PNAS

Supporting Information for

Unnexin is a protein subunit of a large-pore channel expressed by unicellular organisms.

Juan Güiza, Francisco Solis, Bernardita Valenzuela, Duxan Arancibia, Pedro Zamorano, Jorge González, Jonathan Saavedra, Alan Neely, Magdiel Salgado, Agustín D Martínez, Juan C. Sáez and José L. Vega

corresponding author: Juan Carlos Sáez and José L. Vega
Email: juancarlos.saez@uv.cl or joseluis.vega@uantof.cl

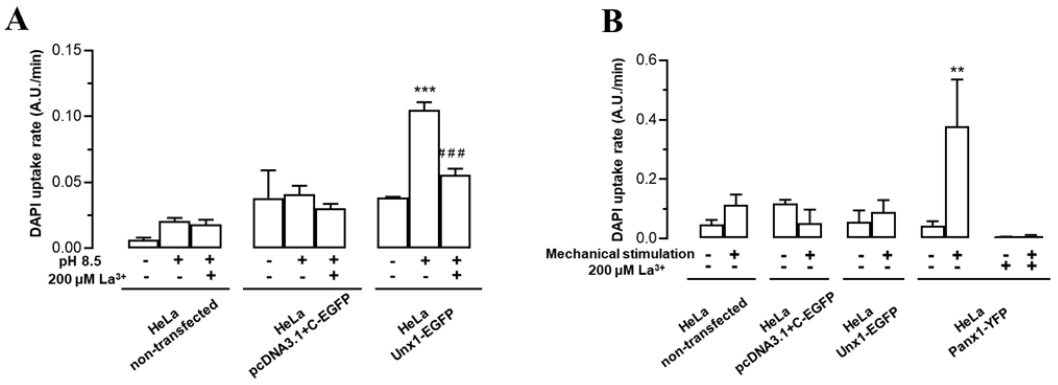

Fig. S1. Alkaline pH but not mechanical stimulation increases the unnexin1 channel activity. (A) DAPI uptake rate of Unx1-EGFP transfected HeLa cells (HeLa Unx1-EGFP cells) exposed to (A) an alkaline extracellular solution (pH 8.5) or (B) mechanical stimulation. During the last 5 min of each experiment, cells were treated with 200 µM lanthanum (La^{3+}). Each value corresponds to the mean ± SEM, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. basal. # # # $P < 0.001$ vs pH 8.5 (n = 3 independent experiments in which a minimum of 20 cells were recorded).

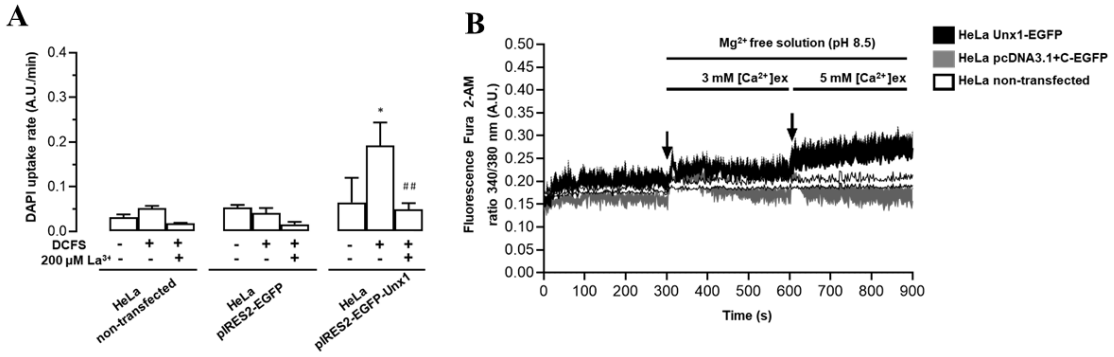

Fig. S2. A low extracellular concentration of divalent cations increases unnexin1 channel activity but does not affect intracellular Ca^{2+} signals. (A). HeLa parental cells or transfected with bicistronic EGFP and Unx1 (HeLa pIRES2-EGFP-Unix1) or transfected with the empty vector (HeLa pIRES2-EGFP) were used to evaluate the DAPI uptake upon exposure to an extracellular solution without Ca^{2+} and Mg^{2+} (divalent cation free solution: DCFS). (B) The absence of extracellular Mg^{2+} did not affect the Fura 2-AM signal in HeLa cells transfected with Unx1-EGFP. Data are presented as the mean ± SEM; $^{*}P<0.05$ vs. basal. $^{\#\#}P<0.01$ vs DCFS (n = 3).

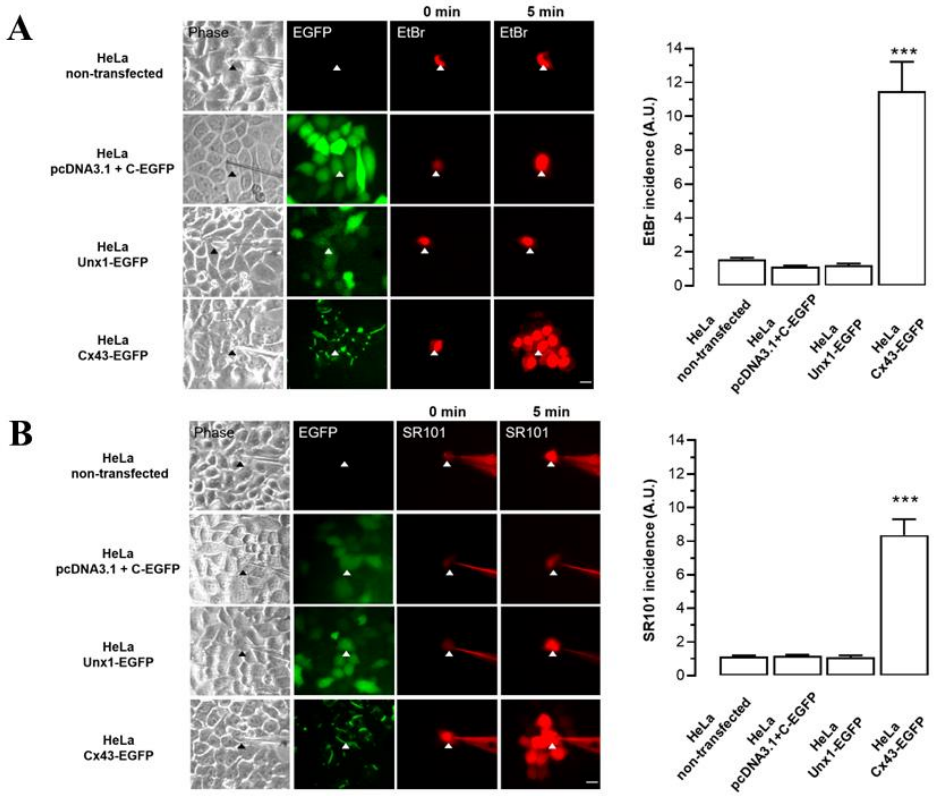

Fig. S3. Unnexin1 does not form gap junction channels. Dye coupling was assayed in monolayers of HeLa cells transfected with Unx1 (HeLa Unx1-EGFP cells). In each assay, one cell was microinjected with (A) ethidium bromide (EtBr) or (B) sulforhodamine 101 (SR101) in a total of 20 cells per experiment. HeLa Cx43-EGFP cells were used as a positive control. The arrowheads (▲) denote the injected cells. Scale bar = 25 μm. Data are presented as the mean ± SEM; $^{***}P<0.001$ vs. non-injected (n = 3).

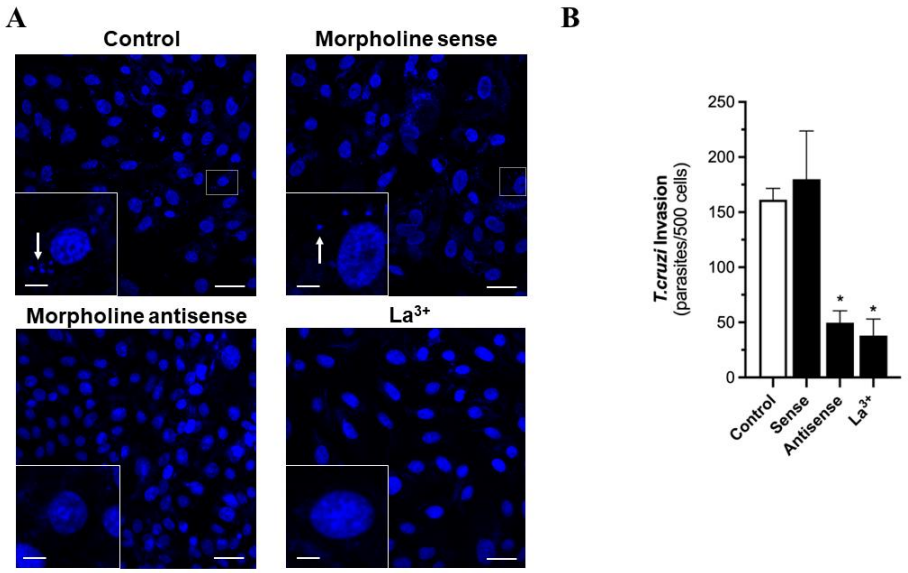

Figure S4. Unnexin1 is required for *T. cruzi* invasion. Representative confocal images of Vero cells with nucleus stained with DAPI. Cells were incubated overnight with Unx1 morpholinos. Vero cells were then incubated with *T. cruzi* trypomastigotes for 4 hours. Arrows in A top panels denote amastigotes, which were absent in the bottom panels. Scale bar = 50 μm. Inset scale bar = 10 μm **(B)** A graph showing the quantification of the number of parasites per 500 cells. Data are presented as the mean ± SEM; $^{*}P<0.05$ vs. control. n=3.

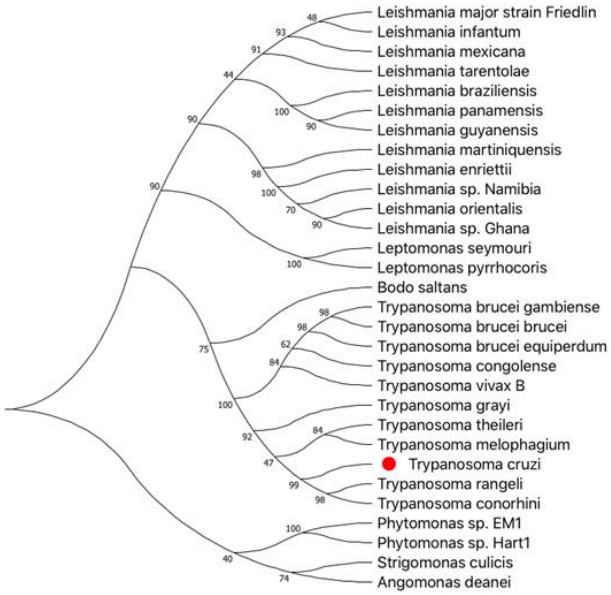

Fig. S5. Phylogenetic tree of putative unnexin sequences. Whole amino acid sequences of putative Unx1 orthologs from 30 protozoan taxa were used for the phylogenetic analysis. The analysis was performed using the neighbor-joining method and Jones–Taylor–Thornton (JTT) model. Numbers at the nodes indicate bootstrap percentages from 500 replicates. The red circle indicates Unx1 from *T. cruzi*, which was functionally characterized in this study and used to retrieve sequences from the NCBI protedatabase via BLASTP.