# Engineered small extracellular vesicle-mediated siRNA delivery for targeted therapy of cardiac hypertrophy

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# **Korean Heart Rhythm Society**
# COI Disclosure

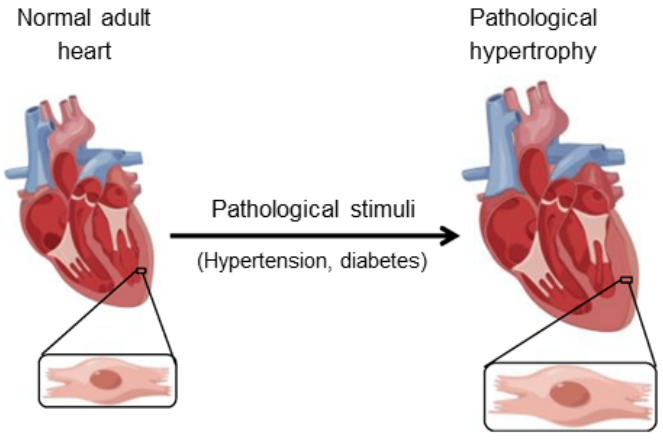
*Ji-Young Kang*

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

# CONTENTS

## [ Small-interfering RNA-based therapy ]

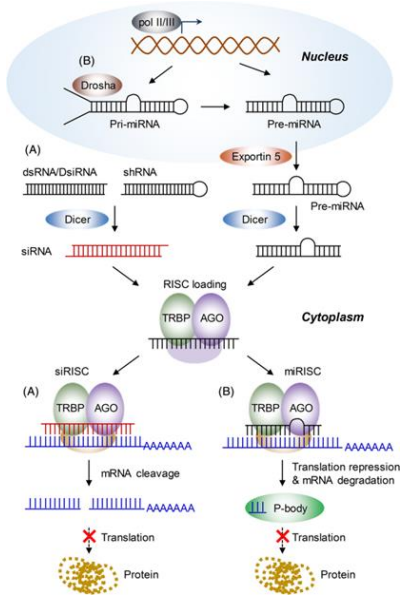
- Cardiac hypertrophy is an independent risk factor for cardiac morbidity and mortality and plays a critical role in the cardiovascular system.
- Small-interfering RNA (siRNA) therapy, which is based on specific post-transcriptional gene silencing, is currently one of the most promising therapeutic platforms for the treatment of cardiovascular diseases, including cardiac hypertrophy.



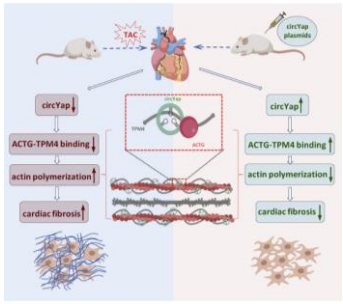

Pan C et al., *Cells* (2022)

✓ Irreversible structural cardiac remodeling

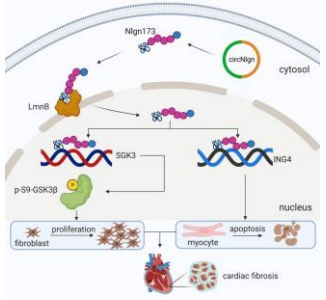
✓ Impairment of cardiac function (eventually resulting in heart failure and arrhythmia)



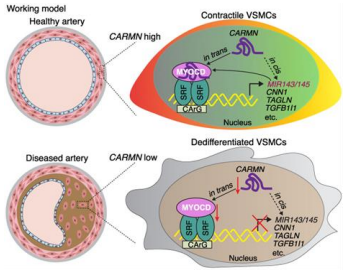

Hu B et al., *J Gene Med* (2019)



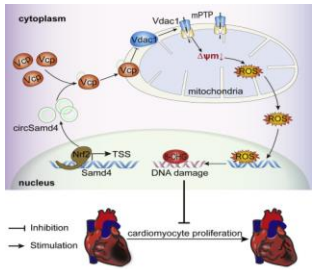

Wu N et al., *Mol Ther* (2021)




Du WW et al., *Circ Res* (2021)

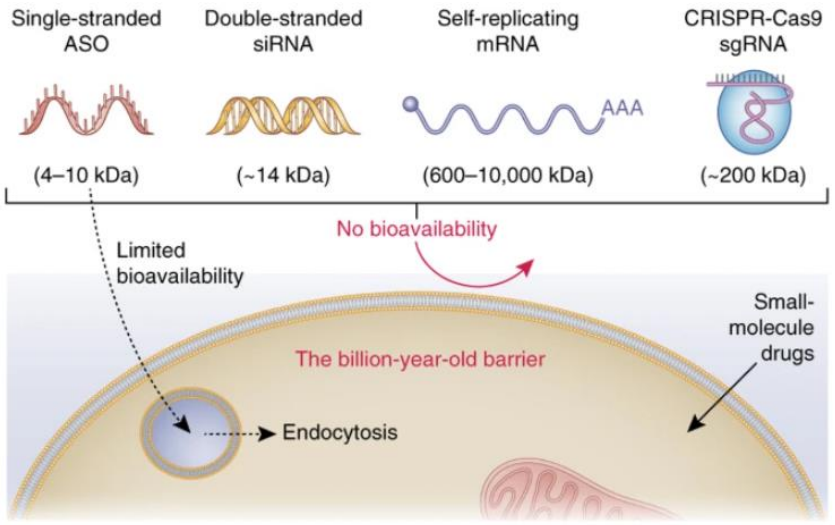



Dong K et al., *Circulation* (2021)




Zheng H et al., *Mol Ther* (2022)

## [ Issues in siRNA delivery ]



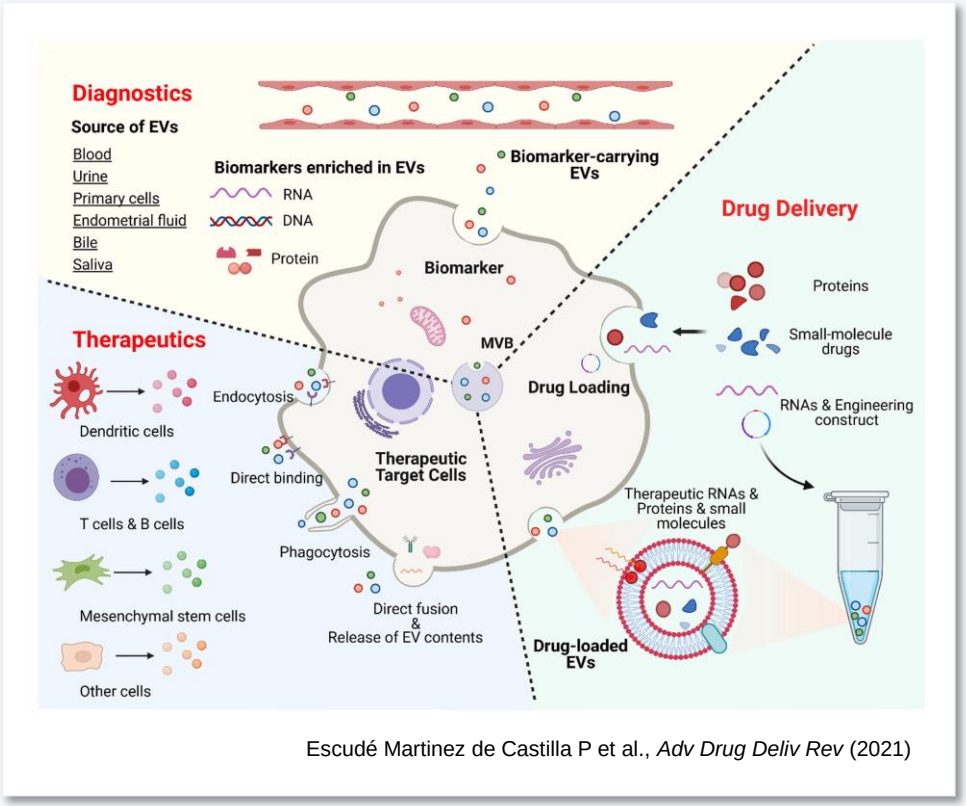

Dowdy S et al., *Nat Biotechnol* (2017)

- Viral vectors, synthetic nanoparticles, and liposomes have been the most commonly studied carriers for siRNA delivery.

Immuno-genicity | Mutagenic toxicity | Poor bio-compatibility | Low efficiency

## [ Small extracellular vesicles ]

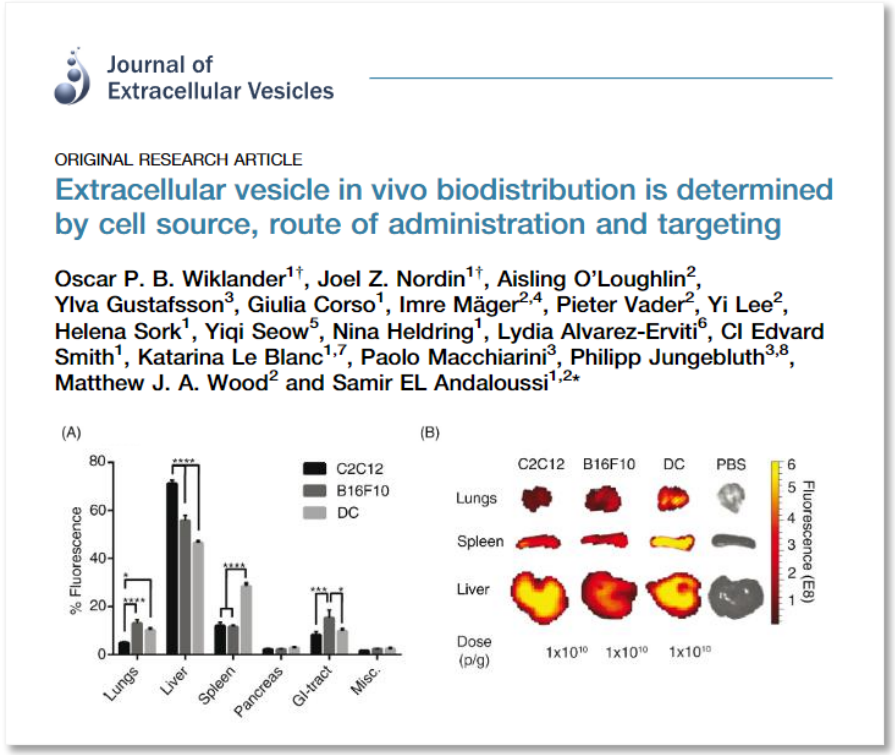
- Small extracellular vesicles (sEVs) with a diameter of 30–200 nm have gained increasing attention as nanocarriers for siRNA delivery.



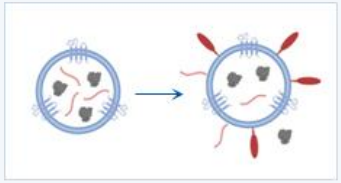

Escudé Martinez de Castilla P et al., *Adv Drug Deliv Rev* (2021)

## [ Limitation of sEV-based delivery carriers ]

- After systemic administration, sEVs are likely to accumulate in non-specific organs, especially in the liver, leading to insufficient delivery to the specific target cell/tissue.

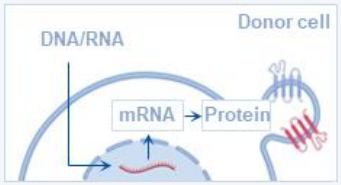



## [ sEVs surface modification methods ]



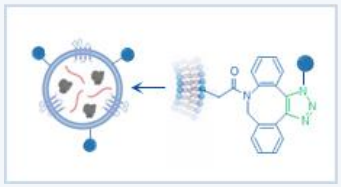
Hydrophobic insertion

- Additional purification process
- Low stability, loss of contents




Cell engineering technique

- Complex, time-consuming
- Cannot be applied to pre-isolated sEVs

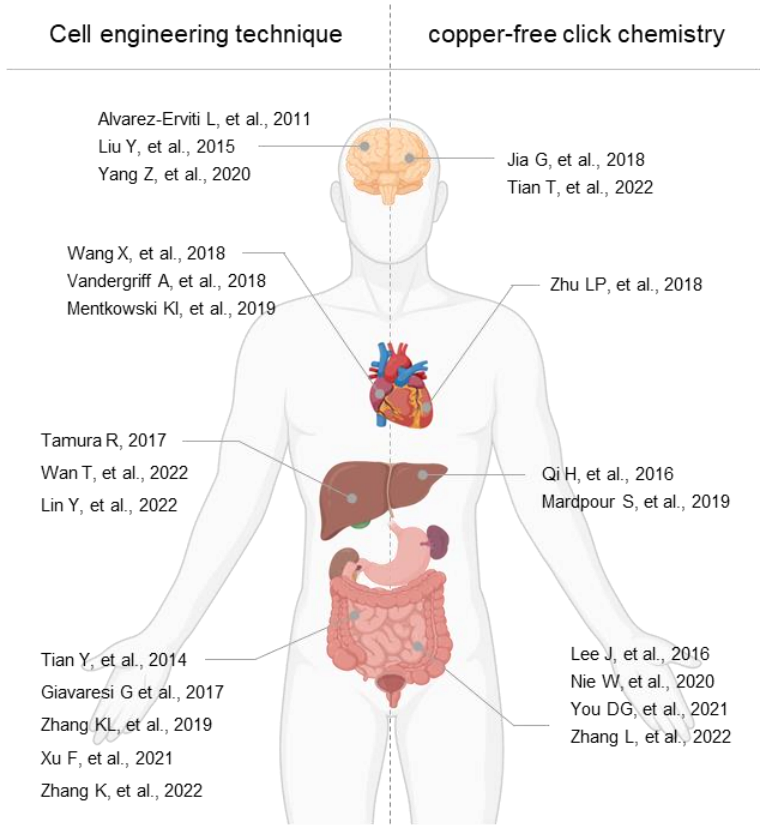


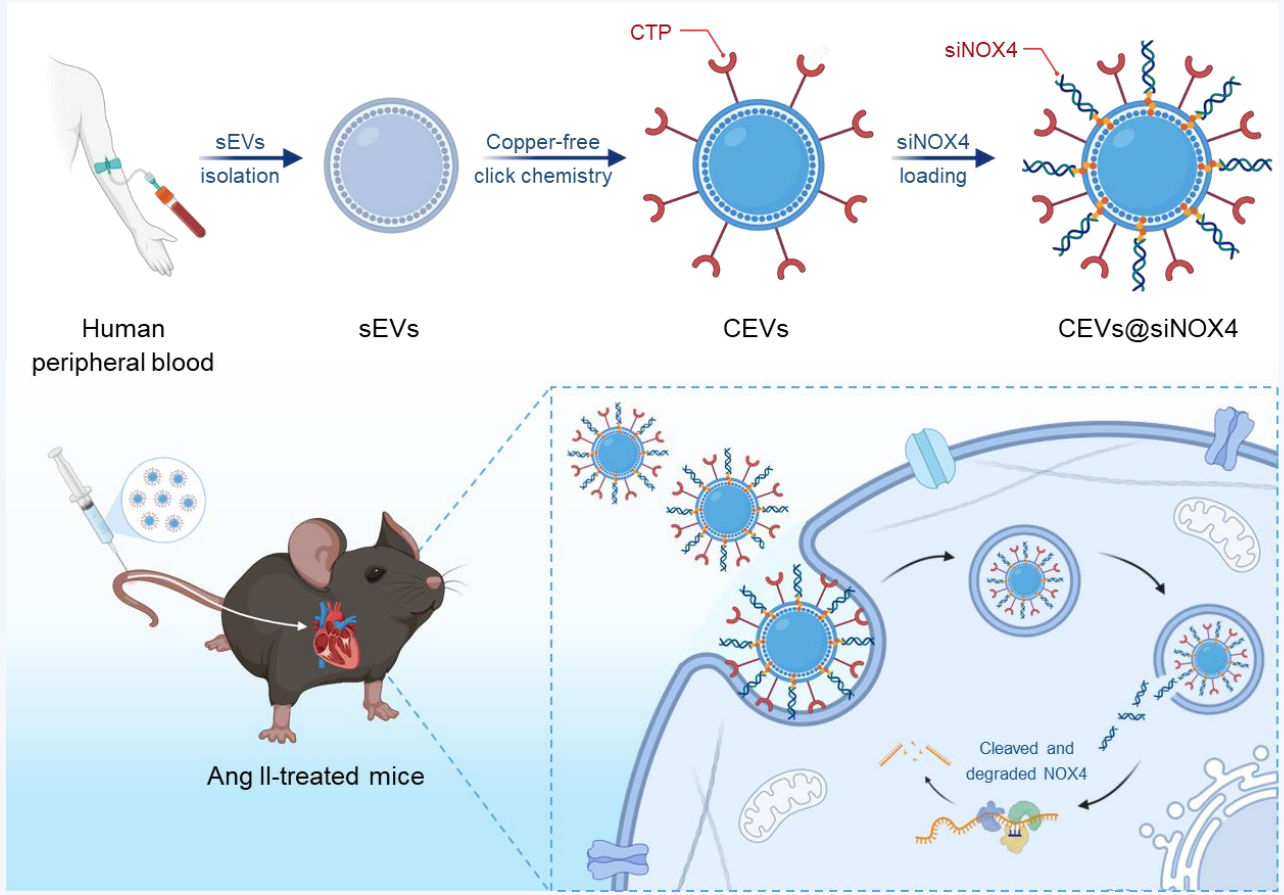
Copper-free click chemistry

- An efficient strategy to simply and rapidly modify the surface of sEVs

# ► Objective

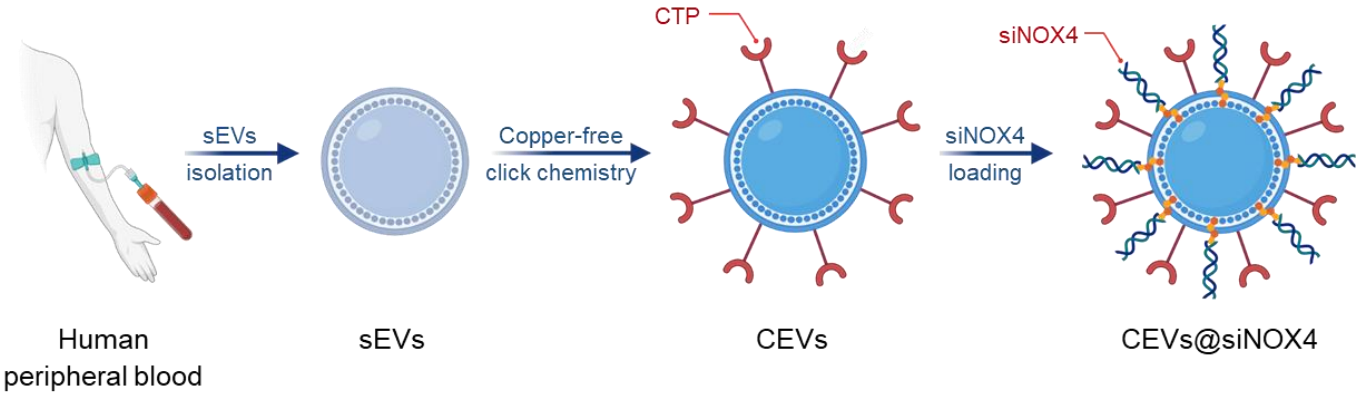
- However, the sEVs currently modified via copper-free click chemistry are mostly designed to treat tumors or cerebrovascular disease.

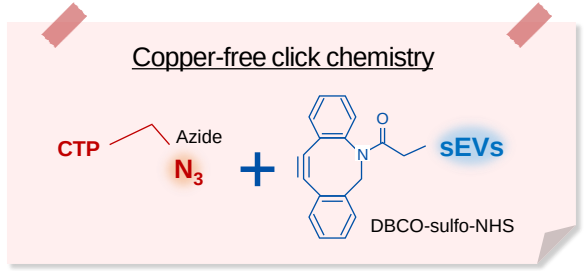



- This study aims to develop a new generation of heart-targeting sEVs (CEVs) by modifying the surface of sEVs using copper-free click chemistry.

## [ Preparation of heart-targeting sEVs ]




* CTP (Cardiac targeting peptide) : a peptide that specifically targets cardiomyocytes

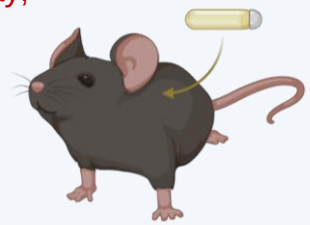
## [ In vitro ]

- The iPSC line CMC-hiPSC-011 was provided by the Korea National Stem Cell Bank, originally provided by Catholic University. Next, we differentiated into ventricular cardiomyocytes (iPSC-vCMs).
- To establish cardiac hypertrophy *in vitro*, iPSC-vCMs were treated with 2.5 μM Ang II for 48 h.

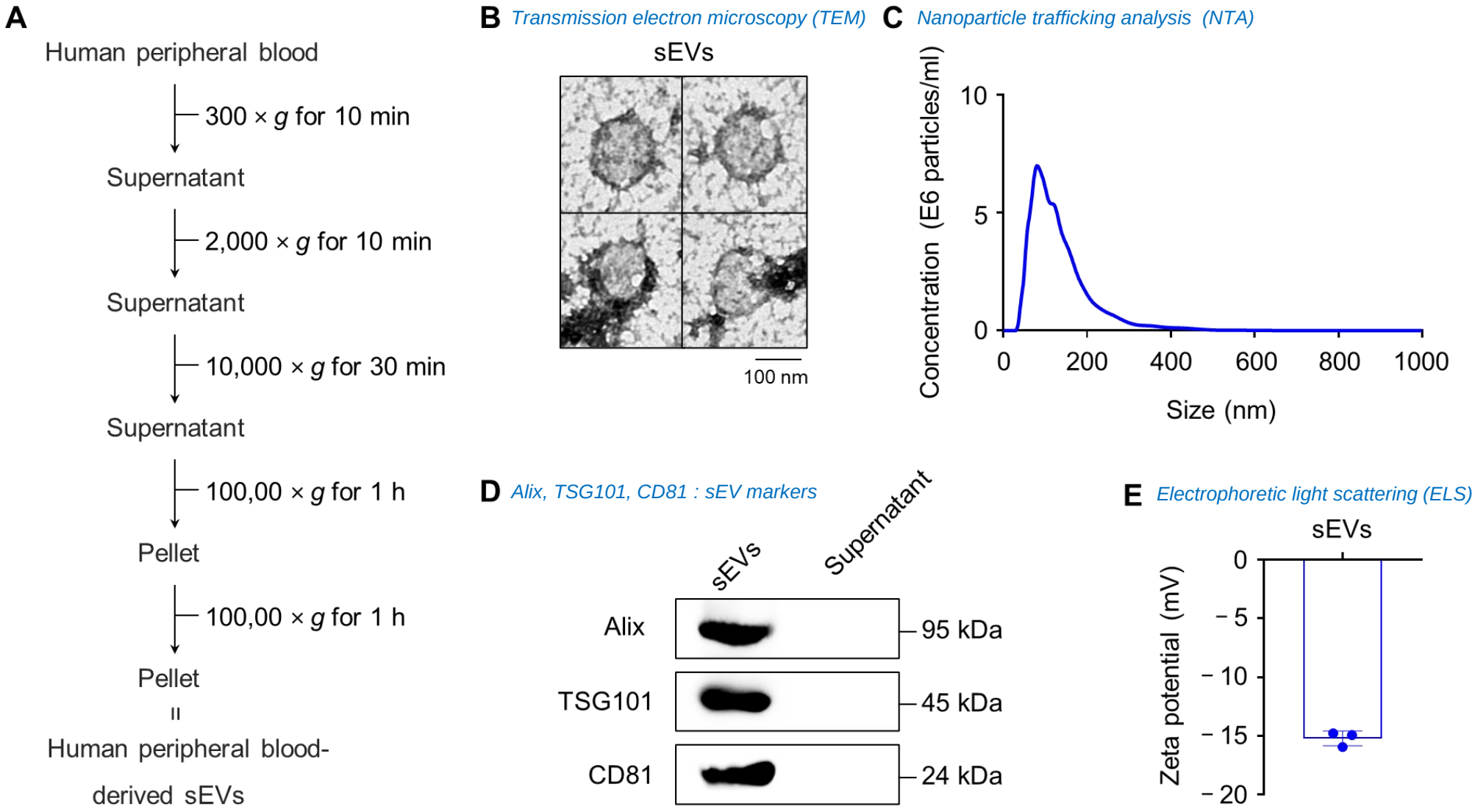
** iPSC = Human induced pluripotent stem cell*

## [ In vivo ]

- The mice were implanted with Ang Ⅱ-containing Alzet® 1002 micro-osmotic pumps (1.5 mg/kg/day; Durect Corp., Cupertino, CA, USA).
- The control mice were implanted with PBS-containing Alzet® 1002 micro-osmotic pumps.

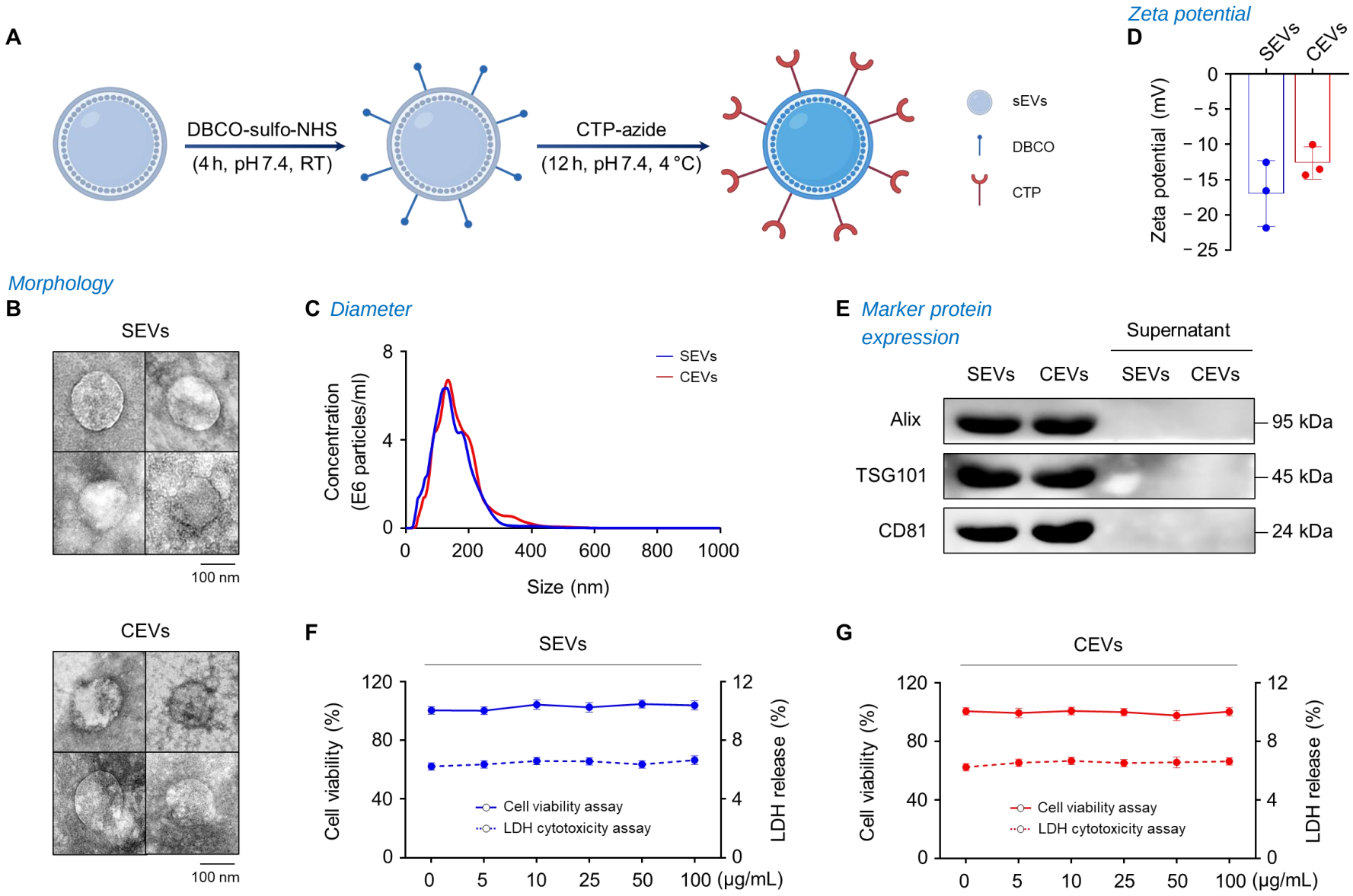


# ► **Results 1.** sEVs were successfully isolated from human peripheral blood.

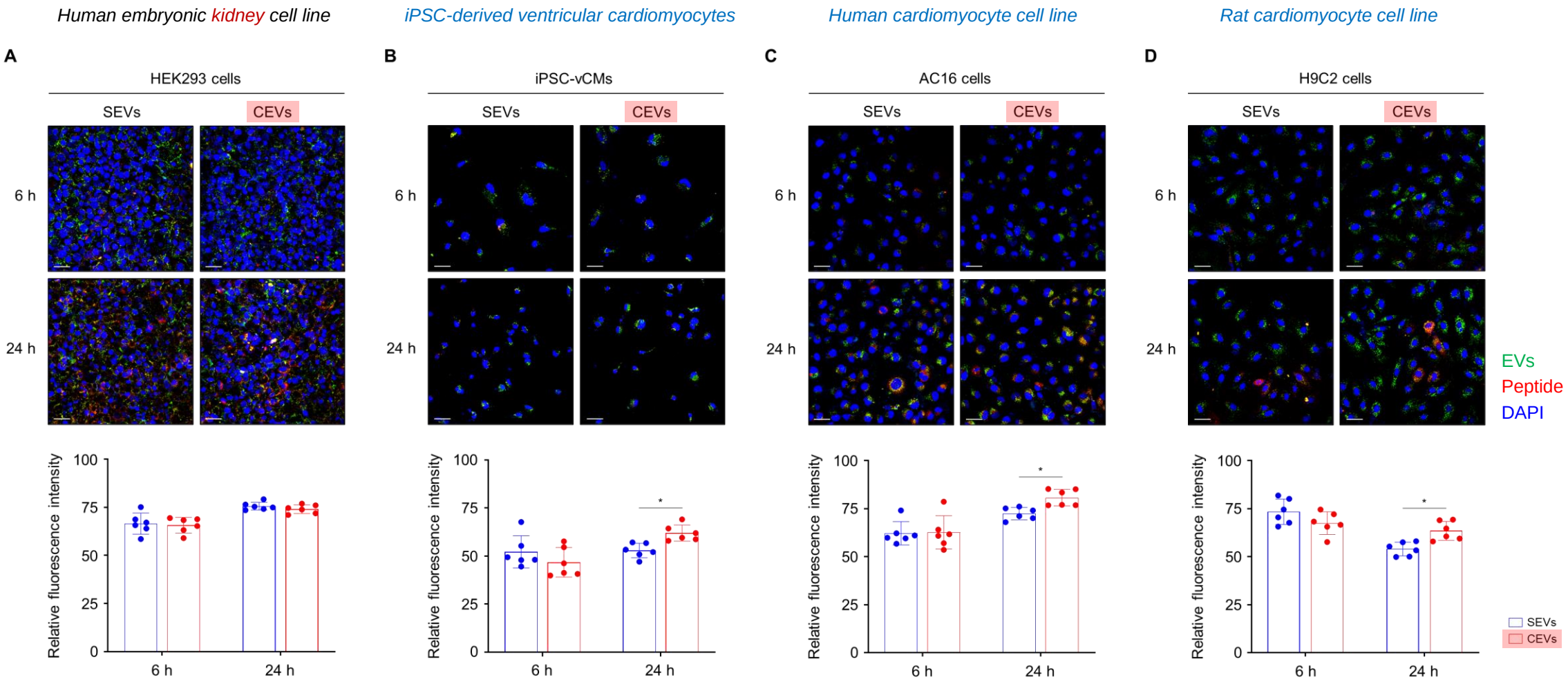


# ► **Results 2.** CEVs were successfully developed using copper-free click chemistry.

* ***CEVs*** *= CTP-modified EVs,* ***SEVs*** *= scrambled peptide (Scr)-modified EVs (as a control)*

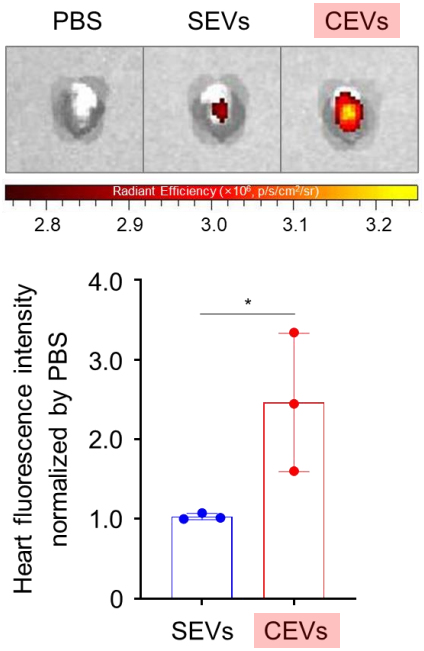


# ► **Results 3.** CEVs exhibited high specificity for cardiomyocytes *in vitro*.

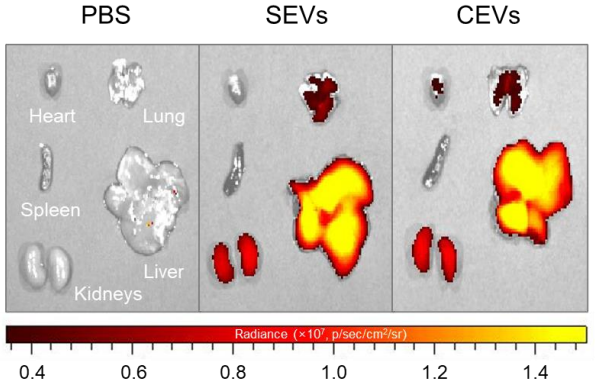


# ► **Results 4.** CEVs had strong tropism for the heart, especially cardiomyocytes *in vivo*.

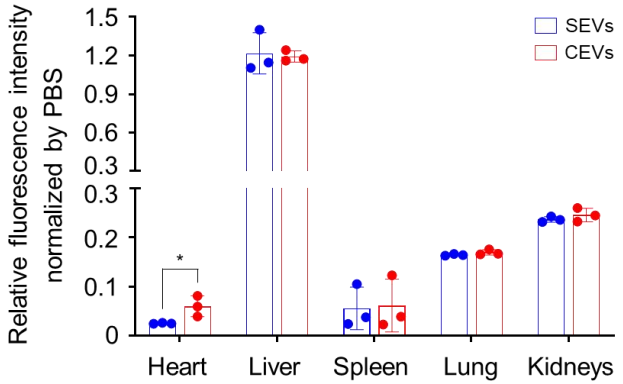
**A** *IVIS® in vivo imaging system*



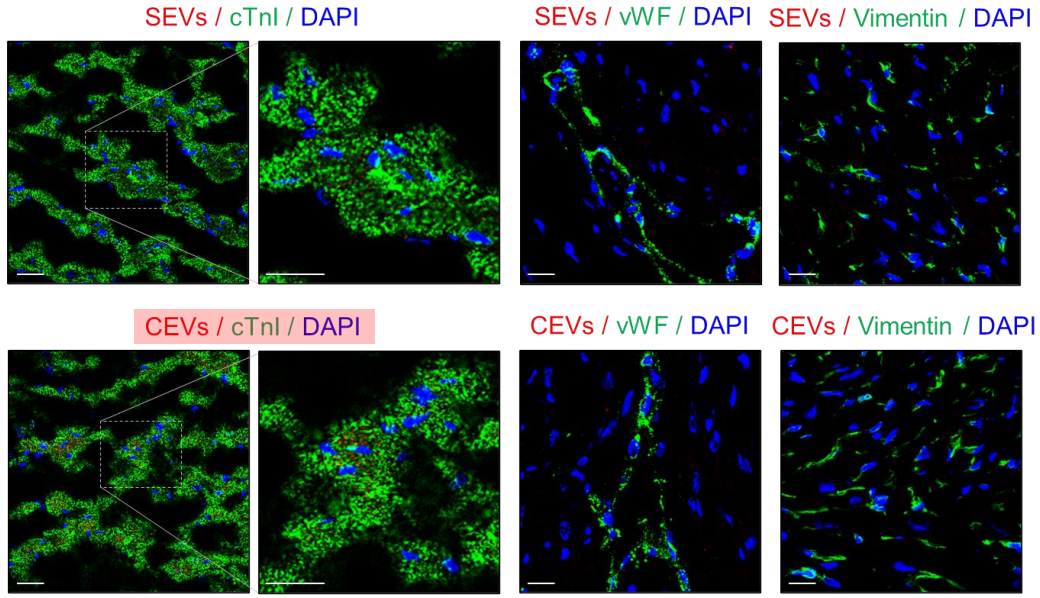

**B**




**C**




**D**

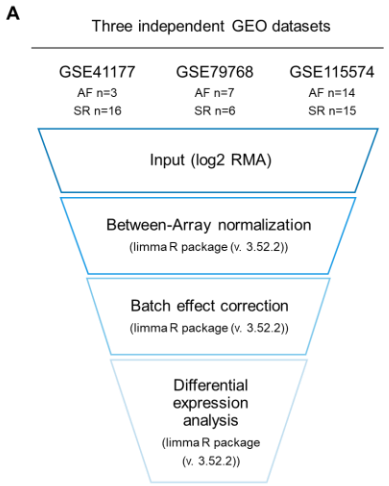


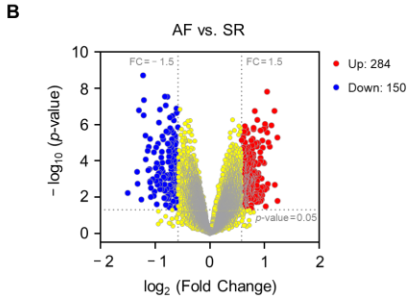

* ***cTnI*** = *Cardiac troponin I, a marker for cardiomyocytes*

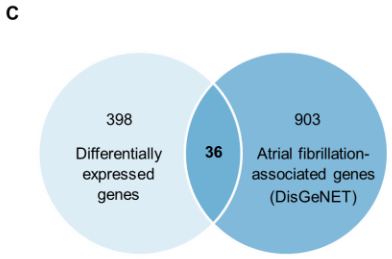
***vWF*** = *von Willebrand factor, a marker for endothelial cells*

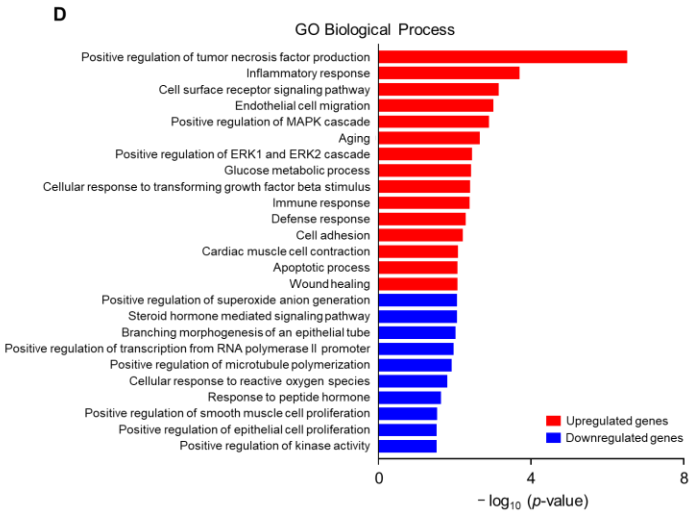
***Vimentin*** = *a marker for cardiac fibroblasts*

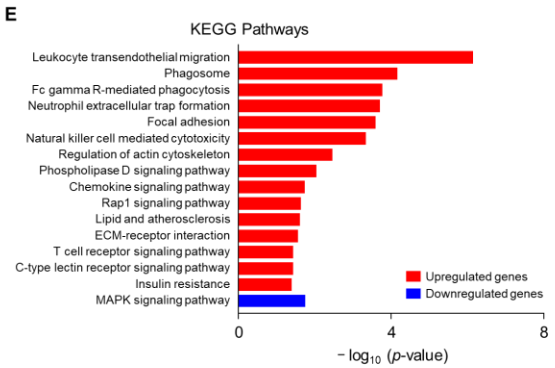
# ► **Results 5.** NOX4 was identified as a molecular target of regulating cardiac hypertrophy.



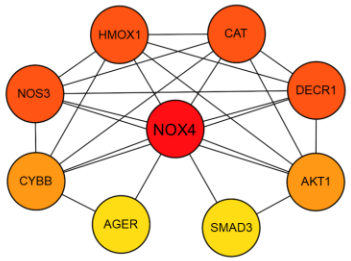



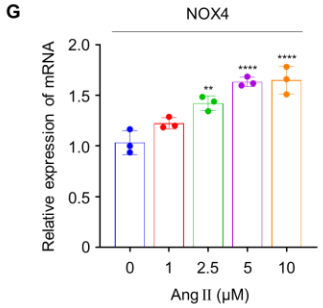



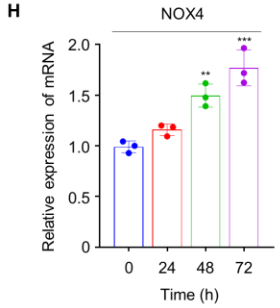



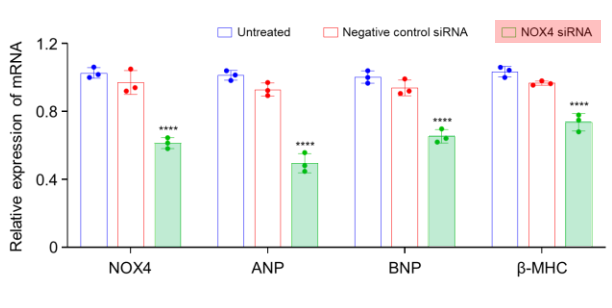




*PPI network*

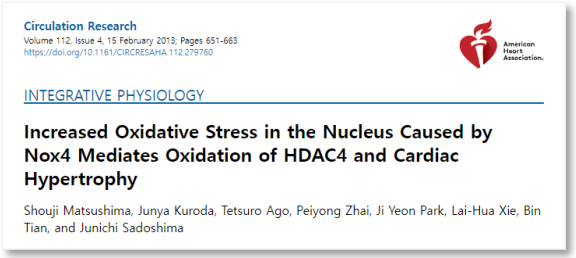


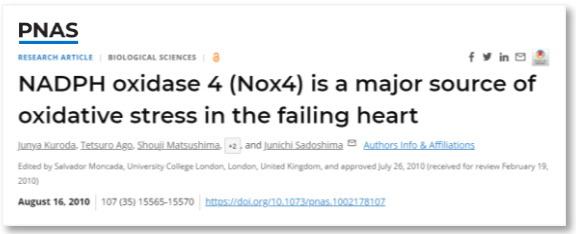

** Previous studies have shown that NOX4 is a key factor that contributes to cardiac hypertrophy, eventually resulting in AF.*


Circulation Research
Volume 112, Issue 4, 15 February 2013; Pages 651-663
https://doi.org/10.1161/CIRCRESAHA.112.279760

INTEGRATIVE PHYSIOLOGY

**Increased Oxidative Stress in the Nucleus Caused by Nox4 Mediates Oxidation of HDAC4 and Cardiac Hypertrophy**

Shouji Matsushima, Junya Kuroda, Tetsuro Ago, Peiyong Zhai, Ji Yeon Park, Lai-Hua Xie, Bin Tian, and Junichi Sadoshima


PNAS

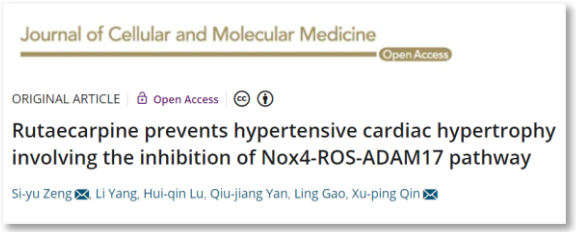
RESEARCH ARTICLE | BIOLOGICAL SCIENCES

**NADPH oxidase 4 (Nox4) is a major source of oxidative stress in the failing heart**

Junya Kuroda, Tetsuro Ago, Shouji Matsushima, +2, and Junichi Sadoshima Authors Info & Affiliations

Edited by Salvador Moncada, University College London, London, United Kingdom, and approved July 26, 2010 (received for review February 19, 2010)

August 16, 2010 | 107 (35) 15565-15570 | https://doi.org/10.1073/pnas.1002178107


Journal of Cellular and Molecular Medicine

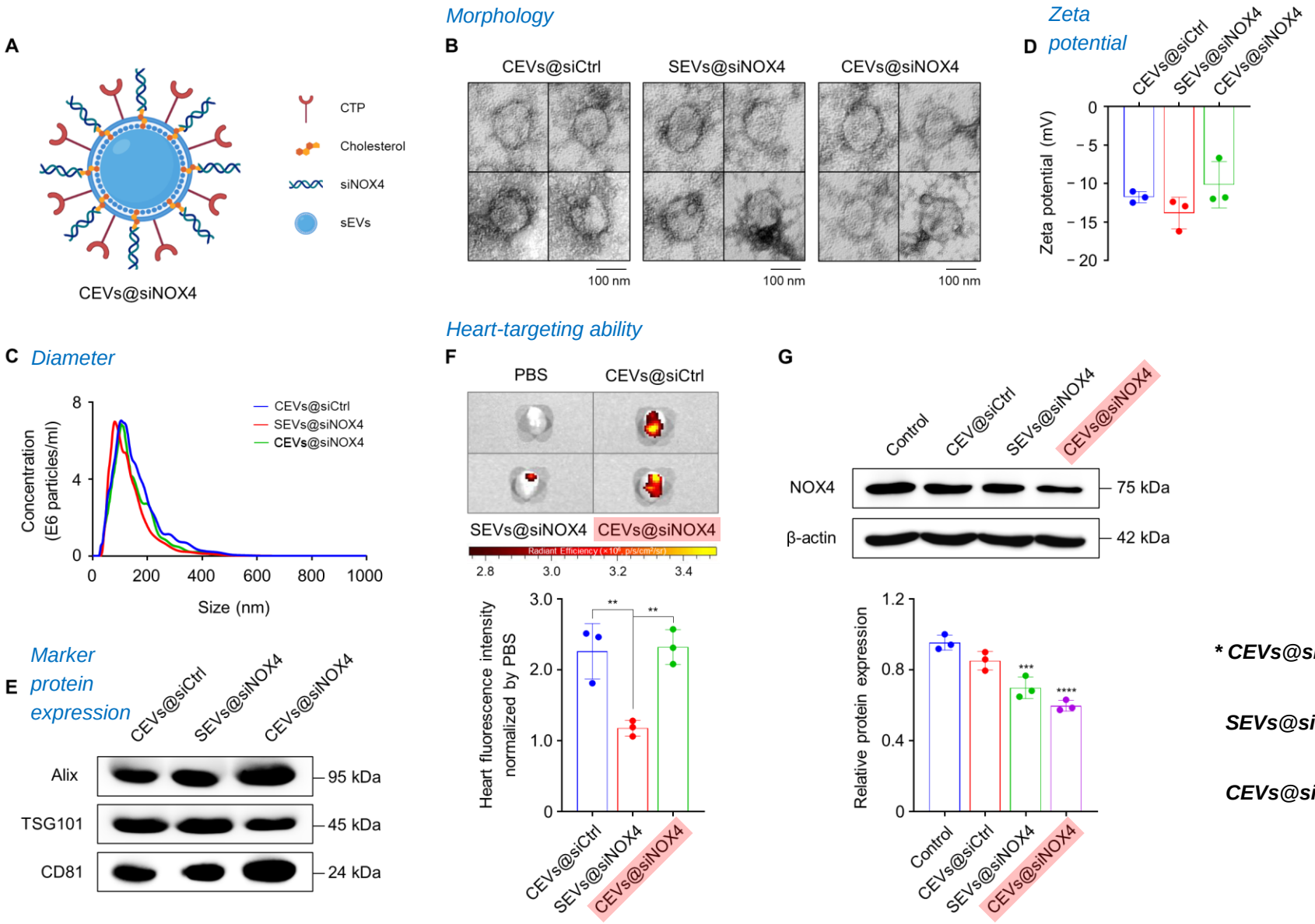
Open Access

ORIGINAL ARTICLE | Open Access

**Rutaecarpine prevents hypertensive cardiac hypertrophy involving the inhibition of Nox4-ROS-ADAM17 pathway**

Si-yu Zeng, Li Yang, Hui-qin Lu, Qiu-jiang Yan, Ling Gao, Xu-ping Qin

# ► **Results 6.** CEVs efficiently contained siNOX4 and successfully delivered it to the cells.

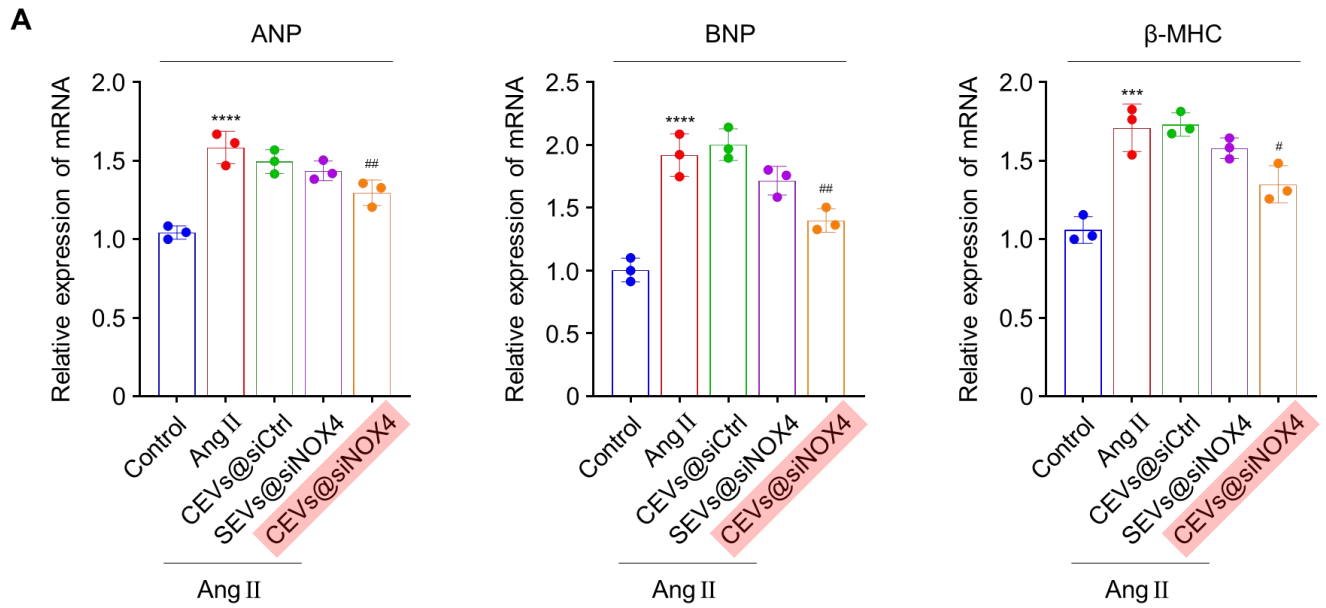



* ***CEVs@siCtrl*** = *Scrambled siRNA (siCtrl) -loaded CEVs*

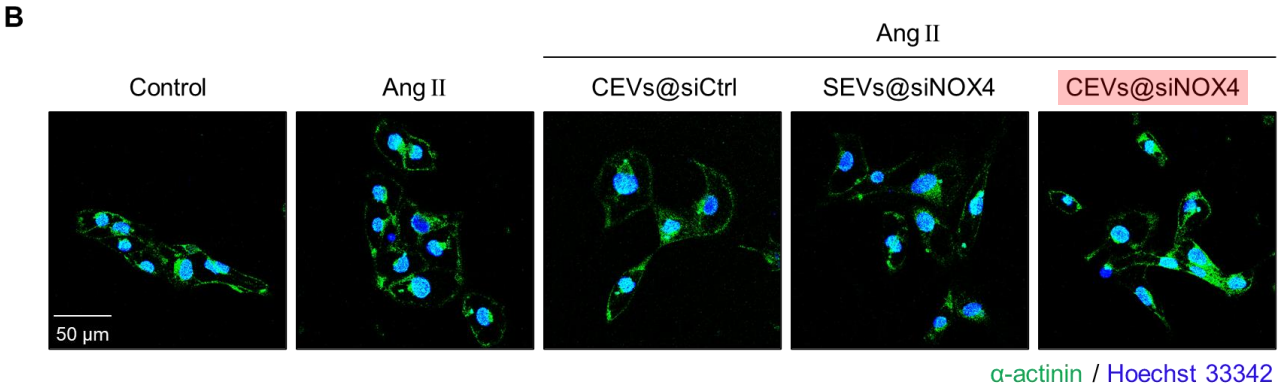
***SEVs@siNOX4*** = *NOX4 siRNA (siNOX4) -loaded SEVs*

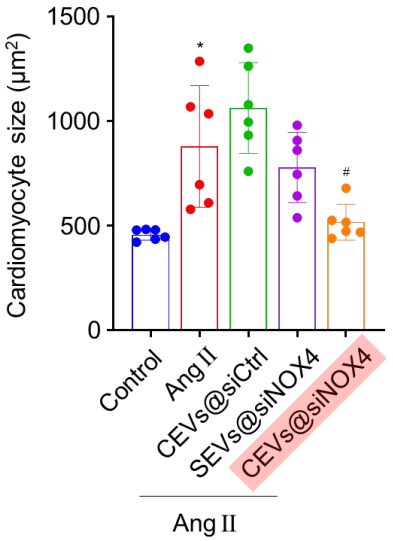
***CEVs@siNOX4*** = *siNOX4-loaded CEVs*

# ► **Results 7.** CEVs@siNOX4 exerted an enhanced protective effect against Ang II-induced hypertrophic responses.

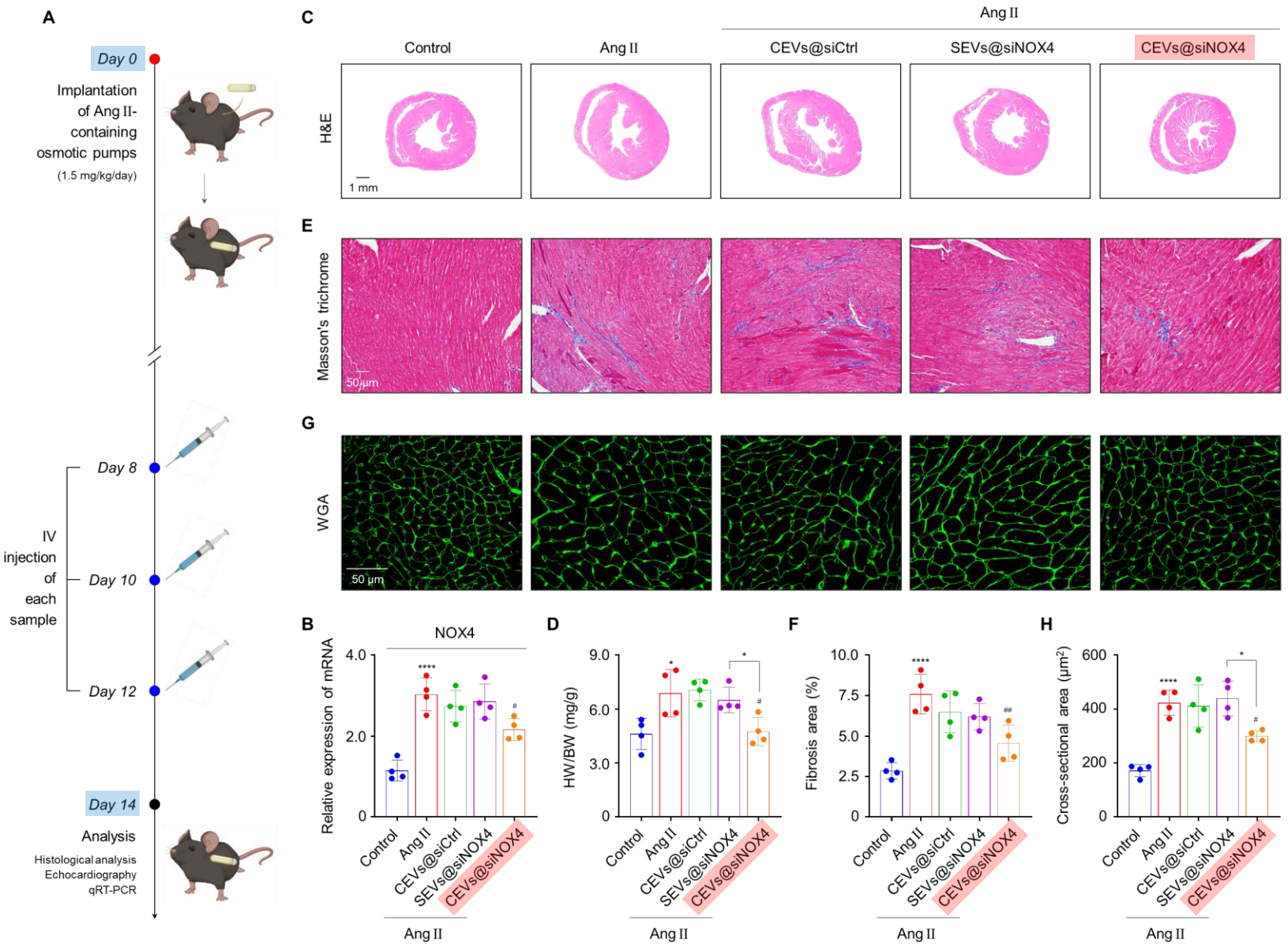



*** *ANP, BNP, β-MHC***
*= hypertrophic markers*



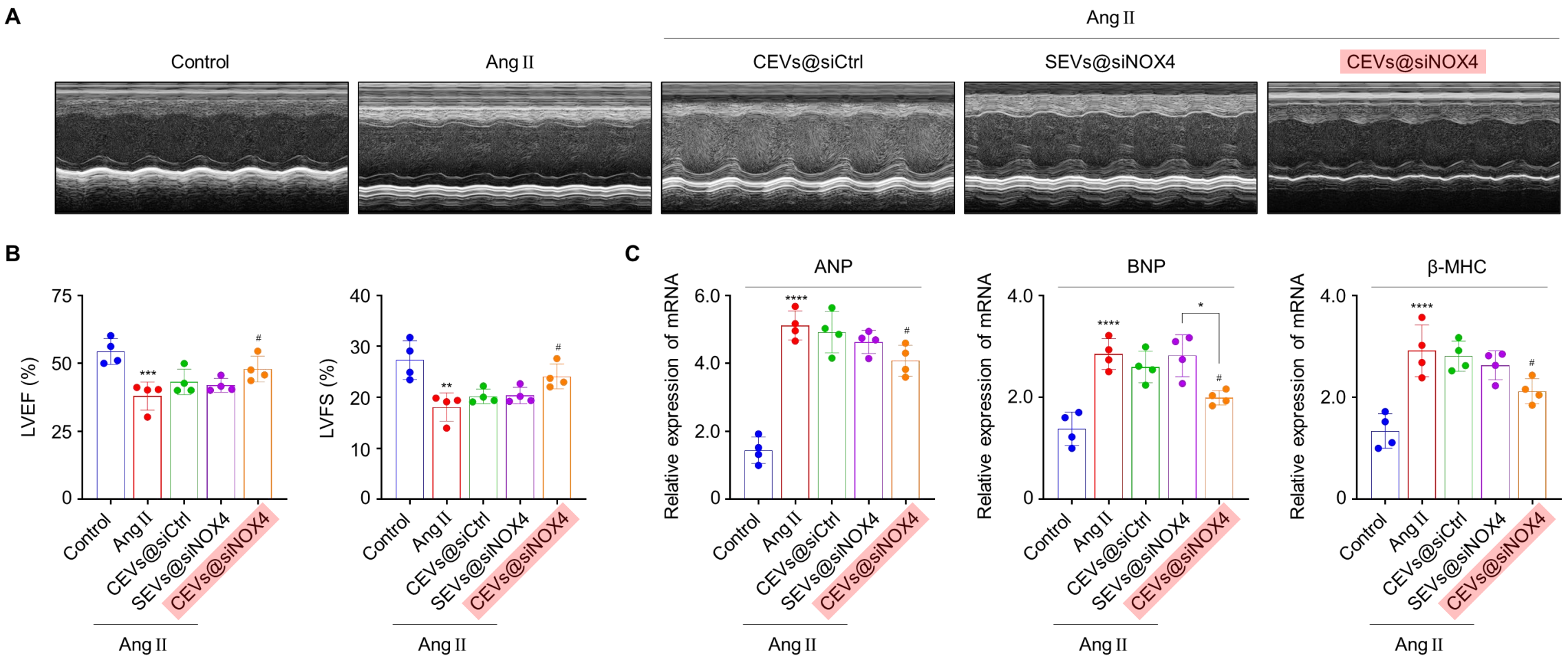
# ► **Results 8.** CEVs@siNOX4 injection resulted in a greater decrease in HW/BW, fibrotic area, and CSA.



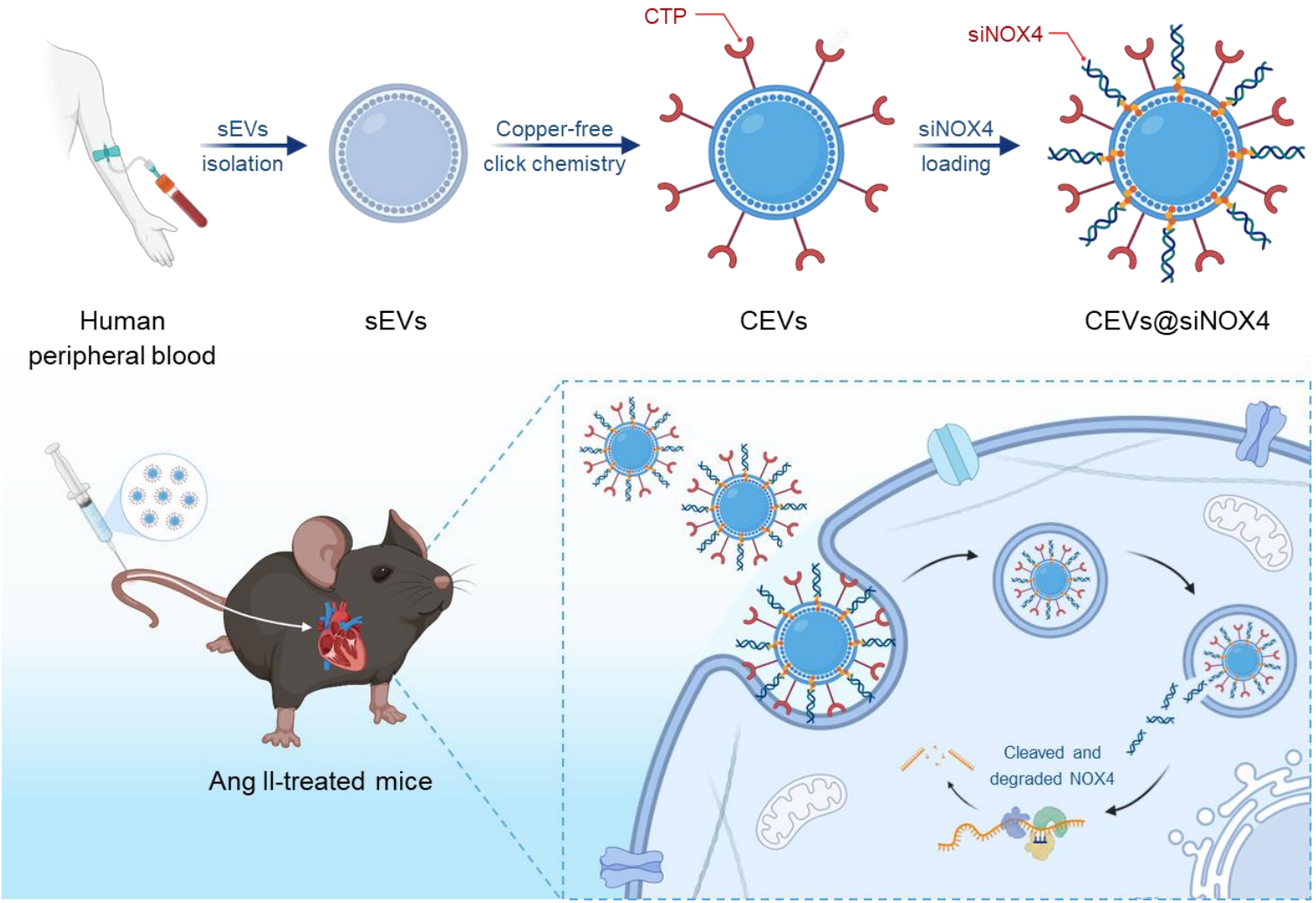

* ***HW/BW*** *= heart weight/body weight ratio*

***CSA*** *= cardiomyocyte cross-sectional area*

► **Results 9.** CEVs@siNOX4 injection significantly improved cardiac function and decreased levels of hypertrophic markers.



# ► Conclusion




In conclusion,

our study suggests that the utilization of CEVs represents an efficient strategy for heart-targeted delivery of therapeutic siRNAs and holds great promise for the treatment of cardiac hypertrophy.

# Thank you for your attention.

## Acknowledgments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NRF 한국연구재단