

Cellular uptake of breast milk-derived extracellular vesicles is higher in mothers with asthma in a transwell model of the gastrointestinal barrier

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Introduction

1. Development of asthma in children:

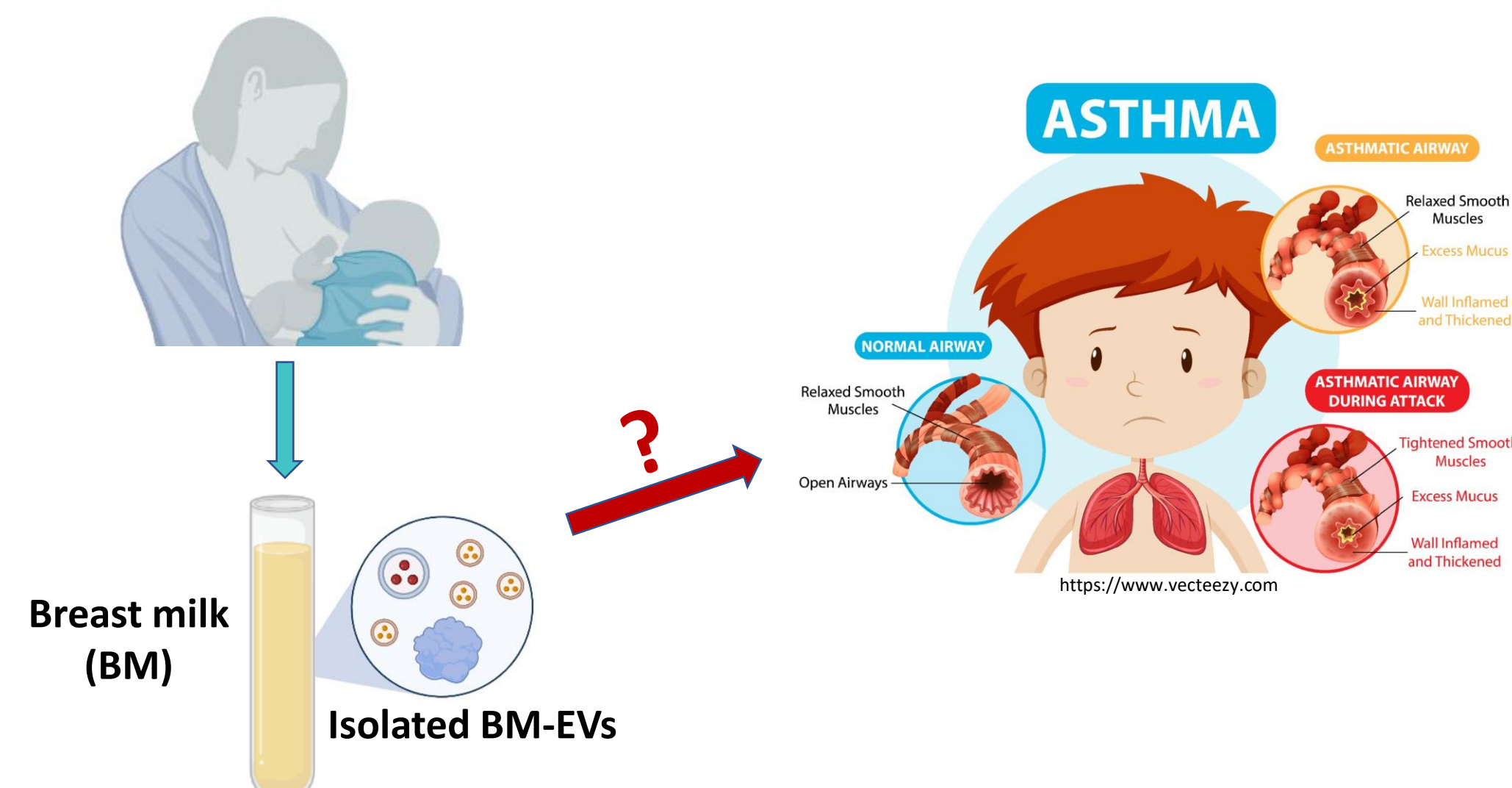
- Asthma is one of the most common chronic diseases in children, characterized by airway inflammation, remodeling and hyperresponsiveness.
- It has a substantial impact on health, quality of life and the economy.

2. Asthma and breastfeeding:

- Asthma in children has been linked with breastfeeding, though evidence is mixed with regards to its effect on asthma development.
- Components of breast milk (BM) likely play a critical role in determining the effect of breastfeeding on asthma development in offspring.

3. Role of BM-extracellular vesicle (EVs) in asthma:

- EVs are small lipid membrane-bound vesicles that enclose biological cargo and constitute a primary form of cellular crosstalk.
- EVs are detected in all biofluids and are an understudied breast milk component.



The specific role of **BM-EVs** in inducing or preventing asthma has not been elucidated to date. Preliminary data from our group illustrate anti-inflammatory effects of BM-EVs from mothers with asthma on primary human airway smooth muscle cells. We do not know if this is due to differential cellular uptake of BM-EVs nor if it is consistent across cell types.

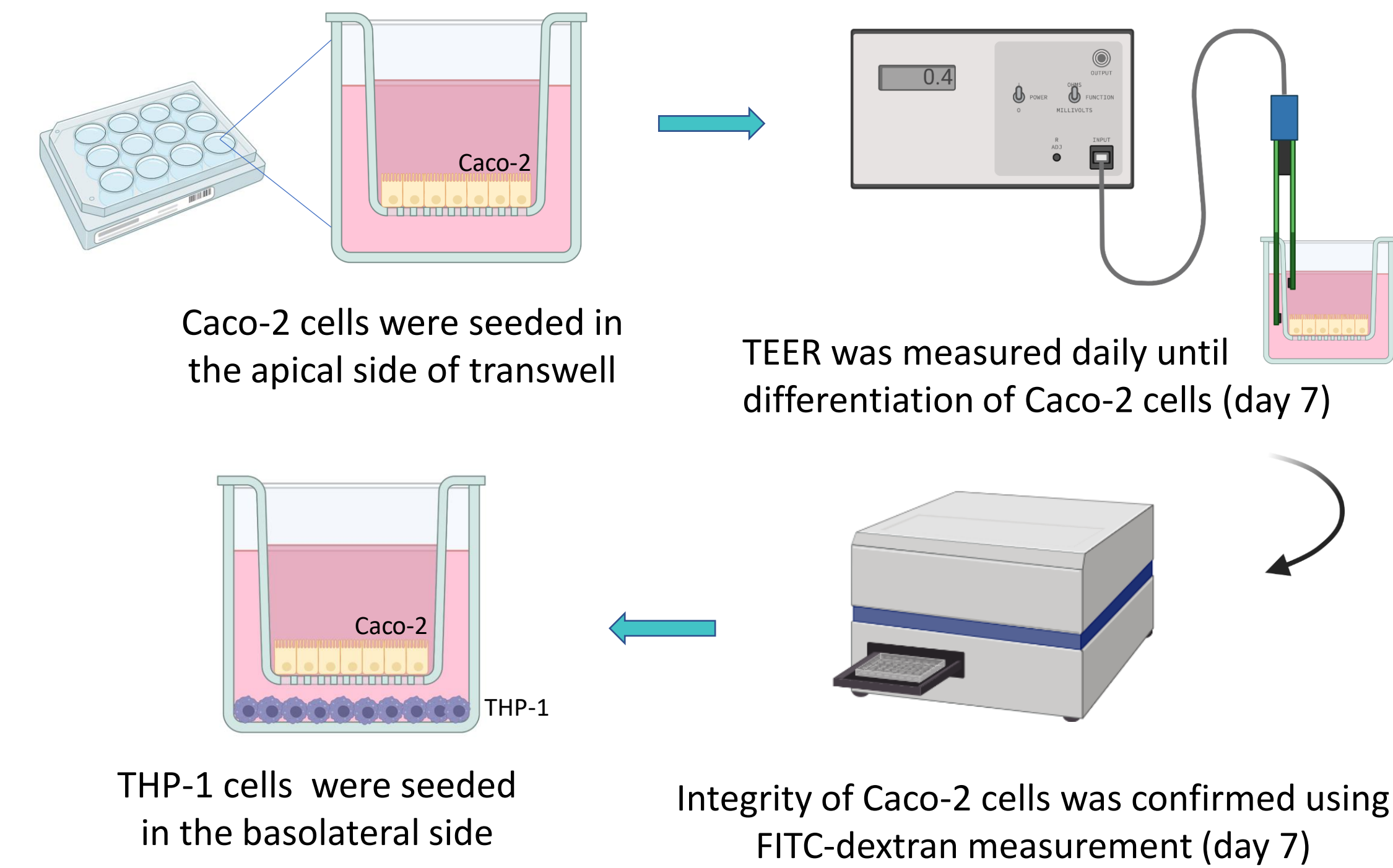
Objectives

To determine whether the effect of BM-EVs is contingent on cellular uptake, we established an *in vitro* transwell co-culture model to elucidate if BM-EVs:

- pass intestinal epithelial cell (Caco-2) barrier,
- are taken up by macrophage cells (THP-1), and
- if uptake is dependent on maternal asthma status.

Methods

A) Caco-2 /THP-1 co-culture in transwell



B) Treatment of Caco-2/THP-1 cells with BM-EVs

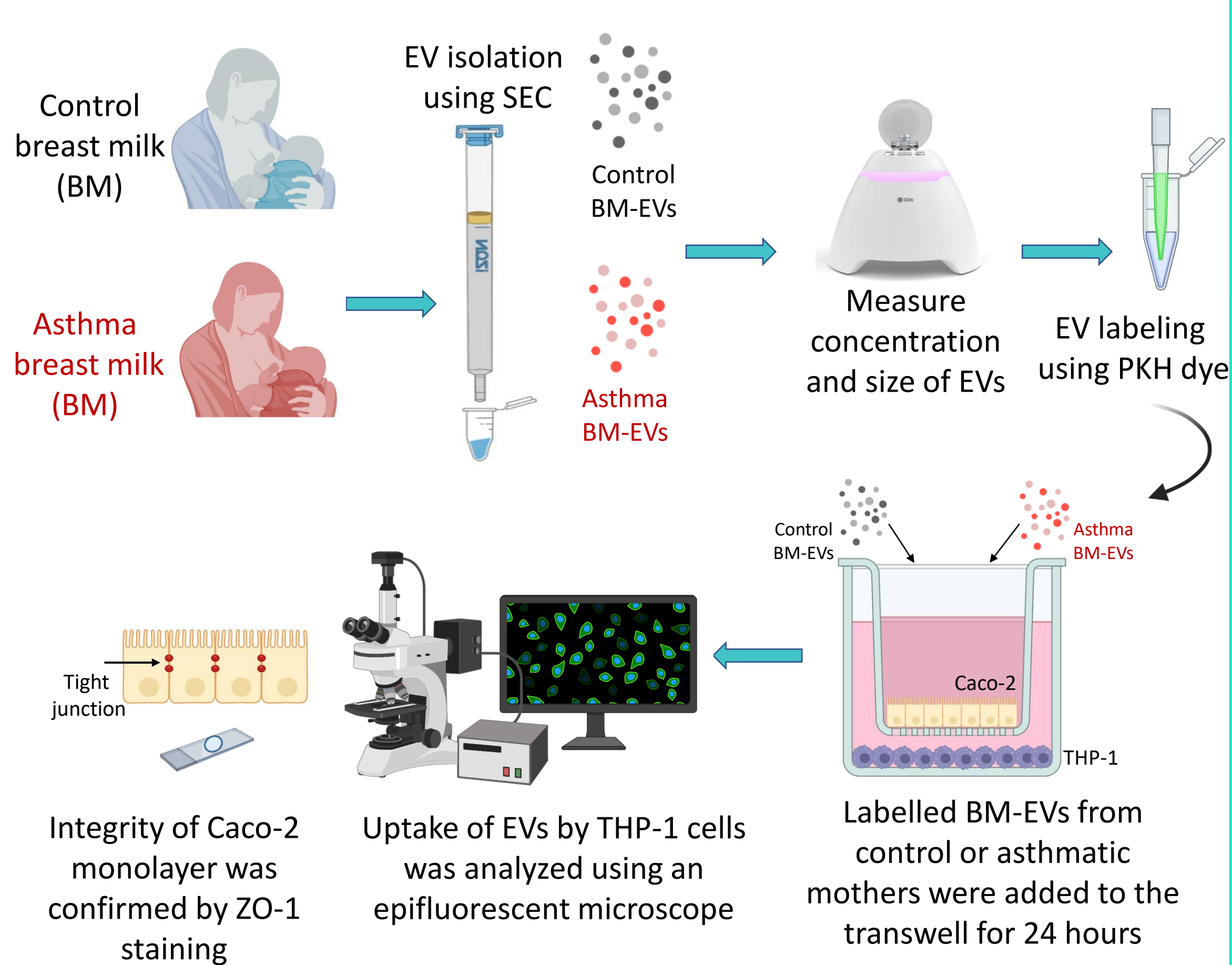


Figure 1. Schematic representation of transwell cell model to mimic the gastrointestinal barrier *in vitro*. (A) Human Caco-2 cells were seeded on the apical side (insert) of transwell. To confirm the integrity of Caco-2 cells, transepithelial electrical resistance (TEER) was measured daily until differentiation of Caco-2 cells reached a steady state plateau on day 7. On the same day, integrity of Caco-2 cells was also confirmed using FITC-dextran measurement. Then, human monocyte (THP1) cells were seeded on the basal side of the transwell. (B) Breast milk (100 μ l) from matched 3-4 months post-partum mothers with/without asthma (N=3, CHILD Cohort Study) was used to isolate BM-EVs by size exclusion chromatography (SEC). Concentration of EVs was measured using the qNano Gold instrument (Izon Science Ltd.). Isolated EVs were labeled using the fluorescent dye PKH67, and the pellet washed and filtered 2x to remove non-specific excess dye. EVs were then added to apical layer with Caco-2 cells for 24hrs. Uptake was measured in THP-1 cells seeded on the basal side using epifluorescent microscopy. The integrity of Caco-2 monolayer was confirmed with immunofluorescent staining of zona occludens-1 (ZO-1). Appropriate negative controls for labelled-EVs were included.

Results

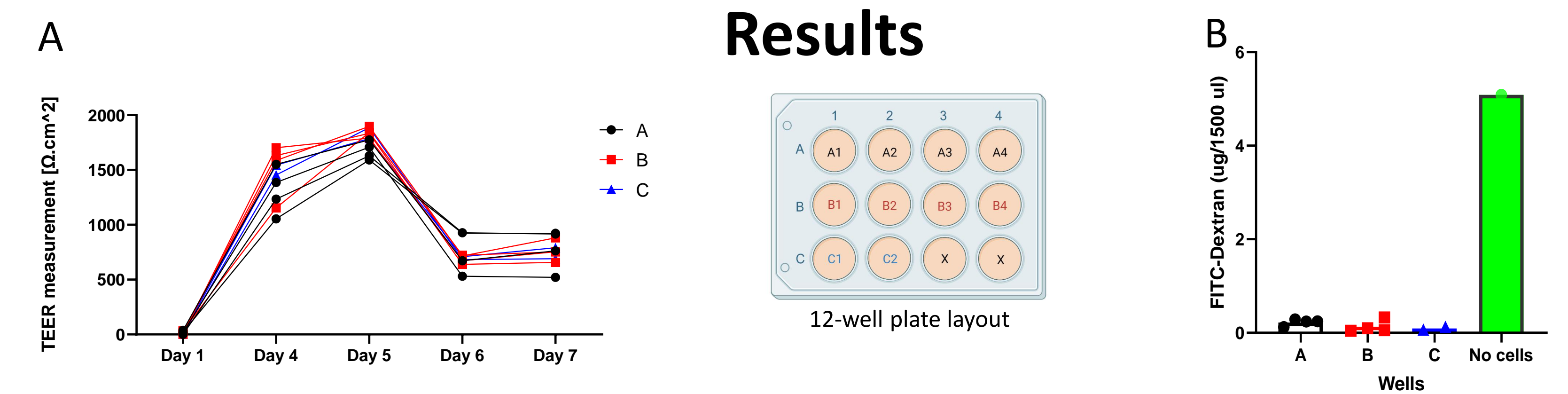


Figure 2. Human intestinal epithelial cells (Caco-2) form a consistent monolayer. (A) TEER measurement was used to confirm: i) integrity of the epithelial Caco-2 cells for 7 days prior to treatment (see schematic plate layout showing wells seeded with Caco-2 cells (A1 to C2); X indicates wells with no cells); and ii) differentiation of Caco-2 cells as shown by a steady state plateau in membrane resistance reached by day 7 (775.62 Ω ·cm²). (B) FITC-Dextran was added to the apical side to measure the impermeability of the Caco-2 cell layer to diffusion of FITC-Dextran dye across the membrane. Uptake of FITC-dextran in the wells without cells was almost 35-fold higher than all other wells with cells (see plate layout in A).

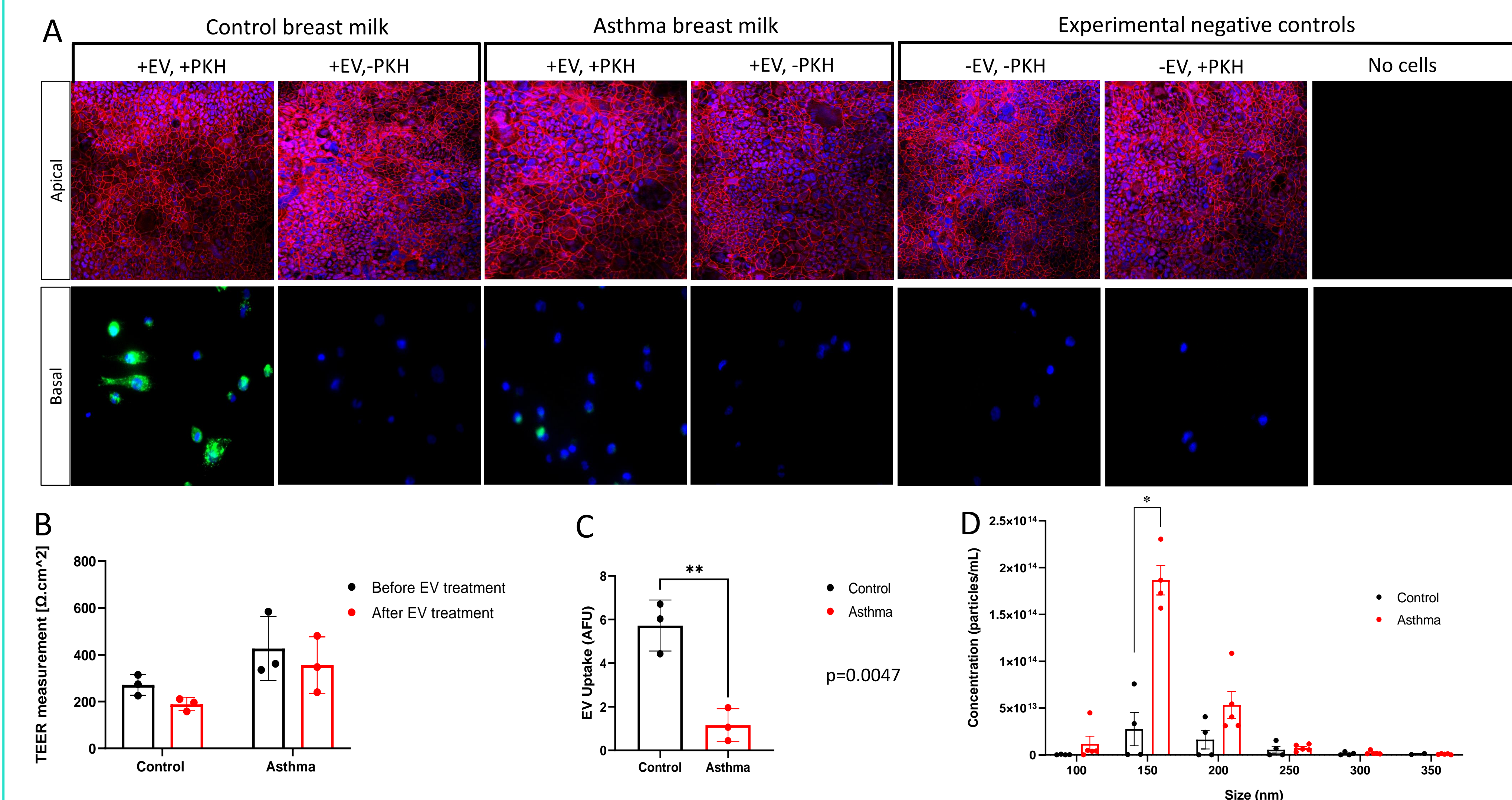


Figure 3. Uptake of BM-EVs by THP-1 cells is lower despite higher BM-EV concentration in mothers with asthma vs. matched controls, in a constant volume of milk. (A) ZO-1 staining was used to confirm an intact Caco-2 layer on the apical side. Uptake of EVs by THP-1 cells was observed on the basal side, and only when EVs were labeled with PKH67. Negative control conditions did not show any green fluorescence staining in the THP-1 cells illustrating the specificity of the model for EV uptake measurements. (B) Apical BM-EV treatment did not change TEER after 24 hours in control or asthma samples, showing no adverse effect of EV treatment on Caco-2 cell integrity. (C) Uptake of BM-EVs derived from a constant volume of milk (100 μ l) by THP-1 cells on basal side was 4.97-fold lower for BM-EVs from mothers with asthma vs. matched controls ($p=0.0047$, N=3). (D) BM-EV concentration (particles/ml) was ~7-fold higher in mothers with asthma vs. matched controls ($p=0.0254$, N=4-5) in a constant volume of milk (200 μ l).

Summary and Conclusion

- Caco-2/THP-1 transwell system is a promising *in vitro* gastrointestinal model to investigate BM-EV uptake and function.
- Uptake of BM-EVs in THP-1 cells is lower in mothers with asthma vs. healthy controls, despite higher BM-EV concentration.
- The mechanisms underlying this effect and functional relevance in offspring asthma development remains to be determined.

Acknowledgements

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