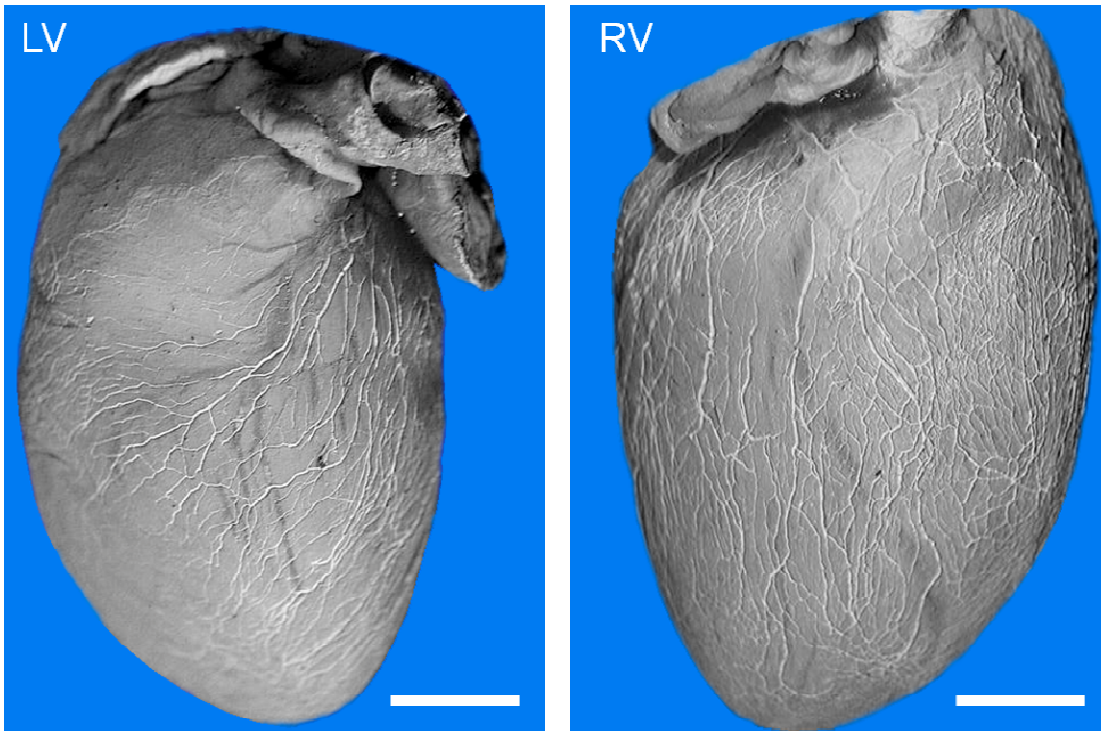


## SUPPLEMENTARY FIGURE



### Cholinergic innervation of the ventricles (Sprague Dawley rat)

Representative photographs of the rat heart stained for acetylcholinesterase (AChE). Left panel shows cholinergic epicardial fibres (stained white) descending from the AV sulcus and innervating the left ventricle (LV). Right panel shows an extensive network of epicardial cholinergic fibres running down from the AV sulcus extensively covering the lateral aspect of the right ventricle (RV). Scale bar – 0.5 cm.

Method: A modified Karnovsky staining method was used to visualize nerve fibres containing AChE. Briefly, the hearts were rinsed in 0.1 M sodium phosphate buffered saline (PBS) and placed in AChE staining solution for 24 hours containing: 0.14% acetylthiocholine iodide, 0.3% glycine and 2%  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 0.1M PBS. The hearts were then air-dried and photographs were captured using a digital camera and a Leica microscope. For presentation purposes artificial shadows were applied on 8-bit images using ImageJ software.

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Karnovsky MJ. The localization of cholinesterase activity in rat cardiac muscle by electron microscopy J Cell Biol. 23:217-32, 1964.