

## Introduction

VivaZome Therapeutics manufactures purified extracellular vesicles (VZT-PEVs) from proprietary cells for therapeutic applications, harnessing their regenerative and anti-inflammatory properties. VivaZome's manufacturing technology is underpinned by a quality control framework to enable clinical translation. The development of a Quality Target Product Profile (QTPP) is critical to establishing and maintaining the characteristics of an EV product for therapeutic use.

## VivaZome EV manufacturing technology

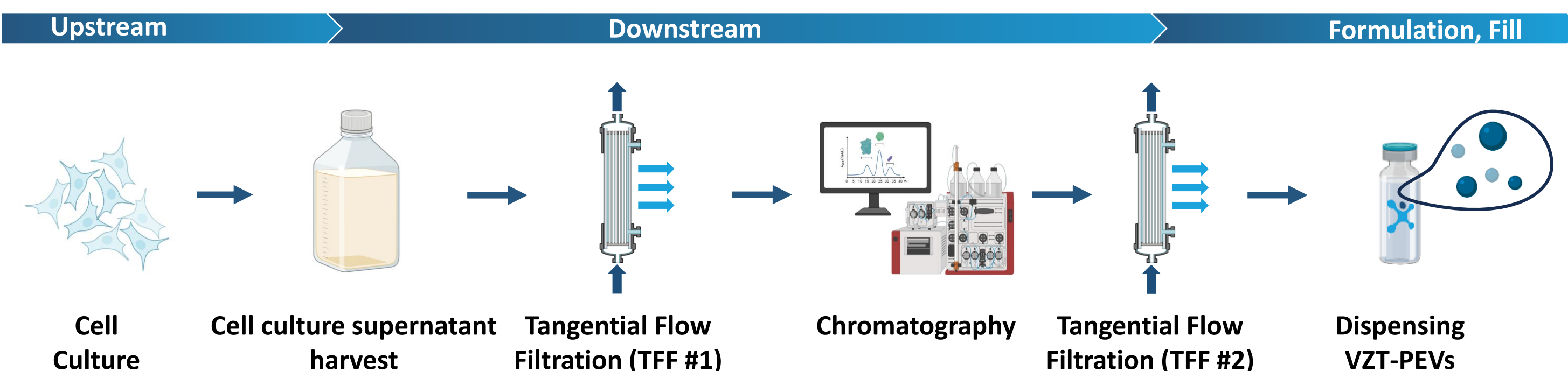
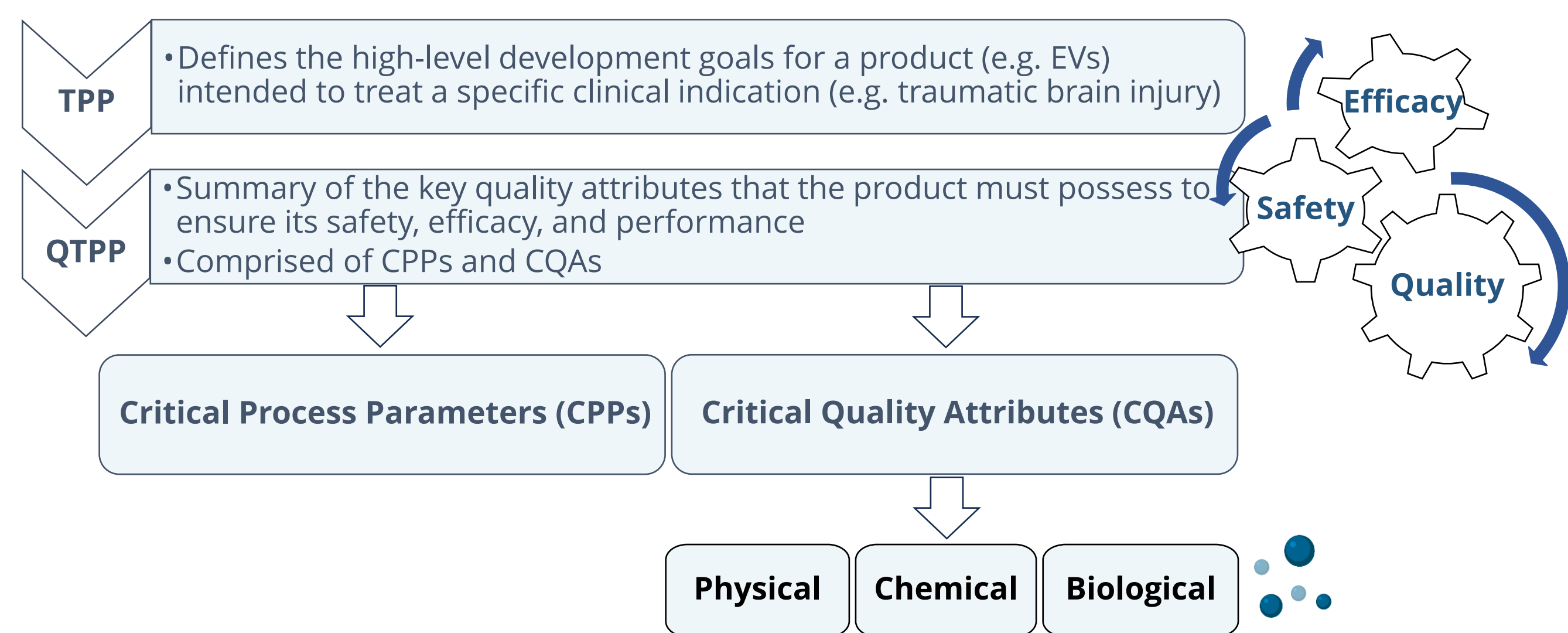


Figure 1. Schematic overview of VivaZome's EV manufacturing process.

## Regulatory guidelines for developing EV products

Regulatory authorities (FDA, EMA, TGA) require documentation to support drug development, which serves as a roadmap from discovery through to clinical trials and registration/marketing approval. The International Council for Harmonisation (ICH) establishes guidelines for the pharmaceutical industry and regulators for documents including the **Target Product Profile (TPP)**<sup>1-3</sup> and the **Quality Target Product Profile (QTPP)**<sup>1,2</sup>.



## QTPP considerations for EV clinical applications

QTPP Element	Description
<b>Appearance</b>	Visual inspection of the EV product
<b>Purity</b>	Defined particle:protein ratio, lack of impurities
<b>Identity</b>	Physical, chemical, and biological CQAs that define the EV product
<b>Stability and Shelf Life</b>	Storage conditions, shelf life duration, stability during transport
<b>Activity and Potency<sup>4</sup></b>	Defined bioactivity relevant to clinical indication
<b>Sterility and Microbial Control</b>	Safety, sterility assurance level, mycoplasma and endotoxin testing
<b>Container Closure System</b>	Compatibility with EVs, integrity and protection from environmental factors

## Critical Process Parameters (CPPs) and Critical Quality Attributes (CQAs) – considerations for an EV product

**CPP:** A critical variable in the production process (e.g. temperature, pH, time).

**CQA:** Attributes that ensure product quality. A CQA may be affected by a CPPs (e.g. a temperature deviation may affect the potency of EVs).

CQAs		
<b>Physical</b> <ul style="list-style-type: none"> <li>- Charge</li> <li>- Size (nm)</li> <li>- Appearance</li> <li>- Morphology</li> <li>- Concentration</li> </ul>	<b>Chemical</b> <ul style="list-style-type: none"> <li>- pH</li> <li>- Buffers</li> <li>- Endotoxin</li> <li>- Excipients</li> <li>- Impurities</li> </ul>	<b>Biological/Microbiological</b> <ul style="list-style-type: none"> <li>- Purity</li> <li>- Cargo composition</li> <li>- Functionality and activity</li> <li>- Adventitious agents</li> <li>- Surface marker expression</li> </ul>

**Analytical assay validation:** necessary to ensure accuracy, precision, reliability, reproducibility, and to support regulatory compliance. The guidelines for analytical assay validation are outlined by the ICH in Q2(R1)<sup>5</sup> and Q2(R2)<sup>6</sup>.

## Acknowledgements

We would like to acknowledge the funding provided by the Australian Government through the CRC-P Grant, as well as the support of our project partners.



## Results

### Identifying and analysing VZT-PEV CQAs

Well-defined CQAs ensure that the EV product has reproducible qualities and functional properties when produced by a defined and robust manufacturing process. VZT-PEVs meet established CQA limits, demonstrating consistent batch-to-batch quality.

### Physical characteristics

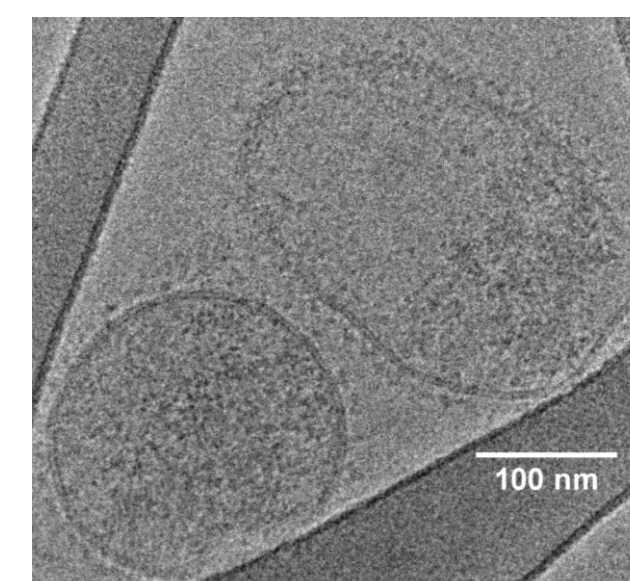


Figure 2. Representative CryoTEM micrograph of VZT-PEVs, demonstrating structural integrity and typical EV morphology. Scale bar = 100nm. Courtesy of La Trobe University Bioimaging Platform.

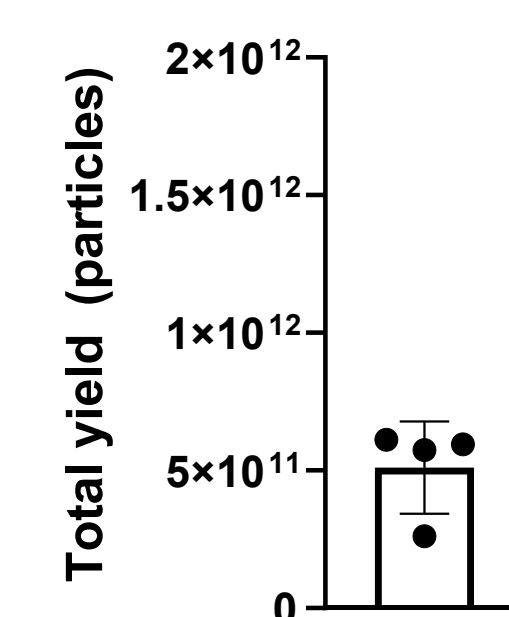


Figure 3. Total yield of VZT-PEVs from four independent batches. Mean ± SD of n=4 biological replicates.

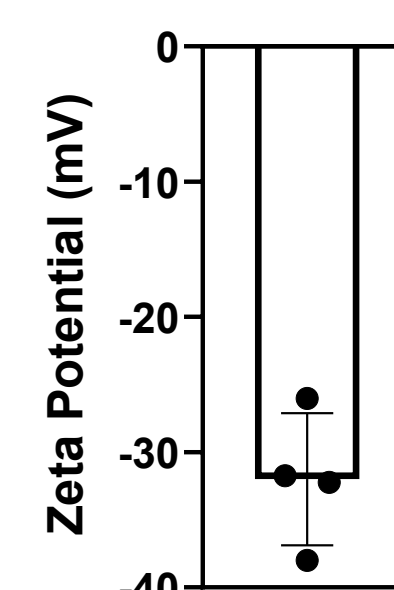


Figure 4. Zeta potential (mV) of VZT-PEVs. Mean ± SD of n=4 biological replicates.

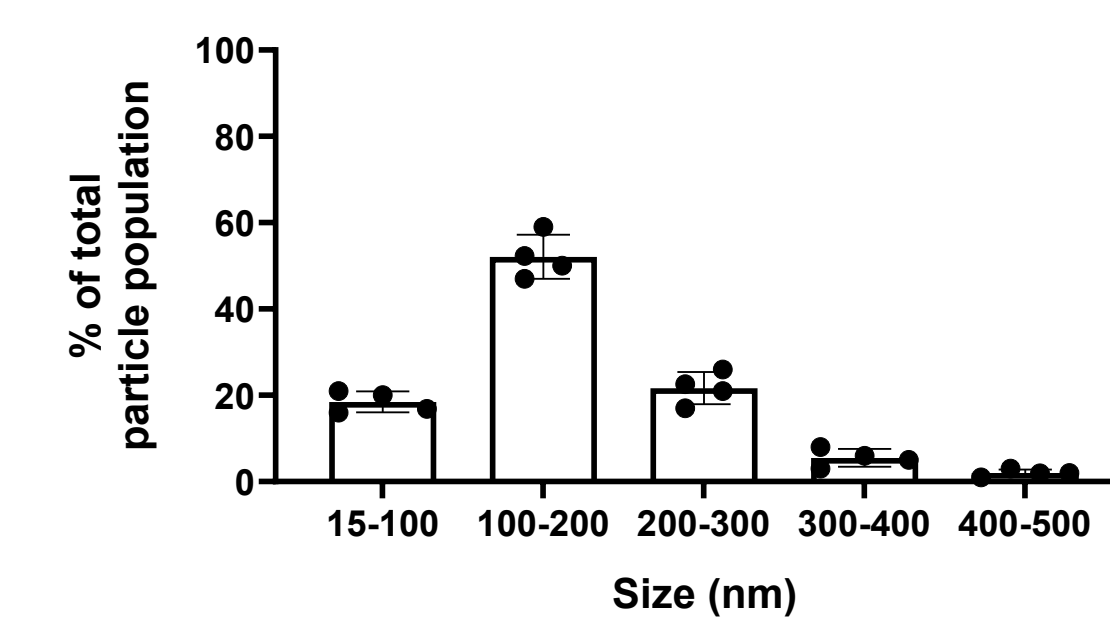


Figure 5. Size distribution of VZT-PEVs from four independent batches. Mean ± SD of n=4 biological replicates.

### Purity and cargo composition

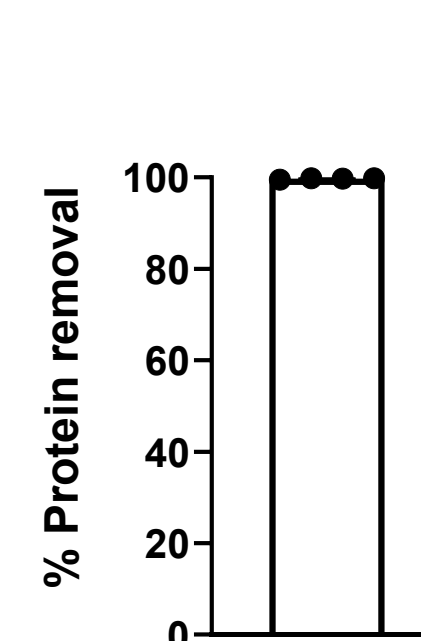


Figure 6. Protein removal (%) during purification of VZT-PEVs. Mean of n = 4 biological replicates.

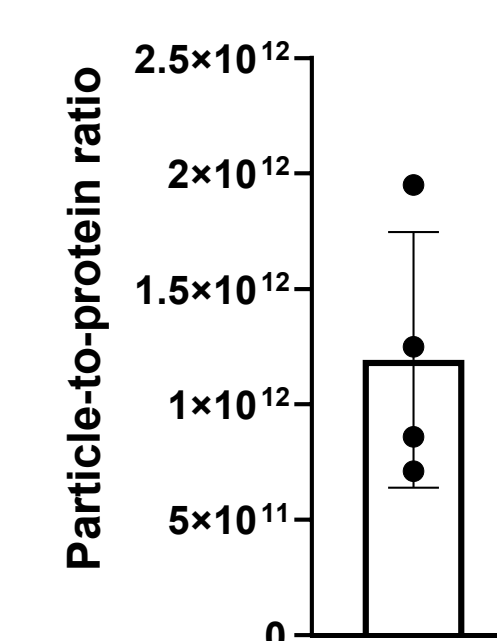


Figure 7. Particle-to-mg protein ratio of VZT-PEVs. Mean ± SD of n = 4 biological replicates.

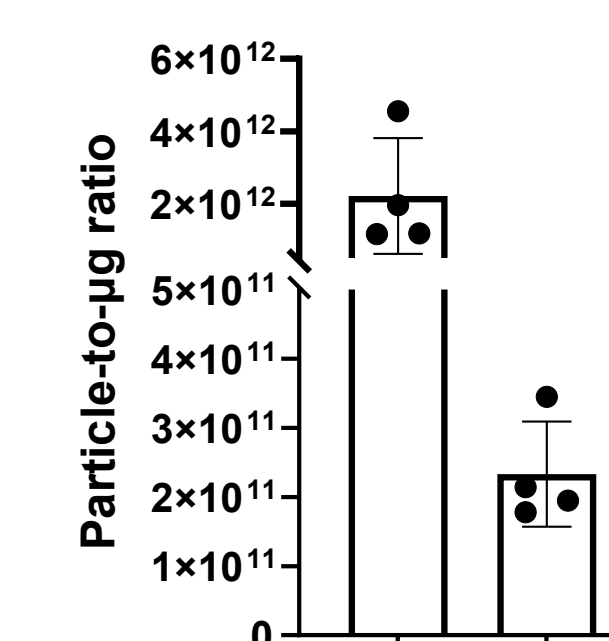


Figure 8. Particle-to-µg nucleic acid (DNA, RNA) ratio of VZT-PEVs. Mean ± SD of n = 4 biological replicates.

### Identity and surface marker expression

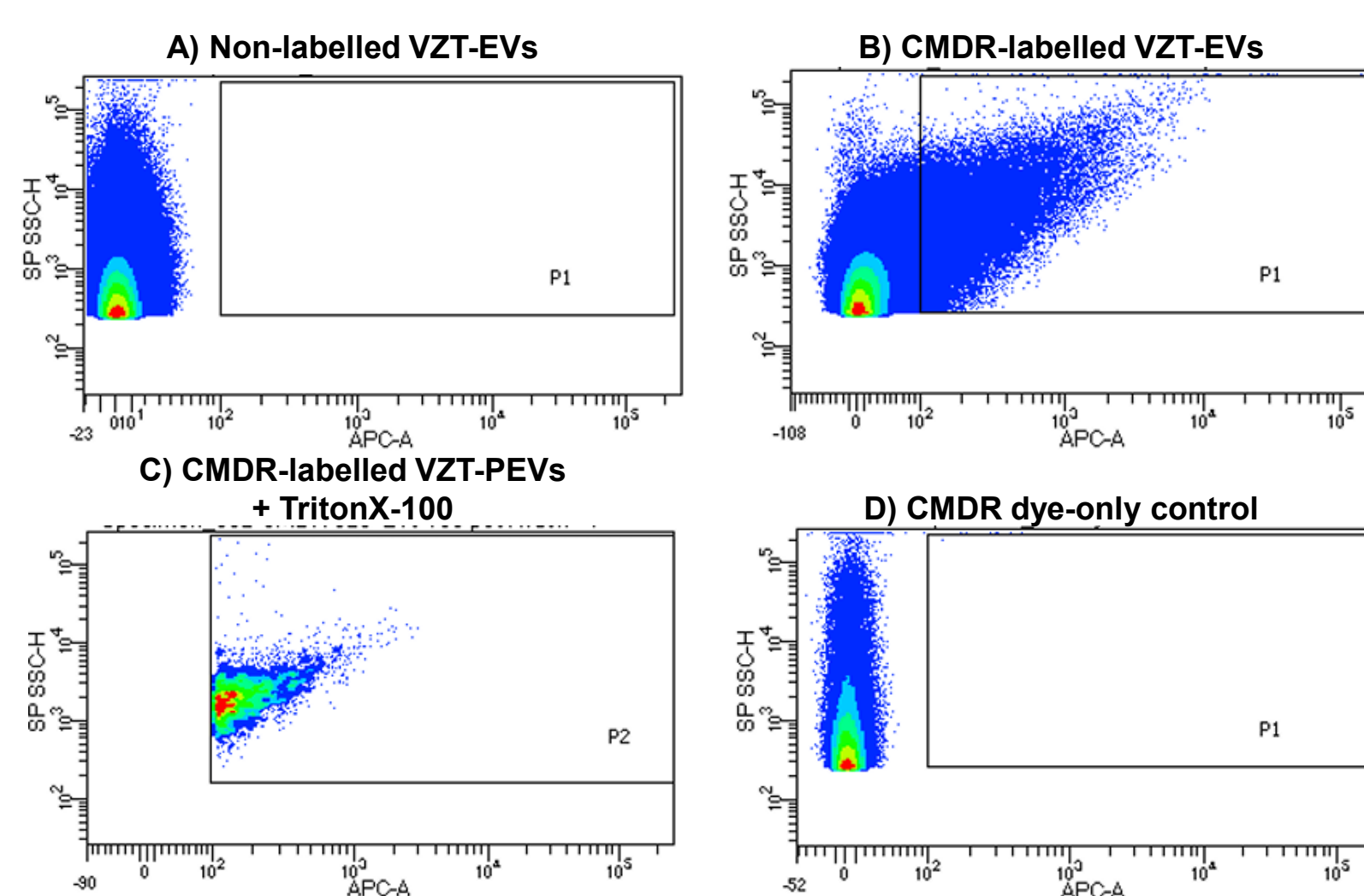


Figure 9. Representative nano-flow cytometry dot plots of A) non-labelled VZT-PEVs, B) CellMask Deep Red (CMDR)-labelled VZT-PEVs, C) CMDR-labelled VZT-PEVs + TritonX-100, and D) CMDR dye alone.

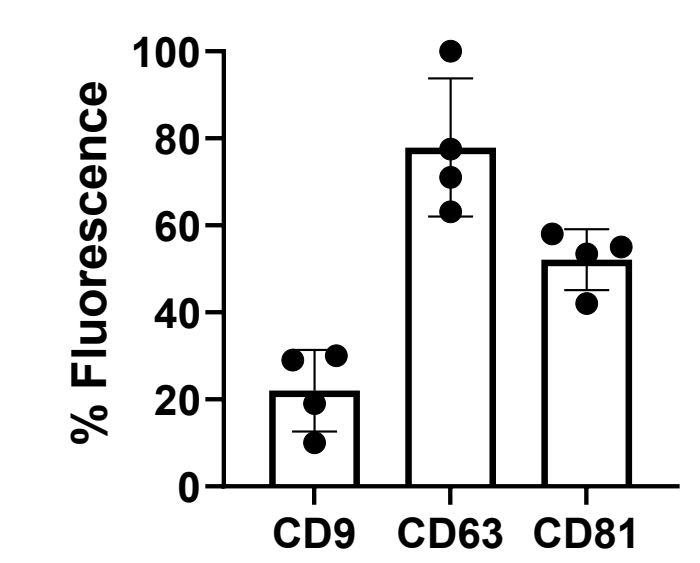


Figure 10. Fluorescence NTA (F-NTA) of VZT-PEV markers as a percentage of the total particle population. Mean ± SD of n=4 biological replicates.

## Summary

This work demonstrates the critical interplay between advanced EV manufacturing, rigorous quality frameworks, and regulatory alignment. By establishing a well-defined QTPP and characterising VZT-PEVs across key critical quality attributes (CQAs), we have established a strong foundation for the clinical translation of VivaZome's EV-based therapeutics.

## References

- International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). Quality Guidelines (2009). *Pharmaceutical Development Q8(R2)*. (4), 1-24.
- World Health Organisation. (2025). *Target product profiles*.
- National Institutes of Health. *Creating a target product profile for new drug products*.
- ISEV Regulatory Task Force: *Development of potency assays for therapeutic EVs: The art of congruence*.
- ICH Q2(R1): *Validation of Analytical Procedures: Text and methodology*.
- ICH Q2(R2): *Validation of Analytical Procedures*.

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