Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Intracellular Delivery of Bacterial Effectors for Cancer Therapy Using Biodegradable Lipid Nanoparticles

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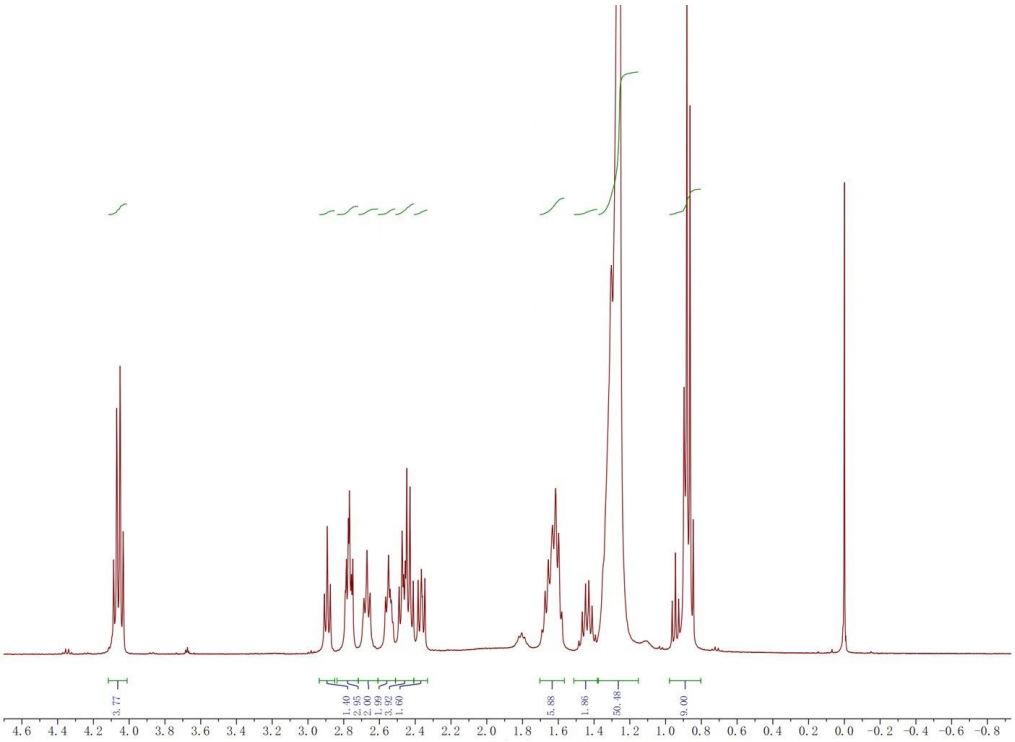
a)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b)

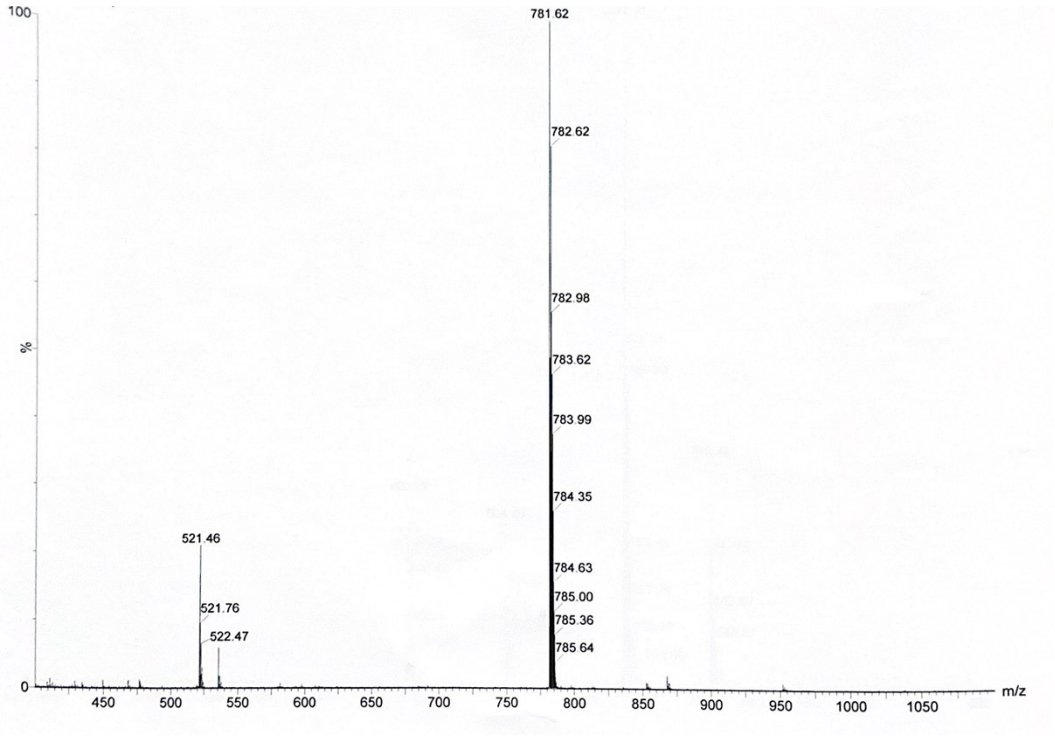

Fig. S1 (a) ^{1}H NMR and (b) MS spectra of PPPDA-O16B.

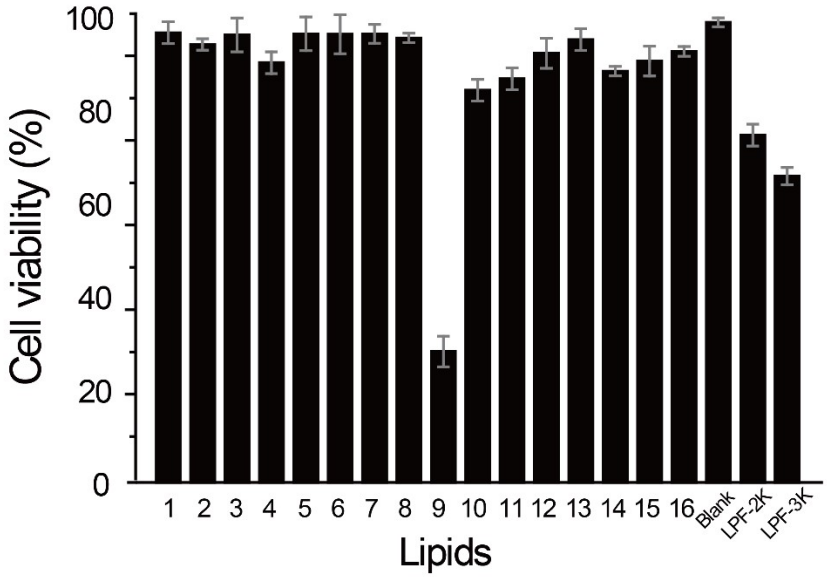

Fig. S2 Cytotoxicity assay of different lipid nanoparticles. HeLa cells seeded in 96-well plate were treated with 4.6 μg/mL of different lipid nanoparticles for 48 h. Cell viability was measured using MTT assay.

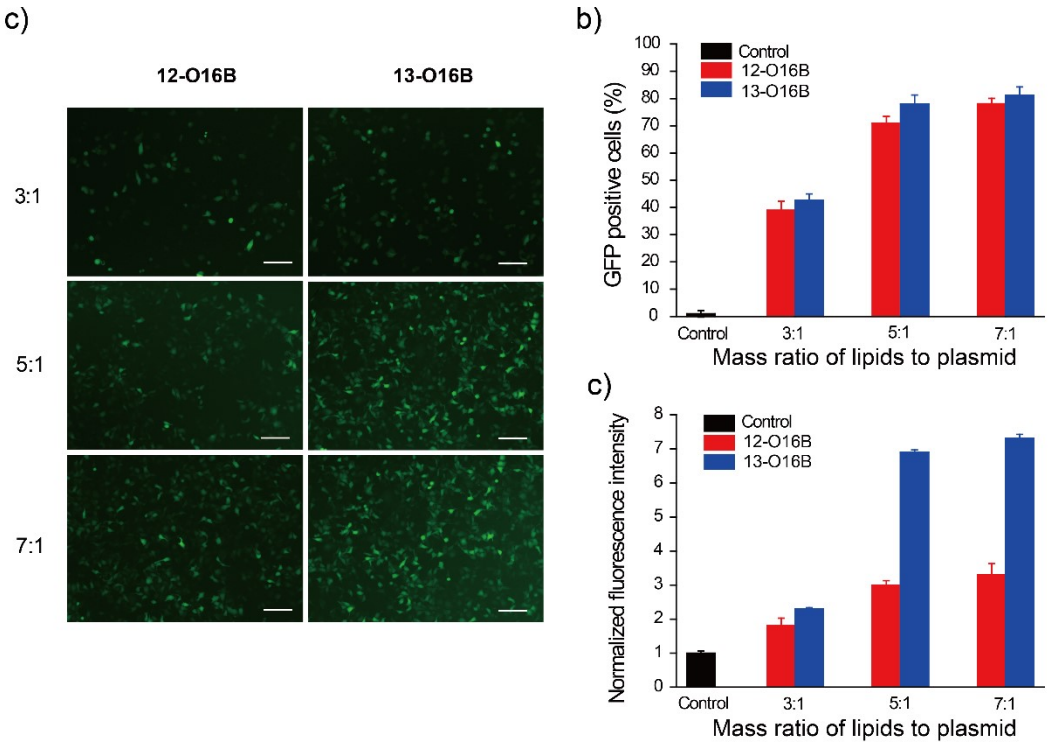

Fig. S3 Optimization of lipid nanoparticle for gene delivery (a) Fluorescence microscope images of HeLa cells transfected with pEGFP-C1(0.33 μg/mL) complexed with 12-O16B and 13-O16B at different ratios (Scale bar: 20 μm), (b) The percentage of GFP-positive cells and (c) Normalized fluorescence intensity of HeLa cells transfected with pEGFP-C1(0.33 μg/mL) and lipid nanoparticles at different ratios as indicated.

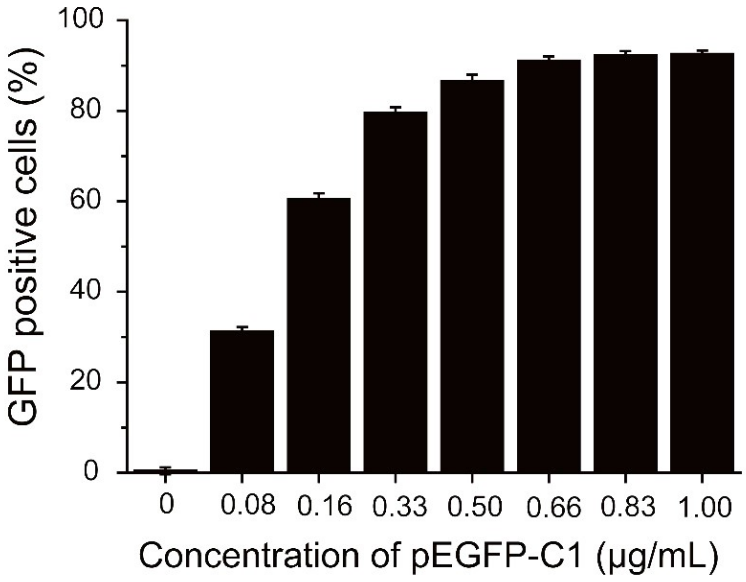

Fig. S4 Flow cytometry analysis of HeLa cells transfected with different concentrations of pEGFP-C1 using PPPDA-O16B.

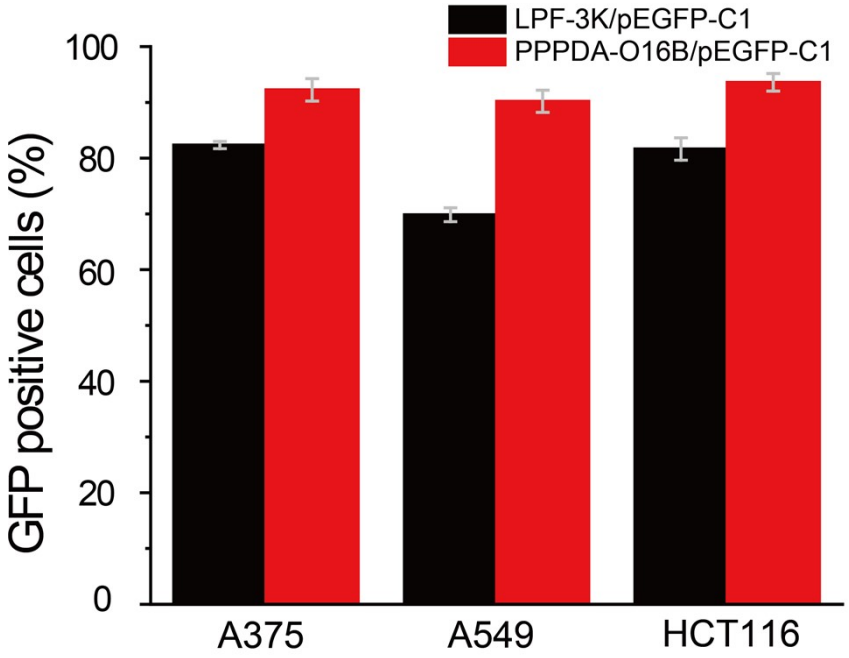

Fig. S5 Flow cytometry analysis of A375, A549 and HCT116 cells transfected with pEGFP-C1 (0.66 μg/mL) complexed with PPPDA-O16B (4.62 μg/mL).

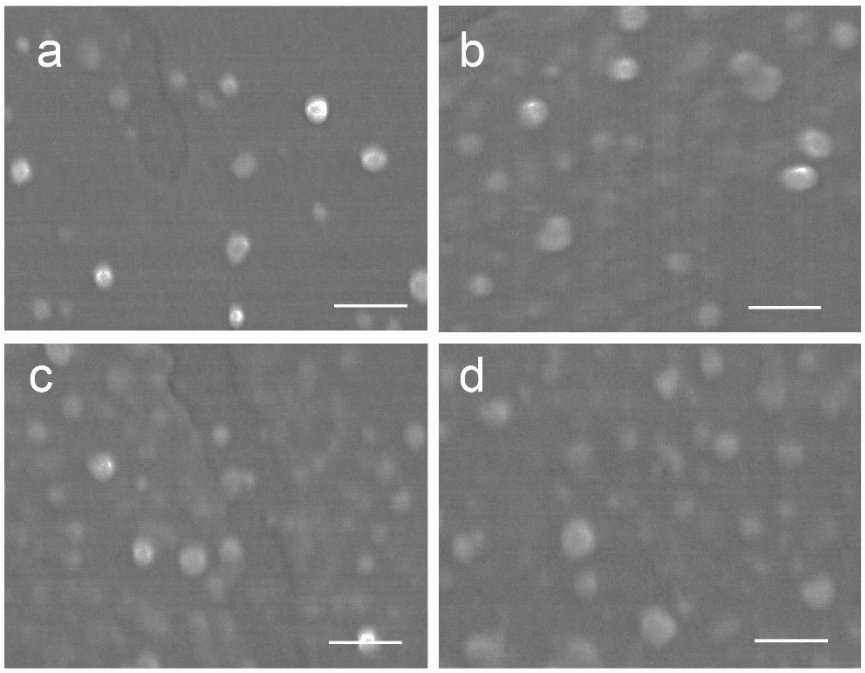

Fig. S6 Scanning electronic microscopy (SEM) images of PPPDA-O16B nanoparticles complexed with different DNA plasmids. (a) PPPDA-O16B, (b) PPPDA-O16B/pEGFP-C1, (c) PPPDA-O16B/OspF and (d) PPPDA-O16B/DUF5 (Scale bar: 300 nm).

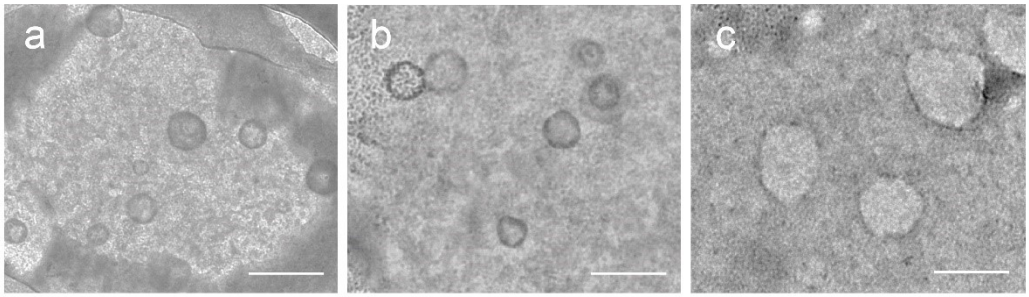

Fig. S7. Transmission electronic microscopy (TEM) characterization of the degradation of lipid nanoparticles by GSH. (a) PPPDA-O16B, (b) PPPDA-O16B/pEGFP-C1, (c) PPPDA-O16B/pEGFP-C1 treated with 5 mM GSH (Scale bar: 300 nm).

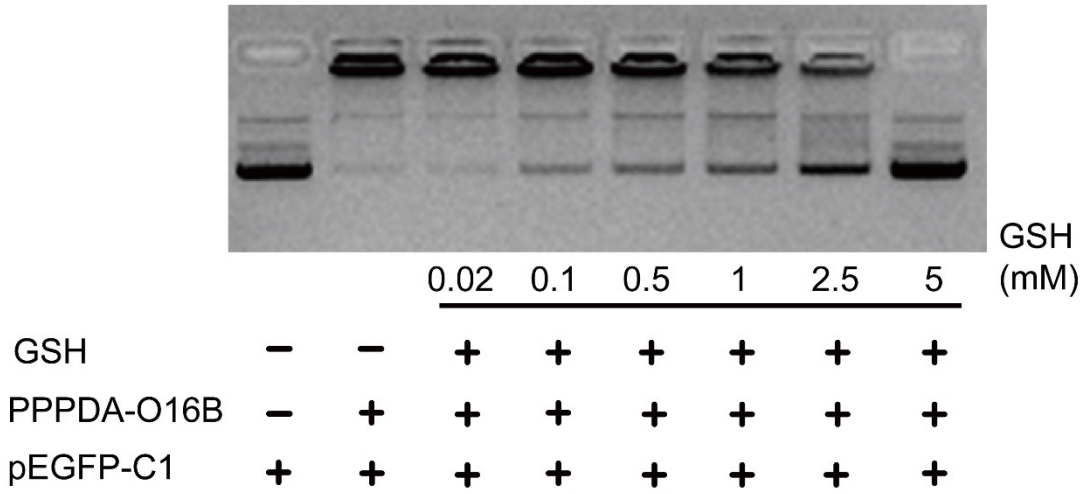

Fig. S8. Gel electrophoresis assay of pEGFP-C1 (6.66 ng/μL), PPPDA-O16B/pEGFP-C1 (lipid concentration was 46.66 ng/μL) and PPPDA-O16B/pEGFP-C1 treated with different concentrations of GSH (0.02, 0.1, 0.5, 1, 2.5, 5 mM).

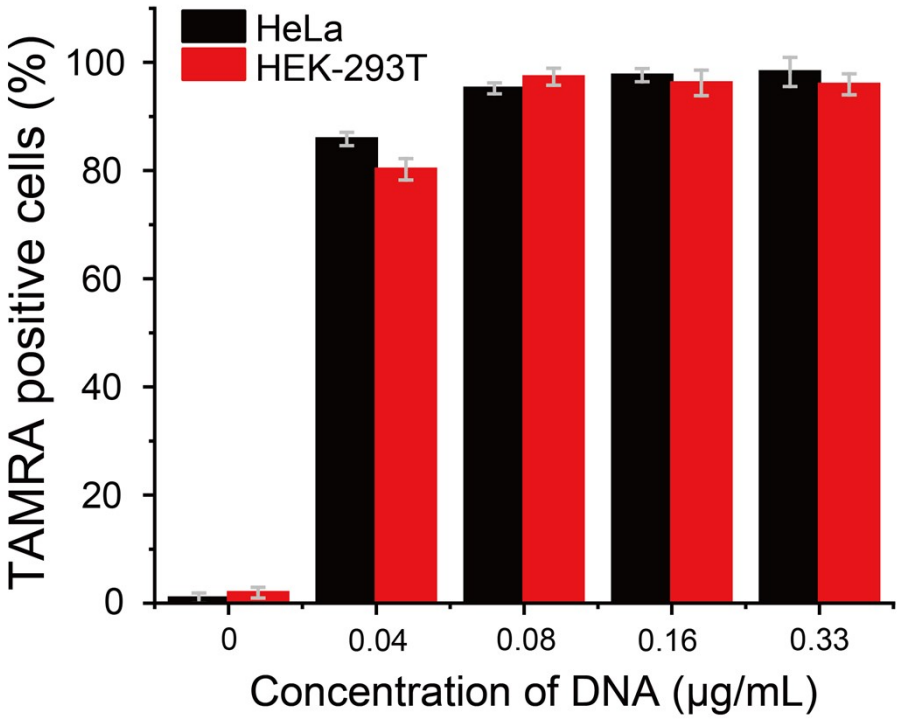

Fig. S9. The comparison of the cellular uptake efficiency of fluorescently labeled PPPDA-O16B/TAMRA-DNA nanoparticles by HeLa and HEK-293T cells. The cells were treated with different concentrations of nanoparticles as indicated for 6 h before quantifying fluorescence-positive cells using flow cytometry.

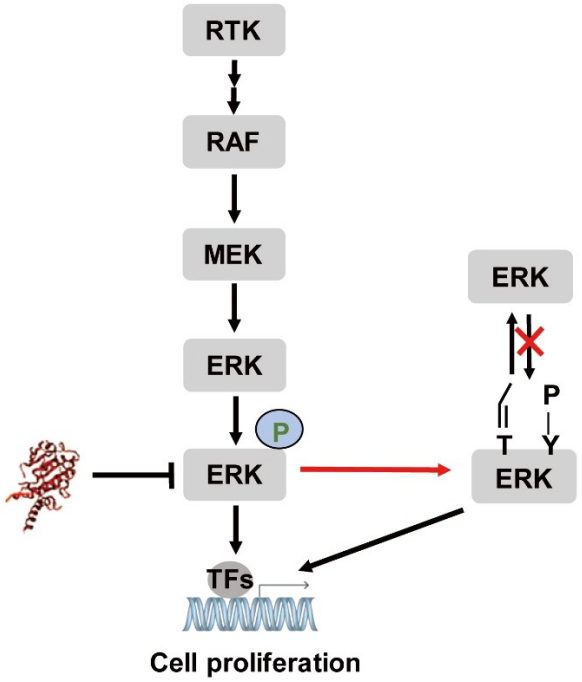

Fig. S10 The mechanism of OspF for ERK dephosphorylation and MAPK signaling inactivation.

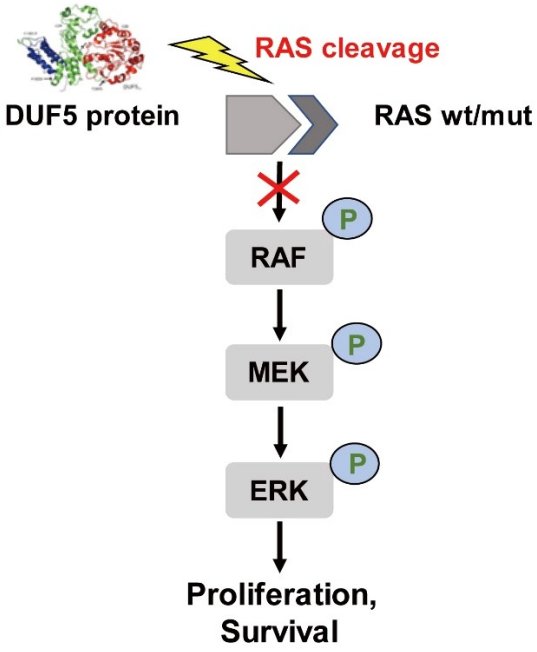

Fig. S11 The mechanism of DUF5 for mutant RAS degradation.

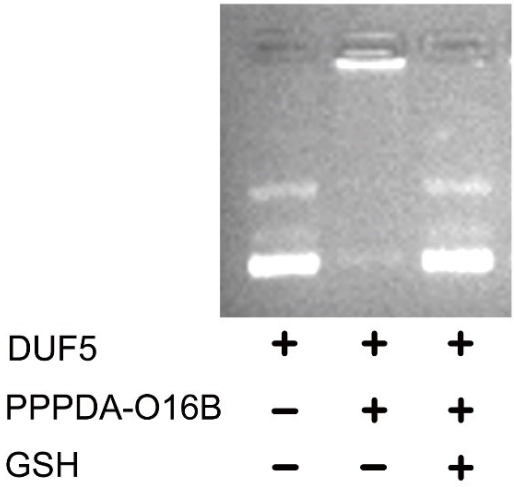

Fig. S12. Gel electrophoresis assay of GSH-triggered DUF5 release from PPPDA-O16B/DUF5 nanoparticles (The concentrations of DUF5 plasmid and lipid were 6.66 ng/μL and 46.66 ng/μL, respectively).

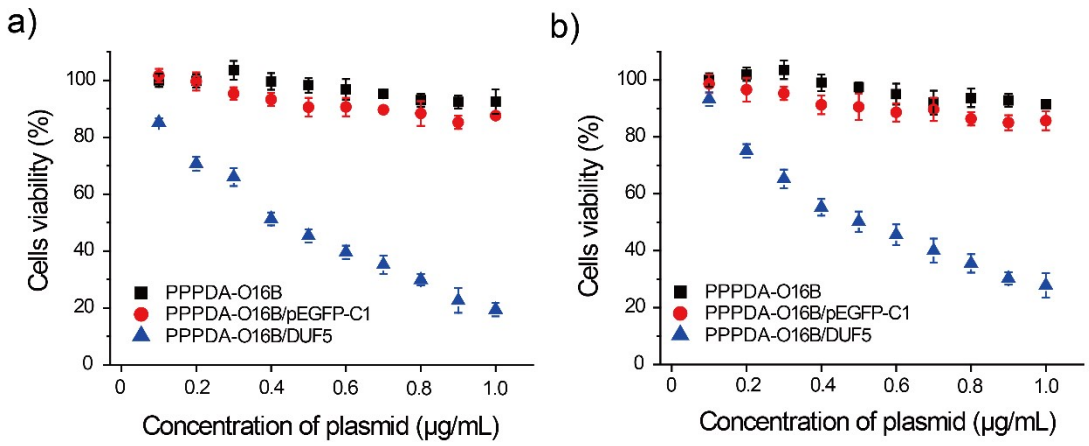

Fig. S13 PPPDA-O16B/DUF5 delivery prohibits the growth of (a) HCT116 and (b) A549 cells

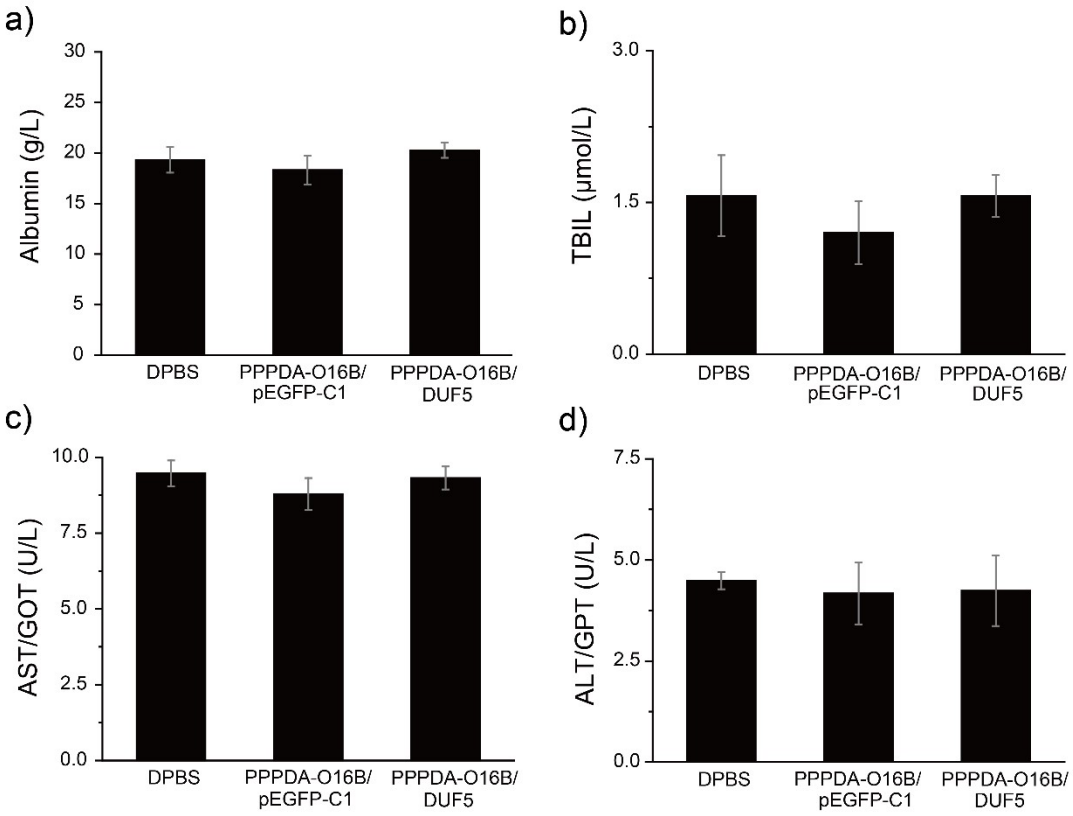

Fig. S14 Liver function tests in mice received different treatments. The concentrations of (a) albumin, (b) total bilirubin, (c) aspartate aminotransferase (AST) and (d) alanine aminotransferase (ALT) in serum of mice injected with DPBS, PPPDA-O16B/pEGFP-C1 and PPPDA-O16B/DUF5 nanoparticles were determined using a quantitative kit according to manufacturer's instruction.

Supplementary Tables

Table S1. Dynamic light scattering (DLS) analysis and Zeta potential of PPPDA-O16B nanoparticles in DPBS at 0 h, 12 h and 24 h.

Time	PBS		PBS+5% serum		PBS+10% serum	
	Size (nm)	Zeta (mV)	Size (nm)	Zeta (mV)	Size (nm)	Zeta (mV)
0 h	129.7 ± 1.8	33.2 ± 0.3	145.8 ± 1.3	14.6 ± 2.0	168.2 ± 2.5	4.2 ± 2.2
12 h	127.3 ± 1.5	33.6 ± 1.8	150.3 ± 2.0	14.2 ± 1.6	173.5 ± 0.8	4.1 ± 1.6
24 h	126.7 ± 0.9	34.6 ± 2.4	163.5 ± 1.8	14.0 ± 3.3	228.1 ± 3.1	3.9 ± 3.0

Table S2. Encapsulation efficiency of pEGFP-C1 by PPPDA-O16B at different ratios

Amount of lipids (μg)	Weight Ratio (lipids:plasmid)	Encapsulation Efficiency (%)
0.3	3:1	43.87
0.5	5:1	66.87
0.7	7:1	79.61

Table S3. Dynamic light scattering (DLS) analysis and Zeta potential of lipid nanoparticles

Samples	Size (nm)	Zeta Potential (mV)
PPPDA-O16B	128.2 ± 3.1	34.6 ± 2.0
PPPDA-O16B /pEGFP-C1	137.3 ± 2.0	19.2 ± 1.6

Table S4. Dynamic light scattering (DLS) analysis and Zeta potential of different lipid nanoparticles with GSH treatment.

Samples	Size (nm)
PPPDA-O16B/pEGFP-C1	137.3 ± 2.0
PPPDA-O16B/pEGFP-C1+GSH	635.8 ± 2.4
PPPDA-O16/pEGFP-C1	156.6 ± 1.5
PPPDA-O16/pEGFP-C1+GSH	165.1 ± 2.3