SFB 852
Ernährung und intestinale Mikrobiota – Wirtsinteraktionen beim Schwein

Feeding *Enterococcus faecium* to piglets affects B-cells on cell, gene and miRNA level

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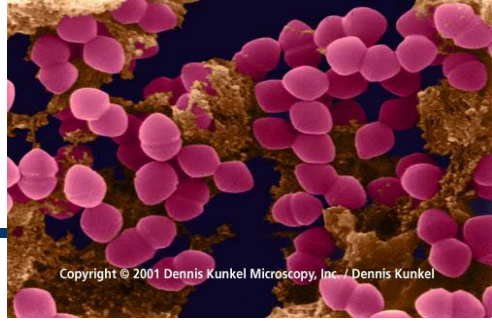
Aim and main question of this study

- Improvement of robustness of piglets through addition of *Enterococcus faecium* NCIMB 10415 to the diet.

➢ How is the intestinal gut immune system affected by the supplementation of *Enterococcus faecium*?

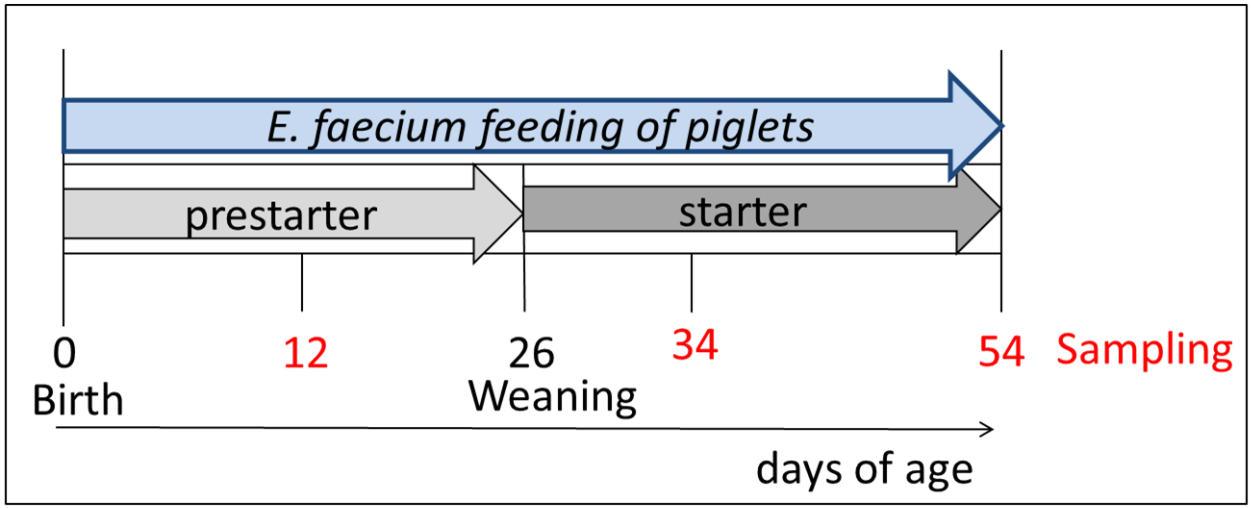
Background



- *Enterococcus faecium* NCIMB 10415: gram positive, lactic-acid producing bacterium
- Licensed probiotic for pigs since 2005
- <u>Known effects:</u>
  - Reduction of incidence and severity of diarrhea (Busing and Zeyner, 2015; Taras et al., 2006; Zeyner and Boldt, 2006)
  - Reduced the number of mucosa-adherent Escherichia Coli pathotypes (Bednorz et al., 2013)
  - Reduction of the *Chlamydia* load of healthy piglets (Pollmann et al., 2005)
  - Modulation of the intestinal immune system (Kreuzer et al., 2012; Scharek et al., 2005; Wang et al., 2014)

Experimental design

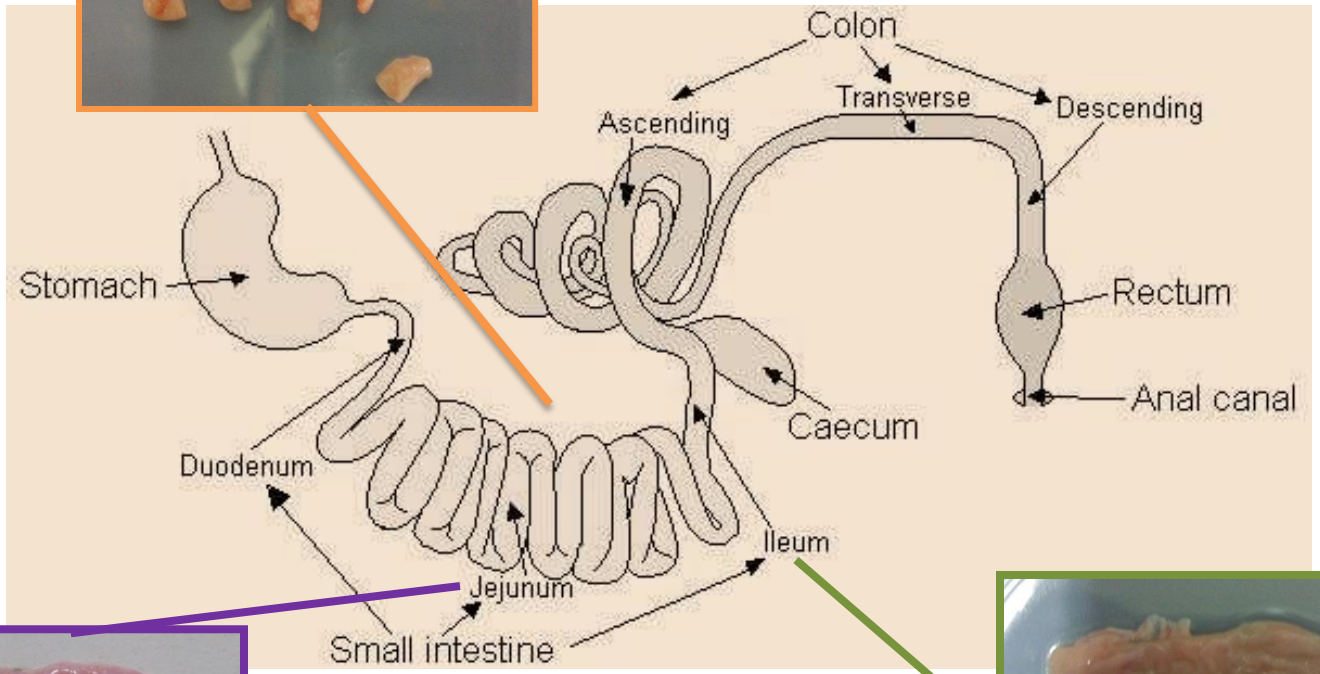
- *Enterococcus faecium* (EF) dietary supplementation (4.2×10^6 cfu/g) for sows and piglets
- EF-feeding of sows beginning 4 wk before partum, piglets all time



- 6 individuals of each group (Control, Probiotic)

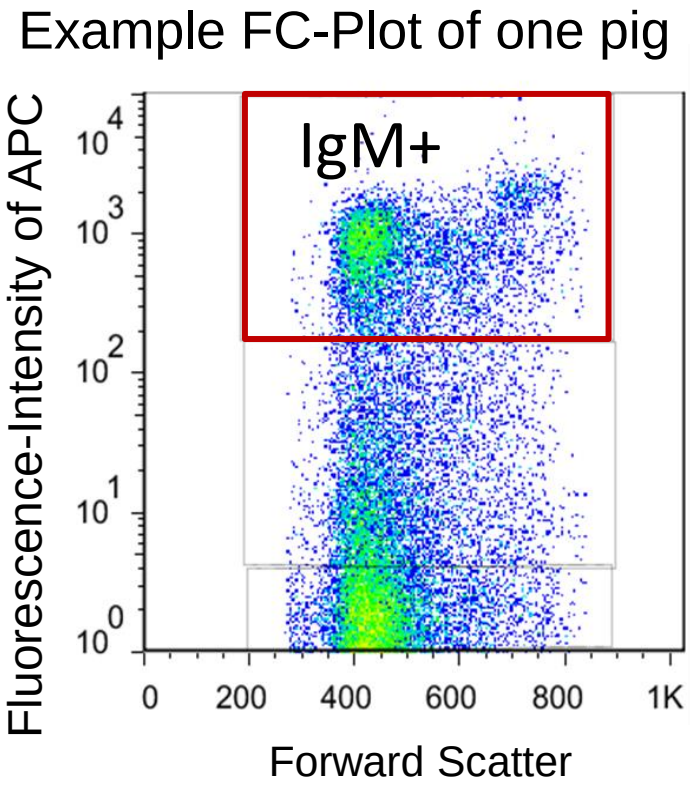
Material

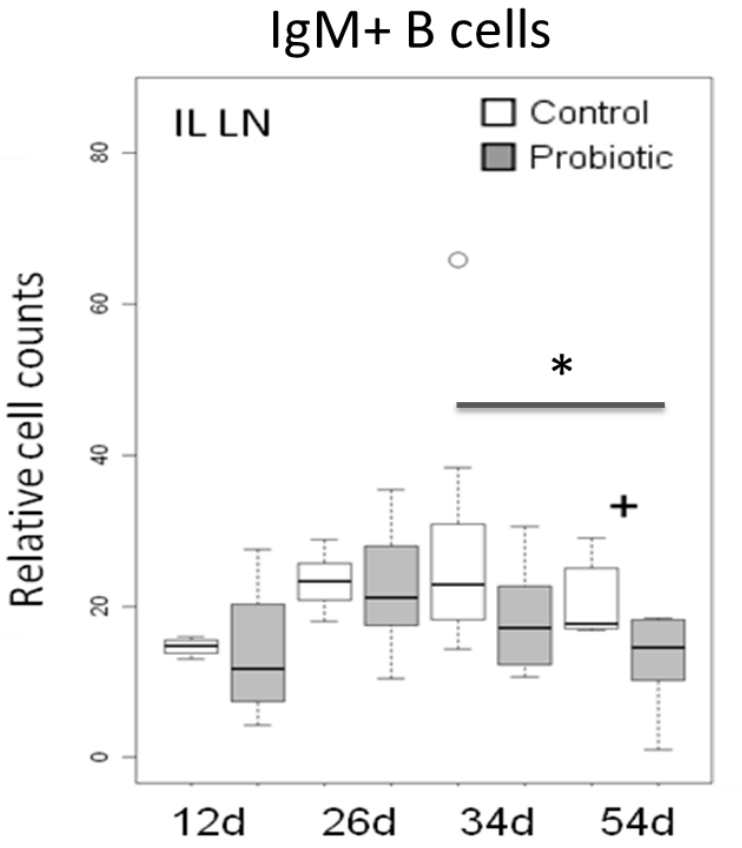
Mesenteric lymph nodes (from Jejunum and Ileum)



Peyer‘s Patch of Jejunum (left) and Ileum (right)

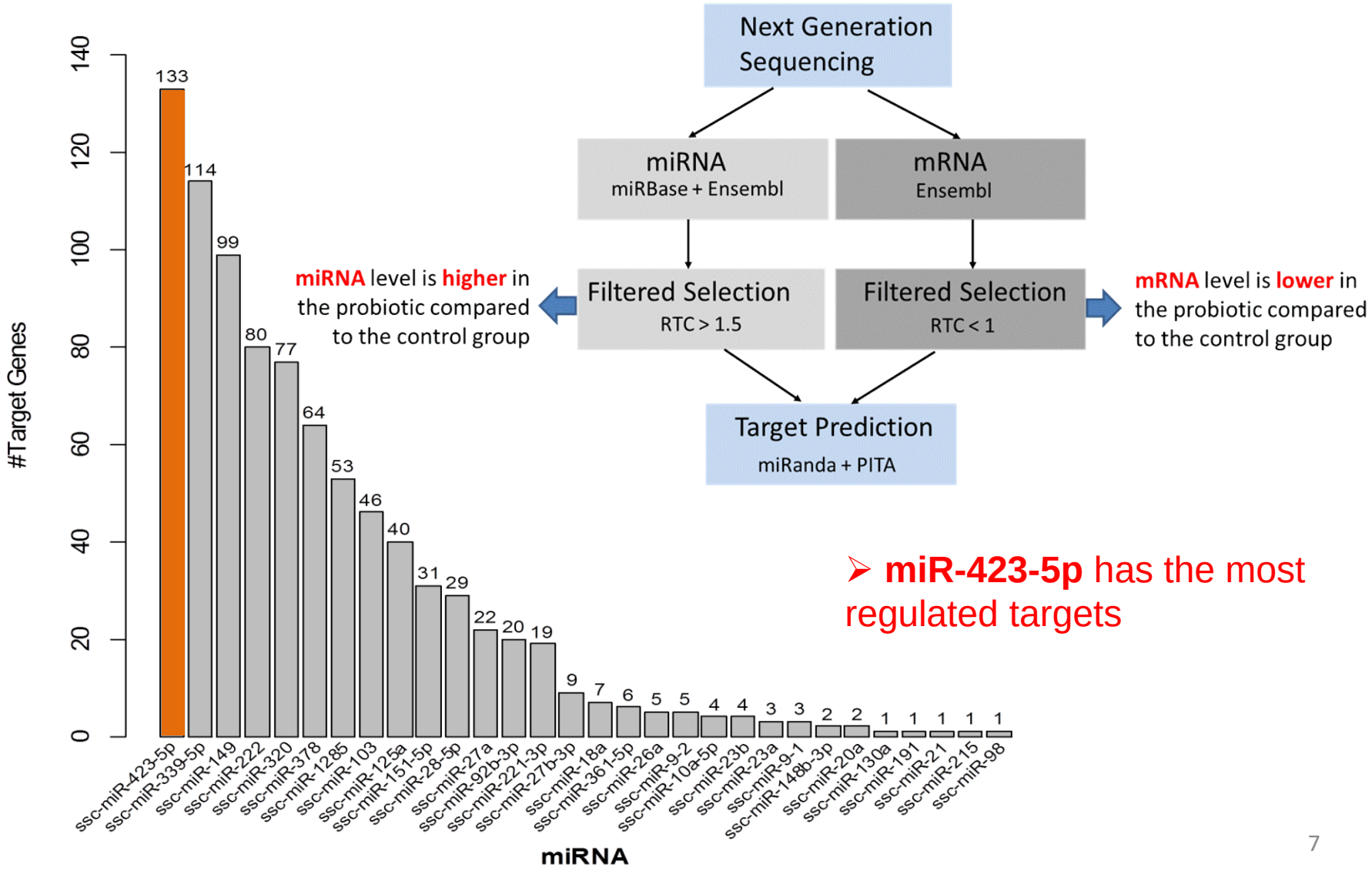
Results: Flow cytometry (FC), cell level





➢ Lower relative frequency of B cells in lymphocytes isolated from ileal mesenteric lymph nodes post weaning in the probiotic group

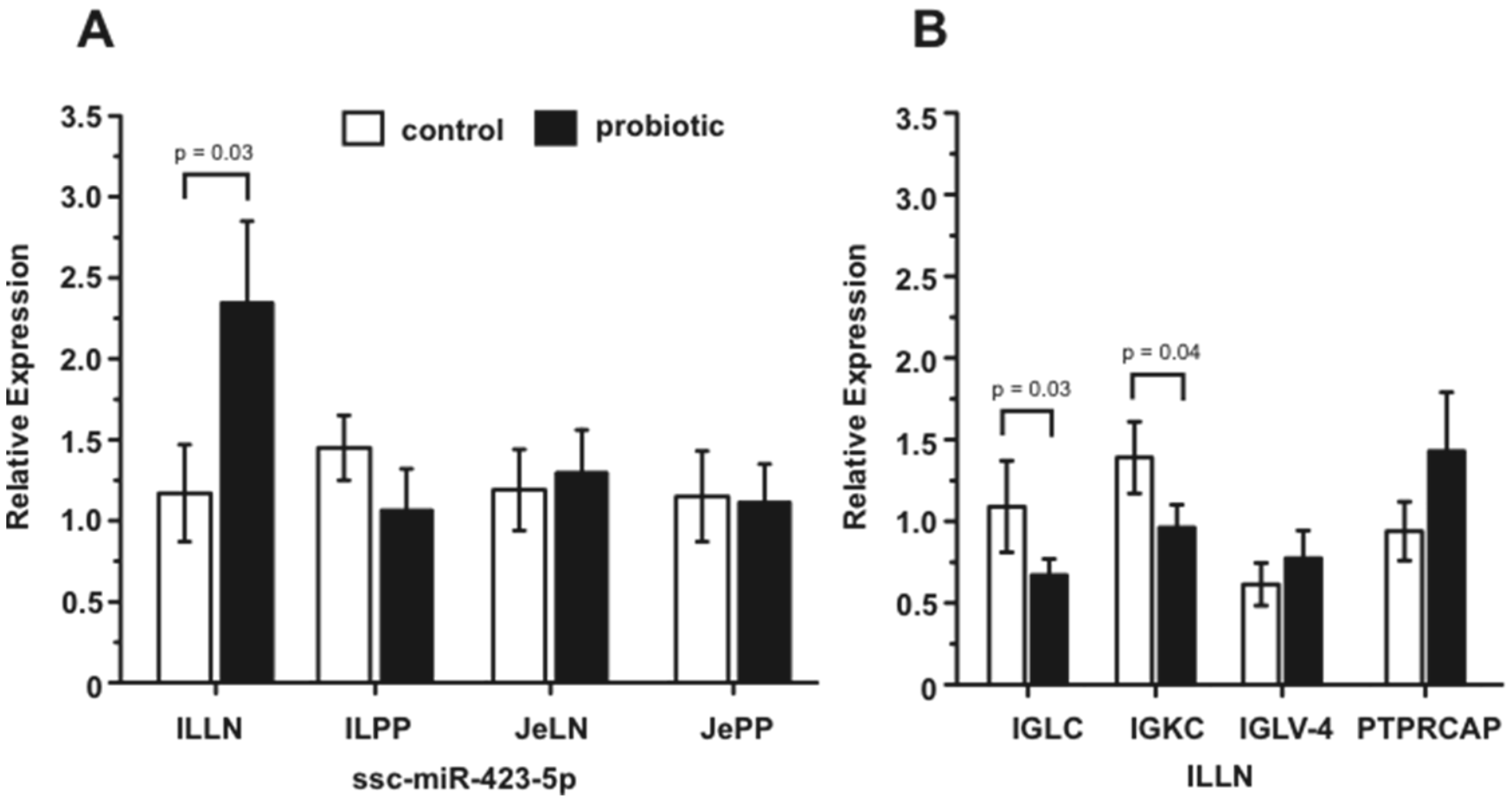
Results: NGS, miRNA and mRNA level



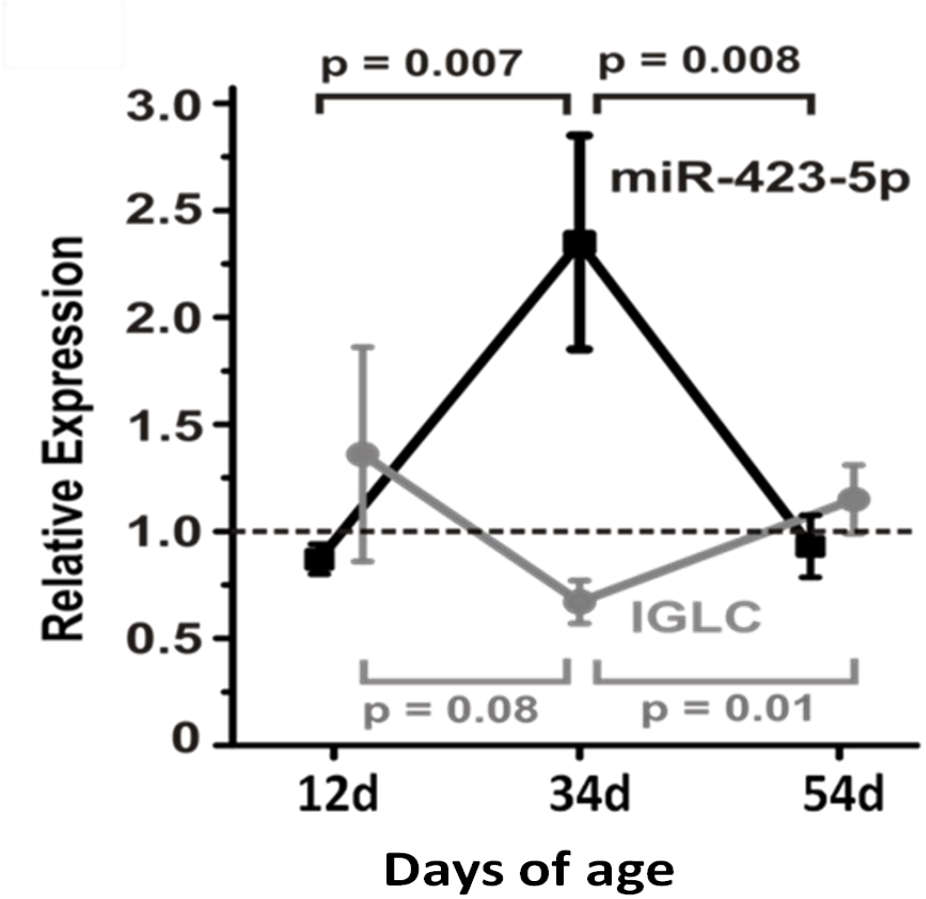
- **miR-423-5p** has the most regulated targets

Results: qPCR, miRNA and mRNA level

- Validation of NGS results by qPCR of 34 day old piglets



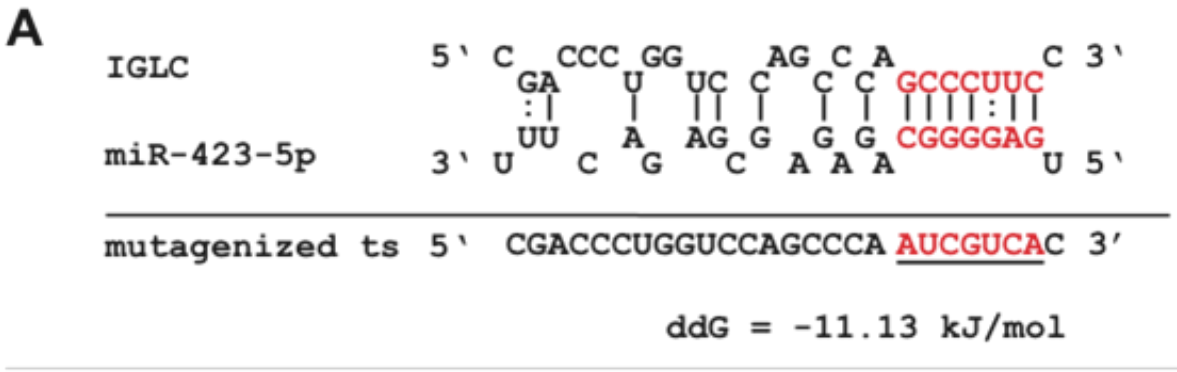
Results: qPCR, miRNA and mRNA level

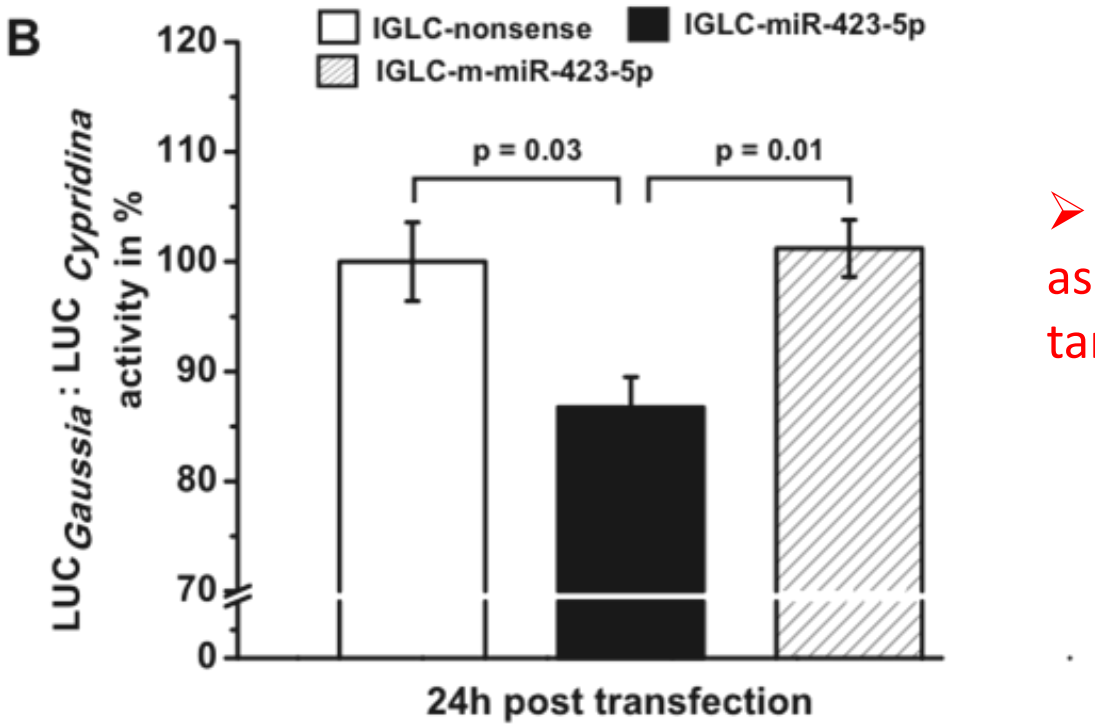


- Additional examination of 12 and 54 days old piglets showed an anti-correlation of miR-423-5p and IGLC

IGLC: Immunglobulin Lambda Light Chain (Constant Region)

Results: miRNA-gene interaction





- Luciferase reporter assays verified IGLC as a target of miR-423-5p

Summary and Conclusion

- The expression of miRNA-423-5p is influenced by *E. faecium* feeding in ileal lymph nodes
- The probiotic *E. faecium* led to lower relative transcript amount of IGLC after weaning
- The probiotic *E. faecium* led to lower relative B cell counts after weaning
- IGLC is likely a target of miRNA-423-5p

➔ There seem to be miRNA mediated effects through *E. faecium* on B cells

Acknowlegment

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http://killerbeesagt.files.wordpress.com/2011/10/schwein.jpg

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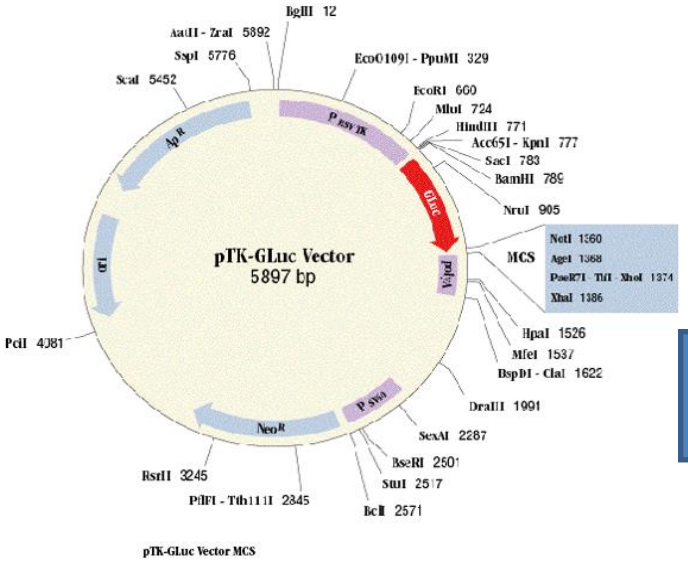
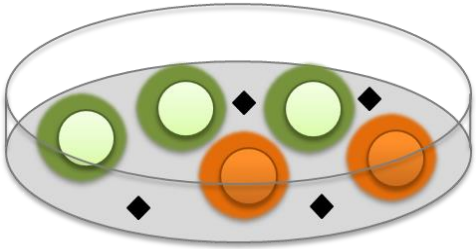
Thank you for your attention!

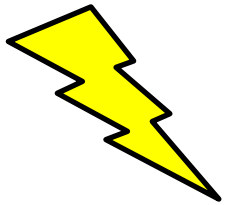
Results – miRNA and gene interaction



1) Identified porcine target sites of IGLC were cloned into the reporter plasmid pTK-Gluc (as well as a mutagenized control)

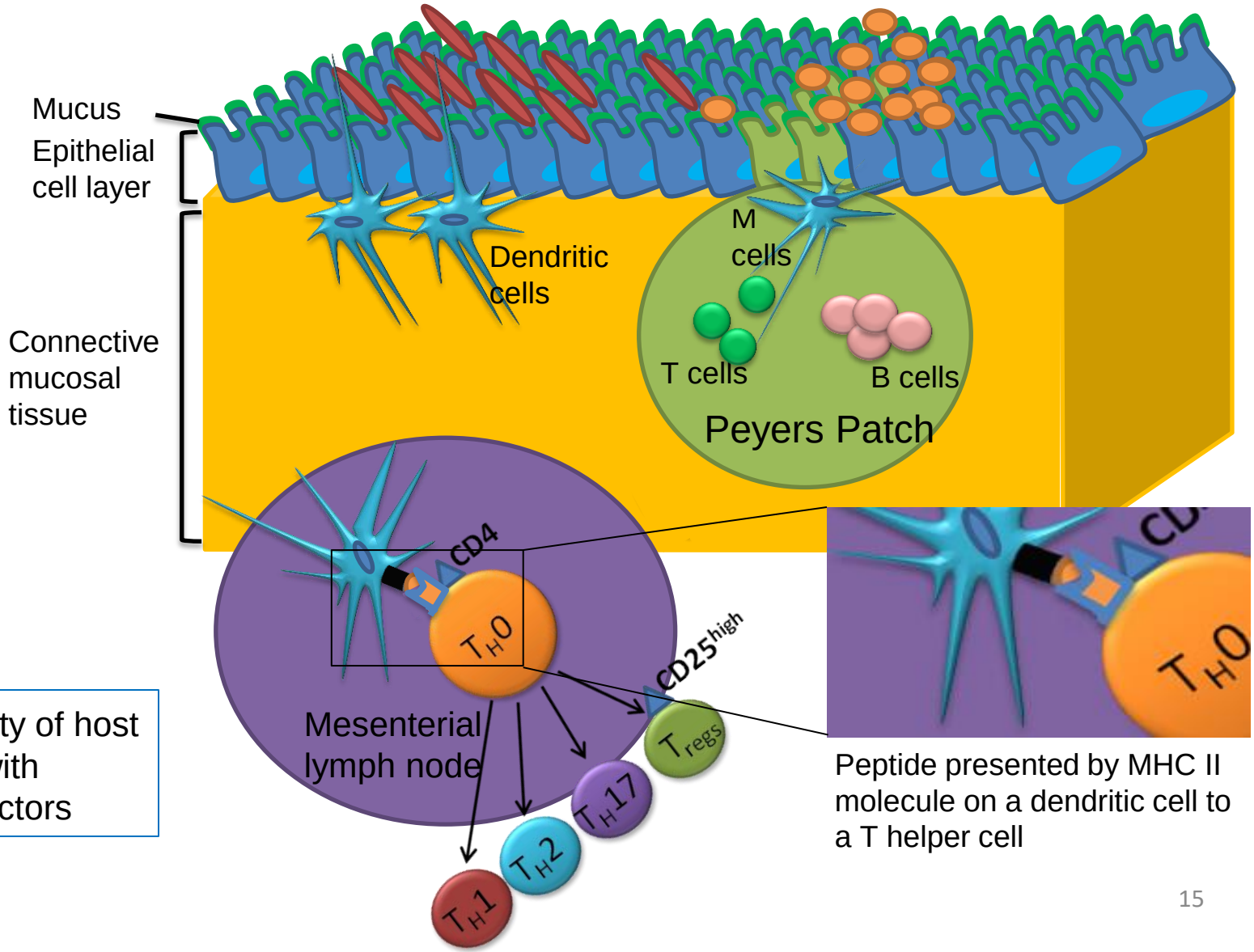


2) Reporter plasmids were co-transfected with pre-miR miRNA-423-5p precursors and nonsense miRNA pre-miR miRNA precursor negative control



3) Detection of Luciferase activity

Introduction - the gut immune system



➔ Possibility of host interaction with nutritional factors

Peptide presented by MHC II molecule on a dendritic cell to a T helper cell