Supplementary Materials and methods

Hemodynamic Studies
At the end of the CH the rats were anesthetized by sodium pentobarbital (50 mg/kg i.p.) and placed on the operating table. The trachea was intubated, and the rats were ventilated with 1.5-ml tidal volume and 80 breaths /min in room air. A parasternal thoracotomy from the xiphoid to the pretracheal region was performed. A needle (O.D. 0.6 mm) connected to a silastic catheter was placed into the RV cavity. The catheter was connected to a pressure transducer. After stabilization for 3 min, the RV systolic and diastolic pressures were recorded during a 5 min period, using a polygraph recorder (RM6240 transducer polygraph; Chengdu Instrument Co., Chengdu, China).

Immunohistochemistry
Serial paraffin sections (4μm thick) were obtained on silanized slides. After deparaffinization and hydration with xylene and graded alcohols, the antigens were unmasked by pressurized heating for 2 min in a 0.01 mol/L citrate buffer at pH 6.0. The endogenous peroxidase activity was inhibited using 3% hydrogen peroxide for 10 min. After washing with phosphate-buffered saline (PBS), the sections were incubated overnight at 4 °C in humidified atmosphere with primary antibody: mouse-anti-rat α-SMA (1:200, Santa Cruz, CA, USA) for pulmonary artery smooth muscle cells; and rabbit anti-rat GHSR-1a (1:200, Alpha Diagnostic International, San Antonio, TX) for pulmonary vascular endothelial cells. Then polymer HRP IHC kit (DAKO, Glostrup, Denmark) was used for immunopositive cells following the manufacturer’s instructions. Finally, the sections were stained with 3, 3′-diaminobenzidine, and counterstained with hematoxylin. As negative controls, sections were incubated without the primary antibody. To examine medial thickening of small pulmonary arteries in response to CH, stainings were checked under light microscopy and photographed at a final magnification of ×200. Both the area of the α-SMA immunoactivity and lumen diameter (LD) of each small pulmonary artery were measured by using Image-Pro plus 5.0 image analysis. Each slide was measured under identical light conditions. Three slides from each lung were analyzed.
RNA isolation and preparation of cDNA

RNA quality and quantity were assessed using a spectrophotometer by measurement of $A_{260}/A_{280}$. RNA quality was confirmed by electrophoresis on a 1.2% agarose-0.66 M formaldehyde gel. One microgram of RNA was reverse transcribed to cDNA using a high-capacity cDNA archive kit (Applied Biosystems, Foster City, CA).

**PCR Array**

This PCR array was chosen because it includes 84 key genes representative of 18 different signal transduction pathways, including Mitogenic Pathway (n=4), Wnt Pathway (n=11), Hedgehog Pathway(n=7), TGF-β Pathway (n=4), Survival Pathway(n=11), p53 Pathway(n=7), Stress Pathway(n=6), NFκB Pathway(n=13), as well as other signal transduction pathway genes. Quantitative real-time reverse transcriptase PCR (qRT-PCR) amplification was performed with an Applied Biosystems ABI 7500 real-time PCR machine (Applied Biosystems, Foster City, CA) using the appropriate program described in the instructions manual of the RT²ProfilerPCR array kit. The data were analyzed using the SuperArray (now SABiosciences, Frederick, MD) PCR array data analysis software at [http://www.sabiosciences.com/pcr/arrayanalysis.php](http://www.sabiosciences.com/pcr/arrayanalysis.php).

**Wnt Pathway verification by qRT-PCR analysis**

To analyze gene expression at selected signal transduction pathway, qPCR was used with gene-specific primers. Primers were designed with Primer3 and IDT tools (http://fokker.wi.mit.edu/primer3/input.htm and http://www.idtdna.com/Scitools/Applications/Primerquest/). PCR was performed with an ABI 7500 real-time PCR machine using 25-µl reactions containing 2.5 ng of first strand cDNA, 0.5 µM forward and reverse primers, and 2×SYBR Green PCR mix (TaKaRa Biotechnology, Dalian, China). PCR reactions were performed in duplicate at 94°C for 2 min, 40 cycles at 94°C for 15 s, and 60°C for 60 s, then dissociated to verify a single amplicon. For each gene, standard curves were generated from a 10-fold serially diluted cDNA. The Ct value of each gene should be normalized to the levels of the housekeeping gene β-actin as an endogenous control, so the ΔCt value was defined as the absolute value of the difference between the Ct value of the detected genes and β-actin for each sample, and approved only when the slope of the standard curve for each targeting gene was parallel with the standard curve for the housekeeping gene (slope difference -0.1). Gene levels were expressed as fold-change, calculated using the $2^{-\Delta\Delta Ct}$ method with β-actin as the internal control.
Protein extraction and Western blot analysis

Lung tissues were homogenized on ice with lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China), according to the manufacturer's instructions. Each protein extract (20 µg) was loaded onto a 10% polyacrylamide gel and electrophoresed at 100 V at room temperature, and then transferred to nitrocellulose membranes (Immobilon-P; Millipore, Bedford, MA). Blots were probed with primary antibodies with gentle agitation overnight at 4°C, followed by secondary antibodies conjugated to horseradish peroxidase (HRP), and last developed with enhanced chemiluminescence (ECL) reagent and exposed to X-ray film. Primary Antibodies include: β-catenin, glycogen synthase kinase (GSK)3β, phospho-GSK3β (ser9) (p-GSK3β), phospho-Akt (ser473) (p-AKT) (1:2,000, 1:2,000, 1:1,000, 1:1,000 respectively; from Cell Signaling Technology, Beverly, MA), and GHSR-1a (1:500, Alpha Diagnostic International, San Antonio, TX). Optical density of the films was quantified by the free software (Image J 1.4) and calculated as a value of ID. Data were converted to relative units with β-actin (1:2000, Sigma Chemical Co., St. Louis, MO) designated as 1. To ensure reproducibility of results, each sample was performed at least twice.