



UMC Utrecht

# EVs or LNPs for RNA delivery?

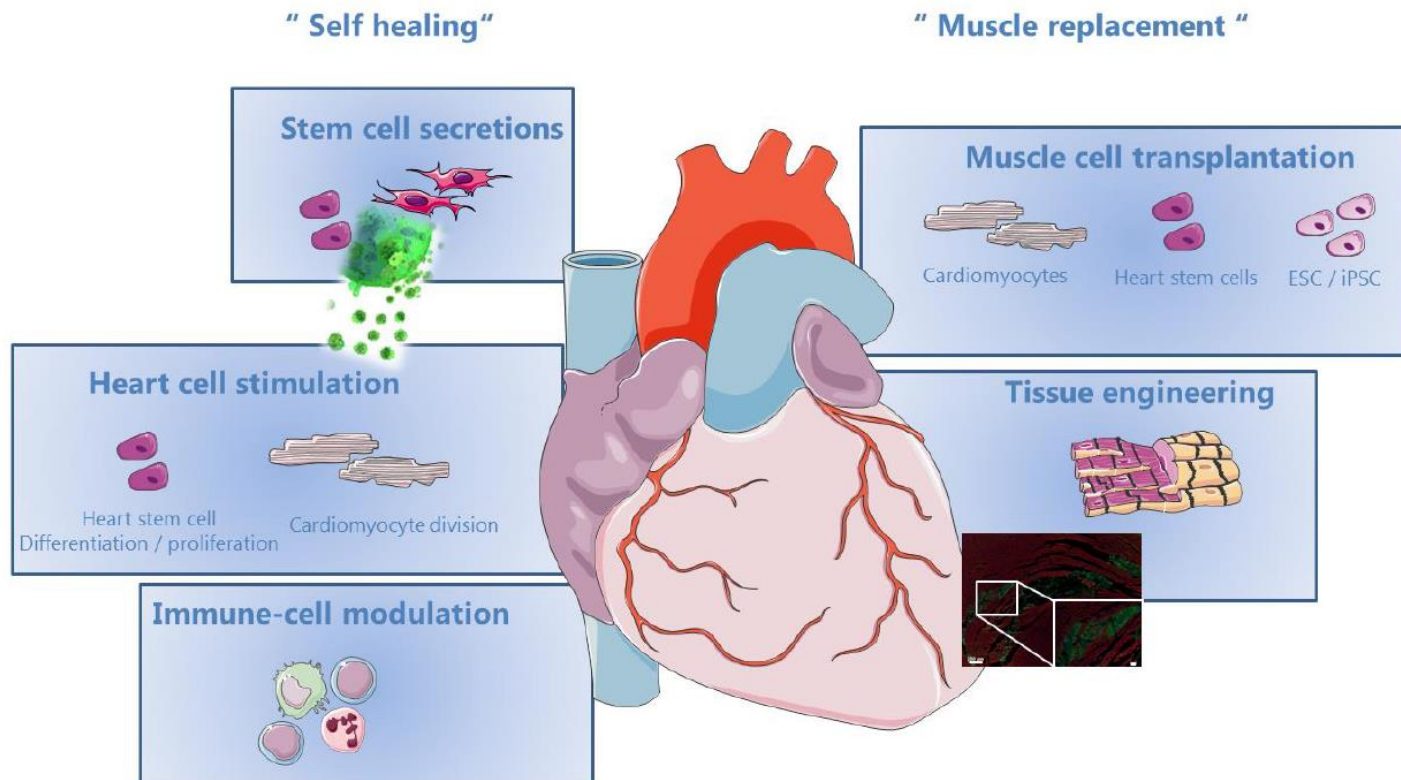
Raymond Schiffelers  
CDL Research



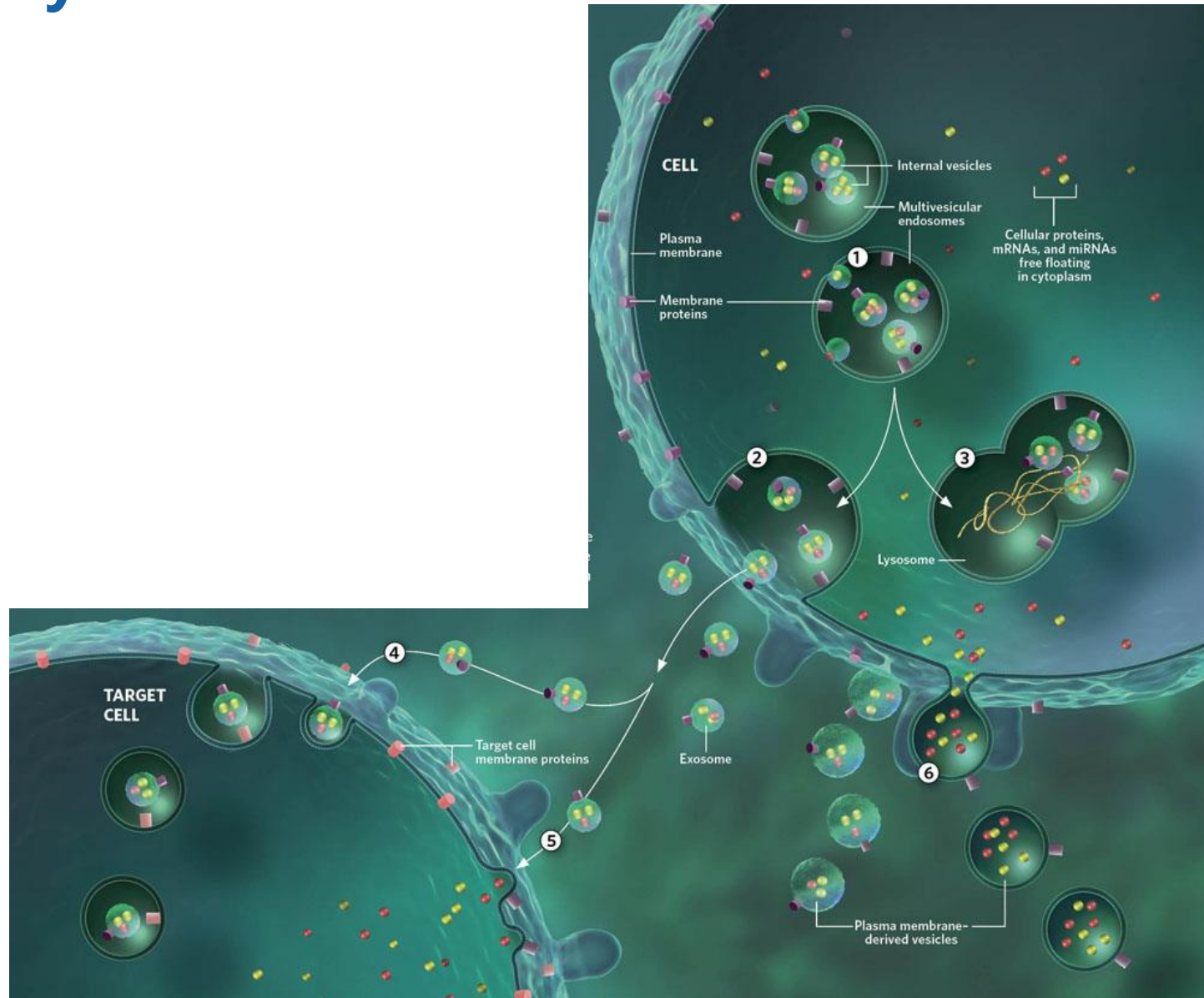
# Regenerating the failing heart

- Prof. Sluijter (UMCU)

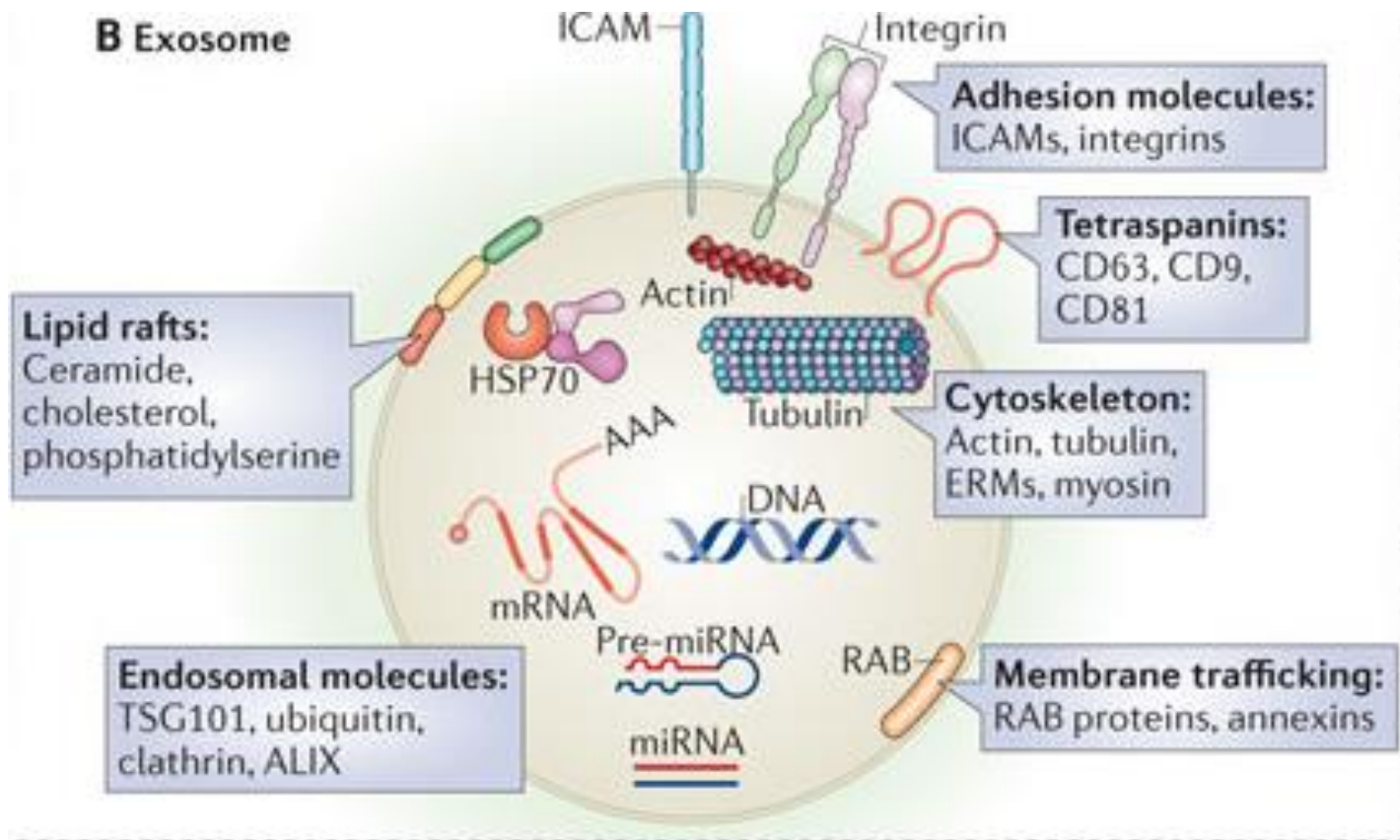
## Empowering the heart



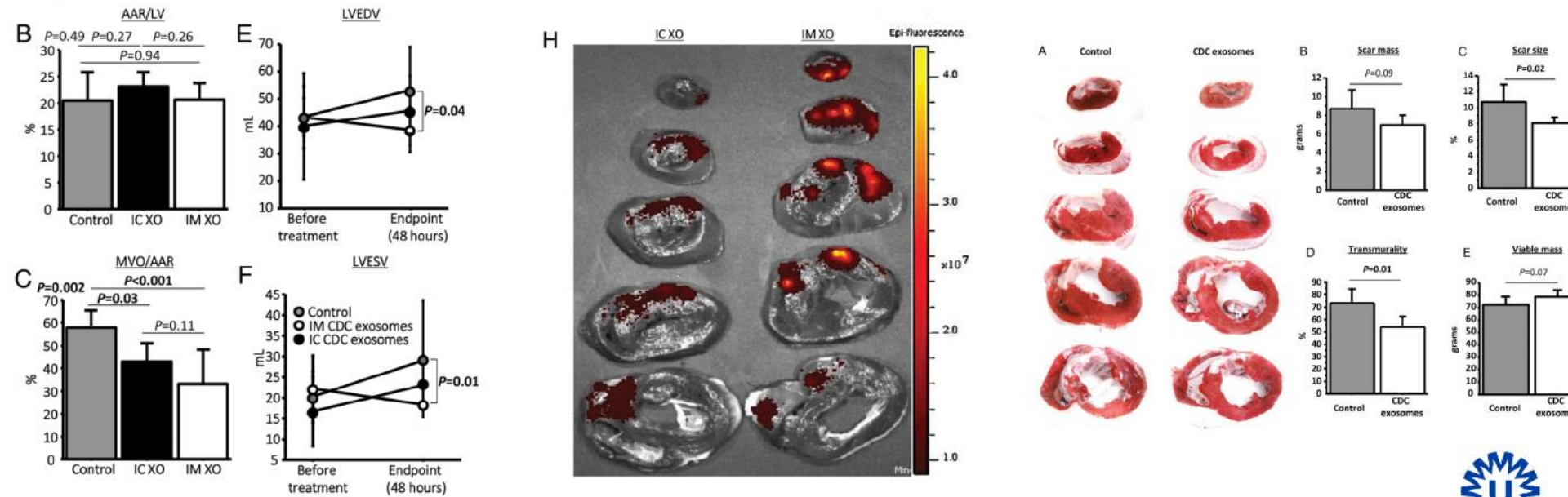
# Extracellular vesicles could be natural drug delivery systems



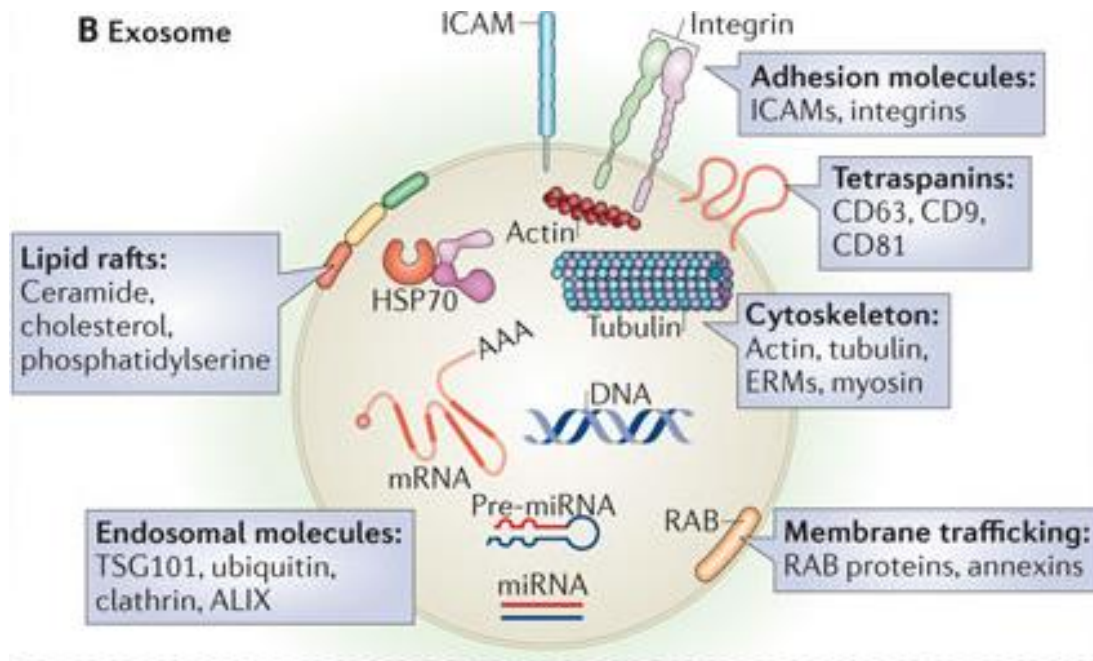
# Extracellular vesicles



## Exosomes by CDCs improve function in acute and chronic porcine myocardial infarction



# Thousands of bioactive compounds



# 20 flasks = $10^{12}$ vesicles

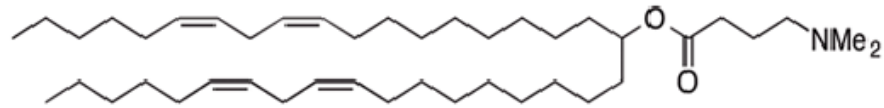


- Donor cell dictates composition
- Changes in culture conditions change composition
- Different isolation protocols result in different populations

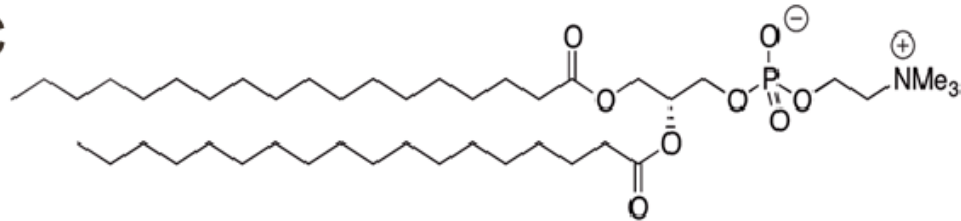


# LNP

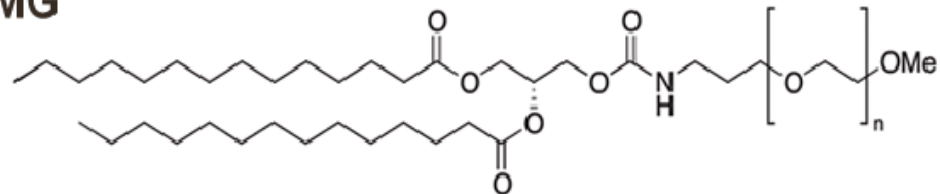
**DLin-MC3-DMA**



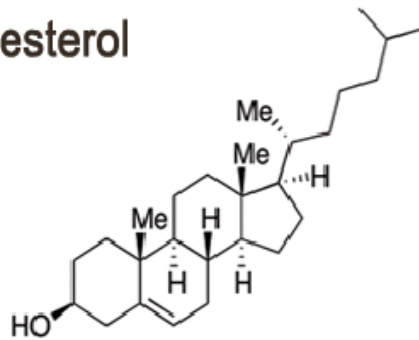
**DSPC**

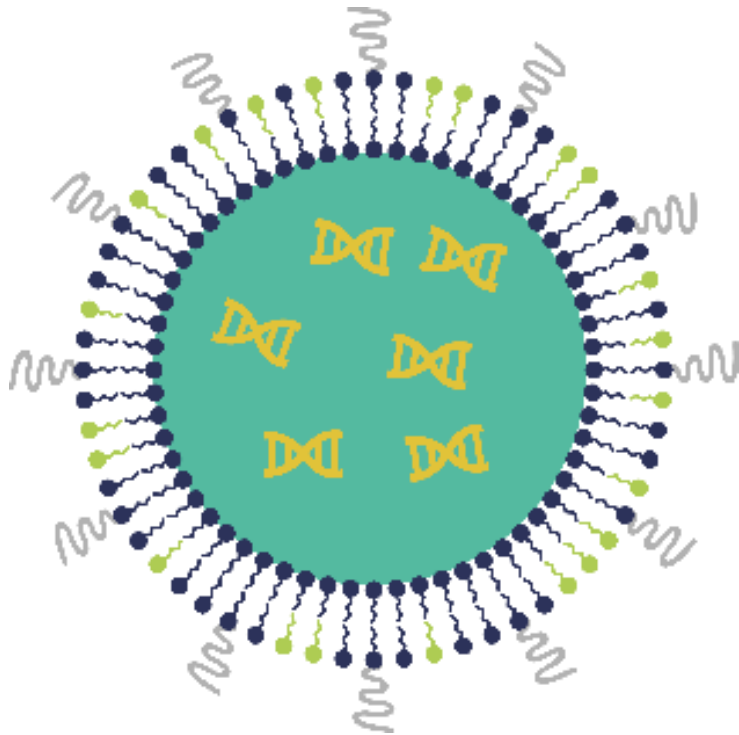


**PEG-DMG**

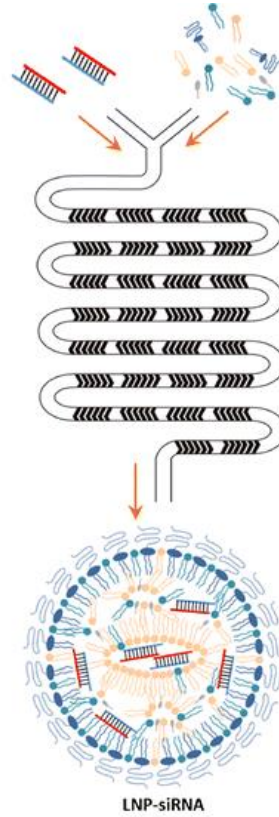


**Cholesterol**





Aqueous Phase  
(contain siRNA)



Lipid in ethanol

LNP-siRNA



# How to make $10^{14}$ LNPs in 30 sec

- Size 92 +/- 3 nm,
- zeta -10 +/- 2 mV
- EE 92 +/- 3%
- 4 excipients
- 1 API



- How to compare?



ARTICLE



<https://doi.org/10.1038/s41467-020-14977-8>

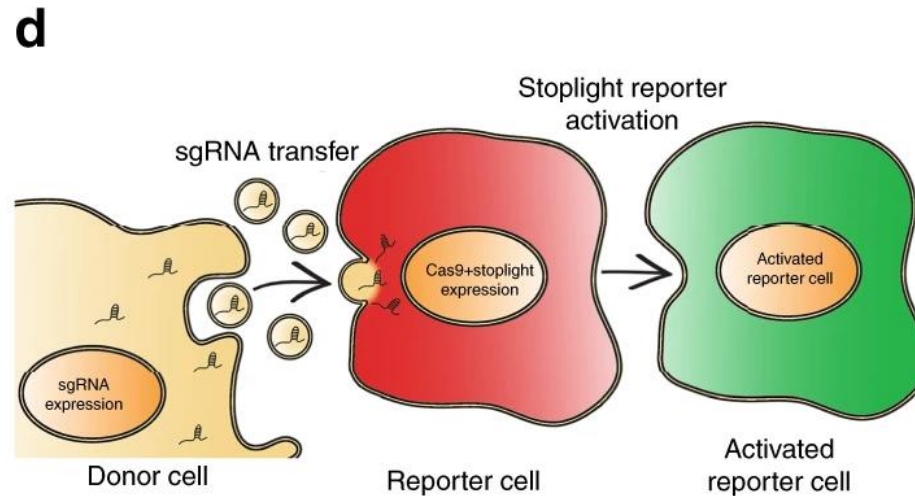
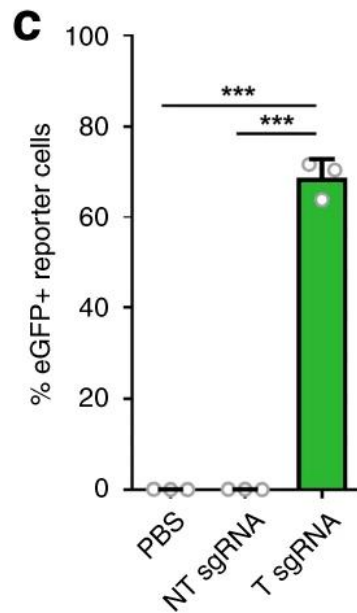
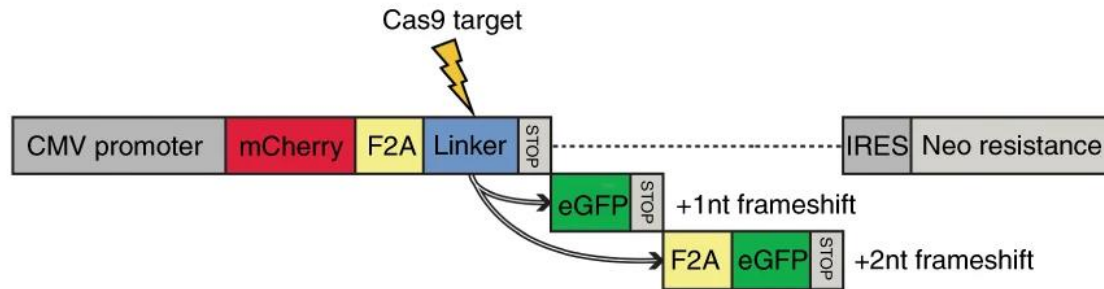
OPEN

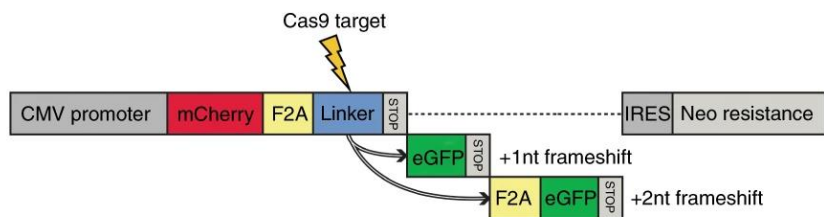
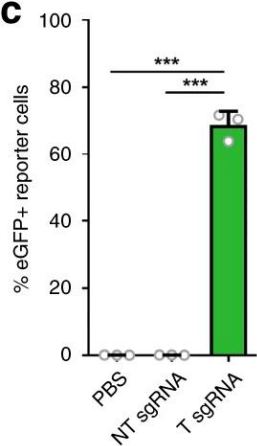
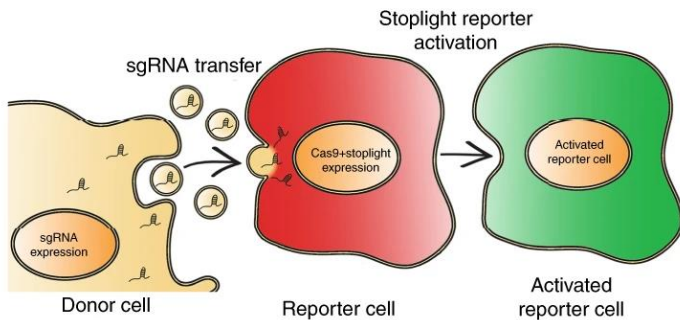
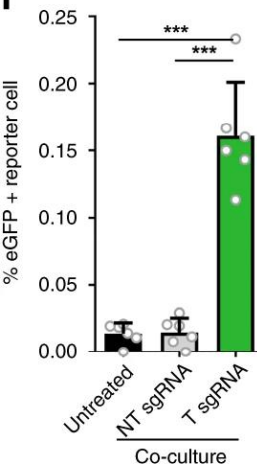
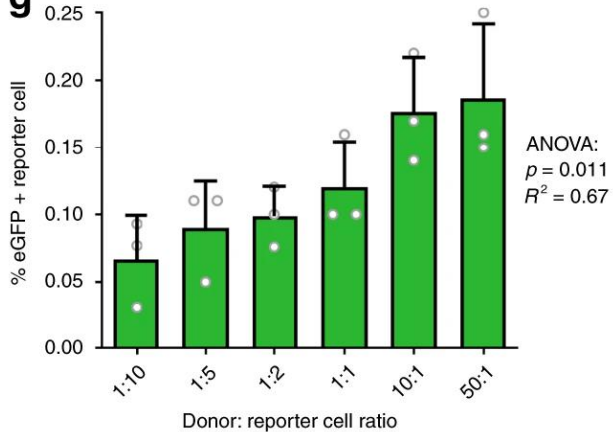
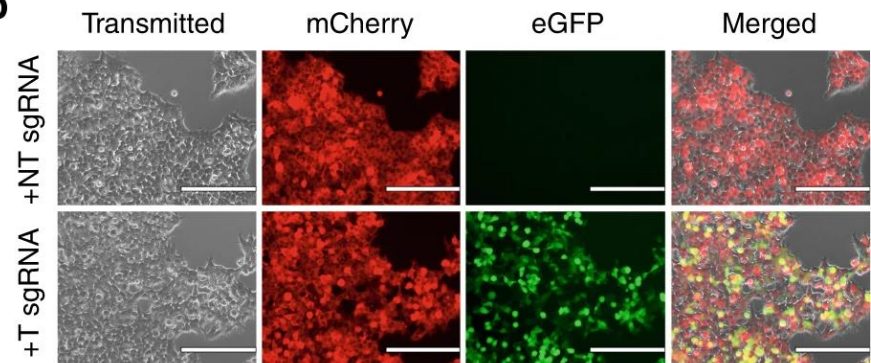
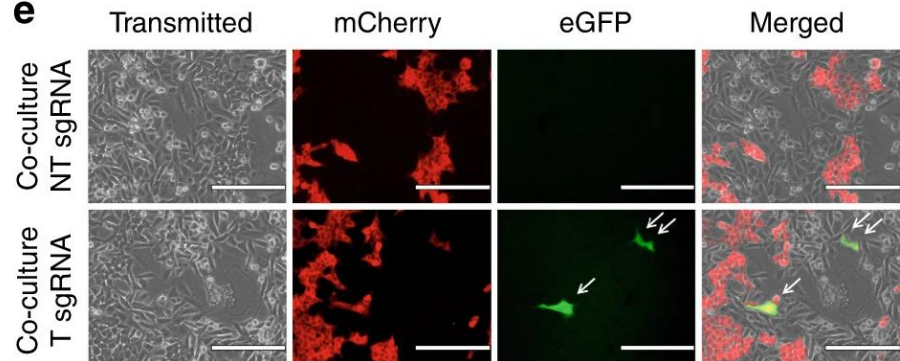
# A CRISPR-Cas9-based reporter system for single-cell detection of extracellular vesicle-mediated functional transfer of RNA

Olivier G. de Jong <sup>1,2</sup>, Daniel E. Murphy<sup>1</sup>, Imre Mäger <sup>2</sup>, Eduard Willms<sup>2,3</sup>, Antonio Garcia-Guerra<sup>2,4</sup>, Jerney J. Gitz-Francois<sup>1</sup>, Juliet Lefferts <sup>5</sup>, Dhanu Gupta <sup>6</sup>, Sander C. Steenbeek<sup>7</sup>, Jacco van Rheenen<sup>7</sup>, Samir El Andaloussi<sup>6</sup>, Raymond M. Schiffelers<sup>1</sup>, Matthew J.A. Wood<sup>2</sup> & Pieter Vader <sup>1,8</sup>✉

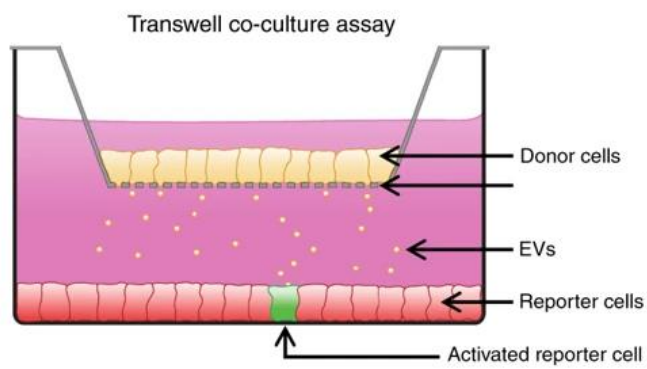
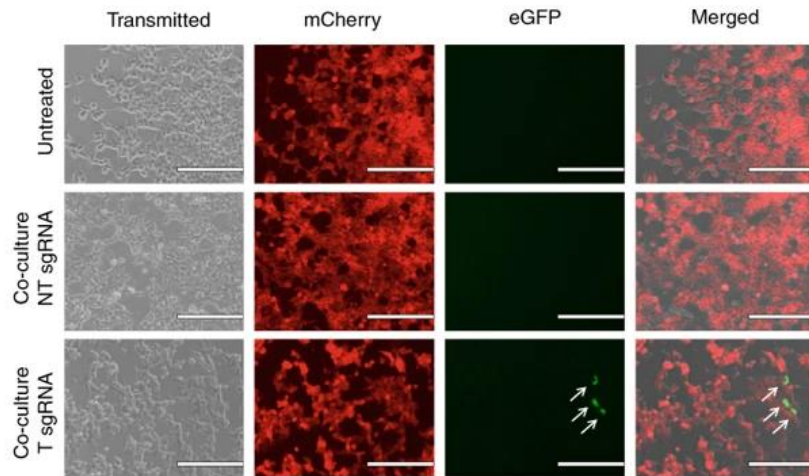


# CRISPR-Cas9 reporter

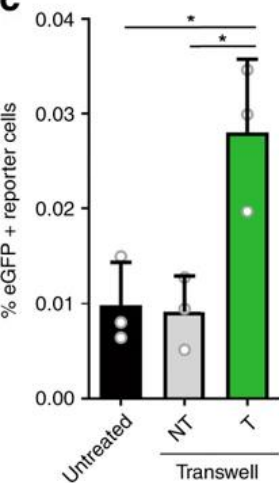
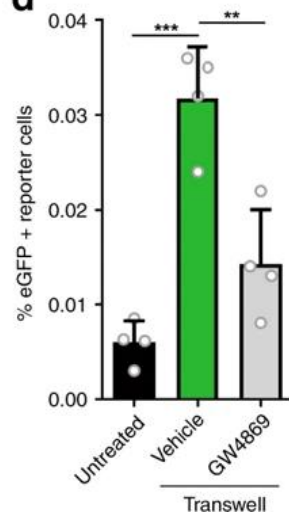
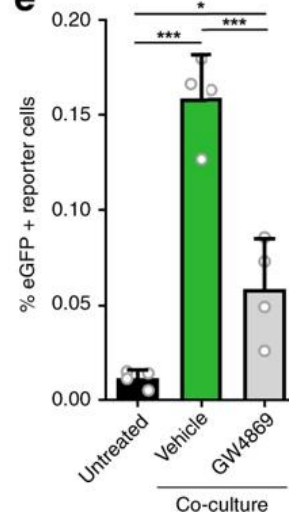


**a****c****d****f****g****b****e**

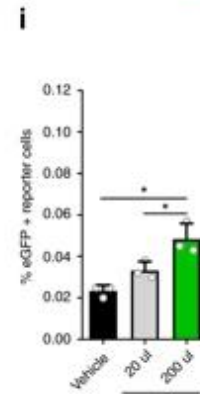
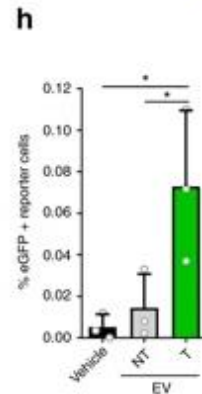
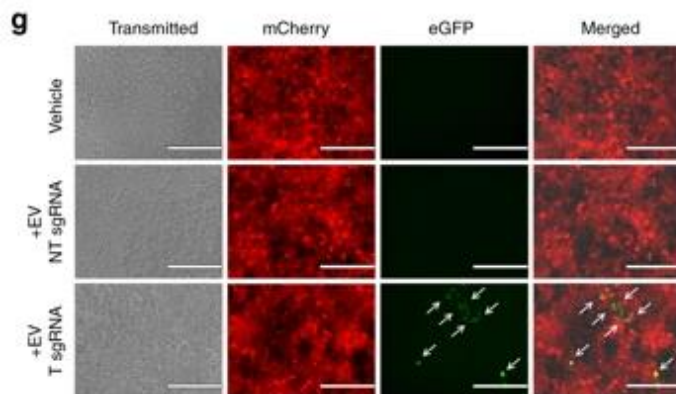
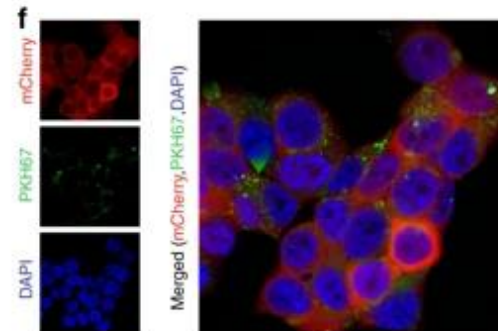
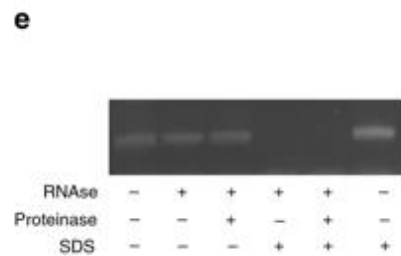
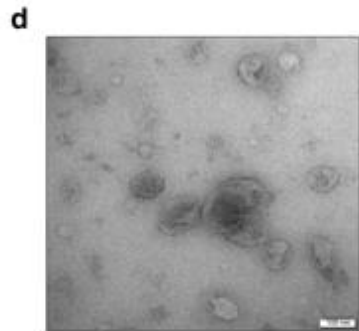
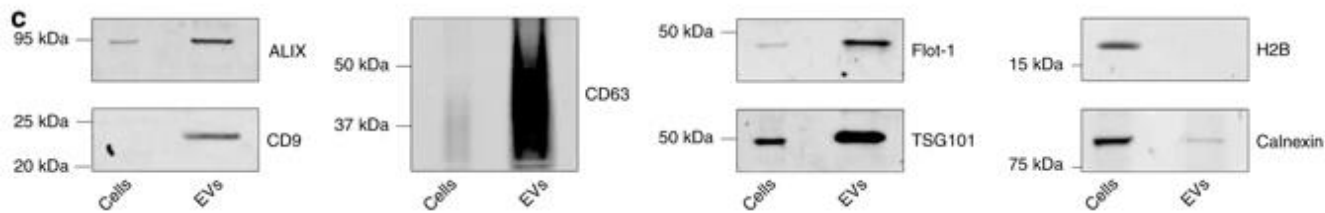
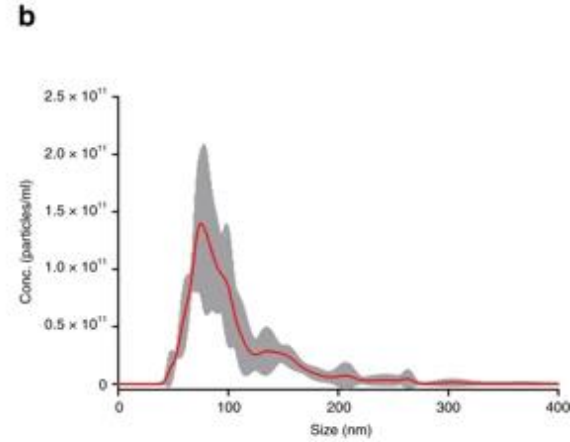
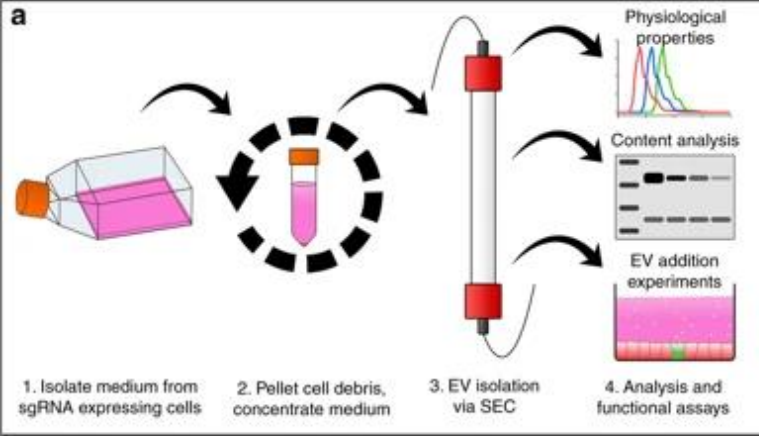
# Co-culture reporter activation

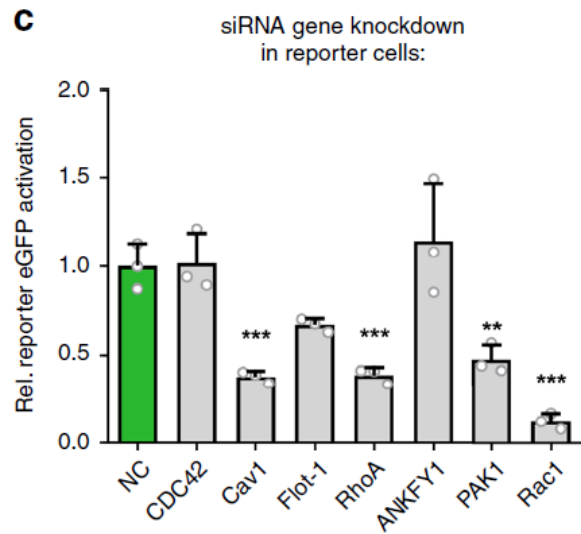
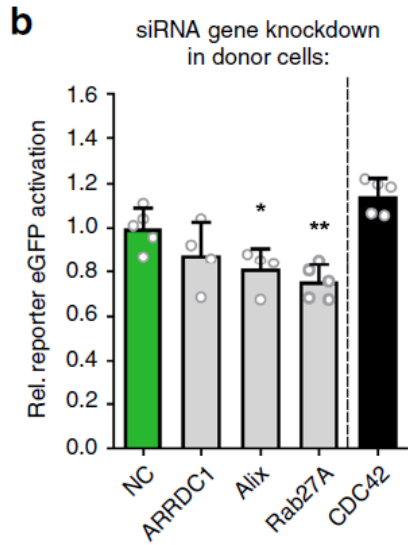
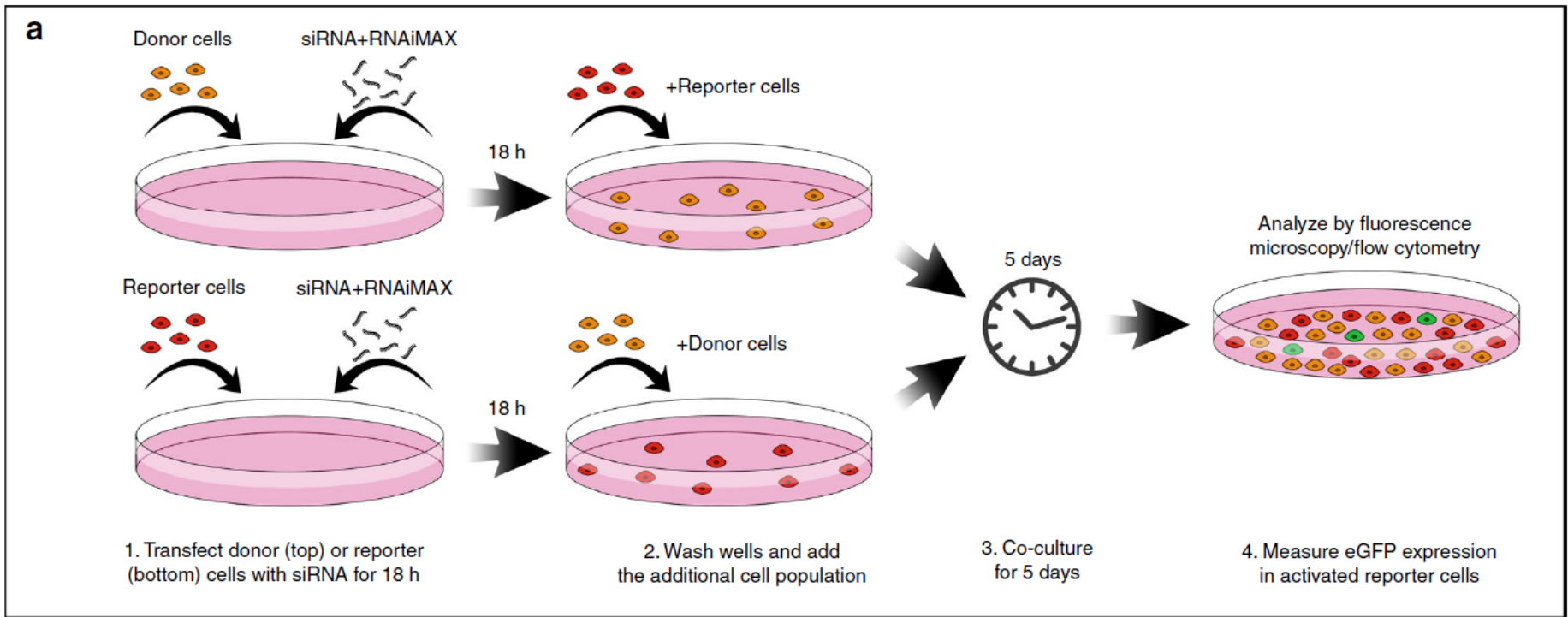
**a****b**

# Without cell-cell contact

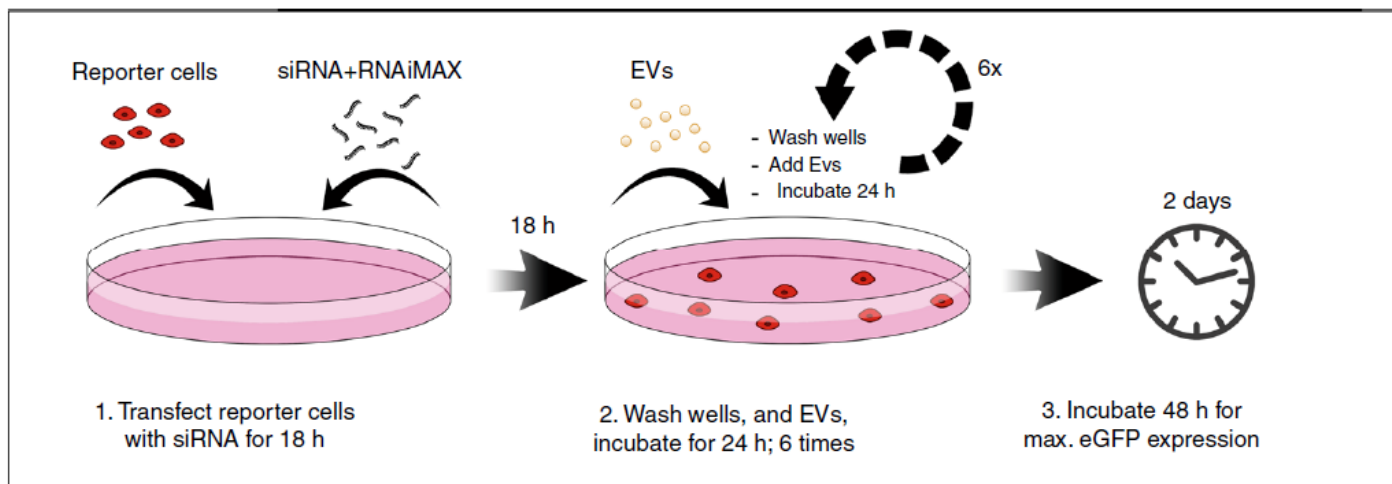
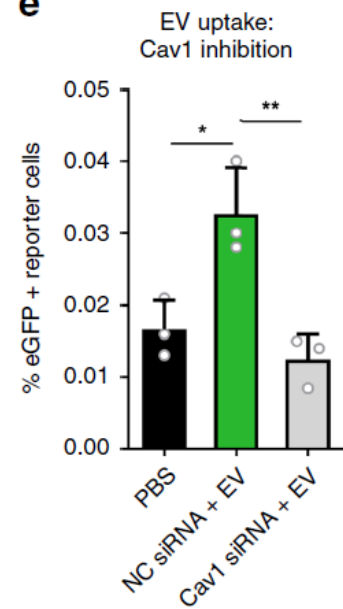
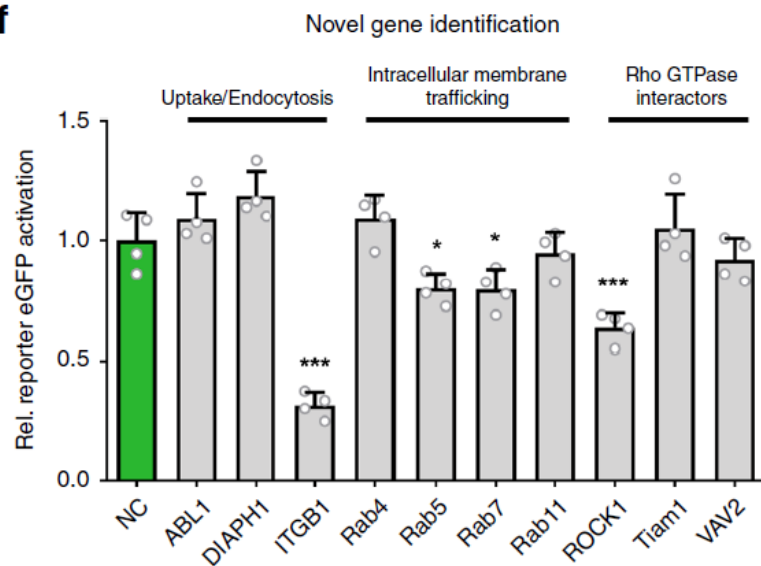
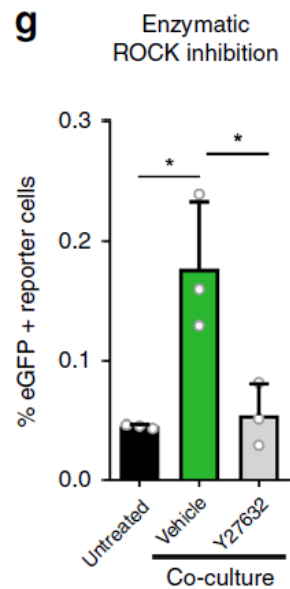
**c****d****e**

# EV addition causes reporter activation

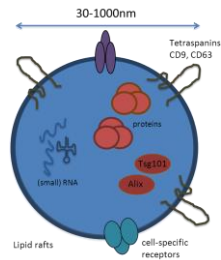




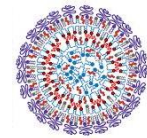
**Dependent on specific uptake pathways**

**d****e****f****g**

# Functional comparison: EVs vs LNPs



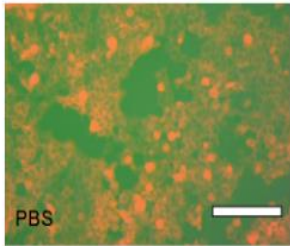
VS



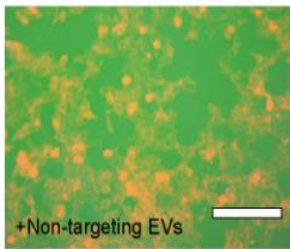
*Murphy et al., in preparation*



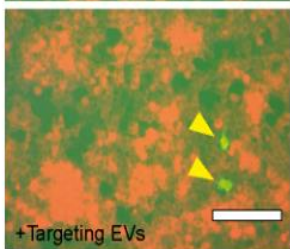
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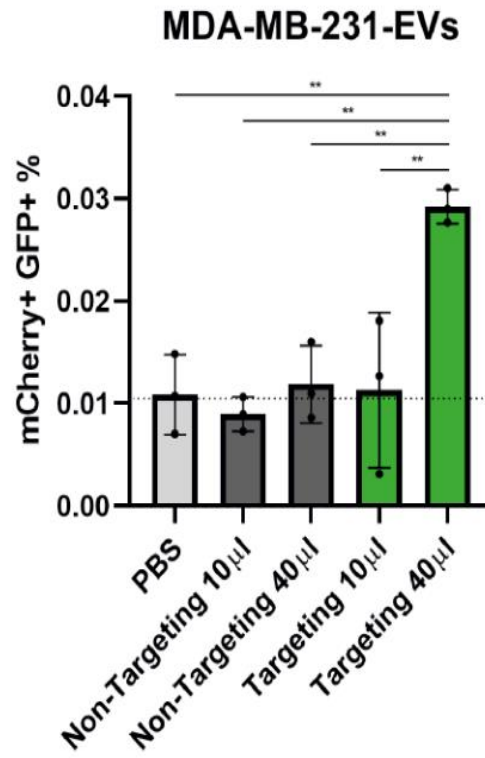
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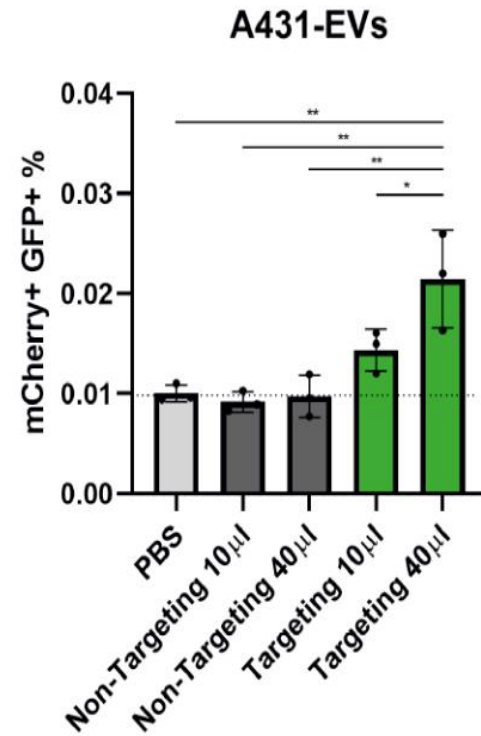
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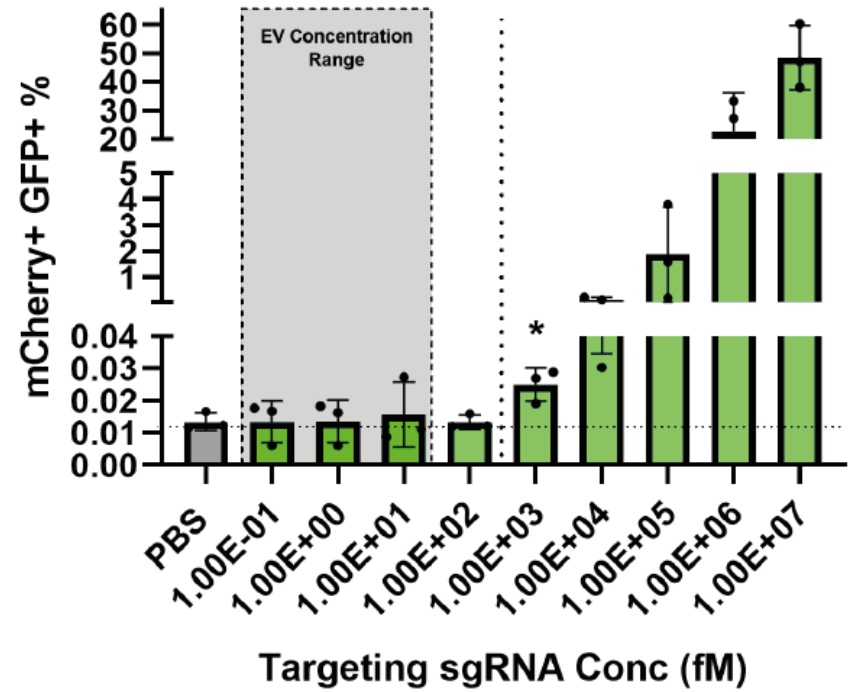
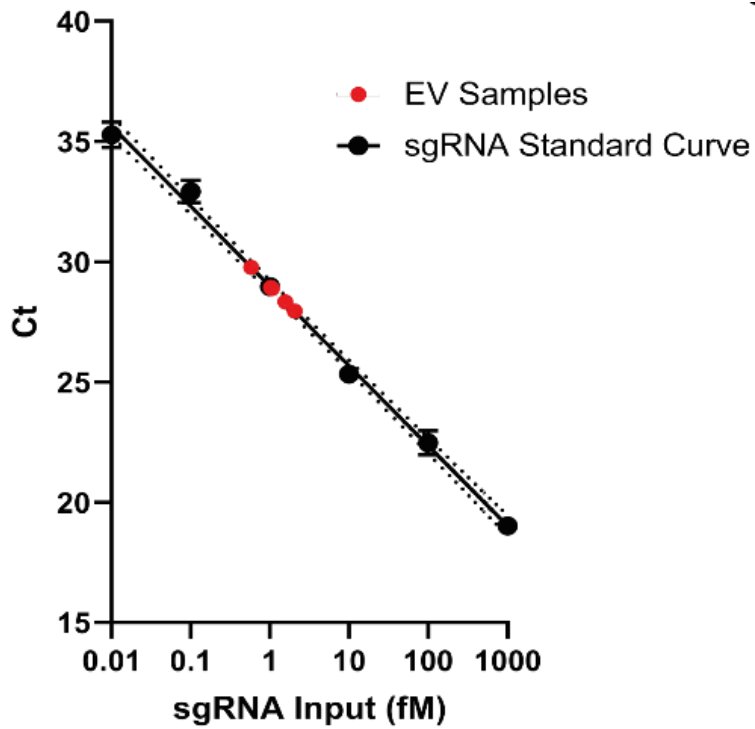
b



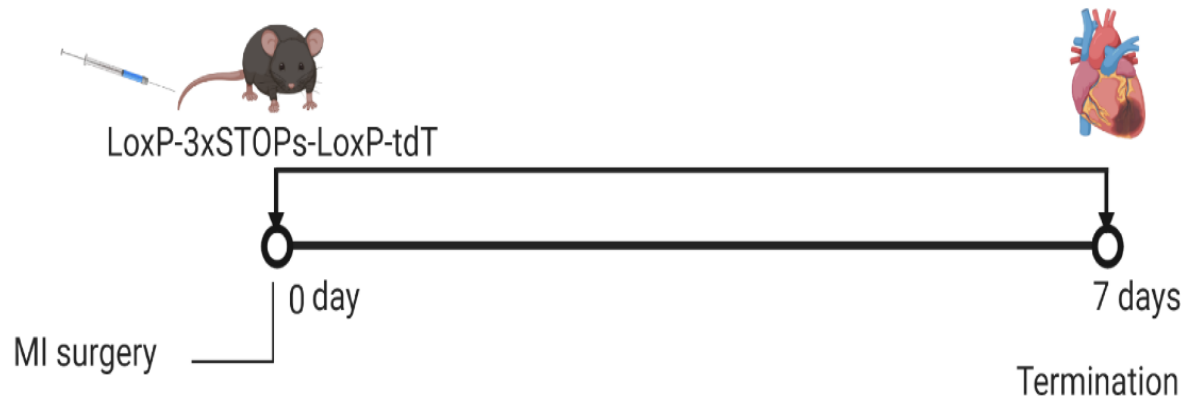
c

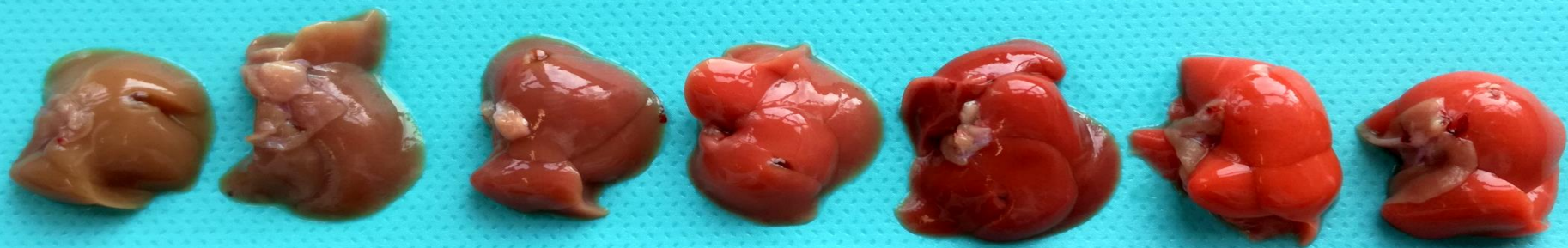


1 in every  $\sim 10^5$  EVs contains sgRNA



A

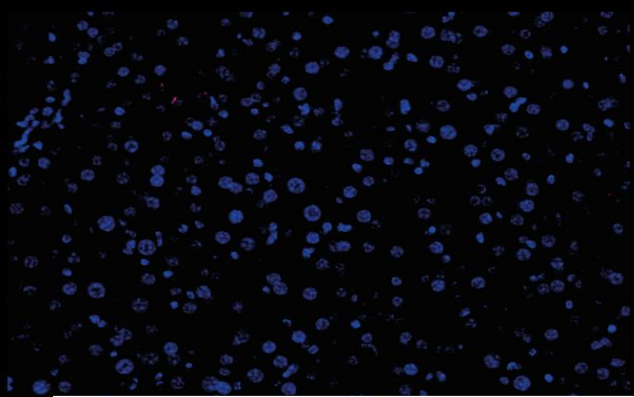




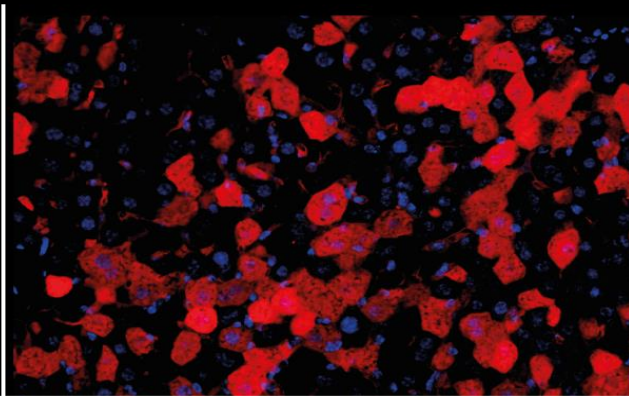
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100 $\mu$ g

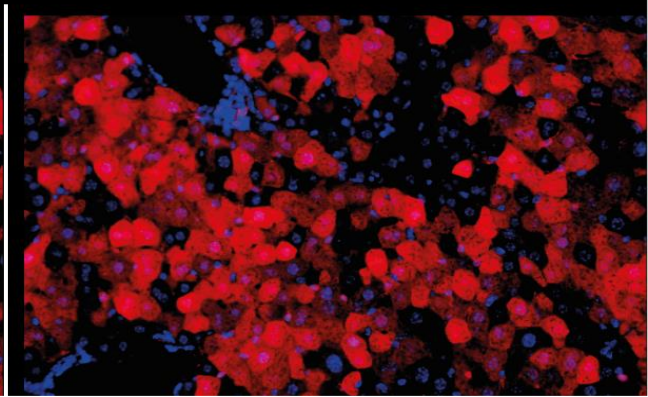
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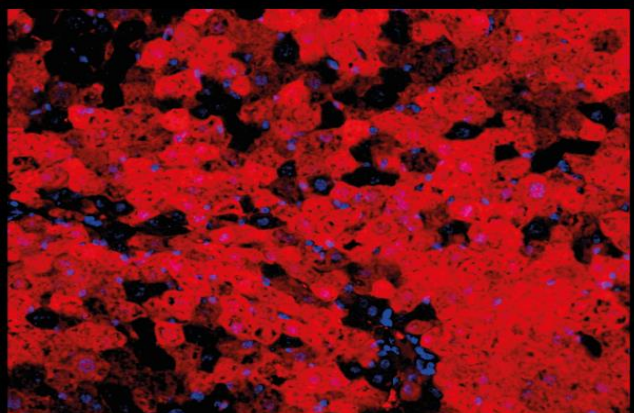
5 $\mu$ g



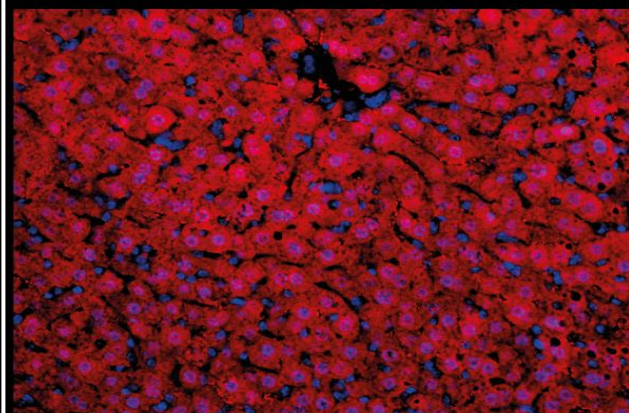
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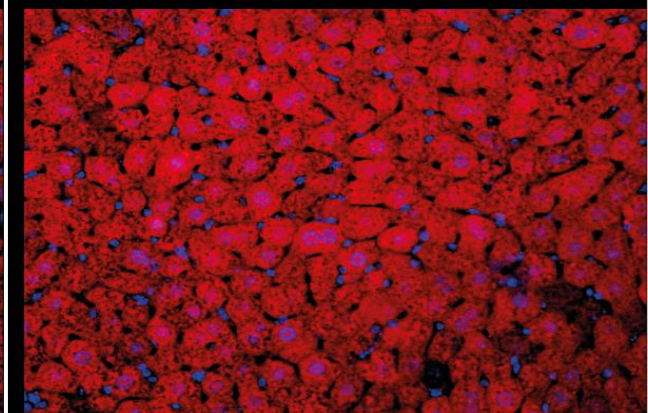
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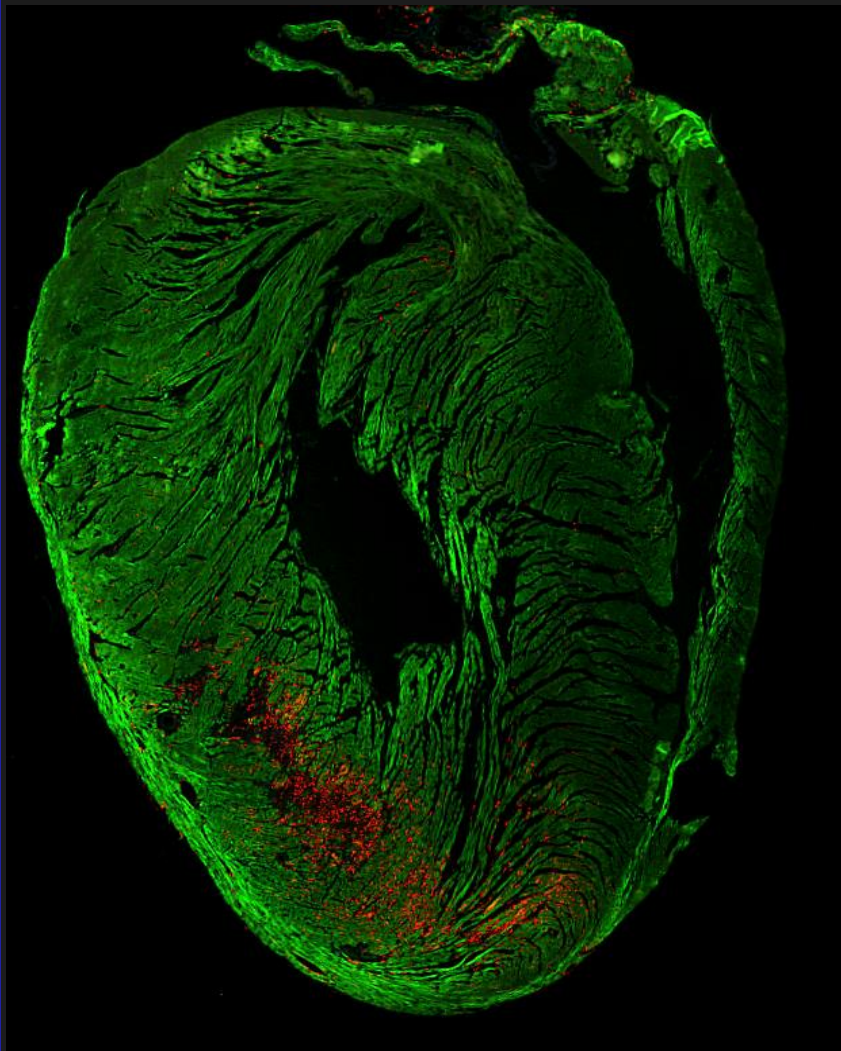


50 $\mu$ g



100 $\mu$ g



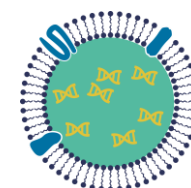
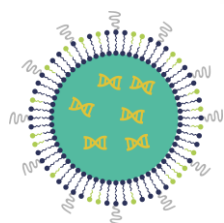
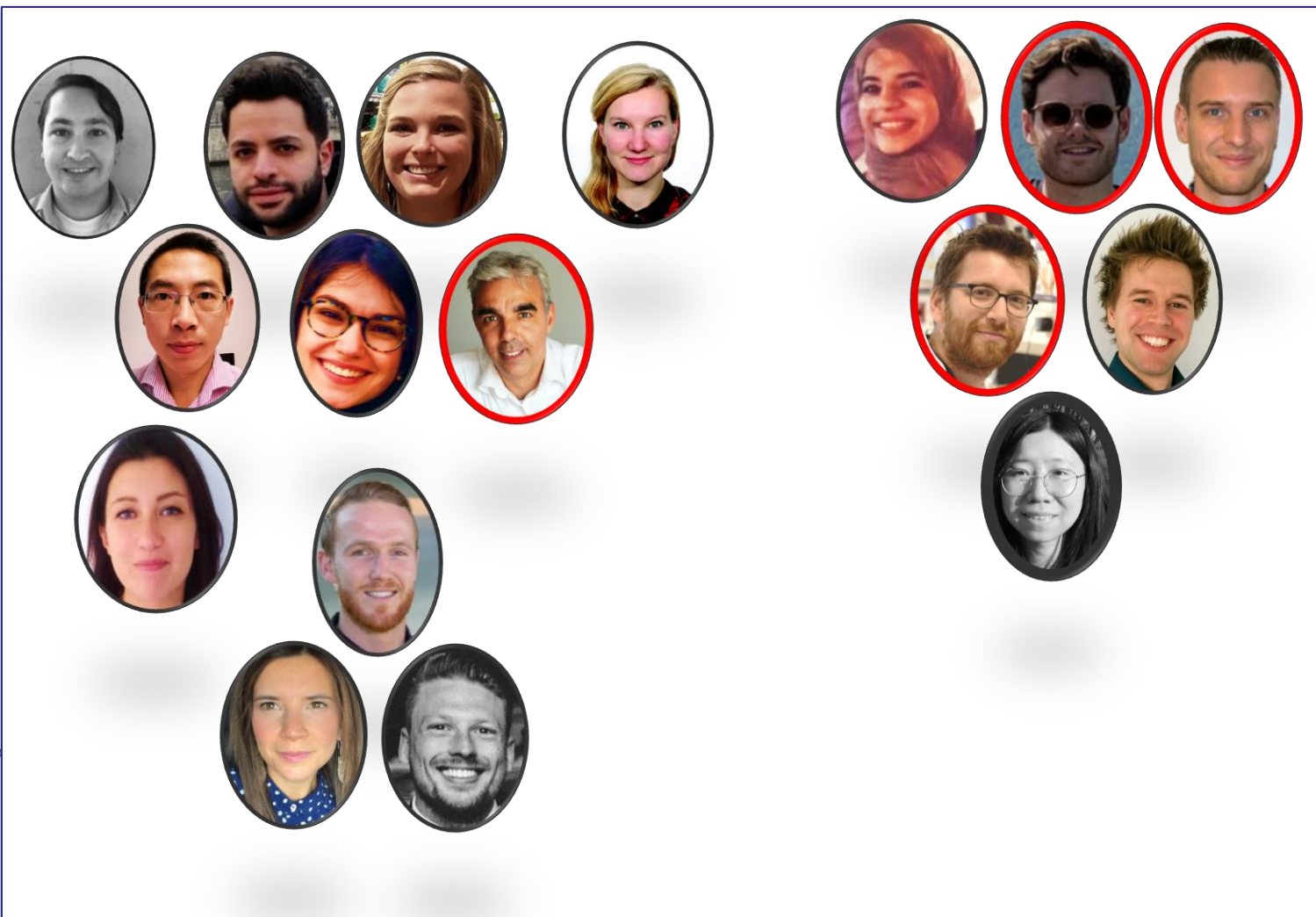
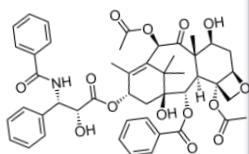
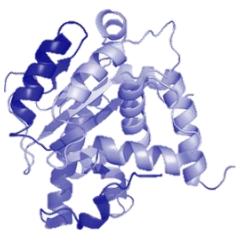
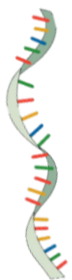


5 $\mu$ g  
IR  
birdview

# EVs vs LNPs

- Evs behave like other nanocarriers regarding tissue distribution
- Remarkable transfer of RNA in vivo
- Less efficient loading than LNPs
- LNPs possess advantageous characteristics for consistent production, characterization and performance





# Funding

