



S3 Fig. Typical chromatograms of 4'-HW in (A)serum and (B) lung tissue of ALI mice.

LC-MS analysis

Serum (100 μ l) and lung homogenate (150 mg) was extracted with 200 μ l and 750 μ l methanol, respectively. Following centrifugation and separation, the supernatant was filtered and injected to UHPLC directly. Stock solution of 4'-HW was prepared by dissolving 1.0 mg in 1.0 ml methanol (1 mg/ml). Calibration curves were prepared by diluting the stock solution. The calibration for 4'-HW was constructed by plotting the peak area against the analyte concentrations.

Quantification of 4'-HW was carried out on an ultrahigh-performance liquid chromatograph hyphenated with triple quadrupole tandem mass spectrometer (UHPLC-QTOF-MS) (Agilent 6540, Agilent Technologies, USA). Chromatographic separations of 4'-HW were performed on an Agilent 1290 Infinity UHPC system using a Xterra MS C18 column (2.1 mm x 150 mm, I.D., 5 μ m, Waters). A gradient program was used with mobile phase consisting of water (solvent A, containing 0.1% formic acid) and methanol (solvent B) as follows: 0-5 min, 50% B-95% B; 5-15 min, 95% B; 15.1-25 min, 50% B. The flow rate was 0.2 ml/min and the injection volume was 3 μ l. The mass spectrometer was equipped with an electrospray ionization (ESI) source. The mass spectrometer conditions were as follows: Nebulizer (N_2) was set at 35 psi. The dry gas was set to 8 L/min. The gas temperature was set to 350 °C. The capillary voltage was set to 3500V. The collision energy was 30 eV. MS scan ranged from 100 to 1000 m/z, and the MS/MS scan ranged from 50 to 500 m/z. Spectra were collected in the positive ESI mode. Data was processed using Agilent Masshunter Workstation Qualitative Analysis.