Supporting Information

None Nuclear Localization Signal-guided CRISPR/Cas9 Ribonucleoproteins for Translocation and Gene Editing Via Apoferritin Delivery Vectors

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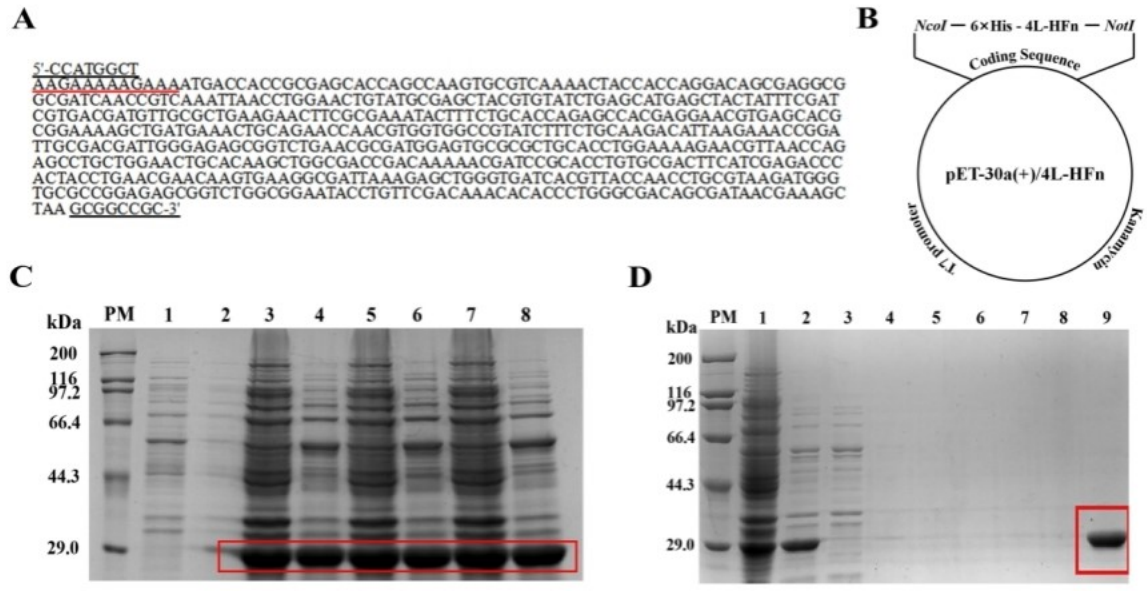

Figure S1. Preparation and characterization of 4L-HFn. (A) Optimized 4L-HFn gene Sequence (5 'to 3' direction). (B) Plasmid of pET-30a(+)/4L-HFn. (C) SDS-PAGE of construction of 4L-HFn engineering bacteria. PM:Protein Marker; 1-2:ArcticExpress(DE3) negative control bacteria total bacteria and supernatant; 3-8: three batch of genetic engineering bacteria total bacteria and supernatant. (D) SDS-PAGE of purified 4L-HFn. 1: total bacterial solution before bacterial breakdown; 2: supernatant after bacterial breakdown; 3: sample penetration; 4:Binding Buffer runoff; 5-9: 30, 50, 70, 90, 300 mM imidazole eluent.

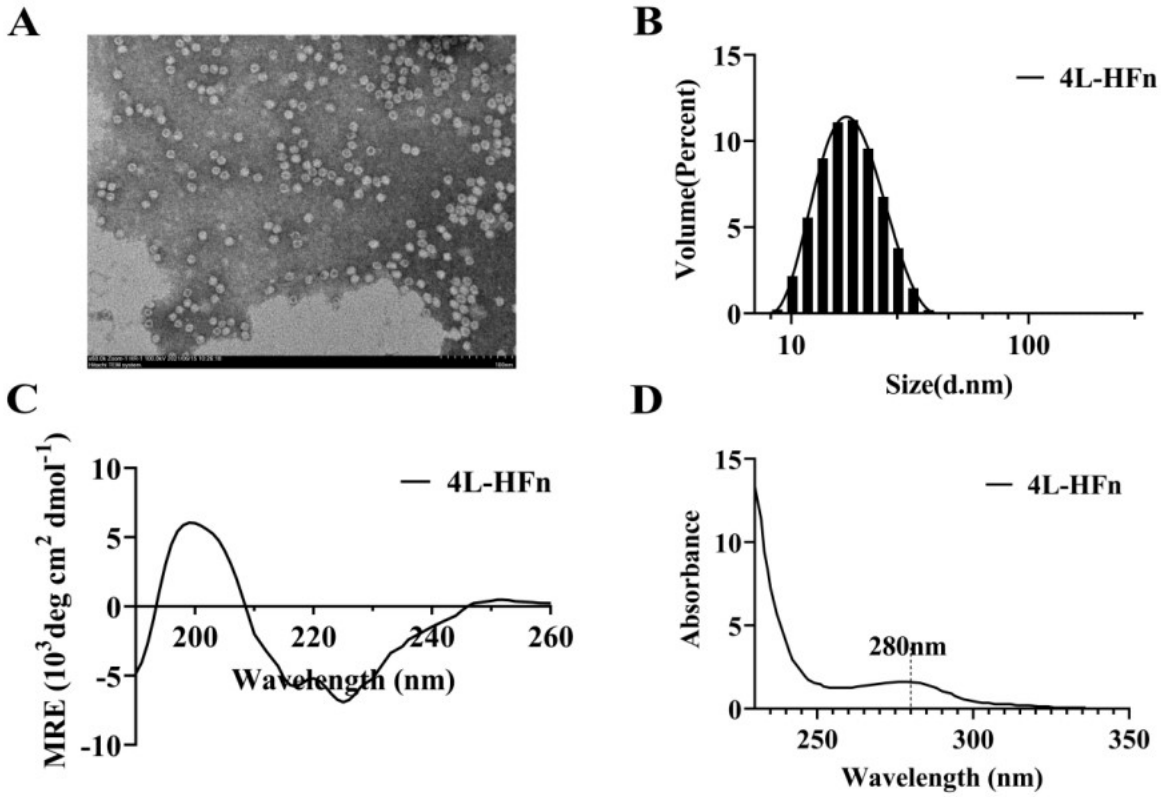

Figure S2. The characterizations of 4L-HFn. (A) TEM. (Scale: 100 nm). (B) Distribution of hydrodynamic diameter. (C) CD spectra. (D) UV-visible spectra.

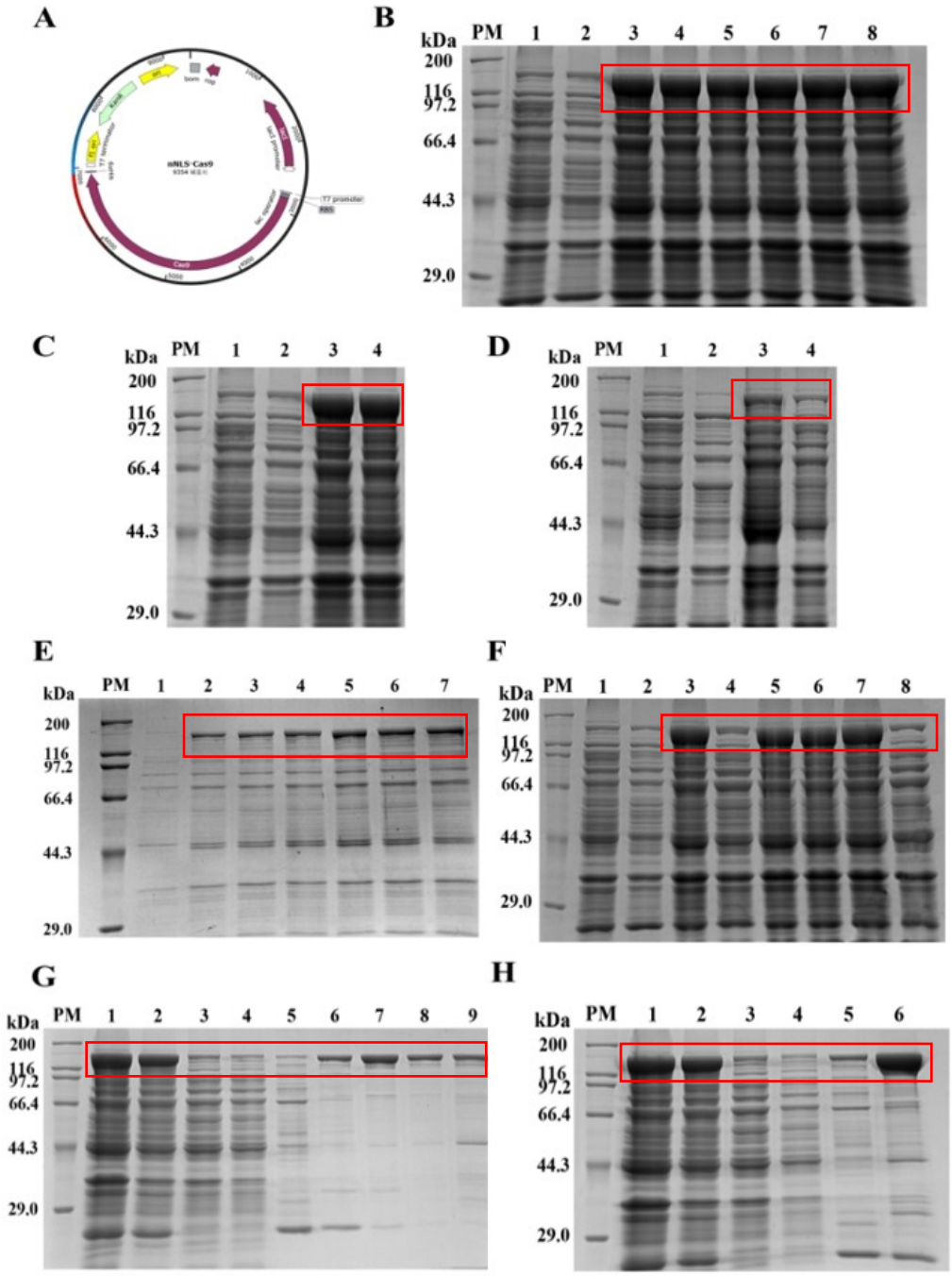

Figure S3. Preparation and characterization of NLS^{-}-Cas9. (A) Plasmid of pET28b-NLS^{-}-Cas 9-His. (B) SDS-PAGE of construction of NLS^{-}-Cas9 engineering bacteria. PM:Protein Marke r; 1-2:ArcticExpress(DE3) negative control bacteria total bacteria lysate and supernatant; 3-8: three batch of genetic engineering bacteria total bacteria lysate and supernatant. (C-D) SDS-P AGE of induction temperature at 18℃ (C) and 37℃ (D). PM: Protein Marker, 1-2: Rosetta (DE3) pLysS negative control bacteria total bacteria lysate and supernatant 3-4: genetically en gineered bacteria total bacteria and supernatant. (E) SDS-PAGE of induction time screening.

1-7: 0, 2, 4, 6, 8, 10 and 12 h. (F) SDS-PAGE of bacteria breaking power screening. 1-2: Rose tta(DE3)pLysS negative control bacteria total bacteria and supernatant 3-4,5-6,7-8: Total bact eria and supernatant of 400 W,500 W,600 W. (G) Screening of imidazole concentration for el uting heteroprotein. 1: bacteria lysate 2: supernatant of bacteria lysate; 3: sample effluent; 4: Binding Buffer effluent; 5-9: 30, 50, 70, 90, 300 mM imidazole eluate. (H) The purification of Cas9 protein using nickel chelation chromatography. 1: bacteria lysate; 2: sample effluent; 3: Binding Buffer effluent; 4-5: Cas9 effluent eluted by 30 and 300 mM imidazole respectively; 6: Protein after ultrafiltration concentration.

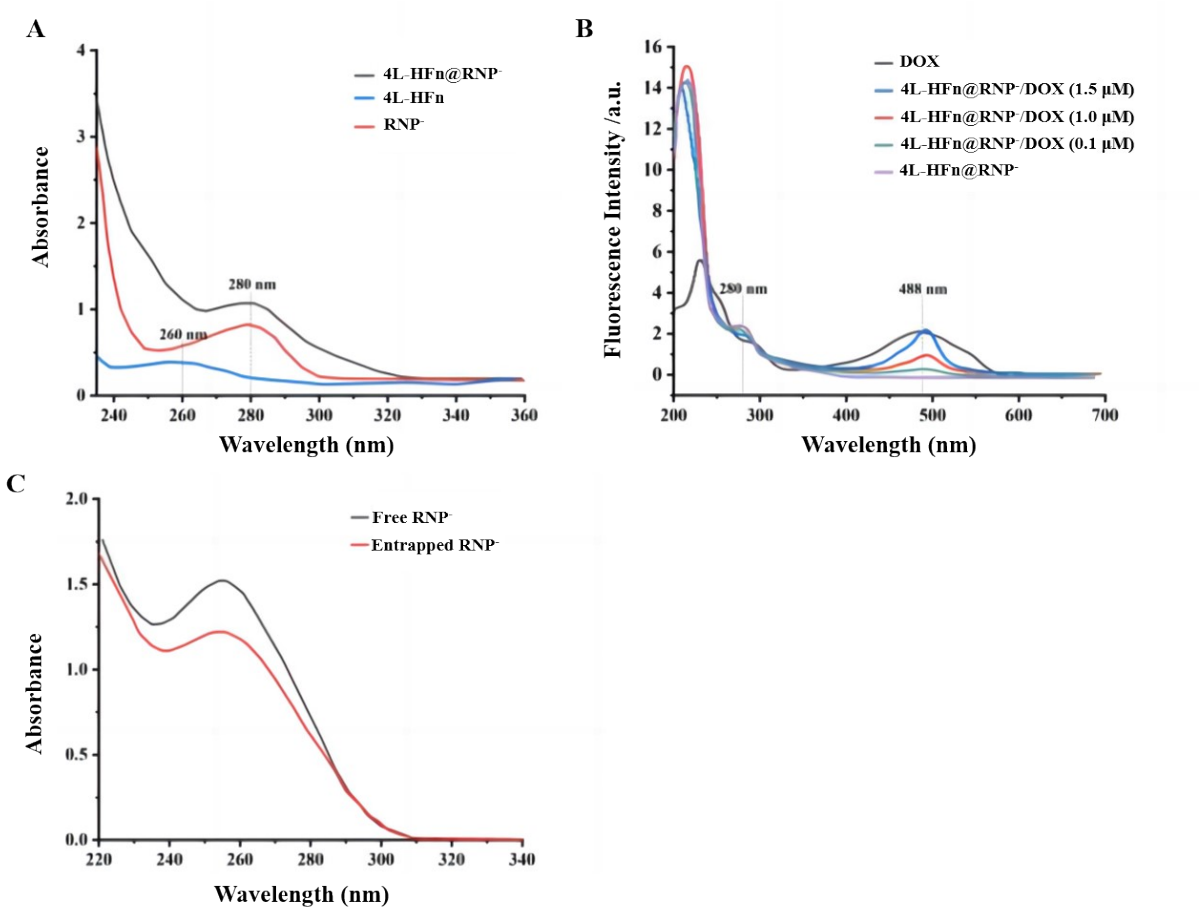

Figure S4. Preparation and characterization of 4L-HFn@RNP-/DOX. (A) Preparation and UV-vis of 4L-HFn@RNP-. (B) Preparation and UV-vis of 4L-HFn@RNP-/DOX. (C) Determination of RNP- encapsulation rate and drug loading in 4L-HFn@RNP-/DOX NPs.

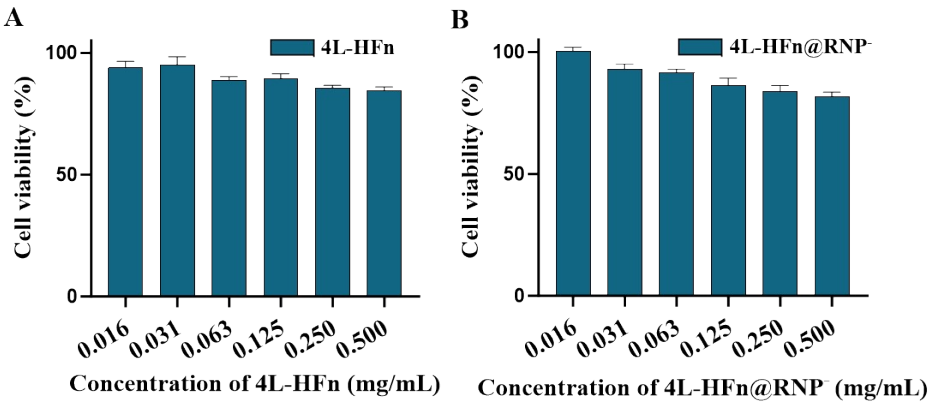

Figure S5. MTT test of 4L-HFn and 4L-HFn@RNP-/DOX. (A) Cytotoxicity of 4L-HFn on MDA-MB-231 cells (n = 6). (B) Cytotoxicity of 4L-HFn@RNP- on MDA-MB-231 cells (n = 6).

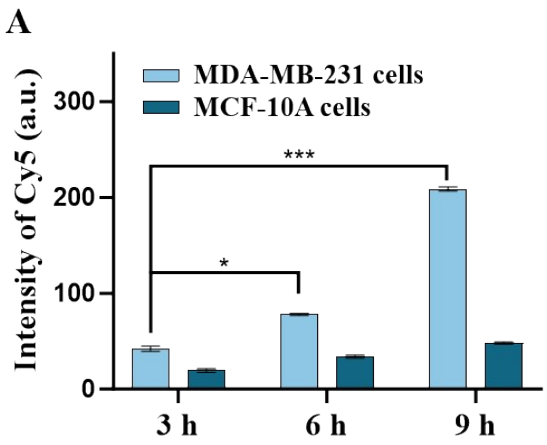

Figure S6. Efficiency of 4L-HFn@RNP^{-}/DOX delivery of RNP^{-}. (A) MDA-MB-231 cells and MCF-10A cells uptake of CLSM mean fluorescence intensity.

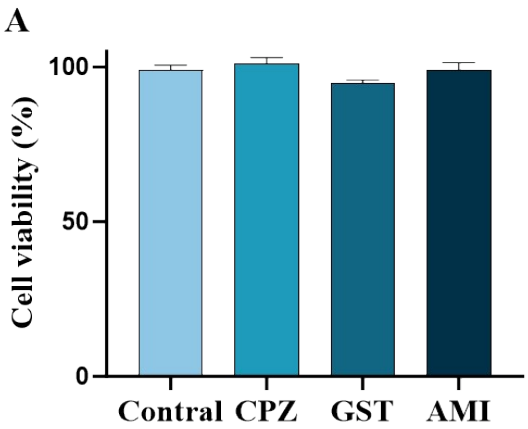

Figure S7. MTT test of endocytosis inhibitors. (A) Cytotoxicity of endocytosis inhibitors on MDA-MB-231 cells (n = 6).

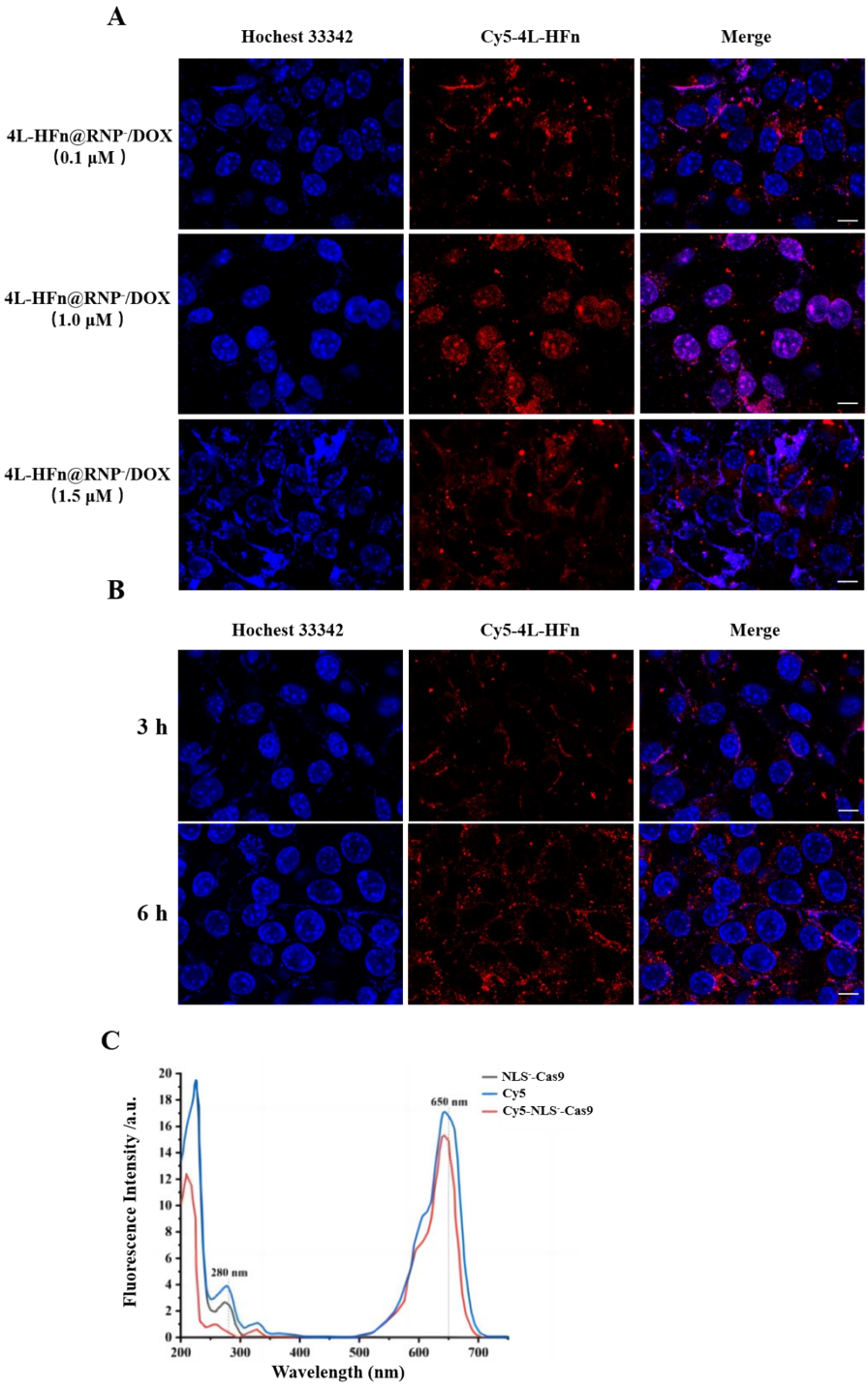

Figure S8. Triggering of 4L-HFn into the nucleus at different concentrations of DOX. (A) Following a 9-hour treatment with low, medium, and high concentrations of DOX, the self-triggered nuclear entry and nuclear co-localization of 4L-HFn/DOX were observed. (Scale bar: 10 μm). (B) Self-triggered nuclear entry and nuclear co-localization of 4L-HFn/DOX in medium DOX concentration. (Scale: 10 μm). (C) Cy5 fluorescent dye labeled NLS⁻-Cas9.

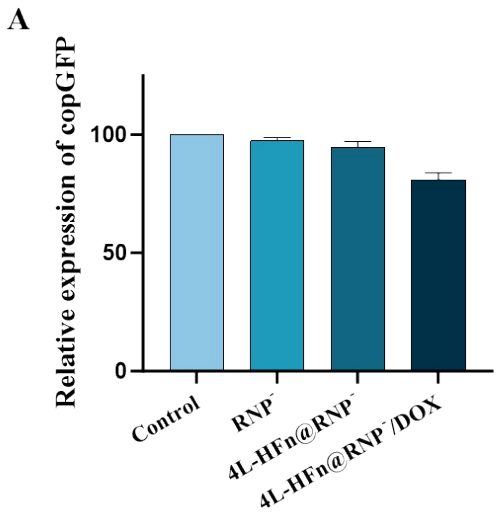

Figure S9. Relative expression of copGFP *in vivo* analysis using Image J.

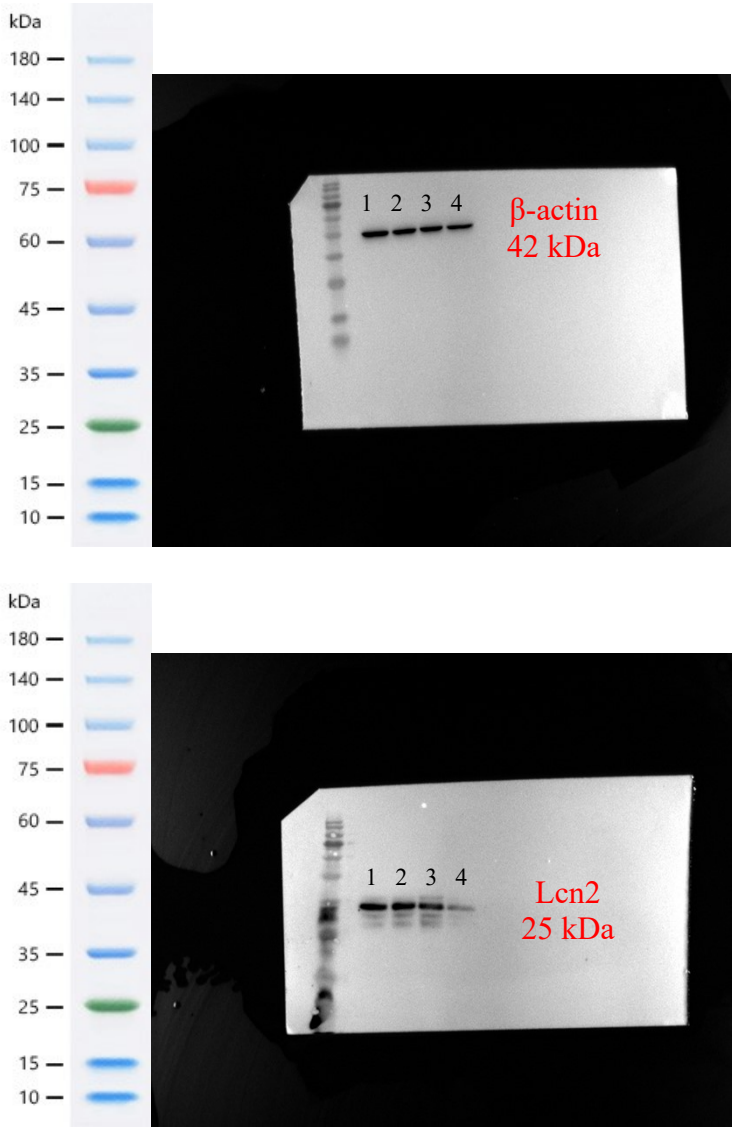

Figure S10. The raw data for the western blotting protein bands for Fig.5D (β-actin and Lcn2): 1.Control; 2. RNP-; 3. 4L-HFn@RNP-; 4. 4L-HFn@RNP-/DOX.

Table S1. Sequence of NLS^{-}-Cas9 used.

Sequence	Note
MDKKYSIGLDIGTNSVGWAVITDDYKVPSKKFKVLGNTDRHSIKKN LIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKV DDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKK LVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLV QTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGL FGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGD QYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDL TLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPIL EKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQE DFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITP WNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVY NELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKED YFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDIL EDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRL SRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQK AQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKP ENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENT QLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRK FDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKY DENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLN AVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKY FFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVR KVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNEL ALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQIS EFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPA AFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDSR AD（PKKKRKV）AAALEHHHHHH	Red for deleted SV40-NLS; Blue for 6 × His

Table S2. Encapsulation rate and drug loading of DOX for 4L-HFn@RNP-/DOX at low, medium and high DOX concentrations (n = 3)

4L-HFn@RNP$^-$/DOX (Concentration of DOX, μM)	Encapsulation efficiency（%）	Loading Content（%）	Quantity of trigger agent DOX contained in each 4L-HFn@RNP$^-$/DOX
0.1	91.20 ± 0.38	0.77 ± 0.03	0.91 ± 0.37
1.0	90.58 ± 0.19	1.53 ± 0.11	1.81 ± 0.38
1.5	91.46 ± 0.26	3.86 ± 0.09	4.57 ± 0.13

Table S3. Sequences of sgRNA and T7E1 primers used.

	Sequence(5’ to 3’)
sgRNA (Targeting Lcn2)	ACGAGGTAACTCGTTAATCCGTTTTAGAGCTAGA AATAGCAAGTTAAAATAAGGCTAGTCCGTTATCA ACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTT
sgRNA (Targeting copGFP)	AGTGCGCCGGCCGGAGCATCCAAAATCTCGATCT TTATCGTTCAATTTTATTCCGATCAGGCAATAGTT GAACTTTTTCACCGTGGCTCAGCCACGAAAA
T7E1 primer (Forward)	GGCAACATTAAGAGTGAGTC
T7E1 primer (Reverse)	TTGGAGAAGCGGATGAAG