

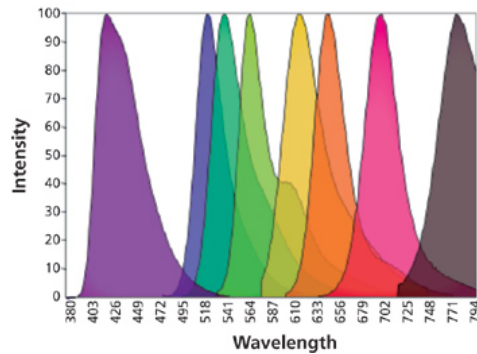
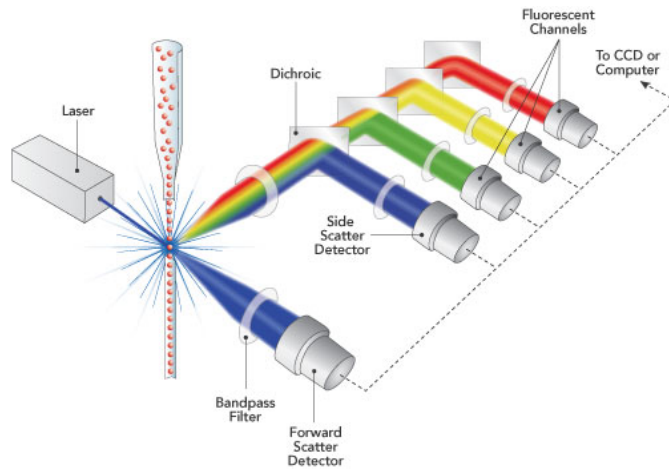
Imaging the unimaginable with (imaging) mass cytometry

Frits Koning

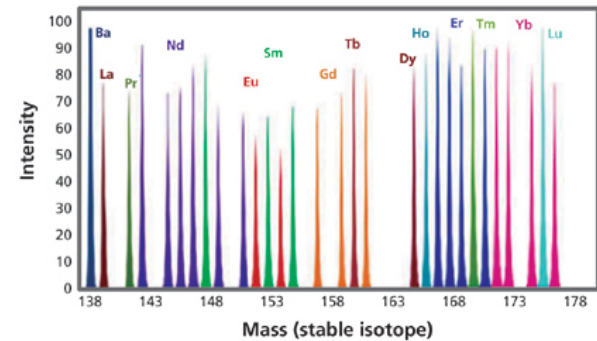
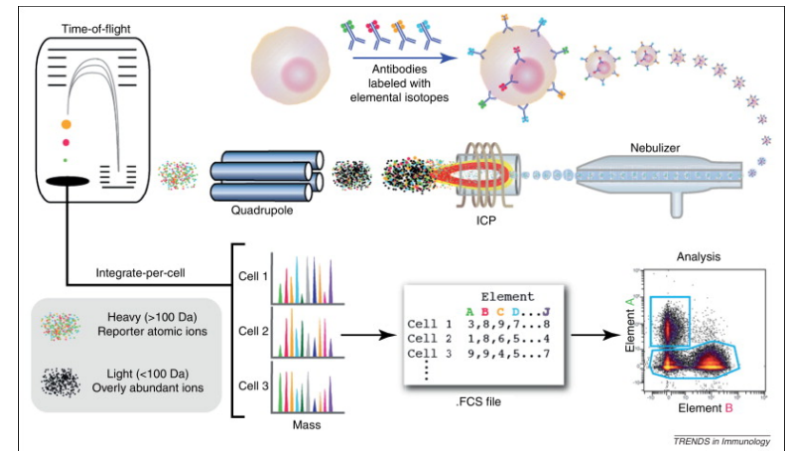
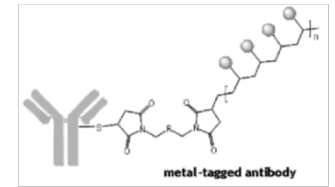
LUMC, Leiden

Celiac Disease Consortium

Flow cytometry

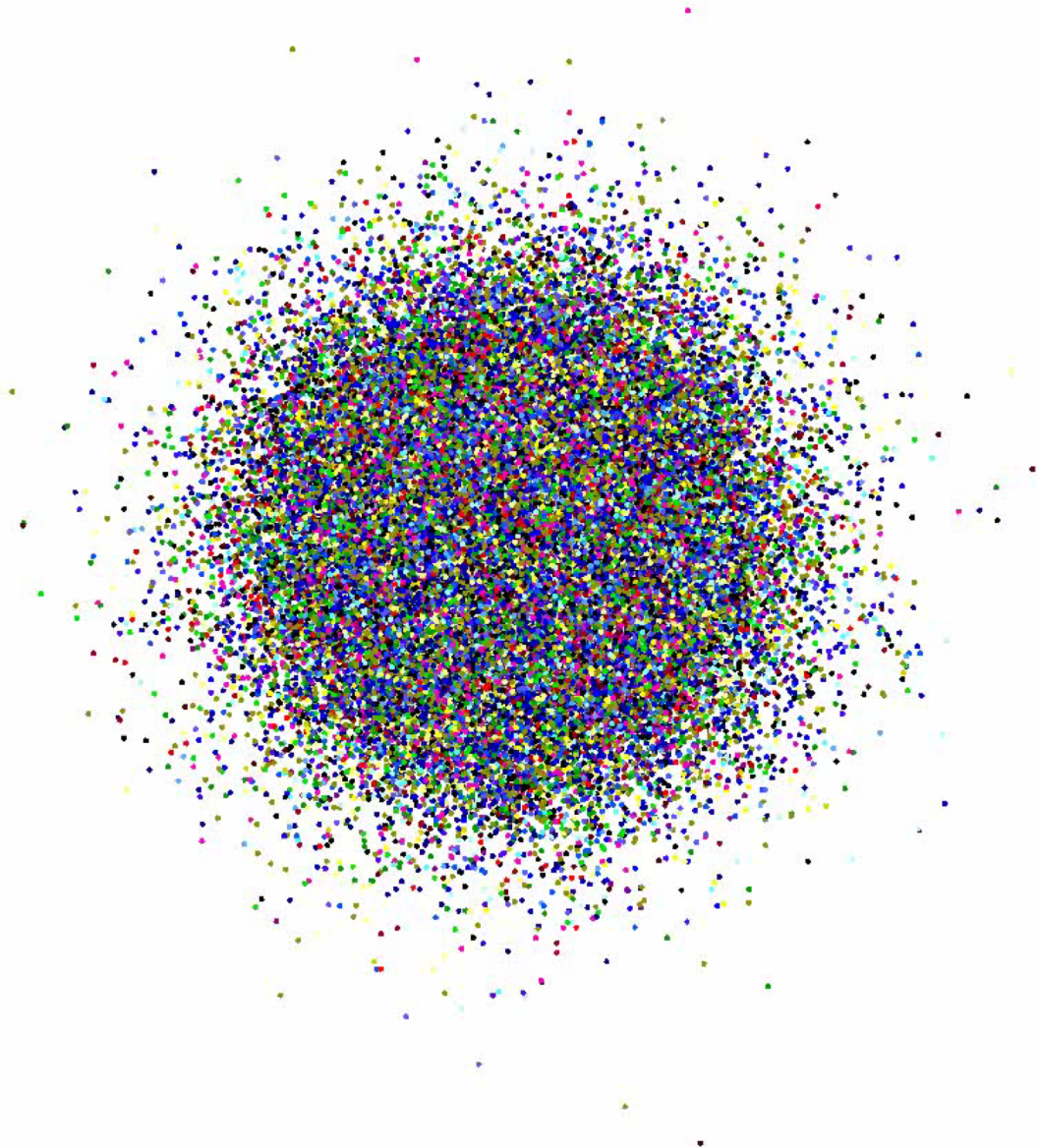


CyTOF

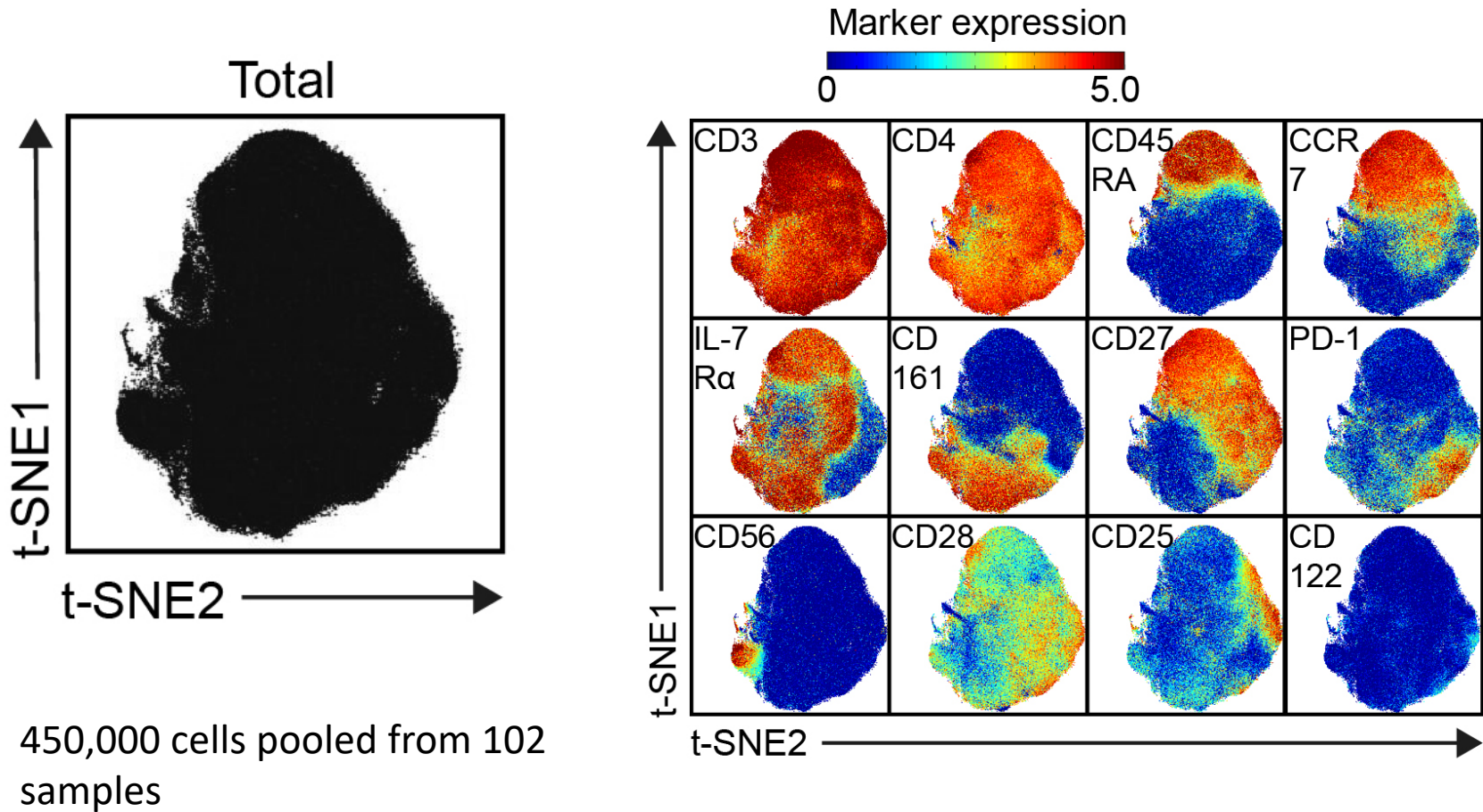


39 markers simultaneously!!!!

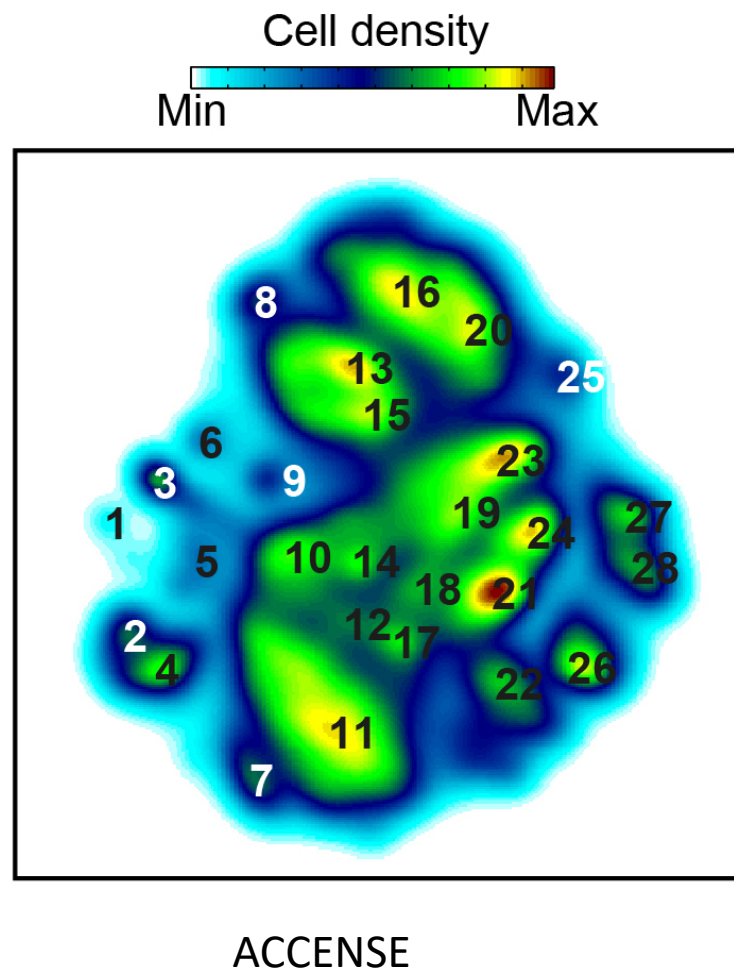
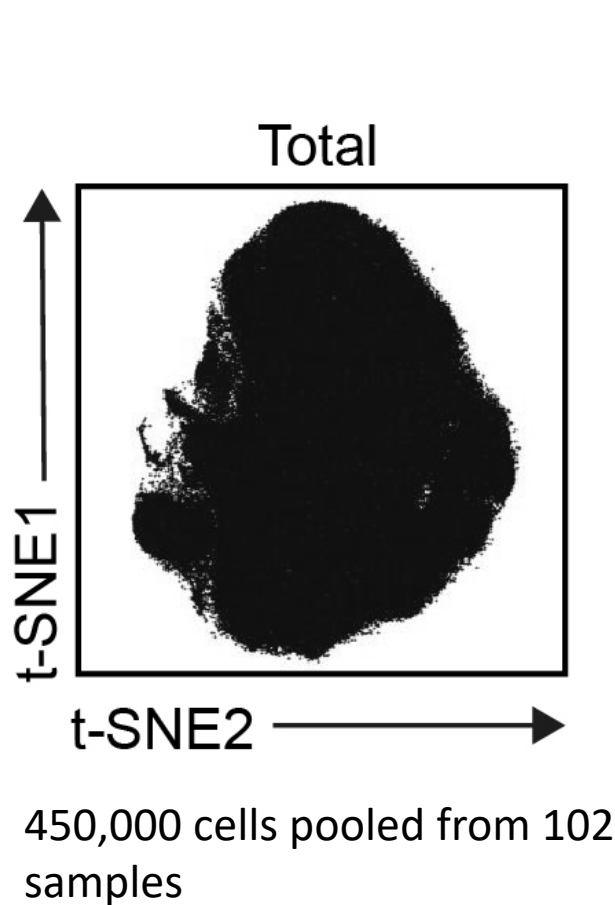
- 39 dimensions
- New approaches to data analysis
- Dimensionality reduction techniques
- t-SNE: stochastic neighborhood embedding

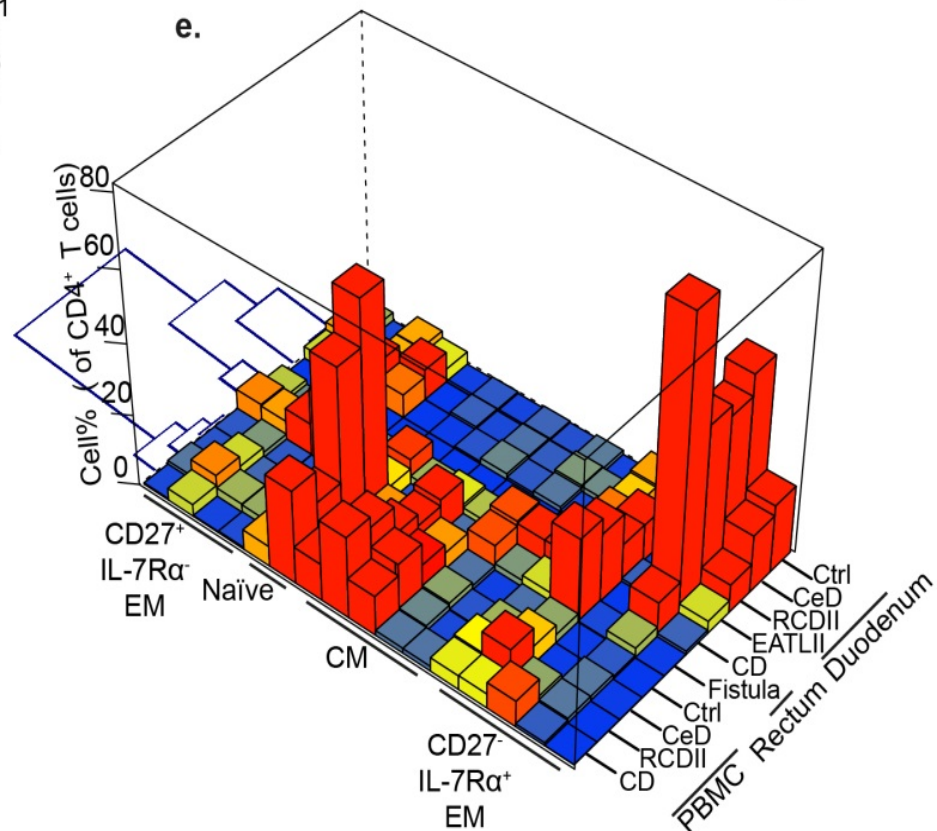
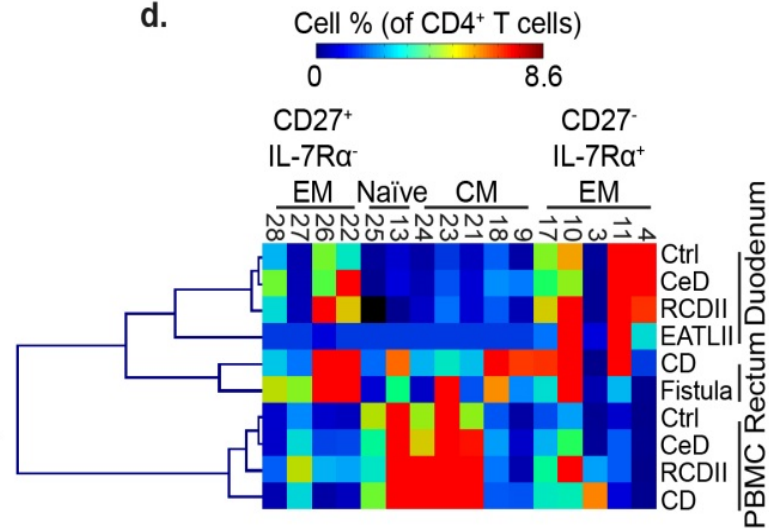
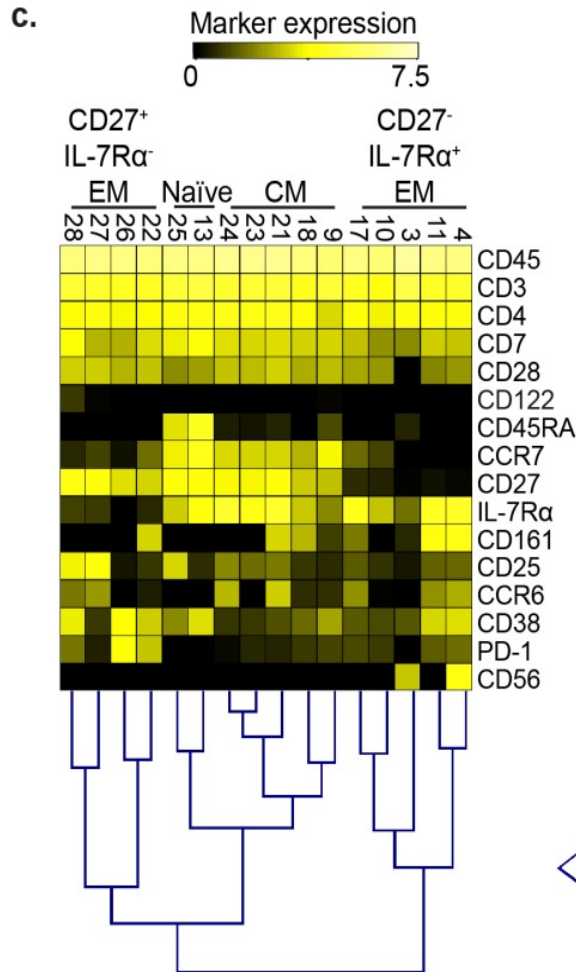


t-SNE analysis of CD4⁺ T cells



Identification of distinct subsets within the CD4⁺ T cell compartment

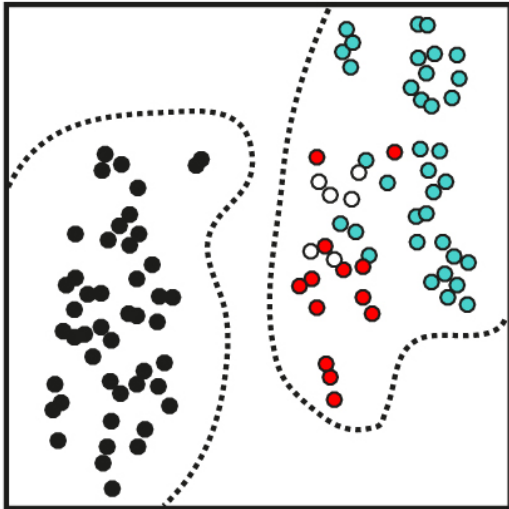




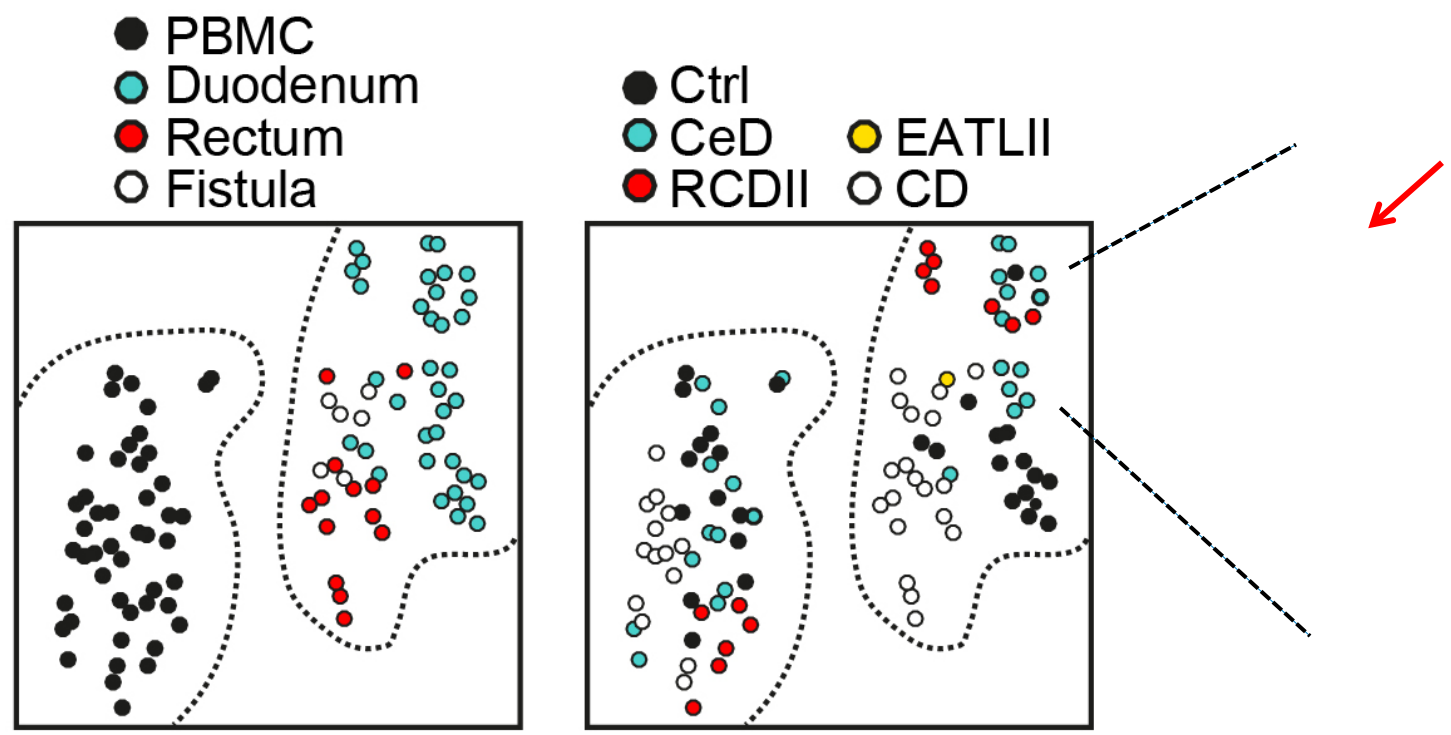
**Specific subsets
reside in
tissue and blood**

Clustering samples on the basis of immune compositions

- PBMC
- Duodenum
- Rectum
- Fistula



Clustering samples on the basis of immune compositions

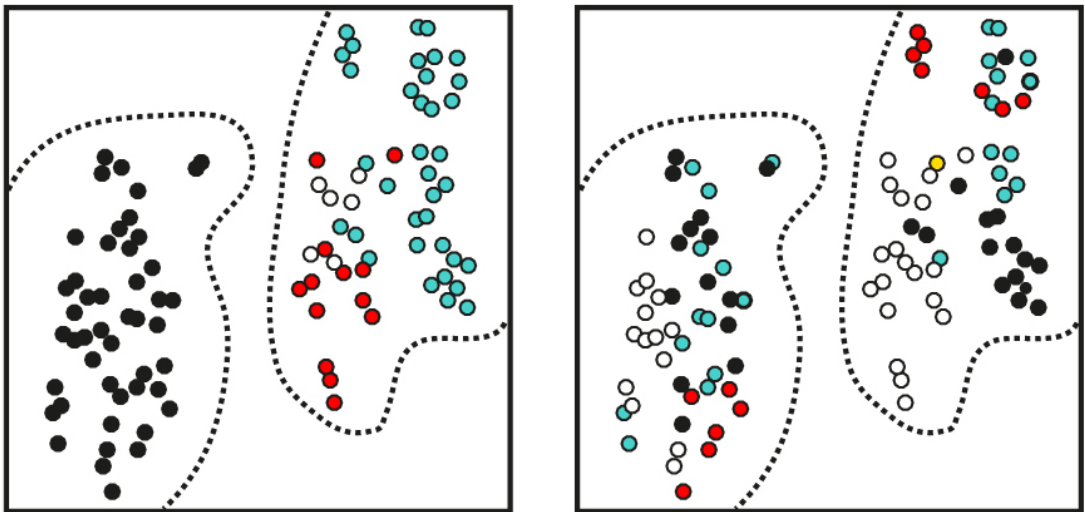


Clustering samples on the basis of immune compositions

- PBMC
- Duodenum
- Rectum
- Fistula

- Ctrl
- CeD
- RCDII
- EATLII
- CD

“A slightly optimistic view of the data”

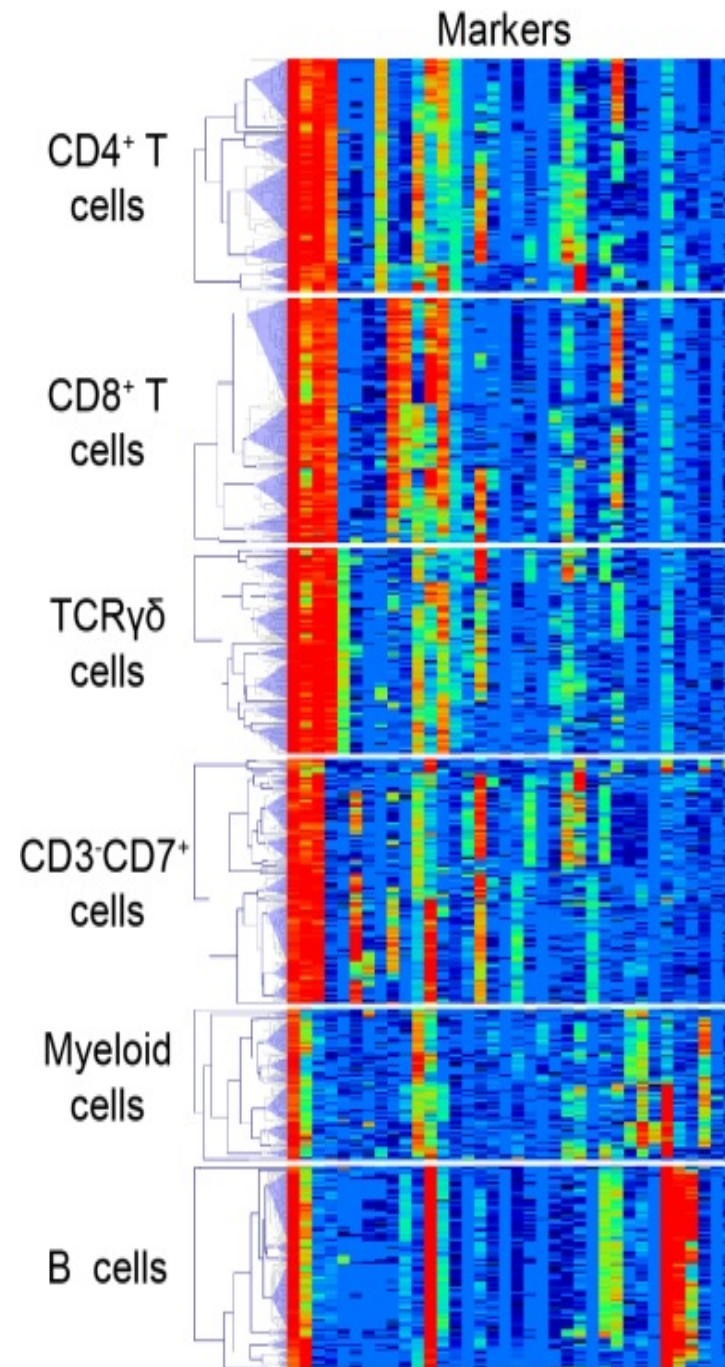


Human fetal intestine

36 antibody panel

Spade/t-SNE/ACCENSE

Unbiased hierarchical clustering

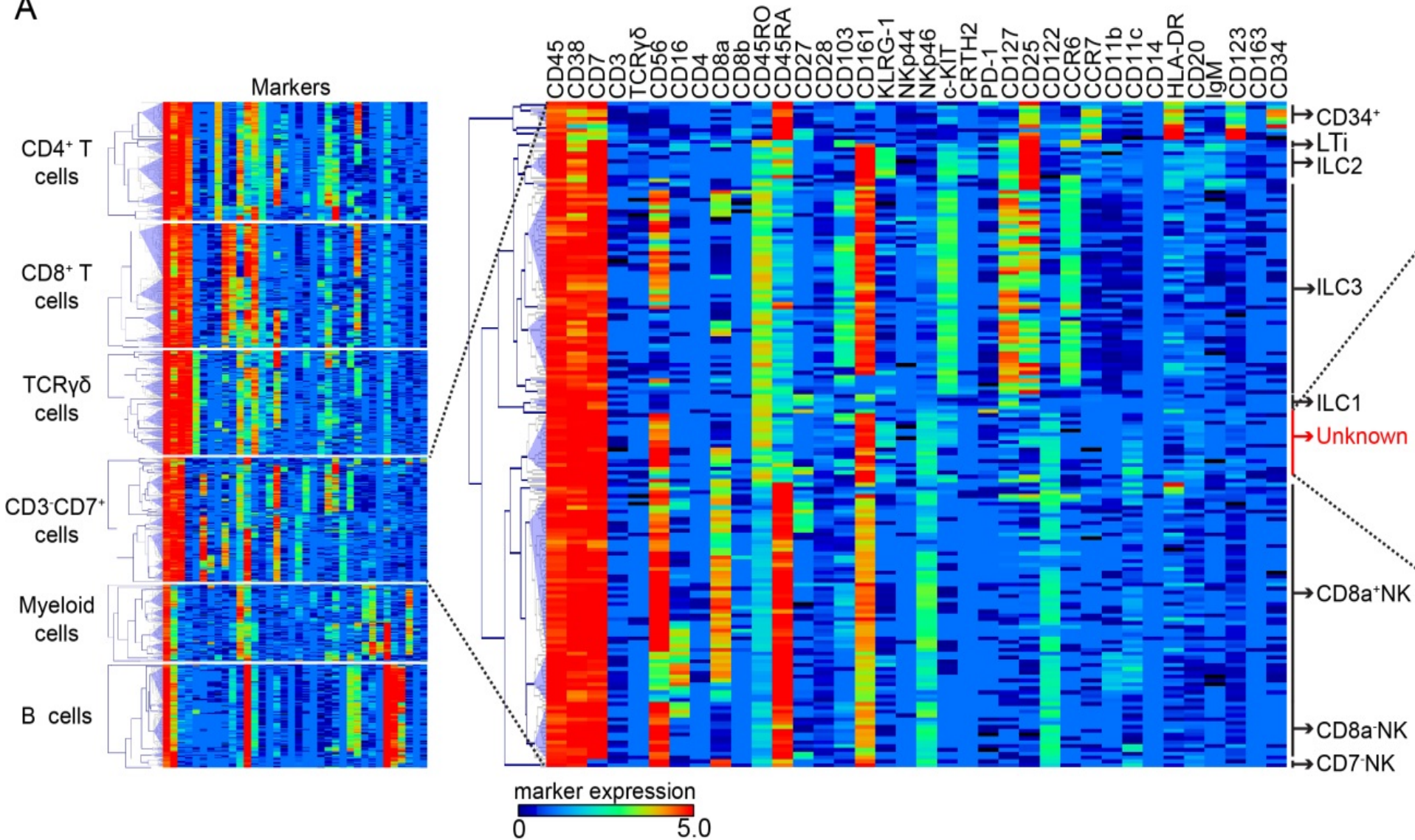


Li, van Unen et al. J. Exp. Med. 2018

Li, van Unen et al. Nat. Immunol, in press

Mass Cytometry

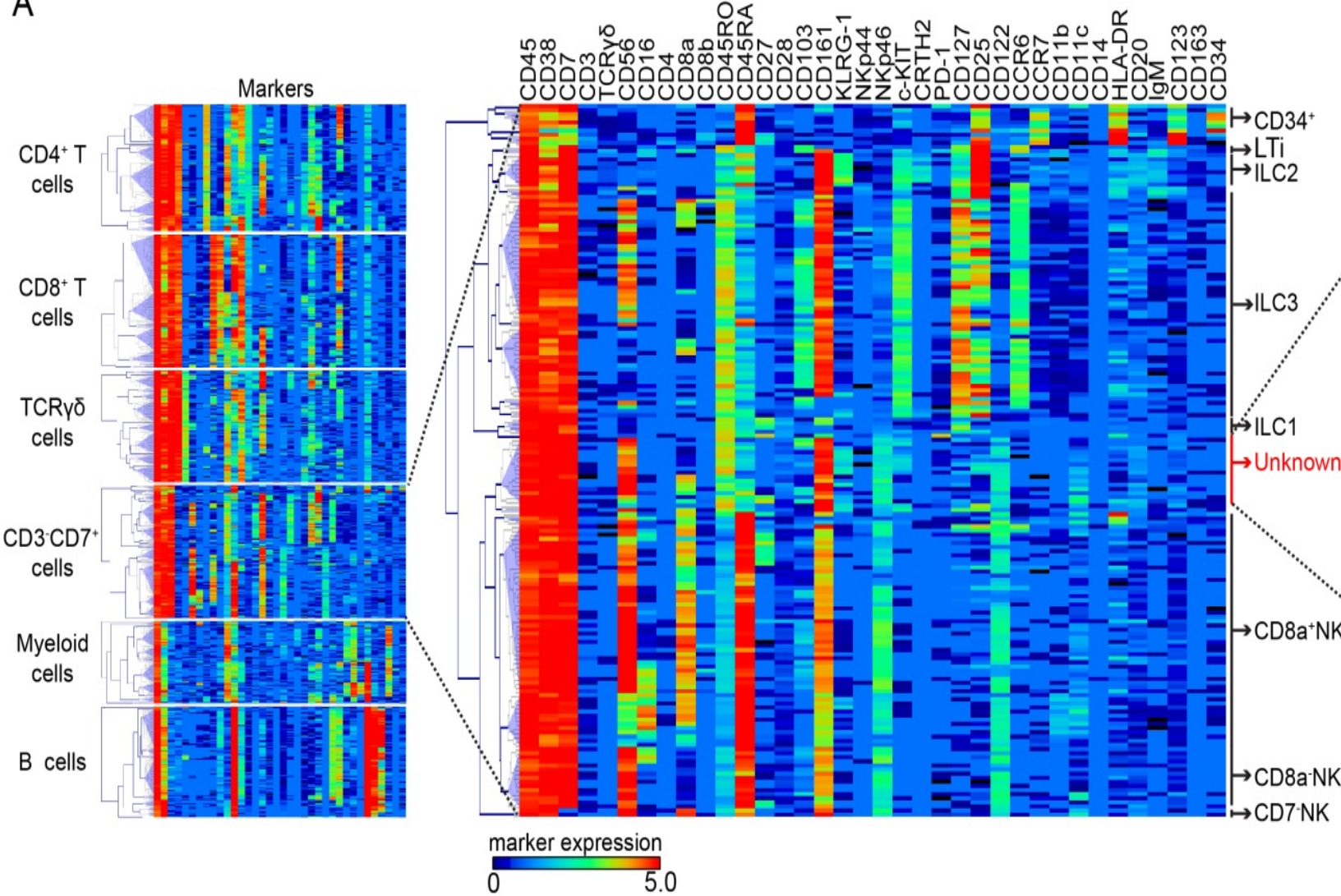
A



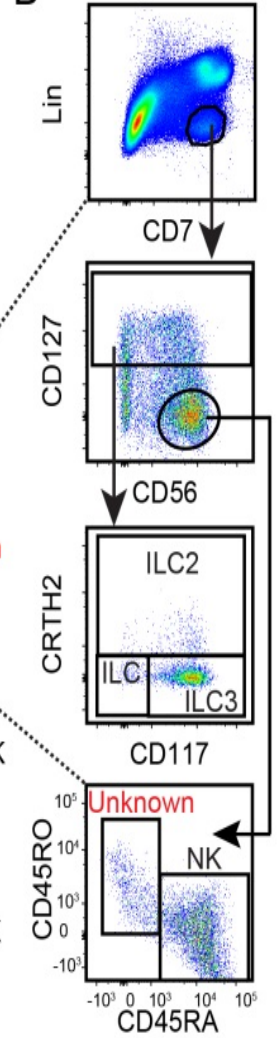
Mass Cytometry

Flow Cytometry

A

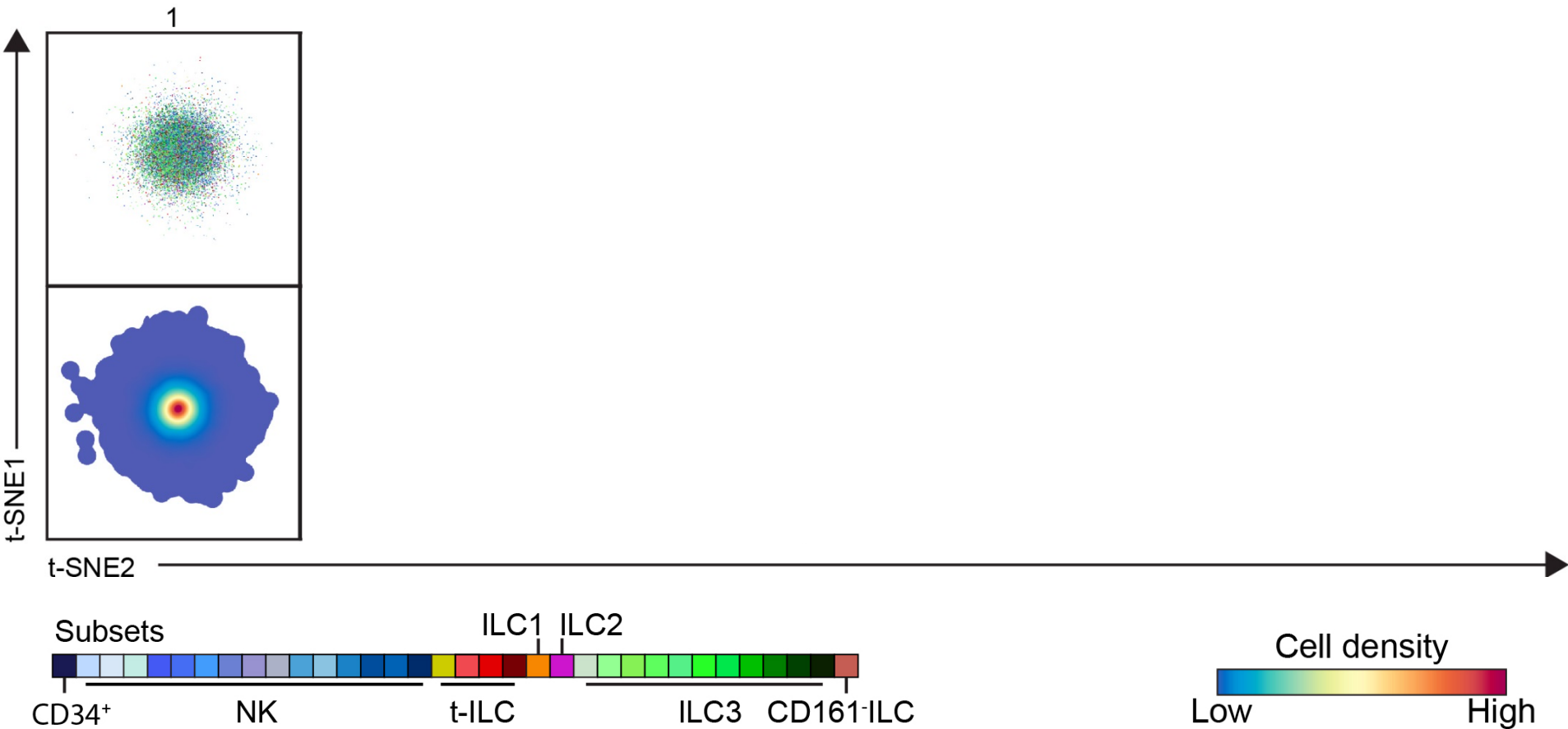


B



Monitoring t-SNE calculation indicates potential differentiation paths of innate lymphocytes

t-SNE calculation over time \longrightarrow

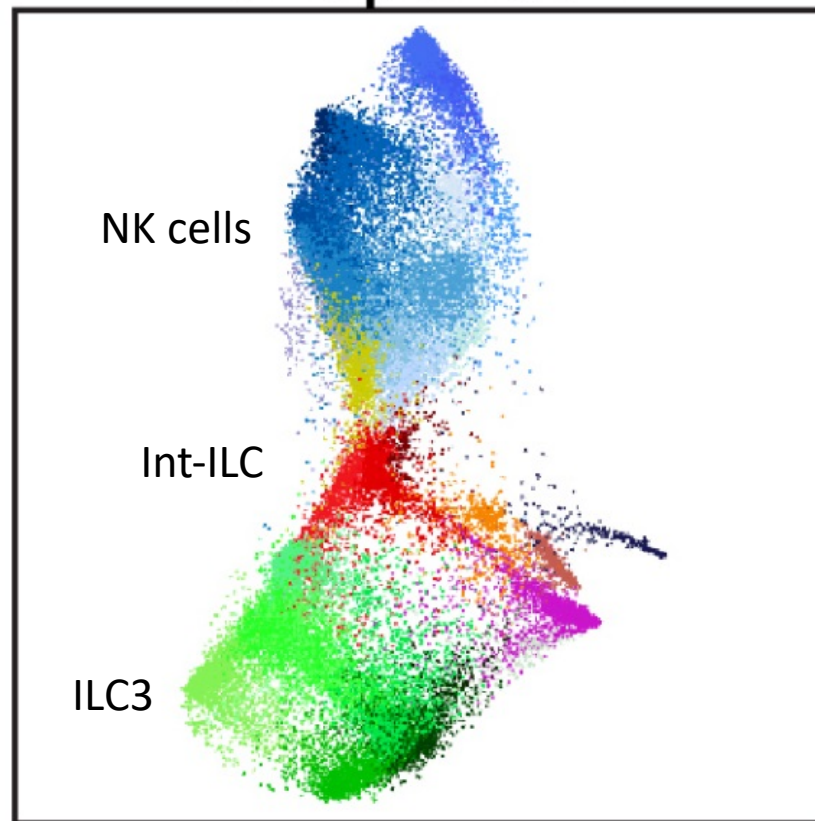


single cell.avi



Stack.avi

Int-ILC: a precursor that can give rise to NK cells and ILC3



Hollt et al, COMPUTER GRAPHICS FORUM 2016
Li, van Unen et al. J. Exp. Med. 2018

Aim of the study

To unravel local and systemic immune profiles in colorectal cancer using high-dimensional system-wide analyses by mass cytometry

Characterization of immune cells using a mass cytometry antibody panel of 39 markers

Major lineage markers	
CD45	Pan immune
CD3	T cells
CD4	CD4 T cells
CD8α	CD8 T cells
TCRγδ	γδ T cells
CD20	B cells
CD14	Monocytes
CD11c	Myeloid cells
CD7	ILCs

Fc/complement receptors	
CD16	Low affinity FCγR3α
CD11b	Complement R3

Apoptosis	
CD95	FASR/TNFRSF6

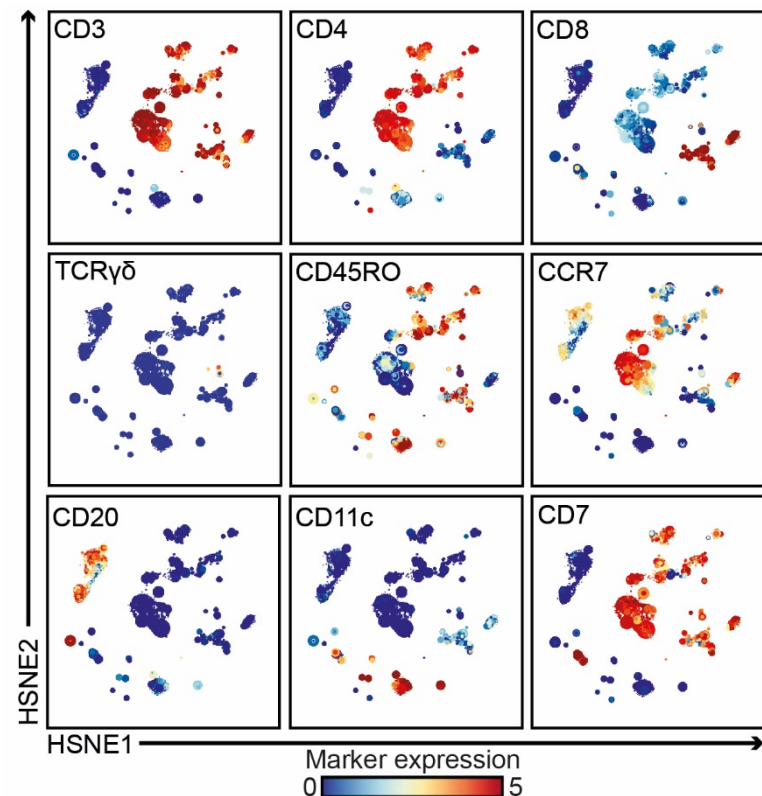
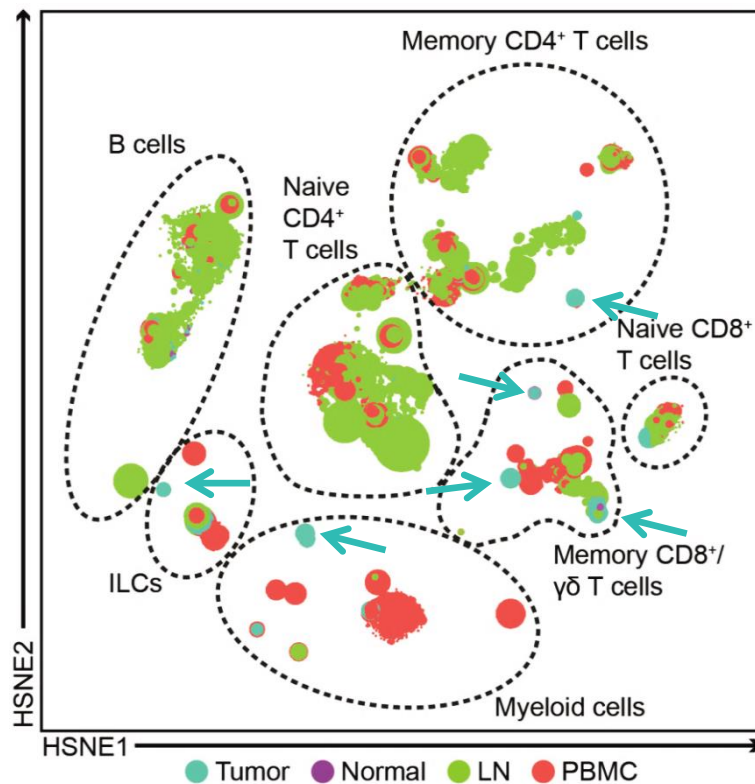
Differentiation/activation markers	
CD45RO	CD45 isoform
CD38	cADP ribose hydrolase
CD161	KLRB1
HLA-DR	Ag presentation
CD335	NKp46

Cytokine/chemokine receptors	
CD123	IL-3Rα
CD127	IL-7Rα
CD25	IL-2Rα
CD122	IL-2Rβ
CCR6	Chemokine R6
CCR7	Chemokine R7
CXCR3	CXC chemokine R3
CD115	CSF1R

Immunomodulatory molecules	
PD-1	Co-inhibitory R
PD-L1	Co-inhibitory ligand
CD27	Co-stimulatory R
CD28	Co-stimulatory R
CD40	Co-stimulatory R
ICOS	Co-stimulatory R
CD86	Co-stimulatory ligand

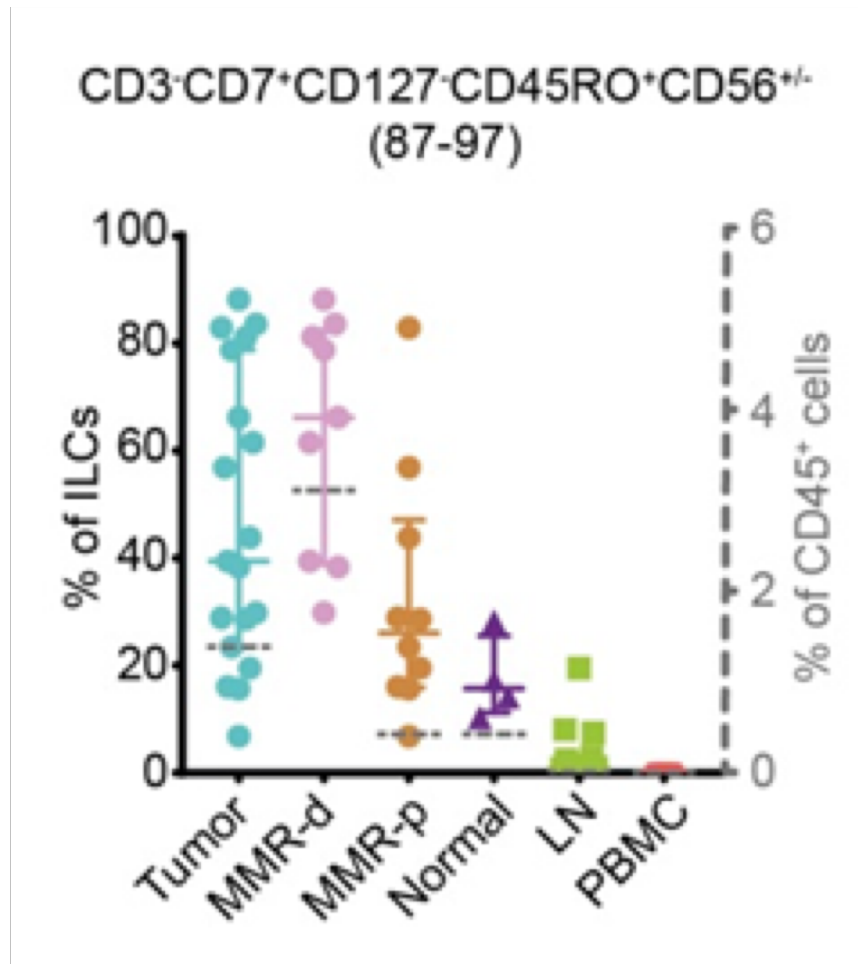
Adhesion/homing markers	
CD44	Glycoprotein
CD54	ICAM-1
CD56	NCAM
CD69	Glycoprotein
CD103	Glycoprotein
CD163	High-affinity scavenger R
KLRG-1	Glycoprotein

High-dimensional immunophenotyping reveals tumor-resident immune subsets across multiple lineages



Int-ILC pop up in CRC!!!

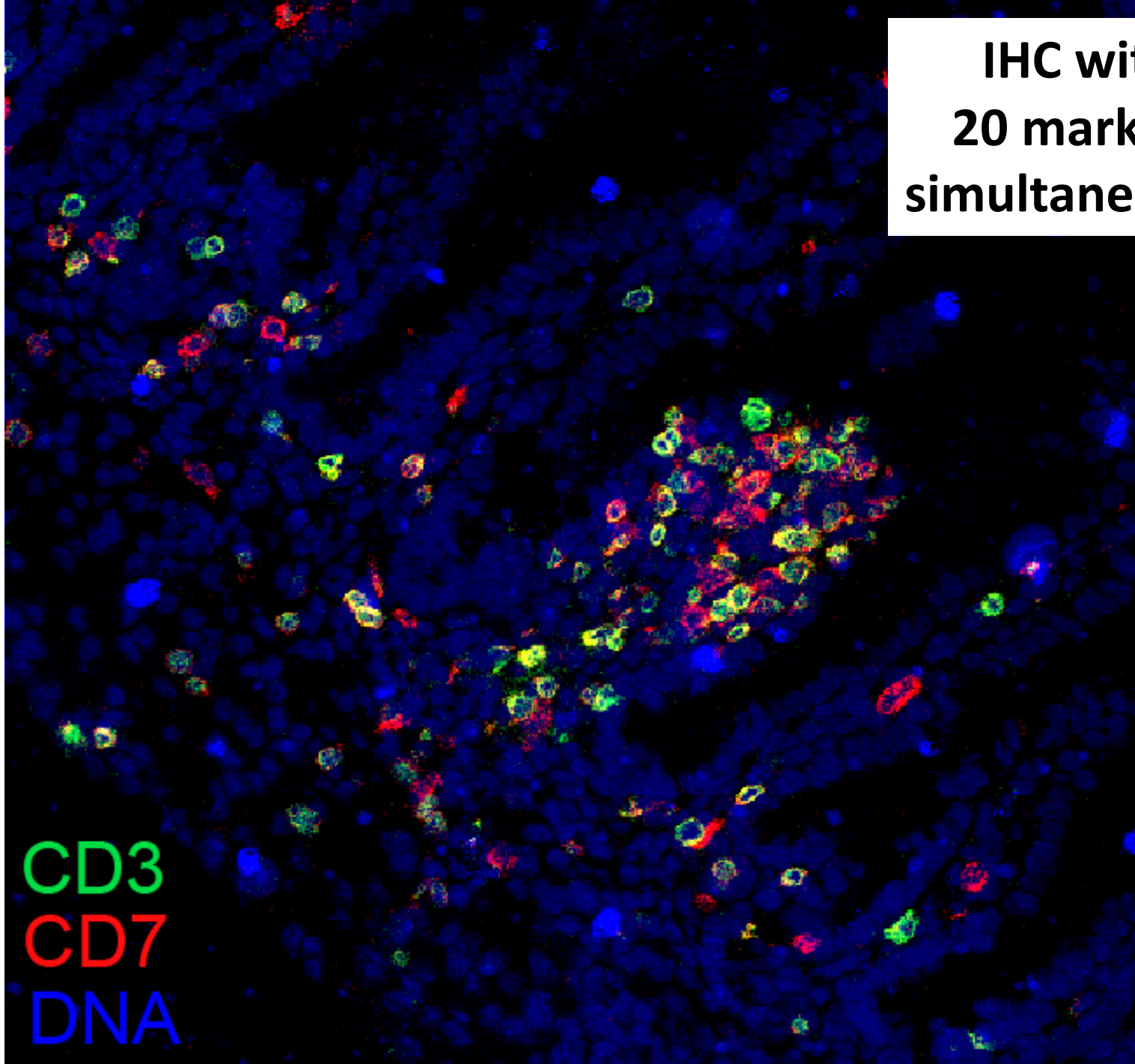
CD3-CD7+CD127-CD45RO+



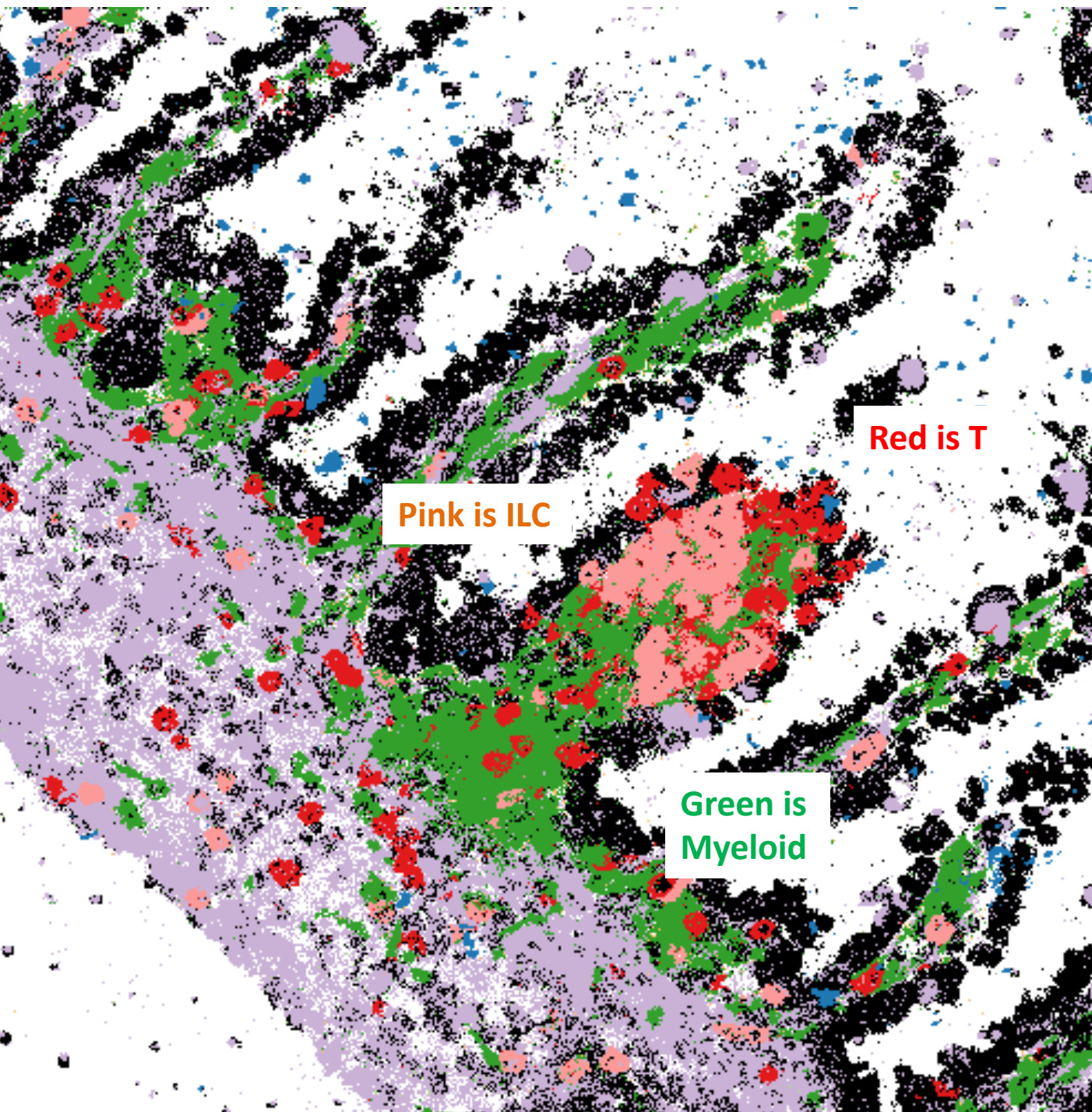
Imaging Mass Cytometry

Immunohistochemistry with 39 markers
simultaneously

IHC with
20 markers
simultaneously



CD3
CD7
DNA



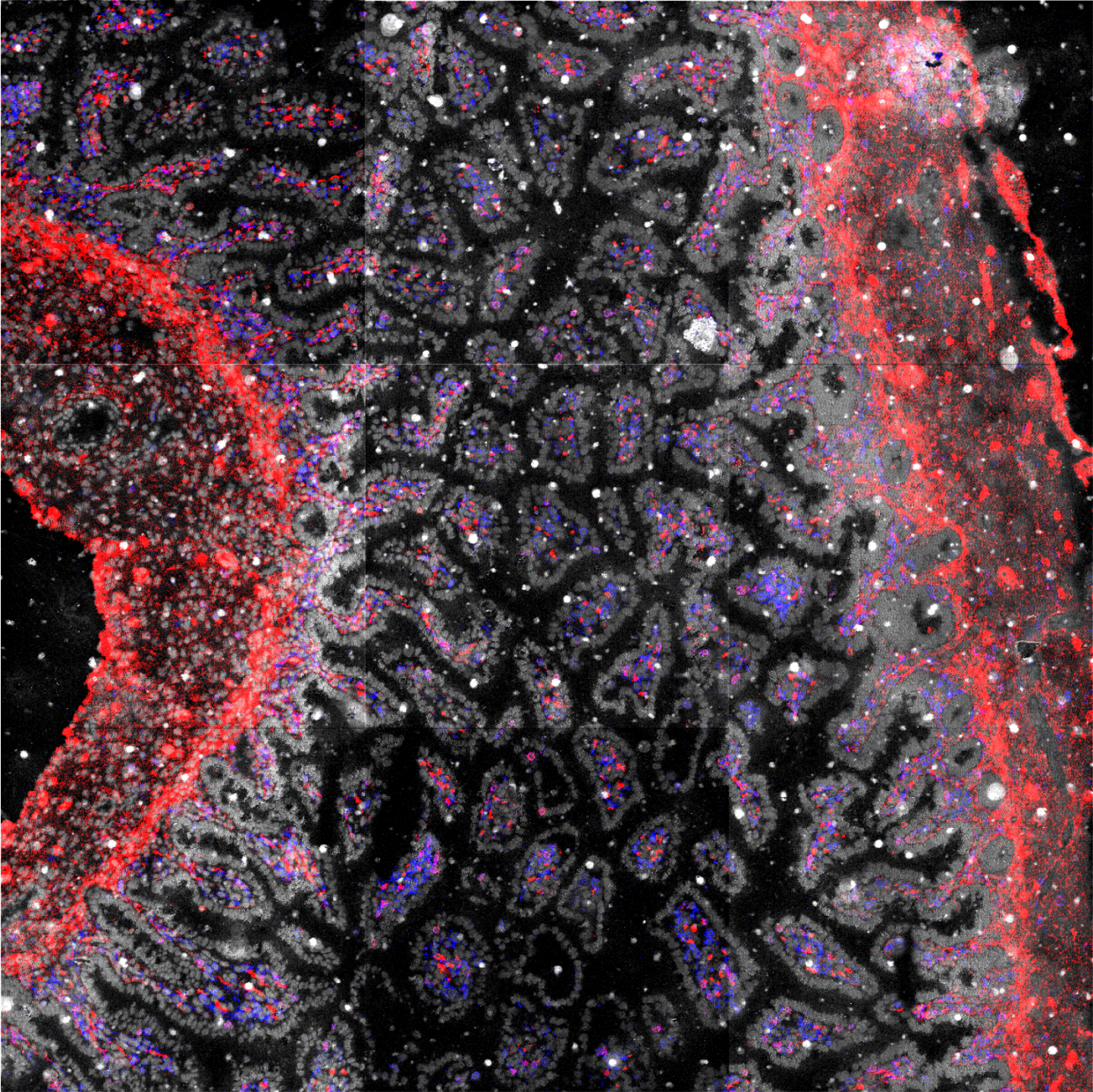
Pink is ILC

Red is T

Green is Myeloid

IHC with
20 markers
simultaneously

HSNE analysis



It is all about discovery!

To boldly go where no one
has gone before

Research positions open

Currently

- Inflammatory bowel disease
- Celiac disease and refractory celiac disease
- Colorectal cancer/Pancreatic cancer
- Transplant rejection
- Pulmonary disease
- Psoriasis and atopic dermatitis
- Parasitology
- Lymphocyte development in the fetal intestine

- PPS-Immunology grant, IMI-BIOMAP grant, CCFA grant, Chinese PhD students, two ZonMW TAS grants

- Research positions open!

Acknowledgements

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- Chris Mulder
- Andrea van der Meulen

Natasja



Vincent



Li Na

