Supplemental Material

Differential role of TGF-β in extracellular matrix regulation during *Trypanosoma cruzi* - host cell interaction

Tatiana Araújo Silva, Luis Felipe de Carvalho Ferreira, Mirian Claudia de Souza Pereira and Claudia Magalhães Calvet*.

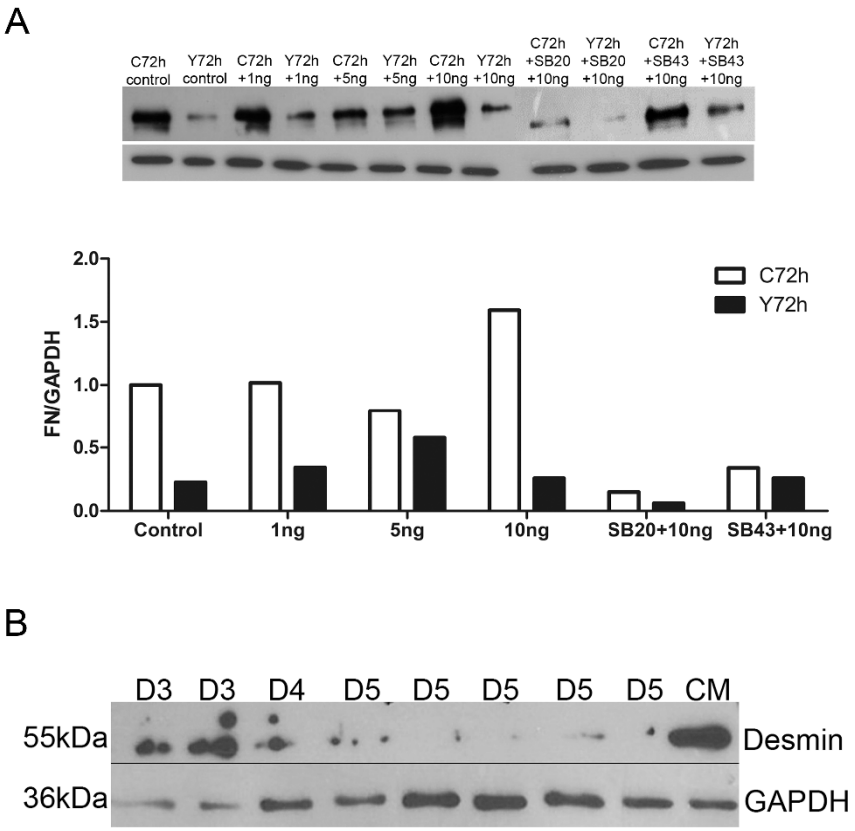
Cellular Ultrastructure Laboratory, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, RJ, 21040-360, Brazil

*Corresponding author: Dr Claudia M. Calvet, Laboratório de Ultraestrutura Celular, Instituto Oswaldo Cruz, FIOCRUZ, Av. Brasil 4365, Manguinhos, 21040-362 Rio de Janeiro, RJ, Brazil, e-mail: cmcalvet@ioc.fiocruz.br

Characterization of cardiac fibroblasts.

Cardiac fibroblasts have the largest cell nucleus when compared to cardiomyocyte (CM), this morphology being a distinctive feature in this cell type. Initially, CF purification obtained from CM cultures was evaluated only by morphology. CF obtained in the third dissociation of CM cultures were infected with *T. cruzi* and treated with TGF-β for 48 hours and were subjected to Western blot to analyze the FN expression under these conditions. Our data revealed an increase in FN expression only with 10 ng/ml TGF-β. Furthermore, third dissociation from CM cultures had reduced FN expression compared to normal control even after the addition of TGF-β. The addition of inhibitors SB431542 and SB203580 (SMADS pathway and p38 MAPK inhibitor respectively) in this culture led to inhibition of FN stimulation by TGF-β, since in infected cultures treated with 10 ng/ml TGF-β (48h) reduced FN expression after pre-treatment with inhibitors signaling (S1A) was seen. The data obtained in the third dissociation from CM culture extracts with the FN expression correspond with that of cardiomyocytes [1], suggesting that CF purification was needed adjustments and that FN response could not be displayed properly when CF is present in CM culture, suggesting that morphological analysis under the microscope do not guarantee good purification of CF.

Thus, to assess the degree of CF purification from CM cultures, desmin, a specific cytoskeletal protein of cardiomyocytes, was evaluated in CF extracts after different dissociations. CF extracts obtained in the third and fourth dissociation revealed that cardiomyocytes are still present in CF culture, since the extracts also had high expression of desmin, demonstrating that these dissociations are not viable for conducting experimental tests. Pure CF was obtained only from the fifth dissociation from CM culture in the absence of cardiomyocytes, with desmin undetectable in this culture only after the 5th passage (S1B).



S1 - FN expression in cells obtained in the third CM culture passage. **(A) Control cultures show FN stimulation only with the treatment with 10 ng/ml TGF-β (48h).** Doses of 1ng/ml and 5ng/ml did not induce increase of FN expression. *T. cruzi* infection leads to a reduction in FN expression after addition of TGF-β. Inhibitory action of SMADs and p38 MAPK pathway prevents increase in FN expression even after stimulation with 10 ng/ml. These results obtained with cell extracts after third CM culture passage are similar to previously published data in cardiomyocyte cultures under the same conditions. **(B) Characterization of the culture of heart fibroblasts**. Rate of CF purification from cardiac culture. The degree of CF purification was evaluated by detection of desmin in cell extracts obtained in the 3rd, 4th and 5th dissociation. CF purification was obtained from the fifth dissociation, as shown by the absence of desmin.

References

1. Calvet, C.M.; Oliveira, F.O.R.; Araújo-Jorge, T.C.; Pereira, M.C.S. Regulation of extracellular matrix expression and distribution in Trypanosoma cruzi-infected cardiomyocytes. *Int. J. Med. Microbiol.* **2009**, *299*, 301–12.