

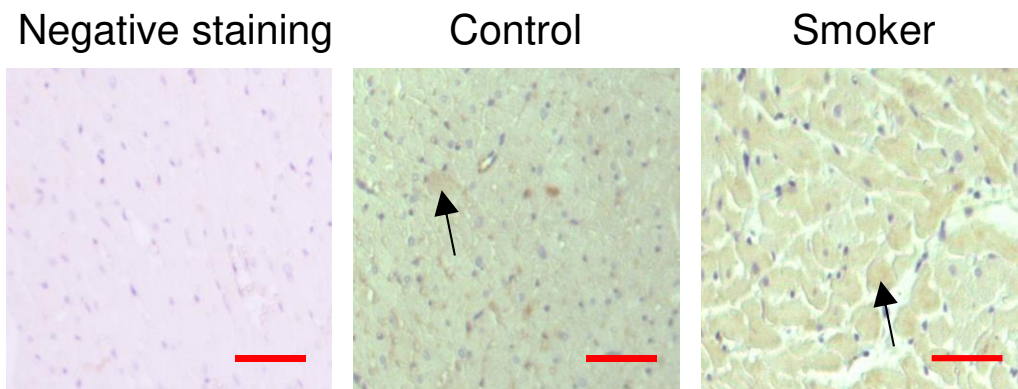
Supplemental Material

Evaluation of the Diaphragm Muscle Remodeling, Inflammation, Oxidative Stress and Vascularization in Smokers: An Autopsy Study

Ricardo Aparecido Baptista Nucci^a Laura Beatriz Mesiano Maifrino^b
Alexandre Leopold Busse^{a,c} Romeu Rodrigues de Souza^{d,e}
Carlos Augusto Pasqualucci^a Carlos Alberto Anaruma^f
Renata Elaine Paraizo Leite^c Roberta Diehl Rodriguez^c
Claudia Kimie Suemoto^c Wilson Jacob-Filho^{a,c}

^aDepartment of Pathology, University of São Paulo Medical School, São Paulo, Brazil, ^bLaboratory of Morphological and Immunohistochemical Studies, São Judas Tadeu University, São Paulo, Brazil, ^cLaboratory of Medical Research in Aging (LIM-66), Division of Geriatrics, University of São Paulo Medical School, São Paulo, Brazil, ^dDepartment of Anatomy, Institute of Biomedical Sciences of the University of São Paulo, São Paulo, Brazil, ^eDepartment of Anatomy, 9 de Julho University (UNINOVE), São Paulo, Brazil, ^fLaboratory of Morphology and Physical Activity, Department of Physical Education, São Paulo State University "Júlio de Mesquita Filho", Rio Claro, Brazil

Positive staining



Supplementary Fig. 1 Negative and positive staining for technical feasibility. We used the reagent negative control (i.e. no primary antibody). In the negative control we incubated the sample with the antibody diluent alone and no primary antibody, followed by incubation with secondary antibodies, detection reagents (i.e. DAB - brown stain), and counterstained with hematoxylin to elucidate the nuclei. This ensures that staining is produced from detection of the antigen by the primary antibody and not by the detection system or the specimen. In positive staining we used both primary and secondary antibodies (specific for each parameter, in this case metalloproteinase type 2 - Santa Cruz Biotechnology, sc-10736), DAB, and counterstained with hematoxylin. DAB was quantitatively evaluated with both volume density and its intensity using ImageJ software. Control: subject number 1. Smoker: subject number 8. Arrow: DAB. Scale bars = 50 μ m