Cellular Adaptive Immunity to Control HIV Infection: Manipulation of T Cell Trafficking in SIV-Infected Macaques

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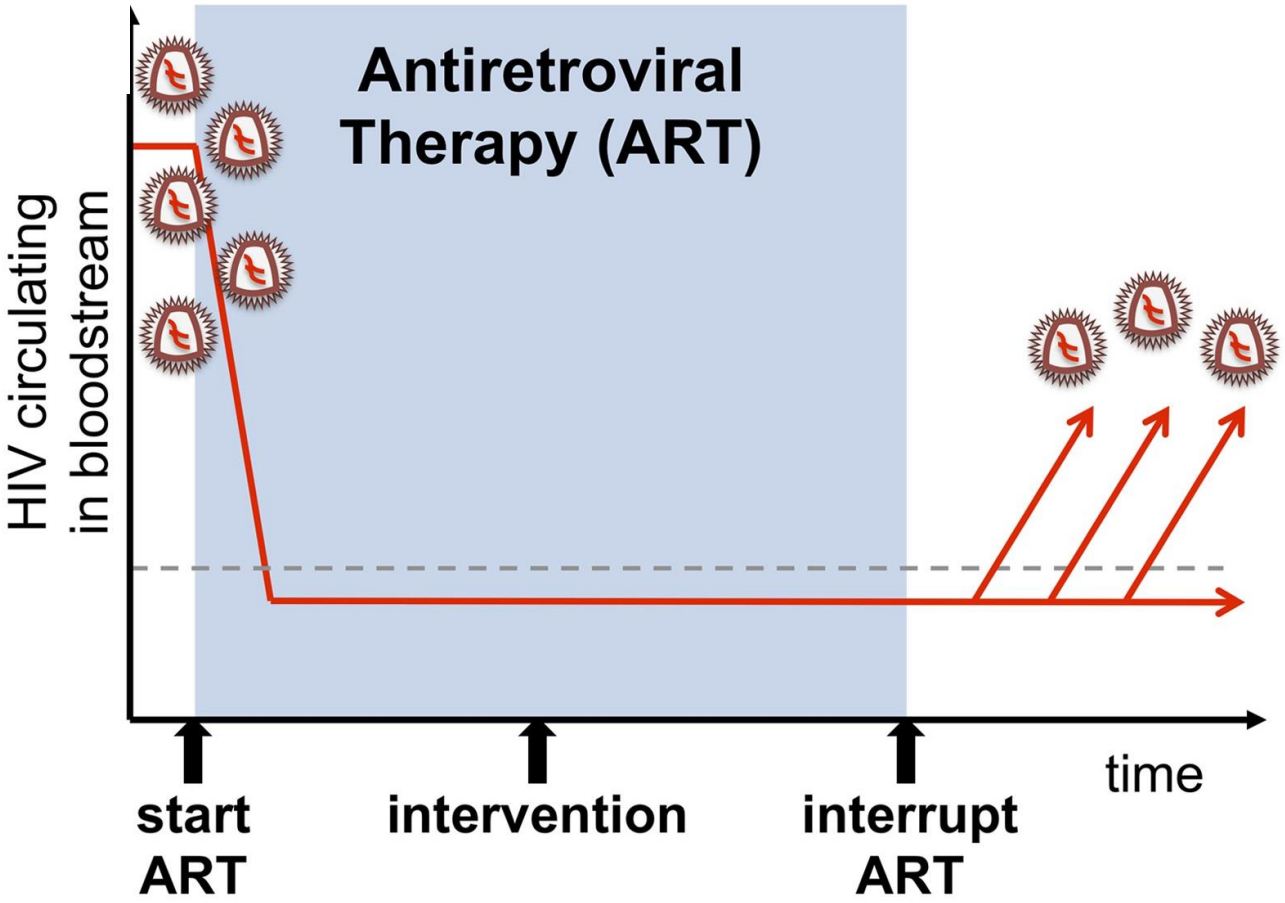
Disclosures:

- MRB is consultant for Interius Biotherapeutics

CD8+ T cell activity is attributed to control of HIV/SIV disease progression

- Depletion of CD8+ T cells from monkeys during chronic SIV infection results in a rapid and marked increase in plasma viremia
- HIV-specific CD8+ T cells from non-progressor PLWH maintain high proliferative capacity coupled to increases in perforin expression
- HIV-specific CD8+ T cells from non-progressor PLWH display an enhanced ability to express perforin directly *ex vivo*
- The ability of CD8+ T cells to upregulate perforin following in vitro stimulation correlates inversely with viral load

The latent HIV+ CD4+ T cell reservoir in tissues is the major barrier to cure



Cockerham & Deeks 2014, eLife

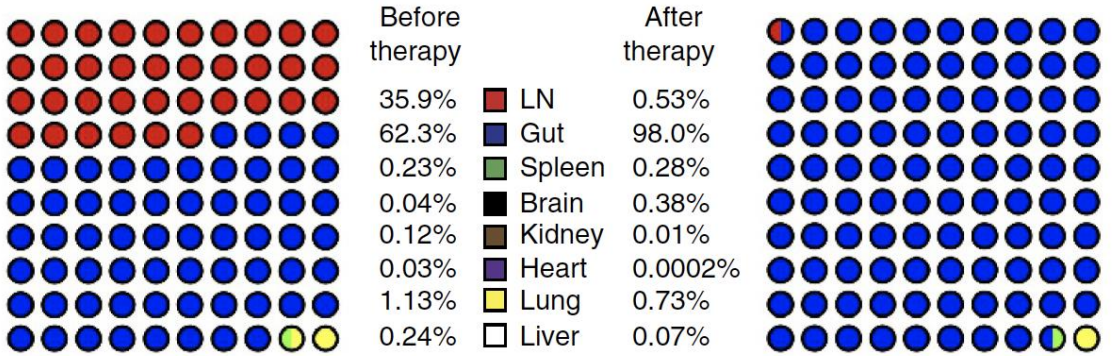
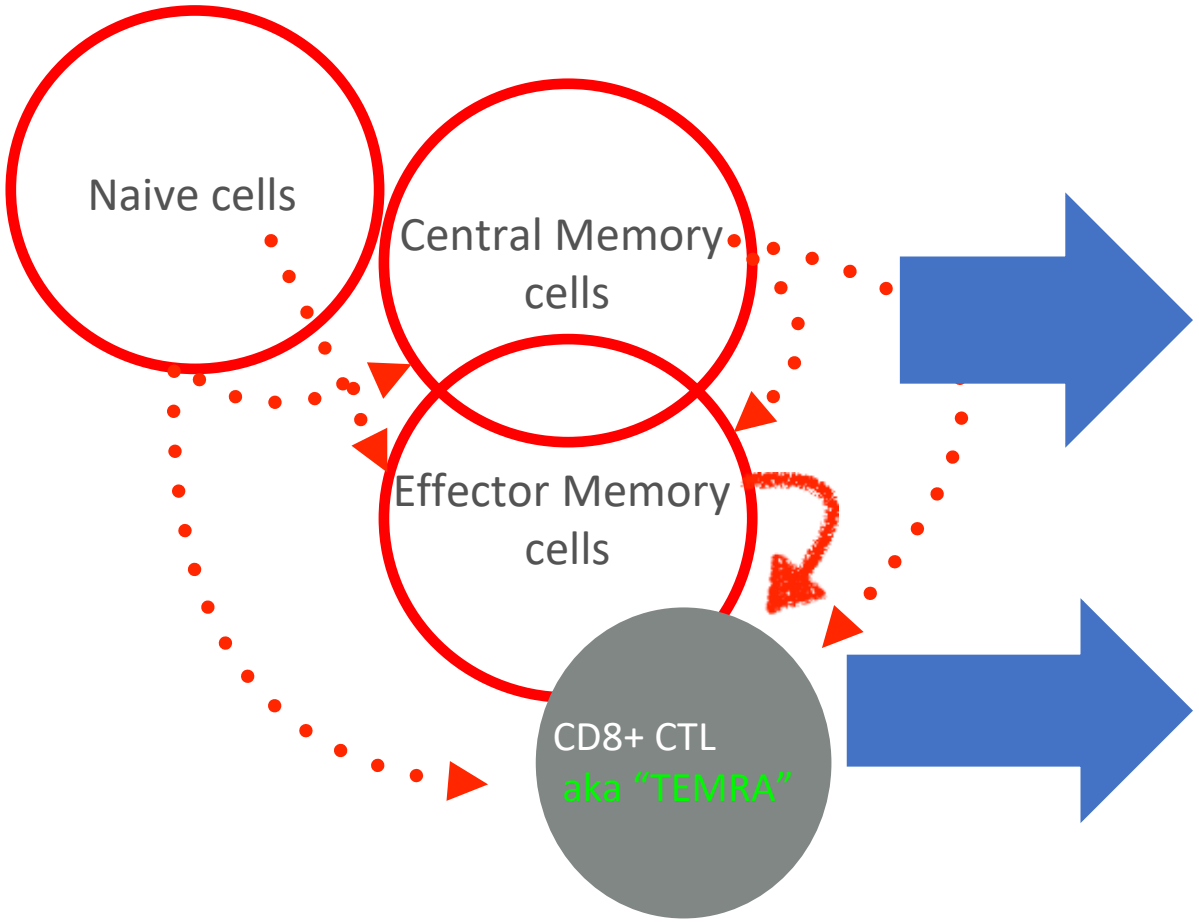


Figure 1 Graphical representation of the proportion of vRNA+ cells in each organ system before and during suppressive ART.

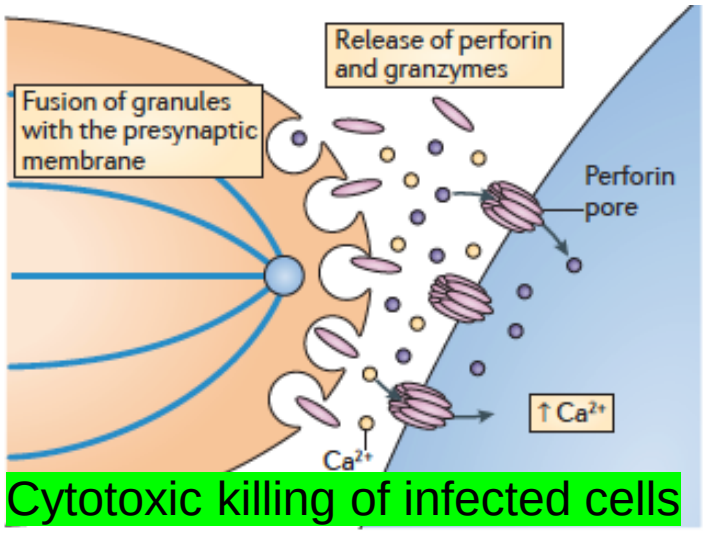
Estes et al 2017, Nat Med

Why can't CD8+ T cells clear infected cells from tissues?

Not all CD8+ T cells are classical "CTL" (cytotoxic T lymphocytes)

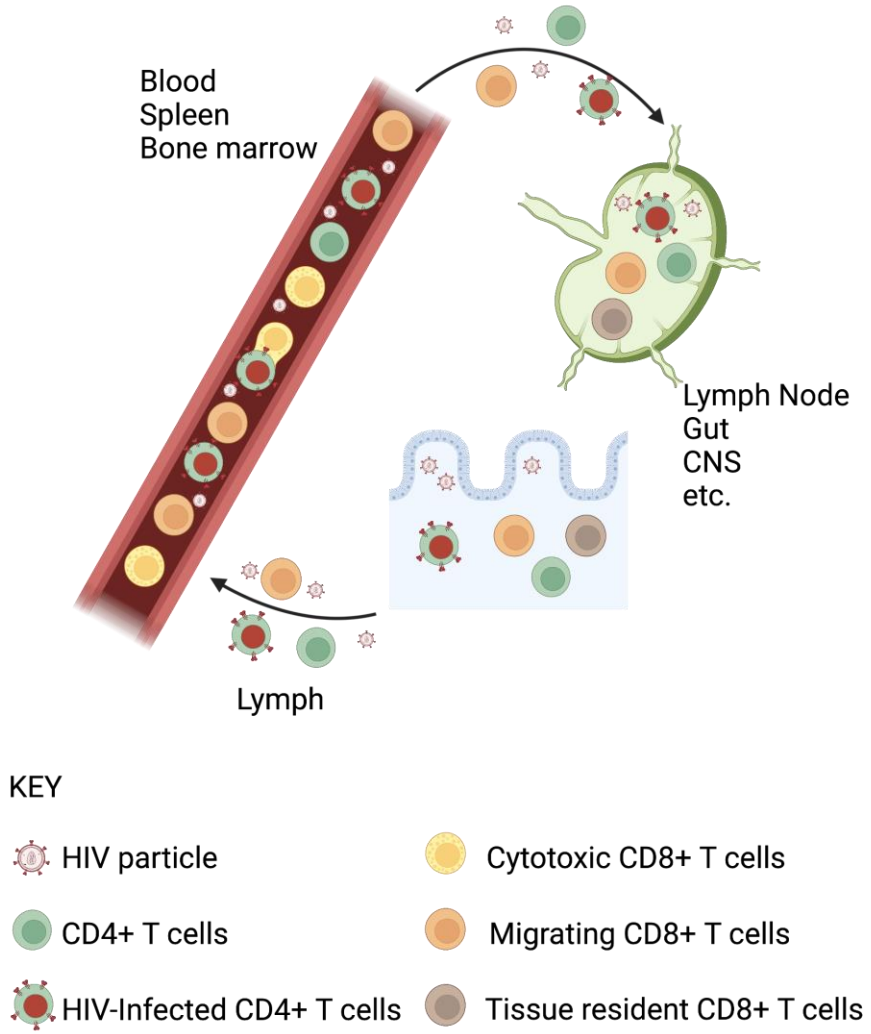


Cytokine producers: TNF, IFNg, IL-2, chemokines, etc.



Cytotoxic killing of infected cells

CD8+ T cell function differs between the vasculature and tissues



- Cytotoxic CD8+ T cells from blood are lost from lymphoid tissues after acute HIV/SIV infection and excluded from lymph nodes and tissues in chronic infection
  - *Buggert, SI 2018, Cell 2020; Reuter, Cell Reports 2017; Roberts, PlosPath 2016; Kiniry, Muc Immunol 2017, JI 2018; Nguyen, STM 2019*
- CD8+ T cells in lymph nodes and tissues are poorly cytotoxic and may function primarily by non-cytotoxic mechanisms
  - *Kiniry, Muc Immunol 2017, JI 2018; Nguyen, STM 2019; Buggert, SI 2018, Cell 2020*
- Underlying differential transcriptional and epigenetic regulation drives tissue vs. vascular CD8 function
  - *Buggert, SI 2018, Cell 2020*

How does this all apply to the role of CD8s in controlling - or not controlling- HIV?

- Are cytotoxic HIV-specific CD8+ T cells lost from tissues after acute infection due to trafficking or regulation?
- Can HIV-specific CD8+ T cells from the blood enter tissues and become cytolytic if activated?
- Can HIV-specific CD8+ T cells in tissues control plasma viremia?

How can we address these questions in vivo?

Study Concept

Failure to effectively control or eliminate HIV may be due to the inability to induce or maintain CD8+ T cell cytotoxicity in lymphoid tissues

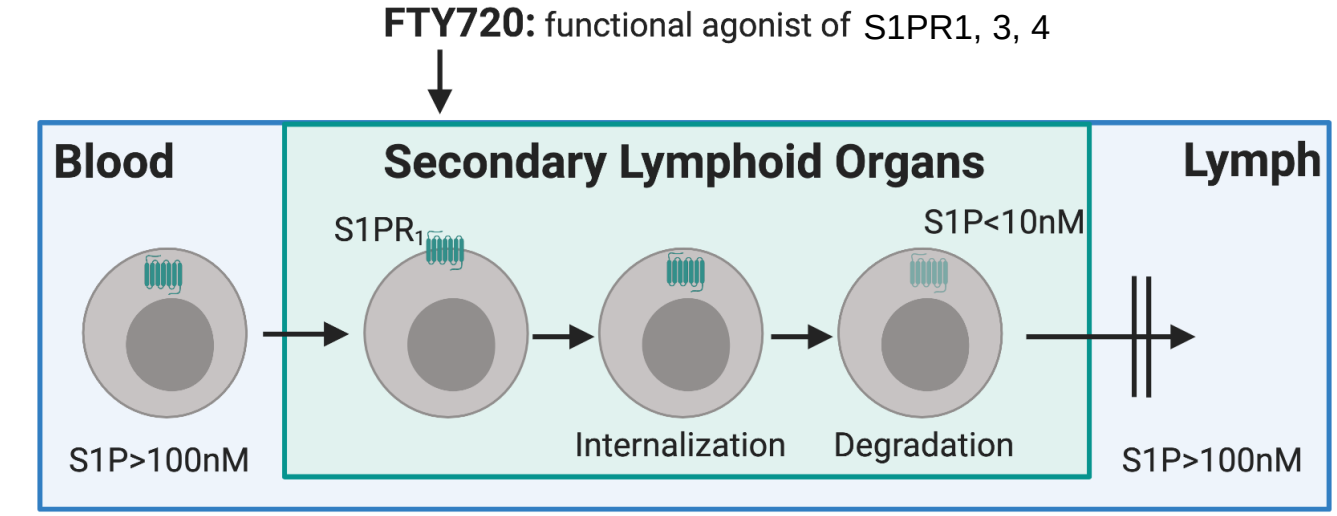
Goals of the study

- *Define the differential roles of tissue vs vascular CD8+ T cells in control of SIV infection*
- *Determine whether lymphoid tissues CD8+ T cells become cytotoxic after encounter with infected cells in lymph node*
- *Determine whether immunomodulation of lymphoid tissues CD8+ T cells enable cytotoxicity and control of viremia*

Strategy

Manipulate T cell trafficking to determine whether CD8+ T cells can become cytotoxic in tissues of SIV-infected RM and control of viral replication during ATI

FTY720 inhibits the S1P-dependent lymphocyte egress from tissues, including lymphoid tissues

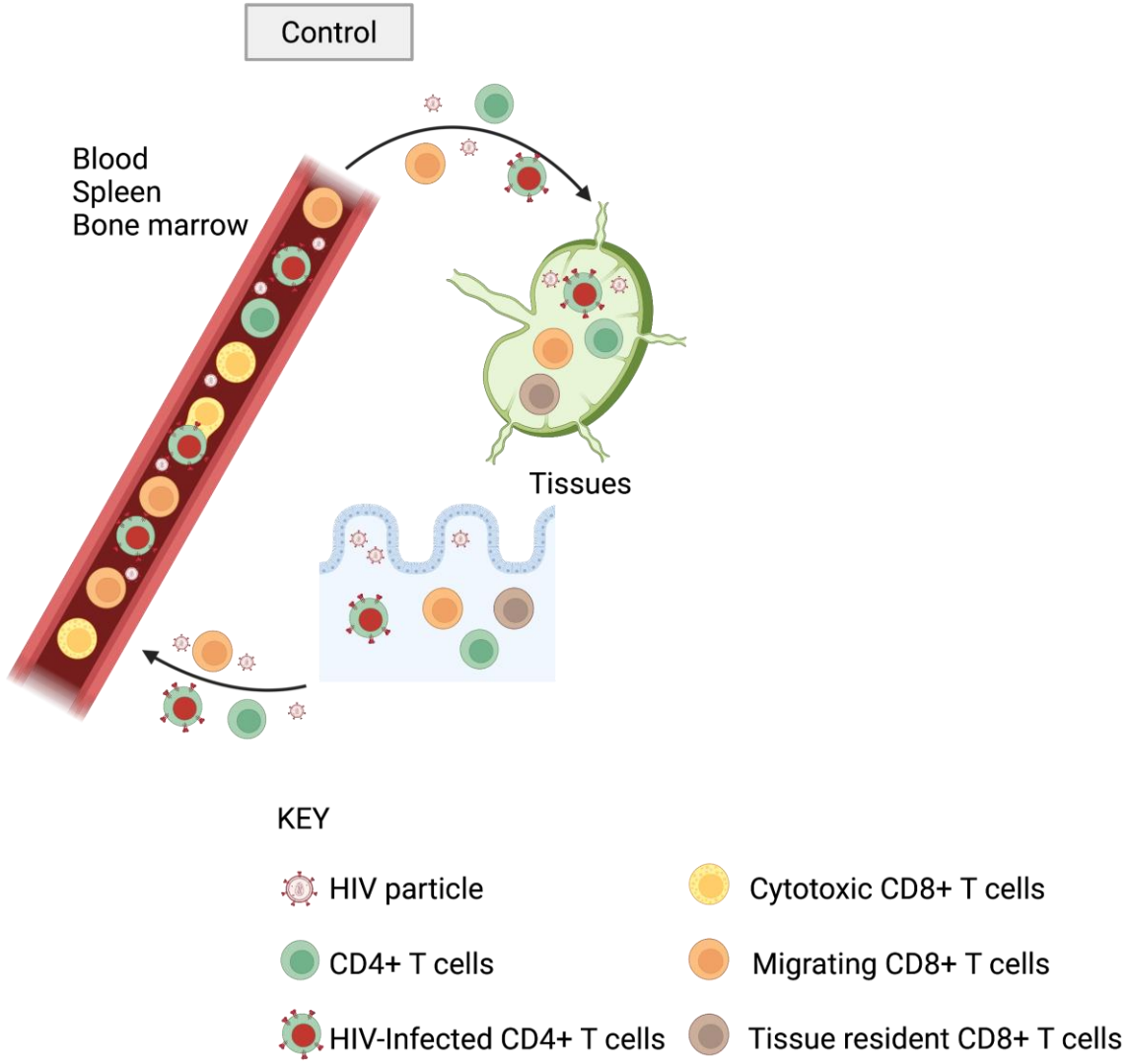


Made with BioRender, taken and modified from Chiba et al 2012, Pharmaceuticals

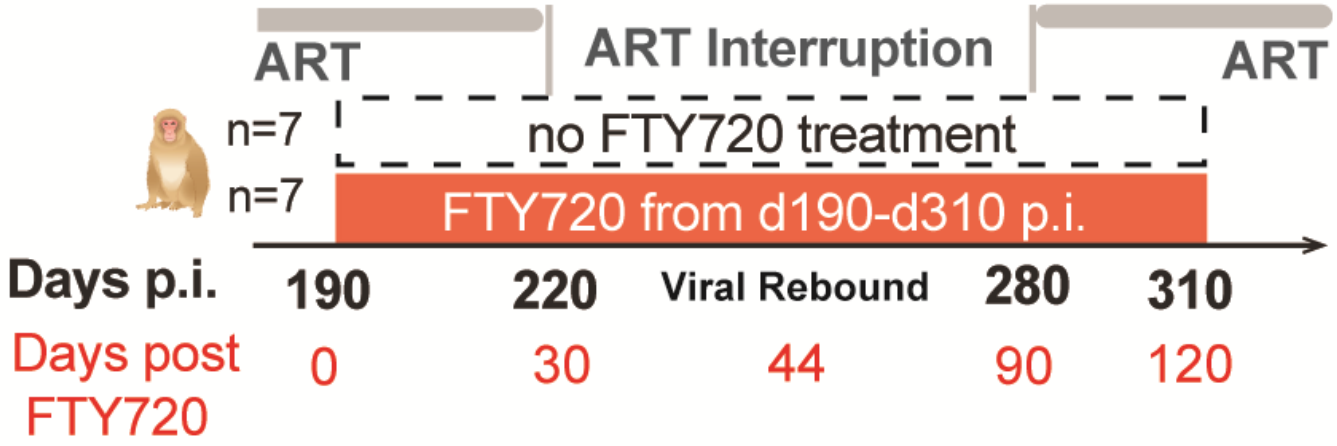
- All lymphocytes capable of trafficking to tissues cannot return to blood under FTY720; this includes the majority of infected CD4+ T cells
- Fully differentiated cytotoxic (Perforin^{+}) CD8+ T cells are retained in blood during FTY720 treatment *(Buggert et al.2020, Cell)*
- Known to be safe in rhesus macaques in the context of SIV infection; during ART does not cause viral rebound; retains CD8s in LN of SIV infected macaques; and administration at ART initiation does not imprint immunological control of viremia at a later ART interruption (Pino et al., PloS Path 2019 and NComm 2022)

What will happen to T cells during FTY720 treatment?

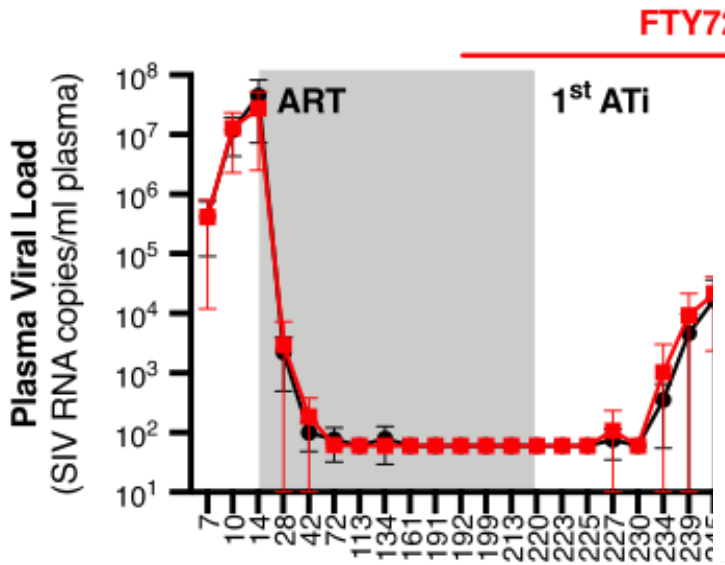
1. CTL remain in blood (both CD4 and CD8)
2. Migratory CD4s and CD8s recirculate
3. Infected CD4s recirculate
4. Resident CD4s and CD8s remain in tissues



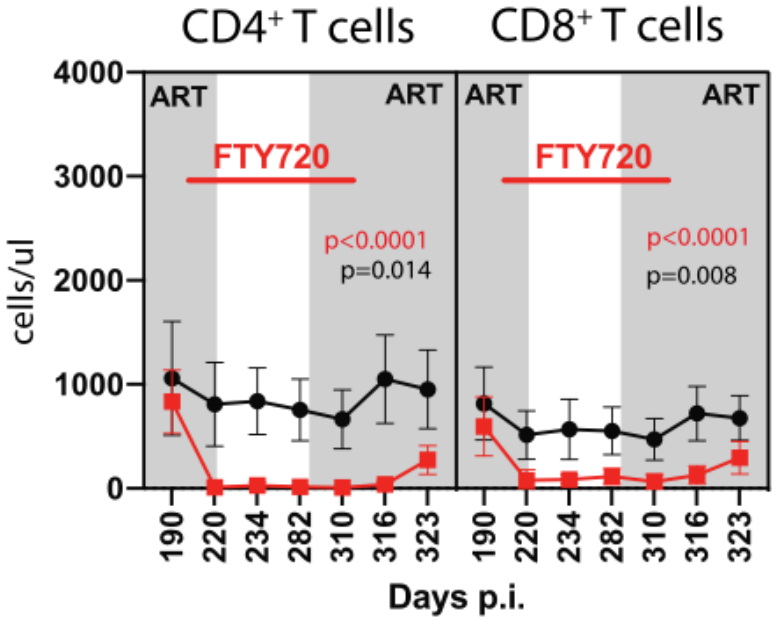
NHP study design



n=14 MamuA*01+ female RM
Challenge virus: SIVmac239M
Studies conducted at Emory Primate Center

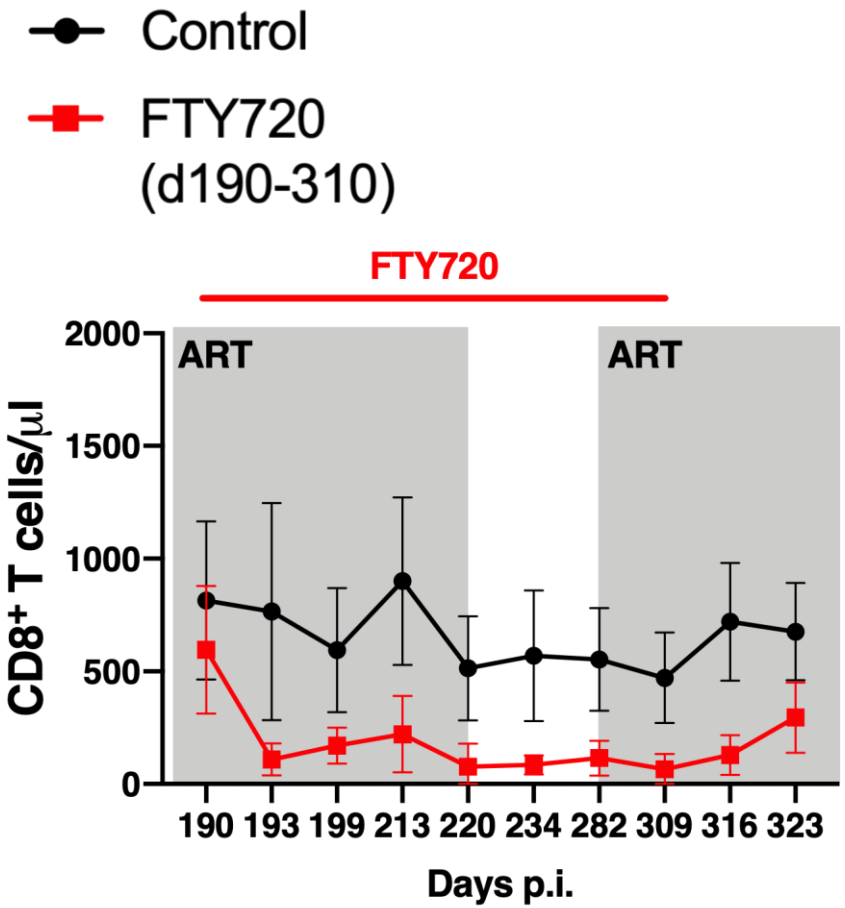


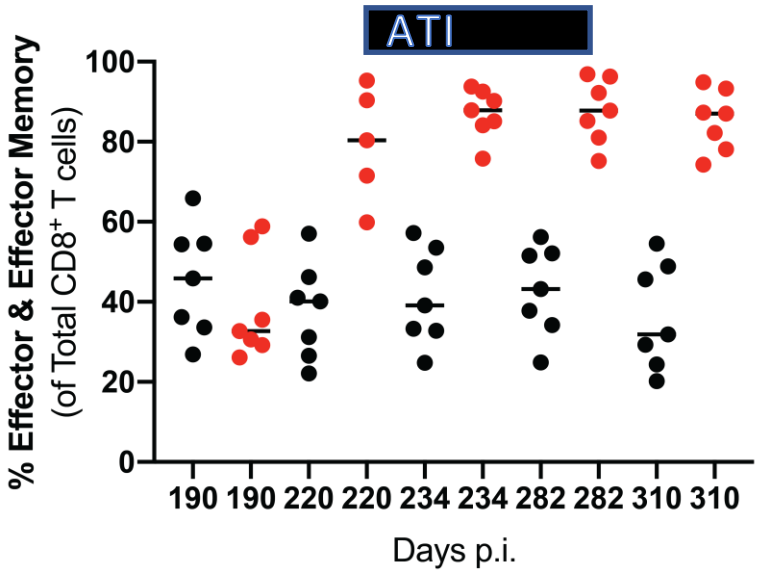
- SIVmac239M achieved high viremia and was rapidly controlled with ART at d14 p.i.
- FTY720 alone did not induce viral replication during ART
- All animals rebounded during ATI



Redistribution… NOT depletion

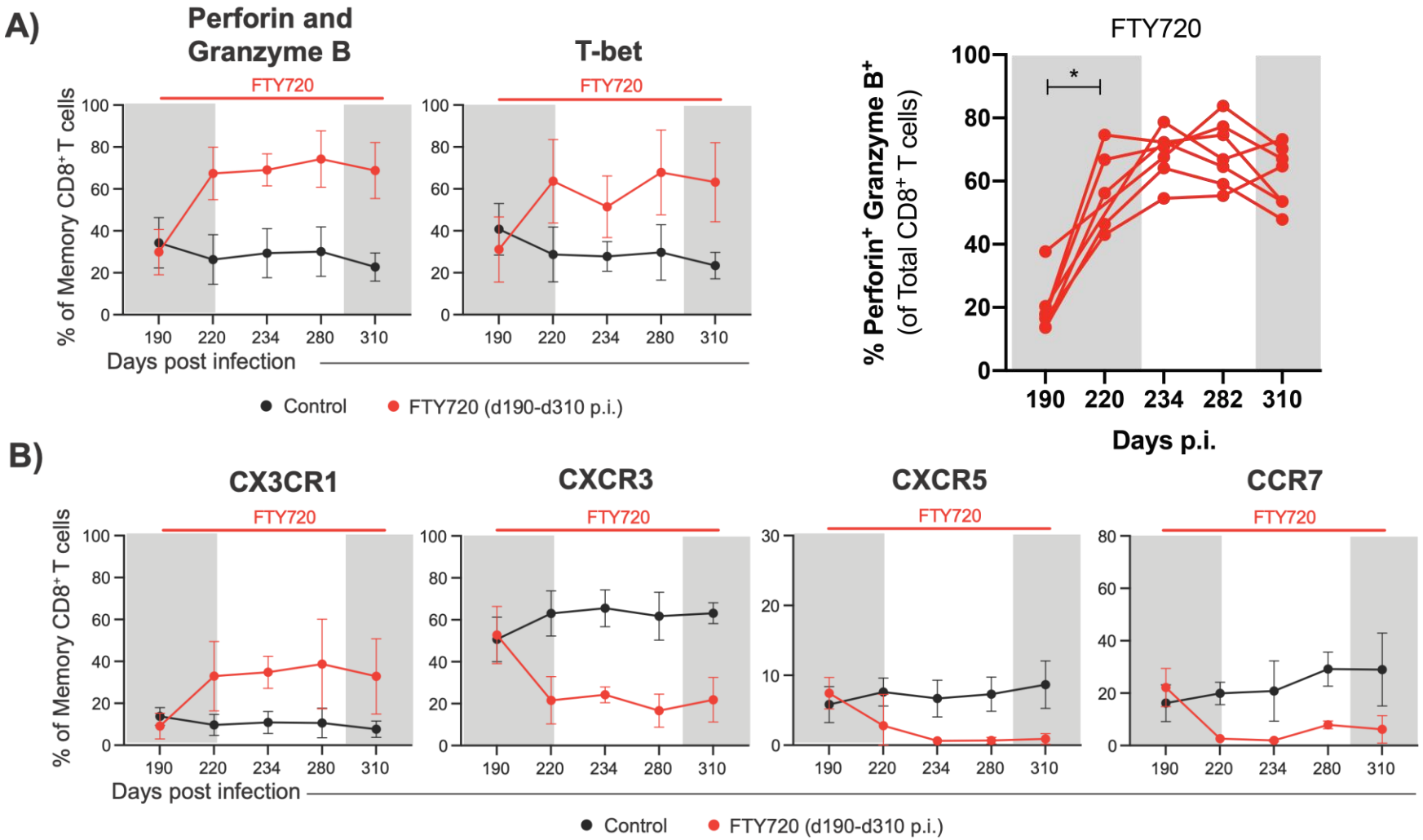
FTY720 purifies for effector/effector memory CD8+ T cells in blood



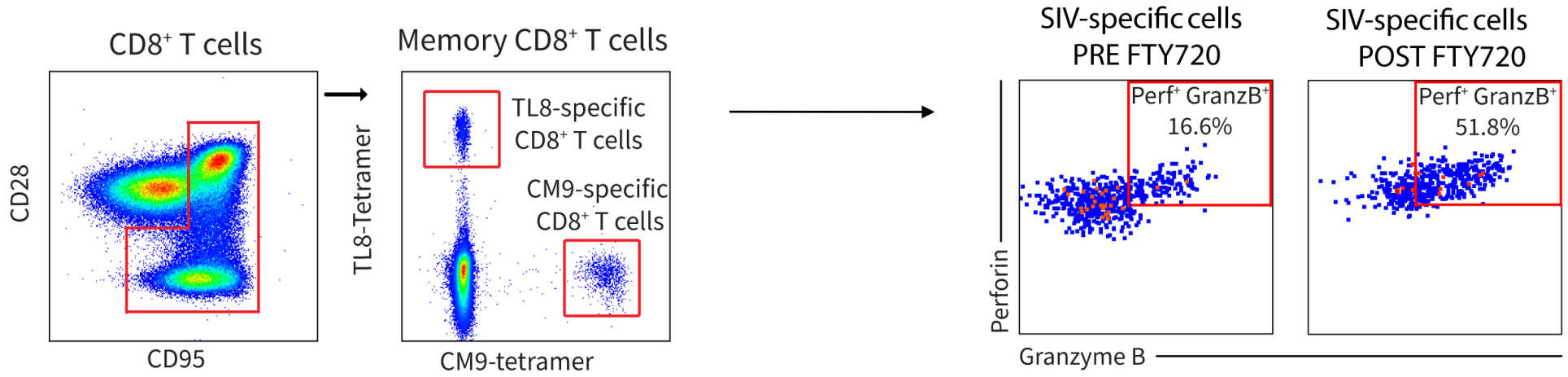


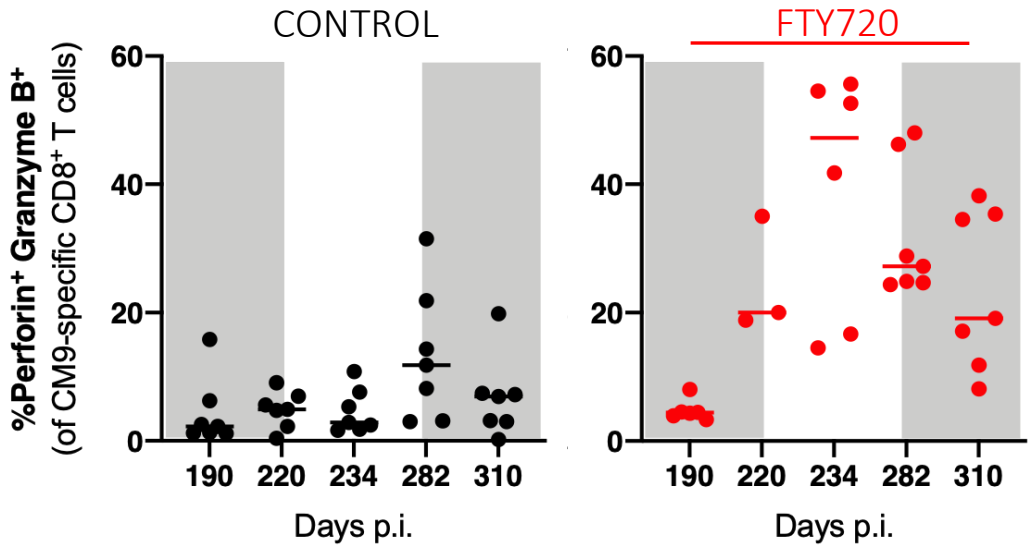
Phenotype determined by CD28/CD95 expression

Cytotoxic non-tissue trafficking CD8s remain in blood during FTY720 treatment

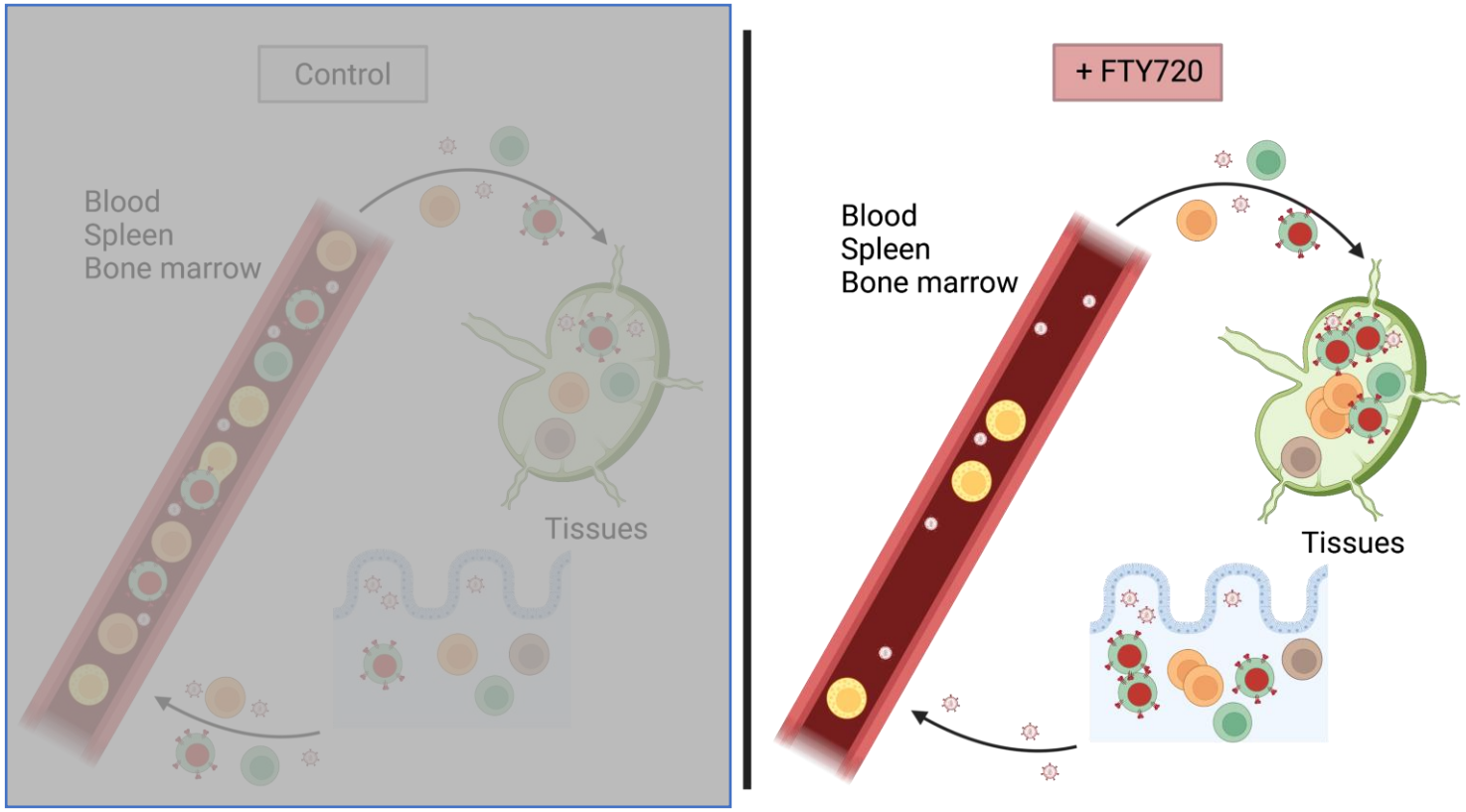


FTY720 purifies for cytotoxic SIV-specific CD8⁺ T cells in blood





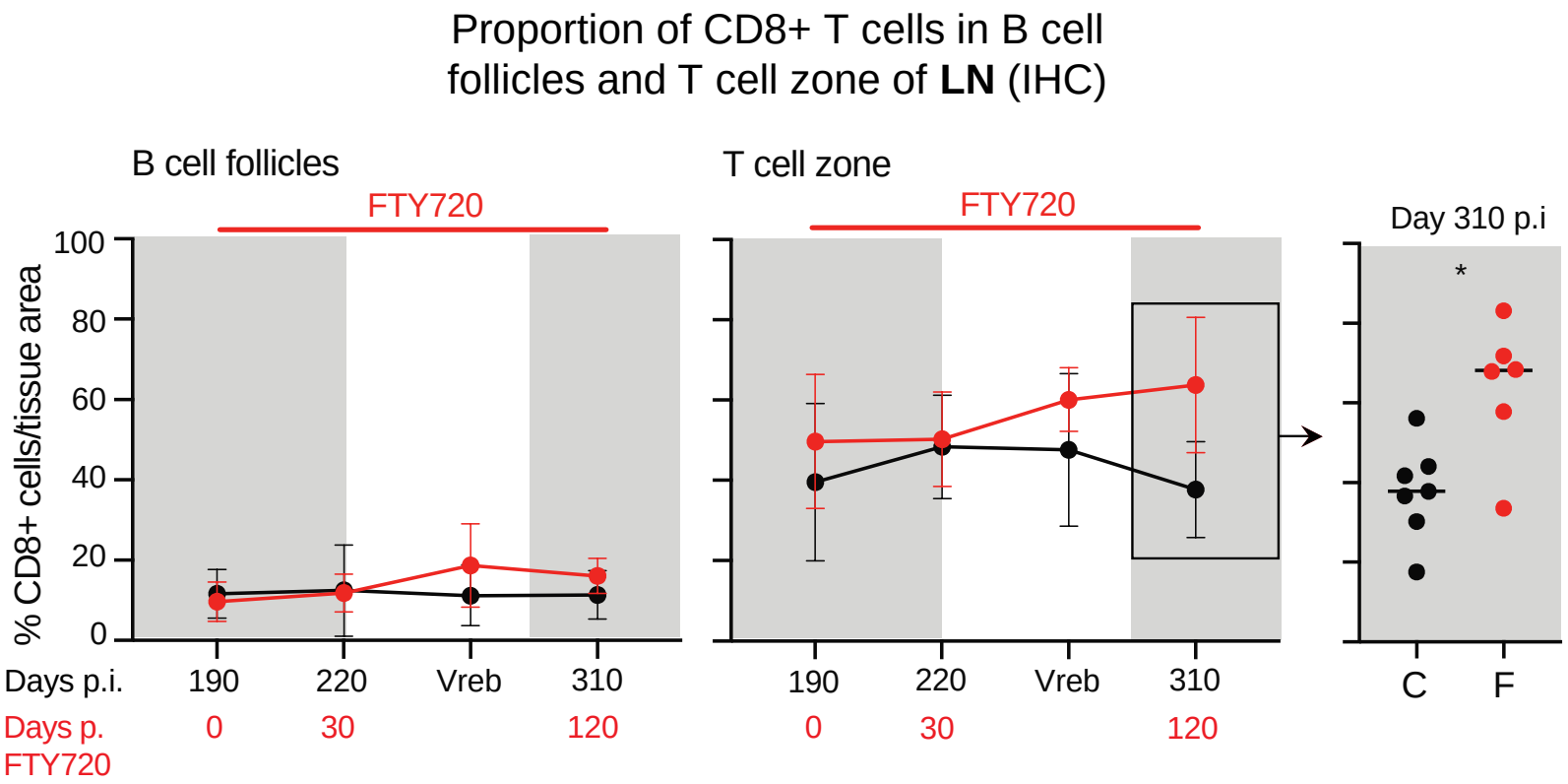
FTY720 treatment separates SIV-infected CD4+ T cells from cytotoxic CD8+ T cells in blood

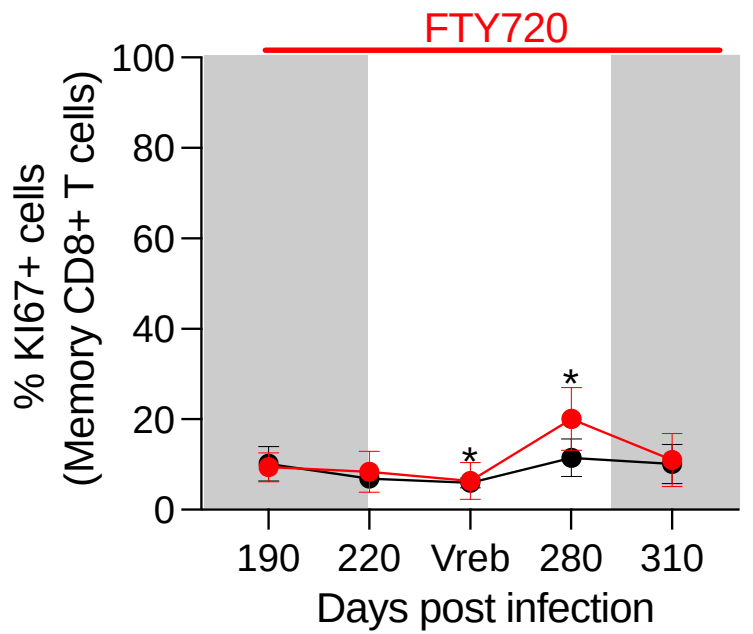


1. Nearly all CD4+ T cells leave blood
2. Only cytotoxic CD8+ T cells remain in blood
3. Only CD8+ T cells in tissues can encounter infected CD4+ T cells

Do CD8+ T cells in tissues become cytotoxic after encountering infected cells during therapy interruption?

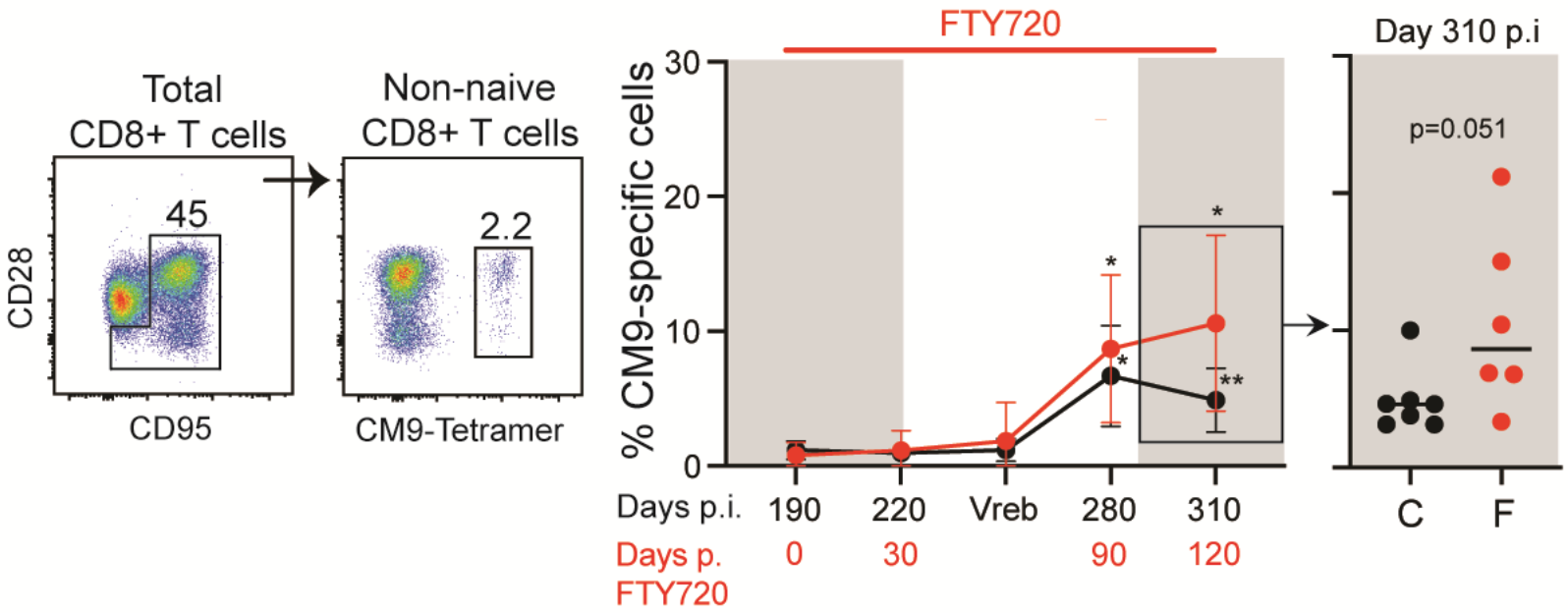
Increased accumulation and activation of LN CD8+ T cells during ATI in FTY720-treated NHP



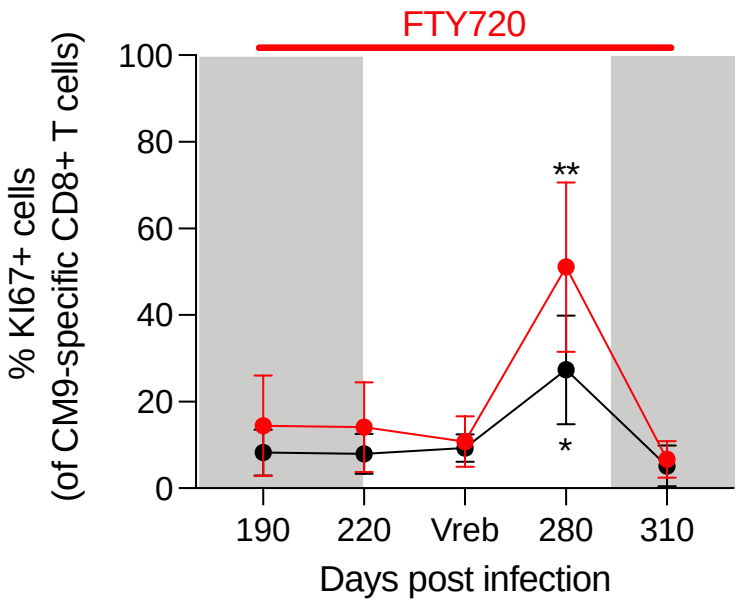


SIV-specific CD8+ T cells in LT of FTY-treated RM expand and get activated after ATI

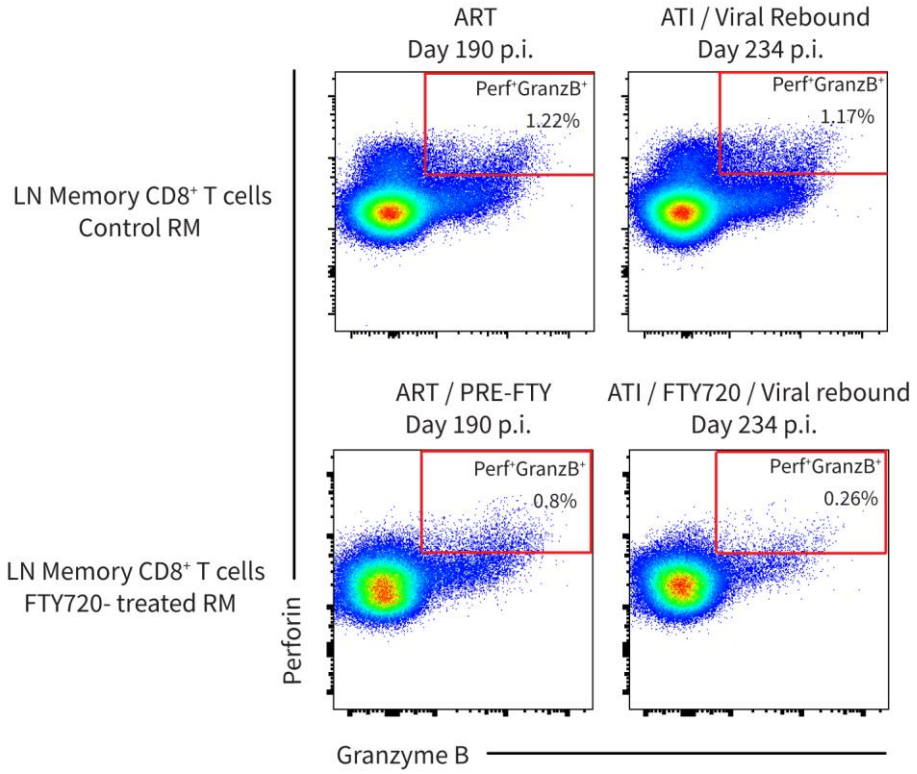
Proportion of CM9-specific CD8+ T cells in **LN**

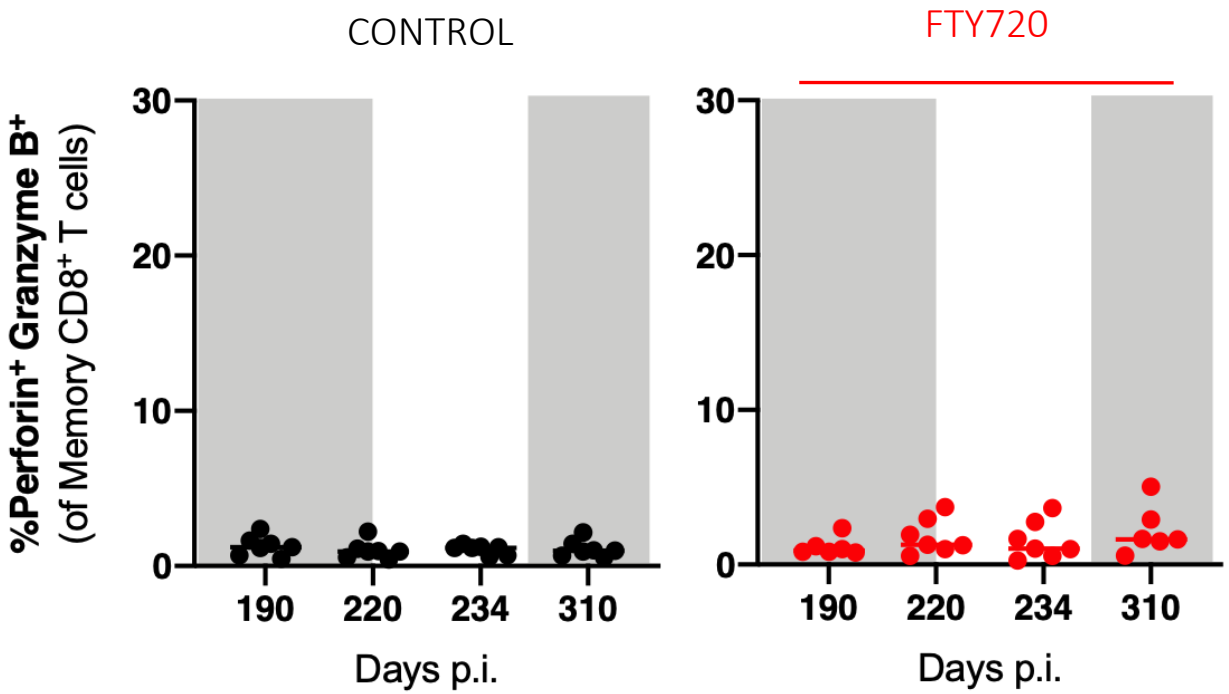


Proportion of proliferating **LN** SIV-specific CD8+ T cells

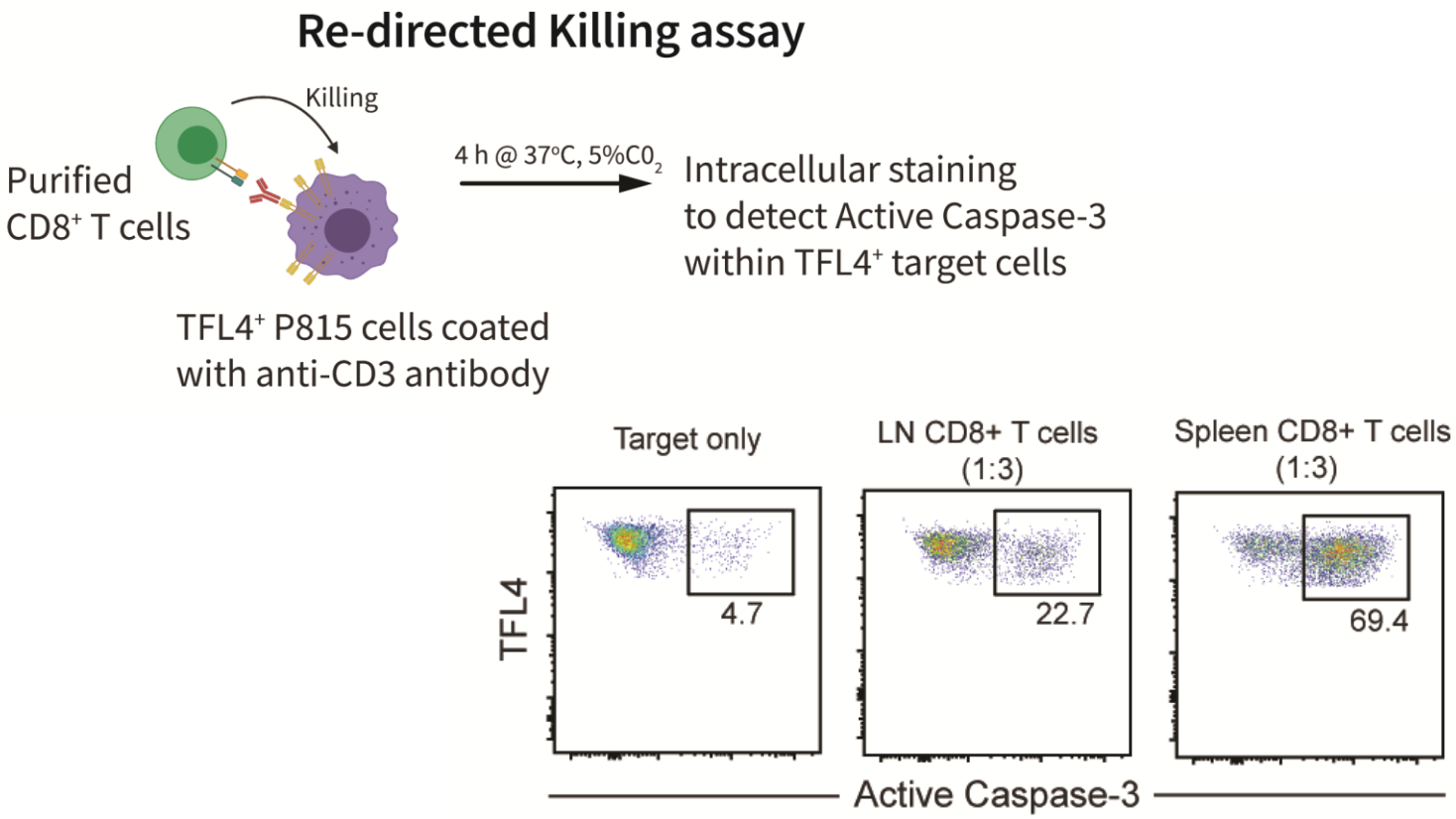


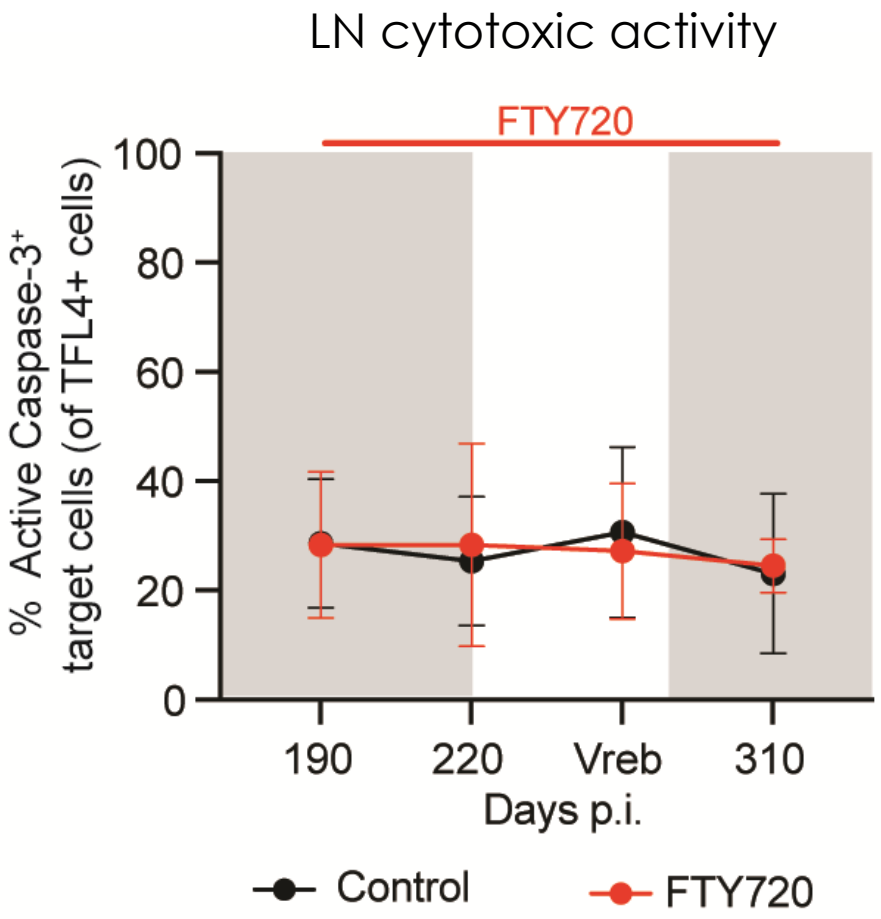
FTY720 during treatment interruption does not induce cytotoxicity in lymph node CD8+ T cells



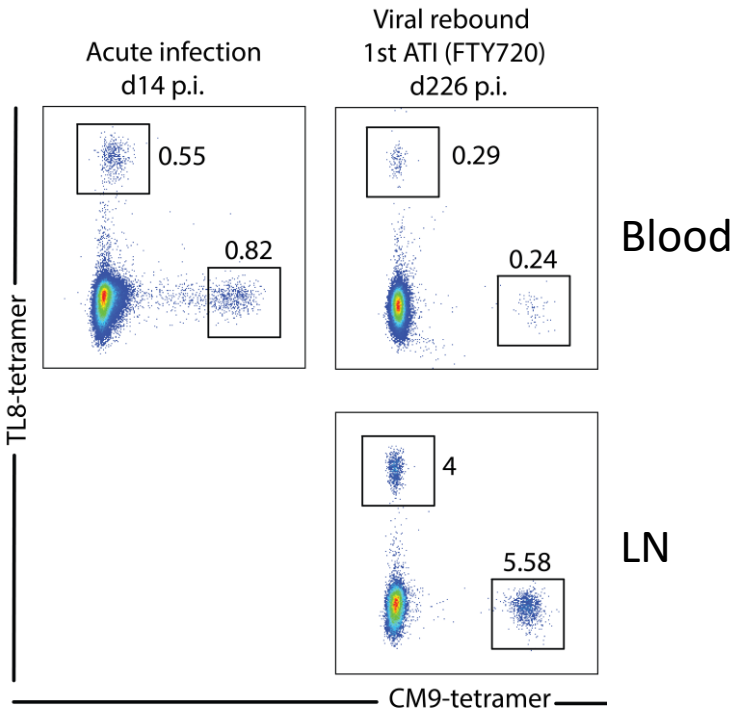


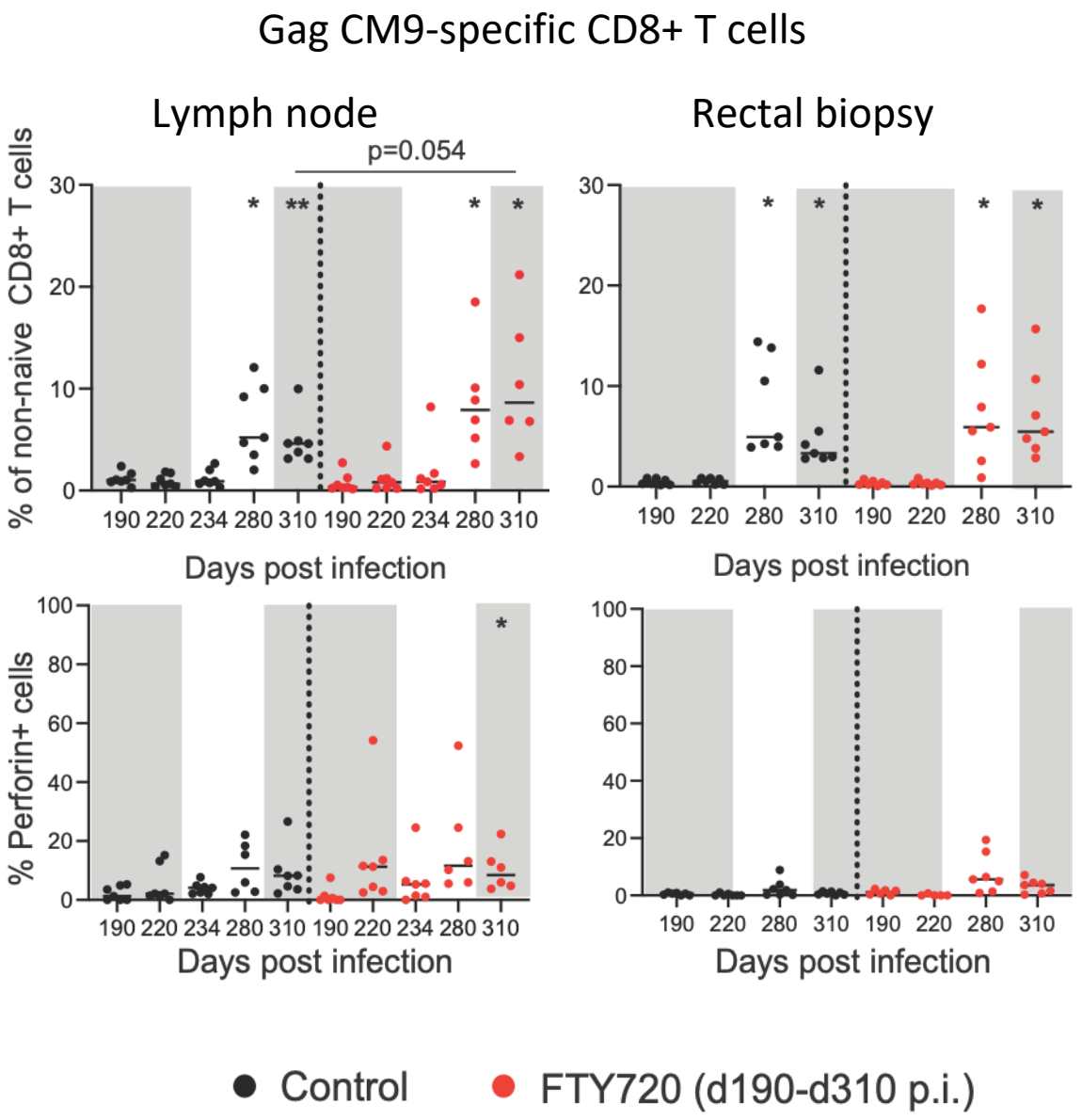
No increase in cytotoxic function of LN CD8+ T cells during ATI in FTY720-treated RMs



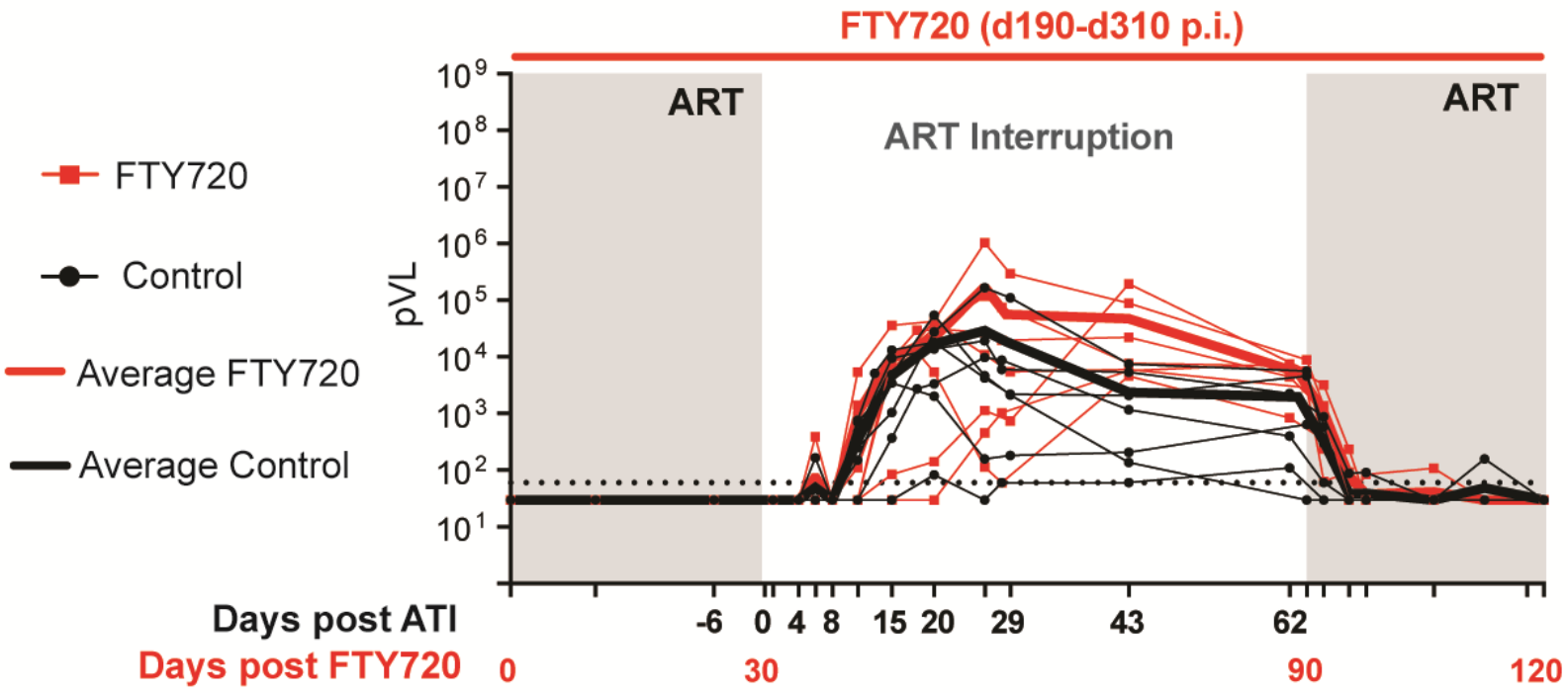


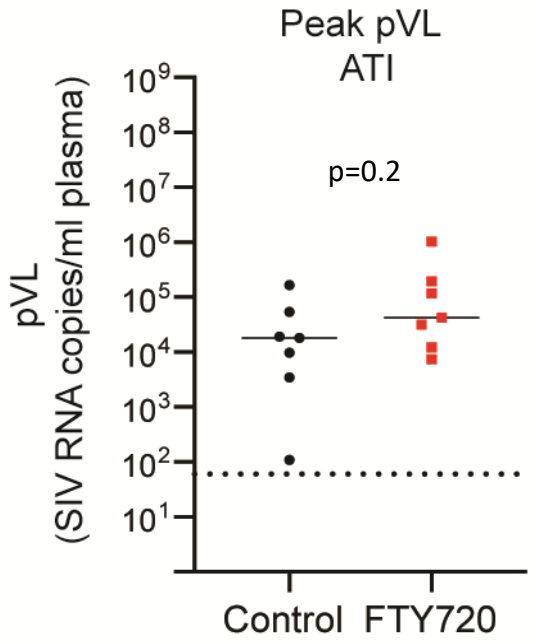
SIV-specific CD8+ T cells in tissues of FTY720-treated RM expand after interruption, but do not become cytotoxic





FTY720 treatment during ATI did not enable control of plasma viremia

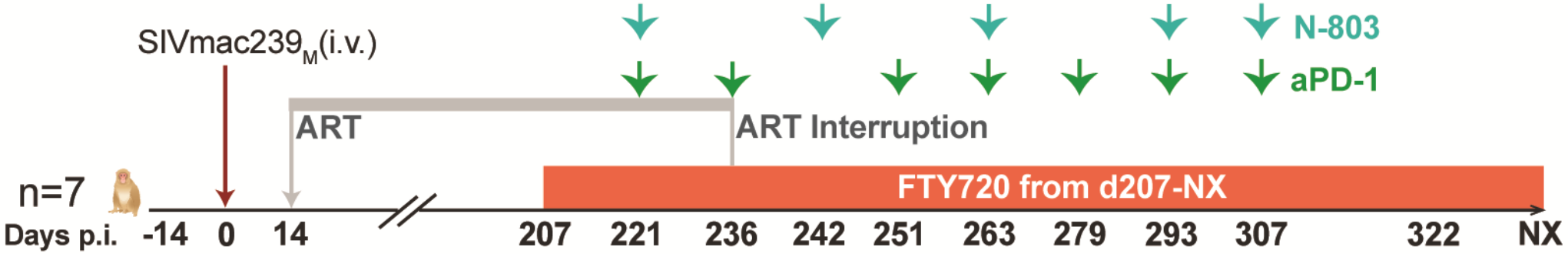




Can additional immunomodulation during FTY720 treatment induce LN CD8+ T cell cytotoxicity?

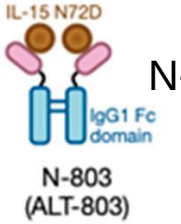
- FTY720 alone traps recirculating CD8+ T cells in tissues, but does not modulate functional properties or transcriptional programming
- The immunomodulatory agents anti-PD-1 and N-803 IL-15 superagonist have individually shown modest beneficial effects on CD8+ T cell function (including in lymph nodes) and plasma viremia
- Without preventing T cell trafficking, the beneficial effects of anti-PD-1 or IL-15SA may be transient in tissues, and the modulated cells may not be in the right place at the right time

Does immunomodulation during FTY720 treatment improve LN CD8 function or immune control?



FTY720 (0.5 mg/kg): daily administration, from day 207 p.i. until necropsy.

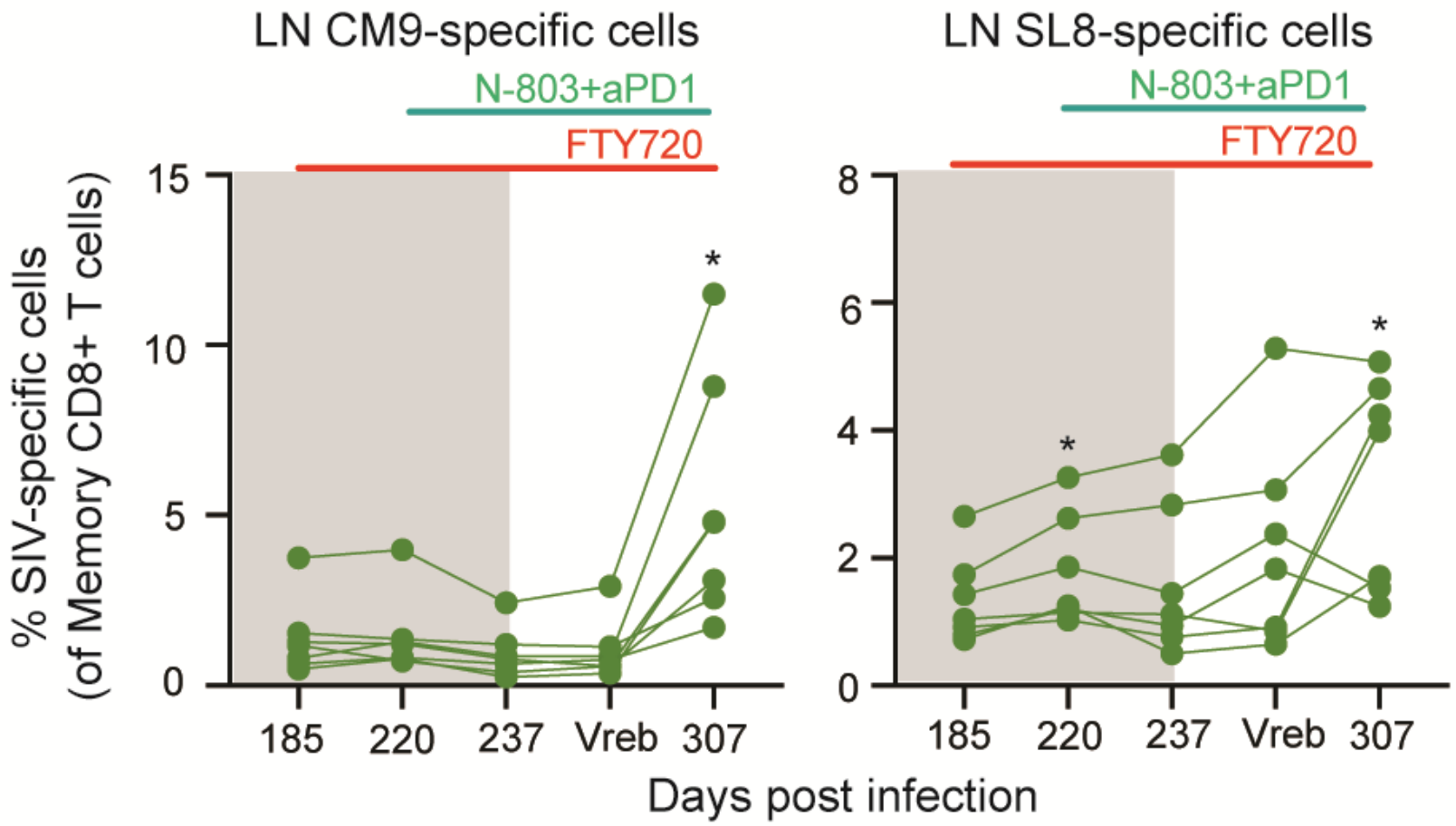
αPD-1 (NIH repository): 7 infusions (3 mg/kg), 4 combined with N-803 → PD1 blockade enhanced T cell responses and viral control *(Barber et al 2006, Nature; Velu et al 2009, Nature)*



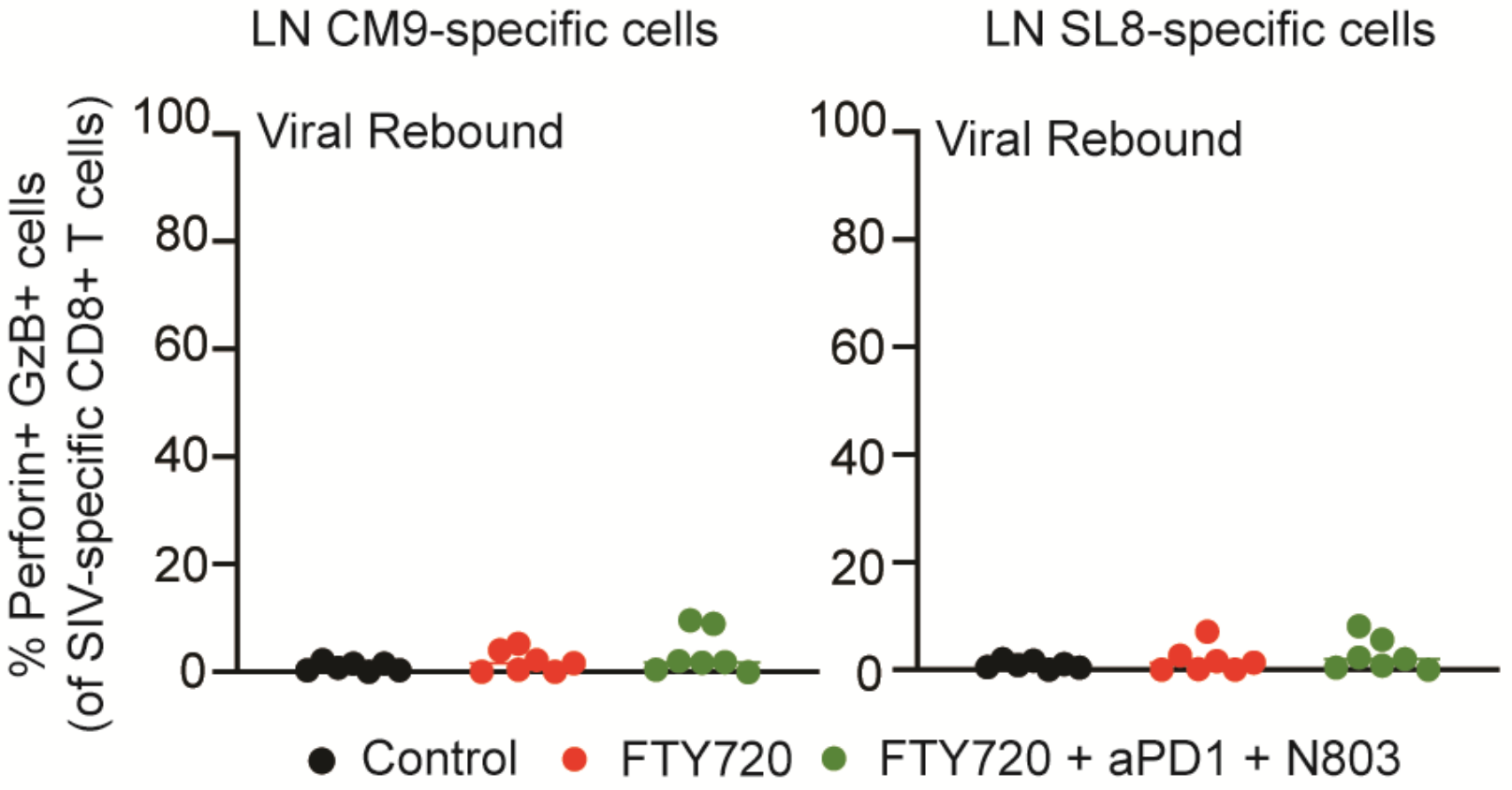
N-803 (IL-15 superagonist, ImmunityBio): 5 infusions (100 ug/kg) → Activate and direct effector NK and SIV-specific CD8+ T cells into B cell follicles *(Webb et al 2020, Plos Path)*

Combined immunotherapy with FTY720, anti-PD-1, and N-803 (FNP) was safe and well tolerated

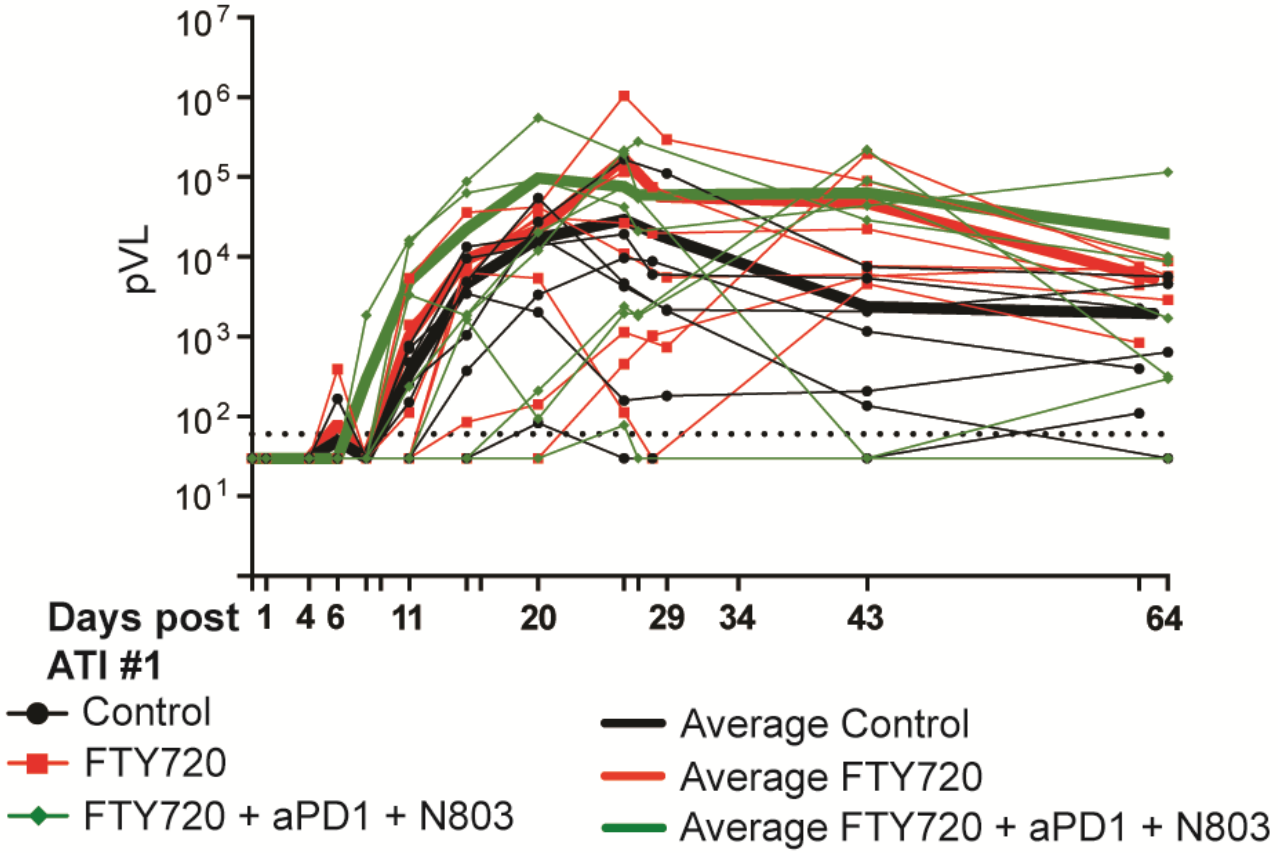
LN SIV-specific responses increase after viral rebound during combined immunotherapy



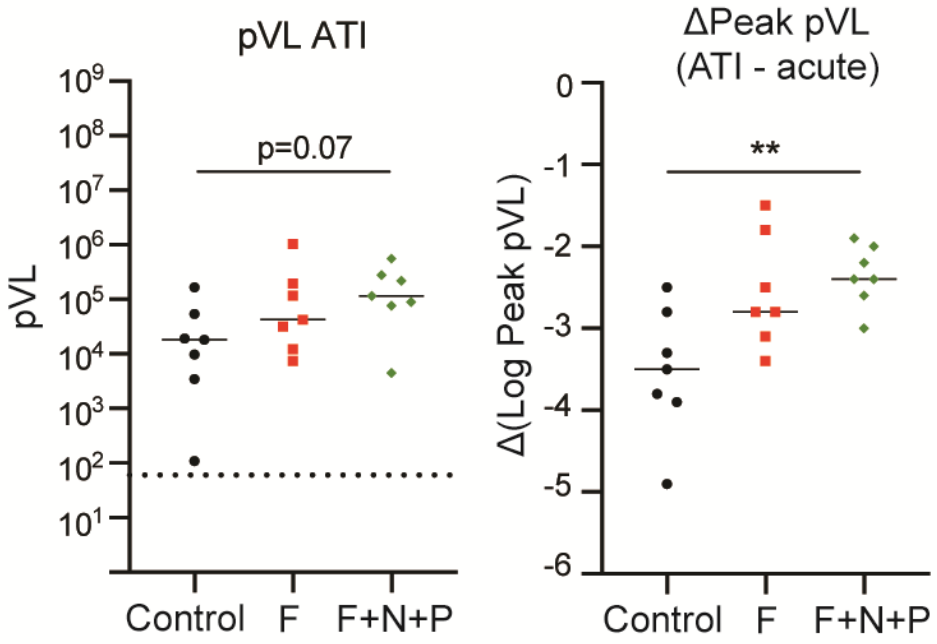
Combined immunotherapy during FTY720 did not increase CD8+ T cell perforin and granzyme B expression in LN



Combined immunotherapy during FTY720 administration did not improve viral control after ATI



… There was a trend towards a higher peak pVL during ATI in FNP-treated compared to control RMs

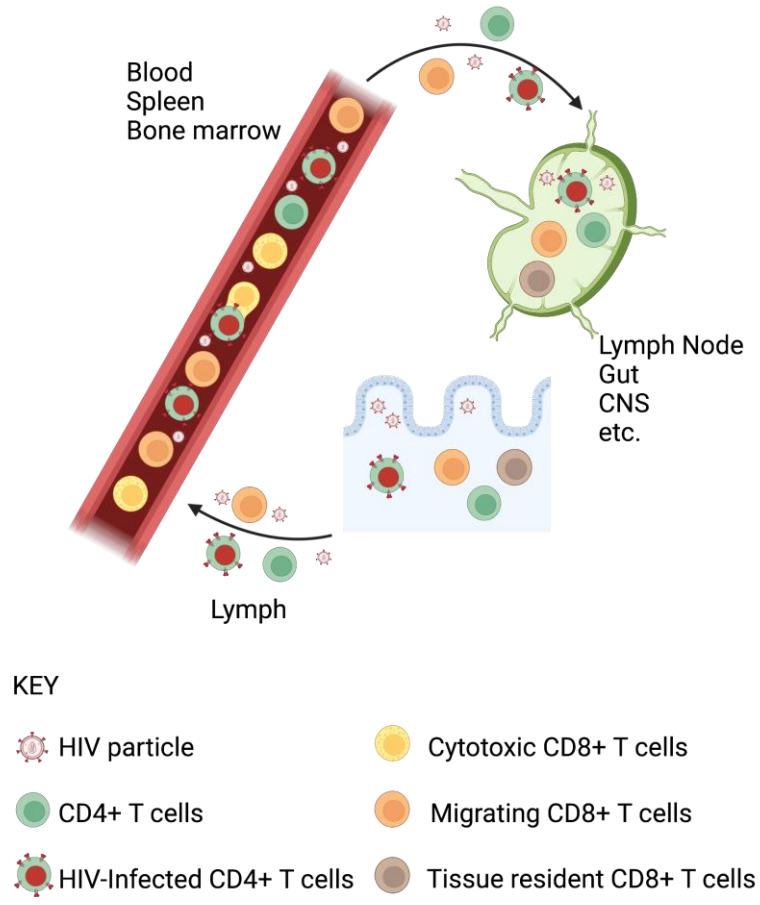


Interpretations and next steps

- CD8+ T cytotoxic function in tissues is not simply controlled by trafficking or lack of exposure to antigen; rather tissue-specific regulation dictates functional properties
- Tissue-localized CD8+ T cells are unable to prevent, delay, or reduce plasma viremia rebound after ATI, even when treated with anti-PD1+IL-15SA
- Tissue-localized CD8+ T cells are unable to eliminate infected cells, or infection propagates too fast for CD8+ T cell suppression
- Indirectly implicates an important role for circulating cytotoxic CD8s in elimination of migrating infected CD4+ T cells

Multi-tier approach to safely awaken and eradicate the virus from tissue reservoirs

1. Latency-reversal agents that reach tissue sites and reawaken proviral expression
2. Immune modulation to enable target cell access and elimination in tissues
   - Increase cytotoxic function of CD8+ T cells in tissues, both residents and tissue migrators
   - Mobilize circulating cytotoxic cells into tissues
   - Engage/enable NK cell cytotoxic activity in tissue sites; and/or
3. Reservoir modulation to 'flush' tissue reservoirs into blood, to allow access by circulating cytotoxic CD8+ T cells/NK cells
4. Protect against new rounds of infection/reservoir formation, assuming incomplete control after latency reversal/ATI



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Emory Yerkes National Primate Research Center

BEAT-HIV Delaney Collaboratory