**Supplemental Digital Content 1**

**Additional Methods**

*Protocol for PaCO2-matched experiments*

To exclude the potential effects of a mismatch in PaCO2 between high-power and low-power groups on the expression of biomarkers of inflammation, 10 additional animals were ventilated with high mechanical power and low as well as high VT, but with the dead space adapted to yield normocapnia.

Ten Wistar rats (weight 386±37g) were anesthetized by inhalation of sevoflurane 2% (Sevorane®; Cristália, Itapira, SP, Brazil) and underwent intratracheal (i.t.) instillation of *Escherichia coli* lipopolysaccharide (O55:B5, LPS Ultrapure, Invivogen), 200 µg suspended in saline solution to a total volume of 200 L1, to induce mild-to-moderate ARDS. After 24 h, animals were premedicated intraperitoneally (i.p.) with 10 mg/kg diazepam (Compaz, Cristália, Itapira, SP, Brazil), followed by 100 mg/kg ketamine (Ketamin-S+, Cristália, Itapira, SP, Brazil) and 2 mg/kg midazolam (Dormicum, União Química, São Paulo, SP, Brazil). After local anesthesia with 2% lidocaine (0.4 mL), a midline neck incision and tracheostomy were performed.

An intravenous (i.v.) catheter (Jelco 24G, Becton, Dickinson and Company, New Jersey, NJ, USA) was inserted into the tail vein, and anesthesia induced and maintained with midazolam (2 mg/kg/h) and ketamine (50 mg/kg/h). Additionally, 10 mL/kg/h Ringer’s lactate (B. Braun, Crissier, Switzerland) was administered i.v., and Gelafundin® (B. Braun, São Gonçalo, RJ, Brazil) was infused i.v. in 0.5-mL boluses to maintain mean arterial pressure (MAP)>70 mmHg. A second catheter (18G, Arrow International, USA) was then placed in the right internal carotid artery for blood sampling and arterial blood gas analysis (Radiometer ABL80 FLEX, Copenhagen NV, Denmark), as well as monitoring of MAP (Networked Multiparameter Veterinary Monitor LifeWindow 6000 V; Digicare Animal Health, Boynton Beach, FL, USA). A 30-cm-long water-filled catheter (PE-205, Becton, Dickinson and Company) with side holes at the tip, connected to a differential pressure transducer (UT-PL-400, SCIREQ, Montreal, QC, Canada), was used to measure the esophageal pressure (Pes). The catheter was passed into the stomach and then slowly returned into the esophagus; its proper positioning was assessed with the “occlusion test”2. Heart rate (HR), MAP, and rectal temperature were continuously monitored (Networked Multiparameter Veterinary Monitor LifeWindow 6000V, Digicare Animal Health, Florida, USA). Body temperature was maintained at 37.5±1°C using a heating bed.

Animals were paralyzed with pancuronium bromide (2 mg/kg, i.v.) and their lungs mechanically ventilated (Servo-i, MAQUET, Solna, Sweden) in volume-controlled mode (VCV), with VT = 6 mL/kg, RR to maintain normocapnia (PaCO2 = 35-45 mmHg), PEEP = 3 cmH2O, and FiO2 = 1.0 (Fig. 1). After 5 minutes, arterial blood gases (300 µl) were determined using a Radiometer ABL80 FLEX (Copenhagen NV, Denmark) (INITIAL). FiO2 was reduced to 0.4 to prevent possible iatrogenic effects, and lung mechanics were assessed. Rats were then assigned to two groups of low (6 mL/kg) or high (11 mL/kg) VT at high mechanical power (n=8/group): high power/low VT, CO2-matched; or high power/high VT, CO2-matched. PaCO2 matching was achieved by serial, incremental increases in dead space by placing polystyrene tubes between the Y-piece and the pneumotachograph. The PaCO2 target was set according to the low mechanical power groups described in the main text. Settings were kept for 2 hours. Heparin (1000 IU) was injected into the tail vein. Animals were euthanized by overdose of sodium thiopental (150 mg/kg i.v.), and their lungs extracted at PEEP = 3 cmH2O for histology and molecular biology analysis.

Lung histology1,3-5, as well as biological markers associated with inflammation, alveolar stretch, and epithelial and endothelial cell damage,6 were analyzed as described in the main text.

**References**

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