Supplementary Materials

Ultra-efficient Delivery of CRISPR/Cas9 Using Ionic Liquid Conjugated Polymers for Genome Editing-Based Tumor Therapy

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Experimental Section

Synthesis and characterization of PFP, PPE, PPV, and PBF

PFP, PPE, PPV, and PBF were prepared according to the previously reported procedures. [1-4]

Characterization of conjugated polyelectrolyte/DNA complexes

The sizes and zeta potential of conjugated polyelectrolyte/DNA complexes at various N/P ratios were measured using a Zetasizer Nano ZS 90 (Malvern, UK) at room temperature. Complex solutions containing 1μg of DNA were prepared and diluted with 1 ml of ultrapure water before characterization.

Western blotting analysis

Bio-Rad image analysis software was used to analyze the intensity of western blotting bands. The band intensity ratio of PLK1 to the internal reference GAPDH was obtained and the experimental groups were compared to group (1) to determine the relative expression level of the PLK1 protein.

***In vivo* experiment**

BALB/c nude mice (age 6-8 weeks) for *in vivo* treatment were maintained in Peking University Laboratory Animal Center, which is an AAALAC-accredited and specific pathogen-free (SPF) experimental animal facility. All the experimental animals in our study were treated in accordance with protocols approved by the Institutional Animal Care and Use Committee of Peking University.

***In vivo* toxicity evaluation**

The concentrations of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate transaminase (AST), creatinine (CREA), and blood urea nitrogen (UREA) in serum, were quantified using an automated spectrophotometric analyzer (Beckman Coulter, UniCel DxC 600 synchron clinical system). For histological examination, the main organs including the liver, the heart, the spleen, the lung, the kidneys, and the tumor were collected after administration. Organs were fixed with 4% paraformaldehyde and embedded in paraffin,

followed by sectioning and staining with H&E and analyzing with an inverted microscope (Olympus X71, Olympus, Tokyo, Japan).

Statistical analysis

All data were analyzed for significance by a one-way analysis of variance (ANOVA) and t-test for comparisons between the two groups using the SPSS software package. In all statistical analyses, $p < 0.05$ was regarded as statistically significant. All values are presented as the means ± standard deviation (SD).

Table S1. Photophysical properties of CPs

CPs	λ (abs/nm)	λ (E_m/nm)	ε ($\times 10^4$)/$M^{-1}\cdot cm^{-1}$	QY (%)
PFP	380	424	4.30	36
PPE	436	514	3.58	8.8
PPV	468	593	1.02	1.5
PBF	550	606	1.54	1.5

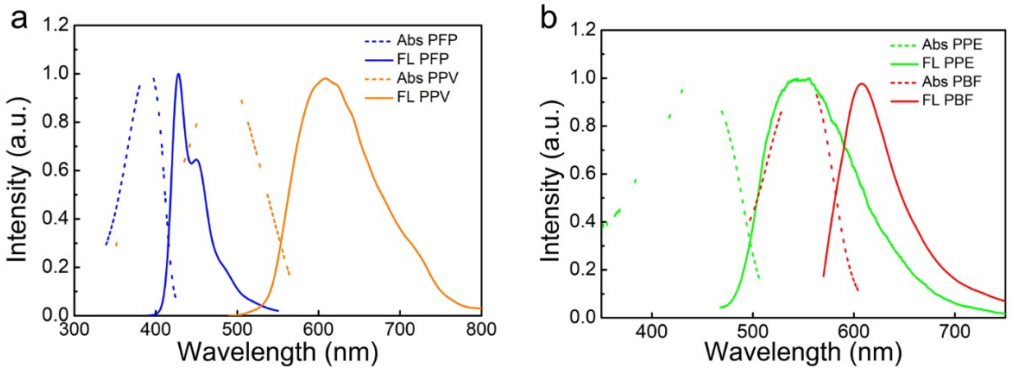

Figure S1. The absorption and emission spectrum characterization. a, b) absorption and emission spectrum of PFP, PPV, PPE, and PBF.

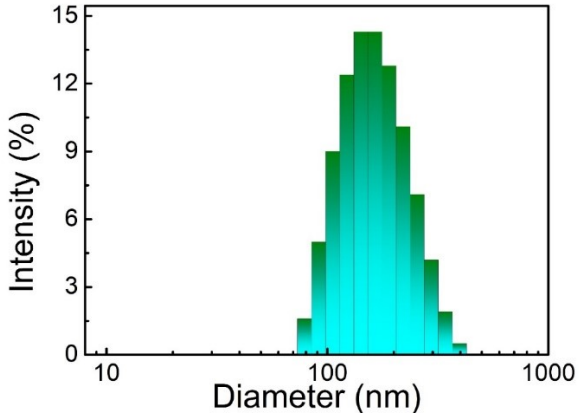

Figure S2. The hydrodynamic diameter of the PBF/DNA complex results by dynamic light scattering (DLS).

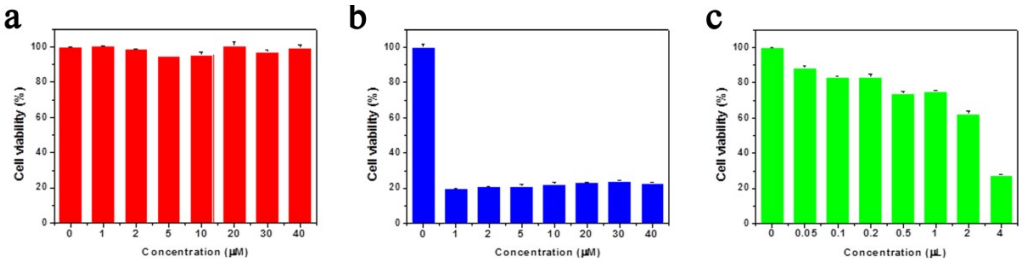

Figure. S3 Cytotoxicity assay of transfection agent at various concentrations. **a**, PBF; **b**, PEI; **c**, Lipofectamine 2000.

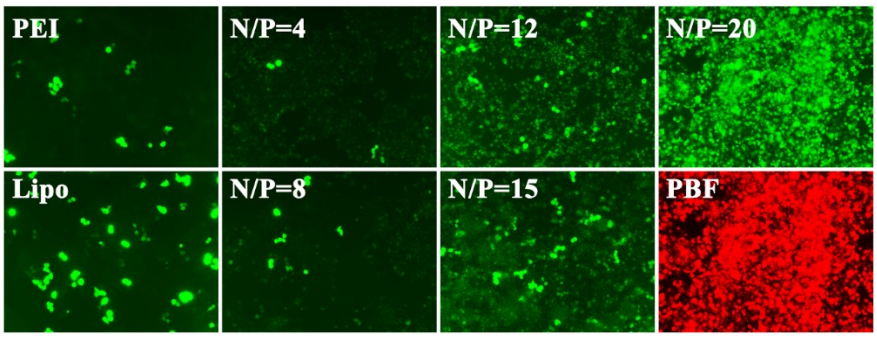

Figure S4. The efficiency of GFP expression under various N/P ratios in hepatoma carcinoma cells HepG2.

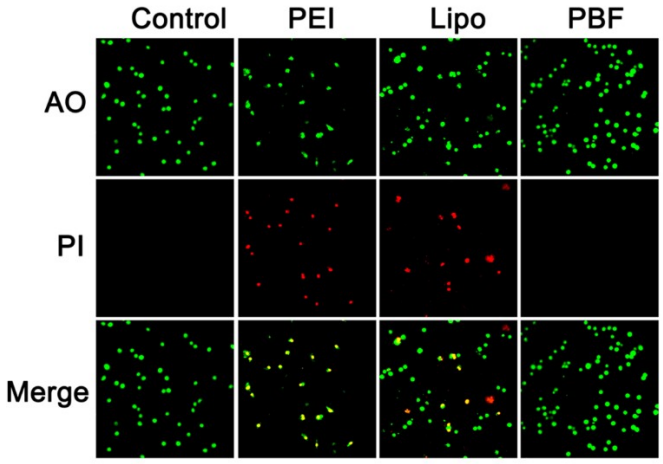

Figure S5. Apoptosis analysis of transfection reagents using AO/PI.

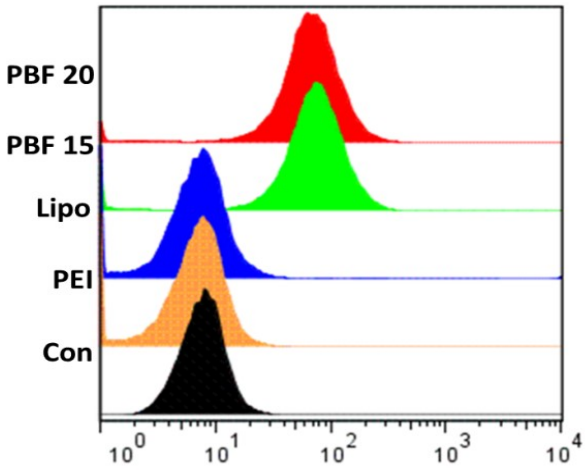

Figure S6. Transfection efficiency of gene vectors in in medium containing 10% fetal bovine serum (FBS) by FACS analysis.

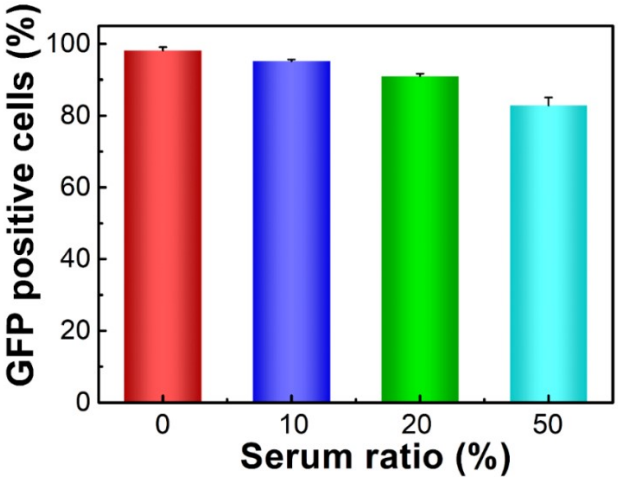

Figure S7. GFP expression level in cells with various concentrations of serum.

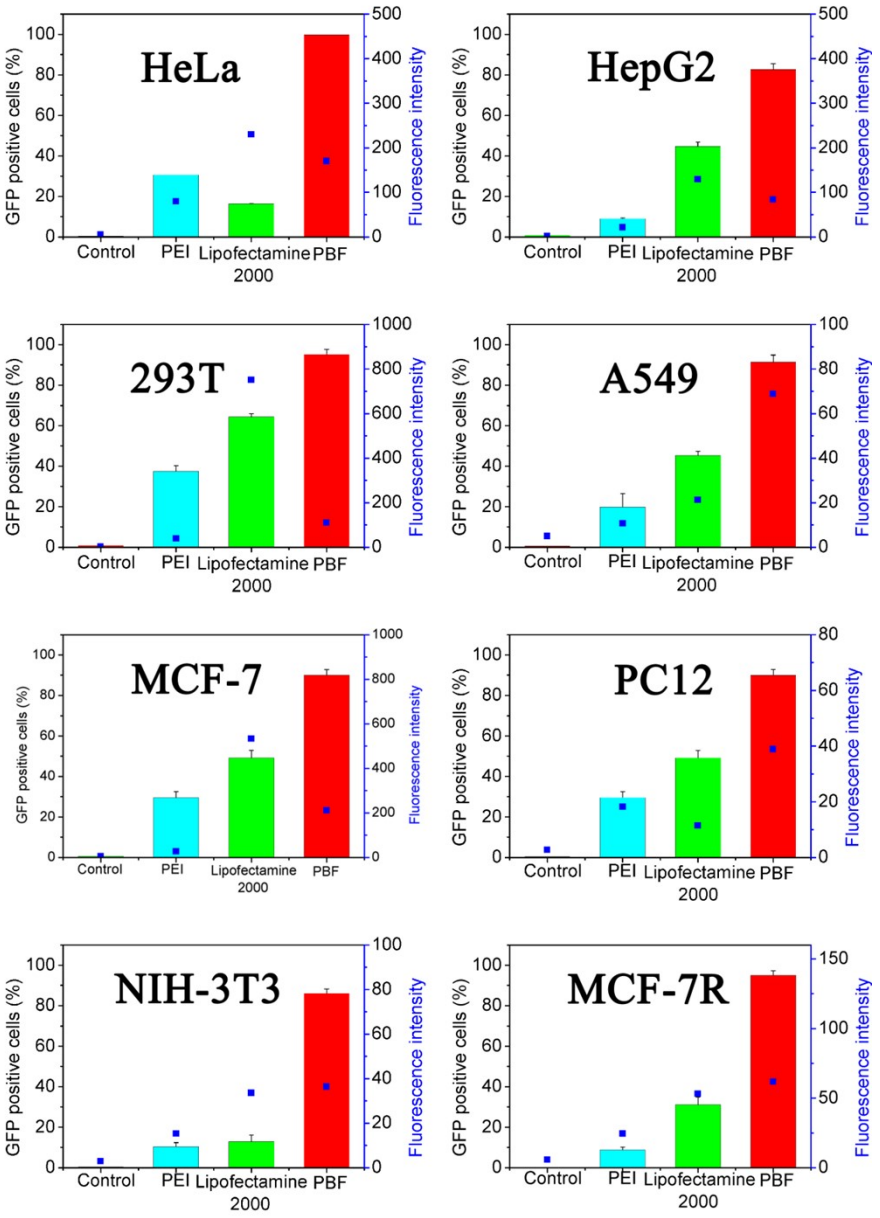

Figure S8. PBF mediated gene delivery in various cells. The transfection efficiency in various cells analysis by FACS, including cervical carcinoma cell HeLa cells, hepatocellular liver carcinoma cell HepG2 cells, embryonic kidney cell 293T, human embryonic kidney cell A549, breast cancer cell MCF-7 cells, primary embryonic fibroblast cell NIH-3T3 cells and neuron-like cells PC12, and multidrug resistance cells MCF-7ADR. Squares represent mean fluorescence intensity.

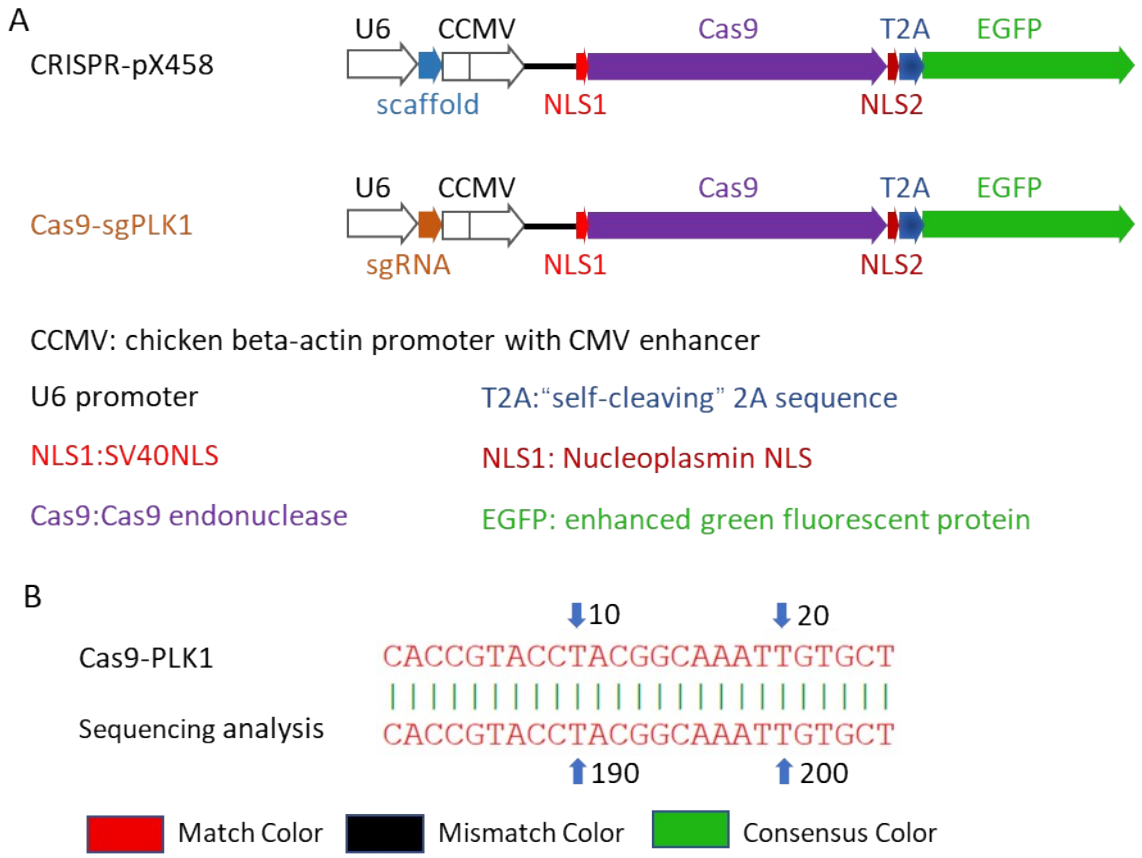

Figure S9. Construction of CRISPR-Cas9 plasmid. (A) The schematic diagram of the CRISPR-PX458 plasmid and the modified Cas9-sgPLK1 plasmid. (B) Sequencing analysis of PLK1 sgRNA.

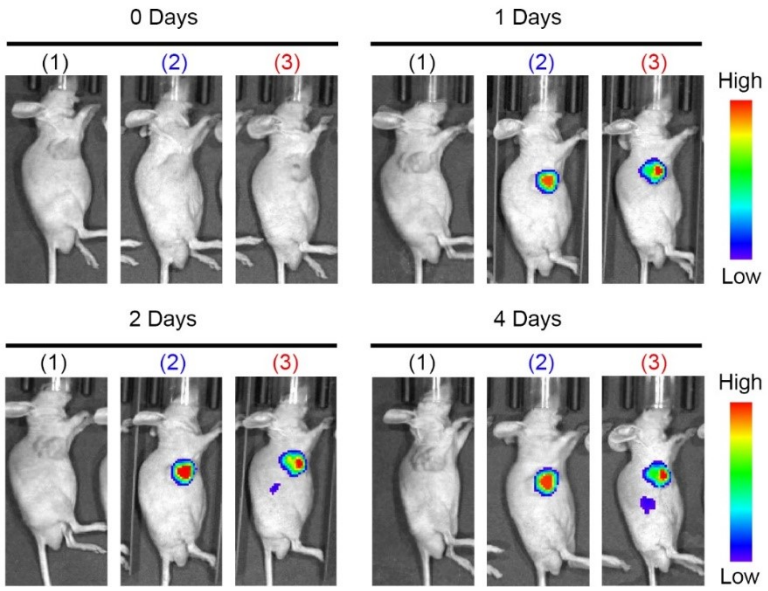

Figure S10. The retention ability of PBF-CRISPR/PLK1 in nude mice bearing tumor by bioluminescence imaging at various time intervals. Intratumor injection of (1) PBS, (2) PBF-CRISPR/PX458, (3) PBF-CRISPR/PLK1.

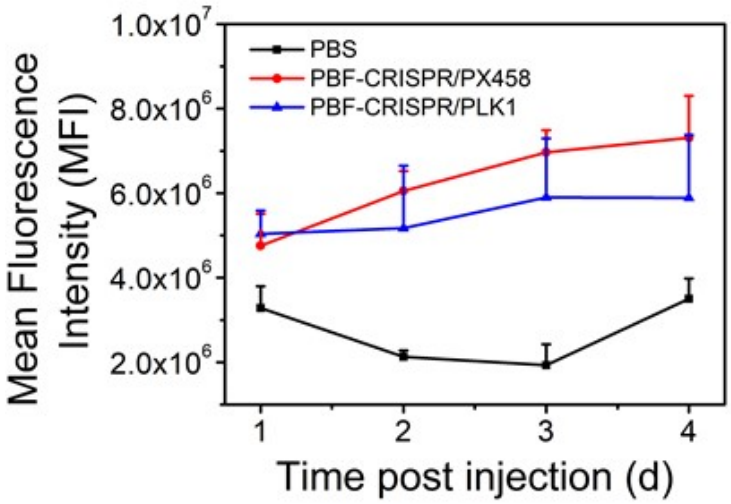

Figure S11. Mean fluorescence intensity analysis of bioluminescence imaging in tumors taken at different time intervals.

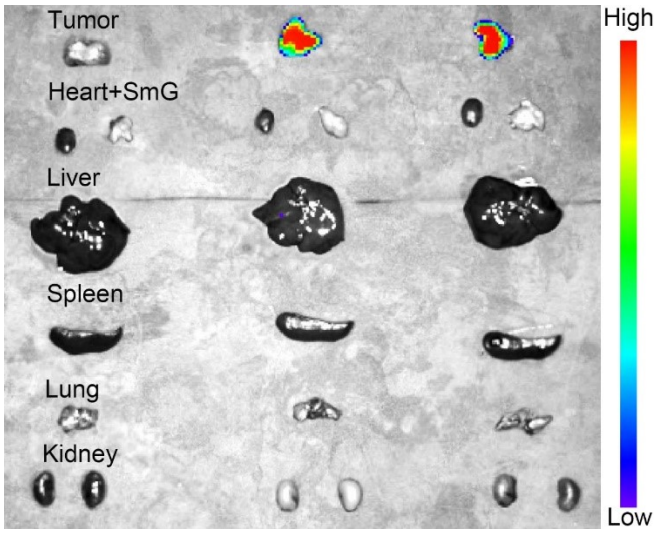

Figure S12. Fluorescence imaging of the tumor and other organs harvested from the athymic nude mice bearing subcutaneous xenograft tumor after 4 days.

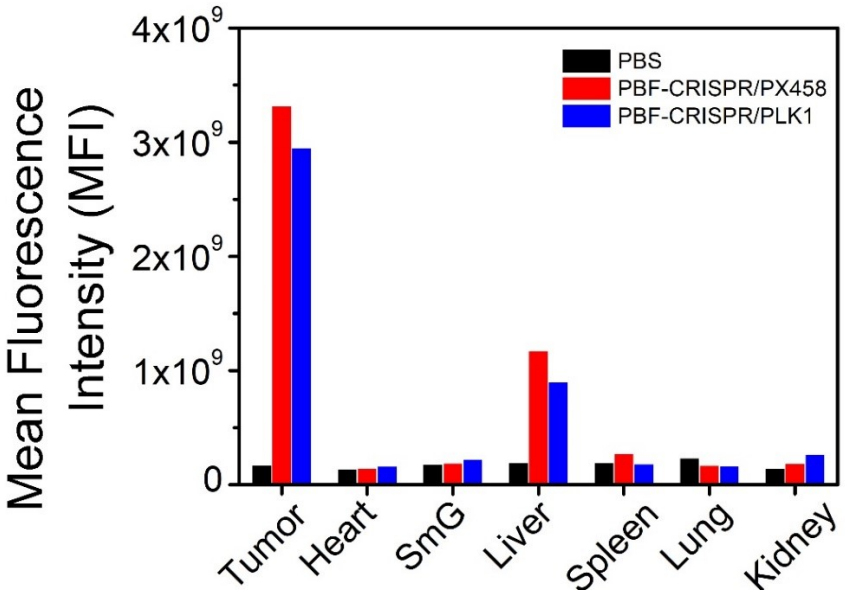

Figure S13. Fluorescence intensity quantification analysis of tumor and other organs harvested from athymic nude mice bearing subcutaneous xenograft tumor after 4 days.

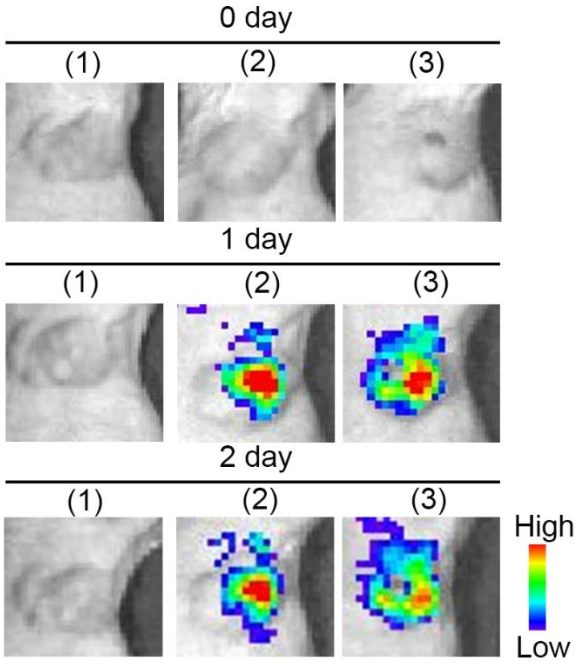

Figure S14. Fluorescence imaging of tumor in living mice after administration of (1) PBS, (2) PBF-CRISPR/PX458, (3) PBF-CRISPR/PLK1 at various time intervals.

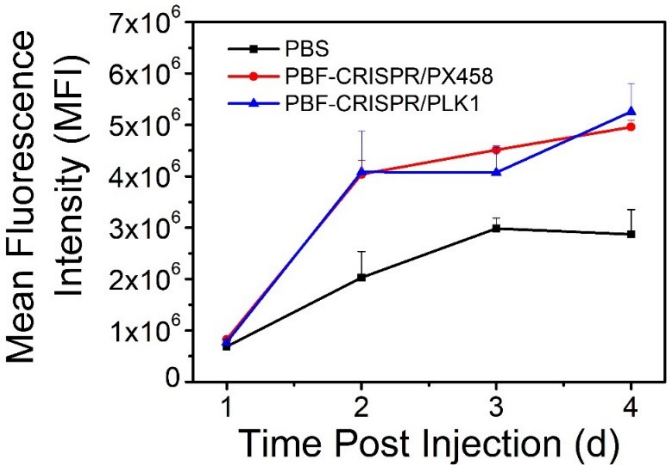

Figure S15. Quantification of total fluorescence intensity of GFP in tumor after PBS, PBF-CRISPR/PX458, and PBF-CRISPR/PLK1 administration.

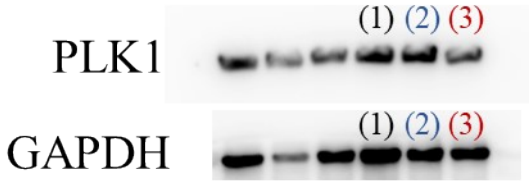

Figure S16. The original WB images of Fig.4i.

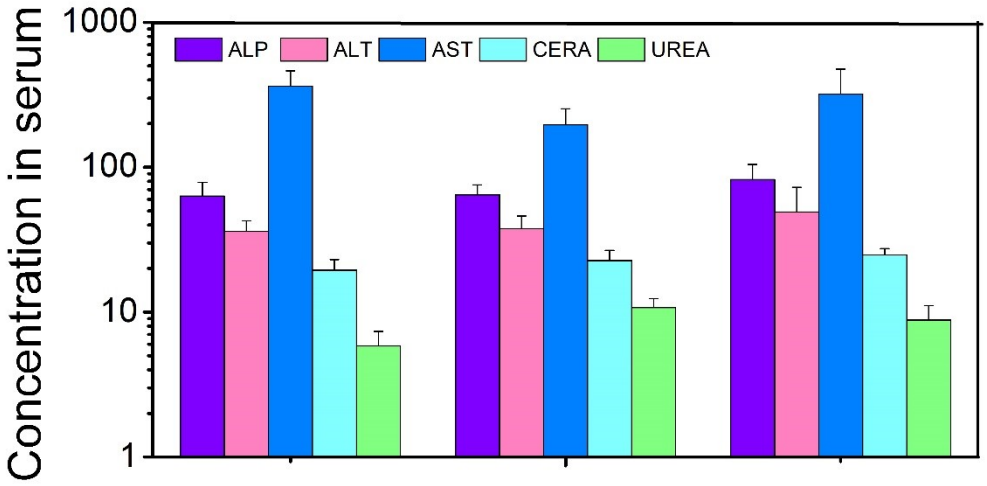

Figure S17. No significant alteration at post-injection of PBF-CRISPR/PLK1 compared with the control group in biochemical blood biomarkers including ALP, ALT, AST, CERA, and UREA (ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CREA, creatinine).

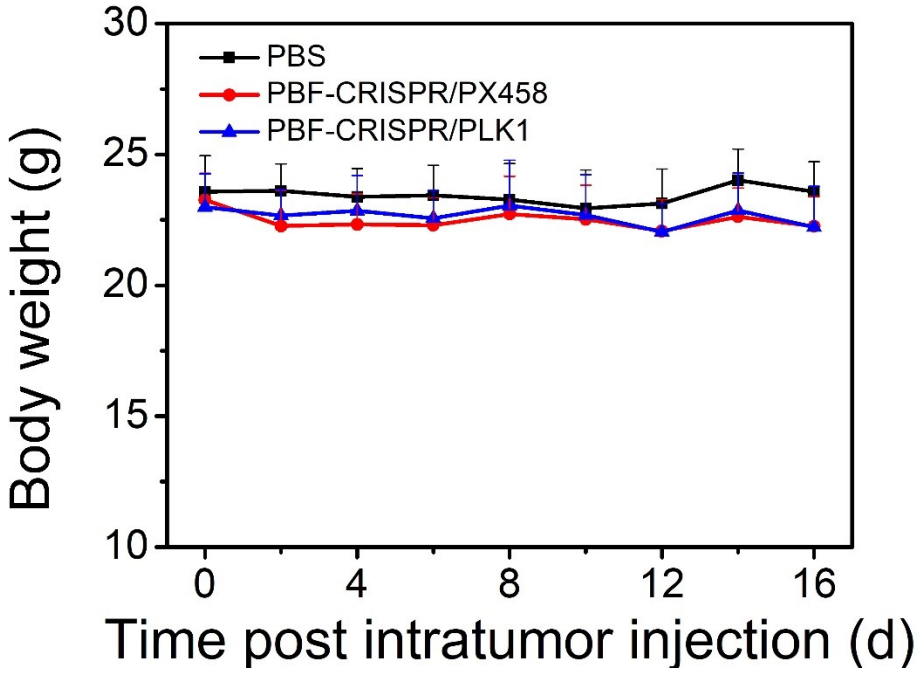

Figure S18. Body weight of mice during the therapeutic period.

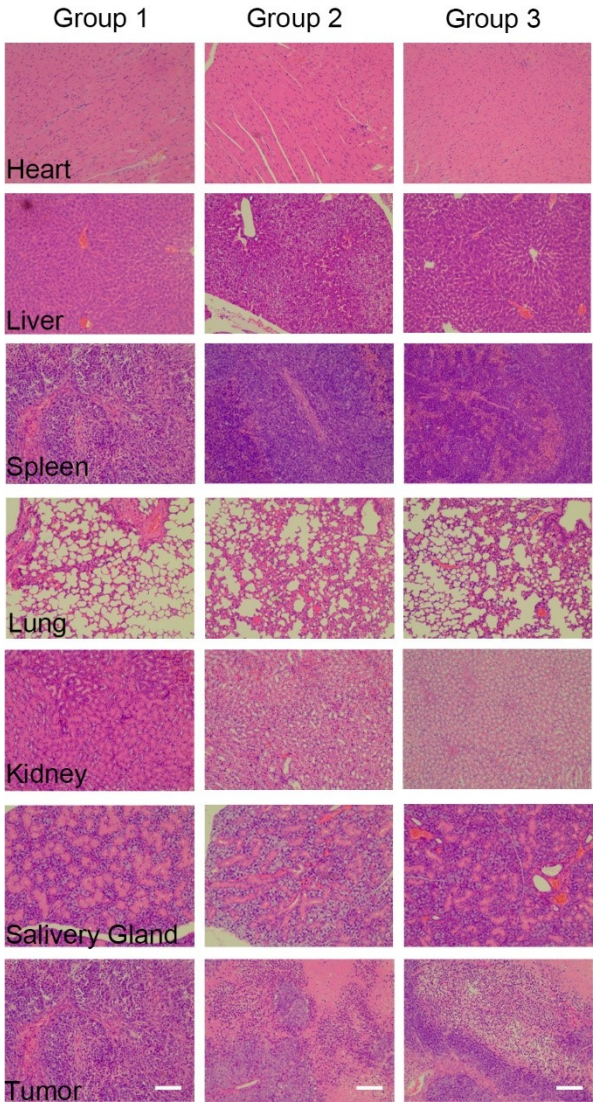

Figure S19. Histological H&E staining of various organs in various therapeutic groups. Group 1: PBS; Group 2: PBF-CRISPR/PX458; Group 3: PBF-CRISPR/PLK1. Scale bar is 200 μm.

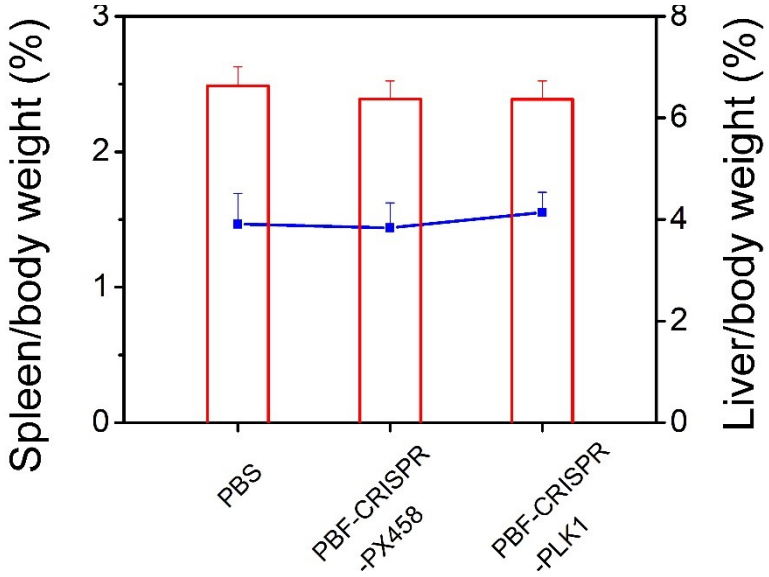

Figure S20. Organ coefficient in various therapeutic groups.

Reference:

[1] H. Yuan, Z. Liu, L. Liu, F. Lv, Y. Wang, S. Wang, *Adv. Mater.* **2014**, 26, 4333-4338.

[2] F. Wang, Z. Liu, B. Wang, L. Feng, L. Liu, F. Lv, Y. Wang, S. Wang, *Angew. Chem. Int. Ed*. **2014**, 53, 424-428.

[3] B. Liu, B. S. Gaylord, S. Wang, G. C. Bazan, *J. Am. Chem. Soc*. **2003**, 125, 6705-6714.

[4] H. Chong, C. Nie, C. Zhu, Q. Yang, L. Liu, F. Lv, S. Wang, *Langmuir* **2012**, 28, 2091-2098.