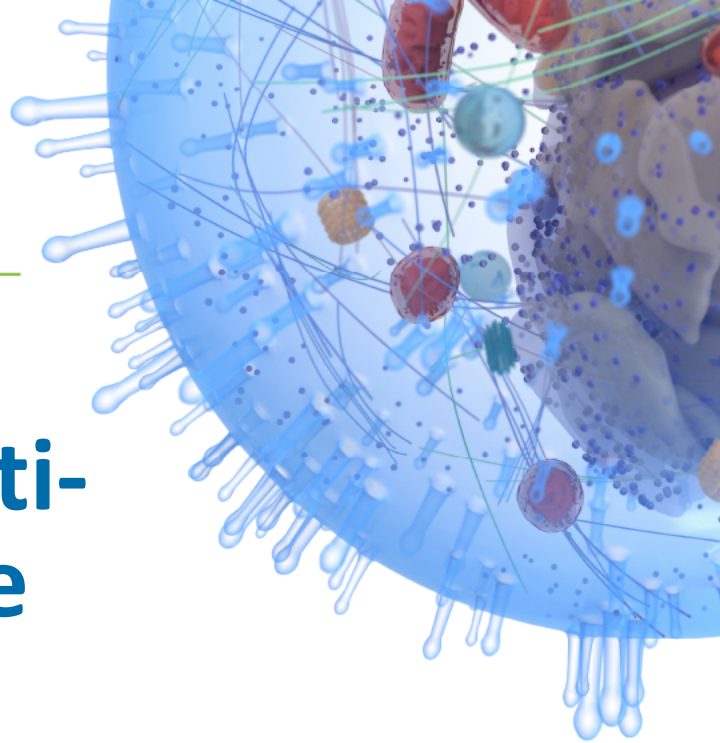
ICoMI VIRTUAL
2nd International Congress of
Micro-immunotherapy
June 2-4, 2022

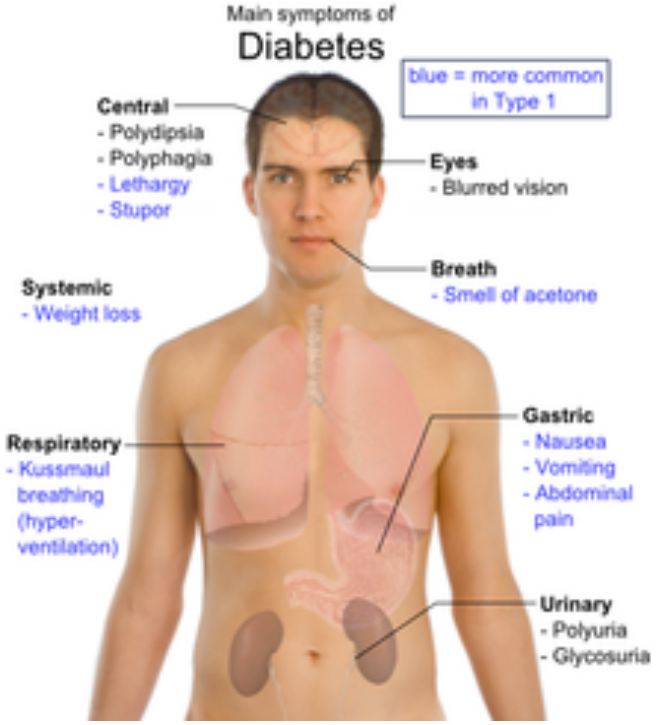


# The effect of low-dose IL-22 & IL-2/anti-IL-2 complex in the prevention of Type 1 diabetes

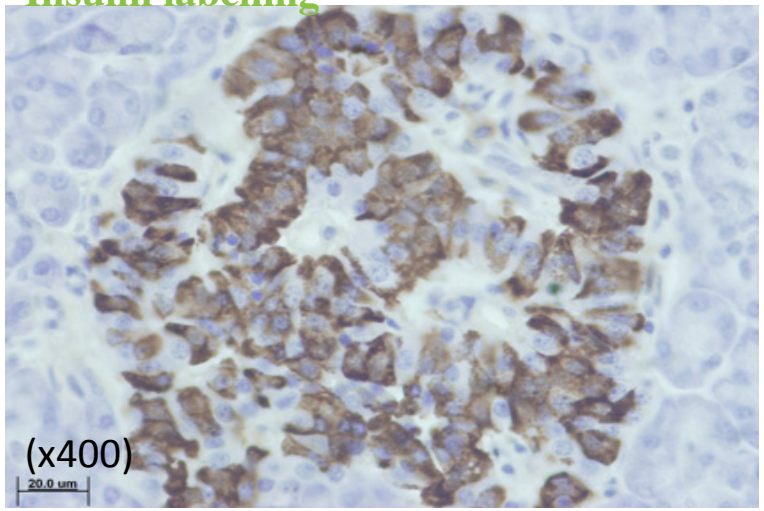
Yu Anne Yap, Sara Bordbar, Chun Wie Wong, Eliana Marino and Pascal Mensah

# What is Type 1 diabetes?

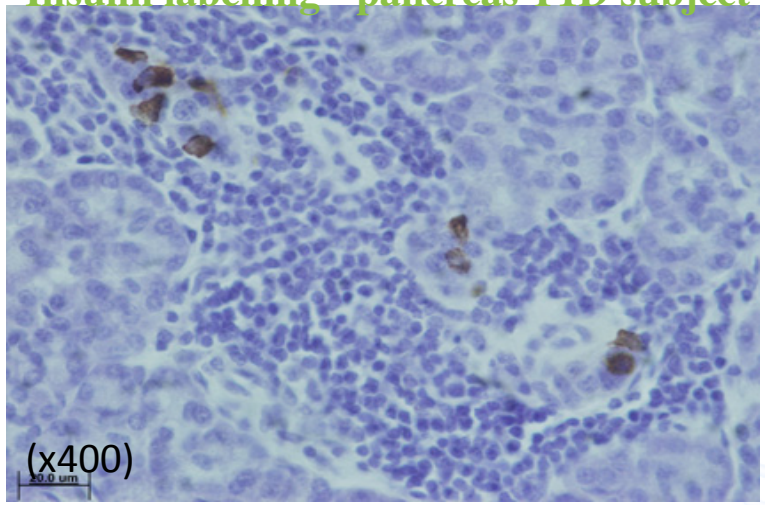
- Blood sugar regulation lost
- Loss of insulin production and Hyperglyceamia
- Insulin producing beta cells destroyed
- Immune attack - *autoimmune disease*
- Controlled with exogenous insulin
- Diabetic complications are a major issue




Insulin labelling




Insulin labelling - pancreas T1D subject






Images: thnx to Pete Campbell & Tom Kay SVI Melbourne

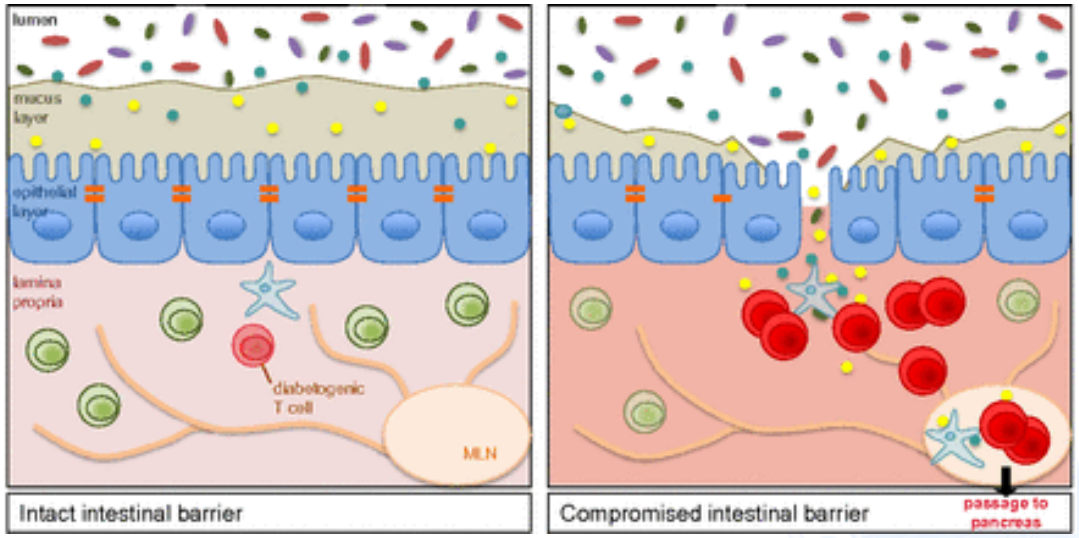
# Autoimmune diabetes and the “leaky” gut

Some environmental risk factors for T1D act at the intestinal level:

- Enteric infections (enterovirus, rotavirus)
- Dietary antigens (cow’s milk, gluten)
- Modification of gut microbiota (diet composition, antibiotics)

Development of clinical diabetes and preclinical models of T1D often preceded by:

- Increased intestinal permeability
- Enteropathy
- Lymphocyte infiltration
- Presence of inflammatory cytokines in intestinal mucosa

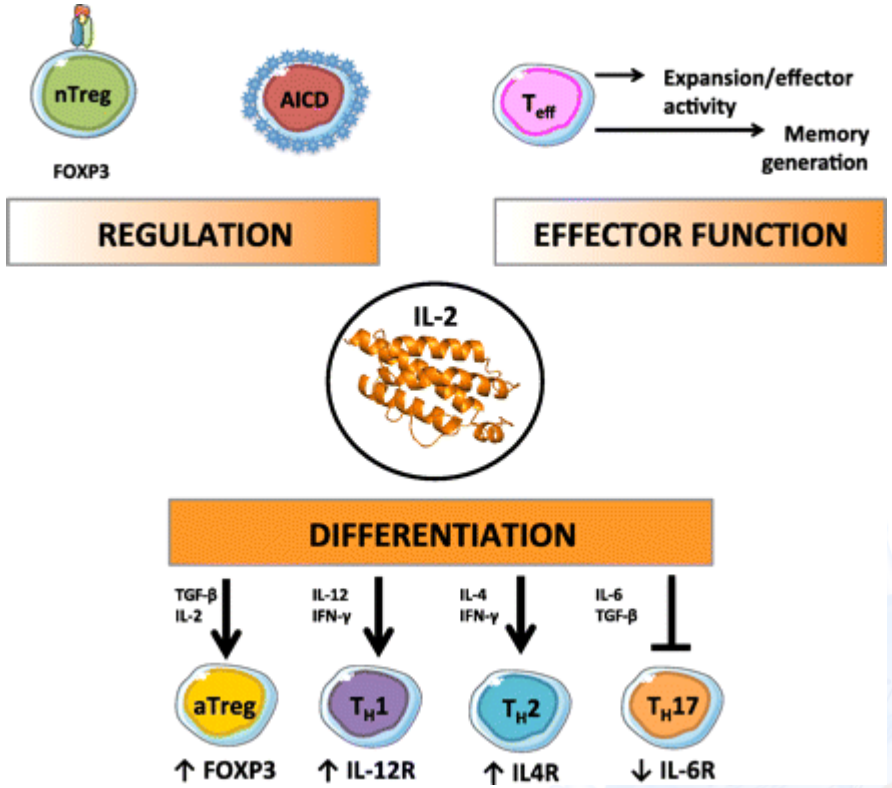



Peters & Wekerle, *PNAS*, 2019

# The beneficial effect of combination immunotherapy using IL2 and IL22 to induce immunoregulation from the gut.

## Interleukin-2

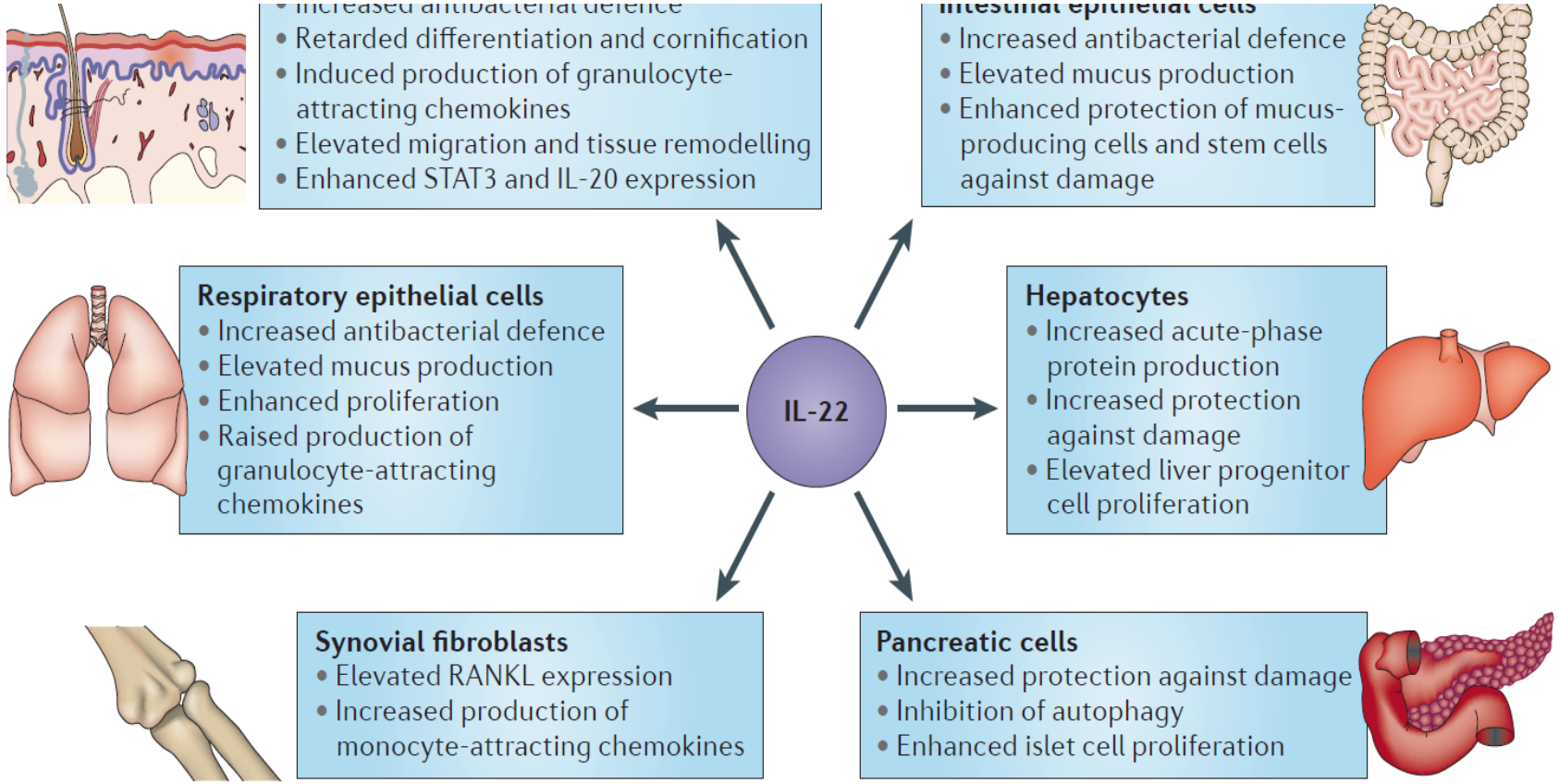
- An essential molecule for immune homeostasis to sustain self-tolerance and prevent autoimmunity
  - CD4+ Foxp3+ Tregs expansion and function
- IL-2 pathway is a genetically validated key pathway with potential therapeutic applications in T1D.
- Binds to Tregs with greater sensitivity due to higher expression of the IL-2 receptor.
- Clinical trials using low or ultra-low dose of Aldesleukin (Proleukin®) induces Tregs in adults and children in a dose-dependent manner without adverse events.
  - DILT1D; DILfrequency (Waldron-Lynch *et al*, BMJ Open 2014; Seelig *et al*, JCI Insight 2018)
  - ITAD (*Currently recruiting*)
  - PROREG (*Currently suspended due to Covid-19*)




Hulme *et al*, Diabetes 2012

# Interleukin-22

- Part of the IL-10 family cytokines
- Predominantly secreted by T cell subsets and ILC3s
- Main effects on tissue epithelial and stroma cells to prevent cell death and tissue damage (Feng *et al* 2012)
- Induce host defense mechanisms against pathogens (Zheng *et al* 2008)
- Facilitates wound healing in diabetic mice (Avitabile *et al* 2015; Kolumam *et al* 2017)
- Promote barrier integrity and tissue homeostasis




Sabat, Ouyang & Wolk, Nat Rev Drug Disc 2014

# Hypothesis

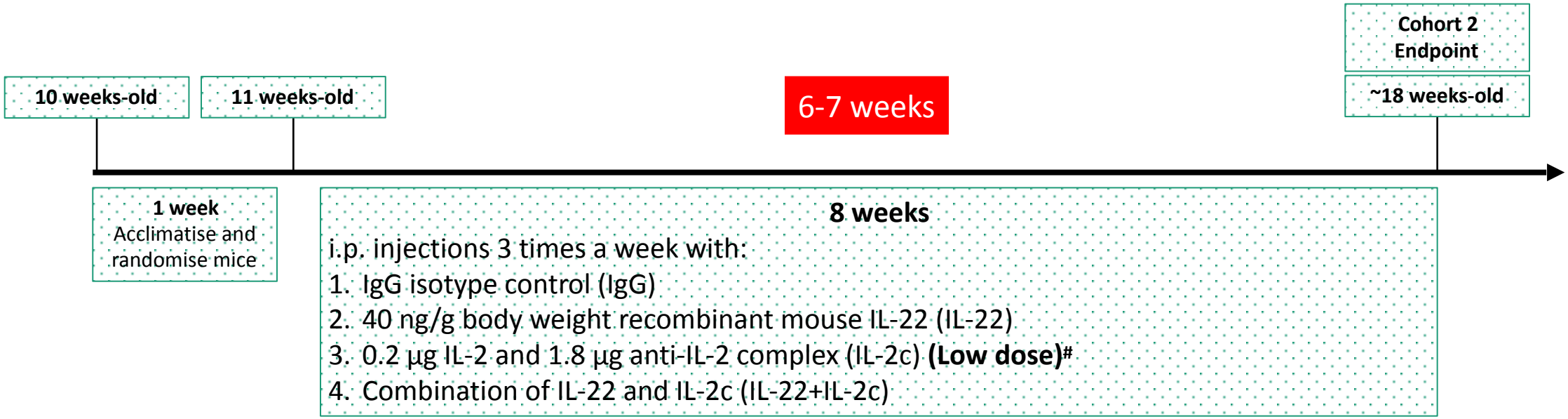
## IF

1. IL-2 promotes self-tolerance to prevent autoimmunity by promoting cell survival, expansion and function of regulatory Tregs (immune regulation).
2. IL-22 reduces stress and inflammation at epithelial sites (barrier integrity & homeostasis).

## THEN

Combination of IL-2 and IL-22 at low doses maybe a potentially safe and effective therapeutic approach for the prevention of T1D

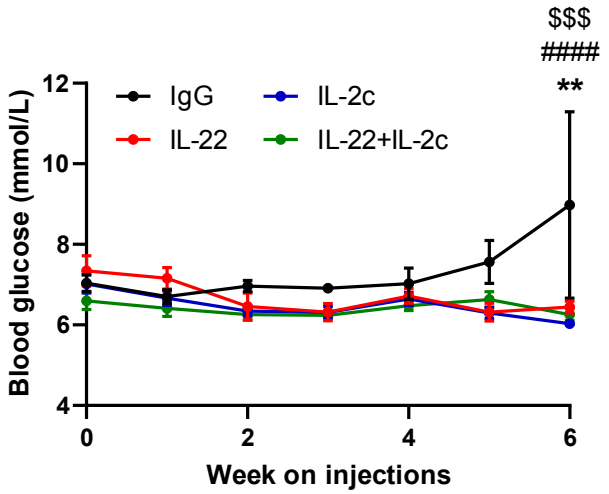
# Experimental design (♀ NOD Mice)




What kind of IgG and why IgG??

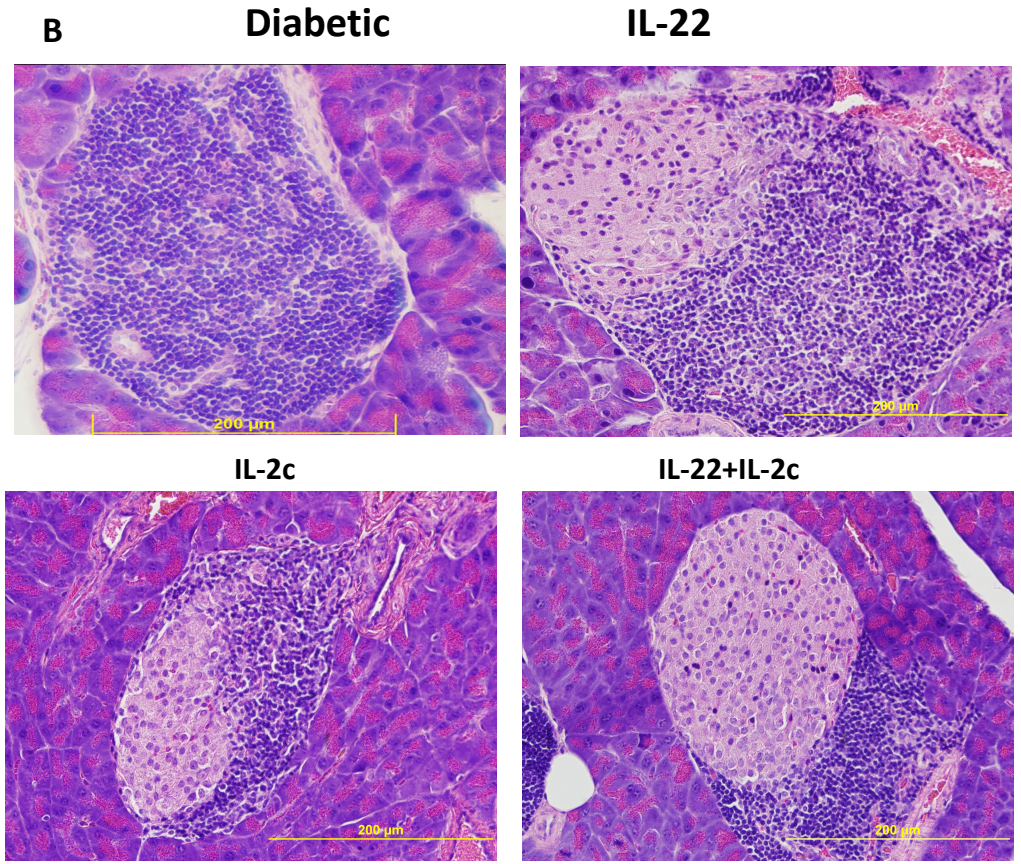
# Combination with low-dose IL2/IL22 preserve insulin producing beta cells and halts the mononuclear infiltration in pancreatic islets

A

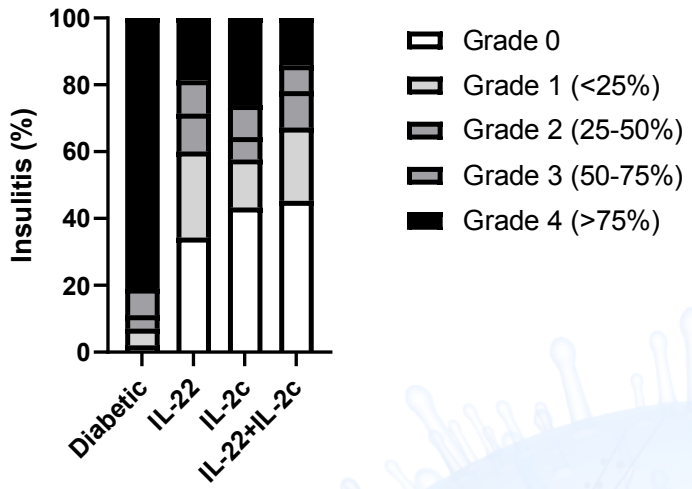



All treatments resulted in better control of blood glucose compared to IgG control in NOD mice.

B

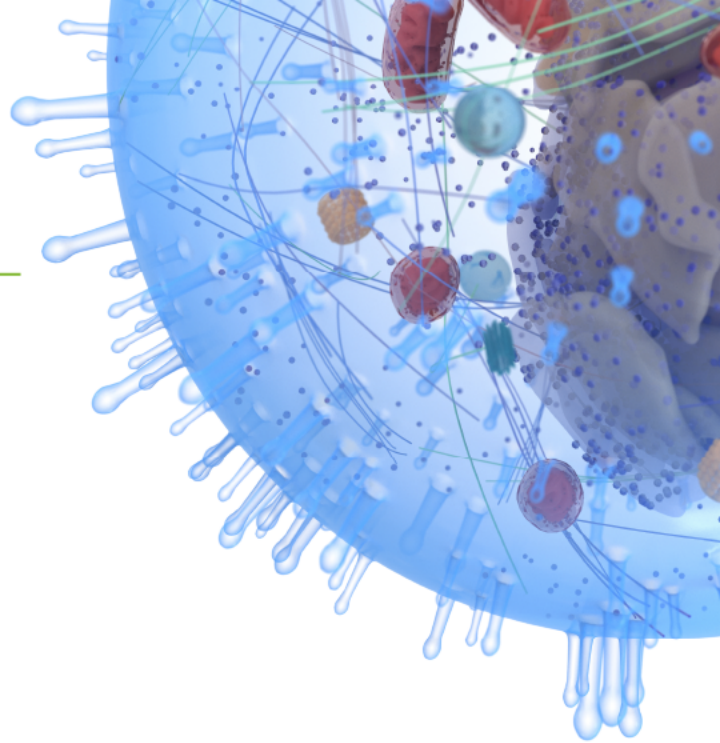



C



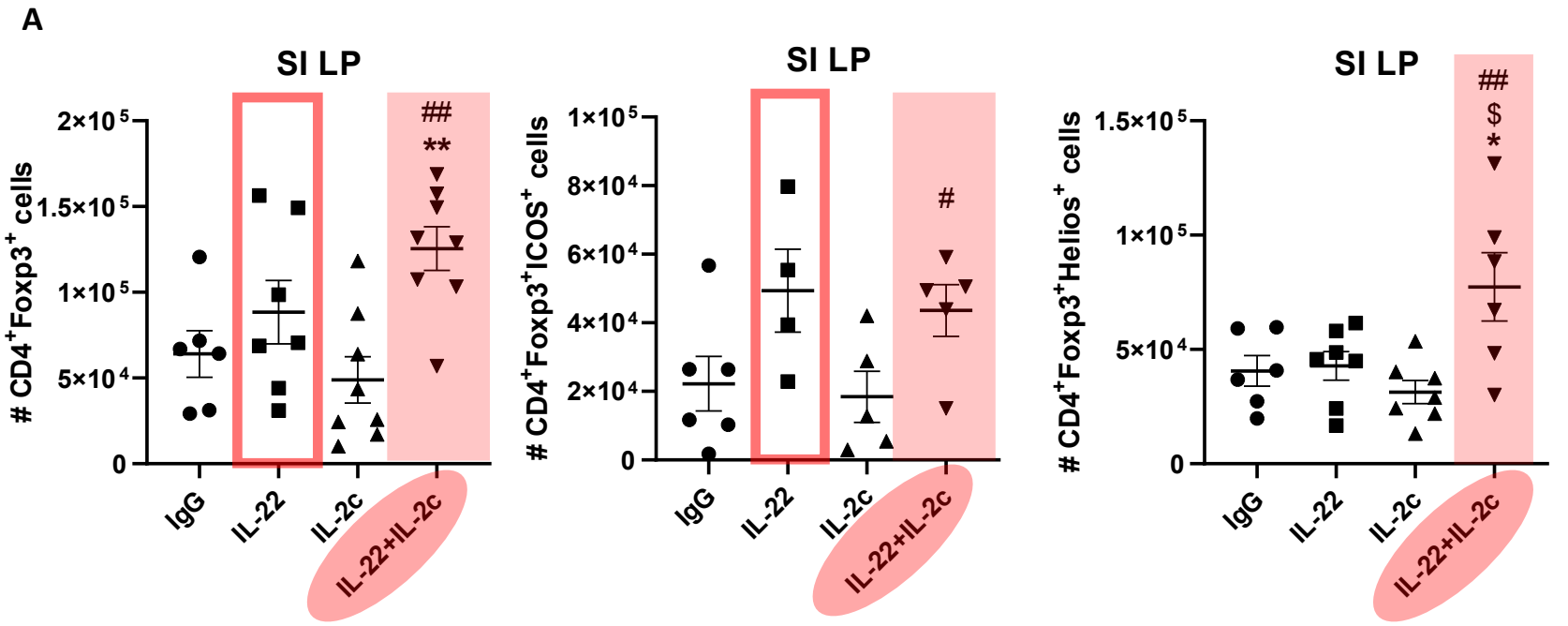

Stats: mean ± SEM; Two-way ANOVA with Bonferroni's correction

*n* = 7-11 per group  *vs IL-22; #vs IL-2c; $vs IL-22+IL-2c

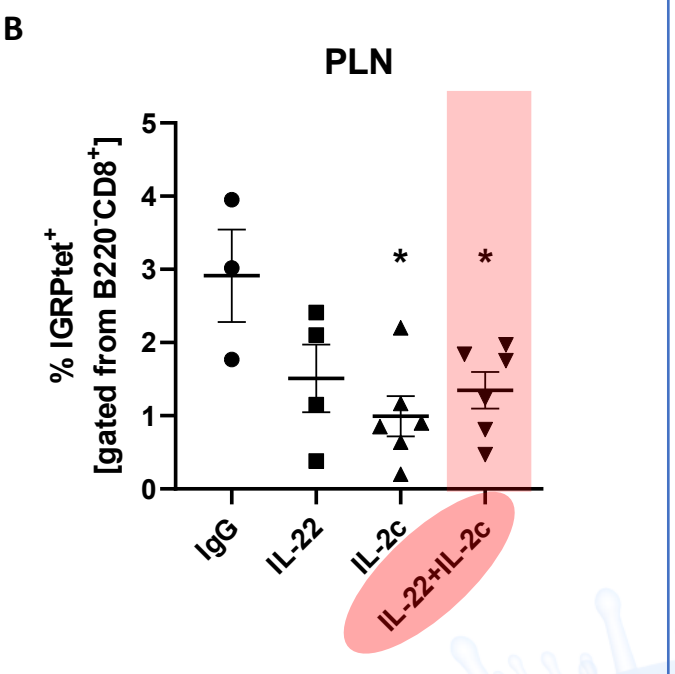


# Immunological data

# IL2/IL22 induce immunoregulatory changes in Small Intestine (SI) and in the Pancreatic Lymph Nodes (PLN)




IL-22+IL-2c significantly increased Foxp3+ Tregs



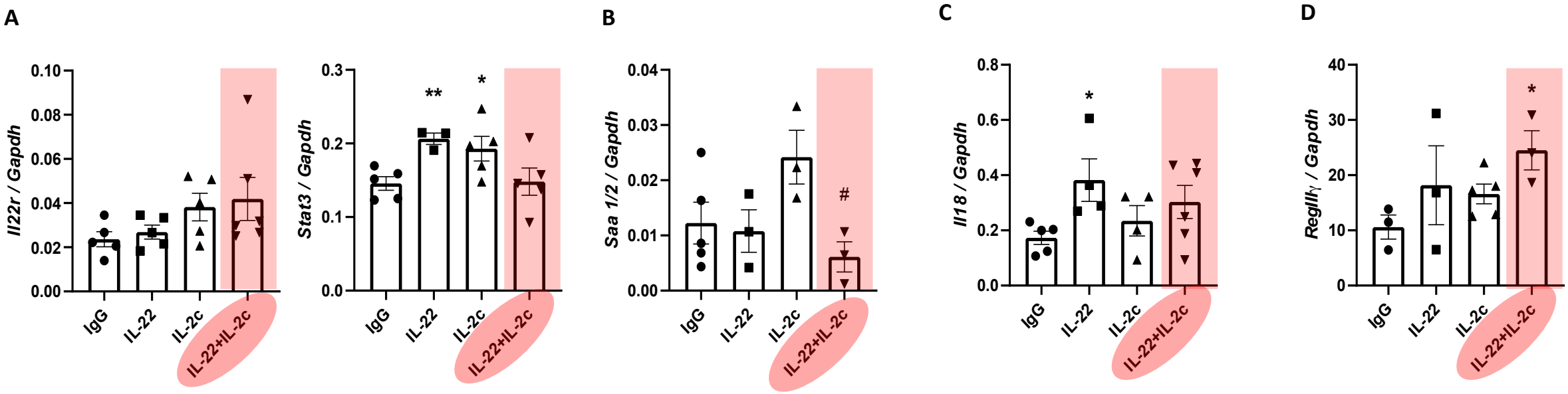

IL-22+IL-2c significantly reduced frequency of autoreactive IGRP+CD8+ T cells

Stats: mean ± SEM; Unpaired student's *t* test

*vs IgG; $IL-22 vs IL-22+IL-2c; #IL-2c vs IL-22+IL-2c

Numbers in FACS plots indicate means for each group

# IL-22+IL-2c induce gut homeostasis in Intestinal Epithelial Cells (IECs)




**SAA 1/2** are stress response genes and **RegIIIγ** is an antimicrobial peptide.

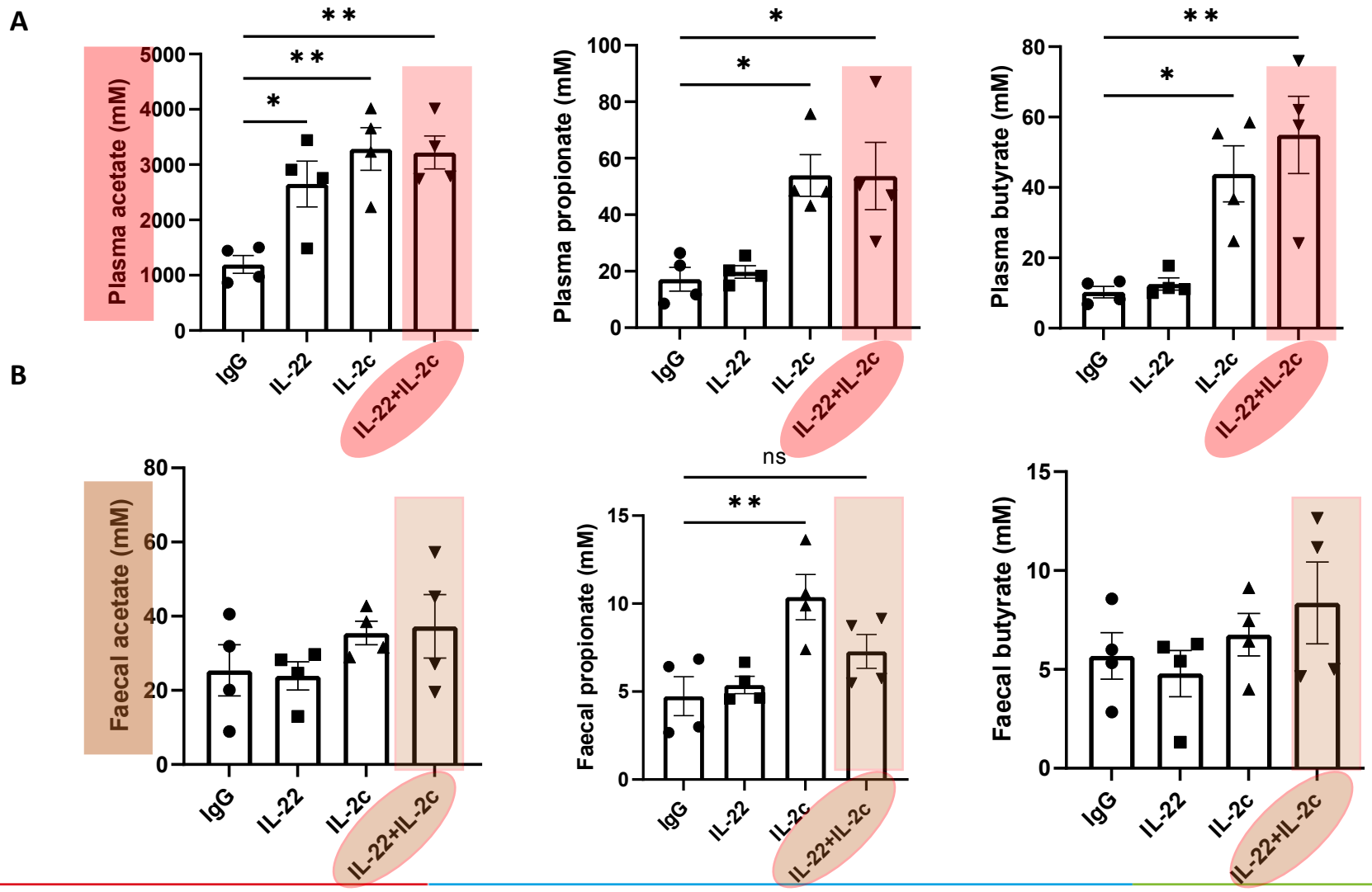
Endotoxemia upregulates mRNA expression and stimulates SAA production in the mucosa of SI (De Beer *et al* 1994; Wang *et al* 1998).

IL-22+IL-2c treatment reduced stress and induced antimicrobial peptides in the mucosa = possibly less endotoxemia.

Stats: mean ± SEM; Unpaired student's *t* test

*vs IgG; $IL-22 vs IL-22+IL-2c; #IL-2c vs IL-22+IL-2c

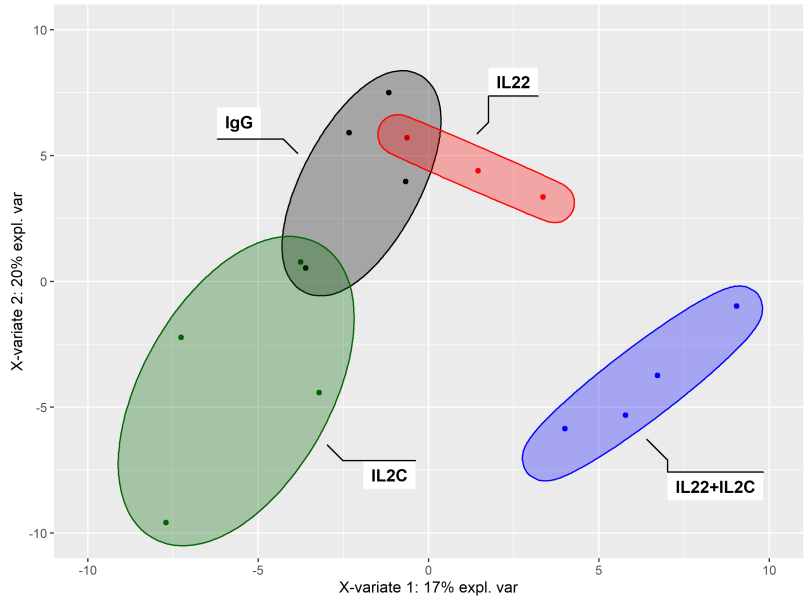
# Il2/IL22 Treatment increased plasma and gut production of anti-inflammatory microbial SCFAs




Stats: mean ± SEM; One-way ANOVA with Bonferroni's correction

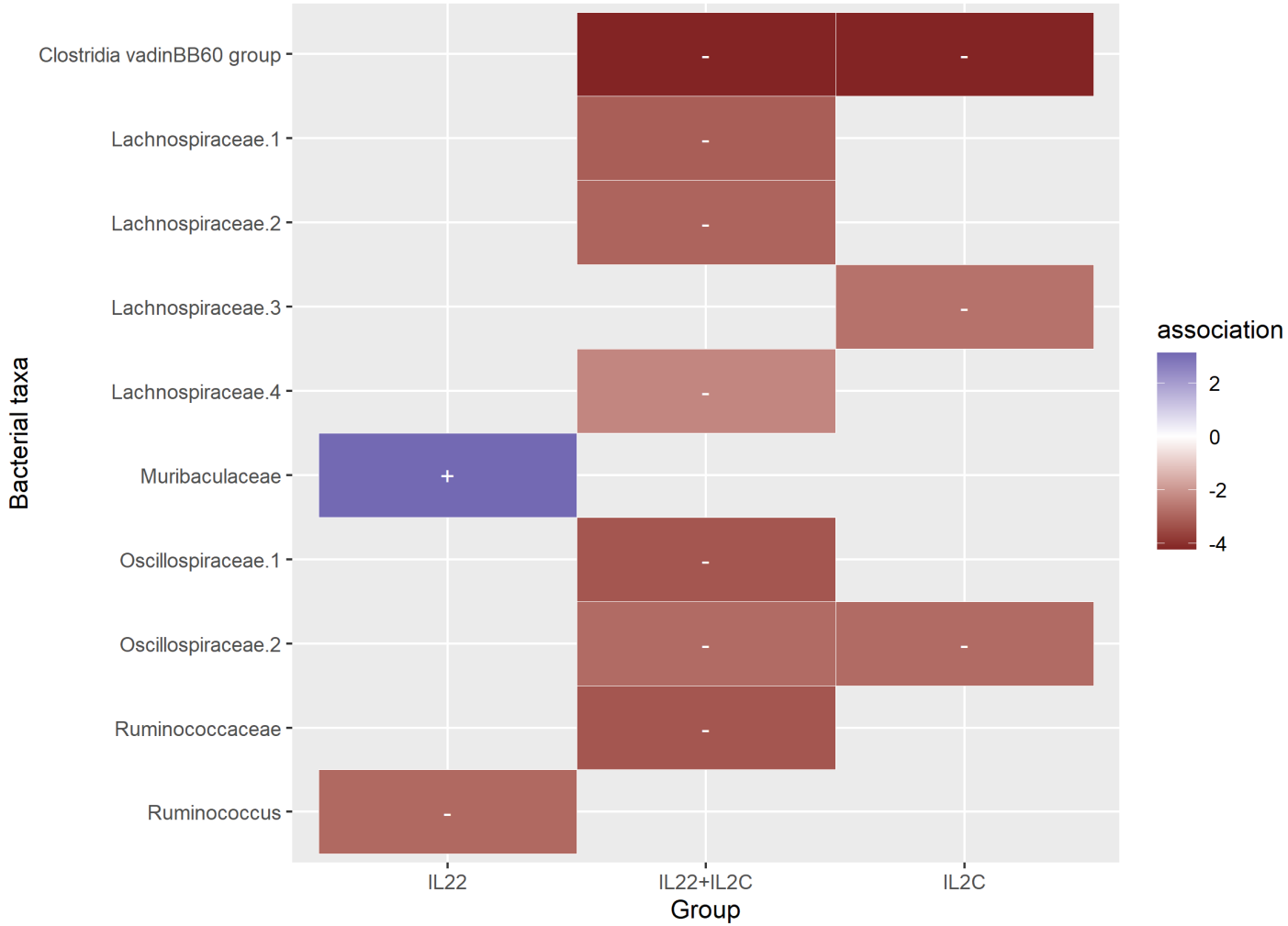
# Low-dose IL2/IL22 significantly change bacterial composition and function (pathways features)

A. Bacteria Taxa



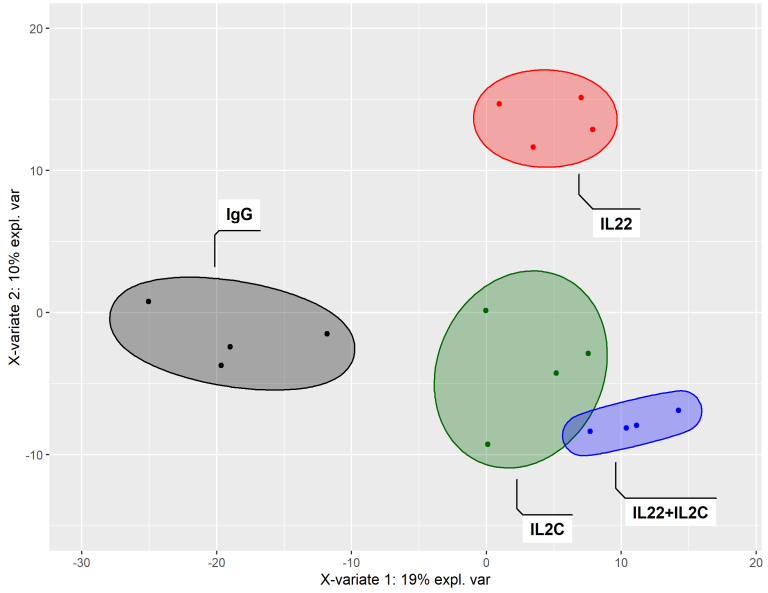

PLSDA

B. Significant associations(-log(qval)*sign(coeff)

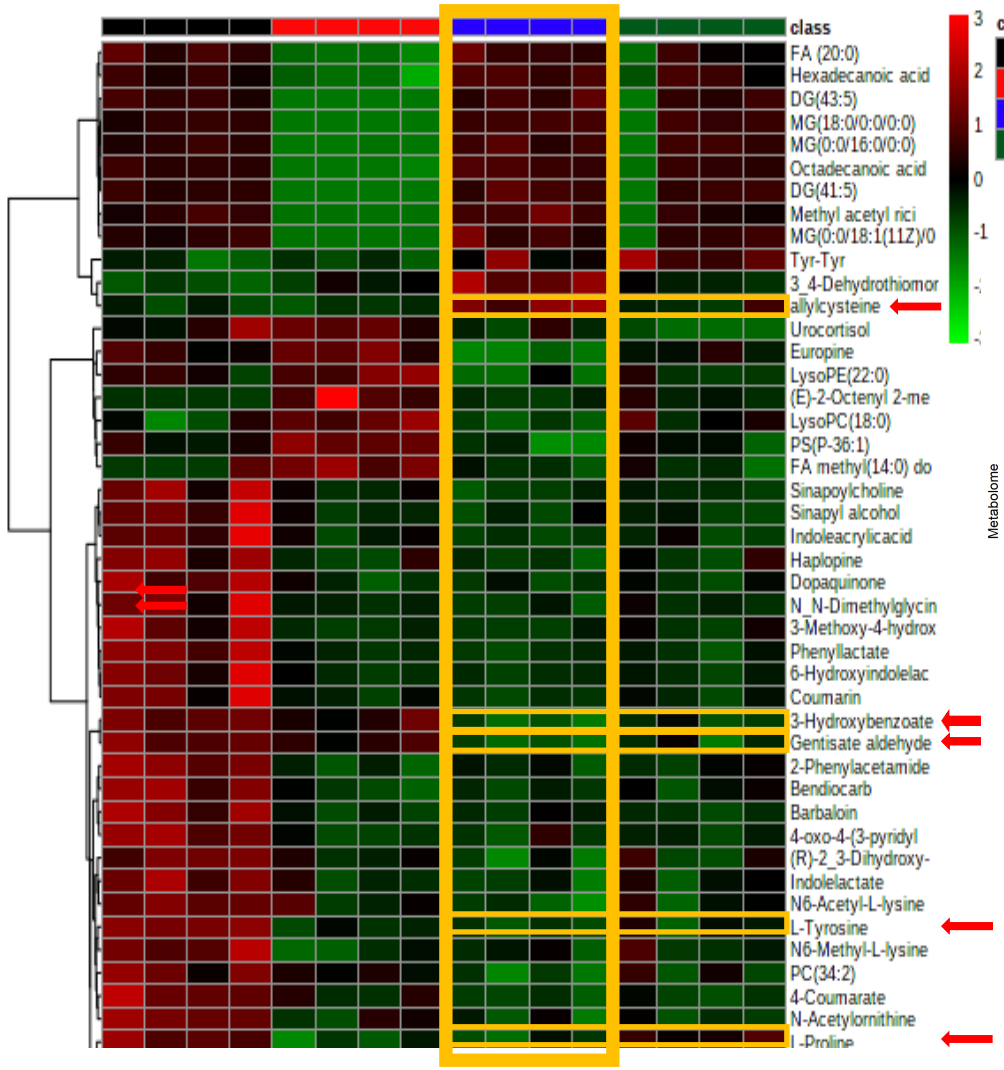


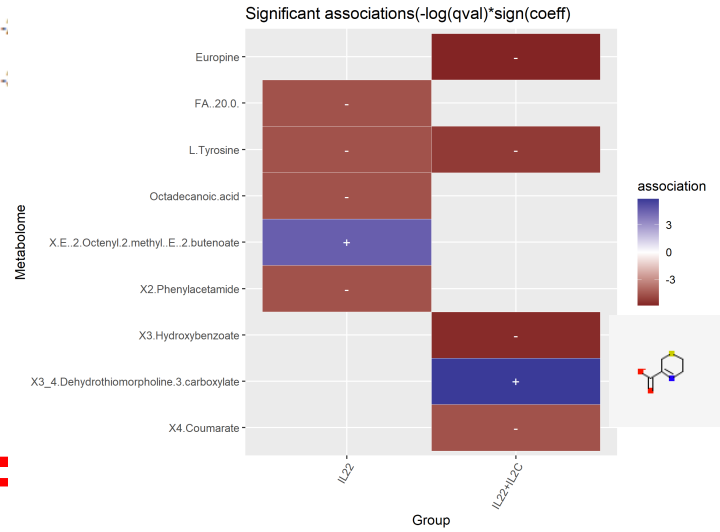
# Low-dose of IL2/IL22 significantly change feature metabolites

A

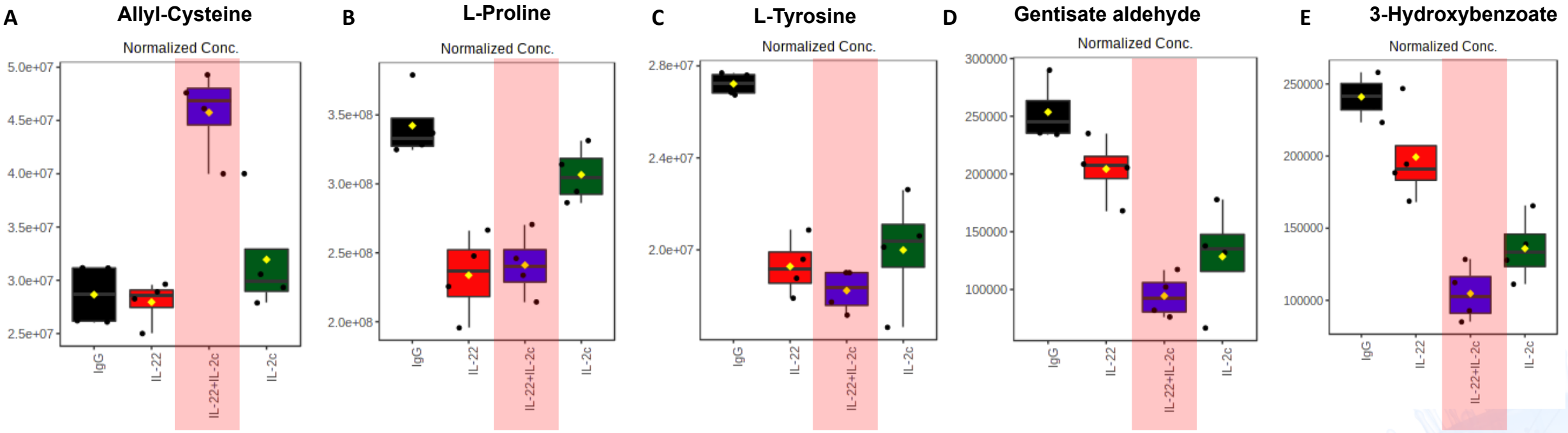



PLSDA plot inferred based on the metabolic profiles



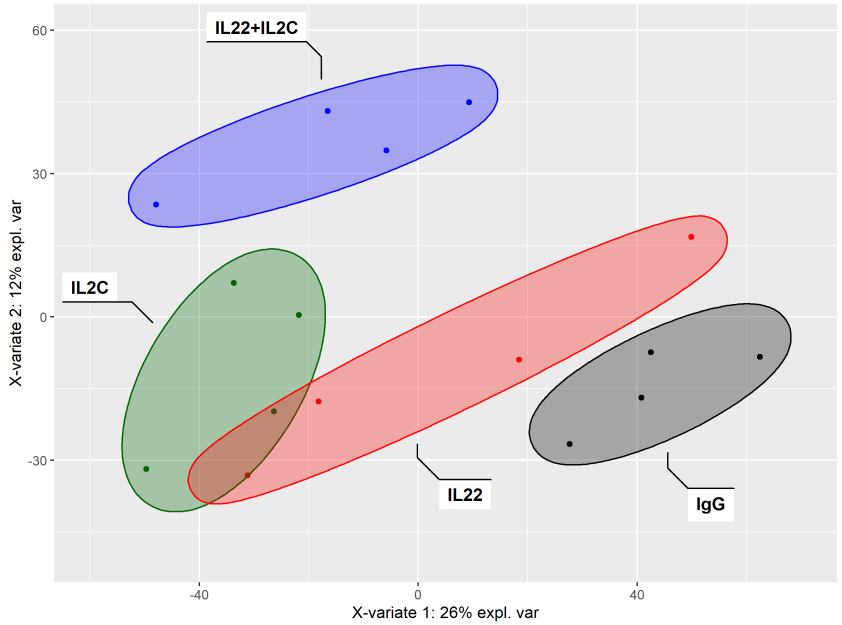



# Most significant metabolites and their impact in health



# Low-dose of IL2/IL22 significantly change feature proteins

A

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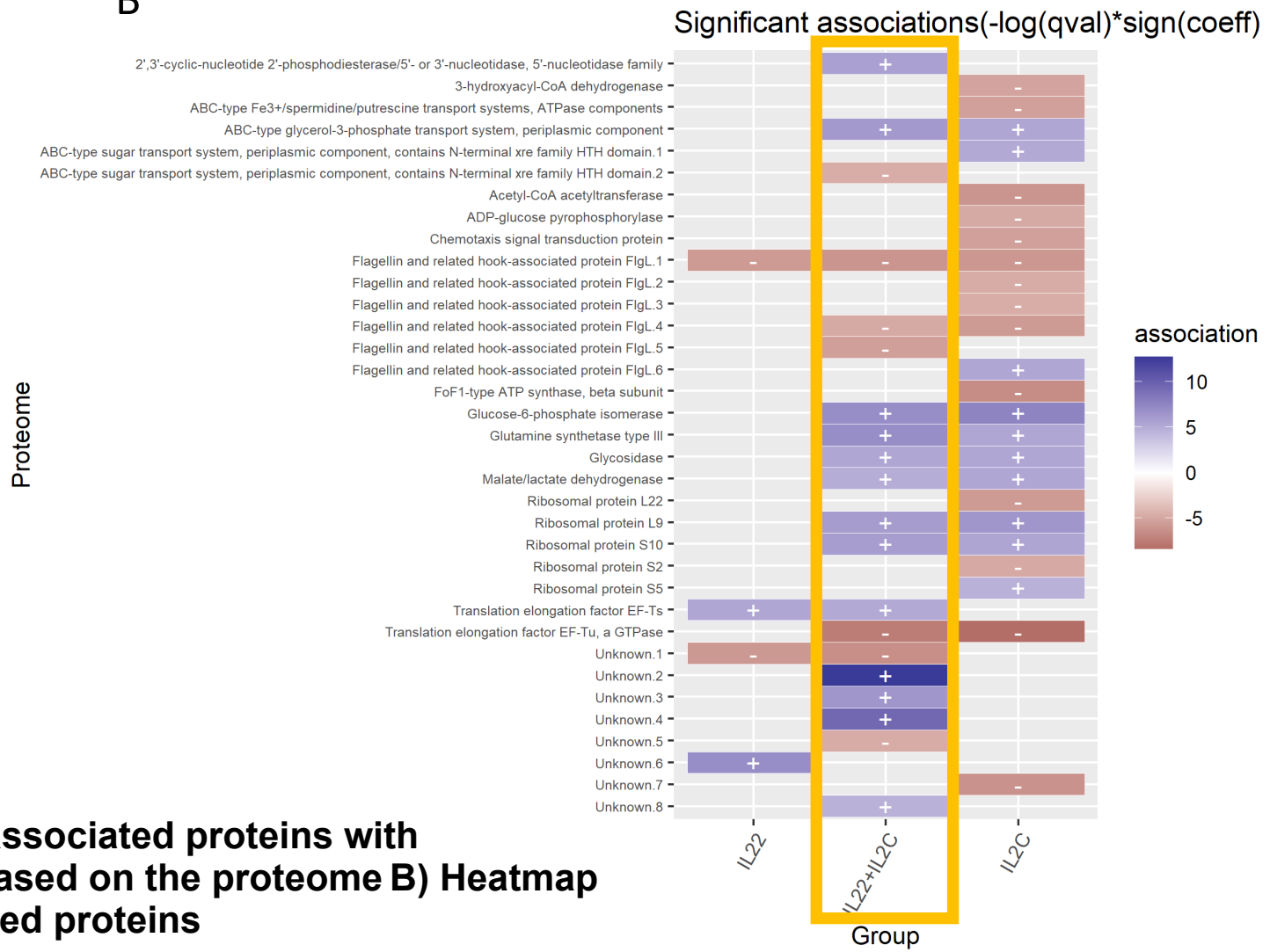
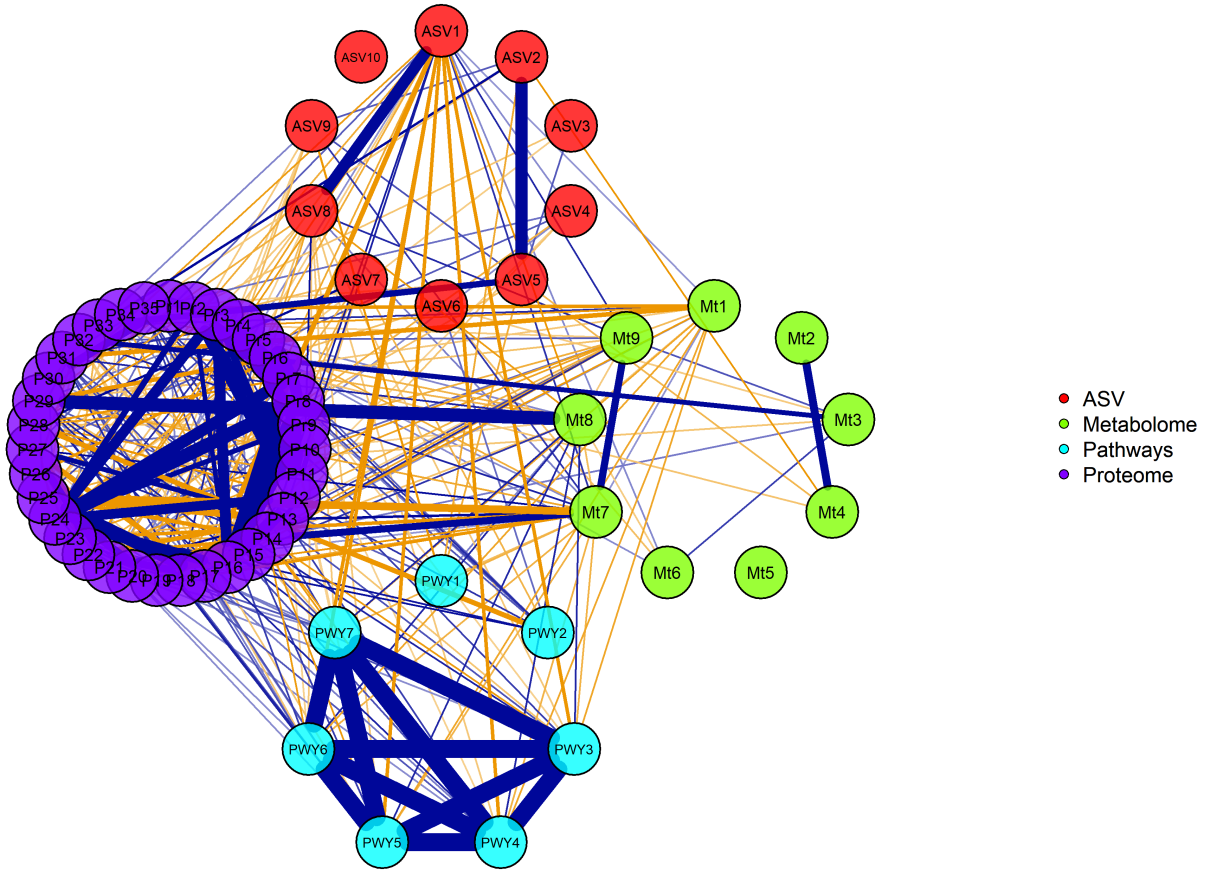



**Distribution of proteome and significantly associated proteins with treatment groups. A) PLSDA plot inferred based on the proteome B) Heatmap of the Top50 proteins Significantly associated proteins**

# Correlation network and selected scattered plots of biologically important features




Blue = positive correlation
Orange = negative correlation

*Check the details from correlation_network.xlsx

# Summary

- Administering low-dose IL-2c and IL-22 or a combination of both improved blood glucose control in NOD mice
- Administering low-dose of all treatments significantly reduced the frequency of autoreactive CD8 T “killer” cells in the pancreatic lymph nodes (the T cell activation place) vs control
- The majority of immunological changes with IL-22+IL-2c were observed in the SI Lamina propria.
  - ↑ $Foxp3^+$ Tregs that are more proliferative and suppressive (↑ ICOS expression)
  - ↑ number of $Helios^+$ Tregs = more stable and functional
  - ↓ expression of SAA 1/2 and ↑ expression of RegIIIγ in the IECs = ↓ endotoxemia?
- Changes in gut microbiota function: metabolome and proteome changes
- Changes in Taken together, these preliminary results show that IL-22+IL-2c treatment for 6 weeks in prediabetic NOD mice
  - Improved immune regulation in the SI mucosa
  - Improved barrier integrity in the SI IECs
  - Reduced autoreactive CD8 T cells in the PLNs
  - Improved blood glucose control

# Thank you for your attention.