Targeting Delivery of CRISPR System into Tumour to Edit Glutamine Metabolism for Cancer Therapy by DPA-Zn-Modified Nanoparticles

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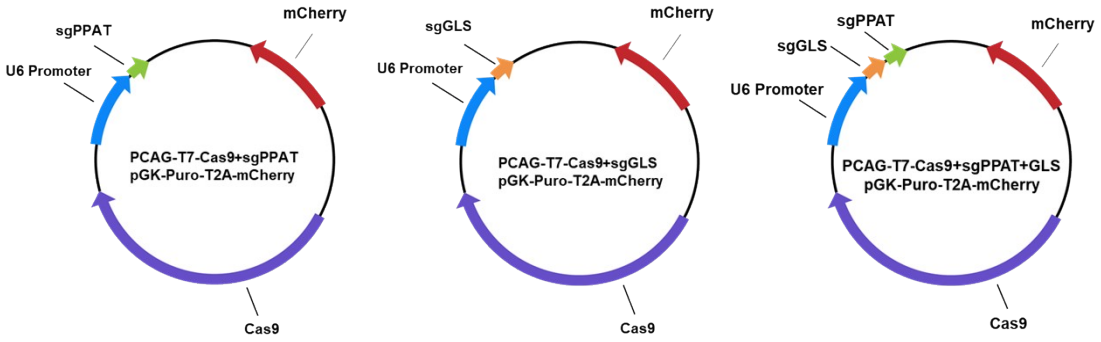

Figure s1. The plasmid maps of Cas9-sgPPAT, Cas9-sgGLS, and Cas9-sgPPAT+sgGLS.

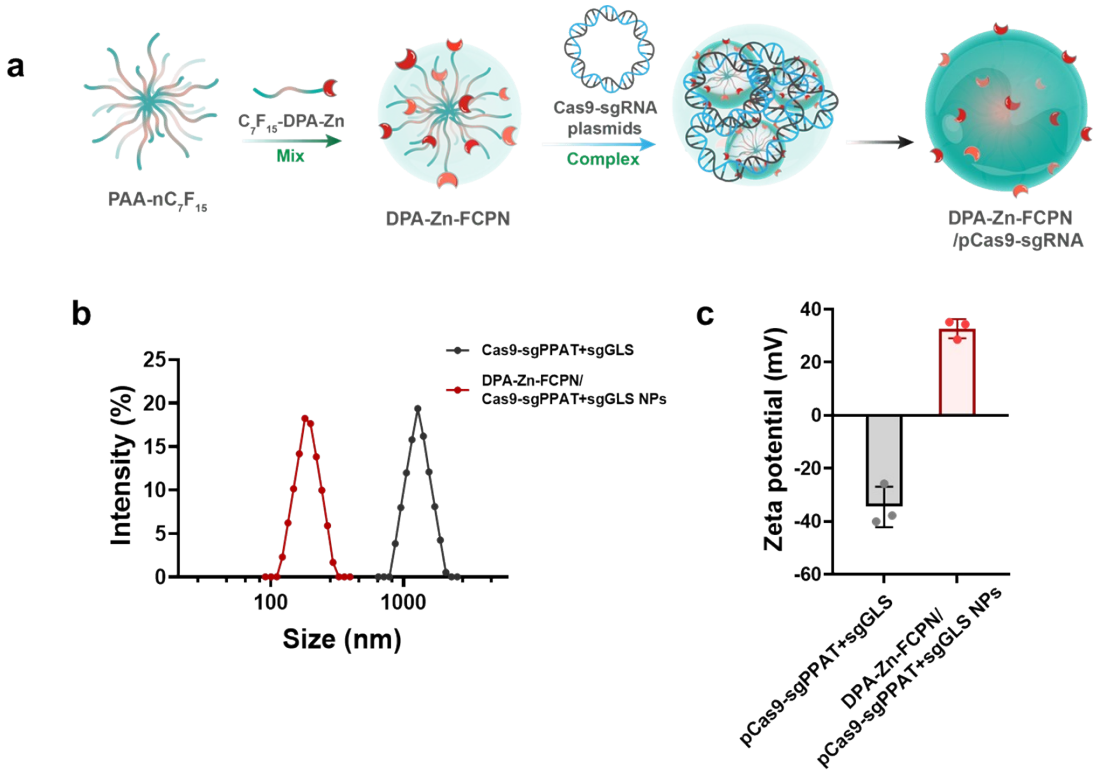

Figure s2. Characterization of DPA-Zn-FCPN/pCas9-sgRNA NPs. (**a**) Illustration of the complexation between DPA-Zn-FCPN and the pCas9-sgRNA plasmid. (**b**)The size distribution and (**c**) Zeta potentials of DPA-Zn-FCPN/pCas9-sgRNA NPs and pCas9-sgRNA plasmid. Data were presented as mean ± s.d (n = 3).

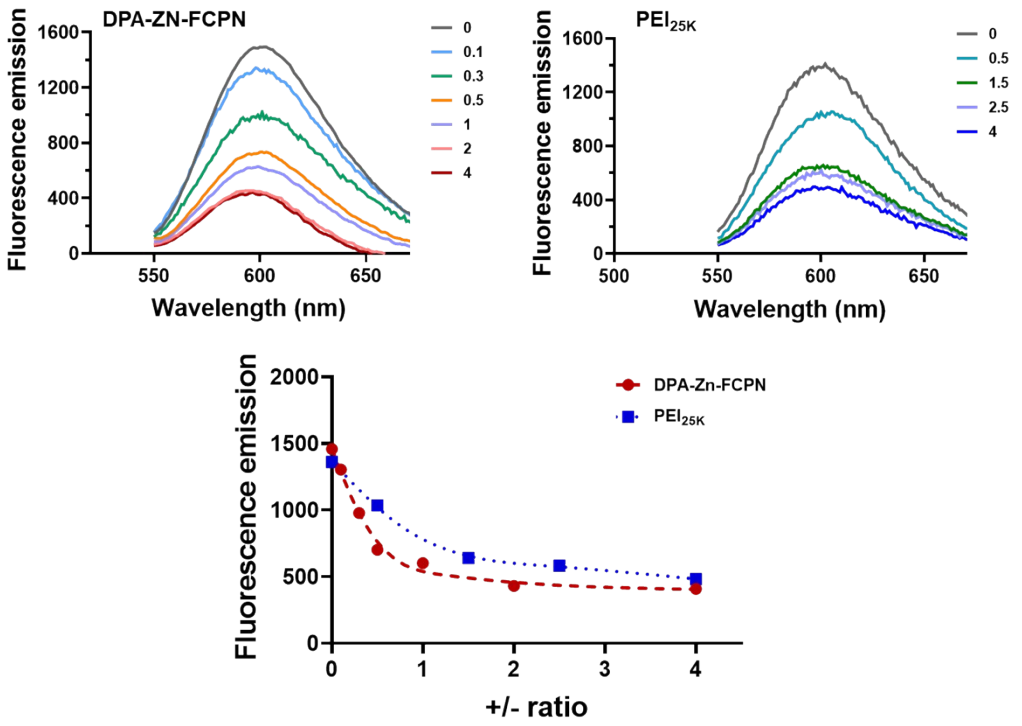

Figure s3. Ethidium bromide displacement assay. Fluorescent quenching assay of EB/DNA by addition of DPA-Zn-FCPN and PEI_{25K}. All the samples were excited at 497 nm and the emission was measured at 600 nm.

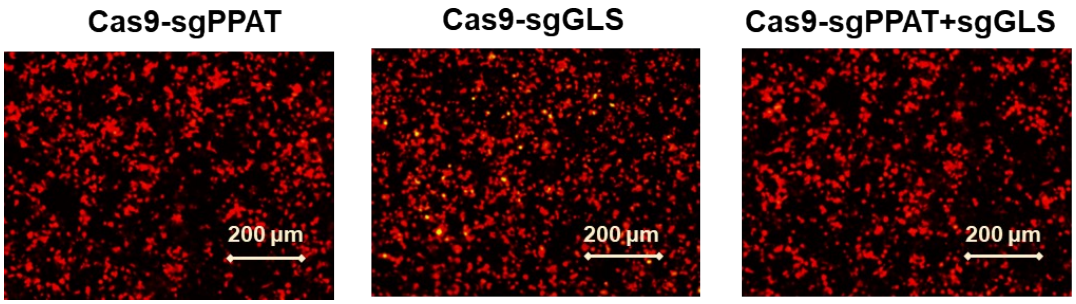

Figure s4. Fluorescence microscopy images of 4T1 cells treated with mCherry (red) coexpression Cas9-sgPPAT, Cas9-sgGLS and Cas9-sgPPAT+sgGLS plasmids delivered by DPA-Zn-FCPN. Scale bar = 200 μm.

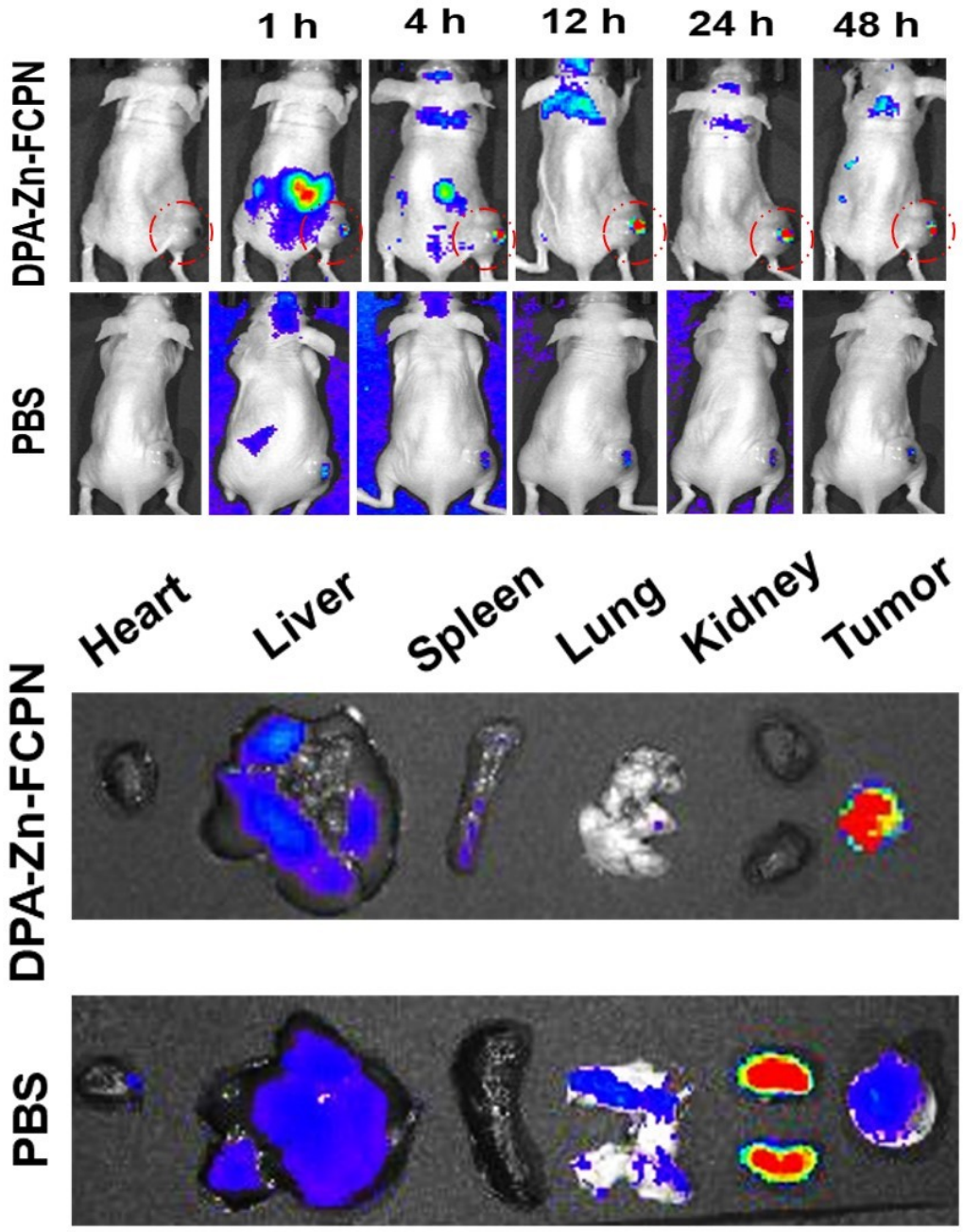

Figure s5. In vivo fluorescence imaging of the nude mice bearing 4T1 tumors at different time points (1, 4, 12, 24 and 48h, respectively) after intravenous injection of PBS and DPA-Zn-FCPN/pCas9-sgRNA NPs, and Cas9-sgRNA plasmids were labeled with Cy5 (0.3 mg Cy5-Cas9 plasmids kg^{-1}, n = 3). Ex vivo fluorescence images of the major organs and tumors 6 h after intravenous injection.

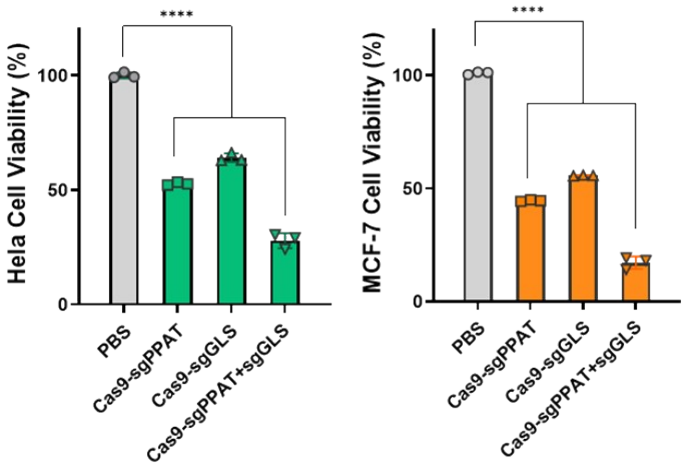

Figure s6. Hela and MCF-7 cells at 72 h after treatment with DPA-Zn-FCPN/pCas9-sgGLS NPs, DPA-Zn-FCPN/pCas9-sgPPAT NPs, and DPA-Zn-FCPN/pCas9-sgGLS+sgPPAT NPs. Data in panels are presented as the mean ± SD (n = 3). P values were determined by Student's t test (NS: not significant, *: P<0.05, **: P<0.01, ***: P<0.001, ****: P<0.0001).

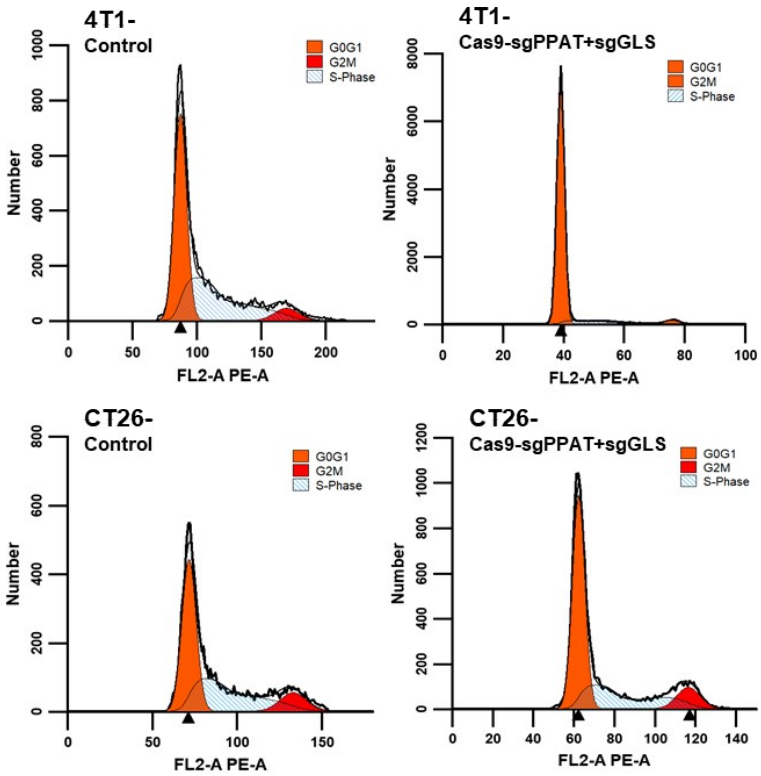

Figure s7. Cell cycle distribution graphs of 4T1 and CT26 cells treated with DPA-Zn-FCPN/pCas9-sgGLS+sgPPAT NPs and control group after 72 hours.

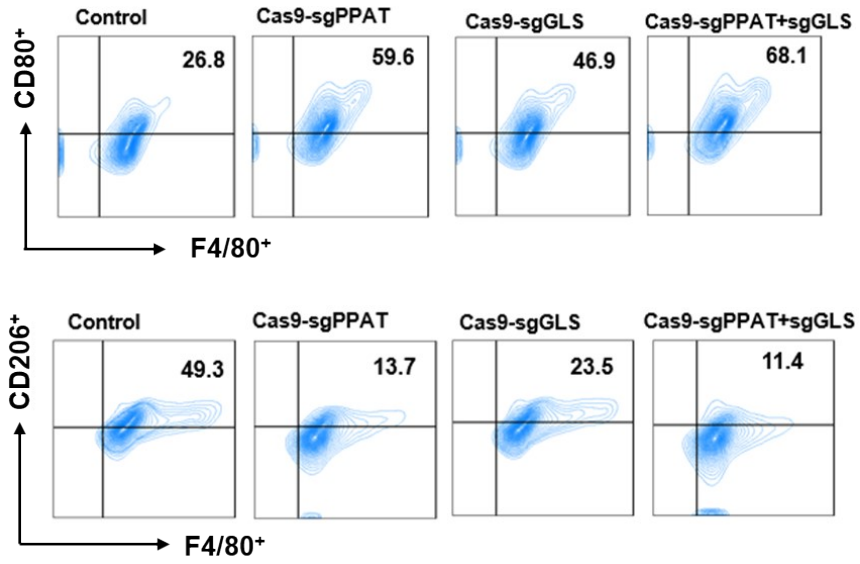

Figure s8. Flow cytometric plots of M1-type (CD80) and M2-type macrophages (CD206) in lymph nodes after various treatments.

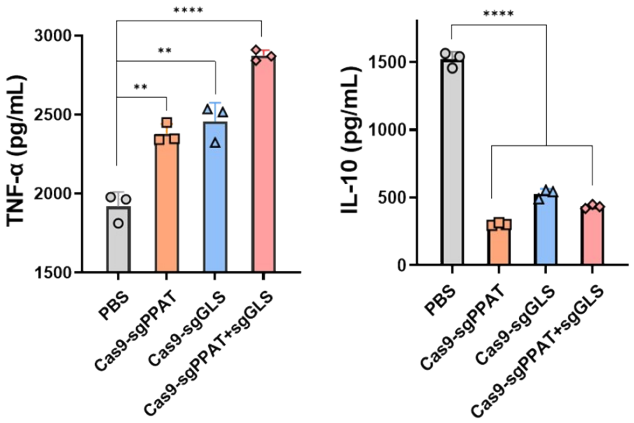

Figure s9. ELISA analysis of TNF-α and interleukin-10 (IL-10) in serum from mice after various treatments. The data are shown as the means ± SDs. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001.

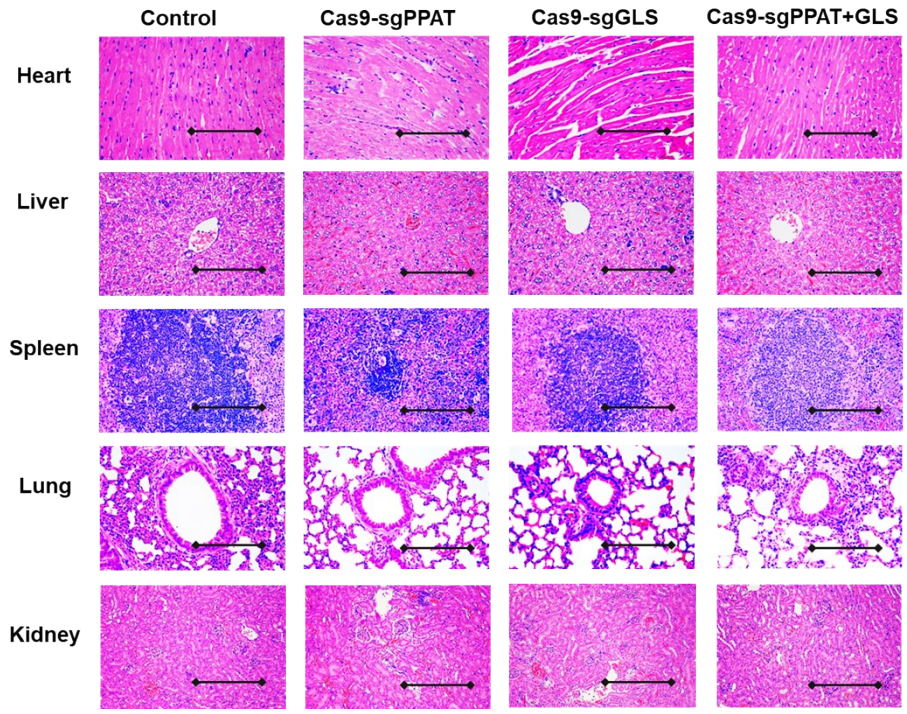

Figure s10. H&E staining analysis of the main organs from the mice in each treatment group. Scale bar = 200 μm.

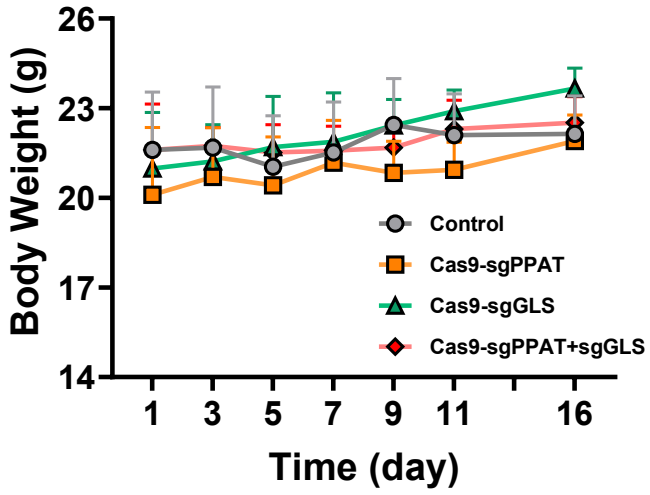

Figure s11. Body weight of the mice injected with PBS, DPA-Zn-FCPN/pCas9-sgGLS NPs, DPA-Zn-FCPN/pCas9-sgPPAT NPs, and DPA-Zn-FCPN/pCas9-sgGLS+sgPPAT NPs. All data in panels are presented as the mean ± SD (n = 5).