Supporting Information

Venetoclax nanomedicine alleviates acute lung injury via increasing neutrophil apoptosis

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Materials and Methods

The antioxidative effect of PEGylated Poly(α-lipoic acid) (mPEG-PαLA) *in vitro*

Briefly, RAW264.7 cells were seeded into 6-well plates at a density of 1×10^5 cells per well and incubated for 12 h. Then, the cells were exposed to 1 μg mL^{-1} lipopolysaccharide (LPS) for 4 h. After that, mPEG-PαLA at a concentration of 1 mg mL^{-1} were added and incubated for another 8 h. The control group was incubated with the equal volume of PBS. Finally, the cells were collected and then incubated with 10 μM DCFH-DA (Sigma, USA) for 30 min at 37 °C. The levels of intracellular reactive oxygen species (ROS) in RAW264.7 cells were determined by flow cytometry (LSRFortessa X-20, BD, USA).

The stability of Nf-venetoclax in different solutions

The hydrodynamic size of Nf-venetoclax in phosphate buffered saline (PBS), 10% fetal bovine serum (FBS), and Dulbecco's Modified Eagle Medium (DMEM) was measured over time, respectively. Briefly, Nf-venetoclax was dispersed in PBS (pH 7.4), 10% FBS, and DMEM, respectively, and then stored in 4 ^oC refrigerator for 8 days. At different time points, 1 mL of the samples were withdrawn in triplicate, and the sizes of Nf-venetoclax in different solutions were measured using an Anton Paar Litesizer 500 particle analyzer (Anton Paar, Austria).

The drug release of Nf-venetoclax

Briefly, aliquots of Nf-venetoclax were prepared in PBS (pH=7.4) with or without DL-Dithiothreitol (DTT) while protected from light. 0.5 mL of the prepared Nf-venetoclax solution was mixed with 0.5 mL of different release mediums. The temperature and agitation were set at 37 °C and 40 rpm. At selected time intervals (4 h, 8 h, 24 h, 48 h, 72 h, and 96 h), the samples were withdrawn in triplicate and filtered through a 0.45 μm filter to separate free venetoclax. Then, the filter was washed with 2 mL deionized water and subsequently 0.5 mL methanol to remove the residual Nf-venetoclax. Finally,

the filter was washed with 0.5 mL DMSO to dissolve free venetoclax. The absorbance of released venetoclax dissolved in DMSO was measured at 423 nm by using microplate reader (Synergy H1, BioTek).

The distribution of mPEG-PαLA nanoparticles in the mouse lung

Nile red (Aladdin, Shanghai, China) loaded mPEG-PαLA nanoparticles were prepared using a method similar to the one used for Nf-venetoclax. Upon the pulmonary delivery of Nile-red alone or Nile-red loaded mPEG-PαLA nanoparticles for 1 h, lung tissues were collected, sectioned, stained with DAPI (Sigma, USA) and imaged using fluorescence microscopy (Zeiss, Germany).

Table. S1 Primer sequences used in this study.

Primers	Sequences (5'–3')
TNF-α	TTCTGTCTACTGAACTTCGGGGTGATCGGTCC GTATGAGATAGCAAATCGGCTGACGGTGTGGG
IL-6	CTGCAAGAGACTTCCATCCAG CTGCAAGAGACTTCCATCCAG
IL-1β	GAAAGACGGCACACCCACCCT GCTCTGCTTGTGAGGTGCTGATGTA
GAPDH	CATGACCACAGTCCATGCCATCAC TGAGGTCCACCACCCTGTTGCTGT

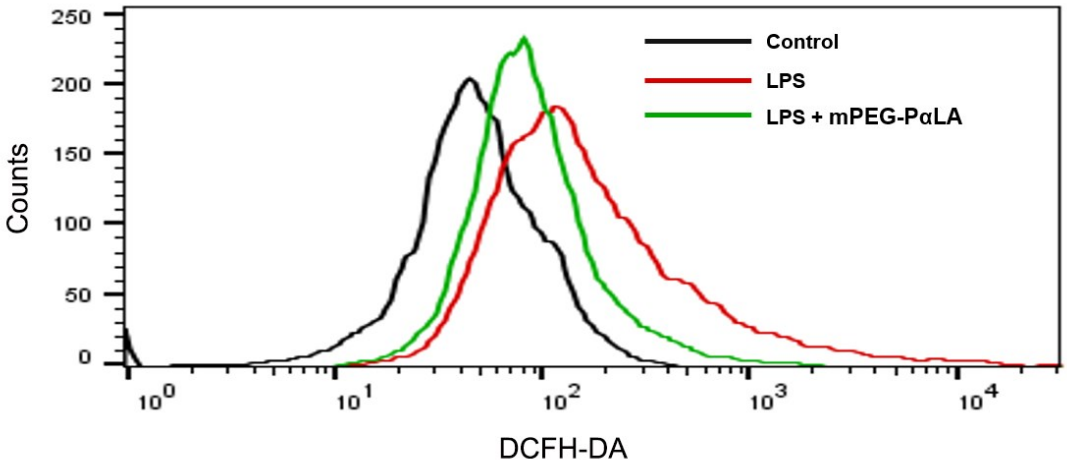

Fig. S1 The antioxidative effect of mPEG-PαLA *in vitro*.

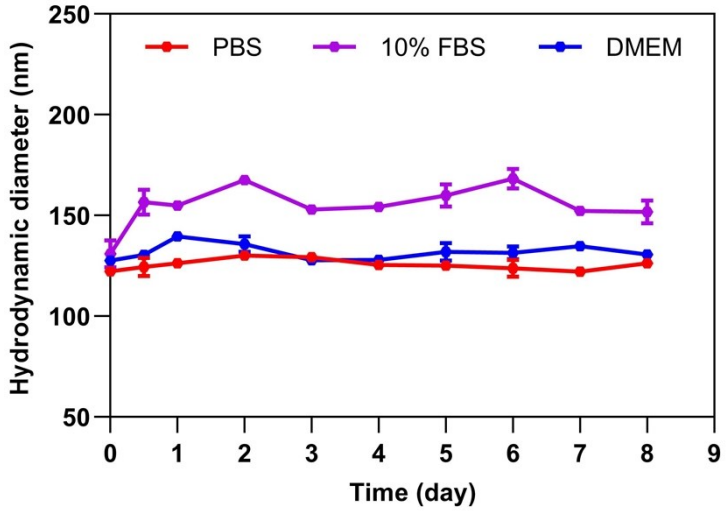

Fig. S2 The stability of Nf-venetoclax in PBS, 10% FBS, and DMEM over time.

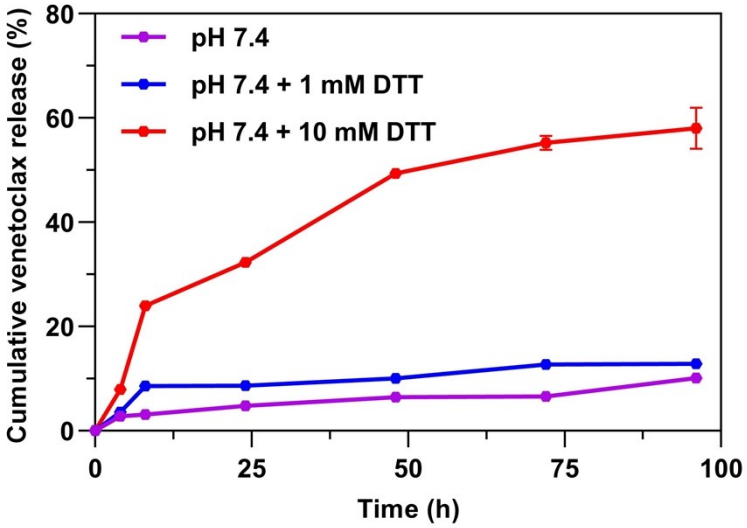

Fig. S3 Release profiles of Nf-venetoclax in PBS with or without DTT.

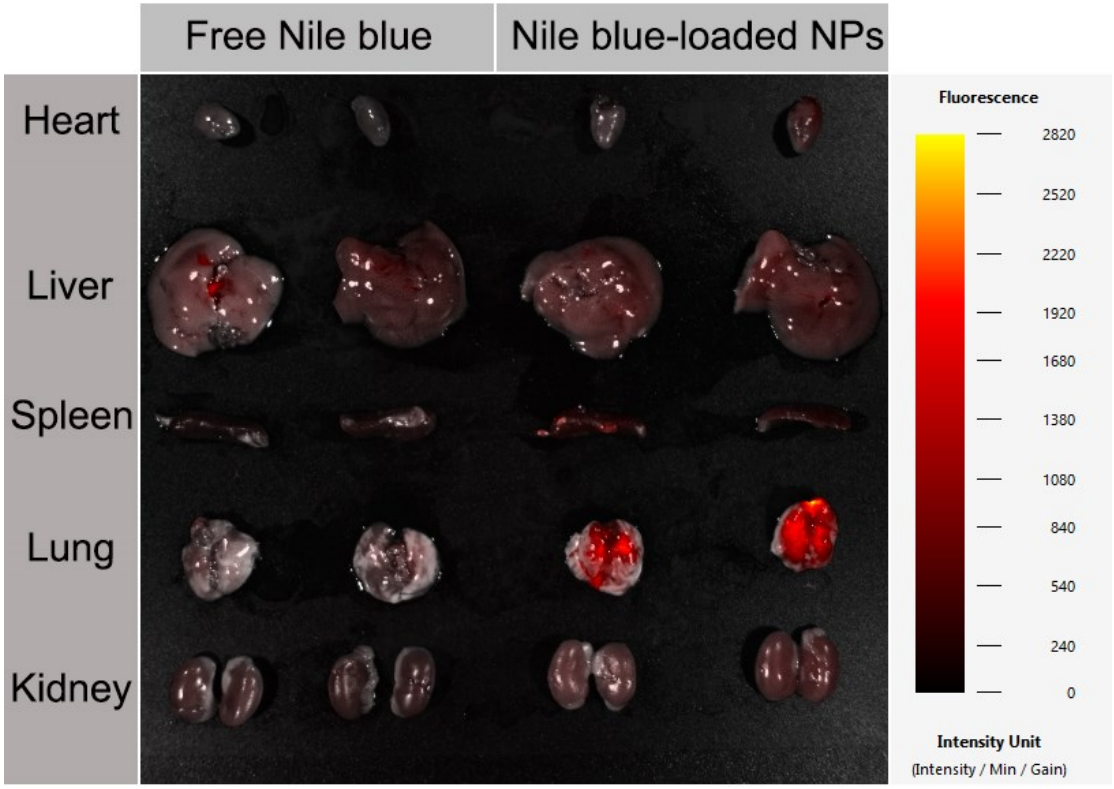

Fig. S4 Amphiphilic PEG modified poly(α-lipoic acid) nanoparticle prolonged the lung tissue-resident time. *Ex-vivo* biodistribution profile of the amphiphilic PEG modified poly(α-lipoic acid) nanoparticle was determined by *in vivo* imaging system.

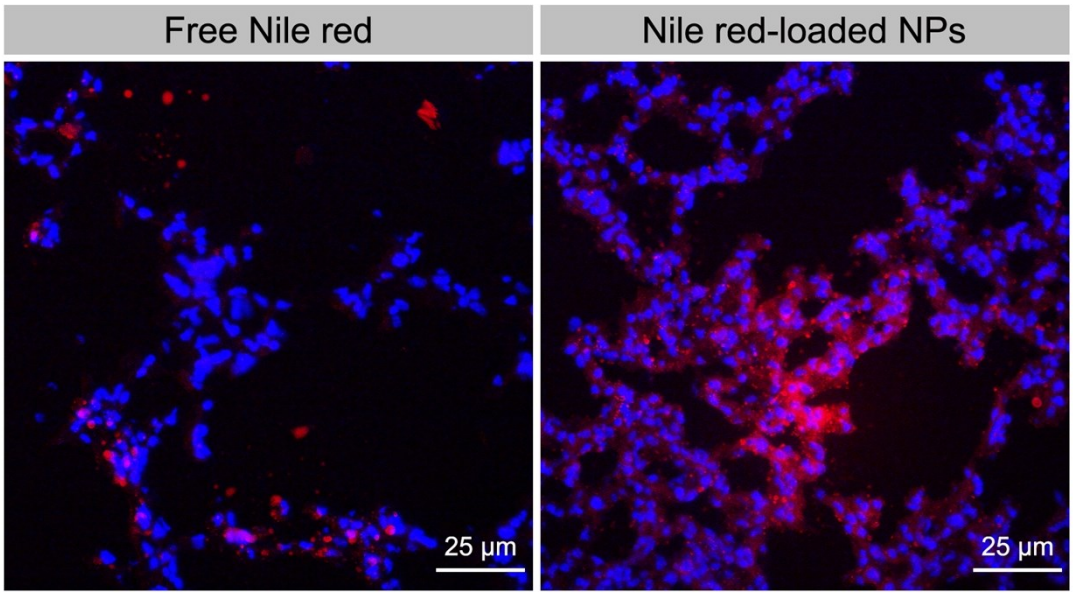

Fig. S5 The distribution of mPEG-PαLA nanoparticles in the mouse lung. Airway distribution of Nile red alone (red) or Nile red-loaded mPEG-PαLA NPs (red) after pulmonary administration. Cell nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) in blue.