

75 HEALTH FOR ALL



Joint TB-Meeting: 32. Tuberkulose-Symposium der LLS 2. Swiss Translational TB Forum

Pause und Gruppenverteilung

Swiss Translational
TB Forum



75 HEALTH FOR ALL



Joint Meeting :
32^{ème} Symposium Tuberculose de la LPS
2^{ème} Swiss Translational TB Forum

Pause et répartition par groupe

Swiss Translational
TB Forum



News aus der Forschung (EN) | Raumwechsel! Actualité de la recherche (ANG) | Changement de salle !

10.00 What are B cells doing during TB, and does it matter?

Carolyn King, Research group Leader

10.30 cMYC expression determines the outcome of macrophages

Edoardo Sarti, University Hospital of Zürich

10.40 Exploring Immune-Stromal cell interactions during Tuberculosis

Tiphaine Camarasa, Infection Immunology Lab, Department of Biomedicine, University of Basel

10.50 Exploring the mechanisms of Mycobacteria-mediated membrane damage: Small or catastrophic, two types of damages leading to different bacterial fates

Céline Michard, Department of Biochemistry, Faculty of Science, University of Geneva

News aus der Praxis | Hauptsaal

Actualités du terrain | Salle principale

10.00 Das neue Handbuch

Otto Schoch, Facharzt Pneumologie und
Schlafmedizin | Kantonsspital St. Gallen

Le nouveau manuel

Otto Schoch, spécialiste en pneumologie et
médecine du sommeil | Hôpital cantonal de St-Gall

10.15 Vorstellung von zwei Fällen komplexer Umgebungsuntersuchungen

Annett Hunger, Lungenliga Ost
Veronica Maglio, Lungenliga Waadt

Présentation de deux cas d'enquêtes d'entourage complexes

Annett Hunger, Ligue pulmonaire Est
Veronica Maglio, Ligue pulmonaire vaudoise

Mit Unterstützung von
Avec le soutien de



Diasorin

Swiss Translational
TB Forum

LUNGENLIGA SCHWEIZ
LIGUE PULMONAIRE SUISSE
LEGA POLMONARE SVIZZERA
LIA PULMUNARA SVIZRA



T-B or not to be: our road to studying tuberculosis immunity

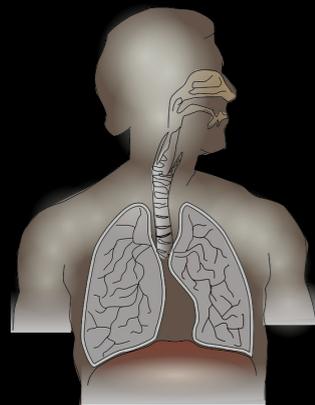
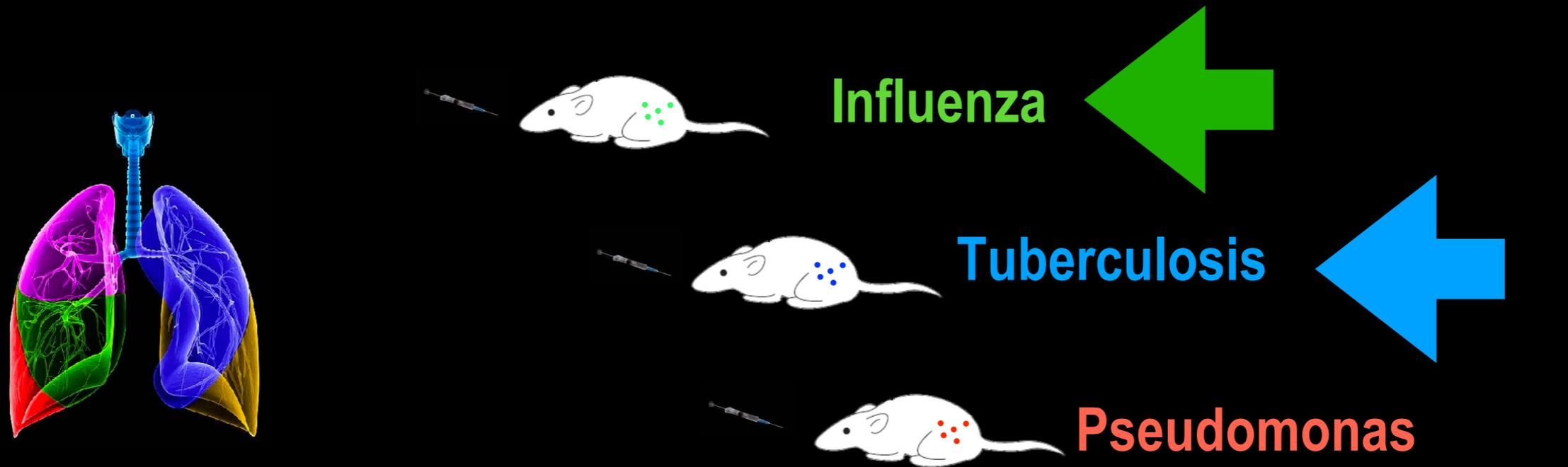
Carolyn King

University of Basel

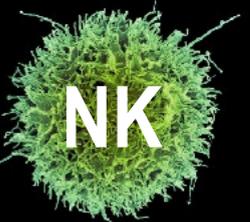
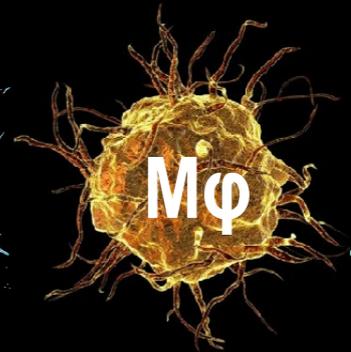
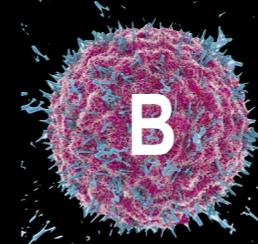
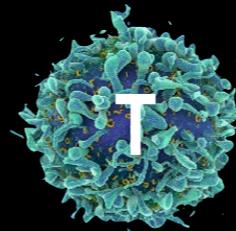
30 October 2024



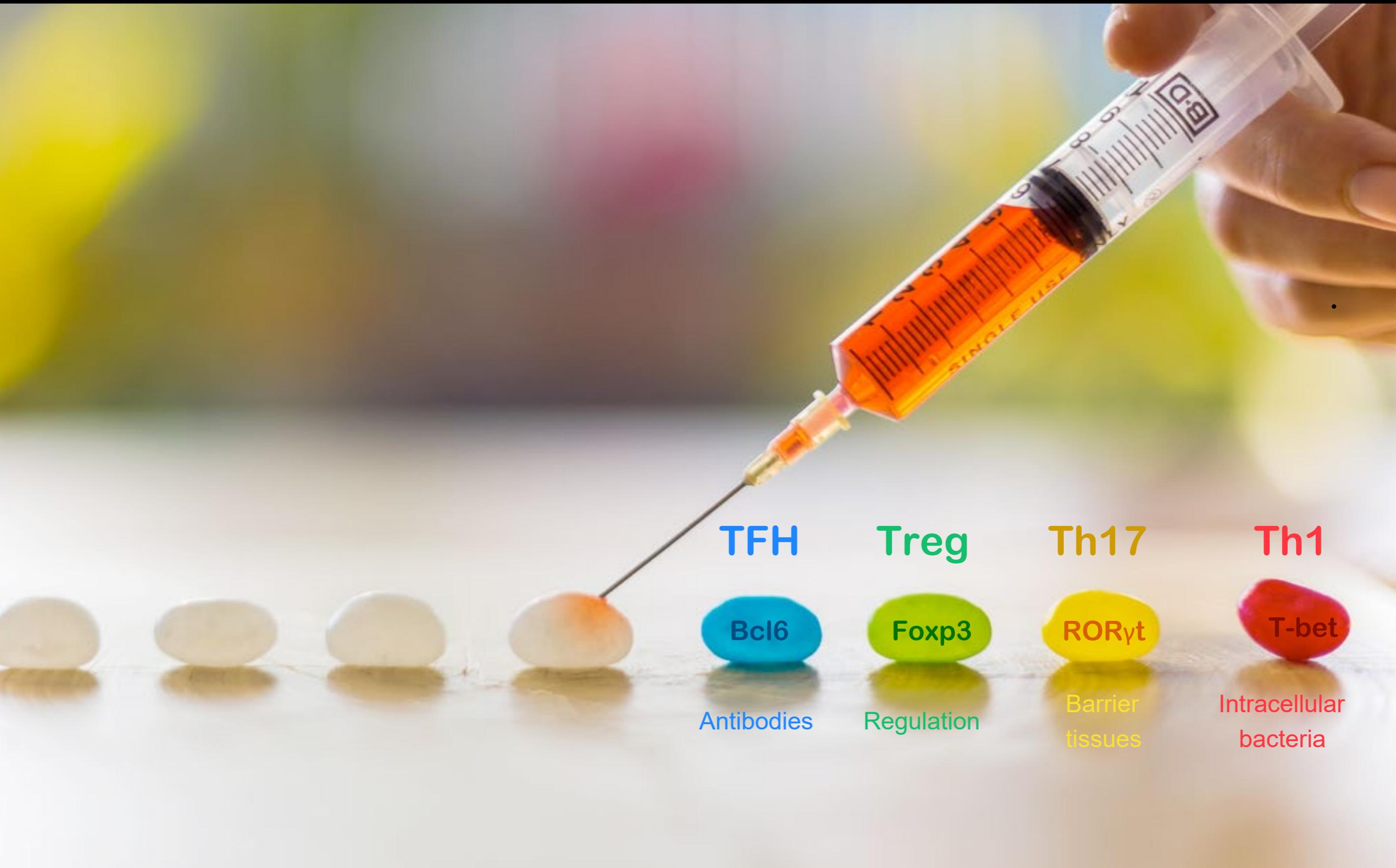
Pulmonary immune responses



BAL fluid
alveolar organoids



CD4 T cells are diverse



TFH

Bcl6

Antibodies

Treg

Foxp3

Regulation

Th17

ROR γ t

Barrier
tissues

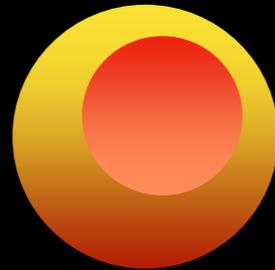
Th1

T-bet

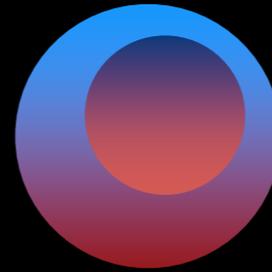
Intracellular
bacteria

CD4 T cell responses are flexible

Tbet
Roryt



Bcl6
Tbet

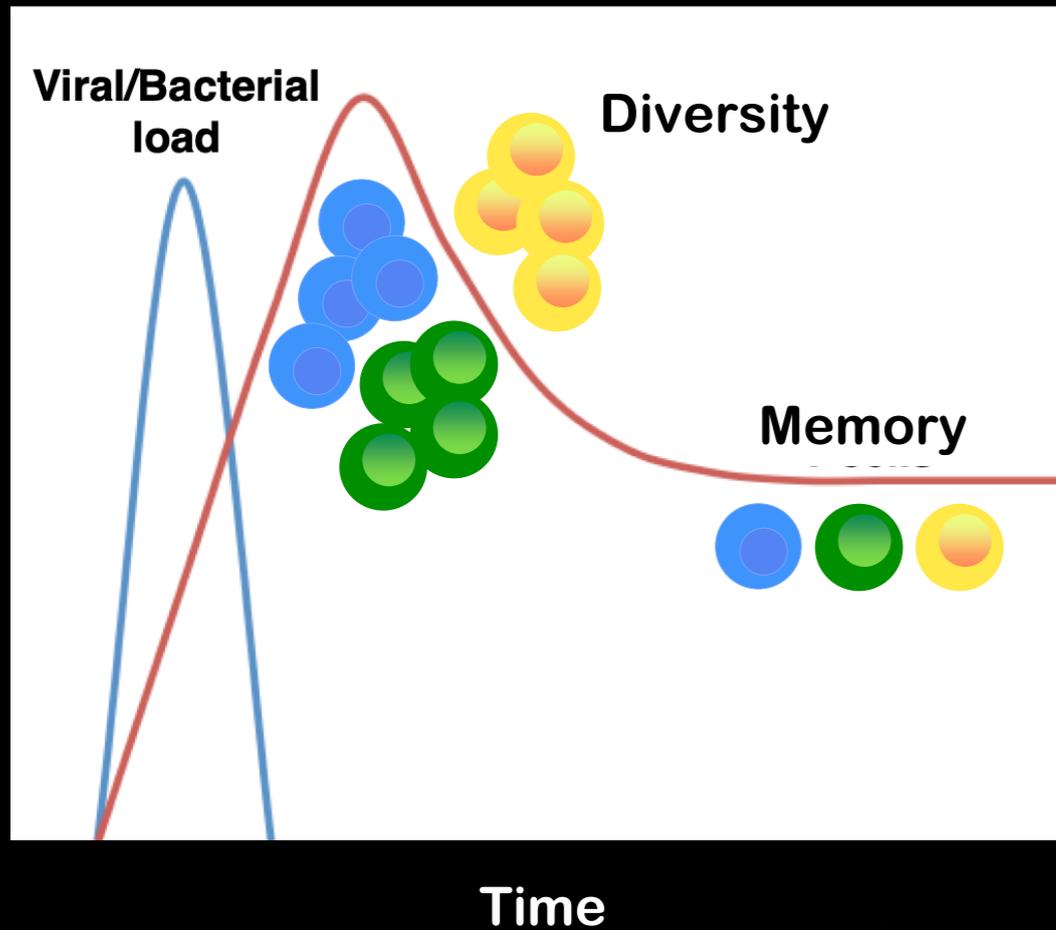


Foxp3
Bcl6



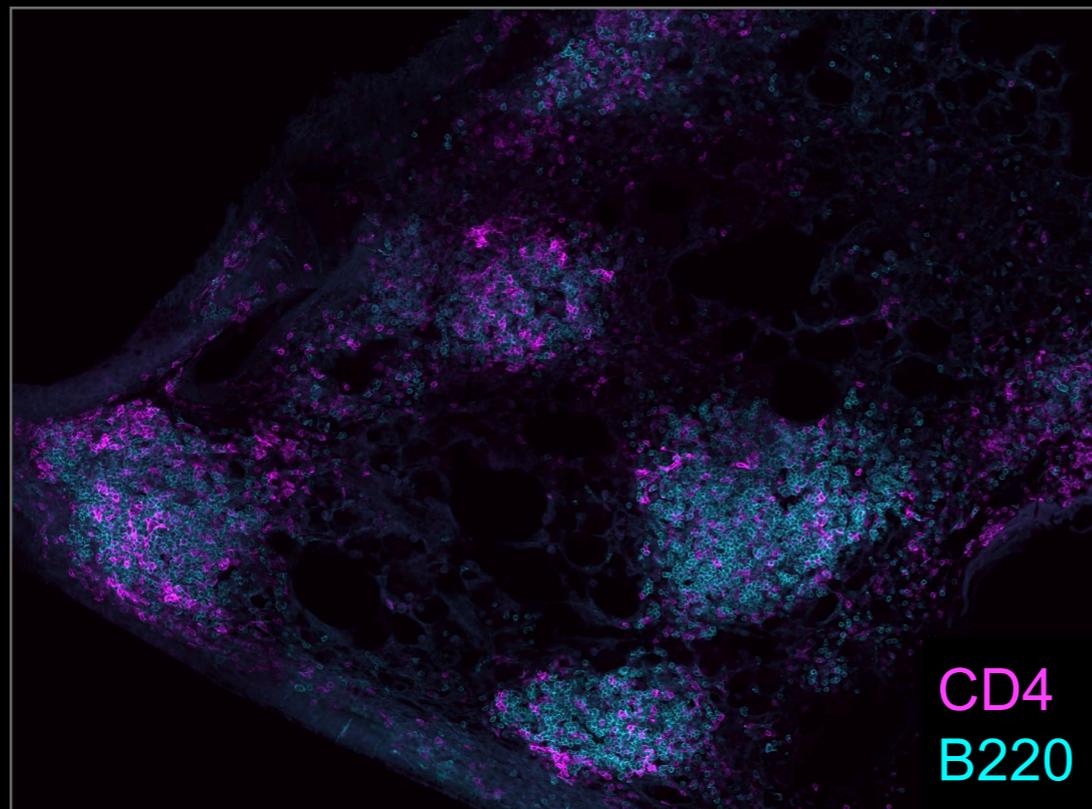
Immune responses are dynamic

Typical T cell response

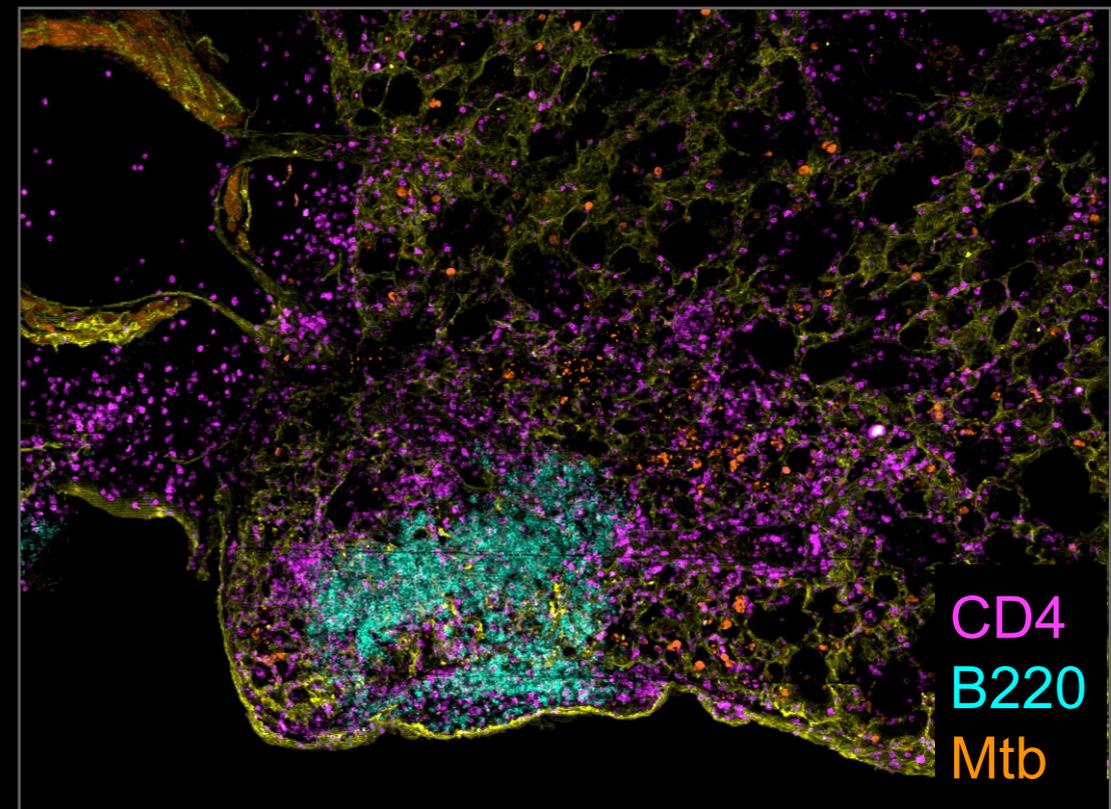


CD4 T cells in the mouse lung

Influenza



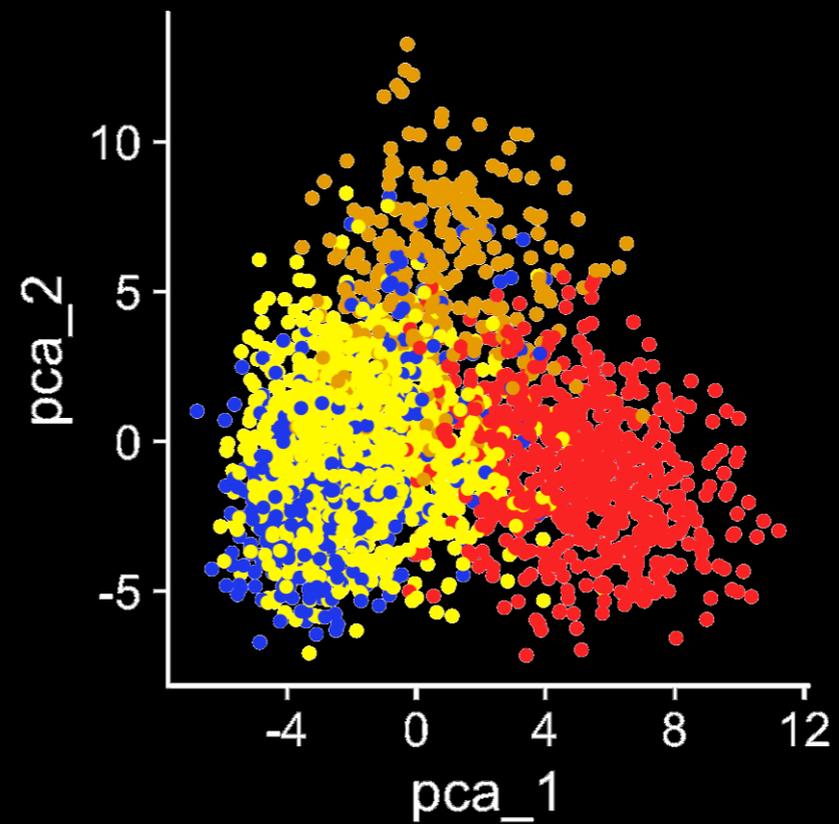
Tuberculosis



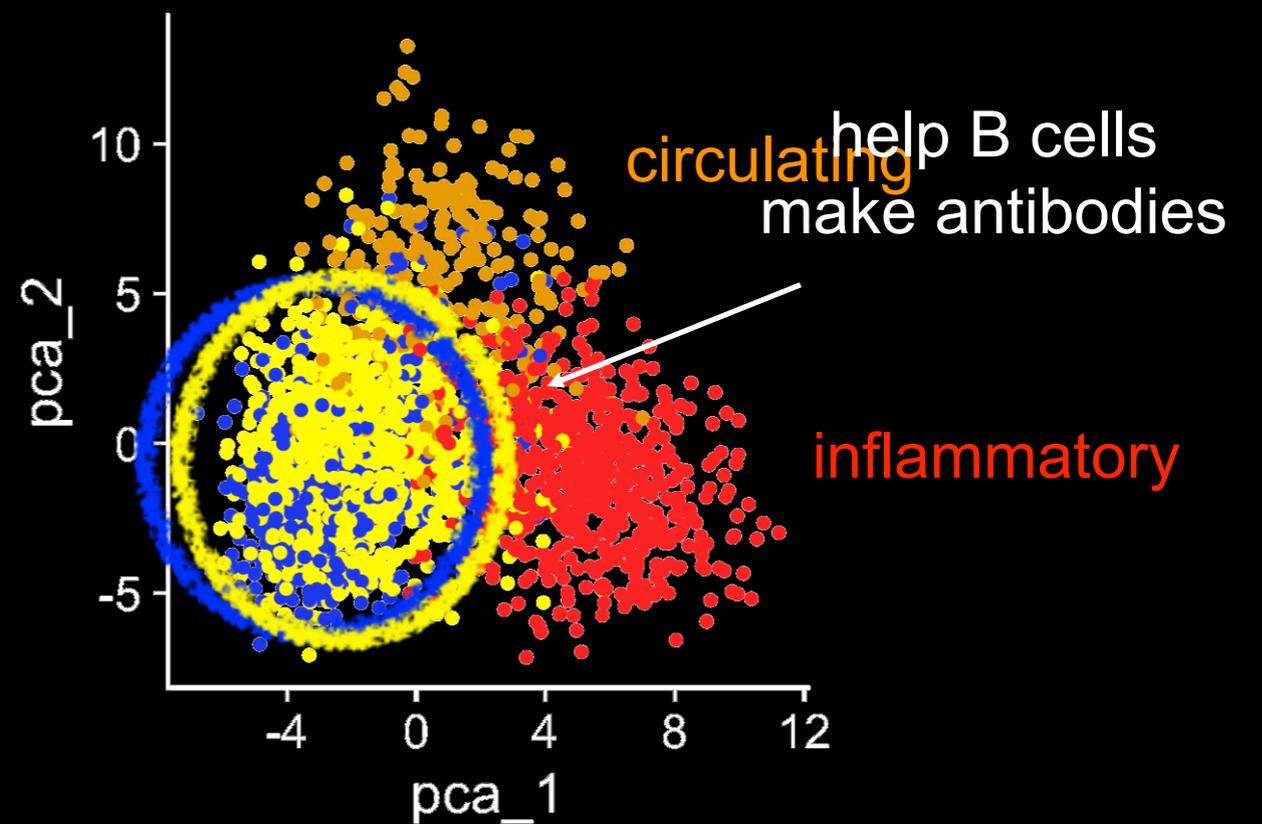
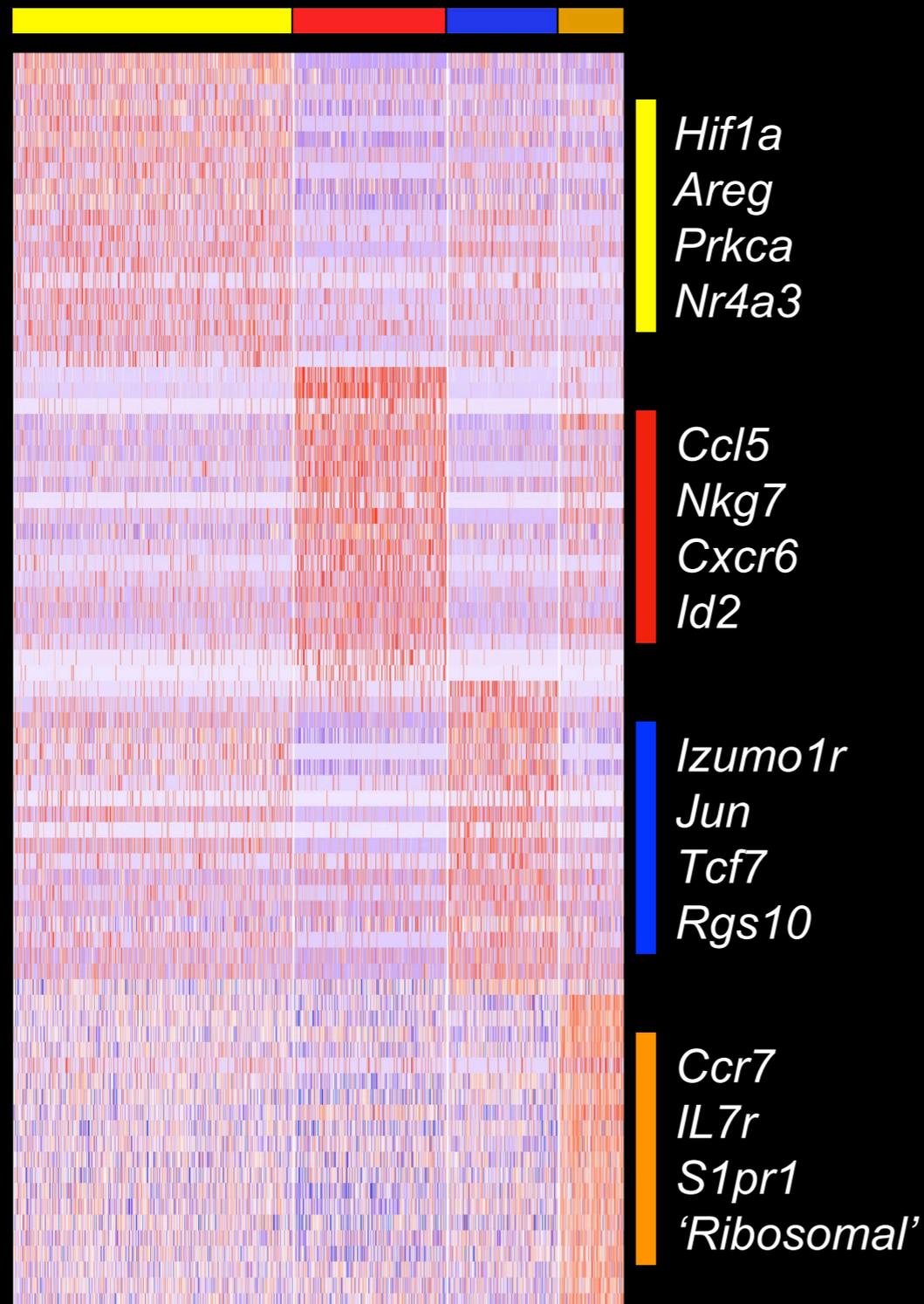
CD4 T cells in the mouse lung



flu-specific
CD4 T cells
scRNAseq

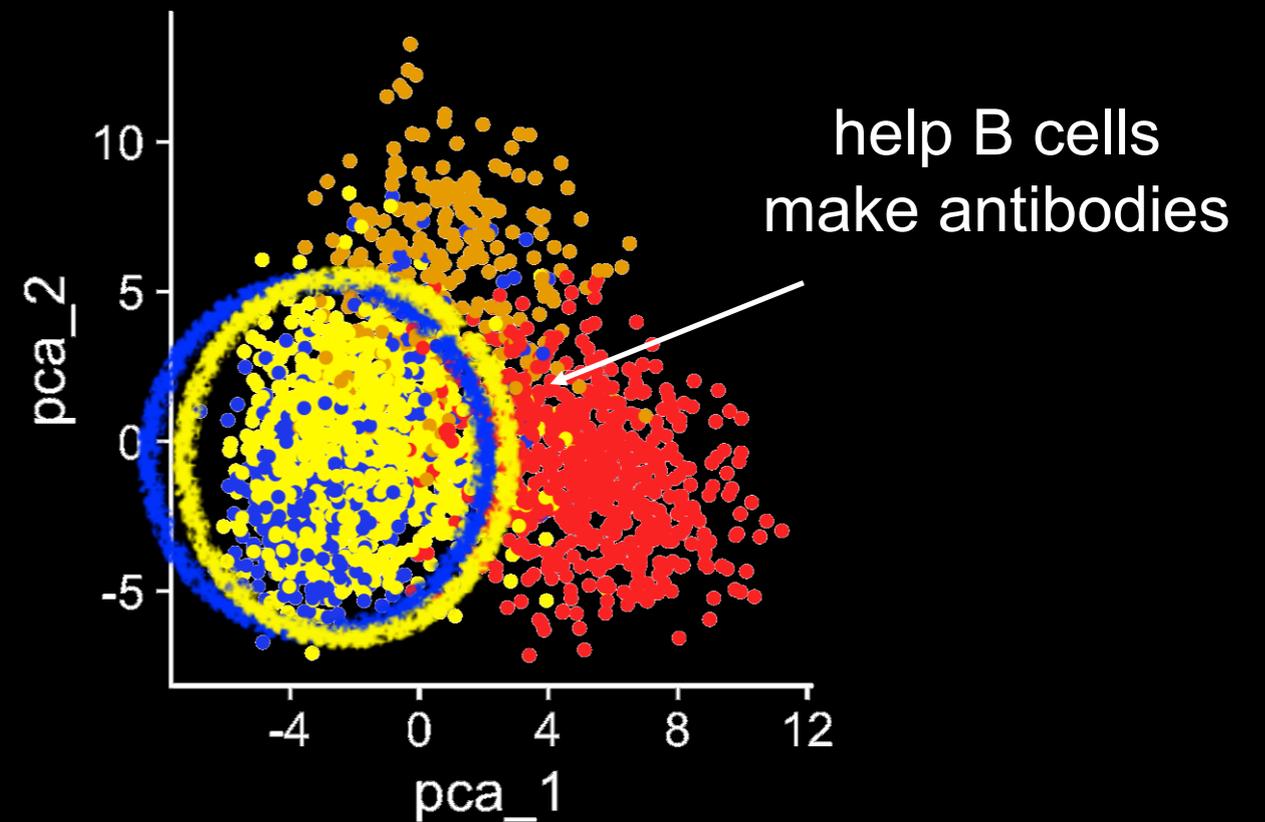
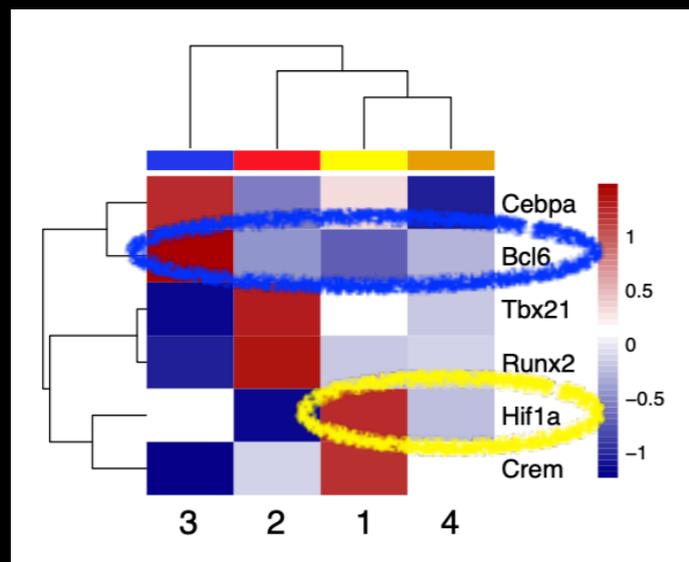


CD4 T cells in the mouse lung

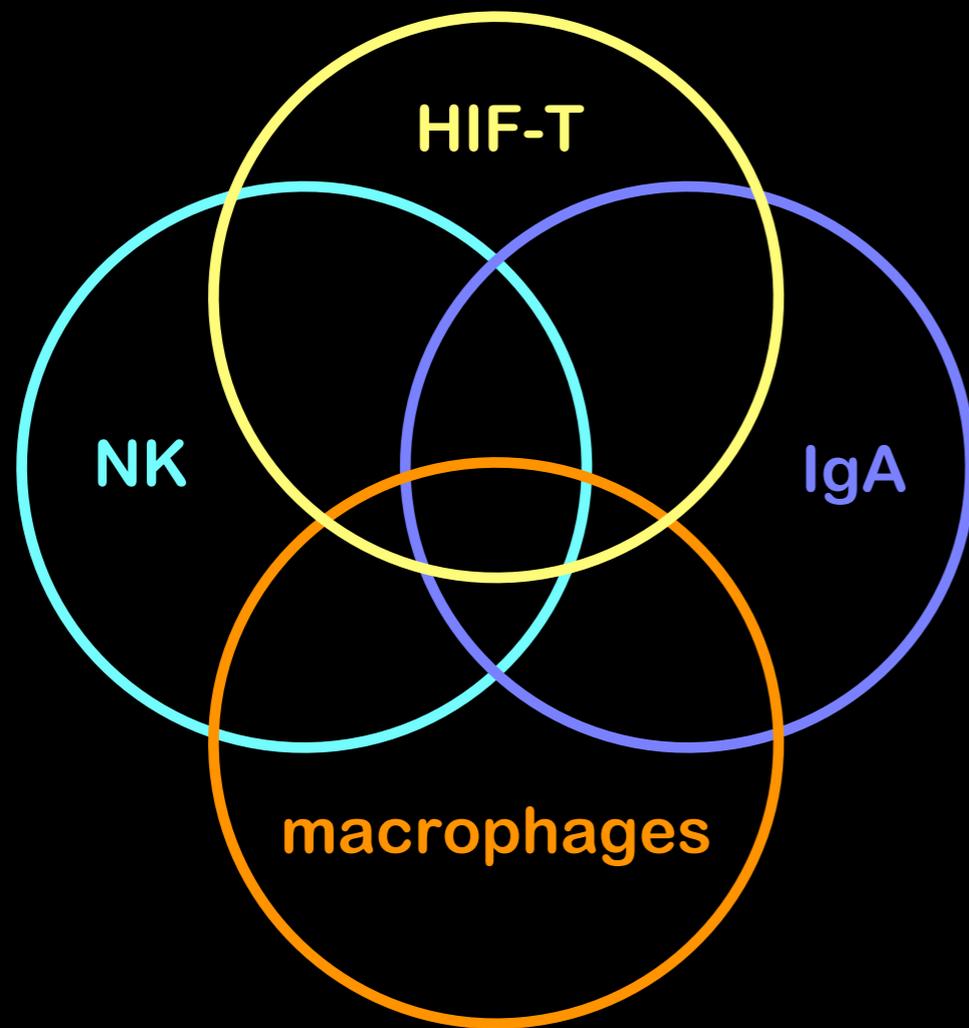


CD4 T cells in the mouse lung

Regulon analysis (SCENIC)



HIF-1 α T cells and the lung immune cell network



Located at perimeter of tertiary lymphoid structures

Tuberculosis?

HIF-1 α • hypoxia
Tcf7 • self-renewal
CD153 • host protection
Areg • tissue remodeling

Tiphaine Camarasa 



#TB team



Wadschma Naderi 



Gian Pietro Pietri



Almin Ljubijankic

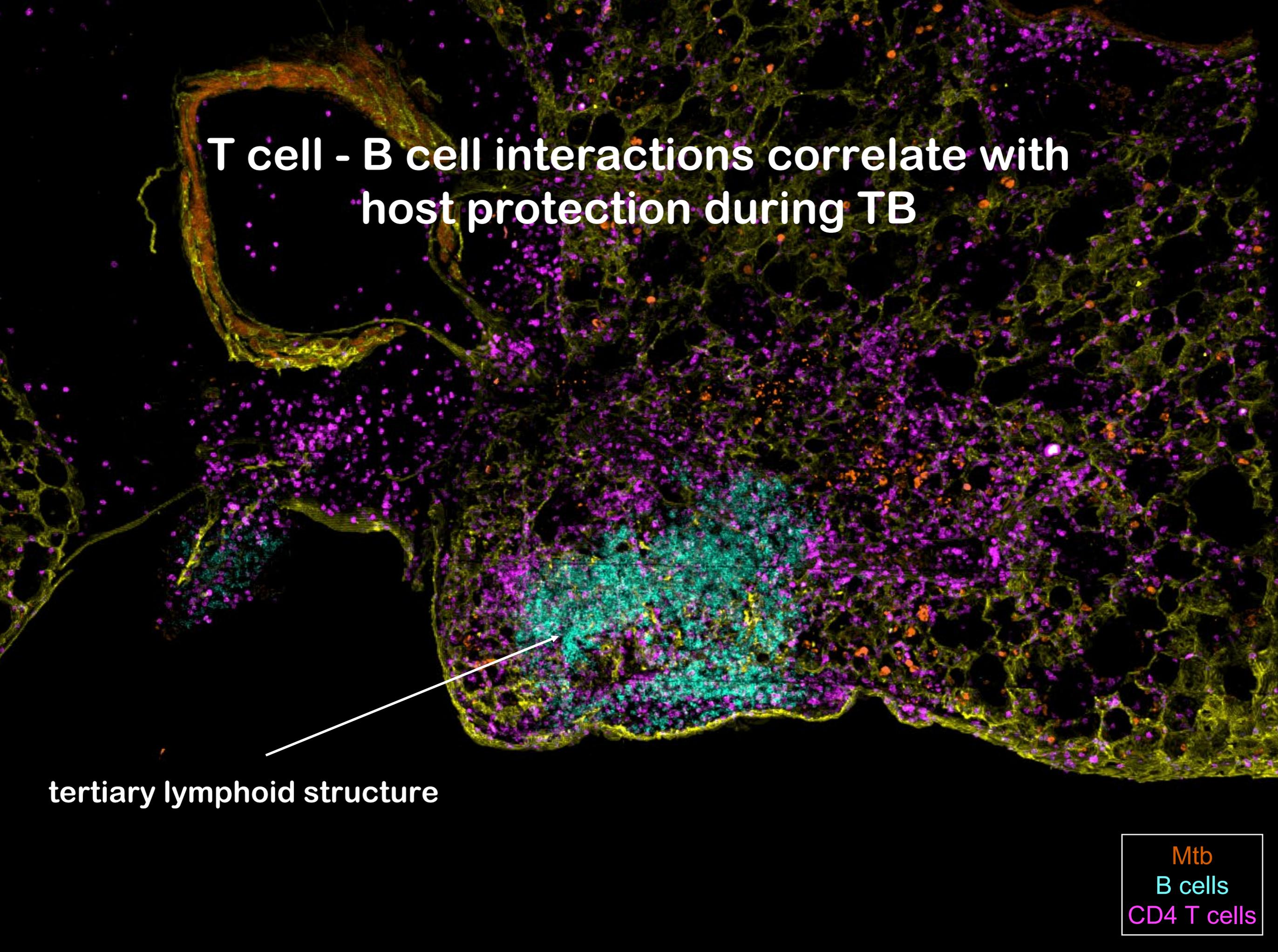


Habiba Soliman 

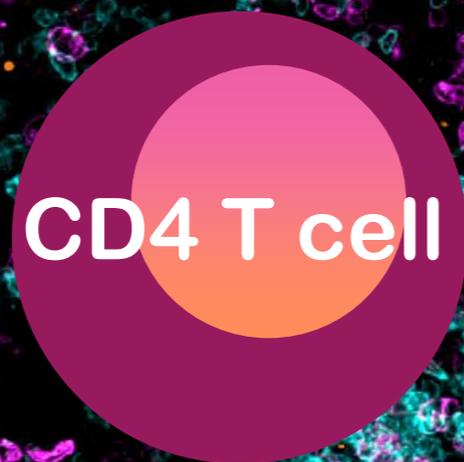
T cell - B cell interactions correlate with host protection during TB

tertiary lymphoid structure

Mtb
B cells
CD4 T cells



T cell - B cell interactions correlate with host protection during TB



stabilization of self-renewing
multipotent T cell reservoir?

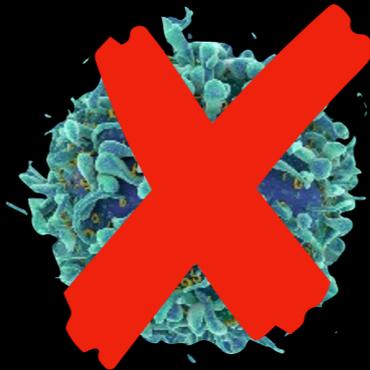
mucosal antibody production
non-antibody mediated functions?

Immune cell depletion to define protection

CD4 T cell

lung macrophage

high
Mtb
CFU

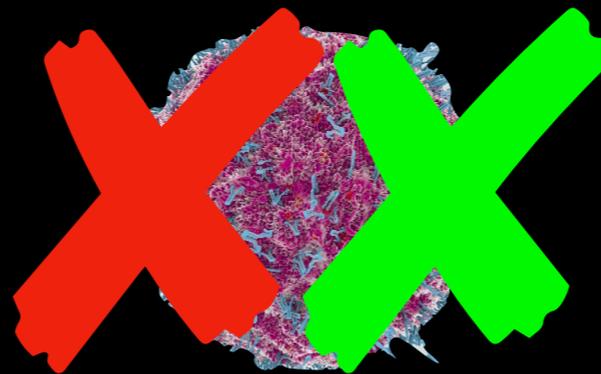


low
Mtb
CFU

?

B cell

MHCII antigen presentation
Bcl6 germinal center reaction
Prdm1 antibody production



Localization?

Do B cells do different things, at different times, in different places?

B cell depletion

before
infection

- **lower bacterial load (spleen / LN)**

Chen J...Chan J, Plos Pathogens 2023

Daniel L..Feng C, JCI 2022

Torrado E..Cooper C, Plos One 2013



after
infection

- **higher lung bacterial load**

Swanson R, Gupta A..Khader S, Nat Immunol 2023

- **increased cachexia/weight loss/death**

Linge R, Apt A, Int J Mol Sci 2023



Assumption:

B cells do the things we expect them to do

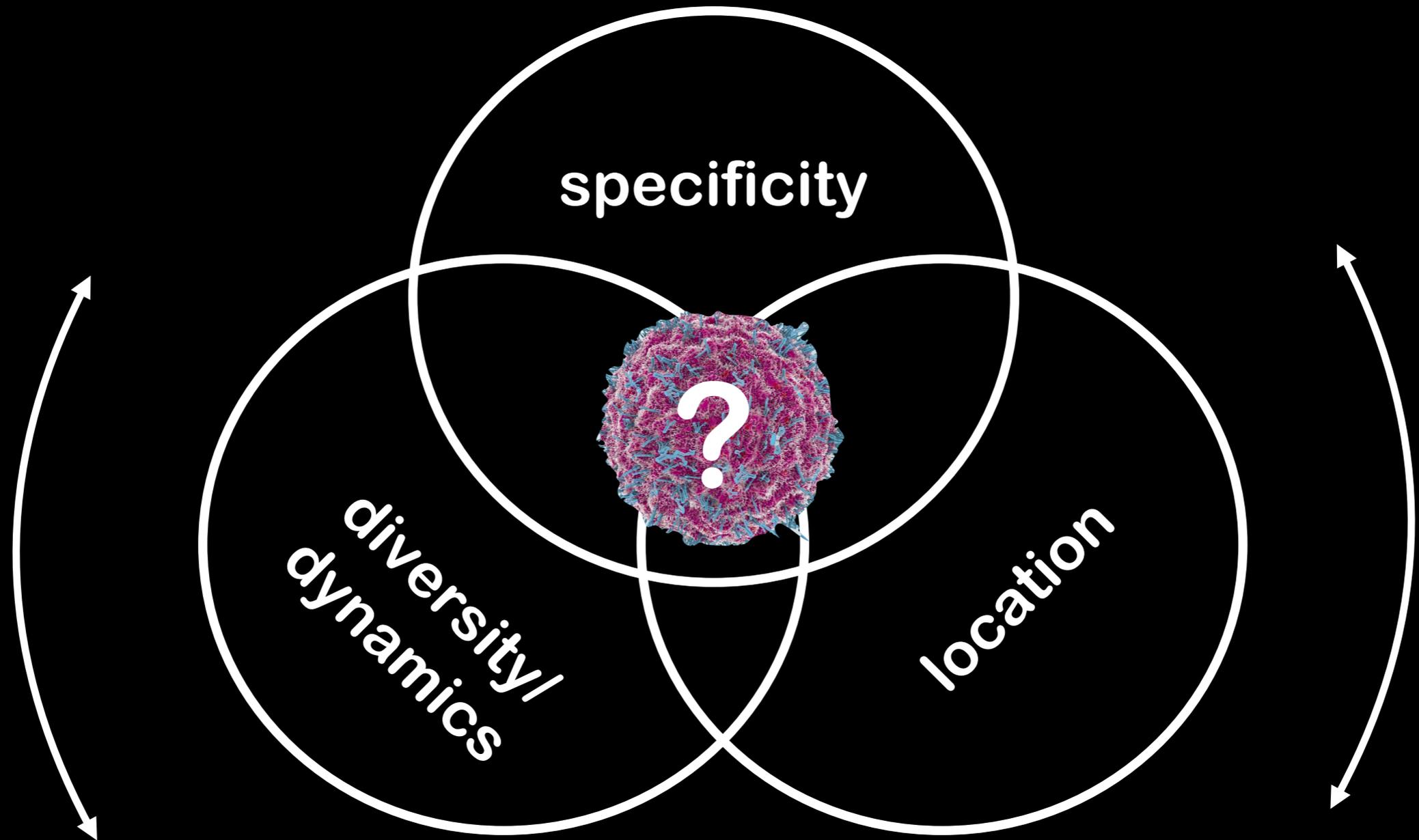
.....B cells are not important

Alternate possibilities:

1. B cells do “other” things (we haven’t measured)*
2. B cells are impaired

Could B cells be harnessed to improve protection?

What do Mtb-B cell responses look like?



Detection of Mtb-surface targeted antibodies

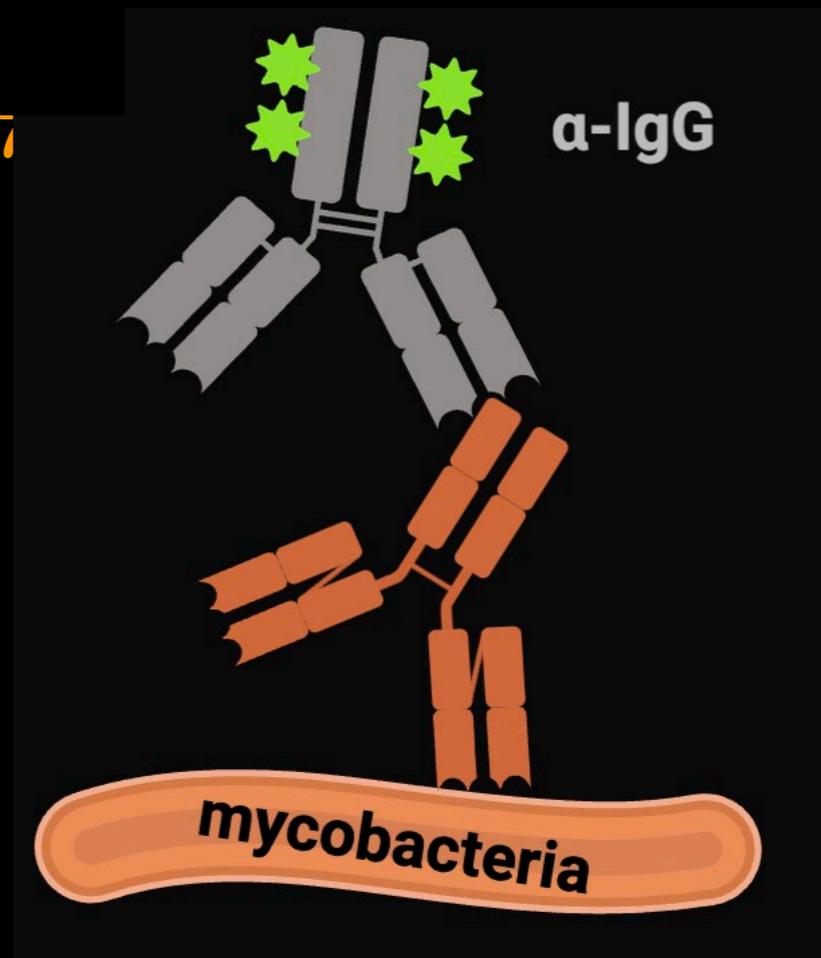


Aerosolized Mtb (HN87)

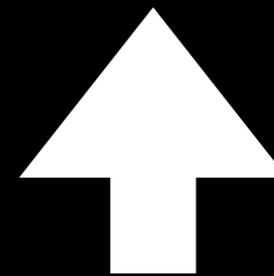
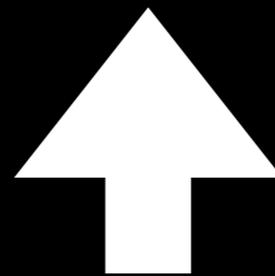
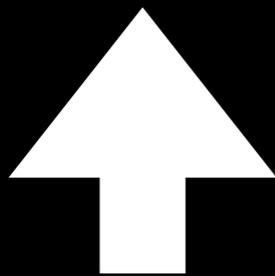
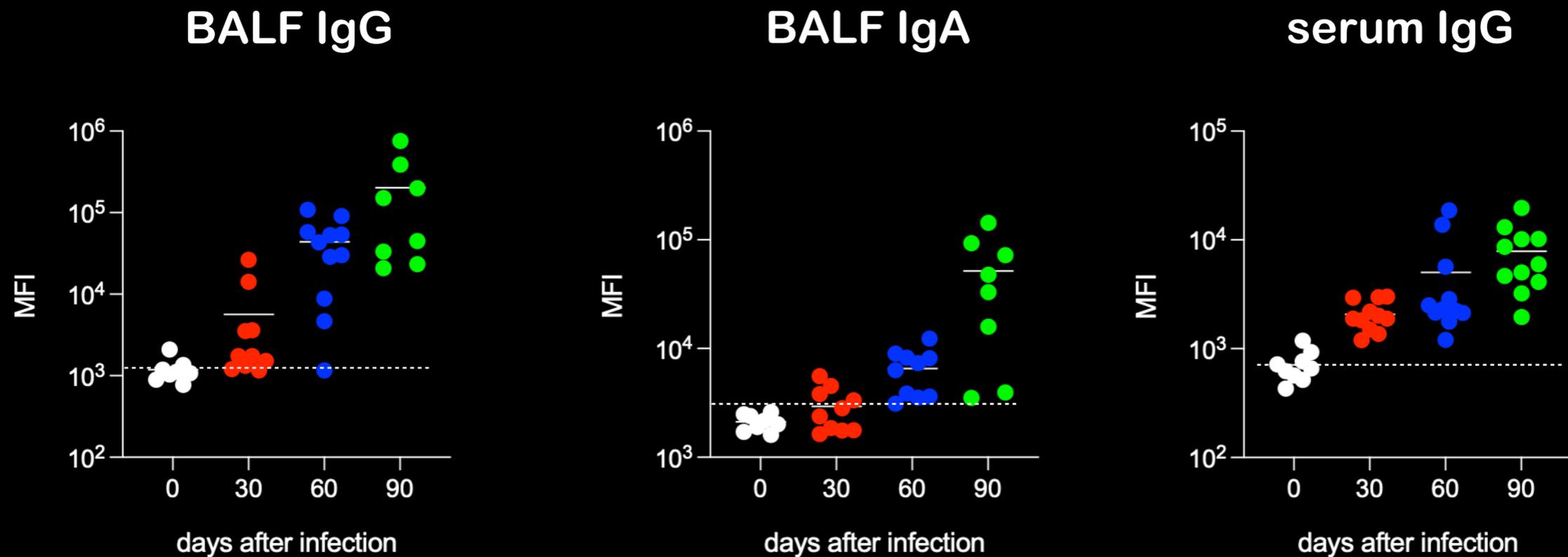
PROTOCOL

Analysis of bacterial-surface-specific antibodies in body fluids using bacterial flow cytometry

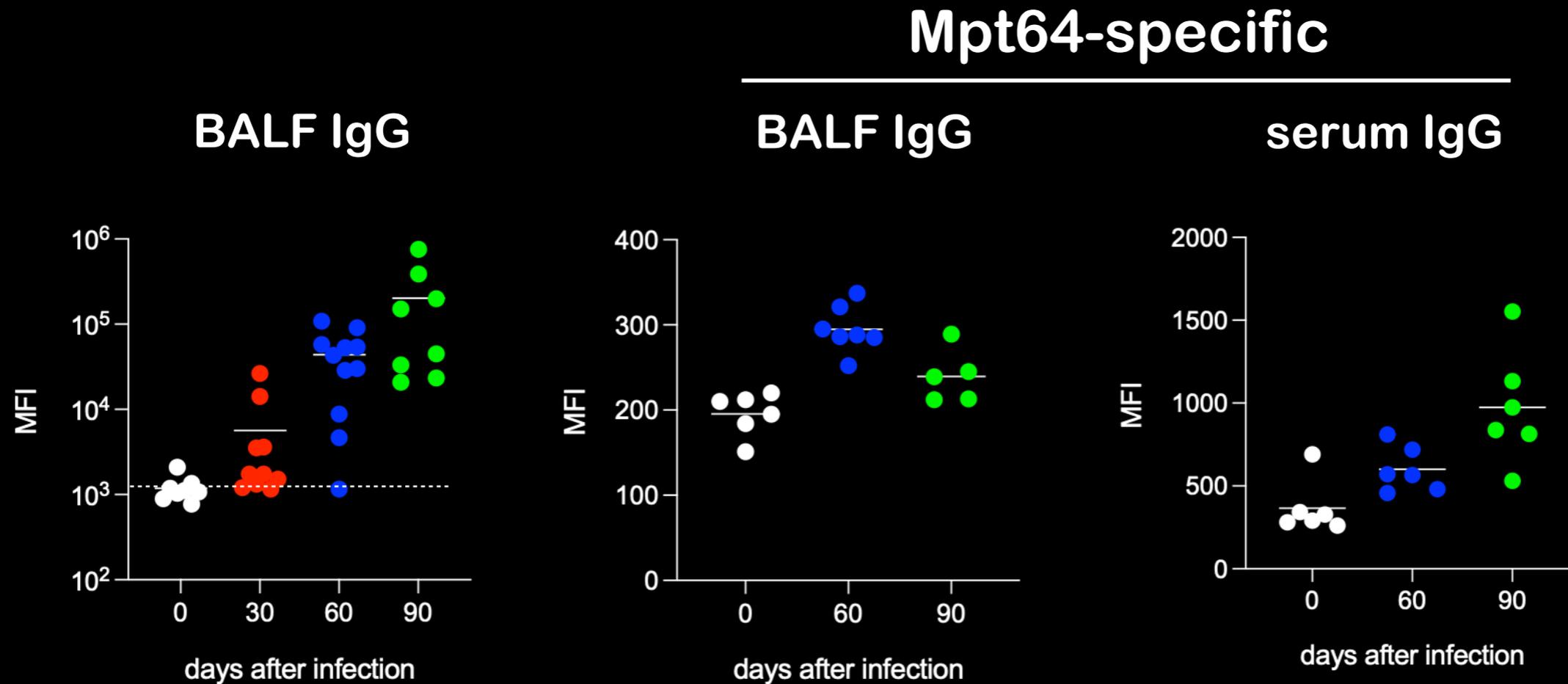
Kathrin Moor^{1,6}, Jehane Fadlallah^{2,6}, Albulena Toska^{1,6}, Delphine Sterlin², Maria L Balmer^{3,4}, Andrew J Macpherson³, Guy Gorochov^{2,5}, Martin Larsen^{2,5,7} & Emma Slack^{1,7}



Detection of Mtb-surface targeted antibodies

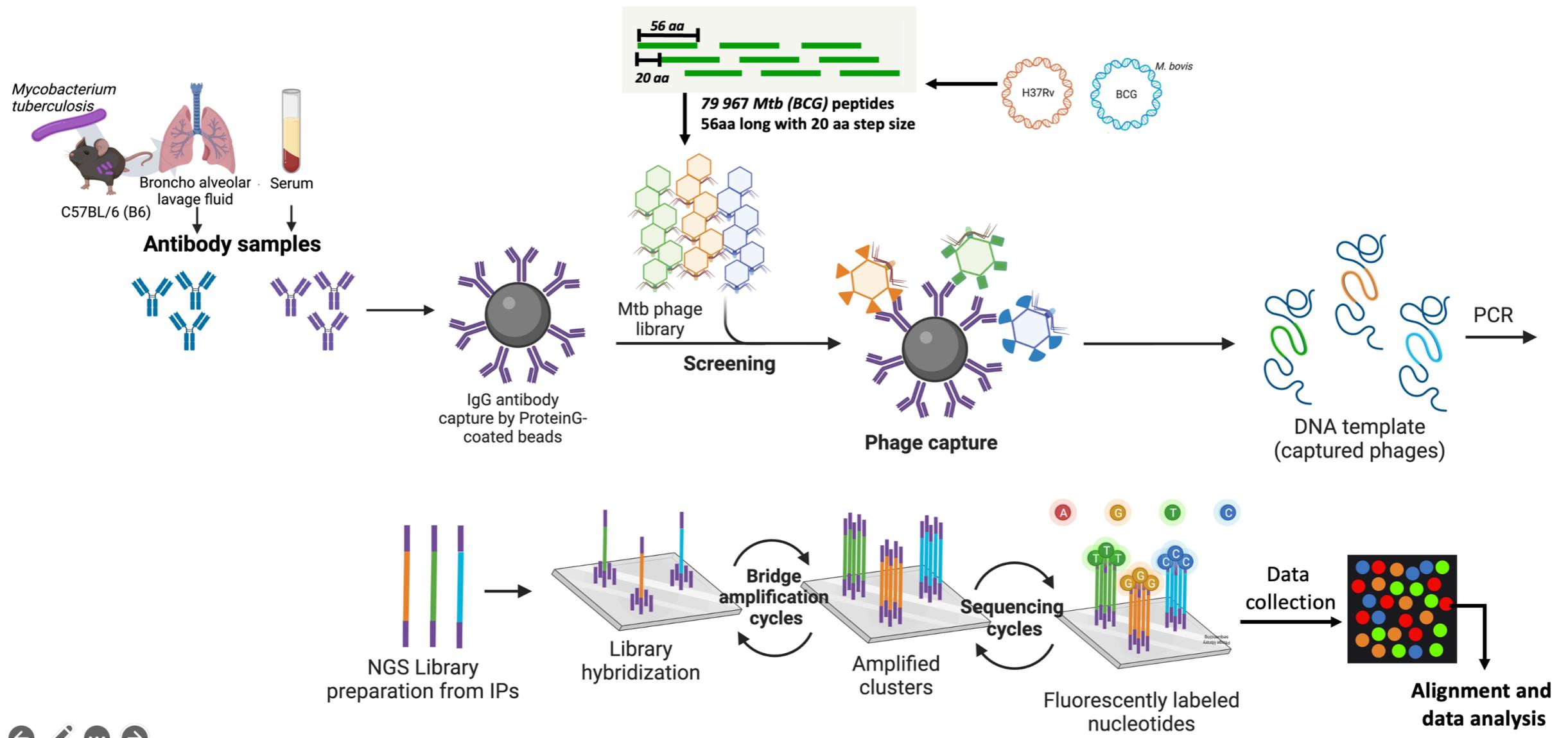


Antibody kinetics may depend on specificity

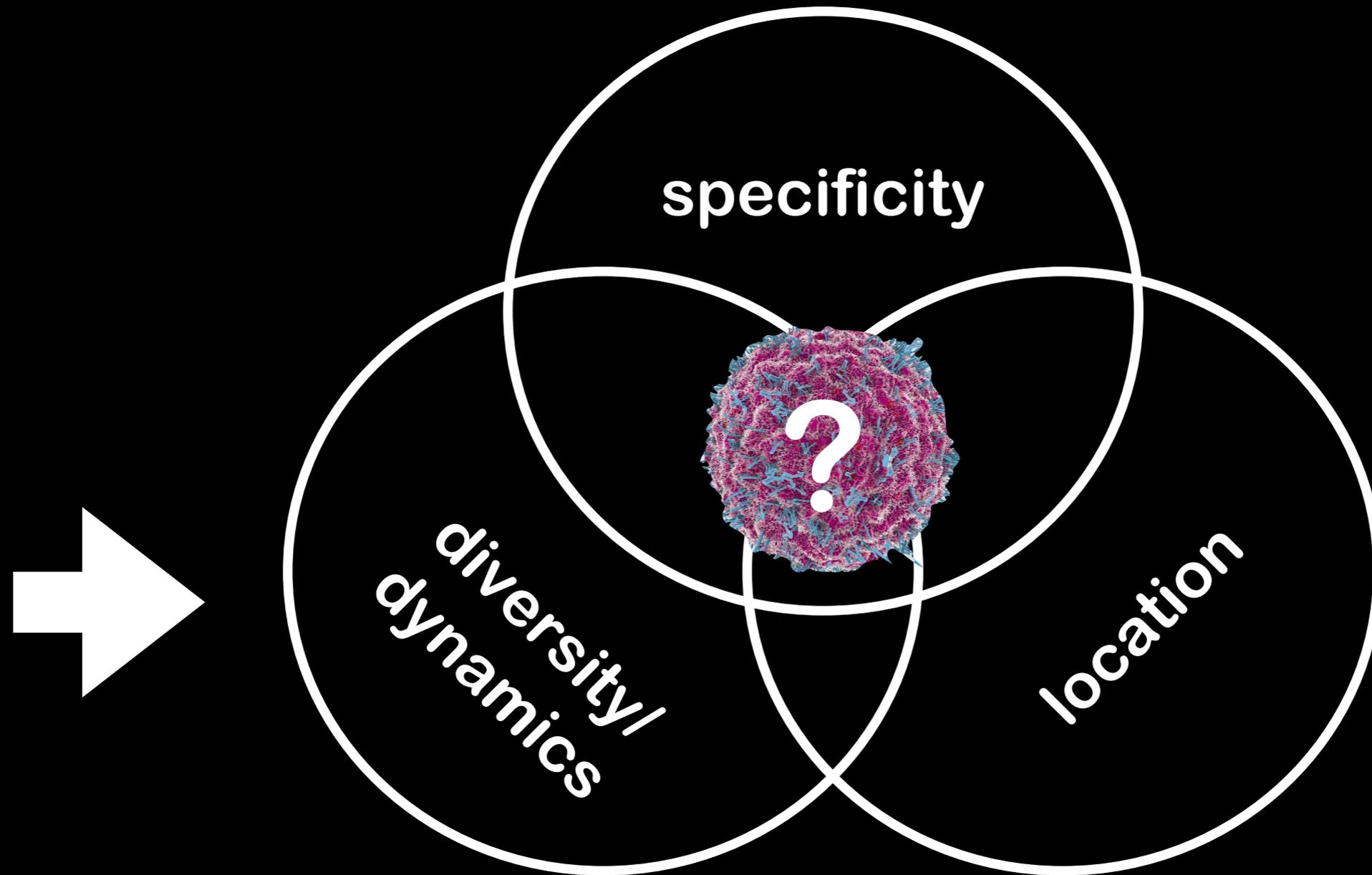


(bead based flow assay)

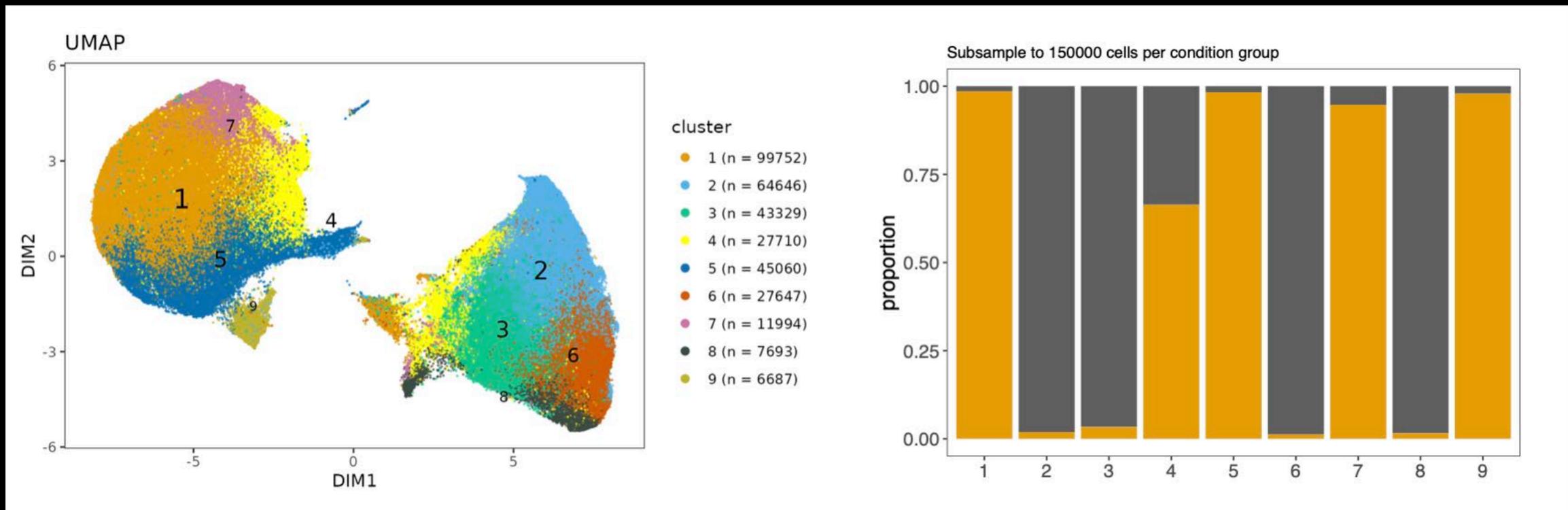
Phage-immunoprecipitation sequencing to characterize Mtb-specific antibodies



What do Mtb-B cell responses look like?



B cell diversity in the lung (HD flow cytometry)



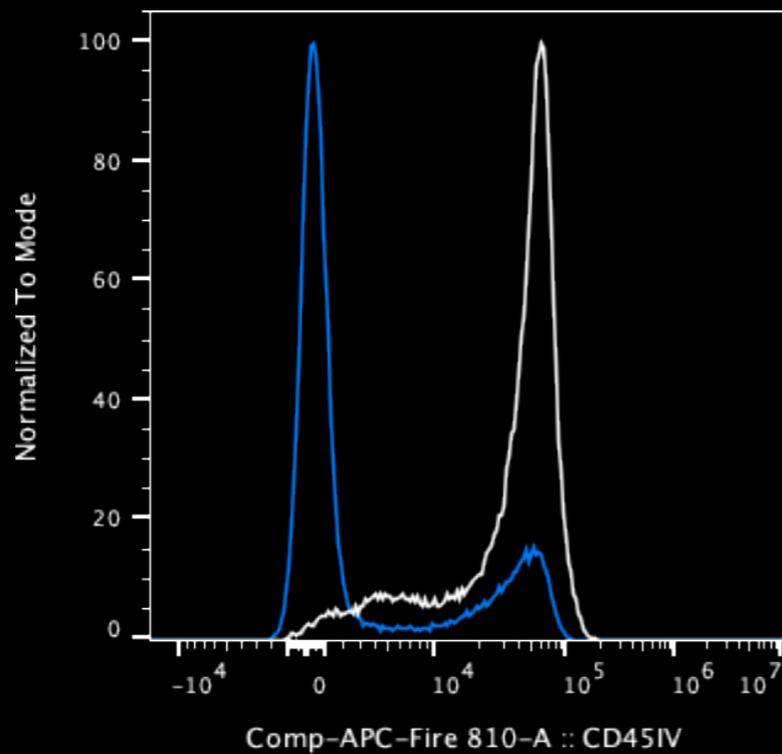
Mtb (d30)
resident

naive
circulating

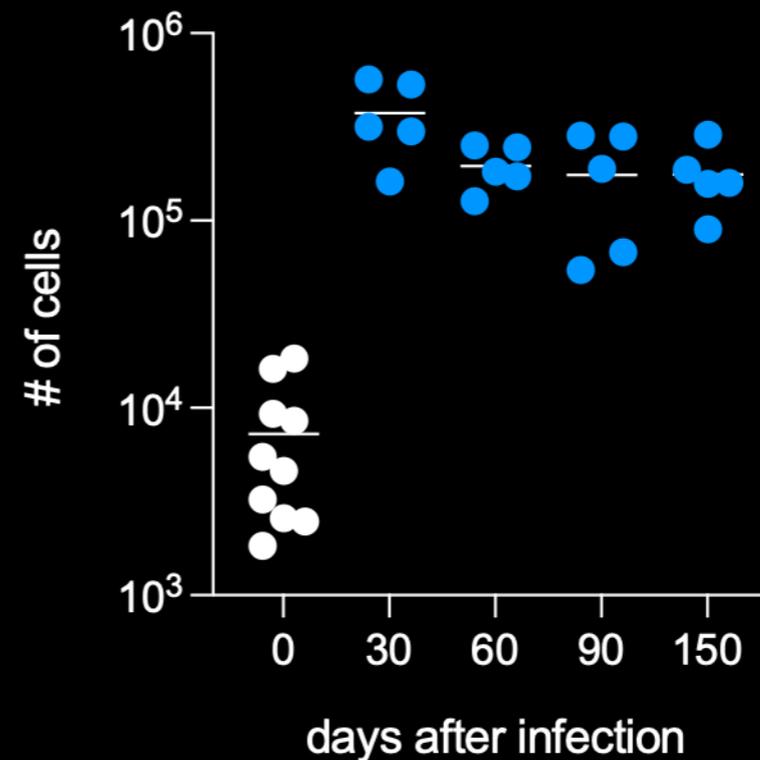
Mtb naive naive **Mtb** **Mtb** naive **Mtb** naive **Mtb**

Lung resident B cells in Mtb infected mice

intravenous anti-CD45

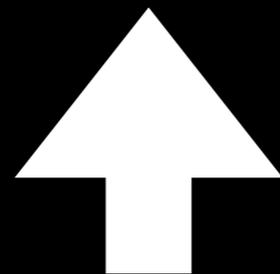
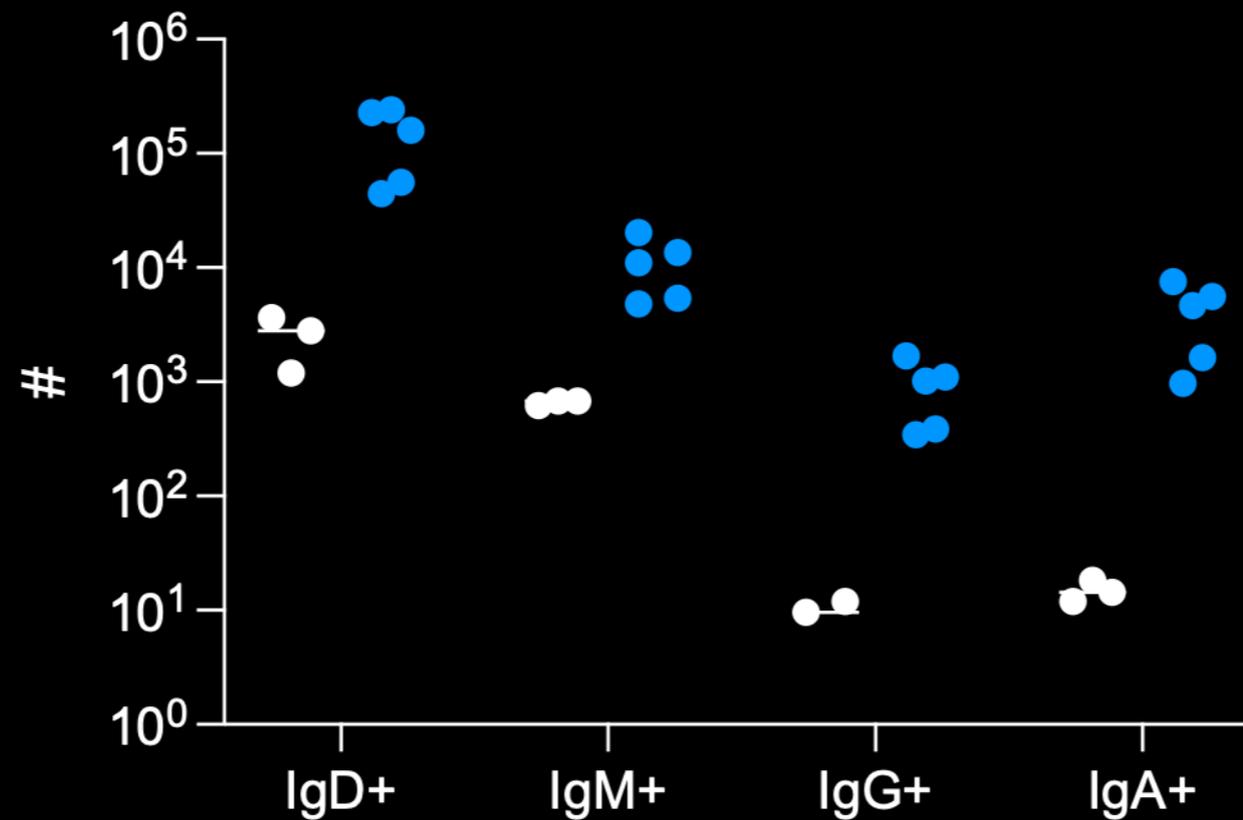


Lung resident B cells
(CD45 i.v. negative)



naive
Mtb HN878

Majority of lung resident B cells are IgD+ (unlikely to be antigen-specific)



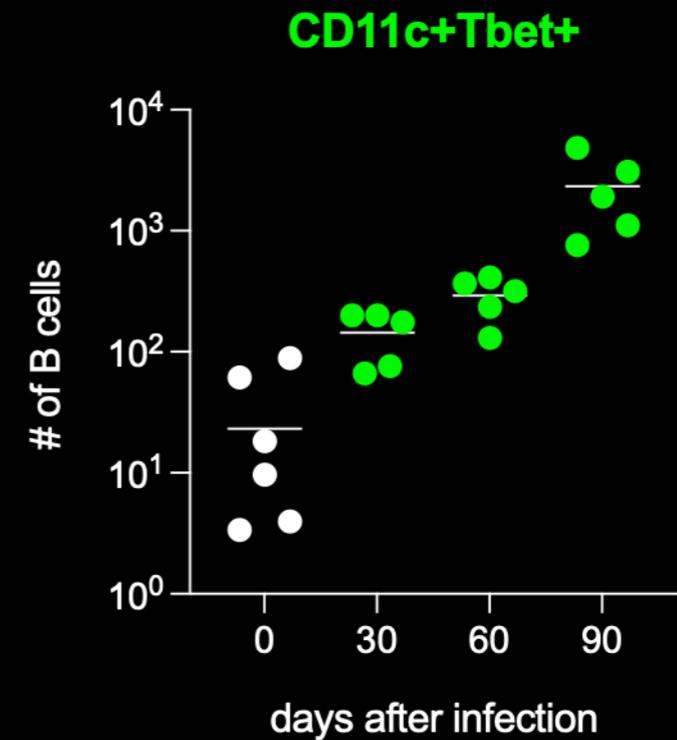
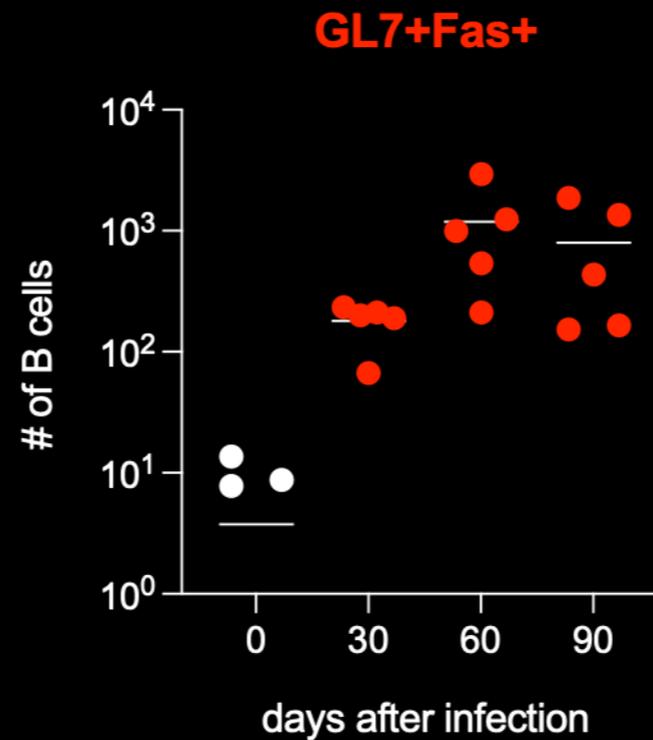
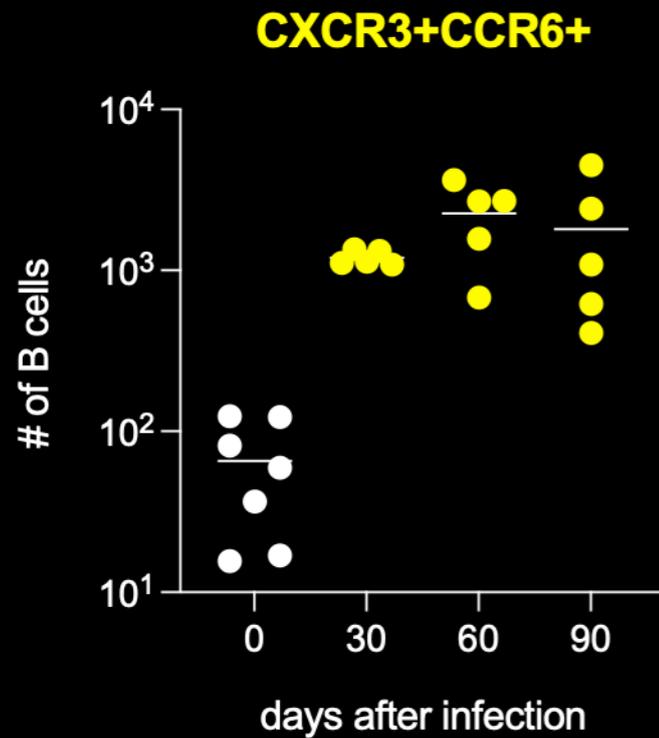
naive
Mtb HN878 (d90)

B cell diversity in the lung (switched Ig)

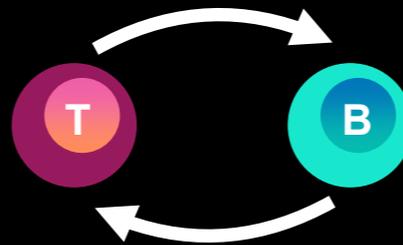
memory

germinal center

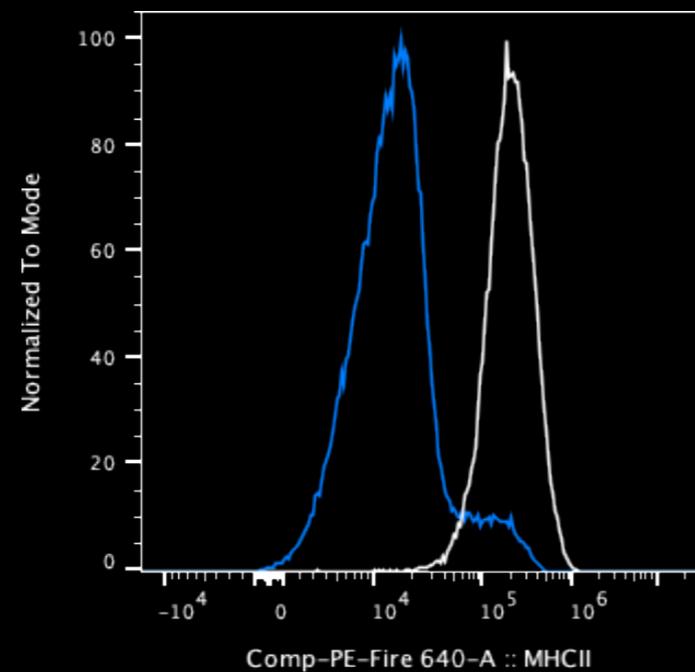
atypical



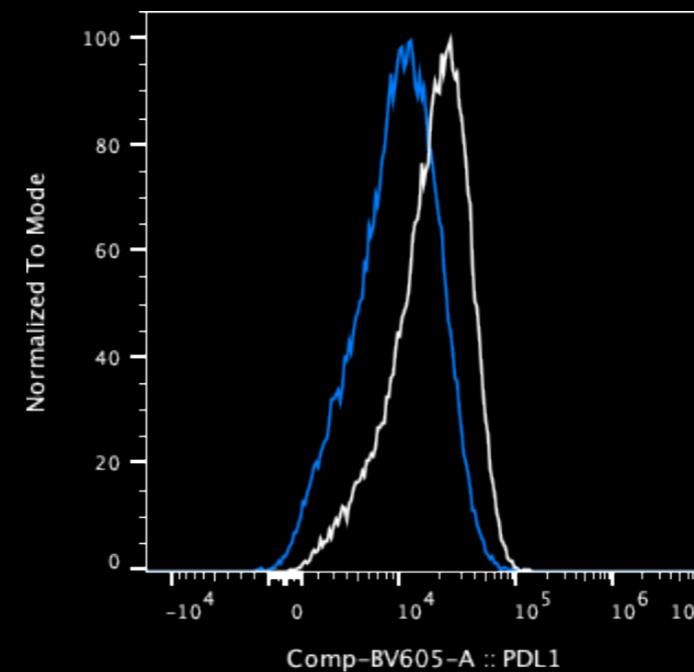
Downregulated MHC-II and PDL1 on lung B cells



MHC2

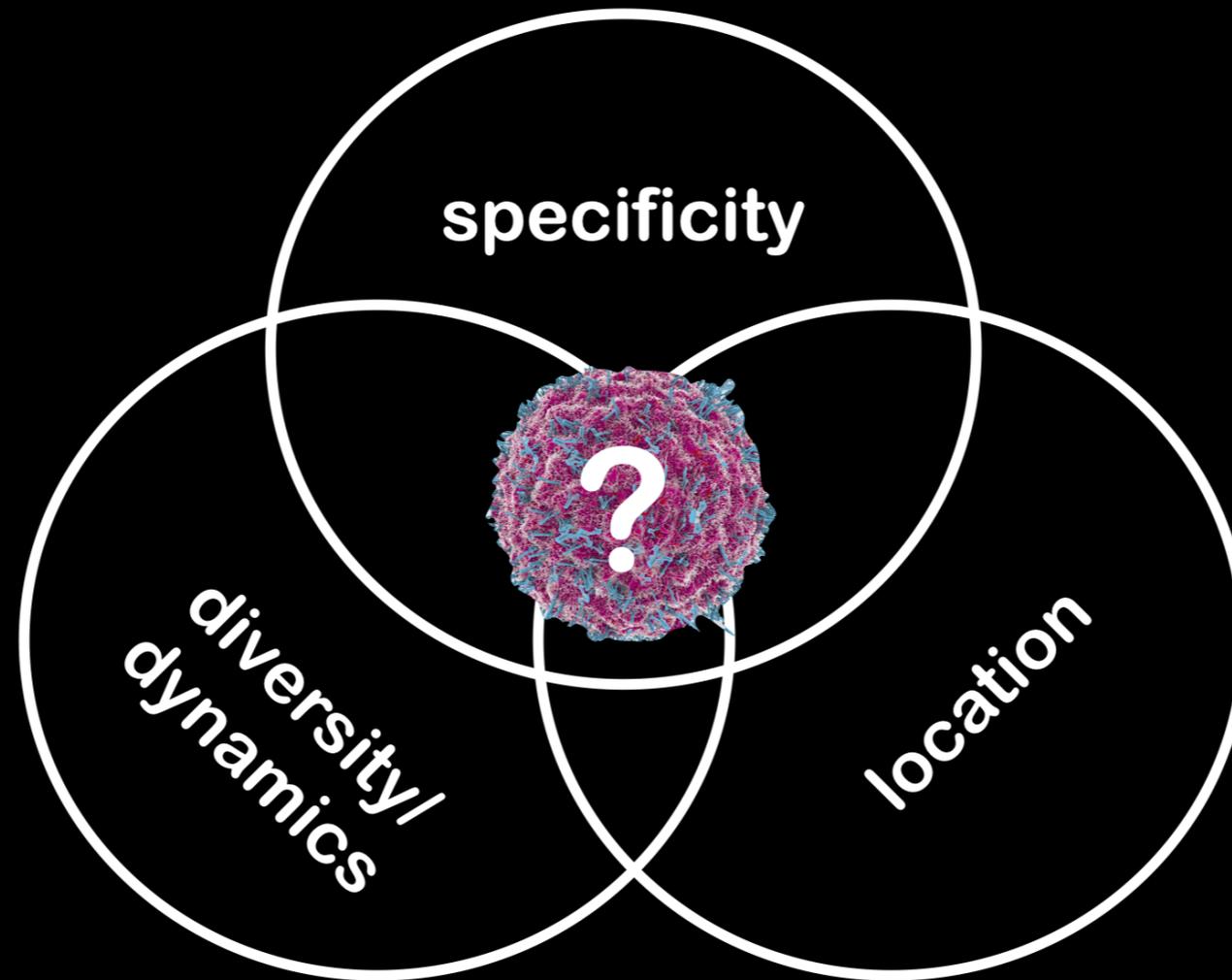


PDL1



naive
Mtb HN878 (d30)

Where do we go from here?



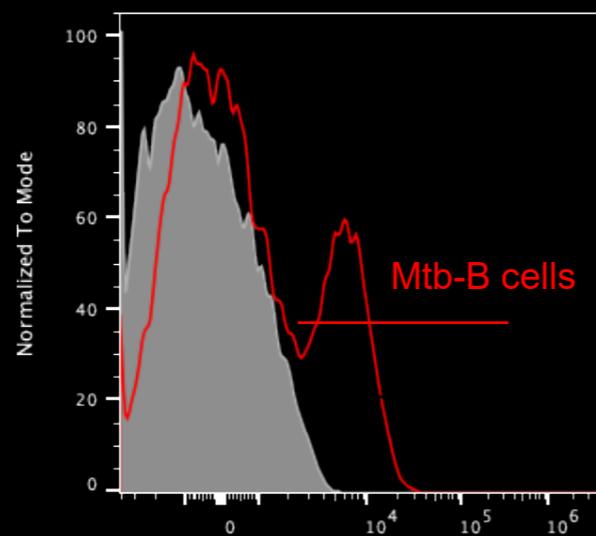
Genetic tools to track Mtb-B cells

GOAL: Mtb-specific B cell repertoire

STRATEGY: **S1pr2**^{ERT2-Cre}-**Rosa26**^{lox-stop-lox-tdTomato}



+ tamoxifen to label
antigen specific B cells



S1pr2 tdTomato

- isolate **tdTomato** B cells
- assess BCR repertoire
- screen for specificity

NEW

Genetic tools to track Mtb-B cells

GOAL: genetic manipulation of lung B cells

STRATEGY: dual recombinase Cre (TB-Cre)

TB-Cre



mb1-Dre (B cell driver)
gene X, gene Y (lung driver)



Thank you

KING LAB

*Tiphaine Camarasa**
Jean de Lima
Claire Depew
Maïke Erber
Mara Esposito
Aaron Forde
Emanuela Kerschbamer
*Almin Ljubijankic**
*Eduardo Moreo **
*Gian Pietro Pietri**
*Wadschma Naderi**
David Schreiner
*Habiba Soliman**

**TB team*

Collaborators

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Rasmus Mortensen, Statens Serum Institut
Keertan Dheda, University of Cape Town
Anil Pooran, University of Cape Town
Vivek Thacker, Uni Heidelberg

Maxime Quebatte, University of Basel
Petra Chiquet, University of Basel

Funding



SWISS NATIONAL SCIENCE FOUNDATION



Horizon Europe



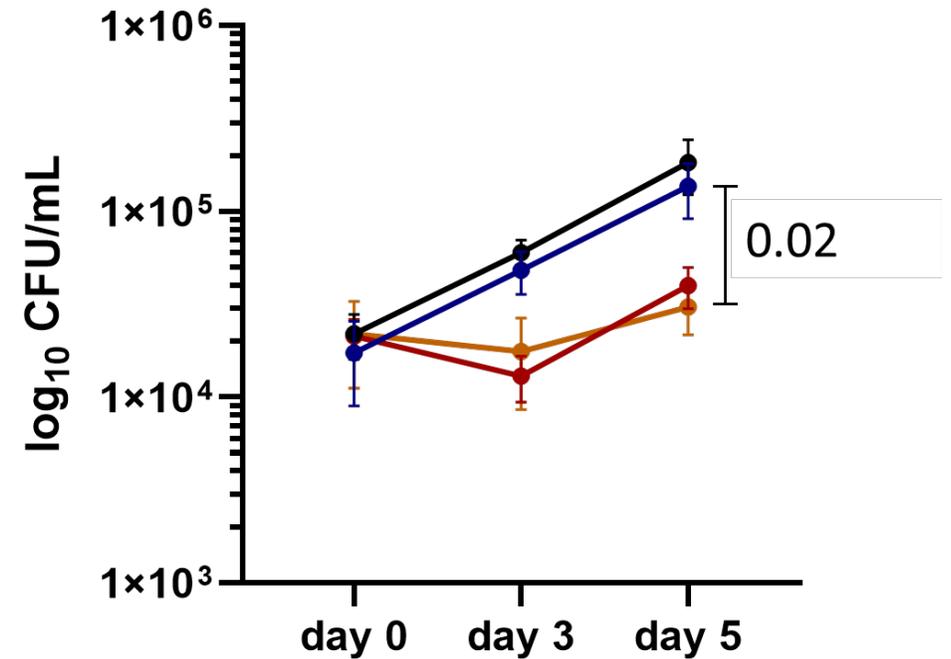
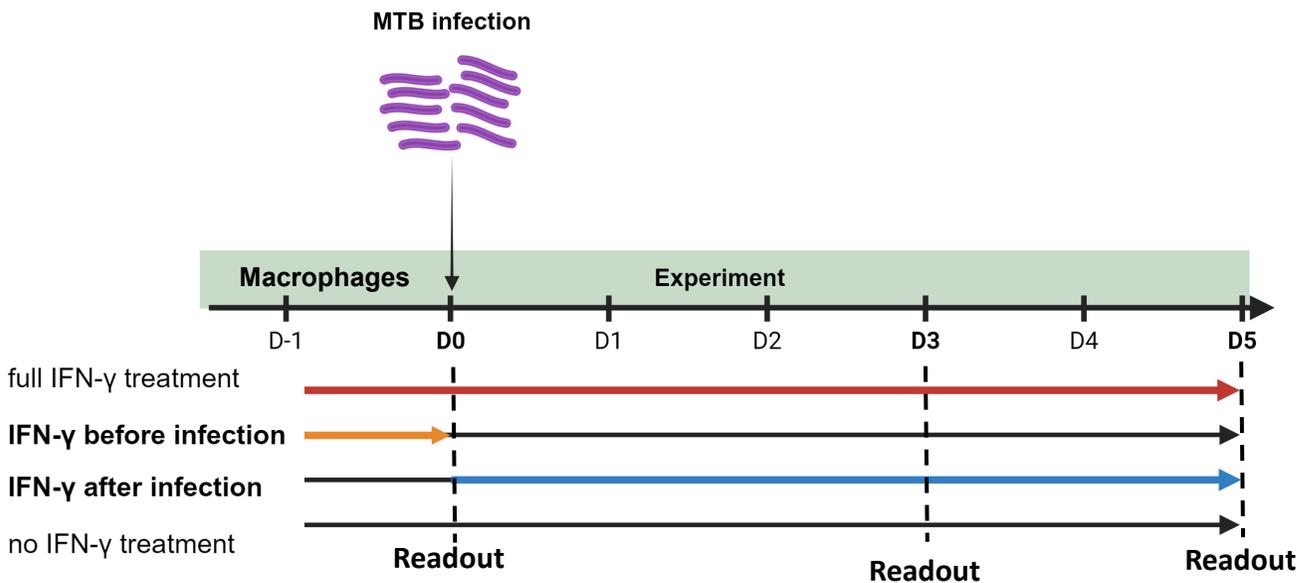
c-MYC expression determines the outcome of macrophages maturation and *Mycobacterium tuberculosis* infection

Joint TB Meeting – 2_{nd} Swiss Translational TB Forum

Edoardo Sarti

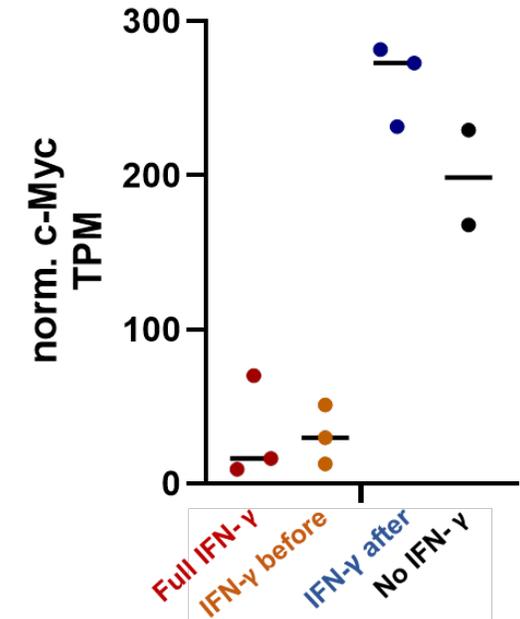
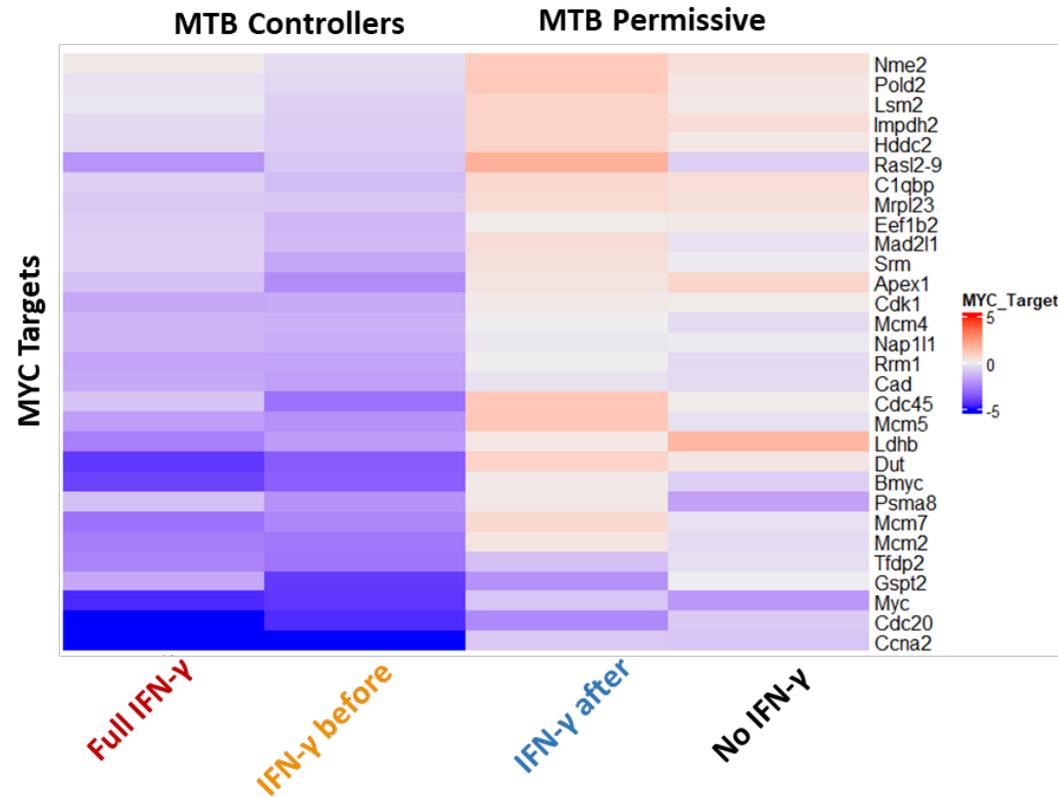
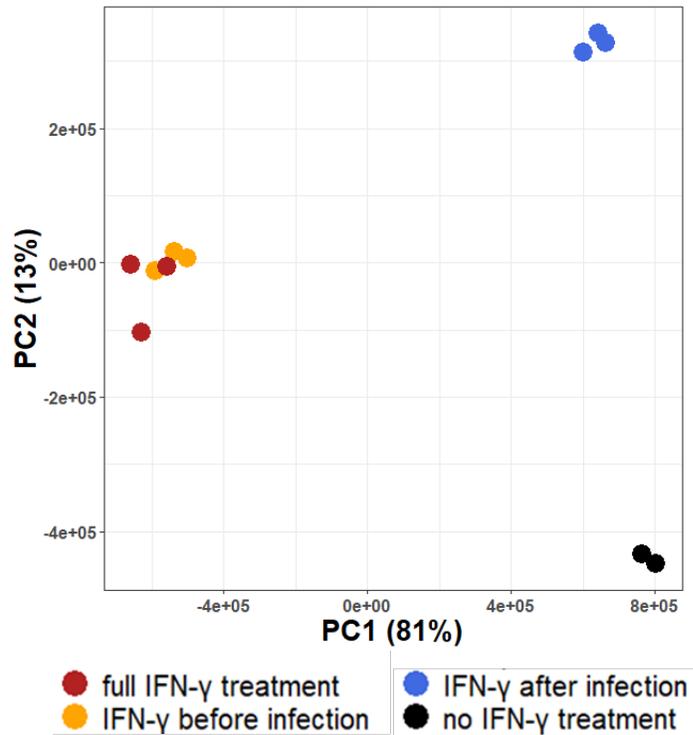
30.10.2024

The timepoint of activation and infection determines the anti-mycobacterial response

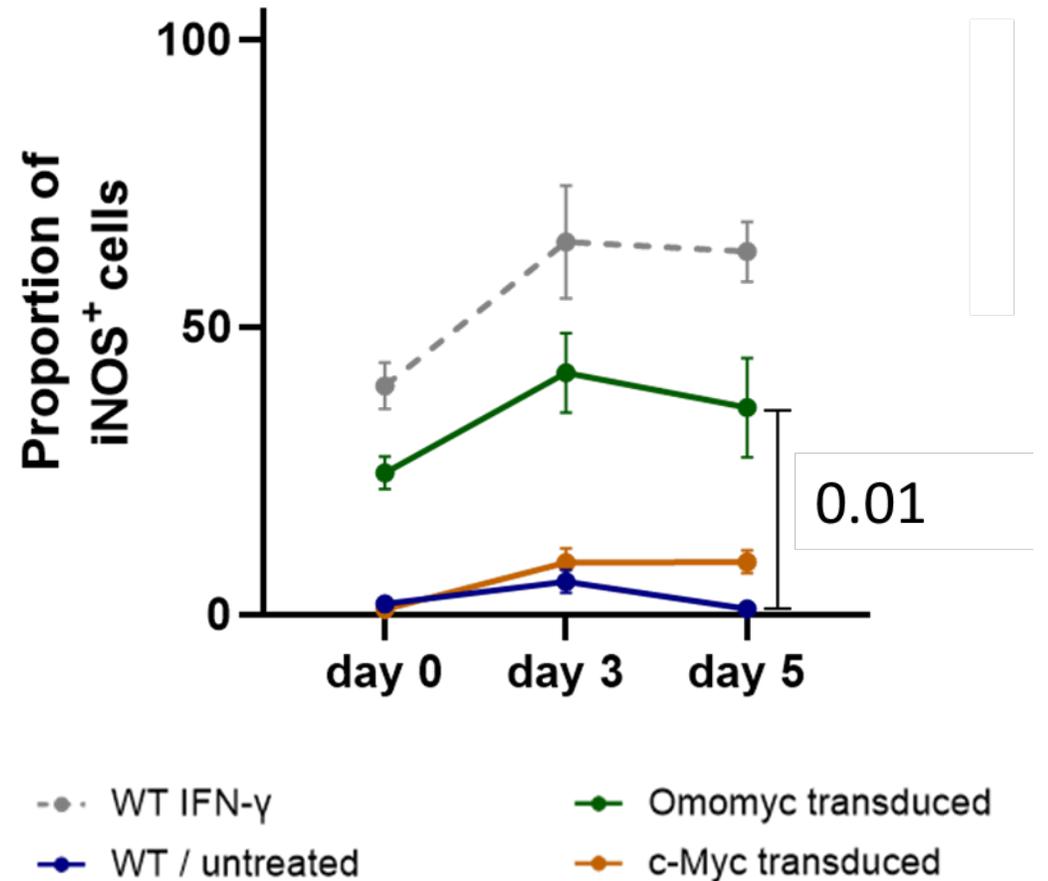
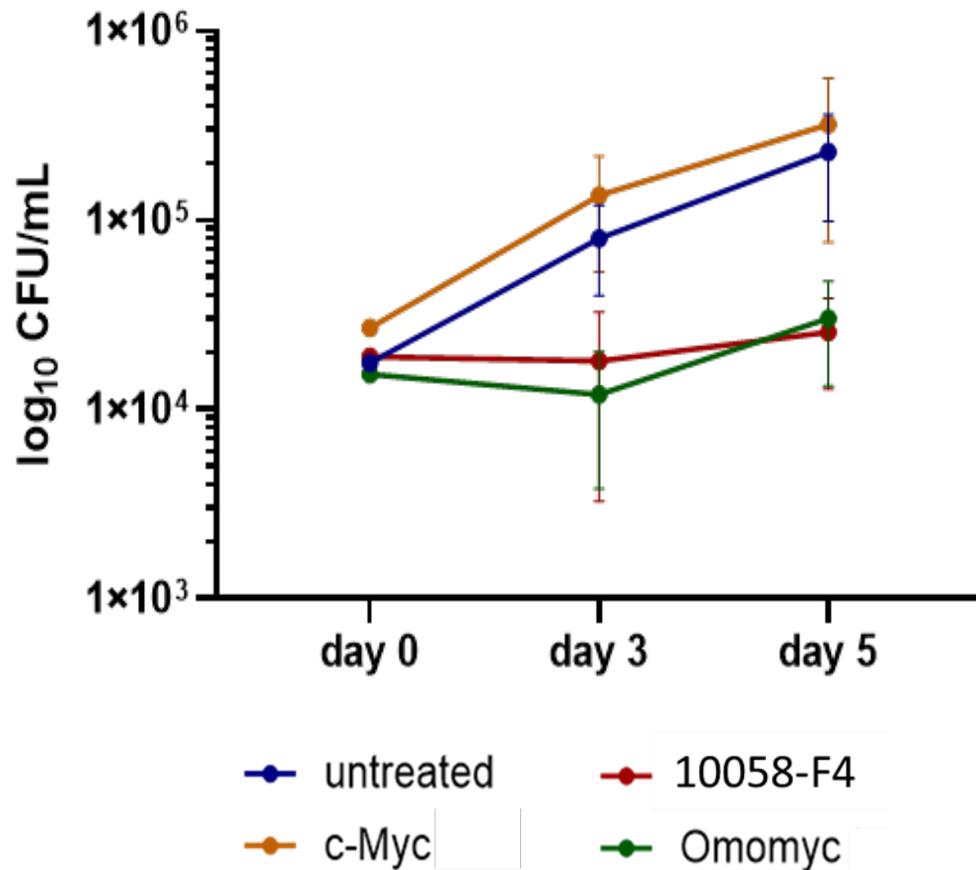


- full IFN-γ treatment
- IFN-γ before infection
- IFN-γ after infection
- no IFN-γ treatment

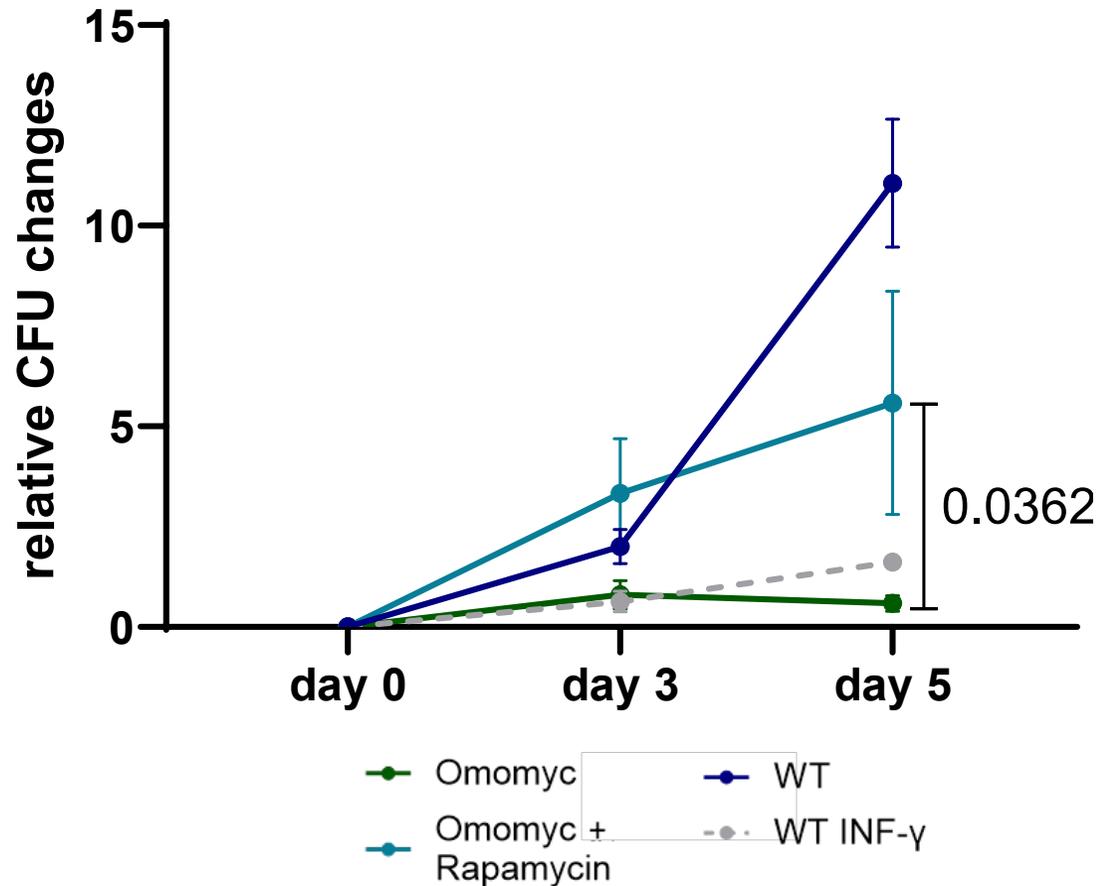
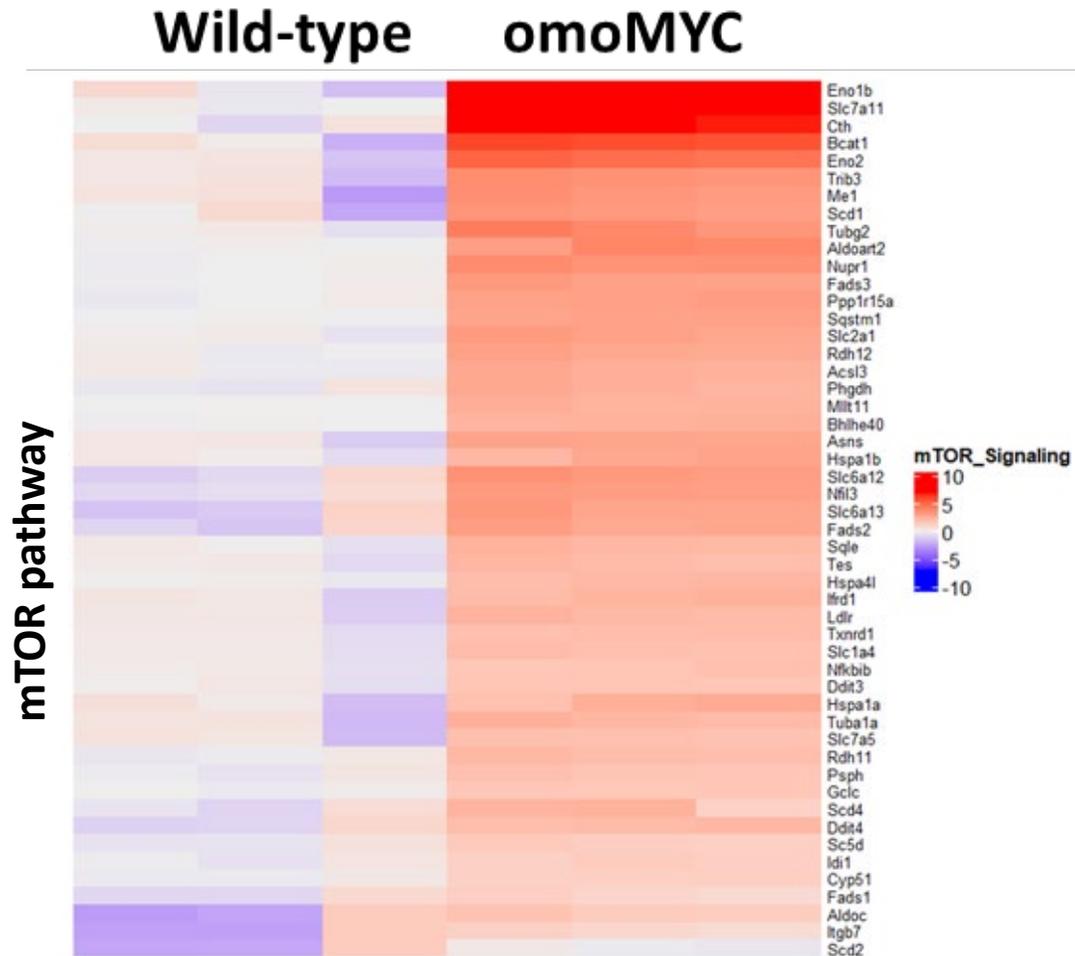
The timepoint of activation and infection determines the anti-mycobacterial response



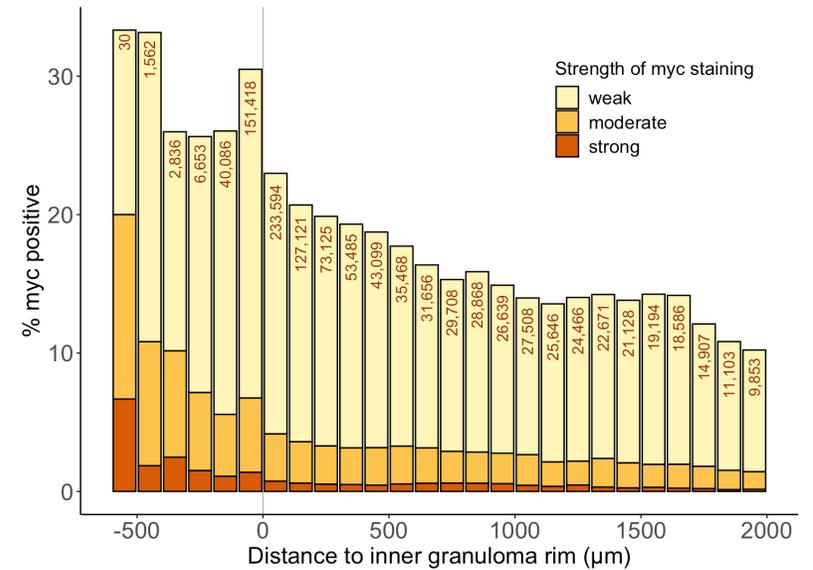
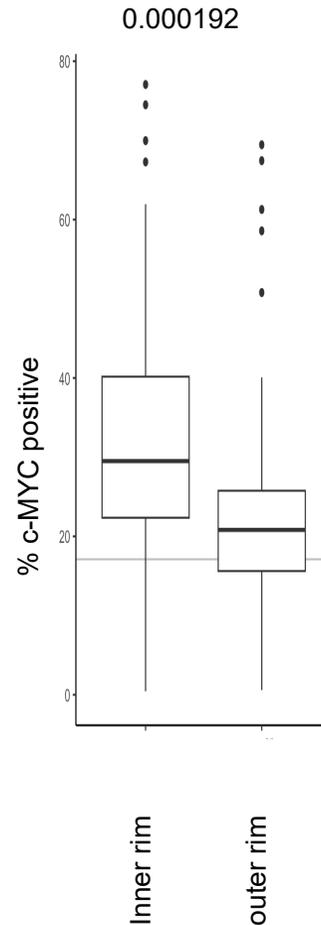
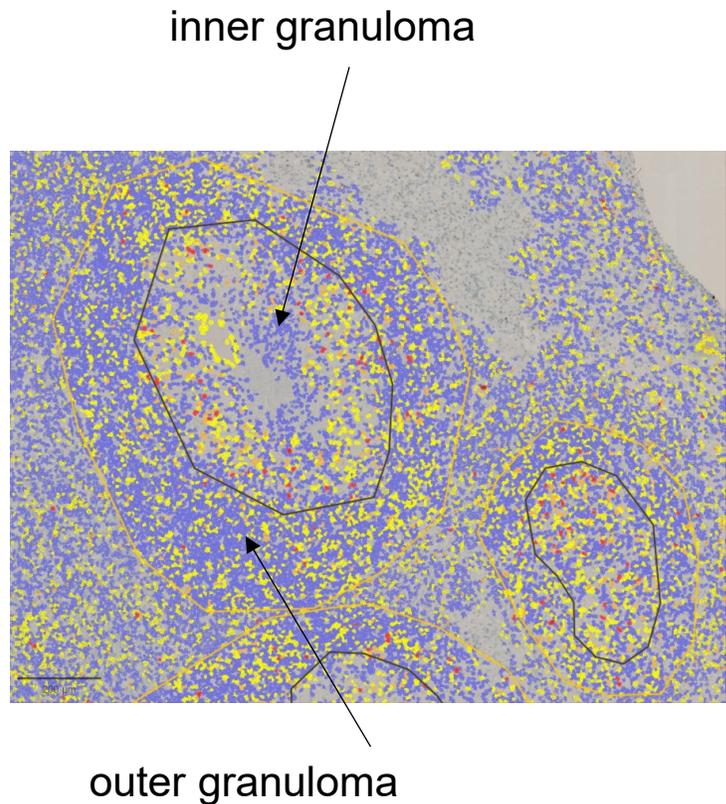
c-MYC inhibition alone increases the anti-mycobacterial activity of macrophages



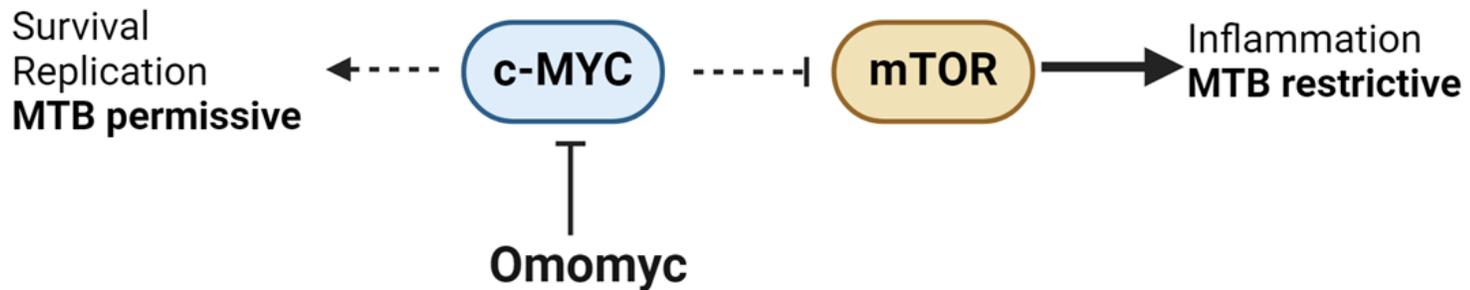
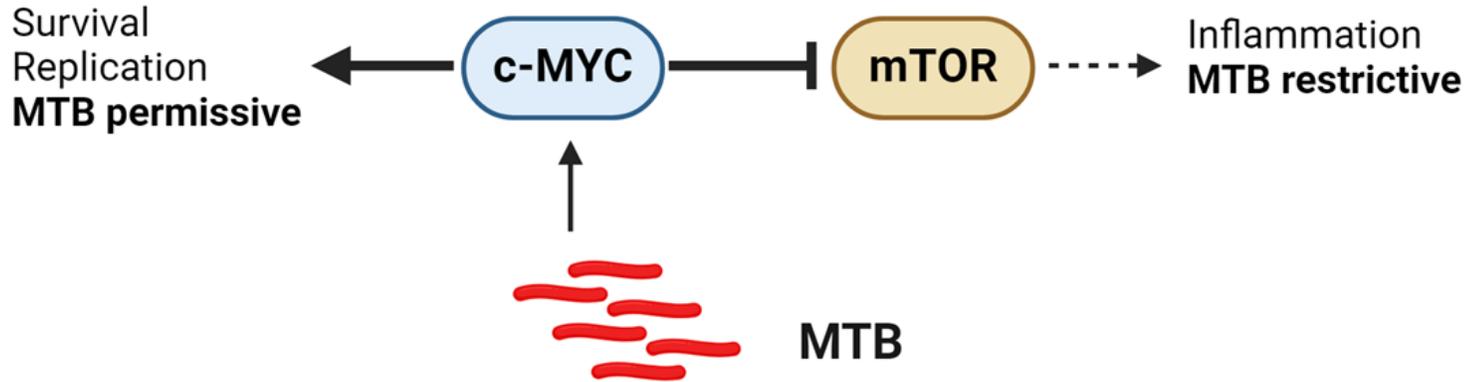
Inhibition of c-MYC activate the mTOR pathway



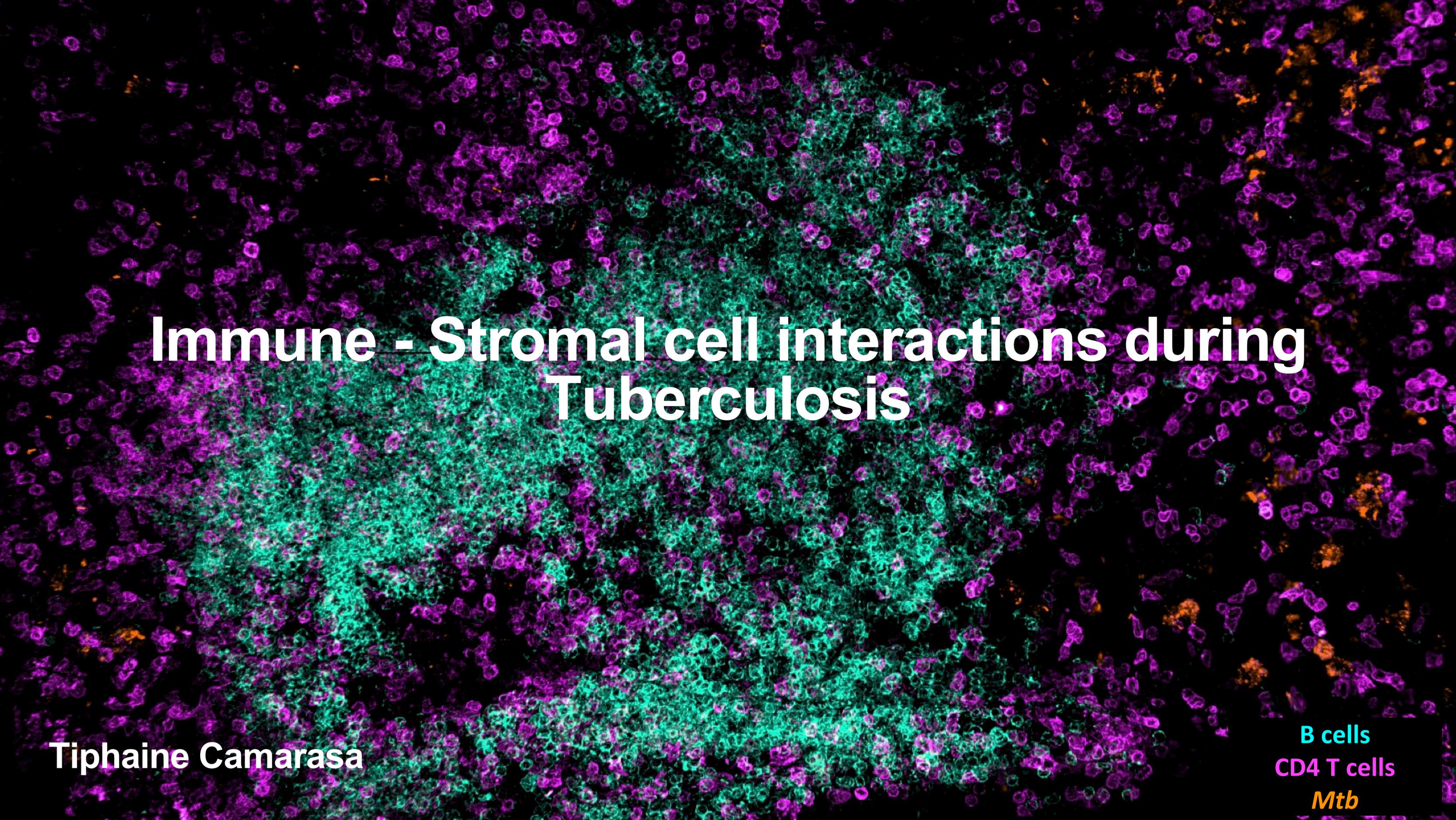
c-MYC is upregulated in the human granuloma



Conclusions



Thank you for your attention!



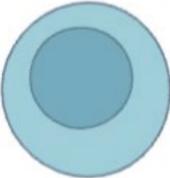
Immune - Stromal cell interactions during Tuberculosis

Tiphaine Camarasa

B cells
CD4 T cells
Mtb

B cells in the lungs

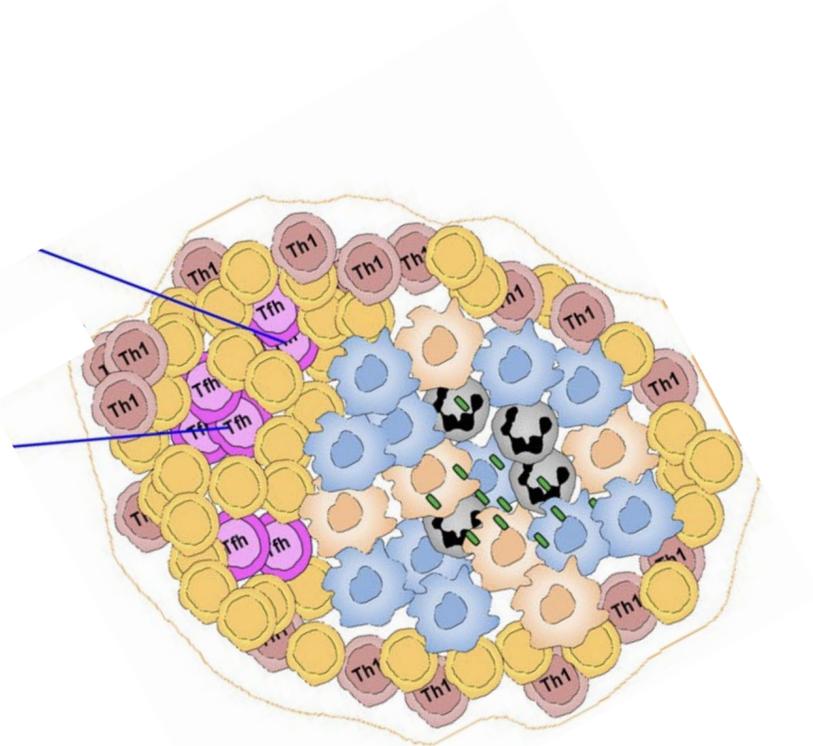
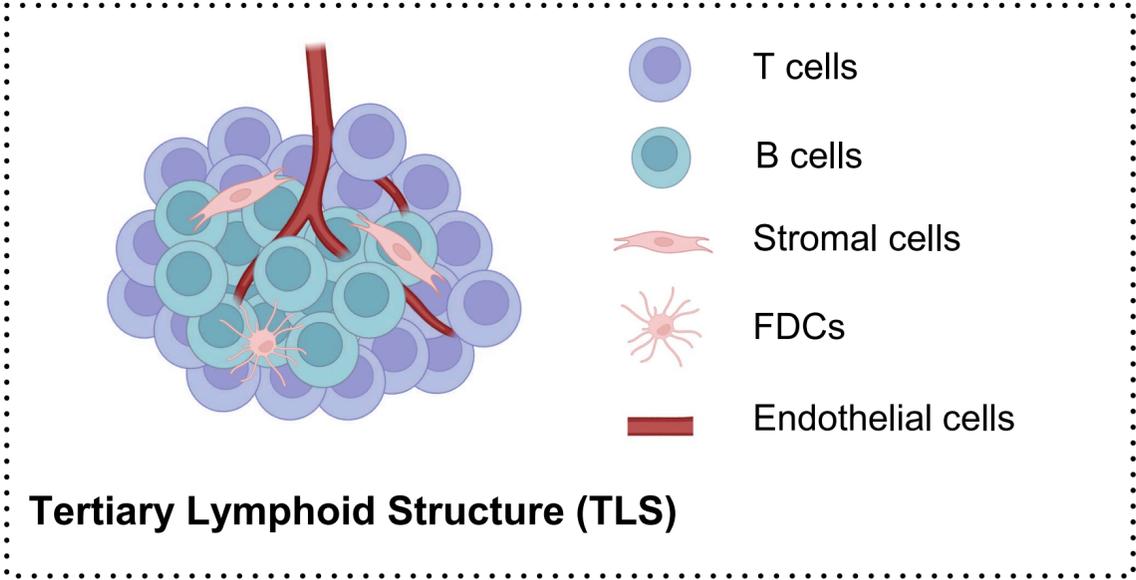
B cells



B cells accumulate in the lungs during tuberculosis

But what roles do they play in the tissue?

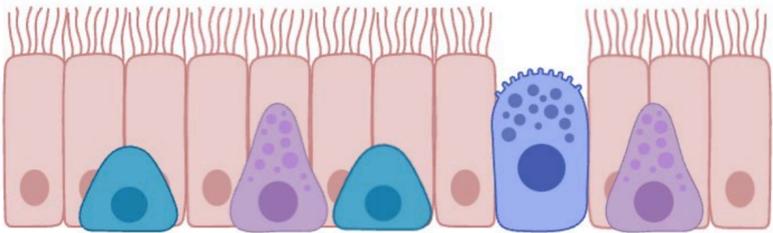
POSITIONING:



Modified from Swanson et al. 2023, Nat Imm

Structural cells

Epithelial cells



Type II, Type I, Club, Multiciliated

STRUCTURE/REPAIR

IMMUNE CELL ACTIVATION

Endothelial cells

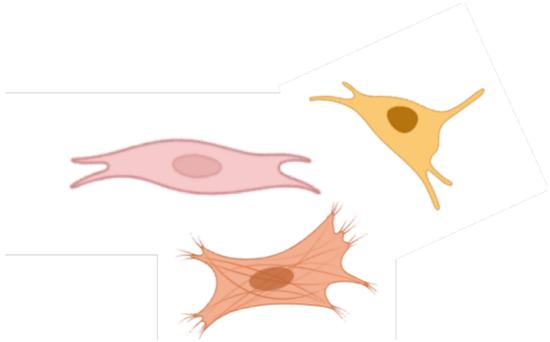


Blood, Lymphatic

STRUCTURE/REPAIR

IMMUNE CELL ACTIVATION

Fibroblasts



Alveolar, Adventitial, Peribronchial

STRUCTURE/REPAIR

IMMUNE CELL ACTIVATION

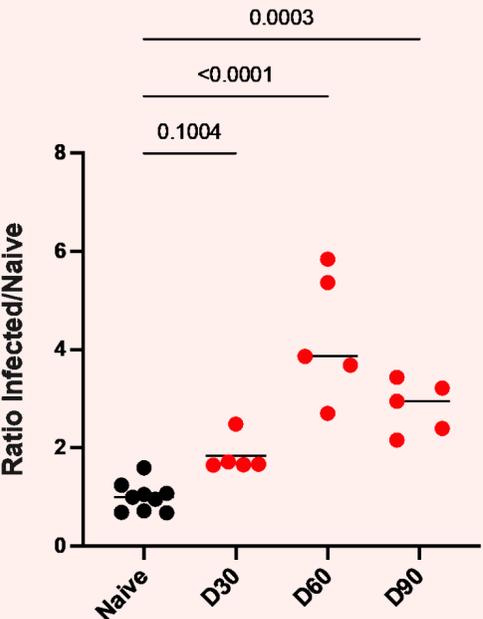
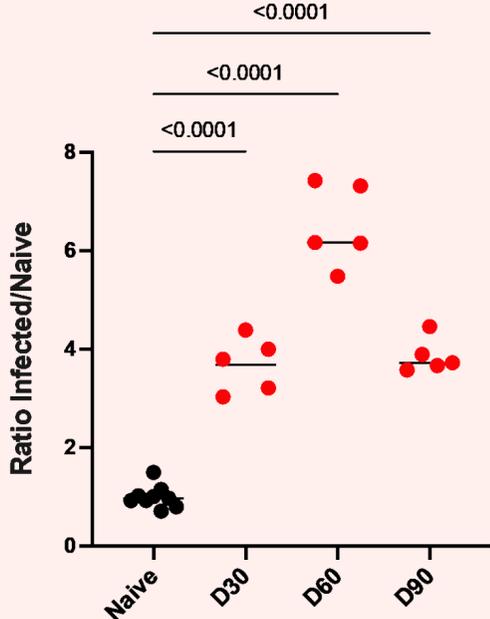
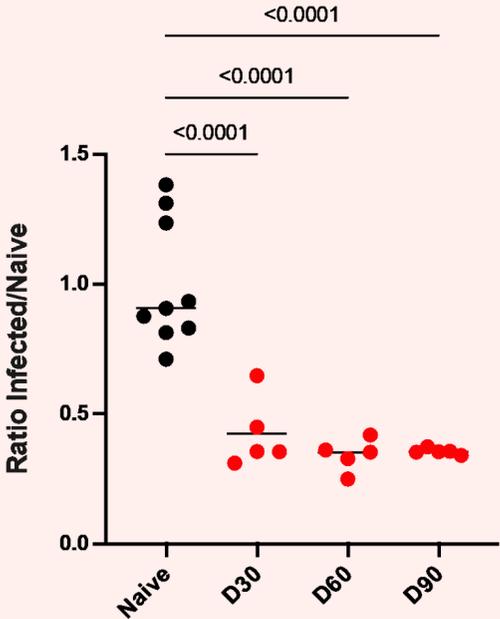
Structural cells

Epithelial cells

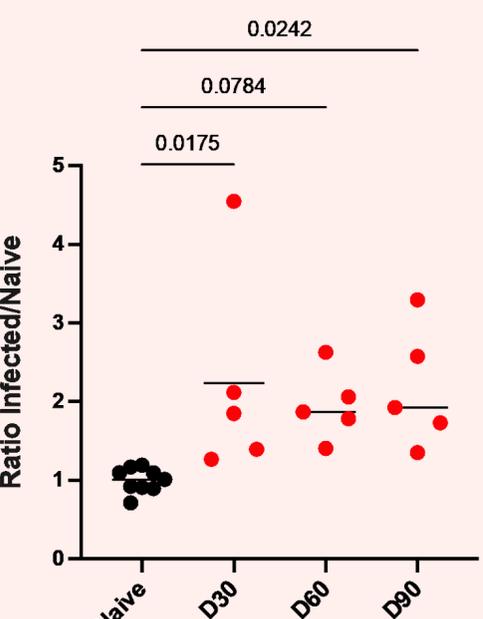
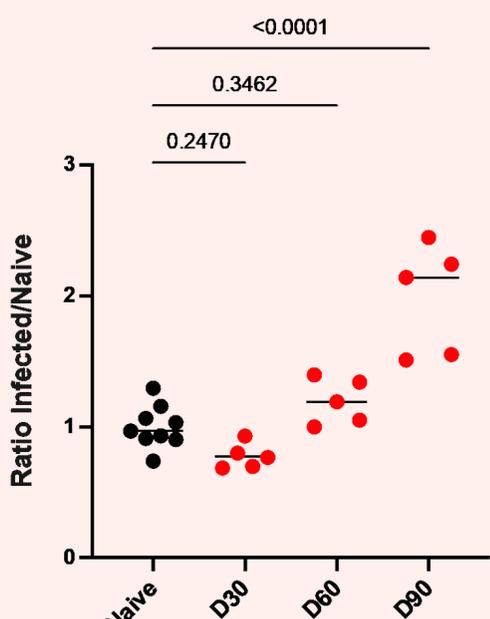
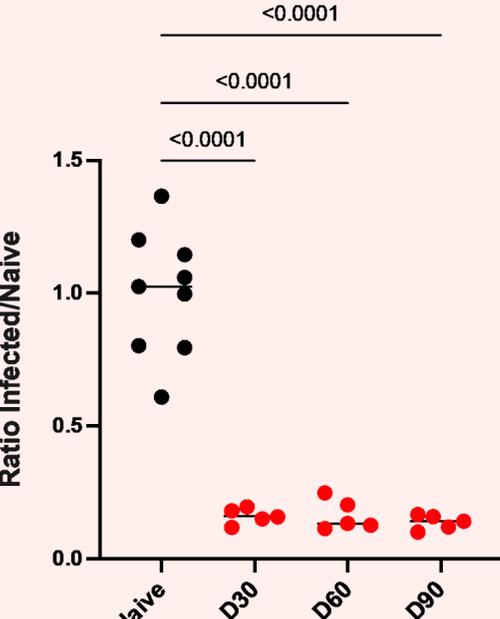
Endothelial cells

Fibroblasts

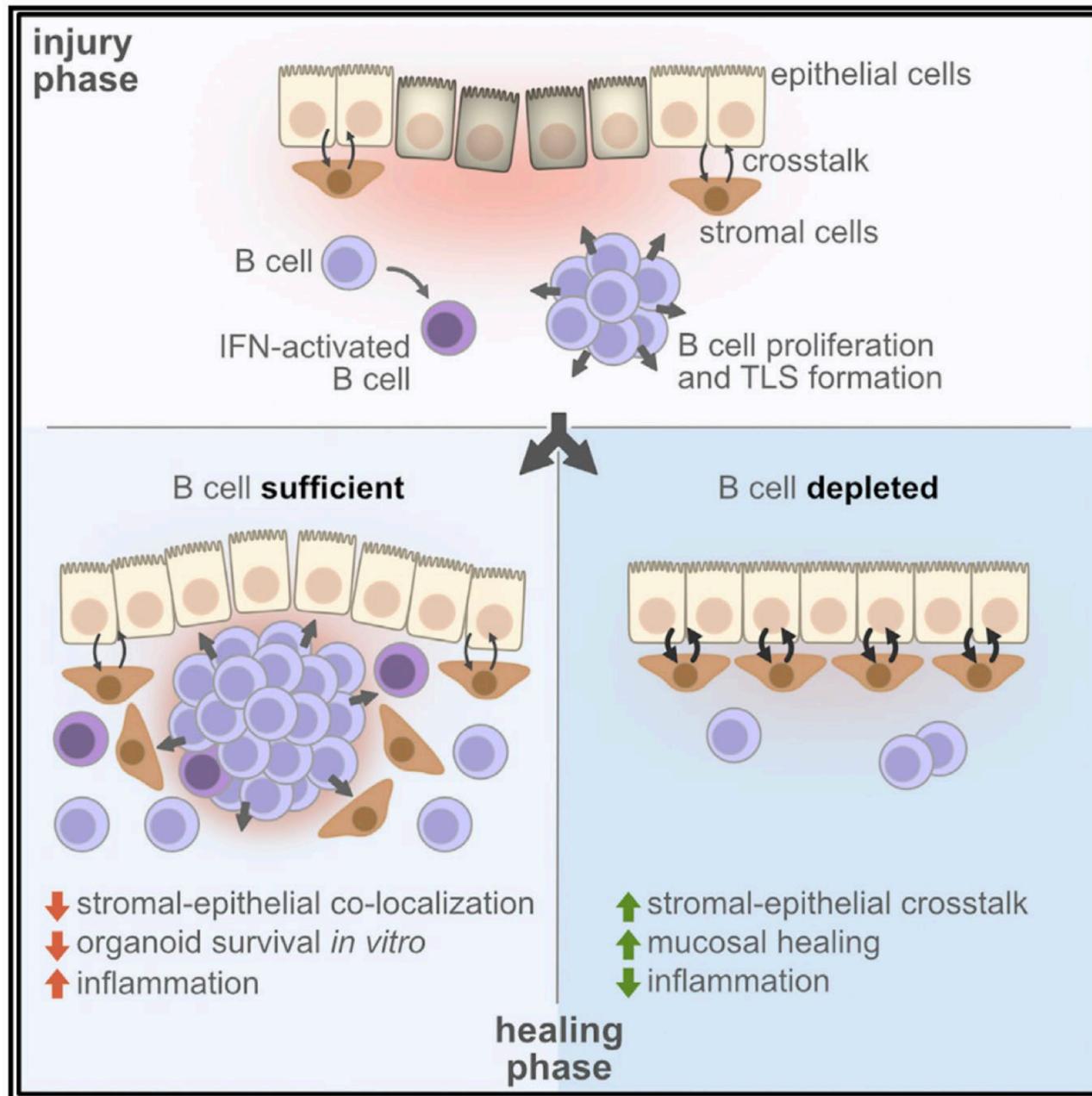
PDL1 (MFI)



MHCII (MFI)



Ulcerative colitis



Frede et al. 2022, Immunity

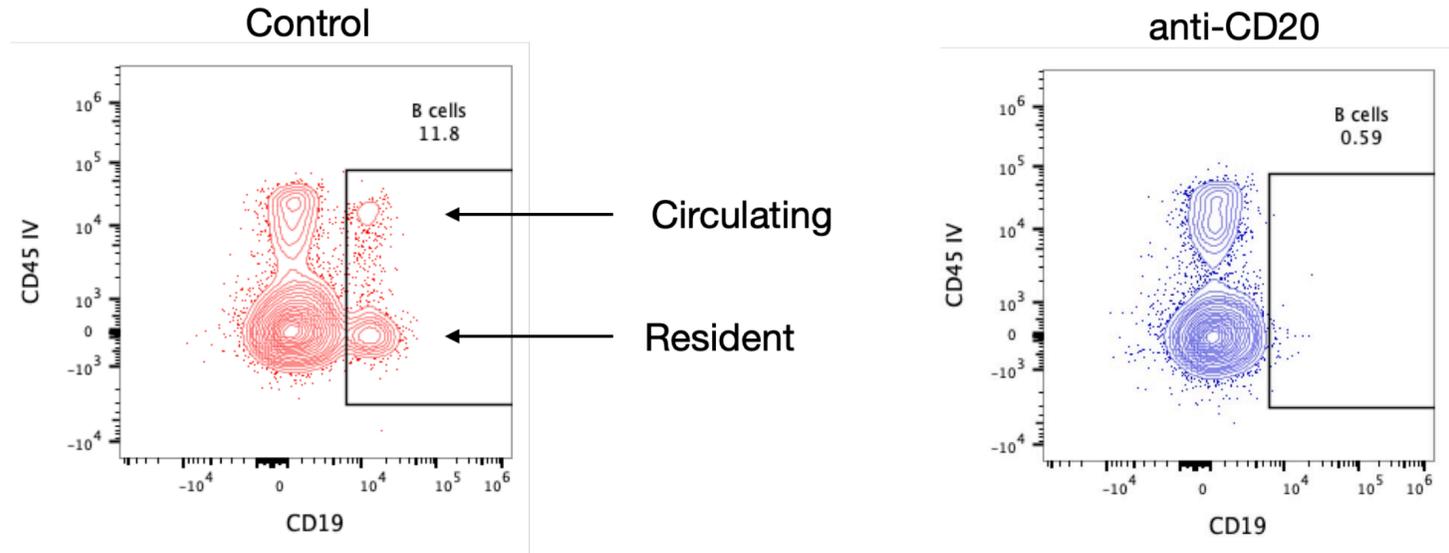
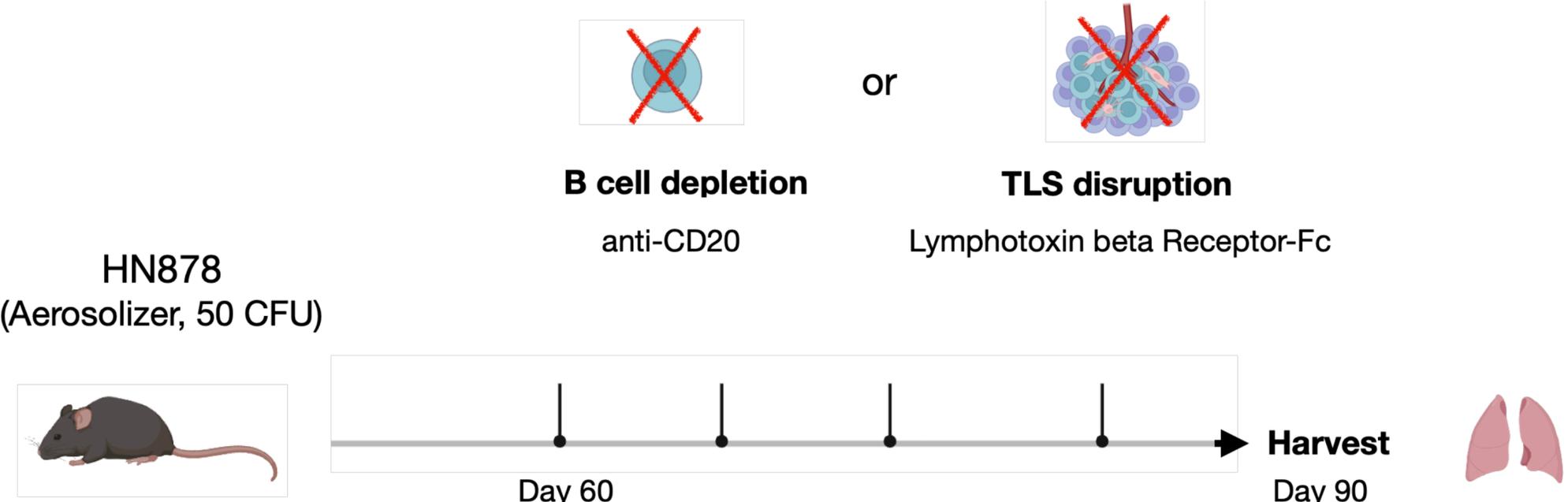
In tuberculosis:

- B cell recruitment in the tissue
- TLS formation
- Type I IFN signature (Akter et al. 2022)
- Stromal – epithelial alterations (phenotype, numbers)

Stromal – epithelial cell remodeling?

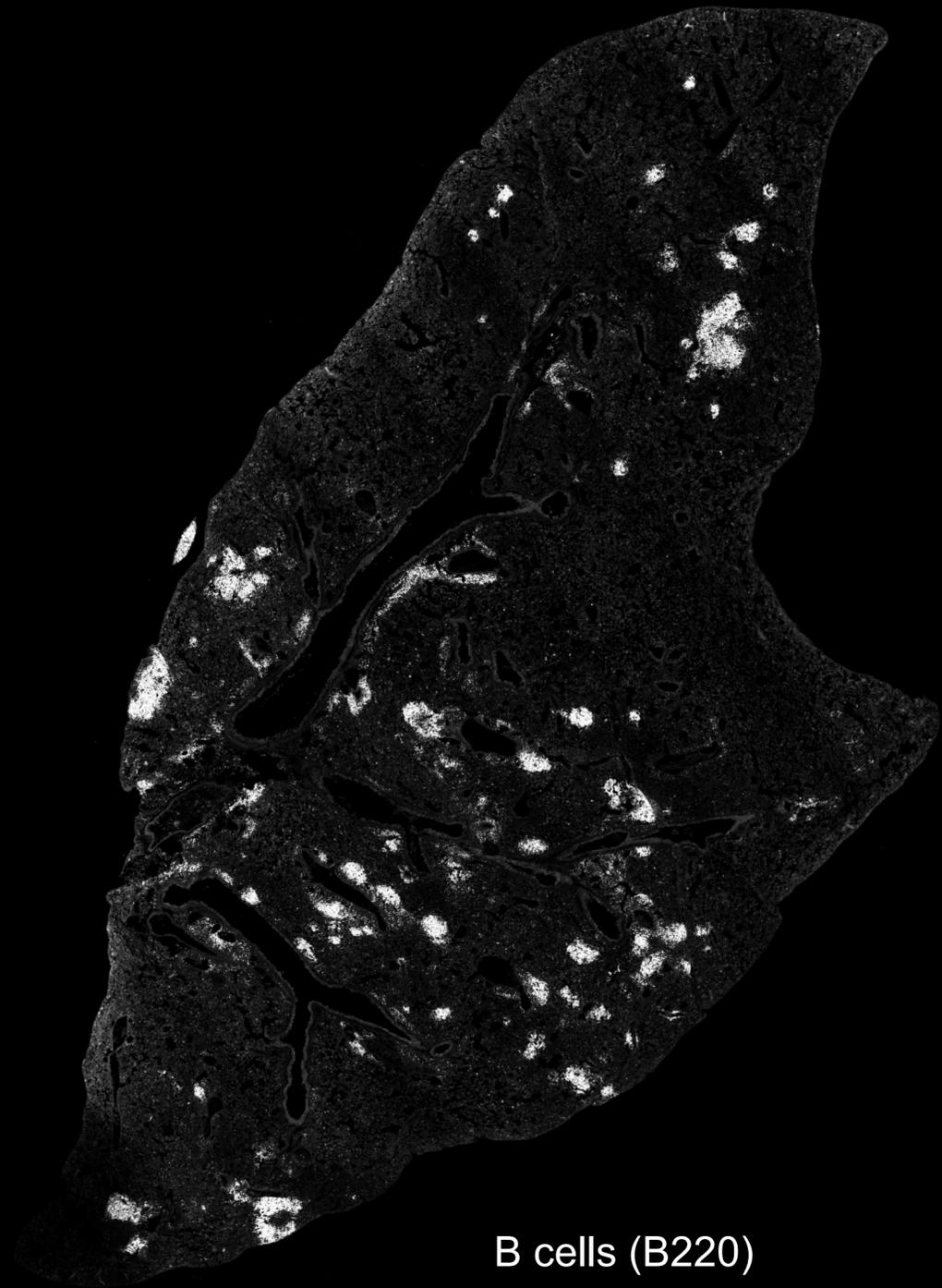
Importance of positioning?

Model

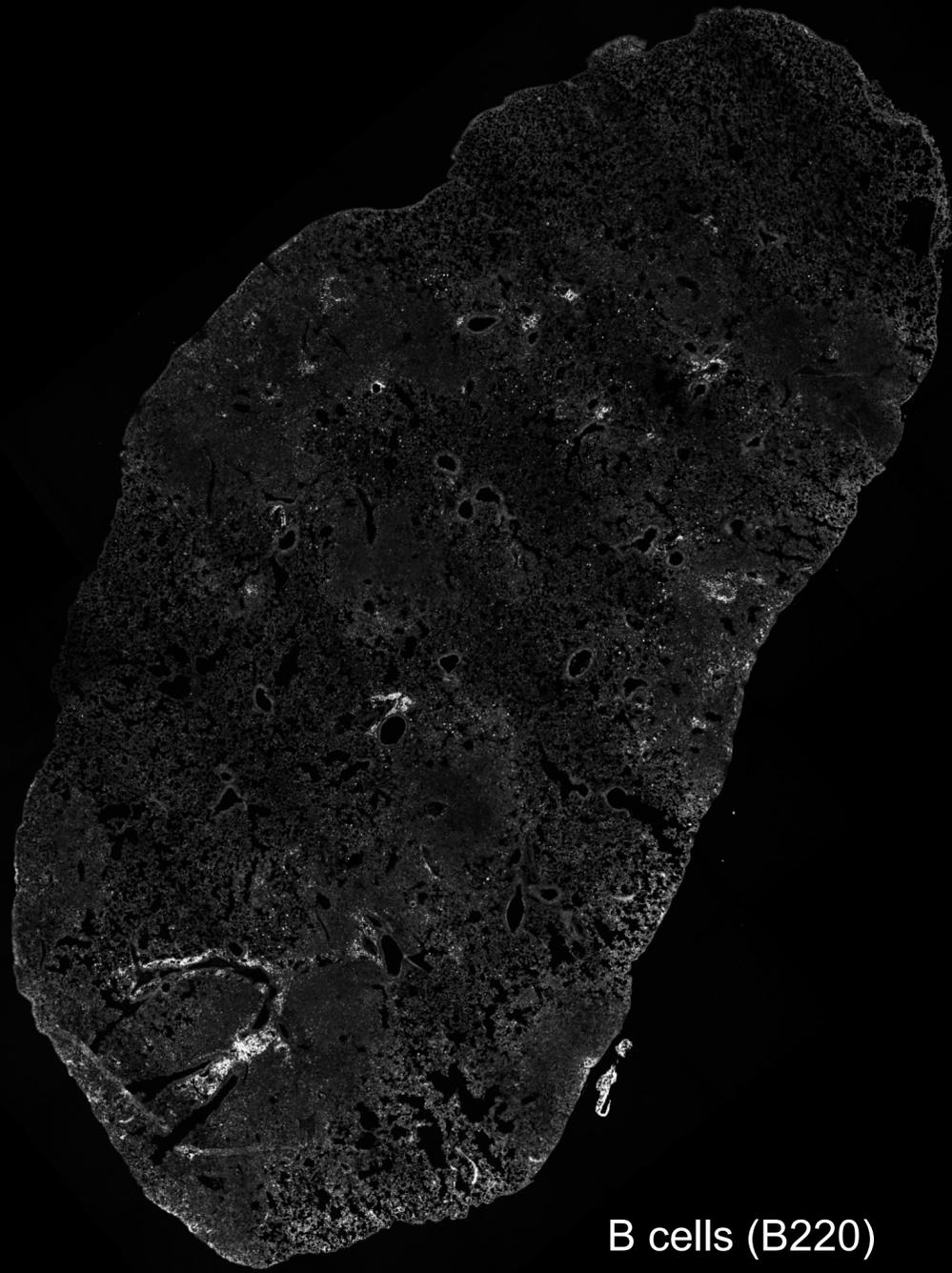


Control

TLS disruption
(Lymphotoxin beta Receptor blocking)



B cells (B220)



B cells (B220)

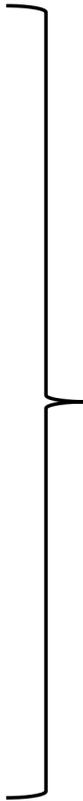
Future directions

Naive

Mtb-infected

Mtb-infected + B cell depletion

Mtb-infected + TLS disruption



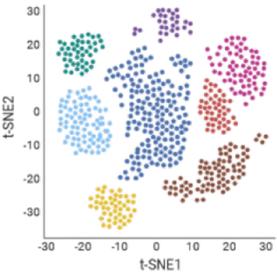
1. Spatial transcriptomic

- positioning
- gene expression



2. High-dimensional flow cytometry

- lymphocytes (30 markers)
- myeloid cells (20 markers)
- structural cells (15 markers)



Infection Immunology Lab

- Carolyn King
- **Wadschma Naderi**
- **Habiba Soliman**
- **Almin Ljubijankic**
- **Gian Pietro Pietri**
- Mara Esposito
- David Schreiner
- Eduardo Moreo
- Jean de Lima
- Maïke Erber
- Claire Depew
- Aaron Forde
- Emanuela Kerschbamer

Collaborators

- Daniel Pinschewer, DBM
- Sitjn Vanhee, Ghent University
- Burkhard Ludewig, Kantonsspital St.Gallen
- Michael Detmar, ETH Zürich

THANK YOU!

BSL3 Facility, Biozentrum

- Maxime Québatte
- Petra Chiquet

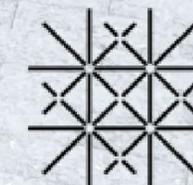
DBM Microscopy Core facility

- Pascal Lorentz

DBM Flow Cytometry Core facility

- Gaël Auray

KING LAB



University
of Basel

Exploring the mechanisms of *Mycobacteria*-mediated membrane damage: Small then catastrophic, two steps in damage leading to different bacterial fates

Céline Michard¹; Angélique Perret¹; Hendrik Koliwer-Brandl²; Hubert Hilbi²; Thierry Soldati¹

¹ Department of Biochemistry, Faculty of Science, University of Geneva, Geneva, Switzerland

² Institute of Medical Microbiology, Faculty of Medicine, University of Zurich, Zurich, Switzerland



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October 30th, 2024

Mycobacterium marinum & *Dictyostelium discoideum*: our model to study the mechanisms of tuberculosis

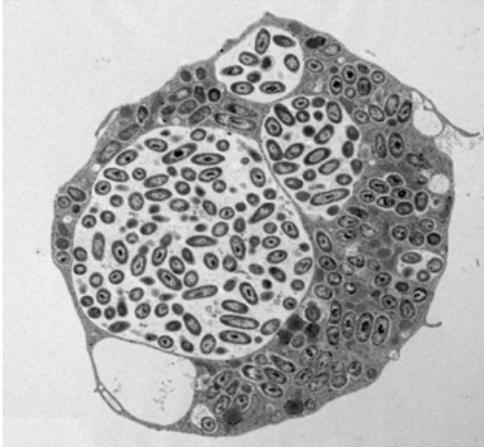
M. marinum

- Close relative to *Mycobacterium tuberculosis* with 85% nucleotide identity
- Shared virulence mechanisms

D. discoideum

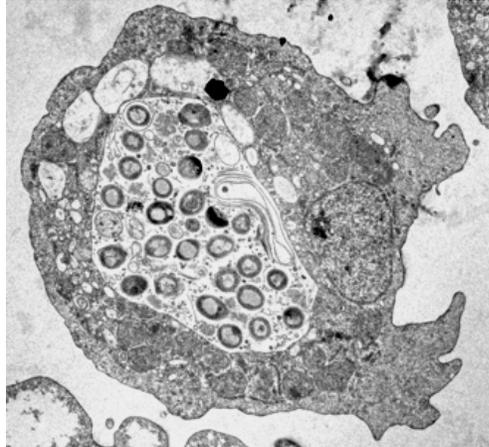
- Cell autonomous defence conserved with macrophages
- Easy to genetically manipulate

Macrophage / *M. tuberculosis*



Russell et al., 2002, J Cell Biol.

D. discoideum / *M. marinum*



Courtesy of M. Hagedorn

Mycobacterium marinum & *Dictyostelium discoideum*: our model to study the mechanisms of tuberculosis

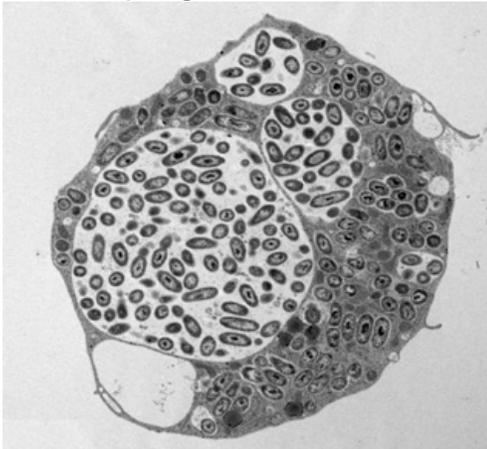
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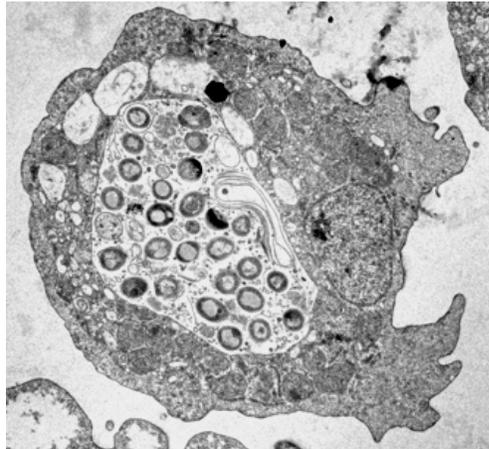
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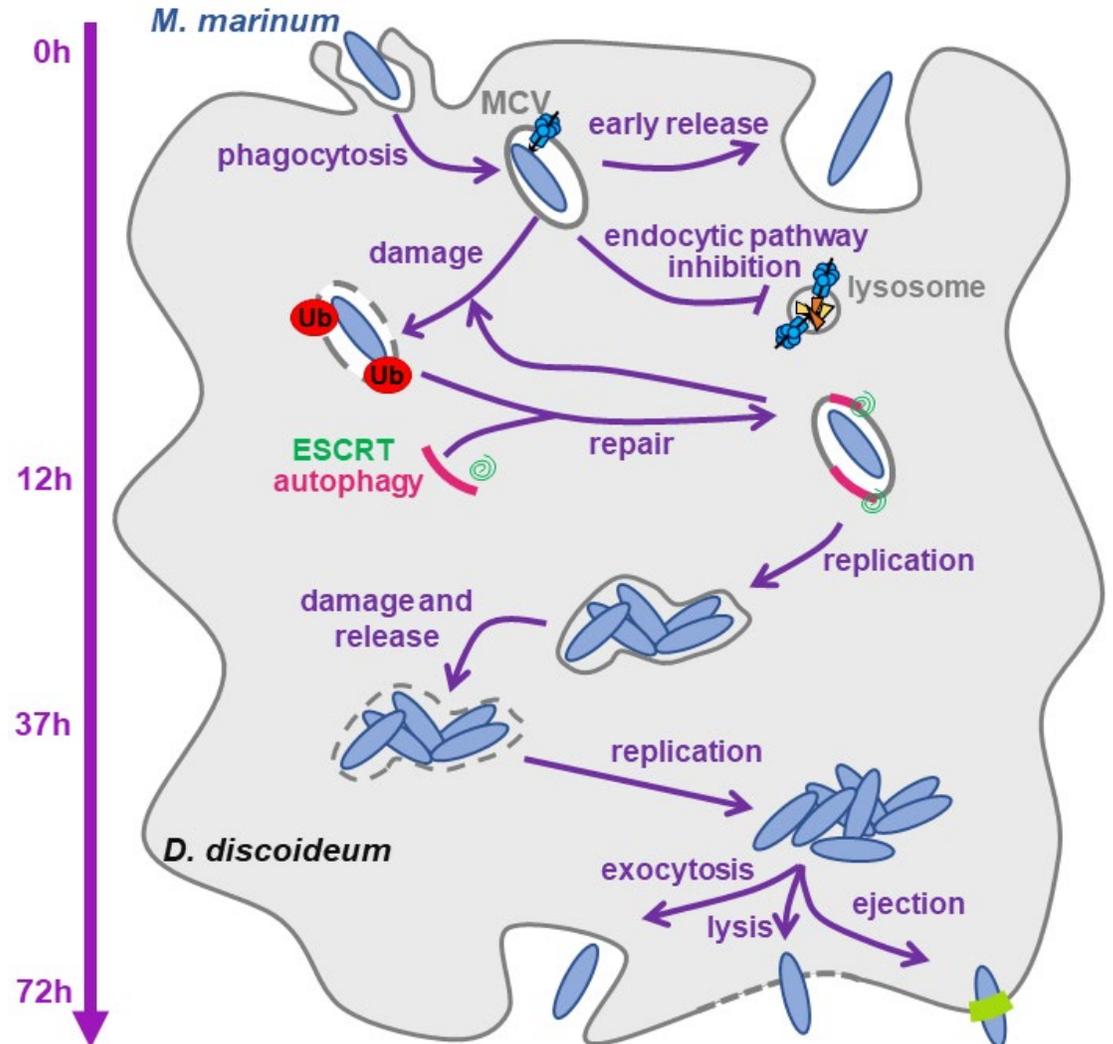


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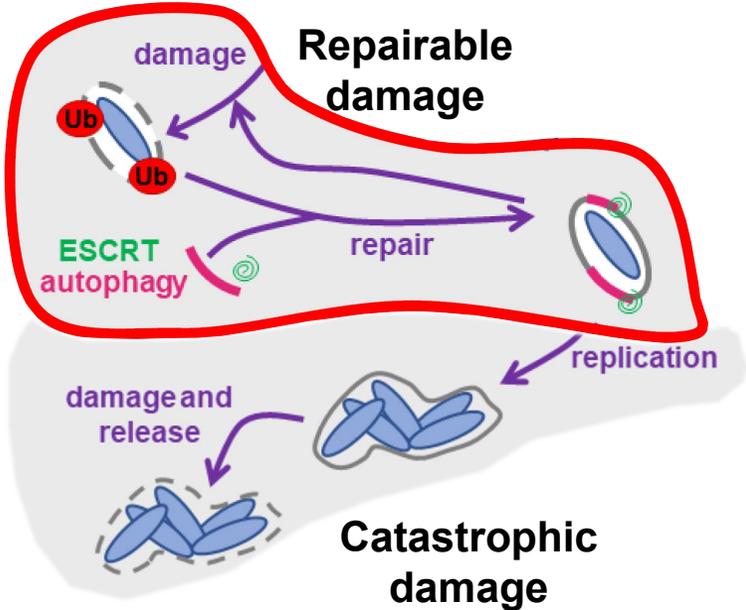
D. discoideum / *M. marinum*



Courtesy of M. Hagedorn

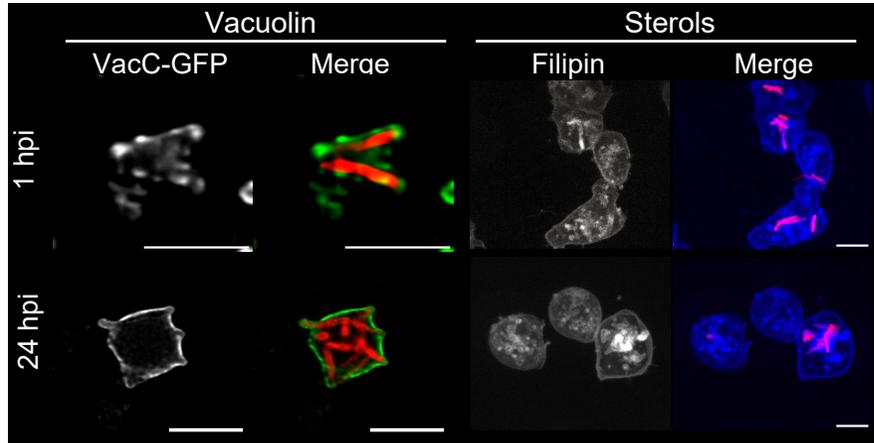


MCV damage/repair cycle

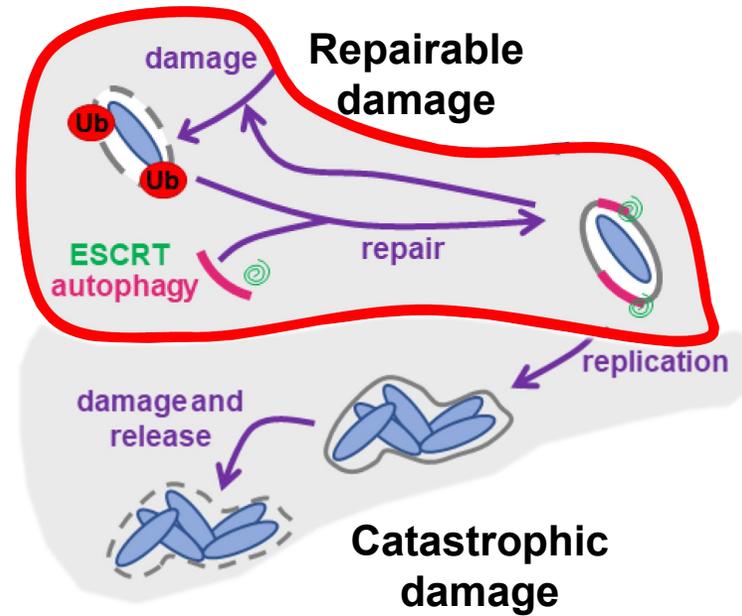


MCV damage/repair cycle

Microdomains necessary for damage

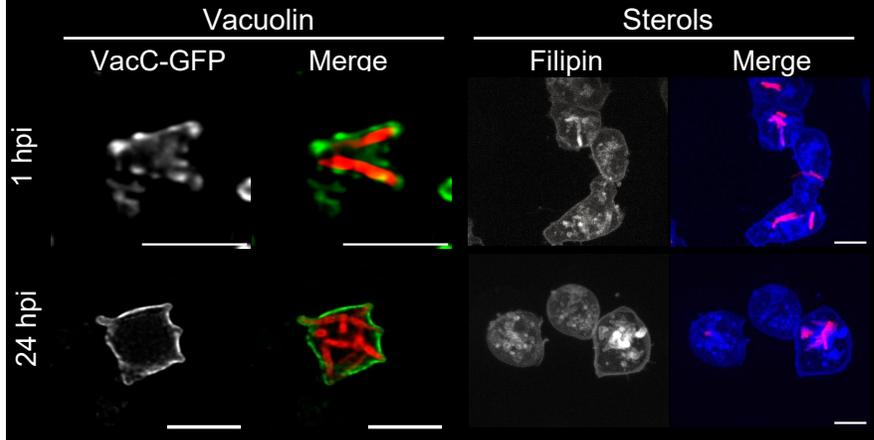


Bosmani & Perret et al., 2024

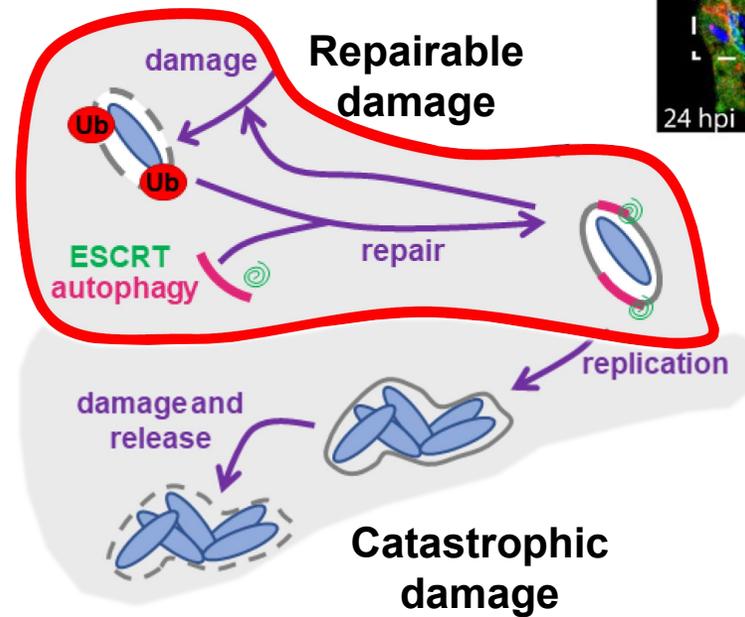


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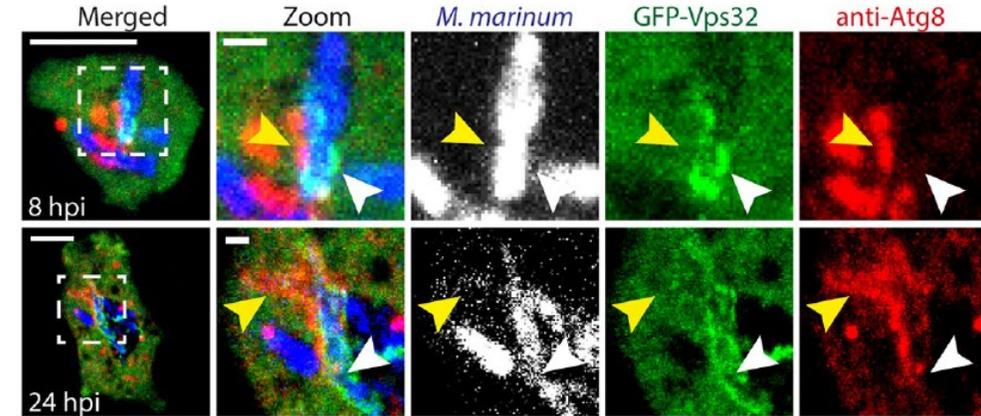
Microdomains necessary for damage



Bosmani & Perret et al., 2024

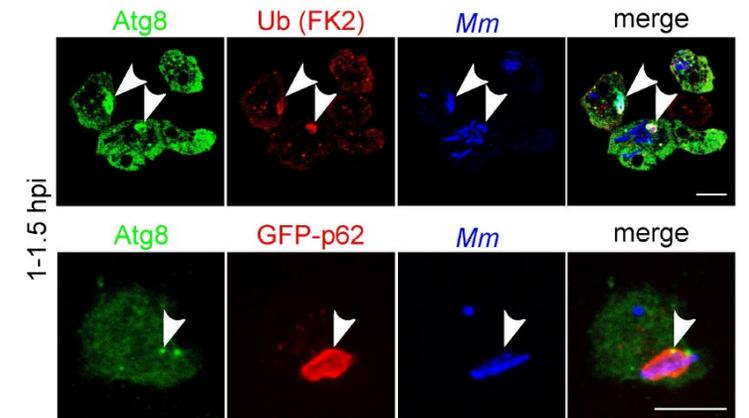


ESCRT recruitment to repair the MCV



López-Jiménez et al., 2018

Autophagy recruitment to repair the MCV

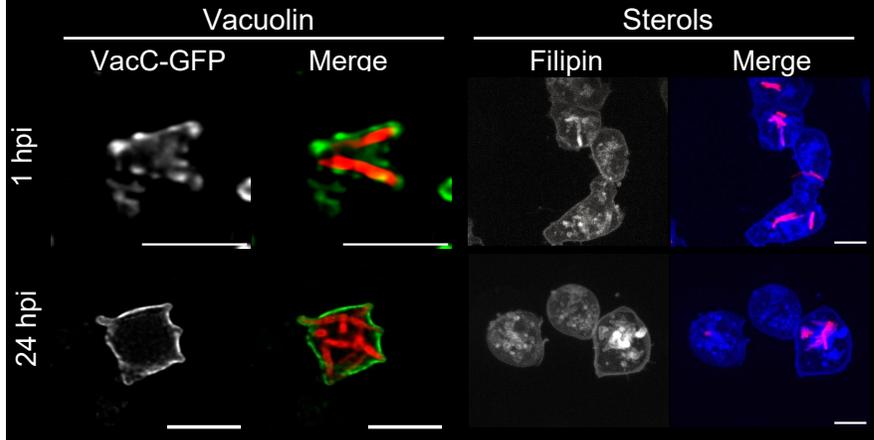


Cardenal-Muñoz et al., 2017

Introduction

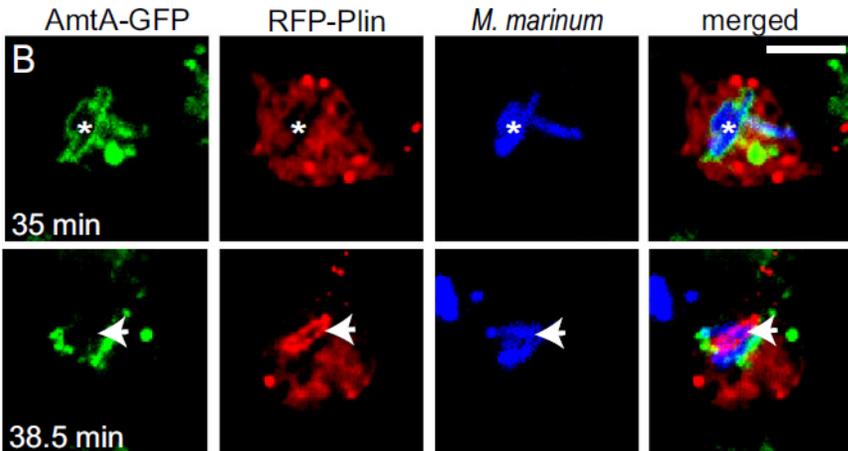
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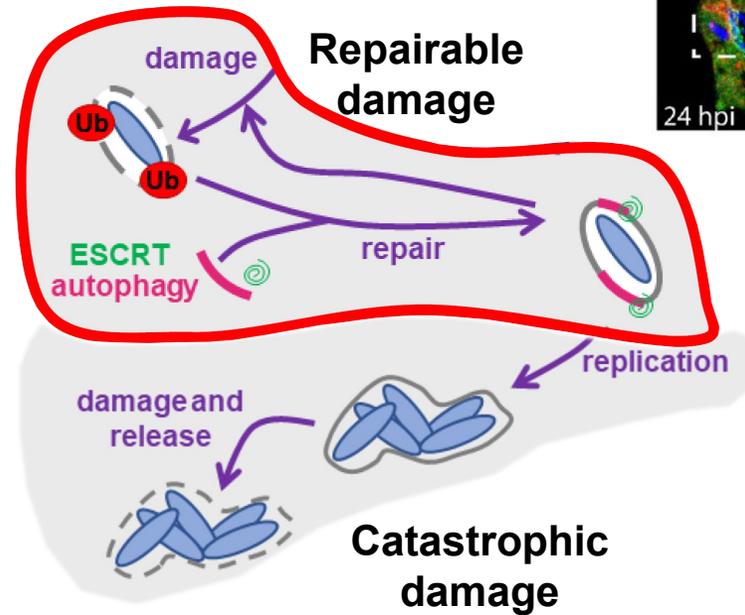


Bosmani & Perret et al., 2024

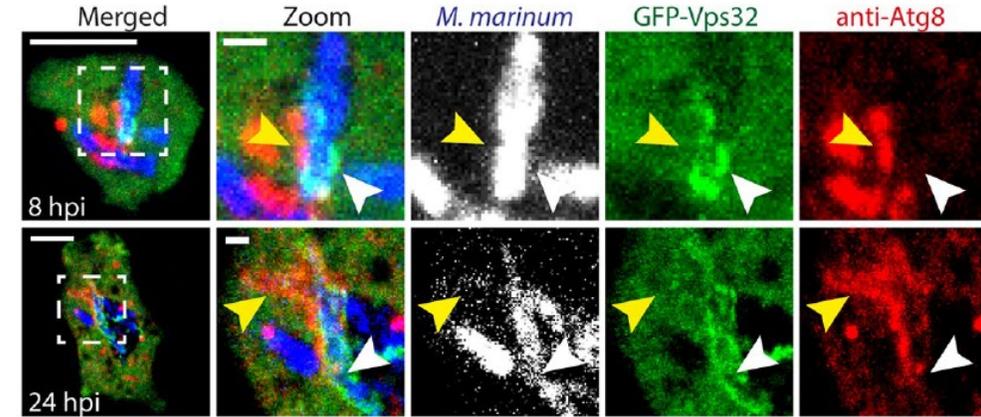
Perilipin recruitment to cytosolic bacteria



Barisch et al., 2015

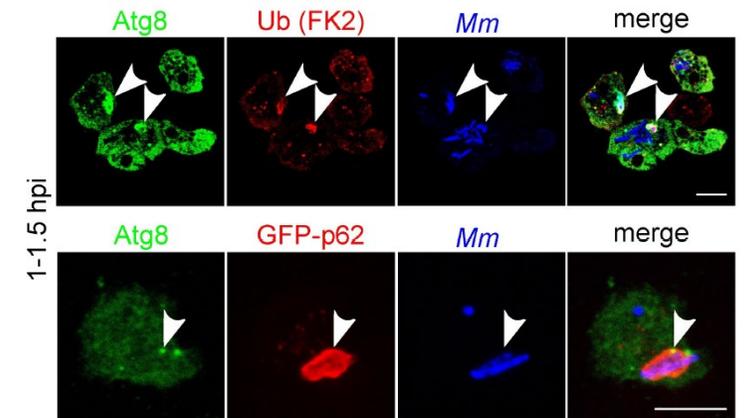


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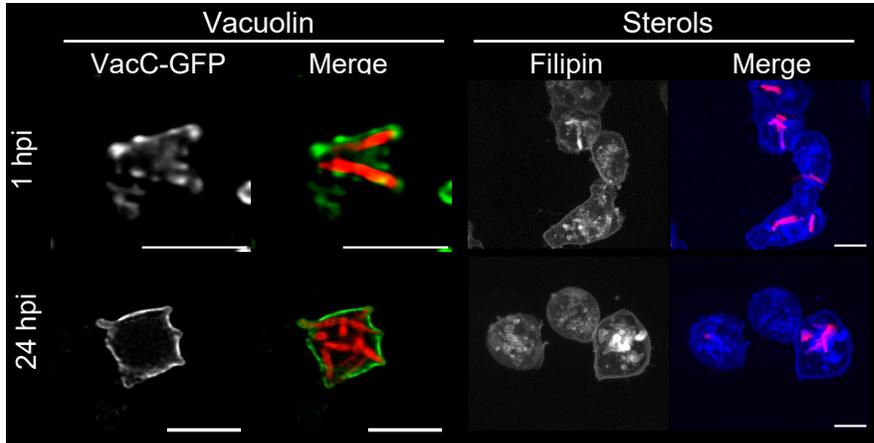
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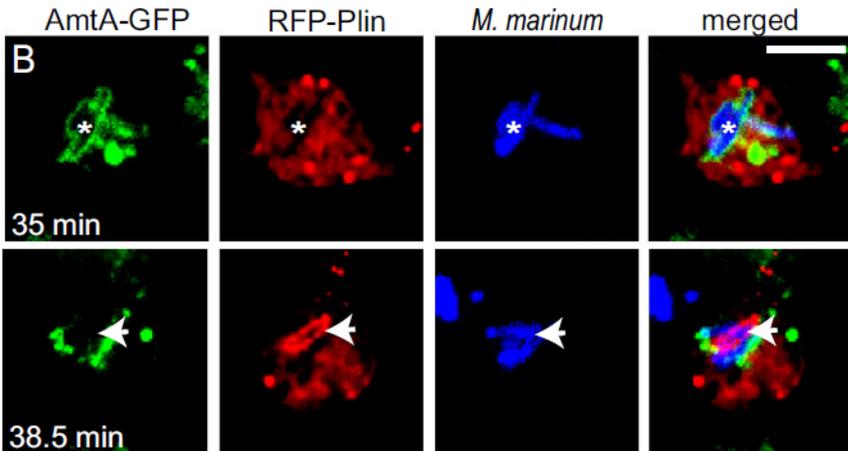
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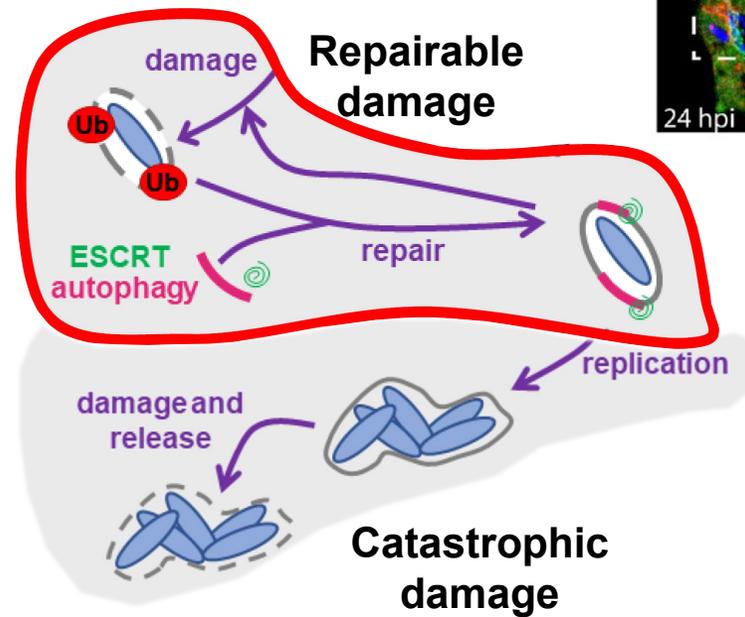


Bosmani & Perret et al., 2024

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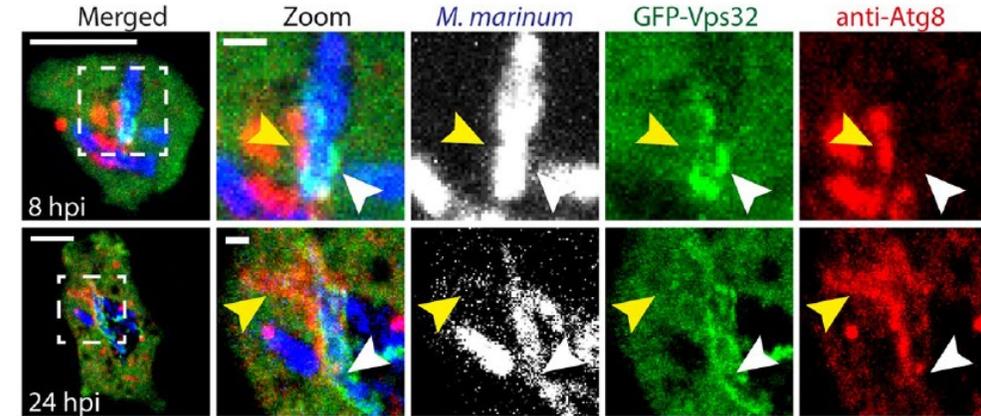


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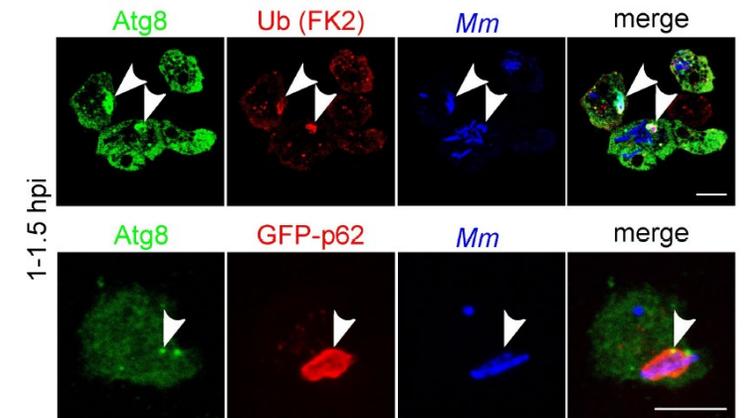
Does the repairable damage progress to catastrophic damage or are they two different types of damage?

ESCRT recruitment to repair the MCV



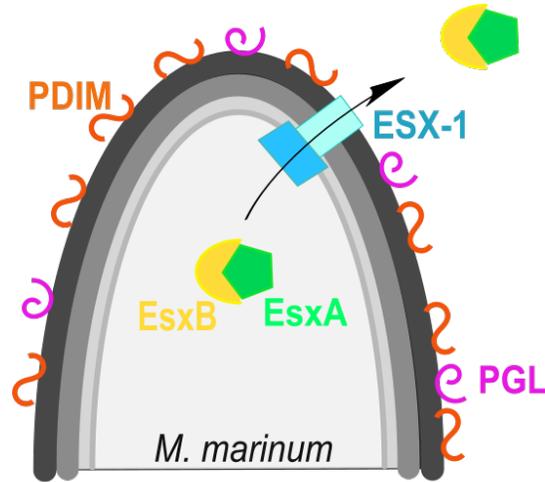
López-Jiménez et al., 2018

Autophagy recruitment to repair the MCV



Cardenal-Muñoz et al., 2017

Bacterial components proposed to damage the MCV



❖ ESX-1

- Type VII secretion system
- EsxA/ESX-1 controversy for MCV damaging activity (Conrad *et al.*, 2017; Lienard *et al.*, 2020)

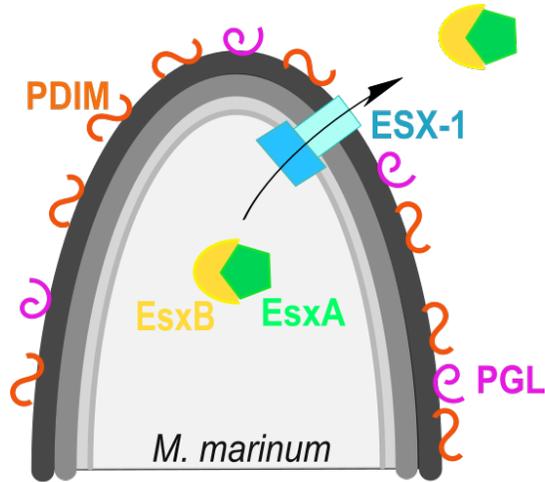
❖ EsxA (or ESAT-6)

- 6kDa early secretory antigenic target
- secreted by *Mycobacterium* through ESX-1 as a heterodimer with EsxB (CFP-10)
- shown to have a vacuole rupture activity (Hsu *et al.*, 2003; Simeone *et al.*, 2012;...)

❖ PDIM (pthiocerol dimycocerosates)

- noncovalently bound lipid in the *Mycobacterium* cell wall
- found only in pathogenic strains
- shown to have a vacuole rupture activity in *Mtb* or *Mm* (Quigley *et al.*, 2017; Augenstreich *et al.*, 2017; Lerner *et al.*, 2018; Osman *et al.*, 2020)

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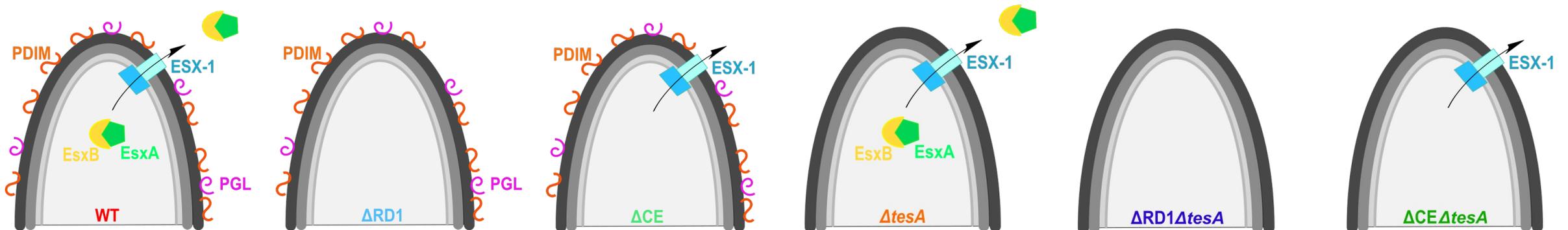
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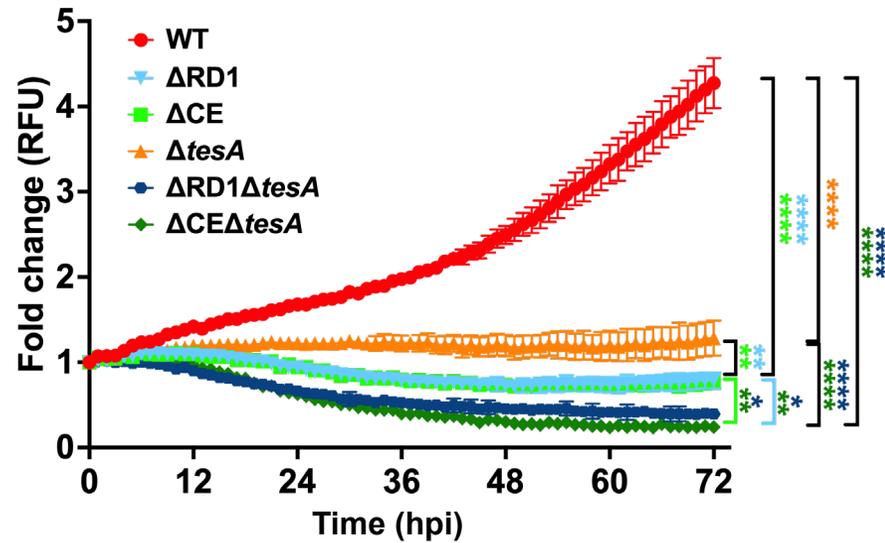
➤ Set of *M. marinum* mutants used in this study



Results

Both PDIMs and EsxA are required for bacterial growth in *D. discoideum* but to different extents

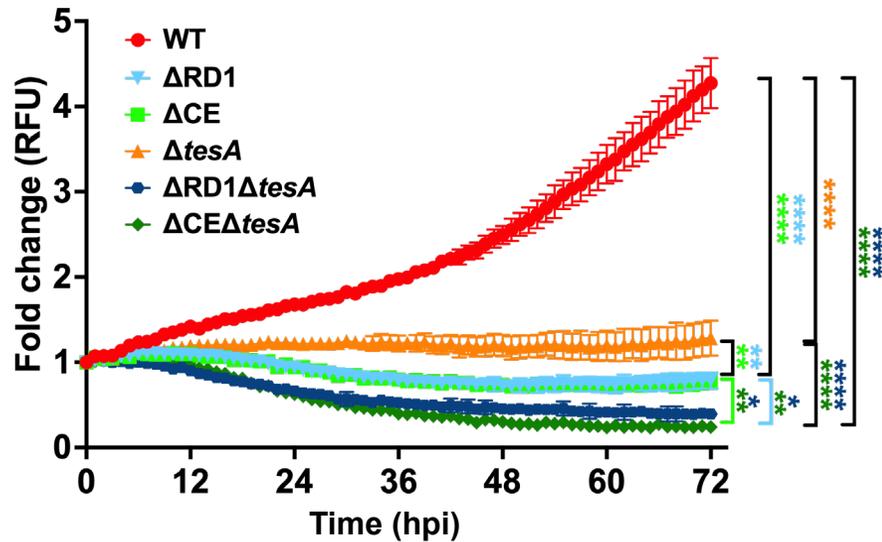
- Intracellular growth of *M. marinum* pGFP strains monitored using a plate reader (fluorescence intensity)



Results

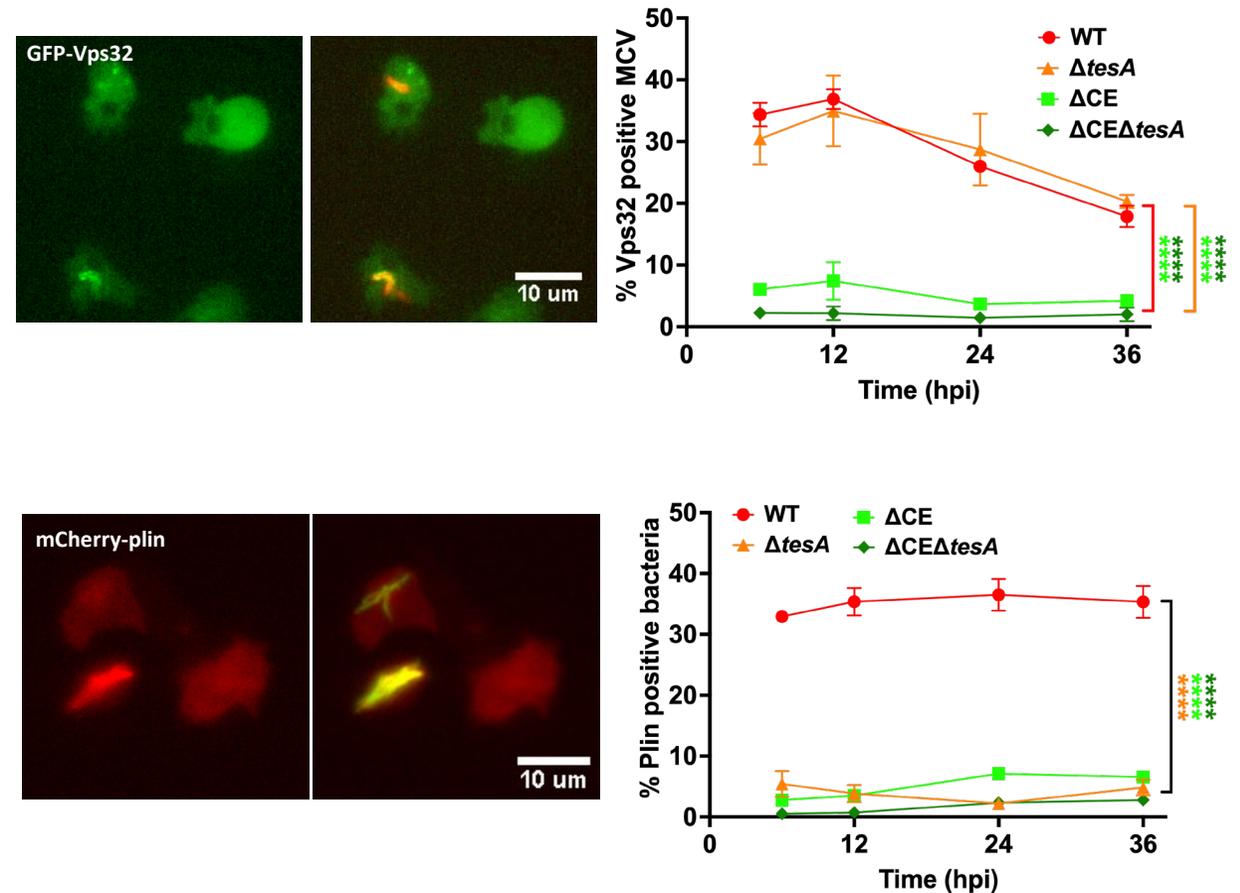
Both PDIMs and EsxA are required for bacterial growth in *D. discoideum* but to different extents depending on their ability to induce membrane damage

➤ Intracellular growth of *M. marinum* pGFP strains monitored using a plate reader (fluorescence intensity)



N=3; 2way ANOVA with Fisher's LSD post hoc

➤ Markers recruitment on MCV after infection of Ax2(Ka) stably expressing GFP or mCherry tagged reporter with Mm pGFP or pmCherry strains (High Content analysis on live samples)

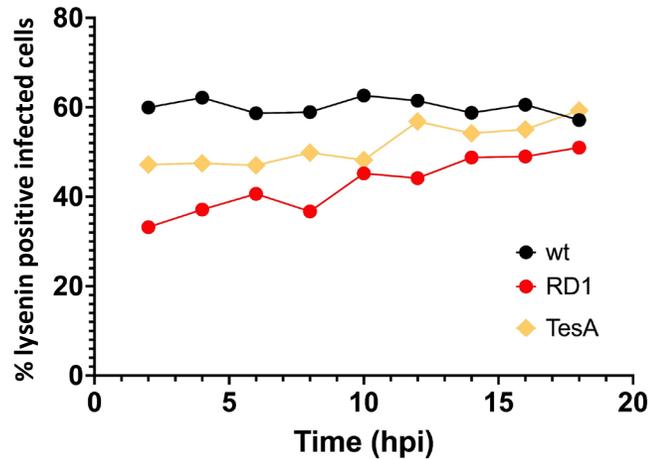
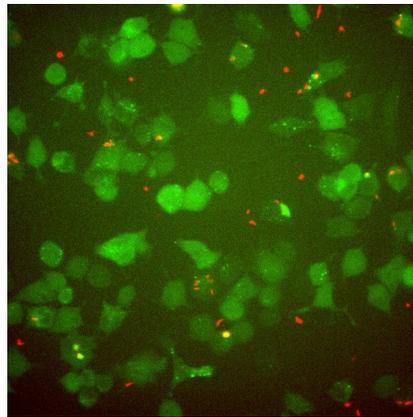


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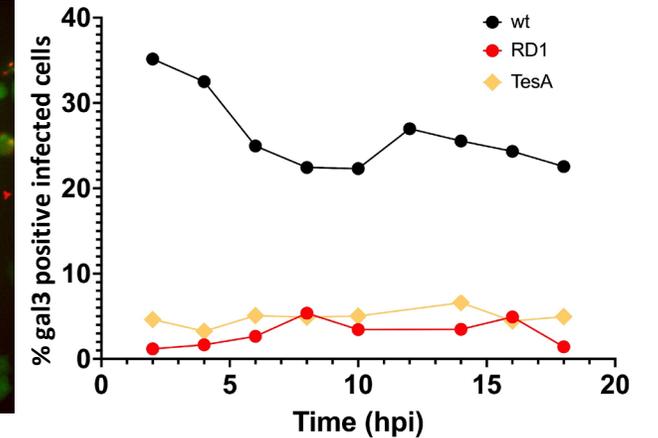
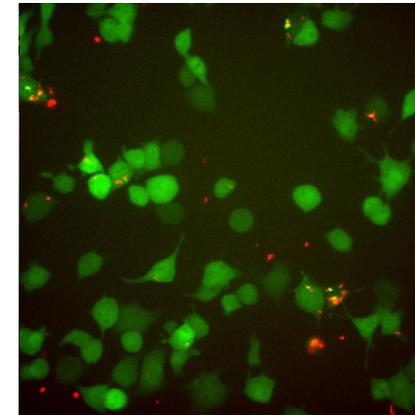
The damage mechanism appears to be conserved in mammalian phagocytes

- Markers recruitment at MCV after infection of BV2 microglial cells stably expressing GFP tagged reporter with Mm pmCherry strains (High Content analysis on live samples)

Lysenin recruitment:
Lysenin binds sphingomyelin
(small damage)



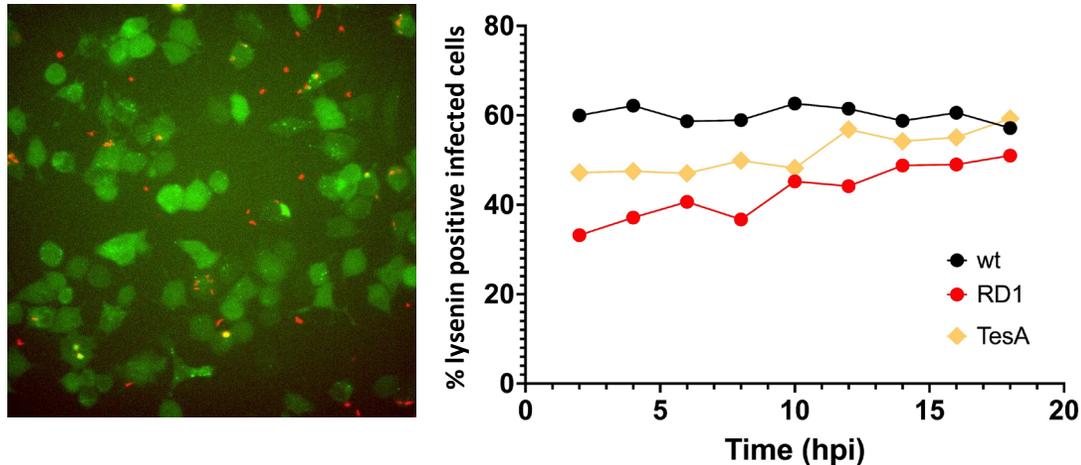
Galectin-3 recruitment:
Galectin-3 binds luminal glycan
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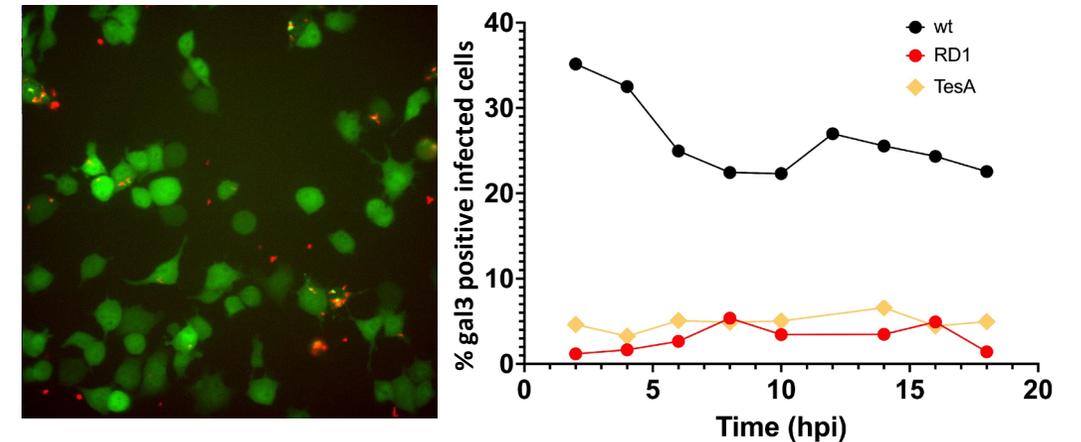
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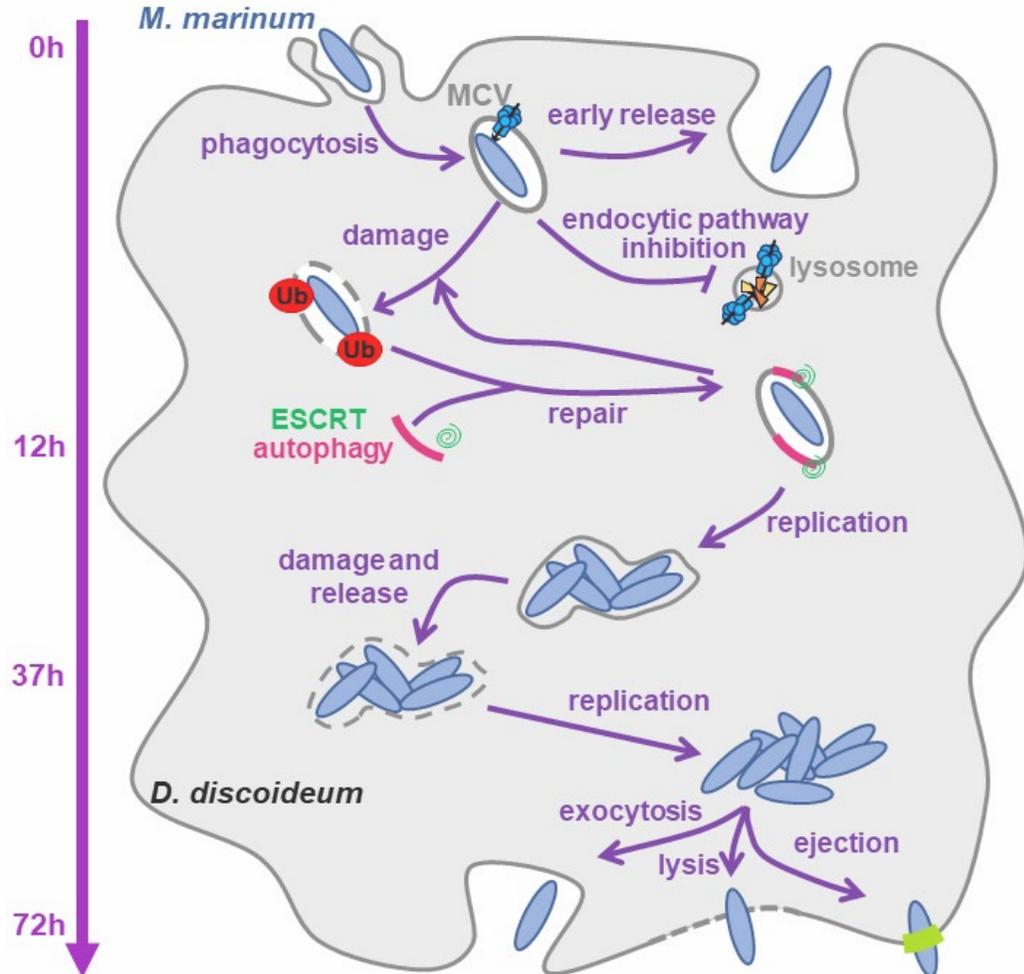
Galectin-3 recruitment:
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Are the intracellular growth defects of the EsxA and PDIM mutants also conserved ?

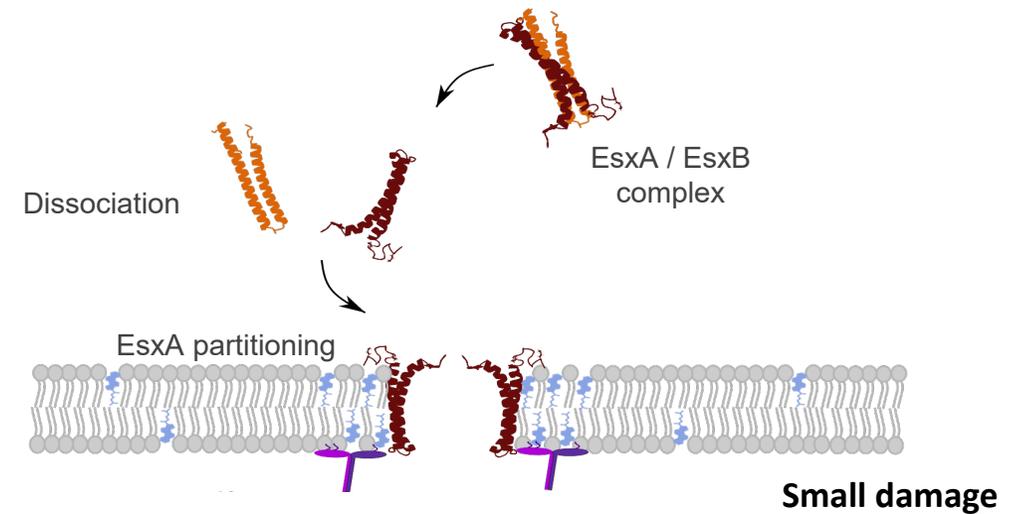
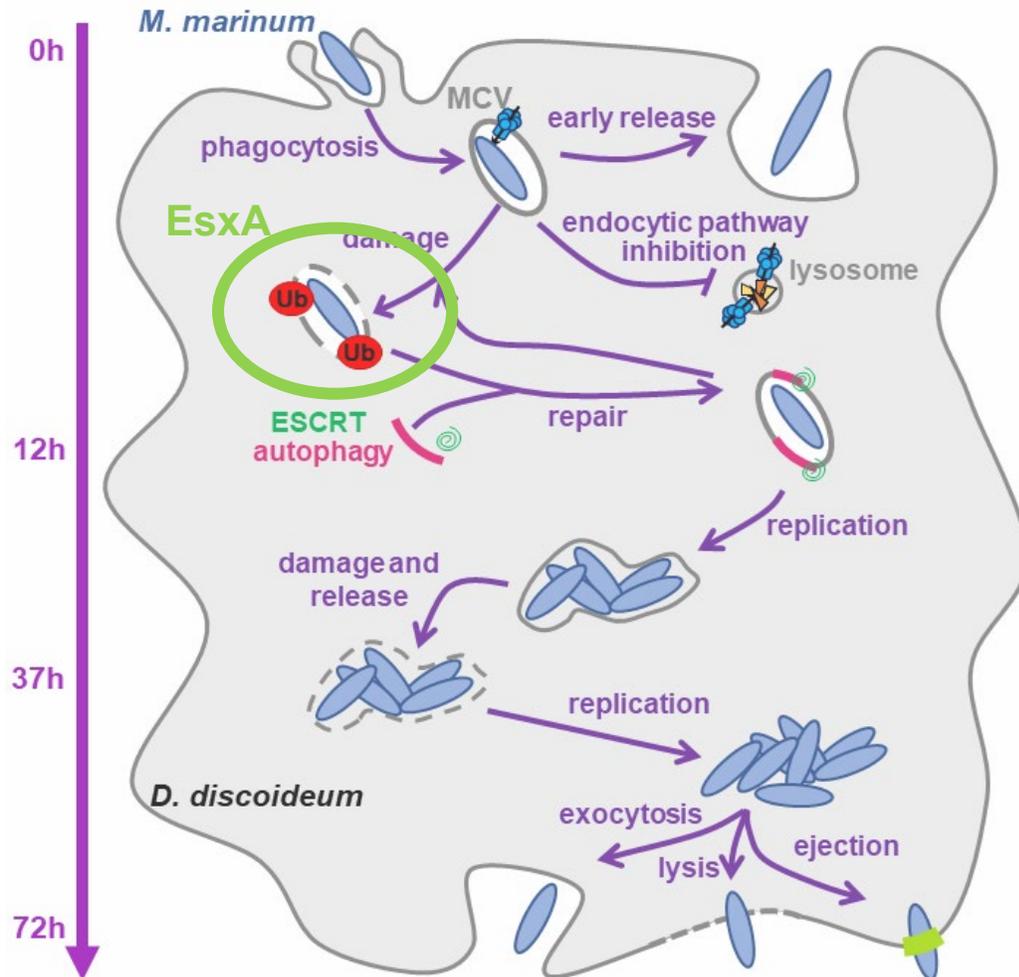
Conclusions

→ PDIMs and EsxA are both required for full virulence during infection but to different extents



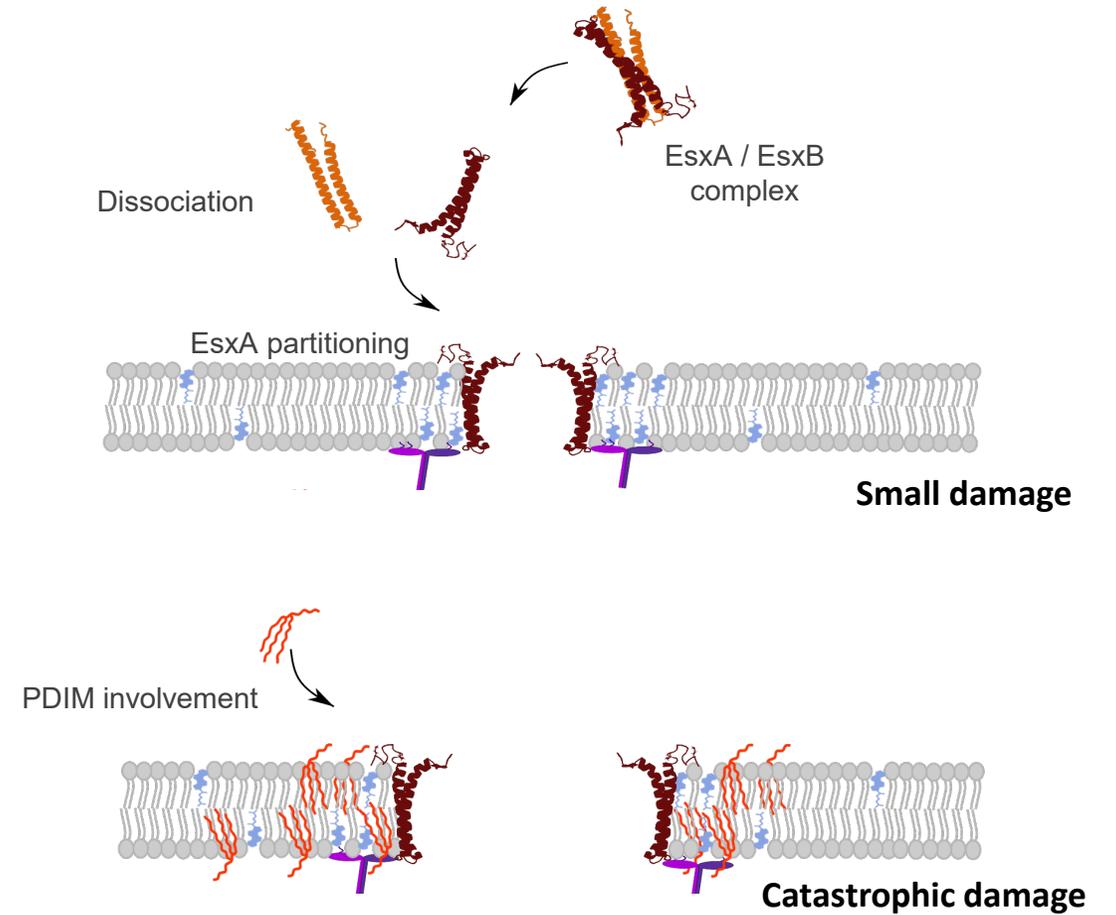
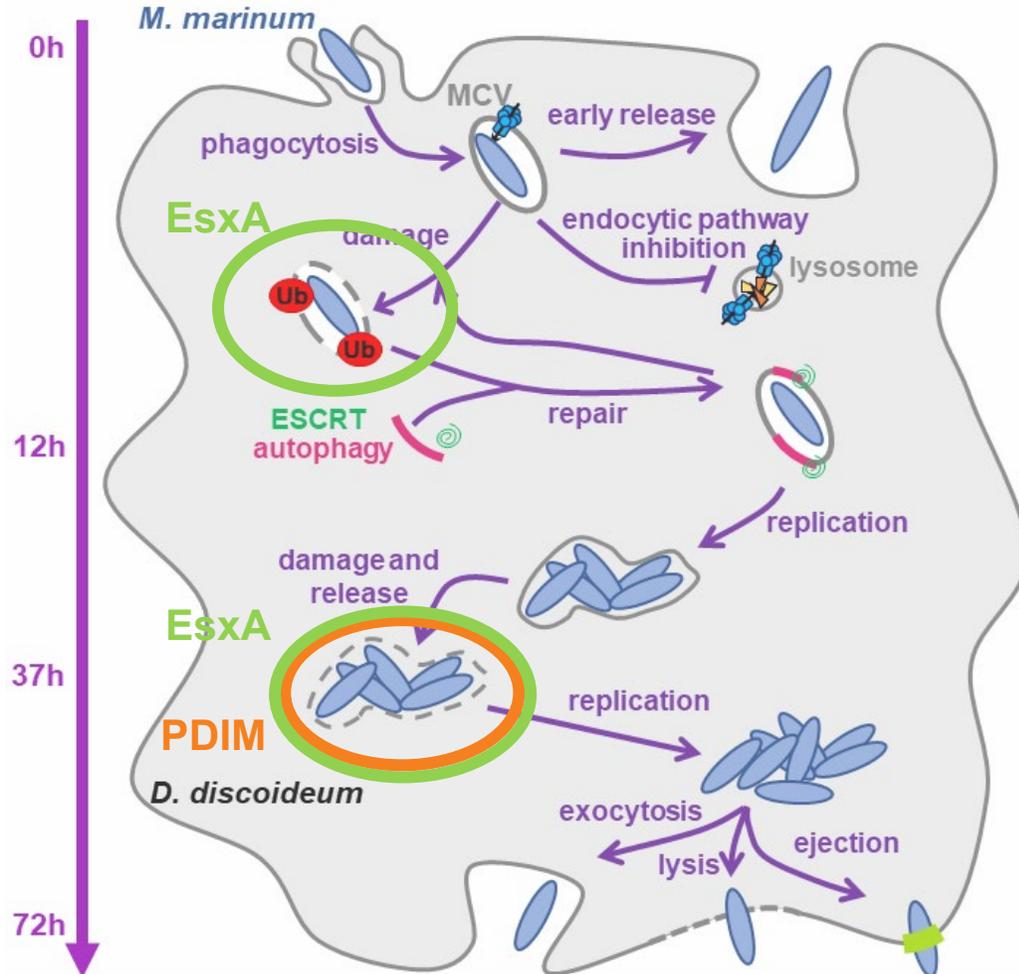
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Conclusions

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Soldati lab:

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Lyudmil Raykov, PhD

Previous member:

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Chiara Toniolo, PhD



Thank you for your attention !!



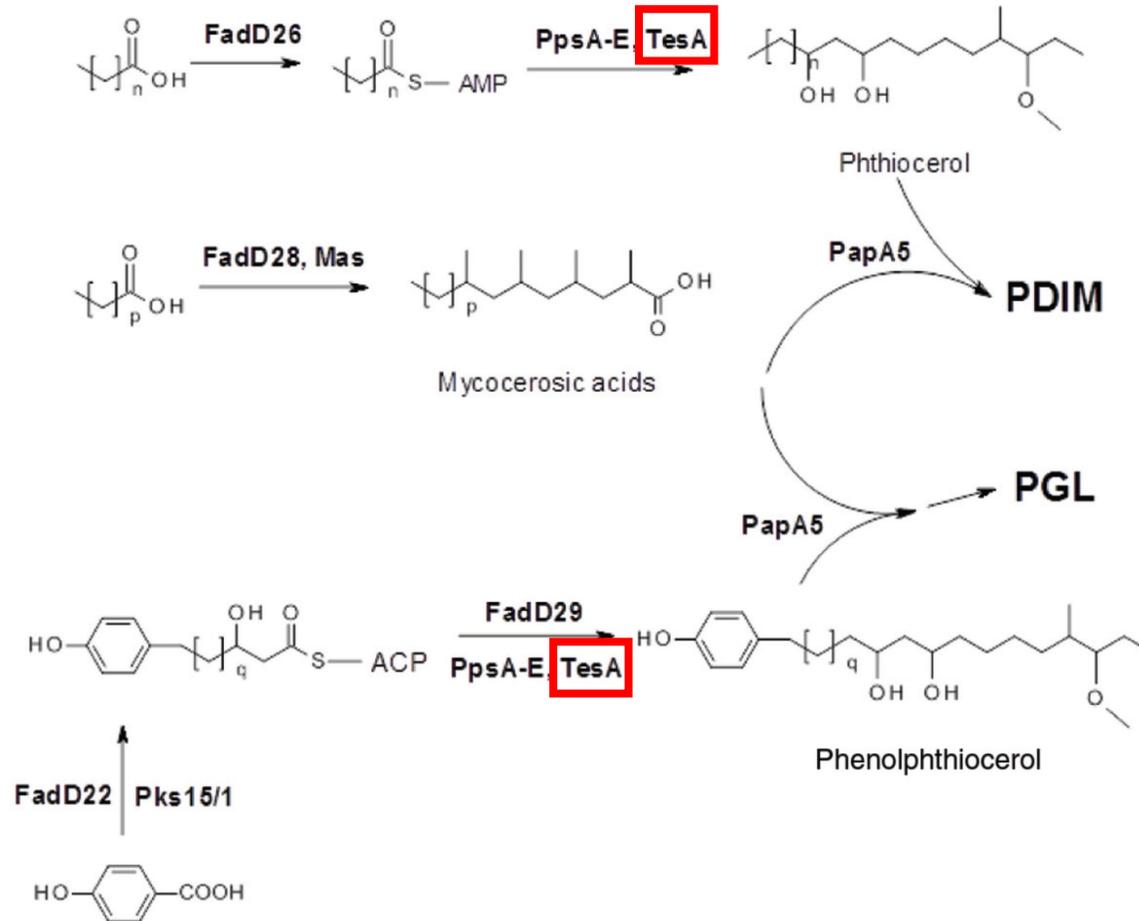
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Could PDIM and PGL have the same role during damage ?

PDIM/PGL synthesis pathway

tesA: type II thioesterase



75 HEALTH FOR ALL



**Joint TB-Meeting:
32. Tuberkulose-Symposium der LLS
2. Swiss Translational TB Forum**

Pause und Zusammenführung

Swiss Translational
TB Forum



75 HEALTH FOR ALL



Joint Meeting :
32^{ème} Symposium Tuberculose de la LPS
2^{ème} Swiss Translational TB Forum

Pause et regroupement

Swiss Translational
TB Forum



Mit Unterstützung von
Avec le soutien de



Diasorin

Swiss Translational
TB Forum

LUNGENLIGA SCHWEIZ
LIGUE PULMONAIRE SUISSE
LEGA POLMONARE SVIZZERA
LIA PULMUNARA SVIZRA

