

Title: Extensive CD8 β depletion does not prevent control of viral replication or protection from challenge in macaques chronically infected with a live attenuated simian immunodeficiency virus.

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Running Head: Importance of CD8 $\alpha\beta$ + T cells during live attenuated SIV infection

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Abstract: (word count: 249)

We evaluated the contribution of CD8 $\alpha\beta$ ⁺ T cells on control of live-attenuated simian immunodeficiency virus (LASIV) replication during chronic infection and subsequent protection from pathogenic SIV challenge. Unlike previous reports with a CD8 α -specific depleting monoclonal antibody (mAb), the CD8 β -specific mAb CD8 β 255R1 selectively depleted CD8 $\alpha\beta$ ⁺ T cells without also depleting non-CD8⁺ T cell populations that express CD8 α , such as natural killer (NK) cells and $\gamma\delta$ T cells. Following infusion with CD8 β 255R1, plasma viremia transiently increased coincident with declining peripheral CD8 $\alpha\beta$ ⁺ T cells. Interestingly, plasma viremia returned to pre-depletion levels even when peripheral CD8 $\alpha\beta$ ⁺ T cells did not. Although depletion of CD8 $\alpha\beta$ ⁺ T cells in the lymph node (LN) was incomplete, frequencies of these cells were three-fold lower ($p=0.006$) in animals that received CD8 β 255R1 compared to control IgG. It is possible that these residual SIV-specific CD8 $\alpha\beta$ ⁺ T cells may have contributed to suppression of viremia during chronic infection. We also determined whether infusion of CD8 β 255R1 in the LASIV-vaccinated animals increased their susceptibility to infection following intravenous challenge with pathogenic SIVmac239. We found that 7/8 animals infused with CD8 β 255R1, and 3/4 animals infused with the control IgG, were resistant to SIVmac239 infection. These results suggest that infusion with CD8 β 255R1 did not eliminate the protection afforded to LASIV vaccination. This provides a comprehensive description of the impact of CD8 β 255R1 infusion on the immunological composition of the host, when compared to an isotype matched control IgG, while showing that the control of LASIV viremia and protection from challenge can occur even after CD8 β 255R1 administration.

Importance: (word count: 124)

Studies of SIV-infected macaques that deplete CD8⁺ T cells *in vivo* with monoclonal antibodies have provided compelling evidence for their direct antiviral role. These studies utilized CD8 α -specific mAbs that target both the major (CD8 $\alpha\beta$ +) and minor (CD8 $\alpha\alpha$ +) populations of CD8⁺ T cells, but additionally deplete non-CD8⁺ T cell populations that express CD8 α , such as NK cells and $\gamma\delta$ T cells. In the current study, we administered the CD8 β -specific depleting mAb CD8 β 255R1 to cynomolgus macaques chronically infected with a LASIV to selectively deplete CD8 $\alpha\beta$ T cells without removing CD8 $\alpha\alpha$ lymphocytes. We evaluated the impact on control of virus replication and protection from pathogenic SIVmac239 challenge. These results underscore the utility of CD8 β 255R1 for studying the direct contribution of CD8 $\alpha\beta$ T cells in various disease states.

Introduction: (word count: 776)

Multiple lines of evidence suggest that CD8⁺ T cells contribute to control of virus replication and subsequently influence disease progression following human immunodeficiency virus (HIV) infection. For instance, the emergence of HIV-specific CD8⁺ cytotoxic T lymphocytes (CTLs) during acute infection is temporally associated with decreases in peak viral load and the decline of viral replication to set point viral load (1, 2). Indeed, CD8⁺ CTLs have been shown to lyse HIV-infected cells *in vitro*, and the selective pressure they exert *in vivo* often leads to the emergence of immune escape variants (3–7). The strongest argument comes from studies of macaques infected with simian immunodeficiency virus (SIV) that are infused with a monoclonal antibody (mAb) that is specific for the CD8 α molecule of CD8⁺ lymphocytes. Following infusion with this antibody, depletion of CD8 α cells persists for approximately 2 to 4 weeks and is

accompanied by a transient increase in virus replication until control is regained coincident with the reemergence of CD8 α ⁺ lymphocytes (8-12, 14, 31, 45-48, 53-54). Of note, control of virus replication is lost following *in-vivo* depletion of CD8⁺ lymphocytes even during antiretroviral therapy (ART), further suggesting that functional CD8⁺ T cells are needed to maintain effective viral control even while on ART (11, 12). Notably, however, CD8 α -specific mAbs deplete not only CD8⁺ T cells, but also a variety of cell populations that express the CD8 α molecule.

The CD8 molecule is expressed as either a CD8 $\alpha\alpha$ homodimer or a CD8 $\alpha\beta$ heterodimer on the cell surface and is present on lymphocytes of both the innate and adaptive immune system (15, 16, 18, 49). The most common lymphocytes to express CD8 are conventional CD8⁺ T cells (TCR $\alpha\beta$ +CD3⁺), which can be divided into a major population that express CD8 $\alpha\beta$ and a minor population that express CD8 $\alpha\alpha$ (13). There also exist populations of TCR $\gamma\delta$ +CD3⁺ T cells and CD3⁻ natural killer (NK) cells that express CD8 $\alpha\alpha$ (17, 18, 52). $\gamma\delta$ T cells (TCR $\gamma\delta$ +CD3⁺+CD8 $\alpha\alpha$ ⁺) which comprise ~ 6% of CD3⁺ T cells (17) can block HIV-1 entry via the secretion of β -chemokines (23), enhance antibody-dependent cellular cytotoxicity (ADCC) (24), and directly lyse HIV-infected cells (25). NK cells (CD3⁻-CD8 $\alpha\alpha$ ⁺) comprise ~16% of peripheral lymphocytes, and have recently been reported to possess traits of adaptive immunity that may contribute to control of HIV-1 replication (19, 20). Accordingly, the contribution of conventional CD8⁺ T cells to viral control are complicated by the depletion of additional cell populations that express CD8 α when using CD8 α -depleting mAbs (10, 14, 53). One approach to better define the antiviral role of CD8⁺ T cells *in vivo* is to administer a CD8 β -specific depleting mAb, as this should selectively deplete CD8 $\alpha\beta$ ⁺ T cells without removing CD8 $\alpha\alpha$ ⁺ lymphocytes or other non-T cell populations. Indeed, two recent studies using the CD8 β -specific mAb CD8 β 255R1 in rhesus macaques provide evidence that CD8 $\alpha\beta$ ⁺ T cells can be specifically depleted *in vivo* (40, 41).

Macaques vaccinated with SIVmac239 Δ nef, a live-attenuated SIV (LASIV) variant of pathogenic SIVmac239, are useful for evaluating the role of CD8 $\alpha\beta$ ⁺ T cells in control of virus replication and protection from SIV challenge. Although rare hosts spontaneously control pathogenic HIV or SIV in a manner dependent on particular major histocompatibility complex (MHC) alleles, control of SIVmac239 Δ nef replication occurs in nearly every vaccinated animal, regardless of host MHC genetics (27-31). These observations question whether the contribution of conventional CD8 $\alpha\beta$ ⁺ T cells to control of SIVmac239 Δ nef is equivalent to their contribution to control of pathogenic SIV. Moreover, vaccination with SIVmac239 Δ nef is the most successful example of vaccine-induced protection from challenge with homologous SIV strains and, less frequently, from challenge with heterologous SIV strains (31–34). After more than 25 years of effort, the precise immune mechanism(s) responsible for this protection are still under debate (35–39). Thus, defining whether SIVmac239 Δ nef-mediated vaccine protection requires CD8 $\alpha\beta$ ⁺ T cells may also help inform either therapeutic or prophylactic HIV vaccine design.

In this study, we utilize the CD8 β -specific mAb CD8 β 255R1 to specifically deplete CD8 $\alpha\beta$ ⁺ T cells in LASIV-vaccinated Mauritian cynomolgus macaques (MCMs) (42), and then measure the impact on control of LASIV replication and protection from pathogenic SIV challenge. In contrast to two recent studies that evaluated the impact of CD8 β 255R1 in SIV-infected or SHIV-infected rhesus macaques (40, 41), we include animals treated with a control IgG to distinguish those immunological effects specific to CD8 β -depletion from those that are a result of infusion of a nonspecific IgG antibody. We also determine whether depletion of CD8 $\alpha\beta$ ⁺ T cells affects the frequency and proliferative capacity of CD8 $\alpha\alpha$ ⁺ T cells, NK cells, and $\gamma\delta$ T cells. Thus, we expand the current knowledge of the immunological effects that follow infusion

with CD8 β 255R1 and provide the first comprehensive comparison to an isotype matched control IgG.

Materials and Methods:

Animal care and use: Two Vietnamese-origin cynomolgus macaques were purchased from Covance Inc. and included in the depletion study of naïve cynomolgus macaques. Both animals were housed and cared for at NIHAC-Poolesville (National Institutes of Health Animal Center) according to protocols approved by the Vaccine Research Center Animal Care and Use Committee. Both animals received a single 50mg/kg intravenous infusion of the anti-CD8 β monoclonal antibody (mAb) CD8 β 255R1.

Eleven Mauritian cynomolgus macaques (MCMs) were purchased from Bioculture Ltd. and included in the depletion/challenge study of cynomolgus macaques chronically infected with a LASIV. These MCMs were housed and cared for by the Wisconsin National Primate Research Center (WNPRC) according to protocols approved by the University of Wisconsin Graduate School Animal Care and Use Committee. All eleven animals were previously infected intravenously with 10ng p27 of wild-type SIVmac239 Δ nef (cy0749 and cy0752) or a variant of SIVmac239 Δ nef containing 10 nonsynonymous mutations (cy0685, cy0688, cy0690, cy0691, cy0750, cy0753, cy0755, cy0756, and cy0757) for 34 to 73 weeks prior to the start of this study (42). All animals were homozygous for the M3 MHC haplotype, with the exception of cy0691 that was heterozygous for the M2 and M3 MHC haplotypes. Seven MCMs received a single 50mg/kg intravenous infusion of the anti-CD8 β monoclonal antibody (mAb) CD8 β 255R1 and four MCMs received a single 50mg/kg intravenous infusion of the DSPR1 rhesus recombinant IgG control mAb, both of which were provided by the NIH Nonhuman Primate Reagent Resource (R24

OD010976, U24 AI126683). All eleven MCMs were challenged intravenously with 100TCID₅₀ SIVmac239 (0.71ng p27) four weeks following mAb infusion.

Antibodies: CD3-AF700 (clone: SP34-2; BD Biosciences), CD4-BV711 (clone: OKT4; BioLegend), CD8 α -PE (clone: DK25; Dako), CD8 β -ECD (clone: 2ST8-5H7; Beckman Coulter), TCR $\gamma\delta$ -FITC (clone: 5A6.E9; Invitrogen), CD95-PE/Cy5 (clone: DX2; BD Biosciences), CD28-BV510 (clone: CD28.2; BD Biosciences), CCR7-Pacific Blue (clone: G043H7; BioLegend), NKG2a-PE/Cy7 (clone: Z199; Beckman Coulter), CD16-BV650 (clone: 3G8; BD Biosciences), CD16-BV786 (clone: 3G8; BD Biosciences), and Ki-67-AF647 (clone: B56; BD Biosciences). LIVE/DEAD Fixable Near-IR Dead Cell Stain (Invitrogen) was used to assess cell viability. All samples were run on the LSR II (BD Biosciences) and analyzed using FlowJo, Version 9.9.6 (BD Biosciences). The presence of the CD8 β 255R1 mAb does not cross-block binding with the anti-CD8 β clone 2ST8-5H7 (40).

Peptides. The NIH AIDS Research and Reference Reagent Program (Germantown, MD) provided 15-mer peptides overlapping by 11 amino acid positions that spanned the entire SIVmac239 proteome. Gag and Env peptides were combined into 2 and 3 pools, respectively, and used at a final pooled concentration of 5 μ M during stimulation.

Lymphocyte isolation and phenotype staining. In the depletion study of naïve cynomolgus macaques, phenotype staining of peripheral blood mononuclear cells (PBMCs) was performed in triplicate on whole blood and in duplicate on lymph node samples. Cells were incubated with a surface antibody mix for 25 minutes in the dark at room temperature. In the depletion/challenge

study, PBMC were isolated from EDTA-anticoagulated blood by ficoll-based density centrifugation as previously described (34). Cells were resuspended in RPMI 1640 (HyClone) supplemented with 10% fetal calf serum (FCS), 1% antibiotic-antimycotic (HyClone), and 1% L-glutamine (HyClone) (R10 medium). Approximately $0.5-1.0 \times 10^6$ PBMC were placed in cluster tubes (Fisher Scientific) and washed with 1x phosphate-buffered saline (PBS) prior to staining. Surface antibodies were added and cells were incubated for 30 min in the dark at room temperature. Cells were then washed twice with 1x PBS supplemented with 10% FCS (10% FCS/PBS), and fixed with 2% paraformaldehyde (PFA) for a minimum of 30 minutes. Fixed cells were then washed once with 1x PBS and then permeabilized with Medium B (Invitrogen, Carlsbad CA) and allowed to incubate with intracellular antibodies for 30 min in the dark at room temperature. Cells were then washed twice with 10% FCS/PBS and resuspended in 2% PFA prior to data collection. Following exclusion of doublets and dead cells, lymphocyte populations were defined as follows: CD8 α β ⁺ T cells: CD3⁺CD4⁻TCR γ δ ⁻CD8 α ⁺CD8 β ⁺; CD8 α ⁺ T cells: CD3⁺CD4⁻TCR γ δ ⁻CD8 α ⁺CD8 β ⁻; γ δ T cells: CD3⁺CD4⁻CD8 β ⁻CD8 α ⁺TCR γ δ ⁺; NK cells: CD3⁻CD4⁻CD8 α ⁺NKG2a⁺CD16⁺; CD4 T cells (naive): CD3⁺CD4⁺CD8 α ⁻CD95⁻CD28⁺CCR7⁺; CD4 T cells (effector memory): CD3⁺CD4⁺CD8 α ⁻CD95⁺CD28⁻CCR7⁻; CD4 T cells (central memory): CD3⁺CD4⁺CD8 α ⁻CD95⁺CD28⁺CCR7⁺.

Intracellular cytokine staining (ICS). Flow cytometry was used to measure intracellular cytokine expression as previously described (59). Cryopreserved cells isolated from LNs were thawed and washed twice in warm R10 medium and allowed to rest at 37°C overnight prior to stimulation. Ki-67 was used to measure the proliferative capacity of lymphocytes freshly-isolated from blood, and IFN- γ , tumor necrosis factor alpha (TNF- α), and CD107a was used to measure SIV-specific

responses in cryopreserved lymphocytes isolated from LNs at week 3 post mAb infusion. LN cells were incubated with CD107a and stimulated with Gag or Env peptide pools for a total of 6 hours, with brefeldin A (Sigma-Aldrich) and monensin (BioLegend) added 1 hour after stimulation. PBMC and LN cells were incubated with LIVE/DEAD fixable near-infrared dead cell stain for 20 minutes, incubated with surface markers for 30 minutes, and fixed with 2% paraformaldehyde (PFA) for at least 20 minutes. Medium B (Invitrogen) was used to permeabilize PBMC and 0.1% saponin was used to permeabilize LN cells. PBMC and LN cells were stained for 30 minutes with Ki-67 or IFN- γ and TNF- α , respectively. Data were collected on an LSR II instrument (BD Biosciences) using 2% PFA-fixed cells and then analyzed using FlowJo version 9.9.6 (BD Biosciences).

Plasma viral load analysis. Plasma was isolated alongside PBMC and cryopreserved at -80°C prior to analysis. SIV *gag* viral loads were determined as previously described (34). Briefly, viral RNA (vRNA) was isolated from plasma, reverse transcribed, and amplified with the Superscript III platinum one-step quantitative reverse-transcription-PCR (RT-PCR) system (Invitrogen). The detection limit of the assay was 100 vRNA copy equivalents per mL of plasma (copies/mL). When the viral load was at or below the limit of detection, the detection limit value of 100 was reported. Full length *nef* and Δ *nef* viral loads were determined as previously described (34). Briefly, highly-specific real-time RT-PCR assays were used with primers that accurately differentiate viruses containing full-length *nef* from those that contain *nef* with a 182 base-pair (bp) deletion, using the methods described above. Serial dilutions of *in vitro* transcripts for both full-length *nef* and *nef* with a 182-bp deletion were used as internal standards for each run. The same machines and

software used for the *gag* viral load assay were used to detect and quantify the *nef* and Δ *nef* viral loads. The limit of detection was identical to that for the SIV *gag* viral load assay.

Virus Neutralization Assays. SIV Env pseudoviruses were produced as previously described (43). Briefly, plasmid DNA encoding SIV gp160 was combined with a luciferase reporter plasmid containing the essential HIV structural genes to produce pseudoviruses expressing SIVmac251.H9, SIVmac251.30, SIVsmE660.CP3C, or SIVmac239 Env. Using TZM-bl target cells, virus neutralization was measured following incubation with SIV-Env pseudovirus and plasma collected from blood. The 50% inhibitory dilution (ID50) was defined as the plasma dilution that caused a 50% reduction in relative light units (RLU) compared to virus control wells following subtraction of background RLU. A nonlinear-regression 5-parameter Hill slope equation was used to calculate plasma ID50 values.

Statistical Analyses. Comparison of two groups in Figures 2b-c, 4c-d, and 5d-f was conducted using Wilcoxon rank sum tests. Comparison of changes from baseline separately for each treatment group shown in Figures 2a, 3, 4a-b, and 5a-c utilized repeated measures ANOVA (RM-ANOVA) to estimate the mean contrasts with animal as a random effect. Comparison between both groups in changes from pre-treatment levels at multiple follow-up time points also utilized RM-ANOVA with treatment, time, and their interaction as fixed effects, and animal as a random effect. Model assumptions of RM-ANOVA were examined and were not deemed to be grossly violated. Dunnett's p-value adjustment for multiple testing over multiple time points was used to keep a family-wise 0.05 type 1 error rate (58). All tests were conducted using a two-sided

225 significance level of 0.05. All analyses were conducted using R for statistical computing version
226 3.3 (44).

Results:

Infusion with CD8 β 255R1 selectively depletes CD8 $\alpha\beta$ ⁺ T cells in naïve cynomolgus macaques. Previous reports of CD8 β -depletion following infusion with the anti-CD8 β mAb CD8 β 255R1 were performed exclusively with rhesus macaques (40, 41). To determine whether CD8 β 255R1 can similarly deplete CD8 $\alpha\beta$ ⁺ T cells in cynomolgus macaques, two naïve animals (A12412 and A12372) were intravenously infused with a single 50 mg/kg dose of the anti-CD8 β mAb CD8 β 255R1 and evaluated for 15 weeks. Flow cytometry was used to monitor CD8 $\alpha\beta$ ⁺ T cells in peripheral blood mononuclear cells (PBMC) and peripheral lymph nodes (LNs) before and after infusion in both animals. Depletion of peripheral CD8 $\alpha\beta$ ⁺ T cells was rapid and sustained (Figure 1a, top) with declines in the percentage of lymphocytes relative to baseline reaching its nadir at week 2 (A12412: 89%, A12372: 99%), and remaining substantially reduced at week 15 (A12412: 75%, A12372: 85%). Levels of circulating CD4⁺ T cells increased transiently following infusion (Figure 1a, middle), while circulating NK cells were relatively unchanged over the course of 15 weeks (Figure 1a, bottom).

Depletion of LN CD8 $\alpha\beta$ ⁺ T cells after administration of the CD8 β 255R1 mAb has been reported in rhesus macaques (<http://www.nhpreagents.org/NHP/availableagents.aspx?Cat=2>). We also observed reductions in LN CD8 $\alpha\beta$ ⁺ T cells (Figure 1b, top) in both cynomolgus macaques at week 2 (mean: 94.5%; range: 94% to 95%) that remained reduced by approximately 84% at week 15 (range: 80% to 88%) when compared to pre-depletion levels. Similar to our observations in the blood, we detected elevated levels of CD4⁺ T cells in LNs (Figure 1b, middle) that persisted for 15 weeks following infusion while levels of NK cells in LNs (Figure 1b, bottom) remained largely unaffected for 15 weeks. These results are not surprising, as a decrease in the frequency of CD3⁺ T cells that are CD8⁺ will correspond to an increase in the frequency of CD3⁺ cells that are

CD4⁺ in the same compartment. To our knowledge, this is the first assessment of the immunological effects following infusion with CD8 β 255R1 in cynomolgus macaques, and these results are similar to those observed in rhesus macaques (40, 41).

Impact of CD8 β 255R1 infusion on CD8 $\alpha\beta$ ⁺ T cells during chronic LASIV infection. Seven MCMs chronically infected with either SIVmac239 Δ nef (n=2) or a SIVmac239 Δ nef variant (n=5) (42) received a single 50 mg/kg intravenous infusion of the anti-CD8 β mAb CD8 β 255R1. Four MCMs infected with the same SIVmac239 Δ nef variant received a single 50 mg/kg intravenous infusion of an isotype-matched rhesus recombinant IgG mAb. All animals had been infected with LASIV for different lengths of time, as they were used in previous studies of T cell mediated control of acute SIV replication (42). Four weeks after the CD8 β 255R1 or IgG control antibody was administered, animals were challenged intravenously with 100TCID₅₀ (0.71ng p27) SIVmac239. Animals were evaluated for an additional 14 weeks before euthanasia, with the exception of animal cy0690, which was sacrificed per veterinarian recommendation at 6 weeks post CD8 β -depletion due to non-SIV-related complications.

Flow cytometry was used to monitor CD8 $\alpha\beta$ ⁺ T cells in PBMC and peripheral LNs of all eleven MCMs. Percent reductions in the absolute count of peripheral CD8 $\alpha\beta$ ⁺ T cells in animals that received CD8 β 255R1 were most prominent at week 2 (median: 94%; range: 70% to 98%); CD8 $\alpha\beta$ ⁺ T cells remained significantly depleted at the time of SIVmac239 challenge (median: 89%; range: 45% to 97%) through the time of necropsy (Figure 2a, blue) (Supplementary Table 1). In control IgG-treated animals, the absolute counts of peripheral CD8 $\alpha\beta$ ⁺ T cells fluctuated around baseline levels for 16 weeks (Figure 2a, black). Shortly after CD8 β 255R1 infusion, the reduction in peripheral CD8 $\alpha\beta$ ⁺ T cell count was statistically significant compared to changes in

the IgG control animals (data not shown). Remarkably, the absolute count of peripheral CD8 $\alpha\beta$ ⁺ T cells at 16 weeks remained depleted by an average of 64% when compared to baseline (median: 67%; range: 28% to 82%) in animals that received CD8 β 255R1. Persistent depletion of CD8 $\alpha\beta$ ⁺ T cells after administration of CD8 β 255R1 lies in stark contrast to the reemergence of CD8 α ⁺ lymphocytes within 2 to 4 weeks after administration of the anti-CD8 α mAb cM-T807 that has been reported in other studies (8, 10, 14, 31, 40, 45-48, 53-54). Our results in both SIV-naïve and LASIV⁺ animals are, however, consistent with another report that observed sustained CD8 $\alpha\beta$ ⁺ T cell depletion in two SIV⁺ rhesus macaques that were treated with CD8 β 255R1 and followed for approximately 18 weeks (40).

The extent to which CD8 β 255R1 depletes LN CD8 $\alpha\beta$ ⁺ T cells in macaques during either chronic LASIV or pathogenic SIV infection has not yet been defined (40, 41). While technical limitations prevented the longitudinal analysis of cell populations in lymph nodes of individual LASIV⁺ animals, we compared the percentage of CD8 $\alpha\beta$ ⁺ T cells in the lymph nodes of animals treated with CD8 β 255R1 to those treated with the control IgG antibody (Figure 2b). At week 3 post infusion, there were threefold fewer ($p < 0.006$) CD8 $\alpha\beta$ ⁺ T cells as a proportion of CD3⁺ T cells in biopsies of axillary lymph nodes isolated from animals infused with CD8 β 255R1 (median: 4%; range: 1.9% to 20%) compared to control IgG animals (median: 27%; range: 21% to 30%). When lymph nodes were assessed 17 to 18 weeks after mAb infusion following necropsy, CD8 $\alpha\beta$ ⁺ T cell frequencies remained substantially lower in the majority of CD8 β 255R1-treated animals compared to control IgG-treated animals (Figure 2c).

Plasma viral load increases transiently following CD8 β 255R1 infusion. To determine the impact of CD8 β depletion on control of chronic LASIV viremia we measured levels of circulating

virus after mAb infusion. Plasma viral load peaked 3 to 11 days after infusion with CD8 β 255R1 and was significantly increased at day 7 ($p<0.001$), day 10 ($p<0.001$), and day 14 ($p=0.006$) compared to baseline (Figure 3a). Surprisingly, at weeks 3 and 4 post infusion with CD8 β 255R1, viral loads returned to levels that were not significantly different from baseline, despite diminished CD8 $\alpha\beta^+$ T cells in peripheral blood and LNs (Figure 2). As expected, minimal changes to peripheral CD8 $\alpha\beta^+$ T cells during the first four weeks after administration of the control IgG antibody (Figure 2) corresponded to viral loads that remained essentially unchanged (Figure 3b).

Minimal changes to CD4 $^+$ T cells following infusion with CD8 β 255R1. One previously reported consequence of depletion of CD8 α^+ cells was a corresponding increase in the frequency or activation state of CD4 $^+$ T cell populations (12, 45–47). We monitored the frequency and proliferation of peripheral CD4 $^+$ naive (T_N), CD4 $^+$ effector memory (T_{EM}), and CD4 $^+$ central memory (T_{CM}) T cells following CD8 β 255R1 infusion. Changes from baseline for both the absolute count (Figure 4a) and percent Ki-67 $^+$ cells (Figure 4b) were compared to baseline within and between each antibody-treated group. Although peripheral CD4 $^+$ T_{EM} cells were significantly increased compared to baseline at two time points in the CD8 β 255R1-treated group (Figure 4a, middle), these changes did not differ significantly when compared to animals that received control IgG (data not shown). Similarly, CD4 $^+$ T cell subpopulations exhibited minimal changes in proliferation compared to baseline within and across groups (Figure 4b and data not shown). We also compared changes in the frequency of total (Figure 4c) and percent Ki-67 $^+$ (Figure 4d) CD4 $^+$ T cells in the lymph nodes isolated at 3 weeks from animals in both groups. We found that the frequency of CD4 $^+$ T cells was higher ($p=0.029$; Figure 4c) in the CD8 β 255R1-treated animals compared to control IgG animals, but that may be attributed to a lower frequency of lymph node

CD8 α β ⁺ T cells detected at this time (Figure 2b). The percent Ki-67⁺ of total CD4⁺ T cells was similar between groups at week 3 post mAb infusion (Figure 4d) (Supplementary Table 2). From these analyses, animals who received an infusion of CD8 β 255R1 exhibited minimal changes in the number and proliferative capacity of CD4⁺ T cells, even while plasma viremia was fluctuating.

Proliferation of CD8 α ⁺ T cells, NK cells, and γ δ T cells after infusion with CD8 β 255R1.

Another consequence of using a CD8 α -specific mAb is the simultaneous depletion of additional cell populations that express CD8 α , such as CD8 α ⁺ T cells, NK cells, and γ δ T cells (10, 12, 14, 31, 40, 47, 53). Following infusion with CD8 β 255R1, we evaluated the absolute count and percent Ki-67⁺ of these three cell populations in the periphery. In animals that received CD8 β 255R1, there was a rapid increase in the absolute count of CD8 α ⁺ T cells (median: 3.6-fold; range: 3.2-fold to 3.8-fold) that differed initially from control IgG animals (Figure 5a, top) (Supplementary Table 3). This was followed by increases in the percent of CD8 α ⁺ T cells expressing Ki-67 (median: 4.5-fold; range: 4.2-fold to 5.3-fold) (Figure 5a, bottom). Control IgG-treated animals did not exhibit significant differences from baseline in the absolute count of CD8 α ⁺ T cells or percent that express Ki-67. Few differences were detected in the absolute count of NK cells (Figure 5b, top) or γ δ T cells (Figure 5c, top) in each group or between groups for four weeks after infusion (Supplemental Table 3). In contrast, we detected significant increases from baseline in the frequency of NK cells and γ δ T cells expressing Ki-67 in the group treated with CD8 β 255R1 that peaked at approximately 7-fold (median: 4.3-fold; range: 1.8-fold to 17.7-fold) and 10-fold (median: 9.9-fold; range: 0.1-fold to 18.8-fold), respectively (Figures 5b and 5c, bottom). The observed proliferation of NK cells in the CD8 β 255R1-treated animals was significantly greater than control IgG-treated animals at days 7, 10, and 14 (Supplementary Table 4).

We also evaluated changes to the frequency and proliferation of CD8 $\alpha\alpha$ + T cells (Figure 5d), NK cells (Figure 5e), and $\gamma\delta$ T cells (Figure 5f) in lymph nodes of animals treated with CD8 β 255R1 or control IgG at week 3 post infusion (Supplementary Table 2). Similar to cells in the periphery, the frequency of CD8 $\alpha\alpha$ + T cells (Figure 5d, top), NK cells (Figure 5e, top), and $\gamma\delta$ T cells (Figure 5f, top) in lymph nodes of animals treated with CD8 β 255R1 was comparable to control IgG animals. At week 3 after CD8 β 255R1 infusion, the frequency of Ki67+ CD8 $\alpha\alpha$ + T cells (Figure 5d, bottom) and $\gamma\delta$ T cells (Figure 5f, bottom) was significantly higher than that observed in control IgG animals. Taken together, our data indicate that CD8 $\alpha\alpha$ + T cells, NK cells, and $\gamma\delta$ T cells exhibited rapid proliferation in either the PBMC or lymph nodes following CD8 β 255R1 infusion, while their absolute cell counts remained relatively unchanged.

Limited SIV-specific T cell responses in LNs following CD8 β 255R1 infusion. To determine the impact of CD8 β 255R1 infusion on SIV-specific T cells within LNs, we performed intracellular cytokine staining (ICS) with cells isolated from LN biopsies taken three weeks after antibody infusion. Multiple pools containing overlapping peptides spanning the entire Gag protein and Env protein were used to stimulate cells, followed by staining with CD107a (Figure 6, top) and IFN γ /TNF α (Figure 6, bottom) to assess degranulation and pro-inflammatory cytokine secretion, respectively. None of the animals that received the control IgG antibody exhibited remarkable SIV-specific responses. This was not surprising, as these animals did not exhibit any marked changes in viremia. We were surprised to find that three of the animals that received CD8 β 255R1 (cy0691, cy0749, and cy0752) exhibited more residual virus-specific CD8 $\alpha\beta$ + T cells than the others (Figure 6). Of note, those three animals were each infected with a LASIV that contained known immunogenic epitopes. Thus, ongoing replication of a virus with intact M3 epitopes may

have provided stronger antigenic stimulation to T cells and therefore driven proliferation in these three animals. The other four animals that received CD8 β 255R1 were homozygous for the M3 MHC haplotype and originally infected with a LASIV that contained point mutations in eight epitopes restricted by MHC molecules expressed by the M3 MHC haplotype (42). Even though it is likely that CD8 β 255R1 infusion alone cannot completely eliminate virus-specific CD8 $\alpha\beta$ ⁺ T cells from lymph nodes, the combination of infecting animals with a minimally immunogenic LASIV and infusion of CD8 β 255R1 was able to limit the frequency of Gag- and Env-specific CD8 β ⁺ T cells at this important tissue site.

Protection from SIVmac239 challenge during depletion of CD8 $\alpha\beta$ ⁺ T cells. To determine whether CD8 β 255R1 infusion increased susceptibility to infection with pathogenic SIV, we performed a high dose intravenous challenge in all 11 animals at 4 weeks post antibody infusion with SIVmac239 (55, 56). Previously, we found that 4/7 MCMs chronically infected with a minimally immunogenic LASIV were protected from intravenous challenge with the same dose of SIVmac239 (34). The 11 animals used in the current study were also chronically infected with a *nef*-deleted SIV, so discriminating qRT-PCR assays were used to differentiate previous Δ *nef* circulating virus from full-length *nef* that was present in the challenge strain. Surprisingly, there was no replicating SIVmac239 in the plasma of six of the seven MCMs infused with CD8 β 255R1 (Figure 7a), despite depletion of peripheral CD8 β ⁺ lymphocytes at the time of challenge (median: 87%; range: 45% to 95%) (Figure 2a). Similarly, there was no replicating SIVmac239 detected in the plasma of three out of four MCMs that received the control IgG antibody (Figure 7b), despite having similar CD8 $\alpha\beta$ ⁺ T cell levels as pre-depletion (Figure 2a). It is possible that residual virus-specific CD8 $\alpha\beta$ ⁺ T cells in the lymph nodes (Figure 6) were sufficient to protect animals from

challenge, as others have found that virus-specific CD8⁺ T cells in lymph nodes are an immune correlate of protection by LASIV (38). However, with so few animals becoming infected with SIVmac239 we could not determine if this was the main mechanism of protection.

Neutralizing antibody titers to SIVmac239 do not predict challenge outcome. We wanted to determine whether depletion of CD8 β ⁺ lymphocytes had a direct impact on neutralizing antibody titers, which may have contributed to protection from pathogenic SIVmac239 infection. To accomplish this, we performed *in vitro* antibody neutralization assays with plasma collected immediately before mAb infusion, as well as immediately before SIVmac239 challenge. We tested neutralization potency against SIV strains that are highly neutralization-sensitive (tier 1; SIVmac251.30 and SIVsmE660.CP3C), moderately neutralization-resistant (tier 2; SIVmac251.H9), and highly neutralization-resistant (tier 3; SIVmac239) (43). In animals that received control IgG, neutralizing antibody titers prior to infusion (Figure 8a, left) were similar to those detected prior to SIVmac239 challenge (Figure 8a, right). Similarly, we did not detect substantial changes to neutralizing antibody titers prior to CD8 β 255R1 infusion (Figure 8b, left) when compared to the titers right before SIVmac239 challenge (Figure 8b, right). Accordingly, we could not conclude protection from challenge following infusion with CD8 β 255R1 was a result of increased *in vitro* SIVmac239 neutralization antibody titers.

Discussion: (word count: 1,087)

Studies of SIV-infected macaques using a CD8 α -specific mAb to deplete CD8⁺ T cells *in vivo* have frequently been interpreted as evidence for a direct antiviral role of CD8⁺ T cells in HIV infection (8, 48). However, these results are complicated by the simultaneous depletion of CD8 α -

expressing NK cells and $\gamma\delta$ T cells (10, 14, 15, 49). Here we showed that a single intravenous infusion of the CD8 β -specific mAb CD8 β 255R1 selectively depleted CD8 $\alpha\beta$ ⁺ T cells in peripheral blood, though to a lesser extent in lymph nodes, from SIV-naïve and LASIV-infected cynomolgus macaques. Following depletion, we observed a transient rise in plasma viremia that was followed by re-establishment of viral control, even when CD8 $\alpha\beta$ ⁺ T cells remained depleted in the peripheral blood. During the time of prolonged peripheral CD8 $\alpha\beta$ ⁺ T cell depletion, we found that CD8 $\alpha\alpha$ ⁺ T cells, NK cells, and $\gamma\delta$ T cells exhibited markers of proliferation regardless of changes to their total frequencies in blood. These observations demonstrated that the CD8 β 255R1 antibody specifically depleted CD8 $\alpha\beta$ ⁺ T cells. CD8 β 255R1 could not fully deplete CD8 $\alpha\beta$ ⁺ T cells from tissue sites, as lymph nodes contained residual CD8 $\alpha\beta$ ⁺ T cells. While our results specifically demonstrated that administration of the CD8 β 255R1 depleting antibody led to a rise in LASIV plasma viremia, we could not conclusively determine whether the residual CD8 $\alpha\beta$ ⁺ T cells in tissues, or the persistence of CD8 $\alpha\alpha$ ⁺ T cells, NK cells, and/or $\gamma\delta$ T cells were responsible for re-establishment of LASIV control and subsequent protection from pathogenic SIVmac239 challenge. Because animals also had very low neutralizing antibody titers against SIVmac239 throughout the study period, it is unlikely that protection against challenge was mediated by neutralization of the challenge virus.

We found that animals treated with the CD8 β 255R1 antibody exhibited demonstrably fewer total CD8 $\alpha\beta$ ⁺ T cells in the periphery and the lymph nodes, when compared to control IgG animals. To determine if we depleted virus-specific CD8 $\alpha\beta$ ⁺ T cells, we initially performed γ -interferon enzyme-linked immunospot assays (IFN- γ ELISPOT) with PBMC prior to, and three weeks after infusion with CD8 β 255R1, but were unable to confirm if the SIV-specific responses that were detected three weeks after infusion were attributed to CD8⁺ T cells or CD4⁺ T cells

(data not shown). While numerous pieces of evidence point towards a critical role of CD8⁺ T cells in long term viral control (8-10, 14, 31, 47, 53), one report suggests that virus-specific CD8⁺ T cells in peripheral blood may not be absolutely required for control of virus replication during chronic SIV infection, even if they are needed to initially suppress viremia (57). While tetrameric reagents are an alternative method to detect virus-specific T cells, these reagents were not available for two reasons: (1) Many of the epitopes that would elicit CD8 $\alpha\beta$ ⁺ T cells were rendered non-immunogenic in the mutant LASIV used to infect several of these animals (42), and (2) tetrameric reagents specific for the CD8 and CD4 T cells that developed in the animals infected with the mutant LASIV have not been produced. We did perform intracellular cytokine staining experiments with lymph nodes that were collected three weeks after antibody infusion. Interestingly, the three animals with the largest frequency of Env-specific CD8 $\alpha\beta$ ⁺ T cells were the three animals that were originally infected with strains of LASIV containing immunogenic epitopes. Two animals were infected with wild type SIVmac239 Δ nef, and the third animal was infected with the mutant LASIV, but expressed some non-M3 MHC alleles. As a result, these three animals likely had the largest pool of virus-specific memory CD8 $\alpha\beta$ ⁺ T cells that could emerge when viremia increased. Together, our data imply that the most effective way to eliminate virus-specific CD8 $\alpha\beta$ ⁺ T cells with currently available technology is to infect animals with a virus whose T cell epitopes are rendered non-immunogenic combined with a CD8 β -specific depleting antibody. Even with this ‘double-knockout approach’, more sophisticated reagents are needed to improve CD8 $\alpha\beta$ ⁺ T cell depletion in tissues to determine if they are required to re-establish viral control.

Even when we minimized the CD8 $\alpha\beta$ ⁺ T cell populations with our interventions, we were surprised to find that six out of seven animals treated with CD8 β 255R1 were resistant to infection with pathogenic SIVmac239. This level of protection was on par with the animals treated with

control IgG. Unfortunately, even when we examined non-CD8 β ⁺ immune cell populations and neutralizing antibody titers, there were no obvious immune correlates of protection. Protection afforded to LASIV has previously been associated with the presence of effector-differentiated T cell responses within the lymph node (37, 38), so it is entirely possible that the residual virus-specific T cells in the lymph nodes or tissues were sufficient to protect animals from SIVmac239 challenge. Continuing to improve the methods to deplete virus-specific immune cells in the LASIV model will be needed to directly demonstrate the immune correlates of protection mediated by LASIV.

One unique attribute of our study was the inclusion of animals treated with an isotype-matched IgG control antibody that was absent in recent reports of CD8 β 255R1 (40, 41). By comparing paired differences of cell populations between animals treated with CD8 β 255R1 and control IgG, we accounted for potential artifacts that may result from infusion of a nonspecific IgG antibody. For example, increases over baseline in the percent of Ki-67⁺ CD8 $\alpha\alpha$ ⁺ T cells detected from weeks 2 to 4 in both groups suggests that IgG infusion alone induces proliferation of CD8 $\alpha\alpha$ ⁺ T cells. Additionally, similar to a previous study using CD8 β 255R1 (40) we observed increased numbers of circulating NK cells in animals receiving CD8 β 255R1, though similar increases were also detected in the control IgG animals. These observations question whether changes to CD8 $\alpha\alpha$ ⁺ cell populations were a direct consequence of administration of the specific CD8 β 255R1 monoclonal antibody, or whether the administration of a control antibody was sufficient to induce this expansion. Thus, including an IgG control group in these types of antibody-infusion studies is critical for identifying non-specific depletion effects.

In this study, we provide a comprehensive comparison of the immunological impact that follows infusion with the anti-CD8 β mAb CD8 β 255R1 compared to an isotype matched control

IgG. The persistent depletion of peripheral CD8 $\alpha\beta$ ⁺ T cells following administration of CD8 β 255R1 lies in stark contrast to the transient depletion of CD8 $\alpha\beta$ ⁺ T cells following infusion with a CD8 α -specific mAb. Unlike studies with the CD8 α -depleting antibody that observed regained control of virus replication coincident with rebound of CD8⁺ T cells, we observed regained control of viral replication, even when peripheral CD8 β ⁺ T cells remained depleted. Moreover, protection from intravenous challenge with pathogenic SIVmac239 was achieved when CD8 $\alpha\beta$ ⁺ T cell magnitude was reduced by administration of CD8 β 255R1 and, in some cases, in combination with a mutant LASIV that failed to elicit many virus-specific T cells (42). Nonetheless, the data we provide demonstrates that the CD8 β 255R1 antibody can be used to specifically deplete CD8 $\alpha\beta$ ⁺ T cells, while leaving other CD8 α ⁺ immune cell populations intact. This may serve to be valuable in future studies evaluating the importance of CD8 $\alpha\beta$ ⁺ T cells in diverse disease models.

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Figure legends:

Figure 1: Infusion with CD8 β 255R1 selectively depletes CD8 $\alpha\beta$ + T cells in naïve

cynomolgus macaques. Two naïve cynomolgus macaques (A12412 and A12372) received a single 50 mg/kg intravenous infusion with CD8 β 255R1. Percent CD8 β + T cells (top), CD4+ T cells (middle), and CD16+ NK cells (bottom) in (a) peripheral blood and (b) lymph nodes were measured by multicolor flow cytometry. Data are shown as mean and standard deviation. Peripheral blood samples from each animal were analyzed in triplicate, while lymph node samples from each animal were analyzed in duplicate.

Figure 2: Depletion of CD8 $\alpha\beta$ + T cells during chronic LASIV infection. (a) Absolute count of CD8 $\alpha\beta$ + T cells were assessed in peripheral blood for 16 weeks following infusion with CD8 β 255R1 (blue) or control IgG (black). Percent CD8 $\alpha\beta$ + T cells from lymph node biopsies at (b) 3 weeks post-infusion and (c) at necropsy (week 17-18). Data are represented as mean and standard error of the mean. *Data shown include four IgG-treated animals and seven CD8 β 255R1-treated animals through week 6, at which point six CD8 β 255R1-treated animals are shown for the remainder of the study due to one animal (cy0690) requiring early euthanasia.

Figure 3: Regained control of viral recrudescence following depletion of peripheral CD8 $\alpha\beta$ + T cells.

Plasma viral load was measured for four weeks after mAb infusion. (a) Viral load initially increased following infusion with CD8 β 255R1, but returned to pre-depletion levels by day 18. (b) Viral load did not change significantly in animals that received control IgG. The limit of detection of the plasma viral load assay (100 vRNA copies/mL) is shown with a horizontal dashed line.

Figure 4: Minimal changes in the frequency and proliferation of CD4⁺ T cell populations following administration of CD8 β 255R1. Absolute cell count and percent Ki-67⁺ of CD4⁺ T cells from peripheral blood and lymph nodes were measured by multicolor flow cytometry for 4 weeks following mAb infusion. Within peripheral blood, individual time points represent mean \pm SEM for animals that received CD8 β 255R1 (blue) or control IgG (black) and open circles represent a significant ($p < 0.05$) change from baseline. (a) Absolute cell count and (b) percent Ki-67⁺ of naive (left), effector memory (middle), and central memory (right) CD4⁺ T cells in peripheral blood. Within the lymph nodes, individual animals are represented by a unique symbol and red text represents a significant difference between groups. (c) Percent CD4⁺ T cells of CD3⁺ T cells and (d) percent Ki-67⁺ of CD4⁺ T cells from axillary lymph nodes biopsied at week 3 post mAb infusion.

Figure 5: Proliferation of CD8 $\alpha\alpha$ ⁺, NK cells, and $\gamma\delta$ T cells in peripheral blood following infusion with CD8 β 255R1. Absolute cell count and percent Ki-67⁺ of CD8 α ⁺ lymphocytes from peripheral blood and lymph nodes were measured by multicolor flow cytometry for 4 weeks after infusion with CD8 β 255R1 (blue) or control IgG (black). Within peripheral blood, individual time points represent mean \pm SEM for animals and open circles represent a significant change from baseline. Absolute count (top) and percent Ki-67⁺ (bottom) of (a) CD8 $\alpha\alpha$ ⁺ T cells, (b) NK cells, and (c) $\gamma\delta$ T cells in peripheral blood. Within the lymph nodes, individual animals are represented by a unique symbol and red text represents a significant difference between groups. (d) Percent CD8 $\alpha\alpha$ ⁺ T cells of CD3⁺ lymphocytes (top) and percent Ki-67⁺ CD8 $\alpha\alpha$ ⁺ T cells (bottom) in lymph nodes. (e) Percent NK cells of CD3⁻ lymphocytes (top) and percent Ki-67⁺ NK cells

(bottom) in lymph nodes. (f) Percent $\gamma\delta$ T cells of CD3+ lymphocytes (top) and percent Ki-67+ $\gamma\delta$ T cells (bottom) in lymph nodes.

Figure 6: Env-specific antiviral responses in lymph nodes following infusion with CD8 β 255R1. At 3 weeks post-infusion we performed intracellular cytokine staining to assess Gag-specific and Env-specific responses from lymph node biopsies for (a) CD8 $\alpha\beta$ + T cells, (b) CD8 $\alpha\alpha$ + T cells, and (c) CD4+ T cells. We assessed degranulation (CD107a, top) and pro-inflammatory cytokine secretion (IFN γ and TNF α , bottom) for seven animals infused with CD8 β 255R1 (blue) and four animals infused with IgG1 control (black). Both single and double positive responses were included as % IFN γ +TNF α +. Symbols represent individual animals.

Figure 7: Protection from intravenous SIVmac239 challenge following infusion with CD8 β 255R1. Four weeks after infusion with CD8 β 255R1 or control IgG, animals were challenged intravenously with SIVmac239. Viral load assays were performed for full-length *nef* (red, closed) and Δ *nef* (red, open) at the indicated time points for each animal and graphed in the context of absolute CD8 $\alpha\beta$ + T cell count (black). The limit of detection for the assay (100 vRNA copies/mL) is represented by a horizontal red dotted line. Animal IDs highlighted in red became infected following SIVmac239 challenge. *cy0690 required necropsy at week 6 following CD8 β 255R1 infusion.

Figure 8. Plasma neutralization potency *in vitro* is not predictive of protection from challenge *in vivo*. Plasma samples collected immediately before mAb infusion and 4 weeks later immediately before intravenous SIVmac239 challenge were used to test antibody neutralization potency against

select tier 1 (SIVmac239.H9, SIVsmE660.CP3C), tier 2 (SIVmac251.30), and tier 3 (SIVmac239) viruses. Neutralizing antibody titers were measured (a) prior to infusion with control IgG and (b) SIVmac239 challenge. Neutralizing antibody titers were measured (c) prior to infusion with CD8 β 255R1 and (d) SIVmac239 challenge. Titers are shown as 50% inhibitory dilution (ID50). Animals that became infected following SIVmac239 challenge are highlighted in red.

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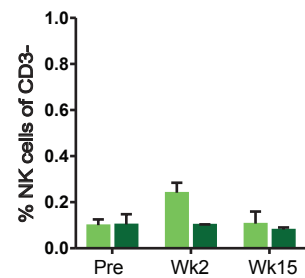
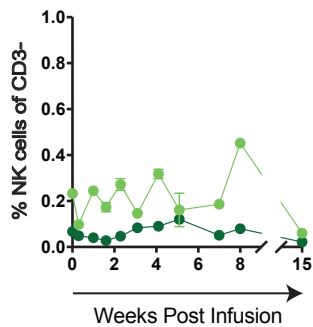
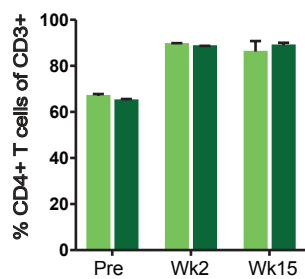
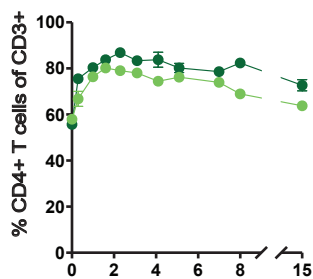
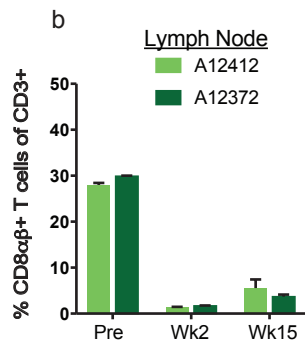
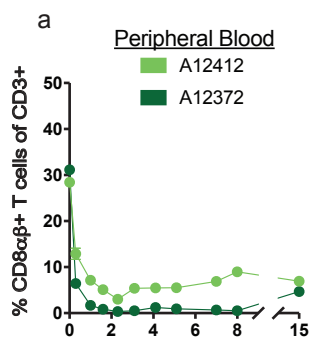
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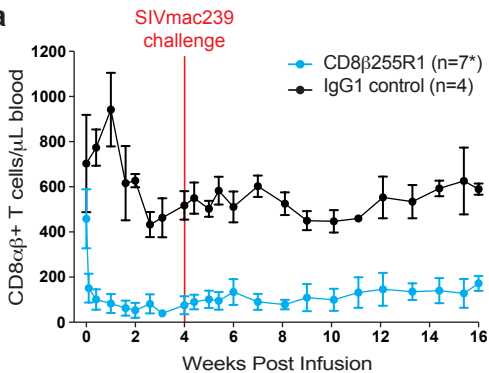
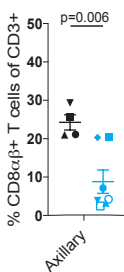
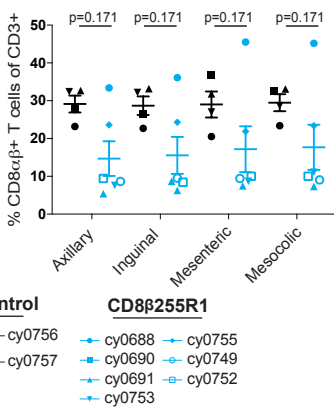
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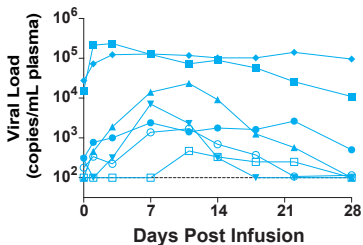


a**b****Week 3 LN****c****Necropsy LN**

a

CD8 β 255R1-treated

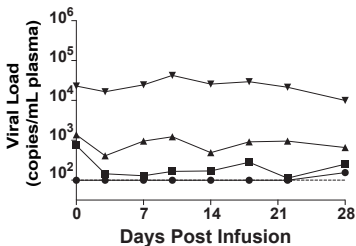
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b

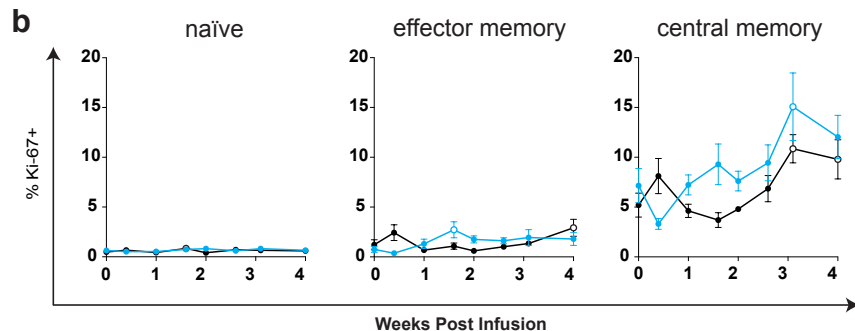
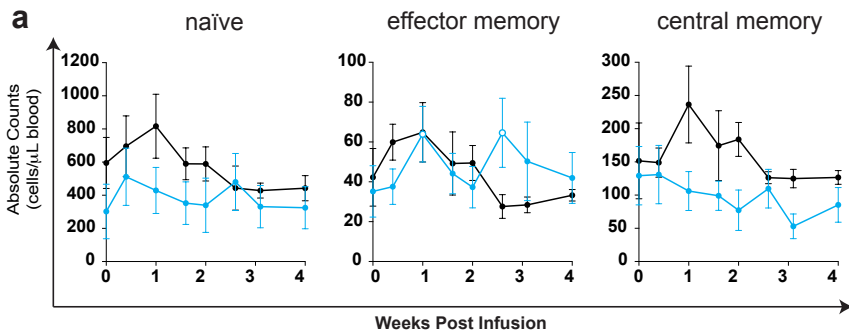
Control IgG-treated

cy0685 cy0756
 cy0750 cy0757



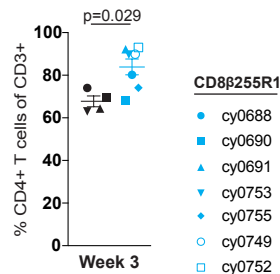
Peripheral Blood

CD8 β 255R1 —
Control IgG —

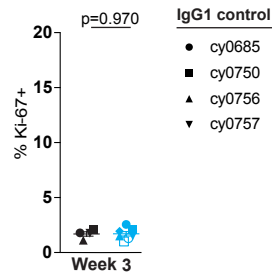


Lymph Node

c



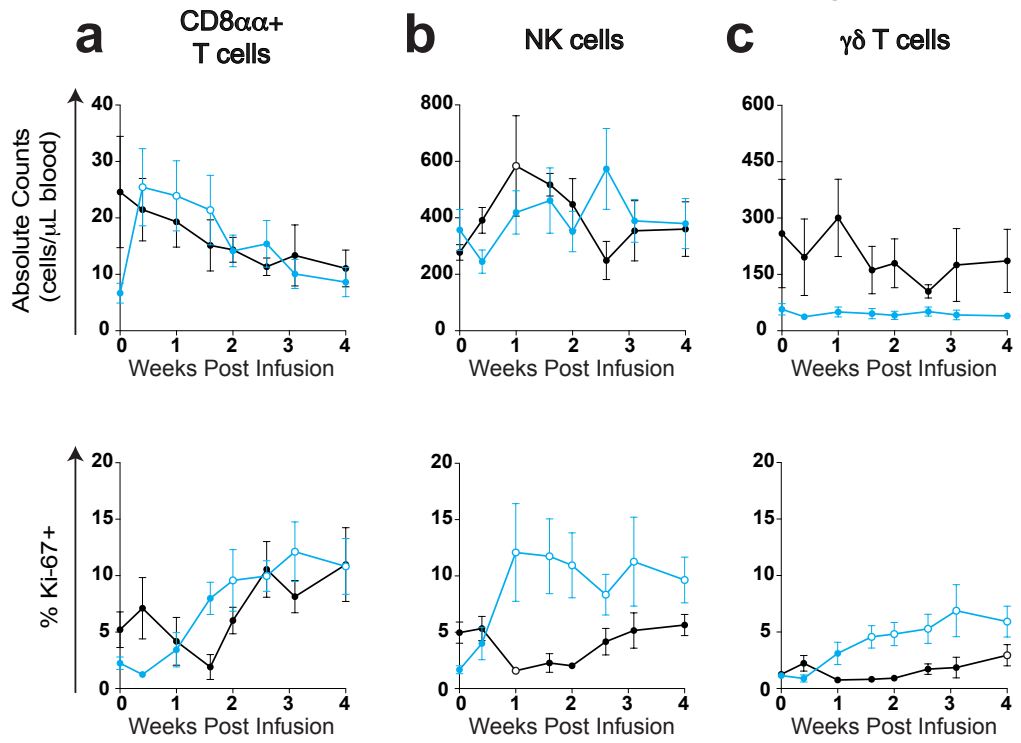
d



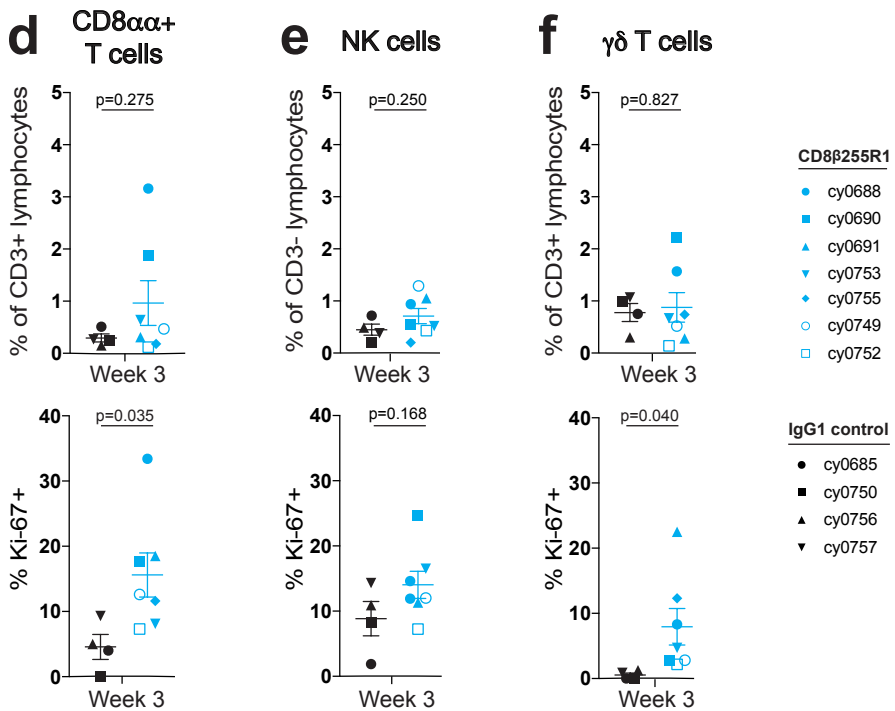
Peripheral Blood

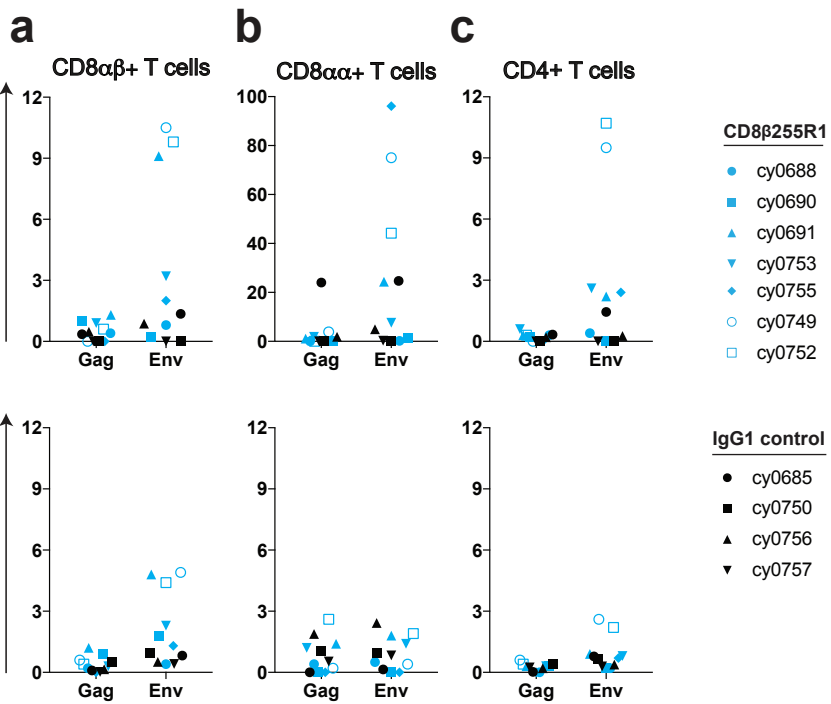
CD8 β 255R1

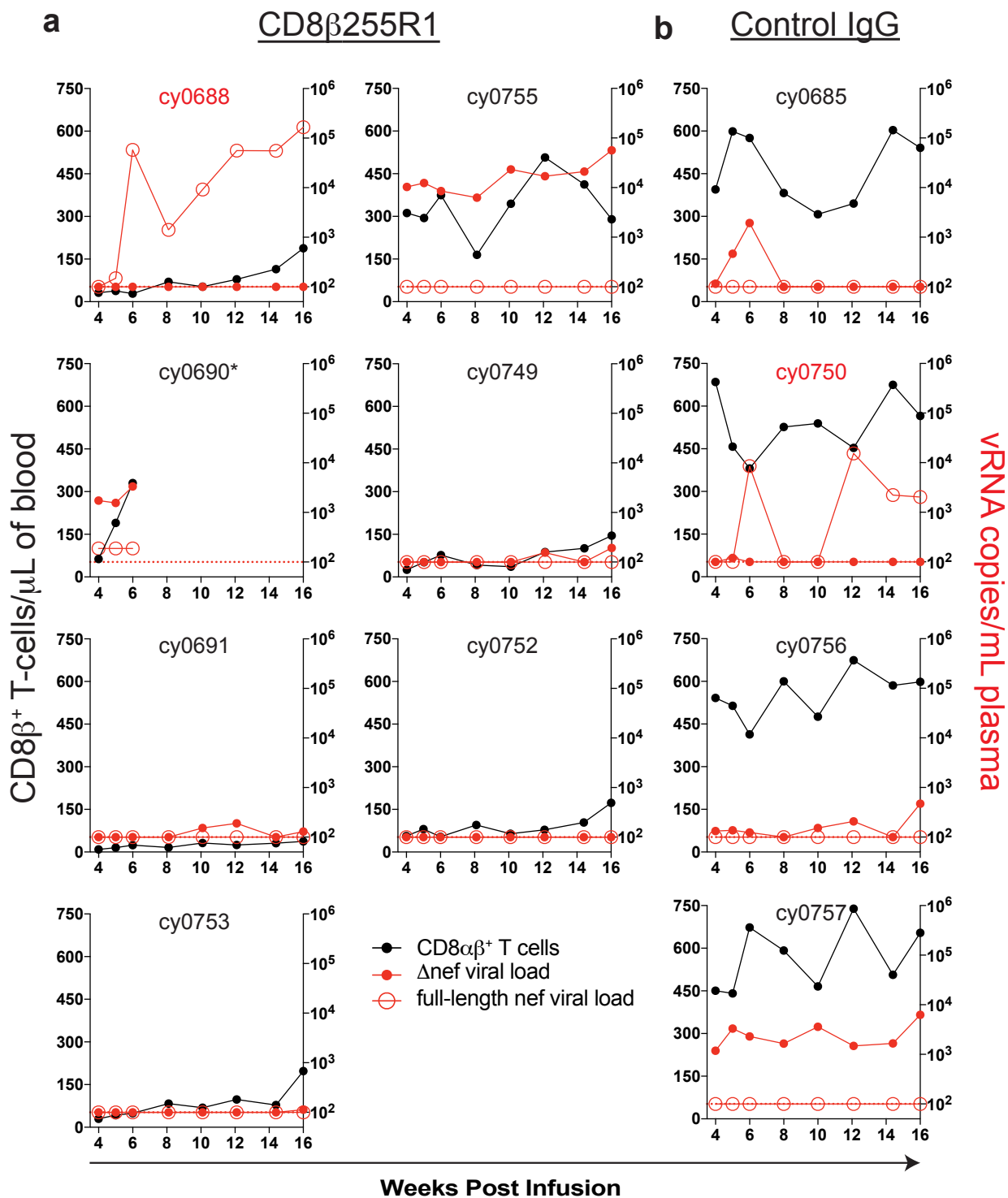
Control IgG



Lymph Node







a Control IgG

Pre-Infusion

	SIVmac251.H9	SIVsmE660 CP3C-A8	SIVmac251.30	SIVmac239
cy0685	14005	12796	9	<5
cy0750	52671	30452	<5	<5
cy0756	29731	14817	44	7
cy0757	101344	50291	<5	<5

Pre-Challenge

	SIVmac251.H9	SIVsmE660 CP3C-A8	SIVmac251.30	SIVmac239
cy0685	14802	14726	<5	<5
cy0750	60539	35916	25	5
cy0756	23512	14805	39	<5
cy0757	142641	56282	353	<5

b CD8β255R1

Pre-Infusion

	SIVmac251.H9	SIVsmE660 CP3C-A8	SIVmac251.30	SIVmac239
cy0688	14474	8448	38	6
cy0690	196887	163639	176	32
cy0691	18082	18310	<5	<5
cy0753	16320	5987	<5	<5
cy0755	196391	170995	37	11
cy0749	63018	36702	50	9
cy0752	14370	8016	46	<5

Pre-Challenge

	SIVmac251.H9	SIVsmE660 CP3C-A8	SIVmac251.30	SIVmac239
cy0688	46026	19218	<5	5
cy0690	241367	191707	105	35
cy0691	81148	79374	<5	<5
cy0753	89786	40222	337	25
cy0755	331729	233600	48	<5
cy0749	152644	93467	76	15
cy0752	20639	16873	59	9

ID50

<5	5-10	10-100	100-1000	1000-10,000	10,000-100,000	>100,000
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