Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Genome editing of PD-L1 mediated by nucleobase-modified polyamidoamine for cancer immunotherapy

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Materials and methods

1. Cytotoxicity analysis of AP-PAMAM and PAMAM

B16F10 cells were inoculated in 96-well plates at a density of 7,000 cells/well. After the culture for 12 h, different concentrations of PAMAM and AP-PAMAM were added into each well, and the cells were incubated for 48 h. Subsequently, MTT solution was added into each well at a final concentration of 0.5 mg/mL. After the incubation for additional 4 h, the supernatant was discarded, and 150 μL dimethyl sulfoxide was used to dissolve the formazan crystals. After 10 min, the absorbance at 490 nm of each well was measured using HBS-1096A microplate reader (Detie, Nanjing, China) to calculate the cell viability.

2. *In vitro* transfection efficiency analysis of AP-PAMAM/pX458 nanoparticles

B16F10 cells were inoculated in 6-well plates at a density of 2.0×10^5 cells/well. After the culture for 12 h, the medium was replaced with FBS-free DMEM containing AP-PAMAM/pX458 or PAMAM/pX458 nanoparticles with different N/P ratios. The final concentration of plasmid in all supernatants was 2 μg/mL. After the incubation for 4 h, the supernatant was discarded and replaced with 10% FBS-containing DMEM. Finally, the expression level of GFP protein after 48 h was detected using Olympus IX73P1F fluorescence microscope (Tokyo, Japan) and CytoFLEX flow cytometer (Beckman Coulter Inc., Kraemer Boulevard Brea, CA, USA).

3. Measurement of serum biochemical indicators

Whole blood was allowed to stand at room temperature for 2 h and then centrifuged at 3,000 rpm for 15 min (4 °C) to obtain the supernatant. The samples were treated with the appropriate assay kits according to the manufacturers' instructions, and the serum biochemical indicators were analyzed on a Chemray-800 Full Automatic Biochemistry Analyzer (Rayto Life Sciences, Shenzhen, China).

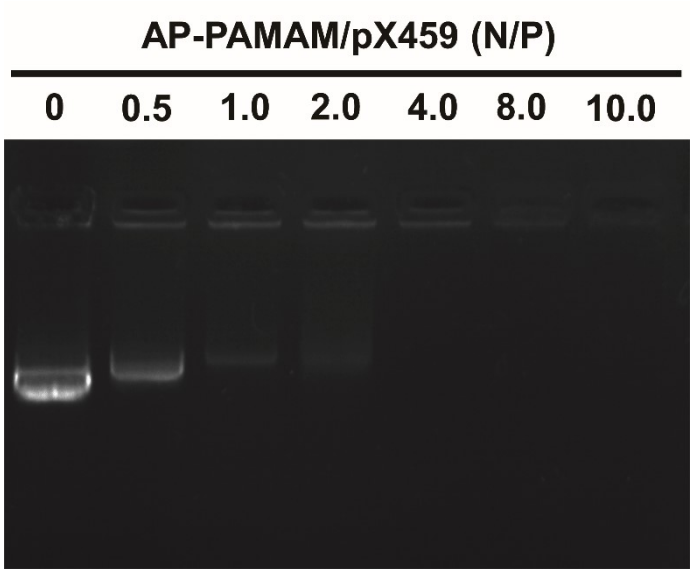

Figure S1. Gel retardation assay of AP-PAMAM/pX459 nanoparticles at different N/P ratios.

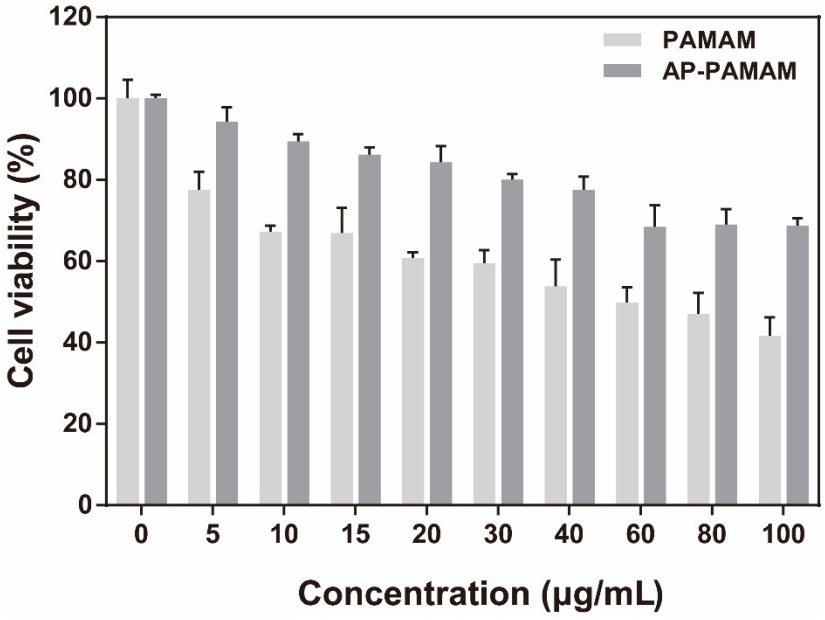

Figure S2. Cell viability of B16F10 cells after the treatment with PAMAM and AP-PAMAM at different concentrations. Data were represented as mean value ± SD (n = 3).

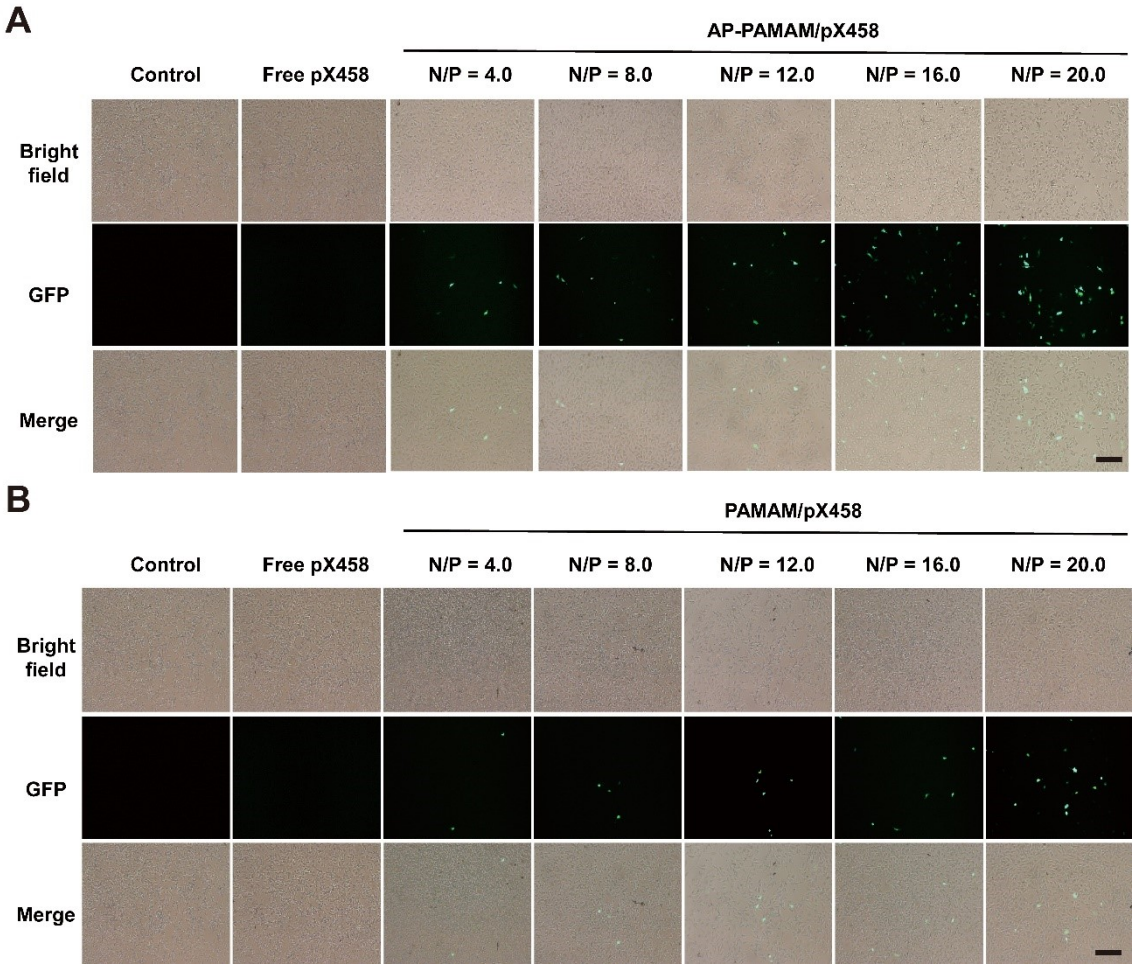

Figure S3. The fluorescence images of B16F10 cells after the carriers-mediated transfection of pX458. The scale bar was 200 μm.

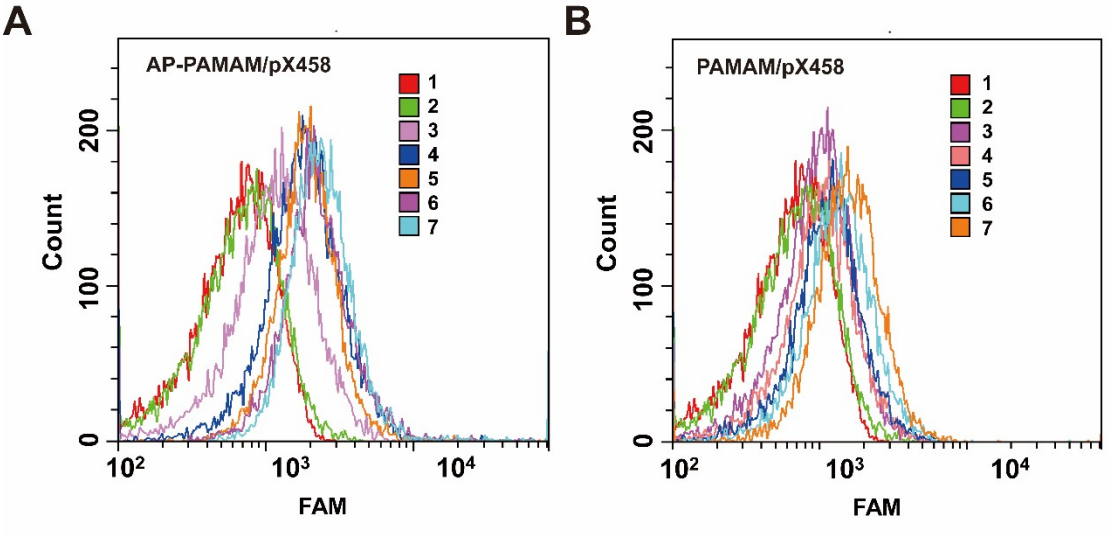

Figure S4. Flow cytometric analysis of GFP expression in B16F10 cells after the transfection of AP-PAMAM/pX458 (A) and PAMAM/pX458 (B): (1) untreated cells, (2) free pX458 group, and AP-PAMAM/pX458 or PAMAM/pX458 transfection at N/P ratios of 2.0 (3), 4.0 (4), 8.0 (5), 16.0 (6) and 20.0 (7).

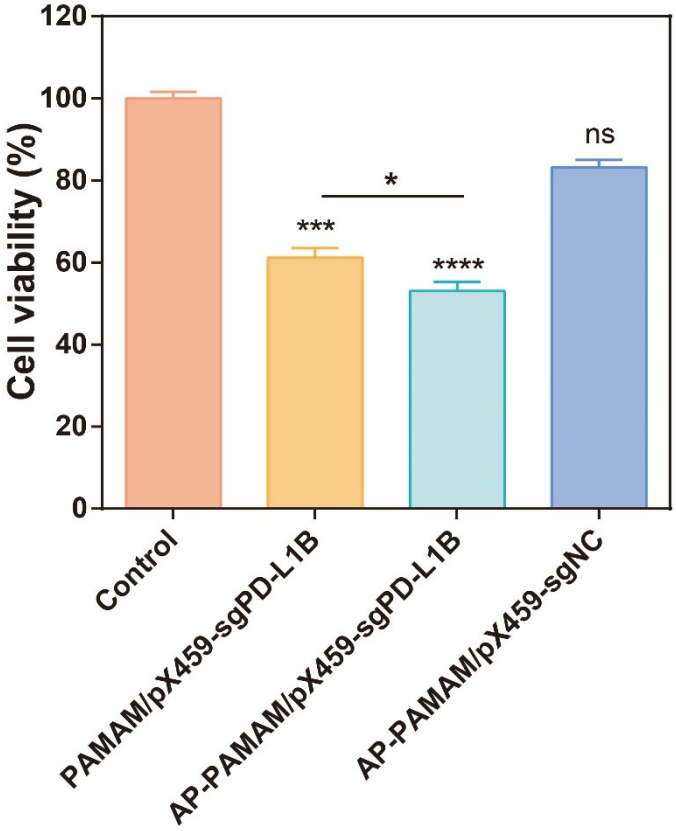

Figure S5. The cell viability of B16F10 cells after the transfection of different nanoparticles for 48 h. Data were represented as mean value ± SD (n = 3; ns, not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$).

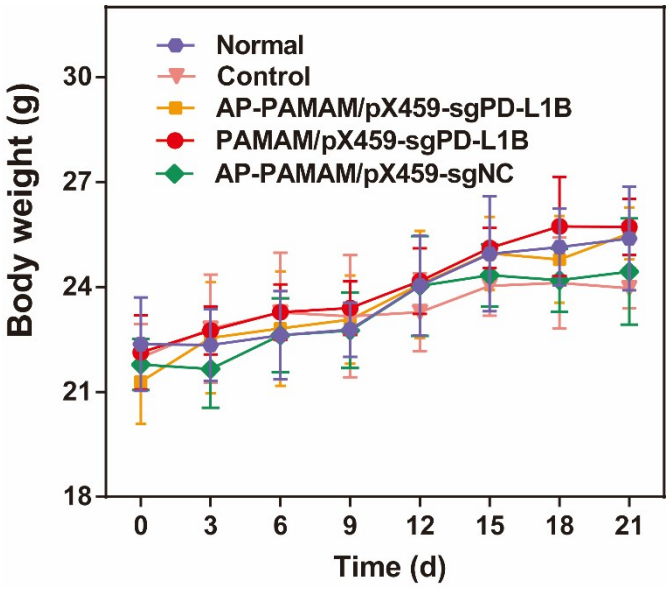

Figure S6. Body weight changes during the treatment with different nanoparticles. Data were represented as mean value ± SD (n = 6).

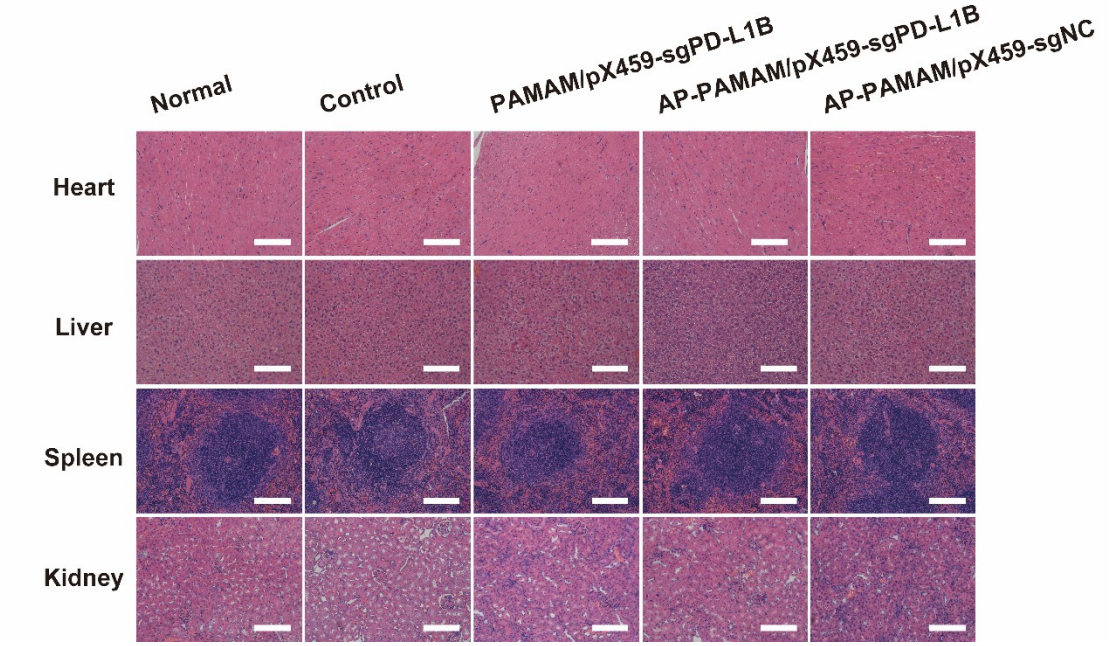

Figure S7. H&E staining of different organs (heart, liver, spleen, lung and kidney) after the treatment with different nanoparticles for 21 days. The scale bar was 100 μm.

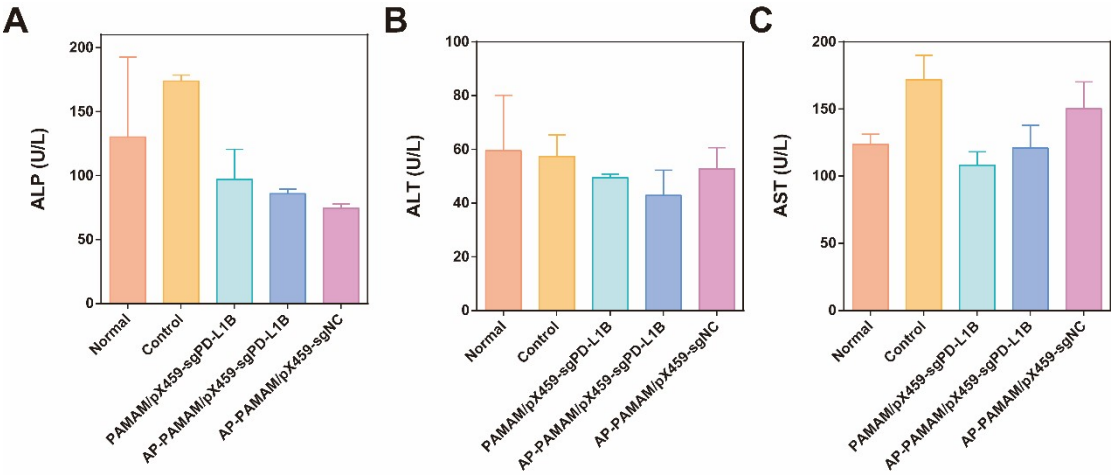

Figure S8. Physiological function assessment of liver toxicity after the treatment with different nanoparticles. Data were represented as mean value ± SD (n = 3).

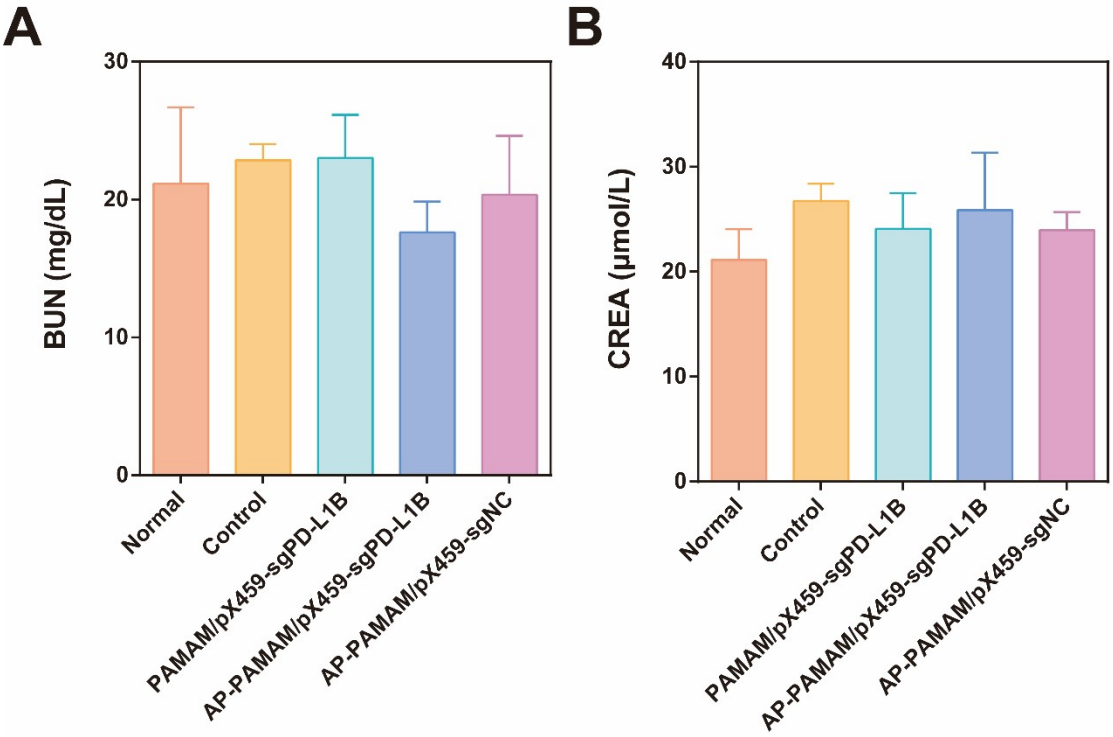

Figure S9. Physiological function assessment of kidney toxicity after the treatment with different nanoparticles. Data were represented as mean value ± SD (n = 3).