

# Investigating the role of the *Spp1* gene in cardiac hypertrophy and fibrosis utilising normotensive and spontaneously hypertensive rat strains



University  
of Glasgow

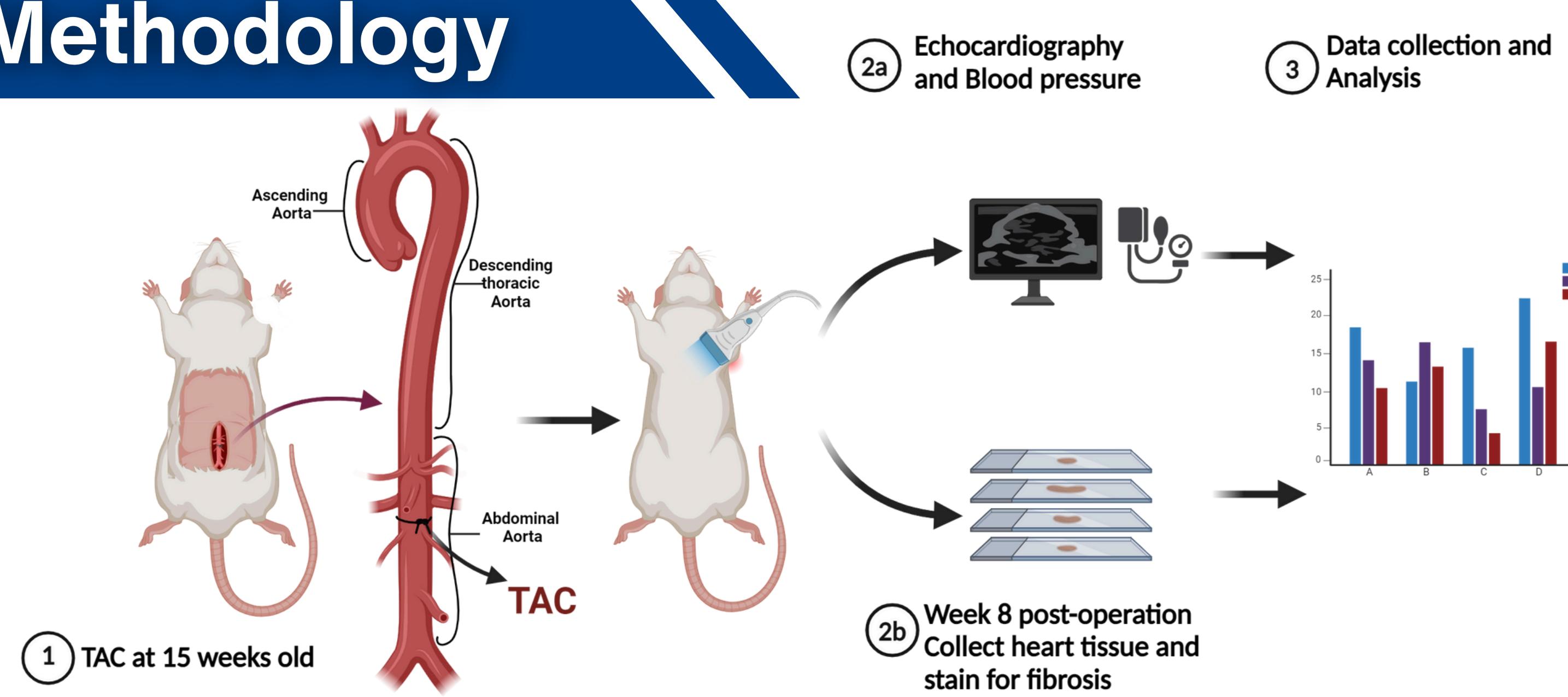
Atheer Al Hefzi, Amrita Asirvatham, Martin McBride, Delyth Graham  
School of Cardiovascular and Metabolic Health, University of Glasgow, Glasgow, Scotland, United Kingdom

## Introduction

A quantitative trait locus (QTL) for left ventricular mass index (LMI) was previously identified on chromosome 14 in an F2 cross of the stroke-prone spontaneously hypertensive (SHRSP) and Wistar Kyoto (WKY) rat strains. Congenic strains (SP.WKYGla14a and WKY.SPGla14a) were constructed on both SHRSP and WKY genetic backgrounds respectively to confirm the LMI QTL. Microarray gene expression profiling identified *Spp1* as a candidate gene for the increased LMI phenotype. *Spp1* expresses the Osteopontin phosphoprotein and plays a role in cardiac fibrosis and hypertrophy.

We aimed to investigate the role of *Spp1* in the development of cardiac hypertrophy and fibrosis using transaortic constriction (TAC) in CRISPR Cas9-generated SHRSP *Spp1* knockout (KO) and wildtype (WT) rats, as well as WKY and WKY.SPGla14a congenic rats.

## Methodology



## Results

### Echocardiography parameters

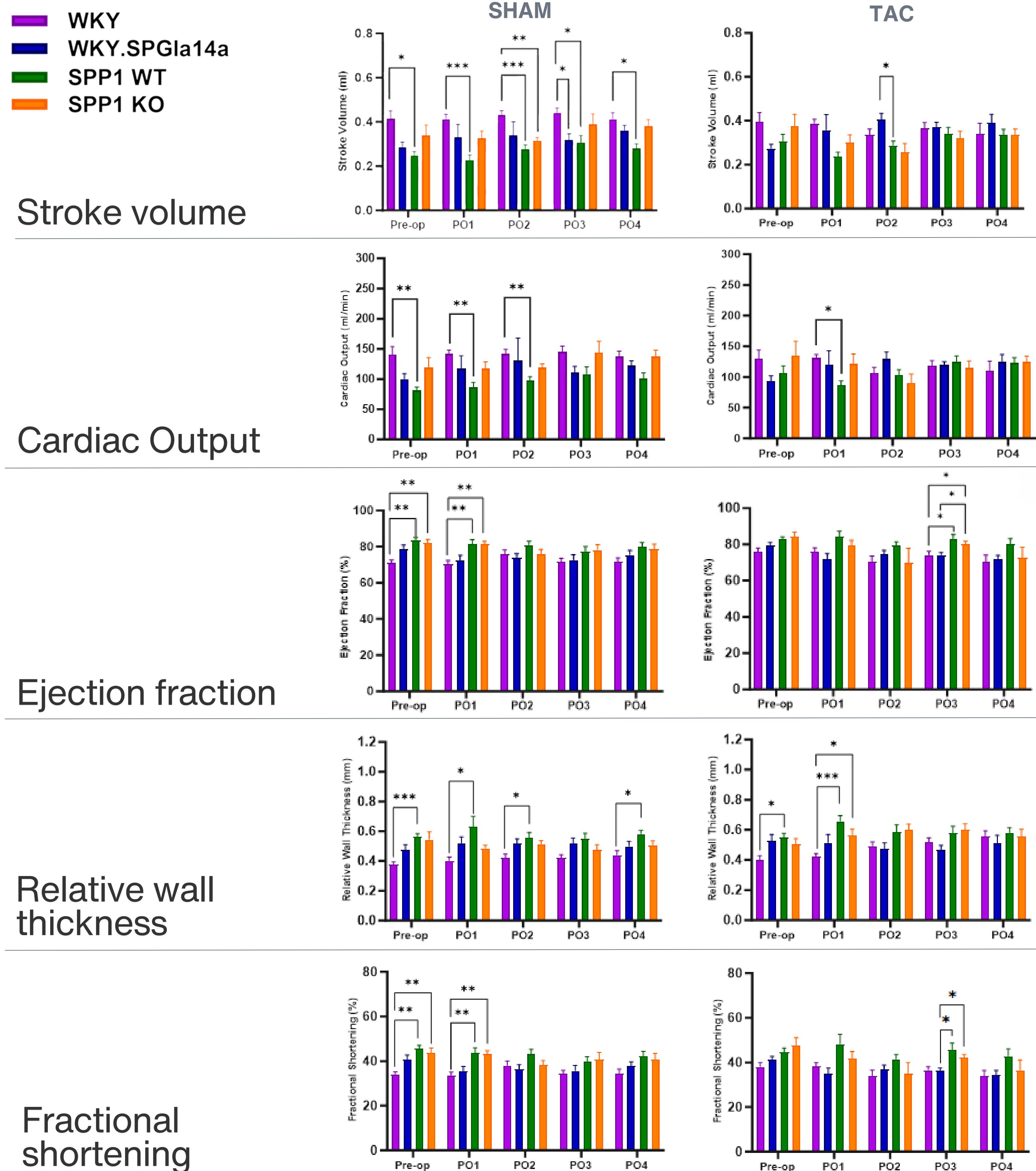


Figure 1: Echocardiography Parameters across all strains post-SHAM and post-TAC over the study period. Pre-operative (Pre-op) and at post-operative 2-8 weeks through (PO1 to PO4). 2-way ANOVA used to analyse differences. Statistically significant differences are indicated as follows: \*P ≤ 0.05, \*\*P ≤ 0.01, \*\*\*P ≤ 0.001, \*\*\*\*P ≤ 0.0001.

## Histology

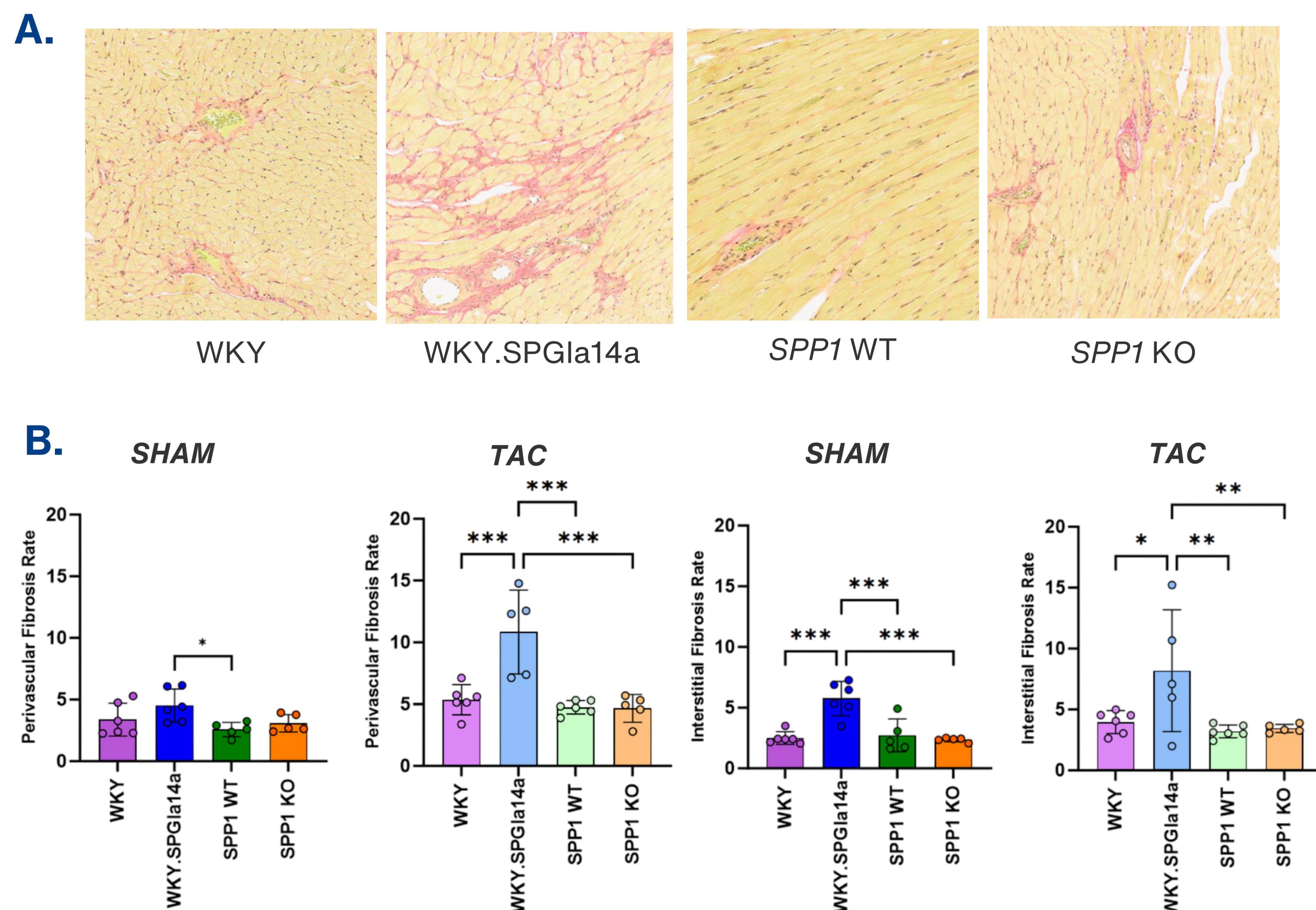


Figure 2: Histology Findings. A) Picosirius red staining for heart tissues TAC . B) Perivascular and interstitial fibrosis rate sham and TAC. (\* P ≤ 0.05, \*\*P ≤ 0.01, \*\*\*P ≤ 0.001, \*\*\*\*P ≤ 0.0001).

## Tissue Harvest data

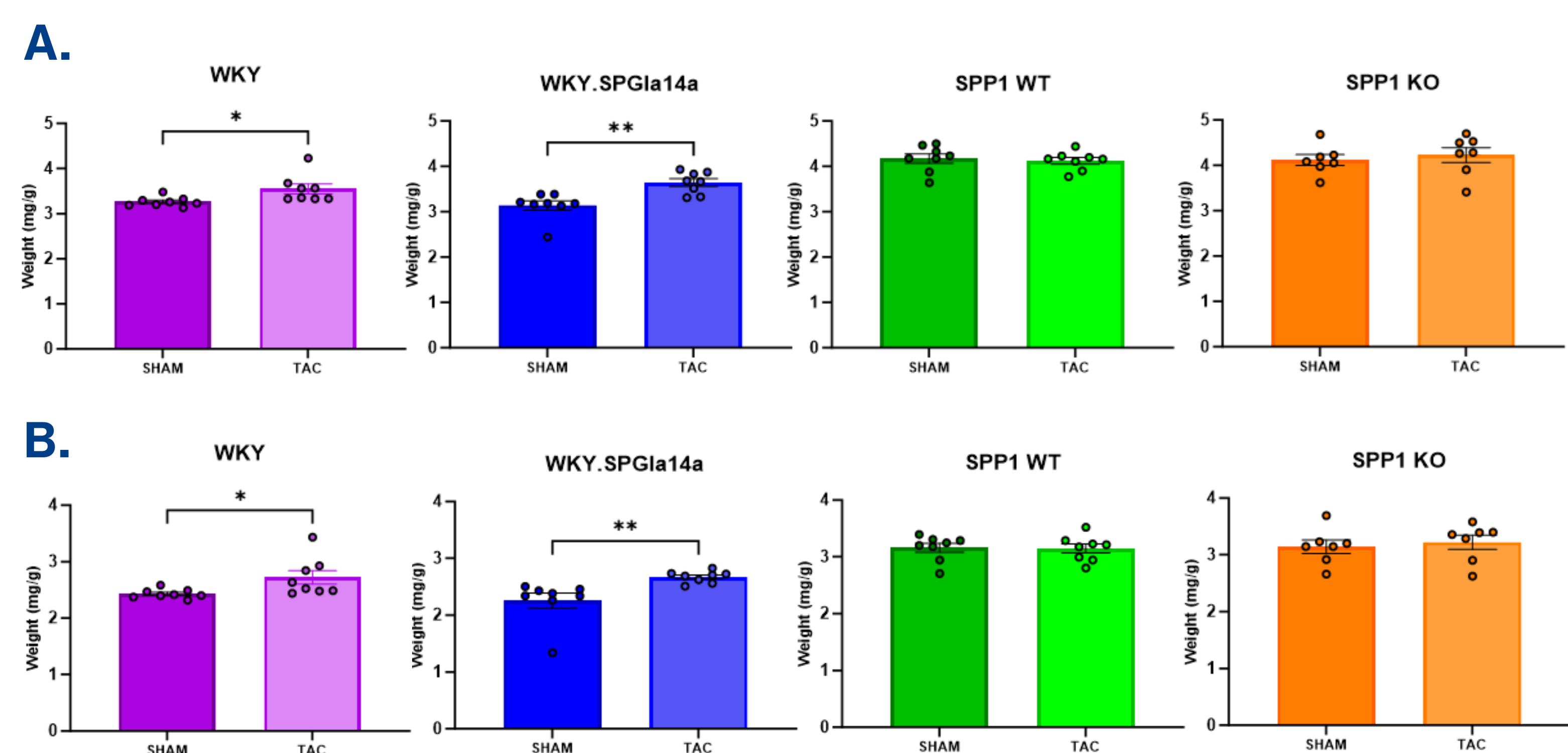


Figure 3: Tissue Harvest data. Heart (A) and left ventricle (B) weights data normalized to body weight (mg/g). Paired t-tests were used to analyse differences within each strain between SHAM and TAC data are presented as mean ± SEM. Statistically significant differences are indicated as follows: \*P ≤ 0.05, \*\*P ≤ 0.01, \*\*\*P ≤ 0.001, \*\*\*\*P ≤ 0.0001.

## Conclusions

- TAC successfully induced hypertrophic responses in normotensive genetic background rat strains, whereas *Spp1* WT and KO rats exhibited pre-existing hypertrophy, potentially limiting further cardiac remodelling.
- Under sham conditions, *Spp1* KO rats show a pattern of consistently higher SV and CO when compared to *Spp1* WT. This 'healthier' cardiac phenotype profile did not translate into protection against TAC challenge since these distinct cardiac function parameters were lost post-TAC.
- Histological analysis revealed significantly increased perivascular fibrosis in all TAC-operated strains. Interstitial and perivascular fibrosis were significantly increased in WKY.SPGla14a rats compared to all other strains post-TAC.

WKY.SPGla14a congenic and *Spp1* KO rat strains are important resources for investigating the genetic factors contributing to cardiac function, hypertrophy and fibrosis in the SHRSP rat.