

Alveolar Stretch Activation of Endothelial Piezo1 Protects Adherens Junctions and  
Lung Vascular Barrier

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Online Data Supplement

## Supplemental methods

Reagents and antibodies. We obtained primary antibodies against VE-Cadherin (ab33168), p-VE-Cadherin Y685 (ab119785), Na<sup>+</sup>-K<sup>+</sup>-ATPase (ab76020) from abcam; Src (2109S), p-Src(6943S) from Cell Signaling; Piezo1 (15939-1-AP), GAPDH (60004) from Proteintech; p-VE-Cadherin Y731 (SAB4301448) from Sigma; CD31 (M0823) from Dako; beta-actin (sc47778) from Santa Cruz. Evan Blue Dye (E2129-10G), Yoda1 (SML-2558), A23187 (C7522) were purchased from Sigma; GsmTx-4 (STG-100) from Alomone labs; PP2 (529573) from Calbiochem; PD150606 (1269) from TORCIS. Pierce BCA protein Assay Kit (23225) and Subcellular Protein Fractionation Kit (78840) were obtained from Thermo Scientific; Calpain Activity Assay Kit (ab65308) from abcam. Piezo1SiRNA (ON-TARGETplus SMARTpool) was purchased from Dharmacon.

*Immunoblotting.* Homogenized lung tissue or cells were lysed with RIPA buffer and proteins were separated by SDS-PAGE gel electrophoresis and transferred onto membranes (Bio-Rad). Blots were developed using secondary antibodies conjugated with horseradish peroxidase and chemiluminescence using the Western blot detection kit.

*Microscopy.* HLMVECs subjected to 18% CS for 30min were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, and stained using indirect immunofluorescence. Fluorescent images of ECs were collected using LSM880 confocal microscope (Zeiss).

*Human samples.* Lung tissue samples were collected from patients admitted to Zhongshan Hospital Fudan University, Shanghai, PRC with lung cancer who needed a surgery (SMV group) or from organ donors after brain death whose lungs were not deemed suitable for lung transplantation (LMV group). Any history of pulmonary diseases or recently diagnosed lung inflammation met exclusion criteria. Data on demographic characteristics, diagnosis, and duration of mechanical ventilation are provided in Supplemental Table E1. All patients were recruited into the study under informed consent guidelines approved by the Ethics Committee of Zhongshan Hospital, Fudan University.

## Supplemental table and figures

Supplemental Table E1. Demographic data of human samples. Corresponds with Figures 6. Human lung samples were collected from 4 patients undergone lobectomy for lung cancer and 3 organ donors whose lung was deemed not suitable for transplantation or could not match a lung transplantation recipient within the timeframe of lung preservation. \*Carrico index was measured at timepoint of sample collection.

## Supplemental Figure Legends

Supplemental Figure E1. High volume mechanical ventilation elicits augmented lung microvascular hyper-permeability in *Piezo1<sup>iEC-/-</sup>* and pharmacological Piezo1-inhibited mice. Corresponds with Figure 1. A-B. *Piezo1<sup>fl/fl</sup>* and *Piezo1<sup>iEC-/-</sup>* mice were mechanically ventilated with  $V_t$  of 20 ml/kg (A) and 60 ml/kg (B) for 2 hours. Pulmonary transvascular albumin permeability induced by HVMV is shown for each group. The data points depicting individual mice; n=4-5. \* $P < 0.05$  by ANOVA. C. Representative photographs of EBA leakage in lungs in A-B. D. Corresponds with Figures 1. *Piezo2<sup>fl/fl</sup>* and *Piezo2<sup>iEC-/-</sup>* mice were mechanical ventilated with  $V_t$  of 40 ml/kg for 2hrs. Pulmonary transvascular albumin permeability for each group is shown. The data points depicting individual mice; n=3-4. *Piezo2<sup>fl/fl</sup>* and *Piezo2<sup>iEC-/-</sup>* mice shown no significant difference in lung capillary permeability in response to HVMV. E-F. GsmTx-4 (1mg/kg) was intravenously injected prior to HVMV. Albumin extravasation (E) and wet/dry ratio (F) as the measurements of lung microvascular permeability and

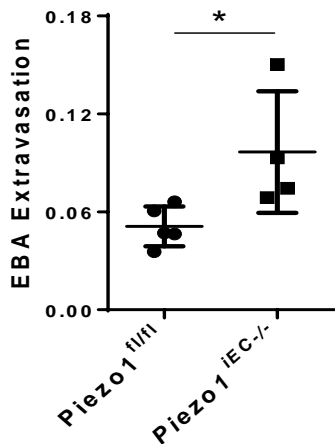
pulmonary edema. The data points depicting individual mice; n=3-4. \* $P<0.05$  and \*\* $P<0.01$  by ANOVA.

Supplemental Figure E2. Genetic depletion of *Piezo1* in human lung endothelium. Corresponds with Figure 2. Western blot analysis of Piezo1 in HLMVECs transfected with control or *Piezo1* siRNA. The arrowhead indicates Piezo1.

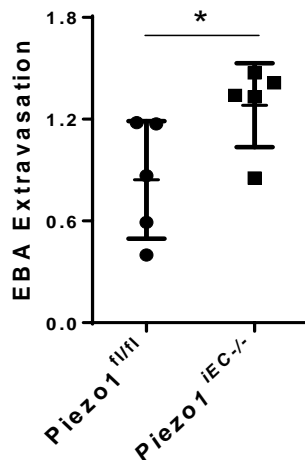
Supplemental Figure E3. Depletion of extracellular  $Ca^{2+}$  increases CS-induced phosphorylation of VE-Cadherin and Src. Corresponds with Figure 4. WB analysis of VE-cadherin and Src phosphorylation in HLMVEC monolayers treated with 5mM EDTA or vehicle control and exposed to 18%CS for 30 min. Chelation of extracellular calcium augments phosphorylation of both VE-cadherin and Src in HLMVEC monolayers exposed to 18%CS for 30 min.

No.	Sex	Age (y)	Primary Diagnosis	Mechanical Ventilation Duration	Carrico index (mmHg)*
<b>Lung surgery controls</b>					
1	Male	68	Lung Adenocarcinoma, Grade II	35 min	475
2	Male	55	Lung Adenocarcinoma, Grade I	30 min	587
3	Male	60	Lung Adenocarcinoma, Grade II	42 min	572
4	Female	47	Lung Adenocarcinoma, Grade I	51 min	397
<b>Organ donors</b>					
1	Male	52	Traumatic brain injury	108 hrs	190
2	Male	47	Spontaneous intracranial hemorrhage	38 hrs	375
3	Male	39	Spontaneous intracranial hemorrhage	252 hrs	154

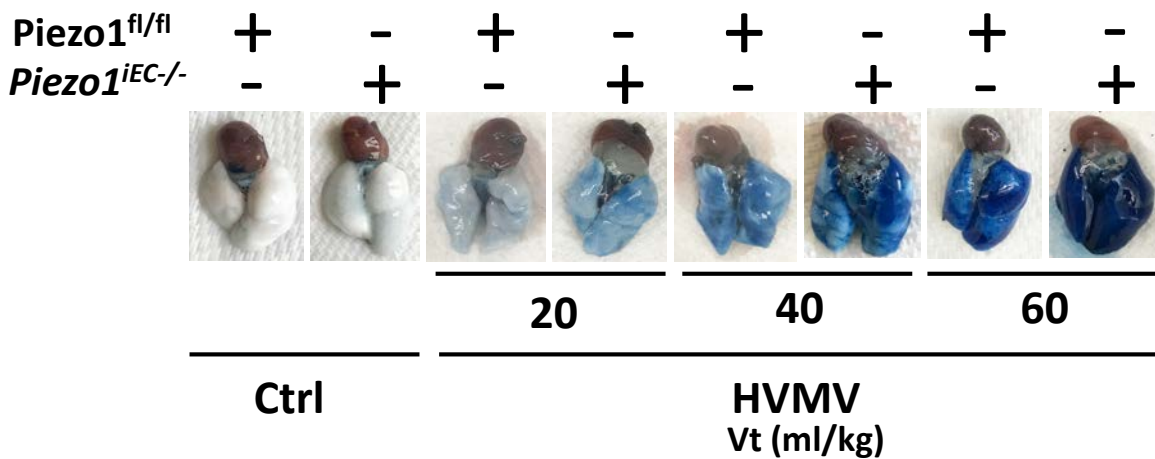
**A**  
HVMV  
( Vt: 20ml/kg)



**B**  
HVMV  
( Vt: 60ml/kg)

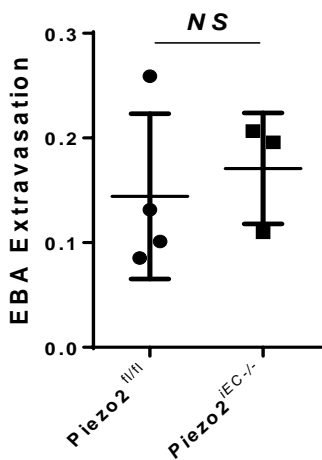


**C**

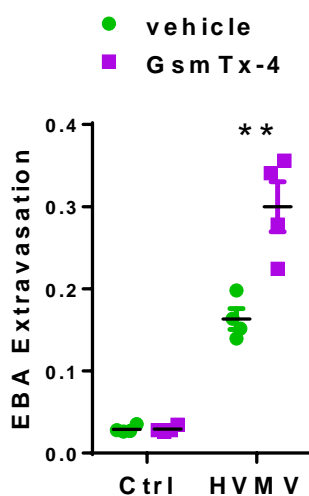


**D**

HVMV  
( Vt: 40ml/kg)



**E**



**F**

