Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Coassembly of nucleus-targeting gold nanoclusters with CRISPR/Cas9 for simultaneous bioimaging and therapeutic genome editing

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Experimental Section

Preparation of all-in-one Cas9-gRNA plasmids. Human codon-optimized Cas9 and guide RNA expression plasmids pX330 and pX458 were purchased from Addgene (Cambridge, MA, USA). The all-in-one Cas9-gRNA plasmid constructs were established by following the previously published protocol without a significant revision.[1] The gRNA-encoding dsDNA for cloning were synthesized by IDT (Coralville, IA). The cloned plasmids were transformed into One Shot Stbl2 competent cells (Thermo Fisher) and then purified using the NucleoBond Xtra Midi Plus EF kit (Clontech, Mountain View, CA). The gRNA sequences were verified by Eton Bioscience (Union, NJ). The Cas9-gRNA$_{E7}$ plasmid and Cas9-control gRNA plasmid were prepared according to our previously established protocol.[2]

Cell cultures.

HeLa cells (a human cervical carcinoma cell line) and U2OS cells (a human osteosarcoma cell line) are purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). HeLa cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco) with 10% FBS (Gibco), 1 × penicillin-streptomycin (HyClone), and 1 × MEM non-essential amino acids (Gibco). We evaluated the HPV oncogene disruption using the AuNCs/Cas9-gRNA plasmid nanocomplexes in HeLa cells. The U2OS and U2OS.EGFP cells were cultured in DMEM (Gibco) with 10% FBS, 2 mM GlutaMAX (Life Technologies), and 1 × penicillin-streptomycin (HyClone). We used the U2OS.EGFP cells for the Cas9-induced reporter gene disruption assay. All cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2. Before each experiment, the cell density was determined using a hemocytometer.

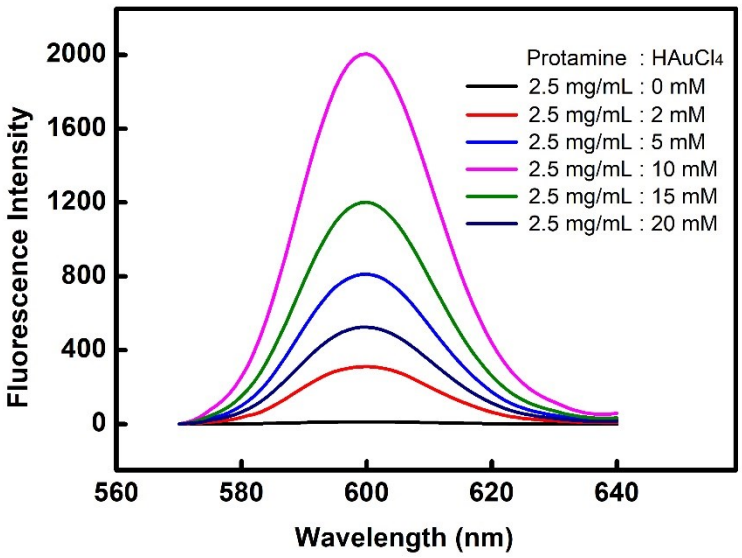

Fig. S1 The fluorescence intensities of the protamine-AuNCs at different ratios of protamine and $HAuCl_4$.

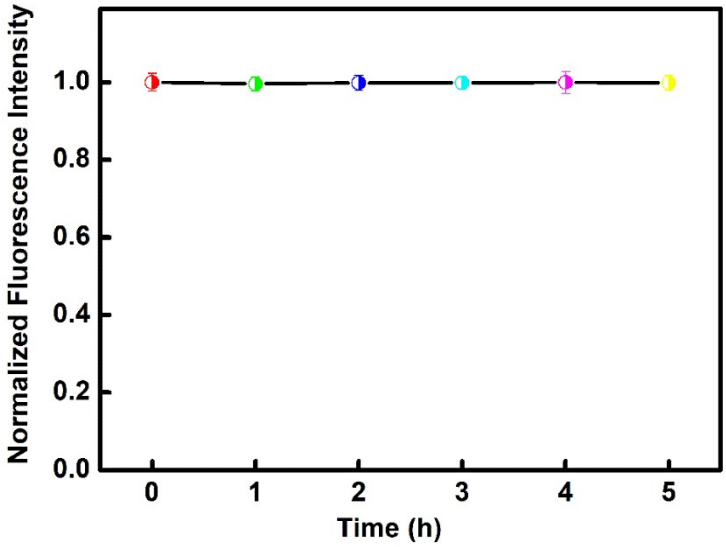

Fig. S2 The fluorescence intensities of the protamine-AuNCs conjugates as a function of time in 50% FBS. The error bars represent variations among three independent measurements.

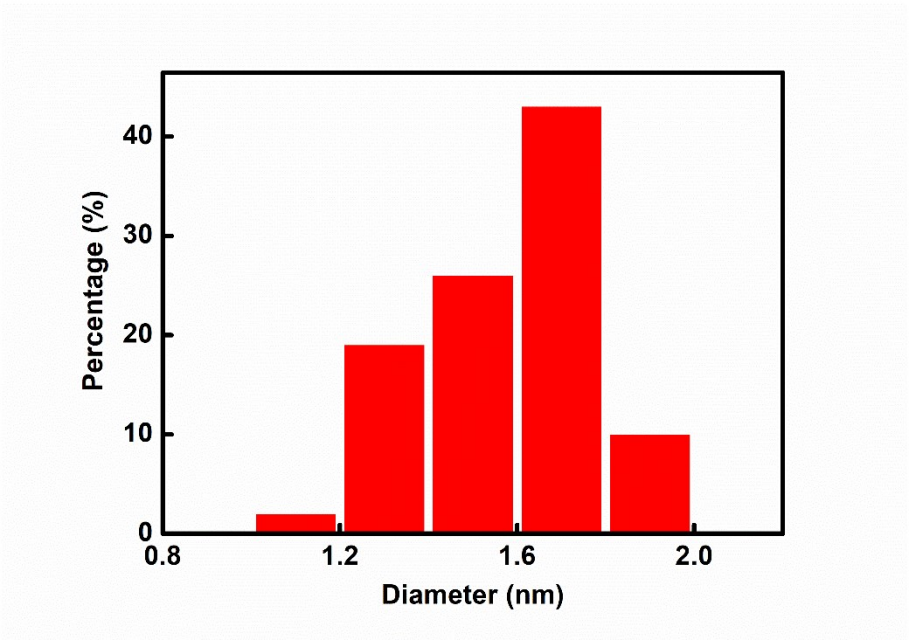

Fig. S3 Size distribution histogram of the protamine-AuNCs. The total number of clusters counted for the histogram was 100.

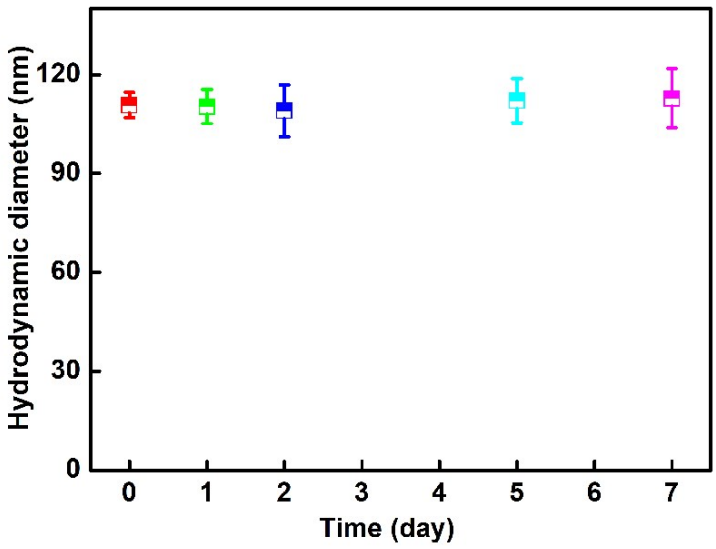

Fig. S4 The hydrodynamic diameter of AuNCs/pDNA complexes after storage at 4 ℃ on days 0, 1, 2, 5, and 7.

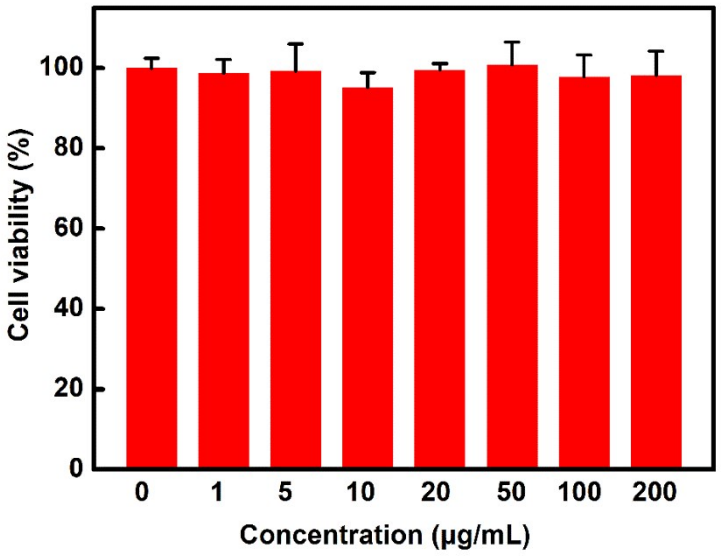

Fig. S5 Cytotoxicity testing results of the protamine against U2OS cells by MTT assay.

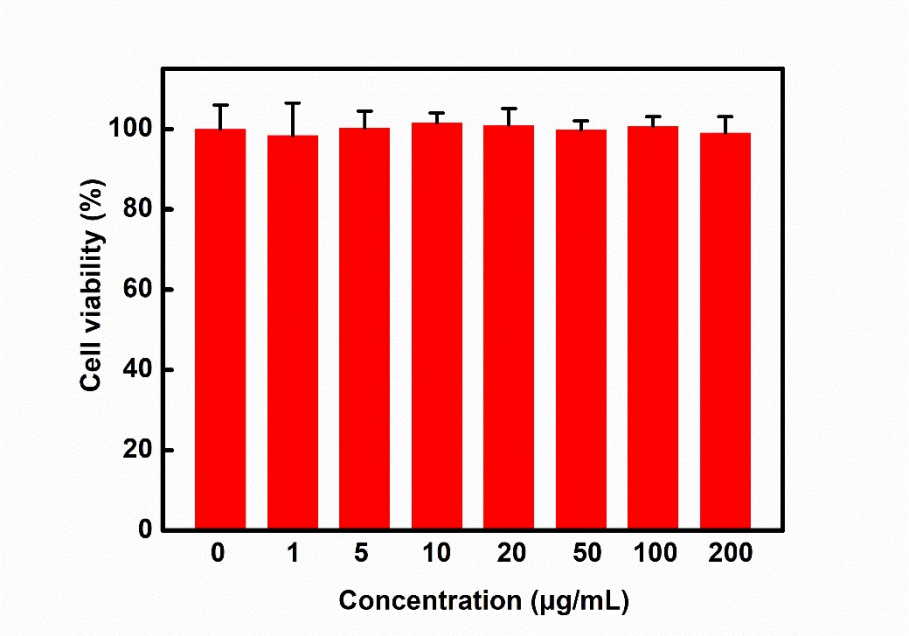

Fig. S6 *In vitro* cytotoxicities of protamine-AuNCs to U2OS cells by MTT assay.

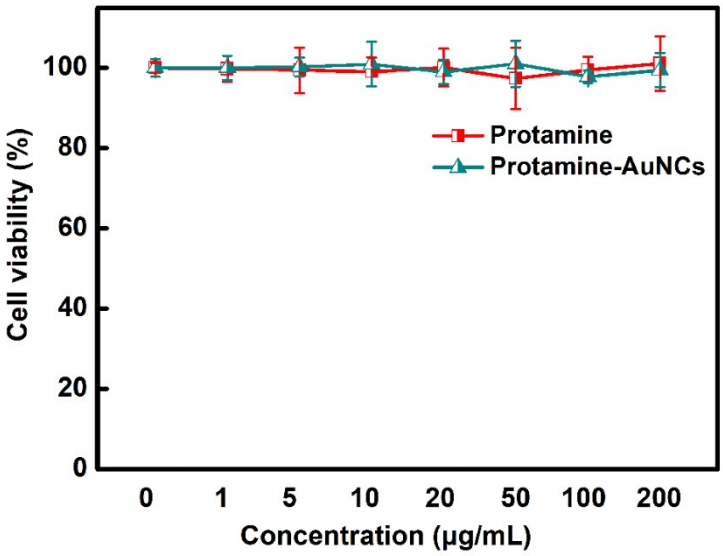

Fig. S7 Cytotoxicity testing results of the protamine and protamine-AuNCs against U2OS cells by LDH assay.

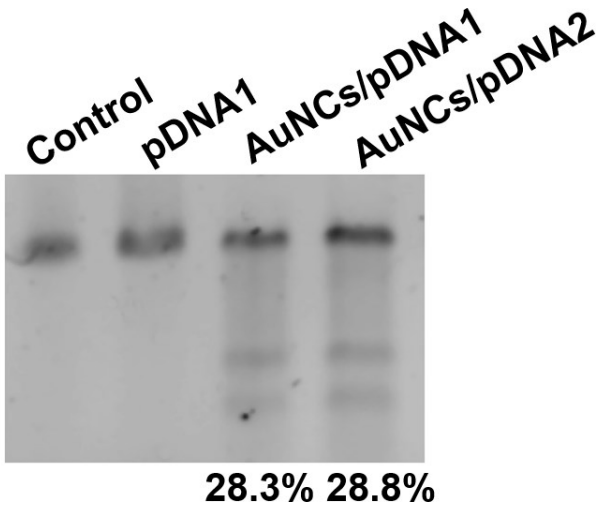

Fig. S8 T7EI assay to verify GFP gene disruption in U2OS.EGFP cells.

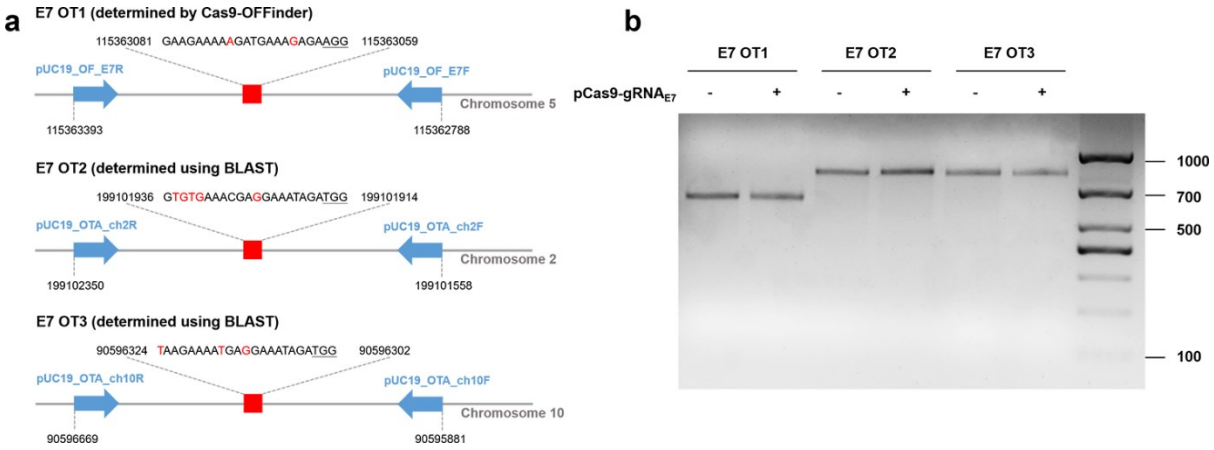

Fig. S9 T7EI assay to verify the off-targeting based on the prediction using Cas9-OFFinder and BLAST.

References

1. F. A. Ran, P. D. Hsu, J. Wright, V. Agarwala, D. A. Scott and F. Zhang, *Nat. Protoc.,* 2013, **8**, 2281-2308.
2. Y. H. Lao, M. Li, A. Gao Madeleine, D. Shao, C. W. Chi, D. Huang, S. Chakraborty, T. C. Ho, W. Jiang, H. X. Wang, S. Wang and K. W. Leong, *Adv. Sci.,* 2018, **5**, 1700540.