# Supplementary files

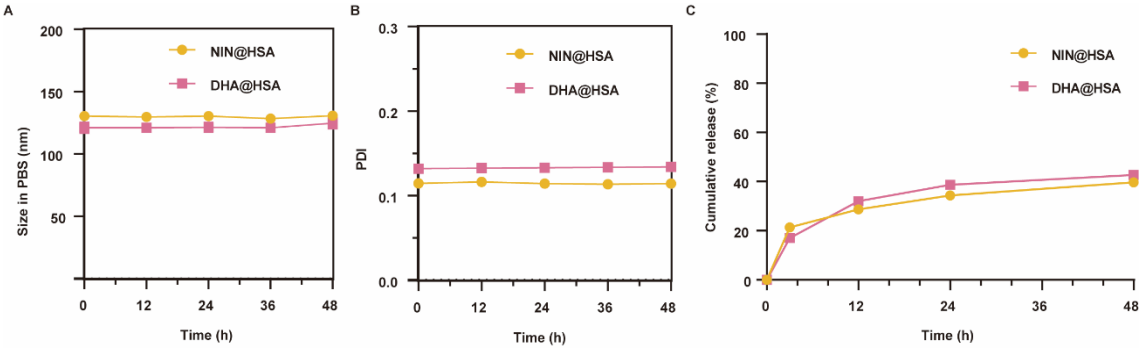


**Figure S1. Stability and in vitro release kinetics of DHA@HSA and NIN@HSA nanoparticles.**

Hydrodynamic diameter (A), polydispersity index (B), and cumulative drug release profiles (C) of DHA@HSA and NIN@HSA nanoparticles were evaluated in PBS (pH 7.4) at 37°C over 48 h. Both formulations showed stable particle size and low PDI values, along with controlled and sustained drug release. Data are shown as mean ± SD (n = 3).

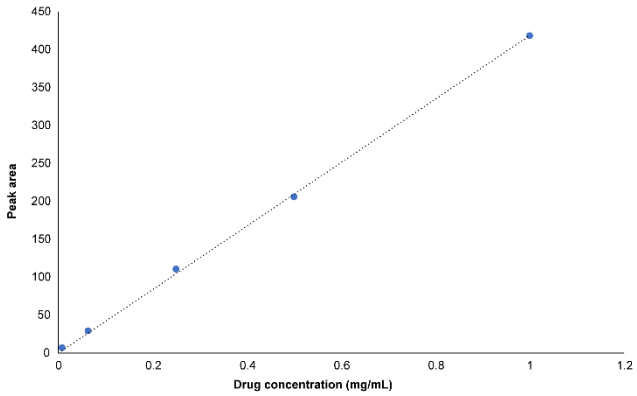


**Figure S2 Standard calibration curve for DHA determination by HPLC.**

The calibration curve was constructed by plotting the peak area against the drug concentration. The linear regression equation is $y = 414.6904x + 2.8628$ ($R^2 = 0.9998$). In this equation, $y$ represents the peak area, and $x$ represents the concentration of DHA (mg/mL).

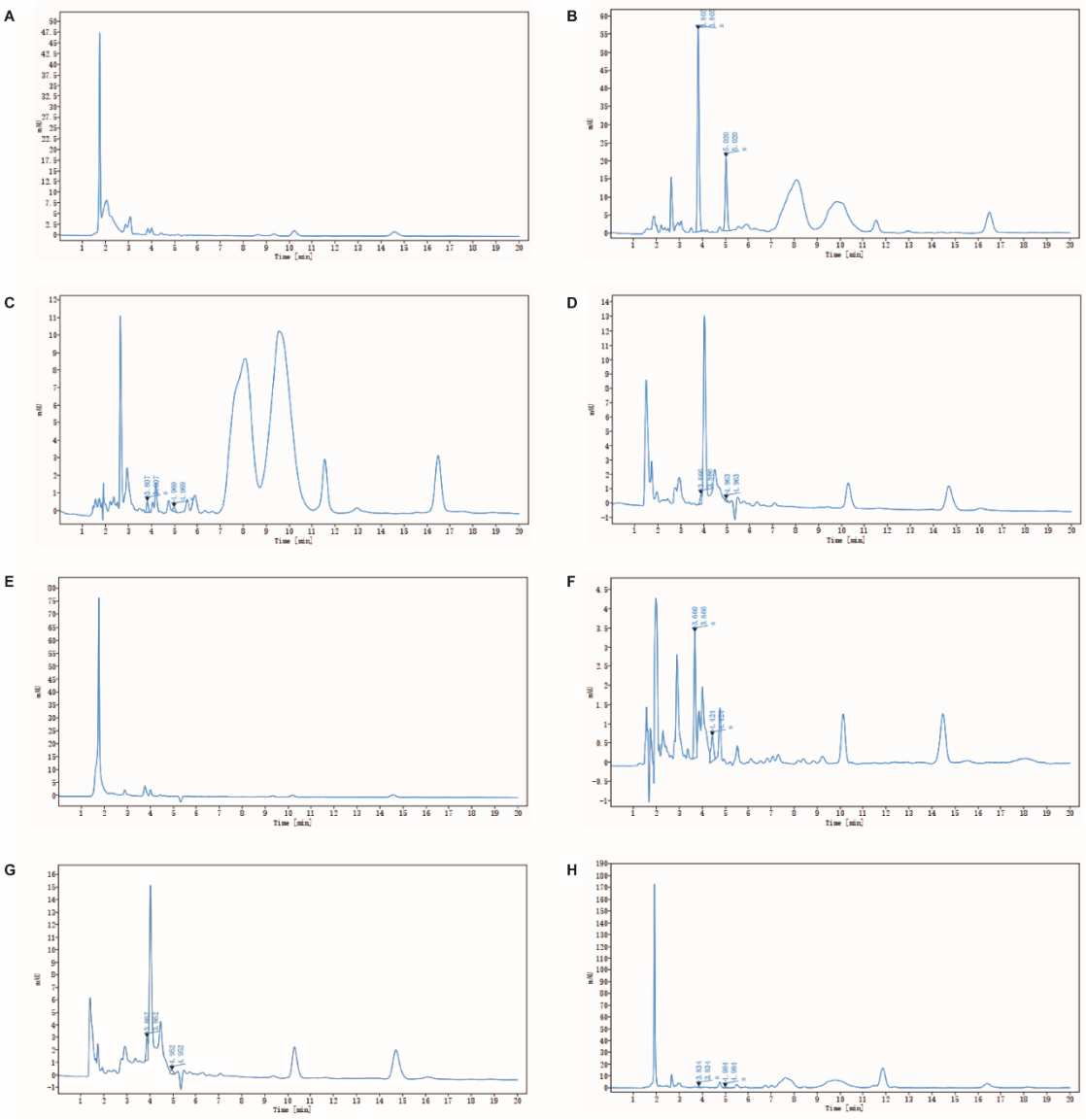


**Figure S3 Representative HPLC chromatograms of DHA in lung tissue samples following intratracheal administration.**

(A) Blank lung tissue homogenate showing no endogenous interference at the DHA retention time. (B) HPLC chromatogram of blank lung homogenate spiked with DHA standard, confirming the characteristic DHA peak with a retention time ranging from 3.5 to 5.2 min. (C–E) Lung tissue samples collected at 12 h (C), 24 h (D), and 48 h (E) after intratracheal administration of free DHA. (F–H) Lung tissue samples collected at 12 h (F), 24 h (G), and 48 h (H) after intratracheal administration of DHA@HSA.

Representative chromatograms from n = 3 biological replicates per time point are shown.

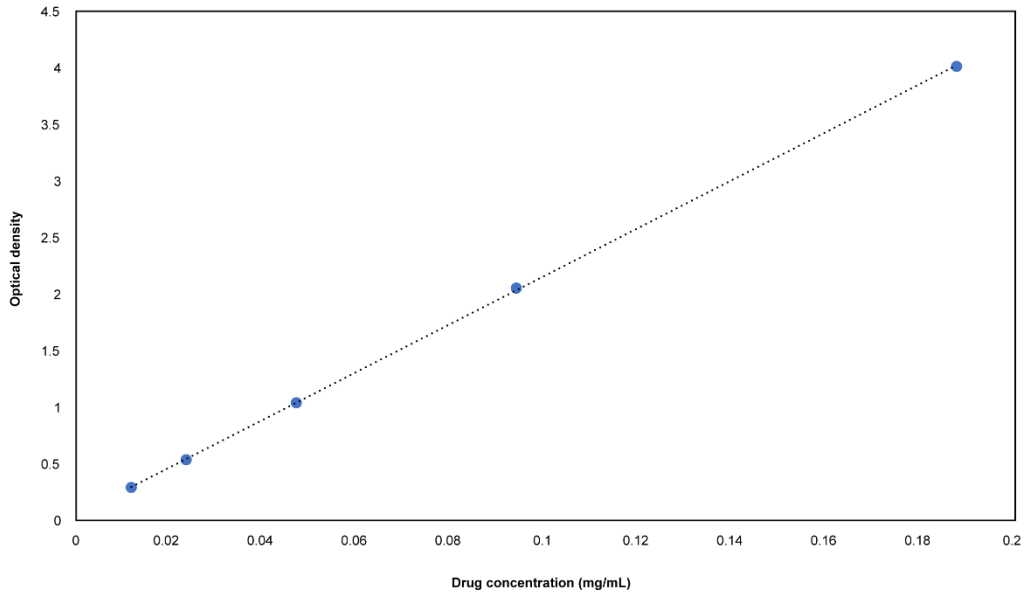


**Figure S4 Standard calibration curve for NIN determined by ultraviolet–visible spectrophotometry.**

Ultraviolet–visible (UV–Vis) spectrophotometry was employed to measure the absorbance of NIN standard solutions at the characteristic wavelength of 385 nm. A calibration curve was constructed by plotting absorbance against the nominal NIN concentrations. Linear regression analysis yielded the equation $y = 21.211x + 0.0464$ with a coefficient of determination of $R^2 = 0.9999$, where $y$ represents absorbance (optical density) and $x$ represents the NIN concentration (mg/mL). This calibration curve was used for subsequent quantification of NIN in lung tissue samples.

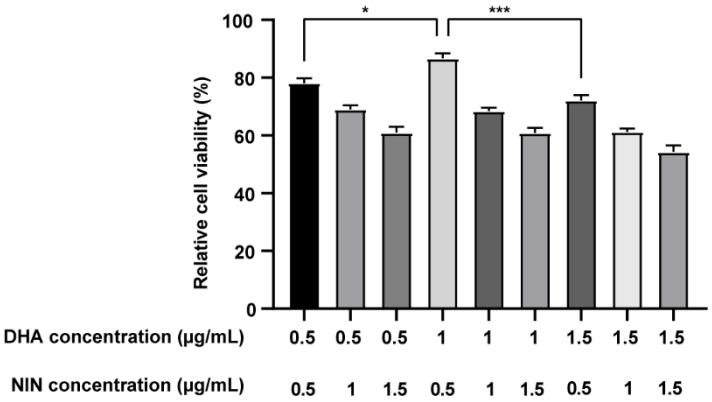


**Figure S5 Determination of the optimal concentration combination of DHA@HSA and NIN@HSA nanoparticles for NIH/3T3 cells.**

NIH/3T3 cells were treated with different concentration combinations of DHA@HSA and NIN@HSA nanoparticles for 48 h. Relative cell viability was assessed using the CCK-8 assay (n = 3). $^{*}p < 0.05$and $^{***}p < 0.001$ (one-way ANOVA). Data are presented as mean ± standard deviation (SD).

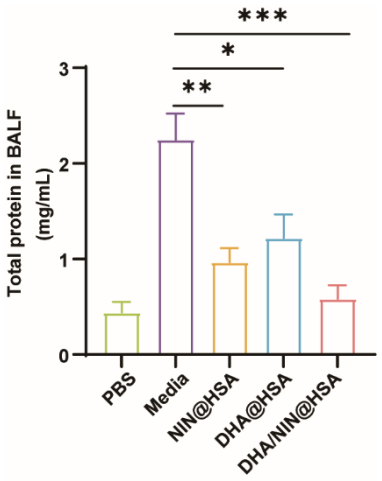


**Figure S6 DHA/NIN@HSA significantly reduced total protein levels in bronchoalveolar lavage fluid.** Total protein concentration in bronchoalveolar lavage fluid (BALF) from different treatment groups (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ (one-way ANOVA). Data are presented as mean ± standard deviation (SD).

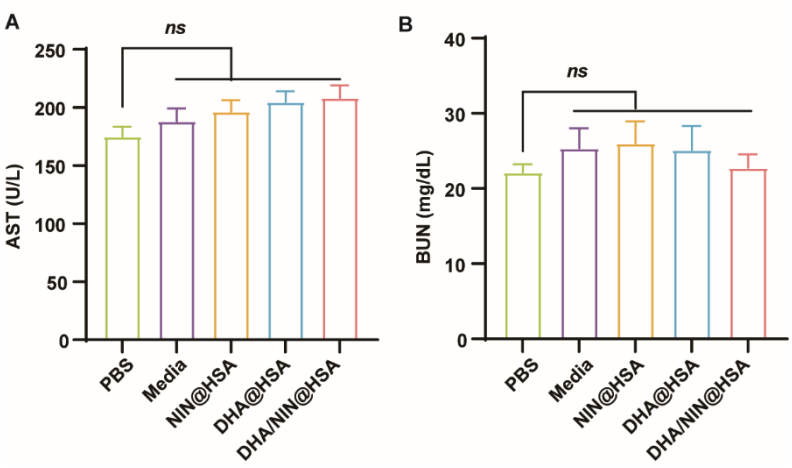


**Figure S7 Serum biochemical evaluation of systemic biosafety after different treatments.** (A-B) Serum aspartate aminotransferase (AST) and blood urea nitrogen (BUN) levels in mice from different groups (n = 6). ns, not significant; $p \geq 0.05$ (one-way ANOVA). Data are presented as mean ± standard deviation (SD).

**Table S1 Pulmonary retention data (%) of DHA and NIN formulations at different time points after intratracheal administration.**

| Formulation | Time (h) | Replicate 1 (%) | Replicate 2 (%) | Replicate 3 (%) | Mean ± SD (%) |
|---|---|---|---|---|---|
| DHA | 12 | 13 | 23 | 16 | 17.33 ± 5.13 |
| | 24 | 11 | 7 | 9 | 9 ± 2 |
| | 48 | 0 | 0 | 0 | 0 ± 0 |
| DHA@HSA | 12 | 72 | 83 | 69 | 74.67 ± 7.37 |
| | 24 | 54 | 59 | 44 | 52.33 ± 7.64 |
| | 48 | 12 | 19 | 27 | 19.33 ± 7.51 |
| NIN | 12 | 30 | 24 | 16 | 23.33 ± 7.02 |
| | 24 | 13 | 19 | 10 | 14 ± 4.58 |
| | 48 | 0 | 0 | 0 | 0 ± 0 |
| NIN@HSA | 12 | 76 | 85 | 70 | 77 ± 7.55 |
| | 24 | 56 | 68 | 50 | 58 ± 9.17 |
| | 48 | 35 | 26 | 20 | 27 ± 7.55 |

Footnotes: Pulmonary retention is expressed as percentage of the administered dose (%ID) and was calculated as Drug retention (%ID) = ($A_{lung}$ / $D_{admin}$) × 100%, where $A_{lung} = C_{ext} \times V_{ext.}$ DHA concentrations were determined by HPLC, and NIN concentrations were determined by UV–Vis spectrophotometry. $D_{admin}$ was 40 μg for DHA (2 mg/kg, mean body weight 20 g) and 80 μg for NIN per mouse per administration. Data represent individual values from n = 3 biological replicates per time point and are presented as mean ± standard deviation (SD).