

1



Research Institute for
Farm Animal Biology

2

INRAE

3



UNIVERSITÄT
HOHENHEIM

4



HOCHSCHULE
NEUBRANDENBURG
University of Applied Sciences

5&6



UNIVERSITÄT
LEIPZIG

A proteomics-machine learning approach to unravel the impact of protozoan infection on calves' intestinal morphology and development

Arash Veshkini¹, Muriel Bonnet², Franziska Dengler³, Lisa Bachmann^{1,4},

Wendy Liermann¹, Christiane Helm⁵, Reiner Ulrich⁵, Cora Delling⁶, Harald M. Hammon^{1*}



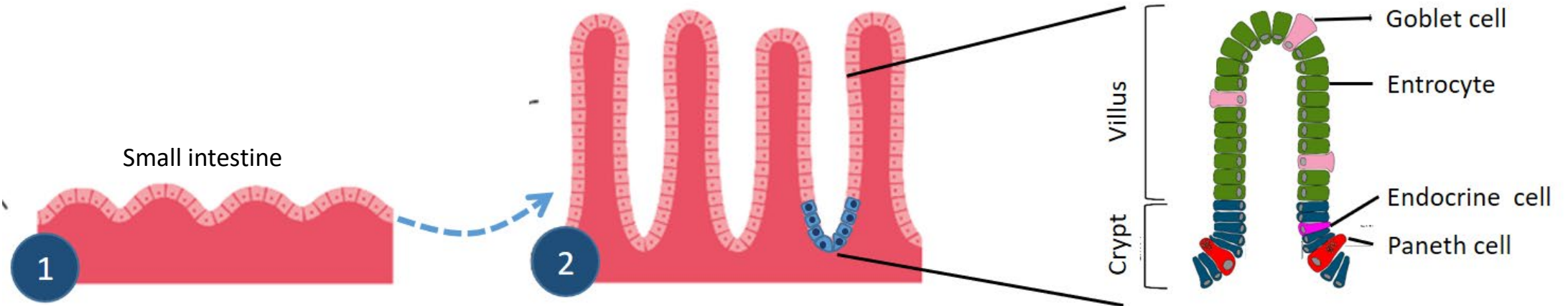
EAAP
European Federation
of Animal Science



#2212979

18(51): CODABE, Reggiana

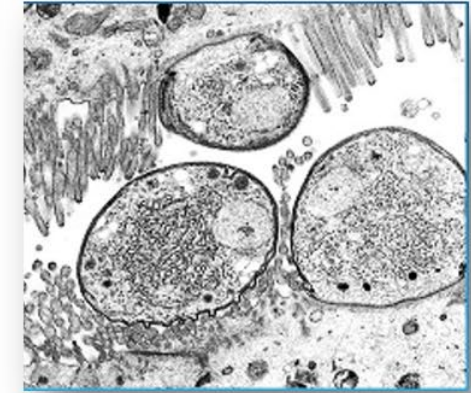
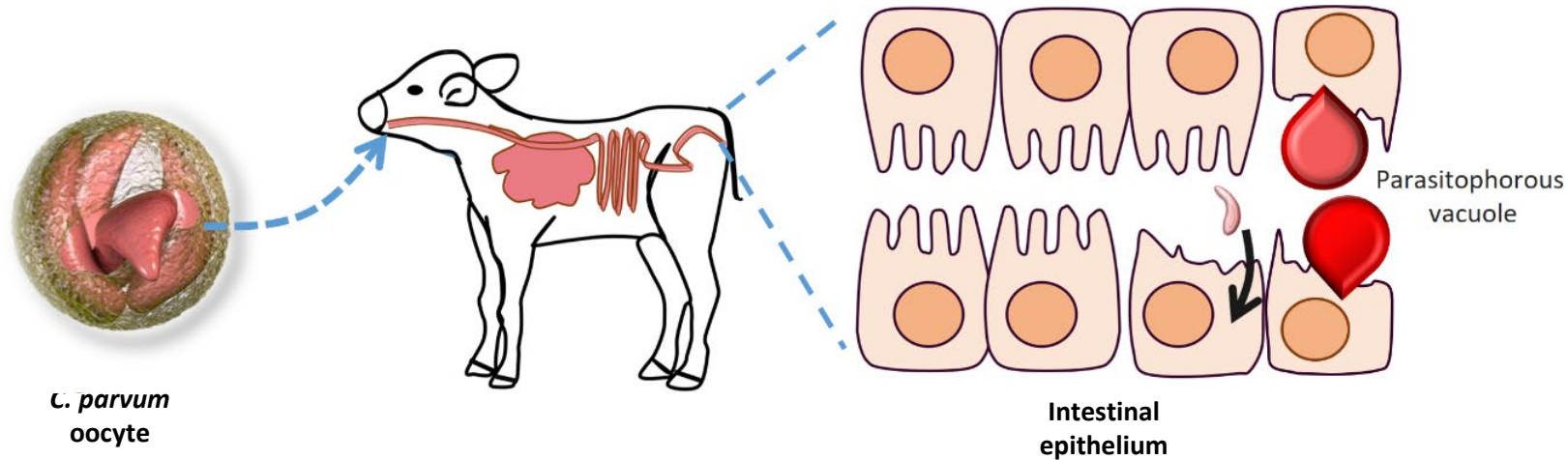
Neonatal calf intestinal development



- A key factor in the health and disease of calves is the proper gastrointestinal development.
- At birth, calves have an underdeveloped digestive system that undergoes significant morphological and developmental changes during the neonatal period, including the formation of crypts. (Du et al 2023)
- The maturation of the epithelium and mucosal immune system may take place during or after weaning stage. (Díez-Sánchez et al., 2024)
- Pathogens utilize this gap to invade the mucus membrane and cause infection.



Cryptosporidium parvum: infection, causes, and treatment



(Humagaine, 2021)

- *Cryptosporidium parvum* (*C. parvum*) is a significant cause of neonatal calf diarrhea.
- Transmission is through a faecal-oral route.
- Cause of stunted growth, morbidity and mortality in newborn.
- Very limited therapeutic options.

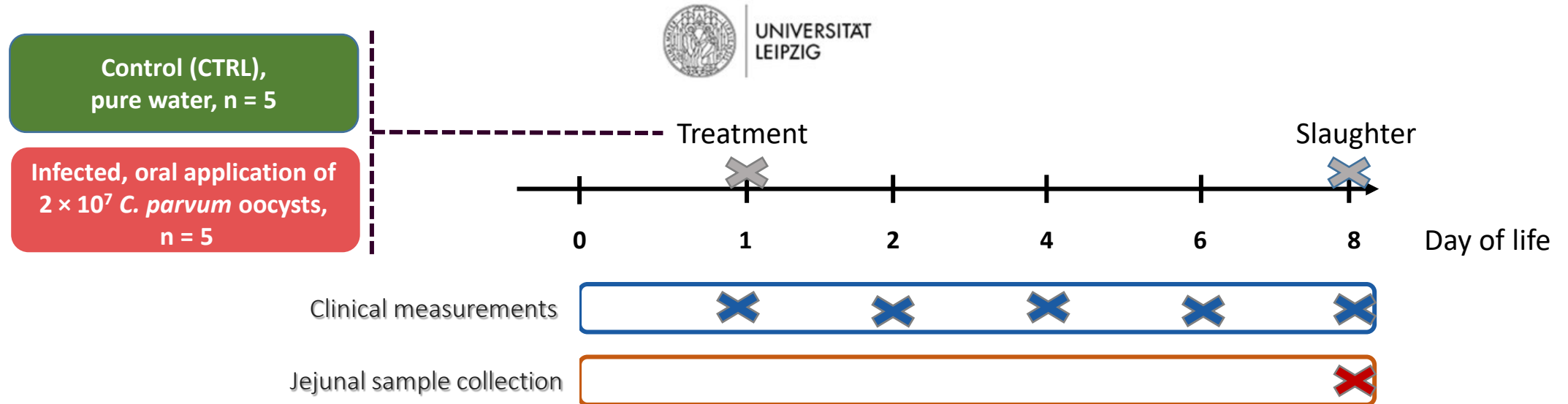
(Brook et al., 2008)



Necessity and Aim

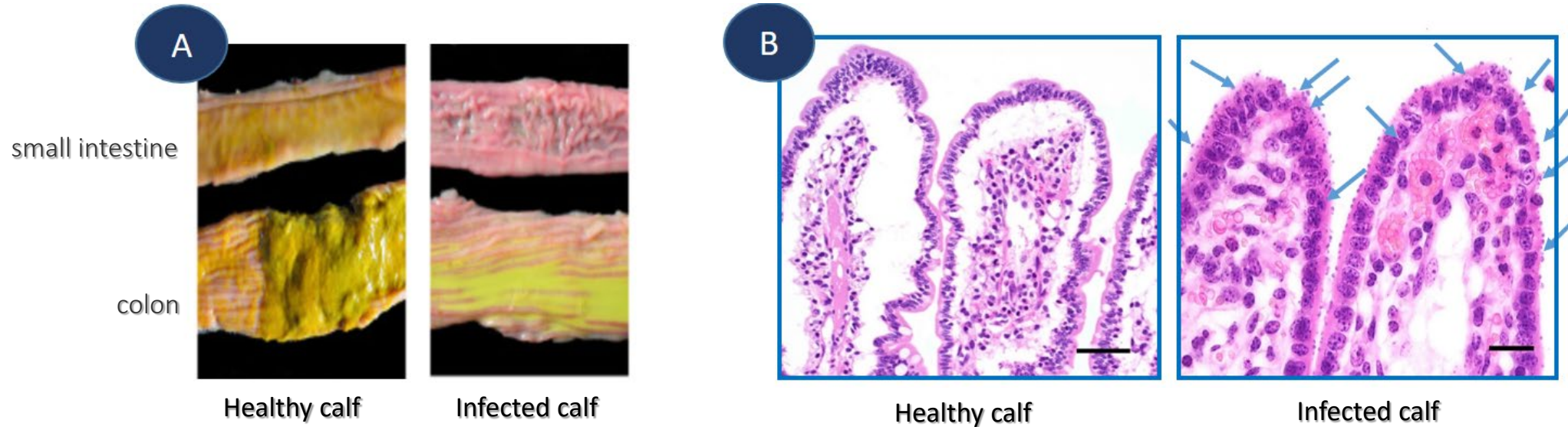
- *C. parvum* invades the intestinal epithelium and disrupts the intestinal morphology and absorption mechanisms. However, Different aspects of intestinal development, such as enterocyte maturation in villus structures, is still largely unknown in dairy calves.
(Jessop et., 2024)
- Understanding the host-pathogen interaction is crucial to optimizing calves' early gut health and preventing infections that have long-term consequences on their growth and development.
- This study aimed to investigate at molecular level the effect of *C. parvum* infection on calves' epithelium mucosa using untargeted proteomics approach combined with bioinformatics and machine learning (ML) analysis.

Experimental design



- The in-house strain of *C. parvum* (LE-01-Cp-15).
- The feeding regime was 3 l pooled colostrum after birth followed by 3 x 2 l milk replacer daily for all calves.
- Calves were stalled in one of two separate barns (control and infection).
- in accordance with the German legislation on the protection of animals and were licensed by the Landesdirektion Leipzig as TVV 19/20.

Macroscopical (A) and histopathological (B) examinations



- The infected calves had significantly higher feces scores (diarrhea) than the control group (from day 4 post-infection).
- Macroscopic examination (A) of intestine revealed differences between groups in the consistency of the ingesta.
- Histopathological examination showed mild to moderate villus atrophy and numerous oval apicomplexan cysts in the infected calves.

(Dengler et al., 2023)

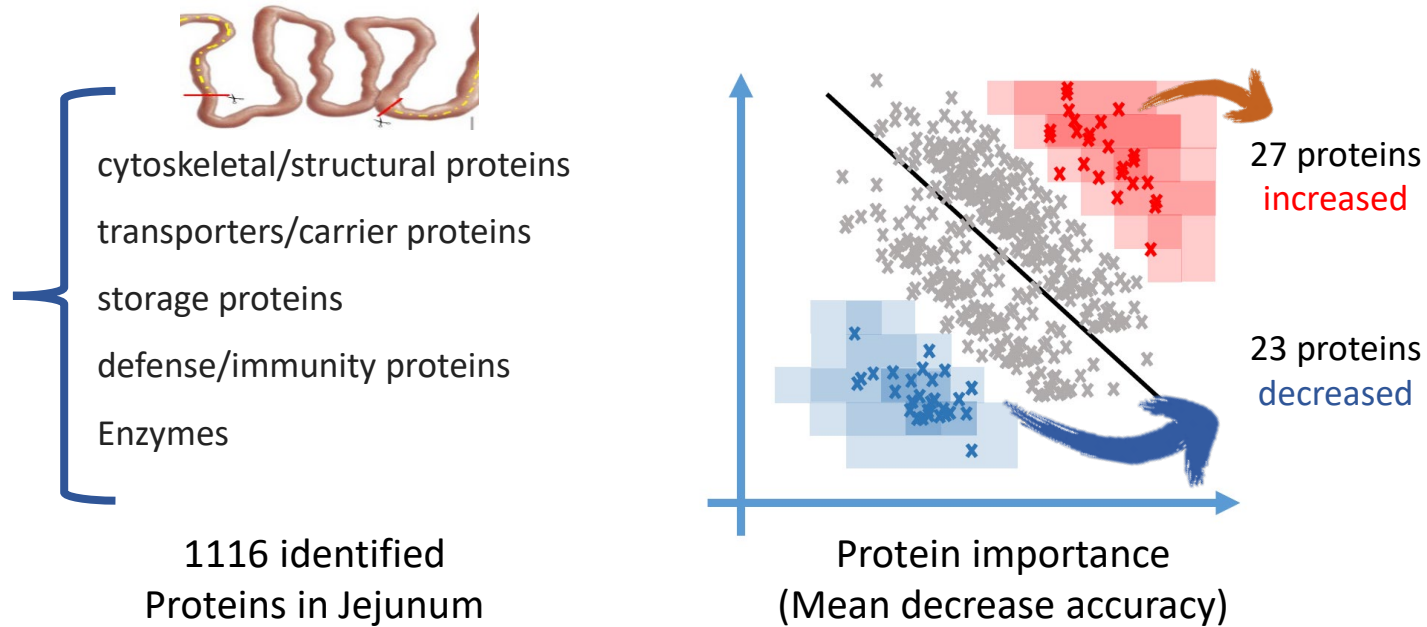
Proteomics analysis



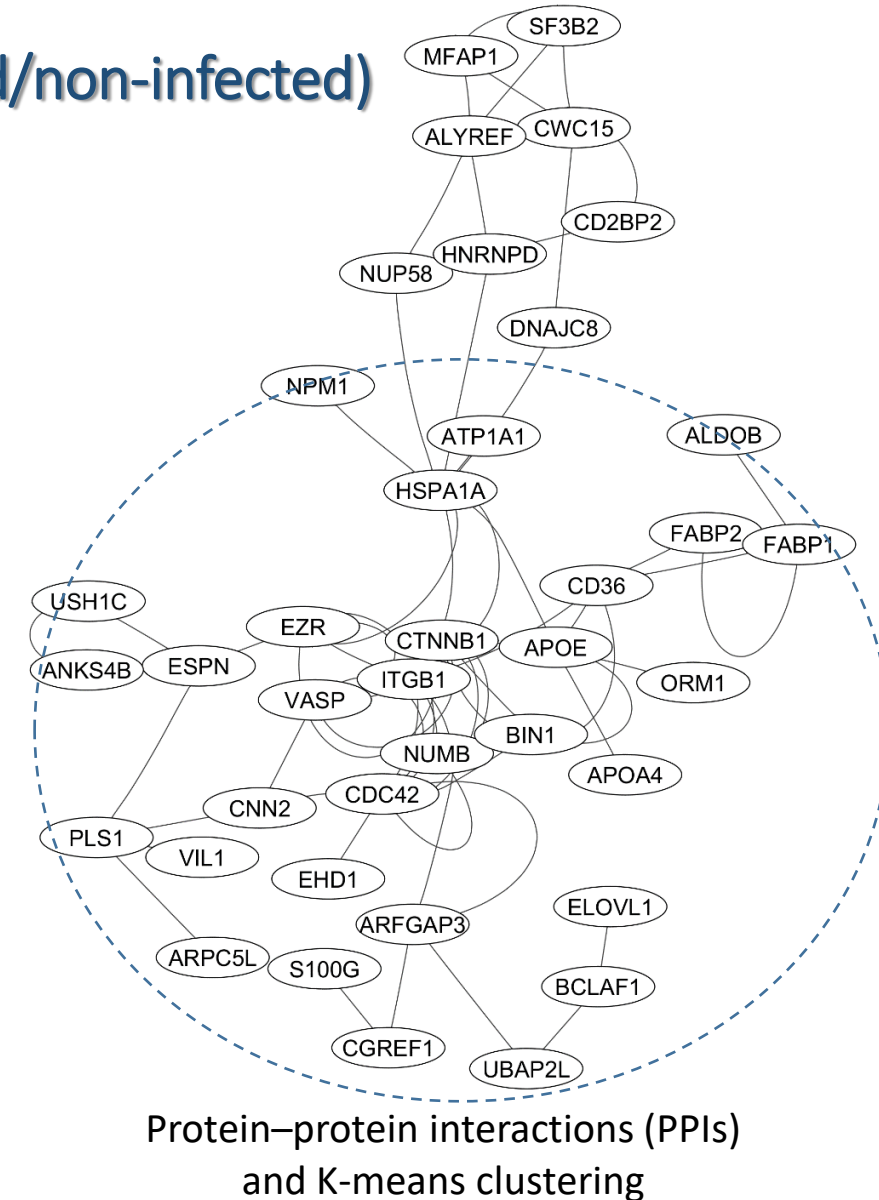
- Total proteins were extracted from the jejunum samples of slaughtered calves on day 8 post infection and subjected to label-free LC–MS/MS quantitative proteomics approach using an Ultimate 3000 RSLCnano system coupled to an Orbitrap Q Exactive HF-X mass spectrometer (Thermo Fisher Scientific).
- Top 50 discriminative proteins (DP) between the infected and control groups were identified using “randomforest” supervised ML algorithm in R (v4.0) setting a single run of a forest with 1,000 trees.
- Identified DAP were used for gene ontology (GO) and pathway enrichment analysis in STRING (v12.0).



Top 50 discriminative proteins (DP) (infected/non-infected)

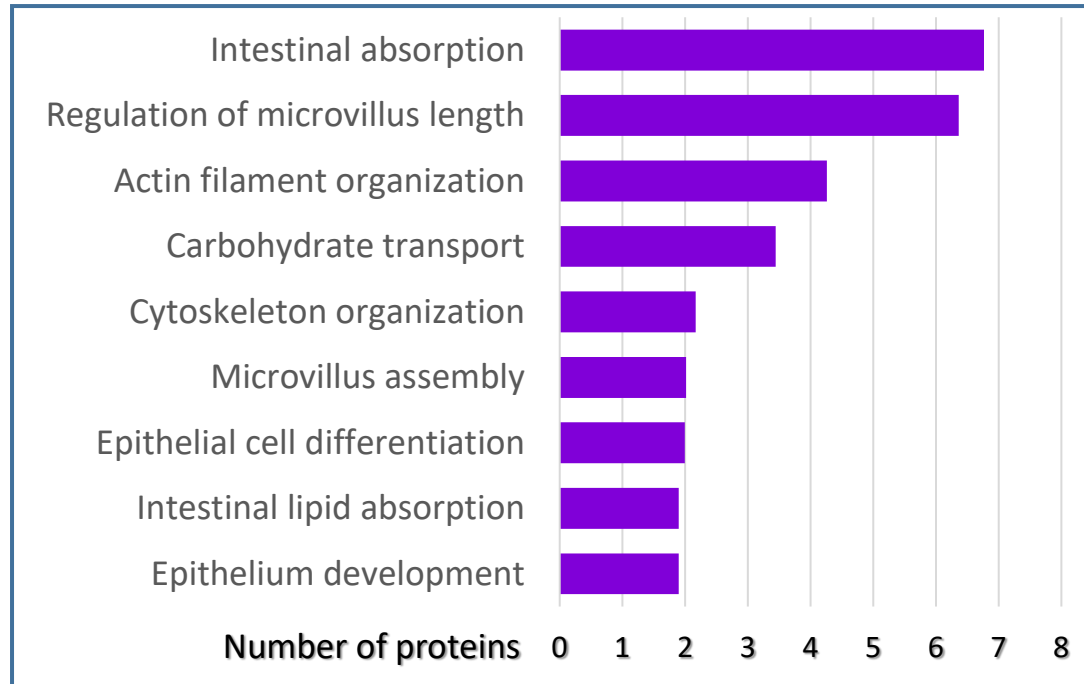


- Among the DP, villin 1 (VIL1), plastin 1 (PLS1), ezrin (EZR), Epidermal growth factor receptor kinase substrate 8 (EPS8), catenin beta-1 (CTNNB1), Harmonin (USH1C), and fatty acid-binding proteins (FABP1 & 2) were clustered to “actin based cell projection”.

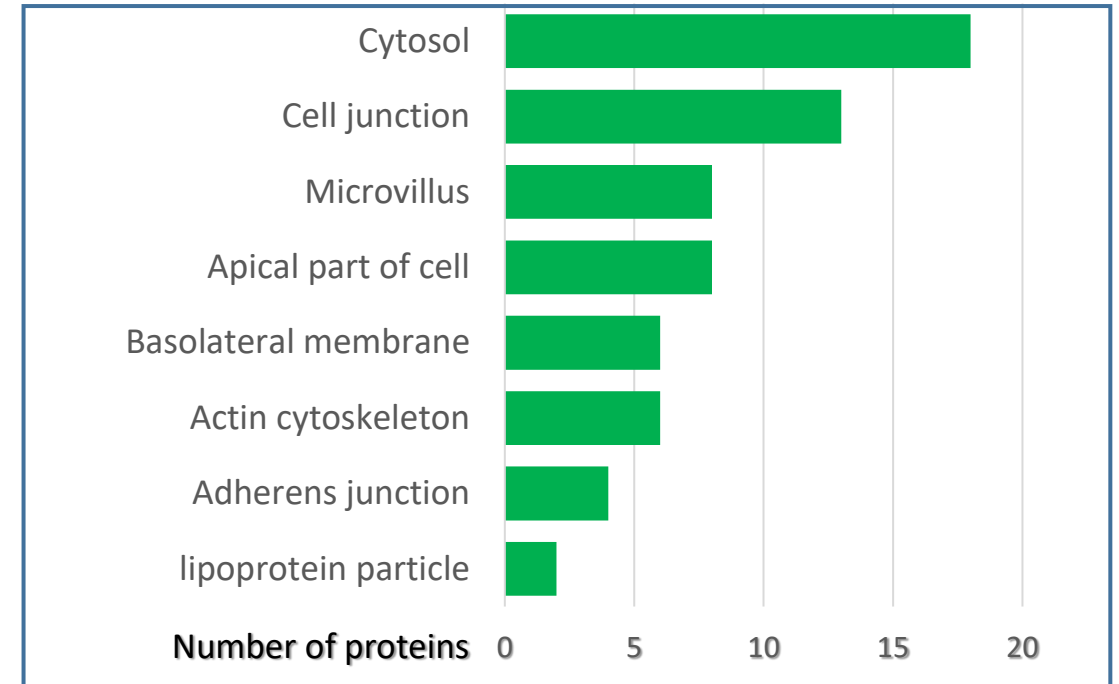




Gene ontology (GO) analysis of discriminative proteins



A. Biological process (BP)

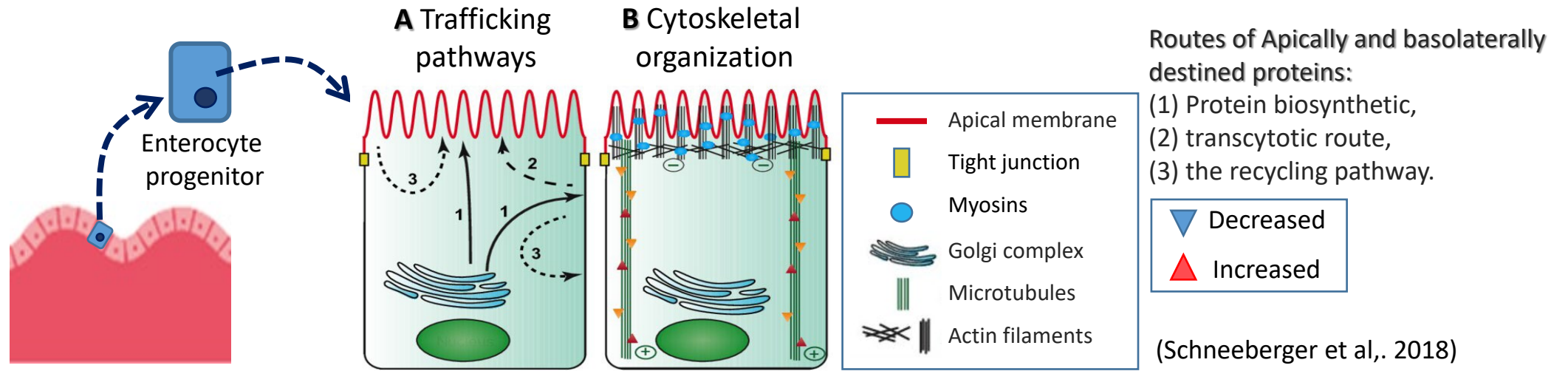


B. Cellular component (CC)

- GO analysis of (A) BP and (B) CC based on the discriminative proteins identified by the network analysis.



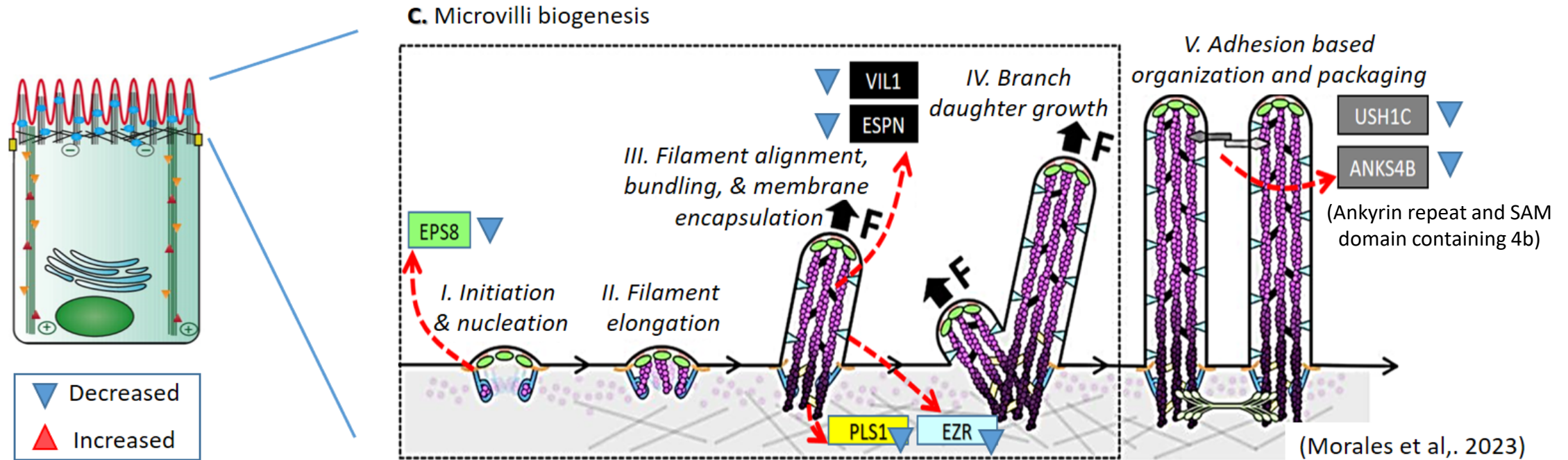
Discriminative proteins in the intestinal epithelial cells development and trafficking machinery



- ▼ CDC42 (Cell division control protein 42) is involved in Notch signaling and modulates cell migration during metaphase.
- ▼ CTNNB1 (Catenin beta-1) functions as a transcriptional regulator in the Wnt signaling pathway.
- ▼ Numb an important cell fate determinant, modulates intestinal epithelial cells towards the goblet cell phenotype.
- ▲ CNN2 (Calponin 2) regulators actin cytoskeleton functions and the rate of cell proliferation.
- ▼ VASP (Vasodilator-stimulated phosphoprotein) regulate epithelial junctional reassembly and permeability.
- ▼ CAMSAP3 (calmodulin-regulated-spectrin-associated protein) regulate longitudinal orientation of microtubules in cells.



Discriminative proteins in the molecular machinery of microvillar biogenesis, stabilization, and organization



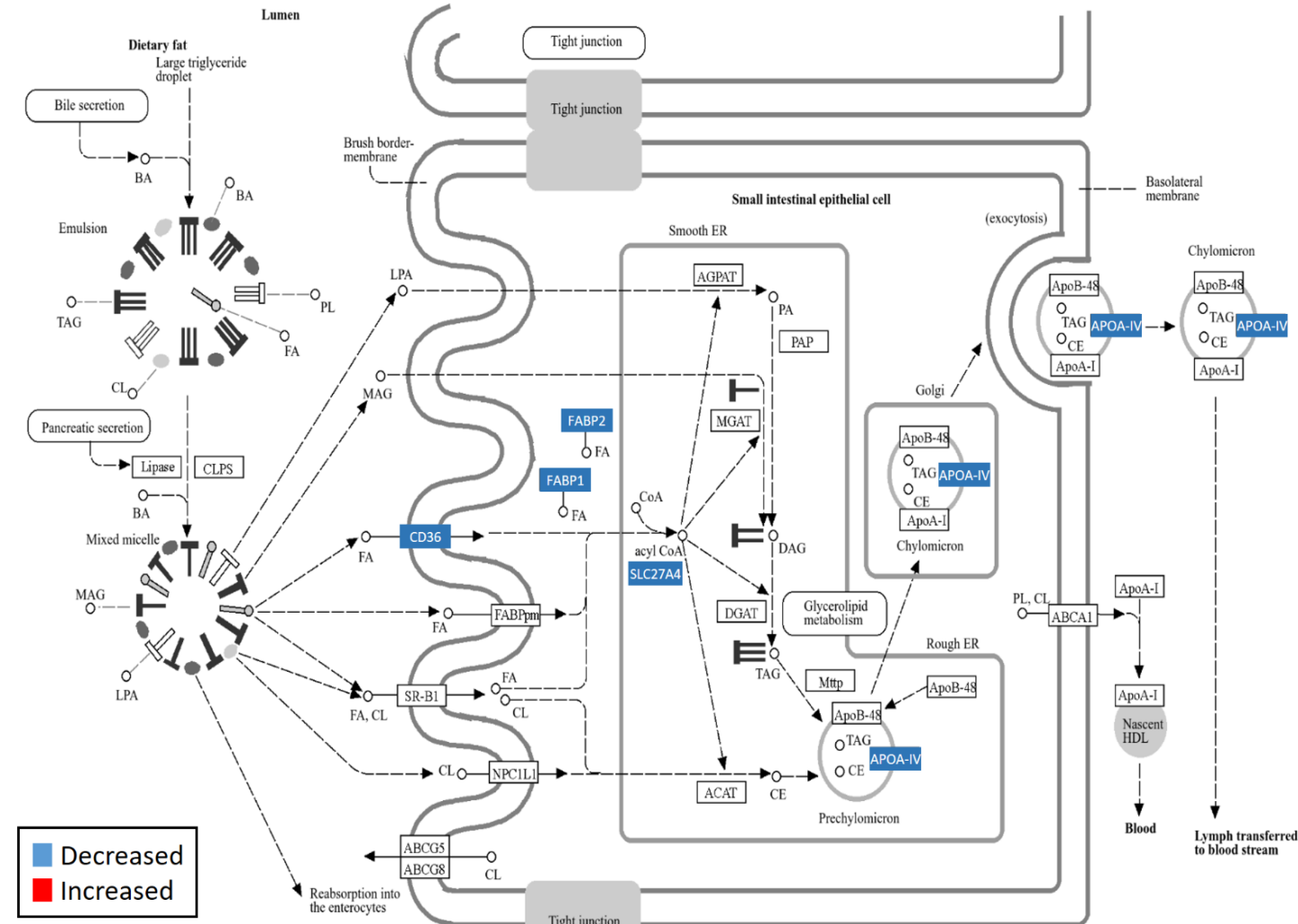
- Discriminative proteins are involved in the following steps:
 - The sites where microvilli grow are marked by distinct spots containing EPS8.
 - Filament alignment enhanced by the bundling activities of PLS1, VIL1, and ESPN.

EZR directs membrane encapsulation of core actin bundles, by linking membrane to cytoskeleton.
 - The packing of microvilli is guided by the intermicrovillar adhesion complex, which consists of USH1C and ANKS4B.



Discriminative proteins in fat digestion and absorption

- Several discriminative proteins have been implicated in fatty acid uptake and metabolism, including:
 - (1) Membrane scavenger fatty acid translocase (FAT)/CD36,
 - (2) Cytosolic fatty acid binding protein 1 & 2 (FABP),
 - (3) Fatty acid transport proteins (FATP)/SLC27A4,
 - (4) Apolipoprotein A4 (APOA4), a lipid-binding protein, which plays a role in modulating triglyceride-rich lipoprotein assembly and metabolism.





Conclusions and take home messages

- The results of this study suggest that *C. parvum* infection may have affected calves' intestinal development by interfering with a hierarchy of molecular interactions, which include:
 - ✓ A reduction in the relative abundance of proteins that are involved in the structural development of epithelial cells, junctional assembly, and microvillus formation.
 - ✓ The relative abundance of proteins involved in the absorption of organic substances, carbohydrates, and fatty acids was reduced.
- As calves are already well beyond the peak of infection (< day 6), it might be hypothesized that *C. parvum* infection stunted epithelial cells proliferative capacity.
- The exact underlying (direct or indirect) mechanism by which *C. parvum* stunts epithelization and whether this effect has long-term consequences is yet to be elucidated.

Thank you for your attention!



Forschungsinstitut für Nutztierbiologie
Wilhelm-Stahl-Allee 2
18196 Dummerstorf

www.fbn-dummerstorf.de

veshkini@fbn-dummerstorf.de

hammon@fbn-dummerstorf.de