# Biased Relaxin-RXFP1 Agonist ML290 Attenuates the Development of Pulmonary Hypertension

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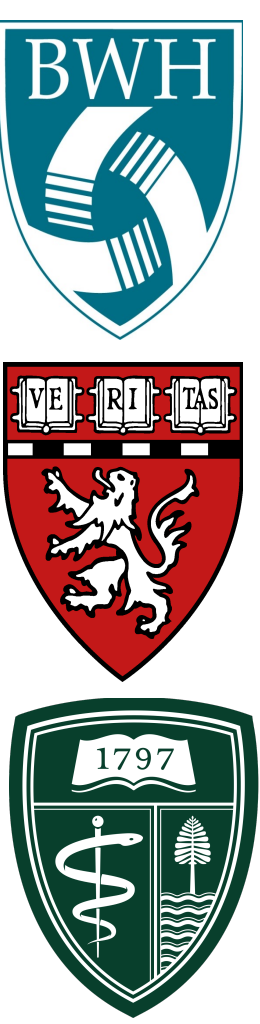
Abstract

Introduction

- Relaxin-2 is an endogenous agonist of the RXFP1 receptor

- Relaxin-2 has vasodilatory and anti-fibrotic properties and has been investigated as therapy for cardiovascular and fibrotic disease.

- RXFP1 is expressed in the vascular media of pulmonary arteries and arterioles, especially the hypertrophied media of pulmonary arteries affected by pulmonary arterial hypertension (PAH).

- Due to its short half-life and need for infusion therapy, the therapeutic potential of RXFP1 signaling in cardiopulmonary disease is not established.

- ML290 is a biased, allosteric agonist of RXFP1 with favorable pharmacokinetics that activates a subset of RXFP1 signaling effectors.

- Consistent with specificity for human RXFP1, ML290 elicits tachycardia in humanized RXFP1 knock-in but not wild-type mice.

Hypothesis:

- Biased modulation of relaxin-RXFP1 signaling via ML290 attenuates experimental pulmonary hypertension (PH) in humanized RXFP1 knock-in mice.

Results:

- Both relaxin and ML290 inhibited TGFβ1-mediated signaling and phenotypic modulation in PASMC (p<0.05).

- Chronic administration of relaxin did not attenuate PH in monocrotaline-exposed rats but was associated with the development of neutralizing antibodies.

- ML290 dose-dependently attenuated PH, RV hypertrophy and pulmonary arteriolar muscularization (p<0.05 for all) in SU5416/hypoxia-exposed humanized mice.

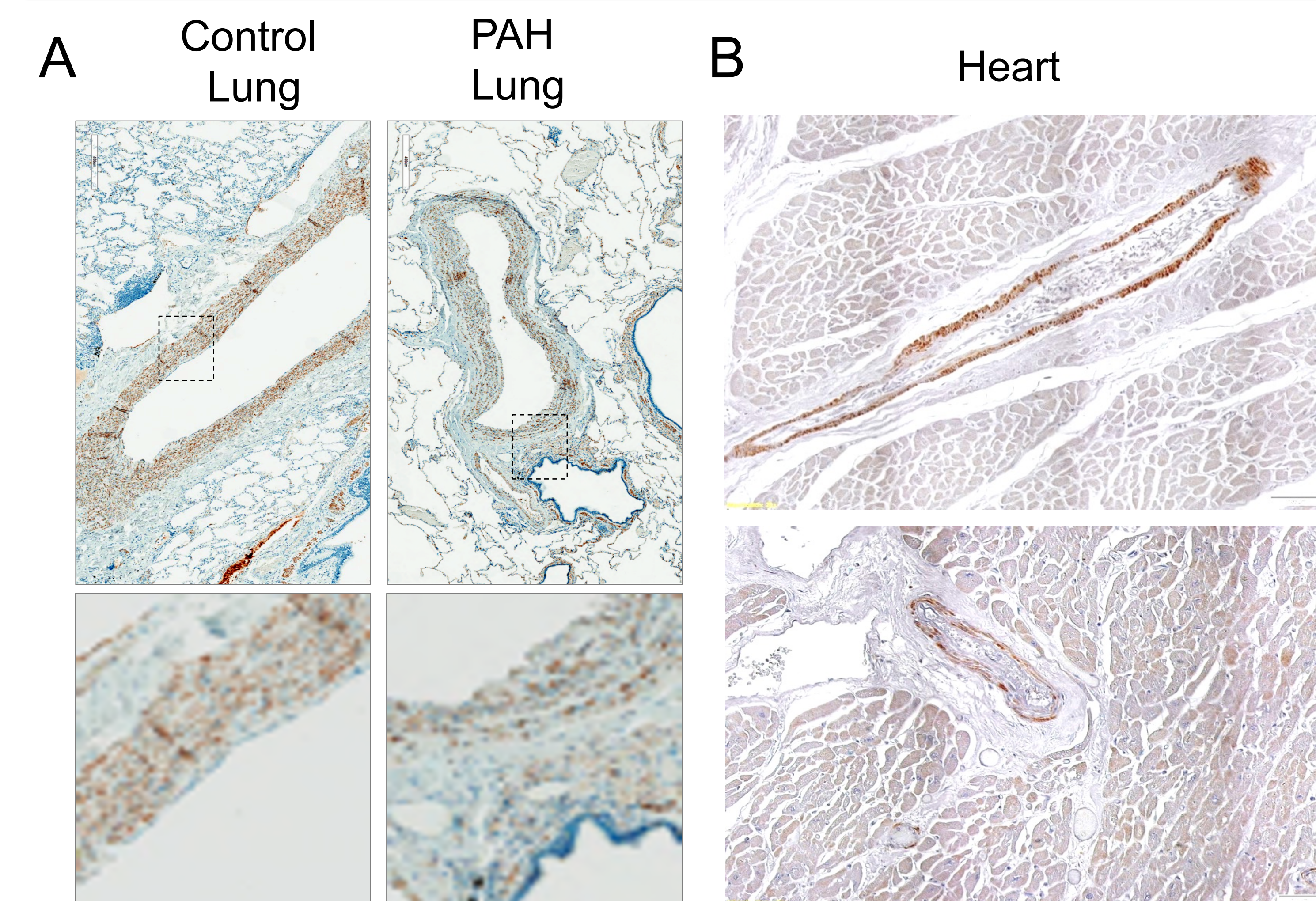## Expression of RXFP1 in human cardiopulmonary tissues

Figure 1. Representative photomicrographs showing RXFP1 immunoreactivity (brown) in vascular and bronchial smooth muscle in (A) human lung and (B) heart (n ≥ 5), counter-stained with hematoxylin (blue). Inset areas of the vascular wall are magnified below.

Relaxin modulates TGF-β signaling activity in human PASMC
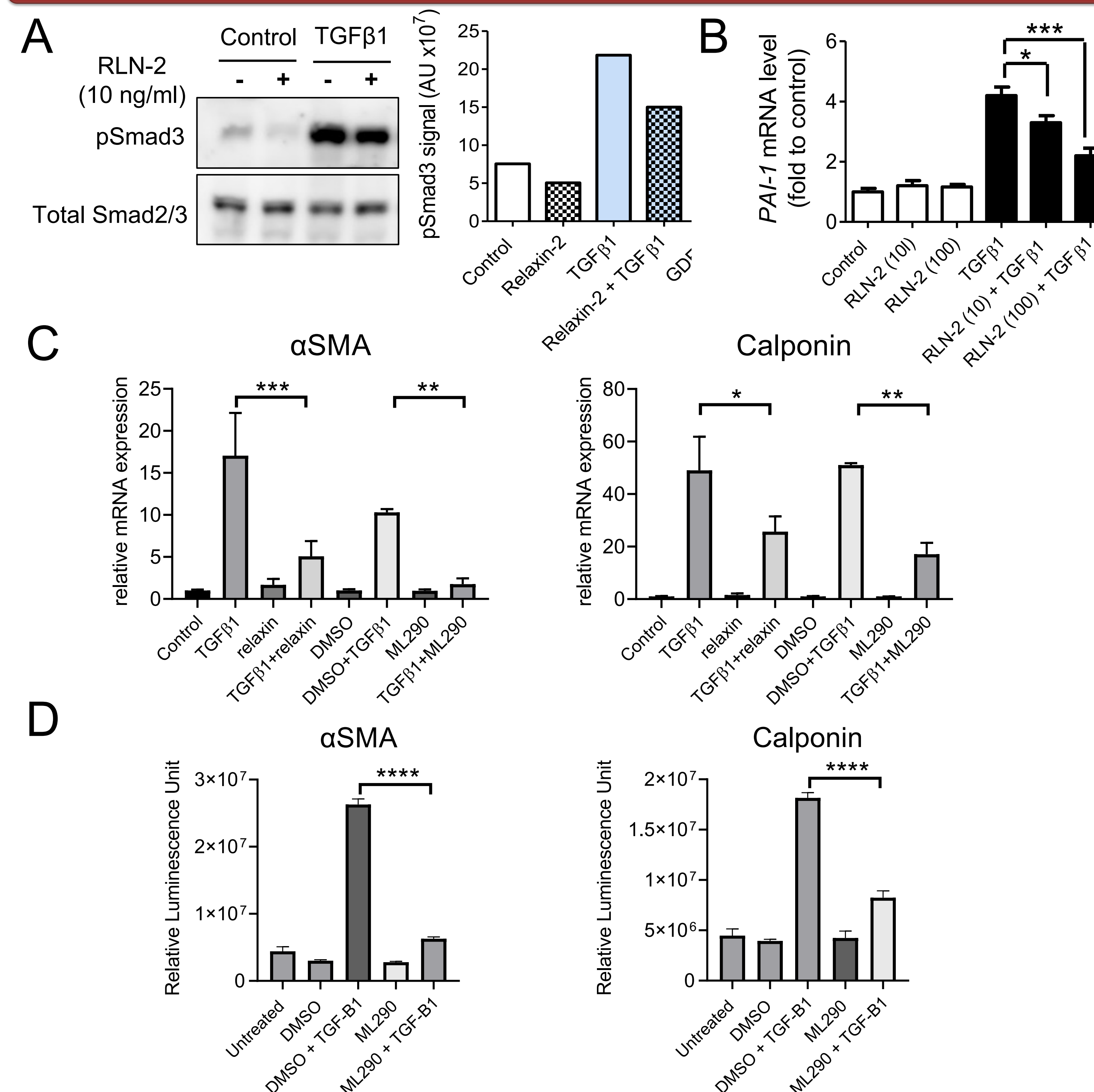

Figure 2. Relaxin-2 and ML290 attenuate TGFβ1-induced signaling and phenotypic modulation in human pulmonary artery smooth muscle cells
(A) Pre-treatment with relaxin-2 (RLN-2, 10 ng/ml, 0.5 h)-induced phosphorylation of Smad3. (B) RLN-2 (10 or 100 ng/ml) suppressed TGFβ1 (1 ng/ml, 3 h)-induced expression of target gene *PAI-1* in a dose-dependent manner. (C) RLN-2 (100 ng/ml) and ML290 (10µM) attenuated TGFβ1 (1 ng/ml, 24 h)-induced mRNA expression of α-smooth muscle actin and calponin measured by qPCR. (D) ML290 (10µM) attenuated TGFβ1 (1 ng/ml, 24 h)-induced protein expression of α-smooth muscle actin and calponin measured by in-cell Western. Data are mean ± SEM, n=3. *p <0.05, **p<0.01, ***p<0.001, ****p<0.0001.

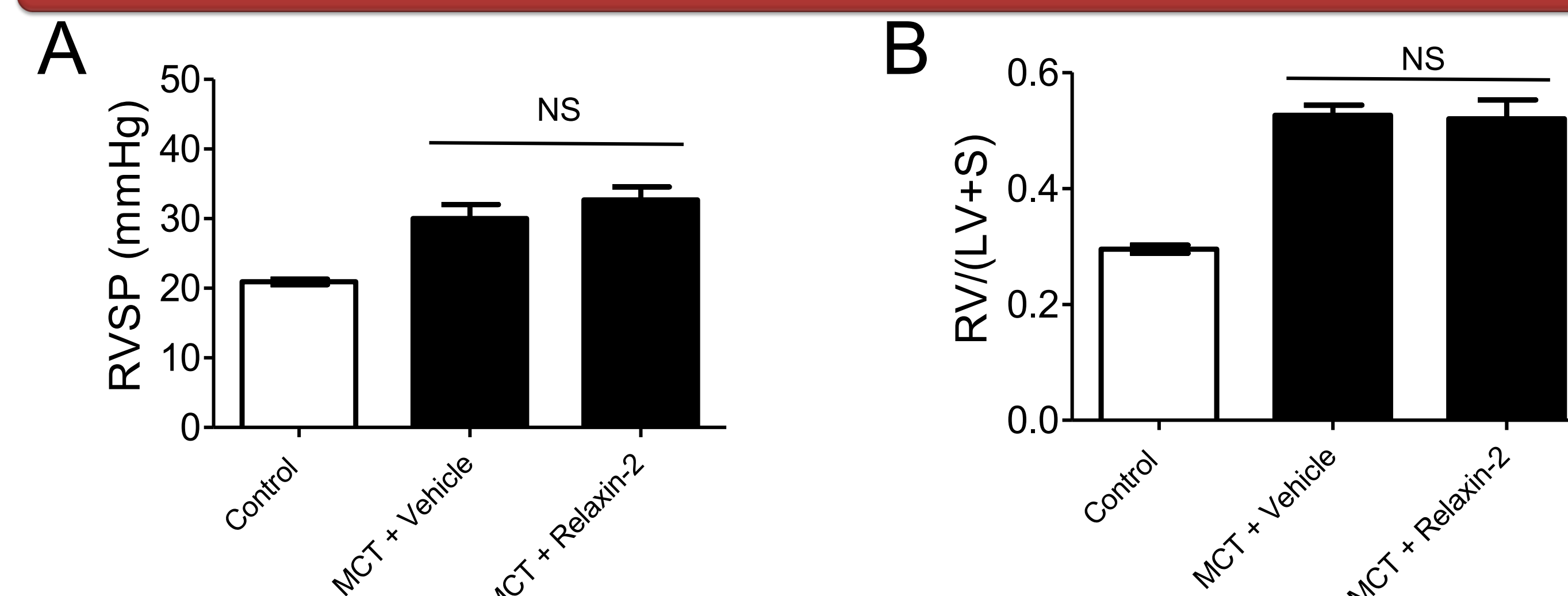## Relaxin-2 does not attenuate MCT-induced PAH

Figure 3. Monocrotaline induced PAH is not ameliorated by administration of relaxin-2. Administration of relaxin-2 (250 µg/kg/day S.C., 14 days, minipump) did not impact experimental PH induced by monocrotaline (MCT, 40 mg/kg, s.c., 21 days), as indicated by (A) right ventricular systolic pressure (RVSP) and (B) hypertrophy (RV/(LV+S), Fulton's index). Data are as mean ± SEM, n=4-8. NS = non-significant.

Chronic dosing of relaxin generates neutralizing antibodies
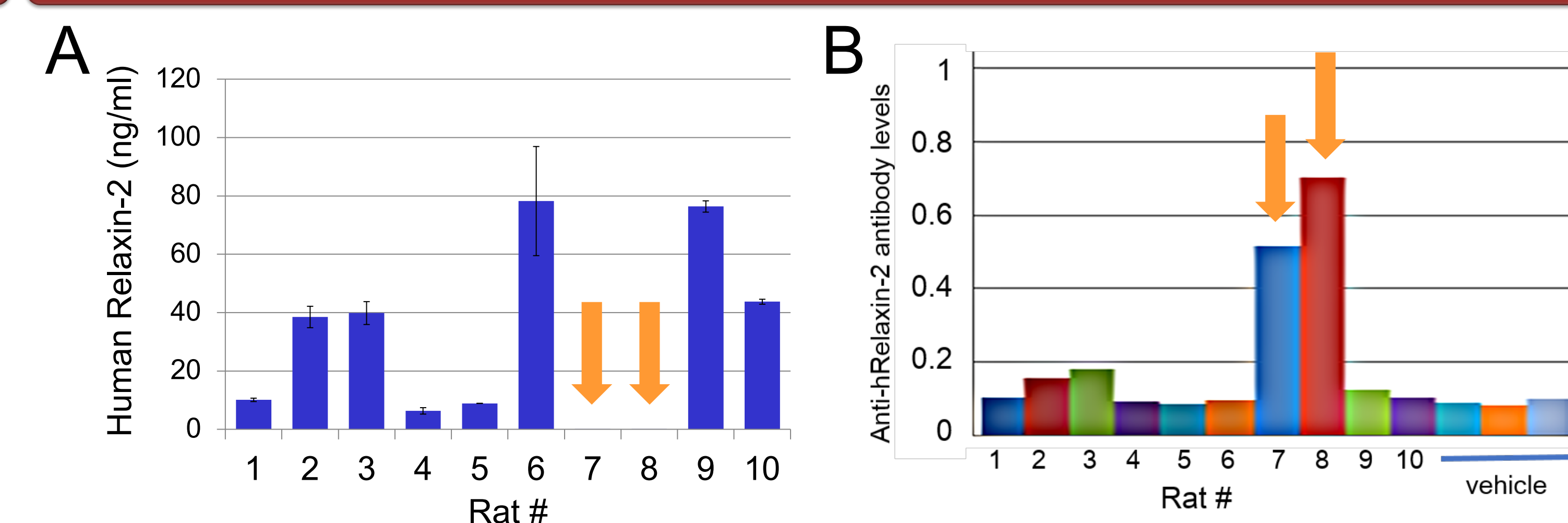

Figure 4. Potential immunogenicity of relaxin-2 peptide. Chronic administration of relaxin-2 resulted in variable plasma levels of relaxin after 14 days of continuous infusion, with several rats (#7, #8) having nearly undetectable levels. (B) Four of the ten rats tested after two weeks of administration had measurable titers of anti-relaxin antibodies.

ML290 attenuates PAH in humanized RXFP1 knock-in mice
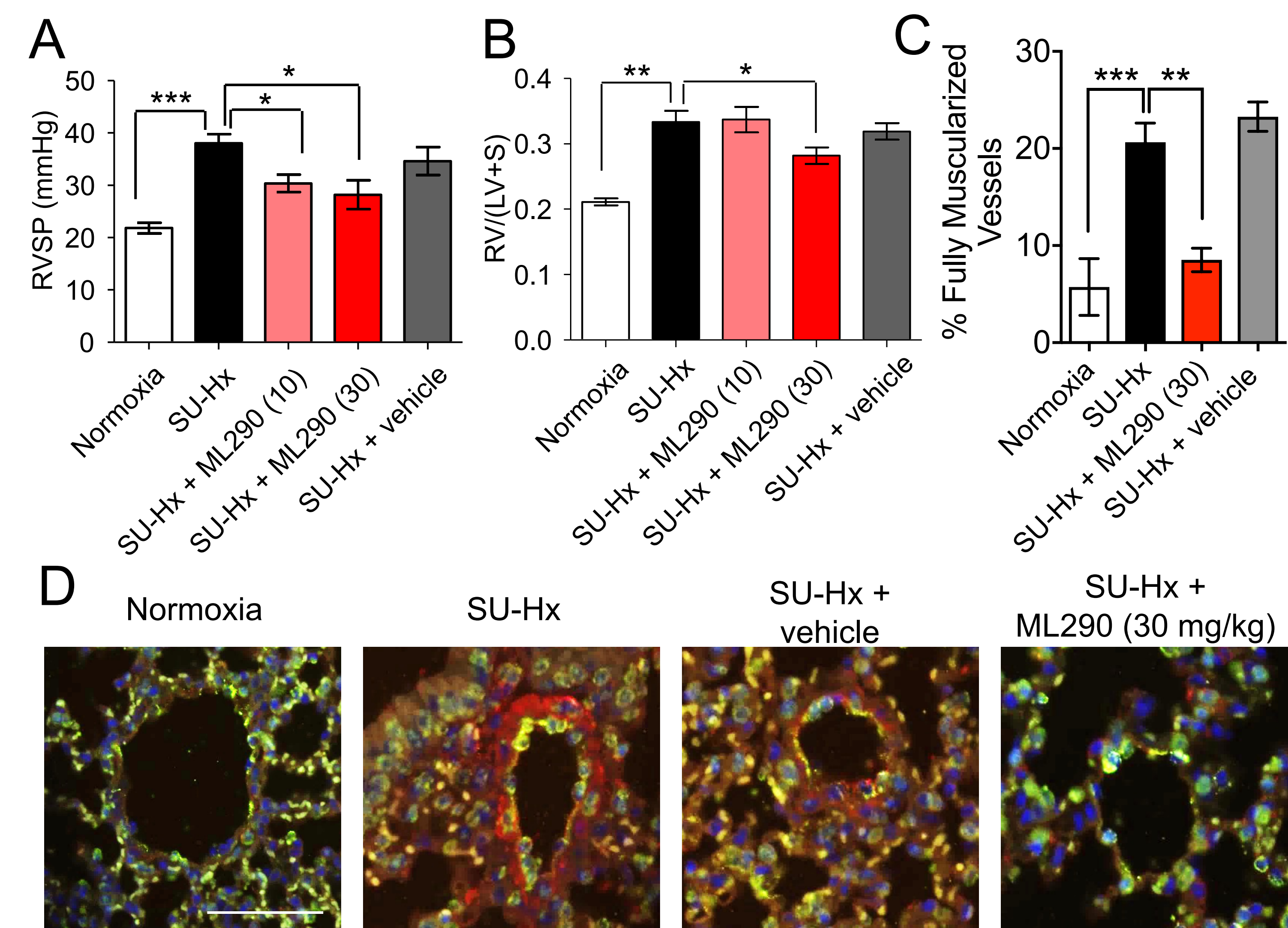

Figure 5. ML290 attenuates SU5416-hypoxia (SU-Hx) induced PAH in humanized RXFP1 knock-in mice.
Exposure to SU5416 (20 mg/kg weekly) and normobaric hypoxia (FIO$_2$ = 0.10) resulted in pulmonary hypertension. Treatment of SU-Hx rats with ML290 (10 or 30 mg/kg/d i.p.) attenuated the increase in (A) RVSP, (B) right ventricular hypertrophy and (C-D) pulmonary vascular remodeling caused by SU-Hx exposure, with representative photomicrographs of the pulmonary vasculature showing the endothelial marker vWF (green), smooth muscle marker αSMA (red) and nuclei (blue). bar = 50 µm; data are expressed as mean ± SEM, n=6-8, * p<0.05, **p<0.01, ***p<0.001.

Conclusions

Relaxin-RXFP1 signaling inhibits canonical TGFβ1-induced signaling and fibrogenic phenotypic plasticity. However, chronic administration of relaxin peptide *in vivo* does not improve experimental PH, and may be limited by immunogenicity. Activation of RXFP1 with small molecule allosteric agonist ML290 ameliorates experimental PH and vascular remodeling, and may represent an alternate strategy for therapeutic RXFP1 modulation.