

[Supplemental Materials]

Blockade of Interplay between IL-17A and Endoplasmic Reticulum Stress Attenuates LPS-Induced Lung Injury

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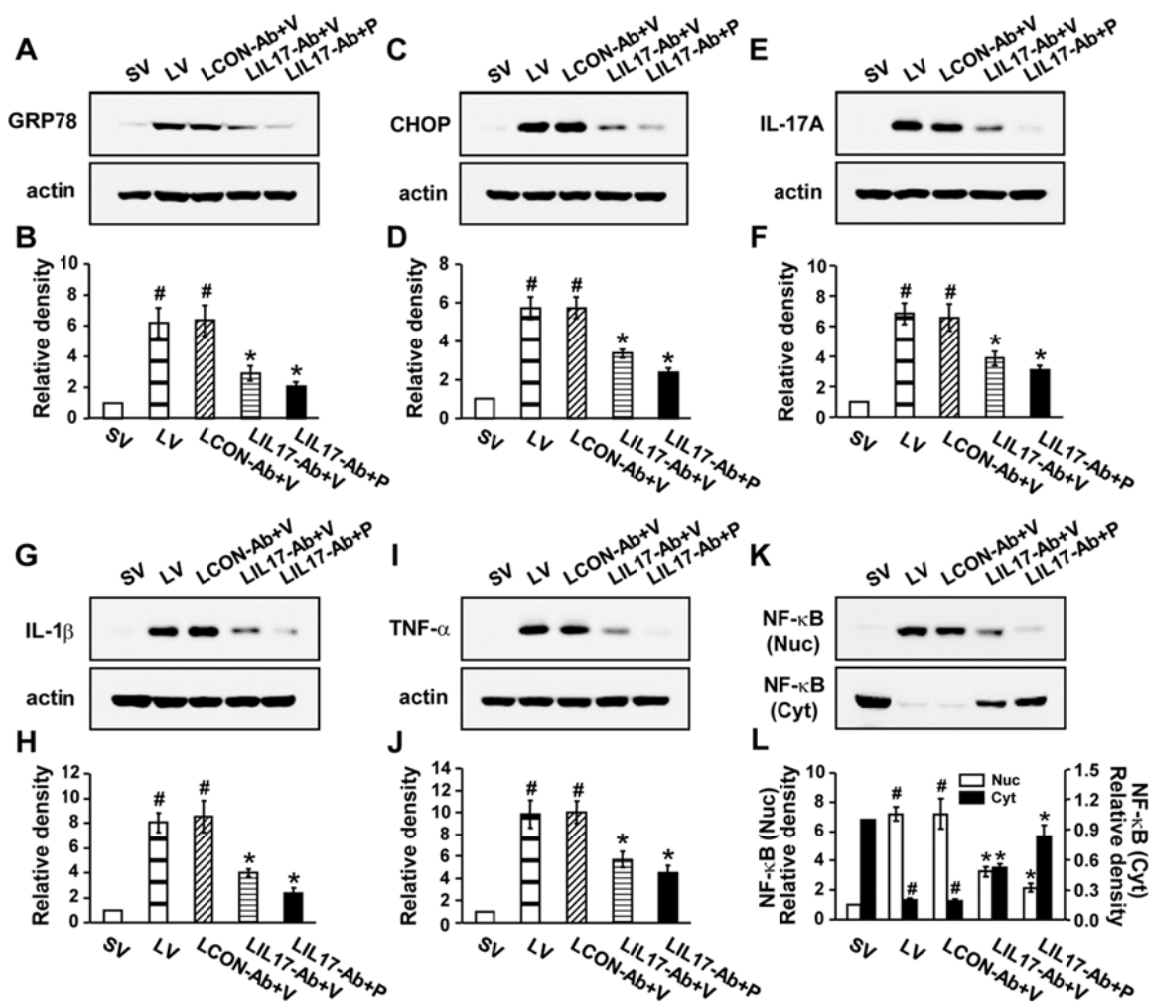


Figure S1: Effects of combination treatment with anti-IL-17A antibody and 4-PBA on ER stress, IL-17A production, expression of TNF- α and IL-1 β , and nuclear translocation of NF- κ B in lung tissues of LPS-instilled mice. Representative immunoblots of GRP78 (A), CHOP (C), IL-17A (E), IL-1 β (G), TNF- α (I), and NF- κ B p55 (K) in lung tissues from SV, LV, LCON-Ab+V, LIL17-Ab+V, or LPS-instilled mice given intraperitoneal injections of anti-IL-17 antibody (5 mg/kg) and 4-PBA (200 mg/kg) (LIL17-Ab+P) and densitometric analysis of GRP78 (B), CHOP (D), IL-17A (F), IL-1 β (H), TNF- α (J), and NF- κ B p55 (L). Sampling was performed at 48 hours after the instillation of LPS. Bars represent mean \pm SEM from 5 mice/group. # P < 0.05 versus SV; * P < 0.05 versus LV or LCON-Ab+V.

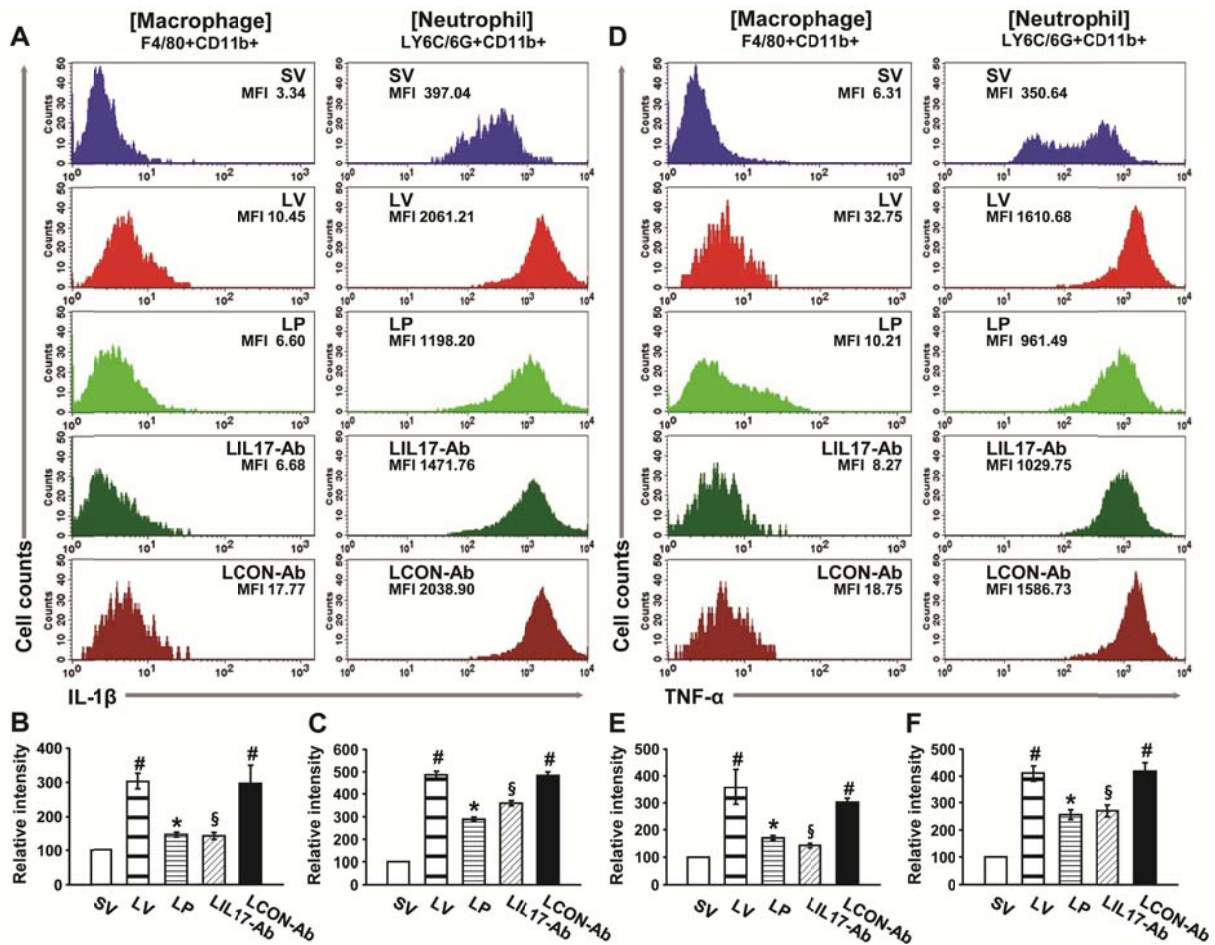


Figure S2: Levels of IL-1 β and TNF- α in macrophages and neutrophils of the lung from LPS-instilled mice. (A and D) Representative histogram for the expression of IL-1 β (A) and TNF- α (D) in lung cells from LPS-instilled mice. **(B and C)** Fluorescence intensity for IL-1 β in macrophages (B) and neutrophils (C) from the lung of LPS-instilled mice. **(E and F)** Fluorescence intensity for TNF- α in macrophages (E) and neutrophils (F) from the lung of LPS-instilled mice. Data are presented as the ratio of the levels of IL-1 β and TNF- α in each group relative to those in SV mice. Sampling was performed at 48 hours after the instillation of LPS. Bars represent mean \pm SEM from 5 mice/group. # $P < 0.05$ versus SV; * $P < 0.05$ versus LV; § $P < 0.05$ versus LCON-Ab.

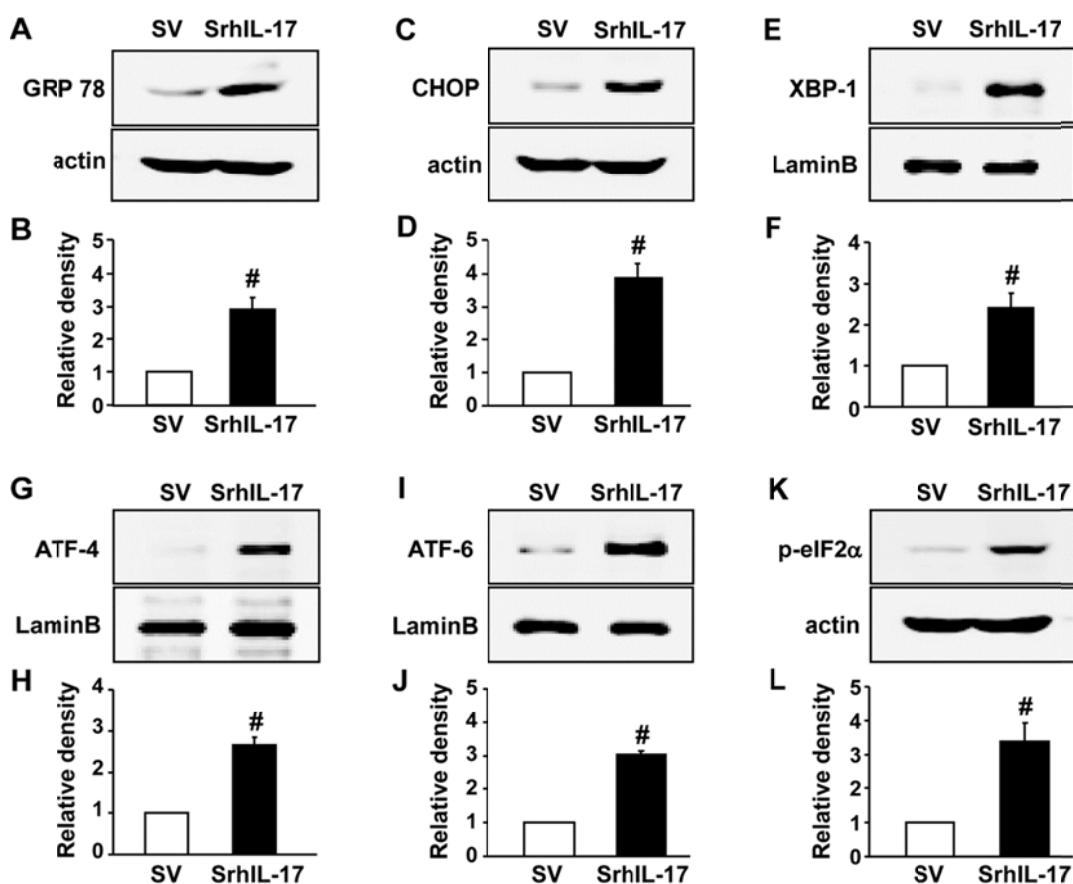


Figure S3: Changes of ER stress and UPR in lung tissues of rhIL-17A-instilled mice. Representative immunoblots of GRP78 (A), CHOP (C), XBP-1 (E), ATF-4 (G), ATF-6 (I) and p-eIF2 α (K) in lung tissues of SV and SV mice and saline-instilled mice given intratracheal instillation of rhIL-17A of 5 μ g/mouse (SrhIL-17) and densitometric analyses of GRP78 (B), CHOP (D), XBP-1 (F), ATF-4 (H), ATF-6 (J), and p-eIF2 α (L). Sampling was performed at 24 hours after the instillation of rhIL-17A. Bars represent mean \pm SEM from 5 mice/group. # $P < 0.05$ versus SV.

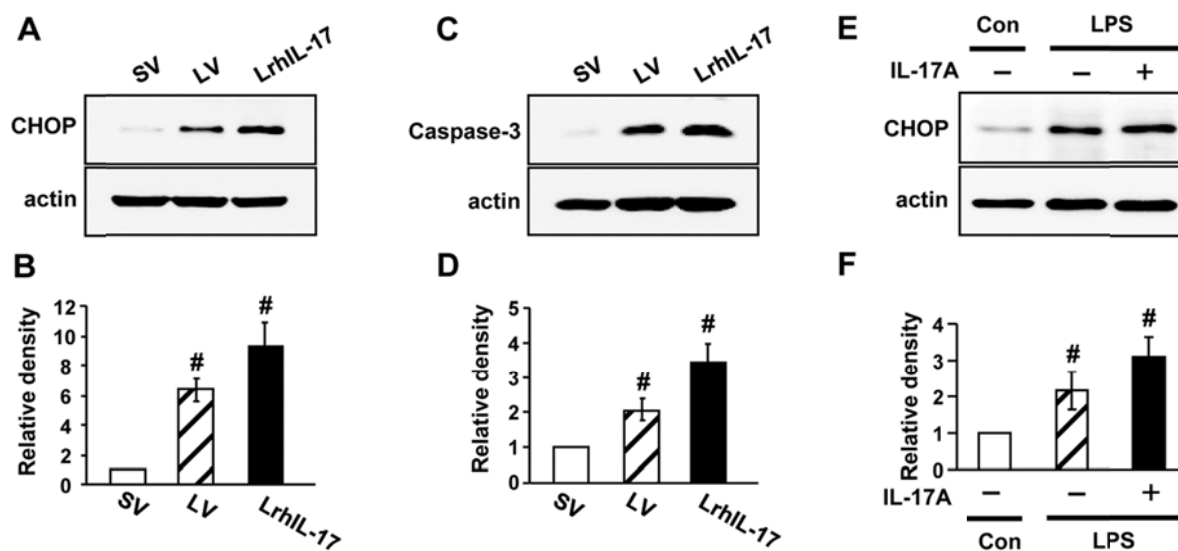


Figure S4: Levels of CHOP and caspase-3 in lung tissues of LPS-instilled mice and LPS-stimulated NHBE cells. Representative immunoblots of CHOP (A) and caspase-3 (C) in lung tissues of SV, LV, LPS-instilled mice given intratracheal instillation of rhIL-17A of 5 $\mu\text{g}/\text{mouse}$ (LrhIL-17) and densitometric analyses of CHOP (B) and caspase-3 (D). Bars represent mean \pm SEM from 5 mice/group. [#] $P < 0.05$ versus SV. (E) Representative immunoblot of CHOP in LPS-stimulated NHBE cells. (F) Densitometric analyses of CHOP are presented as the relative ratio of the protein to actin. The relative ratio of CHOP in control group is arbitrarily presented as 1. Cells were stimulated with 100 $\mu\text{g}/\text{ml}$ of LPS and/or 100 ng/ml of IL-17A for 12 hours. Bars represent mean \pm SEM from 3 independent experiments. Con, control. [#] $P < 0.05$ versus Con.

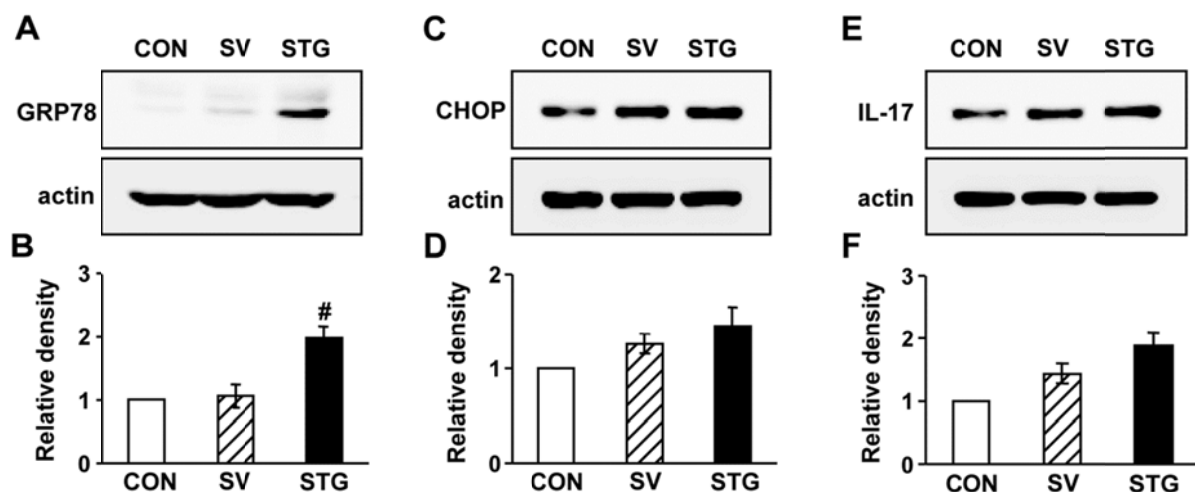


Figure S5: Effects of thapsigargin on the production of IL-17A in lung tissues of saline-instilled mice. Representative immunoblots of GRP78 (A), CHOP (C), and IL-17A (E) in lung tissues from mice without any treatment (CON), saline-instilled mice given injections of vehicle (SV), or saline-instilled mice given intraperitoneal injection of thapsigargin of 20 µg/mouse (STG) and densitometric analysis of GRP78 (B), CHOP (D), and IL-17A (F). Sampling was performed at 8 hours after the injection of thapsigargin. Bars represent mean ± SEM from 5 mice/group. [#] $P < 0.05$ versus CON.

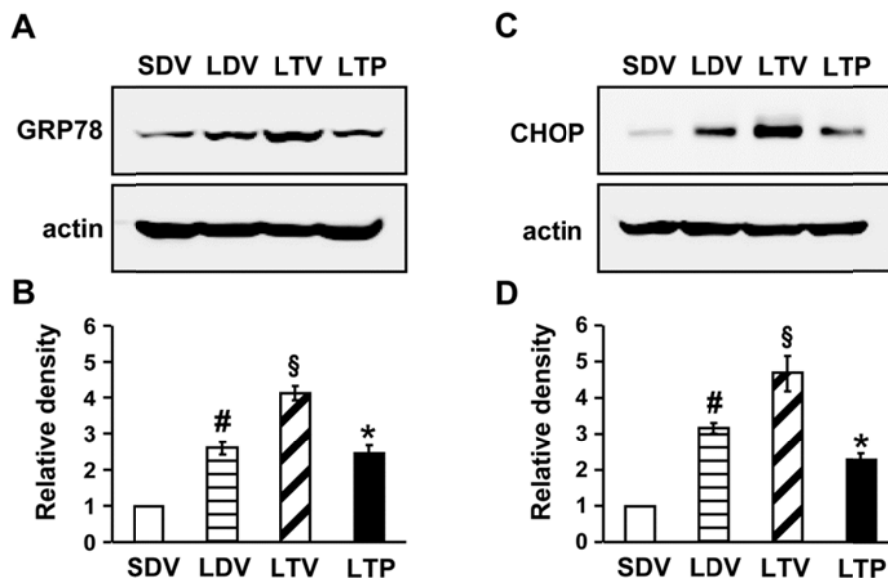


Figure S6: Effects of thapsigargin on ER stress in lung tissues of LPS-instilled mice. Representative immunoblots of GRP78 (**A**) and CHOP (**C**) in lung tissues from saline-instilled mice given injections of DMSO and vehicle (SDV), LPS-instilled mice given administrations of DMSO and vehicle (LDV), LPS-instilled mice given intratracheal instillation of thapsigargin (20 $\mu\text{g}/\text{mouse}$) and injections of vehicle (LTV), or LPS-instilled mice given intratracheal instillation of thapsigargin (20 $\mu\text{g}/\text{mouse}$) and intraperitoneal injections of 4-PBA (200 mg/kg) (LTP) and densitometric analyses of GRP78 (**B**) and CHOP (**D**). Sampling was performed at 48 hours after the instillation of LPS. Bars represent mean \pm SEM from 5 mice/group. # $P < 0.05$ versus SDV; § $P < 0.05$ versus LDV; * $P < 0.05$ versus LTV.

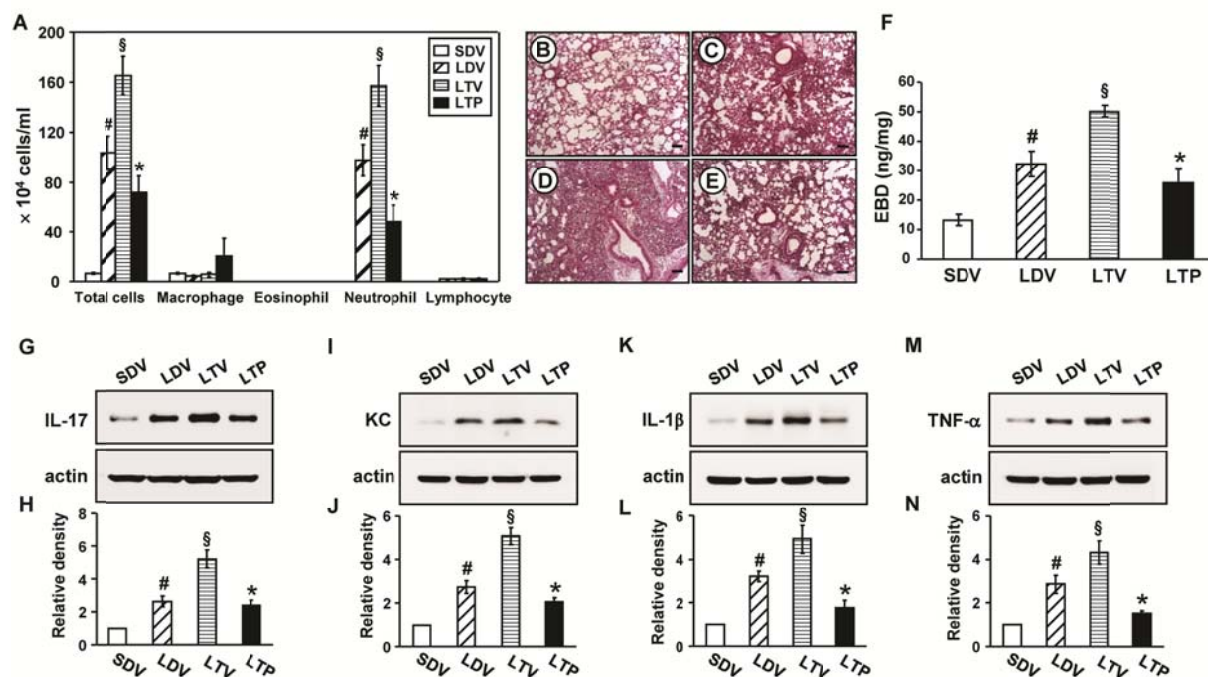


Figure S7: Effects of thapsigargin on the features of LPS-induced lung injury. (A) Cellular changes in BAL fluid from LPS-instilled mice. Representative H&E stained sections of the lungs from SDV (B), LDV (C), LTV (D), and LTP (E). Bars indicate 100 μ m. (F) EBD assay of lung tissues of LPS-instilled mice. Representative immunoblots of IL-17A (G), KC (I), IL-1 β (K) and TNF- α (M) in lung tissues from LPS-instilled mice and densitometric analyses of IL-17A (H), KC (J), IL-1 β (L), and TNF- α (N). Sampling was performed at 48 hours after the instillation of LPS. Bars represent mean \pm SEM from 5 mice/group. [#] $P < 0.05$ versus SDV; [§] $P < 0.05$ versus LDV; ^{*} $P < 0.05$ versus LTV.

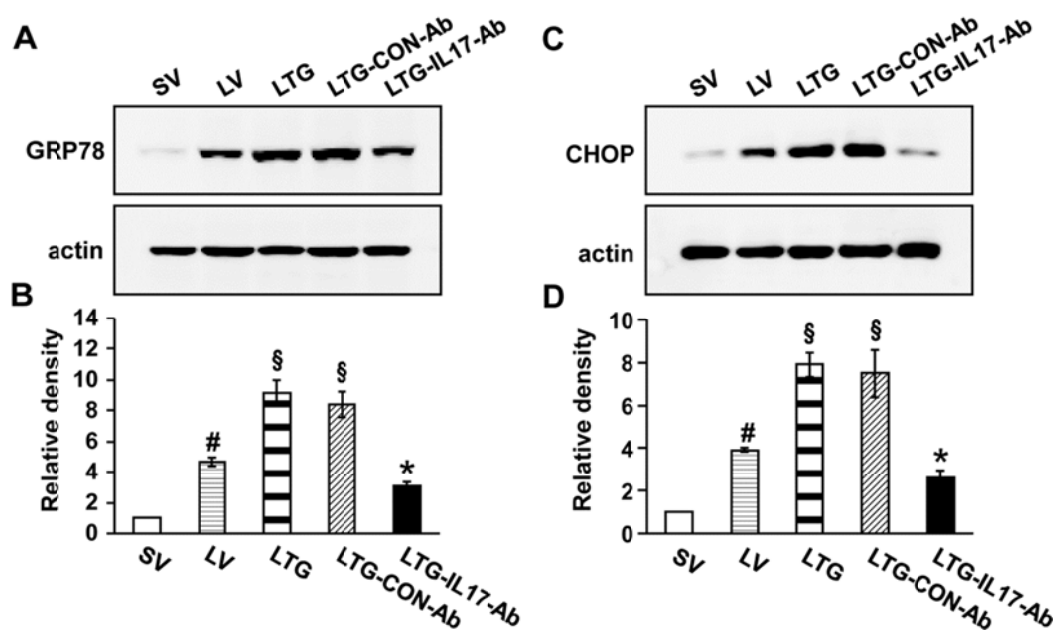


Figure S8: Effects of anti-IL-17A antibody on thapsigargin-induced ER stress in the lung tissues of LPS-instilled mice. Representative immunoblots of GRP78 (A) and CHOP (C) in lung tissues from SV, LV, LPS-instilled mice given intratracheal instillation of thapsigargin of 20 $\mu\text{g}/\text{mouse}$ (LTG), LPS-instilled mice given instillation of thapsigargin and intraperitoneal injections of isotype control monoclonal antibody of 5 mg/kg (LTG-CON-Ab), or LPS-instilled mice given instillation of thapsigargin of 20 $\mu\text{g}/\text{mouse}$ and injections of anti-IL-17A antibody of 5 mg/kg (LTG-IL17-Ab) and densitometric analyses of GRP78 (B) and CHOP (D). Sampling was performed at 48 hours after the instillation of LPS. Bars represent mean \pm SEM from 5 mice/group. [#] $P < 0.05$ versus SV; [§] $P < 0.05$ versus LV; ^{*} $P < 0.05$ versus LTG or LTG-CON-Ab.

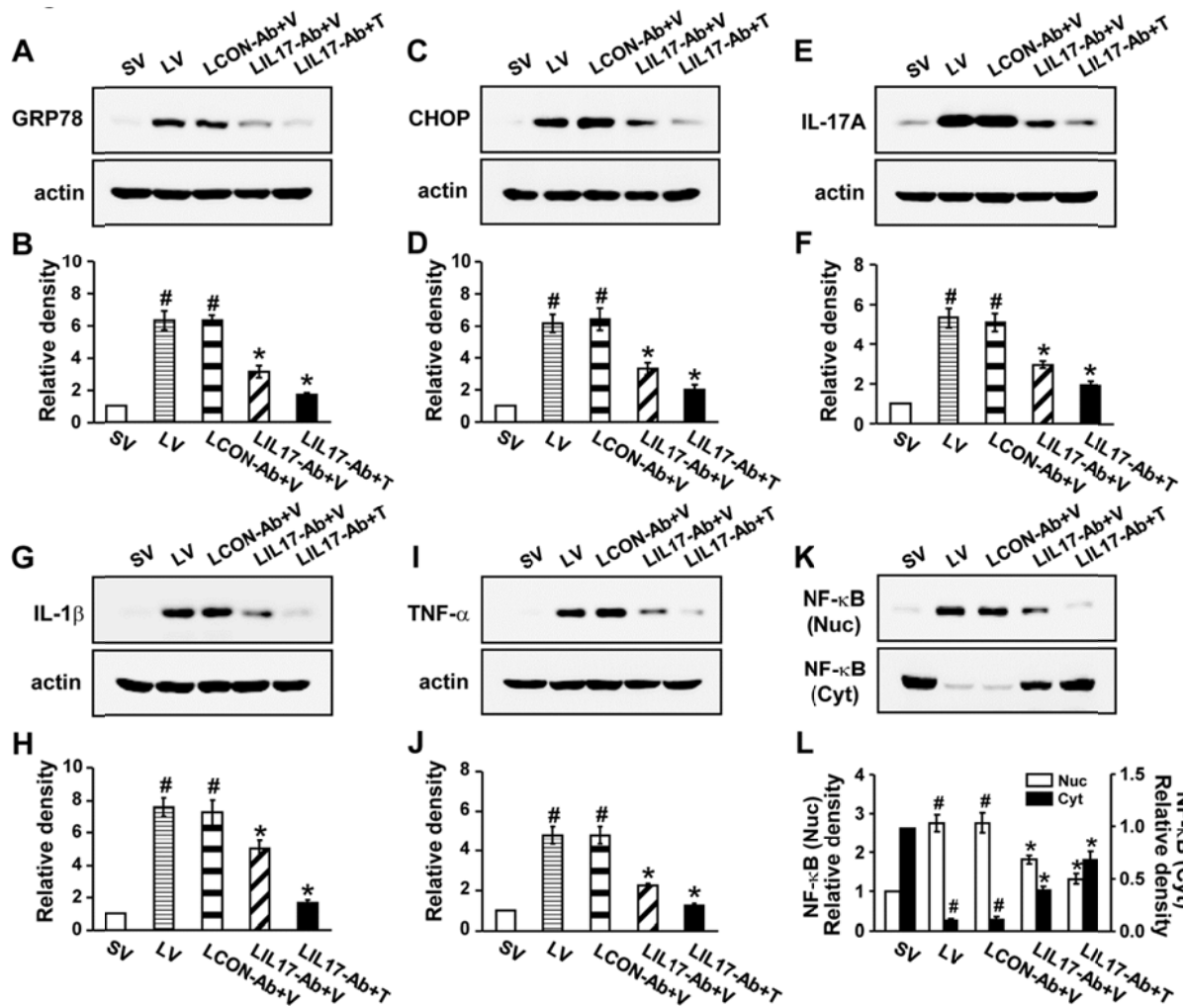


Figure S9: Effects of combination treatment with anti-IL-17A antibody and TAK-242 on ER stress, IL-17A production, expression of TNF- α and IL-1 β , and nuclear translocation of NF- κ B in lung tissues of LPS-instilled mice. Representative immunoblots of GRP78 (A), CHOP (C), IL-17A (E), IL-1 β (G), TNF- α (I), and NF- κ B p65 (K) in lung tissues from SV, LV, LCON-Ab+V, LIL17-Ab+V, or LPS-instilled mice given intraperitoneal injections of anti-IL-17 antibody (5 mg/kg) and TAK-242 (200 μ g/mouse) (LIL17-Ab+T) and densitometric analysis of GRP78 (B), CHOP (D), IL-17A (F), IL-1 β (H), TNF- α (J), and NF- κ B p65 (L). Sampling was performed at 48 hours after the instillation of LPS. Bars represent mean \pm SEM from 5 mice/group. # P < 0.05 versus SV; * P < 0.05 versus LV or LCON-Ab+V.

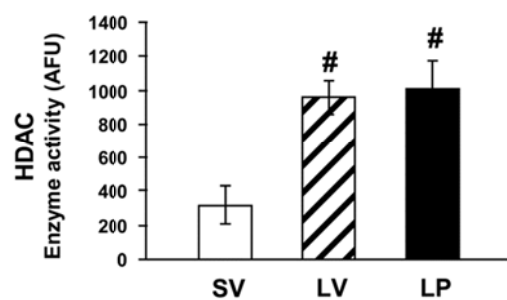


Figure S10: Effects of 4-PBA on HDAC activity in lung tissues of LPS-instilled mice. HDAC activity (arbitrary fluorescent units [AFU]) was determined in lung tissues of SV, LV, LP as described in Materials and Methods. Bars represent mean \pm SEM from 5 mice/group. $\#P < 0.05$ versus SV.