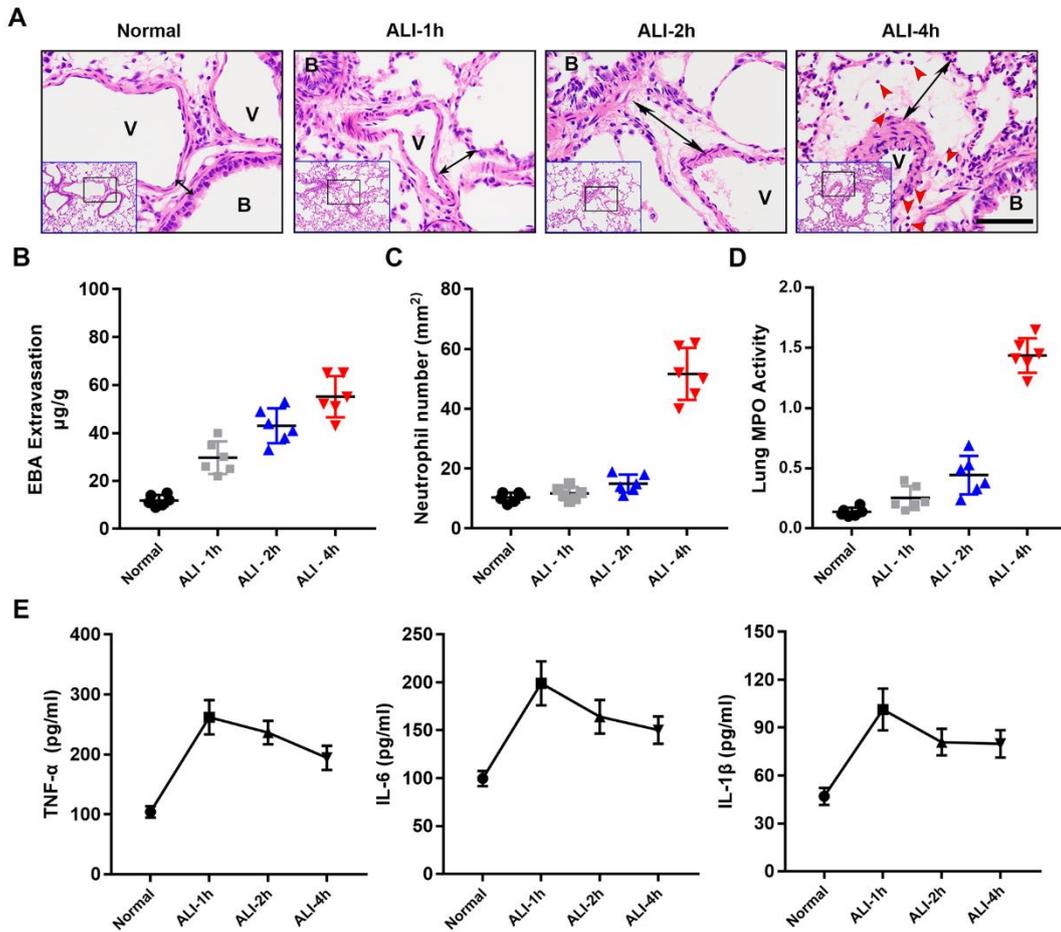
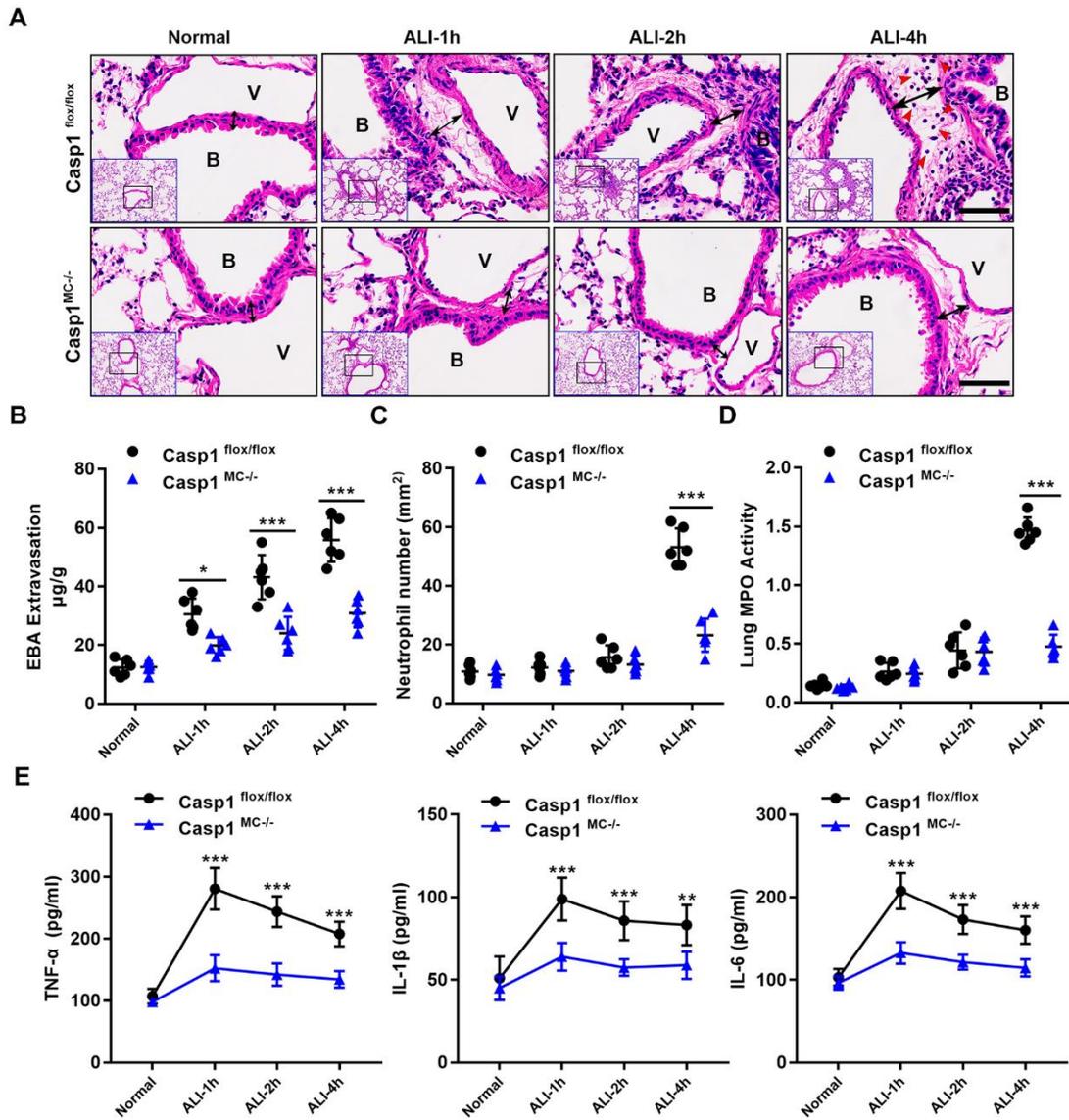


Supplemental Figure 1



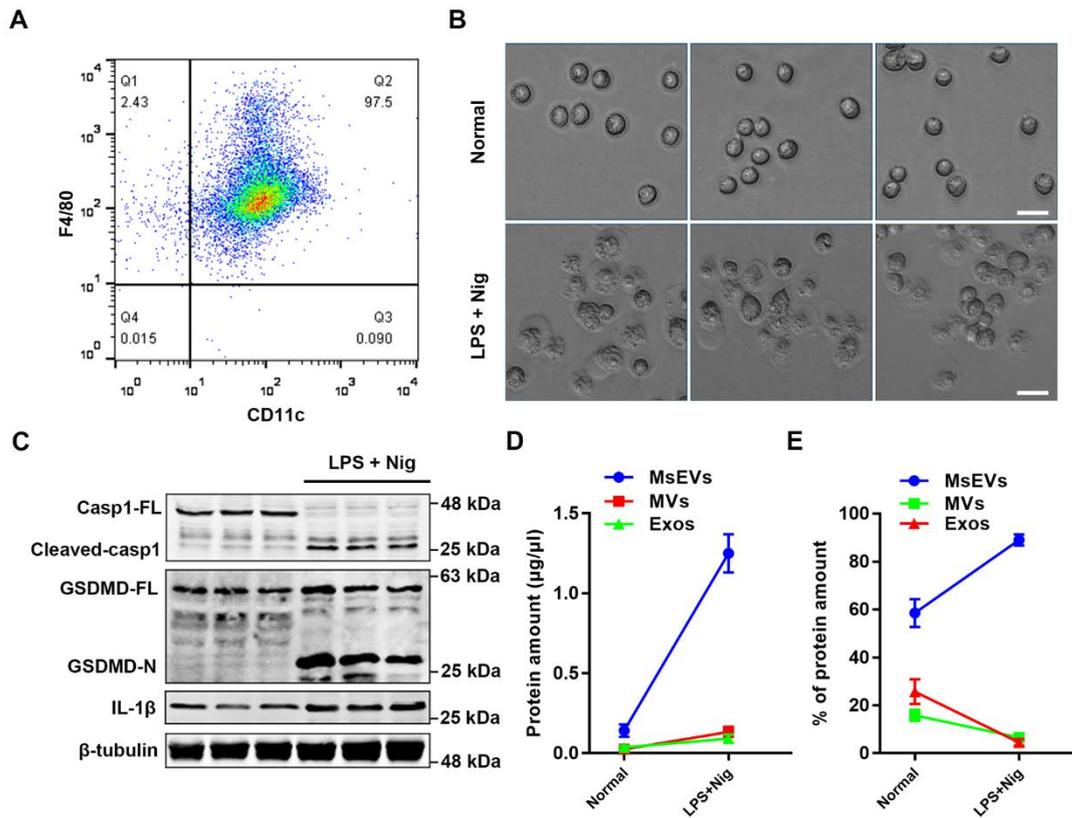
Supplemental Figure 1: Pathological characteristics of LPS-induced acute lung injury in mice. (A) H&E-stained cross-sections of the lung from control mice and LPS-exposed mice at 1, 2, and 4 h show interstitial edema (indicated by black arrows) in the ALI groups. The red arrow points to neutrophils. Scale bars: 50 µm. B, trachea; V, blood vessel. Images are representative of six animals. (B) Assessment of the degree of lung vascular leakage (EBA extravasation). (C) Quantitative analysis of neutrophils infiltration of the lungs. (D) Quantitative analysis of lung tissue MPO activity. (E) Quantification of cytokines (n=6). TNF- α , IL-1 β , and IL-6 expression levels increased in the ALI groups, and the highest expression was observed in the ALI-1h group. Data are expressed as the mean \pm SD.

Supplemental Figure 2



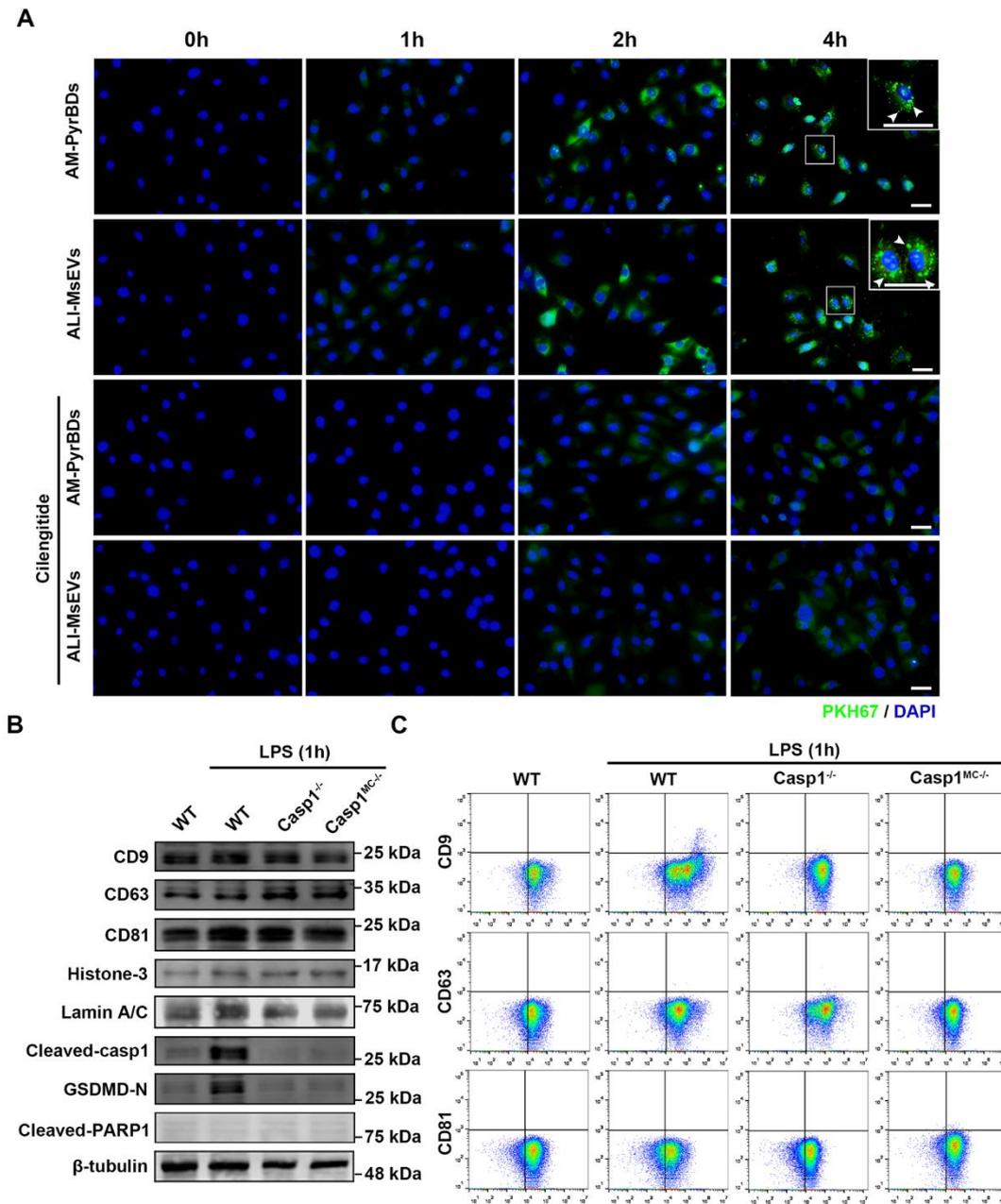
Supplemental Figure 2: Effect of LPS on the lung tissue of mice lacking caspase-1 in macrophages. (A) H&E-stained cross-section of the lung from LPS-exposed Casp1^{flox/flox} and Casp1^{MC-/-} mice at 1, 2, and 4 h shows interstitial edema (indicated by black arrows) in the ALI groups. The red arrow points to neutrophils. Scale bars: 50 μm. B, trachea; V, blood vessel. Images are representative of six animals. Quantitative analysis of (B) lung vascular leakage (EBA extravasation), (C) neutrophil infiltration, and (D) lung tissue MPO activity. Lack of caspase-1 in macrophages significantly reduced LPS-induced vascular leakage and neutrophil infiltration. (E) Quantification of cytokines. TNF-α, IL-1β, and IL-6 expression increased in the Casp1^{flox/flox} groups compared with the Casp1^{MC-/-} groups (n=6). Data are expressed as the mean ± SD. ***P* < 0.01; ****P* < 0.001.

Supplemental Figure 3



Supplemental Figure 3: Primary alveolar macrophages release large amounts of MsEVs during pyroptosis in vitro. (A) Identification of primary alveolar macrophages using flow cytometry. CD11c⁺ and F4/80⁺ events stand for primary alveolar macrophages. (B) Observation of the pyroptosis of alveolar macrophages by microscopy. The cells were primed with LPS (1 μg/mL) for 4 h and subsequently treated with Nig (5 μM) for 30 min or no treatment. (C) Immunoblot analysis for caspase-1 full length (Casp1-FL), cleaved caspase-1, GSDMD full length (GSDMD-FL), GSDMD-N-terminal (GSDMD-N), and IL-1β. The related proteins representing the degree of pyroptosis significantly increased. (D) Protein concentration of MsEVs, microvesicles and exosomes. (E) The protein concentration percentages of each type of EVs.

Supplemental Figure 4



Supplemental Figure 4: Characterization of PyrBDs. (A) PKH67-labeled PyrBDs were collected from LPS + Nig-treated alveolar macrophages (AM-PyrBDs) or mice exposed to LPS for 1 h (ALI-MsEVs); immunofluorescence staining showed the localization of PyrBDs in the cytoplasm. Cilengitide significantly suppressed this phenomenon. The white arrow points to undegraded PyrBDs (green); bar = 20 μm; DAPI (blue). (B) Immunoblot analysis for CD9, CD63, CD81, Histone 3, Lamin A/C, cleaved caspase-1, GSDMD-N, and cleaved PARP1 in PyrBDs isolated from multiple genotype mice. (C) Flow cytometry detected the expression of transmembrane proteins CD9, CD63, and CD81 in PyrBDs isolated from multiple genotype mice.