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PRIFYSGOL CAERDYDD

Dietary gluten permanently reconfigures gastrointestinal tissue-resident immunity in celiac disease

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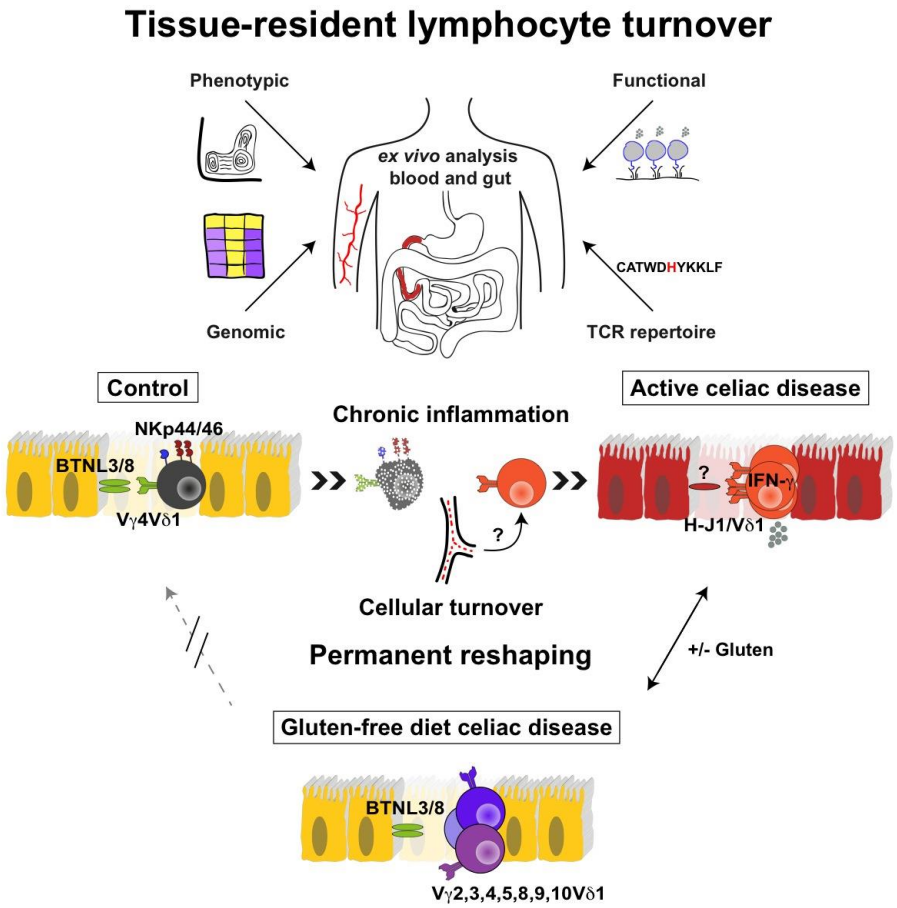
Cardiff University School of Medicine, UK
&
Vaccine Research Center, NIH, USA

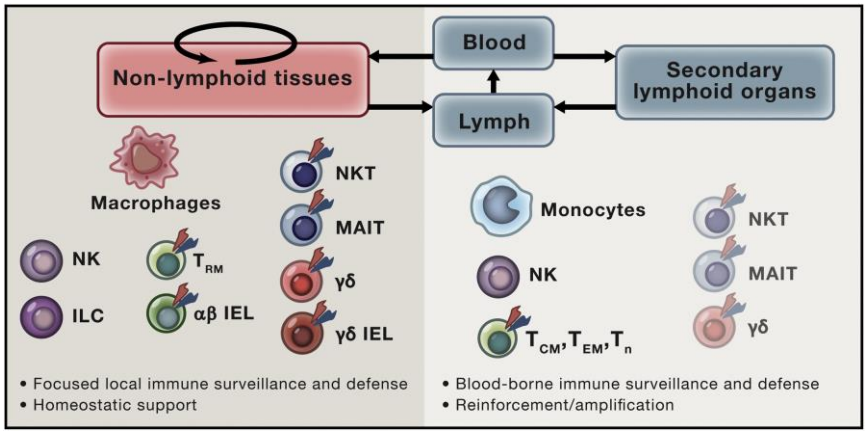
The video

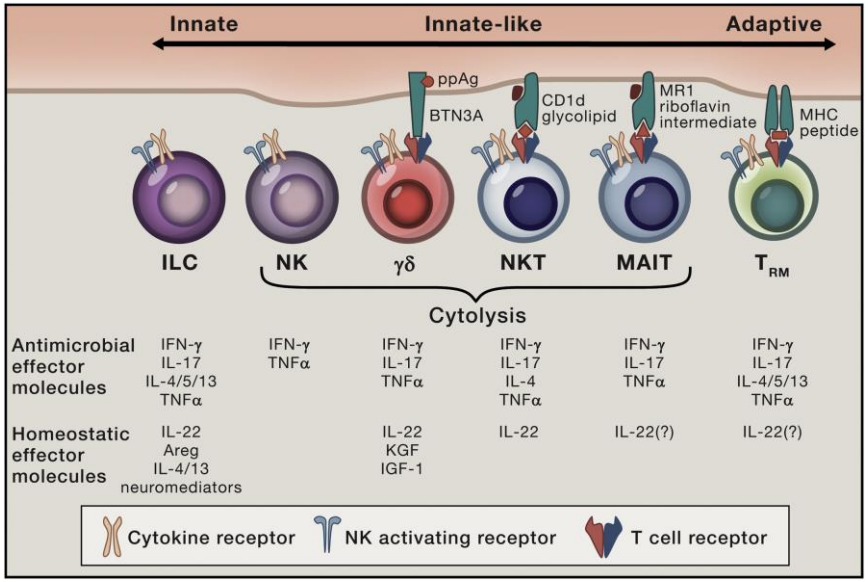
Chronically inflamed then never the same…

https://www.youtube.com/watch?v=nSkrQgugrc8



Tissue-resident lymphocytes





Fan and Rudensky, *Cell* **11**, 1281-1289 (2016)

Tissue-resident lymphocytes come in many different flavours (innate through adaptive)

Tissue-resident lymphocytes provide local immune surveillance and homeostatic functions

T_{RM} cells lack the capacity for tissue egress ($CD62L^-$ $CD69^+$ $CD103^+$ $S1PR1^-$)

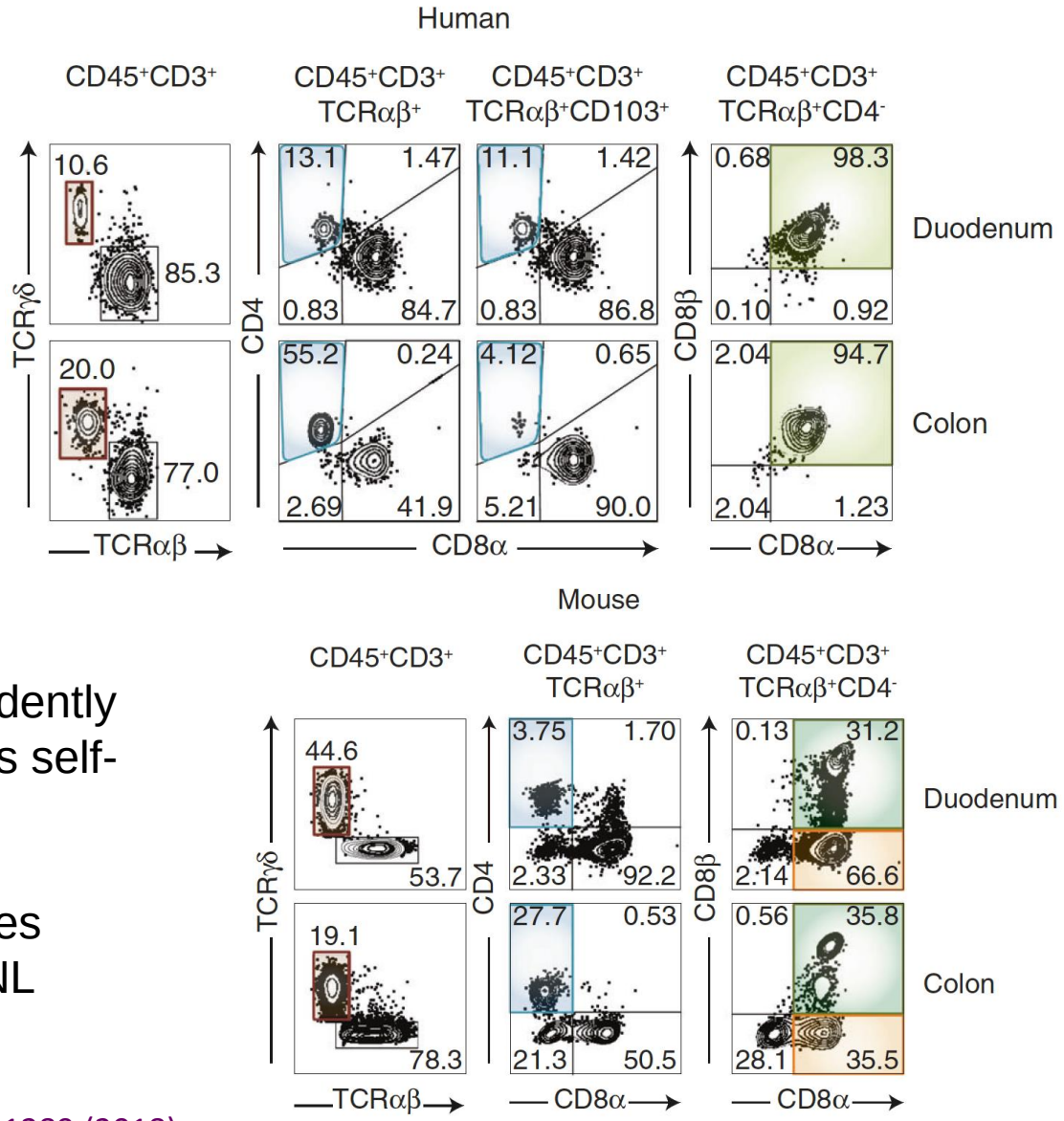
T_{RM} cells proliferate *in situ* to generate tissue-specific immunological memory

T_{RM} cells are “new”

Intraepithelial lymphocytes (IELs) are “old”

Gastrointestinal IELs

- TCRγδ⁺ T IEL (% of CD3⁺ cells)
- TCRαβ⁺ CD8αβ⁺ IEL (% of TCRαβ⁺ CD4⁻ IEL)
- TCRαβ⁺ CD8αα⁺ IEL (% of TCRαβ⁺ CD4⁻ IEL)
- TCRαβ⁺ CD4⁺ IEL (% of TCRαβ⁺ IEL)



The TCRγδ IEL compartment is established early in life, independently of dietary/microbial insults, and is self-sustaining/stable (in mice)

The TCRγδ IEL compartment does NOT form in the absence of BTNL molecules (in mice)

Mayassi and Jabri, *Mucosal Immunol.* **11**, 1281-1289 (2018)

TCRγδ IELs in CeD

Active disease is characterized histologically by villous atrophy and immunologically by expanded populations of IELs

Adherence to a GFD leads to resolution of the villous abnormalities and decreased frequencies of $CD4^+$ and $CD8^+$ TCRαβ IELs, but NOT decreased frequencies of TCRγδ IELs

Jabri and Sollid, *Nat. Rev. Immunol.* **11**, 1281-1289 (2009)
Kutlu *et al.*, *Gut* **34**, 208-214 (1993)

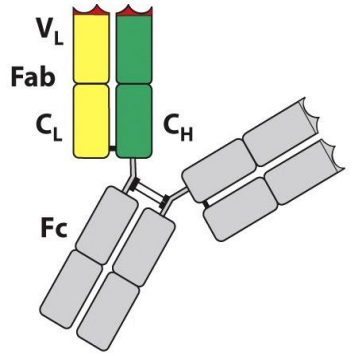
"The persistent increase in TCRγδ IELs in coeliac disease patients who have recovered a normal mucosa suggests that TCRγδ IEL do not induce directly epithelial damage."

Kutlu *et al.*, *Gut* **34**, 208-214 (1993)

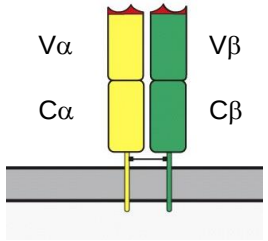
"By whatever means, the end-result of type B IEL activation may again be to suppress tissue infiltration by systemic T cells, as peak type B cell representation seems to correlate inversely with disease symptoms."

Hayday *et al.*, *Nat. Immunol.* **2**, 997-1003 (2001)

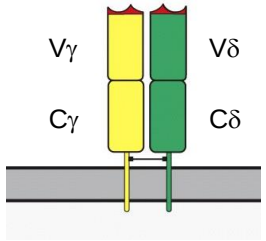
All pure speculation in the absence of defined antigens!!



B cells

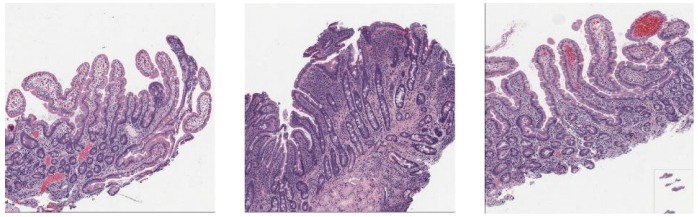


αβ T cells



γδ T cells

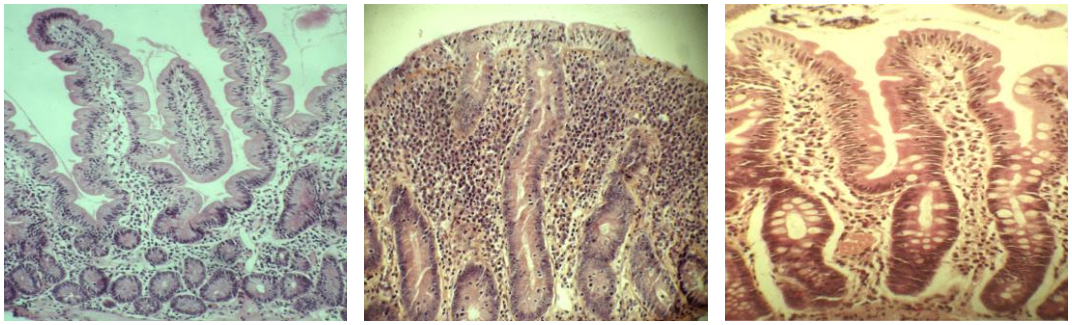
The cohort

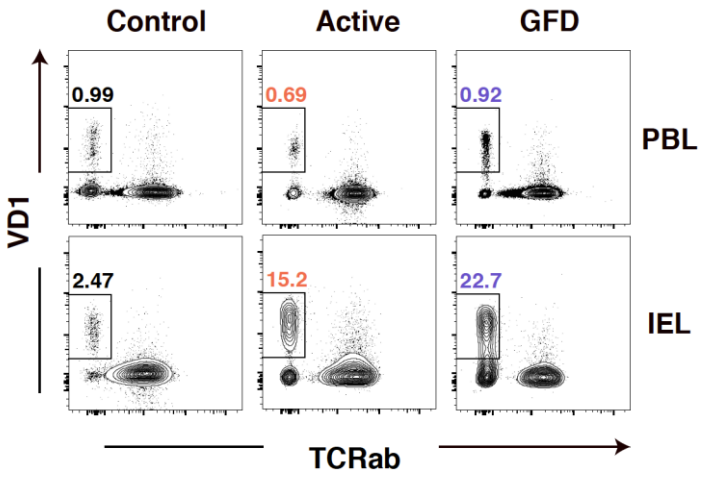


	Control	Active	GFD
Overall study cohort Figure S2A, 5	n = 102 Avg age = 28.3 yrs Male = 29 Female = 73	n = 66 Average age = 24.2 Male = 26 Female = 40	n = 59 Average age = 34.8 Male = 16 Female = 43
NCR cohort Figure 1, S4	n = 31 Average age = 25.3 Male = 8 Female = 23	n = 25 Average age = 19.2 Male = 9 Female = 16	n = 24 Average age = 35.7 Male = 7 Female = 17
Cytokine cohort Figure 2A	n = 8 Average age = 39.6 Male = 5 Female = 3	n = 8 Average age = 29.9 Male = 3 Female = 5	n = 16 Average age = 44.3 Male = 5 Female = 11
Gluten challenge cohort Figure 2B, S5			n = 4 Average age = 51.8 Male = 1 Female = 3
TCR sequencing cohort Figure 3, S6, S7	n = 8 Average age = 24.1 Male = 2 Female = 6	n = 8 Average age = 27.9 Male = 3 Female = 5	n = 8 Average age = 36.3 Male = 2 Female = 6
BTNL expression cohort Figure 4A	n = 16 Average age = 23.9 Male = 5 Female = 11	n = 16 Average age = 26.0 Male = 6 Female = 10	n = 16 Average age = 33.0 Male = 6 Female = 10

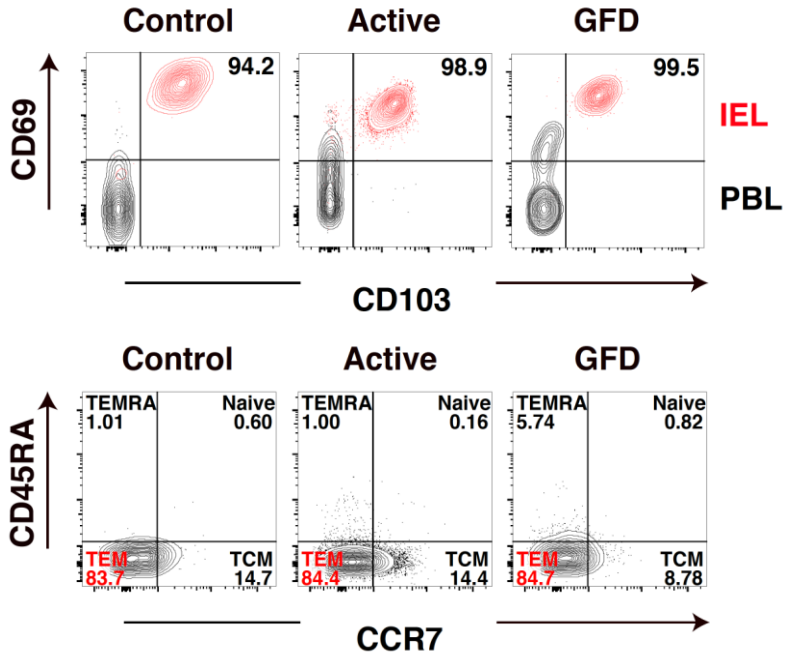
Vδ1⁺ IELs are expanded in active and GFD-treated CeD

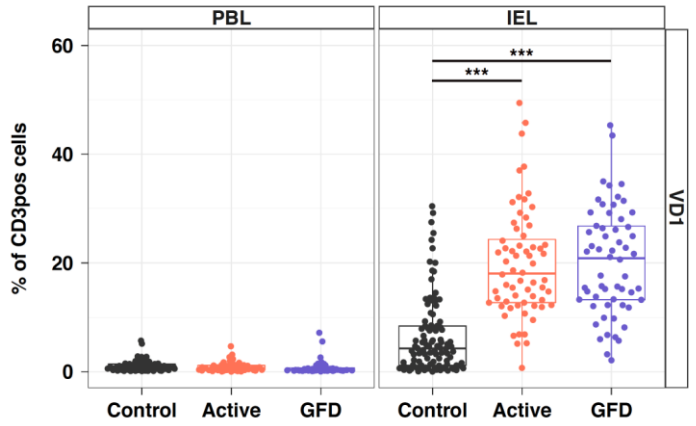
Control | Active | GFD





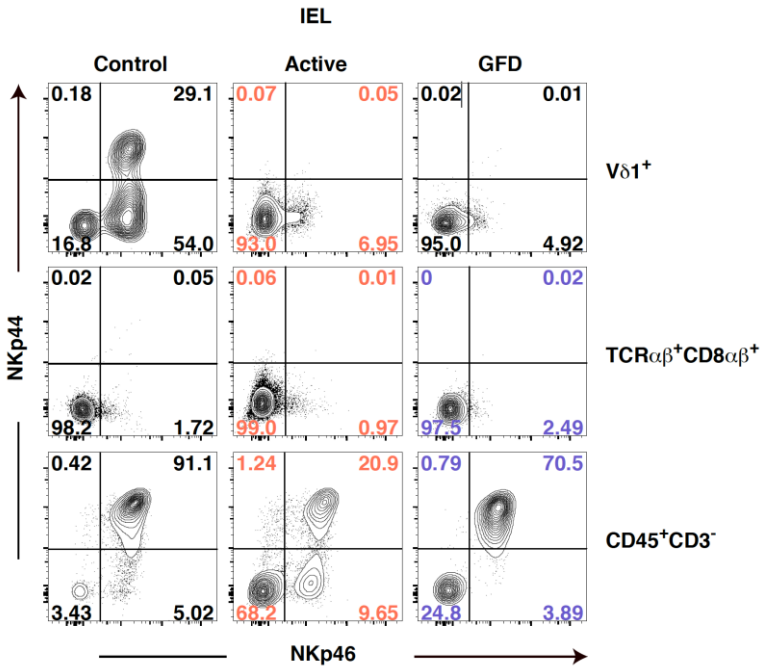
…and exhibit a T_{RM} phenotype. But why?

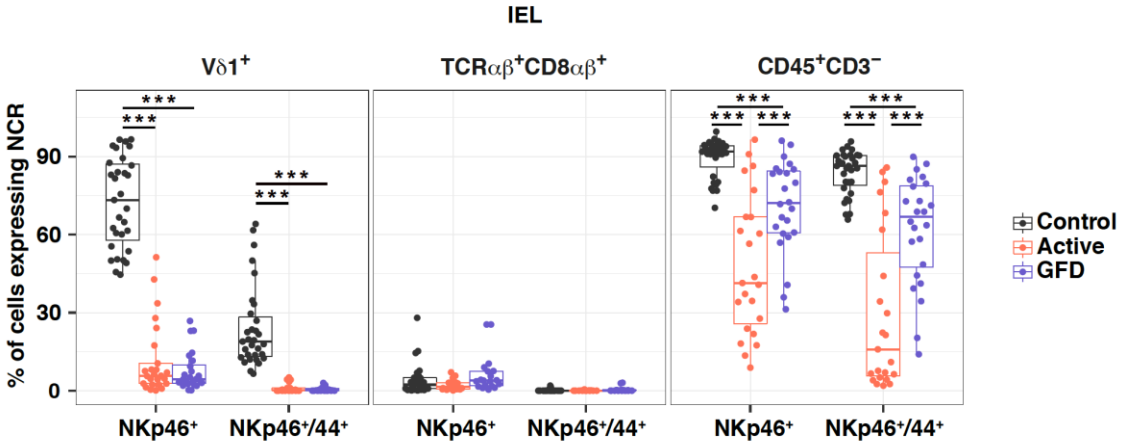




Halstensen *et al.*, *Scand. J. Immunol.* **30**, 665-672 (1989)

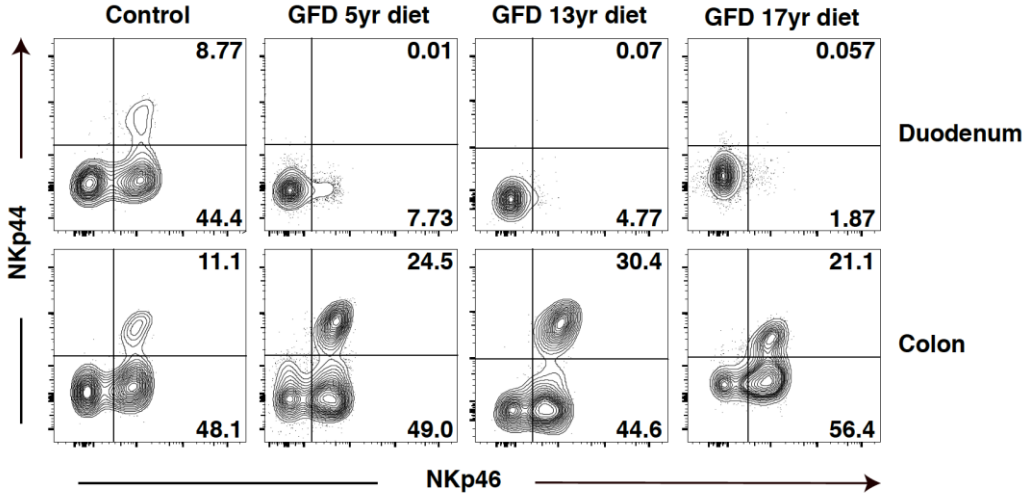
Vδ1⁺ IELs express activating NCRs in the healthy state but not in CeD



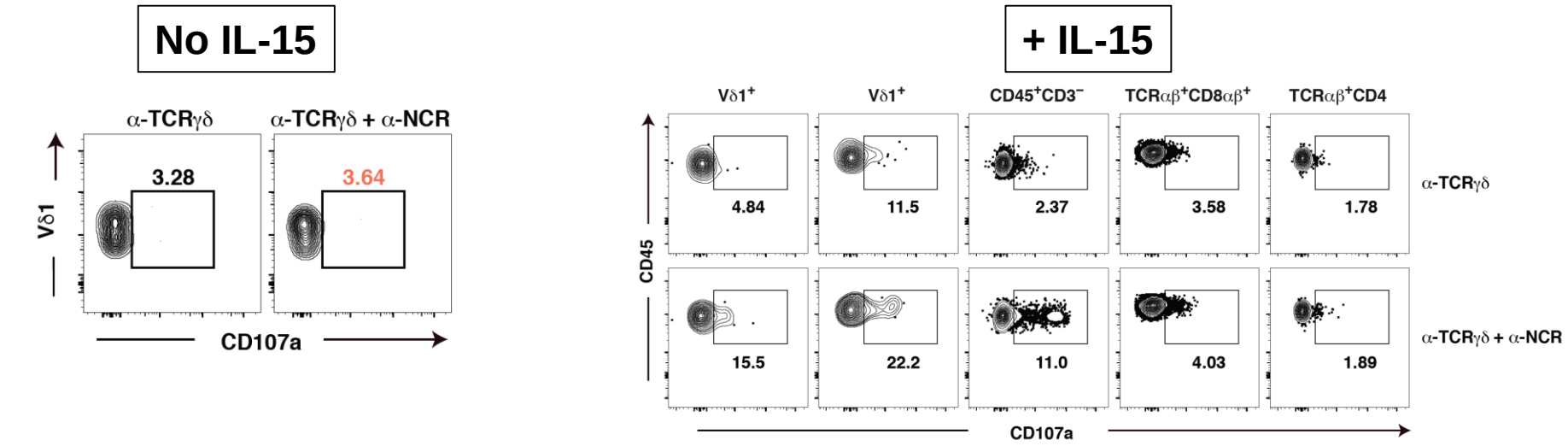


Natural cytotoxicity receptors (NCRs) bind heparan sulphate (HS) and “innately” trigger the cytolytic functions of NK cells

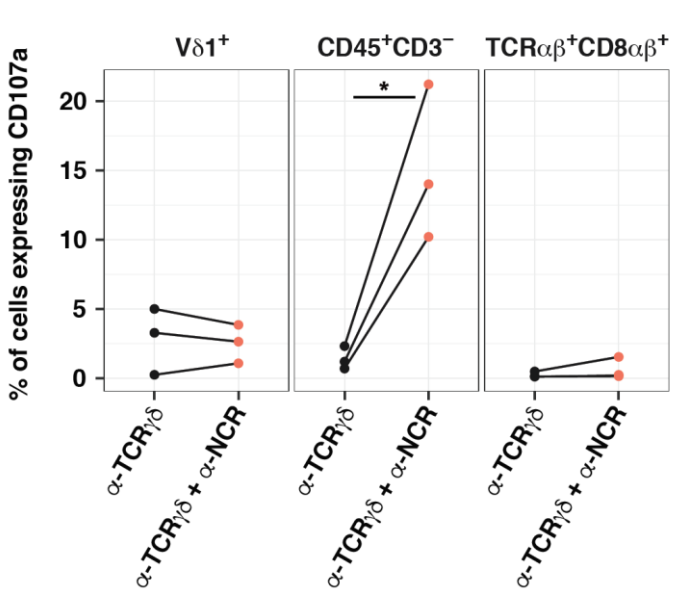
HS has been implicated in the biology of several cancers…

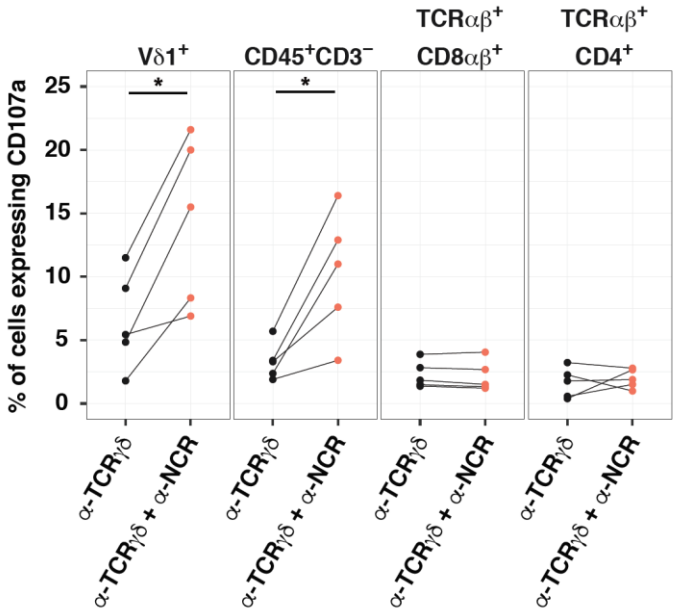


Vδ1⁺ IELs exhibit weak cytolytic activity in CeD

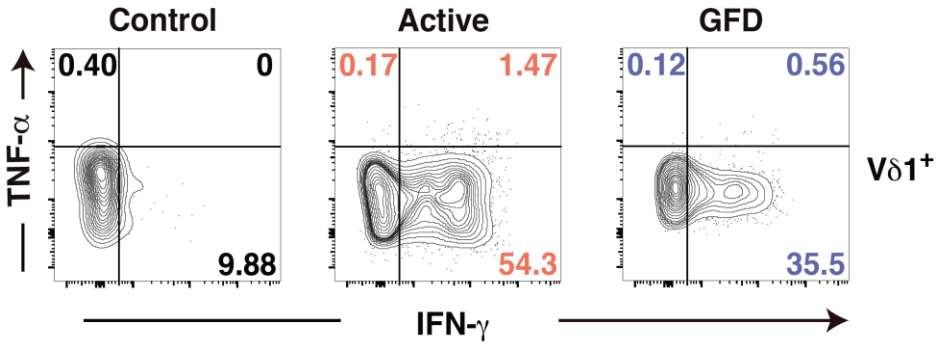


…as measured by surface mobilization of CD107a (LAMP). Enhanced by IL-15 and NCRs.

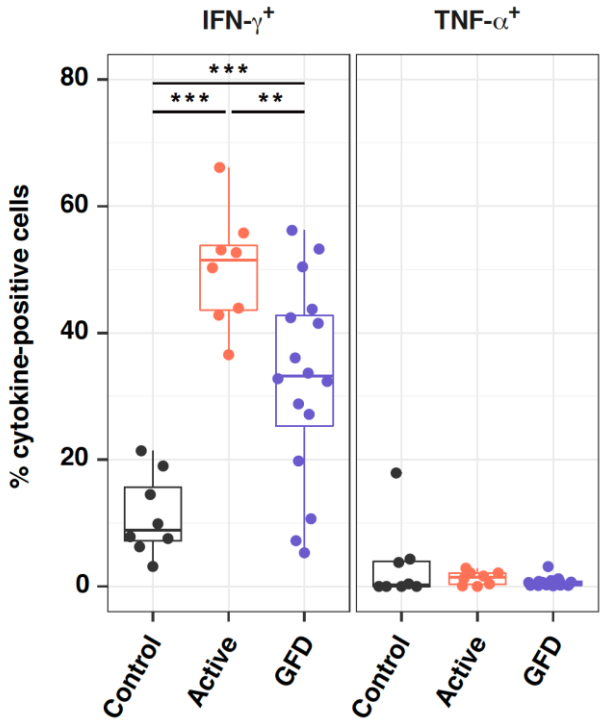




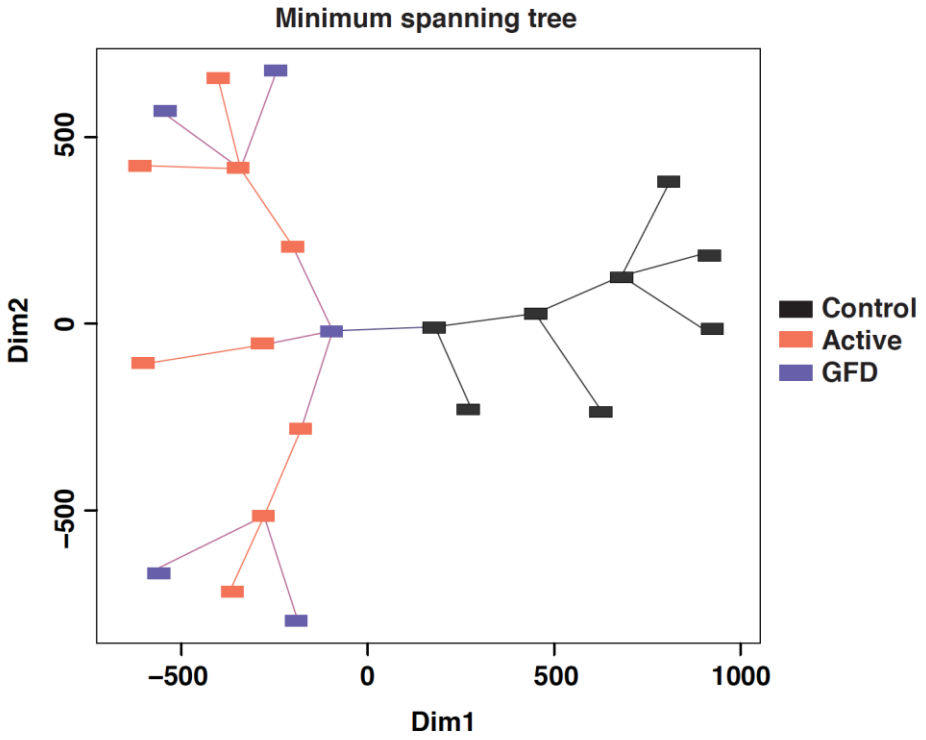
Vδ1⁺ IELs produce IFN-γ in active and GFD-treated CeD

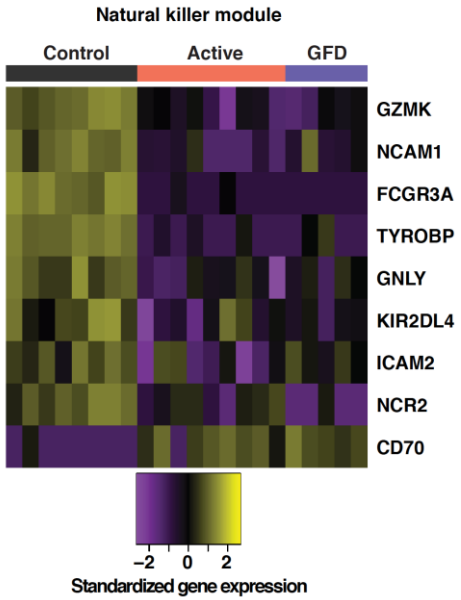


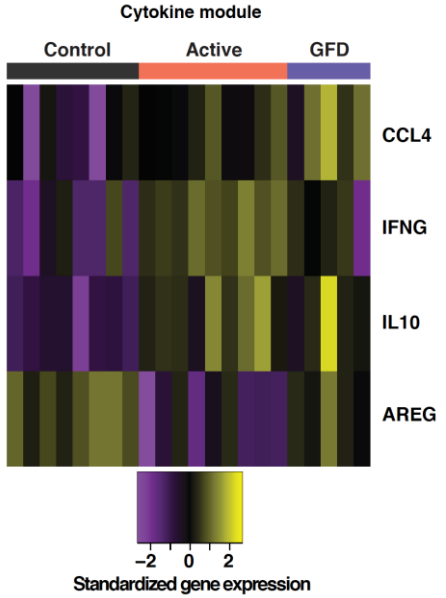
…in response to PMA/IONO. I.e. Vδ1⁺ IELs acquire a proinflammatory phenotype in CeD.

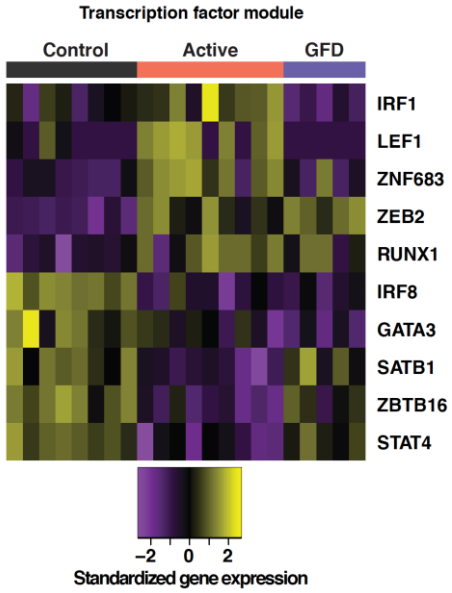


Vδ1⁺ IELs display a permanently altered transcriptional profile in CeD







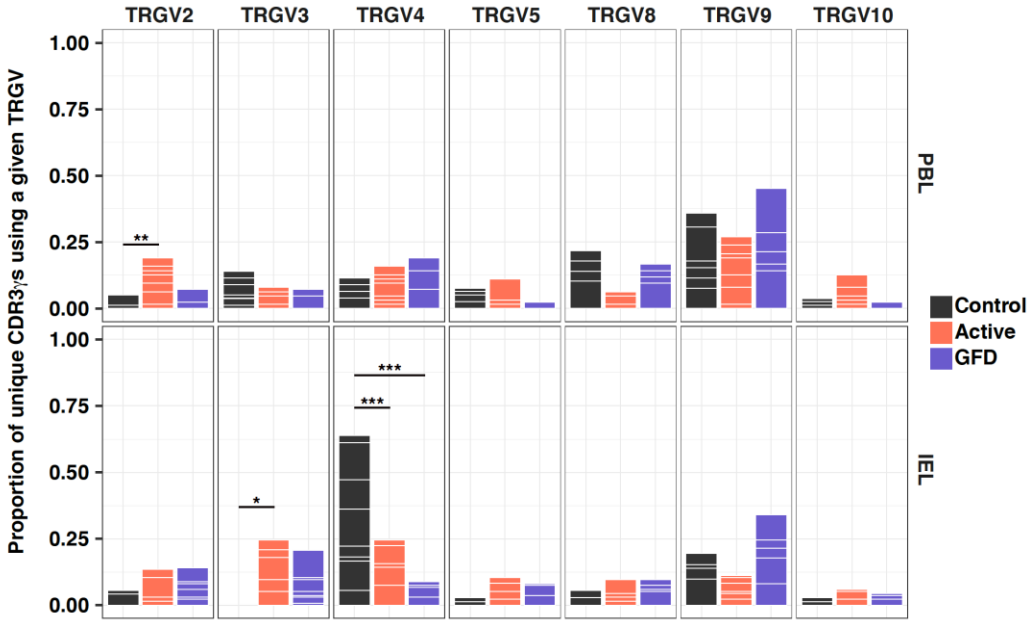


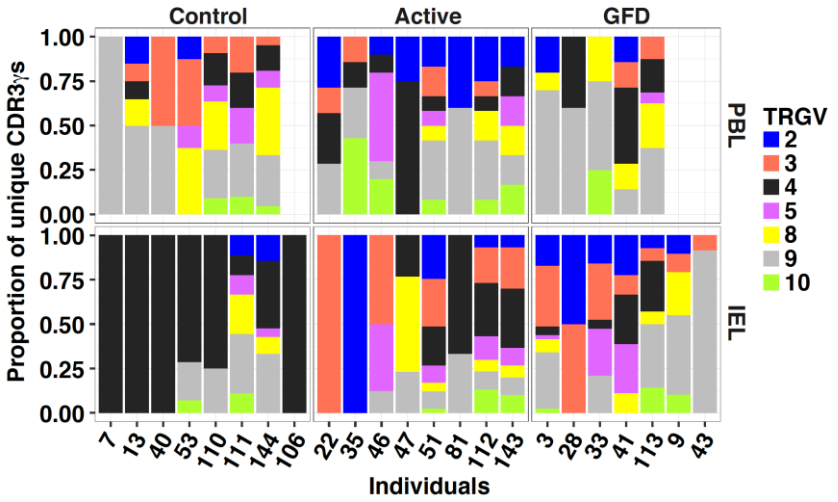
NK module − control Vδ1⁺ IELs preferentially express archetypal NK receptor and cytolytic molecules

Cytokine module − control Vδ1⁺ IELs preferentially express genes associated with tissue, whereas Vδ1⁺ IELs from patients with CeD preferentially express proinflammatory cytokines

Transcription factor module − it's complicated but consistent!!

Vδ1⁺ IELs permanently lose the Vγ4⁺ "gut signature" in CeD

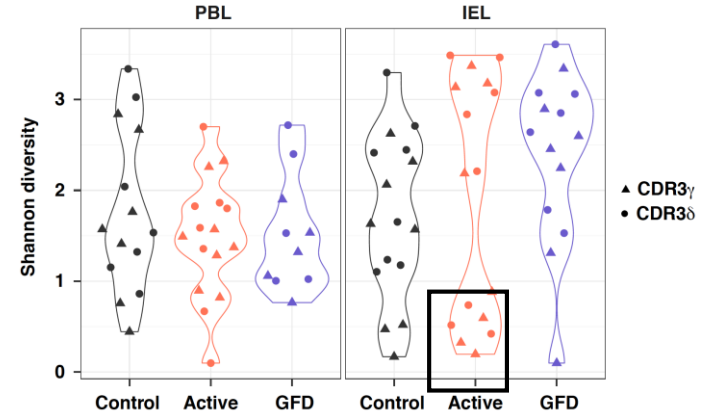


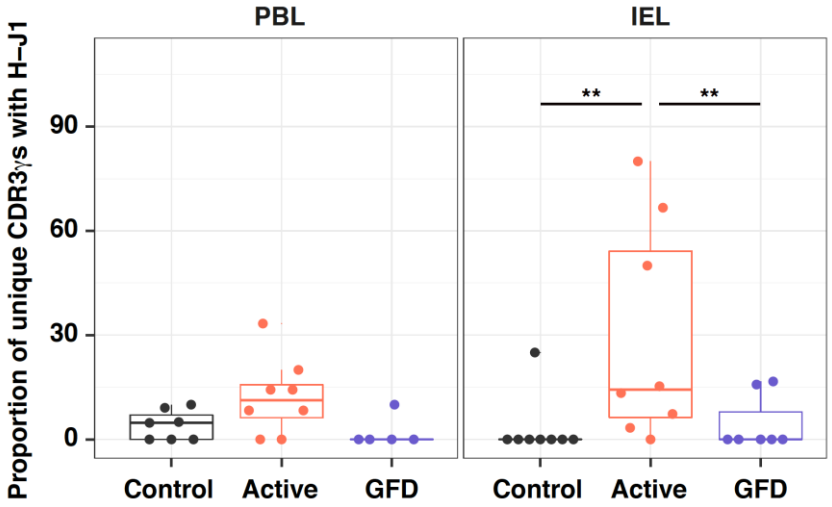


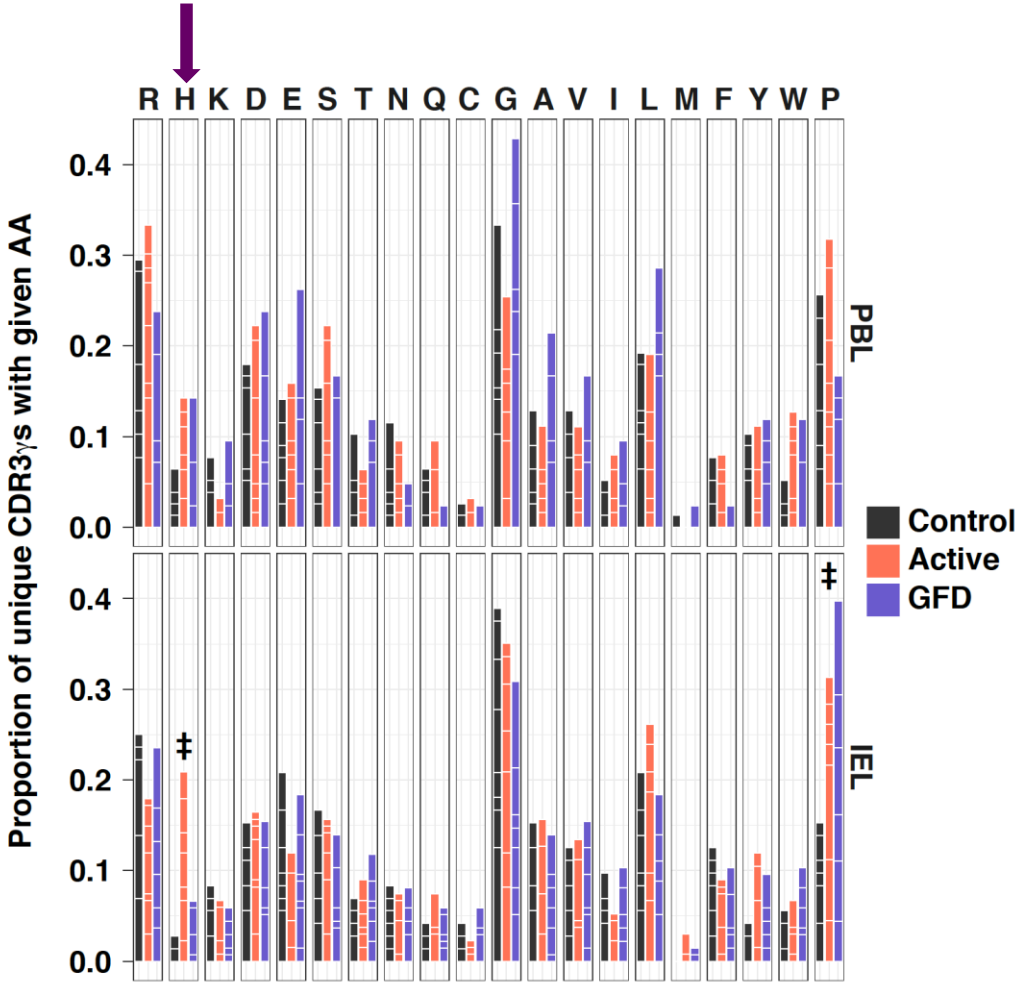
Vδ1⁺ IELs preferentially and in some cases exclusively pair with Vγ4⁺ chains in healthy controls, suggesting germline-driven anatomical localization to the gut

This Vγ4⁺ "gut signature" is permanently lost in patients with active or GFD-treated CeD, suggesting a coincident loss of an associated tissue-specific ligand for this component of the TCR

Vδ1⁺ IELs show evidence of clonal expansions in CeD





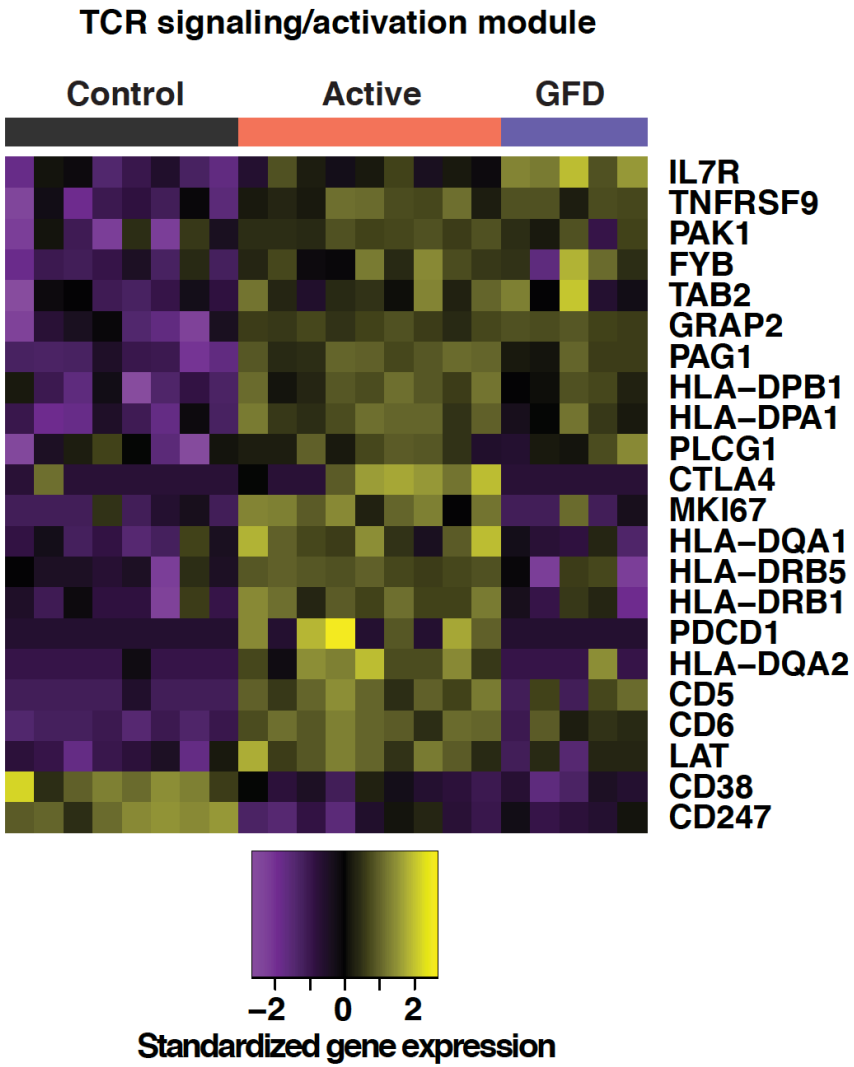


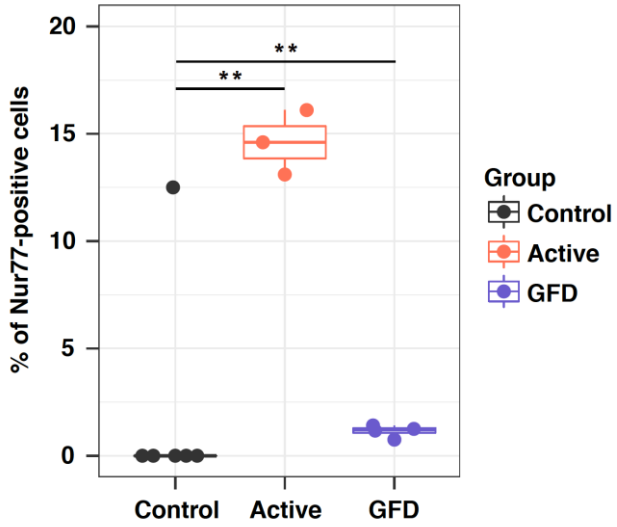
The Vδ1⁺ IEL repertoire is less diverse in a subset of patients with active CeD

The somatically rearranged CDR3γ loop is enriched for H at position J−1 in patients with active or GFD-treated CeD

Patterns typically seen in adaptive TCRαβ responses…is there a specific “driver” antigen?

Vδ1⁺ IELs display hallmarks of TCR-mediated signaling in active CeD





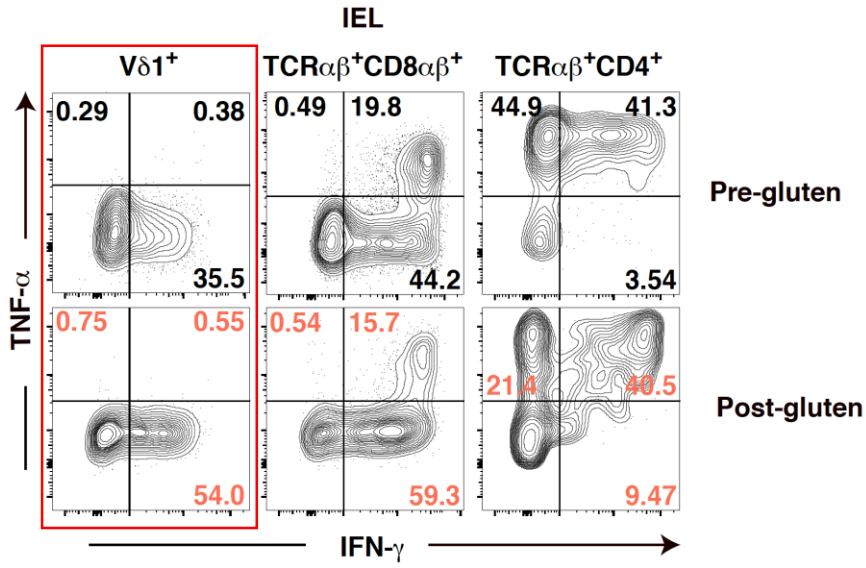
…again consistent with cognate recognition of antigen. But what?

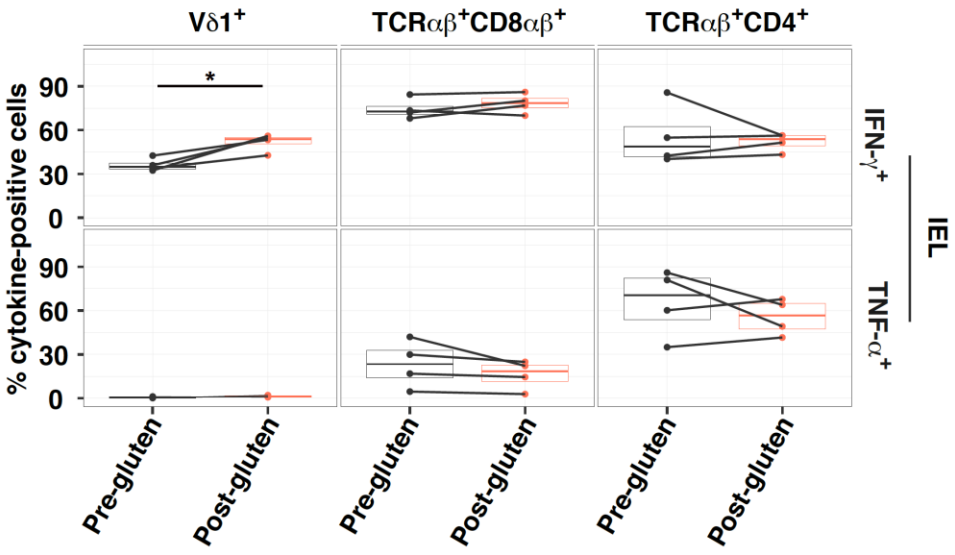
Gluten challenge enhances IFN-γ production by Vδ1⁺ IELs in GFD-treated CeD

D0 — **1 piece of bread a day for 6 weeks** — **D42**

GFD-treated CeD patients (healed mucosa)
Pre-gluten challenge biopsy

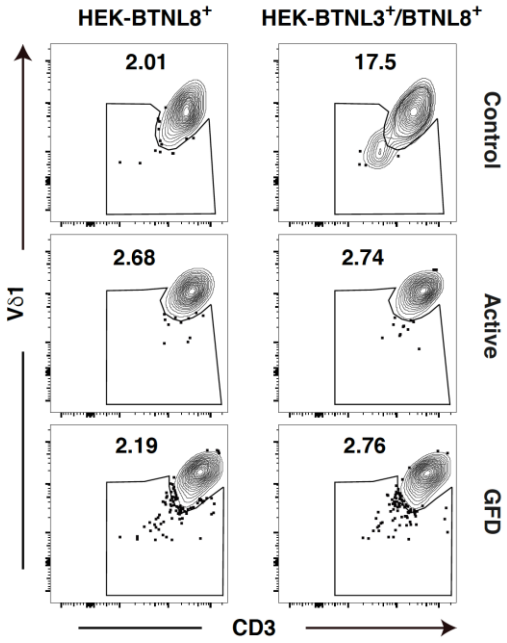
Post-gluten challenge biopsy

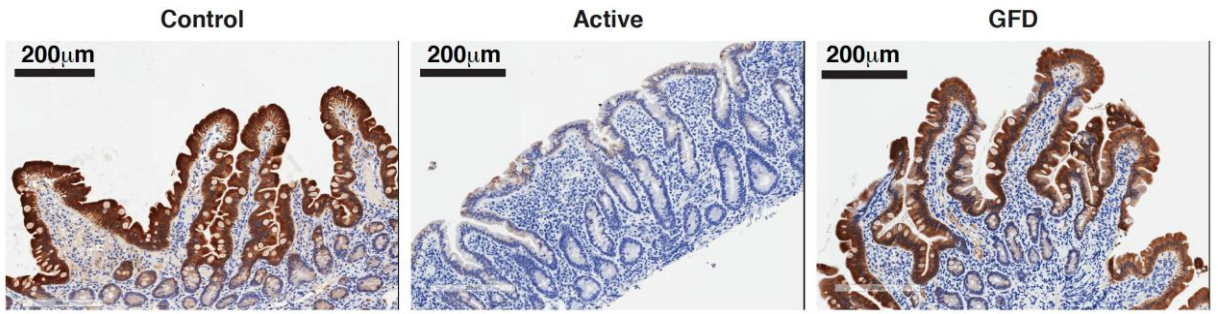




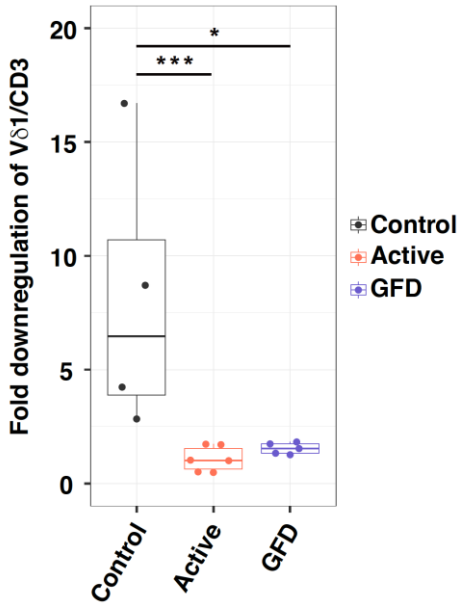
…ligand X? I.e. Specific recognition of a gluten-derived antigen or a self-molecule upregulated in the context of chronic inflammation triggered by gluten exposure?

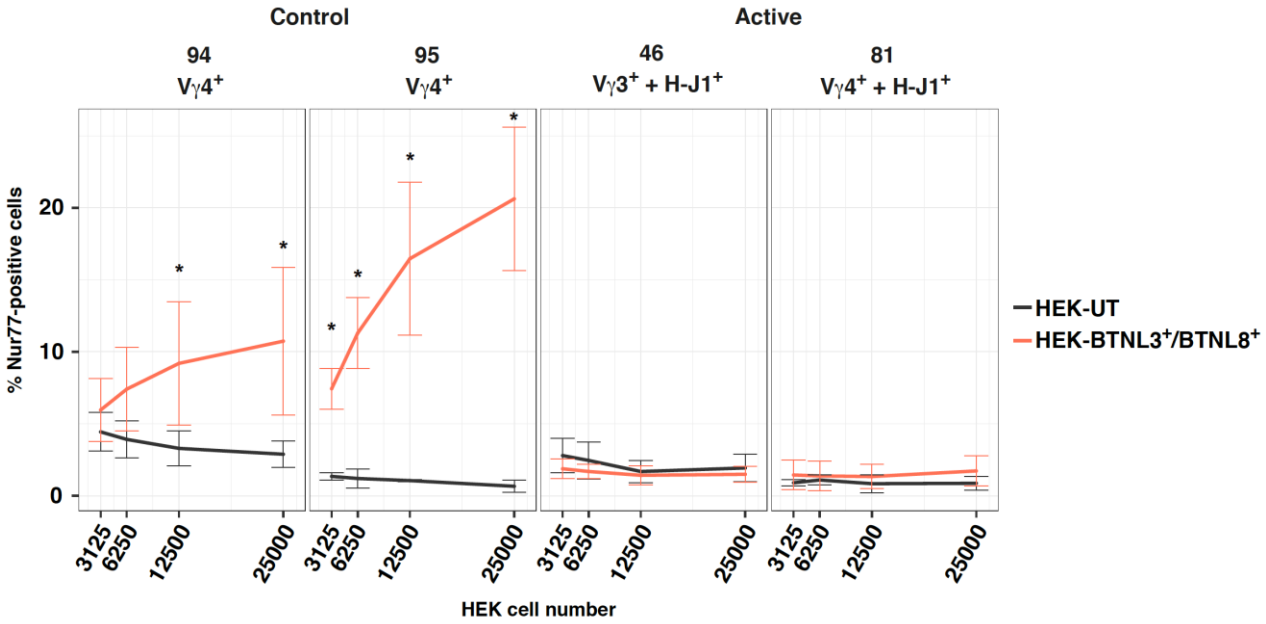
BTNL3/8-reactive Vδ1⁺ IELs are permanently lost in CeD



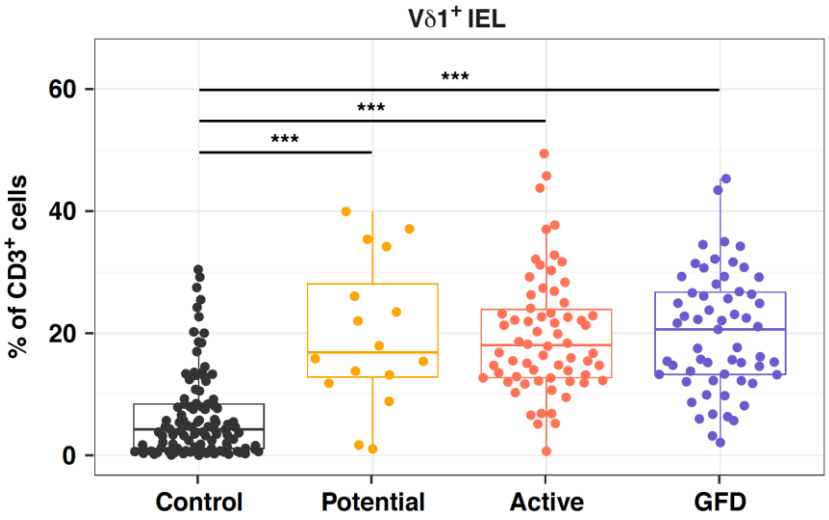


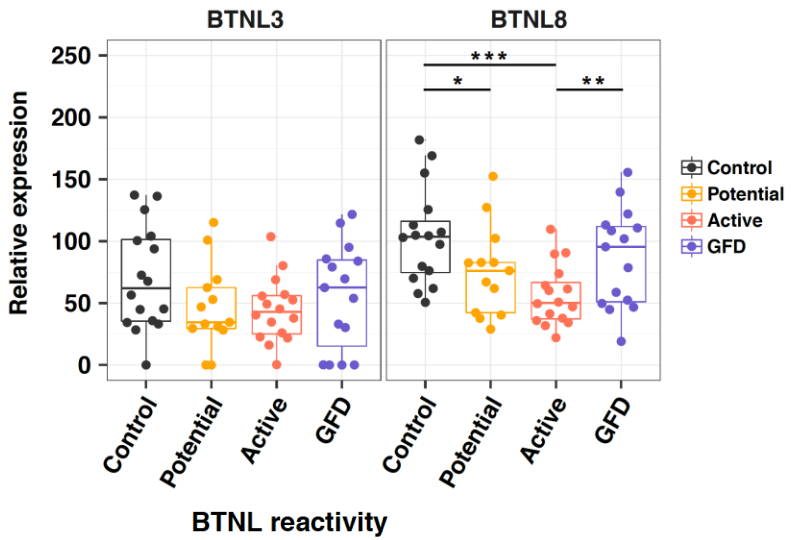
…despite recovery of BTNL8 expression after treatment with a GFD.

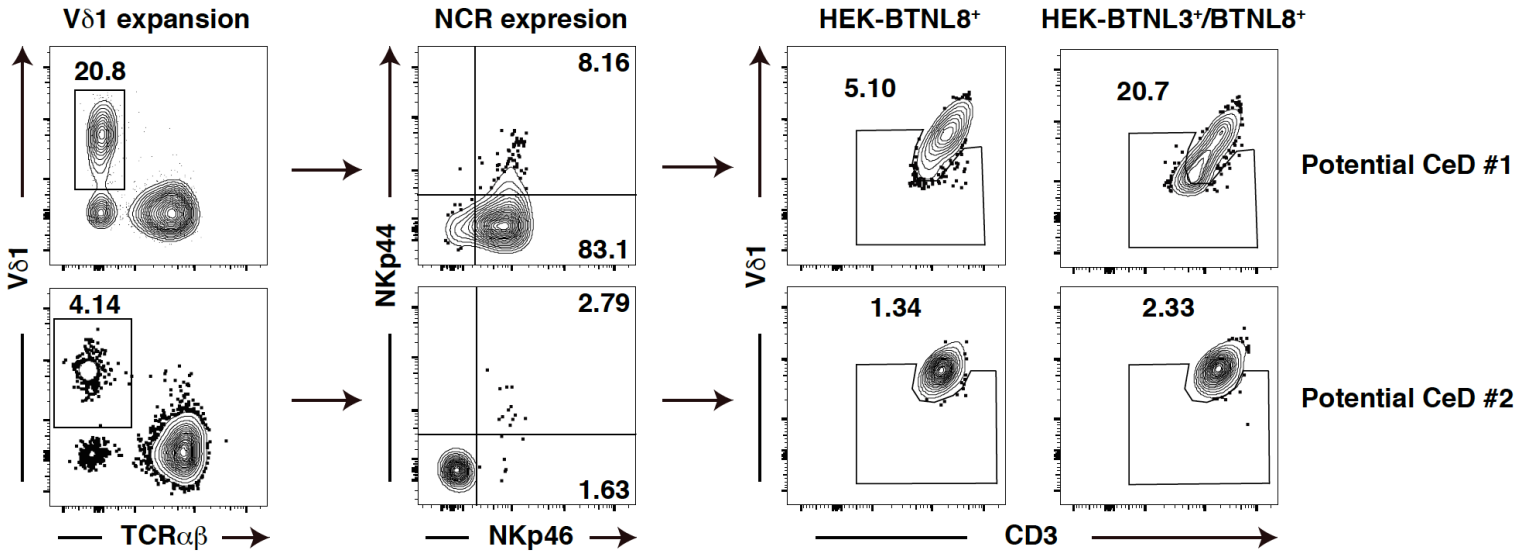




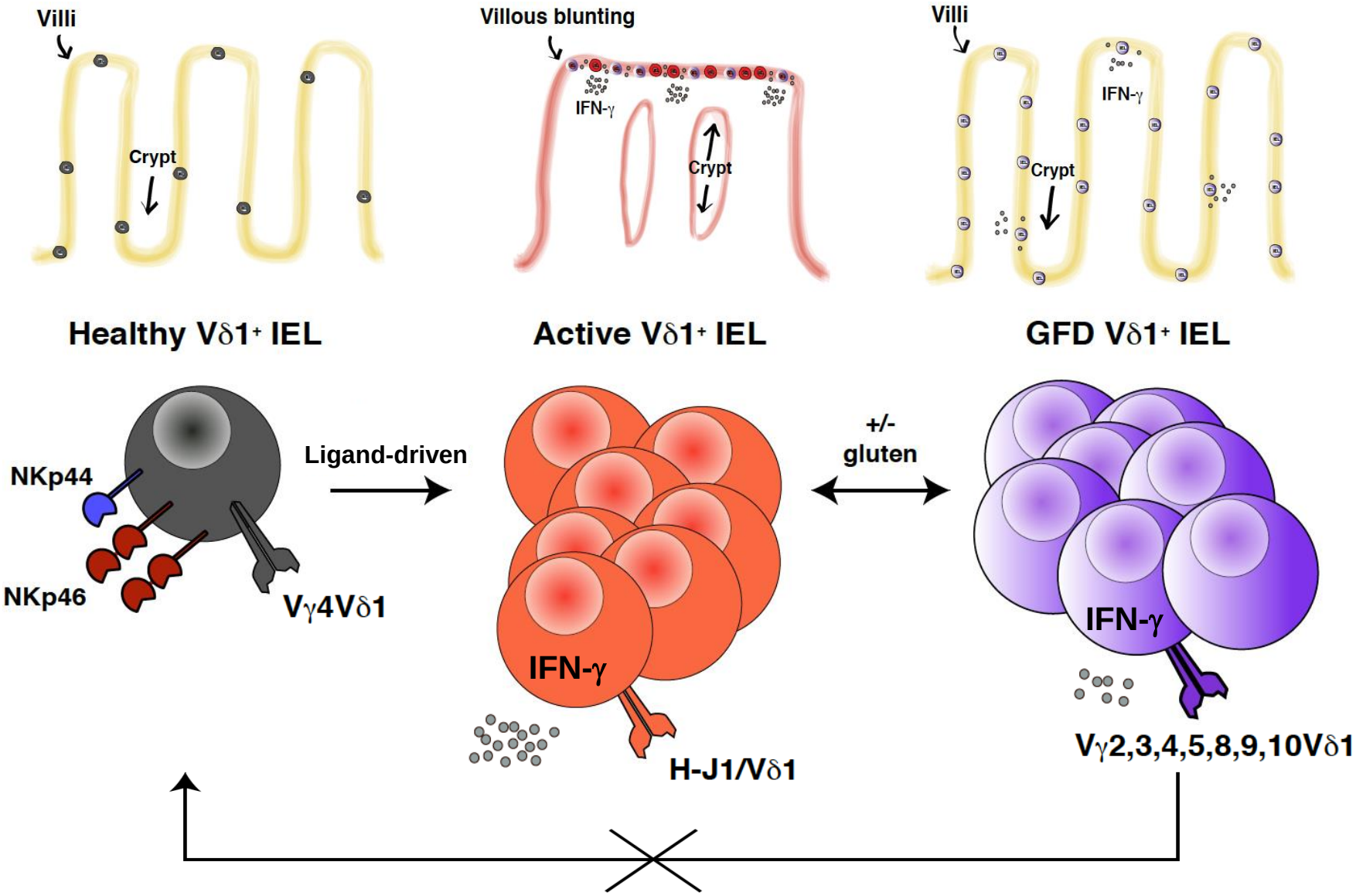
Dynamic remodeling of the Vδ1⁺ IEL compartment precedes tissue damage in CeD







Summary


Villi
Crypt
Villous blunting
IFN-γ
Crypt
Villi
IFN-γ
Crypt
Healthy Vδ1+ IEL
Active Vδ1+ IEL
GFD Vδ1+ IEL
NKp44
NKp46
Vγ4Vδ1
Ligand-driven
IFN-γ
H-J1/Vδ1
+/-
gluten
IFN-γ
Vγ2,3,4,5,8,9,10Vδ1

Acknowledgements

Chicago ❖ USA

Toufic Mayassi
Herman Gudjonson
Dustin Shaw
Jagoda Rokicka
Ian Lawrence
Cezary Ciszewski
Matthew Dimaano
Hoda Sayegh
Ranjana Gokhale
Hilary Jericho
Carol Semrad
Stefano Guandalini
Aaron Dinner
Sonia Kupfer
Bana Jabri

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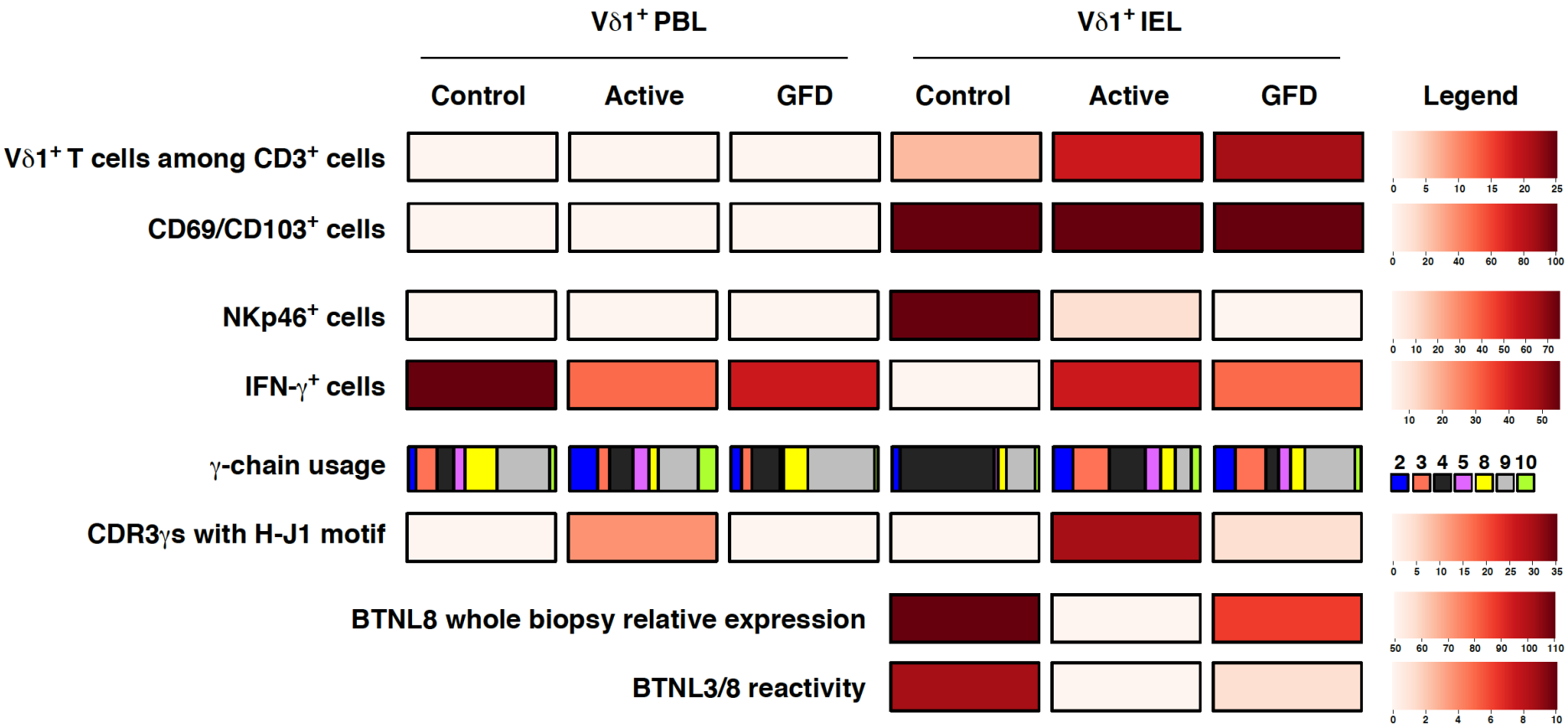
Cisca Wijmenga

Leiden ❖ Netherlands

Vincent van Unen

…and Bob!!

Questions?



Above all, I must beware of all ultimate questions − they are too maddeningly unanswerable.

The Journal of a Disappointed Man (W.N.P. Barbellion)