

## SUPPLEMENTARY MATERIAL

# **Adenine-Induced Chronic Renal Failure in Rats: A Model of Chronic Renocardiac Syndrome With Left Ventricular Diastolic Dysfunction but Preserved Ejection Fraction**

Pavlos Kashioulis<sup>a</sup> Jaana Lundgren<sup>a</sup> Emman Shubbar<sup>a</sup> Lisa Nguy<sup>a,b</sup> Aso Saeed<sup>a</sup> Cecilia  
Wallentin Guron<sup>a</sup> Gregor Guron<sup>a</sup>

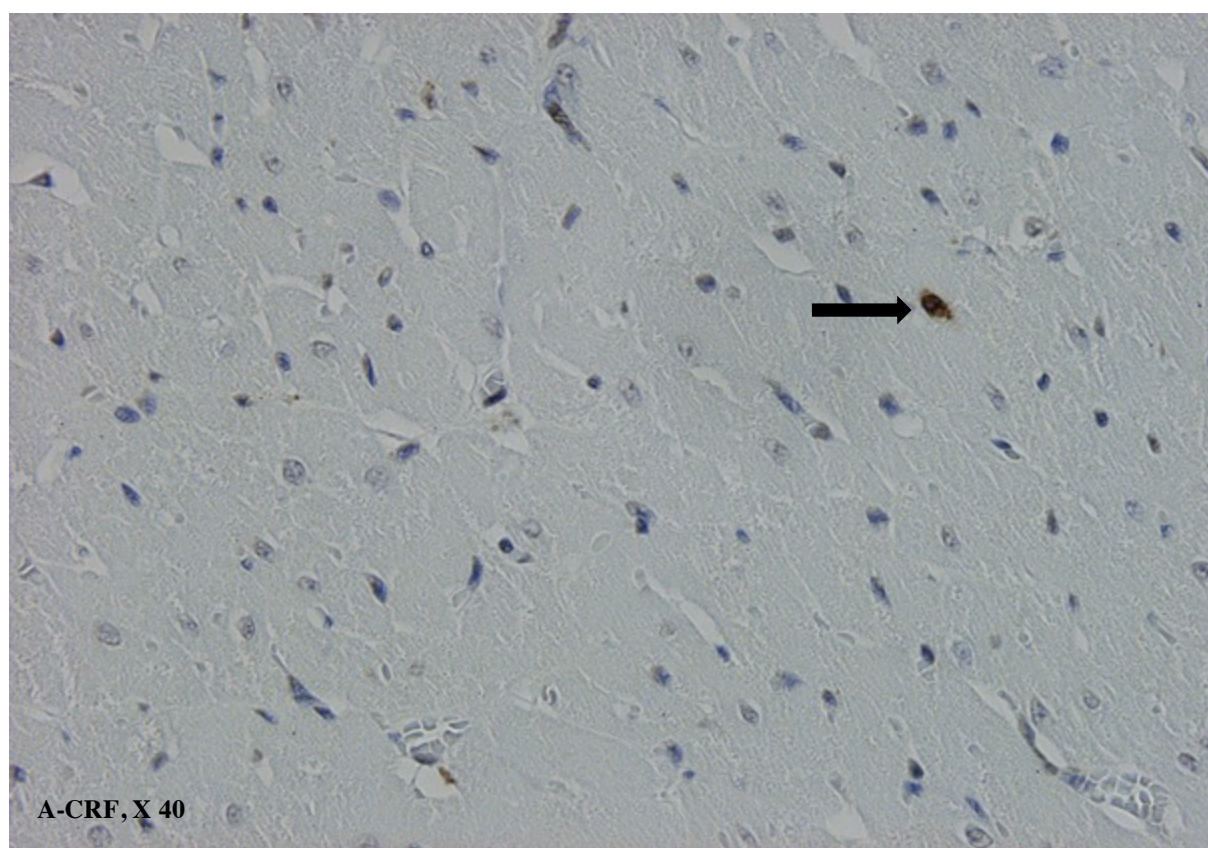
<sup>a</sup>Department of Molecular and Clinical Medicine, Institute of Medicine, the Sahlgrenska Academy at the University of Gothenburg, Gothenburg, <sup>b</sup>Department of Physiology, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

**Supplemental figure 1. TUNEL-staining in the left ventricle of an A-CRF rat.**

Apoptotic cells were detected by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method (see Methods). A TUNEL-positive cardiomyocyte is shown (see arrow) in the left ventricle of a rat with A-CRF (adenine-induced chronic renal failure).

Numerical data on the number of TUNEL positive cells are presented in table 5.

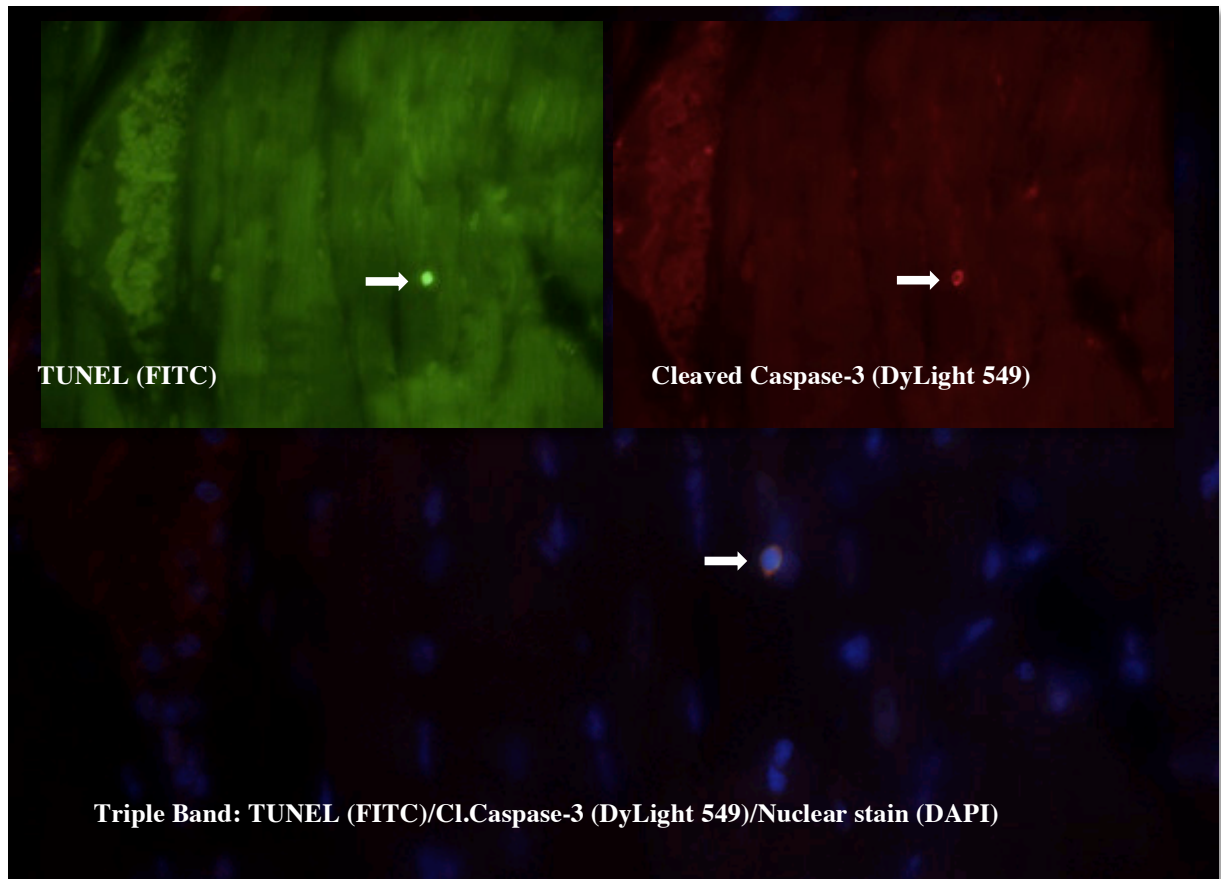
**Supplemental figure 1. TUNEL-staining in the left ventricle of an A-CRF rat.**



**Supplemental figure 2. Co-localization of TUNEL-staining and cleaved caspase-3 in the left ventricle of an A-CRF rat.**

To verify that TUNEL-positive cells in the left ventricle of A-CRF (adenine-induced renal failure) rats were undergoing apoptosis some sections were also stained for cleaved caspase-3 (see Methods). Upper panels show immunostaining demonstrating a cardiomyocyte that is positive for both TUNEL (upper left panel) and cleaved caspase-3 (upper right panel). The lower panel demonstrates that immunostaining for TUNEL and cleaved caspase-3 was co-localized in the same cardiomyocyte clearly indicating that this cell was undergoing apoptosis.

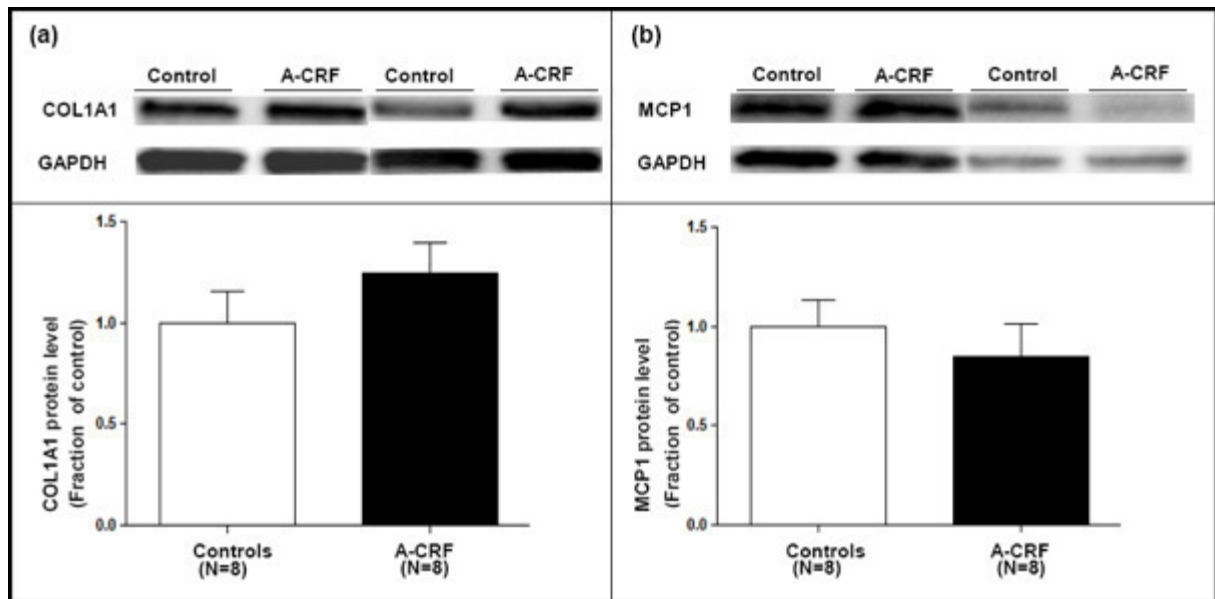
**Supplemental figure 2. Co-localization of TUNEL-staining and cleaved caspase-3 in the left ventricle of an A-CRF rat.**



**Supplemental figure 3. Protein levels of collagen-1 alpha-1 and monocyte chemotactic protein-1 in the left ventricle.**

Representative immunoblots and densitometric data of collagen-1 alpha-1 (COL1A1, left panel) and monocyte chemotactic protein-1 (MCP1, right panel) from the left ventricle of control rats and rats with adenine-induced chronic renal failure (A-CRF). Rats were sacrificed 10 weeks after study start (n=8 per group). Data are normalized to GAPDH and expressed as fraction of control values. Values are means  $\pm$  SD. There were no statistically significant differences between groups.

**Supplemental figure 3. Protein levels of collagen-1 alpha-1 and monocyte chemotactic protein-1 in the left ventricle.**

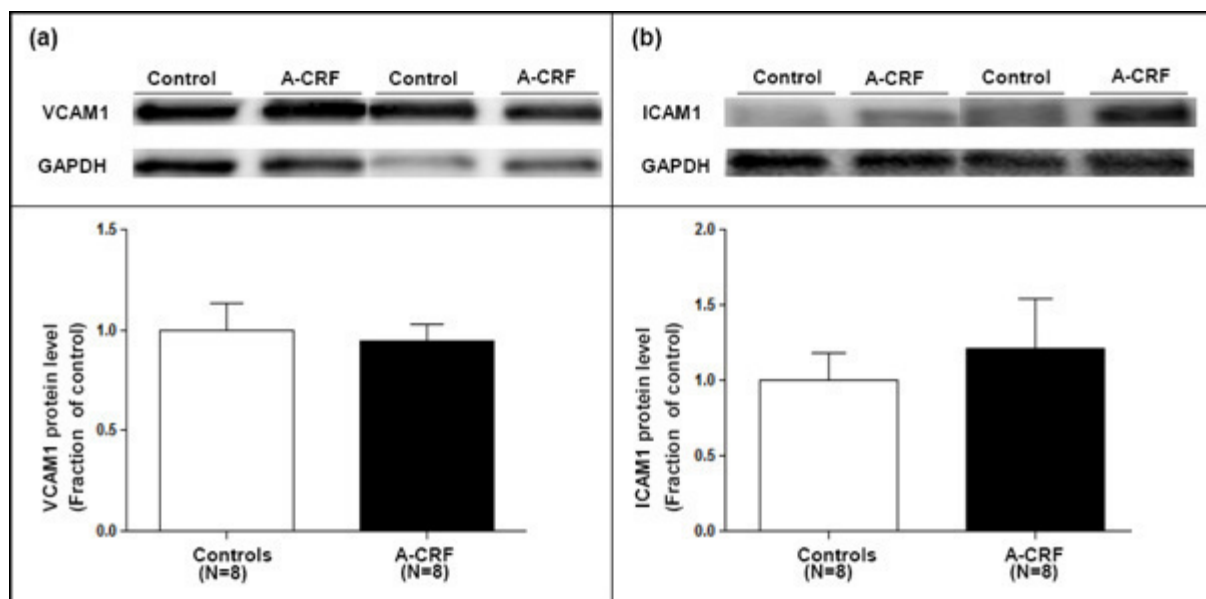


**Supplemental figure 4. Protein levels of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 in the left ventricle.**

Representative immunoblots and densitometric data of vascular cell adhesion molecule-1 (VCAM1, left panel) and intercellular adhesion molecule-1 (ICAM1, right panel) from the left ventricle of control rats and rats with adenine-induced chronic renal failure (A-CRF). Rats were sacrificed 10 weeks after study start (n=8 per group). Data are normalized to GAPDH and expressed as fraction of control values. Values are means  $\pm$  SD. There were no statistically significant differences between groups.



**Supplemental figure 4. Protein levels of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 in the left ventricle.**



**Supplemental figure 5. Protein levels of NCX-1, SERCA2 and BMP4 in the left ventricle.**

Representative immunoblots and densitometric data of sodium-calcium exchanger-1 (NCX-1, left panel), sarcoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase (SERCA2, middle panel) and bone morphogenetic protein-4 (BMP4, right panel) from the left ventricle of control rats and rats with adenine-induced chronic renal failure (A-CRF). Rats were sacrificed 10 weeks after study start (n=8 per group). Data are normalized to GAPDH and expressed as fraction of control values. Values are means  $\pm$  SD. There were no statistically significant differences between groups.

**Supplemental figure 5. Protein levels of NCX-1, SERCA2 and BMP4 in the left ventricle.**

