Supplemental Data

INTRAPHAGOSOMAL PEROXYNITRITE AS A MACROPHAGE-DERIVED CYTOTOXIN AGAINST INTERNALIZED *TRYPANOSOMA CRUZI*
Consequences for oxidative killing and role of microbial peroxiredoxins in infectivity

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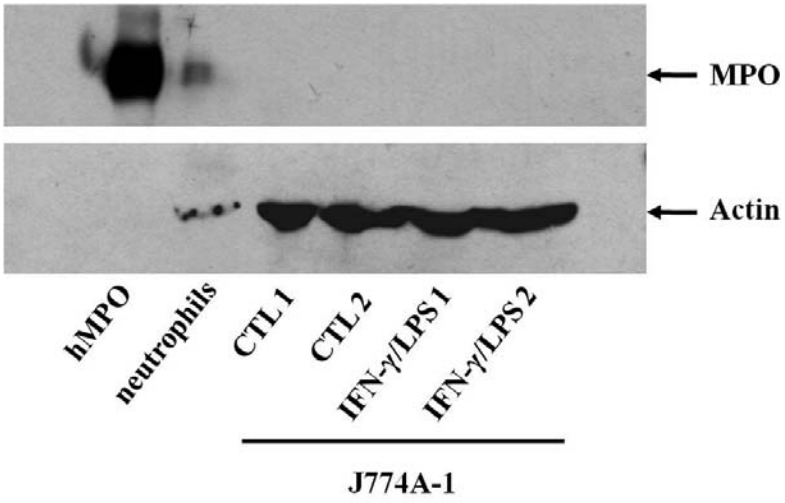
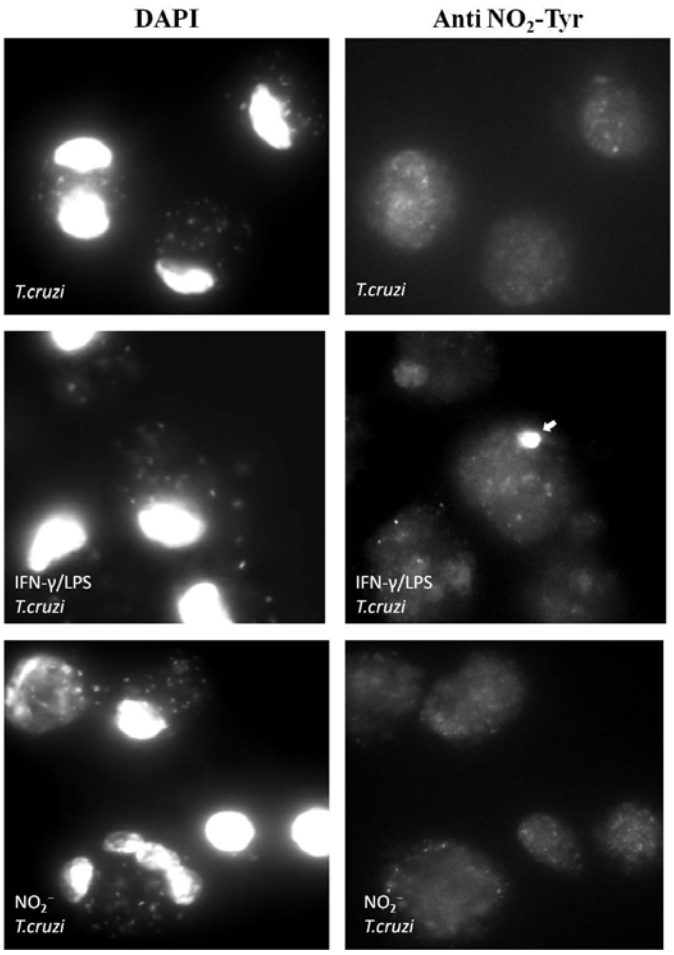
SD_Fig 1. Western blot analysis of myeloperoxidase in J774A-1 macrophages



In order to explore the presence of minimal amounts of MPO in J774A-1 macrophages, a western blot analysis of cells lysates was performed. An anti human-MPO antibody known to react also with murine MPO was used. As a positive control, recombinant human MPO and an extract from mice neutrophils were used. The presence of MPO in J774A-1 macrophages was explored in unstimulated (CTL) and INF-γ/LPS- activated cells. An anti-actin antibody was used for load control of total proteins and for comparative purposes. Protein load was 15 μg for neutrophils and 60 or 80 μg for macrophages in (1) and (2), respectively.

No signal for macrophage MPO protein was detected even though protein load compared to mice neutrophils was notably higher as indicated by the actin signal.

SD_Fig 2 . Immunocytochemical evaluation of protein tyrosine nitration in macrophages



To further evaluate a heme peroxidase-dependent protein tyrosine nitration mechanism (including MPO) which depends on the presence of H_2O_2 and NO_2^- as substrates, macrophages were challenged with *T.cruzi* in the presence of 10 μM NO_2^-; this amount of NO_2^- doubles the expected formation from the decay of $^{\bullet}NO$ in activated macrophages and H_2O_2 is provided by the respiratory burst upon *T. cruzi* internalization. Unstimulated macrophages in the absence (*T.cruzi*) and presence of 10 μM nitrite (NO_2^- + *T.cruzi*) and activated (*T.cruzi* + IFN-γ/LPS) macrophages were infected with trypomastigotes (20:1). Following phagocytosis (2 h), macrophages were incubated with anti-3-nitrotyrosine antibody and visualized with Alexa-488-conjugated anti-rabbit antibody (right column) and nuclei were stained with DAPI (Left column) (magnification, ×1000).

Arrow shows an internalized nitrated *T.cruzi* in stimulated macrophages. No parasite protein tyrosine nitration was observed in unstimulated macrophages in the presence of NO_2^-, ruling out a peroxidatic mechanism.