Supplementary material

Supplementary Figure Legends

Fig. SP1: The suppressive effects of C16 and Ang-1 on inflammatory cell accumulation in BALF. (A-Q) Wright-Giemsa staining of inflammatory cells in BALF. Aliquots of 300 μl of BALF were spread on a clean glass slide and stained with Wright-Giemsa stain solution. Obvious macrophage (arrow in D) and neutrophil (arrow in P) accumulation was initially and subsequently observed in the LPS group (A, B, C, D, I, J, K, L). C16 and Ang-1 treatment significantly decreased the number of inflammatory cells at each time point (E to A, F to B, G to C, H to D, M to I,N to J, O to K, P to L) and only a small portion of **e**xfoliated alveolar epithelium remaining in BALF of normal group(as normal control, Q). The arrow in panel E points to pulmonary alveolar epithelial cell detached in the BALF. Scale bar = 100 μm, (n=5).

Fig. SP2: **The suppressive effects of C16 and Ang-1 on the inflammatory response in vivo**. (A-Q) Lung tissues of ARDS rats were stained with TNF-α (red) to assess pulmonary inflammation. Compared with the normal control (Q), TNF-α-positive inflammatory cells gradually accumulated within the alveolar septal tissue of vehicle treated rats from 1 h to 1 week after LPS insult (A, B, C, D, I, J, K, L). However, in C16 and Ang-1-treated rats, infiltration of visible TNF-α-positive cells was not obvious until 12 hours after LPS insult (M), with weaker expression of TNF-α than that in the PBS treated LPS group (E to A, F to B, G to C, H to D, M to I,

N to J, O To K, P to L). Scale bar = 100 μm, (n=5).

Fig. SP3: The promotive effect of C16 and Ang-1 on pulmonary ultrastructural morphology in ARDS rats. (A-W) Electron micrographs showing perivascular edema, inflammatory cell infiltration, and apoptosis/necrosis in alveolar epithelial cells (AECs) and blood vessel epithelial cells (ECs). The arrow in panel A points to a normal AECs with clear lamellar bodies; the arrow in panel B points to a normal EC. Panels C and D show the LPS group at 1 hour post LPS insult, and the arrow in panel C points to an inflammatory cell; arrow in panel D points to a typical segmented neutrophil; However, in panel E, the C+A treatment abolished inflammatory cell infiltration. In panels F, I, K (low magnification) and G (high magnification), we found that edema appeared in the areas surrounding AECs in the LPS group (arrow in G), but there was no obvious edema in the C16 and Ang-1-treated group (H, J, L). In panel M, the arrow points to severe edema in the surrounding area of blood vessels. The arrow in panel N points to alveolar septal cells exhibiting necrotic signs, and the alveolar septal cells in the C+A treated group (O) are normal. The arrow in panel P points to pulmonary interstitial edema. The arrow in panel R points to AECs with apoptotic features. The arrow in panel T indicates an apoptotic EC, and the arrow in panel V points to an apoptotic AEC with visible empty lamellar bodies, swollen cell organs, and enlarged mitochondria (red arrow). However, at the same time points, the interstitial cells (Q), ECs (U) and AECs (S, W) in the C+A treated group were morphologically normal (n=5).

Fig. SP4: The inhibitory effect of C16 and Ang-1 on neutrophil activity. (A-Q) MPO immunohistochemical staining of lung tissues in LPS-induced ARDS rats. In LPS group, the infiltration appeared in 1 hour after insult (A) compared with the normal control (Q) and C+A treated group (E), and was more severe in B and C. At 6 hours after insult, extensive interstitial infiltration of neutrophils was present in PBS-treated LPS rats (D), whereas there were only a few infiltrating neutrophils in C+A-treated rats 1–6 hours after the insult (E-G). Clear interstitial neutrophil infiltration appeared from 6–24 hours after LPS insult (D, I, J). In the C+A group, the infiltration was obviously less extensive than in the LPS group (H, M, N). Moreover, at 24 hours after the insult, the degree of neutrophil infiltration in both PBS- and C+A-treated LPS rats was evidently decreased, and there was no remarkable neutrophil infiltration surrounding the blood vessel (green) or within the interstitial tissue (K, L, O, P). (R): Enlarged image of MPO-positive neutrophils. (S): cultured endothelial cells that could express CD31 (green) and integrin αvβ3 (red). (T): Double staining of CD31 and integrin αvβ3 in blood vessels and alveolar cell basal membrane of normal lung tissue. Scale bar = 100 μm (n=5).

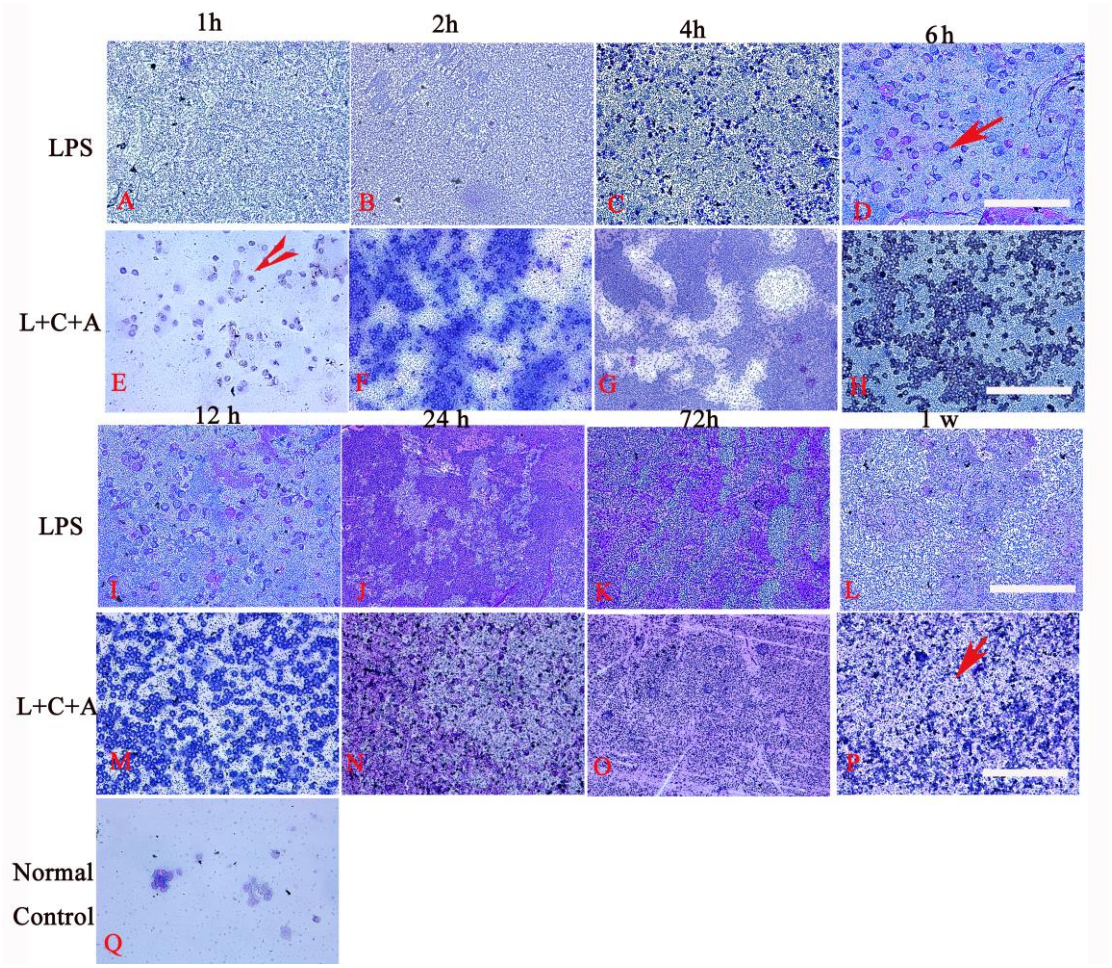
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Fig. SP1

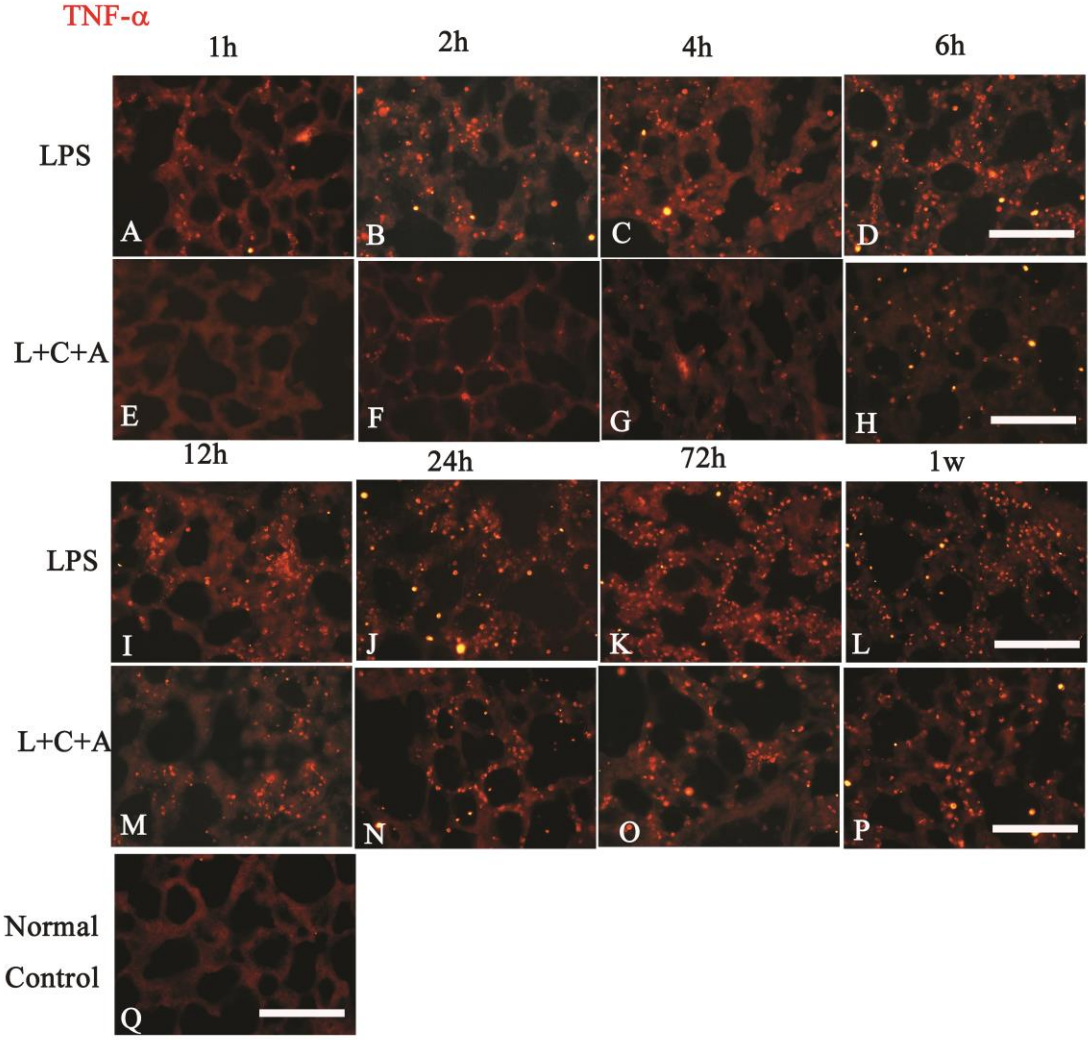
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Fig. SP2

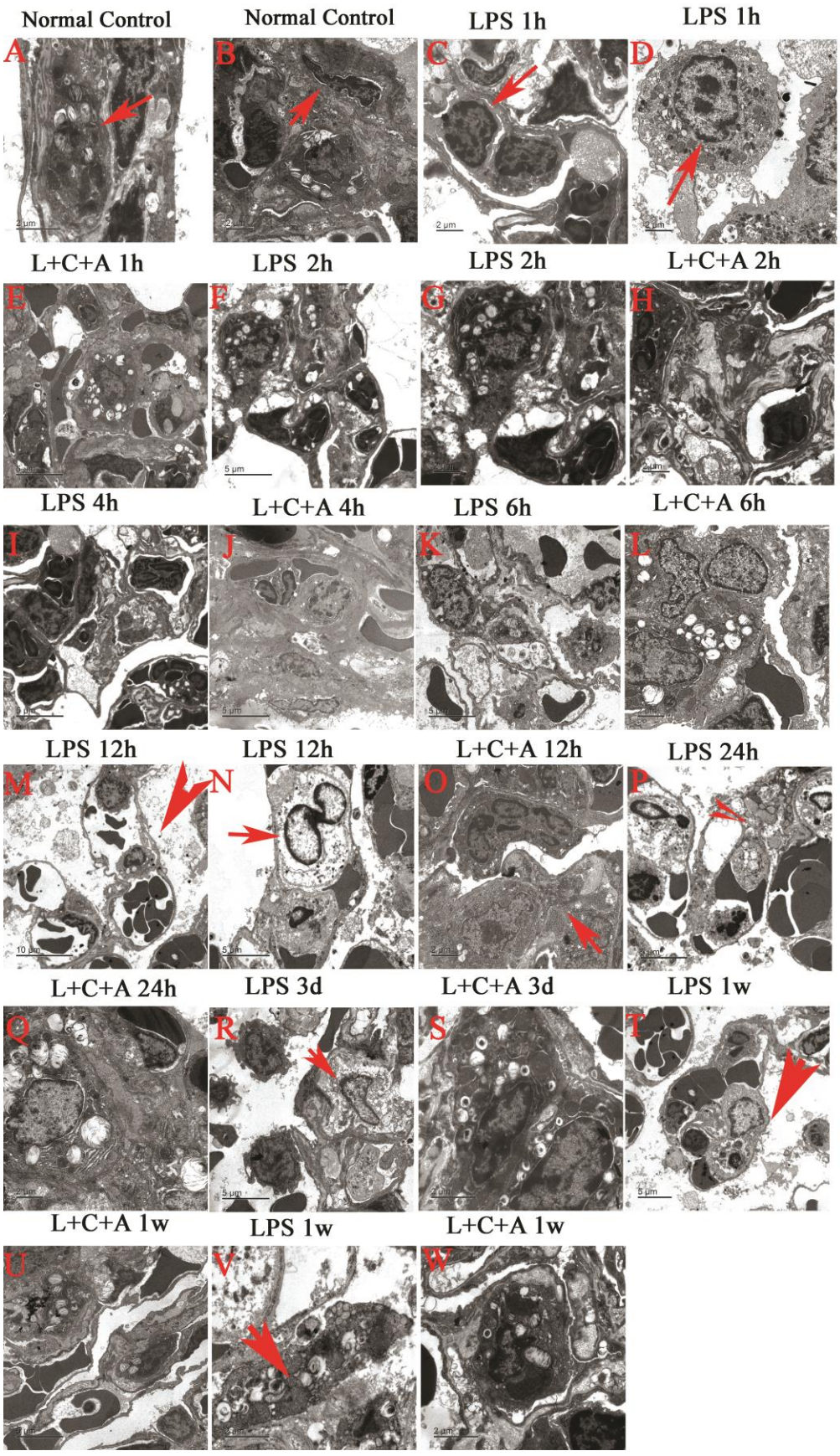
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Fig. SP3

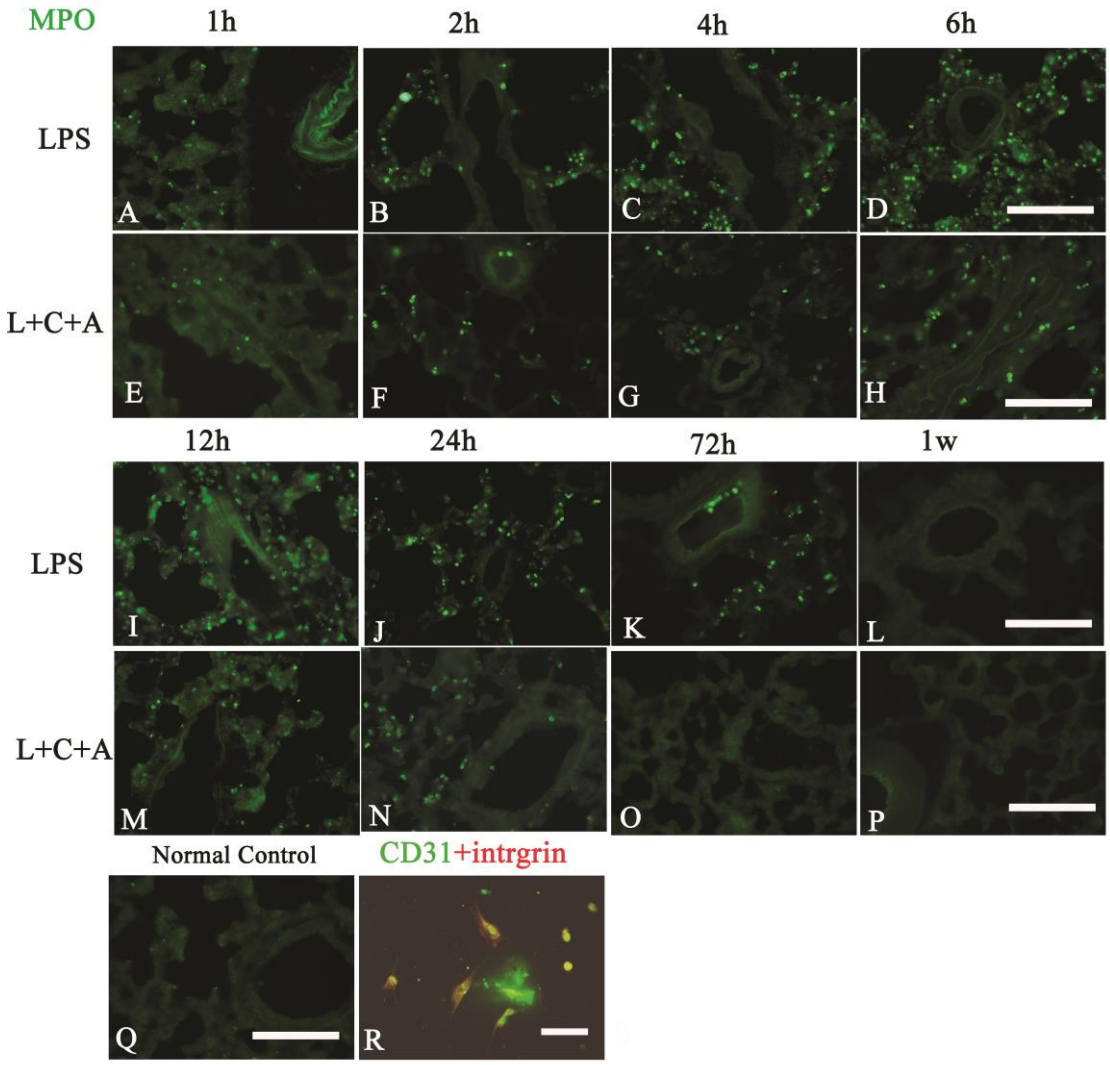
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Fig. SP4