

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

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4 Authors:

5 Matthew S. Sutton^a, Amy Ellis-Connell^a, Alexis J. Balgeman^a, Gabrielle Barry^b, Andrea M.
6 Weiler^b, Scott J. Hetzel^c, Yan Zhou^e, Annie Kilby^e, Rosemarie D. Mason^e, Kristin K. Biris^e, John
7 R. Mascola^e, Nancy J. Sullivan^e, Mario Roederer^e, Thomas C. Friedrich^{b,d}, and Shelby L.
8 O'Connor^{a,b}.

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10 Affiliations

11 ^a Department of Pathology and Laboratory Medicine, UW-Madison, Madison, WI

12 ^b Wisconsin National Primate Research Center, UW-Madison, Madison, WI

13 ^c Department of Biostatistics and Medical Informatics, UW-Madison, Madison, WI

14 ^d Department of Pathobiological Sciences, UW-Madison, Madison, WI

15 ^e Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National
16 Institutes of Health, Bethesda, MD

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

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20 #Address correspondence to Shelby L. O'Connor, slfeinberg@wisc.edu, (608) 890-0843

21 **Abstract: (word count: 249)**

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

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44 **Importance: (word count: 124)**

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

55

56 **Introduction: (word count: 776)**

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

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

73 The CD8 molecule is expressed as either a CD8 $\alpha\alpha$ homodimer or a CD8 $\alpha\beta$ heterodimer on
74 the cell surface and is present on lymphocytes of both the innate and adaptive immune system (15,
75 16, 18, 49). The most common lymphocytes to express CD8 are conventional CD8+ T cells
76 (TCR $\alpha\beta$ +CD3+), which can be divided into a major population that express CD8 $\alpha\beta$ and a minor
77 population that express CD8 $\alpha\alpha$ (13). There also exist populations of TCR $\gamma\delta$ +CD3+ T cells and
78 CD3- natural killer (NK) cells that express CD8 $\alpha\alpha$ (17, 18, 52). $\gamma\delta$ T cells (TCR $\gamma\delta$ +CD3+CD8 $\alpha\alpha$ +)
79 which comprise ~ 6% of CD3+ T cells (17) can block HIV-1 entry via the secretion of β -
80 chemokines (23), enhance antibody-dependent cellular cytotoxicity (ADCC) (24), and directly
81 lyse HIV-infected cells (25). NK cells (CD3-CD8 $\alpha\alpha$ +) comprise ~16% of peripheral lymphocytes,
82 and have recently been reported to possess traits of adaptive immunity that may contribute to
83 control of HIV-1 replication (19, 20). Accordingly, the contribution of conventional CD8+ T cells
84 to viral control are complicated by the depletion of additional cell populations that express CD8 α
85 when using CD8 α -depleting mAbs (10, 14, 53). One approach to better define the antiviral role of
86 CD8+ T cells *in vivo* is to administer a CD8 β -specific depleting mAb, as this should selectively
87 deplete CD8 $\alpha\beta$ + T cells without removing CD8 $\alpha\alpha$ + lymphocytes or other non-T cell populations.
88 Indeed, two recent studies using the CD8 β -specific mAb CD8 β 255R1 in rhesus macaques provide
89 evidence that CD8 $\alpha\beta$ + T cells can be specifically depleted *in vivo* (40, 41).

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

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

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

114

115 **Materials and Methods:**

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

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

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

137

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

148

149 **Peptides.** The NIH AIDS Research and Reference Reagent Program (Germantown, MD) provided
150 15-mer peptides overlapping by 11 amino acid positions that spanned the entire SIVmac239
151 proteome. Gag and Env peptides were combined into 2 and 3 pools, respectively, and used at a
152 final pooled concentration of 5 μ M during stimulation.

153

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

158 study, PBMC were isolated from EDTA-anticoagulated blood by ficoll-based density
159 centrifugation as previously described (34). Cells were resuspended in RPMI 1640 (HyClone)
160 supplemented with 10% fetal calf serum (FCS), 1% antibiotic-antimycotic (HyClone), and 1% L-
161 glutamine (HyClone) (R10 medium). Approximately 0.5-1.0 x 10⁶ PBMC were placed in cluster
162 tubes (Fisher Scientific) and washed with 1x phosphate-buffered saline (PBS) prior to staining.
163 Surface antibodies were added and cells were incubated for 30 min in the dark at room temperature.
164 Cells were then washed twice with 1x PBS supplemented with 10% FCS (10% FCS/PBS), and
165 fixed with 2% paraformaldehyde (PFA) for a minimum of 30 minutes. Fixed cells were then
166 washed once with 1x PBS and then permeabilized with Medium B (Invitrogen, Carlsbad CA) and
167 allowed to incubate with intracellular antibodies for 30 min in the dark at room temperature. Cells
168 were then washed twice with 10% FCS/PBS and resuspended in 2% PFA prior to data collection.
169 Following exclusion of doublets and dead cells, lymphocyte populations were defined as follows:
170 CD8αβ+ T cells: CD3+CD4-TCRγδ-CD8α+CD8β+; CD8αα+ T cells: CD3+CD4-TCRγδ-
171 CD8α+CD8β-; γδ T cells: CD3+CD4-CD8β-CD8α+TCRγδ+; NK cells: CD3-CD4-
172 CD8α+NKG2a+CD16+; CD4 T cells (naive): CD3+CD4+CD8α-CD95-CD28+CCR7+; CD4 T
173 cells (effector memory): CD3+CD4+CD8α-CD95+CD28-CCR7-; CD4 T cells (central memory):
174 CD3+CD4+CD8α-CD95+CD28+CCR7+.

175
176 **Intracellular cytokine staining (ICS).** Flow cytometry was used to measure intracellular cytokine
177 expression as previously described (59). Cryopreserved cells isolated from LNs were thawed and
178 washed twice in warm R10 medium and allowed to rest at 37°C overnight prior to stimulation. Ki-
179 67 was used to measure the proliferative capacity of lymphocytes freshly-isolated from blood, and
180 IFN-γ, tumor necrosis factor alpha (TNF-α), and CD107a was used to measure SIV-specific

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

191

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

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

205

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

215

216 **Statistical Analyses.** Comparison of two groups in Figures 2b-c, 4c-d, and 5d-f was conducted
217 using Wilcoxon rank sum tests. Comparison of changes from baseline separately for each
218 treatment group shown in Figures 2a, 3, 4a-b, and 5a-c utilized repeated measures ANOVA (RM-
219 ANOVA) to estimate the mean contrasts with animal as a random effect. Comparison between
220 both groups in changes from pre-treatment levels at multiple follow-up time points also utilized
221 RM-ANOVA with treatment, time, and their interaction as fixed effects, and animal as a random
222 effect. Model assumptions of RM-ANOVA were examined and were not deemed to be grossly
223 violated. Dunnett's p-value adjustment for multiple testing over multiple time points was used to
224 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).

227 **Results:**

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

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

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

253

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

265 Flow cytometry was used to monitor CD8 $\alpha\beta$ + T cells in PBMC and peripheral LNs of all
266 eleven MCMs. Percent reductions in the absolute count of peripheral CD8 $\alpha\beta$ + T cells in animals
267 that received CD8 β 255R1 were most prominent at week 2 (median: 94%; range: 70% to 98%);
268 CD8 $\alpha\beta$ + T cells remained significantly depleted at the time of SIVmac239 challenge (median:
269 89%; range: 45% to 97%) through the time of necropsy (Figure 2a, blue) (Supplementary Table
270 1). In control IgG-treated animals, the absolute counts of peripheral CD8 $\alpha\beta$ + T cells fluctuated
271 around baseline levels for 16 weeks (Figure 2a, black). Shortly after CD8 β 255R1 infusion, the
272 reduction in peripheral CD8 $\alpha\beta$ + T cell count was statistically significant compared to changes in

273 the IgG control animals (data not shown). Remarkably, the absolute count of peripheral CD8 $\alpha\beta$ +
274 T cells at 16 weeks remained depleted by an average of 64% when compared to baseline (median:
275 67%; range: 28% to 82%) in animals that received CD8 β 255R1. Persistent depletion of CD8 $\alpha\beta$ +
276 T cells after administration of CD8 β 255R1 lies in stark contrast to the reemergence of CD8 α +
277 lymphocytes within 2 to 4 weeks after administration of the anti-CD8 α mAb cM-T807 that has
278 been reported in other studies (8, 10, 14, 31, 40, 45-48, 53-54). Our results in both SIV-naïve and
279 LASIV+ animals are, however, consistent with another report that observed sustained CD8 $\alpha\beta$ + T
280 cell depletion in two SIV+ rhesus macaques that were treated with CD8 β 255R1 and followed for
281 approximately 18 weeks (40).

282 The extent to which CD8 β 255R1 depletes LN CD8 $\alpha\beta$ + T cells in macaques during either
283 chronic LASIV or pathogenic SIV infection has not yet been defined (40, 41). While technical
284 limitations prevented the longitudinal analysis of cell populations in lymph nodes of individual
285 LASIV+ animals, we compared the percentage of CD8 $\alpha\beta$ + T cells in the lymph nodes of animals
286 treated with CD8 β 255R1 to those treated with the control IgG antibody (Figure 2b). At week 3
287 post infusion, there were threefold fewer ($p<0.006$) CD8 $\alpha\beta$ + T cells as a proportion of CD3+ T
288 cells in biopsies of axillary lymph nodes isolated from animals infused with CD8 β 255R1 (median:
289 4%; range: 1.9% to 20%) compared to control IgG animals (median: 27%; range: 21% to 30%).
290 When lymph nodes were assessed 17 to 18 weeks after mAb infusion following necropsy, CD8 $\alpha\beta$ +
291 T cell frequencies remained substantially lower in the majority of CD8 β 255R1-treated animals
292 compared to control IgG-treated animals (Figure 2c).

293

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

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

303

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

319 CD8 $\alpha\beta$ + T cells detected at this time (Figure 2b). The percent Ki-67+ of total CD4+ T cells was
320 similar between groups at week 3 post mAb infusion (Figure 4d) (Supplementary Table 2). From
321 these analyses, animals who received an infusion of CD8 β 255R1 exhibited minimal changes in
322 the number and proliferative capacity of CD4+ T cells, even while plasma viremia was fluctuating.
323

324 **Proliferation of CD8 $\alpha\alpha$ + T cells, NK cells, and $\gamma\delta$ T cells after infusion with CD8 β 255R1.**
325 Another consequence of using a CD8 α -specific mAb is the simultaneous depletion of additional
326 cell populations that express CD8 α , such as CD8 $\alpha\alpha$ + T cells, NK cells, and $\gamma\delta$ T cells (10, 12, 14,
327 31, 40, 47, 53). Following infusion with CD8 β 255R1, we evaluated the absolute count and percent
328 Ki-67+ of these three cell populations in the periphery. In animals that received CD8 β 255R1, there
329 was a rapid increase in the absolute count of CD8 $\alpha\alpha$ + T cells (median: 3.6-fold; range: 3.2-fold to
330 3.8-fold) that differed initially from control IgG animals (Figure 5a, top) (Supplementary Table
331 3). This was followed by increases in the percent of CD8 $\alpha\alpha$ + T cells expressing Ki-67 (median:
332 4.5-fold; range: 4.2-fold to 5.3-fold) (Figure 5a, bottom). Control IgG-treated animals did not
333 exhibit significant differences from baseline in the absolute count of CD8 $\alpha\alpha$ + T cells or percent
334 that express Ki-67. Few differences were detected in the absolute count of NK cells (Figure 5b,
335 top) or $\gamma\delta$ T cells (Figure 5c, top) in each group or between groups for four weeks after infusion
336 (Supplemental Table 3). In contrast, we detected significant increases from baseline in the
337 frequency of NK cells and $\gamma\delta$ T cells expressing Ki-67 in the group treated with CD8 β 255R1 that
338 peaked at approximately 7-fold (median: 4.3-fold; range: 1.8-fold to 17.7-fold) and 10-fold
339 (median: 9.9-fold; range: 0.1-fold to 18.8-fold), respectively (Figures 5b and 5c, bottom). The
340 observed proliferation of NK cells in the CD8 β 255R1-treated animals was significantly greater
341 than control IgG-treated animals at days 7, 10, and 14 (Supplementary Table 4).

342 We also evaluated changes to the frequency and proliferation of CD8 $\alpha\alpha$ + T cells (Figure
343 5d), NK cells (Figure 5e), and $\gamma\delta$ T cells (Figure 5f) in lymph nodes of animals treated with
344 CD8 β 255R1 or control IgG at week 3 post infusion (Supplementary Table 2). Similar to cells in
345 the periphery, the frequency of CD8 $\alpha\alpha$ + T cells (Figure 5d, top), NK cells (Figure 5e, top), and $\gamma\delta$
346 T cells (Figure 5f, top) in lymph nodes of animals treated with CD8 β 255R1 was comparable to
347 control IgG animals. At week 3 after CD8 β 255R1 infusion, the frequency of Ki67+ CD8 $\alpha\alpha$ + T
348 cells (Figure 5d, bottom) and $\gamma\delta$ T cells (Figure 5f, bottom) was significantly higher than that
349 observed in control IgG animals. Taken together, our data indicate that CD8 $\alpha\alpha$ + T cells, NK cells,
350 and $\gamma\delta$ T cells exhibited rapid proliferation in either the PBMC or lymph nodes following
351 CD8 β 255R1 infusion, while their absolute cell counts remained relatively unchanged.

352

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

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

373

374 **Protection from SIVmac239 challenge during depletion of CD8 $\alpha\beta$ + T cells.** To determine
375 whether CD8 β 255R1 infusion increased susceptibility to infection with pathogenic SIV, we
376 performed a high dose intravenous challenge in all 11 animals at 4 weeks post antibody infusion
377 with SIVmac239 (55, 56). Previously, we found that 4/7 MCMs chronically infected with a
378 minimally immunogenic LASIV were protected from intravenous challenge with the same dose of
379 SIVmac239 (34). The 11 animals used in the current study were also chronically infected with a
380 *nef*-deleted SIV, so discriminating qRT-PCR assays were used to differentiate previous Δ *nef*
381 circulating virus from full-length *nef* that was present in the challenge strain. Surprisingly, there
382 was no replicating SIVmac239 in the plasma of six of the seven MCMs infused with CD8 β 255R1
383 (Figure 7a), despite depletion of peripheral CD8 β + lymphocytes at the time of challenge (median:
384 87%; range: 45% to 95%) (Figure 2a). Similarly, there was no replicating SIVmac239 detected in
385 the plasma of three out of four MCMs that received the control IgG antibody (Figure 7b), despite
386 having similar CD8 $\alpha\beta$ + T cell levels as pre-depletion (Figure 2a). It is possible that residual virus-
387 specific CD8 $\alpha\beta$ + T cells in the lymph nodes (Figure 6) were sufficient to protect animals from

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

391

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

406

407 **Discussion: (word count: 1,087)**

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

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

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

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

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

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

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

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

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

492

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507 **Figure legends:**

508

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

510 **cynomolgus macaques.** Two naïve cynomolgus macaques (A12412 and A12372) received a

511 single 50 mg/kg intravenous infusion with CD8 β 255R1. Percent CD8 β + T cells (top),

512 CD4+ T cells (middle), and CD16+ NK cells (bottom) in (a) peripheral blood and (b) lymph

513 nodes were measured by multicolor flow cytometry. Data are shown as mean and standard

514 deviation. Peripheral blood samples from each animal were analyzed in triplicate, while lymph

515 node samples from each animal were analyzed in duplicate.

516

517 **Figure 2: Depletion of CD8 $\alpha\beta$ + T cells during chronic LASIV infection.** (a) Absolute count of

518 CD8 $\alpha\beta$ + T cells were assessed in peripheral blood for 16 weeks following infusion with

519 CD8 β 255R1 (blue) or control IgG (black). Percent CD8 $\alpha\beta$ + T cells from lymph node biopsies at

520 (b) 3 weeks post-infusion and (c) at necropsy (week 17-18). Data are represented as mean and

521 standard error of the mean. *Data shown include four IgG-treated animals and seven CD8 β 255R1-

522 treated animals through week 6, at which point six CD8 β 255R1-treated animals are shown for the

523 remainder of the study due to one animal (cy0690) requiring early euthanasia.

524

525 **Figure 3: Regained control of viral recrudescence following depletion of peripheral CD8 $\alpha\beta$ +**

526 **T cells.** Plasma viral load was measured for four weeks after mAb infusion. (a) Viral load initially

527 increased following infusion with CD8 β 255R1, but returned to pre-depletion levels by day 18. (b)

528 Viral load did not change significantly in animals that received control IgG. The limit of detection

529 of the plasma viral load assay (100 vRNA copies/mL) is shown with a horizontal dashed line.

530

531 **Figure 4: Minimal changes in the frequency and proliferation of CD4+ T cell populations**
532 **following administration of CD8 β 255R1.** Absolute cell count and percent Ki-67+ of CD4+ T
533 cells from peripheral blood and lymph nodes were measured by multicolor flow cytometry for 4
534 weeks following mAb infusion. Within peripheral blood, individual time points represent mean \pm
535 SEM for animals that received CD8 β 255R1 (blue) or control IgG (black) and open circles
536 represent a significant ($p < 0.05$) change from baseline. (a) Absolute cell count and (b) percent Ki-
537 67+ of naive (left), effector memory (middle), and central memory (right) CD4+ T cells in
538 peripheral blood. Within the lymph nodes, individual animals are represented by a unique symbol
539 and red text represents a significant difference between groups. (c) Percent CD4+ T cells of CD3+
540 T cells and (d) percent Ki-67+ of CD4+ T cells from axillary lymph nodes biopsied at week 3 post
541 mAb infusion.

542

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

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

555
556 **Figure 6: Env-specific antiviral responses in lymph nodes following infusion with**
557 **CD8 β 255R1.** At 3 weeks post-infusion we performed intracellular cytokine staining to assess Gag-
558 specific and Env-specific responses from lymph node biopsies for (a) CD8 $\alpha\beta$ + T cells, (b) CD8 $\alpha\alpha$ +
559 T cells, and (c) CD4+ T cells. We assessed degranulation (CD107a, top) and pro-inflammatory
560 cytokine secretion (IFN γ and TNF α , bottom) for seven animals infused with CD8 β 255R1 (blue)
561 and four animals infused with IgG1 control (black). Both single and double positive responses
562 were included as % IFN γ +TNF α +. Symbols represent individual animals.

563
564 **Figure 7: Protection from intravenous SIVmac239 challenge following infusion with**
565 **CD8 β 255R1.** Four weeks after infusion with CD8 β 255R1 or control IgG, animals were challenged
566 intravenously with SIVmac239. Viral load assays were performed for full-length *nef* (red, closed)
567 and Δ *nef* (red, open) at the indicated time points for each animal and graphed in the context of
568 absolute CD8 $\alpha\beta$ + T cell count (black). The limit of detection for the assay (100 vRNA copies/mL)
569 is represented by a horizontal red dotted line. Animal IDs highlighted in red became infected
570 following SIVmac239 challenge. *cy0690 required necropsy at week 6 following CD8 β 255R1
571 infusion.

572
573 **Figure 8. Plasma neutralization potency *in vitro* is not predictive of protection from challenge**
574 ***in vivo*.** Plasma samples collected immediately before mAb infusion and 4 weeks later immediately
575 before intravenous SIVmac239 challenge were used to test antibody neutralization potency against

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

581

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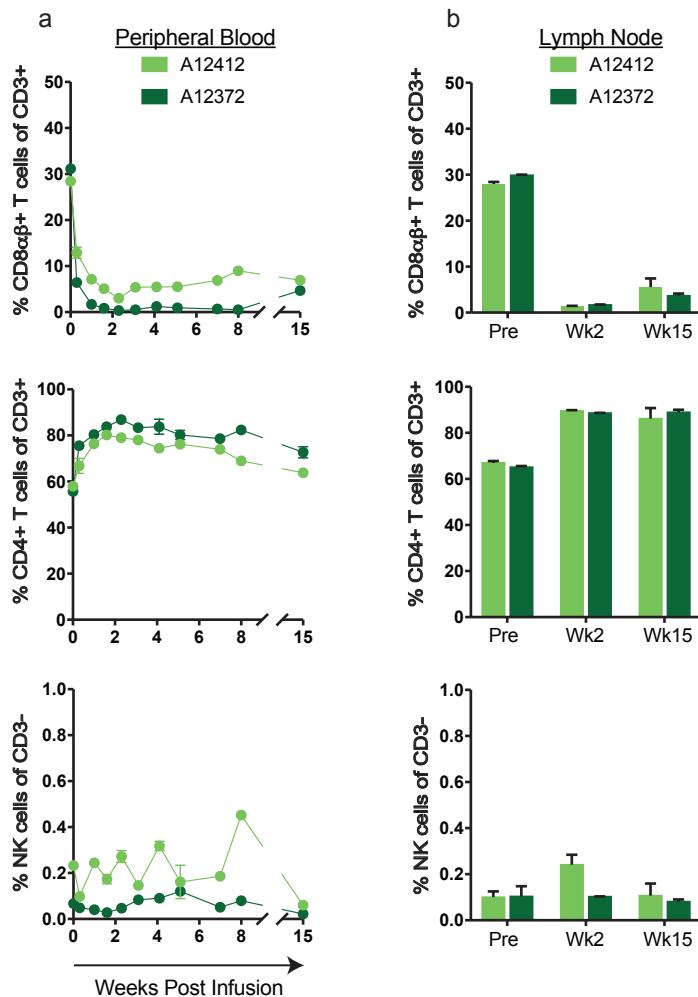
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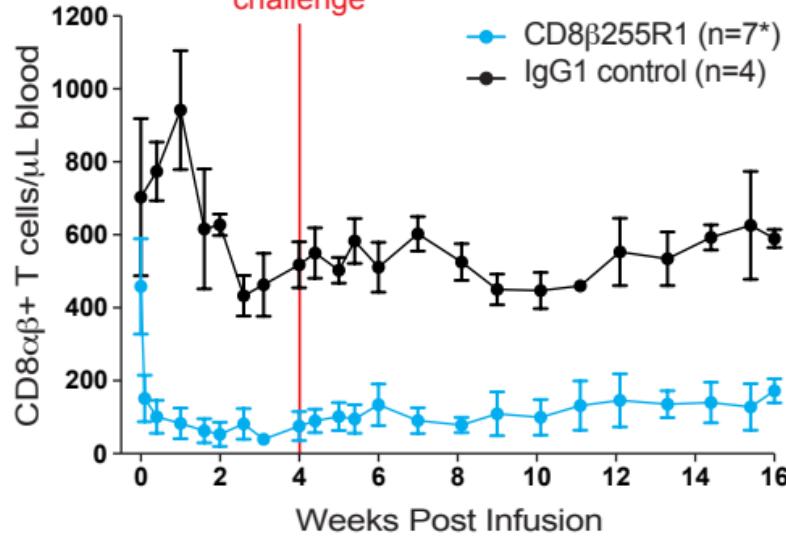
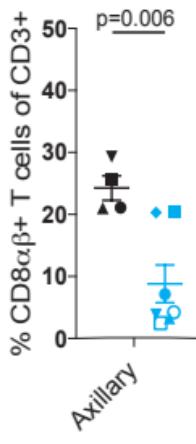
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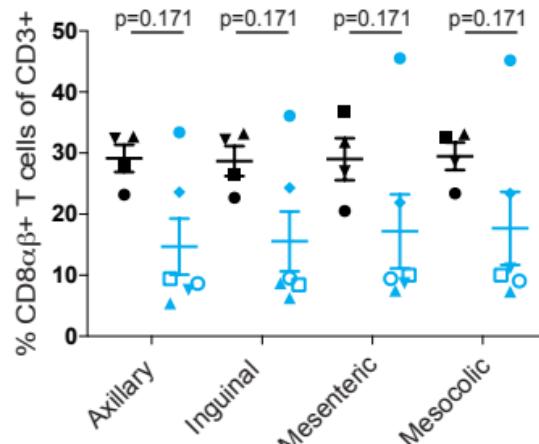
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aSIVmac239
challenge**b** Week 3 LN**c**

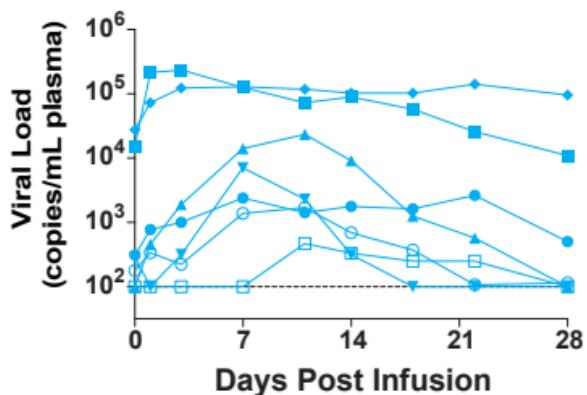
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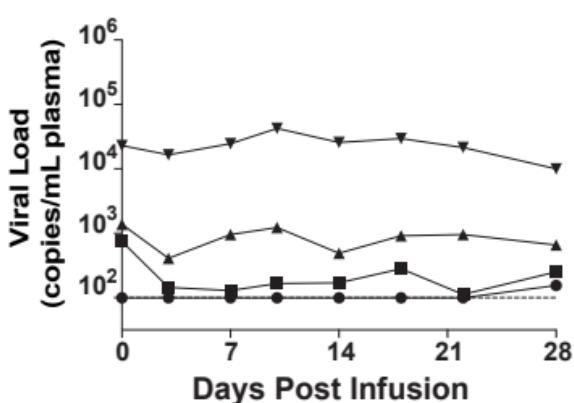
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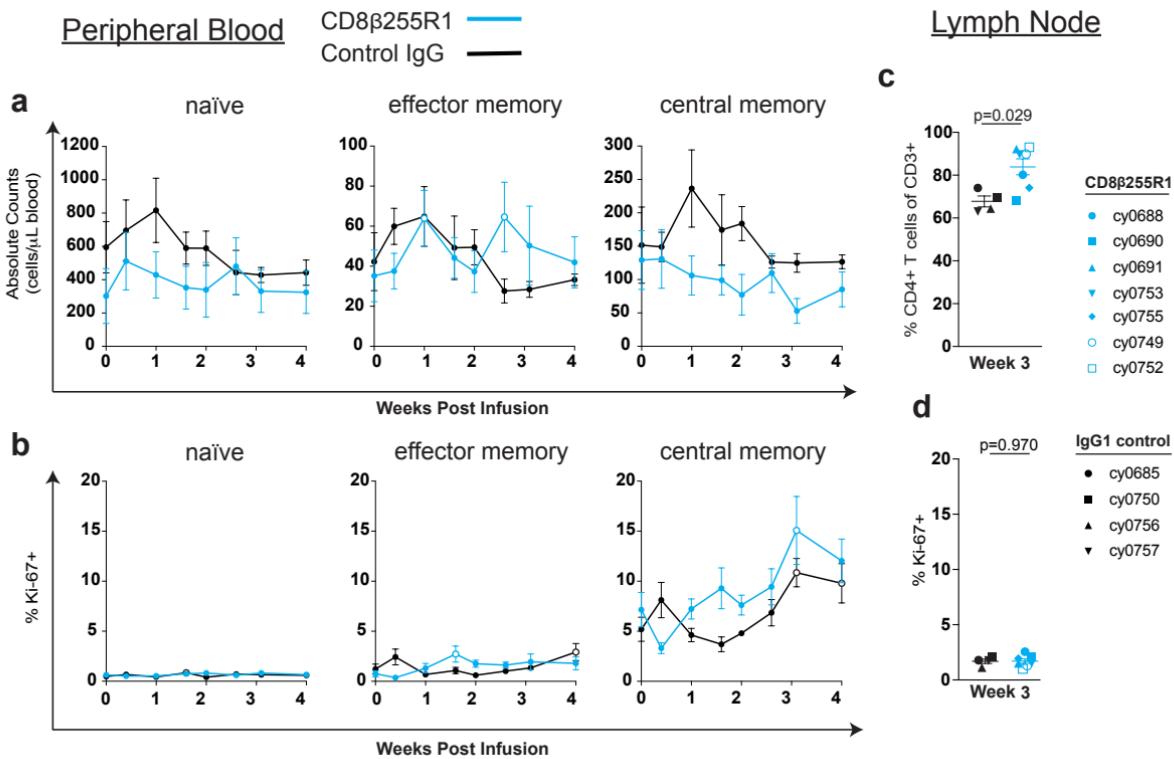
CD8 β 255R1

- cy0688
- cy0690
- ▲ cy0691
- ▼ cy0753
- cy0755
- cy0749
- ▲ cy0752
- ▼ cy0754

a**CD8 β 255R1-treated****Change from baseline**

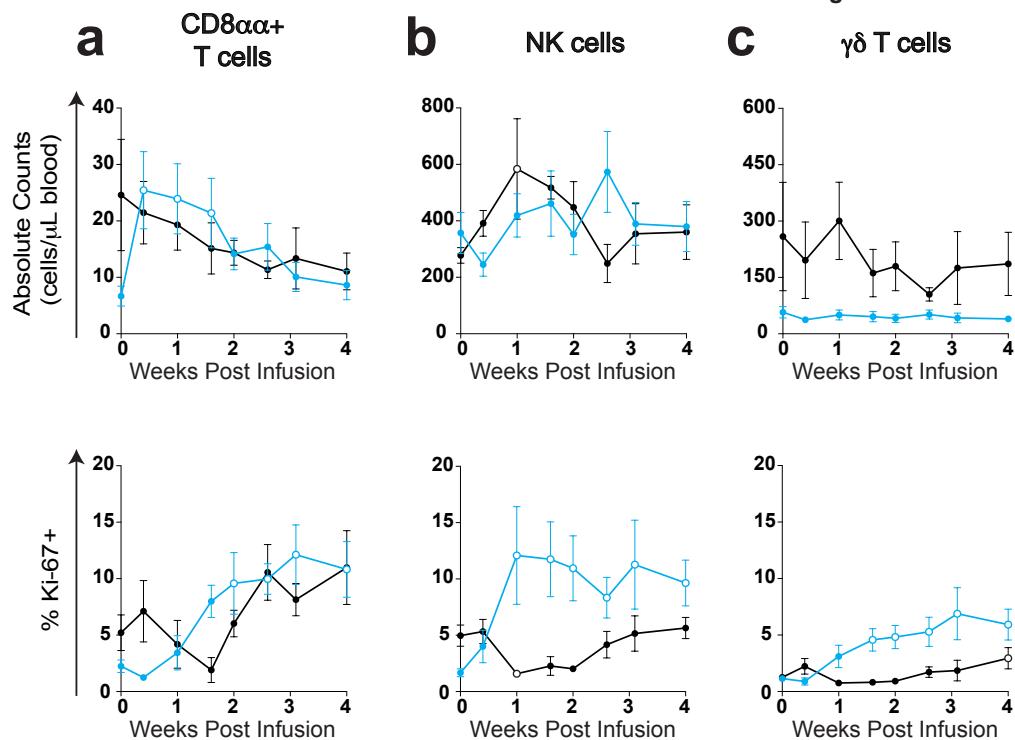
Day	p-value
3	0.078
7	< 0.001
10	< 0.001
14	0.006
18	0.173
22	0.470
28	1.000

b**Control IgG-treated**

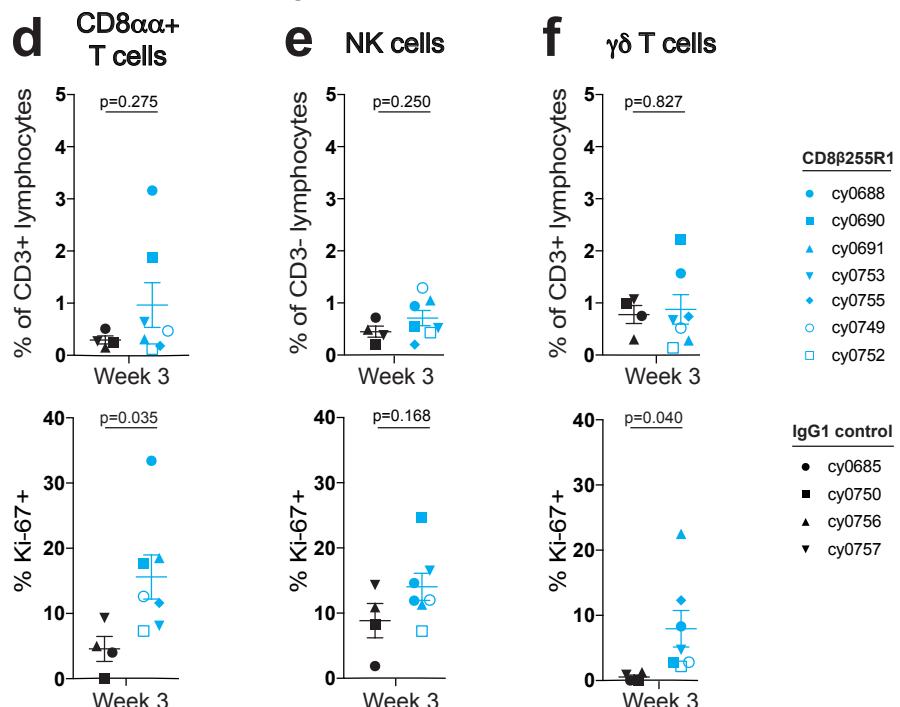


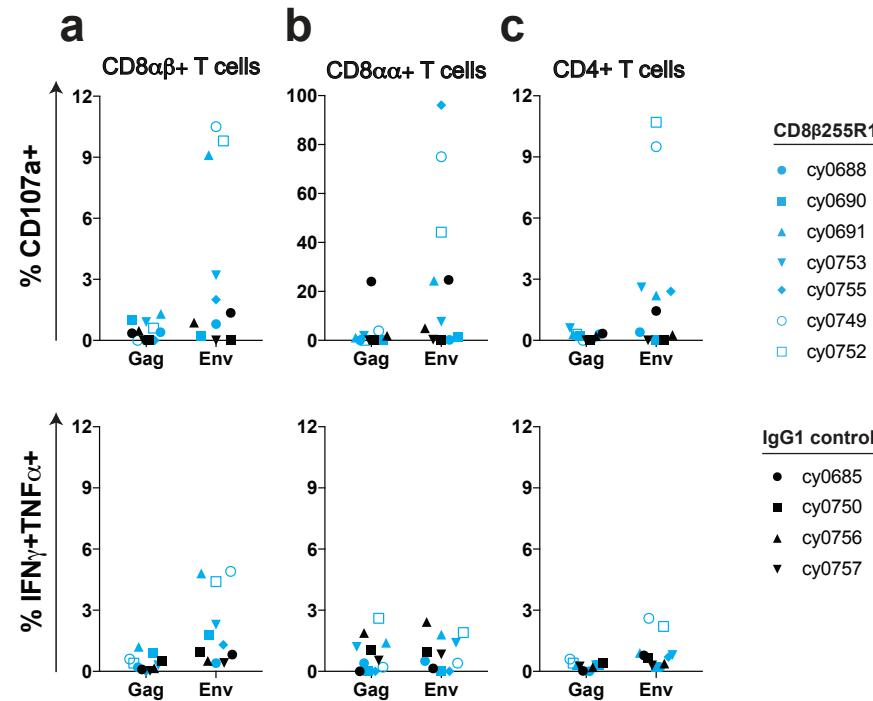
Peripheral Blood

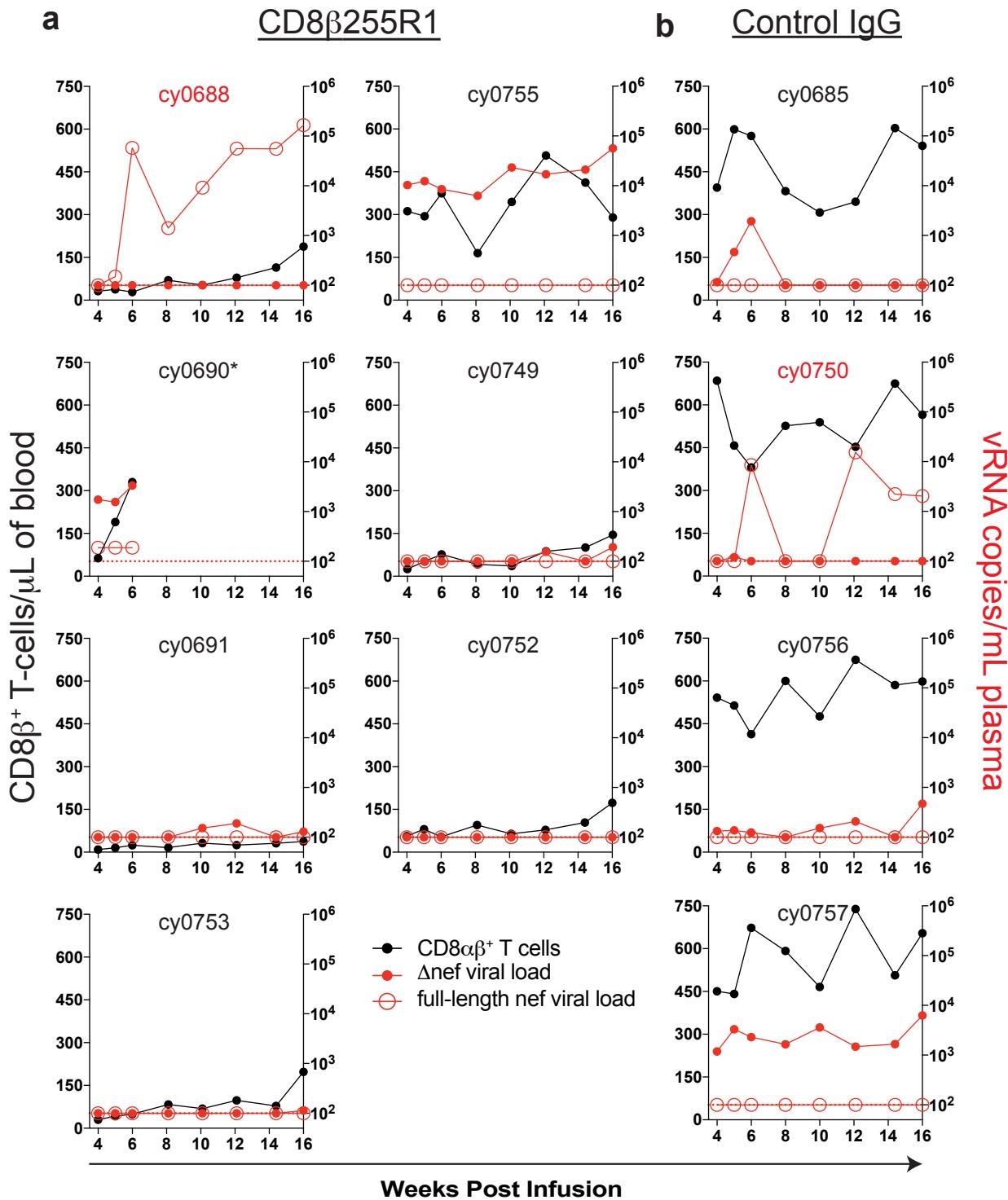
CD8 β 255R1 —
Control IgG —



Lymph Node







a Control IgG

Pre-Infusion					Pre-Challenge				
	SIVmac251.H9	SIVsmE660 CP3C-A8	SIVmac251.30	SIVmac239	SIVmac251.H9	SIVsmE660 CP3C-A8	SIVmac251.30	SIVmac239	
cy0685	14005	12796	9	<5	cy0685	14802	14726	<5	<5
cy0750	52671	30452	<5	<5	cy0750	60539	35916	25	5
cy0756	29731	14817	44	7	cy0756	23512	14805	39	<5
cy0757	101344	50291	<5	<5	cy0757	142641	56282	353	<5

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Pre-Infusion					Pre-Challenge				
	SIVmac251.H9	SIVsmE660 CP3C-A8	SIVmac251.30	SIVmac239	SIVmac251.H9	SIVsmE660 CP3C-A8	SIVmac251.30	SIVmac239	
cy0688	14474	8448	38	6	cy0688	46026	19218	<5	5
cy0690	196887	163639	176	32	cy0690	241367	191707	105	35
cy0691	18082	18310	<5	<5	cy0691	81148	79374	<5	<5
cy0753	16320	5987	<5	<5	cy0753	89786	40222	337	25
cy0755	196391	170995	37	11	cy0755	331729	233600	48	<5
cy0749	63018	36702	50	9	cy0749	152644	93467	76	15
cy0752	14370	8016	46	<5	cy0752	20639	16873	59	9

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