

Supplementary Material; Piñeyro et al. Figure S1.

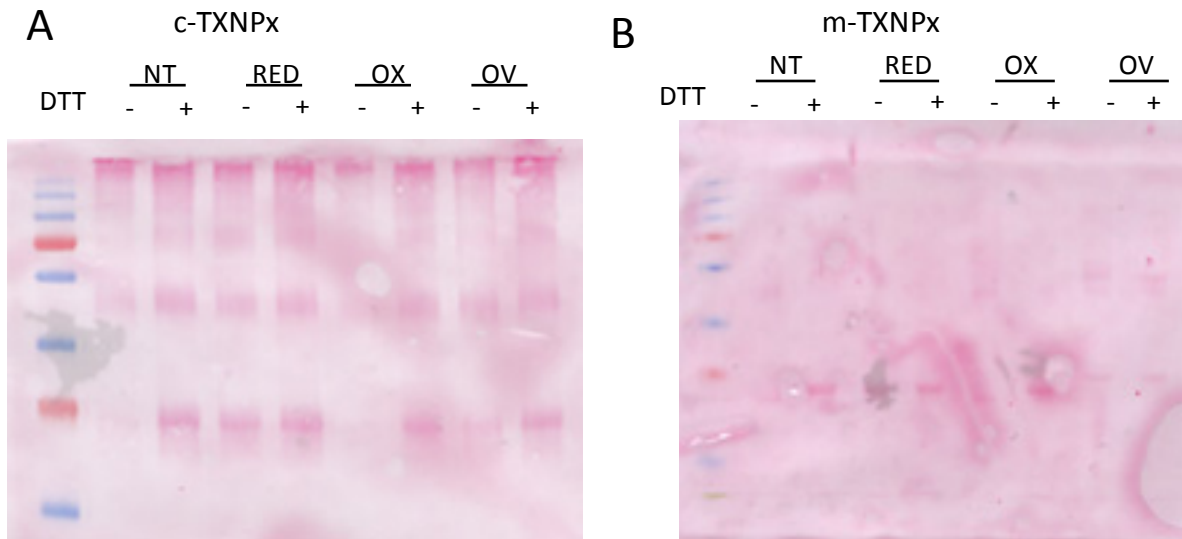


Figure S1. Loading control of western blots shown in Figure 1A.

3 μ g of proteins, A: c-TXNPx and B: m-TXNPx, treated: (RED): 10mM DTT; Oxidation (OX): H_2O_2 (1:1 molar ratio); Overoxidation (OV): 10mM DTT and 10mM H_2O_2 ; NT: proteins without treatment, were resolved by SDS-PAGE, transferred to nitrocellulose membranes and stained with Ponceau-S red as loading control.

Supplementary Material; Piñeyro et al. Figure S2.

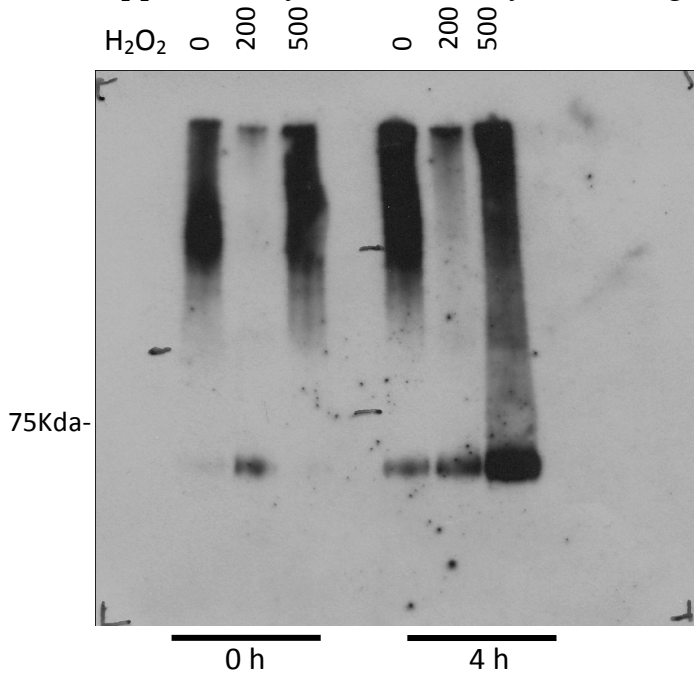


Figure S2. Loading control of western blots shown in Figure 4.

Protein extracts from exponentially growing epimastigotes incubated in PBS-1% glucose at two concentrations of H₂O₂ (200 and 500 μ M) and lysed at different times (0 or 4 h) were resolved by Native Page followed by Western blot using anti tubulin antibody (Sigma). The membrane was analyzed with anti-c-TXNPx and anti-m-TXNPx antibodies, stripped and analyzed with anti-tubulin antibody.

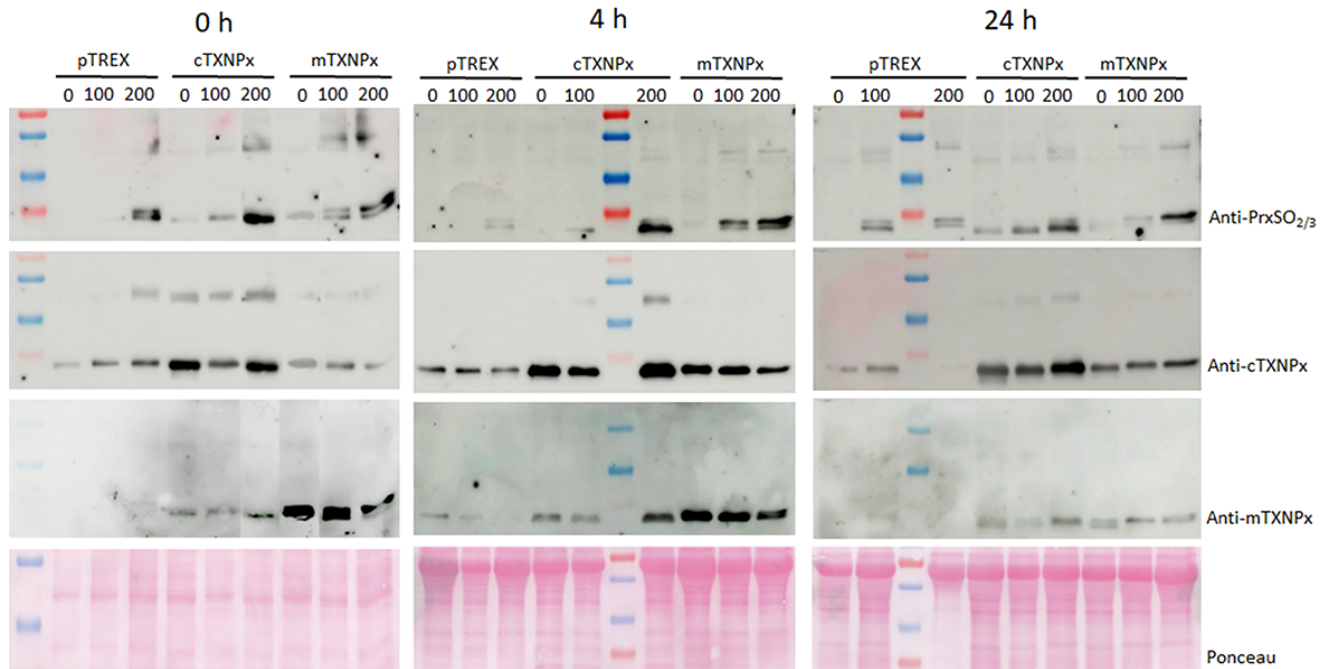


Figure S3. Analysis of the dynamics of overoxidation over time in epimastigotes.

Overexpressing c-TXNPx and m-TXNPx lines were treated with 100 and 200 μM H_2O_2 for 30 min in PBS-1% glucose. Parasites were lysed at different times (0, 4, and 24 h) after growth in LIT medium and analyzed by Western Blot with anti- PrxSO_{2/3} (1/1000) and 15 μg of each extract. The membrane was stripped and analyzed for other antibodies: anti-c-TXNPx (1/5000), anti-m-TXNPx (1/1000). The loading control was performed by Ponceau-S red staining of the membrane.

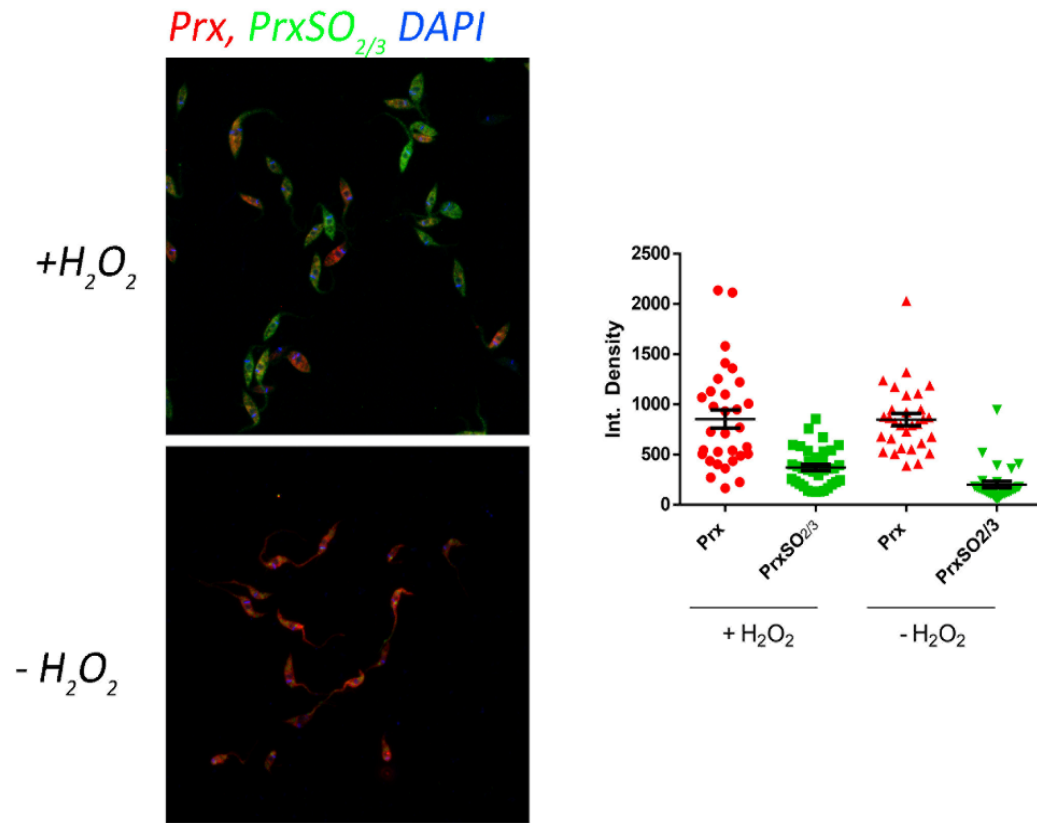


Figure S4. Immunofluorescence of epimastigotes.

Epimastigotes incubated with or without 500 μM H₂O₂ and stained with anti cTXNPx, anti PrxSO_{2/3} or DAPI for nuclear and kinetoplast visualization. The graph shows the integrated density for c-TXNPx fluorescence (red) and PrxSO_{2/3} (green) after incubation with or without 500 μM H₂O₂. Integrated density was calculated with FIJI.

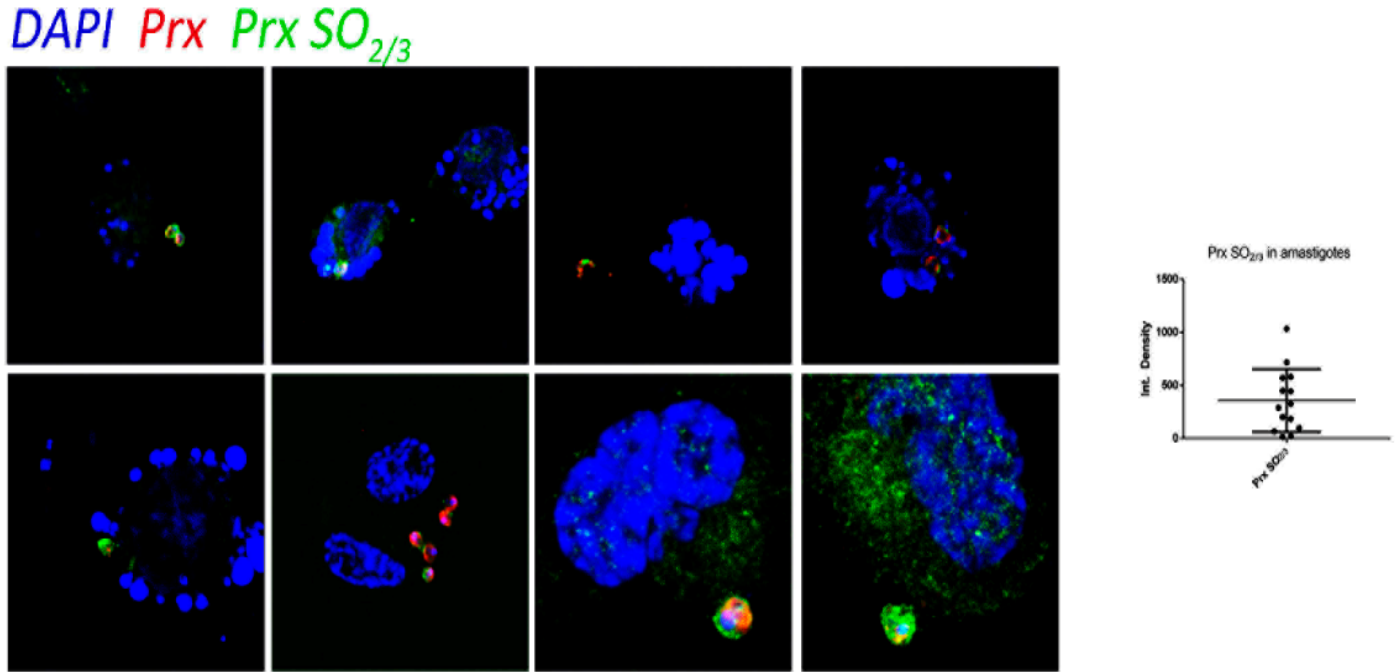


Figure S5. Immunofluorescence of THP1 macrophages infected with trypomastigotes and fixed after internalization.

Cells were stained with DAPI for nuclear and kinetoplast visualization, with anti c-TXNPx and with anti PrxSO_{2/3} antibody. The graph shows the integrated density of PrxSO_{2/3} fluorescence in intracellular amastigotes obtained with FIJI software.

Supplementary Material; Piñeyro et al. Figure S6.

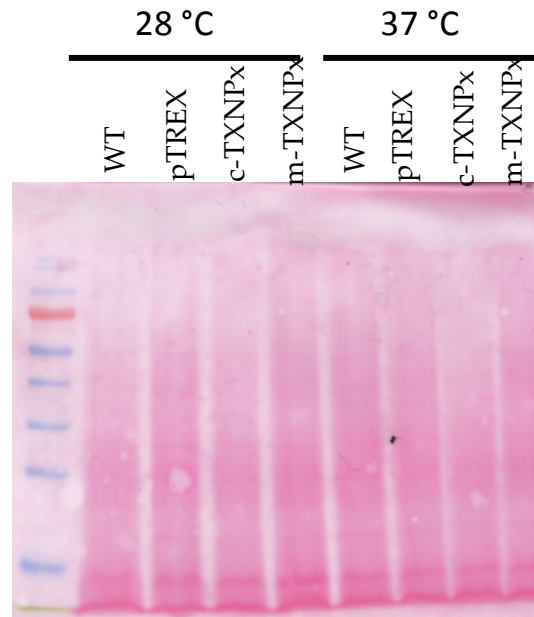


Figure S6. Loading control of western blot shown in Figure 8B.

Proteins extracts (20 μ l of 3.10⁶ cells/mL) of parasites grown at 28°C or 37°C for 72 h, were resolved by SDS-PAGE, transferred to nitrocellulose membranes and stained with Ponceau-S red as loading control.