Small extracellular vesicle-mediated delivery of CRISPR-Cas9 ribonucleoproteins for heart-specific genome editing

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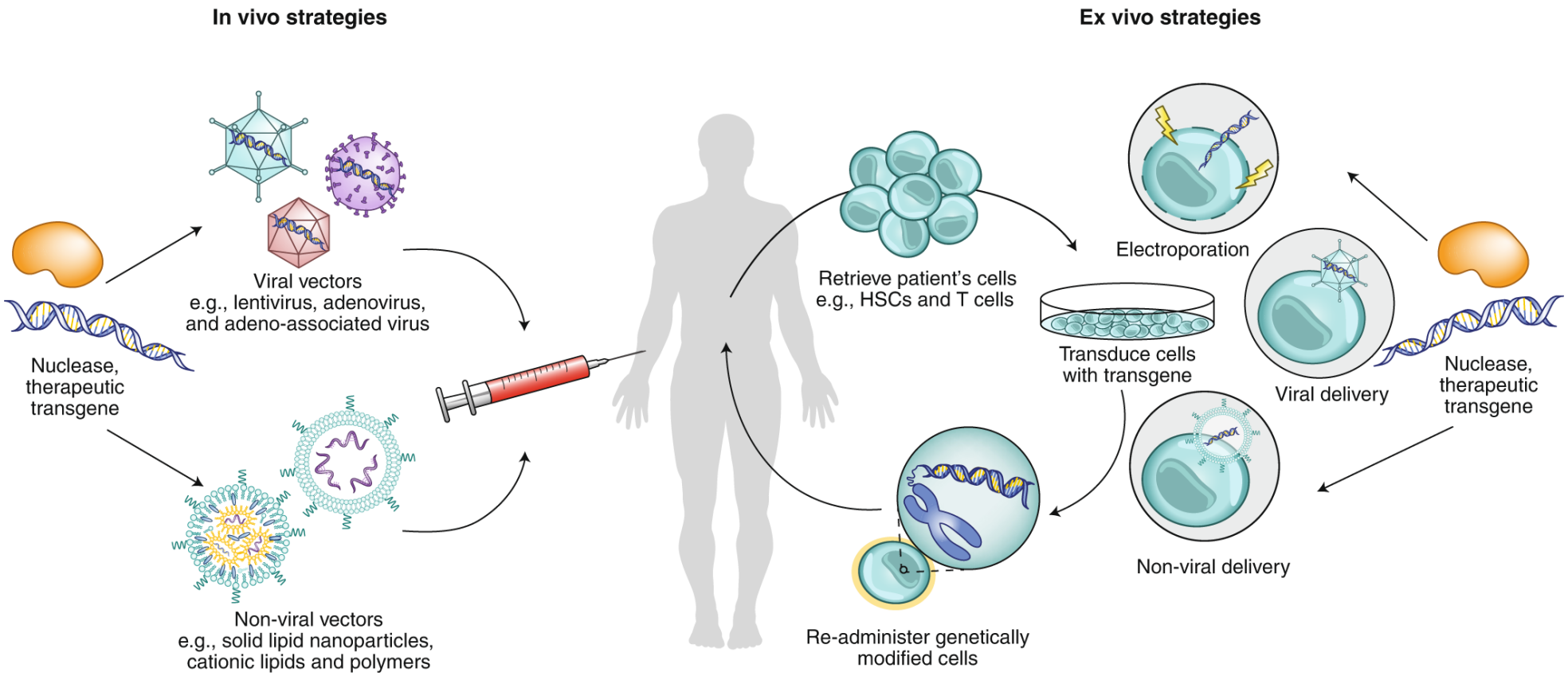
COI Disclosure

The authors have no financial conflicts of interest
to disclose concerning the presentation

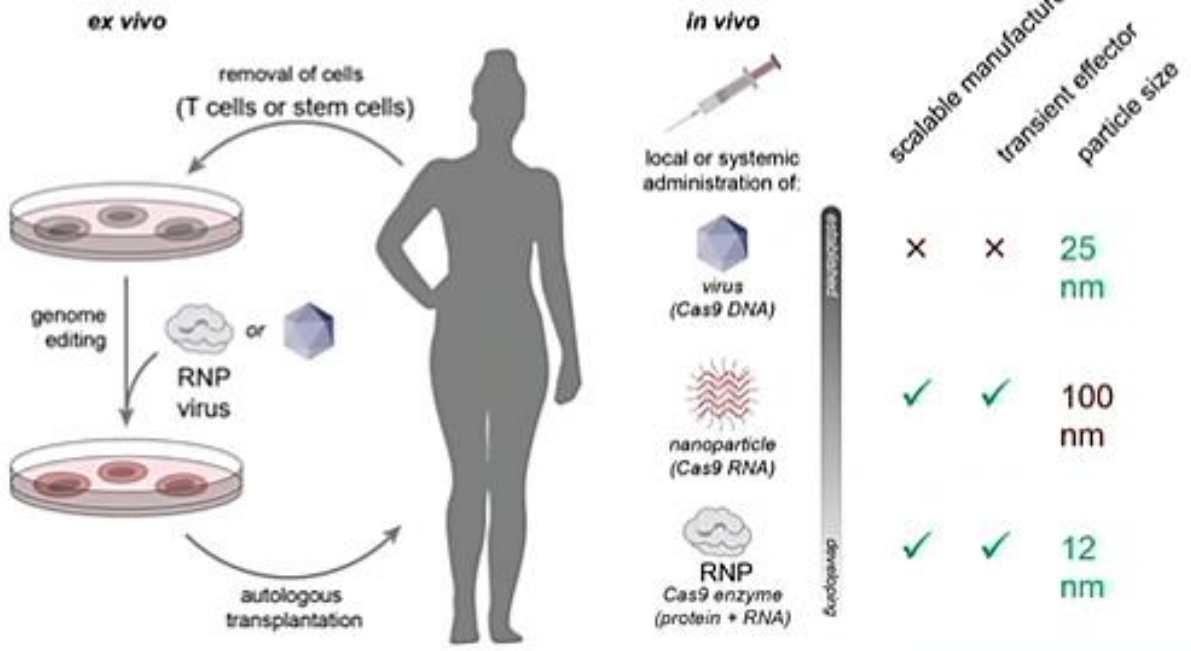
Overview of therapeutic genome editing strategies

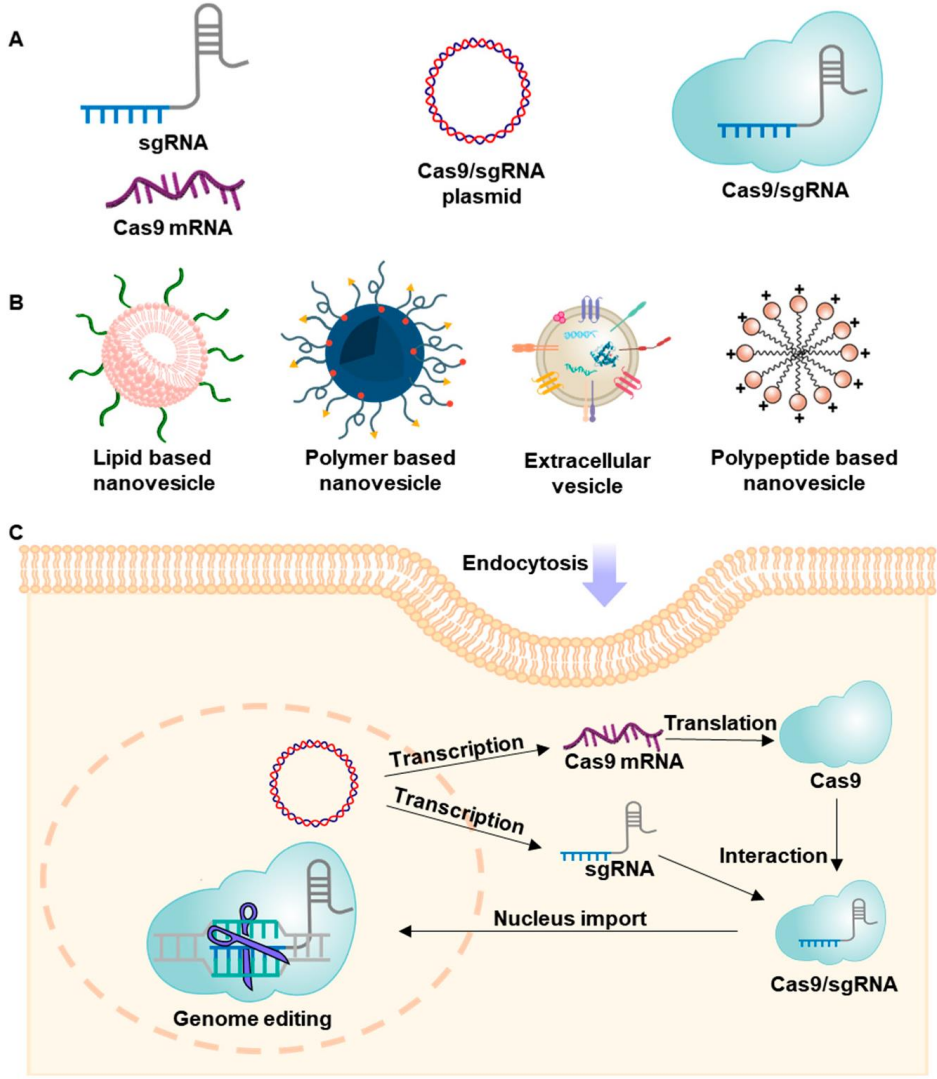


Joost van Haasteren. et al. *Nature biotechnology.* (2022)

- Genome editing can be categorized into two types based on where the editing is performed: in vivo and ex vivo.
- For ex vivo editing therapy, cells are isolated from the patient to be treated, edited, and re-engrafted into the patient.
- For in vivo editing therapy, the tool is delivered by viral or non-viral and injected directly into the patients, but the potential for off-target mutations is a major concern.

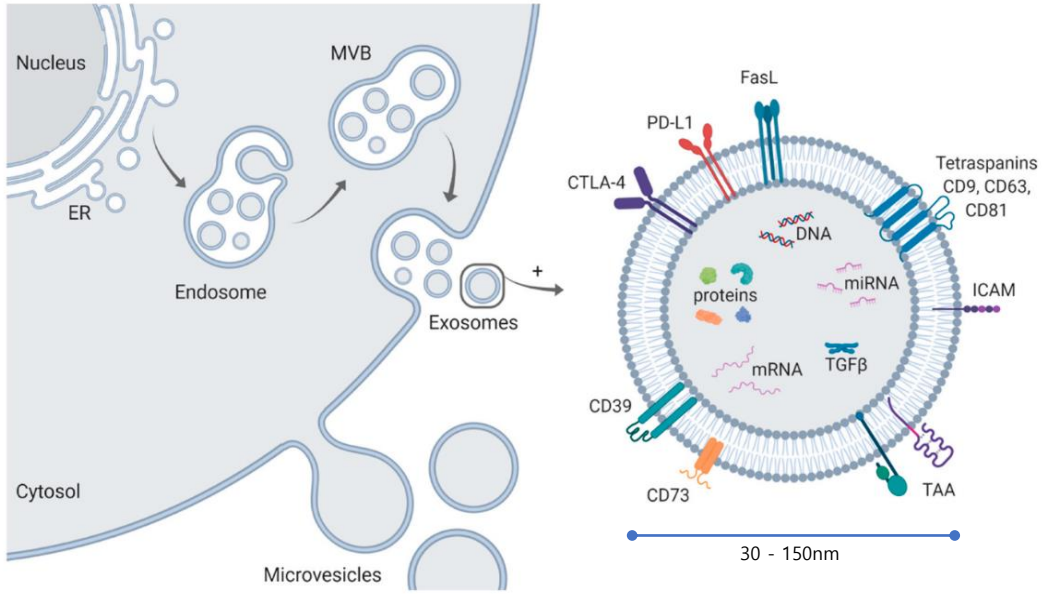
Therapeutic genome editing is limited by delivery options



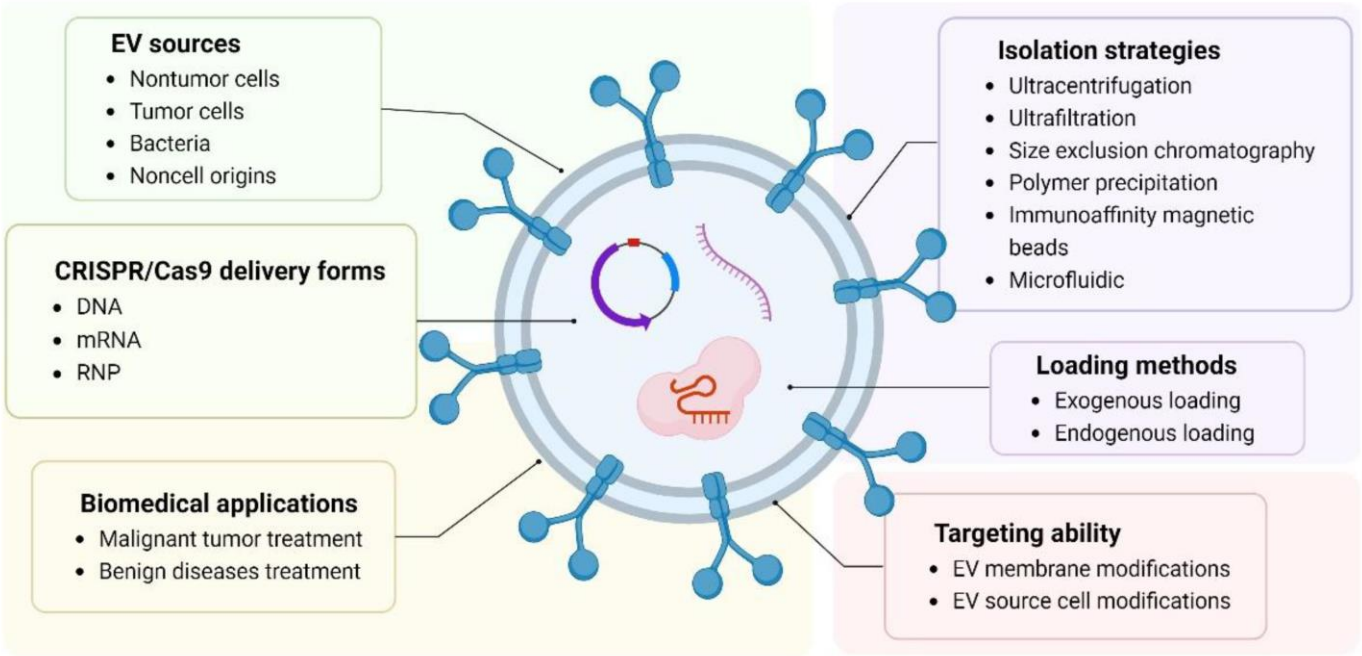


- Therapeutic genome editing holds great promise, but it lacks an efficient in vivo delivery system and faces limitations in loading capacity.
- Despite the advancements in CRISPR-Cas9 systems, their application of CRISPR/Cas9 gene-editing technologies in cardiac diseases poses challenges.

Small Extracellular Vesicle (Exosome)



The CRISPR/Cas9 System Delivered by Extracellular Vesicles

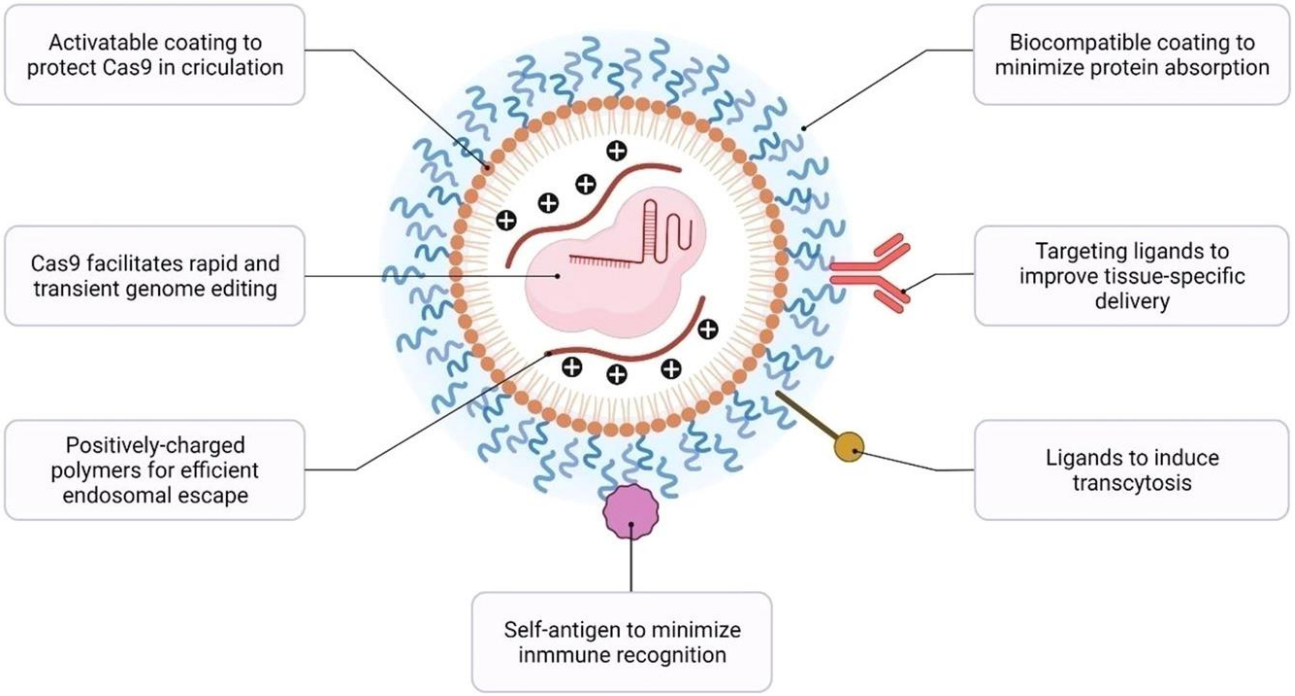


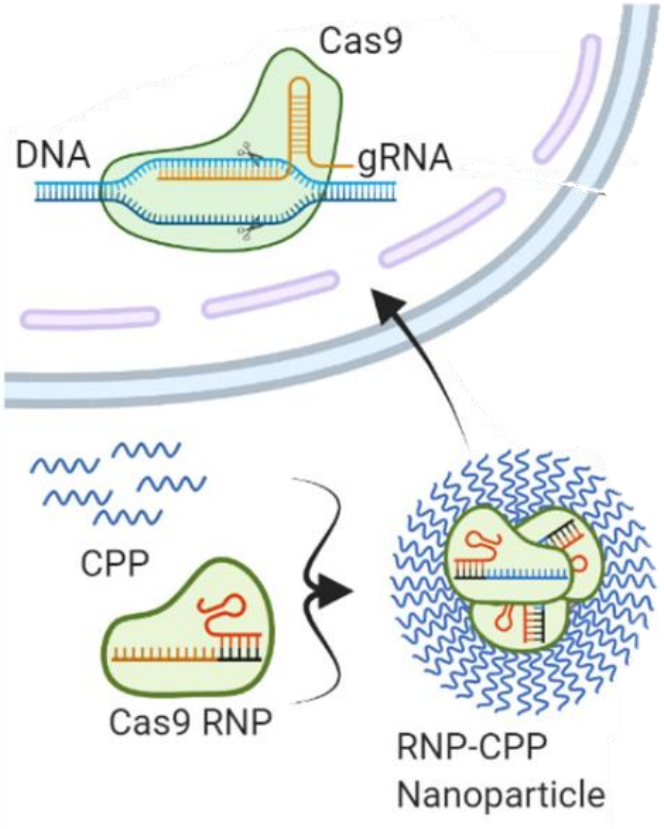
- Small extracellular vesicles (sEVs), especially exosomes, are nanosized membrane vesicles that play an essential role in cell-cell communications and biological functions.
- Therefore, there is emerging research on nanovesicle-mediated delivery systems for CRISPR/Cas9 genome editing.

Linda Hofmann et al. *Int. J. Mol. Sci.* (2020)

Xinglong Zhu et al. *Pharmaceutics*. (2023)

CRISPR/Cas9 Genome Editing for Tissue-specific In Vivo Targeting





- Cell-penetrating peptides (CPPs) are small peptides capable of crossing cellular membrnases and can be enhanced with target ligands for tissue-specific In vivo targeting.
- The ability of CPPs to transduce a wide variety of tissue types in vivo, leading to higher chances of off-target adverse side effects.
- Due to their unique characteristics, CPPs represent a promising strategy to deliver CRISPR/Cas9 systems into tissue-specific invivo targeting

Amit Khurana et al. *J. Cell Biochem.* (2022)

Oskar Gustafsson et al. *Pharmaceutics.* (2021)

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

SCIENCE ADVANCES | RESEARCH ARTICLE

HEALTH AND MEDICINE

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

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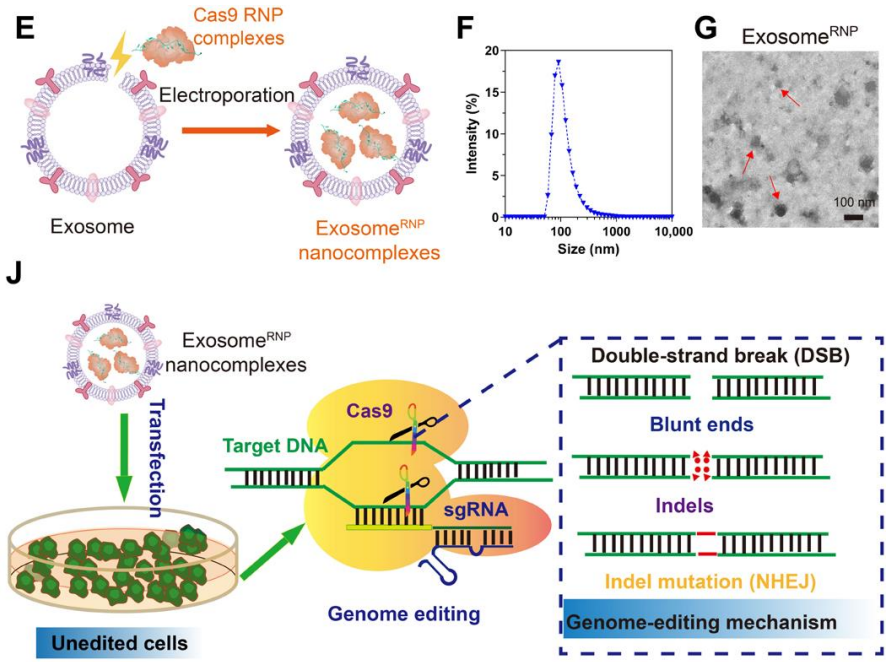
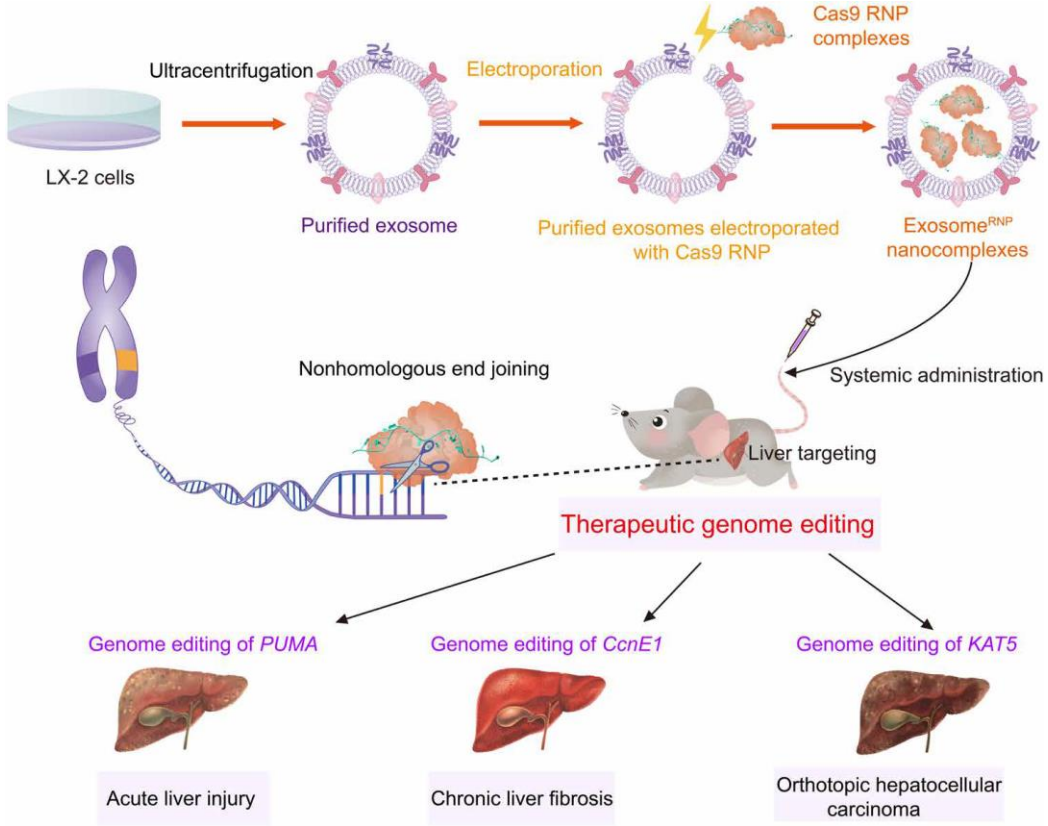
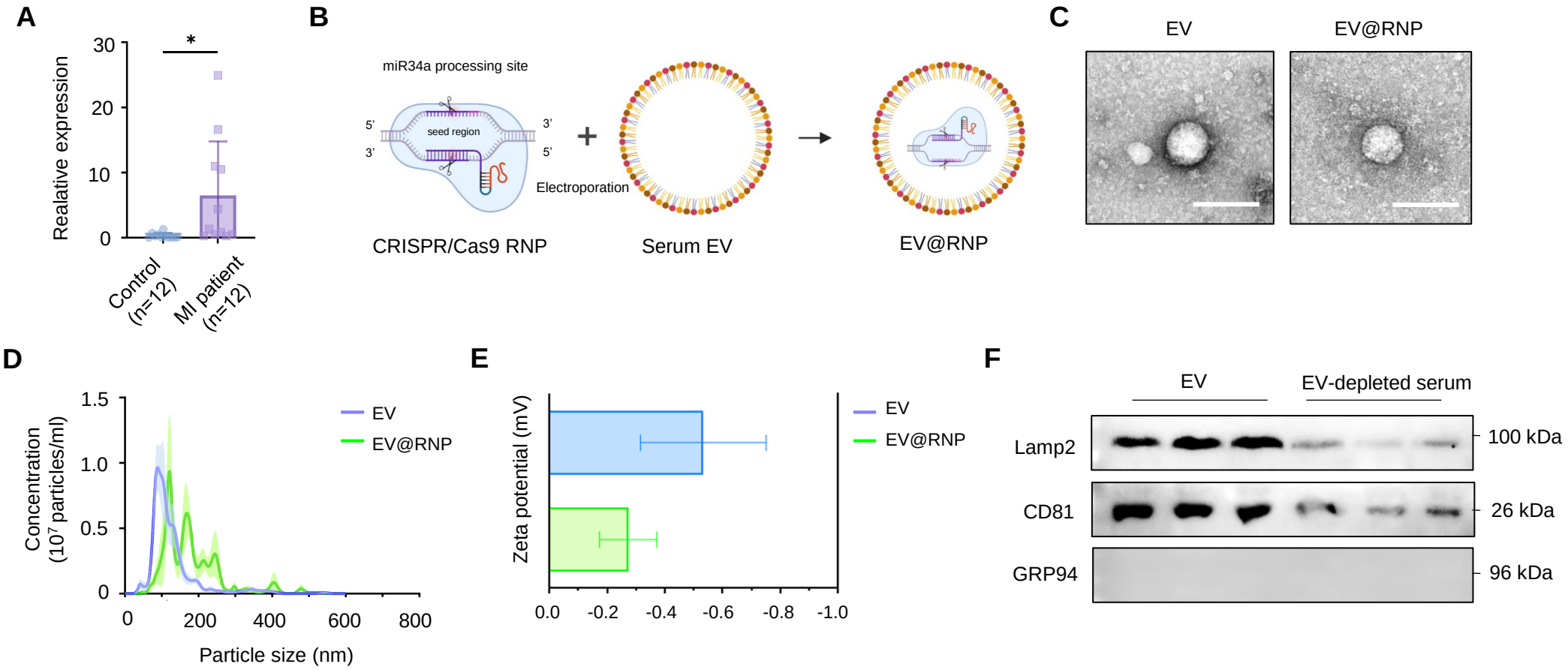




Tao Wan. et al. *Science advances.* (2022)

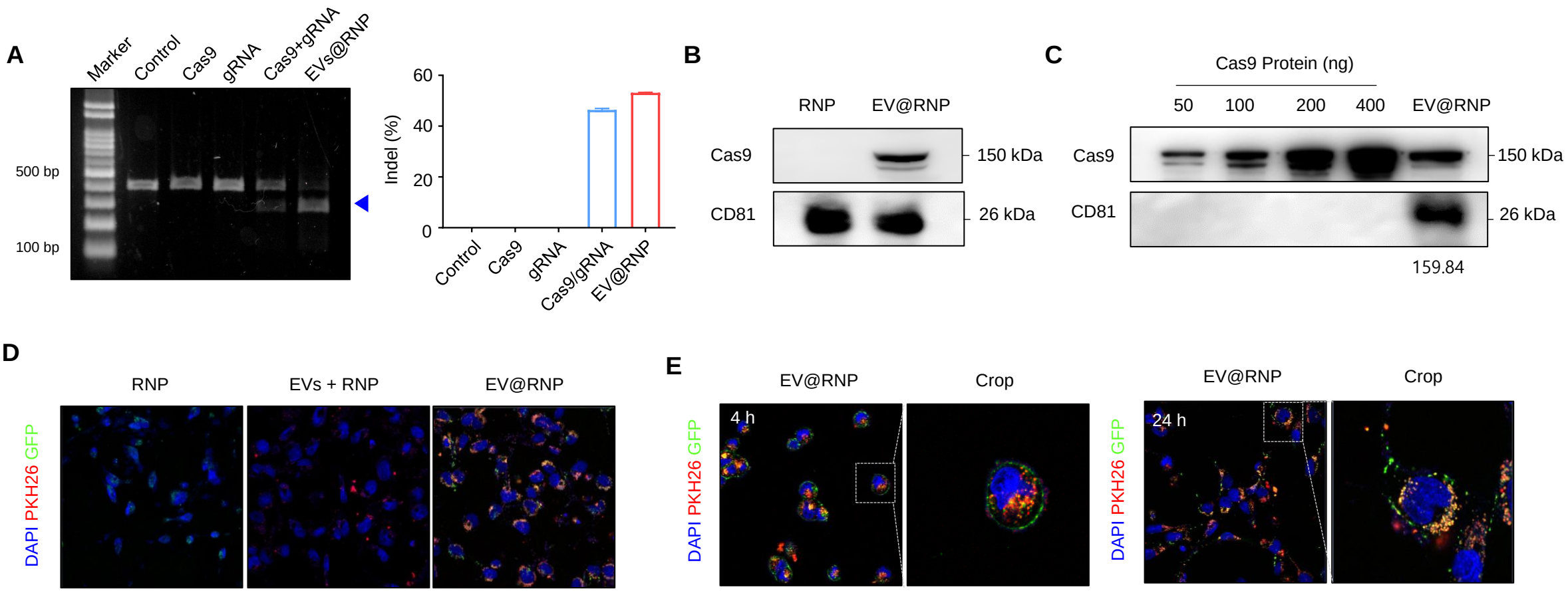
- Recently, exosome-mediate delivery of CRISPR/Cas9 ribonucleoprotein (RNP) complexes for tissue-specific gene therapy in liver diseases; however, the application of CRISPR/Cas9 gene-editing technologies in cardiac diseases still needs to be explored.
- Herein, we present a small extracellular vesicle (EVs)-mediated Cas9/sgRNA RNP complexes for cardiac specific gene therapy.

Characterization of purified EV and EV@RNP complexes



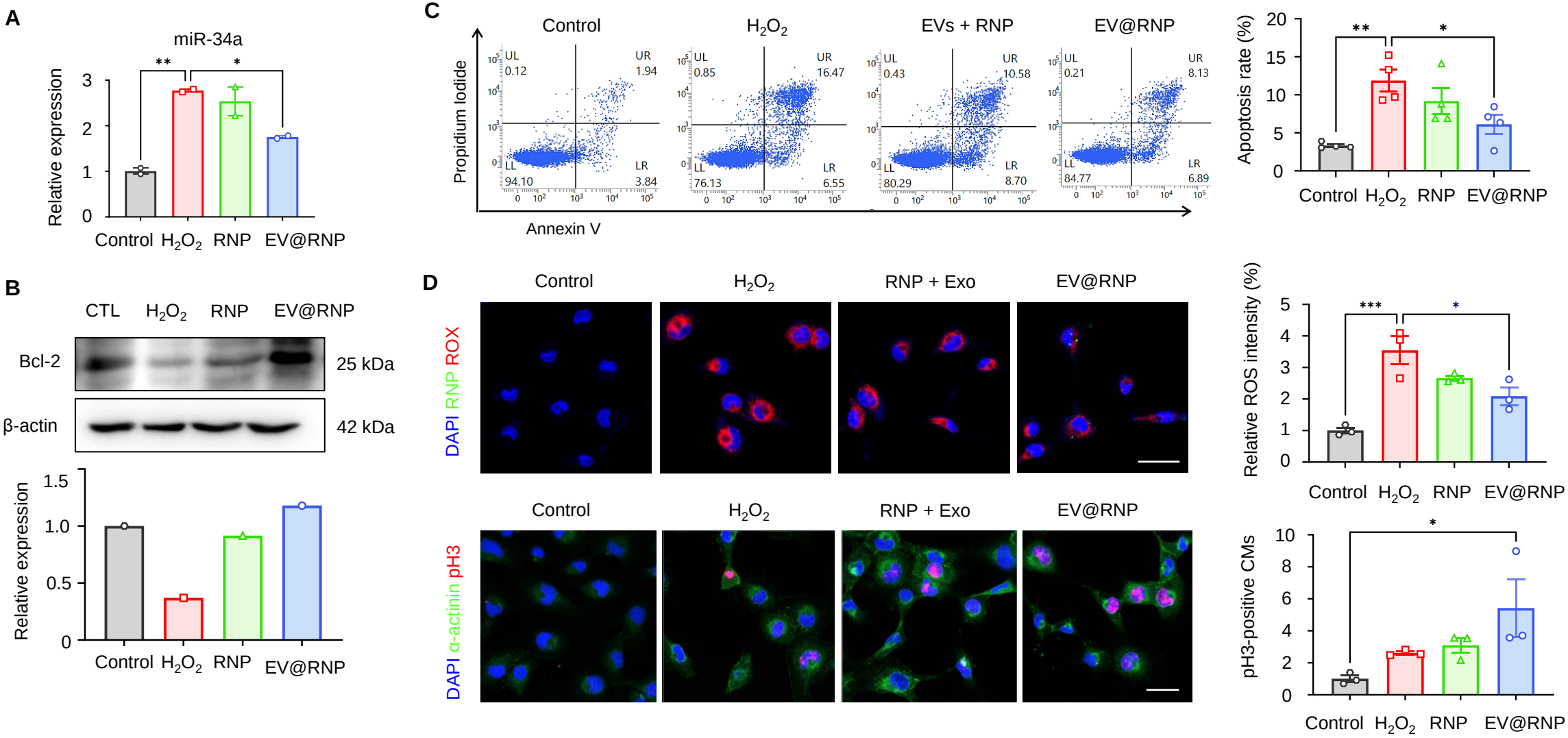
- We loaded EVs with miR-34a sgRNA as a therapeutic agent, which was identified as a molecular target for the treatment of myocardial infarction.
- We detected typical EV's shape, size, zeta poteintial and protein markers indicated that EVs were successfully isolated.

Genome-editing activity and cellular uptake of EV@RNP



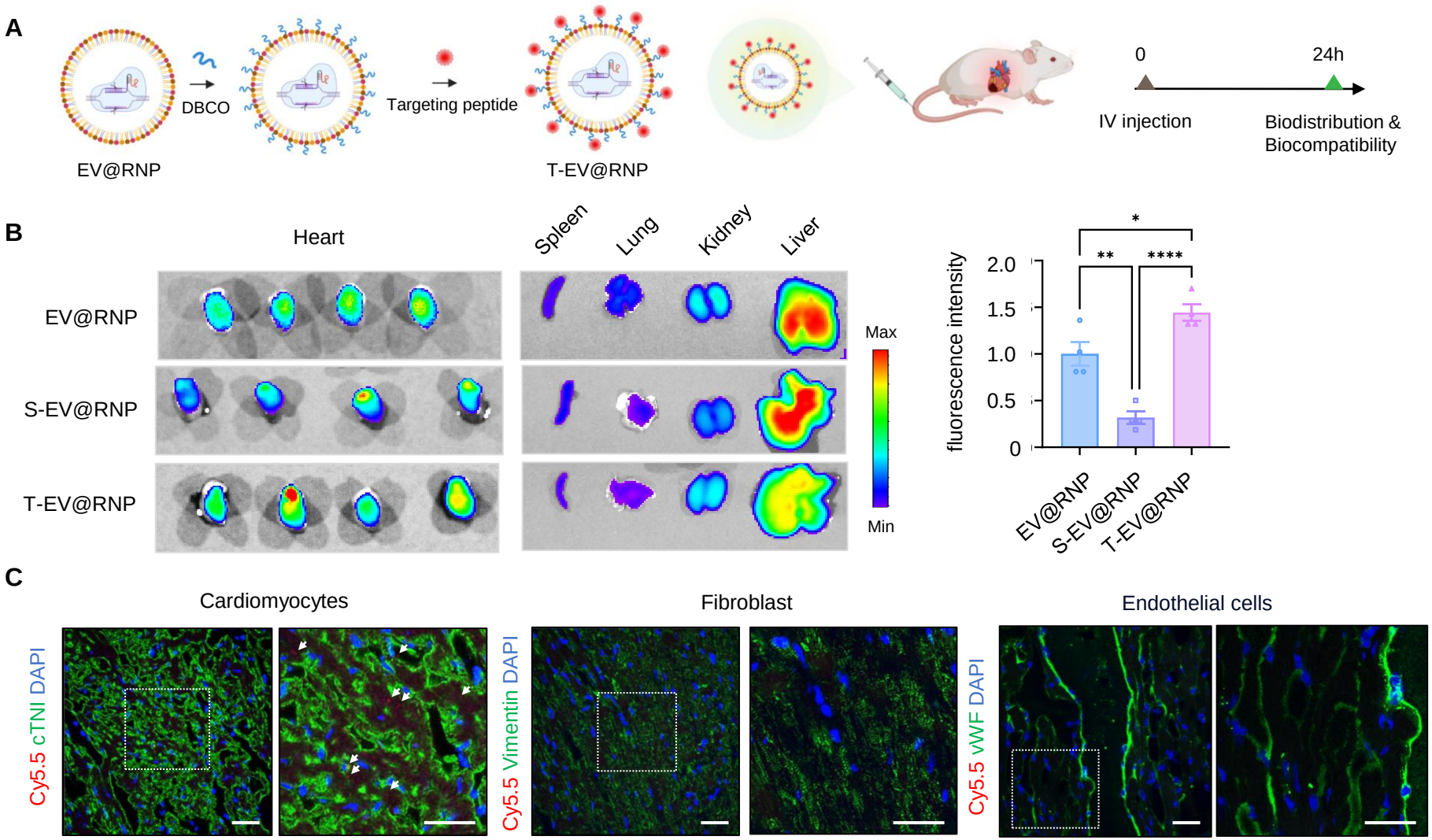
- After loading the RNPs, we confirmed the genome editing activity in the genomic locus targeted by EV@RNP.
- EV@RNP labeled with red fluorescence were successfully internalized into human cardiomyocytes.

EV@RNP protect cardiomyocytes against H_2O_2-induced cardiac injury



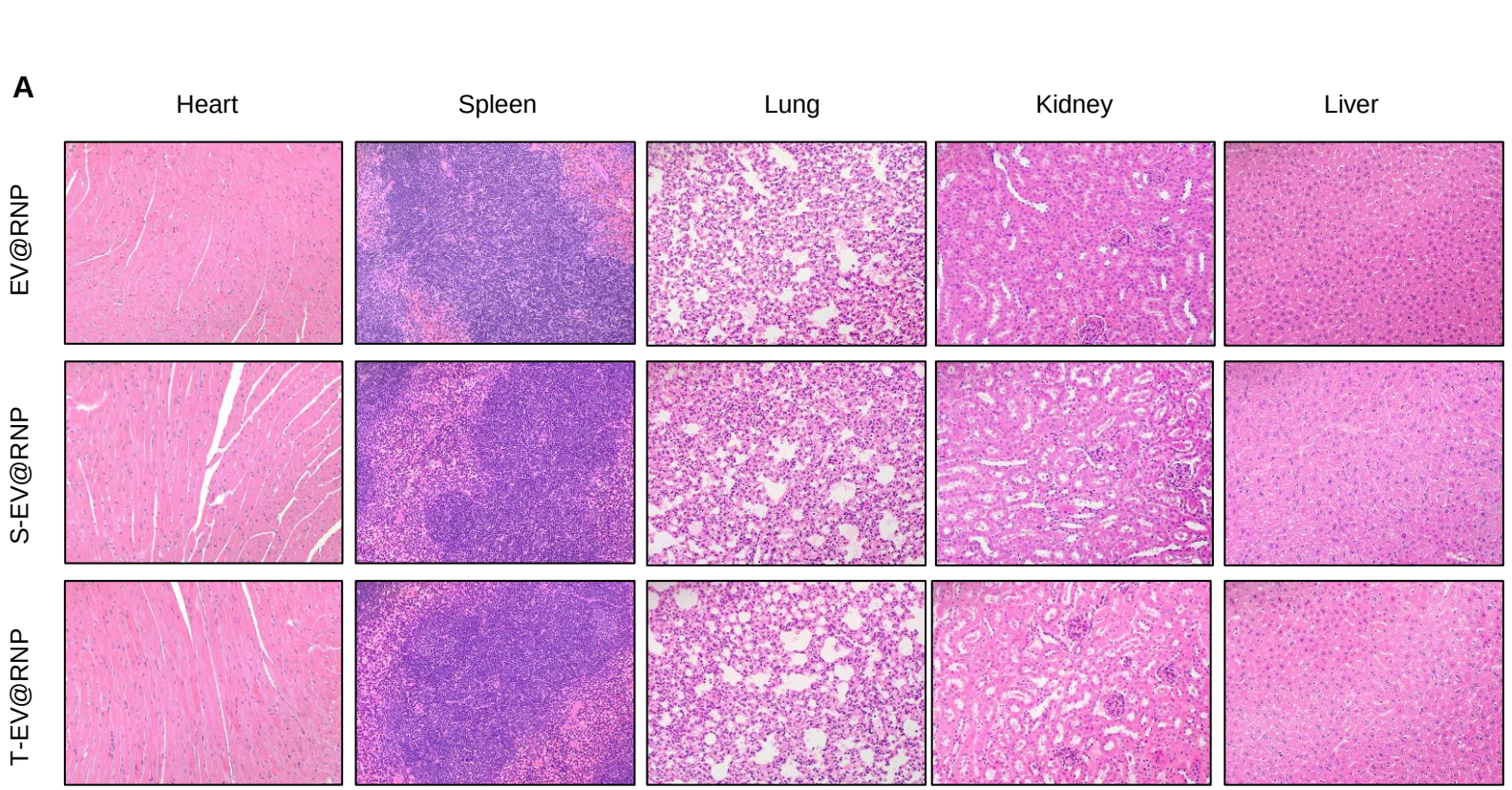
- EV@RNP targeting miR34a led to reducing in miR-34a expression by EV@RNP in H_2O_2 treatment cardiomyocytes.
- EV@RNP protect cardiomyocytes against H_2O_2 -induced damage via miR-34a inhibition.

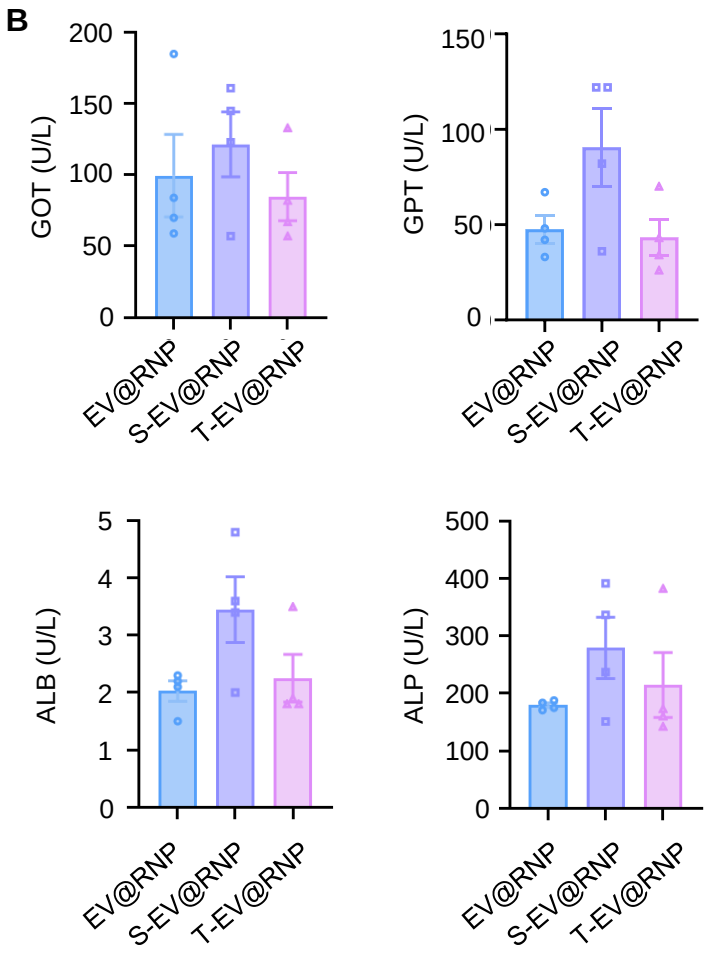
In vivo distribution and cellular localization of T-EV@RNP



- To enhance cardiac targeting, cardiac targeting peptide which was previously identified to target cardiomyocytes modified functional sEVs were loaded as RNPs (T-EVs@RNPs).
- T-EVs@RNP nanoparticles loaded with cardiac targeting peptides were efficiently accumulated in cardiomyocytes.

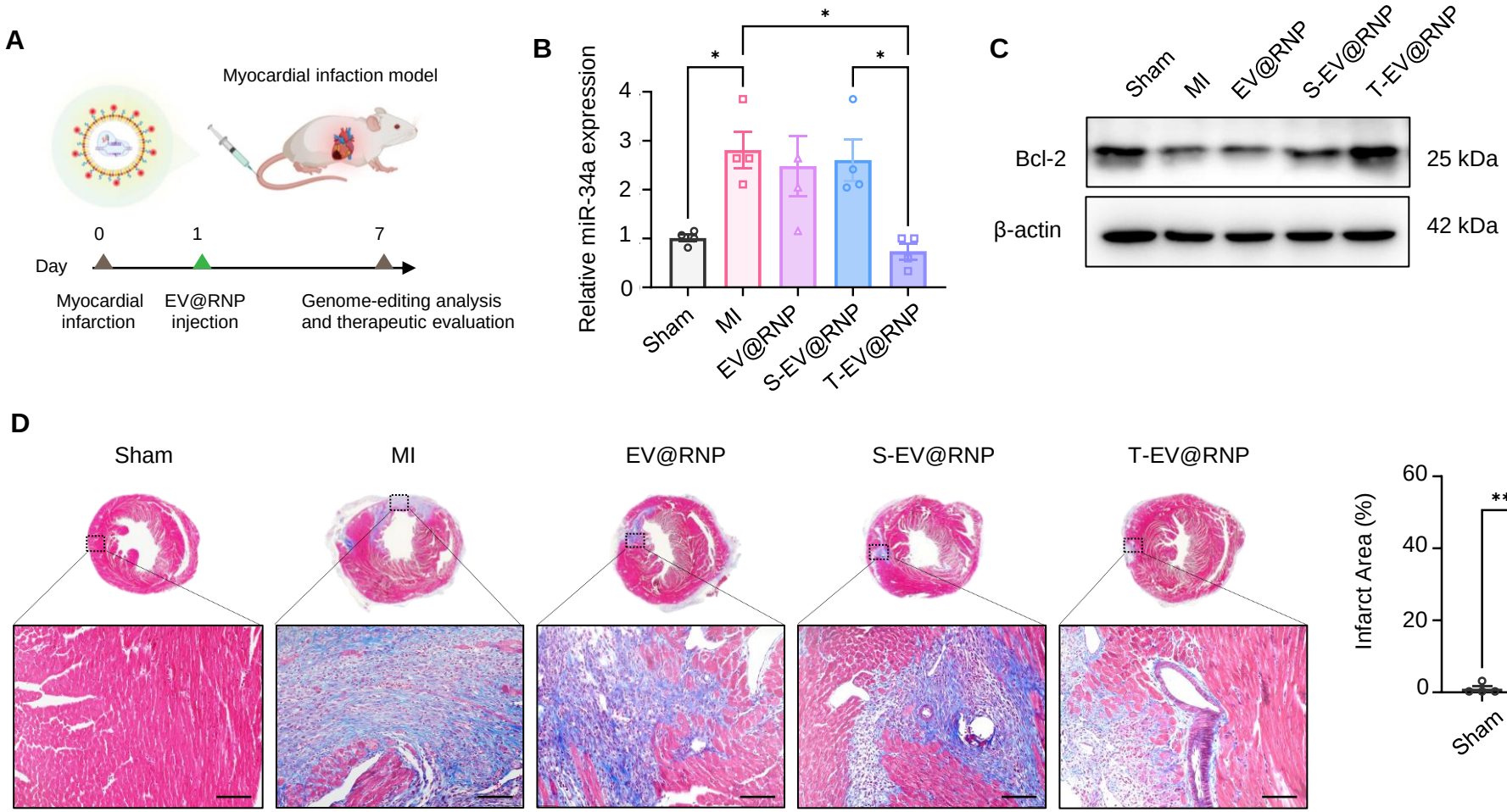
Biocompatibility of T-EV@RNP nanoparticles.





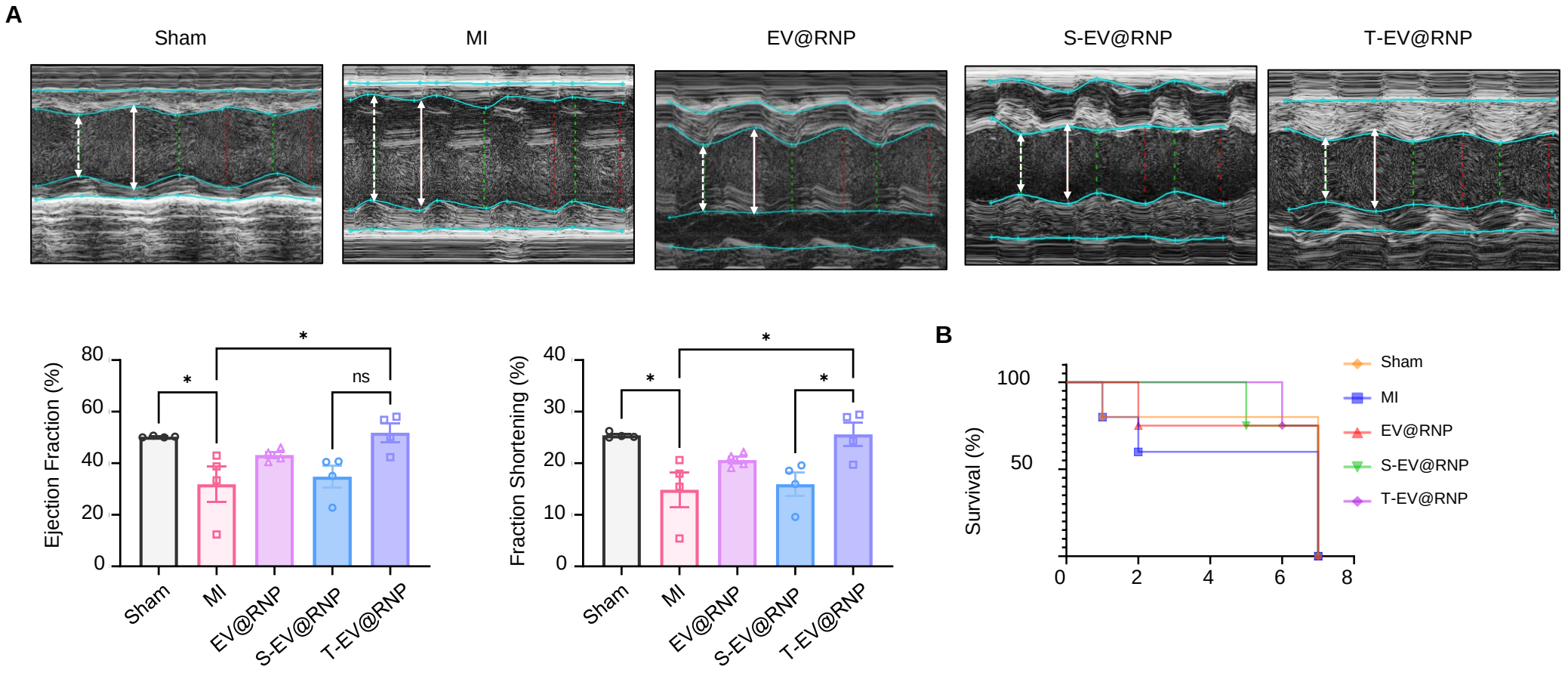
- Histological analysis and blood test parameters indicate that T-EV@RNP was not toxic and had no effect on hematological parameters.

Therapeutic effects of T-EV@RNP against myocardial infarction injury



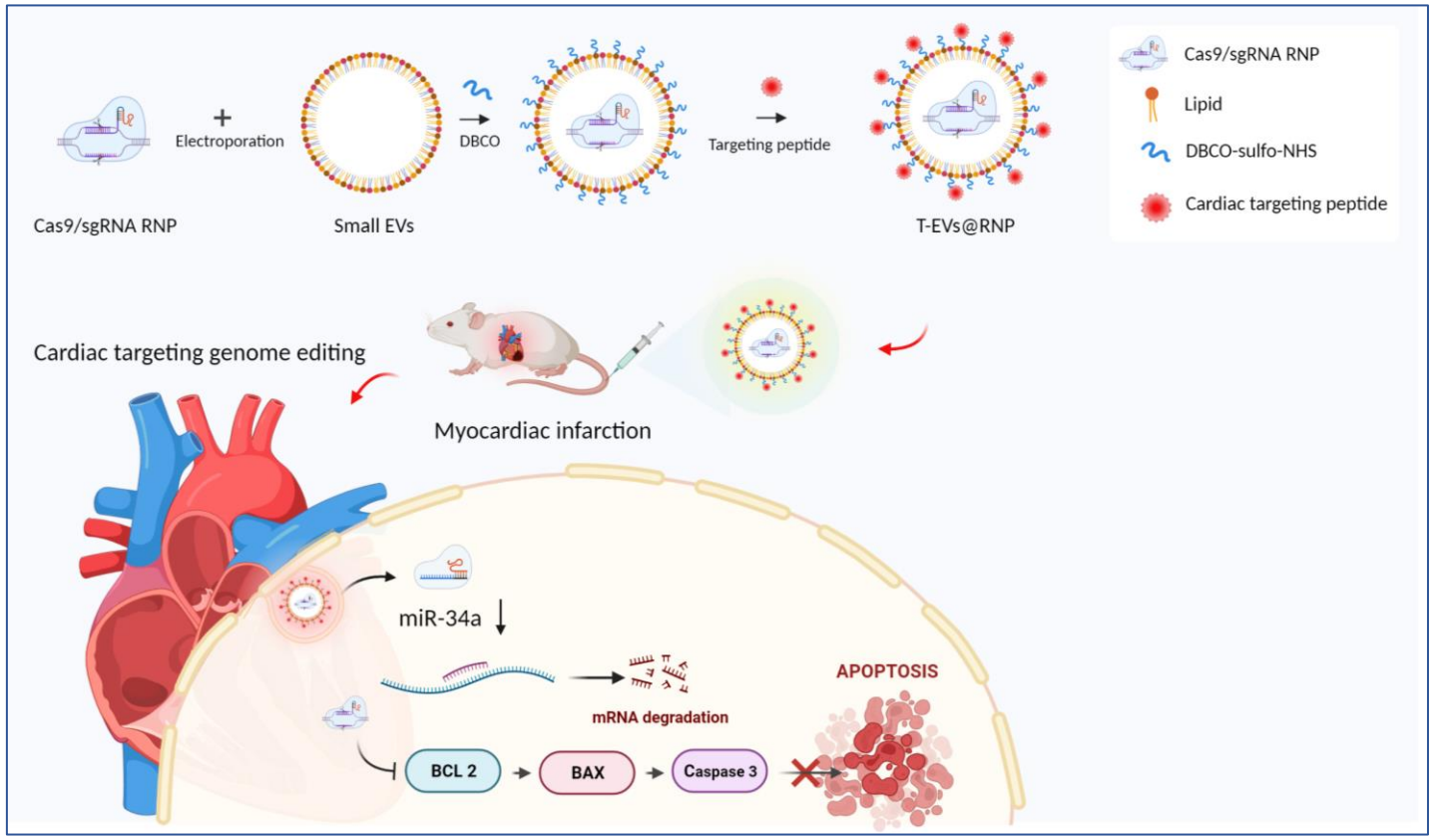
- We observed that miR34a expression was reduced, and their target Bcl2 also was attenuated by T-EV@RNP treatment MI mice.
- Masson trichrome staining confirmed that the infarct region was reduced in T-EV@RNP-treated MI mice.

Therapeutic effects of T-EV@RNP against myocardial infarction injury



- T-EV@RNP-treated mice significantly attenuated ejection fraction (EF) and fractional shortening (FS) compared to MI mice, indicating functional recovery from MI injury.

Summary



- The developed genome-editing delivery system by loading CRISPR/Cas9 RNPs with EVs provides a viable platform for precise and tissue-specific gene therapies for cardiovascular disease.

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NRF National Research Foundation of Korea

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과학기술정보통신부 Ministry of Science and ICT

Small extracellular vesicle-mediated delivery of CRISPR-Cas9 ribonucleoproteins for heart-specific genome editing.

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Objectives

CRISPR-Cas9 gene editing promises to revolutionize genetic manipulation, but the lack of in vivo delivery systems. Here, we developed a cardiac-targeted small extracellular vesicle (EVs)-based nanomedicine by fabricating Cas9/sgRNA ribonucleoprotein (RNP)-loaded EV nanoparticles decorated cardiac-targeted peptides for cardiac-specific genome editing.

Materials and Methods

EVs were isolated from human peripheral blood by using ultracentrifugation. Cas9 proteins were mixed with sgRNA from RNP complexes. RNP complexes were added to EVs at a weight ratio of 1:5, and then the mixture was electroporated. To enhance cardiac targeting, Dibenzocyclooctyne-sulfo-N-hydroxysuccinimidyl ester (DBCO-sulfo-NHS) was added to EVs and allowed to react on a rotating mixer. Cardiac targeting peptide (T), scrambled peptide and Cy5.5 with an azide were added to DBCO-EVs in PBS on a rotating mixer at 4 ℃ for 12 h. The T-EVs@RNP were IV injected into the MI mouse model.

Results

We loaded EVs with miR-34a sgRNA as a therapeutic agent, which was identified as a molecular target for the treatment of myocardial infarction. Characterization, biodistribution, and cellular uptake mechanism of EVs were not altered after loading the RNPs. Then, a surveyor mismatch detection assay confirmed the genome editing activity in the genomic locus targeted by EVs@RNP. Next, gene editing of EVs@RNP attenuates the H_2O_2-induced apoptosis of cardiomyocytes. To enhance cardiac targeting, we loaded cardiac targeting peptide-modified functional sEVs with RNP (T-EVs@RNP) using click chemistry. Cardiac-specific delivery of T-EVs@RNP showed a protective effect attenuating apoptosis to ameliorate myocardial infarction injury.

Conclusions

The developed genome-editing delivery system by loading Cas9 RNPs with EVs provides a feasible platform for precise and tissue-specific gene therapies for cardiovascular disease