Supplementary Information for

Targeting Delivery of Dexamethasone in Acute Pneumonia

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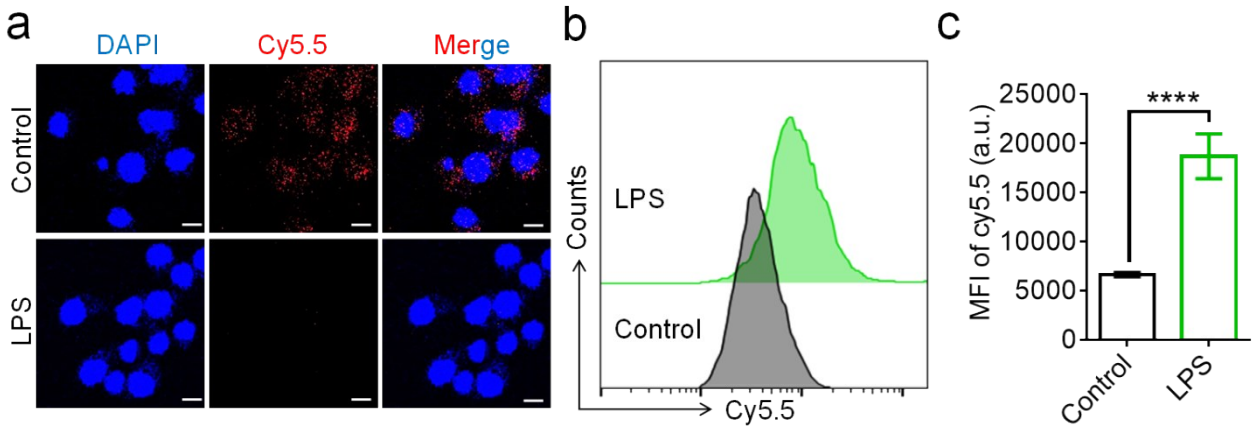
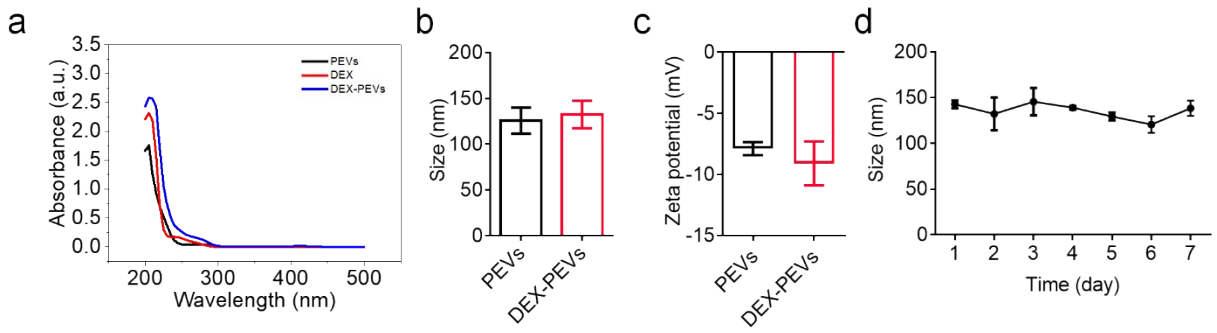
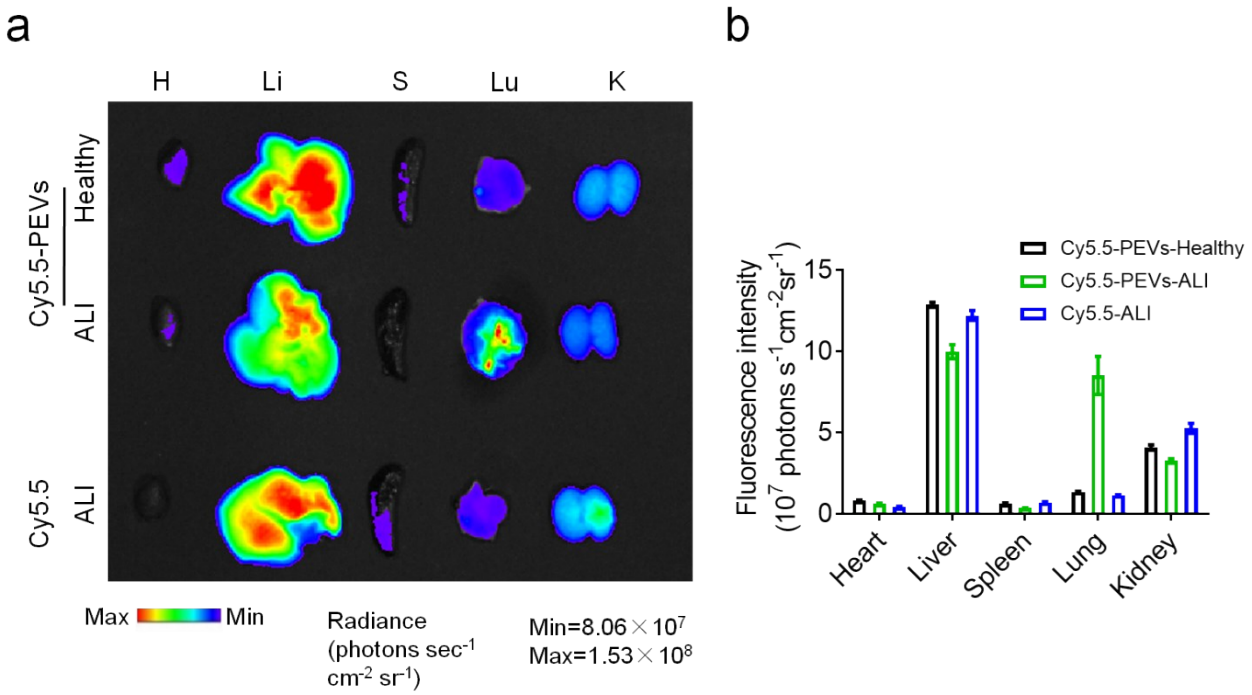
# Equally contribution



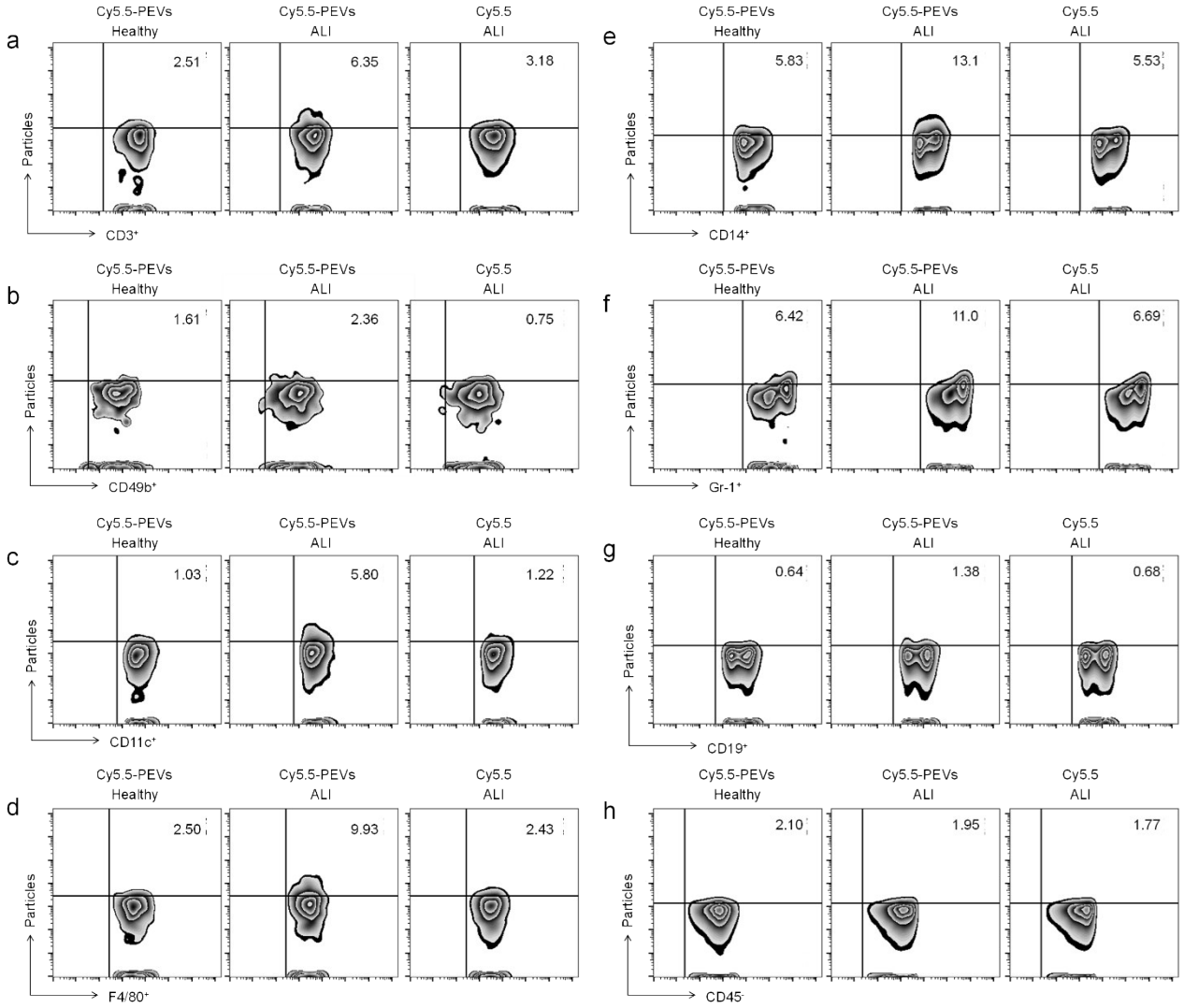
Supplementary Figure S1. (**a**) Confocal fluorescence imaging of RAW264.7 macrophages treated with or without LPS followed by treatment of Cy5.5-labeled PEVs. Scale bars, 20 μm. (**b**) Representative flow cytometric analysis of untreated and LPS-treated RAW264.7 macrophages adhered Cy5.5-labeled PEVs, and (**c**) corresponding quantification results of MFI of Cy5.5. Statistical significance was calculated by one-way ANOVA using the Tukey post-test (n=3). P-value: ****P<0.001. Data are means ± SEM.



Supplementary Figure S2. (a) Representative UV-vis absorption peaks of DEX, DEX-PEVs, PEVs in PBS. **(b)** The size of the PEVs and DEX loaded PEVs. (**c**) The zeta potential of the PEVs and DEX loaded PEVs. (**d**) Particle size of PEVs during incubation at 4 °C for 7 days in PBS. Data are means ± SEM. (n=3)



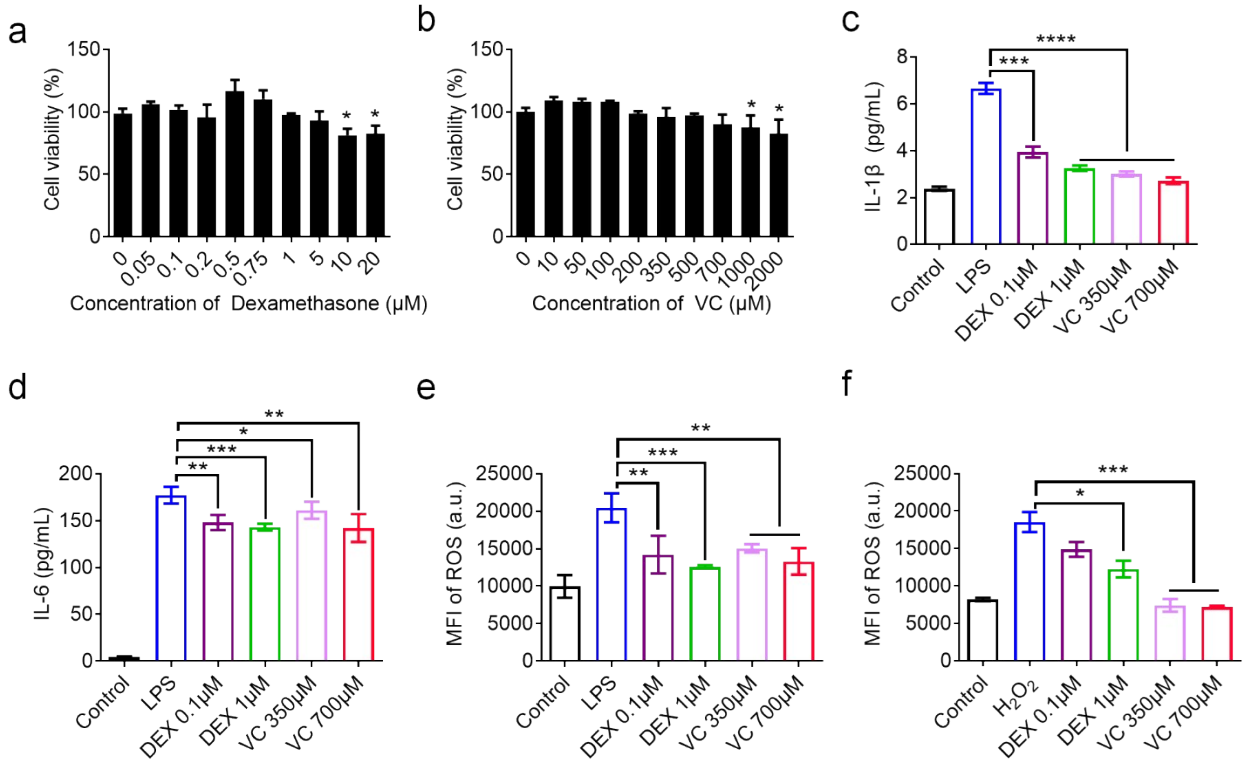
Supplementary Figure S3. (**a**) *Ex vivo* imaging showed biodistribution of Cy5.5 labeled PEVs and (**b**) corresponding quantitative data. Statistical significance was calculated by one-way ANOVA using the Tukey post-test (n=3-5). Data are means ± SEM.



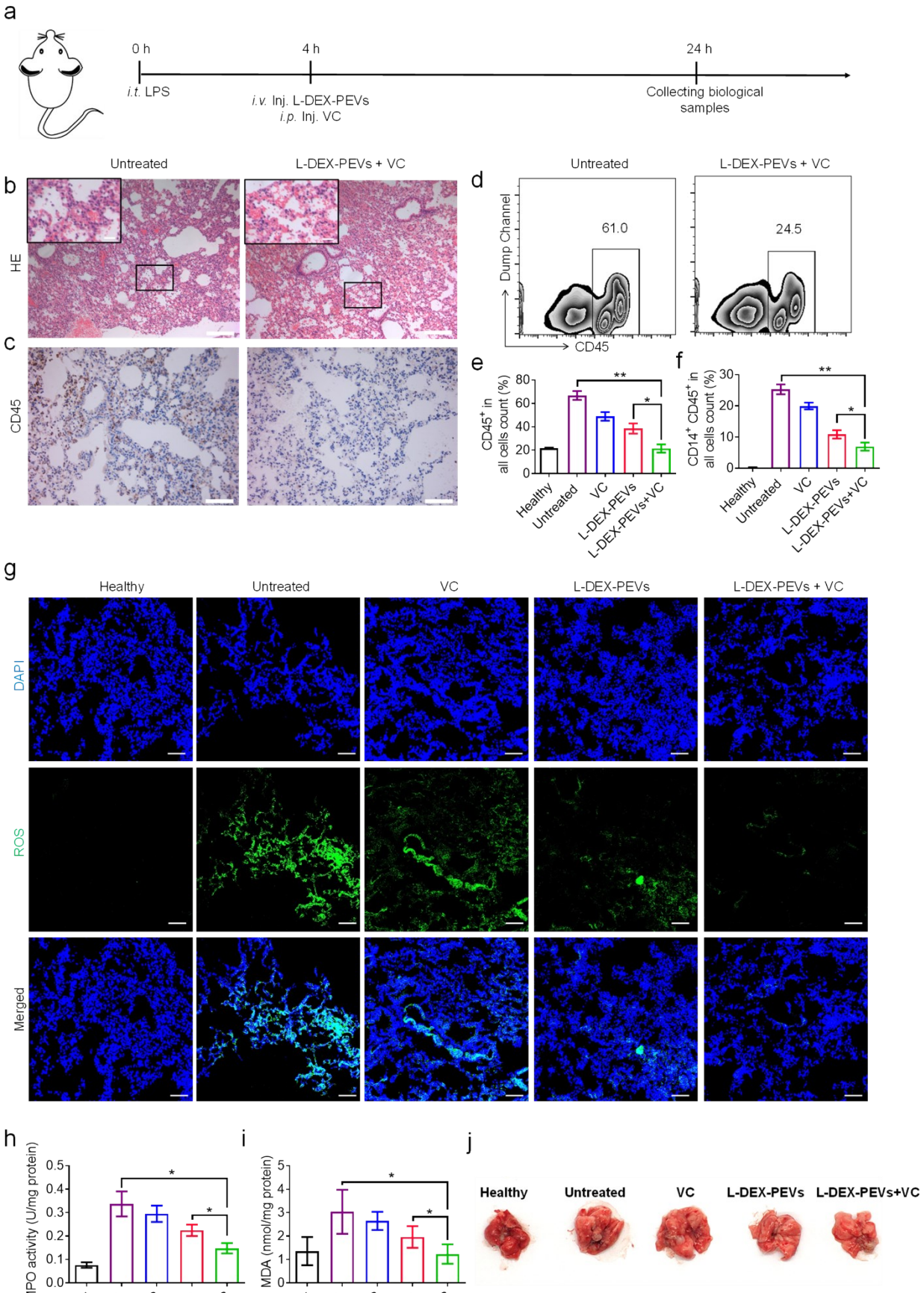
Supplementary Figure S4. (**a-h**) Representative plots of distribution of PEVs of each cellular component of the lungs by flow cytometry. (**a**) T cells ($CD45^+CD3^+$), (**b**) NK cells ($CD45^+CD49b^+$), (**c**) DCs ($CD45^+$ $CD11c^+$), (**d**) macrophages ($CD45^+$ $F4/80^+$), (**e**) monocytes ($CD45^+$ $CD14^+$), (**f**) granulocytes ($CD45^+CD11b^+$ $Gr\text{-}1^+$), (**g**) B cells ($CD45^+CD19^+$), (**h**) Other cells ($CD45^-$).


a
Healthy
Untreated
DEX+P
DEX-P
L-DEX-P
25.4
44.7
41.4
29.0
33.1
CD3
CD45
b
1.52
3.63
3.48
1.76
1.91
CD49b
CD45
c
0.74
2.29
1.91
1.42
1.81
CD11c
CD45
d
16.6
29.4
26.2
20.4
24.3
F4/80
CD45
e
2.01
59.0
42.6
24.5
33.2
CD14
CD45
f
2.27
7.87
12.9
77.0
38.0
32.2
9.43
20.4
32.8
29.7
9.71
27.9
37.8
18.9
18.3
24.9
29.0
24.6
12.1
34.3
Gr-1
CD11b
g
3.85
9.16
6.53
4.23
6.54
CD19
CD45

Supplementary Figure S5. (**a-g**) Representative plots of immune cells by flow cytometry. (**a**) T cells ($CD45^+$ $CD3^+$), (**b**) NK cells ($CD45^+$ $CD49b^+$), (**c**) DCs ($CD45^+$ $CD11c^+$), (**d**) macrophages ($CD45^+$ $F4/80^+$), (**e**) monocytes ($CD45^+$ $CD14^+$), (**f**) granulocytes ($CD45^+$ $CD11b^+$ $Gr\text{-}1^+$), (**g**) B cells ($CD45^+$ $CD19^+$).



Supplementary Figure S6. (**a**) Cell viability of RAW264.7 macrophages untreated or treated with different concentrations of dexamethasone. (**b**) Cell viability of RAW264.7 macrophages untreated or treated with different concentrations of vitamin C. (**c-d**) Production of IL-1β (**c**) and IL-6 (**d**) from activated macrophages supernatant after incubated with combinations of LPS (100 ng/mL), dexamethasone, vitamin C. (**e-f**) Representative expression of ROS in macrophages after various treatment as indicated. Statistical significance was calculated by one-way ANOVA using the Tukey post-test (n=3-5). P-value: **P*<0.05; ***P*<0.01; ****P*<0.005; *****P*<0.001. Data are means ± SEM.


a
0 h
4 h
24 h
i.t. LPS
i.v. Inj. L-DEX-PEVs
i.p. Inj. VC
Collecting biological samples
b
Untreated
L-DEX-PEVs + VC
HE
c
CD45
d
Untreated
L-DEX-PEVs + VC
Dump Channel
CD45
61.0
24.5
e
CD45+ in all cells count (%)
f
CD14+ CD45+ in all cells count (%)
Healthy
Untreated
VC
L-DEX-PEVs
L-DEX-PEVs+VC
g
Healthy
Untreated
VC
L-DEX-PEVs
L-DEX-PEVs + VC
DAPI
ROS
Merged
h
MPO activity (U/mg protein)
i
MDA (nmol/mg protein)
j
Healthy
Untreated
VC
L-DEX-PEVs
L-DEX-PEVs+VC

Supplementary Figure S7. Combined therapy of Vitamin C and DEX-PEVs. (**a**) Experimental schedule of the ALI model study. ALI mouse model was generated by intratracheally administration of LPS (8 mg/kg). Four hours after LPS challenge, mice were intravenously infusions with PBS, or L-DEX-PEVs, intraperitoneally injection vitamin C. Twenty hours post-administration of therapeutics, the mouse lungs were collected for therapeutic analysis. (**b**) Representative HE stained of lung sections after various treatment as indicated. Scale bars, 200 μm. (**c**) Representative immunohistochemistry images of CD45 immunoreactive cells in lung sections of different groups. Scale bars, 100 μm. (**d**) Representative plots of $CD45^+$ cells as a percentage of the total cell population and (**e**) corresponding quantification results. (**f**) The proportion of $CD14^+$ $CD45^+$ in all cells after various treatment. (**g**) Representative fluorescence images visualizing ROS. DCFH-DA and DAPI staining of lung tissues (blue, nuclei; green, ROS). Scale bars, 50 μm. (**h-i**) MPO, MDA level of lung tissue homogenate after various treatments as indicated. (**j**) Representative lung photographs. Statistical significance was calculated by one-way ANOVA using the Tukey post-test (n=3-5). P-value: **P*<0.05; ***P*<0.01. Data are means ± SEM.