# Poly(beta aminoester) – extracellular vesicles hybrids for Antitumor Nucleic Acid Vaccination

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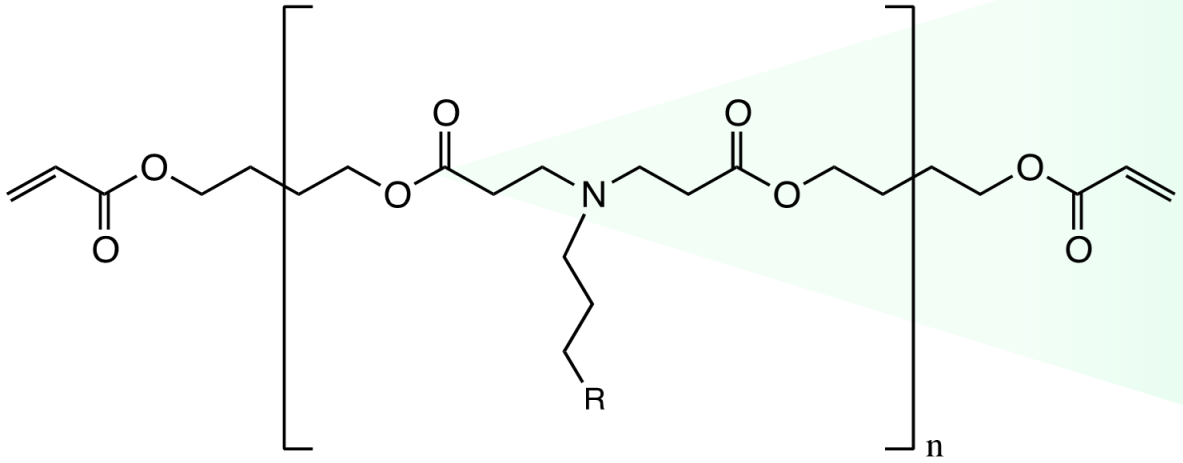
July, 2025

# Poly(beta aminoester) polymer

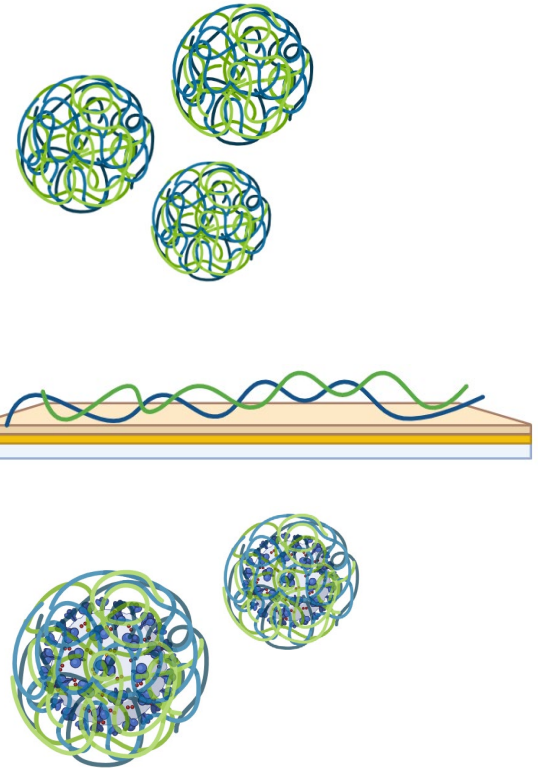
Experts on biomaterials engineering




Nanoparticle formulation

**Surface modification**

Viral coating



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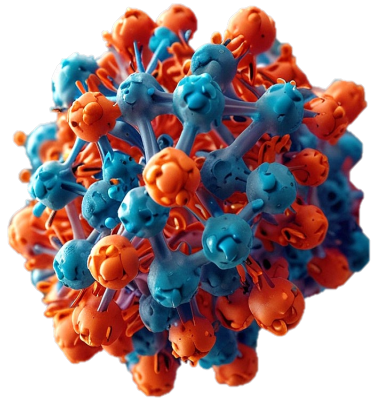
# pBAE NPs

The medicines of the future



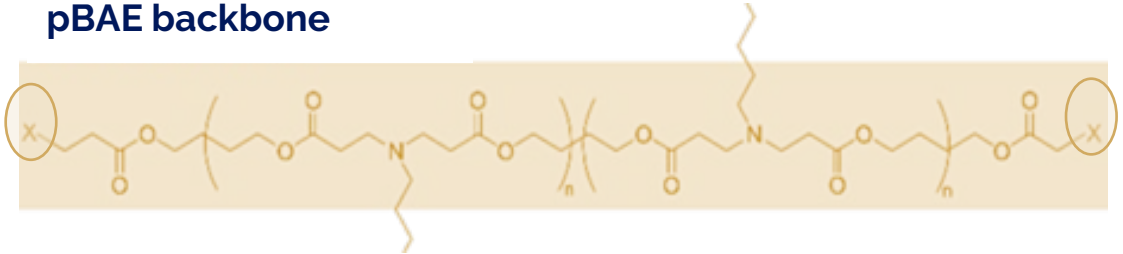
## Formulation advantages

- Easy and scalable synthesis
- Easy manufacturing
- Highly safe: no nuclear penetration
- High immunogenicity control

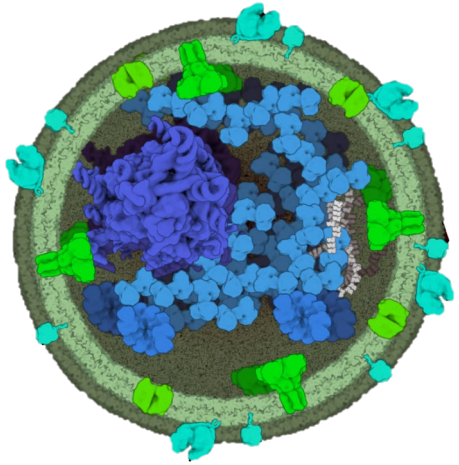
## Challenges

- Need of a targeting moiety
- Slight polymer toxicity

**pBAE backbone**



# Solution: extracellular vesicles (EVs)



The promised natural delivery systems

## EVs advantages

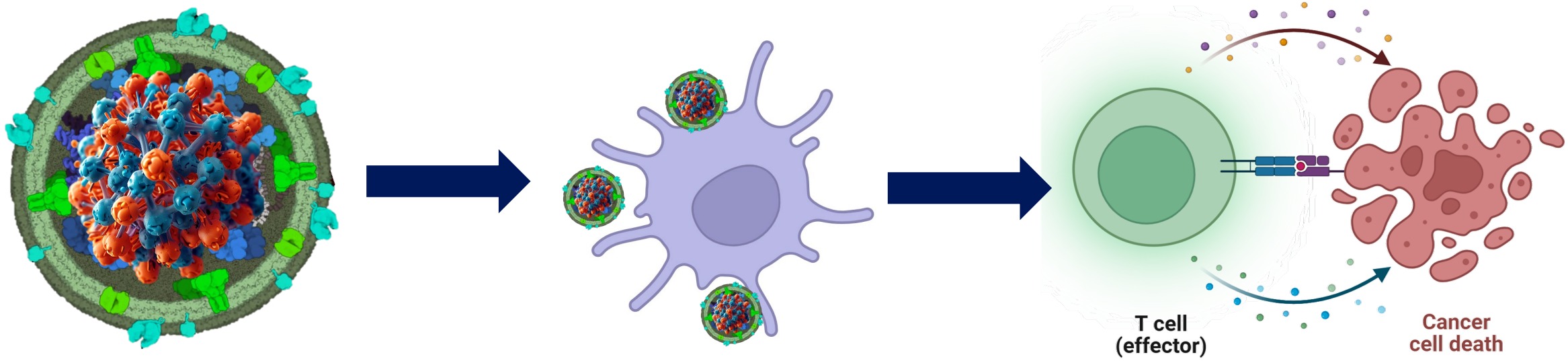
- Natural communication vesicles
- Many sources
- Highly safe
- High immunogenicity control

## Challenges

- Need of a targeting moiety
- Slight polymer toxicity

# AIM

## Cancer cell killing by biomimetic nucleic acid vaccines




**1** Formulation

**2** DC activation

**3** Tumor cell death

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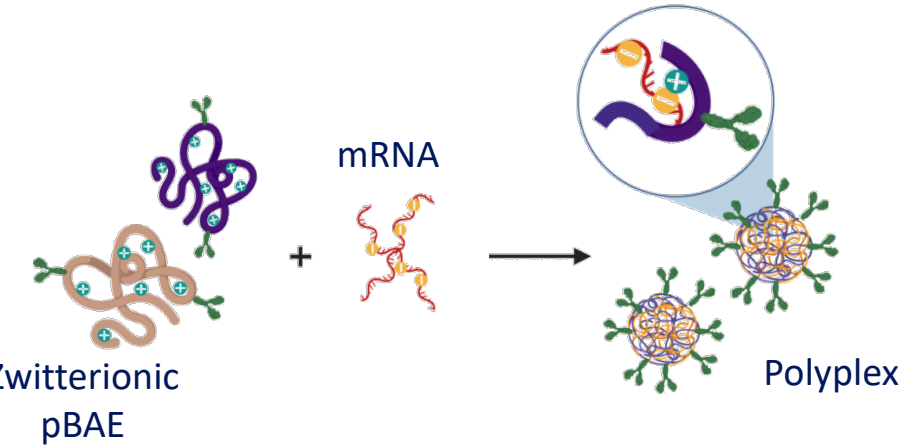
# pBAE-EV hybrids formulation

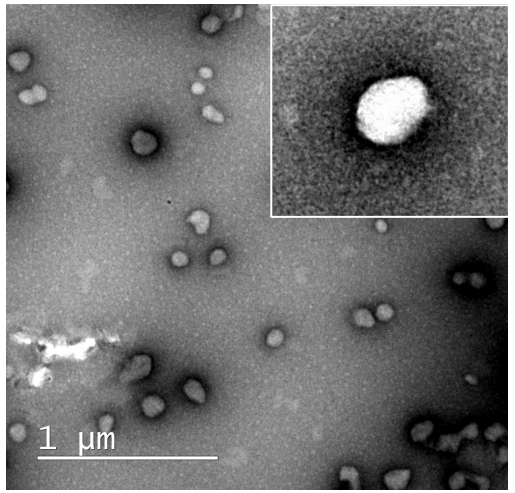
## Method of preparation: 1. pBAE NPs

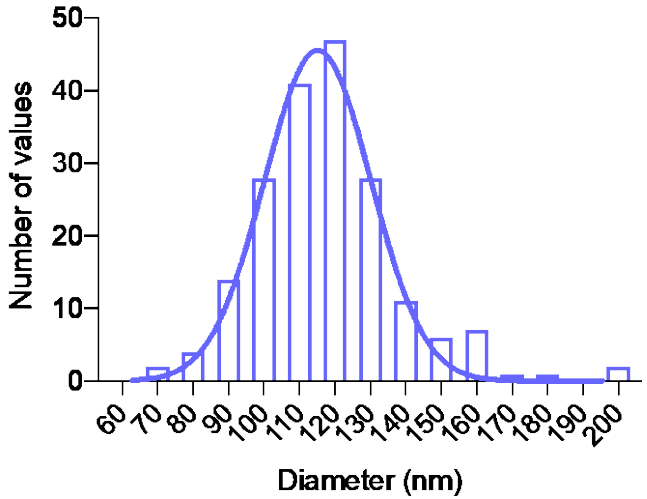



**Polyplex formulation scheme for nucleic acid encapsulation**

| NPs | Size (nm) | Z-pot (mV) | Encaps. eff. pDNA (%) |
|---|---|---|---|
| pBAE | 198.2±15.3 | 32.2±4.5 | 89.2±2.5 |

**TEM**

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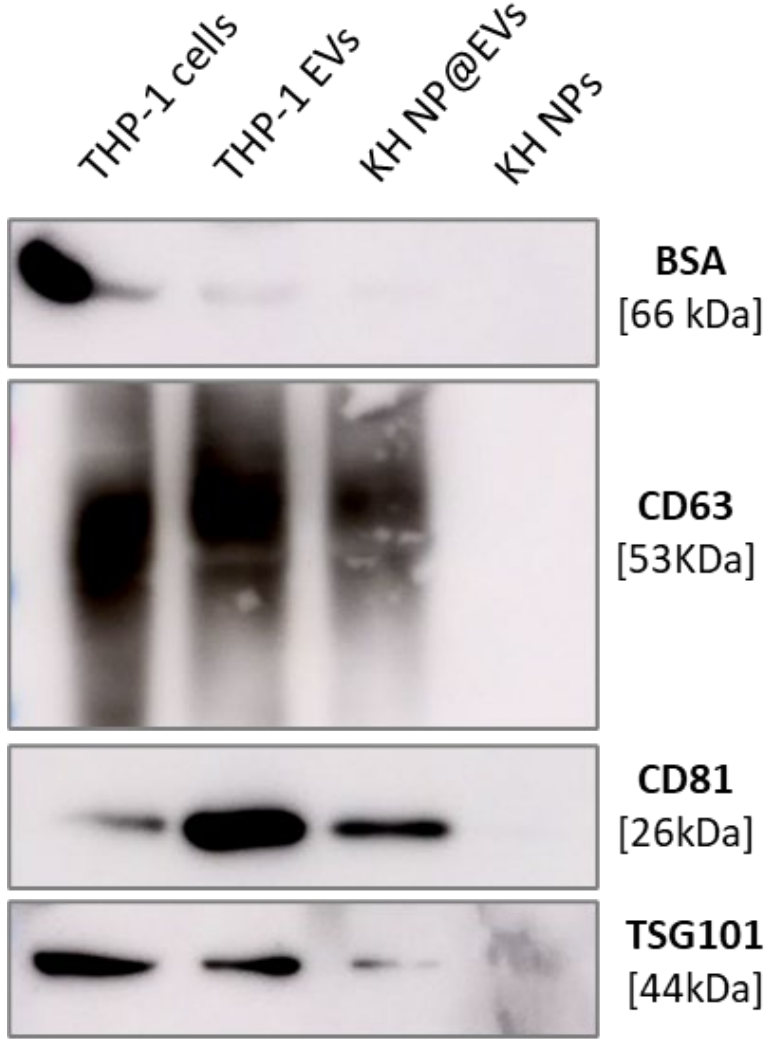
*Coral García et al. J Control Release, 2024*

# pBAE-EV hybrids formulation

## Method of preparation: 2. EVs isolation

To characterize the EVs and complexes formed, three different proteins were quantified by western blot, tetraspanins (CD63, CD81) and a membrane protein (TSG101).

**Their presence confirmed the EVs intact structure.**



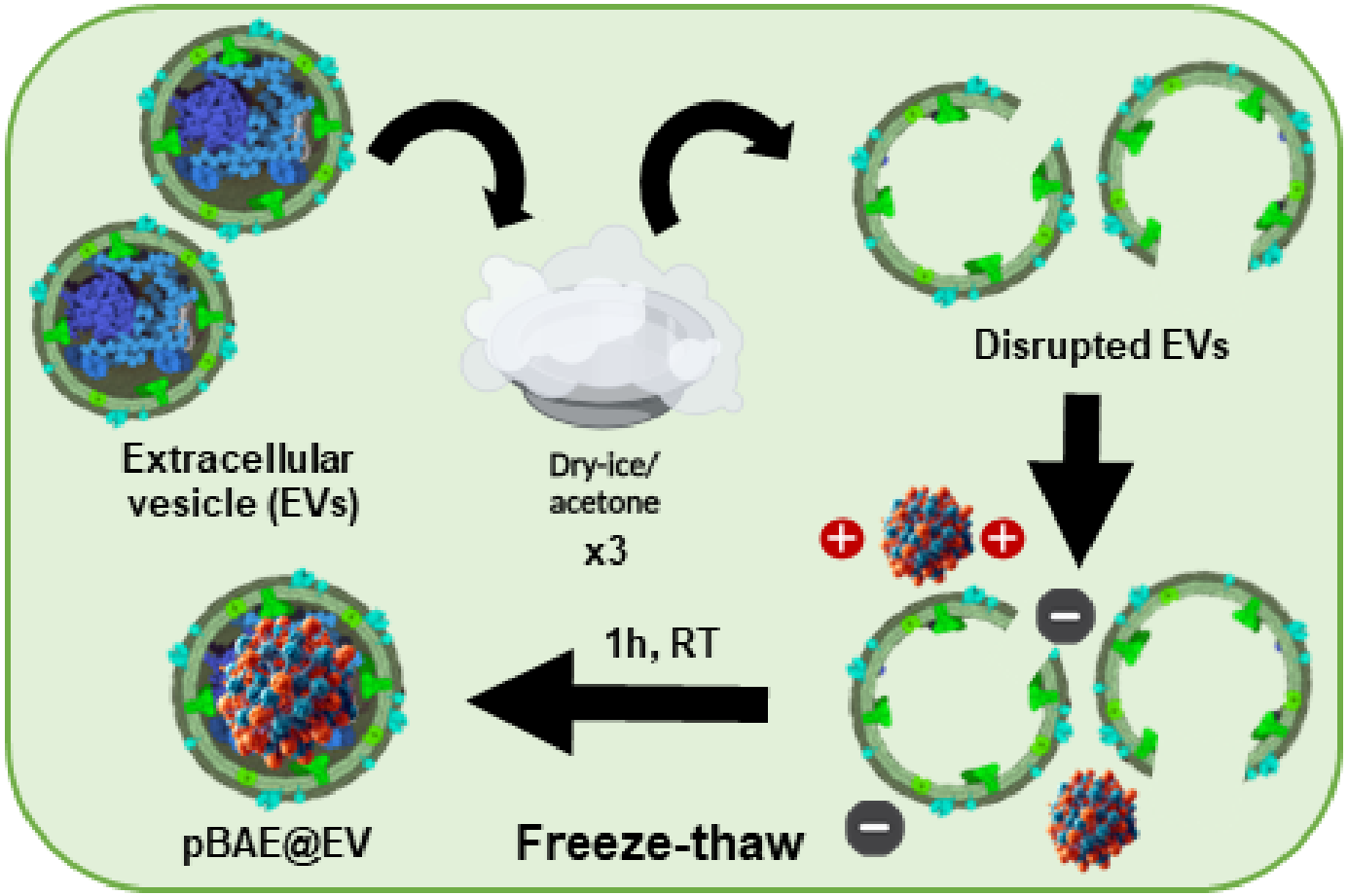



*Coral García et al. J Control Release, 2024*

# pBAE-EV hybrids formulation

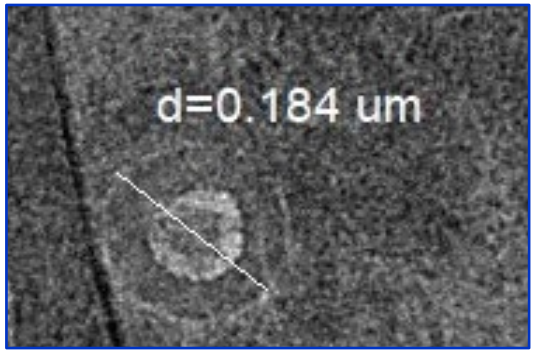
Method of preparation:
3. Coating of pBAE with EVs

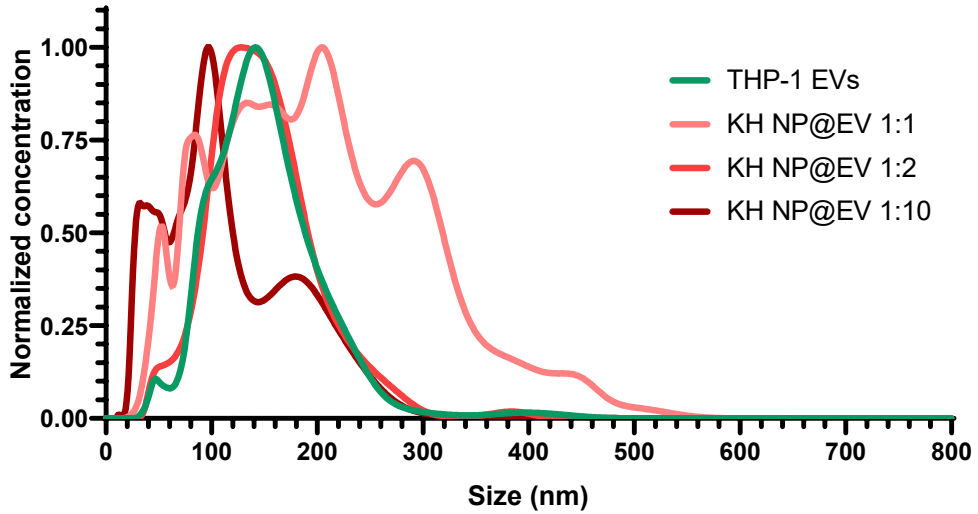
- Biodegradable
- Biocompatible
- Reproducible

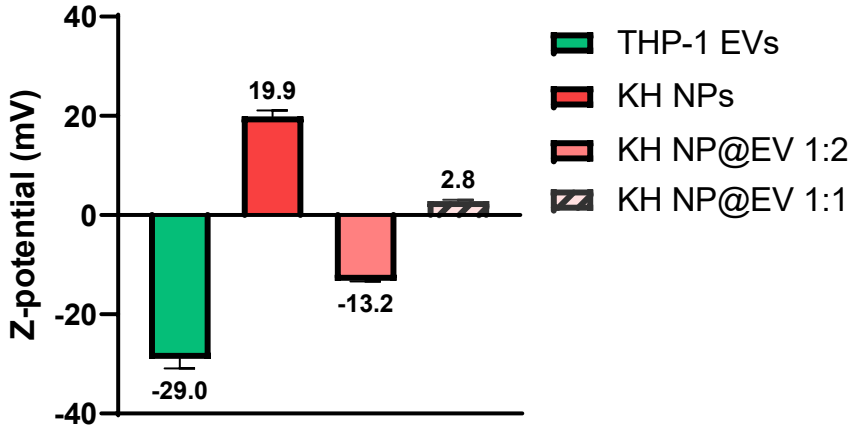


# pBAE-EV hybrids formulation

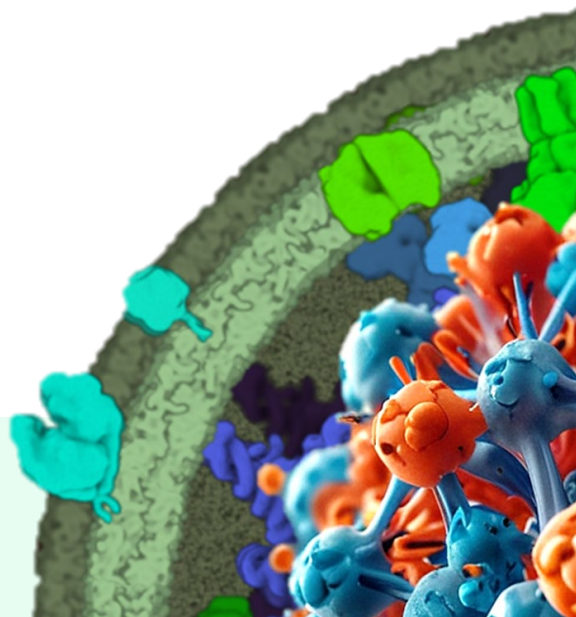
## Characterization




The complexes presented low polidispersity and were negatively charged.



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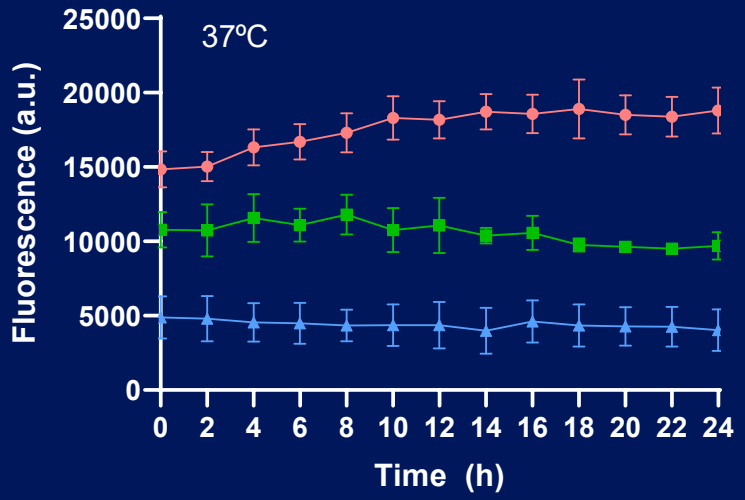
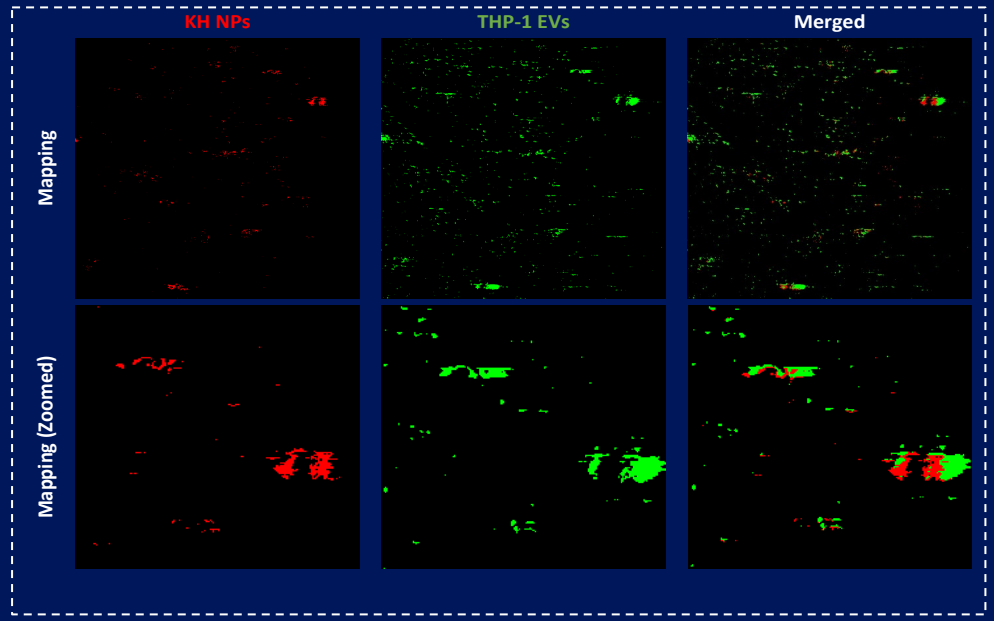
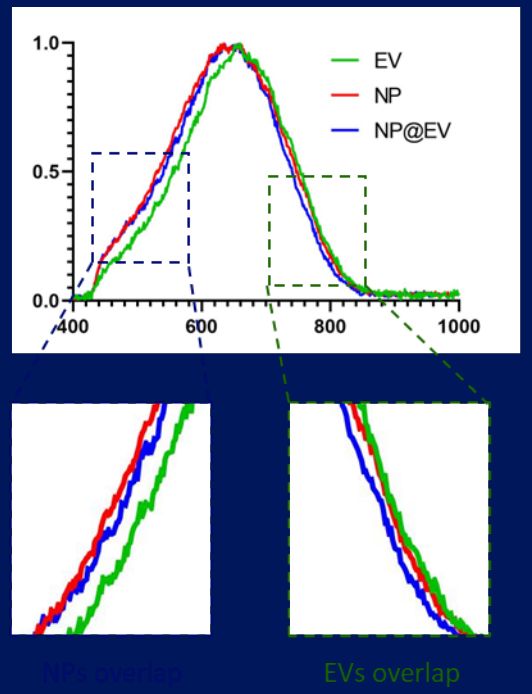
# pBAE-EV hybrids formulation

## Characterization

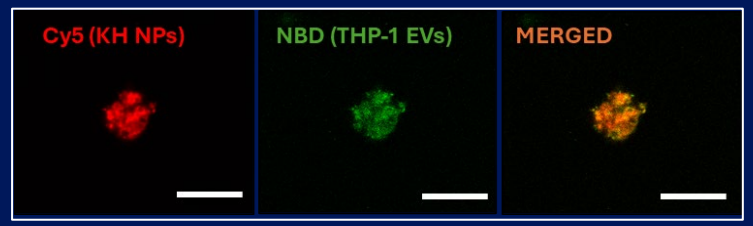


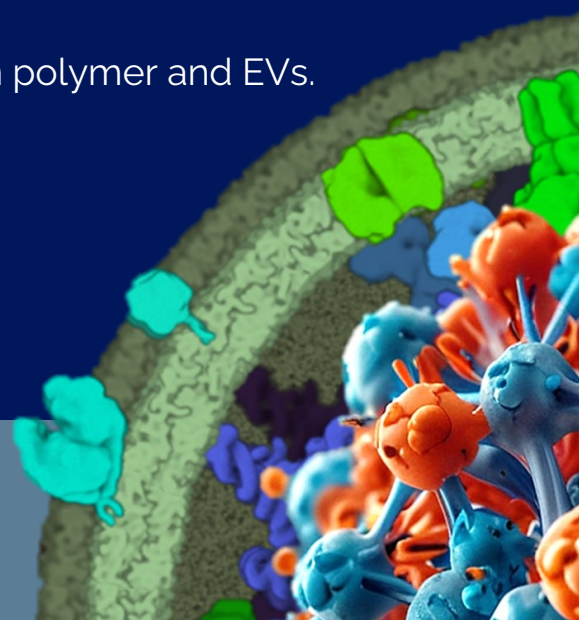

Cy3-THP-1 EVs with Cy5-KH NPs presented the highest FRET, confirming the complex formation.




Hyperspectral microscopy confirmed the colocalization between polymer and EVs.



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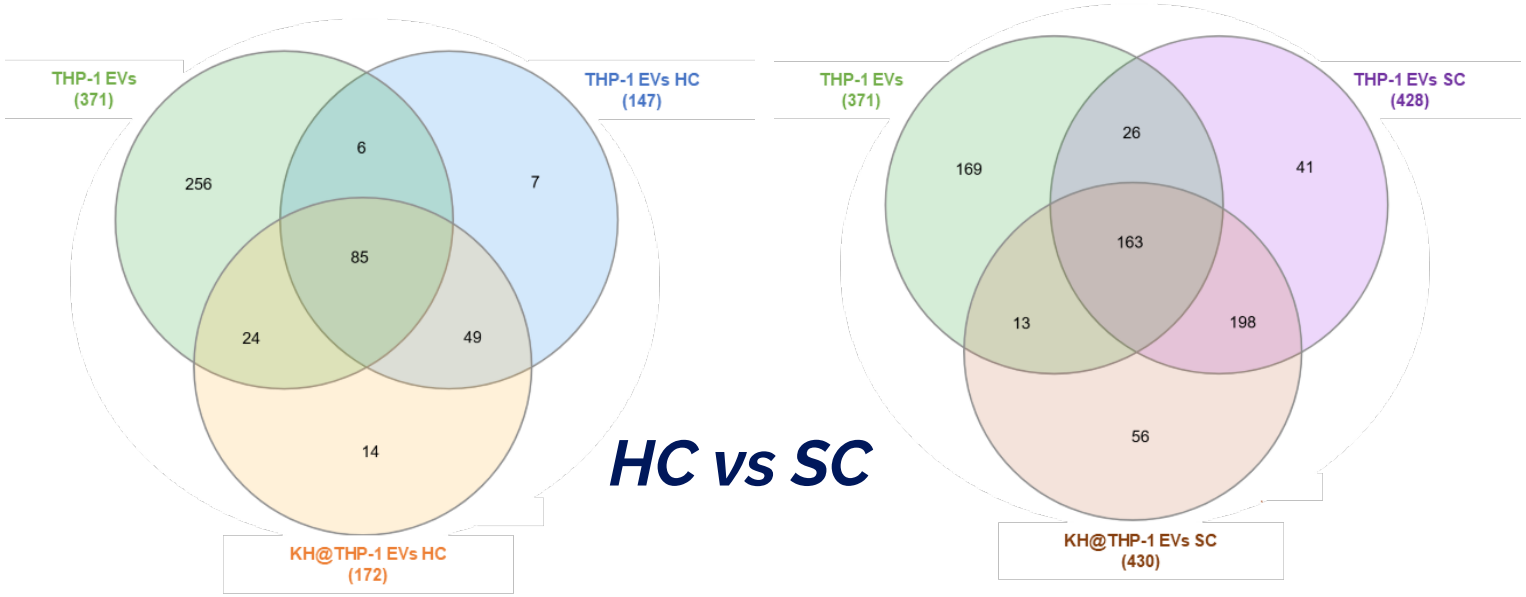
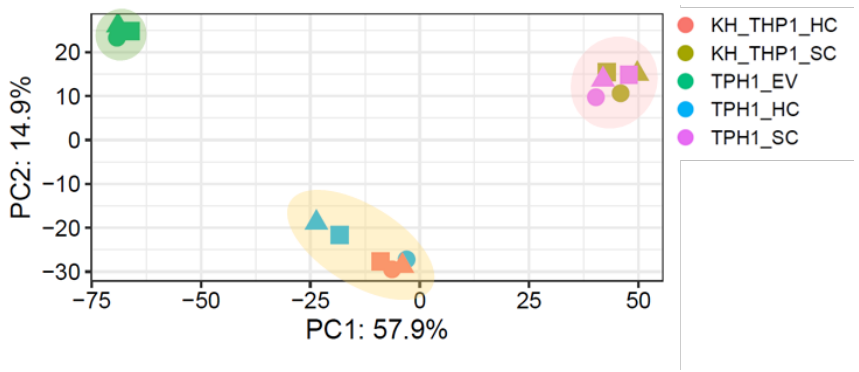
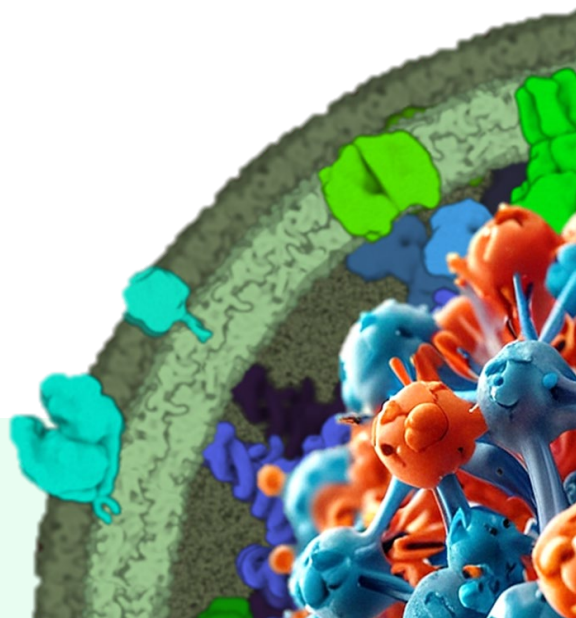
# pBAE-EV hybrids formulation

## Proteomics analysis – hard vs soft corona




SC presented a higher amount of proteins in comparison with HC. Furthermore, the protein corona of THP-1 EVs and KH@THP-1 EVs presented similar properties and protein coronas.



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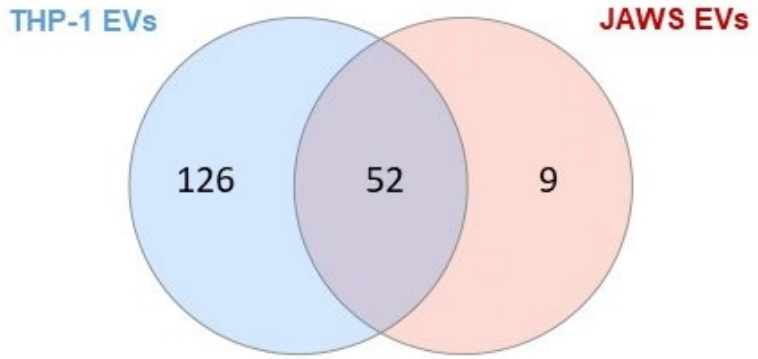
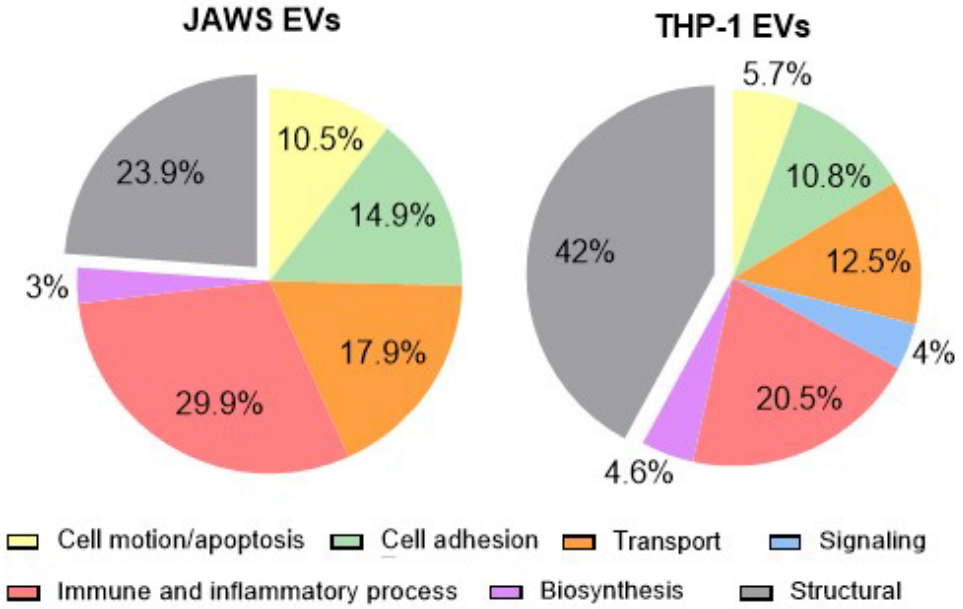
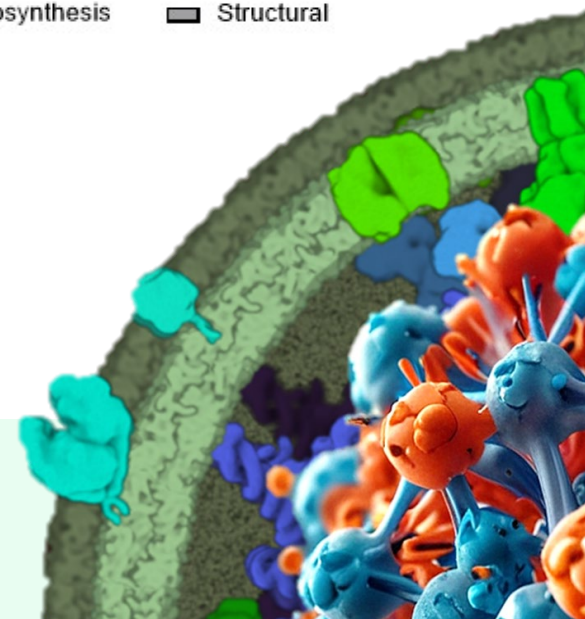
# pBAE-EV hybrids formulation

## Proteomics analysis – human vs mice EVs




No significant differences, although human Evs more structural and signalling.



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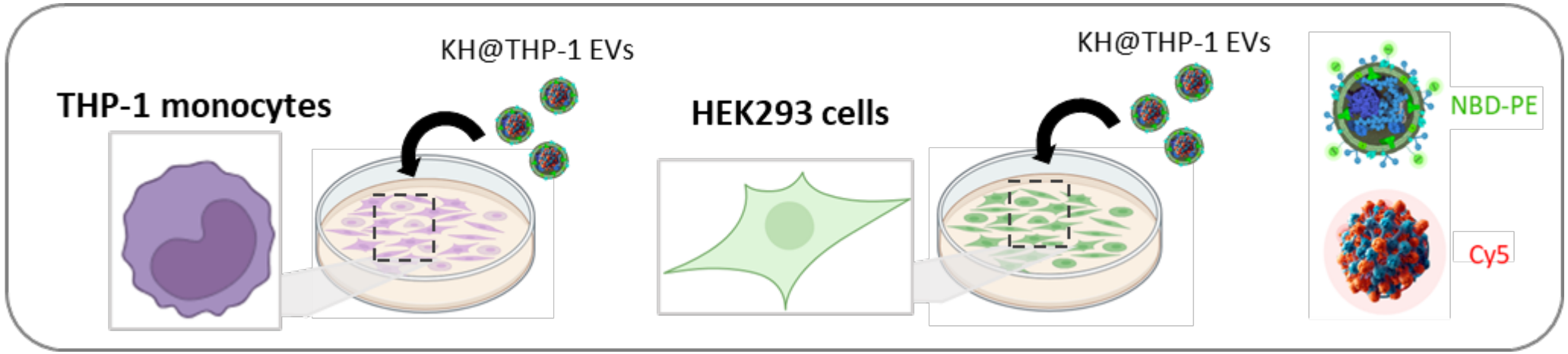
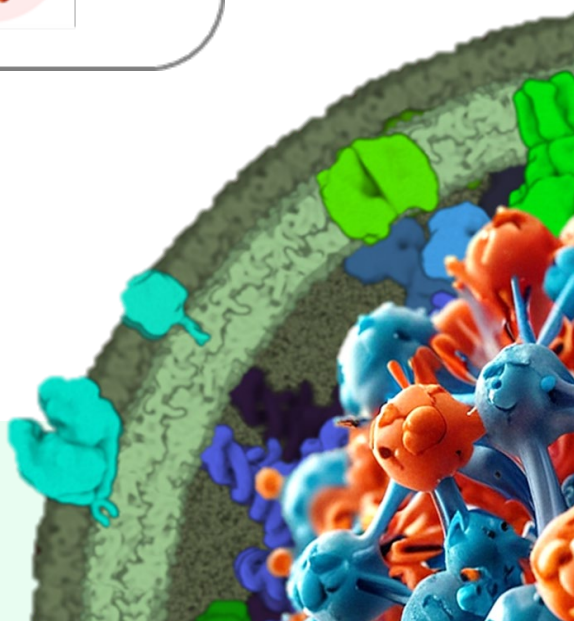
# pBAE-EV hybrids formulation

*In vitro* evaluation



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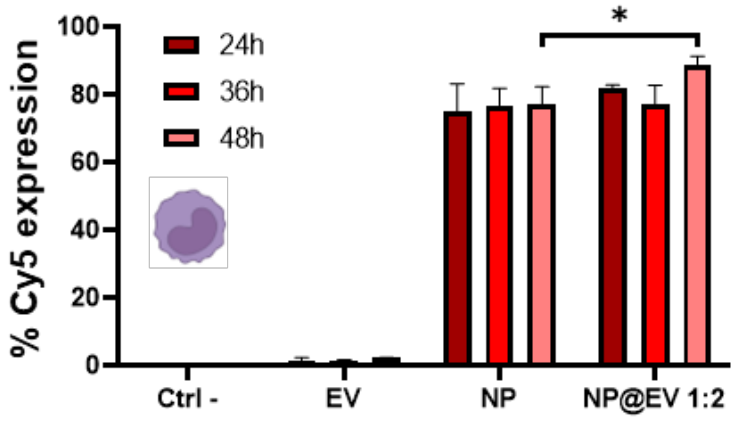
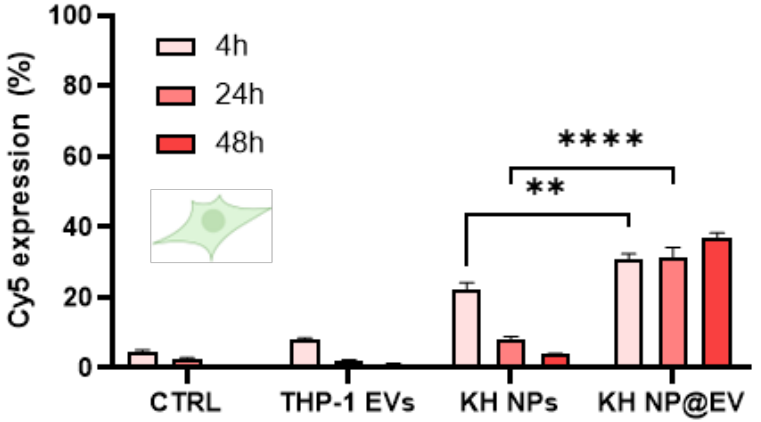
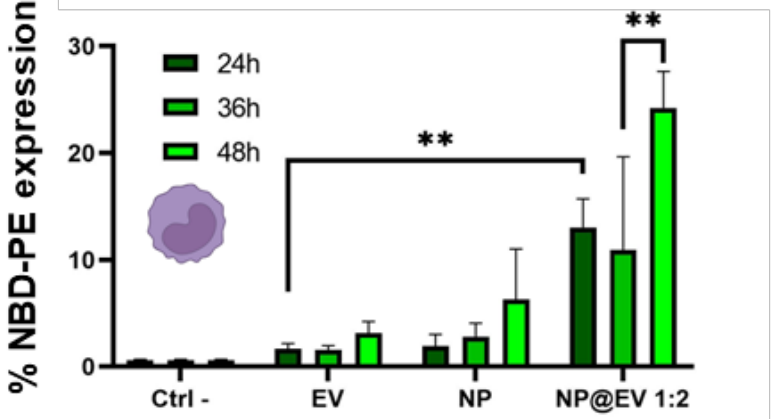
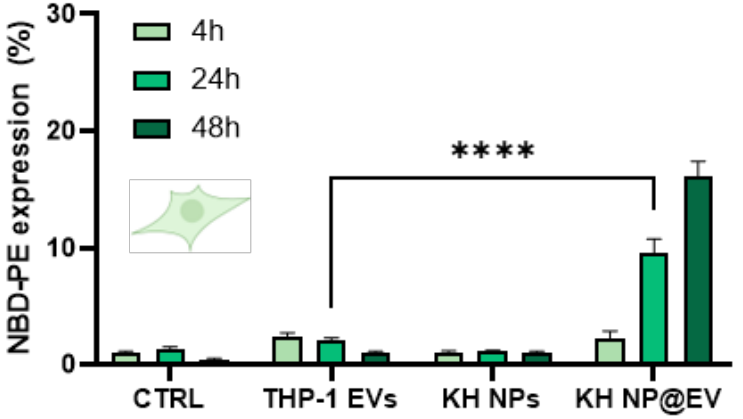
# pBAE-EV hybrids formulation

## *In vitro* evaluation of selectivity

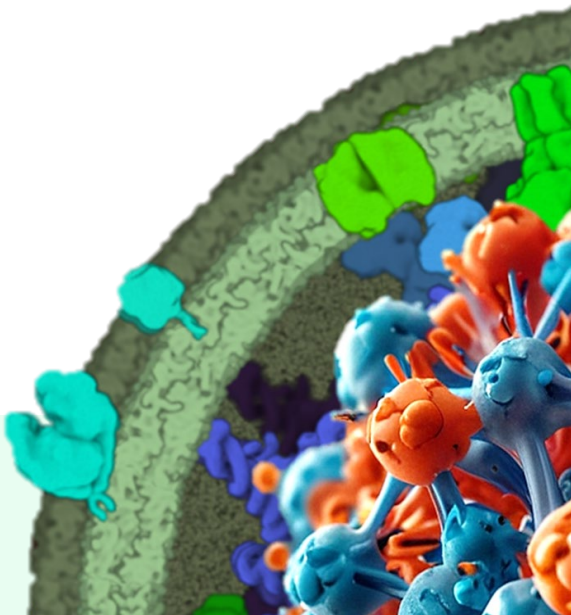



THP-1 EVs presented a higher selectivity towards THP-1 monocytes than HEK293 cells .



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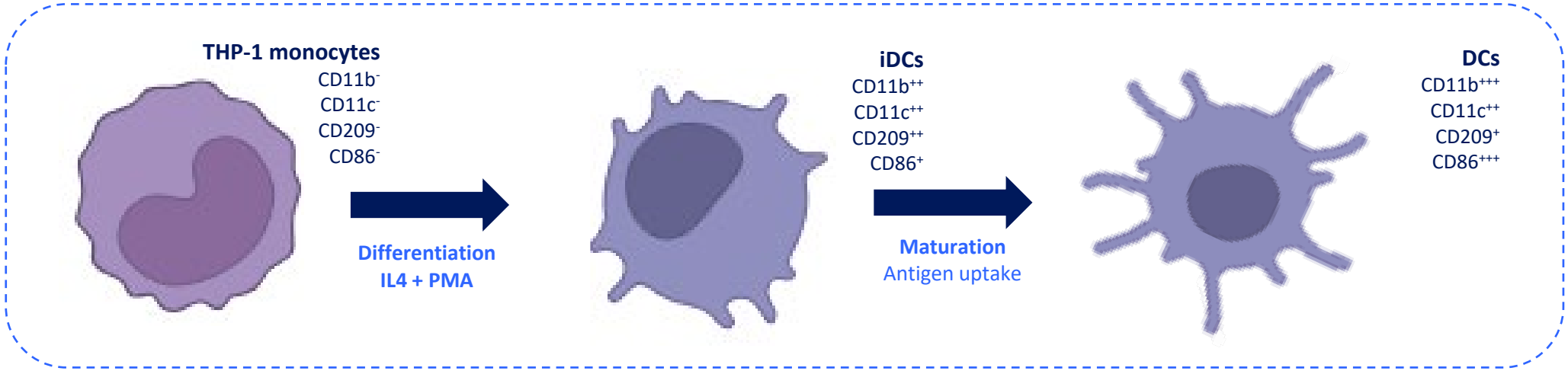
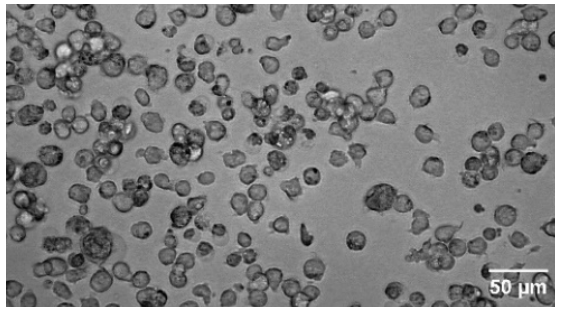
# pBAE-EV hybrids formulation

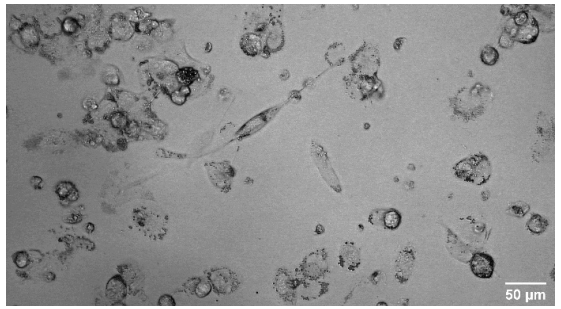
## *In vitro* evaluation of cell differentiation

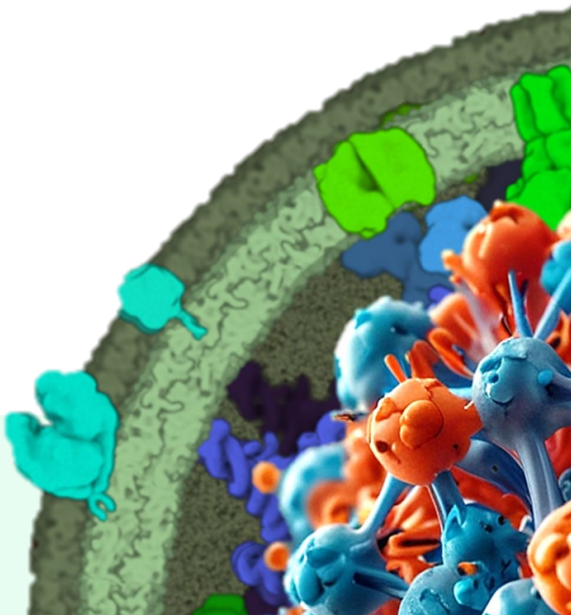



THP-1 monocytes




iDCs



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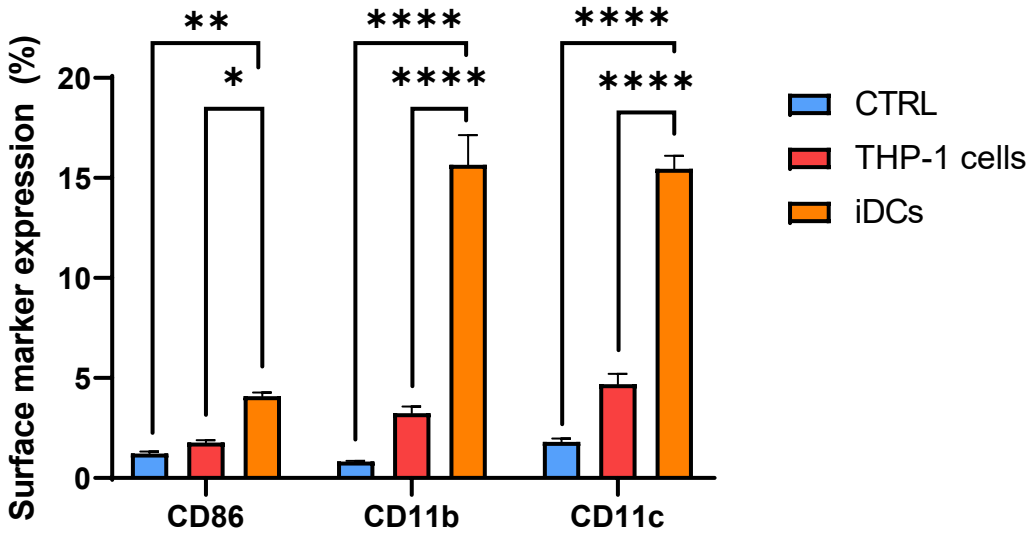
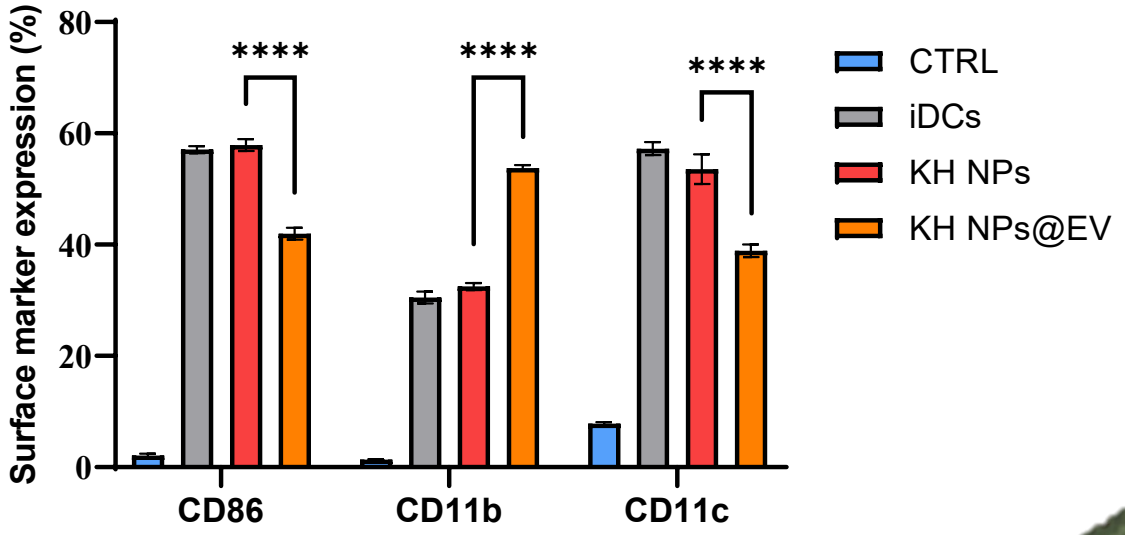
# pBAE-EV hybrids formulation

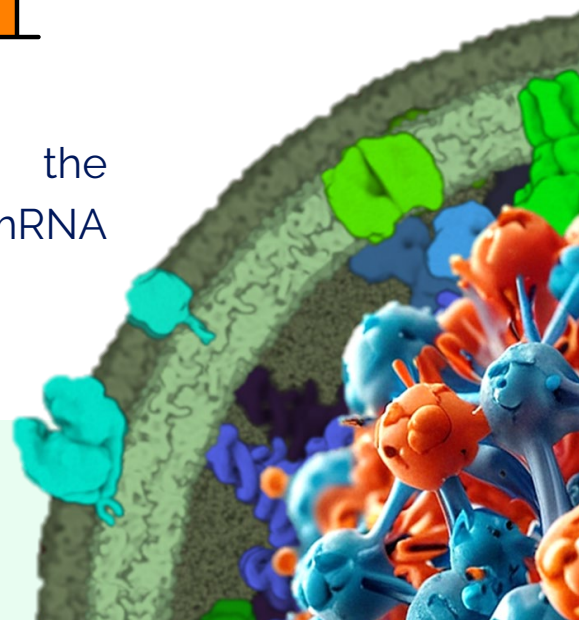
## *In vitro* evaluation of cell differentiation




THP-1 monocytes treated with IL-4 and PMA can differentiate into DCs.




Exposure of THP-1 monocytes with the complex KH NPs@EV containing OVA mRNA also differentiates the monocytes into DCs.



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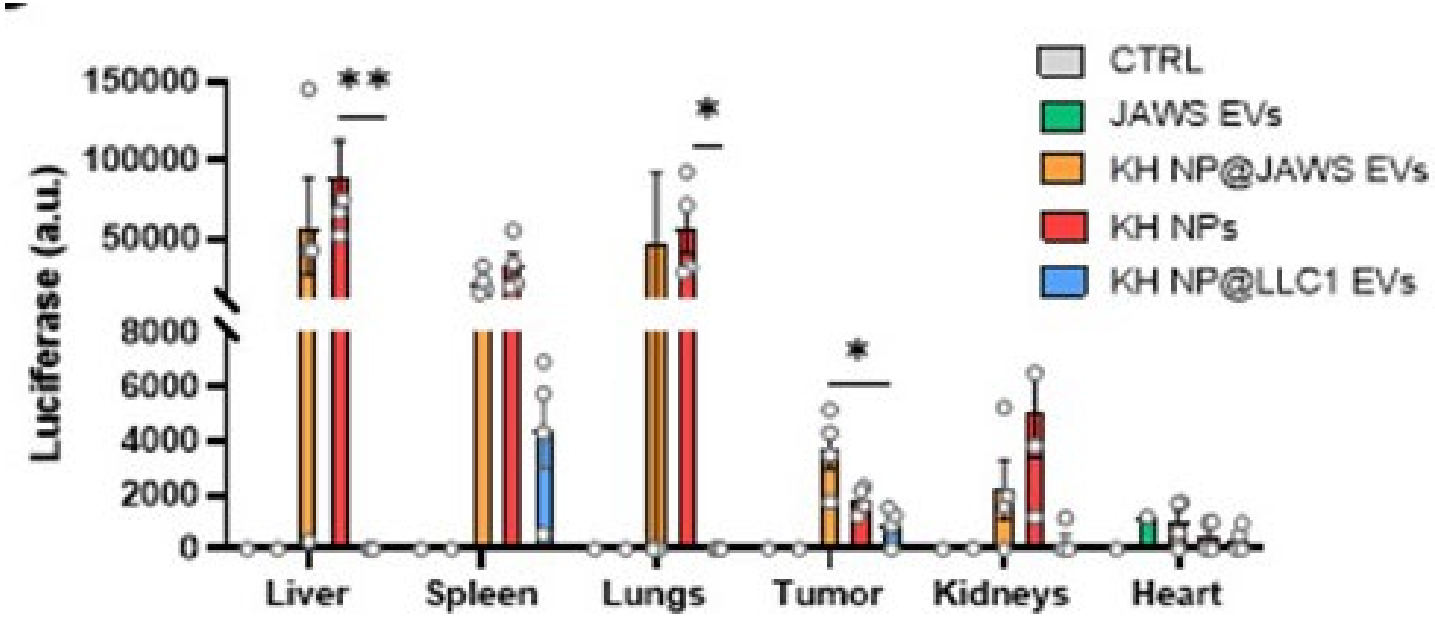
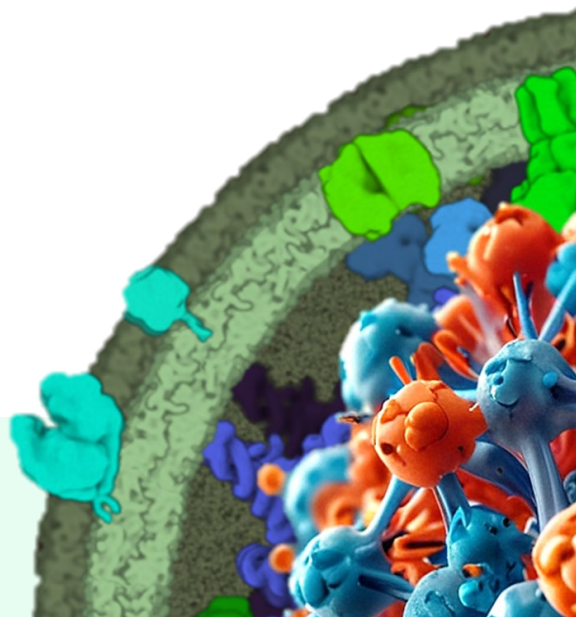
# pBAE-EV hybrids formulation

## *In vivo* analyses - biodistribution


Most in liver, spleen and lungs. Only DC Evs in tumors.



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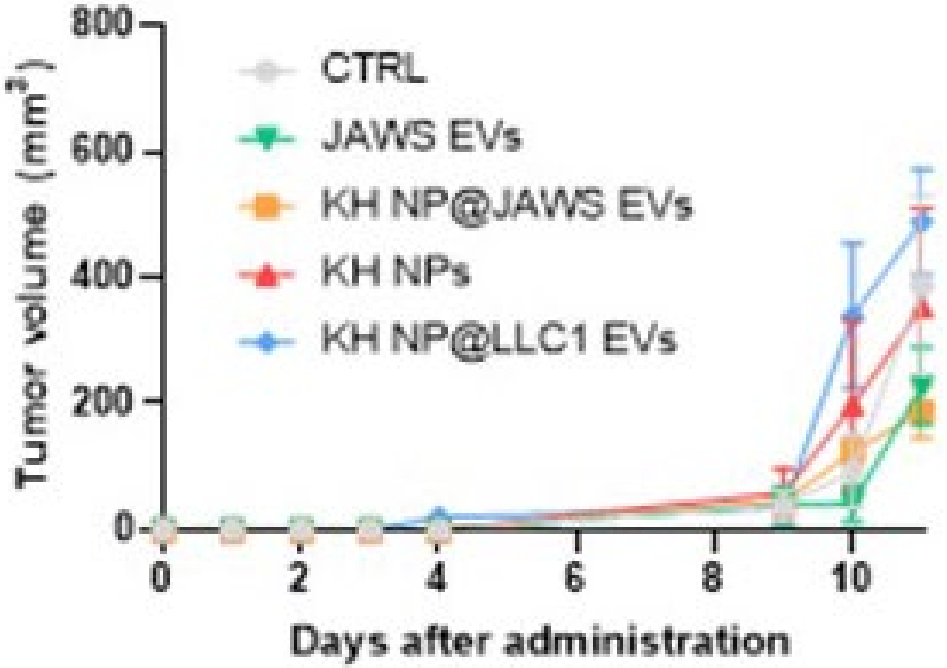
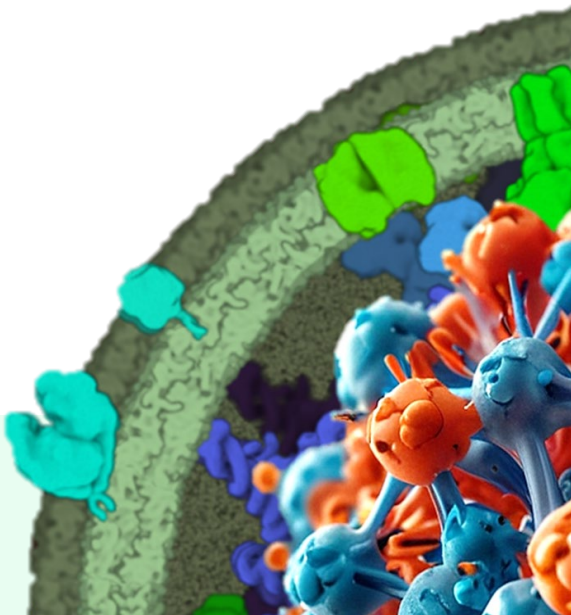
# pBAE-EV hybrids formulation

*In vivo* analyses – tumor growth




Source EV-dependent effect: only DC-derived EVs reduced tumor growth



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# Conclusions

Our results confirmed the formulation of hybrid pBAE-EV nanosystems.

These biomimetic hybrids outperform the functionality of the individual systems *in vitro*.

These novel nanosystems hold potential for use in nucleic-acid-based vaccination strategies.

# Acknowledgements

**Thank you for your attention!**

**Do you want to join us?**
**Open positions available!**

cristina.fornaguera@iqs.url.edu

# Funding


TRANSCAN

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Fondo Europeo de Desarrollo Regional

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