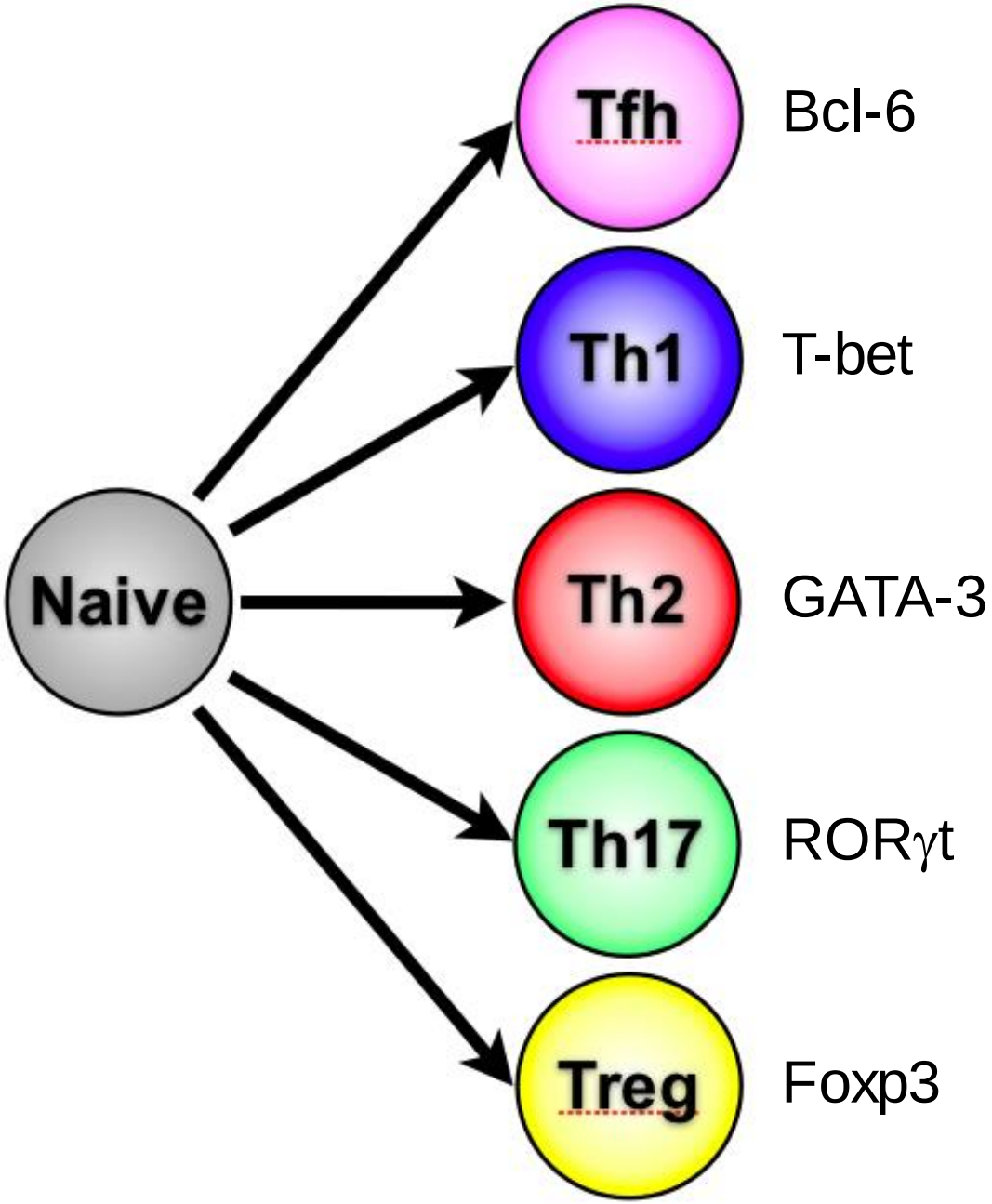
IMMUNOLOGICAL MEMORY

- **CD4 T Follicular Helper Cells**
- **Memory CD8 T Cell Differentiation**

CD4 T Cell Differentiation



CD4 T follicular helper (Tfh) cells

FUNCTION

Provide essential help for germinal center formation and maintenance

Required for affinity maturation and differentiation of memory B cells and long-lived plasma cells

PHENOTYPE

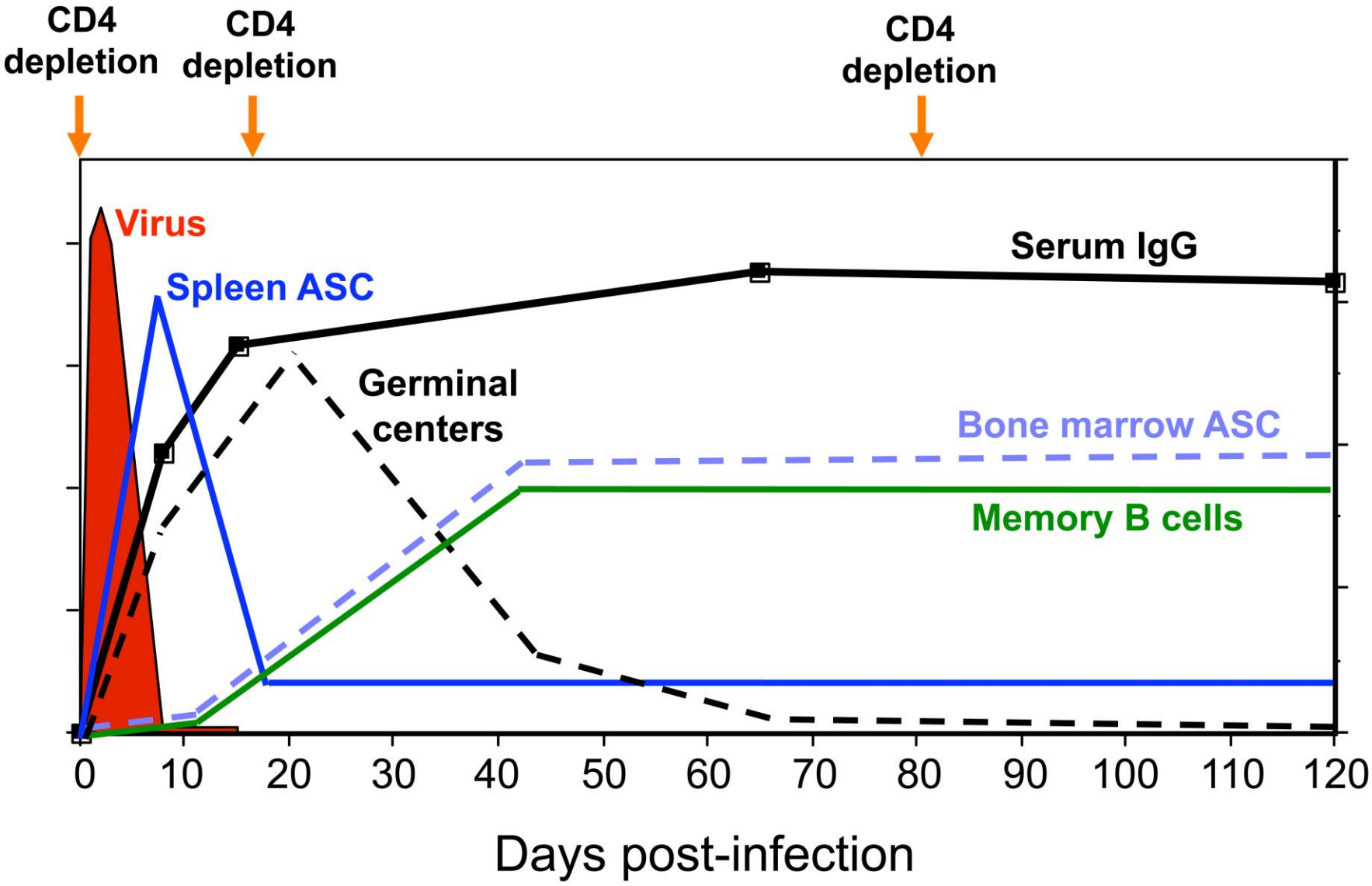
The chemokine receptor CXCR5 is a functional marker for CD4+ Tfh cells

Bcl6 is a lineage regulator of Tfh differentiation in CD4 T cells

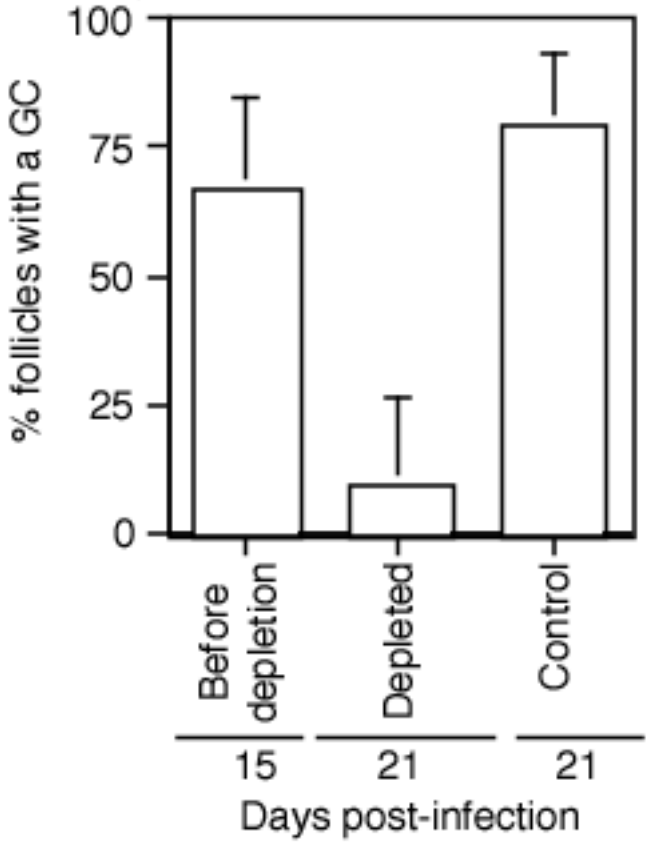
PD-1, ICOS – other phenotypic markers

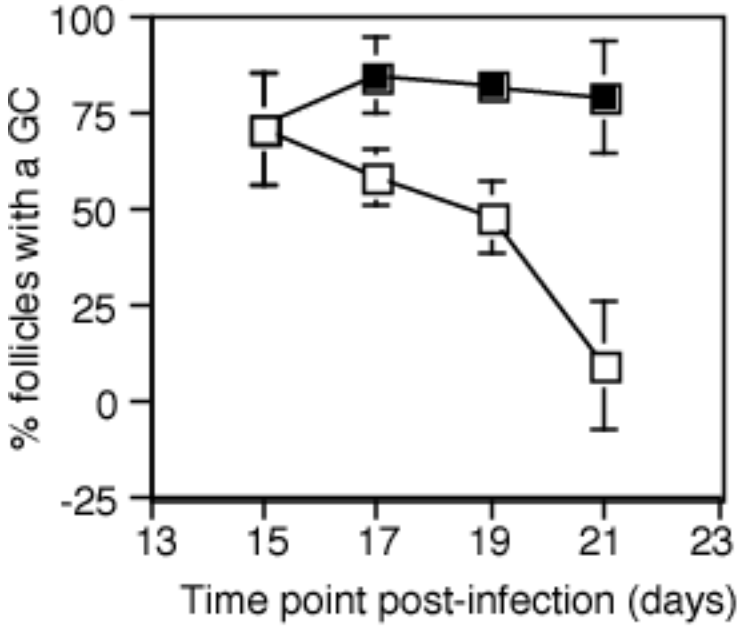
Cytokines – IL-21, (IL-4)

Role of CD4 T cell help in antibody responses



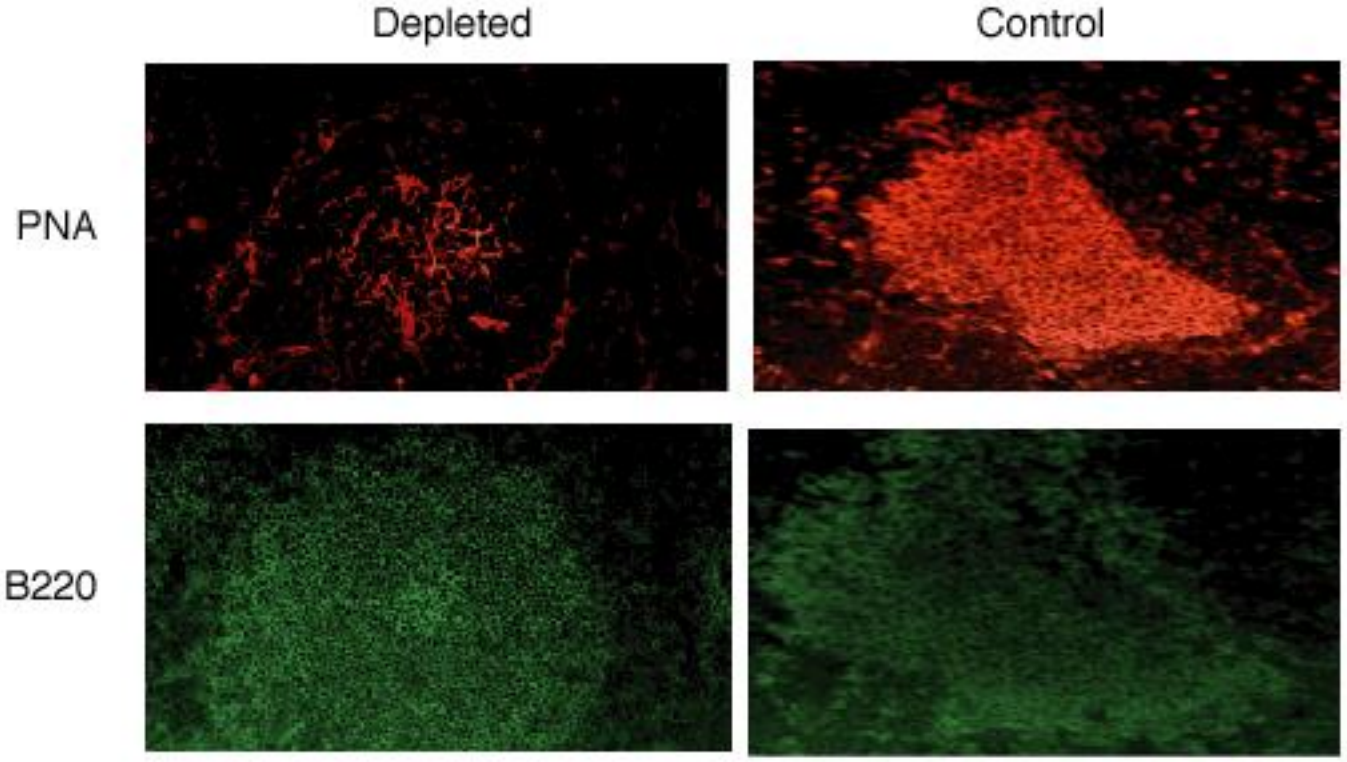
CD4 T cells are required for maintenance of the GC reaction





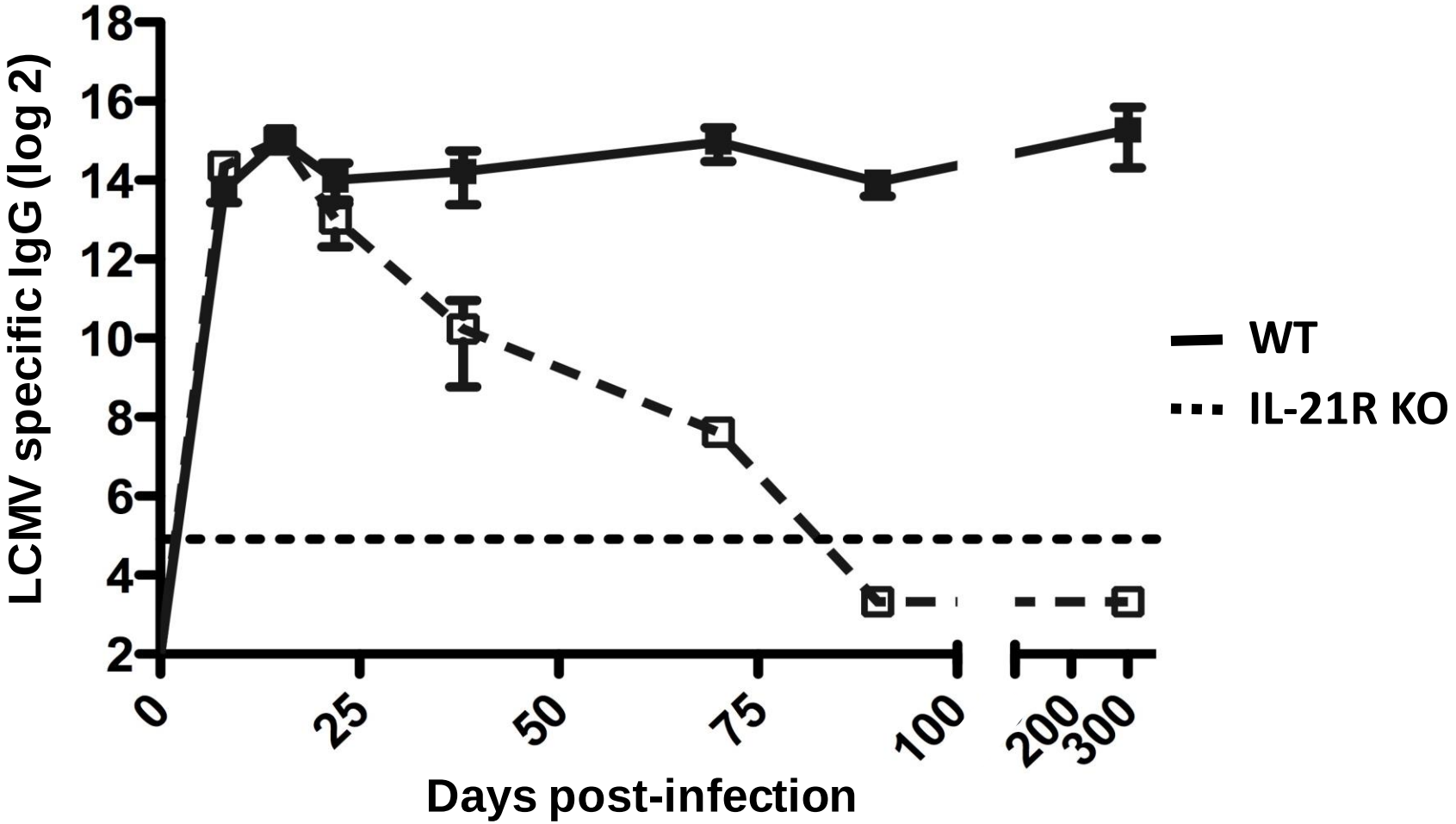
CD4 T cells were depleted when GC were established (Day 15 post-LCMV infection)

“Ruins” of GC remain after CD4 depletion



CD4 T cells were depleted when GC were established (Day 15 post-LCMV infection). Mice were sacrificed 7 days after CD4 T cell depletion.

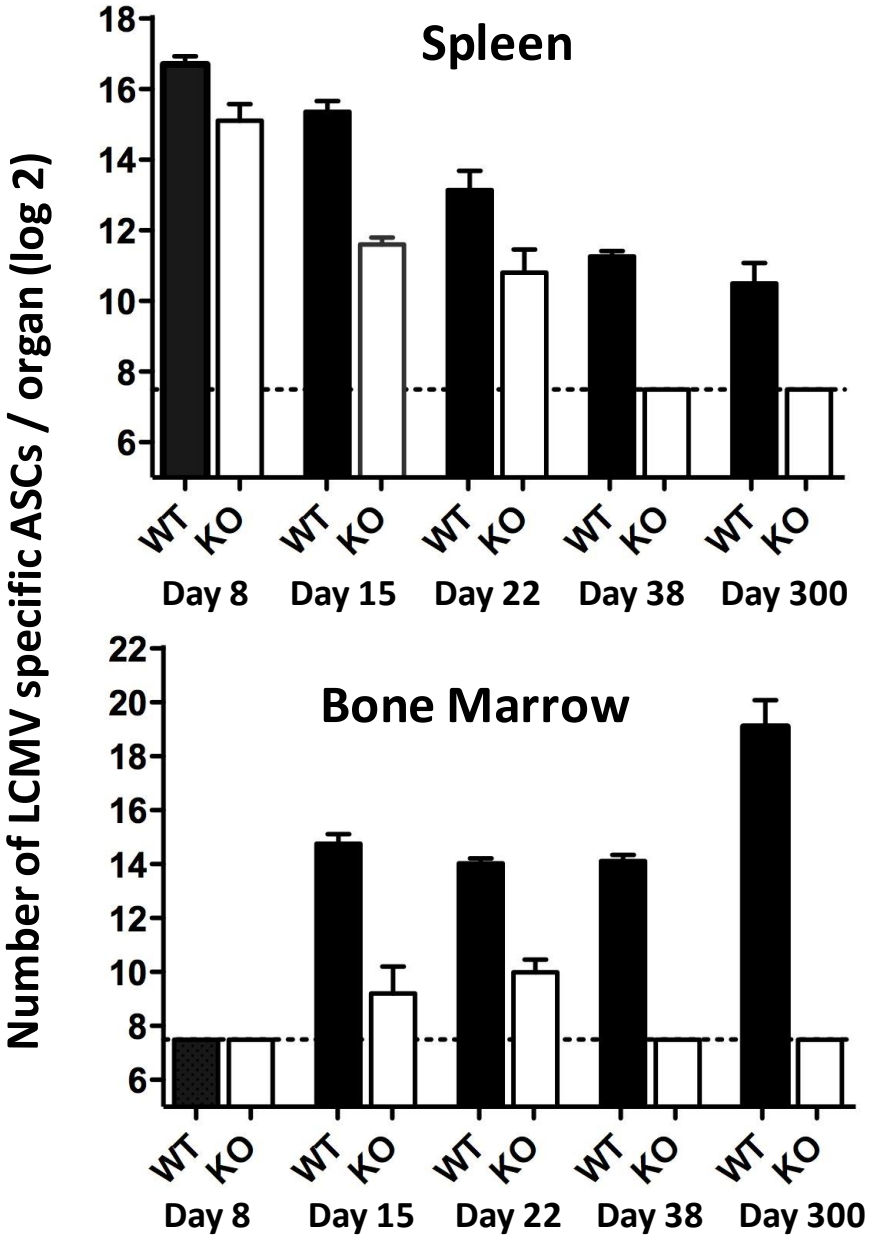
IL-21 is needed for long-term humoral immunity after acute viral infection



Similar results seen with influenza virus and VSV

Mohammed et al. *J Virol*, 2013

Importance of IL-21 for generation of long-lived plasma cells



ASC in spleen (day 300)

WT KO

ASC in BM (day 300)

WT KO

Is there a Tfh memory cell?

Central Questions:

1. **Are there memory CD4 T cells that are poised to recall Tfh lineage-specific functions upon reencounter with antigen?**

2. **What are markers that define Tfh memory cells?**

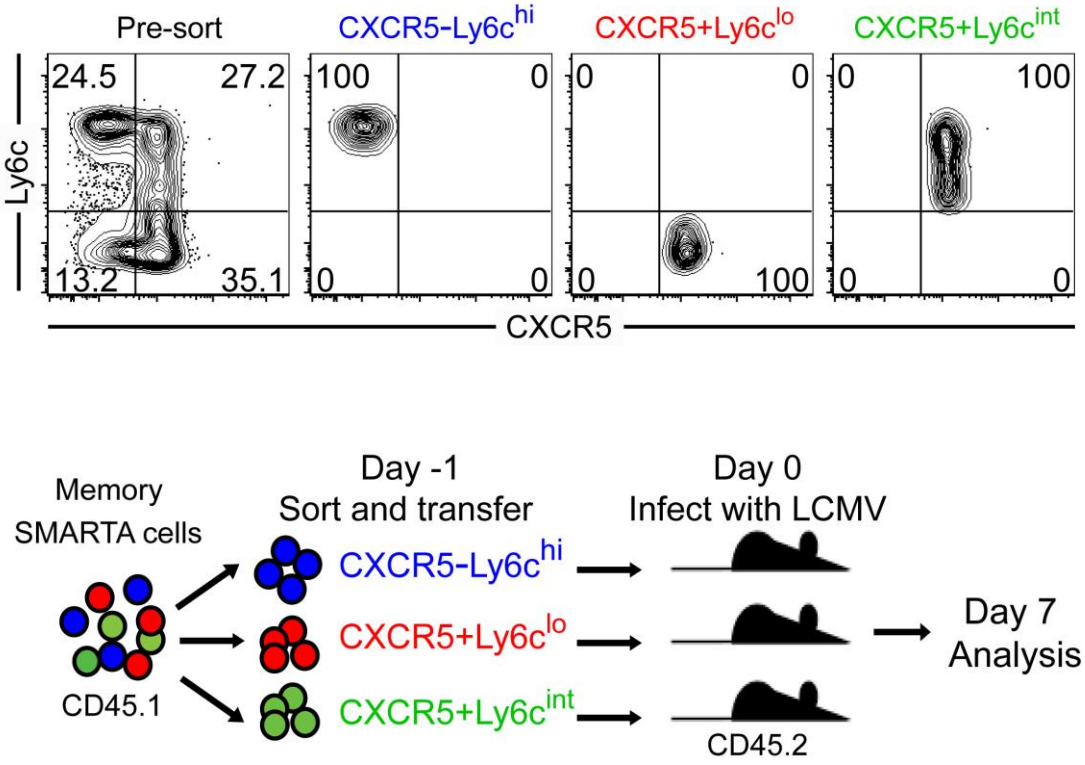
3. **What is the tissue distribution of Tfh effector and Tfh memory cells?**

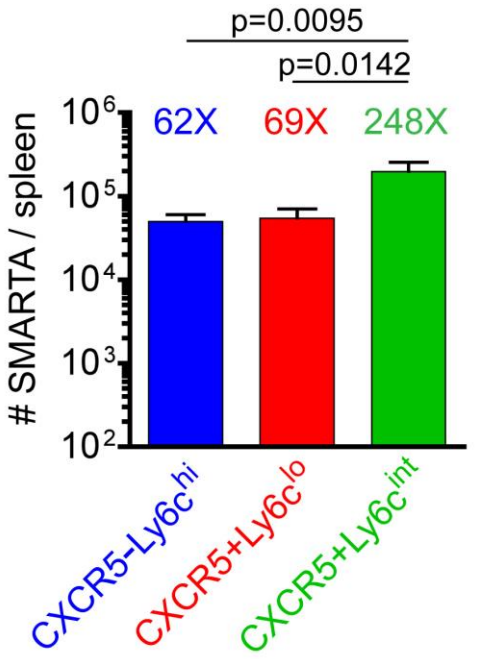
1. **What are mechanisms that reinforce the lineage-specific functions of memory Tfh cells?**

These questions are important for rational vaccine design, where improving the generation and engagement of memory Tfh cells could enhance vaccine-induced immunity.

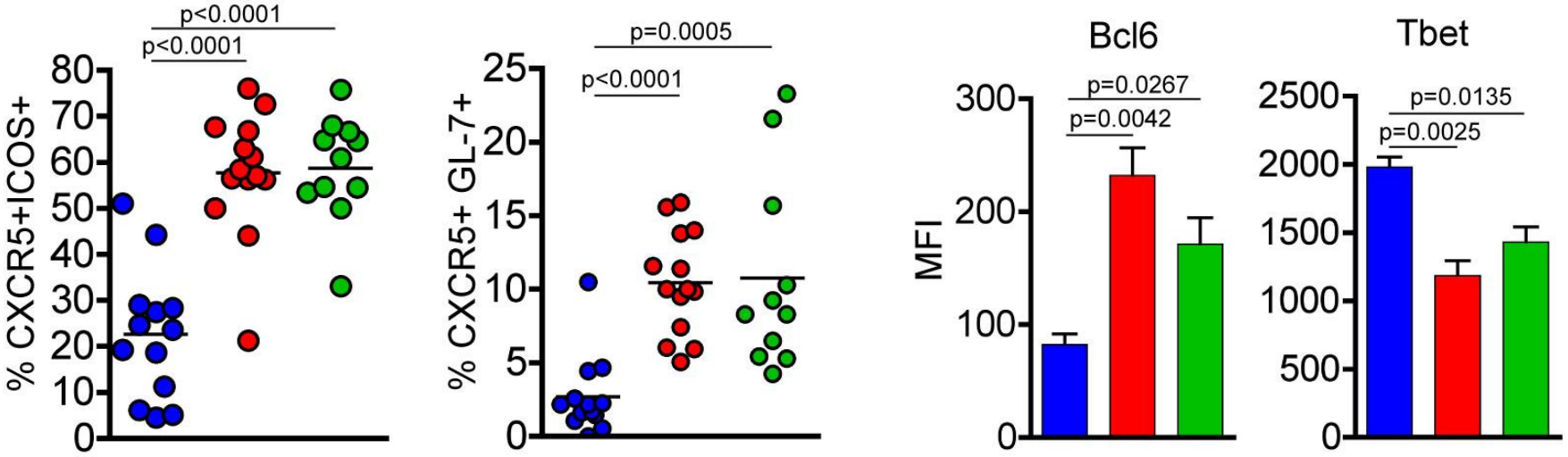
Hale et al. *Immunity* 2013

Do CXCR5+ memory cells preferentially recall Tfh effector responses following antigen reencounter?





CXCR5+ memory cells recall Tfh secondary effector cells

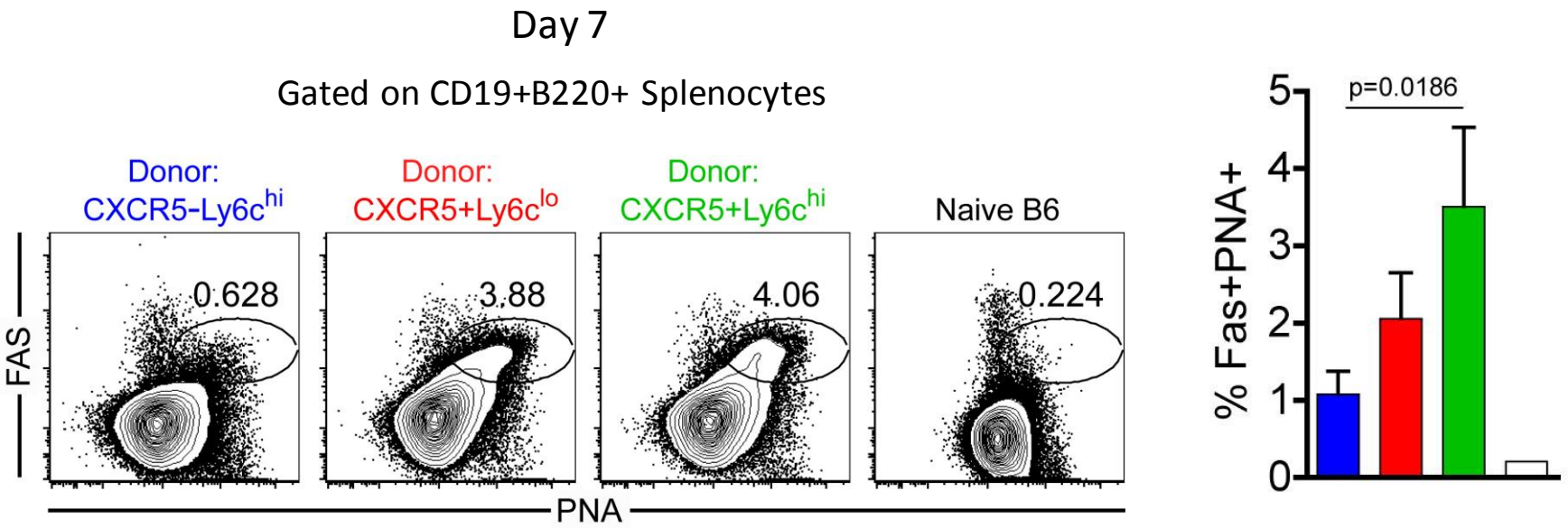


CXCR5-Ly6c^{hi}

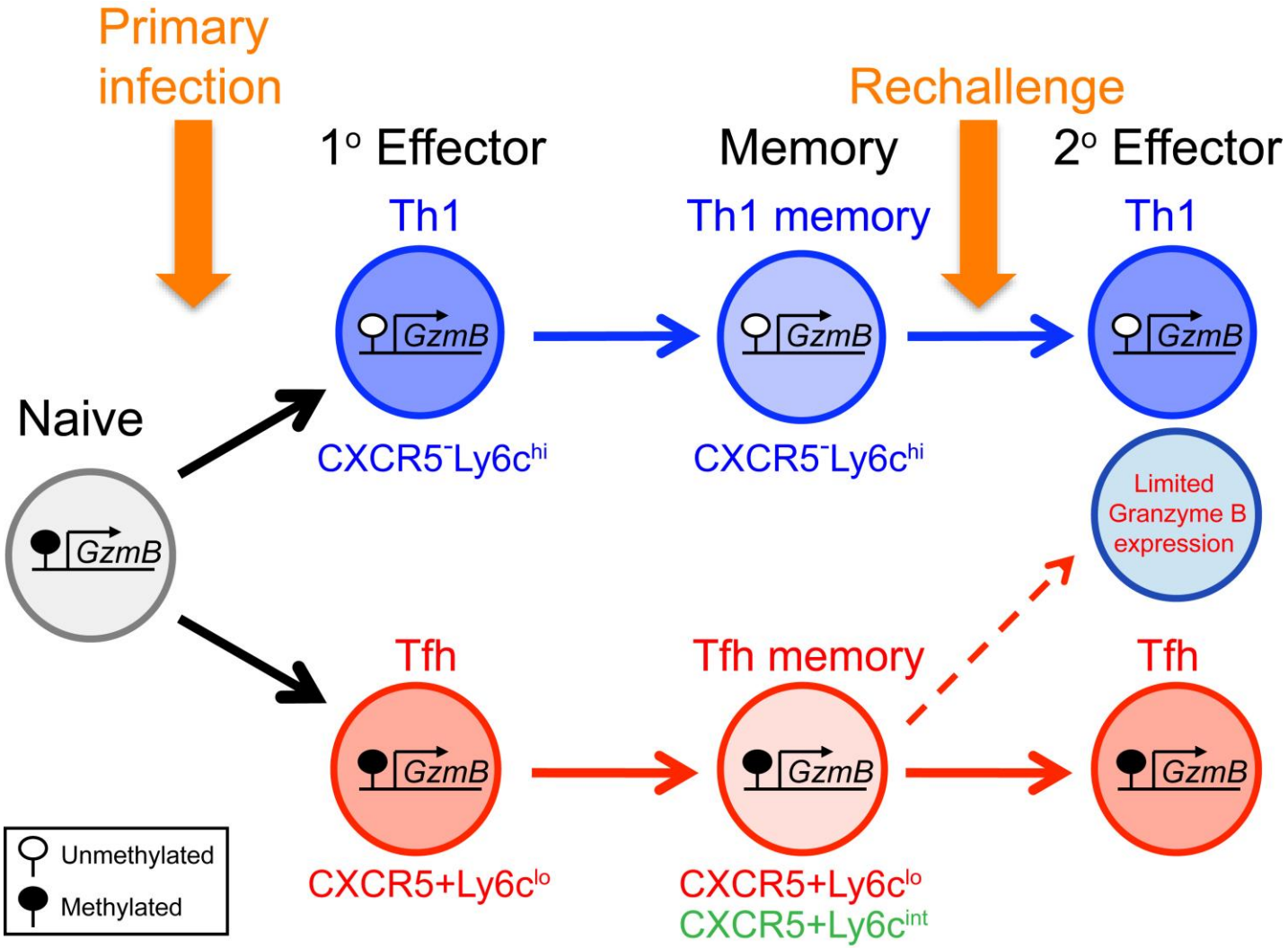
CXCR5+Ly6c^{lo}

CXCR5+Ly6c^{int}

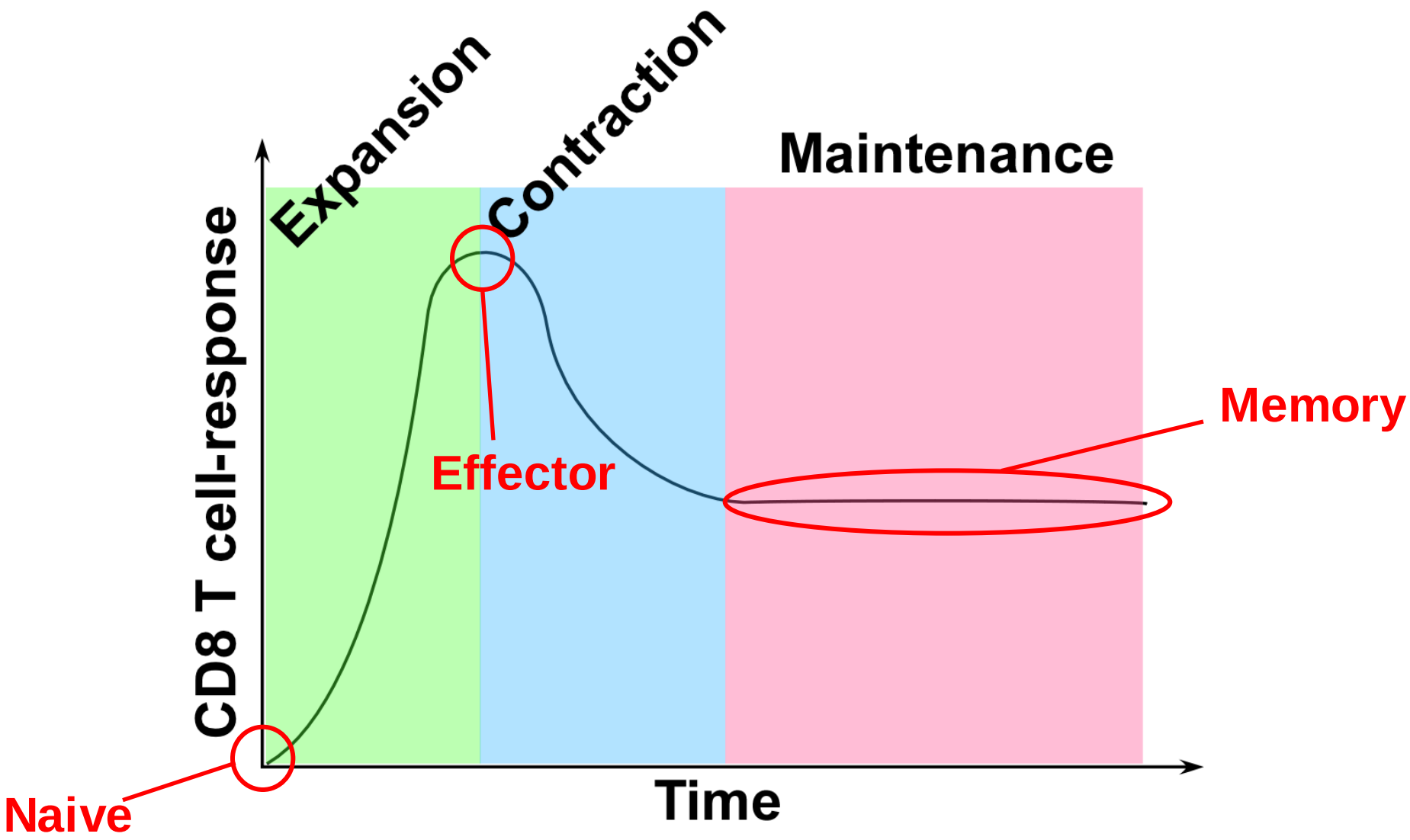
CXCR5+ memory cells promote enhanced germinal center B cell responses following viral challenge



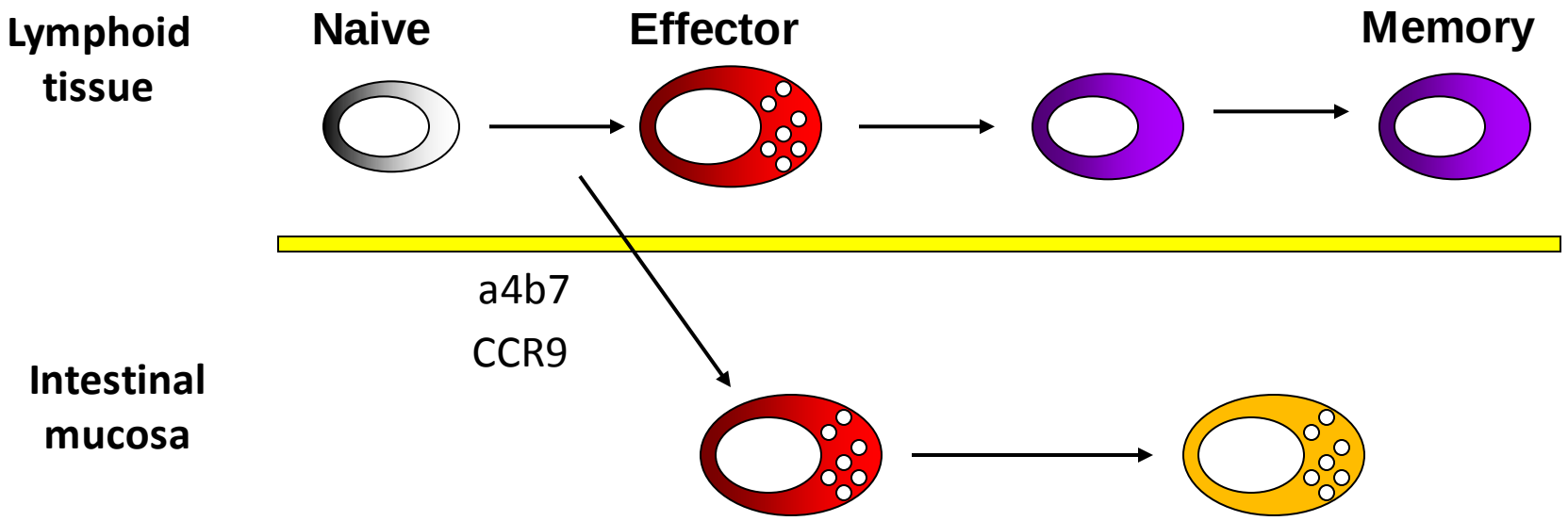
Distinct Tfh and Th1 memory CD4 T cell subsets exist that recall their lineage-specific functions upon reencounter with antigen



Memory CD8 T cell differentiation



CD8 T cells migrate to many non-lymphoid tissues while effectors

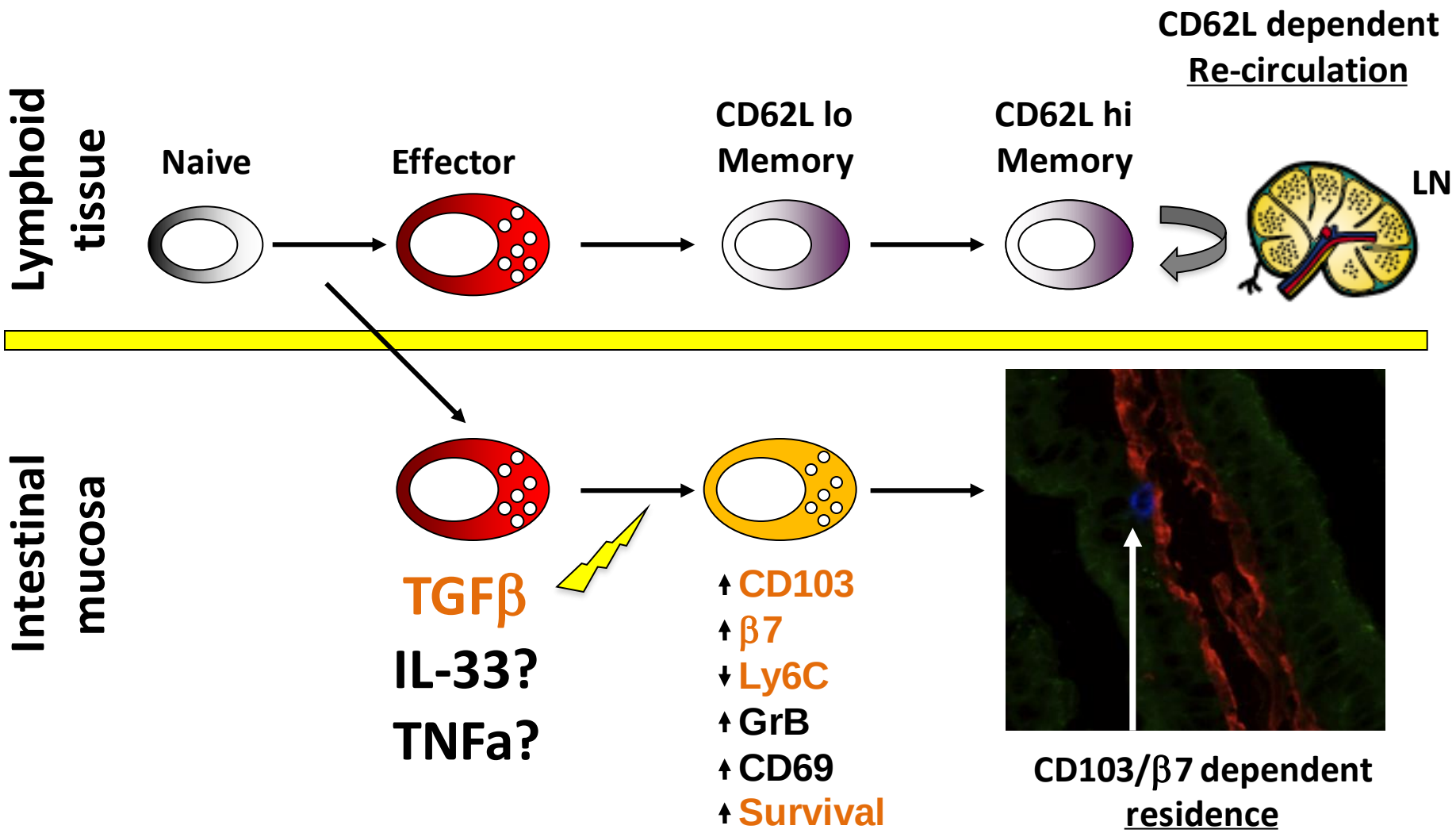


And become resident

Resident memory T cells

- Observed in many tissues
  - Small intestine epithelium
  - Skin
  - Lung
  - Salivary gland
- Do not recirculate with other compartments
- Are not found in blood

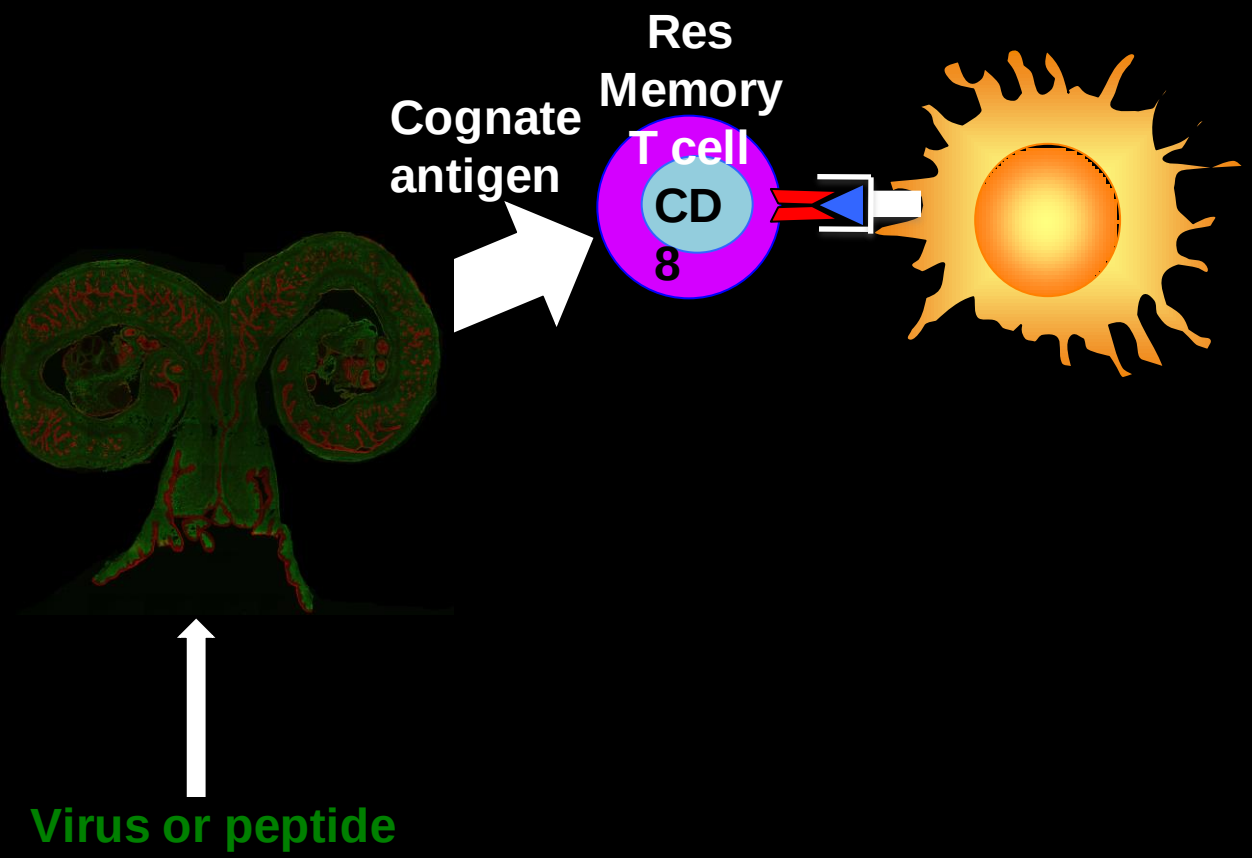
Environmental milieu regulates phenotype and maintenance of resident memory CD8 T cells



Casey, et al., J Immunol 2012

Function of resident memory CD8 T cells extend beyond local killing of infected cells

Sensing and alarm function: when resident memory CD8 T cells encounter Ag in tissues:



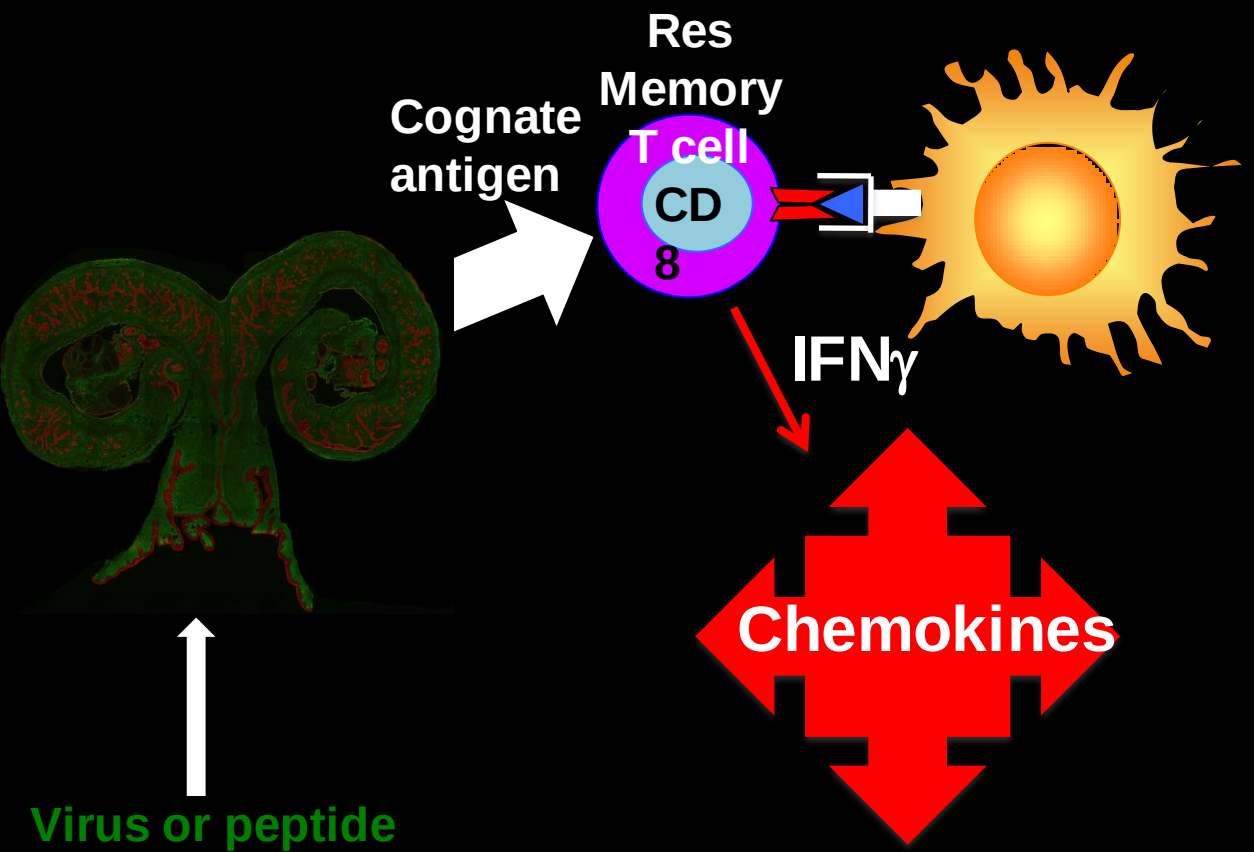
Blood

CD
8

CD
8

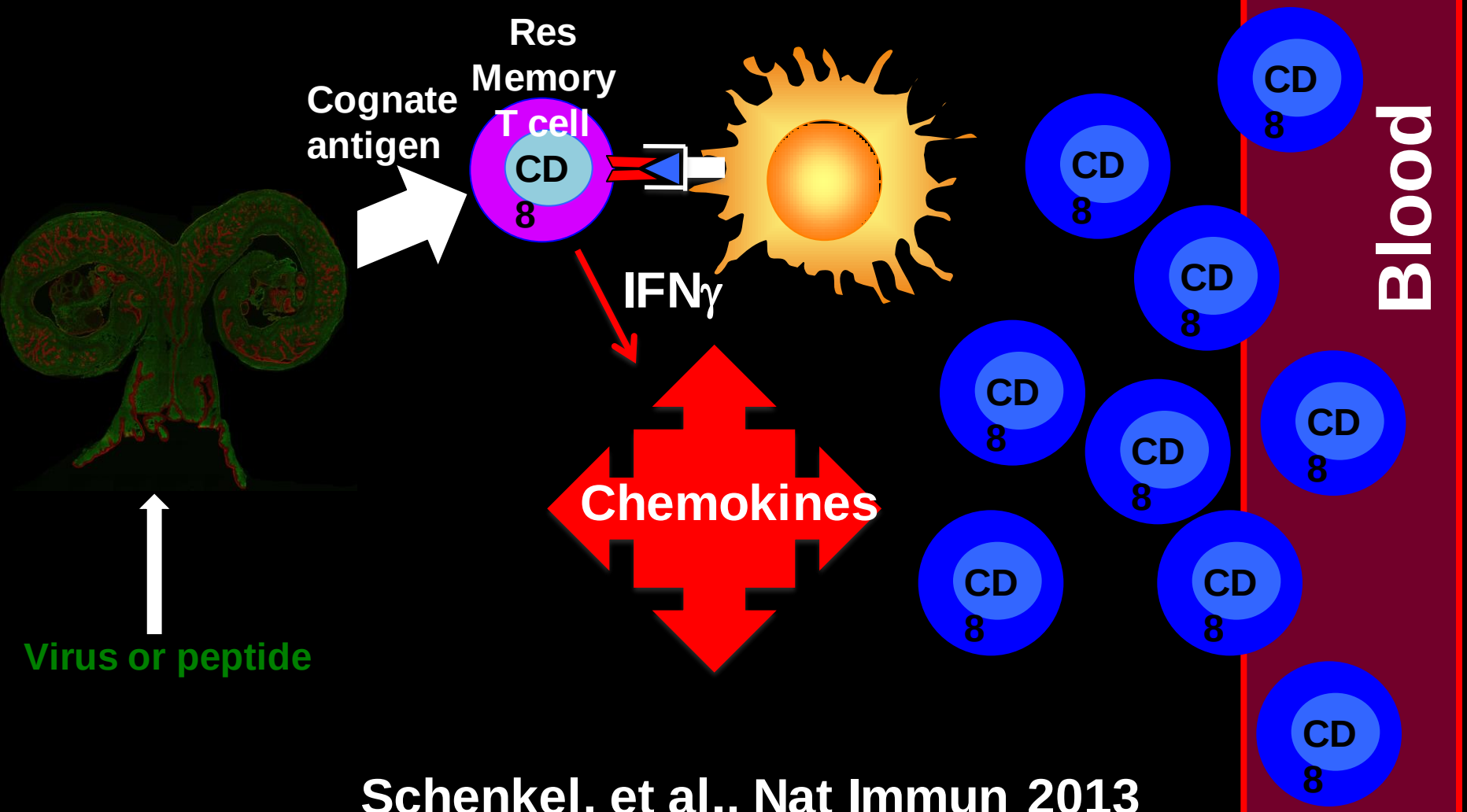
Schenkel, et al., Nat Immun 2013

Sensing and alarm function: when resident memory CD8 T cells encounter Ag in tissues: they rapidly induce local chemokine expression



Schenkel, et al., Nat Immun 2013

Sensing and alarm function: when resident memory CD8 T cells encounter Ag in tissues: they rapidly recruit additional memory CD8 T cells



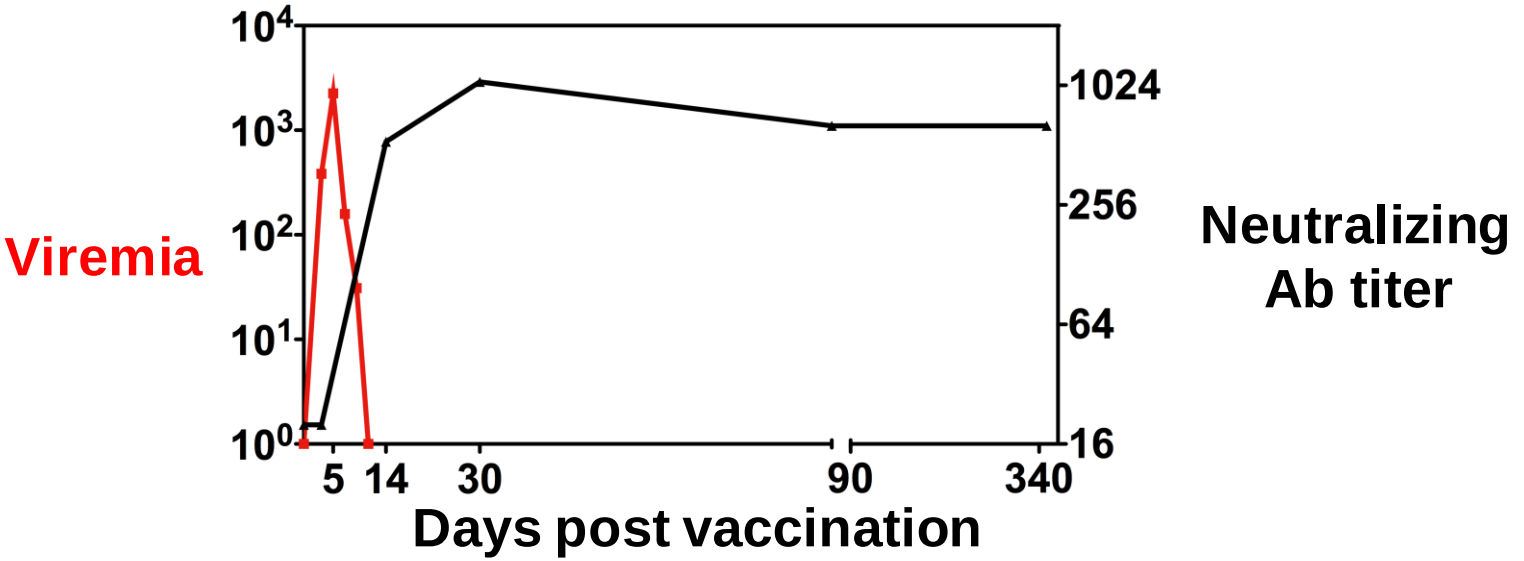
Schenkel, et al., Nat Immun 2013

Protective Immunity by Memory CD8 T cells:

Division of Labor

- **Resident memory CD8 T cells at mucosal sites**
  - **immediate effector function**
  - **limited proliferative capacity**
- **Circulating memory CD8 T cells in blood and lymphoid tissue**
  - **can differentiate into effectors rapidly**
  - **substantial proliferative capacity**

Yellow Fever Vaccine

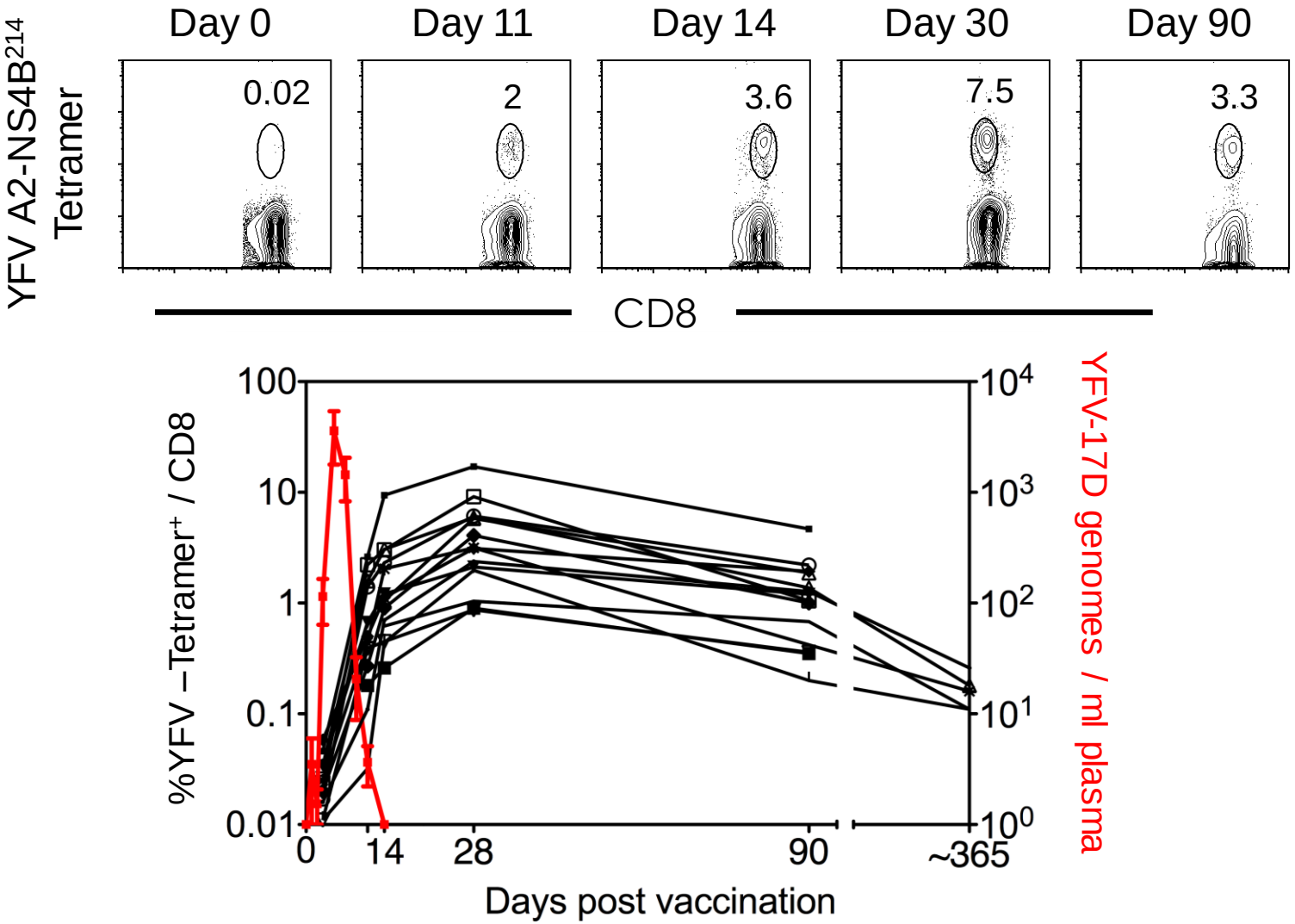


- YFV-17D is a **live** attenuated vaccine.
- A single immunization (~10^5 pfu, subcutaneous) generates **long-term immunity** with neutralizing antibodies appearing by day14.
- Ideal model to study CD8 T cell responses to a **primary**, **acute** viral infection in humans. (Miller et al. *Immunity* 2008)

Yellow Fever Virus Vaccine

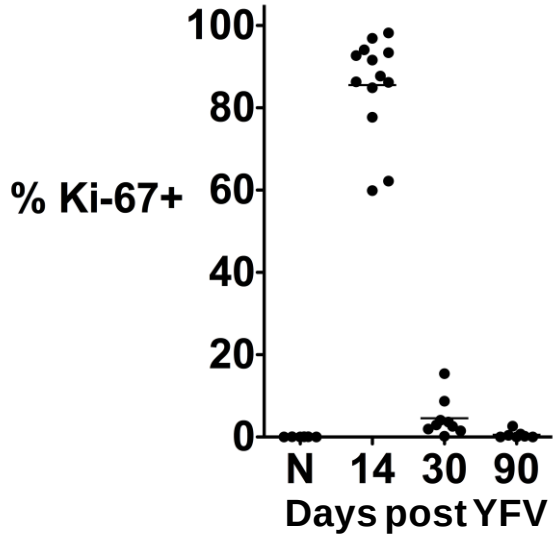
Max Theiler receives the Nobel Prize in Physiology or Medicine from the hands of His Majesty the King Gustaf Adolf VI on December 10, 1951.

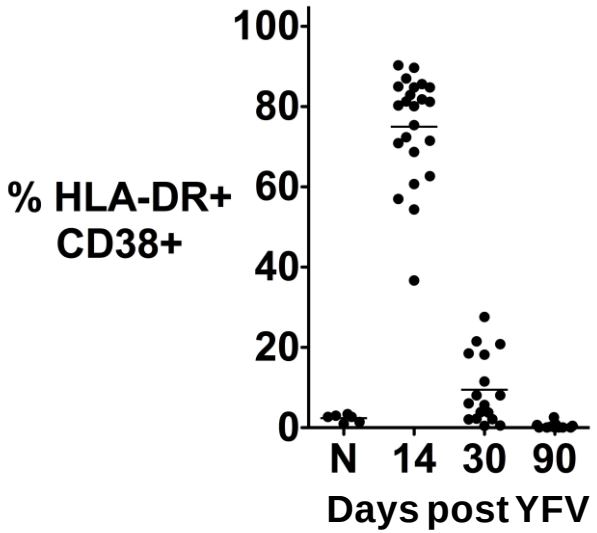
Tracking YFV-specific CD8 T cells

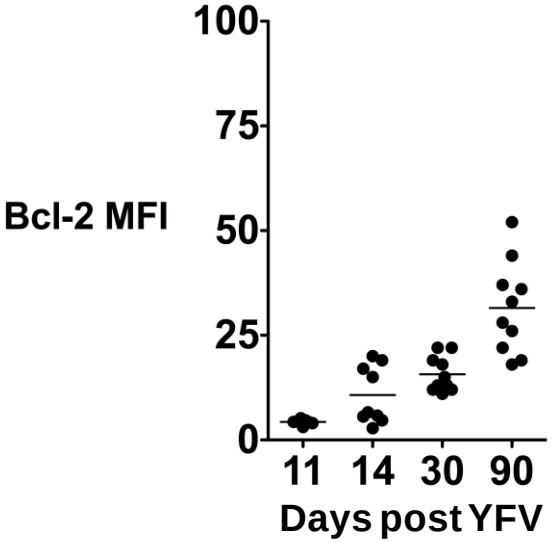


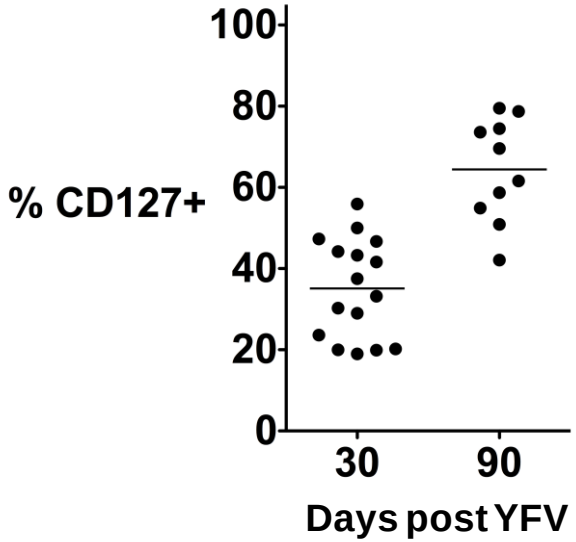
Akondy et al *J.Immunol*, 2009

Differentiation of YFV specific memory CD8 T cells

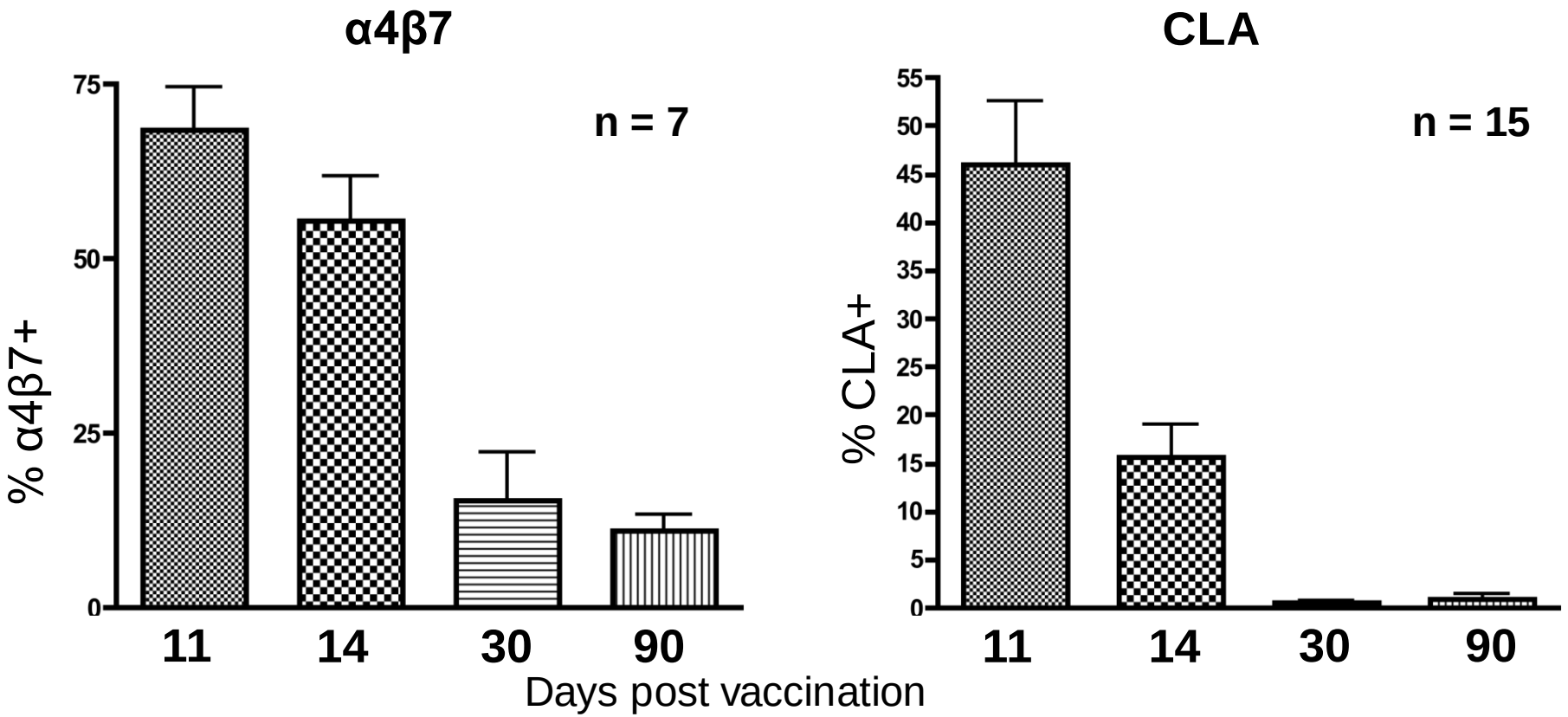






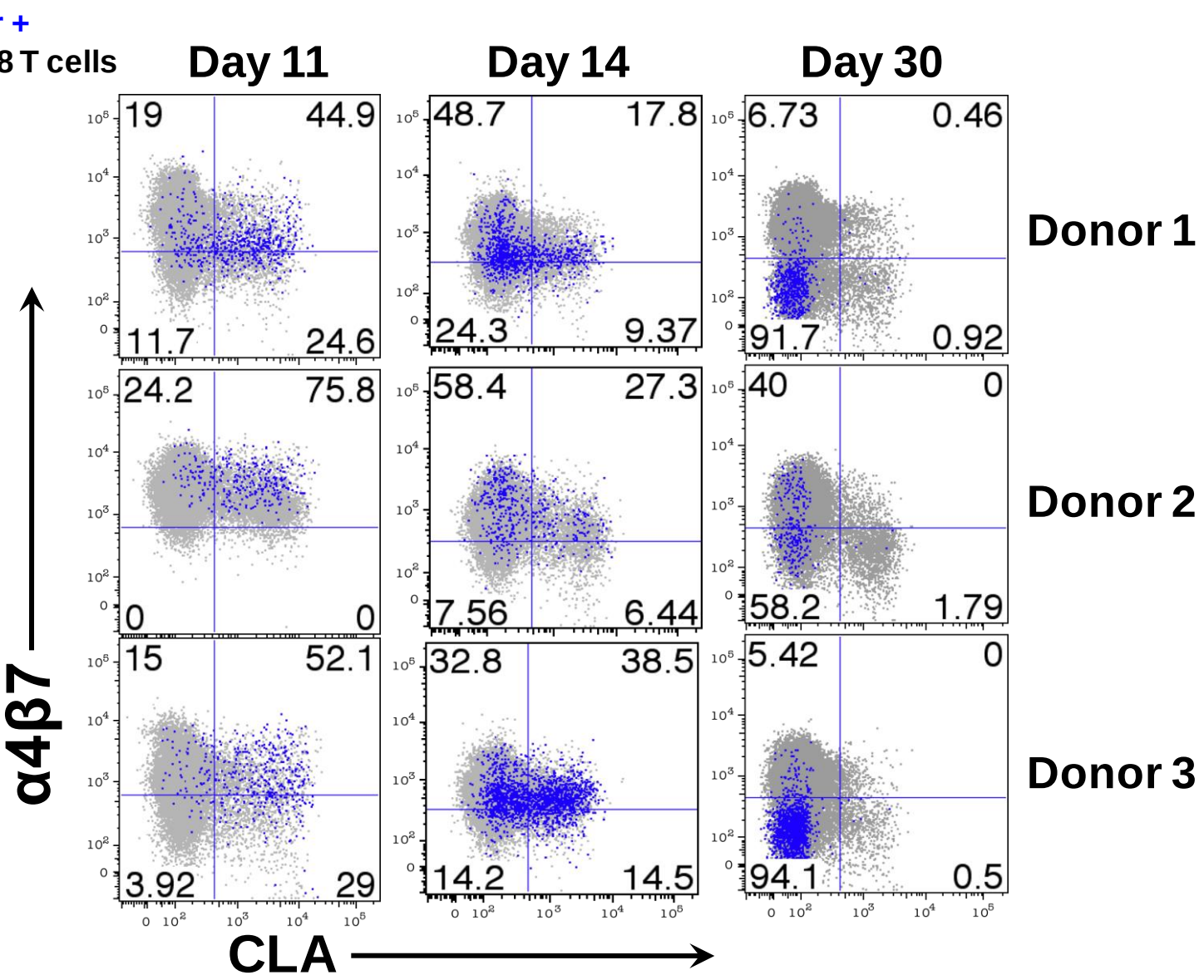


Expression of homing receptors on YFV specific CD8 T cells



α4β7 and CLA are transiently expressed by YFV specific effector CD8 T cells

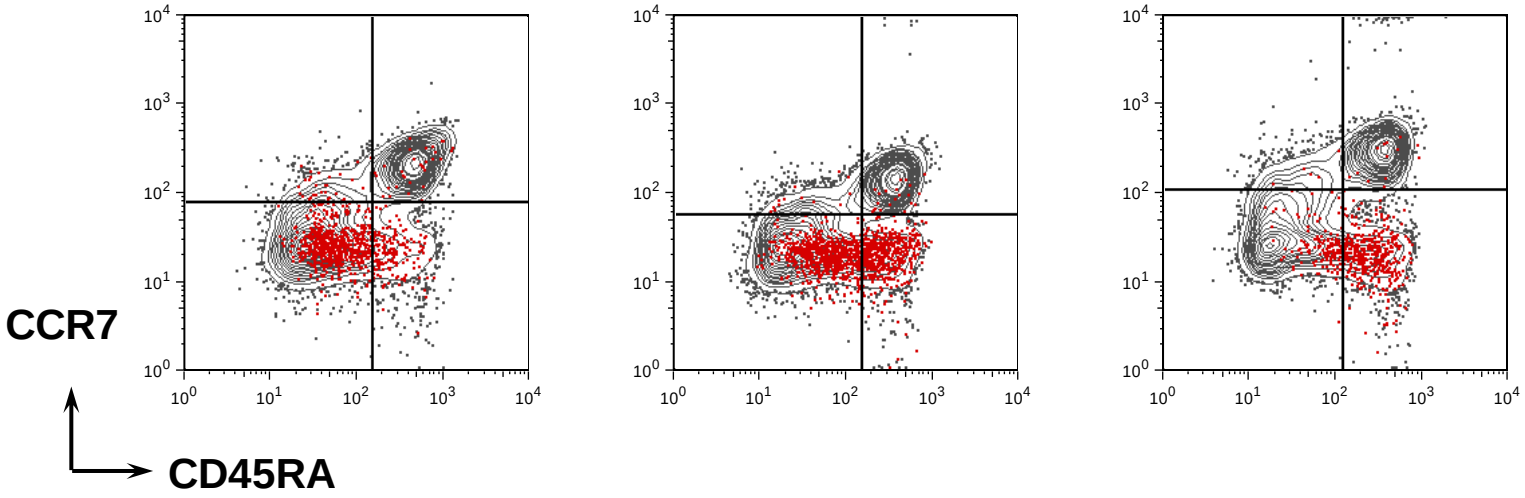
Co-expression of α4β7 and CLA on YFV specific CD8 T cells

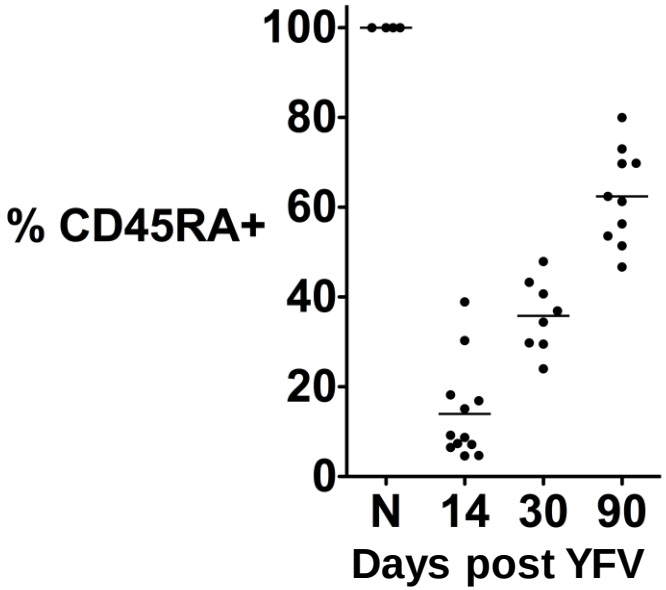


YFV memory CD8 T cells re-express CD45RA

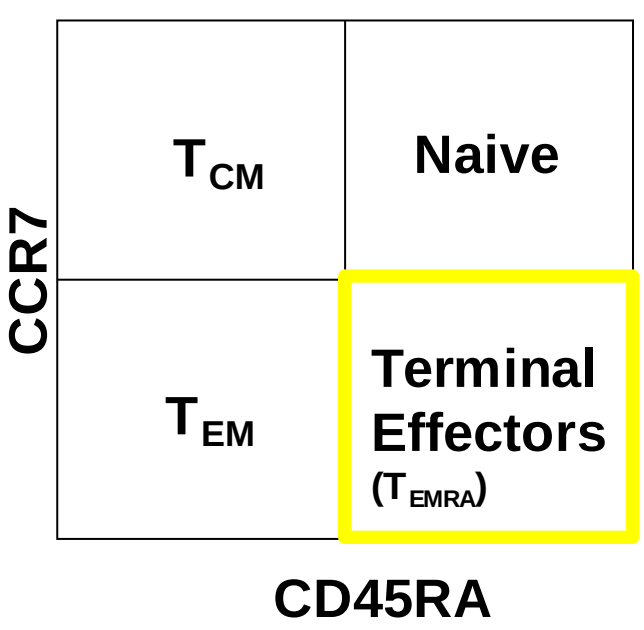
Tetramer positive cells

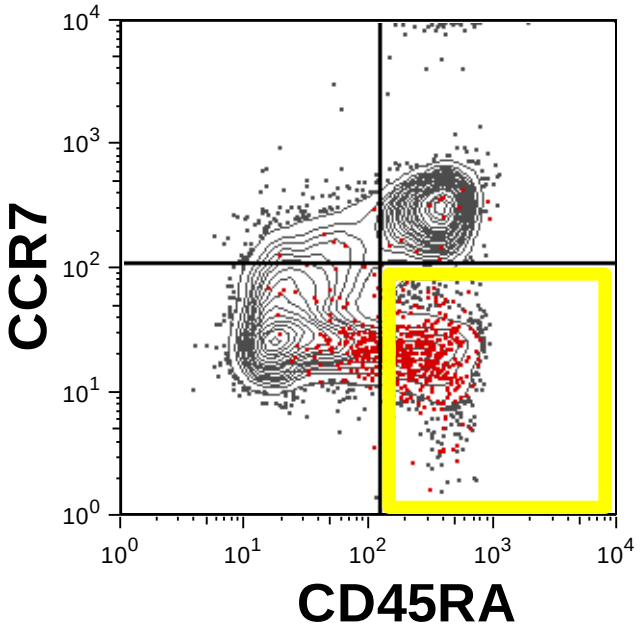
Total CD8 T cells



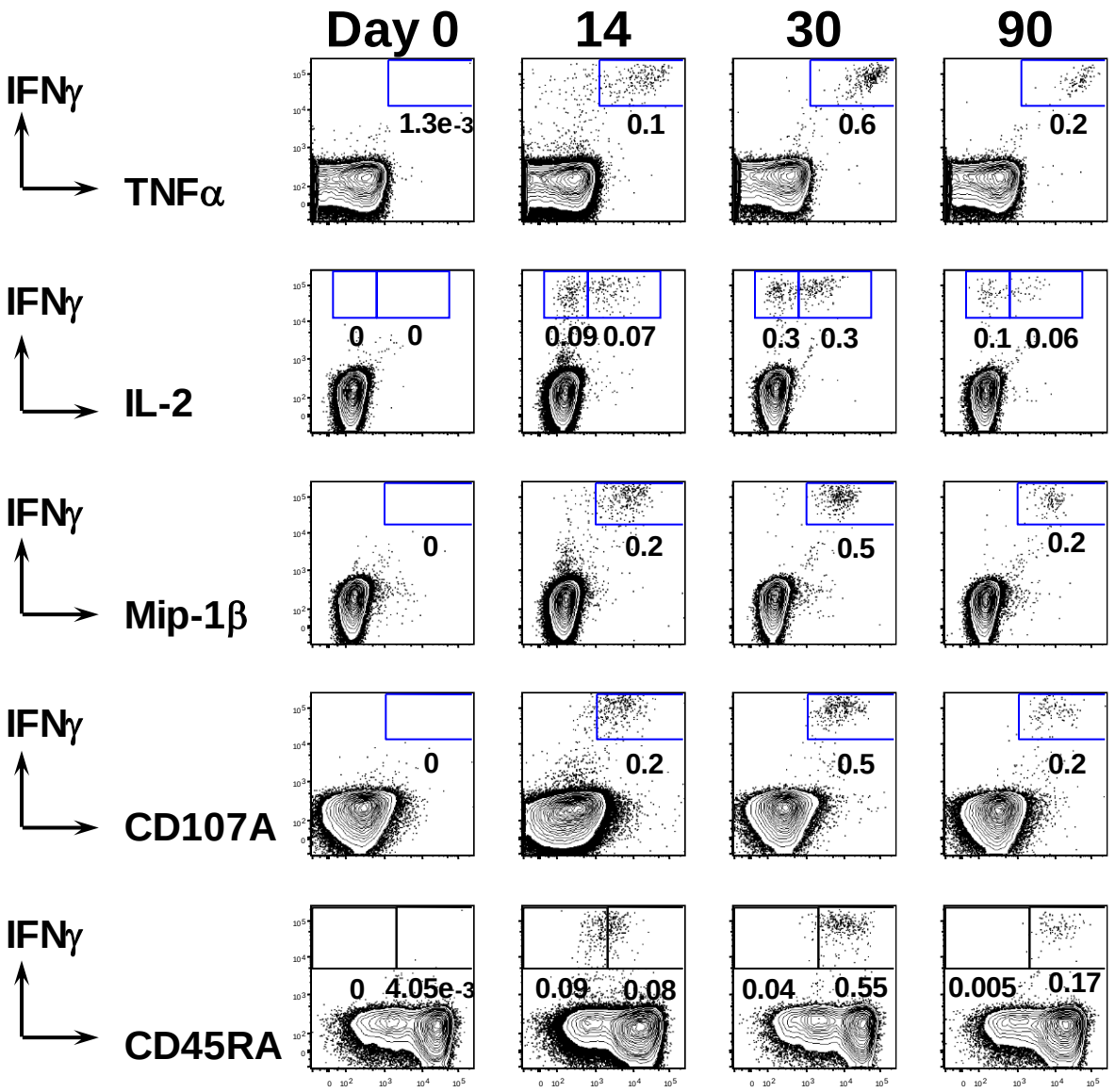


YFV memory cells ‘look like’ terminal effectors

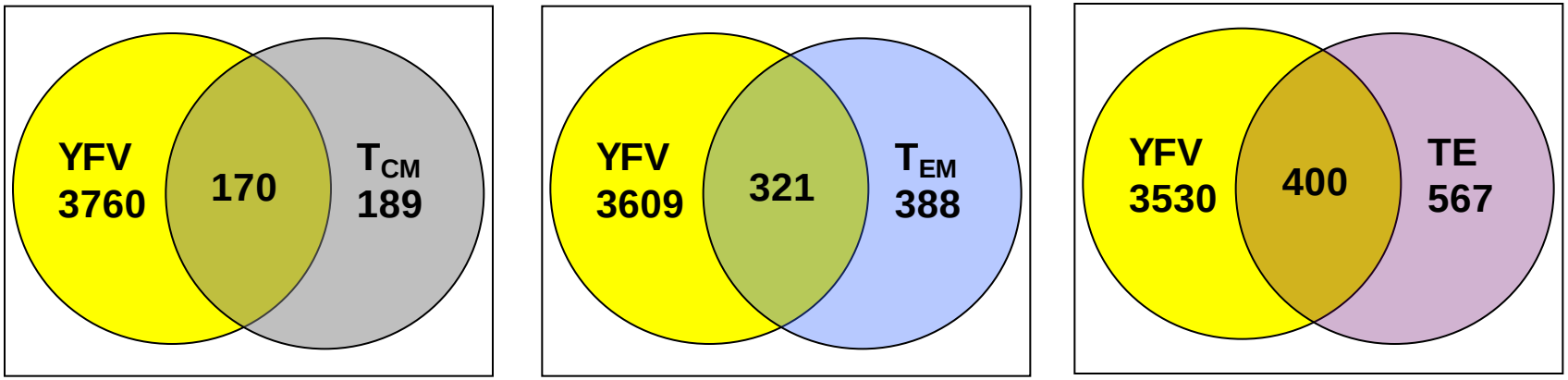




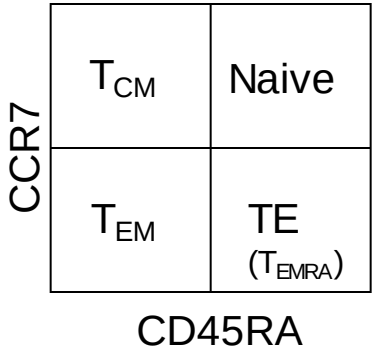
YFV specific CD8 T cells are polyfunctional



YFV specific effector CD8 T cells have an unique gene expression signature



The number of genes that are modulated relative to naïve CD8 T cells (Fold change cutoff 1.8, $p < 0.05$)



Summary

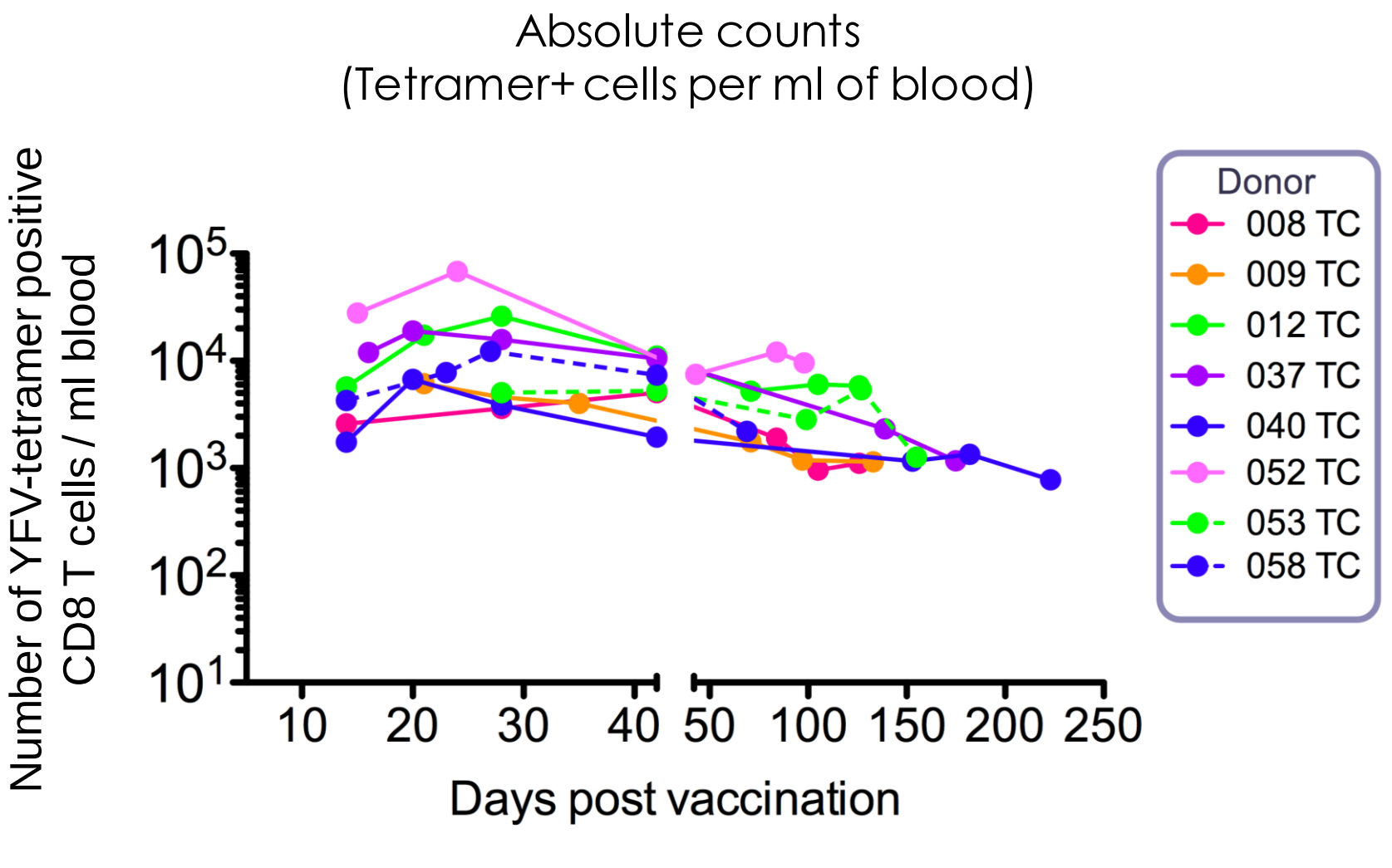
- YFV specific CD8 T cells appear to pass through an effector phase before undergoing memory differentiation.

- YFV specific effector CD8 T cells transiently express homing molecules to not only the skin (CLA) but also the gut (α4β7) even though the vaccine is given subcutaneously. A “window of opportunity” for effector cells to migrate to the mucosal sites.

- Effector CD8 T cells are characterized by a gene expression signature that is distinct from that of memory and effector T cell subsets.

- YFV specific memory cells re-express CD45RA and continue to be CCR7 negative, a phenotype associated with terminal differentiation. However, they are polyfunctional and exhibit high proliferative potential.

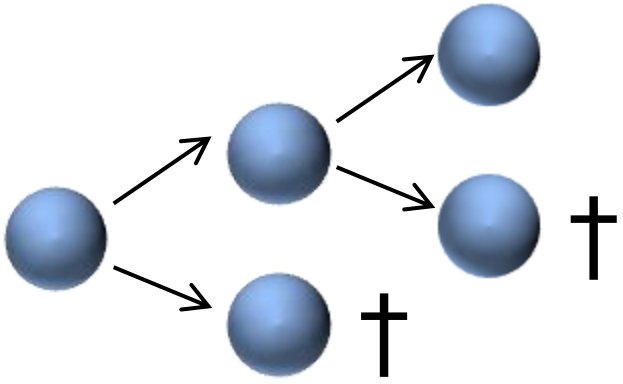
Persistence of the YFV-specific CD8 T cell response



How are memory CD8 T cells maintained?

Truly long-lived, quiescent cells

Rapid replenishment (division and death)

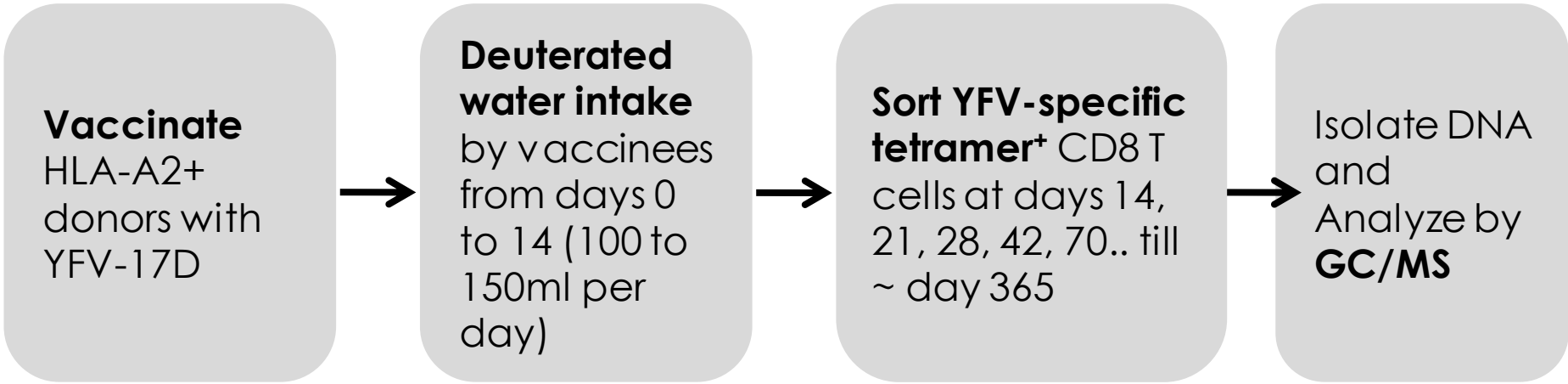


Using ^2H_2O to determine the lifespan and turnover of YFV-specific CD8 T cells

- Deuterium ($^2H_2$) is a non-radioactive isotope of hydrogen.
- ^2H_2O intake is safe and has no effect on physiologic processes at the dose used.
- Labeling can be monitored by sampling body water from saliva, urine or plasma.
- **GC/MS** used to quantify labeled DNA is **sensitive** and **reproducible** (inter-experiment variation s.d < 0.1%).
- **It makes *in vivo* analysis in humans possible.**

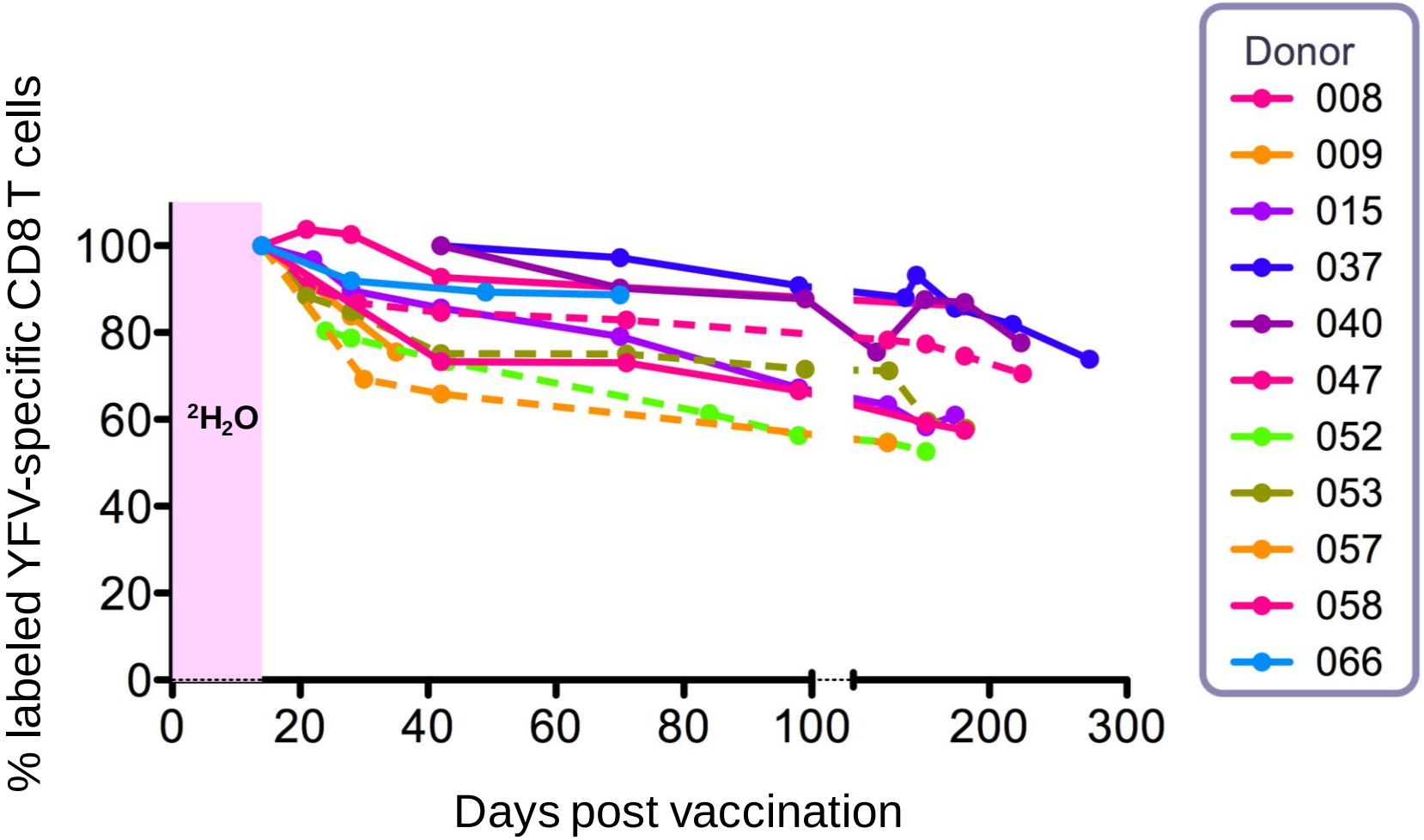
Hellerstein MK, et al. *J Clin Invest*, 2003

Experimental design



Life-span and turnover of YFV-specific CD8 T cells

$^2H_2$ labeled cells can be seen more than 1 year after vaccination



Conclusions

- **Label die-away is minimal with a half life of ~500 days.**

- **YFV-specific memory CD8 T cells are dividing once every 500 days with a very long inter-mitotic phase.**

- ***Thus, the YFV vaccine is inducing truly long-lived memory CD8 T cells in humans.***