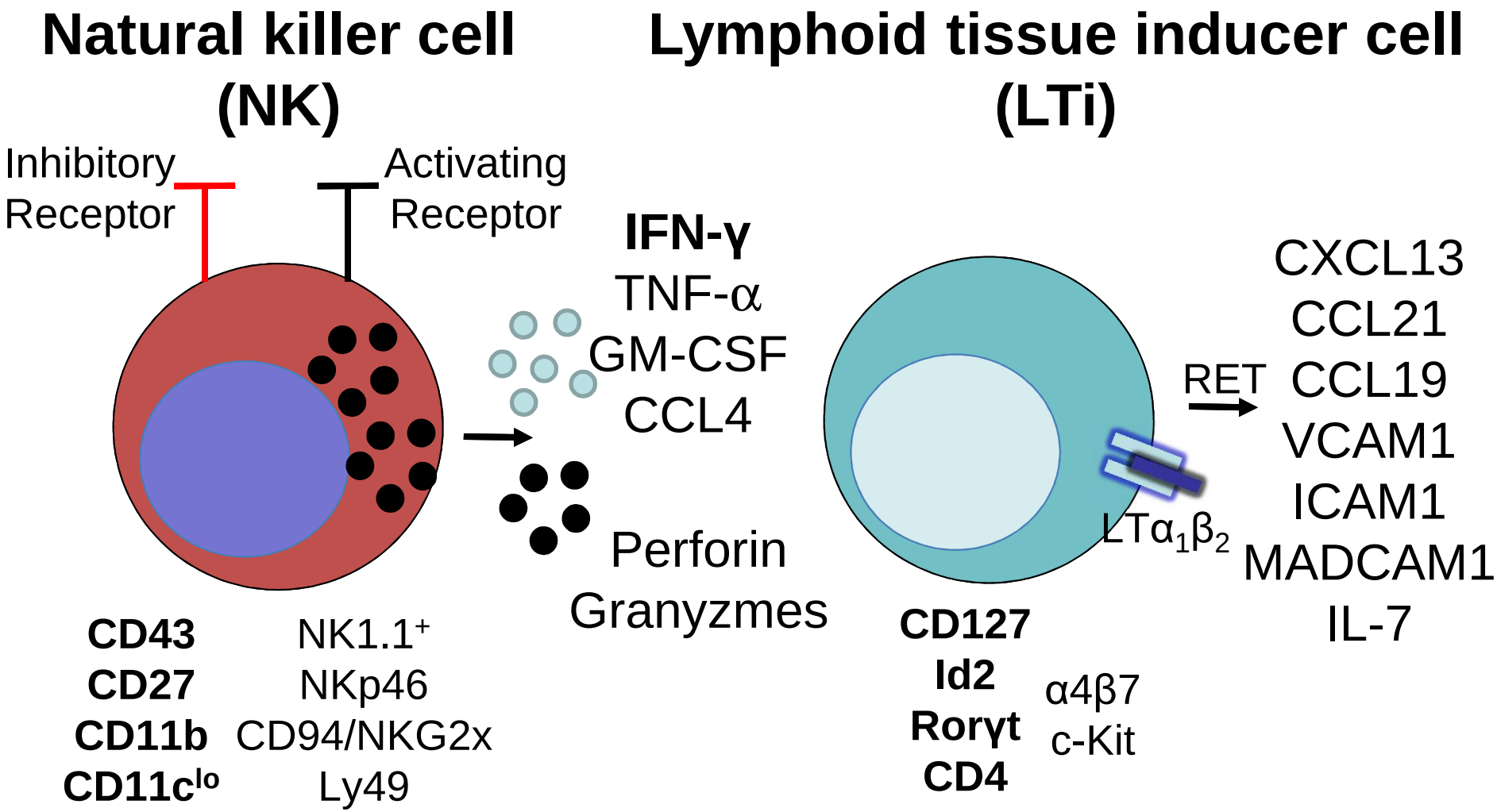
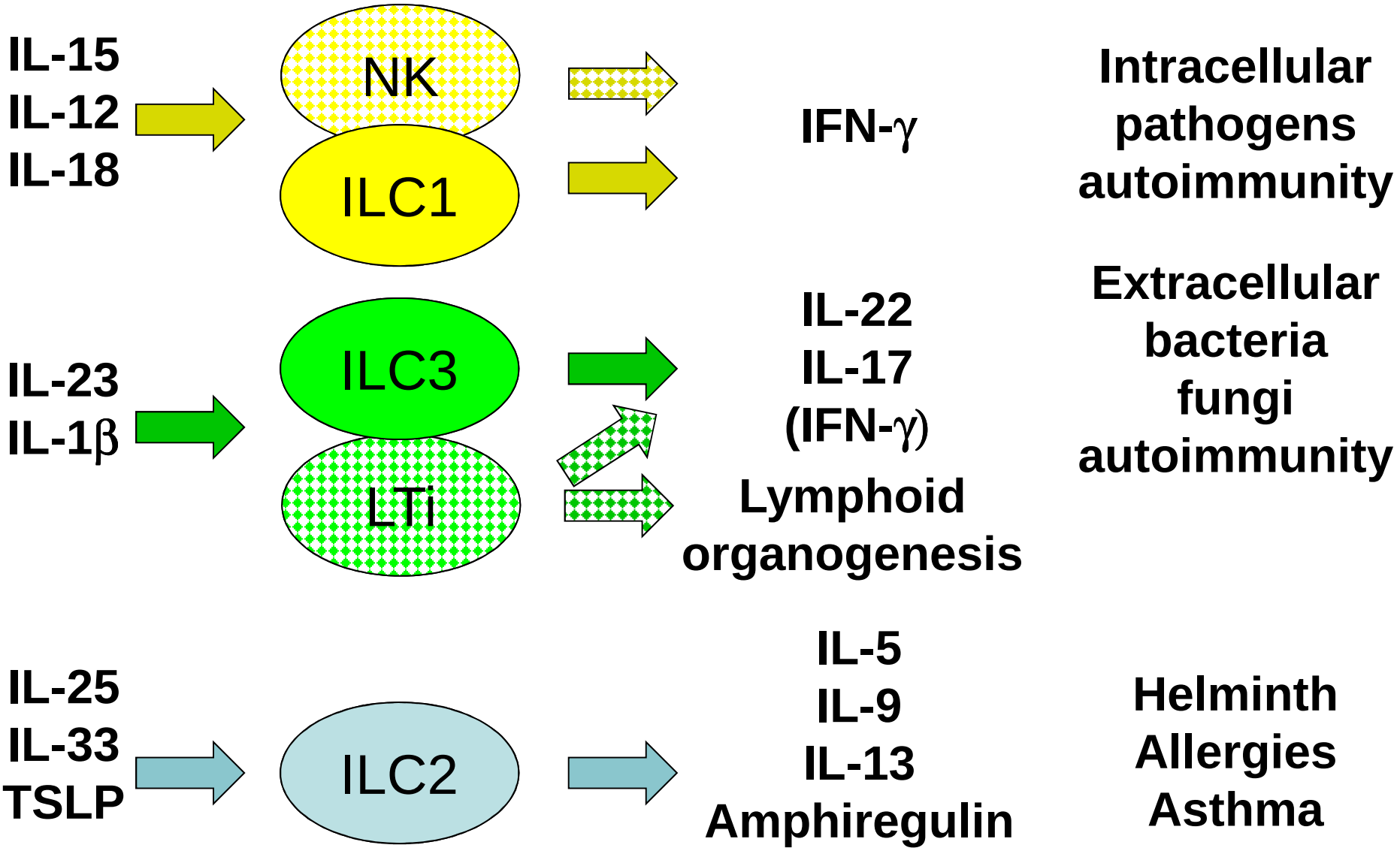
Innate lymphoid cells (ILC) in immunity

Marco Colonna
Washington University in St Louis

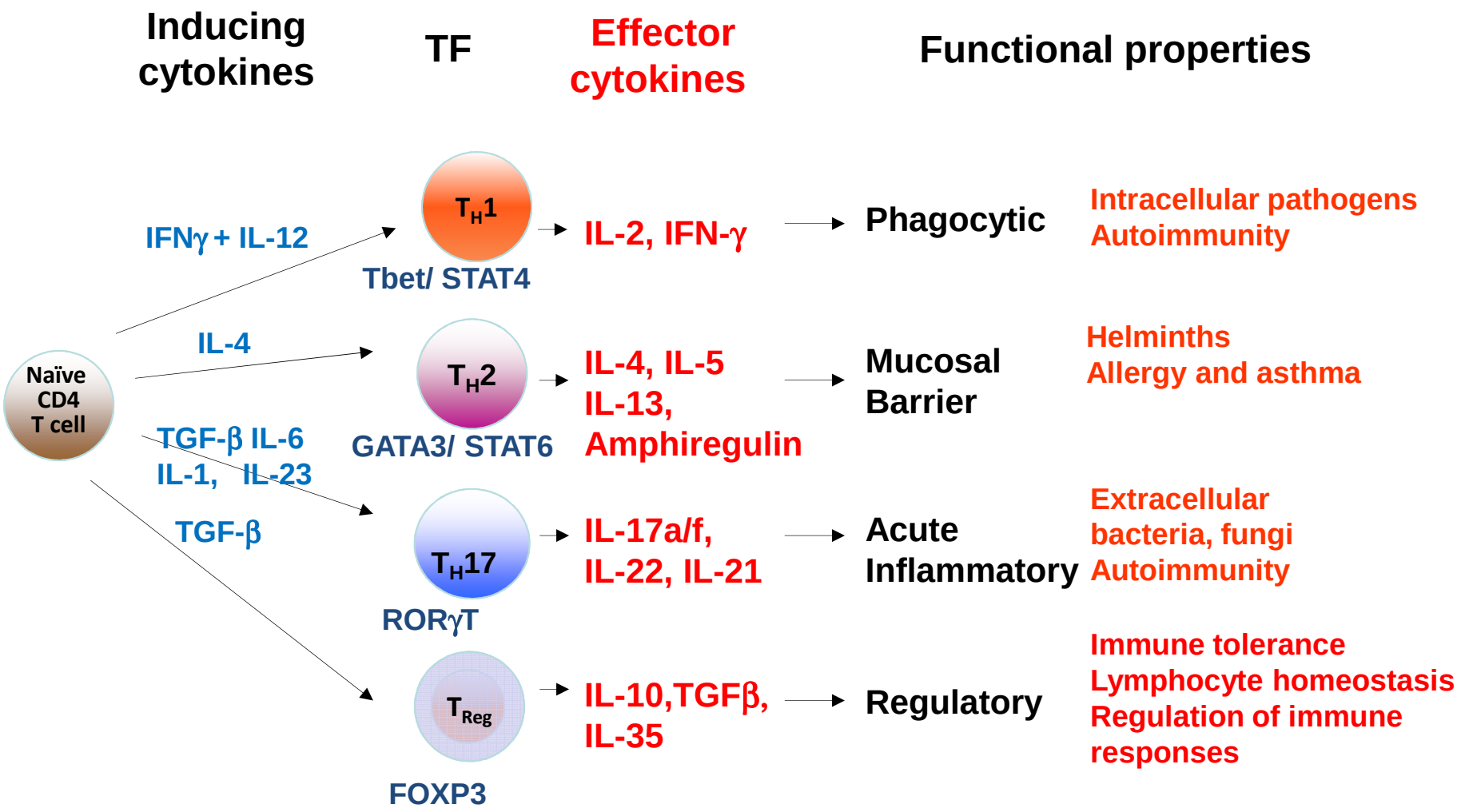
Innate Lymphoid Cells (ILC) before 2009:



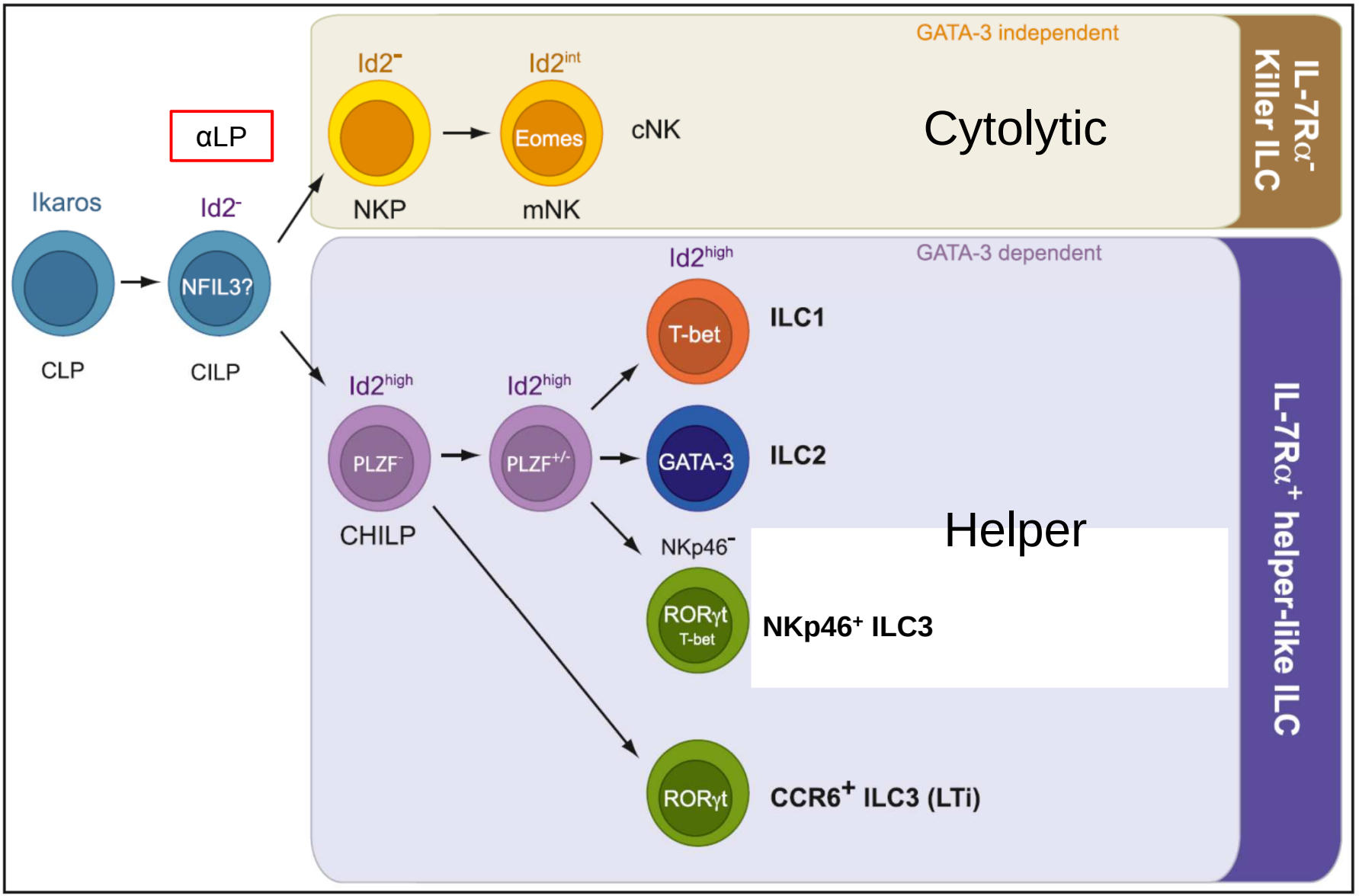
Innate Lymphoid Cells (ILC) after 2009



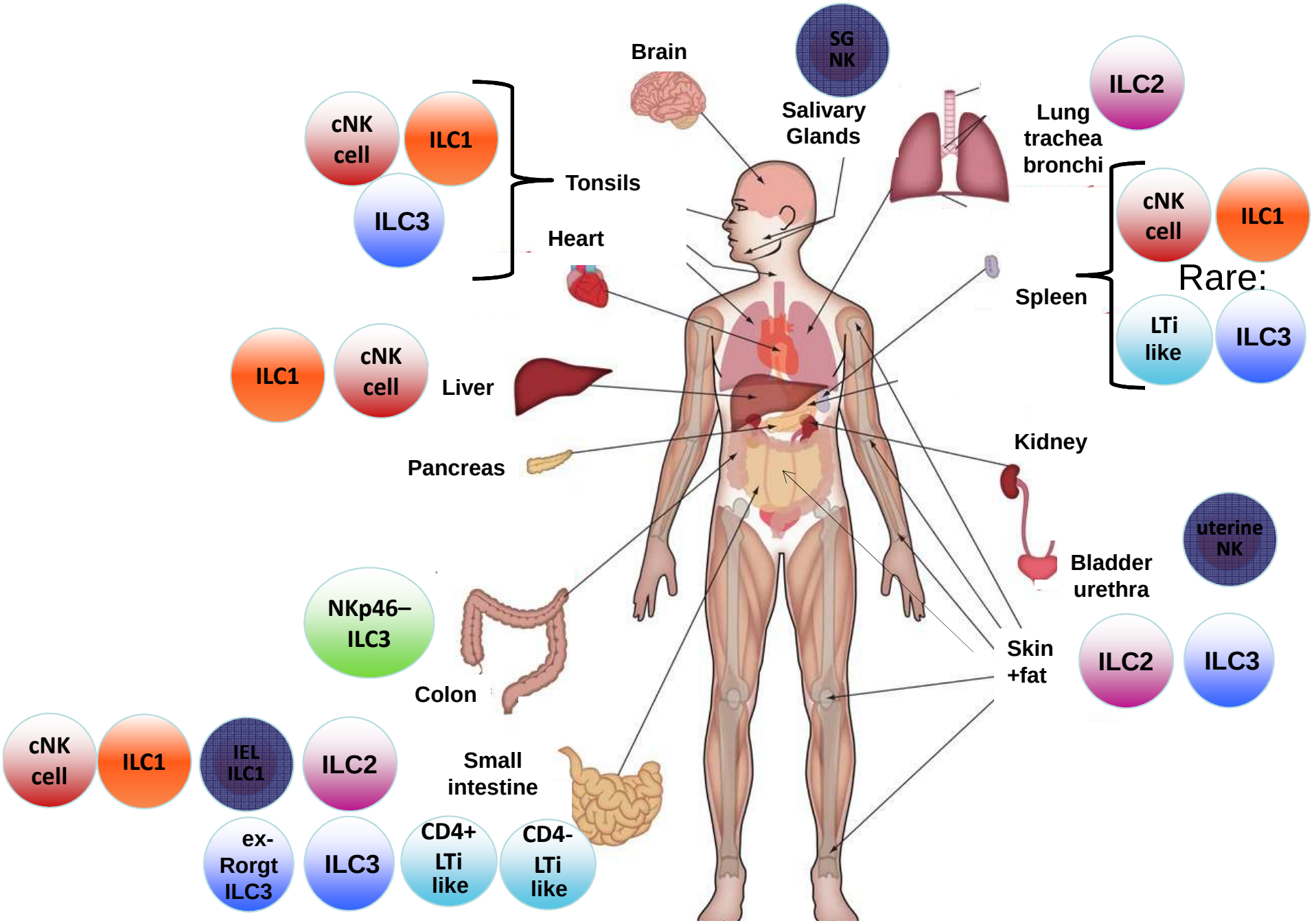
Functional modules of CD4 Th cells



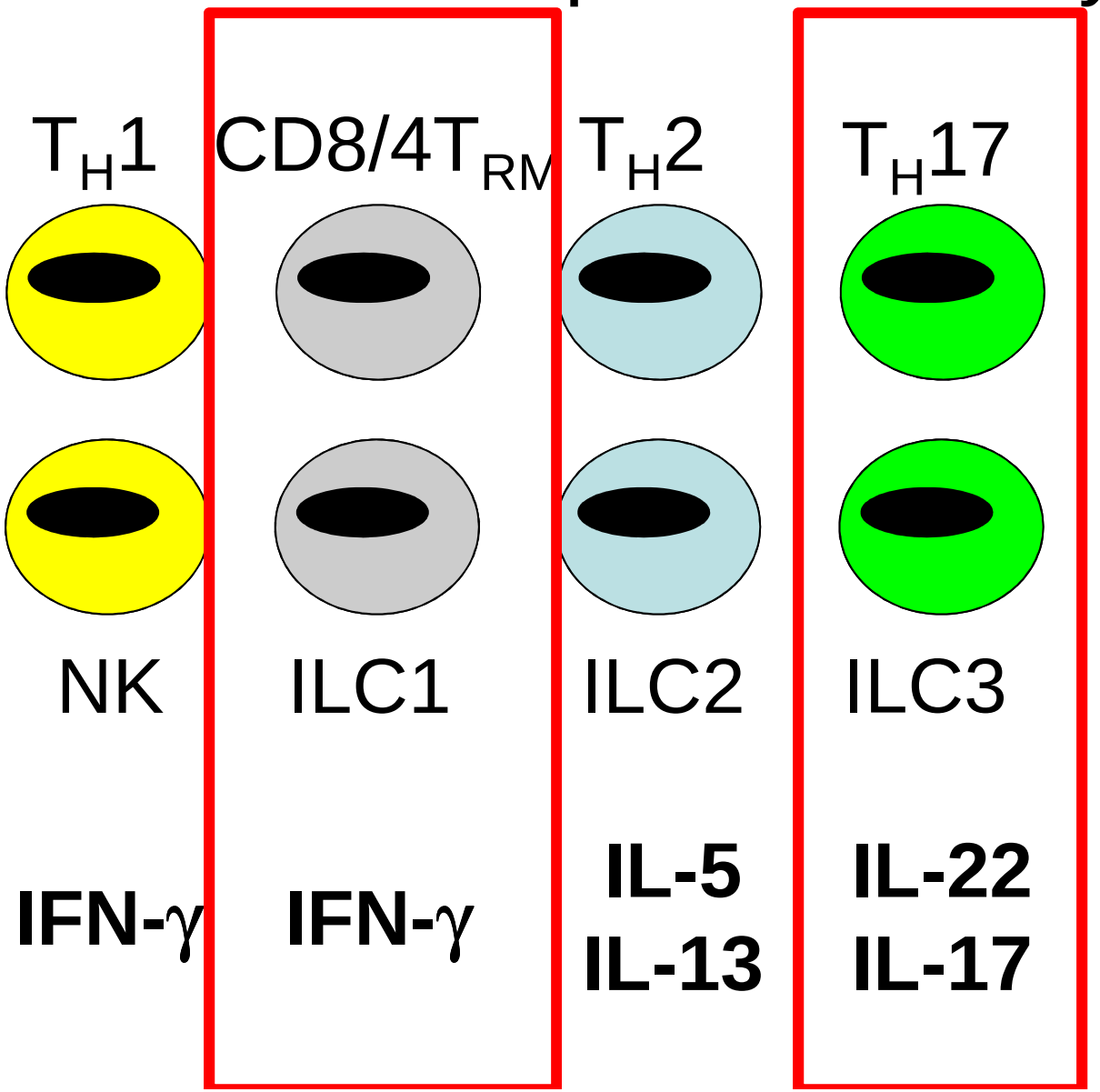
Transcription factors and cytokines in ILC development



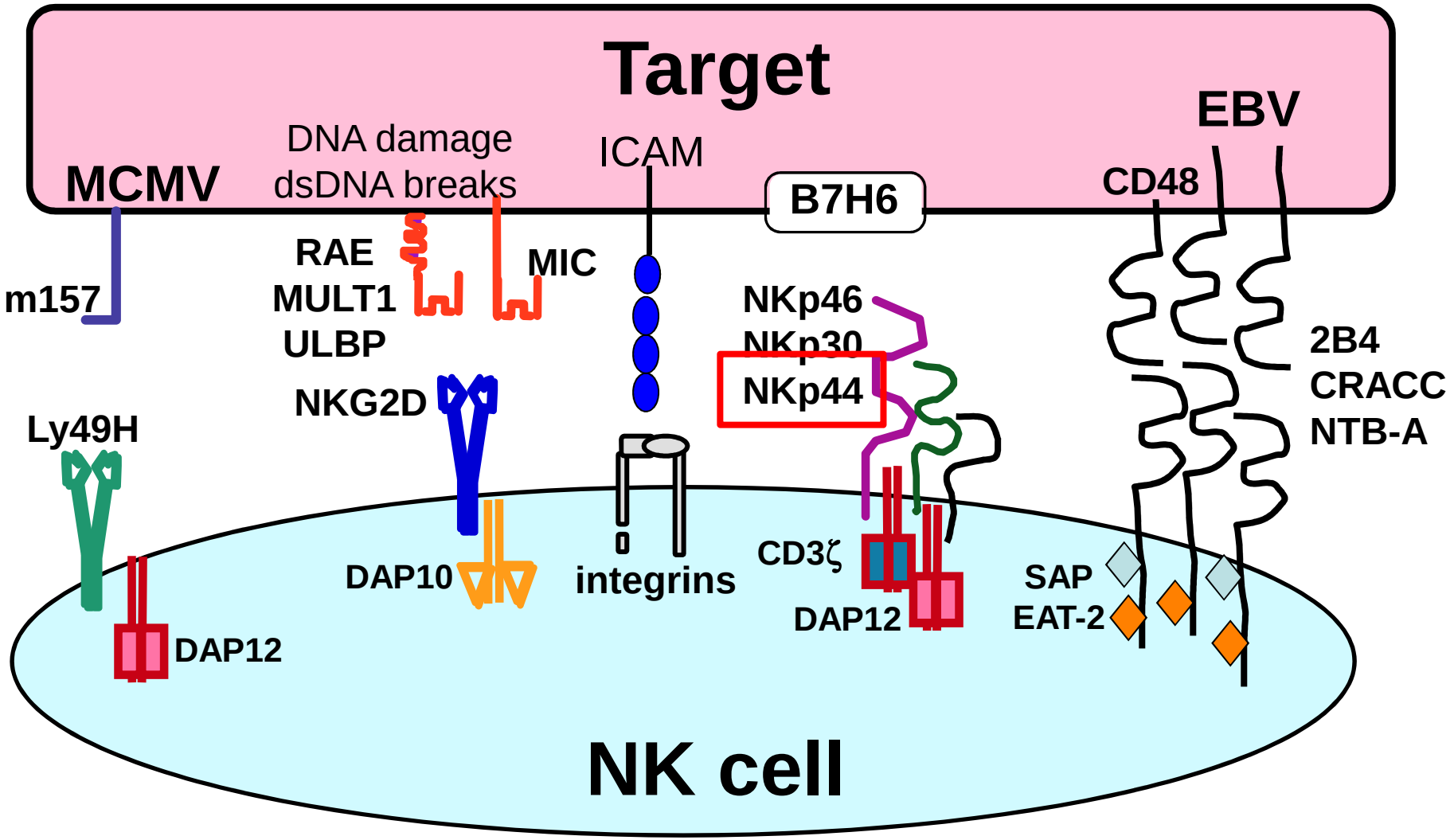
ILCs are tissue resident cells



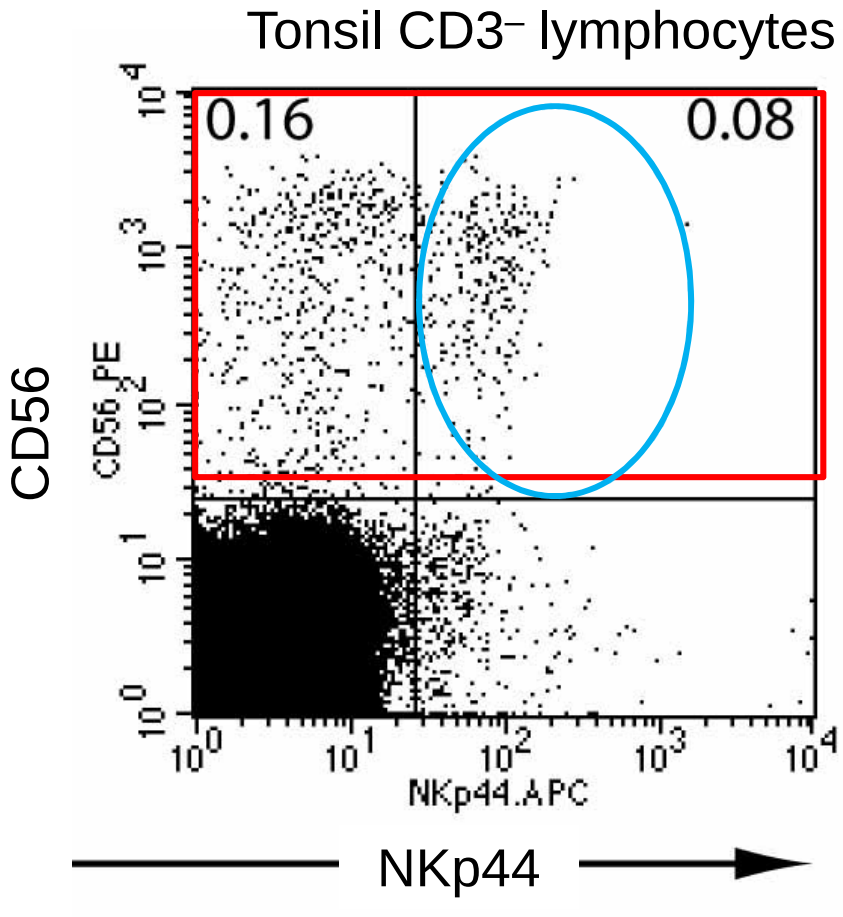
Similarity of functional modules in innate and adaptive immunity



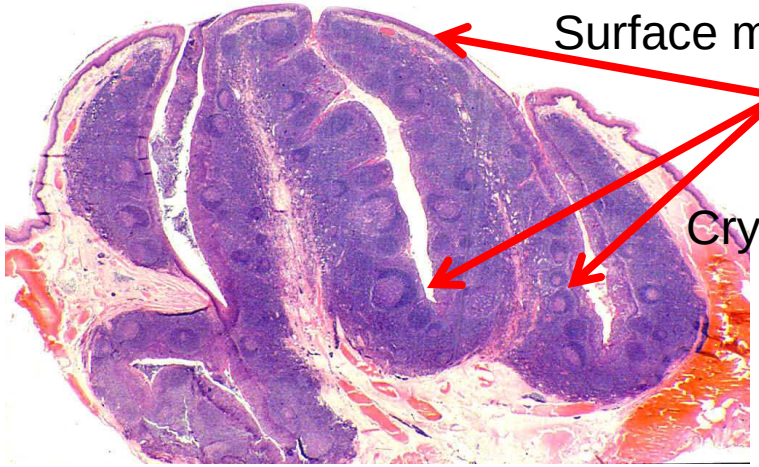
NK cells have multiple receptors

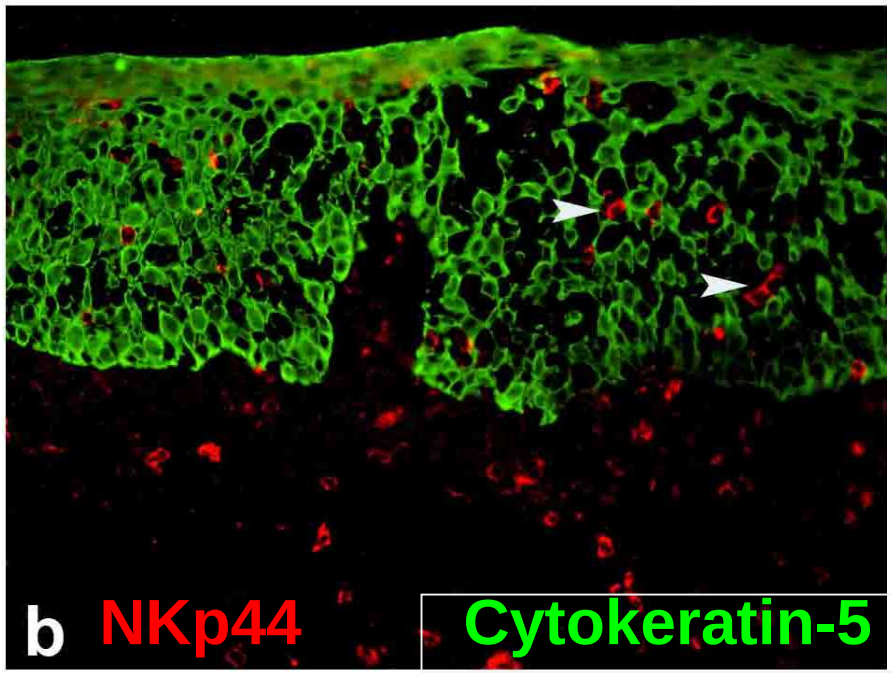


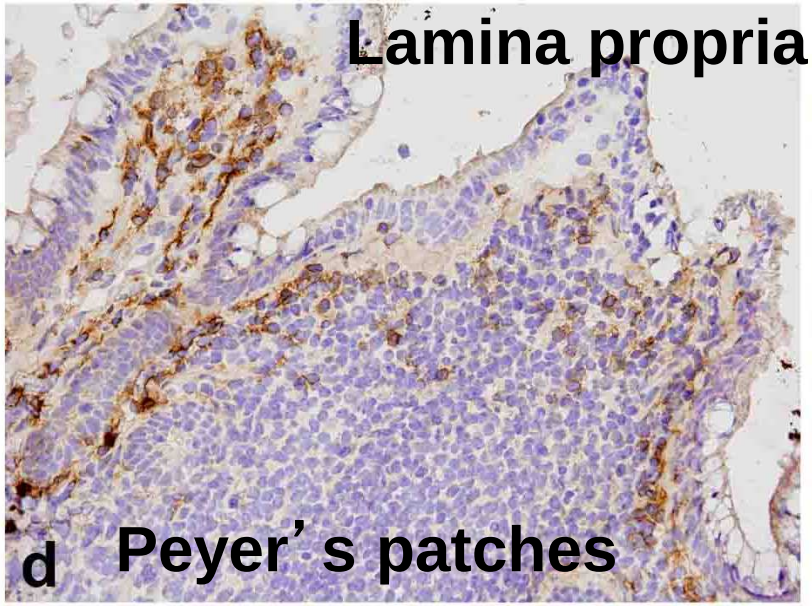
NKp44 is expressed in the human tonsil

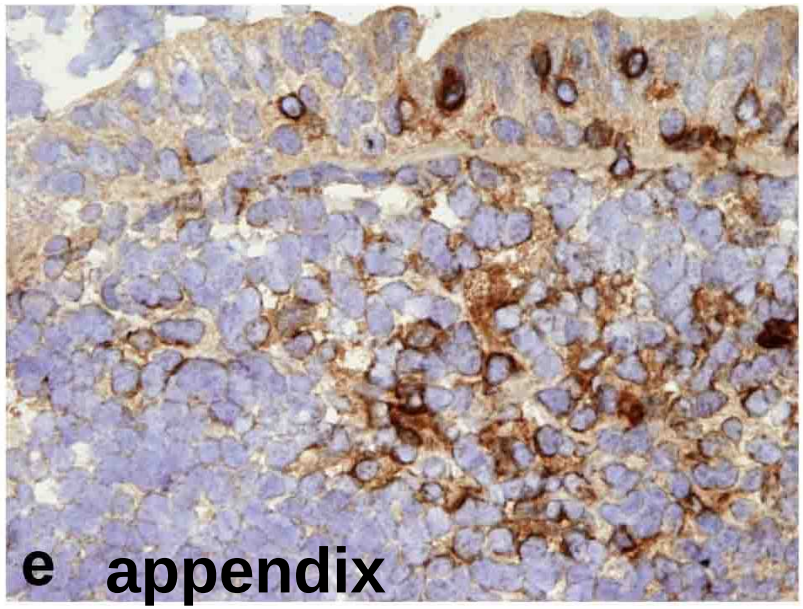


CD56+NKp44+ cells are found in human oral and gastrointestinal mucosae



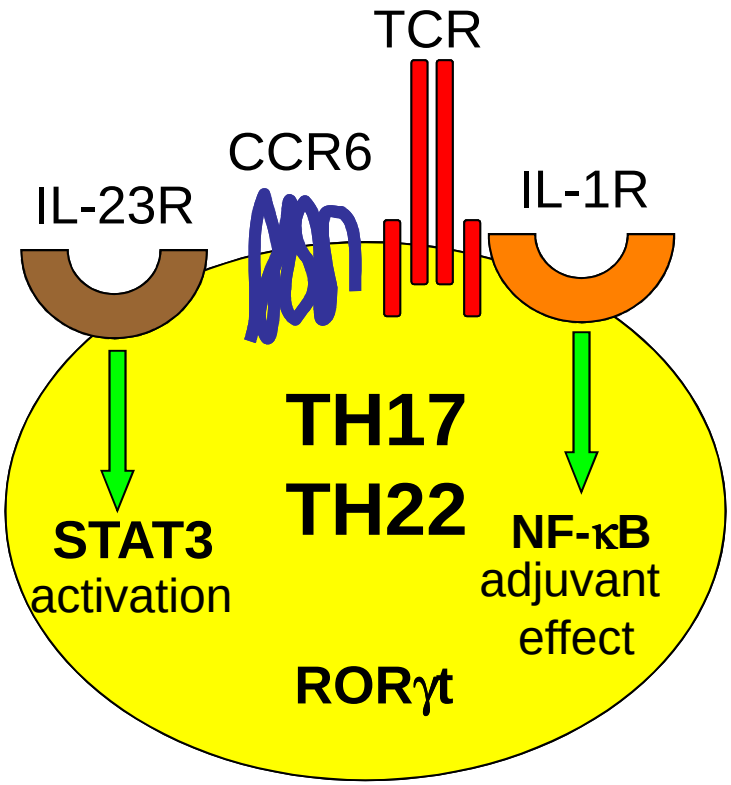






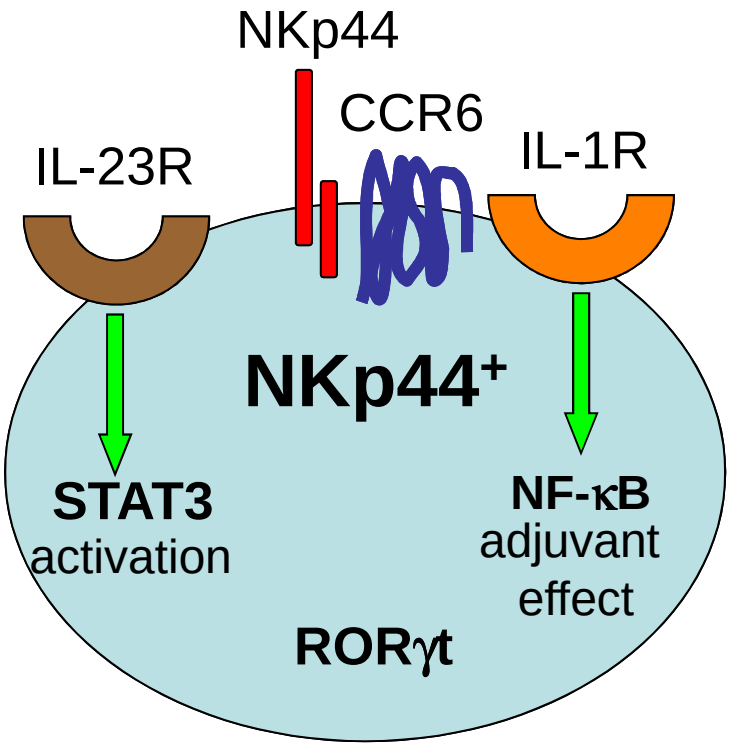
Similarity between human NKp44+ cells and TH17/TH22

Type IL-17/22 CD4 T cells



IL-17 and/or IL-22

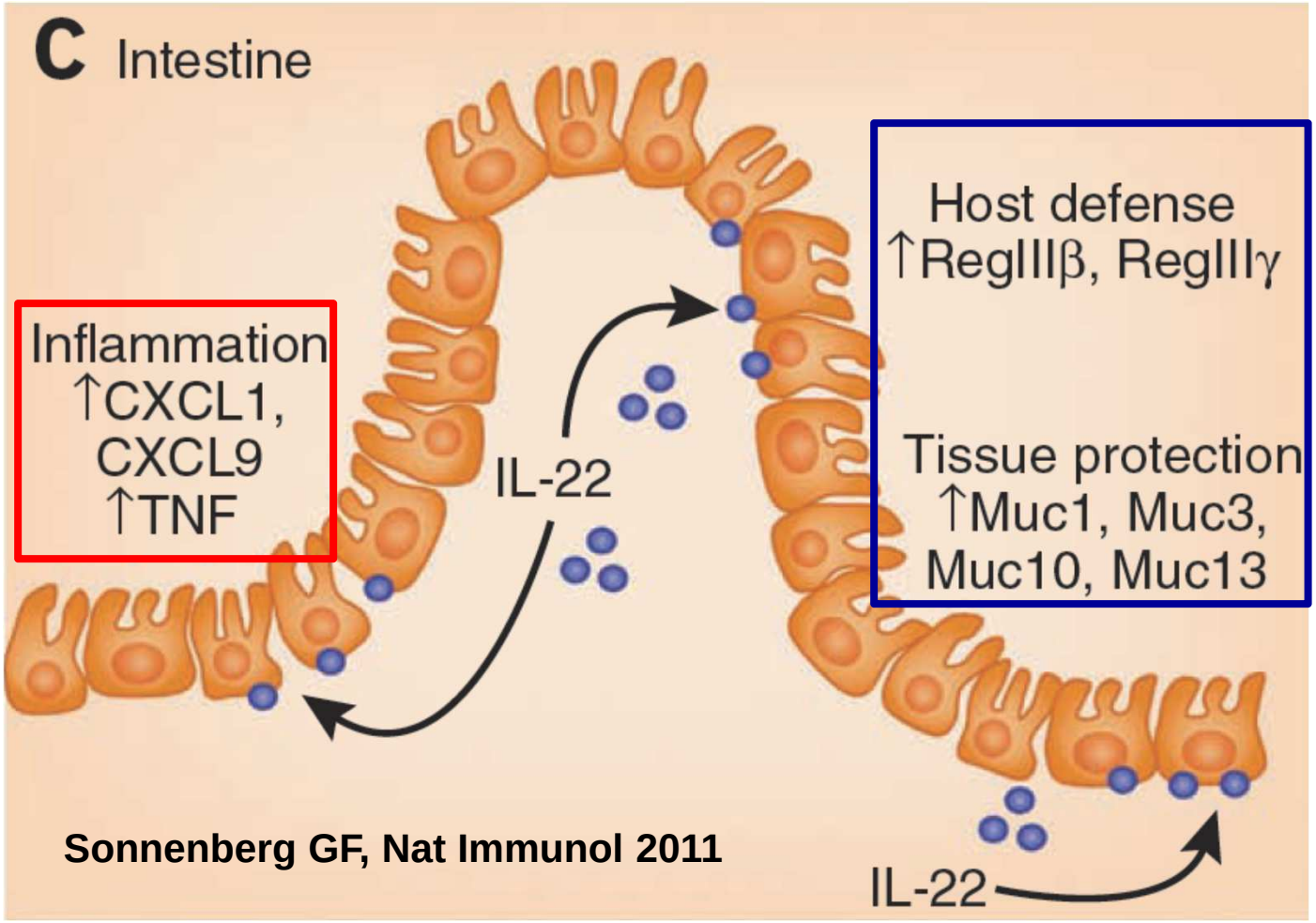
NKp44+ cells Innate lymphoid cells type 3 (ILC3)



IL-22

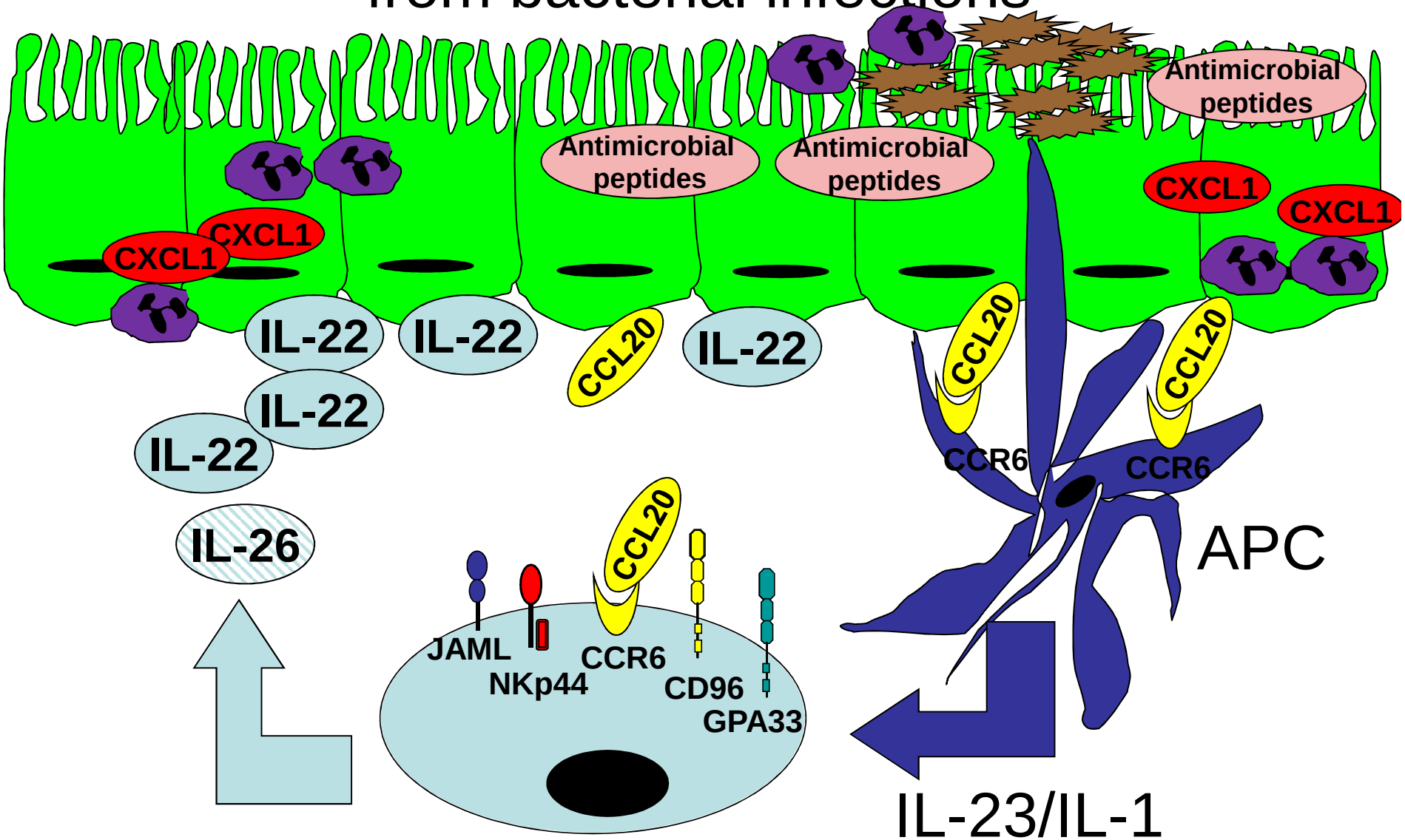
Cella, Fuchs et al. Nature. 2009;457:722

IL-22 triggers epithelial cell secretion of antimicrobial peptides and chemokines



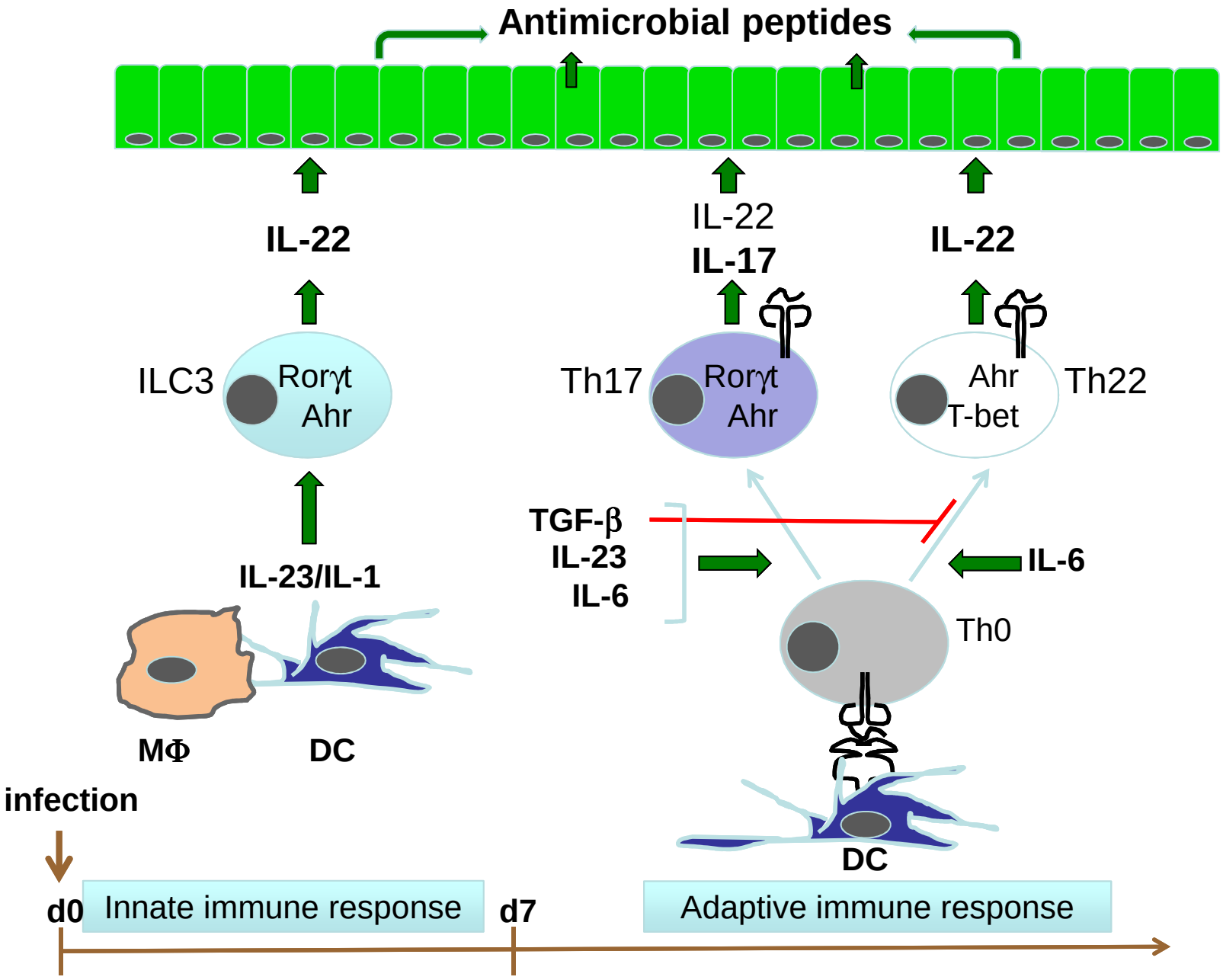
IL-22 protects from bacterial infections but excessive secretion can cause inflammation (psoriasis)

Hypothesis: ILC3 protect the intestinal mucosa from bacterial infections



Cella, Fuchs et al.
Nature. 2009;457:722

Cooperation between ILC3 and T cells



Mouse models for understanding the function of ILC3

- **Genetic models:** *Rag*-/- x γc-/-
- **Antibody depletion in RAG-/- mice**
  - anti-NK1.1 in *Rag*-/- mice
  - anti-CD90 (anti-Thy1) in *Rag*-/- mice

Use of antibodies in *Rag*-/- mice or *Rag*-/- x γc-/- will deplete the cell population in question but will also eliminate other populations as well

Need of lineage-specific deletion mutants

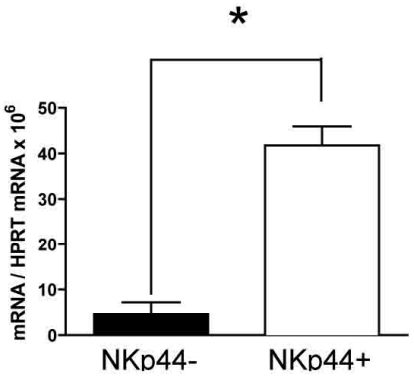
Which transcription factors are required for ILC3 development?

Jacob Lee

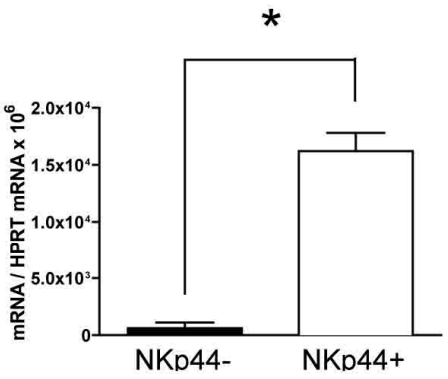
NKp44+ cells express the transcription factor AHR

Retinoic acid-Related Orphan Receptors

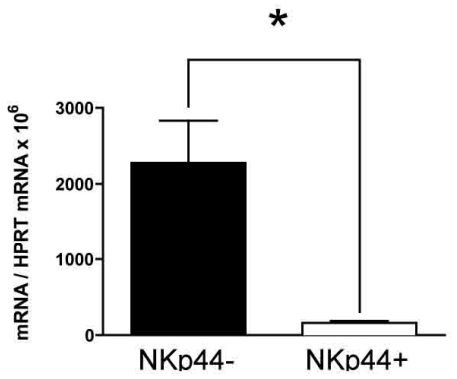
Rora



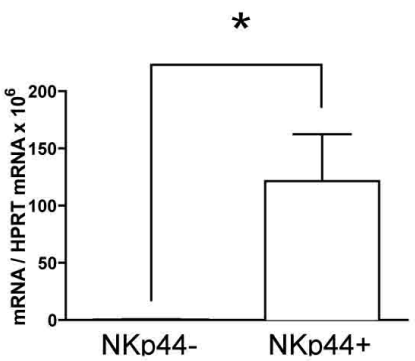
Rorc



Foxp3

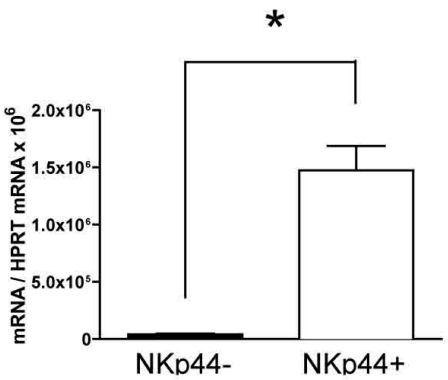


Irf4

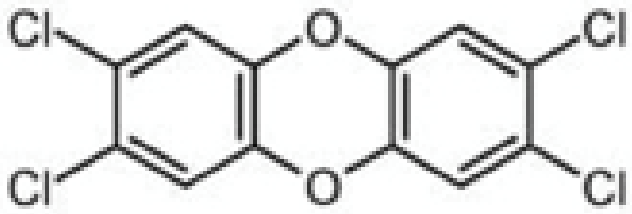


Aryl Hydrocarbon Receptor

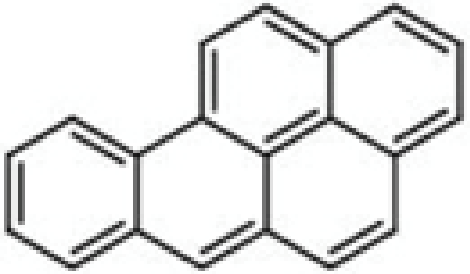
Ahr



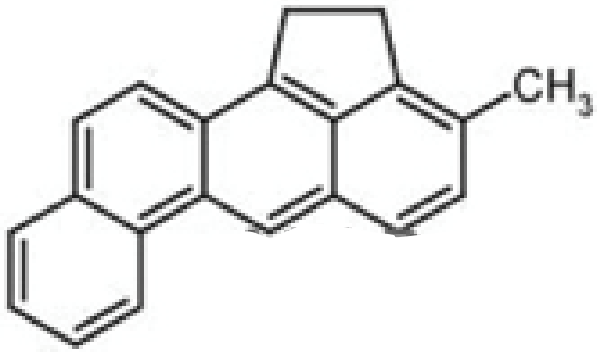
Aryl Hydrocarbon Receptor (AHR): a sensor of polycyclic aromatic hydrocarbons



2,3,7,8-tetrachlorodibenzo-p-dioxin

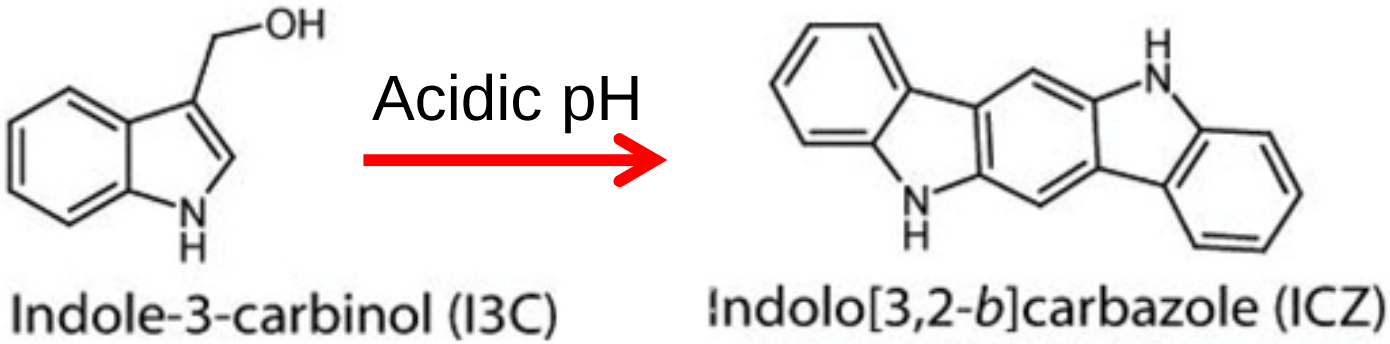


Benzo[a]pyrene



3-methylcholanthrene

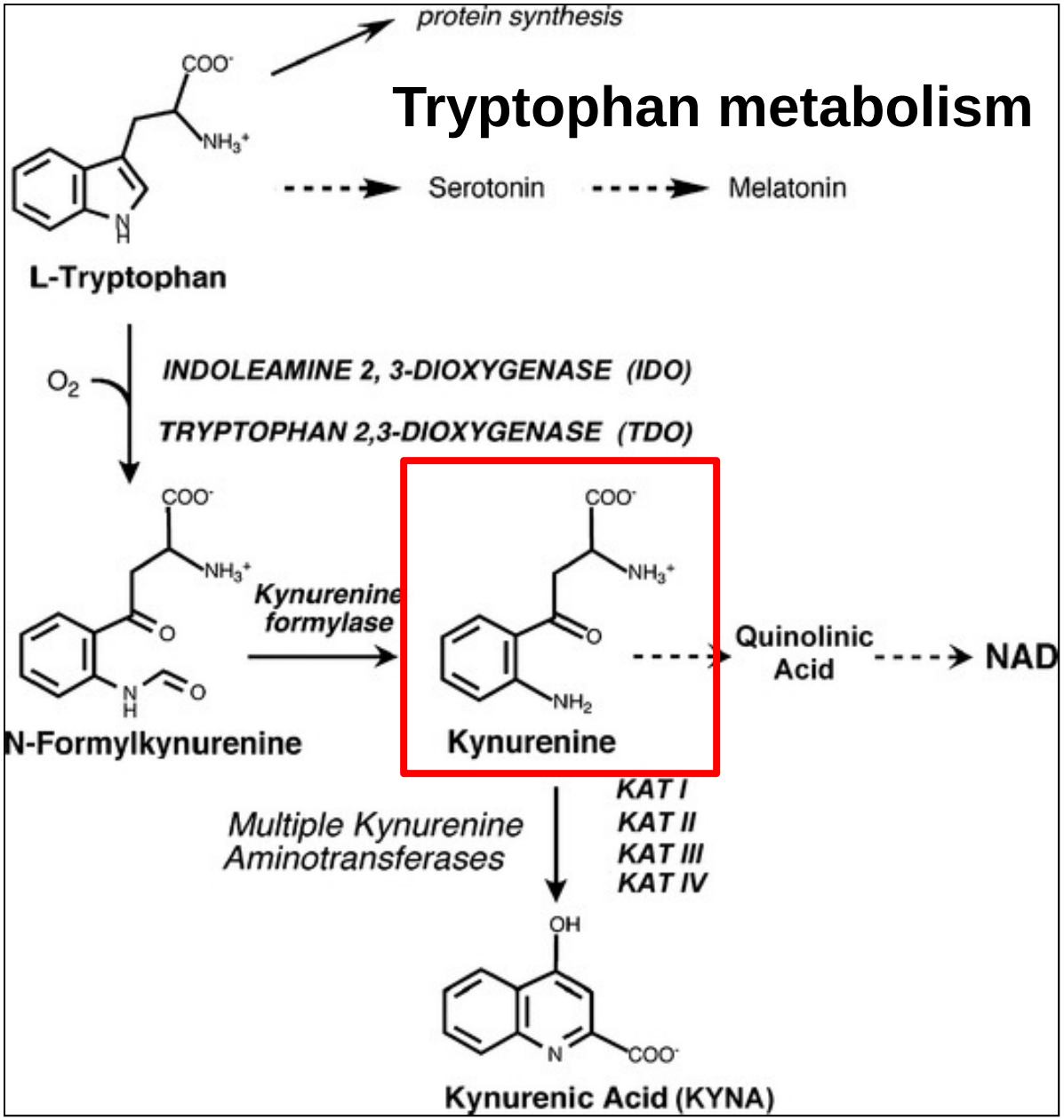
AHR ligands include nutritional components



Indole-3-carbinol (I3C)

Indolo[3,2-*b*]carbazole (ICZ)

Endogenous AHR ligands

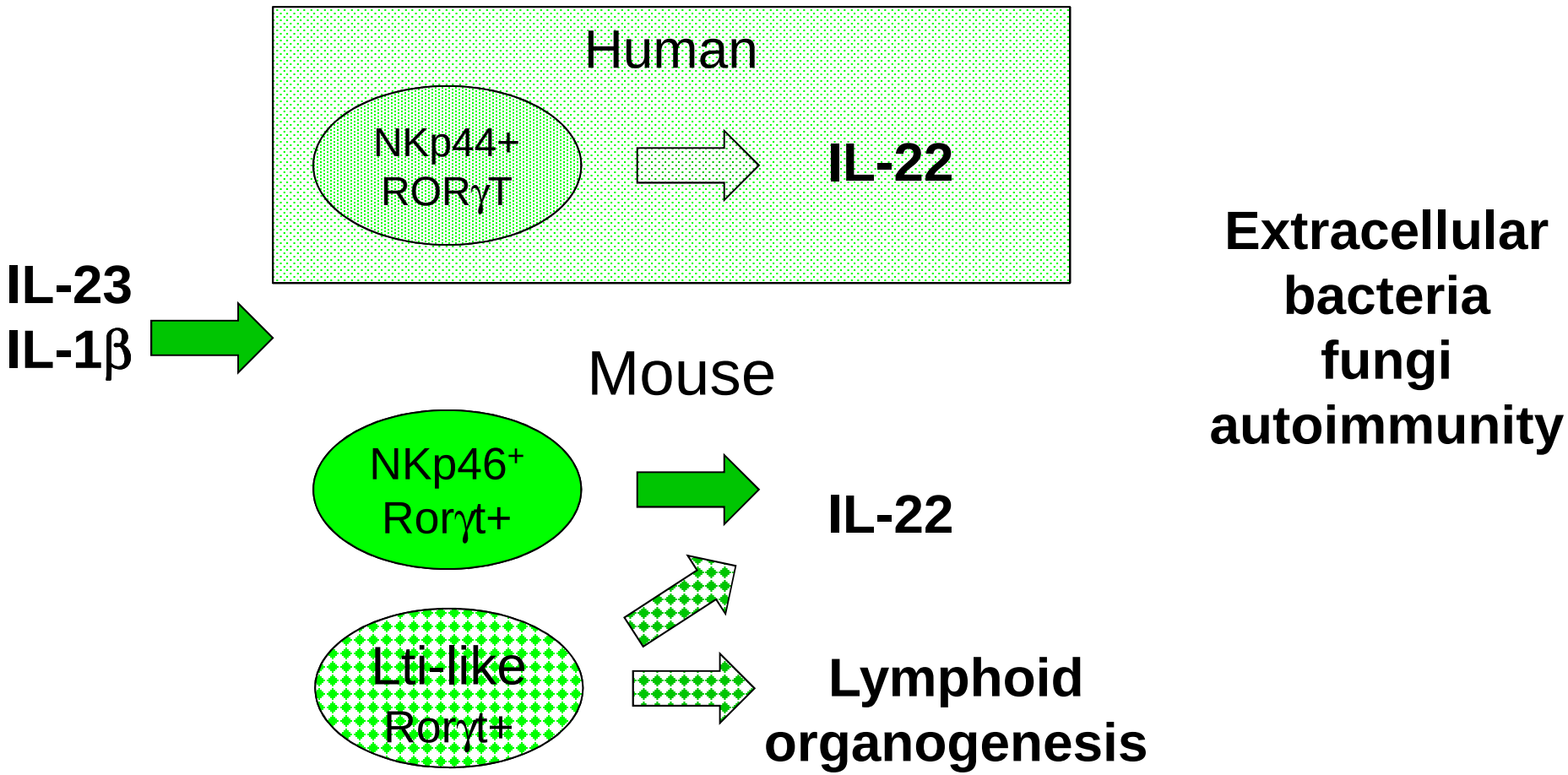


Can we target AHR to block the development of ILC3?

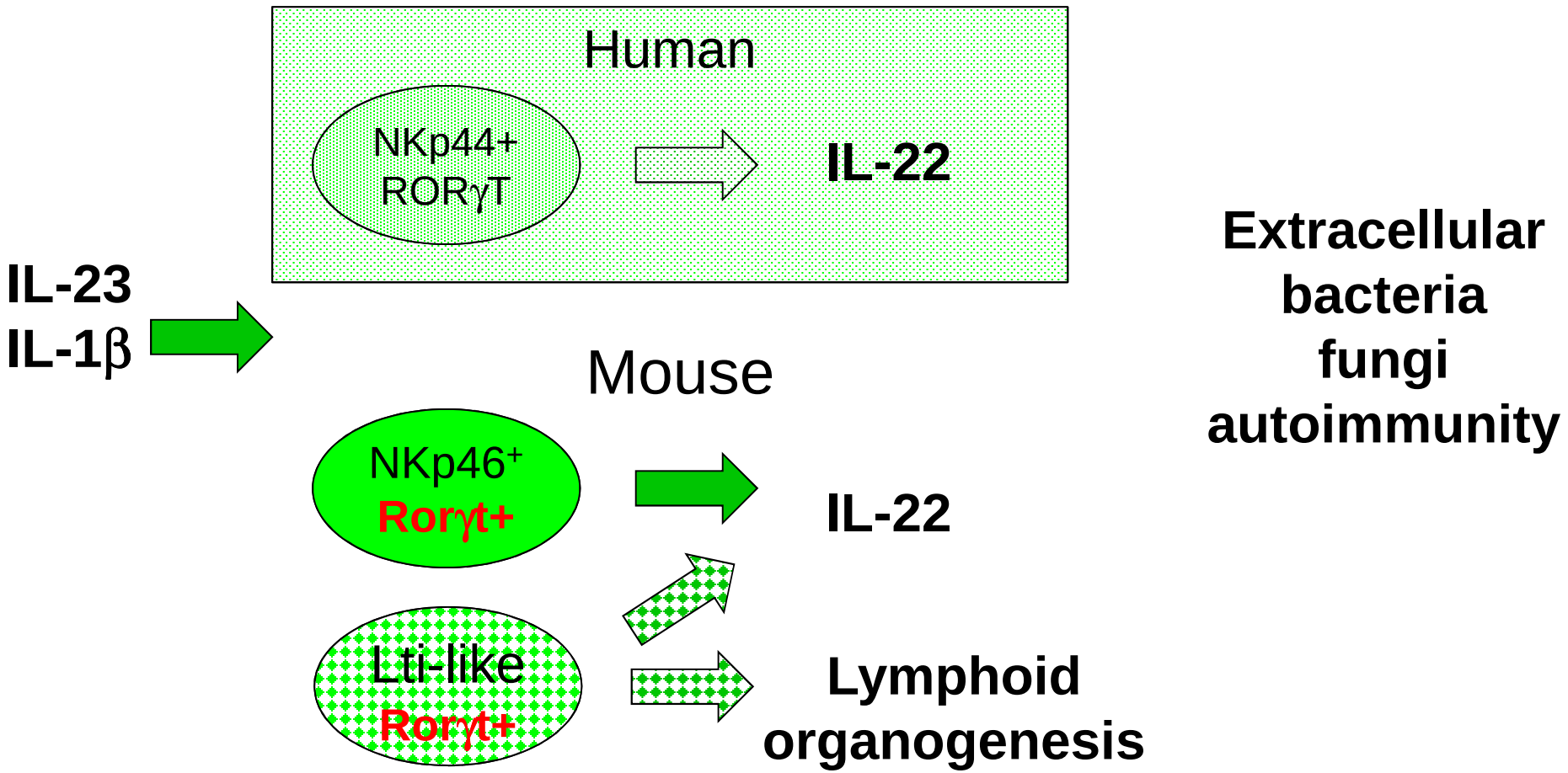
Analysis of conditional *Ahr-/-* mice

Chris Bradfield

ILC3 subsets in mouse small intestine

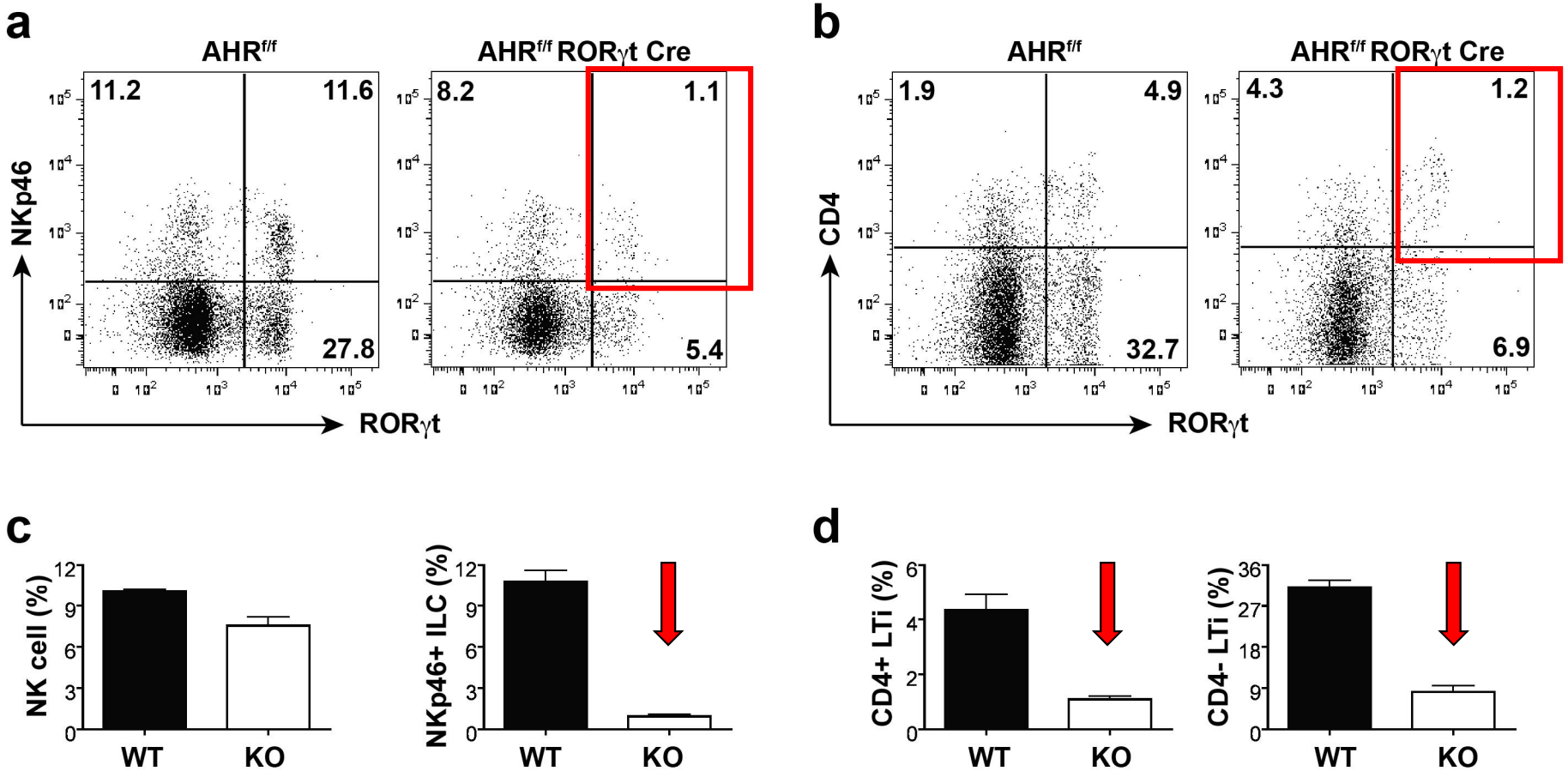


ILC3 subsets in mouse small intestine

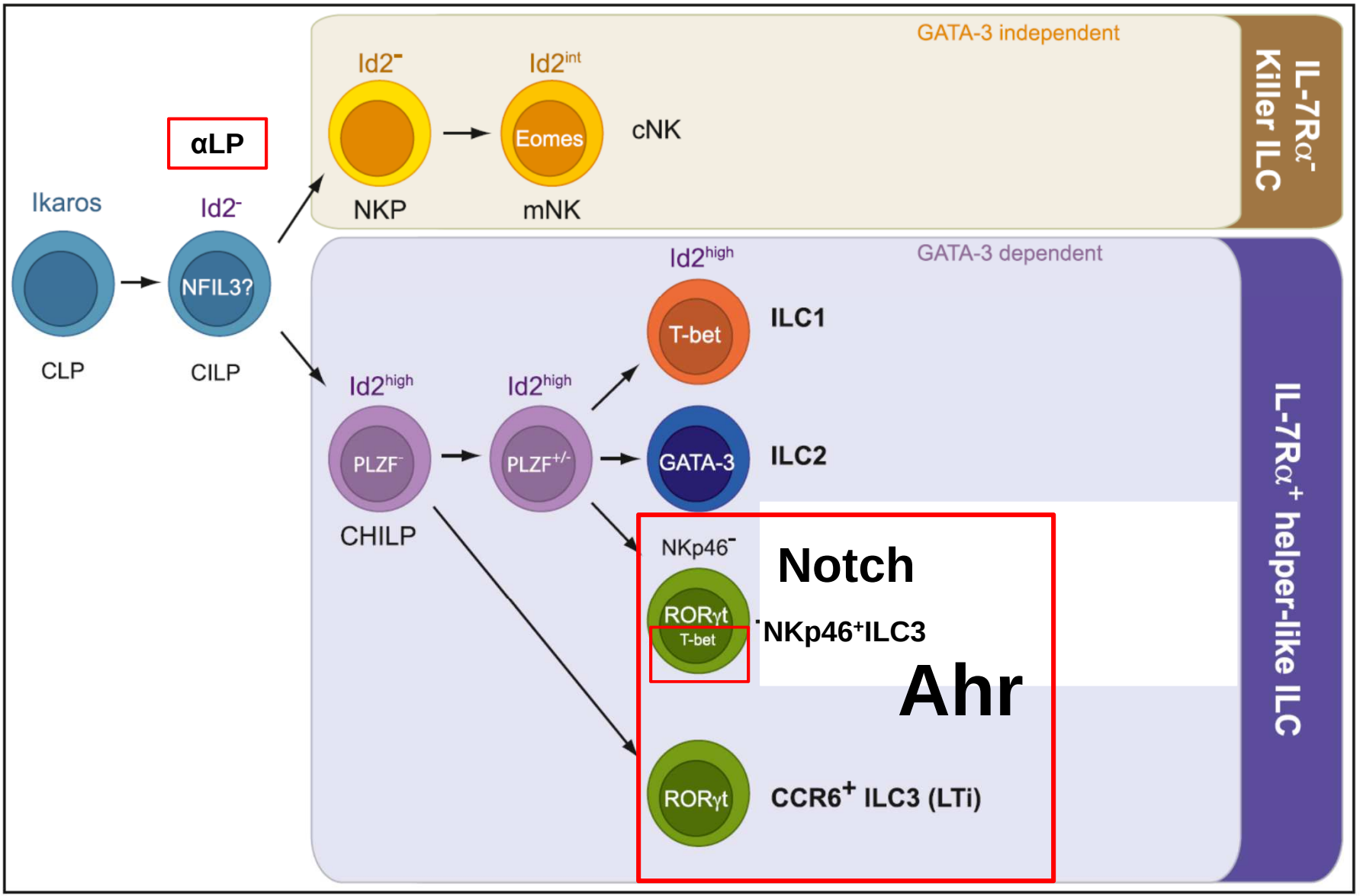


Conditional *Ahr*-deficient mice

$Ahr^{fl/fl}$ x *Roγt-cre*



AHR is required for ILC3 development

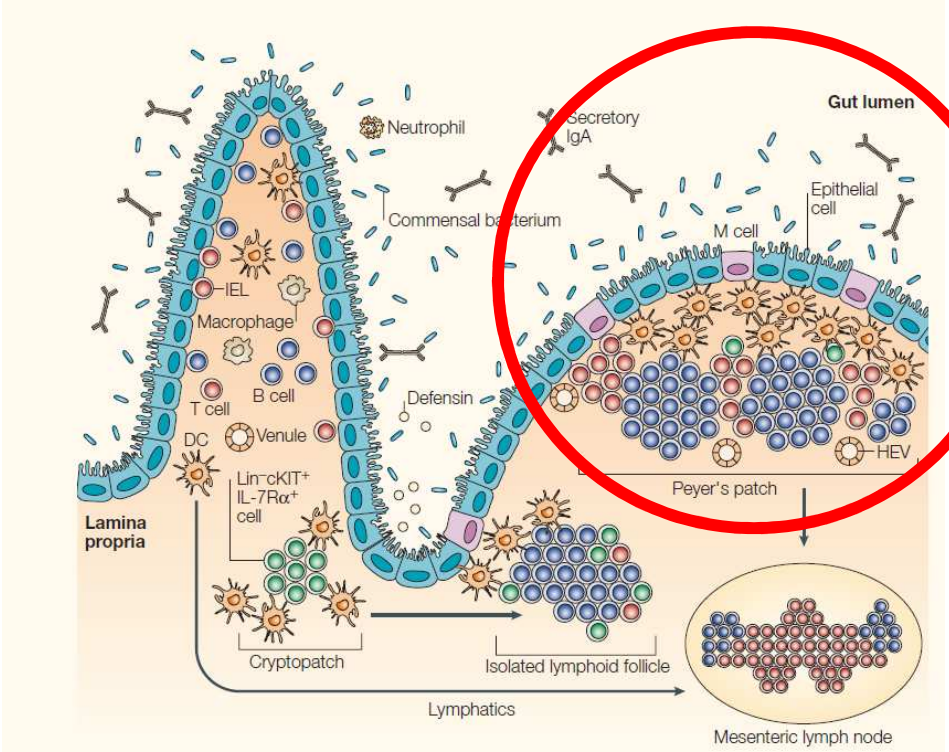


ILC3-deficient mice

Mice	Affected cells
$AHR^{f/f}$ x RORγt-Cre	ILC3
$AHR^{f/f}$ x RORγt-Cre x TCRβδ-/-	ILC3, T cells

Are ILC3 important for intestinal homeostasis?

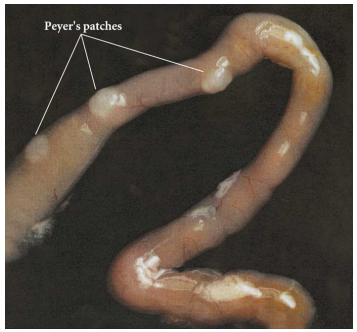
Intestinal lymphoid tissues

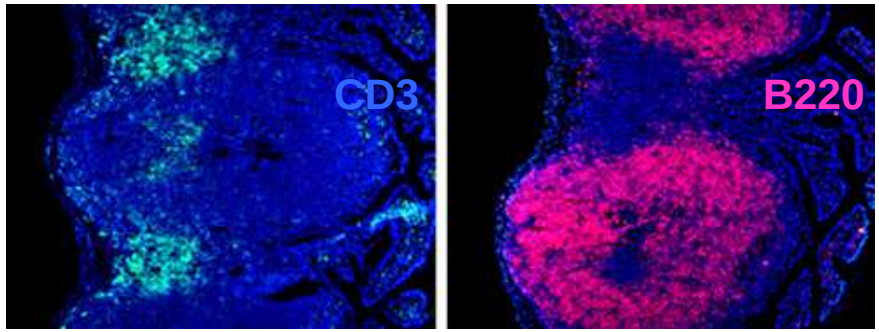


Peyer's Patches

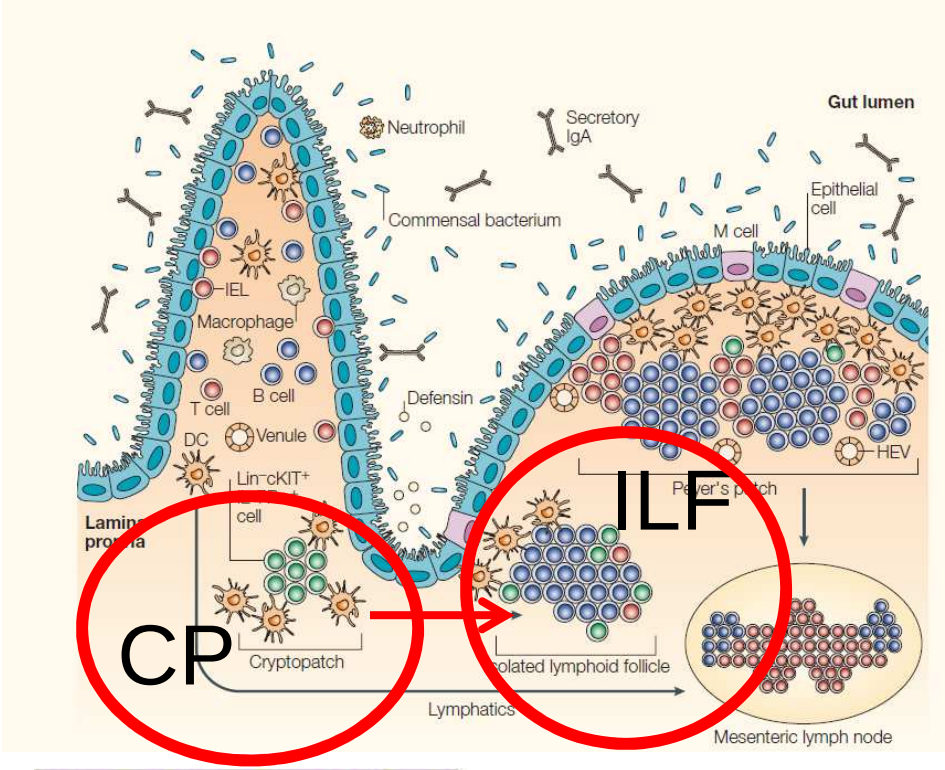
Develop pre-natally

Eberl, Nat Rev Immunol 2005



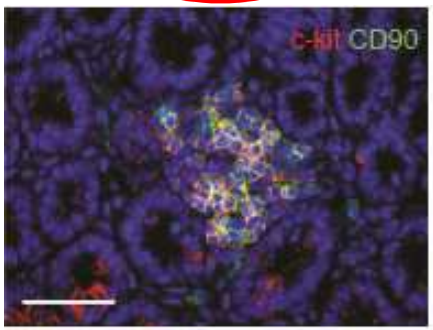


Intestinal lymphoid tissues



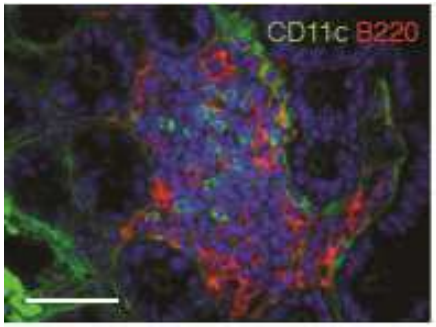
Develop post-natally

Eberl, Nat Rev Immunol 2005



cryptopatches

CD90+(Thy1+) c-Kit+ Lti-ILC



Isolated lymphoid follicles

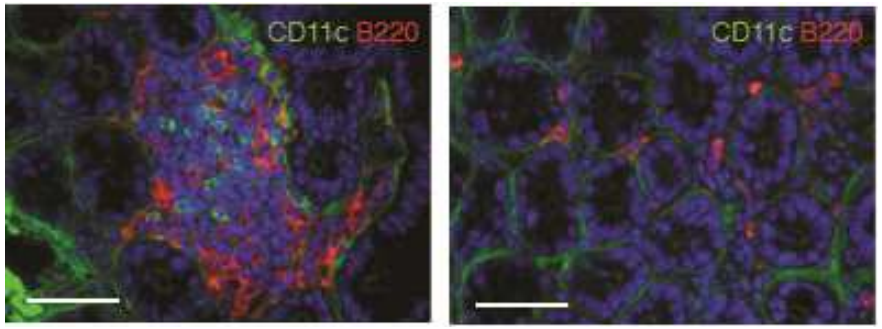
B220+/CD11c+

ILC3-deficient mice lack cryptopatches and isolated lymphoid follicles

WT ILC3-deficient

- CD90+ (Thy1+) clusters identifying CP are absent in

Prenatal lymphoid tissues (Peyer's Patches) are conserved



- CD11c+B220+ clusters identifying ILFs are absent in Ahr-deficient mice

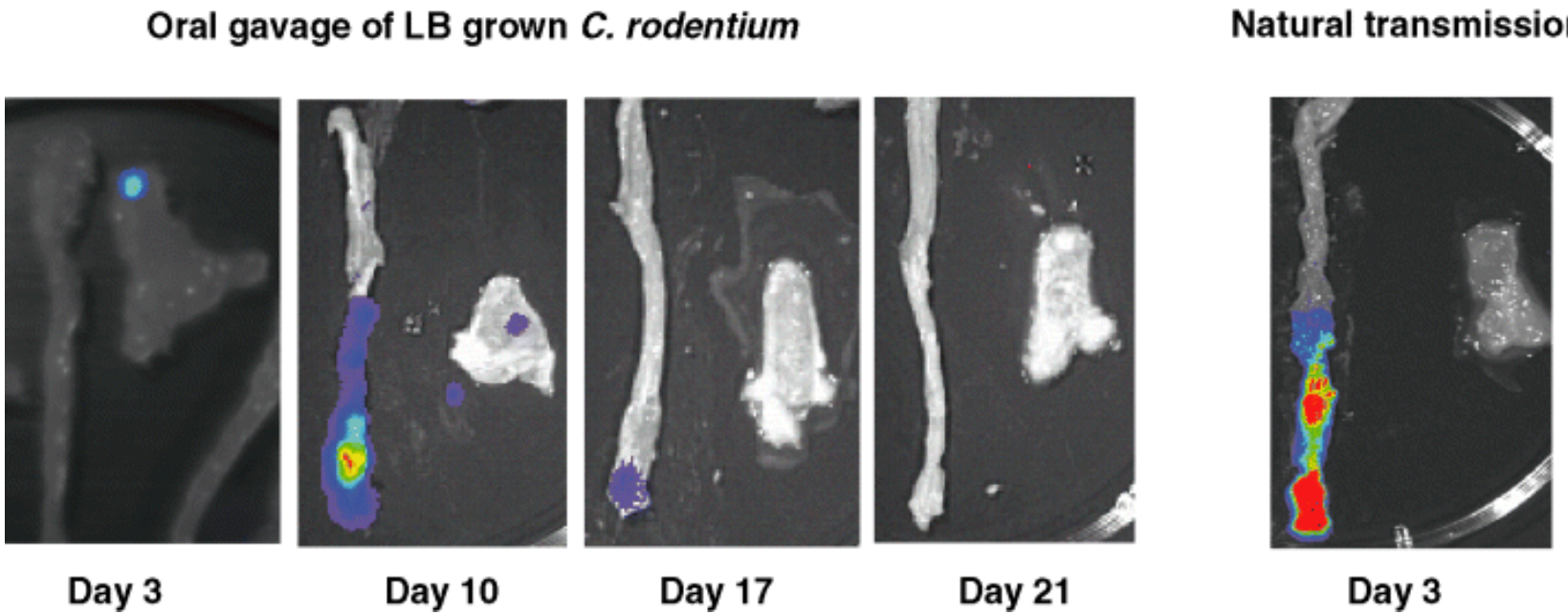
Rodney Newberry
Keely McDonald

Are ILC3 important in host response to pathogenic bacteria?

Christina Song

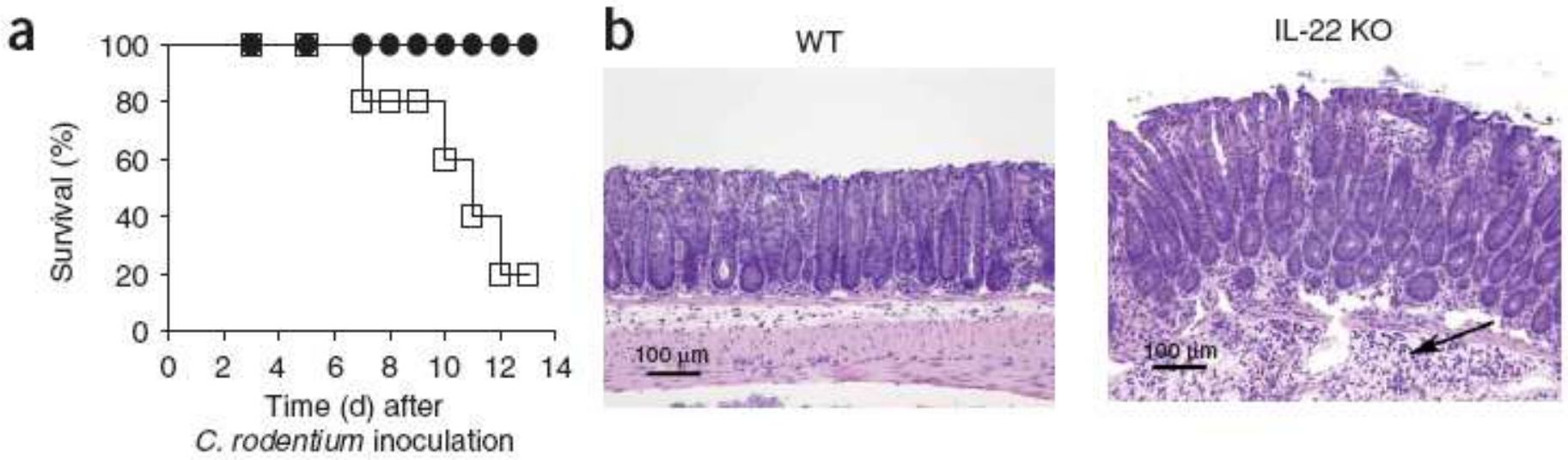
Citrobacter rodentium infection

- *C. rodentium* is a natural mouse pathogen related to enteropathogenic *E. coli*
- The infection is restricted to the lumen of the intestine
- In B6 mice, *C. rodentium* infection initiates on the surface of caecal patches, peaks at d7-14 pi and is cleared by d21-28 pi.



Mundy R et al. Cell Microbiol. 2005;7:1697

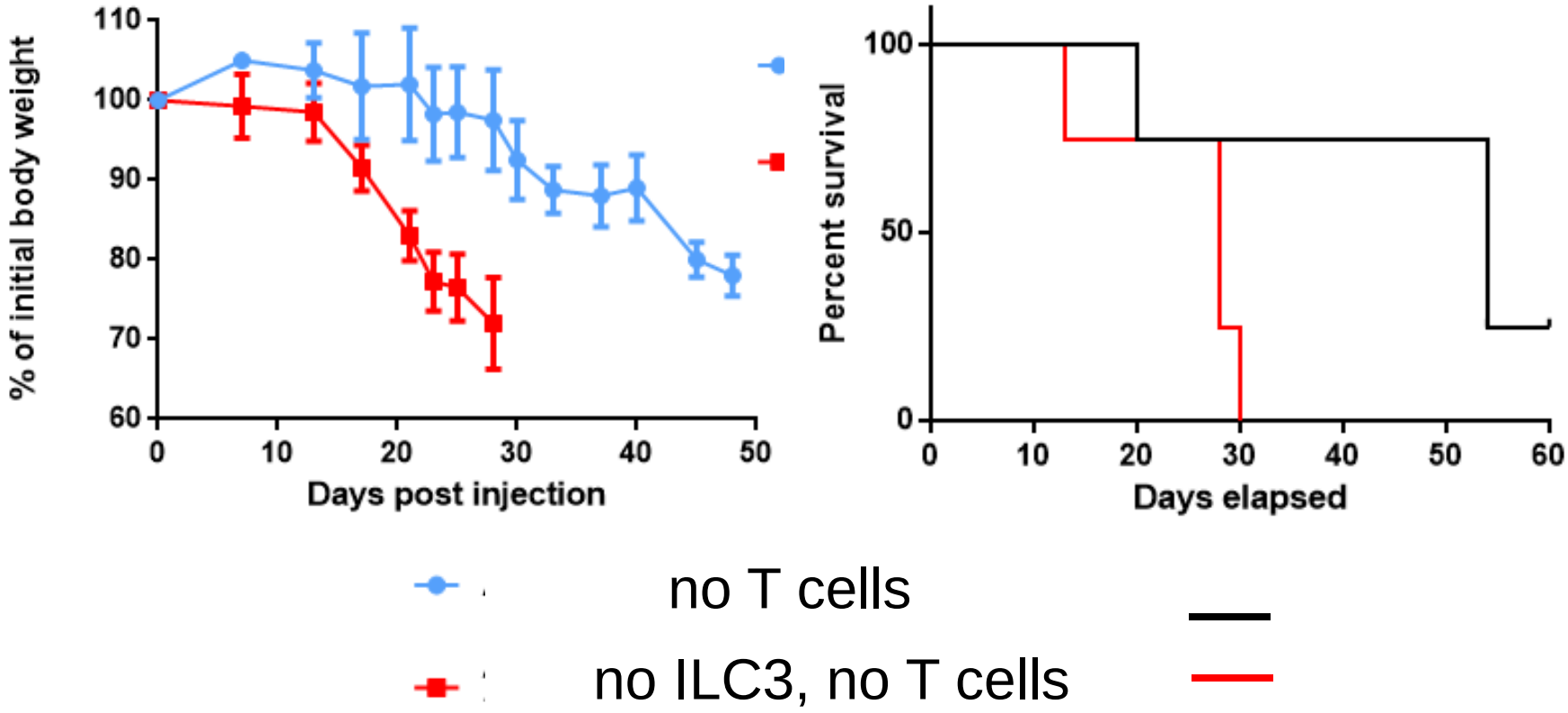
Host defense against *C. rodentium* infection depends on IL-22



Zheng Y, et al. Nat Med. 2008;14:282.

C. rodentium infection in the absence of ILC3 and T cells

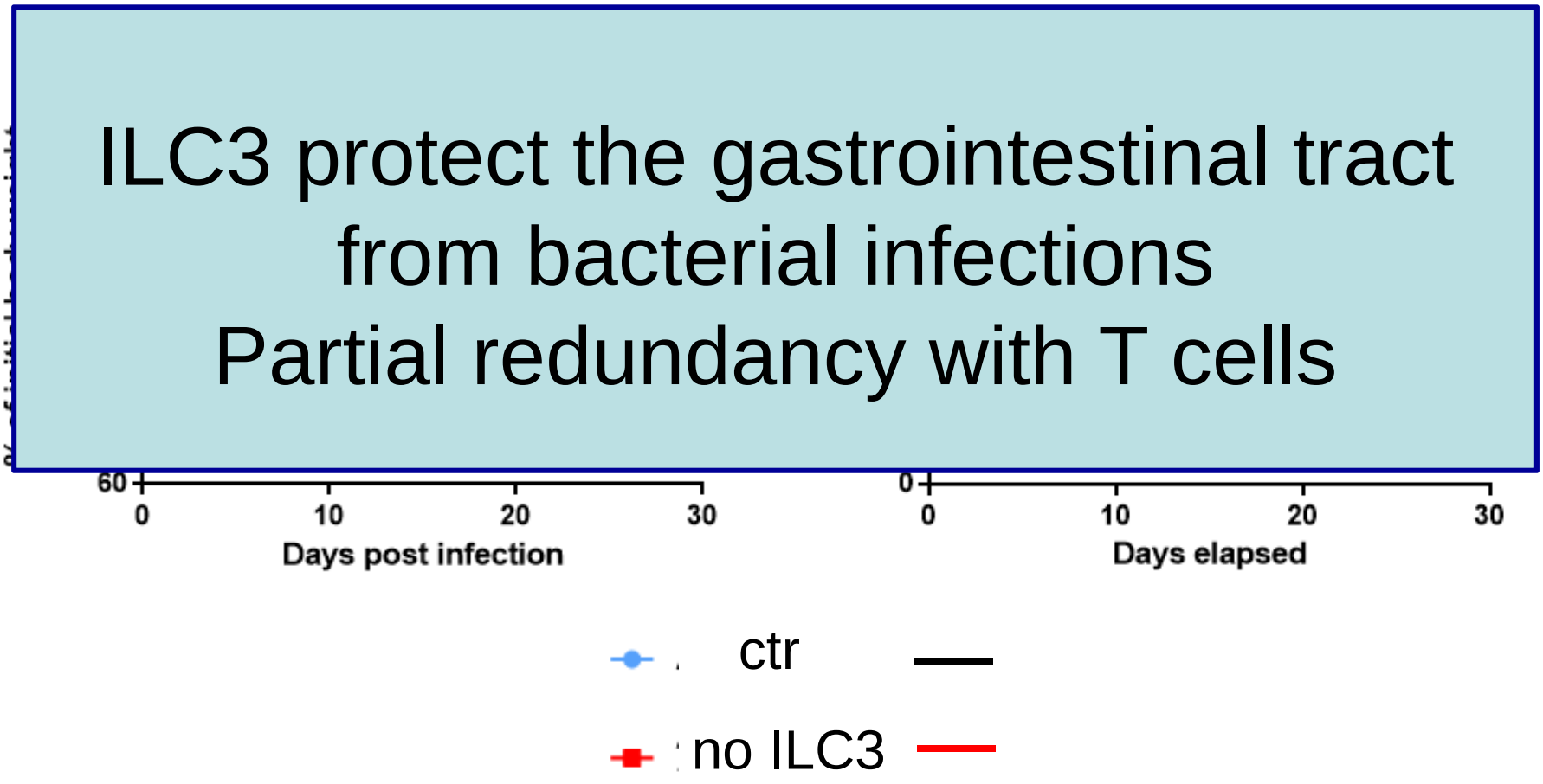
AHR$^{f/f}$ x RORγt-cre x TCRβ/δ ko (no ILC3, no T cells) vs
AHR$^{f/f}$ x TCRβ/δ-/- (no T cells)



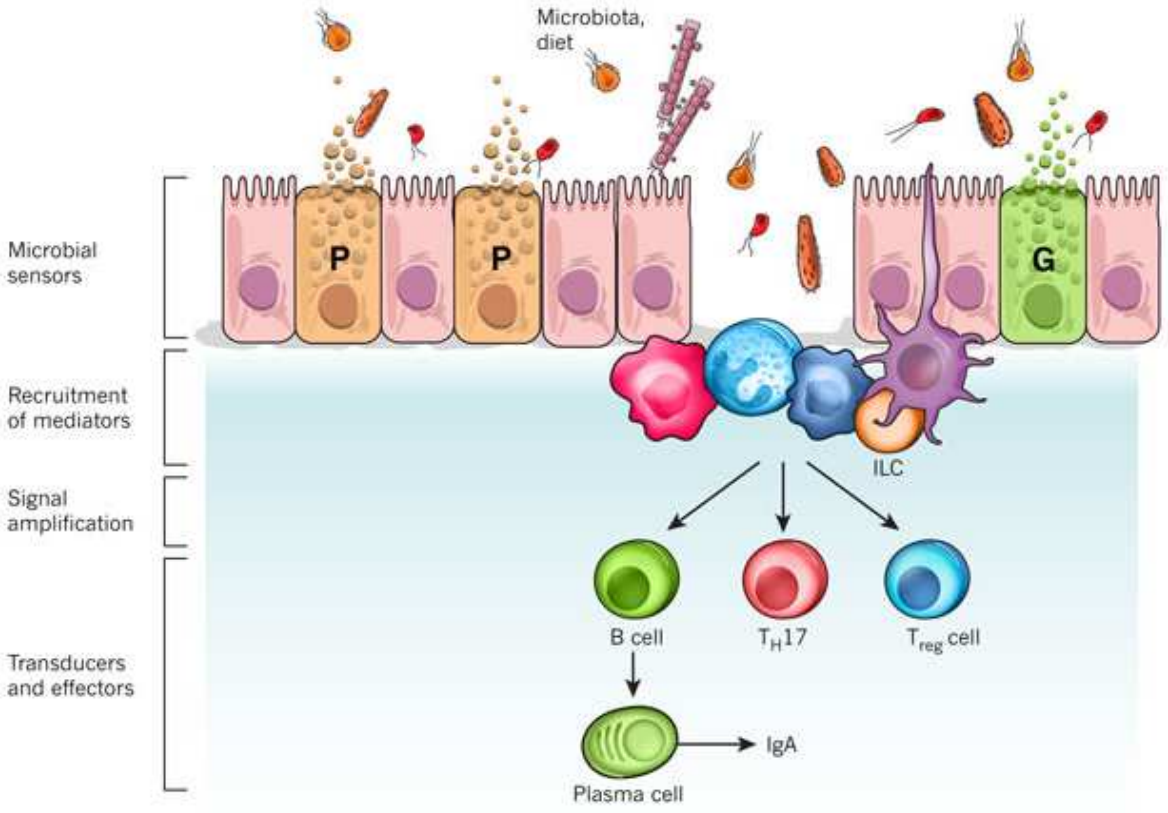
C. rodentium infection in mice lacking ILC3 only

$AHR^{f/f}$ x RORγt-cre (no ILC3) vs
$AHR^{f/f}$

ILC3 protect the gastrointestinal tract
from bacterial infections
Partial redundancy with T cells



Do ILC3 contribute to IBD?



IBD-related processes

Epithelial barrier
GNA12, HNF4A, CDH1, ERRFI1, MUC19, ITLN1**

Restitution
REL, PTGER4, NKX2-3, STAT3, ERRFI1, HNF4A, PLA2G2A/E

Solute transport
SLC9A4, SLC22A5, SLC22A4, AQP12A/B, SLC9A3, SLC26A3*

Paneth cells
ITLN1, NOD2*, ATG16L1*, XBP1**

Innate mucosal defence
NOD2, ITLN1*, CARD9*, REL, SLC11A1, FCGR2A*/B*

Immune cell recruitment
*CCL11/CCL2/CCL7/CCL8, CCR6, IL8RA/IL8RB, MST1**

Antigen presentation
ERAP2, LNPEP, DENND1B*

IL-23/T_H17
IL23R, JAK2, TYK2*, STAT3, ICOSLG, IL21, TNFSF13*

IL-23R
STAT3

T-cell regulation
NDFIP1, TNFSF8, TAGAP, IL2, IL2RA TNFRSF9, PIM3, IL7R, IL12B, IL23R*, PRDM1, ICOSLG, TNFSF8, IFNG, IL21*

B-cell regulation
IL5, IKZF1, BACH2, IL7R, IRF5*

Immune tolerance
IL10, IL27, SBNO2, CREM, IL1R1/IL1R2, NOD2**

Cellular responses

Autophagy
ATG16L1, IRGM, NOD2*, LRRK2, CUL2, PARK7, DAP*

ER stress
*CPEB4, ORMDL3, SERINC3, XBP1**

Intracellular logistics
VAMP3, KIF21B, TTLL8, FGFR1OP, CEP72, TPPP

Cell migration
ARPC2, LSP1, AAMP

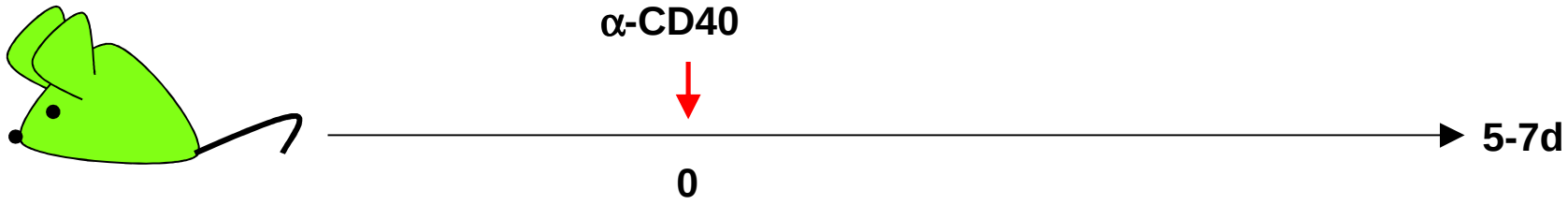
Apoptosis/necroptosis
FASLG, THADA, DAP, PUS10, MST1**

Carbohydrate metabolism
GCKR, SLC2A4RG*

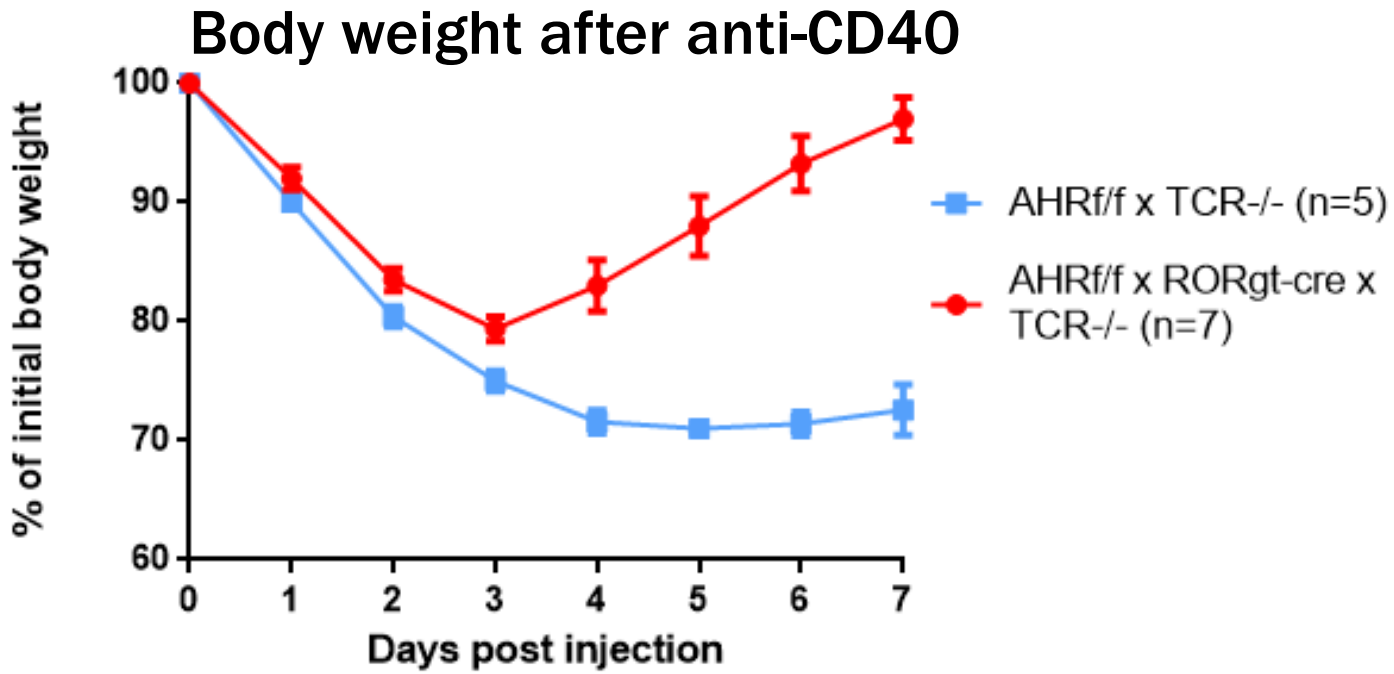
Oxidative stress
PRDX5, BACH2, ADO, GPX4, GPX1, SLC22A4, LRRK2, NOD2*, CARD9*, HSPA6, DLD, PARK7, UTS2*, PEX13*

UC
CD
UC/CD
cis-eQTL
*Coding mutation

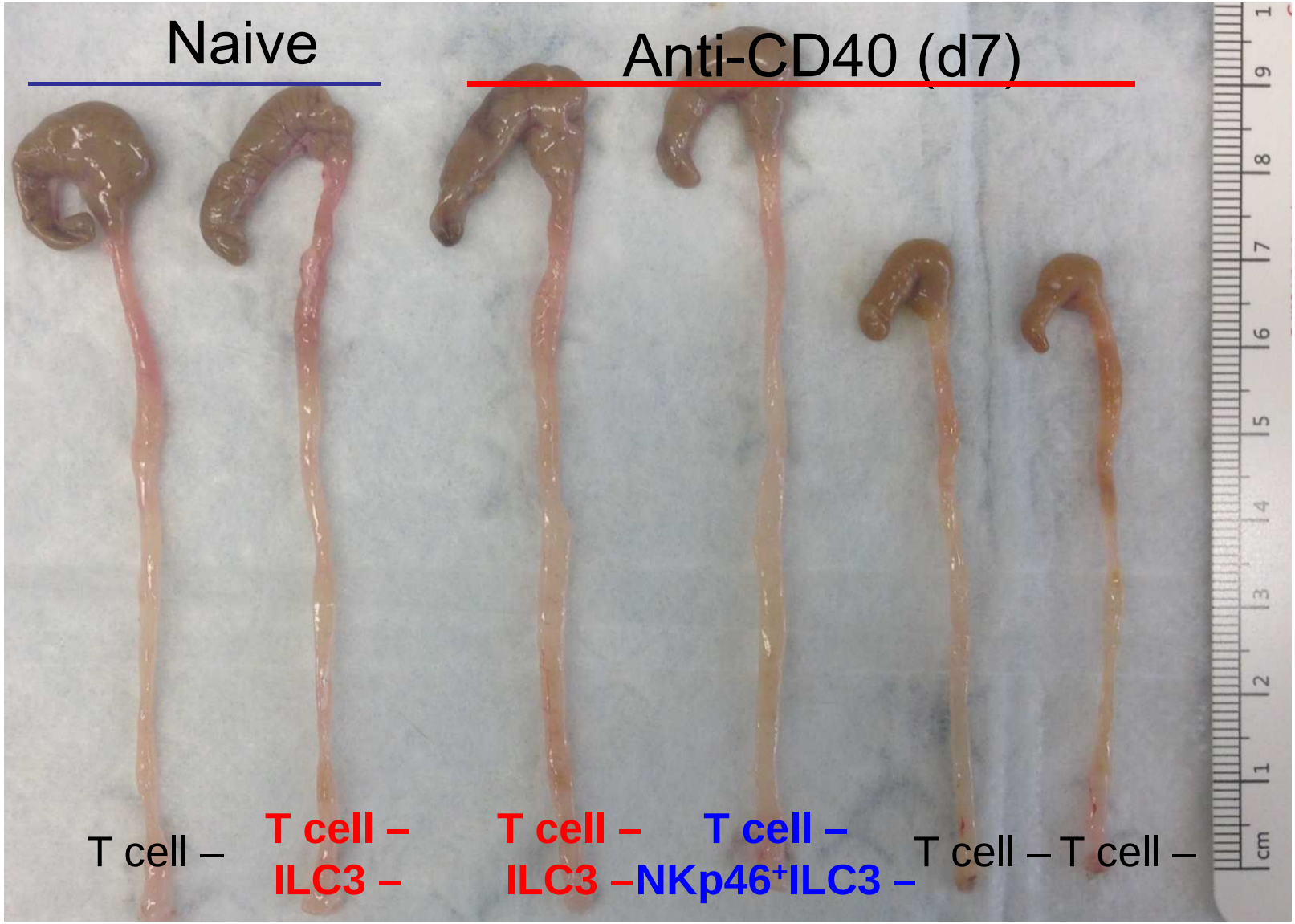
ILC3 worsen anti-CD40-induced colitis



AHR$^{f/f}$ x RORγt-cre x TCRβ/δ ko (**no ILC3, no T cells**) vs
AHR$^{f/f}$ x TCRβ/δ-/- (**no T cells**)



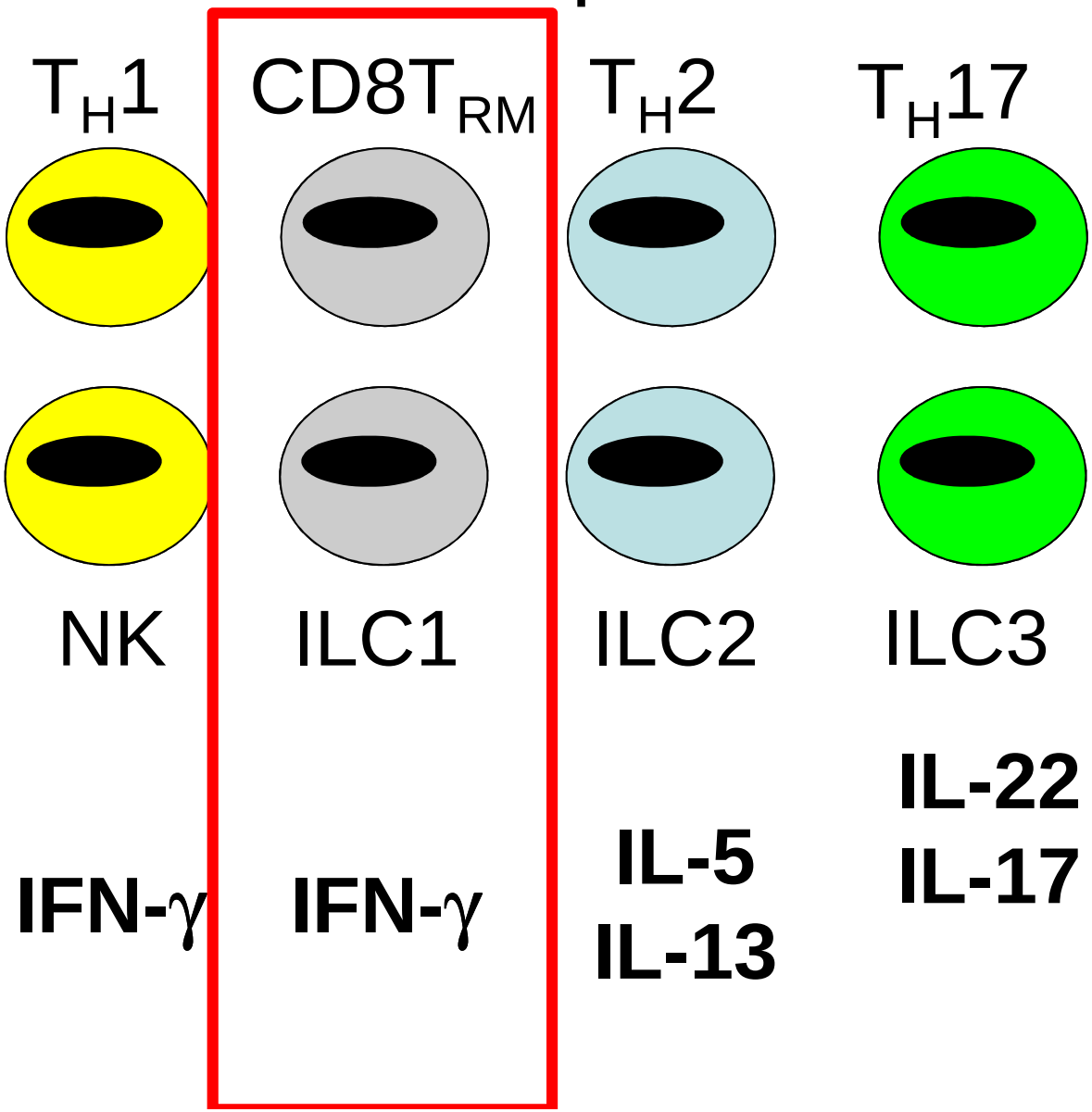
Colon length 7 days after anti-CD40



ILC3: two-edged sword

- Induce the development of ILF but have no obvious role in containment of commensal microbiota
- Protect from *C. rodentium* infections together with T cells
- Promote inflammation in a model of IBD

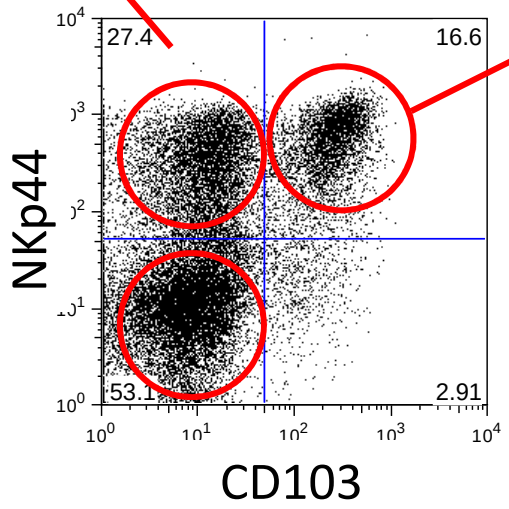
Similarity of functional modules in innate and adaptive immunity



Human tonsils harbor 2 major subsets of NKp44^{+} ILCs

Produce IL-22 upon stimulation with IL-23

NKp44^{+} ILC3



Localized within tonsil surface and crypt epithelium

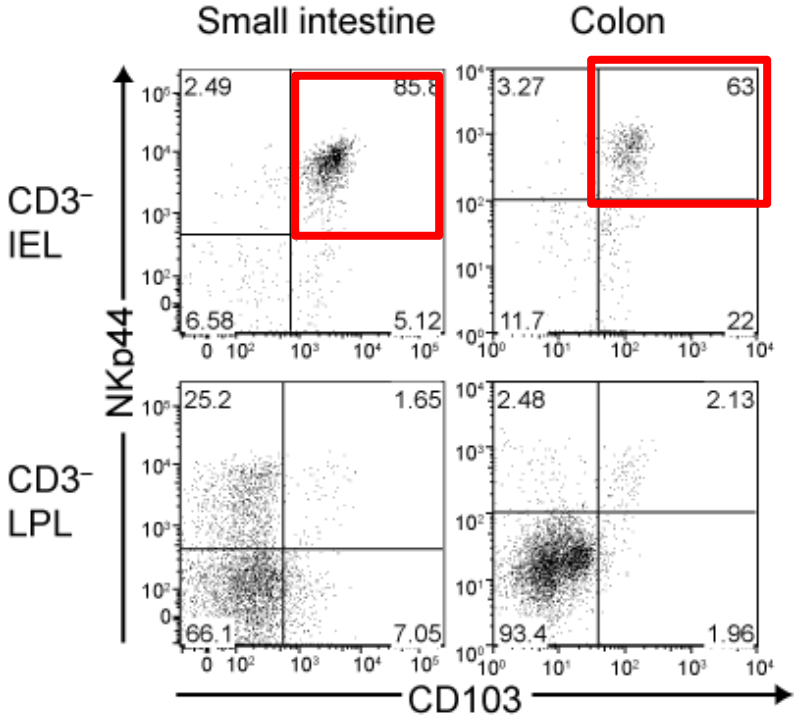
Secrete IFN-γ upon stimulation with IL-12 + IL-15

Limited cytolytic capacity

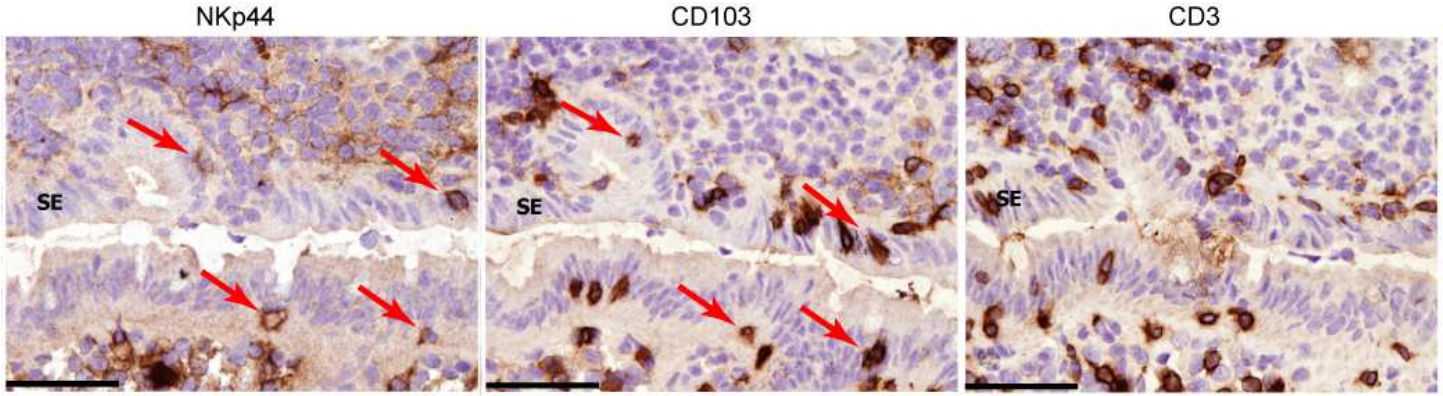
Gated on CD56+

Anja Fuchs

Intraepithelial CD103^{+} ILC1 are present in human gastrointestinal tract

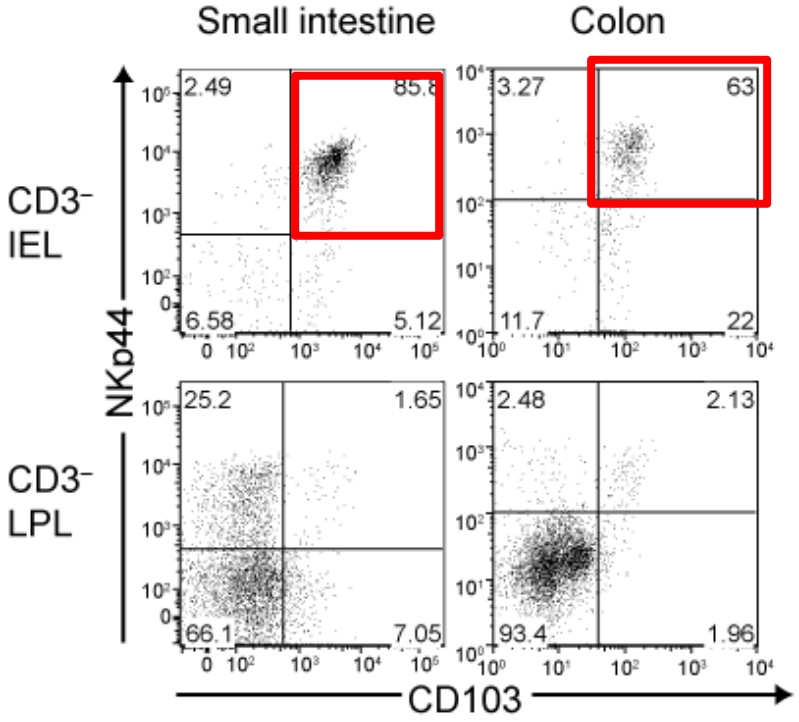


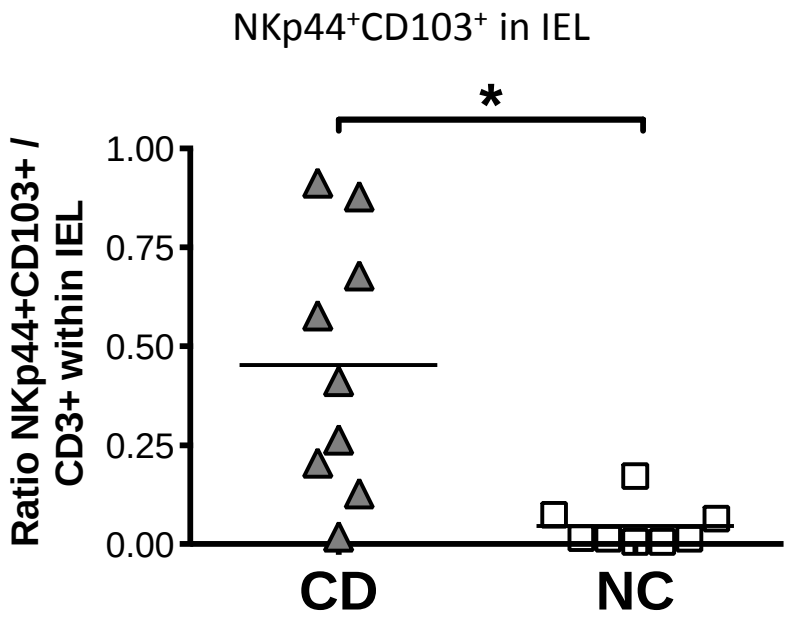
Gated on CD45+CD3- lymphocytes



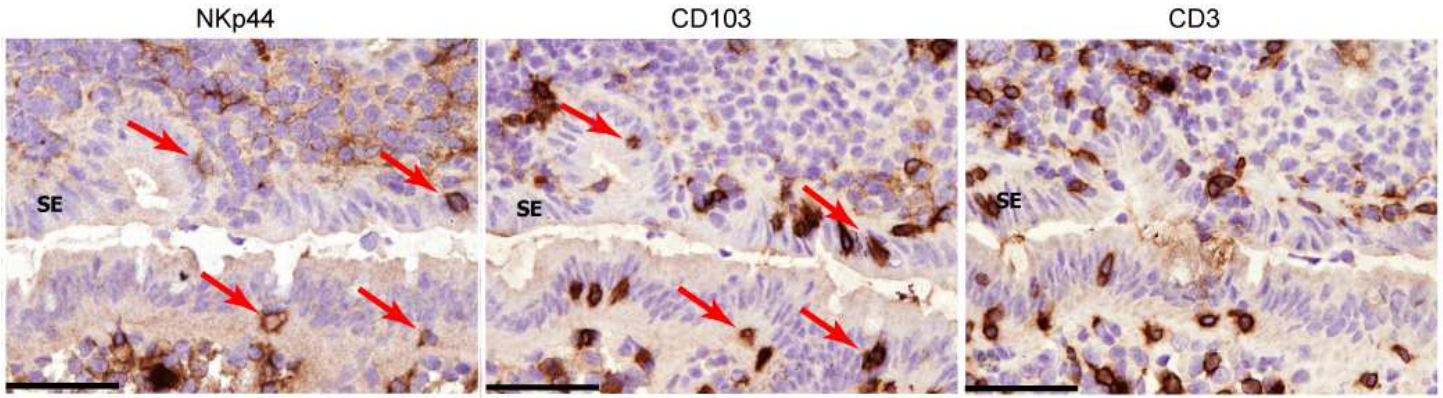
Human appendix

Increased CD103^{+} ILC1 frequencies in the ileum of individuals with Crohn's disease



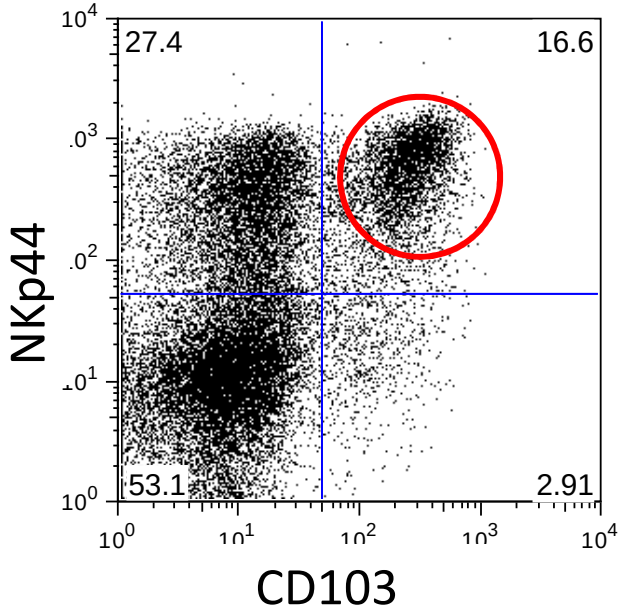


Gated on CD45+CD3- lymphocytes

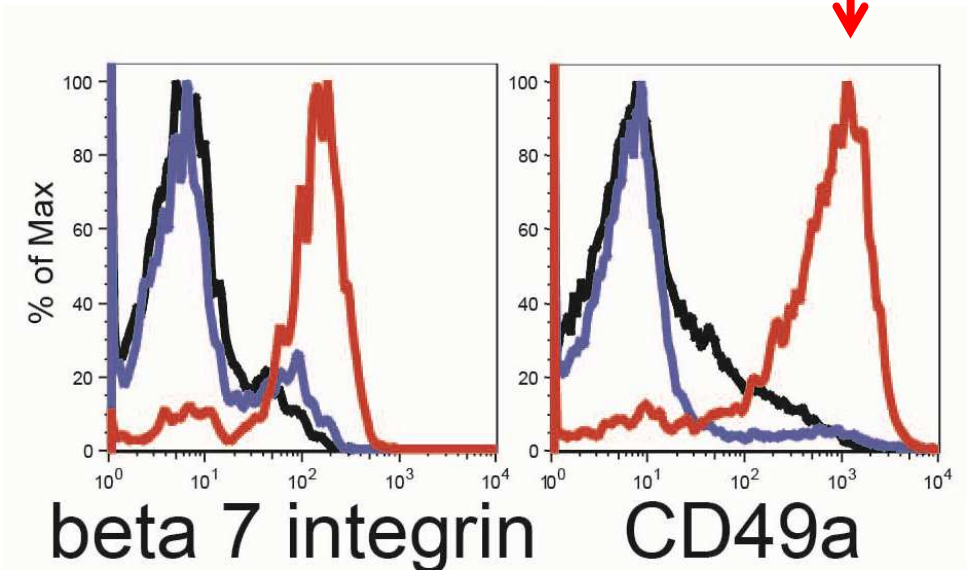


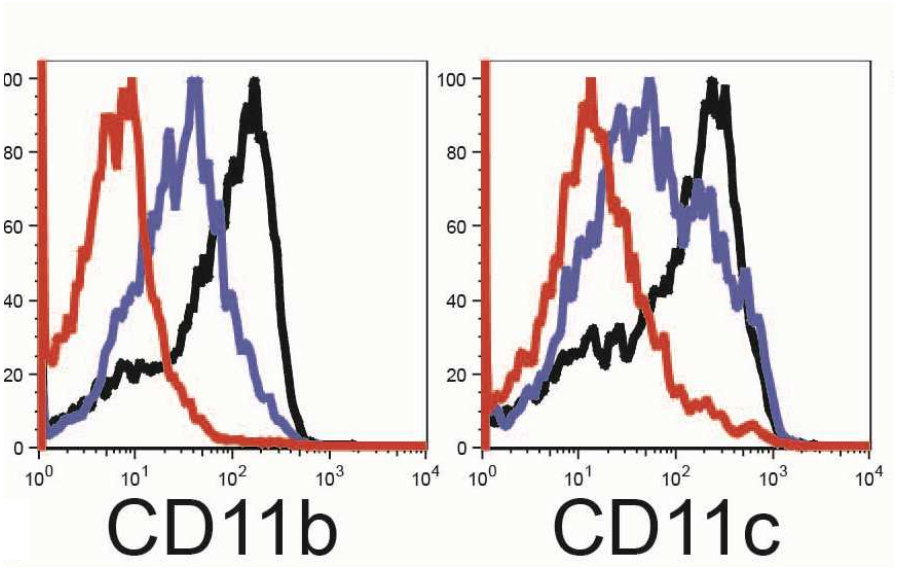
Human appendix

NK cells or distinct ILC subset?



Unique integrin pattern (VLA1+, CD103/Beta7+)



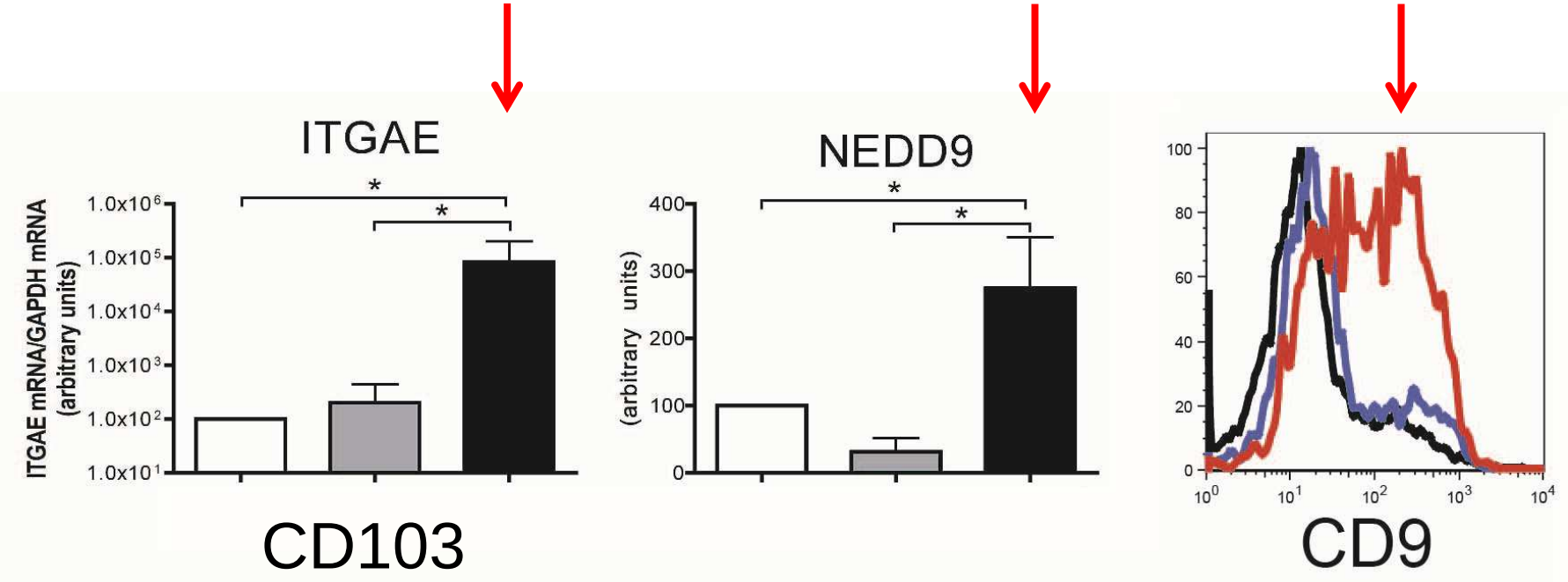


NKp44+CD103+

ILC3

NKp44- (conventional NK)

Unique features: TGF-beta imprinting

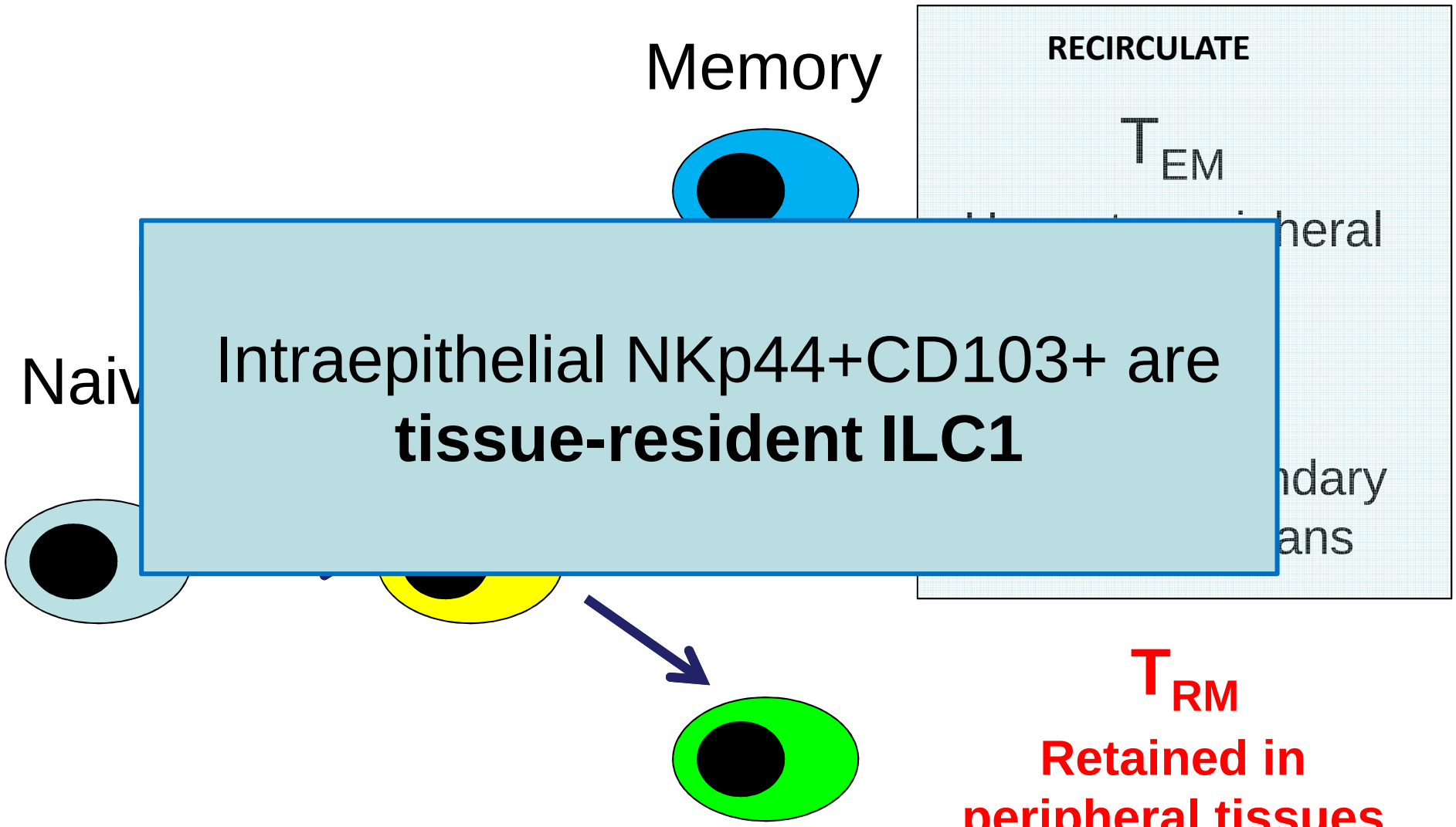


NKp44+CD103+

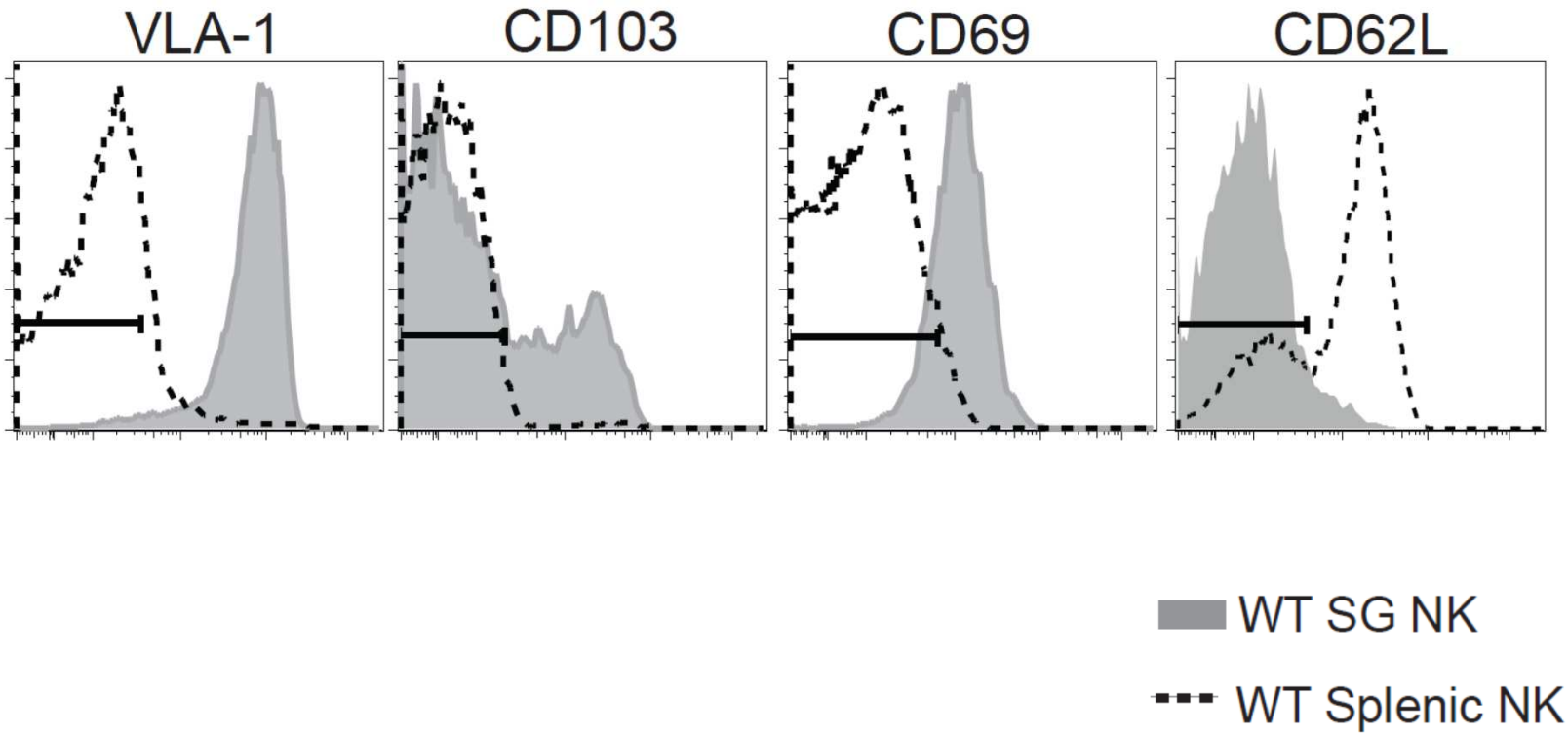
ILC3

NKp44- (conventional NK)

Similarity with Tissue Resident Memory CD8 T cells (T_{RM})



Salivary gland NK cells are very similar to intestinal intraepithelial ILC1

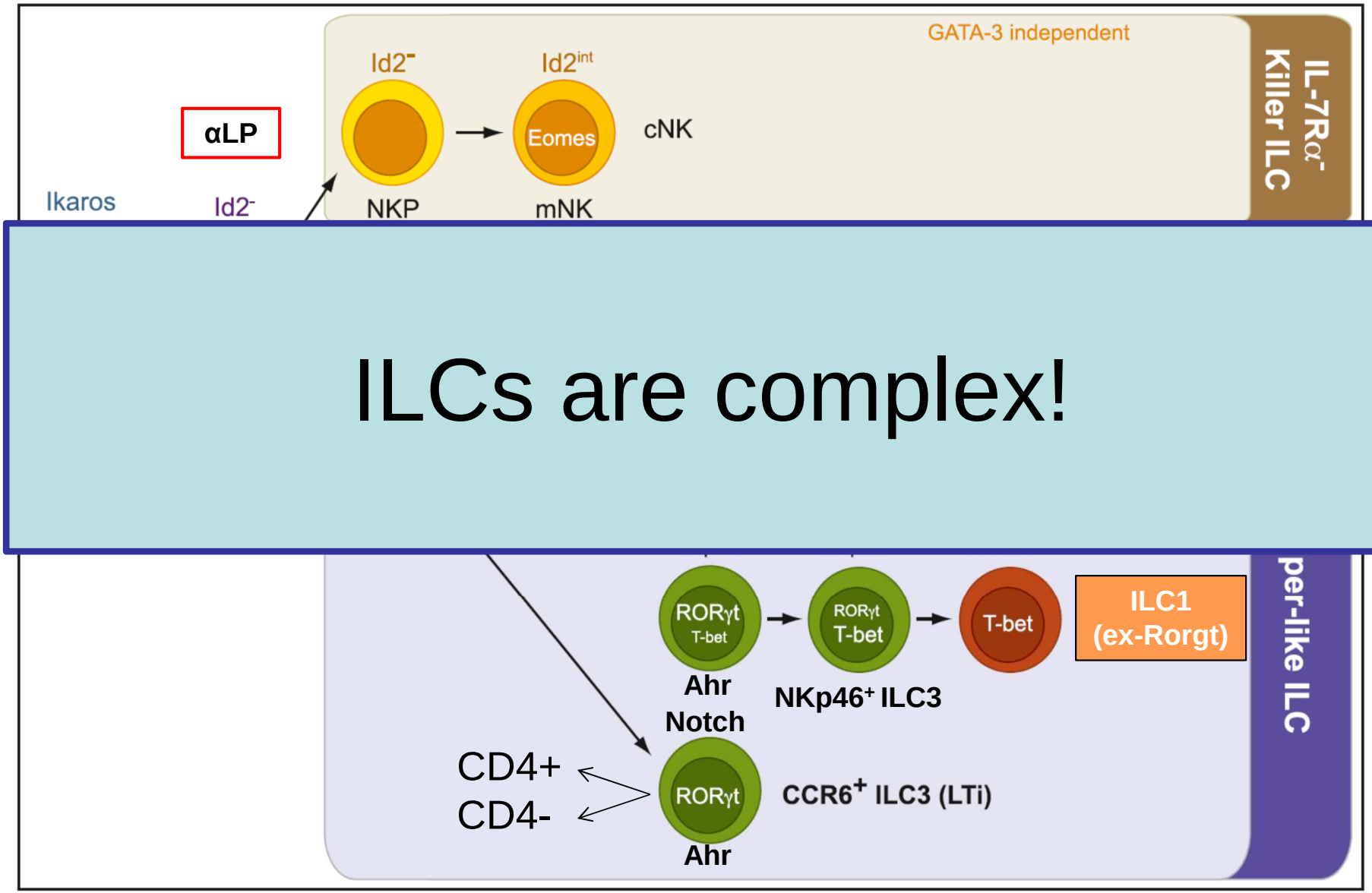


Victor Cortez

NK cells vs ILC1

	Recirculating	Tissue resident		
	NK	IEL ILC1	uterus	Liver ILC1
	BM, spleen, LN, siLP	**Intestinal epithelium**	**also SG**	**also siLP and skin**
VLA2	**positive**	**negative**	**positive***	**negative**
VLA1	negative	**positive**	**positive**	**positive**
TRAIL	negative	**positive**	**positive**	**positive**
CD127	negative	**dim**	negative	**positive***
IL-15	**Dependent**	**Partially independent**	**Dependent**	Dependent
Tbet	Dependent	**Dependent**	**Dependent**	**Dependent**
Eomes	**Dependent**	**Independent**	**Independent**	**Independent**
Nfil3	**Partially independent**	**Dependent**	**Independent**	**Dependent**

Heterogeneity of ILC



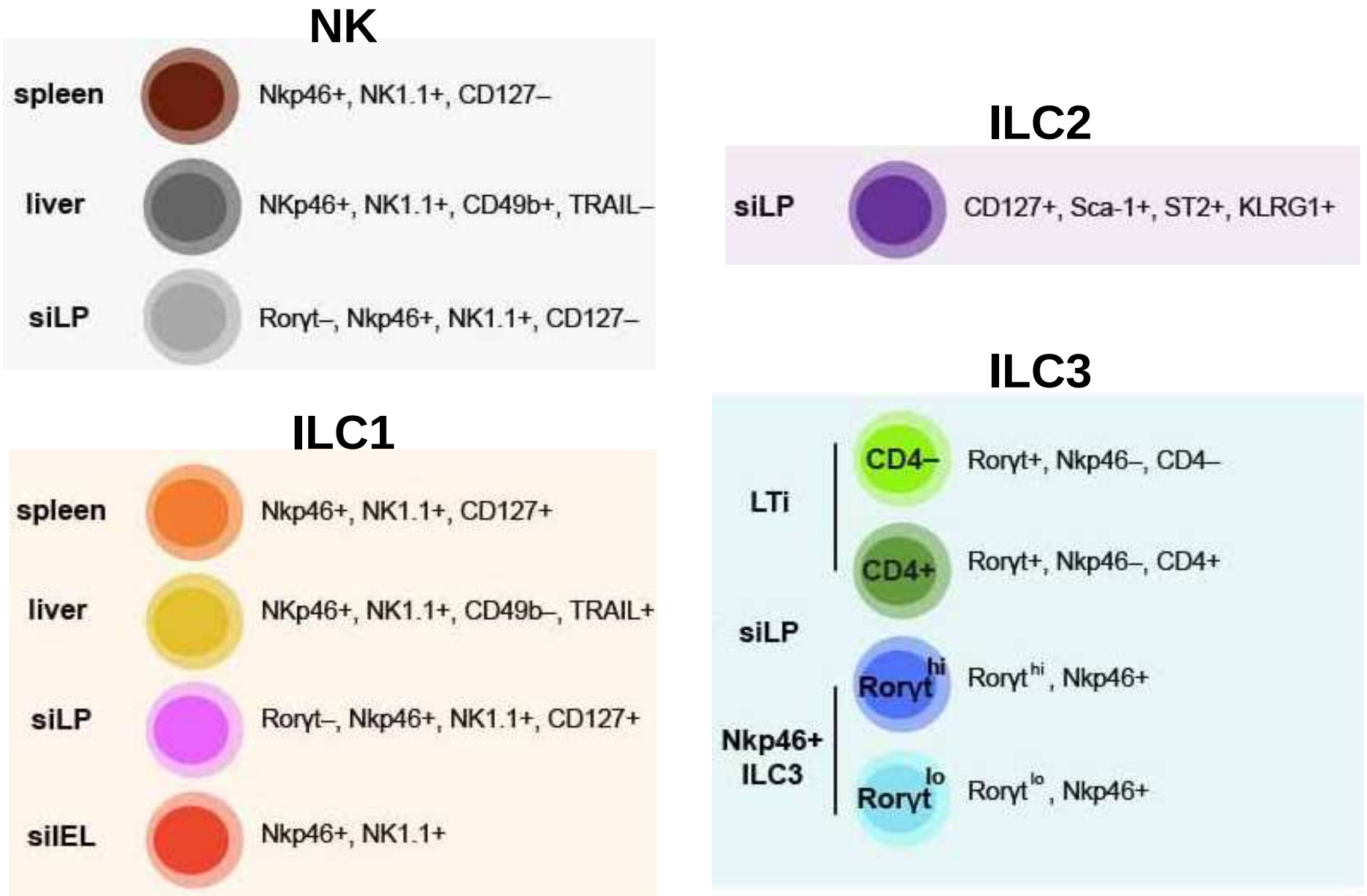
Understanding ILC diversity
Unique and shared transcriptional programs of mouse ILCs

In collaboration with the Immgen Consortium

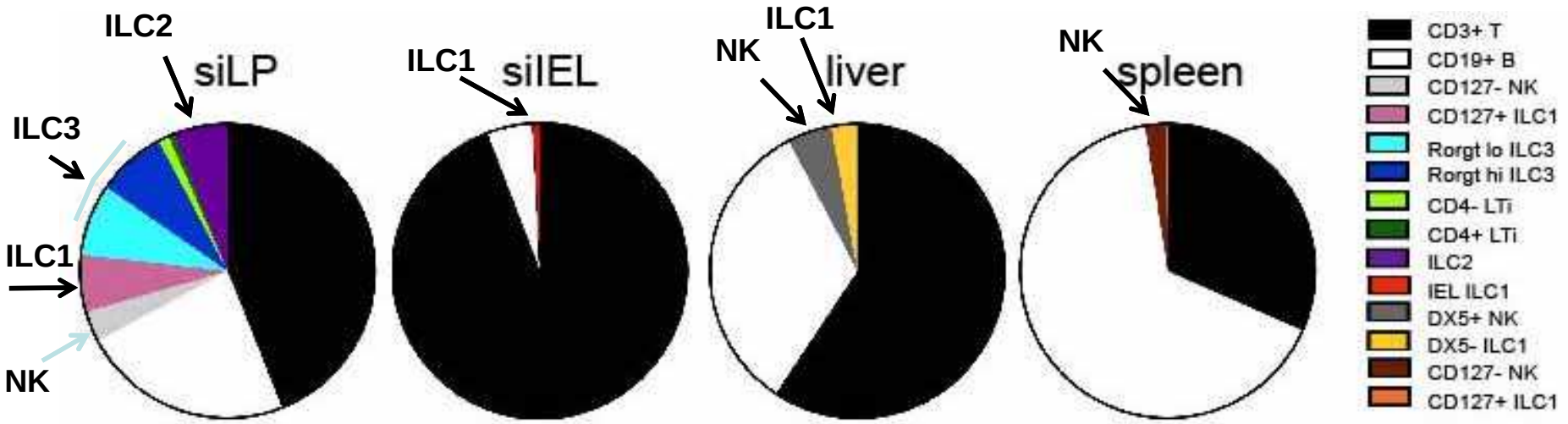
http://www.immgen.org/

Michelle Robinette

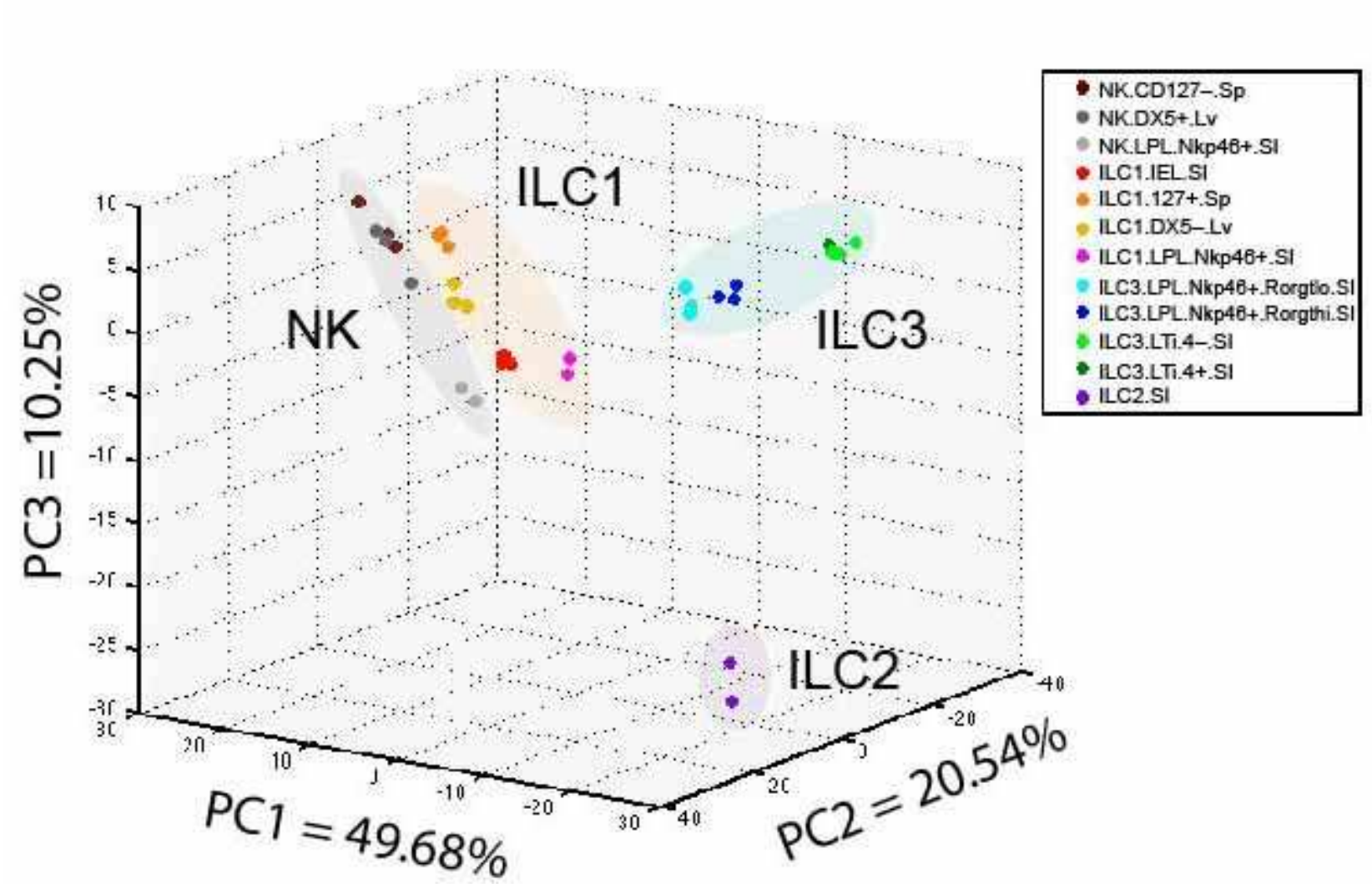
ILC classes and subsets



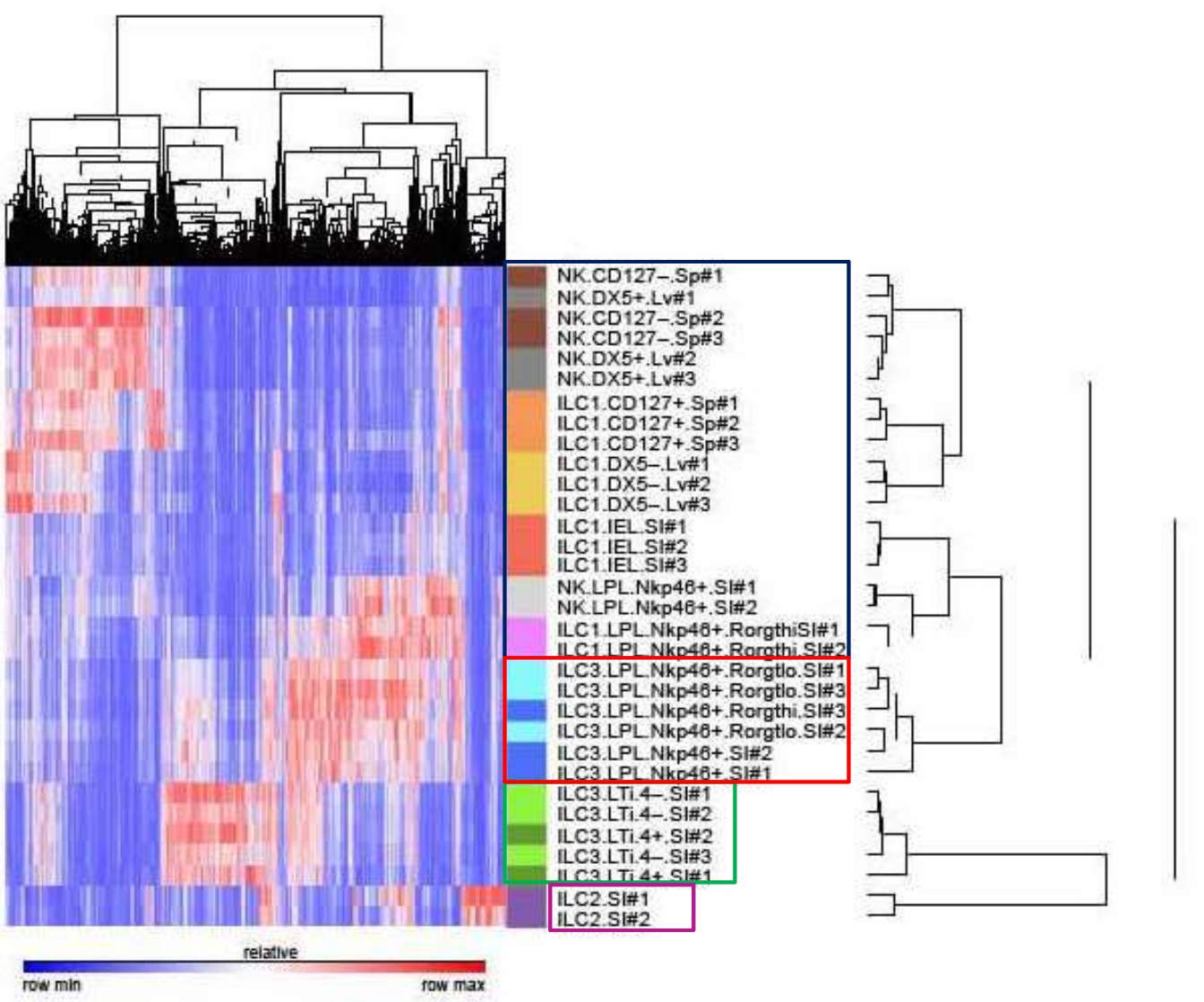
ILC subsets frequency



Principal component analysis of NK cell and ILC diversity

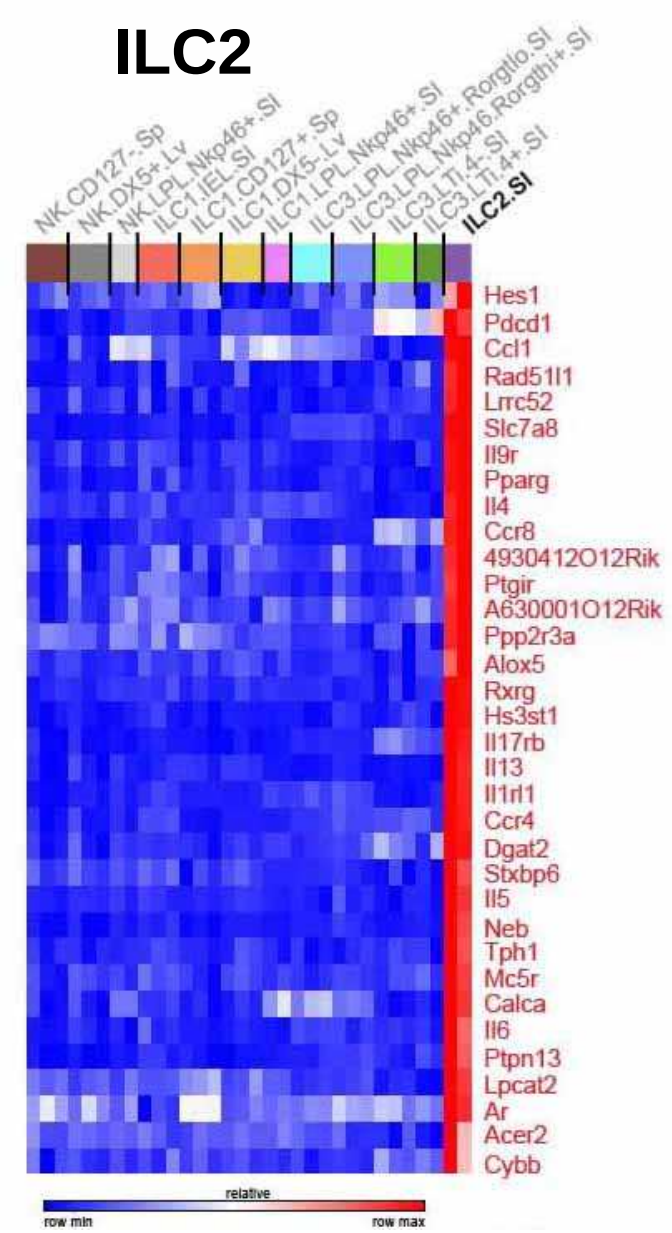


ILC1 and NK cells are intermixed



Hierarchical clustering of the top 10% differentially expressed genes

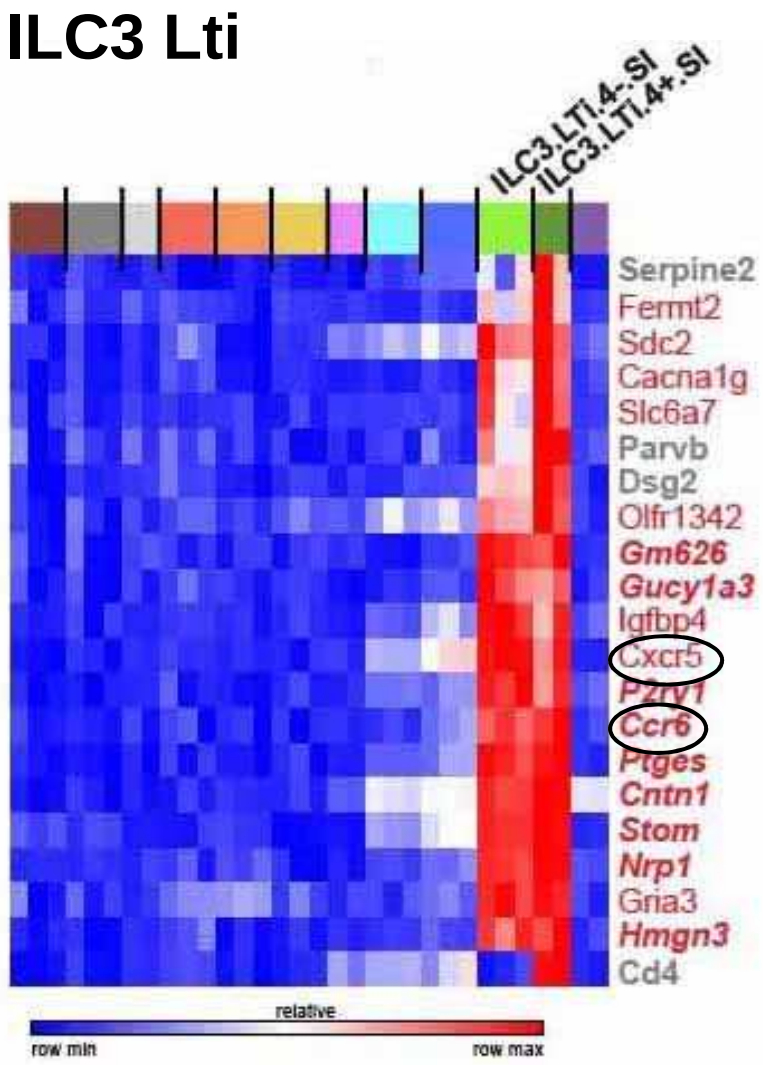
Heatmap of ILC2 unique transcripts

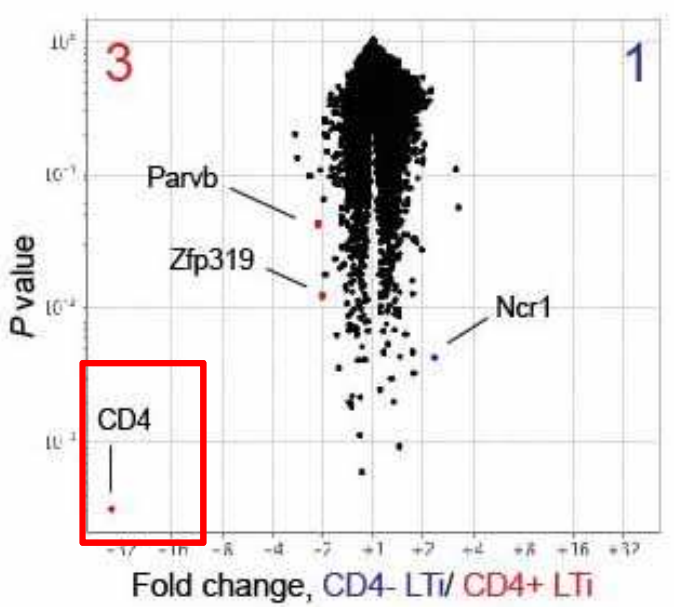


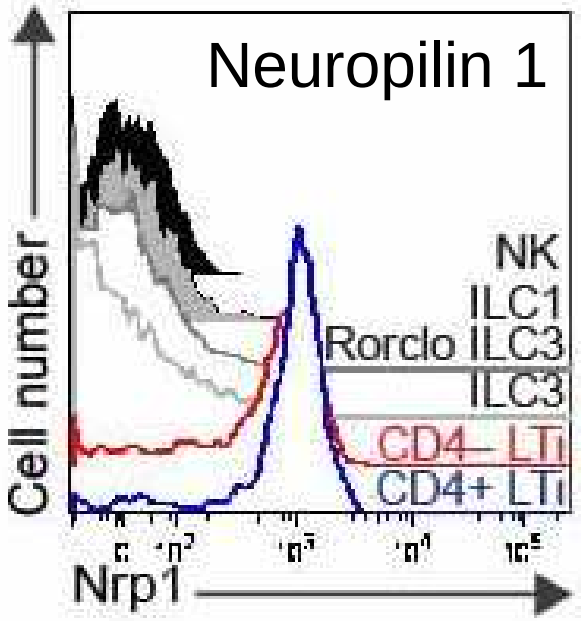
IL4
IL5
IL13
IL9
IL9R
IL33R

M2-like polarization
Arginase 1
PPARγ
Dgat2
Lpcat2

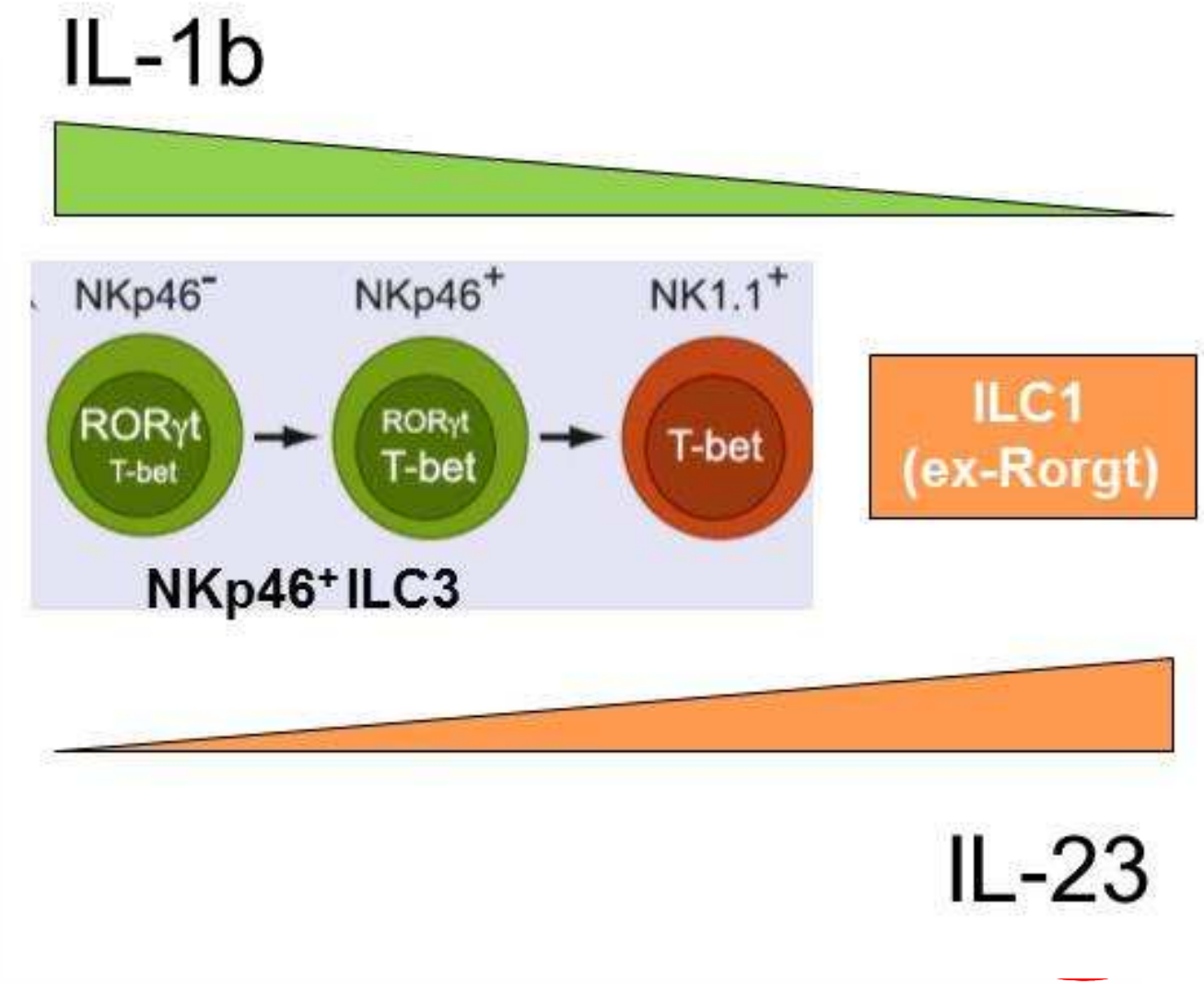
Heatmap of ILC3 (Lti-like) unique transcripts







NKp46+ILC3 share transcripts with Lti ILC3 but also ILC1



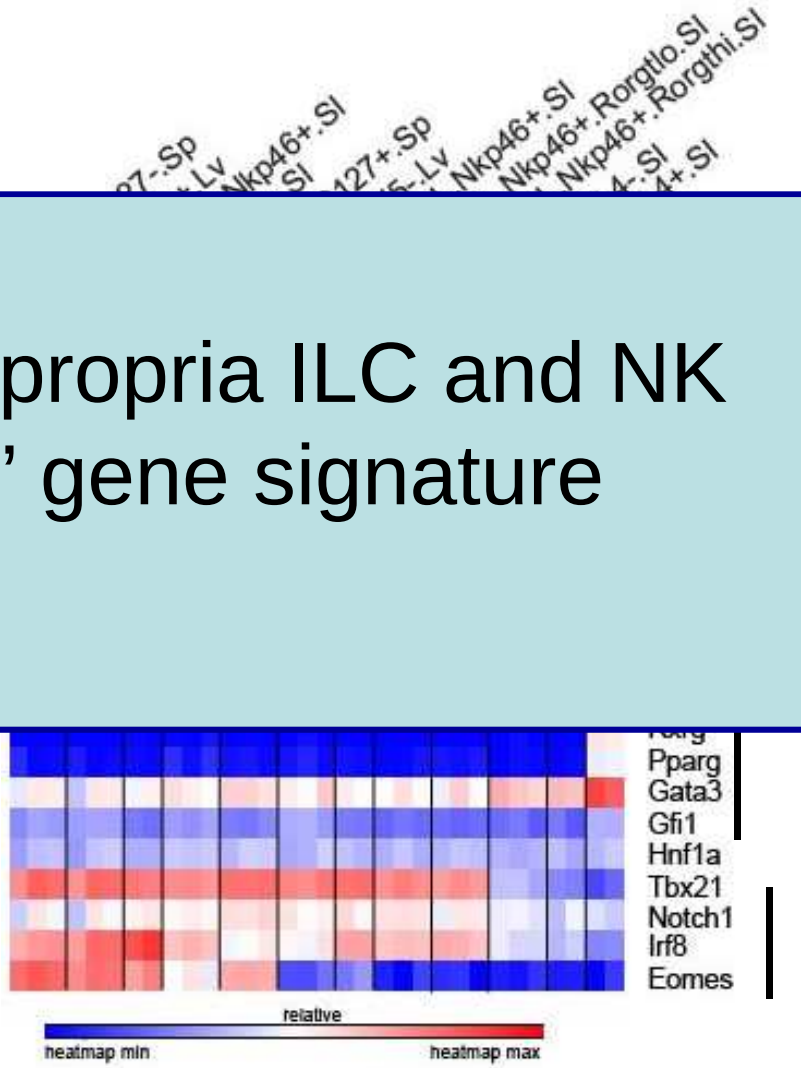
Lti ILC3

ILC1

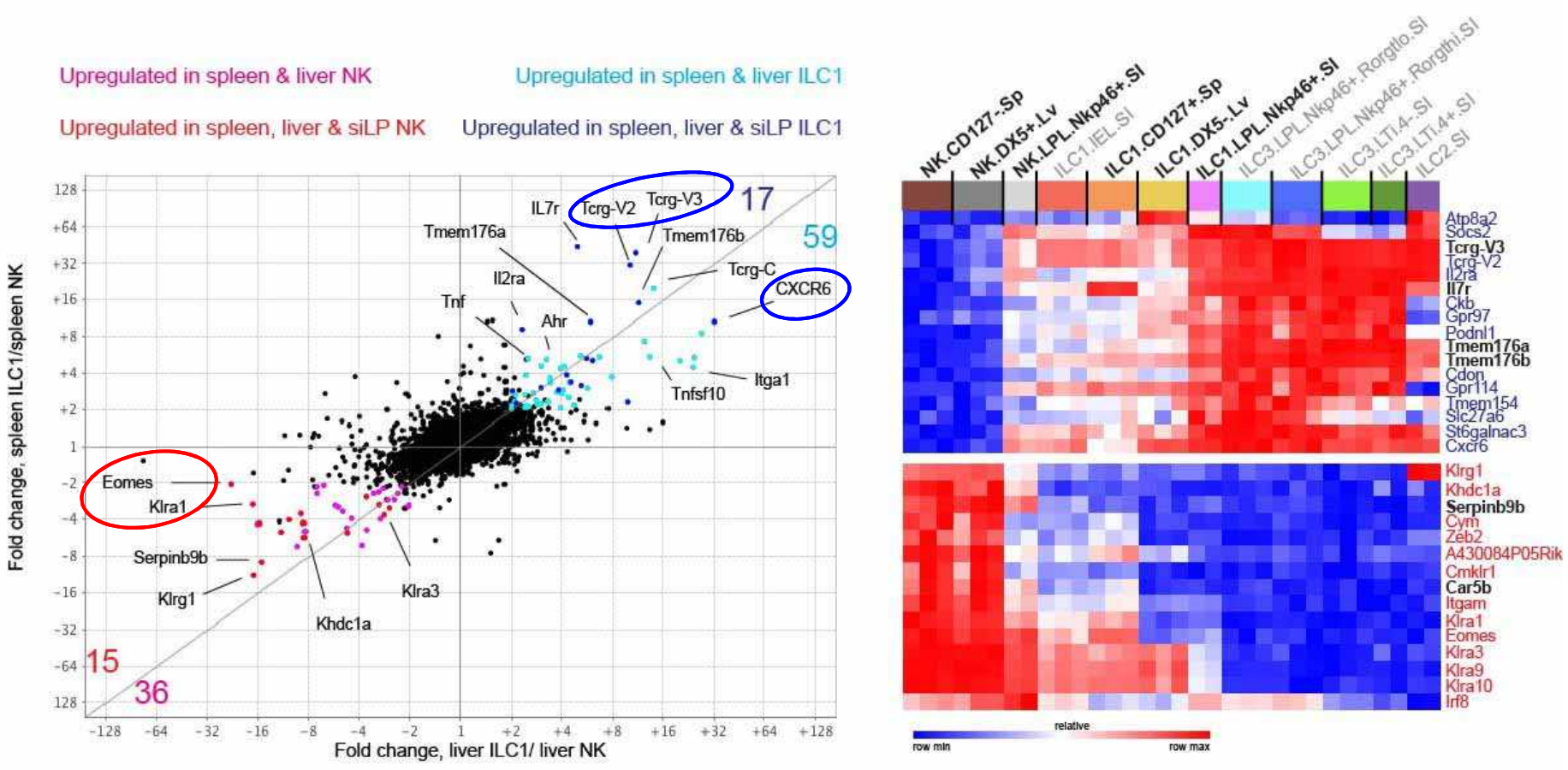
ILC3 Nkp46+

Signature Transcription Factors

Small intestine lamina propria ILC and NK share an “intestinal” gene signature

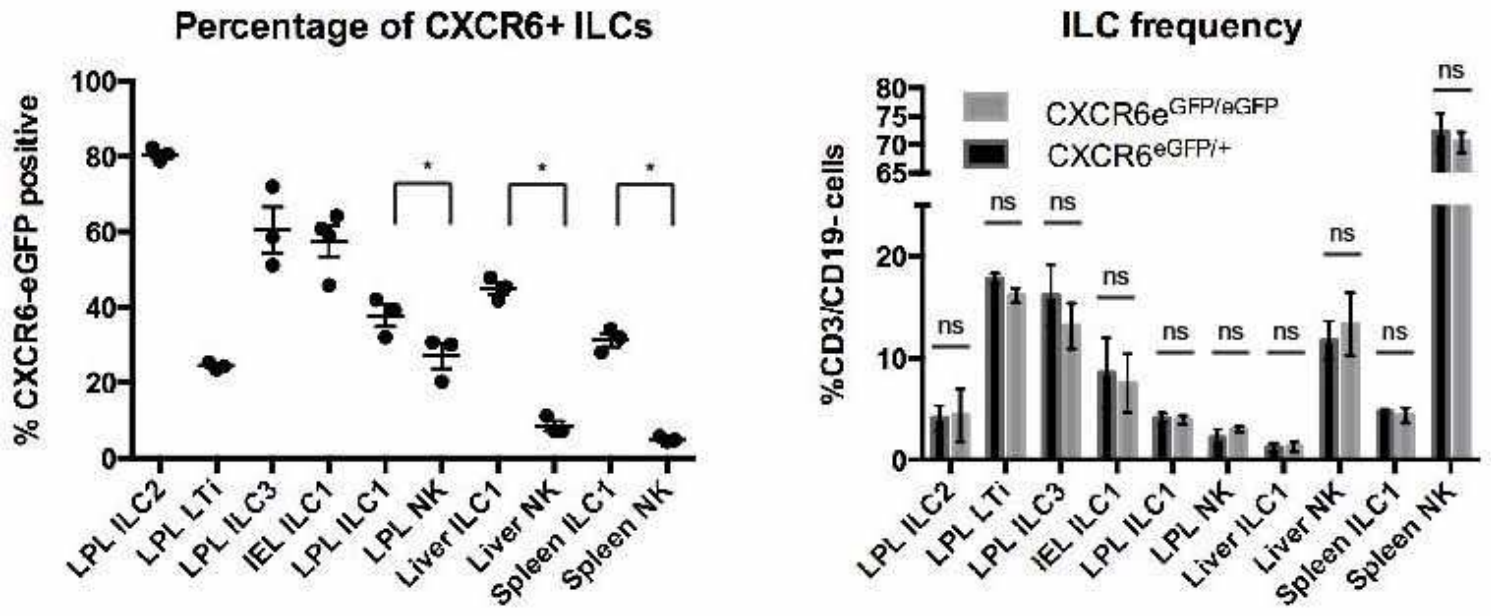


ILC1 transcriptional profile is intermediate between NK cells and ILCs



Core ILC signature

ILC core signature: CXCR6



CXCR6 deficiency has no impact on ILC frequencies but influences cytokine responses

Conclusions

- **ILC2** express transcripts indicative of M2 polarization
- Neuropilin1 is defining marker of Lti **ILC3**
- NKp46+ **ILC3** may have functional plasticity and convert into ILC1
- **Intestinal ILC and NK** share an “intestinal” gene signature probably reflecting environmental exposure
- There is a major functional overlap between **ILC1** and **NK** cells
- A core signature for ILC distinct from NK:
  - CXCR6, IL-7R, GLT of TCRγ, TMEM176a/b

Lab 2014

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