

# Mapping the immune system in people with Down syndrome

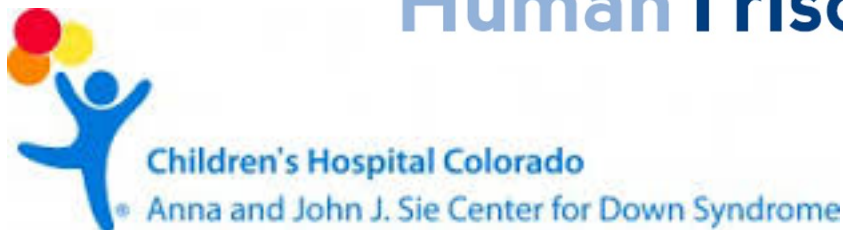
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Lab of Joaquin Espinosa



HumanTrisomeProject

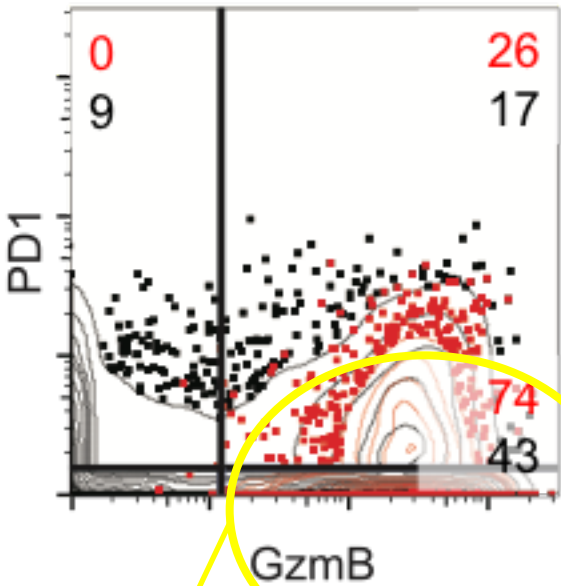
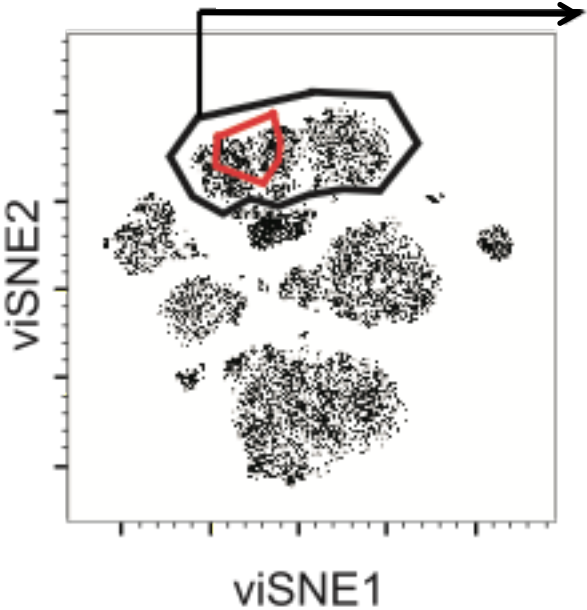
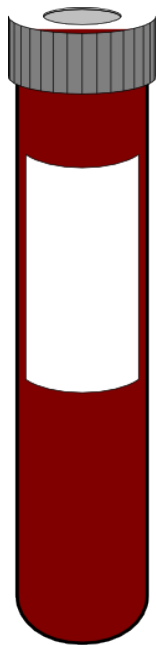


**SPOILER ALERT**

1. Blood collection

2. Look at cells in blood

CD8+ T cells  
Increased in Trisomy 21

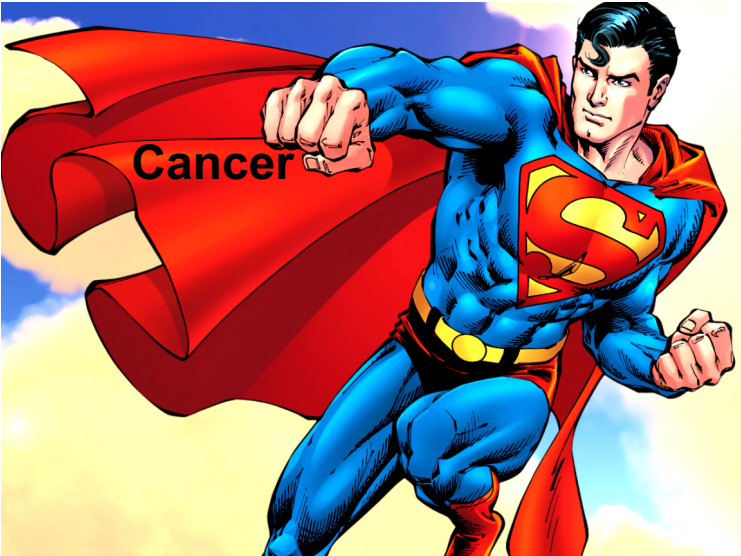


3. Analyze

These red dots are cells that can be like **superman cells** fighting solid cancers.

New immunotherapy drug behind Jimmy Carter's cancer cure

Former president given pembrolizumab, one of the most promising new drugs in the treatment of cancer



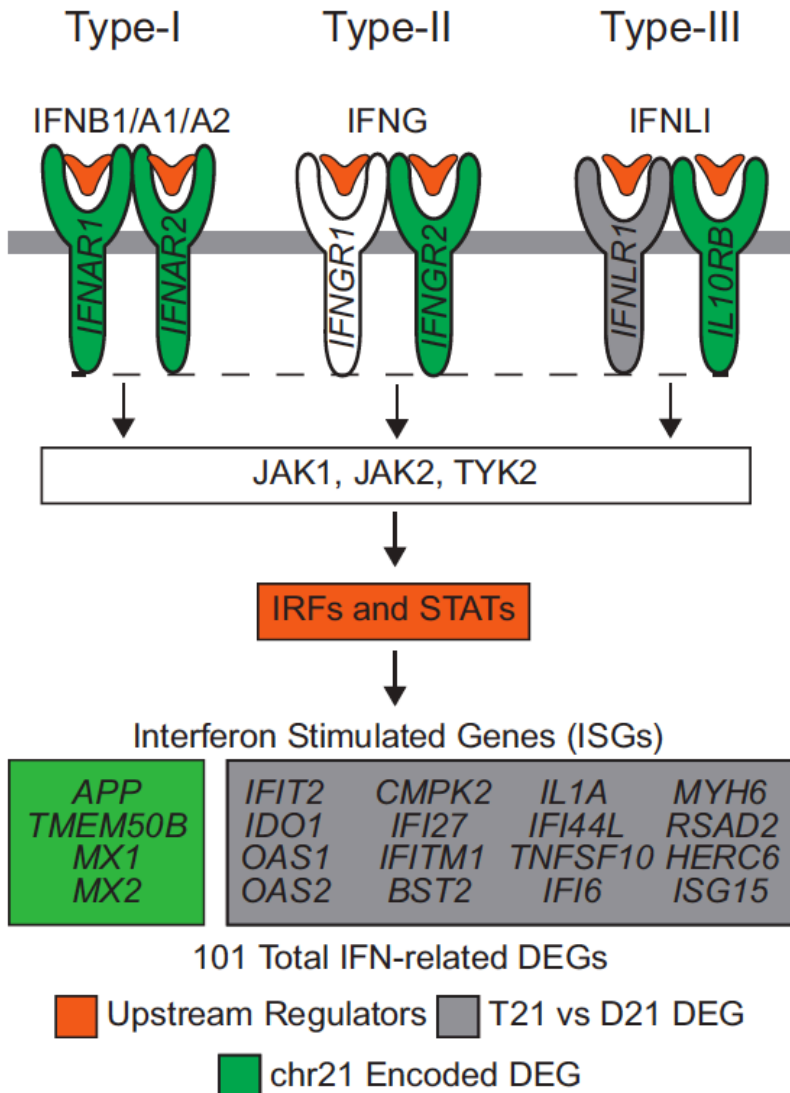
The underlying science...

# Individuals with trisomy of chr. 21 (T21) have alterations in disease incidence



**The >400,000 Americans with Trisomy 21 may hold solutions to major medical conditions**

# Aberrant interferon (IFN) signaling is conserved across individuals with T21



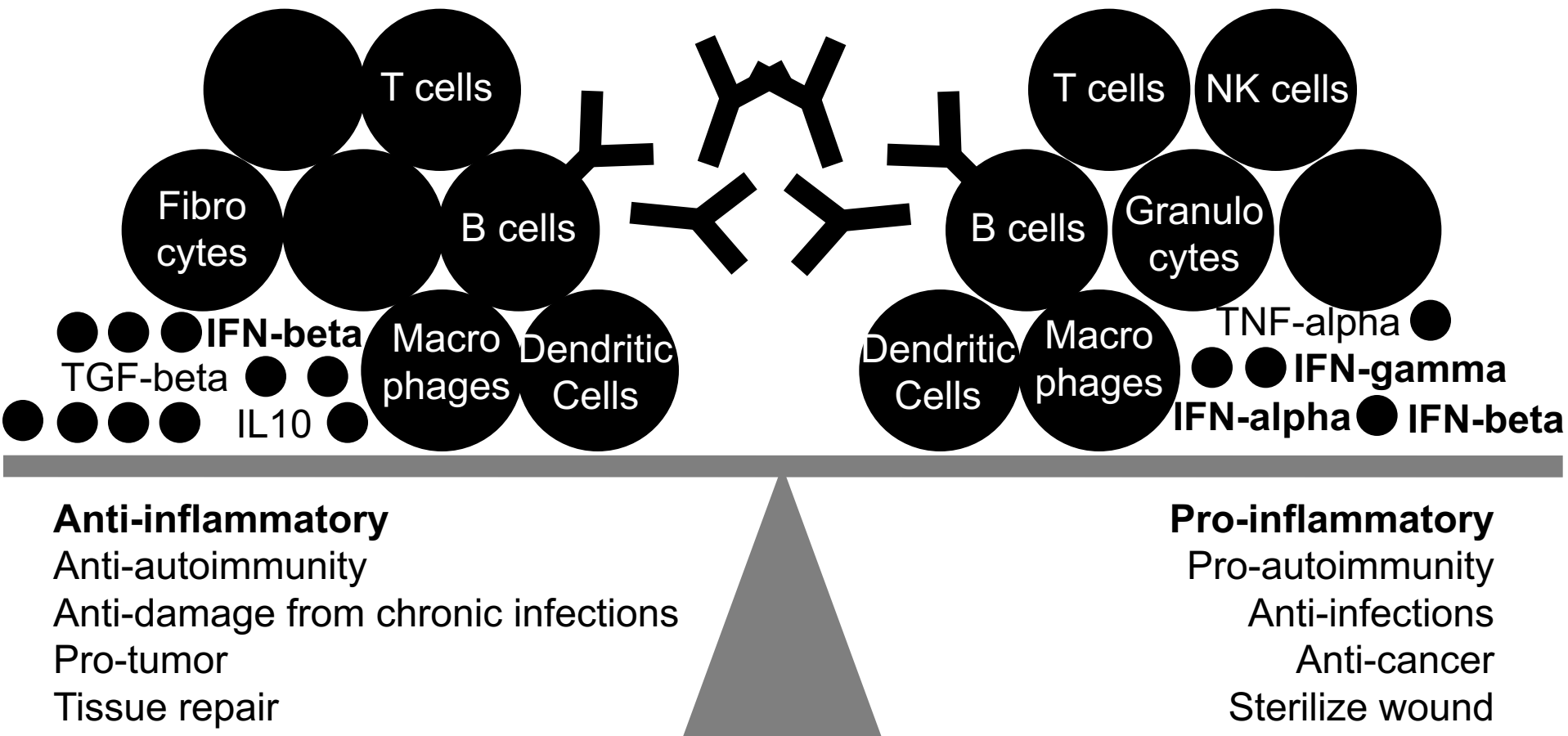
- **Increased IFN signaling has been noted in T21 for over 40 years**  
 (Tan 1974, Maroun 1979, Weil 1980, Zihni 1974, Maroun 1996, Hallam 1998, Maroun 2000, Ling 2014, Lee 2016)

- **IFNs identified as core signaling pathways activated among individuals with T21**
  - Genome-wide mRNA expression
  - shRNA loss-of-function screening

- **As with clinical manifestations of T21, increased ISG expression varied**  
 (de Sola S 2015)

(Sullivan KD 2016)

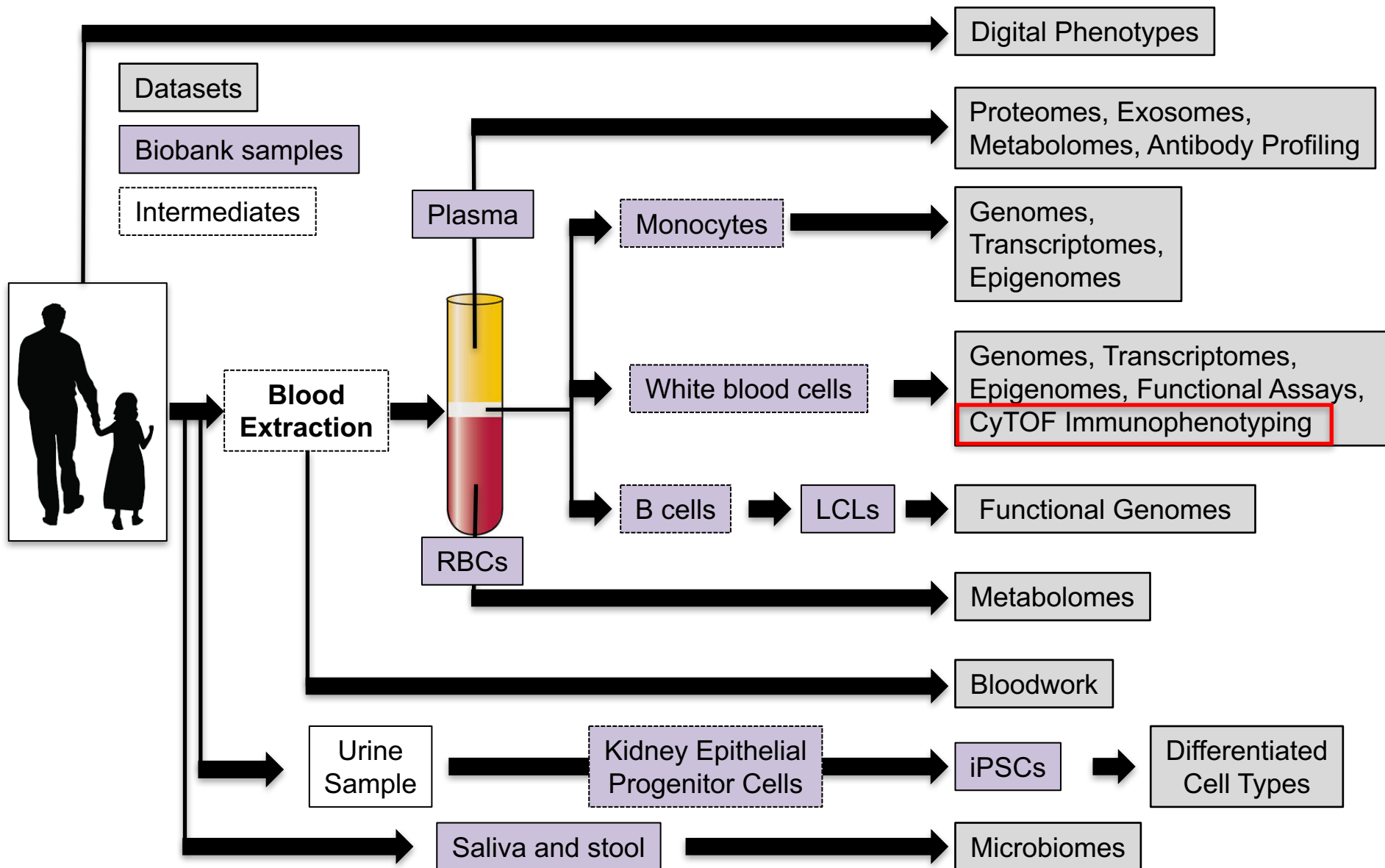
# IFN signaling can instruct the immune system to drive or restrict disease



# Previous studies of the immune system in adults with T21 are contradictory

- Contradictory literature  
(reviewed in Ram G & Chinen J 2011)
  - Reduced frequencies of T cells and B cells (Martinez E 2016)
  - Alterations in B cell phenotype and antibody production (Carsetti R 2015)
  - Functional impairment of T cells
  - Functional impairment in NK cells (Nurmi T 1982)
  - Functional impairment of granulocytes
  - Immunodeficiency (Burgio GR or Ugazio AG 1970-1990s, Lima FA 2011)
  - Thymus and immune cells similar to “older” people  
(Schlesinger M 1976, Cossarizza A 1990s)
- Small N's, limited evaluations, and times have changed

# The Human Trisome Project (HTP)



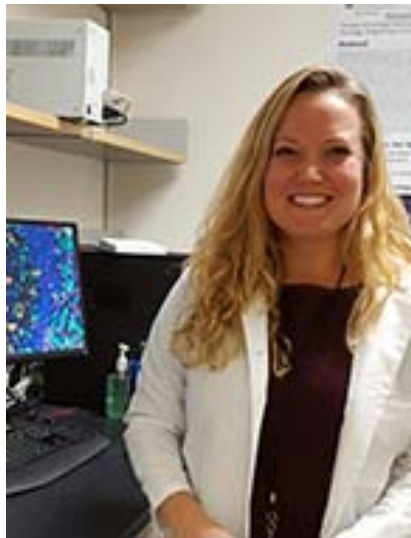
LCLs: lymphoblast cell lines, iPSCs: induced pluripotent stem cells, RBCs: red blood cells

# Overall Hypothesis

Individuals with T21 have a generalized immune dysregulation



Elena Hsieh

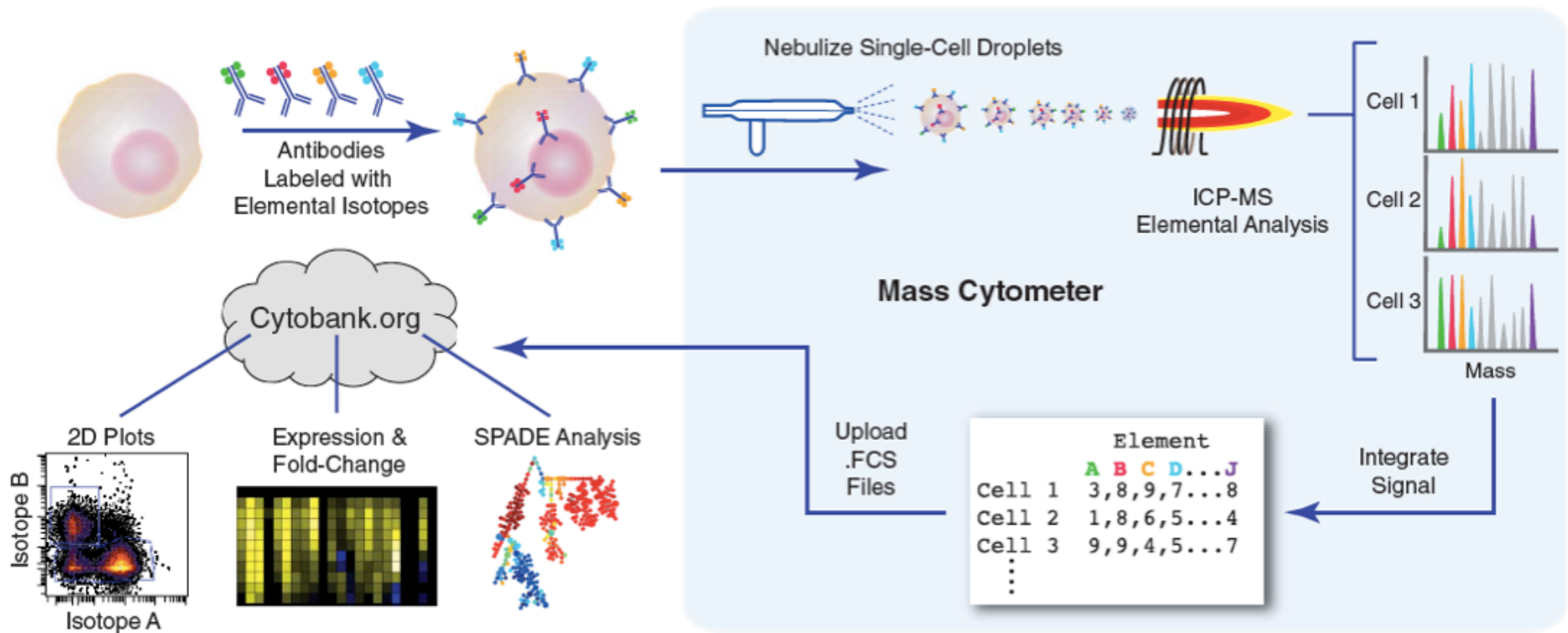


Kim Jordan



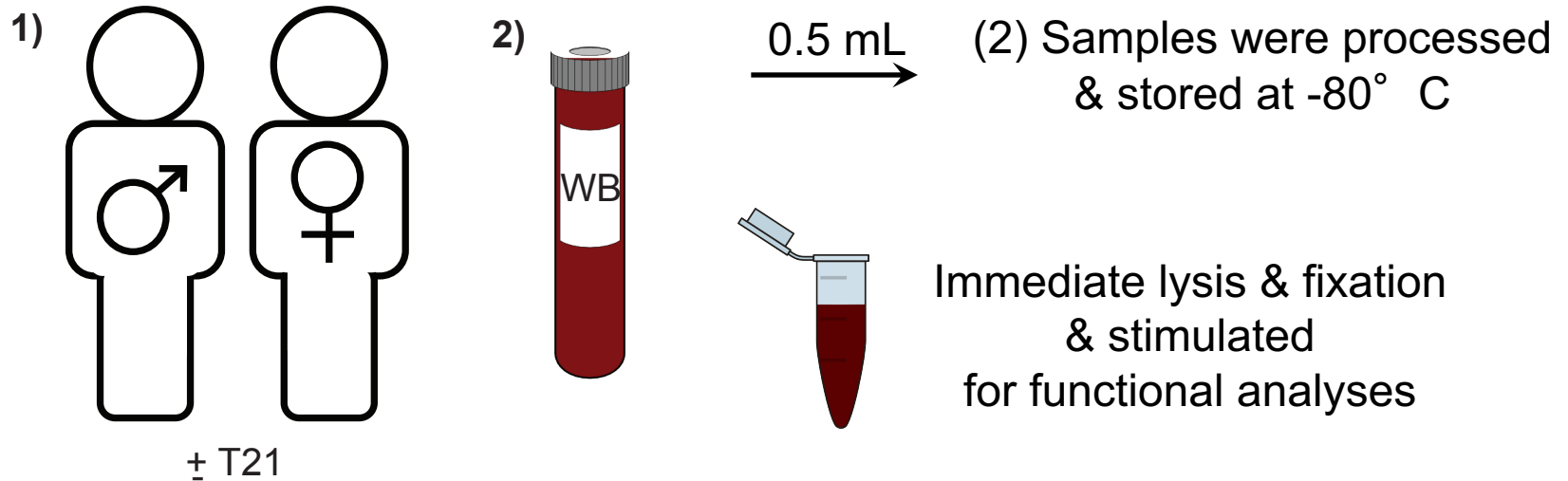
Jen McWilliams

# Methods: Single cell analysis by mass cytometry (CyTOF)



- Limited overlap between channels compared to flow cytometry
- Up to ~40 antibodies per staining panel

# HTP samples processed for mass cytometry (CyTOF)



# Technical variation minimized within and between batches to focus on biology

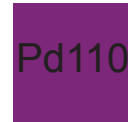
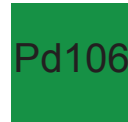
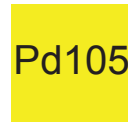
3) Samples barcoded with Pd isotopes

Sample #: 1

2

3

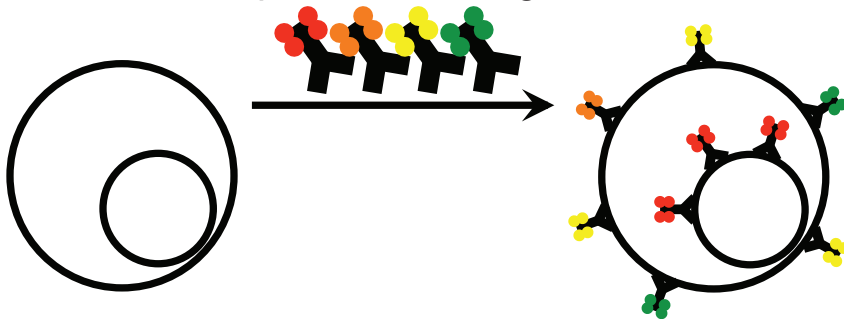
4



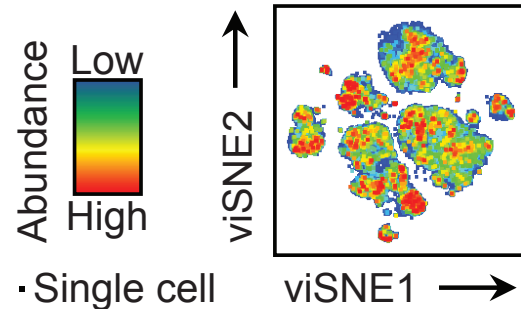
4) Samples combined



5) Combined samples stained together

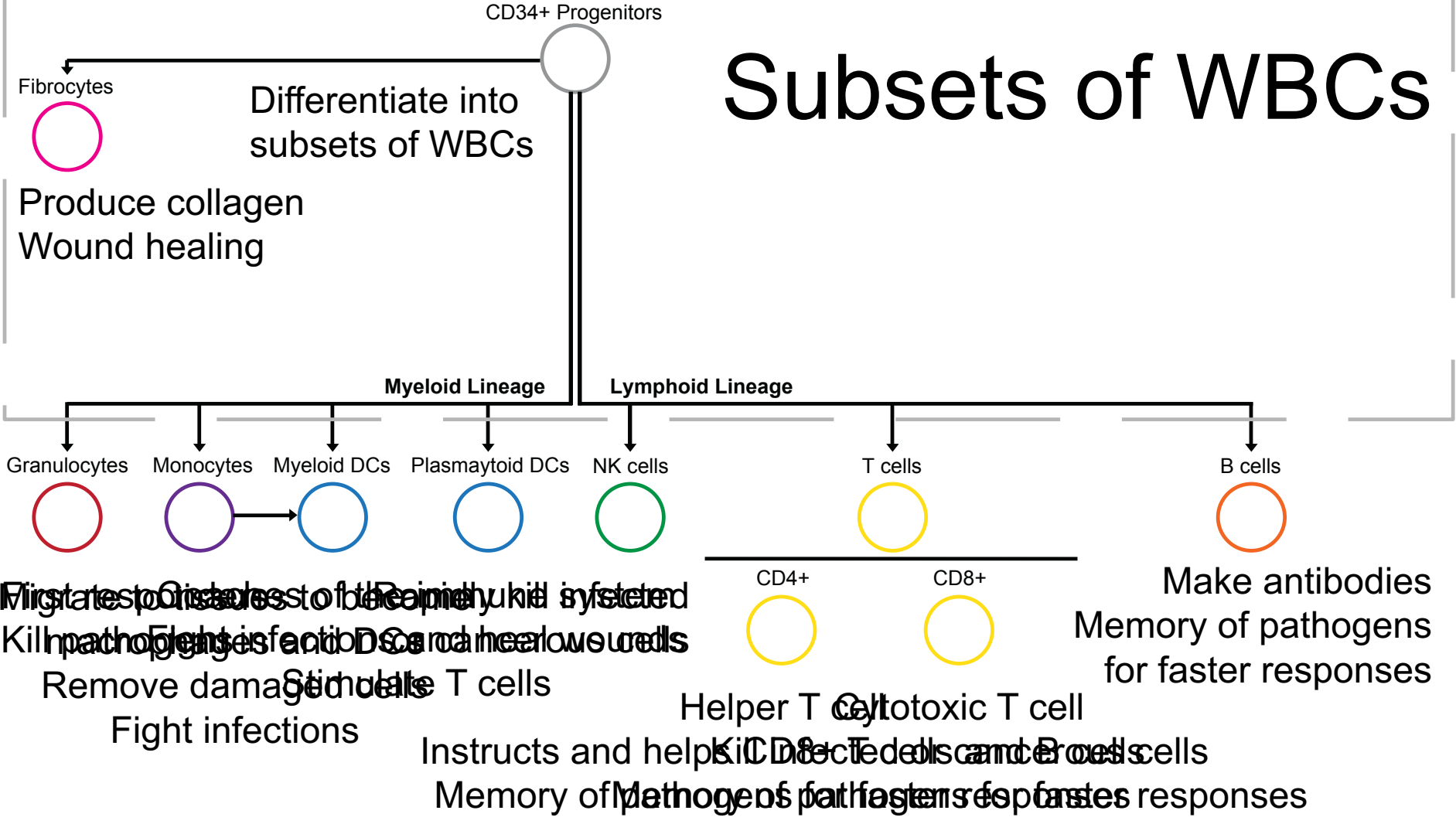


7) Normalized between batches & analyzed



6) Samples run together on mass cytometer with normalization beads

# Subsets of WBCs



First responders  
Kill pathogens  
Remove damaged cells  
Fight infections

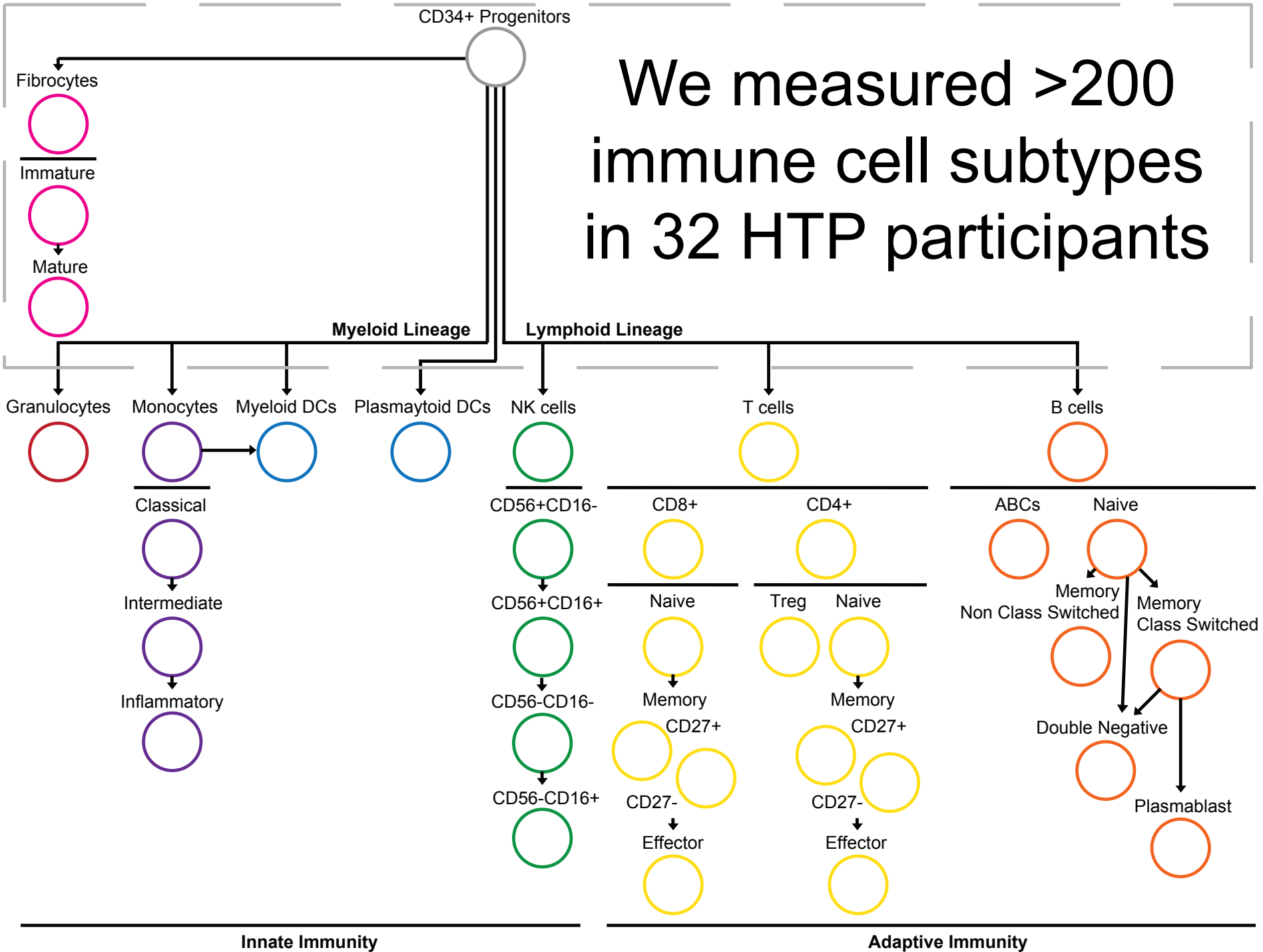
CD4+  
CD8+  
Helper T cells  
Cytotoxic T cells

Make antibodies  
Memory of pathogens  
for faster responses

Instructs and helps kill infected cells  
Memory of pathogens for faster responses

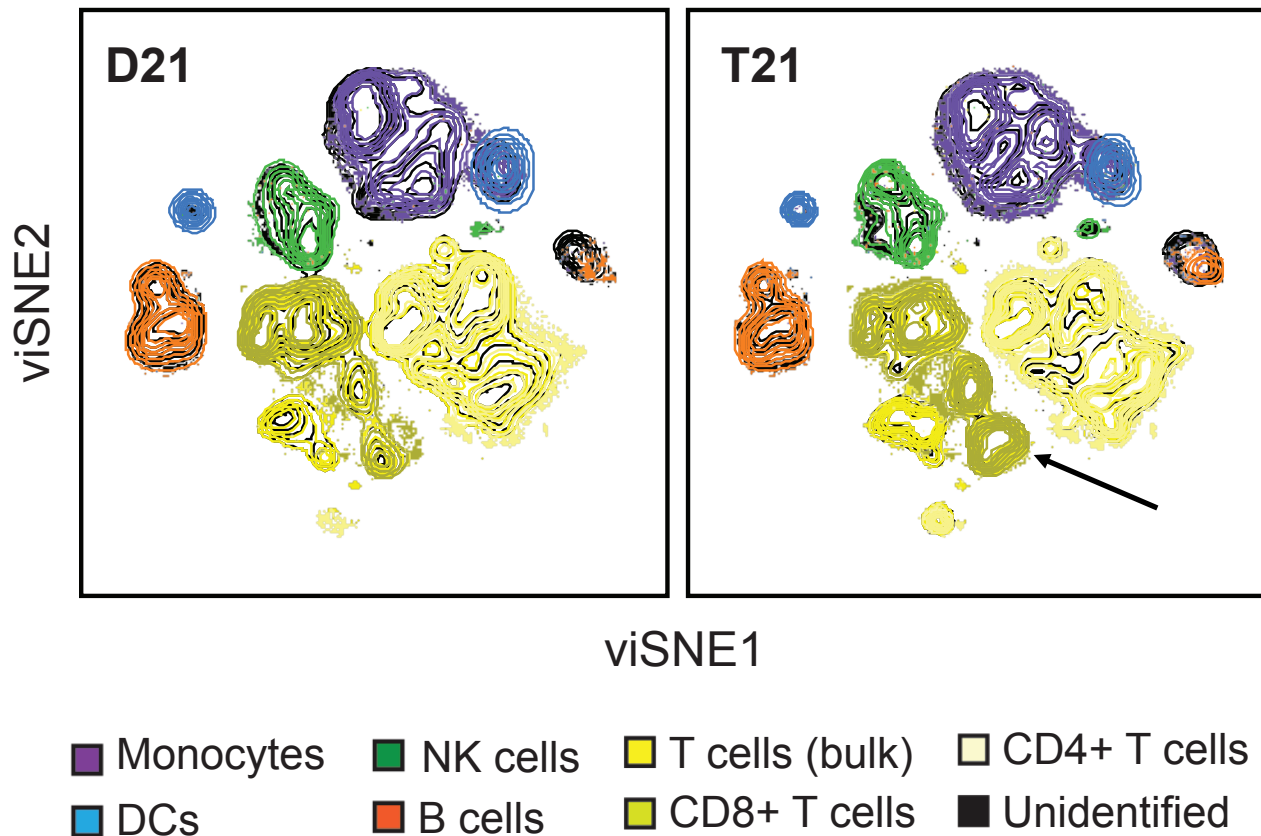
Staining panel focuses on “cellular and humoral immunity”

We measured >200  
immune cell subtypes  
in 32 HTP participants



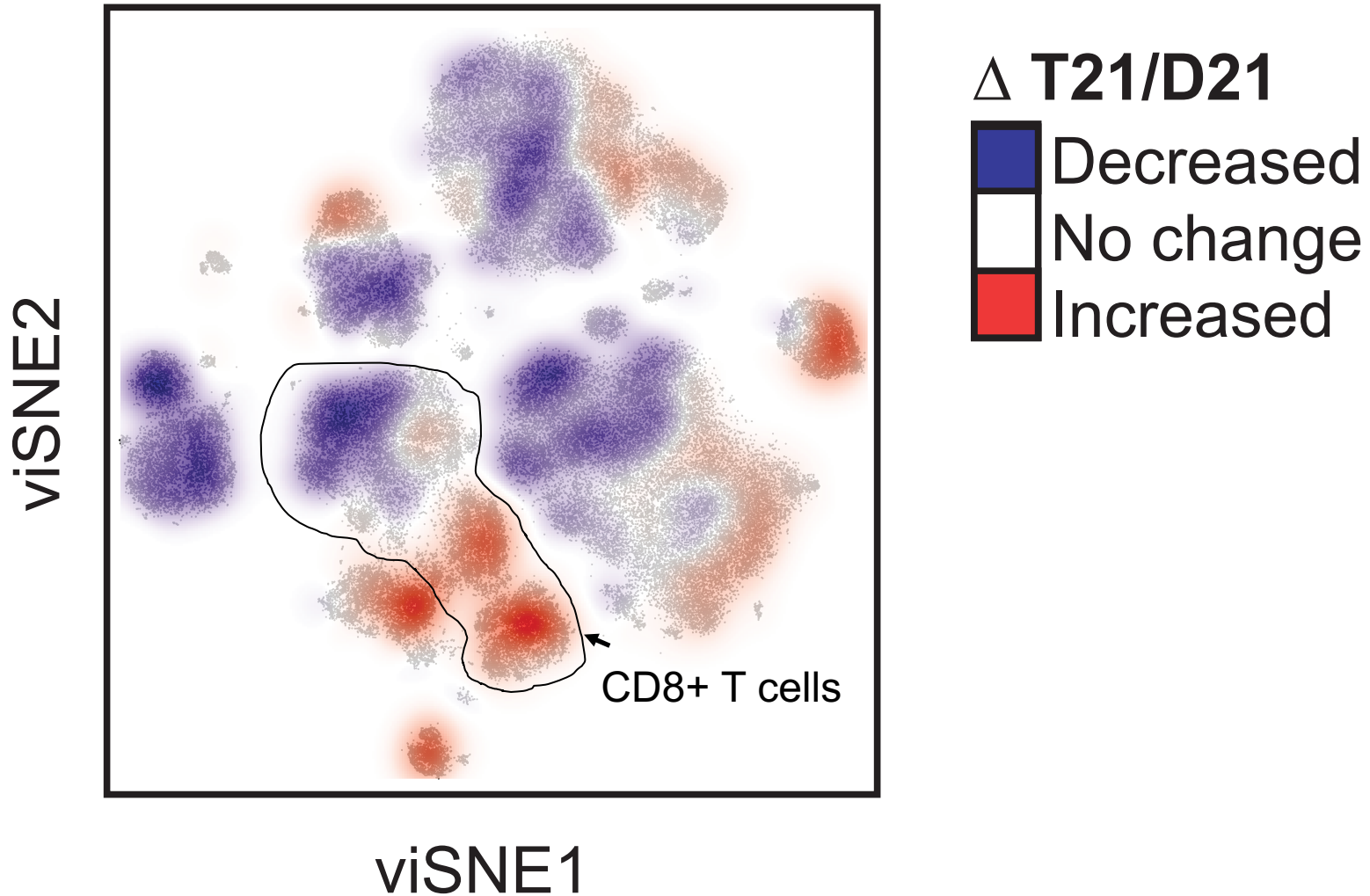
# viSNE maps appear highly similar between individuals with Trisomy 21 and controls (D21)

viSNE enables visualization of global structure within high-dimensional single-cell data.  
(Amir et al. 2014)



# Topographic analysis highlights global immune dysregulation among individuals with Trisomy 21

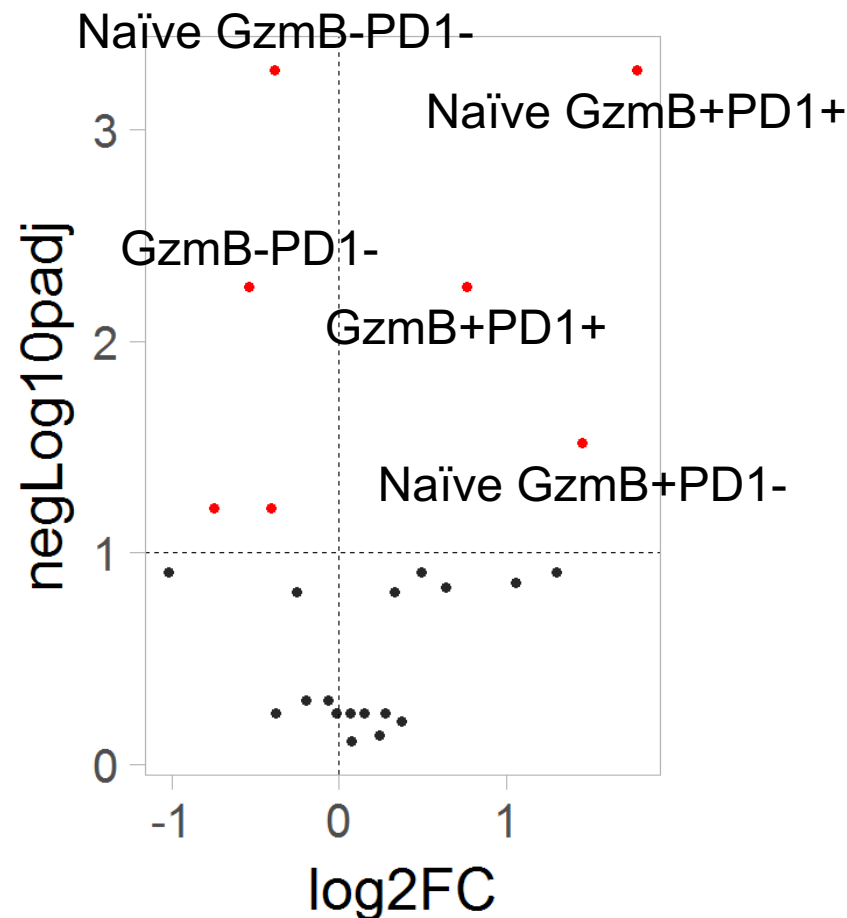
Kernal Density Estimate (KDE) of viSNE plots quantitatively compares cell density.



# Every detected branch of the immune system is altered among individuals with Trisomy 21

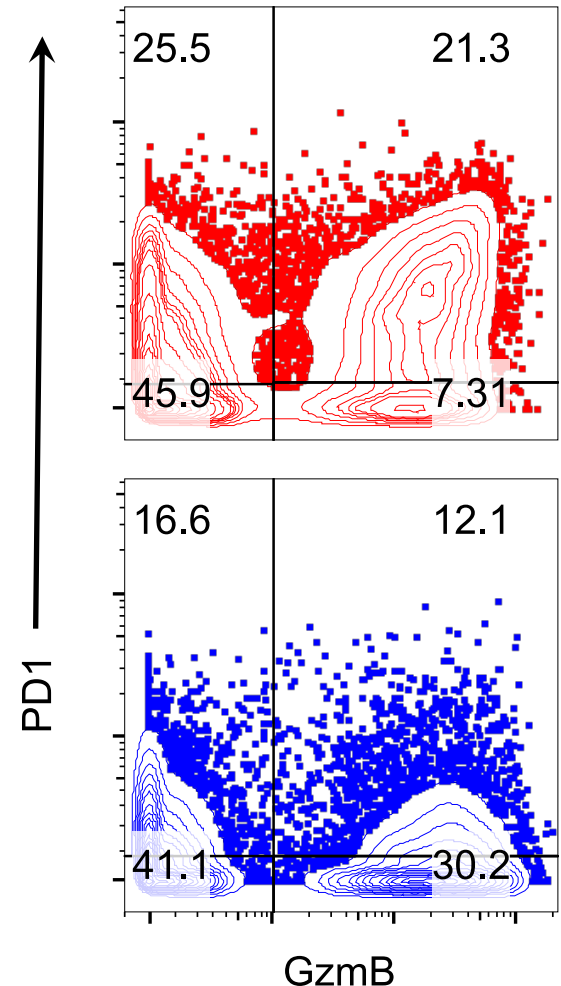
Student's unpaired  $t$  test, Benjamini-Hochberg adjusted  $p < 0.05$

## CD8+ T cells – one example

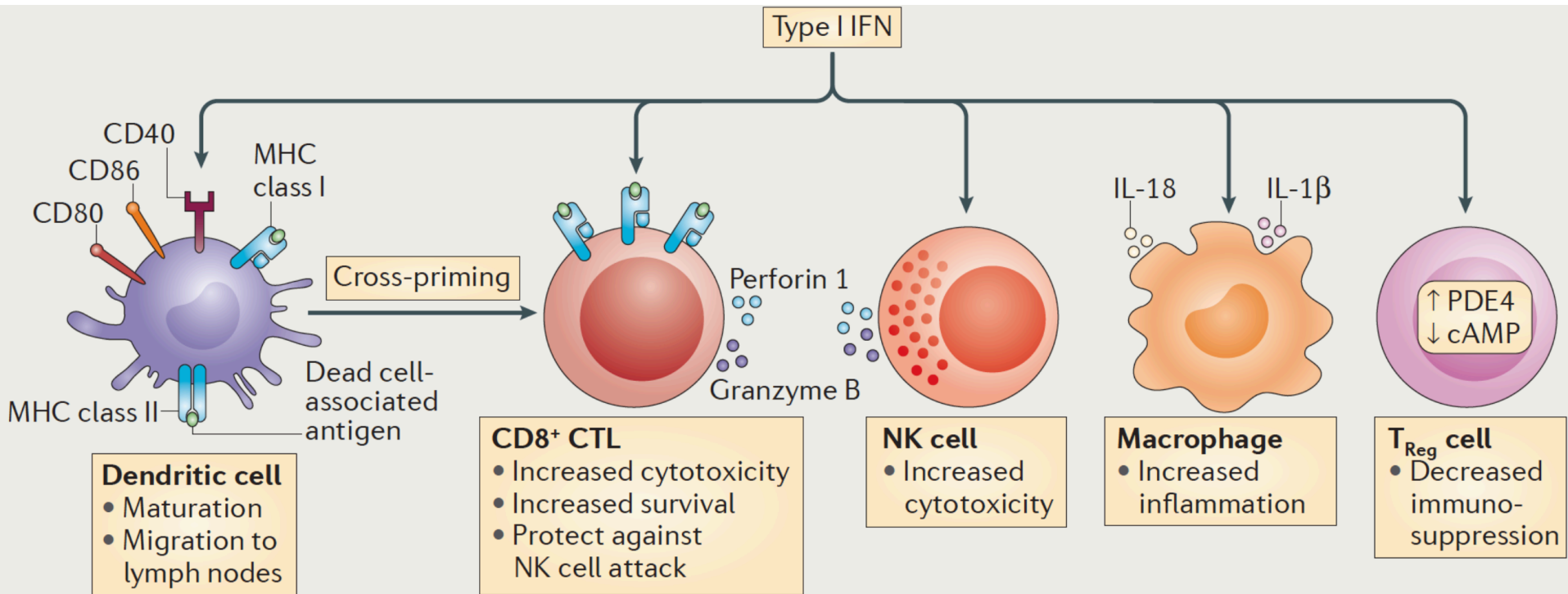


T21

D21



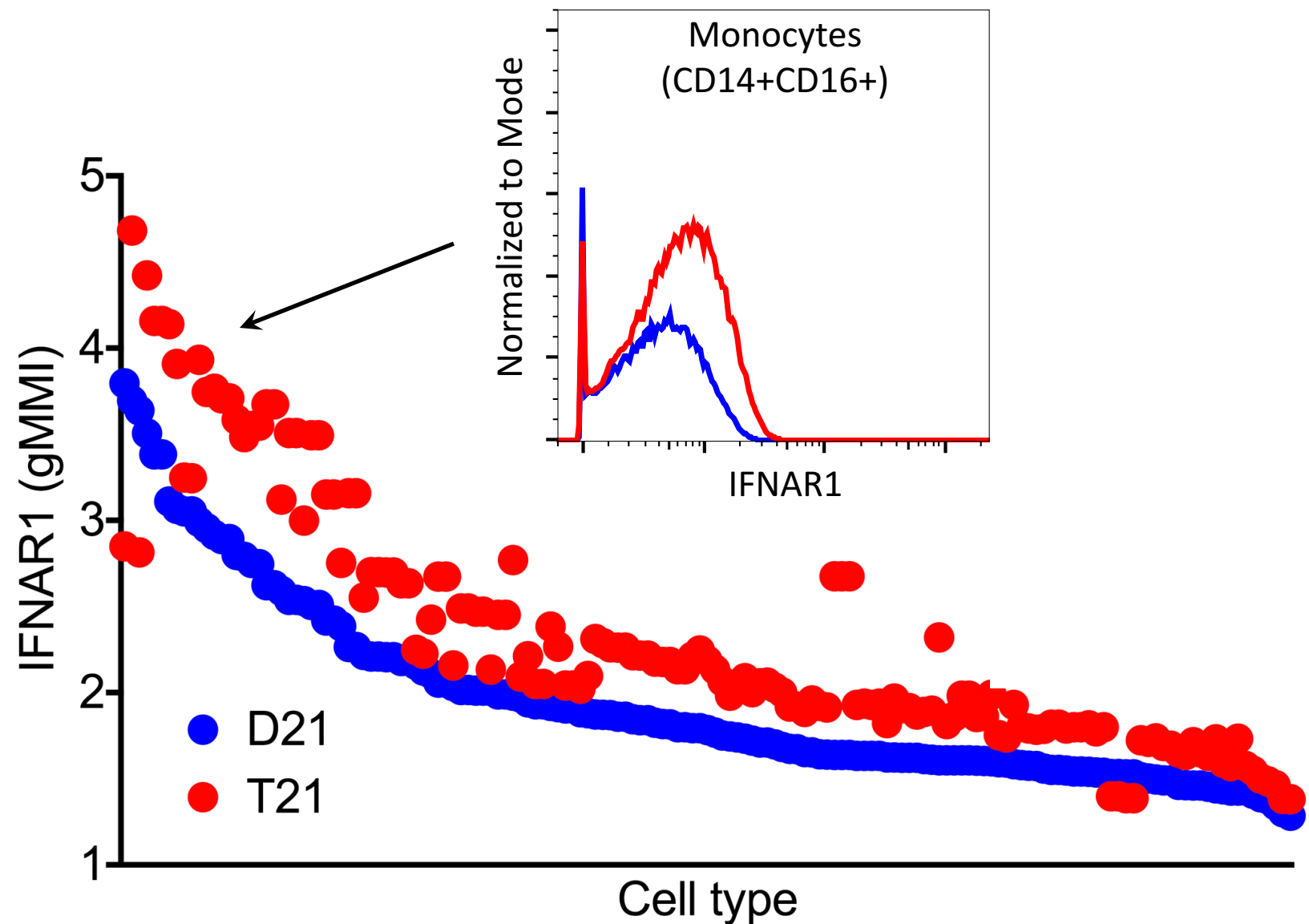
# Solid tumor protection by CD8+ T cell cytotoxicity is mediated by IFN alpha



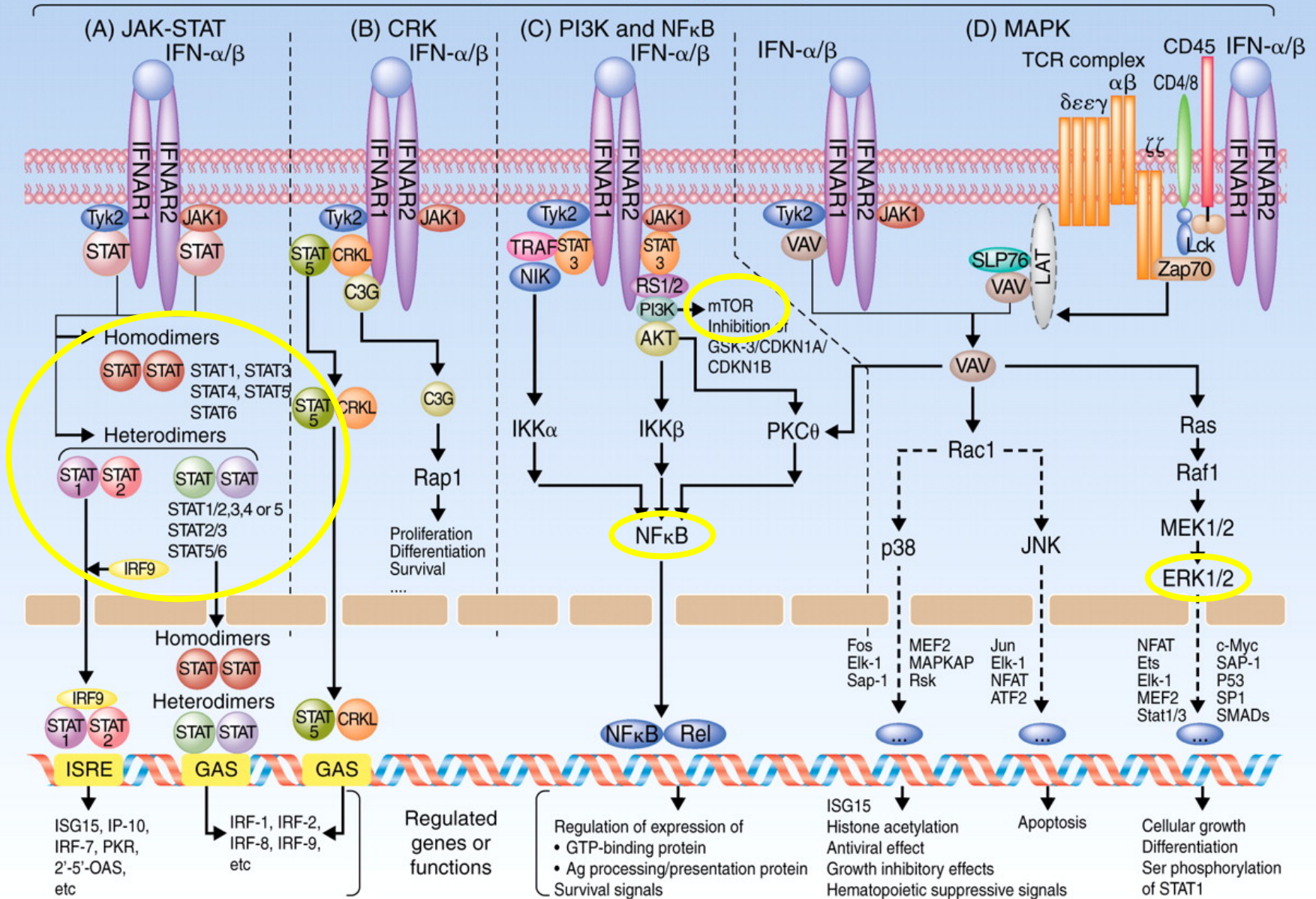
reviewed in Zitvogel L 2015 & Ikeda H 2002

Type 1 interferon licenses naïve CD8 T cells to mediate anti-viral cytotoxicity.  
Urban SL et al. 2016

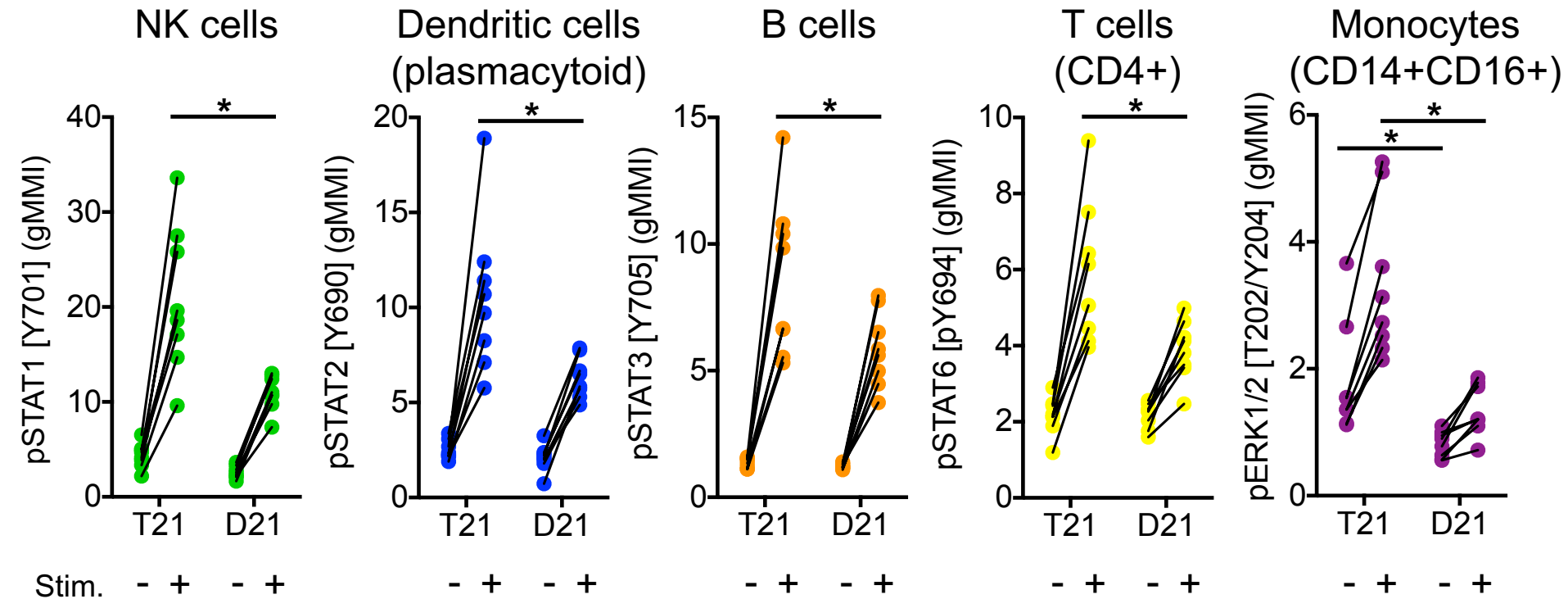
# Distinct immune cell subsets have higher surface protein expression of IFNAR1 among individuals with T21



# Type I IFNs signaling pathways

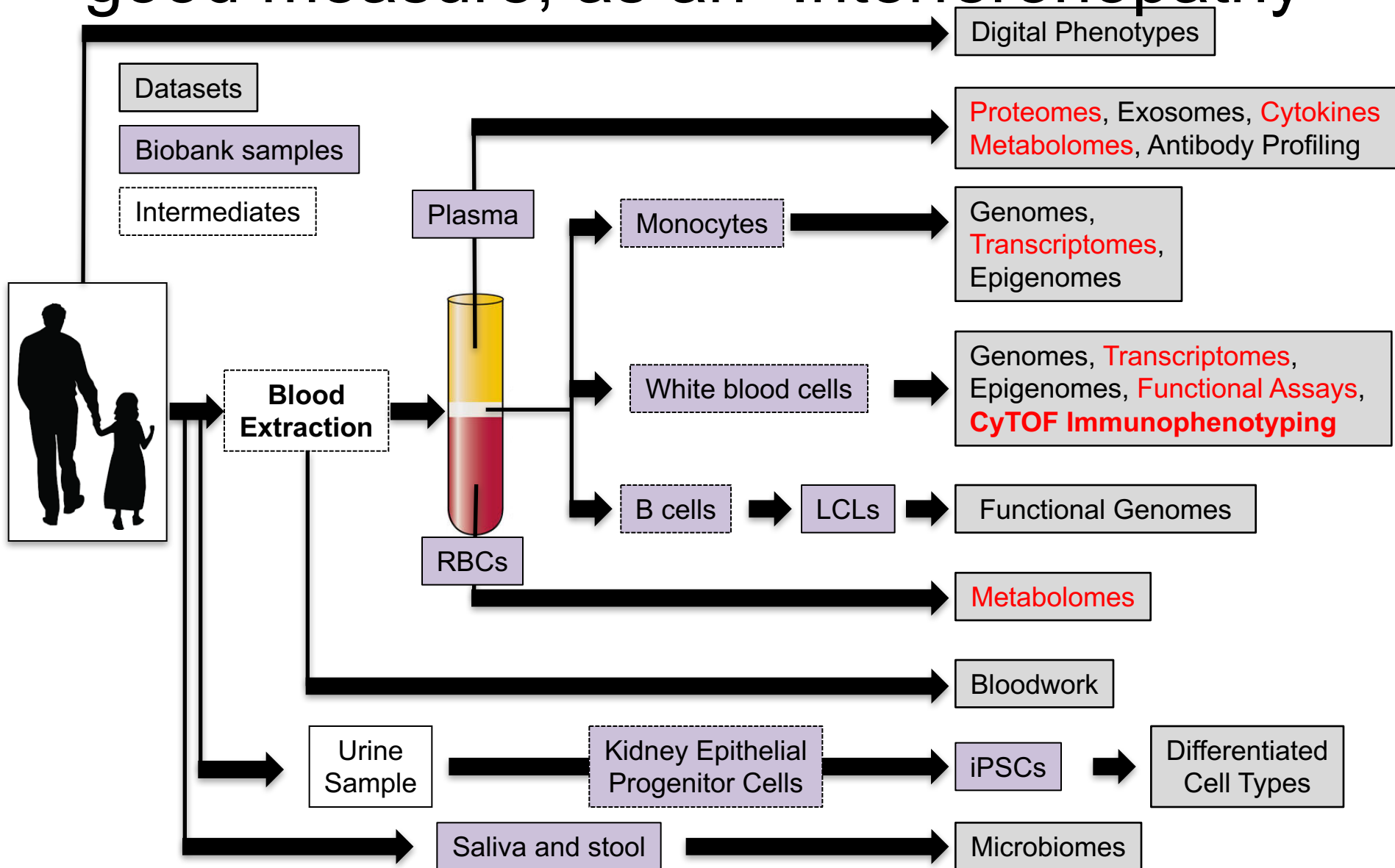


Among individuals with Trisomy 21, all measured branches of the immune system have increased signaling in response to IFN alpha stimulation



Student's unpaired *t* test, \* $p < 0.05$

# Down syndrome could be understood, in good measure, as an “Interferonopathy”



# Conclusions

- Healthy adults with Trisomy 21 have global immune dysregulation in steady state.
- Every detected branch of the immune system has multiple statistically significant differences between individuals with Trisomy 21 and disomic controls.
  - For example, individuals with Trisomy 21 have an increased frequency of CD8<sup>+</sup> T cells that highly express the anti-cancer protein Granzyme B.
- Among individuals with Trisomy 21, distinct immune cell subtypes have higher surface protein expression of an interferon receptor, IFNAR1, that is located on chromosome 21.
- All measured branches of the immune system had heightened signaling after IFN alpha stimulation among individuals with Trisomy 21.

# Future directions

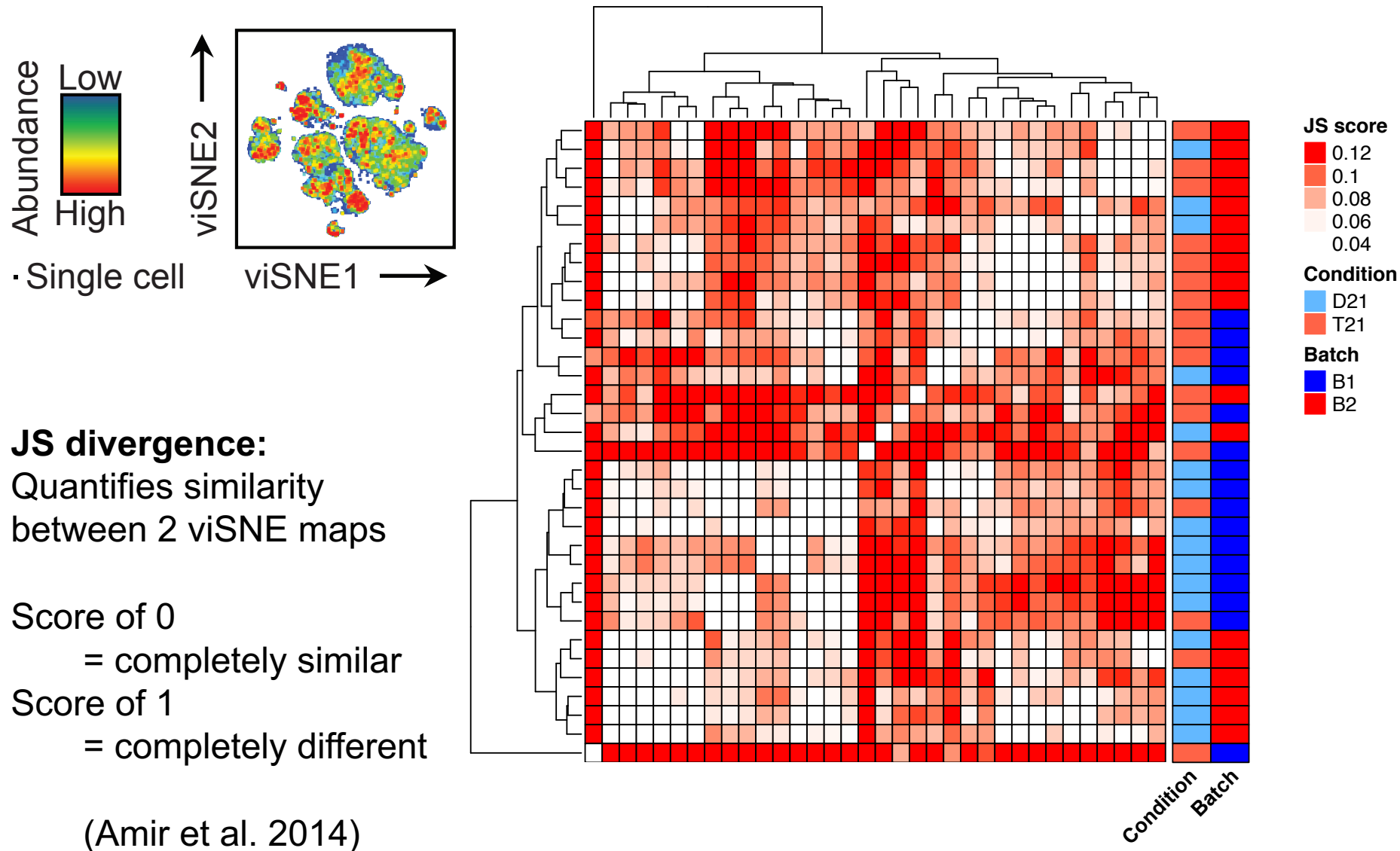
To scale up CyTOF measurements to hundreds of HTP participants with the ultimate goal of identifying immune signatures associated with specific phenotypes.

Key co-morbidities that we are pursuing:

1. Various autoimmune conditions (celiac, Hashimoto's, alopecia, vitiligo, type I diabetes)
2. Regression, Depression, Autism, early onset Alzheimer's disease, Infantile Spasms, Epilepsy

**As of today, we have 68 T21 and 75 D21 CyTOF-ready biological replicates ready for analysis.**

# Analysis pipelines are built to identify immune signatures associated with T21



# Acknowledgements

## **Espinosa Lab & HTP Team**

- [Ahwan Pandey](#)
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## **Porter lab**

- *Dmitry Baturin*

## **Funding**

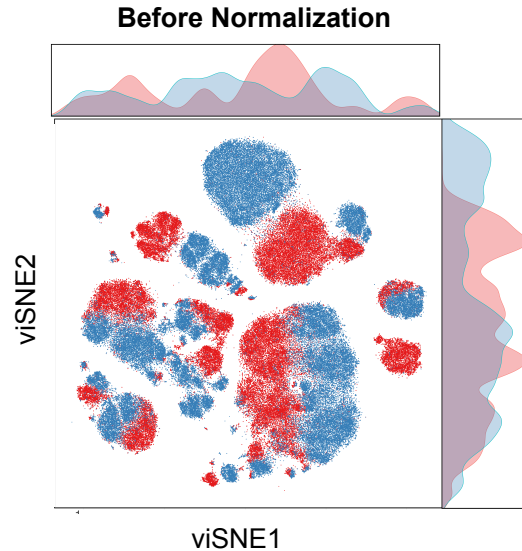
- University of Colorado Cancer Center T32
- Blumenthal Fellowship
- Global Down Syndrome Foundation
- Linda Crnic Institute for Down Syndrome
- *NIH/NCI (Porter)*
- *Cancer League of Colorado (Porter)*

# Questions?



Extra slides

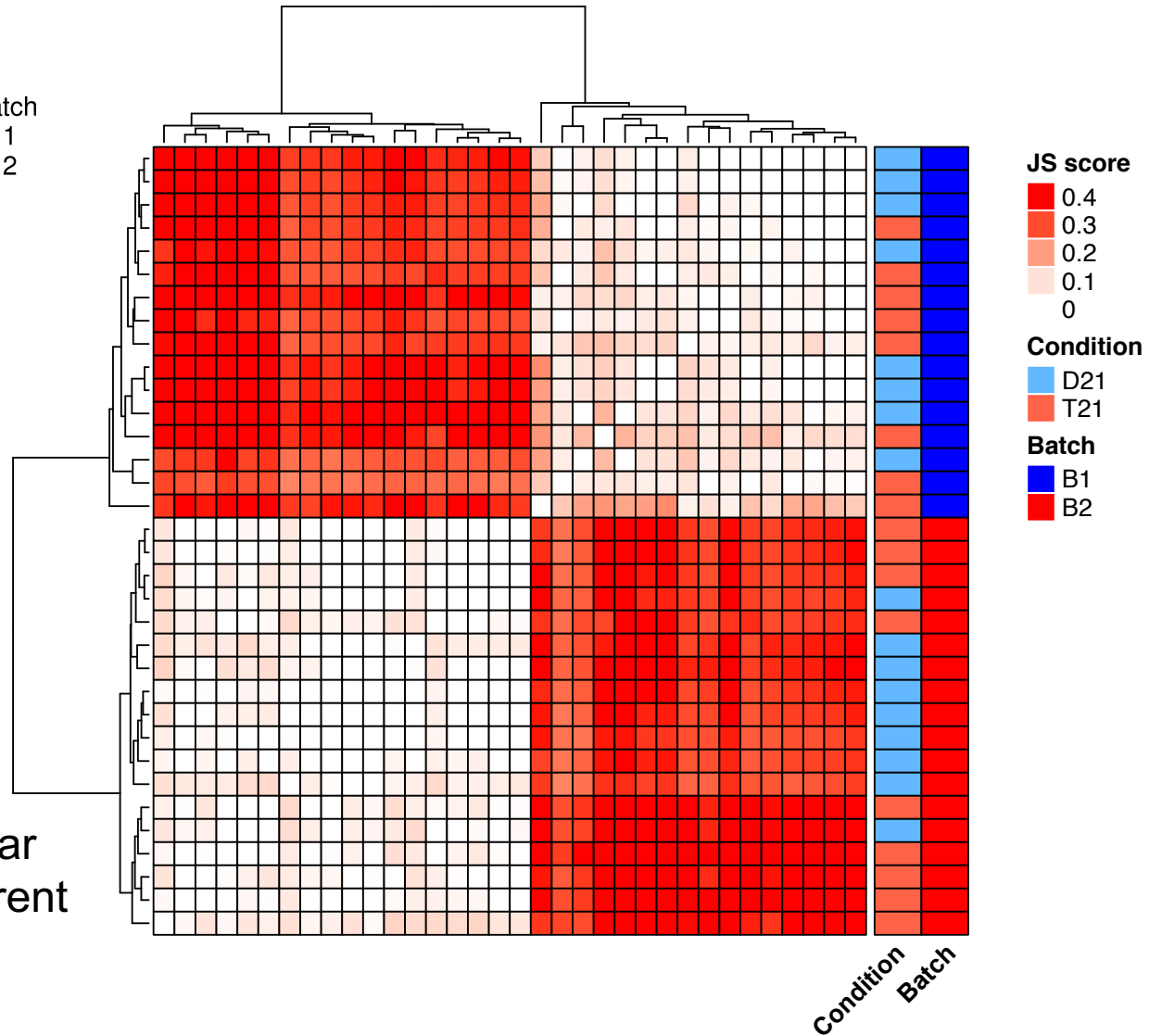
# Technical variation minimized between batches for high-throughput analyses



Batch  
● 1  
● 2

**JS divergence:**  
Quantifies similarity between  
two viSNE maps

Score of 0 = completely similar  
Score of 1 = completely different



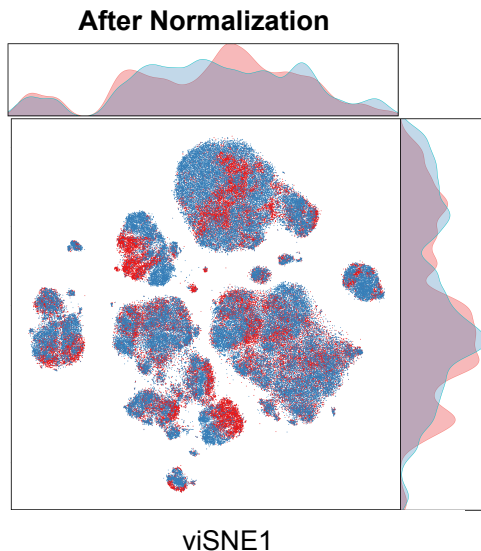
(Amir et al. 2014)

# Technical variation minimized between batches for high-throughput analyses

## SVA ComBat:

Reduces batch effects in high-throughput experiments

(Leek et al. 2012)



Batch  
● 1  
● 2

