

## SUPPLEMENTARY DATA

# **Galectin-3 Mediates Endothelial-to-Mesenchymal Transition in Pulmonary Arterial Hypertension**

**Tangzhiming Li<sup>1</sup>, Lihuang Zha<sup>1</sup>, Hui Luo<sup>1</sup>, Suqi Li<sup>2</sup>, Lin Zhao<sup>3</sup>, Jingni He<sup>1</sup>,  
Xiaohui Li<sup>4</sup>, Qiangqiang Qi<sup>1</sup>, Yuwei Liu<sup>1</sup>, ZaixinYu<sup>1</sup> \***

<sup>1</sup>Department of Cardiology, Xiangya Hospital, Central South University, Changsha, China

<sup>2</sup>State Key Laboratory of Cardiovascular Disease, Fu Wai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

<sup>3</sup>Centre for Pharmacology and Therapeutics, Division of Experimental Medicine, Imperial College London, Hammersmith Hospital, London W12 0NN, UK

<sup>4</sup>Department of Pharmacology, School of Pharmaceutical Sciences, Central South University, Changsha, China

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**Supplementary Table 1.** Gal-3 knockdown and overexpression sequences.

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**Gal-3 knockdown sequence (lentivirus)**

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CAATCATAGGCACAGTGAA

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**Gal-3 overexpression sequence (lentivirus)**

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ATGGCAGACAATTTTTTCGCTCCATGATGCGTTATCTGGGTCTGGAAACC  
CAAACCCTCAAGGATGGCCTGGCGCATGGGGGAACCAGCCTGCTGGGG  
CAGGGGGCTACCCAGGGGCTTCCTATCCTGGGGCCTACCCCGGGCAGG  
CACCCCCAGGGGCTTATCCTGGACAGGCACCTCCAGGCGCCTACCCTGG  
AGCACCTGGAGCTTATCCCGGAGCACCTGCACCTGGAGTCTACCCAGG  
GCCACCCAGCGGCCCTGGGGCCTACCCATCTTCTGGACAGCCAAGTGCC  
ACCGGAGCCTACCCTGCCACTGGCCCCTATGGCGCCCCTGCTGGGCCAC  
TGATTGTGCCTTATAACCTGCCTTTGCCTGGGGGAGTGGTGCCTCGCAT  
GCTGATAACAATTC TGGGCACGGTGAAGCCCAATGCAAACAGAATTGC  
TTAGATTTCCAAGAGGGAATGATGTTGCCTTCCACTTTAACCCACGC  
TTCAATGAGAACAACAGGAGAGTCATTGTTTGC AATACAAAGCTGGAT  
AATAACTGGGGAAGGGAAGAAAGACAGTCGGTTTTCCCATTTGAAAGT  
GGGAAACCATTCAAAAATACAAGTACTGGTTGAACCTGACCACTTCAAG  
GTTGCAGTGAATGATGCTCACTTGTGTCAGTACAATCATCGGGTTAAAA  
AACTCAATGAAATCAGCAAAC TGGGAATTTCTGGTGACATAGACCTCA  
CCAGTGCTTCATATACCATGATA

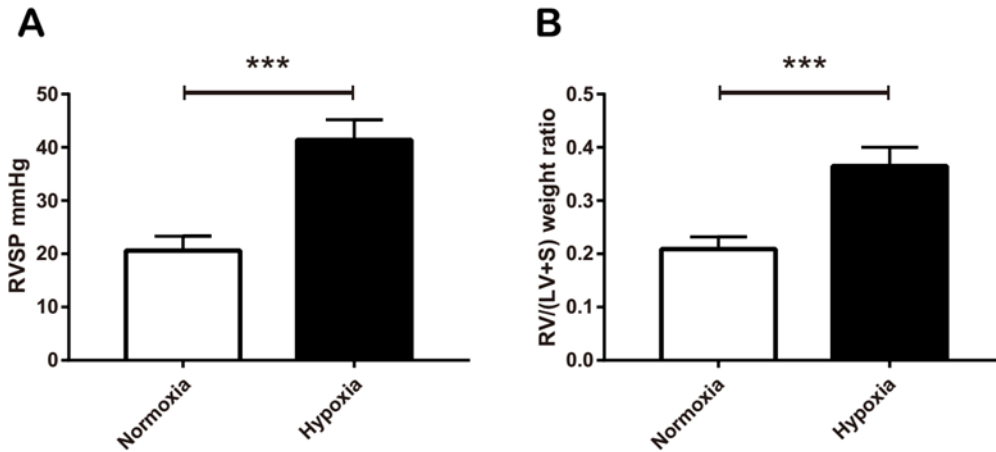
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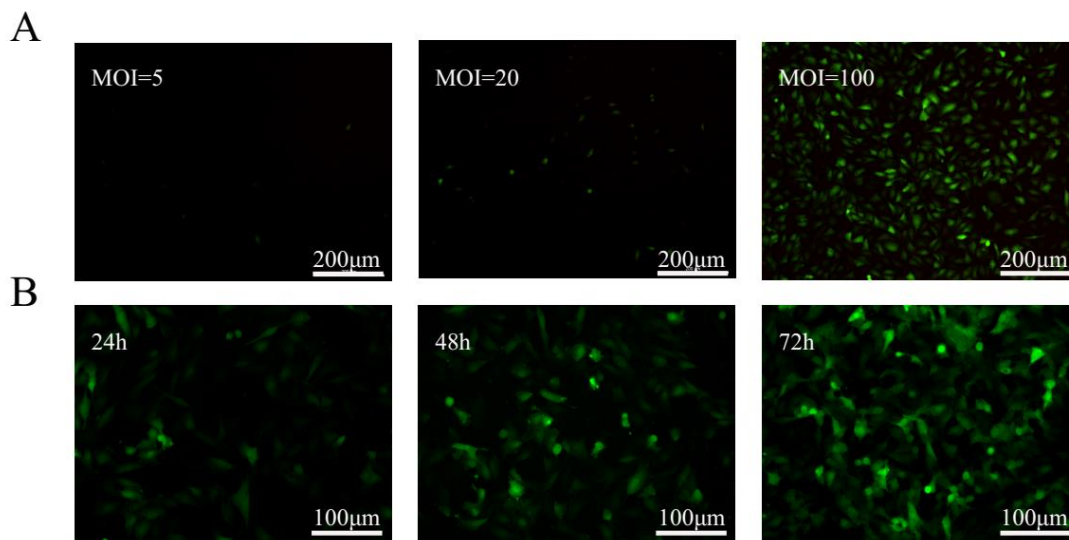
**Supplementary Table 2.** The primers for Quantitative Real-Time PCR were listed as followed

<b>Galectin 3</b>	Rat	<b>Former</b>	<b>5'-TGCTACTGGCCCCTTTGGTG-3'</b>
		<b>Reverse</b>	<b>5'-AAGTGGAAGGCGATGTCGTT-3'</b>
<b>GAPDH</b>	Rat	<b>Former</b>	<b>5'-CTCTACCCACGGCAAGTTC -3'</b>
		<b>Reverse</b>	<b>5'-GCCAGTAGACTCCACGACATA-3'</b>
<b>Slug</b>	human	<b>Former</b>	<b>5'-CATTAGAACTCACACGGGGGA-3'</b>
		<b>Reverse</b>	<b>5'-CGAGTAAACATTGATTGCGTCAC-3'</b>
<b>Snail</b>	human	<b>Former</b>	<b>5'-CCCTCAAGATGCACATCCGAA-3'</b>
		<b>Reverse</b>	<b>5'-GGCACTGGTACTTCTTGACAT-3'</b>
<b>Twist</b>	human	<b>Former</b>	<b>5'-GGCCGGAGACCTAGATGTCA-3'</b>
		<b>Reverse</b>	<b>5'-ATGCAGAGGTGTGAGGATGGTG-3'</b>
<b>Zeb-1</b>	human	<b>Former</b>	<b>5'-GATGATGAATGCGAGTCAGATGC-3'</b>
		<b>Reverse</b>	<b>5'-ACAGCAGTGTCTTGTTGTTGT-3'</b>
<b>IL-6</b>	human	<b>Former</b>	<b>5'-GGTACATCCTCGACGGCATC-3'</b>
		<b>Reverse</b>	<b>5'-GTCAGGGGTGGTTATTGCAT-3'</b>
<b>IL-8</b>	human	<b>Former</b>	<b>5'-TGAAACATGACTTCCAAGCTG-3'</b>
		<b>Reverse</b>	<b>5'-TTCCTGATTCTTGGATACCAC-3'</b>
<b>NF-κB</b>	human	<b>Former</b>	<b>5'-TGGCAGCTCTTCTCAAAGCA-3'</b>
		<b>Reverse</b>	<b>5'-AGCTGTTTCATGTCTCCTTGTGC-3'</b>
<b>β -Actin</b>	human	<b>Former</b>	<b>5'-ACCCAGATCATGTTTGAGACC -3'</b>
		<b>Reverse</b>	<b>5'-TCATGAGGTAGTCAGTCAGGT -3'</b>

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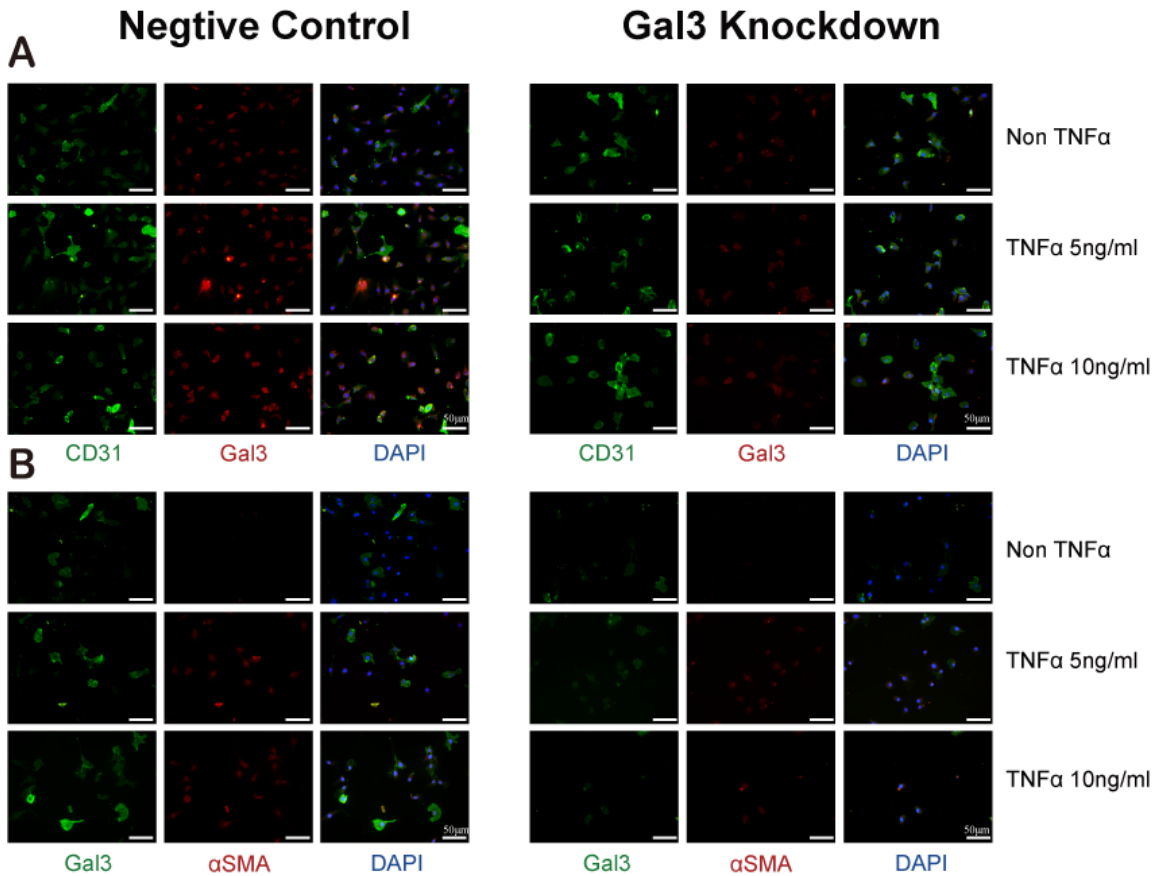


**Supplementary Figure 1. Hypoxia leads to rat PAH.** (A) Hemodynamic analysis between the normoxia and hypoxia group, (B) right ventricular hypertrophy (right ventricular/ (left ventricular + septum) (RV/LV+S)), ### indicate  $P < 0.001$ , compare to normoxia. Student's t-tests were used for comparisons of normoxia and hypoxia groups. Analyses performed by one-way ANOVA and Bonferroni post hoc.

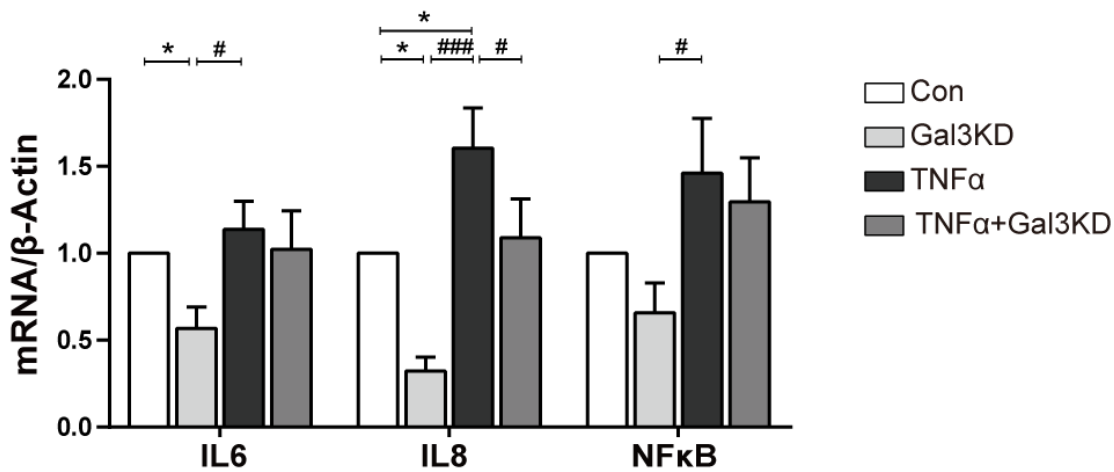


**Supplementary Figure 2. In vitro determination of lentivector titre and transfection time.** Cultured endothelial cells are seeded at  $5 \times 10^5$  on  $10 \text{ cm}^2$  plate and conducted viral vector transfection at 70 - 80% confluence. (A) endothelial cells transfected with different titre (MOI 5, 20, 100 respectively). Twenty-four hours after transduction, EGFP positive fluorescent cells were observed using fluorescent microscope (Olympus FV500, Japan) to evaluate proper MOI for cell transduction. (B) Assessment transduction efficiency of different lentivector incubation time (24, 48, 72 hours respectively) by density of fluorescent.

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**Supplementary Figure 3. Gal-3 siRNA partially reverse TNF $\alpha$  and Hypoxia leading to mesenchymal marker upregulation.** Endothelial cells hardly expressed  $\alpha$ SMA, while TNF $\alpha$  and Hypoxia enhanced  $\alpha$ SMA expression. Gal-3 SiRNA (right) decreased injury stimulus caused  $\alpha$ SMA upregulation.



**Supplementary Figure 4. Gal-3 siRNA partially inhibit TNF $\alpha$  induced inflammation cytokine.** Gal-3 SiRNA reduced IL-8 upregulation induced by TNF $\alpha$ , IL-6 and NF $\kappa$ B have slight downregulation (not significantly). n=3, \* indicate  $p < 0.05$  comparing Control group; #, and ### indicate  $p < 0.05$  and  $p < 0.001$  respectively, comparing the TNF $\alpha$  administrated group Student's t-test was used for comparing differences in normoxia and hypoxia groups. Analyses performed by one-way ANOVA and Bonferroni post hoc.