Supporting Information

Reversal of tumor malignization and modulation of cell behaviors through genome editing mediated by a multi-functional nanovector

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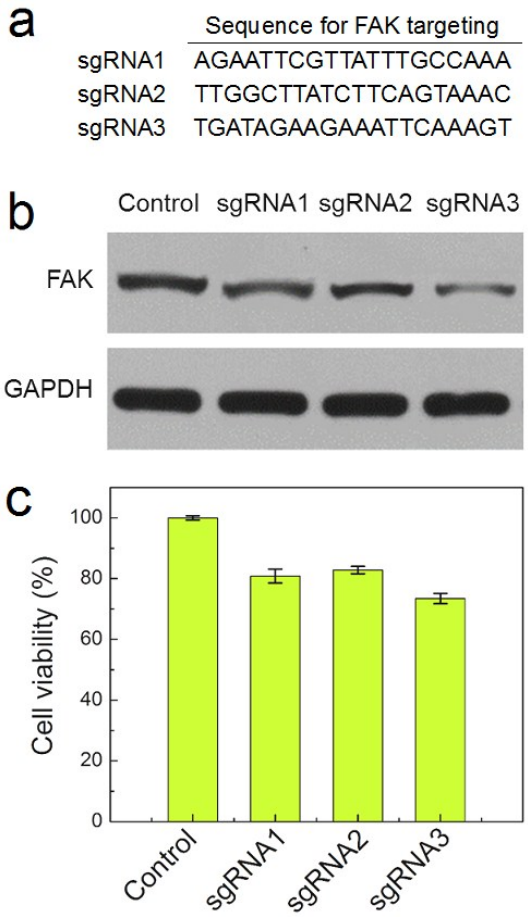
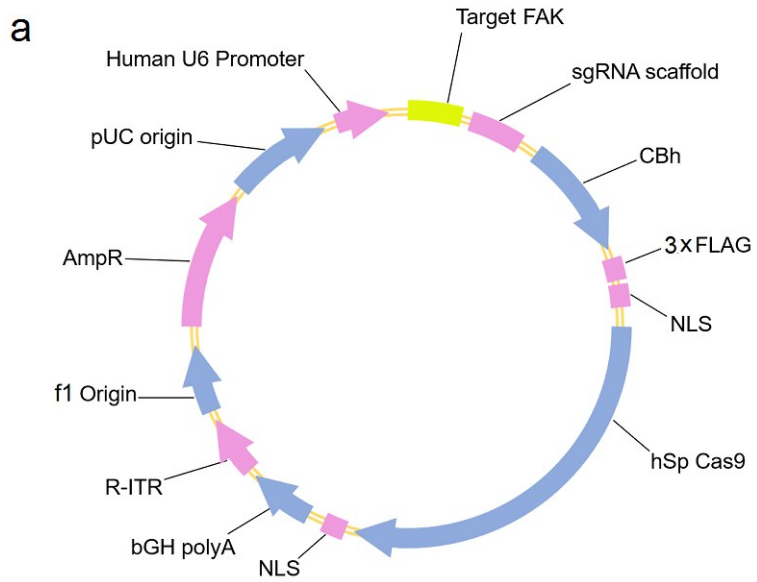


Figure S1. Effect of sgRNA sequences on genome editing. (a) Sequences for FAK targeting. (b) Expression of FAK in HeLa cells after genome editing for 48 h by the CRISPR-Cas9 plasmids with different sgRNA sequences using Lipofectamine 2000 as a vector. (c) HeLa cell viability after genome editing for 48 h by the CRISPR-Cas9 plasmids with different sgRNA sequences using Lipofectamine 2000 as a vector.

Candidate plasmids with different sgRNAs were constructed and their efficacy in FAK knockout was evaluated. As demonstrated in Figure S1, after 48 h, the plasmid with sgRNA3 has the highest efficiency in inhibition of FAK expression and cell growth. Therefore, plasmid with sgRNA3 was selected for the further study, and all data were obtained using the plasmid with sgRNA3 unless otherwise mentioned.



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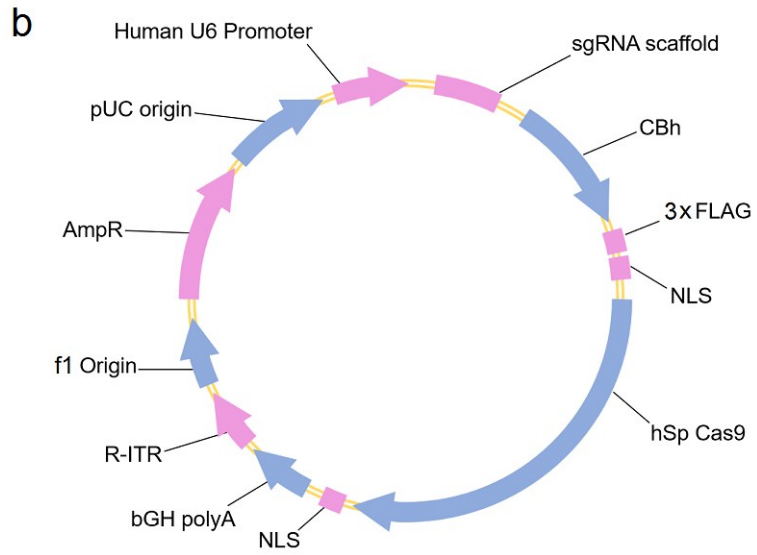

Figure S2. The structure of (a) plasmid FAK and (b) plasmid null.

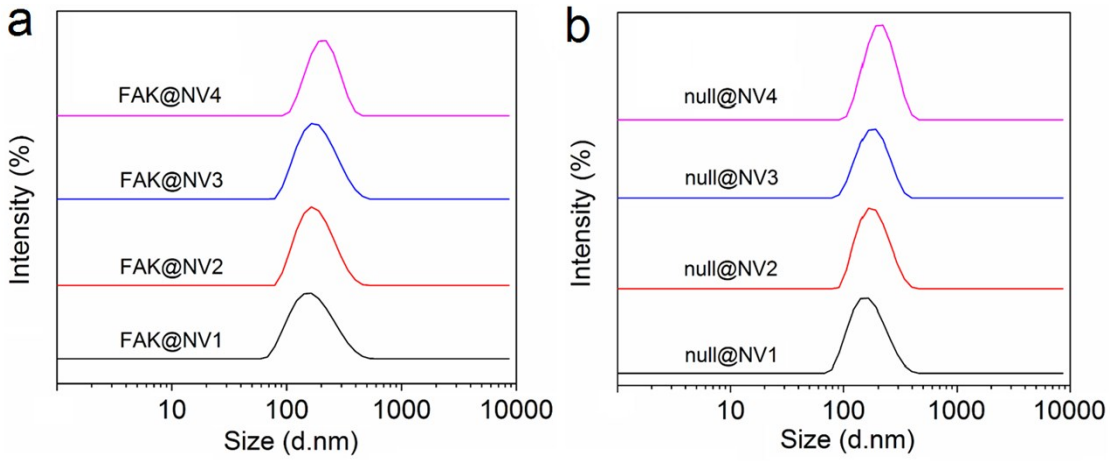

Figure S3. Size distribution of (a) plasmid FAK loaded nanovectors and (b) plasmid null loaded nanovectors measured by DLS.

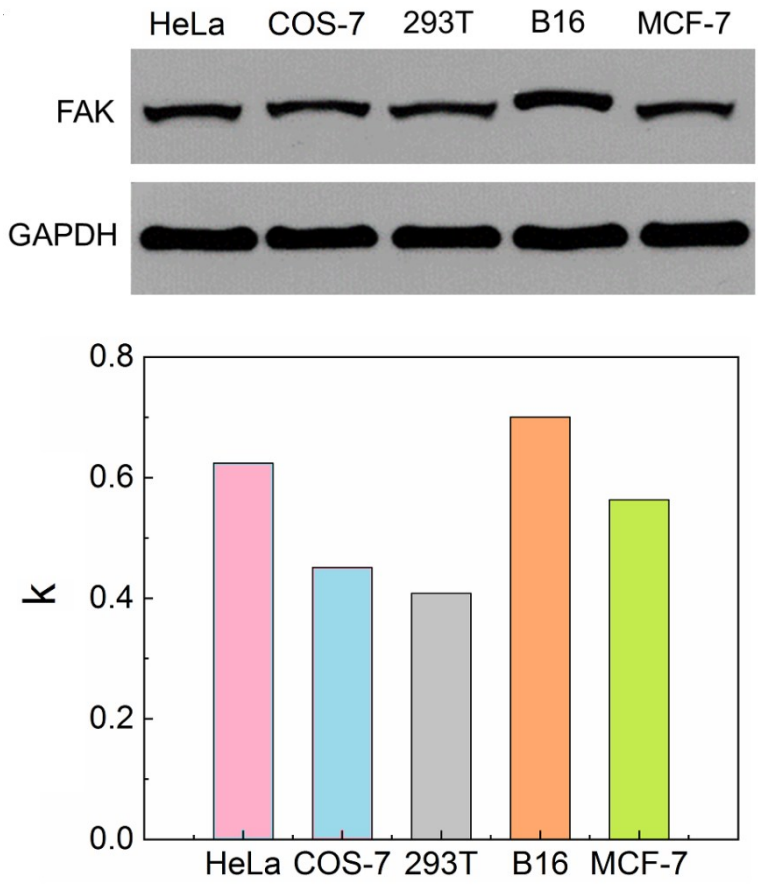

Figure S4. FAK expression in different cells determined by western blot analysis. k is the ratio of FAK expression to GAPDH expression in the same cell line.

As shown in Figure S4, FAK expression in tumorous HeLa, B16 and MCF-7 cells is higher than that in non-tumorous COS-7 and HEK 293T cells.

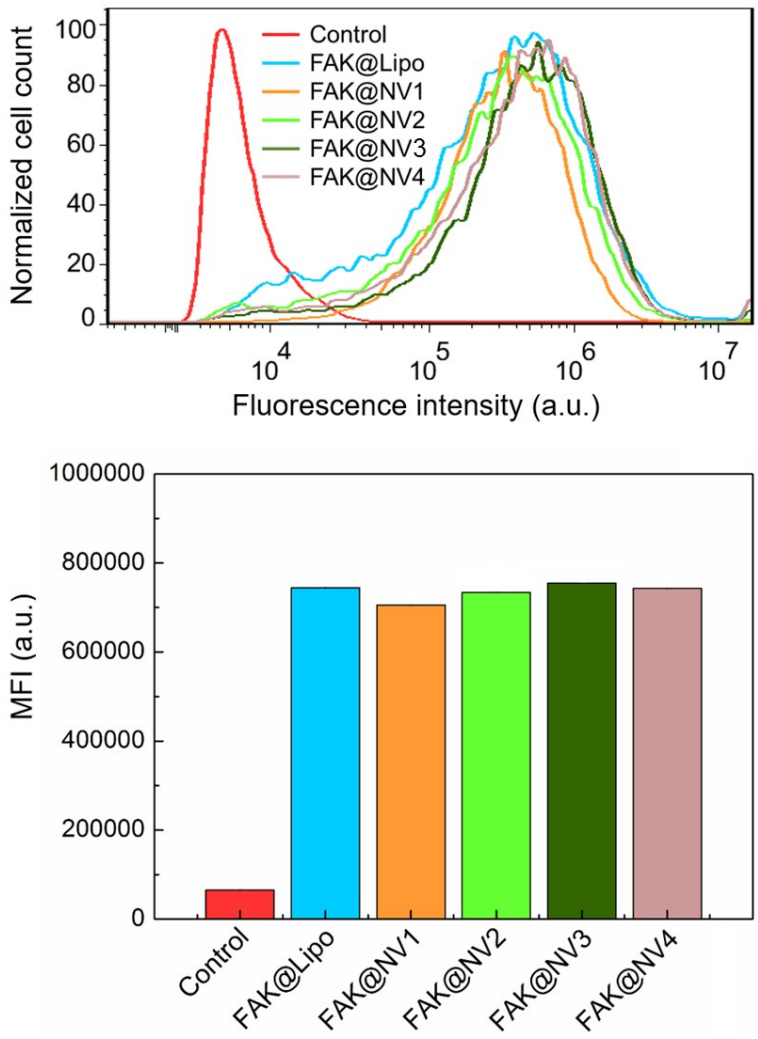

Figure S5. Cellular uptake of YOYO-1 labeled plasmid in HEK 293T cells after being co-incubated with different plasmid loaded nanovectors (4 h) as studied by flow cytometry. Untreated cells were used as a control.

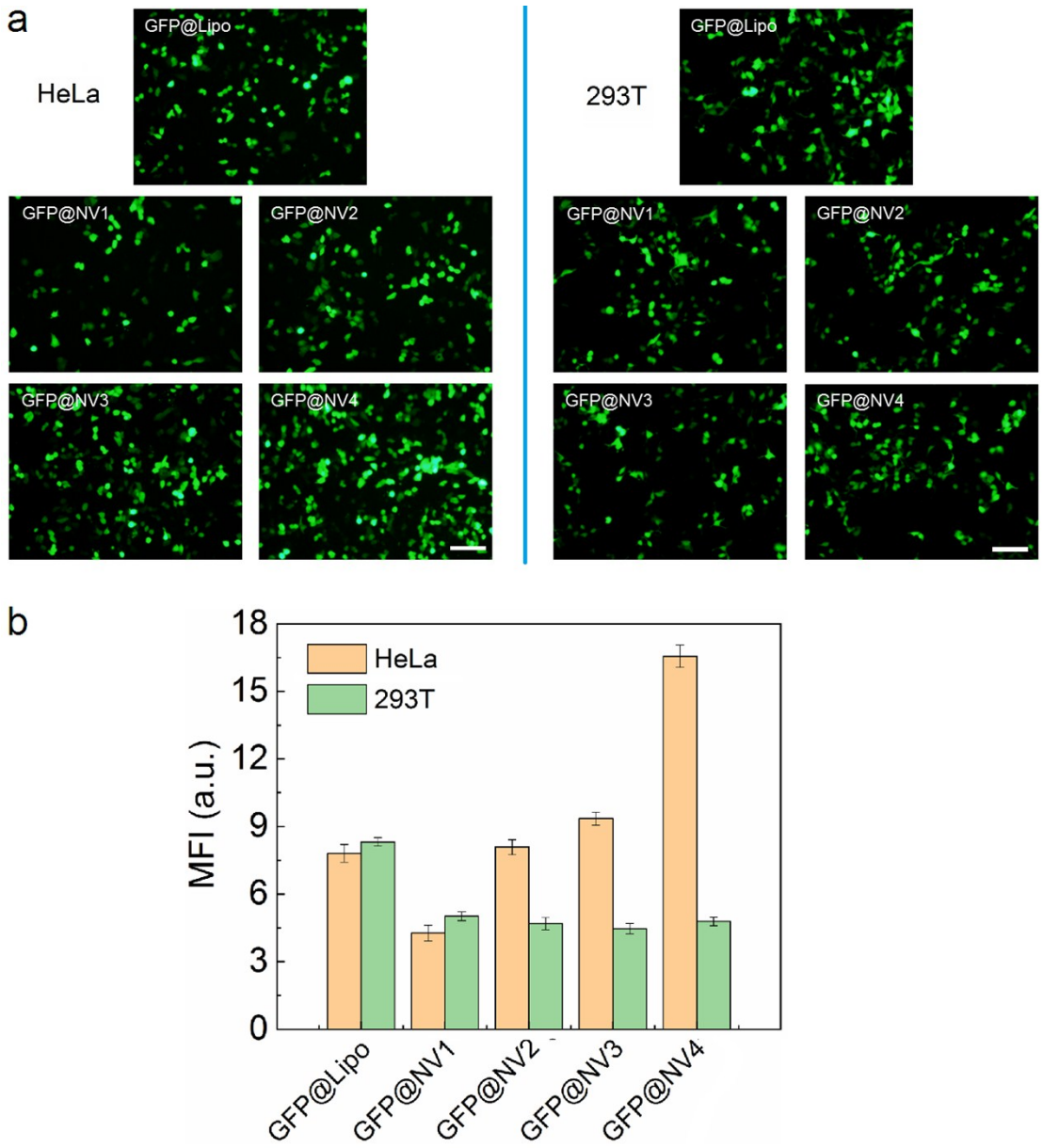

Figure S6. (a) Expressions of GFP in HeLa and HEK 293T cells observed by a fluorescence microscope. Scale bar: 100 μm. (b) The intensity of GFP in HeLa and HEK 293T cells. The cells were treated by different nanovectors loaded with plasmid GFP for 48 h.

As shown in Figure S6, the GFP expression level in HeLa cells increases in the order of FAK@NV1<FAK@NV2, FAK@NV3<FAK@NV4, which is in accordance with the cell uptake study (Figure 2). The GFP expression levels in 293T cells treated by

different gene delivery systems are almost the same since 293T do not overexpress either MUC1 or nucleolin. The GFP expression in HeLa cells is much higher than that in 293T cells after the treatment by the multi-functional delivery system (FAK@NV4).

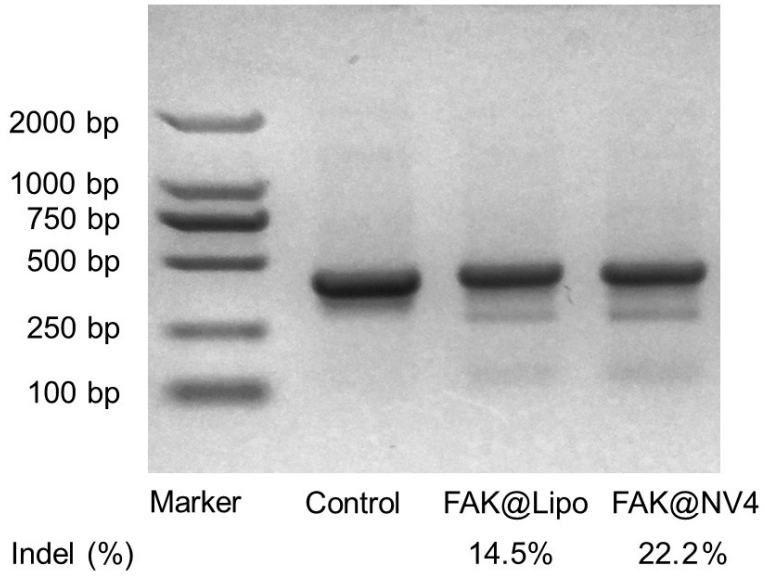

Figure S7. Genome editing efficacy in HeLa cells studied by Surveyor nuclease assay. Untreated cells were used as a control.

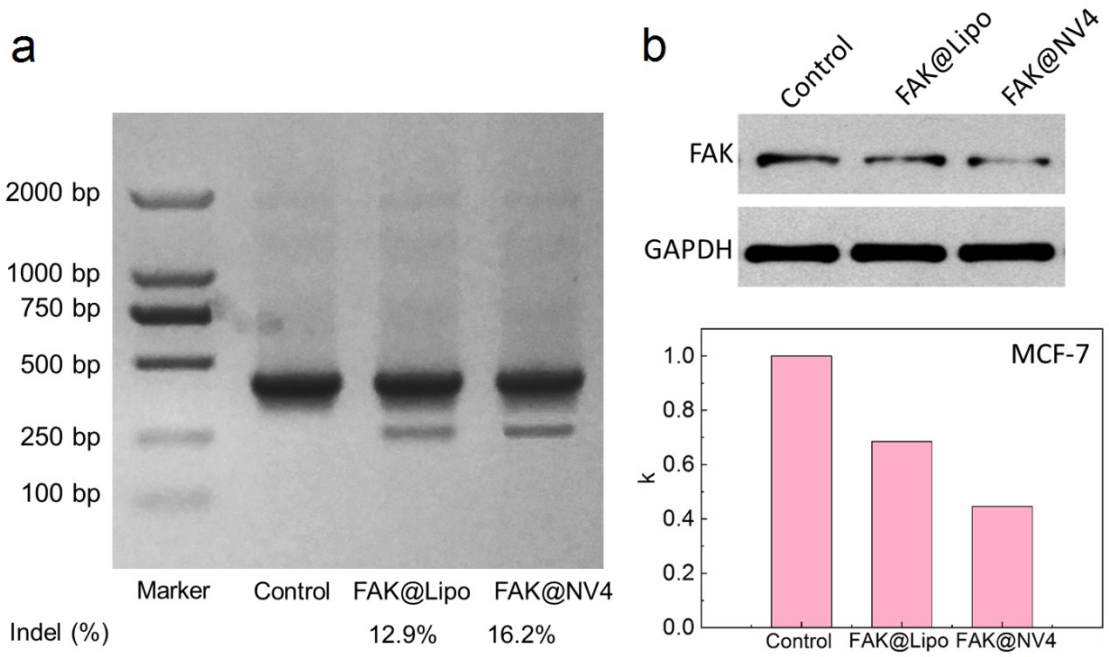

Figure S8. (a) Genome editing efficacy in MCF-7 cells studied by Surveyor nuclease assay. (b) Evaluation of FAK knockout in MCF-7 cells after genome editing for 48 h by western blot analysis. k is the ratio of the expression after genome editing to the expression without genome editing. Untreated cells were used as a control.

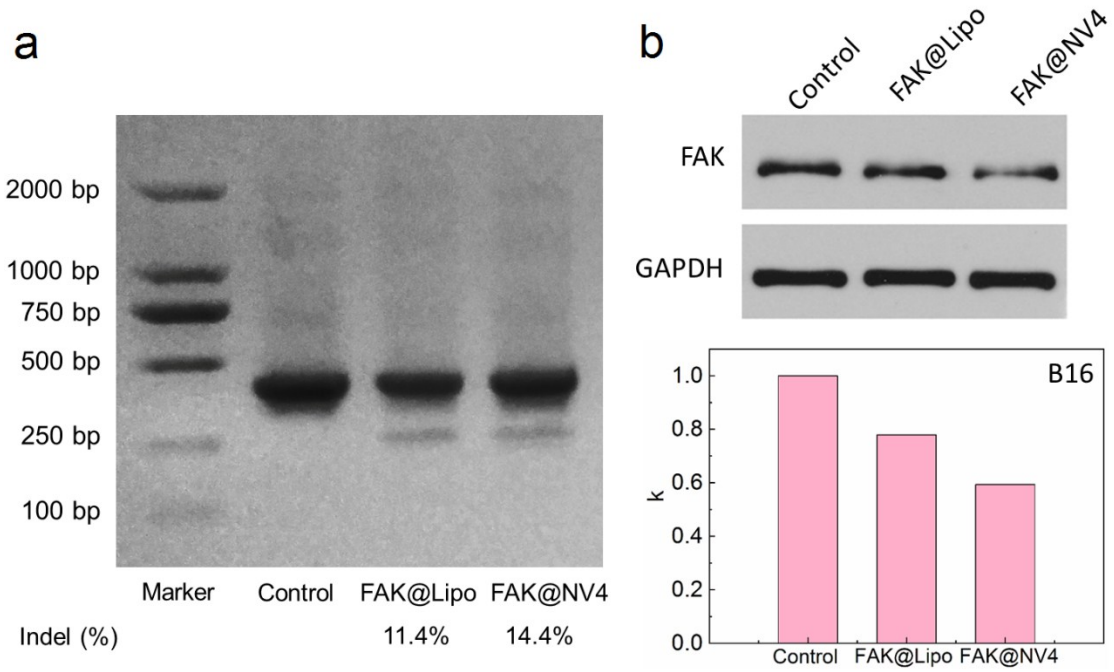

Figure S9. (a) Genome editing efficacy in B16 cells studied by Surveyor nuclease assay. (b) Evaluation of FAK knockout in B16 cells after genome editing for 48 h by western blot analysis. k is the ratio of the expression after genome editing to the expression without genome editing. Untreated cells were used as a control.

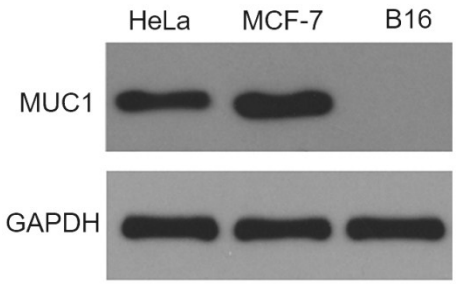

Figure S10. Expression of MUC1 in different tumor cells determined by western blot analysis.

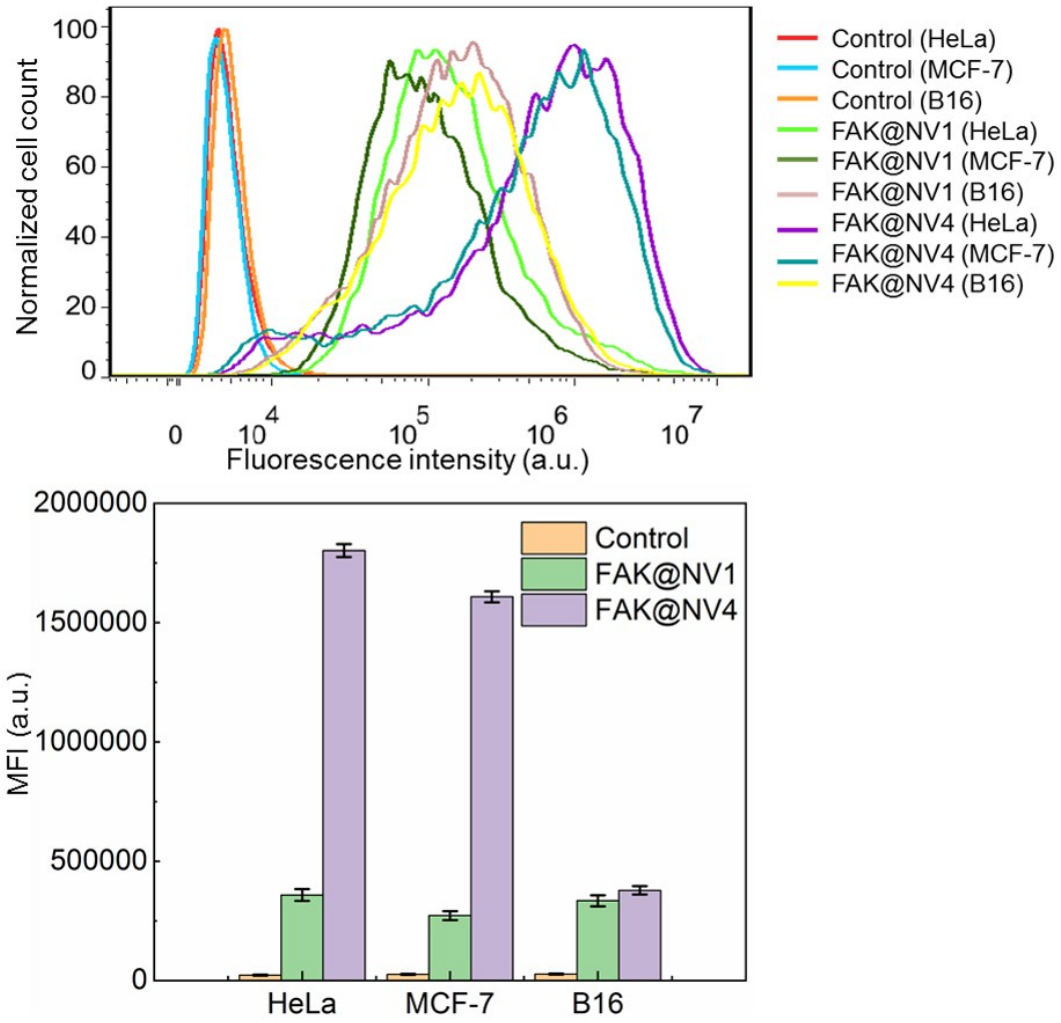

Figure S11. Cellular uptake of YOYO-1 labeled plasmid in different tumor cells after being co-incubated with different plasmid loaded nanovectors (4 h) studied by flow cytometry. Untreated cells were used as a control.

As shown in Figure S11, the targeting delivery system (FAK@NV4) results in much higher intracellular plasmid concentrations as compared with FAK@NV1 for HaLa cells and MCF-7 cells with overexpression of MUC1 and nucleolin. While for B16 cell without overexpression of MUC1 or nucleolin, FAK@NV4 does not lead to apparently enhanced cellular uptake.

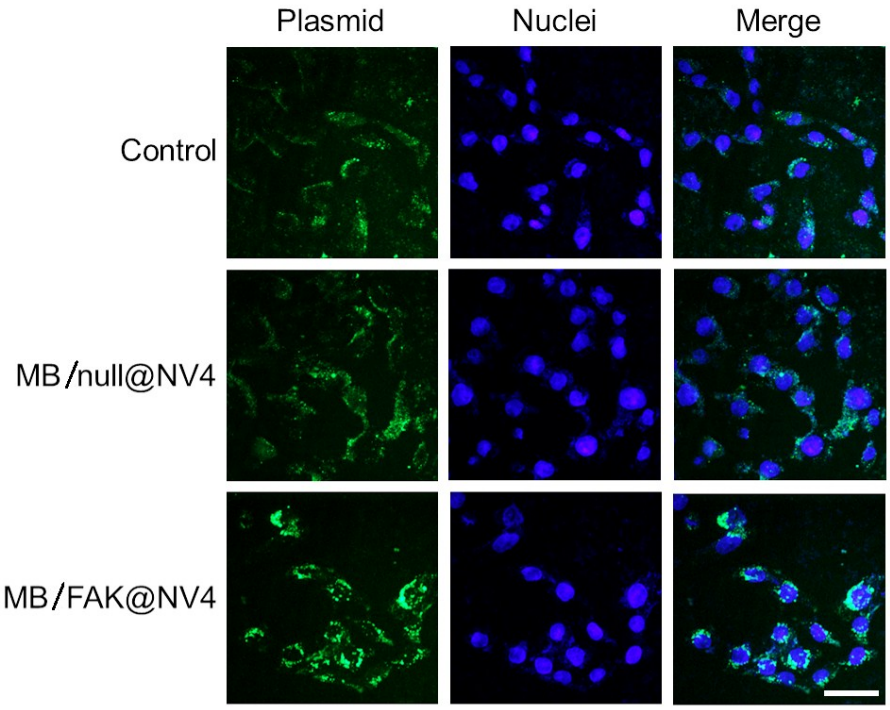

Figure S12. The detection of mRNA of p53 in HeLa cells treated by MB/null@NV4 and MB/FAK@NV4. The cells were observed by confocal microscopy under magnification of 400. Untreated cells were used as a control. Scale bar: 50 μm.

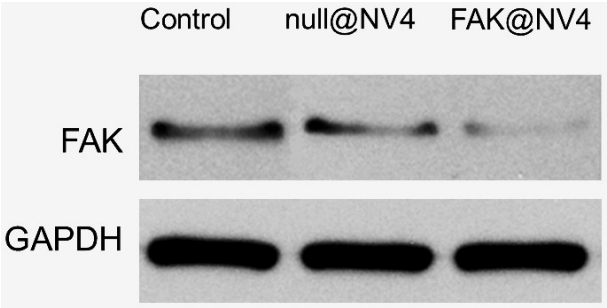

Figure S13. FAK expression in edited HeLa after three subcultures. Untreated cells were used as a control.