Extracellular vesicles from *Gardnerella vaginalis* and *Mobiluncus mulieris* induce host immune responses from the cervicovaginal epithelium

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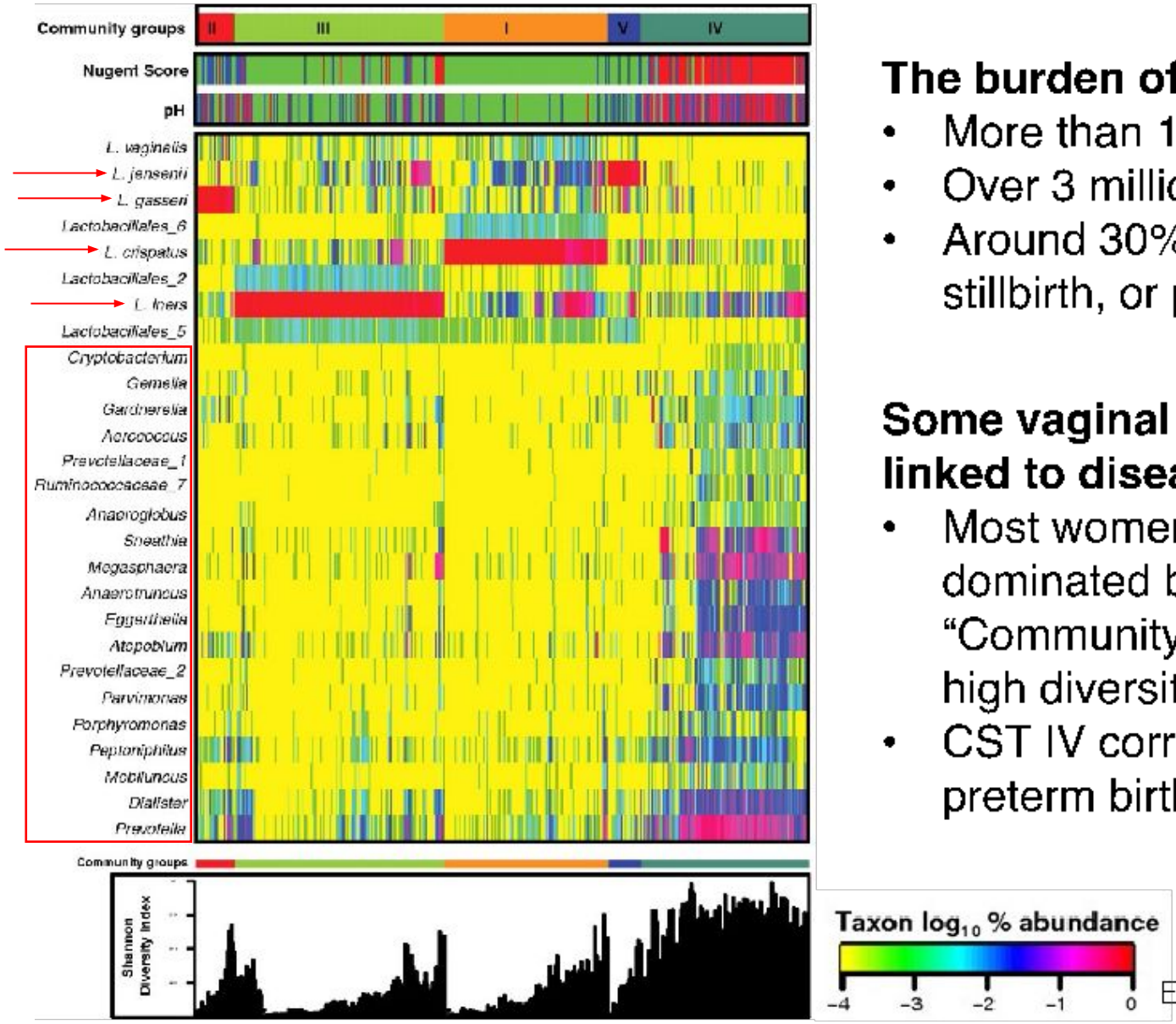
Vaginal microbes & reproductive outcomes



The burden of reproductive disease is high

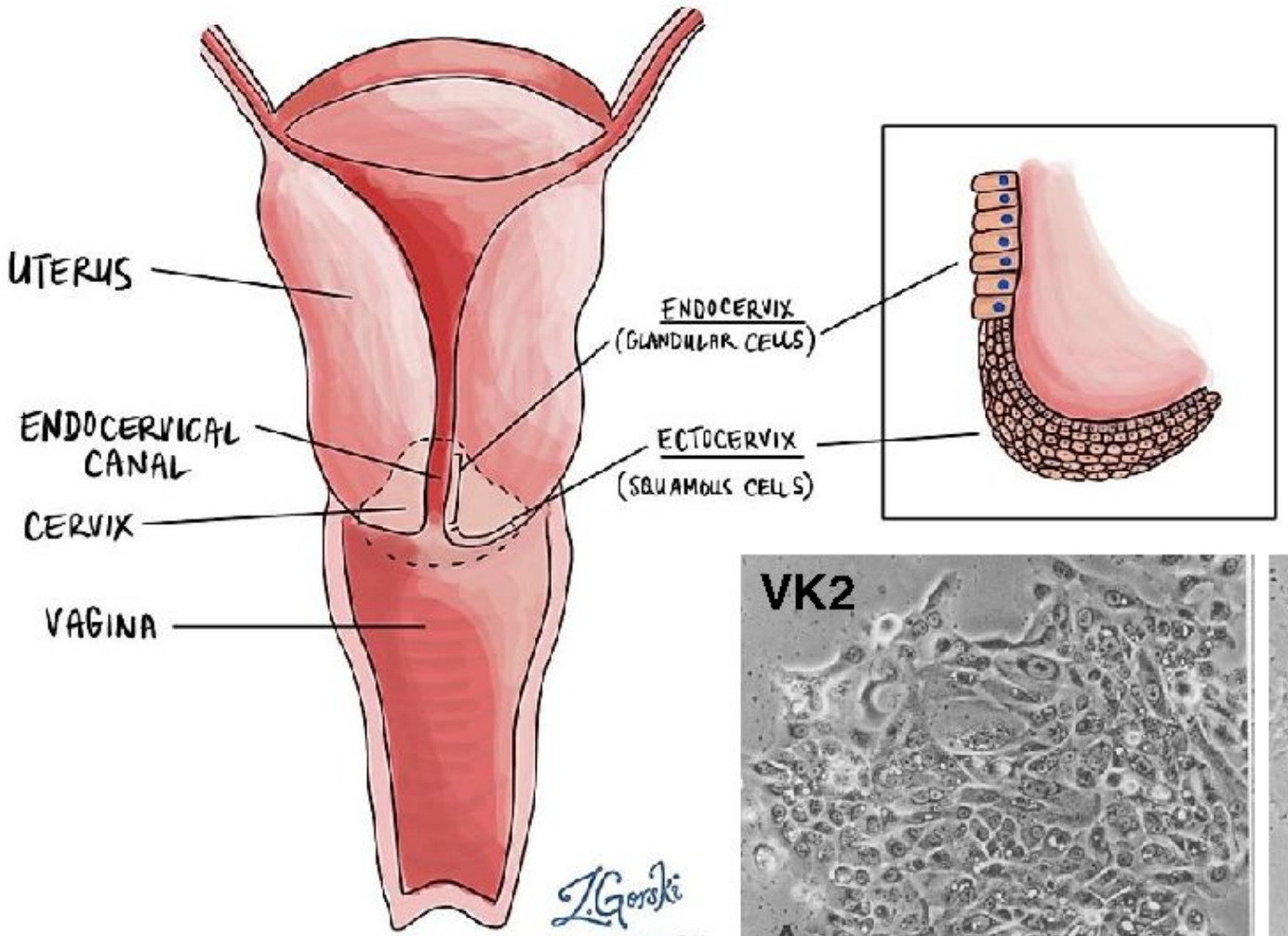
- More than 1 in 5 women are affected by STIs
- Over 3 million women are diagnosed with BV annually
- Around 30% of pregnancies end in miscarriage, stillbirth, or preterm delivery

Some vaginal microbes & microbial communities are linked to disease risk

- Most women have vaginal microbial communities dominated by *Lactobacillus*, but other women host "Community State Type (CST) IV" characterized by high diversity and anaerobic bacteria
- CST IV correlates highly with risk of STIs, HIV, BV, and preterm birth

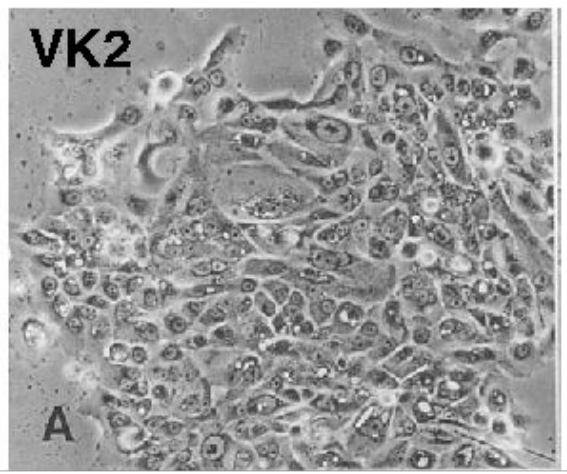
Ravel et al. PNAS (2011) doi.org/10.1073/pnas.1002611107
Elovitz et al. Nat Comm (2018) doi.org/10.1038/s41467-019-09285-9

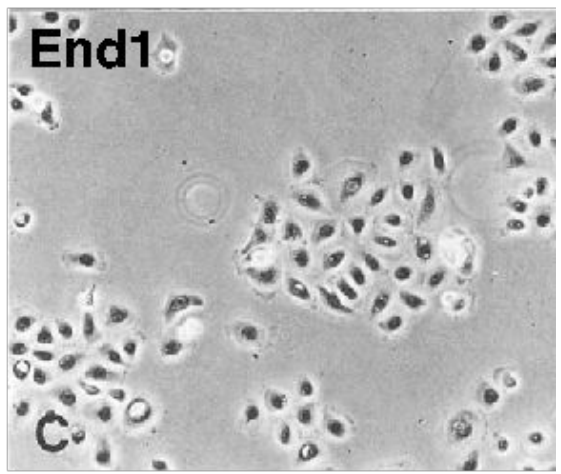
Cervicovaginal epithelial cell types



Fichorova et al. Biol Reprod (1997)
10.1095/biolreprod57.4.847

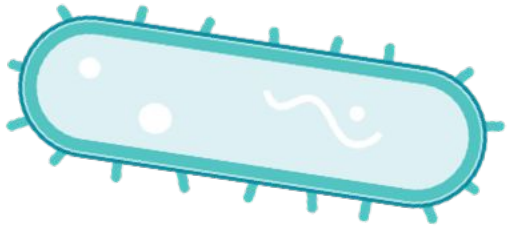
- Cervical and vaginal epithelial cells have different embryological origins, epithelial structures, and functions in the reproductive tract
- Our lab uses three immortalized cell lines to model the CV epithelium:



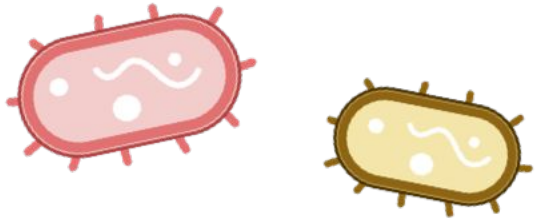


Microbes can damage epithelial function

Lactobacillus crispatus (LC)

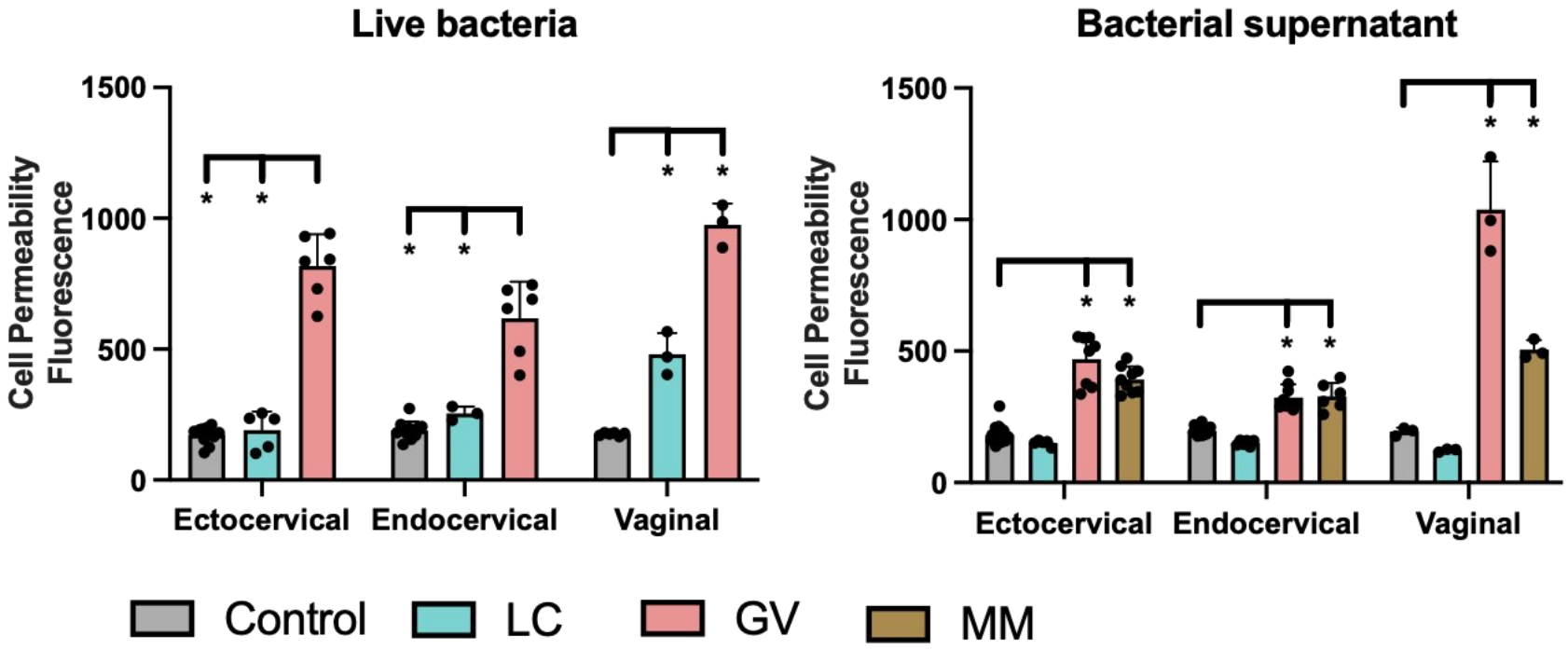


Gardnerella vaginalis (GV)



Mobiluncus mulieris (MM)

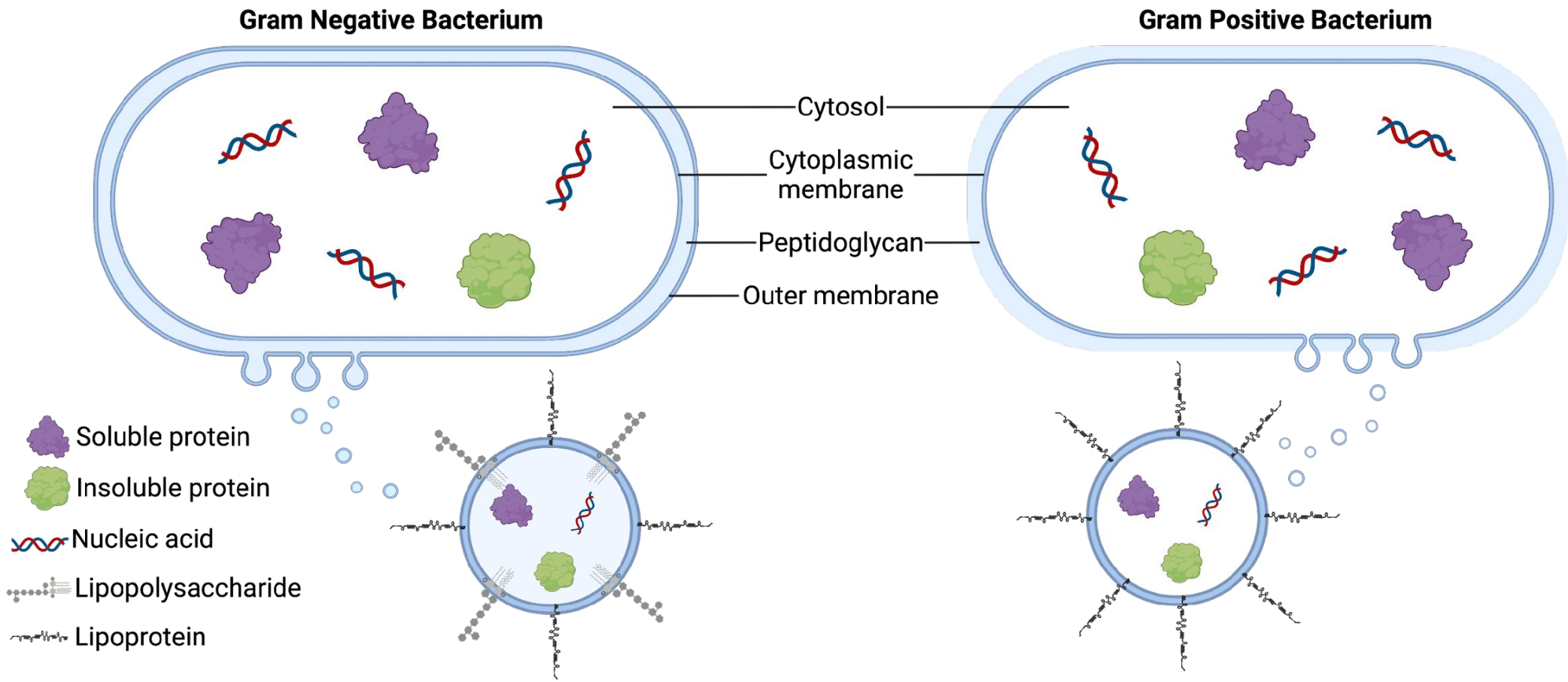
All are Gram-positive bacteria, i.e. contain a thick cell wall and no LPS.



What are these vaginal microbes producing and releasing into the supernatant that are capable of altering the epithelial barrier?

Anton et al. Microbiome (2021, preprint) 10.21203/rs.3.rs-1251543/v1
Anton et al. Front Microbiol (2018) 10.3389/fmicb.2018.02181
Dude et al. Anaerobe (2020) 10.1016/j.anaerobe.2019.102127

Bacterial extracellular vesicles

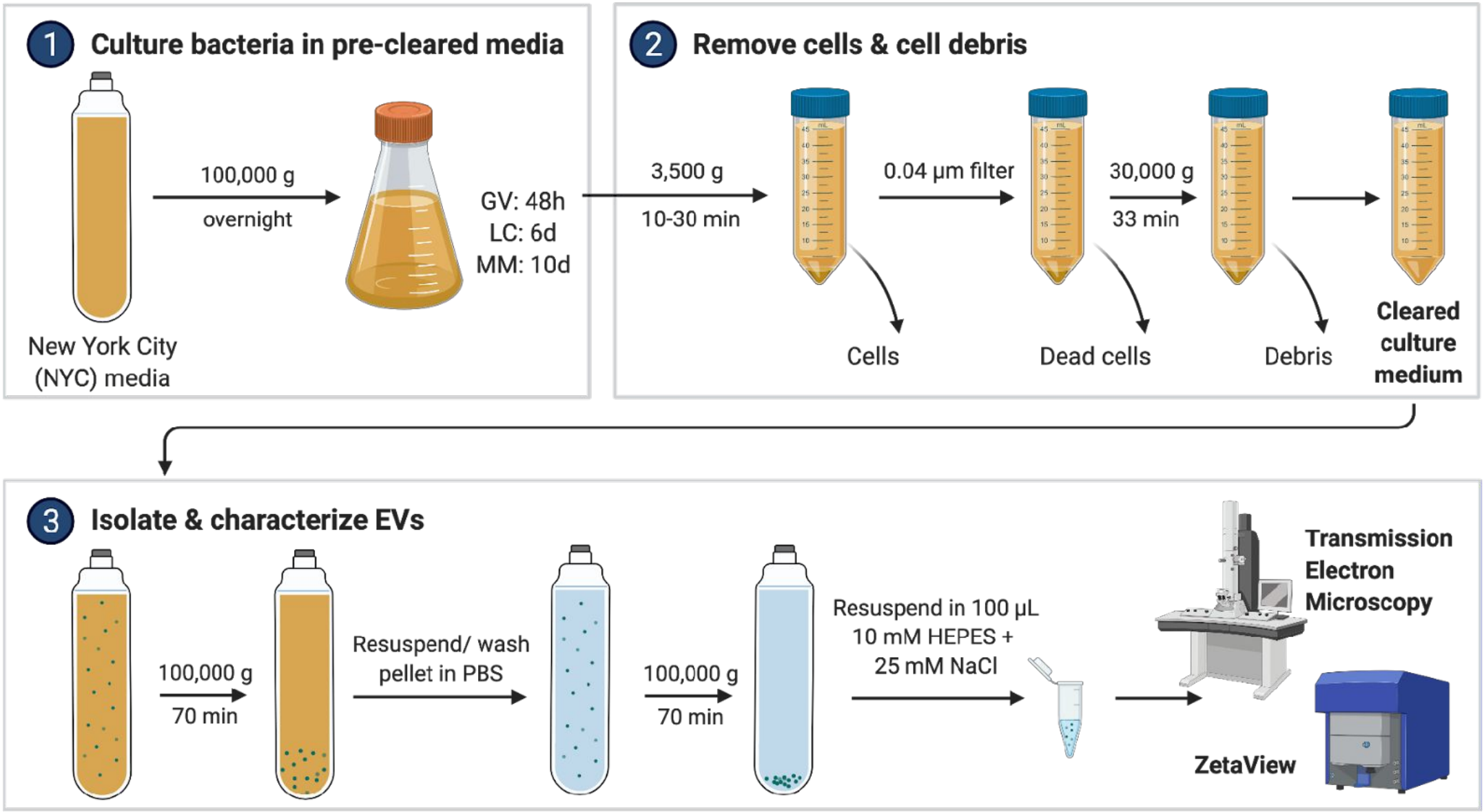


Bacterial EVs are known to play a role in: intercellular communication, intercellular competition, stress response, lateral gene transfer, and pathogenicity

The objectives of our study were to:

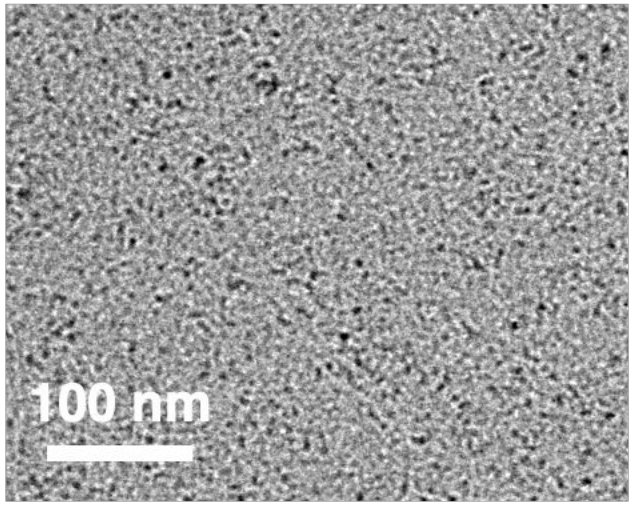
1. characterize EVs from vaginal microbes,
2. track EVs with epithelial cells, and
3. assess functional effects on epithelial cells

EV isolation: sequential ultracentrifugation

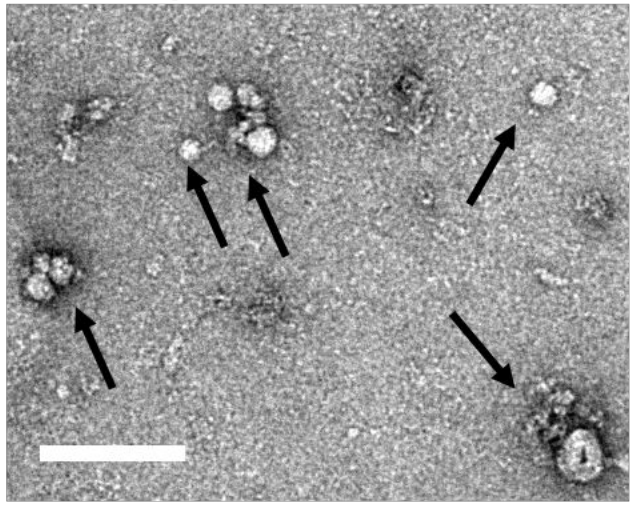


EV characterization: NTA and TEM

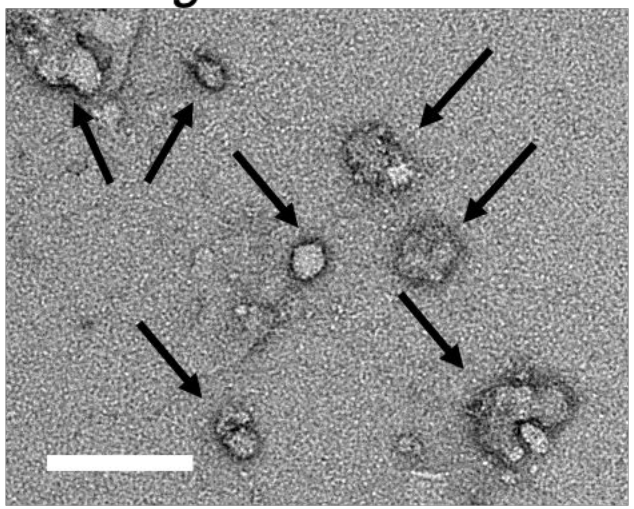
NYC Media



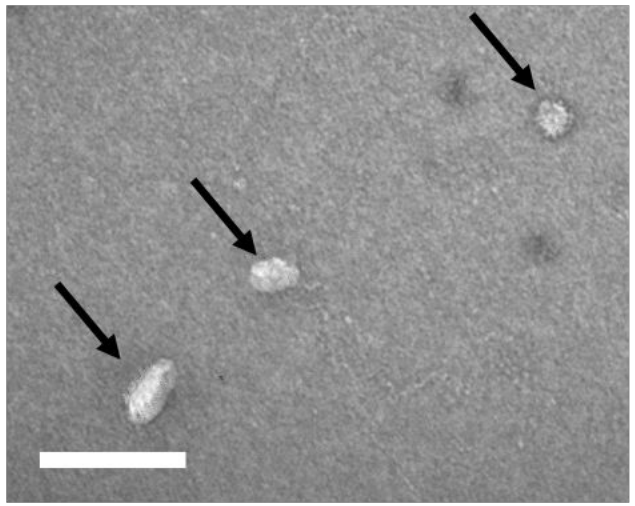
L. crispatus EVs

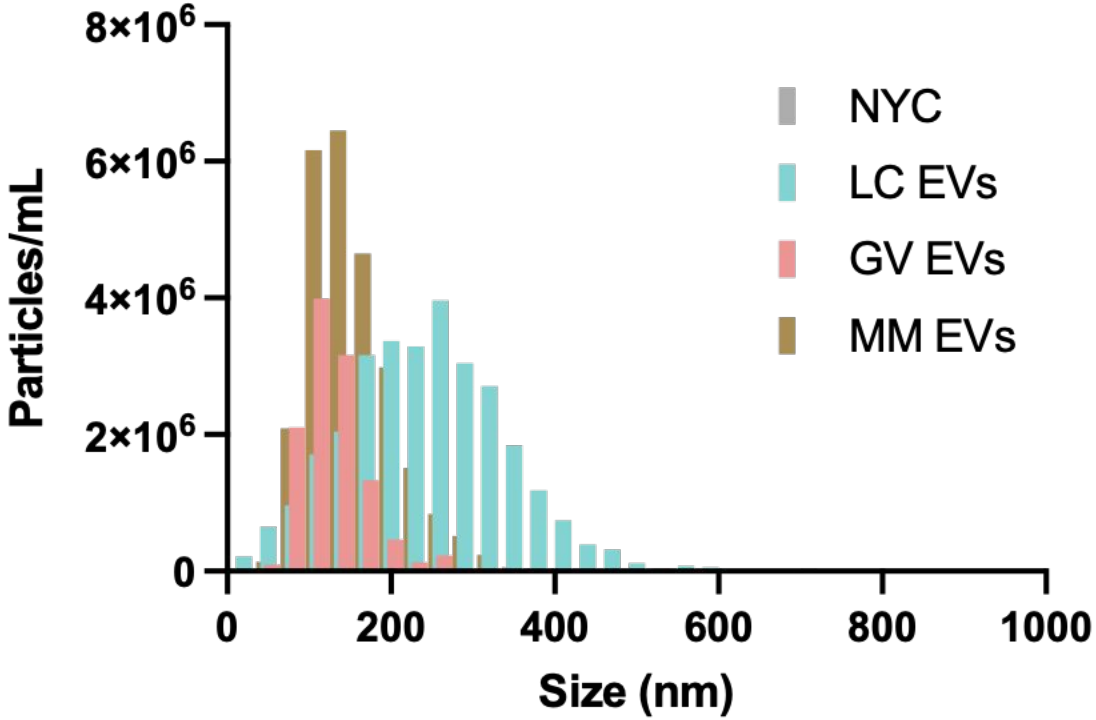


G. vaginalis EVs



M. mulieris EVs





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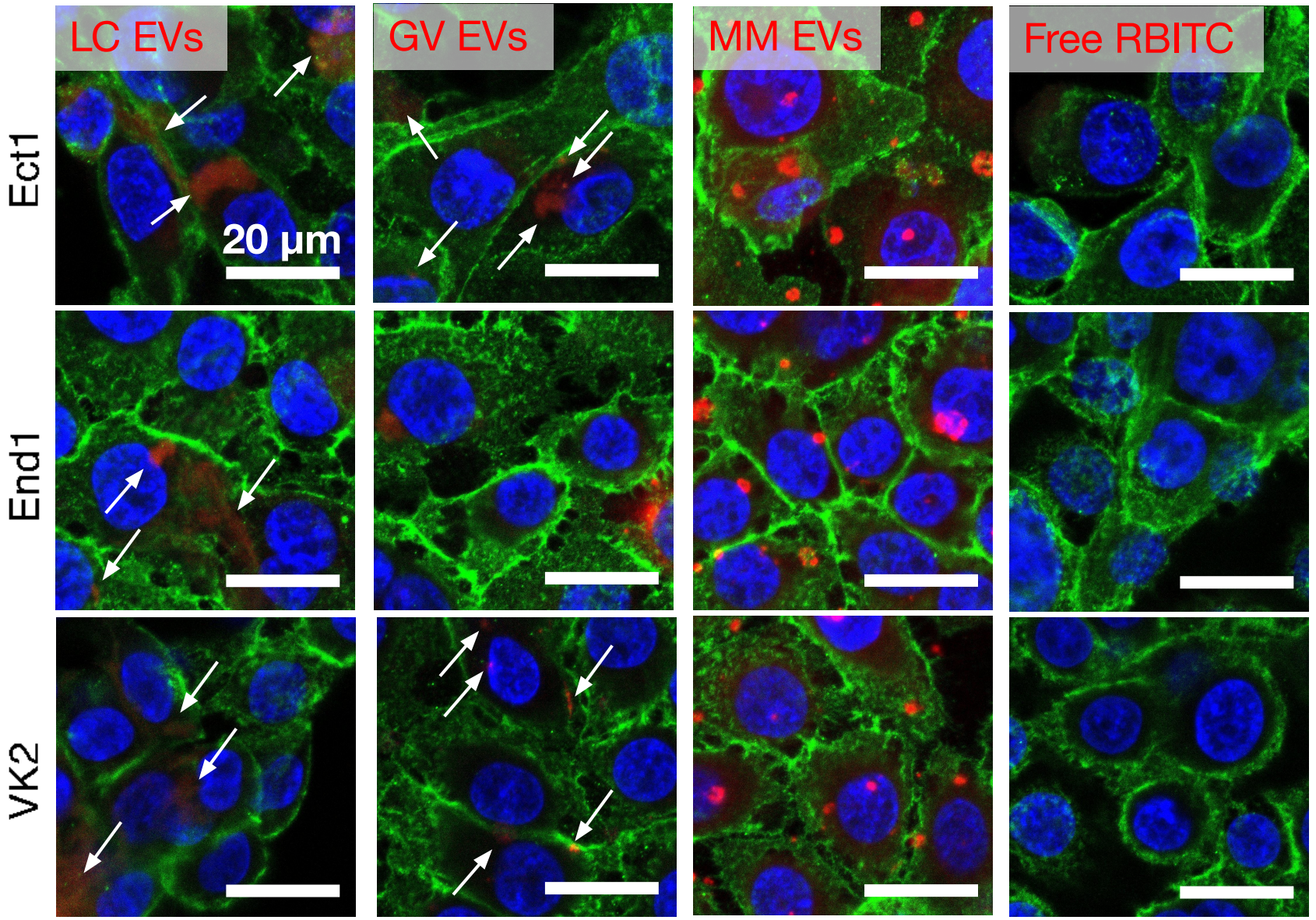
EV uptake by cervicovaginal epithelial cells



Within 24 hours, EVs from all 3 bacteria can uptake into epithelial cells.

DAPI / E-cadherin

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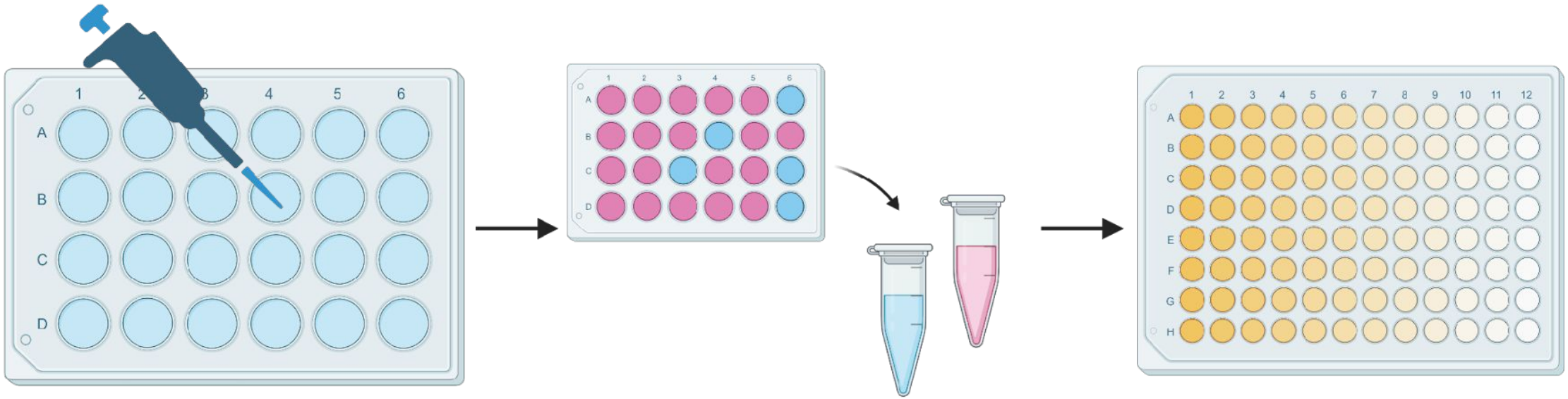
Do bEVs induce a host immune response?



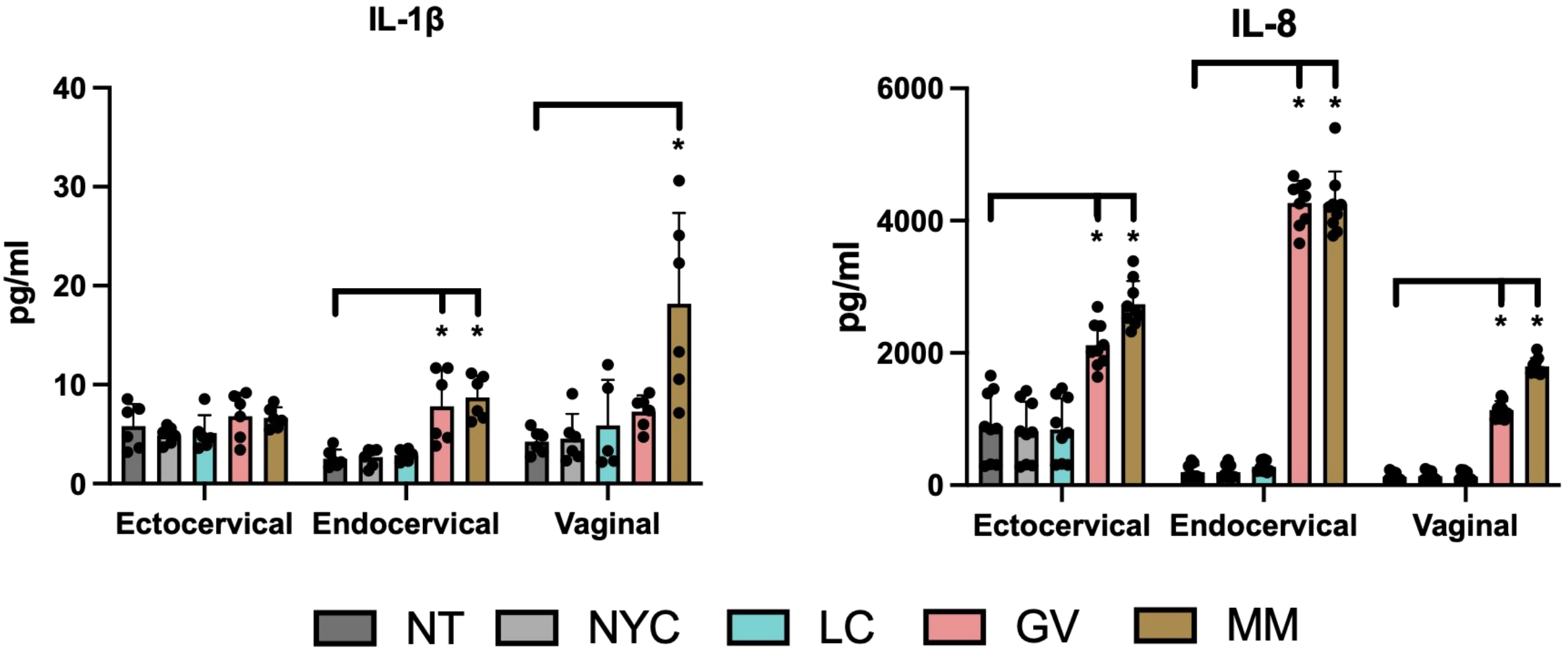
1. Plate cells at 150,000 per well.

2. After 24h, add 10^7-10^9 EVs per well.

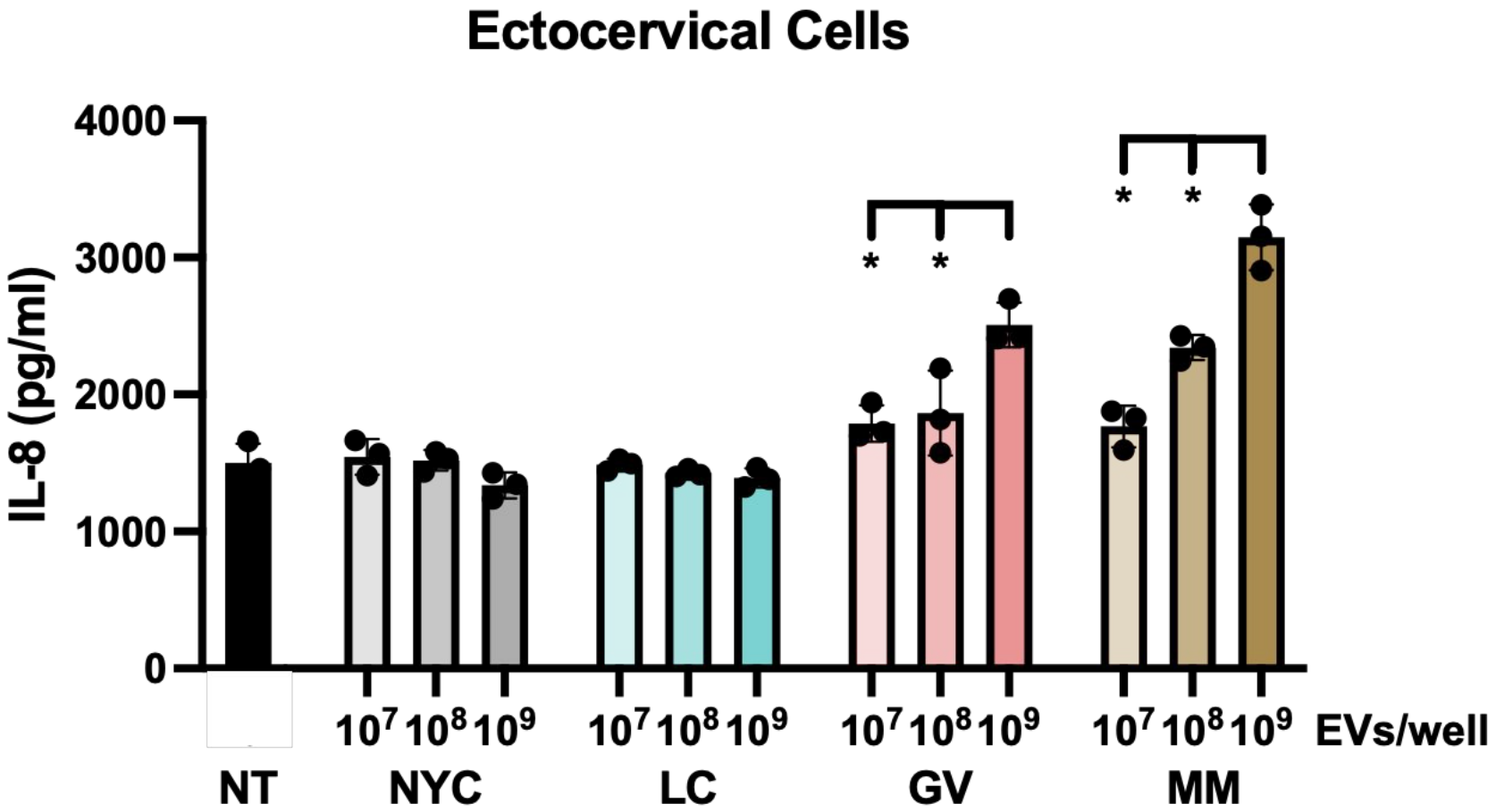
3. After another 24h, collect cell culture media.

4. Run ELISAs with media samples.

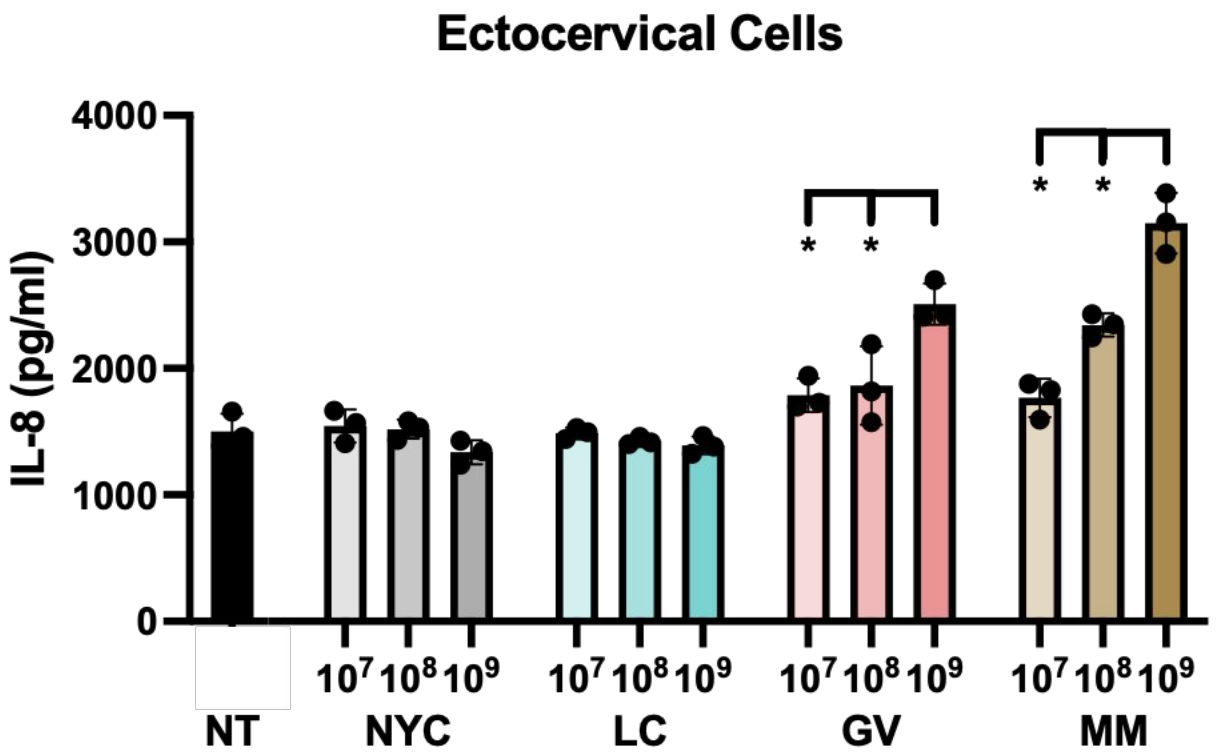
GV & MM EVs induce immune activation

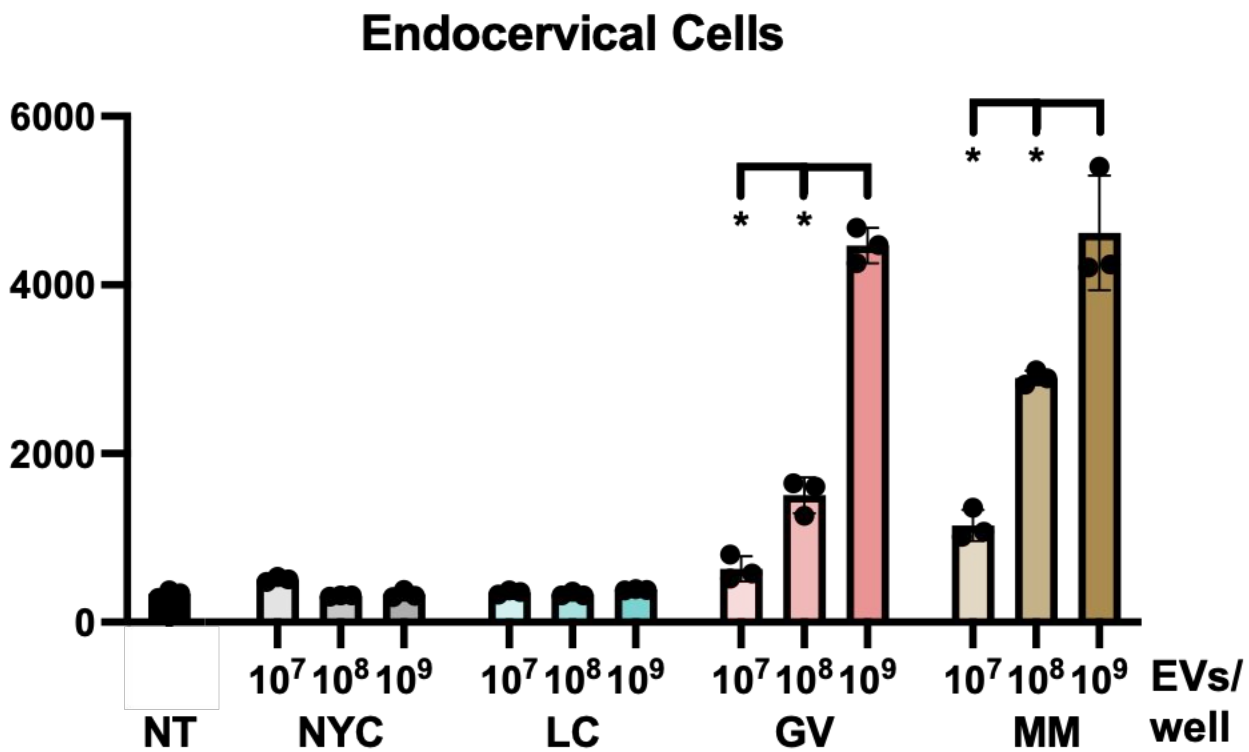


Immune response is dose-dependent

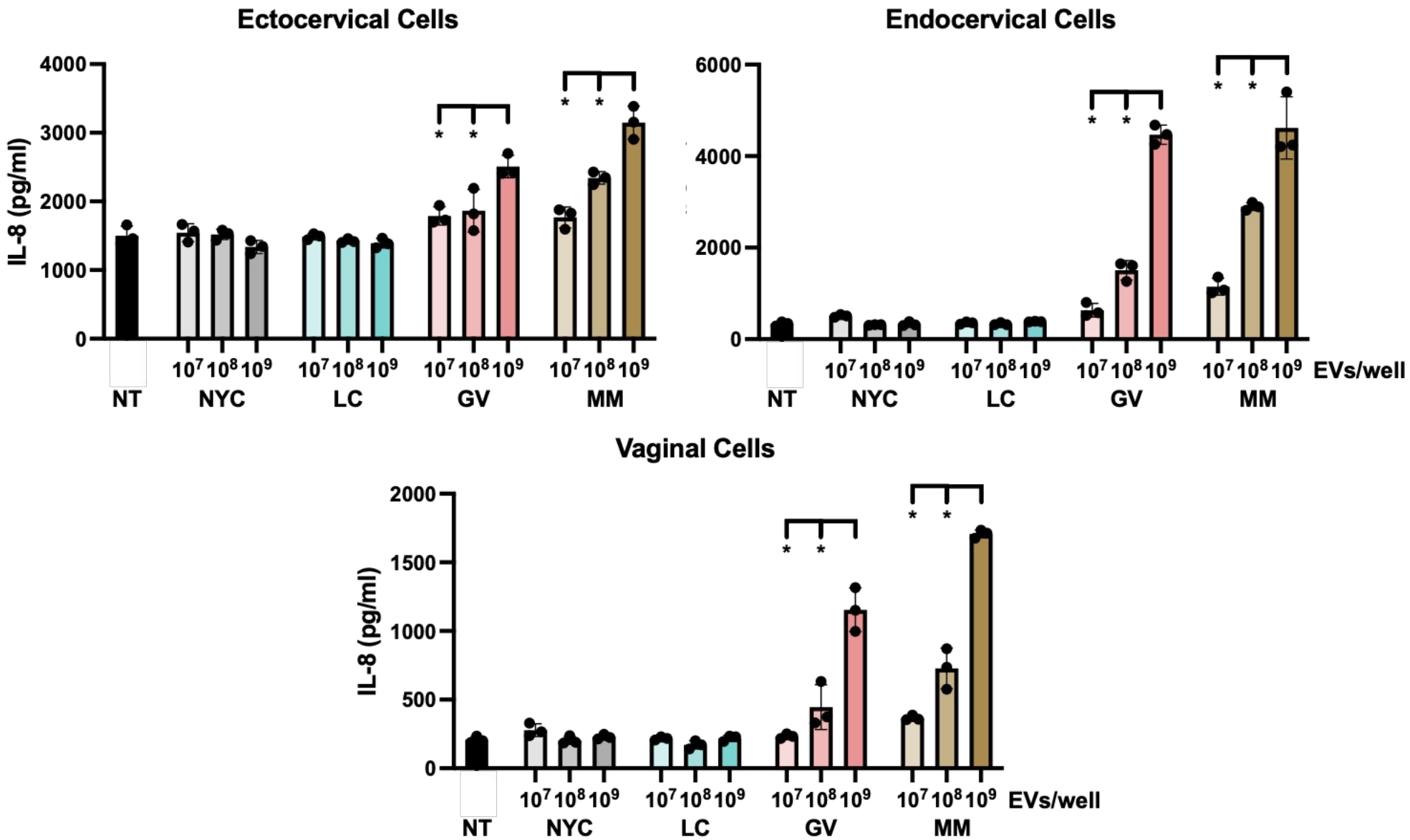


Immune response is dose-dependent

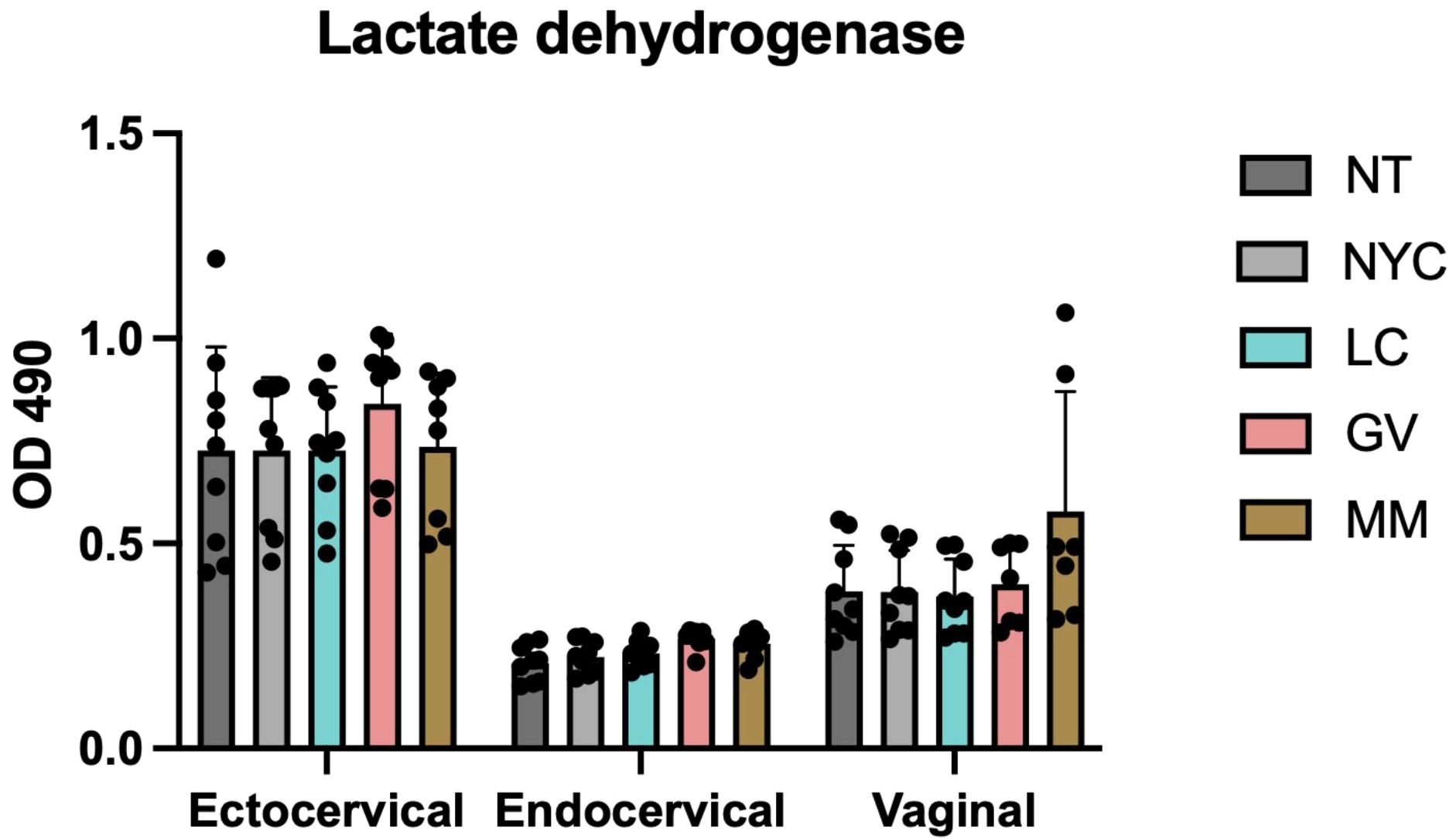




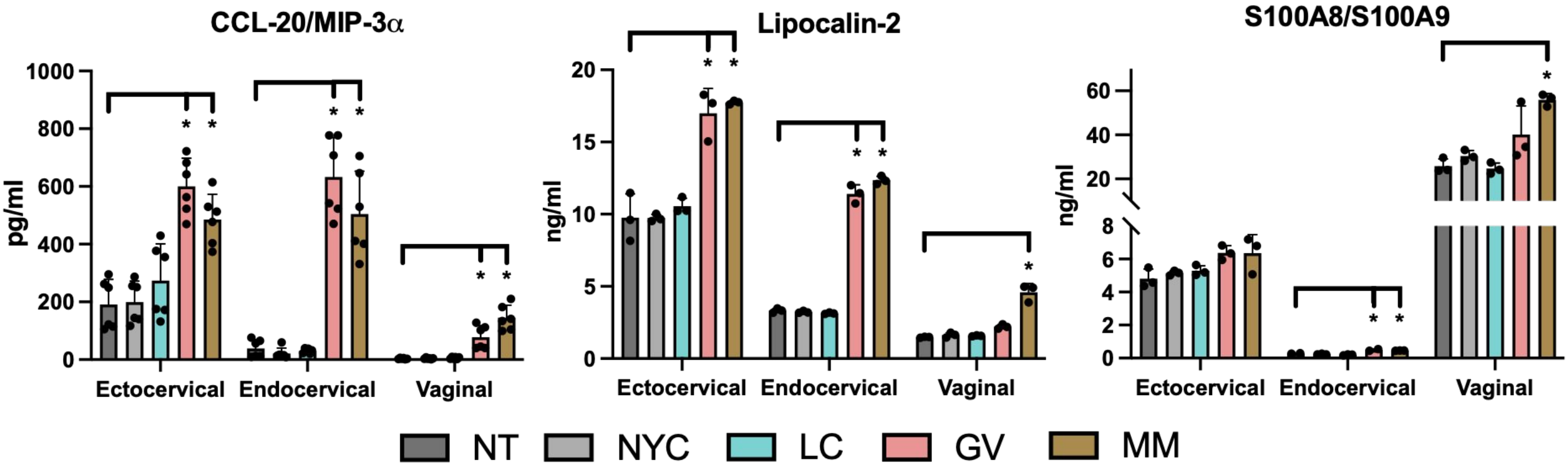
Immune response is dose-dependent



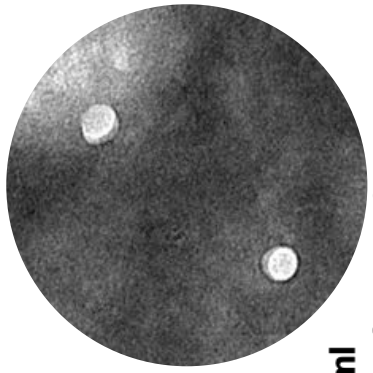
EVs do not induce cytotoxicity

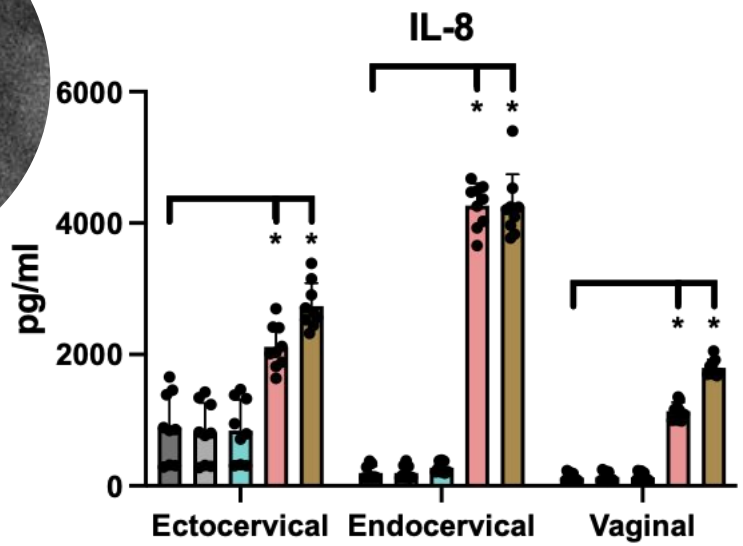


EVs induce an anti-microbial response

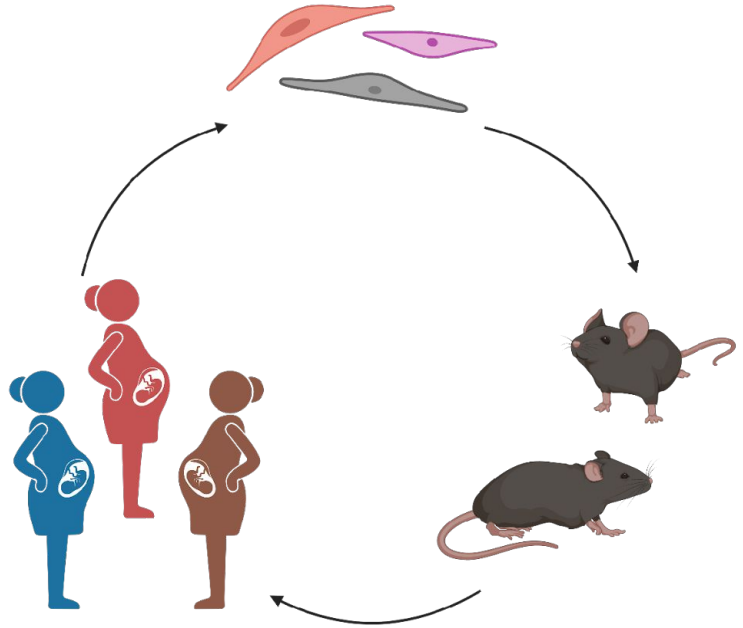


Conclusions & future directions





EVs were successfully isolated from common cervicovaginal microbes. EVs play a role in microbial-immune interaction, which has important consequences for STIs, PTB, and more.



Further *in vitro* and *in vivo* investigation may reveal microbial-host responses with additional complexity. Our findings will drive the understanding, prevention, and treatment of adverse reproductive outcomes.

Thanks to our team & funding!

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