SUPPLEMENTAL DATA

Fig. S1. TbTOR-like 1 does not colocalize with cytosolic, glycosome, acidocalcisome and P-bodies markers in PCF. Exponentially growing PCF (29-13) were collected and processed for immunofluorescence using mouse anti-TbTOR-like 1 fragment 2 together with the following markers: rabbit anti-HSP70 (A), anti-aldolase (B), anti-Dhh1 (C), anti-TbVP1 (D), anti-DHLADH (E) or anti-BIP (F). The antigens were localized by staining with anti-mouse IgG Alexa 488 (green), anti-rabbit IgG 594 (red) and DAPI staining (blue). The bright intensity of colors in merged images were proportionally reduced as compared to the original ones. The images are sections of Z series processed by 3D-deconvolution using Autoquant 2.1 software. Scale bars = 10 μm.

Fig. S2. TbTOR-like 1 protein is soluble and is not found or interacts with membranous cell structures. PCF trypanosomes were lysed in a phosphate buffer in the absence (Control), or absence of 6 M urea (used for destabilizing non-covalent protein-protein interactions). Whole cell lysate was subjected to a first centrifugation step (13K) (13000 g, 15 min). The 13K control pellet contains plasma membrane and heavy organelles like mitochondria and nucleus. The 13K supernatants were subjected to a second centrifugation step (100,000 *g*, 60 min) in order to sediment light organelles and light membranes (100K pellets). The soluble fractions correspond to the 100K supernatant. All samples where resuspended in the original volume and content of TbTOR-like 1 in each fraction was assessed by western blot analysis using F3 antibodies.

Fig. S3. TbTOR2 knocked down cells present same levels of poly P as control cells. The levels of short-chain (SC) poly P were measured in non-induced (-Tet) and induced (+Tet) RNAi PCF 6 days after induction with tetracycline. The figure shows mean values ± standard deviation of three experiments measured in triplicate. The differences between non-induced and induced cells were found not significant by using t-Student test ($p < 0.005$).

Fig. S4. Aldolase (A), DHLADH (B) and BIP (C) localization in PCF treated at different osmotic conditions. PCF (29-13) at exponential growth were collected by centrifugation and resuspended with isosmotic (Iso), hyposmotic (Hypo) and hyperosmotic (Hyper) buffers as described under Experimental Procedures. Ten seconds after the different buffers treatment, the cells were fixed and processed for immunofluorescence with anti-aldolase antibody (green) in the presence of DAPI (blue). The merged fluorescence images and the merged fluorescence and DIC images are shown as well. Scale bars = 10 μm.

Fig. S5. Parasites maintained at high osmotic conditions shown a more sensitive phenotype for TbTOR-like 1 ablation by RNAi. Growth curves of PCF 29-13 (A) and PCF containing the integrated p2T7-177 TbTOR-like 1 (B) in the absence of tetractyclcine (-Tet) or in the presence of tetracyclicne (+Tet) and/or 0.3 M mannitol. At day 3 cells were diluted again. The numbers are mean values ± standard deviation of triplicate experiments, ($p < 0.05$).

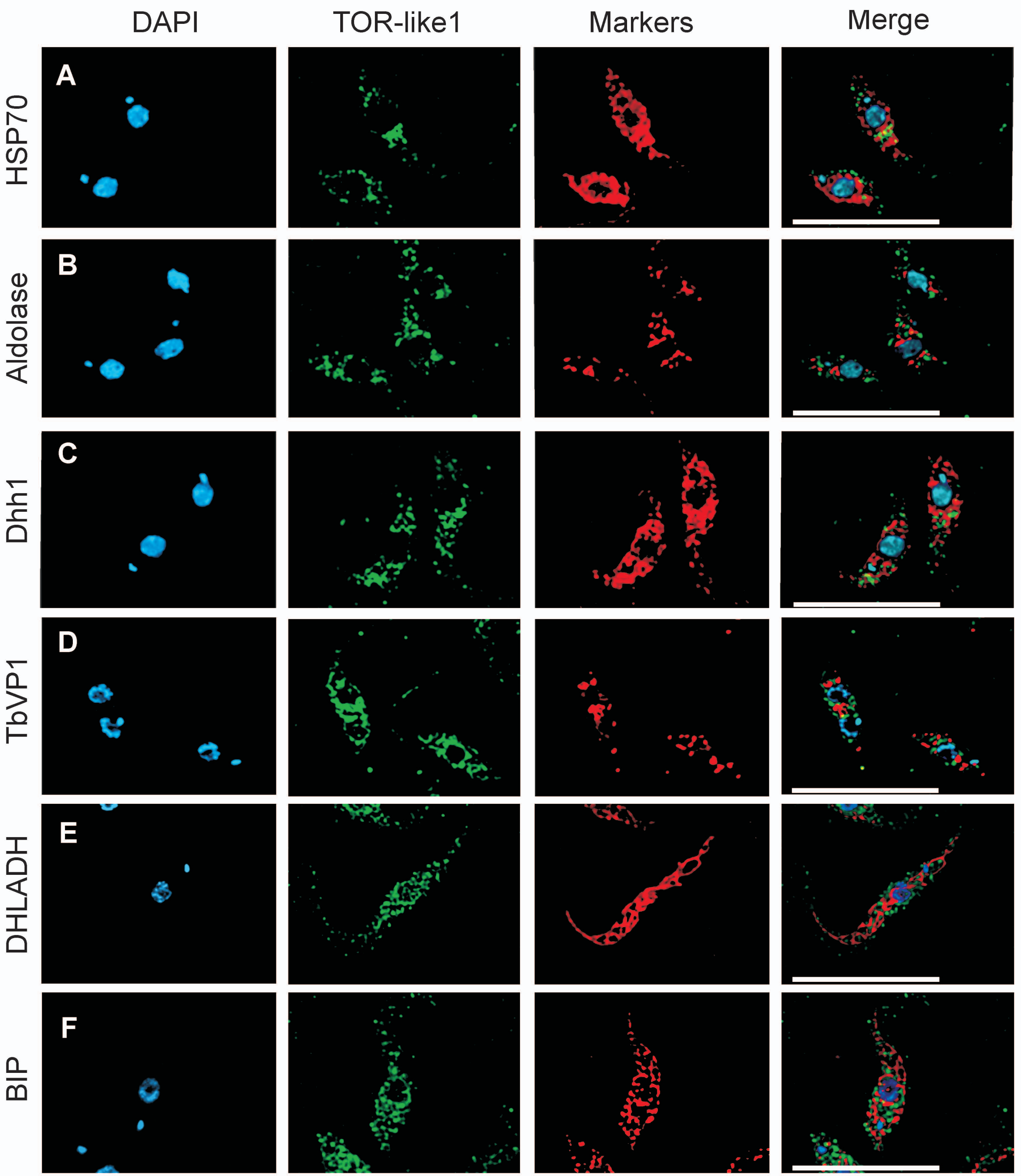

DAPI
TOR-like1
Markers
Merge
HSP70
A
Aldolase
B
Markers
Dhh1
C
TbVP1
D
DHLADH
E
BIP
F

Figure S1

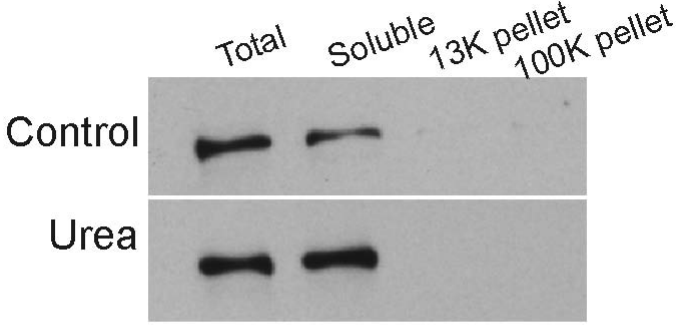
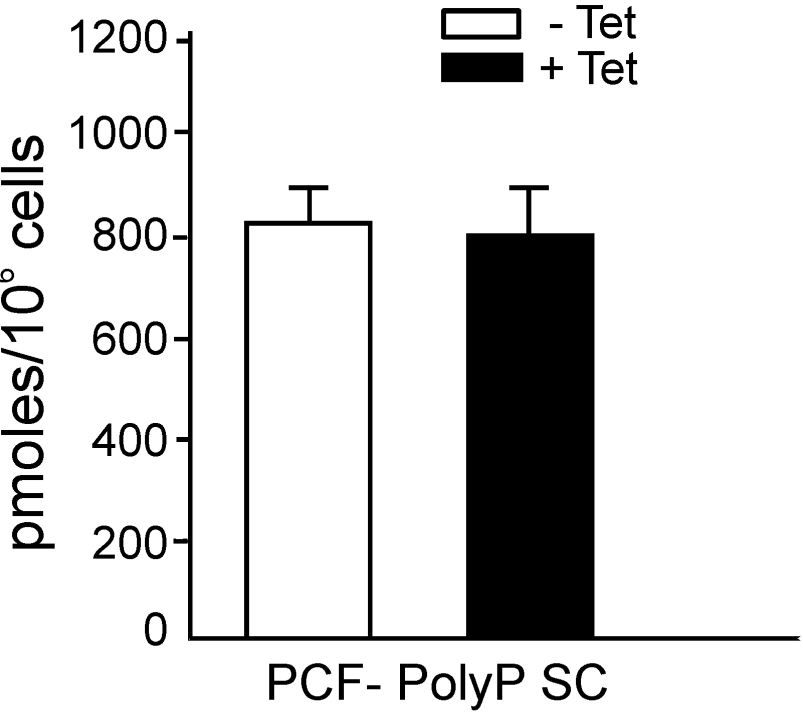


Fig. S2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+ Tet
1200
1000
800
600
400
200
0
pmoles/10^6 cells
PCF- PolyP SC

Figure S3

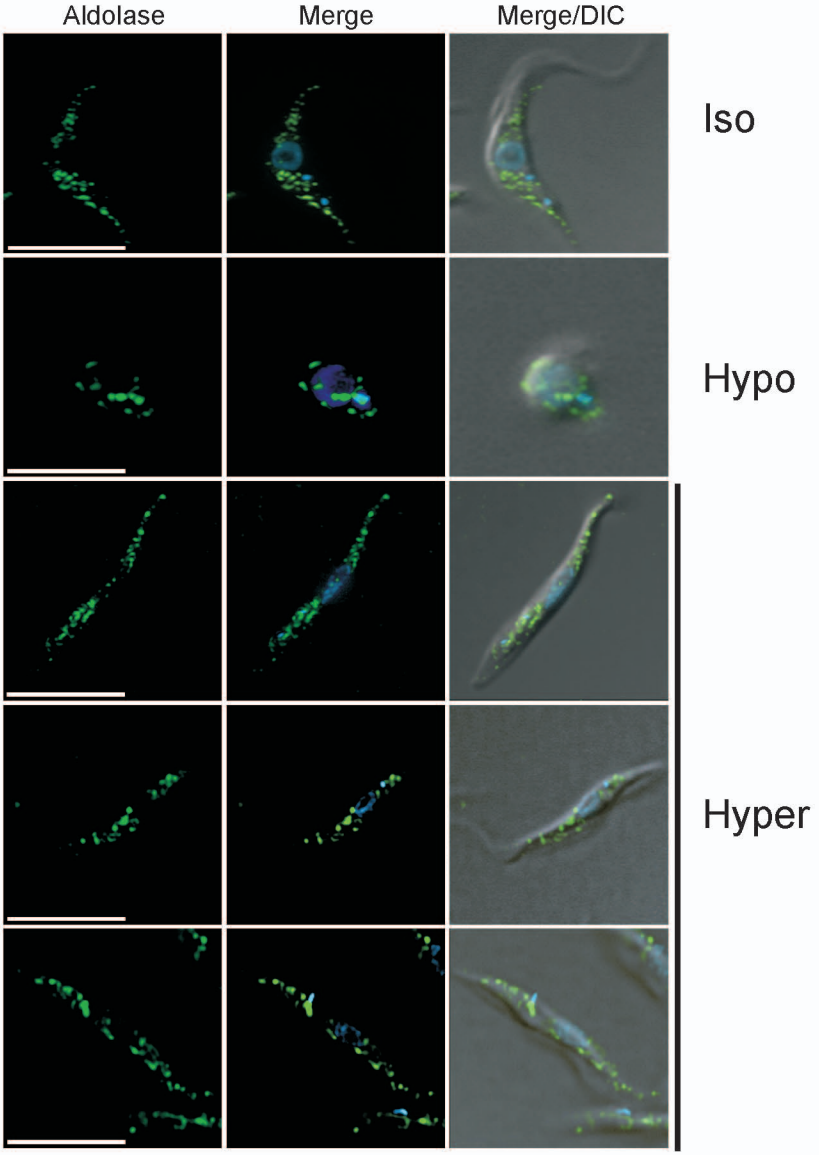

Figure S4A

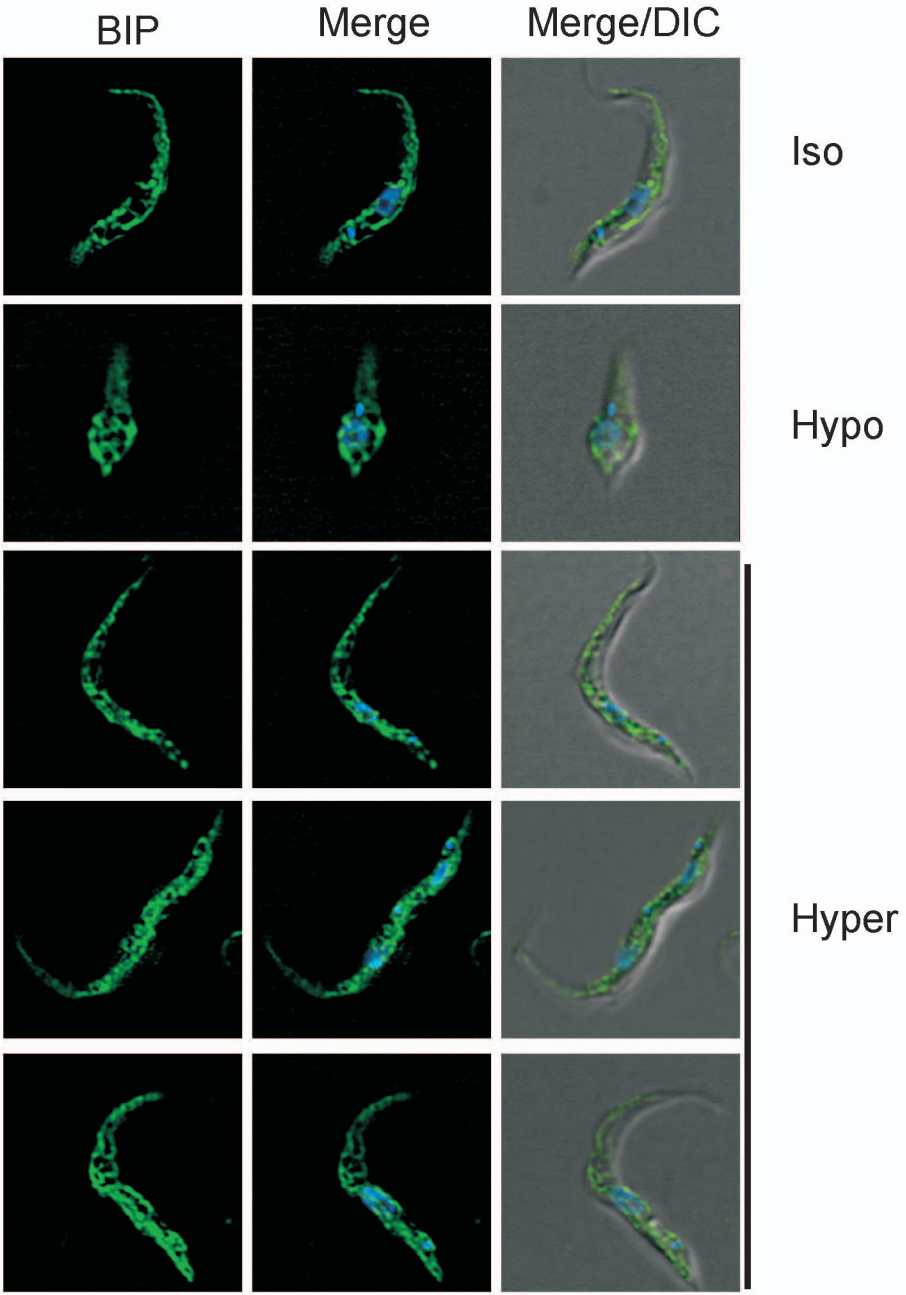

Figure S4B

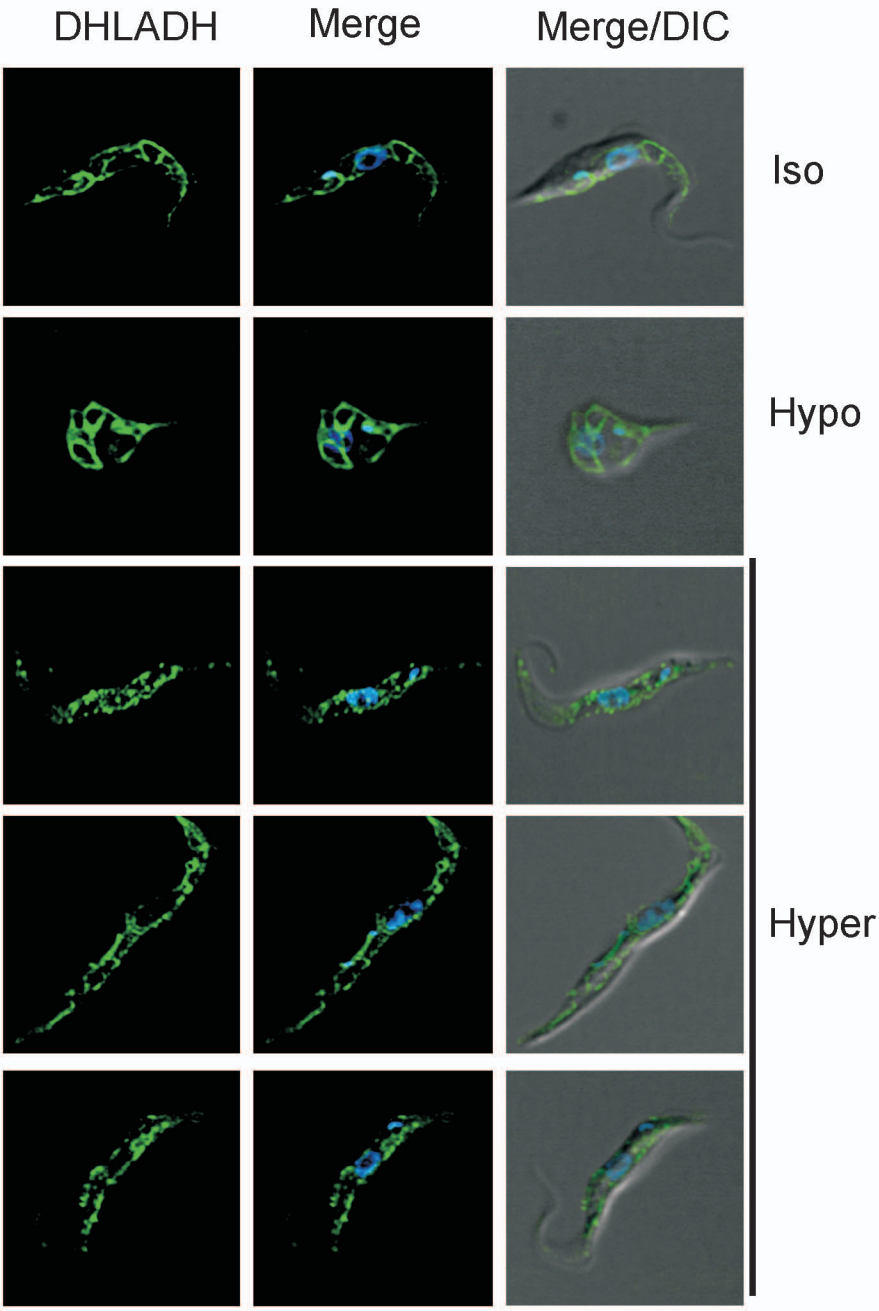

Figure S4C

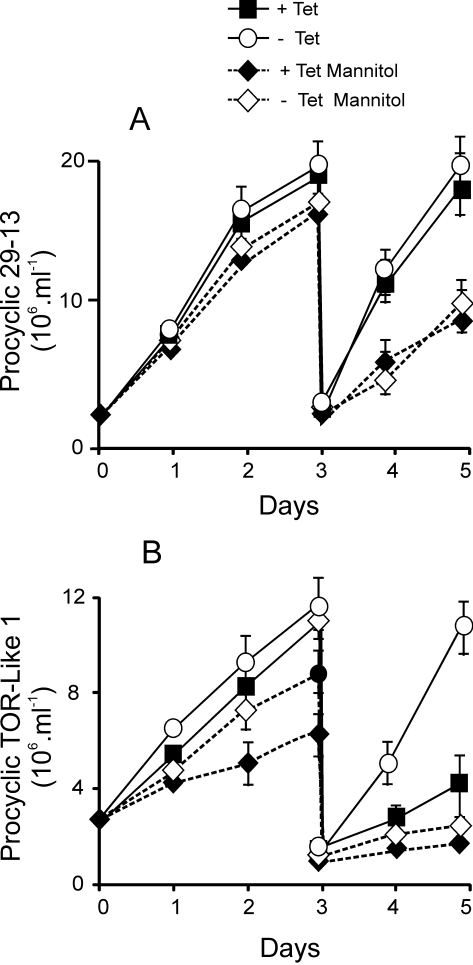
+ Tet
- Tet
+ Tet Mannitol
- Tet Mannitol
A
Procyclic 29-13
(10^6.ml^{-1})
20
10
0
0
1
2
3
4
5
Days
B
Procyclic TOR-Like 1
(10^6.ml^{-1})
12
8
4
0
0
1
2
3
4
5
Days

Figure S5