Supporting Information

Enzyme-responsive micellar JQ1 induces enhanced BET proteins inhibition and immunotherapy of malignant tumor

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Materials

α-Methoxy-ω-amino-poly(ethylene glycol) (mPEG-NH_2, M_n = 5.0 kg/mol) purchased from Xiamen Sinopeg Biotechnology Co., Ltd., tyrosine obtained from Gill Biochemical Shanghai Co., Ltd., proteinase K (> 40 U/mg) provided by Thermo Fisher Scientific Co., Ltd., and JQ1 purchased from MCE (Shanghai, China) were used directly without purification. Anti-BRD4, anti-c-MYC, and anti-PD-L1 antibodies were obtained from Abcam (Shanghai, China). CD3, CD4, CD8, and CD45 antibodies and their homotypic antibodies used for flow cytometry were purchased from BD Biosciences (Shanghai, China). Melanoma cancer cells (B16F10) were provided by the National Collection of Authenticated Cell Cultures (Shanghai, China) and cultured in Dulbecco's modified Eagle's medium (DMEM) culture medium with 1% penicillin/streptomycin and 10% FBS in a humidified incubator at 37 °C under a 5% CO_2 atmosphere. Fetal bovine serum (FBS) and DMEM were purchased from Gibco (Shanghai, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was provided by KeyGEN Biotech. Co., Ltd. (Jiangsu, China). The ELISA kits for the detection of the tumor necrosis factor (TNF-α), interferon γ (IFN-γ), and interleukin-6 (IL-6) were obtained from Invitrogen (Shanghai, China).

Enzyme-triggered release of JQ1

For *in vitro* drug release, mJQ1 was placed in a dialysis tube (MWCO 12000) at a micelle concentration of 0.1 mg/mL under shaking at 37 °C in two different media: (i) PB (10 mM, pH 7.4), and (ii) PB (10 mM, pH 7.4) containing proteinase K (6 U/mL). Typically, 0.5 mL of mJQ1 dispersion was dialyzed against PB buffer (10 mM, pH 7.4, 25 mL). At predetermined time points, 5.0 mL of release medium was withdrawn and refilled with fresh medium. The JQ1 in release medium was quantified by high

performance liquid chromatography (HPLC). The results were presented as mean ± SD (n = 3).

In vitro cytotoxicity of mJQ1

L929 and B16F10 cells were cultured in 96-well plates at 2×10^3 per well under 5% CO_2 at 37 °C in 100 μL of 1640 and DMEM media, respectively. After treating with different concentrations of Ms or mJQ1 for 48 h, the cell viability was assessed by 3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenytetrazolium bromide (MTT) assays. Results are presented as the mean ± SD (n = 3).

Cell cycle arrest and cell apoptosis evaluation

Briefly, B16F10 cells (2×10^5 cells/well) were seeded in 6-well plates and incubated with mJQ1 or free JQ1 at specified concentrations (JQ1 concentration: 0.25-0.5 μg/mL) for 48 h. For detection of apoptosis, the cells were harvested and stained by Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) at RT for 30 min in dark. The apoptosis was analyzed using flow cytometry (FACS Calibur, BD Biosciences, USA) within 1 h. All data were analyzed with the FlowJo V10 software.

Cell cycle analysis was performed following incubation with mJQ1 or free JQ1 (JQ1 concentration: 0.25-0.5 μg/mL) for 24 h. The collected cells were slowly added into 95% cold ethyl alcohol on ice, stored at 4 °C for 24 h, and then resuspended in PI staining solution containing RNase A. After incubation for 15 minutes at RT in dark, the cells were analyzed using flow cytometry (FACS Calibur, BD Biosciences, USA) and flow cytometry data were analyzed using the FlowJo V10 software.

Statistical analysis

Unless indicated, data were expressed as mean ± S.D. Differences between groups were analyzed by one-way analysis of variance (ANOVA) tests (GraphPad Prism Software). $^{*}p < 0.05$ was considered significant, and $^{**}p < 0.01$ as well as $^{***}p < 0.001$ were considered highly significant. The log-rank test was used to compare Kaplan–Meier Survival curves (GraphPad Prism Software).

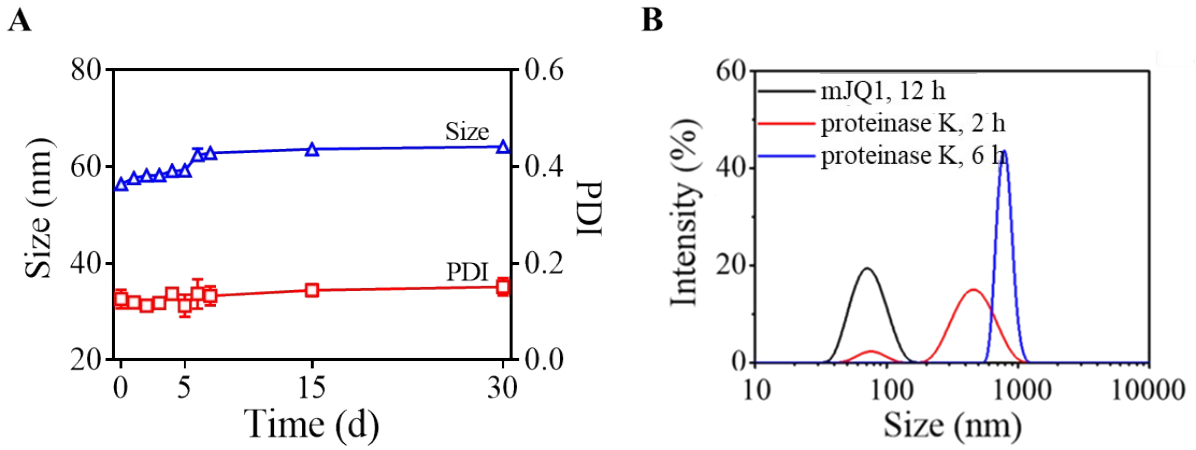

Figure S1. Characterization of JQ1-loaded micelles (mJQ1). (A) Size change profiles of mJQ1 (0.1 mg/mL) in response to proteinase K (6.0 U/mL) solution in PB at 37 °C. (B) Long-term storage stability of mJQ1 monitored by DLS.

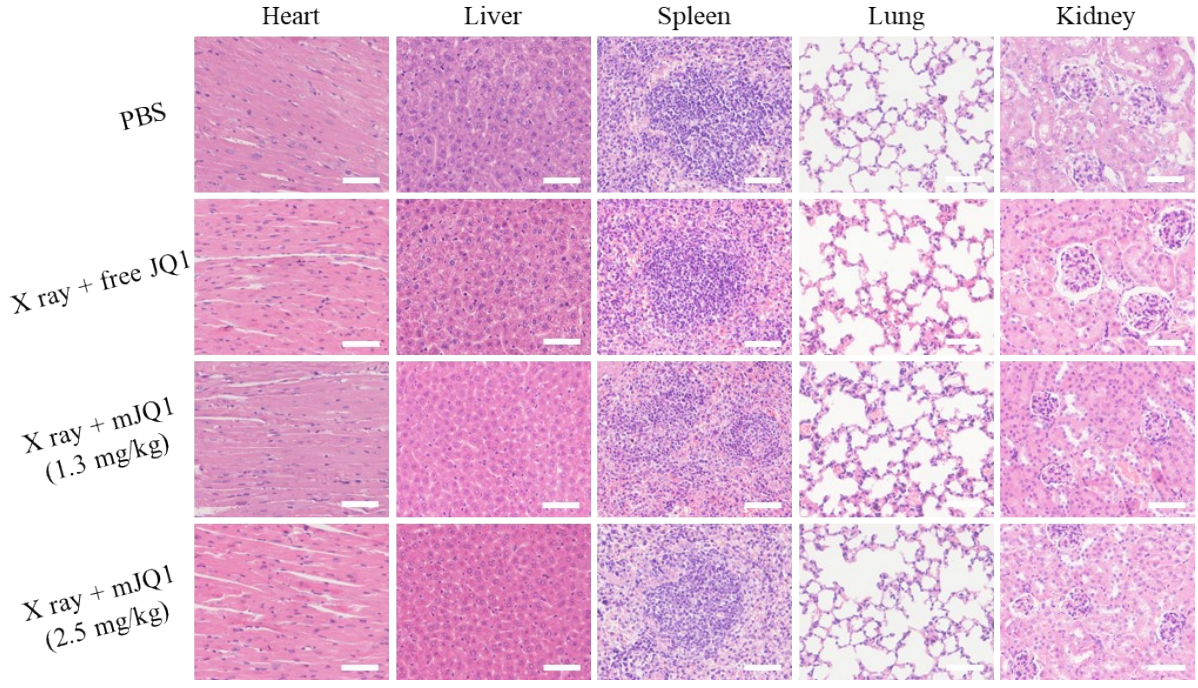

Figure S2. H&E stained sections of major organs (heart, liver, spleen, lung and kidney) of B16F10 melanoma-bearing mice after the end of administration (day 16), Scale bar: 50 μm.