

Supporting Information for

Combination of DNA demethylation and chemotherapy to trigger cell
pyroptosis for inhalation treatment of lung cancer

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Materials

Carboxyl terminated poly (lactic acid-glycolic acid) copolymer OH-PLGA-COOH50/50 (DG-50DLGH018) was obtained from Jinan Daigang Biomaterial Co., Ltd. Decitabine (DAC, 1026705) was purchased from Leyan Company (China). Doxorubicin (DOX, MFCD00077757) was purchased from Macklin Company (Shanghai). RPMI 1640 medium (L210KJ), fetal bovine serum (FBS), phosphate buffered saline (PBS), and Dimethyl sulfoxide (DMSO) were obtained from BasalMedia. Annexin V-FITC/PI kit (E606336-0100) were purchased from BBI CO., LTD. Enhanced ATP Assay Kit (S0027), LDH Release Assay Kit (C0016), Trypsin-EDTA Solution (C0201), BeyoECL Plus (P0018), and Penicillin-Streptomycin Solution (100X, C0222) were purchased from Beyotime Company (China). PolyJet™ In *Vitro* DNA Transfection (SL100688) was purchased from SignaGen laboratories. Anti-Cleaved Caspase-3 antibody [EPR21032] (ab214430), Anti-cleaved N-terminal DFNA5 / GSDME antibody [EPR20867-248] (ab222408), Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602), and Goat Anti-Mouse IgG H&L (HPR) ab6789 were obtained from Abcam Company.

Characterization

The morphology of MPs was observed by scanning electron microscope (SEM, Quattro S, Thermo Fisher Scientific, USA). Before the analysis, The MPs in the aqueous phase were dropped onto a silicon wafer and allowed to dry in air. After the samples were coated under vacuum with 1 nm Au/Pd layer, the MPs were examined and photographed by SEM. The size and size distribution of MPs were analyzed via a Mastersizer (Mastersizer 3000, Malvern Panalytical, China). The lyophilized powder

of MPs were diluted by ultrapure water, and the size and size distribution were detected by a Mastersizer. The aerodynamic diameter (d_{aero}) of the MPs can be calculated by the following formula

$$d_{aero} = d(\rho/\rho_o X)^{1/2}$$

In the formula above, a , ρ_1 , ρ_2 , X respectively are geometric diameter, tapped density, mass density of water, shape factor, the shape factor of a sphere is usually 1. The encapsulation efficiency (EE%) and drug loading capacity (DL%) of MPs encapsulated DAC and DOX were obtained by HPLC (Agilent 1260 HPLC010502, Agilent, USA). The synthesized MPs was centrifuged to determine DAC and DOX in the supernatant. The HPLC conditions of DAC: mobile phases were acetonitrile/water (5:95 v/v), flow rate was 1 mL/min and the retention time was 4-5 min at 244 nm. The HPLC conditions of DOX: mobile phases were acetonitrile/water (65:35 v/v), flow rate was 1 mL/min and the retention time was 2-3 min at 490 nm.

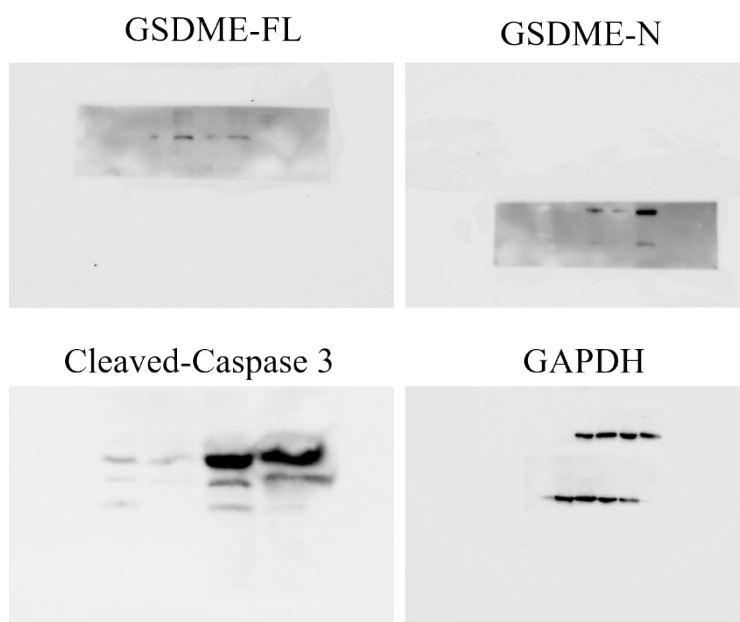
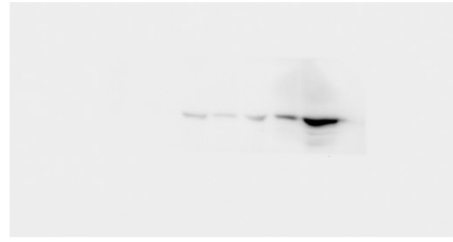


Figure S1. Pyroptosis-related proteins expression in 4T1 cells induced by microsphere release

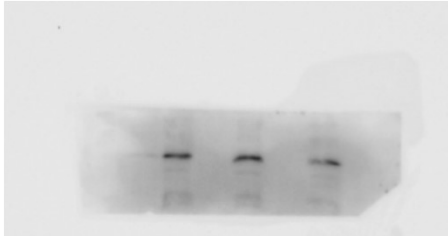
Cleaved-Caspase 3



GSDME-N



GSDME-FL



GAPDH



Figure S2. Western blotting analysis of related proteins expression in lungs