

# Supplementary Materials

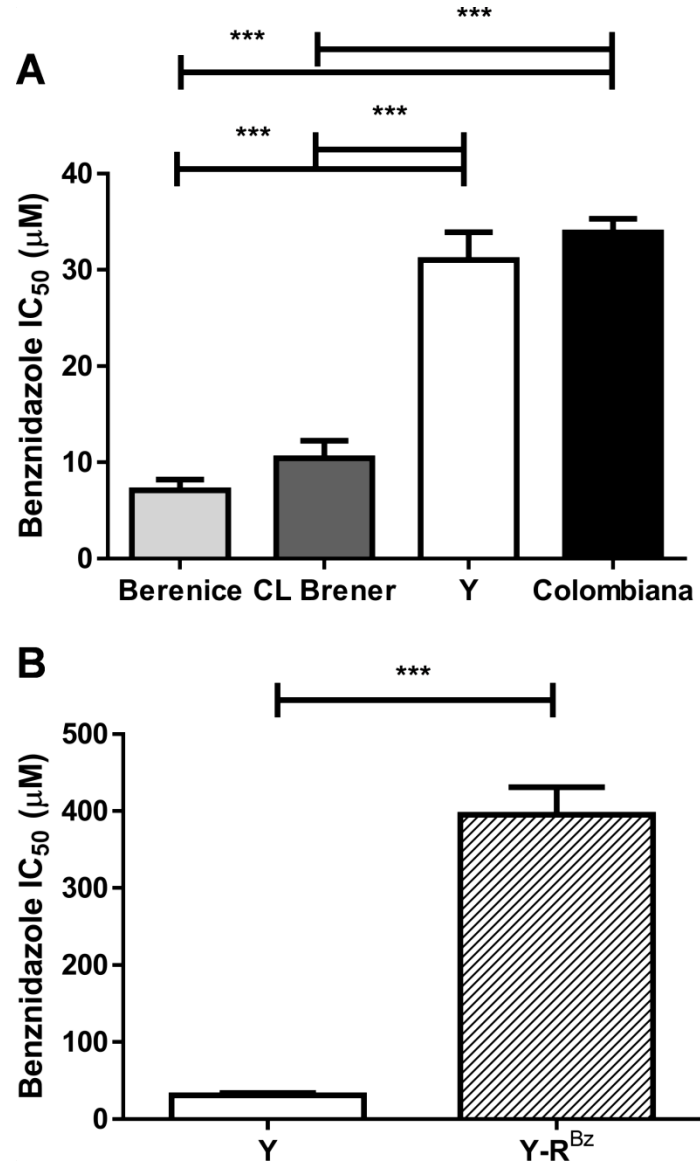
## Intrinsic and Chemotherapeutic Stressors Modulate ABCC-Like Transport in *Trypanosoma cruzi*

Kelli Monteiro da Costa <sup>1,\*</sup>, Eduardo J. Salustiano <sup>1</sup>, Raphael do Carmo Valente <sup>2</sup>,  
Leonardo Freire-de-Lima <sup>1</sup>, Lucia Mendonça-Previato <sup>1,\*</sup> and José Osvaldo Previato <sup>1</sup>

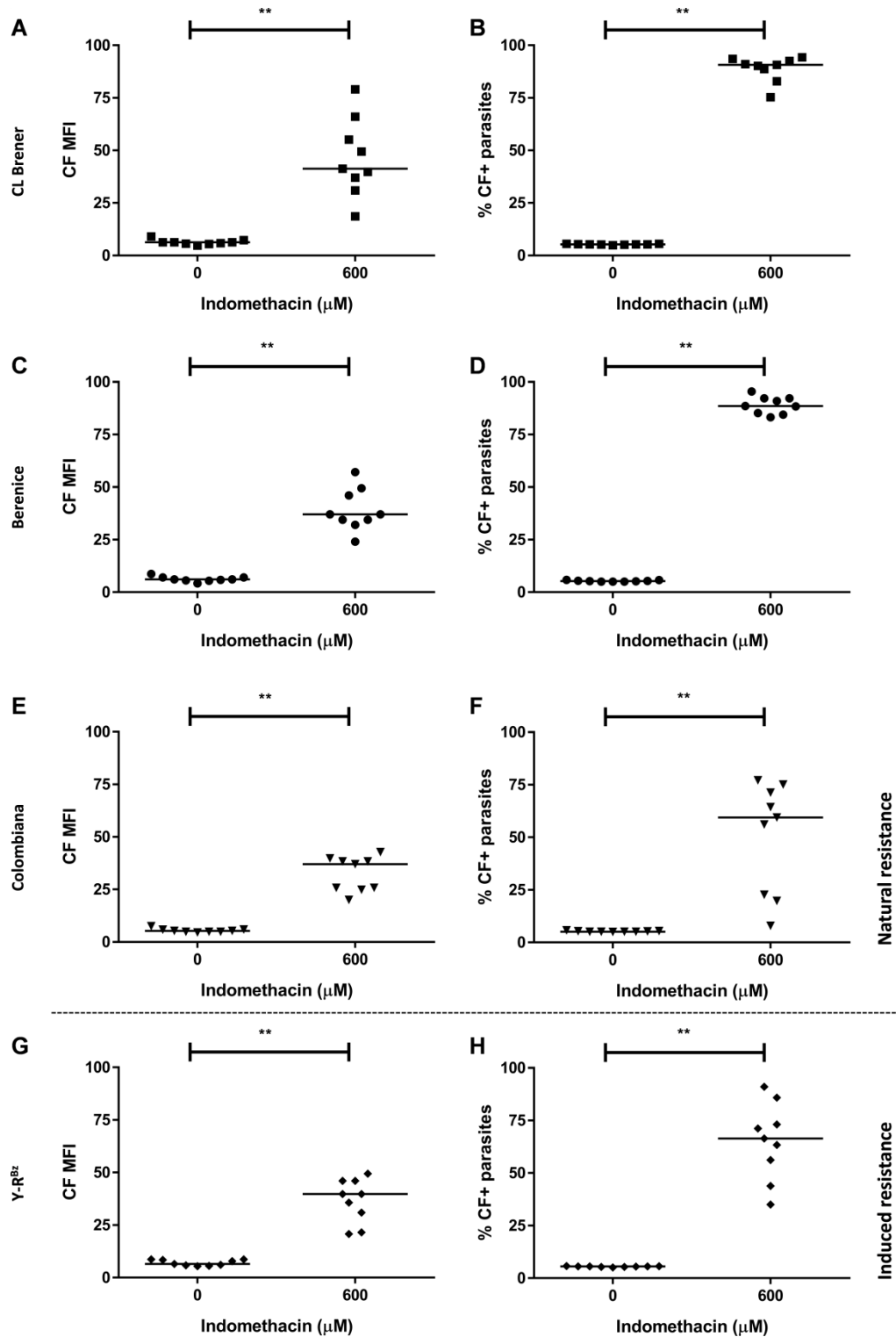
<sup>1</sup> Laboratory of Glycobiology, Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro 21941-902, RJ, Brazil; salustiano@bioqmed.ufrj.br (E.J.S.); leolima@biof.ufrj.br (L.F.L.); previato@biof.ufrj.br (J.O.P.)

<sup>2</sup> Nucleus of Multidisciplinary Research in Biology (Numpex-Bio), UFRJ Duque de Caxias Campus—Professor Geraldo Cidade, Federal University of Rio de Janeiro (UFRJ), Duque de Caxias 25250-470, RJ, Brazil; raphael.valente@xerem.ufrj.br

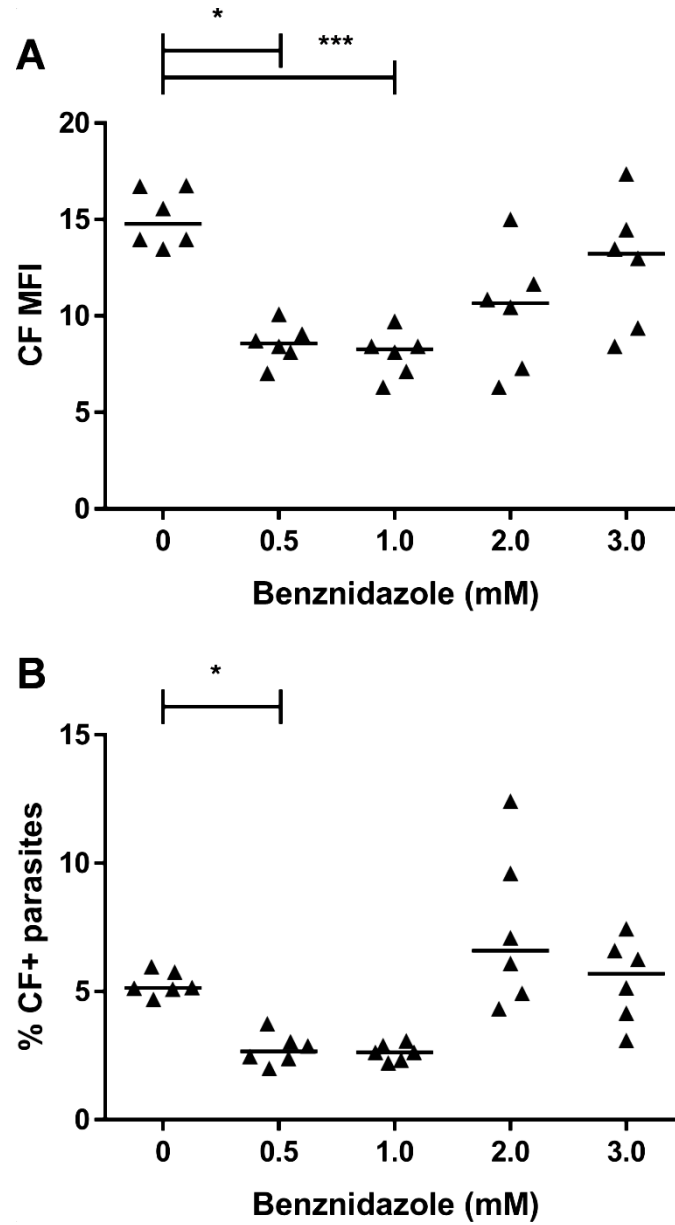
\* Correspondence: kellimc85@biof.ufrj.br (K.M.C.); luciamp@biof.ufrj.br (L.M.P.)



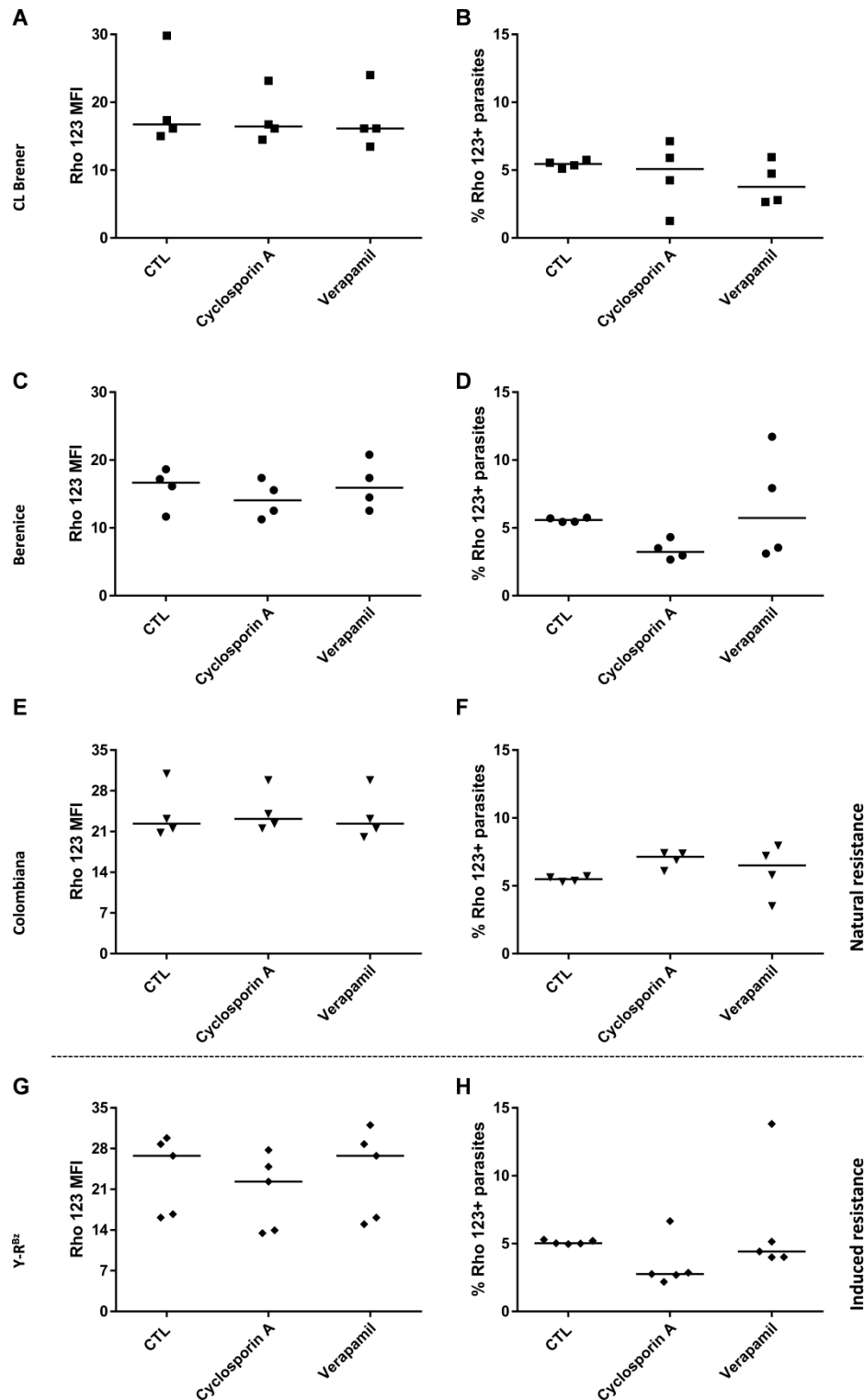
**Figure S1.** IC<sub>50</sub> for benznidazole in *T. cruzi* strains and Y-R<sup>Bz</sup> parasites. In MTT assay, the half-maximum inhibitory concentration (IC<sub>50</sub>) of mitochondrial reducing activity was determined from logarithmic regression as described in Material and Methods in the CL Brener, Berenice, Colombiana and Y strains and Y-R<sup>Bz</sup> parasites. Bars represent the mean + SEM. Significance values were represented by (\*\*\*) for  $p < 0.001$ ,  $n = 4$  (Colombiana) and  $n = 6$  (others) independent experiments.



**Figure S2.** CF accumulation in the presence of the inhibitor indomethacin in *T. cruzi* sensitive and resistant to benznidazole. ABCC-like activity was evaluated by the CF efflux assay in the presence of 600  $\mu\text{M}$  indomethacin. Graphs exhibit CF MFI (left panel) and percentage of CF+ parasites (right panel) from (A and B) CL Brener, (C and D) Berenice, (E and F) Colombiana strains and (G and H) Y-R<sup>Bz</sup> parasites. Lines represent the median and values of significance were represented by (\*\*) for  $p < 0.01$  from  $n = 9$  independent experiments.



**Figure S3.** CF accumulation in the presence of benznidazole in *T. cruzi*. ABCC-like activity was evaluated by the CF efflux assay in the presence of benznidazole. Graphs represent (A) the CF MFI and (B) percentage of CF+ parasites in the Y strain. Lines represent the median and values of significance were represented by (\*) for  $p < 0.05$  and (\*\*\*)  $p < 0.001$ ,  $n = 6$  independent experiments.



**Figure S4.** Rho 123 accumulation the presence of ABCB1 inhibitors in *T. cruzi* sensitive or resistant to benznidazole. ABCB1-like activity was evaluated by rhodamine 123 (Rho 123) efflux assay in the presence or absence of 50  $\mu$ M cyclosporin A or 10  $\mu$ M verapamil. Graphs exhibit Rho 123 median fluorescence intensity (MFI) (left panel) and percentage of CF+ parasites (right panel) for epimastigote forms from (A and B) CL Brener, (C and D) Berenice and (E and F) Colombiana strains and (G and H) Y-R<sup>Bz</sup> parasites. Lines represent the median and values of significance were represented by (\*) for  $p < 0.05$  and (\*\*)  $p < 0.01$ ,  $n = 4$  (strains) and  $n = 5$  (Y-R<sup>Bz</sup>) independent experiments.