# Supplemental Materials

# Methods

***Model induction and functional analysis***

The surgical procedure for inducing chronic pulmonary hypertension has been reported elsewhere(1,2). Briefly, animals underwent left lateral thoracotomy at the fifth intercostal space under isoflurane anesthesia. The left superior pulmonary vein and the common inferior veins were carefully dissected and banded with umbilical tapes to a fixed diameter (radius: 1.5mm). The chest was then closed and the animals were recovered. Intravenous furosemide (4 mg/kg) was administered to prevent acute lung congestion.

Functional analysis included echocardiography and catheter-based hemodynamic measurements. The echocardiography was conducted under propofol anesthesia, and two-dimensional as well as Doppler images were stored for both right ventricle and left ventricle images. For the hemodynamic assessment, a Swan-Ganz catheter was inserted to measure pulmonary arterial (PA) wedge pressure, pulmonary arterial pressure, right ventricular pressure and the right atrial pressure. Thermodilution method was used to obtain cardiac output (CO). Pulmonary vascular resistance index (PVRI) was calculated as

(mean PA pressure – mean PA wedge pressure) / (CO/body surface area).

***Vector and dye delivery***

Echocardiographic pulmonary vein velocity before gene therapy was used for stratified randomization, so that the animals have equal disease model presentation. Animals were intubated with standard tracheal tube (6.5-7.5Fr, Mallinckrodt) and ventilated using veterinary ventilator (SurgiVet, CDS 9000, Smith medical). Position of the tracheal tube tip (4-5cm proximal of tracheal bifurcation) was confirmed by fluoroscopy before gene delivery. For determining the distribution of injectates, Evans blue solution (2%) was either nebulized or delivered using a MicroSprayer® Aerosolizer (Model IA-1B, Penn-Century, Inc.). The pigs were ventilated for 30 minutes after the completion of the delivery and were euthanized to examine the dye distribution. For the gene therapy arm, adeno-associated virus (AAV)-1 encoding sarco/endoplasmic reticulum $Ca^{2+}$-ATPase (SERCA2a) or same amount of saline was delivered through the airway using a

nebulizer. A clinically used nebulizer (Aeroneb Solo, Aerogen) was connected to the tracheal tube with a filter to prevent the vector backflow into the ventilation system. A total of $2.0x10^{13}$ of AAV-1 .SERCA2a in 3ml solution was nebulized and flushed with 2 ml of saline nebulization. The animals were ventilated with ~3 mmHg of positive end-expiratory pressure for 15 minutes after the end of nebulization delivery.

***Post-mortem tissue processing***

After euthanasia, the lung was immediately explanted and perfused with cold lactate linger through the pulmonary veins. Lung tissues from the peripheral part of the left and right lower lobes were collected for molecular (snap frozen) and histological (formalin) analyses. Amount of vector genome in the lung tissues was analyzed using real time qPCR using an in-house AAV reference standard. For histology, lung tissue samples were placed in 10% formaldehyde solution. Formalin-fixed tissues were embedded in paraffin, and sectioned into 8 mm thick sections and mounted on positively charged microscope slides. The slides were stained with hematoxylin and eosin. We measured randomly identified 20-30 peripheral pulmonary arteries in each lower lobes of both lungs by a microscope **(**LSM 880; Carl Zeiss Microscopy, Jena, Germany). Then medial wall thickness and external diameter were analyzed using ImageJ software (National Institutes of Health).

The percentage of medial wall thickness (MT%) was measured and calculated as: MT%=([Wall thickness×2]/external diameter)×100.

**Statistical analysis**

Data are showed as mean ± standard deviation. The unpaired-Student T test was used to compare difference between 2 groups. ANOVA was used to compare the difference between three groups with correction for multiple comparisons. Kaplan-Meier curve was drawn for survival with a Cox regression analysis.

**Supplemental data**

| Supplemental Table 1. Echocardiographic parameters before gene therapy | | | |
|---|---|---|---|
| | Saline (n=7) | AAV1.SERCA2a (n=6) | p value |
| Body weight (Kg) | 13.1 ± 2.9 | 13.1 ± 3.2 | 1 |
| Pulmonary vein velocity (cm/s) | 220 ± 31 | 208 ± 15 | 0.31 |
| PA acceralation time (ms) | 76 ± 36 | 102 ± 55 | 0.77 |
| PA VTI (cm) | 18.2 ± 3.0 | 18.5 ± 3.1 | 0.32 |
| TAPSE (mm) | 12.2 ± 5.7 | 9.8 ± 6.6 | 0.97 |
| PA= pulmonary artery, TAPSE= tricuspid annular plane systolic expcursion, VTI= velocity time integral | | | |

Pulmonary vein velocity was used for stratified randomization to include similar degree of PH in both groups.

| Supplemental Table2. Echocardiographic and hemodynamic parameters 2 months after gene therapy | | | |
|---|---|---|---|
| | Saline (n=4) | AAV1.SERCA2a (n=4) | p value |
| Body weight (Kg) | 15.0 ± 4.8 | 20.0 ± 5.0 | 1 |
| PA acceralation time (ms) | 69 ± 20 | 81 ± 34 | 0.77 |
| PA VTI (cm) | 14.8 ± 5.4 | 20.1 ± 1.8 | 0.32 |
| TAPSE (mm) | 12.9 ± 3.1 | 16.6 ± 1.7 | 0.97 |
| Heart rate (bpm) | 97 ± 36 | 85 ± 18 | 0.58 |
| Cardiac index (L/min/m2) | 4.1 ± 1.9 | 6.3 ± 0.9 | 0.08 |
| Systolic PA presssure (mmHg) | 62 ± 37 | 48 ± 7 | 0.53 |
| Mean PA pressure (mmHg) | 45 ± 28 | 36 ± 6 | 0.55 |
| mean aortic pressure (mmHg) | 54 ± 14 | 83 ± 28 | 0.11 |
| PA= pulmonary artery, TAPSE= tricuspid annular plane systolic expcursion, VTI= velocity time integral | | | |

Due to the hemodynamic instability in a portion of the animals in the saline treated group, there was a wide variability in pressure parameters in saline group.

## Supplemental references


1. Aguero J, Hadri L, Hammoudi N, Leonardson L, Hajjar RJ, Ishikawa K. Inhaled Gene Transfer for Pulmonary Circulation. Methods Mol Biol 2017;1521:339-349.
2. Aguero J, Ishikawa K, Hadri L et al. Intratracheal Gene Delivery of SERCA2a Ameliorates Chronic Post-Capillary Pulmonary Hypertension: A Large Animal Model. J Am Coll Cardiol 2016;67:2032-46.