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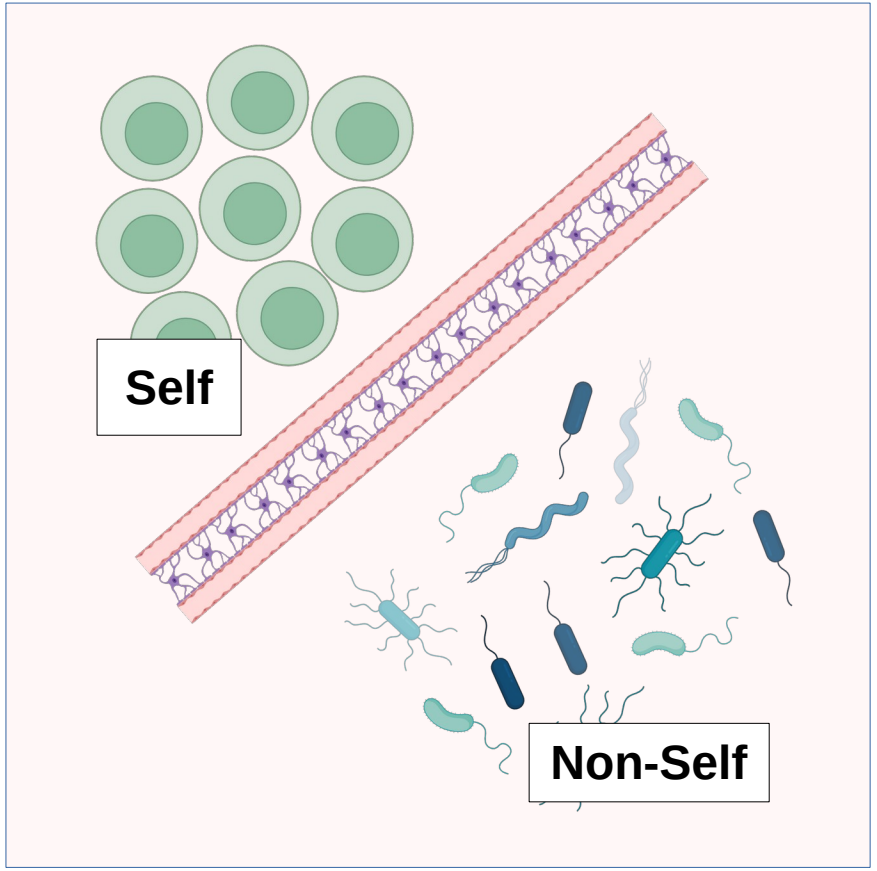


Characterization of Cord Blood Innate-Like T cell responses in neonates born to healthy women and women living with HIV

David Rach
PhD Candidate - Molecular Microbiology & Immunology
Chairs: Cristiana Cairo & Kirsten E. Lyke
June 17th, 2026

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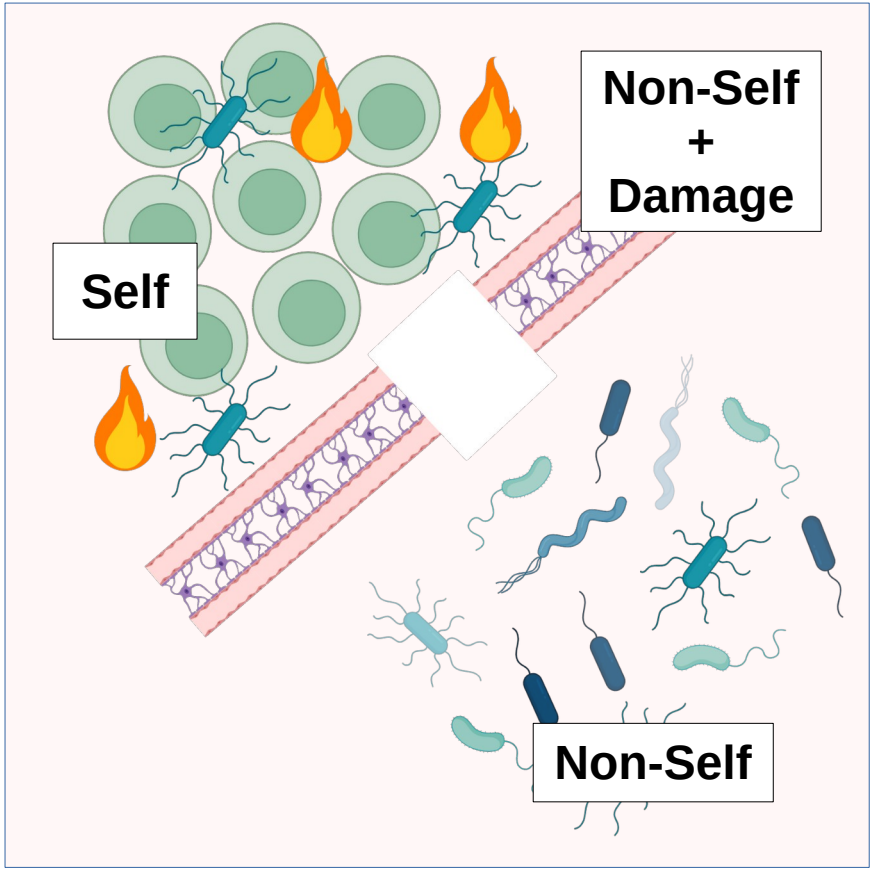
Immune Responses



- Immune systems recognize **self** and **non-self**
- When both **remain separated**, **no immune activation** occurs

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Immune Responses

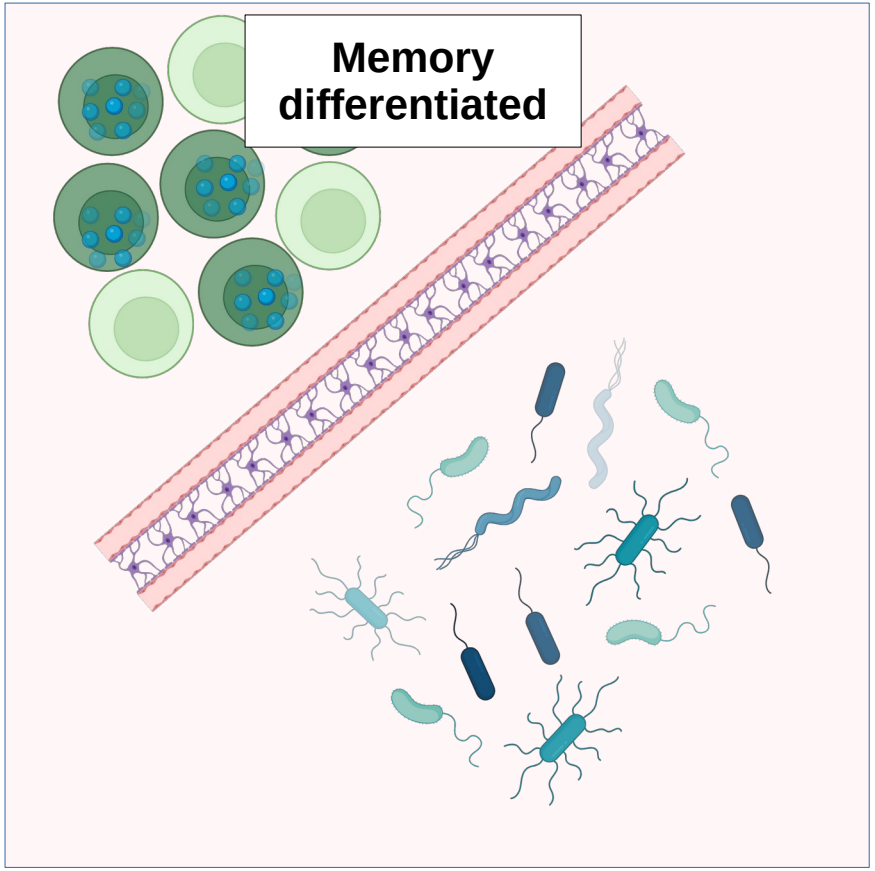


- Immune systems recognize **self** and **non-self**
- When both **remain separated**, **no immune activation** occurs

- **Immune system activation** occurs when **non-self + damage/danger** is present

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Immune Responses

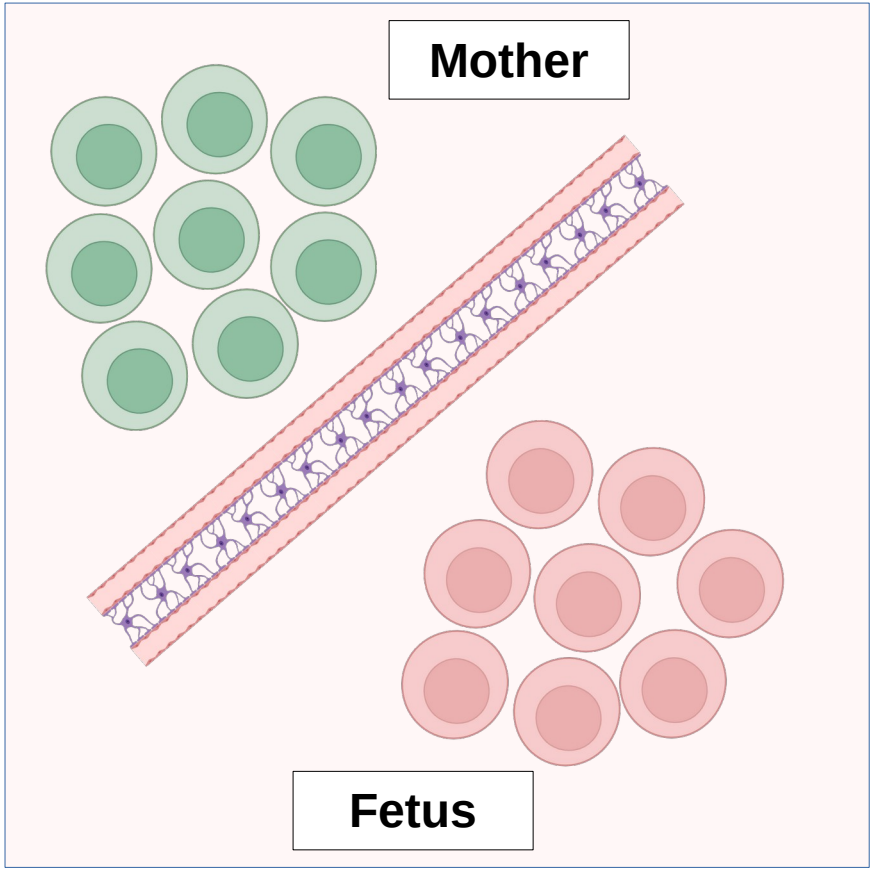


- Immune systems recognize **self** and **non-self**
- When both **remain separated**, **no immune activation** occurs

- **Immune system activation** occurs when **non-self + damage/danger** is present
- Following initial response, **memory cells** capable more rapidly responding persists

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What about during pregnancy?



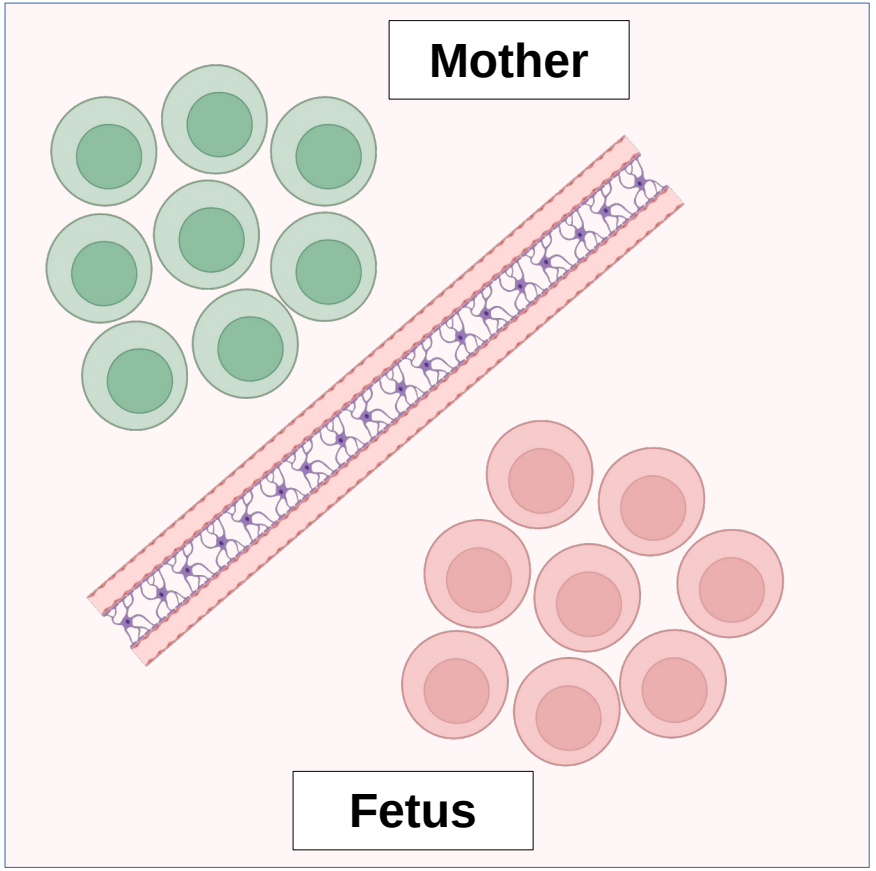
As viewed by maternal immune systems perspective:

Fetus:

- 50% Mom (**self**)
- 50% Dad (**non-self**)

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What about during pregnancy?



As viewed by maternal immune system

Fetus:
- 50% Mom (**self**)
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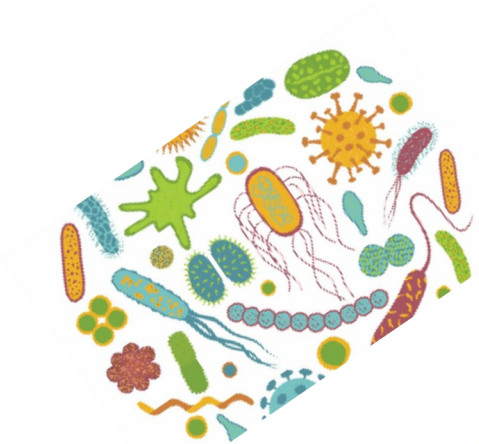
<u>Does the maternal immune system respond?</u>
- Is there interaction?
- Is there damage/danger?

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Neonatal immunology

- Fetal-maternal separation (placenta)
  - Shared circulation, cells mostly excluded
- Maternal antibody transfer

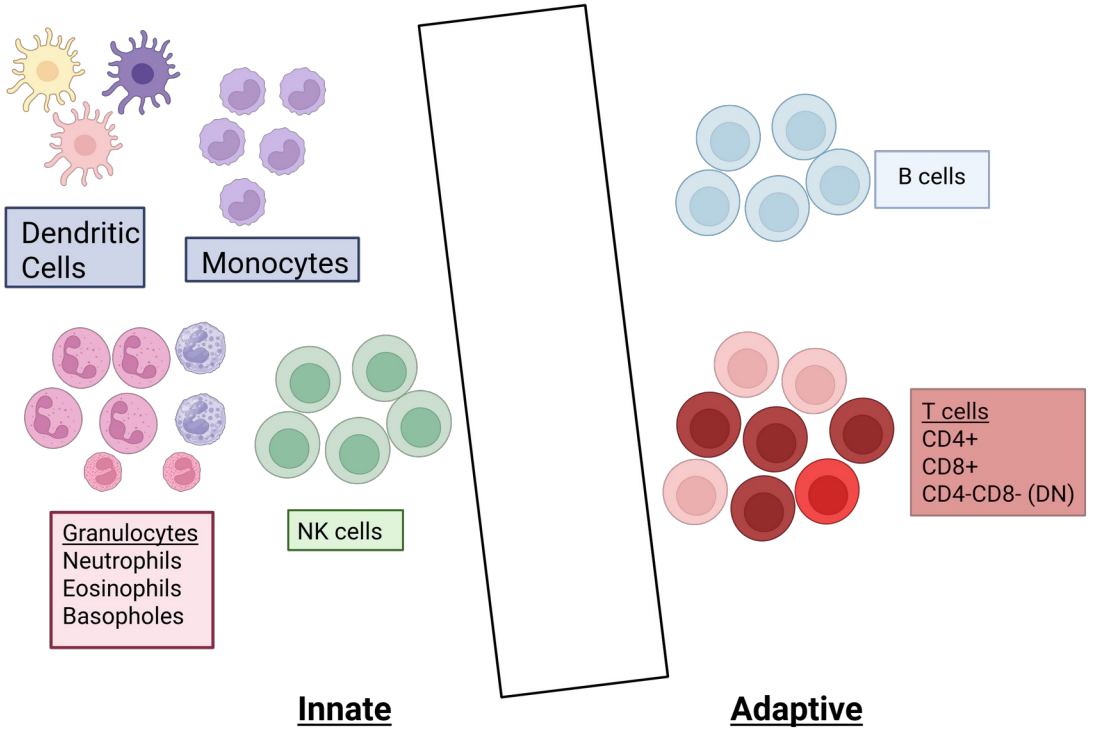
- Microbial exposure
- Ramping up of infant immune responses





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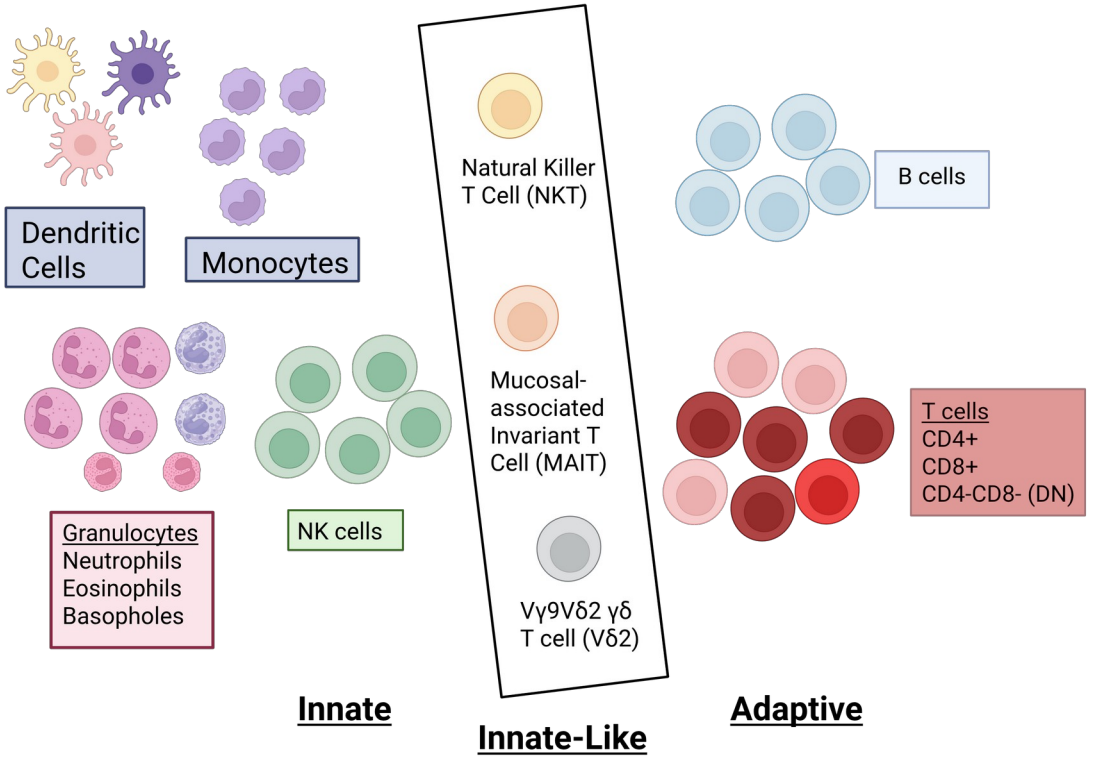
Cells of the Immune System



Innate:
- Rapid response
- Conserved patterns

Adaptive:
- Slower response
- Antigen-specific

Innate-like T cells



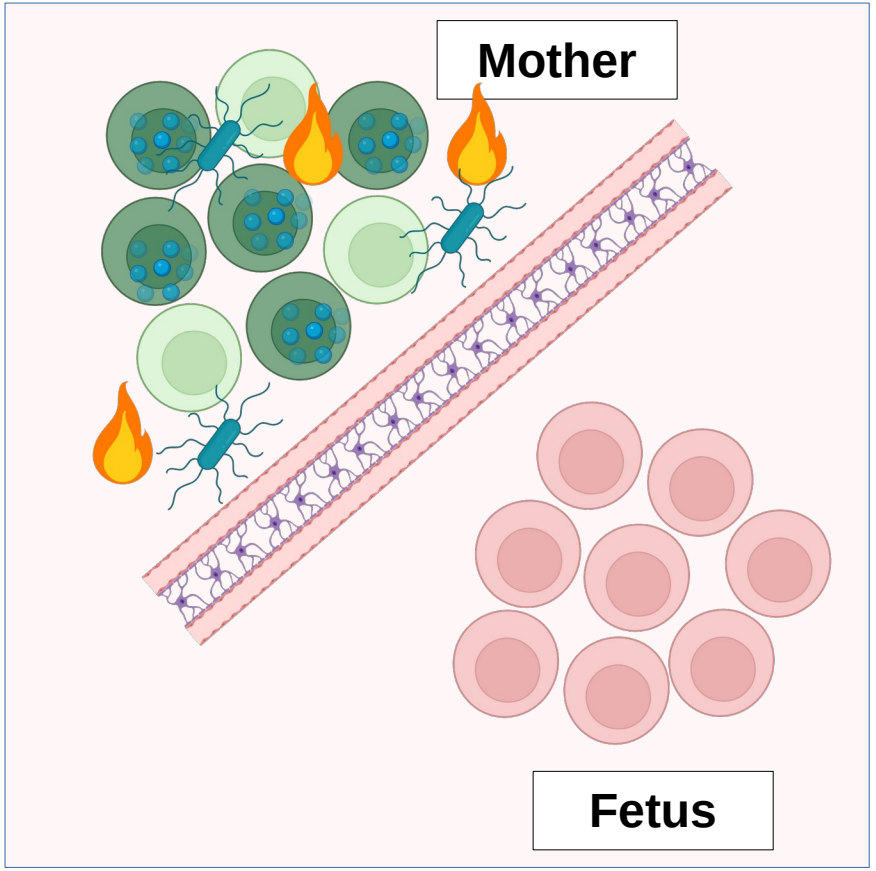
Natural Killer T cells (NKTs)
- Vα24Jα18+ (antibody)
- CD1d (tetramer) with PBS-57 (Lipid)

Mucosal-Associated Invariant T cells (MAITs)
- Va7.2+CD161hi (antibody)
- MR1 (tetramer) + OP-RU-56

$V_\gamma9+V\delta2+$ γδ T cells (Vδ2)
- $V_\gamma9+V\delta2+$ (antibody)

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Infection during pregnancy



Does the maternal immune system respond?

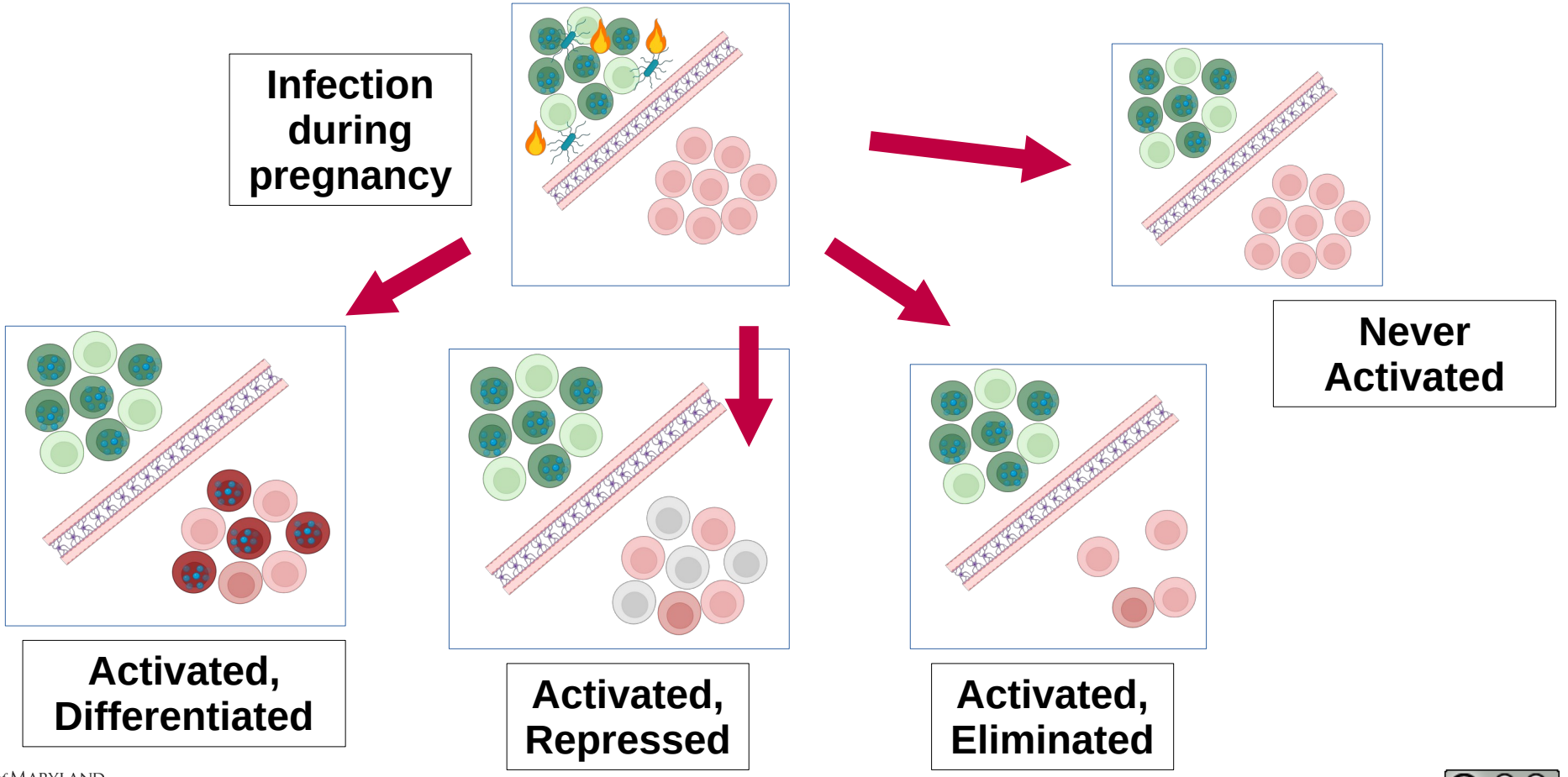
- Is there interaction?
- Is there damage?

Is the developing fetal immune system affected?

- Shared circulation
- Location, Scale, Timing
  - Cold, Upper Nasal
  - Influenza, Systemic

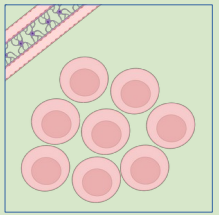
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What effect does early exposure have on fetal immune system?

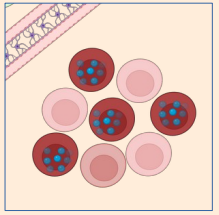


University of Maryland School of Medicine

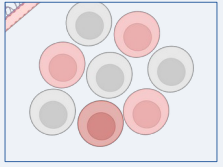
Does this fetal exposure matter impact immune responses during early life?



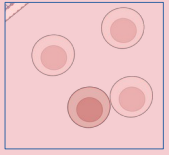
Never Activated



Activated, Differentiated



Activated, Repressed



Activated, Eliminated



Health ?

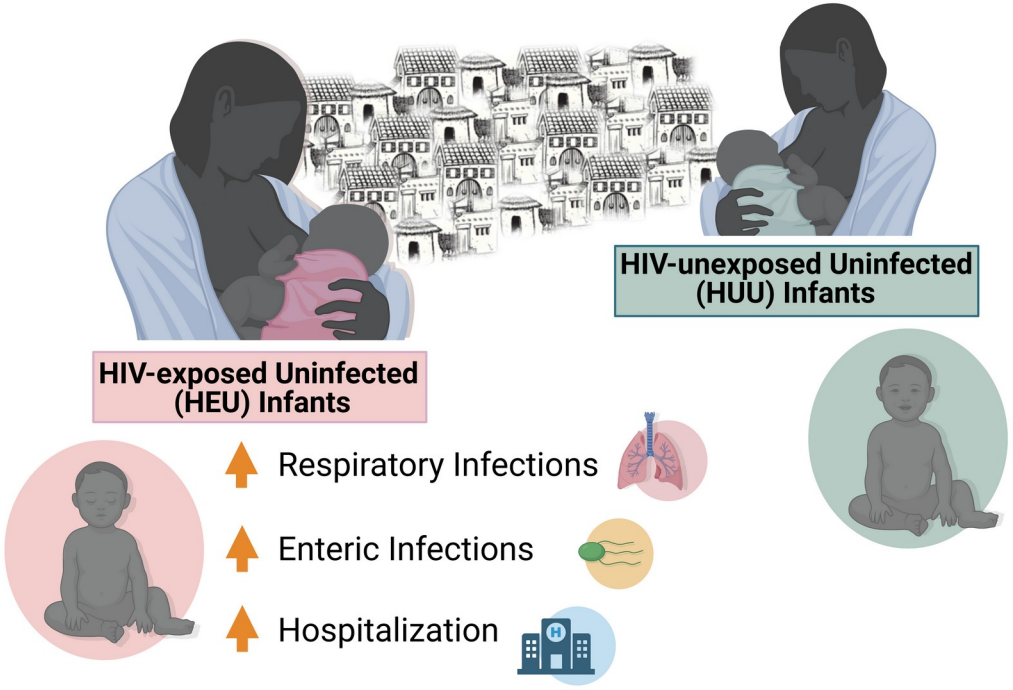
Sickness ?

Hospital ?

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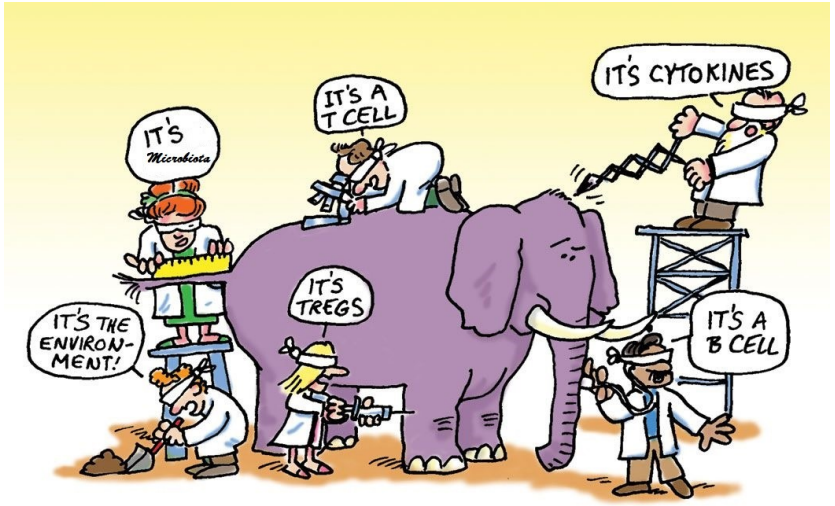
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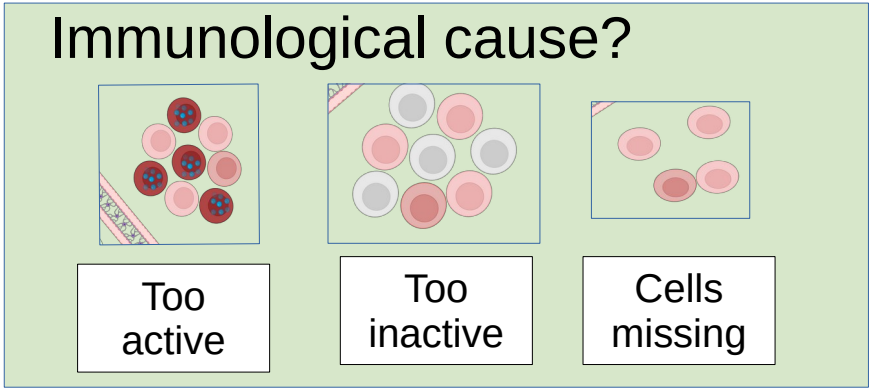
HIV-exposed Uninfected Infants



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Is there an immunological explanation?





Conflicting Studies

Some Studies:

- Increased frequency of Th1-like cytokines in T cells *ex-vivo*
- Decreased proliferation in culture
- Lower antibody titers for maternal antibodies

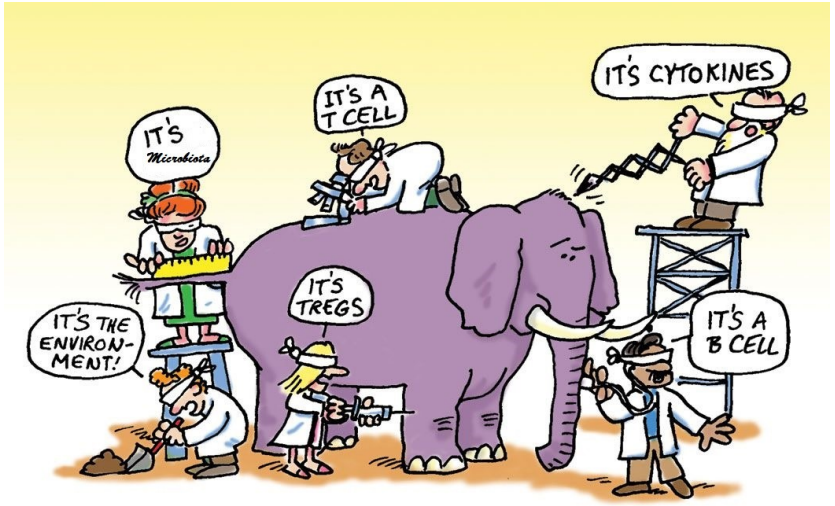


Other Studies:

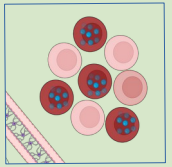
- Same (or lower) frequency of Th1-like cytokines in T cells *ex-vivo*
- No change in proliferation in culture
- Higher antibody titers for maternal antibodies

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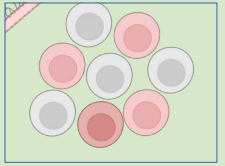
Is there an immunological explanation?



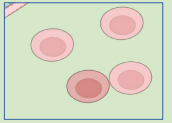
Immunological cause?



Too active



Too inactive



Cells missing

Other factor?



Formula vs. Breastfeeding



Maternal Antiretrovirals

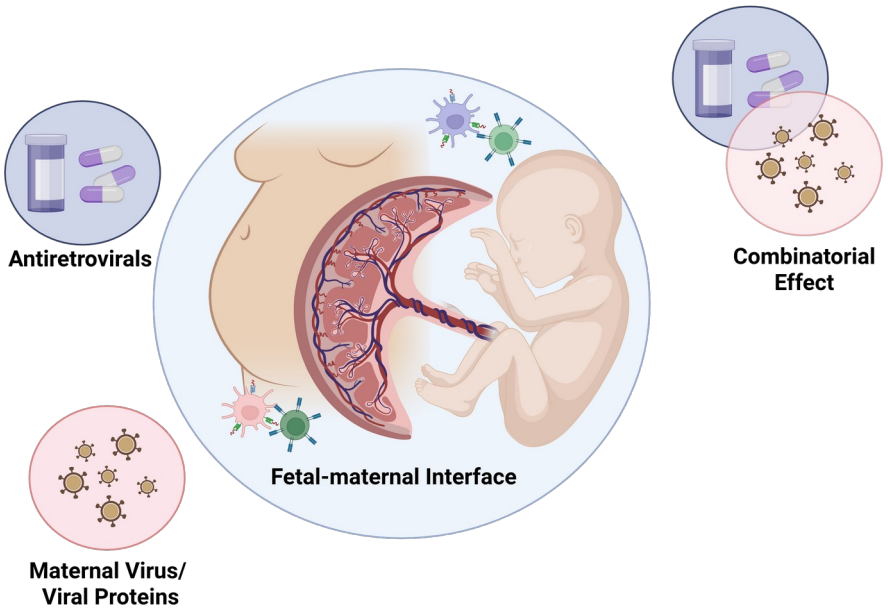
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Increased anti-retroviral availability

Policy timeline:

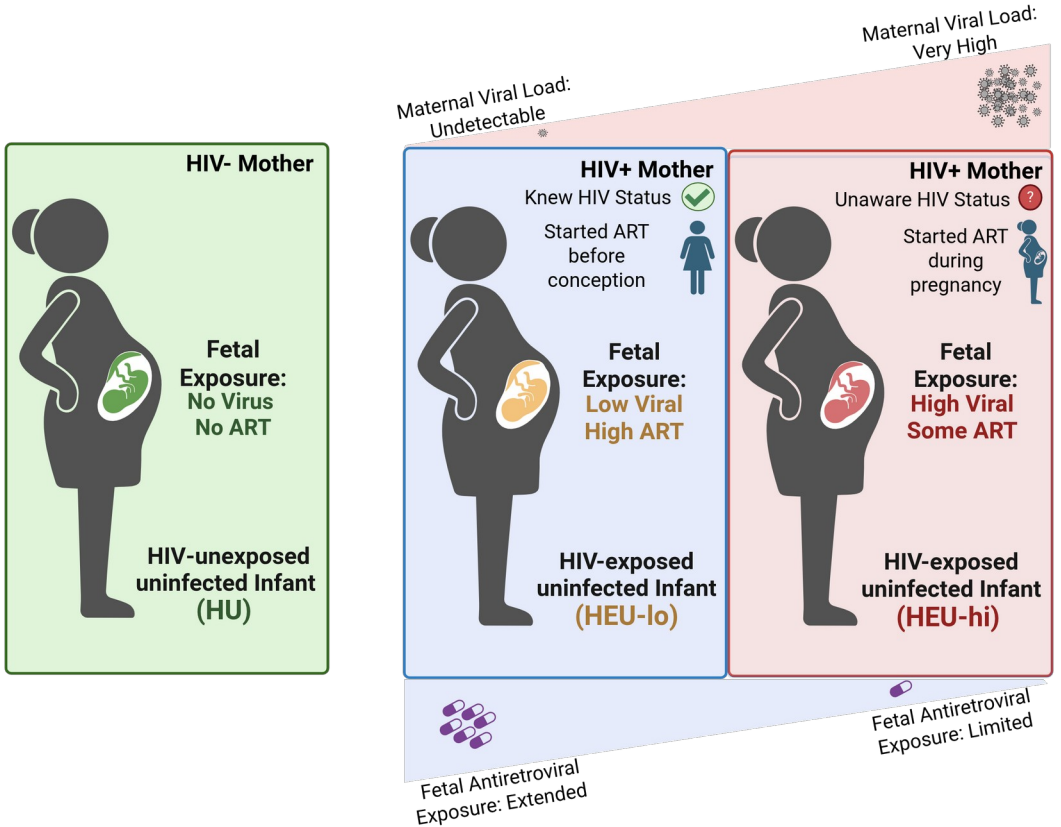
- Maternal antiretroviral until birth
- Single antiretroviral for life
- Combined antiretroviral for life

Prior studies may have grouped infants with varied viral and antiretroviral exposures together



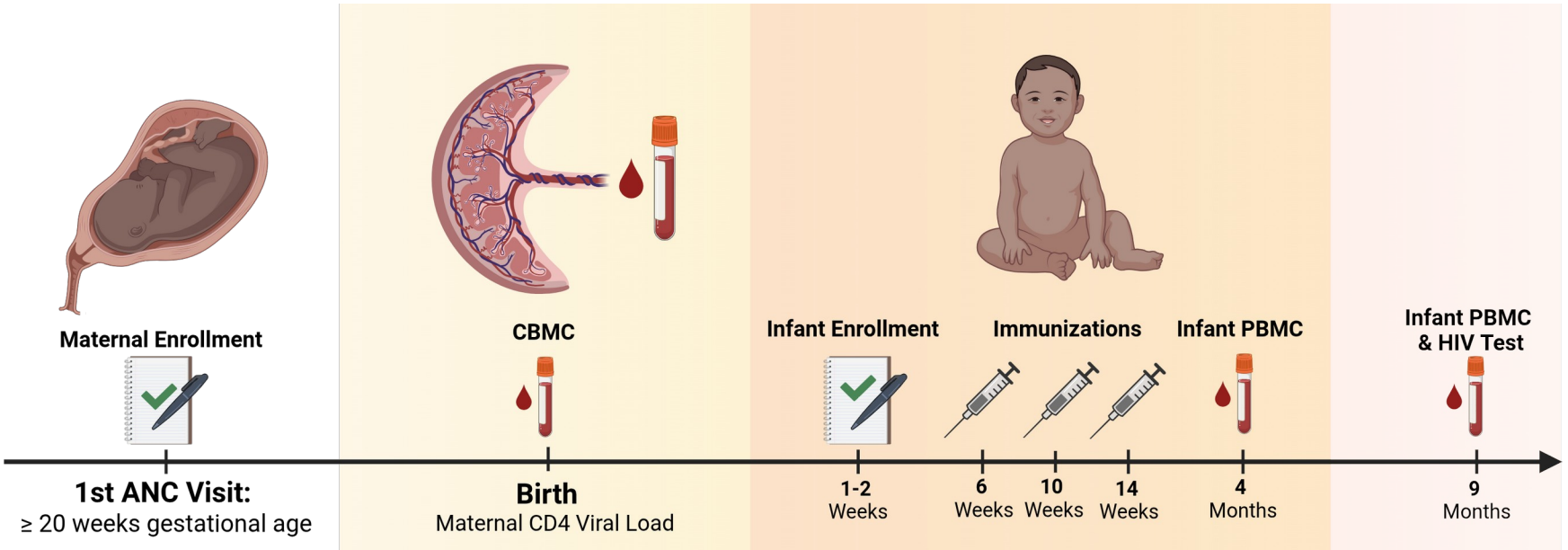
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Our Malawian Cohort



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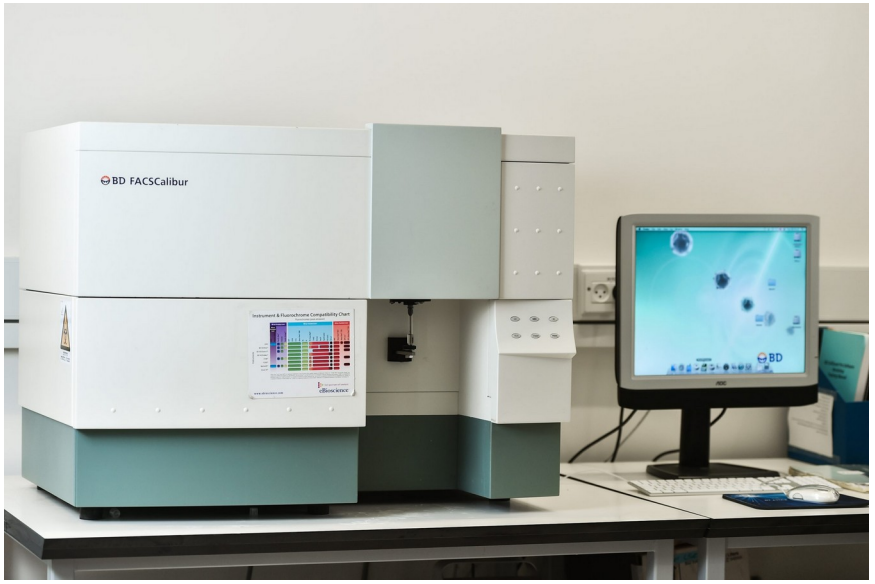
Study Timeline



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Snapshots in Time



- Even carefully controlled ground-breaking studies are defined by the technology and resources available at time

Conventional Flow Cytometers

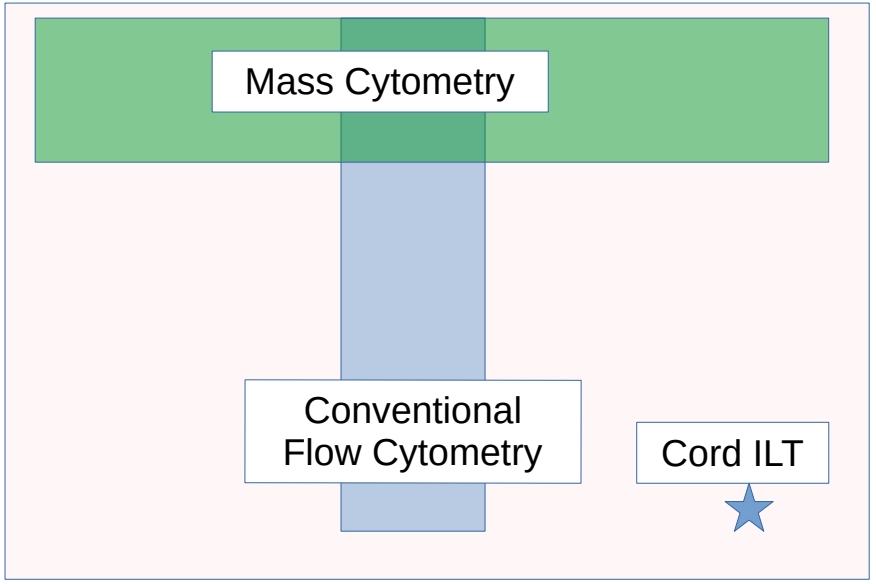
- Calibur (2007) (4-fluorophores)
- LSR II (2017) (14-fluorophores)

Spectral Flow Cytometers

- Up to 60! fluorophores now

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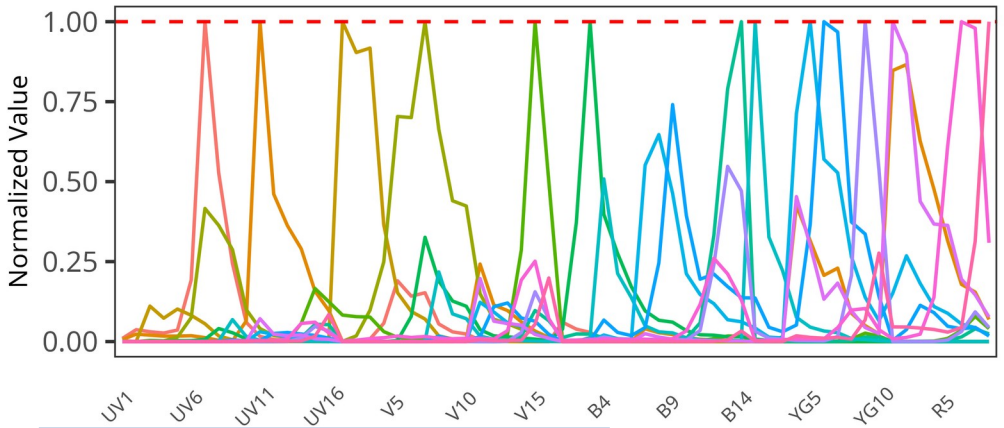
Little is known about Cord Blood ILTs



- Additional lineage markers
  - Vδ2+, Vα24Jα18+, Vα7.2+
- Unfeasible for Conventional Flow Cytometry due to smaller panels

- Rare in Cord Blood!
  - < 0.1% of T cells
- Unfeasible for Mass Cytometry due to low acquisition rate

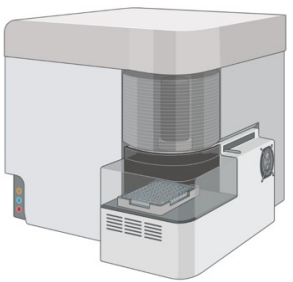
Flow Cytometry



Conventional Flow Cytometry

- One Fluorophore, One Detector
- Signal in given detector attributed to respective marker (after compensation)
- Smaller Panels (<20 fluorophores)
- Fast Acquisition

- ***What fluorophores are present on a cell?***
- ***And in what amount?***

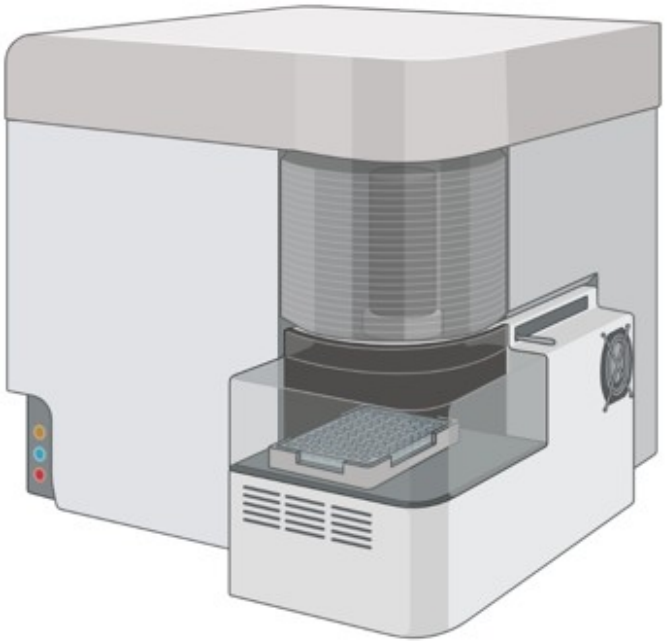


Spectral Flow Cytometry

- One Fluorophore, All Detectors
- Respective fluorophore contributions **unmixed** out on basis of the full reference control signatures
- Large Panels (>40 fluorophores
- Fast Acquisition

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Before we could leverage the full biological discovery potential, the pink elephants in the room



☐

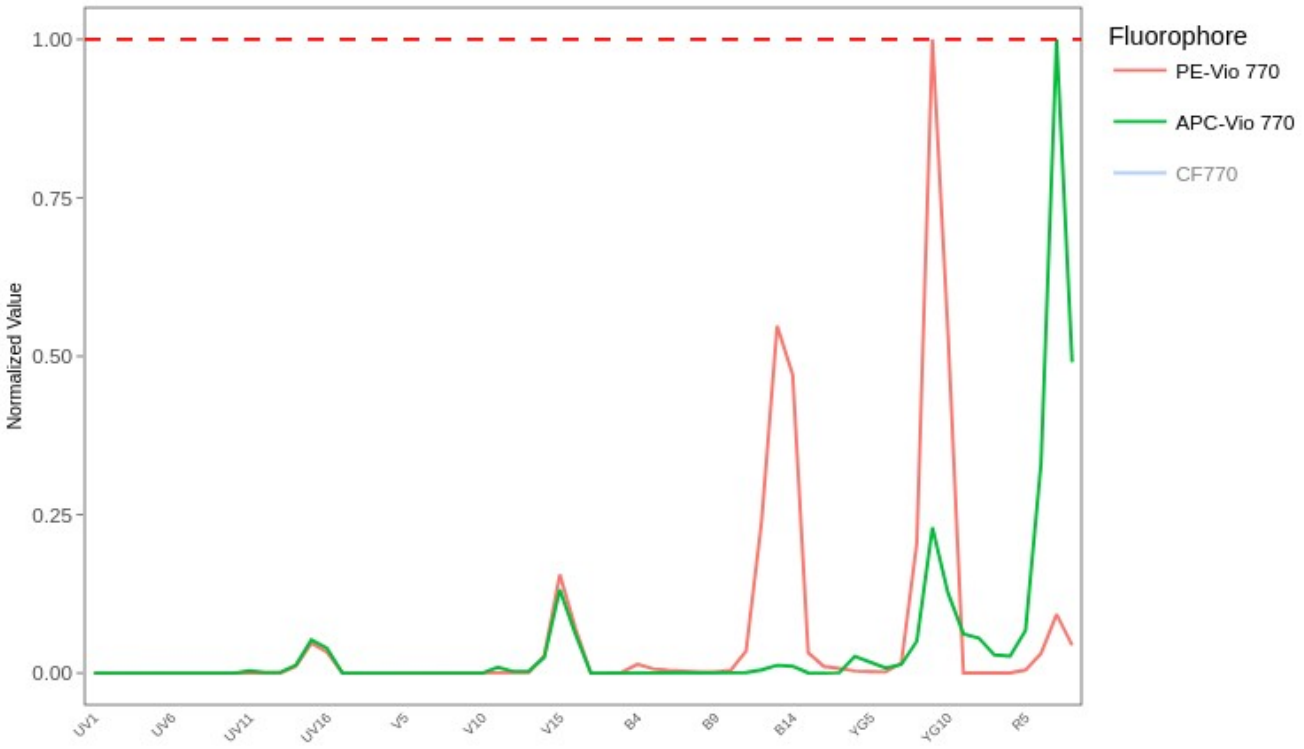
Quality of the unmixed full-stained data is linked to the quality of our single-color controls and ability to provide appropriate autofluorescence control(s)

☐

In acquiring 3 million cells to detect 100 cord blood ILTs, a ton of useful data is gathered. How to analyze it all?

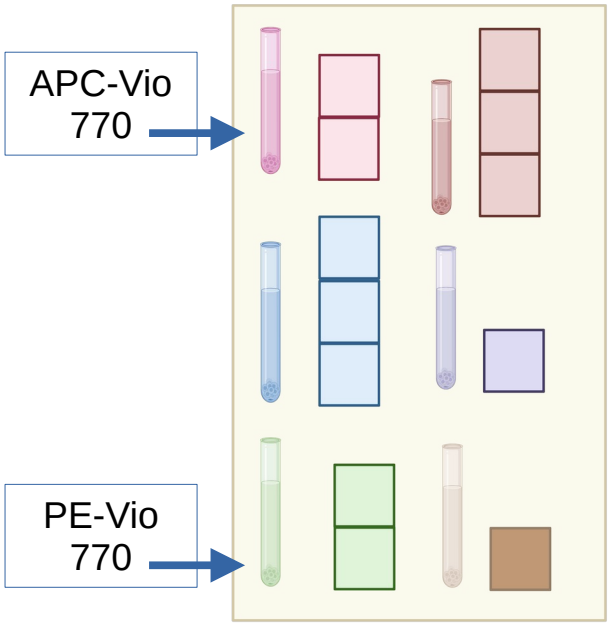
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Spectral Signatures

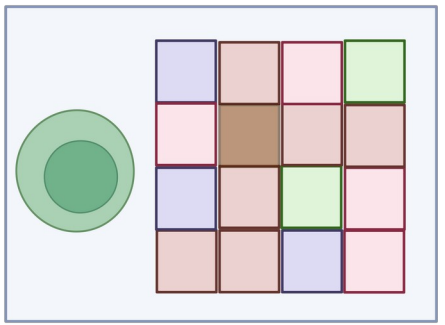


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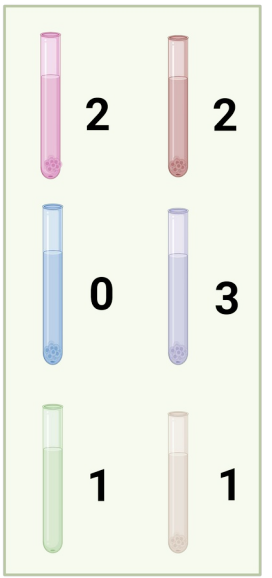
Spectral Unmixing



Unmixing Controls (Single Color and Unstained)



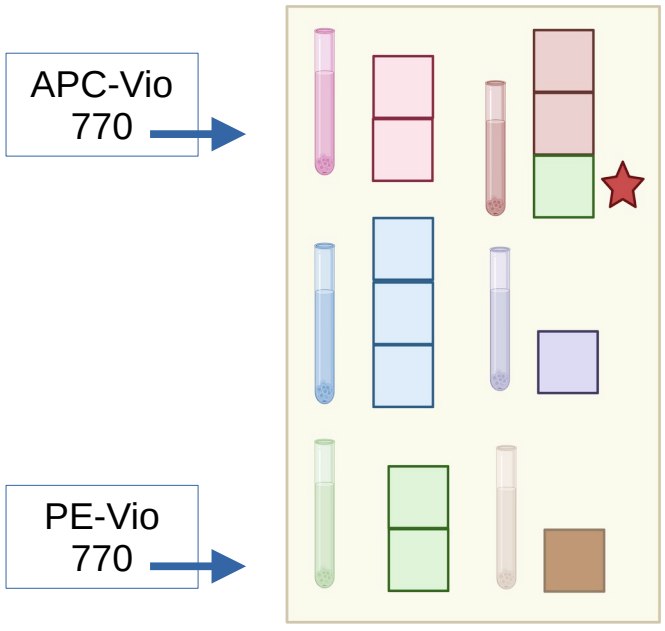
Single cell from full-stained sample



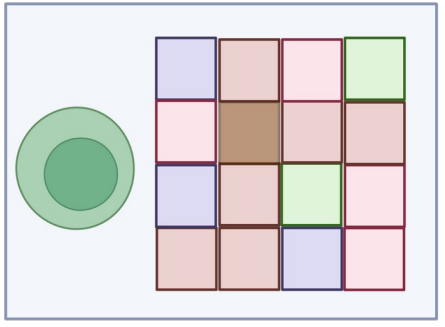
What fluorophores are present, and roughly how much

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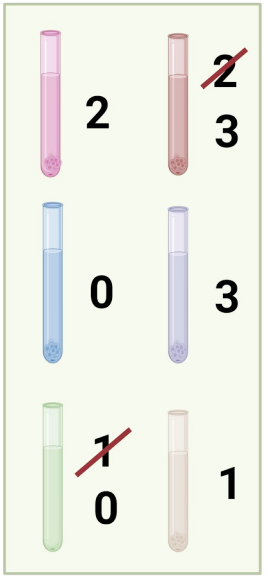
Mismatched References, errors in attribution



Unmixing Controls (Single Color and Unstained)



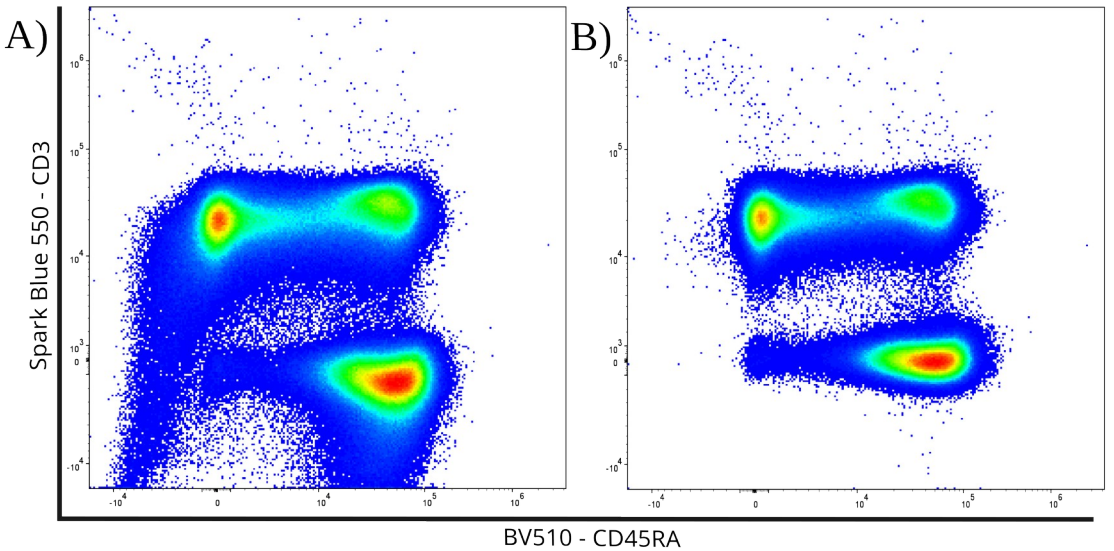
Single cell from full-stained sample



What fluorophores are present, and roughly how much

CC BY SA

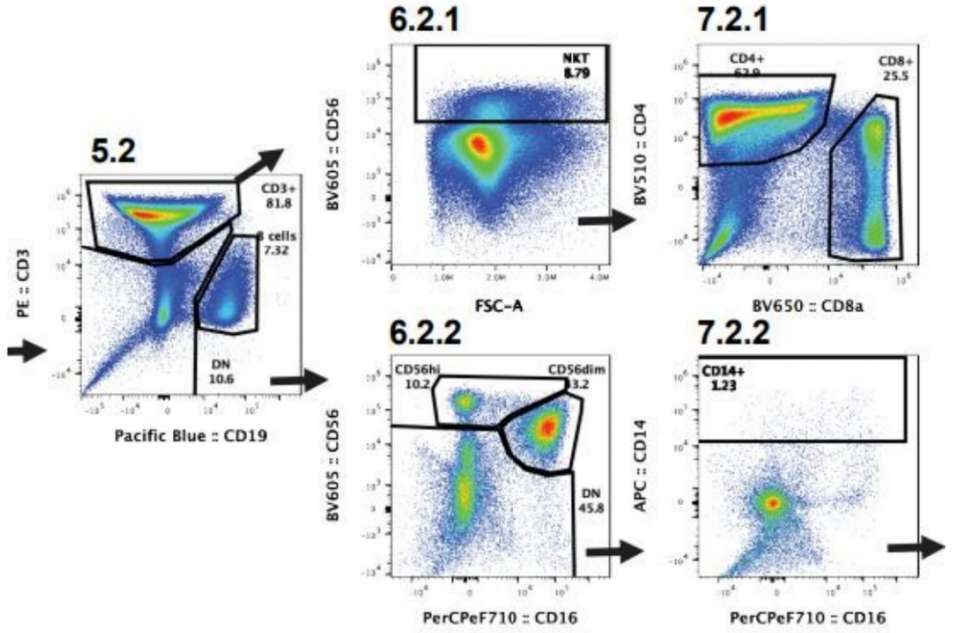
Unmixing Errors



Is the population you are seeing biological, or just a technical artifact?

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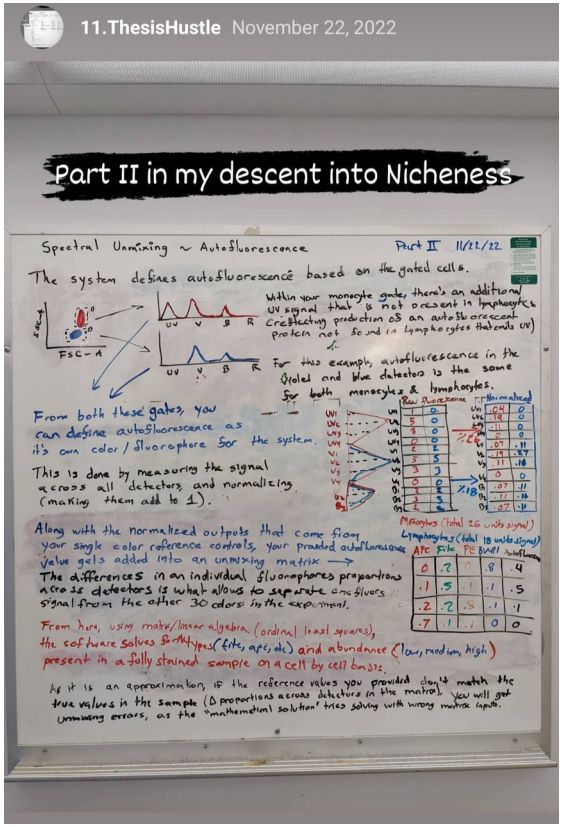
Why does this matter?



- The power to shoot yourself in the foot
- Garbage In, Garbage Out
- Adding to the reproducibility crisis
- Wasting time, effort and funds

* Paper used an identical viability dye signature as the fluorophore of their main marker of interest. No way to tell if their entire paper is based on necrotic cells

Breaking open the black box



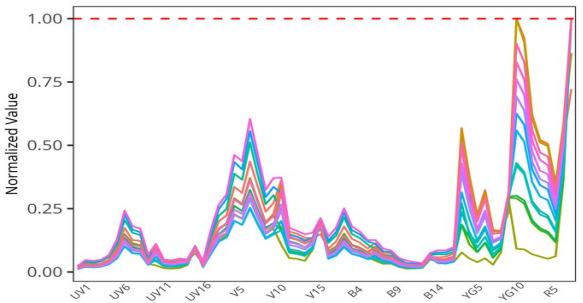
Central Issue:

- Reference signatures extracted from unmixing controls **<u>are critical</u>** to **<u>final data quality</u>**
- No tools existed to screen for signature quality pre-unmixing
- Hours wasted as a result

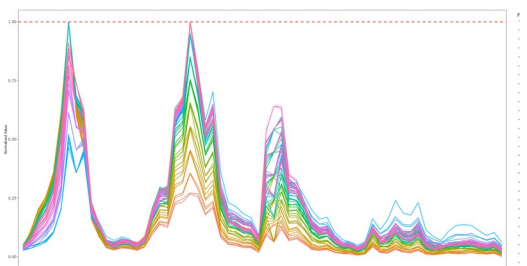
CC BY SA

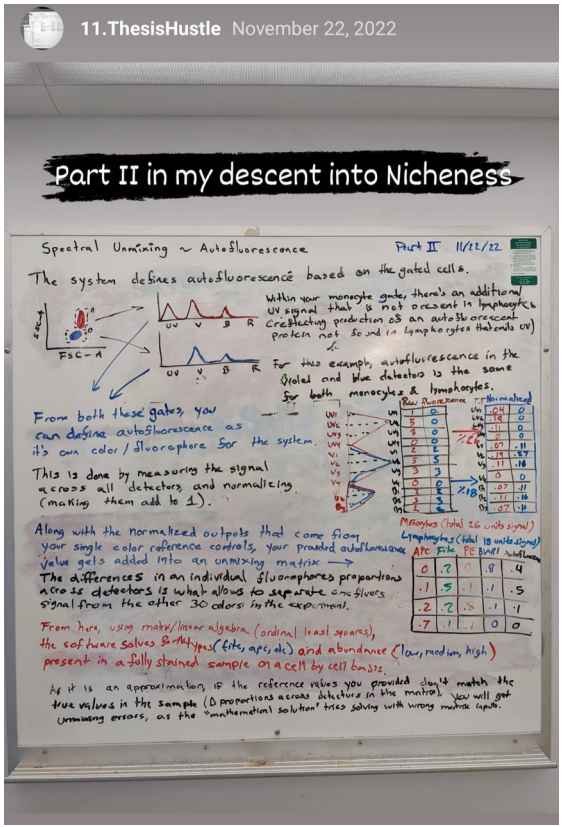
Breaking open the black box

Tandem Degradation



Multiple Autofluorescence





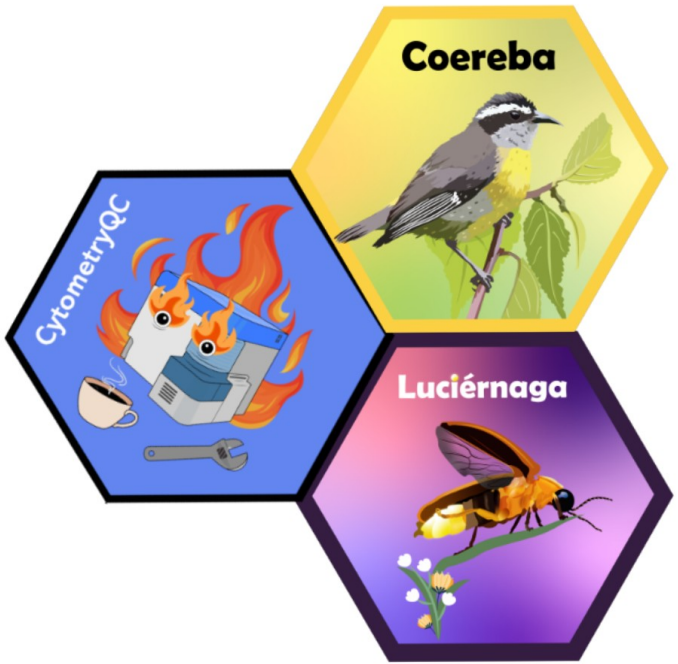
Central Issue:

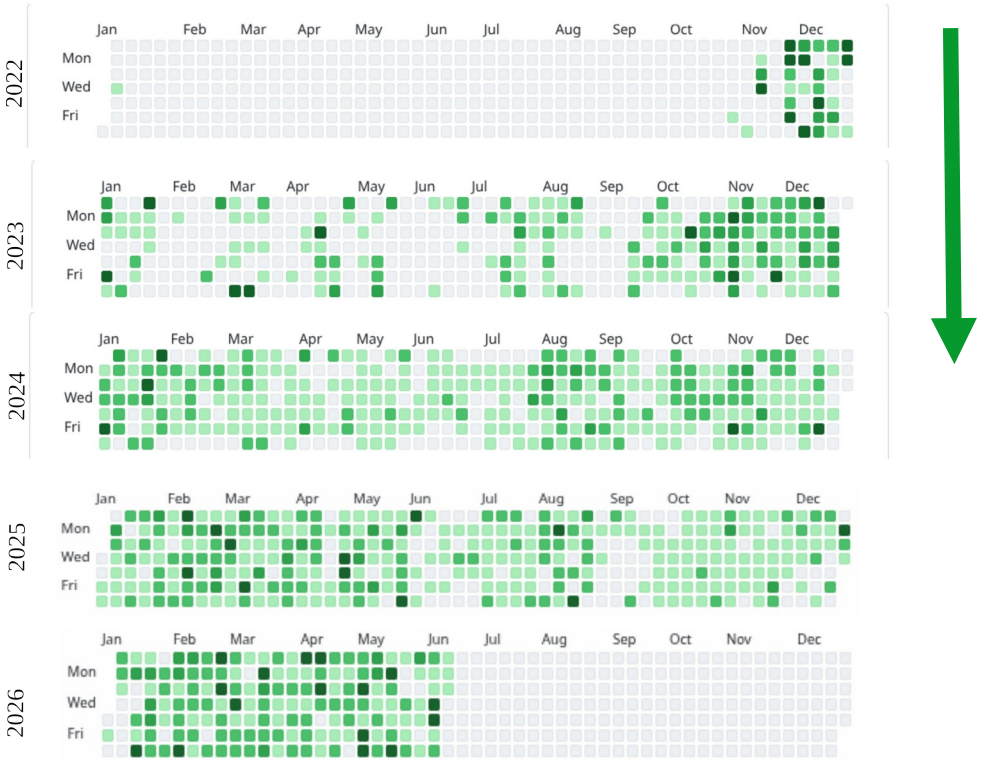
- Reference signatures extracted from unmixing controls **<u>are critical</u>** to **<u>final data quality</u>**
- No tools existed to screen for signature quality pre-unmixing
- Hours wasted as a result

→ What if instead of looking at averaged population signature we looked at that of individual cells?

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Sparked journey into R programming



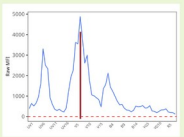


University of Maryland
School of Medicine

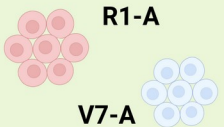
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Luciernaga





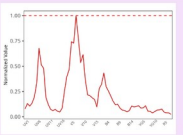
Identify detector with brightest MFI for each cell



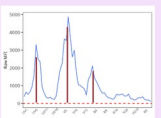
Group cells with shared peak detectors



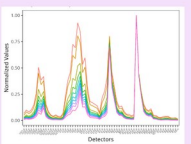
Subtract Out Autofluorescence



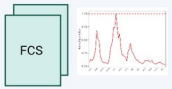
Normalize individual cell fluorescent signatures



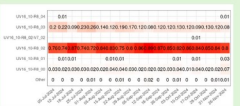
Identify peaks using local maxima



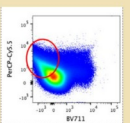
Group cells with shared peaks & heights



Enumerate and export out cells that share signature as .fcs files



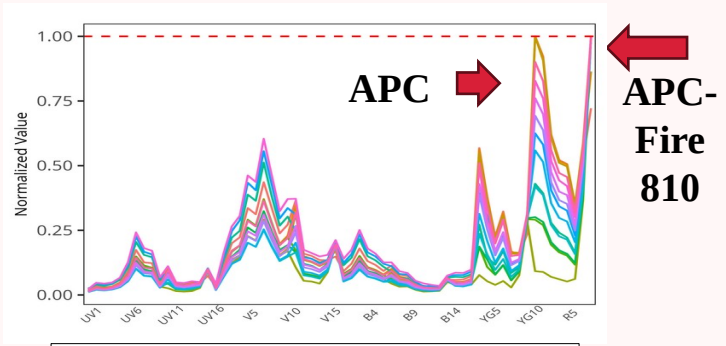
Checks for tandem degradation



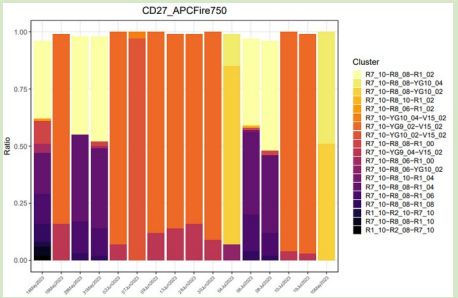
Autofluorescence Isolation

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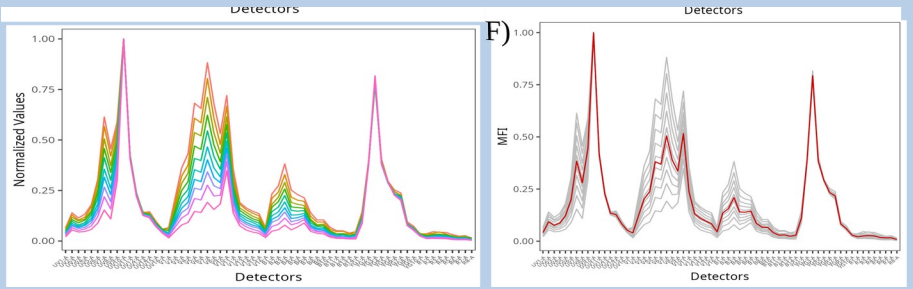
Automated tools for screening unmixing controls



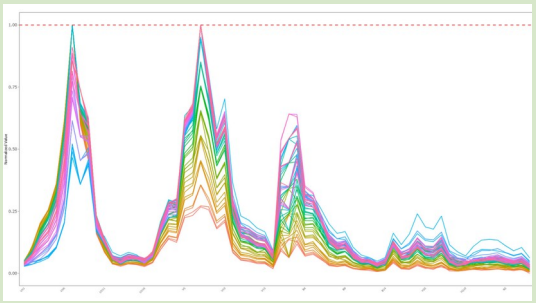
Tandem Degradation



Cell Subset ROS



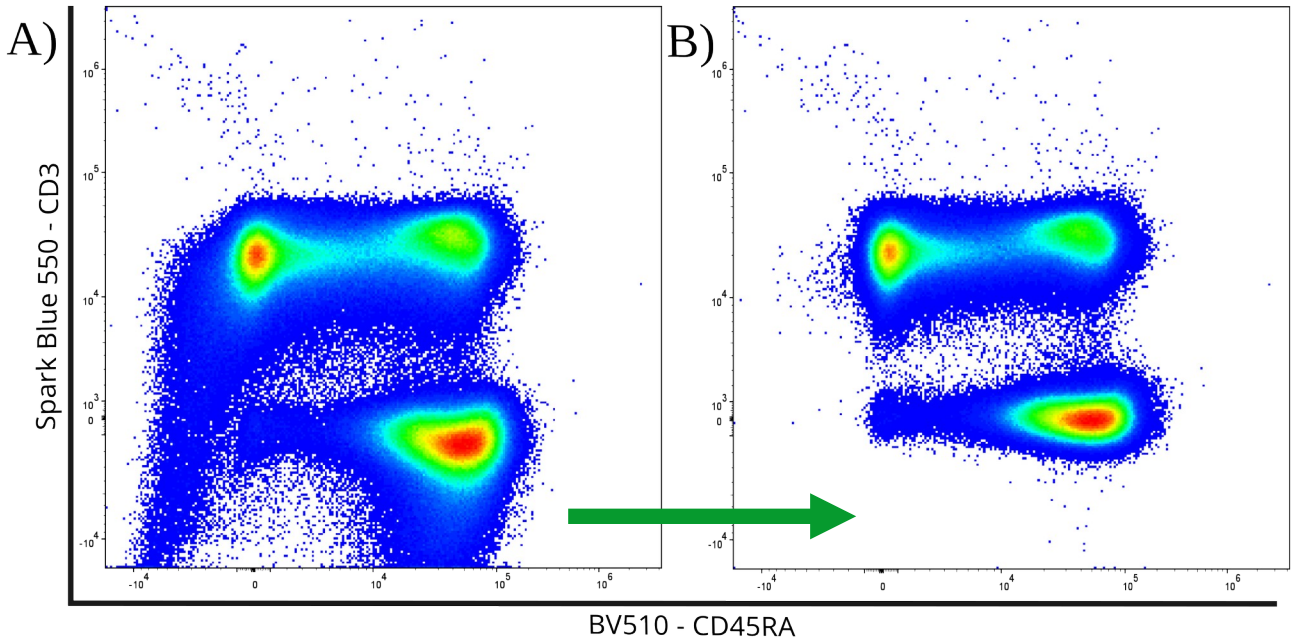
Residual Autofluorescence



Autofluorescence Isolation

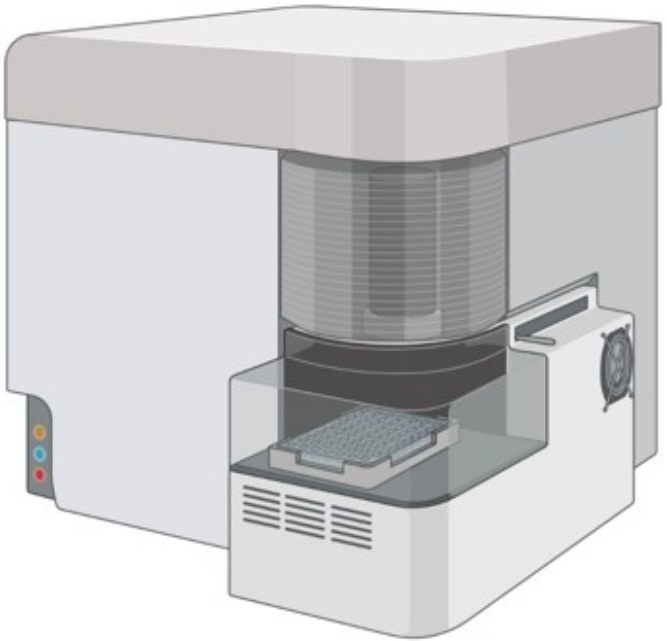
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Dramatic improvement in data quality



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Before we could leverage the full biological discovery potential, the pink elephants in the room



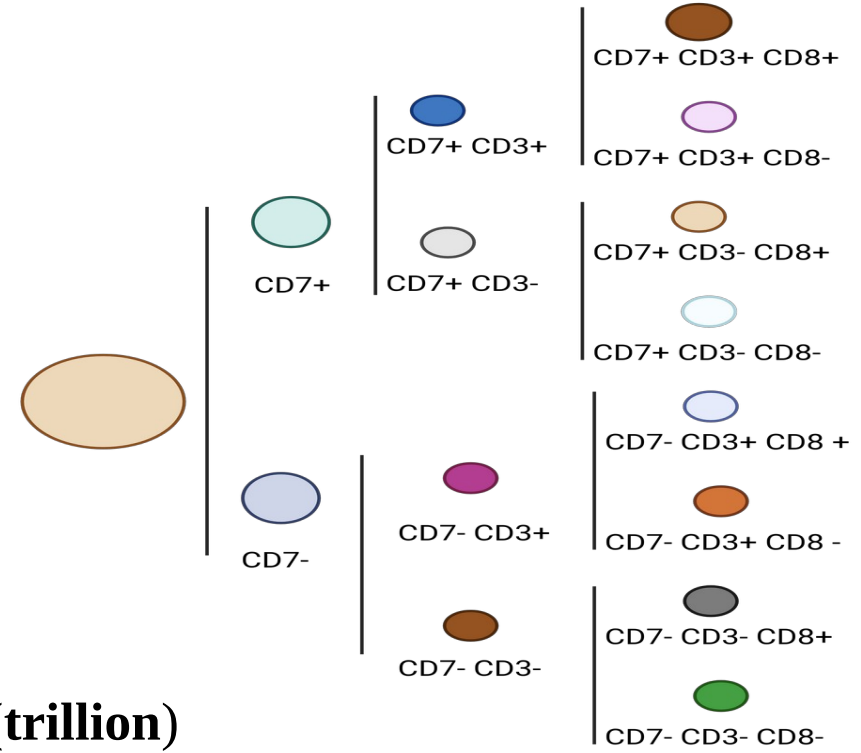
☑ Quality of the unmixed full-stained data is linked to the quality of our single-color controls and ability to provide appropriate autofluorescence control(s)

☐ In acquiring 3 million cells to detect 100 cord blood ILTs, a ton of useful data is gathered. How to analyze it all?

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Complex Datasets, Combinatorially Exponential Clusters

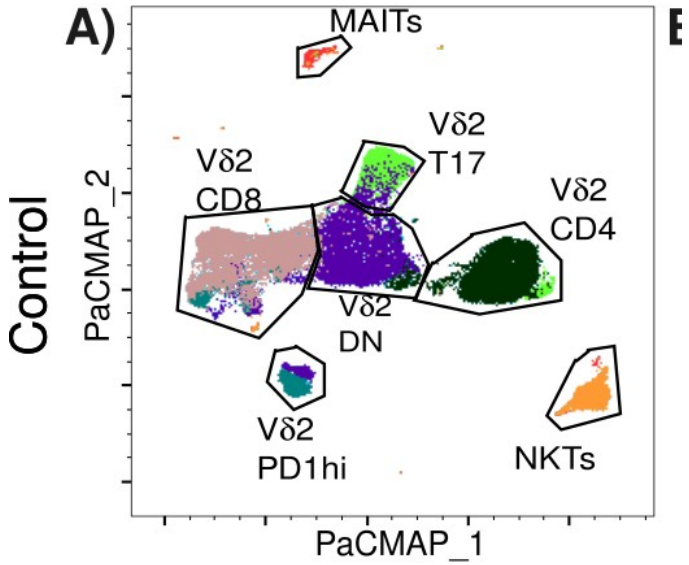
- If every marker in your panel split into **positive** and **negative** populations
- And you repeated this splitting process for **every marker**
- And **grouped cells** that shared the **same expressed markers**
- $2^{\text{NumberOfMarkers}}$

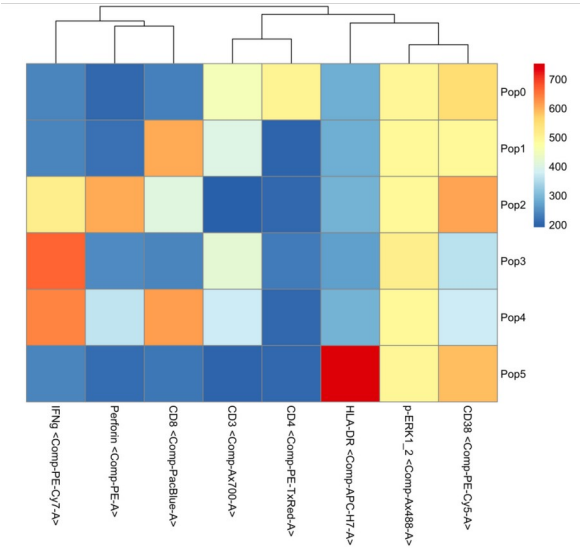


- **9-color** CFC panel: **512** groups at most
- **40-color** SFC panel: **1.1e+12** clusters at most (**trillion**)
- **60-color** SFC panel: **1.2e+18** clusters at most (**quintillion**)

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Unsupervised Analysis (Algorithms)



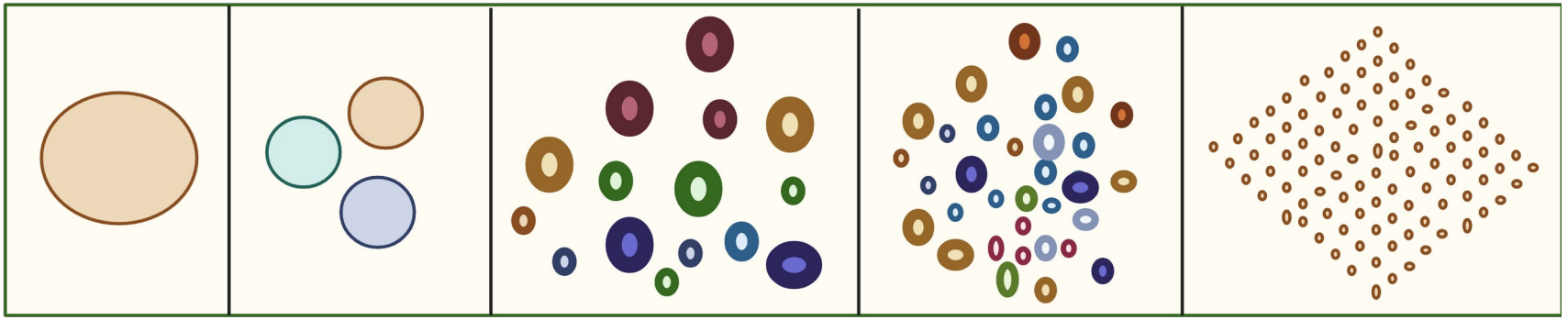


Dimensionality Visualization/Reduction (tSNE, UMAP, PaCMAP, PHATE)

Clustering (FlowSOM, Phenograph, Xshift)

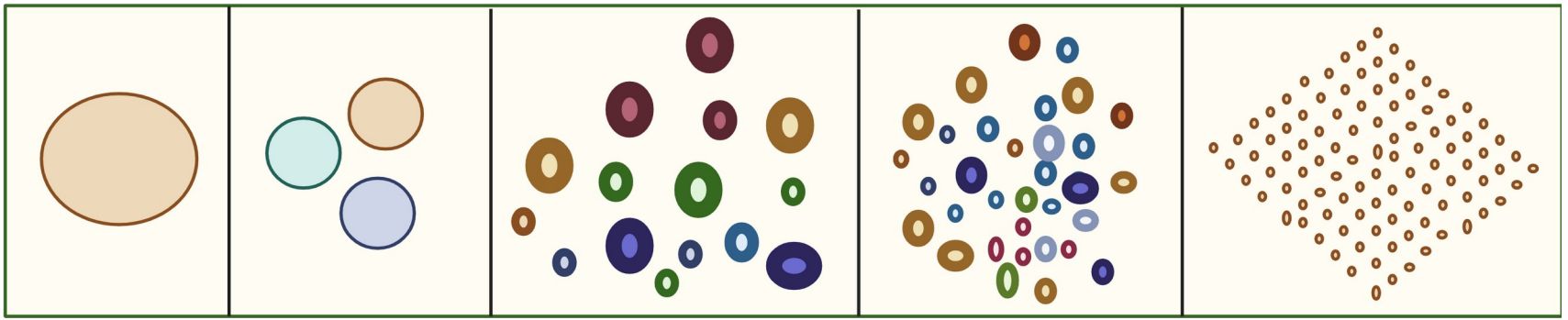
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Unsupervised Analysis



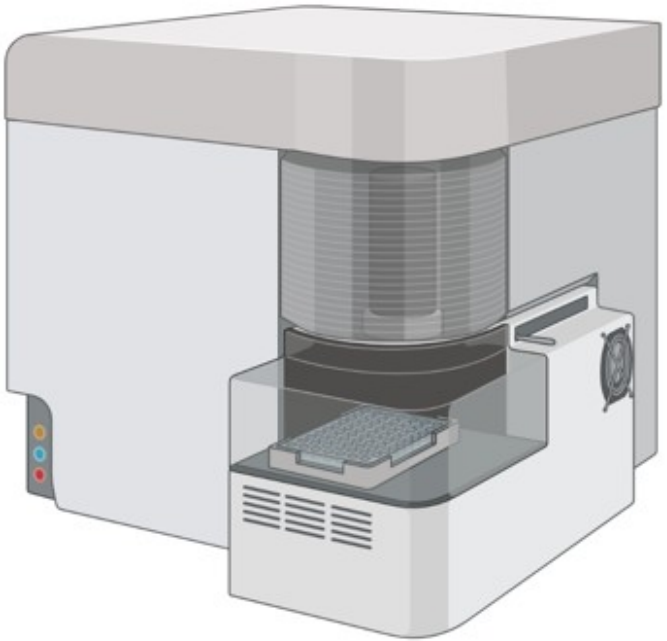
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Unsupervised Analysis



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Put a pin in this one for now

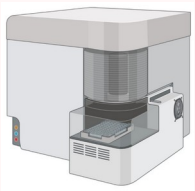


☑ Quality of the unmixed full-stained data is linked to the quality of our single-color controls and ability to provide appropriate autofluorescence control(s)

☐ In acquiring 3 million cells to detect 100 cord blood ILTs, a ton of useful data is gathered. How to analyze it all?

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Optimized a 29-fluorophore SFC panel

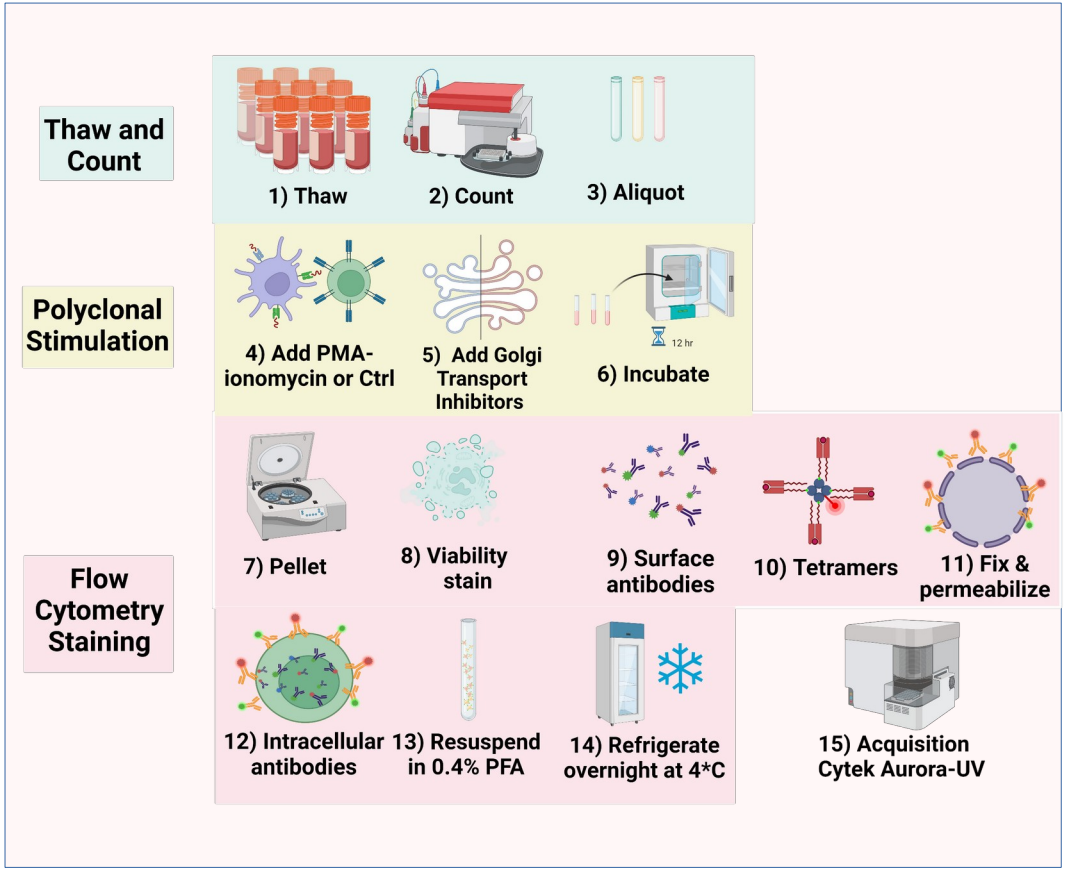


Laser	Wavelength	Detector	Fluorochrome	Marker
355 nm	388	UV2	BUV395	CD62L
	473	UV6	AlexaFluor350	AF-UV6
	514	UV7	BUV496	CD8
	582	UV9	BUV563	CD69
	613	UV10	BUV615	CCR4
	664	UV11	BUV661	Vd2
	750	UV14	BUV737	CXCR3
	812	UV16	BUV805	CD4
405 nm	428	V1	BV421	CD127
	458	V3	Pacific Blue	Lin-
	508	V5	BV480	CD161
	542	V7	BV510	CD45RA
	581	V8	BV570	CD16
	615	V10	BV605	CD56
	664	V11	BV650	CCR7
	720	V13	BV711	CD7
	750	V14	BV750	IFNg
	780	V15	BV786	CCR6
488 nm	525	B2	AlexaFluor 488	hCD1d
	542	B3	Spark550	CD3
	581	B4	PE	NKG2D
	615	B6	PE-CF594	CD26
	679	B8	PE-Cy5	CD25
	697	B9	PerCP-Cy5.5	TNFa
	783	B13	PE-Vio770	PD1
640 nm	664	R1	APC	CD16
	679	R2	AlexaFluor647	hMR1
	717	R4	APC-R700	CD107a
	760	R6	Zombie NIR	Viability
	783	R7	APC/Fire 750	CD27
	812	R8	APC/Fire 810	CD38

- Lineage markers for ILTs
- Phenotypic markers for T and NK cells
- Cytokines (TNFα, IFN$_\gamma$)
- Tetramer or Antibody for MAITs and NKTs

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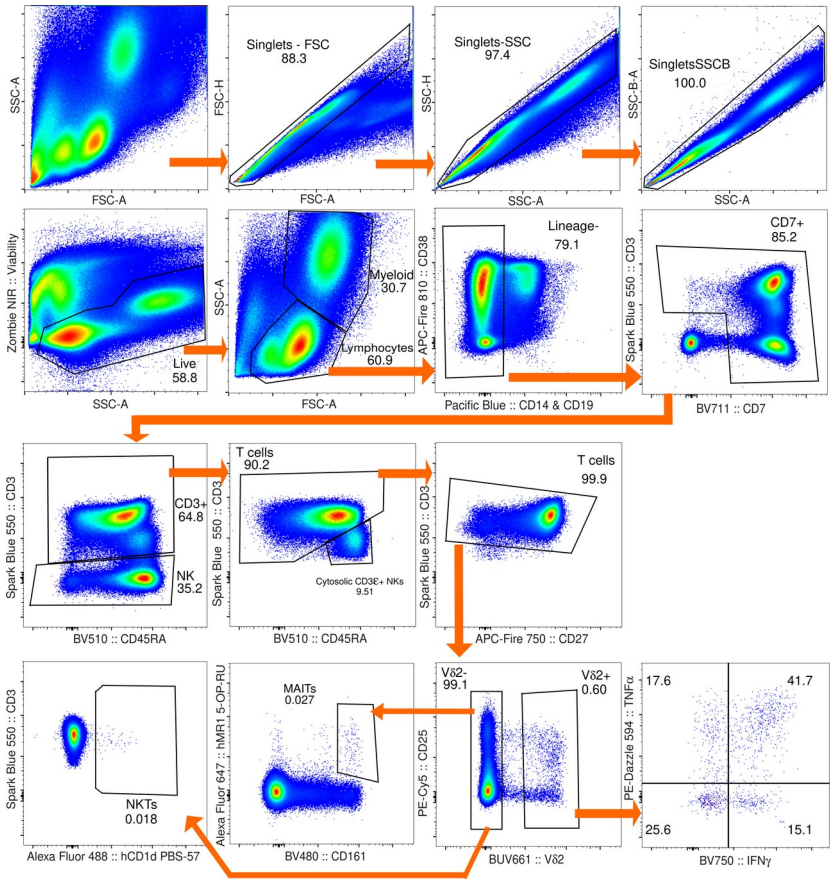
Processing and Staining



- Thawed
- Stimulated
- Stained (surface & intracellular)
- Acquired following day

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Gating Strategy



Good Resolution

- Cord Blood ILTs
- T cells
- NK cells

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Balanced Cohort

Cohort Characteristics

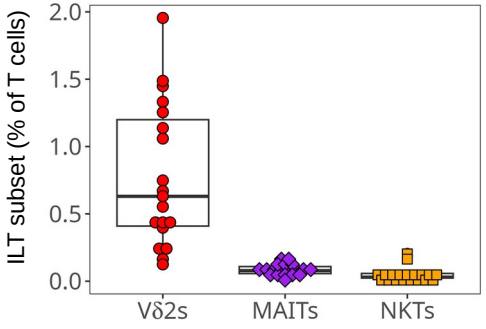
Demographics	HU N = 26[1]	HEU-lo N = 24[1]	HEU-hi N = 25[1]
Maternal Age (years)	24 (19, 32)	27 (24, 35)	26 (22, 30)
Maternal viral load (screening)	NA (NA, NA)	0 (0, 0)	26,915 (15,732, 93,422)
Detectable viral load (delivery)	0 (NA%)	0 (0%)	5 (20%)
Maternal CD4+ cells/μL (delivery)	NA (NA, NA)	613 (503, 769)	489 (391, 576)
Days between screening and delivery	102 (75, 122)	116 (95, 134)	97 (73, 121)
Infant sex (female)	14 (54%)	12 (50%)	13 (52%)
Estimated Gestational Age, weeks	39.79 (38.14, 40.14)	39.79 (39.07, 40.36)	39.57 (38.71, 40.57)
Parity			
0	7 (27%)	0 (0%)	2 (8.0%)
1	10 (38%)	12 (50%)	11 (44%)
2+	9 (35%)	12 (50%)	12 (48%)

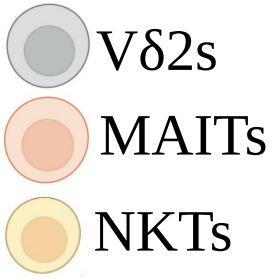
[1] Median (Q1, Q3); n (%)

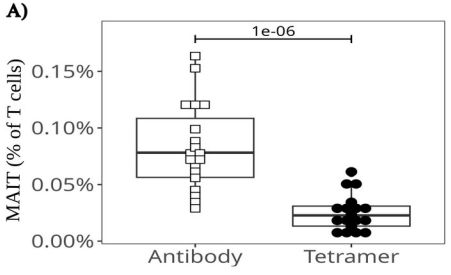
- 80% mothers of HEU-hi infants had undetectable viral loads by time of delivery
- Specimens selected were matched by sex and gestational age

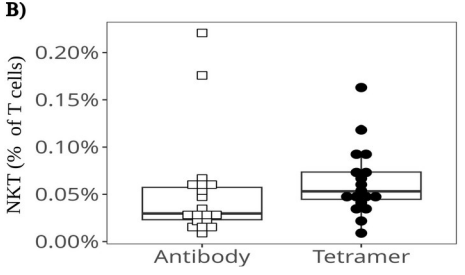
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ILT are rare in Cord Blood Mononuclear Cells









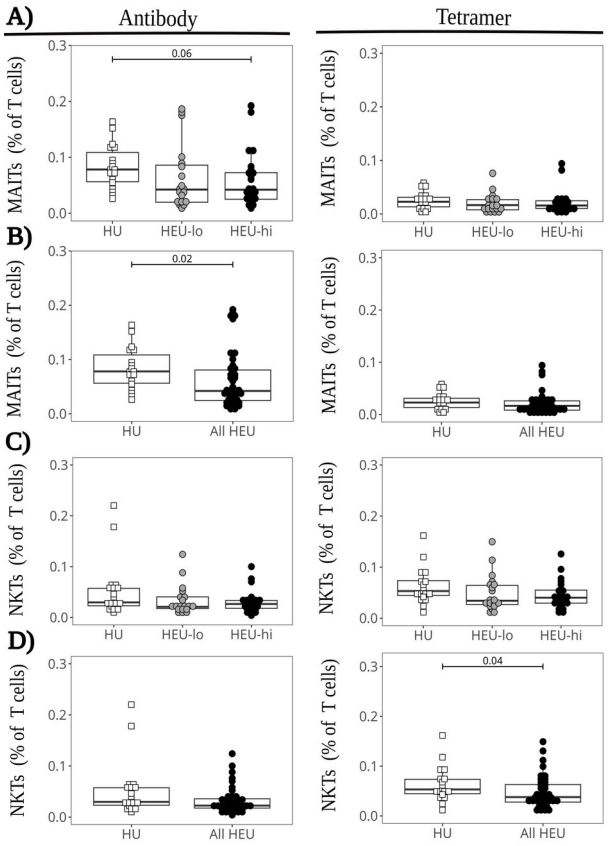
- Vδ2s most abundant of the cord blood ILT subsets (0.6% of T cells")
- Greater frequency of MAITs by antibody-staining than by tetramer-staining

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Decrease frequency of MAITs and NKTs in HEU

MAITs

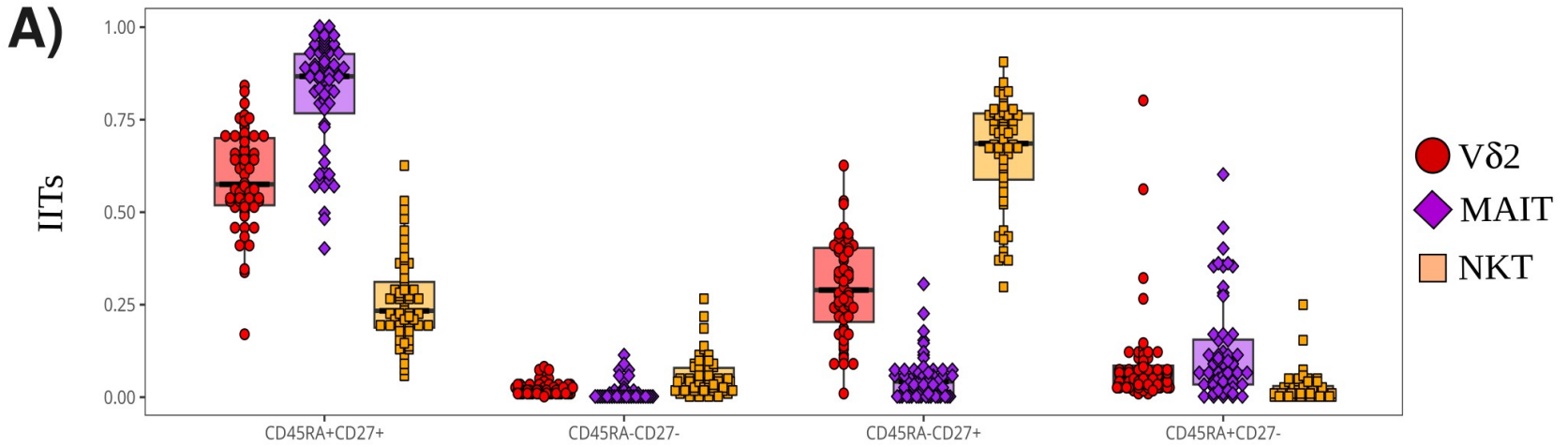
NKTs



- Lower frequency of MAITs (and to lesser extent NKTs) in HEU infants

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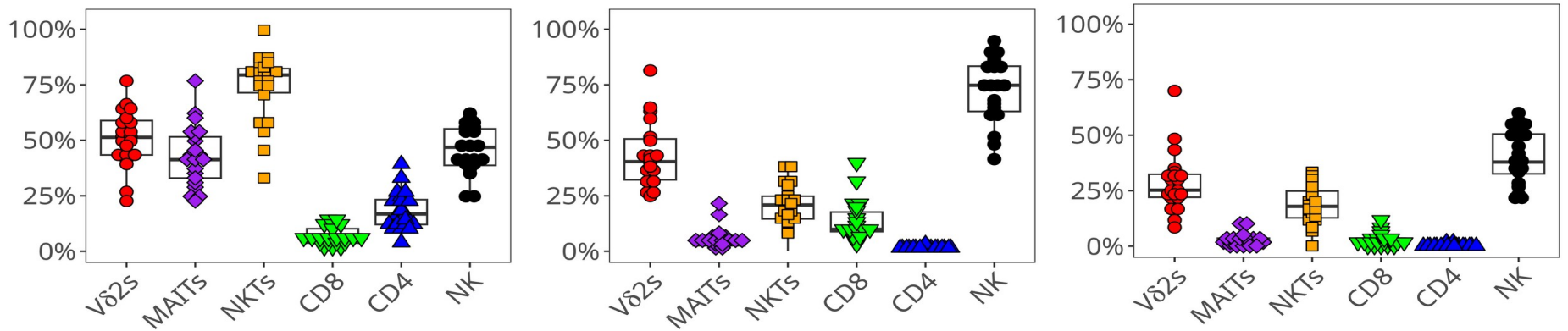
Memory differentiation by ILT subset



- NKTs showed greatest extent of memory differentiation
- MAITs were mostly naive

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Greater frequency of cytokine-producing cells in innate-like T cell subsets

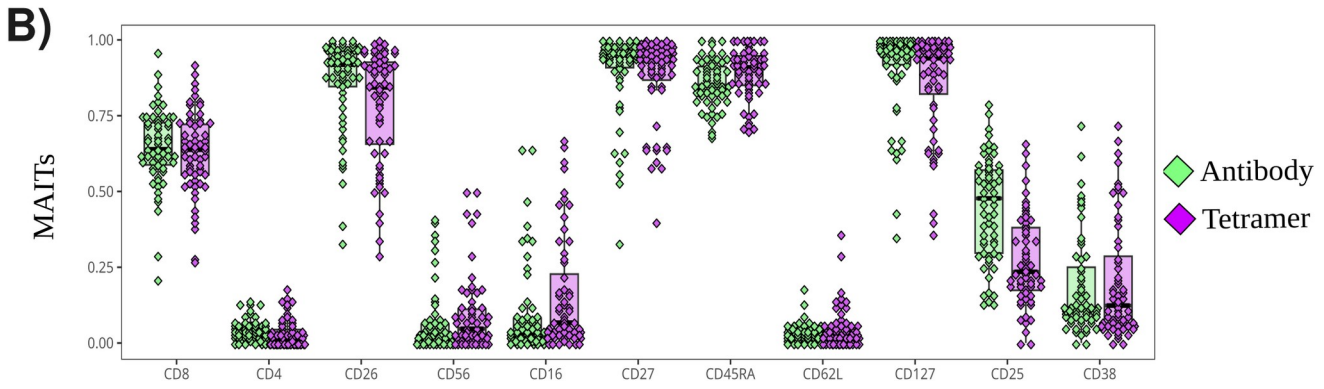


TNFα+ **IFNγ+** **TNFα+ IFNγ+**

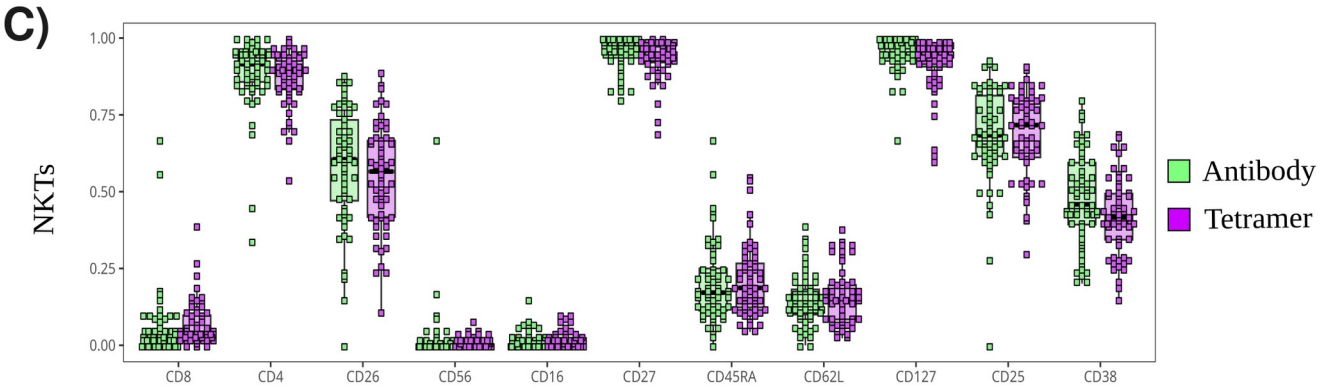
- Cord blood ILT subsets more similar to NK cells in frequency of cytokine expression compared to adaptive T cells

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Similar marker expression profiles by both antibody and tetramer-staining for MAITs and NKTs



No statistically significant difference

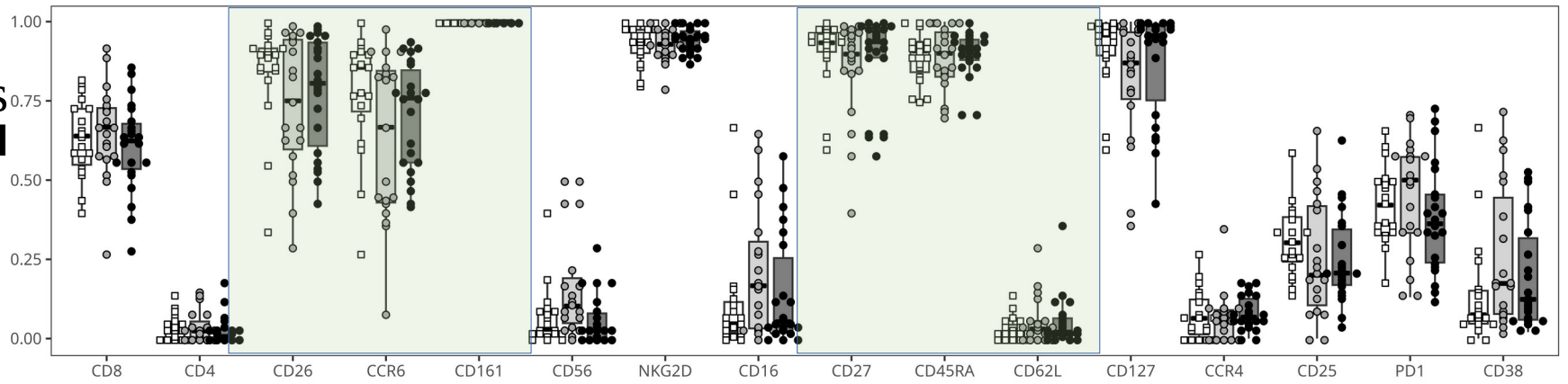


No statistically significant difference

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Cord blood MAITs - Baseline

MAITs
Control



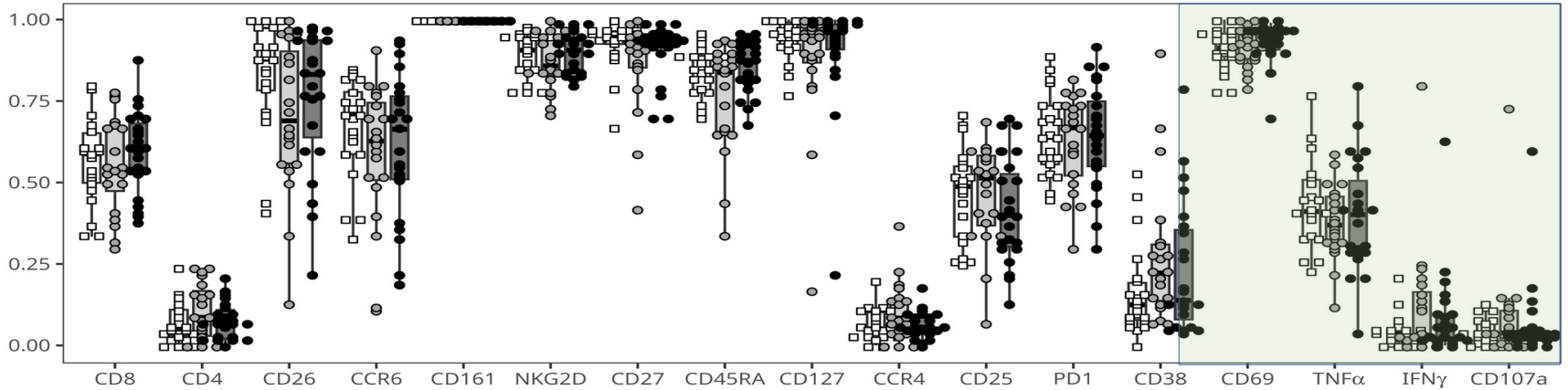
- High expression of CD26, CCR6, CD161
- Mostly CD8+ or CD4-CD8-
- Naive (CD45RA+CD27+)

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Cord blood MAITs - Activated

MAITs

PMA

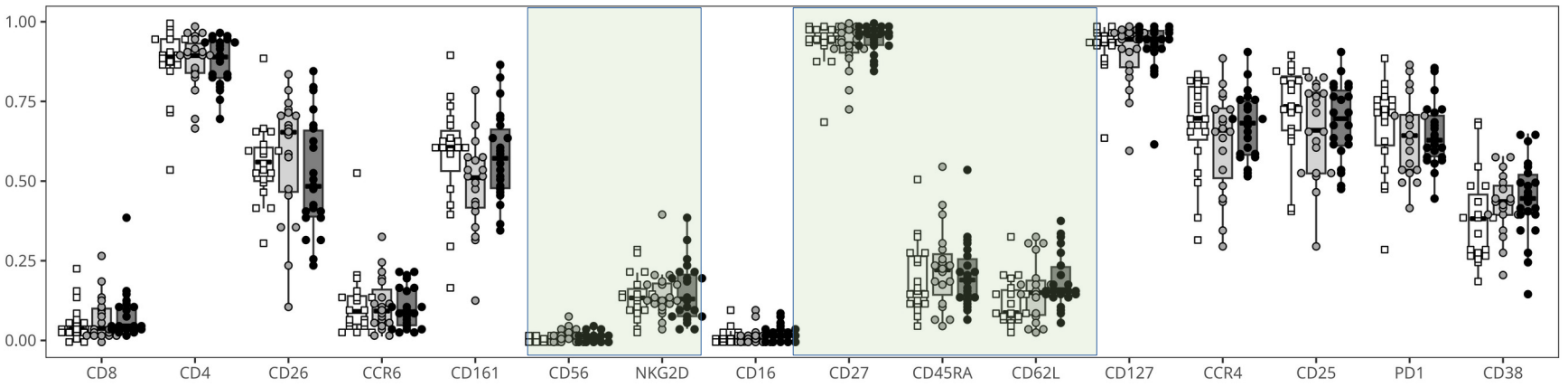


- Following activation, predominantly make TNFα
- Minimal IFNγ or CD107a

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Cord blood NKTs - Baseline

NKTs
Control

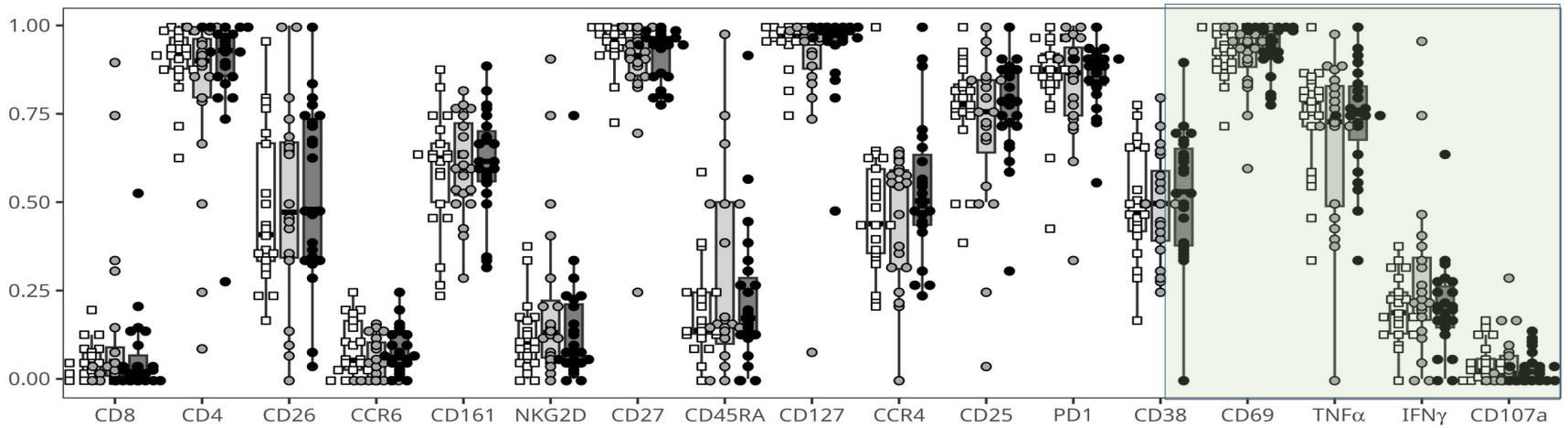


- Mostly CD56 and NKG2D negative
- Memory Differentiated (CD45RA-CD27+)
- Expression of CCR4 & CD25

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Cord blood NKTs - Activated

NKTs PMA

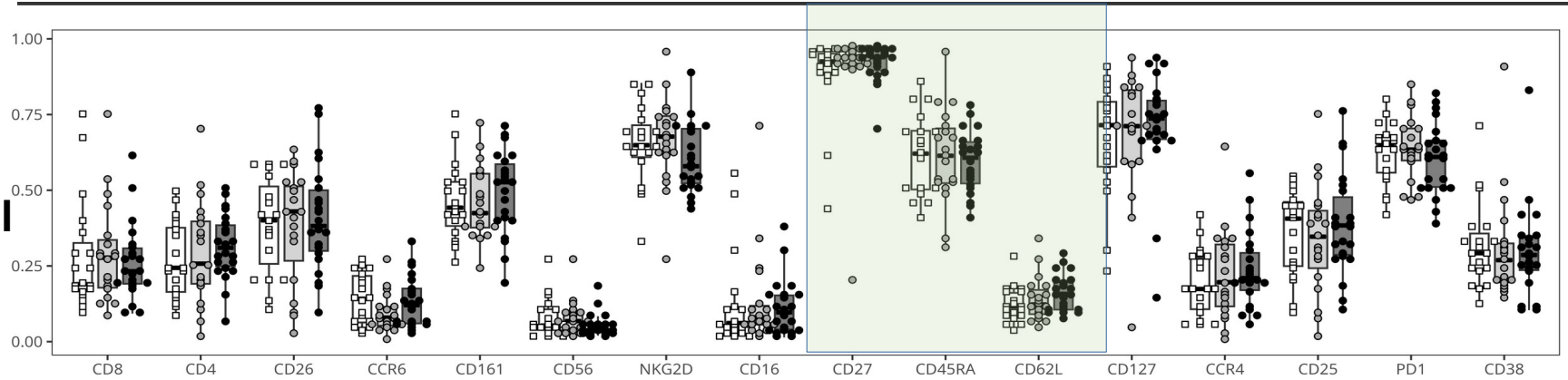


- Following activation, mostly make TNFα, and some IFN$_\gamma$
- Upregulate PD1 vs. baseline

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Cord blood Vδ2s - Baseline

Vδ2 Control

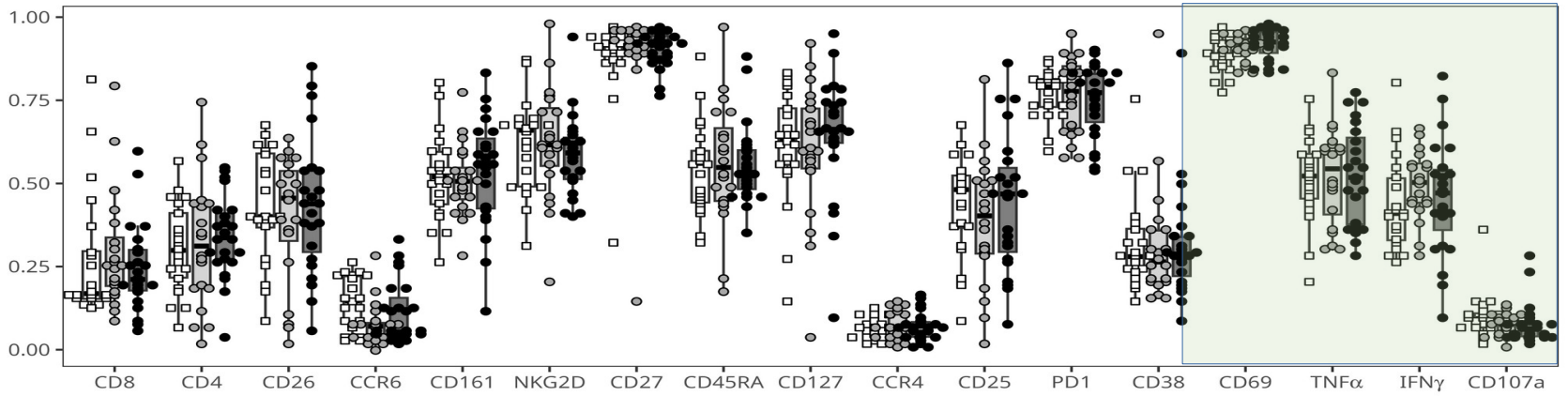


- At the entire subset level, uniformly CD27+
- Additional heterogeneity?

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Cord blood Vδ2s - Activated

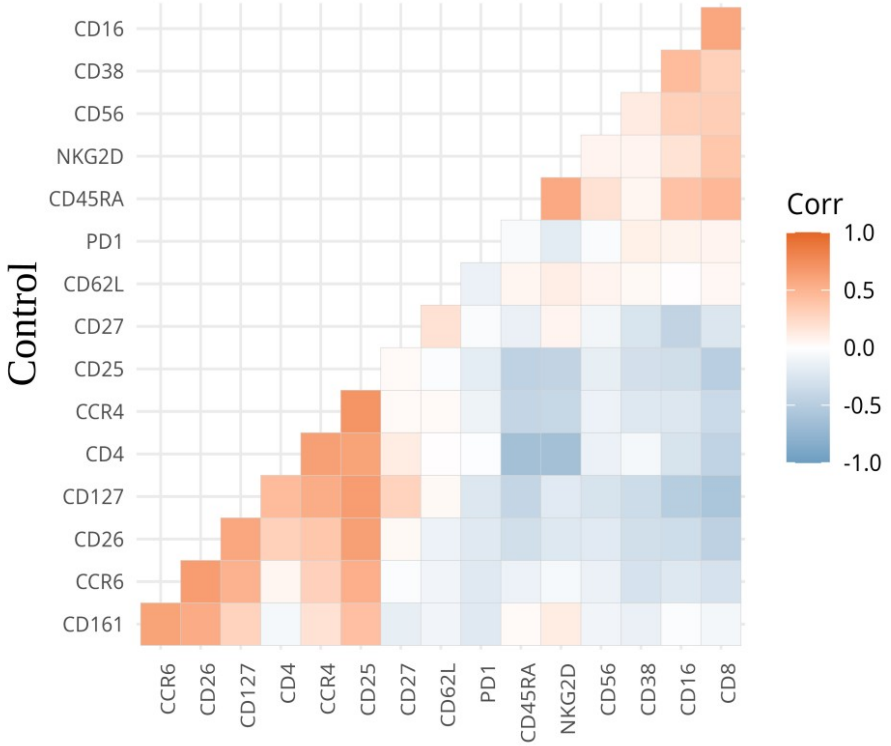
Vδ2
PMA



- Mix of TNFα and IFN$_\gamma$ after activation

Evidence for additional heterogeneity in Vδ2

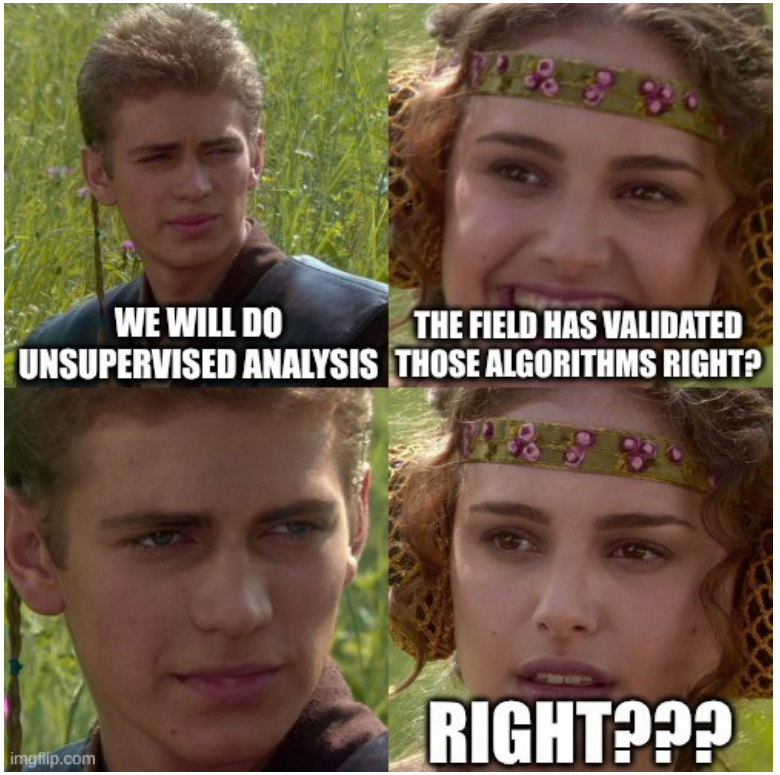
A)



Unlike what we saw for NKTs and MAITs, marker co-expression matrices suggested additional heterogeneity within the Vδ2s

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Before we could leverage the full biological discovery potential, the pink elephants in the room

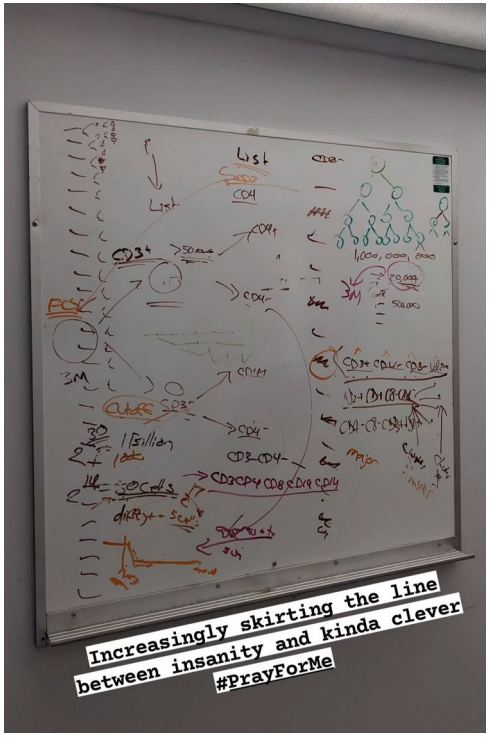


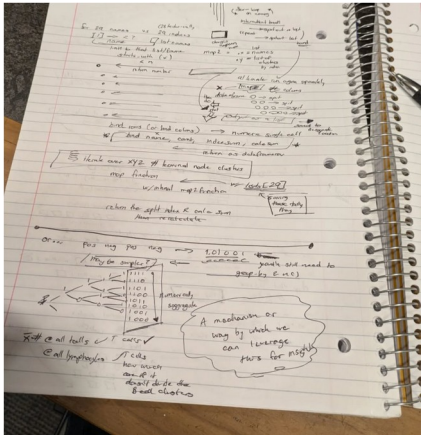
☑ Quality of the unmixed full-stained data is linked to the quality of our single-color controls and ability to provide appropriate autofluorescence control(s)

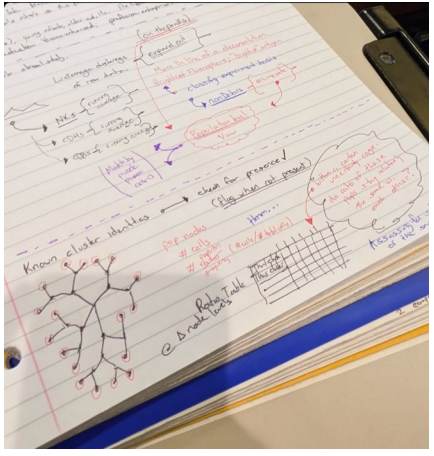
☐ In acquiring 3 million cells to detect 100 cord blood ILTs, a ton of useful data is gathered. How to analyze it all?

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Grouping for shared marker expression







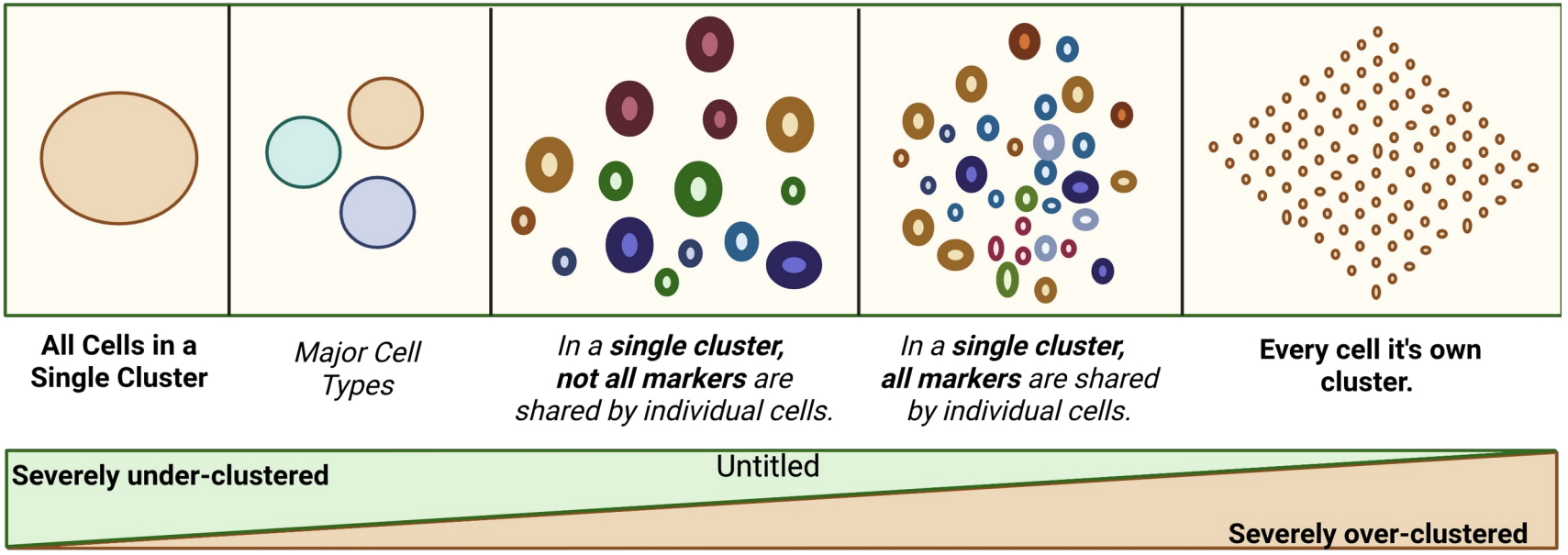
Central Issue:

- Different clustering methods tend to inappropriately group or split
- Manual gating doesn't scale
- Automated gates aren't easily adjustable
- Unclear what drives various unsupervised algorithms

→ What if we just annotated manual-gating information in before passing off to the unsupervised algorithms?

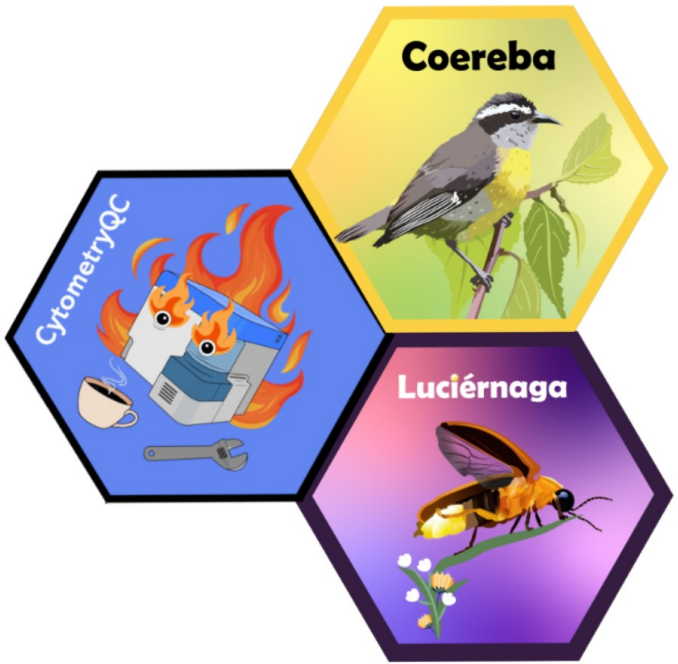
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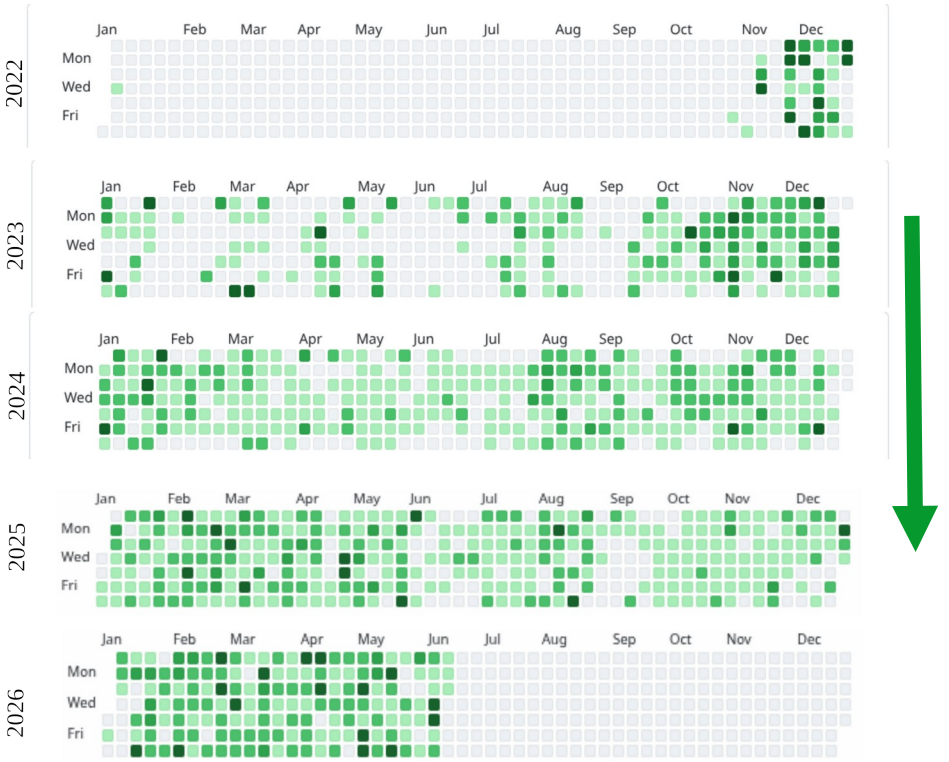
Grouping for shared marker expression



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Continued journey into R programming



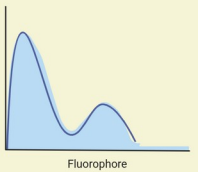


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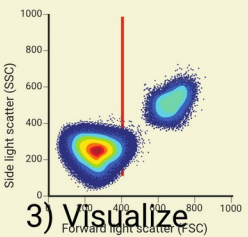
Coereba



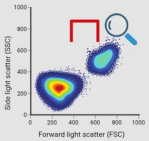
1) Load into R
(flowWorkspace)



2) Calculate Splitpoints

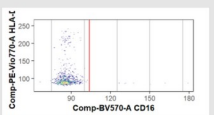


3) Visualize

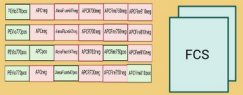


4) Gate Constraints

5) Shiny App adjustments



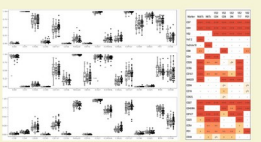
6) Splitpoints updated



7) CoerebaID

Bioconductor
OPEN SOURCE SOFTWARE FOR BIOINFORMATICS

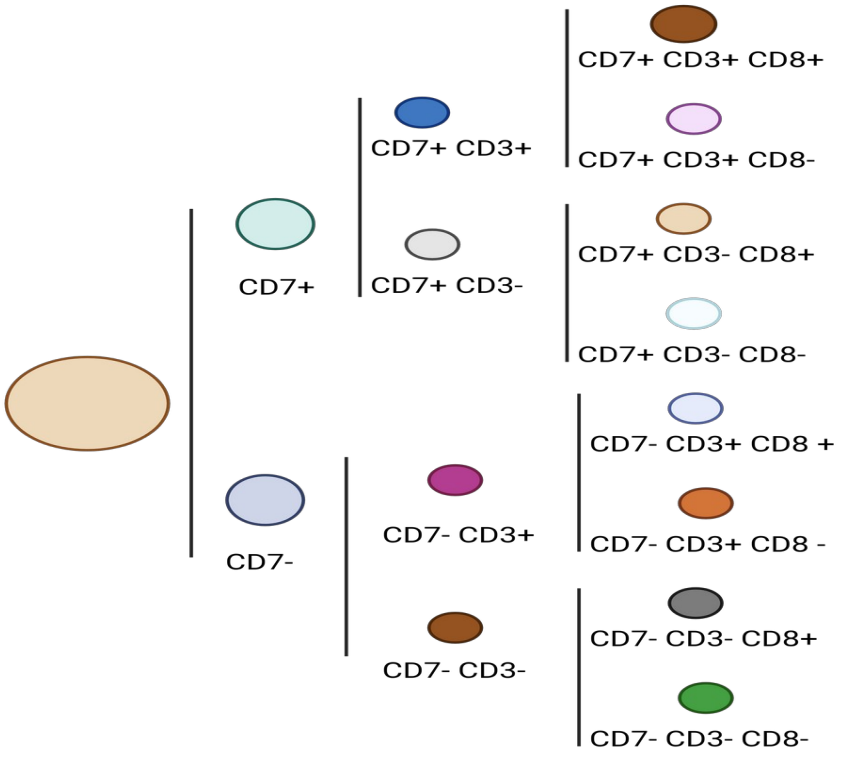
8) Summarized Experiment



9) Visualize

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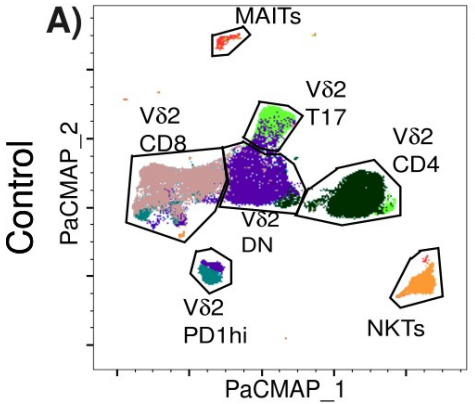
Manually-annotated Gating Information



PEVio770pos	APCneg	AlexaFluor647neg	APCR700neg	APCFire750neg	APCFire810neg
PEVio770pos	APCneg	AlexaFluor647neg	APCR700neg	APCFire750neg	APCFire810neg
PEVio770pos	APCpos	AlexaFluor647neg	APCR700neg	APCFire750pos	APCFire810neg
PEVio770pos	APCneg	AlexaFluor647pos	APCR700neg	APCFire750neg	APCFire810pos

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Retrieving and re-enumerating

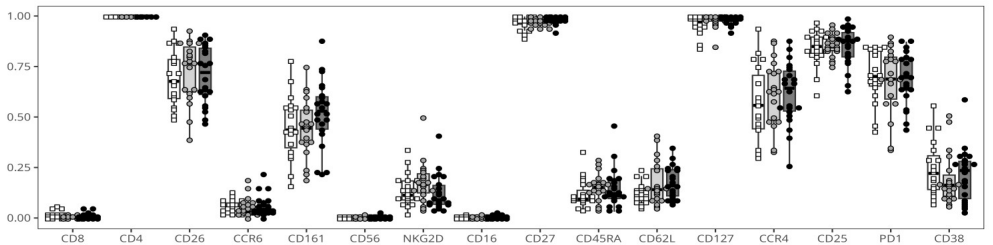


1) Gate

2) Retrieve manual-annotated gates

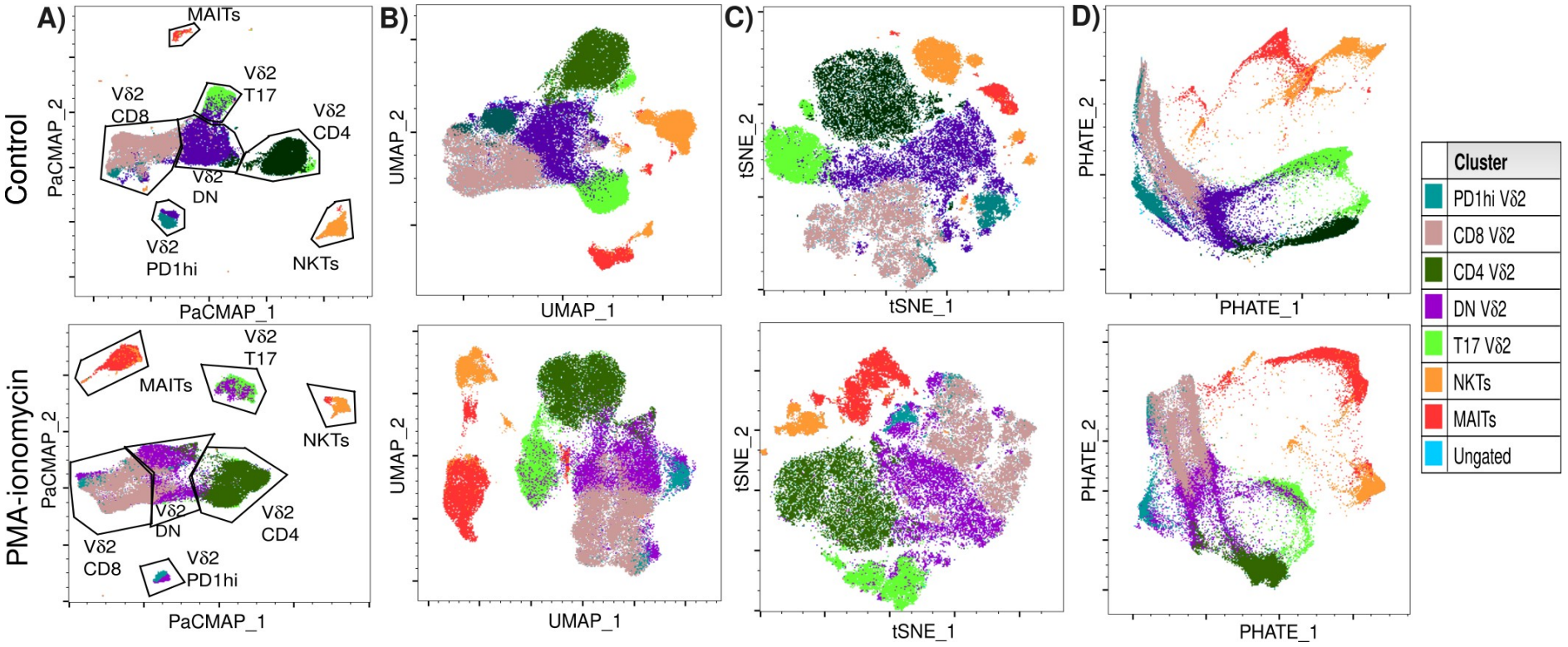
PEVio770pos	APCneg	AlexaFluor647neg	APCR700neg	APCFire750neg	APCFire810neg
PEVio770pos	APCneg	AlexaFluor647neg	APCR700neg	APCFire750neg	APCFire810neg
PEVio770pos	APCpos	AlexaFluor647neg	APCR700neg	APCFire750pos	APCFire810neg
PEVio770pos	APCneg	AlexaFluor647pos	APCR700neg	APCFire750neg	APCFire810pos

3) Re-enumerate and plot



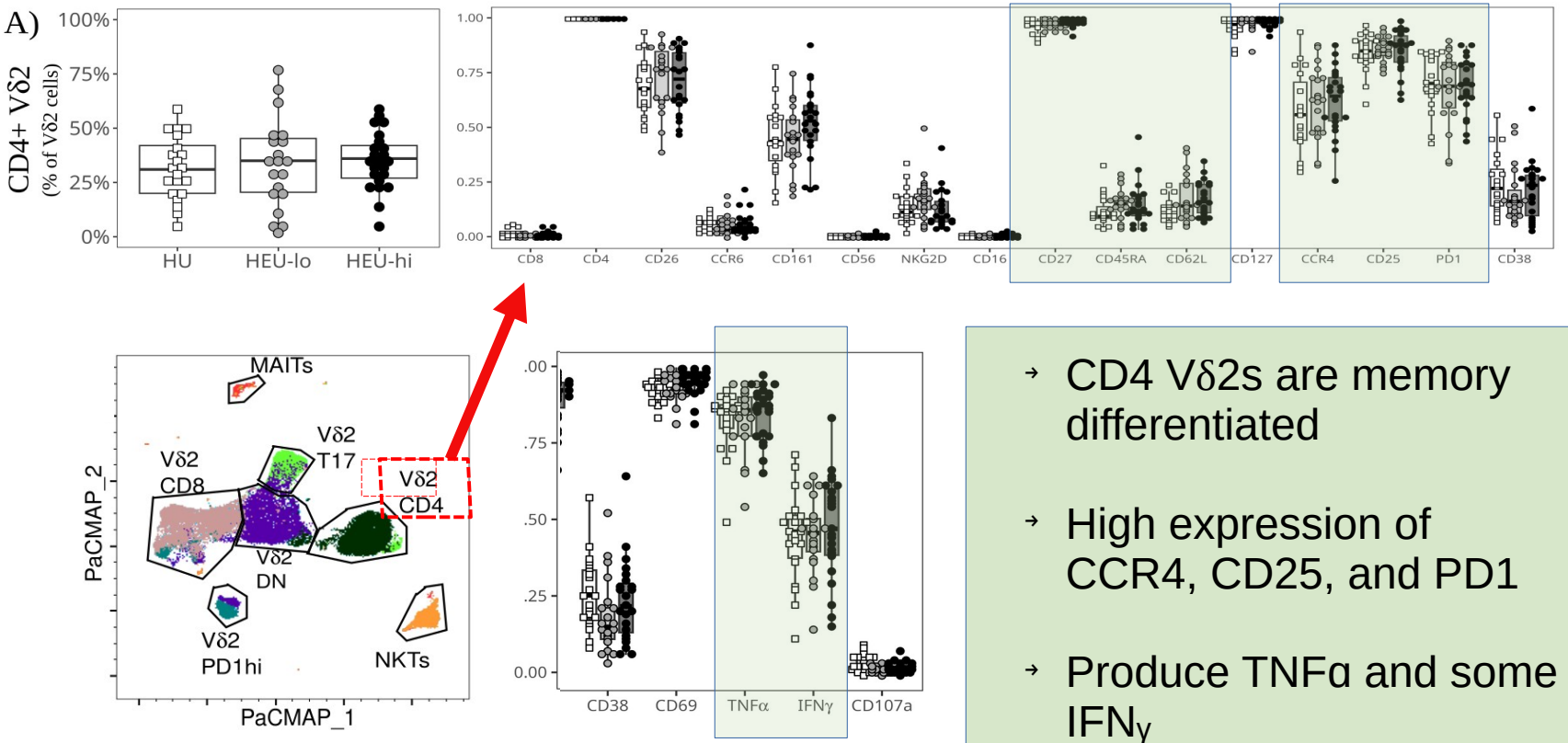
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Dimensionality Visualization



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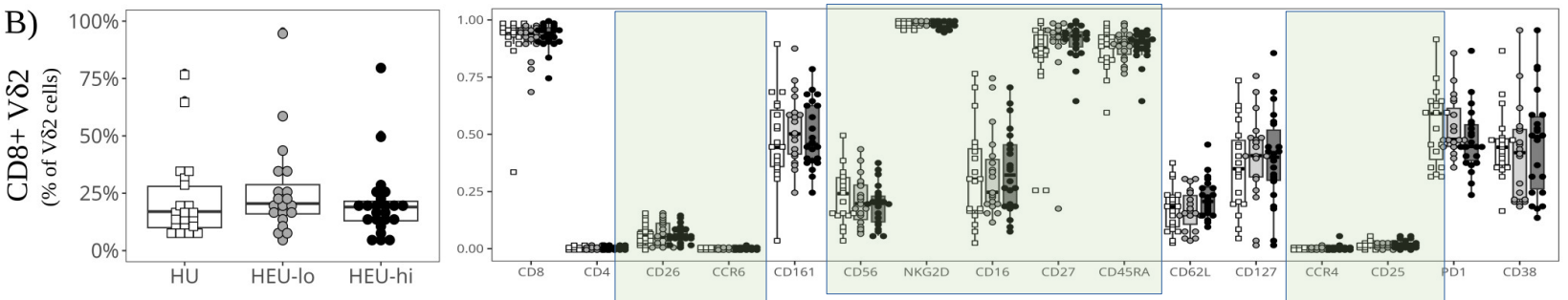
CD4+ Vδ2s

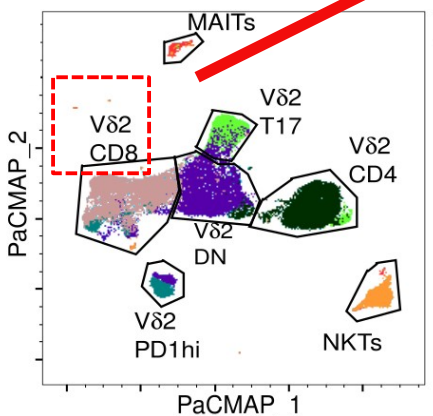


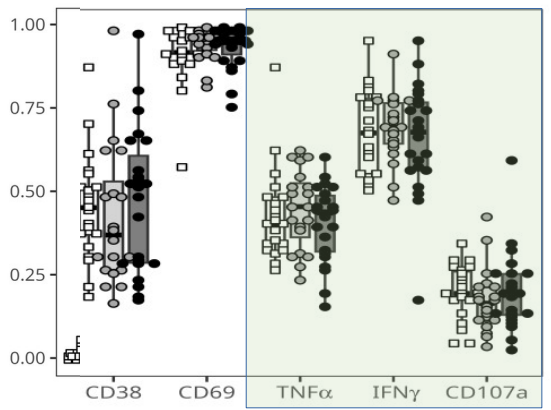
- CD4 Vδ2s are memory differentiated
- High expression of CCR4, CD25, and PD1
- Produce TNFα and some IFNγ

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CD8 Vδ2s



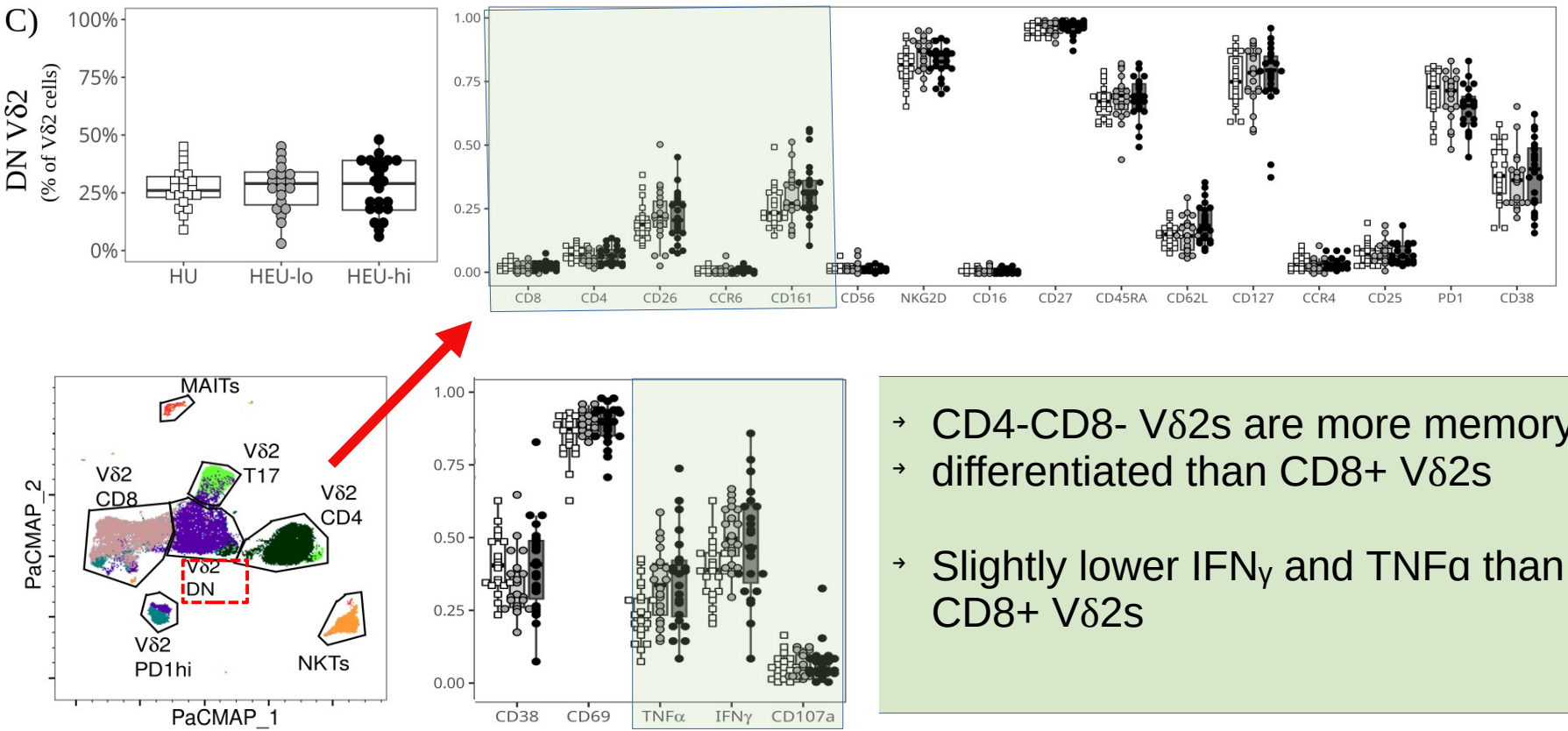




- CD8 Vδ2s are naive
-
- NKG2D+, but CD26-
- Produce IFNγ and some TNFα

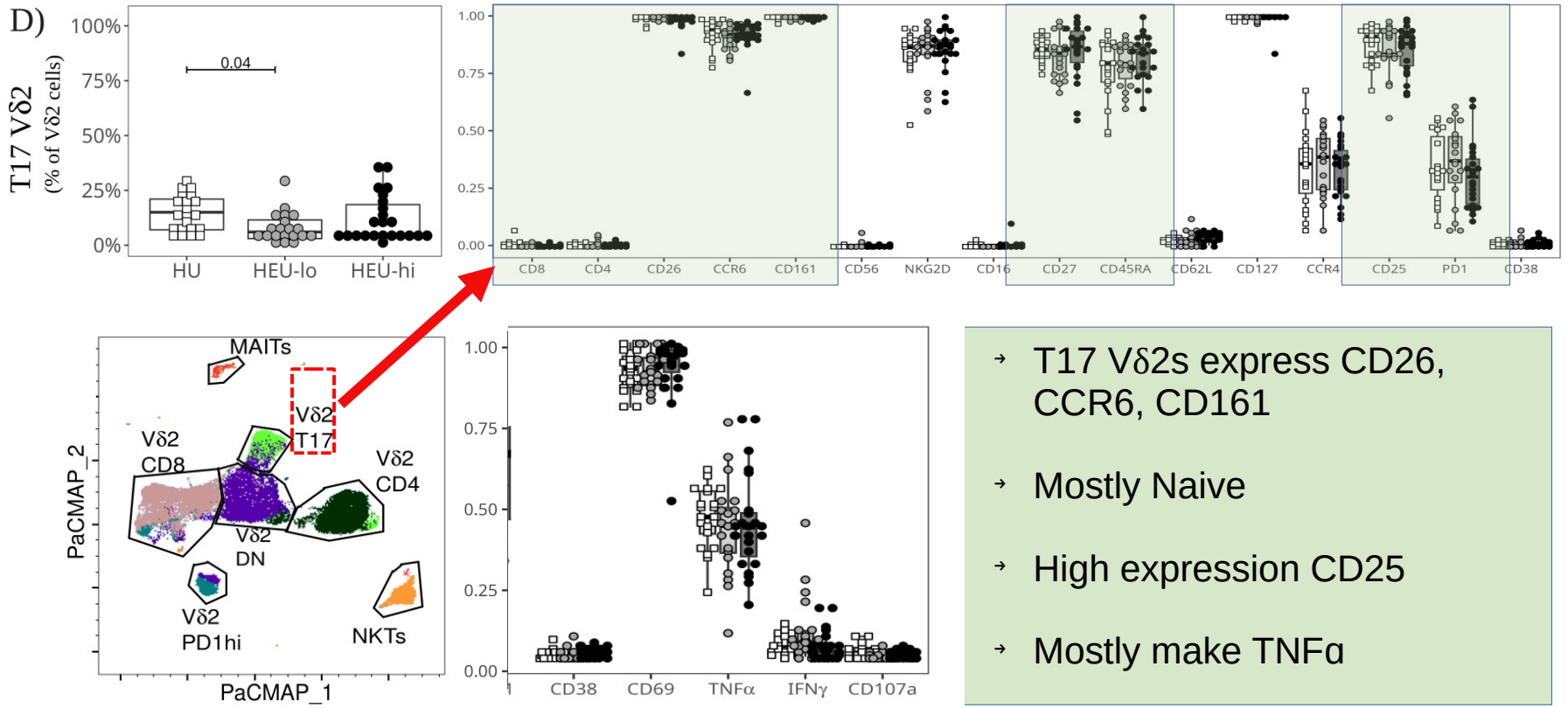
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DN Vδ2s



- CD4-CD8- Vδ2s are more memory differentiated than CD8+ Vδ2s
- Slightly lower IFNγ and TNFα than CD8+ Vδ2s

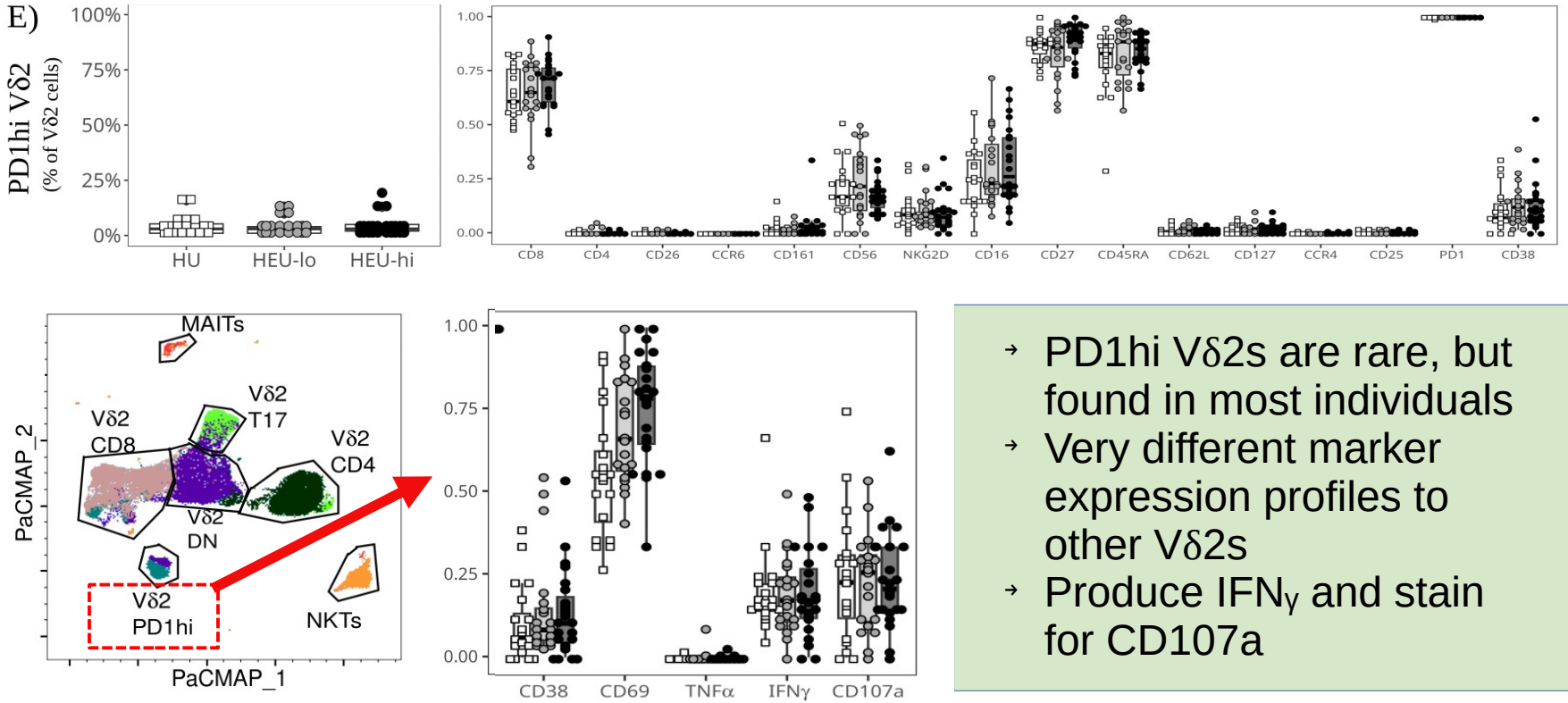
T17 Vδ2s



- T17 Vδ2s express CD26, CCR6, CD161
- Mostly Naive
- High expression CD25
- Mostly make TNFα

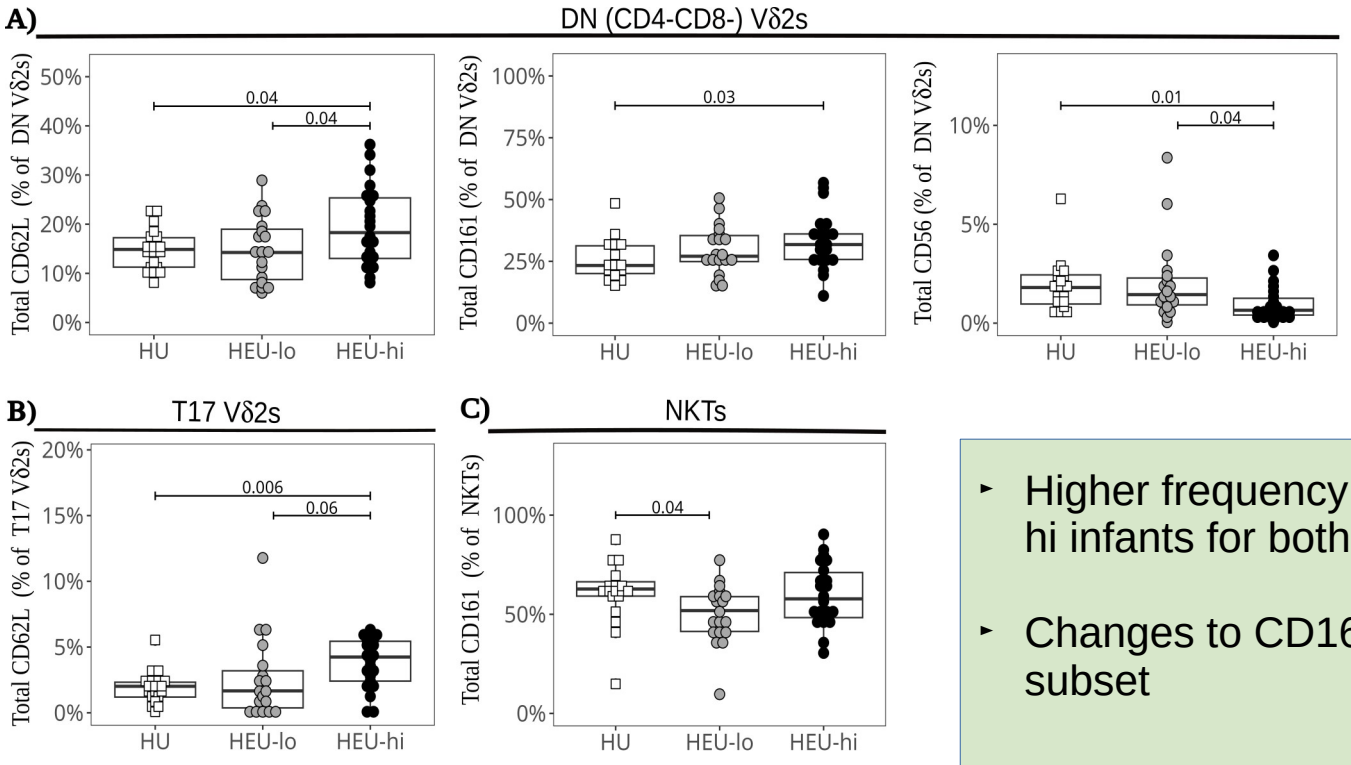
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PD1 high Vδ2s



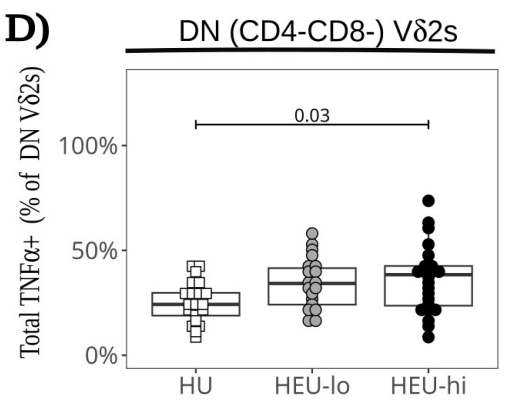
- PD1hi Vδ2s are rare, but found in most individuals
- Very different marker expression profiles to other Vδ2s
- Produce IFNγ and stain for CD107a

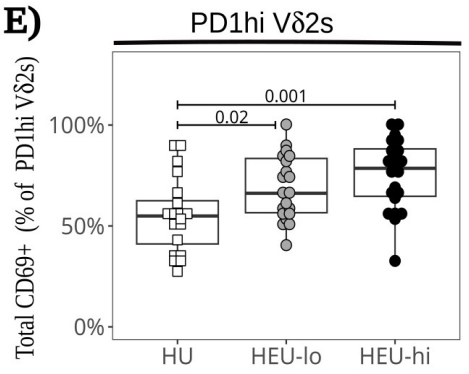
Differential Results - Control

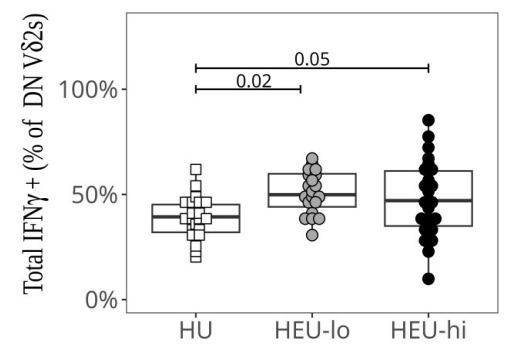


- Higher frequency of CD62L in HEU-hi infants for both DN and T17 Vδ2s
- Changes to CD161 and CD56 by subset

Differential Results - Activated







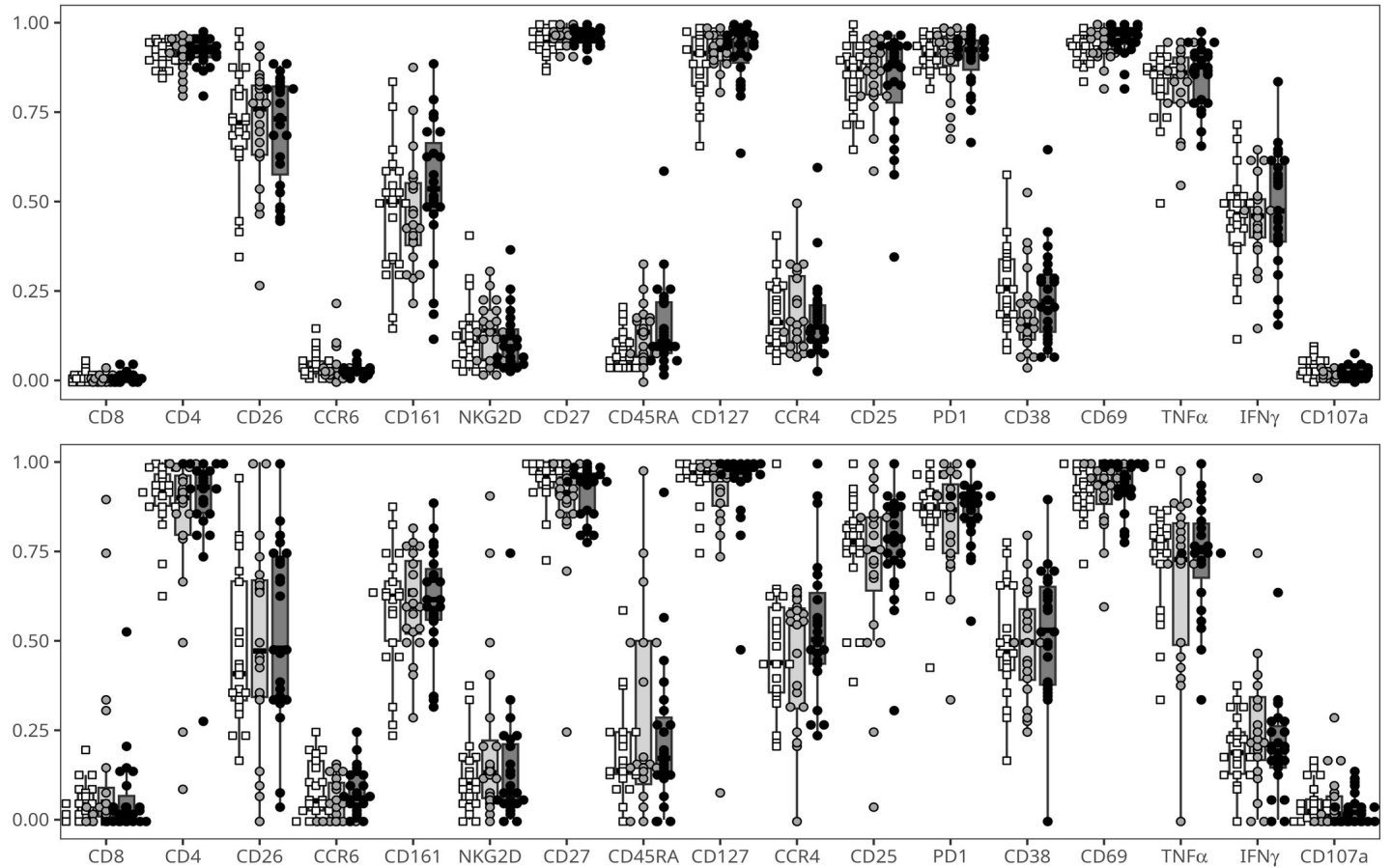
- Higher frequency of TNFα, IFN$_\gamma$ in HEU-hi infants compared to the HU infants for the DN Vδ2s
- Similar pattern for CD69 in PD1hi Vδ2s

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Similar Phenotype and Function

CD4 Vδ2s

NKTs

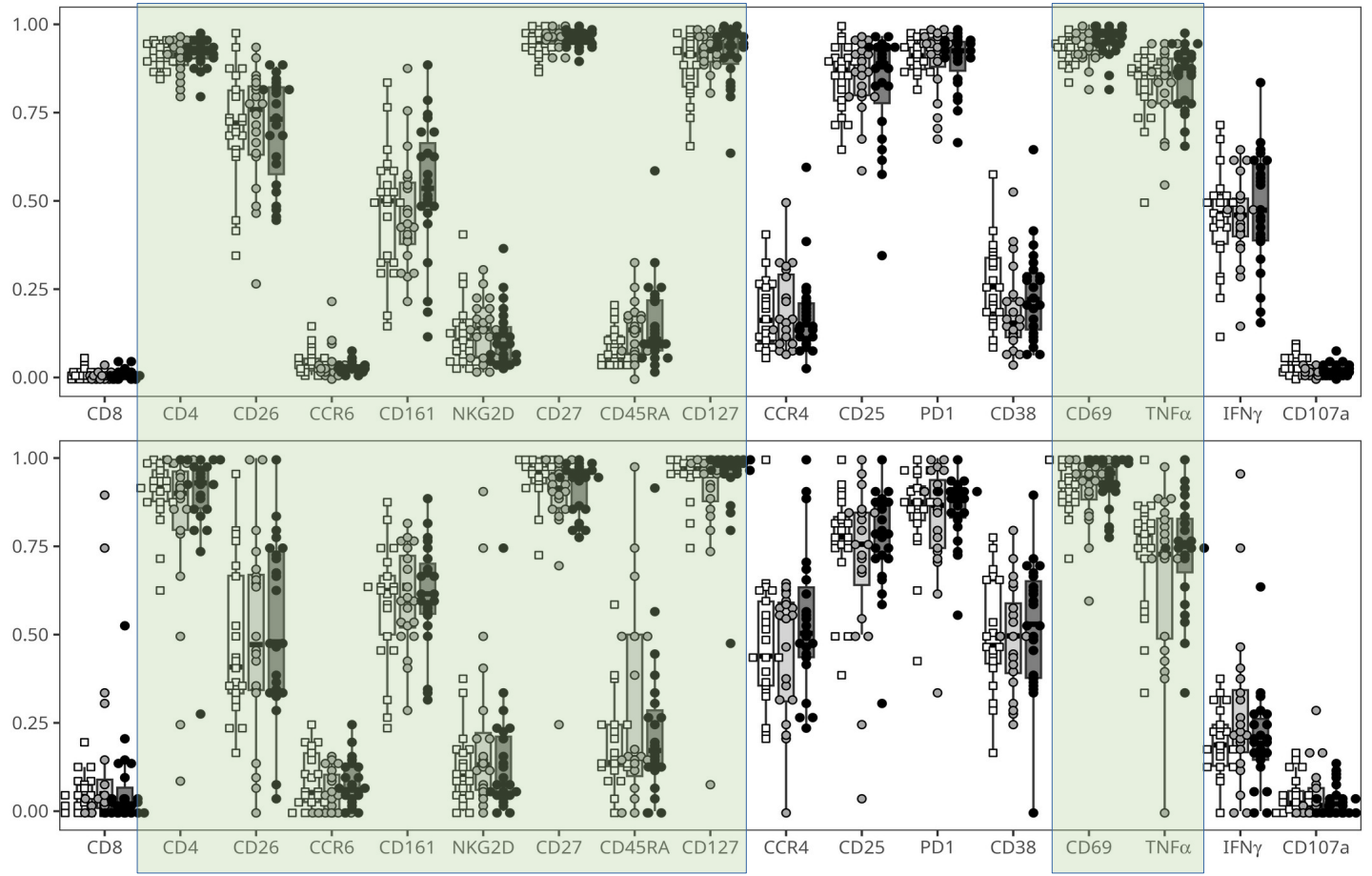


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Similar Phenotype and Function

CD4 Vδ2s

NKTs

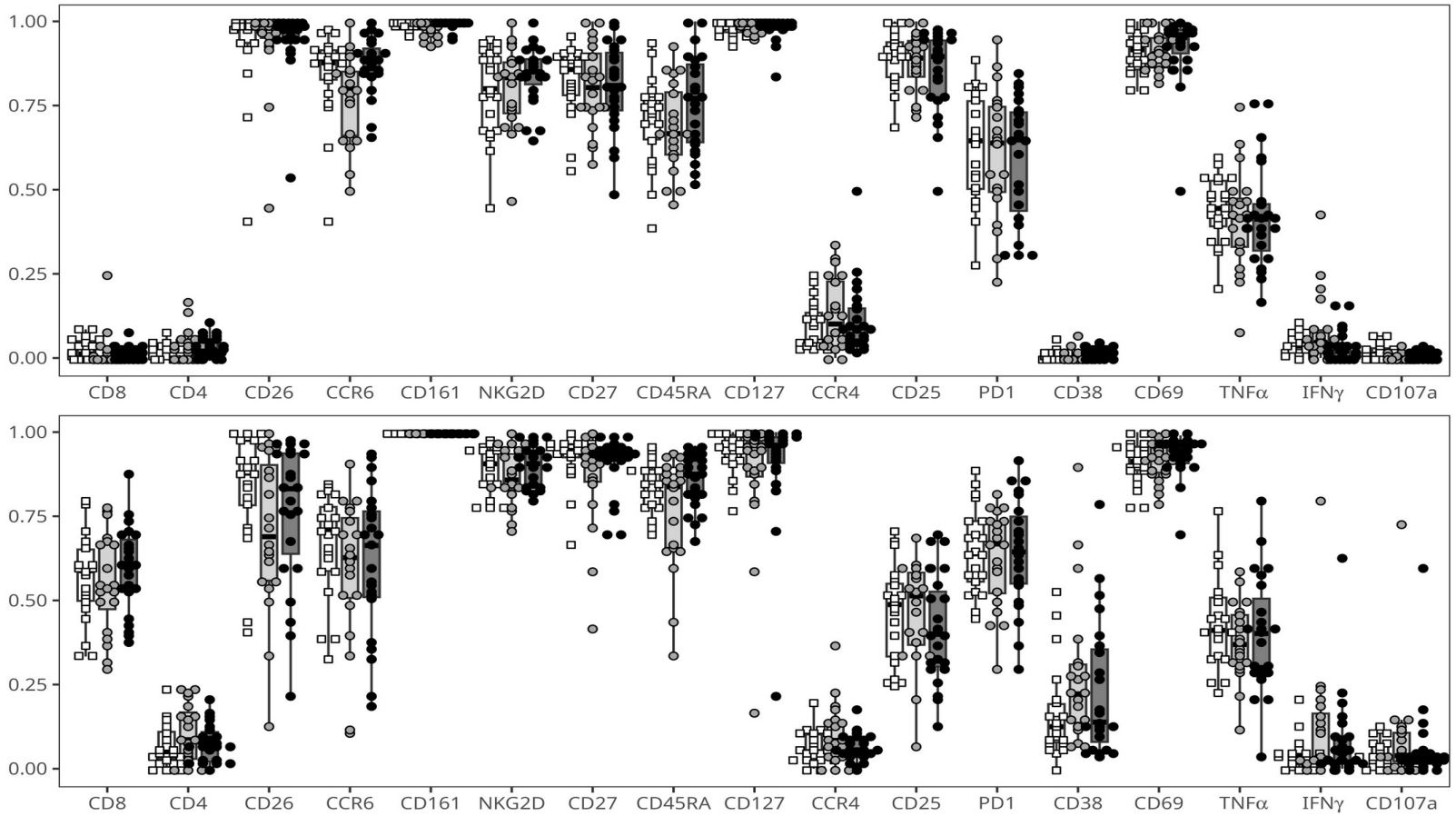


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Similar Phenotype and Function

T17 Vδ2s

MAITs

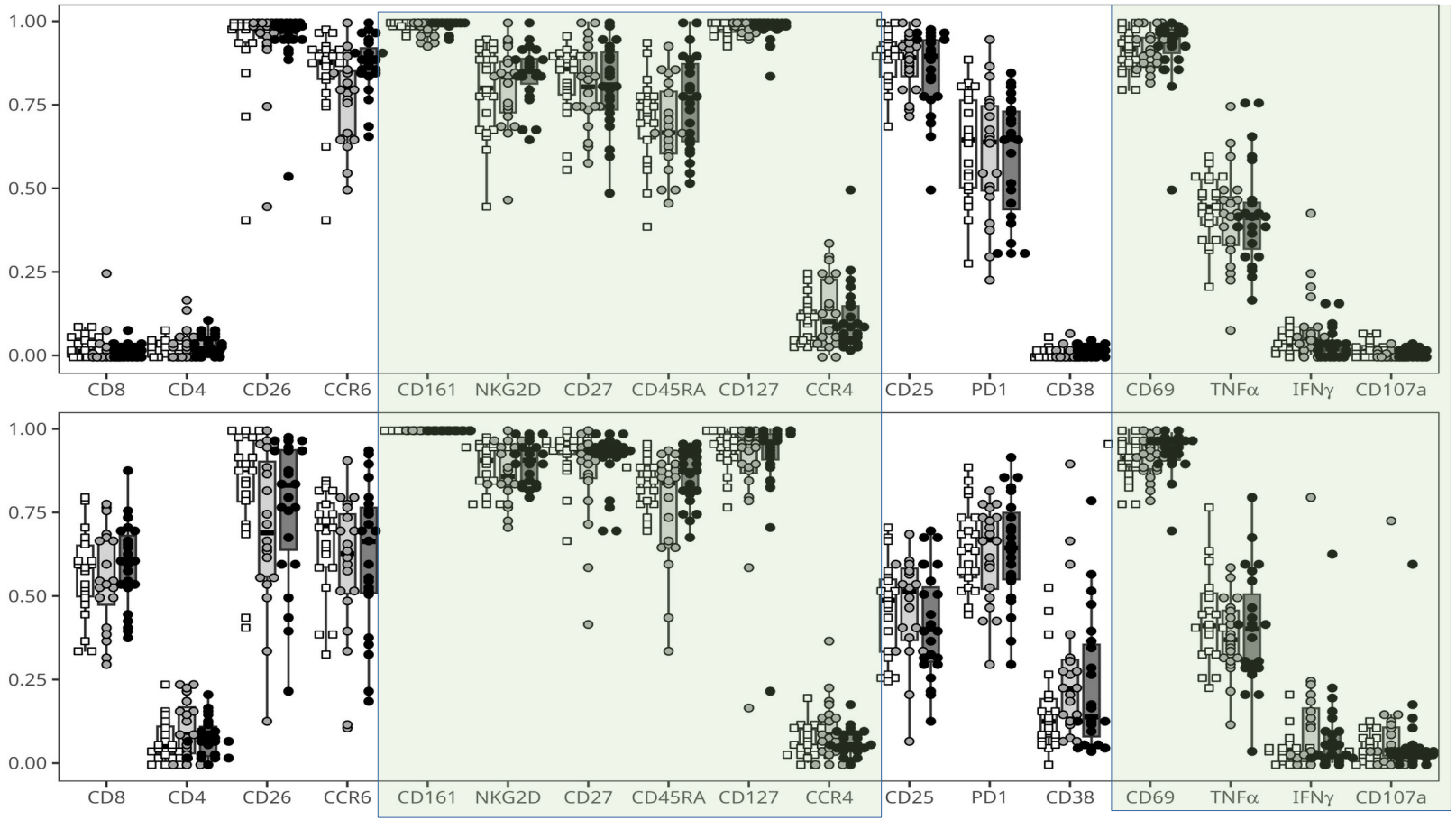


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Similar Phenotype and Function

T17 Vδ2s

MAITs



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Cord Blood ILTs - Marker Expression

Control

Marker	MAITs	NKTs	Vδ2 CD4	Vδ2 CD8	Vδ2 DN	Vδ2 T17	Vδ2 PD1
CD7	+++	+++	+++	+++	+++	+++	+++
CD3	+++	+++	+++	+++	+++	+++	+++
Vδ2	-	-	+++	+++	+++	+++	+++
Vα7.2	+++	-	-	-	-	-	-
Vα24Jα18	-	+++	-	-	-	-	-
CD8	++	-	-	+++	-	-	++
CD4	-	+++	+++	-	-	-	-
CD26	+++	++	++	-	-/+	+++	-
CCR6	+++	-	-	-	-	+++	-
CD161	+++	++	++	++	+	+++	-
NKG2D	+++	-	-	+++	+++	+++	-
CD56	-	-	-	-/+	-	-	-/+
CD16	-	-	-	+	-	-	-/+
CD62L	-	-	-	-/+	-	-	-
CD27	+++	+++	+++	+++	+++	+++	+++
CD45RA	+++	-	-	+++	++	+++	+++
CD127	+++	+++	+++	+	+++	+++	-
CD25	+	+++	+++	-	-	+++	-
CCR4	-	++	++	-	-	+	-
PD1	+	++	++	++	++	+	+++
CD38	-	+	-/+	+	+	-	-

Median Frequency: - 0-10% | -/+ 10-30% | + 30-50% | ++ 50-80% | +++ 80-100%

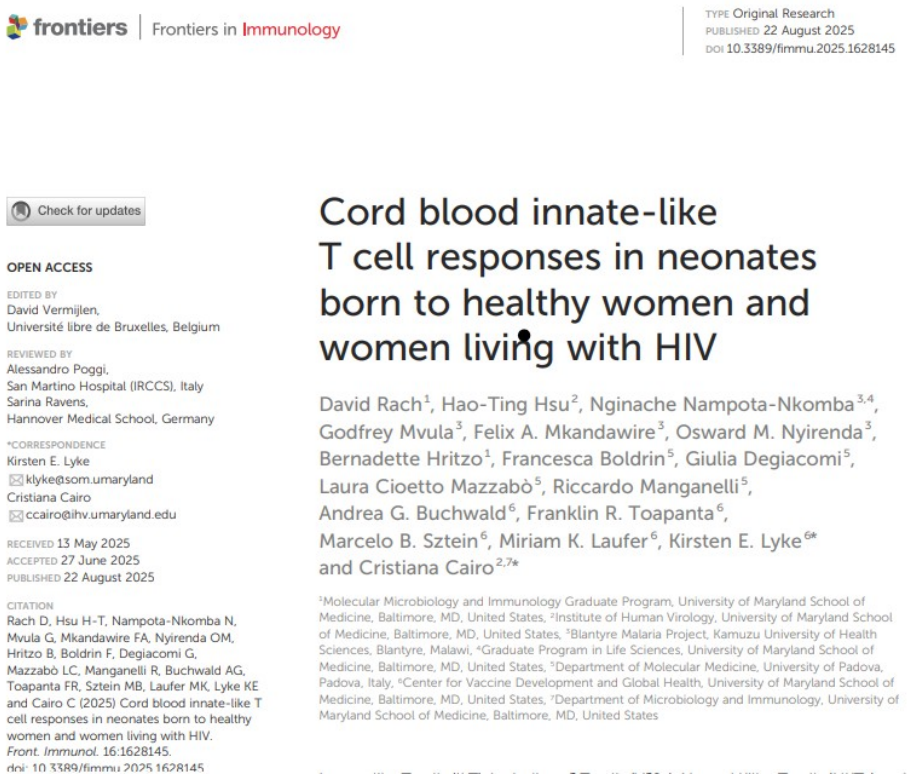
PMA-Ionomycin

Marker	MAITs	NKTs	Vδ2 CD4	Vδ2 CD8	Vδ2 DN	Vδ2 T17	Vδ2 PD1
CD7	+++	+++	+++	+++	+++	+++	+++
CD3	+++	+++	+++	+++	+++	+++	+++
Vδ2	-	-	+++	+++	+++	+++	+++
Vα7.2	+++	-	-	-	-	-	-
Vα24Jα18	-	+++	-	-	-	-	-
CD8	++	-	-	+++	-	-	++
CD4	-	+++	+++	-	-	-	-
CD26	+++	++	++	-	-/+	+++	-
CCR6	++	-	-	-	-	+++	-
CD161	+++	++	++	++	+	+++	-
NKG2D	+++	-	-	+++	+++	+++	-
CD56	-	-	-	-	-	-	-
CD16	-	-	-	-	-	-	-
CD62L	-	-	-	-	-	-	-
CD27	+++	+++	+++	+++	+++	+++	+++
CD45RA	+++	-	-	+++	++	++	+++
CD127	+++	+++	+++	-/+	++	+++	-
CD25	+	+++	+++	-	-	+++	-
CCR4	-	++	-/+	-	-	-	-
PD1	++	+++	+++	++	+++	++	+++
CD38	-	++	-/+	+	+	-	-
CD69	+++	+++	+++	+++	+++	+++	++
TNFα	+	+++	+++	+	+	+	-
IFNγ	-	-/+	++	++	+	-	-/+
CD107a	-	-	-	-/+	-	-	-/+

Median Frequency: - 0-10% | -/+ 10-30% | + 30-50% | ++ 50- 80% | +++ 80-100%

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And additional things covered in our paper



frontiers | Frontiers in Immunology

TYPE Original Research
PUBLISHED 22 August 2025
DOI 10.3389/fimmu.2025.1628145

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RECEIVED 13 May 2025
ACCEPTED 27 June 2025
PUBLISHED 22 August 2025

CITATION
Rach D, Hsu H-T, Nampota-Nkomba N, Mvula G, Mkandawire FA, Nyirenda OM, Hritzo B, Boldrin F, Degiacomi G, Mazzabò LC, Manganelli R, Buchwald AG, Toapanta FR, Sztein MB, Laufer MK, Lyke KE and Cairo C (2025) Cord blood innate-like T cell responses in neonates born to healthy women and women living with HIV. *Front. Immunol.* 16:1628145. doi: 10.3389/fimmu.2025.1628145

Cord blood innate-like T cell responses in neonates born to healthy women and women living with HIV

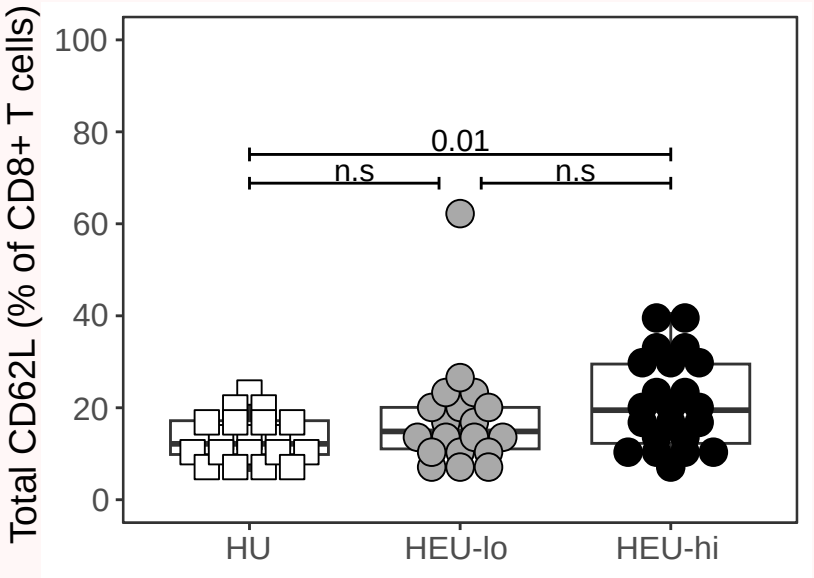
David Rach[1], Hao-Ting Hsu[2], Nginache Nampota-Nkomba[3,4], Godfrey Mvula[3], Felix A. Mkandawire[3], Osward M. Nyirenda[3], Bernadette Hritzo[1], Francesca Boldrin[5], Giulia Degiacomi[5], Laura Cioetto Mazzabò[5], Riccardo Manganelli[5], Andrea G. Buchwald[6], Franklin R. Toapanta[6], Marcelo B. Sztein[6], Miriam K. Laufer[6], Kirsten E. Lyke[6*] and Cristiana Cairo[2,7*]

[1]Molecular Microbiology and Immunology Graduate Program, University of Maryland School of Medicine, Baltimore, MD, United States, [2]Institute of Human Virology, University of Maryland School of Medicine, Baltimore, MD, United States, [3]Blantyre Malaria Project, Kamuzu University of Health Sciences, Blantyre, Malawi, [4]Graduate Program in Life Sciences, University of Maryland School of Medicine, Baltimore, MD, United States, [5]Department of Molecular Medicine, University of Padova, Padova, Italy, [6]Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States, [7]Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, United States

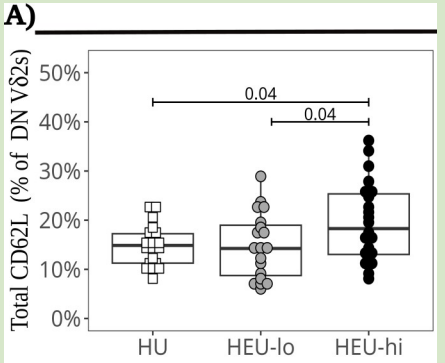
- Reduced Total IFN$_\gamma$ in HEU-hi infants at Day 17 in culture with BCG & IL-2
- Sex-specific differences in marker expressions

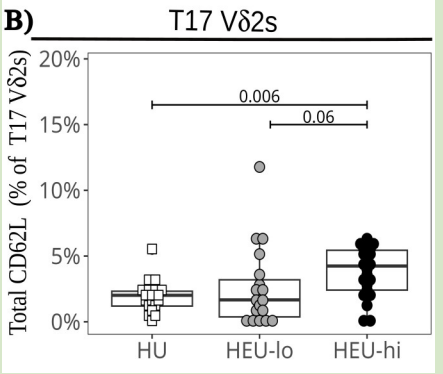
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Intriguingly: Similar for Adaptive T cells



CD8 T cells



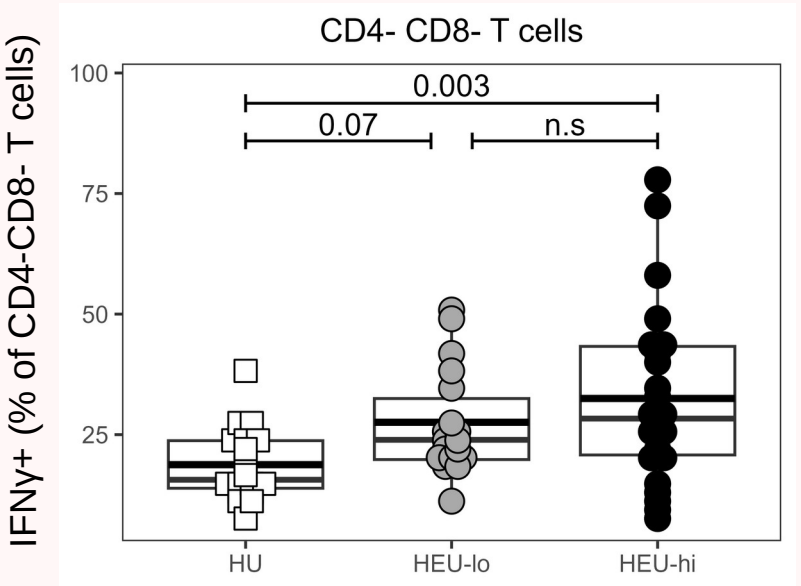


CD4-CD8- Vδ2 T cells

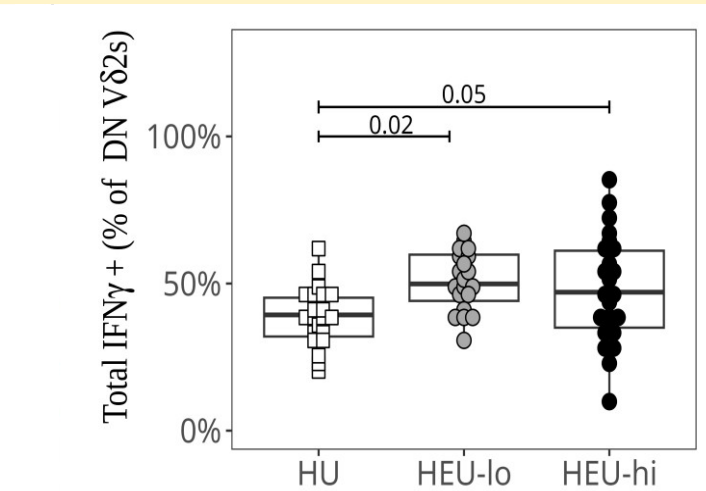
T17 Vδ2 T cells

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Intriguingly: Similar for Adaptive T cells

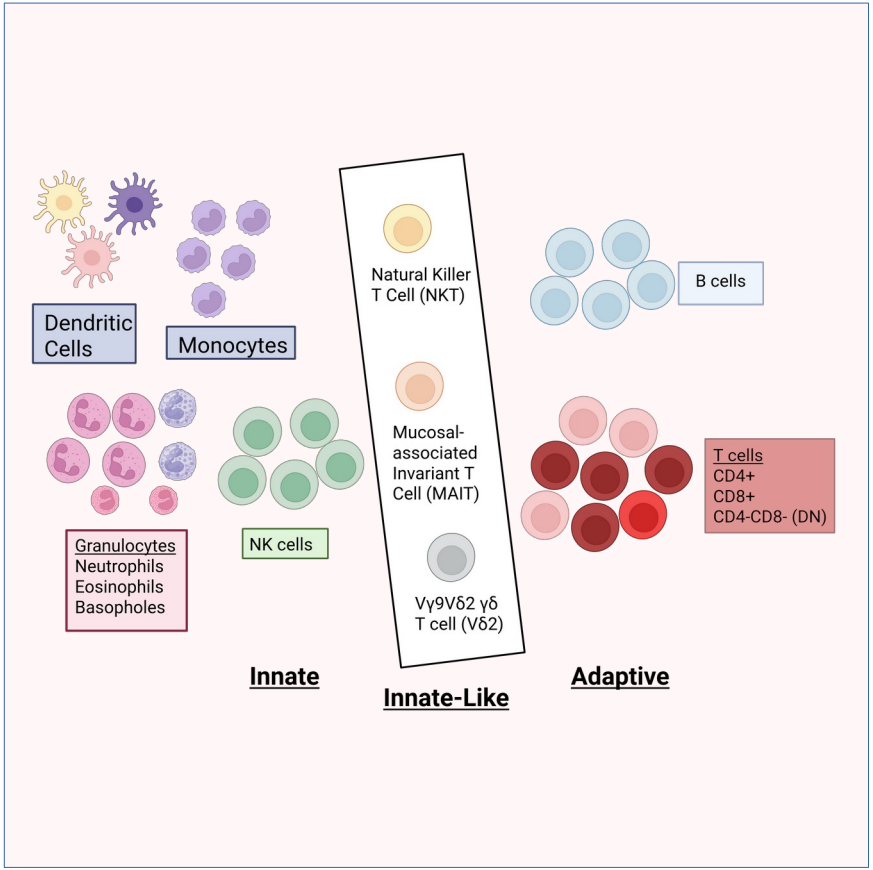


CD4-CD8- T cells



CD4-CD8- Vδ2 T cells

Future Directions



Done (or in analysis)

- Myeloid
- NKs
- ILTs
- Alpha Beta T cells

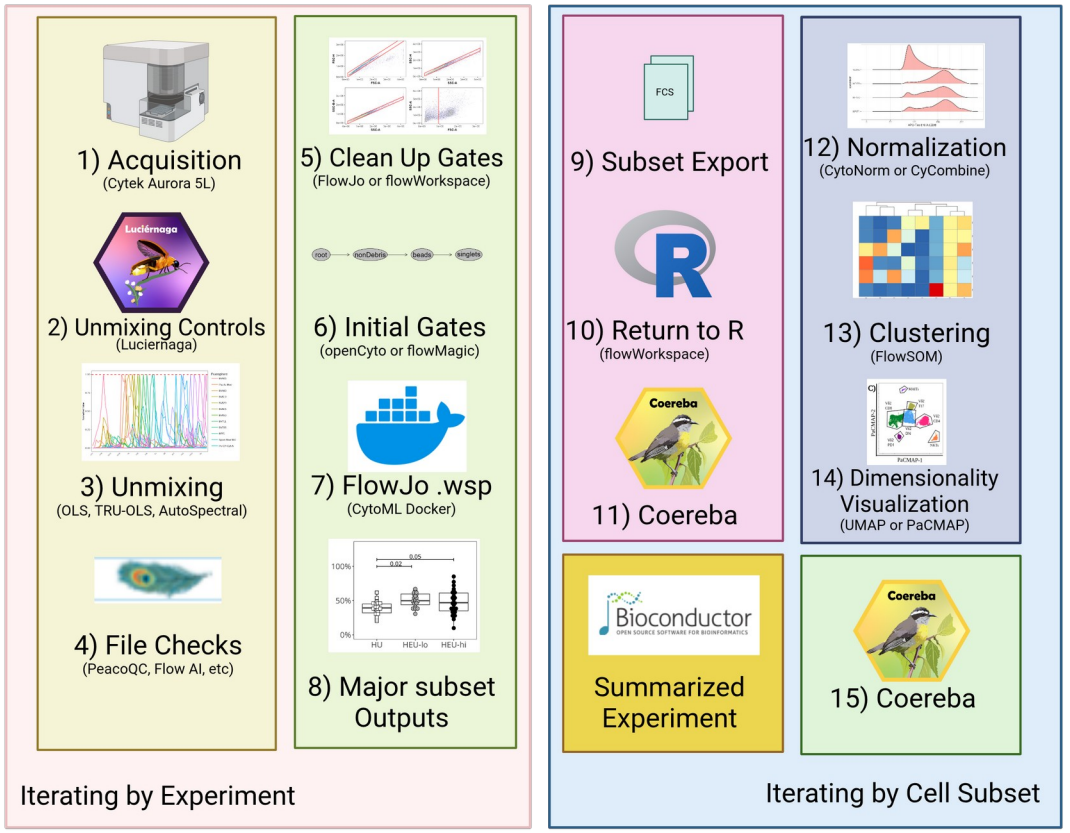
More HEU papers on way

On the plate:

- B cells

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No cell left behind



- Semi-supervised pipeline
- Testing on additional ImmPort datasets

<u>On way to Bioconductor:</u>

- Luciernaga
- Coereba

<u>On Way to CRAN</u>

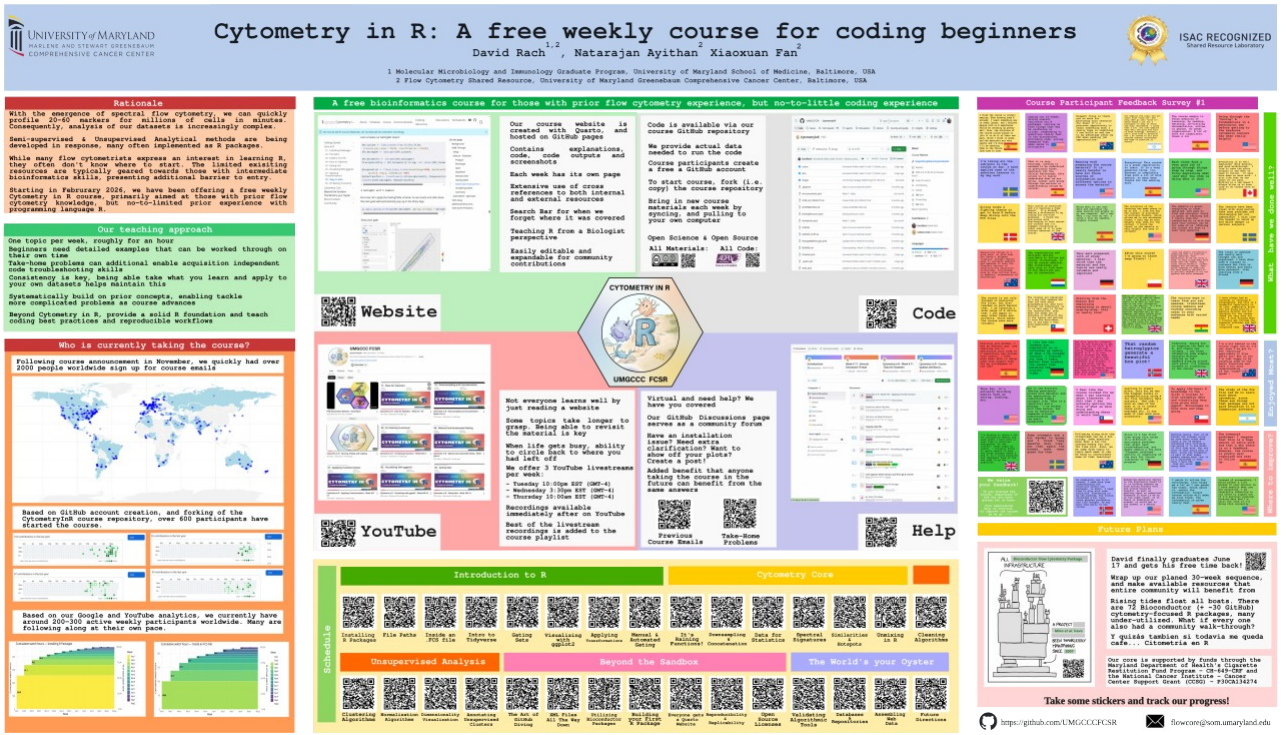
- CytometryQC
- Bucephala

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Cytometry in R

YouTube

Website



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A rather weird PhD


EL PAÍS 50 Ciencia / Materia OFERTA ÚNICA INICIAR SESIÓN

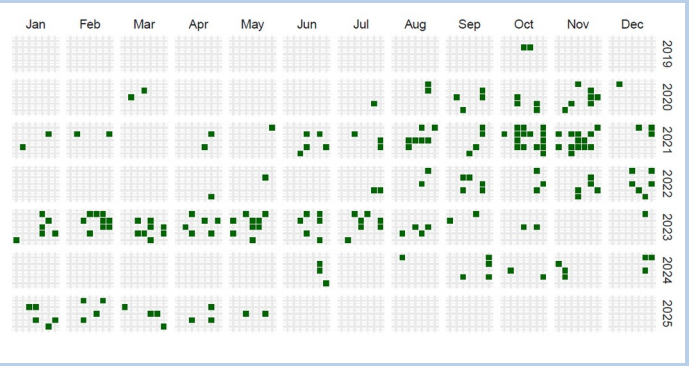
LA CRISIS DEL CORONAVIRUS >

Pfizer anuncia un 95% de eficacia de su vacuna y solicitará su aprobación de emergencia

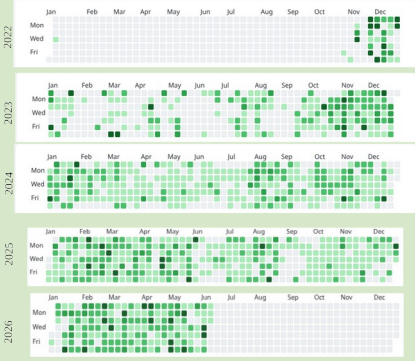
La inyección, desarrollada en colaboración con BioNTech, es la primera en demostrar plena validez estadística

Un voluntario de EE UU recibe la vacuna contra la covid desarrollada por BioNTech y Pfizer.
AP

Flow



Code (and writing via Quarto)



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Acknowledgements

Our Lab:
Dr. Cristiana Cairo
Dr. Kirsten E. Lyke

Dr. Hao-Ting Hsu
Dr. Winter Okoth
Lena King
Bernadette Hritzo
Esosa Omorogbe
Mehnaz Falguni
Julianna Marshall

MMI
Dr. Eileen Barry
Bess Tracey
Dr. Heather Ezelle
Dr. Brett Hassel

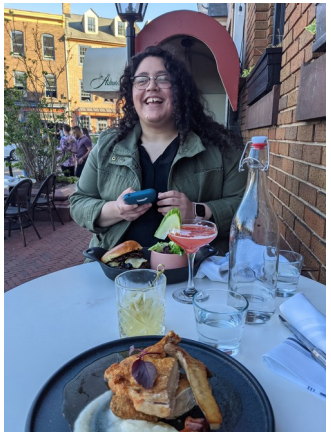


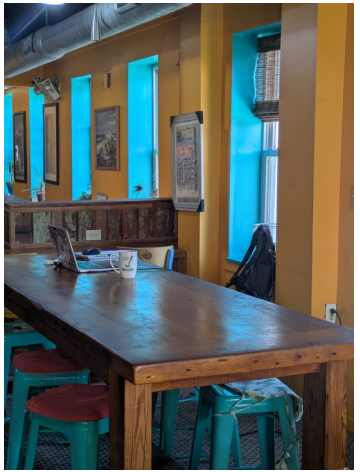
Thesis Committee:
Dr. Margaret Feeney
Dr. Yutaka Tagaya
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Dr. Franklin Toapanta
Dr. Tonya Webb
Dr. Arlene Dent

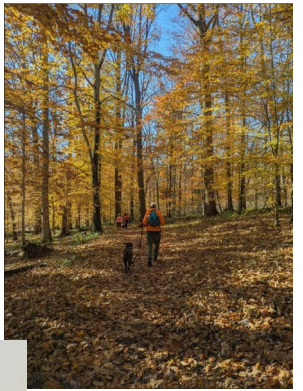
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Mikayla Trainor
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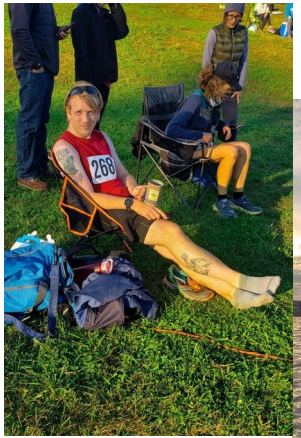
Questions?

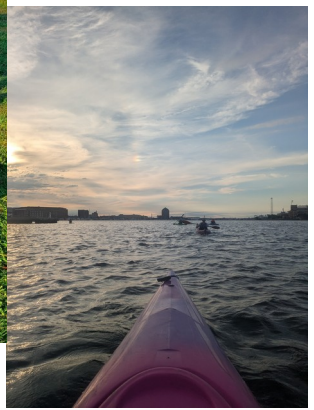








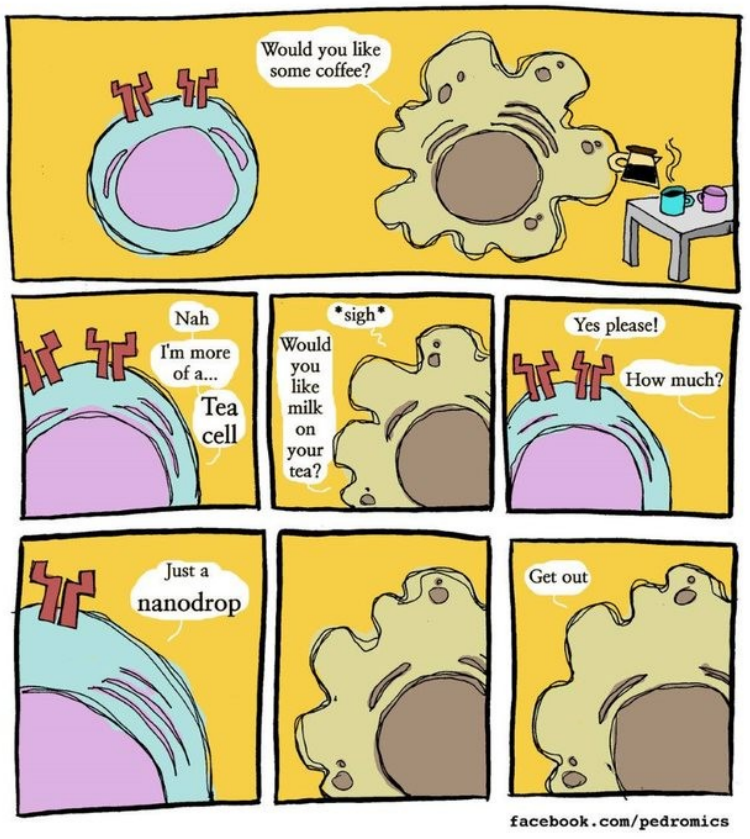


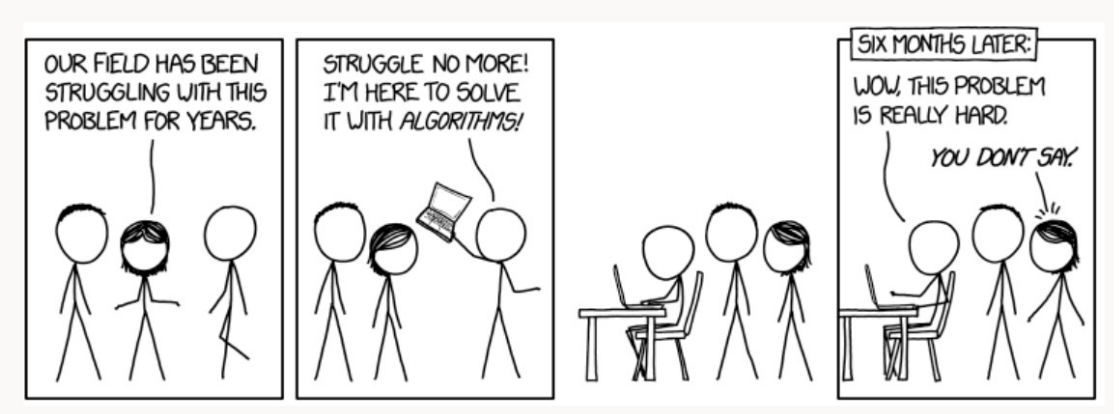




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Questions?

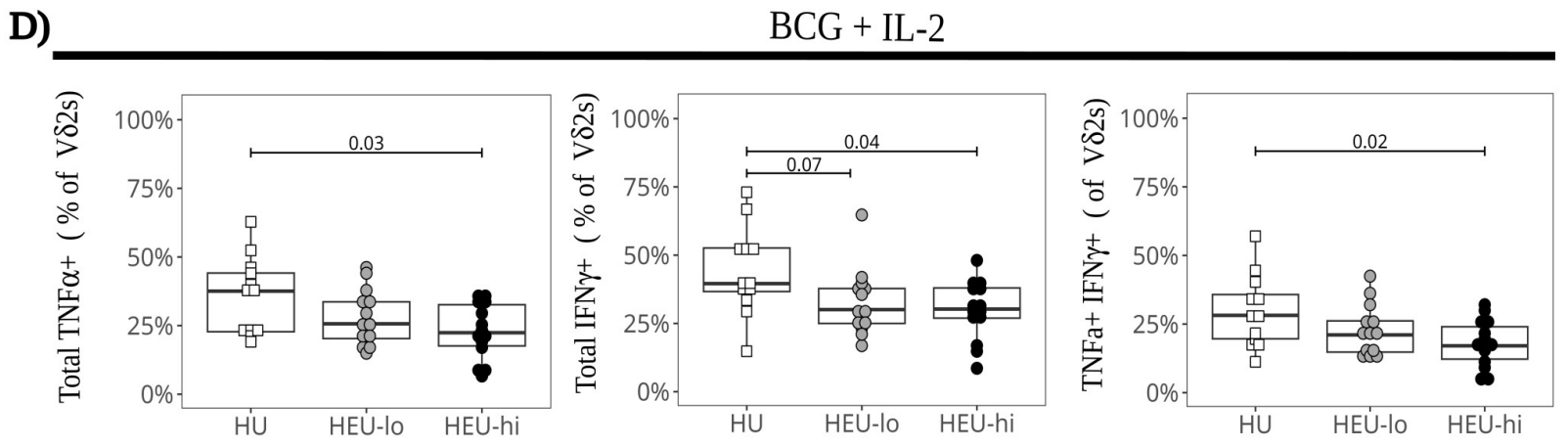




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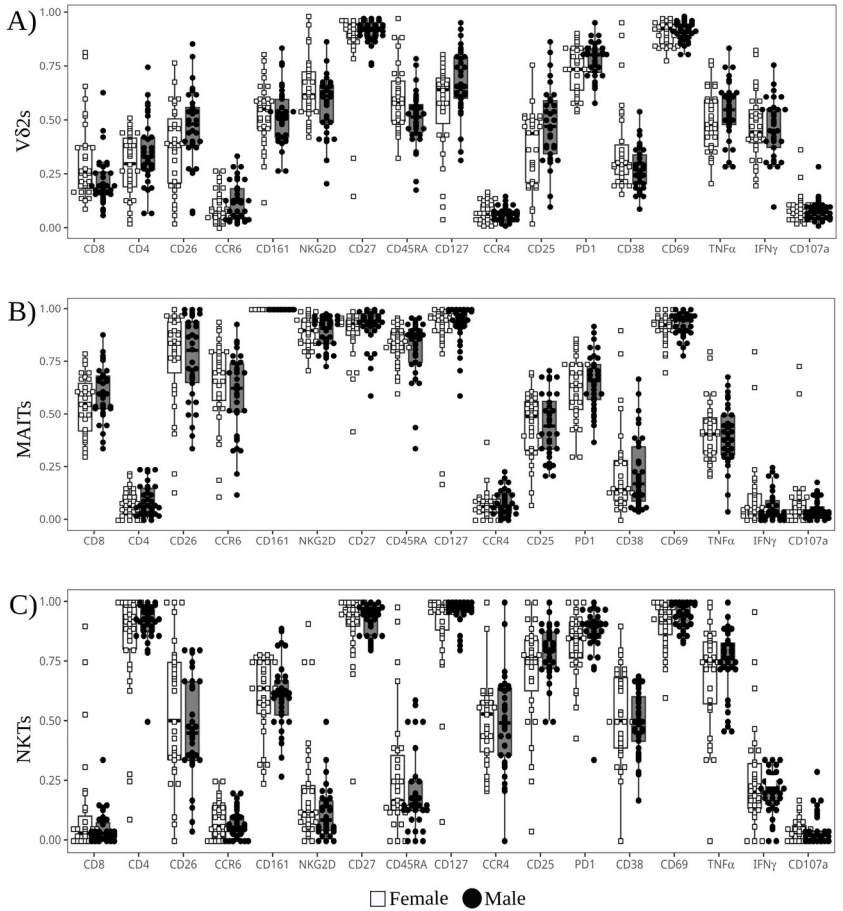
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Day 17 BCG + IL-2 Cultures



University of Maryland School of Medicine

Sex-specific Differences - ILT



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Immunology: Self or not-Self

Supplementary Table 3. Generalized Linear Model Results

Marker ~ Infant Sex + HIV/ART Exposure
Separate models for each dependent variable

Tested	Predictor	OR	SE	z-value	p-value	OR [95% CI]	[1]
Vδ2							
NKG2D	Intercept	2.01	0.03	20.62	**< 0.001**	2.01 [1.88–2.15]	***
NKG2D	Sex: Male	0.92	0.03	-2.55	**0.014**	0.92 [0.87–0.98]	*
NKG2D	Exposure: HEU-lo	1.01	0.04	0.20	0.845	1.01 [0.93–1.09]	
NKG2D	Exposure: HEU-hi	0.95	0.04	-1.43	0.158	0.95 [0.88–1.02]	
CD8	Intercept	1.40	0.04	8.18	**< 0.001**	1.40 [1.29–1.52]	***
CD8	Sex: Male	0.90	0.04	-2.61	**0.012**	0.90 [0.84–0.97]	*
CD8	Exposure: HEU-lo	1.01	0.05	0.18	0.855	1.01 [0.92–1.11]	
CD8	Exposure: HEU-hi	0.97	0.05	-0.55	0.582	0.97 [0.89–1.07]	
CD45RA	Intercept	1.93	0.03	18.99	**< 0.001**	1.93 [1.80–2.07]	***
CD45RA	Sex: Male	0.93	0.03	-2.29	**0.026**	0.93 [0.87–0.99]	*
CD45RA	Exposure: HEU-lo	0.99	0.04	-0.13	0.896	0.99 [0.92–1.08]	
CD45RA	Exposure: HEU-hi	0.97	0.04	-0.66	0.509	0.97 [0.90–1.05]	
CD62L	Intercept	1.11	0.02	5.98	**< 0.001**	1.11 [1.07–1.15]	***
CD62L	Sex: Male	1.04	0.02	2.18	**0.034**	1.04 [1.00–1.07]	*
CD62L	Exposure: HEU-lo	1.02	0.02	0.95	0.346	1.02 [0.98–1.06]	
CD62L	Exposure: HEU-hi	1.04	0.02	2.10	**0.04**	1.04 [1.00–1.08]	*
CD25	Intercept	1.36	0.04	7.07	**< 0.001**	1.36 [1.25–1.48]	***
CD25	Sex: Male	1.09	0.04	2.04	**0.046**	1.09 [1.00–1.18]	*
CD25	Exposure: HEU-lo	0.99	0.05	-0.19	0.852	0.99 [0.90–1.10]	
CD25	Exposure: HEU-hi	1.04	0.05	0.74	0.464	1.04 [0.94–1.14]	
MAIT							
PD1	Intercept	1.46	0.04	8.69	**< 0.001**	1.46 [1.34–1.58]	***
PD1	Sex: Male	1.09	0.04	2.00	**0.05**	1.09 [1.00–1.18]	*
PD1	Exposure: HEU-lo	1.03	0.05	0.60	0.554	1.03 [0.93–1.14]	
PD1	Exposure: HEU-hi	0.96	0.05	-0.73	0.468	0.96 [0.88–1.06]	

[1] ***p < 0.001; **p < 0.01; *p < 0.05

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