Supporting Information for

纳米粒子包封硝基化梅曲奈促进辐射治疗

Nanoparticles Encapsulating Nitrosylated Maytansine to Enhance Radiation Therapy

纳米粒 ……装入胶囊 硝化作用

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放射致敏剂 非小细胞肺癌

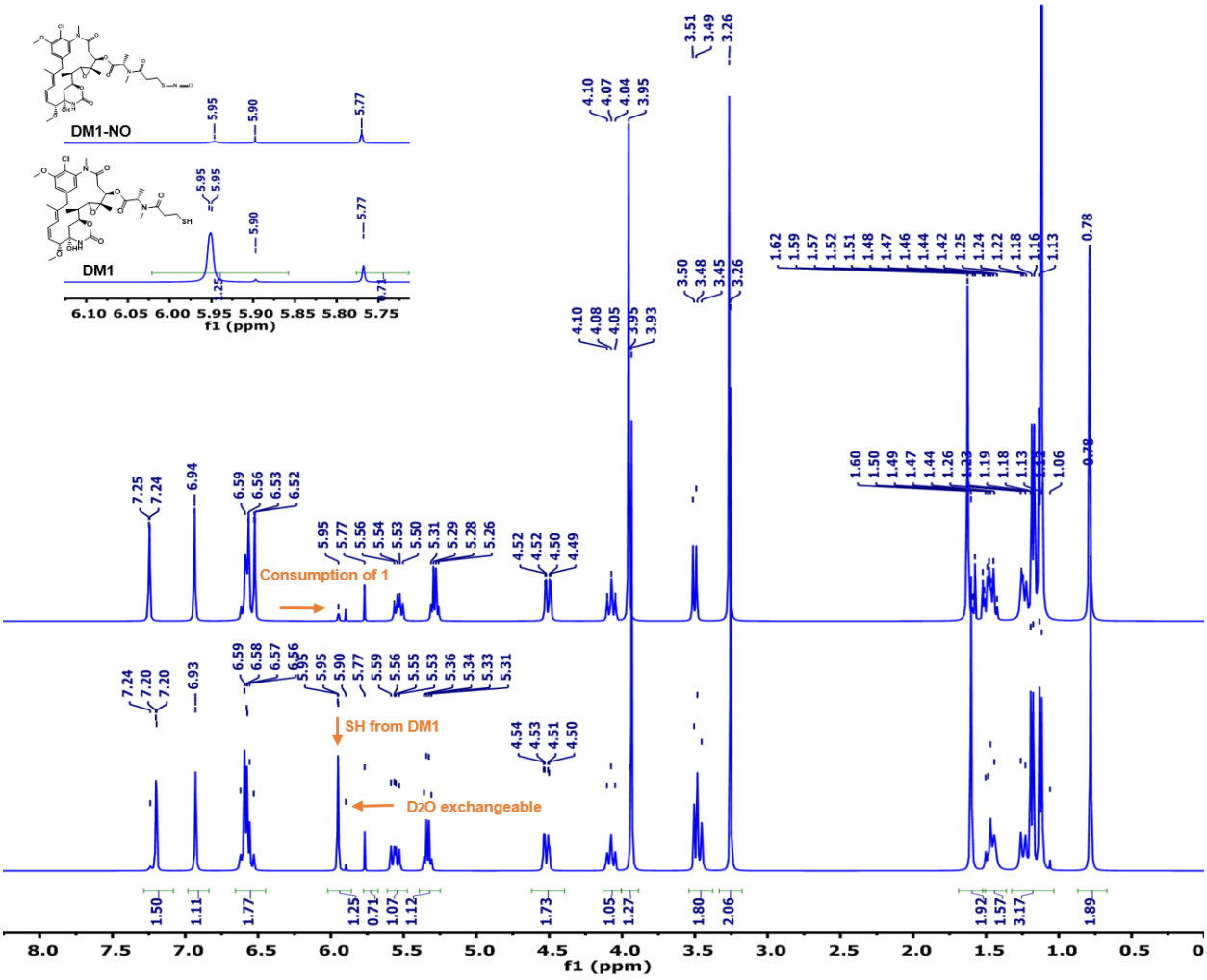


Figure S1: ^{1}H NMR spectra of DM1-NO prodrug (top) and DM1 (bottom) in deuterated dimethyl sulfoxide-d6 (DMSO-d6).

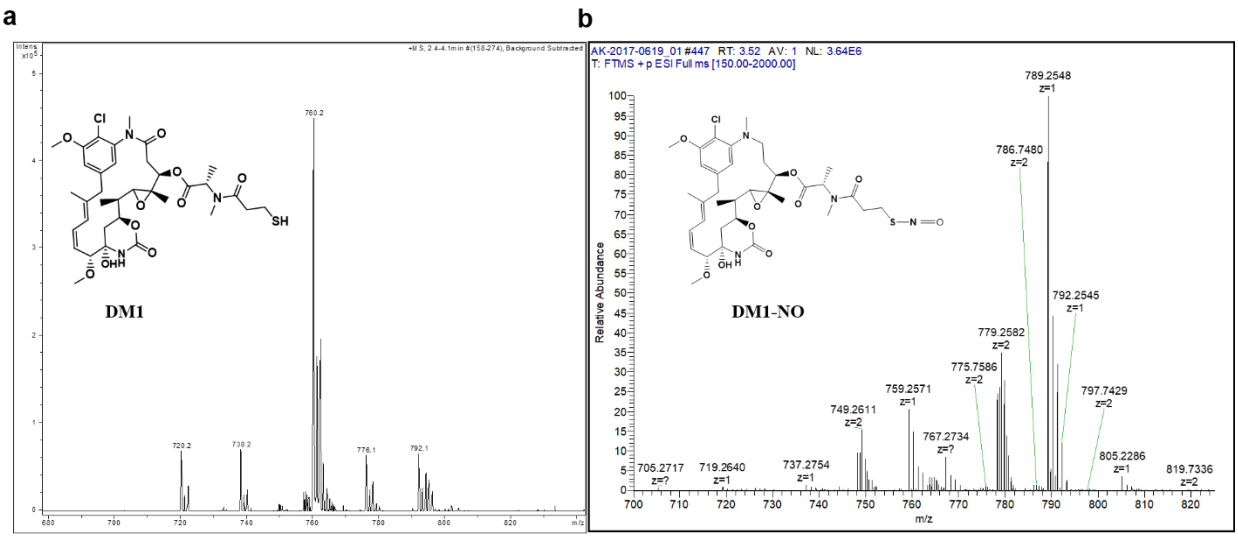
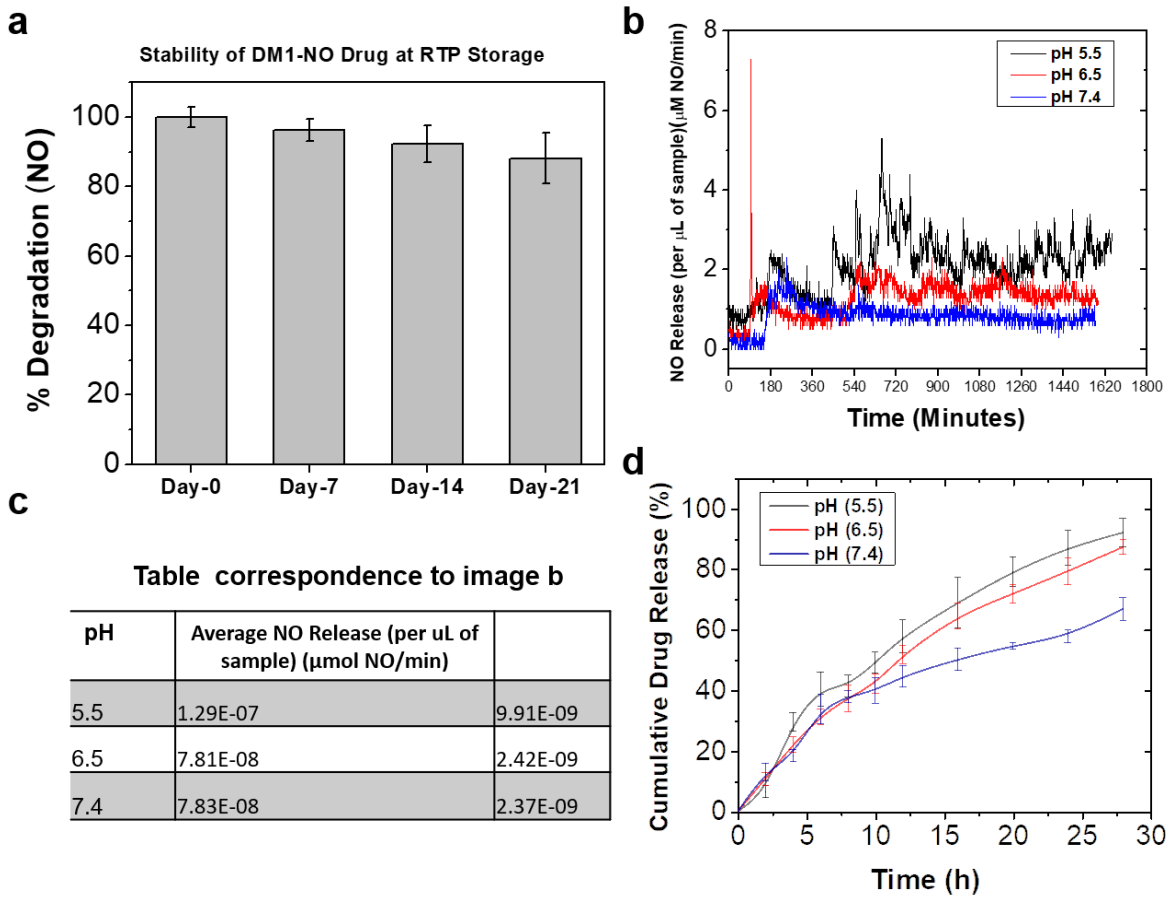


Figure S2: High-resolution mass spectrometry (HRMS) data of DM1 and DM1-NO.



pH	Average NO Release (per uL of sample) (µmol NO/min)	
5.5	1.29E-07	9.91E-09
6.5	7.81E-08	2.42E-09
7.4	7.83E-08	2.37E-09

Figure S3: (a) Stability of DM1-NO under ambient conditions. After synthesis, dry product of DM1-NO was kept at room temperature and the total amount of remaining NO was quantified using Griess assay on Day 0, 7, 14, and 21). (b-c) Stability of DM1-NO in solutions. DM1-NO (20.09 µmol) was dissolved in PBS of different pH (5.5, 6.5, and 7.4). Time-dependent NO release was measured on a Sievers NOA 280i system, which assesses NO based on gas phase chemluminescent reaction between NO and ozone. (d) Release profiles for DM1-NO-NPs, tested in PBS of different pH at 37 °C by Griess assays.

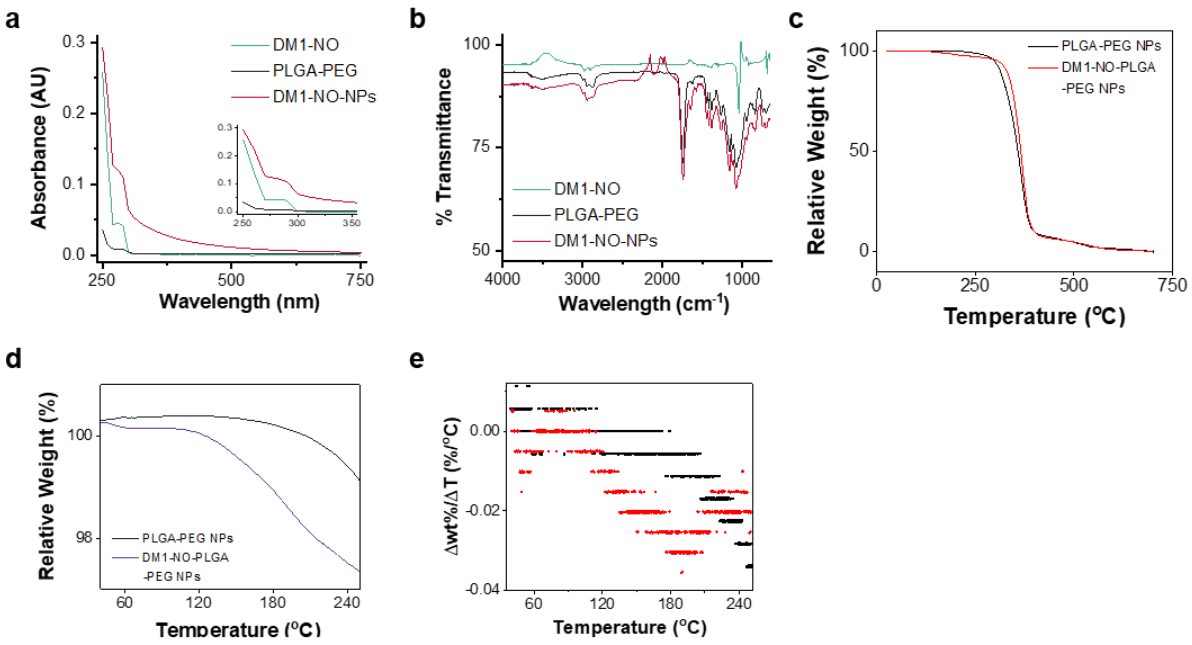


Figure S4: (a) Uv-vis analysis of DM1-NO, PLGA-b-PEG and DM1-NO-NPs. The presence of a shoulder at ~290 nm, which corresponds to the absorbance of DM1-NO, indicates successful drug loading. (b) FT-IR analysis of DM1-NO, PLGA-b-PEG and DM1-NO-NPs. (c) TGA of PLGA-*b*-PEG nanoparticles (PLGA-PEG NPs) and DM1-NO encapsulated PLGA-*b*-PEG nanoparticles (DM1-NO-PLGA-PEG NPs). Fast weight loss was observed with DM1-NO-NPs relative to PLGA-*b*-PEG NPs between 40 °C and 250 °C. Relative weight change vs. temperature plot (d) and relative weight change per temperature increase ($d$wt%/dT) vs. temperature plot (e), based on results from (c).

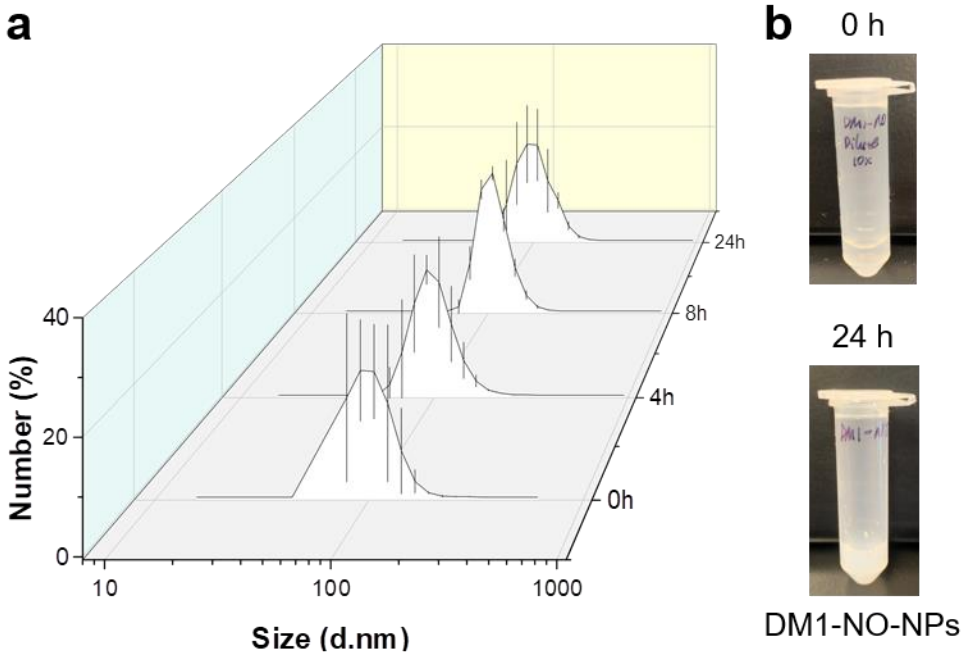


Figure S5: Stability of DM1-NO-NPs. (a) DLS found minimal hydrodynamic size change with DM1-NO-NPs that had been incubated in PBS for 24 h. (b) Digital images showing the dispersion of DM1-NO-NPs at 0 and 24 h.

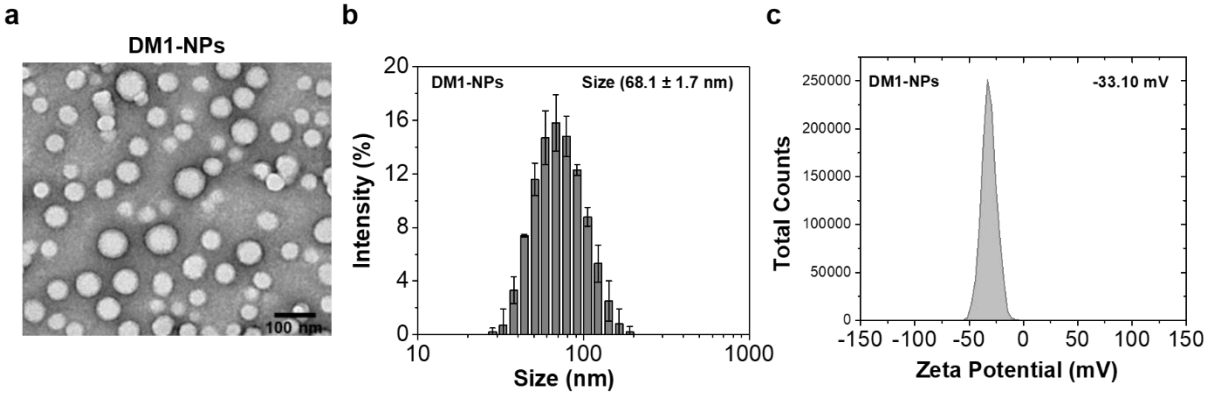


Figure S6: (a) TEM image of DM1-NPs. (b) DLS of DM1-NPs. (c) Zeta potential of DM1-NPs.

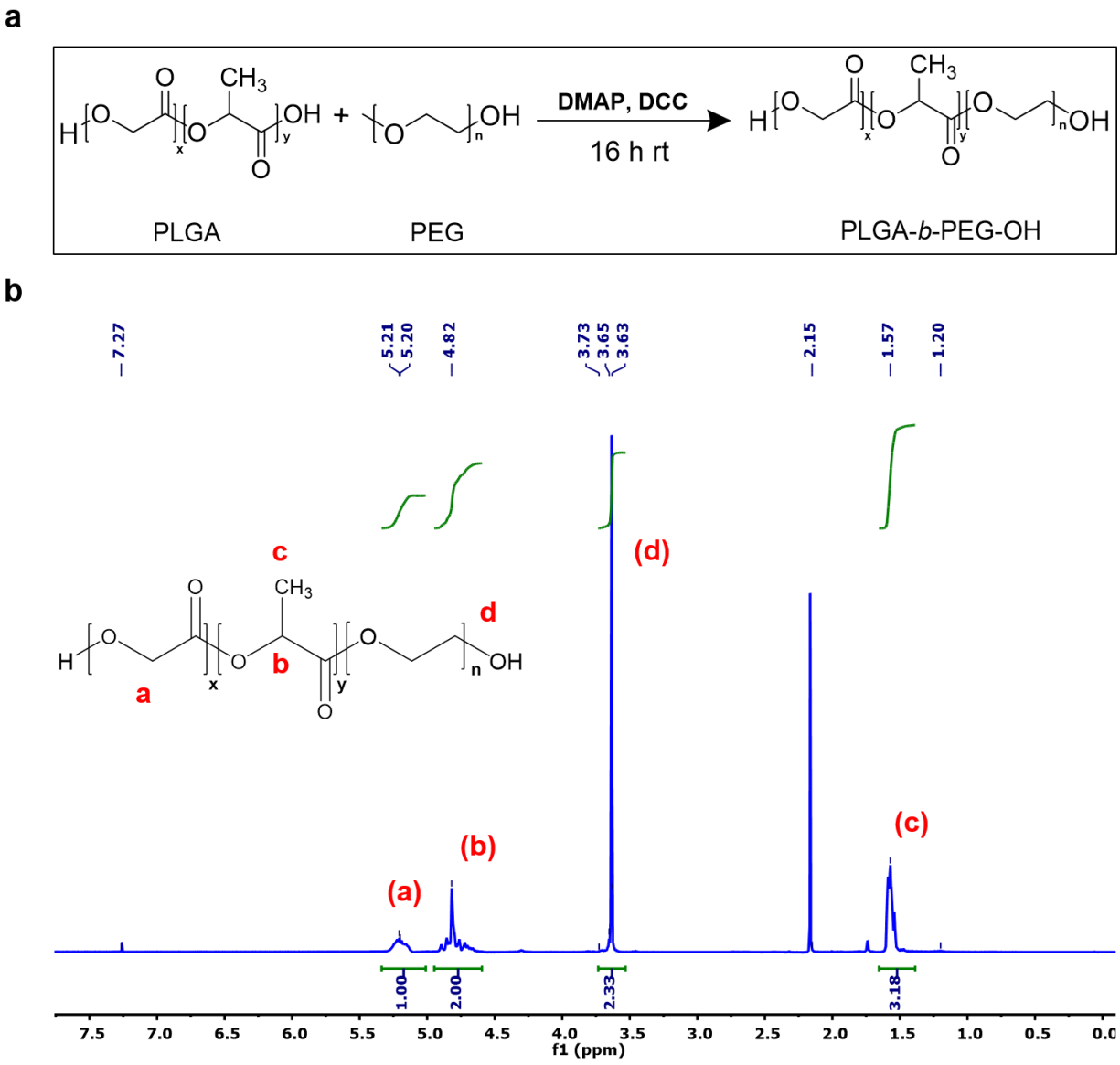


Figure S7: (a) Reaction scheme for PLGA-PEGylation. (b) ^{1}H NMR spectrum of PLGA-*b*-PEG-OH in $CDCl_3$.

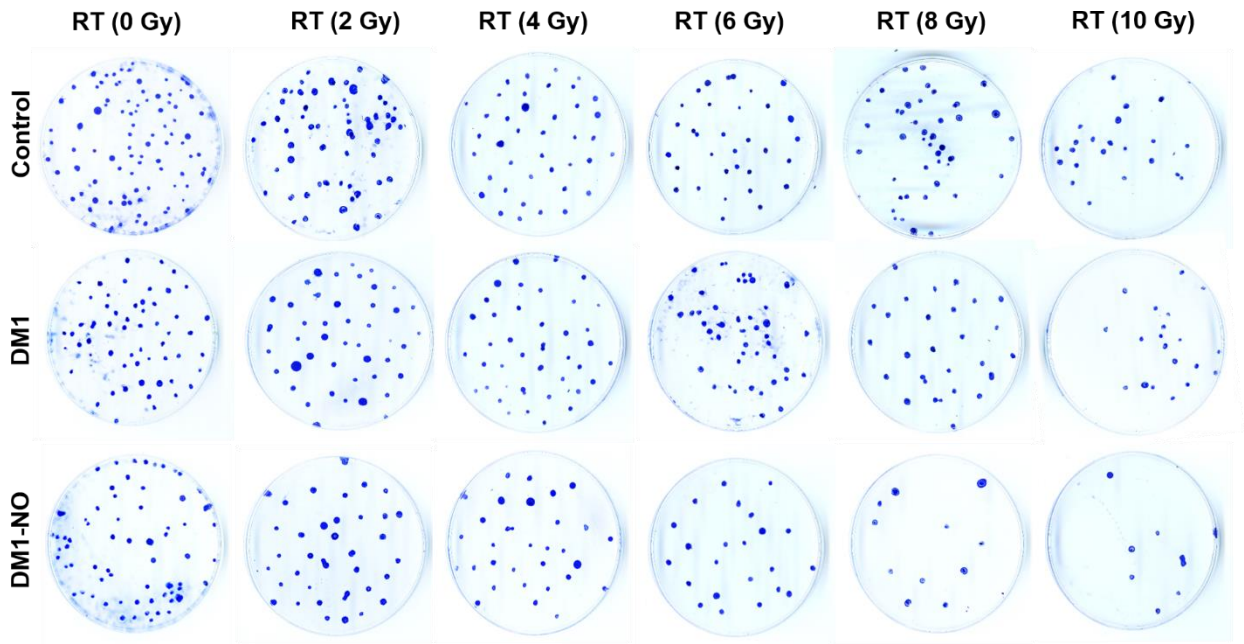
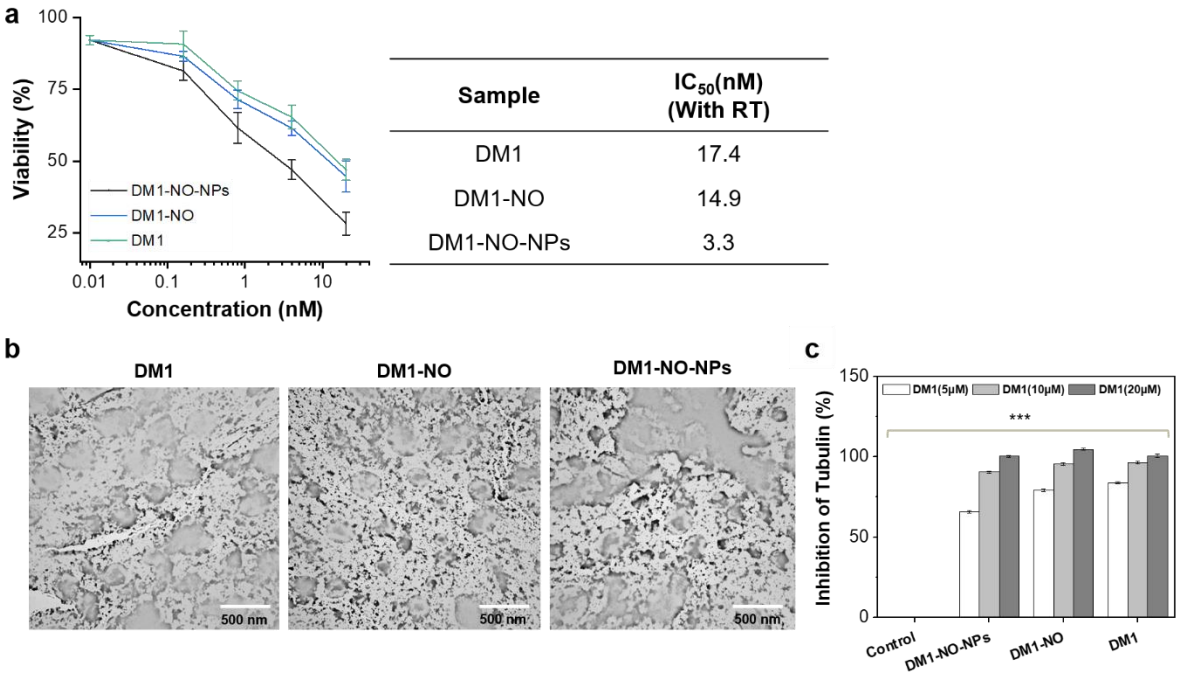


Figure S8: Representative photos of clonogenic assays. H1299 cells were treated with 20 nM of DM1 and DM1-NO for 12 h, followed with X-ray irradiation (6 Gy). The resulting cells were plated for clonogenic assays [number of cells plated: 100 (0 Gy), 100 (2 Gy), 500 (4 Gy), 1000 (6 Gy), 3000 (8 Gy), and 5000 cells (10 Gy) per dish correspondingly].



Sample	IC_{50}(nM) (With RT)
DM1	17.4
DM1-NO	14.9
DM1-NO-NPs	3.3

Figure S9: (a) Cell viability, tested with H1299 cells using 72-h MTT in the presence of RT. The corresponding IC_{50} values are shown to the right. DM1, DM1-NO, and DM1-NO-NPs were studied. (b-c) Inhibition of tubulin polymerization. (b) TEM images show aggregation of microtubules upon treatment with DM1, DM1-NO, or DM1-NO-NPs at high concentrations (> 20 μM). Scale bars, 500 nm. (c) Percentage of tubulin inhibition. Tubulin polymer was collected by centrifugation (35000×*g* for 1 h at 30 °C) and the amount of tubulin protein sediment was quantified by measuring protein concentration. ***, $P < 0.001$.

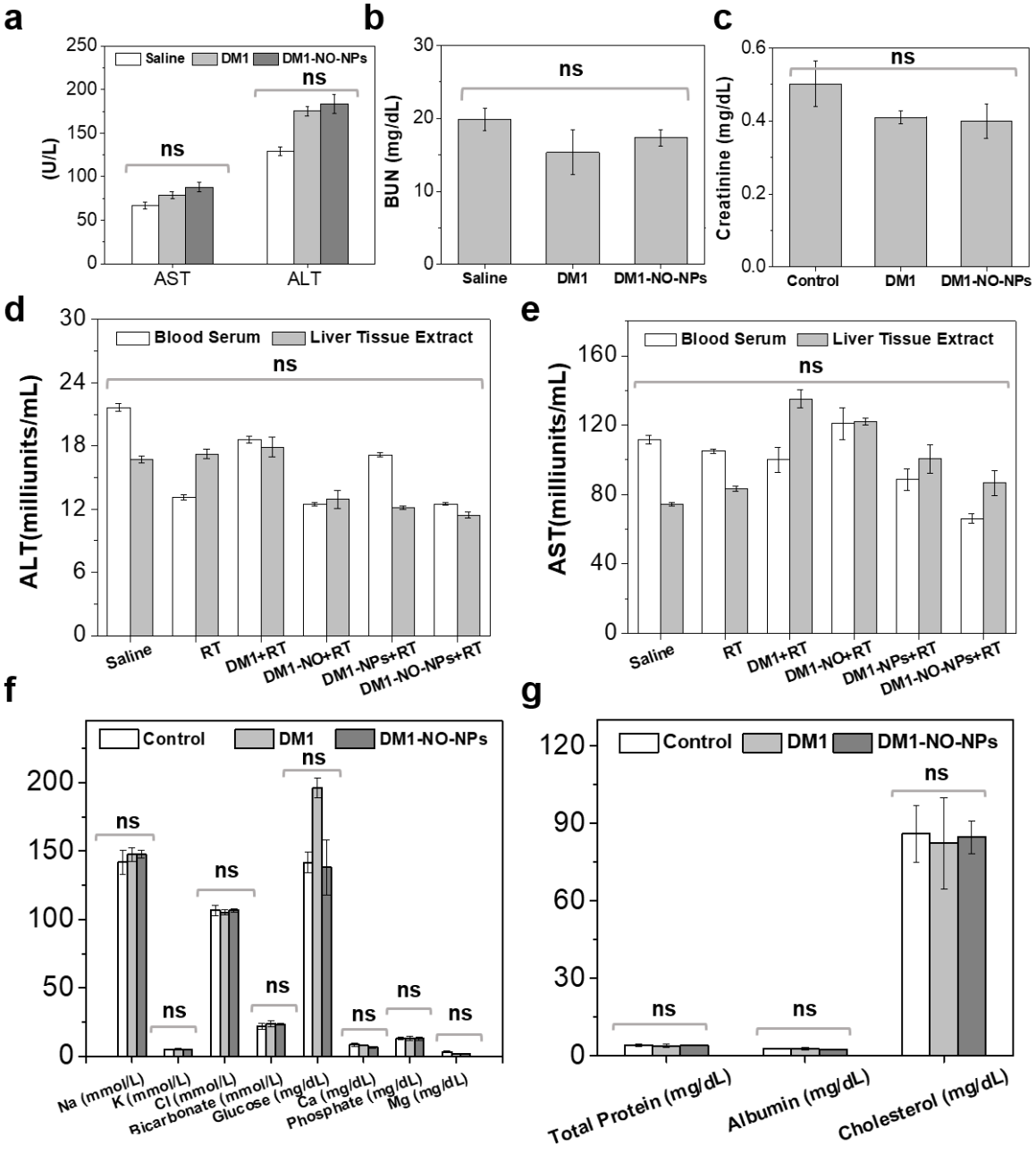


Figure S10: *In vivo* toxicity studies. Blood or tissue samples were taken from balb/c mice receiving i.v. injection of 206.8 nmol/kg (equivalent DM1 dose) DM1-NO-NPs or DM1, or PBS on Day 10 (n=3). Blood biochemical analysis of (a) alanine transaminase (ALT) and aspartate transaminase (AST), (b) blood urea nitrogen (BUN), and (c) creatinine (CR). Liver tissue ALT (d) and AST (e) levels were also tested. (f) Blood electrolyte levels (sodium, potassium, chloride,

bicarbonate, glucose, calcium, inorganic phosphate, and magnesium. (g) Total proteins, albumins, and lipids (cholesterol) levels in the blood. ns, no significant difference.