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Supplemental Information

Precise editing of FGFR3-TACC3 fusion genes with CRISPR-Cas13a in glioblastoma

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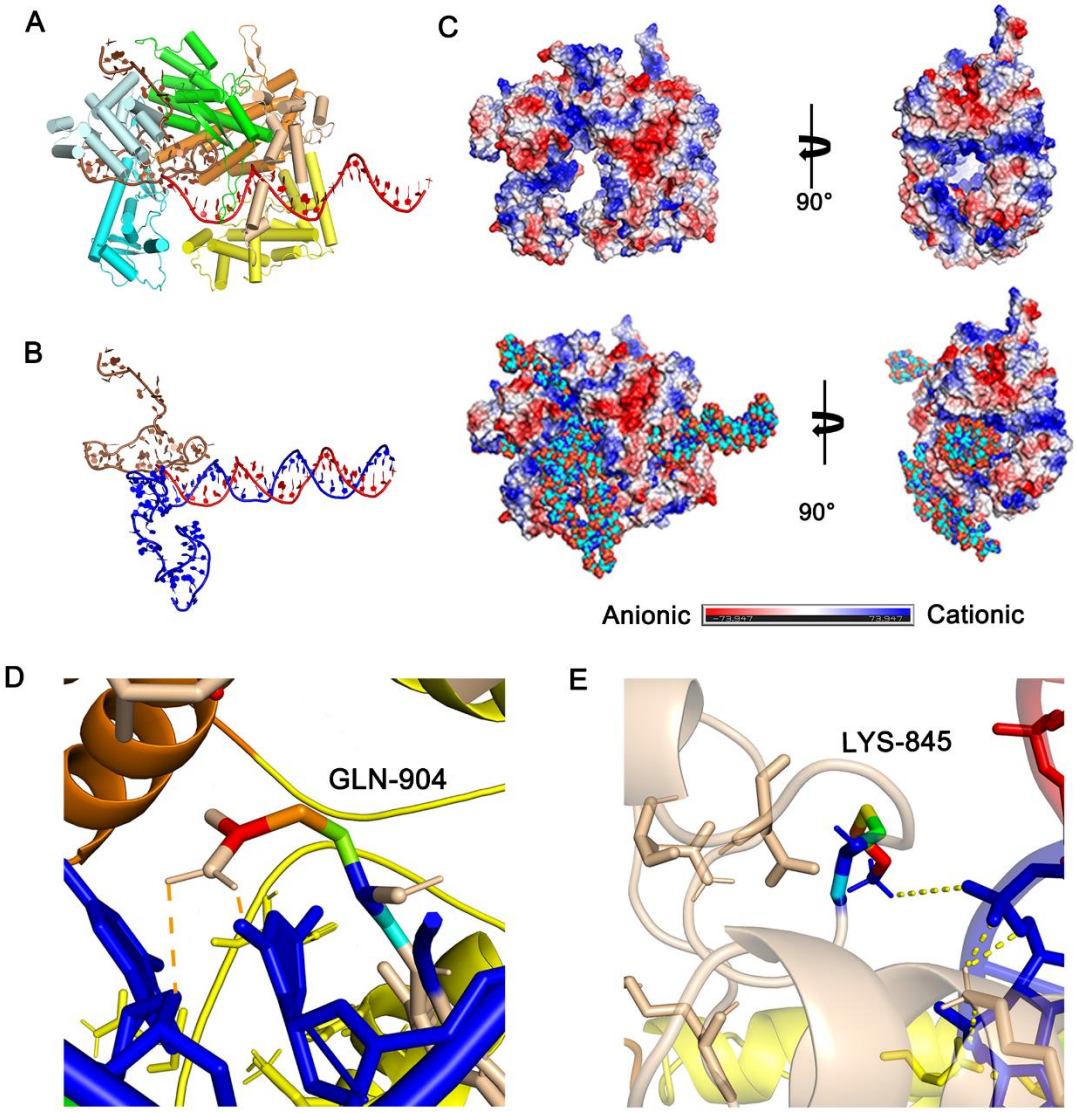

Figure S1. Analysis of the interaction between Cas13a and RNA1. (A) 3D structure of the Cas13a-cr1 duplex. (B) 3D structure of the crRNA1-F3-T3 duplex. F3-T3 is in blue. (C) Electrostatic binding surface analysis indicating that the negatively charged surface of RNA1 matches well with the positively charged region in the active cavity of Cas13a. (D) Partially enlarged details of the hydrogen-bond interactions of Cas13a with the bases in the 38–50-bp segment of RNA1. (E) Partially enlarged details of the hydrogen-bond interactions of Cas13a with the bases in the 51–65-bp segment of RNA1.

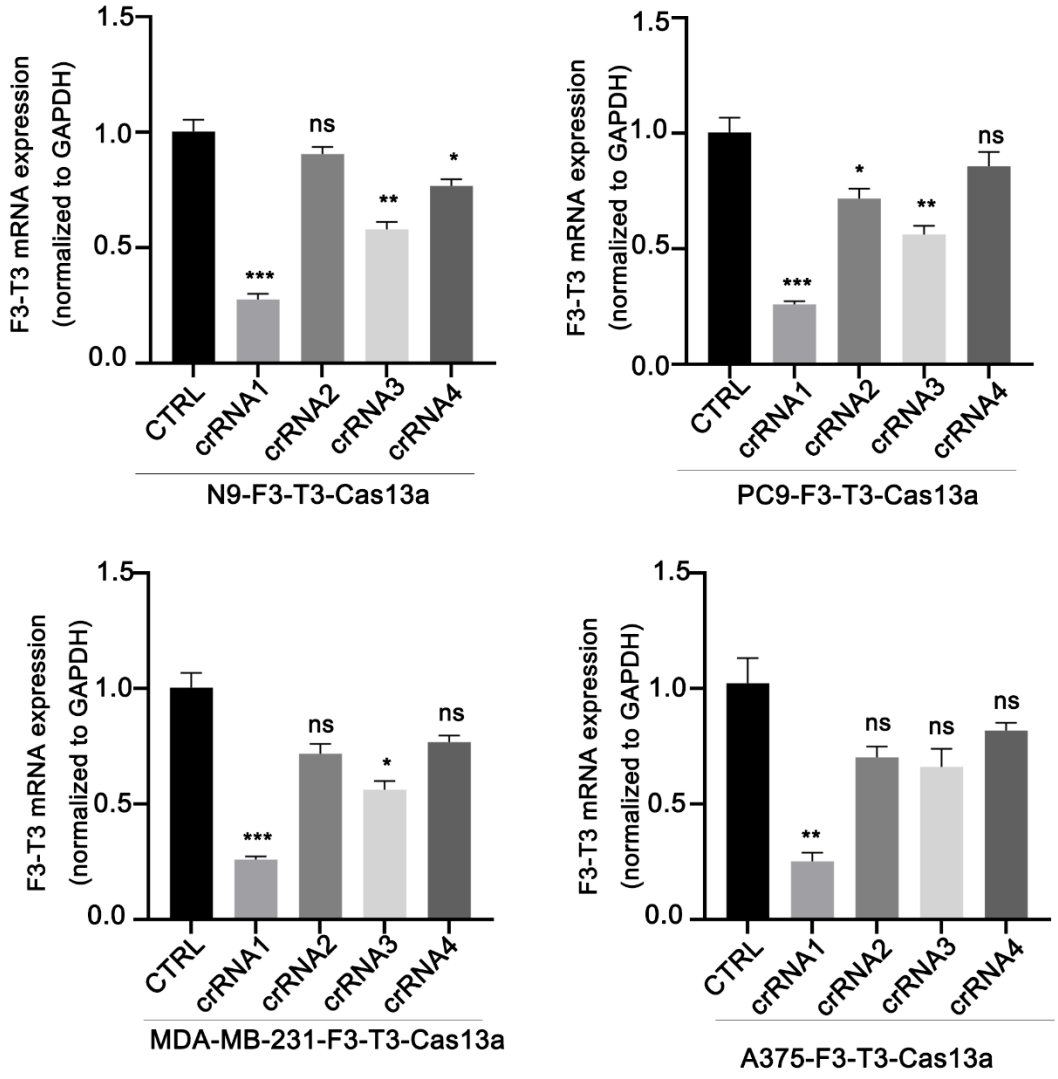

Figure S2. Verification of Cas13a-cr1 knockdown efficiency. Real-time PCR to assess F3-T3 mRNA levels in N9, PC9, MDA-MB-231 and A375 cells. ns represents $p > 0.05$. *$p<0.05$, **$p < 0.01$ and ***$p < 0.001$.

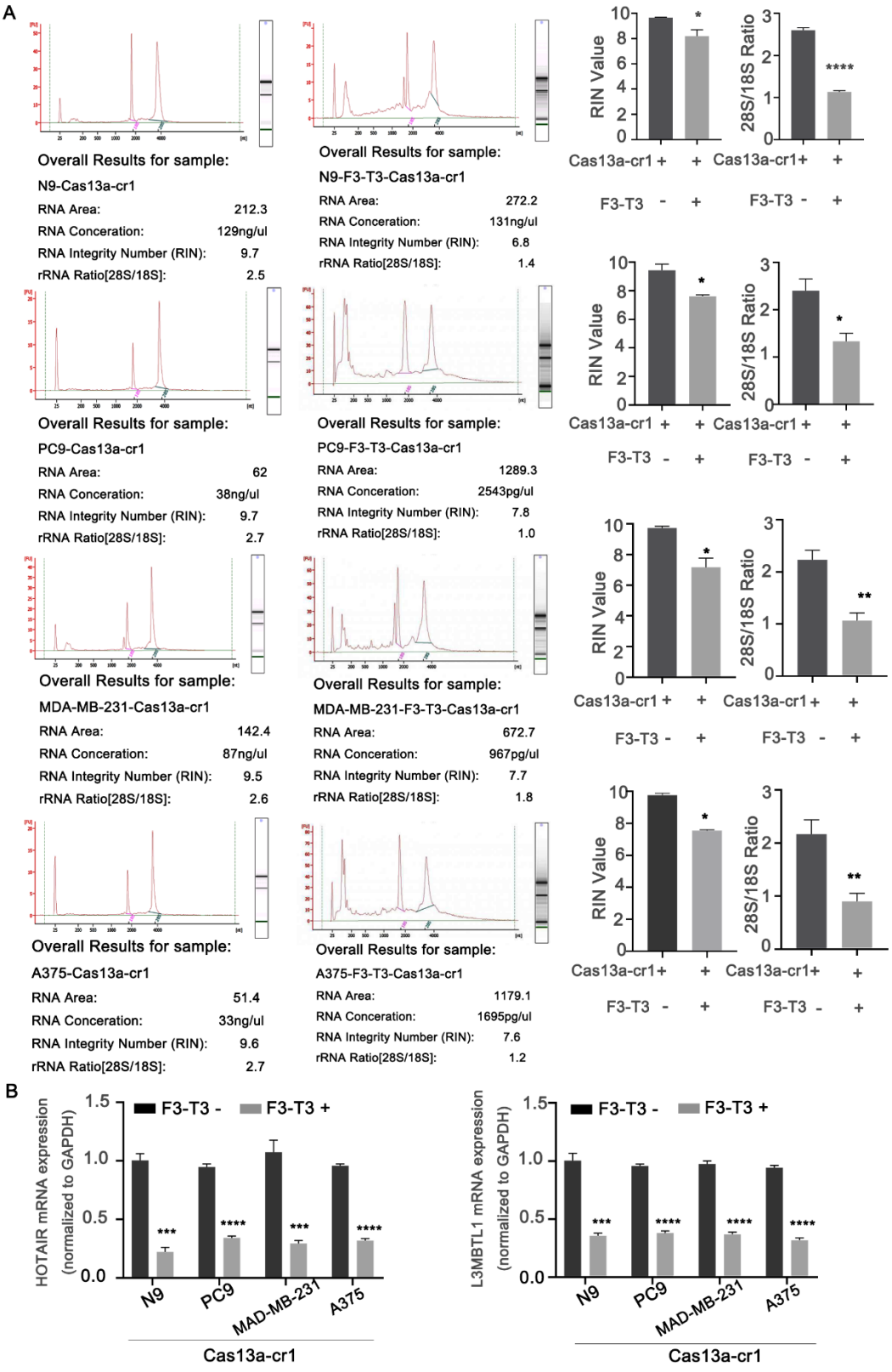

Figure S3. Specificity of Cas13a-cr1 in various tumor cell lines. (A) RNA integrity as assessed on the Agilent 2100 Bioanalyzer. Bars represent the mean ± SEM (n = 3). *p < 0.05, **p < 0.01 and ****p < 0.0001. (B) Real-time PCR to assess HOTAIR and L3MBTL1 mRNA levels in N9, PC9, MDA-MB-231 and A375 cells. ***p < 0.001 and ****p < 0.0001.

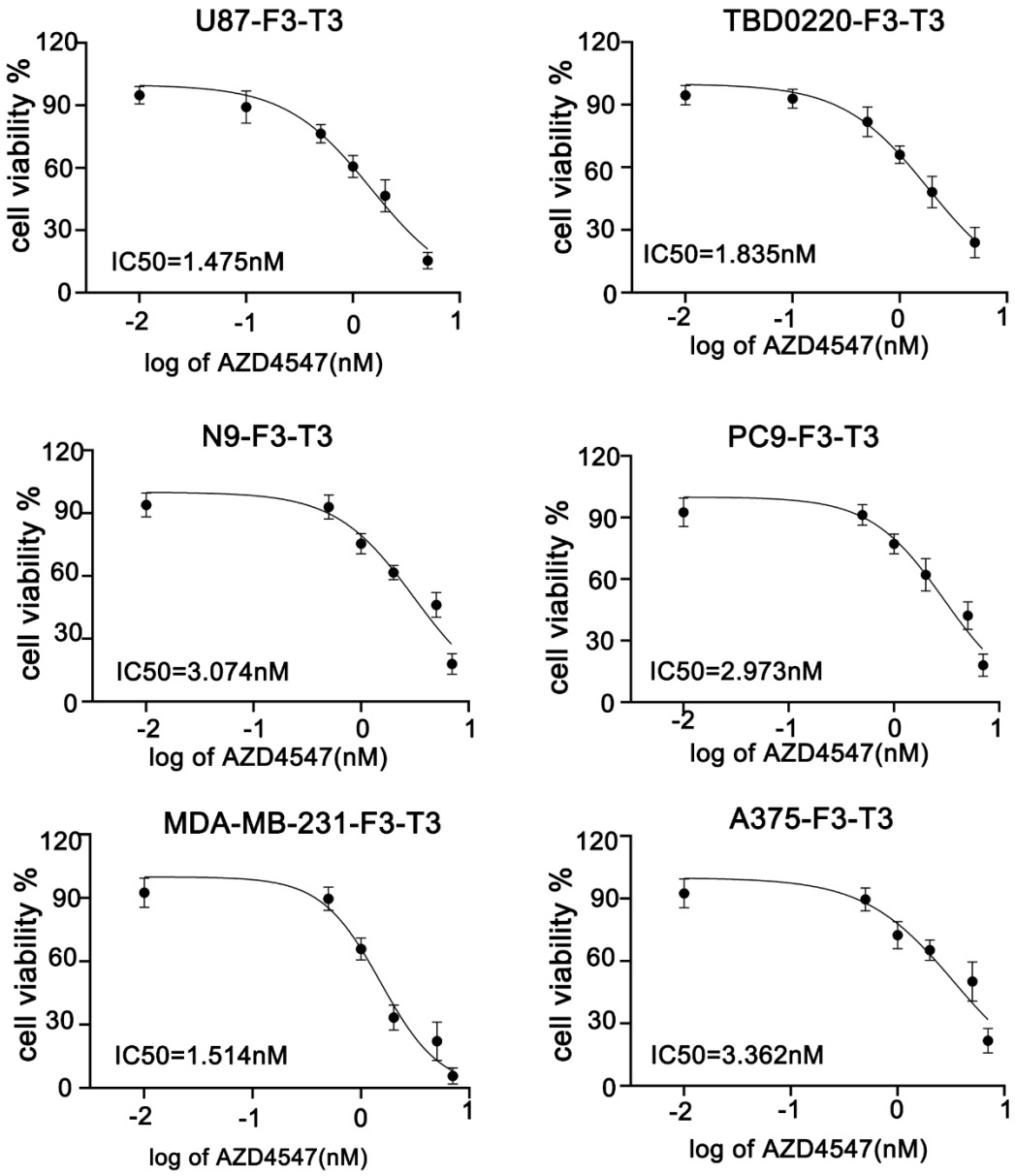

Figure S4. IC50 of AZD4547 in different cell lines. IC50 of AZD4547 in U87, TBD0220, N9, PC9, MDA-MB-231 and A375 cells.

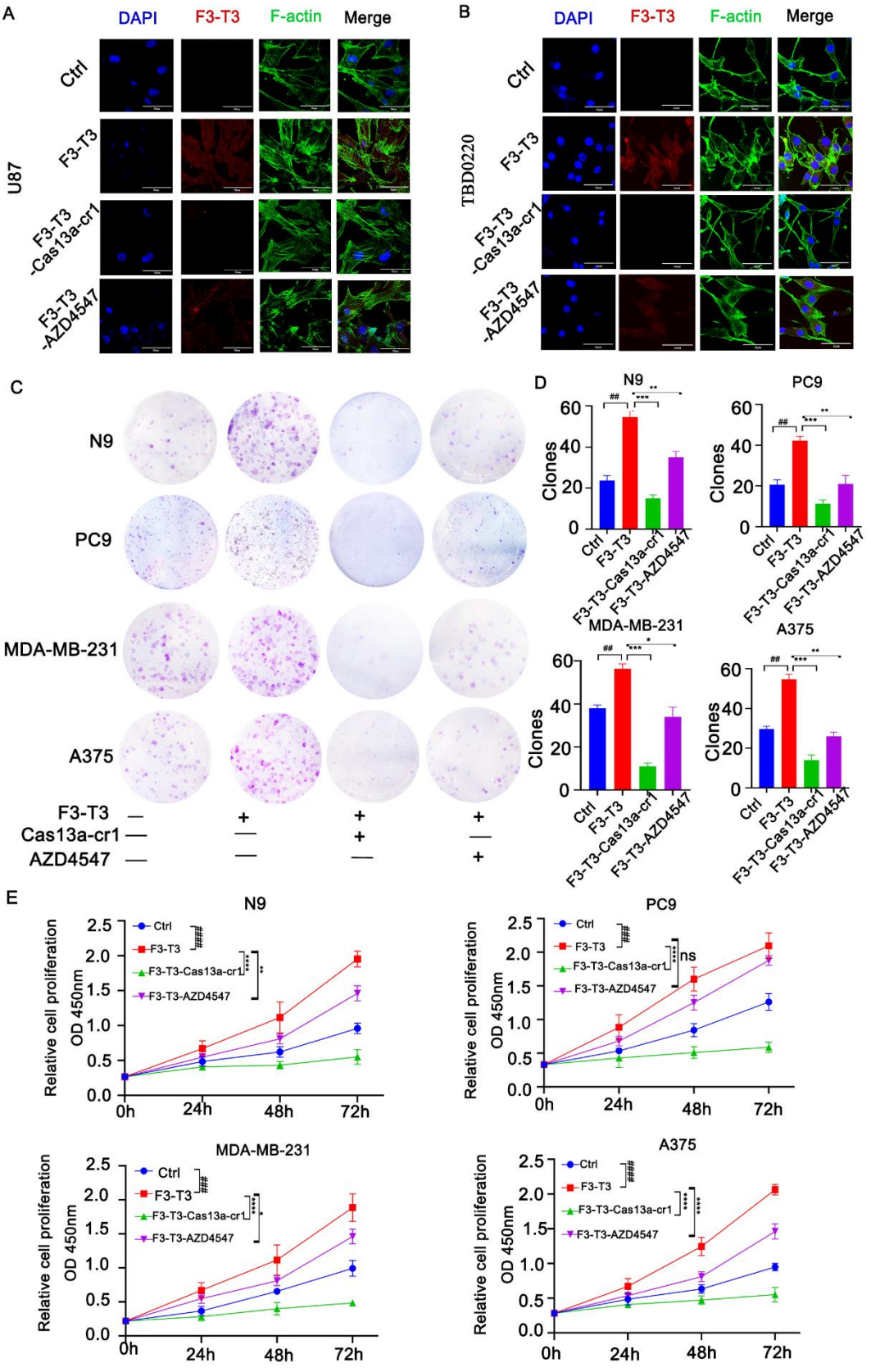

Figure S5. Cas13a-cr1 inhibits cell proliferation in vitro. (A and B) Immunofluorescence

images captured under confocal microscopy to detect expression of the F3-T3 fusion protein. Scale bar, 50 μm. (C) Images of colony formation assays performed in N9, PC9, MDA-MB-231 and A375 cells. (D) Quantification of colony numbers in (C). ##p < 0.01 versus the control group. *p < 0.05, **p < 0.01 and ***p < 0.001 versus the F3-T3 group. (E) Growth curves generated with CCK8 assays to analyze the proliferation of N9, PC9, MDA-MB-231 and A375 cells. ns represents p > 0.05. ###p < 0.001 and ####p < 0.0001 versus the control group. **p < 0.01, ***p < 0.001 and ****p < 0.0001 versus the F3-T3 group.

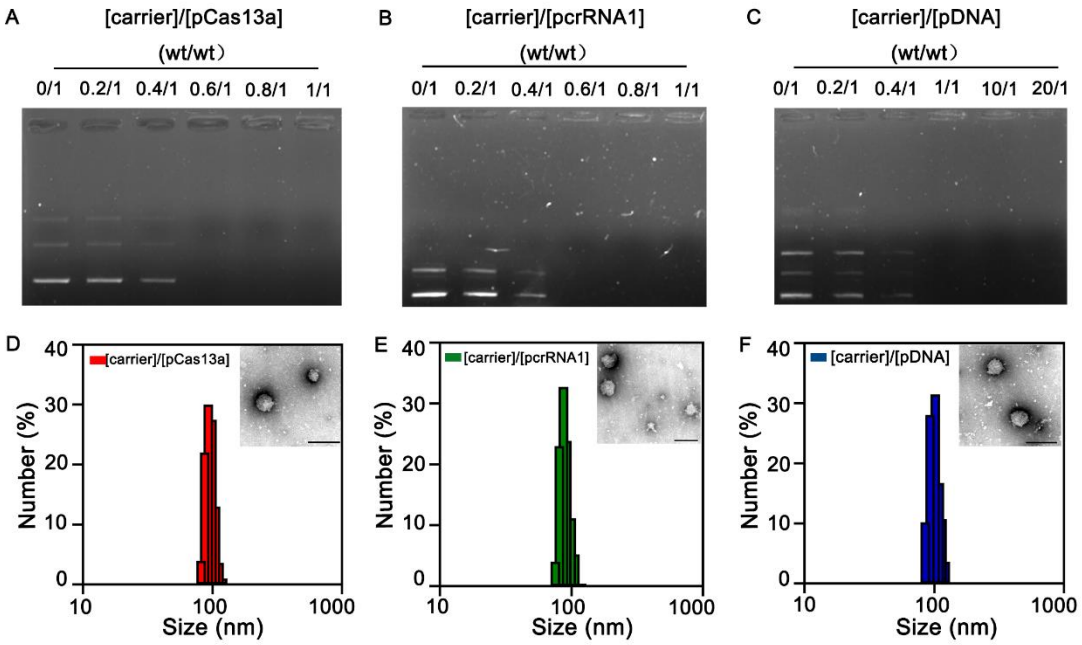

Figure S6. Effective binding capacities of PEI–PBLG (PP) and pDNA. (A - C) Gel retardation assay for PP. pDNA denoted as pCas13a and pcr1 mixed at the same mass ratio. (D - F) Size distribution and transmission electron microscopy images of nanoparticles. PP and plasmid DNA mixed in a 1:1 ratio (wt/wt). The scale bar is 200 nm.