

# **CD4<sup>+</sup> T cells re-wire granuloma cellularity and regulatory networks to promote immunomodulation following *Mtb* reinfection**

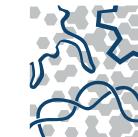
Josh Bromley (he/him/his)

TB Hackday

December 10, 2024



**Massachusetts  
Institute of  
Technology**



**SHALEK LAB**  
from cells to systems

# Natural Infection: *Mtb* Control (or Permissiveness)

## Mechanisms of protection following *Mtb* reinfection?

### Aberrant Inflammatory Response

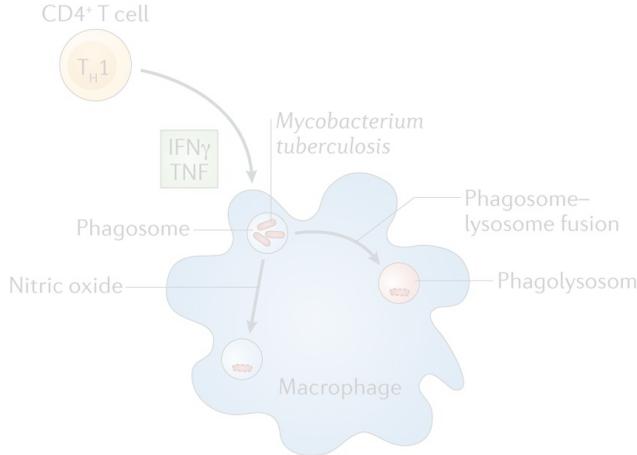
- Neutrophilia
- Type 1 IFN
- Impaired immunoregulation

### Type 1/17 Immunity

- CD4<sup>+</sup> Th1, Th17
- IFN $\gamma$ , TNF $\alpha$ , IL-17
- CD8<sup>+</sup> T cells

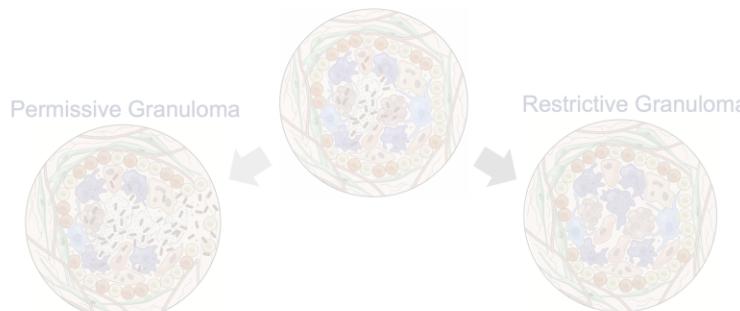
# *Mtb* Reinfection Hypotheses

a Central dogma



## Expectation (from 20,000 feet)

1. Prior infection protects against *Mtb* reinfection
2. Primed CD4<sup>+</sup> T cells mediate protection via IFN $\gamma$  and TNF $\alpha$



### Aberrant Inflammatory Response

- Neutrophilia
- Type 1 IFN
- Impaired immunoregulation

### Type 1/17 Immunity

- CD4<sup>+</sup> Th1, Th17
- IFN $\gamma$ , TNF $\alpha$ , IL-17
- CD8<sup>+</sup> T cells

## I. Infection and in-vivo perturbation

Does primary *Mtb* infection protect against reinfection?



Primary Infection



Reinfection

Following immune priming what is the role of CD4+ T cells during reinfection?



Reinfection-ΔCD4



Reinfection

## II. Microbial assays

Restrictive Granuloma

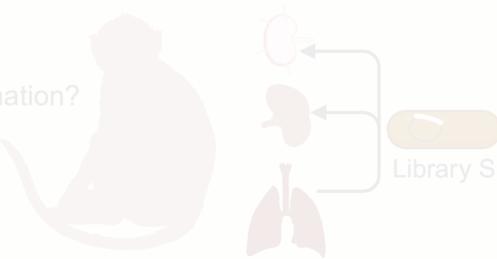


Permissive Granuloma



Bacterial growth (or restriction)?

Bacterial dissemination?



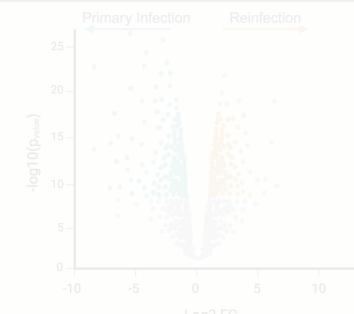
## III. Cellular and molecular correlates of *Mtb* control

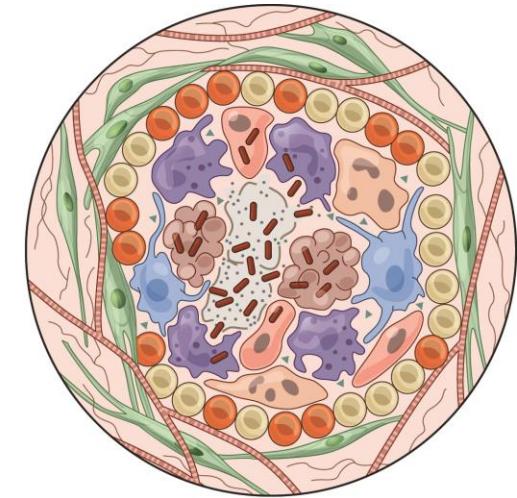
Cellular composition?



- Lymphocyte
- Granulocyte
- Macrophage
- Dendritic
- Epithelial

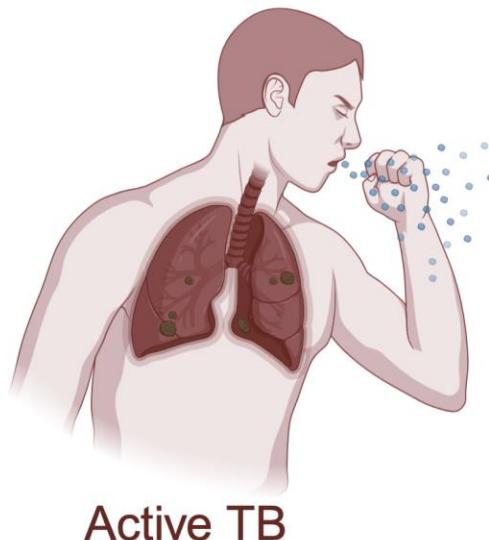
Does reinfection modulate gene expression?



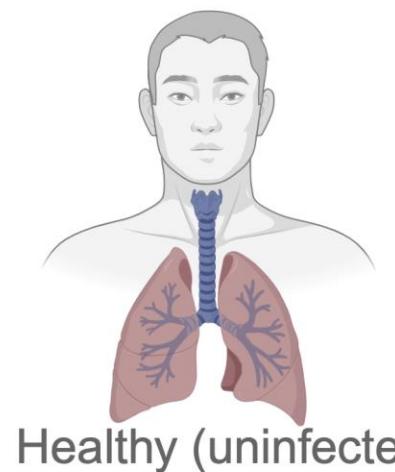
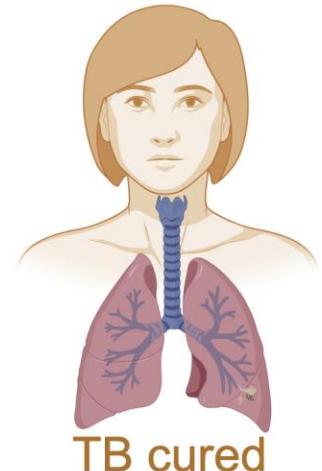


## Background: *Mtb* Reinfection Biology

# Contextualizing *Mtb* Reinfection Events



Active TB



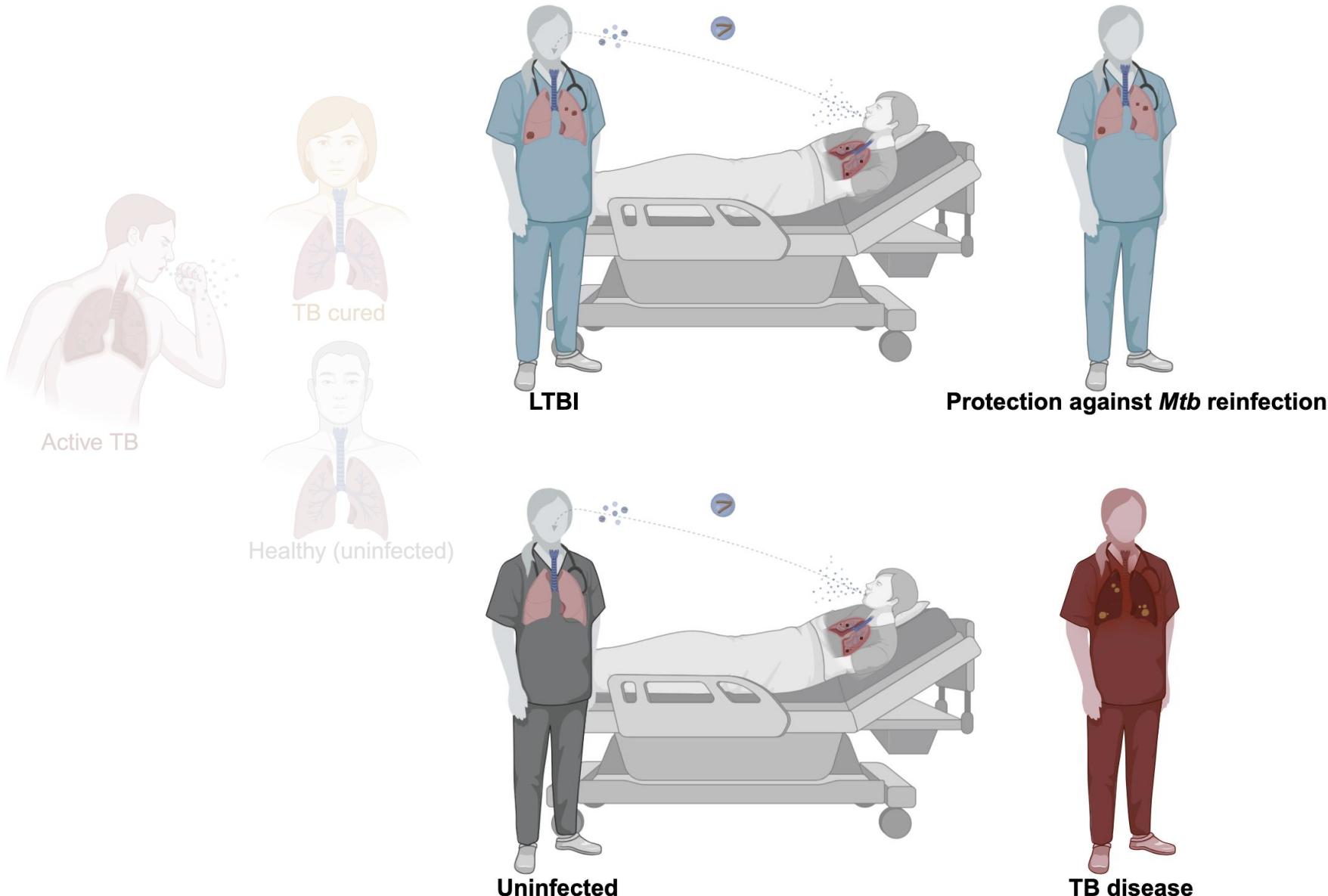
Healthy (uninfected)

Rate of recurrent TB disease (relapse or reinfection) after successful drug treatment:

- 18x China
- 14.6x Spain
- 4x South Africa (*bonafide* reinfection)

J-P Millet et al., 2009; Shen et al., 2017; Verver et al., 2005

# Contextualizing *Mtb* Reinfection Events



LTBI associated with  
a 79% lower risk in  
developing TB

Andrews et al., *Clin. Infect. Dis*  
(2012)

# Contextualizing *Mtb* Reinfection Events

## Mechanism(s) of protection (or lack thereof) following *Mtb* reinfection?



Primary infection protects  
against reinfection

- Robust bacterial killing
- Minimal Th1 response
- Decrease in *IL1B,CXCL8-11*

Cadena et al., *Plos Pathogens* (2018)

# Flynn, Fortune, and Shalek Labs



HI IMPAcTB



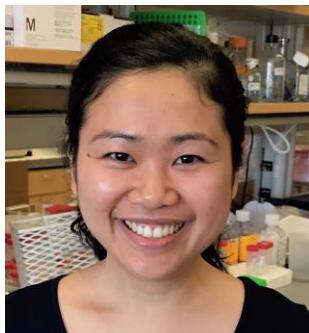
JoAnne Flynn, PhD  
University of Pittsburgh  
Medical School



Sarah Fortune, MD  
Harvard T.H. Chan  
School of Public Health



Alex Shalek, PhD  
M.I.T, Ragon, Broad



Sharie Ganchua, PhD  
(co-first author)



Pauline Maiello, PhD



Sarah Nyquist, PhD

*And many more!*

Dennis Wang  
Douaa Mugahid, PhD  
Mike Chao, PhD  
Son Nguyen, PhD

# I. Infection and in-vivo perturbation

Does primary *Mtb* infection protect against reinfection?

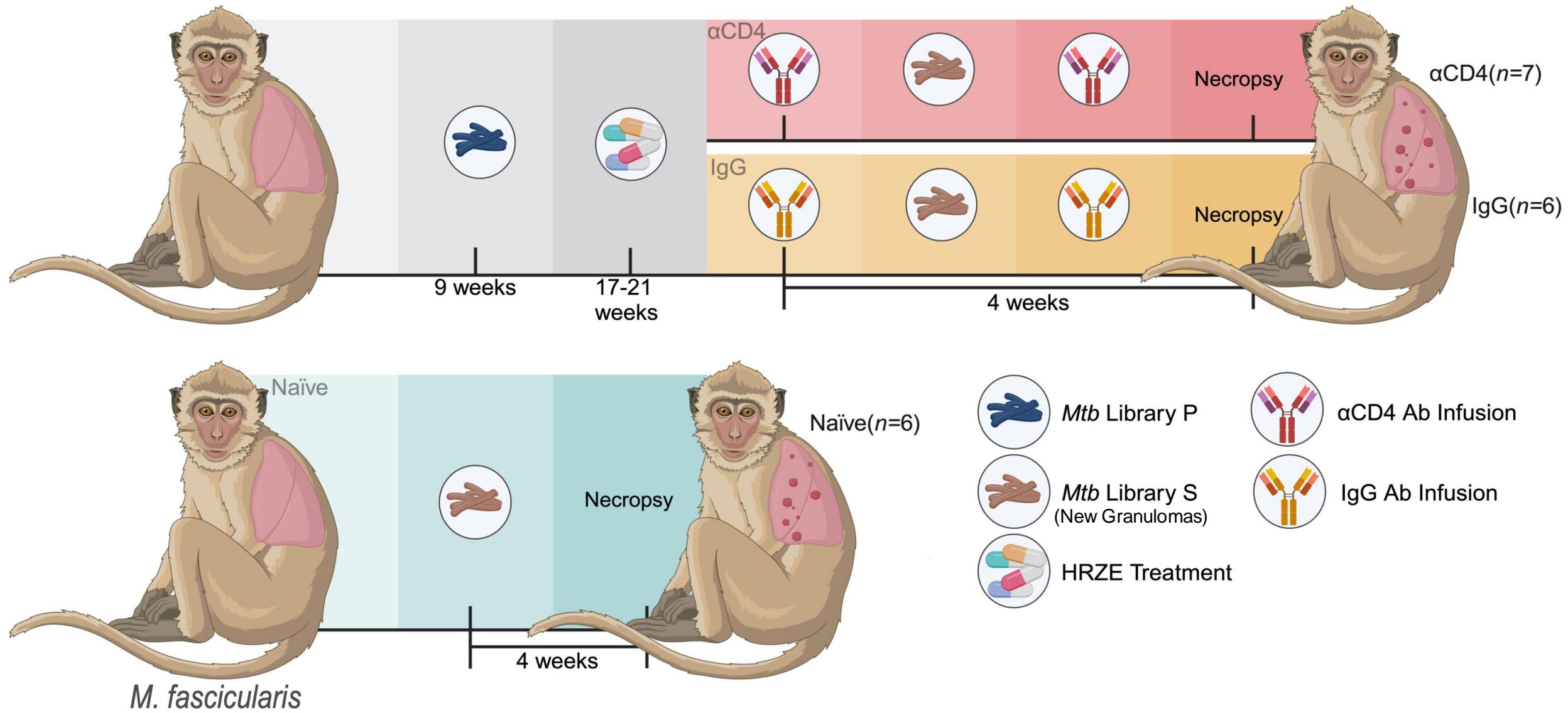


Primary Infection

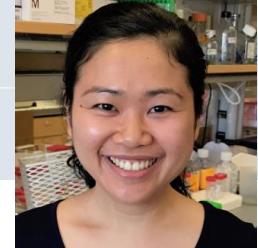


Reinfection

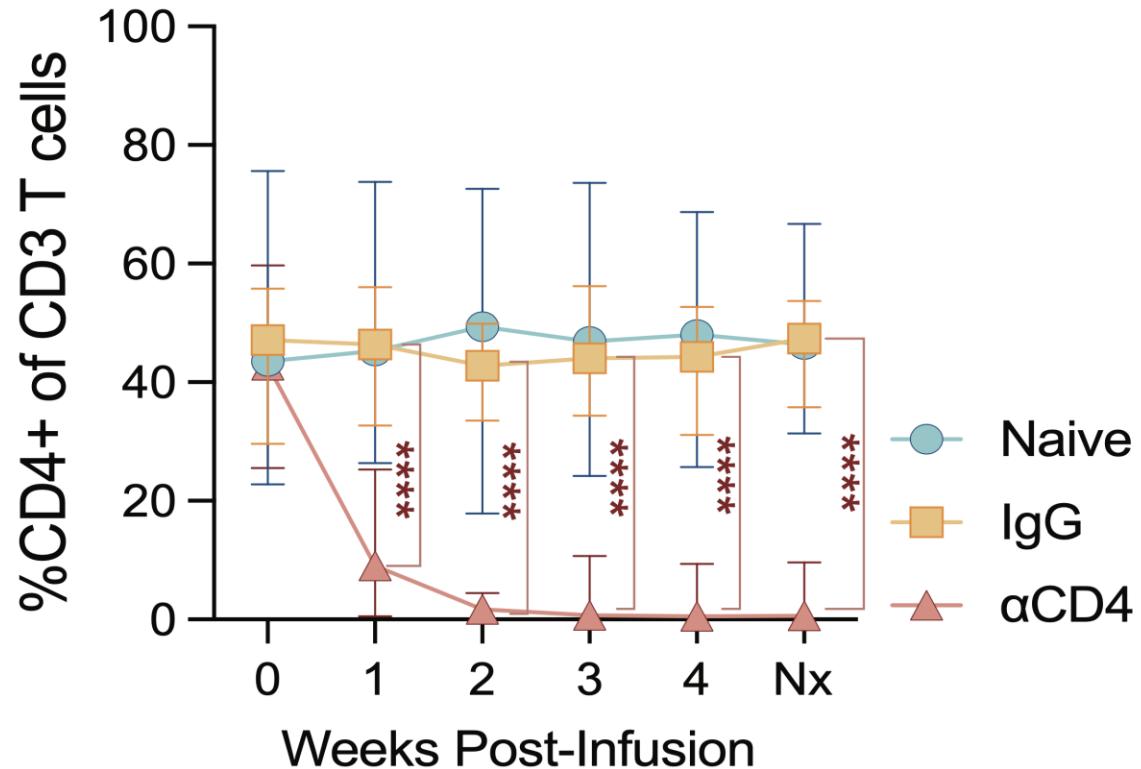
# Experimental Design



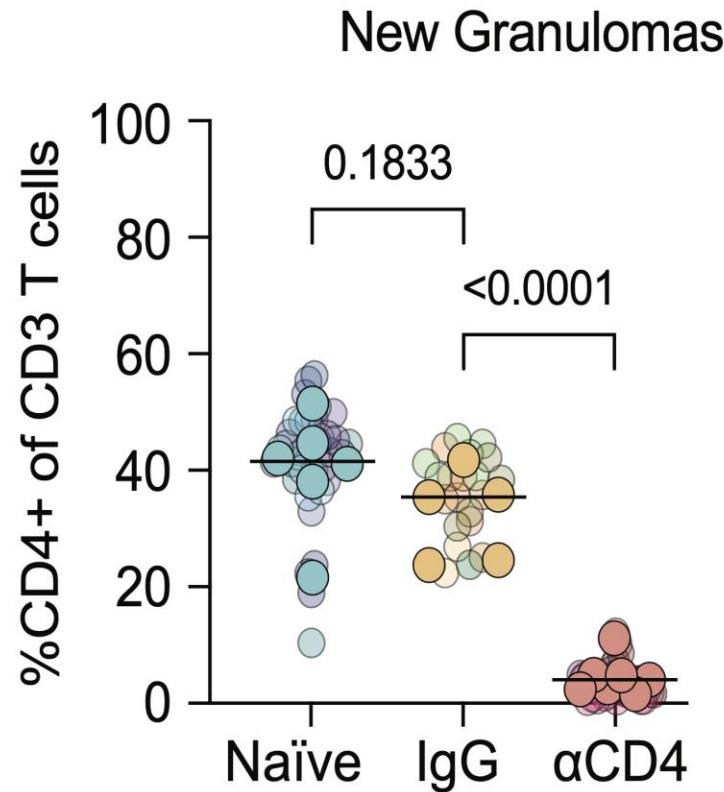
# Antibody-mediated CD4<sup>+</sup> T cell depletion



Sharie Ganchua, PhD

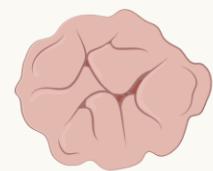


Robust CD4<sup>+</sup> T cell depletion across anatomical sites

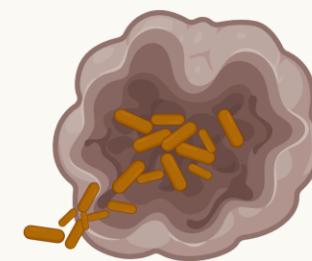


## II. Microbial assays

Restrictive Granuloma

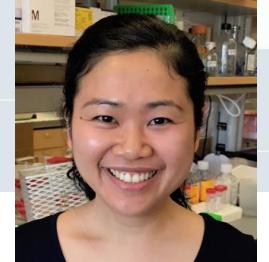


Permissive Granuloma

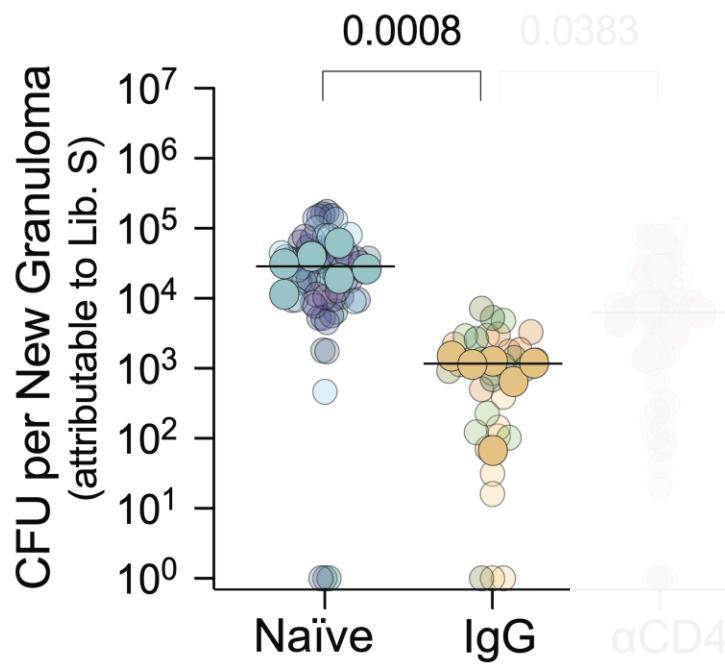


Bacterial growth (or restriction)?

# Reinfection is associated with reduced granuloma formation and bacterial burden in a CD4<sup>+</sup> T-cell dependent manner



Sharie Ganchua, PhD

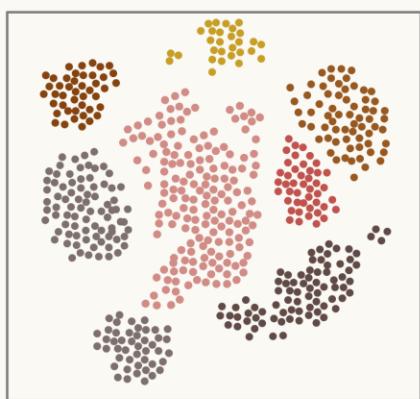


Primary infection confers protection against *Mtb* re-exposure

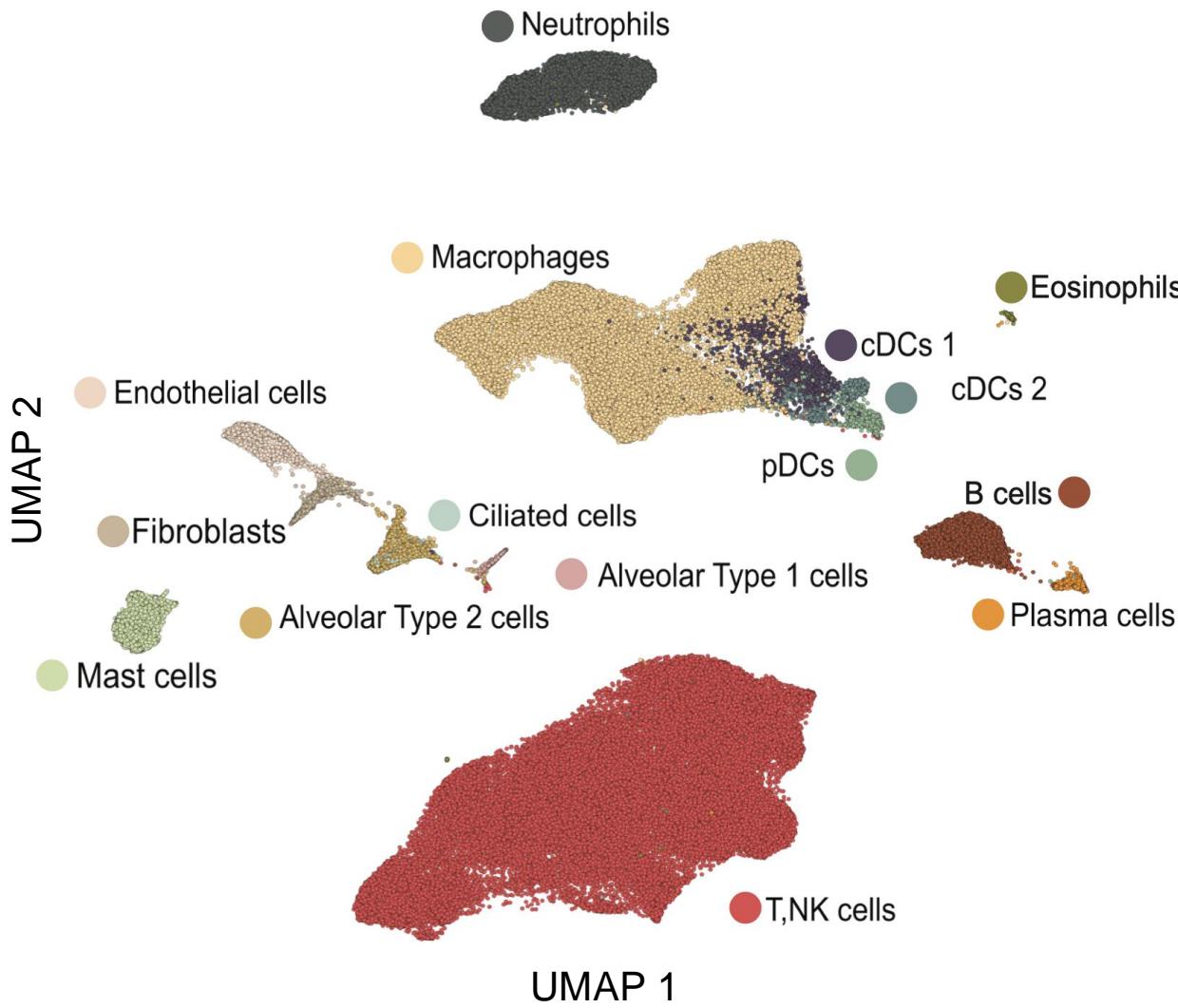
**Reinfection** promotes a growth restrictive niche but not killing

### III. Cellular and molecular correlates of *Mtb* control

Cellular composition?



# Single-cell Census of Reinfestation Granulomas

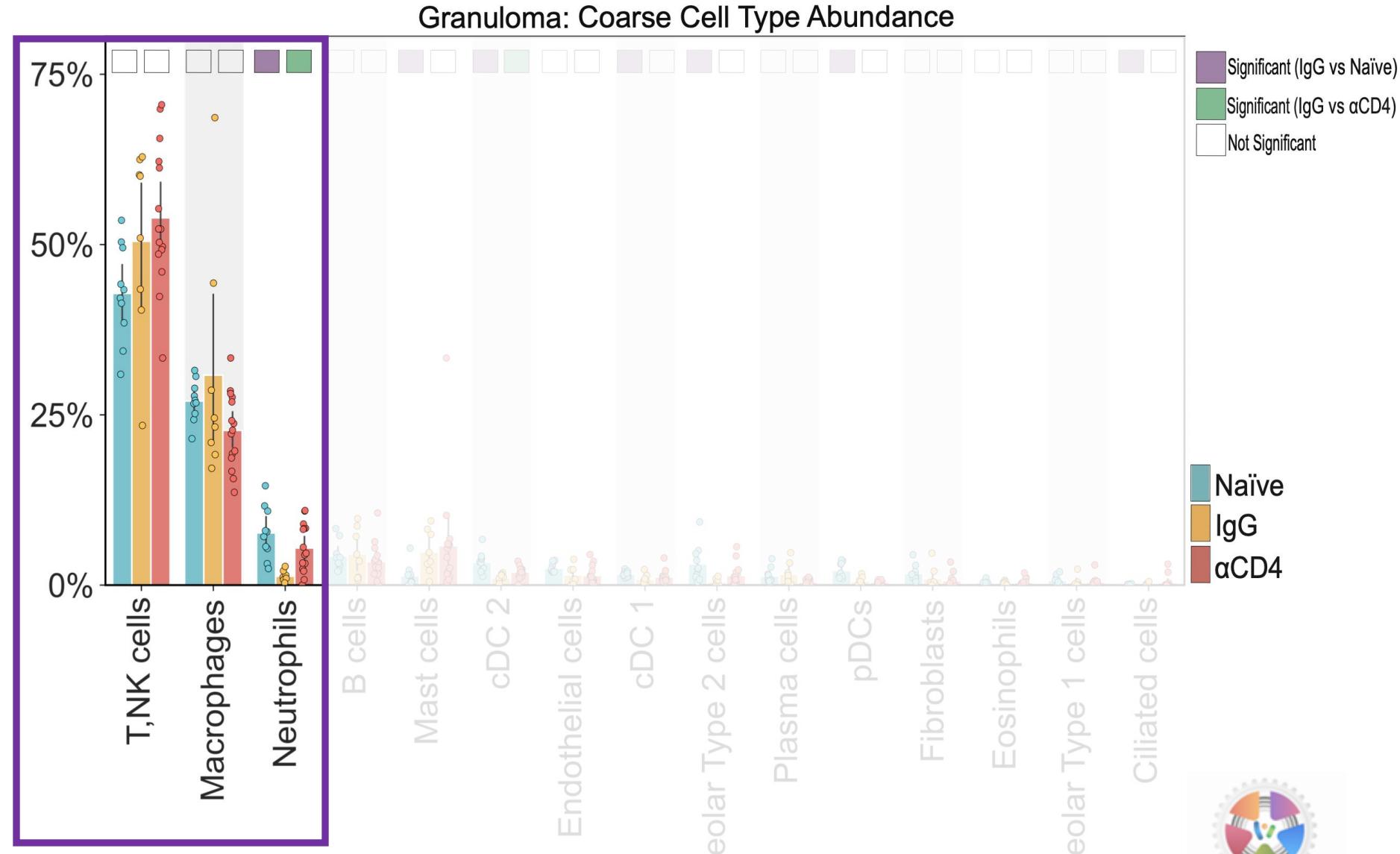


- Seq-Well S<sup>3</sup> scRNA platform\*\*
  - S<sup>3</sup> captures rare + sensitive cell types (e.g., granulocytes)
- 33 granulomas
  - 10 **Naïve**
  - 8 **IgG**
  - 15 **αCD4**
- >88,000 cells
- 15 coarse cell types



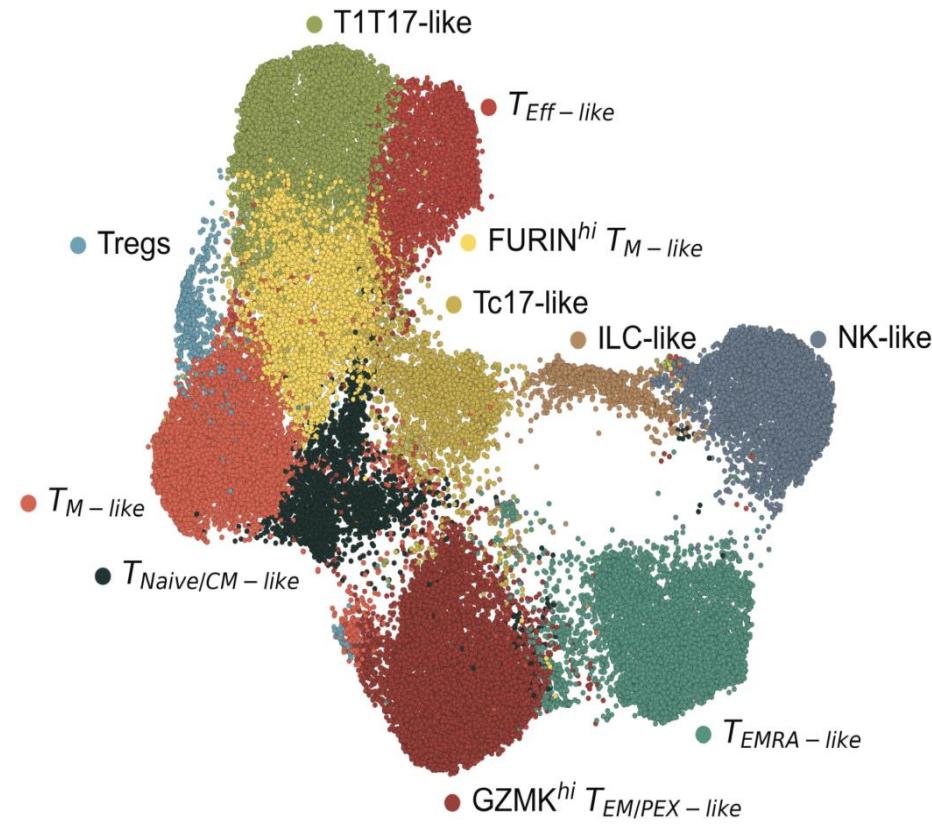
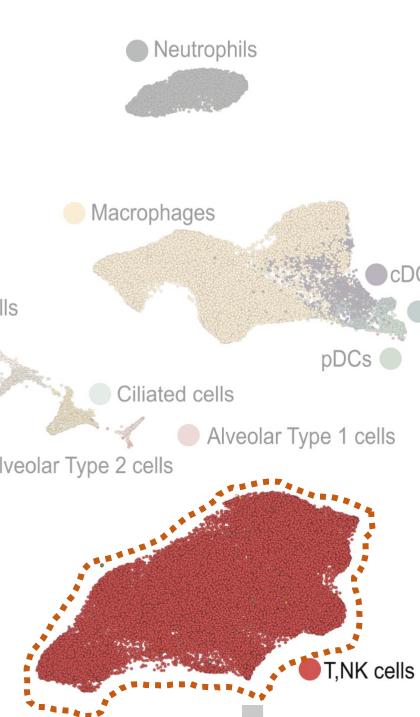
# Suppressed neutrophilic infiltration during reinfection which is CD4-dependent

- Decreased neutrophilia in IgG vs naïve granulomas
- CD4-depletion promotes neutrophilic response

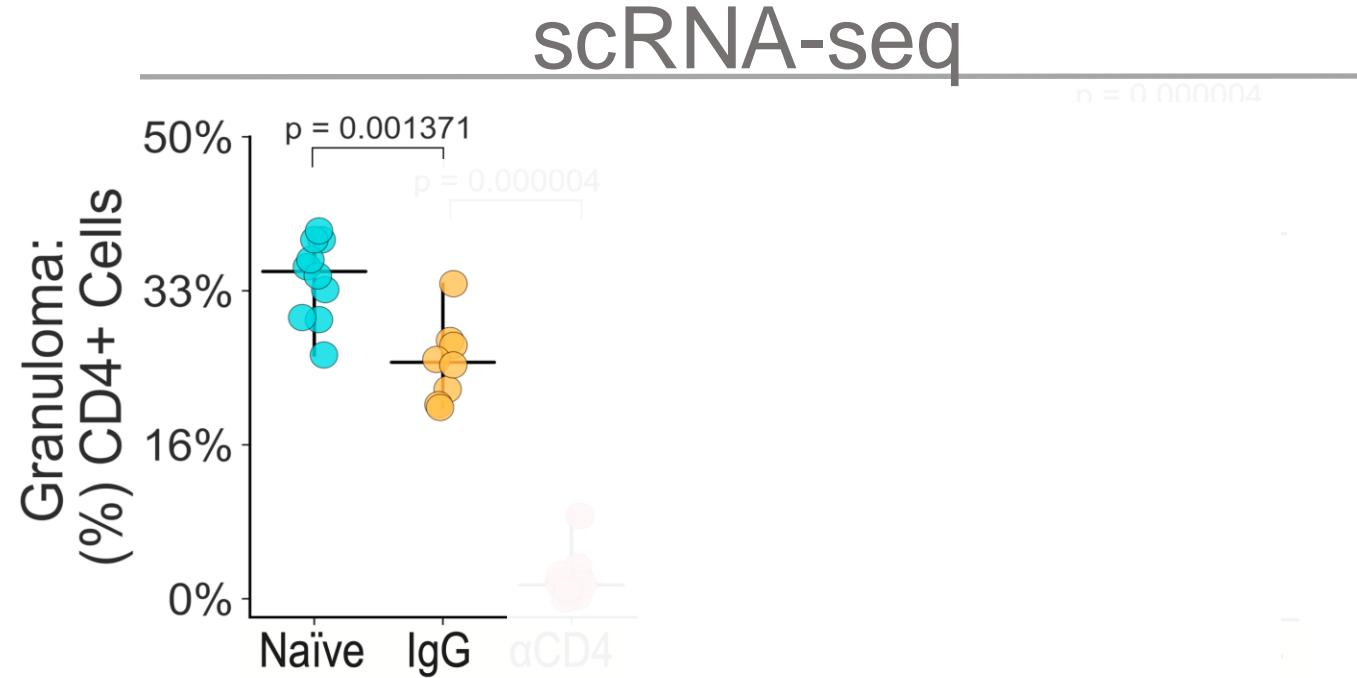


Does *Mtb*  
reinfection alter  
cellularity?

CD4 dependence?

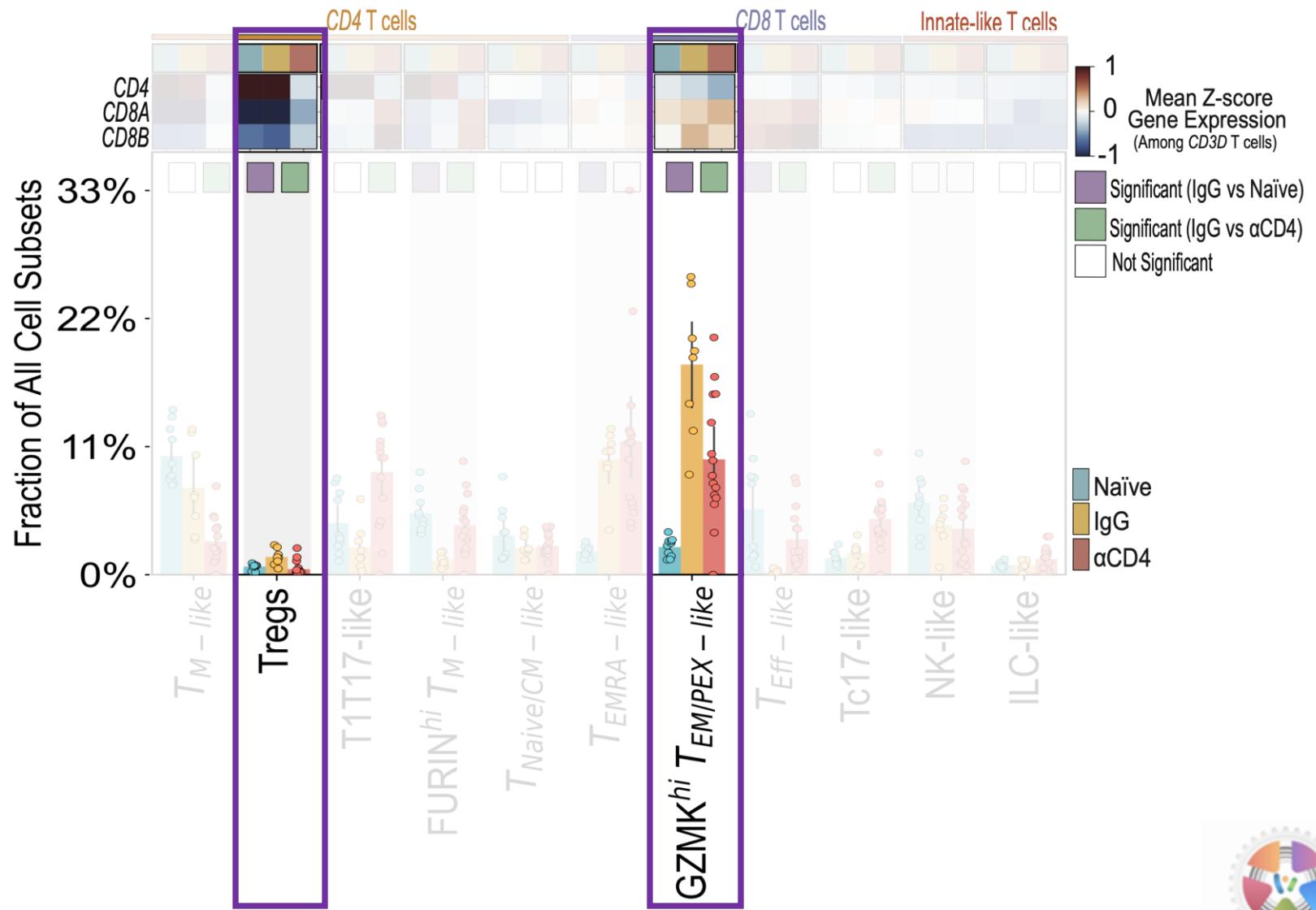


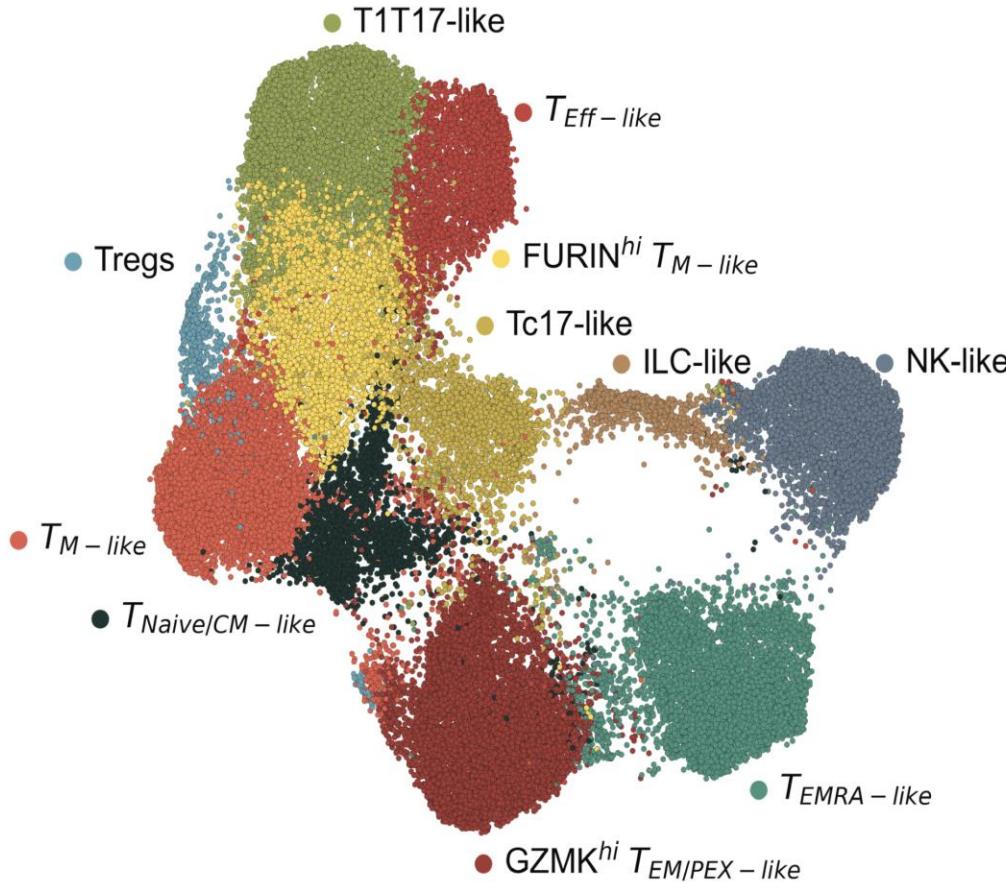
# CD8<sup>+</sup> T cell Enrichment Following *Mtb* Reinfection



# CD4<sup>+</sup> T cells regulate immune tone in granulomas formed after reinfection with *Mtb*

- With CD4 depletion expect decrease in frequency of CD4 enriched subsets and relative increase in other subsets.
  - Tregs
- Subsets that are not CD4 enriched but which also decrease with CD4 depletion may require CD4 function, either directly or indirectly.
  - GZMK<sup>hi</sup> T<sub>EM/PEX-like</sub>

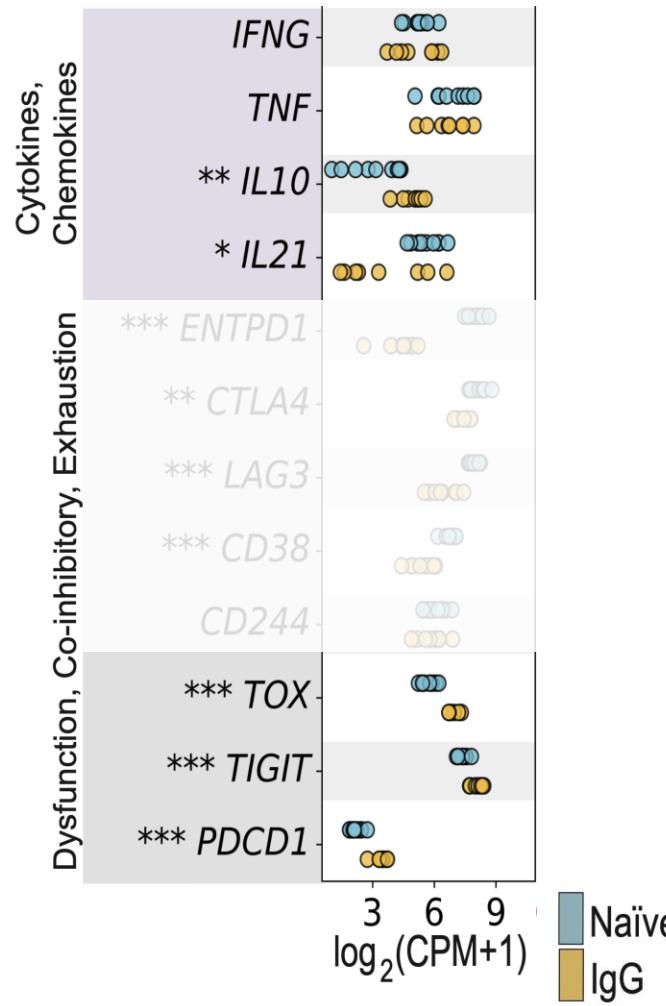




Does *Mtb* reinfection alter immune programming?

CD4 dependence?

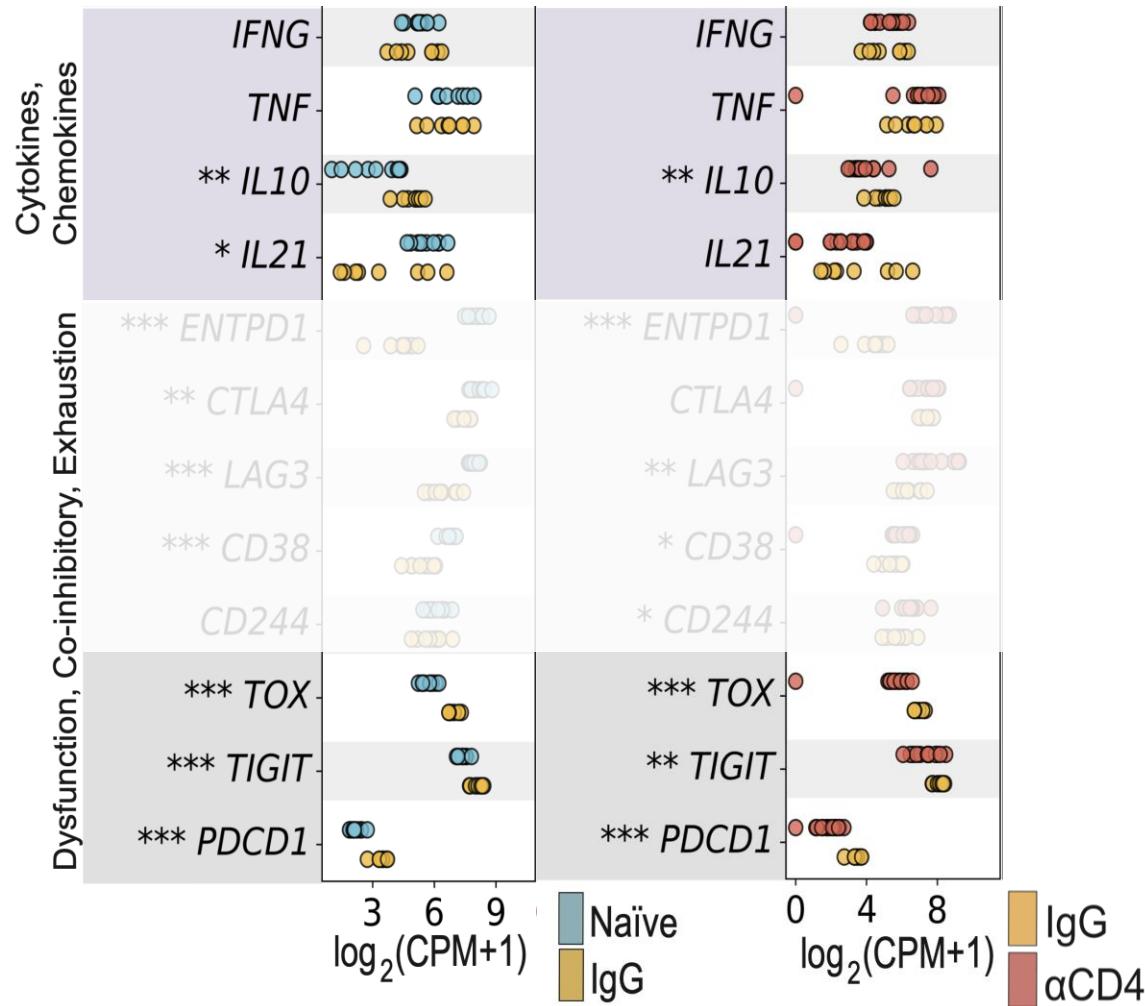
# Immunomodulatory gene programming following *Mtb* reinfection



Pro-inflammatory cytokines (*IFNG*, *TNF*) do not distinguish **reinfection** from **primary infection**

Immunomodulatory molecules *IL10* and *PDCD1* (PD-1) distinguish **reinfection** granulomas from **primary infection**

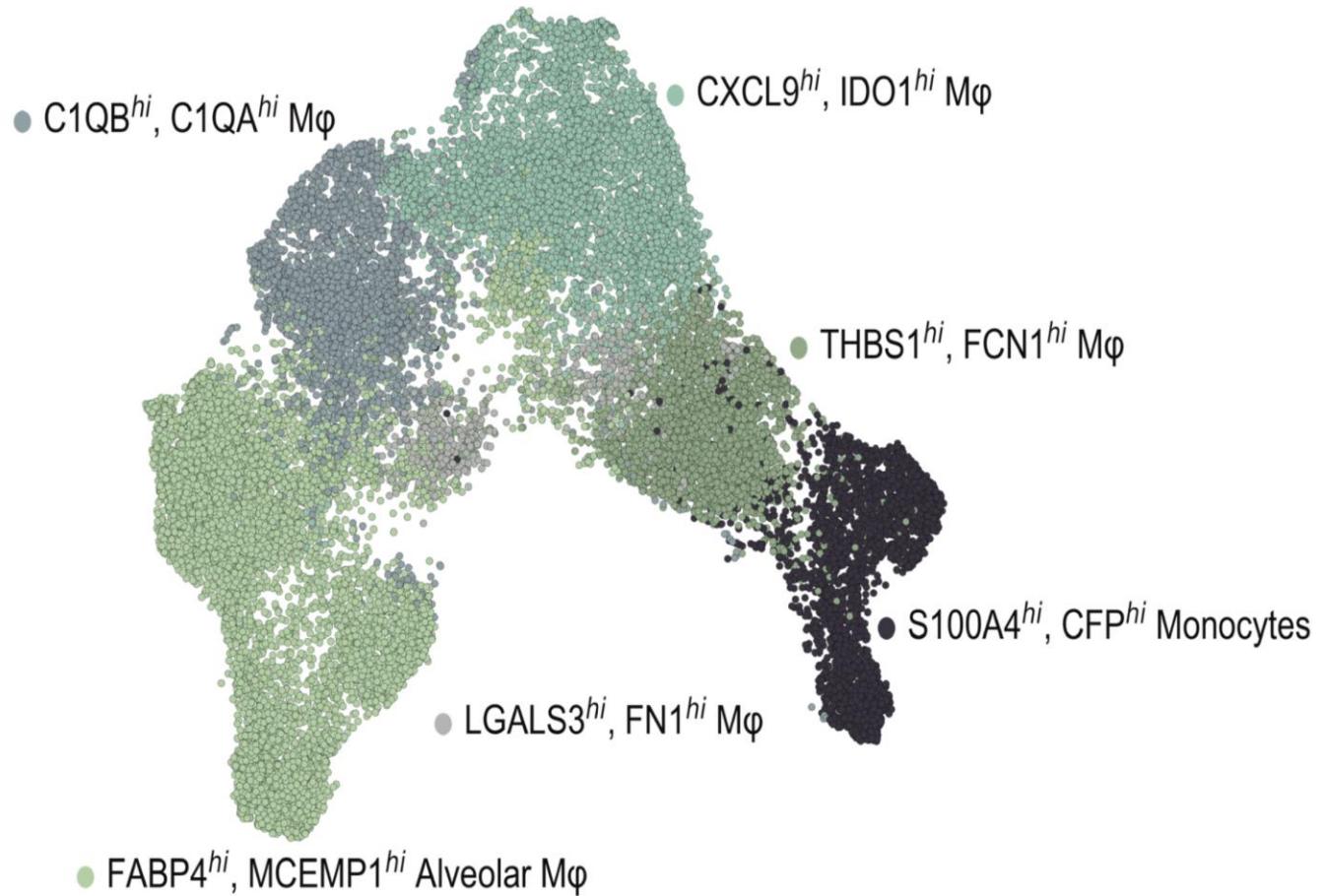
# Immunomodulatory gene programming following *Mtb* reinfection



Pro-inflammatory cytokines (*IFNG*, *TNF*) do not distinguish **reinfection** from **primary infection**

Immunomodulatory molecules *IL10* and *PDCD1* (PD-1) distinguish **reinfection** granulomas from **primary infection**

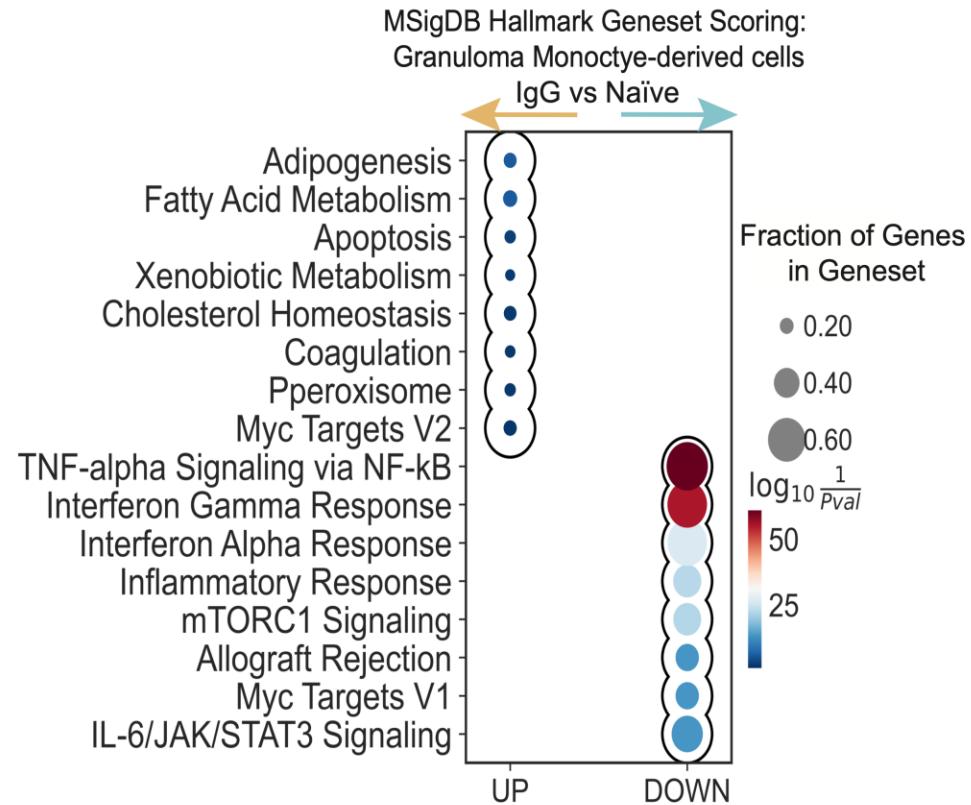
*IL10* and *PDCD1* (PD-1) expression is **CD4-dependent**



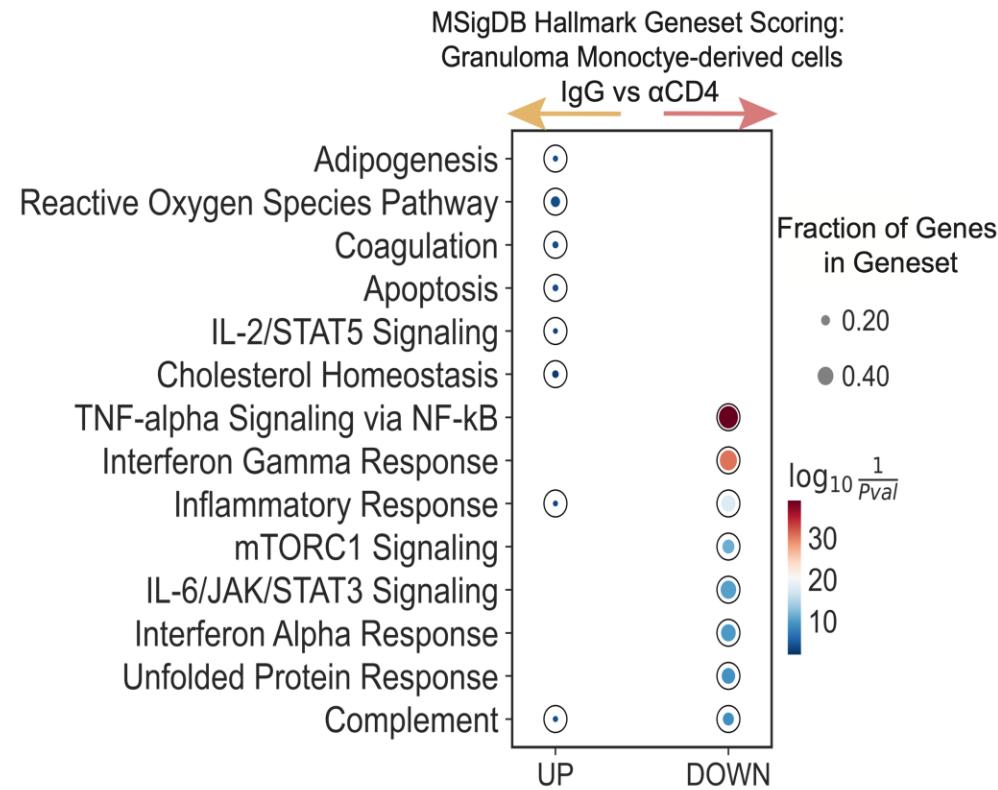
Altered myeloid response  
during reinfection?

CD4<sup>+</sup> dependent  
myeloid activation?

# Attenuated type 1 immunity among monocyte-derived transcriptomes in *Mtb* reinfection granulomas



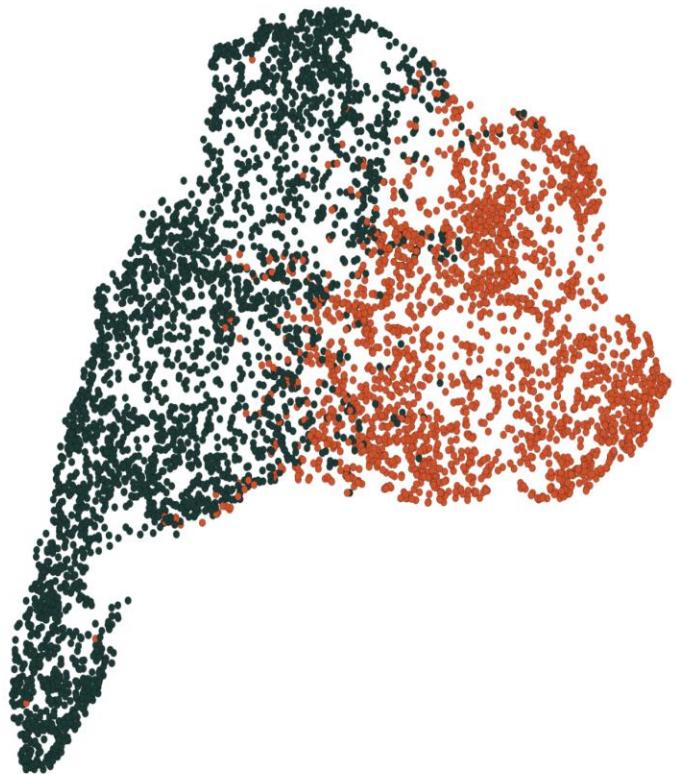
**Naïve** granulomas have an increased inflammatory myeloid response



CD4-independent myeloid inflammation during reinfection

Role for CD4->CD8 regulation of myeloid inflammatory response



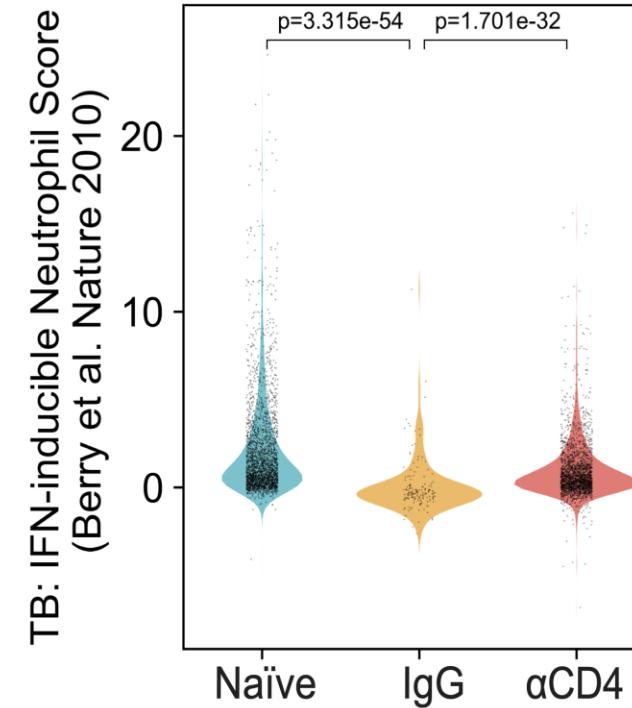
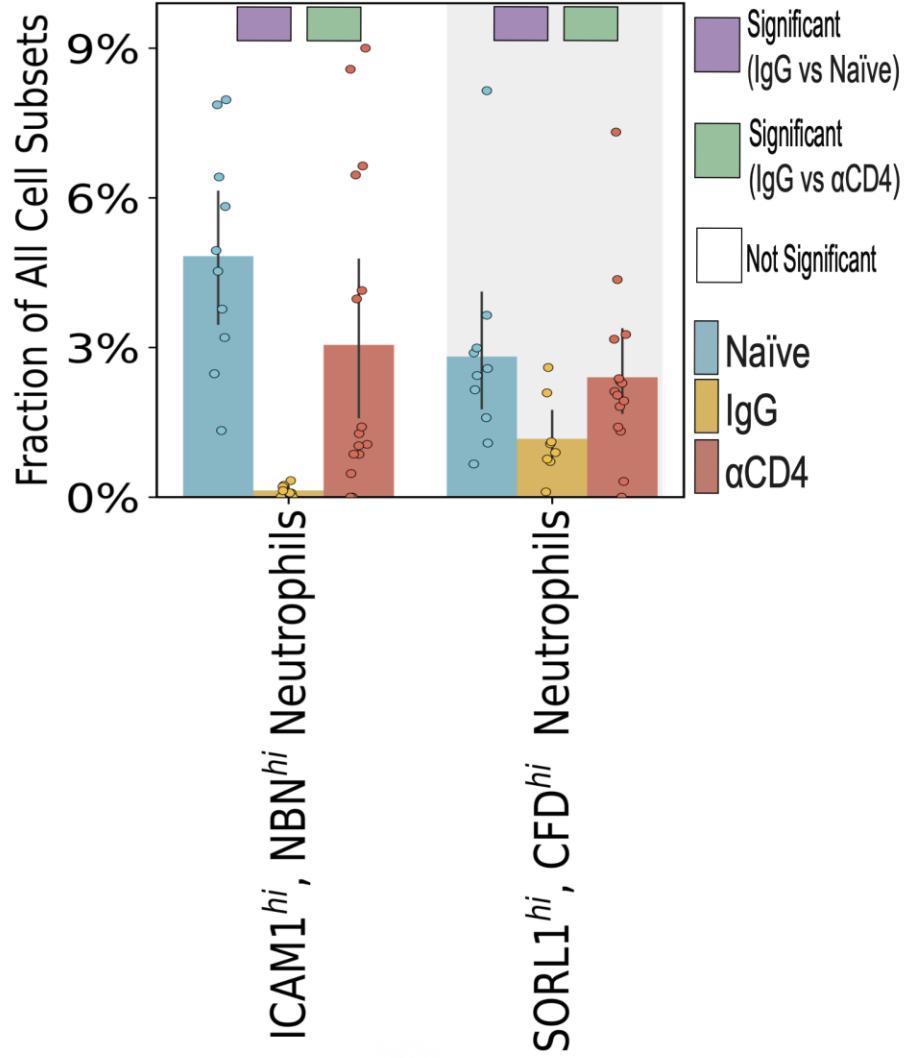


- SORL1<sup>hi</sup>, CFD<sup>hi</sup> Neutrophils
- ICAM1<sup>hi</sup>, NBN<sup>hi</sup> Neutrophils

Neutrophil response during  
reinfection?

Absence of CD4<sup>+</sup> T  
cells?

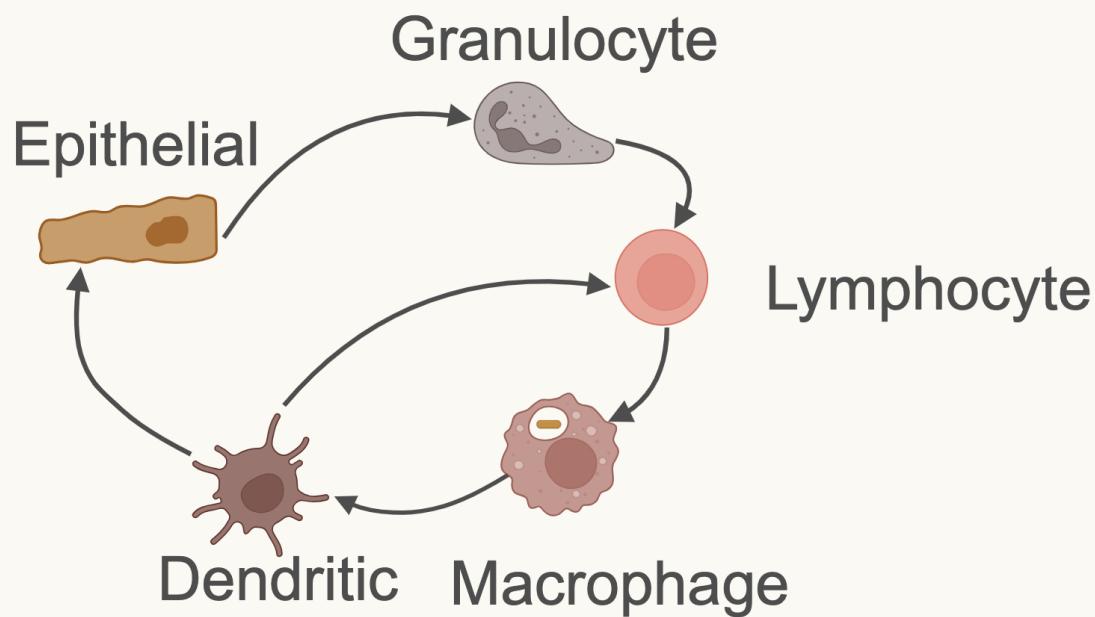
# Blunted neutrophil response following reinfection

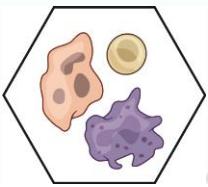


Neutrophilia in **Naïve** and **αCD4** granulomas  
Robust neutrophil interferon induction

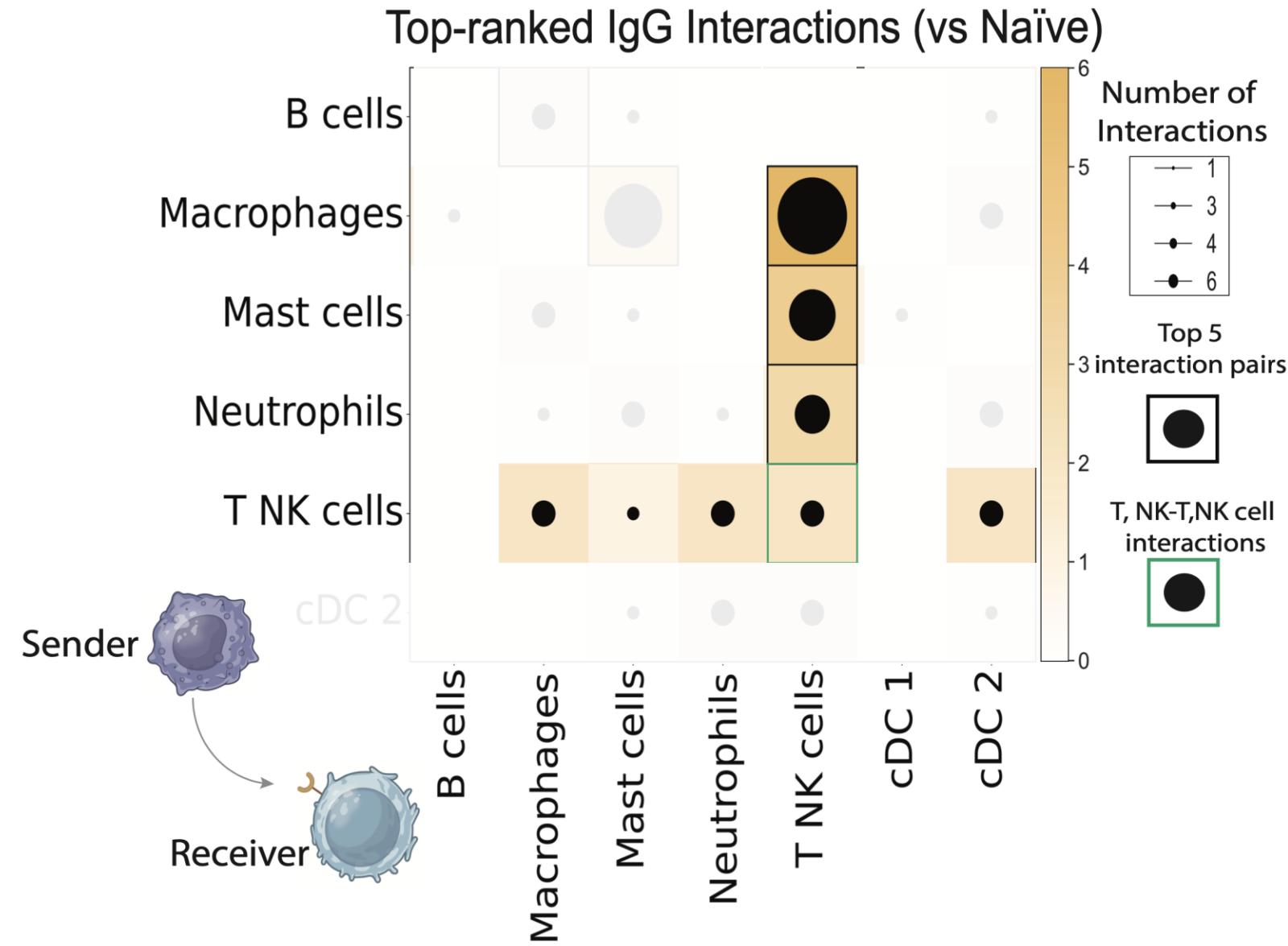
### III. Cellular and molecular correlates of *Mtb* control

Differential cell-cell interactions?





# Differential cell-cell interactions in immunologically primed granuloma



**Reinfection:** T,NK cells are top receivers

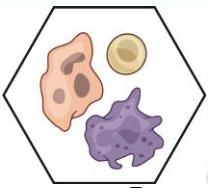
Anti-inflammatory and type II immune cell-cell signaling networks

Senders

- T cell (*IL10, TIGIT*)

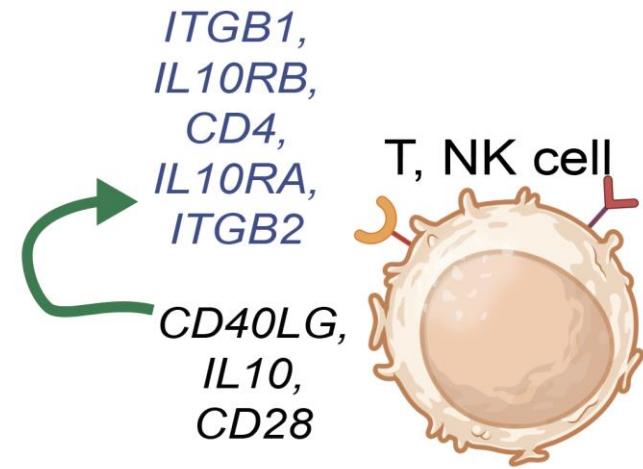
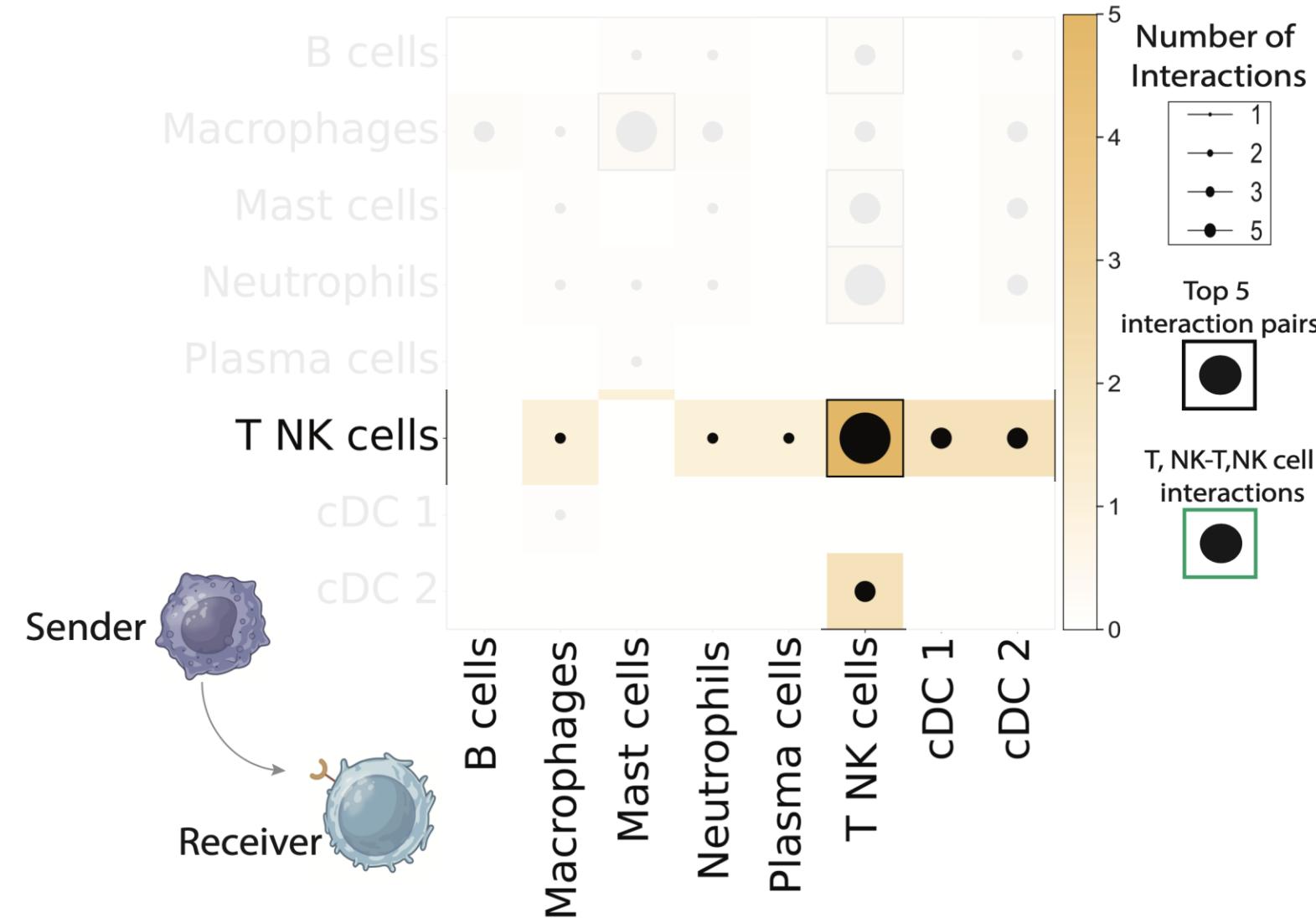
Blunted Type 1 IFN response





# Differential cell-cell interactions in immunologically primed granuloma

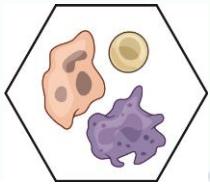
Top-ranked IgG Interactions (vs  $\alpha$ CD4)



Self-reinforcing immunoregulatory T cell circuits in the *presence* of CD4<sup>+</sup> T cells

Suggests CD4-CD8 crosstalk

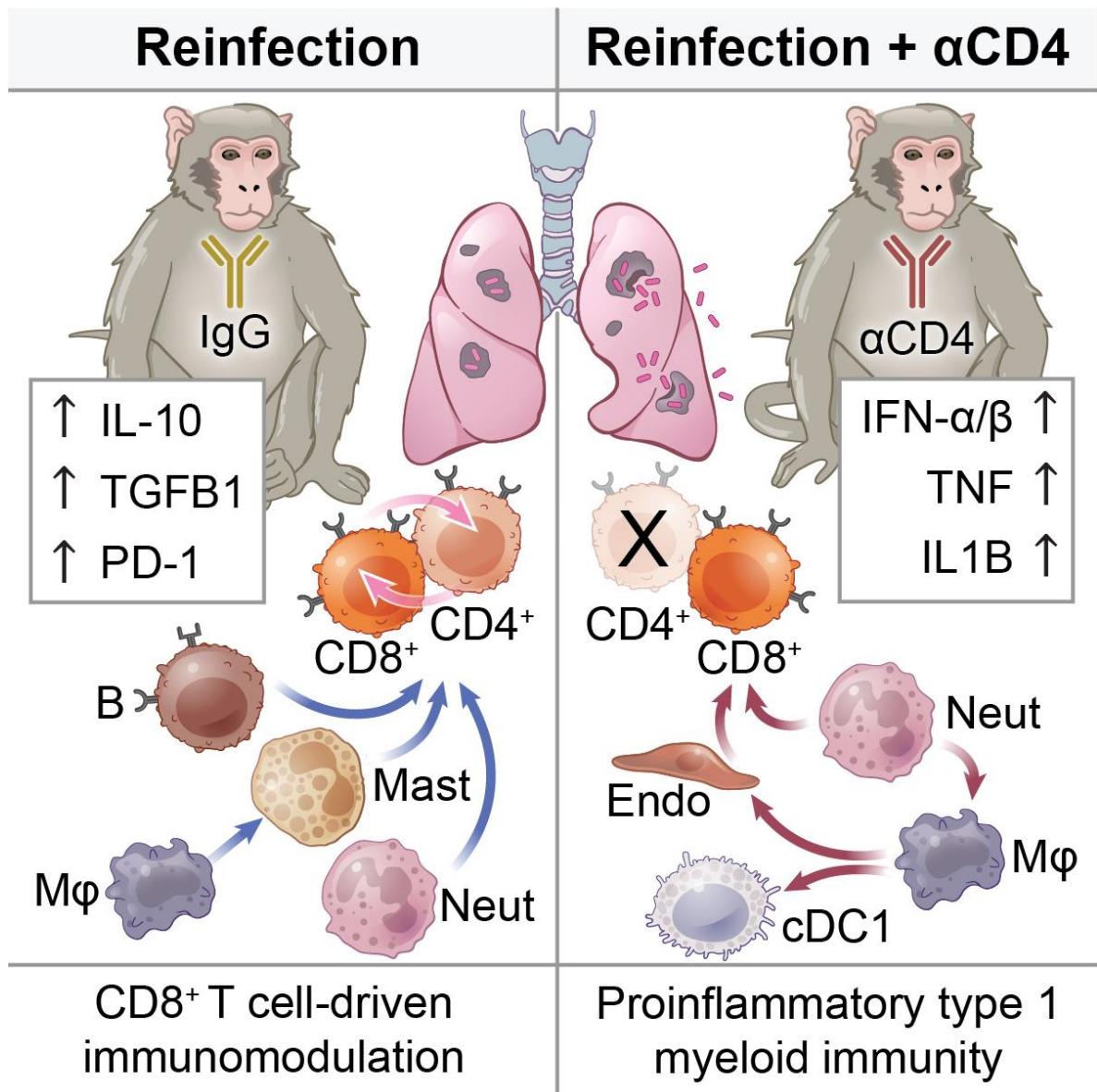




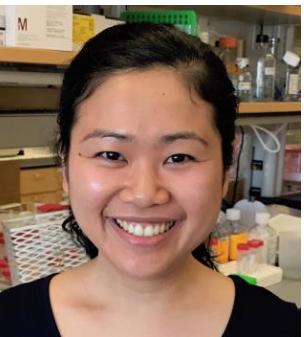
# Immunomodulatory gene and cell-cell interaction networks following *Mtb* reinfection

## Takeaways:

1. CD4 cells required for robust protection.
2. CD4-dependent “tuning” of CD8<sup>+</sup> T cell response.
3. Primed CD4 response downregulates neutrophilic infiltrates.
4. CD4<sup>+</sup> T cells regulate pro- and anti-inflammatory gene programming and cell-cell interaction networks to balance anti-*Mtb* immunity and mitigate TB-associated inflammatory sequelae



# Acknowledgements



JoAnne Flynn

Sharie Ganchua

Sarah Fortune

## Flynn Lab (UPitt)

JoAnne Flynn  
Sharie Ganchua  
Pauline Miaelo

## Fortune Lab (HSPH)

Sarah Fortune  
Douaa Mugahid  
Mike Chao  
Jake Rosenberg

## Shalek Lab (MIT)

Alex Shalek  
Sarah Nyquist  
Andrew Navia  
Carly Ziegler  
Dennis Wang  
Feng Shan  
Nancy Tran  
Samira Ibrahim  
Sarah Quinn  
Son Nguyen  
Tasneem Jivanjee  
Vince Miao

SEARLE SCHOLARS  
PROGRAM  
FUNDING EXCEPTIONAL YOUNG SCIENTISTS

Ragon Institute  
of MGH, MIT and Harvard

National Heart  
Lung and Blood Institute

National Institute  
of Allergy and  
Infectious Diseases

National Human  
Genome Research  
Institute

Chemistry

NOVARTIS

Agilent Technologies

THE ALEXANDER AND MARGARET  
STEWART TRUST

BROAD  
INSTITUTE

KLARMAN  
CELL OBSERVATORY  
AT BROAD INSTITUTE

GORDON AND BETTY  
MOORE  
FOUNDATION

CHAN  
ZUCKERBERG  
INITIATIVE

impactTB

NIH DIRECTOR'S  
NEW INNOVATOR  
AWARD

Arnold and Mabel  
BECKMAN  
FOUNDATION



BILL & MELINDA GATES FOUNDATION



center for  
microbiome  
informatics &  
therapeutics



*And many more!*

**Thank you!**  
**Questions?**

