SUPPLEMENTARY DATA



Fig. S1. Analysis of cell viability and plasma membrane integrity in wild-type (WT) and MDL28170-resistant (MDLR) promastigotes of *P. serpens.* (A)Fluorescence development after incubation with resazurin. Results were expressed as fluorescence arbitrary units (FAU). (B) Colorimetric MTT assay. Cell metabolic viability was determined absorbance (ABS) quantification at 490 nm. Parasites were also treated with sodium azide in order to obtain non-viable cells to use as a positive control. Data shown are the mean ± standard deviation of three independent experiments performed in triplicate. (C,D) Promastigotes were also incubated with propidium iodide (PI) and subsequently analyzed by flow cytometry. Data were expressed as the mean fluorescence intensity (MFI) (C) and as the percentage of fluorescent cells (% FC) (D). Boiled cells were used as positive control of cell membrane disruption. Representative data of the analysis of 10,000 cells in experiments performed in triplicate are shown. The asterisks denote statistic difference to WT promastigotes (*P*<0.05).



Fig. S2. Cell morphology and ultrastructural analysis of wild-type (WT) and MDL28170-resistant (MDLR) promastigotes of *P. serpens.* (A) Both populations were analyzed by flow cytometry in order to measure two cellular parameters, size and granularity, by forward scatter (FSC) and side scatter (SSC) measurements, respectively, and both were expressed as mean of fluorescence intensity (MFI). In the inset, Giemsa-stained smears of WT and MDLR promastigotes of *P. serpens.* k, kinetoplast; n, nucleus; f, flagellum. (B) Scanning electron microscopy analysis of WT and MDLR promastigotes; note the presence of extracellular vesicles in different regions of the cell body, mainly in MDLR cells. Bars: 5 µm. (C) Transmission electron microscopy analysis of WT (a,b) and MDLR promastigotes (c-e). N, nucleus; M, mitochondrion; K, kinetoplast; FP, flagellar pocket, ER, endoplasmic reticulum. MDLR promastigotes (e) presented microvesicules inside the flagellar pocket (white arrows) that were also visible in the extracellular milieu (inset). Bars: 0.5 µm.