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Supplementary Materials for

CRISPR-Cas9 genome editing using targeted lipid nanoparticles for cancer therapy

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This PDF file includes:

Figs. S1 to S6

Supplementary Figures

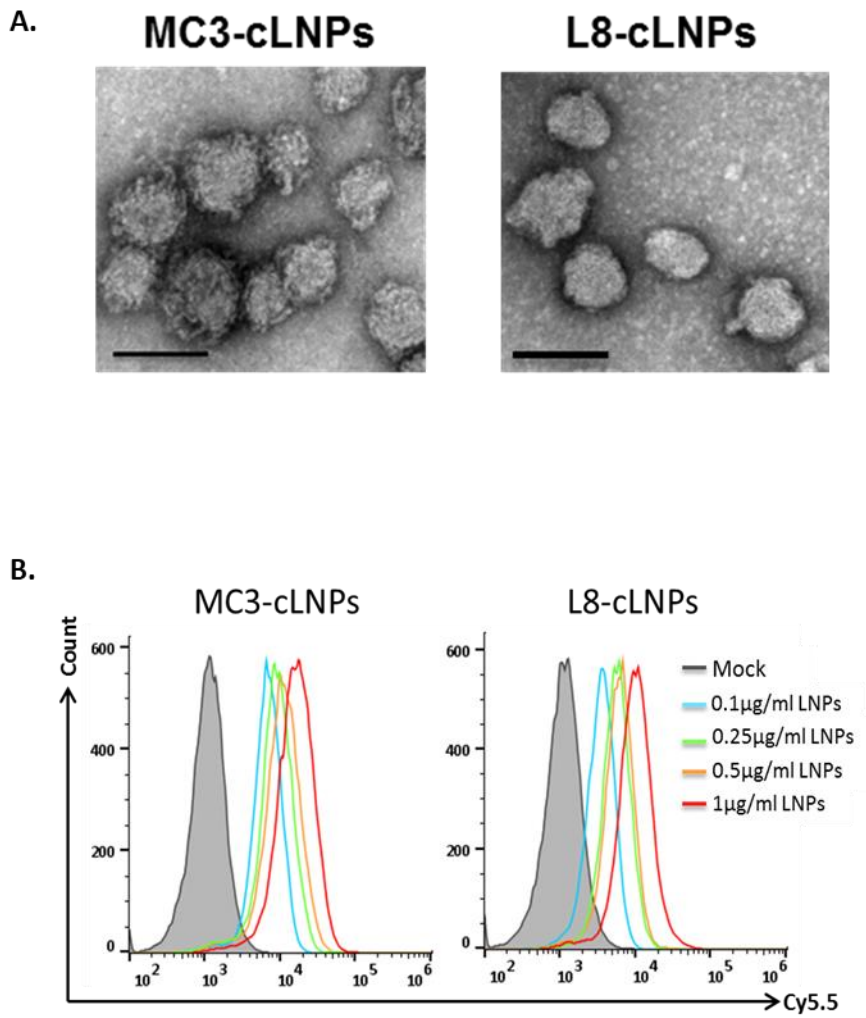

Figure S1. TEM and uptake analysis of cLNPs. (A) Representative transmission electron microscopy (TEM) images of Dlin-MC3-DMA (MC3) (Left) and L8 (right) cLNPs. Scale bars are 100nm. **(B)** Uptake of MC3 or L8 cLNPs by HEK293 cells, cells were transfected with 0.1-1µg/ml (0.7-7nM total RNA) Cy5.5 labeled cLNPs and analyzed by flow cytometry 1 hr later. Representative histograms are presented as the geometric mean of Cy5.5 fluorescence.

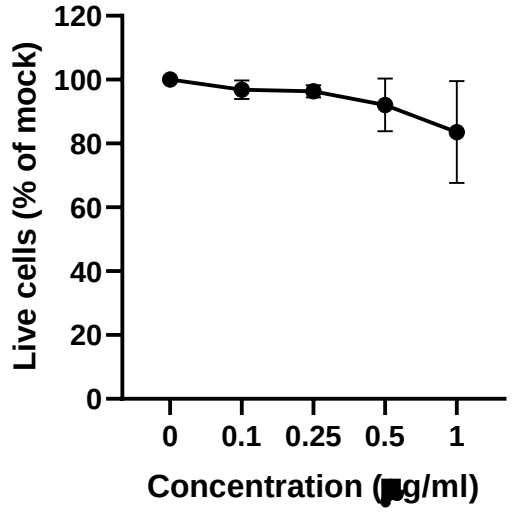

Figure S2. *In vitro* safety study of cLNPs in HEK293. HEK293 cells were treated with 0.1-1µg/ml (0.7-7nM total RNA) cLNPs for 72hrs. Cells were stained with DAPI viability dye and analyzed by flow cytometry. Data are representative of three independent experiments as biological replicates. p=n.s., one-way ANOVA with Tukey multiple comparison test was used to assess the significance

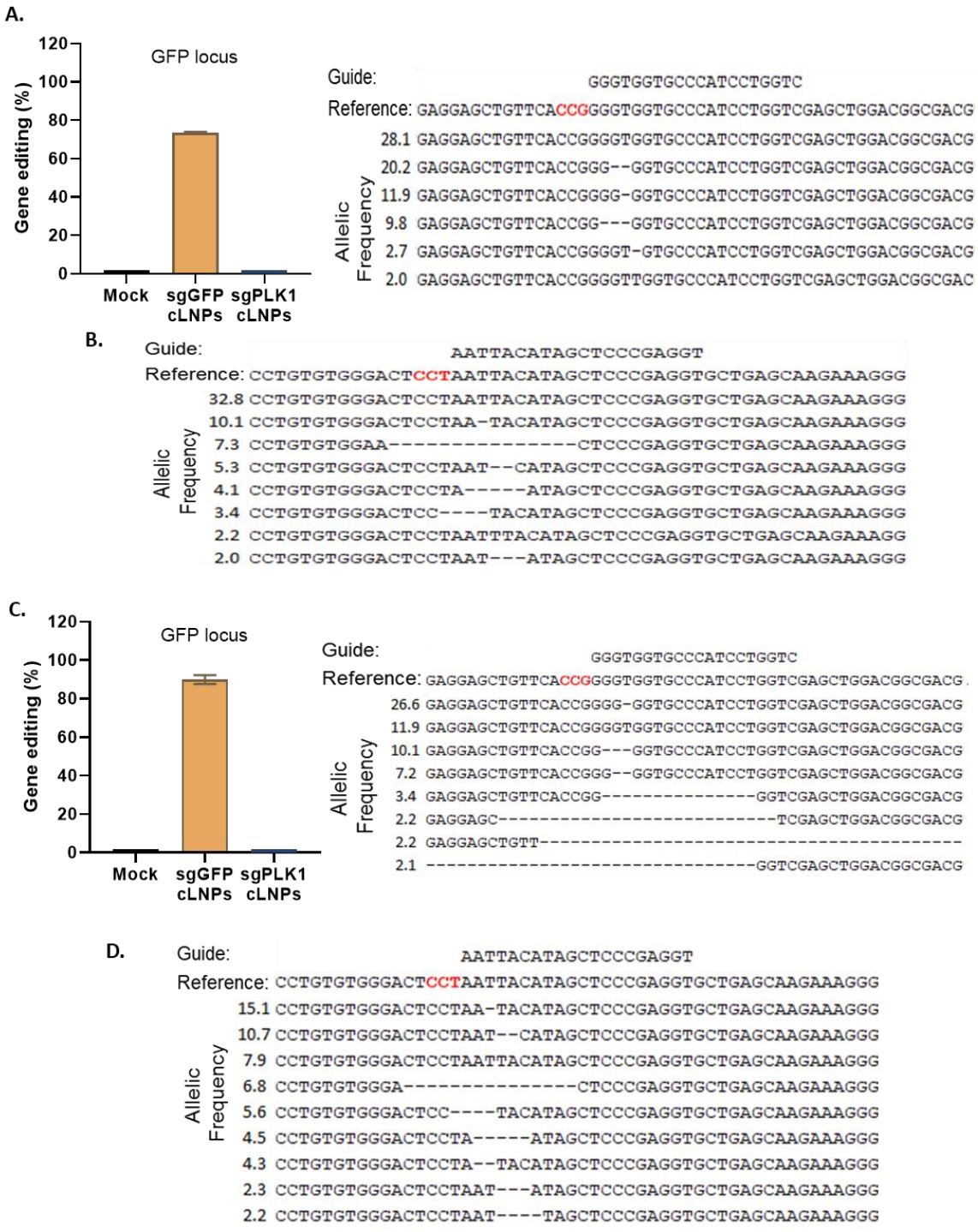

Figure S3. Quantification of gene editing frequency in 005 and OV8 cells by NGS. (A,C) % of gene editing events (left) and allelic frequencies (right) in the GFP loci in 005 (A) and OV8 (C) cells. **(B,D)** allelic frequencies in the PLK1 loci as was determined by next-generation sequencing analysis in 005 (B) and OV8 (D) cells (Allelic frequencies > 2% are presented).

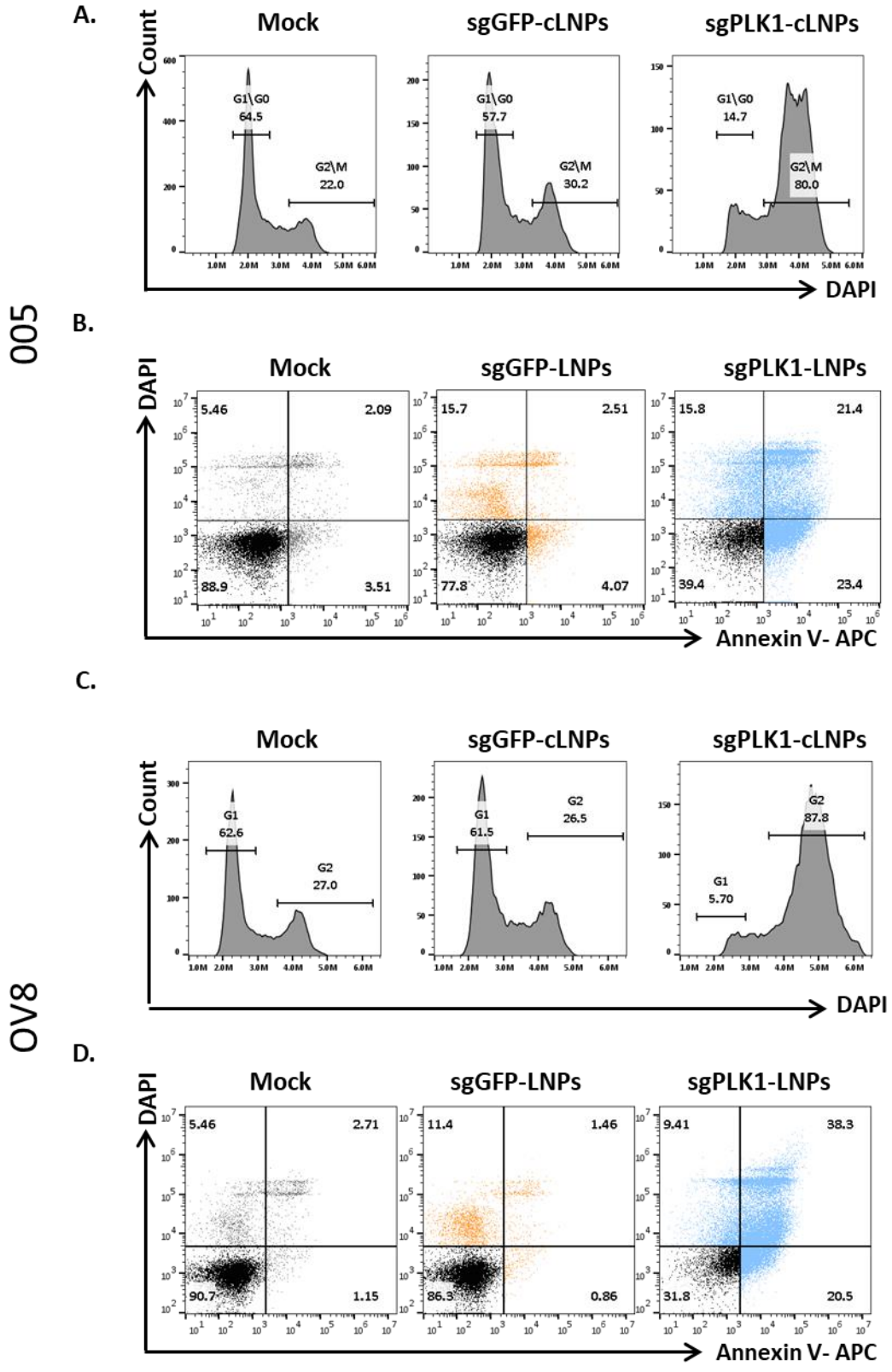

Figure S4. Therapeutic genome editing of PLK1 results in G2/M cell cycle arrest and tumor cell death in 005 and OV8 cells. (A,C) Representative cell cycle analysis diagram of 005 cells (A) or OV8 (C), 48 h post-treatment with Mock, sgGFP, or sgPLK1-cLNPs (005-0.5µg/ml (3.5nM total RNA), OV8-1µg/ml (7nM total RNA)). (**B,D)** Representative

DAPI/AnnexinV apoptosis analysis diagram of 005 (B) or OV8 (D) cells 96 h post-treatment with Mock, sgGFP or sgPLK1-cLNPs (005- 0.5µg/ml (3.5nM total RNA), OV8-1µg/ml (7nM total RNA)). Representative data of three independent experiments.

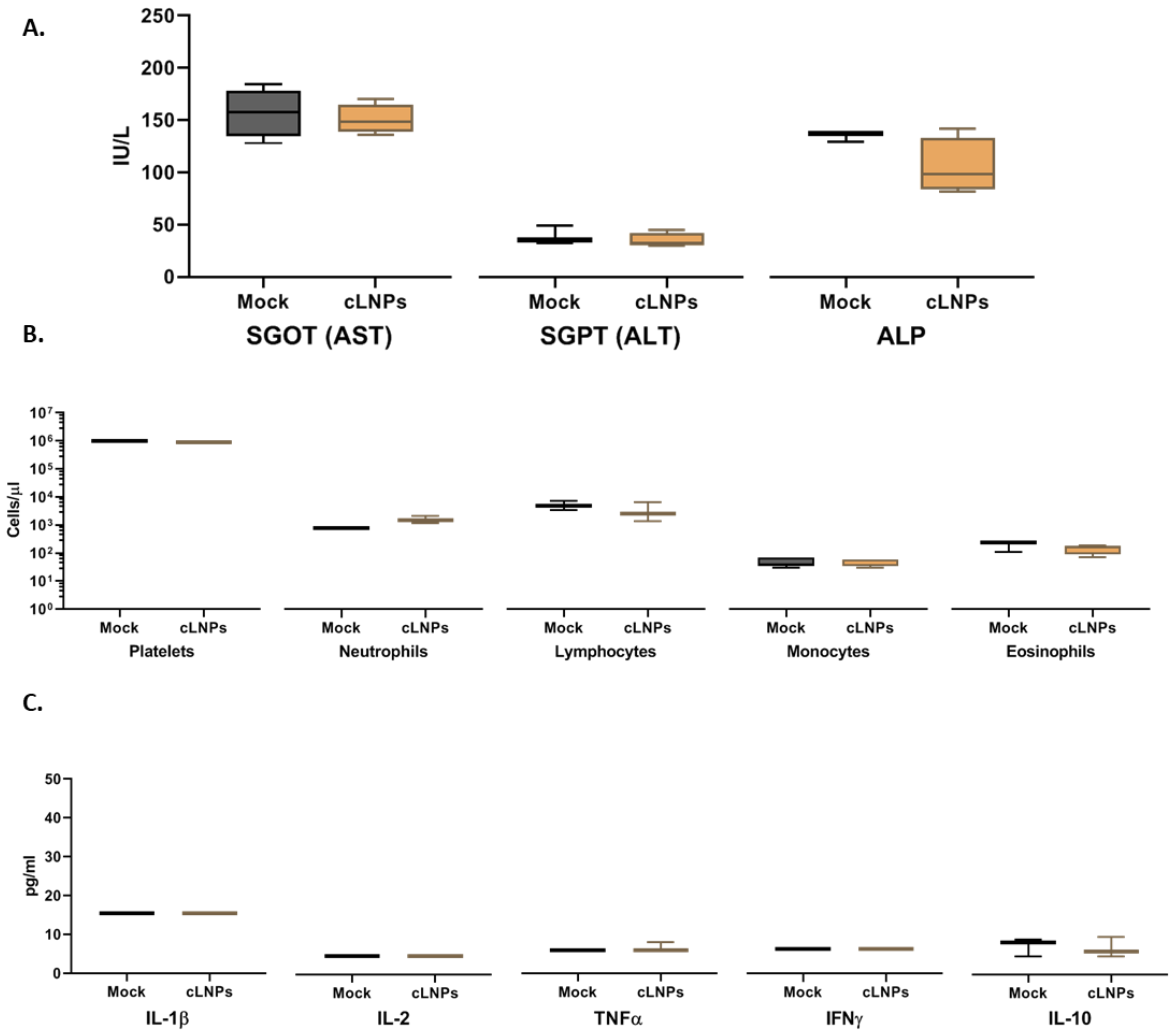

Figure S5. Liver toxicity and Immunogenicity evaluation following intravenous injection of cLNPs. C57/Bl6 Mice were injected intravenously with 1 mg/Kg body cLNP. Liver enzymes elevation in the blood **(A)** (alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)) and total blood counts **(B)** were evaluated 24 h post-injection. Immunogenicity was evaluated by elevation in the blood levels of the pro and anti-inflammatory cytokines (IL-1β, IL-2, TNF-α, IFN-γ, and IL-10) **(C)** Data are representative of 3 independent experiments as biological replicates. Data are IQR with a median center line and min to max error bars (a-b) and mean ± s.d. (c), n = 3, P=not significant.

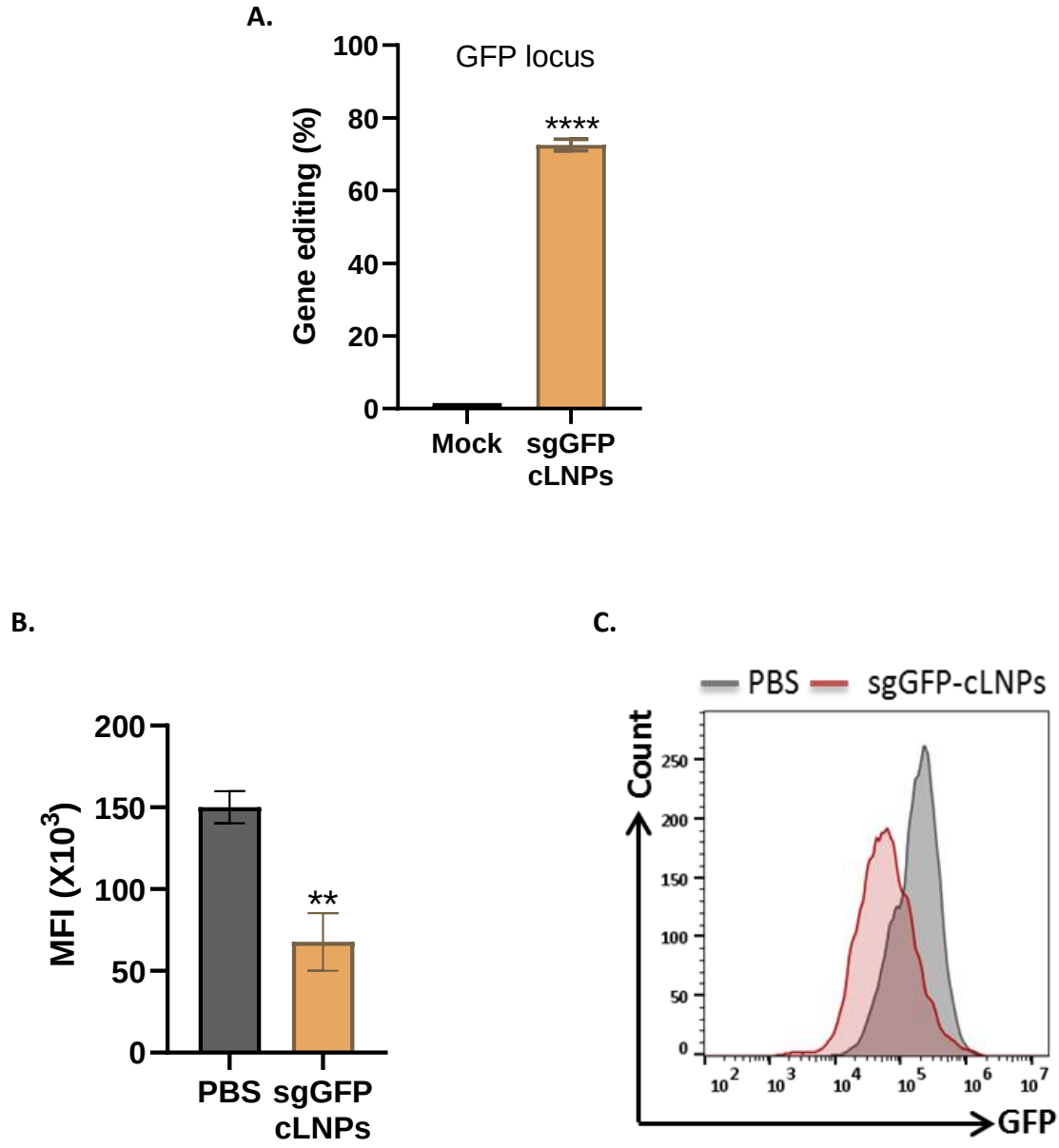

Figure S6. *In vivo* GFP disruption in 005 GBM bearing mice. (A) % of gene editing events in the GFP loci upon injection of either PBS or 0.05 mg/Kg sgGFP-cLNPs to the tumor bed. Forty-eight hours' post-injection, brains were processed to single-cell suspensions, tumor cells were sorted, and the % of gene editing was determined by next-generation sequencing analysis. **(B)** *In vivo* GFP disruption in 005 GBM bearing mice. 0.05mg/Kg of sgGFP-cLNPs were injected into the tumor bed, seven days' post-injection brains were processed to single-cell suspensions, and the reduction in GFP was analyzed by flow cytometry. Mean fluorescent intensity ± SD of three independent experiments. **P<0.005. **(C)** Representative flow cytometry histogram of in vivo GFP disruption in 005 GBM bearing mice.