Supplemental Digital Content 1

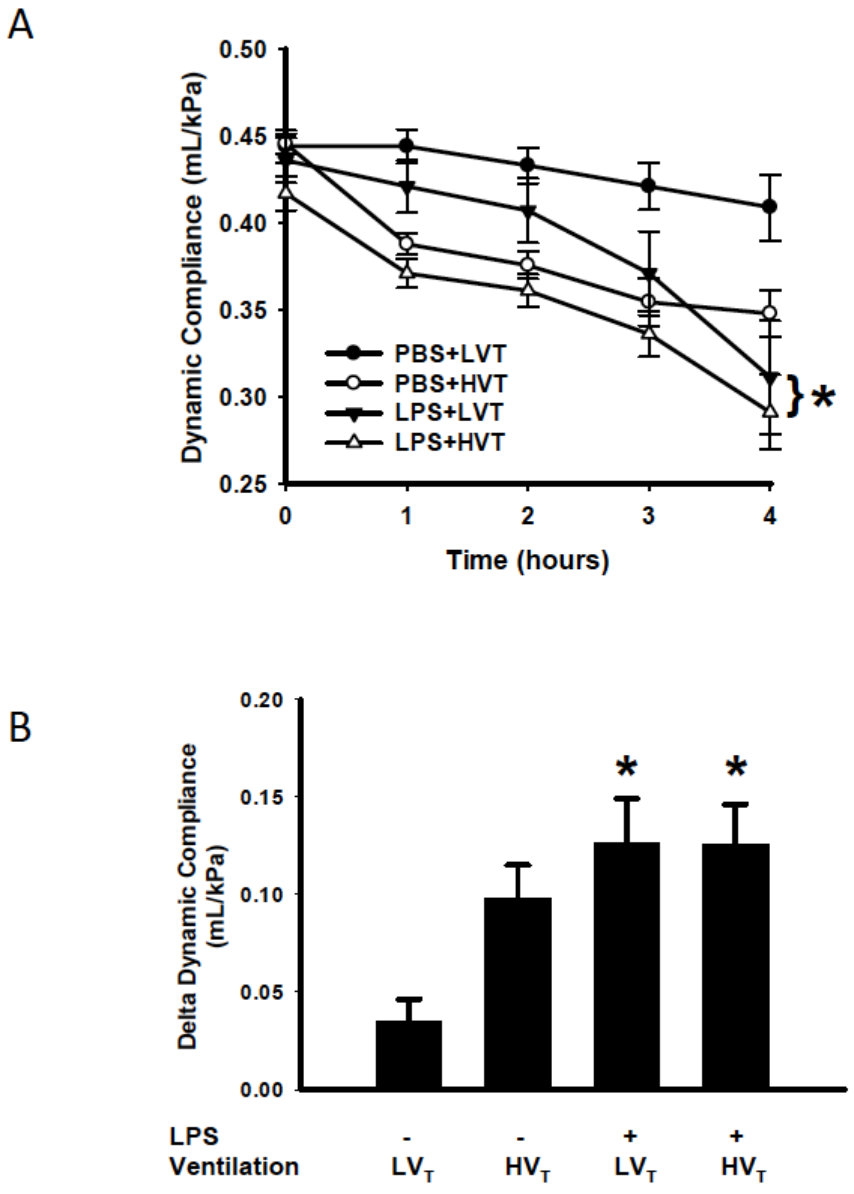

Fig. 1. Dynamic compliance in Two-Hit Model of Lipopolysaccharide and Mechanical Ventilation. (A) Time-course of dynamic compliance in mechanically ventilated mice. Two-way ANOVA on final compliance; pretreatment is significant, ventilation is not. No interaction. * p = 0.002 LPS *vs.* PBS (Holm-Sidak). (B) Delta dynamic compliance calculated by subtracting final from initial values. * p <0.05 *vs.* PBS + LV_T; One-way ANOVA on Ranks. N = 10/group. HV_T = high tidal volume; LPS = lipopolysaccharide; LV_T = low tidal volume; PBS= phosphate-buffered saline.

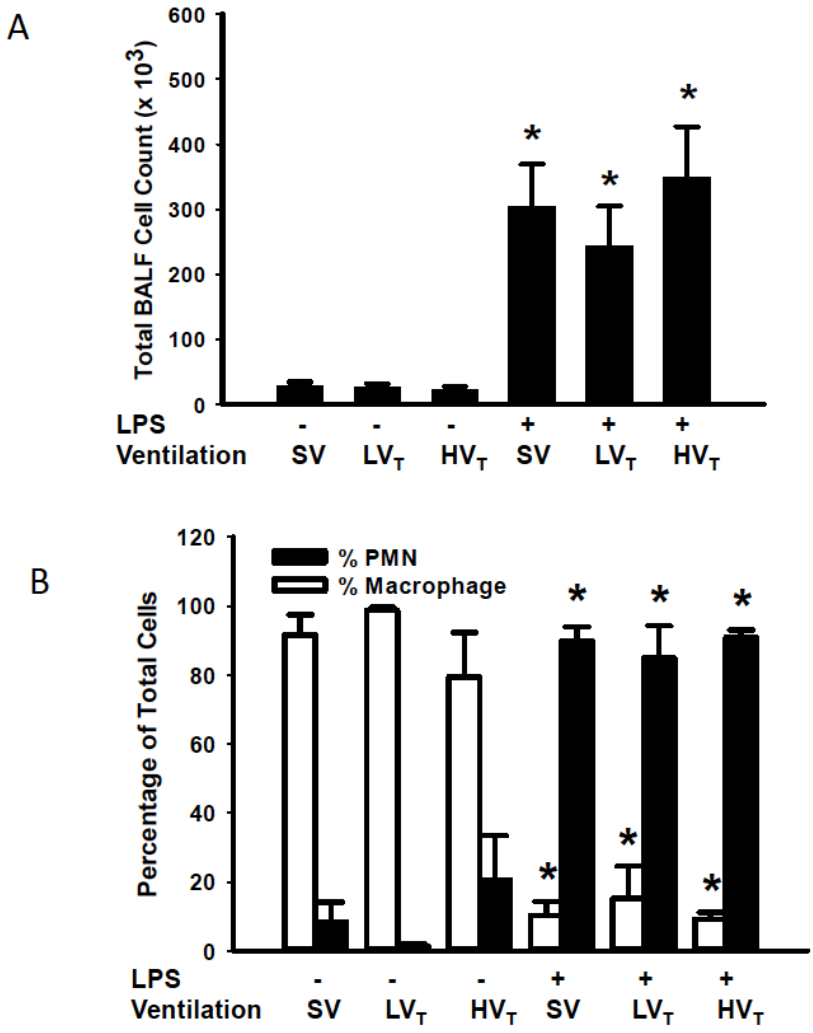

Fig. 2. Leukocyte Recruitment and Differential Cell Percentages in a Two-Hit Model of Lipopolysaccharide and Mechanical Ventilation. (A) Total cell count in BALF. LPS significantly induced leukocyte migration. One-way ANOVA on Ranks; * p <0.05 *vs.* PBS groups. (B) LPS induced an increase in neutrophil percentage and decrease of macrophage percentage in the BALF cell pellet. One-way ANOVA on Ranks; * p <0.05 *vs.* PBS groups. N = 10/group. BALF = bronchoalveolar lavage fluid; HV_T = high tidal volume; LV_T = low tidal volume; LPS = lipopolysaccharide; PMN = polymorphonuclear cells; SV = spontaneous ventilation.

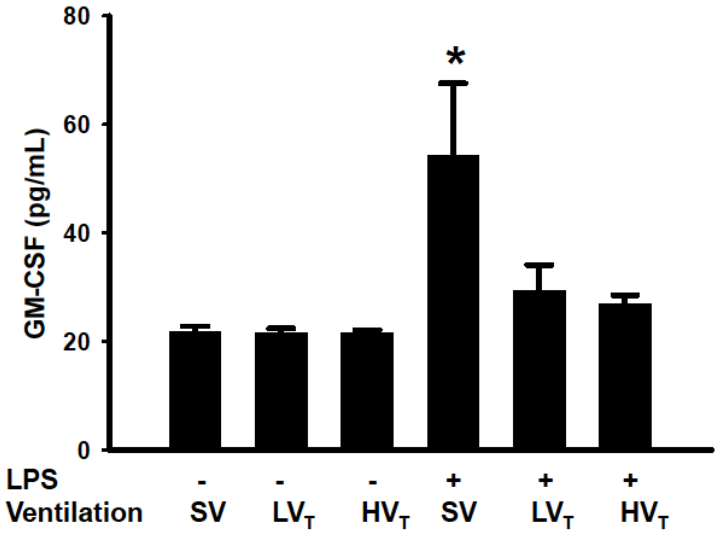

Fig. 3. GM-CSF in BALF. GM-CSF was significantly elevated only in the LPS + SV group. BALF = bronchoalveolar lavage fluid; GM-CSF = granulocyte macrophage colony-stimulating factor; HV_T = high tidal volume; LPS = lipopolysaccharide; LV_T = low tidal volume; SV = spontaneous ventilation. ANOVA on Ranks; * p <0.05 *vs.* PBS groups. N = 10/group.

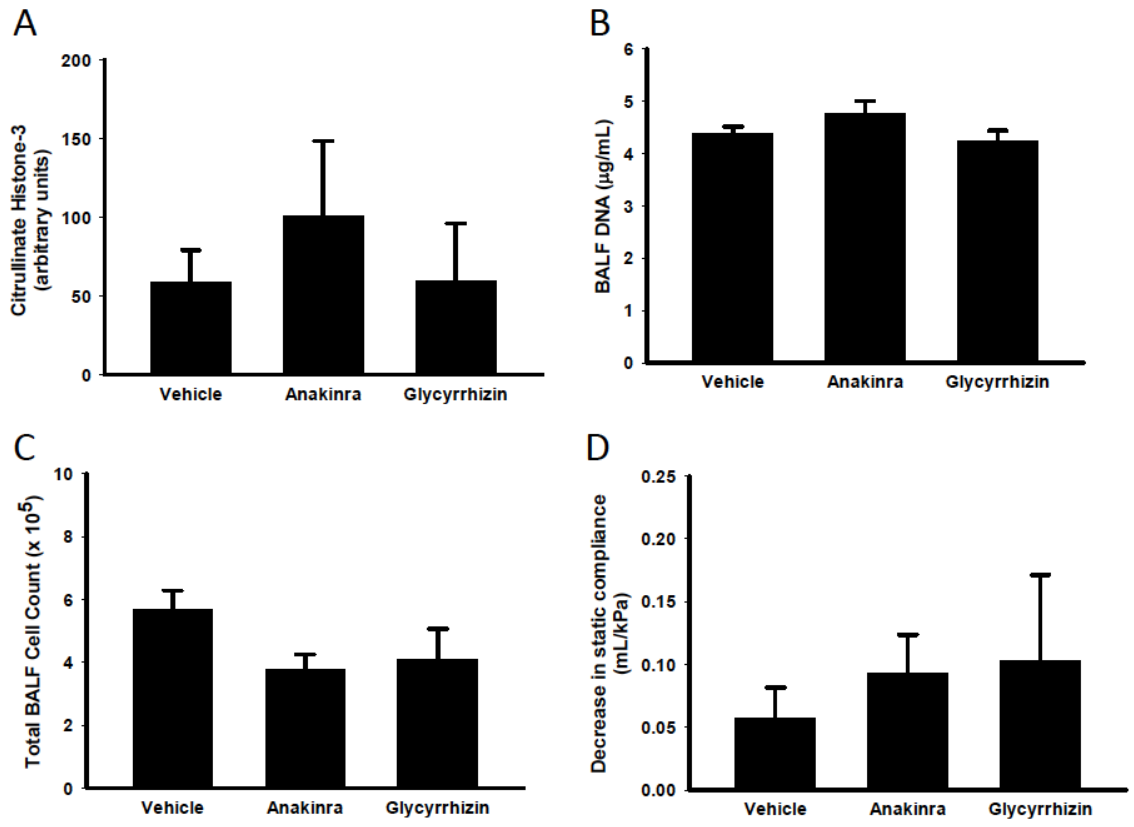

Fig. 4. Results of Pharmacological Intervention Against IL-1β and HMGB1. No significant changes in (A) citrullinated histone-3 (Western blot) , (B) DNA concentration in BALF, (C) BALF total cell count, or (D) attenuation of the decrement in compliance were detected. All by ANOVA, N = 6 per group. BALF = bronchoalveolar lavage fluid; IL-1β = interleukin-1beta; HMGB1 = High Mobility Group Box 1.

Table 1. BALF Cytokine Measurements, DNase Treatment

	Vehicle	**DNase**
G-CSF (ng/mL)	15.7 ± 0.57	16.5 ± 0.58
GM-CSF (pg/mL)	47 ± 8.7	39 ± 7.6
TNFα (ng/mL)	2.53 ± 0.076	2.65 ± 0.088
IL-1β (pg/mL)	49 ± 7.6	62 ± 13.5
IL-6 (ng/mL)	14.5 ± 1.05	16.2 ± 1.30
KC (ng/mL)	11.2 ± 0.40	12.0 ± 0.78
MIP-2 (ng/mL)	10.1 ± 0.65	10.9 ± 1.08
MCP-1 (pg/mL)	230 ± 41.3	470 ± 155.8

Data are mean ± SD. G-CSF = granulocyte colony stimulating factor; GM-CSF = granulocyte macrophage colony stimulating factor; IL-1β = Interleukin 1β; IL-6 = interleukin 6; KC = keratinocyte chemoattractant; MCP-1 = monocyte chemoattractant protein 1; MIP-2 = macrophage inflammatory protein 2; TNFα = tumor necrosis factor α. No significant differences was found between groups.