic residues, and asterisks correspond to NAD+-binding sites. **(C)** Unrooted tree derived from phylogenetic analysis of the above sequences based in the Neighbor-Joining (NJ) method using the Clustal 1.8 and TreeView. *S. cerevisiae* (ScSir2, NP_010242), *H. sapiens* (HsSIRT1, AAH12499.1; HsSIRT2, AAK51133.1; HsSIRT3, NP_036371.1; HsSIRT4, NP_036372.1 and HsSIRT5, Q9NXA8.2); *T. brucei* TbSir2rp1, AAX70528.1; TbSir2rp2, AAZ13079 and TbSir2rp3, AAX79070.1); *L. major* (LmSir2rp1, CAJ040546; LmSir2rp2, CAJ04804 and LmSir2rp3, CAJ07927); *P. falciparum* (PfSir2A, Q8IE47.1; PfSir2B, Q8IKW2.1). (**D**) Western blot of total extracts of 1 x 10^7 epimastigotes of Y, Cl-Brener (CL), Dm28c and promastigotes forms of *Leishmania major* (Leish) probed with anti-Sir2rp1. (**E**) Western blot of total extracts of 1 x 10^7 epimastigotes of wild type Y strain, or the Y strain overexpressing TcSir2rp3 probed with anti-Tc-Sir2rp3. Size markers are shown on the left side of each panel.

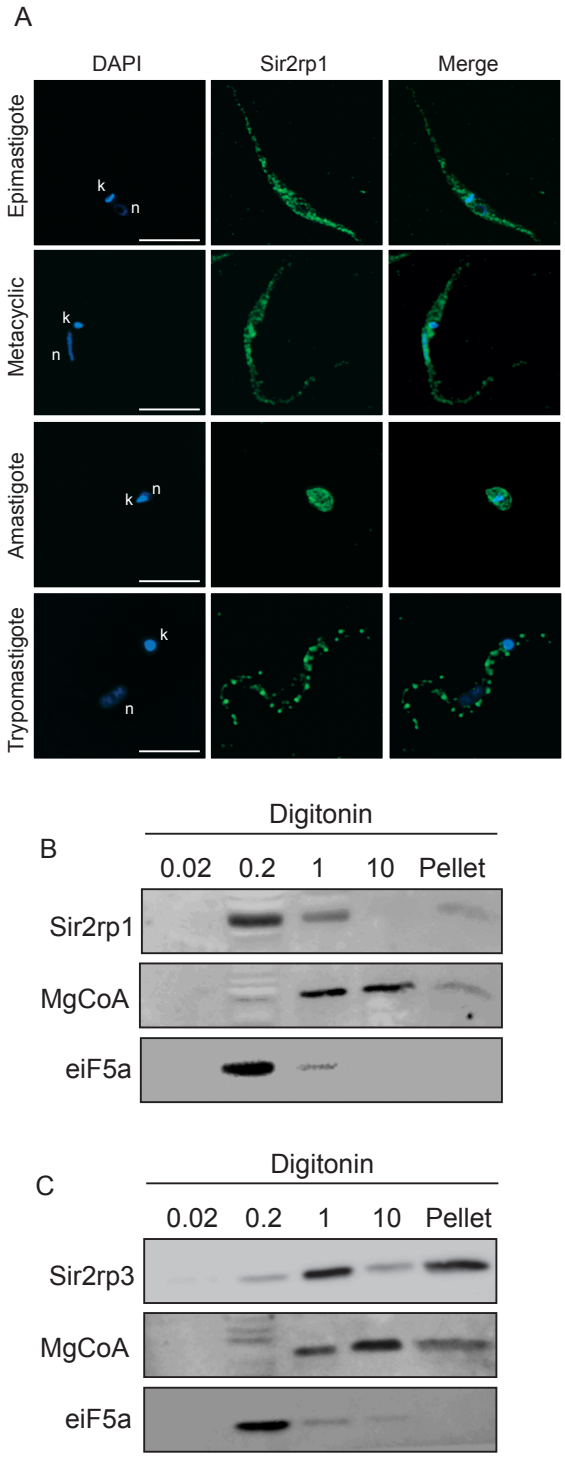

FIG S2. Subcellular localization of TcSir2rp1 and TcSir2rp3. (**A**) Immunofluorescence of epimastigotes, metacyclic-trypomastigotes, intracellular amastigotes and trypomastigotes derived from mammalian cells, probed with anti-Sir2rp1. The TcSir2rp1 labeling is shown in green and the DAPI in blue. Bar = 5 µm. Western blot of solubilized proteins extracted with digitonin at the indicated concentrations in mM of TcSir2rp1-ox (**B**) and TcSir2rp3-ox (**C**) parasites probed with anti-HA antibodies for TcSir2rp1-ox and the indicated antibodies for the other blots.

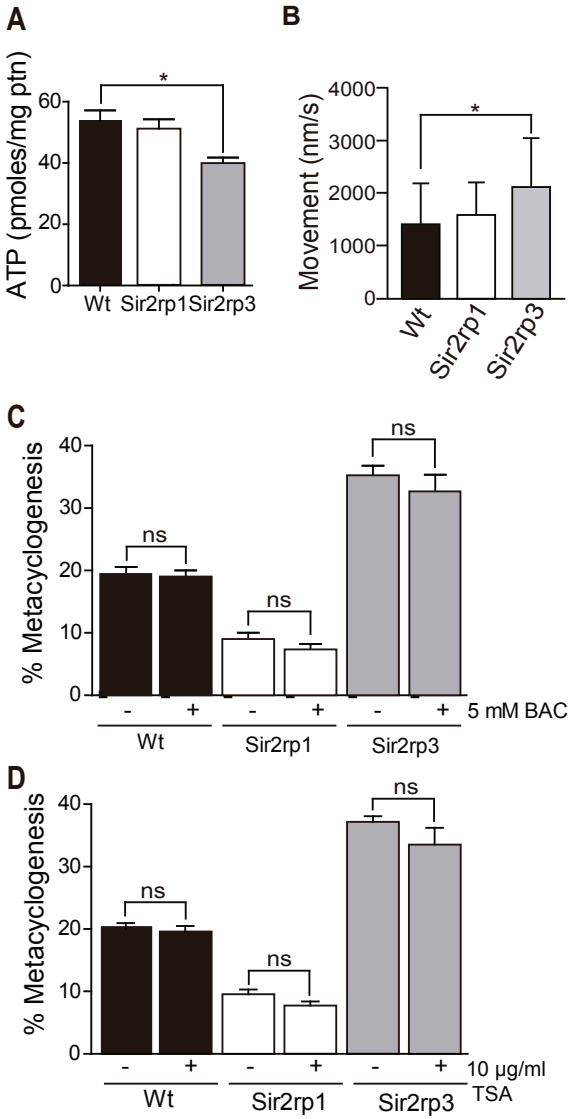

FIG S3. Phenotypic analysis of the sirtuin overexpressors. Panel **(A)** panel (**B**) shows respectively the intracellular ATP levels and the movement of wild type (dark bars), TcSir2rp1-ox (empty bars), and TcSir2rp3-ox (grey bars) epimastigotes. **(C)** and (**D)** show respectively the metacyclogenesis in the absence (-) and presence (+) of 5 mM butyric acid (BAC) or 10 µg/ml trichostatin A using wild type (dark bars); TcSir2rp1-ox (empty bars) and TcSir2rp3-ox (grey bars) epimastigotes. The values are mean ± standard deviation (n = 3) * ($p<0.01$).

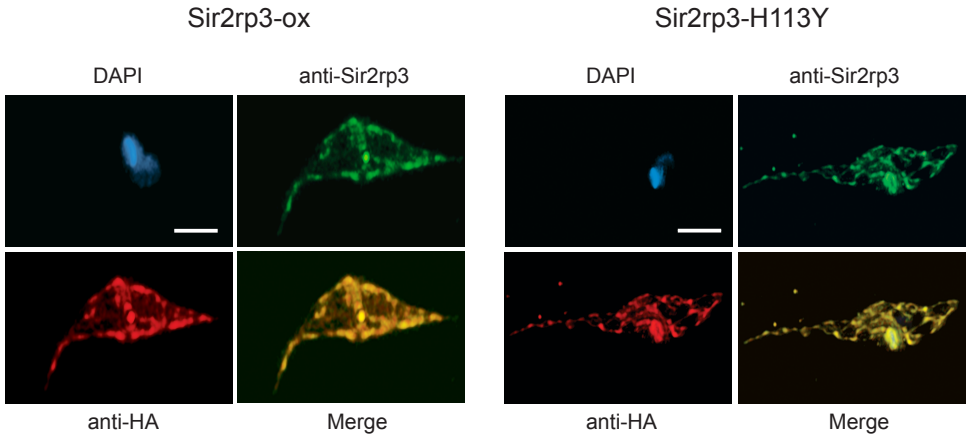

FIG S4. Wild type and overexpressor TcSir2rp3 have similar localizations. Indirect immunofluorescences of TcSir2rp3-ox and TcSir2rp3-His113Y epimastigotes using anti-Sir2rp3 (green) and anti-HA (red). The figures also show the DAPI labeling and the merged fluorescence. Bars = 2.5 μm.

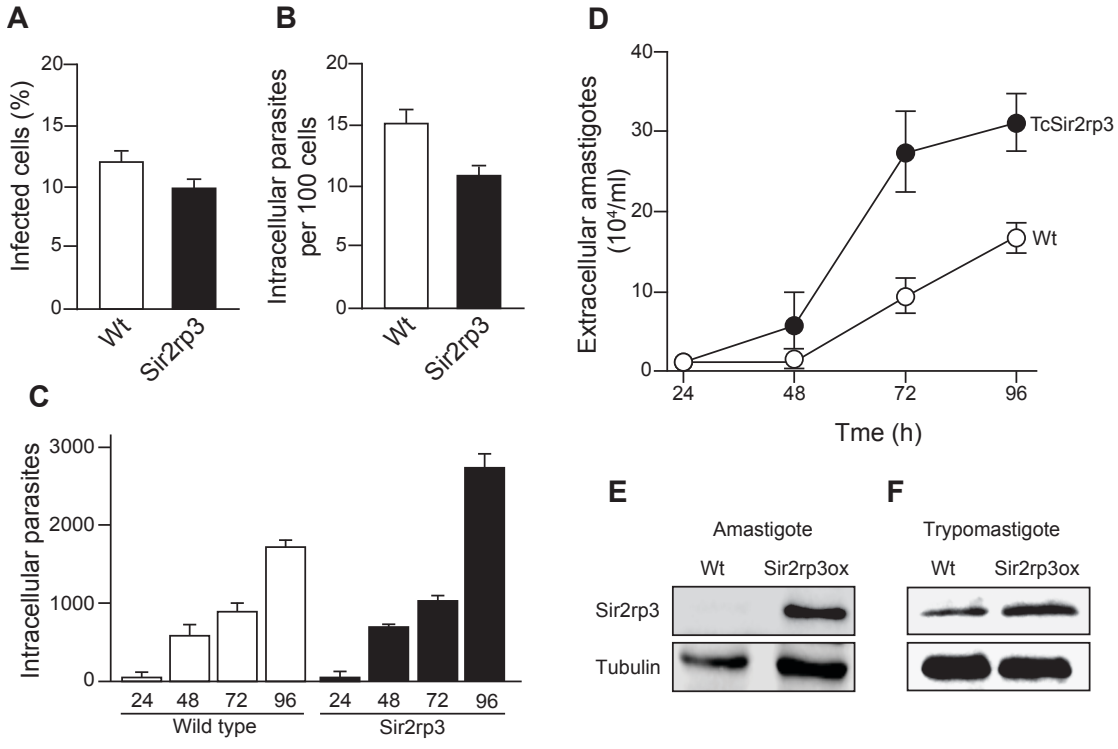

FIG S5. Trypomastigote invasion is decreased while intracellular multiplication is enhanced in TcSir2rp3 overexpressors resulting in the release of amastigotes. Wild type and TcSir2rp3-ox trypomastigotes were incubated for 1h with LLC-MK_2 cells and the number of infected cells **(A)** and intracellular parasites (**B**) measured after infection. Panel **(C)** shows the number of intracellular parasites following invasion of wild type and TcSir2rp3-ox parasites. The numbers are mean ± standard deviation of triplicates. **(D)** Shows the number of extracellular amastigotes released from infected mammalian cells during the progression of infection with trypomastigotes from wild type (empty circles) and TcSir2rp3-ox (dark circles) parasites. **(E)** and **(F)** Western blots of wild type and TcSir2rp3ox intracellular amastigotes and trypomastigotes revealed with anti-TcSir2rp3 and anti-α-tubulin antibodies.

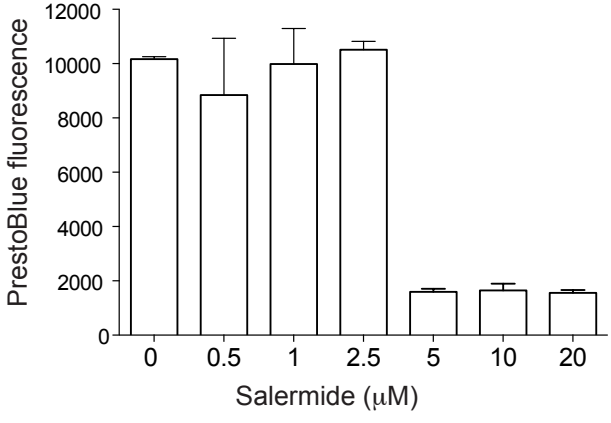

FIG S6 Cytotoxicity of salermide to LLCMK$_2$ cells. Cell viability was determined for the different concentrations of concentrations of salermide incubated after 48 h using PrestoBlue Viability reagent. The numbers are mean ± standard deviation (n = 3), and the experiment was repeated three times.