Szoor et al., http://www.jcb.org/cgi/content/full/jcb.200605090/DC1

Supplemental materials and methods

Generation and recombinant protein expression of *Tb*PTP1 and mutants

The full-length TbPTP1 coding sequence was amplified using PTP BglII 5′ (5′-CCAGATCTATGTCCACAGCGAAGAG-3′) and PTP XhoI 3′ (5′-GGGCTCGAGCGCTTTTAAG-3′) oligonucleotides and cloned into the BamHI and XhoI restriction sites of the bacterial expression construct pET30a (Novagen).

To produce the *Tb*PTP1 mutants, we used the oligonucleotide primers C229S 5′ (5′-TTGGTACATT**C**TAGTG-3′) and C229S 3′ (5′-CCAGCACTA**G**AATGTACC-3′) to introduce a G to C point mutation at position 686 in the cDNA. The C229S 5′ and the PTP BglII 3′ (5′-GGGAGATCTCGCTTTTAAGTTAAG-3′) primers were used to amplify the 3′ end of the C229S mutant gene. The C229S 3′ and the PTP HindIII 5′ (5′-GGGAAGCTTATGTCCACAGCG-3′) primers were used to amplify the 5′ end of the C229S mutant gene. The two PCR products were then combined and used as a template to amplify

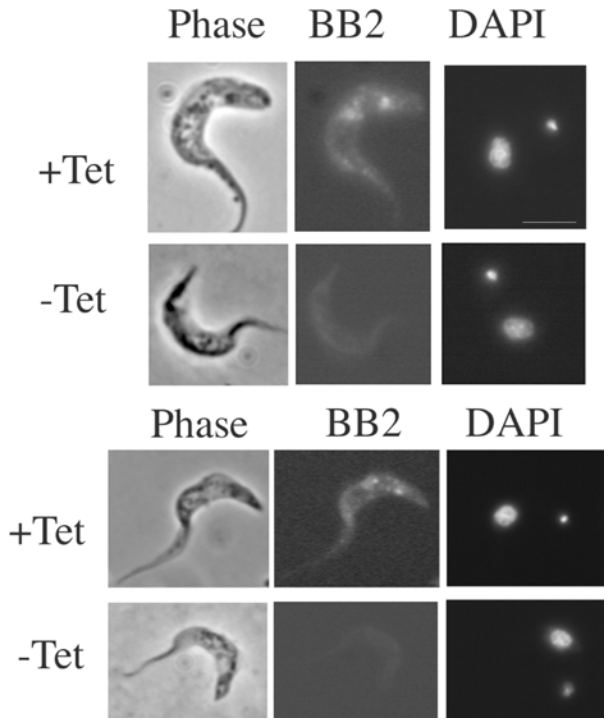


Figure S1. **Localization of TbPTP1 in bloodstream-form cells.** Antibody raised to *Tb*PTP1 did not provide a detectable signal on bloodstream forms because of the low expression level of the protein. Therefore, an ectopic copy of the wild-type protein, with an engineered C-terminal Ty1 epitope tag (Bastin, P., Z. Bagherzadeh, K.R. Matthews, and K. Gull. 1996. *Mol. Biochem. Parasitol.* 77:235–239), was expressed in transgenic parasites under tetracycline regulation. Cells were fixed in Methanol at −20°C and then subject to immunofluorescence using the Ty-1 epitope–specific antibody BB2. A weak fluorescence signal was detected over the trypanosome cell body, but this was not localized to any discernable cytoskeletal structure, for example the flagellum. Bar, 12 μm.

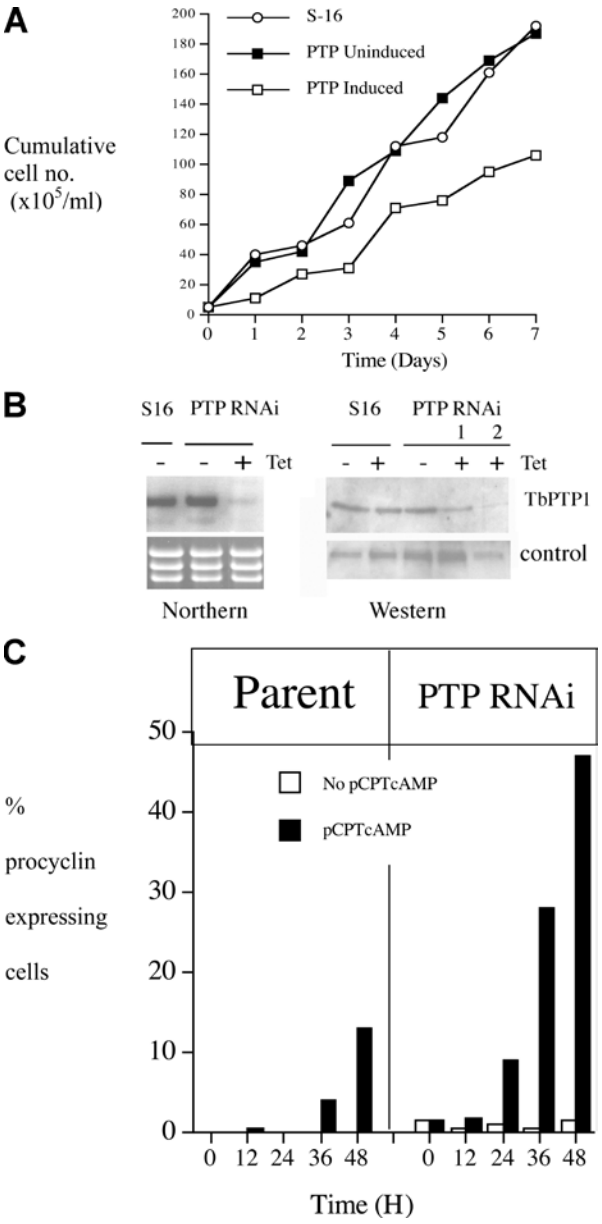
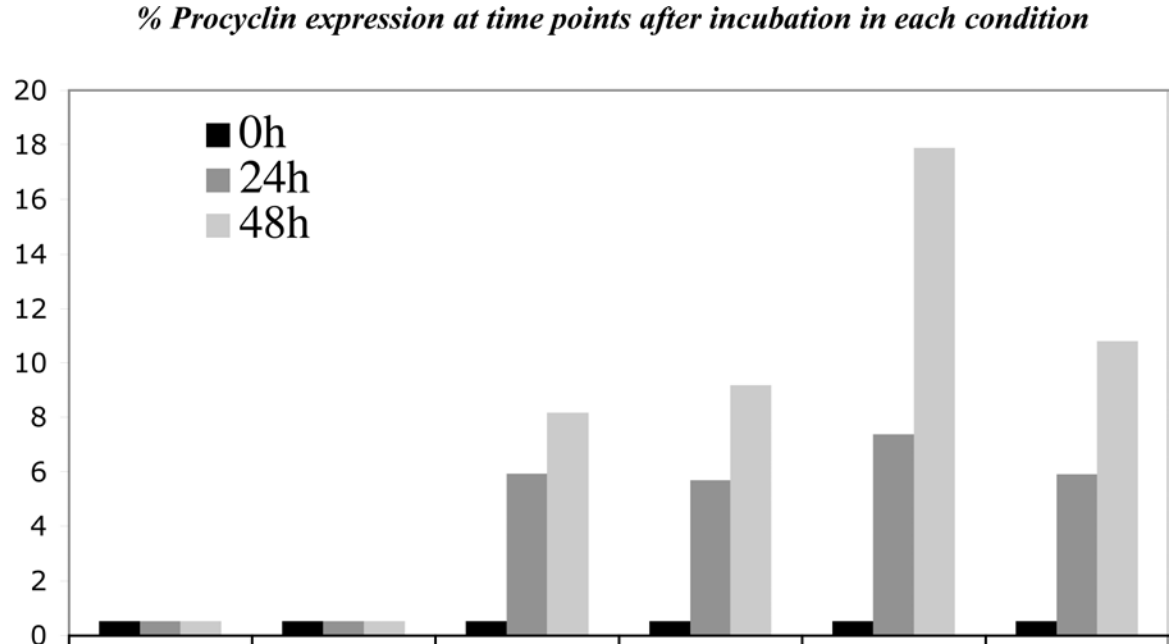


Figure S2. **Molecular and phenotypic characterization of the *Tb*PTP1 RNAi line.** (A) Growth of *Tb*PTP1 RNAi line over 7 d in culture. Induced lines were grown in the presence of 1 μg/ml tetracycline. (B) *Tb*PTP1 RNA and protein levels in the *Tb*PTP1 RNAi line. (left) Northern blot of *Tb*PTP1 in parental cells (S16, single-marker bloodstream forms) or in cells induced or not to undergo RNAi by incubation with tetracycline. (right) Western blot of parental cells or two *Tb*PTP1 RNAi lines. Only line 2 shows significant reduction in *Tb*PTP1 levels. This line exhibits the diferentiation phenotype. (C) Response of the parental or PTP RNAi line to treatment with 250 μM pCPTcAMP (1) over 48 h (Vassella, E., B. Reuner, B. Yutzy, and M. Boshart. 1997. *J. Cell Sci.* 110:2661–2671). At the indicated time points, cells were harvested and subjected to immunofluorescence analysis for the expression of EP procyclin. Although some differentiation is observed in the parental line, considerably enhanced differentiation is observed in TbPTP1 RNAi cells exposed to pCPTcAMP.

the full-length mutant gene using the PTP HindIII 5′ and PTP BglII 3′ primers. The same strategy was used to introduce an A to C point mutation at position 596 in the cDNA. This resulted in the mutation of aspartic acid 199 to alanine. In this case, oligonucleotide primers D199A 5′ (5′-GGTTGGCCCG**C**TCACG-3′) and D199A 3′ (5′-AACAACGT-GA**G**CGGGCC-3′) were used to amplify the 3′ end of the gene, whereas D199A 3′ and the PTP HindIII 5′ primers were used to amplify the 5′ end of the mutant gene.

Purification of recombinant proteins

Recombinant TbPTP1 (wild type and mutants) were expressed as histidine (His) fusions in *Escherichia coli* JM109 by induction with 0.4 mM IPTG at 30°C. After a 3-h induction, cells were harvested and the cell pellet dissolved in 1 ml/g lysis buffer (50 mM Hepes, pH 7.8, 15 0mM NaCl, and 5 mM Imidazole), with 1 mM β-mercaptoethanol, 0.1% Triton X-100, and EDTA-free protease inhibitor mix. (Roche). After sonication, the crude lysates were cleared by centrifugation at 10,000 *g* for 30 min at 4°C and loaded onto a Ni^{2+}-charged Hi-Trap Chelating affinity column (GE Healthcare) equilibrated with lysis buffer using a BioCad 700/Sprint System (PerSeptive Biosystems, Inc.). The column eluted using a linear gradient from 60 mM to 1 M Imidazole. The active fractions were identified by protein-Bradford assay and pNPP assays and stored at 4°C. Enterokinase (New England Biolabs, Inc.) digestion was performed to cleave the N-terminal His and S tags from the recombinant His-TbPTP1 fusion. After the initial wash, enterokinase solution was loaded onto the column (0.5 μg of recombinant enterokinase/1 mg of TbPTP1) and incubated for 5 h at room temperature. The eluted fractions containing tagless TbPTP1 were further purified by gel filtration on a Superdex 75 gel filtration column in 50 mM Hepes, pH 7.8, and 150 mM NaCl and concentrated on Vivaspin concentrator microcolumns.



	DMSO A	DMSO B	Cis A	Cis B	BZ3 A	BZ3 B
0h	0.5	0.5	0.5	0.5	0.5	0.5
24h	0.5	0.5	5.92	5.7	7.38	5.9
48h	0.5	0.5	8.16	9.2	17.9	10.8

Figure S3. **The differentiation response of pleomorphic slender cells to cis aconitate, BZ3 or DMSO over 48 h.** Parasites were harvested 3 d after infection with 1 × 106 *T.b. rhodesiense* EATRO 2340 (which are isogenic with the pleomorphic stumpy lines assayed in Figs. 8 and 9). Differentiation was monitored by immunofluorescence for EP procyclin at 0, 24, and 48 h after incubation of the cells in 6 mM cis aconitate (Cis), BZ3, or DMSO. In each case, cells were maintained in HMI-9 medium at 37°C. Two replicates are shown, A and B. A samples were initiated at a cell density of 2 × 106/ml, and B samples were initiated at a cell density of 2 × 105/ml.