

### **Surfactant protein A /D-CD14 is associated with phagocytosis of nanomaterials and cytokine production by alveolar macrophages**

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This supplementary material contains 6 figures and 1 table:

Figure S1 Shapes of MWCNT-7, TiO<sub>2</sub>, SiO<sub>2</sub> and C60 in the suspension;

Figure S2 Chemical composition of MWCNT-7, TiO<sub>2</sub>, SiO<sub>2</sub> and C60;

Figure S3 Size distribution of MWCNT-7, TiO<sub>2</sub>, SiO<sub>2</sub> and C60 in the suspension;

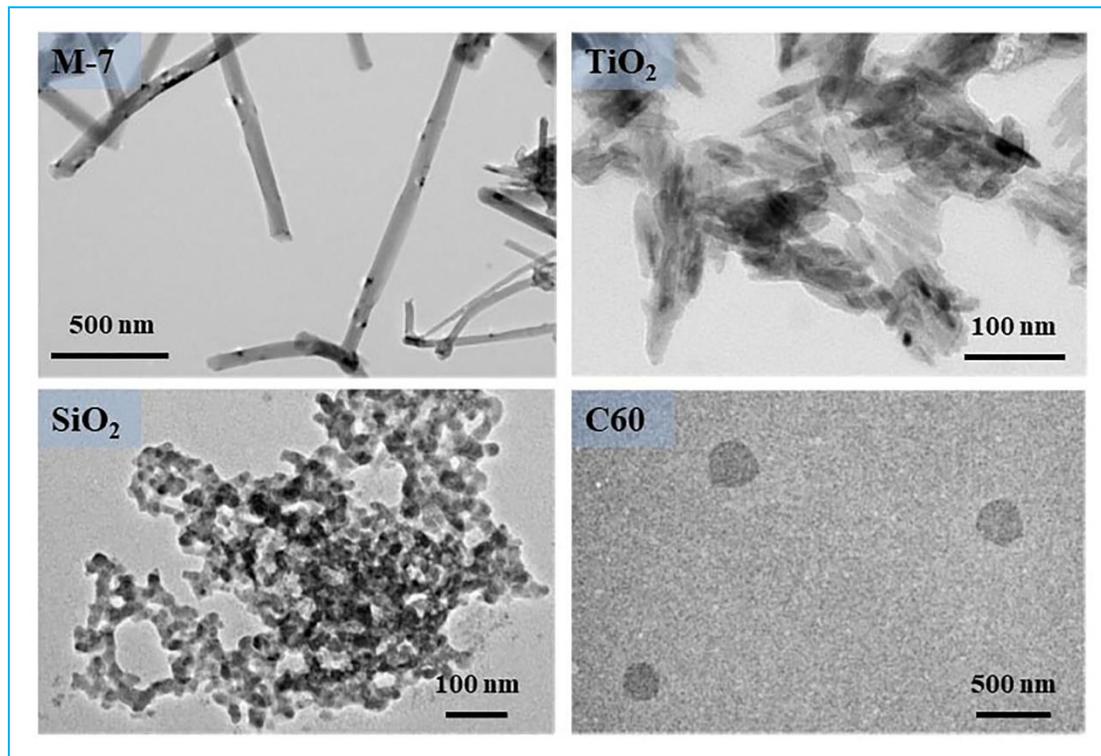
Figure S4 Immunostaining of primary alveolar macrophages

Figure S5 Knockdown of CD14, LRP1 and SIRP $\alpha$  expression in primary alveolar macrophages

Figure S6 summary of the results

Table S1 LC-MS analysis of the nanomaterial-bound proteins

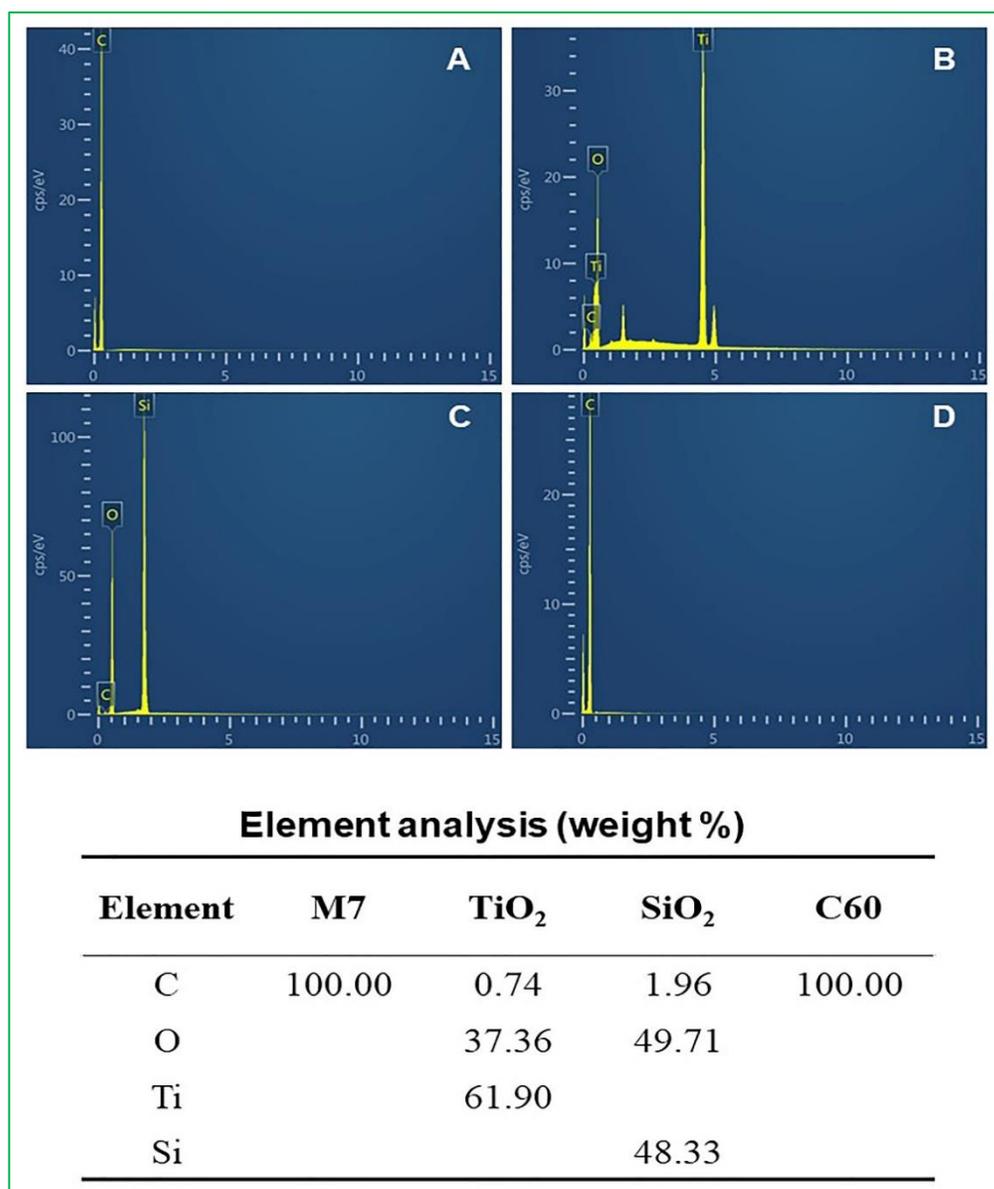
## Supplementary materials for PFT



**Figure S1 Shapes of MWCNT-7, TiO<sub>2</sub>, SiO<sub>2</sub> and C60 in the suspension**

MWCNT-7, TiO<sub>2</sub>, SiO<sub>2</sub> and C60 were suspended in saline containing 0.5% (w/v) Pluronic® F-68 to a final concentration of 500 µg/ml, 1:200 diluted with ddH<sub>2</sub>O, and placed on a carbon sheet. After air-dried, the samples were observed under the JEM-2100 transmission electron microscope (JEOL Co. Ltd, Tokyo, Japan).

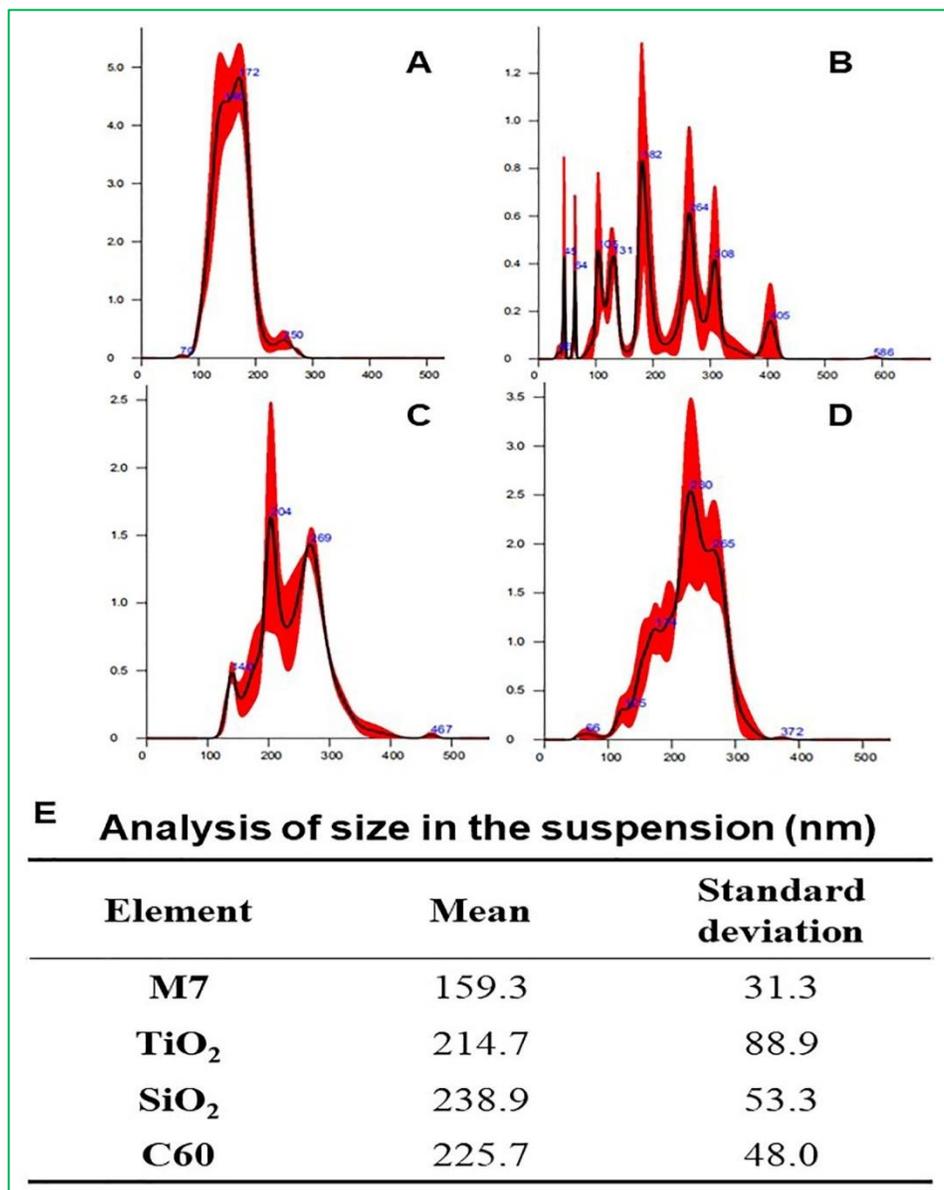
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**Figure S2 Chemical composition of MWCNT-7, TiO<sub>2</sub>, SiO<sub>2</sub> and C60s**

Suspensions of MWCNT-7, TiO<sub>2</sub>, SiO<sub>2</sub> and C60 (500 µg/ml) were 1:200 diluted with ddH<sub>2</sub>O, and placed on an aluminum plate. After air-dried, the samples were observed and analyzed by the FIB-SEM (Helios G4 UC, Aztec) connected with EBSD analysis system (Thermo Scientific). A, B, C and D represent the spectra of MWCNT-7, TiO<sub>2</sub>, SiO<sub>2</sub> and C60, respectively. The table under the spectra is the element composition and ratio of each of the ENMs.

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**Figure S3 Size distribution of MWCNT-7, TiO<sub>2</sub>, SiO<sub>2</sub> and C60 in the suspension**

Size distribution in 500 µg/ml suspensions of MWCNT-7, TiO<sub>2</sub>, SiO<sub>2</sub> and C60 were analyzed with the NanoSight 300 analyzer (Malvern). A, B, C and are the distribution curves of MWCNT-7, TiO<sub>2</sub>, SiO<sub>2</sub> and C60, respectively. Mean size and standard deviation for each kind of the ENMs are shown in the table (E)

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**Table S1 LC-MS analysis of the nanomaterial-bound proteins**

	<b>Source</b>	<b>No.</b>	<b>Proteins</b>
<b>BALF</b>	Blood-derived	26	albumin, hemoglobin subunit alpha-1/2, hemoglobin subunit beta-1, hemoglobin subunit beta-2, hemopexin, Ig gamma-2B chain C region, alpha-1-macroglobulin, murinoglobulin-1, Igh-6, Ig kappa chain C region, IgG-2a, Ig lambda-2 chain C region, immunoglobulin joining chain, serotransferrin, T-kininogen 1, T-kininogen 2, BWK3, globin c2, ceruloplasmin, 14-3-3, apolipoprotein E, apolipoprotein A-I, vitamin D-binding protein, serine protease inhibitor, superoxide dismutase, fibrinogen gamma chain
	Lung/respiratory tract-derived	8	uteroglobin, LOC367586, pulmonary surfactant-associated protein B, lysozyme, pulmonary surfactant-associated protein D, polymeric immunoglobulin receptor, triosephosphate isomerase, beta-actin
	Other	16	11 uncharacterized proteins, alpha-1-antiproteinase, S100-6A, kng2, alpha-2-HS-glycoprotein, haptoglobin
<b>M7</b>	Blood-derived	28	hemoglobin subunit beta-2, hemoglobin subunit alpha-1/2, hemoglobin subunit beta-1, Hemopexin, apolipoprotein C-I, apolipoprotein A-I, apolipoprotein A-IV, apolipoprotein E, Ig gamma-2B chain C region, immunoglobulin joining chain, Ig lambda-2 chain C region, Ig kappa chain C region, Igh-6, albumin, fibrinogen alpha chain, fibrinogen gamma chain, fibrinogen beta chain, 0 beta-2 globin, inter-alpha-trypsin inhibitor heavy chain, serotransferrin, BWK3, T-kininogen 2, T-kininogen 1, complement C3, transthyretin, heat shock cognate 71 kDa protein, HSP 90-alpha, HSP 90-beta
	Lung/respiratory tract-derived	7	uteroglobin, LOC367586, pulmonary surfactant-associated protein D, pulmonary surfactant-associated protein B, lysozyme, destrin, annexin A1,
	Other	15	4 uncharacterized proteins, beta-actin, glyceraldehyde-3-phosphate dehydrogenase, elongation factor 1-alpha, S100-A6, clusterin, alpha-2-HS-glycoprotein, kng2, ras-related C3 botulinum toxin substrate 1, galectin-1, septin-9, fibronectin
<b>TiO<sub>2</sub></b>	Blood-derived	29	hemoglobin subunit alpha-1/2, hemoglobin subunit beta-2, hemoglobin subunit beta-1, Ig gamma-2B chain C region, Ig kappa chain C region, Igh-6, Ig lambda-2 chain C region, complement component C9, complement C4, complement factor B, complement inhibitory factor H, apolipoprotein E, apolipoprotein C-I, apolipoprotein A-IV,

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			apolipoprotein A-I, HSP 90-alpha, HSP 90-beta, heat shock cognate 71 kDa protein, 0 beta-2 globin, fibrinogen gamma chain, albumin, globin a4, fibrinogen beta chain, fibrinogen alpha chain, globin c2, beta-2-glycoprotein 1, inter-alpha-trypsin inhibitor heavy chain, BWK3, serine protease inhibitor
	Lung/respiratory tract-derived	8	LOC367586, s100-A9, lysozyme, annexin A1, adenylyl cyclase-associated protein 1, annexin A2, uteroglobin, beta-actin
	Other	13	3 uncharacterized protein, s100-A6, elongation factor 1-alpha, glyceraldehyde-3-phosphate dehydrogenase, histidine-rich glycoprotein, four and a half LIM domains 1, protein disulfide-isomerase, kng2, elongation factor 2, ras-related C3 botulinum toxin substrate 1, fibronectin
<b>SiO<sub>2</sub></b>	Blood-derived	28	hemoglobin subunit beta-1, hemoglobin subunit alpha-1/2, hemoglobin subunit beta-2, Ig gamma-2B chain C region, Ig kappa chain C region, Igh-6, Ig lambda-2 chain C region, apolipoprotein A-I, apolipoprotein E, apolipoprotein A-IV, apolipoprotein C-I, complement C4, complement component C9, complement inhibitory factor H, heat shock cognate 71 kDa protein, HSP 90-alpha, HSP 90-beta albumin, fibrinogen beta chain, beta-2-glycoprotein 1, fibrinogen alpha chain, fibrinogen gamma chain, 0 beta-2 globin, inter-alpha-trypsin inhibitor heavy chain, globin c2, serotransferrin, transthyretin, serine protease inhibitor
	Lung/respiratory tract-derived	9	lysozyme, LOC367586, adenylyl cyclase-associated protein 1, uteroglobin, pulmonary surfactant-associated protein D, annexin A1, pulmonary surfactant-associated protein B, glyceraldehyde-3-phosphate dehydrogenase, beta-actin
	Other	13	3 uncharacterized protein, elongation factor 1-alpha, histidine-rich glycoprotein, four and a half LIM domains 1, elongation factor 2, s100-A6, ras-related C3 botulinum toxin substrate 1, clusterin, kng2, fibronectin, alpha-2-HS-glycoprotein
<b>C60</b>	Blood-derived	27	hemoglobin subunit beta-2, hemoglobin subunit alpha-1/2, hemoglobin subunit beta-1, apolipoprotein A-I, apolipoprotein C-I, apolipoprotein E, apolipoprotein A-IV, Ig kappa chain C region, Igh-6, Ig gamma-2B chain C region, Ig lambda-2 chain C region, immunoglobulin joining chain, complement C3, complement component C9, beta-2-glycoprotein 1, HSP 90-alpha, heat shock cognate 71 kDa protein, HSP 90-beta, fibrinogen beta chain, fibrinogen gamma chain, fibrinogen alpha chain,

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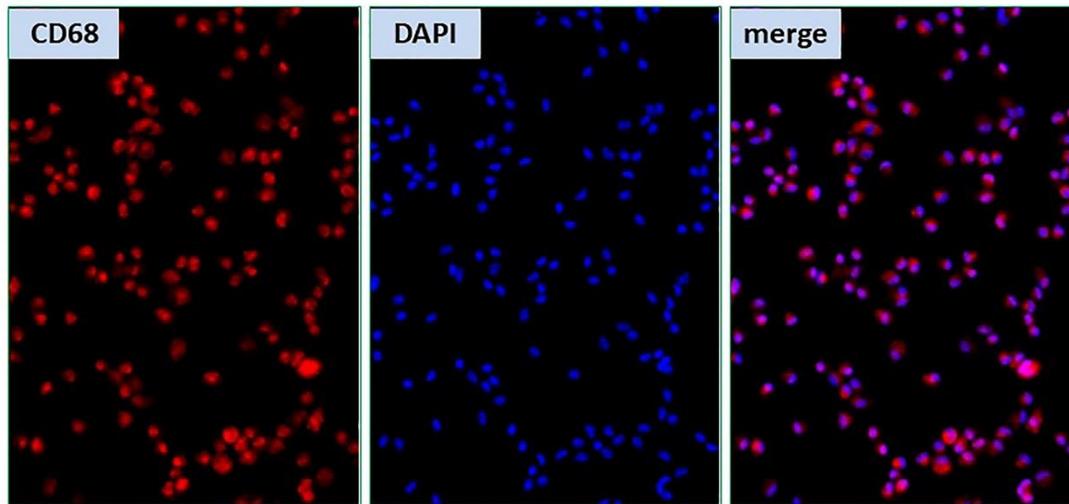
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		albumin, globin c2, 0 beta-2 globin, BWK3, plasminogen, globin a4
Lung/respiratory tract-derived	11	lysozyme, pulmonary surfactant-associated protein D, uteroglobin, LOC367586, annexin A2, annexin A1, pulmonary surfactant-associated protein B, pulmonary surfactant-associated protein A, S100-A9, beta-actin, glyceraldehyde-3-phosphate dehydrogenase
Other	12	3 uncharacterized protein, elongation factor 1-alpha, S100-A6, fibronectin, kng2, clusterin, septin-9, alpha-2-HS-glycoprotein, histidine-rich glycoprotein, ras-related C3 botulinum toxin substrate 1

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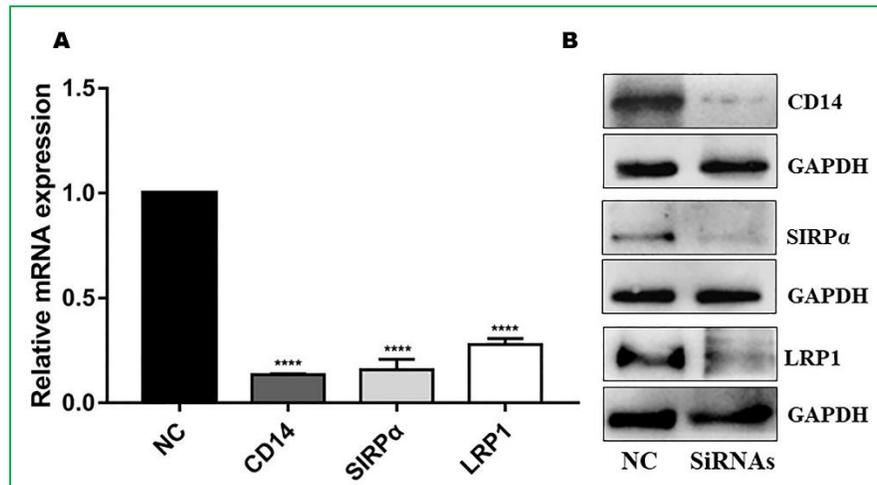
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**Figure S4 Immunostaining of primary alveolar macrophages**

The isolated primary alveolar macrophages were immune-stained with CD68, a macrophage marker, to confirm their identity. The nucleus was stained with DAPI. The images were observed and captured using the fluorescence microscope (ZEISS LSM880+Airyscan, Germany)

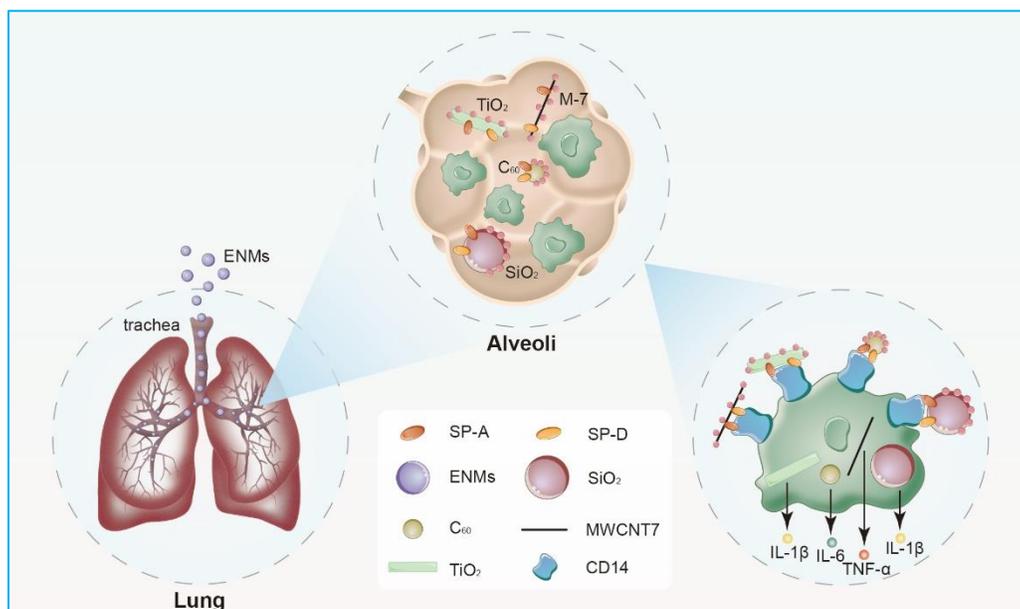
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**Figure S5 Knockdown of CD14, LRP1 and SIRP $\alpha$  expression in primary alveolar macrophages**

CD14, LRP1 and SIRP $\alpha$  expression in primary alveolar macrophages was knocked down by the gene-specific siRNAs. The silencing efficacy for LRP1, CD14 and SIRP $\alpha$  was analyzed by qPCR (A), and confirmed by western blotting (B).

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**Figure S6 Summarized results**