Supplementary Information

Quaternary ammonium-based nanosystem enables delivery of CRISPR/Cas9 for cancer therapy

Mengzhu Zhang,^{a,‡} Siyu Sun,^{a,‡} Xiao Liang,^a Zengguang Liu,^a Jiaxin Yin,^a Quanshun

Li,*a,b and Shengcai Yang*a

^aKey Laboratory for Molecular Enzymology and Engineering of Ministry of Education, School of Life Sciences, Jilin University, Changchun 130012, China

^bCenter for Supramolecular Chemical Biology, Jilin University, Changchun 130012,

China

*Corresponding author.

Tel. and Fax: +86-431-85155200.

Email: <u>yang_shengcai@jlu.edu.cn</u> (S. Yang); E-mail: <u>quanshun@jlu.edu.cn</u> (Q. Li).

[‡]These authors contributed equally to this work.



Figure S1. The synthetic routes of (A) Qua-PLL and (B) Chol-PEG.



Figure S2. The ¹H NMR spectrum of Qua-PLL and PLL in D_2O .



Figure S3. The ¹H NMR spectrum of Chol-PEG in DMSO-*d*.



Figure S4. Hydrodynamic diameter (A) and zeta potential (B) changes of QNP/RNP_{PLK1} and Qua-PLL/Chol-PEG/BSA during the incubation in PBS for 7 days. Data were presented as mean value \pm SD of triplicate experiments.



Figure S5. The SDS-PAGE analysis of purified Cas9. The calculated molecular weight of Cas9 was ~160 kDa. Lane M: protein marker; lane 1: lysates of whole bacterial cells; lane 2: supernatants of lysates; lane 3: effluent fractions of loading sample; lane 4: the fraction eluted with 50 mM imidazole; lane 5: the fraction eluted with 300 mM imidazole; lane 6: the fraction eluted with 500 mM imidazole.



Figure S6. The *in vitro* GFP disruption of HeLa-GFP cells mediated by Lipo2000/RNP_{GFP} without ultrasonication (group 1) and with the ultrasonication for 30 min (group 2). A: fluorescence microscopy analysis; B: quantification of GFP expression in HeLa-GFP cells (***p < 0.001 *vs*. Control group; ns: no significance).



Figure S7. Cell viability of CT26 (A) and HEK293T (B) after the treatment with QNP/RNP_{PLK1} with different QNP concentrations (Cas9 concentration: 2 μ g/mL). Data were presented as mean value \pm SD of five experiments.



Figure S8. T–A cloning sequencing results of the targeted *PLK1* locus in MCF-7 cells treated with PBS and QNP/RNP_{PLK1}.



Figure S9. The changes of body weight within 28 days after different administration.