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## Supplemental Information

# Reversal of Hypoxic Pulmonary Hypertension by Hypoxia-Inducible Overexpression of Angiotensin-(1-7) in Pulmonary Endothelial Cells

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Supplemental figures and legends

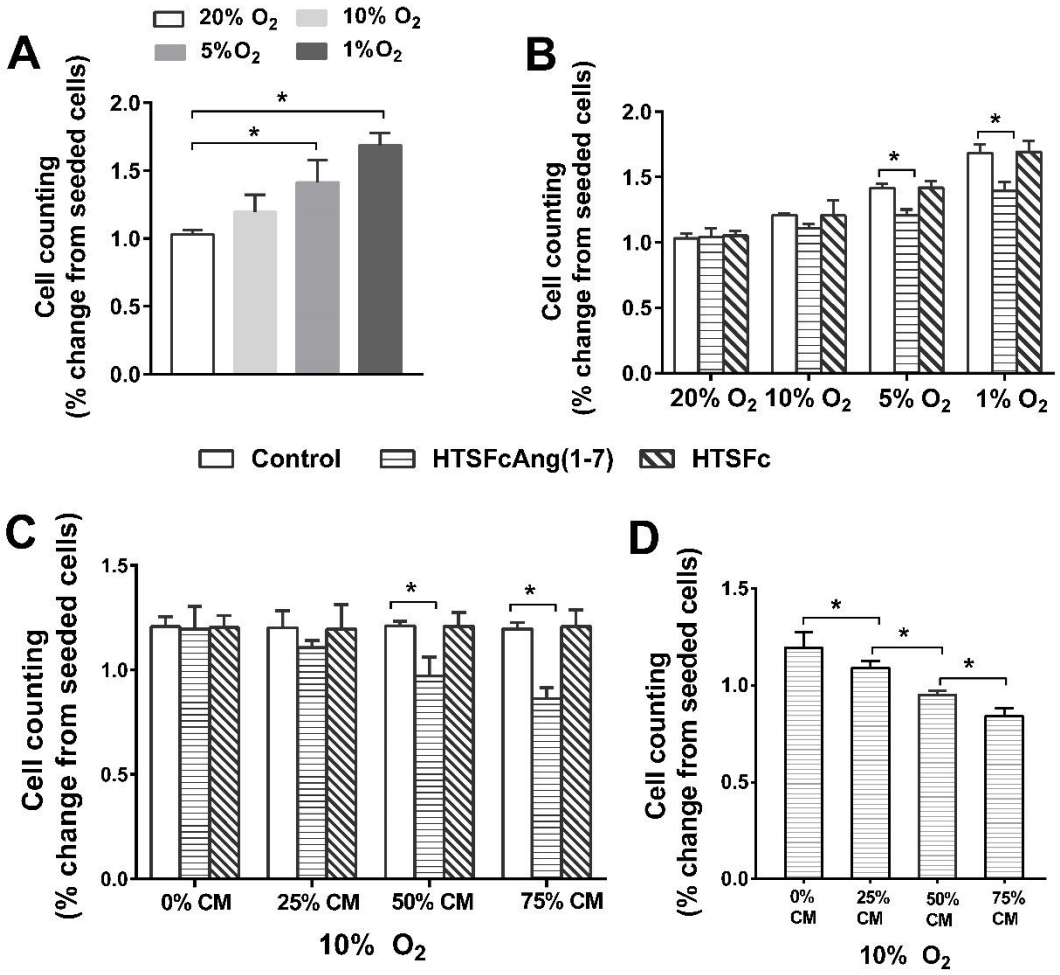


Figure S1. Effects of the condition medium from PMVECs transfected with HTSFcAng(1-7) on hypoxia-induced proliferation of primary PASMCs

PASMCs proliferation was measured by cell counting. (A) : Effects of oxygen concentration on the proliferation of rat primary PASMCs. (B - C) : Inhibition effects of the condition medium from PMVECs transfected with HTSFcAng(1-7) on the proliferation of PASMCs cultured in nomoxia (20% $O_2$) (B) and in hypoxia (10%, 5% and 1% $O_2$) (C). The inhibition showed an oxygen concentration-dependent effect manner. (D and E): Concentration (v/v)-dependent inhibition effects of the condition medium, which is from PMVECs transfected with HTSFcAng(1-7) on the

proliferation of PASMCs cultured in 10% $O_2$ concentration. CM: condition medium. * $P < 0.05$. Data are represented as mean ± SD and from three replicated experiments in each panel.

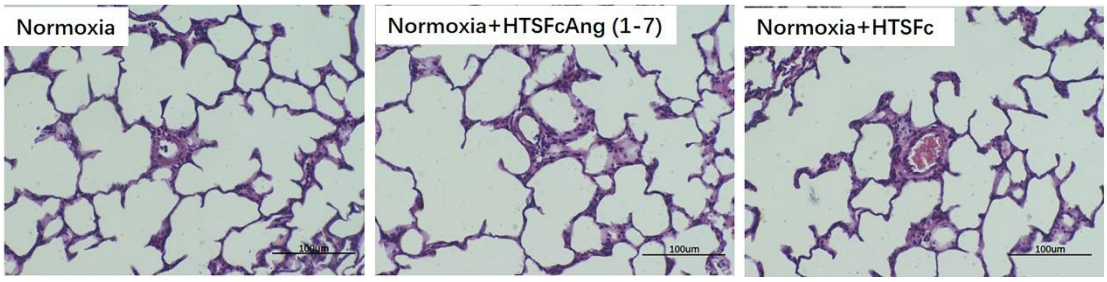


Figure S2. Effects of HTSFcAng(1-7) administration on the lung from rats in normoxia

Representative hematoxylin and eosin staining of the lungs from HTSFcAng (1-7) or HTSFc-administrated rats in normoxia. No obvious edematous change of alveolar walls, swelling of alveolar epithelial cells and massive polymorphonuclear infiltration were observed. The bar is 100 μM.

Table S1 Lung injury score

| Group | Edema | Neutrophil infiltration | Hemorrhage | Epithelial desquamation | Hyaline membranes |
|---|---|---|---|---|---|
| Normoxia | 0.3 ± 0.27 | 0.2± 0.33 | 0.4 ± 0.19 | 0.4 ± 0.43 | 0.0 ± 0.00 |
| Normoxia+ HTSFcAng(1-7) | 0.2 ± 0.37 | 0.4 ± 0.21 | 0.3 ± 0.18 | 0.5 ± 0.25 | 0.1 ± 0.12 |
| Normoxia+ HTSFc | 0.4 ± 0.47 | 0.3 ± 0.39 | 0.5 ± 0.27 | 0.2 ± 0.24 | 0.1 ± 0.15 |
| K-W | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ |

Note: K–W represents Kruskal-Wallis test of analysis of variance.

Supplemental Methods

PASMCs proliferation assay

PASMCs proliferation was measured by cell counting method as previously described[1]. The PASMCs were seeded in 24-well plates at a density of $5\times10^4$ cells /well in DEME supplemented with 10% fetal bovine serum in nomoxia (20% $O_2$). At 60 % confluence, the cells were starved in serum-free medium for 24h to synchronization. Then the cells were cultured in DMEM (containing 10% fetal bovine serum) and the freshly collected condition medium of PMVECs according to the corresponding concentration of condition medium (0%, 25%, 50%, 75%) (v/v) in hypoxia (10% $O_2$) for 24h. At the end of treatment, PASMCs were washed with phosphate buffered solution, harvested by mild trypsinization, and counted with a hematocytometer (QiuJin, Shanghai, China).

Lung histological assay

AAV9 particles packaged with HTSFcAng (1-7) or HTSFc ($3\times10^9$ transduction units) were given to rats in the normoxia condition by nasal drip to check the side effects of the virus. Animals were sacrificed, and the lungs were dissected into 3-mm-thick slices at the same part (the lower lobe of the right lungs) and fixed in 10% formaldehyde, subsequently were embedded in paraffin and sectioned into 4-μm-thick sections. Hematoxylin and eosin staining (HE) were performed and examined by light microscopy to evaluate alveolar and airway inflammation.

The lung injury was scored based on edema, neutrophil infiltration, hemorrhage, bronchiole epithelial desquamation and hyaline membrane formation as previously described[2]. A score scaled from 0 to 4 represents the severity:0 for no injury (a normal appearing lung), 1 for modest injury(limited congestion and interstitial edema but no

interstitial neutrophilic infiltrate with occasional red blood cells and neutrophils in the alveolar spaces), 2 for intermediate injury (mild congestion, interstitial edema, and interstitial neutrophilic infiltrate with occasional red blood cells and neutrophils in the alveolar spaces), 3for widespread injury (more prominent congestion and interstitial edema with neutrophils partially filling the alveolar spaces but with consolidation) and 4 for widespread injury (most prominent, marked congestion and interstitial edema with neutrophilic infiltrate nearly filling the alveolar spaces or with frank lung consolidation). The histological analysis was conducted as double-blind trials.

Statistical analysis

All values were presented as means ± SD. The statistical differences between groups were evaluated by one-way analysis of variance (ANOVA), followed by Dunnett's test for multiple comparisons. For the lung injury scores, a Kruskal – Wallis test was used to detect differences across the groups, and the Wilcoxon – Mann – Whitney test was used to detect differences between two groups. $P$ value $<0.05$ was considered statistically significant.

Reference:


1. Luo, Y, Xu, DQ, Dong, HY, Zhang, B, Liu, Y, Niu, W*, et al.* (2013). Tanshinone IIA inhibits hypoxia-induced pulmonary artery smooth muscle cell proliferation via Akt/Skp2/p27-associated pathway. *PLoS One* **8**: e56774.
2. Zhou, ZH, Sun, B, Lin, K, and Zhu, LW (2000). Prevention of rabbit acute lung injury by surfactant, inhaled nitric oxide, and pressure support ventilation. *American journal of respiratory and critical care medicine* **161**: 581-588.