



# T cell activation and memory formation

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# Topics

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**Differentiation of antiviral effector T cells**

**Stability & plasticity of memory T cell subsets**

**Quantitative cytokine memory of individual T cells**

**Function of quantitative memory & plasticity in inflammation**

# Topics

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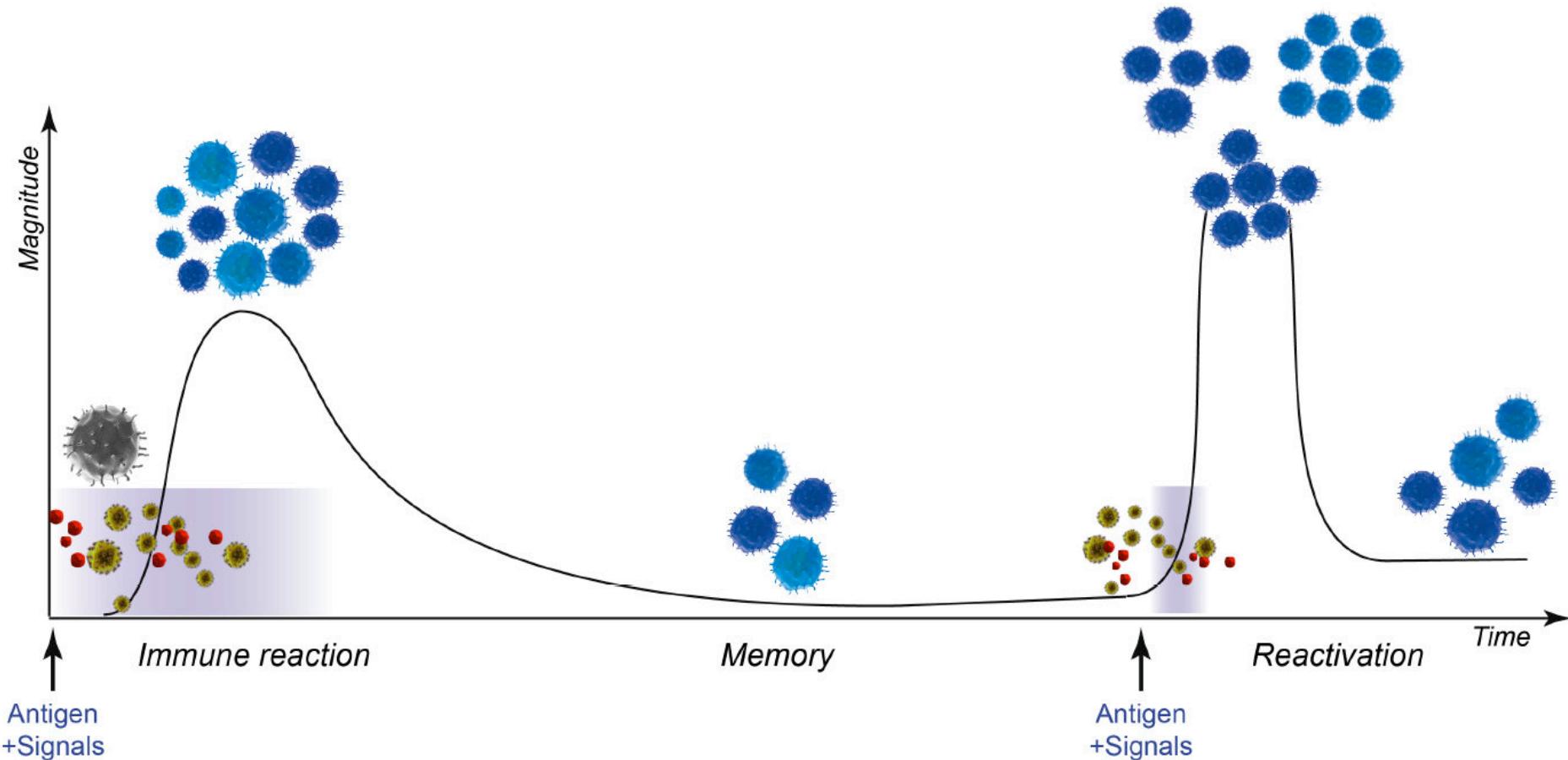
**Differentiation of antiviral effector T cells**

**Stability & plasticity of memory T cell subsets**

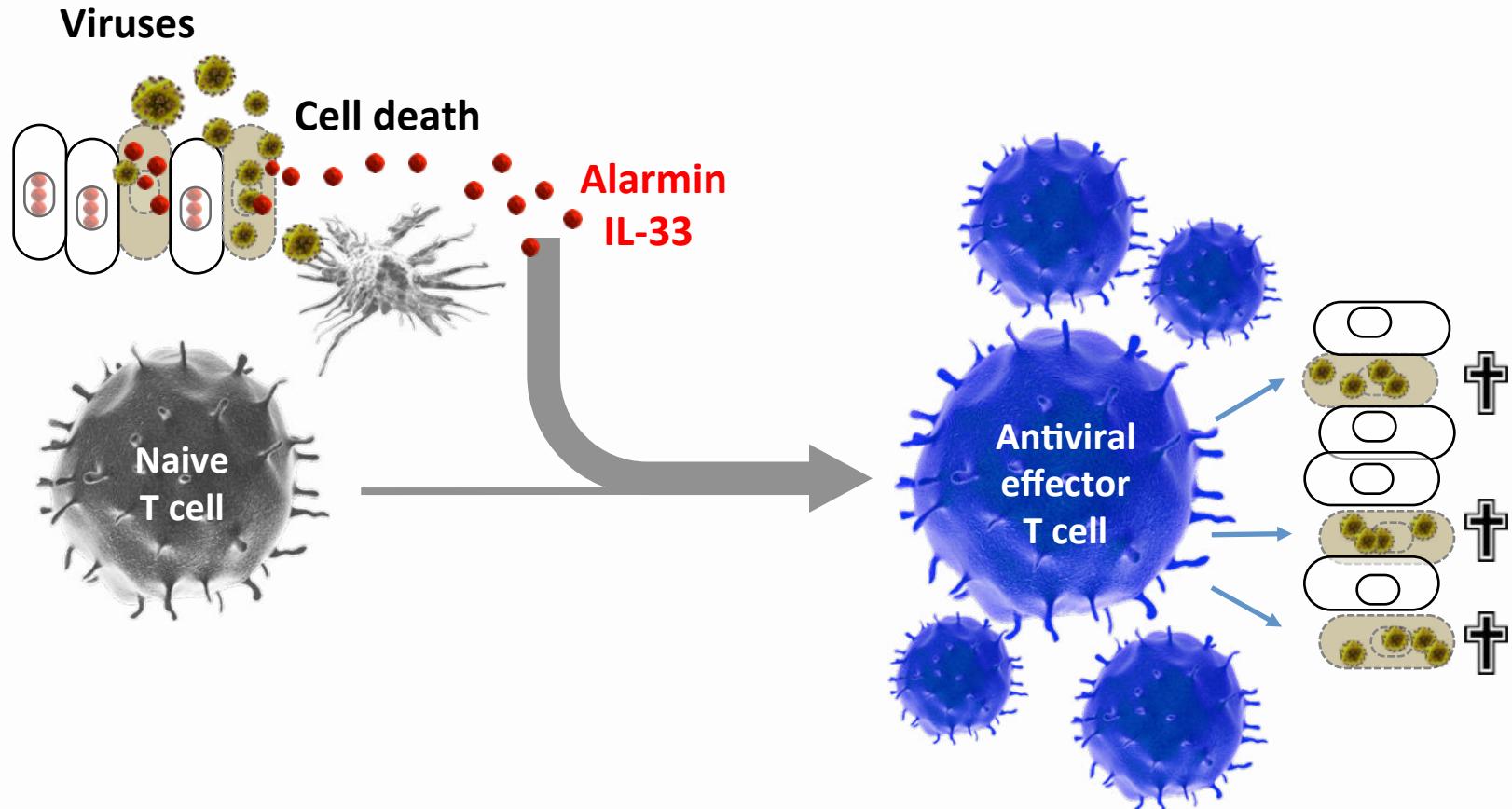
**Quantitative cytokine memory of individual T cells**

**Function of quantitative memory & plasticity in inflammation**

# Effector & memory T cell differentiation

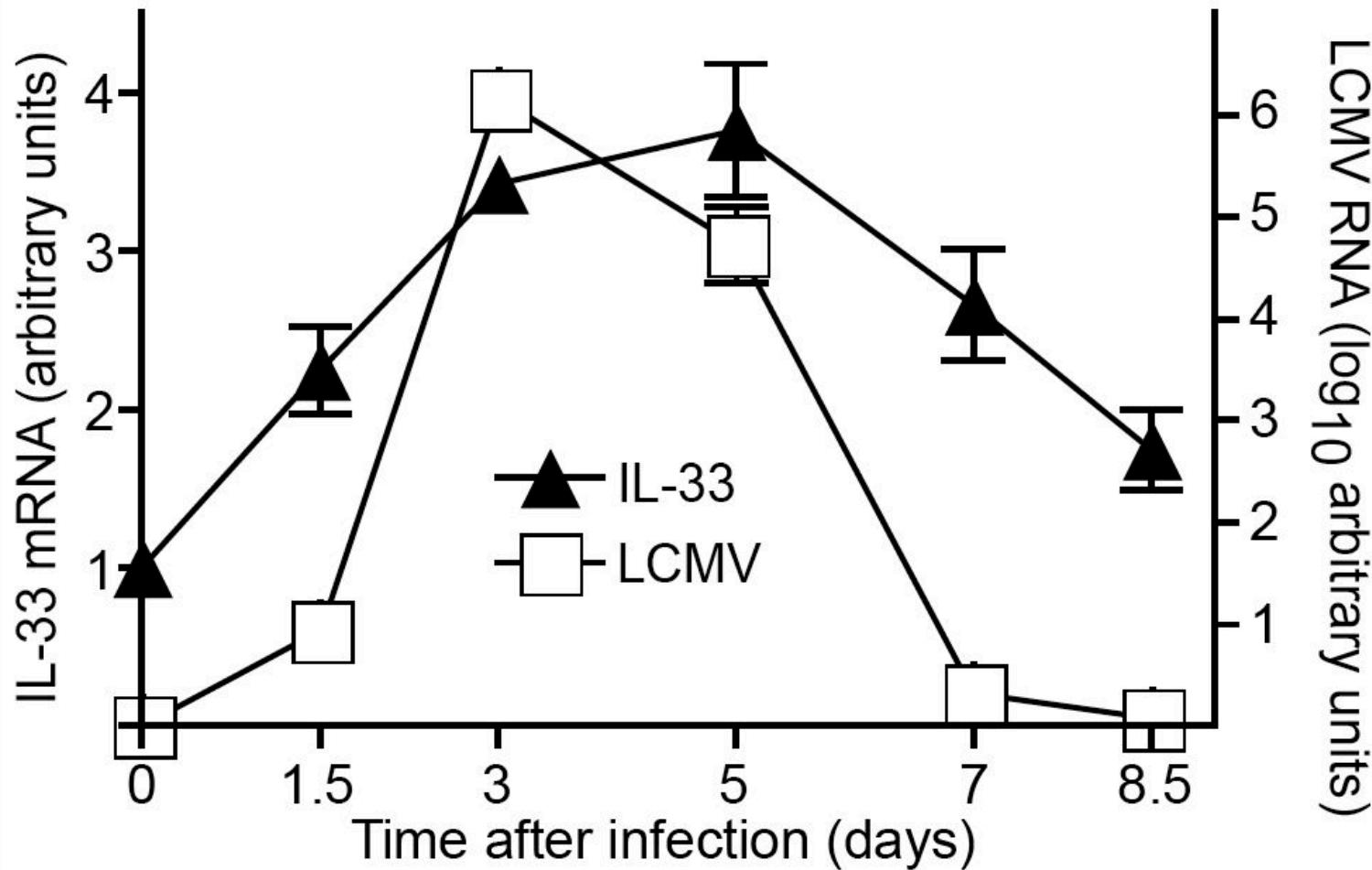


# T cell activation: Enhancement by alarm signals from virus-infected cells?

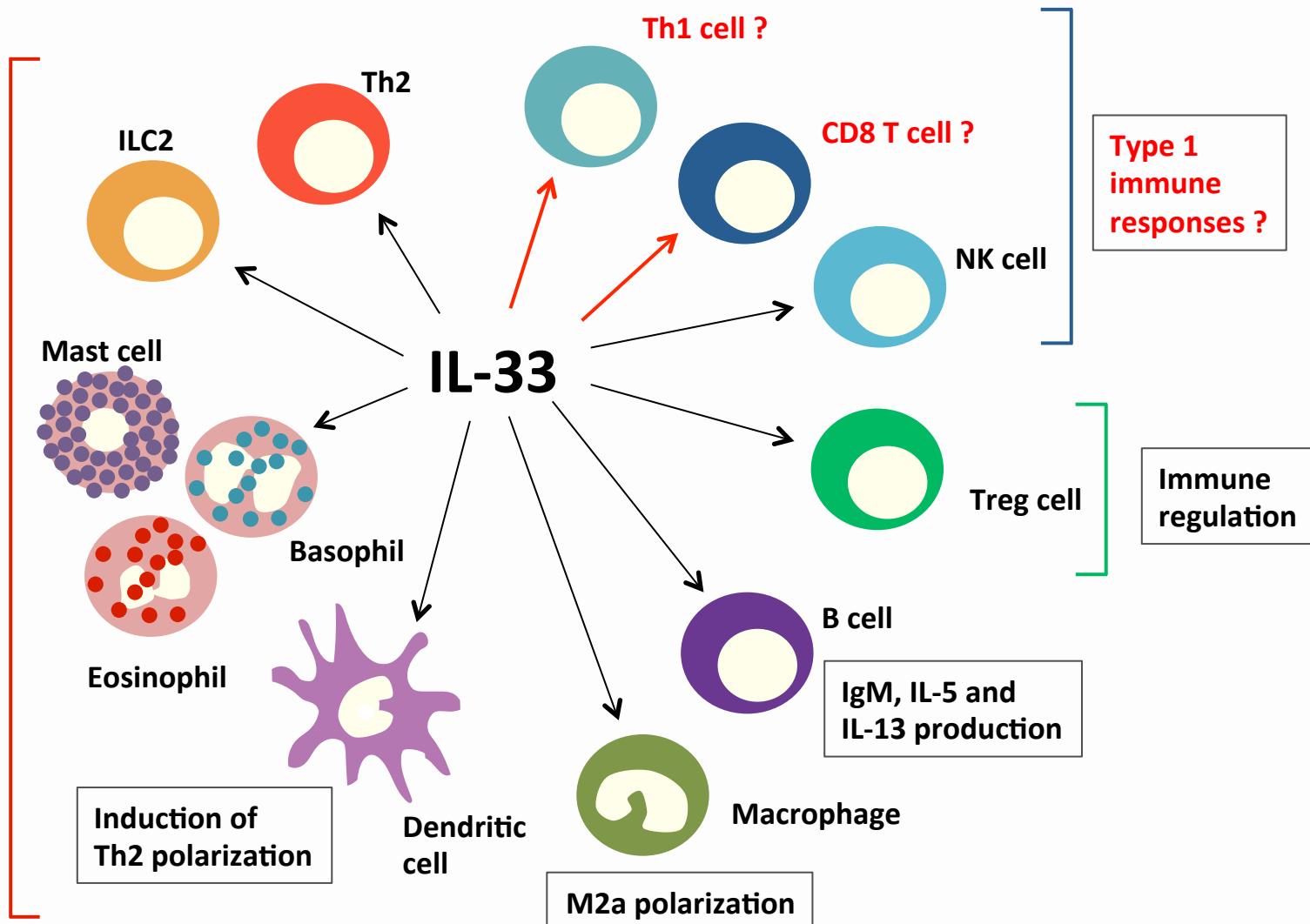


# Viral infection enhances alarmin expression: IL-33

Lymphocytic choriomeningitis virus (LCMV), 200 pfu, spleen analysis

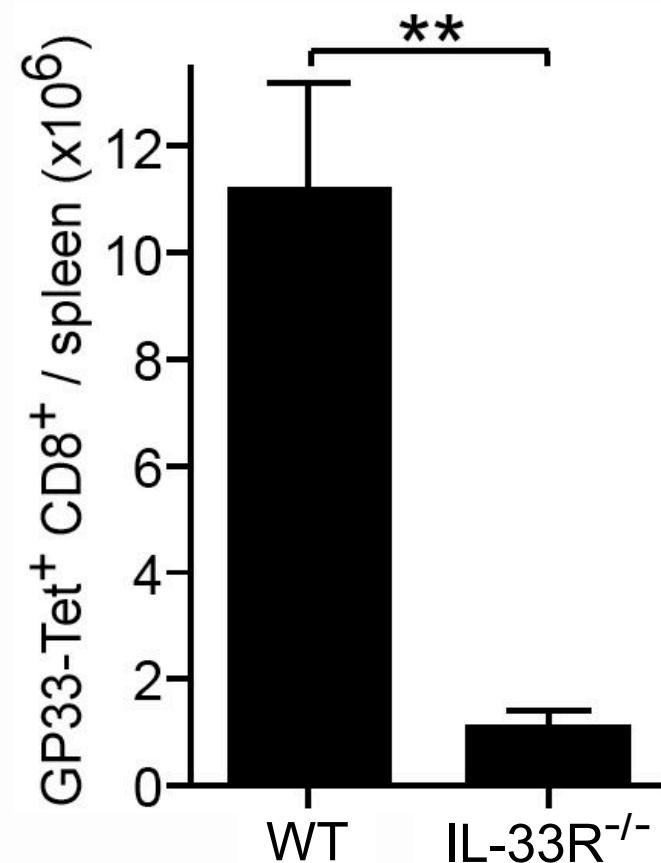
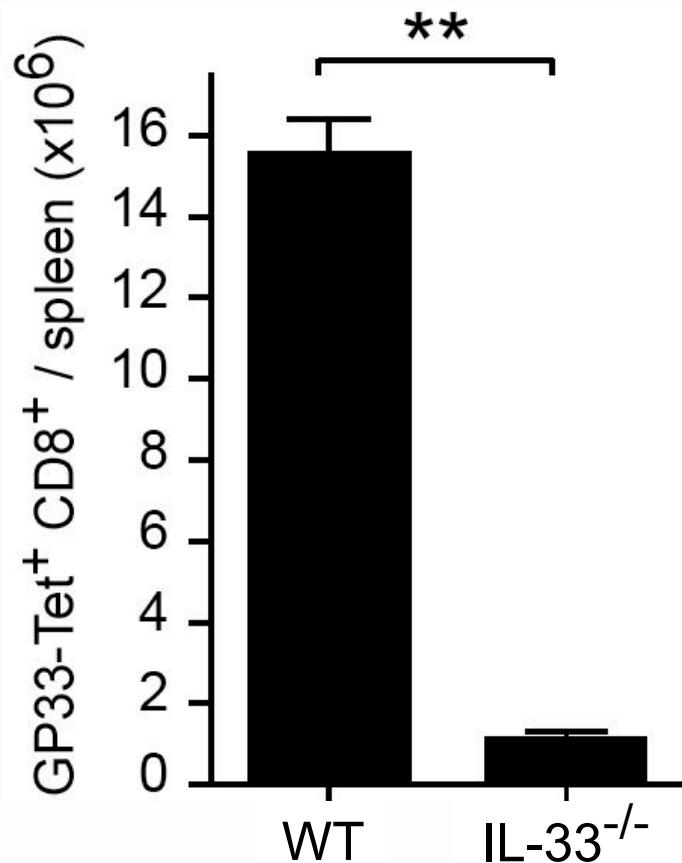


# IL-33 acts on diverse cell types: Enhancement of type 1 immune responses?



# IL-33 signals enhance antiviral CD8<sup>+</sup> T cell responses

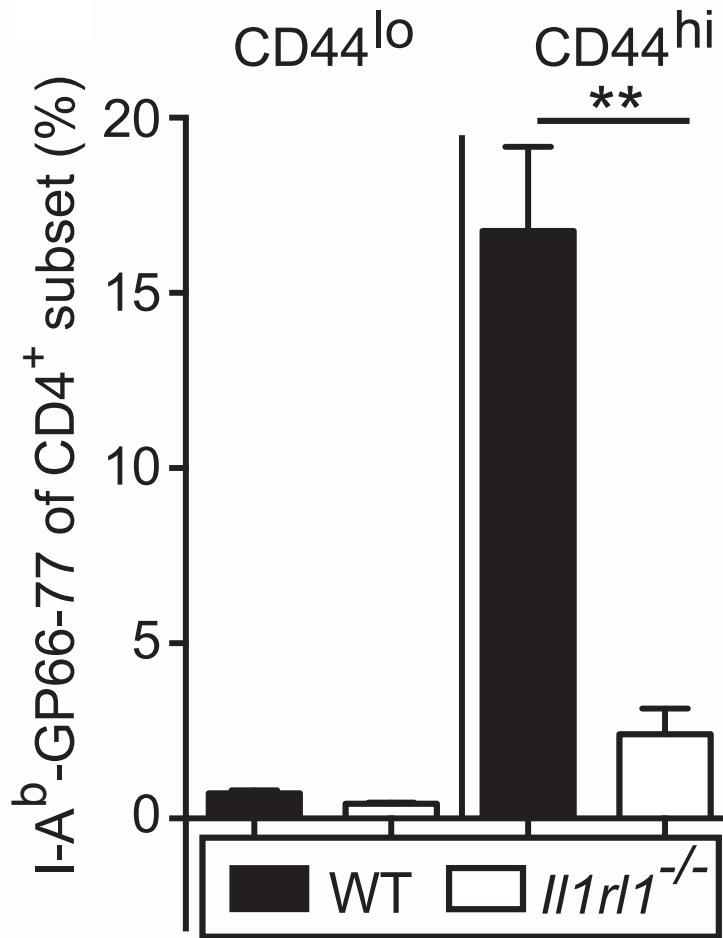
LCMV-WE, 200 pfu, spleen d8



Bonilla\*, Fröhlich\* et al. *Science* 2012

# IL-33 signals enhance antiviral CD4<sup>+</sup> T helper cell responses

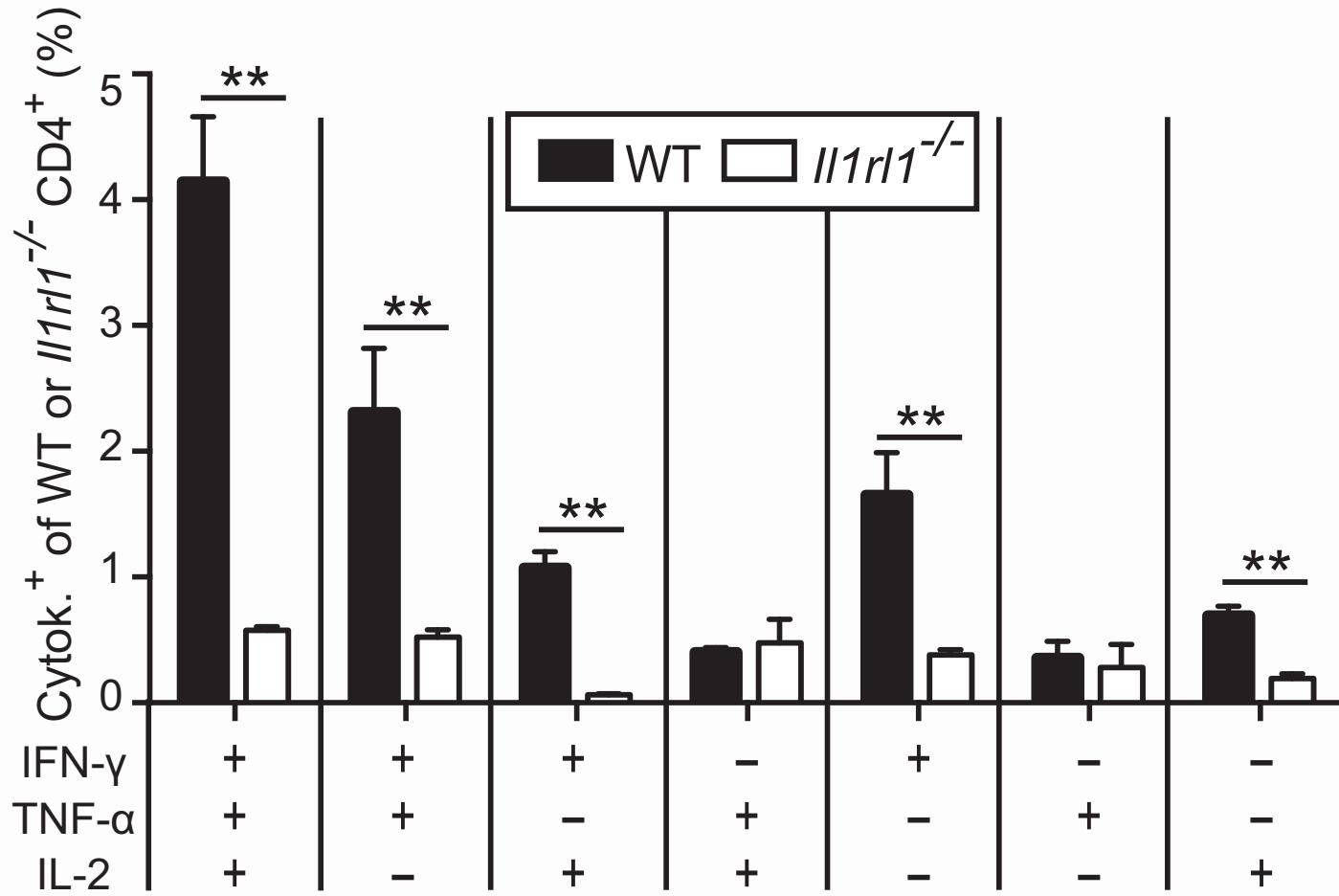
WT + IL-33R<sup>-/-</sup> (= *Il1rl1*<sup>-/-</sup>) BM chimeras, LCMV-WE, 200 pfu, spleen d9



Baumann\*, Bonilla\* et al. PNAS 2015

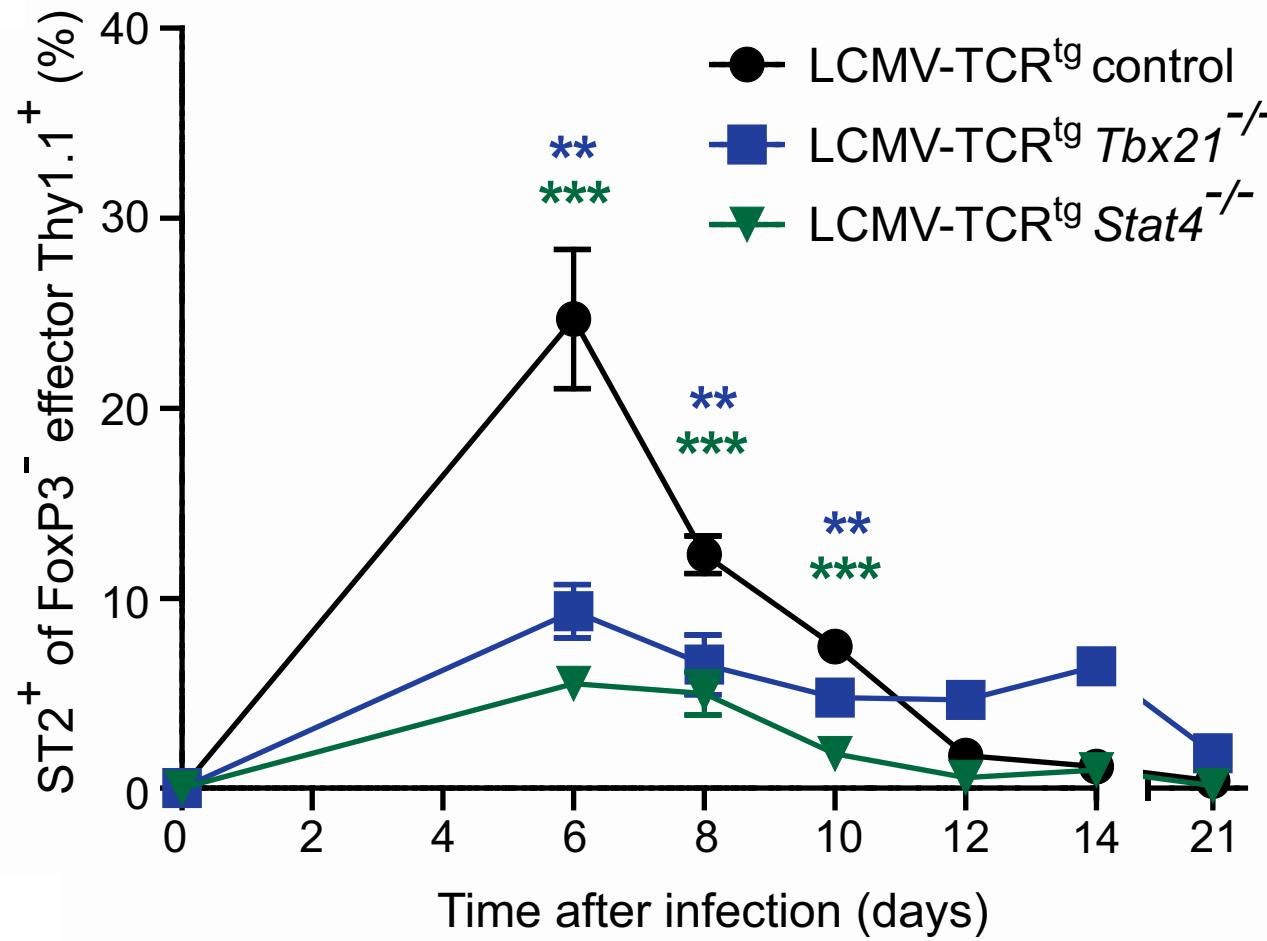
# IL-33 signals enhance polyfunctional Th1 cell responses

WT + IL-33R<sup>-/-</sup> (= *Il1rl1*<sup>-/-</sup>) BM chimeras, LCMV-WE, 200 pfu, spleen d9



# T-bet & STAT4 control transient IL-33R expression on Th1 cells

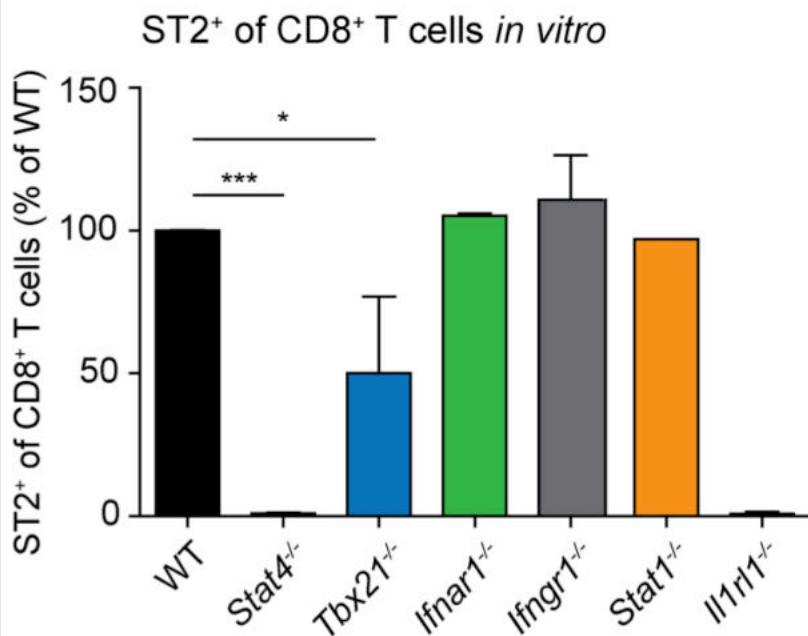
Transfer of naive LCMV-TCR<sup>tg</sup> CD4<sup>+</sup> T cells into WT mice, LCMV-WE, 200 pfu



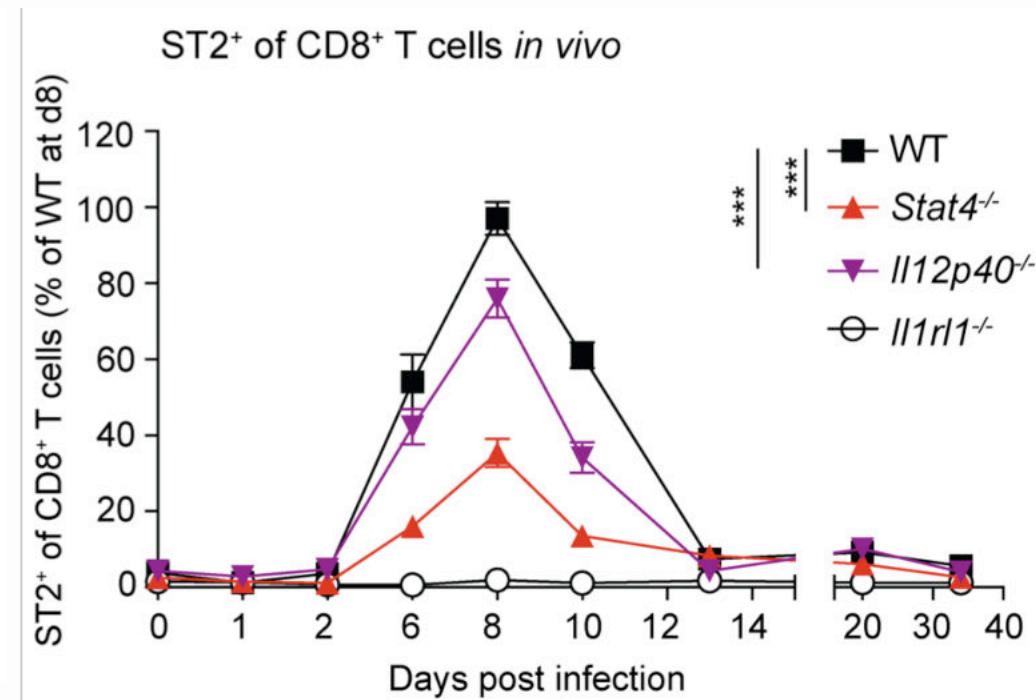
Baumann\*, Bonilla\* et al. PNAS 2015

# STAT4 controls transient IL-33R expression on CD8<sup>+</sup> T cells

Naive CD8<sup>+</sup> T cells,  
anti-CD3/CD28 + APC + IL-12, d5

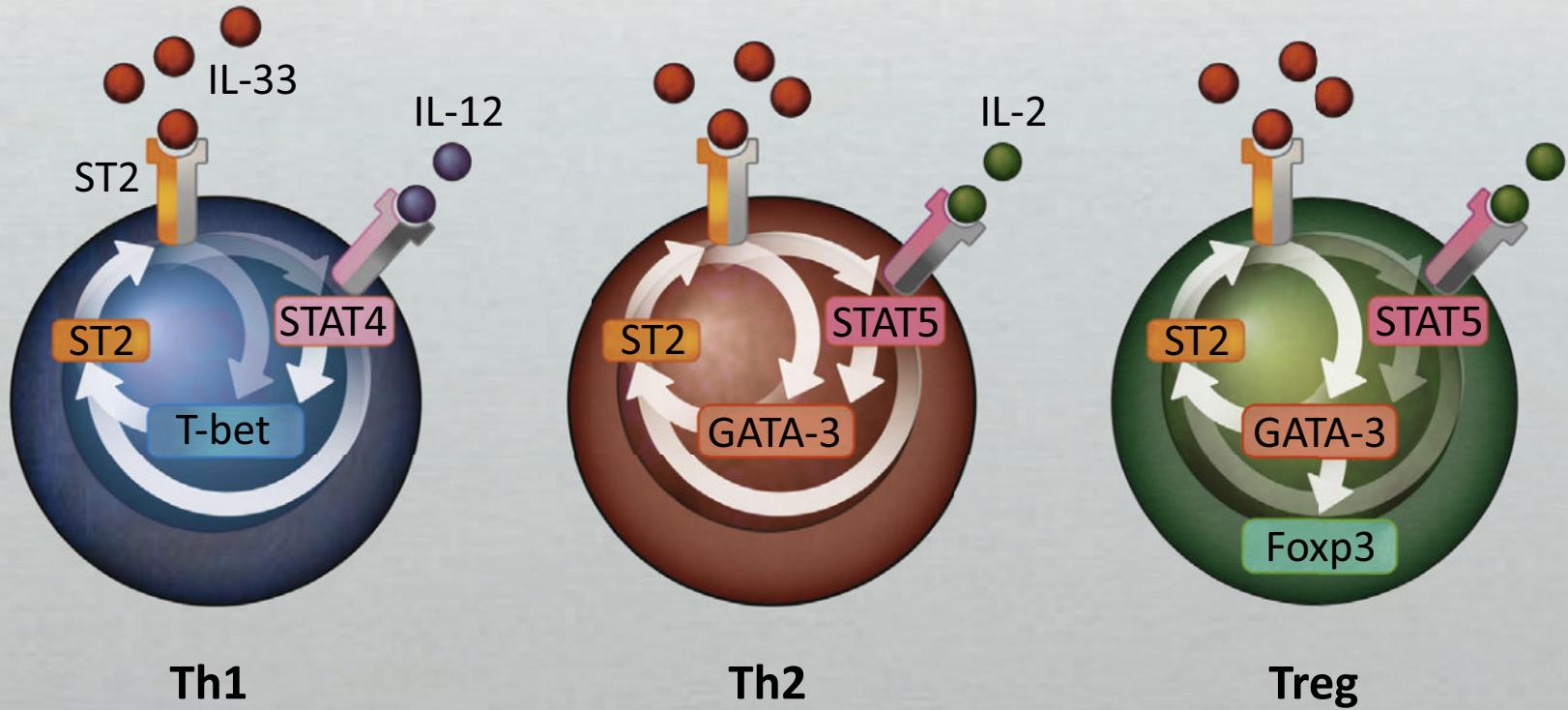


LCMV-WE, 200 pfu



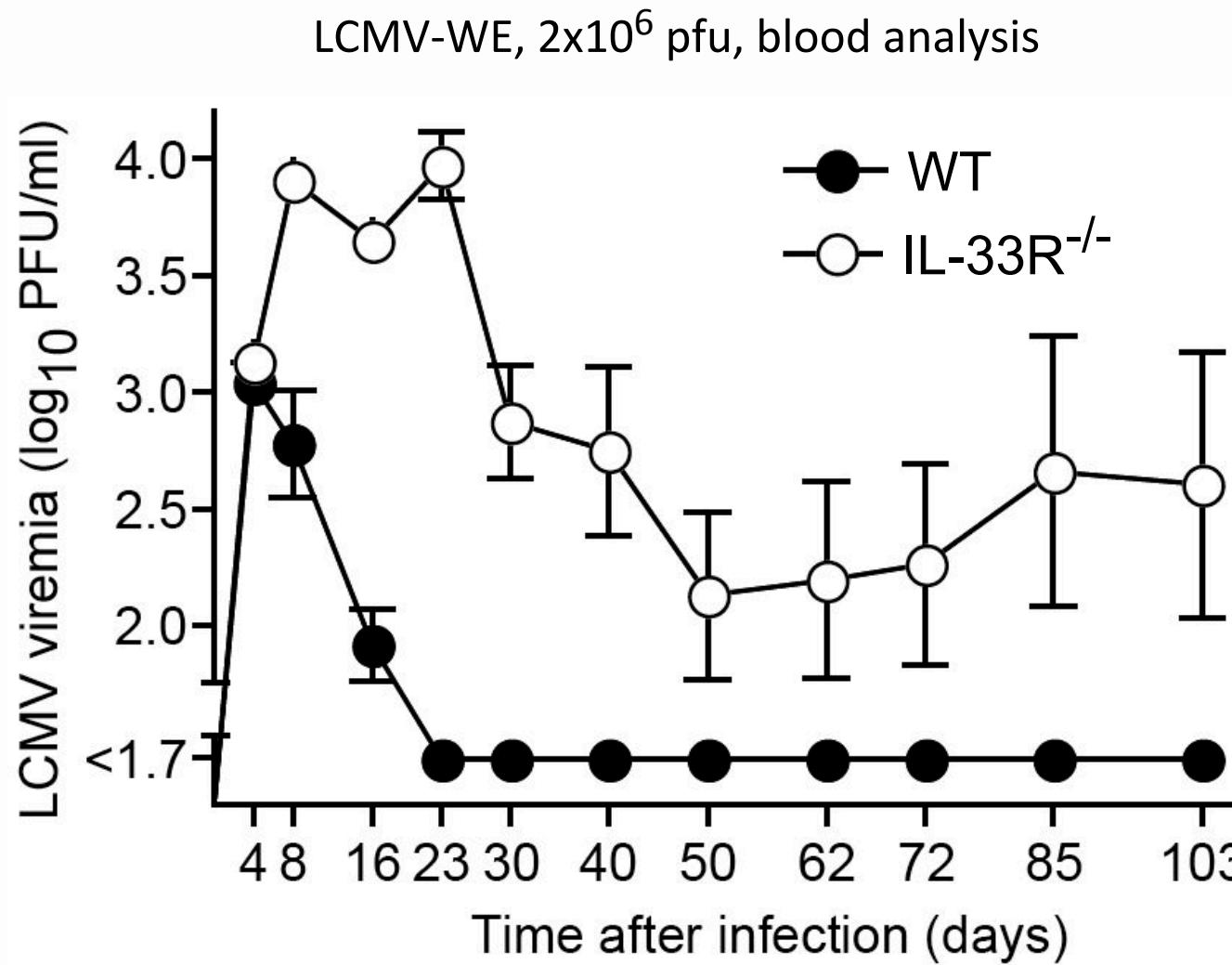
Baumann\*, Fröhlich\* et al. *Front. Immunol.* 2019

# Positive feedback loops link ST2 with key transcription factors



Peine, Marek, Löhning. *Trends Immunol.* 2016

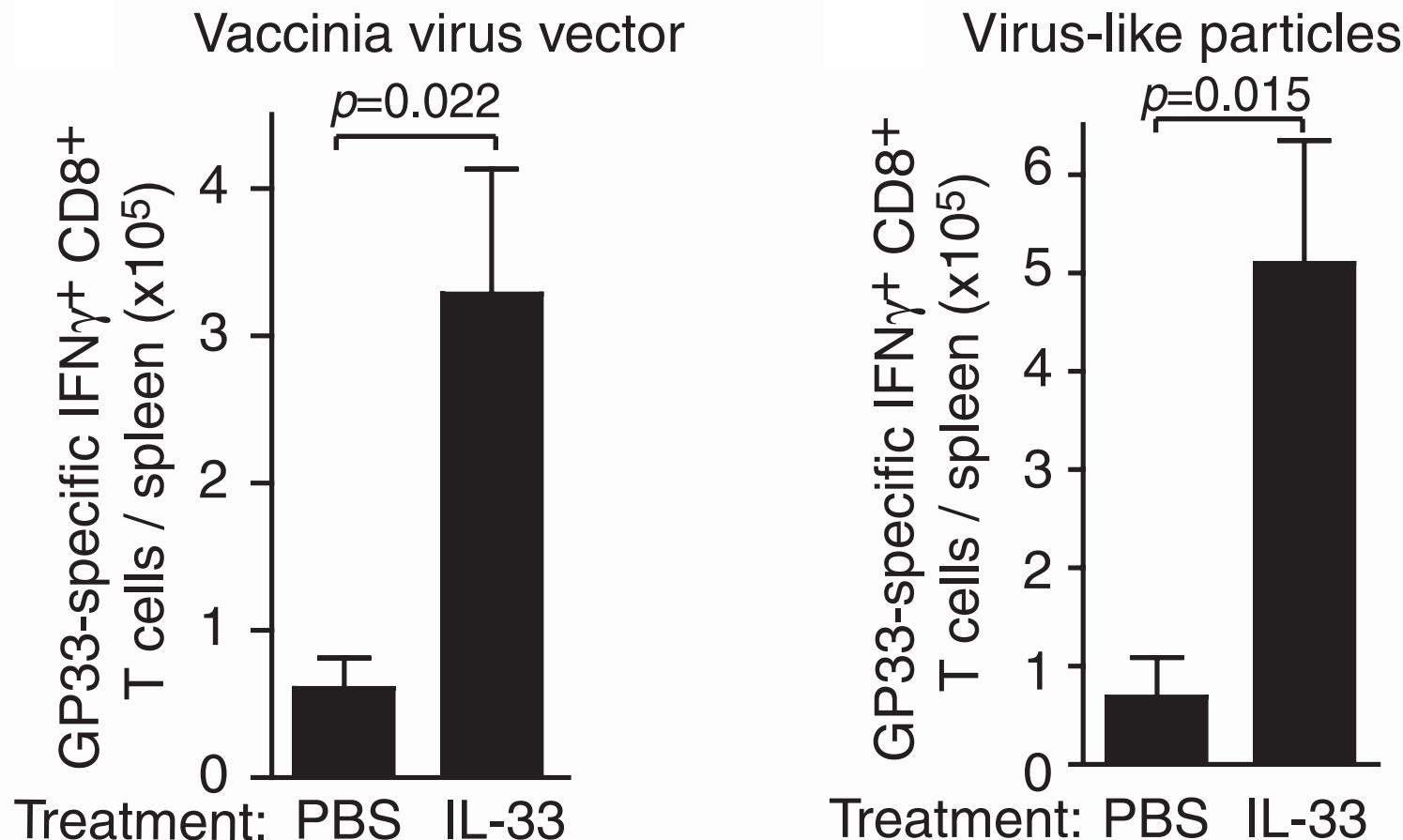
# IL-33 signals drive protective antiviral CD8<sup>+</sup> T cell responses



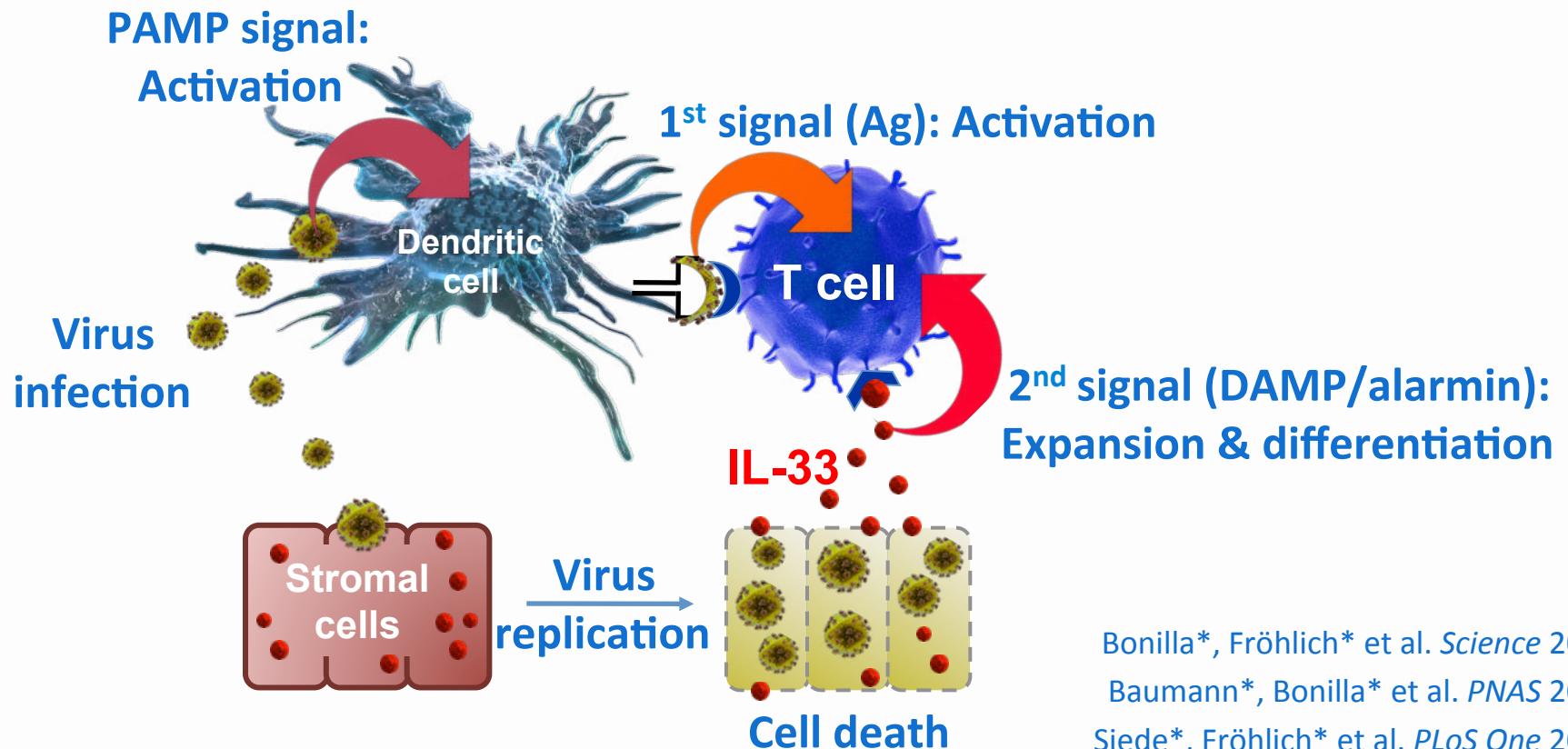
Bonilla\*, Fröhlich\* et al. *Science* 2012

# IL-33 treatment enhances CD8<sup>+</sup> T cell responses

Vaccination with rec. VV vector expressing LCMV-GP or with GP33-carrying VLPs,  
IL-33 treatment: 4 µg i.p. on d1-7 daily, spleen analysis d8



# Virus-infected cells “alarm” T cells by IL-33 release: Induction of protective CD8<sup>+</sup> and CD4<sup>+</sup> effector T cells



Bonilla\*, Fröhlich\* et al. *Science* 2012

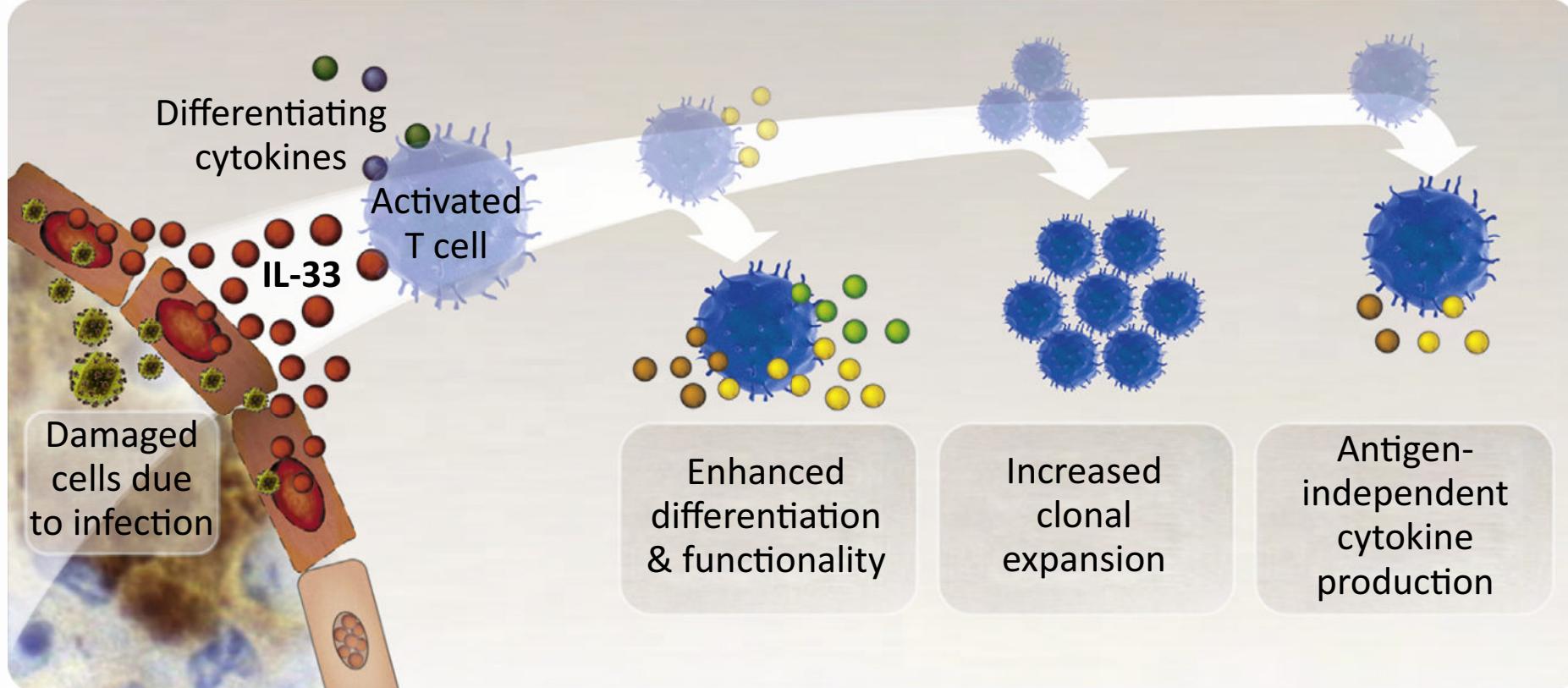
Baumann\*, Bonilla\* et al. *PNAS* 2015

Siede\*, Fröhlich\* et al. *PLoS One* 2016

Peine, Marek, Löhning. *Trends Immunol.* 2016

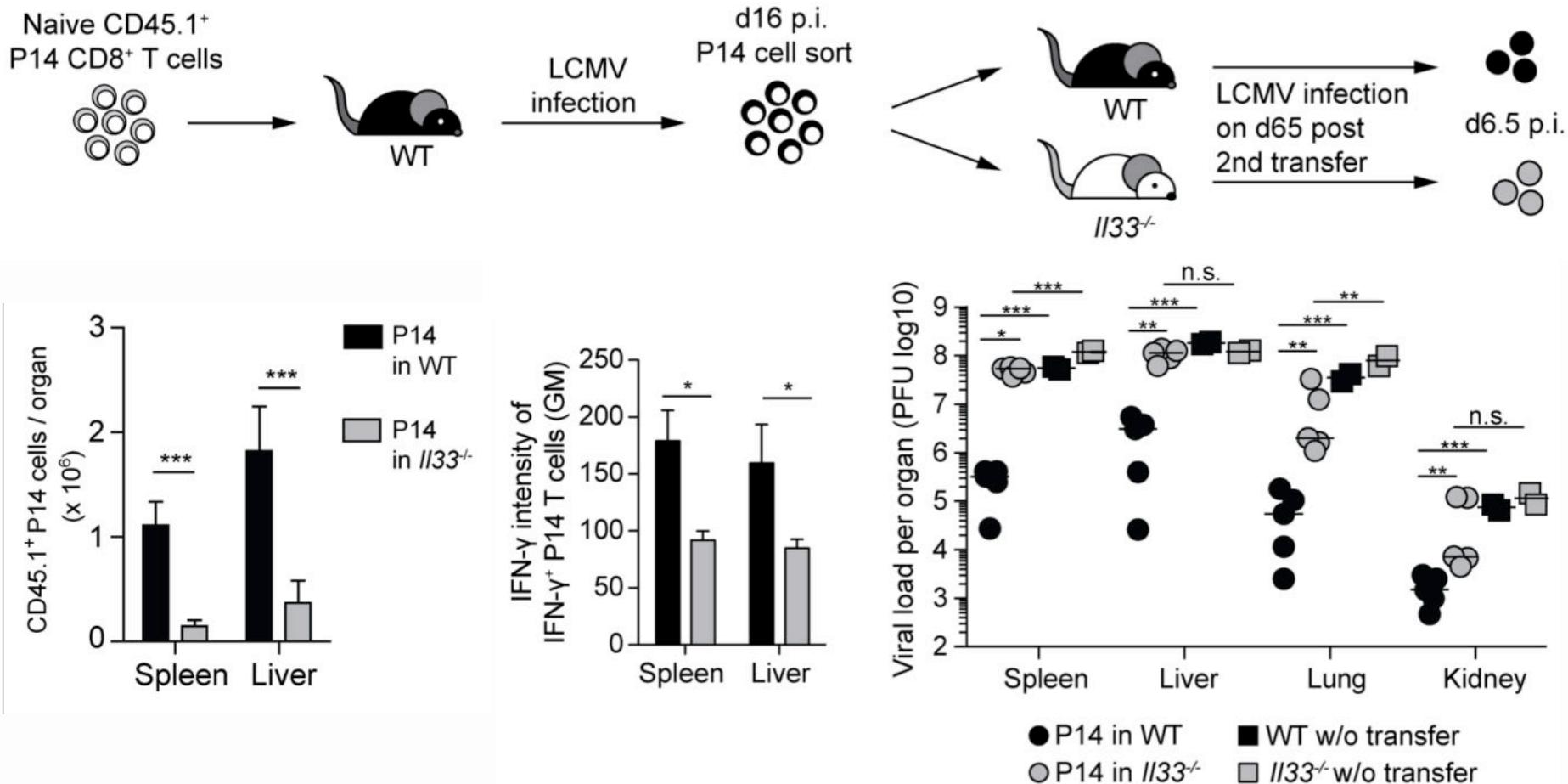
Baumann\*, Fröhlich\* et al. *Front. Immunol.* 2019

# IL-33 enhances T cell differentiation and functionality



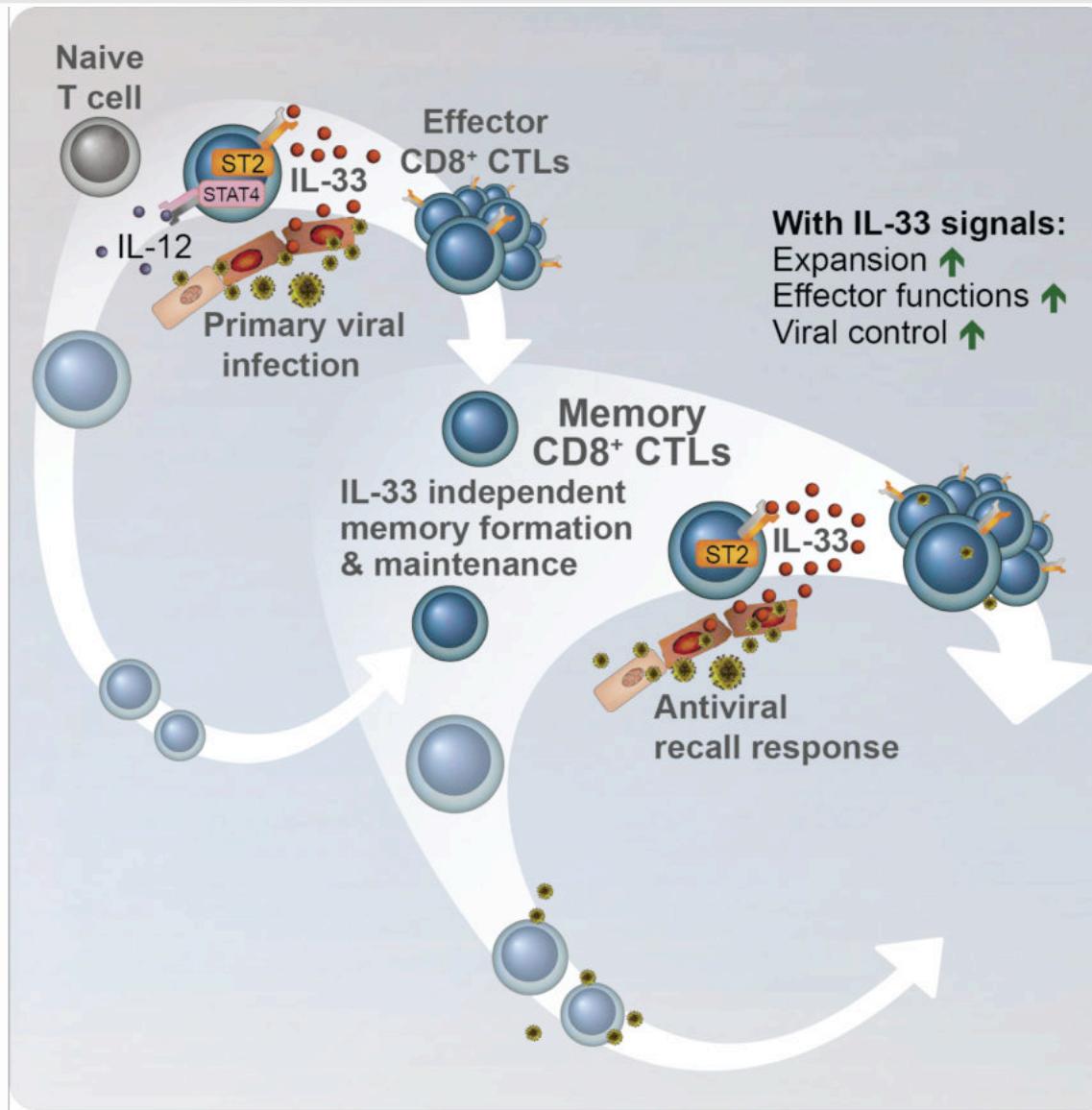
Peine, Marek, Löhning. *Trends Immunol.* 2016

# Memory CD8<sup>+</sup> T cells require IL-33 for protective recall response



Baumann\*, Fröhlich\* et al. *Front. Immunol.* 2019

# Memory CD8<sup>+</sup> T cells require IL-33 for protective recall response



Baumann\*, Fröhlich\* et al.  
*Front. Immunol.* 2019

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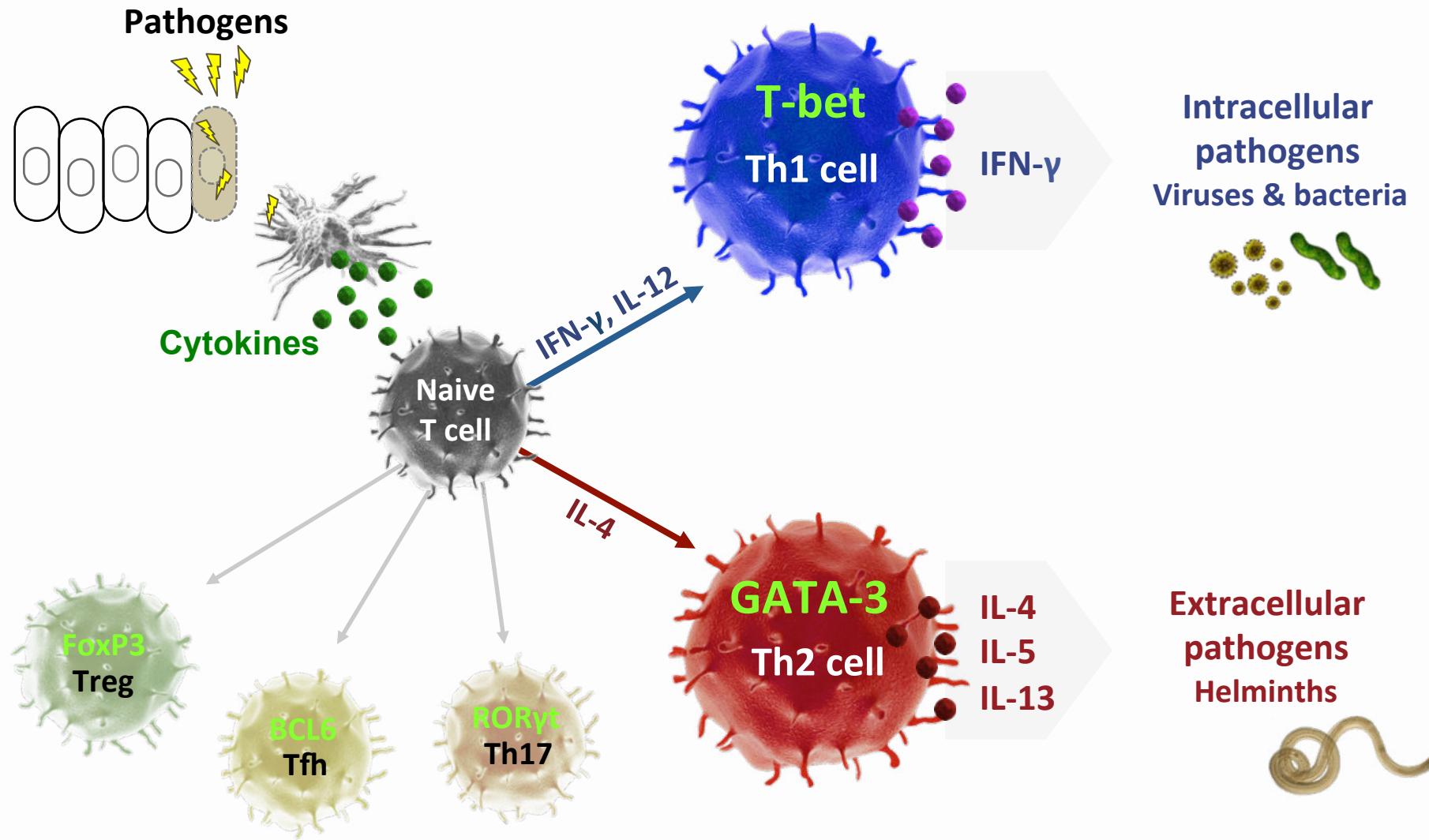
Differentiation of antiviral effector T cells

**Stability & plasticity of memory T cell subsets**

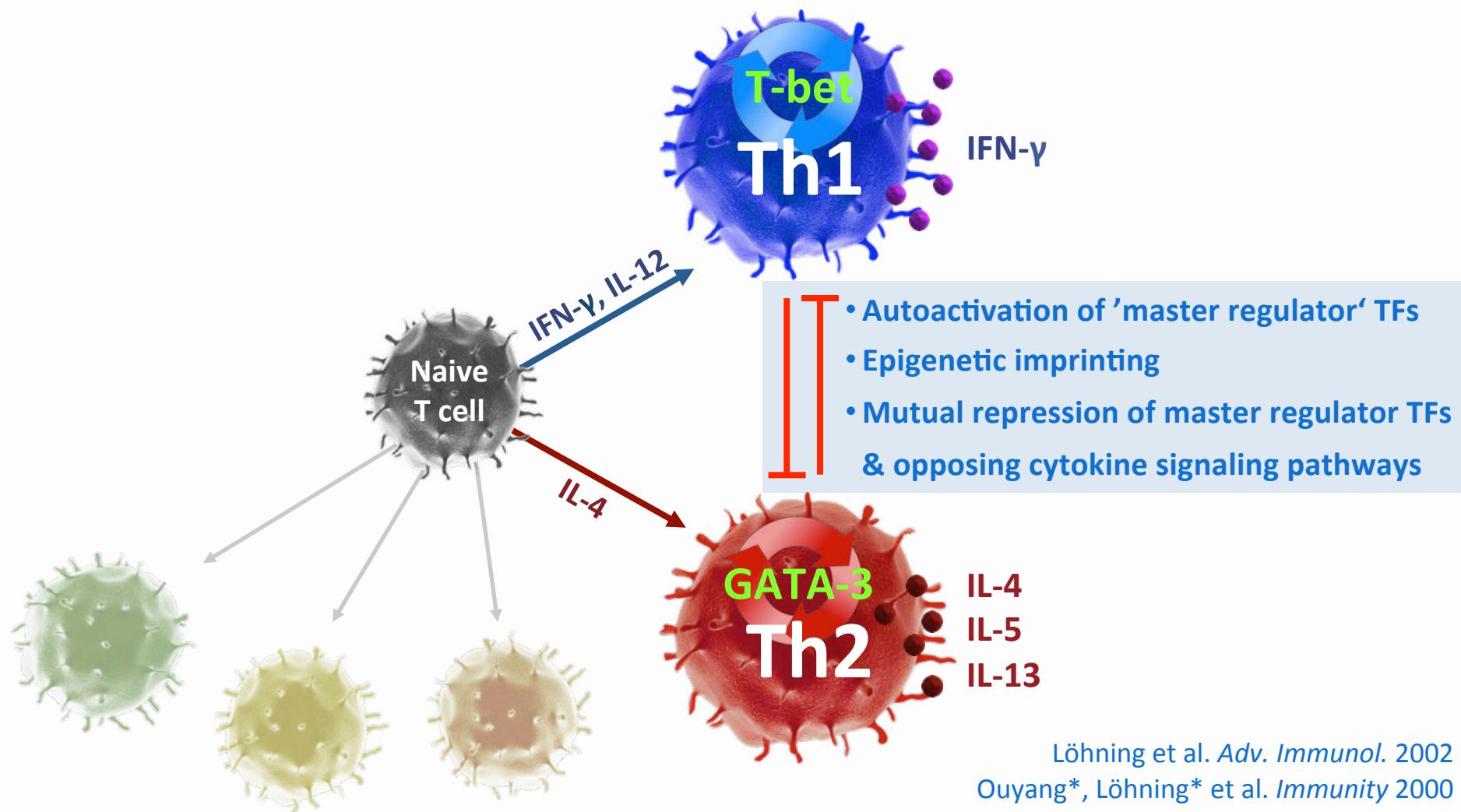
Quantitative cytokine memory of individual T cells

Function of quantitative memory & plasticity in inflammation

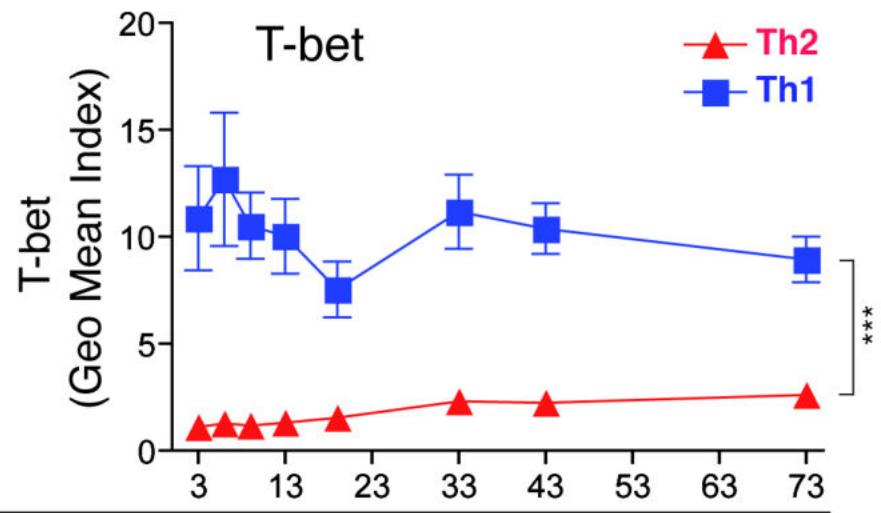
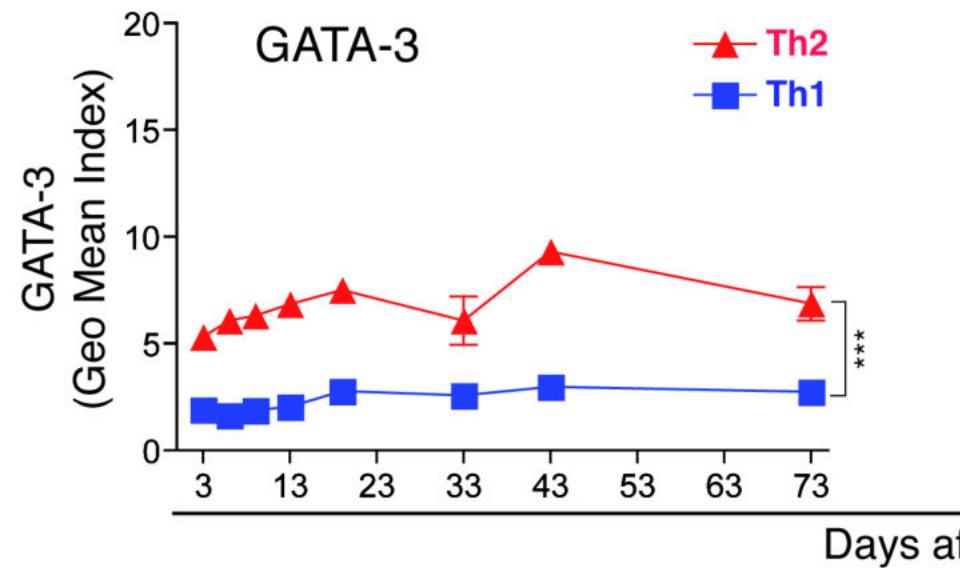
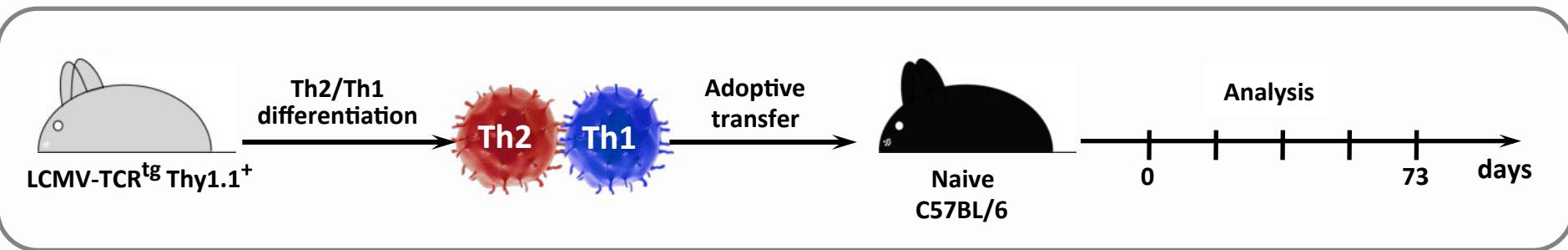
# T helper cell differentiation is directed by pathogen-triggered cytokines



# Positive feedback & mutual inhibition have been thought to achieve mutually exclusive Th1 vs Th2 lineage commitment

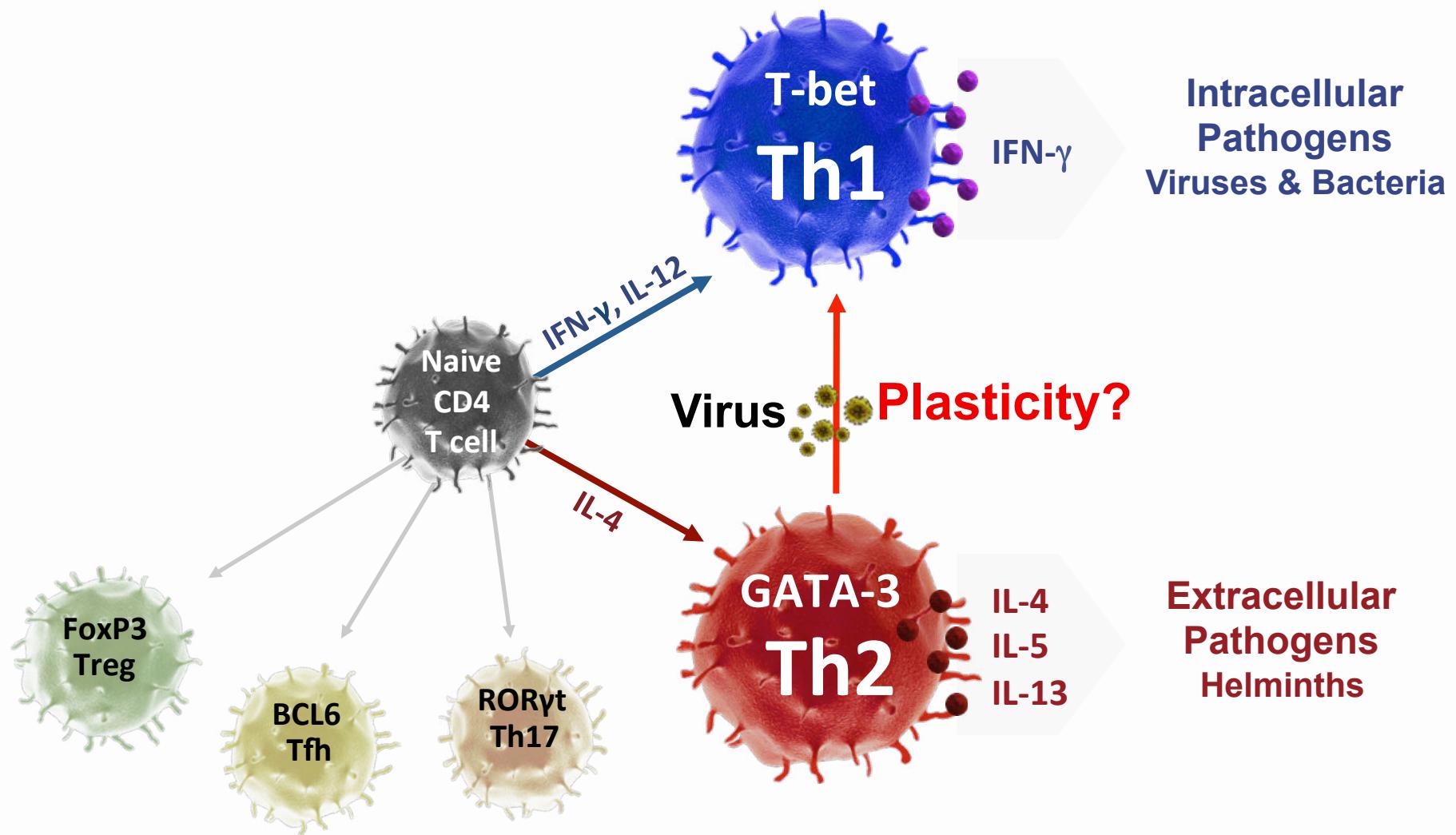


# Stable GATA-3 or T-bet expression in Th2 & Th1 memory cells



Hegazy et al. *Immunity* 2010

# Th2 cell plasticity in viral infections?

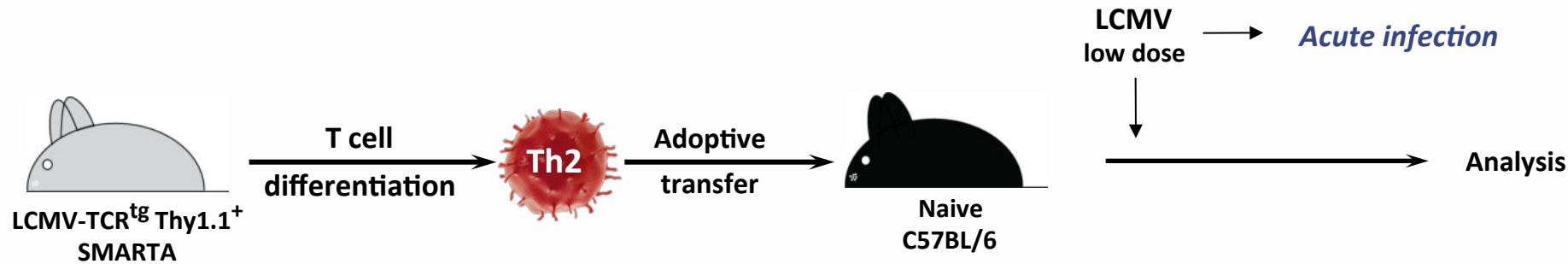


# Th2 cell plasticity in viral infection?

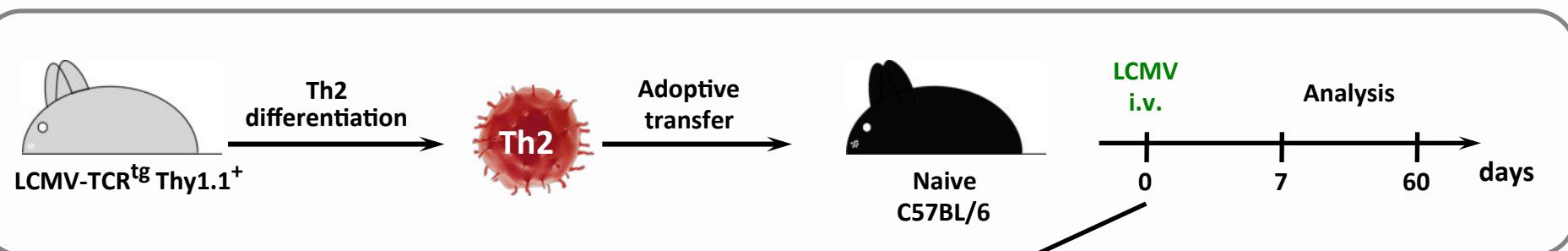
## Lymphocytic choriomeningitis virus (LCMV):

- Noncytopathic ssRNA virus → disease reflects T cell-driven immunopathology
- Strong Th1 & CTL responses

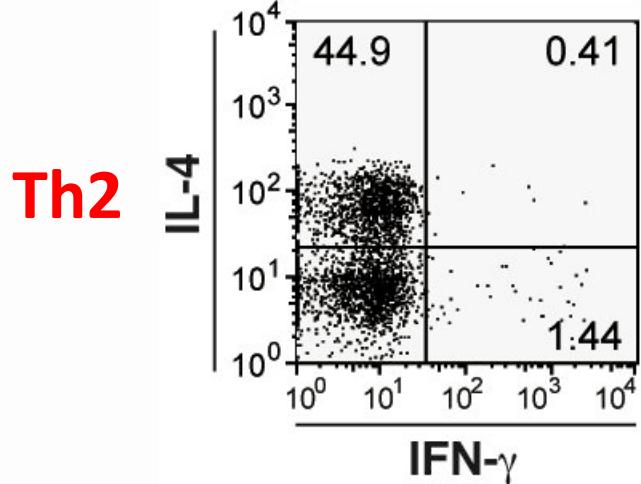
### Animal model



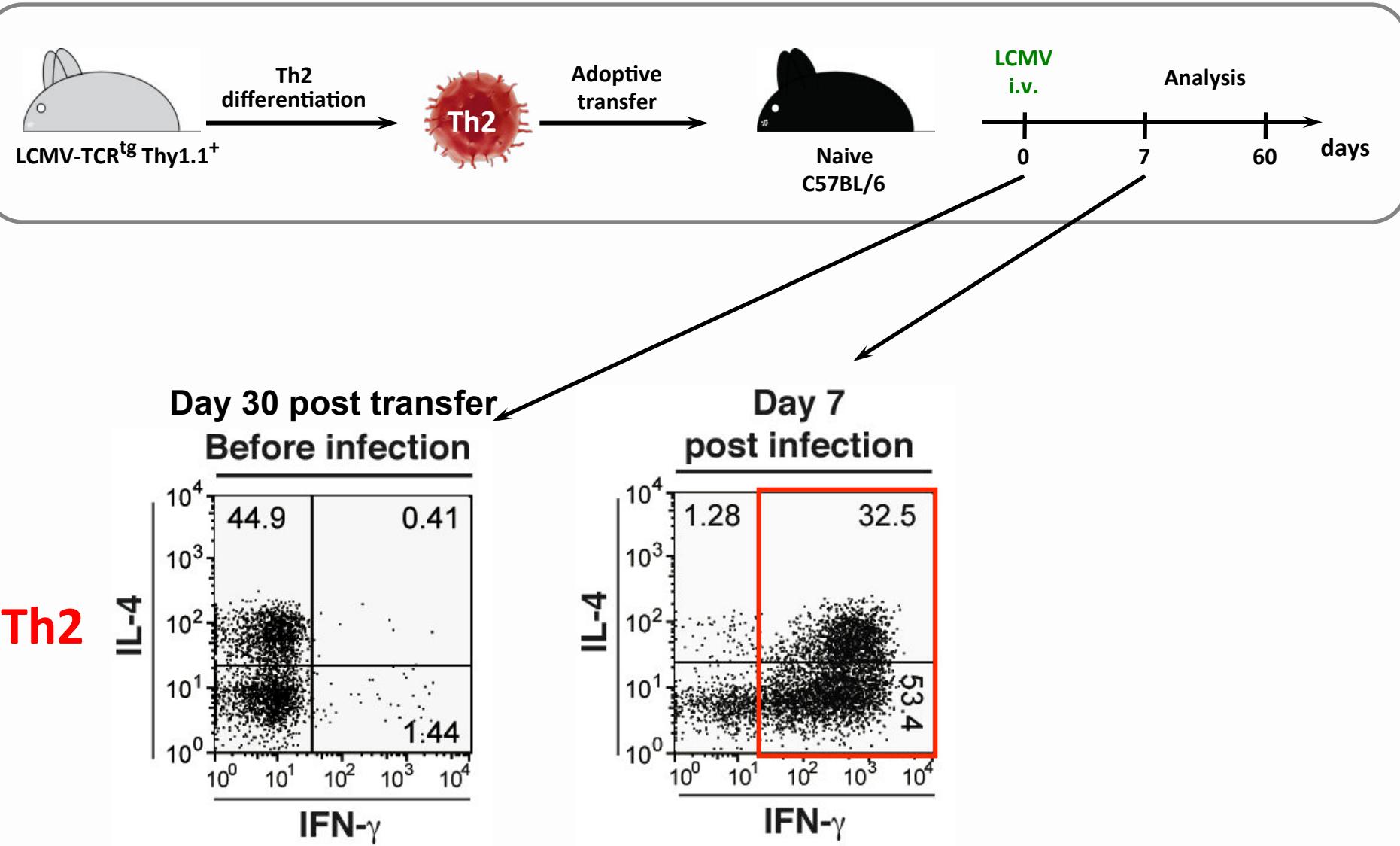
# Stability of Th2 cytokine expression prior to viral infection



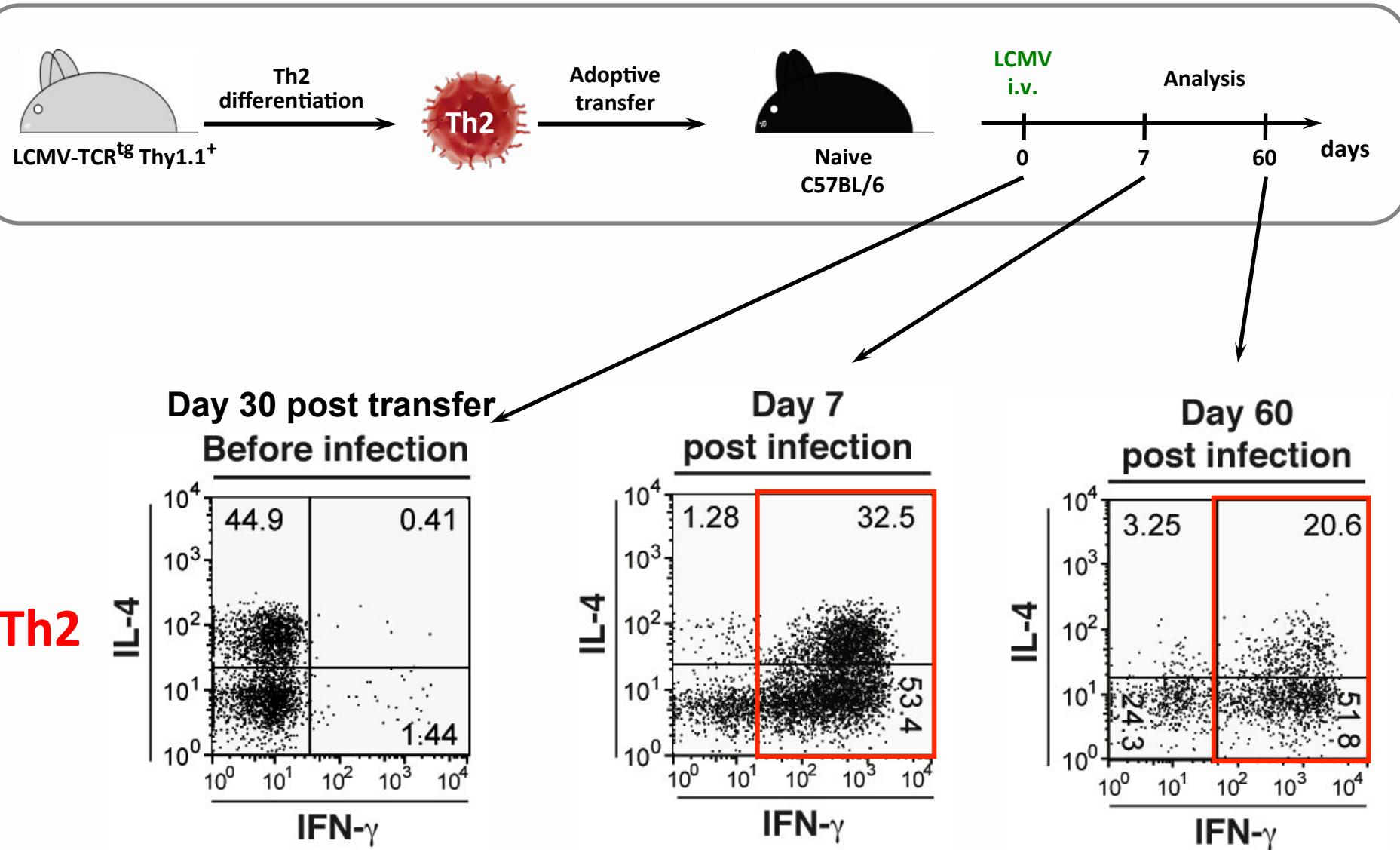
Day 30 post transfer  
Before infection



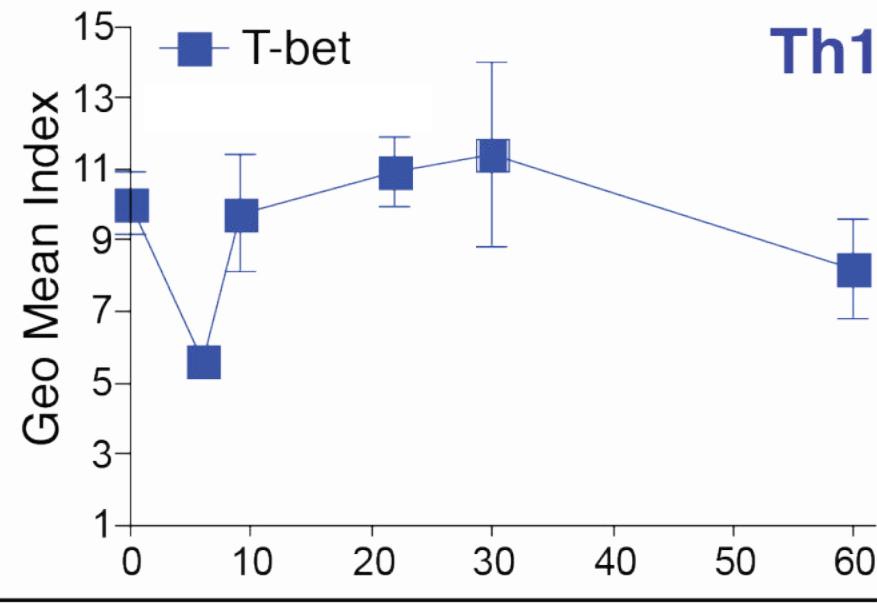
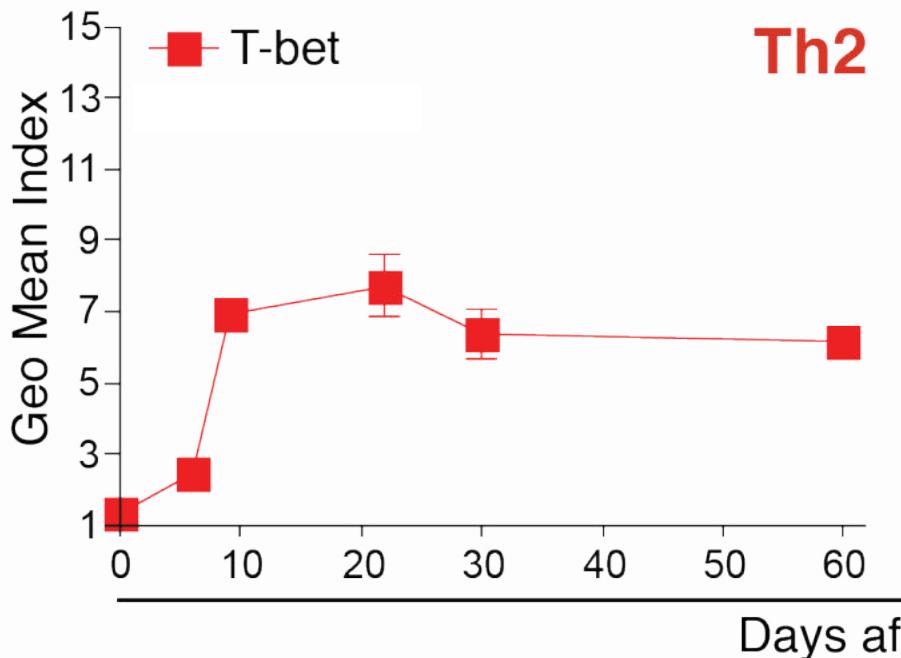
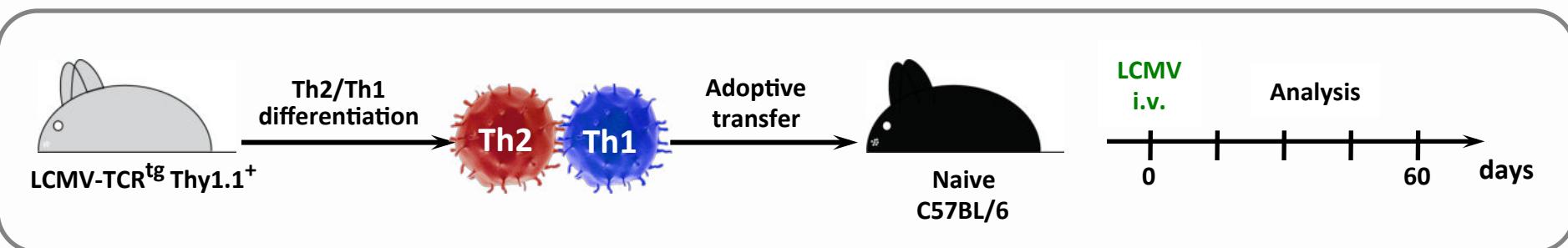
# Th2 cells acquire IFN- $\gamma$ expression upon viral infection



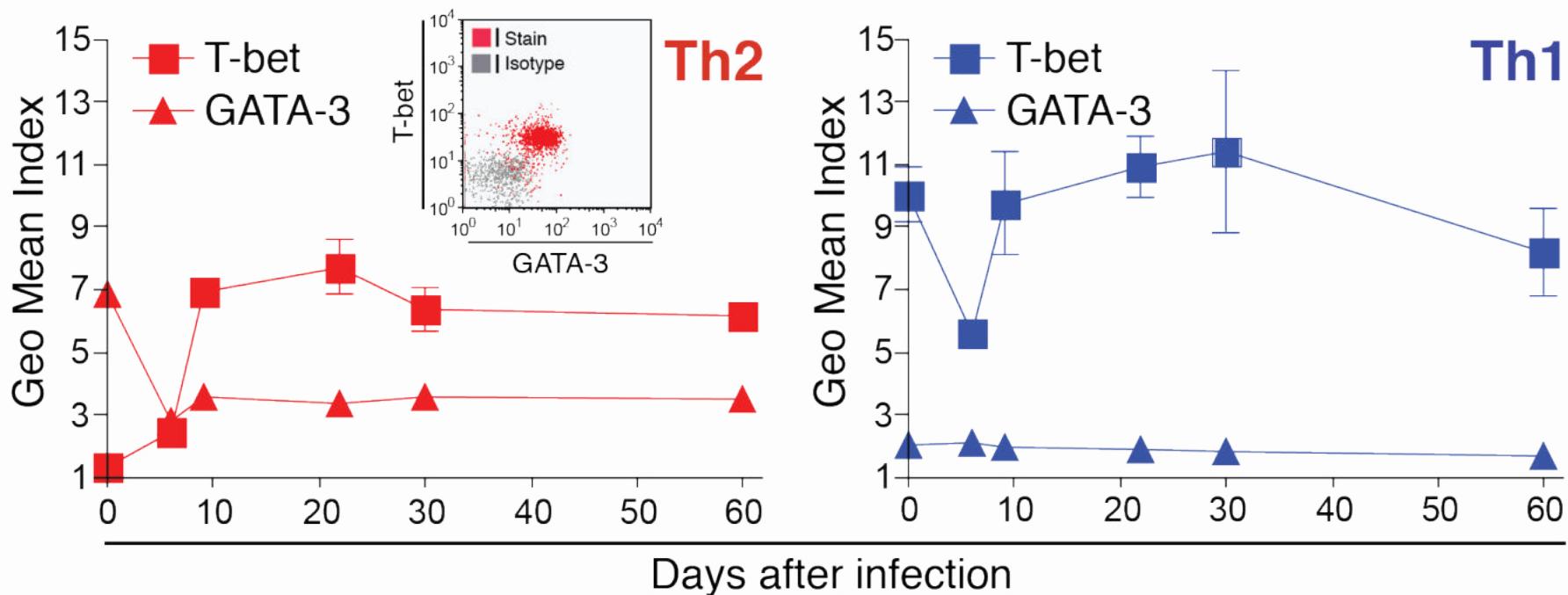
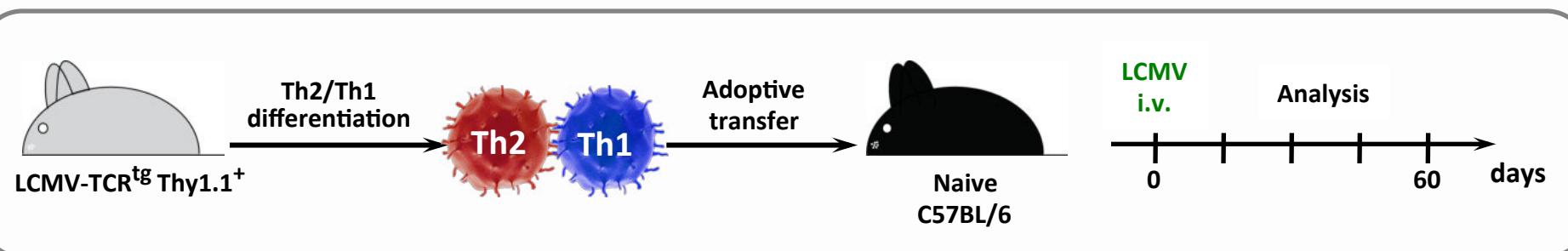
# Th2 cells memorize IFN- $\gamma$ expression after viral infection



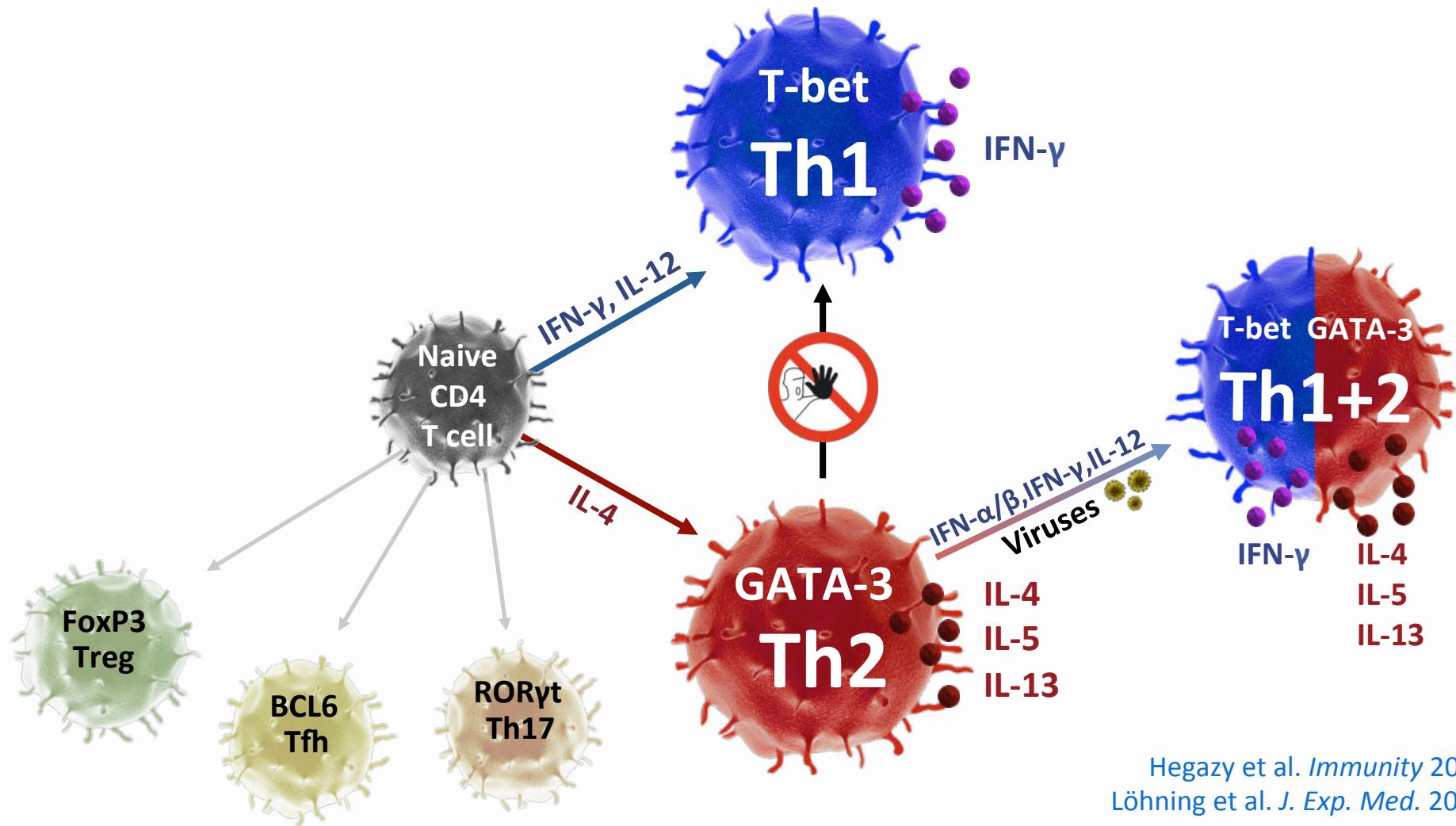
# Viral infection elicits stable T-bet expression in GATA-3<sup>+</sup> Th2 cells



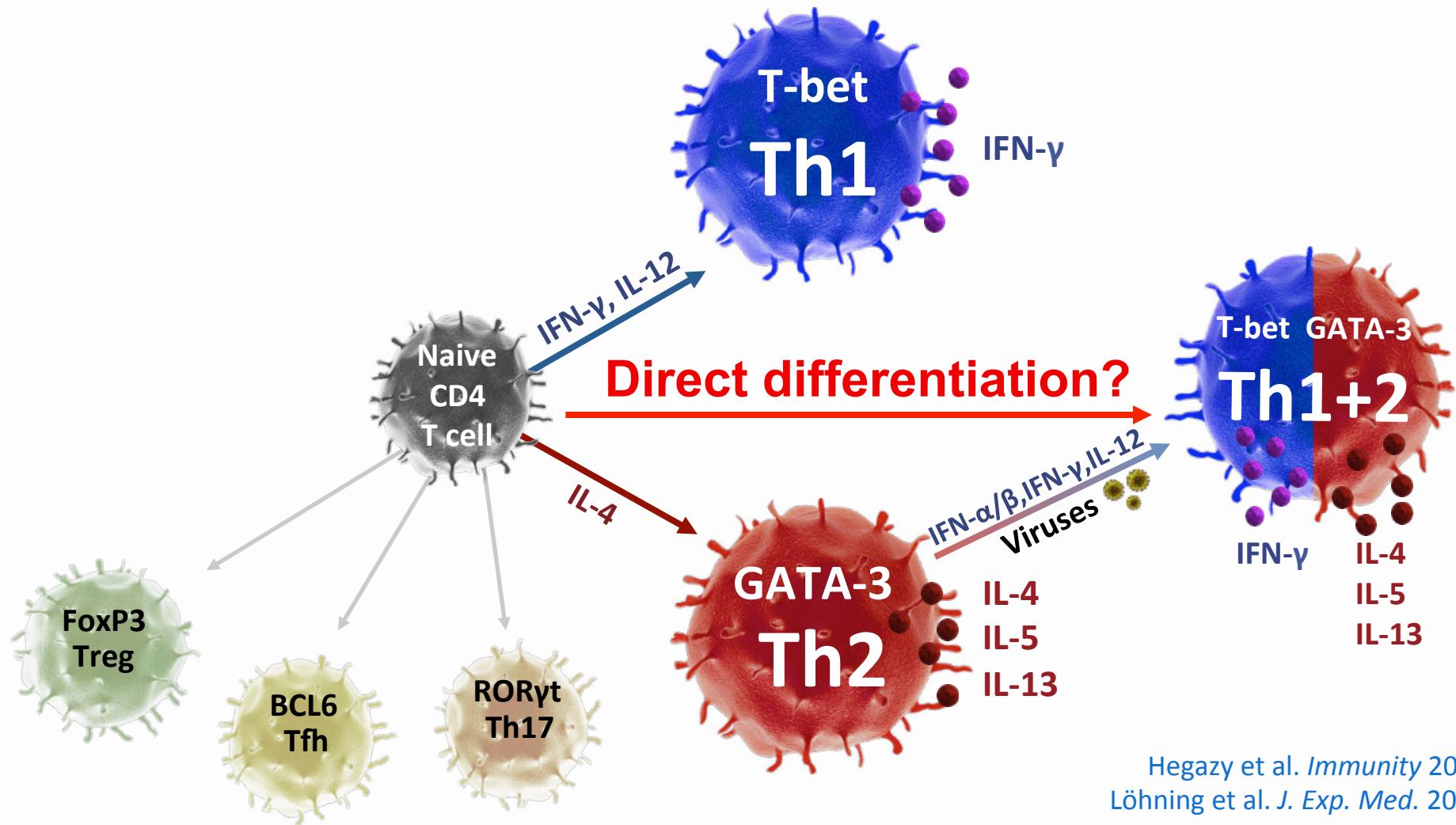
# T-bet<sup>+</sup> GATA-3<sup>+</sup> “Th1+2” phenotype is maintained for months



# Virus-induced reprogramming of Th2 cells: T-bet<sup>+</sup> GATA-3<sup>+</sup> “Th1+2” cells

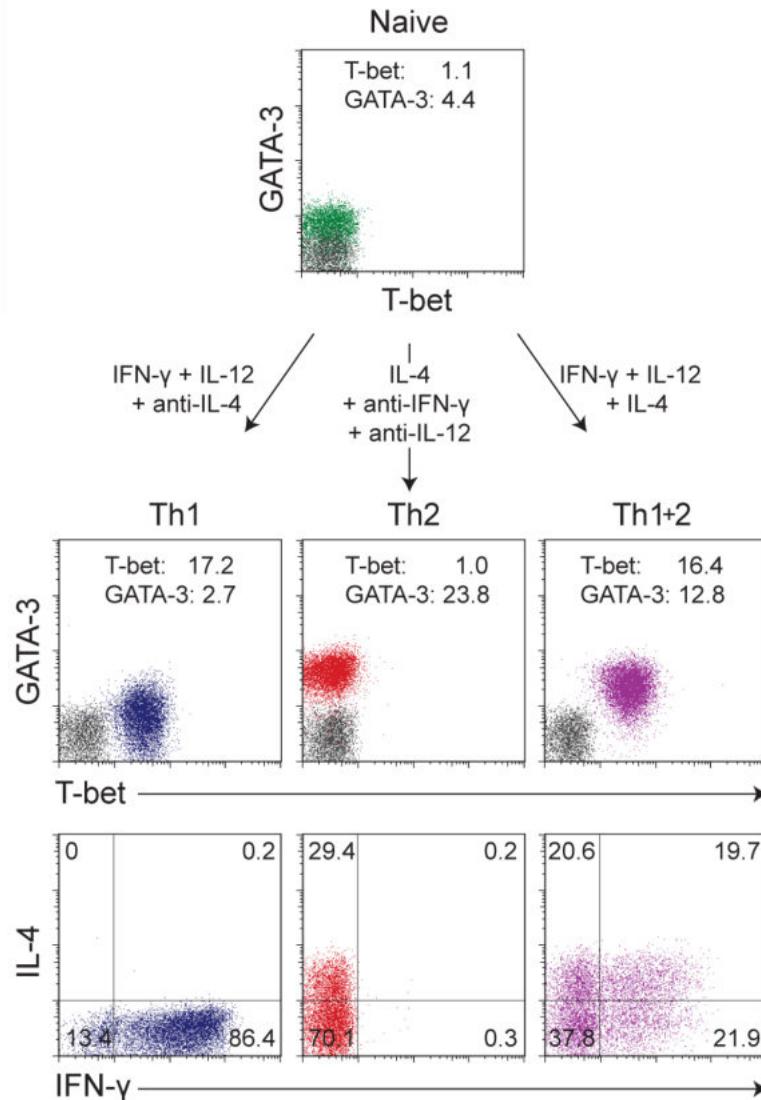


# Differentiation of T-bet<sup>+</sup> GATA-3<sup>+</sup> Th1+2 hybrid cells from naive precursors?

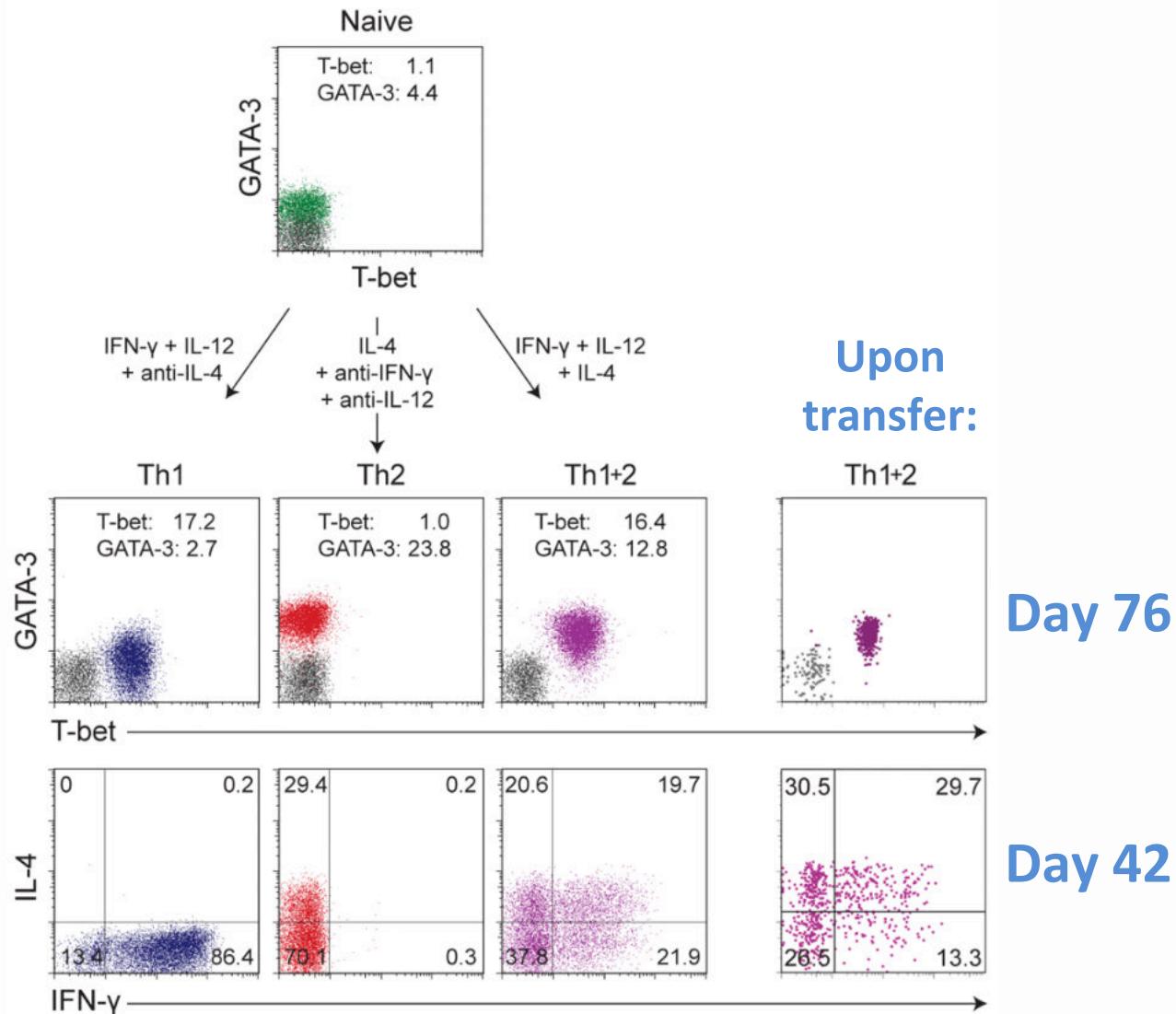


Hegazy et al. *Immunity* 2010  
Löhning et al. *J. Exp. Med.* 2008

# A naive Th cell can commit simultaneously to the Th1 & Th2 differentiation programs

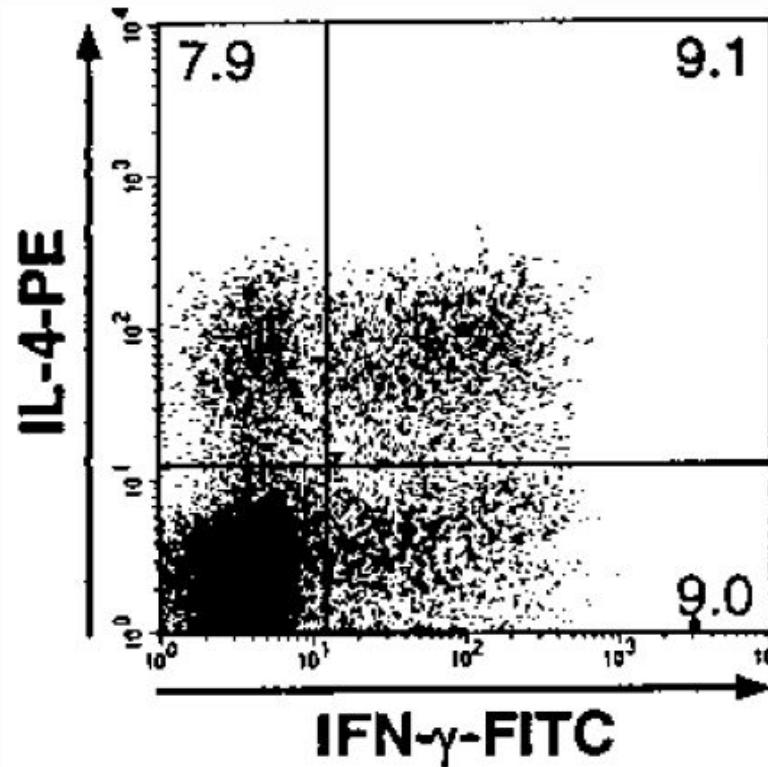


# Hybrid Th1+2 cells are stable



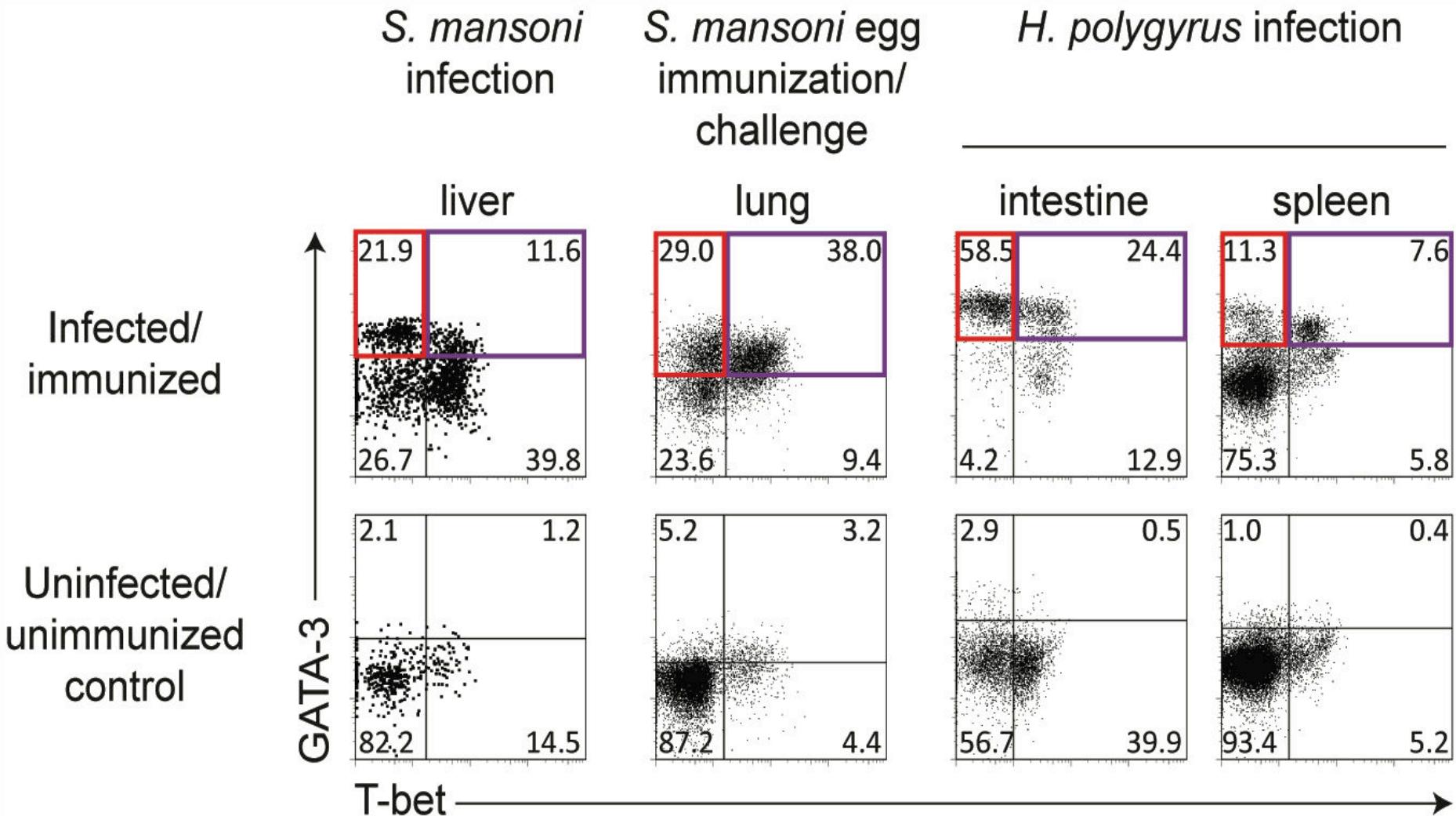
# Do hybrid Th1+2 cells arise *in vivo* during helminth infection? Coexpression of Th1 & Th2 cytokines

CD4<sup>+</sup> T cells from lungs with *Schistosoma mansoni* granulomas



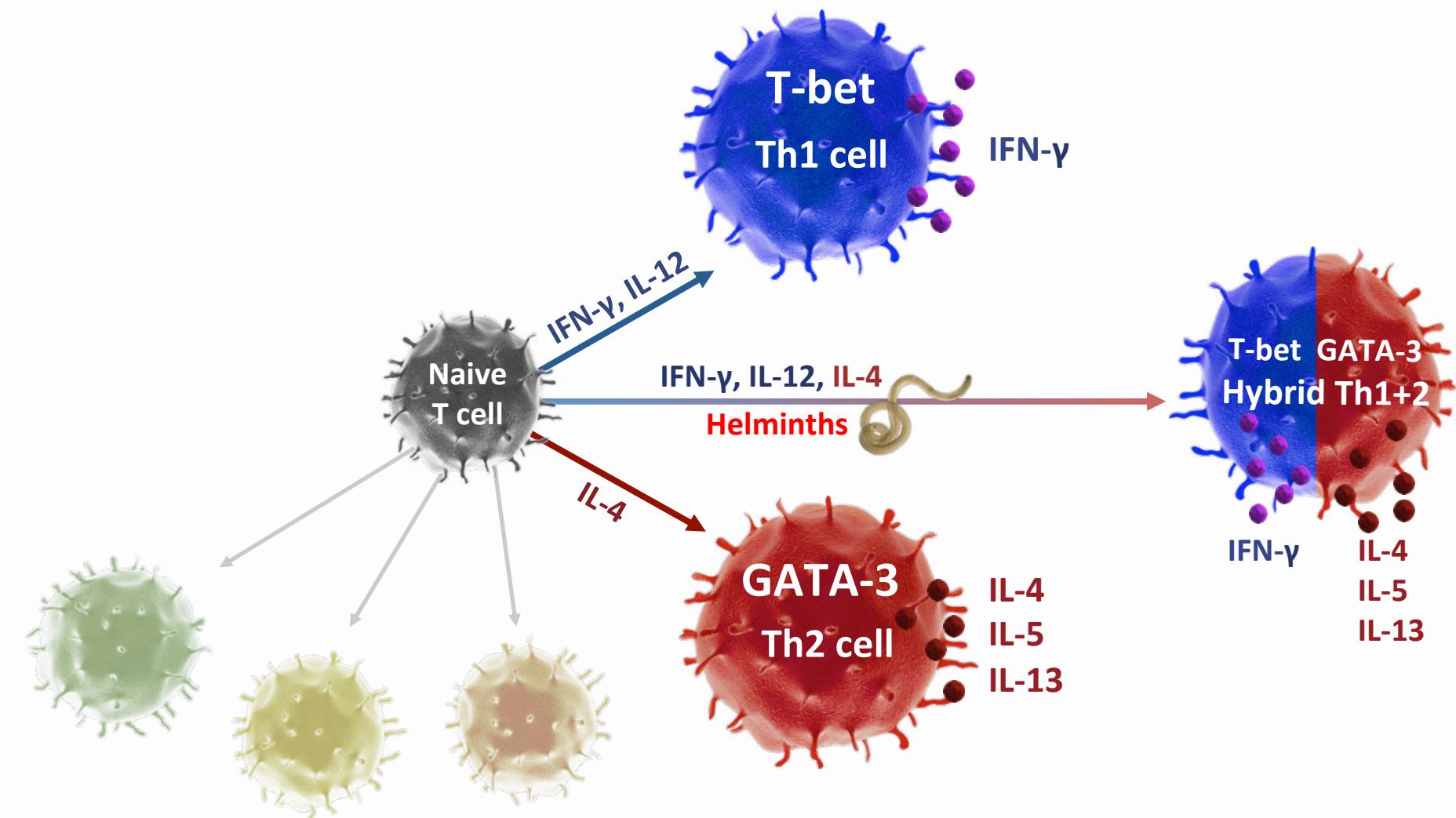
Löhning et al. *J. Immunol.* 1999

# Hybrid Th1+2 cells arise naturally *in vivo* in distinct helminth infections

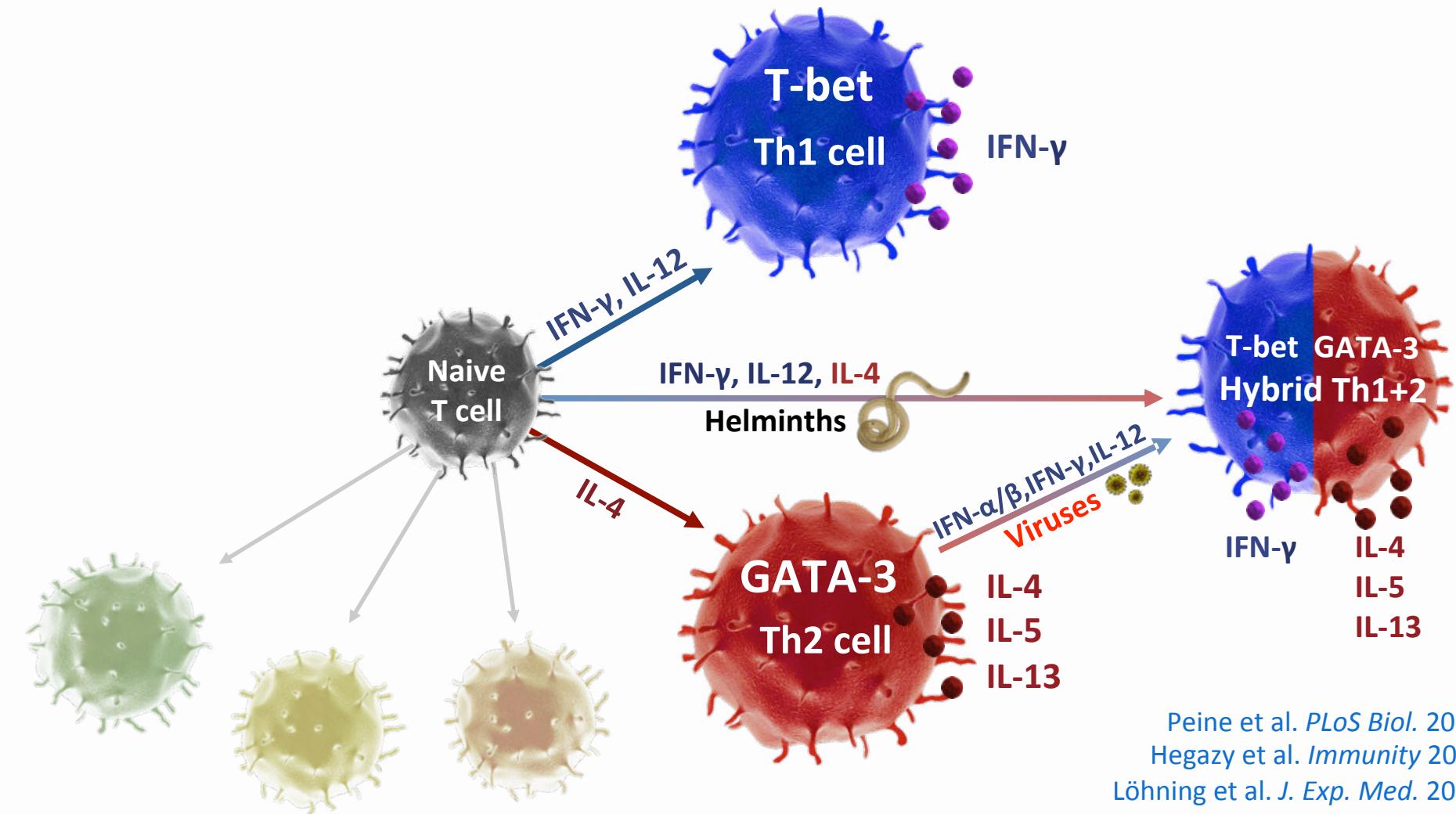


Peine et al. PLoS Biol. 2013

# Differentiation of T cells to a stable hybrid Th1+2 phenotype



# Differentiation & reprogramming of T cells to a stable hybrid Th1+2 phenotype



Peine et al. *PLoS Biol.* 2013

Hegazy et al. *Immunity* 2010

Löhning et al. *J. Exp. Med.* 2008

# Topics

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Differentiation of antiviral effector T cells

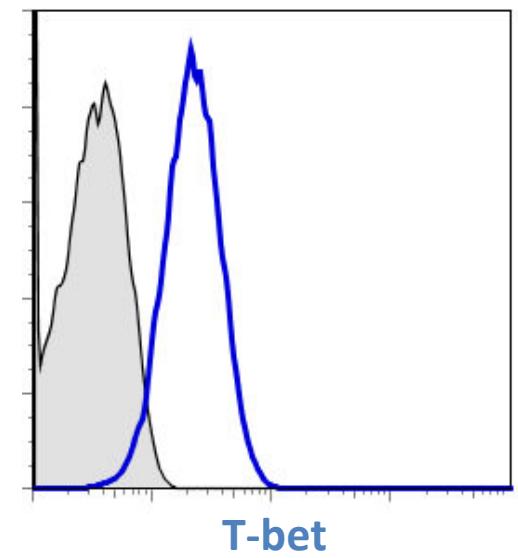
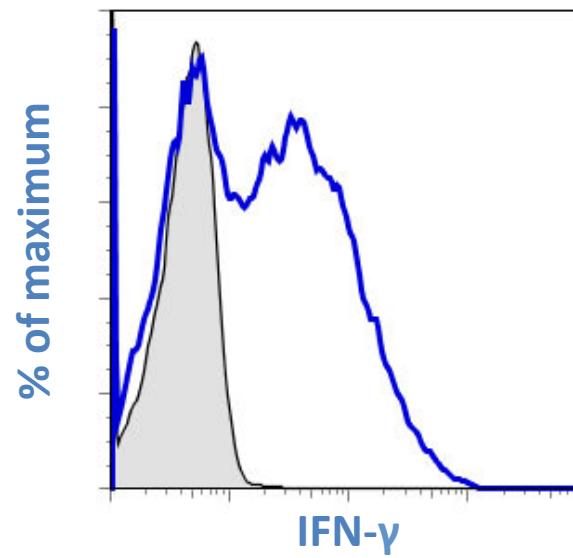
Stability & plasticity of memory T cell subsets

**Quantitative cytokine memory of individual T cells**

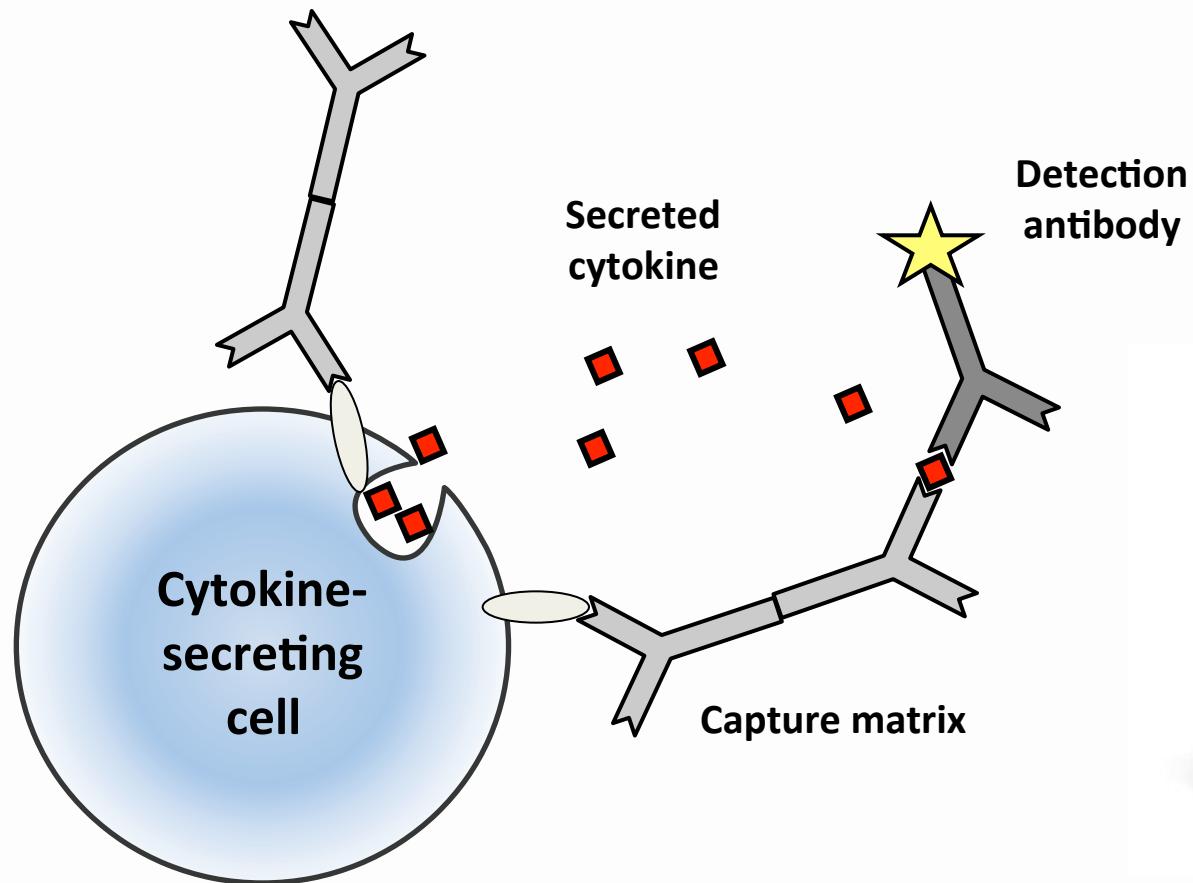
Function of quantitative memory & plasticity in inflammation

# Intra-population heterogeneity of cytokine expression – Stochastic behavior or stable differences?

- IFN- $\gamma$  producers and non-producers in clonal Th1 cell populations  
(Assenmacher, EJI 1994; Bucy, JEM 1994; Openshaw, JEM 1995)
- Large variability of IFN- $\gamma$  expression per cell



# Sorting of live cells by amount of secreted cytokine



Manz et al. *PNAS* 1995

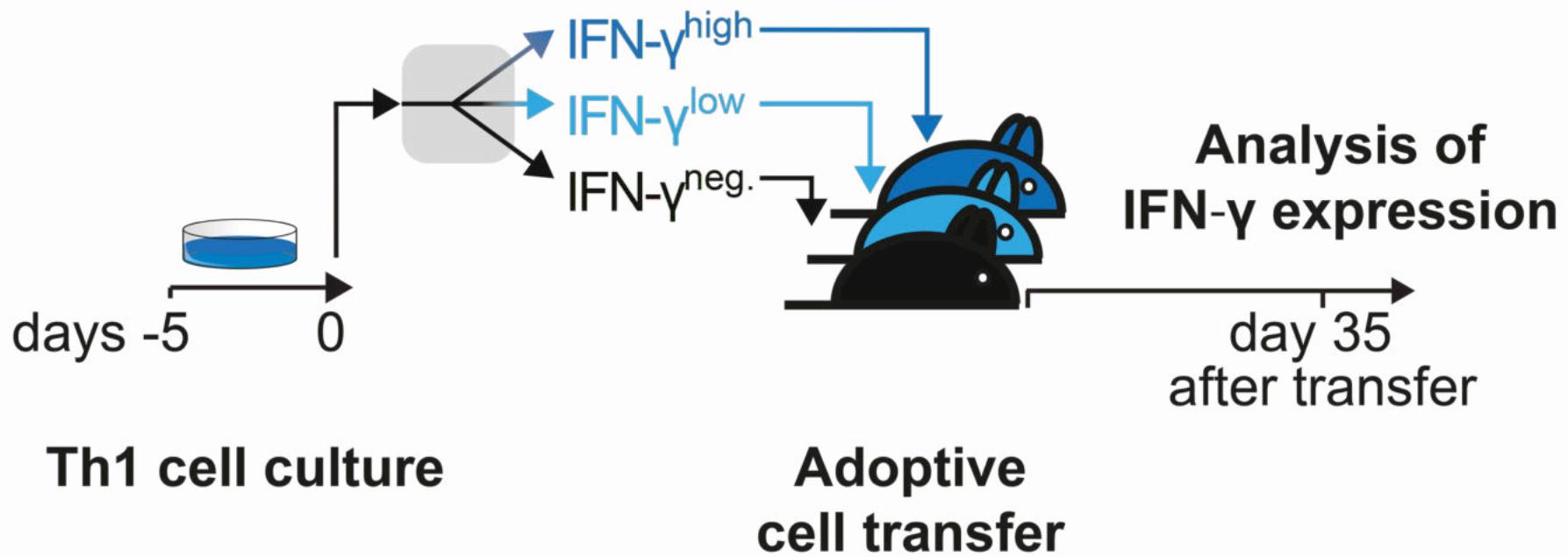
Assenmacher, Löhning, Radbruch. *Curr. Prot. Immunol.* 2001

Löhning et al. *PNAS* 2003

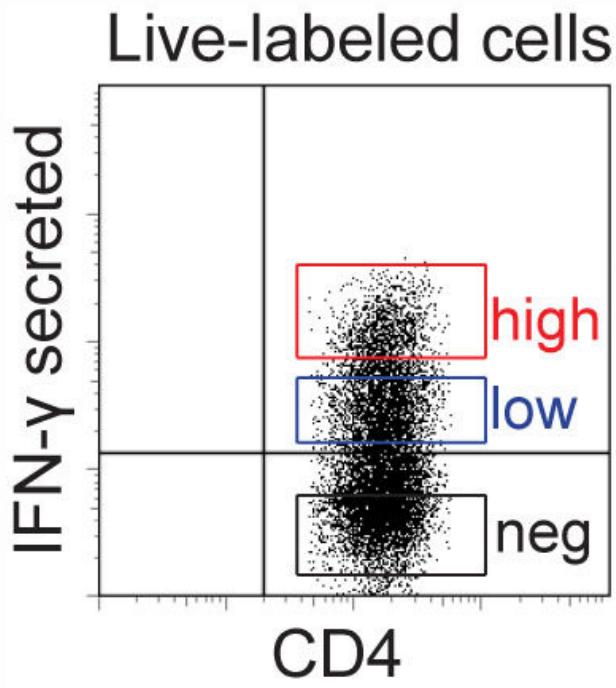
Löhning et al. *J. Exp. Med.* 2008

# Does a Th1 memory cell recall an individual IFN- $\gamma$ production amount?

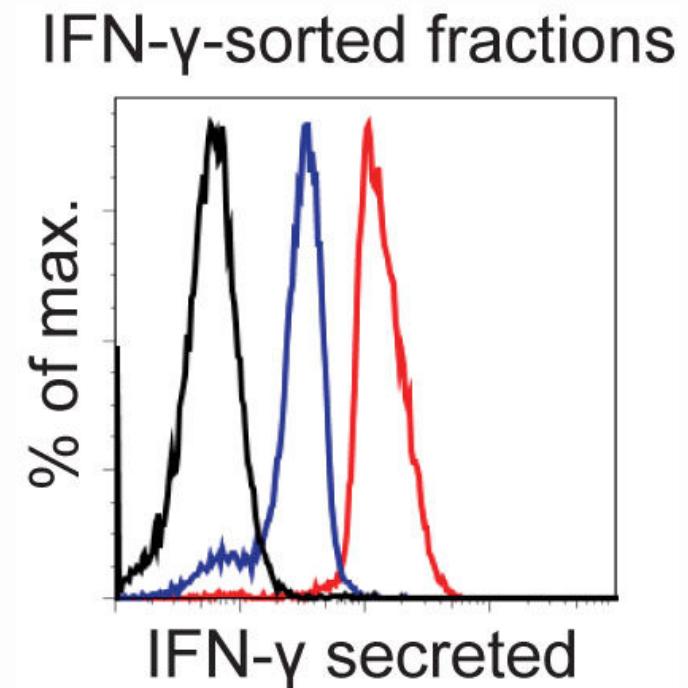
## IFN- $\gamma$ secretion assay + FACSsort



# Sorting of live Th1 cells according to individual IFN- $\gamma$ production amount

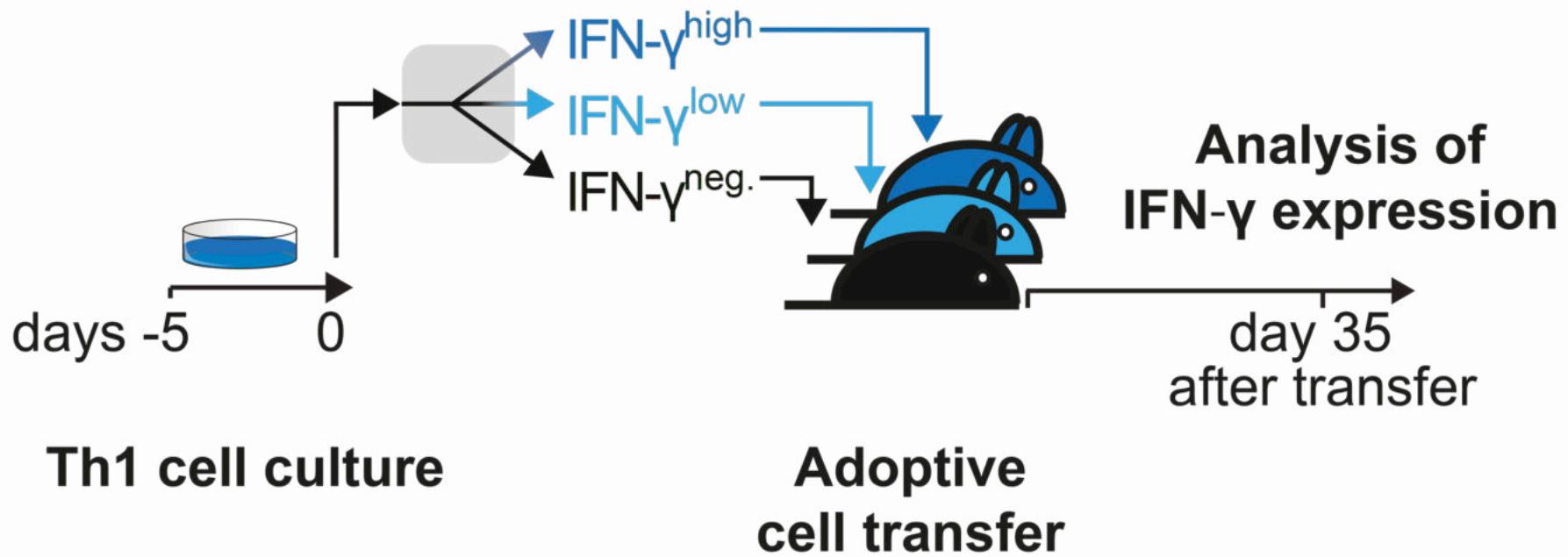


FACSort  
→



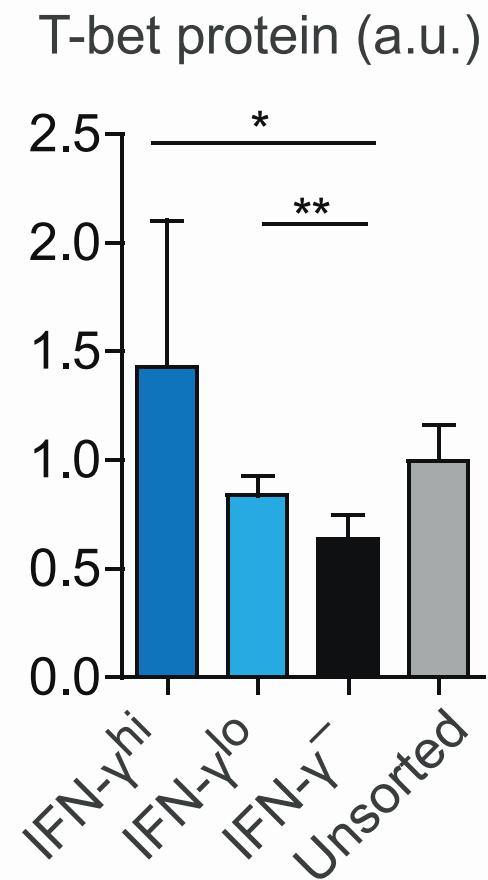
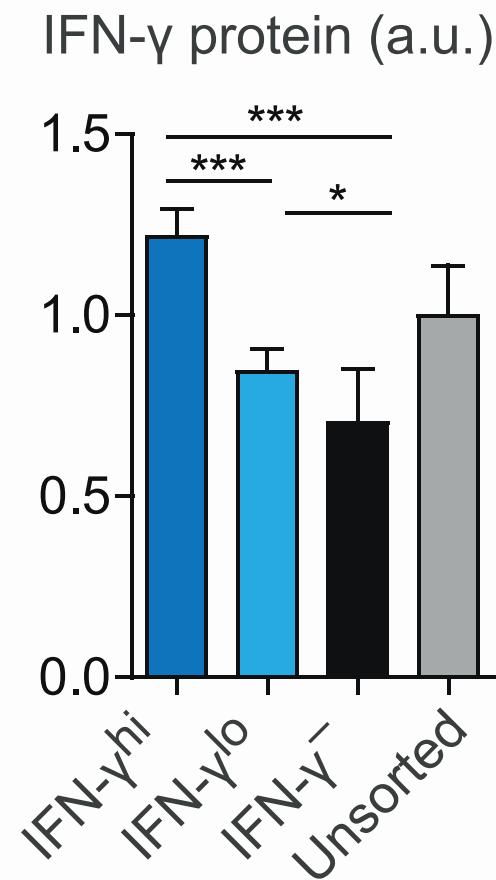
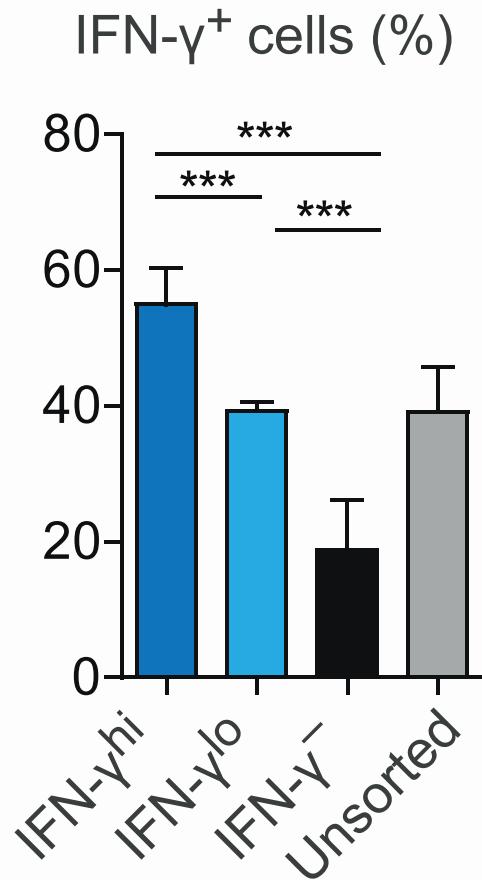
# Does a Th1 memory cell recall an individual IFN- $\gamma$ production amount?

## IFN- $\gamma$ secretion assay + FACSsort



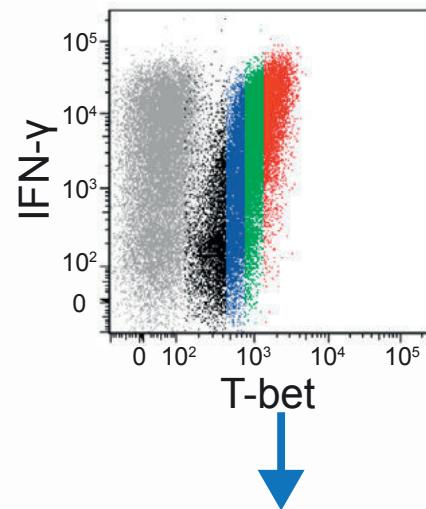
# A Th1 memory cell recalls an individual IFN- $\gamma$ production amount

d35 after transfer

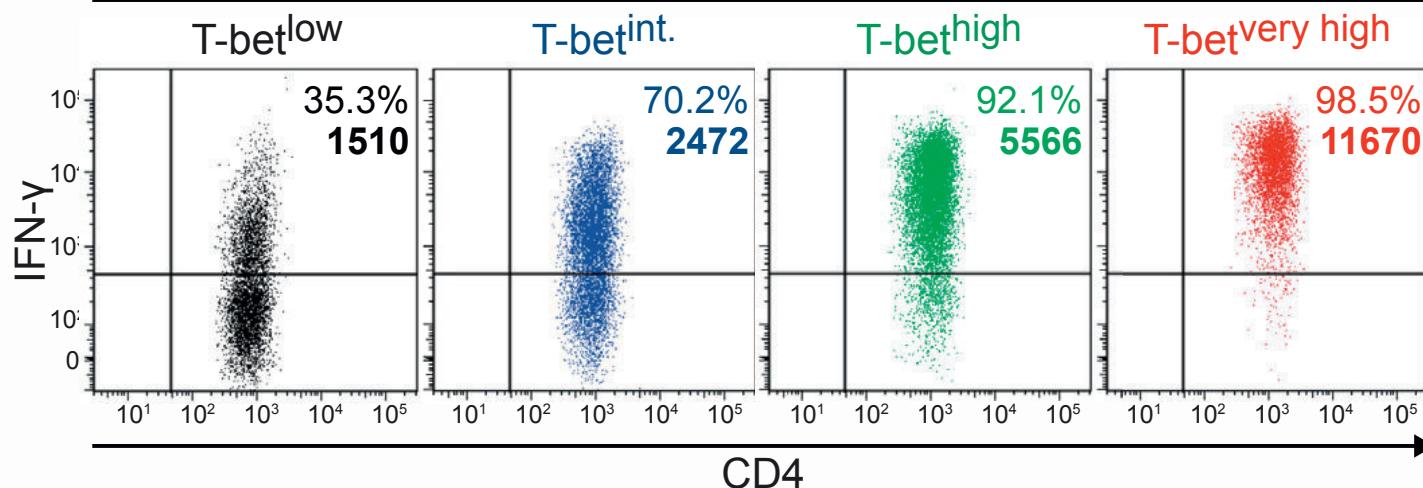


Helmstetter et al. *Immunity* 2015

# T-bet expression level correlates with IFN- $\gamma$ expression level

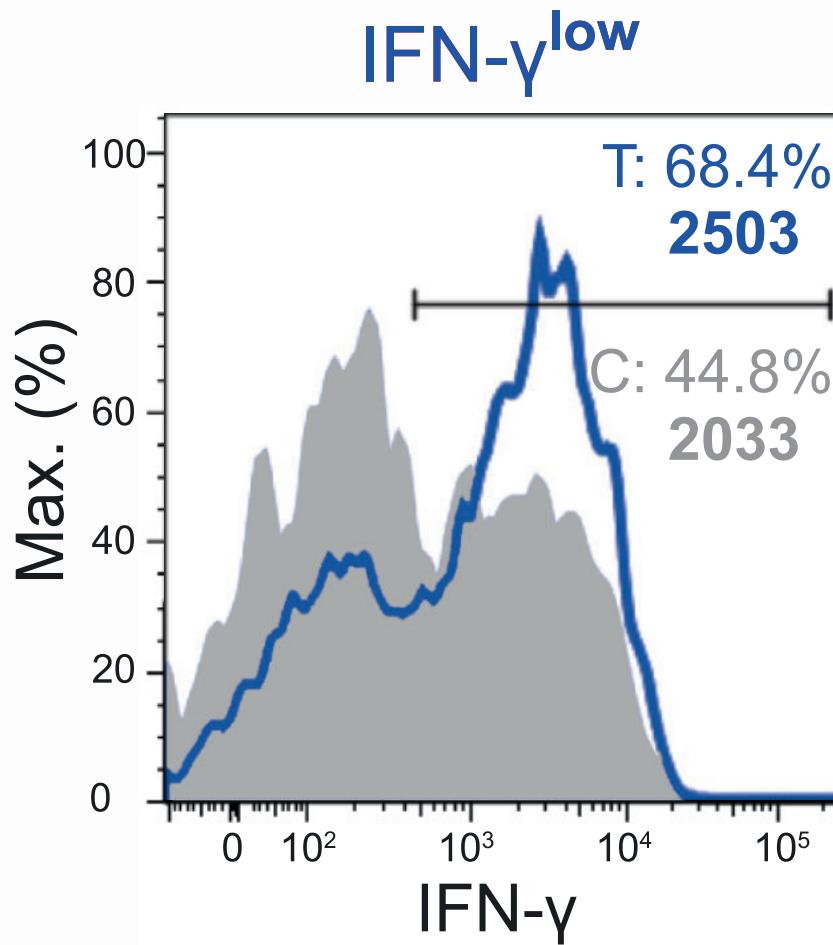


Gated on fractions with different T-bet expression levels



# T-bet expression level controls IFN- $\gamma$ expression level

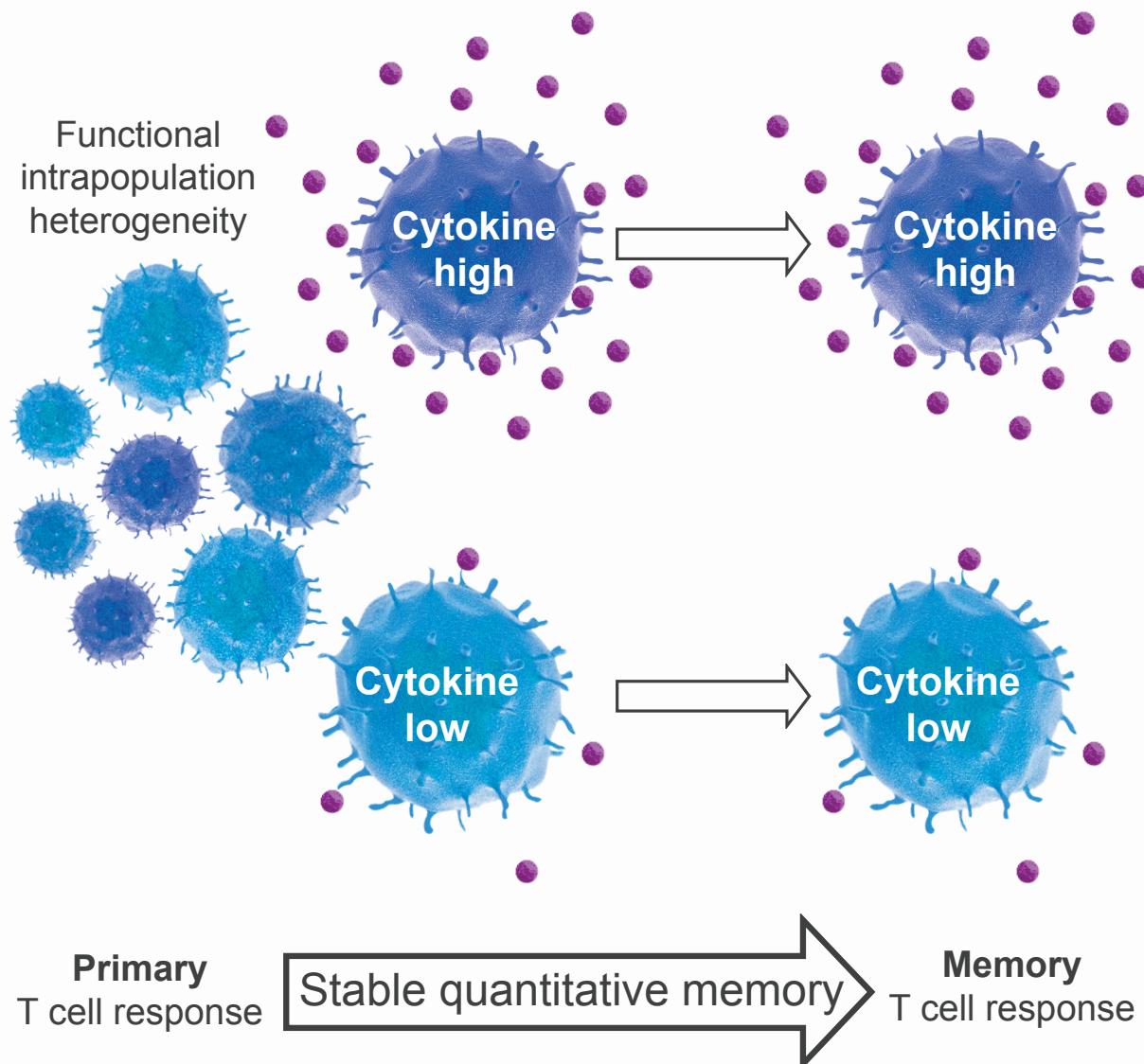
Sorted IFN- $\gamma^{\text{low}}$  Th1 cells, induced overexpression of T-bet



T-bet retrovirus (T)  
Control retrovirus (C)

Helmstetter et al. *Immunity* 2015

# Quantitative cytokine memory of individual Th cells



# Topics

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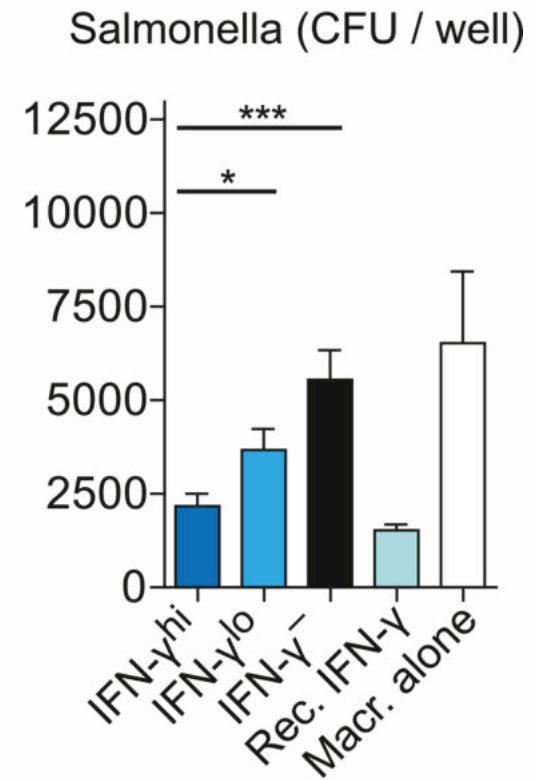
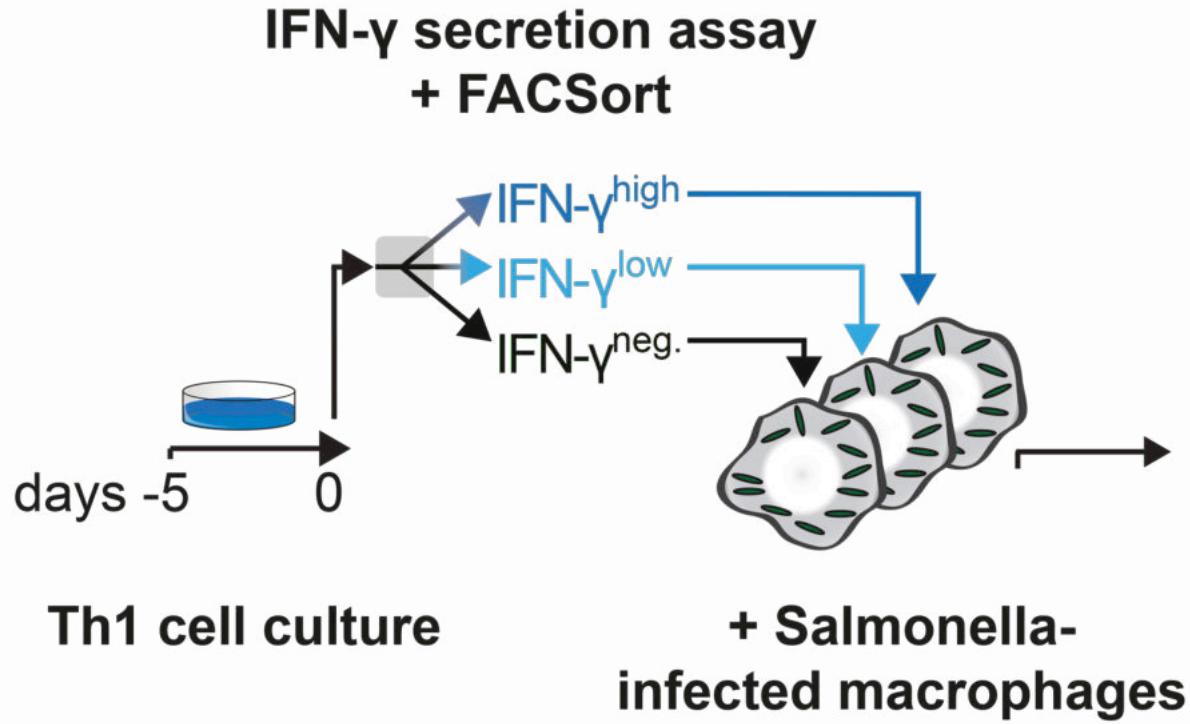
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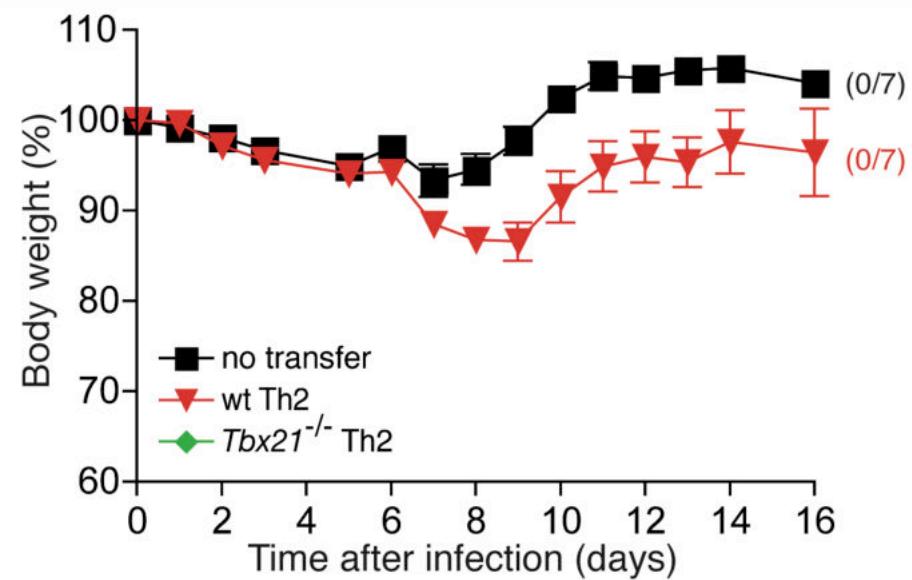
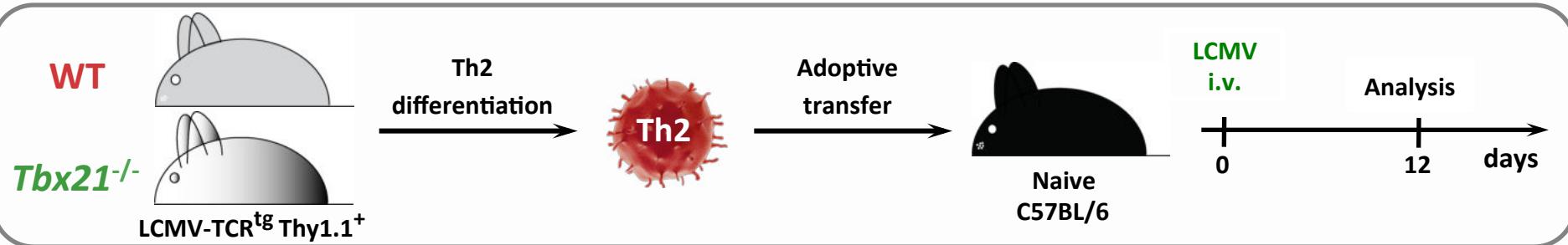
**Function of quantitative memory & plasticity in inflammation**

# Distinct IFN- $\gamma$ production levels of individual Th1 cells translate into graded bacterial killing

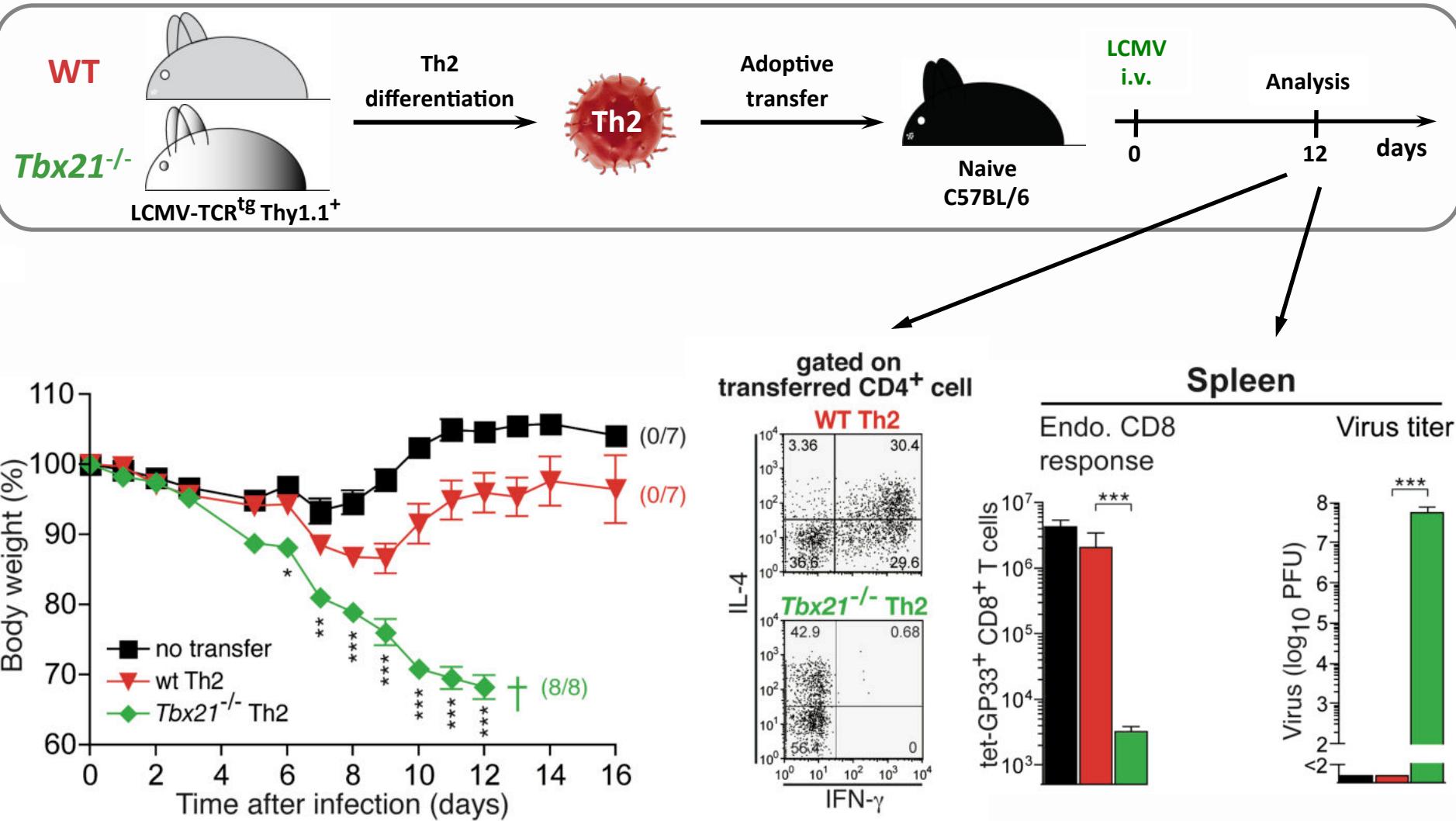


Helmstetter et al. *Immunity* 2015

# Th2 cell reprogramming prevents fatal immunopathology and viral persistence



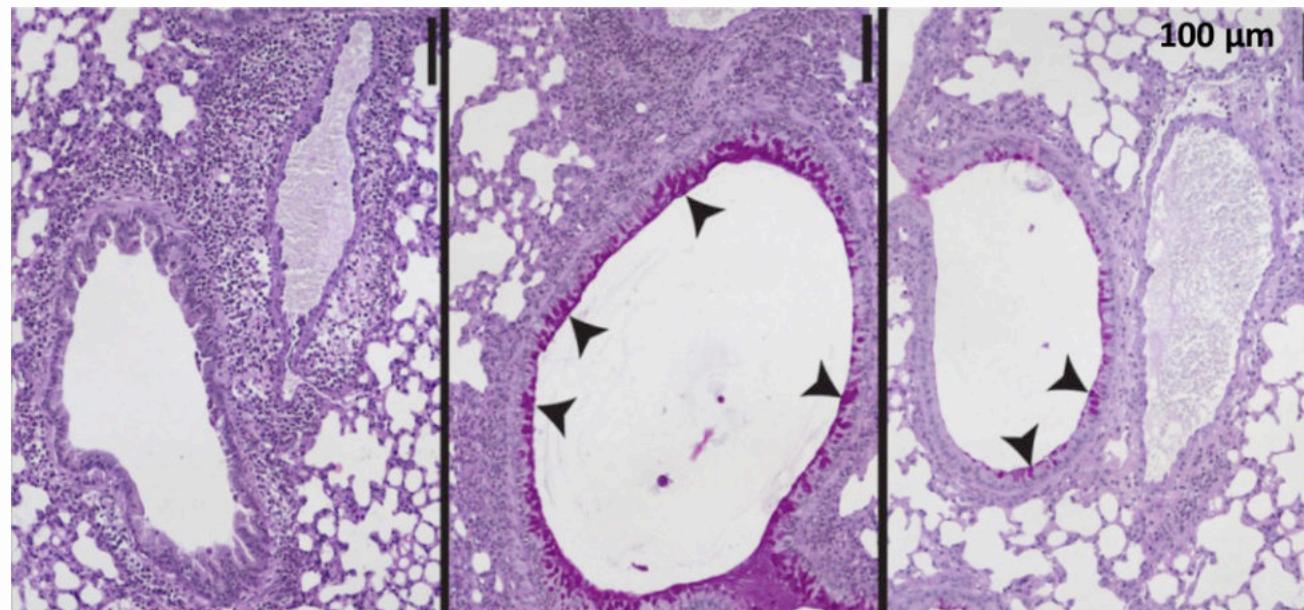
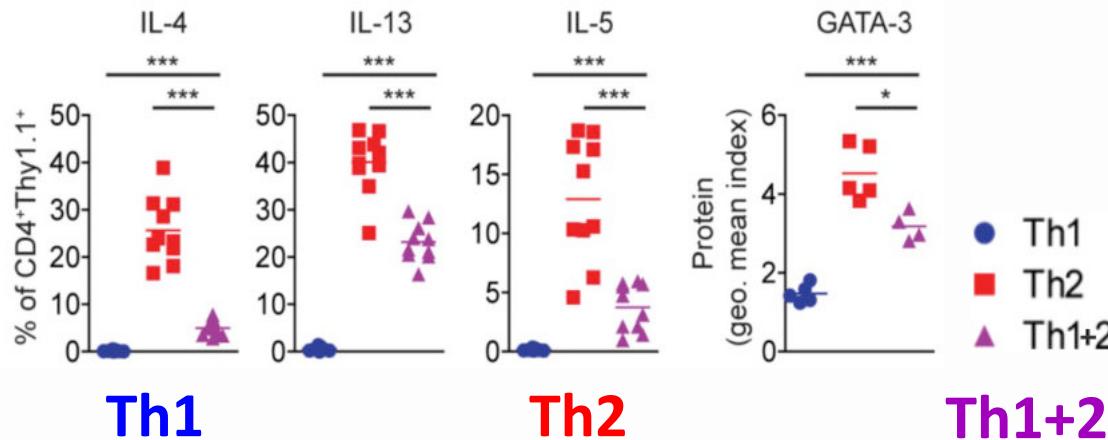
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Hegazy et al. *Immunity* 2010

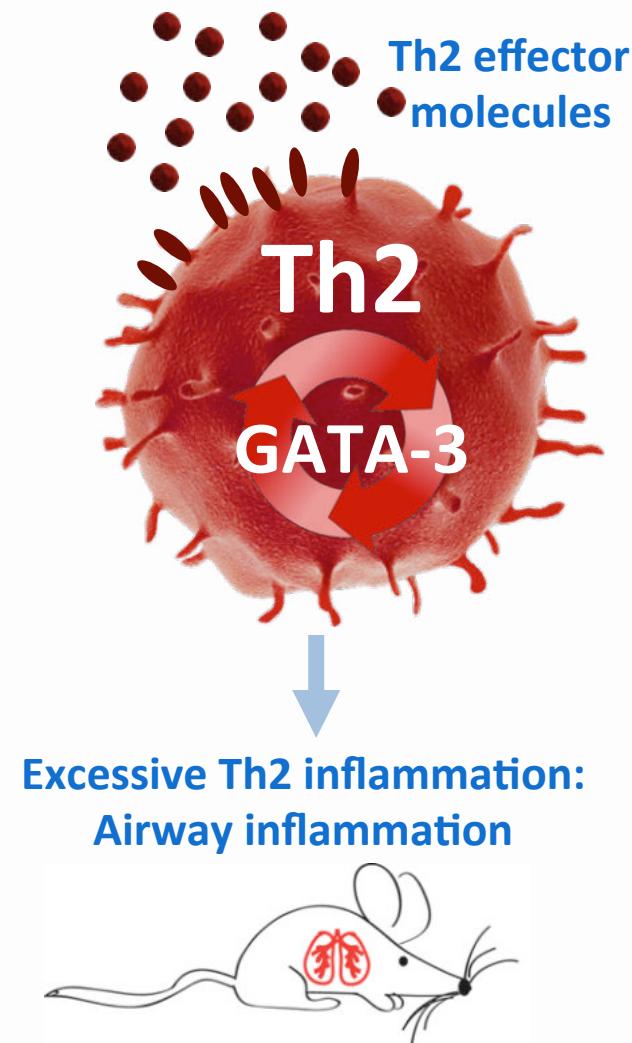
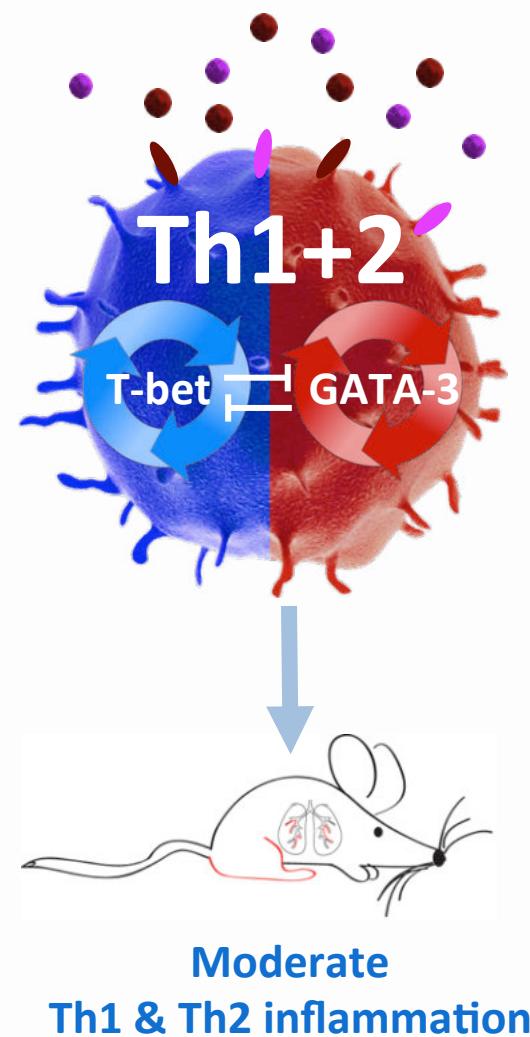
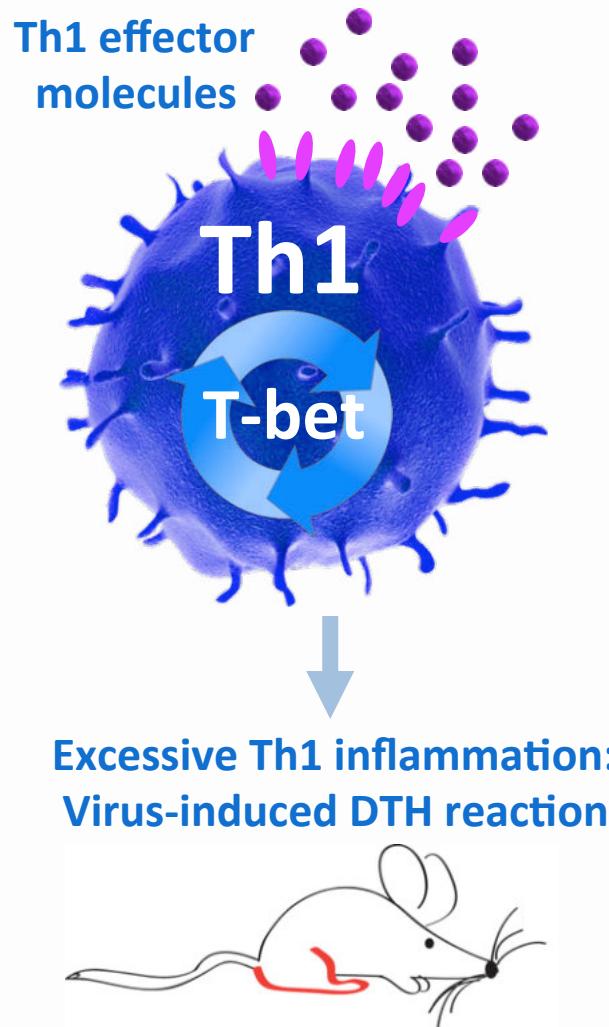
# Balanced opposing differentiation programs in hybrid Th1+2 cells attenuate Th2 immunopathology

## Airway inflammation model



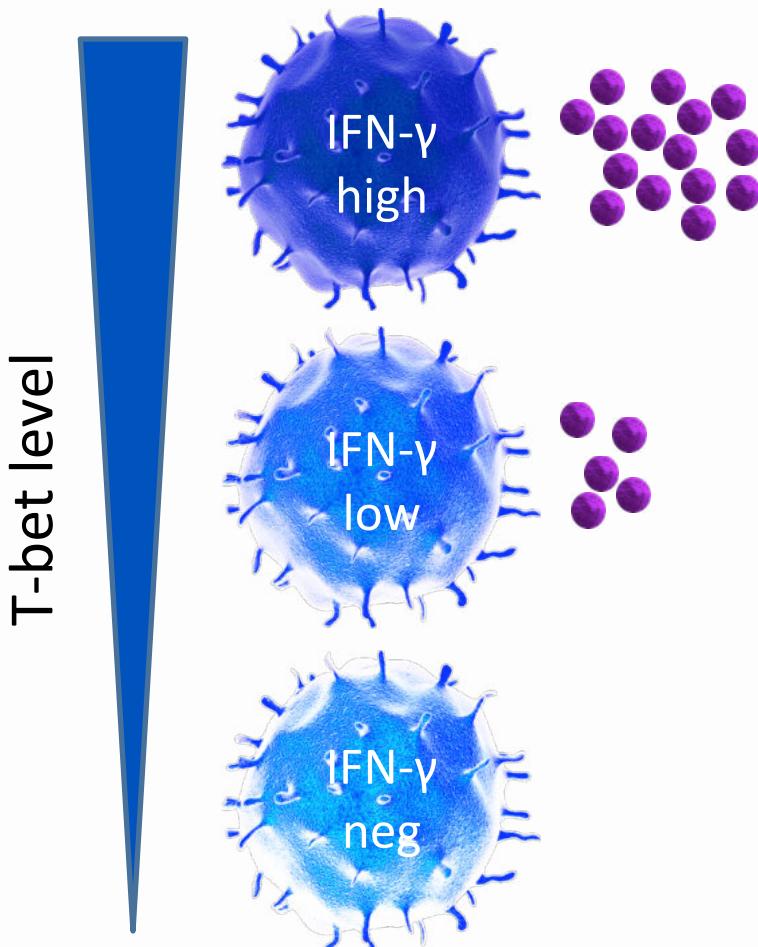
Peine et al.  
*PLoS Biol.*  
2013

# Balanced opposing differentiation programs in hybrid Th1+2 cells attenuate Th1 & Th2 immunopathologies

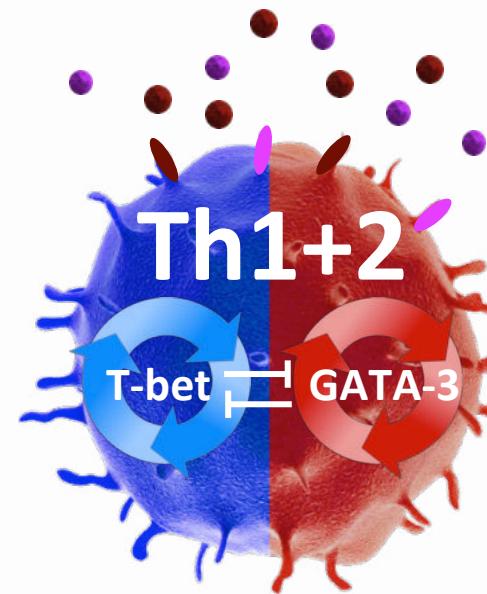


# Alternative molecular mechanisms achieve stable quantitative differences in memory T cell functions

Heterogeneity among classically differentiated Th cell population



Modulation of Th cell function based on antagonistic differentiation programs



# Quantitative memory of individual T cells in infections

- Alarmin IL-33 drives protective antiviral effector & memory T cell responses
- Resting Th2 & Th1 memory cells are functionally stable *in vivo*
- TCR, IFN- $\alpha/\beta$ , IFN- $\gamma$ , IL-12 & T-bet are necessary & sufficient for Th2 cell reprogramming
- Hybrid T-bet $^+$  GATA-3 $^+$  & IFN- $\gamma$  $^+$  IL-4 $^+$  Th1+2 phenotype is stable for months
- Hybrid Th1+2 cells arise *in vitro* & in helminth infections *in vivo*
- A single Th1 cell memorizes an individual amount of IFN- $\gamma$  & T-bet expression
- Distinct IFN- $\gamma$  production amounts of single Th1 cells translate into graded bacterial killing
- Th2 reprogramming prevents viral persistence & fatal immunopathology
- Balanced opposing differentiation programs in hybrid Th1+2 cells limit immunopathology

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