**Supplementary Methods**

***GW 788388 dose-response effect in vivo:***

***Infection and compound administration:*** Male Swiss mice (age 6-8 weeks, weight 18-20 g) were obtained from the animal facilities of CECAL (FIOCRUZ, Rio de Janeiro, Brazil). Infection was performed by intraperitoneal (IP) injection of 104 bloodstream trypomastigotes. Eight mice from each group were used for analysis at each different dpi. The compound GW783388 (GlaxoSmithkline, France) or vehicle dilution buffer (4% DMSO , 96%in [0.5% Hydroxypropylmethylcellulose hydroxypropylmethylcellulose (HPMC), 5% Tween 20, 20% HCl 1M 0.2M in NaH2PO4 0.1M]) was used for oral administration. Mice received GW788388 at 0.3, 3, 6 and 15 mg/kg at 3 dpi by gavage in a single administration (0.2 mL). The control group received vehicle buffer using the same schedule.

***Survival rates and parasitemia:*** Parasitemia was individually checked by direct microscopic counting of parasites in 5 L of blood, as previously described [1]. Mortality was checked daily until 30 dpi and expressed as percentage of survival.

***In vitro* *GW788388 effects on T. cruzi infection*:** Cardiomyocytesfrom mouse embryos were obtained from primary cultures as previously described [2], and maintained in Eagle’s medium (Sigma, Saint-Quentin Fallavier, France) supplemented with 7 % fetal calf serum (FCS) (Sigma), 100 g/ml gentamicin (Sigma), 1mM L-Glutamine (Sigma) and 2.5 mM CaCl2. Cardiomyocytes were seeded in 24-well plates (1x105 cells/well) for 24 h at 37°C in an atmosphere of 5% CO2. Cultures were infected with trypomastigotes of the Y strain in a parasite:host cell proportion of 10:1. After 24 h post-infection cultures were washed and incubated or not with fresh medium containing 10M GW788388 (GlaxoSmithkline, France). 48 h and 96 h post-infection (24 h and 72 h of treatment, respectively), cells were washed with phosphate-buffered saline (PBS), fixed in Bouin’s solution and stained with Giemsa. The percentage of cardiomyocytes containing parasites and the number of parasites/infected cell were determined by counting 400 cells/slide on two distinct coverslips at 48 and 96 h post-infection. Analysis was performed on a Nikon microscope, at magnification 400x.

References:

1. Olivieri BP, de Souza AP, Cotta-de-Almeida V, de Castro SL, Araujo-Jorge T (2006) Trypanosoma cruzi: alteration in the lymphoid compartments following interruption of infection by early acute benznidazole therapy in mice. Exp Parasitol 114: 228-234.

2. Meirelles MN, De Souza W (1986) The fate of plasma membrane macrophage enzyme markers during endocytosis of Trypanosoma cruzi. J Submicrosc Cytol 18: 99-107.