Supporting information

Inorganic Polyphosphate Interacts with Nucleolar and Glycosomal Proteins in Trypanosomatids

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Figs. S1-S9

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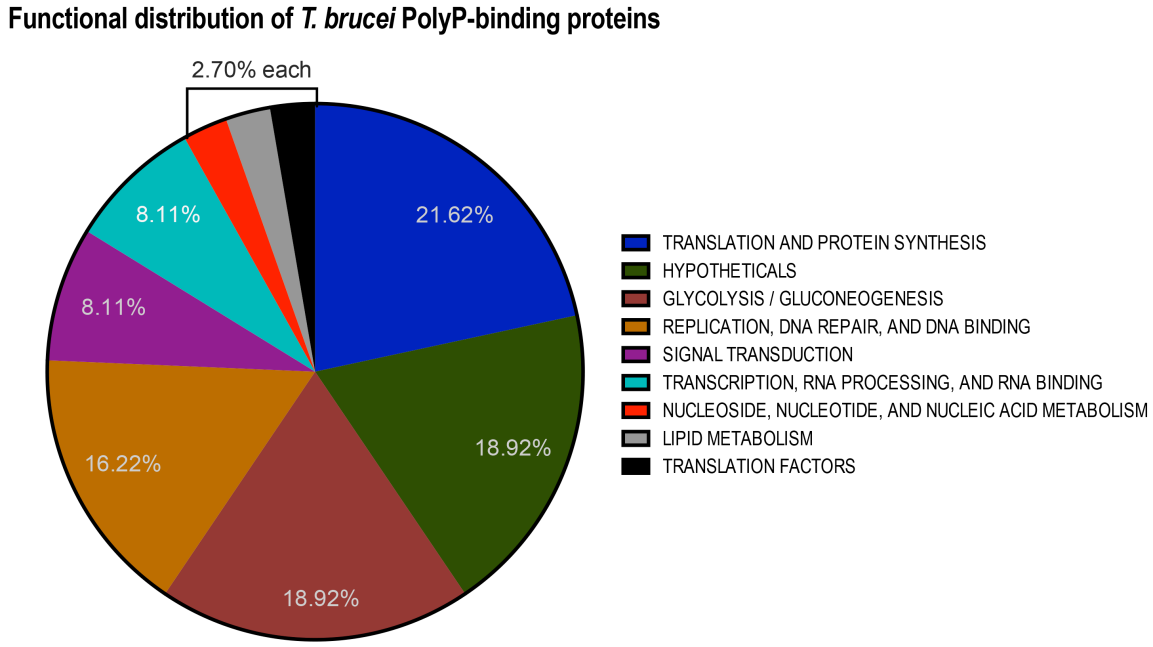

Fig. S1. Functional classification of proteins identified in *T. brucei* polyP-binding proteome.

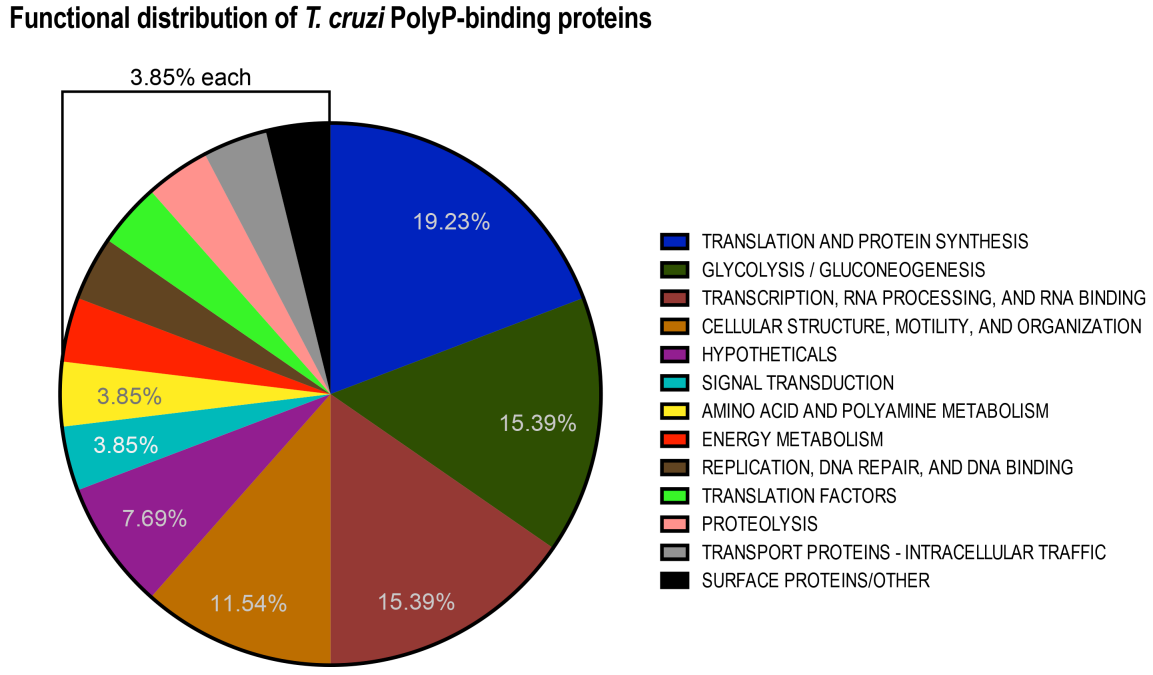

Fig. S2. Functional classification of proteins identified in *T. cruzi* polyP-binding proteome

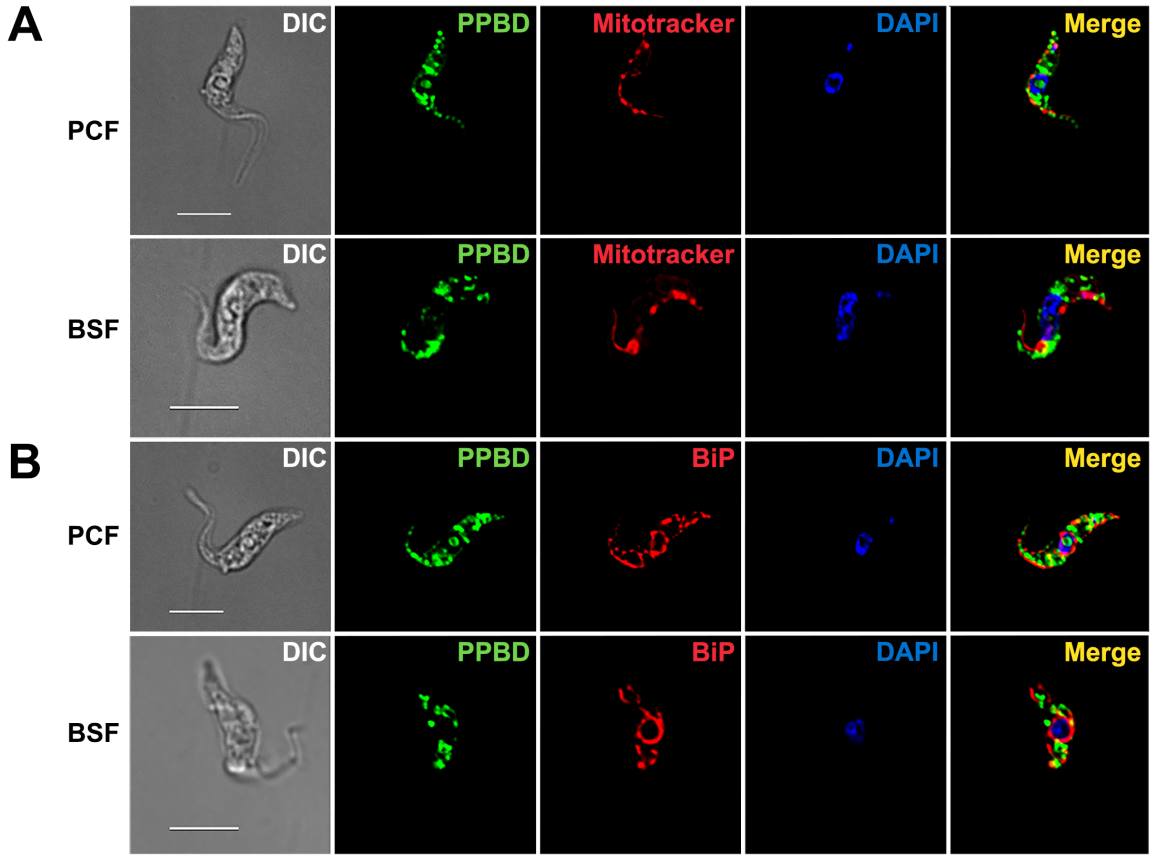

Fig. S3. Immunofluorescence microscopy analysis of polyP localization. (A) PPBD (*green*) does not co-localize with MitoTracker (*red*). (B) PPBD (*green*) does not co-localize with antibodies against BiP (*red*). DIC, differential interference contrast. DAPI staining in *blue*. Scale bars = 5 µm.

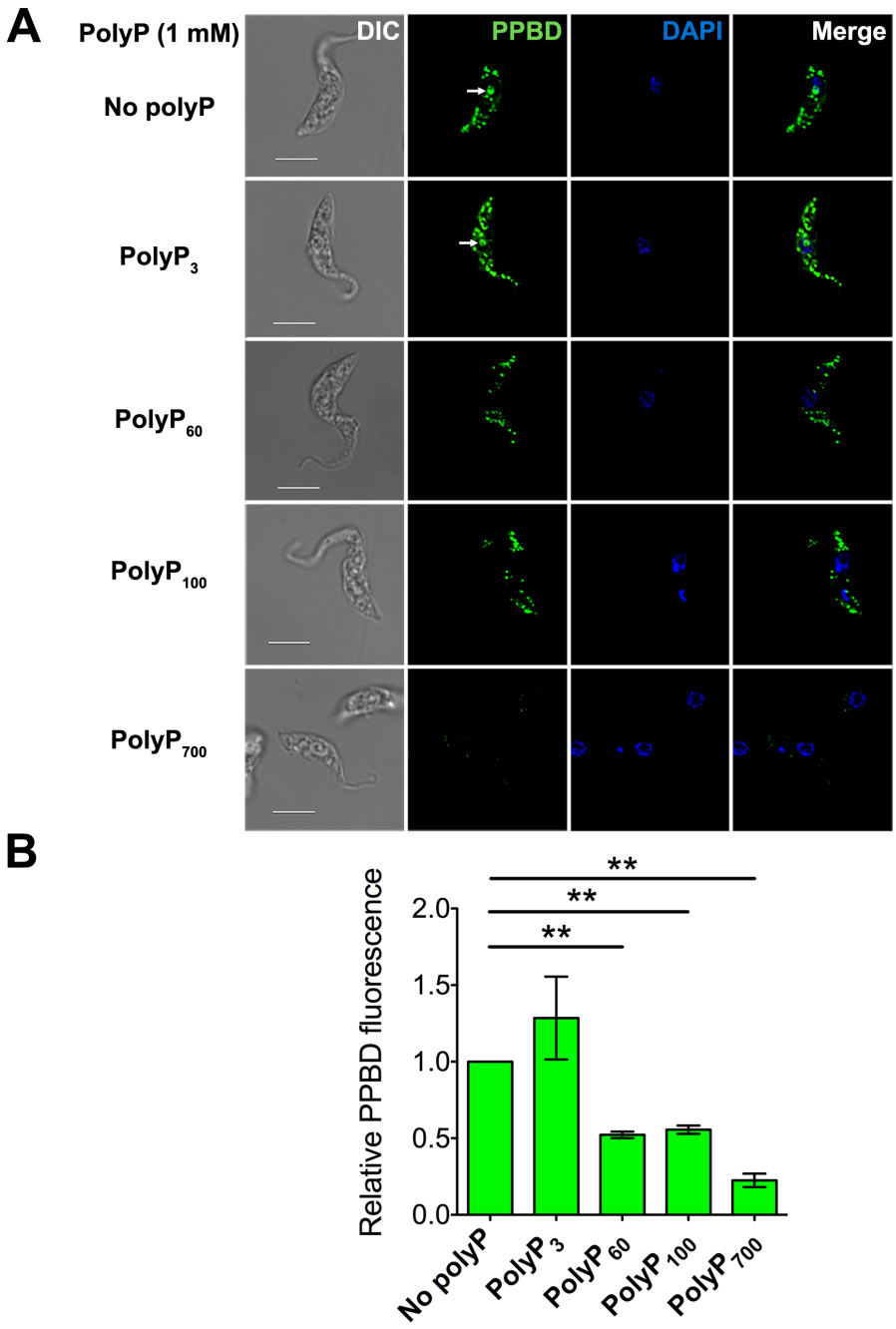

Fig. S4. Fluorescence microscopy analysis of the effect of polyP of different length on PPBD staining in *T. brucei* PCF. (A) Alexa Fluor 488-labeled PPBD was pre-incubated with 1 mM (in phosphate units) $polyP_3$, $polyP_{60}$, $polyP_{100}$, or $polyP_{700}$ for 1 h and then used for fluorescence microscopy. Nucleolus labeling is indicated by *white arrows*. DIC, differential interference contrast. DAPI staining is in *blue*. Scale bars = 5 µm. (B) Quantification of the fluorescence of cells labeled with PPBD previously incubated with polyP of different lengths as compared with control cells. A total of 664 cells were examined in three biological experiments. Values are means ± SEM, n = 3, ** $P < 0.01$, One-way ANOVA test with multiple comparisons.

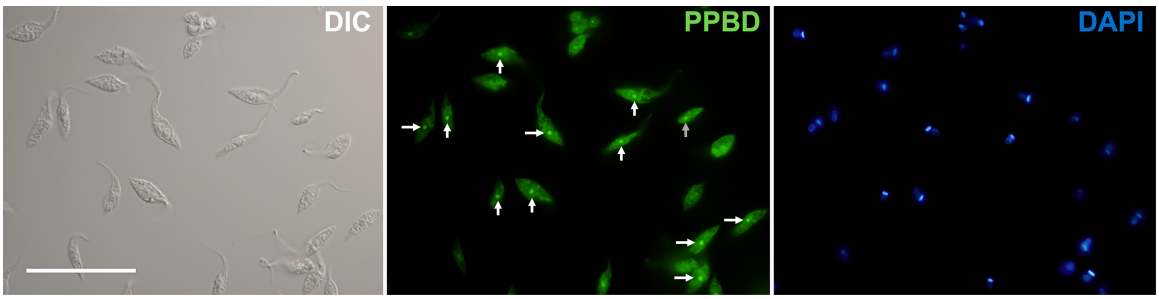

Fig. S5. Conventional fluorescence microscopy analysis of PPBD-labeled *T. cruzi* epimastigotes. PPBD (*green*) shows cytosolic labeling and labels the nucleolus (*white arrows*). DIC, differential interference contrast. DAPI staining is in *blue*. Scale bar = 50 μm.

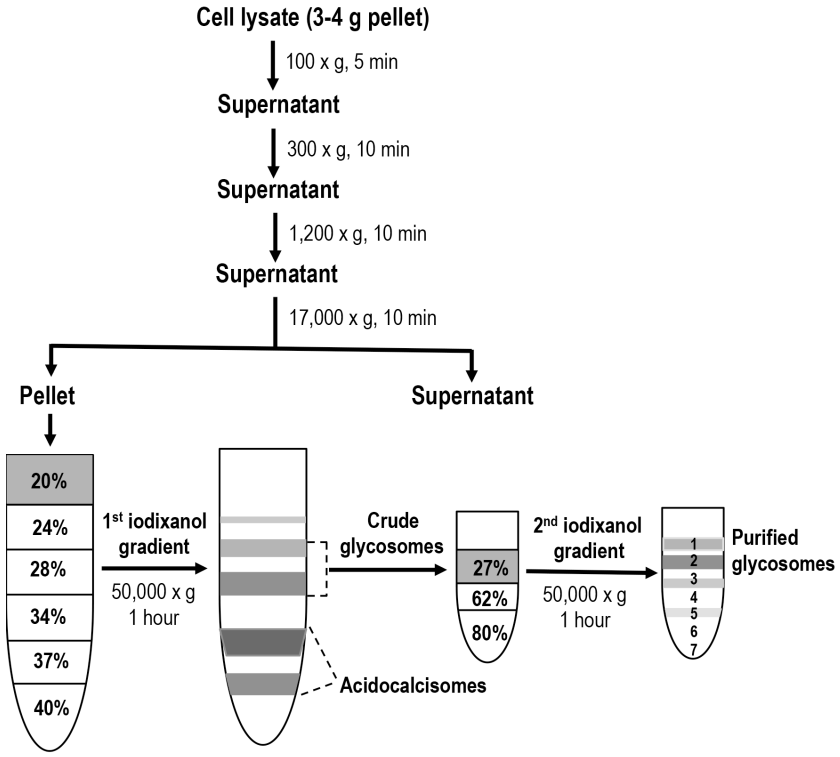

Fig. S6. Subcellular fractionation of glycosomes. Wild type *T. brucei* PCF lysates were obtained by grinding with silicon carbide, decanted by low speed centrifugation to eliminate debris and silicon carbide, and centrifuged at 17,000 x *g* for 10 min to isolate the organellar fraction that was applied to the 20% step of a discontinuous iodixanol gradient. After centrifugation at 50,000 g for 1 h, the fractions containing crude glycosomes were combined, washed, pelleted, resuspended and then applied to the 27% step of a second iodixanol gradient and centrifuged at 50,000 g for 1 h as described under *Experimental Procedures*. Aliquots from each fraction were used for enzymatic assays. Fractions 1 and 2 correspond to the purified glycosomes.

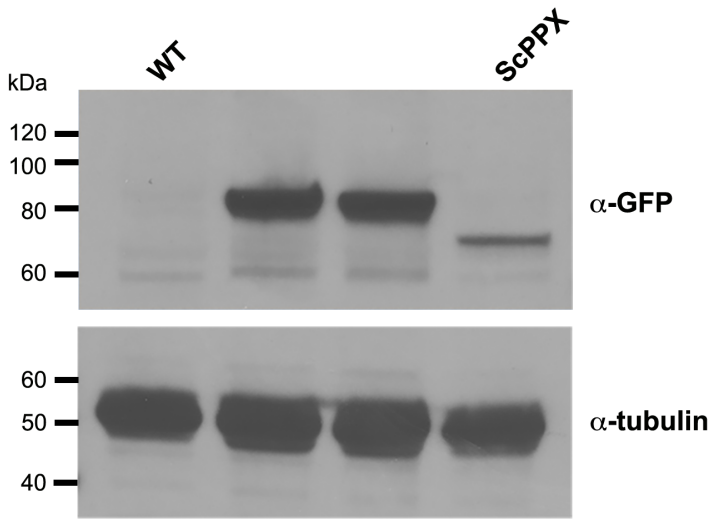

Fig. S7. Complete image of western blot analysis from PCF WT (*left lane*) and PTS2-ScPPX1-eYFP-expressing cells (*right lane*) using polyclonal antibody against GFP. The middle gel lanes correspond to samples not related to this work. Molecular weight markers are at *left*. Tubulin was used as a loading control.

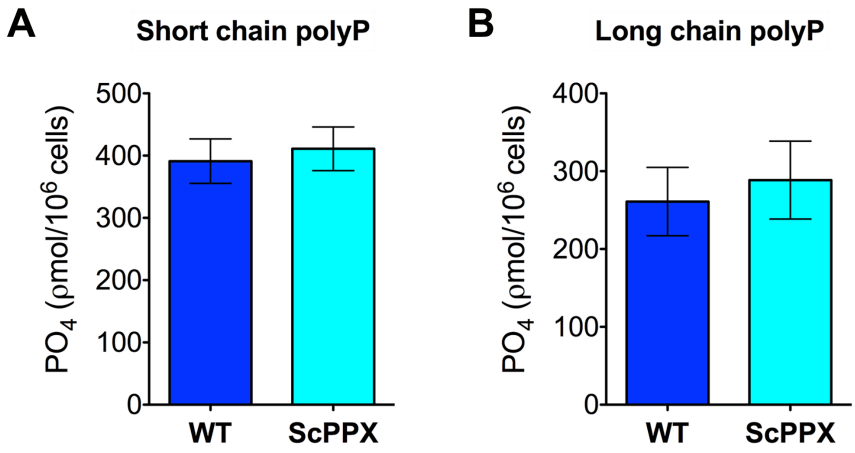

Fig. S8. Quantification of total short and long chain polyP extracted from PCF WT and *PTS2-ScPPX1-eYFP*-expressing cells. (A) Short chain polyP quantification. No significant differences observed, n = 3. (B) Long chain polyP quantification. No significant differences observed, n = 3.

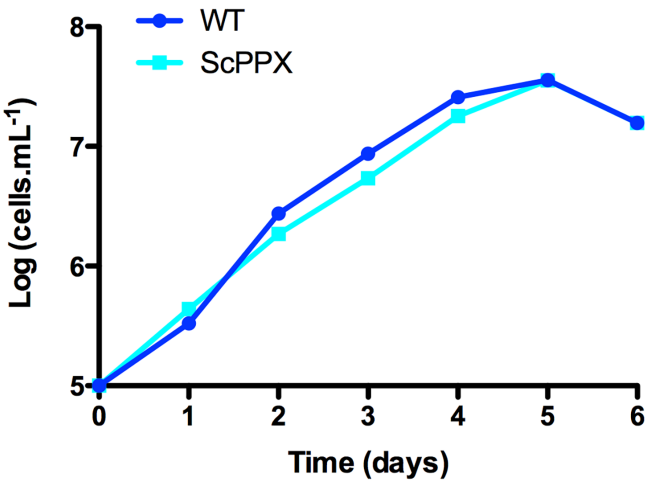

Fig S9. Growth curves of PCF WT and *PTS2-ScPPX1-eY*FP-expressing cells over 6 days.

S1 Table. All proteins identified in *Trypanosoma brucei.*

S2 Table. All proteins identified in *Trypanosoma cruzi.*

S1 Video. Co-localization of PPBD and PPDK in *T. brucei* PCF from Fig 1A. PPBD is shown in *green*, PPDK in *red*, and DAPI in *blue*.

S2 Video. Co-localization of PPBD and VP1 in *T. brucei* PCF from Fig 1B. PPBD is shown in *green*, VP1 in *red*, and DAPI in *blue*.