



Mucosal associated invariant T cells: a new player in antibacterial immunity

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Te Whare Wānanga o Otāgo

Increasing incidence of serious infectious diseases and inequalities in New Zealand: a national epidemiological study

Michael G Baker, Lucy Telfar Barnard, Amanda Kvalsvig, Ayesha Verrall, Jane Zhang, Michael Keall, Nick Wilson, Teresa Wall, Philippa Howden-Chapman

Lancet 2012; 379: 1112-19

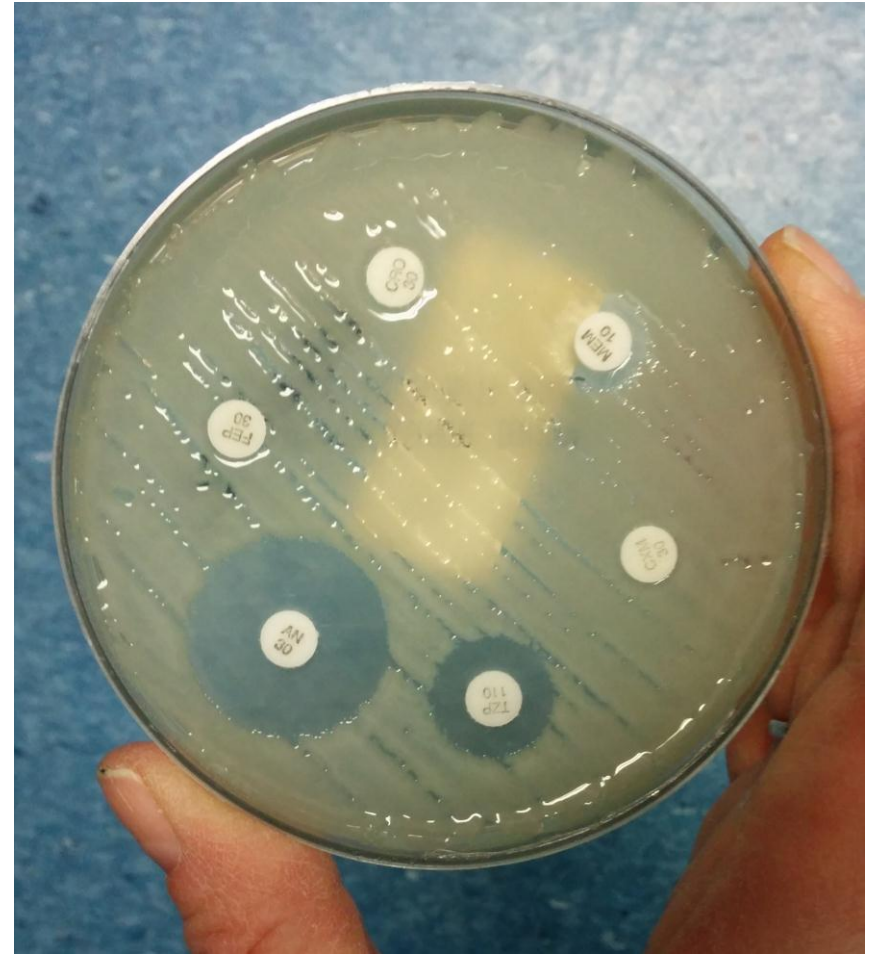
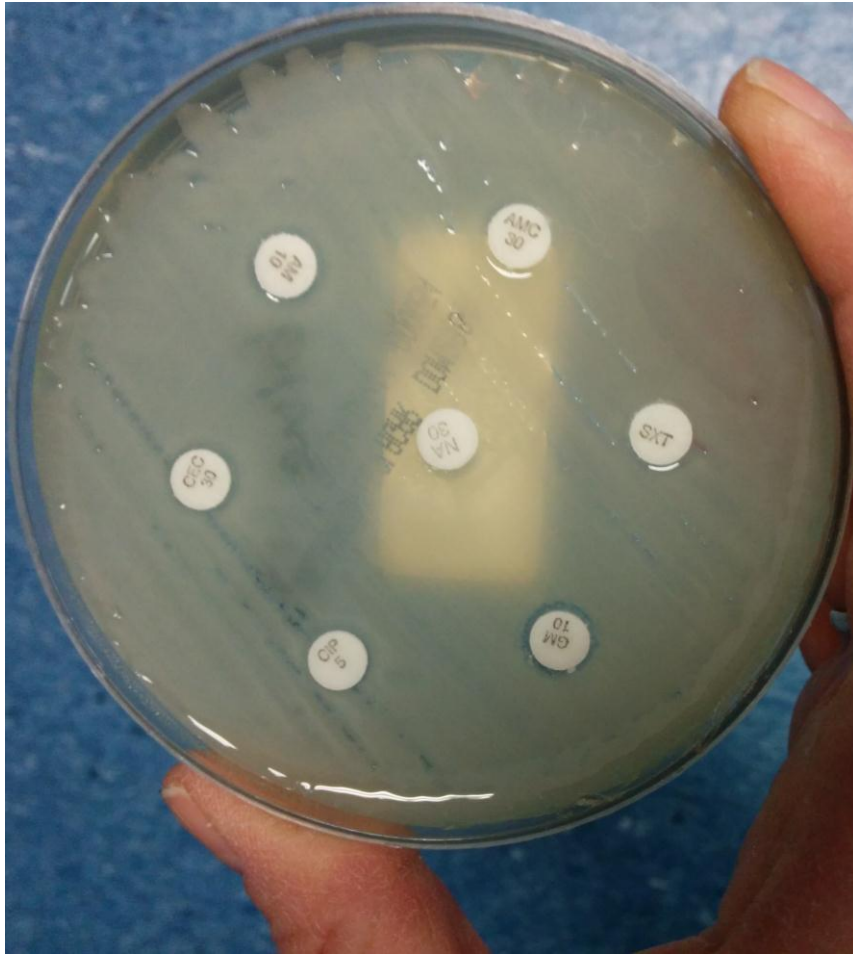
Department of Public Health,
University of Otago,
Wellington, New Zealand

“Infectious diseases made the largest contribution to hospital admissions of any cause”

“increased from 20.5% of acute admissions in 1989-93, to 26.6% in 2004-2008”

“clear ethnic and social inequalities in infectious disease risk”

The growing problem of antimicrobial resistance

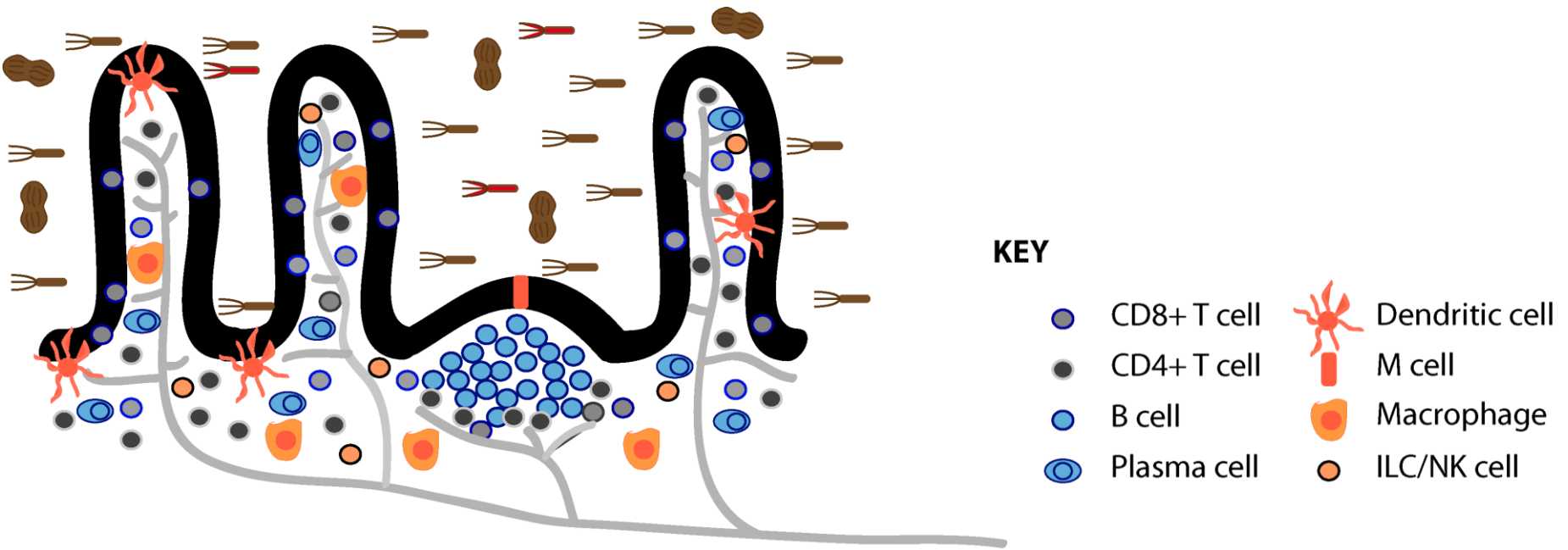


“The problem is so serious that it threatens the achievements of modern medicine. A post-antibiotic era— in which common infections and minor injuries can kill—is a very real possibility for the 21st century.”



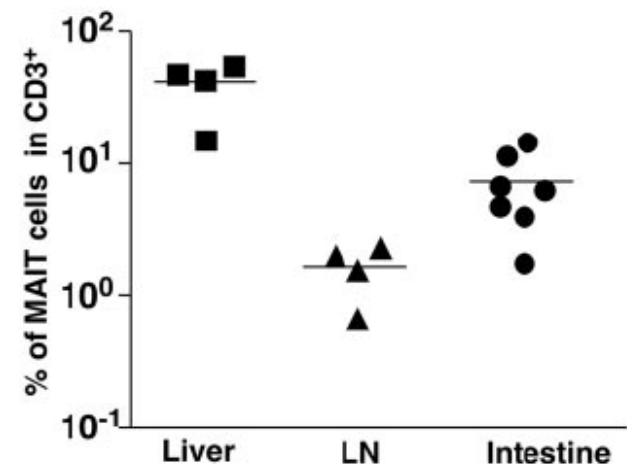
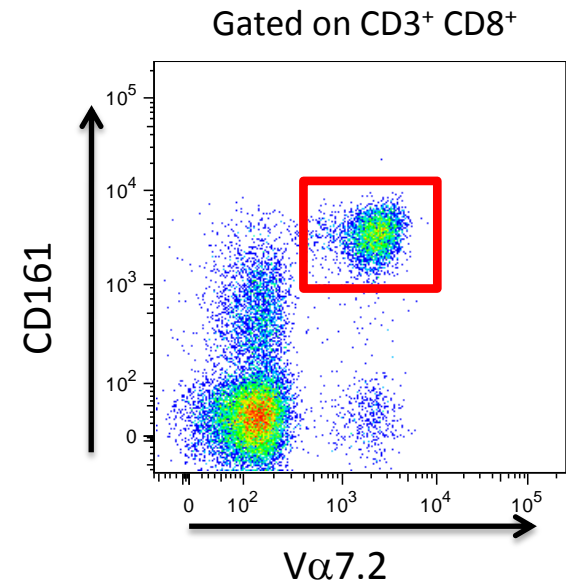
Mucosal surfaces – the central battleground

- Of the top 10 causes of infectious mortality worldwide, 9 are primarily transmitted via a mucosal route
 - >11 million deaths annually
- Commensal flora
- Constant environmental exposure to pathogens



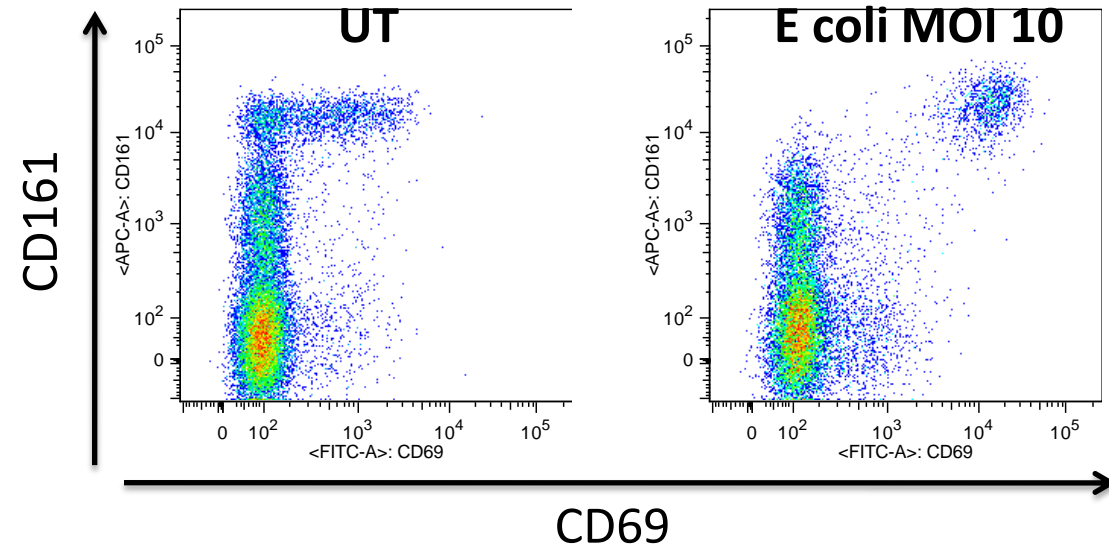
Mucosal Associated Invariant T (MAIT) cells

- Abundant “innate-like” T cell population
- Found at mucosal surfaces
- Enriched in liver
- In blood, ~10% of CD8⁺ T cell population
 - ~100x more common than iNKT cells
- Rare in mice
- Semi-invariant TCR (V α 7.2-J α 33)
- Restricted by MHC related protein 1 (MR1)
 - Non-classical MHC class Ib protein
 - Non-polymorphic
 - Evolutionarily conserved
- Phenotype
 - Effector memory
 - CCR2⁺, CCR5⁺, CXCR6⁺
 - CD161⁺⁺ IL23R⁺ CCR6⁺ ROR γ t⁺
 - IL17, IL22, IFN γ , TNF α



Specific activation of MAIT cells by bacteria

Gated on CD3⁺ CD8⁺



Activated by:

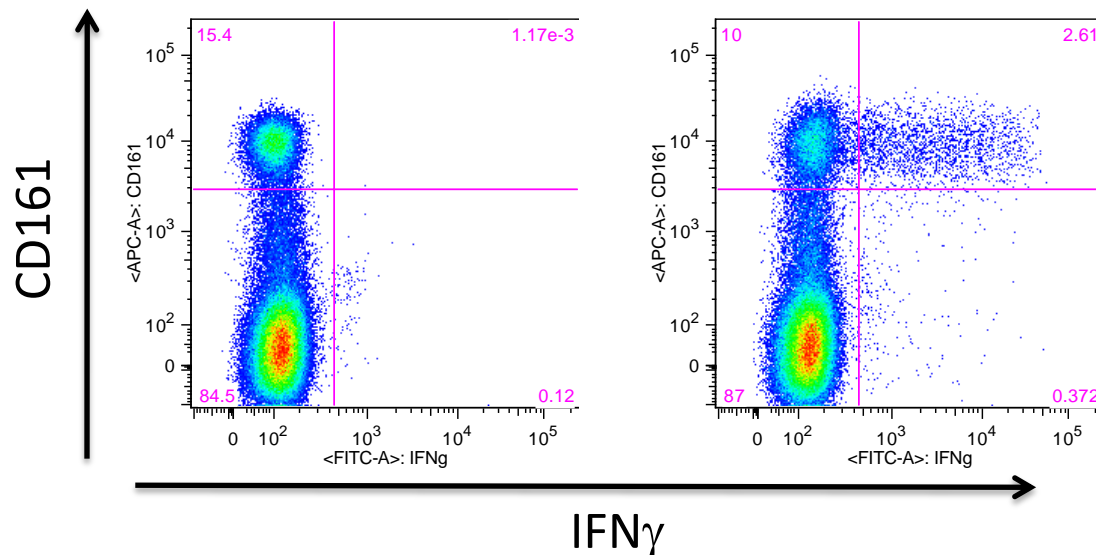
Escherichia coli
Klebsiella pneumoniae
Salmonella spp.
Pseudomonas aeruginosa
Francisella tularensis

Staphylococcus aureus
Staphylococcus epidermidis
Mycobacterium tuberculosis
Mycobacterium abscessus
Lactobacillus acidophilus

Candida albicans
Candida glabrata
Saccharomyces cerevisiae

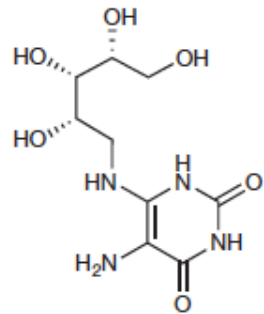
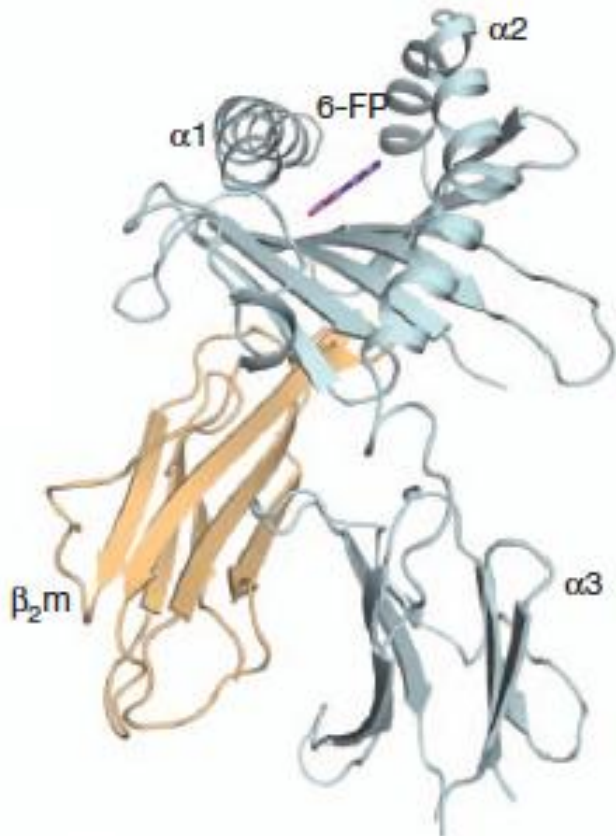
Not activated by:

Streptococcus pyogenes
Enterococcus faecalis



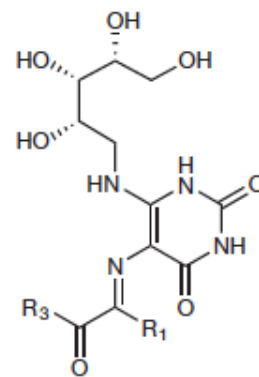
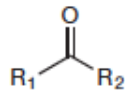
MR1 binds vitamin B metabolites

- Activating ligand in supernatant of *Salmonella* sp. culture
- Intermediate of riboflavin biosynthesis



5-Amino-6-D-ribitylaminouracil

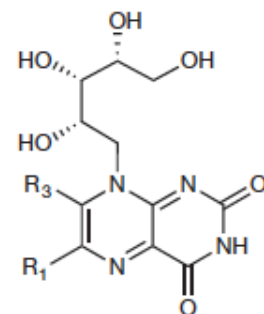
+



Pyrimidines



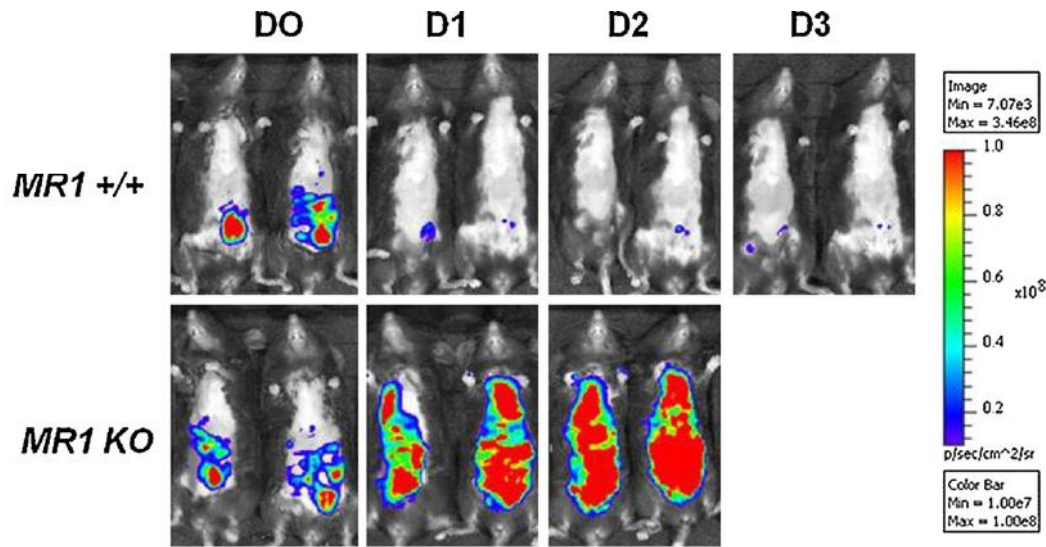
-H₂O



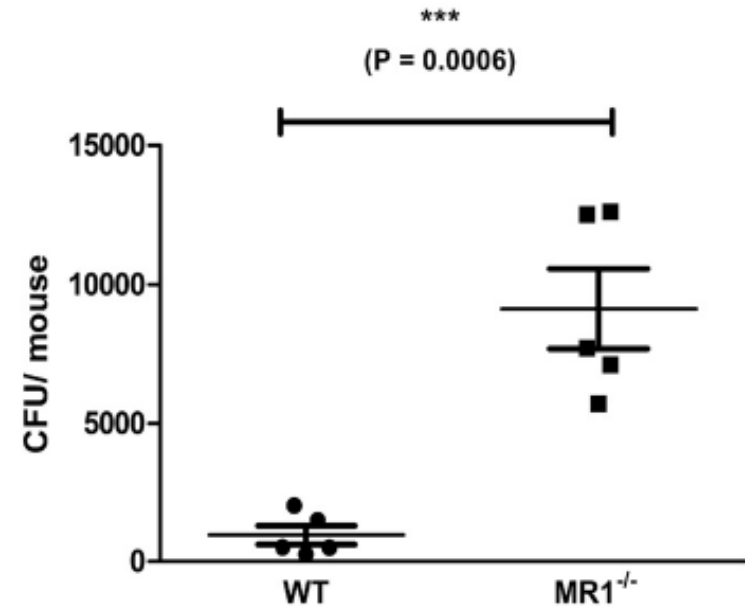
Lumazines

MAIT cells protect against bacterial infection *in vivo*: mice

Wild type vs MR1^{-/-} mice

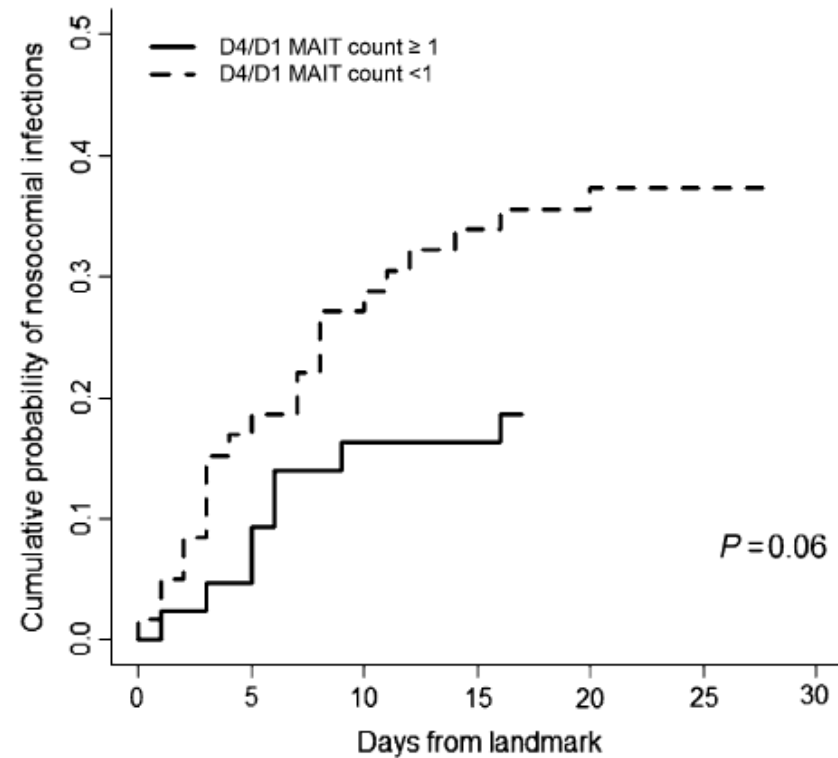
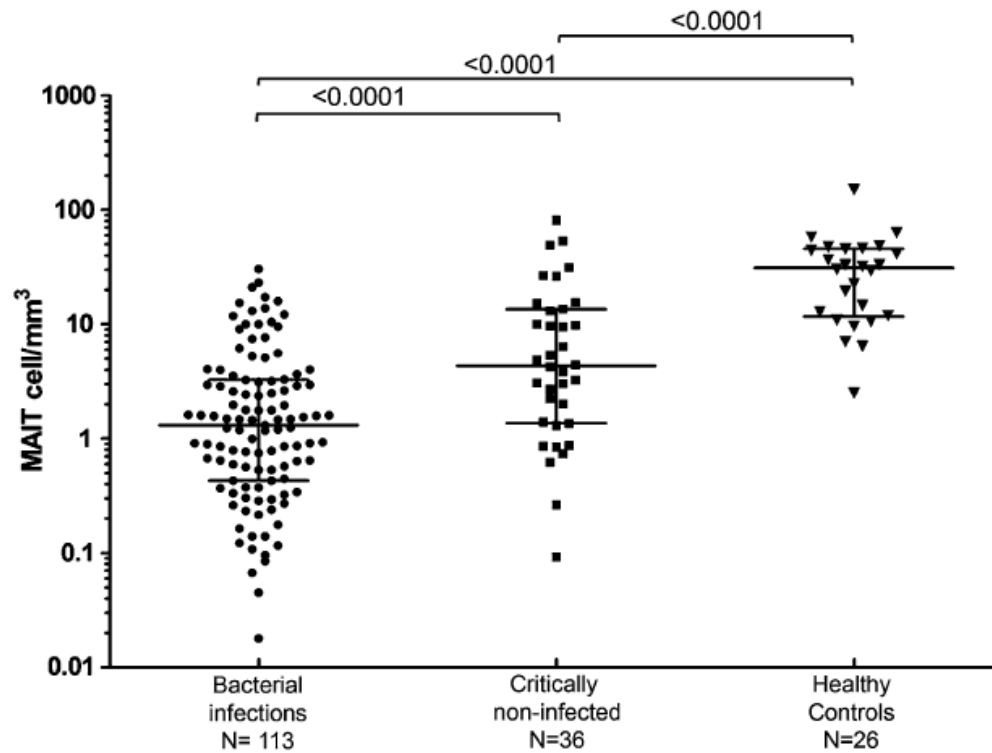


Intraperitoneal injection of luminescent *Klebsiella pneumoniae*

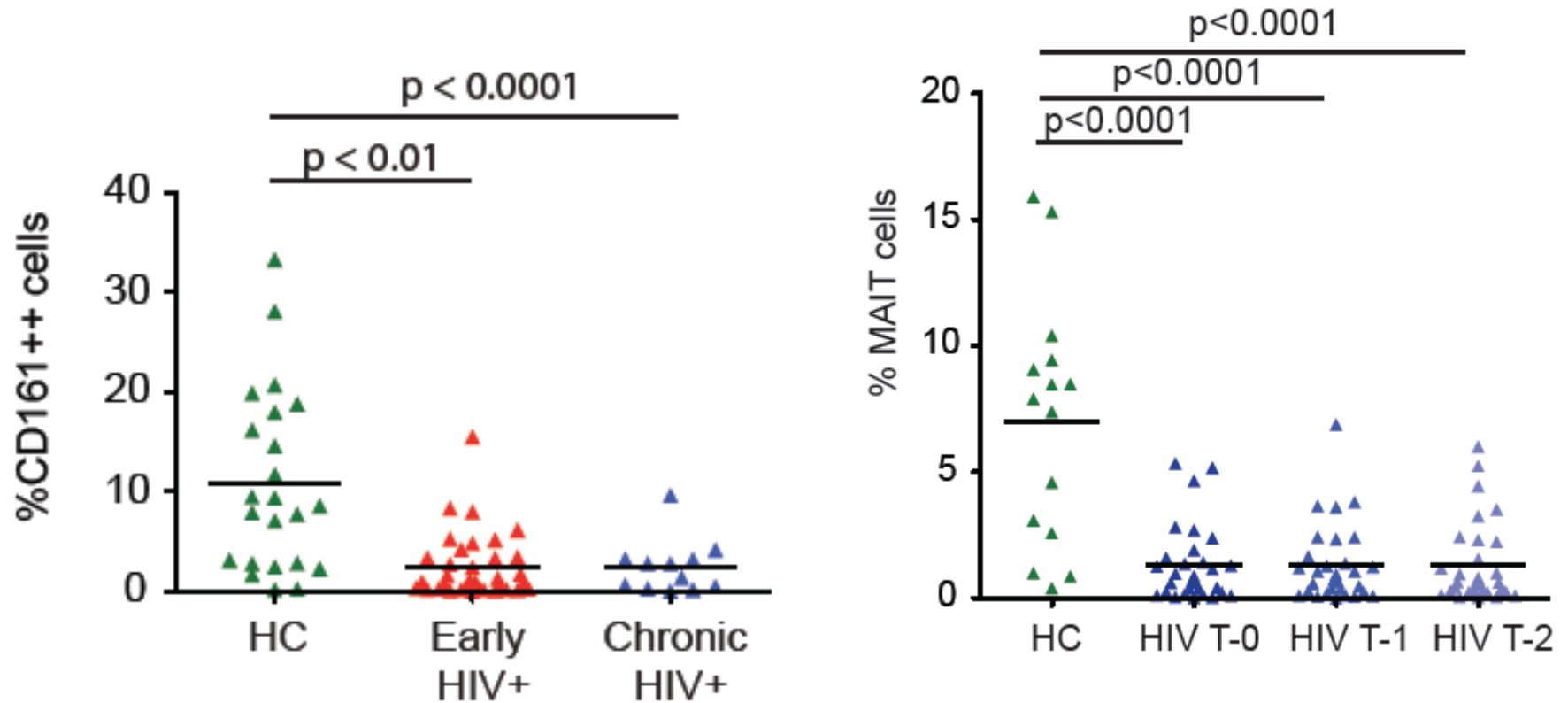


CFU in spleen post aerosol challenge with BCG

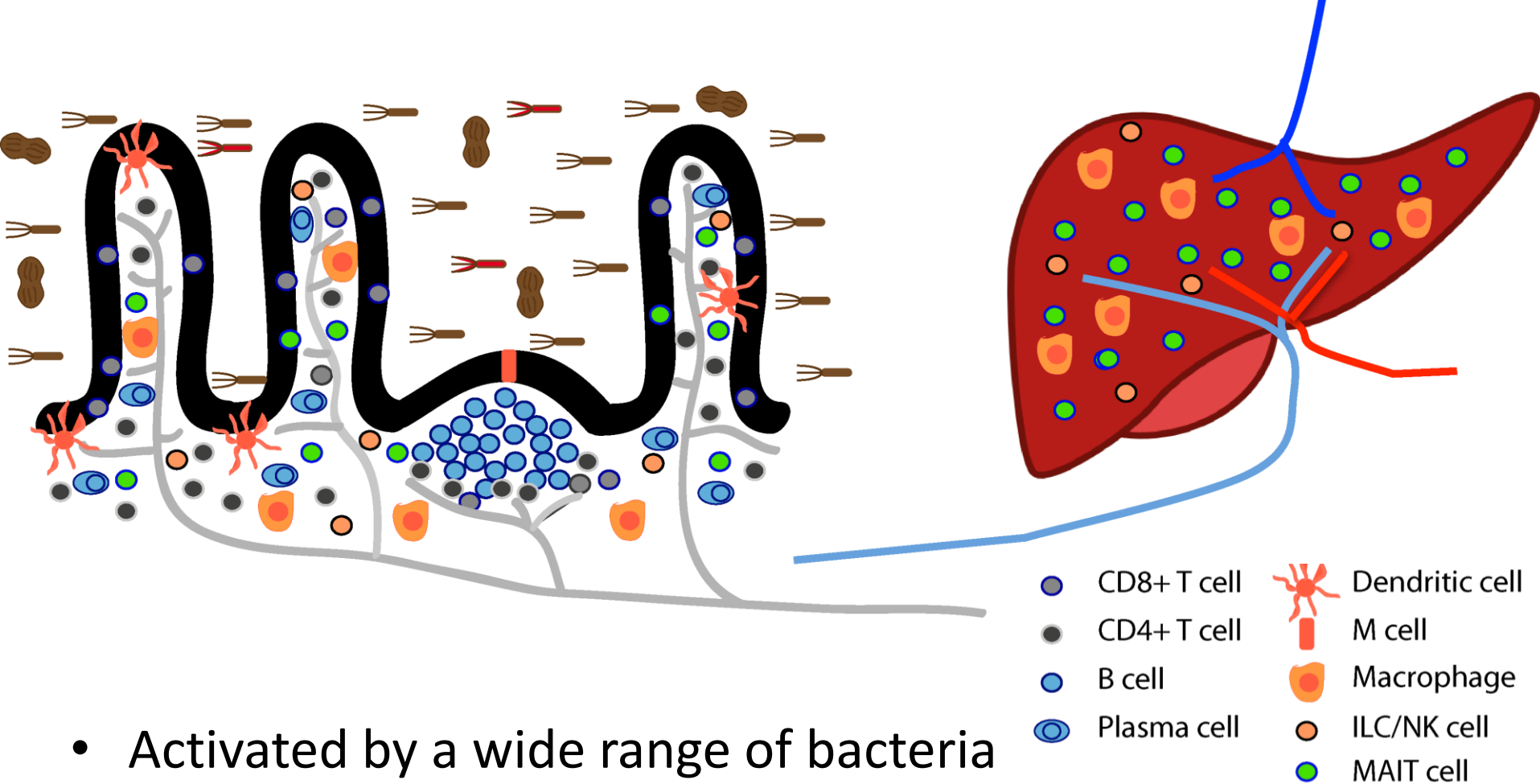
MAIT cells protect against bacterial infection *in vivo*: humans



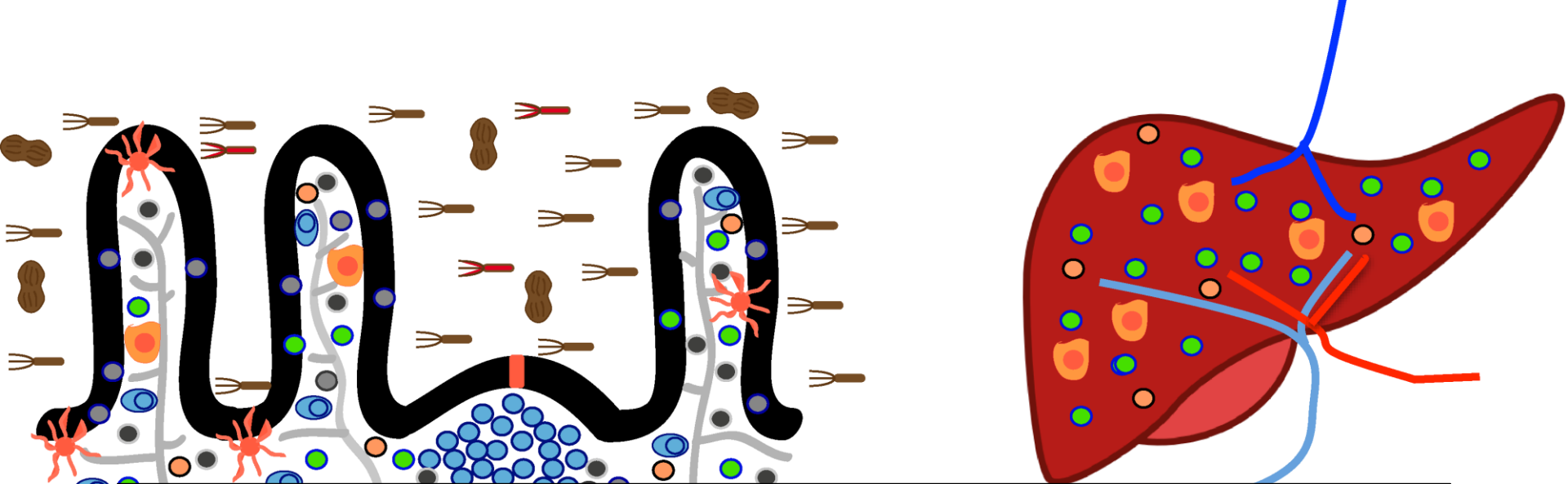
Persistent loss of MAIT cells from the blood in HIV despite HAART



HC = Healthy control



- Activated by a wide range of bacteria
- Probable frequent exposure
 - Ligand is soluble and present in bacterial culture supernatant
- Inappropriate activation could cause immunopathology

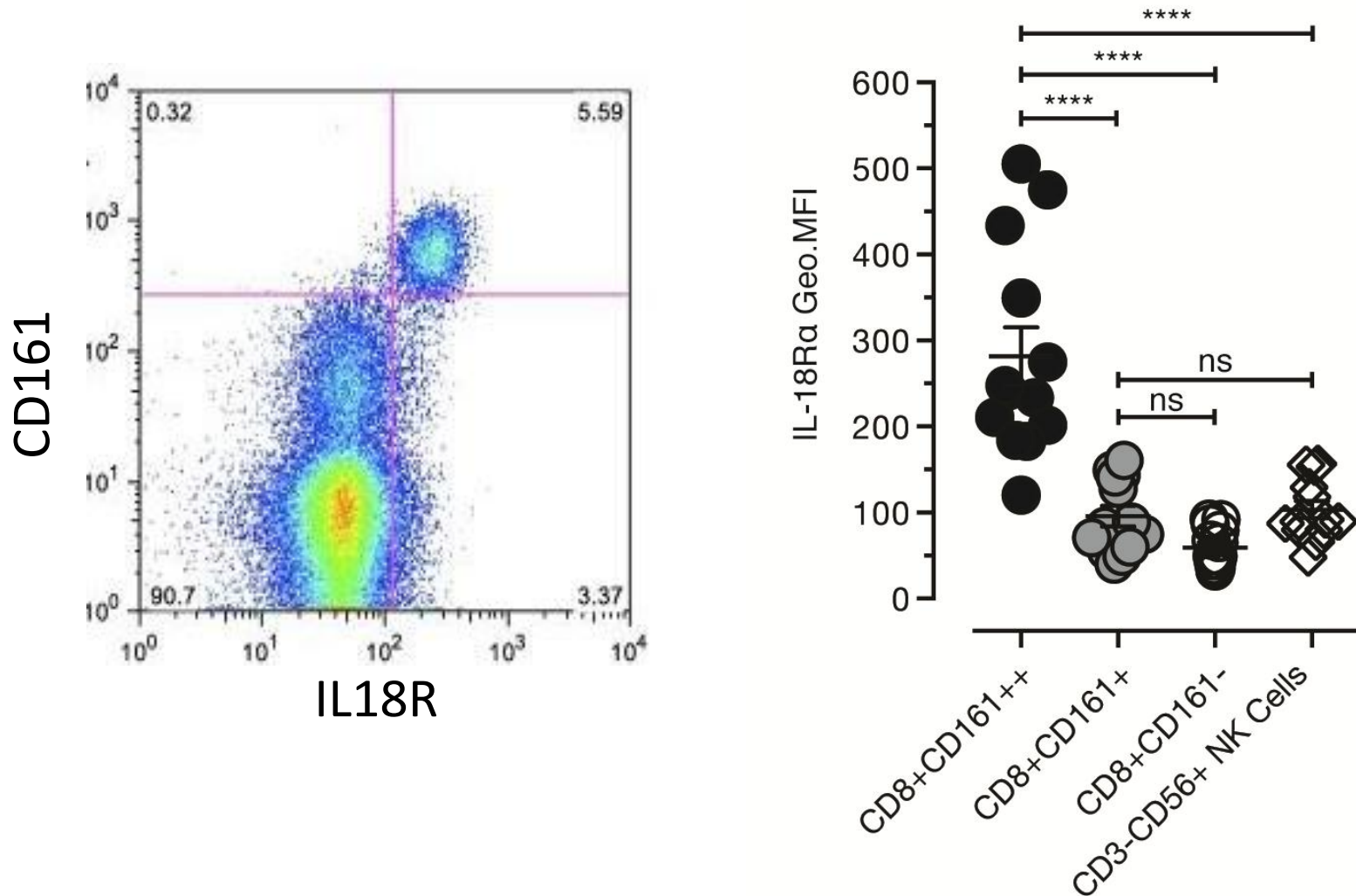


How is MAIT cell activation regulated?

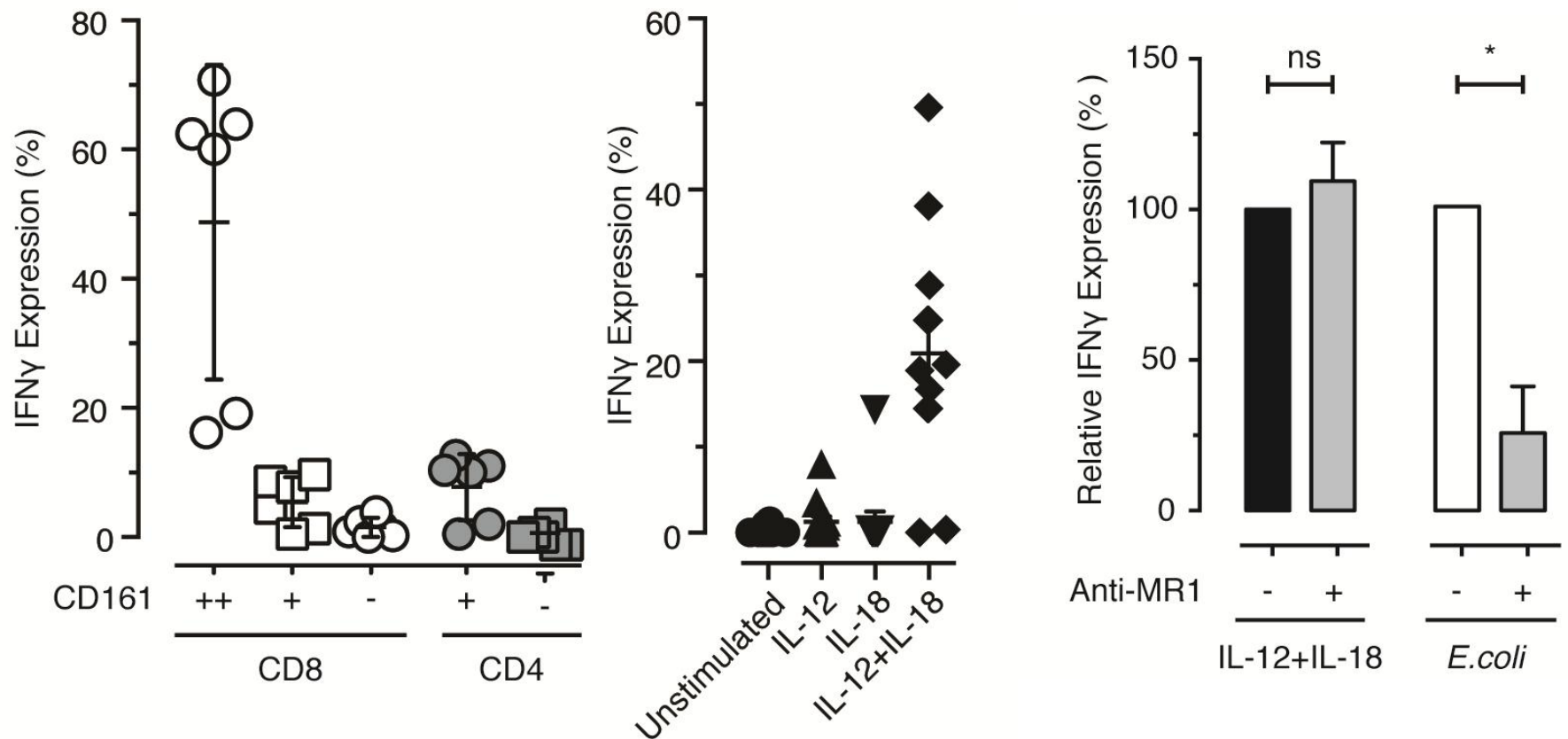
- Activated by a wide range of bacteria
- Probable frequent exposure
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Plasma cell
 Treg/Th17 cell
 MAIT cell

Cytokine-dependent activation of MAIT cells: expression of IL-18 receptor

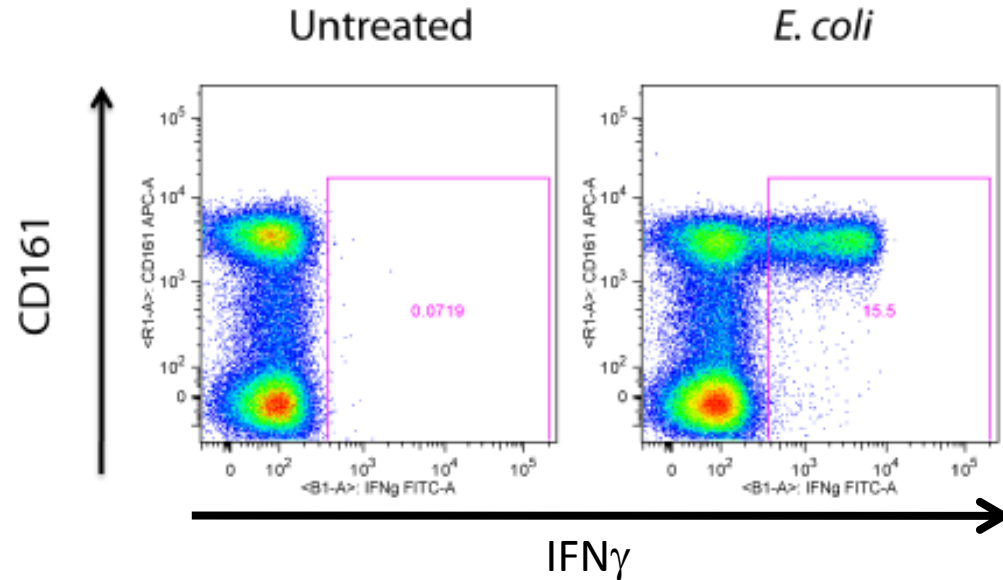


Cytokine-dependent activation of MAIT cells: IL-12+IL-18 specifically induces IFN γ

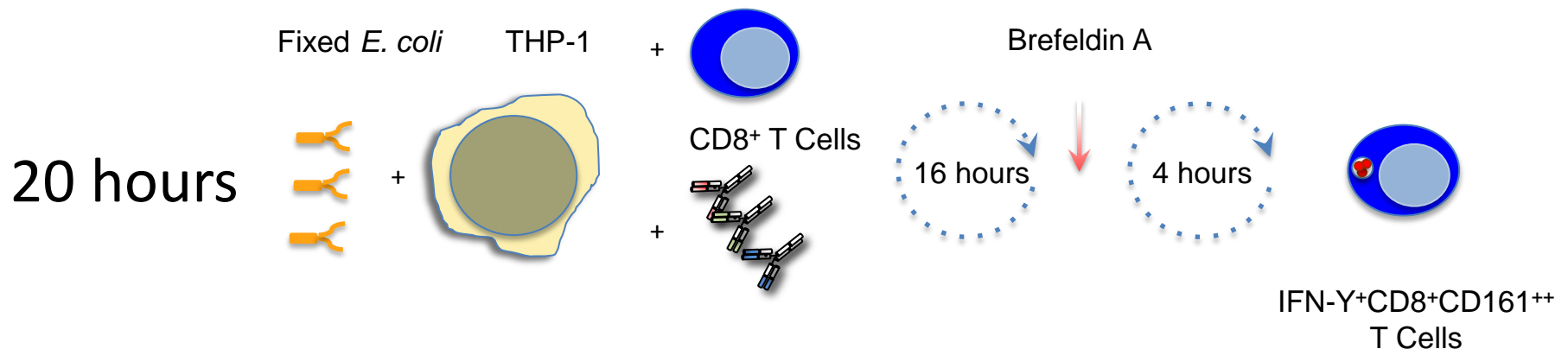


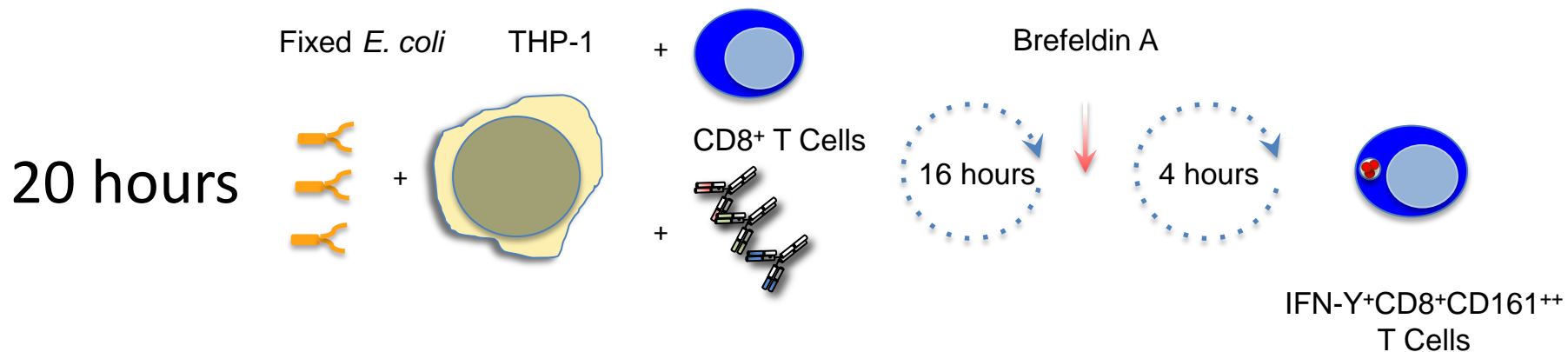
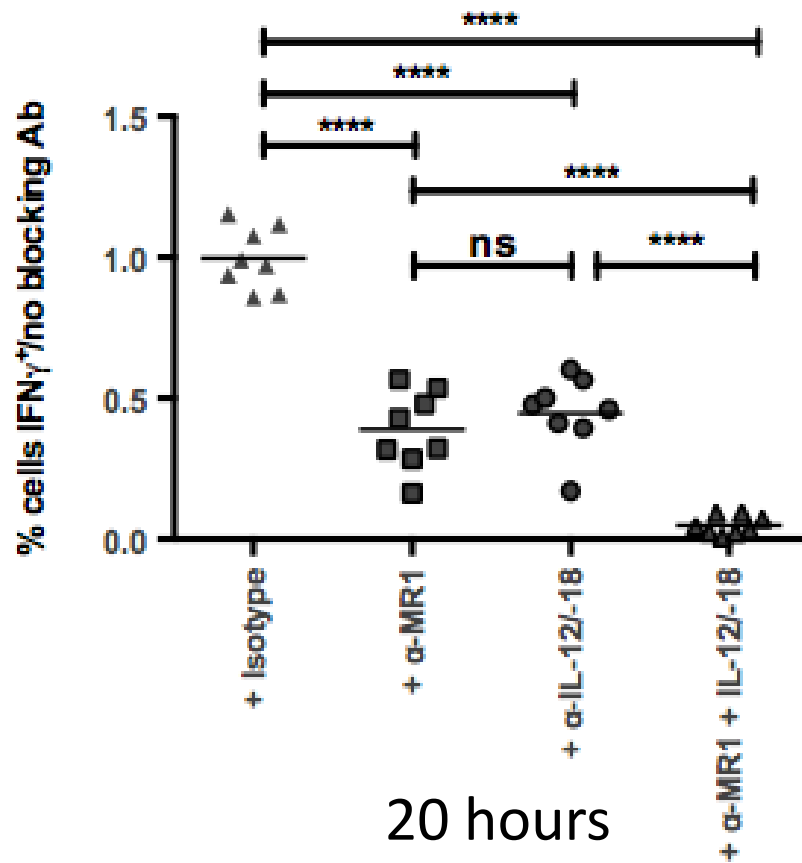
PBMCs, overnight incubation

How do IL-12+IL-18 contribute to MAIT cell activation in bacterial infection?

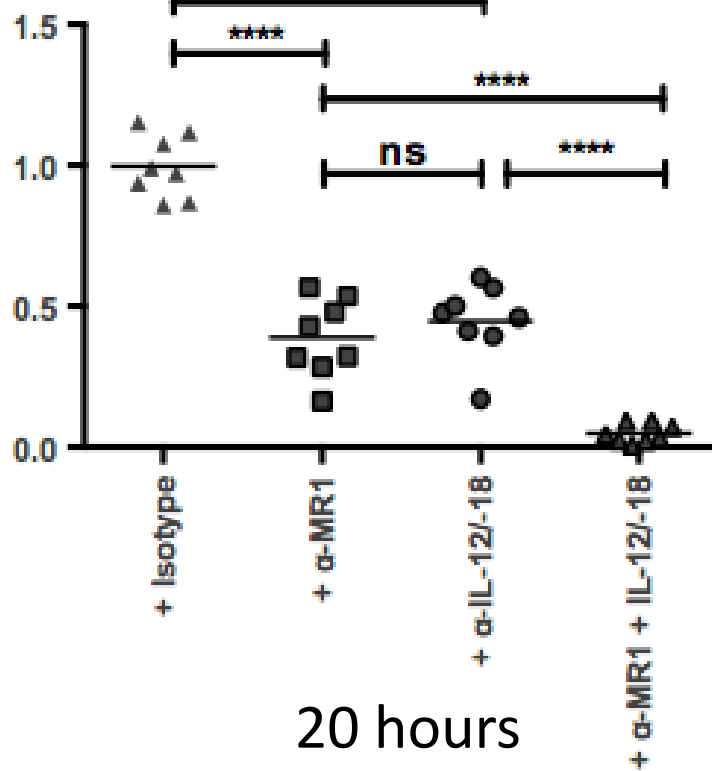


Gated on CD3+CD8+ lymphocytes

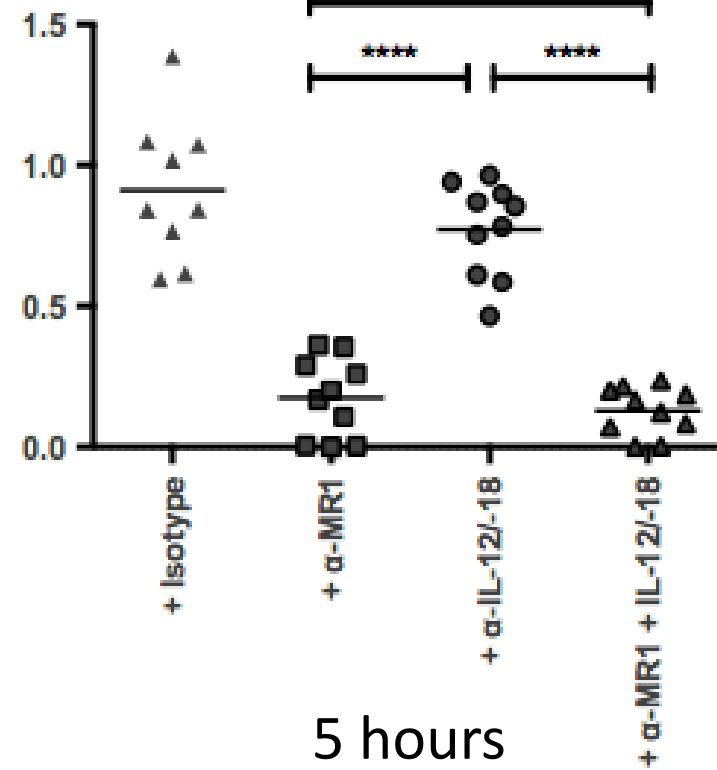




% cells IFN- γ ⁺/no blocking Ab

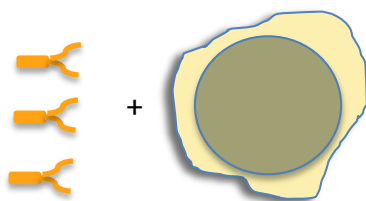


% cells IFN- γ ⁺/no blocking Ab



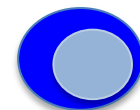
Fixed *E. coli*

THP-1



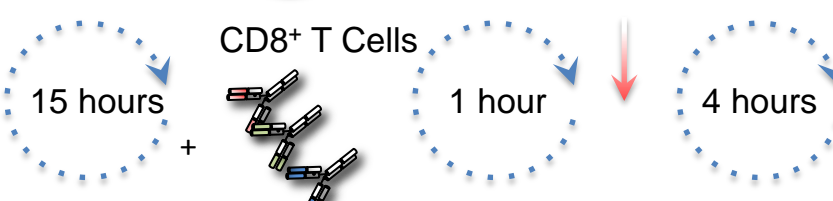
5 hours

+



CD8⁺ T Cells

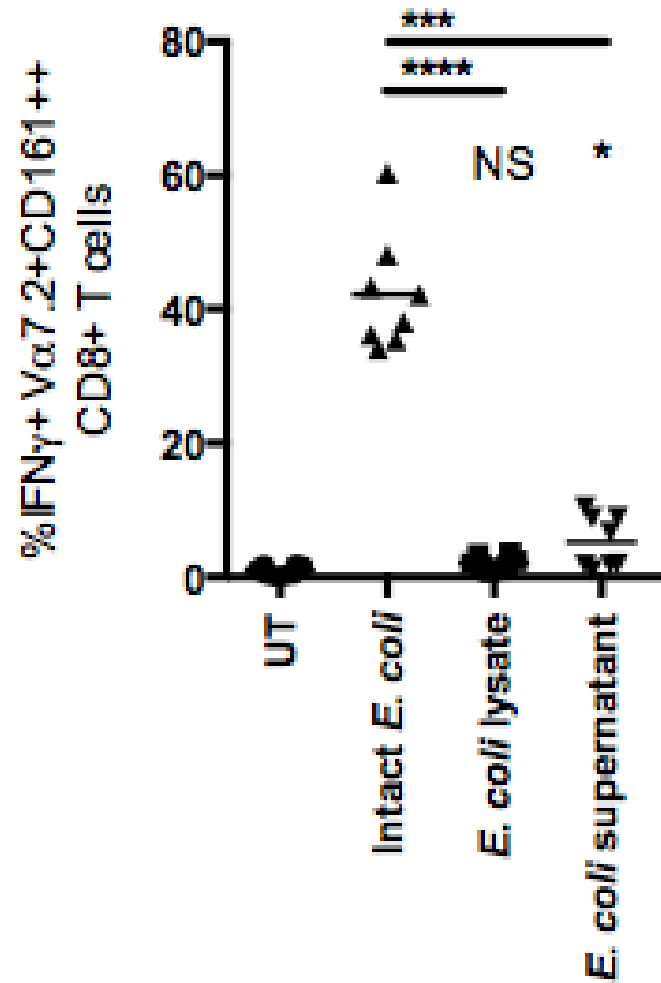
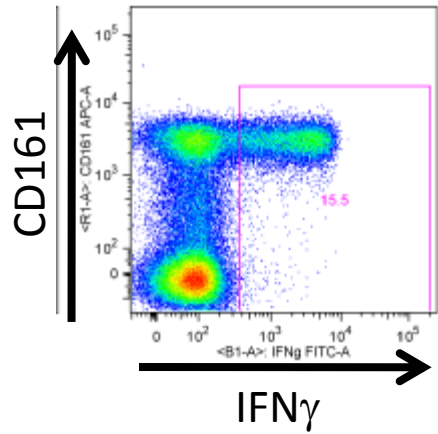
Brefeldin A



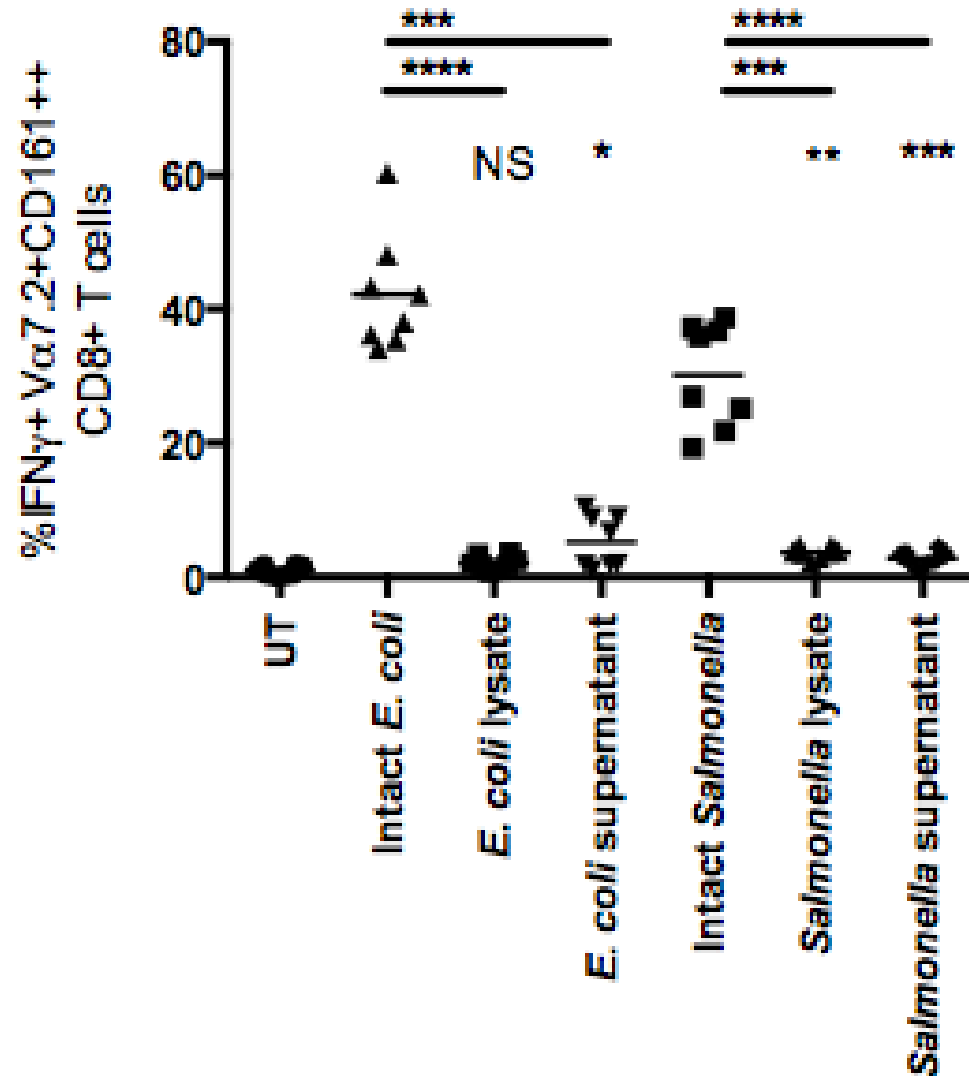
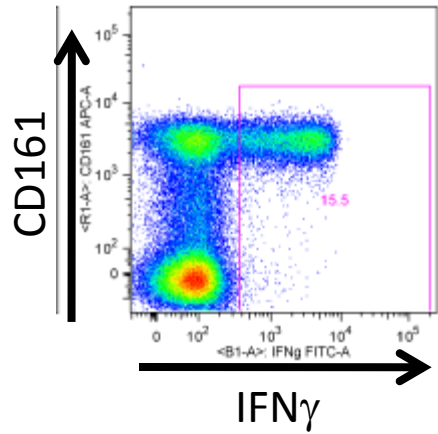
Blocking antibodies

IFN- γ ⁺CD8⁺CD161⁺⁺
T Cells

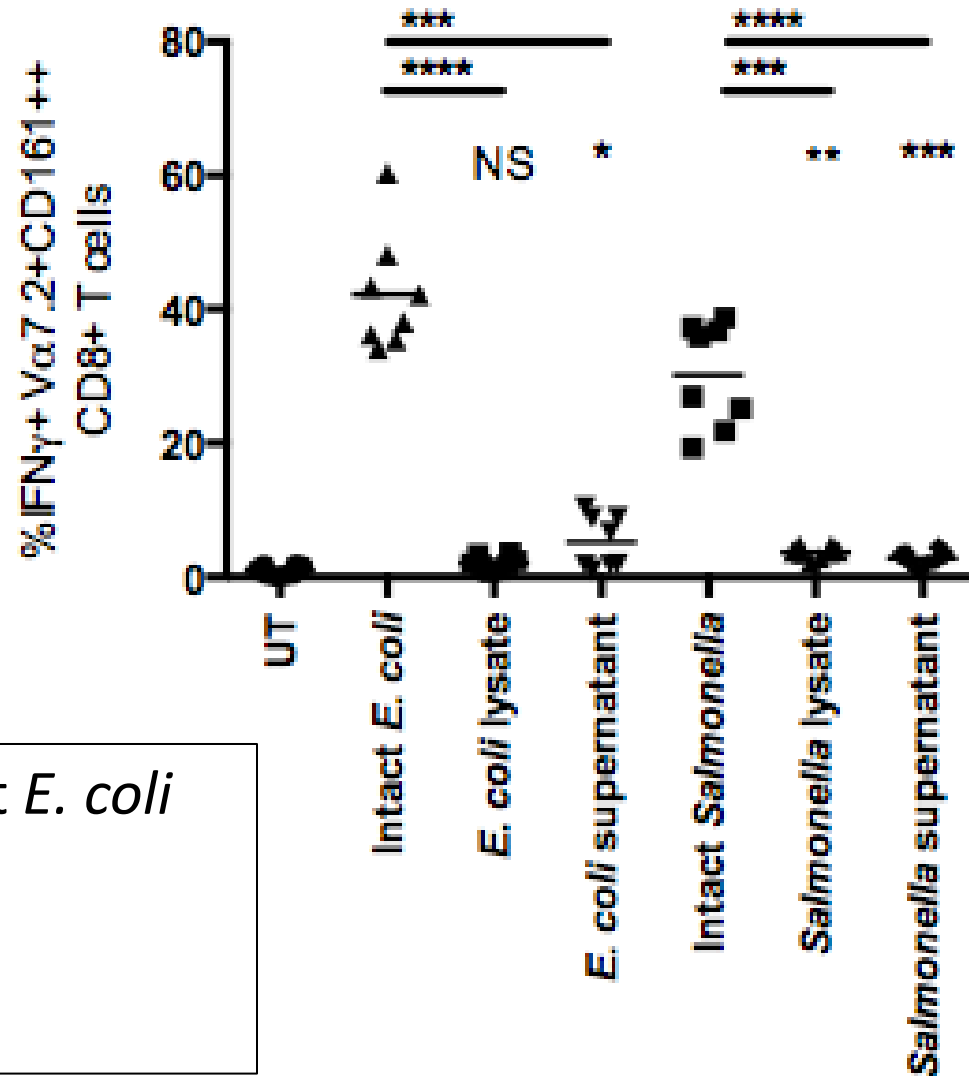
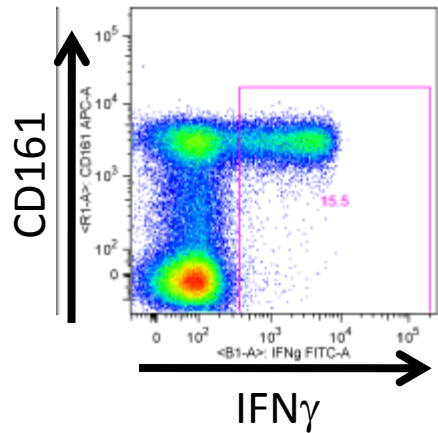
Whole bacteria, but not supernatant or cell lysate, are potent activators of MAIT cells



Whole bacteria, but not supernatant or cell lysate, are potent activators of MAIT cells



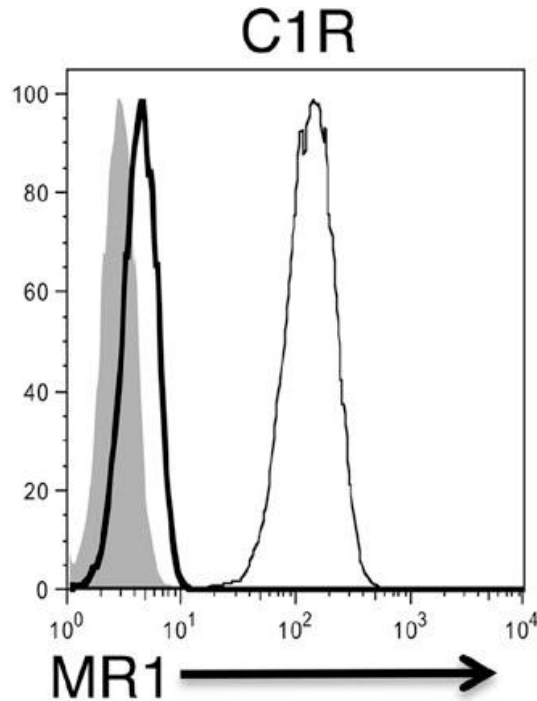
Whole bacteria, but not supernatant or cell lysate, are potent activators of MAIT cells



Activation by intact *E. coli* inhibited by:

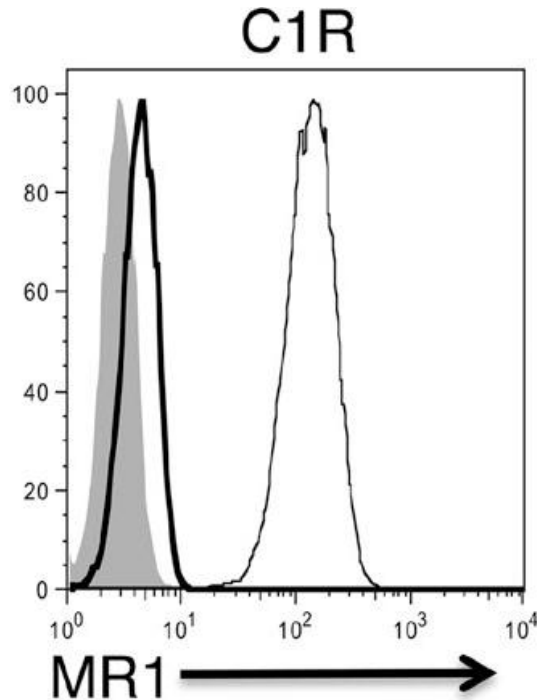
- Cytochalasin D
- Bafilomycin A

Is surface expression of MR1 limiting?

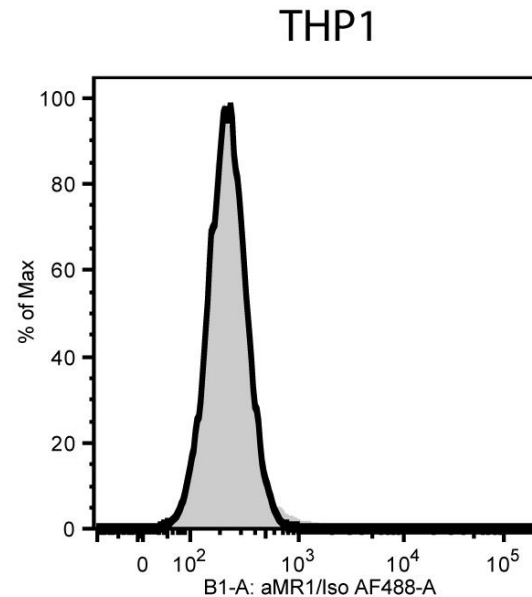


■ +Isotype
— +26.5
— h.MR1 + 26.5

Is surface expression of MR1 limiting?

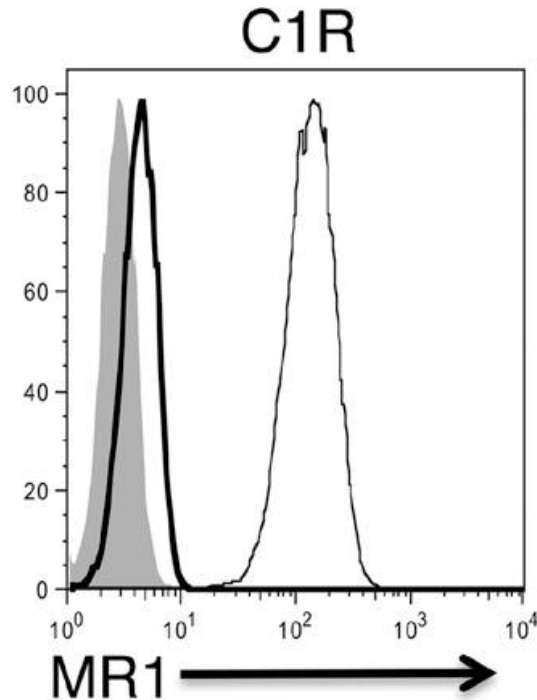


■ +Isotype
— +26.5
— h.MR1 + 26.5

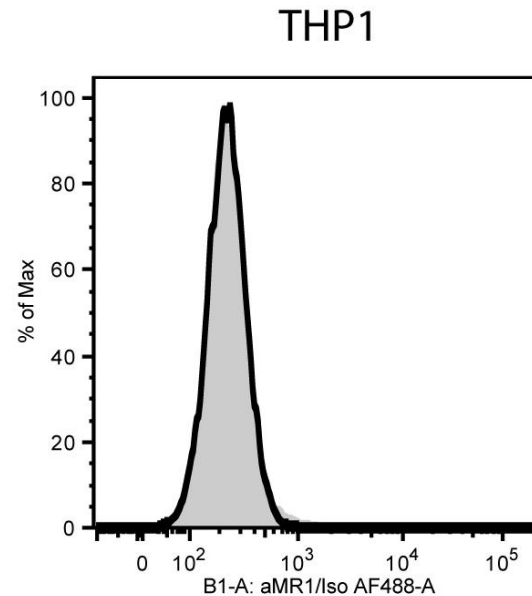


Isotype
αMR1

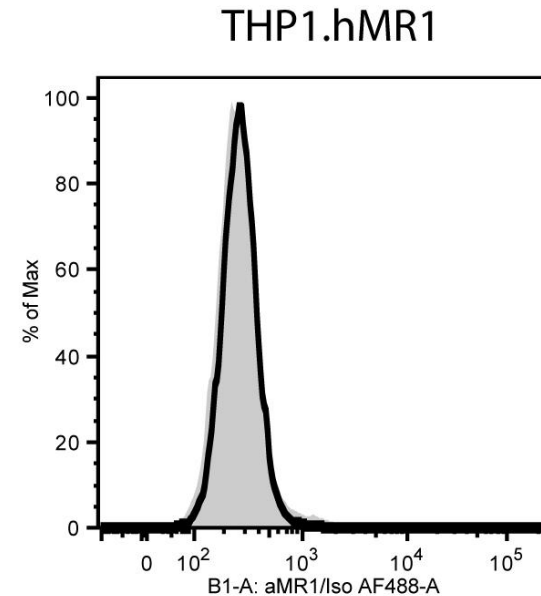
Is surface expression of MR1 limiting?



■ +Isotype
— +26.5
— h.MR1 + 26.5



Isotype
 α MR1



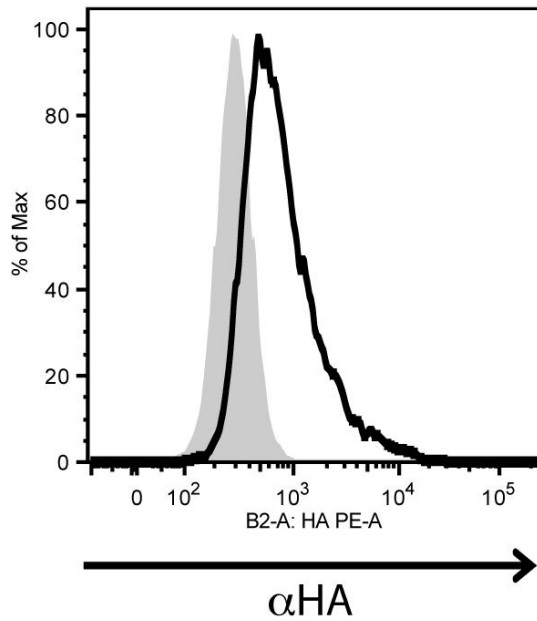
α MR1 →

THP1s transduced
with
MR1 with C-
terminal HA tag

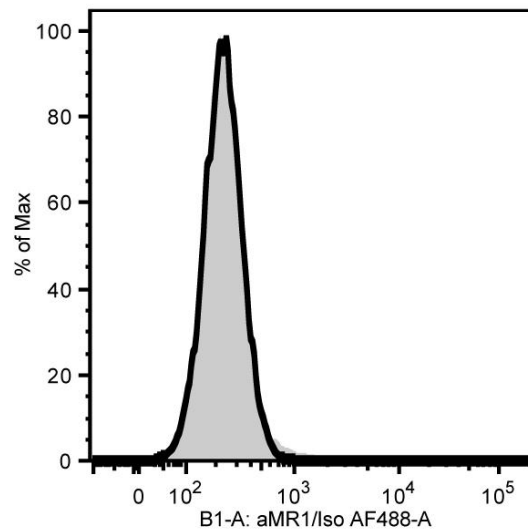
Surface antibody stain

MR1 surface expression in THP1s is tightly regulated, even when over expressed

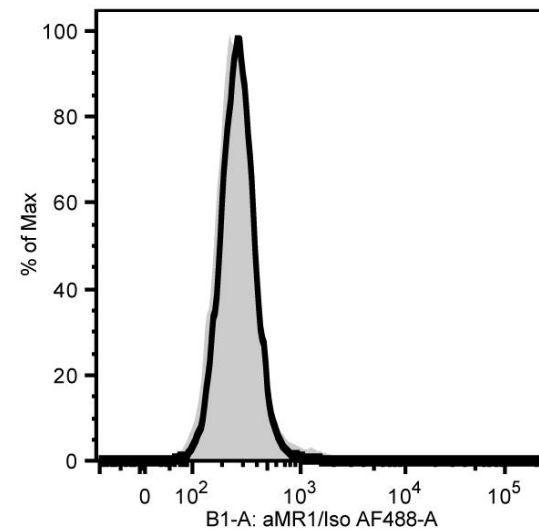
THP1 v THP1.hMR1



THP1



THP1.hMR1



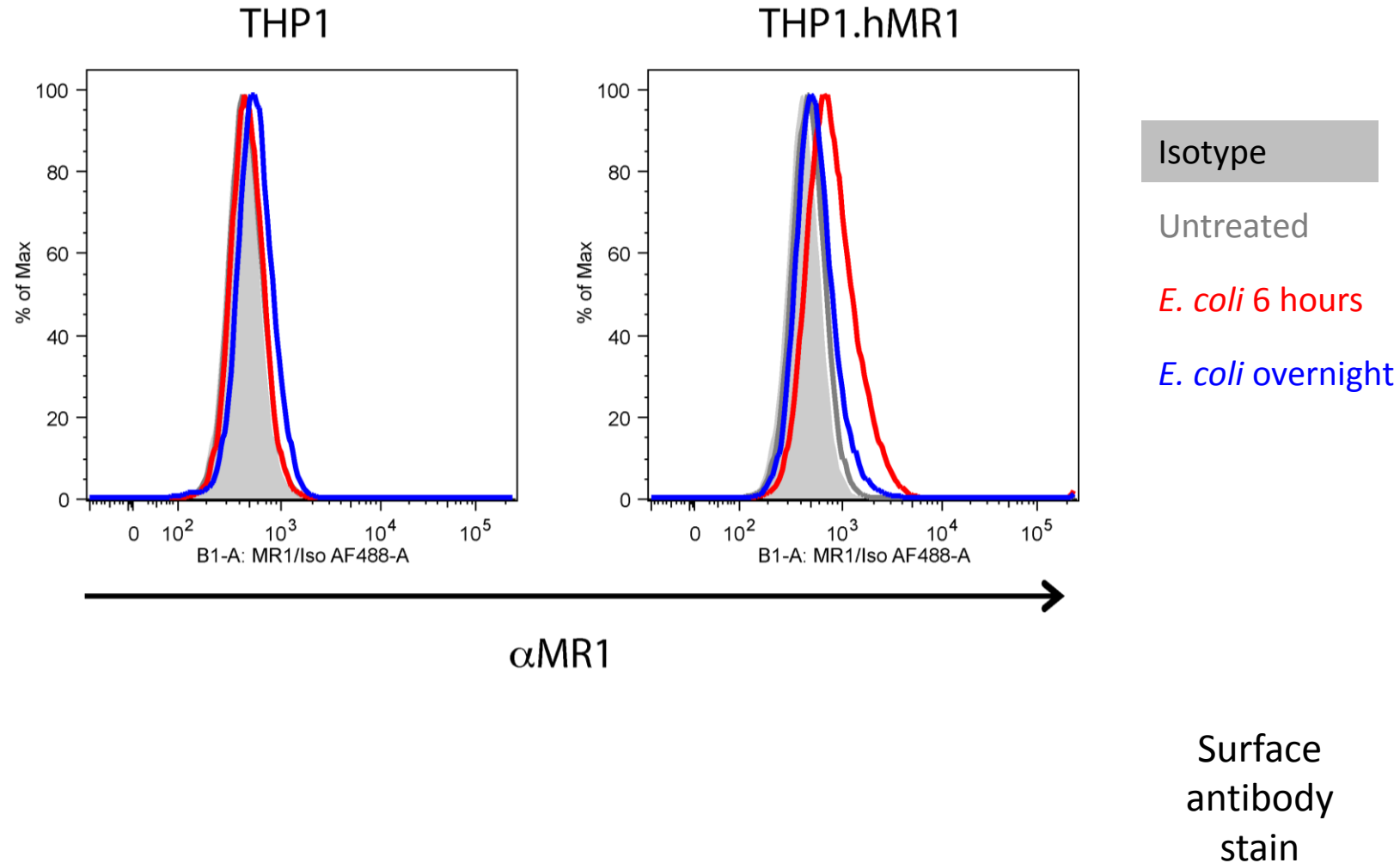
Isotype
αMR1

THP1s transduced
with
MR1 with C-
terminal HA tag

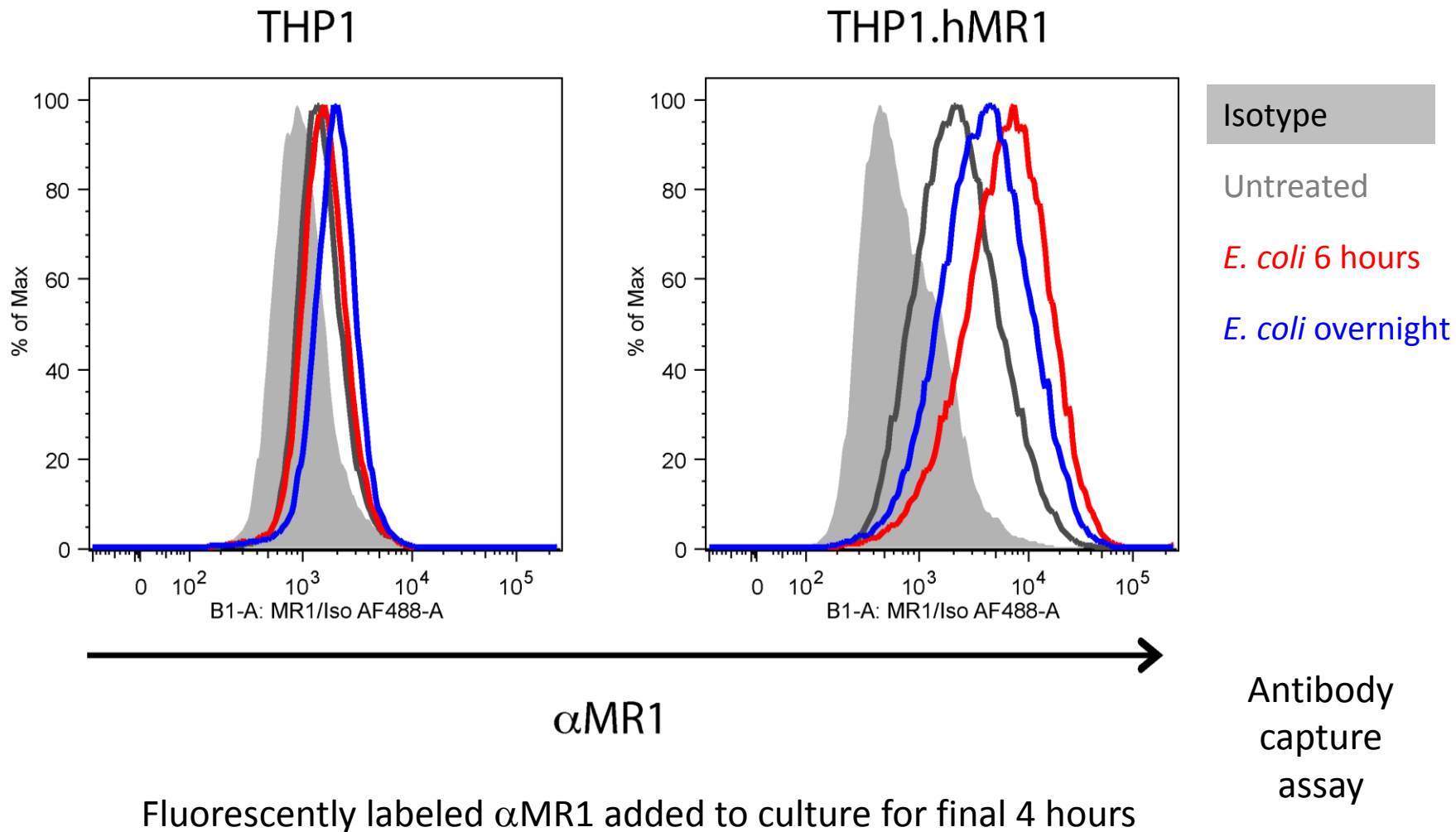
Surface antibody stain

Internal stain

Small increase in MR1 surface expression after treatment with *E. coli*



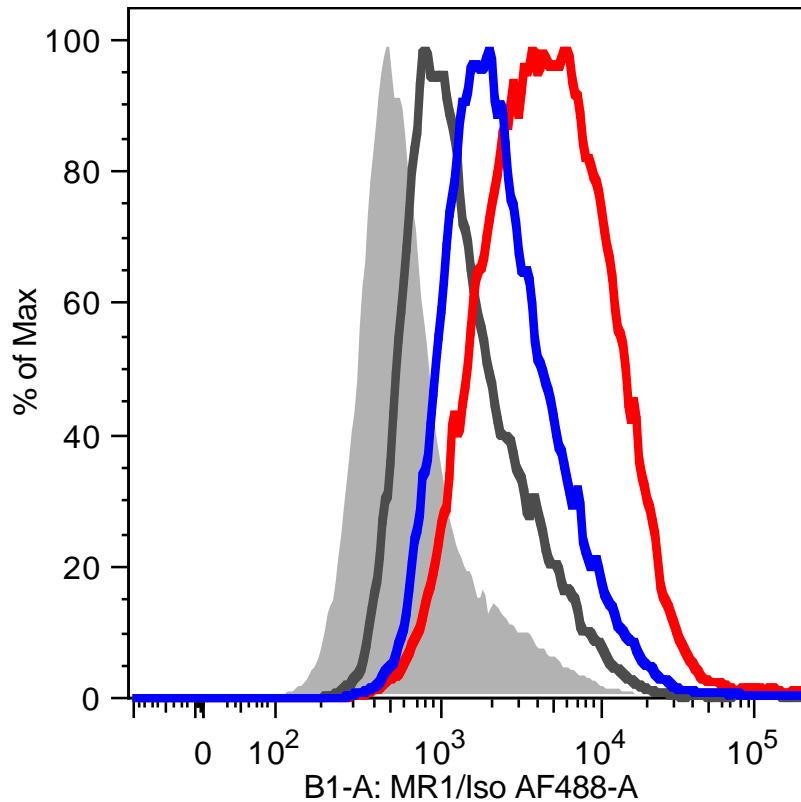
Increased trafficking of MR1 to the cell surface after *E. coli* treatment



Activation induces trafficking of MR1 to the cell surface

THP1.hMR1

+ TLR2
agonist



Isotype

Untreated

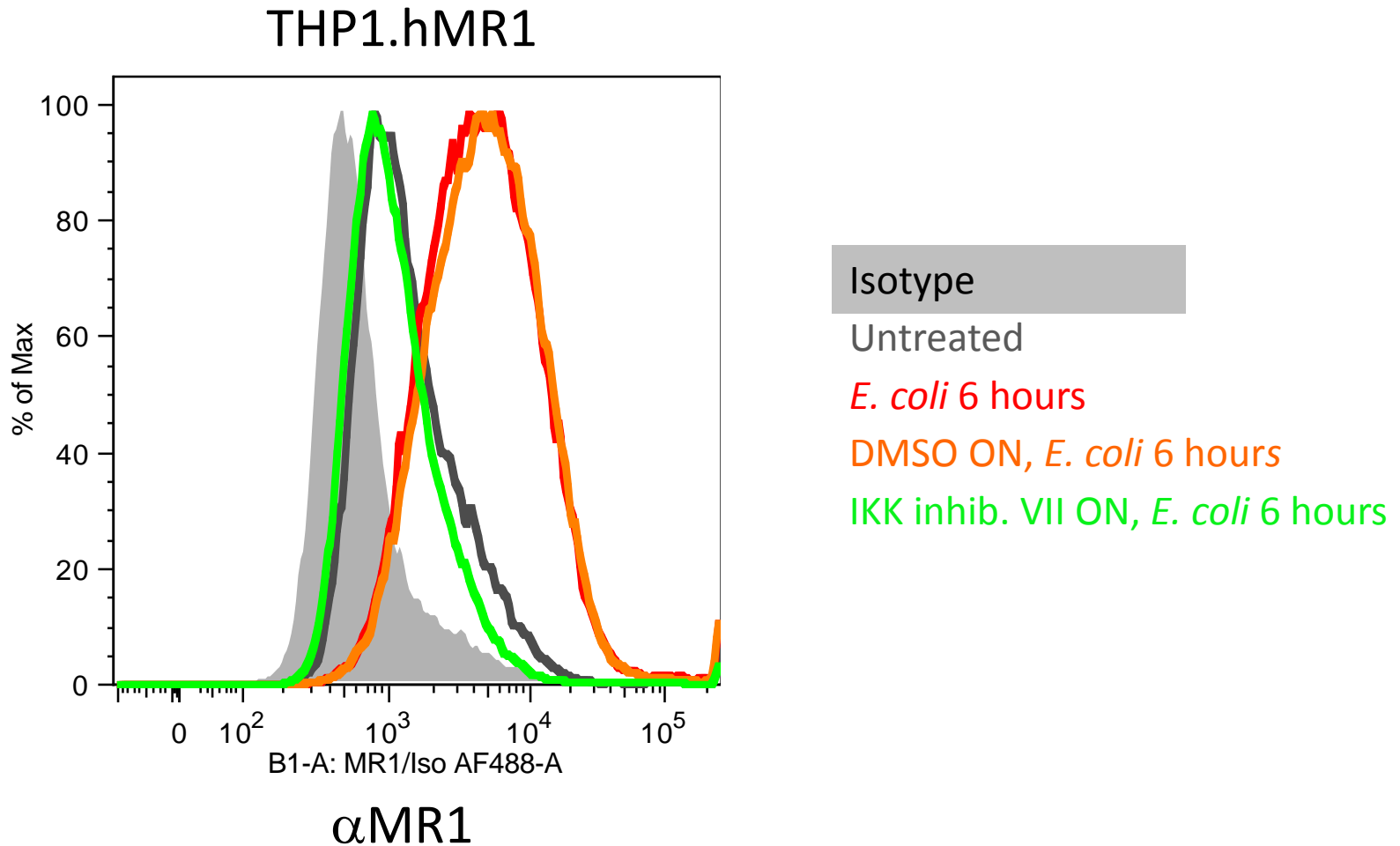
TLR2 agonist 6 hours

TLR2 agonist overnight

α MR1

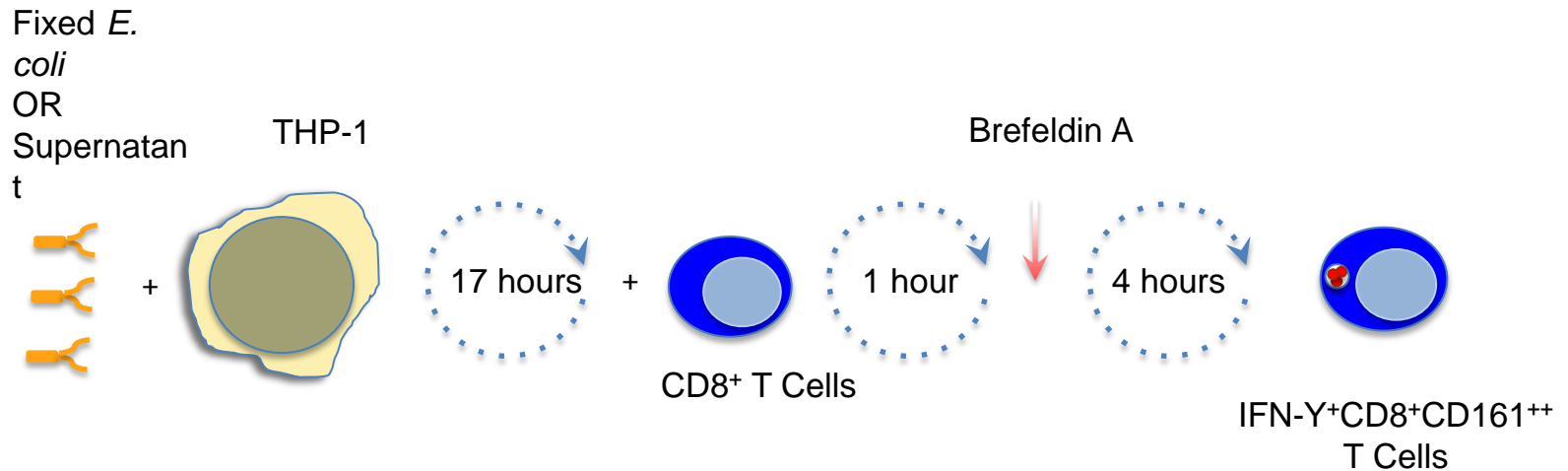
4 hour antibody capture assay

NF κ B signaling is required for activation-induced trafficking of MR1 to the cell surface

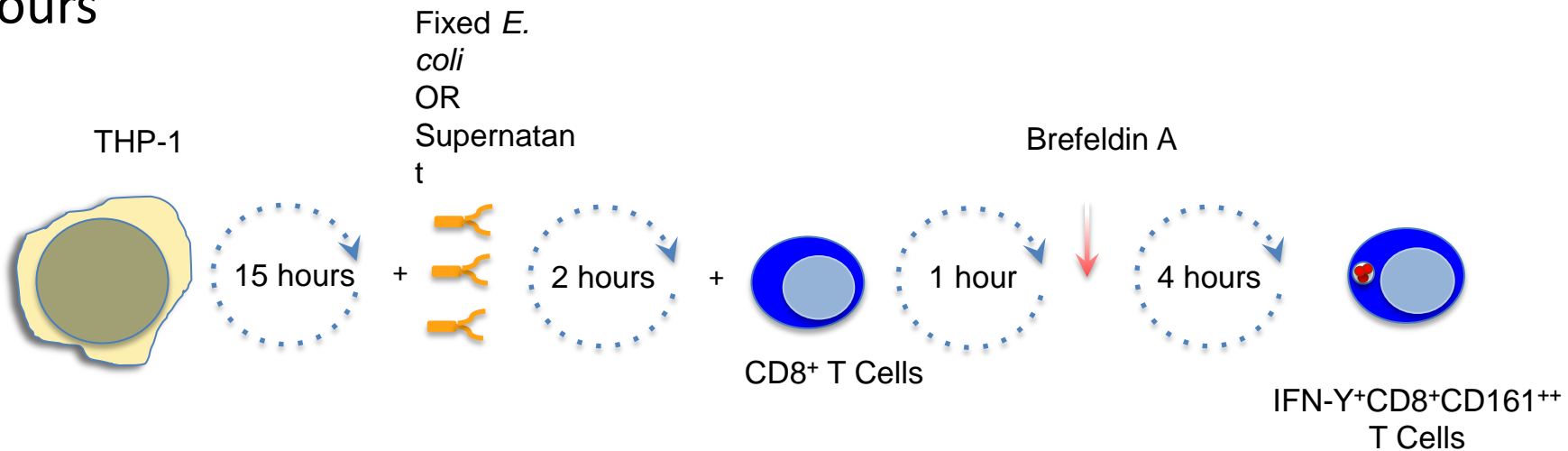


Time of APC exposure to bacteria

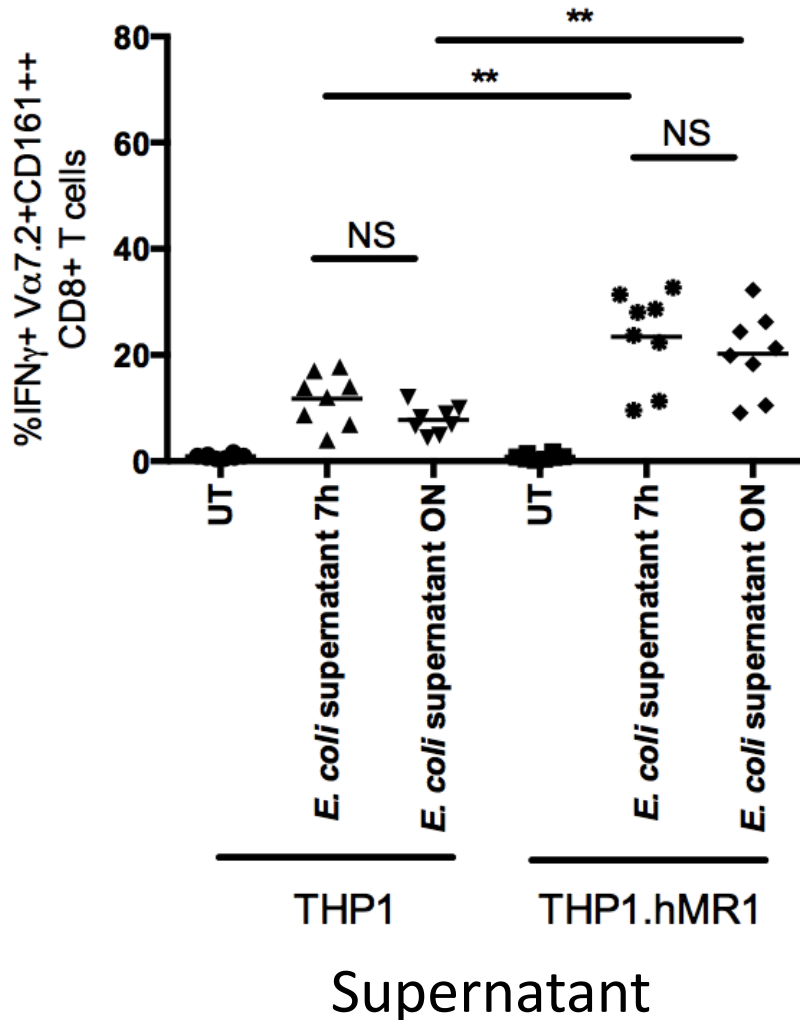
Overnight



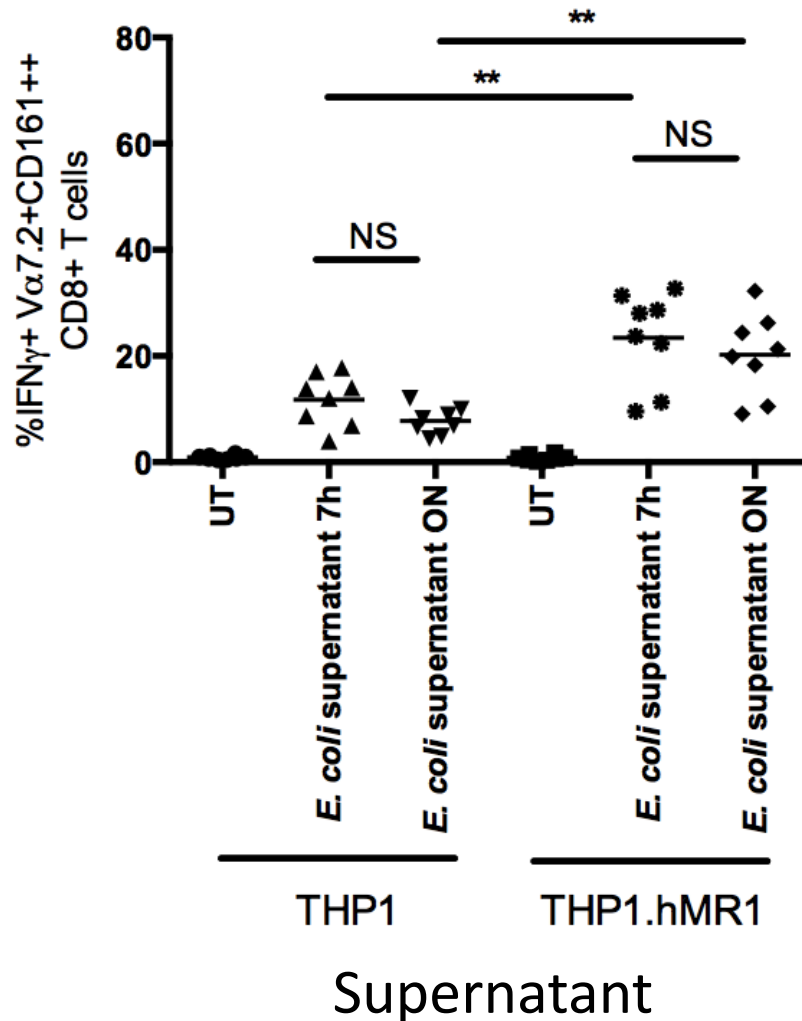
7 hours



Supernatant: MR1 over-expression but not incubation time enhances MAIT cell activation

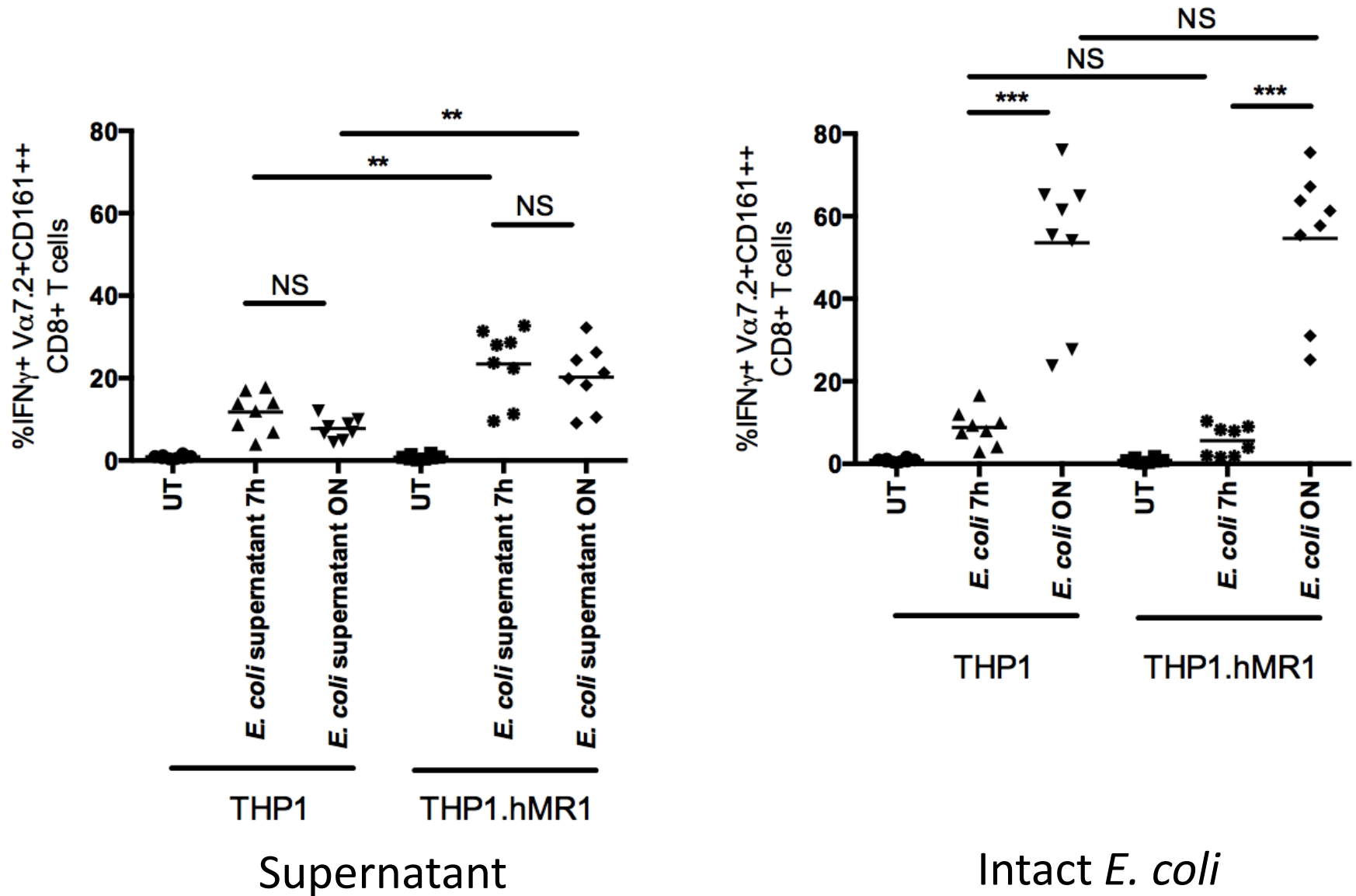


Supernatant: MR1 over-expression but not incubation time enhances MAIT cell activation

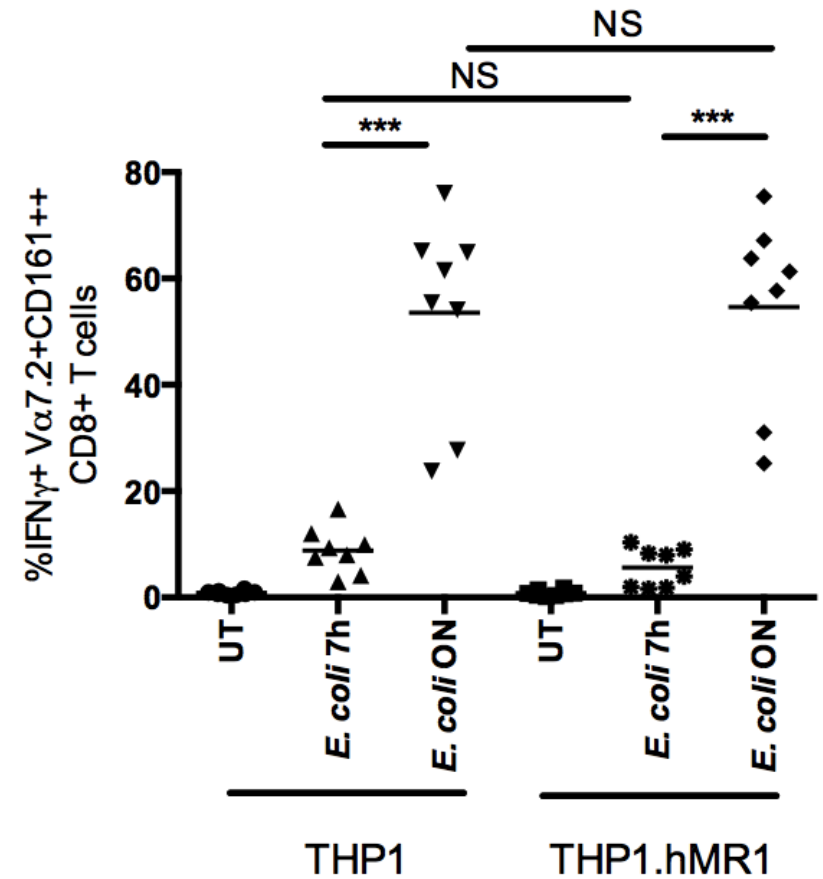
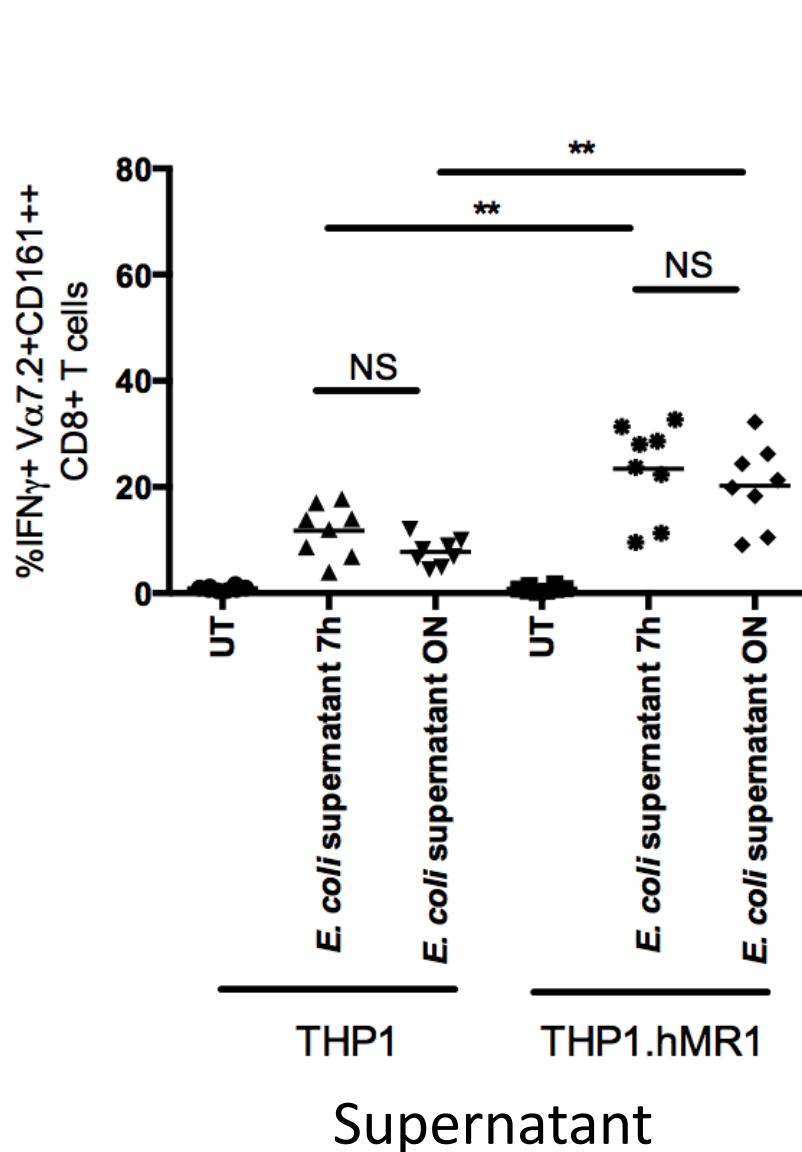


MR1 limiting
No increase with time

Intact *E. coli*: Prolonged incubation but not MR1 expression enhances MAIT cell activation



Intact *E. coli*: Prolonged incubation but not MR1 expression enhances MAIT cell activation

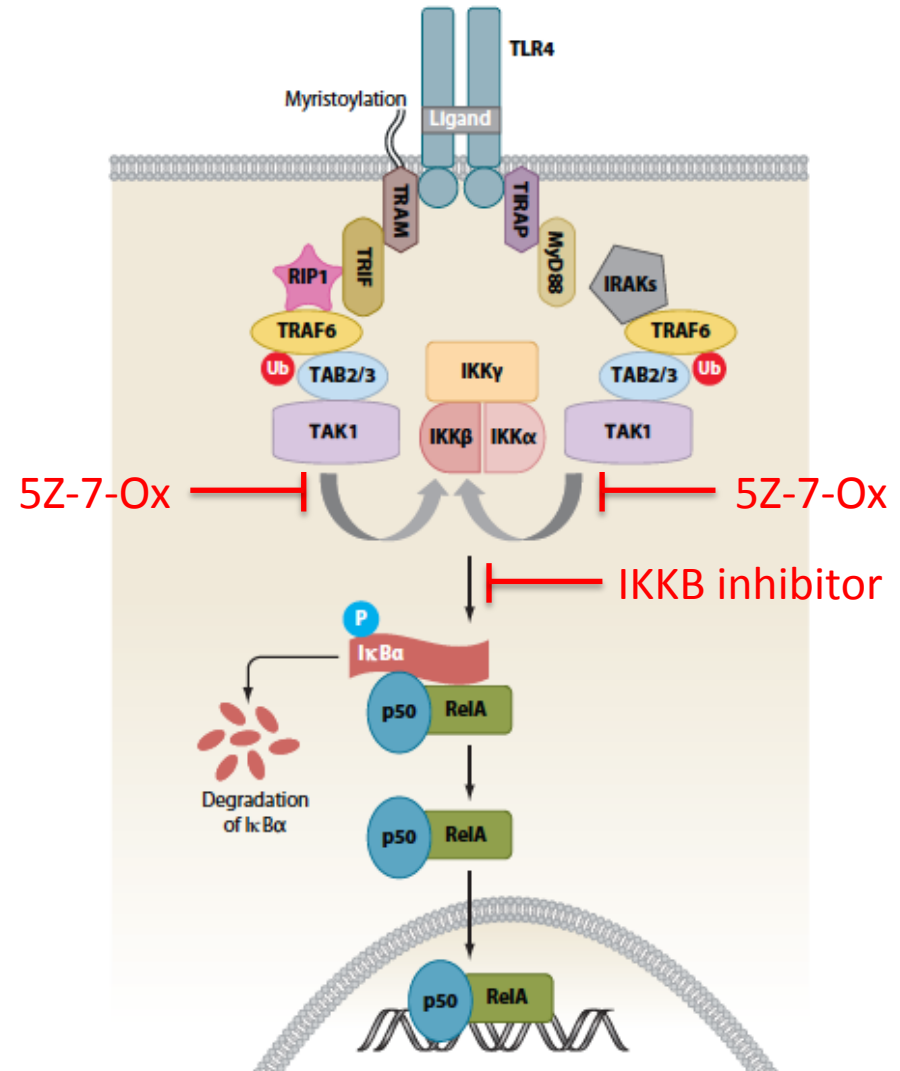
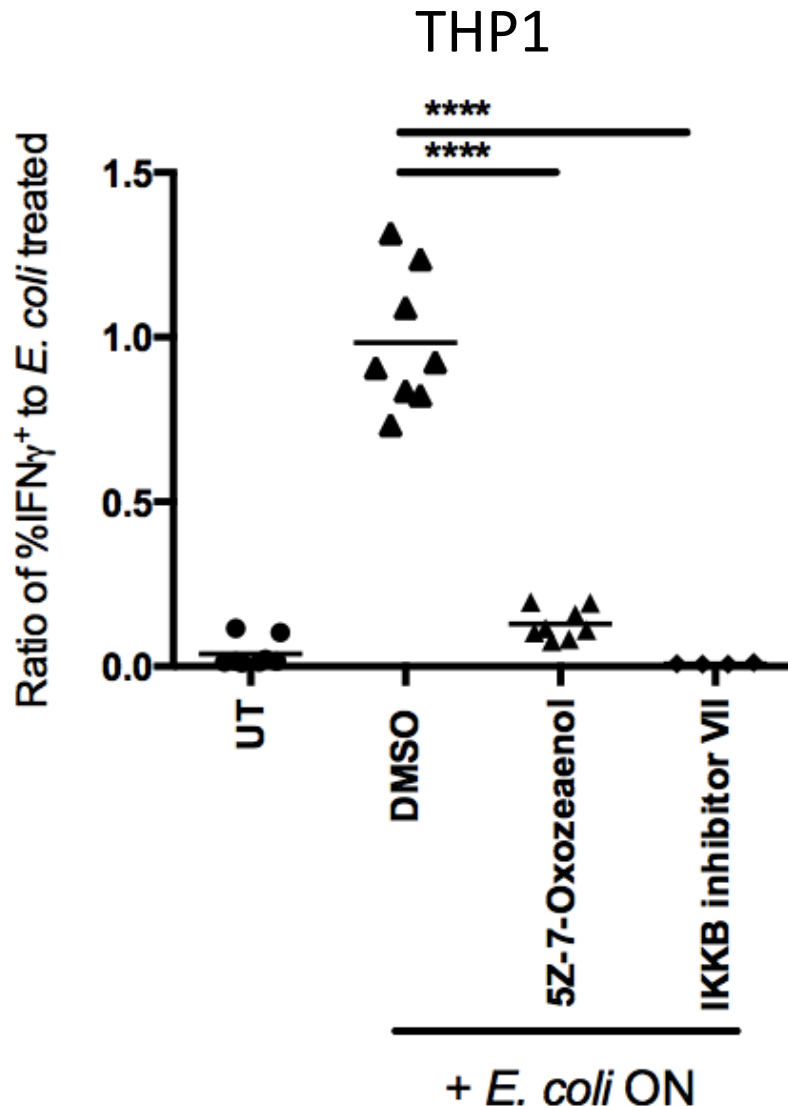


MR1 not limiting

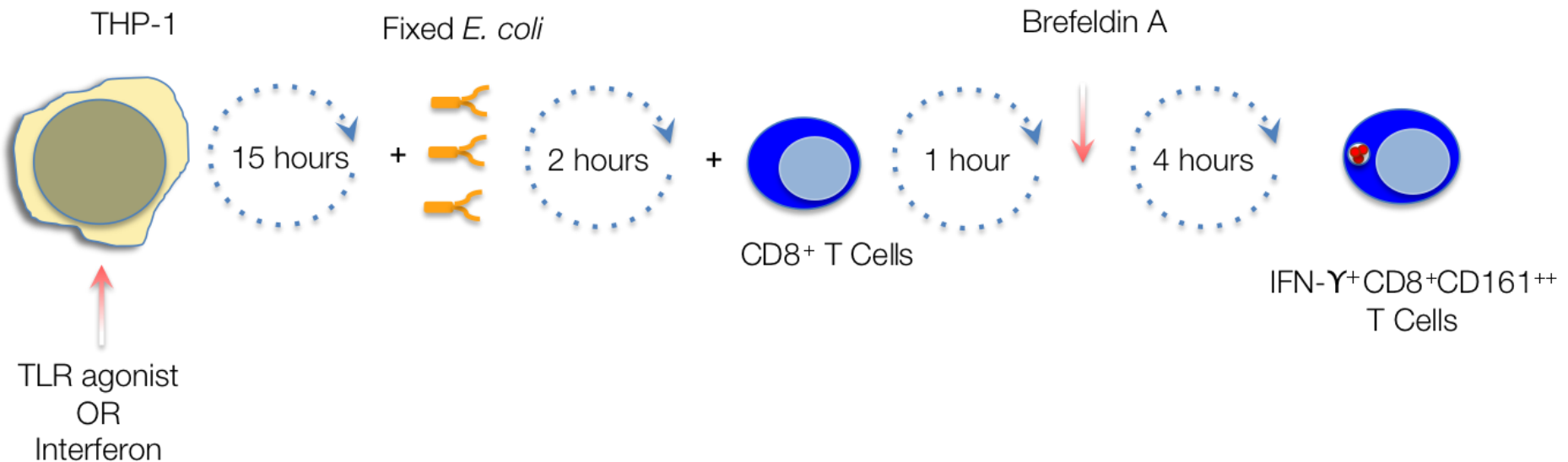
Time-dependent process

Intact bacteria remain more potent

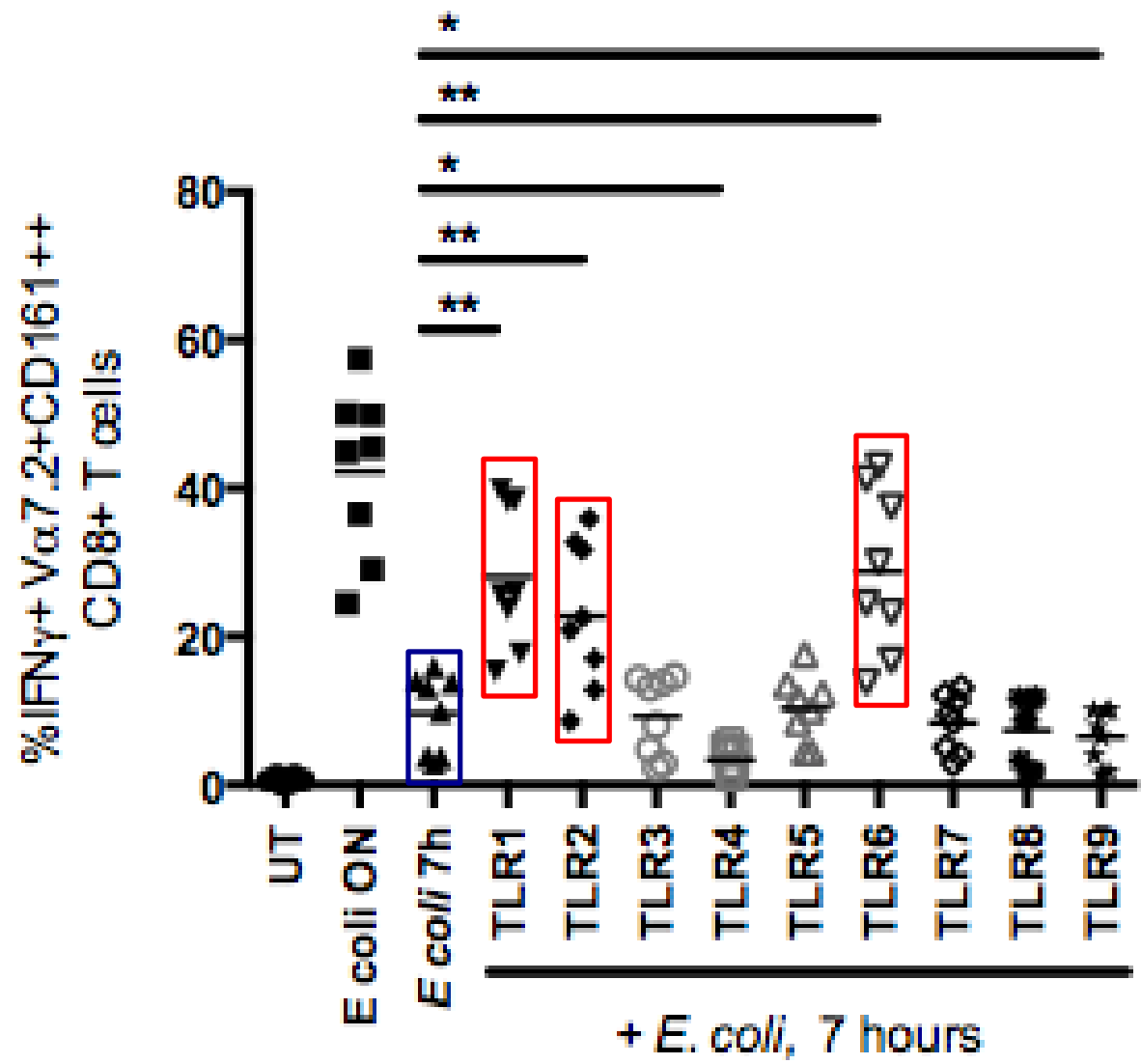
NF κ B signaling in the APC is required for MR1-mediated MAIT cell activation



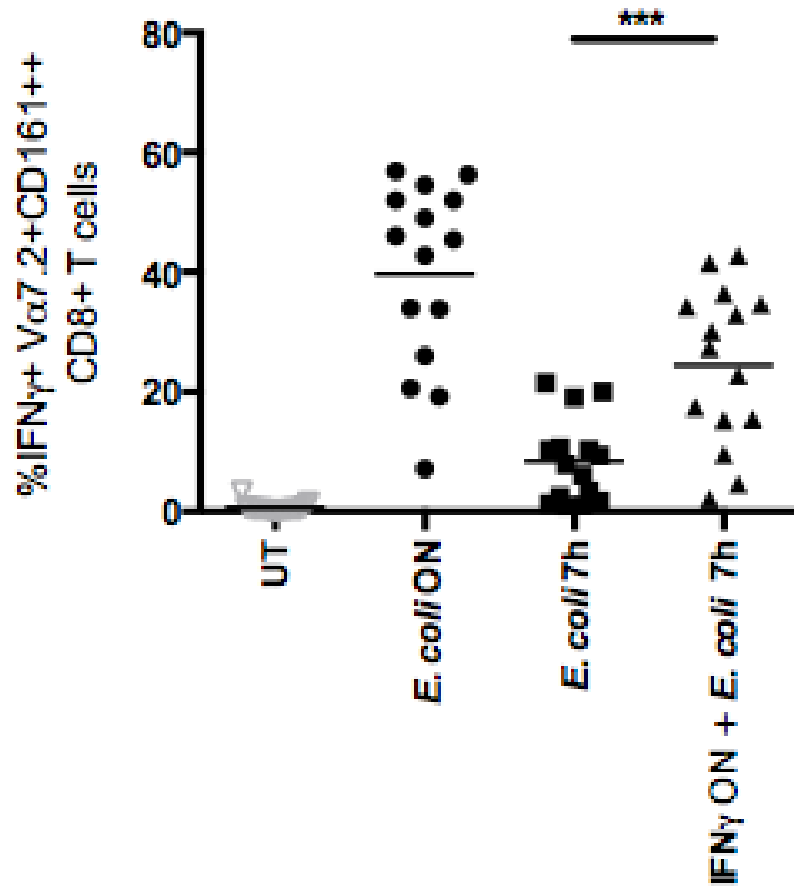
Effect of pre-activation of THP1s on early MR1-mediated MAIT cell activation



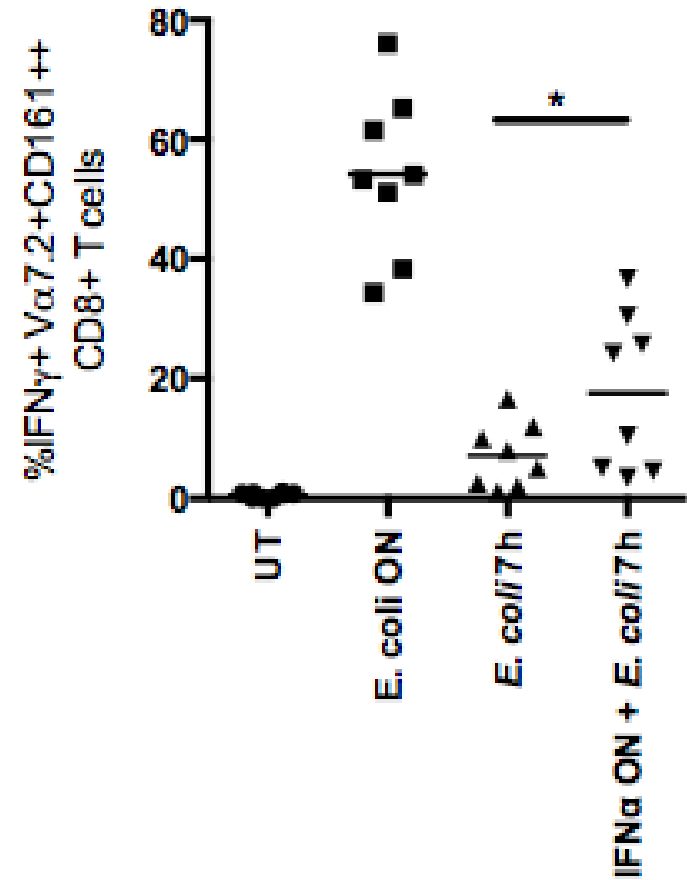
Pre-activation of THP1s with agonists to TLRs 1, 2, or 6 enhances early MR1-mediated MAIT cell activation



Pre-treatment with IFN γ or IFN α also enhances early MR1-mediated MAIT cell activation

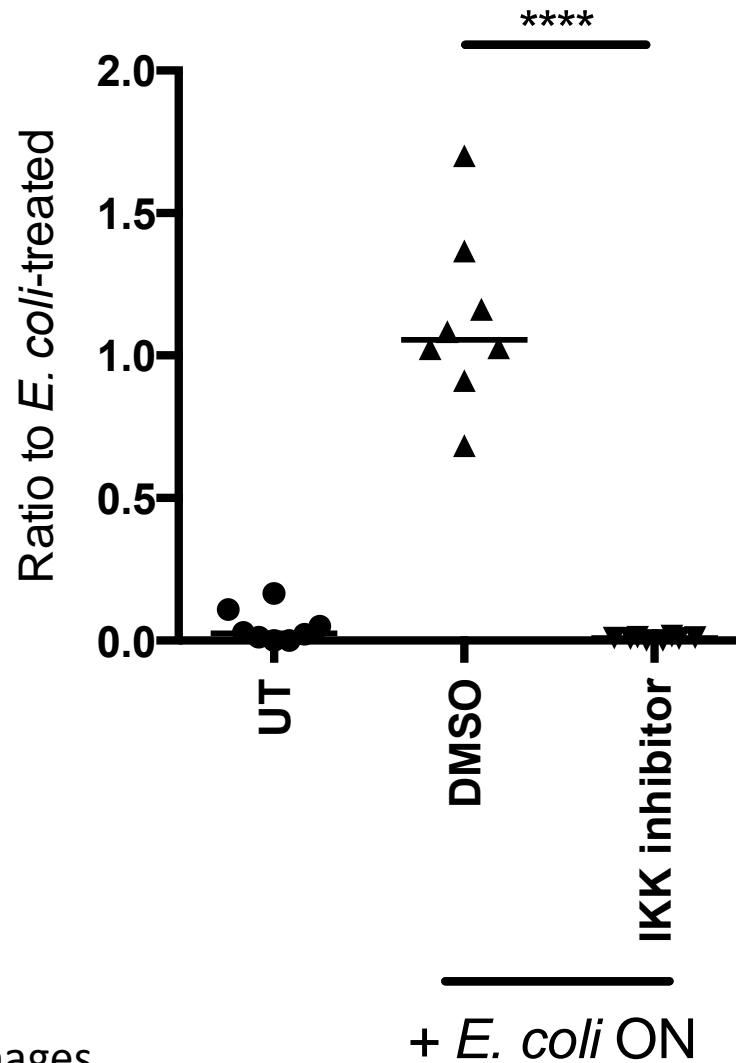


+/- interferon- γ 1000U/ml overnight

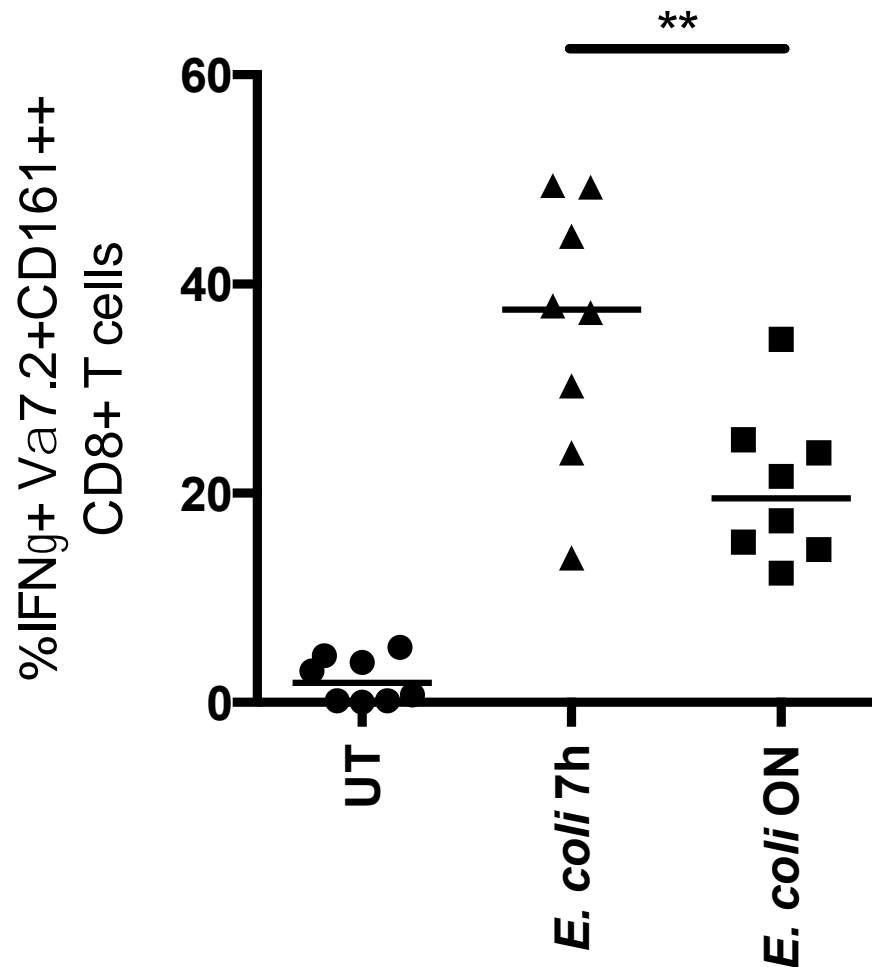


+/- interferon- α 1000U/ml overnight

Monocyte-derived macrophages

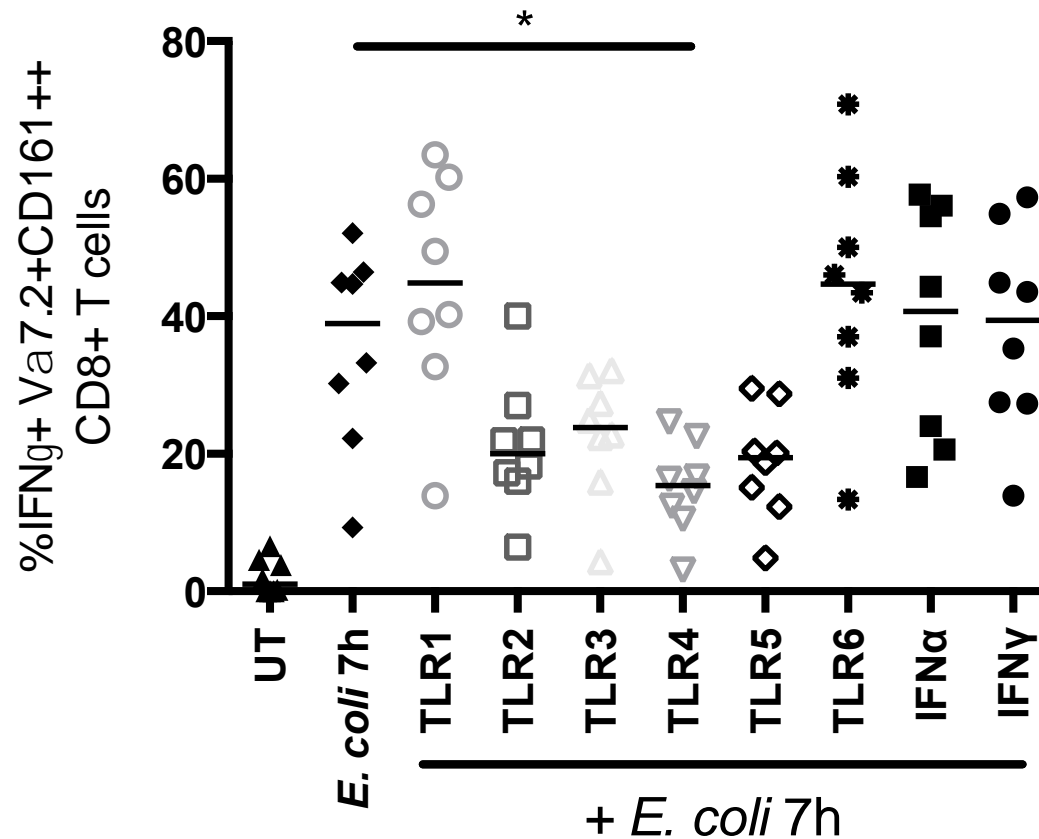


Robust early MR1-mediated activation with MoMo

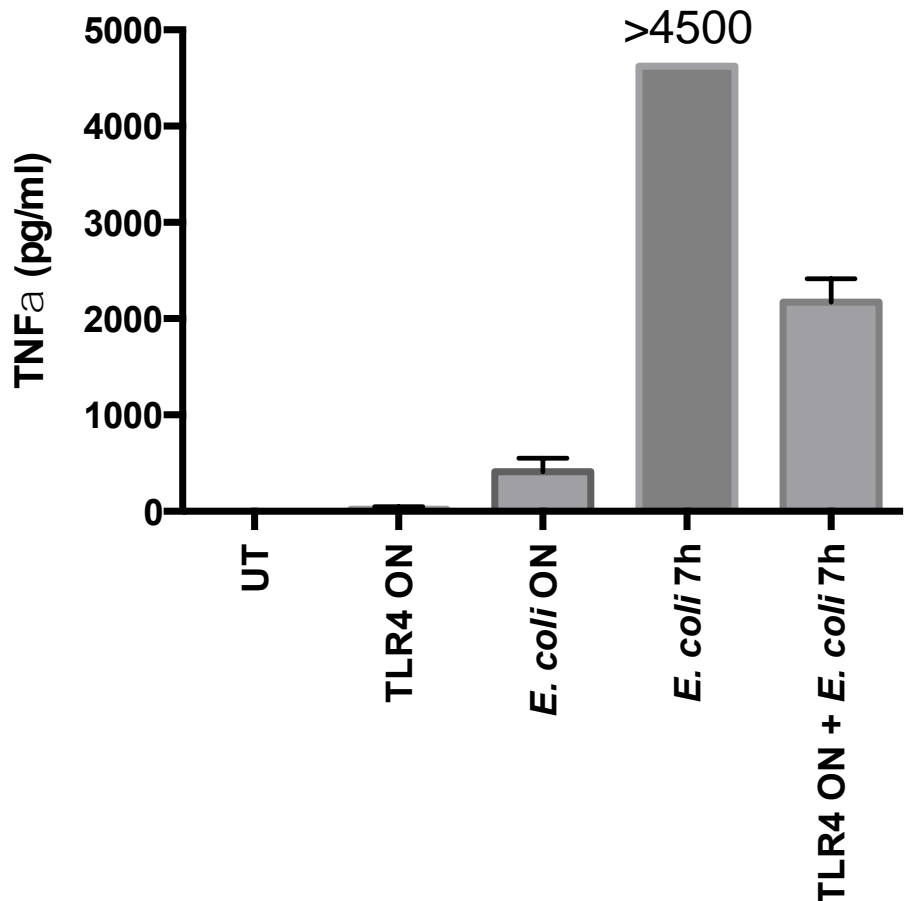
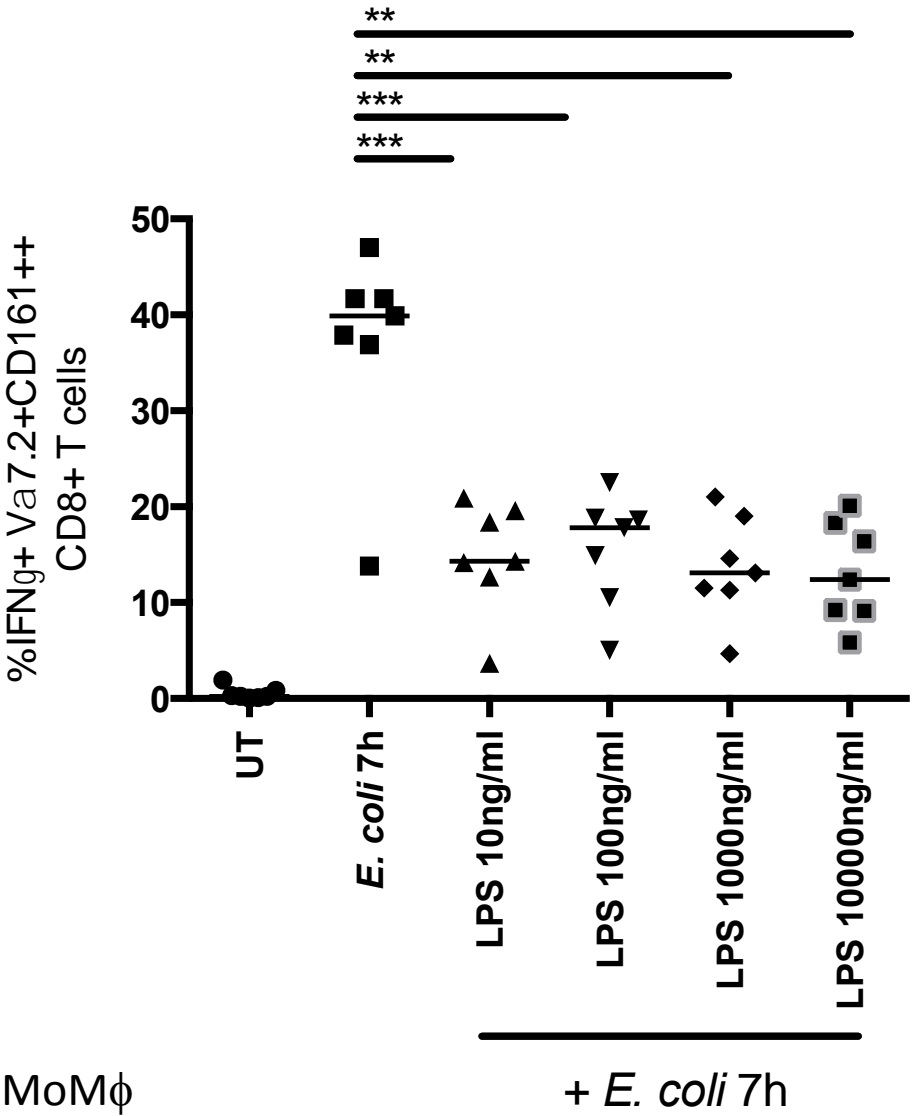


Monocyte-derived macrophages

Pre-activation of MoMφ fails to enhance MR1-mediated MAIT cell activation



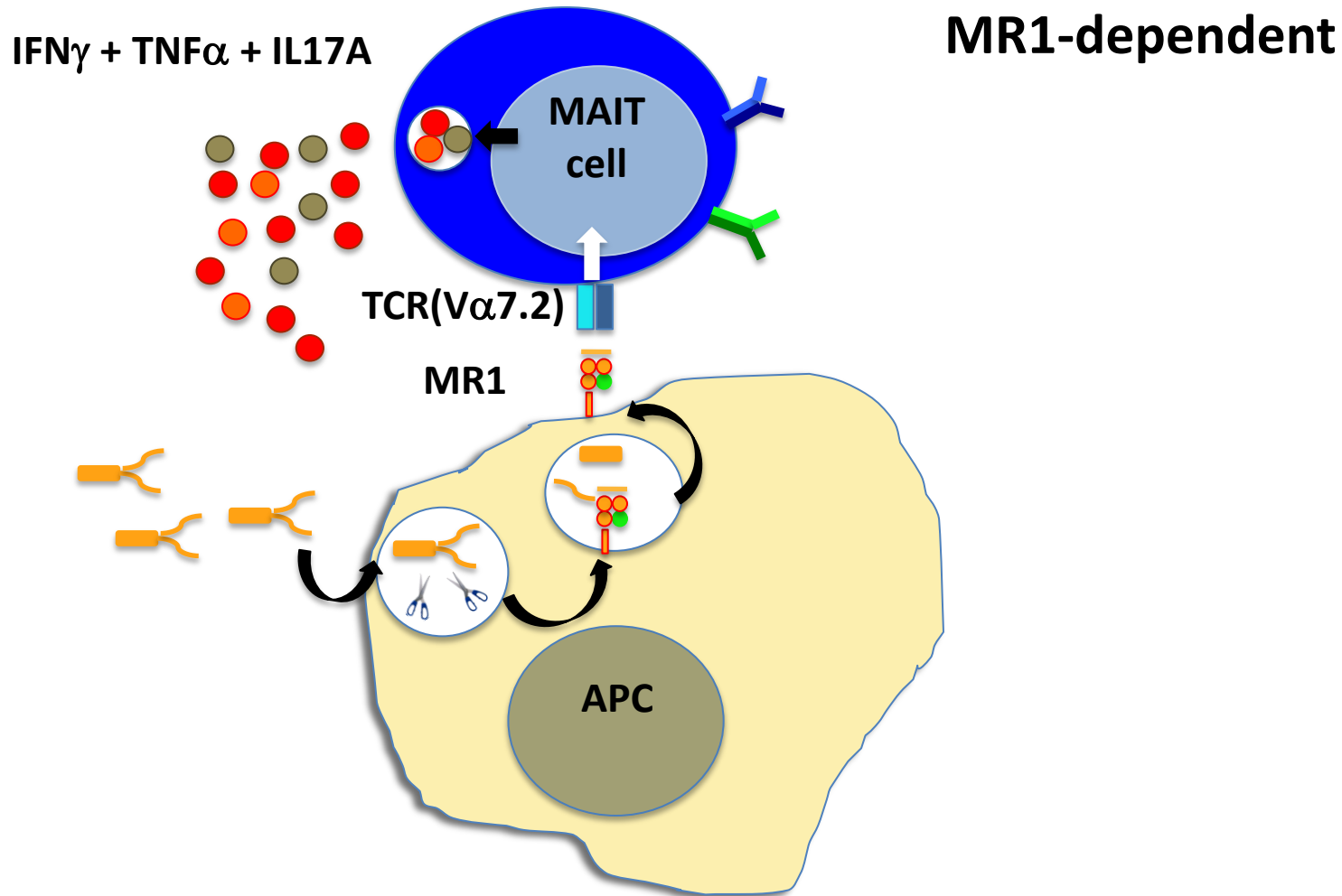
MR1-mediated MAIT cell activation is negatively regulated by endotoxin tolerance



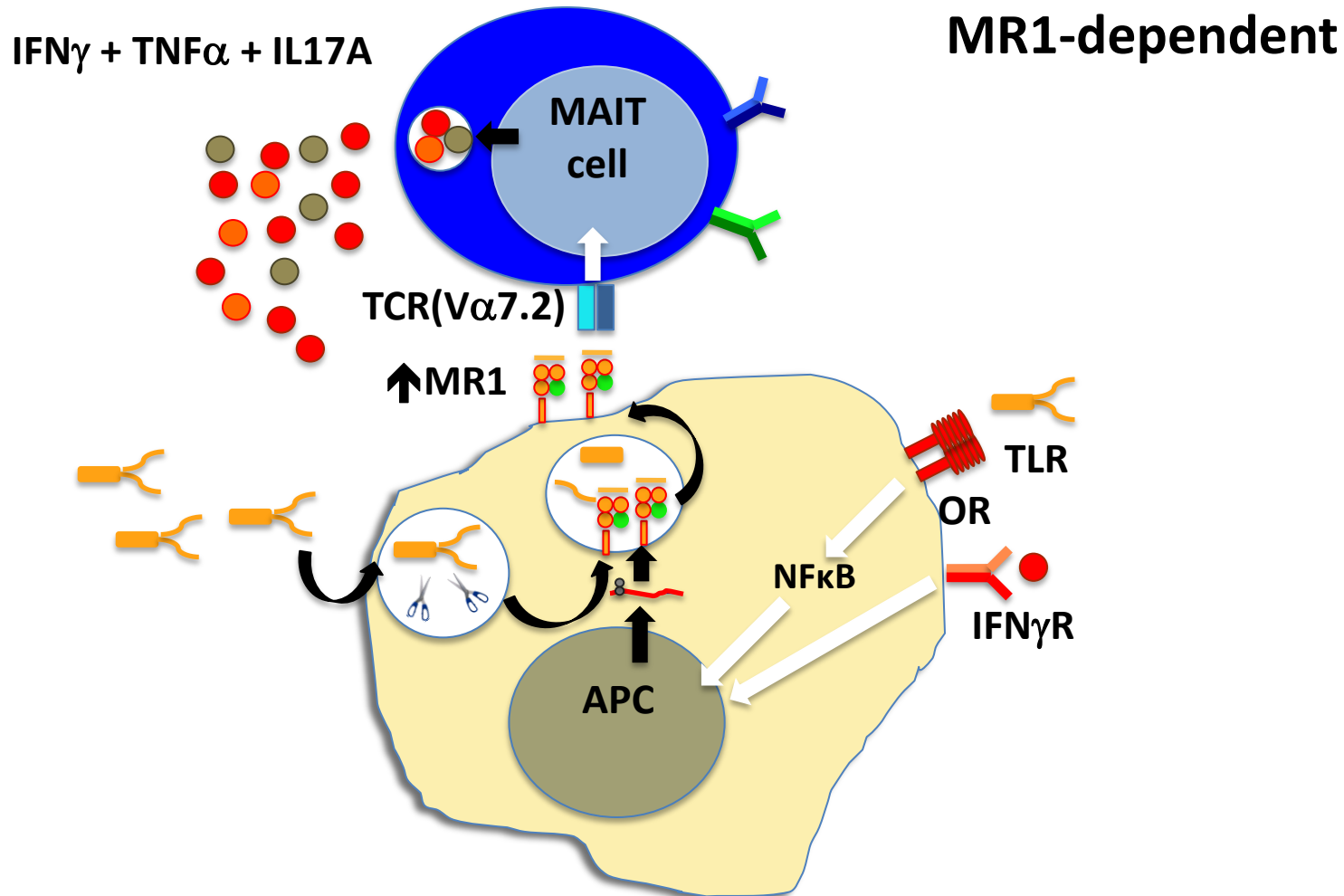
Summary (1)

- Efficient MR1-mediated activation requires APC activation
- LPS-induced tolerance suppresses MR1-mediated activation
- MR1-mediated MAIT cell activation is tightly regulated

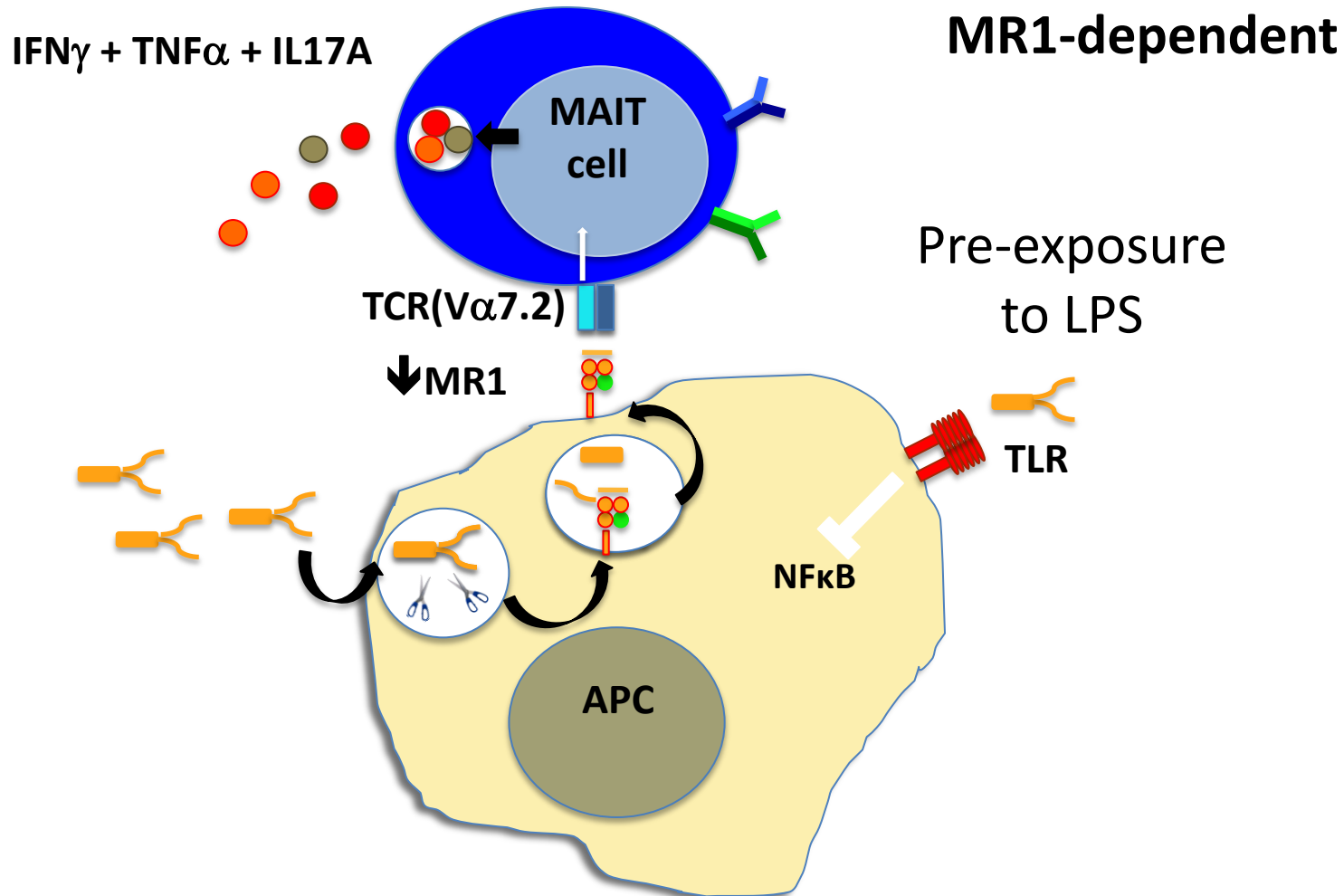
Model of regulation of MAIT cell activation



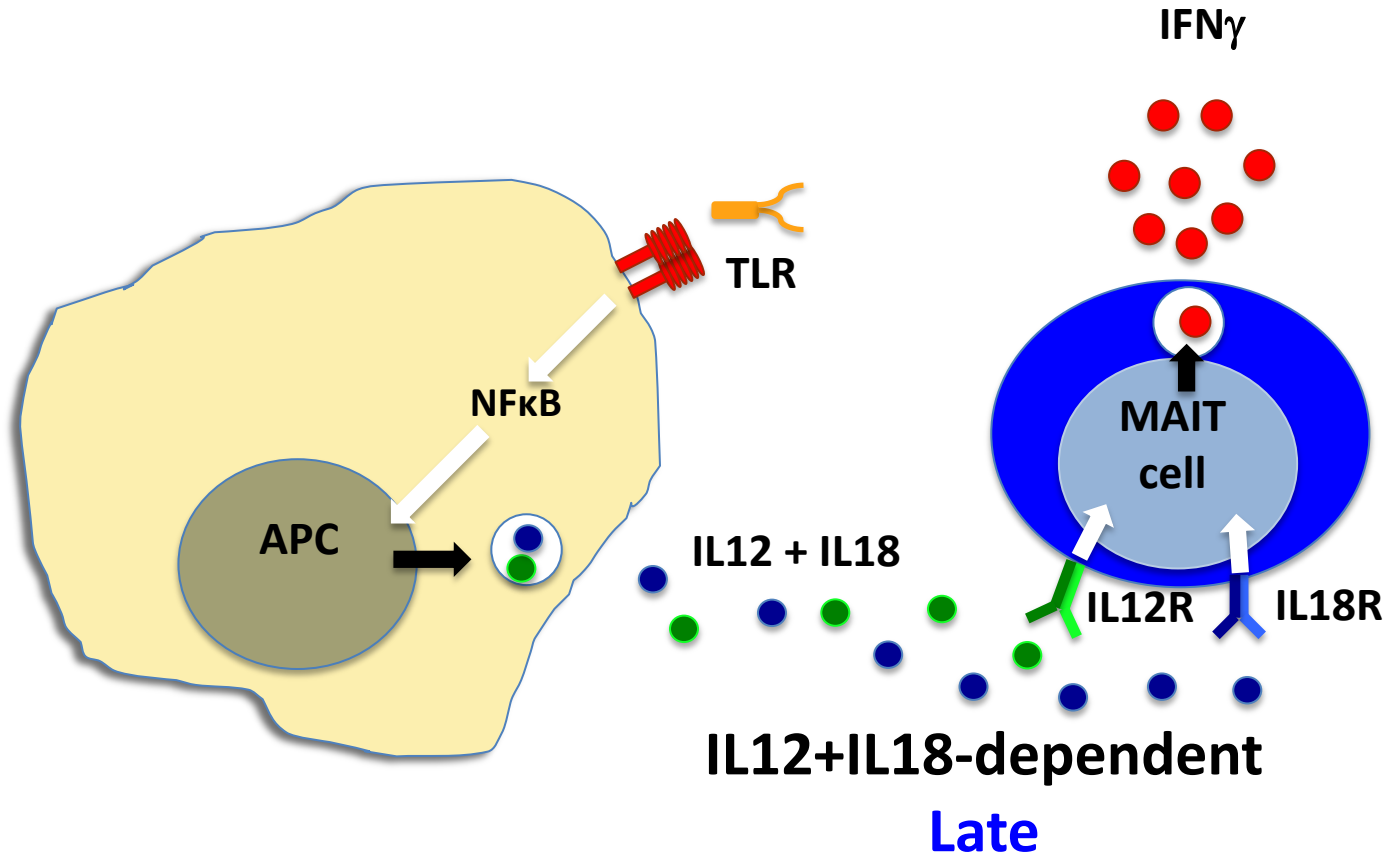
Model of regulation of MAIT cell activation



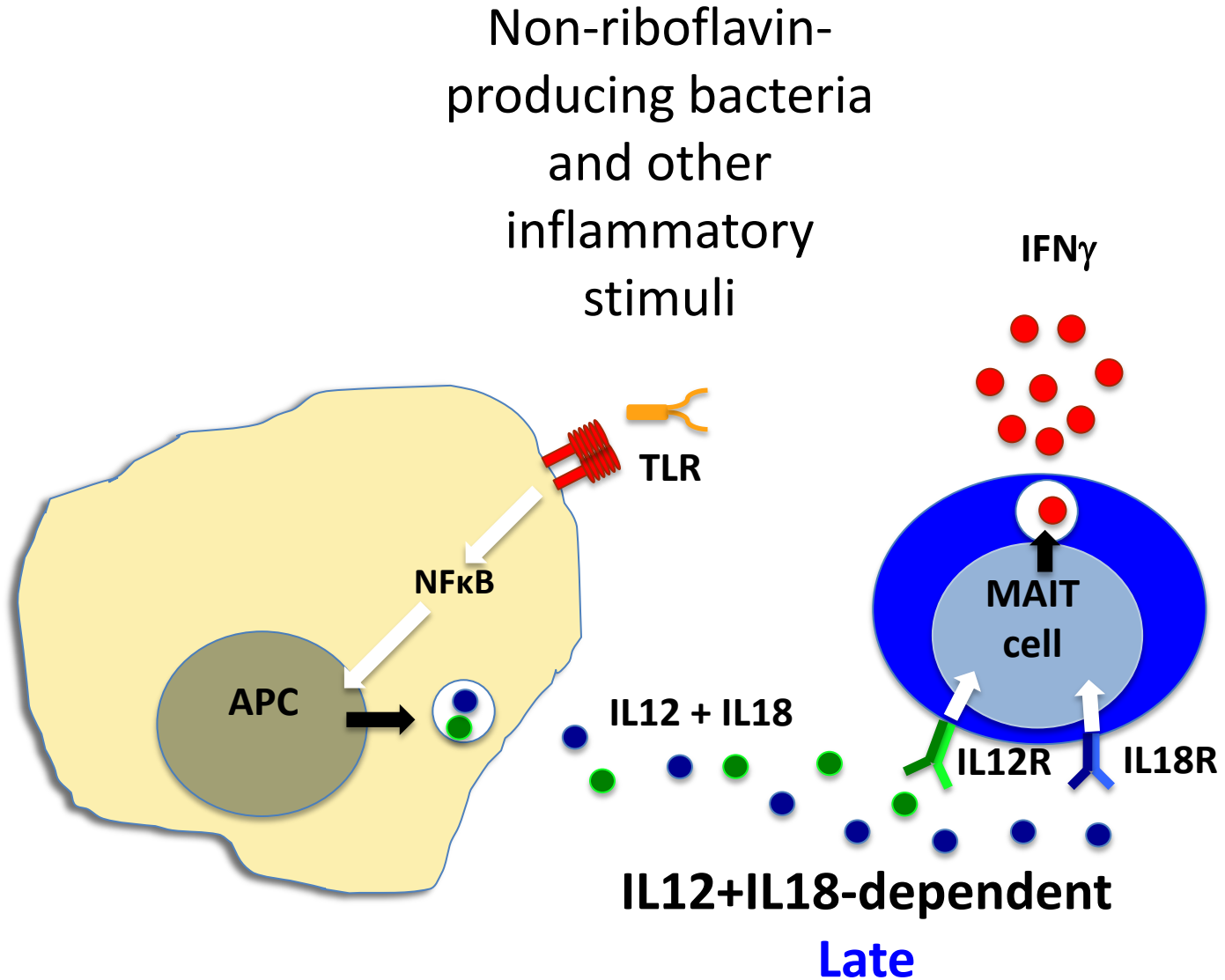
Model of regulation of MAIT cell activation



Model of regulation of MAIT cell activation



Model of regulation of MAIT cell activation



Summary (2)

- Two mechanisms of activation:
 - MR1 (TCR dependent)
 - IL-12+IL-18 (TCR independent)
- Whole bacteria, but not supernatant or cell lysate, are potent activators of MAIT cells via MR1
 - Presentation of supernatant dependent upon surface expression of MR1
 - Presentation of intact bacteria dependent upon time but not level of MR1 expression
- Efficient MR1-mediated activation requires APC activation
 - NF κ B-dependent
- LPS-induced tolerance suppresses MR1-mediated activation
- MR1-mediated MAIT cell activation is tightly regulated

Acknowledgements

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Paul Klenerman

Chris Willberg

Mathew Bilton

Ayako Kurioka

Kerstin Ruustal

Alexander Wilson

Rodney Phillips

Washington University in St. Louis

Ted H. Hansen

Funding Bodies

Wellcome Trust

James Martin School of the
21st Century

NIHR Biomedical Research
Centre Programme

Oxford Dominions Trust

NHS

*National Institute for
Health Research*



JAMES MARTIN 21ST CENTURY SCHOOL

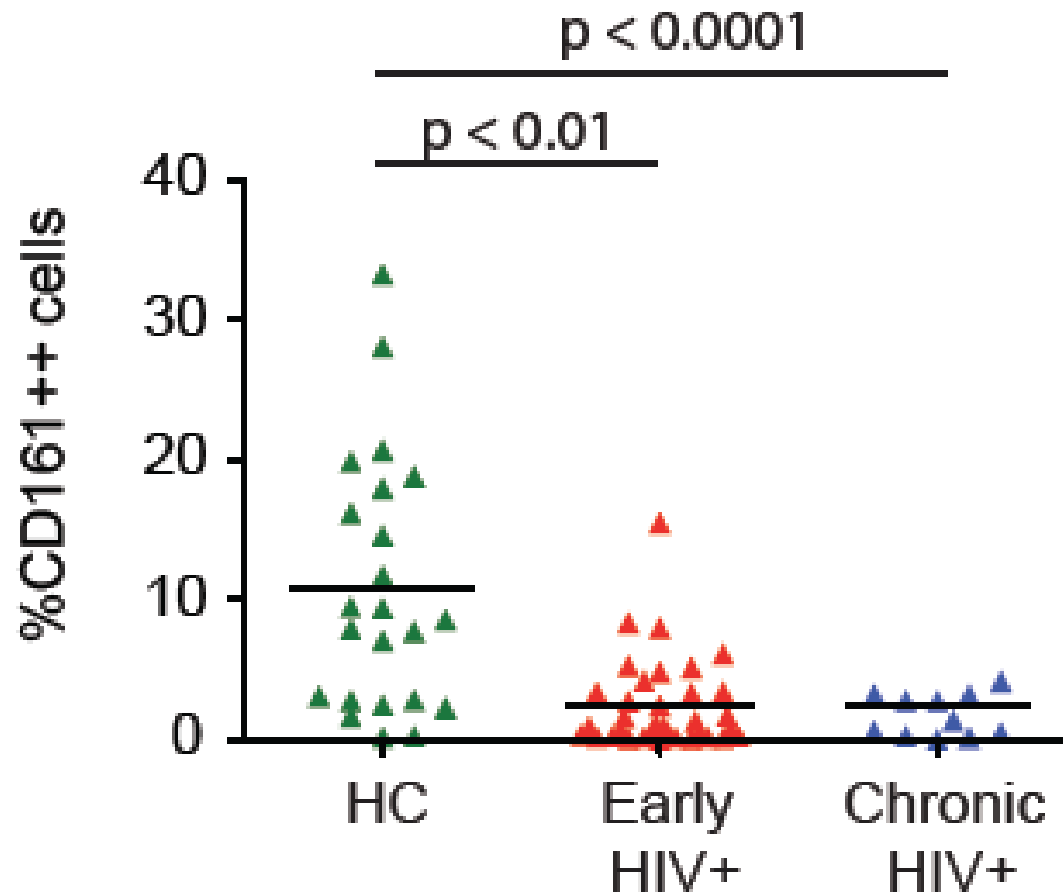
wellcometrust

Is the MAIT cell population
perturbed in HIV infection?

MAIT cells in HIV infection

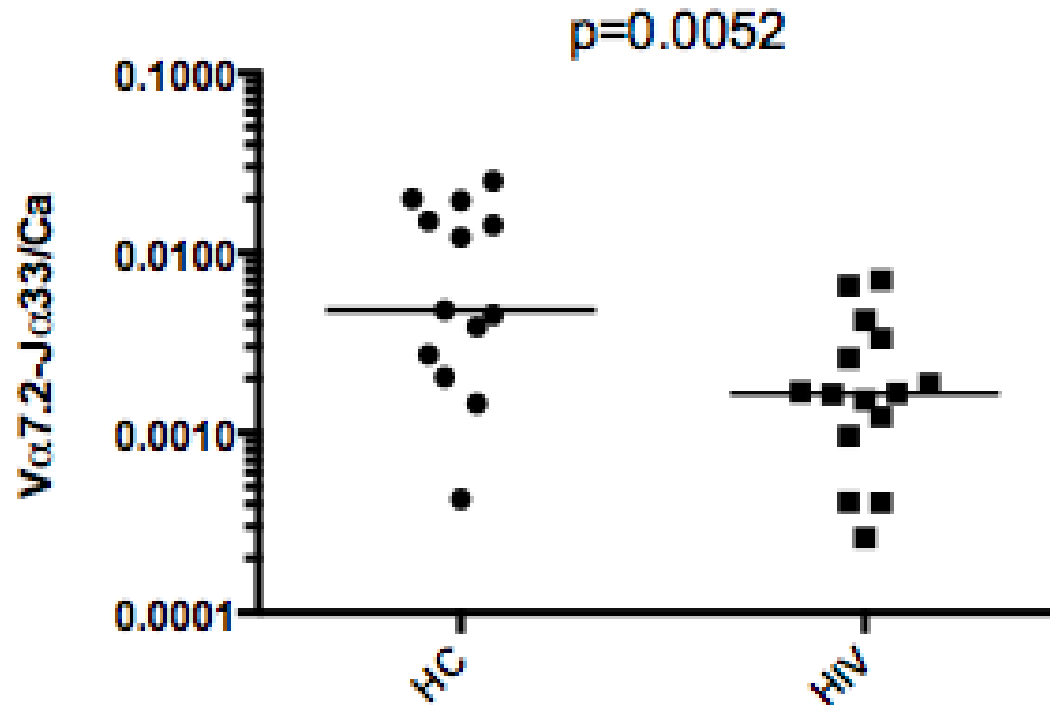
- Early stage HIV infection
 - SPARTAC baseline samples
 - Median CD4 count = 603 cells/ μ l (475, 774)
 - Median viral load = 4.73 \log_{10} copies/ml (3.89, 5.19)
- Chronic untreated HIV infection
 - Kings College London Infectious Diseases Biobank
 - Median CD4 count = 250 cells/ μ l (207, 326)
 - Median viral load = 4.22 \log_{10} copies/ml (3.99, 4.94)

MAIT cells are lost from the blood in HIV



HC = Healthy control

MAIT cells are lost from the blood in HIV

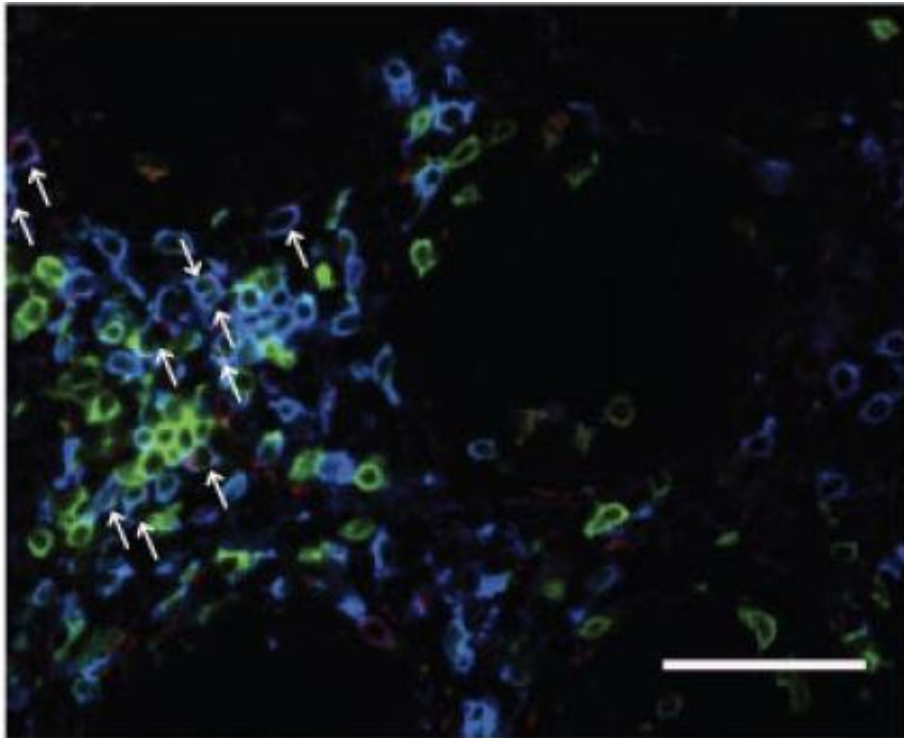


Canonical TCR $V\alpha7.2-J\alpha33$
Normalised against $C\alpha$

MAIT cells are not enriched in colon in HIV

CD3+CD8+MDR1++

HIV+

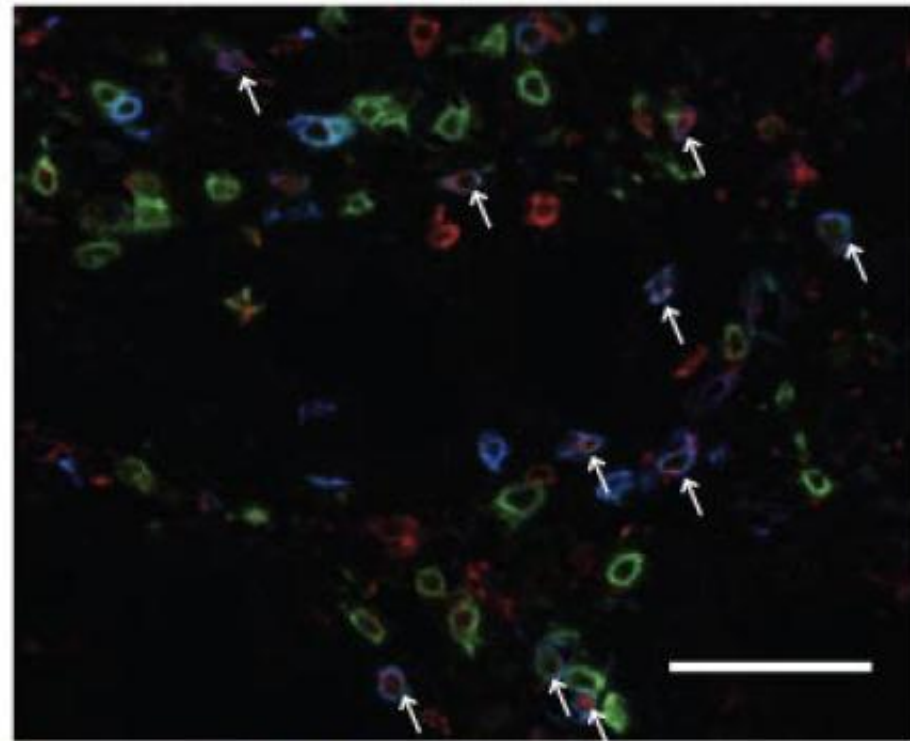


12 HIV⁺ patients

Macroscopically normal colon

7 microscopic colitis

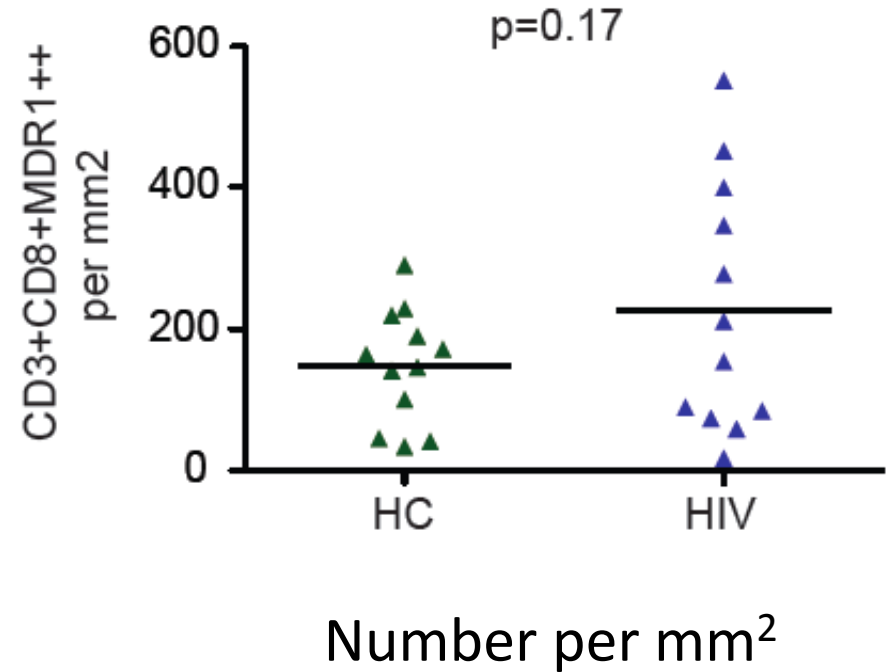
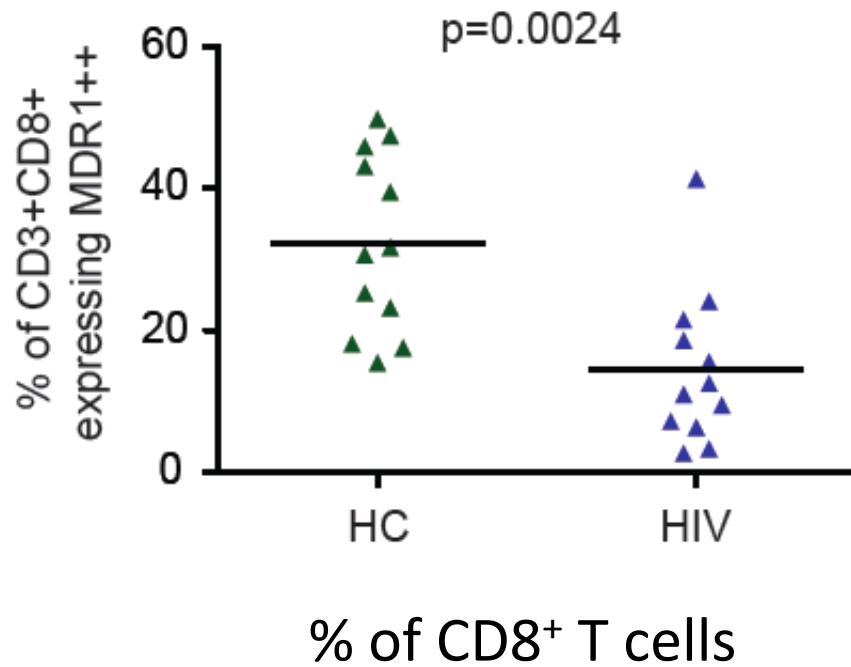
Healthy control



12 age-matched controls

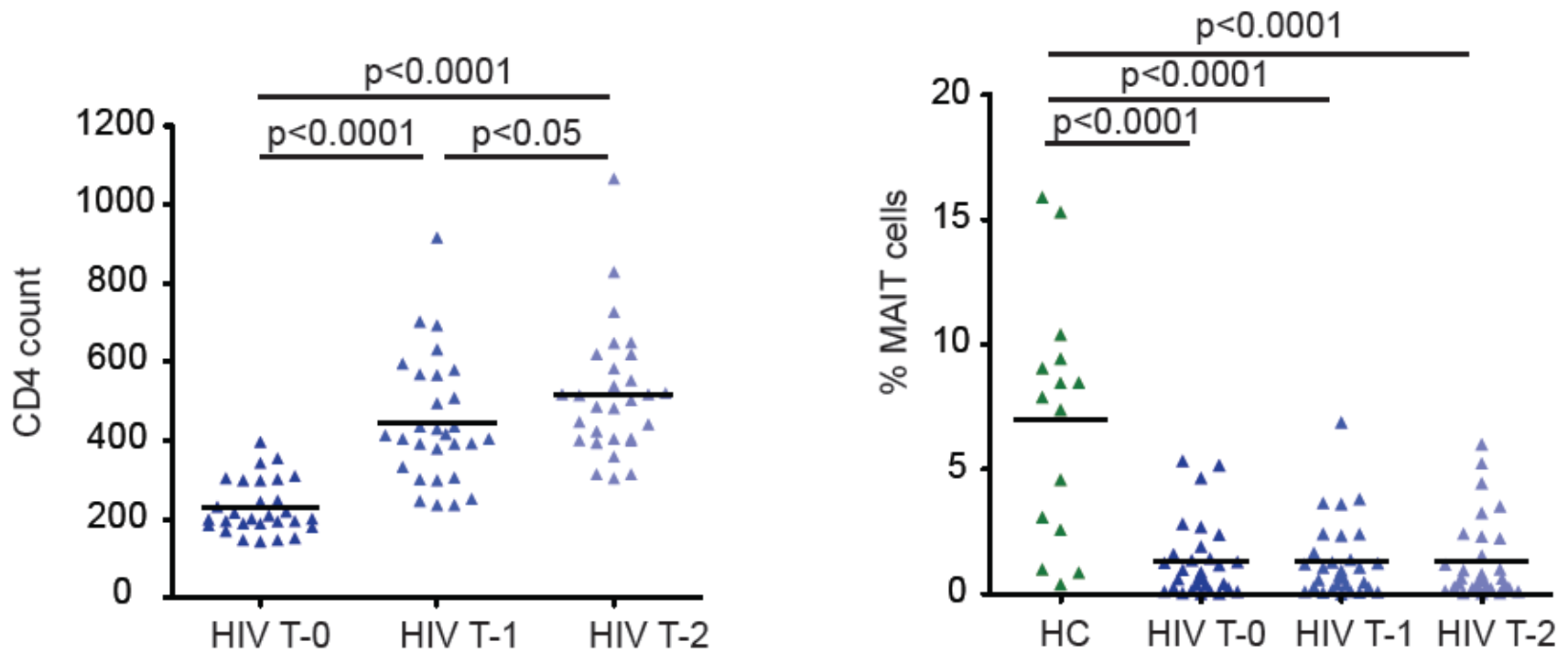
Non-inflamed normal colon

MAIT cells are not enriched in colon in HIV



HC = Healthy control

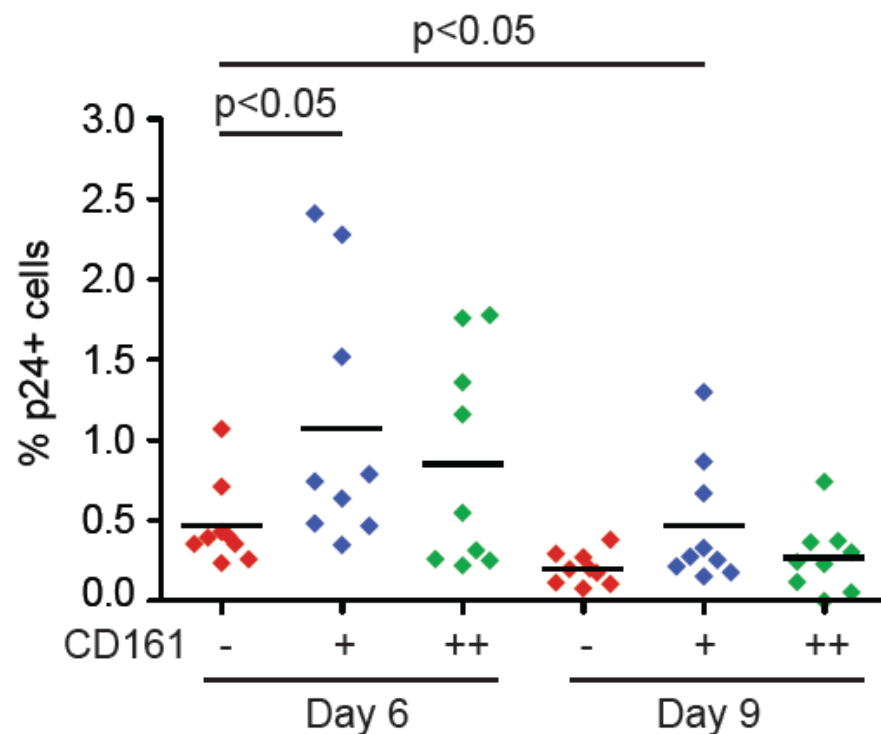
MAIT cells do not recover with HAART



- Swiss HIV cohort study
 - 30 patients
 - Pre-HAART and 1 and 2 years on HAART
 - Fully suppressed viral load

What is the mechanism of MAIT cell loss in HIV infection?

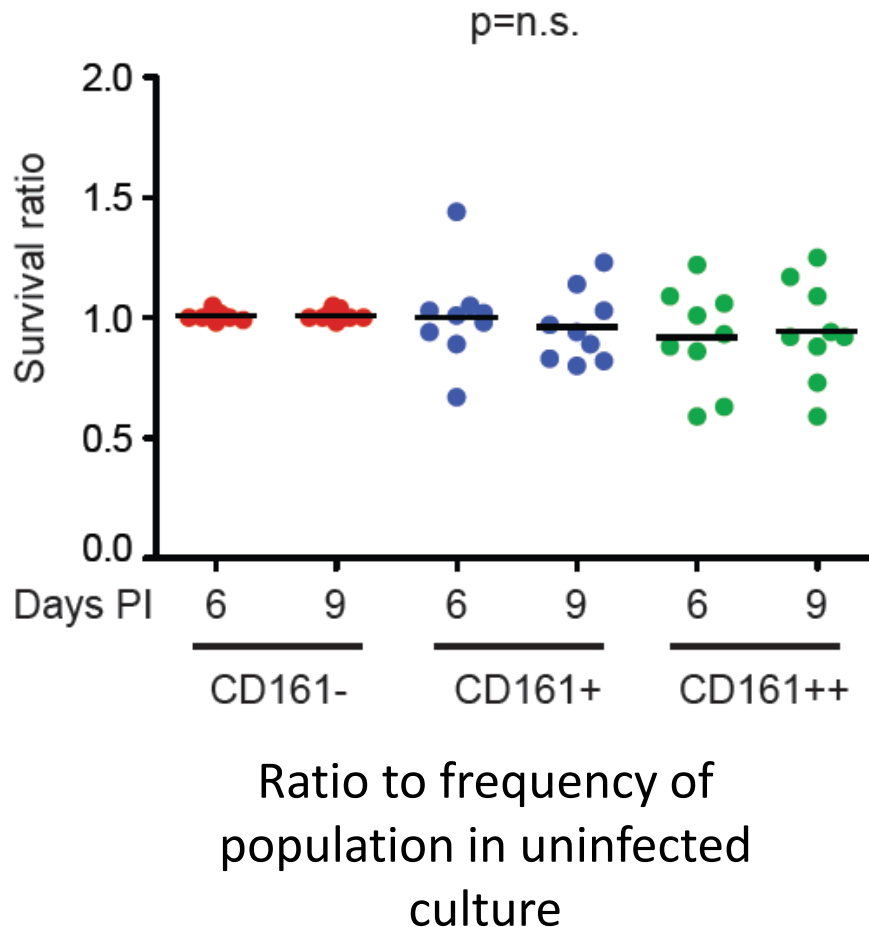
HIV does not preferentially infect MAIT cells



Infected with JR-CSF strain

- PBMCs from healthy subjects
- Activated for 3 days
 - PHA, IL2 and IL7
- Infected with HIV at MOI 10
 - CCR5-tropic virus (JR-CSF)
 - CXCR4 tropic virus (MN)
- p24 detected at days 6 and 9

HIV does not preferentially kill MAIT cells

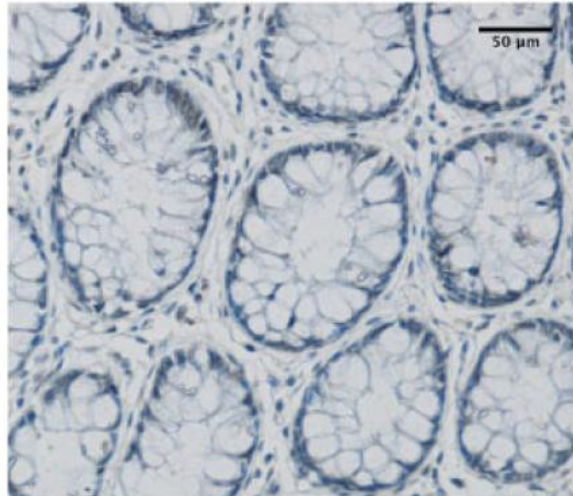


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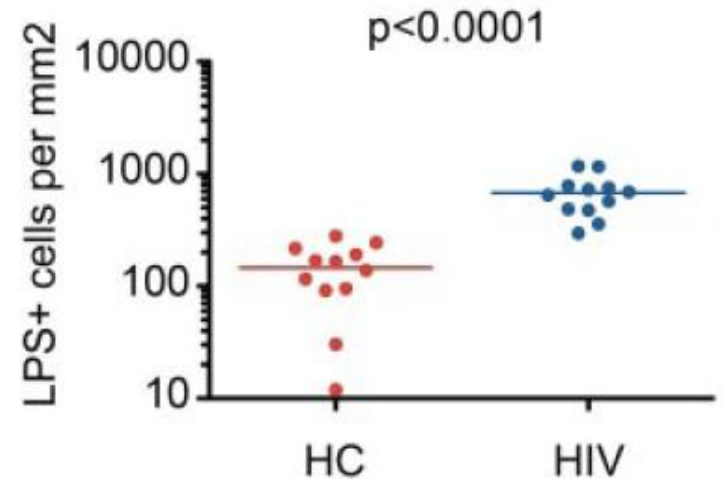
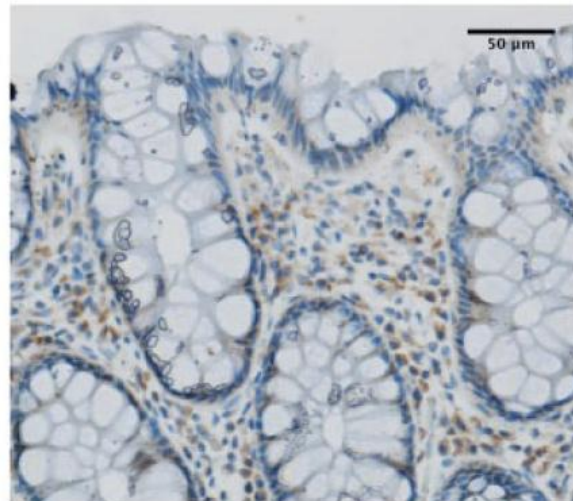
Bacterial lipopolysaccharide is detectable in the lamina propria in HIV infection

Lipopolysaccharide

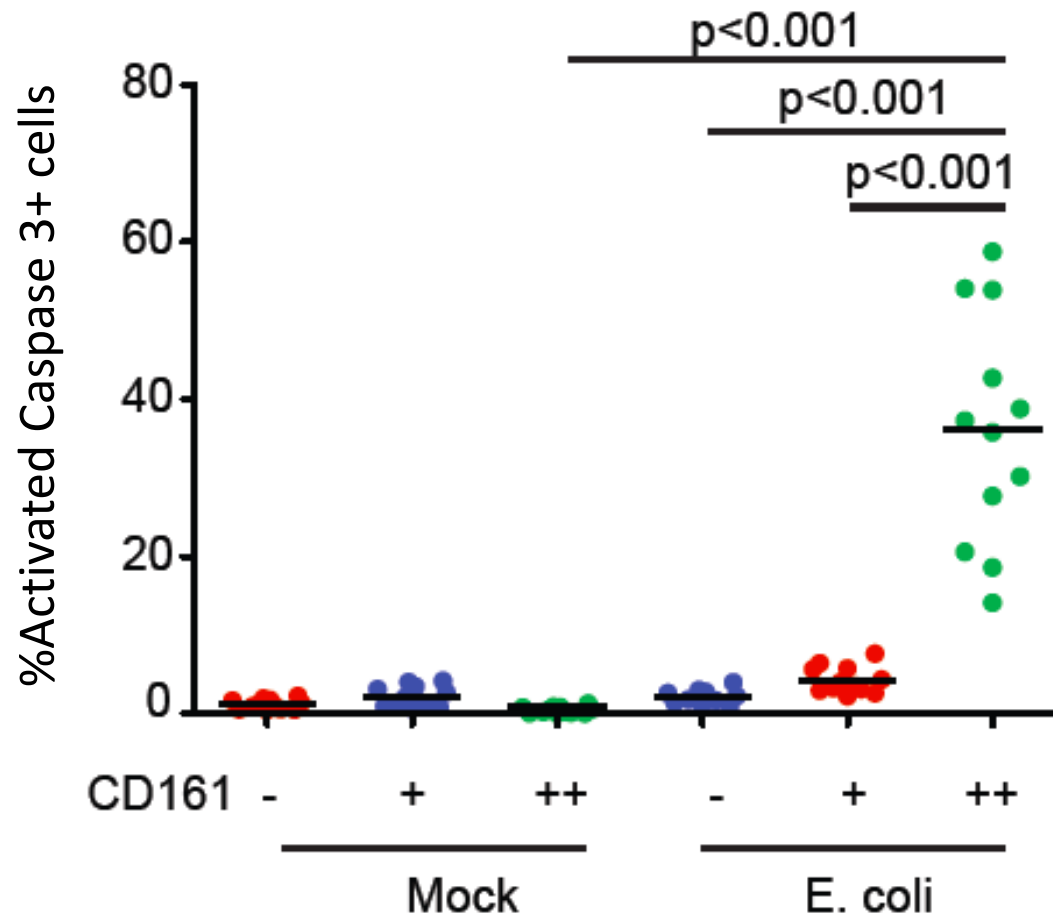
Healthy control



HIV+



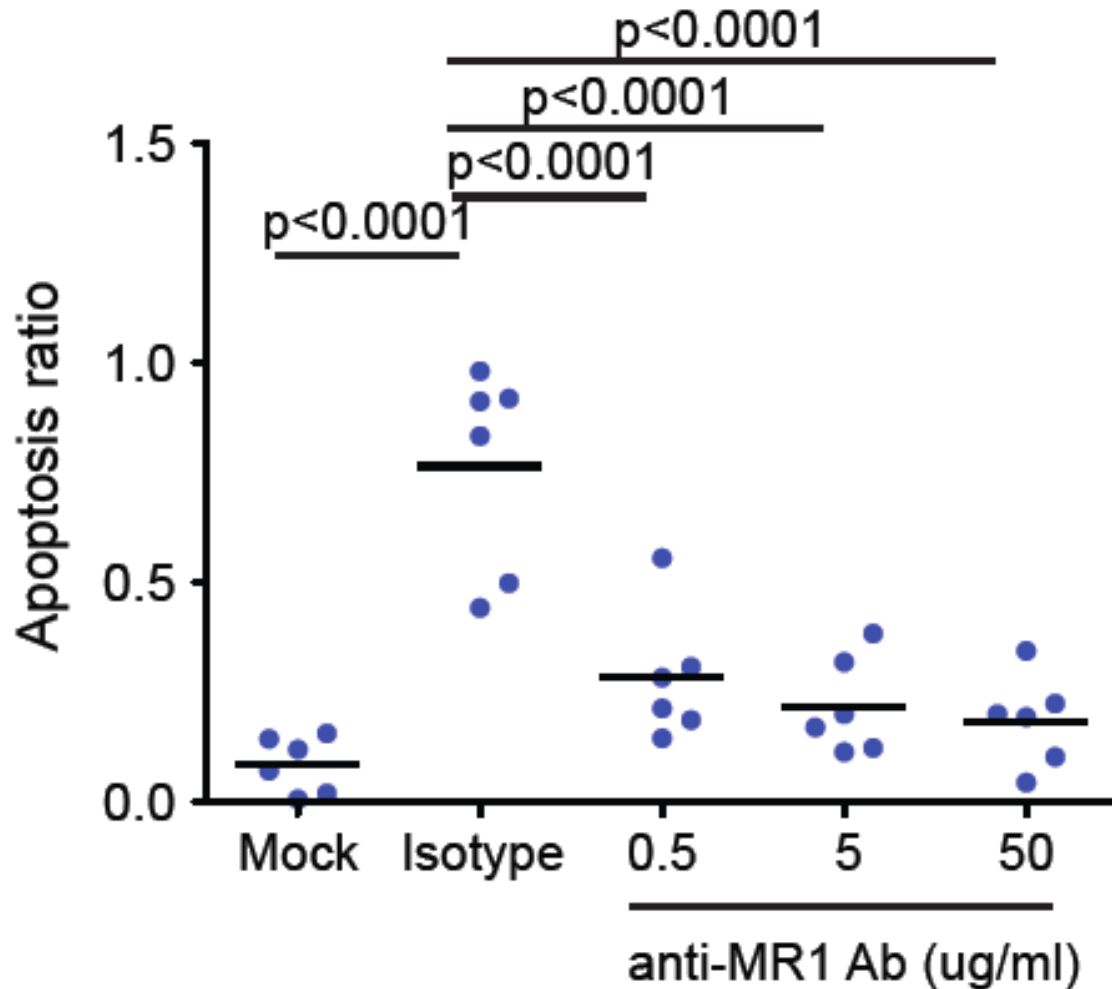
E. coli induces apoptosis of MAIT cells *in vitro*



** $p < 0.01$

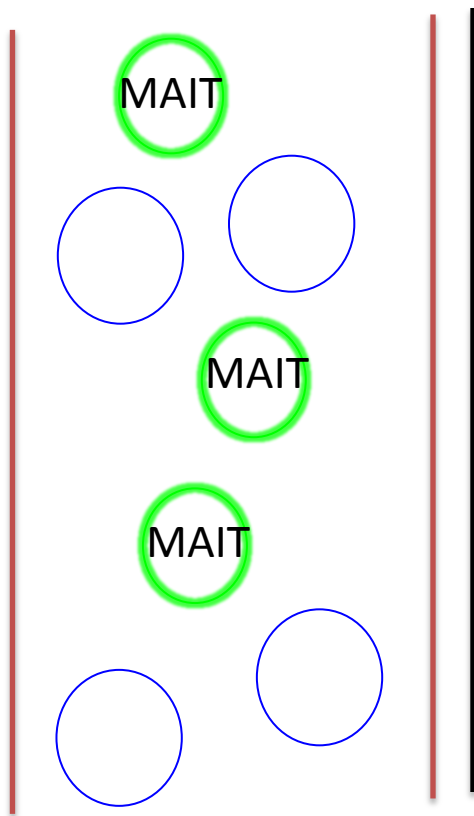
*** $p < 0.001$

Blocking MR1 inhibits *E. coli*-induced apoptosis

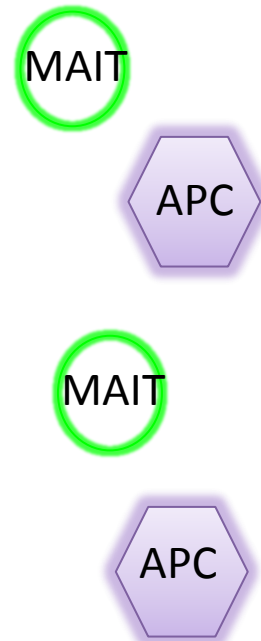


Proposed model for MAIT cell depletion in HIV infection

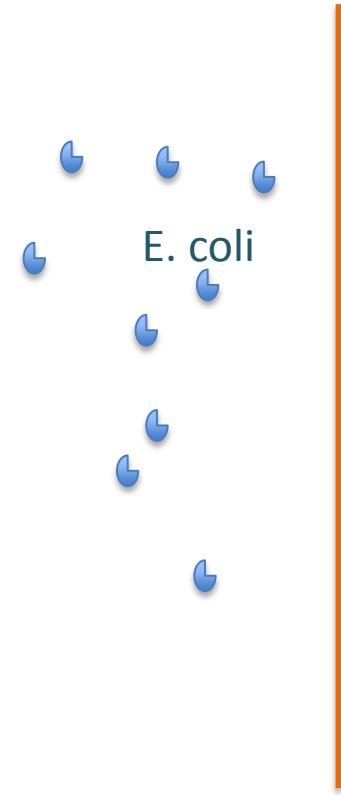
Circulating blood



Lamina propria

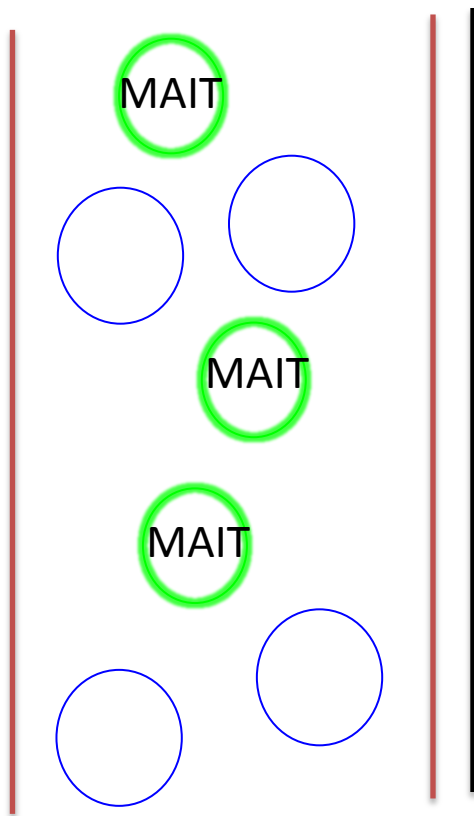


Gut Lumen

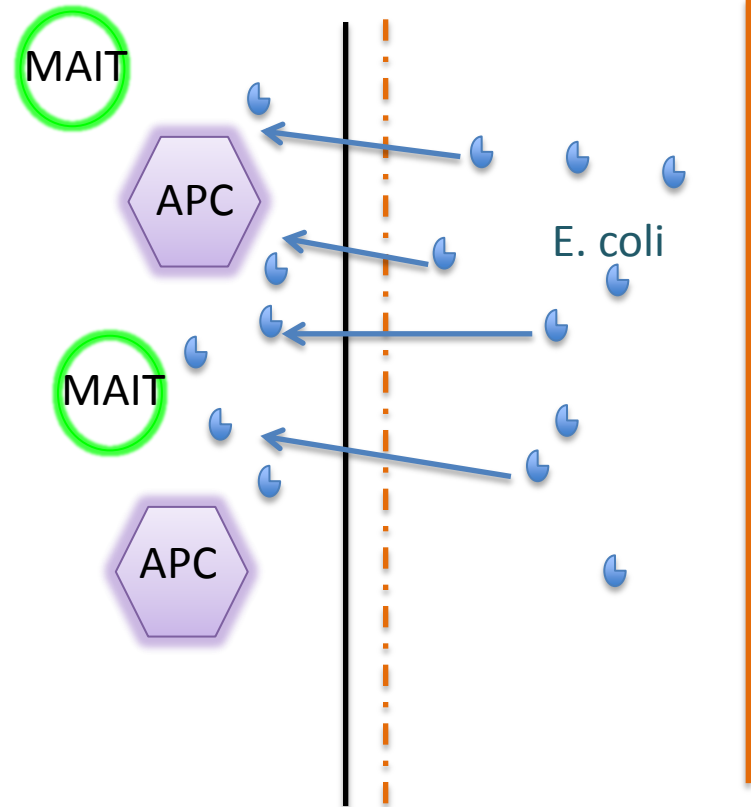


Proposed model for MAIT cell depletion in HIV infection

Circulating blood

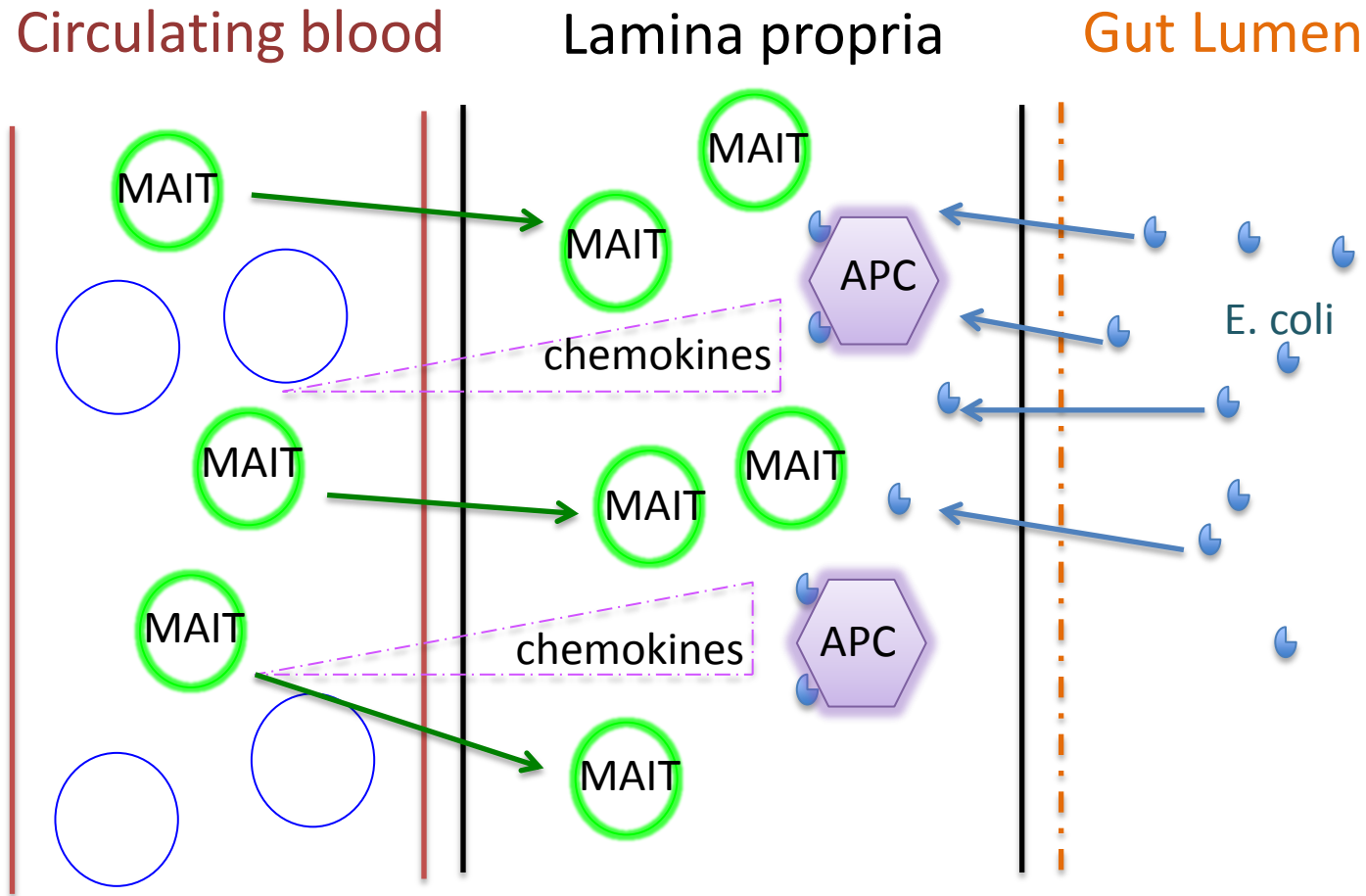


Lamina propria



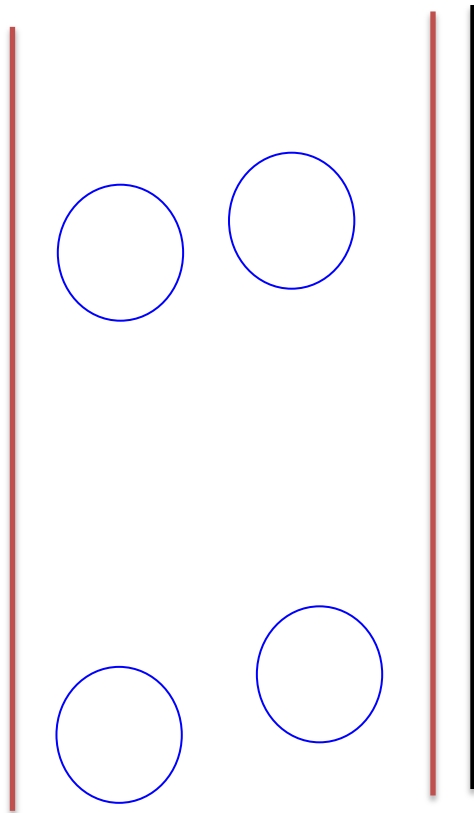
Gut Lumen

Proposed model for MAIT cell depletion in HIV infection

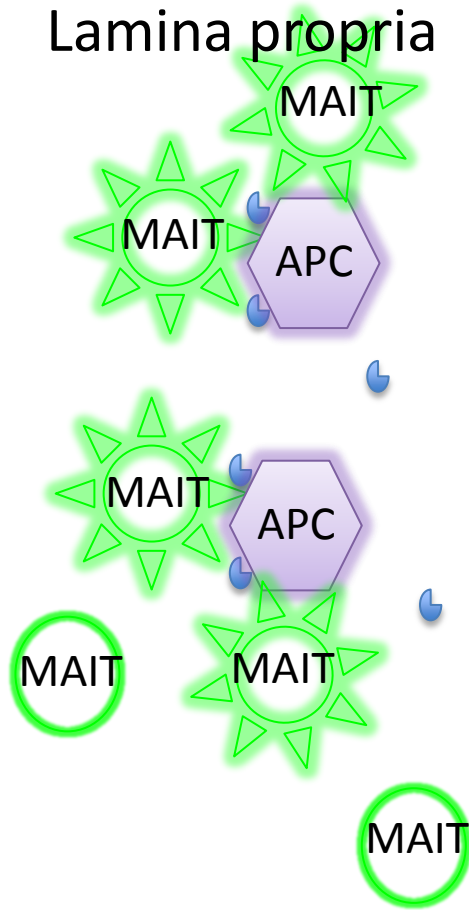


Proposed model for MAIT cell depletion in HIV infection

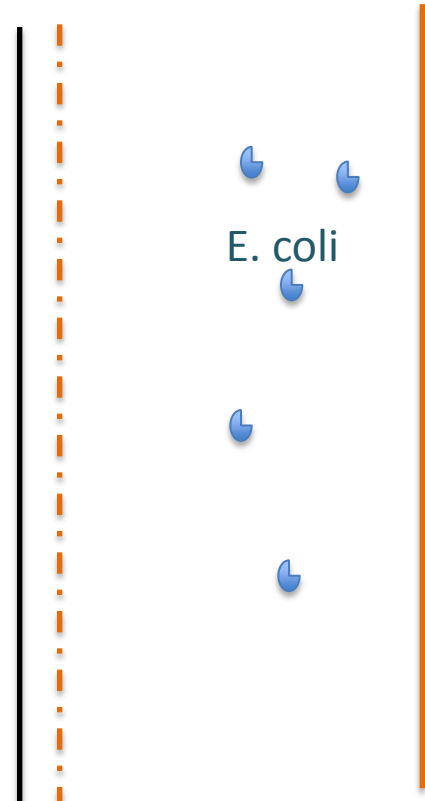
Circulating blood



Lamina propria

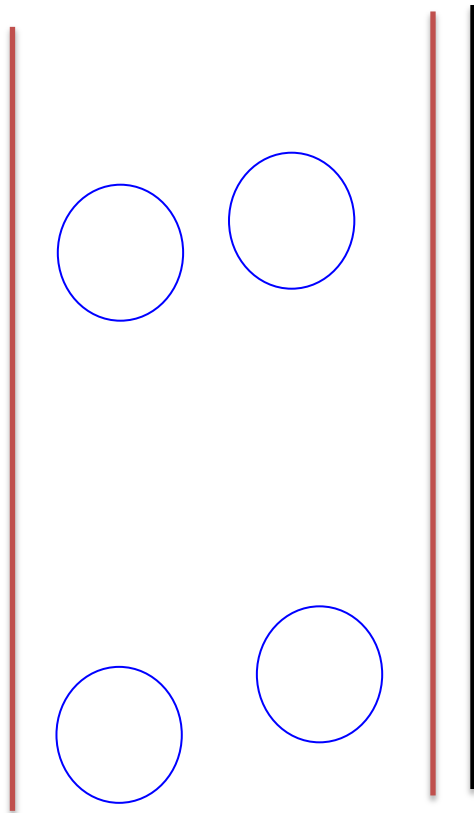


Gut Lumen

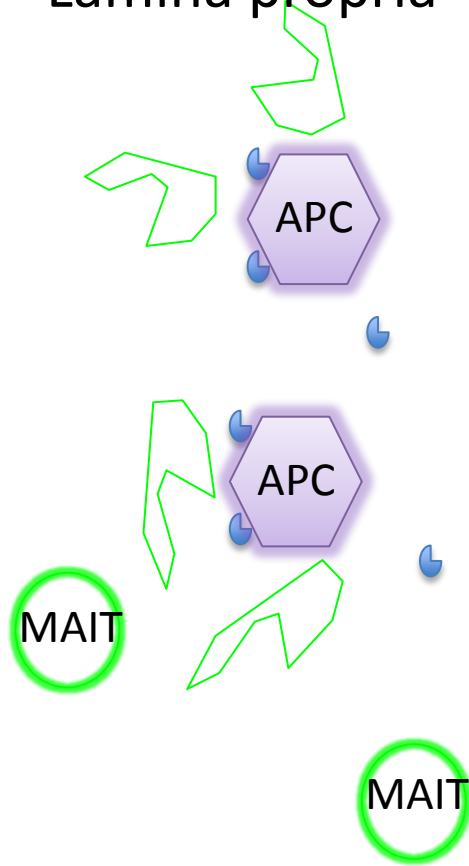


Proposed model for MAIT cell depletion in HIV infection

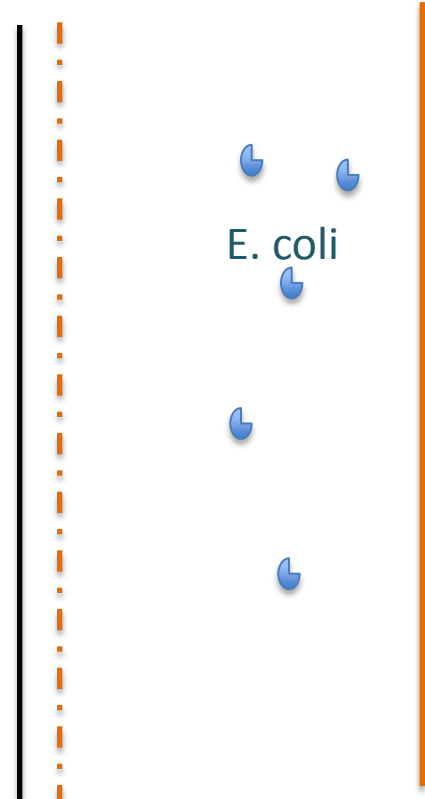
Circulating blood



Lamina propria



Gut Lumen



Summary (1)

- MAIT cells are lost from the blood early in HIV infection
- Not enriched in the colon
- Fail to recover with HAART
- Activation induced cell death potential mechanism of loss
 - Evidence of microbial translocation *in vivo*
 - MR1-dependent cell death *in vitro* following activation by *E. coli*
- Potential implications for control of bacterial infections
 - *Mycobacterium tuberculosis*
 - Non-typhoidal *Salmonella* spp.
 - Invasive pneumococcal disease
- Reconstitution potential therapeutic target