The 3rd International Symposium on Microgenomics 2023

Bacterial Extracellular Vesicles: Spheres of influence within and beyond the gut

Simon R Carding

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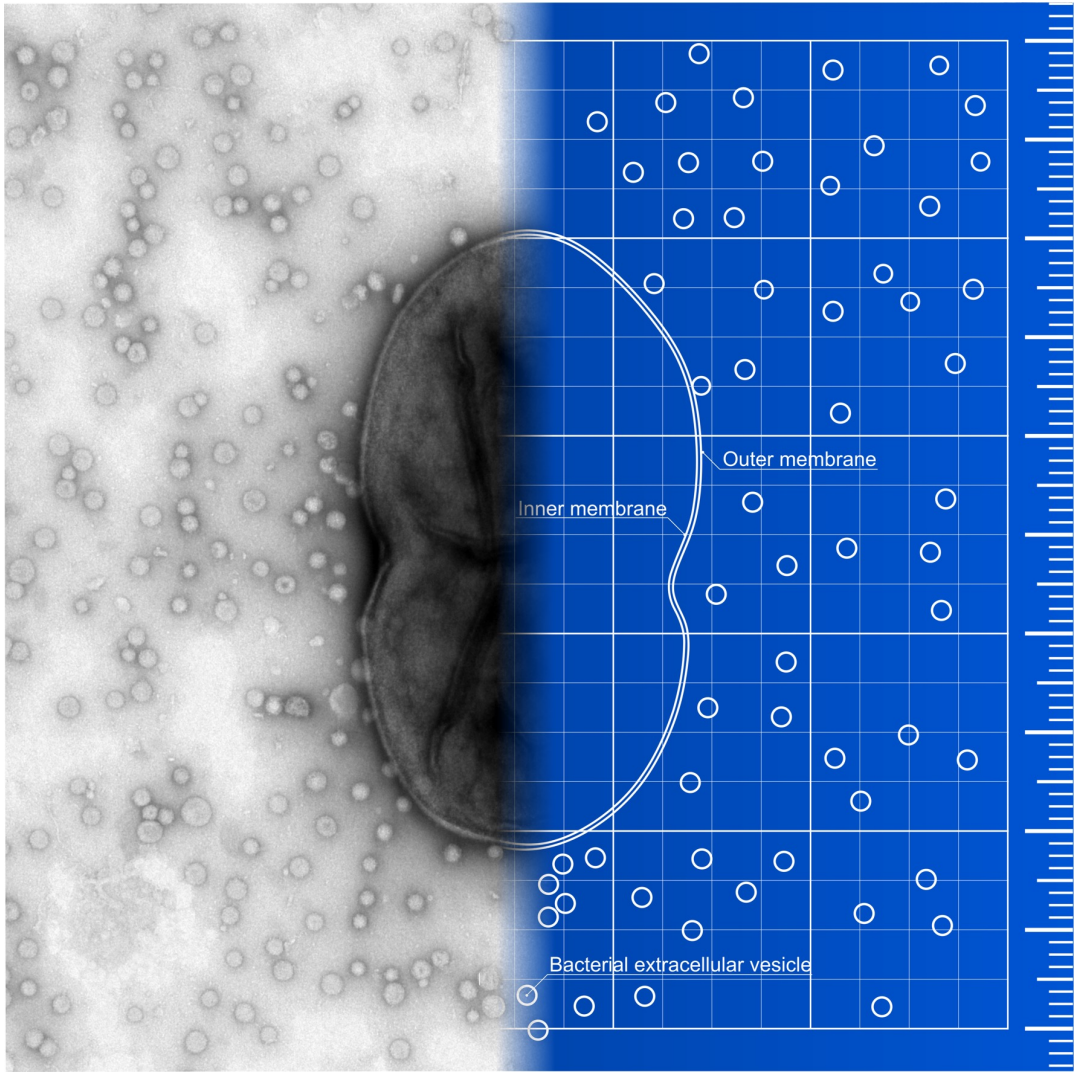
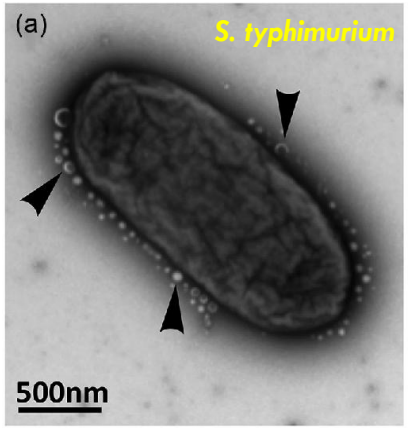
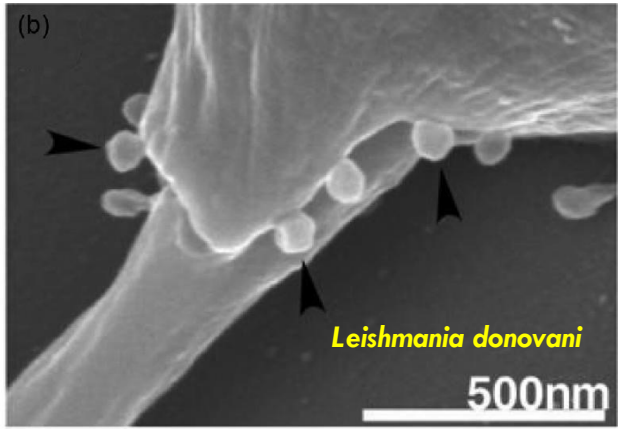
&

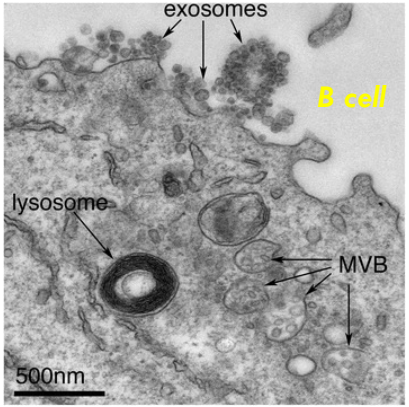
Norwich Medical School, UEA, UK

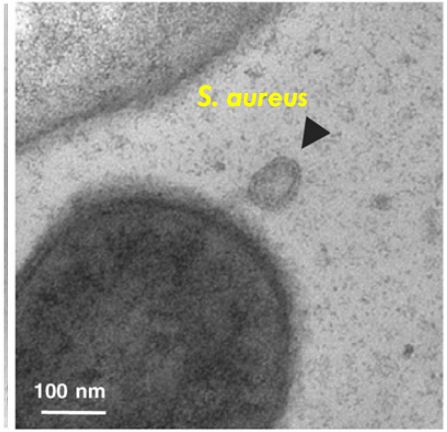


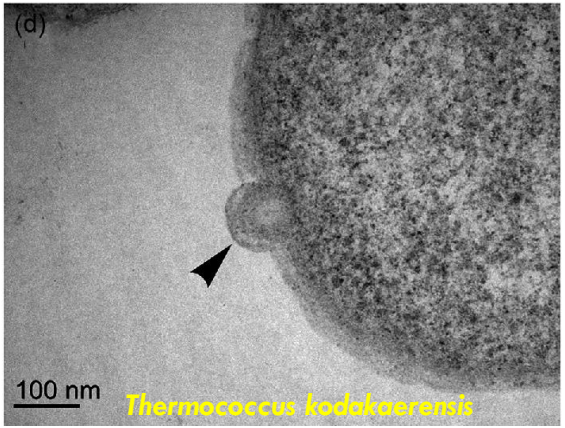
Cells from all three domains of life produce EVs: Evolutionarily conserved mediators of intercellular communication

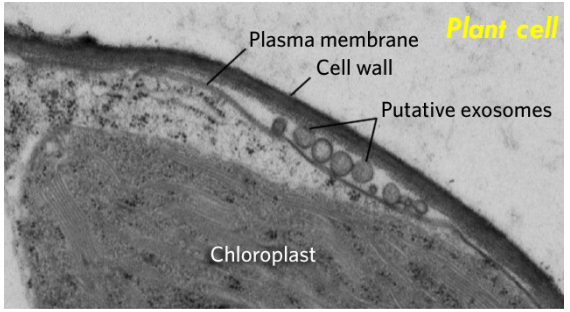








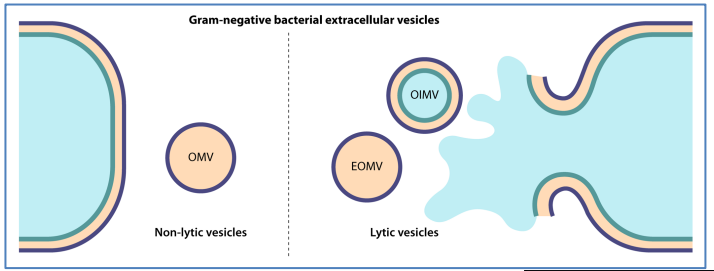




Gill et al., *FEMS Microbiol Rev* 2019; Lee et al., *Proteomics*, 2009; Edgar *BMC Biol.* 2016; Palmer, ASPB, 2019

BEVs: a *bona fide* bacterial secretion system

- First described in 1960's by Bishop and Work for Gram negative bacteria and 30 years later for Gram positive bacteria
- Naturally shed by Gram negative bacteria during normal growth cycle by:
  - Budding from the outer membrane (OMVs)
  - Explosive lysis - outer inner MVs and outer MVs
  - Gram positive bacteria produce cytoplasmic MV

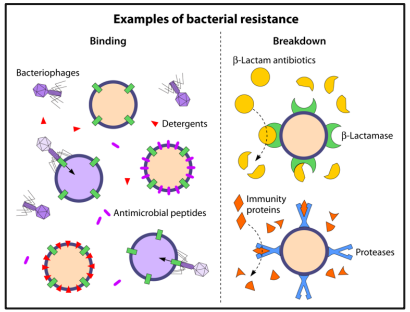


Juodeikis and Carding, 2022

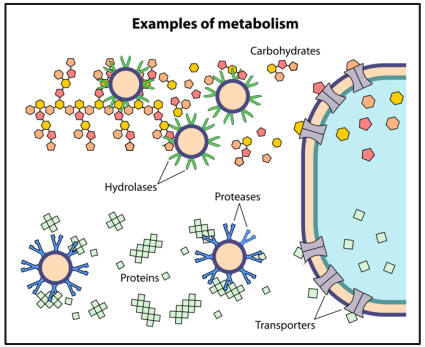
- Genetic basis for regulated production (stress response pathways)
- Selective, non-random, packaging of cytoplasmic and periplasmic contents

BEVs: It's energetically costly so why bother?

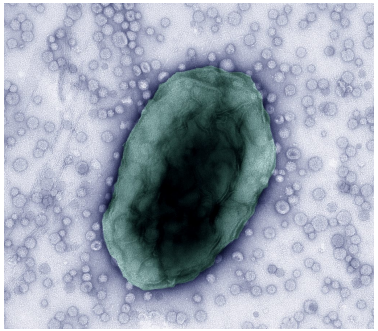
- Garbage disposal
- Re-modelling
- Building fences and self protection
- Export and transport
- Scavenging
- Good neighbors, community development and altruism
- Détente and stabilising cooperative relationships with the host



Juodeikis and Carding, 2022, *MMBR*



Adapted from: Lynch and Alegardo, 2017, J. Bact.; McMillan and Kuehn, 2021, EMBO J.



Bacteroides thetaiotaomicron (Bt): A Model Human GIT Commensal

Obligate anaerobe, non-endospore forming, motile/ immotile:

6.26 MB genome encoding 4776 proteins, ~90% for binding/import of polysaccharides

Wide environmental distribution: Constituent of multiple biomes inc. animal microbiomes

Represents ~10-25% of all anaerobes in the human colonic microbiota

Normally resident at the mucosal interface in the lower GIT

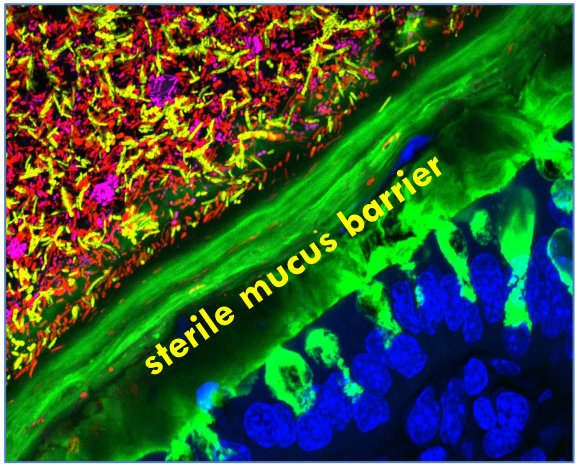
Bacteroides BEVs – Questions of Interest

- Production
- Distribution
- Function
- Exploitation

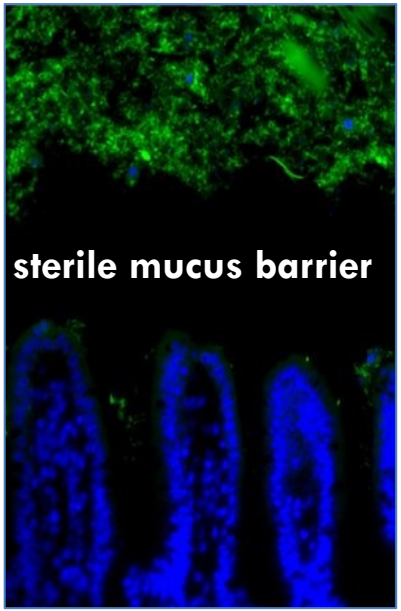
Context

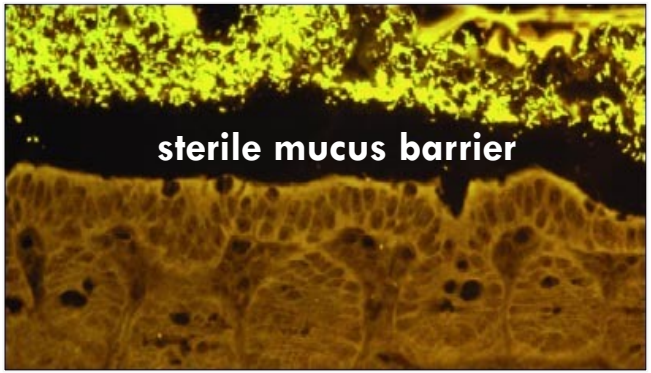
Defining the mechanism and pathways by which gut bacteria communicate with the host to promote health

How do gut microbes communicate with their host?



Vaishnava *et al.* 2009. *J. Physiol. Pharm.*





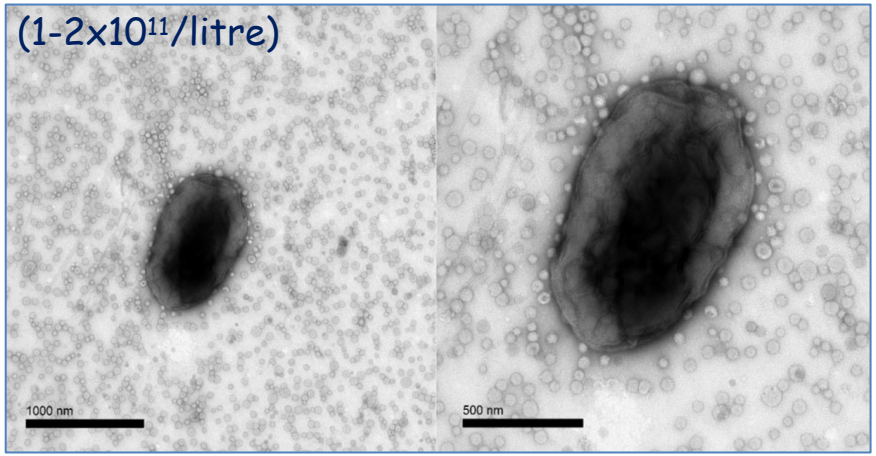
Mucosal barrier crossing by signals is required for microbe-host cell crosstalk

Kamphuis *et al.* 2017. *Scientific Reports*

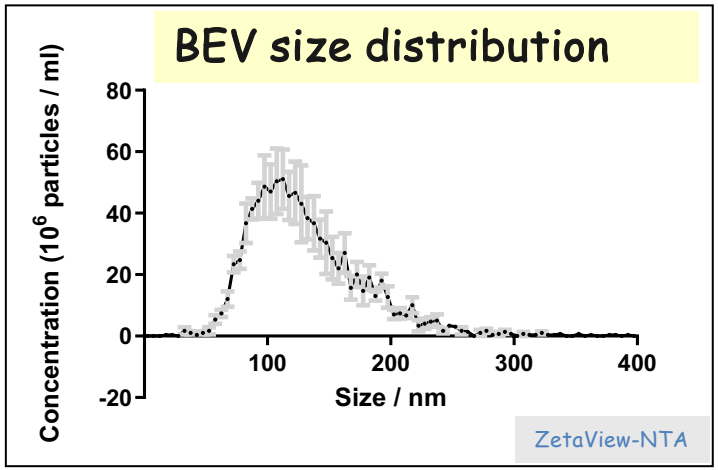
HOW?

- Small Diffusible Molecules
  - Signalling Molecules & Hormones
  - SCFAs
- Nanovesicles (BEVs)

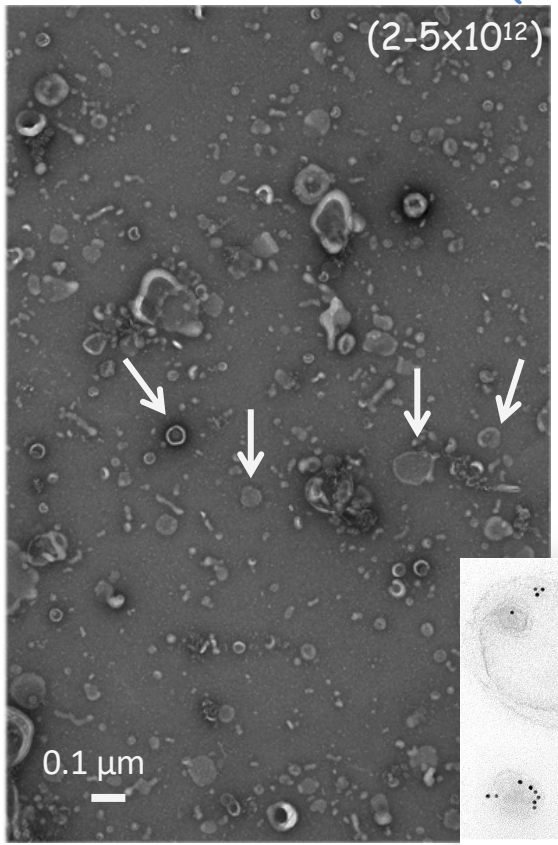
Bt BEVs: Abundant & naturally produced in the GIT

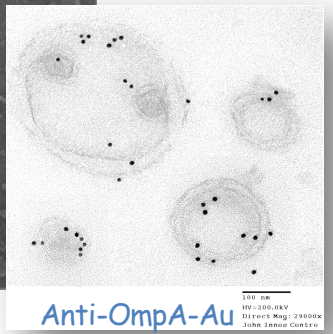


Ian Brown



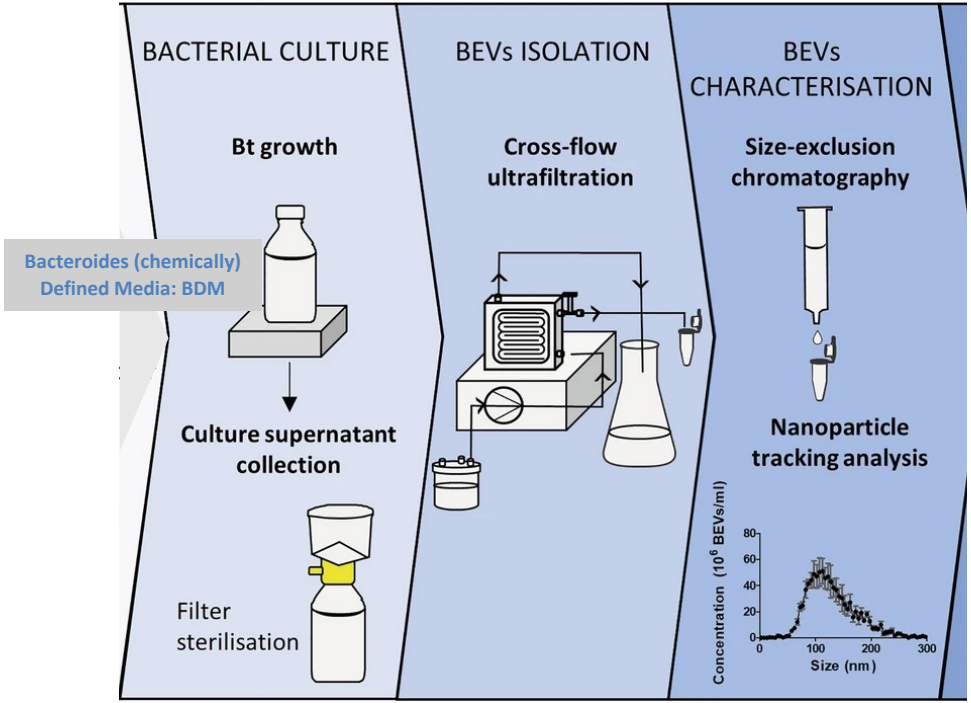
Bt mono-colonised Germfree mice (caecum)





R. Stentz, K. Cross

BEV isolation & characterisation

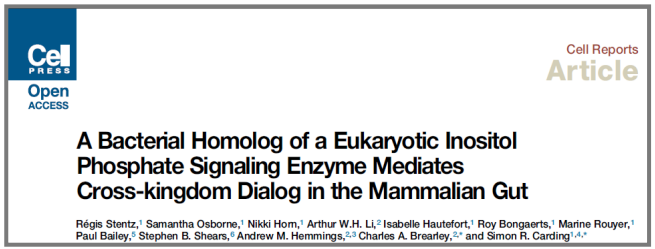


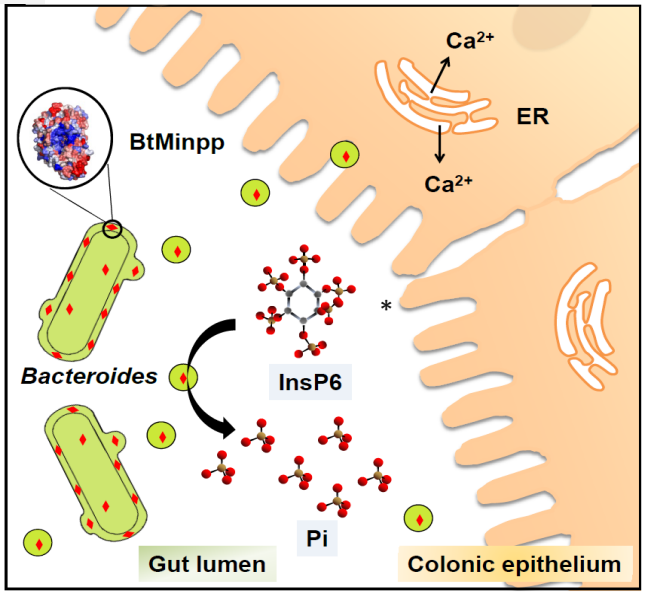
QC

- Size, concentration (NTA)
- Sterility
- Protein content (BCA)
- Lipid content (FM4-64)
- Immunogenicity (THP-1-Blue)

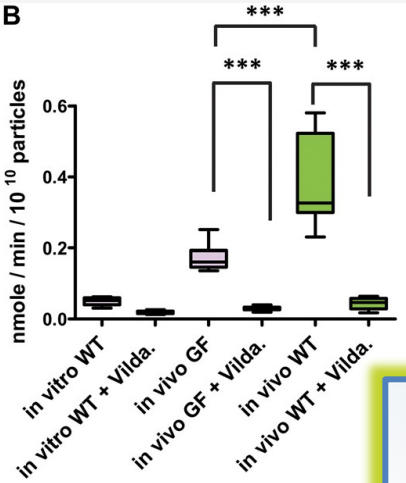
Stentz, Miquel-Clopés and Carding. 2022. *Methods Mol Biol*.

Bt BEVs *in vivo enriched* in metabolic enzymes





BEVs + Ala-Pro-pNa (DPP IV)



*0.33µM Vildagliptin (DPP IV inhib.)

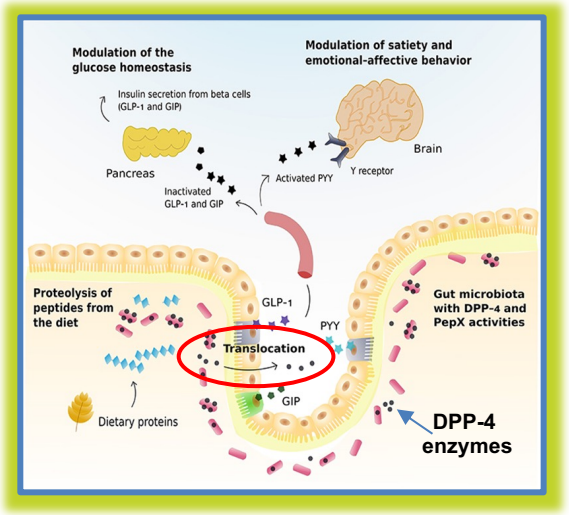
AEM
AMERICAN SOCIETY FOR MICROBIOLOGY

Stentz et al., 2022

Human GLP-1 metabolism (QMUL)

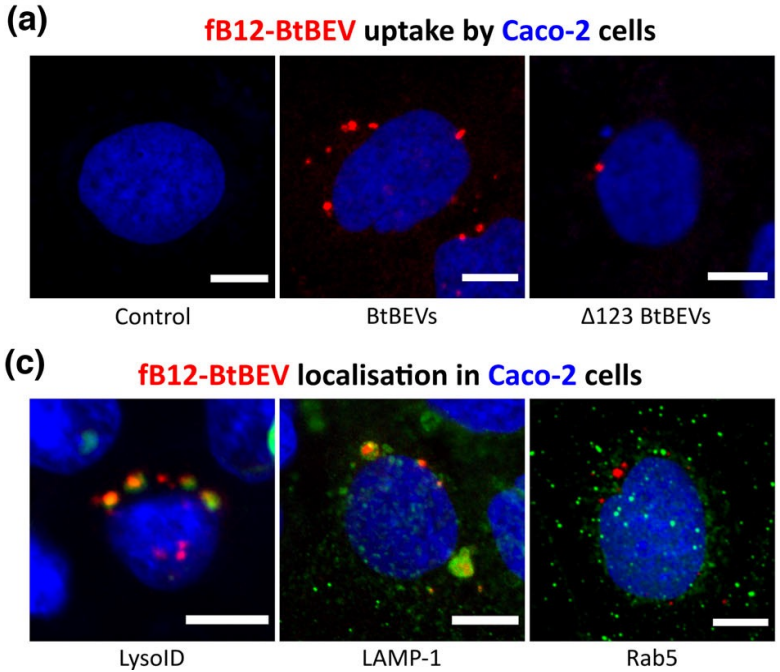
Recombinant DPP IV degrades:

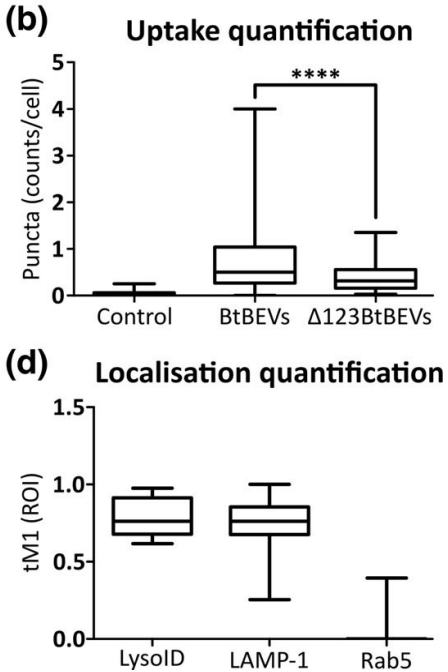
- ✓ **GLP-1**
- ✓ **Substance P**

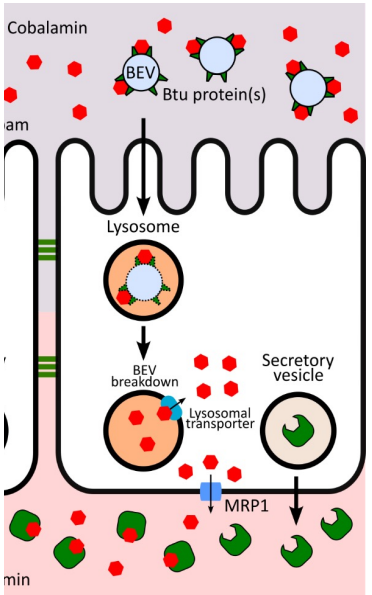


Olivares et al. 2018. *Front. Microbiol.*

Bt BEVS *in vivo* express high levels of BtuG proteins that deliver cobalamin/vit B12 to host cells



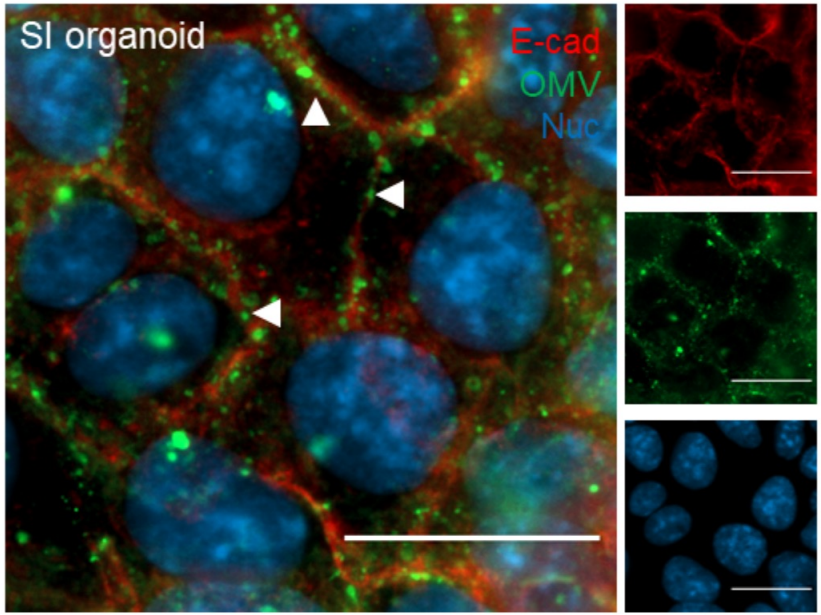


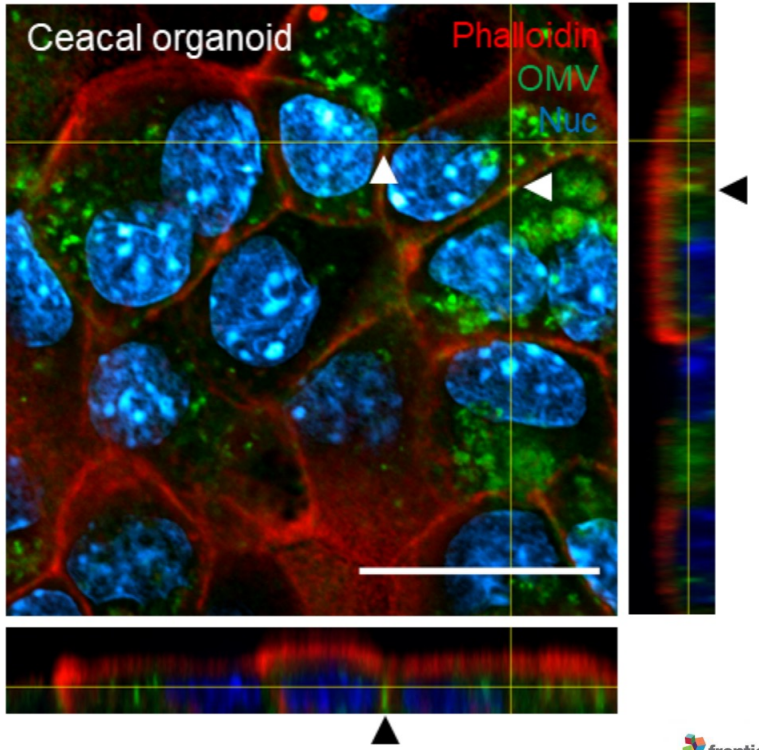


JOURNAL of Extracellular Biology

Juodekis et al., 2022

Bt BEVs access and cross the host GIT





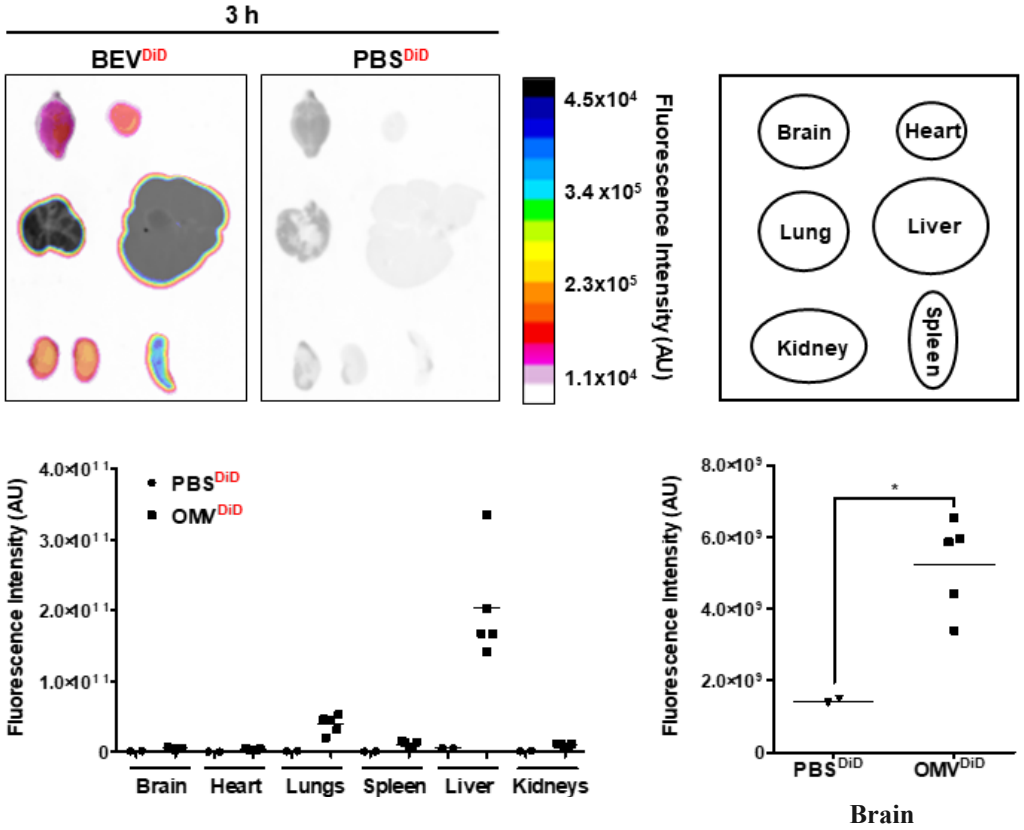
frontiers in Microbiology

Jones et al., 2020

Biodistribution of DiD-labelled Bt BEVs

Post oral administration to adult SPF mice

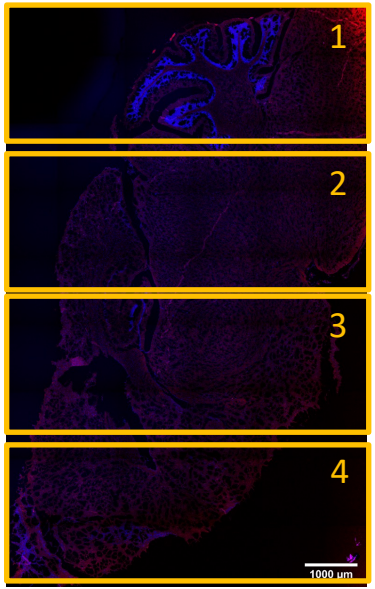
In vivo Xtreme multi-modal optical and x-ray small animal imaging system (Bruker) with a back-illuminated 4MP CCD detector

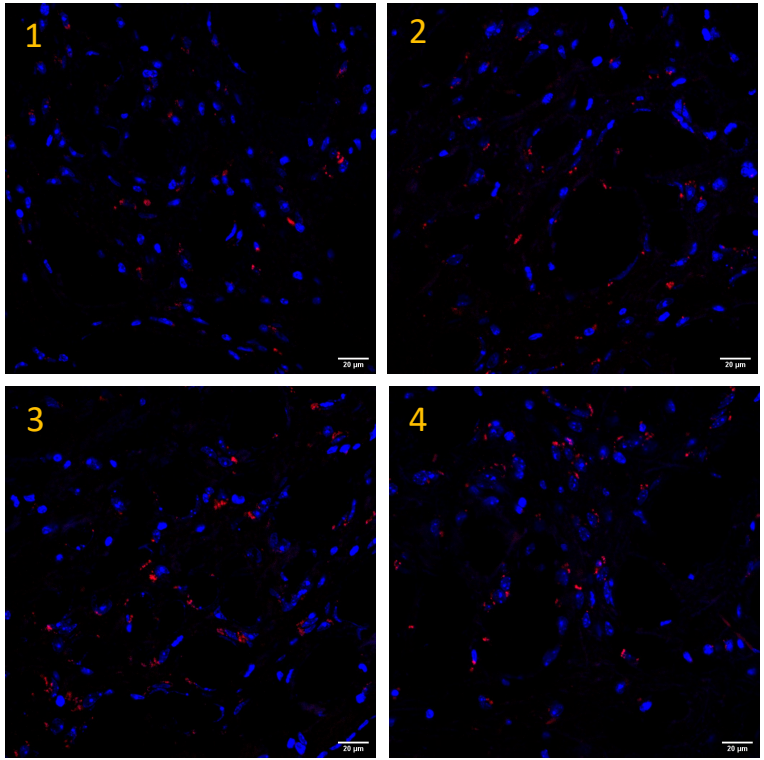


JOURNAL of Extracellular Biology
Modasai et al. 2023

Localisation of DiD-Bt BEVs to the Liver & CNS

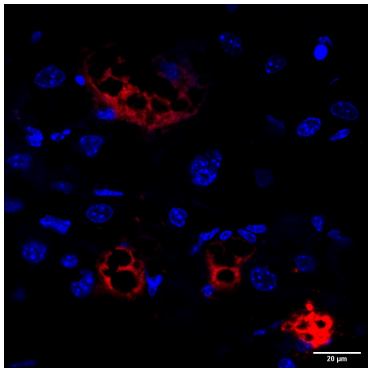
R hemisphere



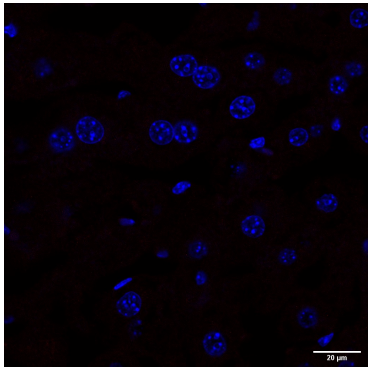


Median lobe

+BEVs

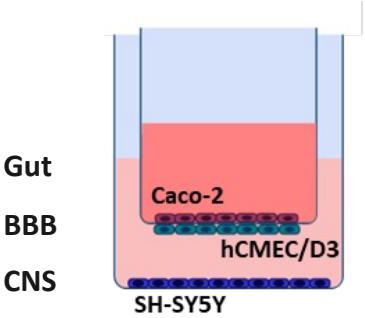


+PBS



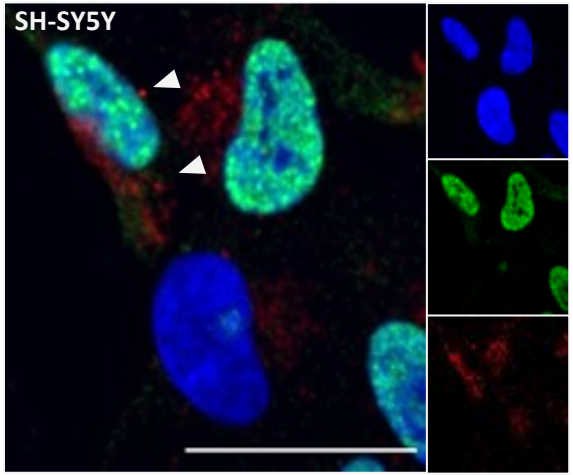
Jones, et al. 2023. In preparation

A simplistic *in vitro* three cell model of the gut-blood-brain axis

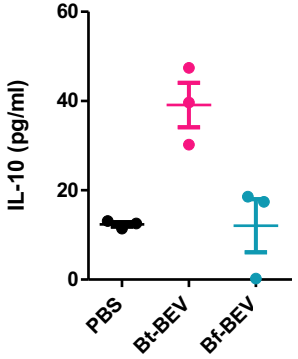


+ Bt-BEV

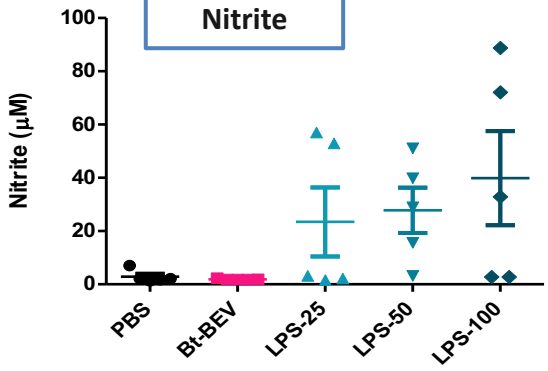
Nuclei
PCNA
DiD-BEVs



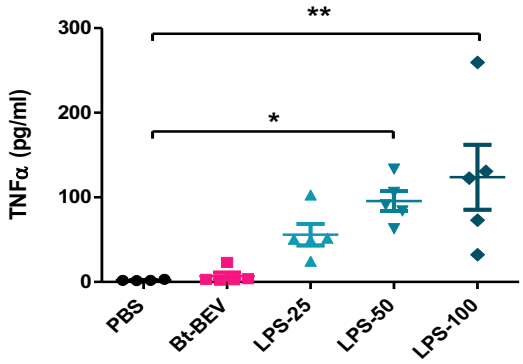
IL-10



Nitrite



TNFα



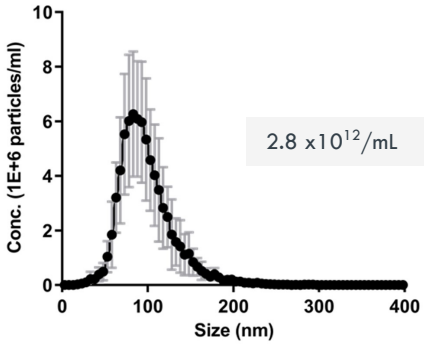
JOURNAL of Extracellular Biology
Modasai et al. 2023

Plasma of healthy individuals contains BEVs

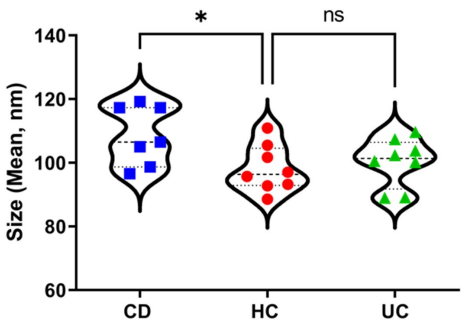
genes
MDPI
Article

The Origin of Plasma-Derived Bacterial Extracellular Vesicles in Healthy Individuals and Patients with Inflammatory Bowel Disease: A Pilot Study

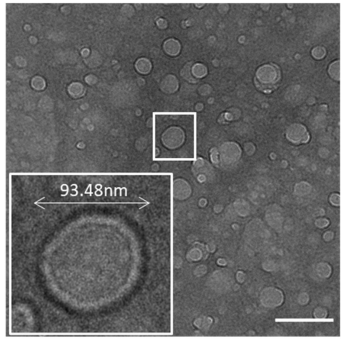
Emily Jones [1], Régis Stentz [1], Andrea Telatin [1], George M. Savva [1], Catherine Booth [2], David Baker [1], Steven Rudder [1], Stella C. Knight [3], Alistair Noble [3,†] and Simon R. Carding [1,4,*]

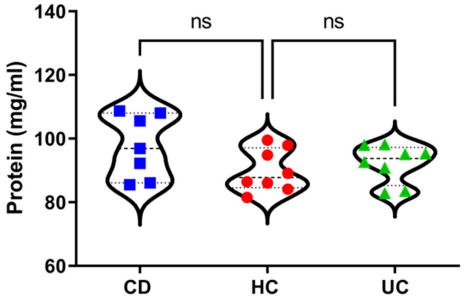


(a)

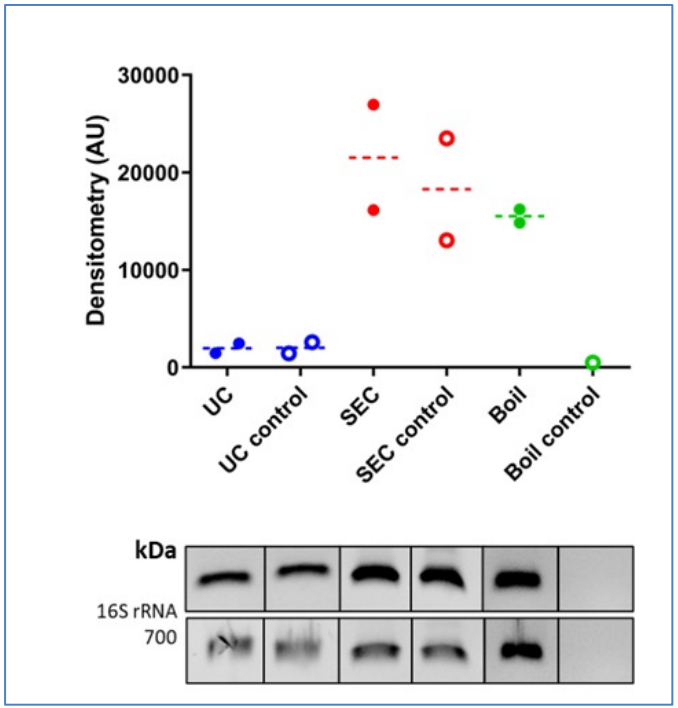


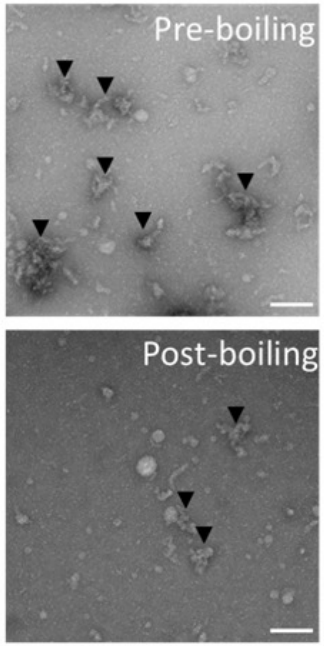
(b)





Optimising bacterial extracellular DNA isolation from plasma BEVs





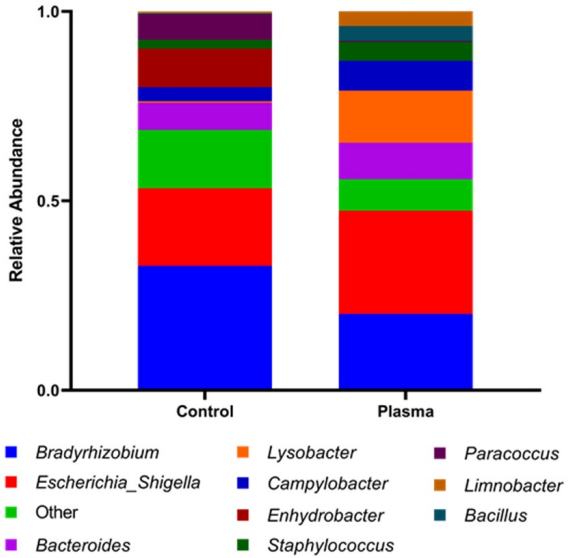
UC = ultracentrifugation (150,000 × *g*, 2 h at 4°C)

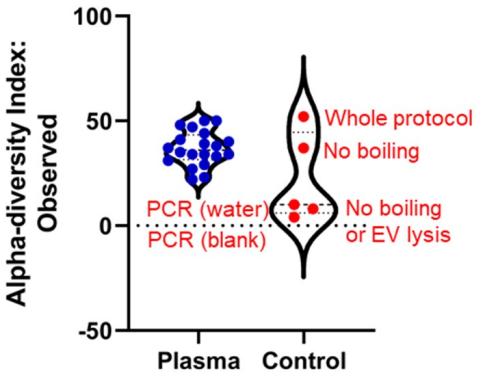
SEC = size exclusion chromatography (qEV/35nm series column)

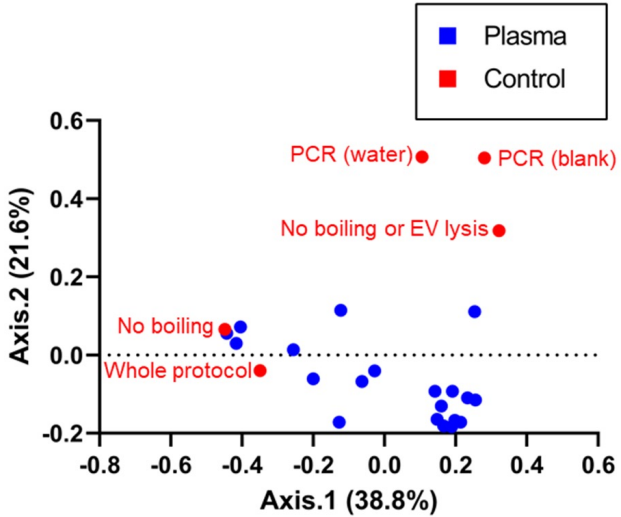
Boil = boiling (100°C for 40 min + centrifugation at 13,000 x g, 30 mins at 4°C

2.9-3.1 ng/ml DNA

Identifying sources of contaminating DNA (kit-omes)

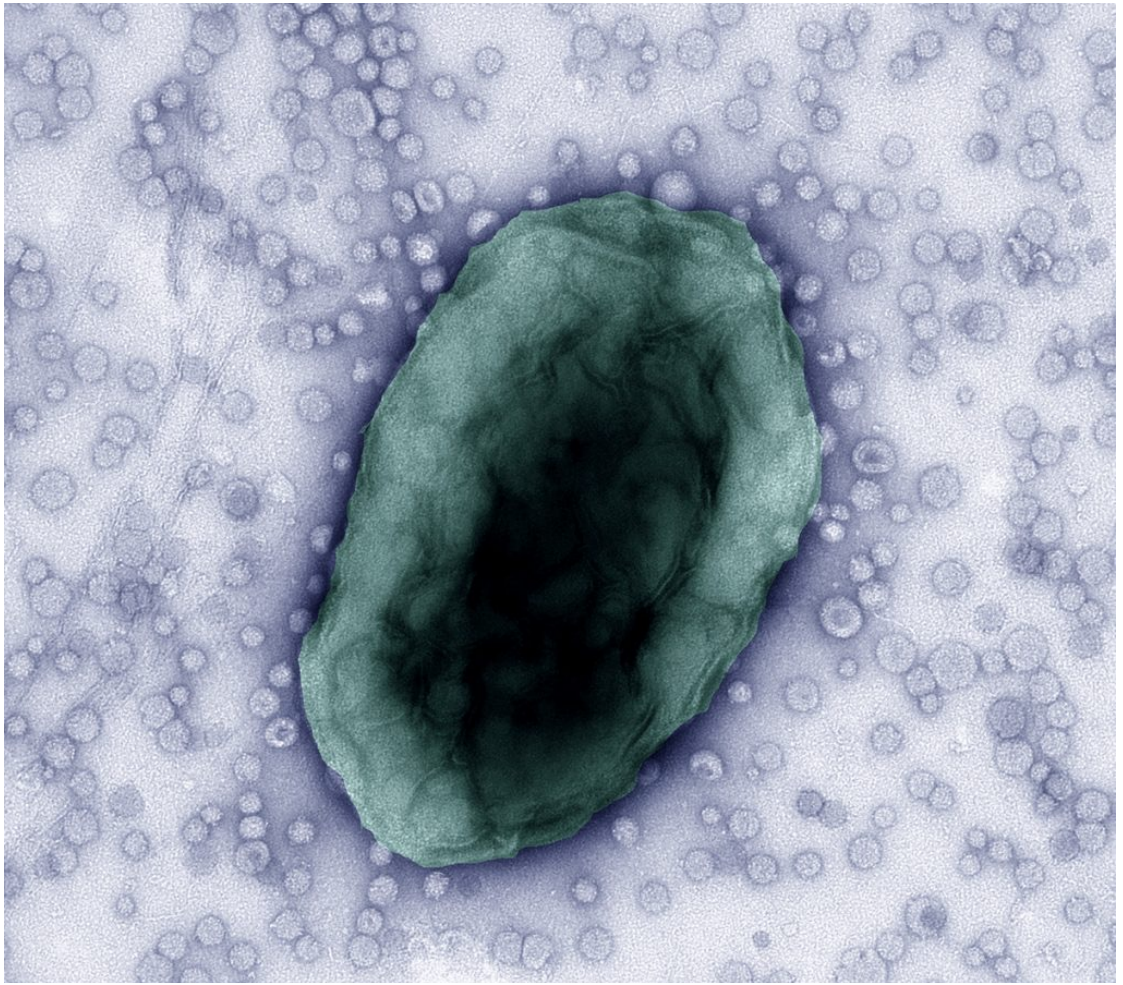




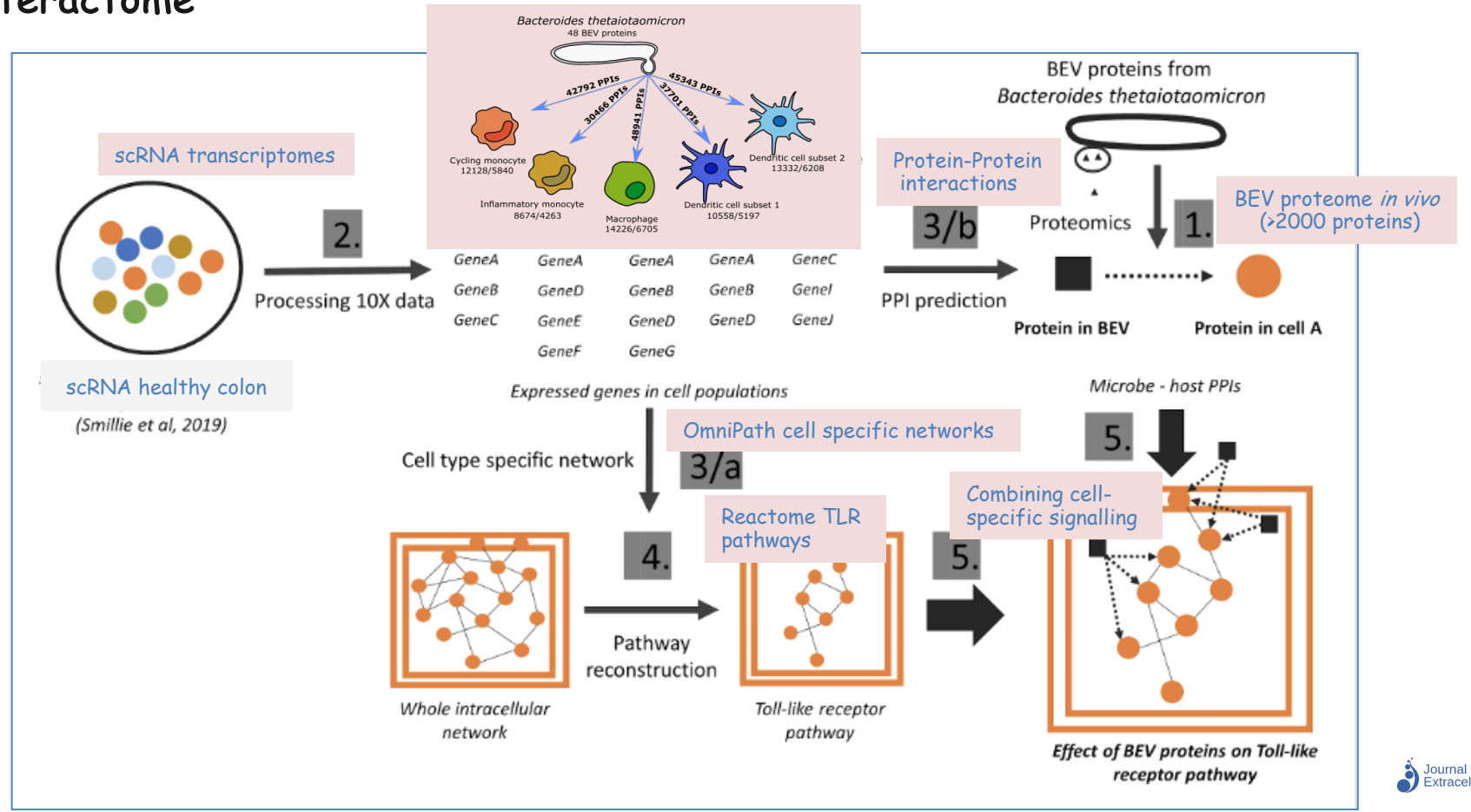


- DNAse I treatment of plasma to eliminate extracellular DNA
- Major source of contamination is post-EV lysis – buffers and kits
- Sequence based filtering to remove protocol control reads >20% prevalence
  - ~15,000 Amplicon Sequence Variants
  - 743 ± 586 average reads/sample

Mediators and outcome of BEV interactions with immune cells



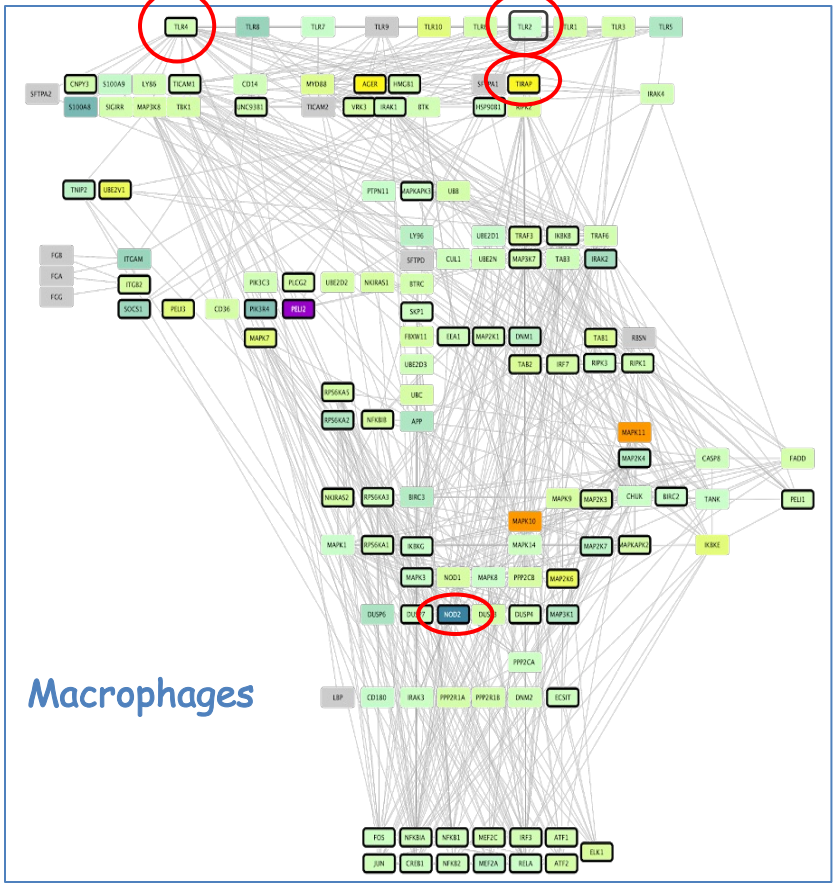
A computational workflow to define the BEV-host cell Interactome



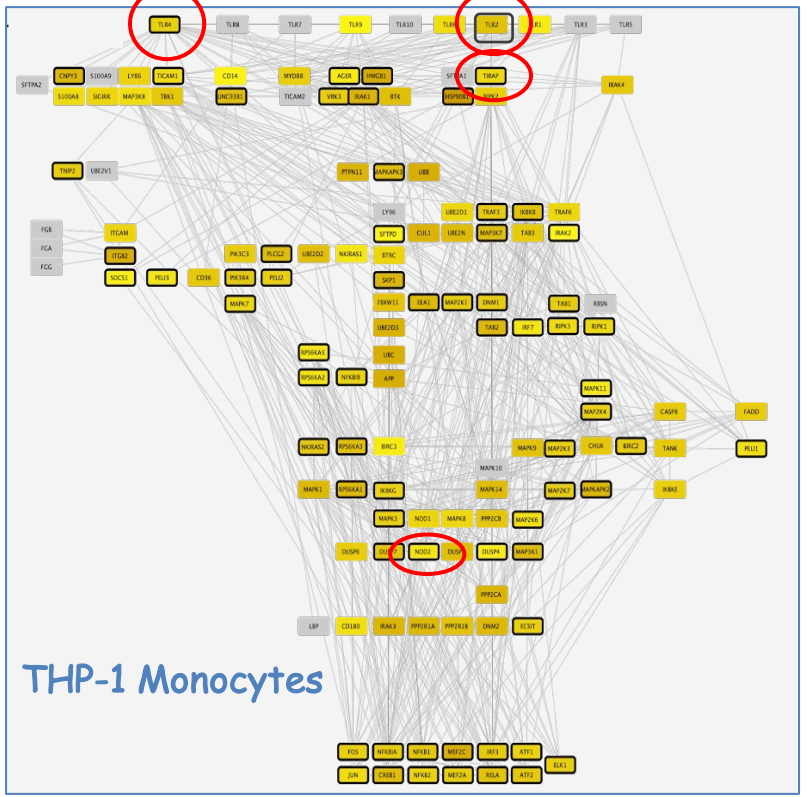
Journal of Extracellular Vesicles

L. Gul, D. Modos.....SR Carding, T Korcsmaros, 2021

TLR Pathway Analysis identifies specific PRRs as a BEV target in human macrophages



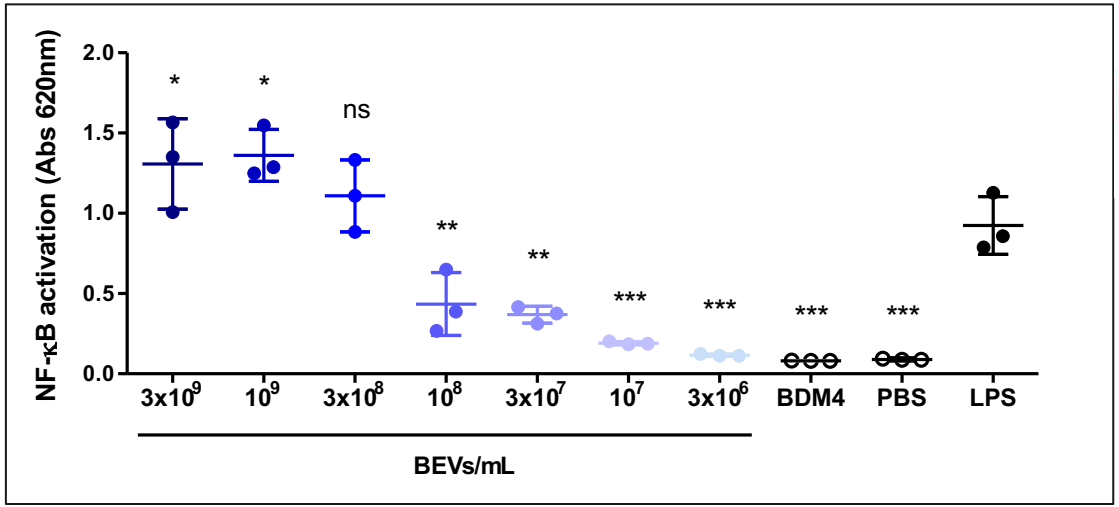
BEV Target

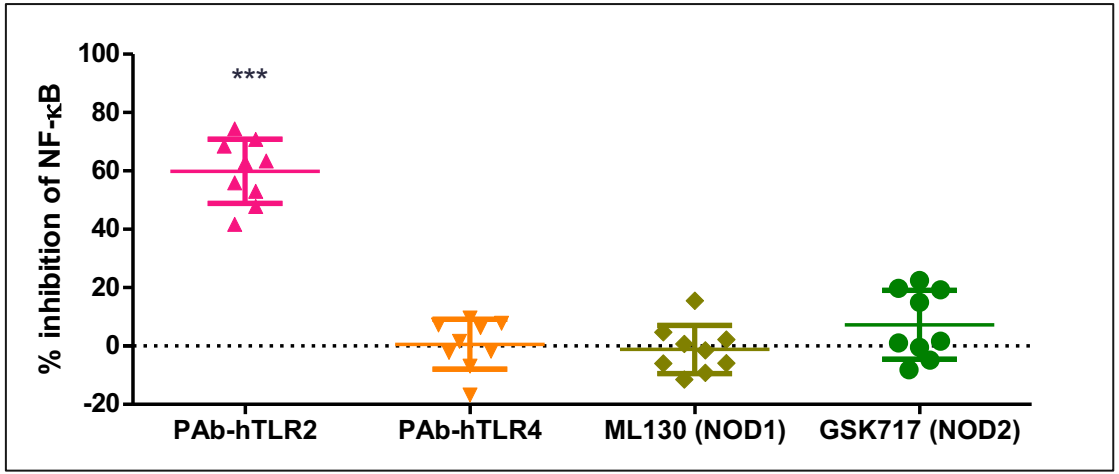


Macrophages: TLR2, TLR4 & NOD2 are candidates for mediating interactions with BEV proteins

TLR2-mediated activation of THP-1 Blue* cells by Bt BEVs

* NF-κB-inducible secreted alkaline phosphatase (SEAP) reporter gene





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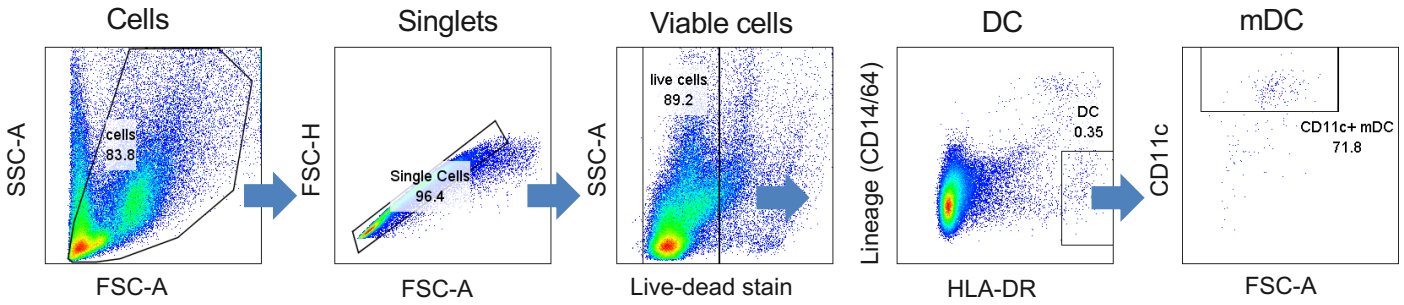
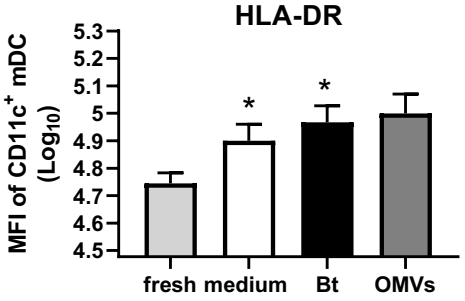
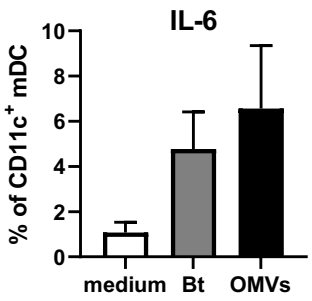
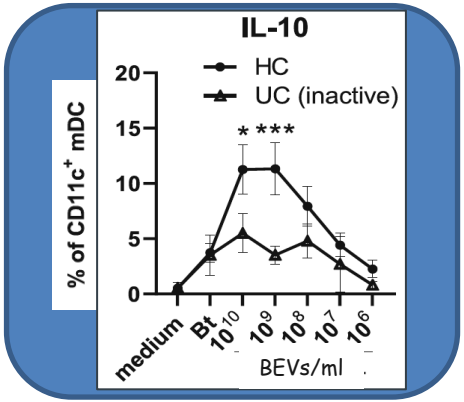
Fonseca et al., 2022

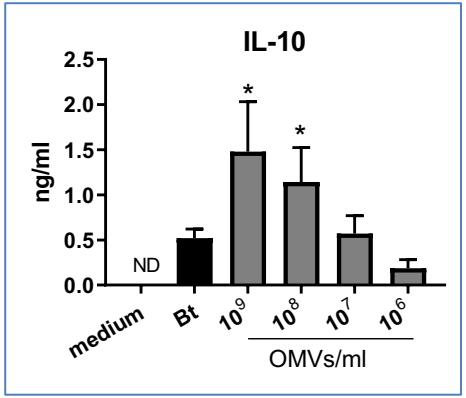
Bt BEVs stimulate production of immunoregulatory IL-10 from mDCs of healthy individuals but not those of IBD patients







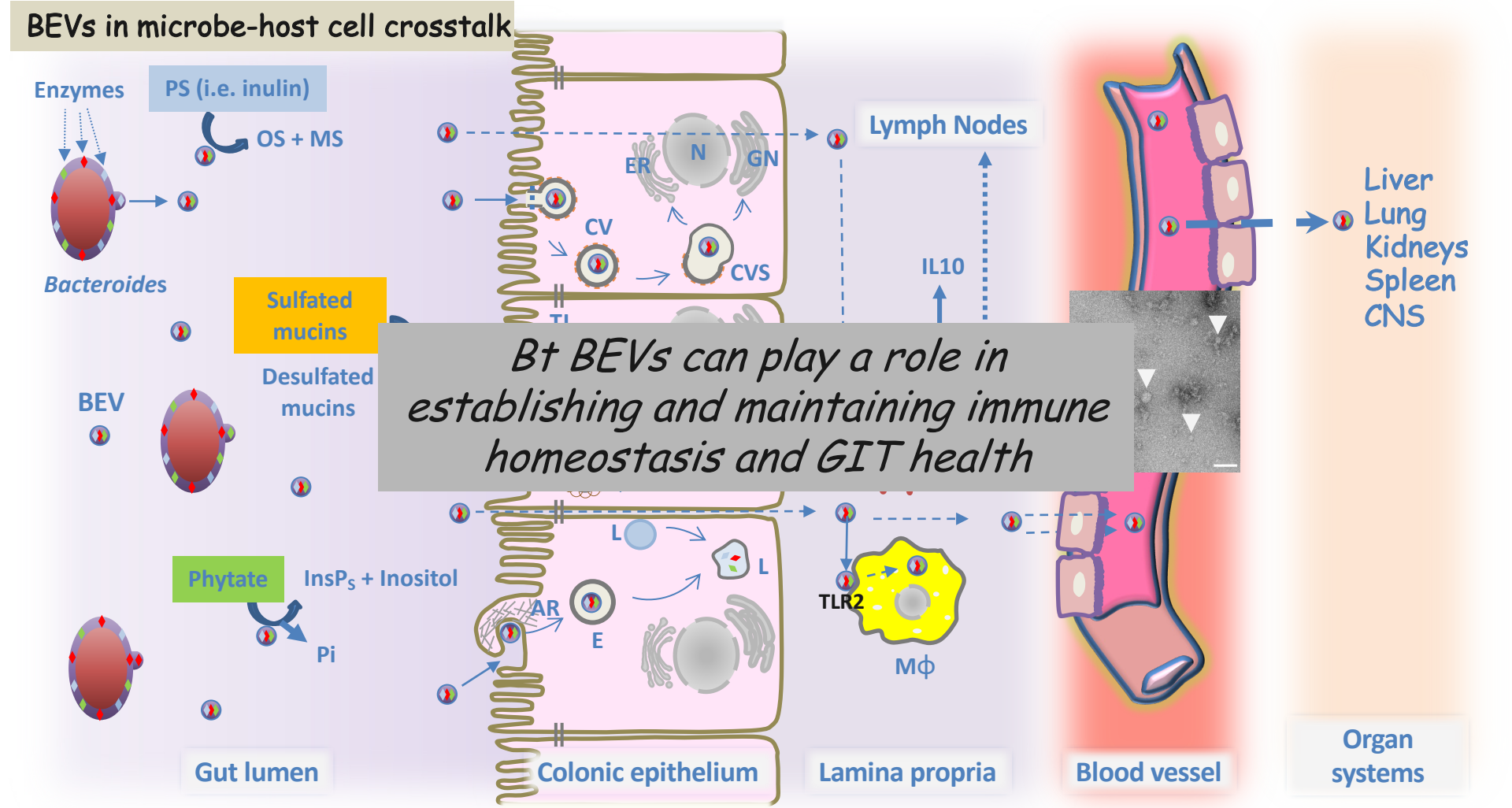




Durant et al., 2020

Microbiome

BEVs in microbe-host cell crosstalk



Stentz et al., Cell Rep. 2014; Stentz et al. 2018, Biochem. Trans.; Jones et al.,2020. Front. Micro; Durant et al., 2020, Microbiome; Jones et al., 2021, Genes

TRANSLATION

Native BEVs

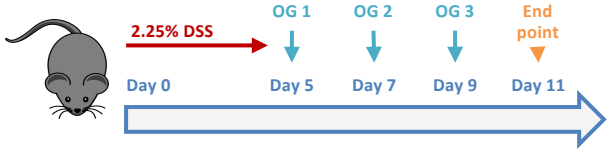
- Limiting/controlling inflammation – IBD (Fonseca et al., 2022, Front. Micro.)
- Cancer therapy
- Adjuvant – boosting natural immunity - Ageing

Engineered BEVs

- Delivery of biologics
  - BEV-Hu.KGF2 for oral treatment of acute colitis (Carvalho et al., 2019, JEV)
- Mucosal vaccines
  - BEV-*Yersina pestis* (pneumonic plague) oral/i.n. vaccine (Carvalho et al., 2019 Clin. Exp. Immunol.)
  - BEV-IAV (influenza virus) i.n. vaccine (Carvalho et al., 20219, JEV)
  - BEV-SARS-CoV-2 i.n. vaccine

Anti-inflammatory effect of Bt BEVs in a pre-clinical model of colitis

A.



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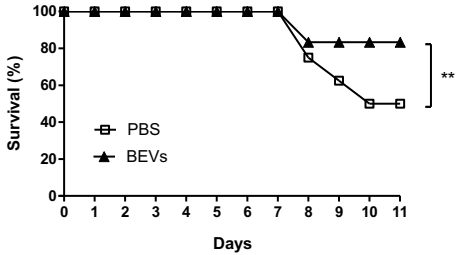

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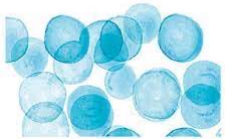
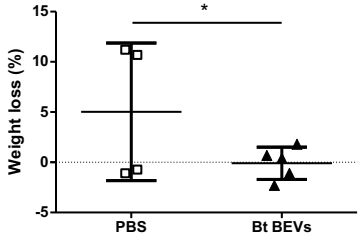


Fonseca et al., 2022

C.



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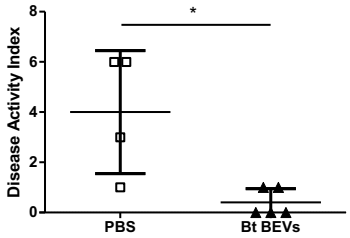

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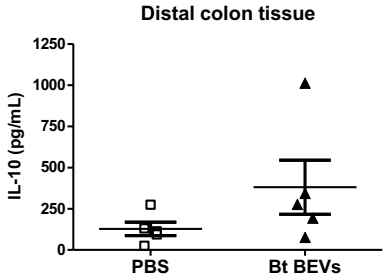

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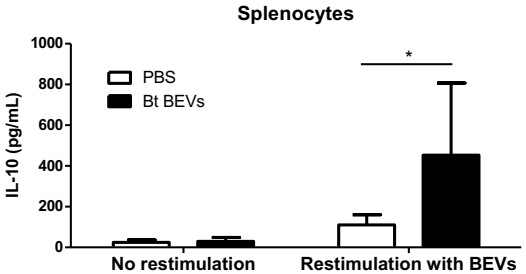
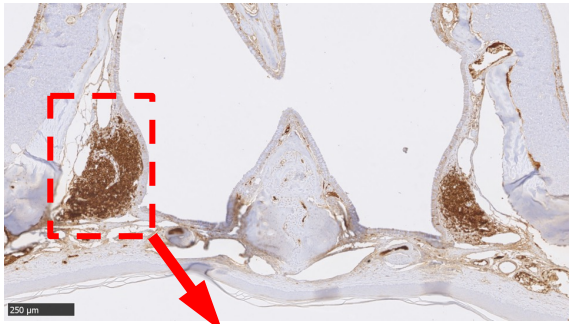
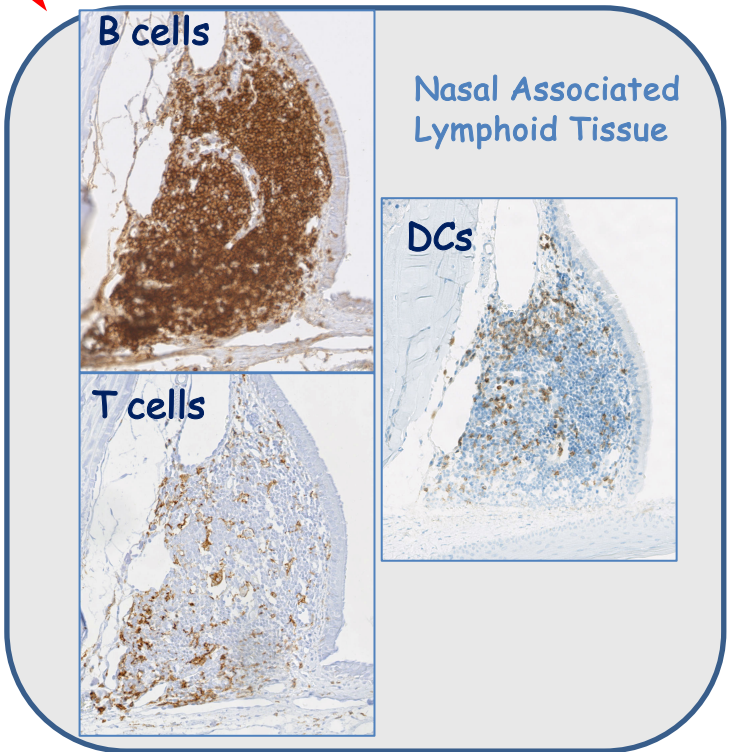
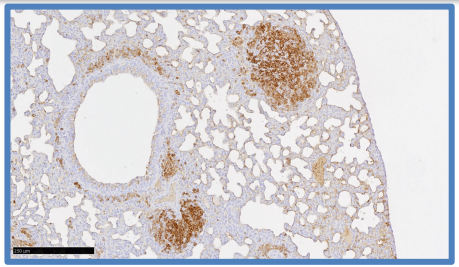
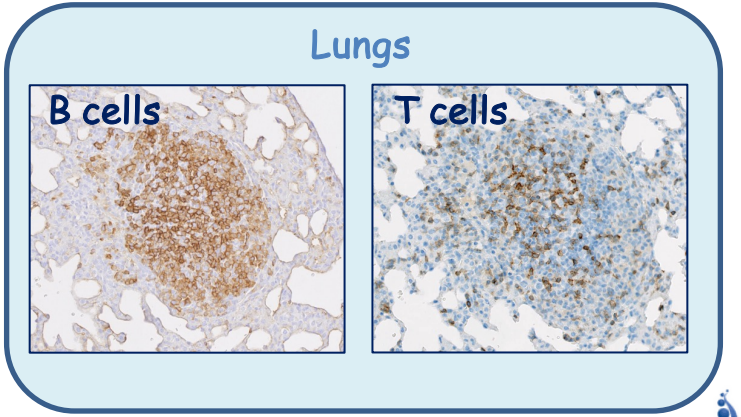


I.N. delivered native BEVs-mediate immune potentiation in the Upper and Lower RT









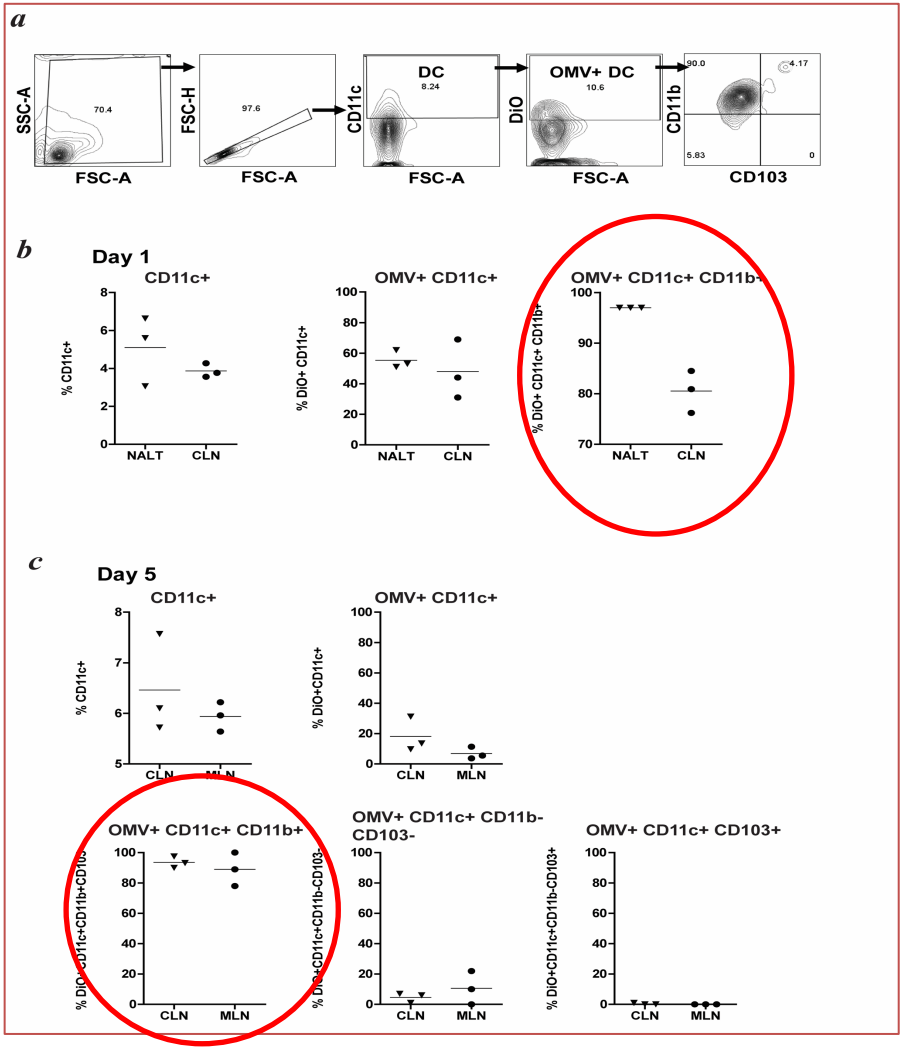
JOURNAL of Extracellular Biology

Carvalho et al., 2019

Rapid (<24h) acquisition of native Bt BEVs by pulmonary-associated DCs and trafficking to cervical and mesenteric lymph nodes (CLN and MLN) after intranasal administration

Carvalho et al., 2019

JOURNAL of Extracellular Biology



Clinical & Experimental Immunology
The Journal of Translational Immunology
British Society for immunology

Mucosal vaccines and technology
Miquel-Clopes, A., et al., 2019. 196:205-214.

Bioengineered Bt BEVs for mucosal delivery of vaccines for respiratory pathogens

Pneumonic plague

Use of bioengineered human commensal gut bacteria-derived microvesicles for mucosal plague vaccine delivery and immunization
Carvalho AL., et al. 2019. 196:287-304.

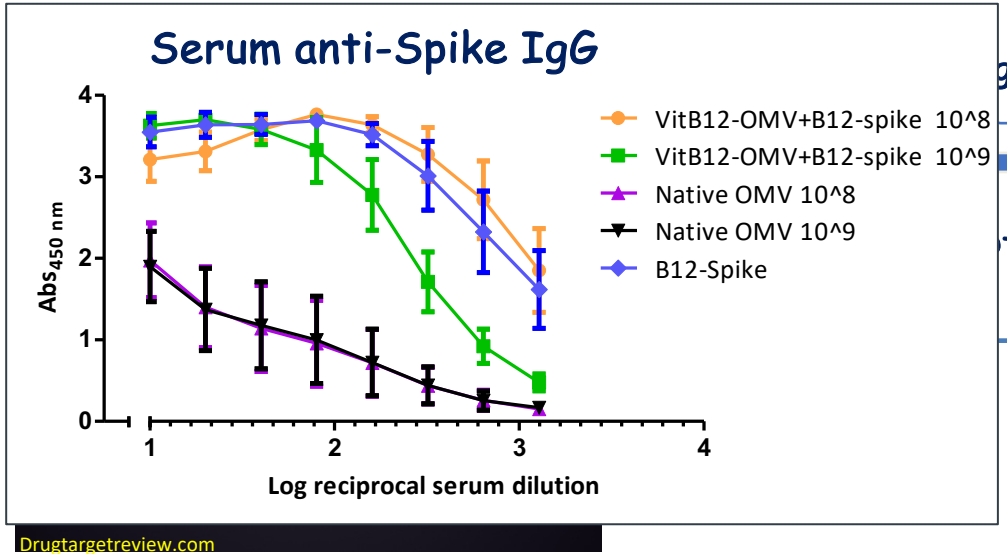
Clinical & Experimental Immunology
The Journal of Translational Immunology
British Society for immunology

Influenza

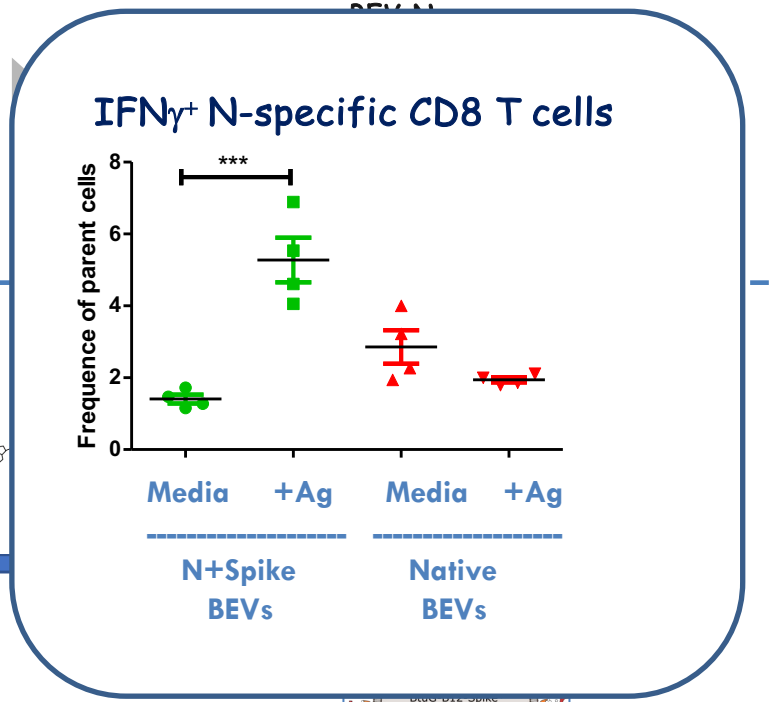
Bioengineering human gut commensal bacteria derived outer membrane vesicles for the delivery of biologics to the gastrointestinal and respiratory tract
Carvalho AL., et al. 2019. 8:1632100.

Journal of Extracellular Vesicles

A multivalent Bt BEV SARS-CoV-2 mucosal vaccine



Drugtargetreview.com

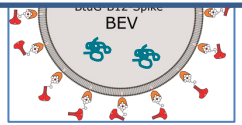


Plug-and-Play System

Based upon using the VitB12-B12-R (BtuG) high affinity covalent interactions to "label" BEVs

Isolation/purification rec. Spike proteins (HEK/CHO cells) + vitB12

Spike



Rokas Juodeikis, Dave Beal, Mark Smales, Martin Warren,

Summary: Revaluating the Biological Role(s) of BEVs

Findings to date challenge established orthodoxies concerning mechanisms of symbiosis in the GI-tract

Roles for bacteria-generated microvesicles and BEVs in:

- Nutrition (e.g., phytate and vitamin metabolism)
- Inter-kingdom communication
- Host cell physiology – adaptation & immune homeostasis
- Ecology of the intestinal microbiota
- Susceptibility vs. resistance to infection (AMR)

Exploiting BEVs and BEV Technology

Native BEVs for:

- Biomarkers (Blood, SNF…)
- Immune-potentiation
- Adjuvant therapy
- Boosting natural immunity:
  - Immunocompromised
  - Immunosenescence
  - Infection – viral/bacteria
  - Cancer

Engineered BEVs for mucosal & systemic delivery:

- Vaccines – human & animal
- Enzymes (replacement therapy)
- Antimicrobials (bacteriocins, anti-virals)
- Cytokines, hormones, neurotransmitters, QS mols
- Anti-tumor agents – angiogenesis inhibitors
- Neutralising antibodies (scFv, sdAb)
- Metabolites, anti-metabolites

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Lydia Durrant

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Innovate UK

Newton Fund

BBSRC

MRC Medical Research Council

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Thank You