Supplementary Material

This supplementary material contains 11 figures and 1 table:

Figure S1 Shapes of MWCNT-7, TiO₂, SiO₂ and C60 in the suspension;

Figure S2 Chemical composition of MWCNT-7, TiO₂, SiO₂ and C60;

Figure S3 Size distribution of MWCNT-7, TiO₂, SiO₂ and C60 in the suspension;

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Figure S8 Effects of SP-A, SD-D, BALF and ENMs on expression of cytokines;

Figure S9 Knockdown of CD14, LRP1, and SIRPα expression in PAMs;

Figure S10 Effects of SP-A, SD-D, BALF and ENMs on expression of cytokines in the receptors-knocked down PAMs;

Figure S11 Summary of the results;

Table S1 LC-MS analysis of the nanomaterial-bound proteins.
Figure S1 Shapes of MWCNT-7, TiO₂, SiO₂, and C60 in the suspension

MWCNT-7, TiO₂, SiO₂, and C60 were suspended in saline containing 0.5% (w/v) Pluronic® F-68 to a final concentration of 500 μg/ml. Suspensions were diluted with ddH₂O 1:200 and placed on a carbon sheet. After air-drying, the samples were observed under the JEM-2100 transmission electron microscope (JEOL Co. Ltd, Tokyo, Japan).
Figure S2 Chemical composition of MWCNT-7, TiO₂, SiO₂, and C60s

Suspensions of MWCNT-7, TiO₂, SiO₂, and C60 (500 µg/ml) were diluted with ddH₂O 1:200 and placed on an aluminum plate. After air-drying, the samples were observed and analyzed by FIB-SEM (Helios G4 UC, Aztec) connected with an EBSD analysis system (Thermo Scientific). A, B, C, and D represent the spectra of MWCNT-7, TiO₂, SiO₂, and C60, respectively. The table under the spectra is the element composition and ratio of each of the ENMs.

| Element | M7   | TiO₂ | SiO₂ | C60   |
|---------|------|------|------|-------|
| C       | 100.00 | 0.74 | 1.96 | 100.00 |
| O       | 37.36 | 49.71|      |       |
| Ti      | 61.90 |      |      |       |
| Si      |       |      | 48.33|       |
Figure S3 Size distribution of MWCNT-7, TiO$_2$, SiO$_2$, and C60 in the suspension

1.6 ml of the four ENM suspensions (equal to 800 µg of each ENM) were added with 1 ml of saline or the 20-fold concentrated rat BALF, and incubated at 37°C in a shaker at 200 rpm for 4-6 hours. Size distribution in 500 µg/ml suspensions of MWCNT-7, TiO$_2$, SiO$_2$ and C60 in the absence (A, C, E and G) or presence (B, D, F and H) of BALF were analyzed with a NanoSight 300 analyzer (Malvern). The representative size distribution curves are shown in figures A and B for MWCNT-7, C and D for TiO$_2$, E and F for SiO$_2$, and G and H for C60, respectively. The mean size and standard deviation (s.d.) for each kind of the ENMs are displayed in the table (E)
1.0 ml of black ink was sprayed into the lung of a 6-week-old SD rat using an intratracheal aerosolizer (series IA-1B, Penn-century, Philadelphia, USA). A. the intratracheal aerosolizer; B. the sprayed ink was evenly distributed in each lobe of the lung; and C. light microscopy showed the sprayed ink in the lung tissue.

**Figure S4 Intratracheal spraying**
The bound proteins in each group were analyzed by LC/MS. The top 120 proteins in the table are listed by values of IBAQ (intensity-based absolute-protein-quantification) indicating abundance of each bound protein.

| No. | MWCNT-7                          | TiO2                      | SiO2                               | C60                                 |
|-----|----------------------------------|---------------------------|------------------------------------|-------------------------------------|
| 1   | Hemoglobin subunit beta-2        | Hemoglobin subunit alpha-1/2 | Glyceraldehyde-3-phosphate          | Hemoglobin subunit beta-2           |
| 2   | Hemoglobin subunit alpha-1/2     | Hemoglobin subunit beta-2  | Hemoglobin subunit beta-1          | Beta-actin                          |
| 3   | Beta-actin                       | Uncharacterized protein    | Elongation factor 1-alpha          | Hemoglobin subunit alpha-1/2        |
| 4   | Glyceraldehyde-3-phosphate       | Hemoglobin subunit beta-1  | Beta-actin                         | Fibrinogen beta chain               |
| 5   | Apolipoprotein A-II              | Beta-actin                | Hemoglobin subunit alpha-1/2       | Anionic trypsin-1                   |
| 6   | Serum albumin                    | Elongation factor 1-alpha  | Protein S100-A8                    | Fibrinogen gamma chain              |
| 7   | Uncharacterized protein          | Glyceraldehyde-3-phosphate dehydrogenase | Apolipoprotein A-I                 | Glyceraldehyde-3-phosphate dehydrogenase |
| 8   | Apolipoprotein C-II              | Uncharacterized protein    | Eukaryotic translation initiation factor | Fibrinogen alpha chain              |
| 9   | Uteroglobin                      | Protein S100-A6           | Uncharacterized protein            | Uncharacterized protein             |
| 10  | Elongation factor 1-alpha        | LOC367586 protein         | T-complex protein 1 subunit beta   | Beta-defensin                       |
| 11  | Apolipoprotein C-I               | Histidine-rich glycoprotein | Apolipoprotein C-III               | Elongation factor 1-alpha           |
| 12  | Hemoglobin subunit beta-1        | Protein S100-A9           | Hemoglobin subunit beta-2          | Apolipoprotein A-II                 |
| 13  | Heat shock 27kDa protein 1       | Lysozyme                  | Transketolase                      | Myosin light polypeptide 6          |
| 14  | LOC367586 protein                | Apolipoprotein H           | Apolipoprotein E                   | Hemoglobin subunit beta-1           |
| 15  | Beta-defensin 4                  | Apolipoprotein A-II       | Histidine-rich glycoprotein        | Lysozyme                            |
| 16  | Protein S100                     | Protein S100-A8           | Tubulin beta chain                 | Heat shock protein beta-1           |
| Number | Protein Name                                   | Protein Name                                      | Protein Name                                      | Pulmonary surfactant-associated protein D          |
|--------|-----------------------------------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
| 17     | Lysozyme                                      | Apolipoprotein E                                 | Apolipoprotein A-IV                               | Myosin regulatory light chain 12B                 |
| 18     | BPI fold-containing family A member 1         | Tubulin polymerization-promoting protein family member 3 | Apolipoprotein C-I                                | Lysozyme                                         |
| 19     | Apolipoprotein A-I                            | Ig gamma-2B chain C region                       | Lysozyme                                         | Apolipoprotein C-II                              |
| 20     | Apolipoprotein C-III                          | Zero beta-globin                                 | LOC367586 protein                                | Myosin, heavy polypeptide 9                      |
| 21     | Glutathione peroxidase 3                      | Four and a half LIM domains 1                    | Serum albumin                                    | Tubulin beta-4B chain                            |
| 22     | Fibrinogen alpha chain                        | Ig kappa chain C region                          | Lipoprotein lipase                               | Apolipoprotein A-I                               |
| 23     | Tubulin beta chain                            | Annexin A1                                       | Heat shock 27kDa protein 1                       | Apolipoprotein C-I                               |
| 24     | Fibrinogen gamma chain                        | Heat shock 27kDa protein 1                       | Adenylyl cyclase-associated protein 1            | Apolipoprotein E                                 |
| 25     | Uncharacterized protein                       | Beta-defensin 4                                  | Beta-defensin 4                                  | Uteroglobin                                      |
| 26     | ADP-ribosylation factor 5                     | Igh-6 protein                                    | Apolipoprotein A-II                              | Protein S100-A6                                  |
| 27     | Fibrinogen beta chain                         | Transketolase                                    | Complement C4                                    | Globin c2                                        |
| 28     | Ig gamma-2B chain C region                    | GTP-binding nuclear protein Ran                  | Uteroglobin                                      | LOC367586 protein                                |
| 29     | Zero beta-globin                              | Fibrinogen gamma chain                           | Transketolase                                    | Fibronectin                                      |
| 30     | Pneumo secretory protein 1                    | Complement component C9                          | Transthyretin                                    | Apolipoprotein A-IV                              |
| 31     | Clusterin                                      | Tubulin beta chain                               | BPI fold-containing family A member 1            | Serum albumin                                    |
| 32     | ADP-ribosylation factor 3                     | Globin a4                                        | Fibrinogen beta chain                            | BPI fold-containing family A member 1            |
| 33     | Ig lambda-2 chain C region                    | Uncharacterized protein                          | ADP-ribosylation factor 5                        | Ribosomal protein S19-like2                      |
| 34     | Uncharacterized protein                       | Fibrinogen beta chain                            | Apolipoprotein C-II                              | Dermcidin                                        |
| 35     | Alpha-2-HS-glycoprotein                       | Apolipoprotein C-I                               | Heat shock cognate 71 kDa protein                | Zero beta-globin                                 |
| 36     | Keratin, type I cytoskeletal                  | Fibrinogen alpha chain                           | Ig gamma-2B chain C region                       | Kng2 protein                                     |
| 37     | Myosin light polypeptide 6                    | Eukaryotic translation initiation factor 5A-1    | Apolipoprotein H                                 | ADP-ribosylation factor 5                        |
| 38     | Inter alpha-trypsin inhibitor, heavy chain 4  | Globin c2                                        | Fibrinogen alpha chain                           | GTP-binding nuclear protein                      |
|   | Protein Name                                      | Protein Name                                      | Protein Name                                      | Protein Name                                      |
|---|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
| 39| Apolipoprotein A-IV                              | Apolipoprotein A-IV                              | Fibrinogen gamma chain                           | Ig kappa chain C region                          |
| 40| Serotransferrin                                  | Coagulation factor XII                           | Four and a half LIM domains 1                    | Barrier-to-autointegration factor                 |
| 41| Apolipoprotein E                                 | Cofilin-1                                        | Beta-2 globin                                    | Lymphocyte antigen 6B                            |
| 42| Kng2 protein                                     | Actin, alpha cardiac muscle 1                    | Serine protease inhibitor                        | Histone cluster 1 H1 family member               |
| 43| Ribosomal protein S19-like2                      | Apolipoprotein A-I                               | Inter alpha-trypsin inhibitor, heavy chain 4     | Apolipoprotein C-III                             |
| 44| Ras-related C3 botulinum toxin substrate         | Peroxiredoxin-2                                  | Adiponectin c                                    | Uncharacterized protein                          |
| 45| Cell division control protein 42 homolog         | Vitronectin                                      | ADP-ribosylation factor 3                        | Clustatin                                        |
| 46| Tubulin beta-5 chain                             | Myosin light polypeptide 6                       | Myosin light polypeptide 6                       | Uncharacterized protein                          |
| 47| BWK3                                             | Peroxiredoxin-4                                  | Actin, alpha cardiac muscle 1                    | 40S ribosomal protein S3                         |
| 48| **Pulmonary surfactant-associated protein D**     | Apolipoprotein C-II                              | 40S ribosomal protein S12                        | Uncharacterized protein                          |
| 49| 40S ribosomal protein S3                         | Complement C4                                    | Uncharacterized protein                          | RAT Igh-6 protein                                |
| 50| Transforming protein RhoA                        | Complement inhibitory factor H                   | Tubulin polymerization-promoting protein family 3| Glutathione peroxidase 3                         |
| 51| 40S ribosomal protein S12                        | Ig lambda-2 chain C region                       | Dermcidin                                        | Transforming protein RhoA                        |
| 52| Uncharacterized protein                          | 40S ribosomal protein S12                        | Complement inhibitory factor H                   | Tissue-type transglutaminase                     |
| 53| Ig kappa chain C region                          | Protein disulfide-isomerase                      | Elongation factor 2                              | RAT Ig gamma-2B chain C region                   |
| 54| Serine/cysteine peptidase inhibitor, clade C, member 1 | 40S ribosomal protein S3                            | Peroxiredoxin-2                                  | BWK3                                             |
| 55| Histone cluster 1 H1 family member               | Serine protease inhibitor                        | Translationally-controlled tumor protein         | High mobility group box 1                        |
| 56| 40S ribosomal protein S4                         | Cysteine and glycine-rich protein 1              | Carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and | Annexin A2                                      |
| 57| Platelet factor 4                                | Ezrin                                            | Protein S100-A6                                  | Ras-related protein Rap-1b                       |
| 58| GTP-binding nuclear protein Ran                  | C-type lectin domain family 3, member B          | Complement component C9                          | Septin-9                                        |
| 59| 40S ribosomal protein S14                        | Kng2 protein                                     | Ribosomal protein S19-like2                      | Protein S100-A11                                 |
| 60| Ras-related protein Rap-1b                       | Elongation factor 2                              | GTP-binding nuclear protein Ran                  | Ig lambda-2 chain C region                       |
|   | Gene Name                                      | Description                                                                 |
|---|-----------------------------------------------|-----------------------------------------------------------------------------|
| 61| Myosin, heavy polypeptide 9                   | Ig gamma-2C chain C region                                                  |
| 62| Actin, alpha cardiac muscle 1                 | Heat shock protein HSP 90-alpha                                             |
| 63| SAR1 gene homolog A                           | BPI fold-containing family A member 1                                      |
| 64| BPI fold-containing family B member 1         | Ras-related C3 botulinum toxin substrate 1                                 |
| 65| Hemopexin                                     | Ribosomal protein S19-like2                                                 |
| 66| Transthyretin                                 | Transforming protein RhoA                                                   |
| 67| Igh-6 protein                                 | Translationally-controlled tumor protein                                    |
| 68| Poly(rC)-binding protein 2                    | Heat shock cognate 71 kDa protein                                           |
| 69| 60S ribosomal protein L11                     | Inter alpha-trypsin inhibitor, heavy chain                                  |
| 70| Destrin                                       | Heat shock protein HSP 90-beta                                              |
| 71| RAB1A, member RAS oncogene family             | Myosin regulatory light chain 12B                                           |
| 72| Uncharacterized protein                       | Protein S100-A11                                                           |
| 73| Cofilin-1                                     | Adenyl cyclase-associated protein 1                                         |
| 74| Uncharacterized protein                       | Glutathione peroxidase 3                                                   |
| 75| RAB5C, member RAS oncogene family             | Serum albumin                                                              |
| 76| Glutathione peroxidase 1                      | Uncharacterized protein                                                     |
| 77| 40S ribosomal protein S5                      | 40S ribosomal protein S18                                                  |
| 78| Dermcidin                                     | Apolipoprotein C-III                                                       |
| 79| Heat shock cognate 71 kDa protein             | Cysteine-rich protein 1                                                     |
| 80| 40S ribosomal protein S7                      | Complement factor B                                                         |
| 81| 40S ribosomal protein S3a                     | Fibronectin                                                                |
| 82| Uncharacterized protein                       | Eukaryotic translation initiation factor 1                                 |

**Notes:**
- **Histone H4**
- **Pneumo secretory protein 1**
- **Pulmonary surfactant-associated protein D**
- **60S ribosomal protein L22**
- **ATP-binding cassette sub-family B member 9**
- **Platelet factor 4**
- **Alpha-2-HS-glycoprotein**
- **40S ribosomal protein S6**
- **Secretoglobin, family 3A, member 1**
- **RAB1A, member RAS oncogene family**
- **Annexin A1**
- **Calcium-binding protein 4**
- **Peroxiredoxin-1**
- **Cell division control protein 42 homolog**
- **Myosin light chain 1/3**
- **Globin c2**
- **Histidine-rich glycoprotein**
| No. | Protein Name                  | Protein Name                  | Protein Name                  |
|-----|------------------------------|------------------------------|------------------------------|
| 83  | Annexin A1                   | Annexin A2                   | Proliferation-associated protein 2G4 |
| 84  | Galectin-1                   | Uncharacterized protein      | Uncharacterized protein      |
| 85  | 60S ribosomal protein L22    | Uncharacterized protein      | Fibronectin                   |
| 86  | Heat shock protein HSP 90-alpha | Moesin                      | Cysteine and glycine-rich protein 1 |
| 87  | Heat shock protein HSP 90-beta | BWK3                        | Annexin A1                   |
| 88  | Protein S100-A11             | Uncharacterized protein      | Alpha-2-HS-glycoprotein       |
| 89  | Prothrombin                  | ADP-ribosylation factor 5    | Histone cluster 1 H1 family member d |
| 90  | Heterogeneous nuclear ribonucleoproteins A2/B1 | Uncharacterized protein | Heat shock protein HSP 90-alpha |
| 91  | Myosin regulatory light chain 12B | Dynein light chain 1         | GTP-binding protein SAR1b     |
| 92  | Kininogen-1                  | Inter-alpha-trypsin inhibitor heavy chain 2 | Uncharacterized protein |
| 93  | Uncharacterized protein      | Uteroglobin                  | Heat shock protein HSP 90-beta |
| 94  | Secretoglobin, family 3A, member 1 | Adiponectin               | Pulmonary surfactant-associated protein B |
| 95  | Uncharacterized protein      | Uncharacterized protein      | TNF alpha-induced protein 8   |
| 96  | Immunoglobulin joining chain | Complement C3                | Myosin, heavy polypeptide 9   |
| 97  | ADP-ribosylation factor 4    | Myristoylated alanine-rich C-kinase substrate | 40S ribosomal protein S4 |
| 98  | 40S ribosomal protein S6     | EH domain-containing protein 1 | Cofilin-1                    |
| 99  | Uncharacterized protein      | 40S ribosomal protein S14    | Uncharacterized protein       |
| 100 | 40S ribosomal protein S25    | Selenoprotein P              | BPI fold-containing family B member 1 |
| 101 | Vimentin                     | Actin-related protein 2      | Serine/cysteine peptidase inhibitor, clade C member 1 |
| 102 | T-kininogen 1                | 40S ribosomal protein S3a    | Uncharacterized protein       |

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| No. | Protein Name                  | Protein Name                  | Protein Name                  |
|-----|------------------------------|------------------------------|------------------------------|
| 103 | Actin, alpha cardiac muscle 1 | Uncharacterized protein      | Uncharacterized protein      |
| 104 | Fibrinogen                    | Uncharacterized protein      | Cytochrome b5                 |
| 105 | Uncharacterized protein       | 40S ribosomal protein S17-like | Myosin heavy chain 14 |
| 106 | Heat shock protein HSP 90-alpha | BWK3                        | 40S ribosomal protein S18     |
| 107 | Uncharacterized protein       | ADP-ribosylation factor 3    | Uncharacterized protein       |
| 108 | ADP-ribosylation factor 4     | ADP-ribosylation factor 4     | Uncharacterized protein       |
| 109 | Pulmonary surfactant-associated protein B | Globin a4 |
| 110 | TNF alpha-induced protein 8   | 40S ribosomal protein S5     |
| 111 | Myosin, heavy polypeptide 9   | 60S ribosomal protein L11    |
| 112 | 40S ribosomal protein S4      | 40S ribosomal protein S4     |
| 113 | Glutathione peroxidase 1      | Pulmonary surfactant-associated protein B |
| 114 | Uncharacterized protein       | Complement C1q subcomponent subunit B |
| 115 | 40S ribosomal protein S3a     | Complement C1q subcomponent subunit B |
| 116 | Uncharacterized protein       | Complement C1q subcomponent subunit B |

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| ID | Name of Protein                                                                 | Description                                                                 | ID | Name of Protein                                                                 | Description                                                                 |
|----|--------------------------------------------------------------------------------|-----------------------------------------------------------------------------|----|--------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| 103| Elongation factor 1-delta                                                      | Serine/cysteine peptidase inhibitor, clade C member 1                        | 107| RCG31562, isoform CRA_c                                                       | Ube2l3 protein                                                               |
| 104| Septin-9                                                                      | Retinal dehydrogenase 1                                                     | 108| Uncharacterized protein                                                        | Actin-related protein 2/3 complex subunit 4                                 |
| 105| T-complex protein 1 subunit beta                                              | Myosin, heavy polypeptide 9                                                 | 109| Myristoylated alanine-rich C-kinase substrate                                 | C4b-binding protein alpha chain                                              |
| 106| Complement C3                                                                 | Inter-alpha trypsin inhibitor, heavy chain 1                                | 110| Similar to 60S acidic ribosomal protein P2                                     | Adiponectin c                                                               |
| 107| RCP31562, isoform CRA_c                                                       | Ube2l3 protein                                                               | 111| Rps16 protein                                                                 | Actin-related protein 3                                                      |
| 108| Uncharacterized protein                                                        | Actin-related protein 2/3 complex subunit 4                                 | 112| Pulmonary surfactant-associated protein B                                     | Pulmonary surfactant-associated protein D                                   |
| 109| Myristoylated alanine-rich C-kinase substrate                                 | C4b-binding protein alpha chain                                              | 113| Vitronectin                                                                   | Plasminogen                                                                 |
| 110| Similar to 60S acidic ribosomal protein P2                                     | Adiponectin c                                                               | 114| Adenine phosphoribosyltransferase                                             | 40S ribosomal protein S25                                                    |
| 111| Rps16 protein                                                                 | Actin-related protein 3                                                      | 115| Uncharacterized protein                                                        | Small ubiquitin-related modifier 2                                           |
| 112| Pulmonary surfactant-associated protein B                                     | Pulmonary surfactant-associated protein D                                   | 116| Fibronectin                                                                   | Coronin-1A                                                                  |
| 113| Vitronectin                                                                   | Plasminogen                                                                 | 117| Dynein light chain 1                                                          | Prothrombin                                                                 |
| 114| Adenine phosphoribosyltransferase                                             | 40S ribosomal protein S25                                                   | 118| Calpain small subunit 1                                                       | Uncharacterized protein                                                      |
| 115| Uncharacterized protein                                                        | Small ubiquitin-related modifier 2                                           | 119| Murinoglobulin-1                                                              | Immunoglobulin joining chain                                                |
| 116| Fibronectin                                                                   | Coronin-1A                                                                  | 120| Barrier-to-autointegration factor                                              | 60S ribosomal protein L5                                                     |
| 117| Dynein light chain 1                                                          | Prothrombin                                                                 | 121| Calpain small subunit 1                                                       | Uncharacterized protein                                                      |
| 118| Calpain small subunit 1                                                       | Uncharacterized protein                                                     | 122| Murinoglobulin-1                                                              | Immunoglobulin joining chain                                                |
| 121| Calpain small subunit 1                                                       | Uncharacterized protein                                                     | 123| Barrier-to-autointegration factor                                              | 60S ribosomal protein L5                                                     |
| 122| Murinoglobulin-1                                                              | Immunoglobulin joining chain                                                | 124| Calpain small subunit 1                                                       | Uncharacterized protein                                                      |
| 123| Barrier-to-autointegration factor                                              | 60S ribosomal protein L5                                                    | 125| Murinoglobulin-1                                                              | Immunoglobulin joining chain                                                |

**Table Notes:**
- **Elongation factor 1-delta**
- **Serine/cysteine peptidase inhibitor, clade C member 1**
- **Eukaryotic peptide chain release factor GTP-binding subunit ERF3B-like**
- **BPI fold-containing family B member 1**
- **Retinal dehydrogenase 1**
- **Ig lambda-2 chain C region**
- **Uncharacterized protein**
- **Myosin, heavy polypeptide 9**
- **Activator of Hsp90 ATPase activity 1**
- **Serine/cysteine peptidase inhibitor, clade C (Antithrombin), member 1**
- **Inter-alpha trypsin inhibitor, heavy chain 1**
- **40S ribosomal protein S25**
- **Heat shock protein HSP 90-alpha**
- **Ube2l3 protein**
- **40S ribosomal protein S5**
- **Poly(rC)-binding protein 2**
- **Actin-related protein 2/3 complex subunit 4**
- **Serotransferrin**
- **40S ribosomal protein S25**
- **Calcium-binding protein 4**
- **Pulmonary surfactant-associated protein A**
- **Adiponectin c**
- **Complement C3**
- **RAB5C**
- **Actin-related protein 3**
- **Vitronectin**
- **Immunoglobulin joining chain**
- **Plasminogen**
- **60S ribosomal protein L22**
- **Complement C3**
- **40S ribosomal protein S25**
- **Protein disulfide-isomerase**
- **Uncharacterized protein**
- **RAB1A**
- **40S ribosomal protein S7**
- **Complement component C9**
- **Small ubiquitin-related modifier 2**
- **Microsomal glutathione S-transferase**
- **Coronin-1A**
- **Prothrombin**
- **Rps16 protein**
- **Histone H2B**
- **Histone H4**
- **Rps16 protein**
- **Plasminogen activator inhibitor 1 RNA-binding protein**
- **Protein S100-A4**
Figure S5 Immunostaining of primary alveolar macrophages

Isolated primary alveolar macrophages were immunostained with CD68, a macrophage marker, to confirm their identity. The nucleus was stained with DAPI. The images were observed and captured using a fluorescence microscope (ZEISS LSM880+Airyscan, Germany)
**Figure S6 Uptake of ENMs by PAMs via phagocytosis**

$5 \times 10^5$ primary alveolar macrophages (PAMs) in a 6-well plate were treated with or without 5μg/ml cytochalasin B (Cyt B, an inhibitor of actin polymerization, Solarbio, Beijing, China) for 1h and then exposed with MWCNT-7, TiO$_2$, or SiO$_2$ pre-incubated with BALF for another 12h. The cells were fixed, eosin-stained and observed under an ECLIPSE polarizing microscope (LV100NPOL, Nikon, Japan). Percentages of the PAMs with burden particles (arrows) were calculated.
Figure S7 Binding of SP-A and SP-D to the ENMs

400 μl of each of 4 ENM suspensions (equal to 200 μg of each ENM) was incubated with 250 μl of the 10-fold concentrated BALF at 37°C in a shaker at 200 rpm for 6 hours, centrifuged at 4°C, 20000 × g, for 10 min. A. the precipitated ENMs were used for immunofluorescence staining and the supernatants were used for ELISA detection of SP-A and SP-D. Briefly, the precipitates were washed 3 times and incubated with rabbit anti-rat SP-A or SP-D antibodies (1:100 dilution, Affinity, Wuhan, China) at 4°C overnight. After washing, the ENMs were visualized with Cy3 labelled goat anti-rabbit IgG (1:100 dilution, Proteintech, Wuhan, China). Images were captured with a fluorescence microscope (ZEISS LSM880+Airyscan, Germany). B. SP-A and SP-D in the supernatants were detected using rat ELISA kits (MLBio, Shanghai, China) according to the manufacturer’s instructions. Two-tailed students’ t-test was used for statistical comparison, and *, ** and *** represent p values less than 0.05, 0.01 and 0.001, respectively.
Figure S8 Effects of SP-A, SD-D, BALF and ENMs on expression of cytokines

$1 \times 10^6$ primary alveolar macrophages (PAMs) in a 6-well plate were treated with saline, $1 \mu g/ml$ SP-A/SP-D, or $20 \mu l$ of 10-fold concentrated BALF for 12h (A); $1 \times 10^6$ PAMs were treated with saline containing 0.5% PF-68 (NC), $1 \mu g/ml$ of MWCNT-7, TiO$_2$, SiO$_2$, or C60 suspensions for 12h (B). Total RNAs were isolated for qRT-PCR analysis of IL-1$\beta$, TNF-$\alpha$ and IL-6, using GAPDH as an internal reference. Two-tailed students’ t-test was used for statistical comparison.
Figure S9 Knockdown of CD14, LRP1, and SIRPα expression in PAMs

CD14, LRP1, and SIRPα expression in primary alveolar macrophages was knocked down by gene-specific siRNAs. The silencing efficacy for LRP1, CD14, and SIRPα was analyzed by qPCR (A) and confirmed by western blotting (B).
Figure S10 Effects of SP-A, SD-D, BALF and ENMs on expression of cytokines in the receptors-knocked down PAMs

1×10^6 rat PAMs in a 6-well plate was transfected with negative control RNA, or gene-specific siRNAs for LRP1, CD14, and SIRPα and cultured at 37°C for 6h (A); and after the culture media was changed to X-VIVO™ serum-free medium (Lonza), the cells were treated with saline, 1μg/ml of SP-A or SP-D and cultured for 12h (B), or treated with saline containing 0.5% (NC), 1μg/ml of MWCNT-7, TiO2, SiO2, or C60 suspended in saline containing 0.5% for 12h. Total RNAs were isolated for qRT-PCR analysis of IL-1β, TNF-α and IL-6, using GAPDH as an internal reference. Two-tailed students’ t-test was used for statistical comparison.
Figure 11 Summarized results