

## Supplementary Digital Content

### **Extracellular histones play an inflammatory role in acid aspiration-induced acute respiratory distress syndrome**

Yanlin Zhang, Ph.D., M.D.<sup>1\*</sup>

Zongmei Wen, Ph.D., M.D.<sup>2\*</sup>

Li Guan, Ph.D.<sup>1</sup>

Ping Jiang, M.D.<sup>3</sup>

Tao Gu, M.D.<sup>4</sup>

Jinyuan Zhao, Ph.D., M.D.<sup>1</sup>

Xin Lv, Ph.D., M.D.<sup>2</sup>

Tao Wen, Ph.D.<sup>5#</sup>

1. Research Center of Occupational Medicine, Peking University Third Hospital, Beijing 100083, P.R. China

2. Department of Anesthesiology, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai 200433, P.R. China

3. Department of Emergency, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai 200433, P.R. China

4. Department of Oncology, First Hospital of Qinhuangdao, Qinhuangdao 066000, Hebei Province, P.R.China

5. Beijing Institute of Hepatology, Beijing Youan Hospital affiliated with Capital Medical University, Beijing 100069, P.R.China

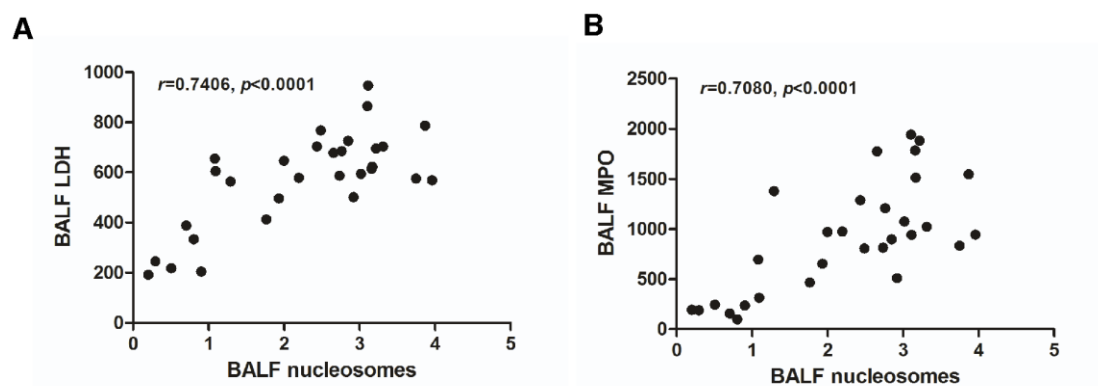
## **Methods**

### **Assay for binding of heparin to histones**

We collected blood samples from acid aspiration-treated mice at 6 h and centrifuged to separate plasma. Then, we incubated the plasma with heparin-sepharose solution (Shanghai Jiangfeng Biocompany, Nanjing, China) at a ratio of 1:1 at room temperature for 1 h. After incubation, the samples were centrifuged at  $3,000 \times g$  at  $4^{\circ}\text{C}$  for 10 min to obtain supernatant. We assayed histone (nucleosome) levels and IL- $1\beta$ , IL-6, IL-10, and TNF- $\alpha$  levels in the plasma before incubation and after incubation, respectively. IL- $1\beta$ =interlukin- $1\beta$ ; IL-6=interlukin-6; IL-10=interlukin-10; TNF- $\alpha$ =tumor necrosis factor- $\alpha$ .

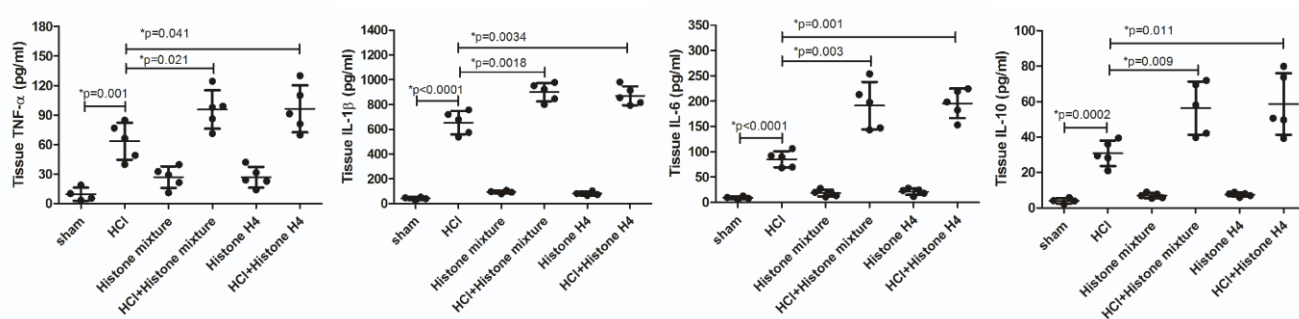
## Supplementary Figures

### Supplementary Figure 1



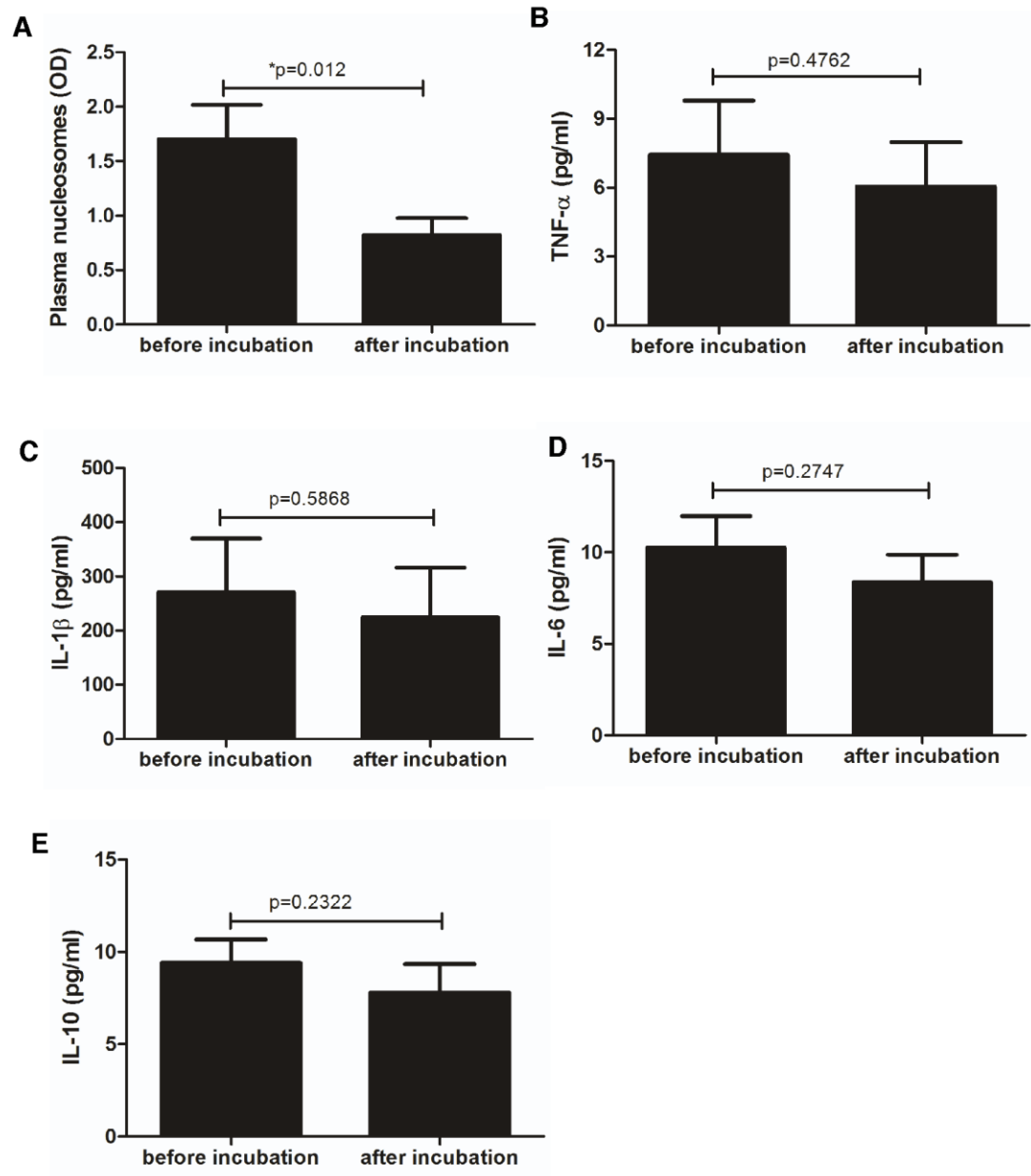
**Figure 1. Elevated BALF nucleosomes correlated positively with BALF LDH, and myeloperoxidase (MPO) activity, respectively.** There was a significant correlation of both BALF (A) LDH and (B) Myeloperoxidase (MPO) with BALF nucleosomes ( $*p<0.001$ ). BALF=bronchoalveolar lavage fluid; LDH= lactate dehydrogenase. MPO=myeloperoxidase.

### Supplementary Figure 2



**Figure 2. Tissue TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-10 levels were all notably increased in histones+HCl groups as compared with HCl-treated mice (mean  $\pm$  SD).** HCl=hydrochloric acid; IL-1 $\beta$ =interlukin-1 $\beta$ ; IL-6=interlukin-6; IL-10=interlukin-10; TNF- $\alpha$ =tumor necrosis factor- $\alpha$ .

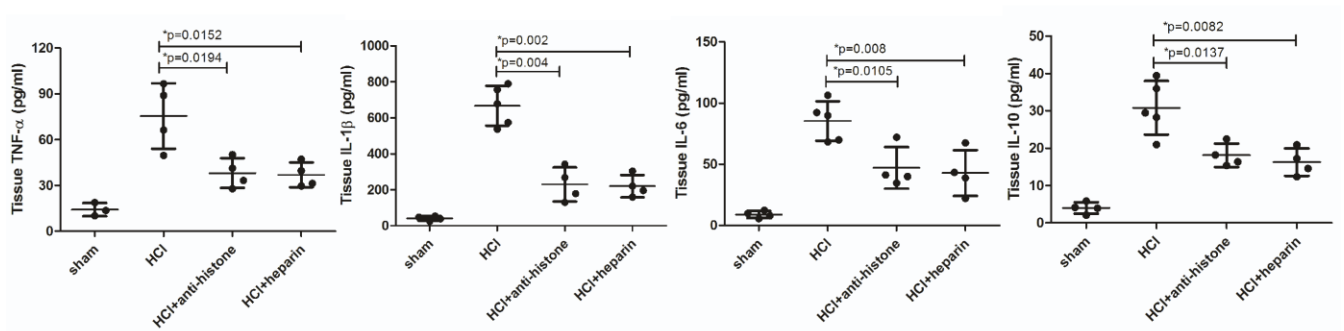
### Supplementary Figure 3



**Figure 3. Assay for the binding of heparin to histones.** It showed that heparin specifically binds to histones, because (A) after incubation with heparin-sepharose solution, the levels of nucleosome in plasma of HCl-treated mice were decreased significantly as compared with the plasma before incubation (\* $p=0.012$ ). However,

there was no statistical difference in the levels of the cytokines including (B) TNF- $\alpha$  (C) IL-1 $\beta$  (D) IL-6 and (E) IL-10 between before and after incubation groups. HCl=hydrochloric acid; IL-1 $\beta$ =interlukin-1 $\beta$ ; IL-6=interlukin-6; IL-10=interlukin-10; TNF- $\alpha$ =tumor necrosis factor- $\alpha$ .

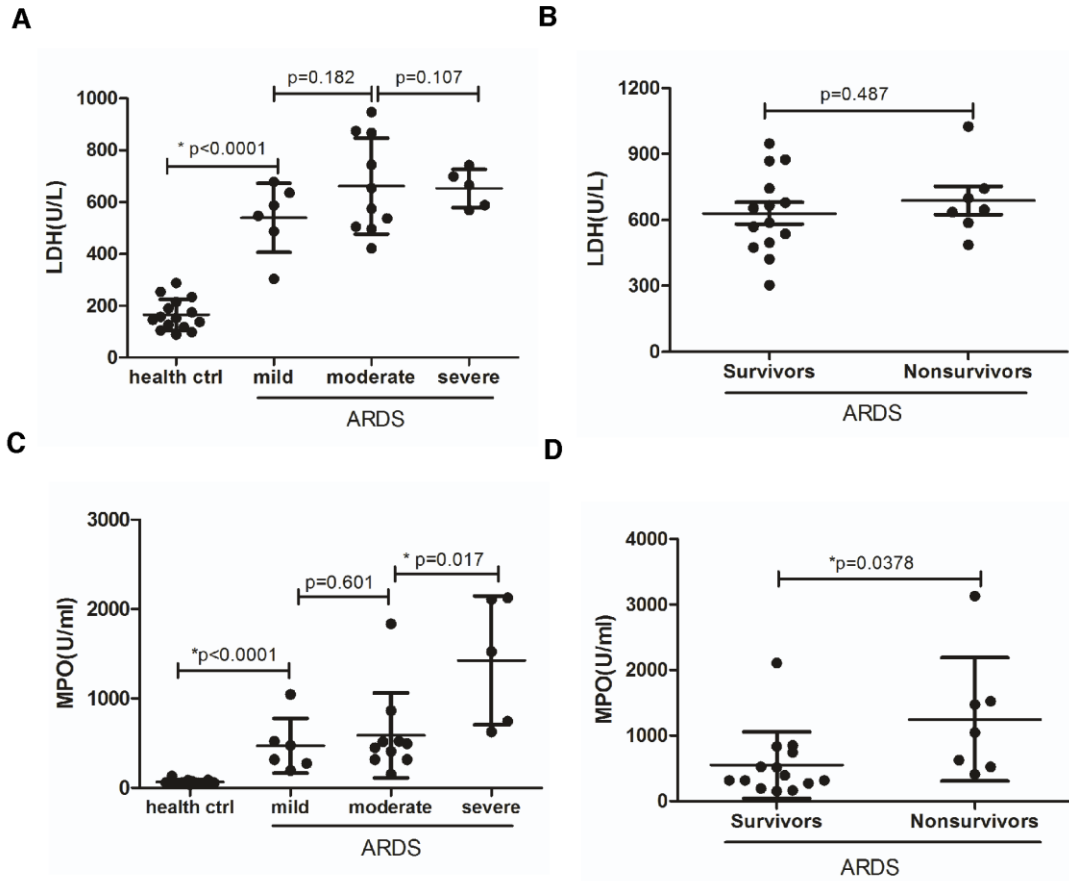
Supplementary Figure 4



**S Figure 4.** Tissue levels of TNF- $\alpha$ , IL-1, IL-6, and IL-10 levels were reduced remarkably with blockade of extracellular histones by anti-histone antibody or heparin.

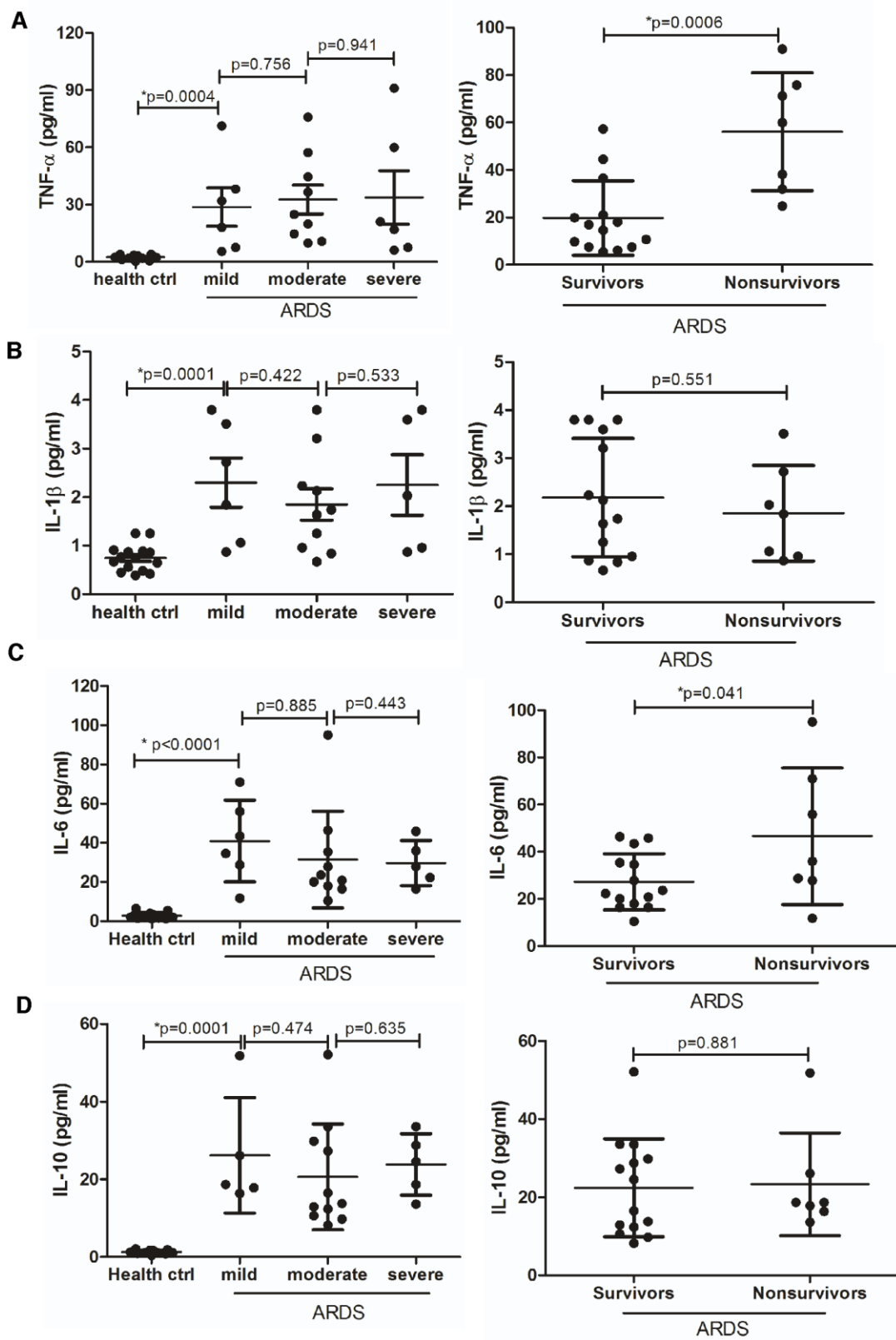
HCl=hydrochloric acid; IL-1 $\beta$ =interlukin-1 $\beta$ ; IL-6=interlukin-6; IL-10=interlukin-10; TNF- $\alpha$ =tumor necrosis factor- $\alpha$

## Supplementary Figure 5



**Figure 5. Elevated plasma LDH and myeloperoxidase activity in patients with ARDS caused by gastric aspiration.** (A, B) LDH activity was observed to increase significantly in ARDS patients compared to healthy controls ( $p<0.0001$ ). There was no difference in LDH activity between mild, moderate and severe ARDS, and between survivors and nonsurvivors ( $p>0.05$ ). (C, D) Myeloperoxidase activity was observed to be much higher in ARDS patients in contrast to healthy controls ( $p<0.0001$ ). In addition, the difference in myeloperoxidase activity was found to be significant between moderate and severe ARDS patients ( $p=0.0174$ ), and between survivors and nonsurvivors ( $p=0.0378$ ). ARDS=acute respiratory distress syndrome; LDH=lactate dehydrogenase; MPO=myeloperoxidase

Supplementary Figure 6



**Figure 6. Elevated plasma inflammatory cytokines in patients with ARDS caused by gastric aspiration. (A) TNF- $\alpha$  levels were increased significantly in**

ARDS patients compared with healthy controls (\* $p=0.0004$ ). There were no statistical differences in TNF- $\alpha$  levels between mild, moderate and severe ARDS ( $p>0.05$ ).

Moreover, TNF- $\alpha$  levels were higher in nonsurvivors than in survivors (\* $p=0.0006$ ).

**(B)** IL-1 $\beta$  levels were found to be higher in ARDS patients than in healthy controls ( $p<0.001$ ), but there was no statistical difference in IL-1 $\beta$  levels between mild,

moderate and severe ARDS, and between nonsurvivors and survivors ( $p>0.05$ ).

**(C)** IL-6 levels were higher in ARDS patients, and in nonsurvivors than healthy controls, survivors ( $p<0.0001$ ,  $p<0.05$ ).

**(D)** IL-10 levels were found to increase significantly in ARDS patients compared with healthy controls ( $p<0.0001$ ). There was no difference in IL-10 levels between mild, moderate and severe ARDS, and between survivors and nonsurvivors ( $p>0.05$ ).

ARDS=acute respiratory distress syndrome; IL-1 $\beta$ =interlukin-1 $\beta$ ; IL-6=interlukin-6; IL-10=interlukin-10; TNF- $\alpha$ =tumor necrosis factor- $\alpha$ .



**Table 1. Demographics of enrolled ARDS patients for sample analysis.**

	Patients
Sample Sizes	21
Sex	
Male	15
Female	6
Age (years)	58.2±8.3
Etiology	Gastric aspiration
Category	
Mild (200mmHg<PaO <sub>2</sub> /FiO <sub>2</sub> ≤300mmHg)	6
Moderate (100mmHg<PaO <sub>2</sub> /FiO <sub>2</sub> ≤200mmHg)	10
Severe (PaO <sub>2</sub> /FiO <sub>2</sub> ≤100 mmHg)	5
Co-morbidities	
COPD	8
Trauma	3
Drunk	3
Cancer (lung, gastric)	3
Postoperative	2
Other	2
Outcome	
Survival	14
Death	7

ARDS=Acute Respiratory Distress Syndrome

COPD=Chronic Obstructive Pulmonary Disease

PaO<sub>2</sub>=Partial Oxygen Tension

FiO<sub>2</sub>=Fraction of Inspired Oxygen