Effects of *Enterococcus faecium* NCIMB 10415 on porcine immune cells

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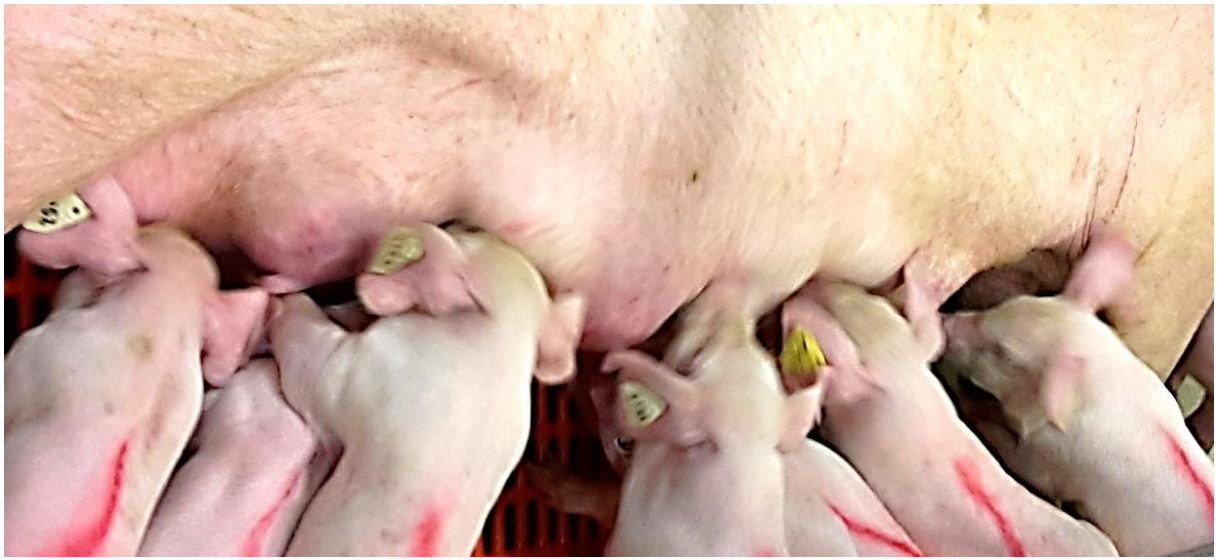
Background

- Improvement of production, health and welfare of weaned piglets
- Reduction of antibiotics and other drugs
- Feeding of probiotics as alternative

➔ What are the mechanisms of action of the probiotics?



Background

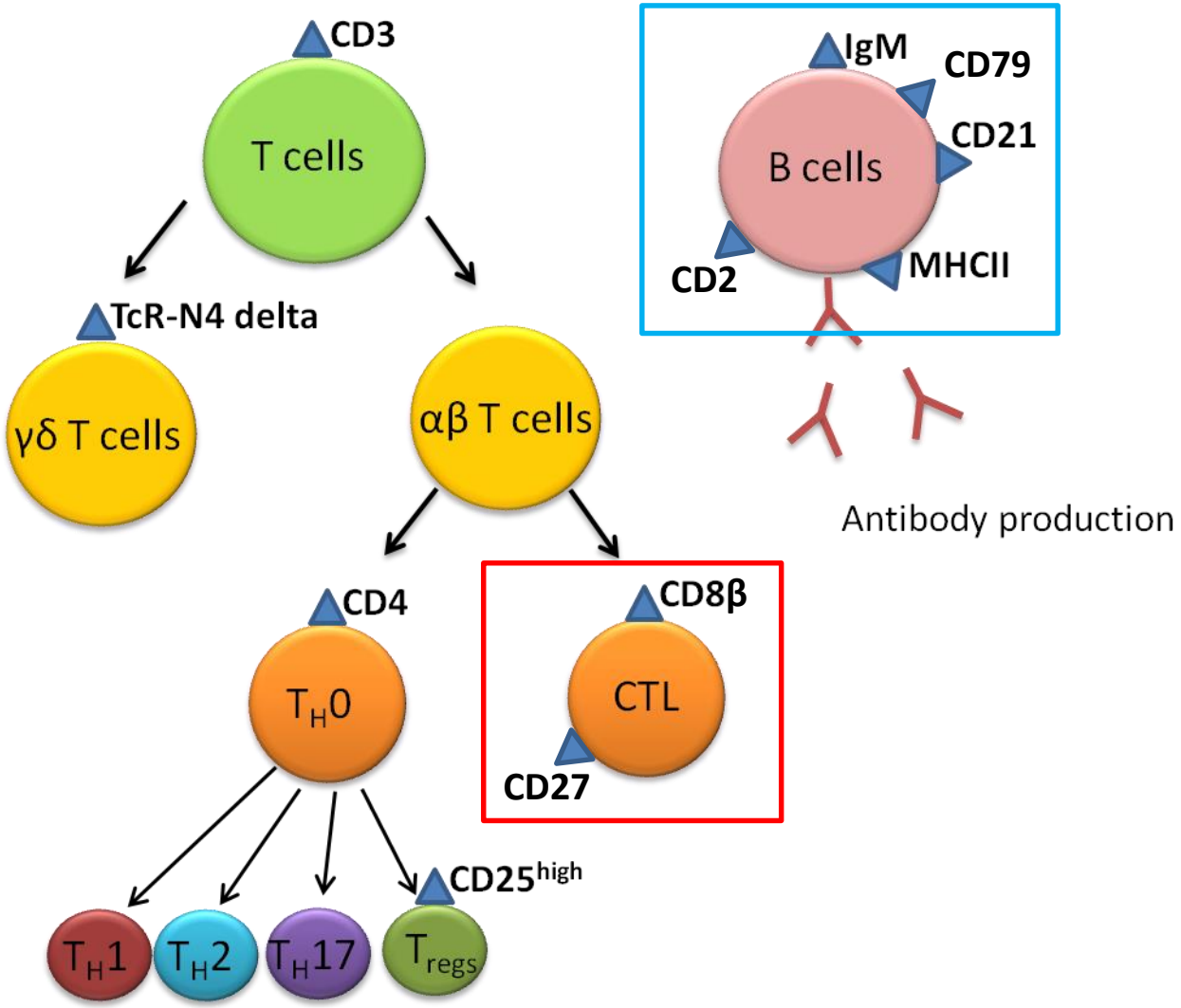
***Enterococcus faecium* NCIMB 10415 (EF):**

- Gram positive, lactic-acid producing bacterium
- Licensed probiotic for pigs since 2005 in Germany
- Pharmaceutical probiotic in humans: „Bioflorin®“ , „Newflora™“

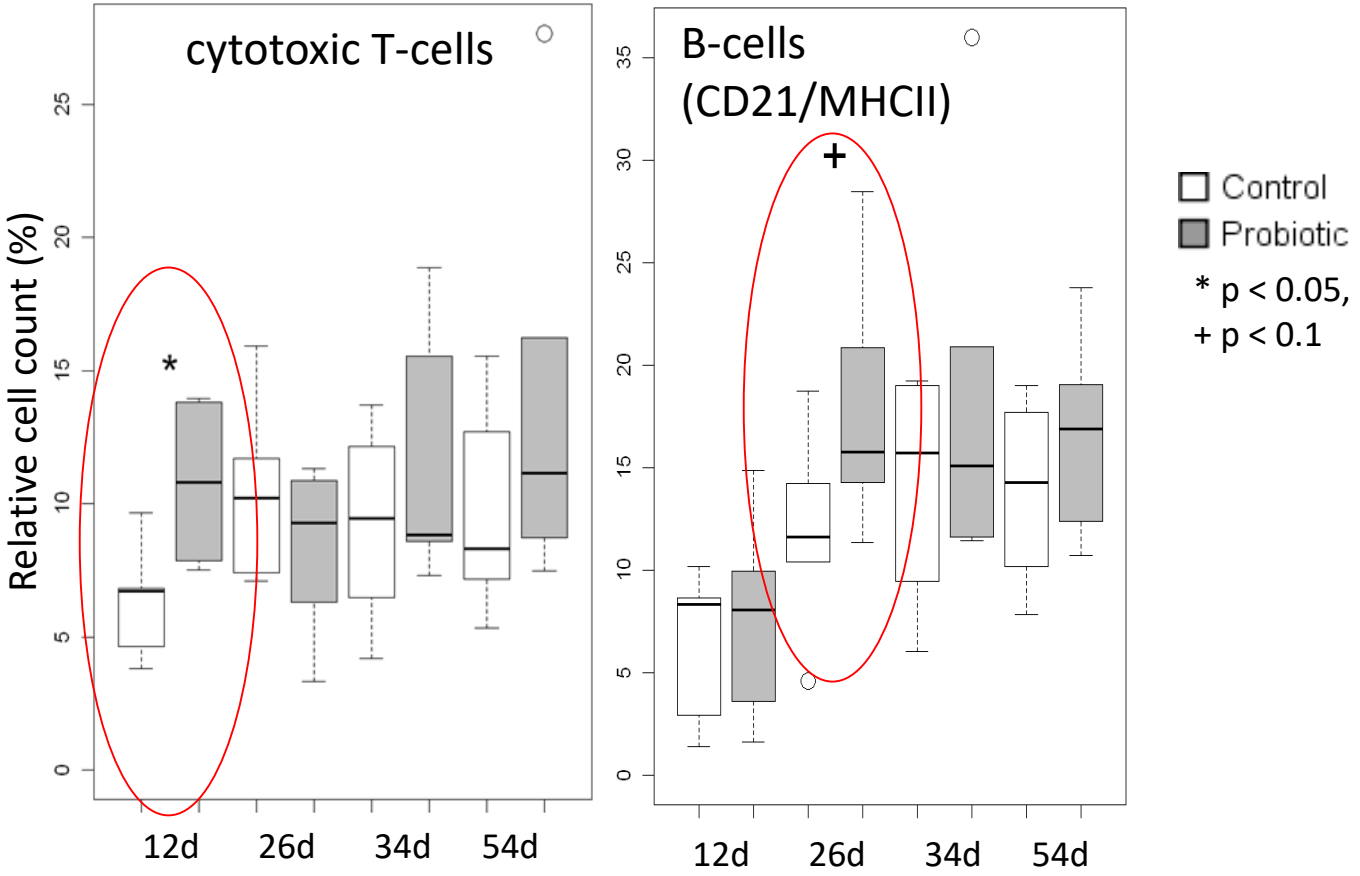
Known effects in swine:

- 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)
- Modulation of the intestinal immune system (Kreuzer et al., 2012; Scharek et al., 2005; Wang et al., 2014)

Background – Adaptive immune cells



Background – *in vivo* EF effects on immune cells in feeding experiments



➢ *E. faecium*-treatment increased the relative cell count of cytotoxic T- and B-cells preweaning in peripheral blood mononuclear cells

Kreuzer et al. *Veterinary Research* 2012, 43:58

Background – Hypothesis

- *E. faecium* is able to directly affect adaptive immune cells.
- *E. faecium* activates cytotoxic T cells and B-cells

1. Does *E. faecium* directly effect the immune system?

2. Which component of *E. faecium* mediates the immunomodulatory effects?

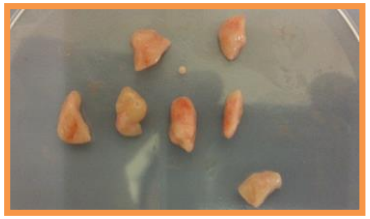
Materials & Methods - Experimental Design

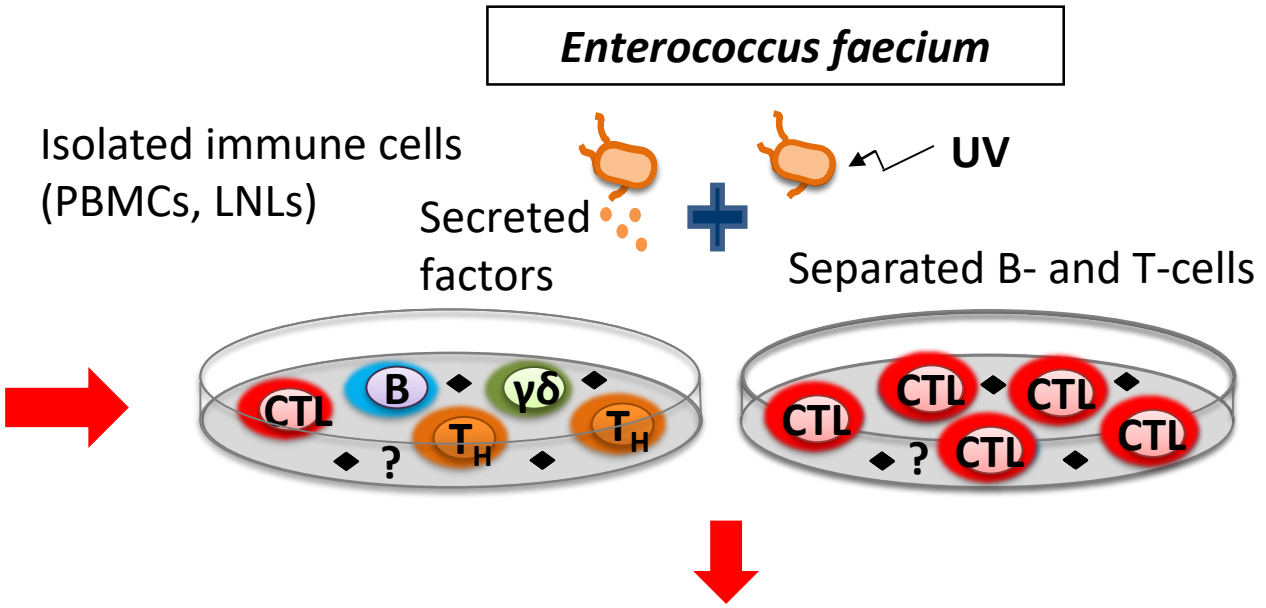
Probiotic strain used: *Enterococcus faecium* NCIMB 10415/SF68

Non-probiotic strains used: *E. faecium* 2918, *E. faecium* 20477

German Landrace pigs

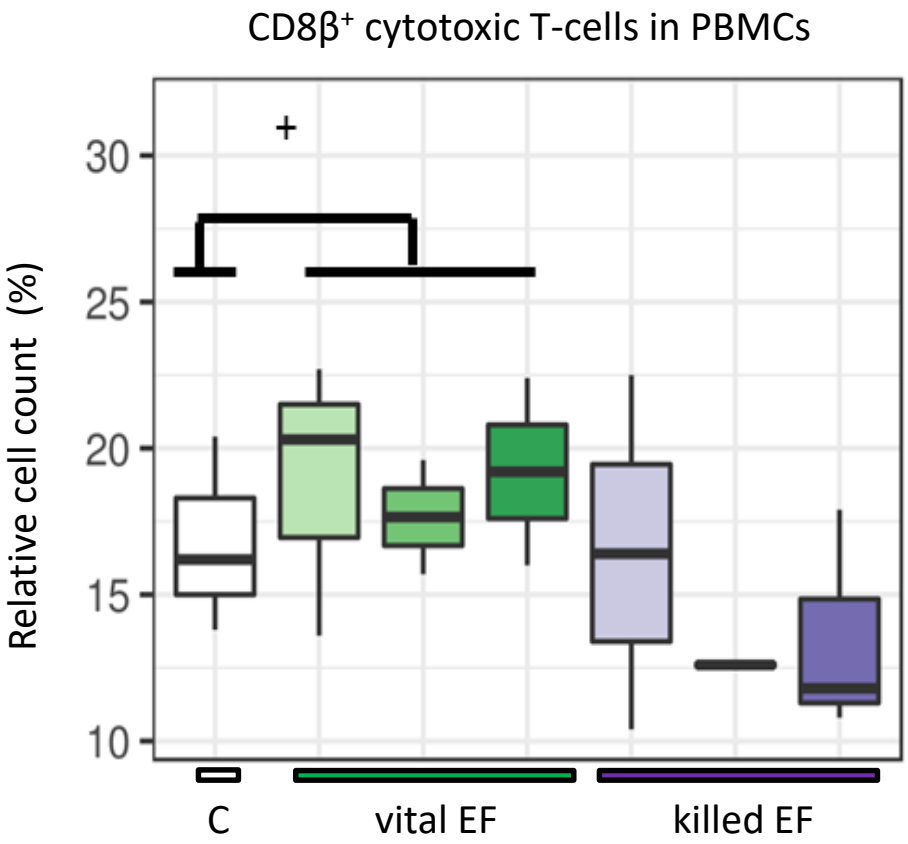
Mesenteric lymph nodes (Slaughter pigs)





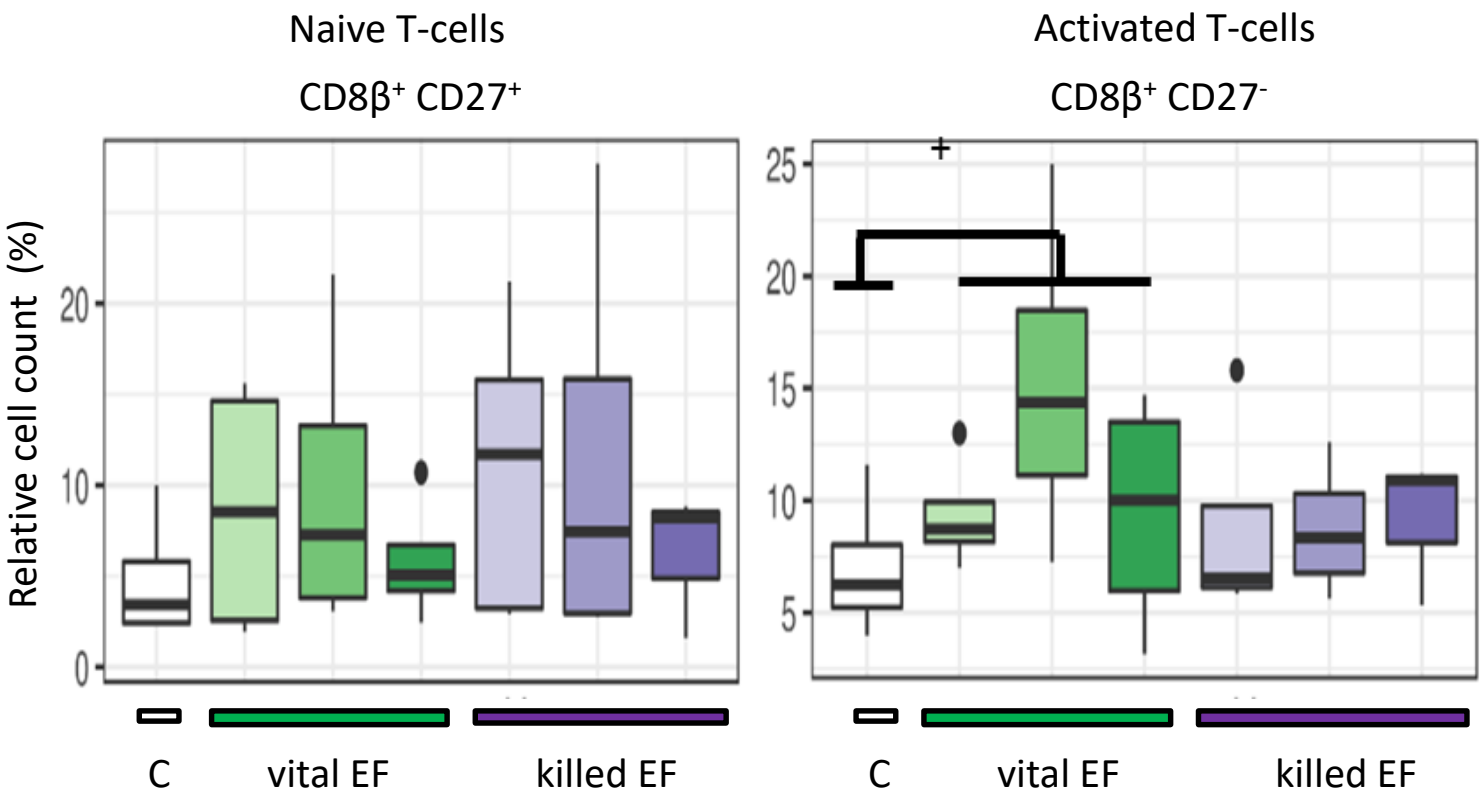
B- and T-cells were analyzed on cell and transcript level by flow cytometry and qPCR

In vitro effects of EF on **cytotoxic T-cells** in PBMCs



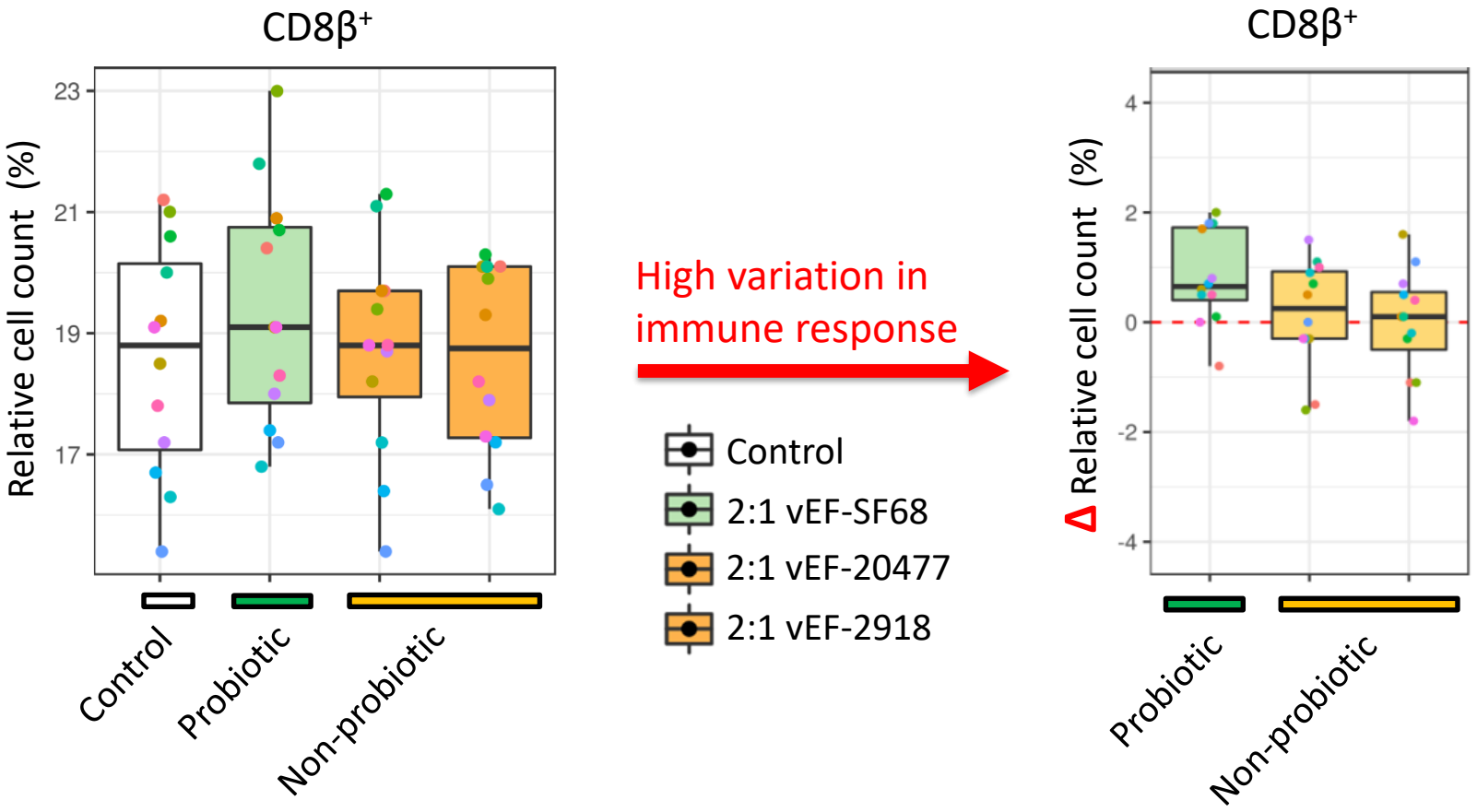
- Tendency towards higher relative cell count of cytotoxic T-cells with vital *E. faecium* treatment suggests an involvement of secreted factors

In vitro effects of EF on **cytotoxic T-cells** in LNLs



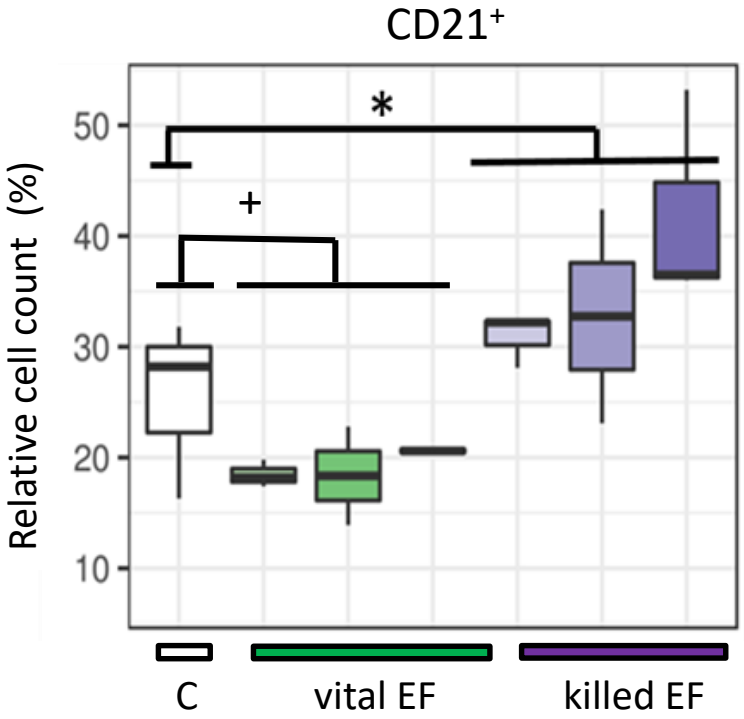
- Tendency towards a higher relative cell count of activated cytotoxic T-cells with vital *E. faecium* treatment suggests involvement of secreted factors

In vitro effects of different EF strains on **cytotoxic T-cells** in PBMCs



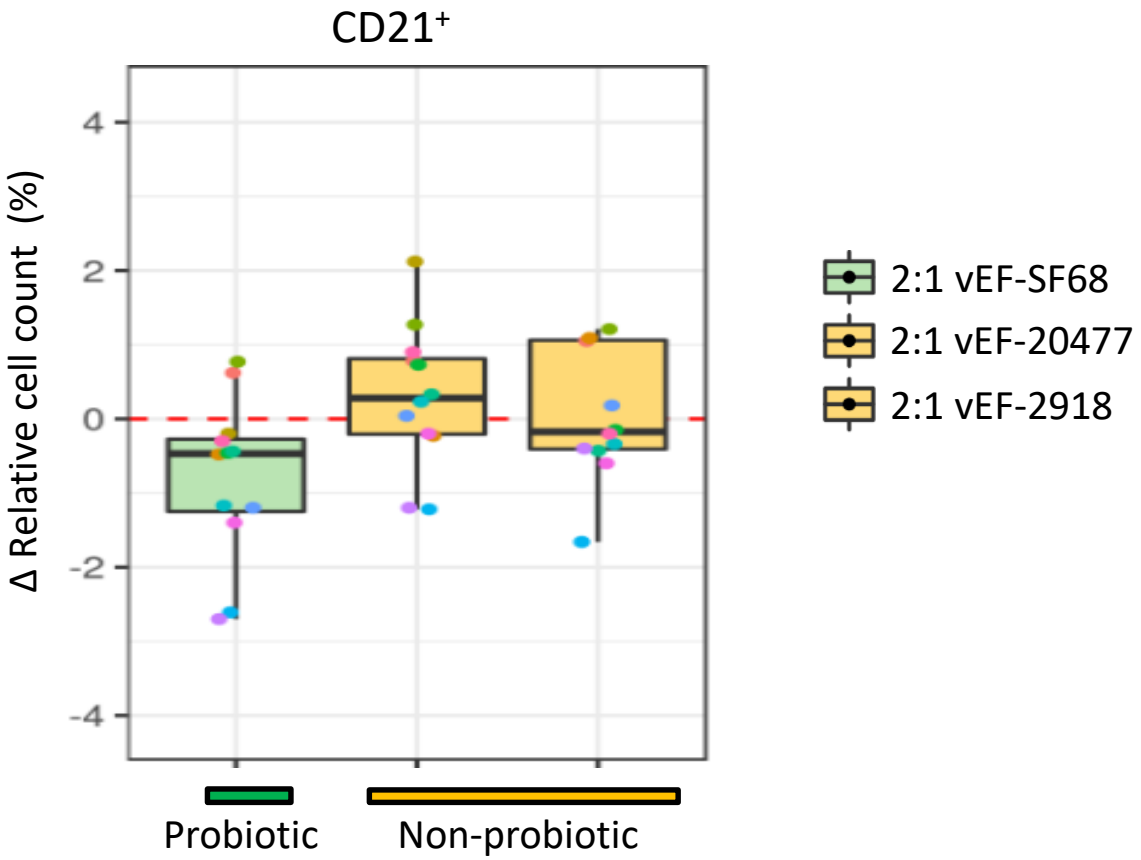
- Higher relative cell count of cytotoxic T-cells with treatment with the probiotic *E. faecium* strain suggests a strain-specific, probiotic effect

In vitro effects of EF on **B-cells** in PBMCs



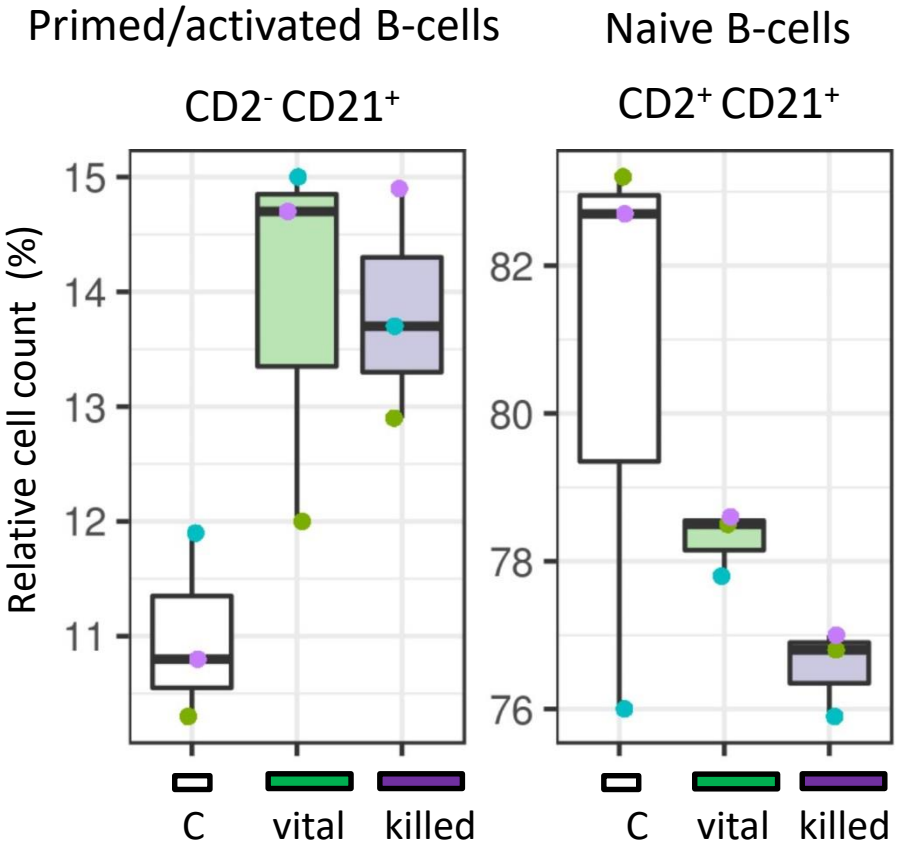
- Higher relative cell count of B-cells with killed *E. faecium* treatment suggests an involvement of a surface compound
- Lower relative cell count of B-cells with vital *E. faecium* suggests an inhibition by secreted factors

In vitro effects of different EF strains on **B-cells** in PBMCs



- Lower relative cell count of B-cells with vital *E. faecium* suggests a strain-specific, probiotic effect

In vitro effects of EF on sorted B-cells



- Higher relative cell count of primed B-cells with vital and killed *E. faecium* treatment suggests a different mode of action on sorted B cells than in a PBMC or LNL composite

Summary & conclusion

- **Vital *E. faecium*** seemed to inhibit B-cells and increased cytotoxic T-cells in PBMCs and LNLs composite, which could be mediated through secreted factors of *E. faecium*
- **Killed *E. faecium*** increased B-cells in PBMCs which might suggest an involvement of a surface compound
- **The effects of *E. faecium* NCIMB 10415 seem to be strain-specific**
- **Vital and killed *E. faecium*** increased primed B-cells in sorted B-cells which might suggest activation via bacterial components as secreted factors or surface molecules
- There is evidence of a direct immunomodulatory effect of *Enterococcus faecium* NCIMB 10415 on adaptive immune cells

Acknowledgements

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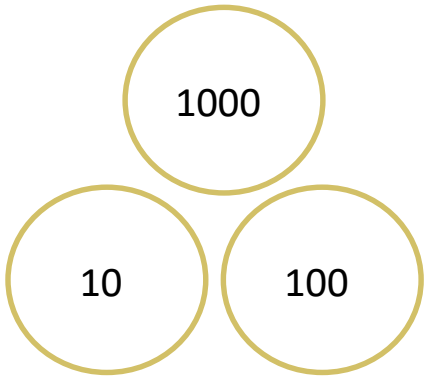
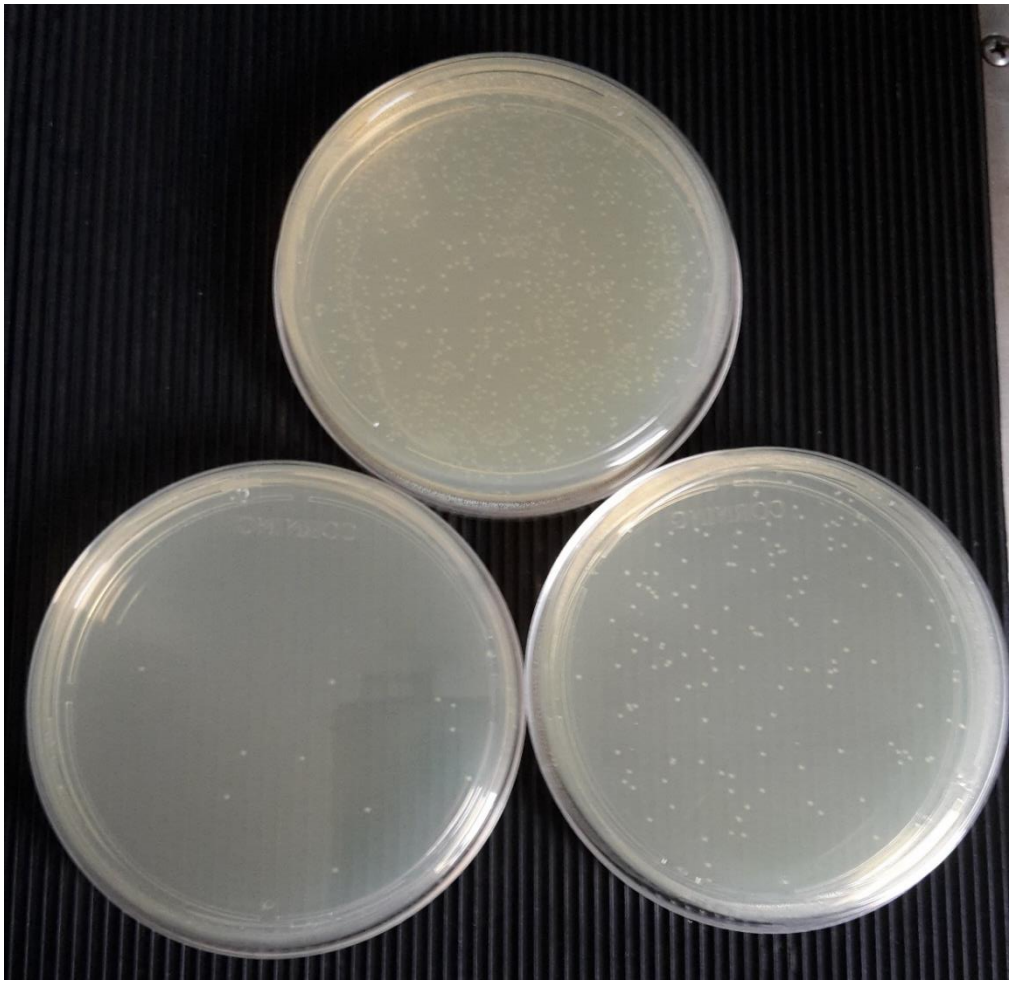
  Albrecht Daniel Thaer-Institut, Humboldt Universität zu Berlin, Germany

THANK YOU FOR YOUR ATTENTION!

Pre-experiments

Testing the cfu (colony forming unit)
of the used product (Cylactin, Cerbios Pharma)





Columbia-Agar plates incubated with EF

Pre-experiments

- Testing the “killing” effect of UV to *Enterococcus faecium*

UV-light for 60 min

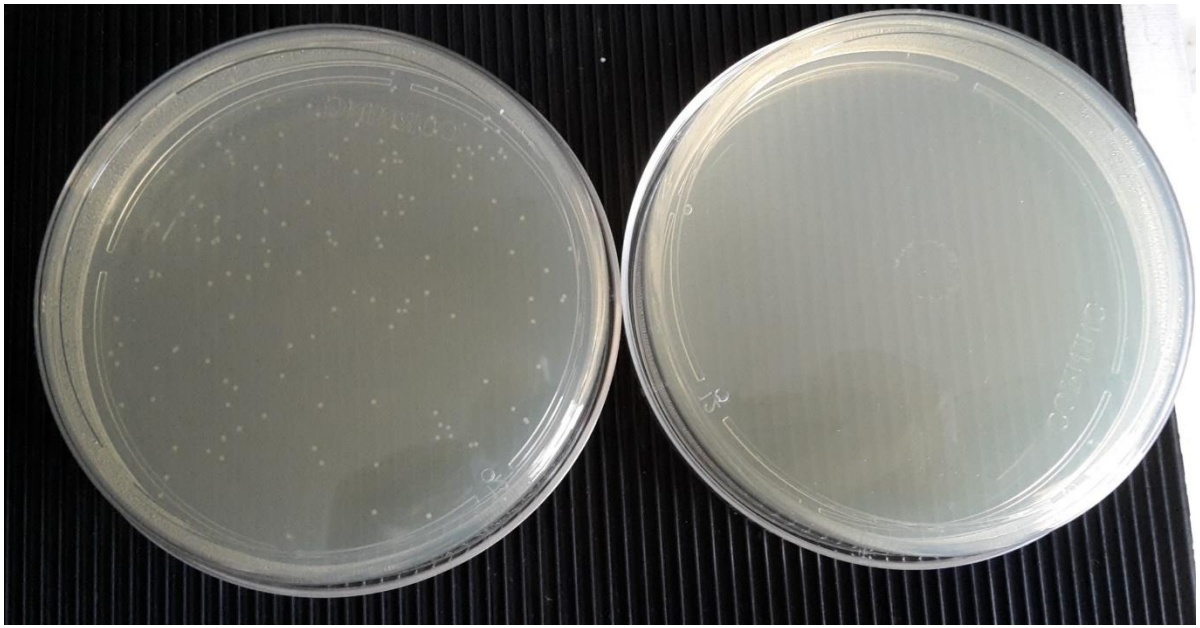
UV-light for 40 min

UV-light for 20 min

live

Pre-experiments

- Testing the “killing” effect of UV to *Enterococcus faecium*



Columbia-Agar plates incubated with EF

Key findings

- *Enterococcus faecium* NCIMB 10415 supplementation affects intestinal immune-associated gene expression in post-weaning piglets (Siepert et al. *Vet Immunol Immunopathol* 2014, 157:65-77).

- Characterization of CD4+ subpopulations and CD25+ cells in ileal lymphatic tissue of weaned piglets infected with Salmonella Typhimurium with or without Enterococcus faecium feeding (Kreuzer et al. *Vet Immunol Immunopathol* 2014, 158:143-155.

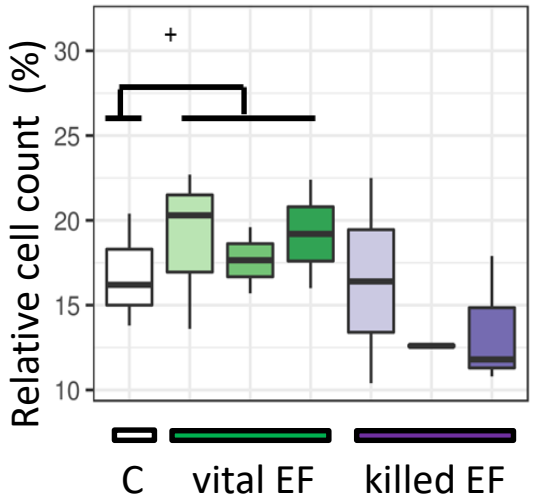
- Feeding of the probiotic bacterium Enterococcus faecium NCIMB 10415 differentially affects shedding of enteric viruses in pigs (Kreuzer et al. *Vet Res* 2012, 43:58).

- Feeding of *Enterococcus faecium* NCIMB 10415 leads to intestinal miRNA-423-5p induced regulation of immune-relevant genes (Kreuzer-Redmer et al. *Appl Environ Microbiol* 2016, 82: 2263-2269).

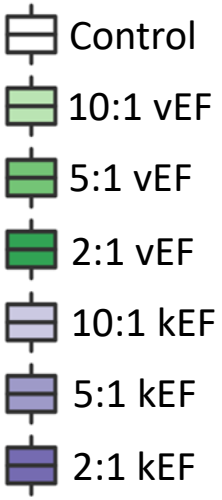
In vitro effects of EF on **cytotoxic T-cells** in a PBMC and LNL composite

CD8β+ cytotoxic T-cells in LNLs

CD8β⁺ cytotoxic T-cells in PBMCs



1.5 h EF-Treatment
Ratio PBMC : EF

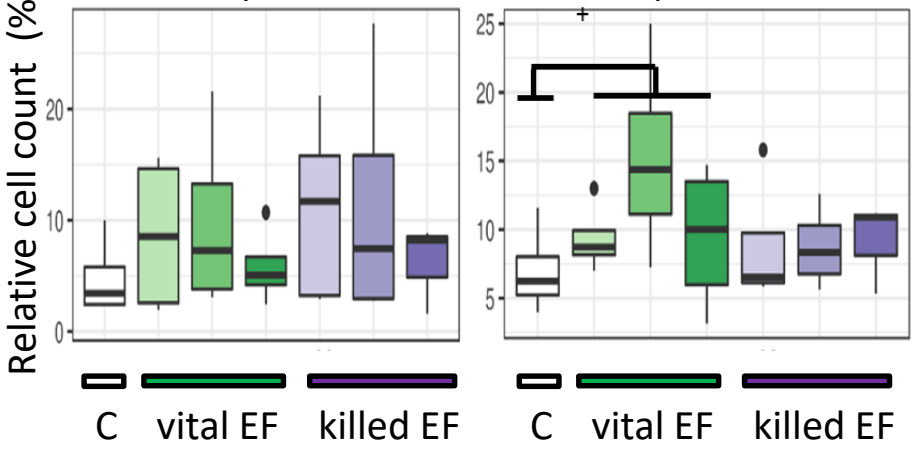


Naive T-cells

CD8β⁺ CD27⁺

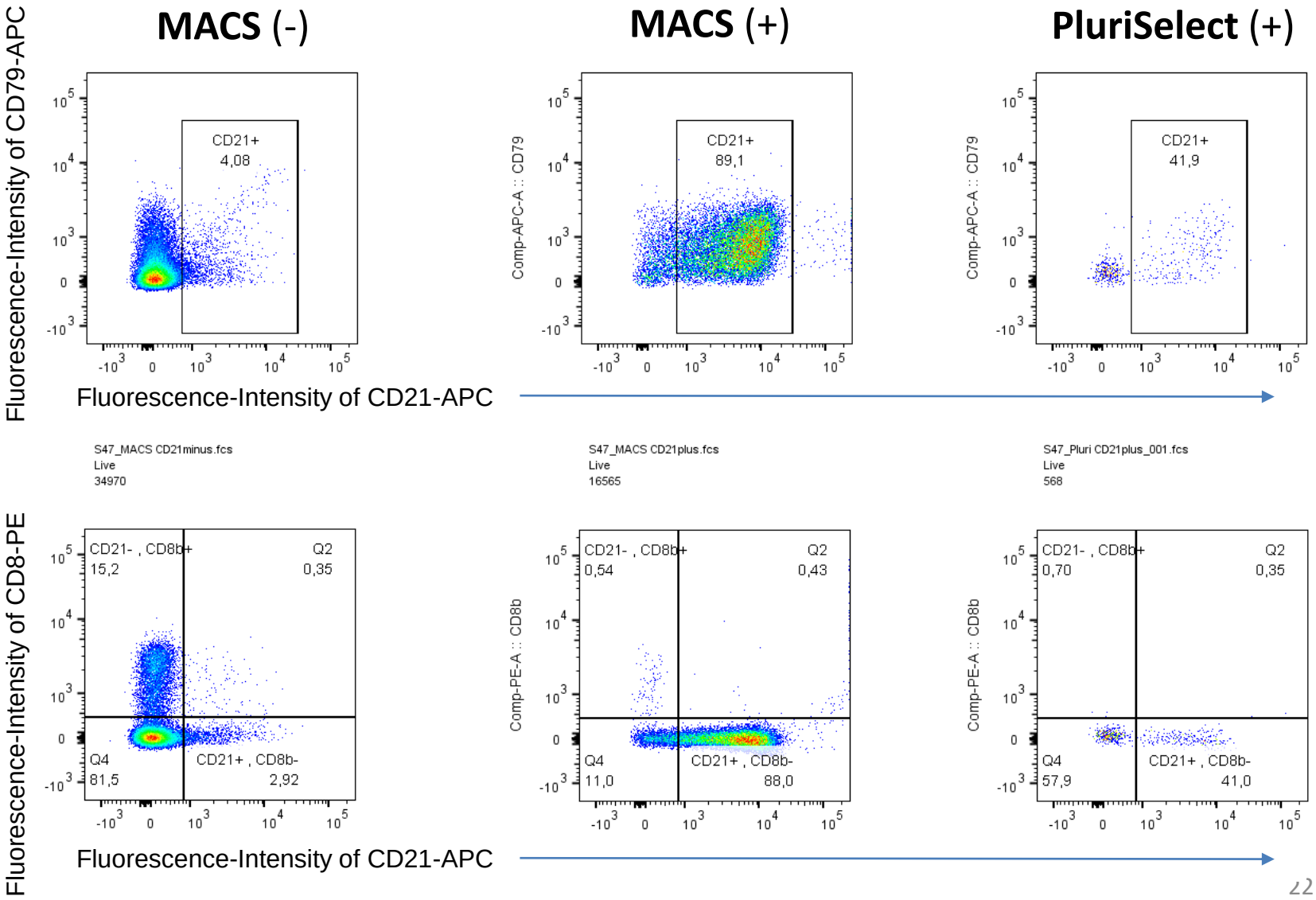
Activated T-cells

CD8β⁺ CD27⁻

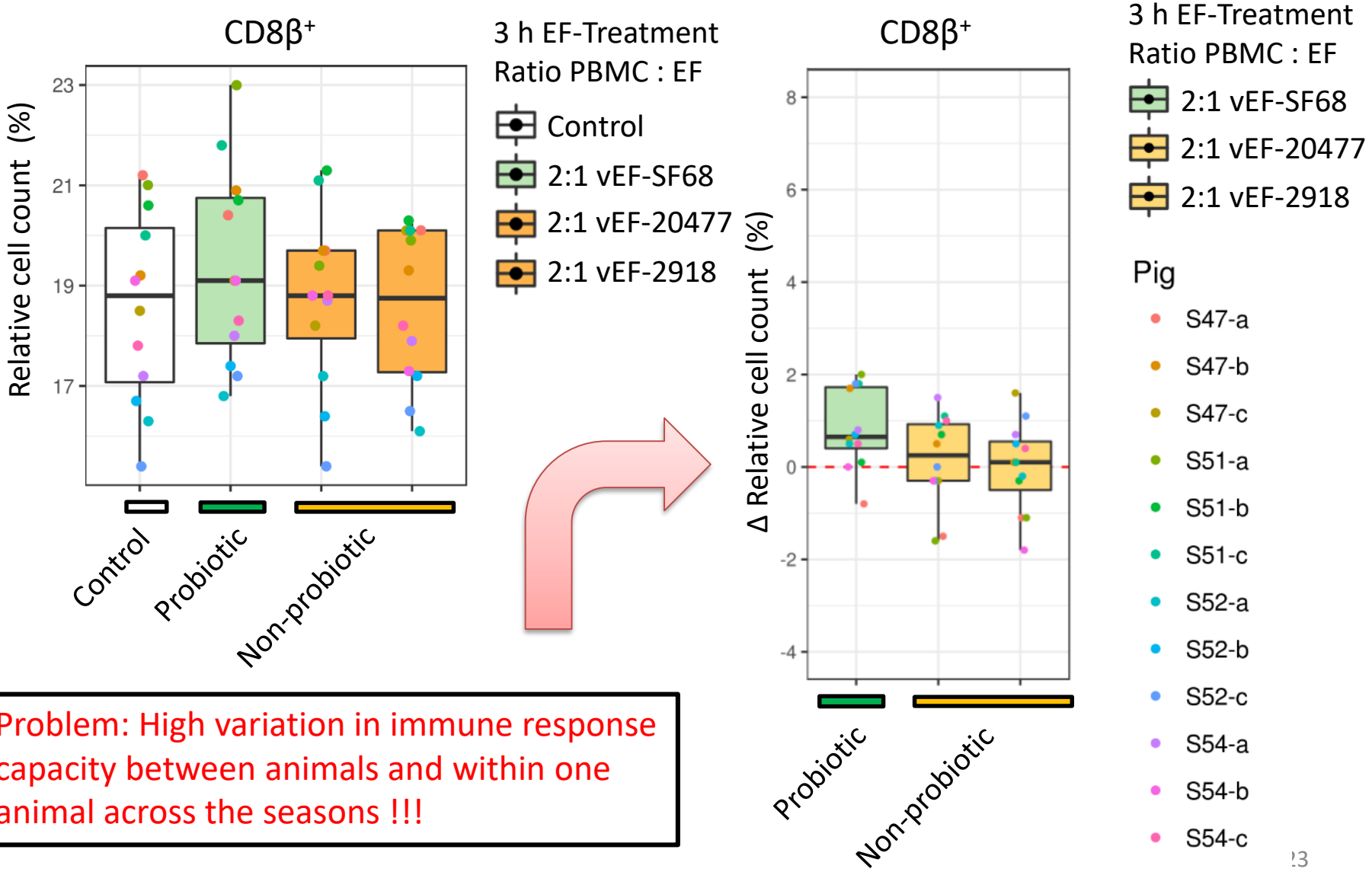


- Tendency towards higher relative cell counts of cytotoxic T-cells (CD8β⁺) with vital *E. faecium*
- Tendency towards a higher relative cell counts of activated cytotoxic T-cells (CD8β⁺ CD27⁺) with vital *E. faecium* bacteria in mesenteric lymph nodes (mLN)

Cellsort – (pre)experiments

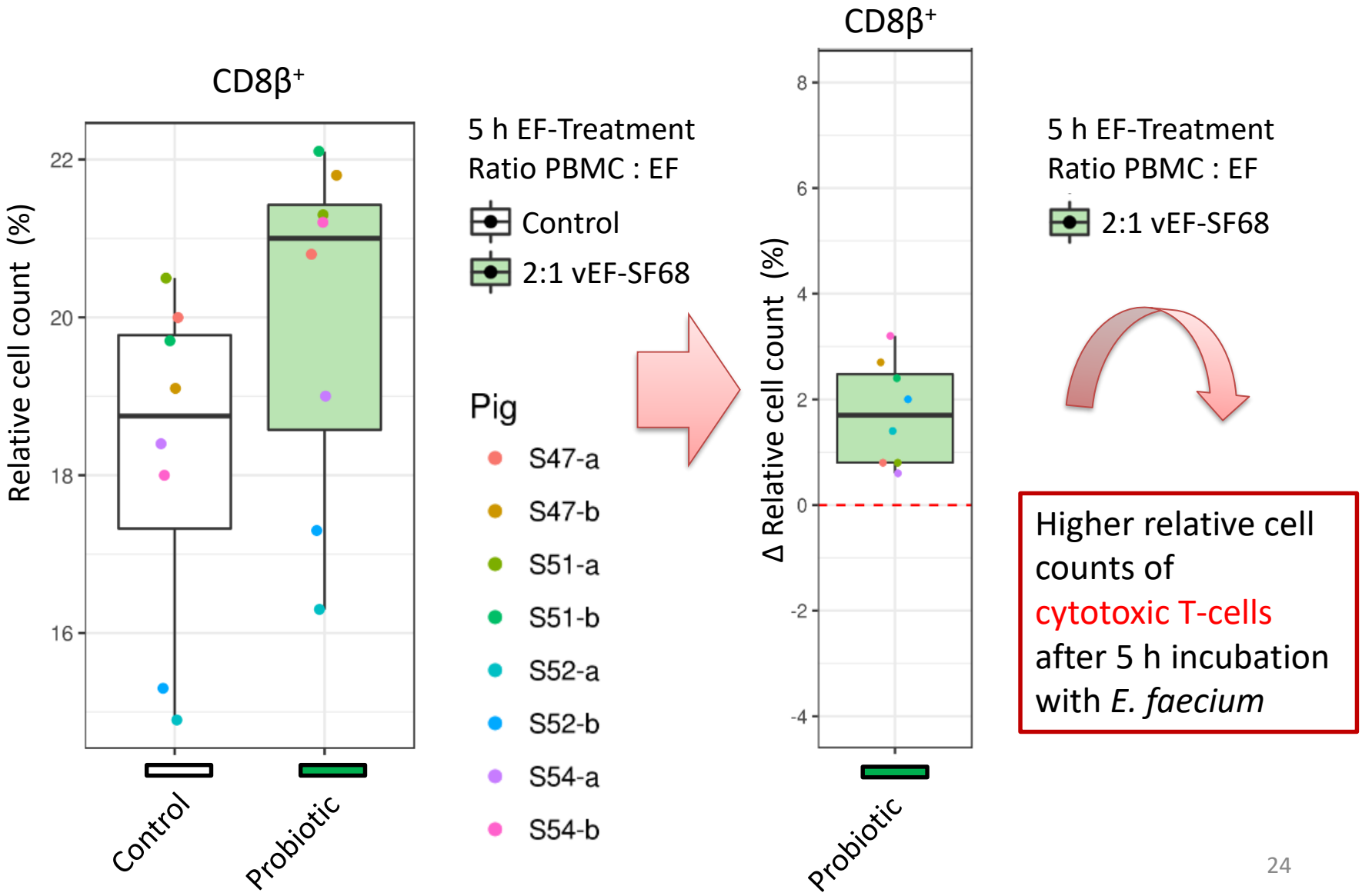


In vitro effects of different EF strains on **cytotoxic T-cells** in a PBMC composite



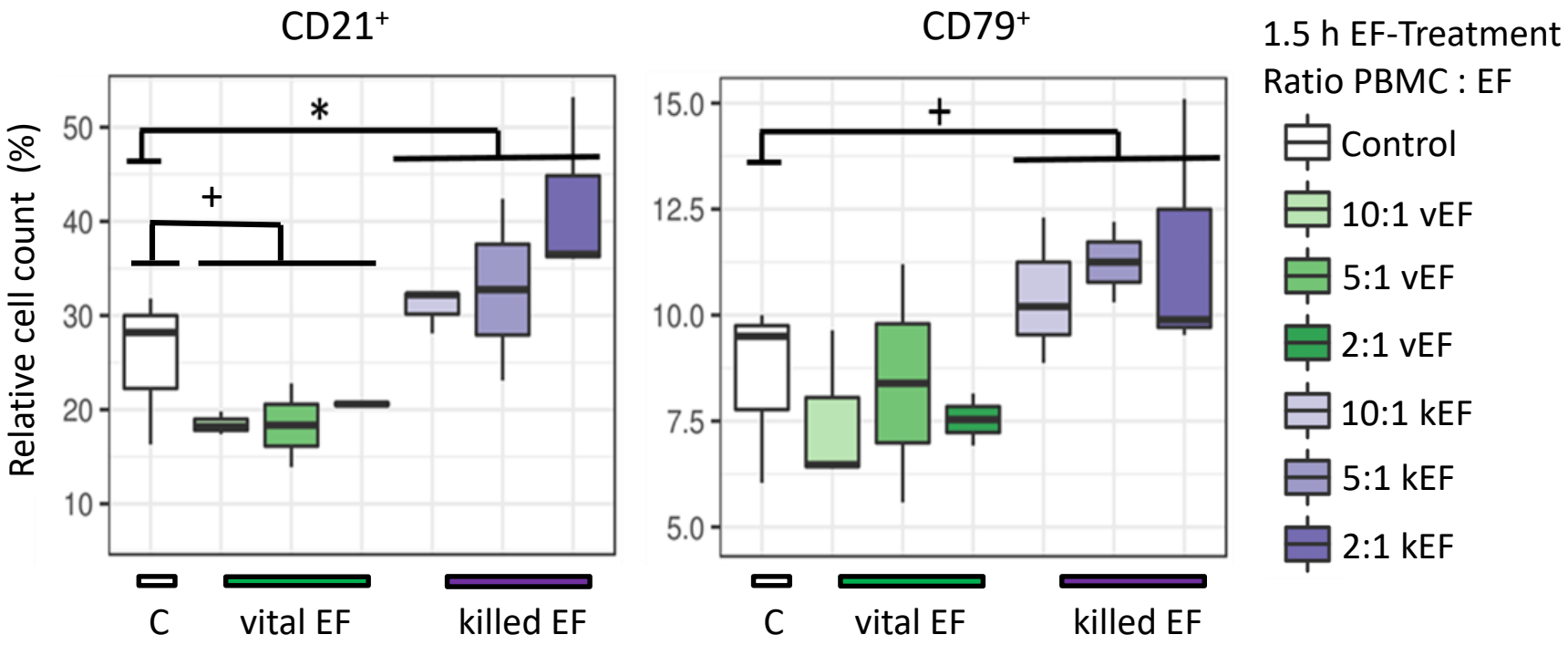
Problem: High variation in immune response capacity between animals and within one animal across the seasons !!!

In vitro effects of EF on **cytotoxic T-cells** in a PBMC composite



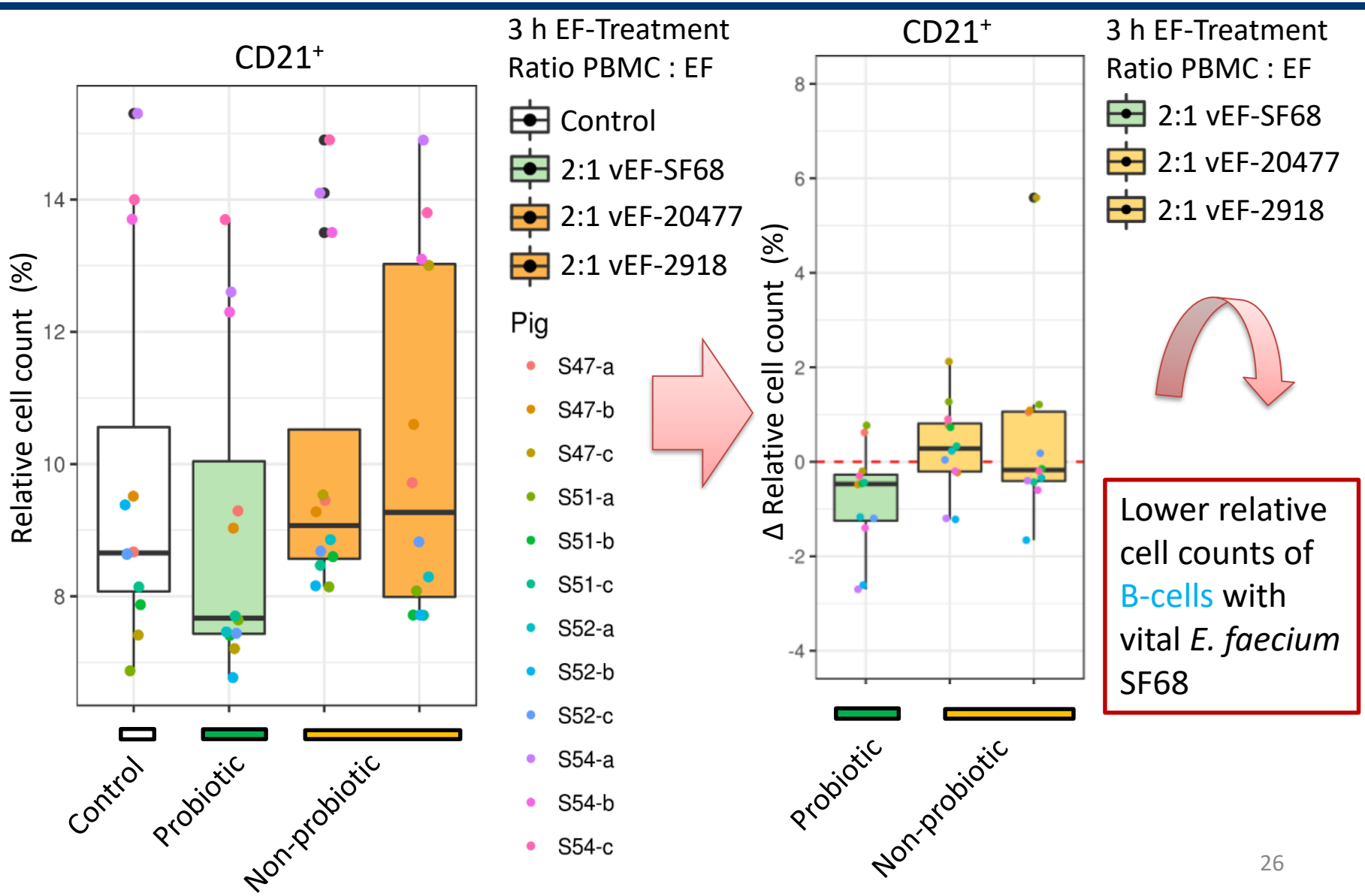
Higher relative cell counts of cytotoxic T-cells after 5 h incubation with *E. faecium*

In vitro effects of EF on **B-cells** in a PBMC composite



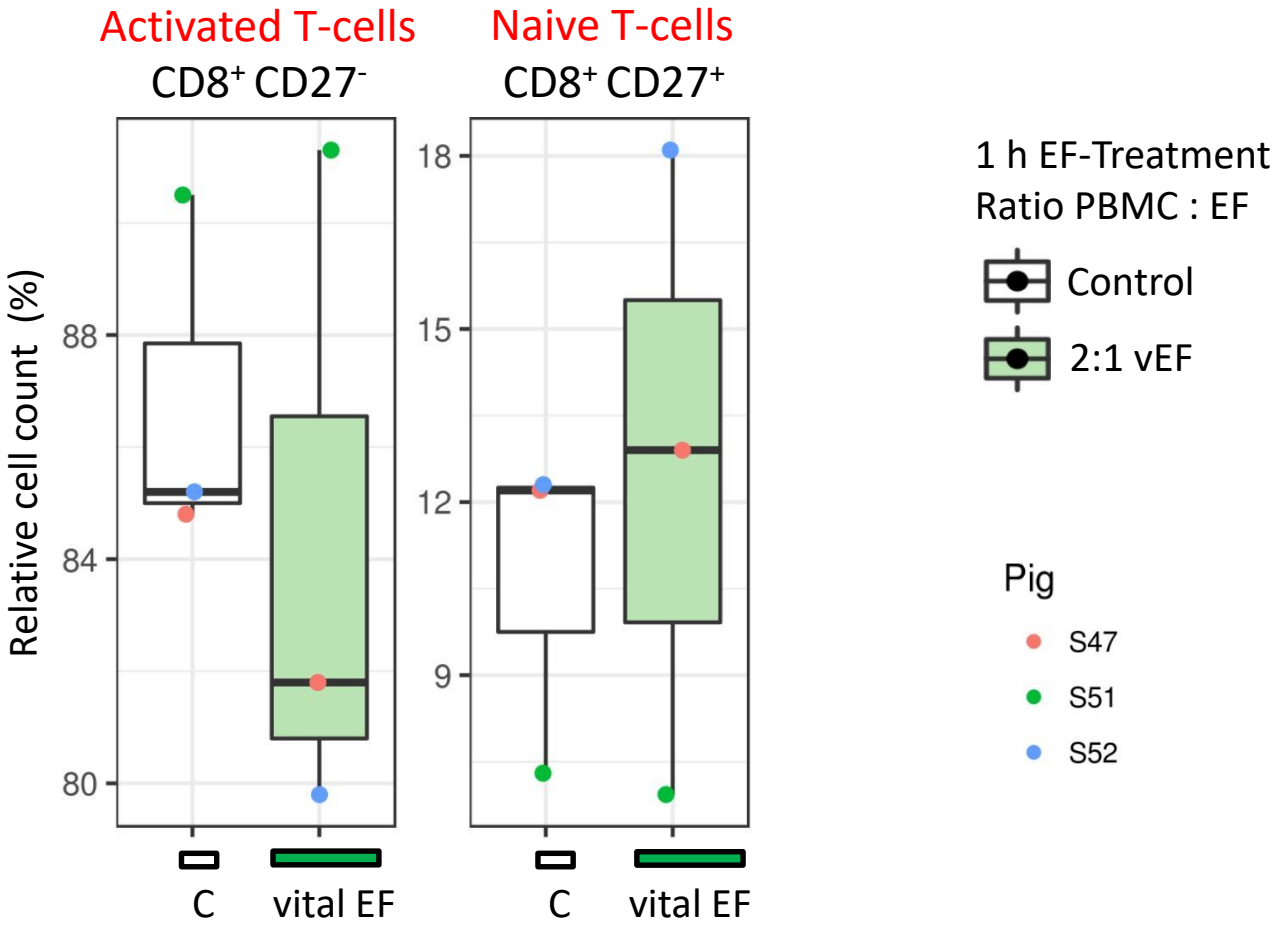
- Trend to lower relative cell counts of B-cells (CD21, CD79) with vital *E. faecium*
- Higher relative cell counts of B-cells (CD21, CD79) in killed *E. faecium*

In vitro effects of different EF strains on B-cells in a PBMC composite



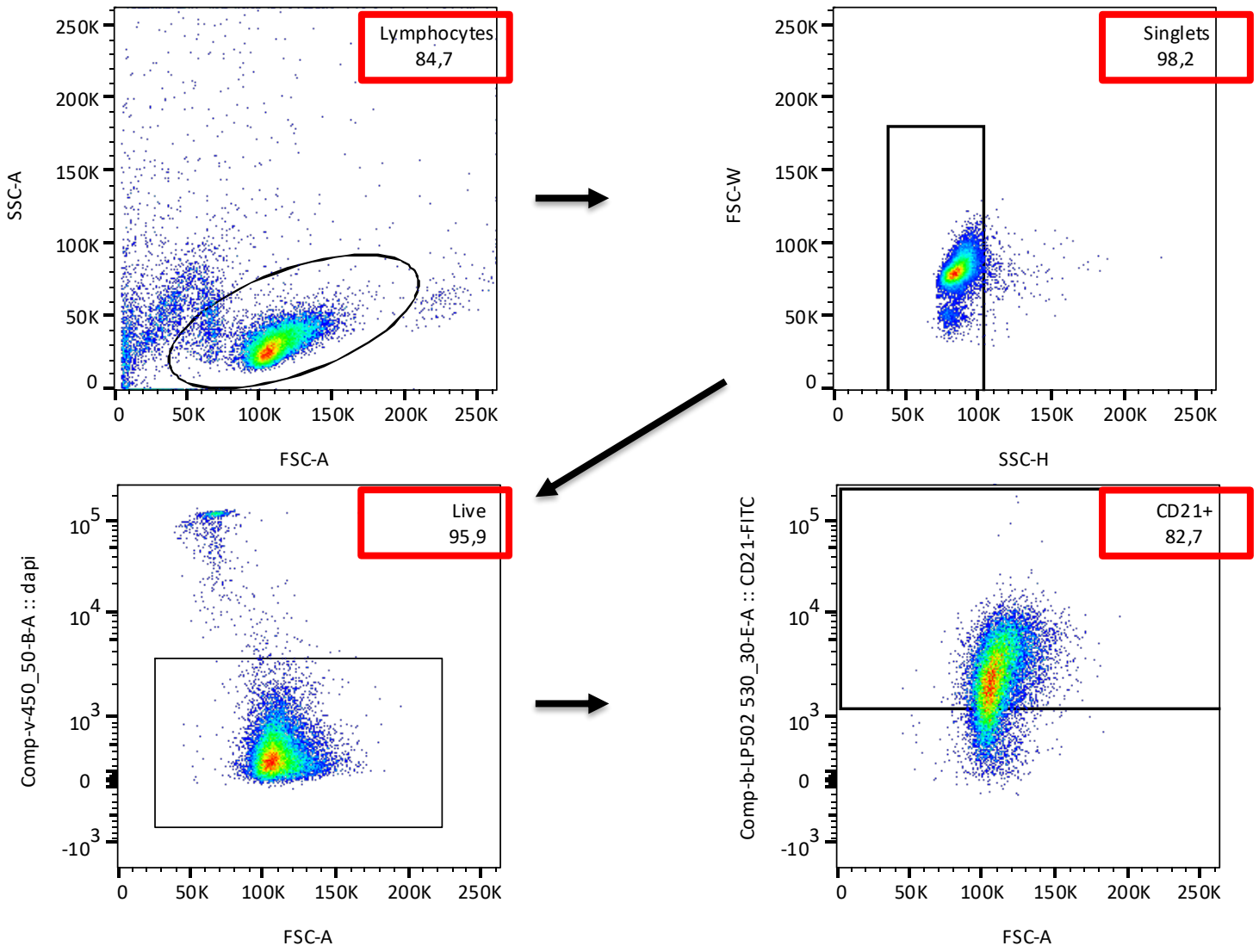
Lower relative cell counts of B-cells with vital *E. faecium* SF68

In vitro effects of EF on separated **cytotoxic T-cells**

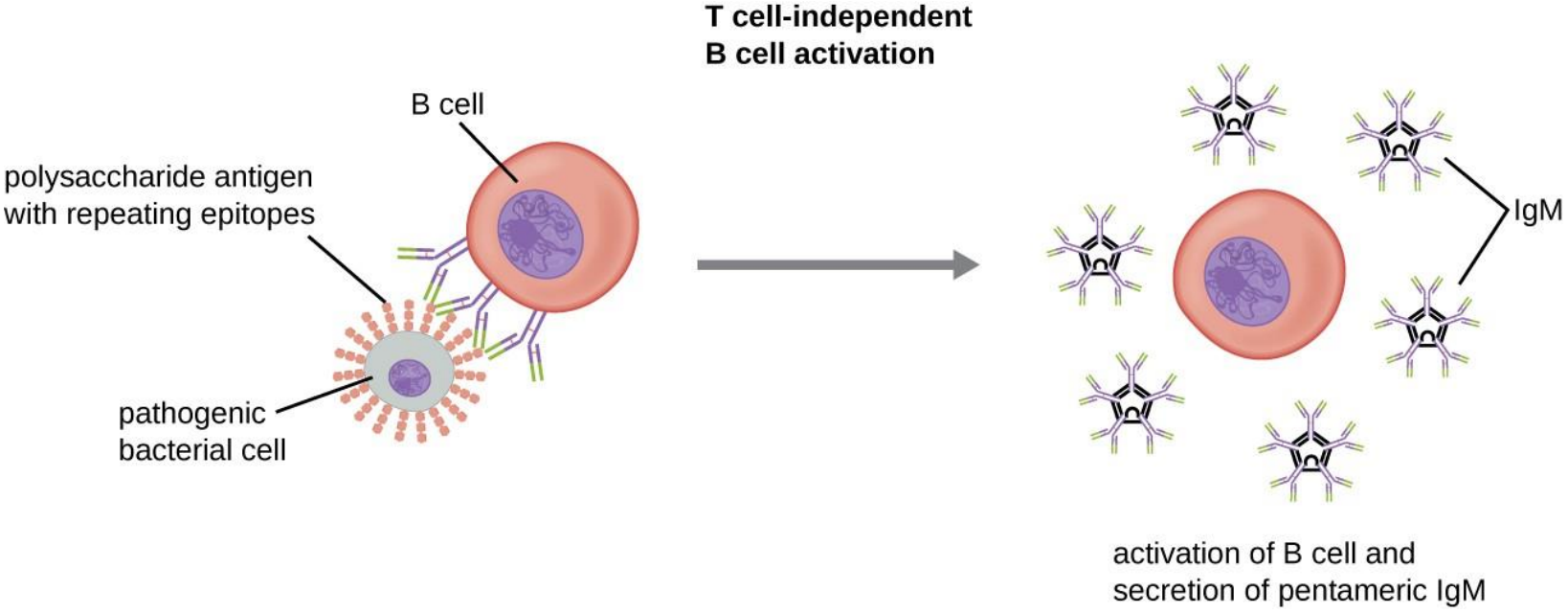


No clear effect on activated and naive cytotoxic T-cells after treatment with vital *E. faecium*

Flow cytometry – Gating strategy



T-cell-independent B-cell activation



https://s3-us-west-2.amazonaws.com/courses-images/wp-content/uploads/sites/1094/2016/11/03172714/OSC_Microbio_18_04_indact.jpg

TI-1 antigens:

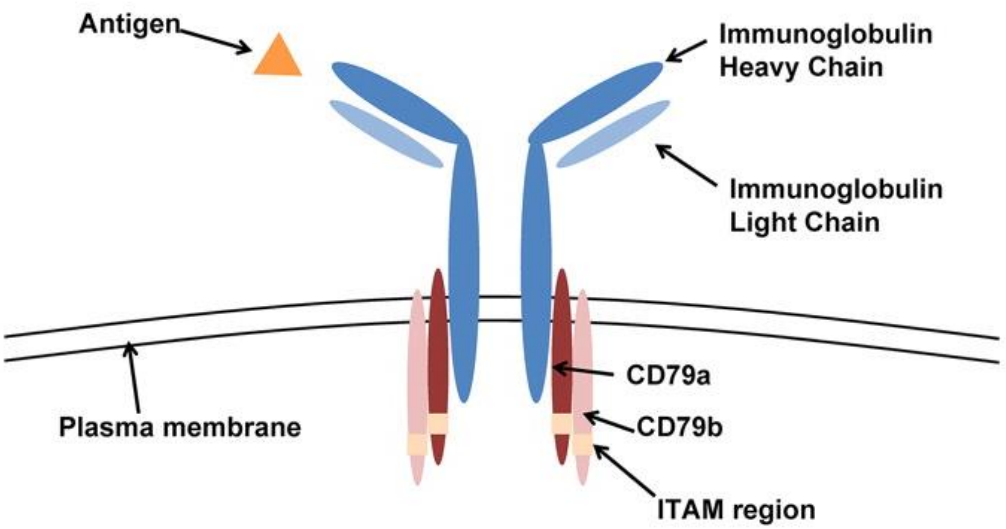
- directly cause proliferation and differentiation (TLR) (polyclonal when high concentration or antigen-specific)
- BCR-crosslink or other forms of costimulation (LPS – LPS-receptor)
- B-cell mitogens
- Examples: LPS, bacterial DNA

TI-2 antigens:

- Highly repetitive surface structures
- Cross-linking of BCRs leading to cross-activation
- Need residual T-cell, DC or MP help for activation (costimulatory signals)

Introduction - CD79

- Encompasses two transmembrane proteins
- Parts of BCR
  - Signal transduction
- First components of BCR expressed developmentally



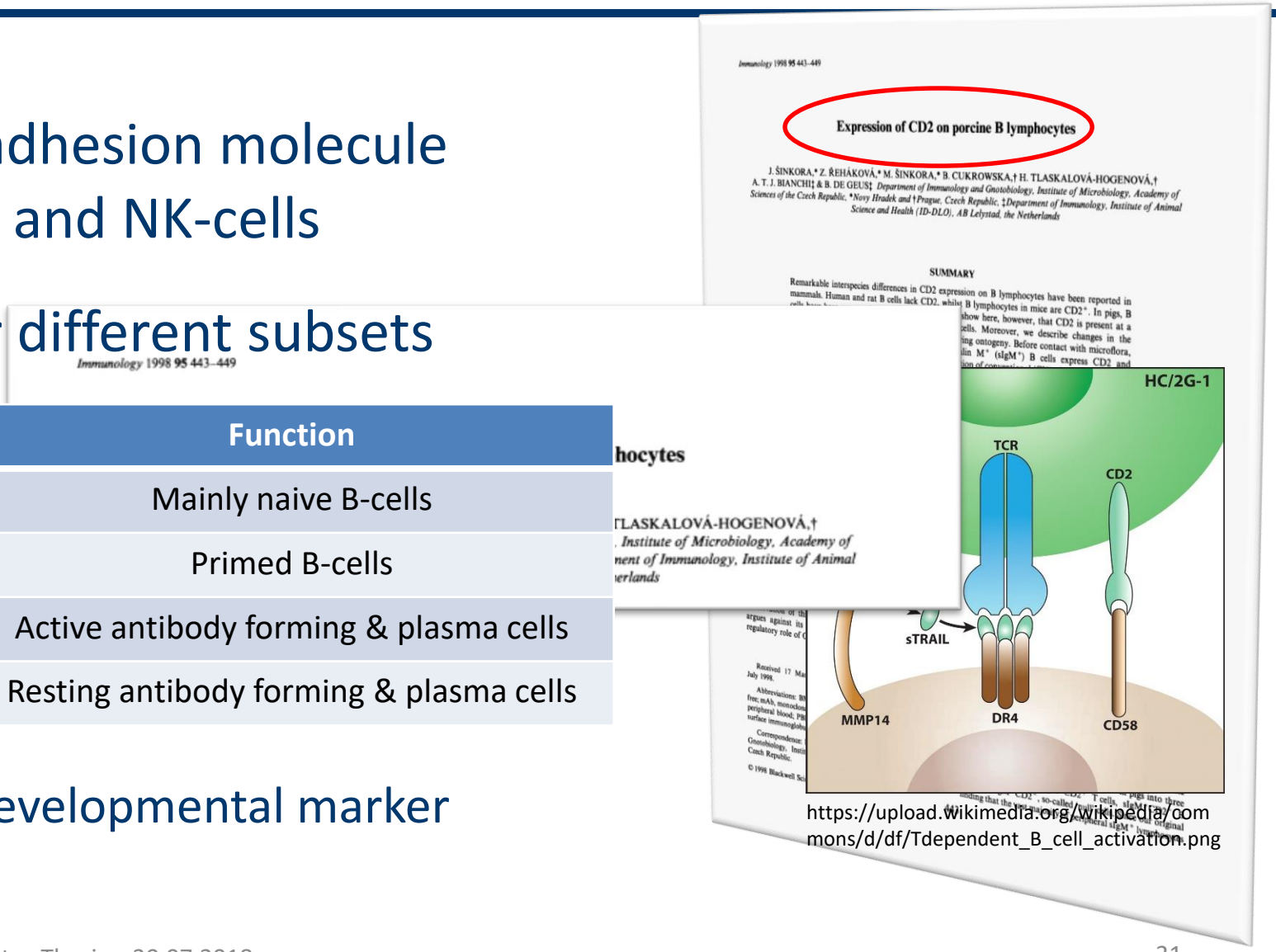
Woyach, J. A. *et al.* (2012)

Introduction - CD2

- Cell adhesion molecule on T- and NK-cells
- Four different subsets

Subsets	Function
$CD2^+CD21^+$	Mainly naive B-cells
$CD2^-CD21^+$	Primed B-cells
$CD2^+CD21^-$	Active antibody forming & plasma cells
$CD2^-CD21^-$	Resting antibody forming & plasma cells

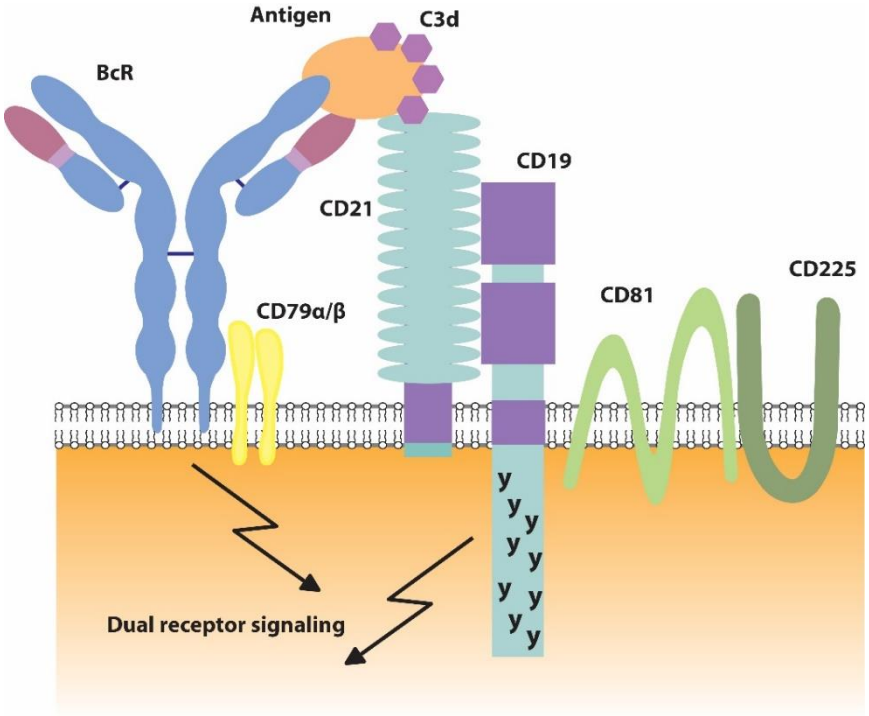
  - Developmental marker

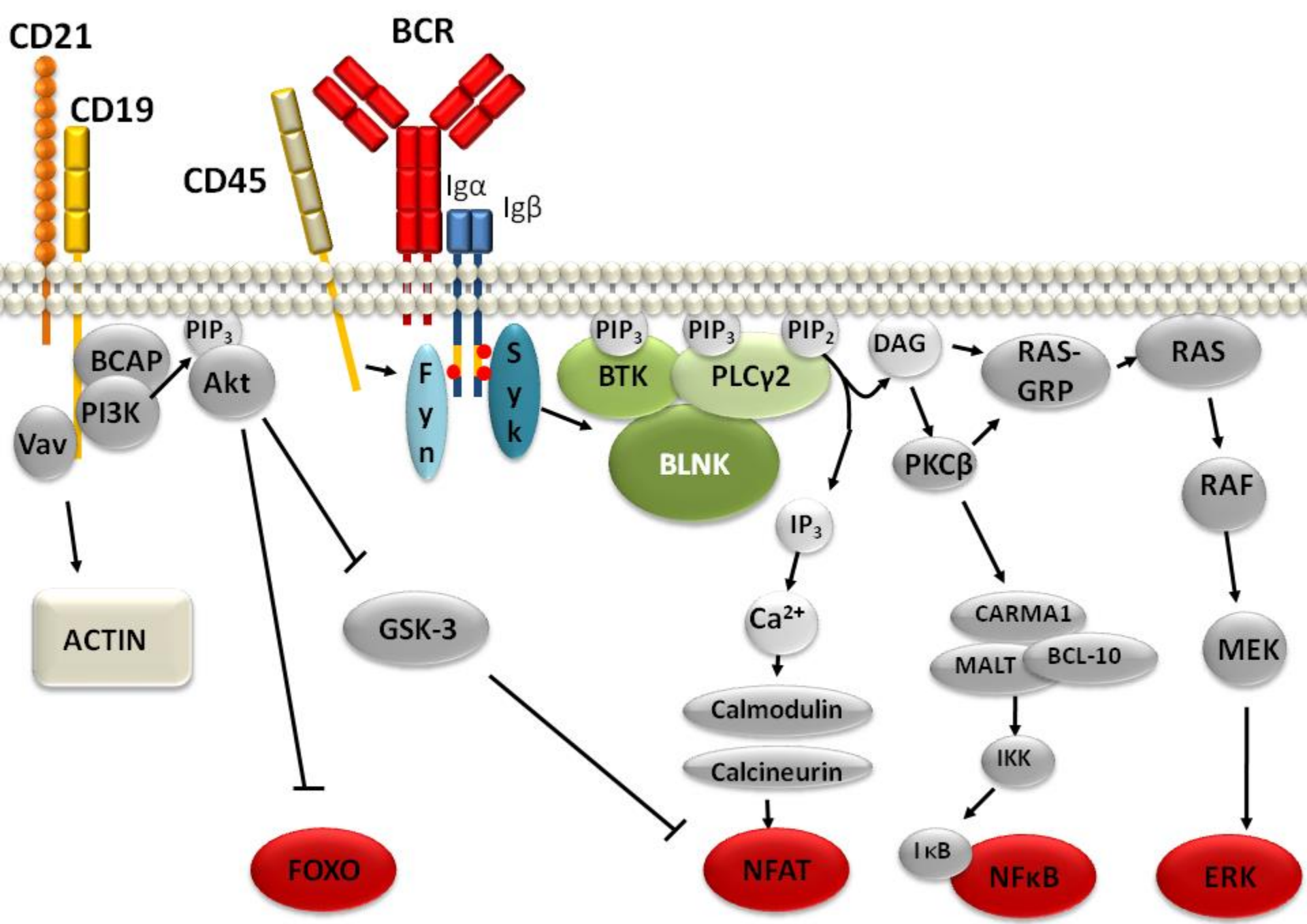


https://upload.wikimedia.org/wikipedia/commons/d/df/Tdependent_B_cell_activation.png

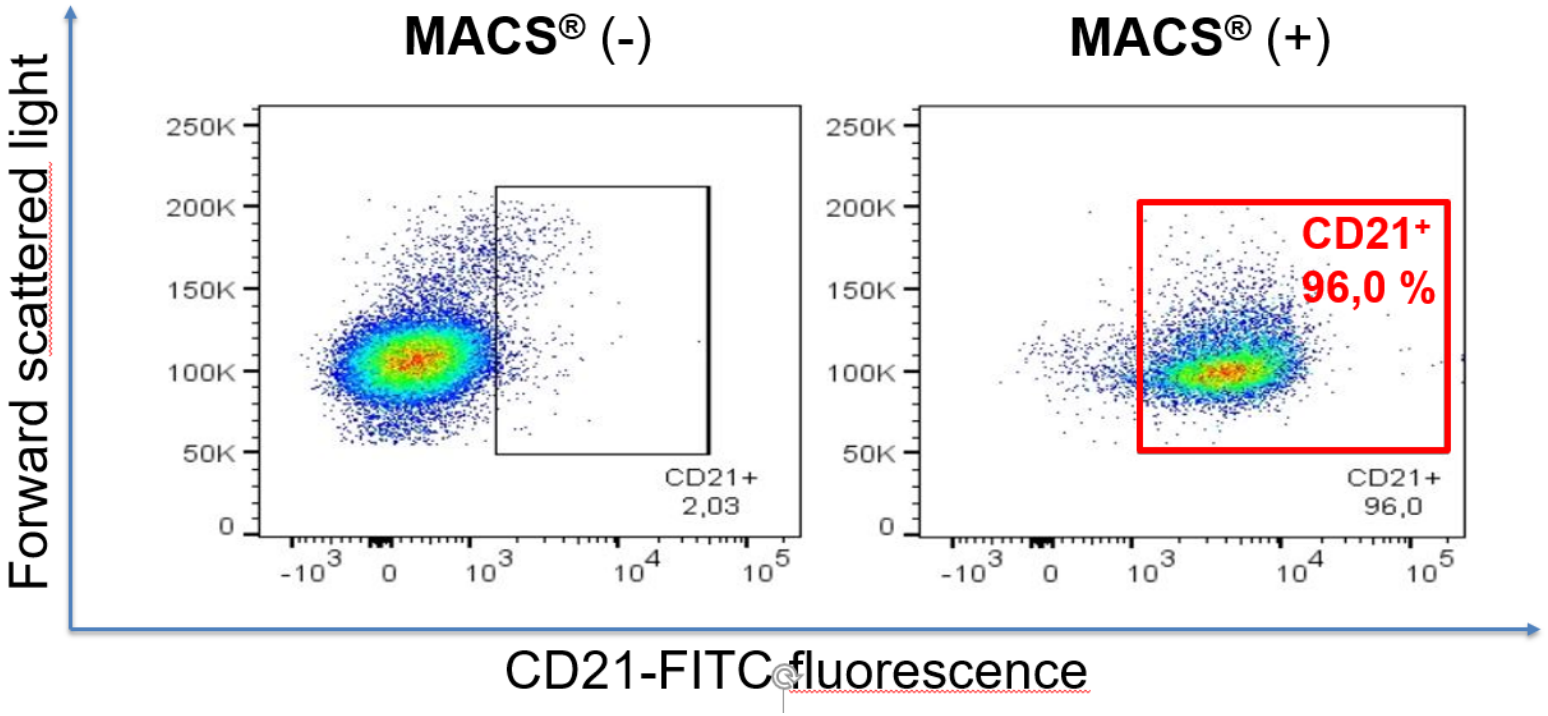
Introduction - CD21

- CD21:
  - Complement receptor type 2 (CR2)
  - All mature B lymphocytes
  - Allows B-cell activation by complement
  - Maturation marker
  - Two differential forms




CD21
CD19
BCR
CD45
Igα
Igβ
BCAP
PI3K
Vav
PIP3
Akt
Fyn
Syk
PIP3
BTK
PIP3
PLCγ2
PIP2
BLNK
DAG
RAS-GRP
RAS
PKCβ
RAF
IP3
Ca2+
CARMA1
MALT
BCL-10
MEK
ACTIN
GSK-3
Calmodulin
Calcineurin
IKK
IκB
NFκB
FOXO
NFAT
ERK

Results - MACS-efficacy



Good B-cell sort-efficacy!