SUPPLEMENTAL MATERIAL

Fernandes et al., http://www.jem.org/cgi/content/full/jem.20102518/DC1

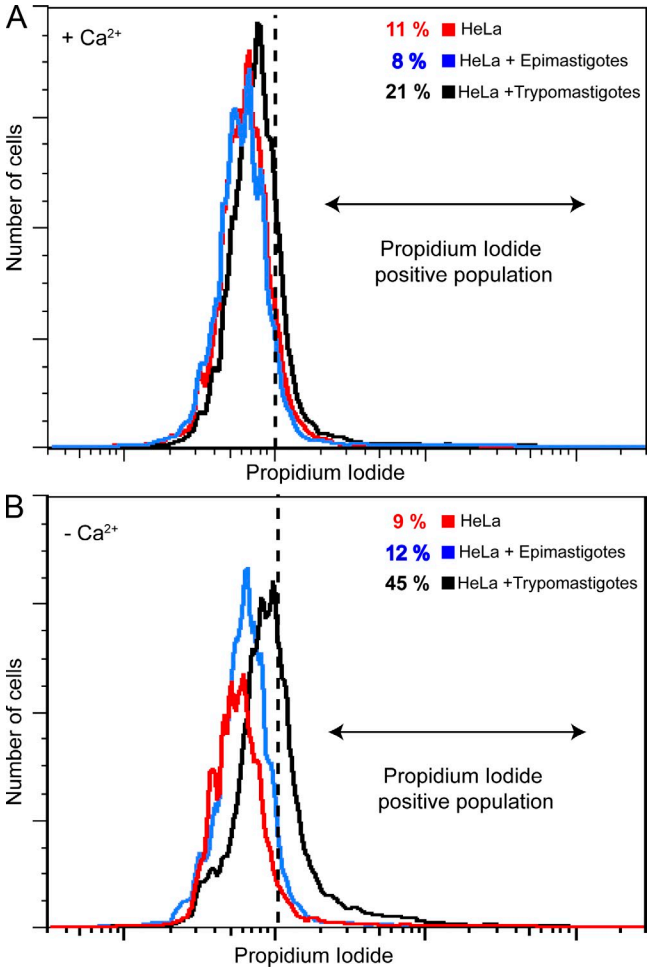


Figure S1. Infective *T. cruzi* trypomastigotes wound host cells. FACS quantification of PI staining in control HeLa cells (red line), Hela cells incubated with noninfective epimastigotes (blue line), and HeLa cells incubated with infective trypomastigotes (black line) and PI for 40 min in the presence (A) or absence (B) of Ca^{2+}. Percentages correspond to the wounded population (PI positive) in the gated region (dashed line). 10,000 events were analyzed. These results are representative of two independent experiments.

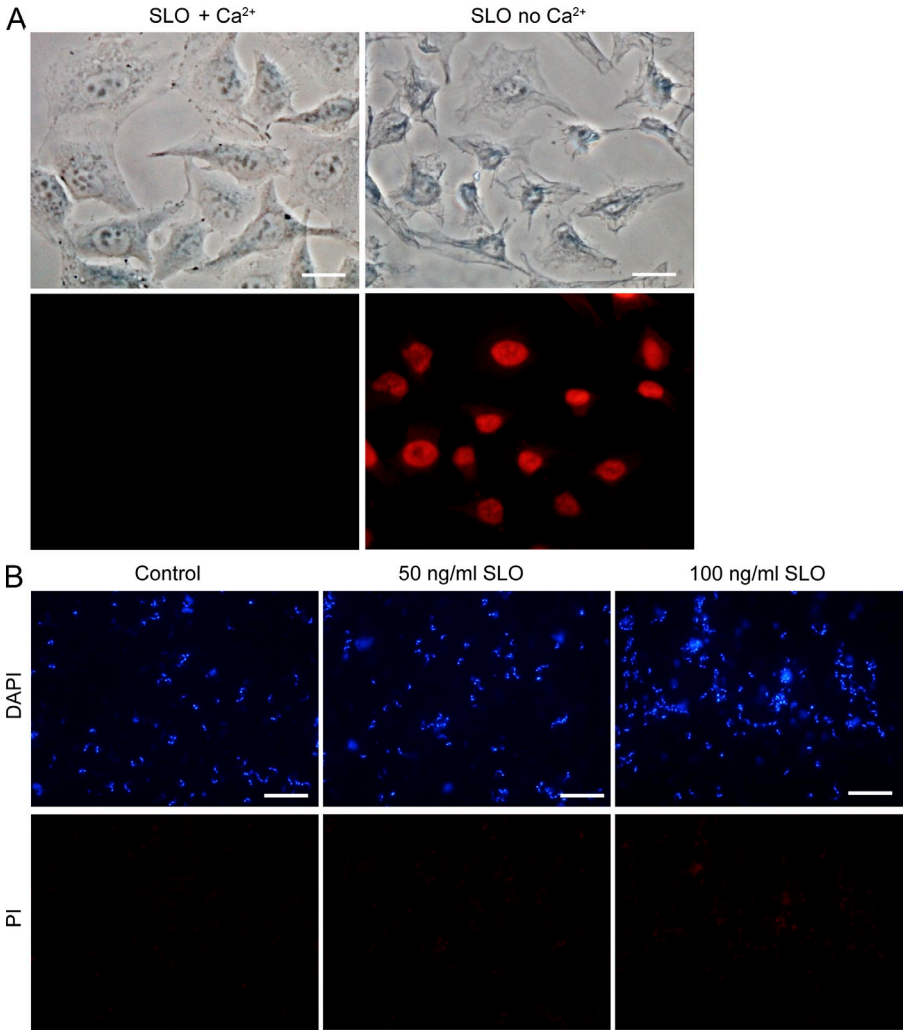


Figure S2. SLO permeabilizes the plasma membrane of mammalian cells but not *T. cruzi* trypomastigotes. (A) HeLa cells were incubated with 50 ng/ml SLO and PI for 20 min under repair (Ca^{2+}) and nonrepair (Ca^{2+} free) conditions, fixed, and imaged. Under nonrepair conditions, PI (red) influx is detected in the majority of cells. Bars, 10 µm. (B) Trypomastigotes were incubated with the indicated concentrations of SLO for 20 min in Ca^{2+}-free medium, fixed, and imaged. No PI influx was detected. Nuclei and kinetoplasts were stained with DAPI (blue). Bars, 20 µm. These results are representative of three independent experiments.

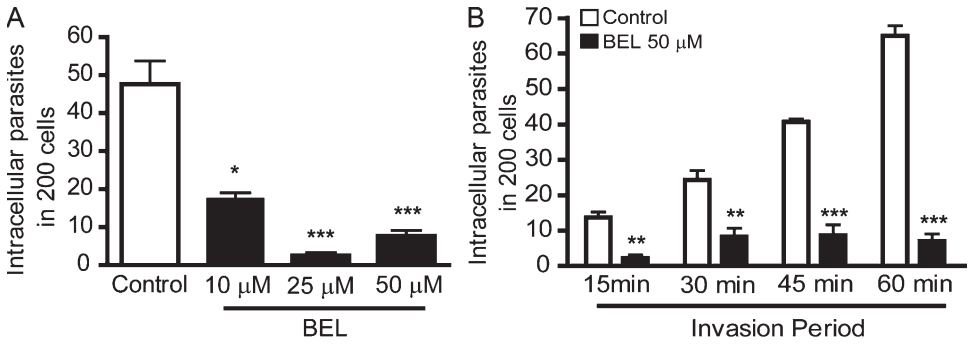


Figure S3. Inhibition of lysosomal exocytosis blocks trypomastigote entry. (A) HeLa cells were pretreated with the indicated concentrations of the lysosomal exocytosis inhibitor BEL for 30 min, washed, and incubated with trypomastigotes. The number of internalized parasites was determined after a 30-min invasion assay. (B) Kinetics of trypomastigote invasion in HeLa cells preincubated for 30 min with 50 µM BEL before exposure to trypomastigotes. The data correspond to the mean of triplicates ± SD. *, P = 0.016; **, P < 0.0051; ***, P < 0.0002, Student's *t* test comparing control (white bars) and treated (black bars) cells. These results are representative of four independent experiments.

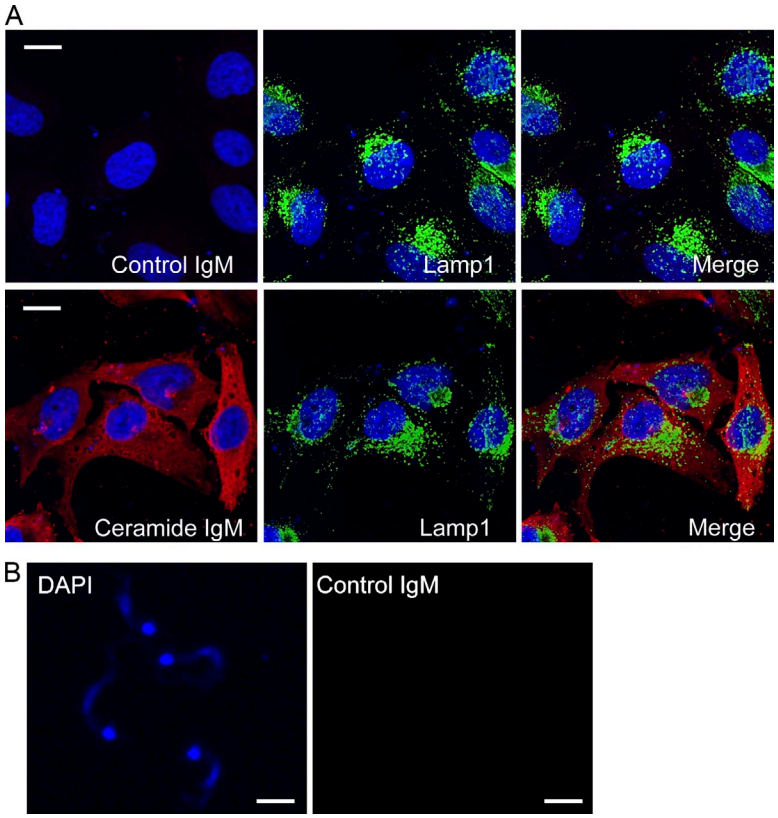


Figure S4. Control assays for ceramide immunolabeling. (A) HeLa cells infected with trypomastigotes were fixed and stained with a mouse IgM isotype control (control IgM) or anti-ceramide IgM 15B4 monoclonal antibodies. Cells were also stained with anti-Lamp1 (green) and DAPI (blue). Bars, 12 µm. (B) Isolated trypomastigotes were fixed and stained with a mouse IgM isotype control. Parasite nuclei and kinetoplasts are shown in blue. Bars, 8 µm. These results are representative of three independent experiments.

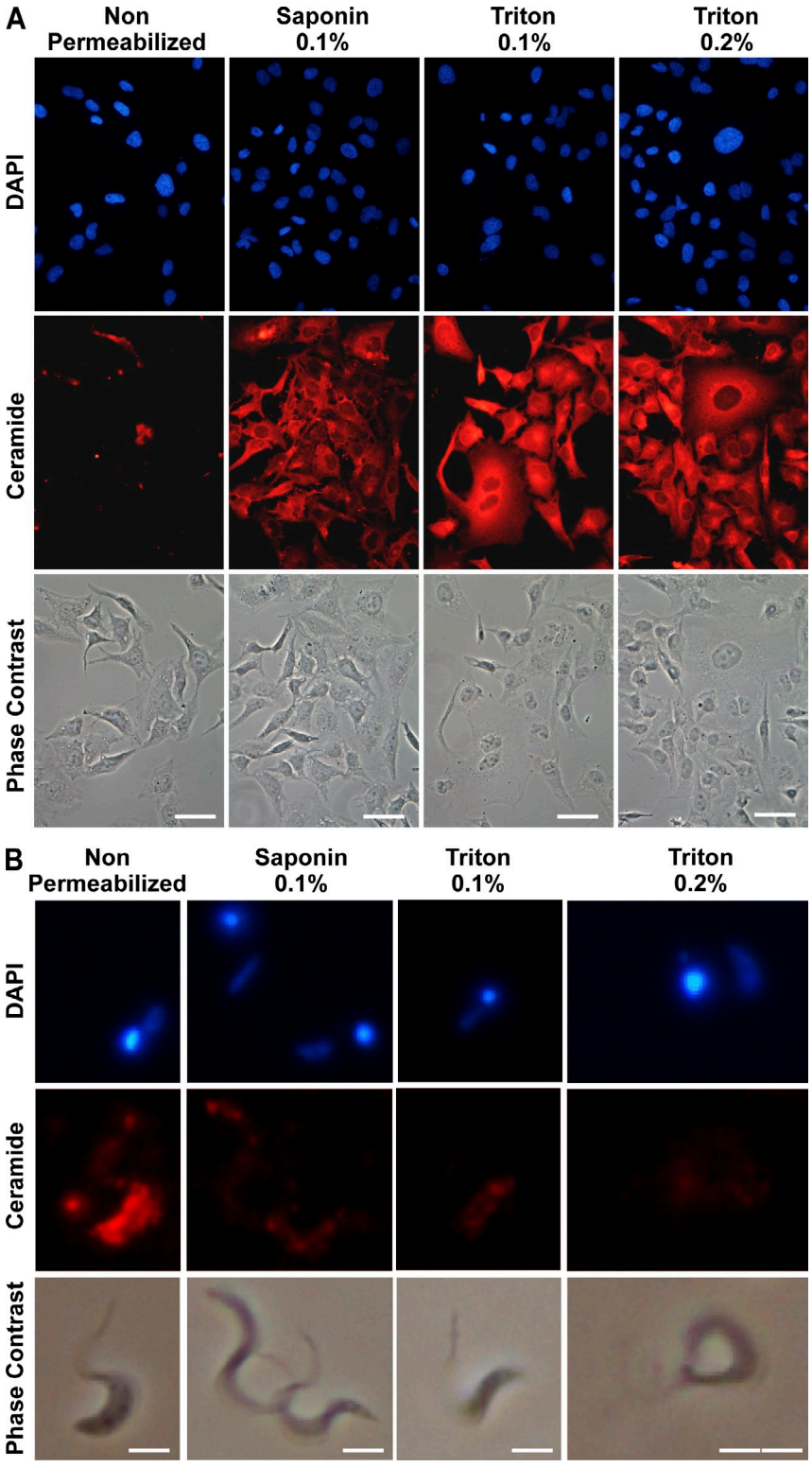
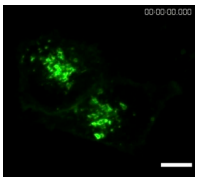
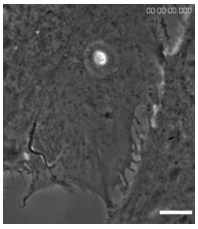


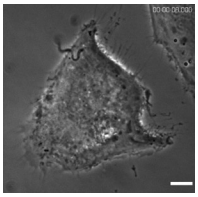
Figure S5. Ceramide is extracted more efficiently from trypomastigotes than host cells after Triton X-100 permeabilization. (A) HeLa cells were fixed with PFA, permeabilized with the indicated concentrations of Saponin or Triton X-100, and incubated with 15B4 anti-ceramide monoclonal antibodies (red). Quantification of the anti-ceramide fluorescence intensity associated with 15 fields of cells did not reveal statistically significant differences between 0.1 and 0.2% Triton X-100 extraction. Nuclei are shown in blue (DAPI). Bars, 20 µm. (B) Trypomastigotes were fixed with PFA, permeabilized with the indicated concentrations of Saponin or Triton X-100, and incubated with 15B4 anti-ceramide monoclonal antibodies (red). Parasite nuclei and kinetoplasts are shown in blue (DAPI). Bars, 5 µm. These results are representative of two independent experiments.



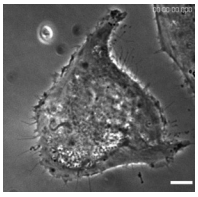
Video 1. Exocytosis of host cell lysosomes during interaction with *T. cruzi* trypomastigotes. (From Fig. 1.) HeLa cells overexpressing Lamp1-RFP (pseudo-colored in green) were incubated with trypomastigotes in a live imaging chamber at 37°C and 5% CO_2 in DME with 2% FBS. Parasites were incubated with cells for 20 min before imaging (0 min). Spinning disk confocal live time-lapse imaging (UltraVIEW; PerkinElmer) was acquired at 1 frame/100 s for the initial 96 min and at 1 frame/10 min for the remaining period. Bar, 10 µm. The video is displayed at 10 frames/s.



Video 2. Extracellular trypomastigotes cause mechanical deformations in the host cell membrane before invasion. (From Fig. 2.) Trypomastigotes were incubated with HeLa cells for 10 min, nonattached parasites were washed out, and cells were then imaged by time-lapse live phase-contrast microscopy (Axiovert 200; Carl Zeiss) in a heated stage and imaged for 7 min at 1 frame/2 s in DME containing 20 mM Hepes at pH 7.2. The video is displayed at 10 frames/s.



Video 3. Invasion and intracellular motility of *T. cruzi*. (From Fig. 7 A.) Trypomastigotes were incubated with HeLa cells for 10 min, nonattached parasites were washed out, and cells were then imaged by time-lapse live phase-contrast microscopy (Axiovert 200) in a heated stage for 15 min at 1 frame every 2 s in DME containing 20 mM Hepes at pH 7.2. The video is displayed at 10 frames/s.



Video 4. Protrusion of motile intracellular trypomastigotes from the surface of host cells. (From Fig. 7 B.) Continuation of the infection event shown in Video 3. After a 15-min interval, the same infected HeLa cell shown in Video 3 was imaged, showing the extensive intracellular movement of the trypomastigote, resulting in the parasite protruding from the surface of the infected cell (bottom right). The cell was imaged by time-lapse live phase-contrast microscopy on a heated stage (Axiovert 200) for 15 min at 1 frame/2 s in DME containing 20 mM Hepes at pH 7.2. The video is displayed at 10 frames/s.