

Asking T cells about what they see in cancer



Immuno-Oncology Biomarkers: State of the Art

Evan W. Newell

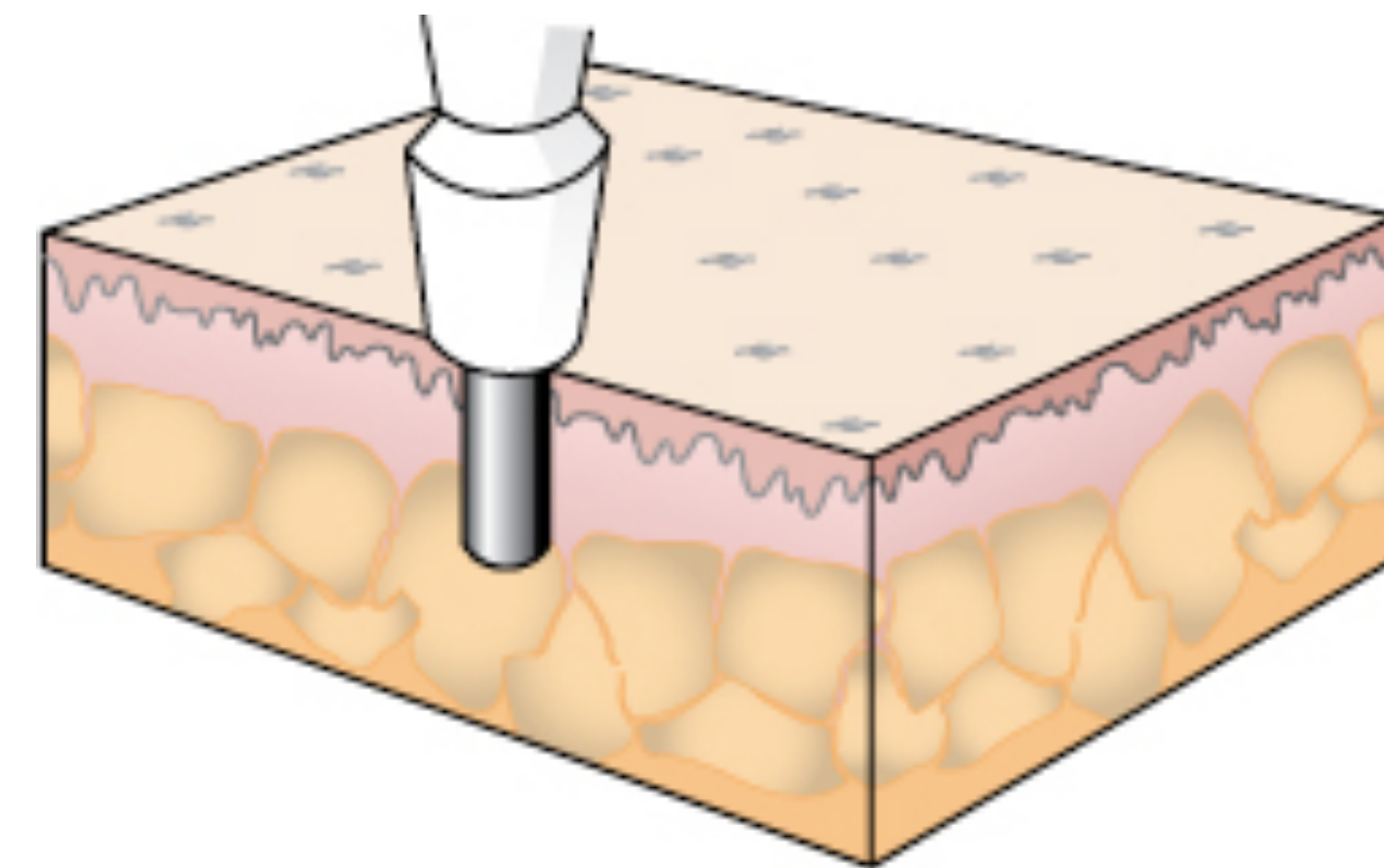
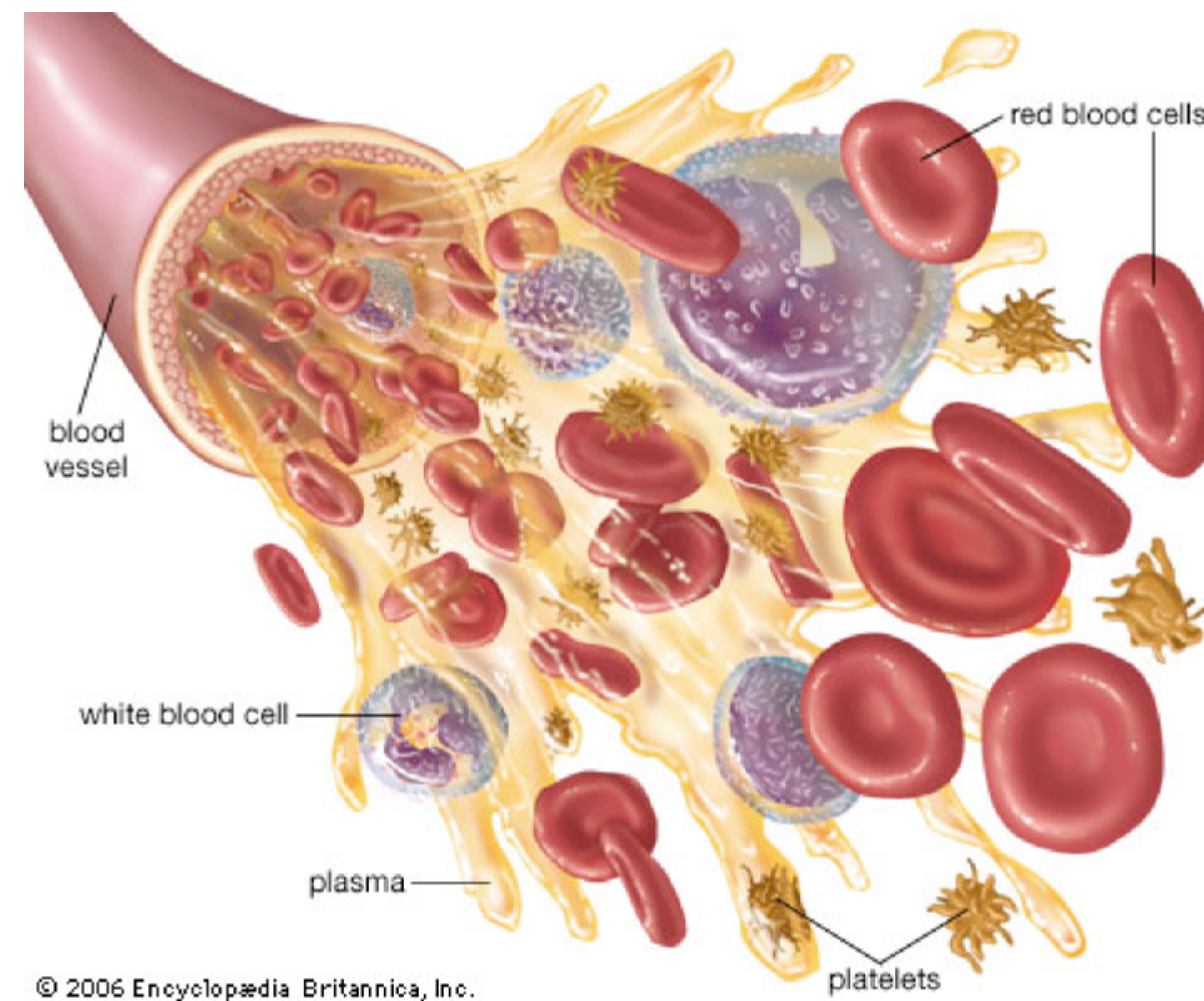
Singapore Immunology Network (SIgN)

Agency for Science, Technology and Research (A*STAR)

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Studying immune responses in humans

- Highly diverse immune cell types in all human tissues confound bulk measures
- Can we do better by focusing attention on **relevant cells**?
- How to focus on the relevant cells?
 - **A broad unbiased perspective**
 - Requires right timing/location
 - **Antigen specificity**
 - Relatively few antigens known



Vision:

Leverage the specificity of T cells to gain insights and develop more accurate methods for personalised medicine

Disclaimer:

Co-founder immune profiling company



www.immunoscape.com



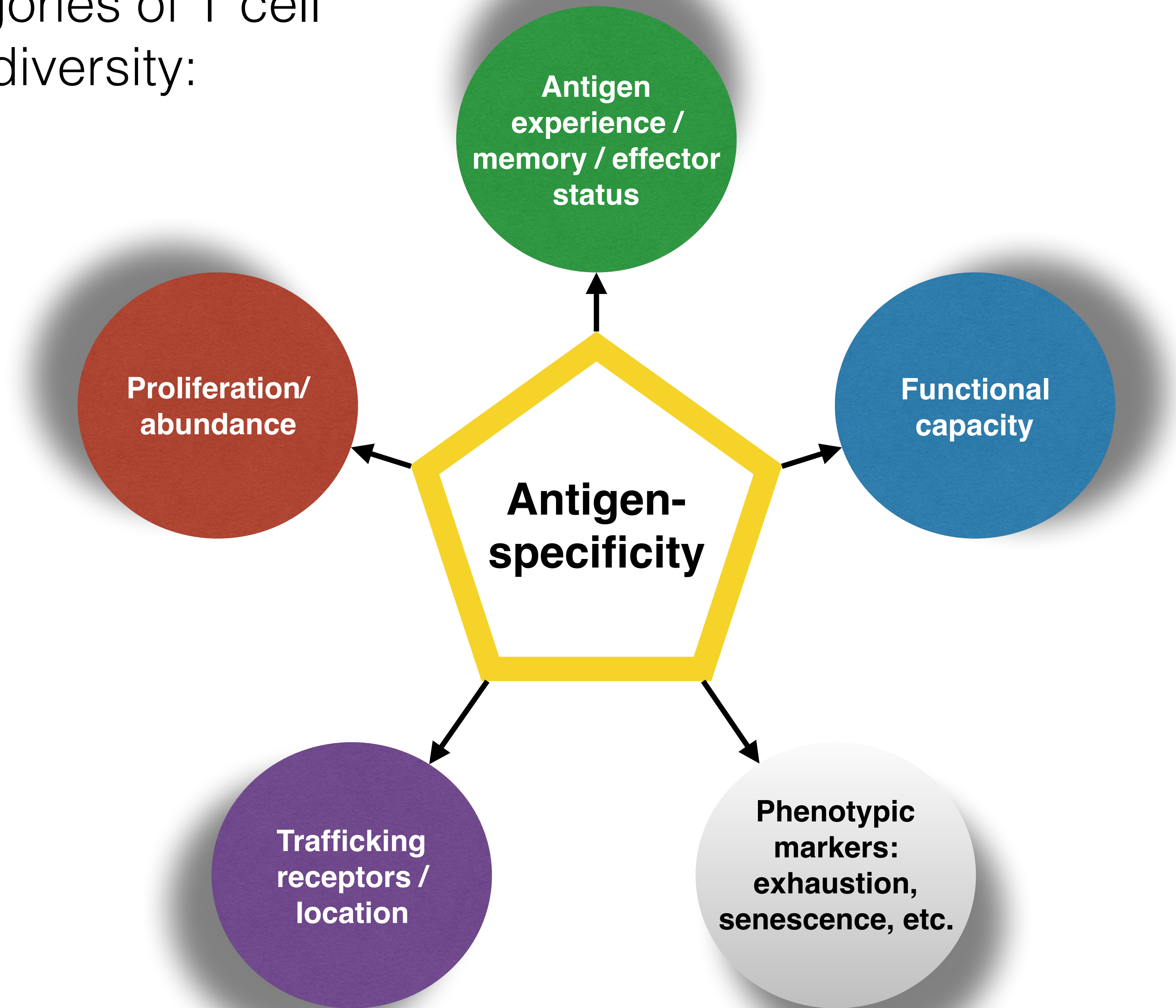
T cell receptor

peptide-MHC

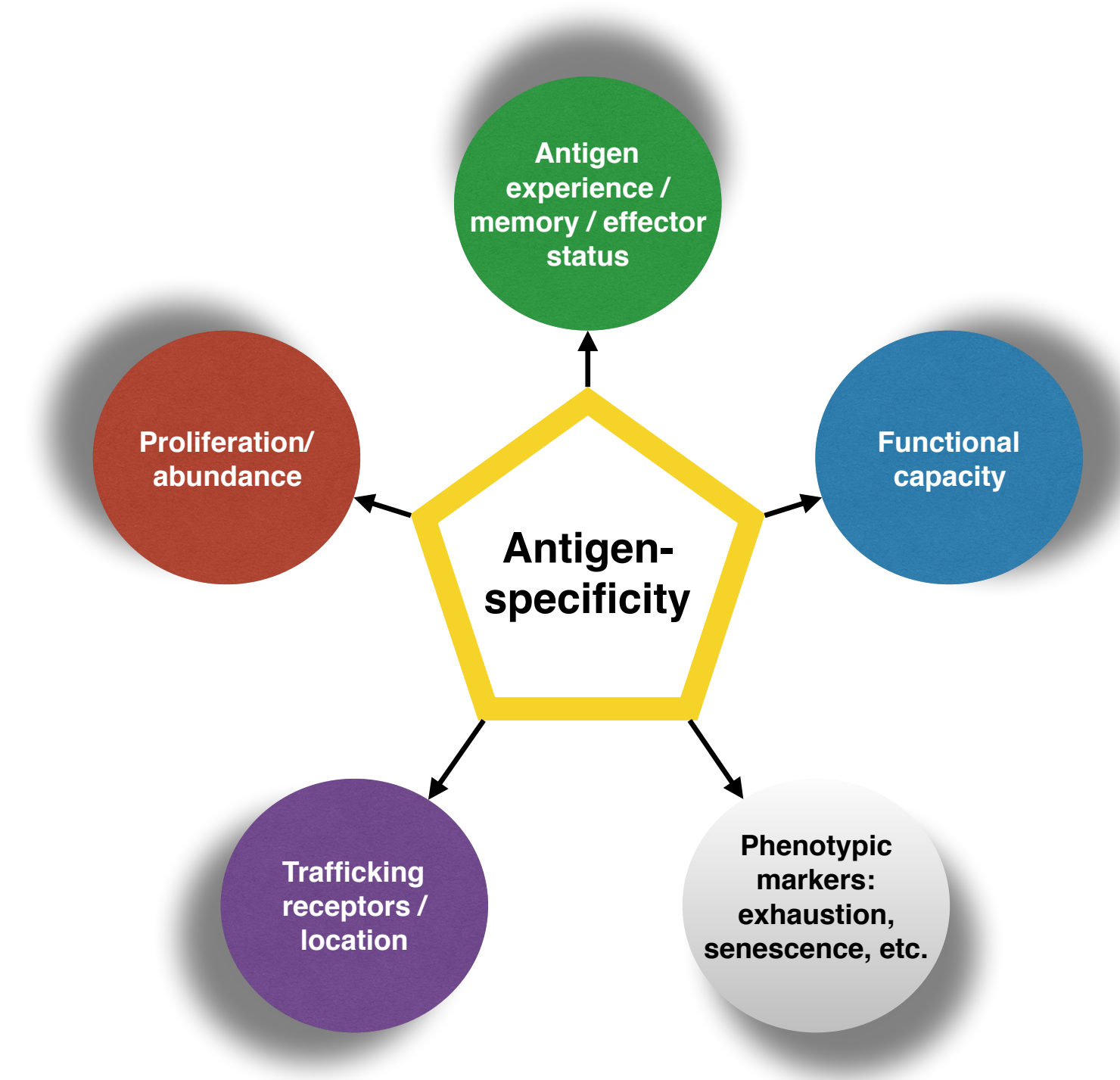
Topics for today

- High-dimensional phenotypic profiling of human T cells
- Identifying and profiling antigen-specific T cells
- Applied to human cancer: using antigen-specificity to decipher tumor infiltrating T cell profiles

Categories of T cell diversity:



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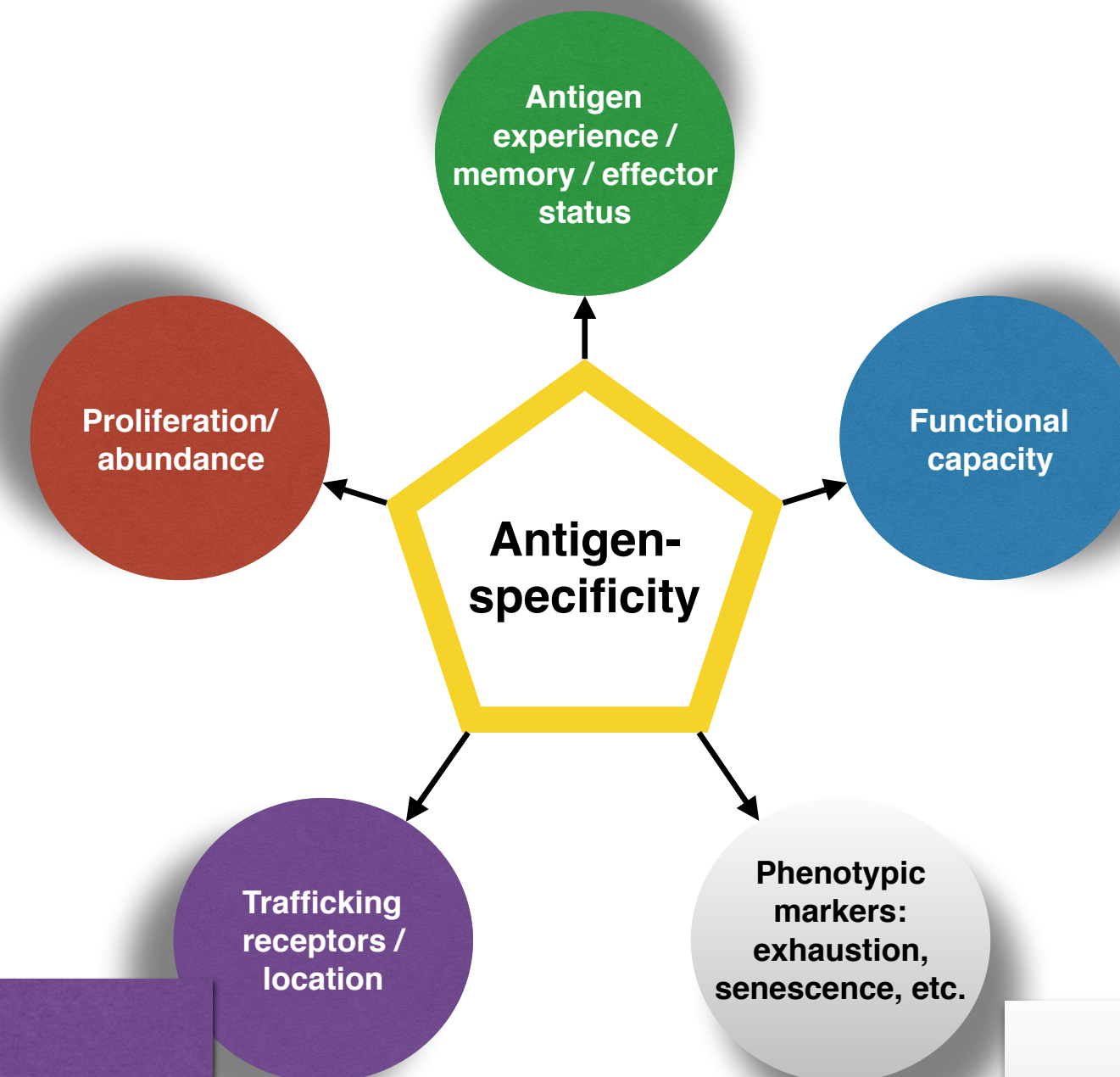
Categories of T cell diversity:

Cell frequency
Ki67, Cell cycle markers,
DNA content, etc.

Surface marker phenotype
“Naive, CM, EM, TEM, Activated,
etc.”
CD45RA, CD27, CCR7, CD62L,
CD38, etc.

Cytokines
Chemokines
Cytotoxic
granules
CD107

Chemokine receptors
Integrins
Other tissue-specific
receptors



Markers associated with
cellular disfunction
PD-1, PD-L1, Tim-3, Lag-3,
2B4, CD57, OX40, Tigit, etc.

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Cytokines
Chemokines
Cytotoxic granules
CD107

Cell frequency
Ki67, Cell cycle markers, DNA content, etc.

Proliferation/
abundance

Antigen
experience /
memory / effector
status

Functional
capacity

Antigen-
specificity

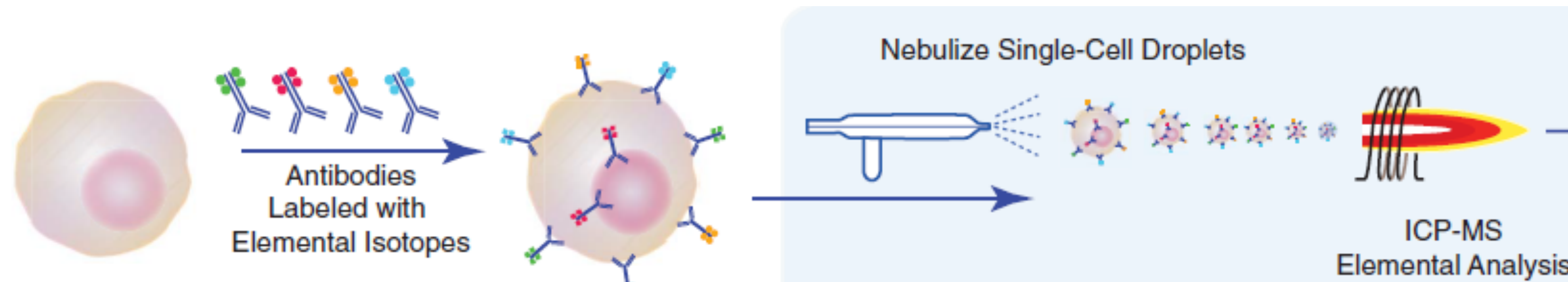
Trafficking
receptors /
location

Phenotypic
markers:
exhaustion,
senescence, etc.

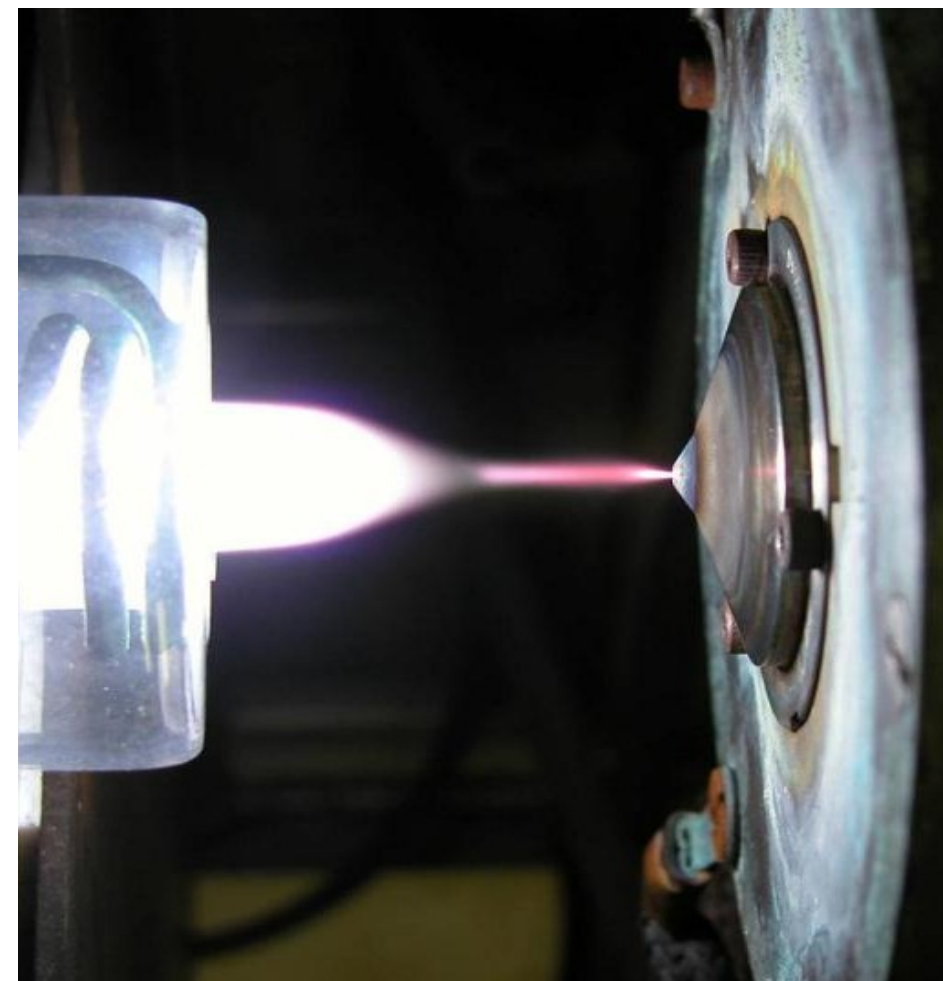
Chemokine receptors
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Single-cell mass spectrometry (CyTOF)

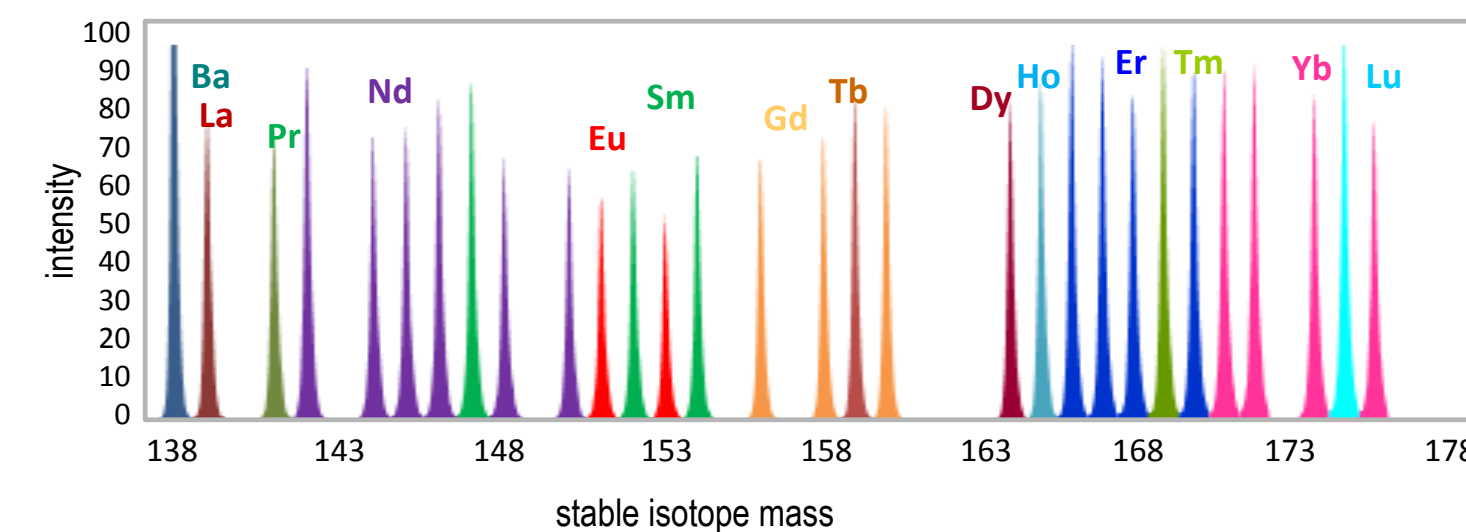


Argon
+
dry cells



>40 Abs simultaneously

Time-of-flight
mass spectrometer

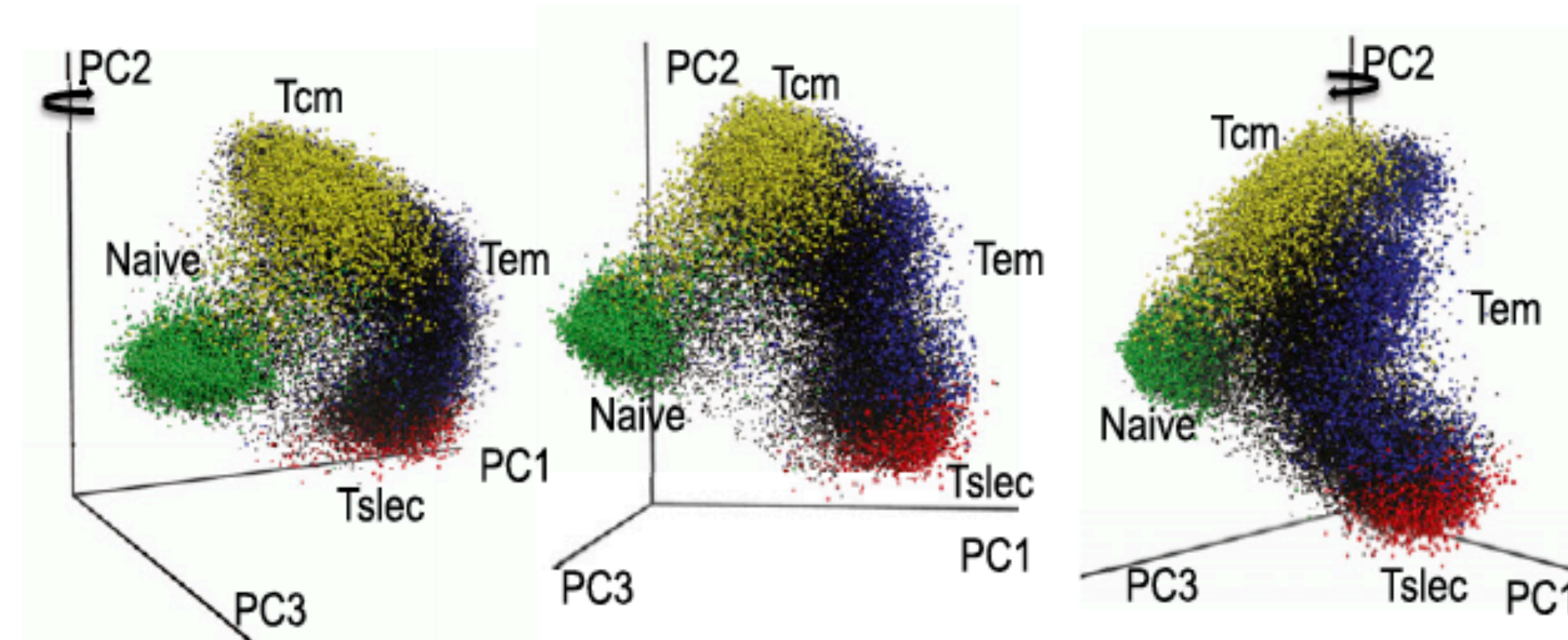


Pros and cons...

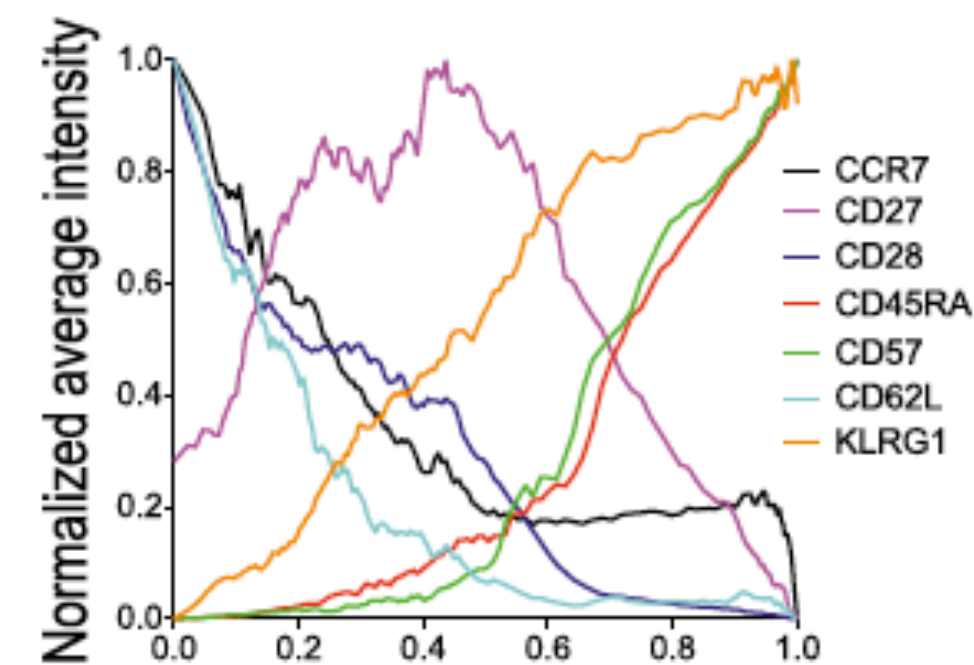
Visualizing high dimensional data

- The problem
- Cluster analysis
- Force directed layout
- **Dimensionality reduction**
 - **PCA**
 - t-SNE
 - UMAP

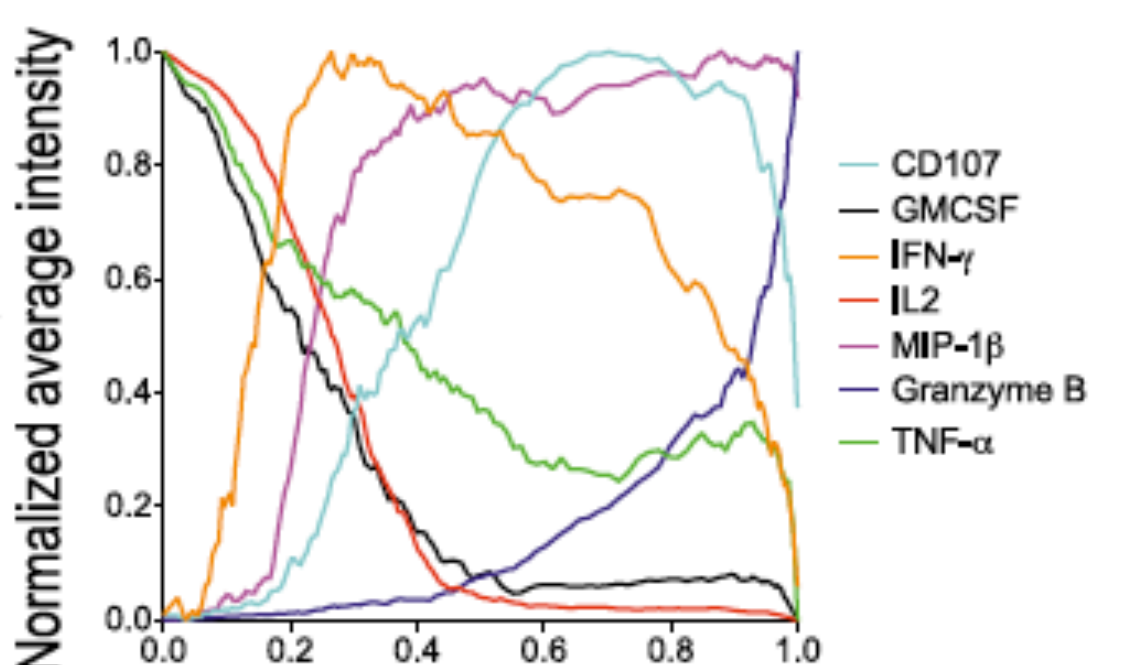
A 3D-PCA view of CD8⁺ T cell 25 parameter data



B Memory cell phenotypic progression



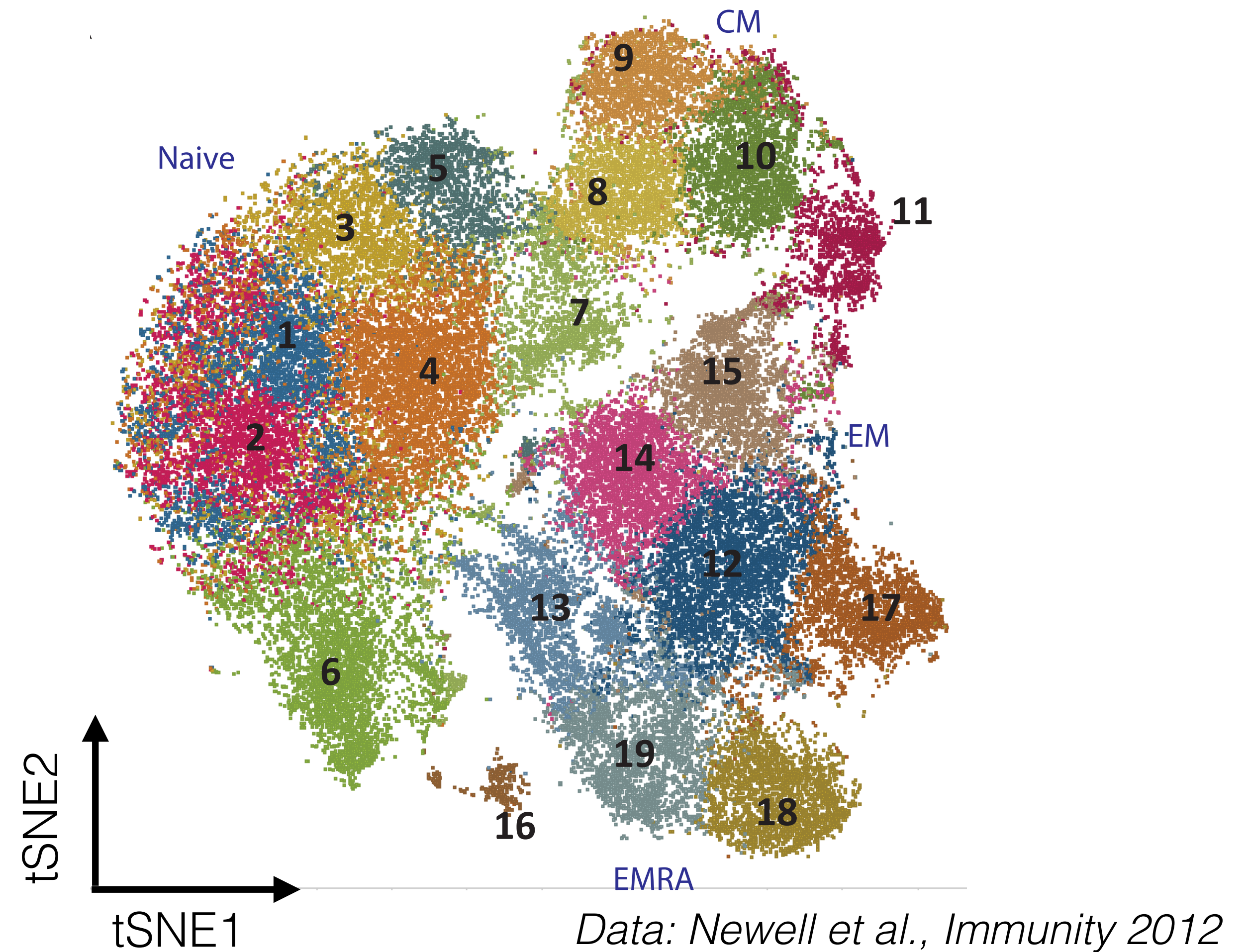
C Memory cell functional capacity progression



Normalized PC2 (memory phenotypic progression)

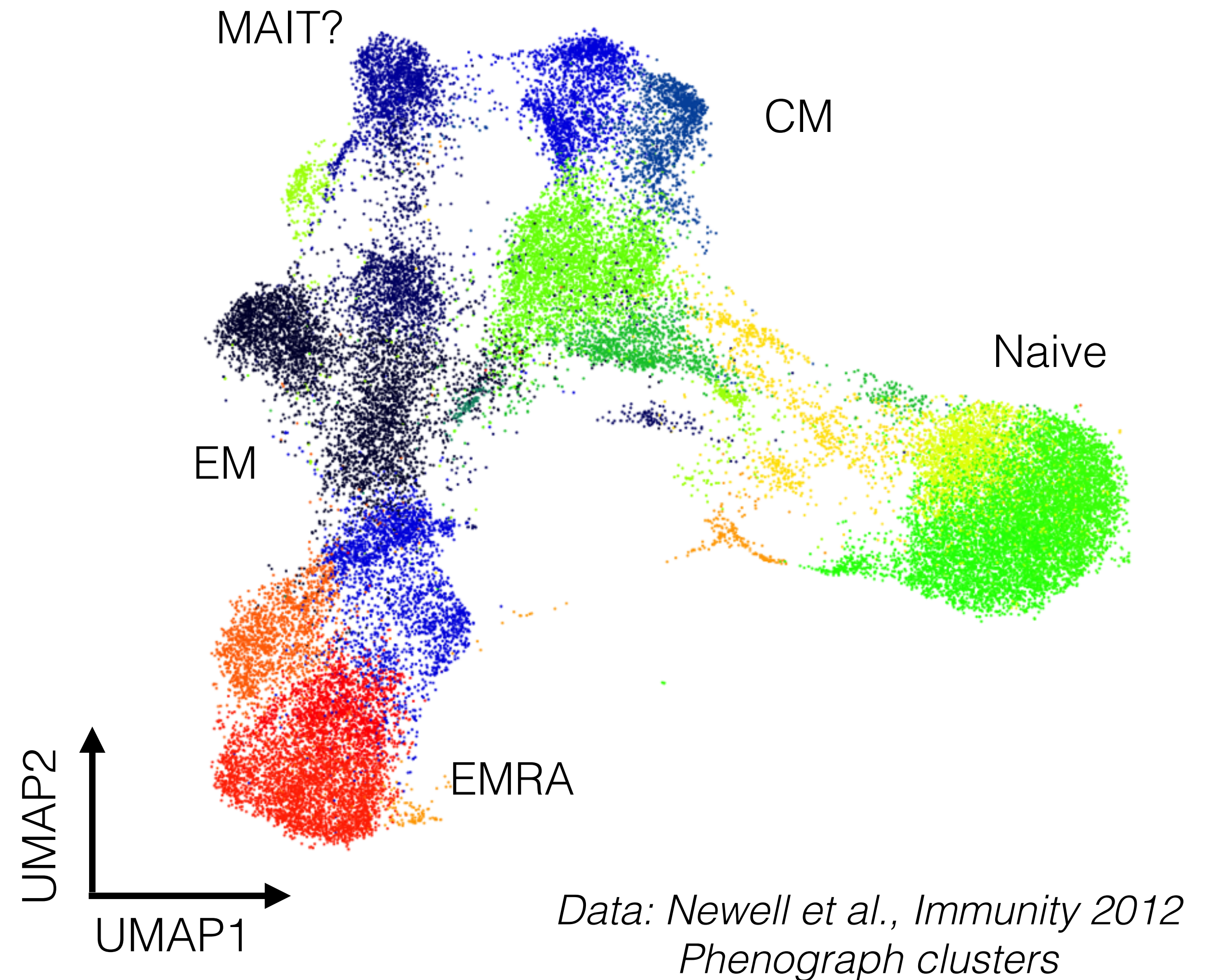
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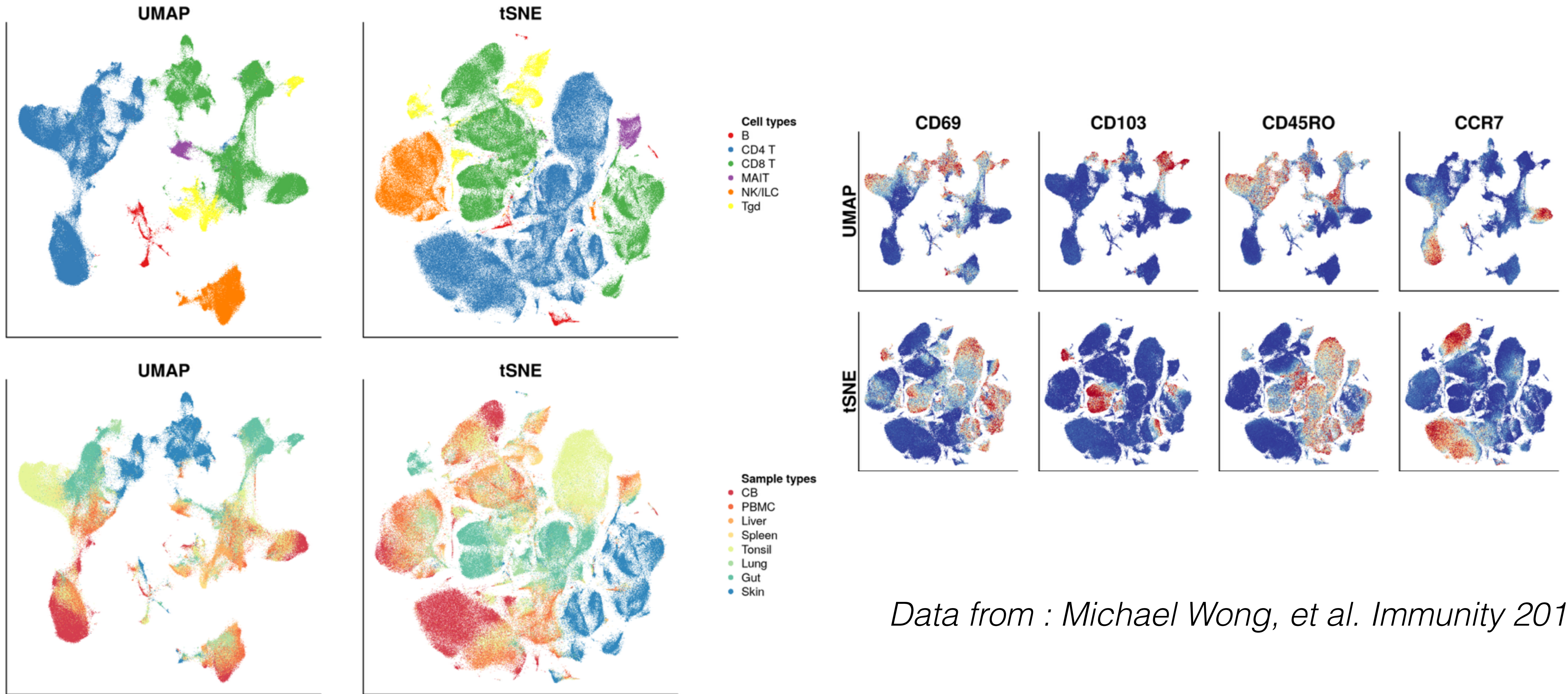


Visualizing high dimensional data

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T and NK cells from 8 human tissues: Wong et al., Immunity 2016



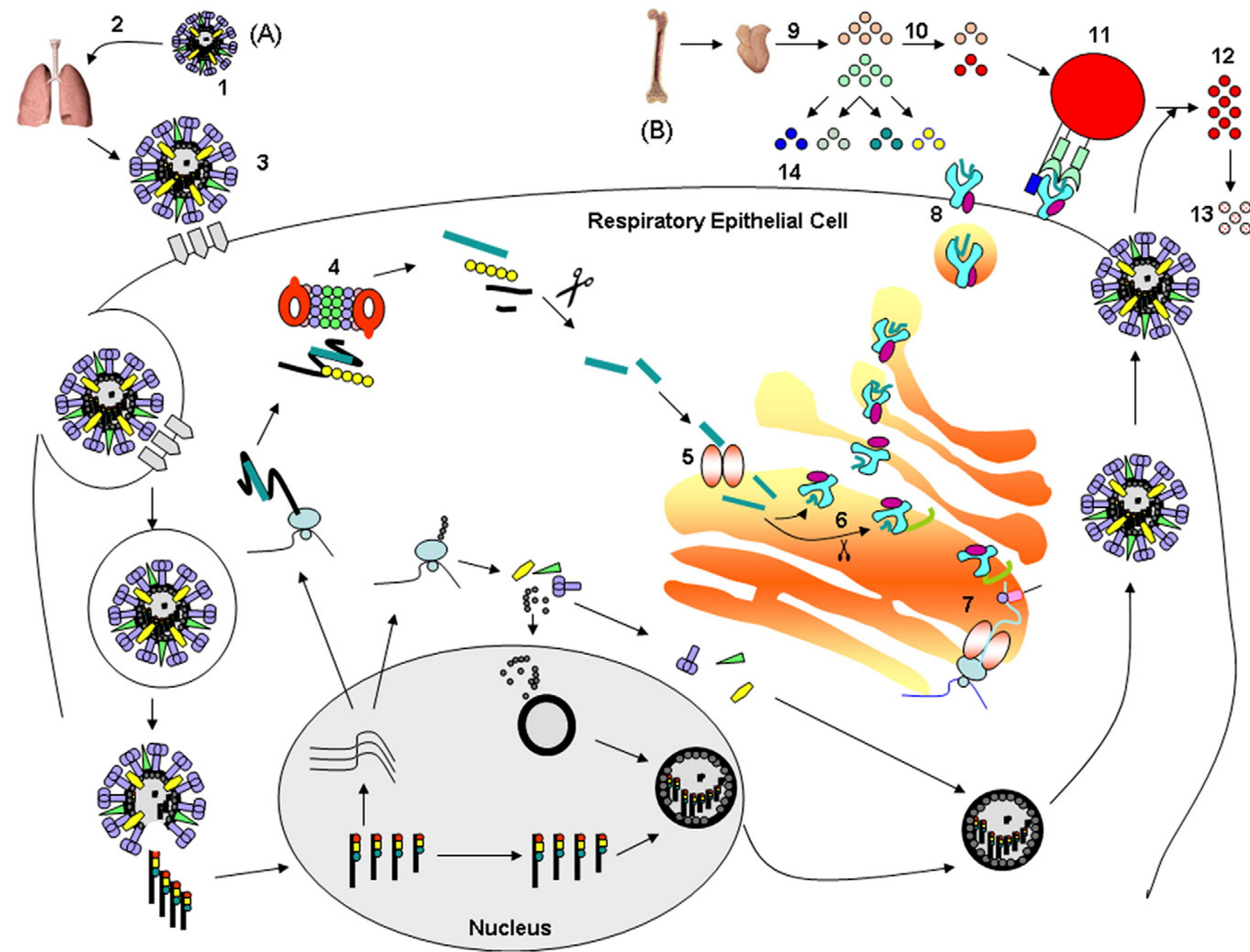
Data from : Michael Wong, et al. Immunity 2016

Topics for today

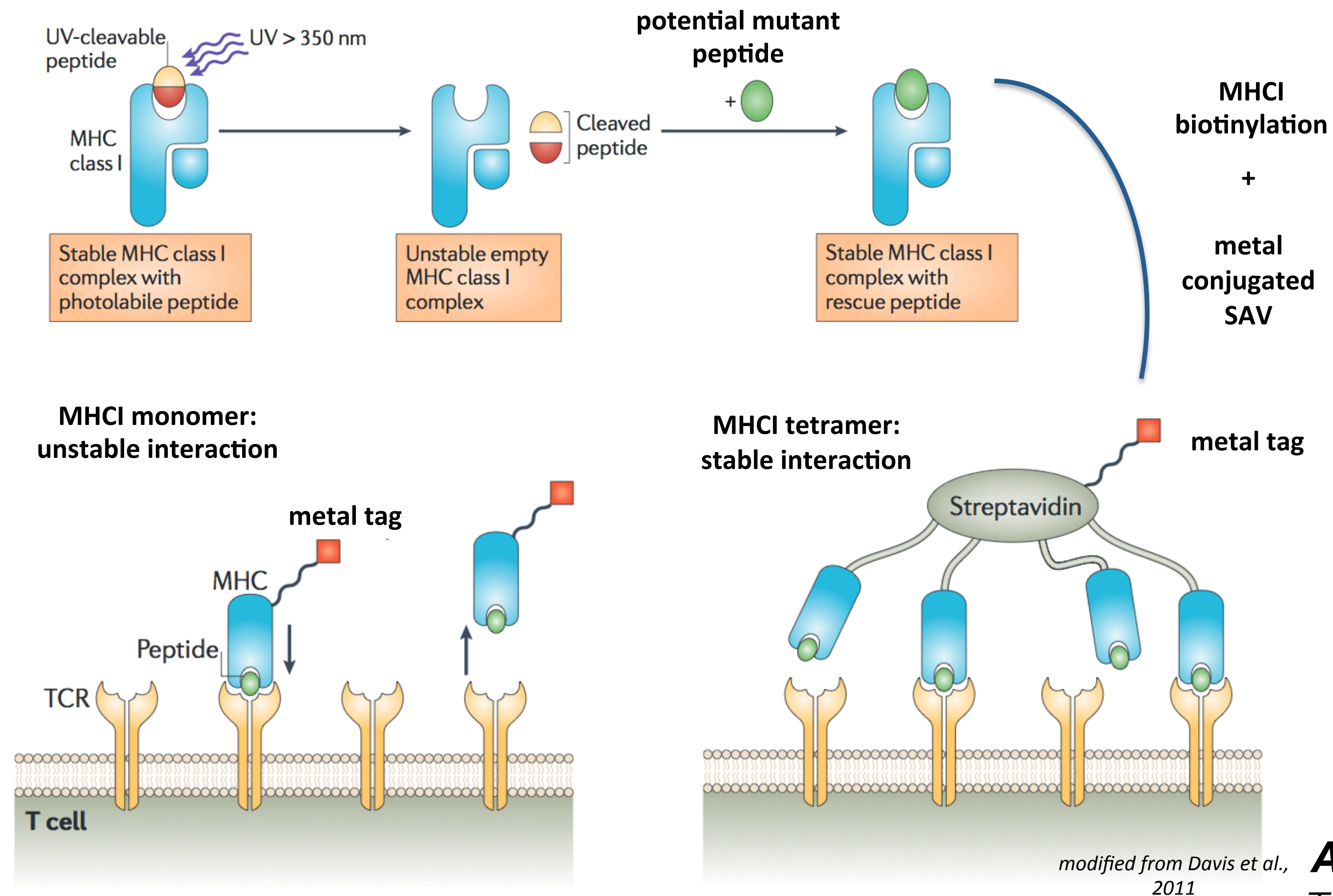
- High-dimensional phenotypic profiling of human T cells
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Many factors influence T cell epitope usage

1. Prior infection status
2. Route of infection
3. Viral mutation
4. Proteasome
5. TAP
6. ER aminopeptidase
7. Calnexin/calreticulin
8. MHC allele
9. TCR recombination
10. T cell precursor frequency
11. TCR:pMHC binding
12. Granzymes
13. Memory T cells
14. Tregs
15. Host age / energy level
16. And more!



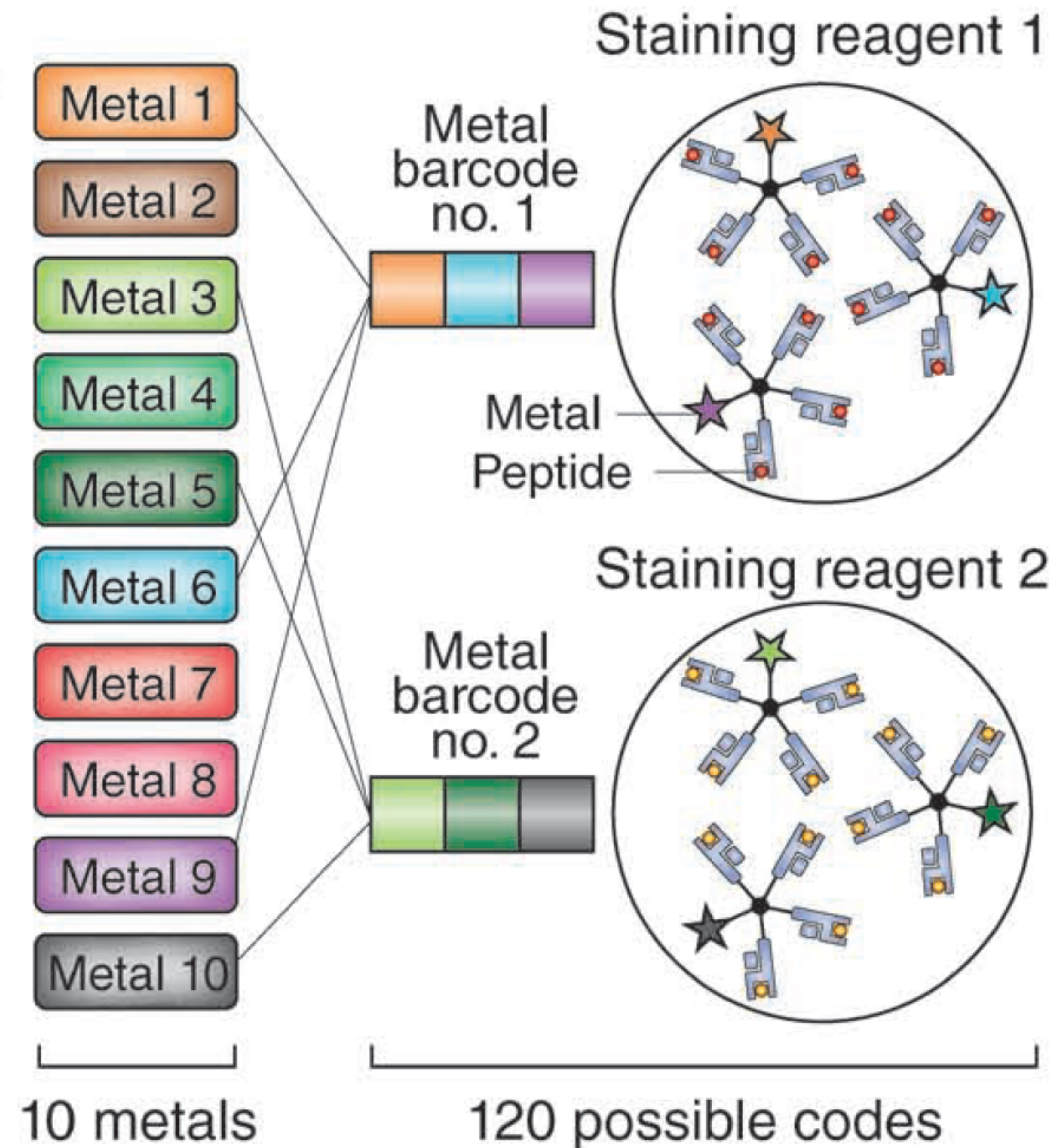
MHC tetramer staining to ID antigen specific T cells



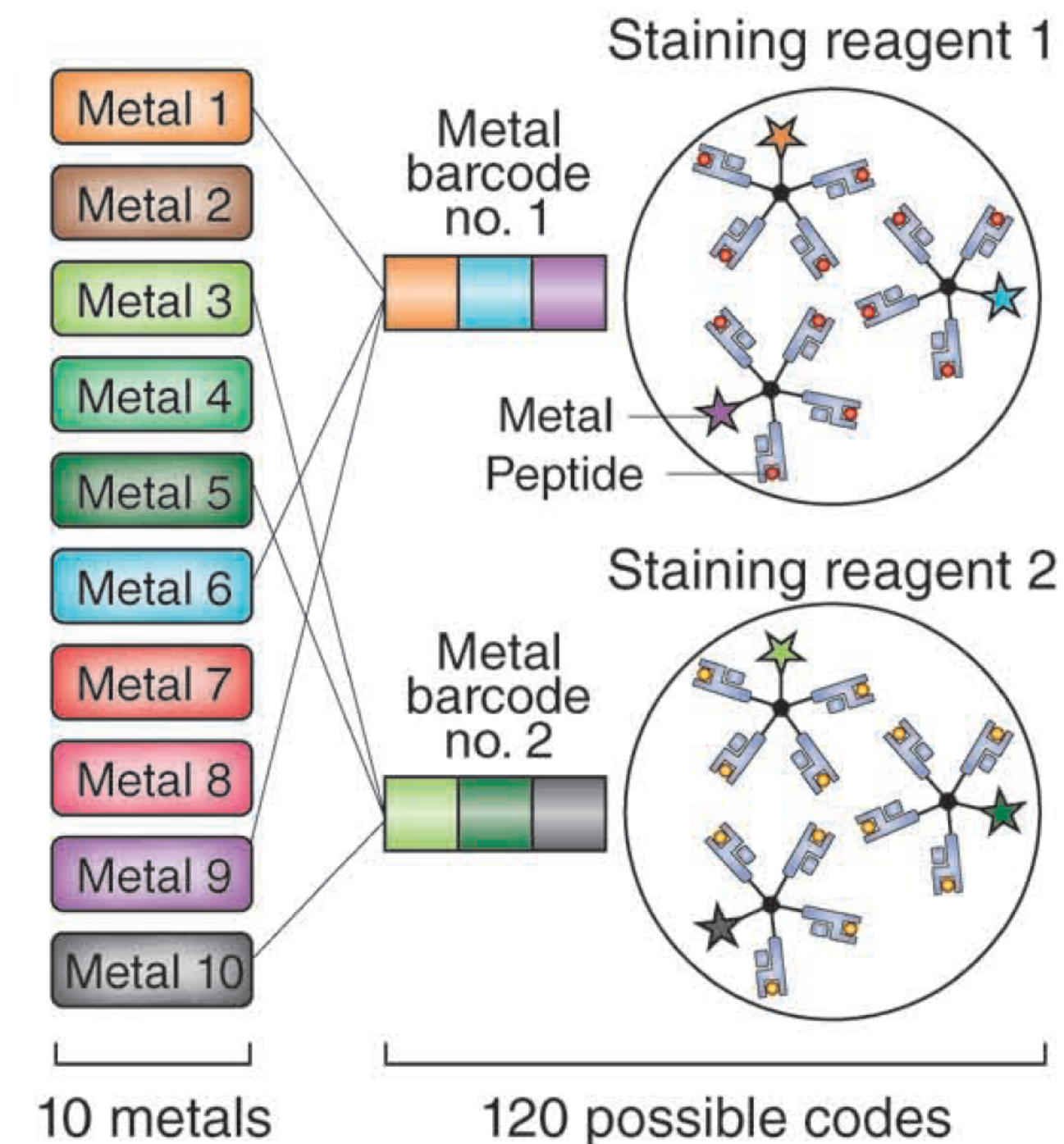
modified from Davis et al.,
2011

Altman et al., Science 1996
Toebe et al., Nat. Med. 2006

CyTOF based combinatorial tetramer staining



CyTOF based combinatorial tetramer staining



Newell & Davis, Nat. Biotech 2014

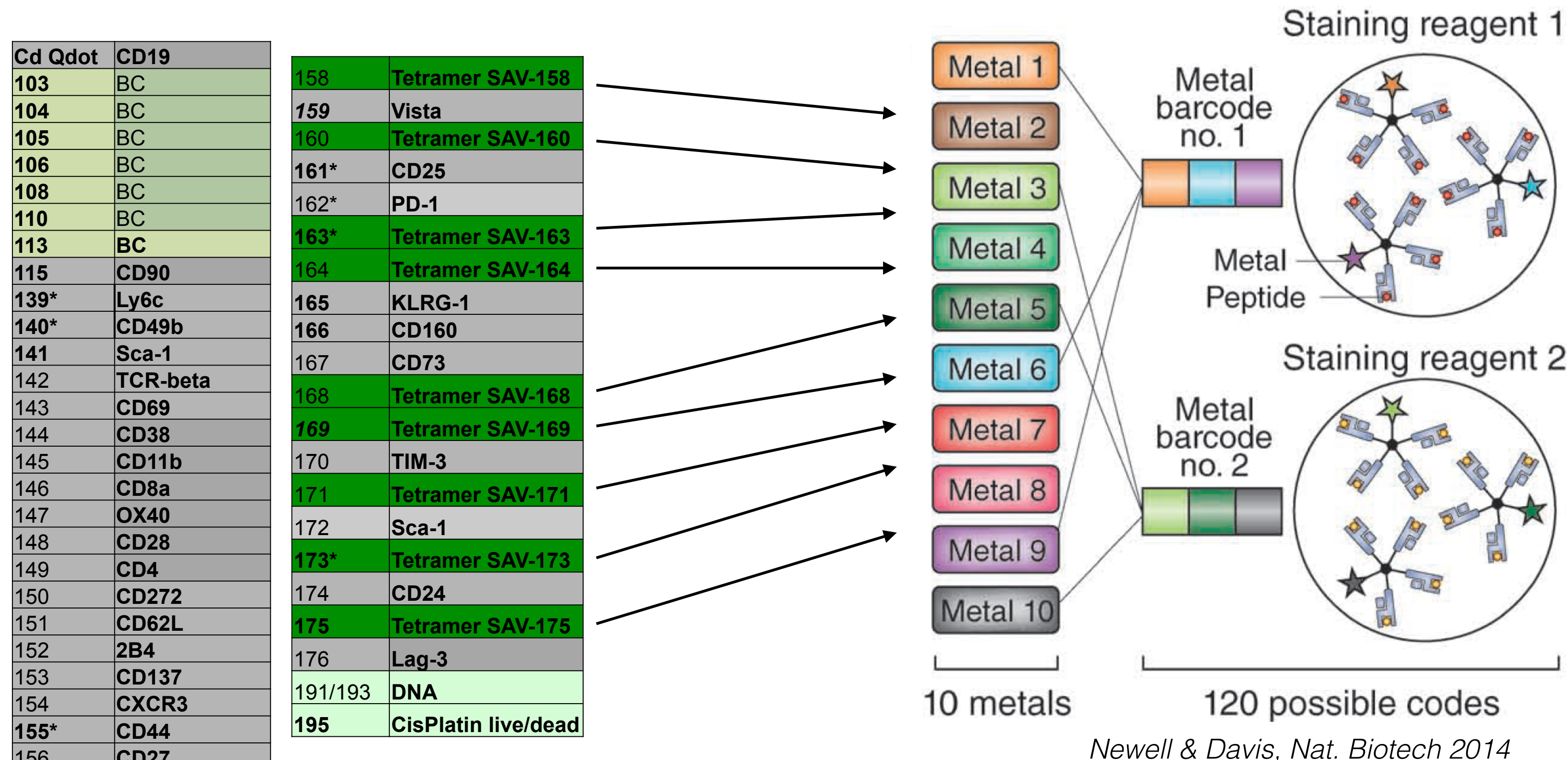
120 ways to choose 3 metals from a list of 10

Each tetramer (peptide) has a unique combination of 3 SAV metal codes

>1000 ways to choose 4 from a list 14

Newell, et al. & Hadrup et al., Nat. Methods 2009 Newell, et al. Nat. Biotech 2013 Fehlings, et al. Nat. Comms. in press

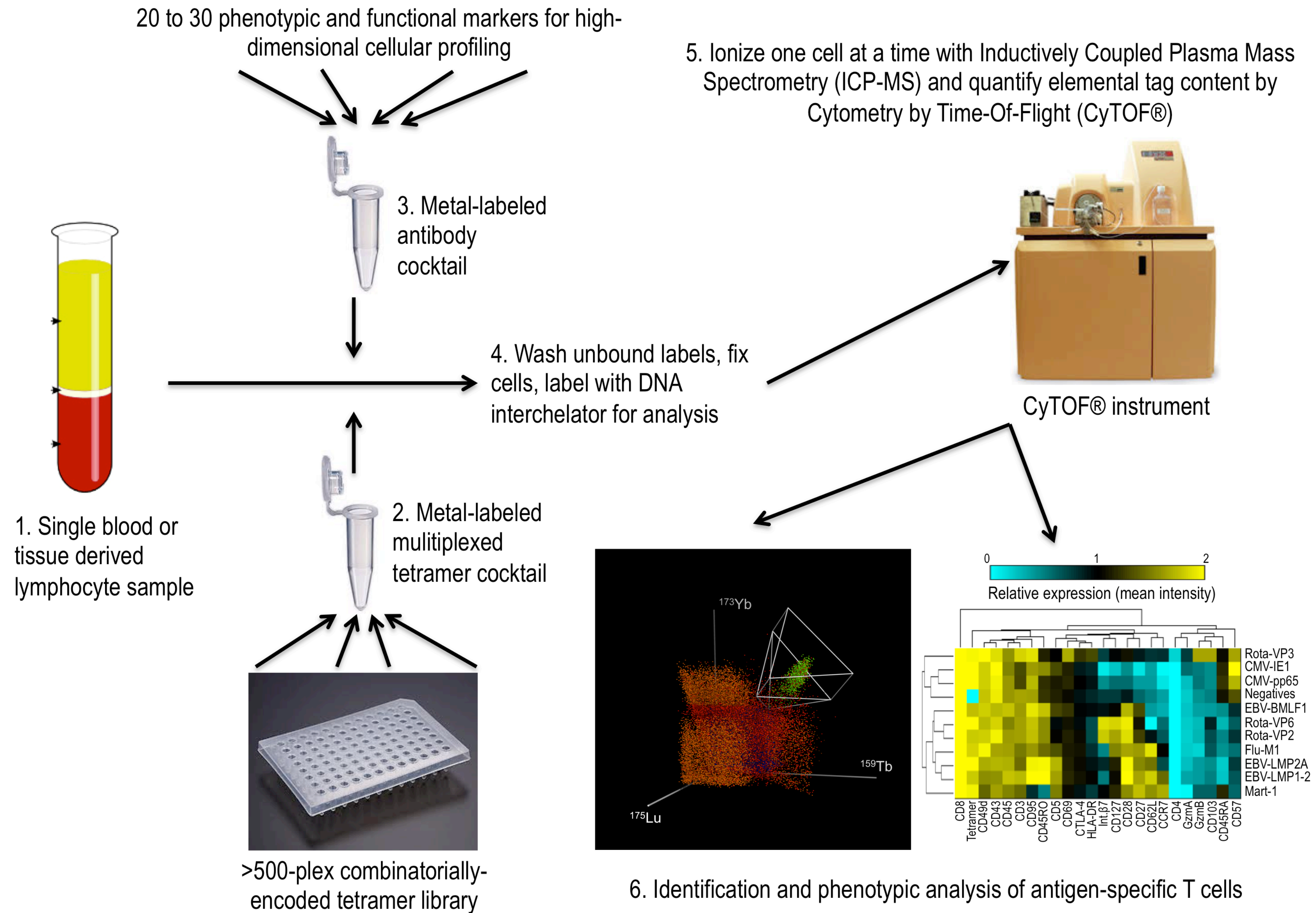
CyTOF based combinatorial tetramer staining



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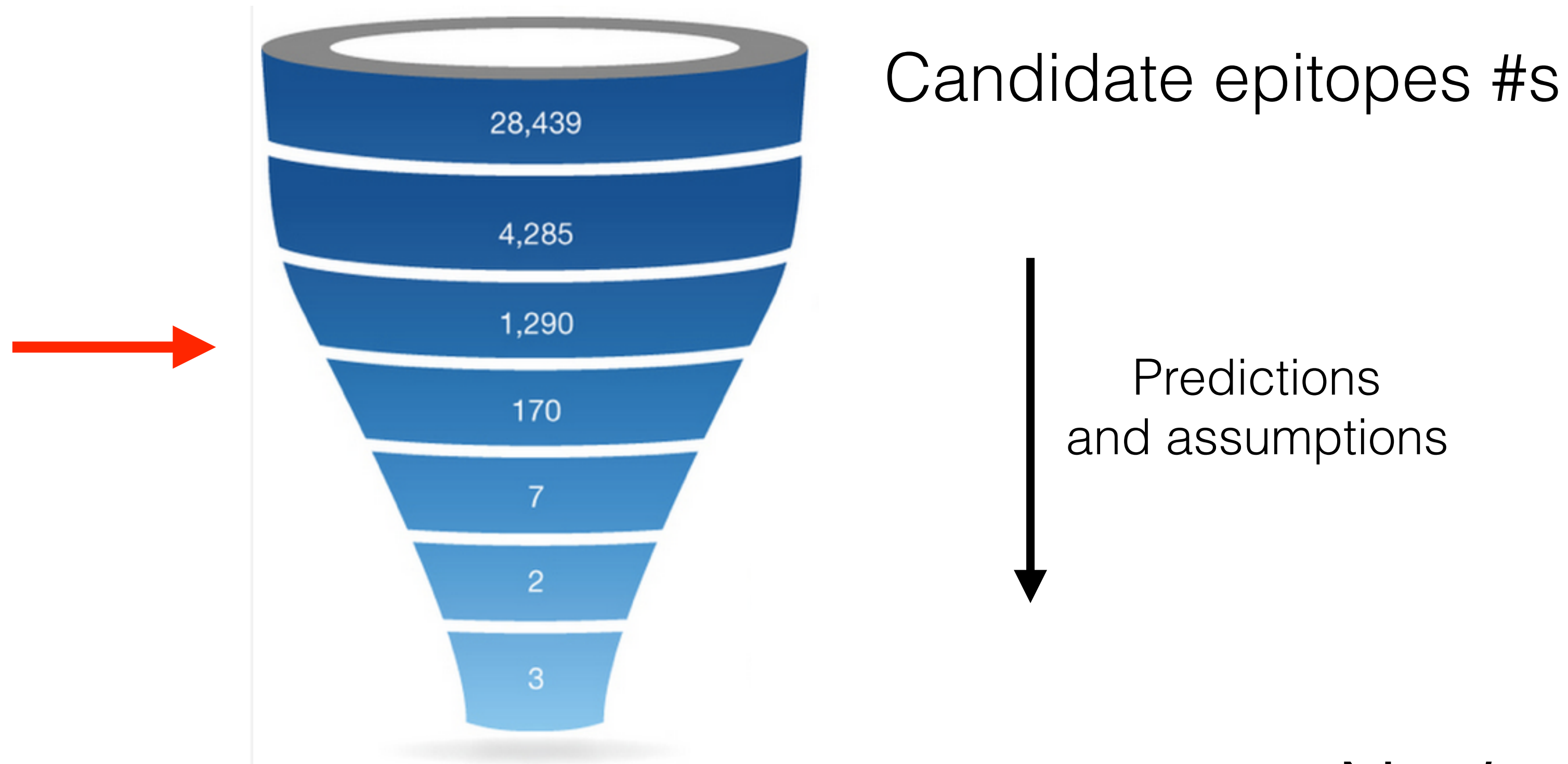
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Types of tumor antigens

Category	Example Antigen	Cancer Histology
Oncofetal	CEA	Colorectal carcinoma
Oncoviral	HPV E6, E7	Cervical carcinoma
Overexpressed/accumulated	Her2/neu	Multi
	Telomerase	Multi
	Mesothelin	Ductal pancreatic carcinoma
	SAP-1	Colorectal carcinoma
	Survivin	Multi
Cancer-Testis	BAGE family	Multi
	MAGE family	Multi
	NY-ESO-1/LAGE-1	Multi
	PRAME	Multi
	SSX-2	Melanoma, Multi
Lineage Restricted	Melan-A/MART-1	Melanoma
	Gp100/pmel17	Melanoma
	Tyrosinase	Melanoma
	Prostate-specific antigen	Prostate
 Mutated	β-catenin	Melanoma, Prostate, HCC
	BRCA1/2	Breast, ovarian carcinoma
	Ras	Multi
	TGF-βRII	Colorectal carcinoma
Posttranslationally altered	MUC1	Ductal carcinoma, RCC
Idiotypic	Ig, TCR	B, T leukemia, lymphoma, myeloma
Endogenous RV	HERV's	esp. ccRCC

Highly multiplex approach



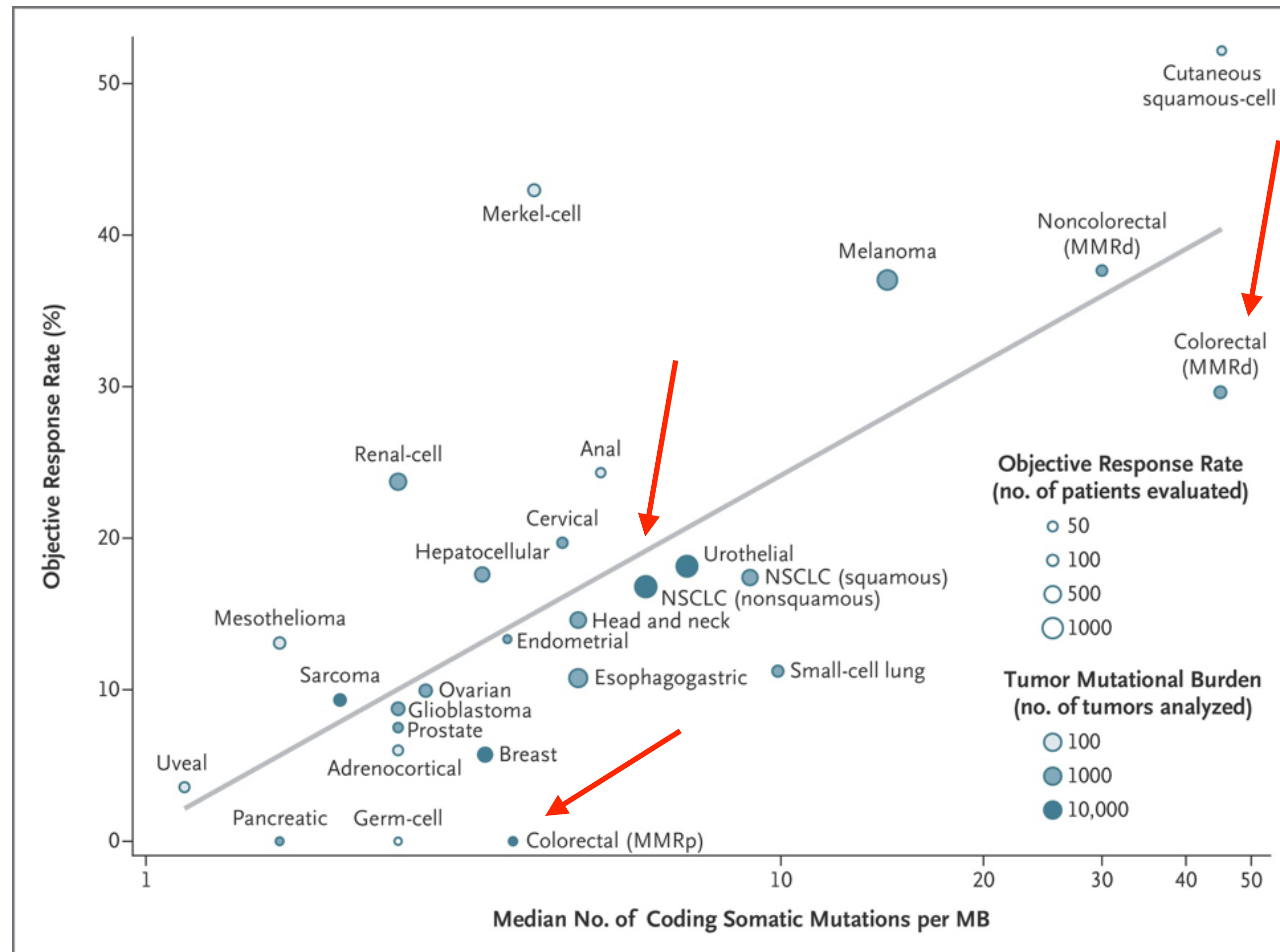
From: Yadav et al., Nature 2014

No *in vitro* expansion required:
phenotypic profiles preserved

Topics for today

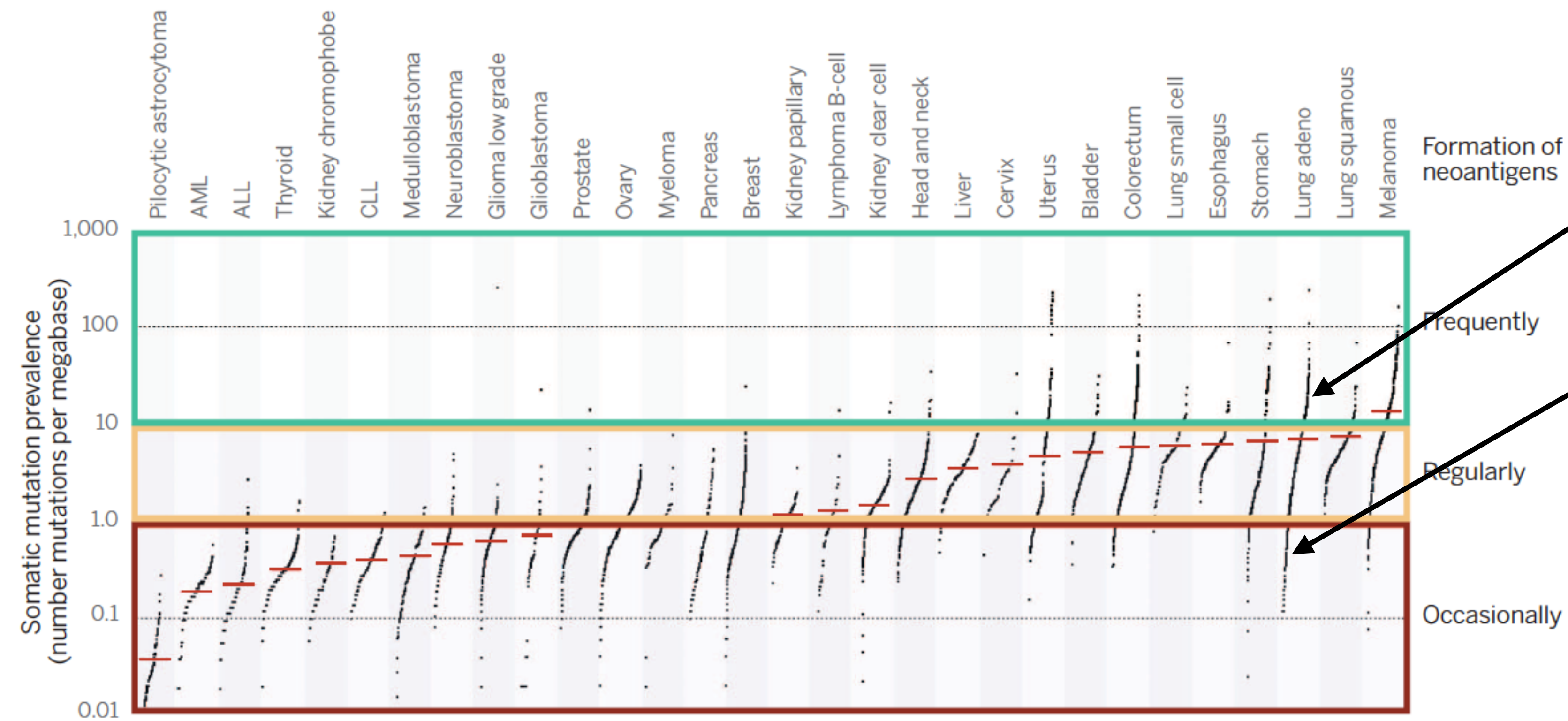
- High-dimensional phenotypic profiling of human T cells
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Evidence for importance of T cells specific for mutation derived 'neo-antigens'



Yarchoan NEJM 2018

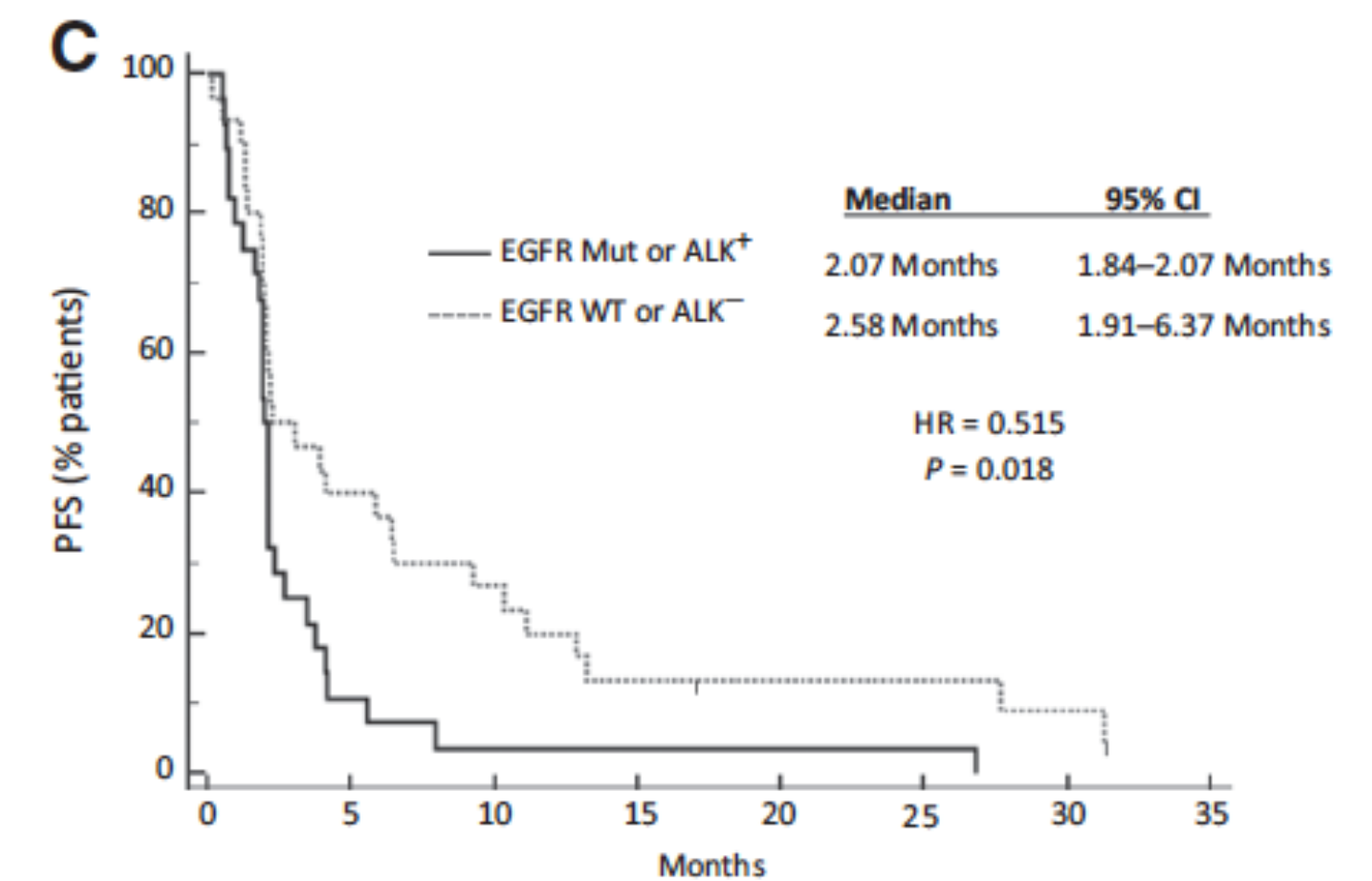
Evidence for importance of T cells specific for mutation derived 'neo-antigens'



EGFR-wt NSCLC

EGFR-mutant NSCLC

Alexandrov et al., Nature 2013,
Schumacher and Schreiber, Science 2017

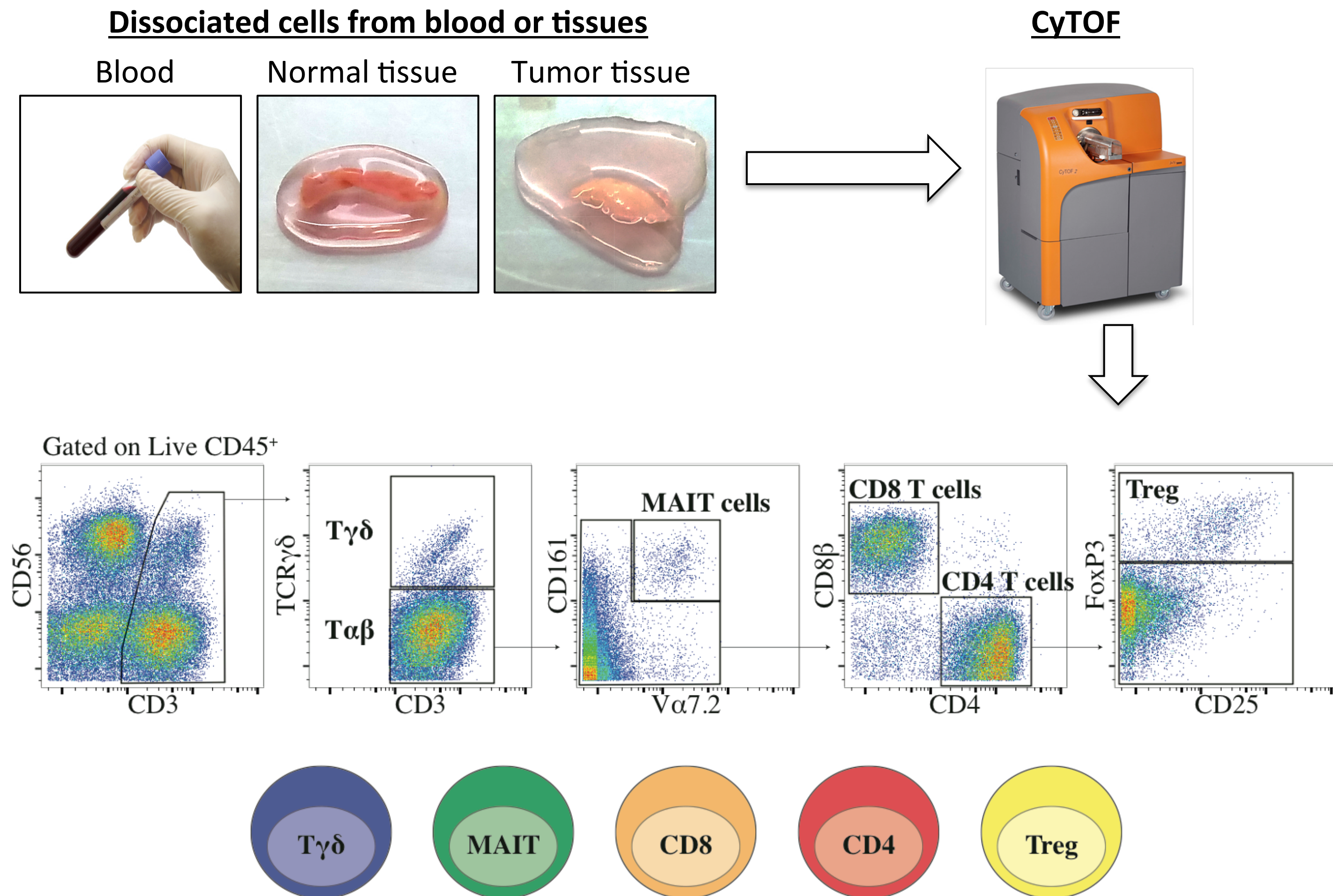


Gainor et al., Can. Res. 2016

Many big questions

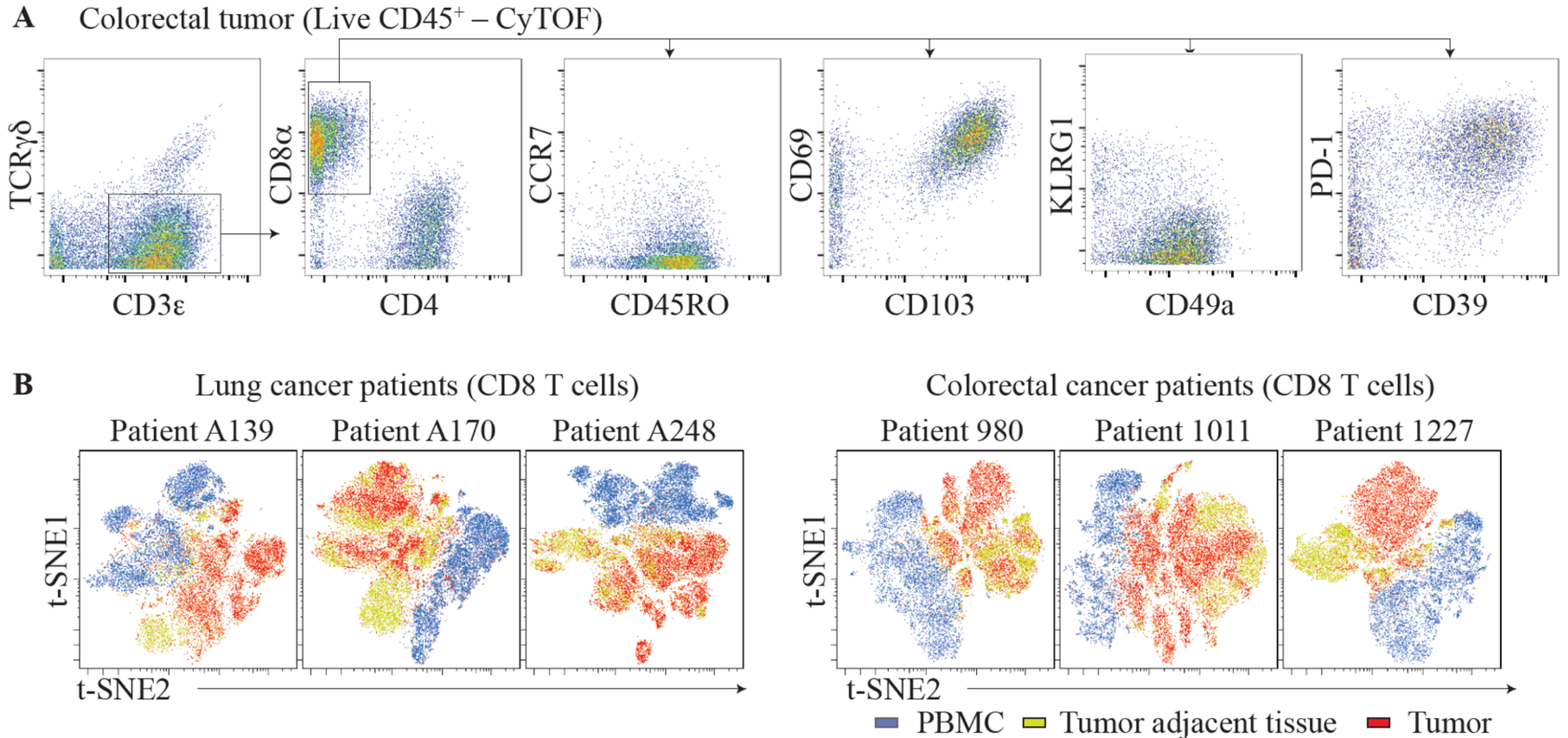
- Why do some patients respond to checkpoint blockade immunotherapy and can they be predicted?
 - Can understanding the profiles of TILs help to solve this problem?
 - Can information about antigen-specificity help to resolve complexity of TIL profiles?

CyTOF analysis of tumor infiltrates in human lung and colorectal cancer

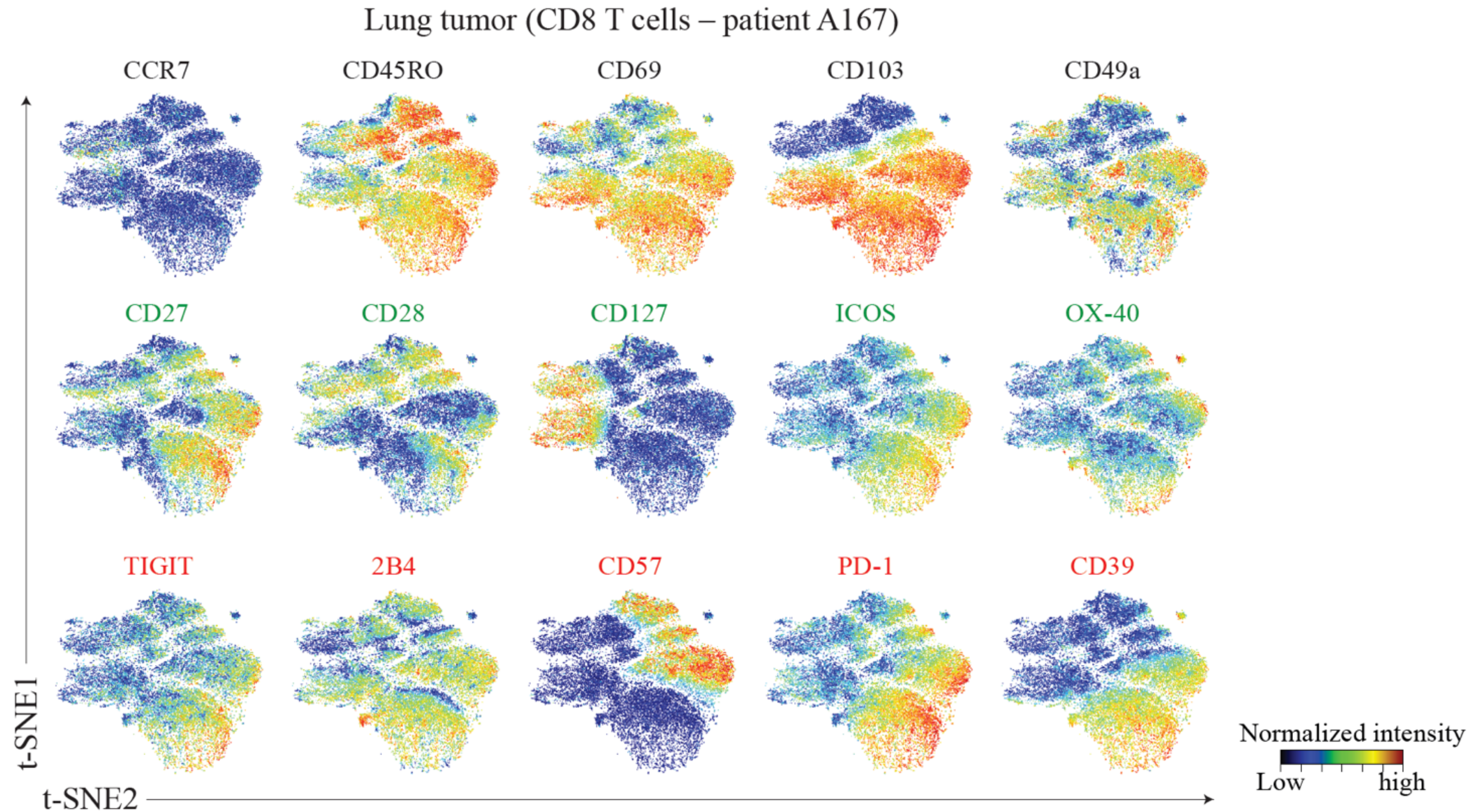


Yannick Simoni, Cheryl Loh
Daniel Tan, Eng Huat Tan et al. LCCS
Iain Tan, et al.

CyTOF analysis of CD8⁺ TILs

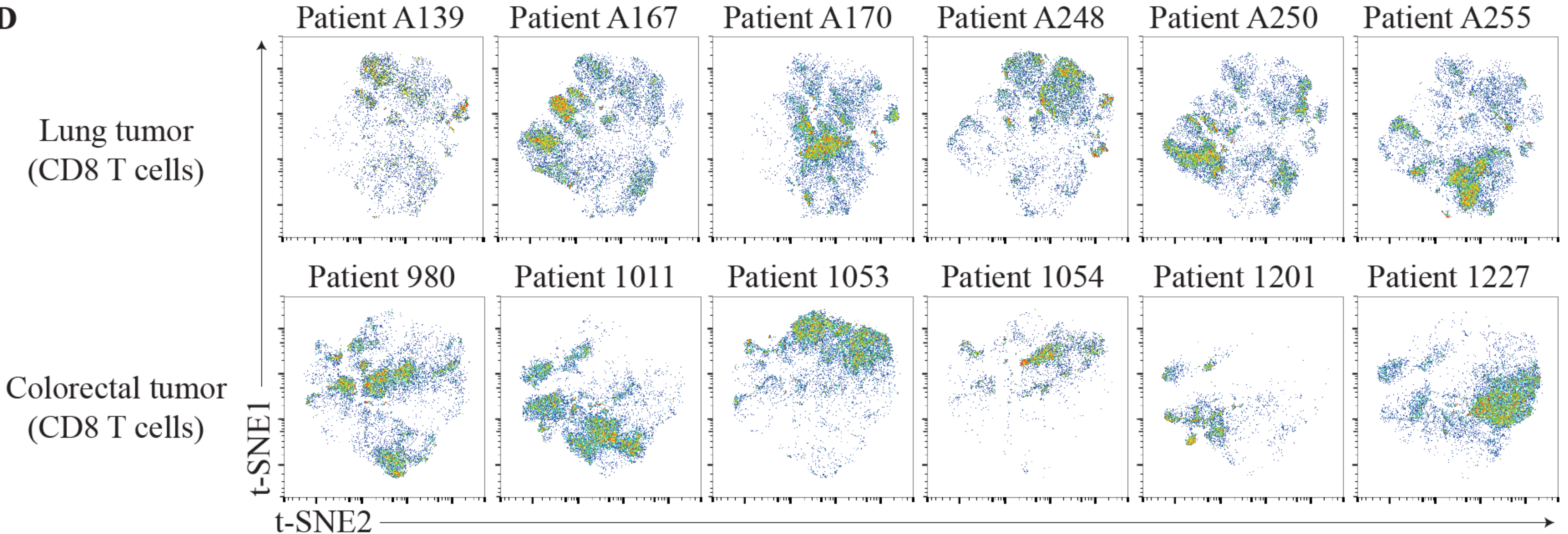


Heterogenous CD8⁺ TILs in one patient



Heterogenous CD8⁺ TILs between patients

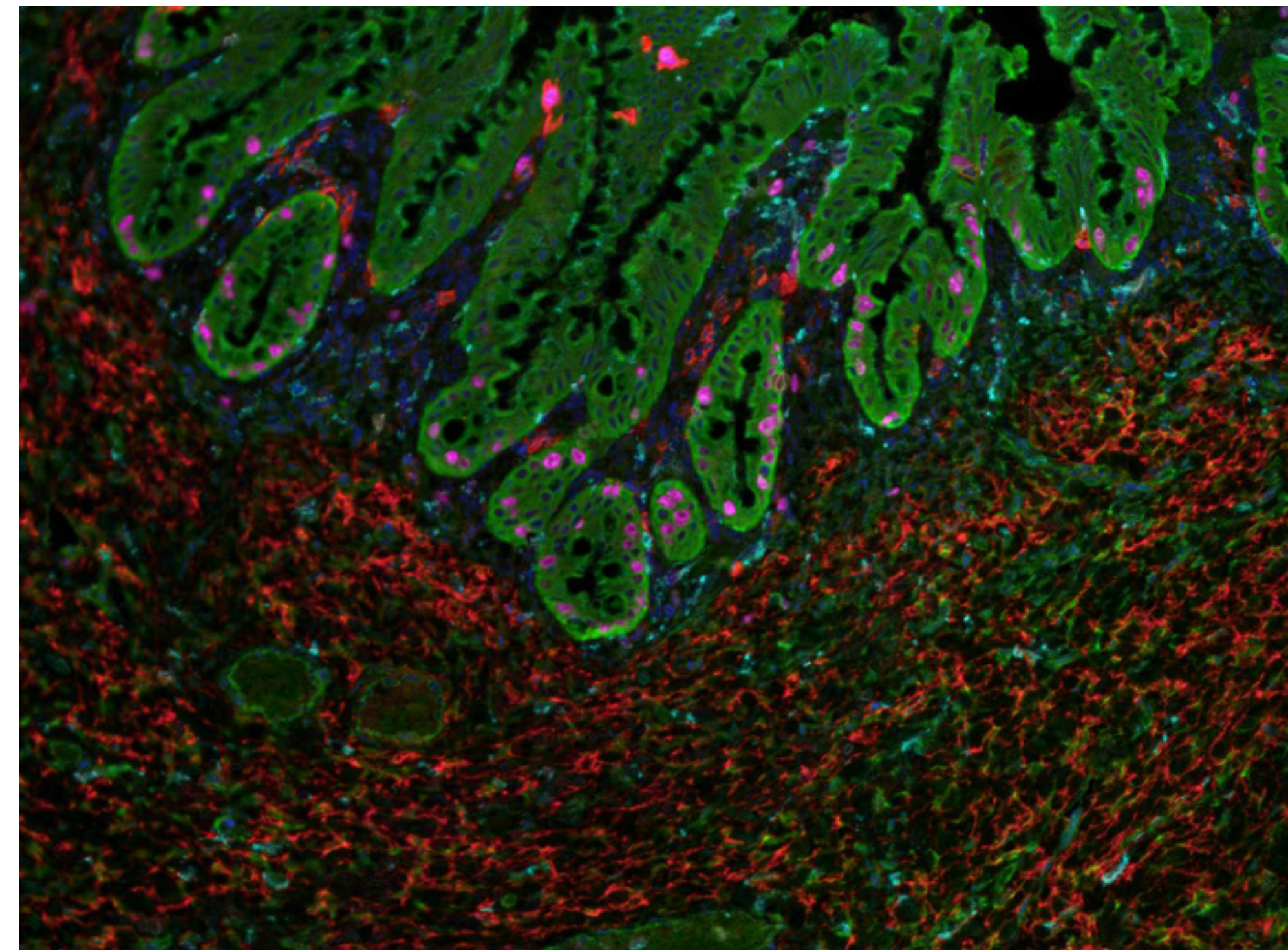
D



>140 tumors analyzed

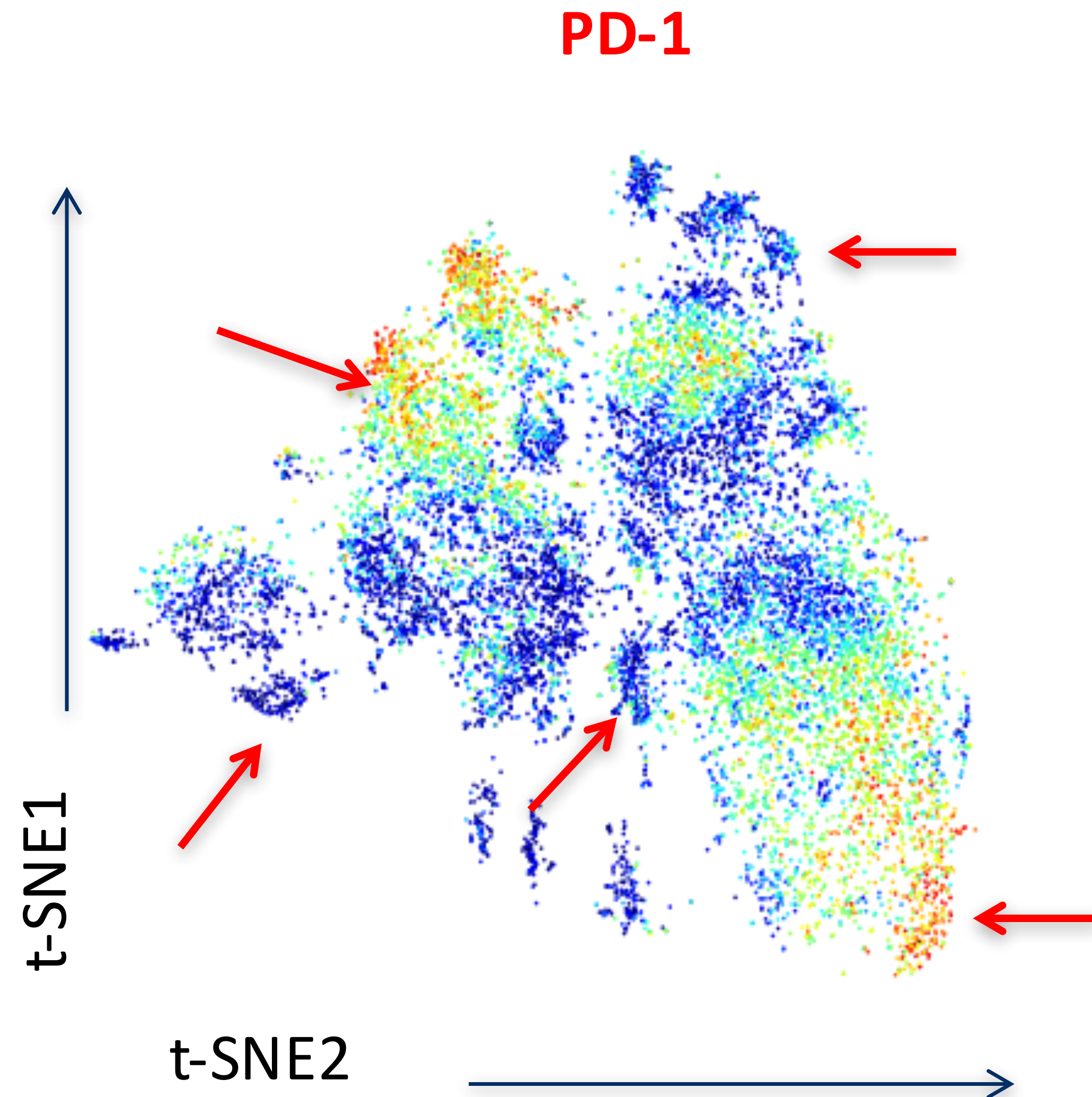
Yannick Simoni, et al. Nature, in press

- Ranges of T cell phenotypes in tumors
- Many samples analyzed/banked (CRC, NSCLC, HCC)
- Correlates
 - Patient status, treatment, outcomes
 - Clinical subtypes (MSS vs. MSI, etc.)
 - Histopathology (Vectra)
 - Molecular subtype (Transcriptomics)
 - Mutational burden (Genomics collab.)
 - Tumor evolution (Genomics collab.)
 - etc.

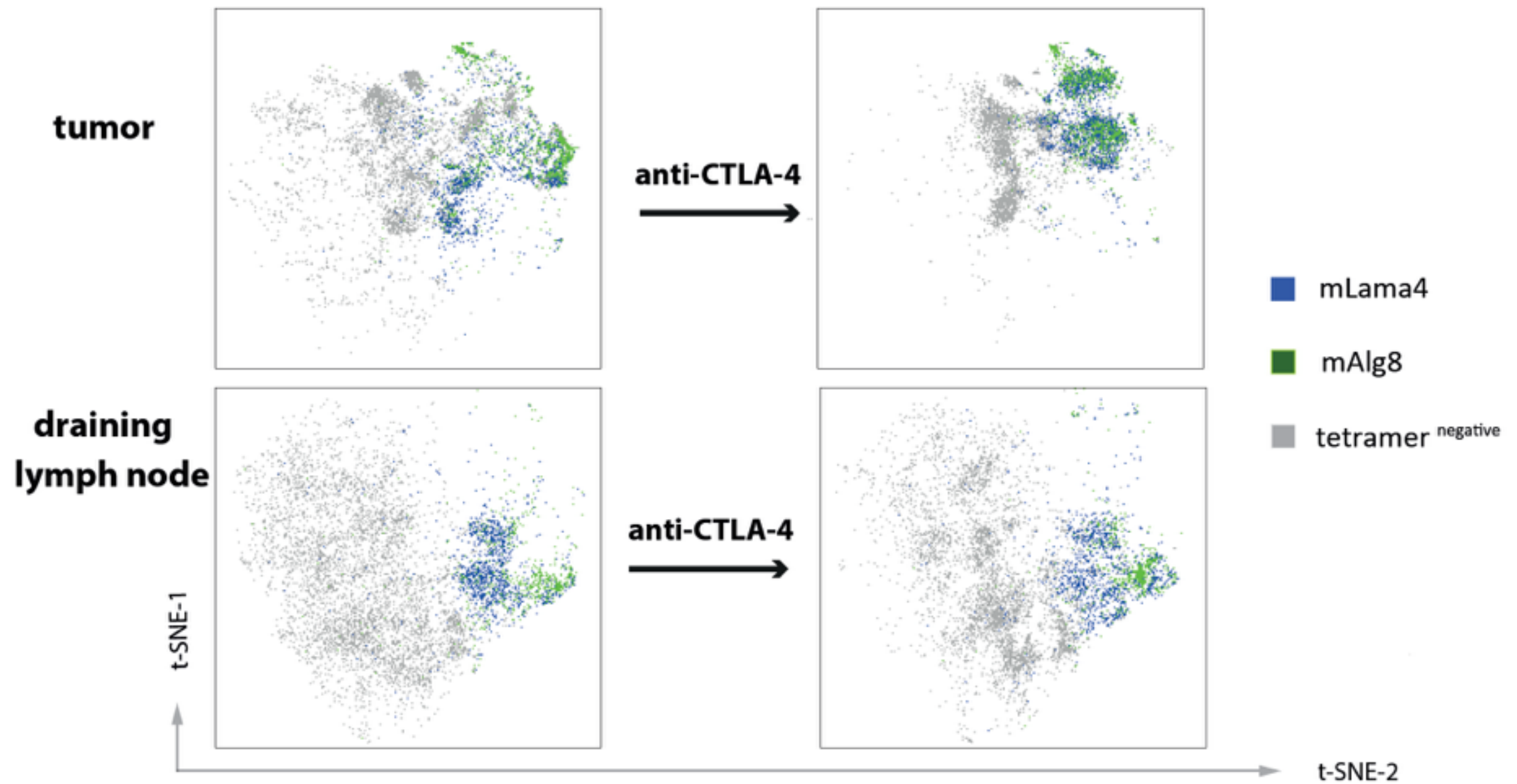
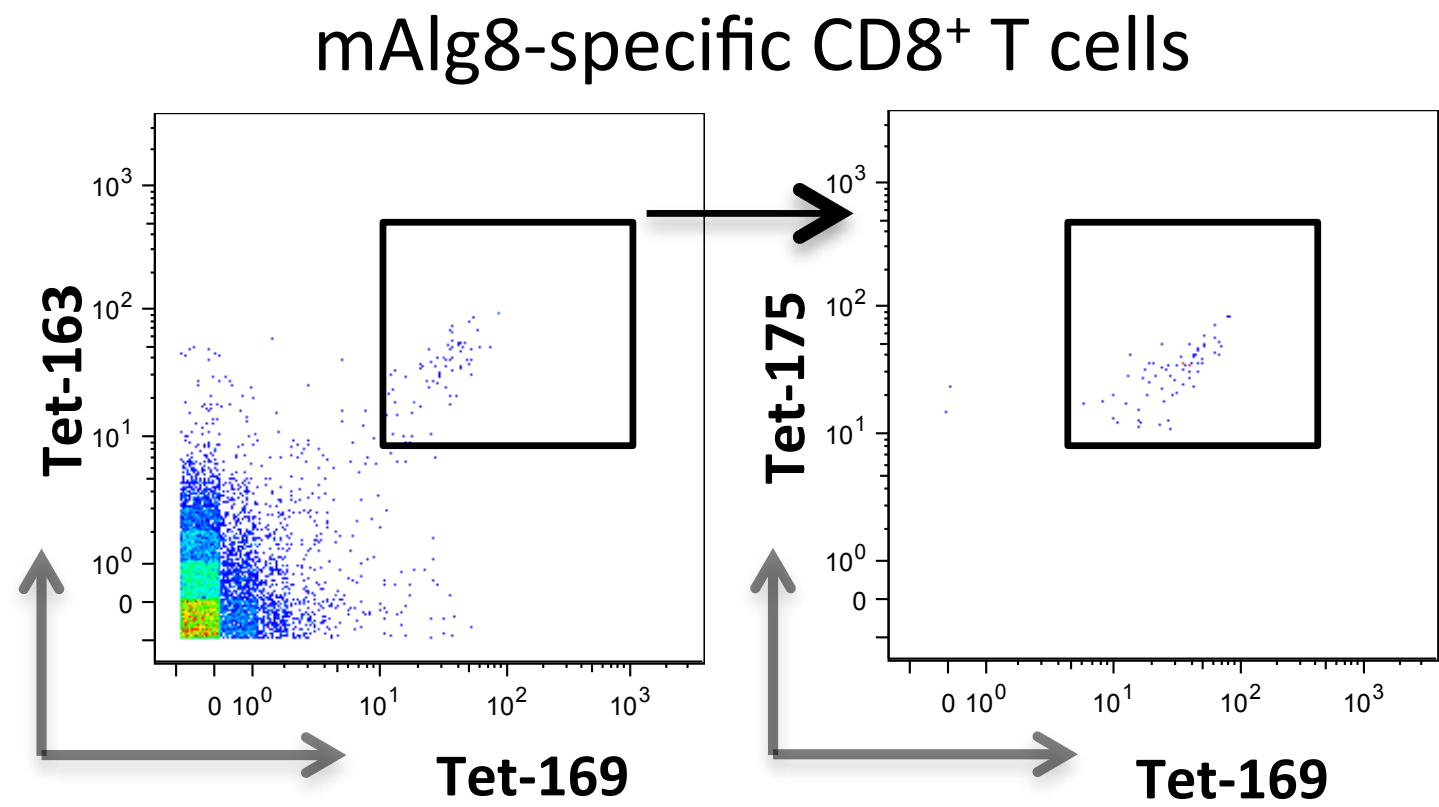
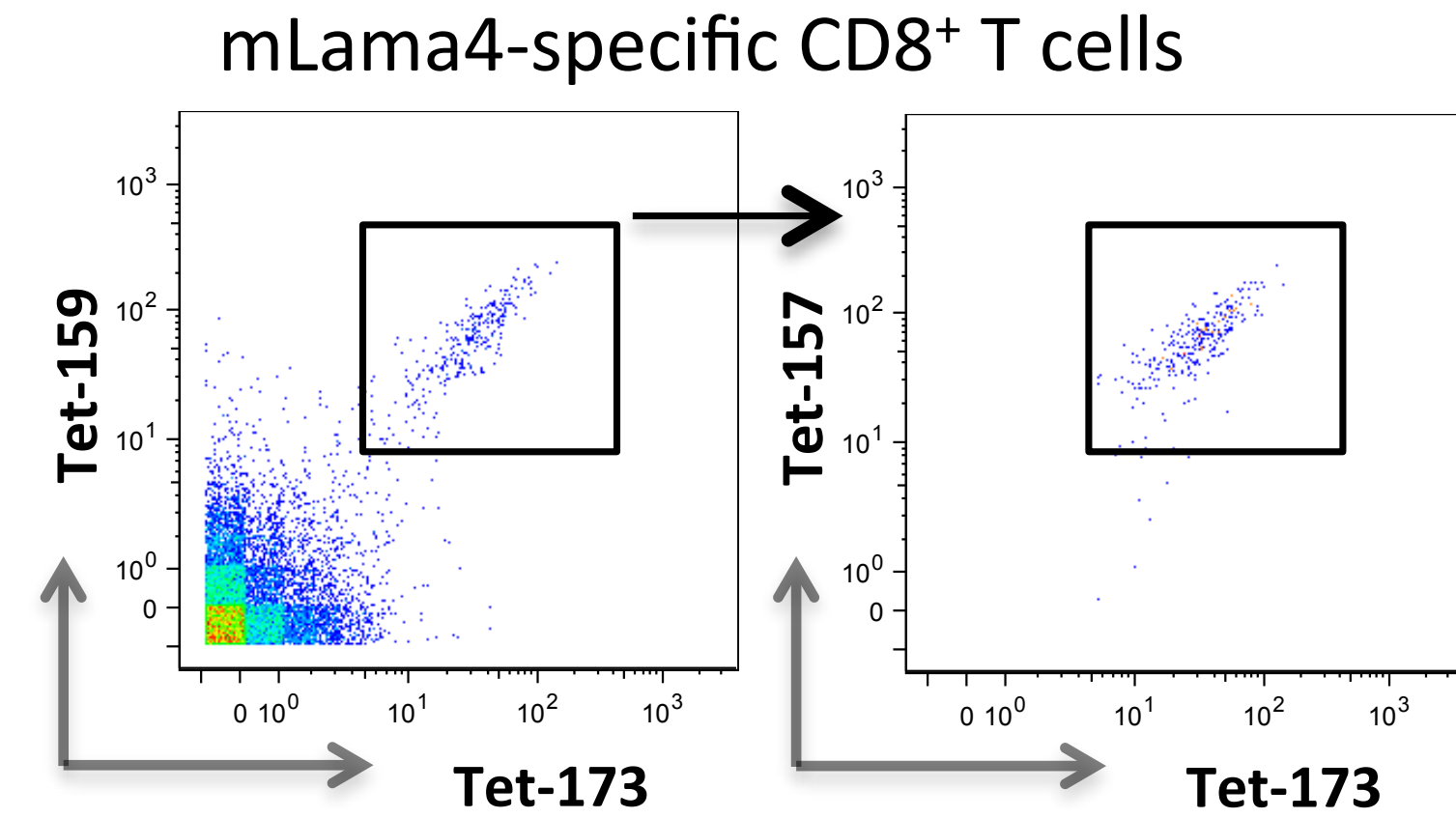


Joe Yeong, et al., unpublished

What about antigen-specificity?
Which are tumor-specific?

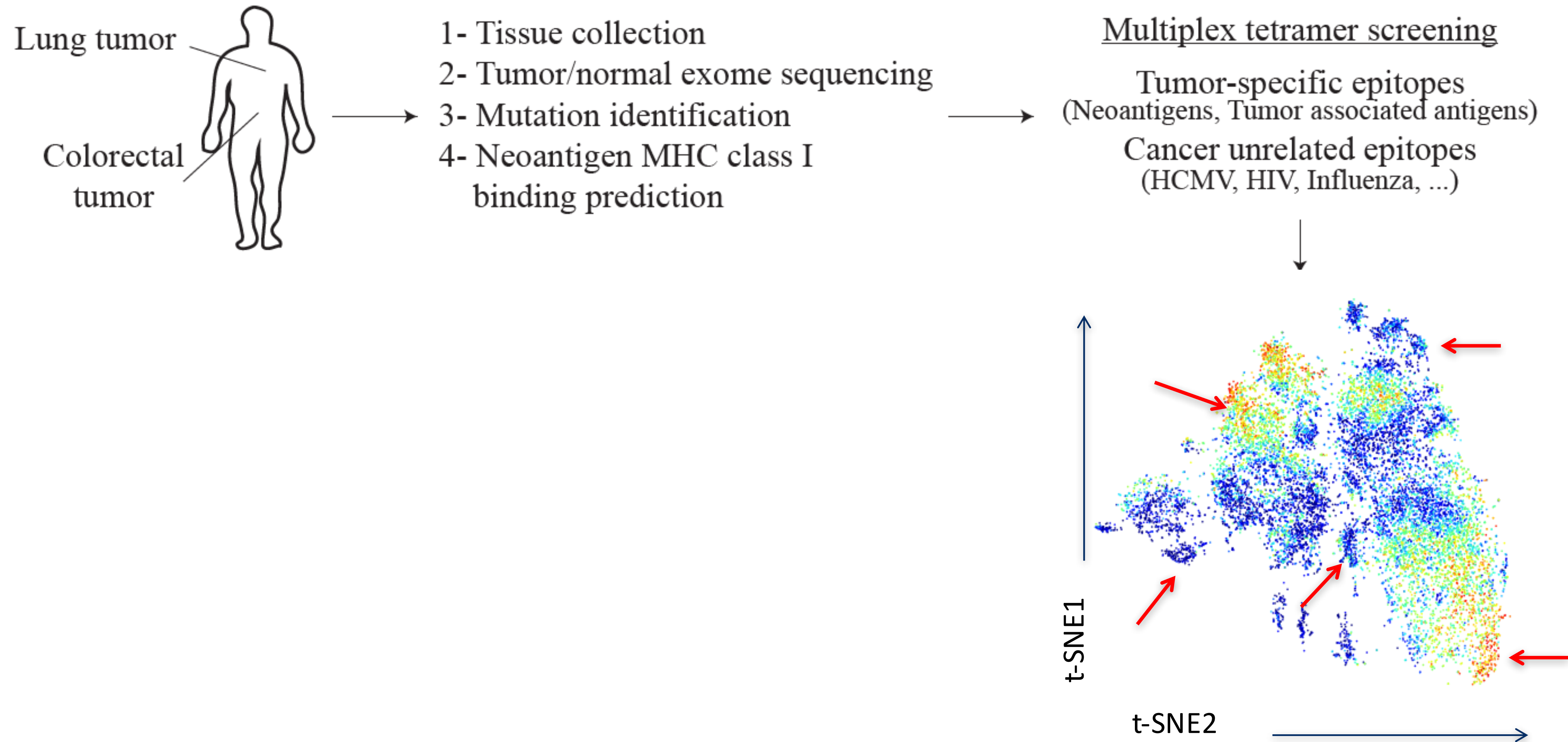


Tumor-antigen epitope screening: Mouse MCA sarcoma

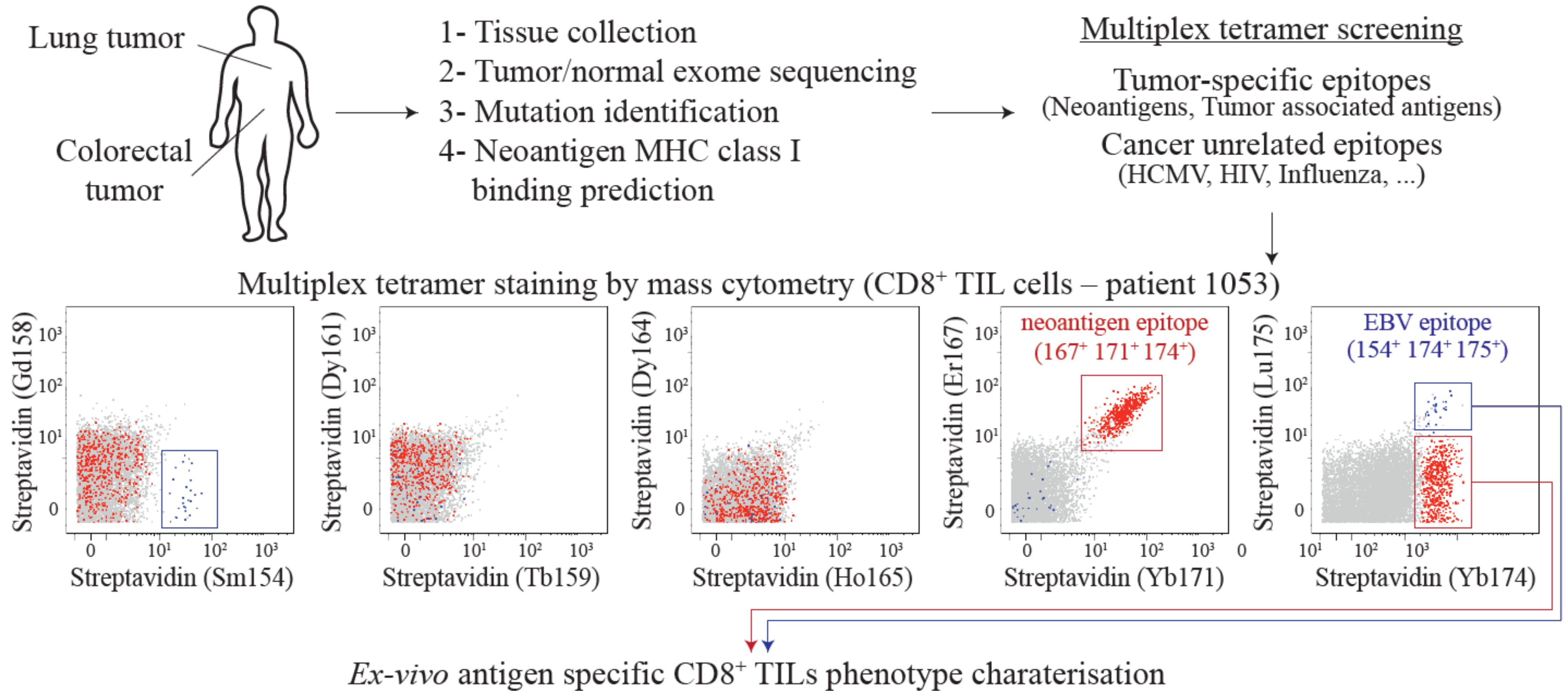


*Michael Fehlings, Schreiber et al.
Nature Communications, 2017*

Approach

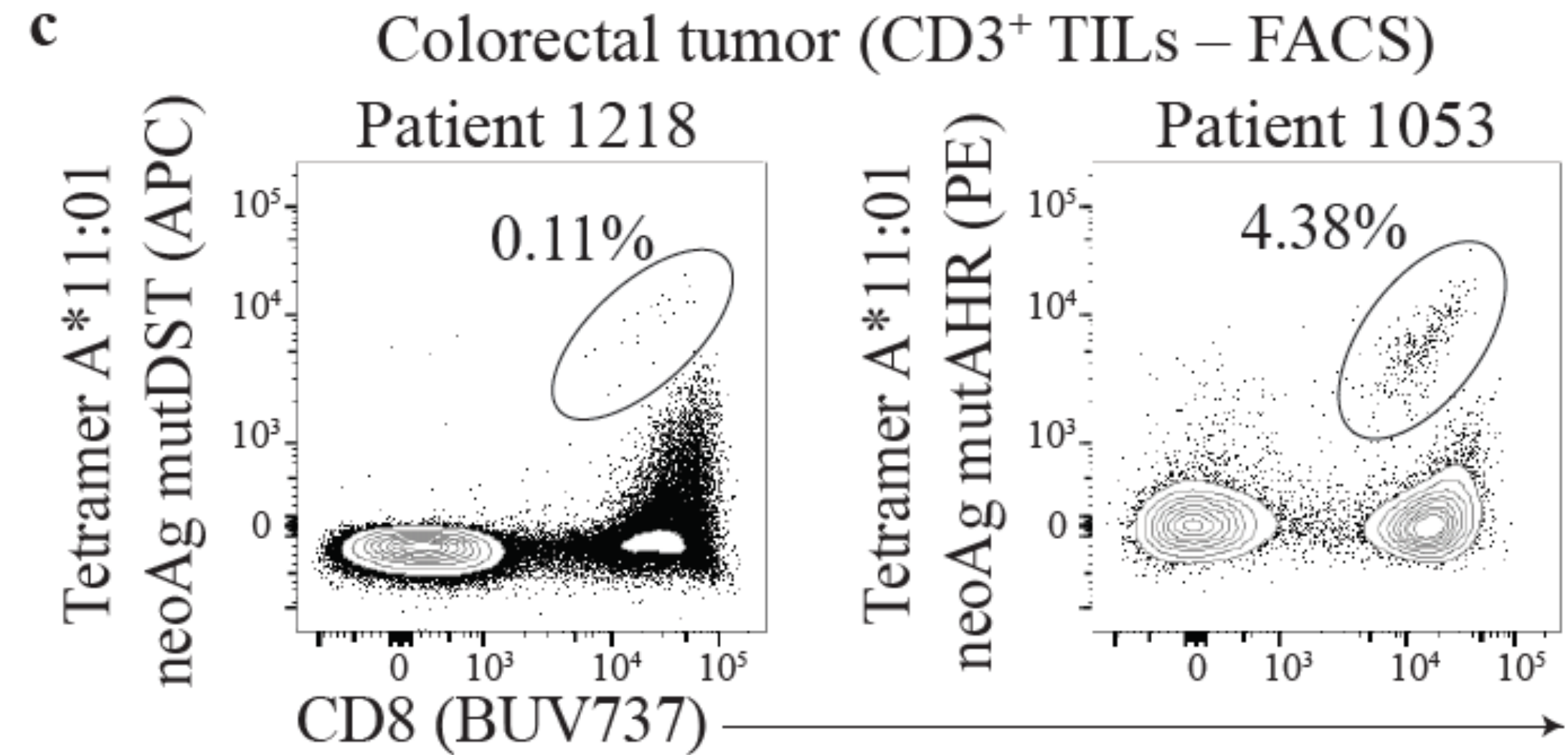
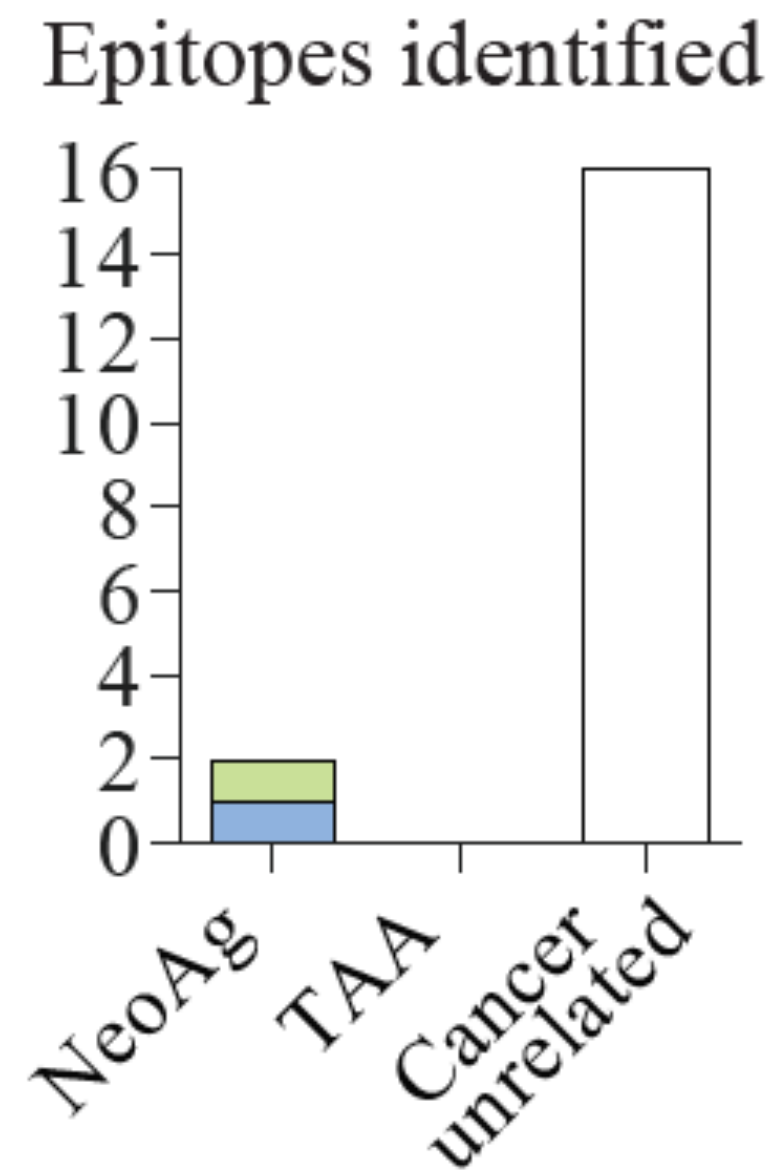
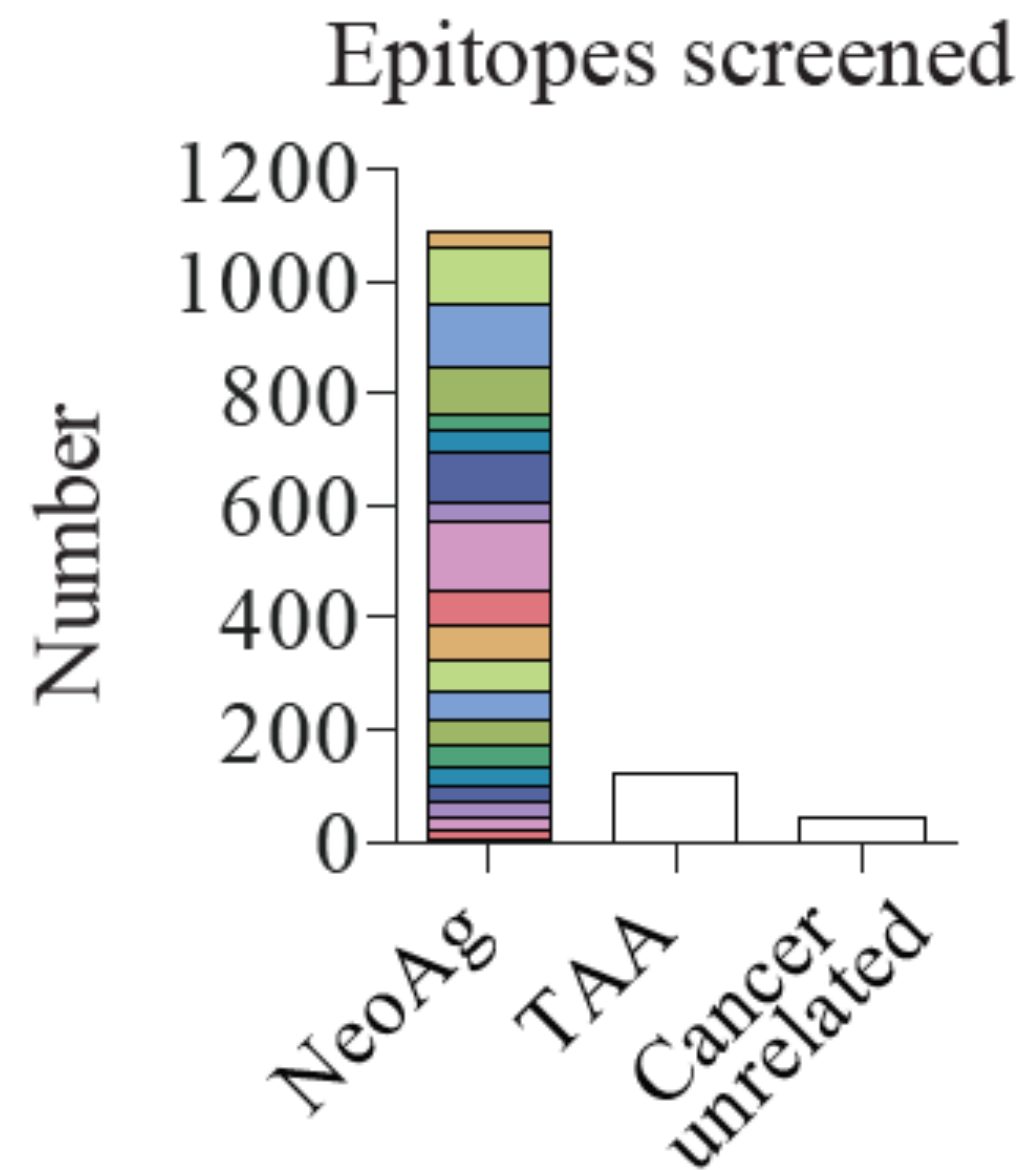


Identifying tumor-specific cells



Identifying tumor-specific T cells

NeoAg x2

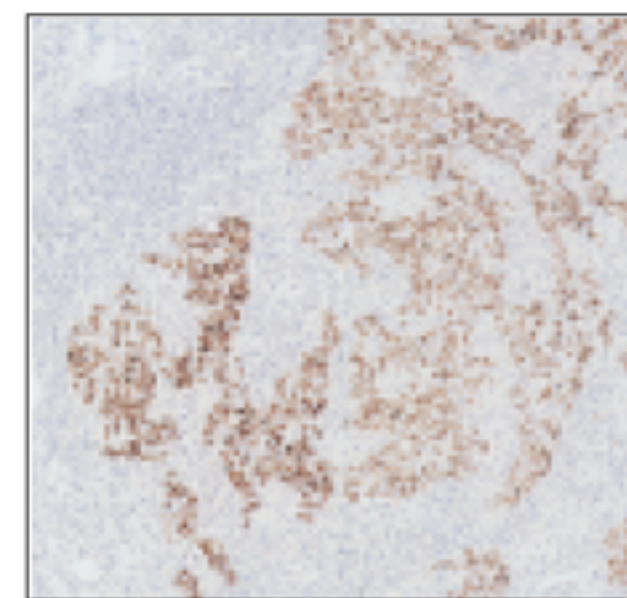


Viral tumor antigen x2

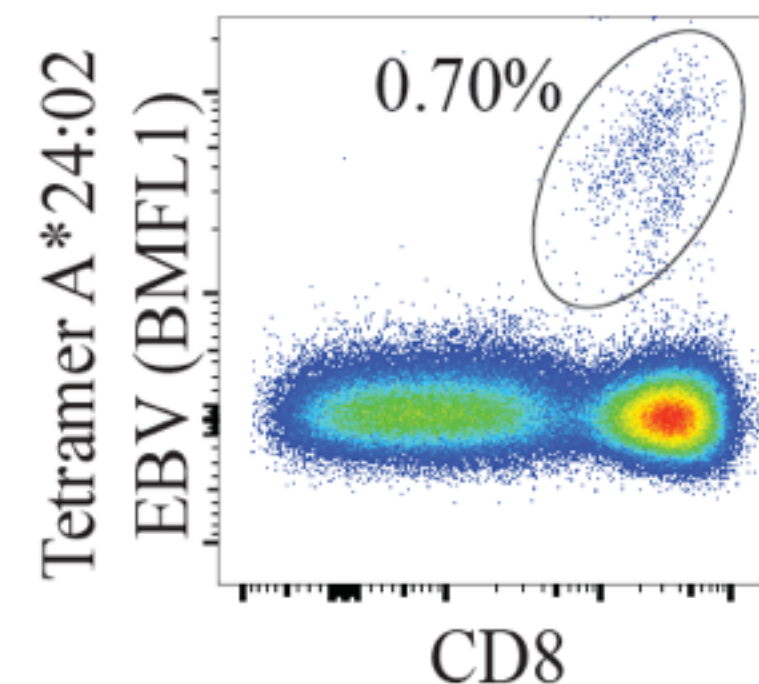
8 NSCLC (low TMB)
15 CRC (MSS)

Only 1 HLA allele
tested per patient

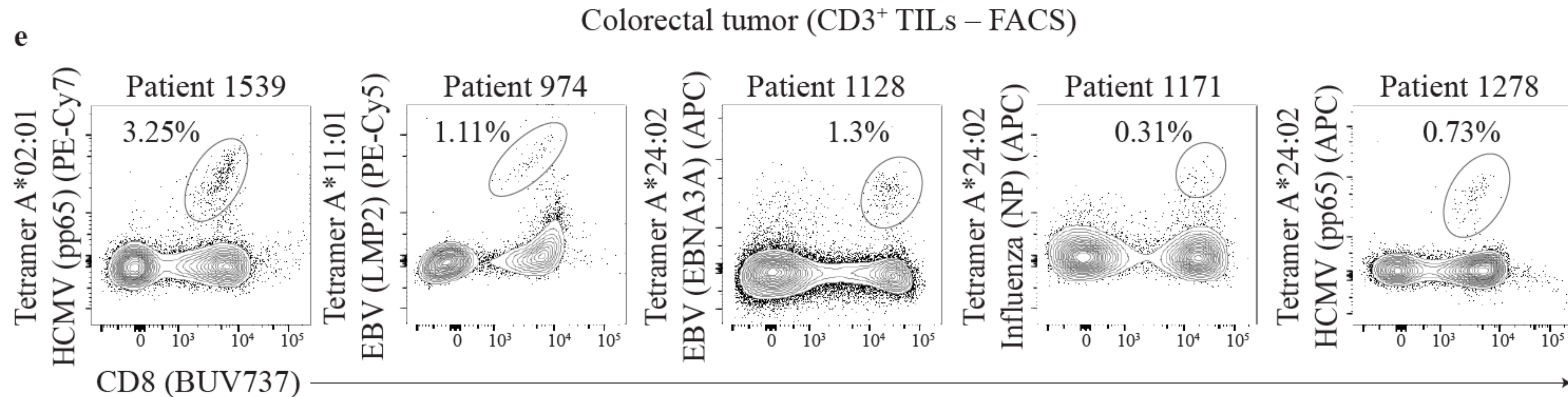
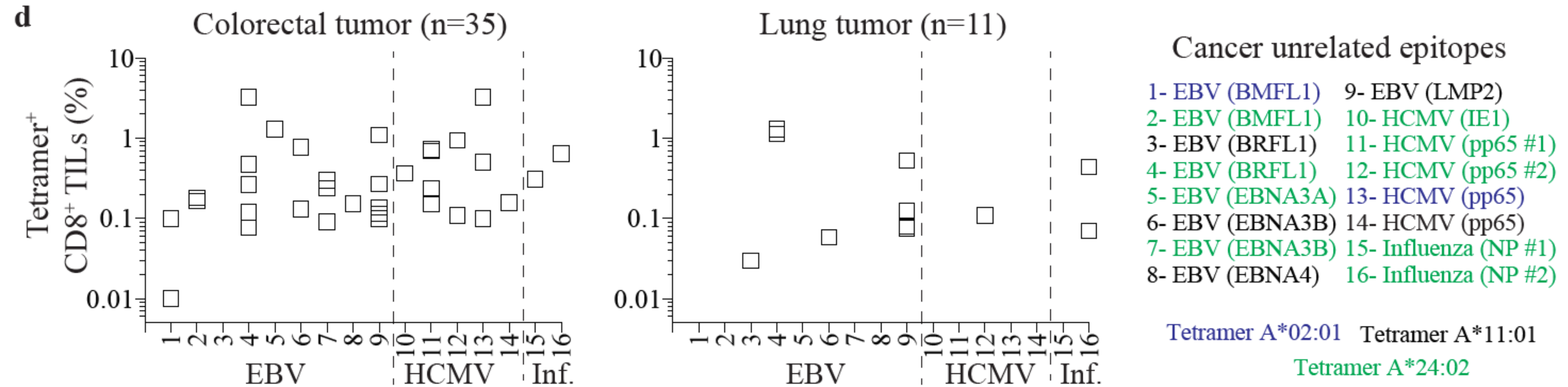
Lung LELC (patient A311)



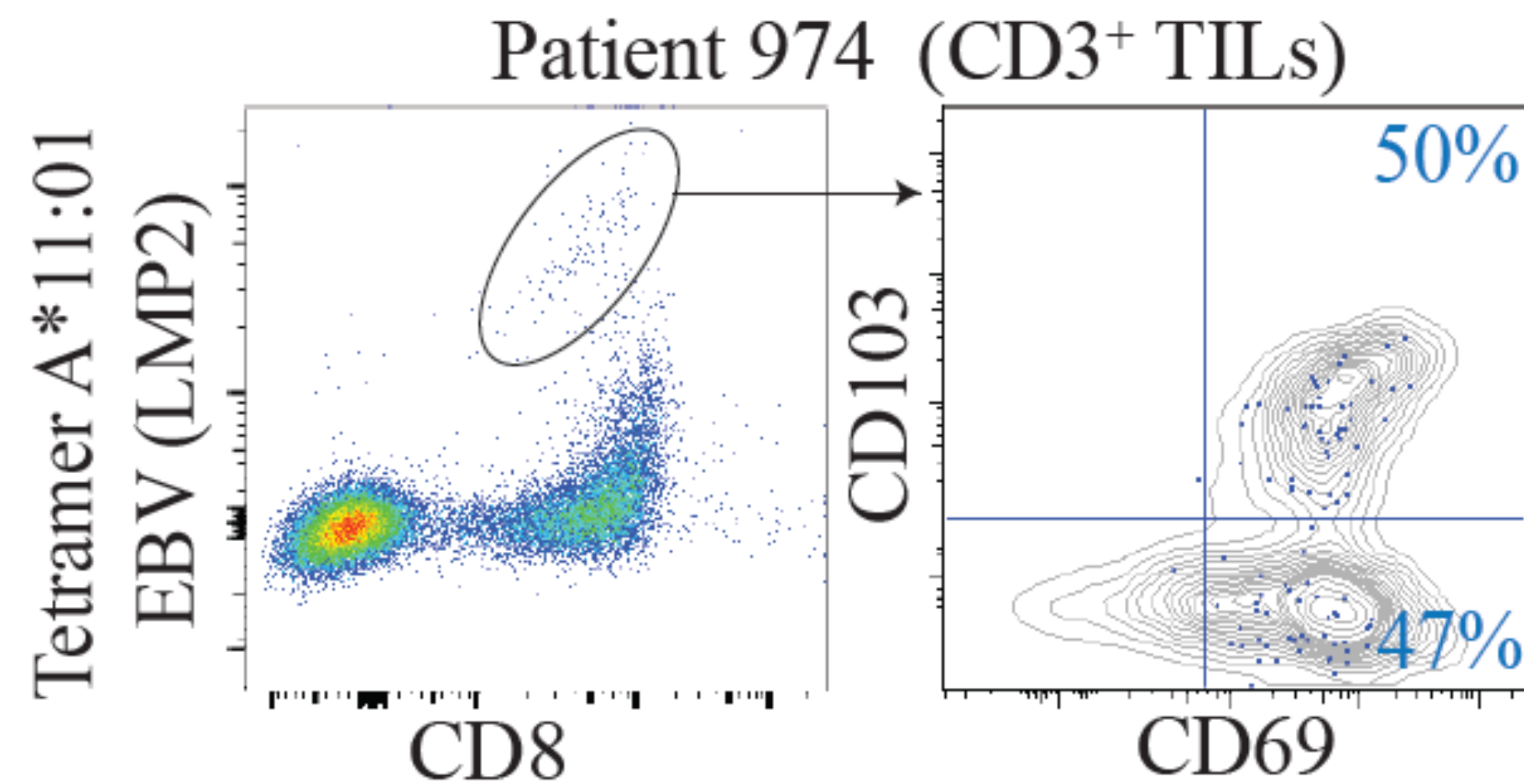
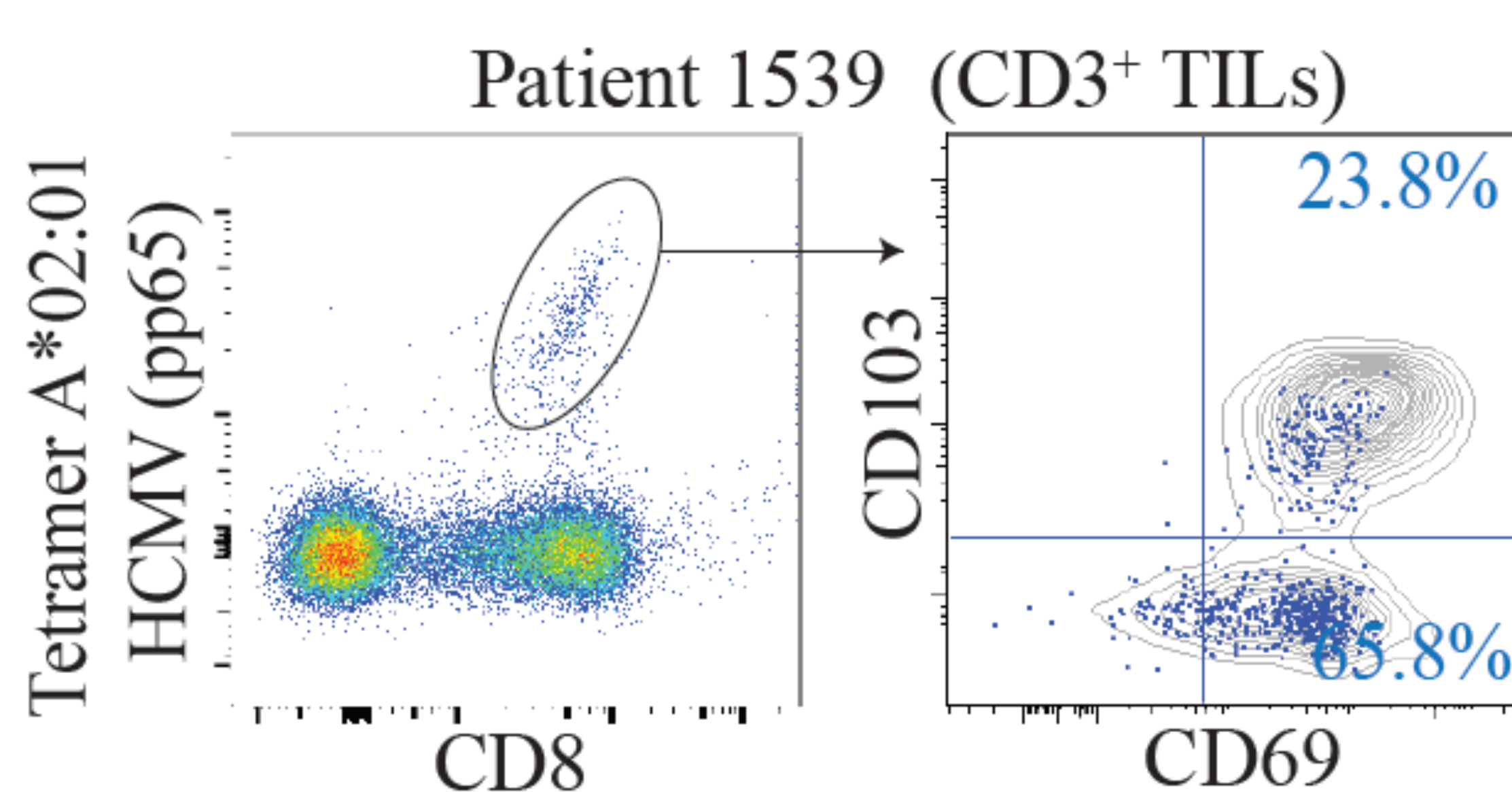
EBERish
Hematoxylin



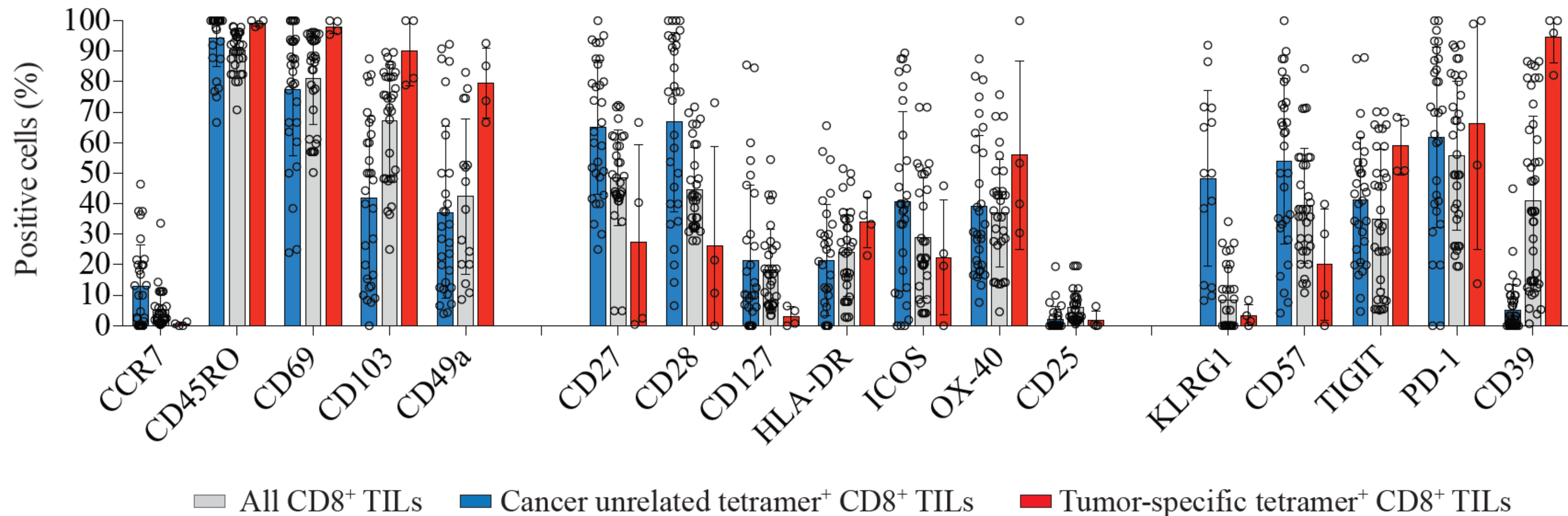
Cancer-unrelated antigen-specific Tumor infiltrating T cells - easier!



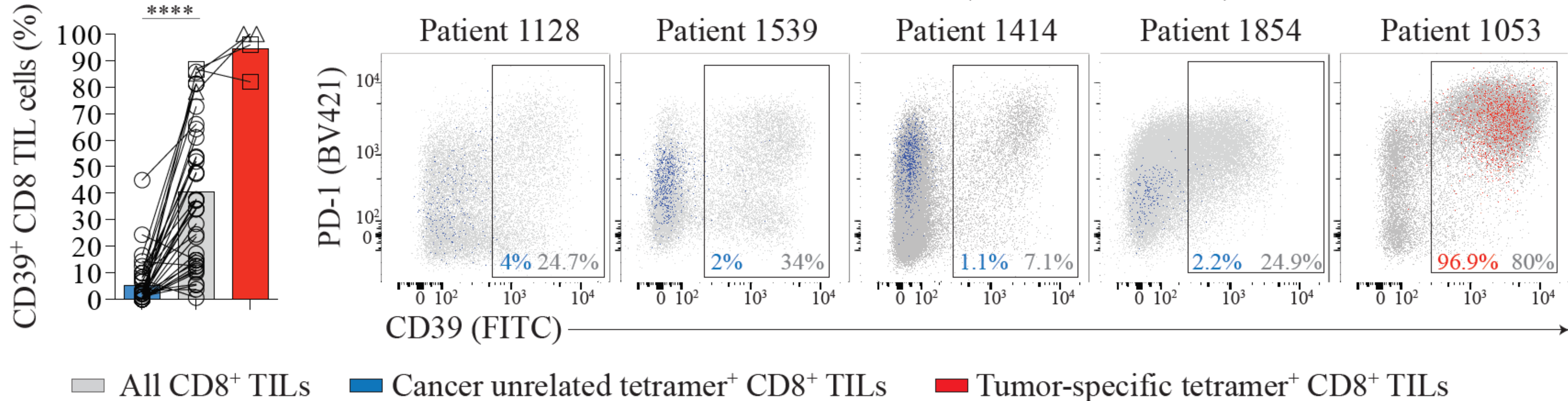
Cancer-unrelated antigen-specific Tumor infiltrating cells - Not just blood contamination



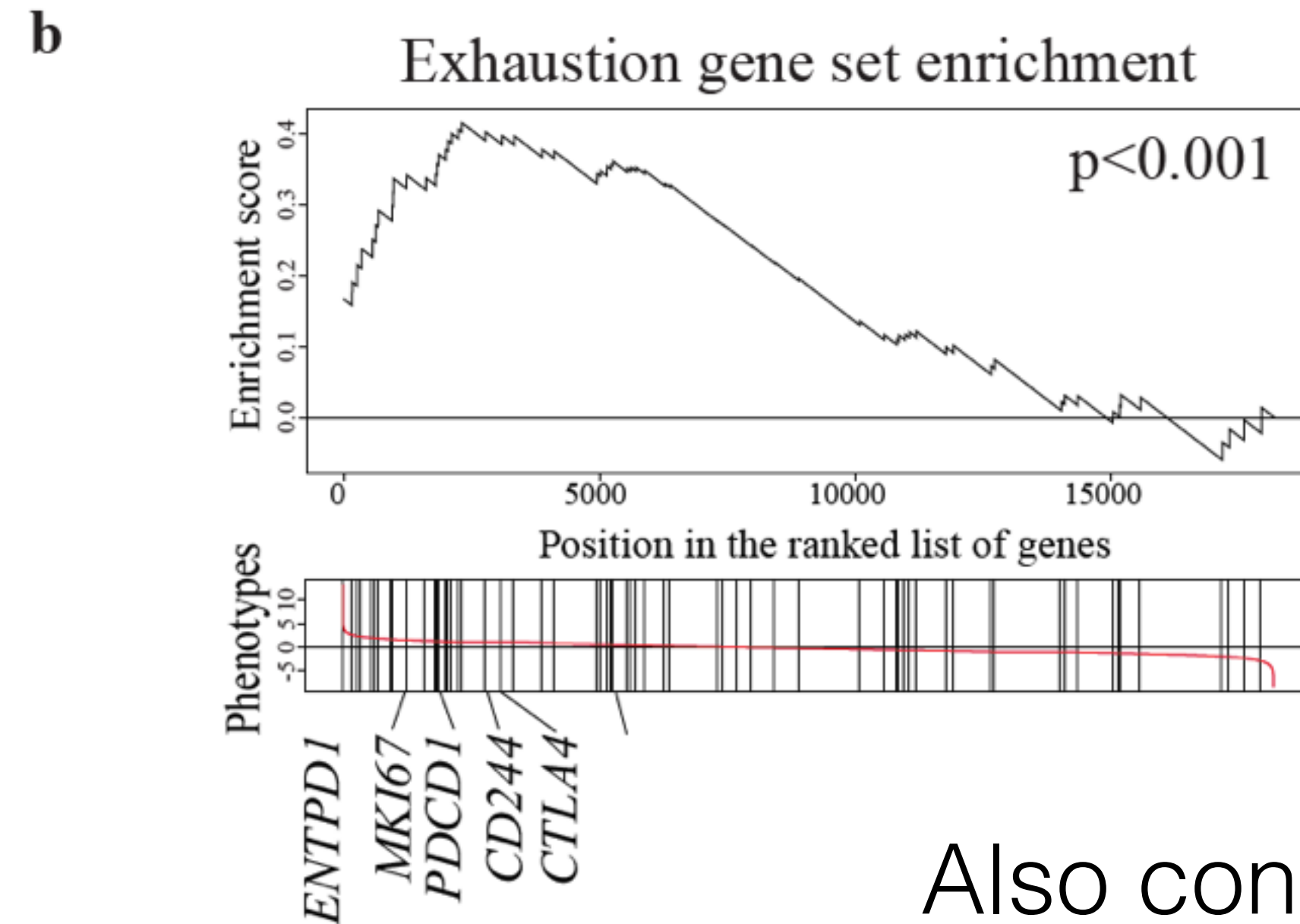
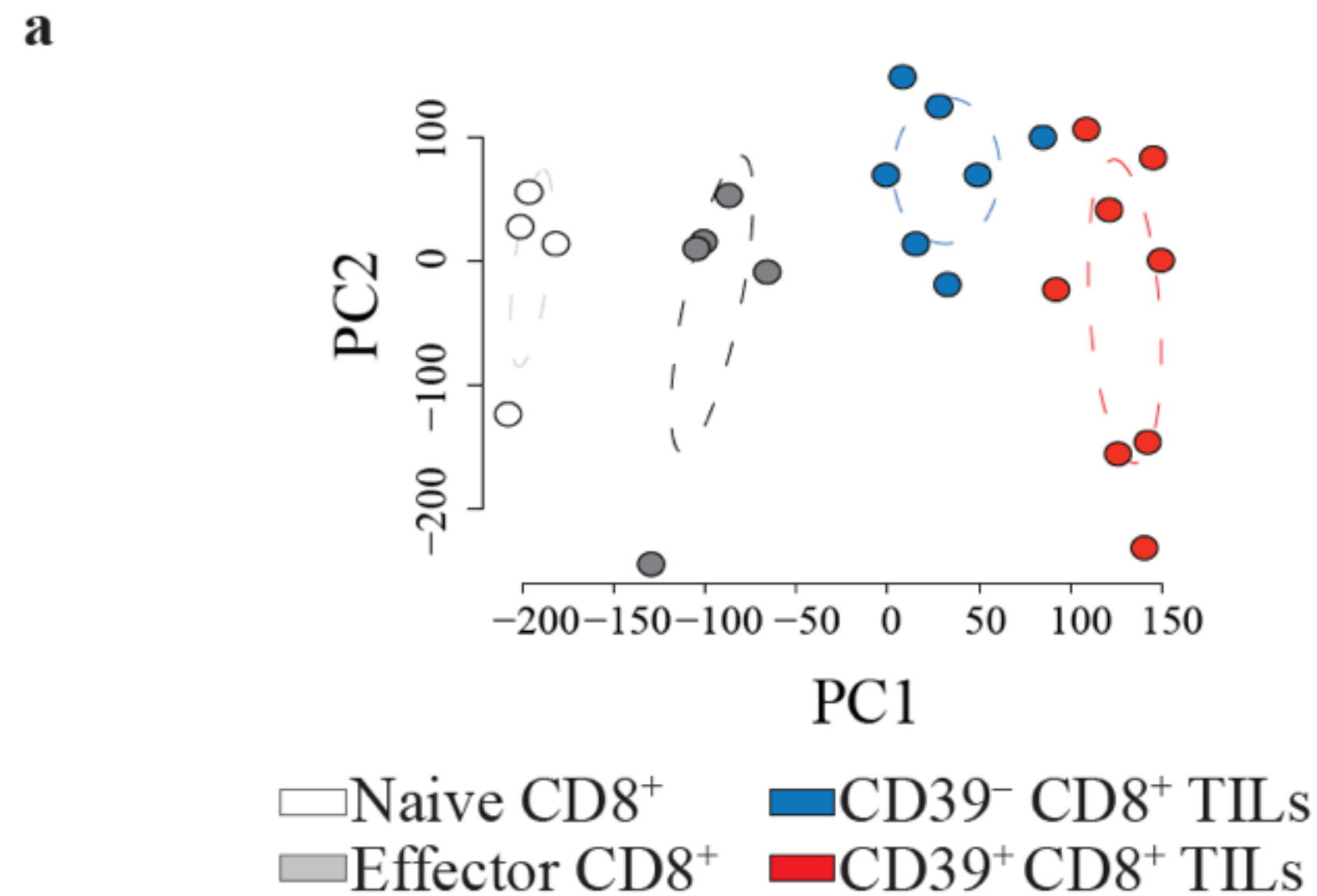
Comparing profiles of bystander vs. unspecific tumor infiltrating T cells (and vs. a few tumor-specific)



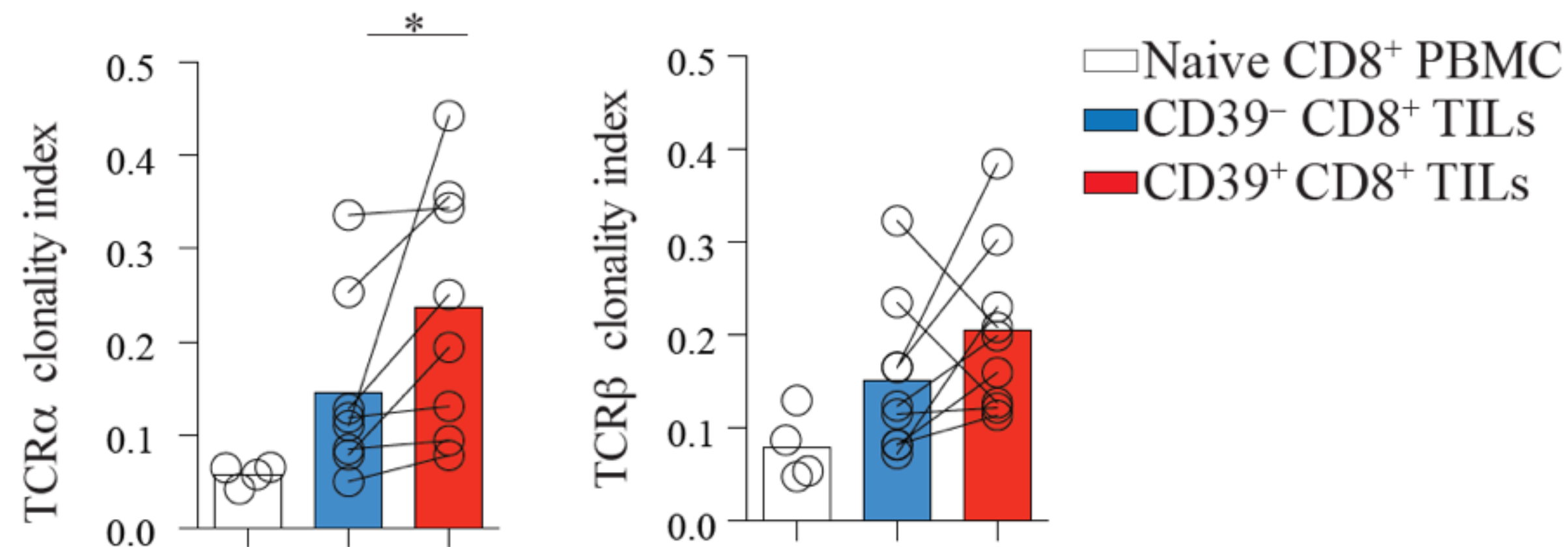
Robust lack of CD39 expression on cancer-unrelated antigen-specific tumor infiltrating cells



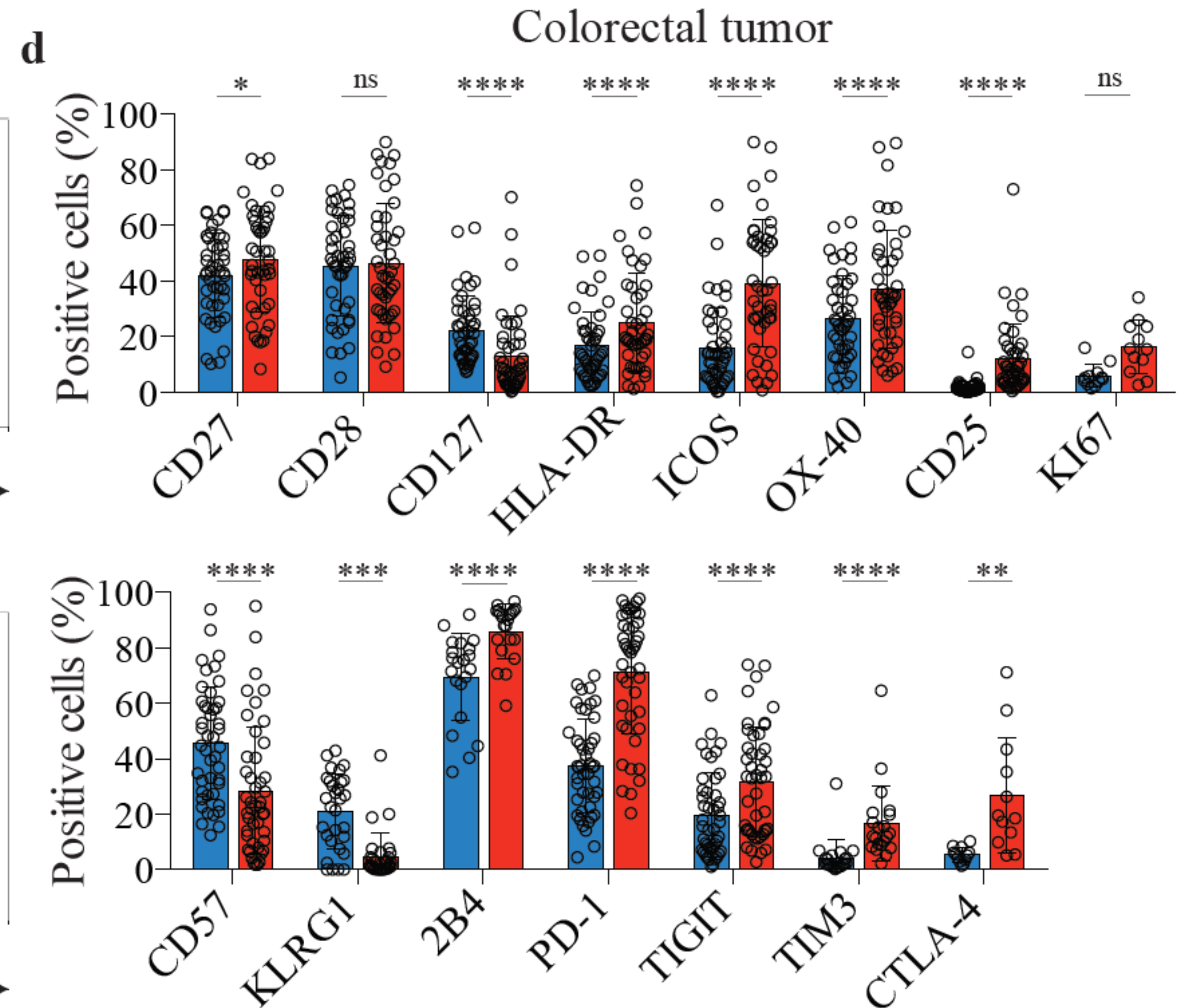
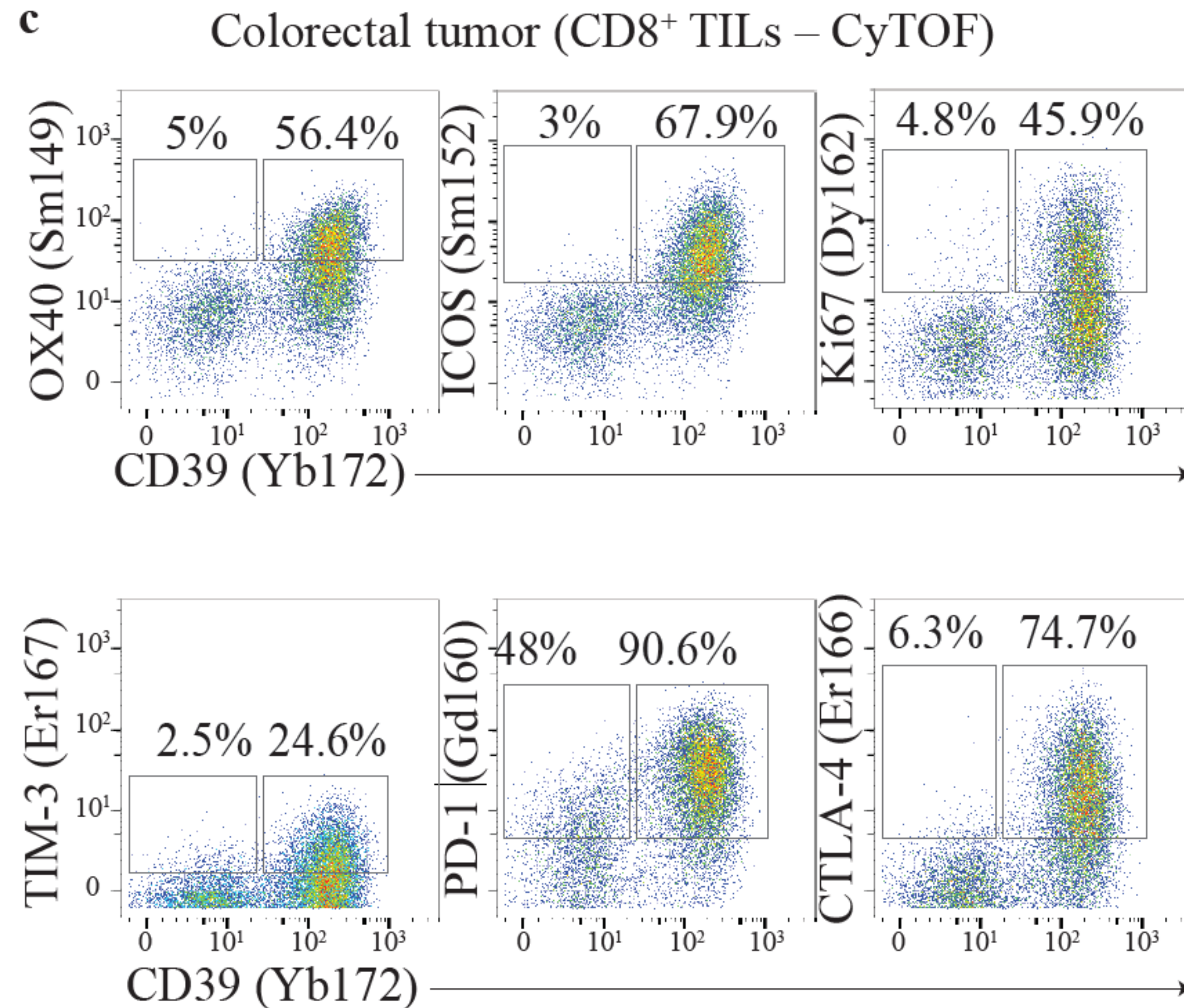
Putatively bystander vs. tumor specific (by CD39) T cell transcriptional and TCR sequence profiles



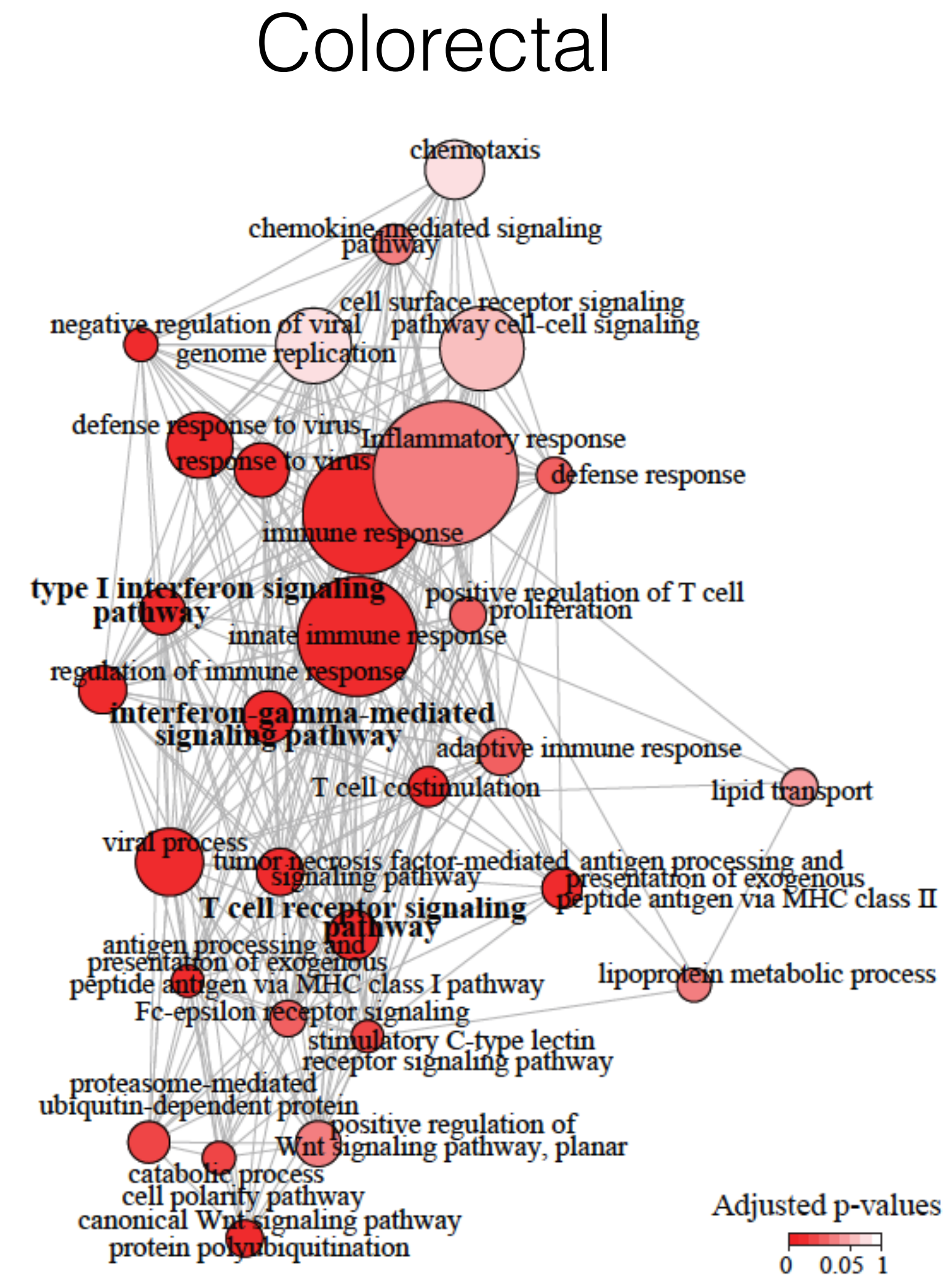
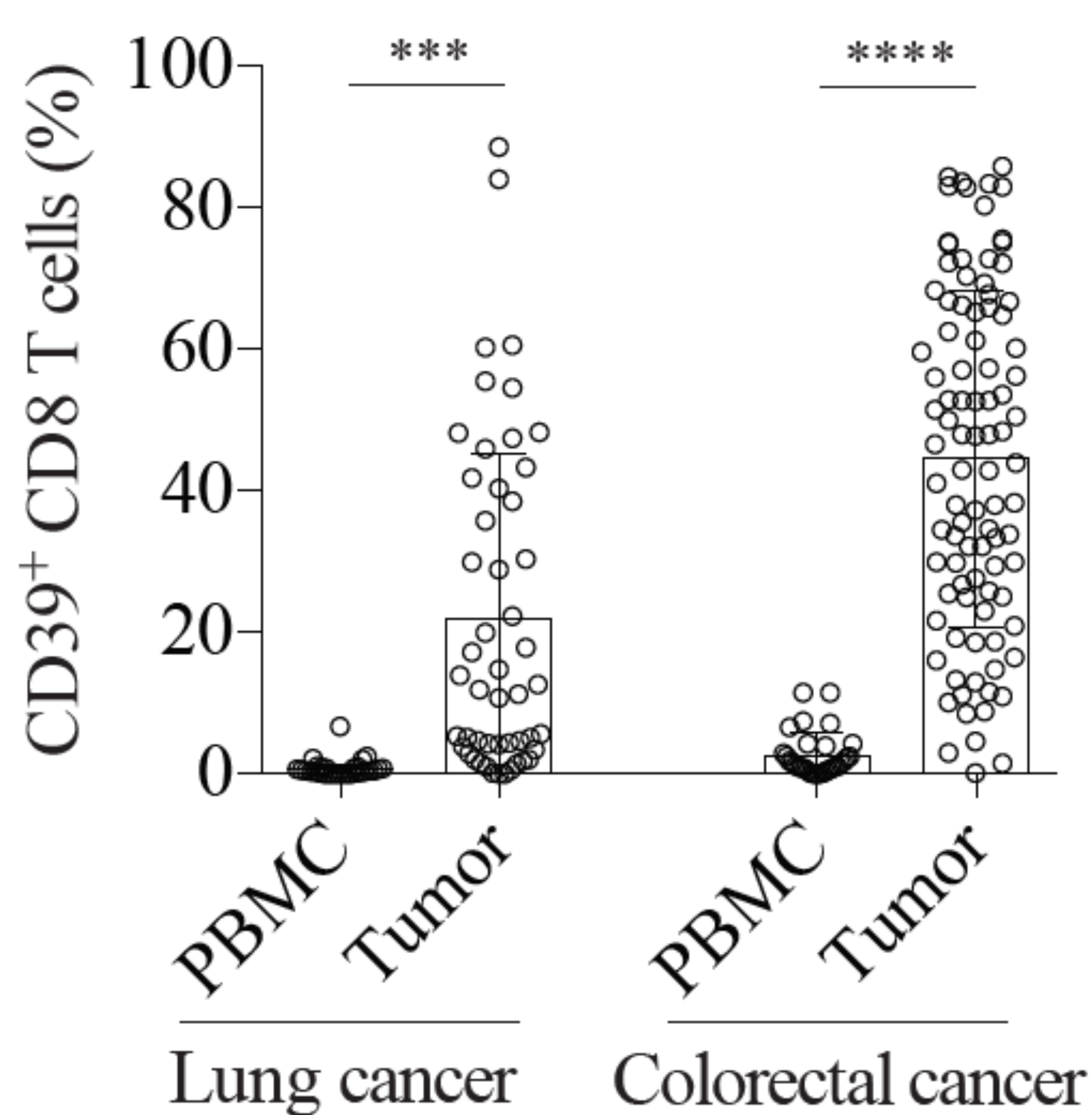
Also confirmed at protein level by CyTOF and functional assays



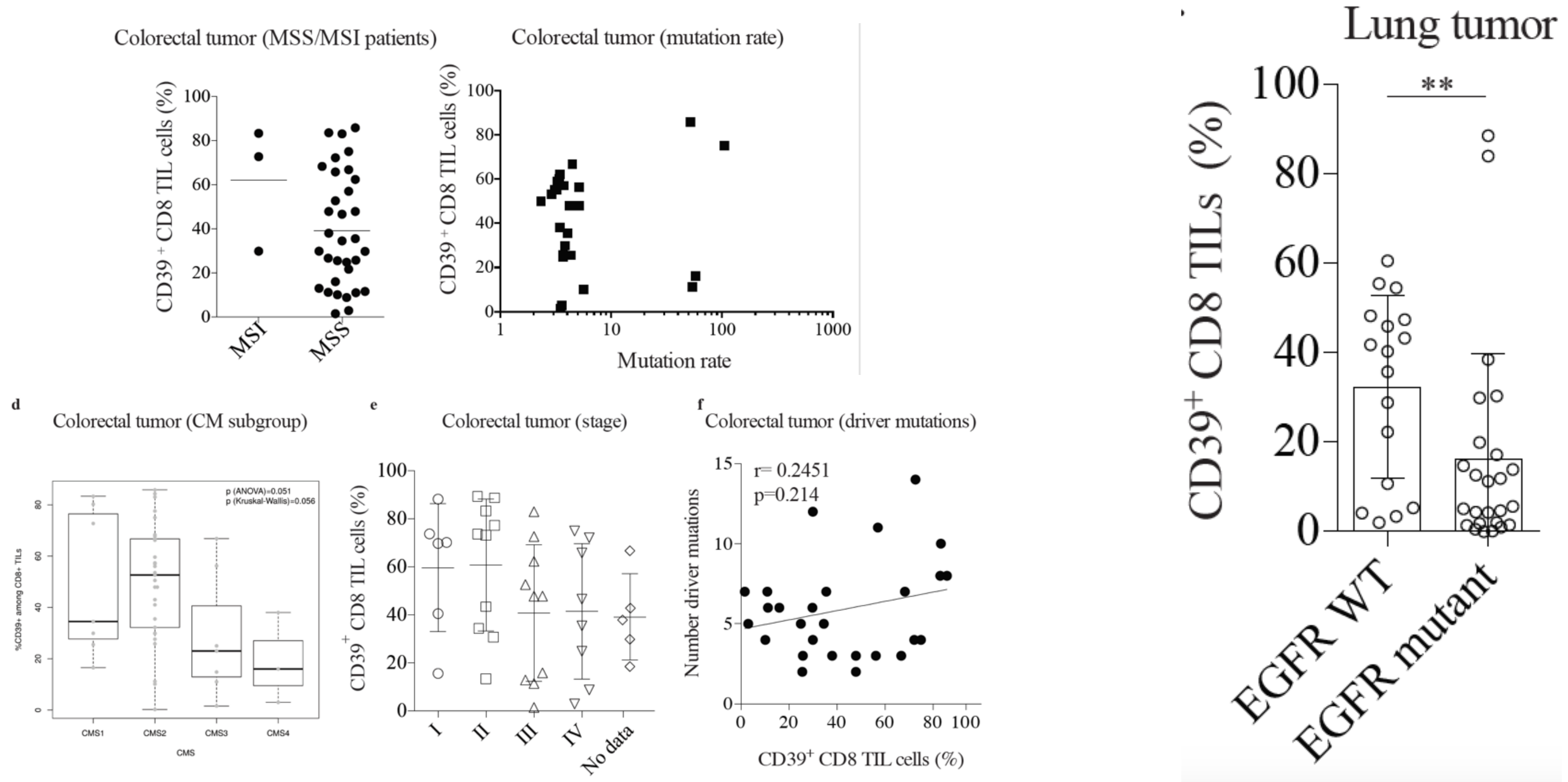
Putatively bystander vs. tumor specific T cell protein expression profiles



High diversity of CD39 frequencies in CD8⁺ TILs and correlation with bulk tumor gene expression



Correlations with clinical parameters

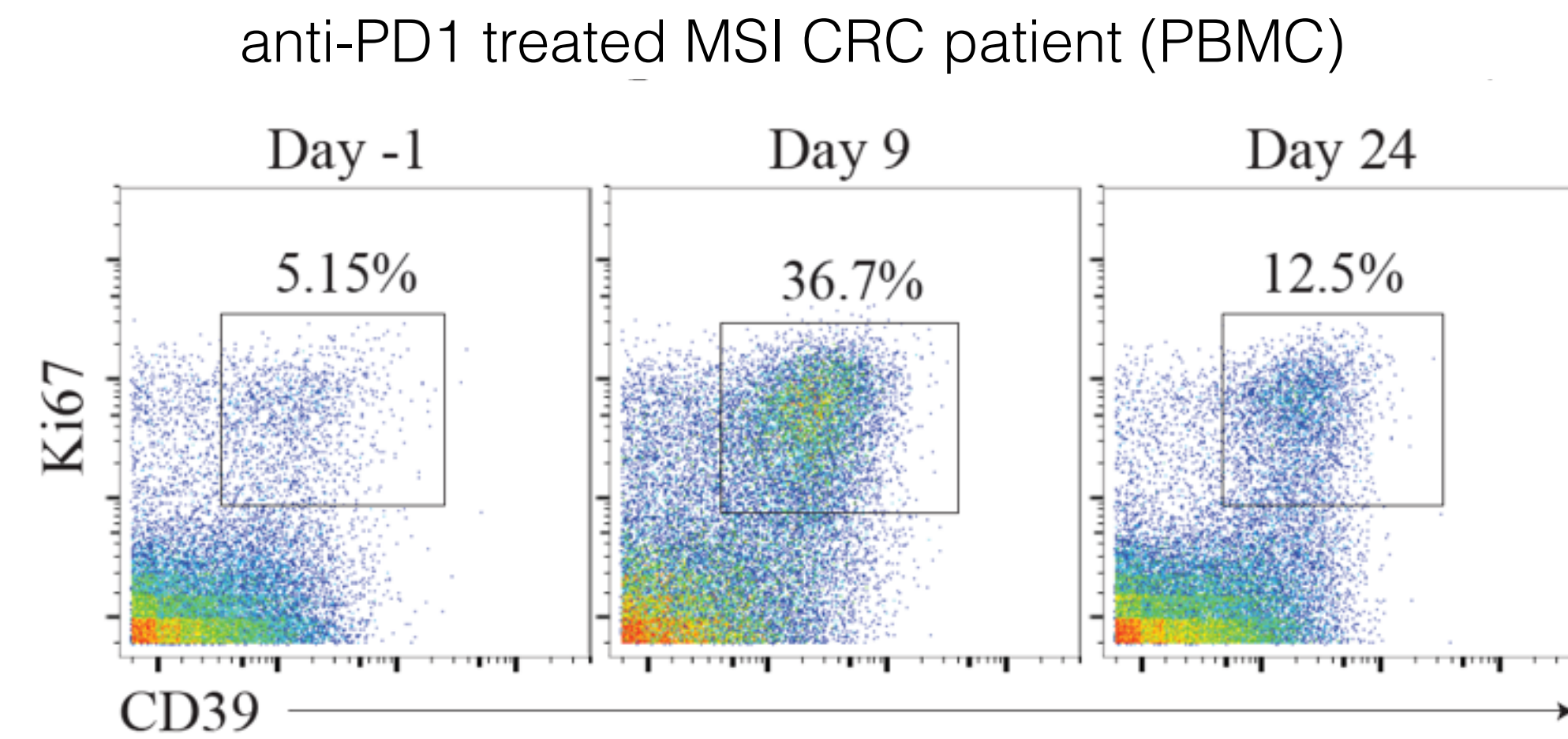


Summary and implications

- Benefits of simultaneous profiling of T cell phenotype and antigen-specificity
- Bystander T cell prevalent: implies that some patient tumors may have very few infiltrating tumor-specific T cells. (e.g., EGFR-mut NSCLC)
- Diverse profiles of bystander T cells: often Trm-like, lack CD39
- CD8⁺CD39⁺ T cell frequencies as biomarker for checkpoint blockade immunotherapy? (also in blood?)
- Selection of CD39⁺ T cells for adoptive therapy approaches?
- Non-responsiveness of MSS CRC not entirely due to lack of tumor-specific cells.
(other types of antigen involved?)

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Thanks!



- **Singapore Immunology Network**
 - EN lab
 - **Yannick Simoni**
 - **Cheryl Loh**
 - **Etienne Becht**
 - **Joe Yeong** (also SGH pathology)
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 - Yang Cheng
 - Melissa Ch'ng
 - Faris Kairi
 - Bavani Gunasegaran
 - **Nicholas Ang**
 - Jackwee Lim
 - **Florent Ginhoux**
 - **Charles Duterte**
 - Immunogenomics platform
 - Brian Abel

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 - **Daniel Tan, Eng Huat Tan (NSCLC)**
 - **Iain Tan, Si Lin Koo (CRC)**
 - Su Pin Choo (HCC)
 - **Tony Lim, Angela Takano** (Pathology)
 - **Joe Yeong**
 - et al.
- **Genome Institute of Singapore (GIS)**
 - **Weiwei Zhai**
 - et al.
- **immunoSCAPE**
 - **Michael Fehlings**
 - Hermi Sumatoh
- **Stanford**
 - Mark Davis
- **Many others with ongoing projects not discussed**





Moving to Seattle...

And thanks to SIgN support
we are still going strong in Singapore:



After August:

