MISSIONE 4
ISTRUZIONE
RICERCA

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NextGenerationEU

Ministero
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e della Ricerca

Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA

WP3 – Medicina personalizzata

Task 2 – Engineering bioinspired and biomimetic nanomedicines for precise drug delivery

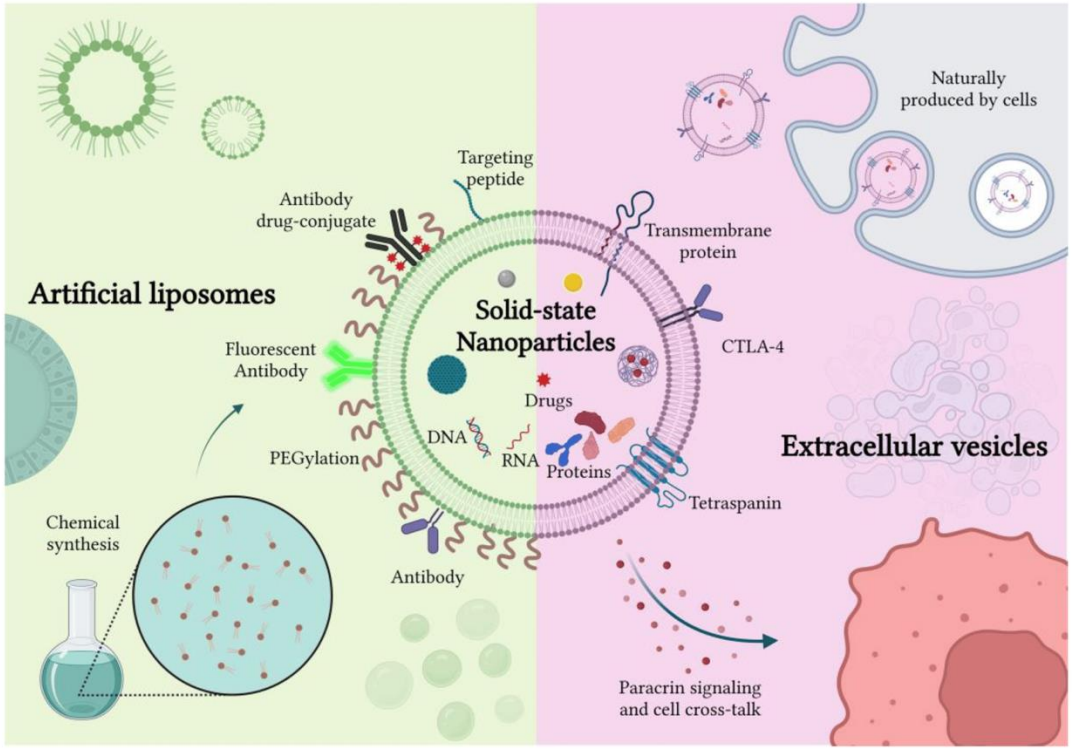
Vescicole extracellulari sintetiche: nuovi vettori per drug delivery tessuto-specifico

Prof. Michele Guescini
(Università di Urbino)

WP3 T3.2 focused on developing high-throughput vesicle assembly technology allowing encapsulation of biomolecules and the incorporation of integral and peripheral membrane proteins to target the vesicular carrier towards a specific recipient cell.

Bottom-up assembly of EV mimics enables stepwise quantitative loading of EVs with different important molecular signaling compounds, which enables exquisite control over their physicochemical properties, mechanical and chemical stability, intracellular admission, and manipulability. Two main approaches have been studied:

The first one consisted in developing specific protocols for the extraction of plasma membrane proteins using mild extraction conditions with the aim to preserve membrane micro-domains, like lipid rafts and/or tetraspanin networks, that subsequently can be reused to assemble synthetic lipid-based vesicles decorated with surface proteins.



An alternative approach was investigated, this strategy is based on exploiting RBC-derived extracellular vesicles (RBCEVs). Indeed, much attention has been paid to RBCEVs and their potential exploitation as a drug delivery system. To address these points, we tried to set up the lab-scale production of RBCEVs by a newly proposed physical vesiculation method and fine-tune the purification process.

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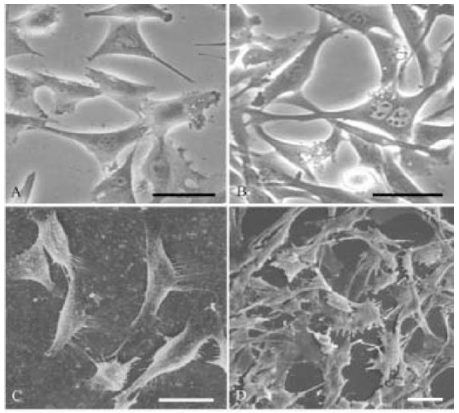
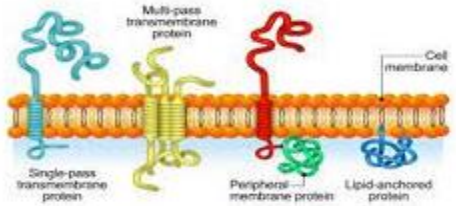
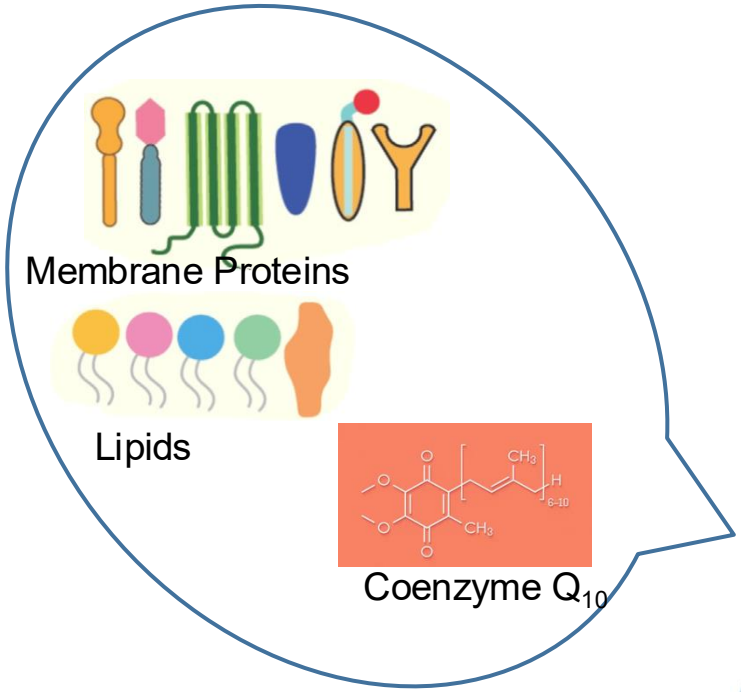
SCHEMATIC ILLUSTRATION OF THE PROCEDURES FOLLOWED TO GENERATE BIOINSPIRED EXOSOME-MIMETIC NANOVESICLES

C2C12 Myocytes

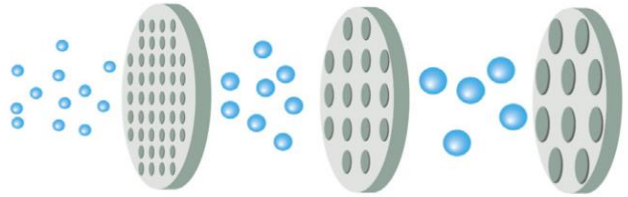


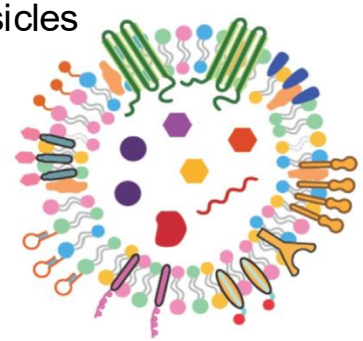


Membrane Protein Exctraction

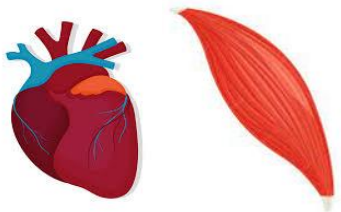


Serial Extrusion





Bioinspired exosome-mimetic nanovesicles



Target tissues

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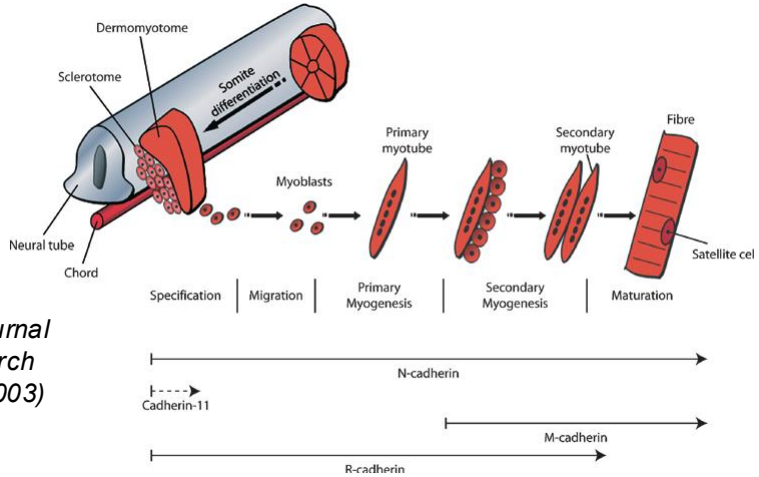
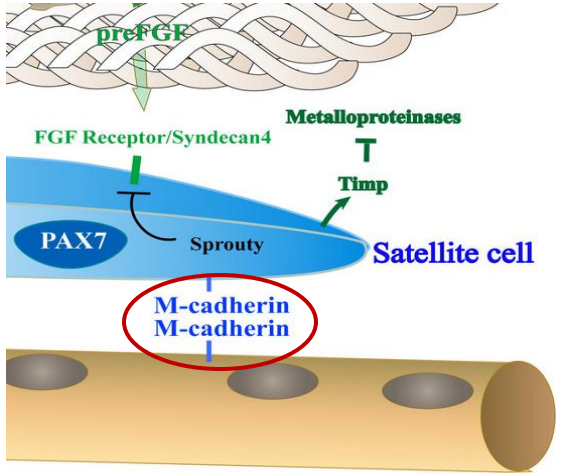
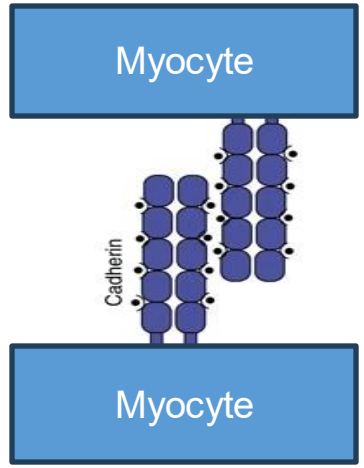
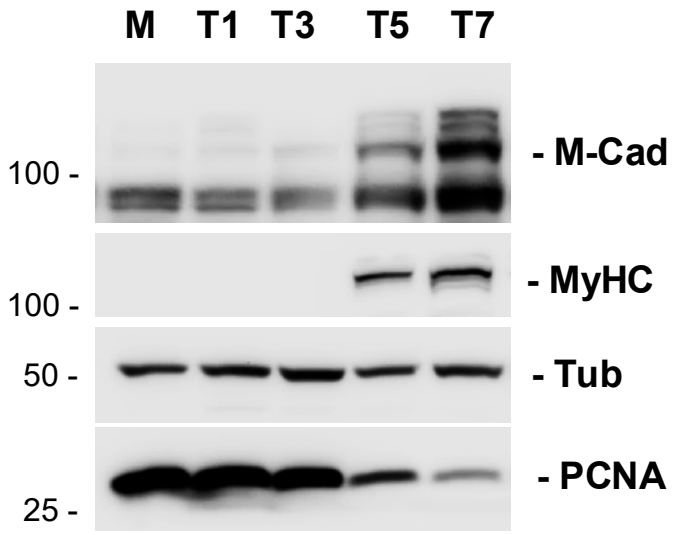
Schematic representation of skeletal muscle formation in vertebrates and cadherin expression during this process.

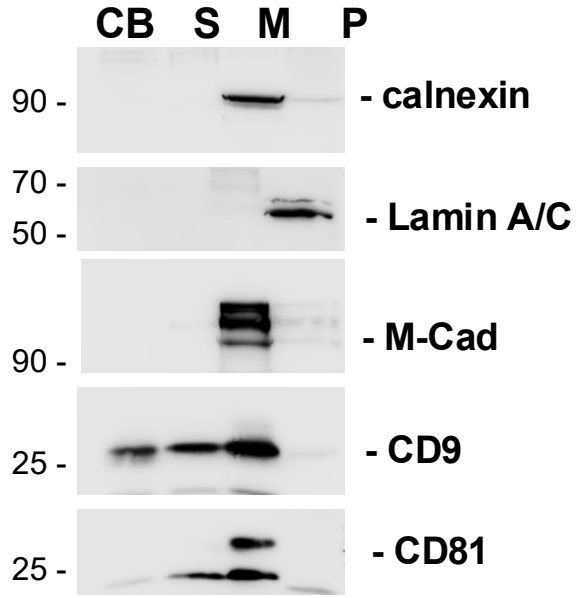


Charrasse S. Journal of Muscle Research & Cell Motility (2003)









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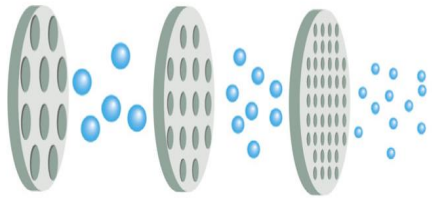
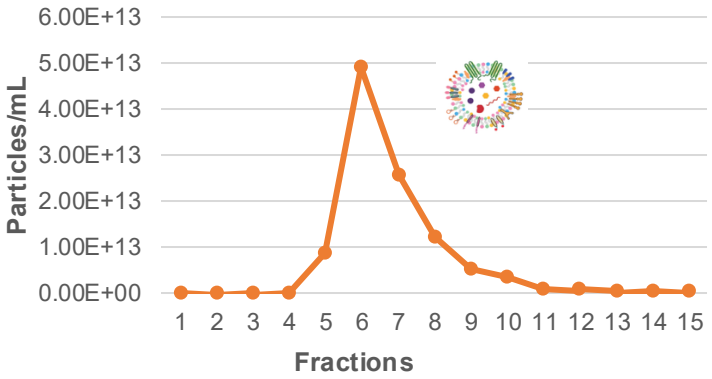
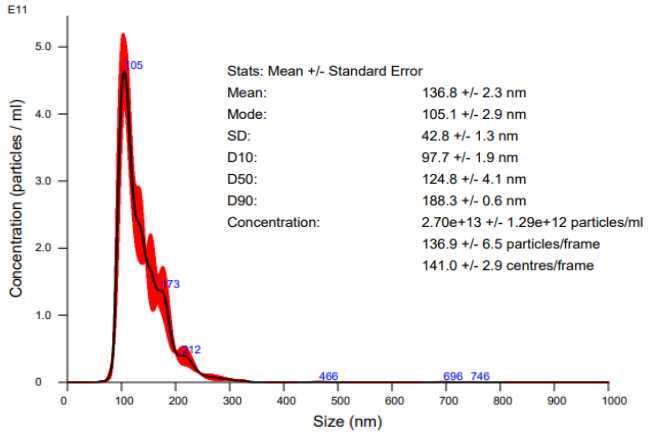


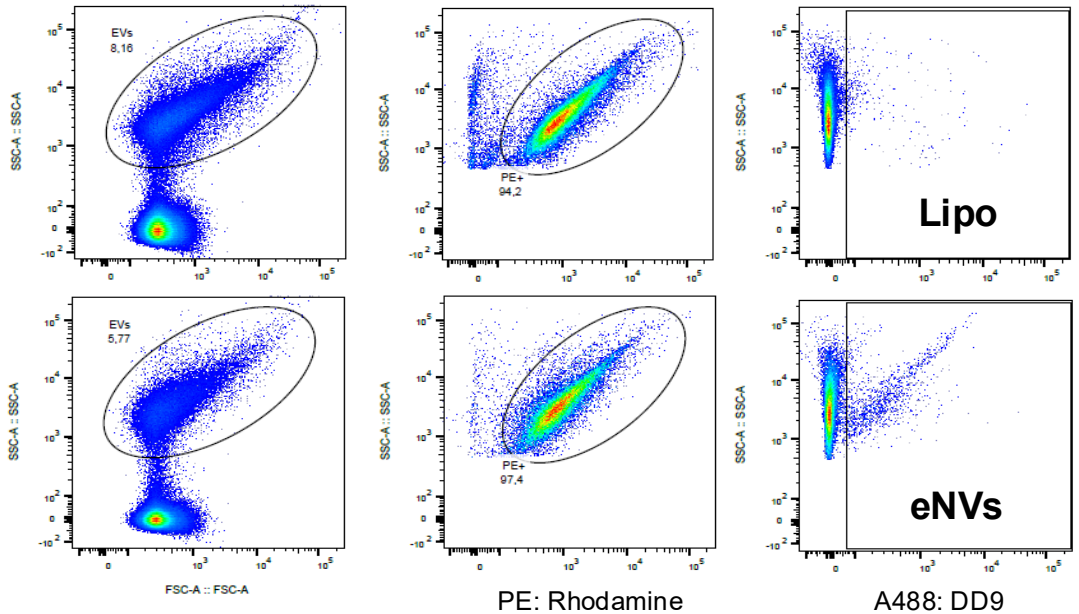
Size-Exclusion Chromatography of Bioinspired exosome-mimetic nanovesicles



Nanoparticle Tracking analysis of Engineered biomimetic nanovesicles



Flow cytometry characterization of Engineered biomimetic nanovesicles



PE: Rhodamine A488: DD9

- SEC of the extruded nanovesicles showed co-elution of labelled lipids and nanoparticles
- NTA demonstrated that the extrusion process allowed us to produce nanovesicles ranging from 90 to 180 nm in size
- Flow cytometry confirmed the presence of CD9 onto the nanovesicle surface

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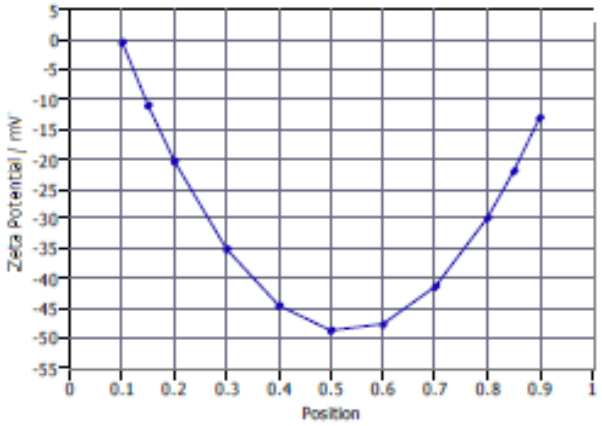
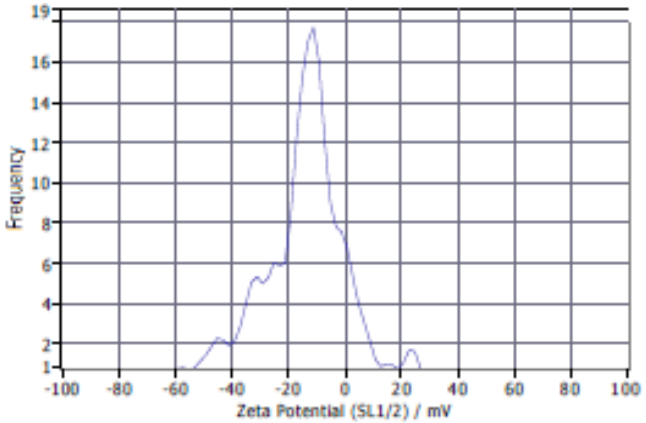
Measurement of the Zeta Potential of Synthetic Vesicles Using Nanoparticle Tracking Analyzer

Result (sizes in nm)

	Number	Concentration	Volume
Median (X50)	126.1	126.1	240.6
StdDev	64.9	64.9	354.7

Concentration: 5.0E+7 Particles / mL
Dilution Factor: 1000000
Original Concentration: 5.0E+13 Particles / mL





Result
Mobility: -1.16 ± 0.06 µm/sec/V/cm, @ 25 °C: -1.16 µm/sec/V/cm
ZP Factor: 12.8 (Smoluchowski)
Zeta Potential @ 25 °C: -14.87 ± 0.79 mV
Zeta Potential Distribution: -14.87 mV FWHM 13.34 (SL1/2)
Concentration: 6.6E+7 Particles / mL
Dilution Factor: 1000000
Original Concentration: 6.6E+13 Particles / mL

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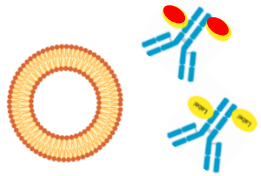
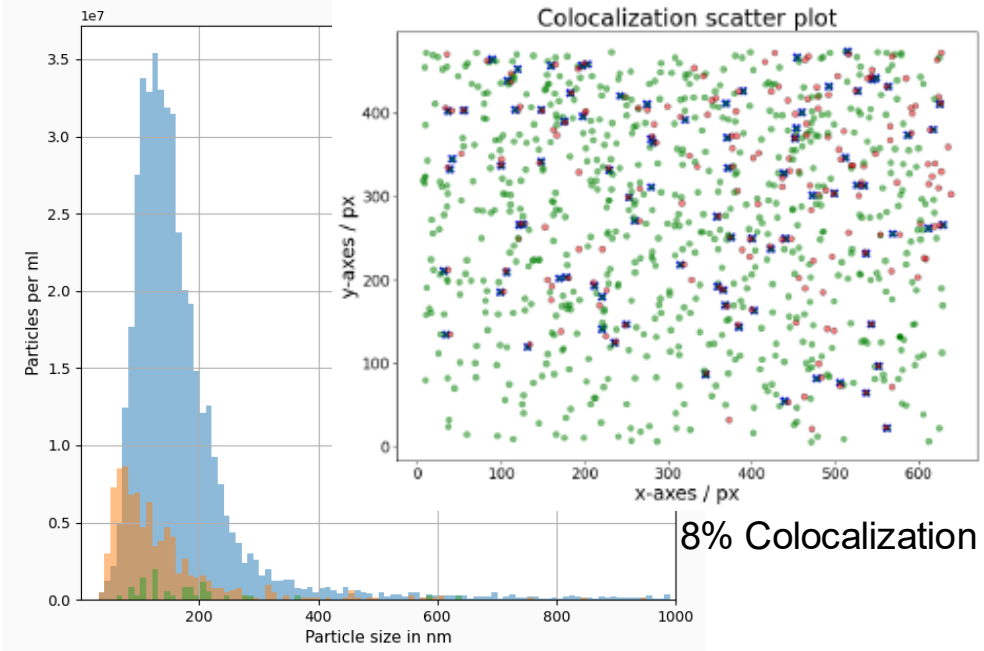
Colocalization with Fluorescence Nanoparticle Tracking (F-NTA)

Liposomes
CD9(AF488)/CD81(AF647)

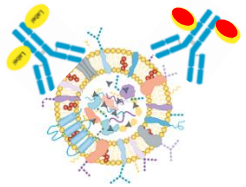


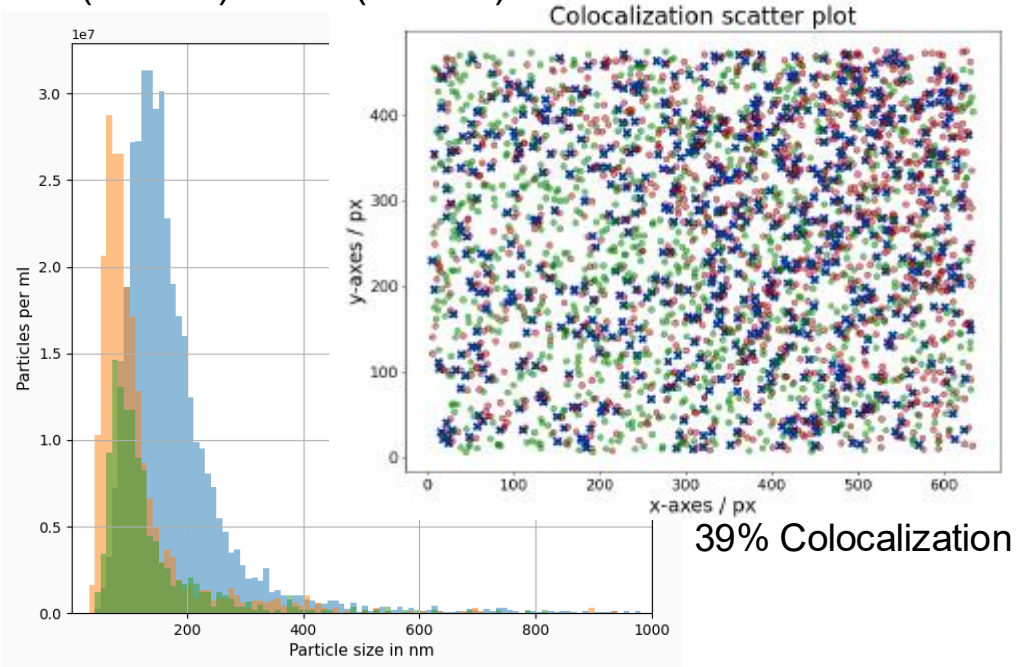


View	Experiment	Peak	Median	#Particles	Avg. Num Det.	FWHM	Concentration
e_id: 50, ZetaScript: NP, Sample name: Z-24-037-A							
	NP_scatter	128.7 (± 6.1)	147.1 (± 11.3)	6499	679.9 (± 141.7)	120.4 (± 29.5)	1.73E+13 (± 3.6E+12)
	NP_fluor_488	90.1 (± 10.8)	108.7 (± 12.9)	560	138.8 (± 37.0)	128.2 (± 25.5)	3.53E+12 (± 9.4E+11)
	NP_fluor_640	128.7 (± 18.2)	152.4 (± 26.7)	39	63.0 (± 5.5)	163.2 (± 7.3)	1.60E+12 (± 1.4E+11)

8% Colocalization

Synthetic Vesicles
CD9(AF488)/CD81(AF647)





View	Experiment	Peak	Median	#Particles	Avg. Num Det.	FWHM	Concentration
e_id: 52, ZetaScript: NP, Sample name: Z-24-037-B2							
	NP_scatter	136.3 (± 5.2)	153.4 (± 10.3)	5925	615.8 (± 160.2)	116.0 (± 18.5)	1.57E+13 (± 4.1E+12)
	NP_fluor_488	76.2 (± 5.0)	88.6 (± 4.5)	1478	342.1 (± 56.9)	72.8 (± 8.8)	8.71E+12 (± 1.4E+12)
	NP_fluor_640	91.3 (± 7.5)	103.2 (± 7.7)	686	185.6 (± 24.2)	83.3 (± 10.7)	4.72E+12 (± 6.2E+11)

39% Colocalization

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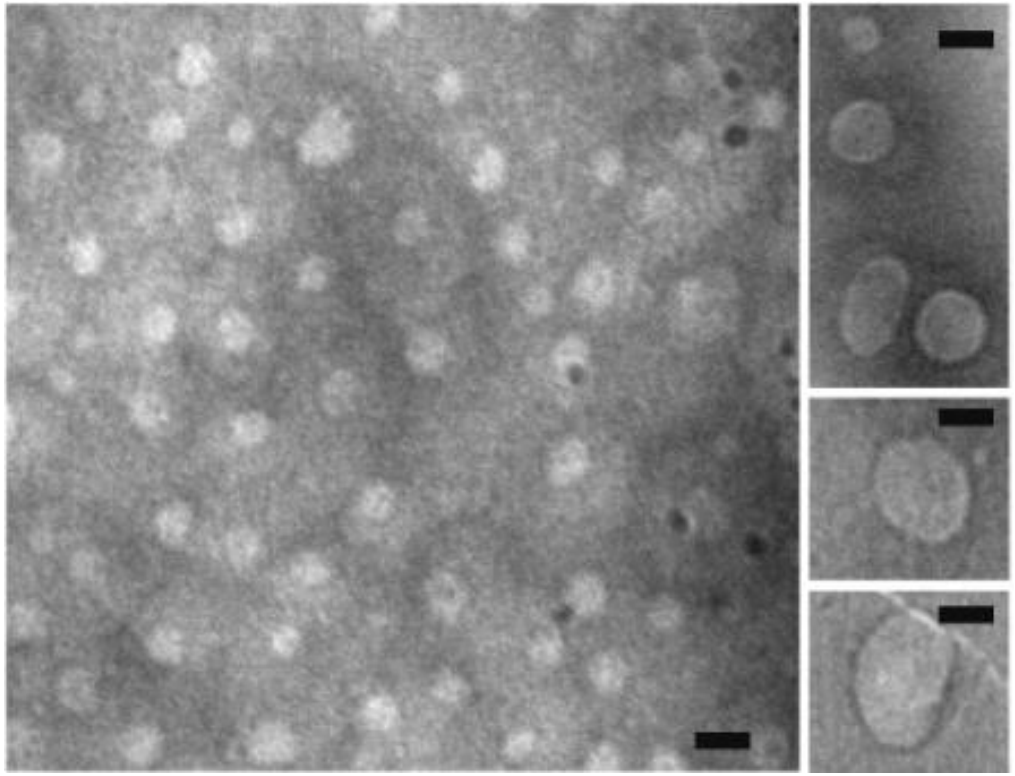
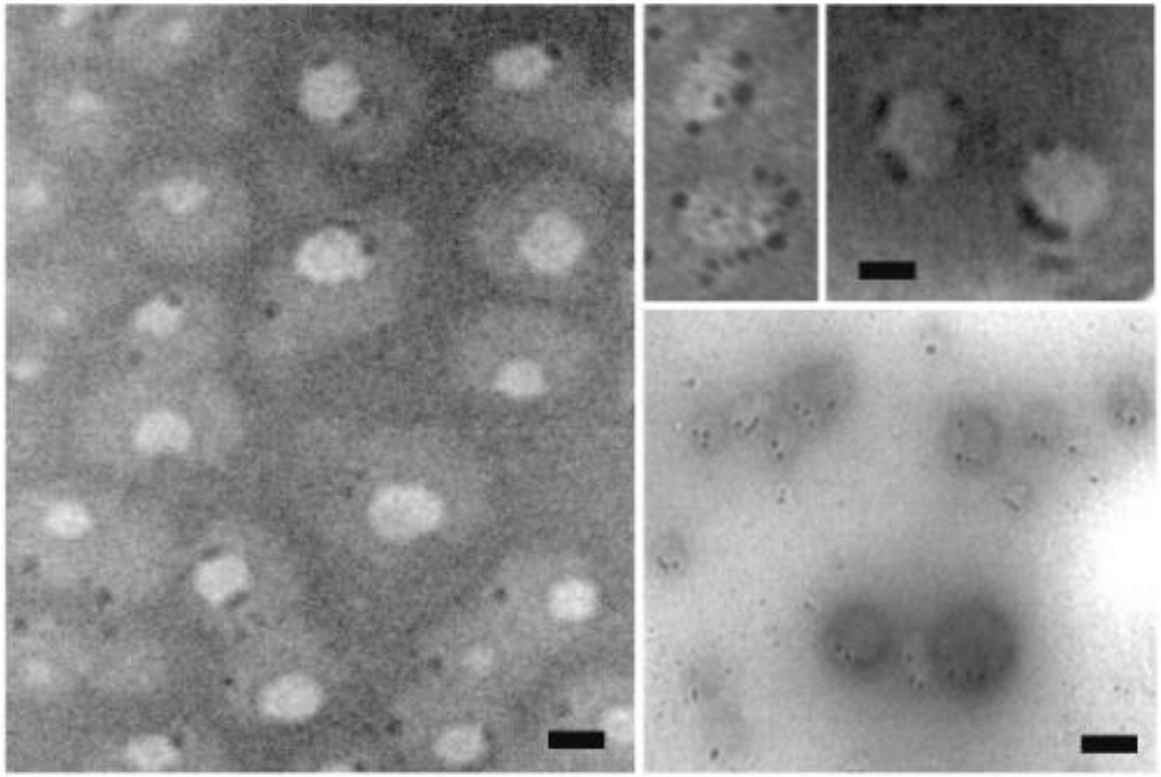
Characterization of Synthetic Vesicles by Immunolabeling Electron Microscopy

Liposomes



Synthetic Vesicles



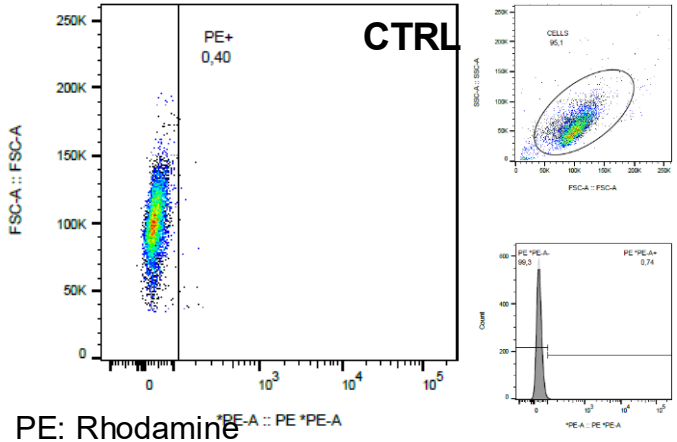
SV UPTAKE IN DIFFERENT TARGET CELLS

Incubation at 37°C for 4 and 24h

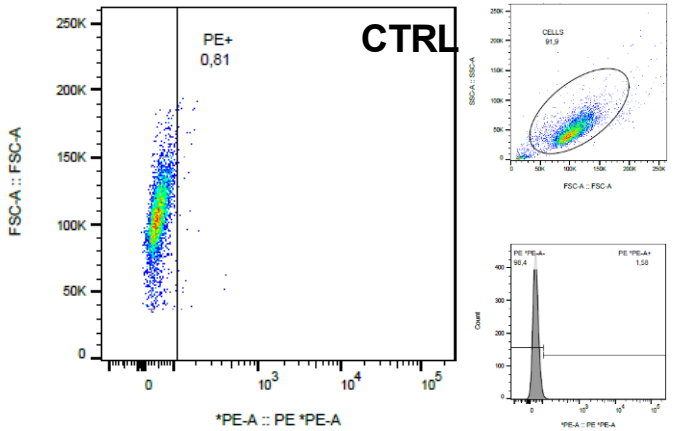
Rhodamine-labeled phospholipids

- C2C12
- H9C2
- AC16
- HeLa

Incubation for 4 h



Incubation for 24 h



PE: Rhodamine

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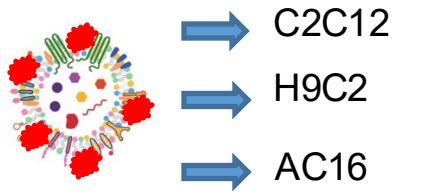


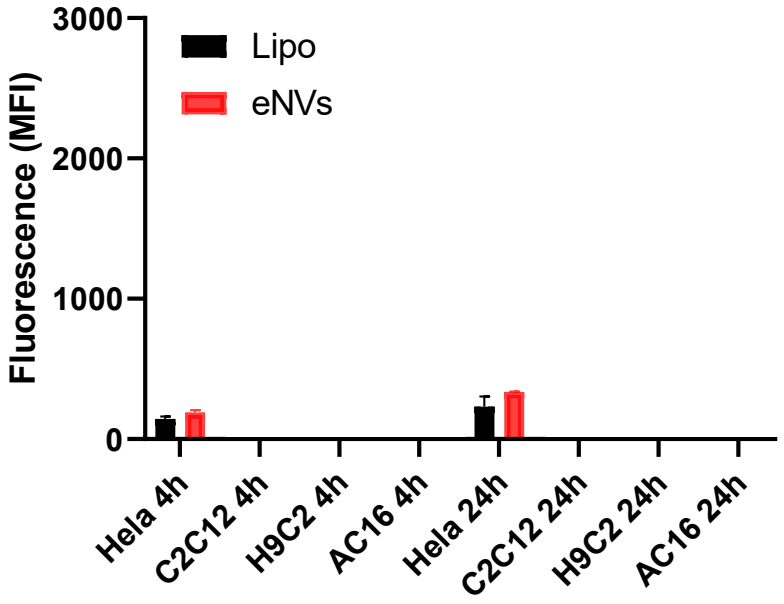
Rhodamine-labeled phospholipids

C2C12

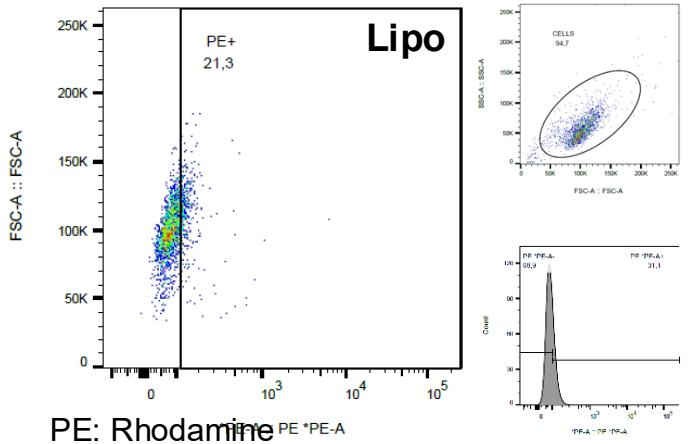
H9C2

AC16

HeLa

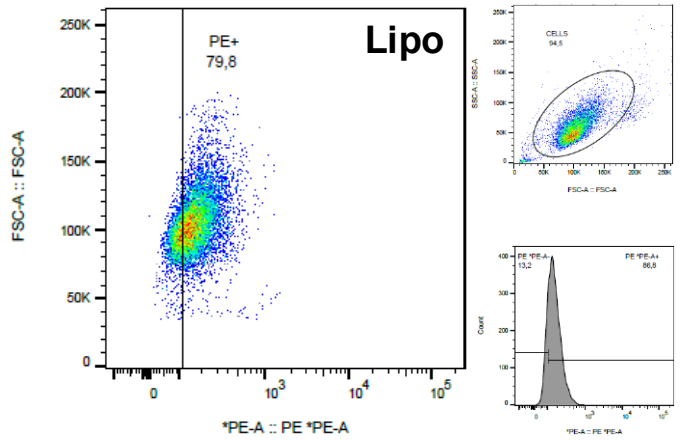


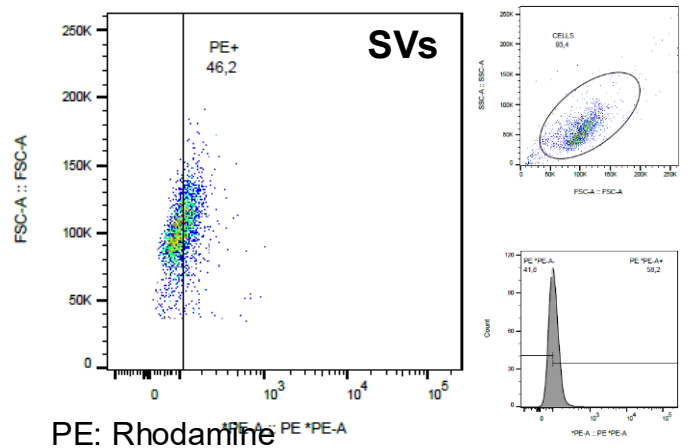
Incubation for 4 h



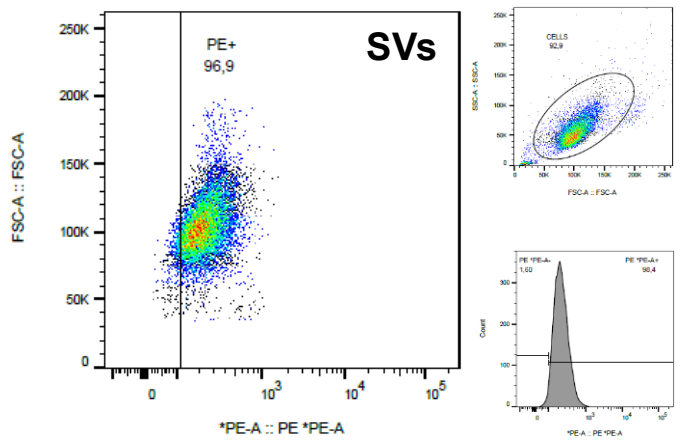
PE: Rhodamine

Incubation for 24 h





PE: Rhodamine



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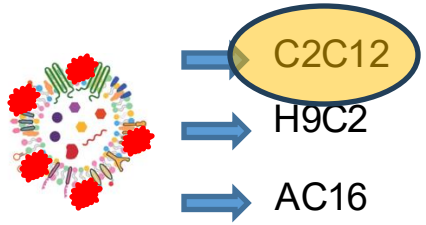
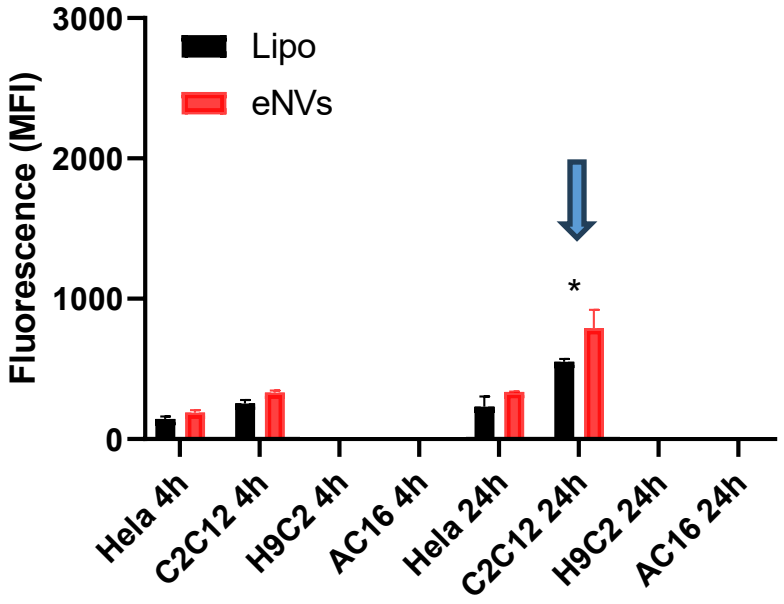
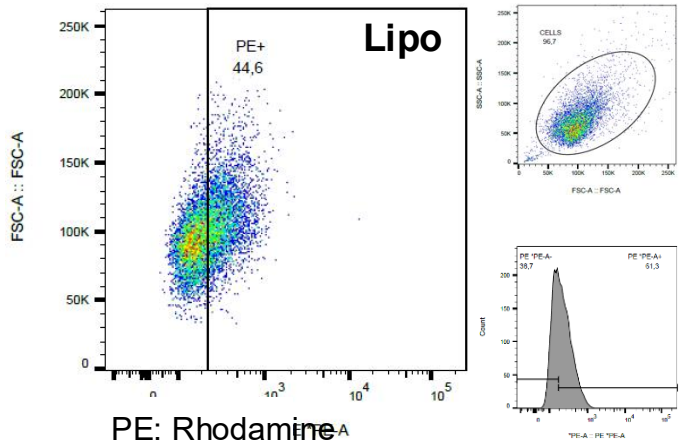


Rhodamine-labeled phospholipids

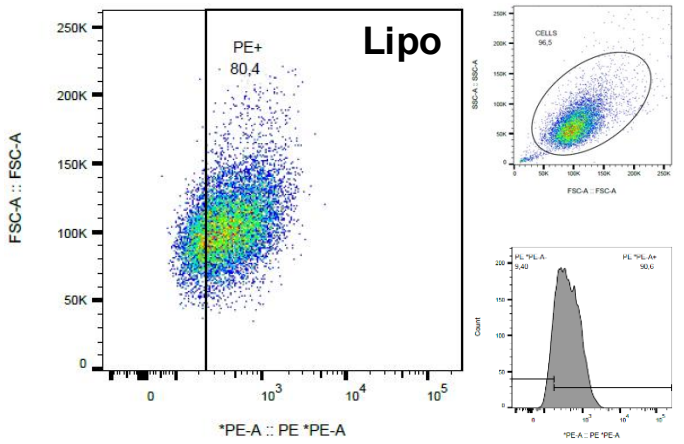
- C2C12
- H9C2
- AC16
- HeLa

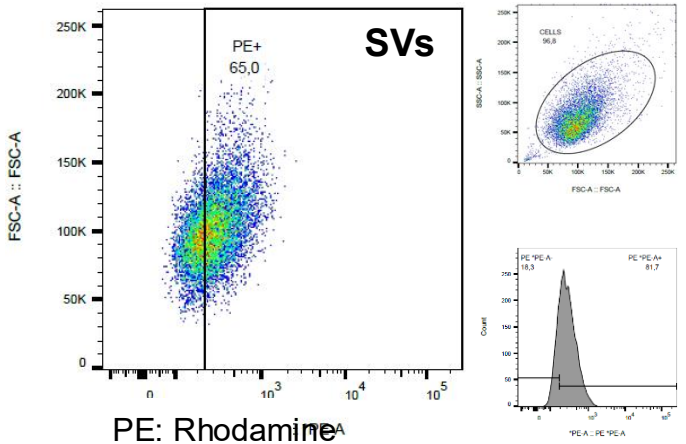


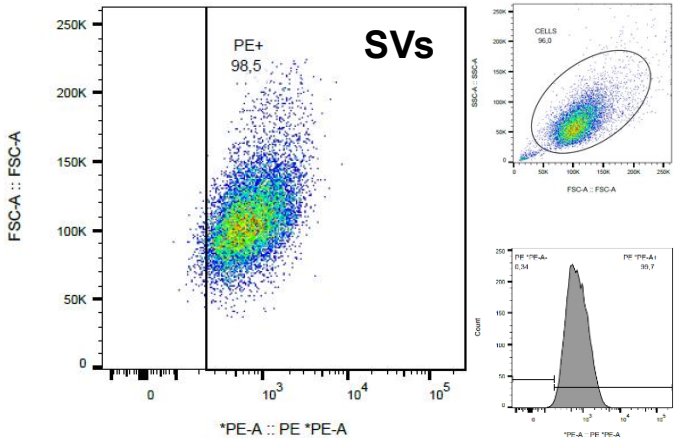
Incubation for 4 h



Incubation for 24 h







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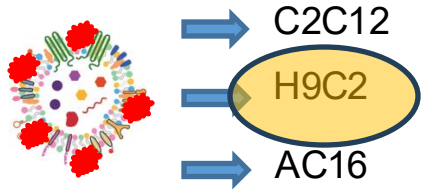
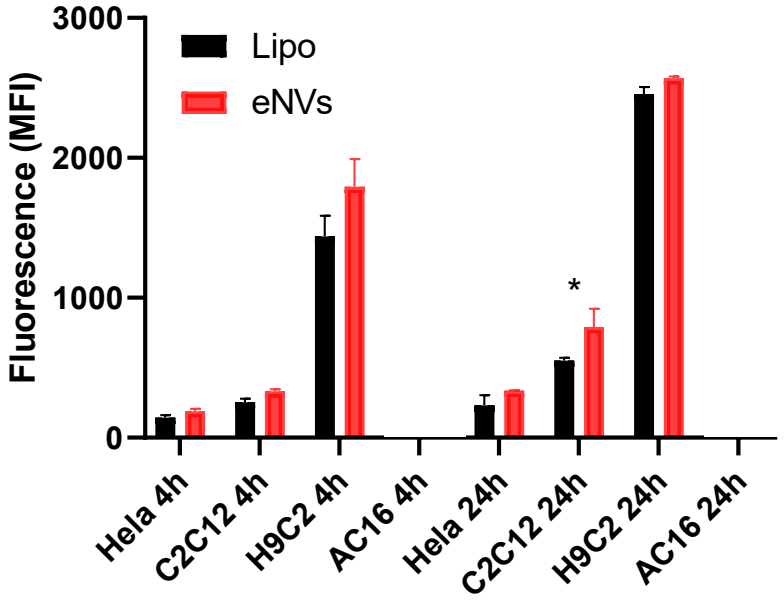
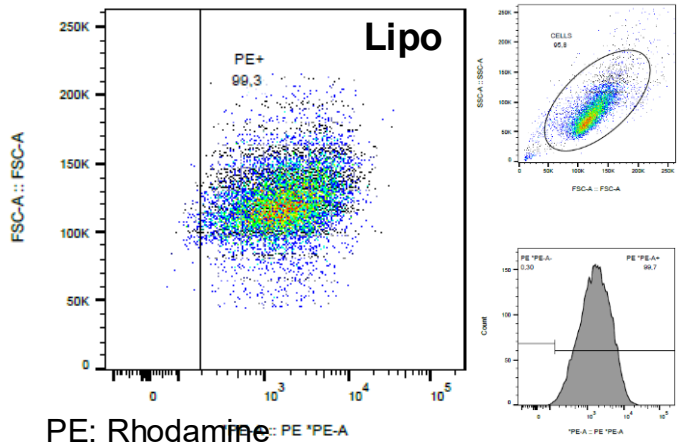


C2C12
H9C2
AC16
HeLa

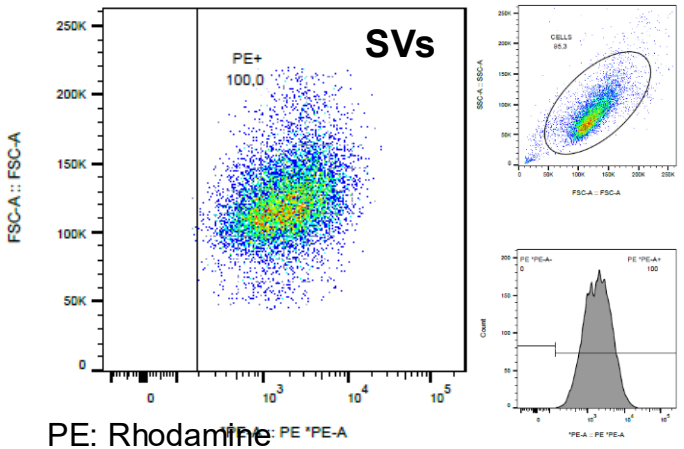
Rhodamine-labeled phospholipids



Incubation for 4 h

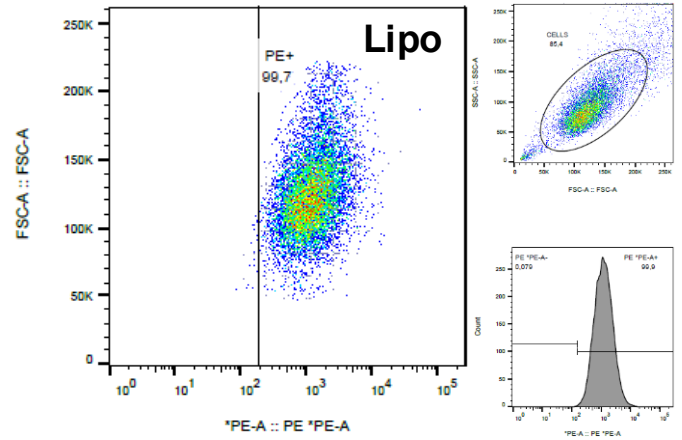


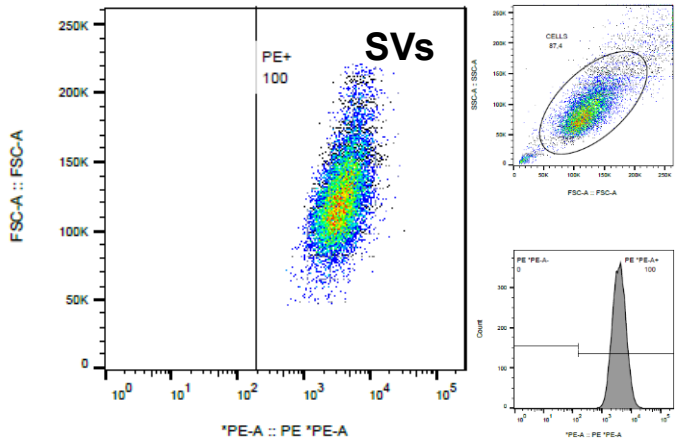
PE: Rhodamine



PE: Rhodamine

Incubation for 24 h





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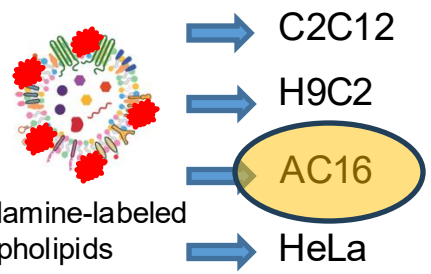
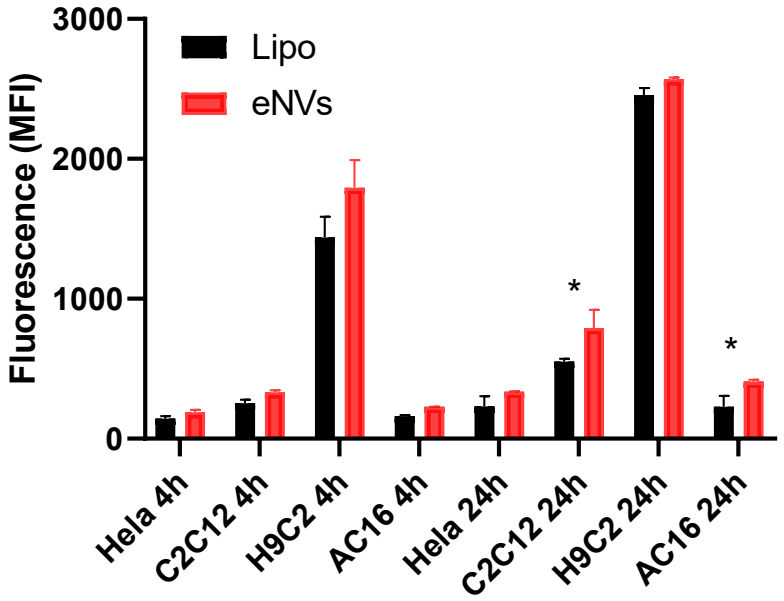
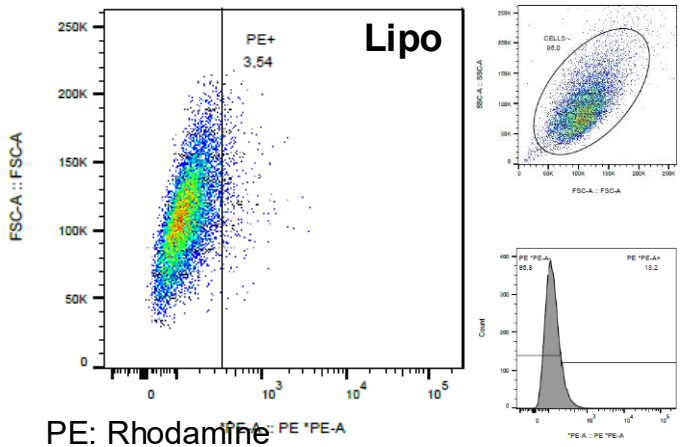
SV UPTAKE IN DIFFERENT TARGET CELLS

Incubation at 37°C for 4 and 24h

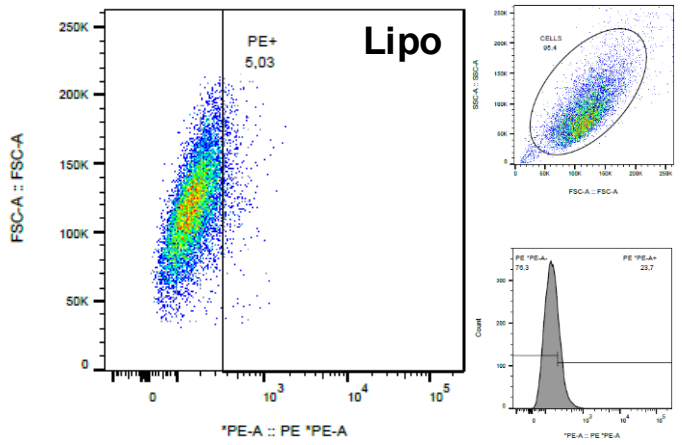




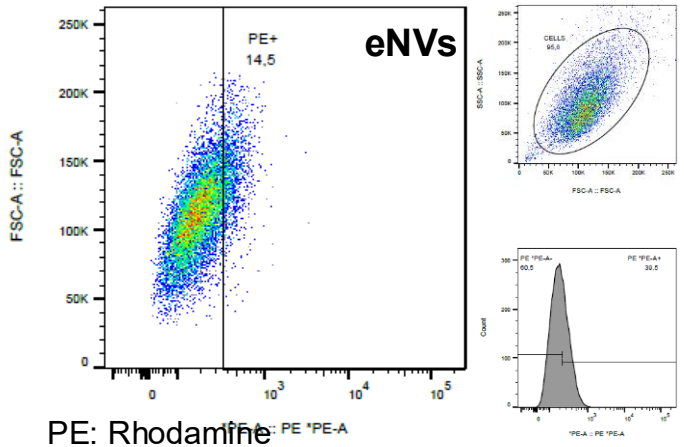
Incubation for 4 h

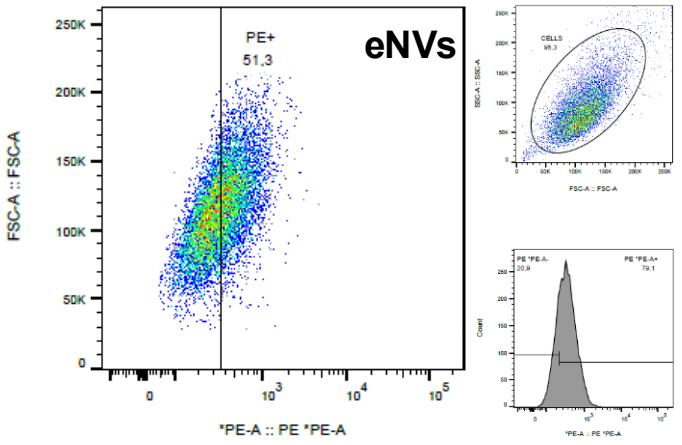


Incubation for 24 h



PE: Rhodamine





PE: Rhodamine

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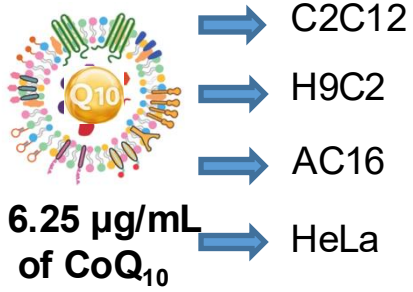
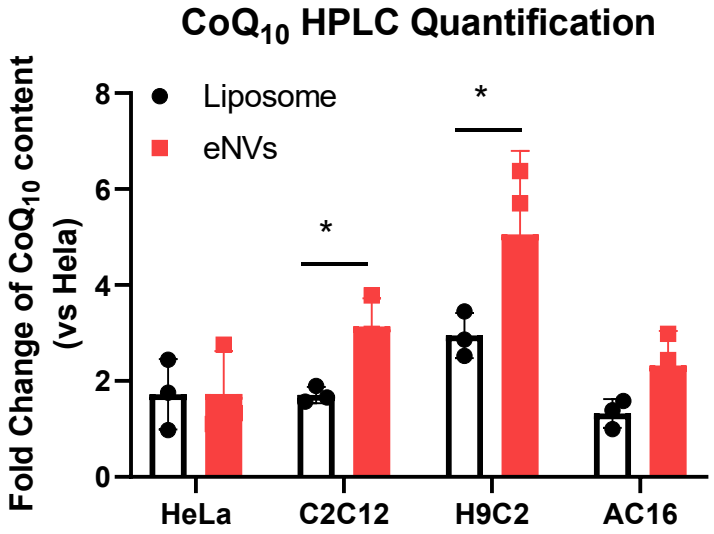
Incubation at 37°C for 24 h

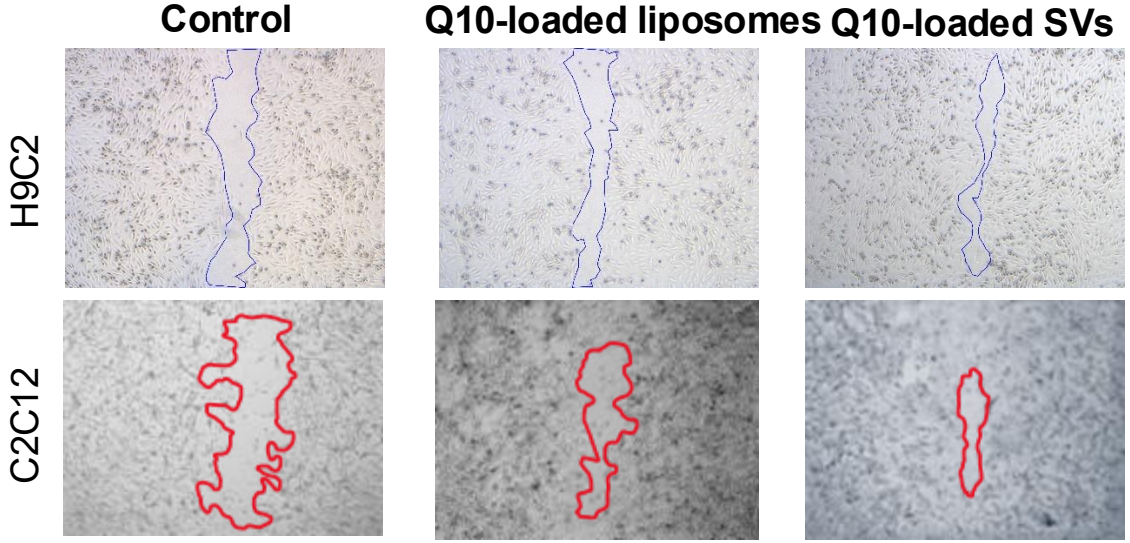




C2C12 and H9C2 cells take up more eNV-derived CoQ_{10} than other cells.

Wound healing assay in H9C2 and C2C12

i. H9C2 and C2C12 cells were pre-incubated with Q_{10}-loaded vesicles;
ii. 0.3 mM H_2O_2 treatment for 1 h;
iii. 18 h recovery after scratching.



Q_{10}-loaded eNVs protect H9C2 cardiomyocytes against oxidative stress.

CONCLUSION

Taken together, the reported data suggest that bioengineered synthetic nanovesicles (SVs), decorated with C2C12 membrane proteins, can serve as novel exosome-mimetics to deliver CoQ_{10} to muscle cells effectively.

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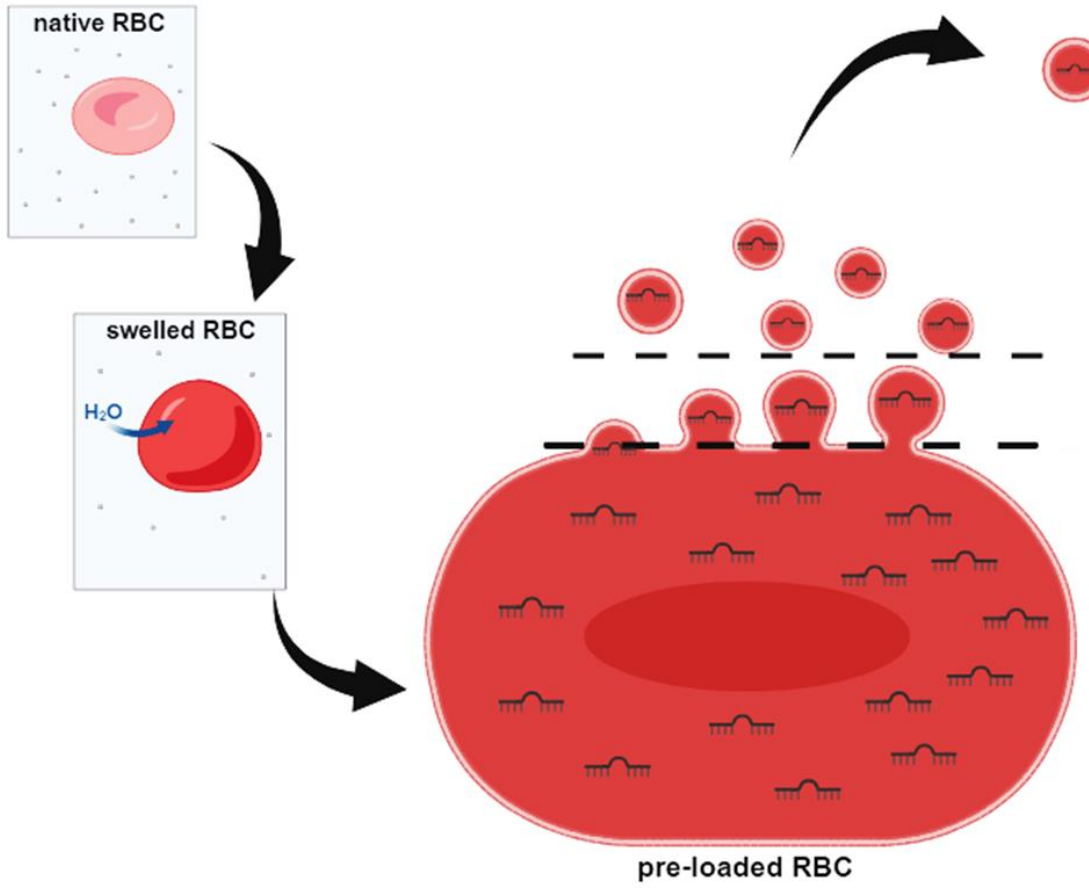
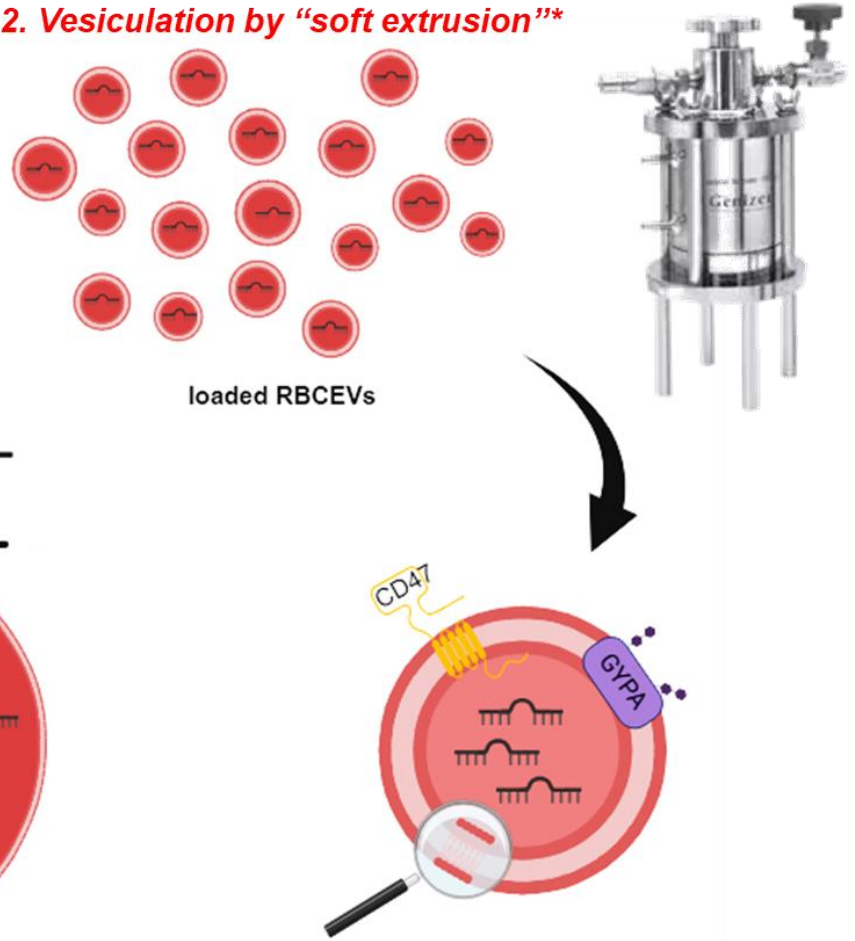
Red Blood Cell-derived Extracellular Vesicle (RBCEV) mimetics

1. RBC pre-loading by hypotonic dialysis



*2. Vesiculation by "soft extrusion"**



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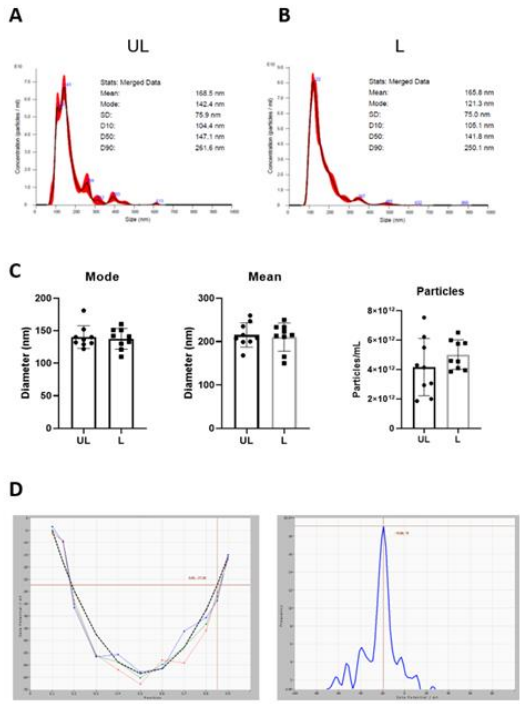
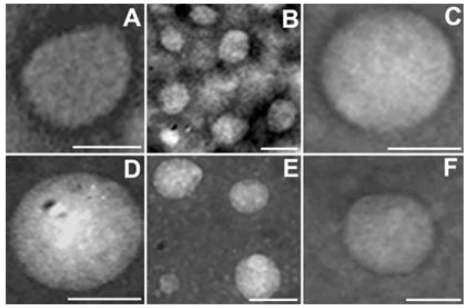
RBCEV characterization according to MISEV2023

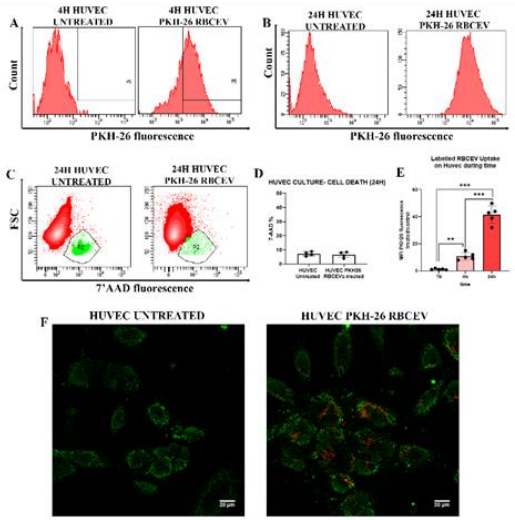
NTA characterization



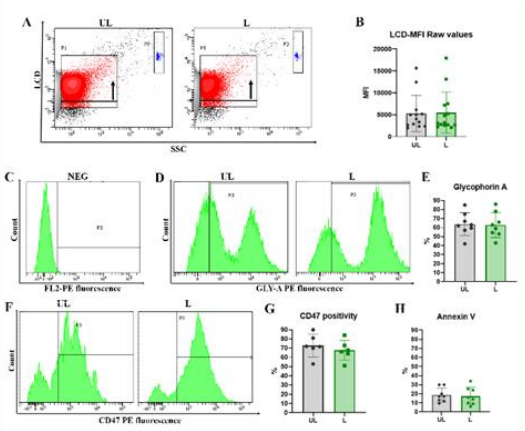
TEM characterization



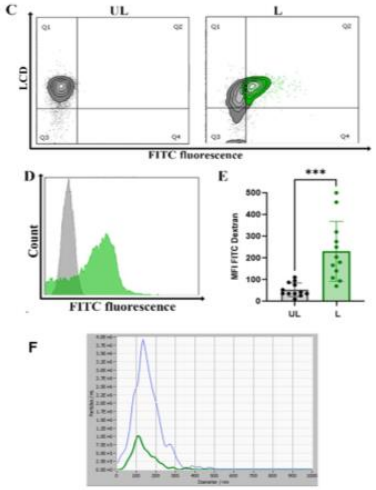
Cell uptake



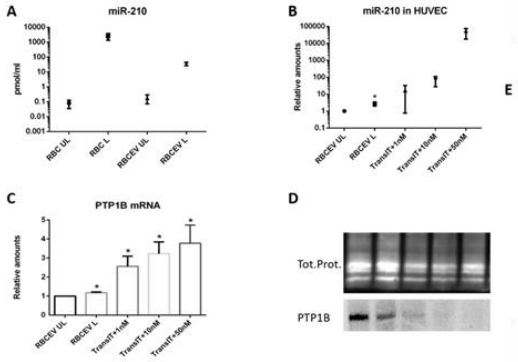
FC characterization



Cargo-loading



Biological effect



Biagiotti S, Canonico B, Tiboni M, Abbas F, Perla E, Montanari M, Battistelli M, Papa S, Casettari L, Rossi L, Guescini M, Magnani M. *Efficient and highly reproducible production of red blood cell-derived extracellular vesicle mimetics for the loading and delivery of RNA molecules*. **Sci Rep. 2024** Jun 25;14(1):14610.

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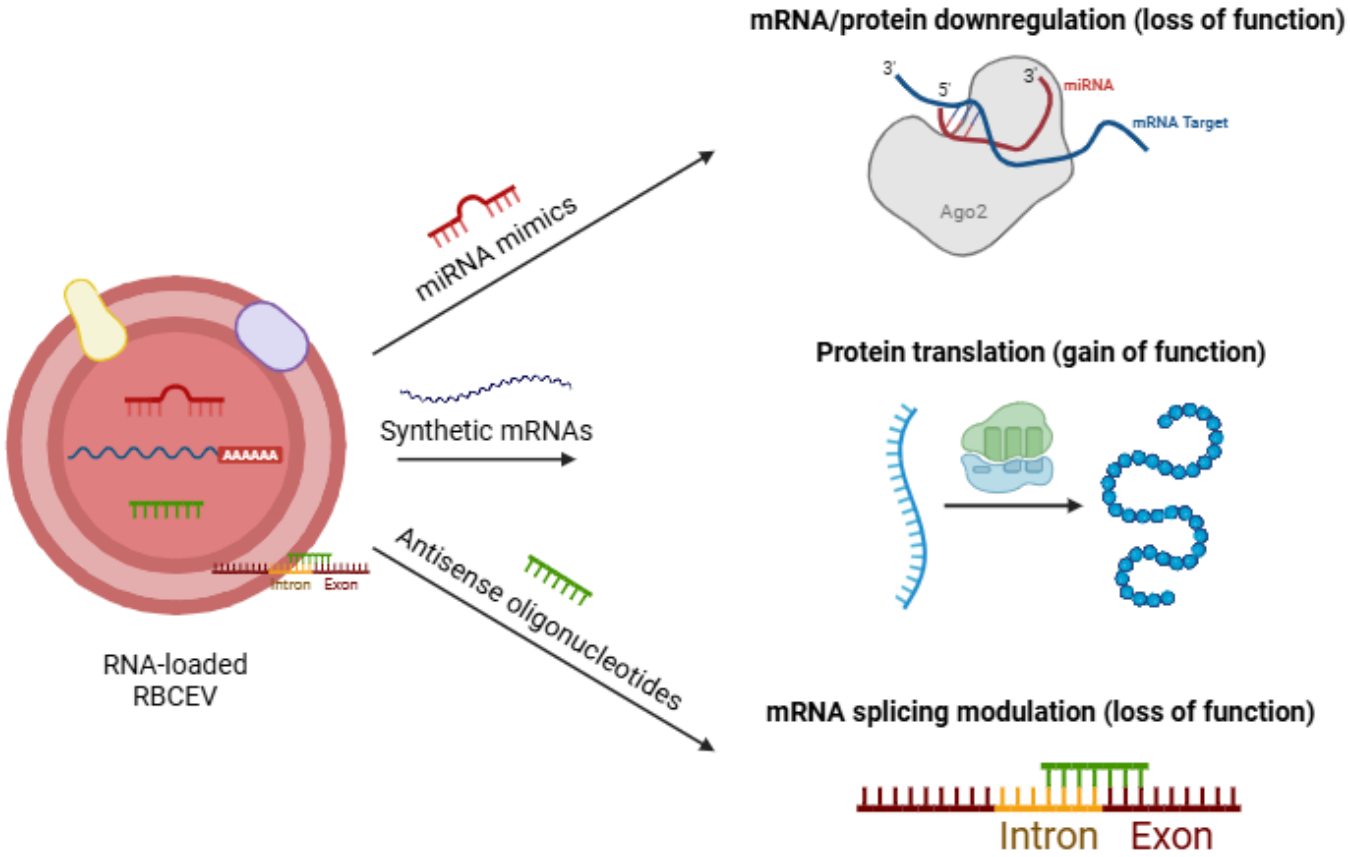
RBCEVs for the loading and delivery of RNA molecules



Ongoing applications: RBCEVs for the delivery of miRNAs and ASO to modulate gene expression or splicing, and of mRNA to treat genetic diseases

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ACKNOWLEDGMENTS

1506 UNIVERSITA DEGLI STUDI DI URBINO CARLO BO

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Department of Biomolecular Sciences
Prof. M. Guescini
Dr. P. Ceccaroli, Dr. E. Polidori, Dr. R. Agostini, Dr. S. Fondi, Dr. S. Silvestri

Prof.ssa S. Biagiotti, Prof.ssa L. Rossi, Prof. M. Magnani, Dr. E. Perla

Prof.ssa M. Battistelli, Prof.ssa B. Canonico Prof. S. Papa, Dr. M. Montanari

Prof. L. Casettari, Dr. M. Tiboni

UNIVERSITÀ POLITECNICA DELLE MARCHE

MARCHE POLYTECHNIC UNIVERSITY

Department of Life and Environmental Sciences Sciences
Prof. A. Frontini, Prof. P. Orlando,

Grazie per l'attenzione