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# Supplementary Materials for

## Exosome-mediated delivery of Cas9 ribonucleoprotein complexes for tissue-specific gene therapy of liver diseases

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**This PDF file includes:**

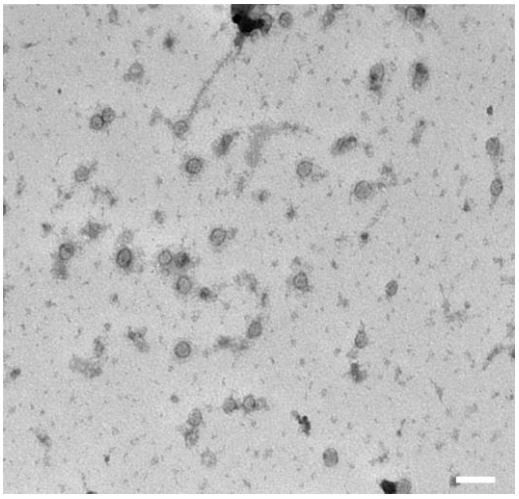

**Fig. S1. The typical contracted TEM image of the exosome in Figure 2D.** Scale bar = 100 nm.

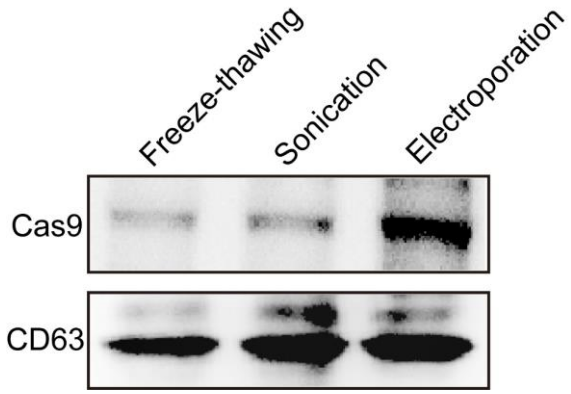


**Fig. S2. Loading capabilities of various exogenous approaches.**

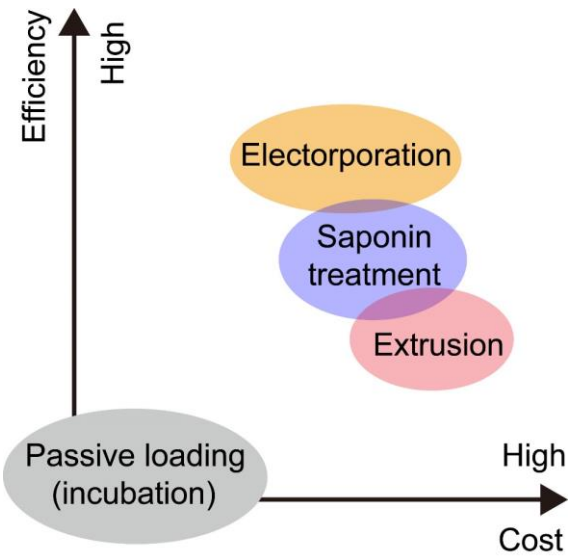


**Fig. S3. Available exogenous loading methods as a function of loading efficacy and costs for large-scale production.**

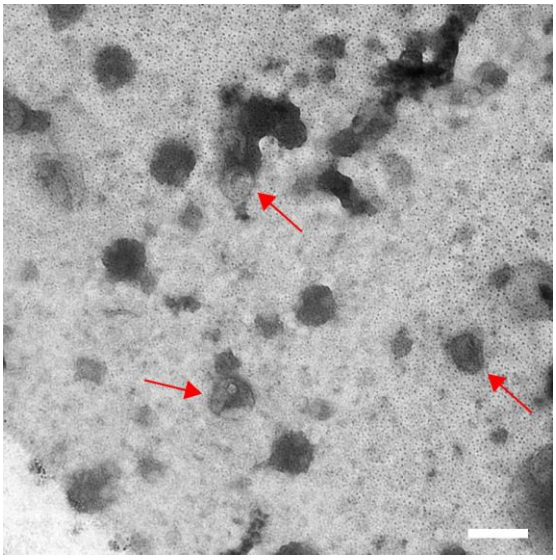

**Fig. S4. The typical TEM image of exosome$^{RNP}$.** The red arrows indicating exosome$^{RNP}$ particles. Scale bar = 200 nm.

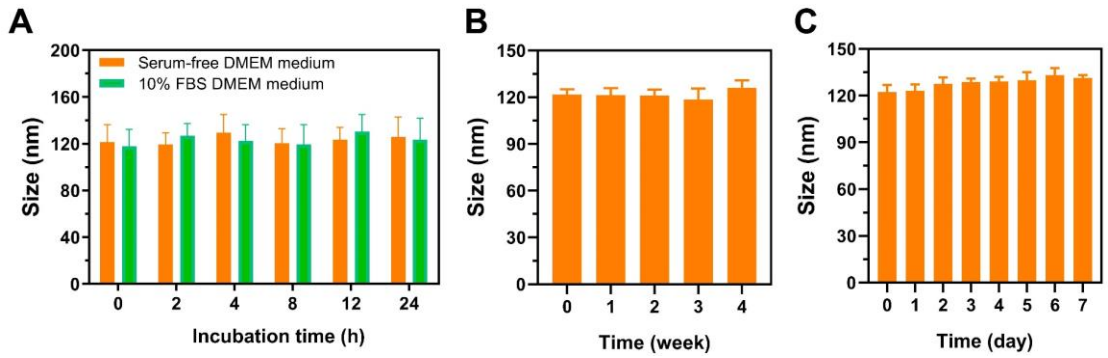


**Fig. S5. Stability of exosome$^{RNP}$ nanocomplexes.** (A) The particle size variation of exosome$^{RNP}$ nanocomplexes in the culture DMEM medium with or without serum. (B-C) Size of exosome$^{RNP}$ nanocomplexes stored for -80 ℃ (B) and 4 ℃ (C). Mean ± SD; $n$ = 3.

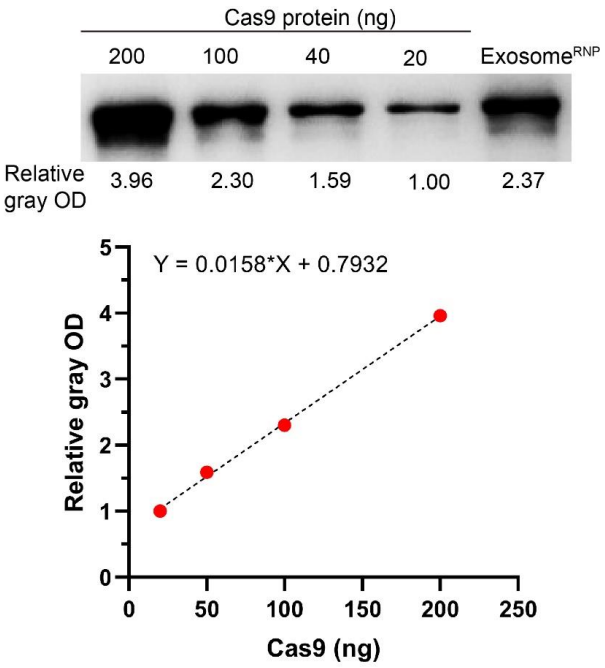


**Fig. S6. Western blot analysis to determine the loading of Cas9 protein in exosome.**

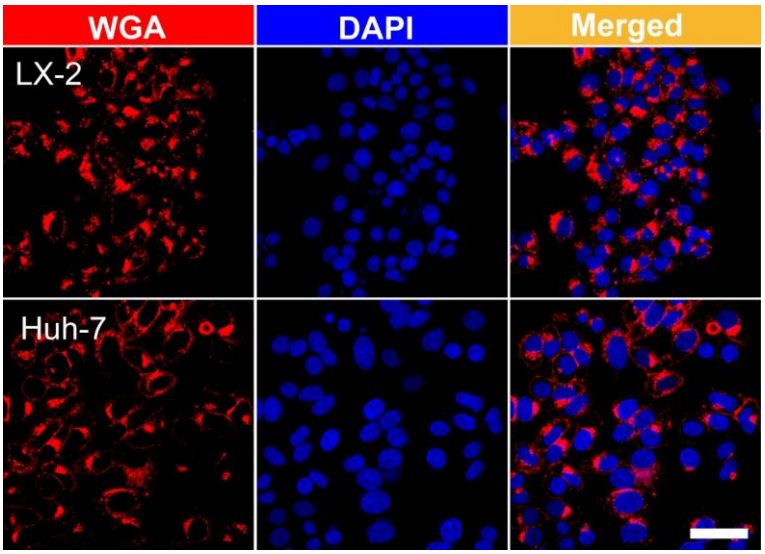


**Fig. S7. Cytosolic delivery of WGA-labeled exosome into LX-2 and Huh-7 cells.** Scale bar = 25 μm.

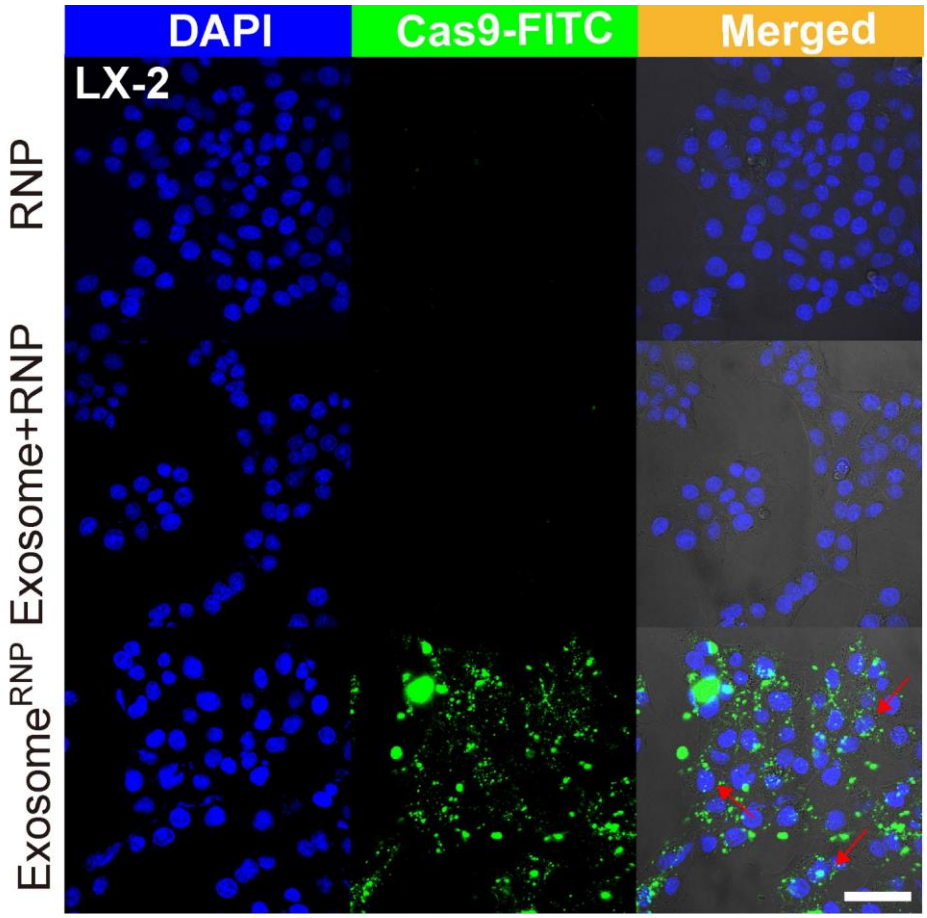


**Fig. S8. Cytosolic delivery of Cas9-FITC into LX-2 cells by exosomes for 4 hours.** Cas9-FITC and exosome+Cas9-FITC were used as negative controls. The red arrows point at the efficient translocation of Cas9-FITC/sgRNA RNP into the nuclei. These pictures correspond to Figure 2H.

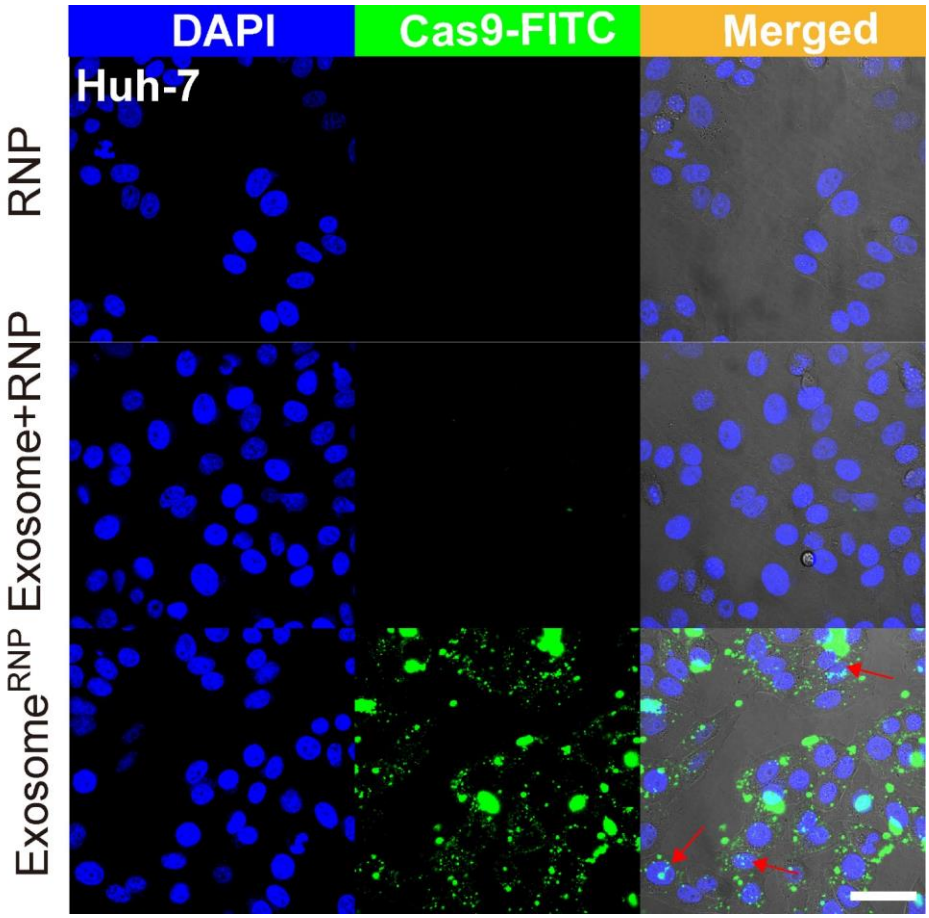


**Fig. S9. Cytosolic delivery of Cas9-FITC into Huh-7 cells by exosomes for 4 hours.** Cas9-FITC and exosome+Cas9-FITC were used as negative controls. The red arrows point at the efficient translocation of Cas9-FITC/sgRNA RNP into the nuclei. These pictures correspond to Figure 2I.

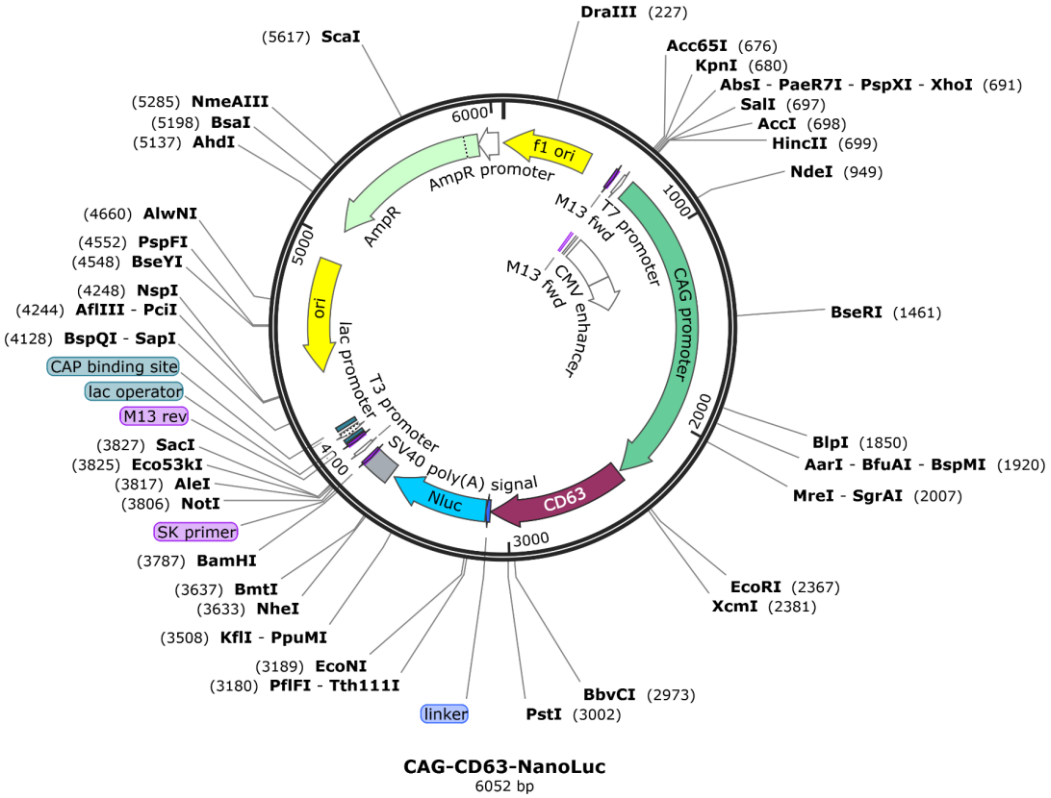


**Fig. S10. Plasmid map of CAG-CD63-NanoLuc DNA vector.**

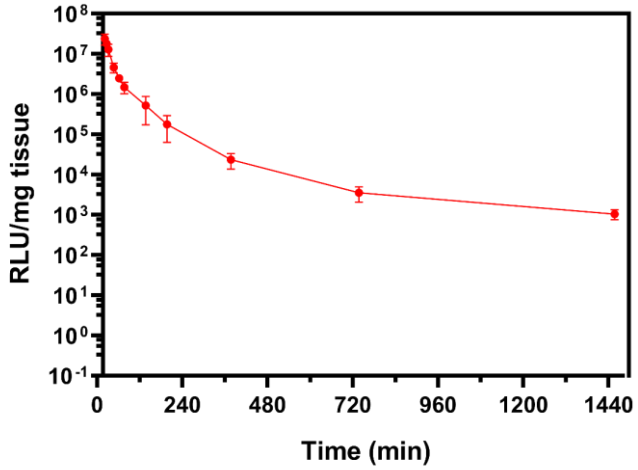


**Fig. S11. Time-course of liver CD63-NanoLuc activity after systemic administration of CD63-NanoLuc-exosome$^{RNP}$ nanocomplexes.** Mean ± SD; *n* = 3.

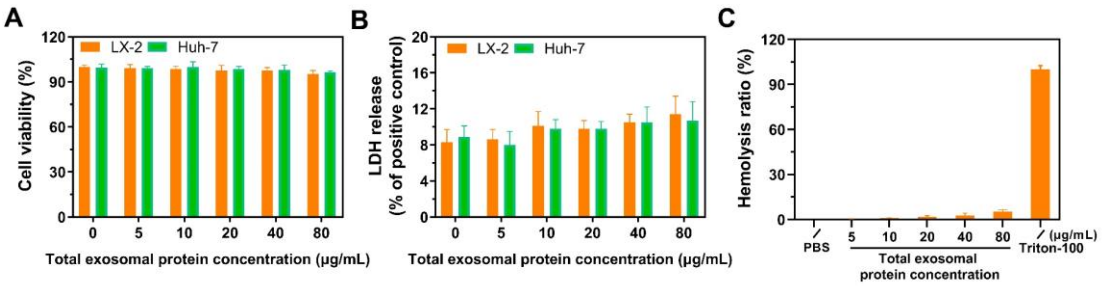


**Fig. S12. Biocompatibility of exosome$^{RNP}$ nanocomplexes *in vitro*.** (A) Cell viability of LX-2 and Huh-7 cells after 24 h incubation with exosome$^{RNP}$ nanocomplexes. Cell viability was determined by MTT assay. (B) LDH release assay. (C) Hemolytic activity of the exosome$^{RNP}$ nanocomplexes. Total exosomal protein concentration ranges from 5 to 80 μg/mL. PBS and Triton-100 (0.5%) were used as the negative and positive controls, respectively. Mean ± SD; $n$ = 6.

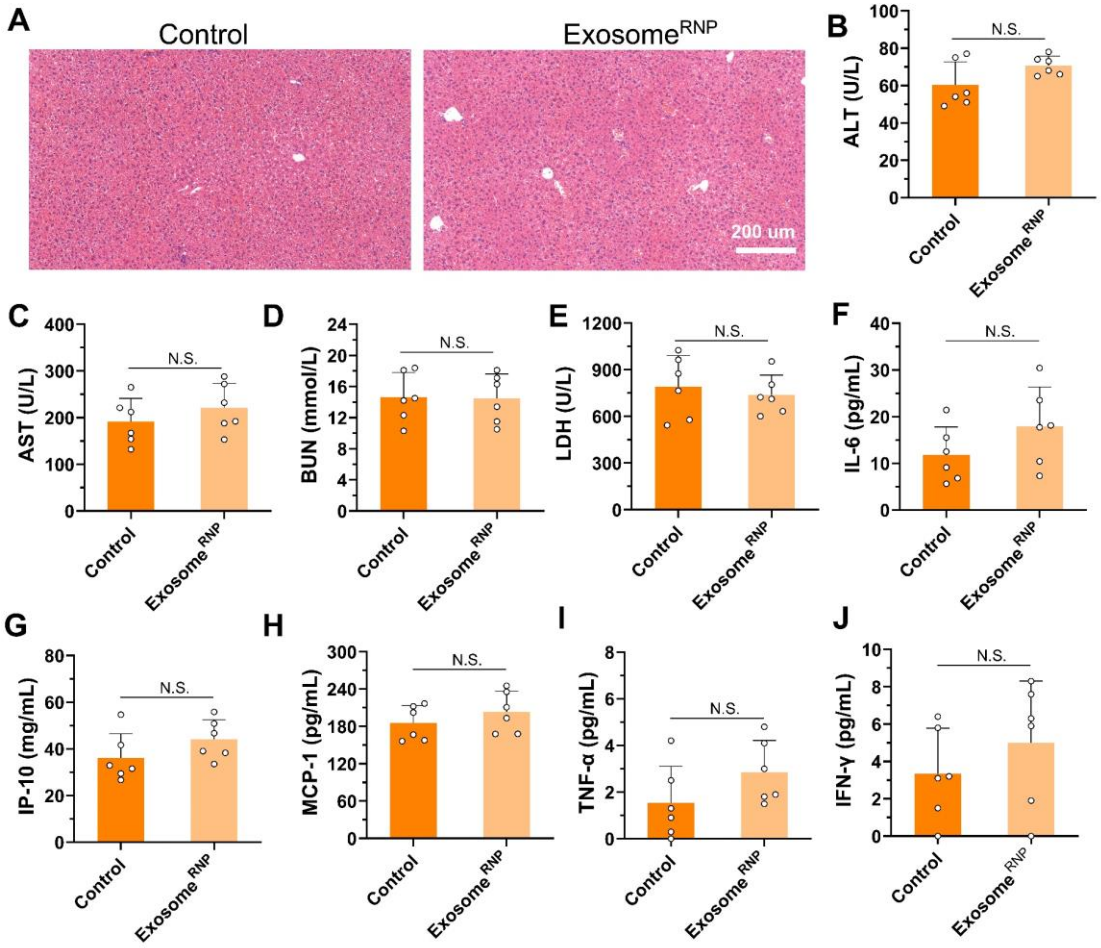


**Fig. S13. Systemic toxicity and immunogenicity evaluation of exosome$^{RNP}$ via intravenous injection.** (A) H&E staining of liver sections from healthy mice after the exosome$^{RNP}$ treatment. (B-E) Serum levels of ALT, AST, BUN, and LDH in healthy mice treated with or without exosome$^{RNP}$. (F-J) Levels of five major inflammatory cytokines in healthy mice treated with or without exosome$^{RNP}$. Statistical significance was calculated by Students' t-test (mean ± S.D., $n$ = 6). N.S. represents no significant difference.

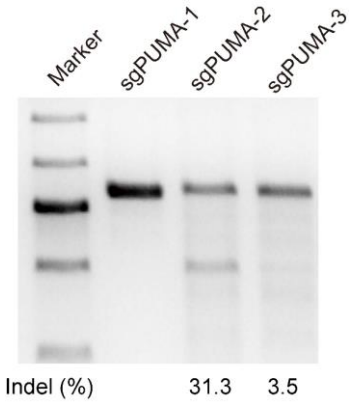


**Fig. S14. Frequency of indel mutation detected by T7E1 assay from AML-12 cells at *PUMA* locus of different sgRNAs.**

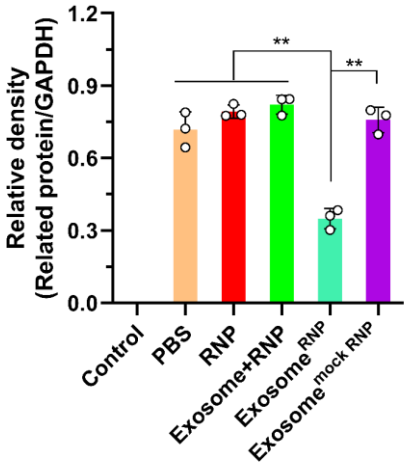


**Fig. S15. Protein levels were quantified by densitometry after the specified treatments.** Mean ± SD; $n = 3$ (one-way ANOVA with a Tukey's post-hoc test, $^{**}P < 0.01$).

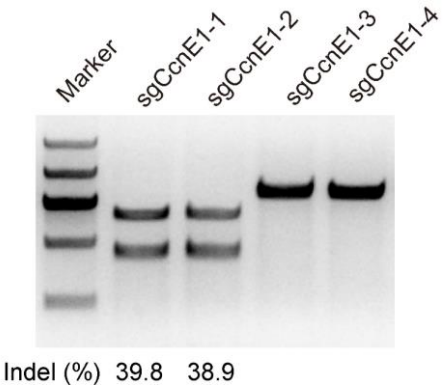


**Fig. S16. Frequency of indel mutation detected by T7E1 assay from AML-12 cells at *CcnE1* locus of different sgRNAs.**

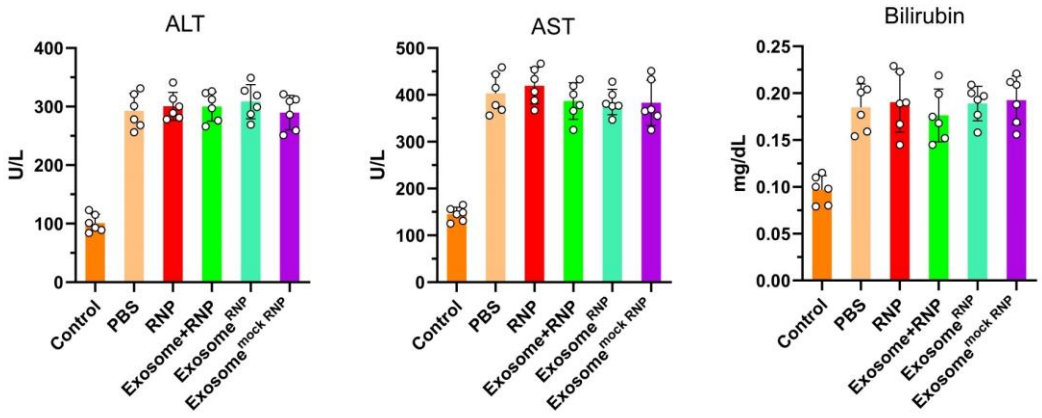


**Fig. S17. Serum ALT and AST activities were measured to evaluate global liver injury. Bilirubin content in serum was determined as a measure of liver function.** Mean ± SD; $n = 6$.

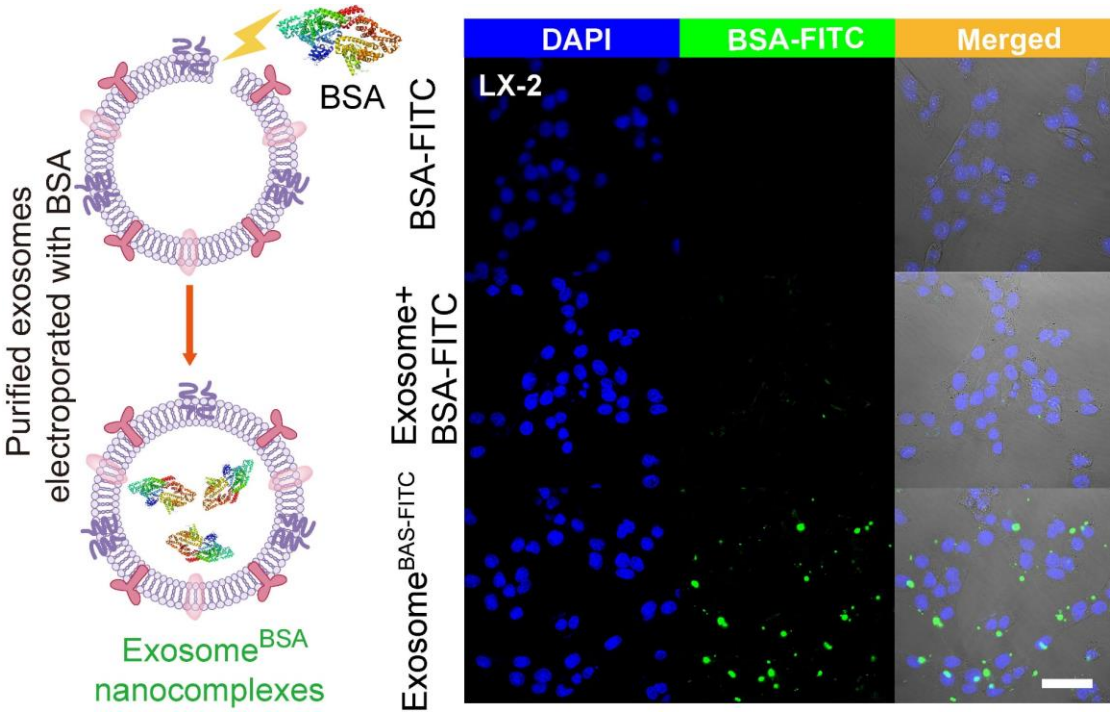


**Fig. S18. Preparation of exosome$^{BSA\text{-}FITC}$ complexes and cytosolic delivery of BSA-FITC into LX-2 cells by exosomes for 4 hours.** Free Cas9-FITC and exosome+Cas9-FITC were used as negative controls.

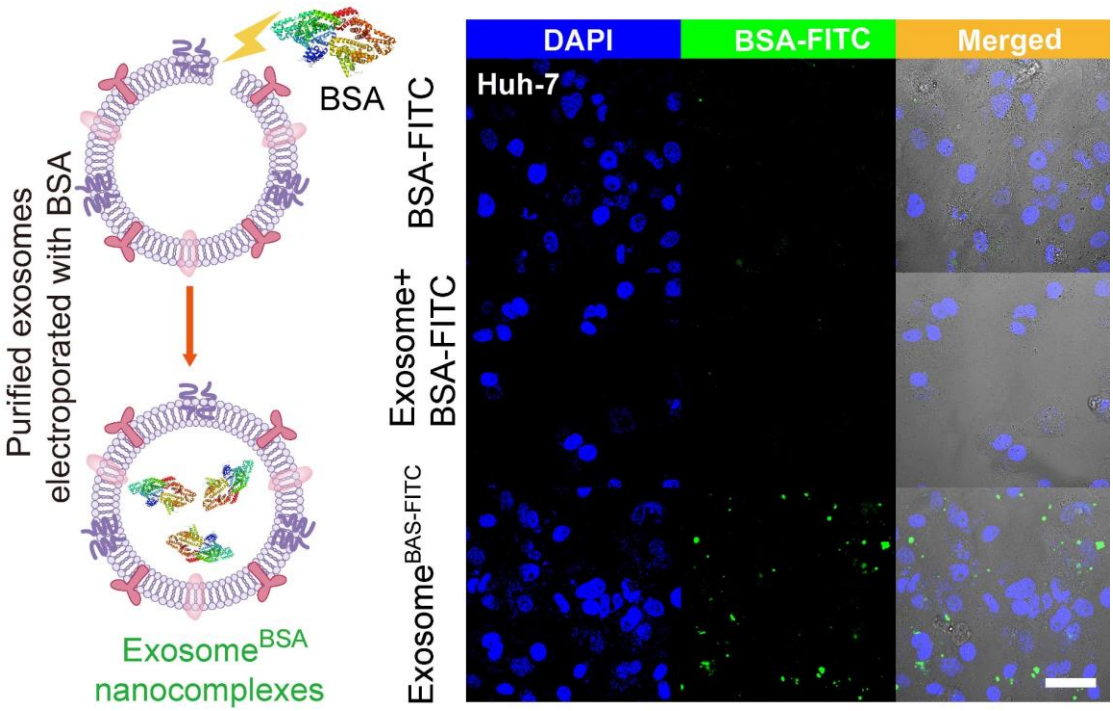


**Fig. S19. Preparation of exosome$^{BSA\text{-}FITC}$ complexes and cytosolic delivery of BSA-FITC into Huh-7 cells by exosomes for 4 hours.** Free Cas9-FITC and exosome+Cas9-FITC were used as negative controls.

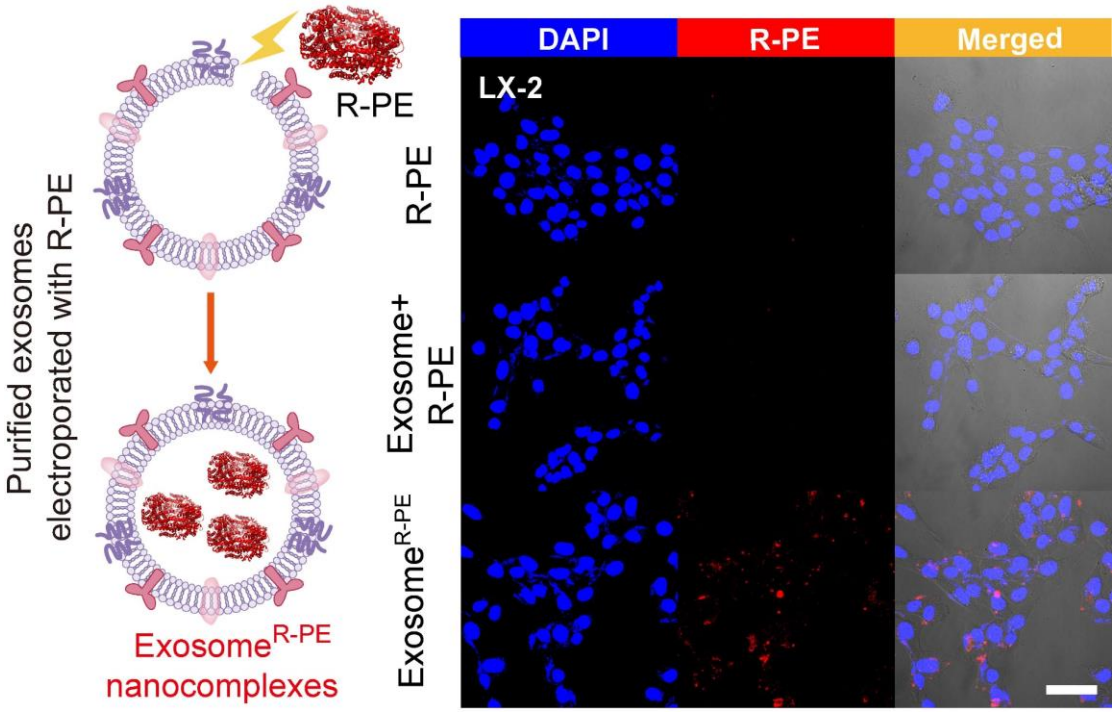


**Fig. S20. Preparation of exosome$^{R\text{-}PE}$ complexes and cytosolic delivery of R-PE into LX-2 cells by exosomes for 4 hours.** Free R-PE and exosome+R-PE were used as negative controls. Scale bar = 25 μm.

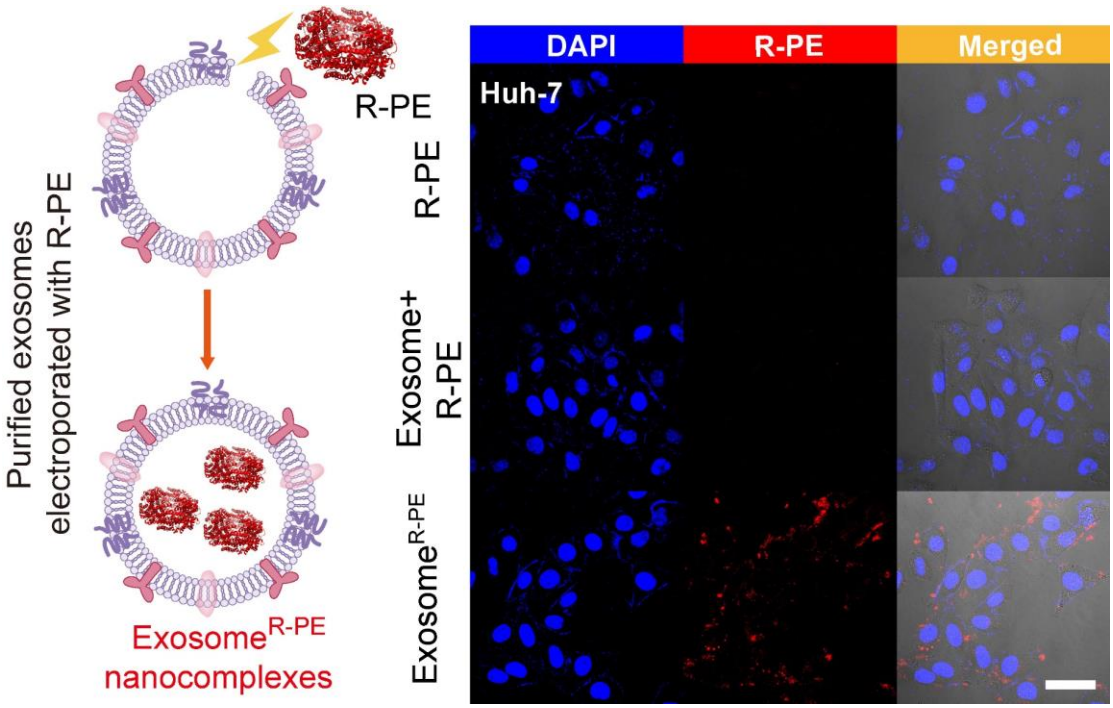


**Fig. S21. Preparation of exosome$^{R\text{-}PE}$ complexes and cytosolic delivery of R-PE into Huh-7 cells by exosomes for 4 hours.** Free R-PE and exosome+R-PE were used as negative controls. Scale bar = 25 μm.

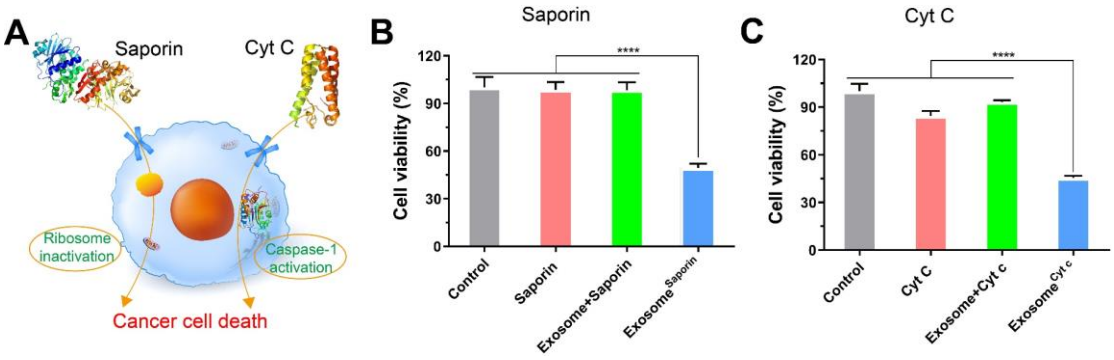


**Fig. S22. Cytosolic delivery of toxic proteins.** (A) Cytosolic delivery of saporin and Cyt C into cancer cells leads to ribosome inactivity and Caspase-1 activation, respectively, and cell death. (B-C) Cell viability of Huh-7 cells treated with exosome$^{saporin}$ (B) and exosome$^{Cyt\ C}$ (C) determined by MTT assay, respectively. Mean ± SD; $n = 6$ (one-way ANOVA with a Tukey's post-hoc test, $^{****}P < 0.0001$).

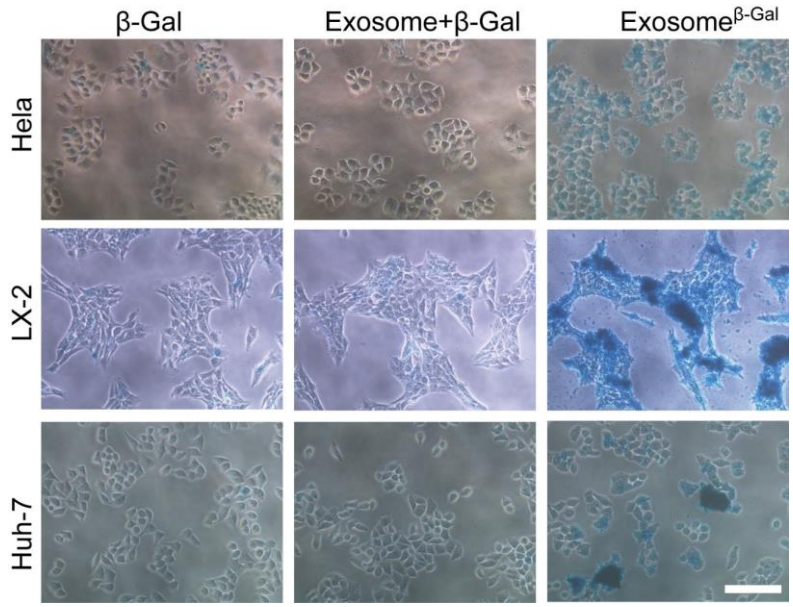


**Fig. S23. Cytosolic delivery of β-Gal into Hela, LX-2, and Huh-7 cells, respectively.** Scale bar = 25 μm.

**Table S1. Sequences of sgRNA used in this study.**

| Nucleic Acid ID | Sequences (5'-3') | Notes |
|---|---|---|
| sgPUMA-1-F | GAAATTAATACGACTCACTATAGGGGGCACTCACCGTCCG GGCGGTTTTAGAGCTAGAAATAGCA | T7 Promoter |
| sgPUMA-2-F | GAAATTAATACGACTCACTATAGGGCCGCTCGTACTGCGCG TTGGTTTTAGAGCTAGAAATAGCA | T7 Promoter |
| sgPUMA-3-F | GAAATTAATACGACTCACTATAGGGTCGCGGGCTAGACCC TCTAGTTTTAGAGCTAGAAATAGCA | T7 Promoter |
| sgCcnE1-1-F | GAAATTAATACGACTCACTATAGGTTTCACAGTCTTGTCAA TCTGTTTTAGAGCTAGAAATAGCA | T7 Promoter |
| sgCcnE1-2-F | GAAATTAATACGACTCACTATAGGTTTCAGTCCGCTCCAGA AAAGTTTTAGAGCTAGAAATAGCA | T7 Promoter |
| sgCcnE1-3-F | GAAATTAATACGACTCACTATAGGGGATGATAATTCAGCA TGCGGTTTTAGAGCTAGAAATAGCA | T7 Promoter |
| sgCcnE1-4-F | GAAATTAATACGACTCACTATAGGACAATGAGCTTGAATA CCCCGTTTTAGAGCTAGAAATAGCA | T7 Promoter |
| sgKAT5-F | GAAATTAATACGACTCACTATAGGGATTGATGGACGTAAG AACAGTTTTAGAGCTAGAAATAGCA | T7 Promoter |
| sgRNA-R | AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGG ACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC | |

**Table S2. Primer sequences for PCR amplification of target genes.**

| Nucleic Acid ID | Sequences (5'-3') |
|---|---|
| PUMA-1-F | ATCCTGCAGCCTTTGCGAGC |
| PUMA-1-R | TAACAGCCCATCAGGCGAGGGA |
| PUMA-2-F | TGGATGGTGACCACGCCCCTTT |
| PUMA-2-R | AACCGGGCTCTGGGGGTTTCAT |
| PUMA-3-F | AGCACCCCTTCTGCGCTCTT |
| PUMA-3-R | AAGACCACACTGGCCACACCCT |
| CcnE1-1-F | TCCAAGCCCAAGTCCTGAGCCA |
| CcnE1-1-R | TGGCCTGCAGCTCTGTTTTGGG |
| CcnE1-2-F | TCCAAGCCCAAGTCCTGAGCCA |
| CcnE1-2-R | TGGCCTGCAGCTCTGTTTTGGG |
| CcnE1-3-F | ACCACCATGTGGTTGCTGGGA |
| CcnE1-3-R | AGCCGGAACCTCCAAGCTCA |
| CcnE1-4-F | ACCACCATGTGGTTGCTGGGA |
| CcnE1-4-R | AGCCGGAACCTCCAAGCTCA |
| KAT5-F | GCTGCCTTCCCAGCACCCTC |
| KAT5-R | GCCTGCTGCTGGGTACTGCC |