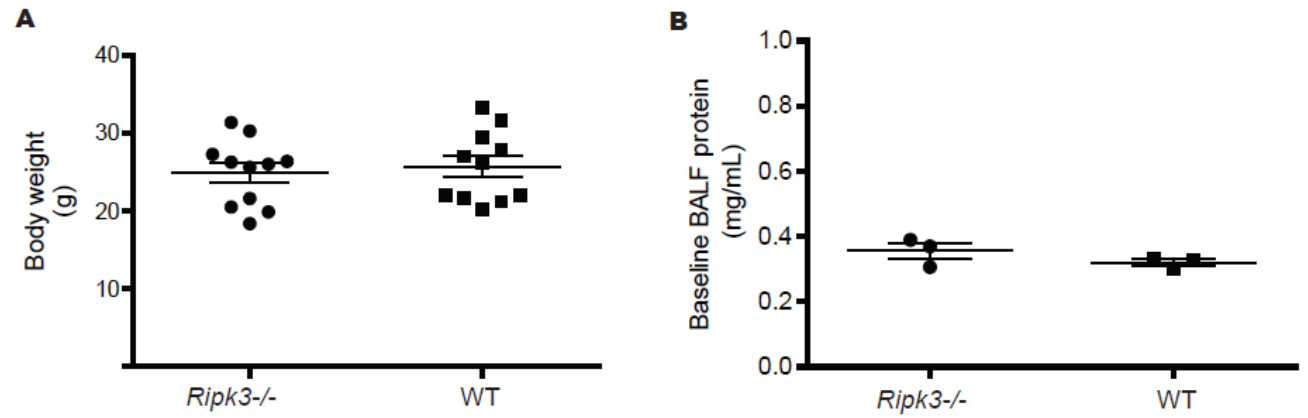


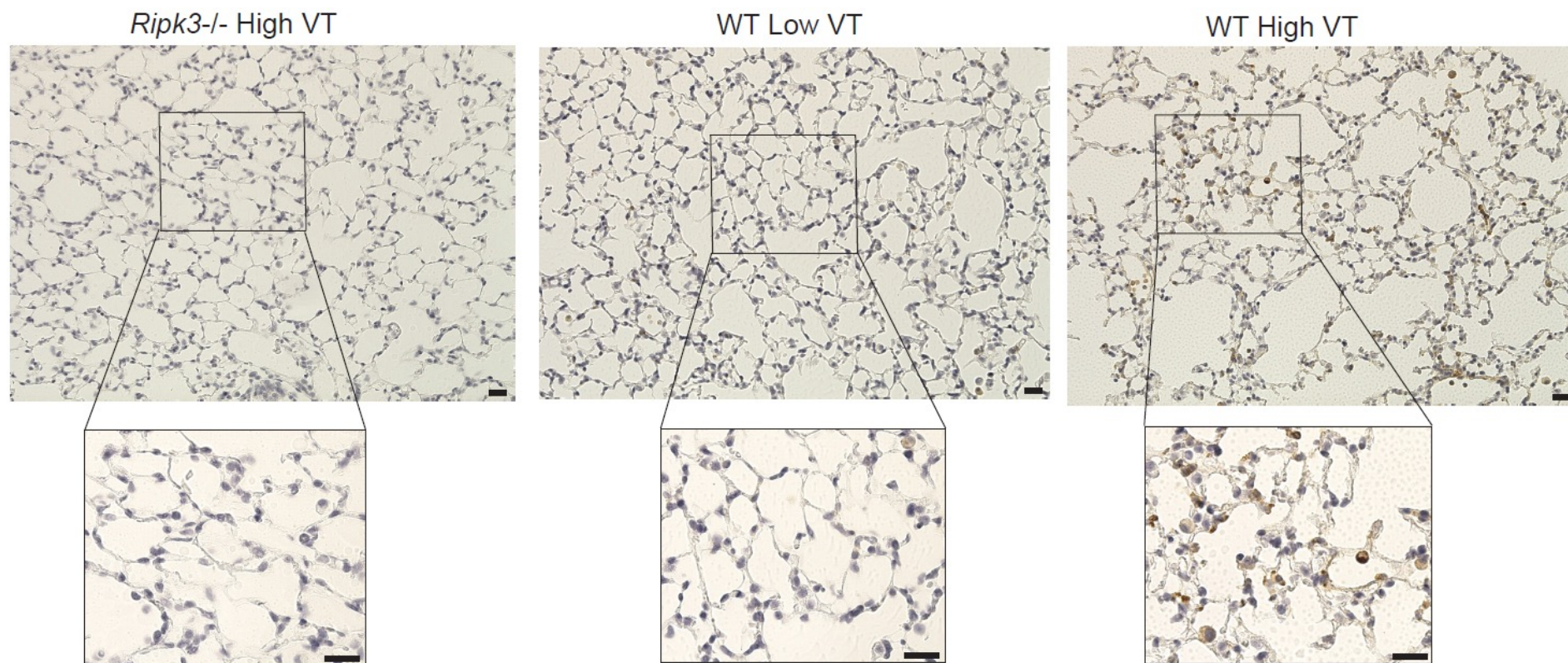
Supplemental Information

RIPK3 mediates pathogenesis of experimental ventilator-induced lung injury

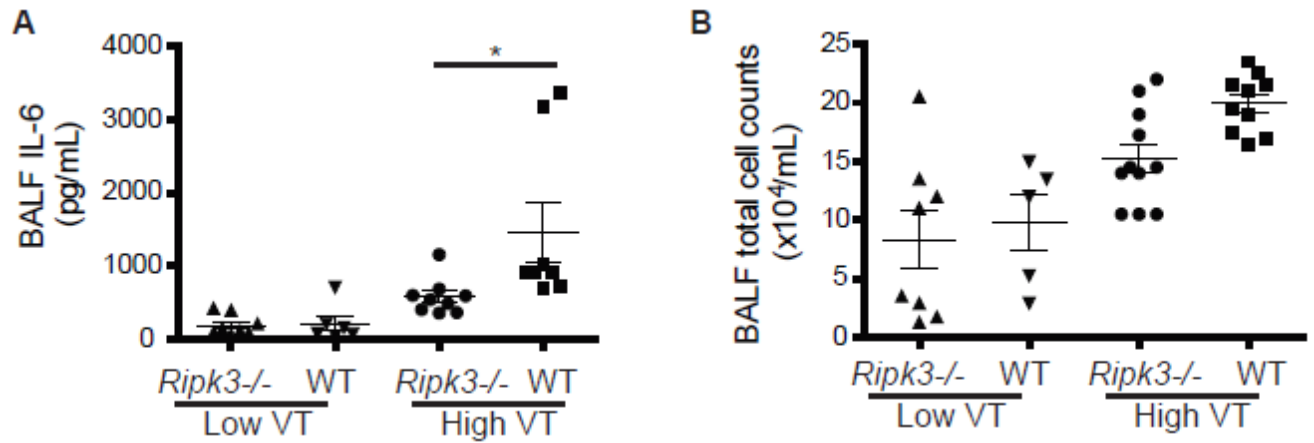
Supplemental Figure 1. Comparisons between *Ripk3*^{-/-} and wild type (WT) mice in terms of (A) body weight and (B) total protein concentration in the bronchoalveolar lavage fluid (BALF) under baseline conditions. There was no difference by using Mann-Whitney U test.



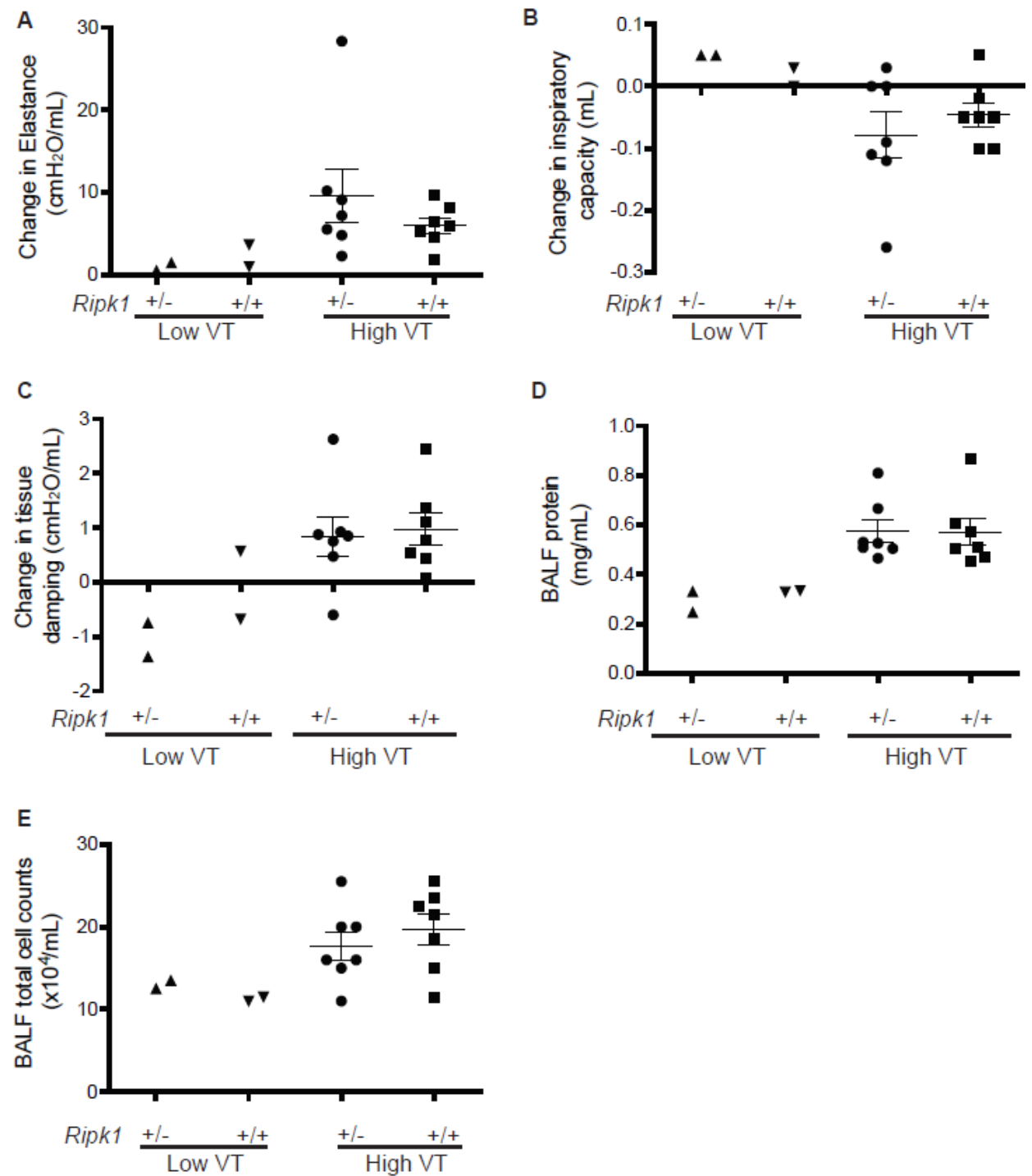
Supplemental Figure 2. Representative images of RIPK3 immunostaining of lung tissue sections from wild type (WT) mice after mechanical ventilation for 7 hours with low or high tidal volume (n=3 mice per group). Lung tissue section from *Ripk3*^{-/-} mouse was used as negative control. Size bar= 20um.



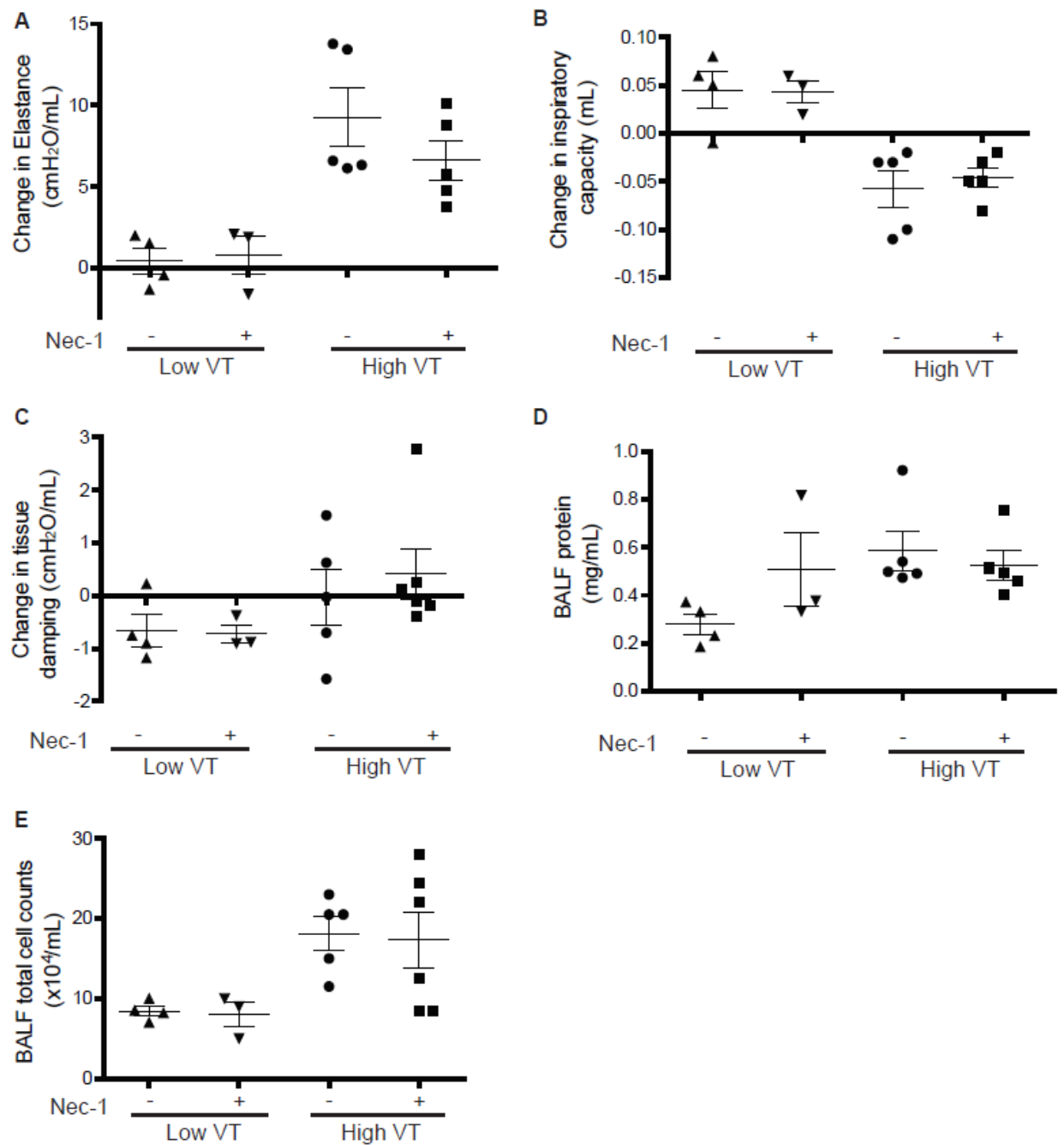
Supplemental Figure 3. Comparisons between *Ripk3*^{-/-} and wild type (WT) mice after mechanical ventilation with low or high tidal volume in terms of (A) IL-6 and (B) total white cell counts in the bronchoalveolar lavage fluid (BALF). *P<0.05, ANOVA with Tukey post-hoc correction.



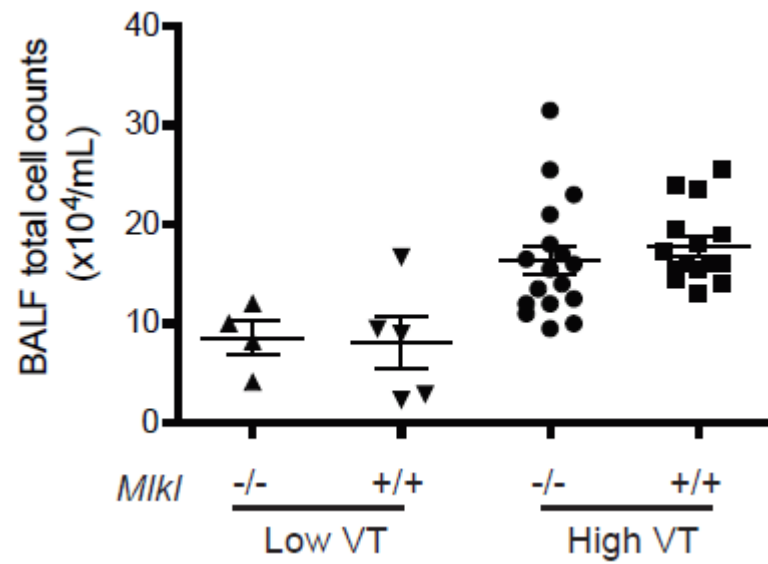
Supplemental Figure 4. Comparisons between *Ripk1*^{+/-} and *Ripk1*^{+/+} mice after mechanical ventilation with low or high tidal volume in terms of (A) change in lung elastance, (B) change in inspiratory capacity, (C) change in tissue damping, (D) total protein concentration and (E) total white cell counts in the bronchoalveolar lavage fluid (BALF).



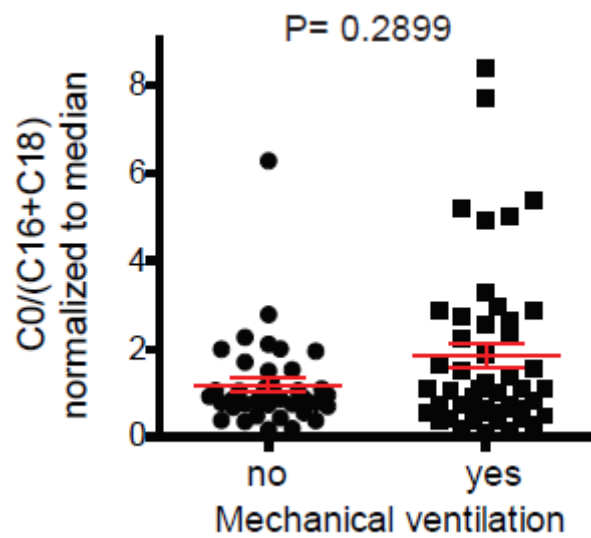
Supplemental Figure 5. Comparisons between wild type (WT) mice receiving or not necrostatin-1 (nec-1) and undergoing mechanical ventilation with low or high tidal volume in terms of (A) change in lung elastance, (B) change in inspiratory capacity, (C) change in tissue damping, (D) total protein concentration and (E) total white cell counts in the bronchoalveolar lavage fluid (BALF). There was no difference between mice receiving or not nec-1 by using ANOVA with Tukey post-hoc correction.



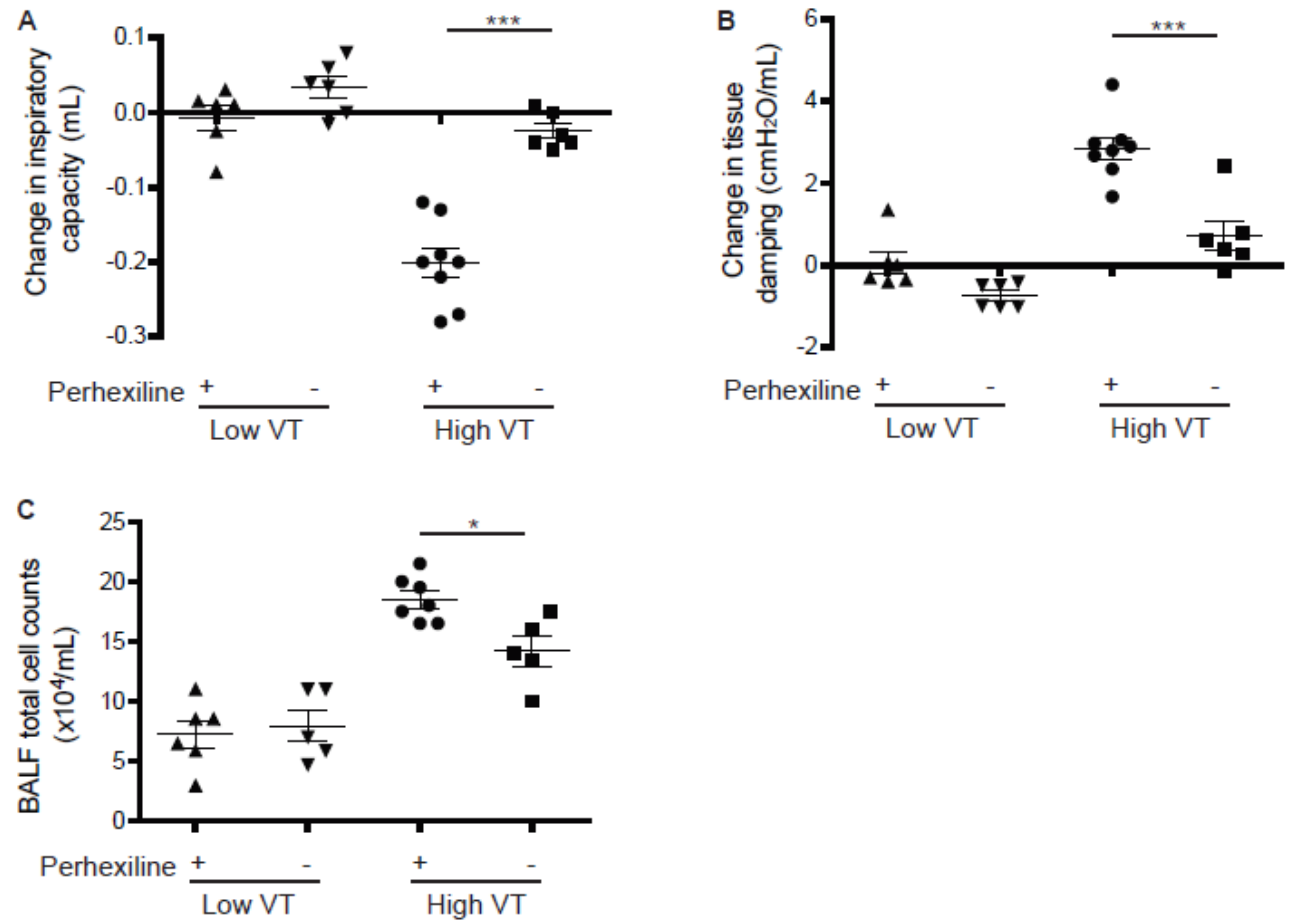
Supplemental Figure 6. Comparison between *Mkl*^{-/-} and *Mkl*^{+/+} mice after mechanical ventilation with low or high tidal volume in terms of total white cell counts in the bronchoalveolar lavage fluid (BALF). There was no difference between *Mkl*^{-/-} and *Mkl*^{+/+} mice by using ANOVA with Tukey post-hoc correction.



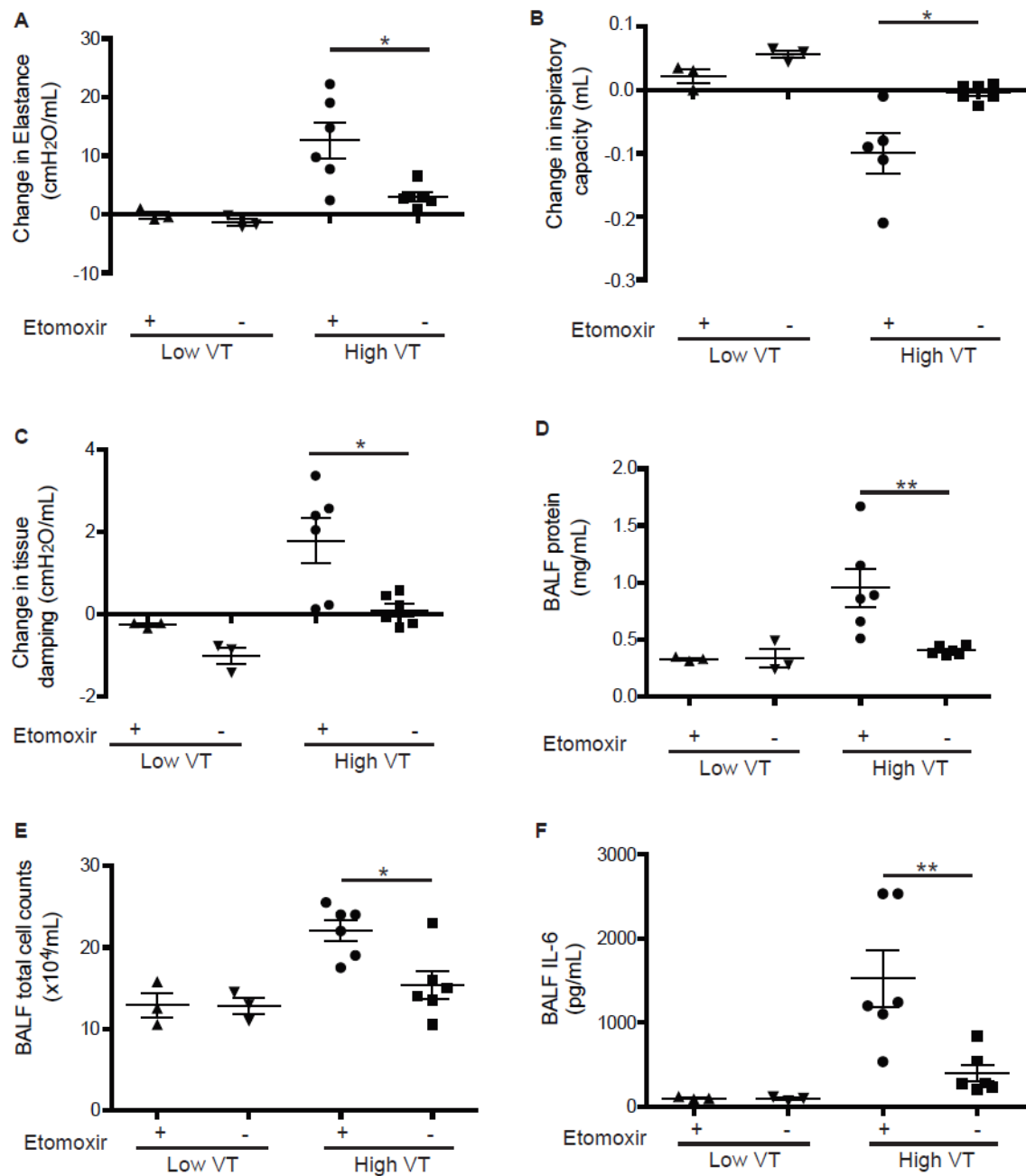
Supplemental Figure 7. Calculation of free carnitine to palmitoylcarnitine plus oleoylcarnitine ratio [$C0/(C16+C18)$], a screening test for deficiency of carnitine palmitoyl transferase (CPT1), which is a key enzyme in the fatty acid oxidation pathway] in plasma of critically ill patients with versus without mechanical ventilation (n=86). This is a secondary analysis of previously acquired metabolomic data (24). A Mann-Whitney U test was used.



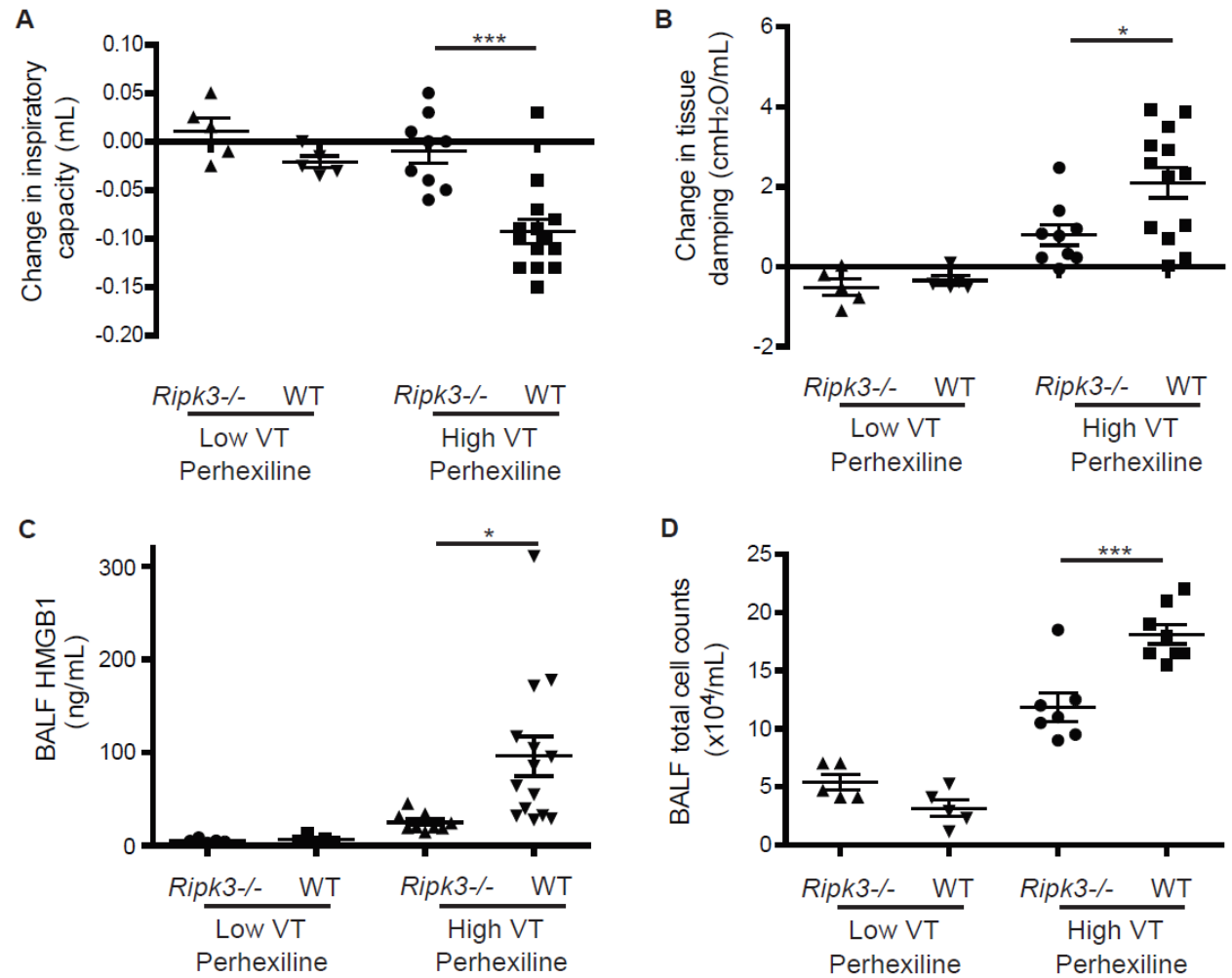
Supplemental Figure 8. Comparisons between wild type (WT) mice receiving or not perhexiline and undergoing mechanical ventilation with low or high tidal volume in terms of (A) change in inspiratory capacity, (B) change in tissue damping, and (C) total white cell counts in the bronchoalveolar lavage fluid (BALF). * $P < 0.05$ and *** $P < 0.001$, ANOVA with Tukey post-hoc correction.



Supplemental Figure 9. Comparisons between wild type (WT) mice receiving or not etomoxir and undergoing mechanical ventilation with low or high tidal volume in terms of **(A)** change in lung elastance, **(B)** change in inspiratory capacity, **(C)** change in tissue damping, **(D)** total protein concentration, **(E)** total white cell counts, and **(F)** IL-6 in the bronchoalveolar lavage fluid (BALF). * $P < 0.05$ and ** $P < 0.01$, ANOVA with Tukey post-hoc correction.



Supplemental Figure 10. Comparisons between *Ripk3*^{-/-} and wild type (WT) mice receiving perhexiline and undergoing mechanical ventilation with low or high tidal volume in terms of (A) change in inspiratory capacity, (B) change in tissue damping, (C) HMGB1 and (D) total white cell counts in the bronchoalveolar lavage fluid (BALF). *P<0.05 and ***P<0.001, ANOVA with Tukey post-hoc correction.



Supplementary Table 1. Multiple linear regression analysis using plasma

RIPK3 as a continuous response variable (BWH cohort).

Explanatory Variable	Coefficient	SE	95% CI	P value
Gender	-155	379	-902 to 592	0.68
Age	23	11	1 to 46	0.037
Malignancy	109	398	-676 to 893	0.79
Mechanical Ventilation*	1273	412	461 to 2086	0.002
Vasopressors*	1025	439	160 to 1890	0.020
Sepsis	1091	437	229 to 1953	0.013

Abbreviations: BWH: Brigham and Women's Hospital; SE: Standard Error; CI: Confidence Intervals

* Within 24 hours after admission in the ICU.

Mechanical ventilation is statistically significant independent predictor of plasma RIPK3 levels.