

## EVs from *Citrus clementina* isolated by scalable methodology show potential as immunomodulatory ingredients for different industrial sectors

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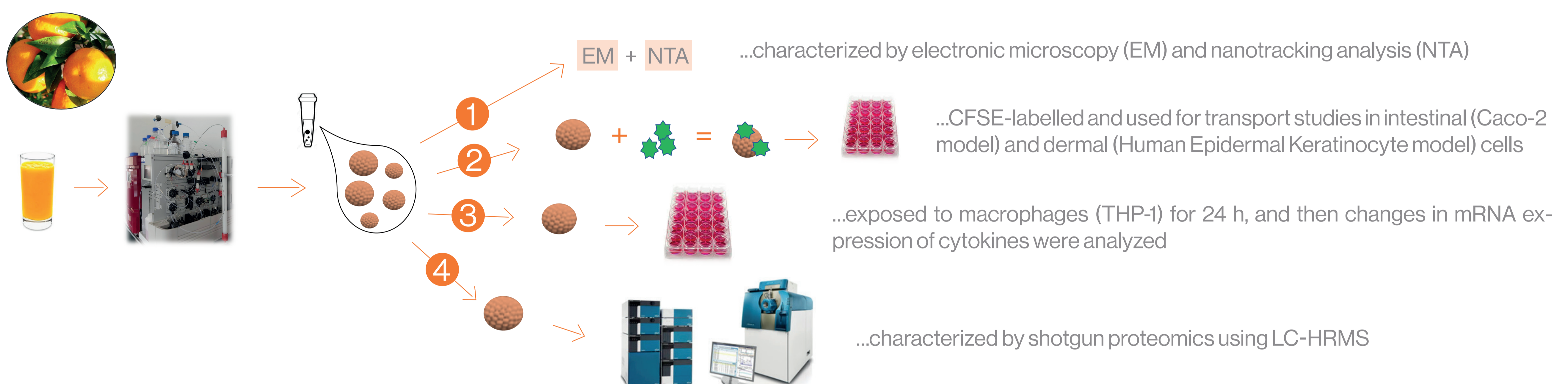
### INTRODUCTION

Extracellular vesicles (EVs) from fruit juices raise as new potential sources to obtain plant-derived functional ingredients or as drug delivery systems.

The objective was to characterize *Citrus clementina* derived EVs, obtained by scalable methodology, in terms of intestinal and dermal absorption and possible immunomodulatory role using different cell models. In addition, proteomic characterization of EVs was performed.

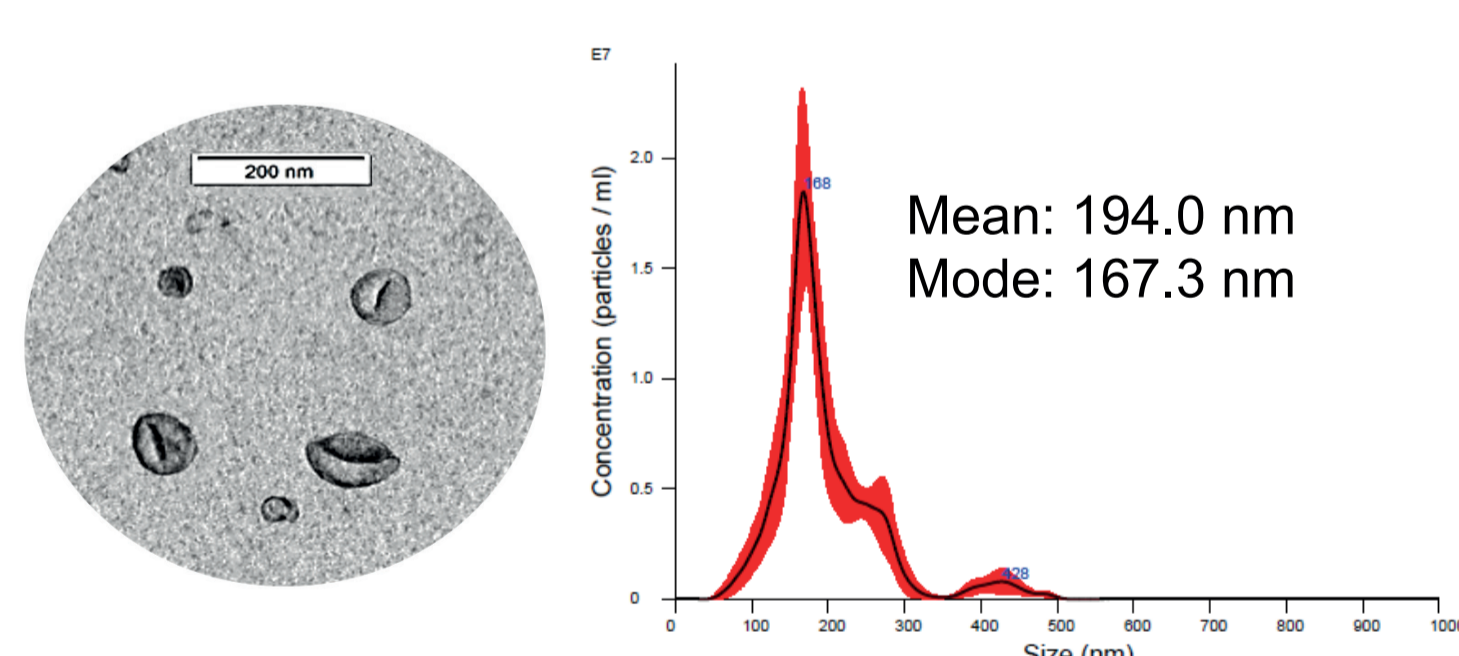
### METHODS

EVs from *C. clementina* were obtained using anion exchange chromatography (AIEX), using an AKTA Avant 150 (Cytiva). The juice was first centrifuged at 10000 g. Then the supernatant was filtered through 0,45 um and diluted with equilibration buffer (50 mM TRIS pH8) prior to AIEX. Obtained EVs were...



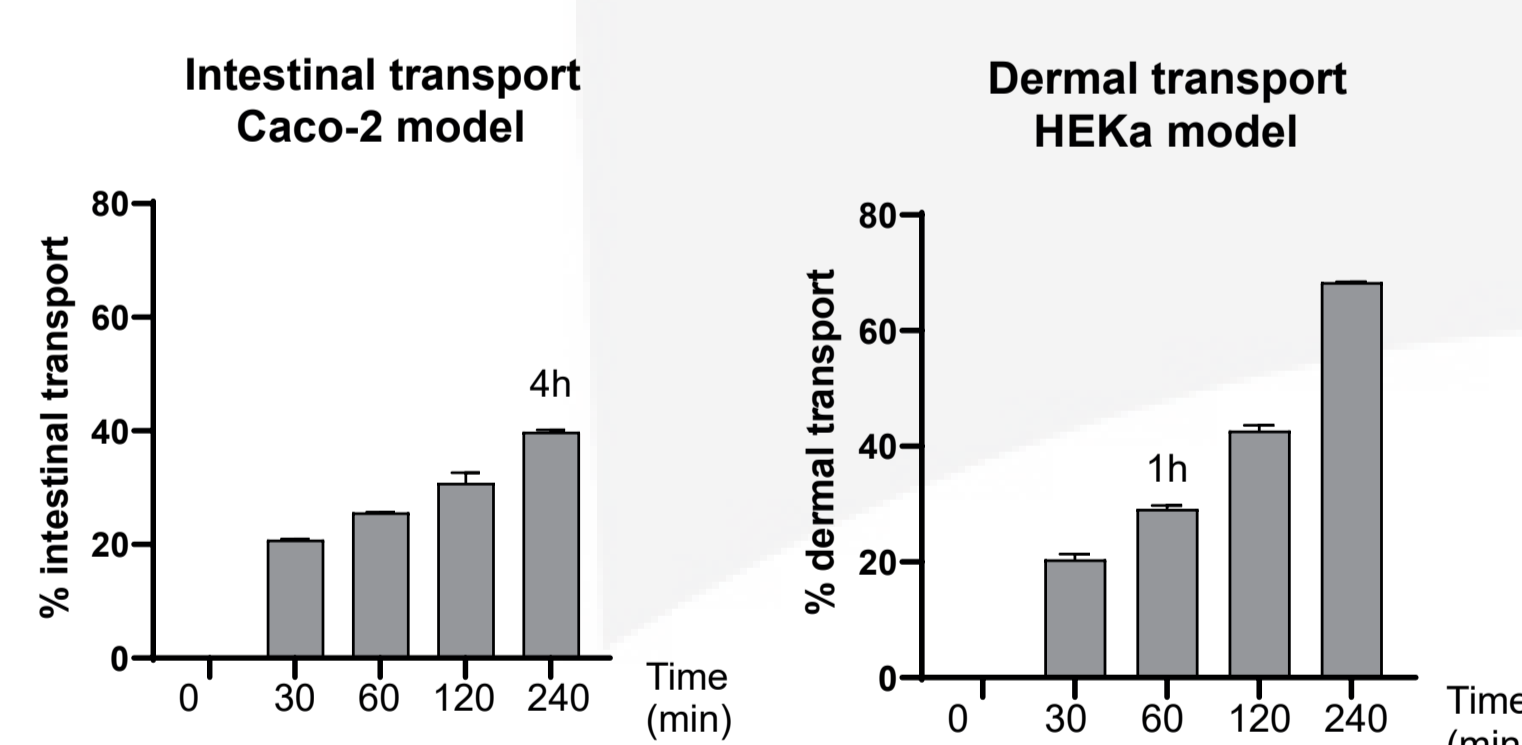
### RESULTS

#### 1 EM and NTA characterization



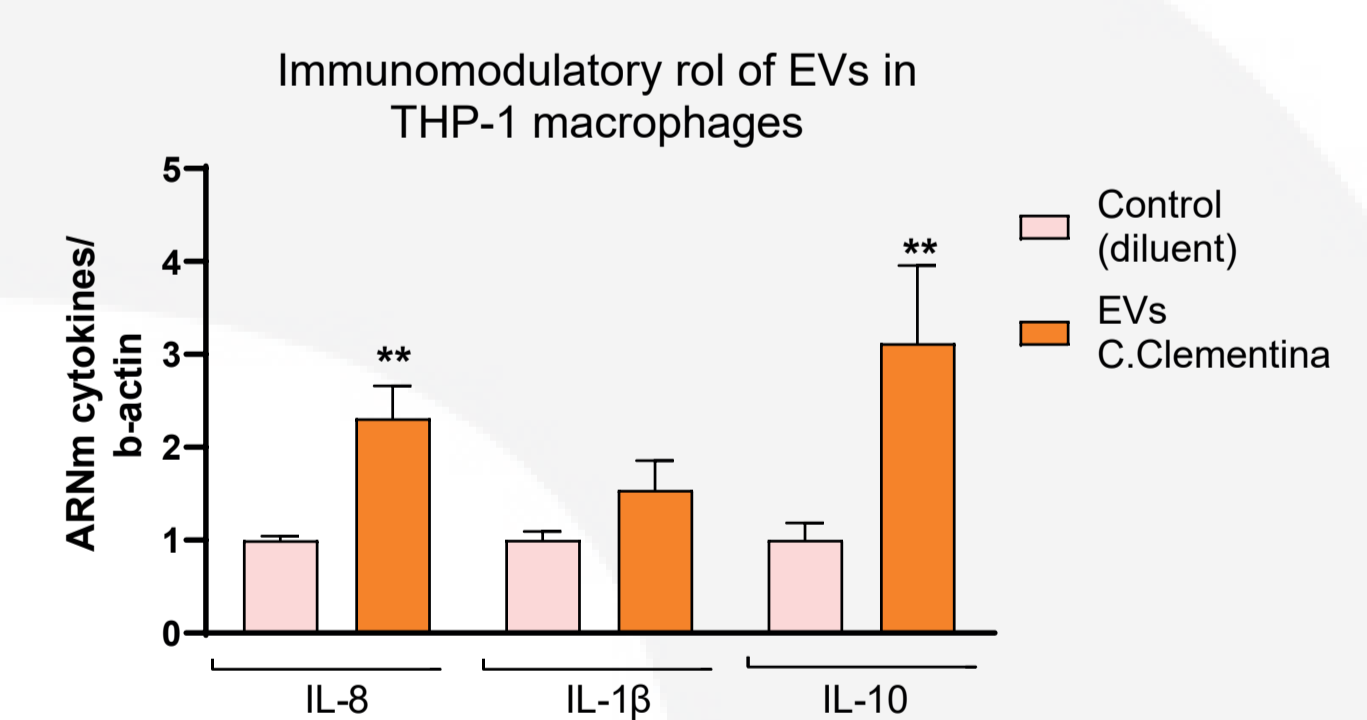
EVs showed normal morphology and a mean size of 194±21 nm

#### 2 In vitro transport of labeled-EVs



The cell assays showed a transport of 39.8% after 4h in contact with intestinal cells and 29.2% after 1h in contact with keratinocytes

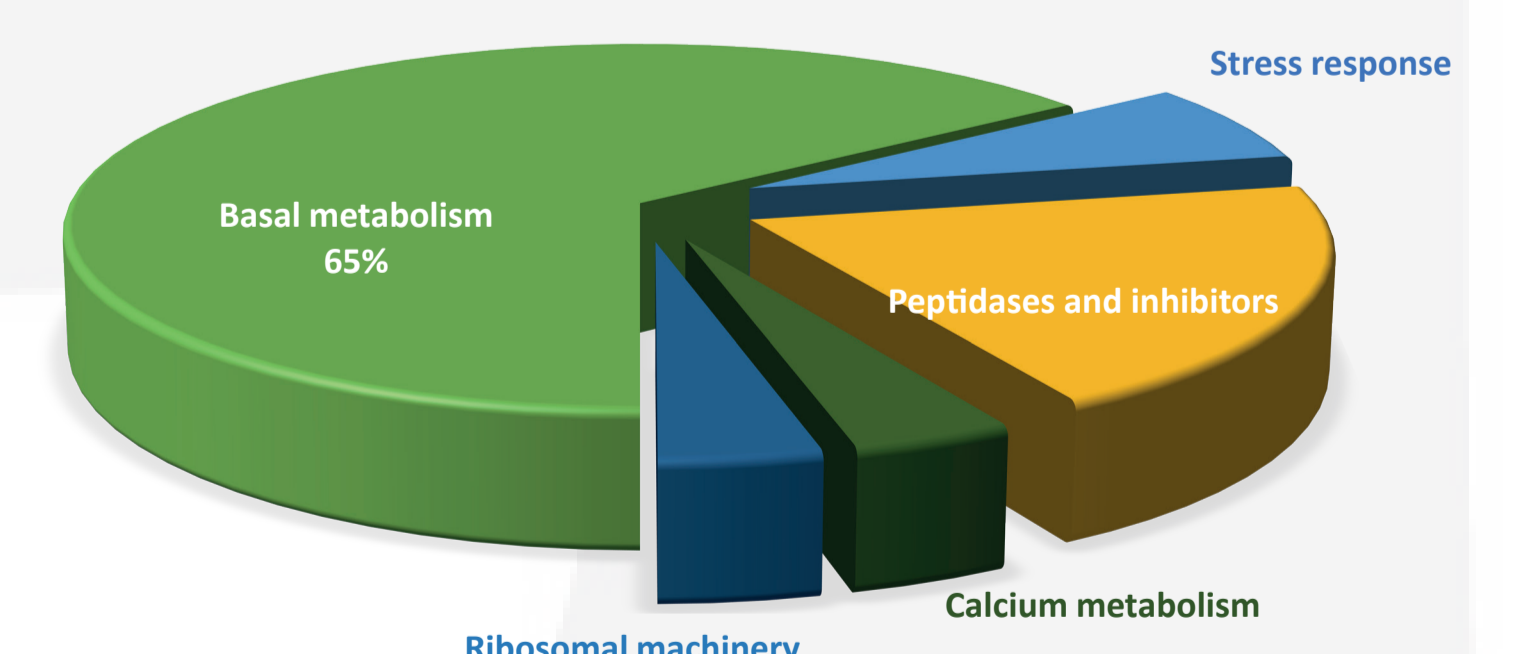
#### 3 Immunomodulatory effect



EVs increased the expression of IL-8 and IL-10 but not IL-1β, showing an immunomodulatory effect

#### 4 Shotgun proteomics

Results showed several protein clusters: ribosomal machinery (4%), stress response (7%), calcium metabolism (4%) or peptidases and inhibitors (25%), including different cysteine peptidases that may explain the biological effect in macrophages.



### CONCLUSIONS

EVs from *Citrus clementina* separated and concentrated by **scalable methodology** for its industrial application, were **transported by intestinal and dermal cells** and showed potential as **functional immunomodulatory ingredients**. A more extensive omic study on its biocargo could identify and explain new potential effects and the mechanism of action of them.