

# CD8+ lymphocytes modulate Zika virus dynamics and tissue dissemination and orchestrate antiviral immunity

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21 **Abstract**

22 CD8<sup>+</sup> lymphocytes are critically important in the control of viral infections, but their roles in  
23 acute Zika virus (ZIKV) infection remain incompletely explored in a model sufficiently similar  
24 to humans immunologically. Here, we use CD8<sup>+</sup> lymphocyte depletion to dissect acute immune  
25 responses in adult male rhesus and cynomolgus macaques infected with ZIKV. CD8 depletion  
26 delayed serum viremia and dysregulated patterns of innate immune cell homing and monocyte-  
27 driven transcriptional responses in the blood. CD8-depleted macaques also showed evidence of  
28 compensatory adaptive immune responses, with elevated Th1 activity and persistence of  
29 neutralizing antibodies beyond the clearance of serum viremia. The absence of CD8<sup>+</sup>  
30 lymphocytes increased viral burdens in lymphatic tissues, semen, and cerebrospinal fluid, and  
31 neural lesions were also evident in both CD8-depleted rhesus macaques. Together, these data  
32 support a role for CD8<sup>+</sup> lymphocytes in the control of ZIKV dissemination and in maintaining  
33 immune regulation during acute infection of nonhuman primates.

34

## 35 **Introduction**

36 ZIKV has been a known pathogen for over half a century (Dick et al., 1952), but severe  
37 manifestations of the disease were not directly associated with the virus for most of its history.  
38 Although recent outbreaks of ZIKV in the Western hemisphere are notorious for neurological  
39 complications including congenital Zika syndrome (CZS) and Guillain-Barré syndrome (GBS),  
40 most cases remain asymptomatic, and when symptoms arise, they are usually mild and self-  
41 limiting (Plourde and Bloch, 2016). Differential immune responses to ZIKV infection may  
42 dictate the severity of accompanying diseases and underlie clinical outcomes.

43 As the immunological correlates of protection against ZIKV are beginning to be explored, the  
44 CD8 T cell response is emerging as an important mediator of viral control, as is true with other  
45 flaviviruses (Slon Campos et al., 2018). Studies in mice have identified CD8 T cell responses to  
46 ZIKV infection, but the induction of ZIKV-associated pathology in these models requires  
47 deficiency in type-I interferon (IFN) signaling (Elong Ngono et al., 2017; Huang et al., 2017),  
48 which is not representative of natural ZIKV infection in humans. This is perhaps an unavoidable  
49 caveat, as ZIKV is incapable of antagonizing type-I IFN signaling in mice as it does in humans  
50 due to a lack of recognition of murine STAT2 by ZIKV NS5 (Grant et al., 2016). Disrupting  
51 IFN-I signaling, either genetically or through antibody blockade is, therefore, necessary to  
52 recapitulate ZIKV neurotropism in mouse models. Importantly, these studies have described dual  
53 protective and deleterious effects of CD8 T cell responses in ZIKV infected mice. While CD8+  
54 lymphocyte infiltration appears to reduce viral burdens in the brain, spinal cord and lymphatic  
55 tissue (Elong Ngono et al., 2017; Huang et al., 2017), under certain circumstances, CD8+ influx  
56 may also promote immunopathology, evidenced by neural damage and paralysis (Jurado et al.,  
57 2018). However, these findings have yet to be replicated in a model sufficiently similar to  
58 humans genetically and immunologically. Given the recent development of rhesus (Coffey et al.,  
59 2017; Dudley et al., 2016; Dudley et al., 2018; Magnani et al., 2018a; Osuna et al., 2016a) and  
60 cynomolgus (Koide et al., 2016; Osuna et al., 2016a) macaque models of ZIKV infection, we  
61 sought to explore the role of CD8+ lymphocytes in acute ZIKV infection by way of CD8+  
62 lymphocyte depletion. CD8+ depletion is a well-established immune manipulation in nonhuman  
63 primates (Schmitz et al., 1999) and is thus a plausible approach to gauge how the absence of  
64 CD8+ cells impacts acute viremia and potentially modulates adaptive responses.

65 In the present study, we infected four adult male rhesus macaques and five adult male  
66 cynomolgus macaques with a minimally passaged Brazilian ZIKV strain. Prior to infection, two  
67 animals of each species were depleted of CD8+ lymphocytes, including CD8 T cells and natural  
68 killer (NK) cells. The absence of CD8+ lymphocytes resulted in striking virus-response patterns  
69 that were not evident in nondepleted macaques, including delayed serum viremia, enhanced viral  
70 dissemination to peripheral tissues, and global repression of antiviral gene transcription. CD8-  
71 depleted rhesus macaques also manifested brainstem lesions that were characterized by increased  
72 inflammation. Finally, the absence of CD8+ lymphocytes appeared to alter patterns of monocyte  
73 expansion and activation and induce compensatory adaptive responses, characterized by  
74 enhanced Th1 phenotypes and prolonged neutralizing antibody production.

## 75 **Methods**

### 76 **Animal experiments**

77 The four adult male Indian origin rhesus macaques (*Macaca mulatta*) and five adult male  
78 cynomolgus macaques (*Macaca fascicularis*) utilized in this study were housed at the Tulane

79 National Primate Research Center (TNPRC). The TNPRC is fully accredited by AAALAC  
80 International (Association for the Assessment and Accreditation of Laboratory Animal Care),  
81 Animal Welfare Assurance No. A3180-01. Animals were cared for in accordance with the NRC  
82 Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act Animal  
83 experiments were approved by the Institutional Animal Care and Use Committee of Tulane  
84 University (protocol P0367).

85 Two rhesus macaques (R25671 and R64357) and two cynomolgus macaques (C78777 and  
86 C18942) were depleted of CD8<sup>+</sup> lymphocytes by administration of the anti-CD8 $\alpha$  antibody  
87 MT807R1 (NHP Reagent Resource; <https://www.nhpreagents.org>) (Schmitz et al., 1999). The  
88 initial subcutaneous administration of 10 mg/kg at 14 days pre-infection was followed by three  
89 intravenous administrations of 5 mg/kg at 11, 7, and 5 days pre-infection, as per the distributor's  
90 protocol. C84545 was treated with the irrelevant control antibody anti-desmipramine (NHP  
91 Reagent Resource; <https://www.nhpreagents.org>) at the same dosages and time intervals pre-  
92 infection. All animals were subcutaneously infected with 10<sup>4</sup> PFU of a Brazilian ZIKV isolate  
93 (Bonaldo et al., 2016) at 0 days post-infection (dpi) (Fig. 1a). As part of a previous study,  
94 C46456 (nondepleted cynomolgus macaque) was splenectomized 9 months and 19 days prior to  
95 inoculation with ZIKV. For data comparison, we included viral loads and complete blood count  
96 (CBC) data from a previous cohort of 4 non-pregnant female rhesus macaques (R32835,  
97 R24547, R25508, R22624) that were similarly infected with the same dose of the same Brazilian  
98 ZIKV isolate that was used in this study (Supplementary Figs. 2a-e).

99 Whole blood, cerebrospinal fluid (CSF), and semen were obtained from animals at the indicated  
100 timepoints (Fig. 1a). Peripheral blood mononuclear cells (PBMCs) were isolated from the blood  
101 of rhesus macaques using SepMate tubes (Stemcell Technologies) according to the  
102 manufacturer's protocol or from the blood of cynomolgus macaques using Lymphoprep  
103 (Stemcell Technologies) for standard density gradient centrifugation. At necropsy, the indicated  
104 tissues were collected and snap-frozen.

### 105 **Virus quantification**

106 Viral RNA was extracted from serum and CSF using the High Pure Viral RNA Kit (Roche).  
107 Semen, as well as the indicated lymphoid, reproductive, GI, and neural tissues were  
108 homogenized in Qiazol (Qiagen) using either disposable tissue grinders (Fisherbrand) or a  
109 TissueRuptor (Qiagen), and RNA was isolated using the RNeasy Lipid Tissue Mini Kit  
110 (Qiagen). Viral RNA from body fluids and tissues was quantified using qRT-PCR as described  
111 previously (Magnani et al., 2018b).

### 112 **Antiviral gene expression assays**

113 2.5 ml whole blood was drawn from each animal at 0, 1, 3, and 15 dpi into PAXgene blood RNA  
114 tubes (PreAnalytiX) and equilibrated to -80°C as per the manufacturer's protocol. RNA was  
115 extracted from blood samples using the PAXgene blood RNA kit (PreAnalytiX), and cDNA was  
116 synthesized using the RT2 First Strand Kit (Qiagen). Transcriptional profiles of immune  
117 signaling were generated using the nCounter NHP Immunology Panel of 770 macaque immune  
118 response genes (NanoString Technologies). In whole blood, transcriptional responses were  
119 assessed at 3 dpi relative to expression levels pre-infection using nSolver software v4.0  
120 (NanoString Technologies). Fold change data were imported into Ingenuity Pathway Analysis  
121 (IPA) (Qiagen) to discern relevant signaling pathways and disease functions. Antiviral  
122 transcriptional responses were confirmed by way of qRT-PCR using a rhesus macaque RT2

123 Profiler PCR Array (Qiagen). Responses within each species and treatment group were analyzed  
124 together to identify expression levels at the indicated timepoints relative to pre-infection.  
125 Heatmaps of gene expression and disease-related pathways were generated using Morpheus  
126 (<https://software.broadinstitute.org/Morpheus>). Principal component analysis (PCA) of antiviral  
127 gene expression was performed using ClustVis (Metsalu and Vilo, 2015).

128 To identify cell populations contributing to antiviral signaling in blood, the CD14 and CD8  
129 MicroBead kits (Miltenyi Biotec) were used to sort CD14<sup>+</sup> monocytes and CD8<sup>+</sup> lymphocytes  
130 from the PBMCs of the indicated animals at peak transcriptional activity (3 dpi). RNA was  
131 isolated from cell fractions using the RNeasy Mini Kit (Qiagen), and cDNA was synthesized  
132 using the RT2 First Strand Kit (Qiagen). Transcriptional profiling was performed using the  
133 nCounter NHP Immunology Panel (NanoString) and verified by RT2 qPCR Primer Assays  
134 (Qiagen) for ISG15, OAS2, and DDX58.

135 To characterize antiviral signaling in myeloid cells, PBMCs were isolated from the whole blood  
136 of ZIKV-naïve colony rhesus macaques as described above, and the CD14 MicroBead kit  
137 (Miltenyi Biotec) was used to isolate monocytes. For the co-culture assay, monocytes were  
138 incubated in the presence or absence of autologous CD8<sup>+</sup> cells and/or ZIKV in a 96-well U-  
139 bottom plate with 50,000 cells of each type plated per well for the indicated timepoints. qPCR  
140 for ISG15 and OAS2 was performed as described above. For antiviral gene screening in  
141 monocyte-derived macrophages (MDMs), monocytes were cultured at  $1 \times 10^6$  cells/ml in  
142 RPMI-1640 medium supplemented with 1% human AB serum (Sigma), 20 ng/ml M-CSF  
143 (Peprotech), 1% L-glutamine, and 1% penicillin/streptomycin. After 7 days of culture,  
144 monocytes were sufficiently differentiated into MDMs and were either infected with the same  
145 Brazilian ZIKV isolate described above or left uninfected. At 24 hpi, RNA was extracted using  
146 the RNeasy Mini Kit (Qiagen), cDNA was synthesized using the RT2 First Strand Kit (Qiagen),  
147 and transcriptional signaling was assessed using the rhesus macaque antiviral response RT2  
148 Profiler PCR Array (Qiagen). Antiviral gene expression in ZIKV-infected monocyte-derived  
149 macrophages (MDMs) was calculated relative to uninfected controls.

#### 150 **Flow cytometry and gating strategy**

151 For absolute lymphocyte counts, whole blood was stained within 2 hours of blood draw for the  
152 surface markers CD45 (PerCP; DO58-1283; BD Biosciences), CD3 (FITC; SP34; BD  
153 Biosciences), CD4 (APC; L200; BD Biosciences), and CD8 (V500; SK1; BD Biosciences). Flow  
154 cytometry was performed on a BD FACSVers instrument, and absolute counts were calculated  
155 using FACS Suite software.

156 For immunophenotyping, PBMCs from the indicated timepoints were thawed, washed, and  
157 stained using Live/Dead Fixable Aqua Dead Cell Stain Kit (Invitrogen). PBMCs were then  
158 stained for the surface markers CD16 (AL488; 3G8; BioLegend), CD169 (PE; 7-239;  
159 BioLegend), CD28 (PECF594; CD28.2; BD Biosciences), CD95 (PCP-Cy5.5; DX2;  
160 BioLegend), CD3 (PE-Cy7; SP34-2; BD Biosciences), CD8 (PacBlue; SK1; BioLegend), CD14  
161 (BV605; M5E2; BD Biosciences), HLA-DR (BV650; L243; BioLegend), NKG2A (APC; Z199;  
162 Beckman Coulter), and CD4 (APC-H7; L200; BD Biosciences). Cells were subsequently fixed in  
163 FluoroFix buffer (BioLegend), permeabilized using Perm/Wash buffer (BioLegend), and stained  
164 intracellularly for CD69 (BV711; FN50; BD Biosciences) and Ki67 (AL700; B56; BD  
165 Biosciences). Flow cytometry was performed on a BD LSRII instrument and data were analyzed  
166 using FlowJo (vX.10.4.2) and visual t-distributed stochastic neighbor embedding (viSNE)

167 (Cytobank) softwares. For viSNE analysis, live singlet monocytes (CD14+ and/or CD16+) or  
168 live singlet CD3+ T cells were gated prior to downsampling at a minimum of 500 cells per  
169 animal in FlowJo v. 10.5.3. Downsampled files for each animal were then concatenated by group  
170 (i.e., species, dpi, and treatment condition). When the number of animals differed per group,  
171 concatenated files were further downsampled to achieve an equal number of cells per group.  
172 viSNE was conducted using Cytobank with the following settings: Perplexity = 30, Iterations =  
173 1000, Theta = 0.5, Seed = random, Compensation = internal file. For the monocyte viSNE, the  
174 following parameters were utilized in the run: Ki67, CD14, HLA-DR, CD69, CD95, CD14, and  
175 CD169. For the CD3+ T cells viSNE, the following parameters were utilized in the run: Ki67,  
176 CD4, HLA-DR, CD69, CD95, CD28, CD3, CD8.

177 For general immunophenotyping analysis, cytometry data were first gated for lymphocytes,  
178 singlets, and live cells. NK cells were considered as CD8+/CD16+. CD4 T cells (CD3+/CD4+)  
179 and CD8 T cells (CD3+/CD8+) were gated into naïve (CD28+/CD95-), central memory (CM)  
180 (CD28+/CD95+), and effector memory (EM) (CD28-/CD95+) subsets. CD3- cells were divided  
181 into B cells (DR+/CD14-/CD16-) and monocytes (classical, CD14++/CD16-; intermediate,  
182 CD14+/CD16+; nonclassical, CD14<sup>low</sup>/CD16+). Cell subsets were analyzed with respect to  
183 frequency, proliferation (Ki67+) and activation (CD69+ or CD169+).

#### 184 **Intracellular cytokine staining**

185 PBMCs from the indicated timepoints were thawed and rested overnight prior to stimulation with  
186 peptide pools comprising ZIKV capsid (C), membrane (M), envelope (E), and nonstructural  
187 protein 1 (NS1) (BEI Resources). On peptide stimulation, cells were also treated with brefeldin  
188 A (BioLegend), GolgiStop (BD Biosciences), anti-CD28 (NHP Reagent Reference Program,  
189 [www.nhpreagents.org/](http://www.nhpreagents.org/)), anti-CD49d (9F10; BioLegend), and anti-CD107a (AL700; H4A3; BD  
190 Biosciences). 24 hours post-stimulation, cells were stained for the surface markers CD3 (PE-  
191 Cy7; SP34-2; BD Biosciences), CD8 (PacBlue; SK1; BioLegend), and CD4 (APC-H7; L200;  
192 BD Biosciences). Cells were also fixed and permeabilized as described above and stained  
193 intracellularly for perforin (FITC; Pf-344; Mabtech), granzyme B (PE; GB12; Invitrogen), CD69  
194 (PE-CF594; FN50; BD Biosciences), IL-2 (PCP-Cy5.5; MQ1-17H12; BD Biosciences), and  
195 IFN $\gamma$  (AL647; 4S.B3; BioLegend). Flow cytometry was performed on a BD LSRII instrument  
196 and data were analyzed using FlowJo software (vX.10.4.2).

#### 197 **Plaque reduction neutralization tests**

198 ZIKV plaque reduction neutralization tests (PRNTs) were conducted according to previously  
199 published protocols (Lieberman et al., 2009; Ward et al., 2018). Briefly, ZIKV MEX-I-44  
200 isolated in Tapachula, Mexico in 2016 was obtained from The University of Texas Medical  
201 Branch, Galveston, TX and cultured to passage 8 in Vero cells. Serum specimens were incubated  
202 for one hour at serial dilutions of 1:10, 1:20...1:320 with a previously frozen virus stock of  
203 known plaque forming unit (PFU). Samples were then inoculated in duplicate onto a mono-layer  
204 of Vero cells grown on 6-well plates and allowed to incubate for an additional hour. Infectious  
205 material was then removed and replaced with a 1:1 mixture of Vero media and Avicel® before  
206 being incubated for 4 days. To read plaques, the Avicel® layer was fixed with 10% neutral  
207 buffered formalin. Finally, the formalin-Avicel® layer was removed and the monolayer was  
208 stained with crystal violet, washed with tap water and allowed to dry before plaques were  
209 counted manually.

210 Percent reduction in observed plaques and a PRNT90 cutoff were used for interpretation. A  
211 PRNT90 titer is the dilution of a sample at which a 90% reduction in possible plaques is  
212 observed. The maximum number of potential plaques was obtained for each run using a  
213 corresponding back-titration and a linear model was fit to the observed number of plaques for  
214 each dilution. A PRNT90 titer was derived for each sample using the linear model and the  
215 equation for a straight line in the statistical program R (R Core Team, 2018). For samples that  
216 were positive but above the resolution of the PRNT assay the value of the greatest number of  
217 possible plaques for that run, as determined by the back titration, was assigned for each dilution  
218 for use with the linear model.

### 219 **Histology**

220 Tissues samples collected at necropsy were fixed in Z-Fix (Anatech), embedded in paraffin and 5  
221  $\mu$  m thick sections were cut, adhered to charged glass slides, and either stained routinely with  
222 hematoxylin and eosin or Prussian blue.

### 223 **Statistical analysis**

224 Statistical analysis was conducted using GraphPad Prism v6.07 (GraphPad Software). A Mann-  
225 Whitney test was used to compare neutrophil-to-lymphocyte ratios (NLRs) among treatment  
226 conditions (CD8-depleted versus nondepleted).

## 227 **Results**

### 228 **Delayed serum viremia and altered leukocyte kinetics**

229 CD8<sup>+</sup> lymphocyte depletion commenced 14 days prior to ZIKV infection (Fig. 1a), and CD8 T  
230 cells were undetectable in all depleted animals well before infection (Fig. 1b & Supplementary  
231 Fig. 1a). To achieve CD8 depletion, we used the MT807R1 antibody (Schmitz et al., 1999) to  
232 target CD8 $\alpha$ , effectively depleting CD8 T cells (Fig. 1b & Supplementary Fig. 1a), CD8<sup>+</sup>/CD4<sup>+</sup>  
233 double-positive T cells (Supplementary Fig. 1b), and NK cells (Fig. 1c) but not CD4 T cells  
234 (Supplementary Fig. 1c) from the blood of all treated animals. As MT807R1 targets NK cells in  
235 addition to CD8<sup>+</sup> lymphocytes, any deficiency in host response following treatment with anti-  
236 CD8 $\alpha$  could indicate that either or both types of cells are important for acute control of ZIKV.  
237 Flow cytometric analysis of NK cell frequency in nondepleted animals revealed expansion early  
238 in infection (Fig. 1c). Intriguingly, the CD8-depleted macaque R64357 recovered CD8 T cells  
239 and NK cells at later timepoints, between 15 and 21 days post-infection (dpi) (Figs. 1b-c).

240 Following subcutaneous inoculation with ZIKV, nondepleted animals and a single CD8-depleted  
241 cynomolgus macaque experienced rapid serum viremia of 3-4.5 logs at 1 dpi (Fig. 1d), consistent  
242 with previous reports of ZIKV in both rhesus and cynomolgus macaques (Dudley et al., 2016;  
243 Koide et al., 2016; Osuna et al., 2016b). Strikingly, serum viremia in 3 of 4 CD8-depleted  
244 macaques was delayed until 2 dpi, when viral RNA was higher than in nondepleted animals (Fig.  
245 1d). Viremia was also delayed until 3 dpi in \*C46456 (Fig. 1d), a nondepleted cynomolgus  
246 macaque that had been previously splenectomized. Perhaps importantly, the spleen is a major  
247 site of replication and spread of the related mosquito-borne flaviviruses dengue virus (DENV)  
248 (Prestwood et al., 2012) and West Nile virus (WNV) (Bryan et al., 2018) and is also an immense  
249 reservoir of monocytes (Swirski et al., 2009), which are permissive to ZIKV replication in  
250 humans (Michlmayr et al., 2017) and macaques (O'Connor et al., 2018). The lack of a spleen in  
251 \*C46456 (nondepleted) might have precluded ZIKV replication in this important target organ,  
252 thereby delaying viral kinetics.

253 By 7 dpi, serum viremia persisted in the CD8-depleted cynomolgus macaques in addition to the  
254 mock-depleted control, while viremia was undetected in both nondepleted animals of the cohort  
255 (Fig. 1d). For the remainder of the study, viral kinetics were similar among cohorts and treatment  
256 conditions, peaking at 3 dpi and dropping to undetectable levels by 10 dpi and beyond (Fig. 1d).  
257 This was again in exception to \*C46456 (nondepleted), which showed a small viral rebound at  
258 10 dpi. A previous cohort of female macaques infected with the identical strain of ZIKV  
259 demonstrated similar patterns of serum viremia to those observed in nondepleted animals  
260 (Supplementary Fig. 2a).

261 The previous female cohort also showed consistent patterns of innate immune cell recruitment in  
262 the blood one day following ZIKV infection, summarized by the biomarker neutrophil-to-  
263 lymphocyte ratio (NLR) (Faria et al., 2016). Patterns of leukocyte mobilization included a spike  
264 in neutrophil frequency (Supplementary Fig. 2c) and a simultaneous drop in lymphocyte  
265 frequency (Supplementary Fig. 2d), resulting in an elevated NLR at 1 dpi (Supplementary Fig.  
266 2e). These findings were generally recapitulated in nondepleted animals but not in CD8-depleted  
267 animals (Figs. 1e-f and Supplementary Figs. 2c-e), potentially linking NLR and acute serum  
268 viremia, although the trend did not hold in the mock-depleted cynomolgus macaque C84545.  
269 This association is strengthened by prototypical patterns of leukocyte homing in C78777 (Fig.  
270 1e), the only CD8-depleted macaque with serum viremia at 1 dpi (Fig. 1d).

### 271 **Differential monocyte-driven transcriptional profiles**

272 To characterize immune responses that might be differentiating patterns of viremia and leukocyte  
273 mobilization, we used the NanoString platform to quantify the expression of macaque immune-  
274 related genes in whole blood. CD8-depleted and nondepleted animals showed highly divergent  
275 profiles in genes related to IFN $\alpha$  signaling (Fig. 2a) and leukocyte homing (Supplementary Fig.  
276 3a), resulting in the robust induction of disease-related pathways in nondepleted macaques that  
277 failed to activate in animals lacking CD8<sup>+</sup> lymphocytes (Supplementary Fig. 3b). To confirm the  
278 transcriptional quiescence evident in CD8-depleted macaques, we used a quantitative real-time  
279 PCR (qRT-PCR) array of 84 antiviral genes in the rhesus macaque genome. Consistent with the  
280 NanoString results, nondepleted rhesus and cynomolgus macaques showed strong induction of  
281 several RIG-I like receptors (RLRs) and type-I IFN stimulated genes (ISGs) at 3 dpi,  
282 synchronous with peak serum viremia (Fig. 2b). The most highly induced genes include the  
283 pattern recognition receptors *TLR3*, *DDX58* (also known as *RIG-I*), and *IFIH1* (also known as  
284 *MDA5*), as well as the ISGs *ISG15*, *MX1*, and *OAS2*. Principal component analysis (PCA) of  
285 antiviral signaling further discriminated the transcriptional phenotypes in CD8-depleted and  
286 nondepleted animals (Fig. 2c). The induction of antiviral genes in nondepleted rhesus macaques  
287 was highest at 3 dpi and was generally followed by a return to near-baseline expression by 15 dpi  
288 (Supplementary Fig. 3c). In contrast to nondepleted animals, CD8-depleted macaques showed a  
289 virtual absence of transcriptional responses in whole blood at all timepoints tested (Figs. 2a-b  
290 and Supplementary Fig. 3d).

291 Although we suspected monocytes to be driving antiviral gene expression owing to their  
292 susceptibility to ZIKV infection (Michlmayr et al., 2017; O'Connor et al., 2018), an important  
293 caveat of probing whole blood is that the identity of the cell populations responding  
294 transcriptionally is unknown. To resolve cell populations contributing to antiviral signaling in  
295 blood, we sorted CD14<sup>+</sup> monocytes from peripheral blood mononuclear cells (PBMCs) at 3 dpi  
296 and profiled their expression of immune genes. Probe hybridization revealed selective antiviral  
297 gene expression in the monocytes of a nondepleted macaque and showed that even purified and



298 sorted PBMCs from a CD8-depleted animal fail to establish transcriptional responses at peak  
299 serum viremia (Fig. 2d). These findings were verified by qRT-PCR (Fig. 2e). Nonetheless, it  
300 remained possible that the lack of a transcriptional response in CD8-depleted macaques could  
301 have been attributed in part to an absence of otherwise responding NK cells. Sorting PBMCs  
302 from a nondepleted rhesus macaque into CD8<sup>+</sup> and CD8<sup>-</sup> fractions, we found similar levels of  
303 gene induction in both populations, although expression was marginally higher in the CD8-  
304 subset, and transcription of *DDX58* was almost exclusive to CD8<sup>-</sup> cells (Fig. 2f). Gene induction  
305 in CD8<sup>+</sup> cells indicates that NK cells may indeed contribute to antiviral signaling, but similar  
306 transcriptional activation in the CD8<sup>-</sup> fraction affirms that the absence of transcriptional  
307 activation in CD8-depleted animals was not simply the product of a lack of NK cells.

308 To further explore transcriptional responses to ZIKV in myeloid cells, we infected monocyte-  
309 CD8 cell co-cultures *ex vivo* and compared antiviral gene expression to monocytes infected in  
310 the absence of CD8<sup>+</sup> cells, finding that the presence of CD8<sup>+</sup> lymphocytes is important in  
311 promoting transcriptional responses (Fig. 2g). We also cultured monocyte-derived macrophages  
312 (MDMs) *in vitro*, infected the macrophages with ZIKV, and profiled antiviral gene expression  
313 using qRT-PCR. We found an overlapping transcriptional fingerprint to those observed in the  
314 blood of nondepleted rhesus and cynomolgus macaques at 3 dpi (Fig. 2h), suggesting that ZIKV-  
315 permissive myeloid cells may be driving antiviral gene induction *in vivo*. Although cultured  
316 MDMs exhibited higher induction of several TLR responsive genes (Fig. 2g), this difference  
317 might be attributed to cell type.

### 318 **Altered monocyte activation and frequency**

319 Divergent transcriptional patterns in CD8-depleted and nondepleted macaques could be induced  
320 by differentially responding monocytes, given that monocytes are known targets of ZIKV  
321 infection (Lum et al., 2018; O'Connor et al., 2018) and contribute to antiviral signaling during  
322 ZIKV infection (Lum et al., 2018). To interrogate the immunophenotypic effects of CD8  
323 depletion, we developed a multicolor flow cytometry panel to track innate and adaptive immune  
324 cells over time. The resulting data were highly dimensional, comprising a variety of surface  
325 markers and sampling animals at multiple timepoints and with respect to different treatment  
326 groups. To survey general immune responses over time, we used an adaptation of t-distributed  
327 stochastic neighbor embedding (tSNE), viSNE (Amir et al., 2013).

328 In both rhesus and cynomolgus macaques, CD8 depletion dysregulated the kinetics of monocyte  
329 activation as measured by CD169 (siglec-1) expression (Biesen et al., 2008; Hirsch et al., 2018;  
330 York et al., 2007). Nondepleted rhesus and cynomolgus macaques showed early activation of  
331 monocytes, peaking at 3 dpi and returning sharply to baseline by 14-15 dpi (Figs. 3a-c).  
332 Upregulation of CD169 in nondepleted animals was affirmed at the RNA level (Fig. 2a).  
333 Although patterns of CD169 induction were consistent in all monocyte subsets (Supplementary  
334 Figs. 4a-c), viSNE analysis indicated that CD169 was most highly expressed on intermediate and  
335 nonclassical monocytes in both cohorts (Figs. 3a-b). Contrasting nondepleted animals, CD8-  
336 depleted rhesus and cynomolgus macaques showed less well-defined monocyte activation at 3  
337 dpi, which was accompanied in rhesus macaques by prolonged monocyte activation beyond 15  
338 dpi (Figs. 3a-c & Supplementary Figs. 4a-d). These findings were consistent transcriptionally, as  
339 whole blood from CD8-depleted animals had muted expression of genes related to myeloid cell  
340 activation (Fig. 3d). Monocyte subsets showed additional nuances in phenotype that appeared  
341 dependent on CD8 depletion: In rhesus macaques, CD95 (Fas) was increased on classical  
342 monocytes in CD8-depleted animals (Supplementary Fig. 4e) and on nonclassical monocytes in

343 nondepleted animals (Supplementary Fig. 4f), although similar patterns were not observed in  
344 cynomolgus monkeys.

345 CD8 depletion also differentially modulated the abundance of monocyte subsets in blood. One  
346 day following ZIKV infection, classical monocytes expanded immediately in nondepleted  
347 animals of both cohorts (Fig. 3e) excluding the mock-depleted cynomolgus macaque C84545.  
348 During acute infection (3-7 dpi), the frequency of nonclassical monocytes increased  
349 preferentially in CD8-depleted rhesus macaques and in nondepleted cynomolgus macaques (Fig.  
350 3f). Nondepleted rhesus macaques showed an expansion of intermediate monocyte frequency at  
351 3-7 dpi (Fig. 3g), although a CD8-dependent effect on intermediate monocyte frequency was not  
352 evident in cynomolgus monkeys.

### 353 **Compensatory adaptive immune responses**

354 Adaptive immune responses to ZIKV were also differentially modulated by CD8 depletion, with  
355 apparent compensatory responses in CD8-depleted macaques of both cohorts. Nondepleted  
356 rhesus and cynomolgus macaques began to mount CD8 T cell responses 7-10 dpi, which were  
357 characterized by proliferation (Ki67) and activation (CD69) of effector memory (EM), central  
358 memory (CM), and naïve CD8 T cell subsets (Figs. 4a-c & Supplementary Figs 5a-e). These  
359 responses were antigen-specific and functional, given that CD8 T cells stimulated with ZIKV  
360 peptides produced IFN $\gamma$  and contained perforin by intracellular cytokine staining (ICS) (Fig. 4e).  
361 Intriguingly, the CD8-depleted rhesus macaque R64357 also showed evidence of a CD8 T cell  
362 response at 21 dpi (Figs. 4c & 4e), concomitant with the recovery of CD8<sup>+</sup> lymphocytes in this  
363 animal (Supplementary Fig. 1a). Although tested, antigen-specific T cell responses were not  
364 detected by ICS in cynomolgus macaques.

365 The absence of CD8 T cells in depleted rhesus and cynomolgus macaques appeared to promote  
366 the reciprocal activation and expansion of CD4 T cell subsets (Figs. 4a-b, 4d and Supplementary  
367 Figs. 5f-j), mirroring the kinetics of CD8 T cell activation in nondepleted animals. CD8-depleted  
368 rhesus macaques began to induce CD4 T cell responses consistent with a Th1 phenotype,  
369 characterized by co-positivity for IL-2 and IFN $\gamma$ , but such responses were not present in  
370 nondepleted animals (Fig. 4f).

371 To gauge humoral responses to ZIKV, we conducted plaque reduction neutralization tests  
372 (PRNTs) using rhesus macaque sera to quantify neutralizing antibody titers. All animals except  
373 R20865 (nondepleted) showed evidence of neutralizing antibodies at 7 dpi, the earliest post-  
374 infection timepoint tested (Fig. 4g). While highly neutralizing titers were detected in all animals  
375 at 15 dpi, antibody concentrations declined in nondepleted animals, but not in CD8-depleted  
376 animals, beyond this timepoint. Strikingly, depleted rhesus macaques retained highly  
377 neutralizing antibody titers until necropsy, a finding consistent with elevated B cell activation  
378 (Fig. 4h) and proliferation (Fig. 4i) in these animals.

### 379 **Enhanced tissue dissemination and neuropathology**

380 Given the persistence of high neutralizing antibody titers in CD8-depleted rhesus macaques, we  
381 suspected that virus might be lingering in the peripheral tissues of these animals. The duration of  
382 infection before necropsy differed among the rhesus (30 dpi) and cynomolgus (14 dpi) macaque  
383 cohorts to identify patterns of viral dissemination and clearance over time. Informed by previous  
384 reports of ZIKV tropism in macaques (Coffey et al., 2017; Hirsch et al., 2017; Osuna et al.,  
385 2016b), we searched for viral RNA in lymphoid, neural, gastrointestinal (GI), and reproductive

386 tissues, as well as in semen and cerebrospinal fluid (CSF) to evaluate viral distribution in these  
387 sites.

388 Relative to nondepleted animals, CD8-depleted cynomolgus macaques had markedly higher  
389 levels of ZIKV RNA in the inguinal, mesenteric, and colonic lymph nodes, as well as in the  
390 spleen and jejunum (Figs. 5a and 5c). All cynomolgus monkeys except C91638 (nondepleted)  
391 harbored virus in the rectum without an obvious difference among treatment groups. Notably, the  
392 trend of higher viral burdens in the lymphatic tissues of CD8-depleted animals was consistent in  
393 rhesus macaques (Fig. 5f). CD8 depletion also appeared to promote ZIKV dissemination in the  
394 semen, with both CD8-depleted cynomolgus macaques presenting semen viral loads and no viral  
395 RNA detected in nondepleted animals of the same cohort (Fig. 5d). Intriguingly, the nondepleted  
396 macaque C84545 (mock-depleted) showed the highest level of viral RNA in the prostate and was  
397 the only animal to present virus in the testes (Fig. 5c), yet no ZIKV was detected in the semen of  
398 this animal (Fig. 5d). These findings too were consistent in rhesus macaques, with virus detected  
399 in the semen (Fig. 5g) and seminal vesicle (Fig. 5f) of a CD8-depleted animal and only a  
400 miniscule quantity of viral RNA detected in the semen of a nondepleted animal (Fig. 5g).

401 ZIKV RNA was detected in the brainstem and subcortical white matter of C84545 (mock-  
402 depleted) (Fig. 5b), and this animal also presented a high magnitude viral load in the CSF early  
403 in infection, which persisted until necropsy (Fig. 5e). Exclusive of C84545 (mock-depleted),  
404 CD8-depleted cynomolgus and rhesus macaques manifested CSF viral loads at least an order of  
405 magnitude greater than nondepleted animals (Figs. 5e and 5h). Although ZIKV was not detected  
406 in the central nervous system (CNS) of any rhesus macaque, R25671 (CD8-depleted) and  
407 R64357 (CD8-depleted) manifested neural lesions at necropsy that were not present in  
408 nondepleted animals. Most strikingly, the brainstem of R25671 showed an area of severe  
409 multifocal to coalescing encephalomalacia which showed evidence of Wallerian degeneration,  
410 characterized by vacuolation, swollen axons, and infiltration by lymphocytes and phagocytic  
411 gitter cells (Fig. 5i). Gitter cells are occasionally found within dilated myelin sheaths. Scant  
412 brown granular pigment (presumed hemosiderin) and a proliferative cerebral vessel adjacent to  
413 the malacia may indicate that the malacia is the result of a vascular event (thromboembolism,  
414 infarct, ischemia, etc.). Additionally, lymphocytic infiltrate was present in the meninges  
415 surrounding the lumbar spinal cord (Fig. 5i). No gross abnormalities were noted in R64357,  
416 although the sciatic nerve exhibited mild lymphocytic perivascularitis. The sciatic nerve is a  
417 known site of ZIKV replication in mice depleted of CD8 cells (Elong Ngonu et al., 2017).  
418 Further, the brainstem contained a localized area of gliosis, an indicator of CNS damage  
419 (Garman, 2011), and dilated myelin sheaths (Fig. 5j). A cause for these neural inflammatory  
420 lesions was not apparent by histology.

## 421 **Discussion**

422 Owing to the importance of CD8+ T cells in the control of ZIKV in mice (Elong Ngonu et al.,  
423 2017; Huang et al., 2017), our aim was to explore whether these findings are consistent in  
424 nonhuman primates. Despite a small sample size in two cohorts, the absence of CD8+  
425 lymphocytes prompted immediately observable host responses that diverged from previously  
426 consistent patterns of viremia and immunity. Contrasting similar experiments with simian  
427 immunodeficiency virus (SIV) (Jin et al., 1999; Klatt et al., 2010), the absence of CD8+  
428 lymphocytes did not overtly affect the control of serum viremia. However, the delay of serum  
429 viremia in CD8-depleted macaques stood in patent contrast to patterns of acute ZIKV infection

430 observed by our own group (Supplementary Fig. 2a) and others (Dudley et al., 2016). Although a  
431 mechanism underlying the delayed serum viremia remains obscure, it is possible that a lack of  
432 NK cell stimulation in CD8-depleted animals may be misfiring viral replication in what would  
433 otherwise be readily permissive monocytes. Indeed, monocytes engage in intercellular crosstalk  
434 with NK cells (Dalbeth et al., 2004; Michel et al., 2012), and IFN $\gamma$  is shown to support ZIKV  
435 replication (Chaudhary et al., 2017). NK cell-derived IFN $\gamma$  may activate ZIKV infected myeloid  
436 cells in nondepleted animals, promoting an inflammatory milieu that favors early viral  
437 replication. Alternatively, NK cells are shown to be minor reservoirs of ZIKV RNA in infected  
438 humans (Michlmayr et al., 2017) and pigtail macaques (O'Connor et al., 2018), so the absence of  
439 this potential target cell may contribute to the delayed serum viremia in CD8-depleted animals.  
440 Additional co-culture assays may elucidate intercellular dynamics important for maintaining  
441 patterns of innate immune regulation.

442 CD8 depletion also impacted the mobilization of leukocyte populations acutely following  
443 infection, contrasting patterns reliably observed by our own group (Figs S2c-e) and others  
444 (Osuna et al., 2016b). Macaques depleted of CD8 T cells and NK cells show little fluctuation in  
445 the biomarker of inflammation NLR, indicating altered innate immune responses immediately  
446 following infection. In line with these observations, mice lacking NK cells exhibit altered  
447 neutrophil recruitment in a variety of infectious and noninfectious conditions (Costantini and  
448 Cassatella, 2011). Neutrophil effector functions are modulated by NK cell-derived cytokines  
449 (Costantini and Cassatella, 2011), a signaling axis which might have been disrupted by the  
450 depletion of NK cells in macaques.

451 Confirming miscommunication within the innate immune system of CD8-depleted macaques,  
452 these animals presented largely muted transcriptional activity in key virus response pathways  
453 during acute infection. In nondepleted macaques, antiviral gene expression was driven  
454 principally by circulating monocytes, which stood in sharp contrast to the transcriptional void  
455 evident in CD8-depleted animals. Importantly, this signaling pattern was replicated in a co-  
456 culture model *ex vivo*. Intercellular crosstalk between monocytes and NK cells affects  
457 transcriptional responses to ZIKV infection (Lum et al., 2018), so the absence of NK cells in  
458 CD8-depleted macaques might have permitted the infection to evade transcriptional induction in  
459 monocytes, complementing the delayed viremia in these animals.

460 Consistent with a model of monocyte-dependent outcomes in acute ZIKV infection, CD8-  
461 depleted and nondepleted macaques also differed in the magnitude and phenotype of their  
462 monocyte responses during acute infection. Intermediate and nonclassical monocytes showed the  
463 greatest degree of activation, agreeing with recent findings that these subsets are primary targets  
464 of ZIKV in the blood (Foo et al., 2017; Jurado and Iwasaki, 2017; Michlmayr et al., 2017). CD8  
465 depletion also impacted the activation of monocytes temporally, further underscoring  
466 dysregulated innate responses in depleted animals. CD169 (siglec-1) is a sialic acid-binding  
467 lectin previously found to be upregulated in acute ZIKV infection in rhesus macaques (Hirsch et  
468 al., 2018; Hirsch et al., 2017). CD169 has important roles in virus capture by myeloid cells  
469 (Sewald et al., 2015) and in the mounting of CD8 T cell responses in viral infection (van Dinther  
470 et al., 2018), so the robust induction of CD169 in nondepleted animals might have promoted  
471 sufficient CD8 T cell responses. CD8 depletion also affected monocyte frequency, possibly  
472 contributing to differential transcriptional responses. The transient increase in classical  
473 monocytes of both cohorts may be analogous to the monocytosis that accompanies acute ZIKV  
474 replication in human patients (Michlmayr et al., 2017). The increase in CD16<sup>+</sup> nonclassical

475 monocytes in CD8-depleted rhesus macaques is an outcome also observed in ZIKV infection of  
476 human blood (Foo et al., 2017), and the expansion of intermediate monocytes in nondepleted  
477 animals resembles ZIKV infection in Nicaraguan patients (Michlmayr et al., 2017). Although  
478 minor species differences were evident in the immunophenotyping of rhesus and cynomolgus  
479 monocytes, our data support a CD8+ lymphocyte-dependent effect in these transitions, possibly  
480 accounting for divergent transcriptional responses in blood.

481 In addition to modulating innate immune responses, the depletion of CD8+ lymphocytes also  
482 promoted compensatory adaptive responses to ZIKV in both cohorts. Nondepleted animals and  
483 even the CD8-recovering rhesus macaque R64357 mounted robust CD8 T cell responses,  
484 affirming the importance of CD8+ lymphocytes in acute infection. There is precedence for CD8  
485 T cell responses to ZIKV in mice (Elong Ngonu et al., 2017; Huang et al., 2017; Pardy et al.,  
486 2017) and humans (Grifoni et al., 2018; Grifoni et al., 2017), which appears to be consistent in  
487 nonhuman primates. Meanwhile, the presence of Th1 responses and prolonged humoral  
488 responses in CD8-depleted animals possibly compensate for the absence of CD8 surveillance.  
489 Such adaptive responses are reported in mice, as Th1 polarization (Pardy et al., 2017), and CD4-  
490 driven humoral responses (Lucas et al., 2018) are important for controlling infection. Our data  
491 support the importance of these adaptive responses in nonhuman primates, especially when the  
492 CD8 arm of adaptive immunity is compromised.

493 The persistence of high neutralizing antibody titers until necropsy in CD8-depleted rhesus  
494 macaques also suggested that there might be virus lingering in the peripheral tissues of these  
495 animals. Indeed, ZIKV RNA was generally more abundant in the lymphatic tissues, semen and  
496 CSF of CD8-depleted rhesus and cynomolgus macaques relative to nondepleted animals,  
497 implying the importance of CD8+ lymphocytes in limiting ZIKV dissemination and/or  
498 persistence in tissues. Lymphatic tissue viral loads were also consistently higher in cynomolgus  
499 compared to rhesus macaques, possibly reflecting the abbreviated time of infection before  
500 necropsy. Despite the detection of viral RNA in semen, ZIKV was scarce in reproductive tissues,  
501 in line with an absence of gross pathological lesions including atrophy of the testes. Although  
502 testicular atrophy is reported in murine models of ZIKV (Uraki et al., 2017), such manifestations  
503 have not been observed in macaques or in clinical cases (Matusali et al., 2018). In rhesus  
504 monkeys, it is possible that viral RNA in neural and reproductive tissues might have been only  
505 transiently present due to viral clearance given the 30-day infection period of these animals.  
506 Previous reports in rhesus (Aid et al., 2017) and pigtail (O'Connor et al., 2018) monkeys have  
507 also shown that ZIKV persists in lymphatic tissues well beyond the clearance of virus from the  
508 serum. It remains unclear whether the ZIKV present in lymph nodes is replication competent, but  
509 our data are nonetheless consistent with a model where the absence of CD8+ lymphocytes  
510 permits the dispersal of ZIKV.

511 CD8-depleted rhesus macaques also presented gross neural lesions at necropsy not seen in  
512 nondepleted animals. The most severe lesion occurred in the brainstem of a depleted animal that  
513 never recovered CD8+ lymphocytes, and similar manifestations of encephalomalacia and axon  
514 degeneration have been reported in ZIKV infection of human fetal brain tissue (Driggers et al.,  
515 2016; Petribu et al., 2018; Vesnaver et al., 2017). Perhaps complementarily, neural lesions in the  
516 CD8-recovering rhesus macaque were less severe. Although it is tempting to speculate that the  
517 absence of CD8+ lymphocytes in R25671 (CD8-depleted) and R64357 (CD8-depleted) allowed  
518 neural dissemination of the virus and thereby promoted neuropathy, our inability to detect ZIKV  
519 RNA in brain sections from these animals precludes this conclusion. Because ZIKV was cleared

520 from the CSF of rhesus monkeys within 15 dpi, it is possible that virus could have also cleared  
521 from the CNS by necropsy and that these lesions were virus associated even if viral RNA was  
522 not detectable late in infection. Supporting this argument, CSF viral loads appear to be associated  
523 with ZIKV dissemination in neural tissue, given that the mock-depleted cynomolgus macaque  
524 C84545 showed the highest and most persistent CSF viremia and was also the only animal with  
525 ZIKV RNA identified in the brain. Despite the presence of CD8+ lymphocytes in C84545, this  
526 animal was the sole example of neural dissemination and occasionally produced responses more  
527 similar to CD8-depleted animals in key immune measures including NLR and classical  
528 monocyte frequency, underscoring the importance of innate immunity in limiting viral  
529 dissemination. Although it remains possible that off-target antibody effects produced  
530 immunological nuances in C84545, this animal ultimately aligned more closely with nondepleted  
531 animals in terms of serum viremia, antiviral gene induction, and adaptive immune activation,  
532 affirming the importance of CD8+ lymphocytes in maintaining immune regulation during acute  
533 ZIKV infection. The general absence of immune surveillance and IFN signaling in CD8-depleted  
534 animals might have permitted initial infection of neural tissues, which might have been transient  
535 due to the eventual priming of compensatory adaptive responses. Additionally, ZIKV localizes as  
536 discrete foci in macaque tissues (Hirsch et al., 2018), complicating the detection of sparse viral  
537 lesions within organs. It is worth noting that CNS localization of ZIKV has been observed as  
538 early as 5 dpi in acutely infected macaques (Osuna et al., 2016b), and a separate study in rhesus  
539 monkeys failed to identify ZIKV RNA in the CNS at 14 dpi, despite diffuse patterns of viral  
540 dissemination (Coffey et al., 2017). These findings, together with our own, establish precedence  
541 for early CNS dissemination of ZIKV in nonhuman primates, which may be cleared later in  
542 infection.

543 In summary, the present study illustrates a pliable dynamic between ZIKV and its hosts. CD8  
544 depletion appears to alter innate immune activation and antiviral signaling and also modulate  
545 viral kinetics without overtly affecting serum viremia. Together with apparently compensatory  
546 adaptive responses and the presence of enhanced viral tissue distribution, these findings suggest  
547 that CD8 T cells provide default adaptive immune responses to ZIKV, a conclusion with  
548 important consequences for immune-based interventions such as vaccine development.

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#### 552 **Author contributions**

553 BS, AP, and NM planned the studies. BS, MF, ES, MW, KH, DS, RB, MG, LDM, and VD  
554 conducted the experiments. DW and MB provided reagents. BS, MF, MW, RB, AP, and NM  
555 interpreted the studies. BS and NM wrote the first draft. AP and NM obtained funding. All  
556 authors reviewed, edited, and approved the paper.

#### 557 **Conflict of Interest**

558 The authors declare no conflicts of interest.

#### 559 **Contribution to the Field**

560 A number of reports have indicated an important role for CD8 T cells in the control of ZIKV  
561 replication in mice, but these models are limited in that the mice need to be

562 immunocompromised for efficient viral infection. Using two established nonhuman primate  
563 models of ZIKV infection, we found that CD8<sup>+</sup> lymphocytes are critical in orchestrating the  
564 earliest immune events during viral infection. The absence of CD8 cells enhanced viral  
565 dissemination into multiple tissues and prompted immediately observable host responses that  
566 diverged from previously consistent patterns of viremia and immunity. Importantly, the  
567 implications of these data may reach beyond ZIKV and are likely instructive to how CD8 cells  
568 interact with other immune cells to coordinate the control of viral infections generally.

## 569 **Materials & Correspondence**

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## 718 **Figure legends**

### 719 **Figure 1**

720 Delayed serum viremia and altered leukocyte kinetics in CD8-depleted macaques. **(A)** Study  
721 design. Two animals of each cohort were depleted of CD8<sup>+</sup> lymphocytes, and all animals were  
722 infected with a Brazilian isolate of ZIKV. Viremia was tracked over 30 days in cohort 1 and 14  
723 days in cohort 2 before necropsy. Colors used in the timeline correspond to cohorts and treatment  
724 conditions. C46456 (nondepleted) was splenectomized in a previous study and is indicated by an  
725 asterisk (\*) throughout. **(B)** Flow cytometric analysis of CD8 T cell frequencies in PBMCs over  
726 time. (*Top*): cohort 1; (*Bottom*): cohort 2 (consistent throughout). **(C)** NK cell frequency, as  
727 measured by flow cytometry. **(D)** Viral RNA in serum over infection (error bars, SD). **(E)** NLR,  
728 derived using total neutrophil and lymphocyte counts in blood from CBC data. **(F)** Fold change  
729 in NLR from 0 to 1 dpi among 4 rhesus and 4 cynomolgus macaques included in the present  
730 study in addition to a previous cohort of 4 ZIKV-infected non-pregnant female rhesus macaques  
731 (center line, mean; error bars, SD). (The blood of one CD8-depleted cynomolgus macaque,  
732 C18942, clotted prior to complete blood count (CBC) analysis, precluding calculation of NLR  
733 for this animal.) The significant ( $p \leq 0.05$ ) difference in NLR fold change was determined using  
734 a Mann-Whitney test.

### 735 **Figure 2**

736 Differential monocyte-driven transcriptional profiles among CD8-depleted and nondepleted  
737 macaques. **(A)** Activation of downstream IFN $\alpha$  effector molecules in whole blood at 3 dpi  
738 relative to pre-infection (cyno. = cynomolgus, consistent throughout). **(B)** Fold regulation of  
739 antiviral gene expression in whole blood at 3 dpi, confirmed using a qRT-PCR array of 84 genes  
740 in the rhesus macaque genome (dep. = CD8-depleted; non. = nondepleted). **(C)** PCA of antiviral  
741 gene expression in whole blood at 3 dpi. **(D)** Activation of downstream IFN $\alpha$  effector molecules  
742 in sorted CD14<sup>+</sup> monocytes and CD14<sup>-</sup> PBMCs at 3 dpi. **(E)** qPCR confirmation of antiviral  
743 gene expression in CD14<sup>+</sup> monocytes and CD14<sup>-</sup> PBMCs at 3 dpi. Gene induction is normalized  
744 to b-actin, and fold regulation is expressed relative to 0 dpi. **(F)** Antiviral gene expression in  
745 CD8<sup>+</sup> and CD8<sup>-</sup> fractions of PBMCs from a nondepleted rhesus macaque at 3 dpi. Gene

746 induction is normalized to b-actin, and fold regulation is expressed relative to 15 dpi. **(G)**  
747 Antiviral gene expression in co-cultured CD14<sup>+</sup> monocytes and autologous CD8<sup>+</sup> cells from  
748 ZIKV-naïve PBMC infected with ZIKV *ex vivo* at 1 and 3 dpi. **(H)** Comparison of antiviral gene  
749 induction in cultured, ZIKV-infected MDMs (red) and the whole blood of nondepleted rhesus  
750 (blue) and cynomolgus (green) macaques at 3 dpi. Genes included in the qPCR array relate to  
751 toll-like receptor (TLR), nod-like receptor (NLR) or type-I interferon (IFN-I) signaling (resp. =  
752 responsive).

### 753 **Figure 3**

754 Altered monocyte activation and frequency in CD8-depleted macaques. **(A-B)** viSNE analyses of  
755 monocyte activation in rhesus (A) and cynomolgus (B) macaques, as measured by CD169 mean  
756 fluorescence intensity (MFI). Dot plots are concatenated for animals within each treatment  
757 condition. The viSNE clustering profile of monocyte subsets (*left*) correspond to cell populations  
758 in the CD169 MFI heatmaps in nondepleted and CD8-depleted animals over time (*right*). **(C)**  
759 summaries of CD169 expression in total monocytes. **(D)** Induction of genes related to myeloid  
760 cell activation at 3 dpi relative to pre-infection. **(E-G)** Frequencies of classical (E), nonclassical  
761 (F), and intermediate (G) monocyte subsets in rhesus and cynomolgus macaques over time.

### 762 **Figure 4**

763 Compensatory adaptive immune responses in CD8-depleted macaques. **(A-B)** viSNE analyses of  
764 T cell activation in rhesus (A) and cynomolgus (B) macaques, as measured by CD69 expression.  
765 Dot plots are concatenated for animals within each treatment condition. The viSNE clustering  
766 profiles of CD4 and CD8 T cell subsets (*left*) correspond to cell populations in the CD69  
767 heatmaps in nondepleted and CD8-depleted animals at 1 & 10 dpi (*right*). **(C)** Proliferation of  
768 EM CD8 T cells in rhesus and cynomolgus macaques over time. CD8-depleted macaques are  
769 excluded owing to the absence of CD8<sup>+</sup> lymphocytes in these animals exclusive of R64357 at  
770 later timepoints. **(D)** Proliferation of EM CD4 T cells in rhesus and cynomolgus macaques over  
771 time. **(E)** CD8 T cell responses in rhesus macaques, assessed by ICS of PBMCs stimulated with  
772 viral peptides derived from the indicated ZIKV proteins (C = capsid; M = membrane; E =  
773 envelope; NS1 = nonstructural protein 1, consistent throughout). CD8 T cell responses were  
774 identified by co-positivity for perforin and IFN $\gamma$ . **(F)** Th1 responses, determined by ICS for IL-2  
775 and IFN $\gamma$  co-positivity. (*Inset*): representative antigen-specific cytometry plots for R64357  
776 (CD8-depleted) at 30 dpi. **(G)** Serum neutralizing antibody titers in rhesus macaques, represented  
777 as PRNT90. **(H)** Activation of B cells in rhesus macaques over time. **(I)** Proliferation of B cells  
778 in rhesus and cynomolgus macaques over time.

### 779 **Figure 5**

780 Enhanced tissue dissemination and neuropathology in CD8-depleted macaques. **(A-E)** Viral  
781 dissemination in cynomolgus macaques, including lymphatic (A), neural (B) and reproductive  
782 (C) tissues, as well as semen (D) and CSF (E) (center line, mean; error bars, SD of two replicates  
783 per sample, LN = lymph node; sub. wt. matter = subcortical white matter, consistent throughout).  
784 **(F-H)** Viral dissemination in rhesus macaques, including lymphatic, neural, and reproductive  
785 tissues (F), as well as semen (G) and CSF (H). **(I)** R25671 (CD8-depleted) brainstem (top) and  
786 lumbar spinal cord (bottom). *Top*: There is an area of encephalomalacia (dotted region, left)  
787 adjacent to a vessel that exhibits medial thickening (arrow, left). The area of malacia is  
788 characterized by dilated myelin sheaths with swollen axons (arrow, right) or gitter cell  
789 infiltration (asterisks, right). H&E, Bar = 100  $\mu$ m. *Bottom*: The meninges surrounding the lumbar  
790 spinal cord are multifocally infiltrated by aggregates of lymphocytes (arrows). H&E, Bar = 1 mm

791 (left) and 100  $\mu\text{m}$  (right). **(J)** R64357 (CD8-depleted) sciatic nerve (top) and brainstem lesions  
792 (bottom). *Top*: Small vessels within the sciatic nerve are surrounded by low numbers of  
793 lymphocytes (arrows). *Bottom*: A focal glial nodule is present within the gray matter of the  
794 brainstem (dotted region, left) with dilation of adjacent myelin sheaths and spheroid formation  
795 (arrowhead, right). H&E, Bar = 100  $\mu\text{m}$ .

#### 796 **Figure S1**

797 MT807R1 depletes CD8<sup>+</sup> lymphocytes with variable recovery. **(A)** Absolute CD8 T cell counts  
798 in blood, as determined by complete blood count (CBC). **(B-C)** Absolute counts of CD4<sup>+</sup>/CD8<sup>+</sup>  
799 double-positive T cells (B) and (C) CD4 T cells prior to infection, determined by CBC.

#### 800 **Figure S2**

801 Comparison of virus and immune cell dynamics to a previous female cohort. Data from a  
802 previous cohort of ZIKV-infected non-pregnant female rhesus macaques is shown in gray, and  
803 data from rhesus macaques of the present study is overlaid. **(A)** Serum viral loads over the course  
804 of the studies (error bars, SD). **(B)** CSF viral loads over the course of the studies. **(C-D)**  
805 Frequencies of neutrophils (C) and total lymphocytes (D) in whole blood over time, determined  
806 by CBC. **(E)** NLR, derived using total neutrophil and lymphocyte CBC data.

#### 807 **Figure S3**

808 Transcriptional responses correlate with serum viremia in nondepleted macaques. **(A-B)** Pathway  
809 analysis of gene expression in whole blood at 3 dpi relative to pre-infection reveals the induction  
810 of genes related to leukocyte homing (A) as well as differentially activated biological functions  
811 and disease-related pathways among CD8-depleted and non-depleted animals (B). **(C-D)**  
812 Patterns of antiviral gene induction at 1, 3, and 15 dpi in the whole blood of nondepleted (C) and  
813 CD8-depleted (D) rhesus macaques relative to pre-infection.

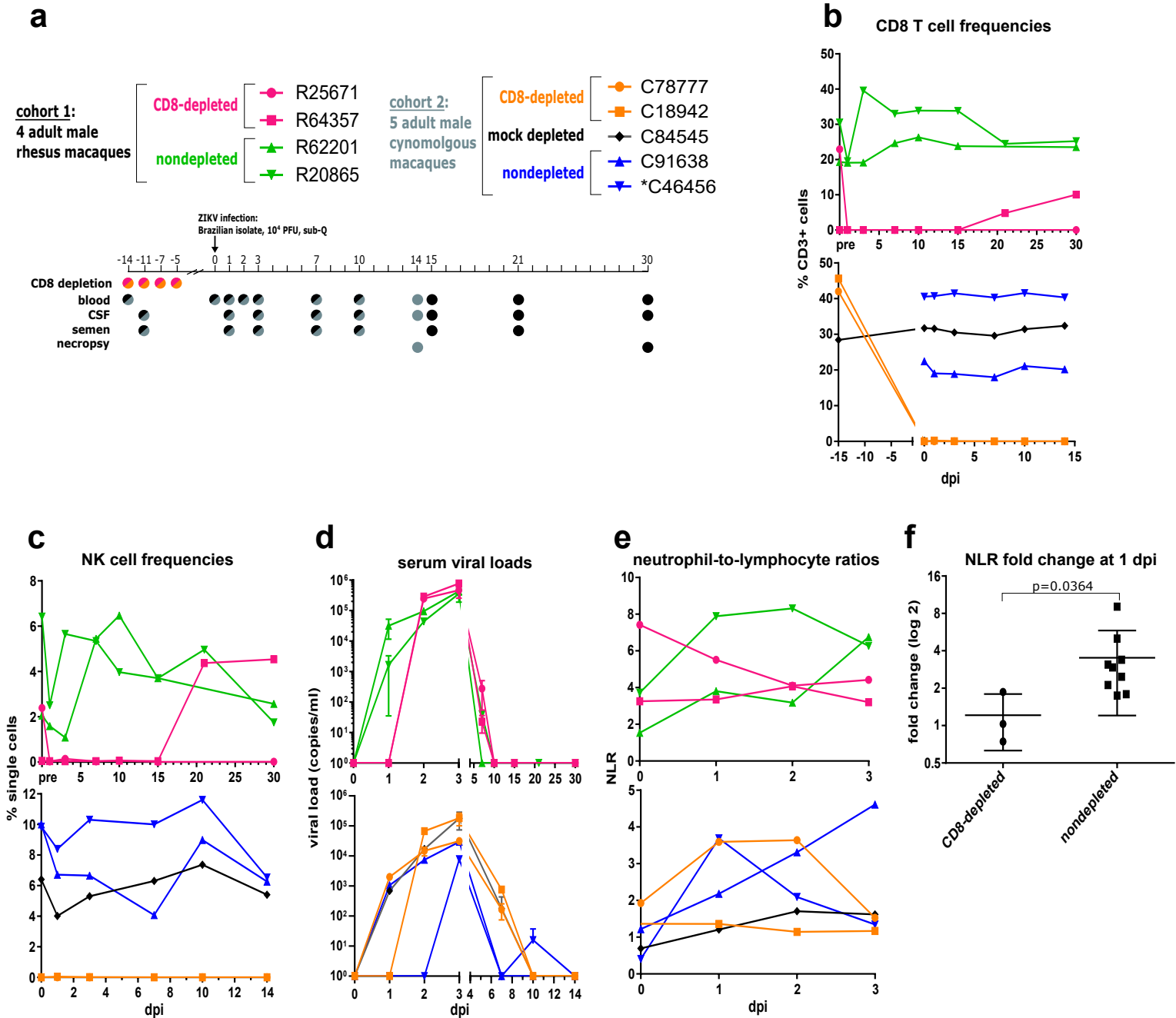
#### 814 **Figure S4**

815 CD8 depletion modulates monocyte phenotype during ZIKV infection. **(A-C)** Flow cytometric  
816 analysis of monocyte activation, as measured by CD169 expression in classical (A), intermediate  
817 (B), and nonclassical (C) subsets in rhesus and cynomolgus macaques. **(D)** Overall monocyte  
818 activation, as measured by CD69 expression. **(E-F)** Expression of CD95 in classical (E) and  
819 nonclassical (F) subsets.

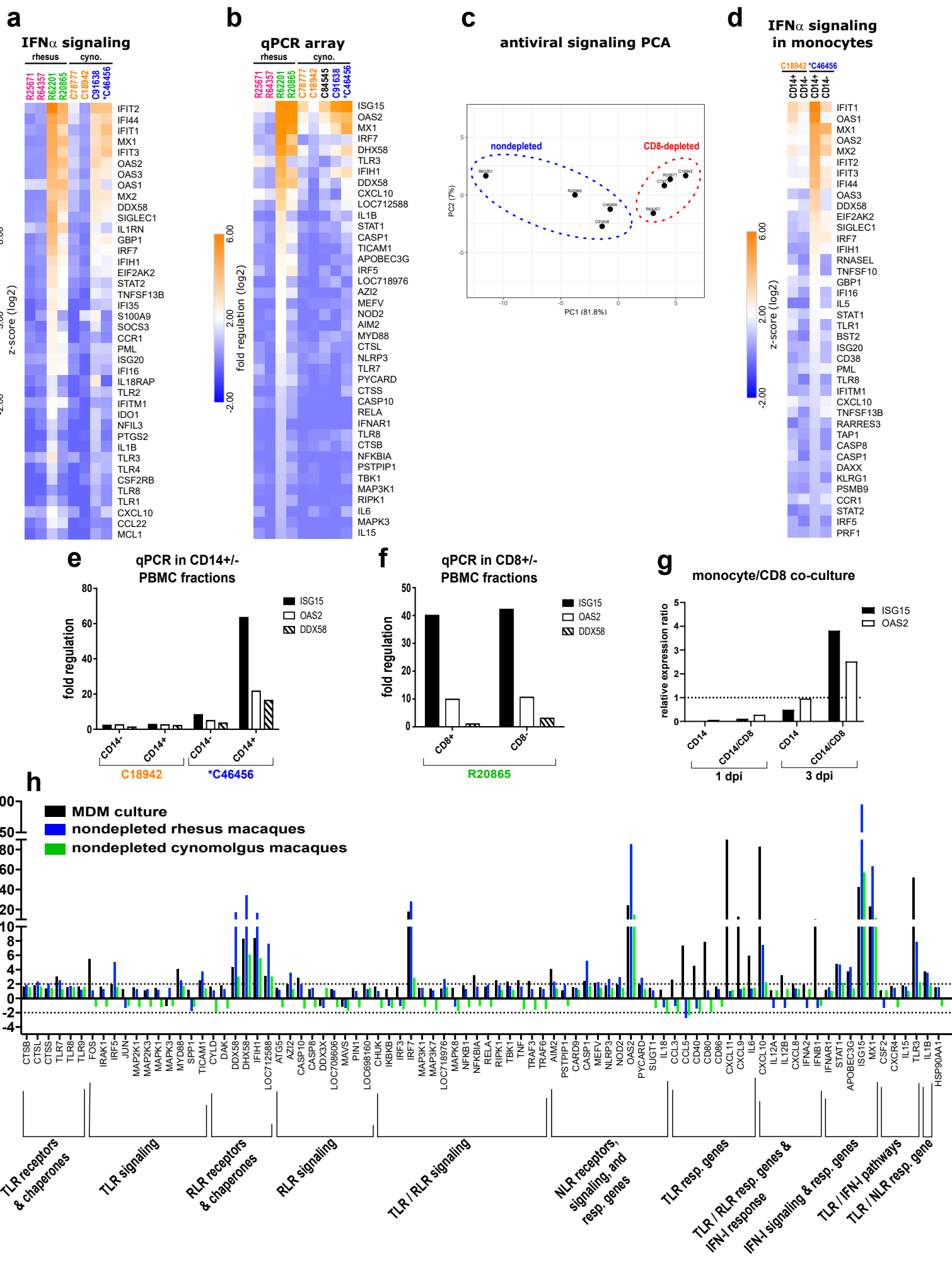
#### 820 **Figure S5**

821 Reciprocal T cell responses are polyphenotypic. **(A-E)** Immunophenotyping of CD8 T cells in  
822 rhesus and cynomolgus macaques, including EM CD8 activation (A), CM CD8 activation (B)  
823 and proliferation (C), and naïve CD8 activation (D) and proliferation (E). **(F-J)**  
824 Immunophenotyping of CD4 T cells, including EM CD4 activation (F), CM CD4 activation (G)  
825 and proliferation (H), and naïve CD4 activation (I) and proliferation (J).

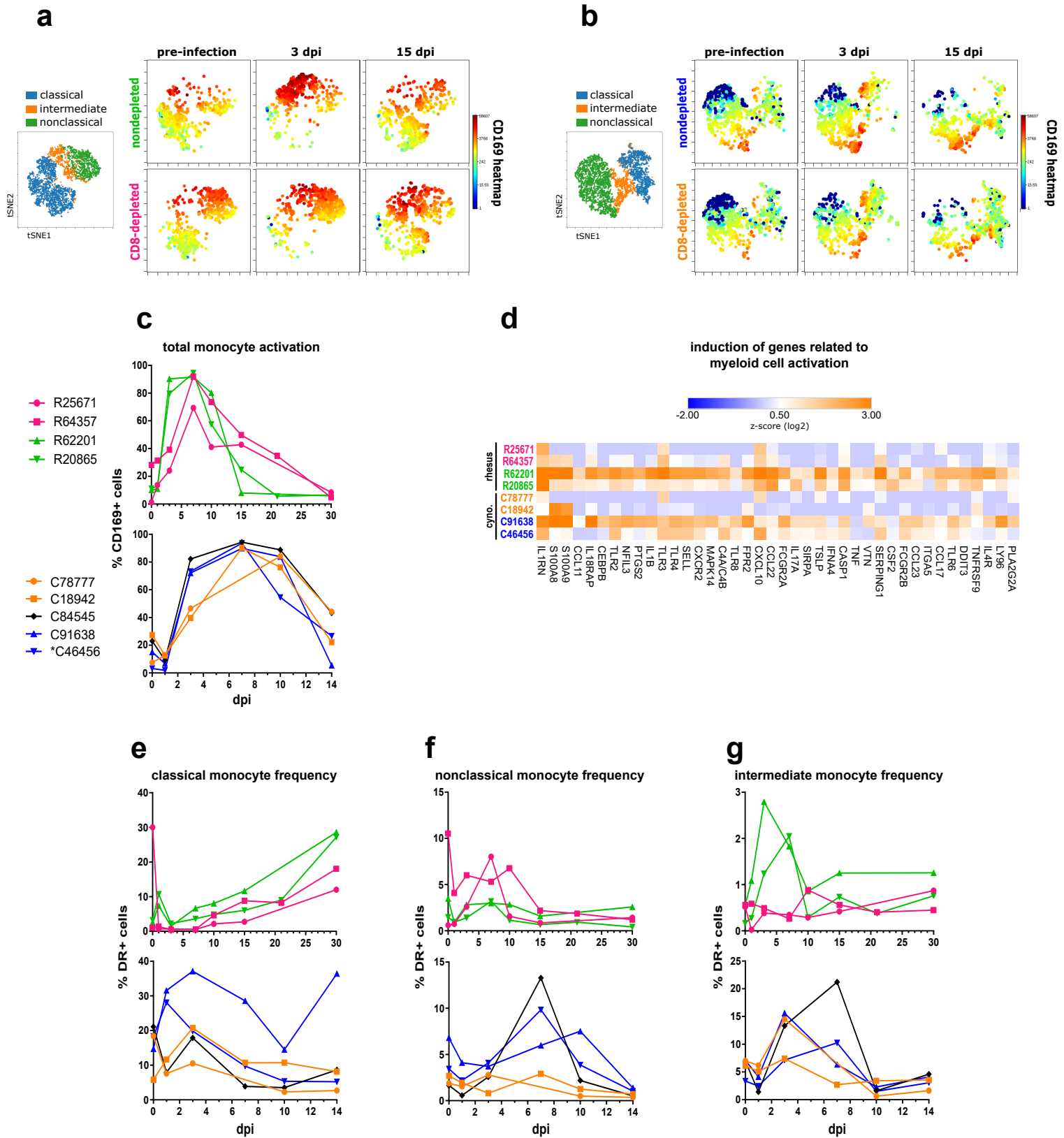
# Fig 1: Delayed serum viremia and altered leukocyte kinetics



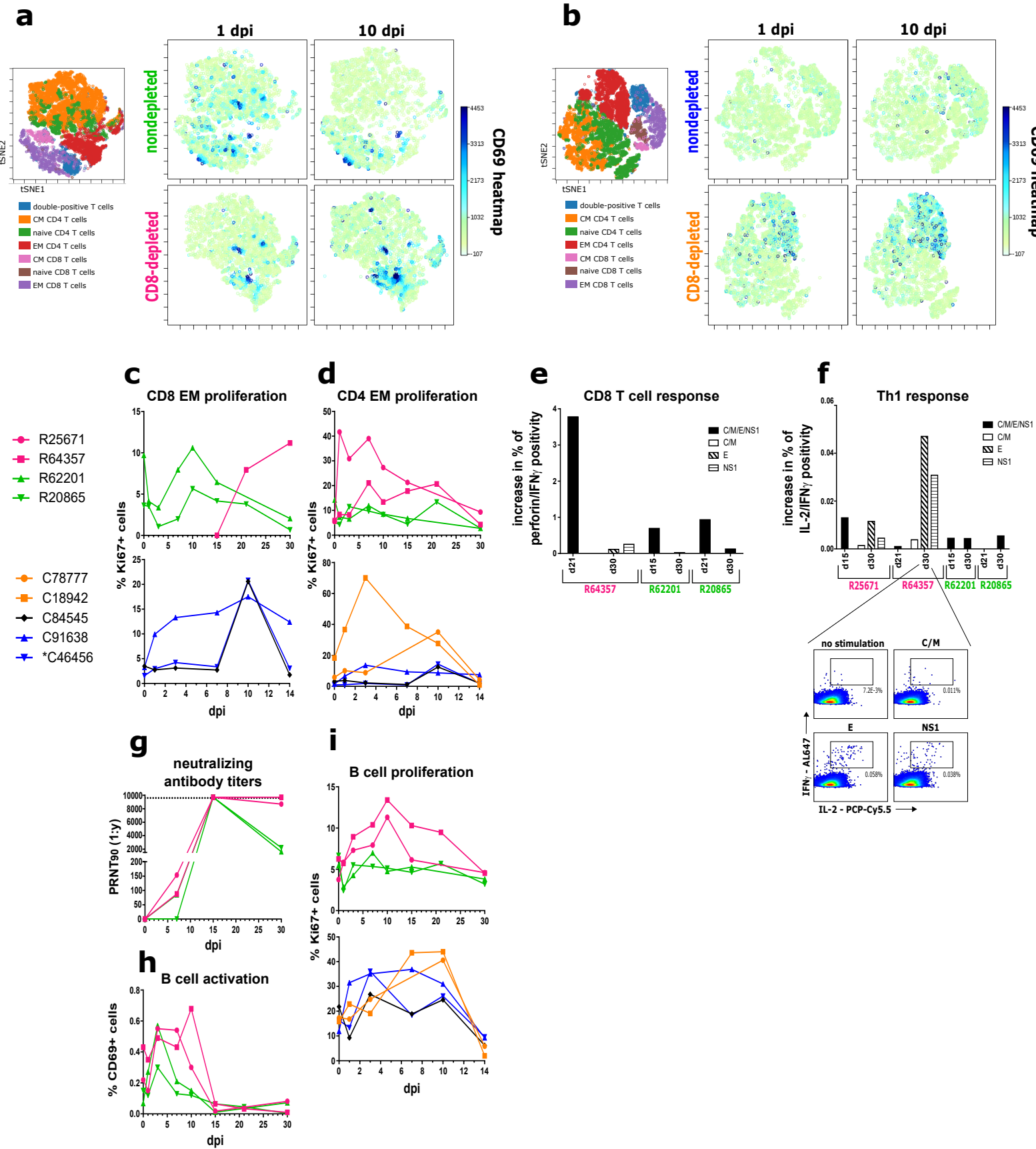
# Fig 2: Differential monocyte-driven transcriptional profiles



## Fig 3: Altered monocyte activation and frequency



## Fig 4: Compensatory CD4 and humoral responses





# Fig 5: Enhanced tissue dissemination and neuropathology

