

# Variability of colonic and ileal intestinal stem cell derived organoids in pigs

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# Acknowledgements



Animal Breeding & Genomics

Animal nutrition



# Content

- What are organoids?
- Objective of this study
- Material & Methods
- RNA sequencing
- Results
- Discussion

# Why did we start working with organoids?

To study complex phenotypes and nutrient transmission

Organoids could be a **powerful tool** to gain insight in complex processes

Could **reduce** the number of animal experiments

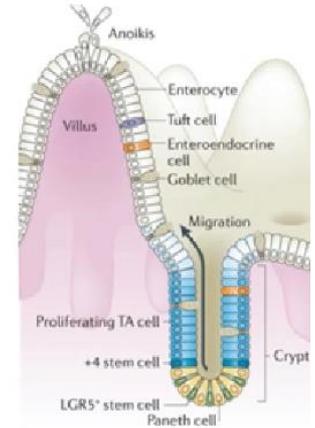
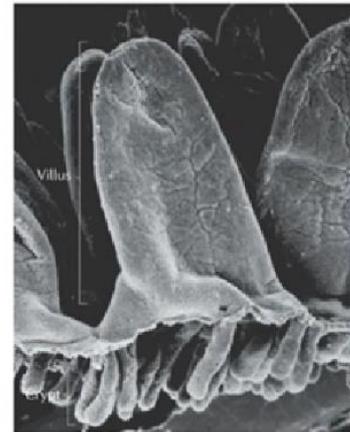
# Organoids

Self-renewing and self-organizing 3D structures



# Organoids

- Tissue collected from organ
- 3-D structure developed *in vitro*
- Contains major cell types of an organ
- Good representation of an organ



(Barker et al., 2014)

# Organs



Lungs



Heart



Intestines



Brains



Kidney



Liver

- Number of organs for organoids culturing is unlimited....

# Lab



# Applications

- 3D organoids
  - Understanding biology
  - Complex phenotypes
  - ...
- 2D monolayers
  - Transport studies
  - Challenge with pathogens
  - ...

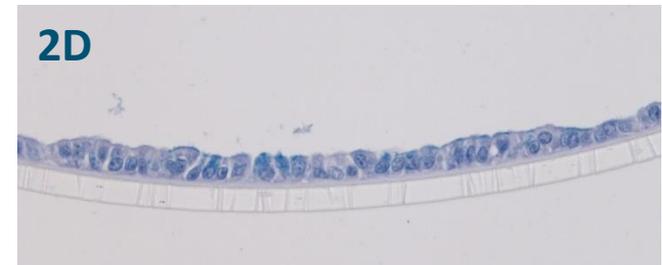


Figure J. Wells, HMI-WUR

# Objective

To pave the road for **high throughput** *in vitro* **organoid** systems of the **pig intestinal tract** to use as a **key technology** in animal breeding and nutrition



# Research questions



Effect of age?

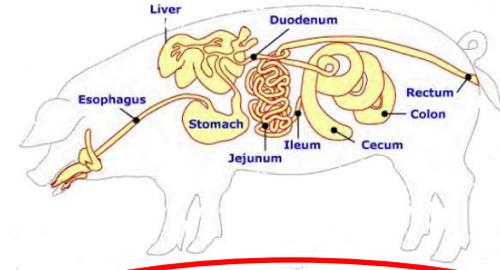


High throughput?



Is there a difference in organoids between phenotypically divergent pigs?

- Histology
- Gene expression

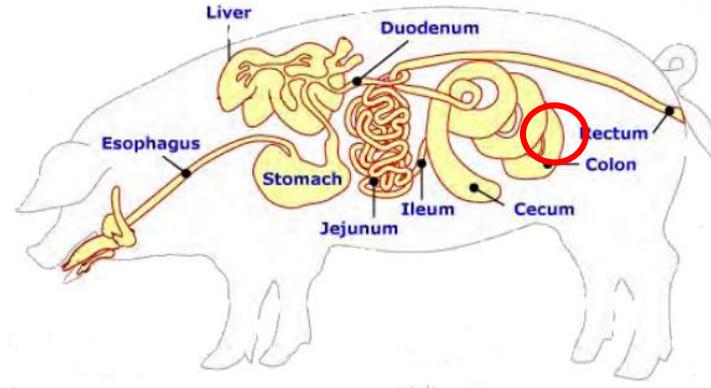


How well do organoids represent the *in vivo* tissue they originate from?

Effect of location of the collected tissue?

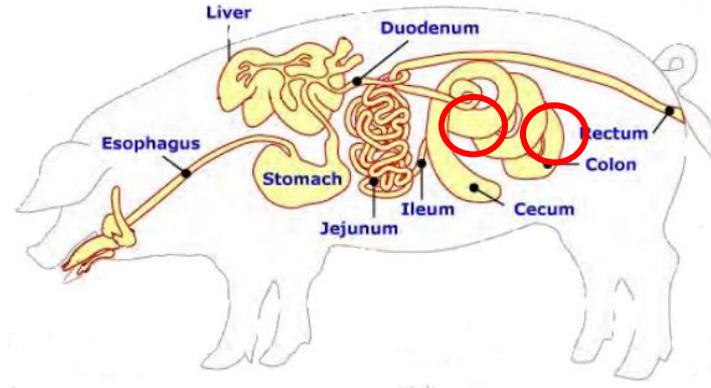
# Effect of location?

- Variation **within** nearby locations
  - Will tissue collected in nearby locations grow out to similar organoids and to what extent do they represent their original tissue?

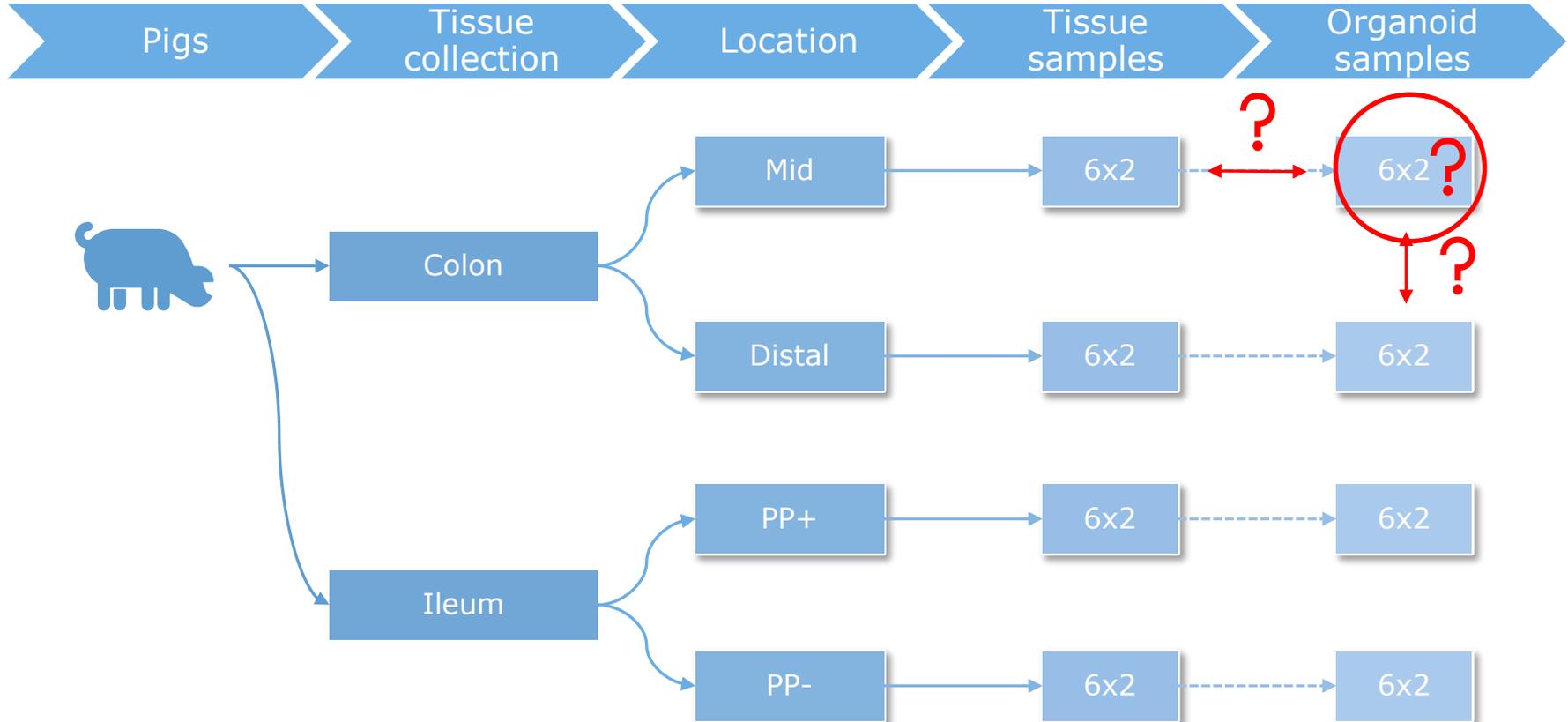


# Effect of location?

- Variation **between** different locations
  - Will tissue collected in different parts of the same organ grow out to similar organoids?



# Material



# Methods to analyse organoids

- Genomics
- Transcriptomics
- Metabolomics
- Proteomics
- Histology
- ...

# Why RNAseq analysis?

- Measure how active genes are and compare this gene activity between groups



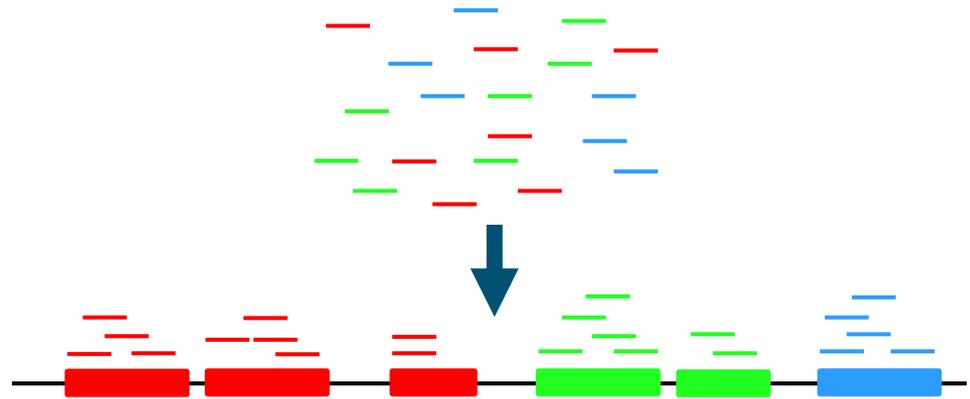
# RNAseq

- Extract and isolate RNA
- Sequence
- Align reads to a reference genome

Sequenced reads (150bp)

Aligned reads to reference genome

Reference genome

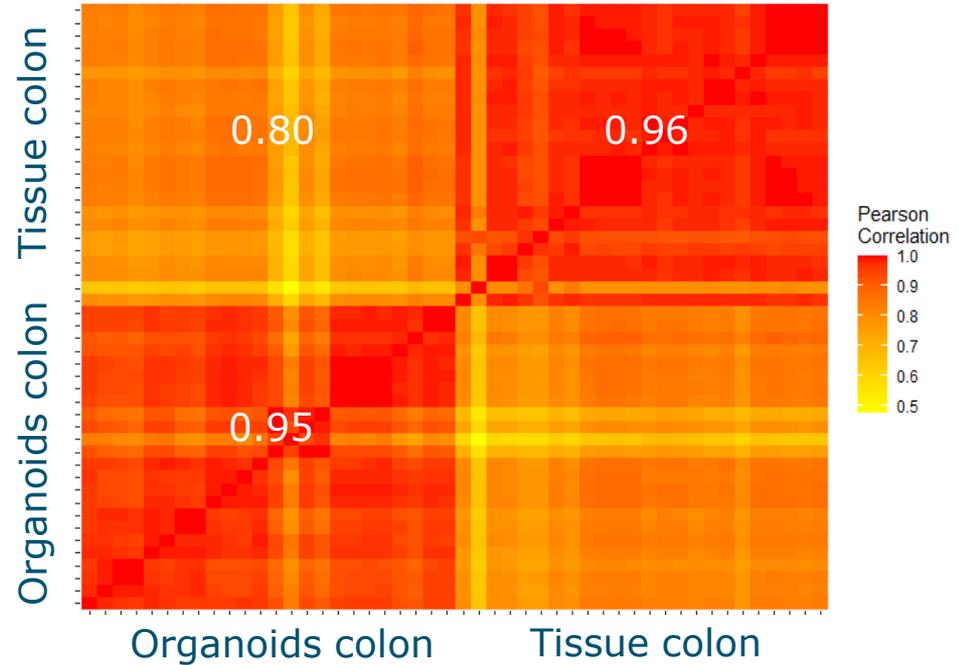
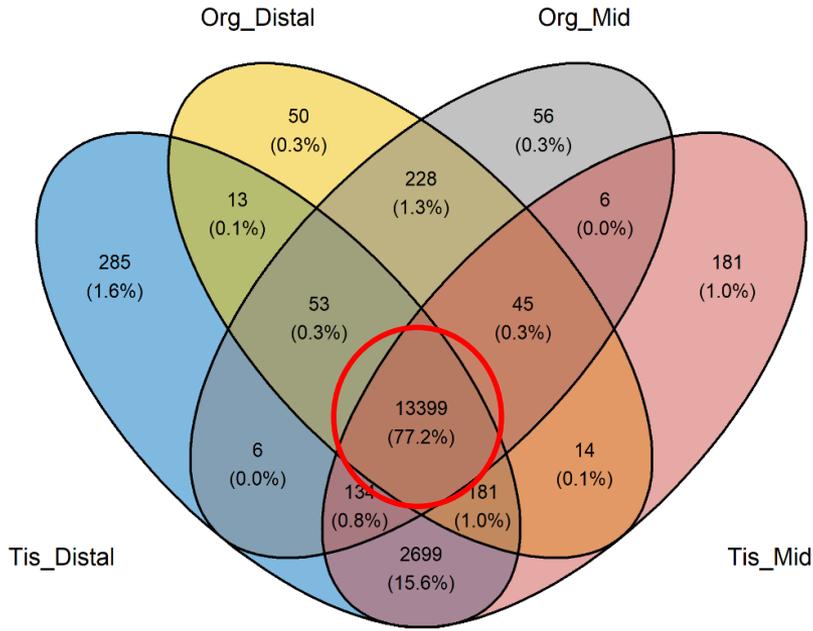


# Results – Colon mid and distal

- Do the organoids **resemble** their original tissue?
- Is there variation **within** the nearby locations?
- Is there variation **between** colon mid and colon distal?

# Results – Colon mid and distal

- Do the organoids **resemble** their original tissue?





# Results – Colon mid and distal

- Is there variation **between** colon mid and colon distal?
- Correlation colon  $0.95 \pm 0.05$
- Number of differentially expressed genes mid vs. distal
  - Tissue 0
  - Organoids 0

# Summary colon results

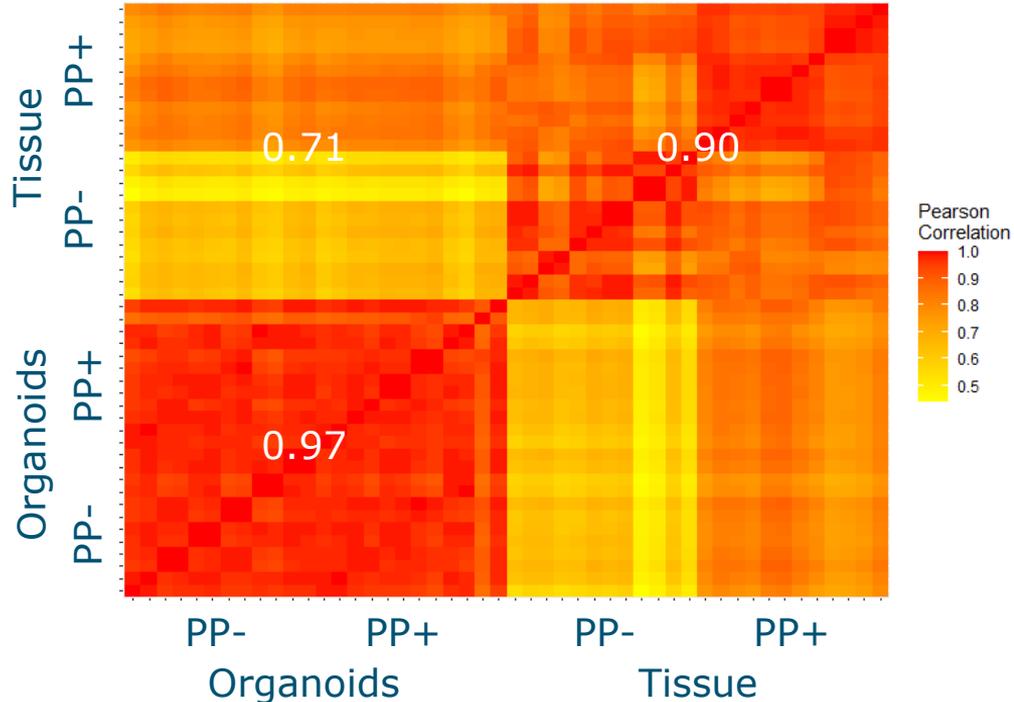
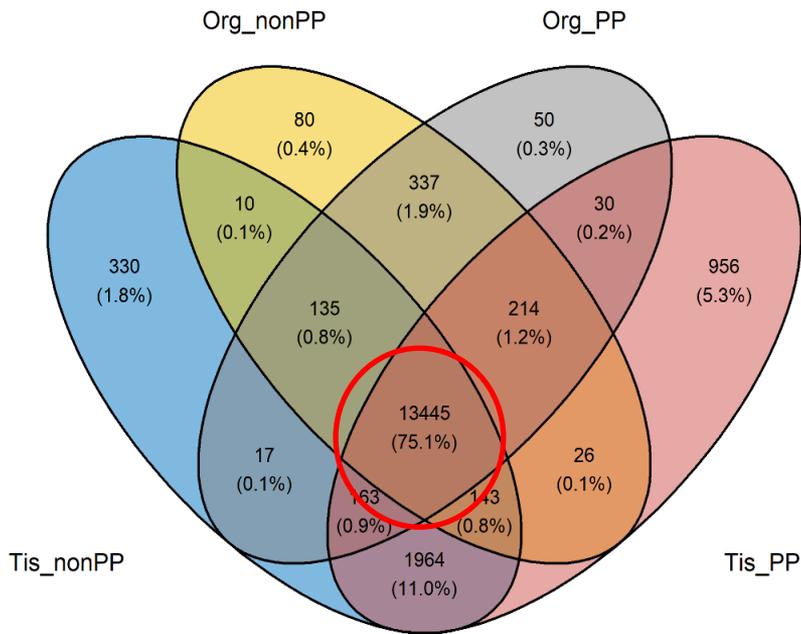
- High correlations
  - Within animal
  - Within locations
  - Between locations (mid and distal)
- No differentially expressed genes
- No effect of location, thus easier to sample and sample live animals

# Results – Ileum PP+ and PP-

- Do the organoids **resemble** their original tissue?
- Is there variation **within** the nearby locations?
- Is there a difference **between** ileum PP+ and PP-?

# Results – Ileum PP+ and PP-

- Do the organoids **resemble** their original tissue?





# Results – Ileum PP+ and PP-

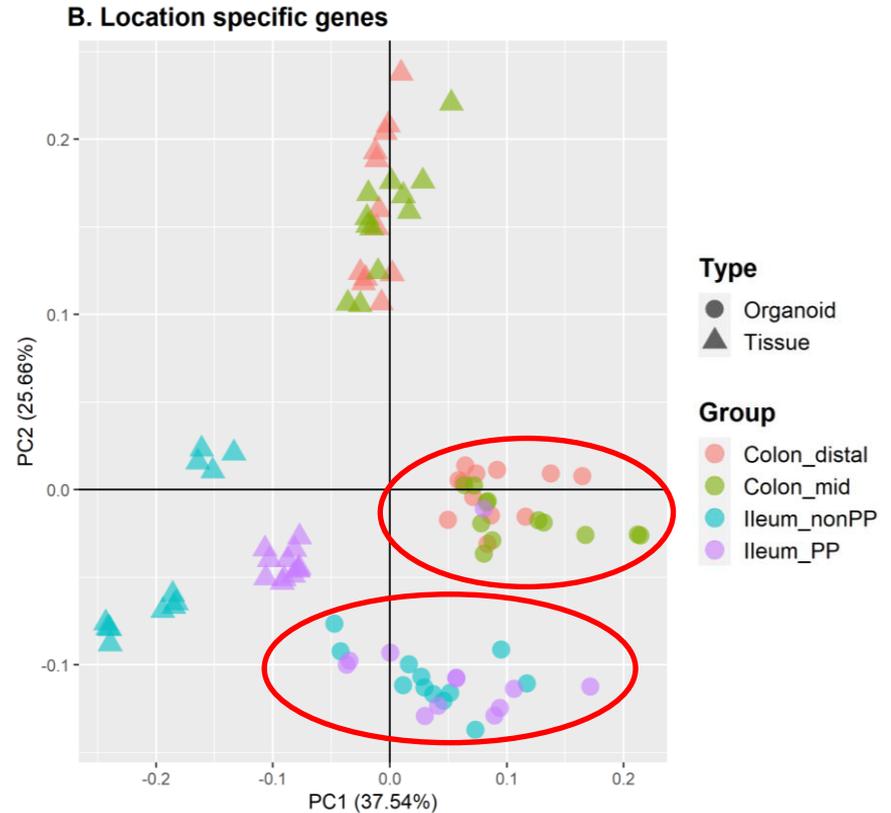
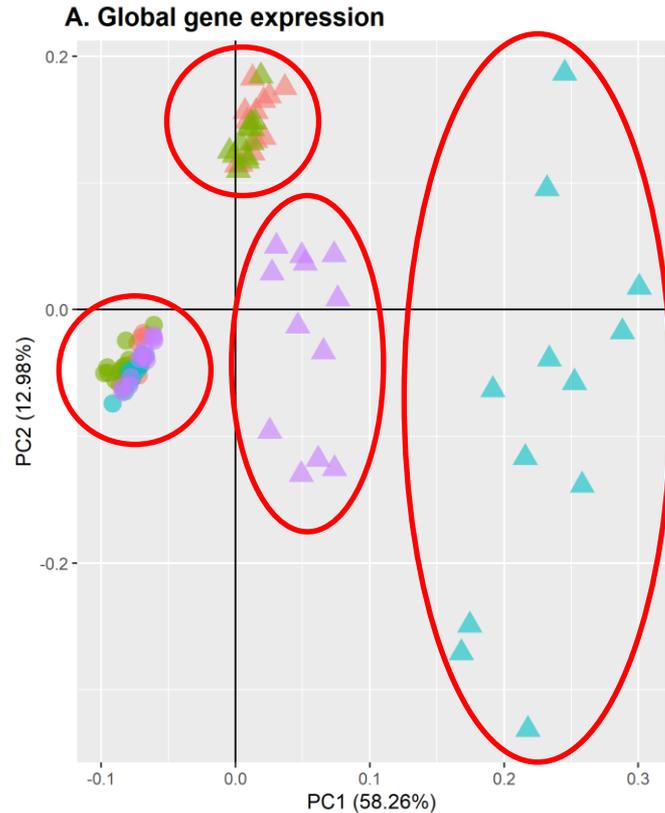
- Is there variation **between** ileum PP+ and PP-?
- Correlation ileum  $0.97 \pm 0.03$
- Number of differentially expressed genes PP+ vs. PP-
  - Tissue 13,399
  - Organoids 5

??? Not enough differentiation yet ???

# Summary ileum results

- High correlations
  - Within animal
  - Within locations
  - Between locations (PP+ and PP-)
- 5 differentially expressed genes
- Might be an effect of location

# Results overall



# Discussion

- No difference mid and distal colon
  - Sample live animals
- 5 DEGs between PP+ and PP- organoids
  - LSP1 gene upregulated in PP+ organoids
  - Immune related gene
- 2D organoids
  - Challenge, nutrient transport, difference between animals, ...

# Conclusion

- Organoids **resemble** their original tissue quite well
- There is little to no variation **within** the nearby locations
- For colon there is no difference **between** the two locations

# Take home message

Organoids are a **promising** tool to better understand whole-tissue functions in animals and could **reduce** the number of animal experiments

# Questions?

Thank you for your attention!

