CD4+ T Cell Depletion Before Primary Dengue and/or Secondary Zika Infection Reveals Mechanistic Correlates of Antibody Functionality in Rhesus Macaques.

Crisanta Serrano-Collazo[1,2], Angel Miranda[1], Lorna A. Cruz[1], Sandra Henein[3], Mitchell Sanchez-Rosado[1], Laura Alvarez[1], Teresa Arana[1], Melween I. Martinez[2], Chiara Roman[2], Armando G. Burgos[2], Aravinda de Silva[3], Carlos A. Sariol[1,2,4*#]

[1] Department of Microbiology and Medical Zoology, University of Puerto Rico-Medical Sciences Campus, San Juan, Puerto Rico, United States of America, [2]Unit of Comparative Medicine, Caribbean Primate Research Center, University of Puerto Rico-Medical Sciences Campus, San Juan, Puerto Rico, United States of America, [3]University of North Carolina Chapel Hill, North Carolina, United States of America, [4]Department of Internal Medicine, University of Puerto Rico-Medical Sciences Campus, San Juan, Puerto Rico, United States of America

Running Title: CD4 T cell depletion before dengue or zika of rhesus macaques

[*]Crisanta Serrano-Collazo, Angel Miranda and Lorna A. Cruz contributed equally to this work. Author order was determined based on data analyses.

[#]Address correspondence to Carlos A. Sariol, carlos.sariol1@upr.edu

Abstract

Dengue (DENV) virus and Zika virus (ZIKV) are two flaviviruses of major public health concern. One drawback designing effective vaccines is our limited understanding of the mechanisms ruling protection or harm among DENV serotypes, or between DENV and ZIKV. Here, we depleted rhesus macaques of $CD4^+$ T cells *in vivo* before primary DENV infection and/or secondary ZIKV challenge to recreate a sub-optimal priming of the humoral immune response. Our results support that $CD4^+$ T cells are needed to induce a quantitative and type-specific effective humoral immune response against primary DENV, but also against secondary ZIKV in DENV-experimented subjects. Our results also indicate a limited contribution of the DENV-Memory B cells to anti-ZIKV response. Furthermore, our results suggest that a suboptimal B cell priming during a primary DENV infection does differentially impact different antibody (Abs) properties. While binding or neutralization of ZIKV or DENV during a subsequent exposure to ZIKV is not affected by the lack of $CD4^+$ T - B cells interaction during a primary DENV infection, that interaction is critical to guarantee the Abs specificity. Also, we found that depleting $CD4^+$ T cells before DENV primary infection but not before ZIKV challenge significantly increases Abs cross-reactivity against DENV-EDIII domain and DENV-NS1 protein but not against ZIKV-EDIII domain or NS1 protein. Furthermore, there was more cross-reactivity among the DENV-NS1 proteins than against DENV-EDIII domains, suggesting that during a primary DENV infection $CD4^+$ T cells have a different weight in the responses against EDIII domain and NS1 protein. The proper Abs binding and neutralization with increased cross-reactivity profile was associated with limited frequency of circulating peripheral T helper cells (pTfh) with T helper 1 phenotype (CD4+/CXCR5+/CXCR3+) and expressing markers related to B cell activation (CXCR5+/CXCR3+/PD-1+/ICOS+) in the group depleted of $CD4^+$ T cells only before primary DENV infection. However, memory B cells – but not Antibody Secreting Cells (ASC) activation 7 days after the infection – positively correlate with those two populations of pTfh. Finally, when Abs cross-reactivity values were incorporated in a Principal Component Analysis (PCA), the DENV- $CD4^+$ T depleted group separates from the other two groups with similar Abs binding and neutralization profiles. Our result strongly suggests that during a heterologous sequential DENV/ZIKV infections Abs binding, and neutralization, may be regulated by different factors than their specificity. Before, the induction of cross-neutralizing Abs has been described in the context of secondary DENV infection. Here, for the first time, we are reproducing the experimental conditions leading to the generation of such Abs population *in vivo*. In summary, we show that suboptimal immune priming during a primary flavivirus infection has functional consequences during a

54 secondary heterologous infection. Finally, we shown that $CD8^+$ T cells are essential to guarantee an
55 optimal Abs neutralization activity. These results have huge implications understanding the immune
56 response to DENV vaccines (and maybe ZIKV), including why an optimal vaccine or natural-induced
57 neutralizing response not necessarily protects or enhances pathogenesis during a subsequent natural
58 heterologous exposure.

Introduction

Dengue (DENV) and Zika (ZIKV) viruses are flaviviruses of significant public health concern (Li et al., 2021; Paixao et al., 2022; Qiao et al., 2021; Tian et al., 2022; Wong et al., 2022). DENV is responsible for about 390-500 million infections per year, of which 96 million present different levels of disease severity (Bhatt et al., 2013) including Dengue Hemorrhagic Fever/ Shock Syndrome with up to 20 % mortality rate if untreated (WHO/TDR, 2009). It is estimated that the population at risk will increase in 60% by 2080 for a total of 6.1 billion people (Messina et al., 2019). ZIKV infections are usually mild but have been associated with unique severe adverse outcomes such as fetal loss (Campos et al., 2016), congenital ZIKV syndrome (CZS) (Brasil et al., 2016), Guillain-Barré syndrome (GBS) (Cao-Lomeau et al., 2016), and rare cases of encephalopathy (Roze et al., 2016), meningoencephalitis (Carteaux et al., 2016), myelitis (Mecharles et al., 2016), uveitis (Furtado et al., 2016), and severe thrombocytopenia (Sharp et al., 2016).

DENV and ZIKV circulate in similar geographic areas (Braga et al., 2017; Rodriguez-Morales et al., 2016), and the effect of prior DENV or ZIKV immunity to each other is subject of an ongoing debate. Some evidence from animal models and humas suggest that prior DENV immunity has a protective role in secondary ZIKV infection (Ana Carolina Bernardes Terzian, 2017; Breitbach et al., 2019; McCracken et al., 2017; Pantoja et al., 2017). Other works propose that prior DENV infection may increase ZIKV disease severity (Bardina et al., 2017; Crooks et al., 2021; Katzelnick et al., 2020b; Katzelnick et al., 2021). Intermediate pre-existing levels of naturally acquired anti-DENV or anti-ZIKV antibodies (Abs) were found to be associated with enhancement of symptomatic and severe DENV. However, subjects with high anti-DENV or anti-ZIKV Abs are at much lower risk of symptomatic and severe DENV2, with rates similar to DENV-naïve individuals (Katzelnick et al., 2020a). To add complexity to the field, recently it has been documented that different DENV and ZIKV interactions can be different or asymmetrical depending on the DENV serotype {Bos, 2023 #2166;Katzelnick, 2020 #2029}. Nevertheless, the mechanisms supporting a proportionate immune response among all four DENV serotypes or DENV and ZIKV after a natural infection – particularly after vaccination – still remains as a critical knowledge gap.

Immunity to one DENV serotype is believed to confer type specific (TS) protective Abs or immunity to that infecting serotype, but can also induce cross-reacting (CR) Abs able to amplify the pathogenesis during a secondary infection with a different serotype – a phenomenon known as antibody-dependent enhancement (ADE) (Guzman et al., 2013; Halstead, 2003; Halstead et al., 1983; Katzelnick et al.,

2017). As a result, DENV vaccines are designed as tetravalent formulations to induce balanced Abs (Abs) response against all four DENV serotypes at the same time. Two of the leading DENV vaccines (Dengvaxia and TAK-003), based on attenuated chimeric viruses (Deauvieau et al., 2007; Guy et al., 2017; Huang et al., 2013; Osorio et al., 2011b) has shown to induce unbalanced immune responses (Biswal et al., 2020; Guirakhoo et al., 2002; Hadinegoro et al., 2015; Halstead, 2018; Sabchareon et al., 2012; White et al., 2021). A third vaccine candidate developed by the Laboratory of Infectious Diseases at the National Institutes of Health (TV003) based on live attenuated DENV viruses (Durbin et al., 2013; Durbin et al., 2011; Whitehead, 2016), induced TS Abs to all four DENV serotypes and protection against challenge (Kirkpatrick et al., 2016; Nivarthi et al., 2021). Recent results from phase 3 are encouraging, but differences in efficacy were still reported between DENV1 [89.5% (95% CI, 78.7 to 95.0)] and DENV2 [69.6% (95% CI, 50.8 to 81.5)] the two serotypes detected in the three years of follow up period (Kallás et al., 2024). The limitations of Dengvaxia and TAK-003 formulations in inducing a balanced immune response to all four DENV serotypes have been associated to unequal replication of vaccine components or to the lack of/incomplete cellular immune response to the nonstructural (NS) proteins, among others (Grifoni et al., 2020; Guy et al., 2008; Guy et al., 2020; Thomas et al., 2009; Waickman et al., 2019a; Waickman et al., 2019b). For Dengvaxia, the DENV4 component was replicating and immunodominant, compared to the other three serotypes (Guy et al., 2009); a similar situation is documented with DENV2 in the TAK-003 vaccine in non-human primates (NHP) and humans (Osorio et al., 2011a; Rupp et al., 2015; Tricou et al., 2020).

Total Abs to each DENV serotypes have been used as a marker of protection for DENV vaccines, as it has shown to correlate with the efficacy of other flavivirus vaccines like yellow fever vaccine (YFV), Japanese encephalitis vaccine (JEV) (Appaiahgari et al., 2006; Goncalvez et al., 2008; Gupta et al., 2008; Raengsakulrach et al., 1999; Saini and Vrati, 2003), and tick-borne encephalitis (TBEV) (Beran et al., 2019; Leonova and Pavlenko, 2009; Maikova et al., 2016; Venturi et al., 2009; Vorovitch et al., 2019). But recent growing evidences point out that TS neutralizing Abs (nAbs) above a certain threshold may be a better indicator of vaccine efficacy than the total Abs (Henein et al., 2021; Nivarthi et al., 2021). Earlier works also provide explanations as of why some Abs might neutralize DENVs in cell culture, but not protect *in vivo* (Raut et al., 2019). Martinez et al. assembled a comprenhensive review on the multiple factors modifiying the efficacy of the immune response to DENV and DENV vaccines, incluidng the intra and inter serotypic viral antigenic diversity and the genotypic diversity among others {Martinez, 2021 #2068}.

121 All those observations and findings from natural infections and vaccines are supported on Abs response
122 mainly against the structural proteins, reinforcing the concept that immunogenicity of the structural
123 proteins does not guarantee protection from natural infection or vaccination efficacy. Most humoral
124 immunity epitopes are located within the domains of the DENV envelope (E) protein, while cellular
125 immunity epitopes are located on the NS proteins (Pinheiro et al., 2021; Rothman, 2010). Additionally,
126 the NS1 protein is particularly interesting and unique, as it is a protein produced and secreted during
127 viral replication, which make it highly immunogenic (Zhang et al., 2023) inducing significant levels of
128 anti-NS1 Abs in DENV- and ZIKV-infected individuals (Aquino et al., 2023; Castro-Trujillo et al., 2023;
129 Churdboonchart et al., 1991; Costa et al., 2007; Petphong et al., 2023). It is documented that DENV E
130 and NS1 proteins equally score 4 – out of the 10 proteins – in an immunogenicity score (Weiskopf et al.,
131 2016), and some reports have pointed NS1 as a target antigen for the development of DENV vaccines
132 (Costa et al., 2007; Costa et al., 2006; Henchal et al., 1988; Wan et al., 2014), while others suggest a role
133 for this protein in the pathogenesis (Perera et al., 2024; Shu et al., 2000; Zhang et al., 2023). Thus, it still
134 remains a puzzle due to its ability to elicit both protective and potentially pathogenic immune responses.
135 On this work, in addition to the conventional Abs to the structural proteins, we explore the contribution
136 of NS1 to the immune response during the primary DENV infection and a secondary ZIKV challenge.
137 Beyond the cannonical humoral component, a robust T cell response is needed to confer protection after
138 a natural DENV or ZIKV infection (Elong Ngono and Shresta, 2019; Roth et al., 2018; Tian et al., 2019;
139 Weiskopf and Sette, 2014; Yauch et al., 2009) and most of the cognates epitopes for the T cells ($CD4^+$
140 and $CD8^+$ T cells) are located in the NS proteins (Grifoni et al., 2017a; Rivino et al., 2013; Rivino and
141 Lim, 2017). Despite this, one common aspect shared among all three leading DENV vaccines is that
142 they do not present NS proteins of all DENV serotypes to the immune system. Dengvaxia is based on a
143 Yellow Fever (YF) 17D backbone and contains no DENV NS proteins (Guirakhoo et al., 2001). The
144 Takeda vaccine uses a DENV-2 backbone and has only DENV-2 NS proteins (Huang et al., 2013; Kinney
145 et al., 1997). The NIH/Butantan/MSD vaccine has full genome DENV-1, -3, and -4 components with a
146 DENV-2/-4 chimera based on a DENV-4 backbone. This vaccine possesses NS proteins from DENV-1,
147 -3, and -4 but not of DENV2 (Blaney et al., 2006; Blaney et al., 2008; Whitehead et al., 2003a;
148 Whitehead et al., 2003b). Further, data on the contribution of T cells and their cognate epitopes on the
149 NS proteins to the vaccine efficacy, and potentially protection, is still restricted (Angelo et al., 2017;
150 Guy et al., 2008; Tricou et al., 2022; Waickman et al., 2019a; Weiskopf et al., 2015a). These data
151 underscore how the contribution of NS proteins and T cells has been understudied in the context of

152 DENV vaccination. Pinheiro et al. completed a comprehensive analysis of B and T cells epitopes shared
153 by all three leading DENV vaccines, and concluded that investing in vaccines that contain most of the
154 epitopes involved in protective immunity (cellular and humoral arms) is an important issue to be
155 considered (Pinheiro et al., 2021). In fact, a few years ago the World Health Organization (WHO)
156 recommended that the cell mediated immunity (CMI) induced by DENV vaccines should be studied in
157 detail (Thomas et al., 2009).
158 Also, it has been shown that $CD4^+$ T cells are essential for inducing a protective immune response against
159 DENV and ZIKV (Elong Ngono et al., 2019; Grifoni et al., 2017b; Grifoni et al., 2017c; Hassert et al.,
160 2018; Manh et al., 2020; Weiskopf et al., 2015b; Wen et al., 2020); these cells play a critical role
161 connecting the cellular and humoral immune responses though unique interactions with B cells (Crotty,
162 2015; Swain et al., 2012). Moreover, immunological characterization of T cells has identified the
163 presence of follicular $CD4^+$ T helper cells (Tfh) or "Tfh-like" in blood, known as circulating (cTfh) or
164 peripheral (pTfh) cells. These cells have been described, in terms of function, as the equivalent
165 counterpart of germinal center (GC) Tfh cells in peripheral blood (Bentebibel et al., 2013; Crotty, 2014;
166 Morita et al., 2011). However, there is limited literature available on the role of pTfh cells in DENV
167 (Haltaufderhyde et al., 2018; Izmirly et al., 2022; Wijesinghe et al., 2020) and ZIKV vaccination or
168 infections (Liang et al., 2019; Pardy et al., 2022). Despite their critical role helping B cells form an
169 optimal humoral immune response, there are scarce works studying the direct interactions of $CD4^+$ T
170 cells/pTfh cells subsets with B cells and NS proteins, and its effect in Abs functionality against DENV
171 and/or ZIKV (Elong Ngono et al., 2019; Rivino et al., 2013; Rouers et al., 2021). Using a NHP model –
172 rhesus macaques – we asked how the lack of essential cells, such as $CD4^+$ T cells, impacts the quality of
173 the humoral immune response (including the response to NS1 protein) during a primary DENV2
174 infection and its consequences in the response to a secondary heterologous ZIKV infection. We believe
175 this is the type of mechanistic questions that needs to be addressed in order to identify correlates of
176 protections and ultimately design more efficient flavivirus vaccines.
177 In this work, we depleted rhesus macaques of $CD4^+$ T cells before a primary DENV2 infection and/or
178 before a subsequent ZIKV infection one year apart. A cohort was depleted of $CD8^+$ T cells before the
179 secondary challenge with ZIKV.
180 We found that the lack of $CD4^+$ T cells before infections results in a significant increase in DENV
181 viremia and DENV-NS1 antigenemia and ZIKV viremia. Lack of $CD4^+$ T cells before DENV or ZIKV
182 also results in a delay in total Abs switch documented by limited IgM and IgG production and in a

183 significantly lower anti-DENV and anti-ZIKV NS1 Abs response. We also found that Abs binding or
184 neutralizing DENV or ZIKV after heterologous challenge with ZIKV secondary infection were
185 minimally impacted by the lack of $CD4^+$ T cells during DENV primary infection. Nevertheless, the
186 presence of these cells during the primary DENV infection were critical to preserve the specificity of
187 the Abs against the DENV, but not ZIKV EDIII domain and NS1 protein after ZIKV challenge.
188 This profile of Abs functionality (proper binding and neutralization with increased cross-reactivity) was
189 associated with a significant lower frequency of pTfh TH1 and expression of markers related to B cell
190 activation – PD-I and ICOS – when $CD4^+$ T cells were not present during the primary DENV infection.
191 Furthermore, the expression of these markers correlates with the activation of the MBC, but not ASC,
192 in the same group. Interestingly, the depletion of $CD8^+$ T cells abrogates the direct correlation between
193 those $CD4^+$ T cells subsets and MBC or ASC activation.
194 The regulation of the antibody response and its quality by $CD8^+$ T cells is significantly less characterized
195 than by $CD4^+$ T cells. While $CD8^+$ T cells are well defined for their cytotoxic response, recent findings
196 have identified a population of $CD8^+$ T cells that show distinct phenotypical characteristics of Tfh cells
197 and can be found in the B cell follicles (Chen et al., 2023). These cell subsets show high expression of
198 markers such as CXCR5 (related to follicular- homing) and CXCR3 while downregulating T zone
199 homing markers such as CCR7 (Quigley et al., 2007). Here we show that depleting $CD8^+$ T cells
200 eliminates the strong correlation between $CD4^+$ T Cells and Abs neutralization activity.
201 With our experimental design, we could reproduce *in vivo*, first the time, the mechanism inducing those
202 CR nAbs. This Abs population was described in the context of secondary DENV infections (Patel et al.,
203 2017) and has been at the center of an active debate on their protective or pathogenic contribution to the
204 flavivirus response. Our findings are critical to understand the efficacy limitations of Abs induced by
205 current approved DENV vaccines or protecting from natural infections and thus are essential to guide
206 the new generations of DENV and ZIKV vaccines. We also provide an animal model to reproduce an
207 immunological signature of the response to flaviviruses that can be used to test and challenge the
208 protective or disease-enhancing attributes of current and new vaccine candidates or, as in this work, to
209 better characterize flavivirus interactions.
210 We are unaware of any prior work addressing the complex interactions among $CD4^+$ T cells, B cells and
211 NS proteins in response to flavivirus infection in NHPs or correlating the $CD8^+$ T cells with the quality
212 of the humoral immune response to flavivirus.
213

214 **Results**

215 **Rhesus macaque cohorts, T cell depletion and sample collection**. Five groups of flavivirus-naïve
216 rhesus macaques (*Macaca mulatta*) (SFig 1). Prior to any experimental activity, all animals were put
217 through a quarantine period of 40 days. Depletion of T cells was performed by initial subcutaneous (s.c.)
218 administration of 50 mg/kg of anti-CD4+ treatment at 15 days pre-challenge, followed by two
219 intravenous (i.v.) administrations of 7.5 mg/kg at 12- and 9-days pre-challenge. Animals were depleted
220 of CD4+ or CD8 T+ cells, or administered phosphate buffer saline (PBS), followed by a primary
221 infection with DENV-2 ($5x10^5$ pfu s.c.) and a subsequent ZIKV challenge (1×10^6 pfu s.c.) one year
222 later, after a second round of T cell depletion – or administration of PBS – before infection. CD4+ T cell
223 depletion was performed on groups 1 and 3 prior to primary DENV2 infection and on groups 1 and 2
224 prior to secondary ZIKV infection (group 1: n=6, denoted CD4-/DV2/CD4-/ZV; group 2: n=6, denoted
225 CD4+/DV2/CD4-/ZV; group 3: n=6, denoted CD4-/DV2/CD4+/ZV). Group 4 is CD4-competent but
226 CD8-depleted prior to secondary ZIKV infection (n=6, denoted CD4+/DV2/CD8-/ZV). Group 5, the
227 control group, was treated with PBS before each infection (n=5, denoted CD4+/DV2/CD4+/ZV).

228

229 **Depletion of CD4+ T cells elicits a CD8+ T cell overcompensation, and vice-versa.** To determine if
230 the depletion treatment was effective, frequency of CD4+ and CD8+ T cells was assessed by
231 immunophenotyping using flow cytometry during pre-treatment (pre-TX), post-treatment (post-TX),
232 and post-primary DENV2 infection. Before depletion, CD4+ T cell frequency was similar in all animals
233 and decreased by day 2 post-TX, resulting in a depletion of 99.8% (SFig 2A). This reduction was
234 consistent until day 7 post infection (p.i.), when they began to slowly recover by day 15 post infection
235 p.i.. Further, the absence of CD4+ T cells in depleted animals promoted an increase in CD8+ T cell
236 frequency (SFig 2B).

237 CD4+ and CD8+ T cell frequencies were also measured before, during and after depletions prior to and
238 during ZIKV challenge (SFig 2C and D). Similar to the prior scenario, CD4+ T cell frequency was
239 similar in all animals, and decreased by day 2 after treatment start (SFig 2C). Interestingly, animals that
240 underwent CD8+ T cell depletion experimented an increase in CD4+ T cell frequency (SFig 2C). Lastly,
241 CD8+ T cells behaved similarly to CD4+ T cells after depletion, with frequency decreasing by day 2
242 post-TX and beginning to recover by day 7 post-challenge (p.c.), while CD4-depleted animals showed
243 an increase in CD8+ T cell frequency (SFig 2D).

244 **CD4+ T cell depletion impairs DENV2 viral clearance in flavivirus-naïve macaques.** To determine
245 if CD4+ T cell depletion enhances or reduces DENV2 replication, and how it changes depending on
246 various depletion conditions, DENV2 RNAemia was measured in serum during the first 15 days and
247 day 30 after primary DENV2 infection. RNAemia was defined as follows: early from days 1 to 3 p.i.,
248 mid from days 4 to 7 p.i., and late from day 7 p.i. onwards. During the early period, viral RNA detection
249 increased similarly in all groups (Fig 1A). By day 5 p.i., the control group showed a decrease in viremia
250 leading to resolution, while the CD4-depleted group still had high detectable RNAemia levels. This
251 difference was statistically significant on days 10 and 11 p.i. ($p= 0.000078$ and $p= 0.001346$,
252 respectively). By day 12 p.i. there was no viral RNA detection in any animal from the control group,
253 indicating a faster RNAemia resolution in this group compared to CD4-depleted animals. This suggests
254 a delay in viral clearance in animals that are CD4-deficient. By day 15 p.i. all animals tested negative
255 for DENV2 (Fig 1A and Table S1).
256 When evaluating the area under the curve (AUC), we found that the CD4-competent animals, although
257 not statistically significant, had lower values, on average, than the CD4-depleted group (Fig 1B). Next,
258 we evaluated the average RNAemia days, defined as the days with detectable viremia divided by the
259 number of animals per group (Fig 1C). The undepleted animals had significantly less mean RNAemia
260 days compared to the depleted animals ($p< 0.0001$). Taken together, these results suggest that $CD4^+$ T
261 cells have an early role controlling DENV2 RNAemia, but it remains unknown if this is due to a direct
262 action or to an interaction with B cells.
263
264 **CD4+ but not CD8+ T cells are necessary to control early ZIKV RNAemia in DENV-immune**
265 **macaques**. To assess the impact of T cell depletions in ZIKV replication in DENV2-immune animals,
266 and how it changes depending on various depletion conditions, ZIKV RNAemia was measured in serum
267 during the first 15 days and on day 30 after secondary ZIKV challenge using qRT-PCR. ZIKV RNAemia
268 was defined as follows: early from days 1 to 3 p.i., mid from days 4 to 7 p.i., and late from day 7 p.i.
269 onwards. Although all groups had a similar increase in early viral RNA detection, numerous significant
270 differences were noted on day 3 p.c. which is also when peak viremia was reached (Fig. 1D). The
271 CD4+/DV2/CD8-/ZV group had the lowest peak viremia among all groups, and it was significantly
272 lower in comparison to the CD4+/DV2/CD4-/ZV, CD4-/DV2/CD4+/ZV and CD4+/DV2/CD4+/ZV
273 groups ($p< 0.0001$, $p< 0.0001$ and $p= 0.0173$, respectively). On the other hand, the CD4+/DV2/CD4-
274 /ZV group had a significantly higher peak viremia compared to groups CD4-/DV2/CD4-/ZV, CD4-

275 /DV2/CD4+/ZV and the control group ($p< 0.0001$, $p= 0.0032$ and $p< 0.0001$, respectively). Lastly, the
276 CD4-/DV2/CD4+/ZV had a significantly higher peak viremia compared to the double-depleted group
277 ($p= 0.0109$) (Fig 1D and Table S2). Of note, historical data from our laboratory shows that ZIKV
278 replication in naive undepleted animals is higher than ZIKV replication in DENV-immune animals
279 regardless of CD4+ T cell status, as well as an earlier peak viremia, which was displaced by one day in
280 the presence of previous DENV2 immunity in the current presented data (Fig. 1D). By day 7 p.c., there
281 was no viral RNA detection in any group.
282 We observed a difference when comparing all five groups based on AUC on different RNAemia periods
283 (Figures 1E and F). During early RNAemia, CD8-depleted animals had lower AUC levels compared to
284 all groups, and they were significantly lower than the double-depleted animals ($p= 0.0029$) (Fig 1 E).
285 However, a shift occurs during mid RNAemia where CD8-depleted animals, although not significantly
286 different, have the highest AUC levels of all groups (Fig 1F). This is further confirmed by evaluating
287 the mean RNAemia days, where it was observed that CD8-depleted animals had the longest RNAemia
288 (Fig 1G). This suggests that early viremia and peak control associates with a late viremia resolution in
289 CD4-competent but CD8-depleted animals, indicating that CD8+ T cells may not play a crucial role
290 controlling early viremia but do so in mid to late viral clearance.
291 Taken together, these results confirm the requirement of CD4+ T cells for an optimal control of DENV2
292 replication during a primary infection. In addition, our findings propose an important role for CD4+ T
293 cells controlling secondary ZIKV infection and that role being independent of prior DENV experience.
294 Lastly, this indicates that CD8+ T cells may not be required for early ZIKV viral replication control in
295 individuals with prior DENV2 immunity but are necessary for mid and late viral clearance.
296
297 **$CD4^+$ T cell depletion results in higher detection of DENV NS1.** To confirm viremia and to explore
298 the role of CD4+ T cells in the generation of NS1 antigen, we measured DENV NS1 levels in serum.
299 Since NS1 antigen is generally detected during days 1 to 9 p.i., we tested samples from baseline and
300 days 3, 5, 7, 10, 15 and 30 post DENV2 infection (SFig 3). As expected, no detectable levels of NS1
301 were observed at day 0 on any of the groups. On days 3, 5, 7 and 10 p.i., an increase in NS1 levels was
302 observed on all animal groups, with CD4-depleted animals having slightly higher NS1 levels in
303 comparison to the undepleted animals by day 10 p.i.. By day 15 p.i., NS1 levels were significantly higher
304 in depleted animals ($p< 0.0001$) compared to undepleted animals, where NS1 levels are completely
305 undetectable. This is of particular interest because by this time, detection of DENV-NS1 is not expected.

306 This suggests that the lack of $CD4^+$ T cells enhances NS1 circulation, which correlates with enhanced
307 pathogenesis, further confirming a protective role for $CD4^+$ T cells during primary DENV infection.
308
309 **Lack of CD4+ T cells during a primary DENV2 infection does not hinder antibody binding to**
310 **ZIKV in a secondary exposure.** We evaluated the impact of $CD4^+$ and $CD8^+$ T cell depletion on the
311 quantity and quality of the humoral response against DENV2 and ZIKV infections. Anti-DENV IgM
312 and IgG levels were measured after primary DENV2 infection (Fig 2). CD4-depleted animals had
313 significantly lower levels of anti-DENV IgM in comparison to the undepleted animals by days 10 and
314 15 p.i. ($p< 0.001$) (Fig 2A). Furthermore, total anti-DENV IgM produced from day 0 to day 15 p.i. was
315 significantly lower in the CD4-depleted group in comparison to the control group ($p= 0.007$) (Fig 2C).
316 On the other hand, anti-DENV IgG levels were statistically different between groups only on day 15
317 p.i., where the CD4-depleted group had lower levels compared to the control group ($p< 0.001$) (Fig 2B).
318 Similar results were observed with the total anti-DENV IgG produced (from day 0 to day 30), where
319 CD4-depleted animals had significantly lower IgG values in comparison to undepleted animals ($p=$
320 0.0153) (Fig 2D). IgG levels by days 60 and 90 p.i. were at similar levels in both groups. These results
321 suggest a delay in B cell activation and in isotype class switching during primary DENV infection related
322 to the lack of $CD4^+$ T cells.
323 Additionally, DENV and ZIKV binding IgM and IgG levels were measured after secondary ZIKV
324 challenge (Fig 3). As expected, all animals had no detectable anti-DENV IgM at baseline and remain
325 consistently low throughout days 7,10 and 15 p.c. (Fig 3A). At day 15 p.c. only one animal from the
326 control group and one from the CD4+/DV2/CD8-/ZV group had values over the assay threshold, further
327 indicating the positive role of $CD4^+$ T cells in the magnitude of the humoral immune response during
328 primary DENV infection. In contrast, all animals had similar detectable anti-DENV IgG levels at
329 baseline (Fig 3B). As expected, ZIKV infection transiently boosted cross-reacting binding Abs which
330 declined by day 30 p.c. in all groups (Fig 3B). However, the magnitude of this boost was significantly
331 impacted on day 15 p.c. by the lack of CD4+ T cells. Lower anti-DENV IgG levels were observed in
332 the two groups lacking CD4+ T cells before ZIKV challenge (double CD4-depleted and
333 CD4+/DV2/CD4-/ZV). The significance between group CD4-/DV2/CD4-/ZV with groups
334 CD4+/DV2/CD4+/ZV, CD4+/DV2/CD8-/ZV and CD4-/DV2/CD4+/ZV was statistically significant
335 ($p< 0.019$, $p< 0.008$ and $p< 0.047$ respectively). The differences between the CD4+/DV2/CD4-/ZV with
336 the CD4+/DV2/CD4+/ZV and CD4+/DV2/CD8-/ZV groups were also significant ($p< 0.018$ and $p<$

337 0.040 respectively). There were no differences among the three groups with $CD4^+$ T cells before ZIKV
338 infection. Altogether, these results indicate that DENV-experimented $CD4^+$ T cells do not contribute to
339 an optimal humoral immune response to ZIKV. This also suggests that DENV MBCs have a limited
340 expansion during a secondary ZIKV infection and if they have, the expansion is CD4-independent.
341 Anti-ZIKV IgM levels became detectable by day 7 p.c. with a peak by day 15 and started declining by
342 day 30 p.c. (Fig 3C). By day 10 p.c., the control group had significantly higher levels than at least two
343 groups, the doubled-depleted group ($p< 0.030$) and the CD4-/DV2/CD4+/ZV group ($p< 0.033$). By day
344 15 p.c., the differences between the control undepleted group and the two other groups undepleted of
345 CD4+ T cells before ZIKV challenge were significant (CD4-/DV2/CD4+/ZV, $p< 0.022$ and
346 CD4+/DV2/CD8-/ZV, $p< 0.028$). However, the two groups depleted of CD4+ T cells before ZIKV
347 challenge have the lowest levels of ZIKV-binding IgM compared to the three undepleted groups (group
348 CD4+/DV2/CD4+/ZV vs. CD4-/DV2/CD4-/ZV and CD4+/DV2/CD4-/ZV $p< 0.0001$ and $p< 0.0001$
349 respectively, group CD4-/DV2/CD4+/ZV vs. CD4-/DV2/CD4-/ZV and CD4+/DV2/CD4-/ZV $p< 0.001$
350 and $p< 0.053$ respectively and group CD4+/DV2/CD8-/ZV vs. CD4-/DV2/CD4-/ZV and
351 CD4+/DV2/CD4-/ZV $p< 0.001$ and $p< 0.004$ respectively).
352 Of note, some of the CD4-/DV2/CD4-/ZV animals had a late modest boost in ZIKV IgM levels by day
353 30 p.c. after the initial observed delay; interestingly, all depleted animals before ZIKV challenge induced
354 the lowest IgM levels. Together, these observations suggest a delayed ZIKV-naïve B cell expansion in
355 absence of CD4+ T cells.
356 Results from ZIKV-binding IgG Abs were similar to the IgM results (Fig 3D). By day 10 p.c. the two
357 groups depleted of CD4+ T cells before the challenge showed the lowest values. The values were
358 significantly lower in the group depleted of CD4+ T cells only before ZIKV challenge
359 (CD4+/DV2/CD4-/ZV) compared to the CD4+/DV2/CD4+/ZV ($p< 0.004$) and CD4-/DV2/CD4+/ZV
360 ($p< 0.0001$) groups. By day 15 p.c. this trend was maintained. Results reached statistical significance
361 between the control undepleted group and the double-depleted group ($p< 0.003$) and the
362 CD4+/DV2/CD4-/ZV group ($p< 0.001$) and between the CD4-/DV2/CD4+/ZV group with the CD4-
363 /DV2/CD4-/ZV and the CD4+/DV2/CD4-/ZV groups ($p< 0.001$ and $p< 0.0007$ respectively).
364 An interesting finding was the presence of detectable cross-reacting anti-ZIKV IgG levels at baseline
365 only in the group depleted of CD4+ T cells before primary DENV infection but not before ZIKV
366 challenge (CD4-/DV2/CD4+/ZV) with significant higher values when compared to the
367 CD4+/DV2/CD4-/ZV and the CD4+/DV2/CD4+/ZV groups ($p< 0.031$ and $p< 0.037$ respectively). The

368 double-depleted group also had higher cross-reactive IgG levels compared to the undepleted groups but
369 did not reach statistical significance. This result suggests an increased expansion of DENV-cross-
370 reactive B cell clones in animals without $CD4^+$ T cells during primary DENV infection.
371 To confirm our results on the anti-ZIKV IgG, we analyzed the total ZIKV-specific IgG produced on
372 days 10, 15 and 30 p.c. (Fig 3E). Double-depleted animals had significantly less total ZIKV IgG
373 production compared to the CD4-competent animals before ZIKV challenge (p= 0.0002 compared
374 to CD4-/DV2/CD4+/ZV group, p= 0.0200 compared to CD4+/DV2/CD8-/ZV group and p= 0.0065
375 compared to CD4+/DV2/CD4+/ZV). Similarly, the CD4+/DV2/CD4-/ZV group had lower IgG
376 production compared to the CD4-/DV2/CD4+/ZV, CD4+/DV2/CD8-/ZV and CD4+/DV2/CD4+/ZV
377 groups (p= 0.0003, p= 0.0322 and p= 0.0108, respectively). In general, CD4-depleted animals before
378 ZIKV challenge produced significantly less total ZIKV-specific IgG.
379 These results indicate that while $CD4^+$ T cells during a primary DENV infection have limited impact in
380 the binding of ZIKV-specific Abs, their absence may result in a significant induction of flavivirus cross-
381 reactive B cell clones (Fig 2D) during a secondary infection. This also indicates that DENV-induced
382 MBC populations generated during a primary DENV infection have limited contribution in the anti-
383 ZIKV-binding Abs after ZIKV infection. In addition, these results suggest that $CD4^+$ T cells are required
384 for a more effective and specific antibody response against ZIKV.
385
386 **Recall memory to DENV NS1 is expanded by lack of CD4+ T cells before DENV2 infection.** Next,
387 we performed an anti-DENV NS1 assay using samples from baseline and days 15, 30 and 90 after
388 primary DENV2 infection (SFig 4A). No DENV NS1 anti-IgG levels were detected on day 0 as
389 expected. However, by day 15 p.i., depleted animals had significantly lower levels in comparison to the
390 undepleted animals, similar to anti- DENV IgG levels previously presented (p= 0.0003). By day 30 p.i.,
391 both groups had reached similar anti-NS1 IgG levels. Interestingly, by day 90 p.i. depleted animals
392 showed significantly higher anti-NS1 IgG levels compared to undepleted animals (p= 0.0003),
393 altogether supporting the delay in Abs switch in this group as seen in the total IgG Abs response.
394 We also measured DENV NS1 anti-IgG levels in samples from baseline and days 15, 30 and 90 after
395 secondary ZIKV challenge (SFig 4B). Of note, on baseline the group depleted of $CD4^+$ T cells before
396 DENV infection but not ZIKV (CD4-/DV2/CD4+/ZV) had higher values when compared to the groups
397 with $CD4^+$ T cells prior to DENV infection, and they were significantly higher particularly in
398 comparison to the CD4+/DV2/CD4-/ZV group (p= 0.002). Although not significant, the double-depleted

399 group had similarly high values in comparison to the groups with CD4+ T cells prior to DENV infection.
400 This implies that the lack of $CD4^+$ T cells before primary DENV infection also results in an increased
401 durability of NS1 Abs. By day 15 p.c., we observed significantly higher values of DENV NS1 anti-IgG
402 in the CD4-/DV2/CD4+/ZV group in comparison to all other groups (CD4-/DV2/CD4-/ZV, p= 0.0124;
403 CD4+/DV2/CD4-/ZV, p= 0.0002; CD4+/DV2/CD4+/ZV, p= 0.0094 and CD4+/DV2/CD8-/ZV, p=
404 0.0152). This trend is maintained by day 30 p.c. (CD4-/DV2/CD4-/ZV, p= 0.0053; CD4+/DV2/CD4-
405 /ZV, p< 0.0001; CD4+/DV2/CD4+/ZV, p< 0.0001 and CD4+/DV2/CD8-/ZV, p< 0.0001). Intriguingly,
406 similar to what we stated on the previous section, these results seem to imply that deficiency of $CD4^+$ T
407 cells during the primary DENV2 infection modify the recall memory quantitatively resulting in an
408 increased DENV NS1-cross-reactivivity induced by ZIKV.
409 Lastly, no significant differences between groups were observed on day 90 p.c.. Of note, some animals
410 belonging to the CD4+/DV2/CD4-/ZV group exhibited a marked separation between IgG values,
411 indicating that antibody repertoire may be different between individuals even sharing similar conditions.
412
413 **Depletion of CD4+ T cells only prior to DENV infection increases ZIKV NS1-binding IgG after**
414 **ZIKV infection.** To further study the impact of CD4+ T cells in the generation of cross-reactive and
415 specific Abs, we measured anti-ZIKV NS1 IgG levels in serum at days 0, 15, 30 and 90 p.c. (SFig 5).
416 Contrary to the total IgG, no animals had, regardless of their CD4+ T cell status, detectable anti-ZIKV-
417 NS1 IgG Abs at baseline, indicating that those Abs are very specific. However, by day 15 p.c., only
418 animals with CD4+ T cells before ZIKV challenge produced detectable IgG levels, with the exception
419 of one animal from the CD4+/DV2/CD4-/ZV group (SFig 5). Double-depleted animals had the lowest
420 IgG levels of all groups, significantly different from the control group (p= 0.0003) and the CD4-
421 /DV2/CD4+/ZV group (p= 0.0004) on day 15 p.c. Additionally, NS1 IgG levels from the double-
422 depleted group were significantly lower in comparison to the CD4-/DV2/CD4+/ZV group (p= 0.0071)
423 also on day 30 p.c.. The latter group was consistently the highest IgG-producing group, even higher than
424 the control group. Unsurprisingly, CD8-depleted animals behaved similarly to the control group as they
425 both have intact CD4 T cells populations before the primary infection and the challenge. By day 90 p.c.
426 almost all animals, regardless of their depletion status, had no NS1-IgG levels. Collectively, these data
427 suggests that DENV-experimented $CD4^+$ T cells have limited or no effect on the production of anti-
428 ZIKV NS1-abs during a secondary ZIKV exposure.
429

430 **Early DENV2 neutralization activity is delayed by lack of CD4+ T cells.** To determine the
431 contribution of Abs to DENV2 and ZIKV neutralization and the impact of CD4+ and CD8+ T cell
432 depletion, all groups were tested using FRNT and PRNT assays against DENV2 and ZIKV respectively
433 (Fig. 2 and 3). Neutralization assays were completed for baseline and days 15, 30 and 90 p.i. for DENV-
434 2 and days 15, 30 and 90 p.c. for ZIKV. The 50% effective concentration (EC) of neutralizing Abs is
435 shown in Figures 3 and 4 as well. As expected, no animals had nAbs against DENV2 at baseline before
436 primary infection (Fig 4A). By day 15 p.i., a significantly lower neutralizing activity is observed in the
437 CD4-depleted animals in comparison to the undepleted group ($p< 0.0001$) (Fig 4B). This trend is not
438 observed on day 30 p.i., when both groups behave similarly (Fig 4C). Endpoint titers dilutions are shown
439 in Figures 3D and 3E in order to facilitate a better interpretation. Similar to the scenario described above,
440 significantly lower EC50 values are observed in the depleted animals ($p= 0.0032$) (Fig 4D).
441 PRNT assays were performed after ZIKV challenge; despite the detection of anti-ZIKV IgG at baseline
442 in some groups, no neutralizing activity was observed at that time point against ZIKV (Fig 5A).
443 However, on day 15 p.c., the CD4-double depleted group was the lowest at neutralizing ZIKV (Fig 5B).
444 Animals belonging to the CD4-/DV2/CD4+/ZV group and interestingly those from group
445 CD4+/DV2/CD8-/ZV, having a higher frequency of CD4+ T cells, have better neutralizing activity by
446 day 15 p.c. compared to the other three groups – even the undepleted control group. Those differences
447 were statistically significant compared to the double-depleted group ($p= 0.0231$ and $p= 0.0155$
448 respectively). Taken together, these results lead us to consider that DENV-primed $CD4^+$ T cells have a
449 limited contribution to an effective early neutralization during a secondary ZIKV infection and that the
450 magnitude of neutralization can correlate with the frequency of those cells.
451 By day 30 p.c., a change in neutralization profile is observed in all groups except the double-depleted
452 animals, which still show a delay in neutralization in comparison to the other groups (Fig 5C). By day
453 90 p.c., all groups behave similarly in terms of neutralization magnitude (Fig 5D). Endpoint titers
454 dilutions are shown in Figures 4F-H in order to facilitate a better interpretation.
455 To further study the impact of CD4+ T cells on the humoral response, neutralization assays were
456 performed against DENV2 using serum samples from 90 days post ZIKV challenge (Fig 5E and 5I). No
457 statistical differences were detected between any of the groups.
458
459 **Lack of CD4+ T cells during a primary DENV2 infection compromises antibody specificity in a**
460 **secondary ZIKV exposure**. To further explore the role of CD4+ T cells in shaping the immune response

461 against DENV and ZIKV, epitope reactivity against EDIII and NS1 was evaluated using a Luminex
462 assay. EDIII was selected as a major neutralizing target and NS1 due to its proposed role in pathogenesis.
463 Type-specific and cross-reactive responses were measured at day 90 p.i. after DENV2 and ZIKV
464 infections against EDIII and NS1 antigens from all four DENV serotypes and ZIKV. To normalize the
465 data a cross-reactivity index (CRI) was calculated by subtracting the background from the homologous
466 value (values against ZIKV) and the heterologous values (values against DENV2) followed by dividing
467 the subtracted homologous by the subtracted heterologous values (Fig 6).
468 As expected, after primary DENV2 infection, both undepleted and depleted animals had a high similar
469 type-specific response against EDIII and NS1 to the infecting serotype DENV2, and a similar limited
470 cross-reactivity against EDIII to the other DENV serotypes, with a significant difference observed in
471 DENV4 EDIII ($p= 0.0265$) (Fig 6A). As reported in the literature, primary DENV infection in flavivirus-
472 naïve individuals triggers minimal or no cross-reactivity to ZIKV (Collins et al., 2017; Duffy et al.,
473 2009). Both groups show a similar high type-specific response against DENV2 NS1 protein. In contrast
474 to the response to EDIII domain, a higher cross-reactive response against NS1 to the other DENV
475 serotypes, particularly against DENV serotype 1, was observed ($p= 0.0045$). Interestingly, there was a
476 limited but detectable cross-reactivity against ZIKV NS1 protein in both groups (Fig 6A). This suggests
477 that during a primary DENV infection, CD4+ T cells have a different weight in the responses against
478 EDIII domain and NS1 protein.
479 A more complex scenario is observed after secondary ZIKV infection (Fig 6B). All animals show an
480 increase in the type-specific response against EDIII and NS1 towards the infecting ZIKV, but their
481 reactivity against other DENV serotypes varies. Additionally, all groups show reactivity against DENV2
482 antigens. Of note, two animals from the undepleted control group NS1 (MA254 and MA264) had a
483 higher type-specific response against DENV2 EDIII, and two had a lower response (MA311 and
484 MA350) (Fig 6B), indicating that antibody repertoire can be different between individuals, a point to
485 take into consideration in vaccine design. Lastly, as expected, the CD4+/DV2/CD8-/ZV group behaved
486 very similar to the CD4-competent groups, demonstrating that $CD8^+$ T cell depletion does not have an
487 impact in epitope reactivity (Fig 6B). Taken together, these results indicate that while $CD8^+$ T cell
488 depletion increases $CD4^+$ T cell frequency and results in better ZIKV viremia control, quantity has no
489 effect on the specificity of epitope recognition. Overall, the cross-reactivity to NS1 protein was more
490 significant among DENV serotypes than to EDIII domain suggesting that, like after a primary DENV
491 infection, CD4+ T cells have a different weight in the response against EDIII domain and NS1 protein

492 after a secondary ZIKV infection in DENV-immune individuals. Animals belonging to all groups,
493 regardless of depletion condition, show an increased but similar response against EDIII and NS1 to the
494 infecting ZIKV. These results suggest that pre-existing DENV immunity has a very limited impact in
495 the cross-reactivity to ZIKV EDIII domain and NS1 protein, and that $CD4^+$ T cells have a limited role
496 regulating that cross-reactivity to those ZIKV targets.
497
498 **CD4+ T cells modulate early response IgG+ MBC activation and functionality during a**
499 **heterologous ZIKV infection with previous DENV-2 immunity**. To determine the contribution of
500 CD4+ T cells in modulating the surface IgG+ memory B cell (IgG MBC) (CD3-CD20+CD27+sIgG+)
501 response to ZIKV, we measured expression levels of surface IgG in total MBCs via Flow Cytometry.
502 Whole blood for Immunostaining was collected on baseline and days 7, 15, and 30 p.c. with ZIKV. By
503 day 15 p.c., a discrete peak in frequencies was observed in all groups, except the CD4+/DV2/CD8-/ZV
504 group which remain at the baseline levels (Fig 7A). On day 15 p.c., the CD4+/DV2/CD4+/ZV and the
505 CD4-/DV2/CD4+/ZV were the only groups showing a significant increase in IgG expression when
506 compared to their baseline status. By day 30 p.c., all groups showed similar levels of IgG expression to
507 their baseline status. We did not detect a significant difference in IgG expression among groups
508 compared at each timepoint suggesting that sIgG expression is not impacted by depletion treatment
509 status. Taken together, this data suggests that a secondary ZIKV infection in individuals with prior
510 DENV-2 immunity, IgG+ MBCs levels suffer limited increase in frequency and if it happens it is CD4+
511 T cell-dependent.
512 To determine if CD4+ T cells have a role in modulating the activation pathways of IgG MBCs after the
513 ZIKV challenge, we surveyed the expression of CD69 in this cell population. Compared to their baseline,
514 a significant increase in the frequency of IgG MBCs expressing CD69 was observed at day 7 p.c. only
515 on those groups with intact CD4+ T cells before ZIKV challenge (CD4-/DV2/CD4+/ZV, $p< 0.034$;
516 CD4+/DV2/CD4+/ZV, $p< 0.024$; and CD4+/DV2/CD8-/ZV, $p< 0.010$) (Fig 7B and 7C). Taken
517 together, this data suggests that IgG MBCs go through $CD4^+$ T cell-dependent interactions for their
518 activation during a secondary ZIKV infection.
519 To determine if CD4+ T cells have a role in the differentiation of activated B cells into Antibody
520 Secreting Cells (ASCs) (possibly through Germinal Centers formation) during a heterologous ZIKV
521 infection we measured CD69 expression in total ASCs ($CD20^{dim}CD3^-CD27^+$). Despite our gating
522 strategy being designed to characterize rhesus macaque plasmablast populations (Silveira et al., 2015)

523 (Zhang et al., 2019), we were unable to segregate that population. However as previously reported by
524 us (Marzan-Rivera et al., 2022), we idetified the total ASC population and observed a trend in activation
525 of that population during the ZIKV infection. At day 7 p.c., we detected a significant increase in CD69
526 expression in the CD4+/DV2/CD4+/ZV and the CD4-/DV2/CD4+/ZV groups, compared to the other
527 groups (Fig 7D, $p< 0.005$). This peak in CD69 expression was maintained up to day 15 p.c. in the
528 CD4+/DV2/CD4+/ZV group followed by a significant drop in CD69 expression levels at day 30 p.c..
529 Levels of expression observed during this timepoint were of similar magnitude as on baseline. On the
530 other hand, groups that lacked CD4+ T cells before the ZIKV infection (CD4+/DV2/CD4-/ZV and CD4-
531 /DV2/CD4-/ZV) showed a trend of lower levels of ASCs expressing CD69 compared to the $CD4^+$ T cell
532 competent groups before the ZIKV infection. Our data points to a *de novo* B cell response pathway that
533 is T cell-dependent to promote the formation of both IgG MBCs and ASC subsets with good neutralizing
534 capacity towards ZIKV.
535
536 **$CD4^+$ Peripheral T helper cells (pTfh) are present in circulating blood of Rhesus macaques during**
537 **heterologous ZIKV infection with prior DENV2 immunity**. To confirm the presence and frequency
538 of pTfh CD4+ T cells, we measured expression levels of surface markers such as CXCR5, CXCR3, PD-
539 1, and ICOS via flow cytometry analysis. Chemokine receptor CXCR3+ expression has been associated
540 with Type 1 T helper immune responses (Th1) (Sánchez-Vargas and Mathew, 2019; Sandberg et al.,
541 2021; White et al., 2021). Moreover, PD-1 and ICOS expression is associated with B cell interaction in
542 order to induce B cell activation (Lei et al., 2021; Shi et al., 2018). This analysis was performed only on
543 groups CD4+/DENV2/CD4+/ZIKV, CD4-/DENV2/CD4+/ZIKV and CD4+/DENV2/CD8-/ZIKV
544 because they have CD4+ T cell populations before ZIKV infection. Frozen PBMCs were used for
545 Immunostaining on day 0 (baseline), day 7, and day 15 p.c. with ZIKV. Surface expression of
546 CD4+/CXCR5+ was detected at similar levels in all three tested groups, confirming circulation of pTfh
547 cells. No significant differences were established among groups after ZIKV challenge on day 7 p.c.. By
548 day 15 p.c. the control undepleted group and the group lacking CD4+ T cells before DENV showed a
549 trend to higher frequencies compared to the group depleted of CD8+ T cells before ZIKV challenge, and
550 that difference reached significant values between that group and the CD4-/DENV2/CD4+/ZIKV group
551 on day 15 p.c. ($p< 0.039$) (Fig 8A). Despite having similar frequency of pTfh T cells (CD4+/CXCR5+),
552 results demonstrate significance on days 0, 7, and 15 p.c. regarding the frequency of total pTfh TH1
553 CD4+ T (CXCR5+ and CXCR3+) among groups with the group depleted of CD4+ T cells before DENV

554 infection, having significant lower frequencies of those cells since baseline ($p < 0.000$ and $p = 0.0034$)
555 and on day 7 p.c. ($p < 0.000$ and $p = 0.0306$) compared to groups CD4+/DENV2/CD4+/ZIKV and
556 CD4+/DENV2/CD8-/ZIKV, respectively. On day 15 p.c. there was a marked decrease in the frequency
557 of TH1 cells in the CD4+/DENV2/CD8-/ZIKV group compared to the control undepleted group ($p =$
558 0.0009). The difference in frequency was also significant between the control group and the CD4-
559 /DENV2/CD4+/ZIKV group ($p = 0.0012$) (Fig 8B). Further, we wanted to assess the co-expression of B
560 cell co-stimulating markers PD-1 and ICOS in order to correlate these results with B cell activation. The
561 frequency of total CD4+ pTfh T cells CD4+/CXCR5+/CXCR3+/PD-1+/ICOS+ showed a trend to
562 increase from baseline to day 7 p.c. in all three groups. We found same profile as with the TH1 pTfh
563 cells were the CD4-/DENV2/CD4+/ZIKV show statistically significantly lower frequency of these co-
564 stimulating markers at baseline and on days 7 and 15 p.c. compared to groups
565 CD4+/DENV2/CD4+/ZIKV and CD4+/DENV2/CD8-/ZIKV ($p<0.0001$ in all comparisons) (Fig 8C).
566 Gating strategy is shown in Figure 6D.
567 When evaluating the total CD4+ T cell memory phenotype subsets (naïve CD28+CD95-, effector
568 memory (EM) CD28-CD95-, and central memory (CM) CD28+CD95+), CM pTfh CD4+ T cells
569 (CD4+/CD28+/CD95+) remain to be the more abundant phenotype of CD4+ memory T cells. (data not
570 shown). To explore the impact of CD4+ and CD8+ T cell depletion on the frequencies of CM CD4+
571 pTfh T cells (CD4+/CD28+/CD95+/CXCR5+) we measured frozen Peripheral Blood Mononuclear
572 Cells (PBMC) collected on day 0 (baseline), day 7, and day 15 p.c. with ZIKV via flow cytometry
573 analysis (Fig 9A). Overall, there was a non-significant trend to increased frequency in all groups from
574 day 0 to day 7 p.c. followed by a contraction on day 15 p.c. On baseline, the frequency was significantly
575 lower in the CD4-/DENV2/CD4+/ZIKV group compared to the CD4+/DENV2/CD8-/ZIKV group ($p <$
576 0.002). However, there were no differences among groups after the challenge. Results also demonstrate
577 a limited expansion of CM CD4+ pTfh T cell subsets co-expressing CD28+/CD95+/CXCR5+/CXCR3+
578 in all groups on day 7 p.c. with a contraction in the CD4+/DENV2/CD8-/ZIKV group by day 15 p.c..
579 As similarly seen with prior CD4+ pTfh profiles, the CD4+ T cell-deficient group before primary DENV
580 infection result in the lowest frequency since baseline ($p < 0.0001$ and $p = 0.0024$), on day 7 ($p < 0.0001$
581 and $p = 0.0209$), and on day 15 p.c. ($p < 0.0001$ and $p = 0.0008$) compared to groups
582 CD4+/DENV2/CD4+/ZIKV and CD4+/DENV2/CD8-/ZIKV, respectively (Fig 9B). We also measured
583 the CM subpopulation co-expressing the B cell co-stimulating markers PD-1+ and ICOS+
584 (CD28+/CD95+/CXCR5+/CXCR3+/PD1+/ICOS+) (Fig 8C). All three groups showed the same trend

585 as in the other CM population with a very limited expansion on day 7 and a trend to contraction on day
586 15 p.c. in the control undepleted and in the CD4+/DENV2/CD8-/ZIKV groups but not in the CD4-
587 /DENV2/CD4+CD4+/ZIKV, which remains with similar frequency as day 7 p.c.. Interestingly, at
588 baseline the CD4+/DENV2/CD8-/ZIKV group showed a frequency of these cells that is significantly
589 higher compared to the control group ($p< 0.035$). But again, the CD4+ T cell-deficient group before
590 primary DENV infection resulted in the lowest frequency since baseline ($p= 0.0412$ and $p< 0.000$), on
591 day 7 ($p= 0.0046$ and $p< 0.0001$), and on day 15 p.c compared to groups CD4+/DENV2/CD4+/ZIKV
592 and CD4+/DENV2/CD8-/ZIKV respectively (Fig 9C). There were no significant differences among
593 groups on day 15 p.c. This data on the pTfh cells subsets show a detectable, but minimal expansion of
594 the pTfh cells, and its CM compartment, during heterologous ZIKV infection in prior DENV2 immune
595 individuals.
596
597 **Total and DENV primed CM pTfh cells modulate both B cell subset activation and serum antibody**
598 **response functionality towards ZIKV.** To determine if the frequencies of peripheral pTfh cells are
599 directly associated with the humoral response to the secondary infection with ZIKV, we performed
600 Pearson correlation and simple linear regression analysis comparing pTfh cell frequencies with the
601 neutralization assays and the IgG^+ MBC immunophenotyping data. For the IgG^+ MBC analysis, we
602 compared three pTfh cell phenotypes: $CXCR5^+$, $CXCR5^+CXCR3^+$, and $CXCR5^+CXCR3^+PD1+ICOS^+$,
603 at days 7 and 15 p.c., with frequencies of activated IgG^+ MBCs, and activated ASCs, at 7 days p.c..
604 While we did perform correlation analysis comparing the pTfh cell frequencies with frequencies of total
605 IgG^+ MBCs, at 15 days p.c., we did not observe any significant correlation (data not shown).
606 Interestingly, we observed a very low yet significant positive correlation ($R^2= 0.3237$, $P= 0.0214$) when
607 we compared frequencies of $CXCR5^+$ pTfh cells from day 7 p.c.. with total IgG^+ MBC frequencies from
608 day 15 p.c. from all animals in the study (SFig 6A). From these observations, we decided to perform
609 these correlation analyses with the experimental groups separately as we observed that correlations vary
610 significantly depending on both cell phenotype and T-cell depletion treatment. When comparing the
611 $CXCR5^+CXCR3^+$ pTfh cell phenotype, from Day 7 p.c., with the activated IgG^+ MBC phenotype, we
612 observed very high and significant correlations in both the CD4+/DV2/CD4+/ZV and the CD4-
613 /DV2/CD4+/ZV groups ($R^2= 0.8790$, $P= 0.0186$ and $R^2= 0.7424$, $P= 0.0274$, respectively) (SFig 6B and
614 6C). In addition, a very positive and significant correlation with activated IgG^+ MBC and $PD1^+ICOS^+$
615 pTfh cell frequencies was observed in the CD4-/DV2/CD4+/ZV group ($R^2= 0.7028$, $P= 0.0371$) (SFig

616 6D). When comparing the CXCR5$^+$ pTfh cells, from Day 7 p.c., with activated ASCs, we only observed
617 a highly negative and significant correlation in the CD4-/DV2/CD4+/ZV group (R^2= -0.7, P= 0.0378)
618 (SFig 6E). On the other hand, we did not observe a significant correlation when performing this
619 comparison in the CD4+/DV2/CD4+/ZV group. Furthermore, when we compared the frequencies of
620 PD1$^+$ICOS$^+$ pTfh cell, from Day 7 p.c., with activated ASCs we observed a very high and significant
621 positive correlation in the CD4+/DV2/CD4+/ZV group only (R^2= 0.8657, P= 0.0218) (SFig 6F) These
622 observations suggest that not only the B cell response is modulated by possible interactions with these
623 pTfh cell subsets, but also these mechanisms are strongly influenced by the nature of the initial priming
624 with DENV. Also, the opposed correlation (MBC vs. ASC) in the CD4-/DV2/CD4+/ZV group strongly
625 suggest that lack of DENV-experimented pTfh modify the interactions with the MBC and ASC in
626 different ways. As part of this analysis, we also measured possible correlations between these B cell
627 subsets and these same pTfh cell subsets but focusing on CM pTfh cells. Here, we only observed very
628 high and significant positive correlations between the CXCR5$^+$CXCR3$^+$ and PD1$^+$ICOS$^+$ CM pTfh cells,
629 from day 7 p.c., and activated IgG$^+$ MBCs and activated ASCs in the CD4+/DV2/CD4+/ZV group (SFig
630 7). This data adds new insight to the impact of the initial DENV priming in the secondary ZIKV infection
631 response, as CM pTfh cells only show a significant correlation in the expansion of activated B cell
632 subsets in animals with DENV- primed CD4$^+$ T cells. Lastly, the CD4+/DV2/CD8-/ZV group showed
633 no significant correlations between any of the pTfh, and B cell subsets analyzed (data not shown). This
634 could suggest a role or possible interactions of CD8+ T cells with either pTfh cells and/or B cell subsets
635 during the ZIKV infection.
636 To characterize the impact of pTfh cells in the functionality of the antibody response towards the ZIKV
637 infection, we performed Pearson correlation and linear regression analysis comparing the previous pTfh
638 cell phenotypes, during day 7 and 15 p.c.., with the EC50 data (from ZIKV and DENV neutralizations)
639 gathered from days 30 and 90 p.c.. Similar to what we observed in the correlation analysis with IgG$^+$
640 MBCs and ASCs, only the CD4+/DV2/CD4+/ZV group showed highly positive and significant
641 correlations between pTfh cell phenotypes and the ZIKV EC50 values (SFig 8). When comparing
642 frequencies of CXCR5$^+$ pTfh cells, from both days 7 p.c. and 15 p.c., with EC50 values from day 30
643 p.c., we observed a high and significant positive correlation at both timepoints (R^2= 0.8317, p= 0.0309
644 and R^2= 0.9115, p= 0.0115, respectively) (SFig 8A and 8B). In the EC50 from day 90 p.c., we only
645 observed significant positive correlations with the frequencies of PD1$^+$ICOS$^+$ pTfh cells in both day 7
646 and 15 p.c. timepoints (R^2= 0.7796, p= 0.0472 and R^2= 0.9182, p= 0.0102, respectively) (SFig 8C and

647 8D). We did not observe any significant correlations with $CXCR5^+CXCR3^+$ pTfh cells (data not shown).
648 As part of this analysis, correlations were performed with CM pTfh cell subsets as well. Similar to
649 previous results, only the CD4+/DV2/CD4+/ZV group showed significant positive correlations between
650 CM pTfh cell frequencies and ZIKV EC50 values from days 30 and 90 p.c.. Interestingly, for both the
651 $CXCR5^+$ from day 15 p.c. and the $CXCR5^+CXCR3^+$ pTfh cells from days 7 and 15 p.c., a strong positive
652 correlation was observed when compared with EC50 values from day 30 p.c. when comparing all
653 animals in the study (R^2= 0.3282, p= 0.0204, R^2= 0.2863, p= 0.0269, R^2= 0.5100, p= 0.0019
654 respectively) (SFig 8E, 8F and 8G). Similar to previous observations, there were significant positive
655 correlations for $CXCR5^+$ CM pTfh cells from days 7 and 15 p.c., when compared to EC50 values from
656 day 30 p.c. (R^2= 0.9505, p= 0.0470, and R^2= 0.9063, p= 0.0125 respectfully) (SFig 8H and 8I). Lastly,
657 the only positive correlation observed in EC50 values from day 90 p.c. was observed when comparing
658 frequencies of $PD1^+ICOS^+$ CM pTfh cells from day 15 p.c. (R^2= 0.8214, p= 0.0339) (SFig 8J). Taken
659 together, this data adds new insight to the importance of the nature of the initial priming with DENV
660 and its impact on the ZIKV infection, as we also saw strong correlations between CM pTfh cell
661 phenotypes and EC50 values in the CD4+/DV2/CD4+/ZV group only. This suggests that DENV-primed
662 CM pTfh cells may play an important role in modulating an efficient antibody response to ZIKV. In
663 addition, this data adds more weight to the role of $ICOS^+PD1^+$ pTfh cells in priming a robust Abs
664 response with efficient neutralizing capacity towards flavivirus. Interestingly, we observed no
665 significant correlations when we compared total and CM pTfh cells with EC50 values, from day 90 p.c.,
666 that neutralized DENV-2 in any experimental group (data not shown). These results suggest that, while
667 possible DENV-primed CM pTfh cells help to modulate the potency of nAbs to ZIKV, these
668 mechanisms between pTfh cells and the B cell response may be only part of the type-specific humoral
669 response to ZIKV. These results further support a T-cell dependent humoral immune response to ZIKV
670 in DENV-experimented individuals. Very interesting and highly relevant is the fact that mechanistically
671 the CD8+ T cell depletion abrogates the significant correlations between pTfh cell phenotypes and the
672 ZIKV EC50 values in the CD4+/DV2/CD4+/ZV group. This strongly indicates a role of that cell
673 population shaping the nAbs properties.
674
675 **Principal component analysis recapitulates infection dynamics.** To reduce the dimensionality of our
676 data – which included ELISAs, neutralization assays, flow cytometry, and cross reactivity indexes – we
677 performed principal component analysis (PCA). Interestingly, PCA projections of days 15 and 30 p.c.

678 corroborate our assay-by-assay results. More specifically, both principal component projections separate
679 most of the CD4+/DV2/CD4-/ZV and the double depleted groups (CD4-/DV2/CD4-/ZV) samples –
680 most samples have positive values on PC2 on days 15 p.i. (Fig 10A and 10B). Also, most of the samples
681 from the undepleted CD4+/DV2/CD4+/ZV group and from the CD4-/DV2/CD4+/ZV and
682 CD4+/DV2/CD8-/ZV groups having in common the presence of $CD4^+$ T cells before ZIKV infection
683 cluster together – having positive values in PC1 and negative value in PC2 on day 15 p.c. and negative
684 values in PC1 on day 30 p.c.–, which corroborates our results that demonstrates that these groups behave
685 similarly at day 15 p.i.. Of particular interest is the group with CD4+ T cells depleted before primary
686 DENV infection but not before ZIKV challenge. On day 15 p.c., half of samples (n=3) clustered very
687 close with the double-depleted group with a positive value in PC1 similar to the other three samples
688 from same group, but with a positive value in PC2 as the double depleted group.
689 PCA projections of day 90 post ZIKV challenge shows how groups return to baseline, as there is no
690 clear separation between groups (Fig 10C). This is in line with our individual assay-by-assay results that
691 show that, after 90 days of infection, there is no significant difference between groups. Eighty three
692 percent (83%) of the samples from the CD4-/DV2/CD4+/ZV showed negative PC1 clustering with
693 100% of samples from the double depleted group and 66% of samples from the group depleted of CD4+
694 T just before ZIKV infection (CD4+/DV2/CD4-/ZV). Nevertheless, after incorporating the CRI to our
695 PCA matrix there is a dramatic shift with the CD4-/DV2/CD4+/ZV group (83% of samples) clustering
696 together with all samples from the CD4-/DV2/CD4-/ZV double-depleted groups with most having now
697 positive PC1 values of over 0.5 between most of the samples. This way those groups remained separated
698 from the other groups (Fig 10D). These results are in line with our individual assay-by-assay results that
699 show that, in most of the results the double depleted group and the CD4+/DV2/CD4-/ZV trend to have
700 similar results while the CD4-/DV2/CD4+/ZV have similar profile from the two undepleted groups
701 (CD4+/DV2/CD4+/ZV and CD4+/DV2/CD8-/ZV). Such a drastic change in the dimensionality of the
702 data reflects the impact of the CRI and most importantly, this analysis strongly suggest a differential
703 impact of CD4+ T cells on the Abs binding and neutralization properties versus specificity generating
704 immune memory during a primary DENV infection.
705 Overall, PCA projections recapitulated the individual trends in the various analysis performed within
706 our dataset. PCA further provides an excellent resource to corroborate the validity and biological
707 importance of our results.
708

709 **Discussion**

710 Despite many years or research, DENV pathogenies is not completely understood. However, it is
711 recognized that a balanced humoral and T cell immune response is essential to drive protection.
712 The role of $CD4^+$ T cells in DENV pathogenesis evolved over the years. Early data and subsequent
713 works highlighted their role in DENV pathogenesis (Duangchinda et al., 2010; Mangada and Rothman,
714 2005; Rivino et al., 2013; Rouers et al., 2021; Yu et al., 2022). However, more recent works show a
715 relationship between these cells and DENV protection (Graham et al., 2020; Weiskopf et al., 2015b).
716 Also, a contribution of $CD4^+$ T cells in protection against ZIKV has been documented (Cimini et al.,
717 2017; Elong Ngono et al., 2019; Hassert et al., 2018; Lucas et al., 2018). Nevertheless, there are currently
718 limited data available on the relationship between the generation of cross-reactive T cells following
719 vaccination and protection from infection or severe DENV disease (Guy et al., 2008; Tricou et al., 2022;
720 Waickman et al., 2019a) or ZIKV (Rivino and Lim, 2017; Wen and Shresta, 2017). In this work, we
721 found that the presence of $CD4^+$ T cells was essential for optimal control of primary DENV infection
722 and an effective Abs switching, as the depletion of those cells resulted in increased mean viremia days
723 and in a delay in anti-DENV IgM and IgG production. These results suggest a delay in B cells activation
724 and in isotype class switching during primary DENV infection related to the lack of $CD4^+$ T cells. Our
725 findings are in contraposition with a prior work in mice showing that depletion of $CD4^+$ T cells did not
726 have a significant effect on viral clearance and were not required for the induction of DENV2-specific
727 Abs or CD8+ T cell responses, and that the early anti-DENV2 Ab response is $CD4^+$ T cells-independent
728 (Yauch et al., 2010). Nevertheless, in agreement with our results, earlier works also performed in mice
729 showed that depletion of $CD4^+$ and $CD8^+$ T lymphocytes significantly reduced survival of ACS46-
730 primed mice challenged with the DENV JHA1 strain, and that Abs were not required to protect against
731 DENV, suggesting that the cellular immune response targeting NS proteins may be a promising route in
732 vaccine development against DENV (Amorim et al., 2016). In addition to their role interacting with B
733 and $CD8^+$ T cells, $CD4^+$ T cells have also been shown to be protective against encephalitis induced by
734 West Nile Virus (WNV), another flavivirus, in RAG(-/-) and C57BL/6 (H-2(b)) mice (Brien et al., 2008).
735 Again, differences in animal models may account for the radical differences in our results from prior
736 published works.

737 During secondary ZIKV challenge we identified a dynamic and sequential effect of prior DENV
738 immunity and of $CD4^+$ T cells as well. First, all groups showed a displacement of ZIKV peak viremia
739 from day 1 and 2 p.c. to day 3 p.c. compared to naïve groups and second, we identified an early ZIKV

740 viremia resolution in the $CD4^+$ T cells undepleted group. This ZIKV viremia profile confirms an adverse
741 effect of prior DENV immunity in ZIKV replication in comparison to DENV naïve animals as reported
742 by our group before (Perez-Guzman et al., 2019; Serrano-Collazo et al., 2020). In addition to the
743 preexisting DENV immunity, the depletions show a striking two-phase effect. Initially we identified an
744 early significant ZIKV viremia control in the group having the higher significant $CD4^+$ T cell frequency
745 (as a compensatory response to the $CD8^+$ T cell depletion), strongly suggesting an early role of DENV-
746 experimented $CD4^+$ T cells. However, during the mid-viremia period (days 5-7 p.c.) we identified a
747 viremia rebound in this group with a strong trend to have higher AUC values compared to the groups
748 having intact $CD4^+$ and $CD8^+$ T cell components. This change in profile, from an early significant ZIKV
749 viremia control followed by a viremia rebound in this $CD4^+/CD8^-$ group, outlines a role for $CD8^+$ T cells
750 controlling viremia at later time points.
751 The result on the contribution of $CD4^+$ T cells is coherent with prior reports confirming the role of those
752 cells controlling ZIKV viremia in naïve but also in DENV-experimented animals (Wen et al., 2020).
753 Mice depleted of $CD4^+$T cells have more severe neurological sequela and significant increases in viral
754 titers in the central nervous system. When those cells were transferred from ZIKV-immune mice, CD4+
755 T cells protected type I interferon receptor-deficient animals from a lethal challenge, showing that the
756 response by these cells is necessary and sufficient for control of ZIKV disease (Hassert et al., 2018). On
757 another work, depletion of CD4+ T cells in mice reduced plasma cell, GC B cell, and IgG responses to
758 ZIKV without affecting CD8+ T cell response, and those cells were required to protect mice from a
759 lethal dose of ZIKV after infection intravaginally, but not intravenously (Elong Ngono et al., 2019).
760 These results are also in line with our finding that depletion of $CD4^+$ T cells before ZIKV challenge in
761 animals with prior DENV immunity significantly dampened B cell immune response, delaying early
762 IgM and IgG responses and ZIKV neutralization, supporting a protective role of $CD4^+$ T cells against
763 ZIKV mediated by different mechanisms.
764 Also interesting is our finding that depleting $CD8^+$ T cells before ZIKV challenge resulted in a strong
765 trend to have lower viremia, suggesting that $CD8^+$ T cells do not play a crucial role in controlling early
766 ZIKV infection. Prior several works support the role of $CD8^+$ T cells and CR DENV virus-specific $CD8^+$
767 T cells controlling ZIKV replication (Regla-Nava et al., 2018; Rivino and Lim, 2017; Wen et al., 2017;
768 Wen and Shresta, 2017; Yauch et al., 2010). Our finding in the NHP model points out that DENV-
769 experimented $CD8^+$ T cells may have a role controlling ZIKV viremia but at later time points of
770 infection. Our model suggests a sequential contribution of the cellular immune response with $CD4^+$ and

771 $CD8^+$ T cells as early and late players controlling ZIKV viremia in DENV-immune individuals
772 respectively.
773 Results from multiple $CD4^+$ T cell depletions also shed light on the quantity and quality of the immune
774 response in sequential DENV/ZIKV infections in the context of an optimal or suboptimal priming of B
775 cells. It is known that $CD4^+$ T cells can have a dissimilar contribution to the humoral immune response
776 depending on the type of prior flavivirus exposure. A prior T cell receptor repertoire analysis revealed
777 preferential expansion of CR clonotypes between JEV and ZIKV, suggesting that pre-existing immunity
778 against JEV may prime the establishment of stronger $CD4^+$ T cell responses to ZIKV purified-inactivated
779 virus vaccination. These $CD4^+$ T cell responses correlated with titers of ZIKV-nAbs in JEV- pre-
780 vaccinated group, but not in flavivirus-naïve or YFV- pre-vaccinated individuals, suggesting a stronger
781 contribution of $CD4^+$ T cells in the generation of nAbs in the context of JEV-ZIKV cross-reactivity
782 (Lima et al., 2021). This finding, together with our results and from others, emphasize that the efficacy
783 of a DENV or ZIKV vaccine may also depend on the nature of prior or post exposure to flavivirus.
784 The binding or magnitude of neutralization against DENV2 or ZIKV after ZIKV challenge was
785 essentially unaffected by the lack of $CD4^+$ T cells before primary DENV infection. These results indicate
786 that DENV-experimented $CD4^+$ T cells do not contribute to an optimal humoral immune response
787 against ZIKV and support prior reports confirming the specificity of ZIKV-induced humoral immune
788 response in naïve (Collins et al., 2017; Duffy et al., 2009), but also in DENV-pre-exposed, subjects
789 (Andrade et al., 2020). However, depletion before primary infection results in a significant increase in
790 ZIKV-cross reactivity prior ZIKV challenge evidenced by the presence of CR ZIKV-binding IgG. This
791 implies an essential role of $CD4^+$ T cells to guarantee a more specific Abs repertoire after the *primo*
792 infection. That role may contribute to avoid potential disease enhancement during a secondary flavivirus
793 exposure, due to generation of wide CR DENV MBC as consequence of a suboptimal priming. In
794 contrast to the total IgG, no animals had detectable anti-ZIKV-NS1 IgG Abs at baseline, indicating that
795 those Abs are very specific, supporting the efforts to use this protein as a diagnostic marker (Castro-
796 Trujillo et al., 2023; Guzman et al., 2010; Petphong et al., 2023; Vazquez et al., 1995), but also
797 considering the antigen as a target for vaccination. However, removing $CD4^+$ T cells prior to primary
798 infection resulted in a significant increase in DENV anti-NS1 Abs durability. The implications of this
799 finding need further considerations to determine their probable or not pathogenic role, during a
800 subsequent natural exposure or DENV or ZIKV vaccine administration.

801 Despite this, NS1 has also been used as a strategy for protection against TBE (Beicht et al., 2023) and
802 against JEV (Li et al., 2020; Nath et al., 2020; Zhou et al., 2020). Gonçalves et al. nicely showed in mice
803 that the cooperation between $CD4^+$ T cells and the humoral response induce a robust protection against
804 DENV mediated by a DNA-NS1 vaccine (Goncalves et al., 2015). Rhesus macaques is a good model to
805 evaluate the resppnse to NS1 protein as prior work using this specie and same DENV2 strain we used,
806 confirmed that $CD4^+$ T cells from these animals targeted NS1, NS3 and NS5 proteins (Mladinich et al.,
807 2012).
808 Our results confirmed an enhanced circulation of DENV-NS1 antigen after DENV infection and
809 significant lower DENV or ZIKV-specific anti-NS1 IgG response after the primary infection of
810 challenge respectivelly, associated to the lack of $CD4^+$ T cells, confriming a protective role for these
811 cells.
812 Consistent with the Abs response, our data on the MBC and ACS after ZIKV challenge points to a *de*
813 *novo* B cell response pathway that is T cell-dependent to promote the formation of both IgG MBCs and
814 ASC subsets with good neutralizing capacity towards ZIKV. Only the groups with $CD4^+$ T cells before
815 the ZIKV challenge recorded a significant activation of both MBC and ACS, while the depleted groups
816 showed a trend to activation but without reaching significant differences with their baseline frequencies.
817 Previosly, we showed a similar profile ($CD4^+$ T cells-dependent MBC and ACS activation) during
818 tertiary flavivirus infection, but only in the sequence where the ZIKV challenge was preceded by a
819 DENV infection and not when two consecutive DENV infections were secondary and terciary exposures
820 (Marzan-Rivera et al., 2022). Our results are in agreetment with data from humans showing that ZIKV
821 infection in DENV-naïve subjects (or with prior DENV immunity) induces both DENV-MBC and naive
822 B cells with the production of ZIKV type-specific Abs in both cases (Andrade et al., 2020; Rogers et al.,
823 2017). The recall memory against DENV2 detected in our work after the ZIKV challenge was
824 signifincantly higher in the undpelted groups suggesting to be $CD4^+$ T cells-dependent. However more
825 specific studies on the BCR and Abs repertoir diversity, which are being imlemented by our group, are
826 needed. A recent work characterized the plasmablast response in rhesus macaques exposed to primary
827 ZIKV or sequential ZIKV/ZIKV or DENV3/ZIKV infections. Authors reported the highest VH and VL
828 diversity levels upon primary ZIKV infection and lower levels in both homologous and heterologous
829 secondary ZIKV or DENV responses (Singh et al., 2024). In addition to the authors discussion, those
830 results also strongly suggest dissimilar T-B cells interactions in those different sequential flavirus
831 infections.

832 A different scenario compatible with our prior findings on sequential DENV infections (Marzan-Rivera
833 et al., 2022), showing that the pool of cross-reactive policlonal Abs were generated mainly from MBC-
834 derived plasmablasts and not from naïve B cells, was characterized for repeated DENV infections (Xu
835 et al., 2012).
836 Cell phenotyping results were consitent with the correlation analysis we performed. Only the groups
837 that were CD4+ before ZIKV challenge showed a significant positive correlation between some pThf
838 phenotypes and MBCs activation. However only the control undepeleted group showed significant
839 correaltion with the activiation of the ACS. Interesting the group depleted of $CD4^+$ T cells only before
840 ZIKV infection showed a significan inverse correlation with the activation of the ACS. This finding and
841 its relationship with the increased cross-reactivity in this group need further considerations.
842 Interestignly, the group depleted of $CD8^+$ T cells before ZIKV challenge – but with intact $CD4^+$ T cells
843 – did not showed any correlation with the activiation of these cells, suggesting a potential role of $CD8^+$
844 T cells in an effective activation of the MBC and the ACS as it has been discussed before (Rouers et al.,
845 2021). Finally we found that pTfh (CXCR5+) and pTfh with TH1 pehonotype (CXCR5+/CXCR3+), but
846 not the the pTfh expressing the B cells markers activation (PD1+/ICOS+), had a strong correlation with
847 EC50 values against ZIKV (but not DENV). However when we split the results by groups, only the
848 control double positive group kept the significant correlation with the EC50 values, sugesting a
849 contribution of the DENV-pre exposed T helper population in an effective neutralization against ZIKV.
850 This also underscores a potential role for $CD8^+$ T cells in quality of neutralization, a field largely
851 unexplored in flavivirus research. In the context of DENV infection, one study showed how levels of
852 CXCR3+CD8+ T cells had a significant positive correlation with levels of antigen specific MBCs at the
853 post febrile phase after a DENV infection in adult patients in Singapore (Rouers et al., 2021).
854 Because $CD4^+$ pTH cells are essential for the formation of germinal centers and to regulate germinal
855 center B cell differentiation into plasma cells and memory B cells (Crotty, 2015), our correlation results
856 support our other findings suggesting that the humoral immune response to ZIKV in DENV-
857 experimented subject is most likelly T-cell dependent; any disruption or suboptimal T-B cells
858 interactions during a primary DENV infection has consequence in the quality of the immune response
859 to a secondary ZIKV exposure. Vaccination of naive HLA-DRB1*0101 transgenic mice with
860 DENV/ZIKV-cross-reactive $CD4^+$ T cell epitopes induced a $CD4^+$ T cell response sufficient to reduce
861 tissue viral burden following ZIKV infection suggesting that those cells expressing a TH1 pehnotype
862 (as we characterized in our model) can suppress ZIKV replication, even in a Abs-independient manner

863 (Wen et al., 2020). A recent work on the response to the TV003 vaccine in phase II clinical trial revealed
864 that sero-positive subjects have increased baseline/pre-vaccination frequencies of circulating T follicular
865 helper (cTfh) cells and, more important and inline we our findings, they found that the baseline/pre-
866 vaccination cTfh profile correlated with the vaccines ability to launch neutralizing antibody response
867 against all four DENV serotypes (Izmirly et al., 2022).
868 The findings on the correlation between the pTfh with the MBC and the magnitude of neutralization
869 form our work have implications for vacines design. Lack of inducing an effective and specific $CD4^+$ T
870 cell (and most likely pTfh cells) responses might thus impair vaccine performance. Our results add on
871 the mechanisms guiding the immune response during heterologous flavivrus infection and may
872 contribute to rational vaccine development efforts.
873 One key finding from our work is the increased cross-reactivity to both DENV-EDIII domain and NS1
874 (but not to similar ZIKV regions) in absence of $CD4^+$ T cells before the primary DENV infection but
875 not before the ZIKV challenge. The fact that the cross reactivity was identified against both the structural
876 EDIII domain and the NS1 protein agrees with the finding that $CD4^+$ T cell epitopes in humans are
877 skewed toward recognition of viral components that are also targeted by B lymphocytes and, from here,
878 guiding the production of Abs mainly against the structural proteins (Rivino et al., 2013) or NS1 protein
879 having also T and B cells epitopes (Aquino et al., 2023; Beicht et al., 2023; Perera et al., 2024; Shu et
880 al., 2000). We do not completely understand why the cross-reactivity was higher against the NS1 protein
881 than to the EDIII domain, but the factors responsible for directing $CD4^+$ T cell to similar sites at the
882 virus protein surface (or to NS1) in different flaviviruses are unknown (Aberle et al., 2018). However,
883 they are most likely related to the structural context of these epitopes and their accessibility during
884 antigen processing (Koblischke et al., 2017; Koblischke et al., 2018; Landry et al., 2017; Schwaiger et
885 al., 2014). The involvement of NS1 can be explained as 50% or more of the MBC repertoire represented
886 in mAbs from DENV patients bind to NS1, NS3, prM, or capsid in addition to the ENV (Beltramello et
887 al., 2010; de Alwis et al., 2011). A prior work showed that half of anti-NS1 mAbs had limited cross
888 reactivity among DENV, while most of the structural Abs showed full cross reactivity against all virus
889 serotypes (Dejnirattisai et al., 2010). Supported in that data, the increased cross-reactivity against NS1
890 we found, reinforce the critical contribution of $CD4^+$ T cells during a primary infection to the Abs
891 specificity during a subsequent flavivirus exposure.
892 Strikingly, that increase in cross-reactivity was not accompanied by a detriment in Abs binding or DENV
893 neutralization after the challenge, indicating that $CD4^+$ T cells were not essential for those properties,

894 but they were required for the Abs specificity. Interestingly, while $CD8^+$ T cell depletion increased $CD4^+$
895 T cell frequency, it had no effect on the specificity of epitope recognition, supporting that our finding is
896 related to the quality of the priming. This suggest that the specificity of DENV Abs needs to be secured
897 during a *primo* infection with an optimal B cell priming and may be regulated through a different
898 mechanism than the binding and neutralization functions. After many years of extensive research, the
899 mechanism regulating the Abs somatic hypermutation and affinity maturation are not well understood
900 (Elsner and Shlomchik, 2020; Stebegg et al., 2018; Victora and Nussenzweig, 2012). How this process
901 occurs in flavivirus is also largely unknown. However, we do know that high affinity Abs with higher
902 SHM rate are generated in the GCs (Fink, 2012; Fink et al., 2007; Godoy-Lozano et al., 2016; Wong et
903 al., 2020) where the encounter with the antigen-presenting cells, including $CD4^+$ T cells) occurs. Our
904 results agree and provide, for the first time, mechanistic evidence to prior findings where a population
905 of broadly cross-neutralizing Abs in addition to the type specific neutralizing Abs was identified after
906 secondary DENV infections (Patel et al., 2017).
907 Currently, our group is performing Ab depletion to characterize the TS vs. CR Abs and ADE experiment
908 to test the potential enhancement of these cross-reactive Abs. We understand that results from these
909 experiment will add instrumental information in support of our results. We also acknowledge that we
910 used samples from day 90 p.c. where cross-reactive Abs start to decline but are still present (Katzelnick
911 and Harris, 2017; Patel et al., 2017; Wahala and Silva, 2011). However, this will not change the fact that,
912 in our experimental conditions, the Abs population after ZIKV challenge, in DENV-experimented
913 individuals with a suboptimal T-B cell interaction, behave differently in their binding and neutralization
914 properties vs. their specificity.
915 Also, experiments reproducing the sequence of infections ZIKV/DENV2 are underway, and exploring
916 DENV/DENV and ZIKV followed by different DENV serotypes is in the pipeline.
917 Before, it has been highlighted that the risk of immunopathology associated with sub-optimal cross-
918 reactive B-cell and T-cell responses to heterologous serotypes represent critical factors for the
919 development of a fully protective DENV vaccine (Rivino and Lim, 2017). Our work adds on the
920 relevance and uniqueness of the contribution of $CD4^+$ T cells to the specificity of the neutralizing Abs
921 to increase vaccines efficacy and to potentially prevent or decrease the risk of pathogenic manifestations
922 mediated by ADE or similar mechanisms.
923 Finally, and not less relevant, our work reinforces the value of RMs as a reliable model with a strong
924 translational capability and a needed complement to the results obtained with other animal models.

925 One limitation to advance our knowledge on the correlates of protection towards DENV is the lack of
926 an adequate animal model to reproduce DENV clinical manifestations. Despite the proven value of the
927 small animal models, most of the studies performing a fine dissection of the mechanisms addressing the
928 protection against DENV and ZIKV use transgenic mice deficient in some essential anti-viral pathways
929 (Yauch and Shresta, 2008; Zompi and Harris, 2012). The genetic modifications in murine models prevent
930 to recognize the contribution of all components of the immune response as a whole and from here, the
931 translation to humans may be narrow. In addition, it is known that the T cell immune response to
932 flavivirus in humans is restricted by the HLA system (Angelo et al., 2017; Sierra et al., 2007; Weiskopf
933 et al., 2016; Weiskopf et al., 2011; Wen et al., 2010; Zivna et al., 2002).
934 The value of rhesus macaues as NHPs to study the pathogenesis and immune response after natural
935 infection, or vaccination, to DENV (Mladinich et al., 2012; Onlamoon et al., 2010; Osorio et al., 2014;
936 Sariol et al., 2011; Sariol et al., 2007; Sariol and White, 2014; White et al., 2021; Young et al., 2023)
937 or ZIKV (Abbink et al., 2017; Aliota et al., 2016; Best et al., 2017; Breitbach et al., 2019; Carroll et al.,
938 2017; Coffey et al., 2018; Coffey et al., 2017; Dudley et al., 2019; Dudley et al., 2016; Haddow et al.,
939 2017) is well documented. Also, our group and others have shown the usefulness of this model to dissect
940 many aspects of DENV and ZIKV interactions (Crooks et al., 2021; George et al., 2017; Larocca et al.,
941 2021; Marzan-Rivera et al., 2022; McCracken et al., 2017; Pantoja et al., 2017; Perez-Guzman et al.,
942 2019; Serrano-Collazo et al., 2020; Valiant et al., 2018; Valiant et al., 2019). RMs have also shown to
943 be very reliable to anticipate vaccine outcomes (Borges et al., 2019). Early immunization studies of
944 RMs with Dengvaxia, either with the tetravalent formulation CYD-TDV (CYD1–4) or with two bivalent
945 formulations (CYD1,2 + CYD3,4), resulted in higher responses against the dominant DENV4 and
946 DENV1, and a very limited response to DENV2 serotypes in both approaches (Guy et al., 2009). Later
947 on, results from humans showed a vaccine efficacy of 55.6%, 75.3% and 100.0% for serotypes 1, 3 and
948 4 respectively. The efficacy for serotype 2 was as low as 9.2 % resulting in an overall efficacy of 30.2%
949 for all serotypes (Sabchareon et al., 2012). RM studies with DENVax (formulation used for TAK-003
950 vaccine) likewise anticipated the findings from the clinical trials. Different DENVax formulations failed
951 to generate adequate responses against DENV4 and stimulated lower levels of nAbs to DENV 1, 3 and
952 4 but higher levels to DENV2 (Osorio et al., 2011a).
953 Overall, our findings have enormous impact for the planning and evaluation of DENV and ZIKV vaccine
954 schedules, design, and monitoring, strongly suggesting the consideration of CD4+ T cell epitopes for
955 the composition of new generation flavivirus vaccines.

Acknowledgments

This work would have not been possible without the dedication and commitment of the Caribbean Primate Research Center and the Animal Resources Center staff. We thank Dr. Daniela Weiskopf for her valuable comments, Dr. Stephanie Dorta for her guidance in cell phenotyping and Dr. Espino for her support with the correlation analysis. The authors also recognize the excellent technical support provided by Dr. Nicole Marzán, Jorge Sánchez and Rafael Ocasio. Funding: This work was supported by R01AI148264 (NIAID) to C.A.S. and by P40 OD012217 to C.A.S. and M.I.M. (ORIP, OD, NIH), and partially by 5-U24-AI152170-04 to A.M.d.S. and R25GM061838 to A.M.-S.

Author contributions

C.A.S., A.M.d.S, C.S.-C., A.M.-S. and L.C. developed the experimental design. C.R., M.I.M. and A.G.B. supervised and performed sample collection and animal monitoring. C.S.-C., A.M.-S., L.C., S.H. and L.A. performed the experiments. C.A.S., C.S.-C, A.M.-S., L.C. and M.S.-R. analyzed the data. C.A.S., C.S.-C, A.M.-S. and L.C drafted the article. C.A.S., C.S.-C., A.M.-S, L.C., S.H., M.S.-R., L.A., T.A., M.I.M., C.R., A.G.B. and A.M.d.S. reviewed and corrected the last version.

Declaration of interests

The authors declare no competing interest.

986 **Materials and Methods**

987 **Experimental model.** Young adult (4–7 years of age) male rhesus macaques (*Macaca mulatta*),
988 seronegative for DENV and ZIKV, were housed in the CPRC facilities at the University of Puerto Rico,
989 San Juan, Puerto Rico. All procedures were reviewed and approved by the Institute's Animal Care and
990 Use Committee at the Medical Sciences Campus, University of Puerto Rico (IACUC-UPR-MSC) and
991 performed in a facility accredited by the Association for Assessment and Accreditation of Laboratory
992 Animal Care (AAALAC) (Animal Welfare Assurance number A3421; protocol number, 7890116). In
993 addition, the study was conducted following the USDA Animal Welfare Regulations, the Guide for the
994 Care and Use of Laboratory Animals, and institutional policies, to ameliorate animal suffering by the
995 recommendations of the Weatherall Report on the use of nonhuman primates in research. All procedures
996 were conducted under anesthesia by intramuscular injection of ketamine at 10–20 mg/kg of body weight,
997 as approved by the IACUC. Anesthesia was delivered in the caudal thigh using a 23-gauge sterile syringe
998 needle. During the period of the entire study, the macaques were under the environmental enrichment
999 program of the facility, also approved by the IACUC. Animals were continuously monitored by trained
1000 veterinarians at the Animal Research Center.

1001

1002 **Viral Stock.** DENV-2 New Guinea 44 (NGC) strain (provided by Steve Whitehead, NIH/NIAID,
1003 Bethesda, MD) and ZIKV PRVABC59 (ATCC VR-1843) were used to infect macaques at different
1004 timepoints. Viruses were expanded and tittered by plaque assay and qRT-PCR using protocols
1005 standardized in our laboratories. In addition, both viruses were used for neutralization assays.

1006

1007 **Depletion treatment, viral infection, and sample collection.** A total of 29 rhesus macaques
1008 seronegative for DENV and ZIKV were housed in the CPRC facilities, at the University of Puerto Rico,
1009 San Juan, Puerto Rico. During the first year, macaques were divided into two cohorts based on their
1010 depletion status. Twelve (12) animals were depleted of CD4+ T cells by administration of anti-CD4 Ab
1011 CD4R1 (NHP Reagent Resource; https://www.nhpreagents.org). Administration of the depletion
1012 treatment consisted of an initial s.c. inoculation of 50 mg/kg at 15 days prior to viral infection, followed
1013 by two i.v. injections of 7.5 mg/kg 12 days and 9 days before viral infection respectively. The seventeen
1014 (17) remaining animals received phosphate-buffered saline (PBS) as a control. Both cohorts were then
1015 infected with DENV-2 NGC strain (5.0×10^5 pfu s.c.), which is denoted from here on as the primary
1016 infection. Following one year after infection, animals were further divided into five cohorts based on

1017 depletion status. Animals were depleted of CD4+ or CD8+ T cells, or treated with PBS, followed by a
1018 secondary infection with ZIKV (1×10^6 pfu s.c.). CD8+ T cell depletion was performed by
1019 administration of anti-CD8 alpha MT807R1 at the same dosage as CD4+ T cells depletion treatment.
1020 CD4+ T cell depletion was performed on groups 1 and 2 (group 1: n=6, denoted CD4-/DV2/CD4-/ZV;
1021 group 2: n=6, denoted CD4+/DV2/CD4-/ZV). Group 3 was depleted of CD8+ T cells (n=6, denoted
1022 CD4+/DV2/CD8-/ZV). Lastly, the remaining two groups were administered PBS (group 4: n=6, denoted
1023 CD4-/DV2/CD4+/ZV; group 5: n=5, denoted CD4+/DV2/CD4+/ZV).
1024 Blood samples were collected on days $^{-}15$, $^{-}12$ and $^{-}9$ pre-infection, and during the first fifteen
1025 consecutive days after infection, followed by a monthly sample collection from there onwards until a
1026 year later when sample collection pattern described is repeated for the secondary infection. Collected
1027 samples included serum, heparinized whole blood, and citrate whole blood for PBMCs isolation.
1028 Macaques were monitored after treatments and infections by trained veterinarians for evidence of
1029 disease and clinical status. Rectal and external temperatures and weights were taken on day 0 and every
1030 other day pre- and post-infection. A summary for the experimental design and groups is provided (SFig
1031 1).
1032
1033 **Immunophenotyping.** Phenotypic characterization of Rhesus macaque's adaptive immune response
1034 was performed by 16- multicolor flow cytometry using fluorochrome-conjugated Abs at several time
1035 points. Depletion efficacy was confirmed by measuring the frequency of $CD4^+$, $CD8^+$ T cells, and $CD20^+$
1036 B cells on the following timepoints: pre-treatment/depletion baseline, and days 2, 5, and 9 post-
1037 treatment. In addition, B and T cell frequencies were measured on baseline and days 1, 2, 3, 7, 15, 30,
1038 60 and 90 p.i. and p.c.. Aliquots of 150 uL of heparinized whole blood were incubated with a mix of
1039 antibodies for 30 min. in the dark at room temperature. After incubation, red blood cells are fixed and
1040 lysed with BD FACS fix and lyse solution, and cells were washed twice with BSA 0.05%. Samples were
1041 analyzed using a MACSQuant® Analyzer 16 Flow Cytometer (Miltenyi Biotec, CA). Antibodies used
1042 in this study were: CD8-FITC (DK25) from Sigma; CD3- PerCP (SP34), Ki67- Viogreen (B56), CD4-
1043 APC (L200), CD69- PeCy7 (FN50), CD95- PE (DX2), IgG- APC- H7 (G18- 145), CD11b- AF 700
1044 (ICRF44), CD80- BV650 (L307.4), CD27- PE (M-T466) from BD Biosciences; CD20- PacBlue (2H7),
1045 HLA-DR- BV570 (L243), CD138- FITC (DL- 101), ICOS- PE/Dazzle 594 (C398.4H), CXCR3- PECy7
1046 (GO25H7), PD-1- BV650 (EH12.2H7) from Biolegend; CD28- APC- Vio770 (I5E8) and CCR7- PerCP

1047 Vio770 (REA546) from Miltenyi; CXCR5- PE (MU5UBEE) from Thermo eBiosciences; and CD38-
1048 APC (AT-1) from Adipogen.
1049 For analysis, lymphocytes (LYM) were gated based on their characteristic forward and side scatter
1050 patterns. B cells were defined as $CD20^{+}CD3^{-}$, Memory (MBC= $CD20^{+}$, $CD3^{-}$, $CD27^{+}$), class-switched
1051 IgG Memory (IgG MBC= $CD20^{+}$, $CD3^{-}$, $CD27^{+}$, surface IgG+ (sIgG), plasmablasts as (PB= $CD20^{-/low}$,
1052 $CD3^{-}$, $CD11b^{-}$, HLA-DR^{+}, $CD80^{+}$) as described by Silveira et al., 2015 (Silveira et al., 2015), plasma
1053 cells as (PC= $CD20^{-/low}$, $CD3^{-}$, $CD138^{+}$) as described by Martinez- Mirullo et al., 2016 (Martinez-
1054 Murillo et al., 2016). Gating strategy for B cells and subsets is presented on Supplementary Figure 9
1055 (SFig 9). T cells were defined as $CD3^{+}CD20^{-}$, $CD4^{+}$ T cells ($CD3^{+}CD20^{-}CD4^{+}$), $CD8^{+}$ T cells
1056 ($CD3^{+}CD20^{-}CD8^{+}$), and subpopulations were defined inside the $CD3^{+}$ T cells. Naïve ($CD3^{+}CD20^{-}$
1057 $CD28^{+}CD95^{-}$), EM ($CD3^{+}CD20^{-}CD28^{-}CD95^{+}$), and CM ($CD3^{+}CD20^{-}CD28^{+}CD95^{+}$) T cell
1058 subpopulations were determined within $CD4^{+}$ and $CD8^{+}$ T cells. Gating strategy for T cells and memory
1059 subsets is presented on Supplementary Figure 10 (SFig 10), while gating strategy for the characterization
1060 of $CD4^{+}$ pTfh cells was represented by selecting CM and EM T cells ($CD4^{+}$/$CXCR5^{+}$/$CXCR3^{+}$)
1061 expressing activation and migration markers such as ($CCR7^{+}$/PD-1^{+}/$ICOS^{+}$) (SFig 11). All data from
1062 cell population frequencies and immune markers expression were analyzed using Flow/Jo software and
1063 GraphPad Prism (FlowJo LLC Ashland, OR).
1064
1065 **qRT- PCR.** DENV-2 and ZIKV viral RNA for real-time PCR assay was extracted from 200uL of virus
1066 isolate (previously tittered by focus reduction neutralization assay) and from acute serum samples using
1067 a MagMax Viral/Pathogen Nucleic Acid Isolation Kit (Applied Biosystems, MA) as per the
1068 manufacturer's instructions. Viral RNA extraction was performed using a KingFisher Duo Prime
1069 Purification System (Thermo Fisher Scientific, MA). In brief, a Viral Pathogen DNA/RNA binding
1070 magnetic bead solution is prepared, 200uL of sera, 500uL of Viral Pathogen Nucleic Acid isolation kit
1071 wash buffer, 5uL of a protein kinase solution, and 1mL of ethanol solution (80% ethanol in DNAse free
1072 water) were added to specified wells in a KF Duo Deepwell 96 plate (Thermo Fisher Scientific, MA)
1073 and loaded on the KingFisher Duo Prime Purification System. Purified RNA was collected and stored
1074 at -80°C in kit elution buffer solution. Real-time PCR for DENV-2 and ZIKV isolated RNA was carried
1075 out using the Gensig Real-Time PCR detection kit for Dengue Virus and the Gensig Real-Time PCR
1076 detection kit for Zika Virus (Primer Design, UK), respectively, as per the manufacturer's instructions.
1077 Briefly, a kit Positive Control is diluted in 500uL of a kit Template buffer and a series of tenfold dilutions

1078 were prepared as follows: 2 x 10^5/uL, 2 x 10^4/uL, 2 x 10^3/uL, 2 x 10^2/uL, 20/uL, 2/uL (copy number/
1079 mL). For the reaction mix, 10uL of oasig 2X Precision one step qRT-PCR was combined with 1uL of
1080 kit probe/primer mix and 4uL of sterile water, for a total volume of 20uL once the RNA sample was
1081 added. The reaction mix was added to a 96 well plate, alongside the experimental RNA samples, a kit
1082 non-template control (NTC), an RNA extraction negative control (DNAse free water), a positive control
1083 (RNA extraction from virus stock), and calibration standards, in specified wells. The plate was then
1084 loaded, and the RT-PCR reaction was performed using a Quant Studio 5 Real-Time PCR Instrument
1085 (Applied Biosystems, MA). For quantification, a standard curve was generated from the ten-fold
1086 dilutions of the kit RNA positive control.
1087
1088 **ELISAs for DENV and ZIKV.** Serostatus of animals for DENV and ZIKV was assessed before and
1089 after DENV-2 infection using DENV IgG/IgM commercial kits (Focus Diagnostics, CA). ZIKV
1090 serostatus was also assessed using a commercial kit for ZIKV IgM (InBios, International Inc, Seattle)
1091 and ZIKV IgG (XpressBio, Frederick, MD). Timepoints measured include baseline and days 7, 10, 15,
1092 30, 60, and 90 post-infection. All assays were performed per the manufacturer's instructions and as
1093 described by our group (Pantoja et al., 2017; Perez-Guzman et al., 2019; Serrano-Collazo et al., 2020).
1094
1095 **ELISAs for detection of DENV NS1 antigen, DENV anti-NS1 IgG and ZIKV anti-NS1 IgG**.
1096 Detection of DENV NS1 antigen after primary DENV-2 infection, ZIKV anti-NS1 IgG after secondary
1097 ZIKV infection, and DENV anti-NS1 IgG after both primary and secondary infections were measured
1098 using commercial kits (InBios, International Inc, Seattle). The assays were performed per the
1099 manufacturer's instructions. Timepoints measured include baseline and days 3, 5, 7, 15 and 30 p.i. for
1100 DENV NS1, and baseline and days 15, 30 and 90 p.c. for ZIKV and DENV anti NS1 IgG.
1101
1102 **DENV neutralization assays.** For the Focus Reduction Neutralization Test (FRNT) for DENV, Vero81
1103 cells (ATCC CCL-81) were seeded at approx. 2.0 x 10^5 per well in 24 well-plates with DMEM
1104 (Dulbecco's Modified Eagle's medium, Thermo Fisher Scientific) for approx. 18 hours. Serum dilutions
1105 (ten-fold) were prepared in diluent medium Opti-MEM (Invitrogen) with 2% FBS (Gibco) and 1%
1106 antibiotic/antimycotic (Hyclone). Virus was added to each dilution and incubated for 1 hour at 37ºC/5%.
1107 Before inoculation, the growth medium was removed, and cells were inoculated with 50 uL per well of
1108 serum-virus dilution in triplicates; plates were incubated for 1 hour at 37ºC/5%CO_2/rocking. After

1109 incubation, 125 uL per well of overlay (Opti-MEM 1% carboxymethylcellulose (Sigma), 2% FBS, 1%
1110 non-essential amino acids (Gibco) and 1% antibiotic/antimycotic (HyClone)) was added to the plates
1111 containing virus dilutions, followed by an incubation period of 48 hours at 37ºC/5%CO_2. After two days,
1112 overlay was removed with PBS and fixed with 4% paraformaldehyde for 30 minutes. Plates were
1113 blocked with 5% non-fat dairy milk in 1X perm buffer (BD Cytofix/Cytoperm) for 10 min and incubated
1114 for 1hr/37ºC/5%CO2/rocking with anti-E mAb 4G2 and anti-prM mAb 2H2 (kindly provided by Dr.
1115 Aravinda de Silva and Ralph Baric, University of North Carolina Chapel Hill, NC, USA), both diluted
1116 1:100 in blocking buffer. Plates were washed three times with PBS and incubated
1117 1hr/37ºC/5%CO_2/rocking with horseradish peroxidase (HRP)-conjugated goat anti-mouse antibody
1118 (Sigma), diluted 1:1,500 in blocking buffer. Foci were developed with TrueBlue HRP substrate (KPL)
1119 and counted using an Elispot reader. Results were reported as FRNT with a 60% or greater reduction in
1120 DENV foci (FRNT60). A positive neutralization titer was designated as 1:20 or greater, while <1:20
1121 was considered a negative neutralization titer.
1122
1123 **ZIKV neutralization assays.** For the Plaque Reduction Neutralization Test (PRNT) for ZIKV, Vero81
1124 cells (ATCC CCL-81) were seeded at approx. 2.0 x 10^5 per well in 24 well-plates with DMEM
1125 (Dulbecco's Modified Eagle's medium, Thermo Fisher Scientific) for approx. 18 hours. Serum dilutions
1126 (ten-fold) were prepared in a diluent medium (Opti-MEM (Invitrogen) with 2% FBS (Gibco) and 1%
1127 antibiotic/antimycotic (Hyclone). Virus was added to each dilution and incubated for 1 hour at
1128 37ºC/5%/CO2. Before inoculation, the growth medium was removed, and cells were inoculated with
1129 100 uL per well of each dilution in triplicates; plates were incubated for 1 hour at 37ºC/5%CO2/rocking.
1130 After incubation, 1mL per well of overlay (Opti-MEM 1% carboxymethylcellulose (Sigma), 2% FBS,
1131 1% non-essential amino acids (Gibco), and 1% antibiotic/antimycotic (HyClone)) was added to the
1132 plates containing virus dilutions, followed by an incubation period of 4 days at 37ºC/5% CO2. After
1133 four days of incubation at 37ºC/5%CO2, the overlay was removed; the cells were washed twice with
1134 phosphate-buffered saline (PBS), fixed in 80% methanol, and stained with crystal violet and foci were
1135 counted. The mean focus diameter was calculated from approx. twenty foci per clone were measured at
1136 35 magnifications. Results were reported as the PRNT with a 60% or greater reduction in ZIKV or
1137 DENV plaques (PRNT60). A positive neutralization titer was designated as 1:20 or greater, while < 1:20
1138 was considered a negative neutralization titer.
1139

1140 **EDIII and NS1 Antigen Production.** ZIKV (H/PF/2013) EDIII antigen used in this study was produced
1141 as previously described (Adams et al., 2021). EDIIIs of DENVs were also cloned and expressed similarly
1142 to ZIKV EDIII. Briefly, codon-optimized genes encoding the EDIIIs of DENV1 (AAB70694·1, E
1143 protein aa 297-394), DENV2 (ADA00411·1, E protein aa 297-394), DENV3 (AAB69126·2, E protein
1144 aa 295-392) and DENV4 (ADA00410·1, E protein aa 297-394) with an N-terminal human serum
1145 albumin signal peptide, a polyhistidine tag, and a HaloTag were cloned into the mammalian expression
1146 plasmid pαH. Recombinant DENV EDIII antigens were expressed in mammalian Expi293 cells and
1147 purified from the culture supernatant using Ni-NTA agarose (Qiagen). Purified EDIII antigens were
1148 biotinylated using HaloTag PEG biotin ligand (Promega), according to the manufacturer's protocol. For
1149 NS1 antigen production, codon-optimized genes encoding the NS1s of DV1 NS1 Region 37, DV2 NS1
1150 Region 39, DV3 NS1 Region22, DV4 NS1 Region 45 and ZKVNS1 Region 47 were used. Mobility-
1151 shift analysis was used to assess the biotinylated EDIII antigens using SDS-PAGE. The mammalian
1152 expression plasmids and sequences will be made available in the plasmid repository and Genbank,
1153 respectively.
1154
1155 **Coupling antigens to beads via avidin-biotin interaction:** Site-specifically biotinylated EDIII
1156 antigens were coupled to unique MagPlex®-Avidin Microspheres (DENV1 (region 12), DENV2 (region
1157 26), DENV3 (region 36), DENV-4 (region 38) and ZIKV (region 30)) at a concentration of 5 µg of
1158 antigen per 106 beads in phosphate-buffered saline, pH 7.4 (PBS, 137 mM NaCl, 2.7 mM KCl, 8 mM
1159 Na2HPO4, and 2 mM KH2PO4) for 1 hour at 37°C with shaking at 700 rpm. Beads were then
1160 washed/blocked three times in block buffer (PBS + 1% BSA) and resuspended in wash buffer at 2x106
1161 beads/mL.
1162
1163 **EDIII and NS1 multiplex Assay:** DENV and ZIKV EDIII coupled beads were combined in equal ratios
1164 by plating 50 µL containing 2,500 beads per antigen into each well of a 96-well assay plate (Cat.
1165 No:655906). Human serum samples were diluted 1:500 in block buffer. Mouse monoclonal antibodies
1166 specific for each EDIII antigen (ZV-67 (ZIKV), E103 (DENV1), 3H5 (DENV2, 8A1 (DENV3), E88
1167 (DENV4)) and NS1 antigen were titrated in each assay as controls for assay consistency across
1168 couplings, plates, and assay days. A magnetic plate separator was used to remove the block buffer from
1169 the assay plate, and 50 µL of diluted serum or mAb was added to the beads. The serum and bead mixture
1170 were incubated for 1 hour at 37°C with shaking at 700 rpm. Beads were washed thrice200 µL block

1171 buffer per well using a magnetic separator. 50 µl of Goat anti-Human IgG Fc Multi-species SP ads-PE
1172 (Southern Biotech, catalog:2014-09) and Goat anti-Mouse IgG Fc Human ads-PE (Southern Biotech,
1173 catalog:1030-09) were added to the beads (6 µg/ml in block buffer) and incubated for 1 hour at 37°C
1174 with shaking at 700 rpm to detect respective antibody species. Beads were washed thrice with 200 µL
1175 block buffer per well using a magnetic separator and resuspended in 100 µL of block buffer before
1176 acquiring data. Fluorescence data were obtained using a Luminex 200 analyzer set to acquire at least 50
1177 beads per bead region.
1178
1179 **Principal Component Analysis (PCA).** All PCA analyses were performed using R statistical software
1180 R version 4.2.3 using the *prcomp* function of the *stats* package (Team et al., 2018; Team, 2010). To
1181 examine global patterns of infection, we carried out a PCA using all the parameters measured during the
1182 infection timeline; these analyses were performed for day 15, day 30 and day 90 of infection. The
1183 parameters used to perform PCA are depicted in Supplementary Table 3 (Table S3).
1184
1185 **Quantification and statistical analysis.** Statistical analyses were performed using GraphPad Prism 9.0
1186 software (GraphPad Software, San Diego, CA, USA). For viral burden analysis, the log titers and levels
1187 of vRNA were analyzed by multiple unpaired t-tests and two-way ANOVA. The statistical significance
1188 between or within groups evaluated at different time points was determined using one-way or two-way
1189 analysis of variance (ANOVA) (Tukey's, Sidak's, or Dunnett's multiple comparisons test) or unpaired
1190 t-test to compare the means. Significant multiplicity-adjusted p-values (*< 0.05, **< 0.01, ***< 0.001,
1191 ****< 0.0001) show statistically significant differences between groups (Tukey test) or time points
1192 within a group (Dunnett test).
1193

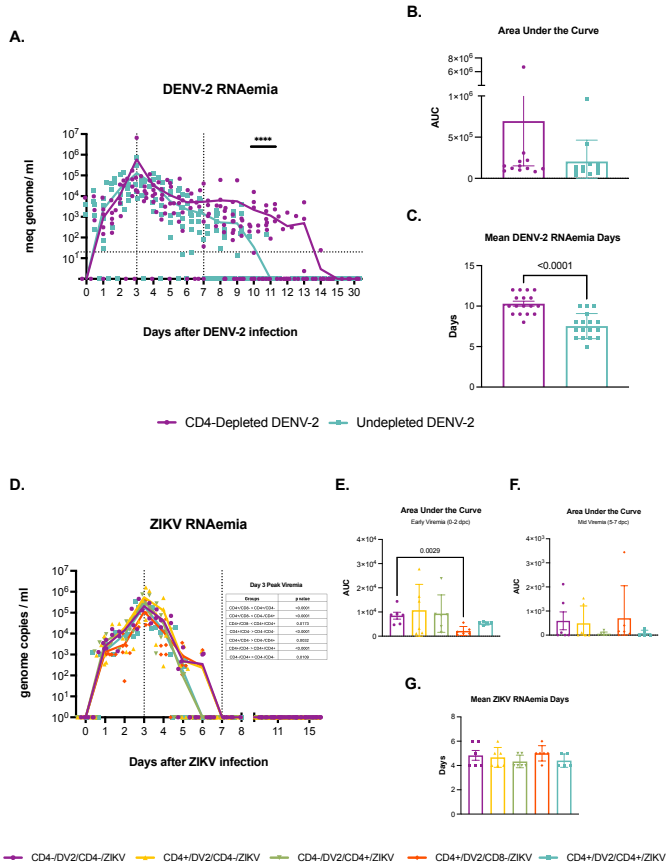
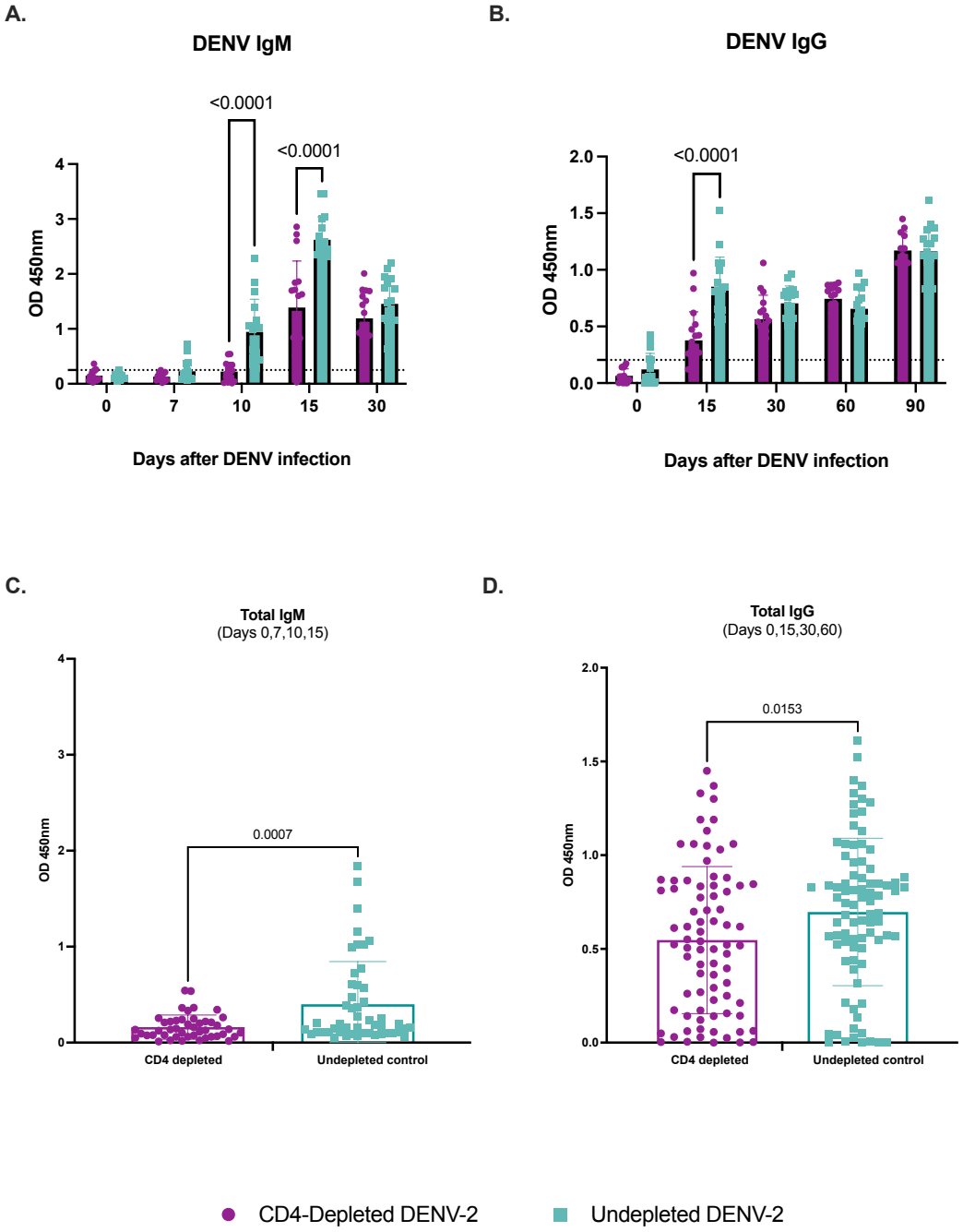


Fig 1. DENV2 and ZIKV RNA kinetics in flavivirus-naïve CD4-depleted and undepleted animals. RNAemia is affected by depletion of CD4+ or CD8+ T cells. **Upper panel**. CD4-depleted animals are depicted in purple and undepleted animals are depicted in turquoise. **(A)** DENV-2 genome copies/mL were measured in serum to monitor viral replication during the first 15 days and day 30 after infection. Genome copies per mL are shown logarithmically. DENV RNAemia was defined as early RNAemia (days 0 to 3 p.i.), mid RNAemia (days 4 to 7 p.i.) and late RNAemia (day 7 p.i. onwards). **(B)** The area under the curve (AUC) was calculated for individual values. **(C)** Average RNAemia days were calculated using the following formula: total viremia days divided by the total number of days during which viremia was monitored. **Lower panel**. Animal cohorts are depicted as follows based on treatment regime: CD4+/DV2/CD4+/ZV (shown in turquoise), CD4-/DV2/CD4+/ZV (shown in green), CD4+/DV2/CD4-/ZV (shown in yellow), CD4-/DV2/CD4-/ZV (shown in purple), and CD4+/DV2/CD8-/ZV (shown in red). **(D)** ZIKV genome copies/mL were measured in serum to monitor viral replication during the first 15 days and day 30 after infection. Genome copies per mL are shown logarithmically. ZIKV RNAemia was defined as early RNAemia (days 0 to 3 p.i.), mid RNAemia (days 4 to 7 p.i.) and late RNAemia (day 7 p.i. onwards). **(E)** The area under the curve (AUC) was calculated for individual values on days 0, 1 and 2 post ZIKV challenge, denominated early viremia. **(F)** The area under the curve (AUC) was calculated for individual values on days 5, 6 and 7 post ZIKV challenge, denominated mid viremia **(G)** Average RNAemia days were calculated using the following formula: total viremia days divided by the total number of days during which viremia was monitored. Statistically significant differences among and within groups were calculated by two-way ANOVA using Tukey's multiple comparisons test and unpaired multiple t-tests followed by Bonferroni's multiple comparisons test.

1218



1219
1220

1221 **Fig 2. T cell depletion modifies serological profile during primary DENV2 infection.** IgM and
1222 IgG response after DENV-2 infection was assessed using commercial ELISA tests. CD4-depleted
1223 animals are depicted in purple and undepleted animals are depicted in turquoise. Dotted lines
1224 indicate the limit of detection for each test. **(A-B)** Binding capacity of DENV IgM and IgG
1225 antibodies from CD4-depleted and undepleted animals after DENV-2 infection. **(C-D)** Total
1226 DENV IgM and IgG levels throughout different timepoints. Statistically significant differences
1227 among and within groups were calculated by two-way ANOVA using Tukey's multiple
1228 comparisons test and unpaired t-tests.

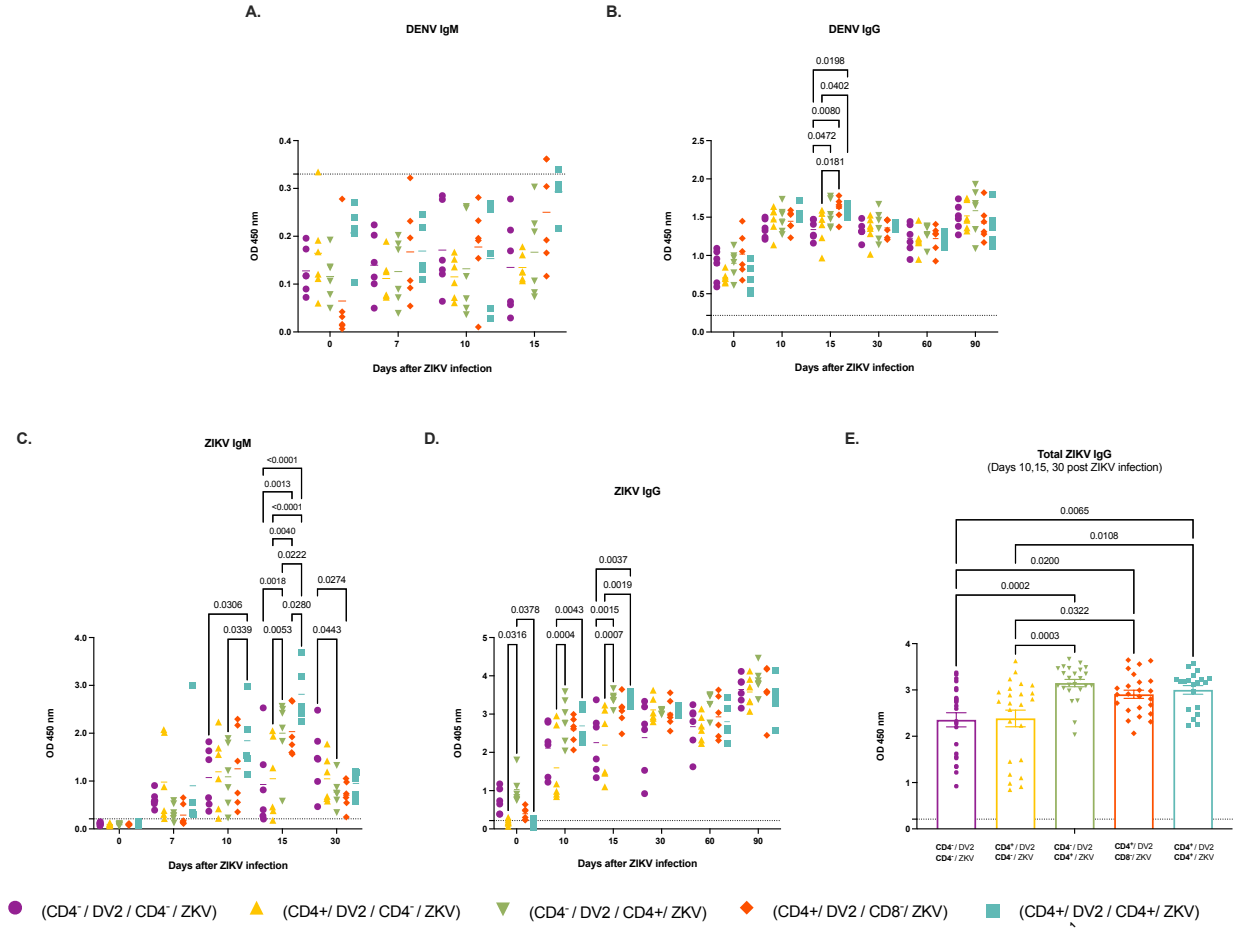


Fig 3. T cell depletion modifies serological profile during secondary heterologous ZIKV infection. IgM and IgG response after ZIKV infection in the presence of DENV immunity was assessed using commercial ELISA tests. Animal cohorts are depicted as follows based on treatment regime: CD4+/DV2/CD4+/ZV (shown in turquoise), CD4-/DV2/CD4+/ZV (shown in green), CD4+/DV2/CD4-/ZV (shown in yellow), CD4-/DV2/CD4-/ZV (shown in purple), and CD4+/DV2/CD8-/ZV (shown in red). Dotted lines indicate the limit of detection for each test. **(A-B)** Binding capacity of DENV IgM and IgG antibodies from CD4-depleted and undepleted animals after ZIKV infection. **(C-D)** Binding capacity of ZIKV IgM and IgG antibodies from CD4-depleted and undepleted animals after ZIKV infection. **(E)** Total ZIKV IgG levels after ZIKV infection were analyzed. Statistically significant differences among and within groups were calculated by two-way ANOVA using Tukey's multiple comparisons test and unpaired t-tests, and by One-way ANOVA using Tukey's multiple comparisons test.

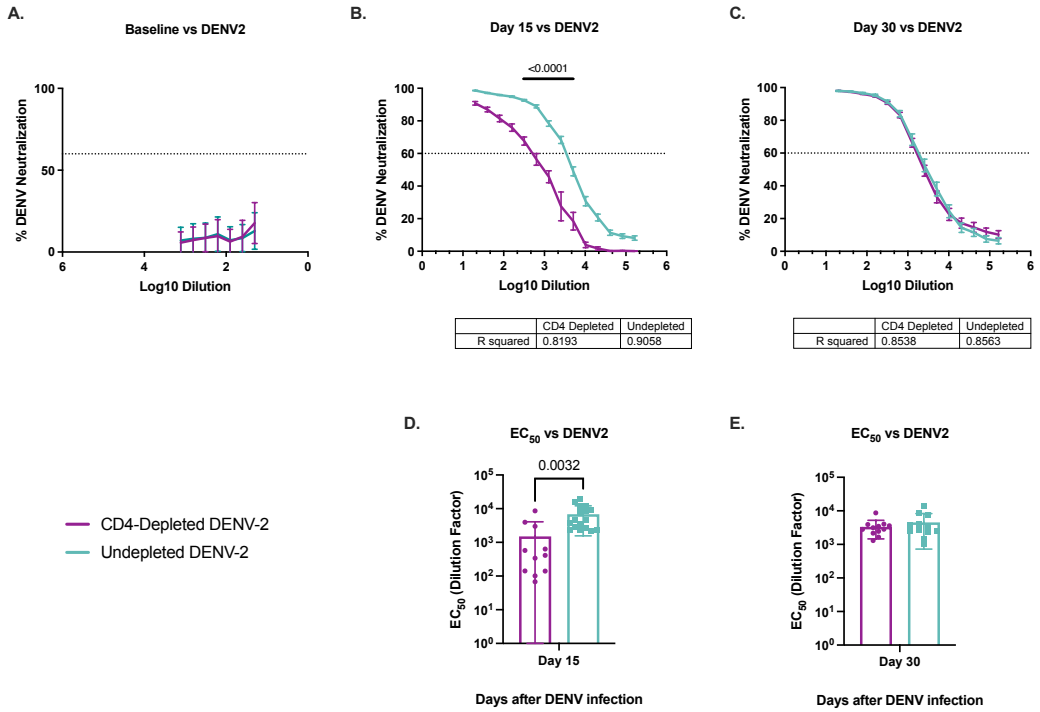
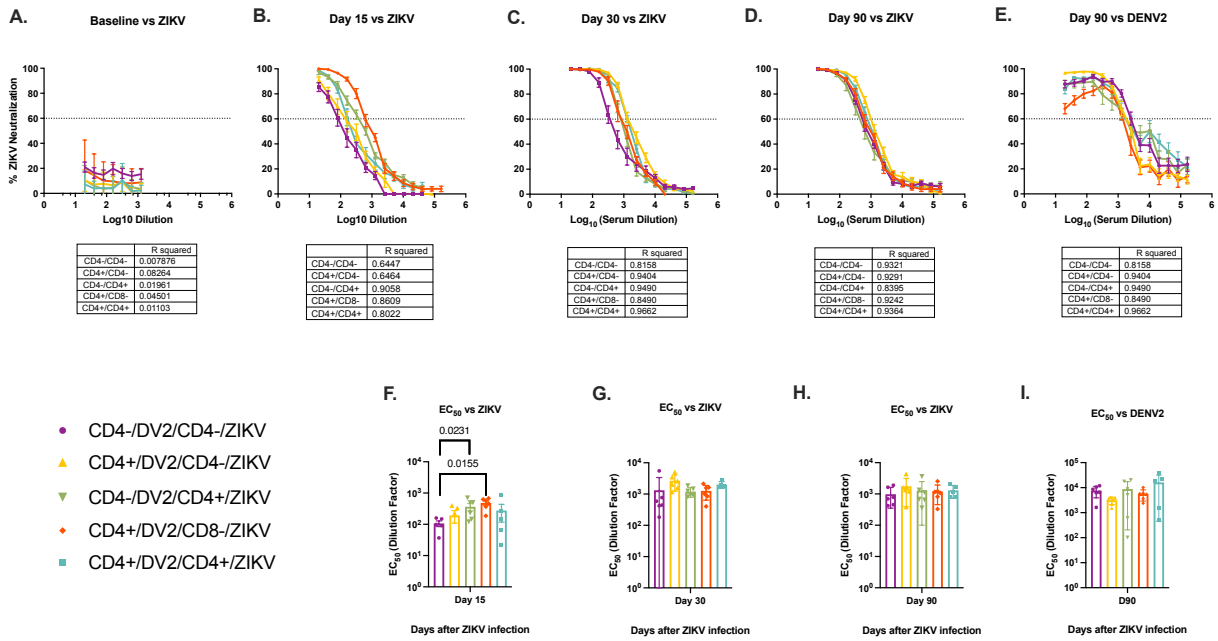


Fig 4. FRNT values of neutralizing antibodies against DENV-2 in depleted or undepleted flavivirus-naïve macaques. Neutralization capacity against DENV-2 is shown. CD4-depleted animals are depicted in purple and undepleted animals are depicted in turquoise. Dotted lines indicate the limit of detection for each test. **(A-C)** FRNT60 values of neutralizing antibodies against DENV-2 after DENV-2 infection are shown. **(D-E)** EC50 values of neutralizing antibodies against ZIKV after ZIKV infection. Statistically significant differences among groups were calculated by one-way and two-way ANOVA using the Tukey's multiple comparisons test and unpaired t-tests.



	R squared
CD4-/CD4-	0.007876
CD4+/CD4-	0.08264
CD4-/CD4+	0.01961
CD4+/CD8-	0.04501
CD4+/CD4+	0.01103

	R squared
CD4-/CD4-	0.6447
CD4+/CD4-	0.6464
CD4-/CD4+	0.9058
CD4+/CD8-	0.8609
CD4+/CD4+	0.8022

	R squared
CD4-/CD4-	0.8158
CD4+/CD4-	0.9404
CD4-/CD4+	0.9490
CD4+/CD8-	0.8490
CD4+/CD4+	0.9662

	R squared
CD4-/CD4-	0.9321
CD4+/CD4-	0.9291
CD4-/CD4+	0.8395
CD4+/CD8-	0.9242
CD4+/CD4+	0.9364

	R squared
CD4-/CD4-	0.8158
CD4+/CD4-	0.9404
CD4-/CD4+	0.9490
CD4+/CD8-	0.8490
CD4+/CD4+	0.9662

Fig 5. PRNT values of neutralizing antibodies against ZIKV in depleted or undepleted DENV-immune macaques. Neutralization capacity against ZIKV is shown. Animal cohorts are depicted as follows based on treatment regime: Animal cohorts are depicted as follows based on treatment regime: CD4+/DV2/CD4+/ZV (shown in turquoise), CD4-/DV2/CD4+/ZV (shown in green), CD4+/DV2/CD4-/ZV (shown in yellow), CD4-/DV2/CD4-/ZV (shown in purple), and CD4+/DV2/CD8-/ZV (shown in red). Dotted lines indicate the limit of detection for each test. **(A-D)** FRNT60 values of neutralizing antibodies against ZIKV after ZIKV infection are shown. **(E-G)** EC50 values of neutralizing antibodies against ZIKV after ZIKV infection. Significant differences among groups were calculated by one-way and two-way ANOVA using the Tukey's multiple comparisons test and unpaired t-tests.

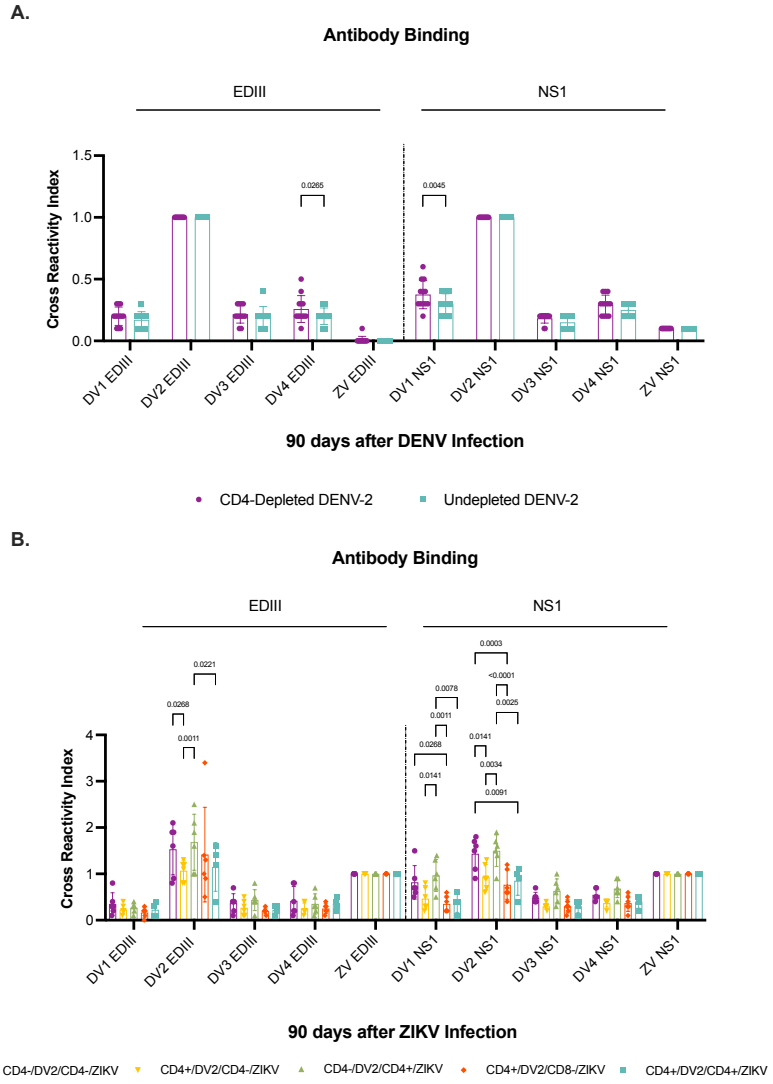


Fig 6. Epitope reactivity against DENV and ZIKV antigens in depleted and undepleted macaques. Antibody type-specific and cross-reactive responses against EDIII and NS1 antigens were assessed. Data generated by Luminex assay was normalized to a cross-reactivity index (CRI) by subtracting background (BSA) from the homologous value (values against infecting virus, HOM) and the heterologous values (values against heterologous virus, HET) followed by dividing the subtracted homologous by the subtracted heterologous values [$\frac{(HOM-BSA)}{(HET-BSA)}$]. **Upper panel.** CD4-depleted animals are depicted in purple and undepleted animals are depicted in turquoise. **(A)** Antibody response to EDIII (left side) and NS1 (right side) antigens from all four DENV serotypes and ZIKV 90 days after DENV2 infection**. Lower panel.** Animal cohorts are depicted as follows based on treatment regime: CD4+/DV2/CD4+/ZV (shown in turquoise), CD4-/DV2/CD4+/ZV (shown in green), CD4+/DV2/CD4-/ZV (shown in yellow), CD4-/DV2/CD4-/ZV (shown in purple), and CD4+/DV2/CD8-/ZV (shown in red). **(B)** Antibody response to EDIII (left side) and NS1 (right side) antigens from all four DENV serotypes and ZIKV 90 days after ZIKV infection**.** Significant differences among groups were calculated by two-way ANOVA using the Tukey's multiple comparisons test.

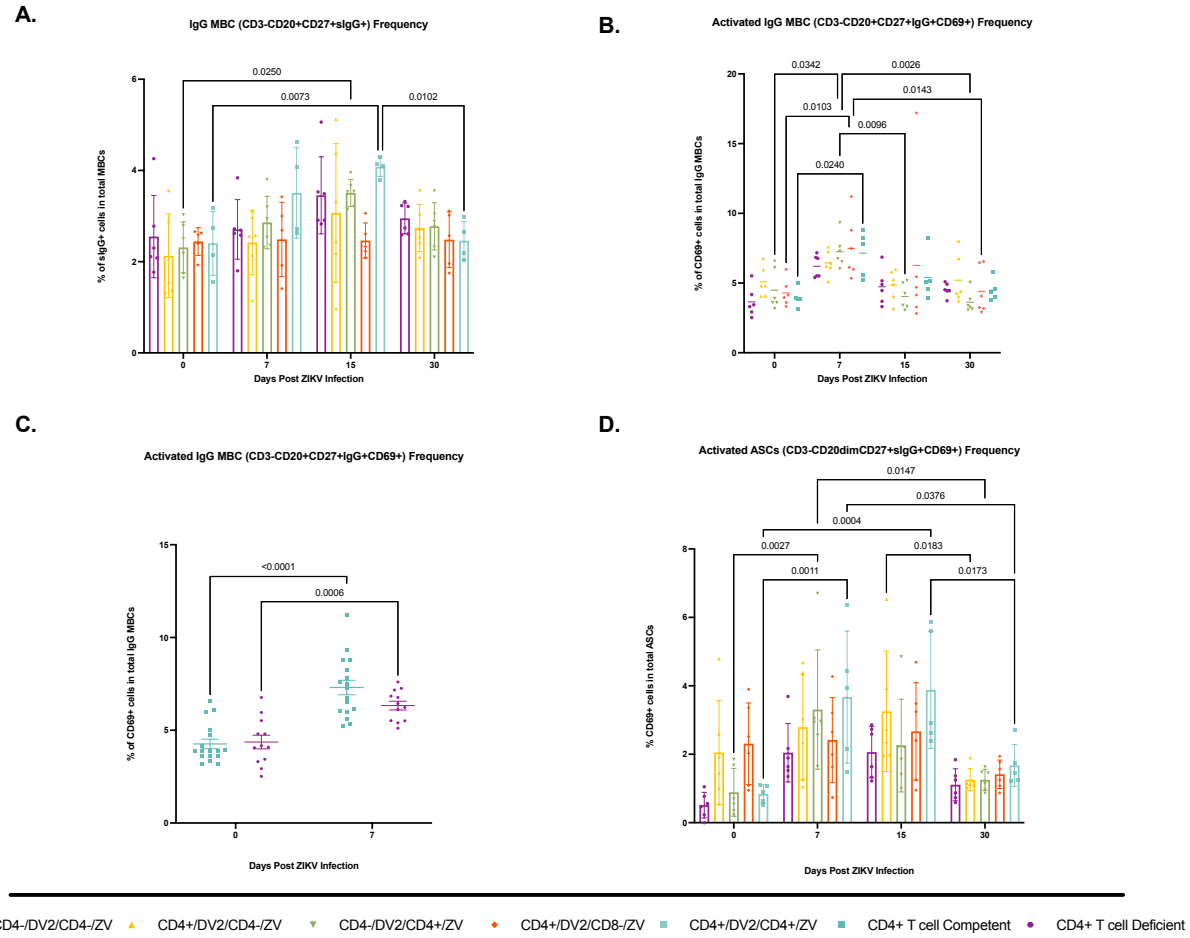


Fig 7. IgG memory B cell and ASC population kinetics after ZIKV infection in CD4+ T cell-competent and -deficient animals with previous DENV-2 immunity. The frequency of IgG MBCs and ASCs was assessed by immunophenotyping using flow cytometry. Animal cohorts are depicted as follows based on treatment regime: CD4+/DV2/CD4+/ZV (shown in turquoise), CD4-/DV2/CD4+/ZV (shown in green), CD4+/DV2/CD4-/ZV (shown in yellow), CD4-/DV2/CD4-/ZV (shown in purple), and CD4+/DV2/CD8-/ZV (shown in red). **(A)** Percentages of surface IgG expression in total MBCs (CD3-CD20+CD27+sIgG+). **(B)** Frequencies of CD69+ cells in total IgG+ MBCs (CD3-CD20+CD27+sIgG+CD69+). **(C)** Frequencies of CD69+ cells in total IgG MBCs with all cohorts organized into CD4+ T cell competent (shown in turquoise) and CD4+ T cell depleted (shown in purple). **(D)** Frequencies of CD69+ cells in total ASCs (CD3-CD20dimCD27+CD69+). Statistically significant differences among groups were calculated by two-way ANOVA using the Tukey's multiple comparisons test and unpaired t-tests.

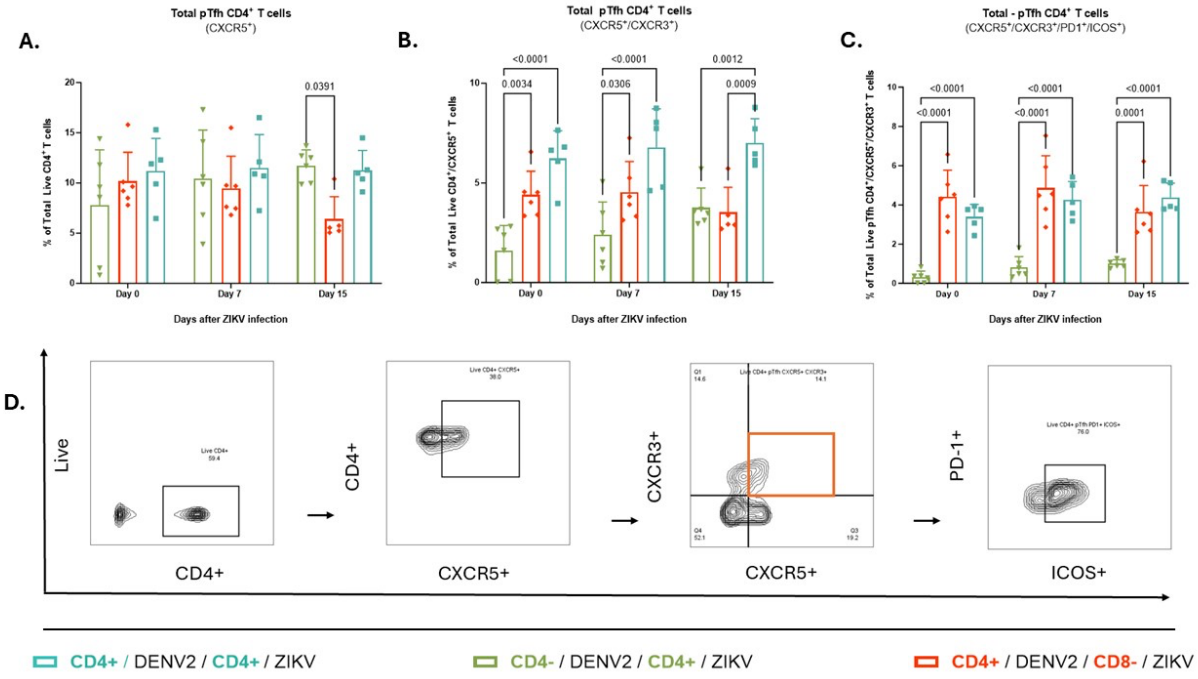


Fig 8. Total pTfh CD4+ T cell characterization. Immunophenotyping of CD4+ pTfh T cells was assessed via flow cytometry using frozen PBMCs collected on baseline, day 7, and day 15 pi with ZIKV. Animal cohorts are depicted as follows based on treatment regime: CD4+/DV2/CD4+/ZV (shown in turquoise), CD4-/DV2/CD4+/ZV (shown in green), and CD4+/DV2/CD8-/ZV (shown in red). **(A)** Frequency of total CD4+ T cells expressing follicular canonical marker CXCR5. **(B)** Frequency of total CD4-pTfh T cells expressing CXCR5 and CXCR3. **(C)** Frequency of total pTfh CD4 T cells expressing B cell co-stimulating surface makers PD1, and ICOS. **(D)** Gating Strategy for total pTfh CD4+ T cells characterization based on CXCR5, CXCR3, PD1, and ICOS surface markers expression. Comparisons between groups are reported as multiplicity-adjusted p values performed by two-way ANOVA using Tukey's multiple comparisons tests and unpaired t-test.

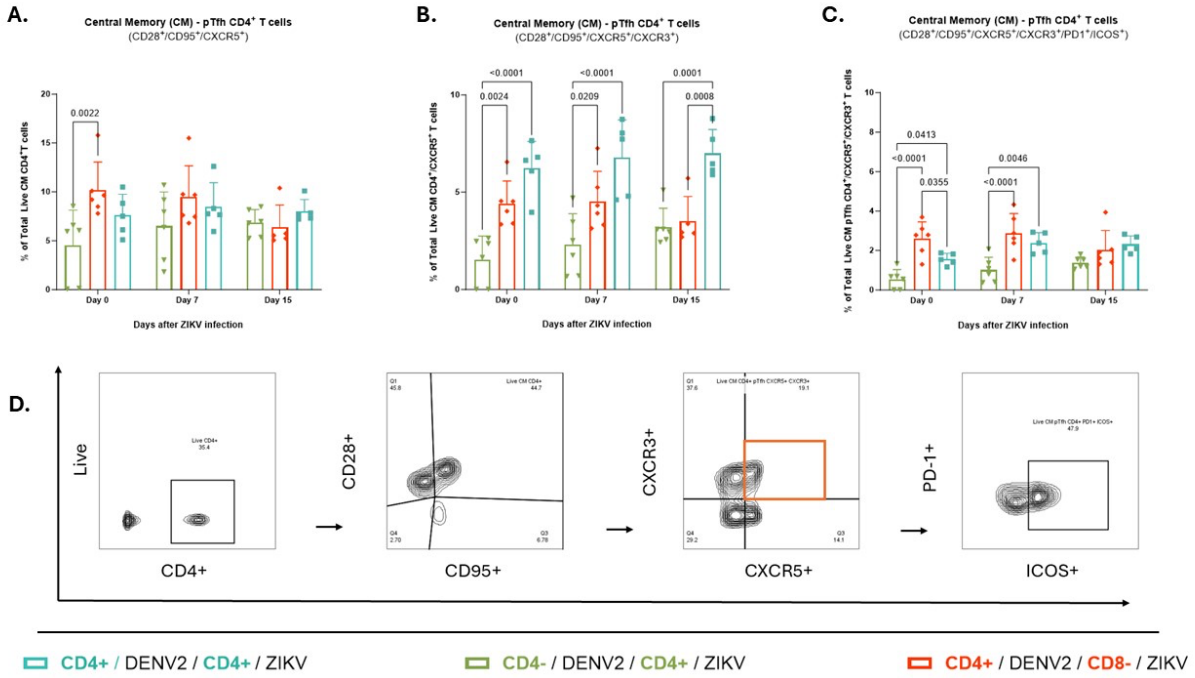


Fig 9. Total CM-pTfh CD4+ T cell characterization. Immunophenotyping of CM-pTfh CD4+ T cells after secondary ZIKV infection was accessed via flow cytometry using frozen PBMCs collected on baseline, day 7, and day 15 pi with ZIKV. Animal cohorts are depicted as follows based on treatment regime: CD4+/DV2/CD4+/ZV (shown in turquoise), CD4-/DV2/CD4+/ZV (shown in green), and CD4+/DV2/CD8-/ZV (shown in red). **(A)** Frequency of total CM CD4+ T cells expressing CXCR5. **(B)** Frequency of total CM- pTfh CD4+ T cells expressing CXCR5 and CXCR3. **(C)** Frequency of total CM- pTfh CD4+ T cells expressing B cell co-stimulating surface makers PD1, and ICOS. **(D)** Gating Strategy for total CM-pTfh CD4+ T cells characterization based on CXCR5, CXCR3, PD1, and ICOS surface markers expression. Comparisons between groups are reported as multiplicity-adjusted p values performed by two-way ANOVA using Tukey's multiple comparisons tests and unpaired t-test.

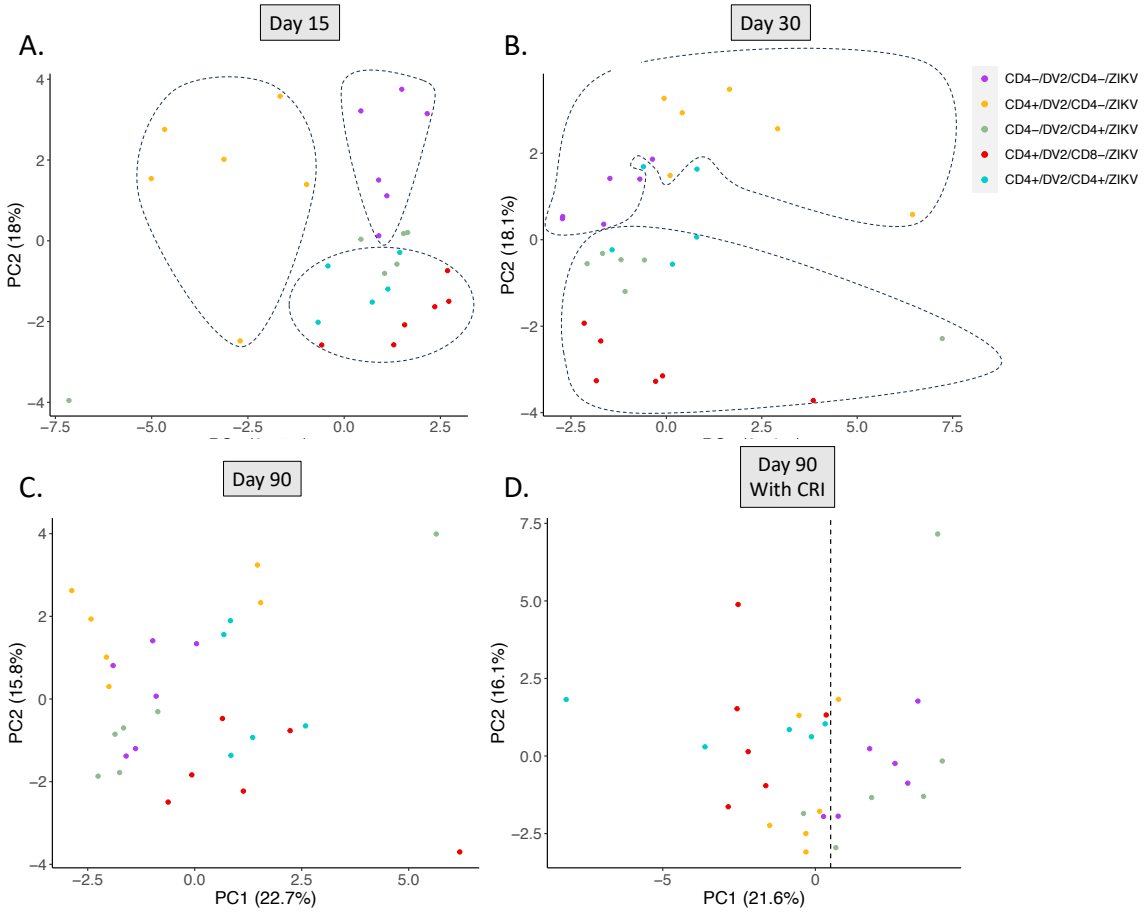
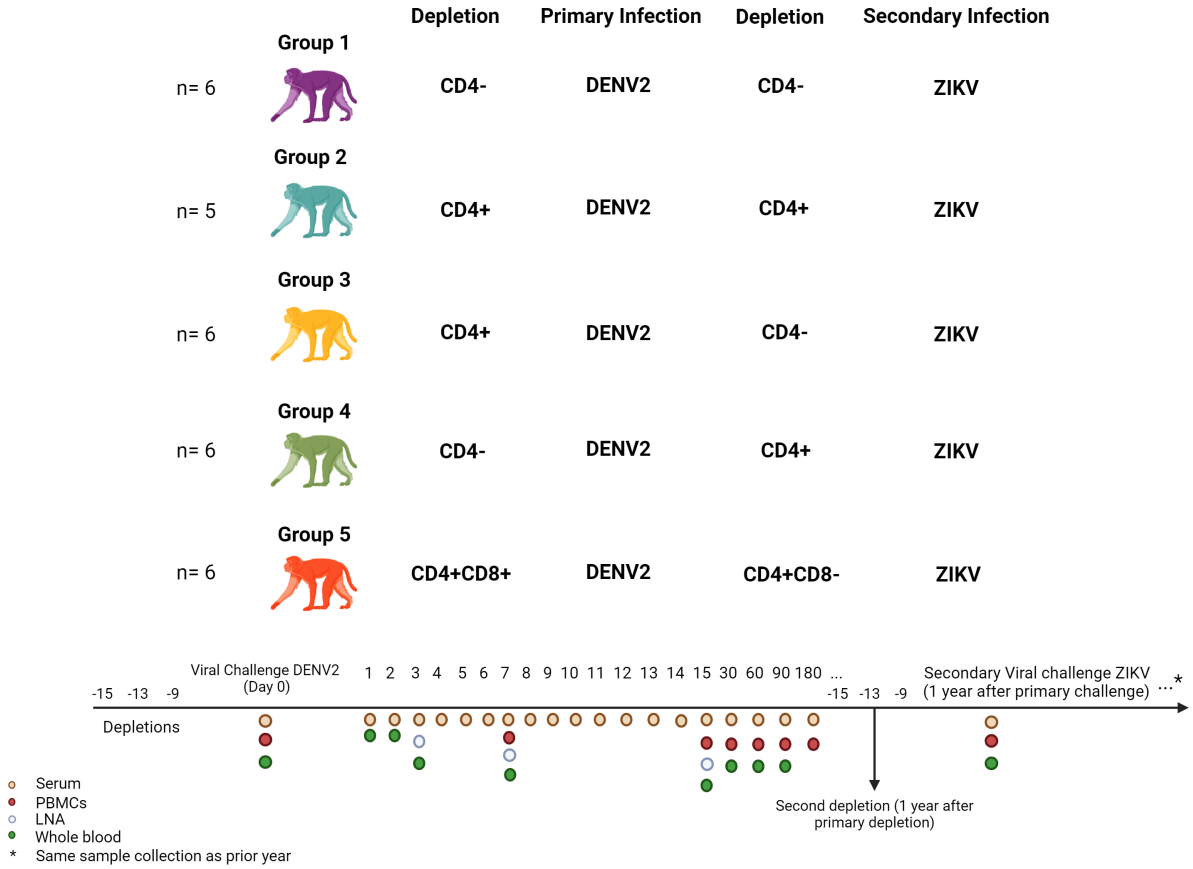
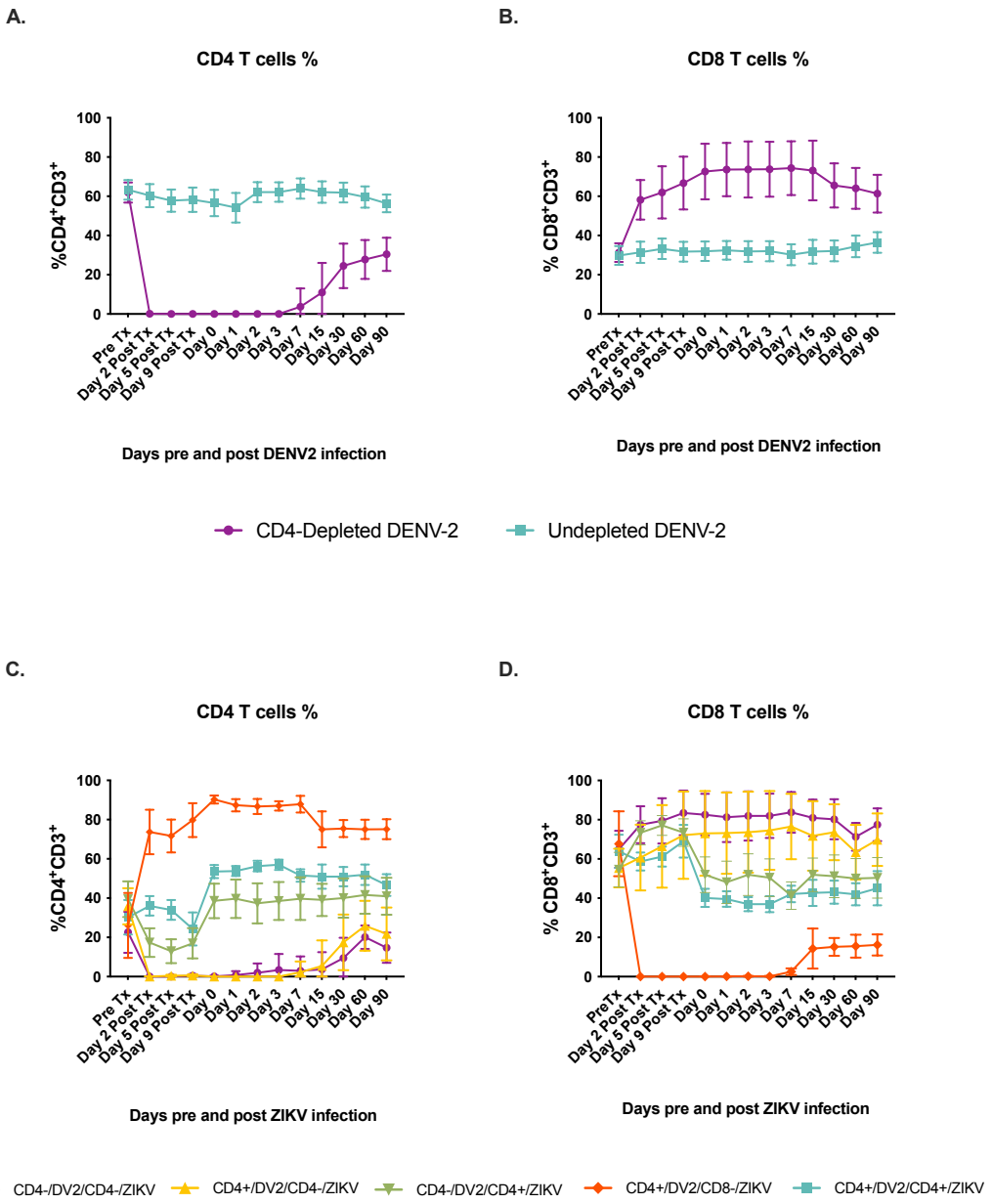


Fig 10. Principal component analysis of the effect of CD4+ T cell depletion on the ZIKV immune response. Principal component analysis was conducted in order to evaluate the overall effect of CD4+ T cells on various elements of the immune response against ZIKV including neutralization activity, IgM and IgG response and B and T cell response. Animal cohorts are depicted as follows based on treatment regime: CD4+/DV2/CD4+/ZV (shown in turquoise), CD4-/DV2/CD4+/ZV (shown in green), CD4+/DV2/CD4-/ZV (shown in yellow), CD4-/DV2/CD4-/ZV (shown in purple), and CD4+/DV2/CD8-/ZV (shown in red). Principal component analysis of day 15 **(A)**, day 30 **(B)** and day 90 **(C)** post ZIKV challenge**.** Principal component analysis of day 90 p.c. including CRI data **(D)**.

SUPPLEMENTARY FIGURES



SFig 1. Experimental design of T cell depletion and heterologous DENV2 and ZIKV infections in flavivirus-naïve macaques. Five cohorts of rhesus macaques (*Macaca mulatta*) underwent depletion of CD4+ T cells (or PBS in control group) followed by DENV-2 (5 x 10^5 pfu s.c.) infection at different timepoints. One year later, all cohorts were exposed to ZIKV strain PRABCV59 (1 x 10^6 pfu s.c.) after a second round of CD4+ or CD8+ T cell depletion (or PBS in control group). Sample collection was performed at various time points up to 90 days post-infection (p.i.) for serum, whole heparinized blood, and PBMC isolation.



SFig 2. CD4+ and CD8+ T cell frequency following depletion treatments prior to DENV2 and ZIKV infections. Frequency of CD4+ and CD8+ T cells was assessed by immunophenotyping before and after depletion treatments using flow cytometry. **Upper panel**. CD4-depleted animals are depicted in purple and undepleted animals are depicted in turquoise. CD4+ **(A)** and CD8+ **(B)** T cell depletion efficacy for depleted and undepleted animals before, during and after DENV2 infection. **Lower panel**. Animal cohorts are depicted as follows based on treatment regime: CD4+/DV2/CD4+/ZV (shown in turquoise), CD4-/DV2/CD4+/ZV (shown in green), CD4+/DV2/CD4-/ZV (shown in yellow), CD4-/DV2/CD4-/ZV (shown in purple), and CD4+/DV2/CD8-/ZV (shown in red). CD4+ **(C)** and CD8+ **(D)** T cell depletion efficacy for depleted and undepleted animals before, during and after ZIKV infection.

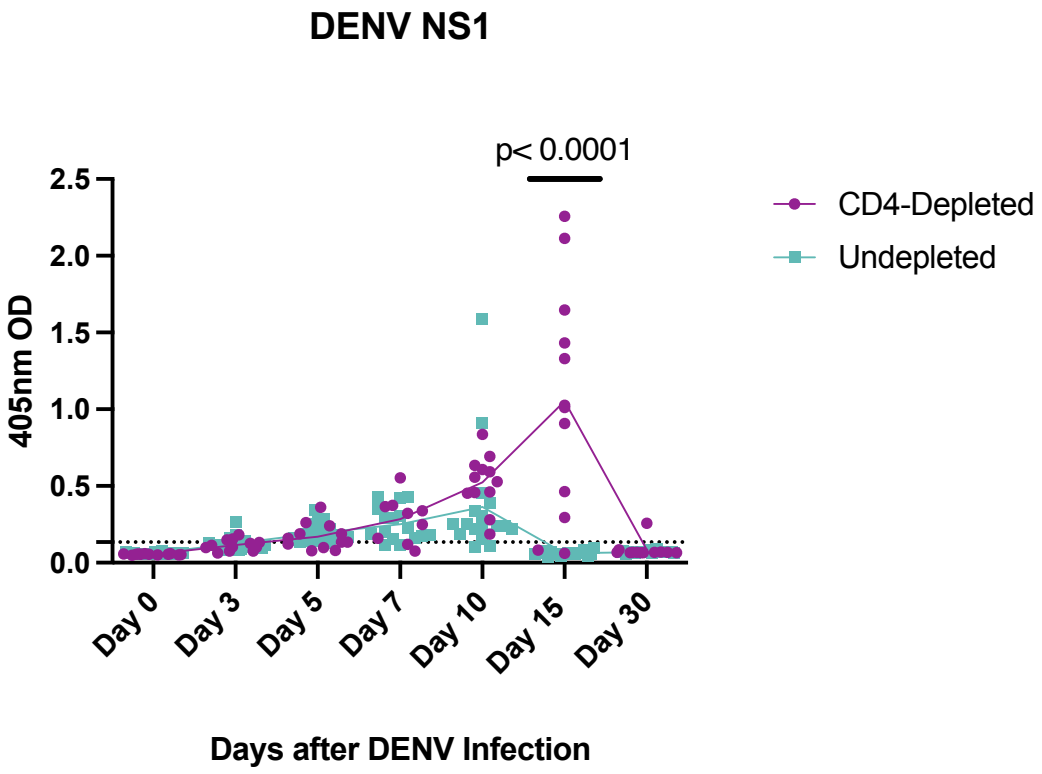
ID	Immune History	RNAemia (Log10 genome copies/mL) Post-DENV Infection																	Days	
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	30	Total	Mean
0Z6	CD4-/DV2	ND	2.905	3.924	4.505	4.245	3.875	3.799	3.040	ND	2.259	3.446	3.615	3.039	ND	ND	ND	ND	117	9.75
AE9		ND	2.834	4.204	4.759	4.059	3.758	3.164	3.545	2.957	2.540	2.623	3.272	3.202	ND	ND	ND	ND		
1Z3		ND	3.120	4.123	4.672	4.528	3.641	3.768	3.566	3.828	2.786	2.720	2.892	ND	ND	ND	ND	ND		
MA317		ND	2.682	4.557	5.235	4.888	4.291	3.176	ND	2.356	2.083	2.293	ND	2.492	ND	ND	ND	ND		
MA312		ND	2.493	3.908	5.060	3.718	3.961	3.217	3.425	3.285	3.107	3.713	ND	ND	ND	ND	ND	ND		
MA209		ND	2.167	3.323	4.003	3.844	2.765	4.283	4.608	4.784	4.765	4.202	3.555	3.018	ND	ND	ND	ND		
5Z1		ND	3.357	3.655	4.967	4.298	2.869	2.325	ND	ND	1.876	ND	ND	ND	ND	ND	ND	ND		
0Z7		ND	3.326	3.991	6.821	4.282	3.663	3.494	3.490	2.549	3.720	2.578	3.362	2.403	ND	ND	ND	ND		
MA333		ND	ND	4.111	4.812	4.456	3.692	3.147	3.429	2.831	2.604	2.368	2.600	ND	ND	ND	ND	ND		
MA321		ND	2.226	3.873	4.753	4.844	4.016	3.659	2.770	2.786	ND	2.294	1.945	ND	ND	ND	ND	ND		
MA267		ND	3.030	3.805	4.459	4.467	4.334	3.236	1.572	ND	2.916	1.257	2.786	ND	ND	ND	ND	ND		
MA316		ND	3.192	3.928	4.785	4.840	4.632	4.127	3.785	3.515	2.220	2.540	ND	ND	ND	ND	ND	ND		
9X5	CD4+/DV2	ND	3.076	3.879	4.741	4.379	3.663	2.551	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	95	7.8
MA341		ND	1.473	5.037	5.855	5.111	4.006	3.174	3.054	3.114	ND	ND	ND	ND	ND	ND	ND	ND		
4Z0		ND	2.466	4.092	4.865	4.364	3.085	3.052	3.474	2.524	2.003	ND	ND	ND	ND	ND	ND	ND		
MA320		ND	3.566	4.598	4.650	4.641	3.674	2.390	ND	2.401	ND	ND	ND	ND	ND	ND	ND	ND		
8X2		ND	3.598	4.078	4.505	3.255	1.983	3.257	2.227	ND	1.119	ND	ND	ND	ND	ND	ND	ND		
MA258		ND	1.853	3.330	4.924	4.692	4.249	3.768	3.313	1.457	ND	ND	ND	ND	ND	ND	ND	ND		
9X7		ND	2.895	4.247	5.148	4.305	2.716	1.266	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
5Z8		ND	3.901	4.555	5.487	4.782	3.123	1.193	1.898	2.797	ND	ND	ND	ND	ND	ND	ND	ND		
9X2		ND	3.638	4.313	4.662	4.704	4.079	3.456	2.340	3.067	ND	ND	ND	ND	ND	ND	ND	ND		
9X1		ND	2.408	3.804	4.242	4.009	3.552	2.940	2.190	2.190	2.421	ND	ND	ND	ND	ND	ND	ND		
2Z4		ND	3.604	3.827	4.477	4.215	3.089	2.754	3.162	2.792	3.159	ND	ND	ND	ND	ND	ND	ND		
MA163		ND	3.103	3.762	4.026	4.581	4.030	4.078	3.911	3.282	3.605	ND	ND	ND	ND	ND	ND	ND		

Table S1. **DENV-2 RNAemia of CD4-depleted and undepleted animals.** DENV-2 RNA detection during the first 15 days and day 30 p.i. Mean viremia days per group was calculated using days with detectable RNAemia divided by the number of animals in each group. ND= viral RNA not detected. Blank spaces represent sample was able to be collected.

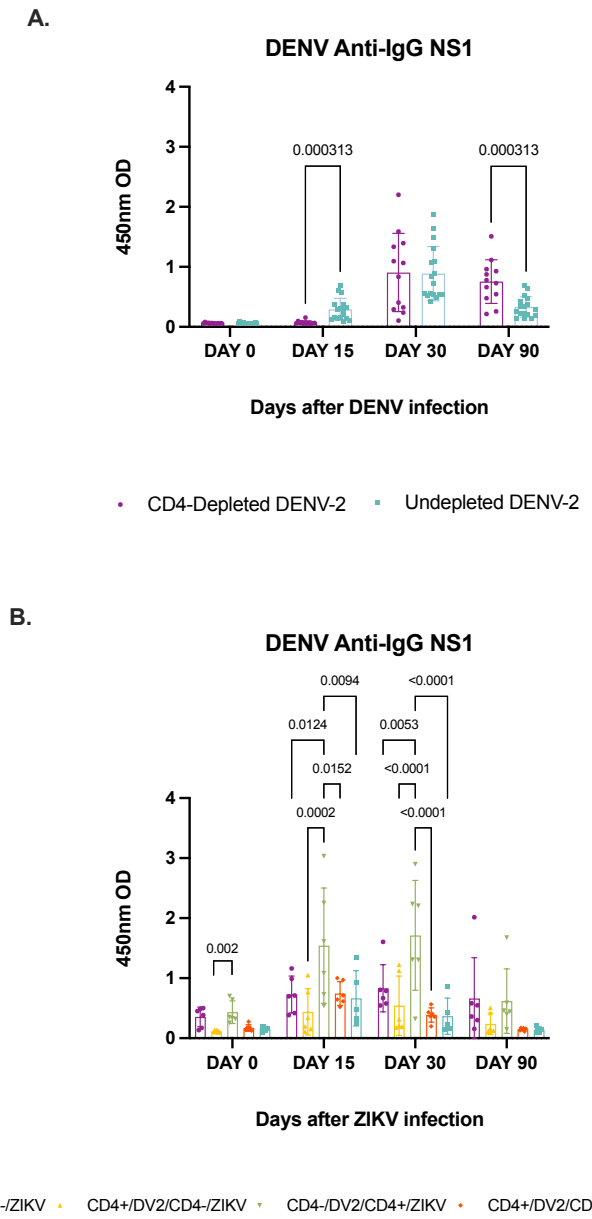
ID	Immune History	RNAemia (Log10 genome copies/mL) Post-ZIKV Infection																	Days	
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	30	Total	Mean
0Z6	CD4-/DV2/CD4-/ZV	ND	3.550	4.401	5.114	4.305	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	29	4.83
AE9		ND	3.277	3.878	5.158	4.496	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
1Z3		ND	3.296	4.213	5.459	4.744	2.313	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
MA317		ND	3.118	4.214	5.663	4.639	2.663	3.042	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
MA312		ND	3.702	4.096	5.188	4.850	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
MA209		ND	3.156	3.939	4.730	4.507	3.347	3.001	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
9X5	CD4+/DV2/CD4-/ZV	ND	3.455	4.477	5.582	4.955	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	28	4.67
MA341		ND	3.884	4.680	6.208	5.709	3.412	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
4Z0		ND	2.962	3.272	4.574	3.473	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
MA320		ND	2.623	3.770	4.662	2.447	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
8X2		ND	2.971	3.122	4.982	3.911	2.326	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
MA258		ND	2.533	4.456	6.038	5.164	2.459	3.143	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
5Z1	CD4-/DV2/CD4+/ZV	ND	3.508	3.686	5.364	3.378	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	26	4.33
0Z7		ND	3.967	4.585	5.828	4.904	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
MA333		ND	3.724	4.097	5.244	4.185	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
MA321		ND	3.468	4.183	5.437	4.873	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
MA267		ND	3.150	3.243	4.994	3.045	2.064	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
MA316		ND	3.578	4.121	5.871	3.820	2.610	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
9X7	CD4+/DV2/CD8-/ZV	ND	3.006	1.732	4.311	3.000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	30	5
5Z8		ND	3.000	4.010	5.605	4.701	2.464	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
9X2		ND	3.455	3.429	4.991	3.458	2.900	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
9X1		ND	3.000	3.410	4.420	3.000	2.042	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
2Z4		ND	3.041	3.109	4.438	3.420	2.495	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
MA163		ND	3.000	3.069	5.038	4.161	3.559	3.212	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
MA254	CD4+/DV2/CD4+/ZV	ND	3.109	3.989	5.012	ND	2.722	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	21	4.2
MA264		ND	3.000	3.874	5.521	4.261	2.447	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
MA311		ND	3.000	3.964	5.271	4.307	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
MA350		ND	3.493	3.901	5.262	4.290	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
MA364		ND	3.000	4.045	5.340	4.059	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		

Table S2. ZIKV RNAemia days of DENV-immune macaques with different T cell depletion status. ZIKV RNA detection during the first 15 days and day 30 p.i. Mean viremia days per group was calculated using days with detectable RNAemia divided by the number of animals in each group. ND= viral RNA not detected.

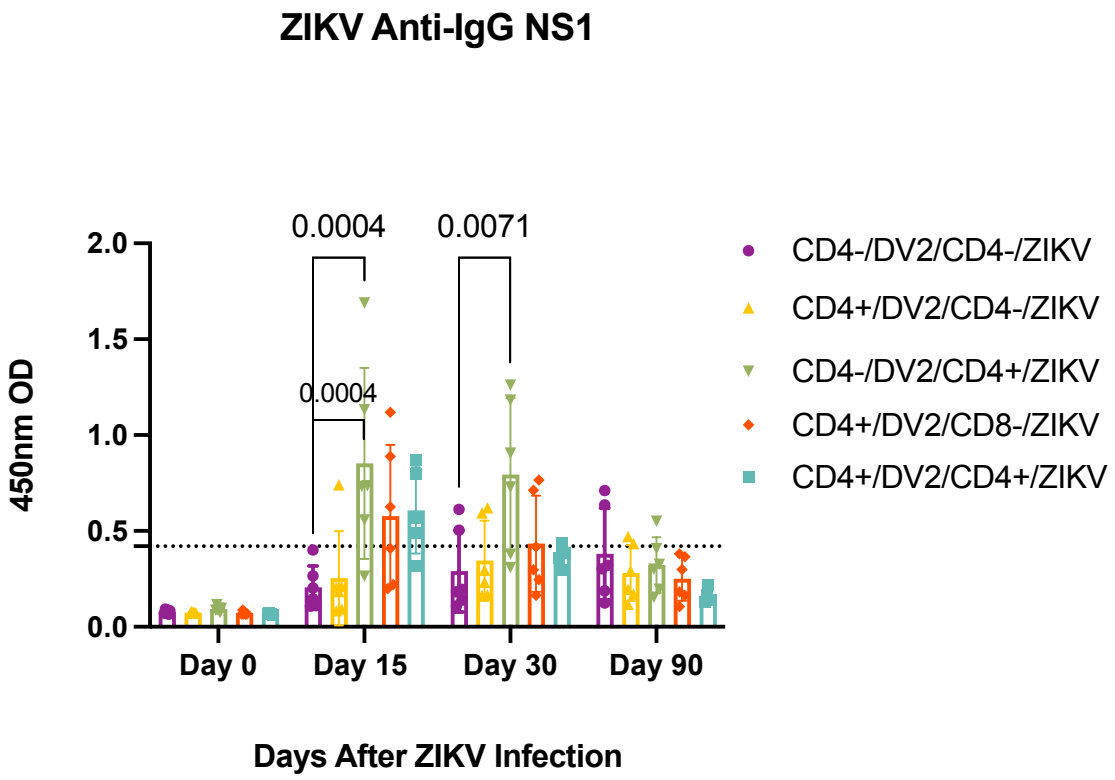
1445



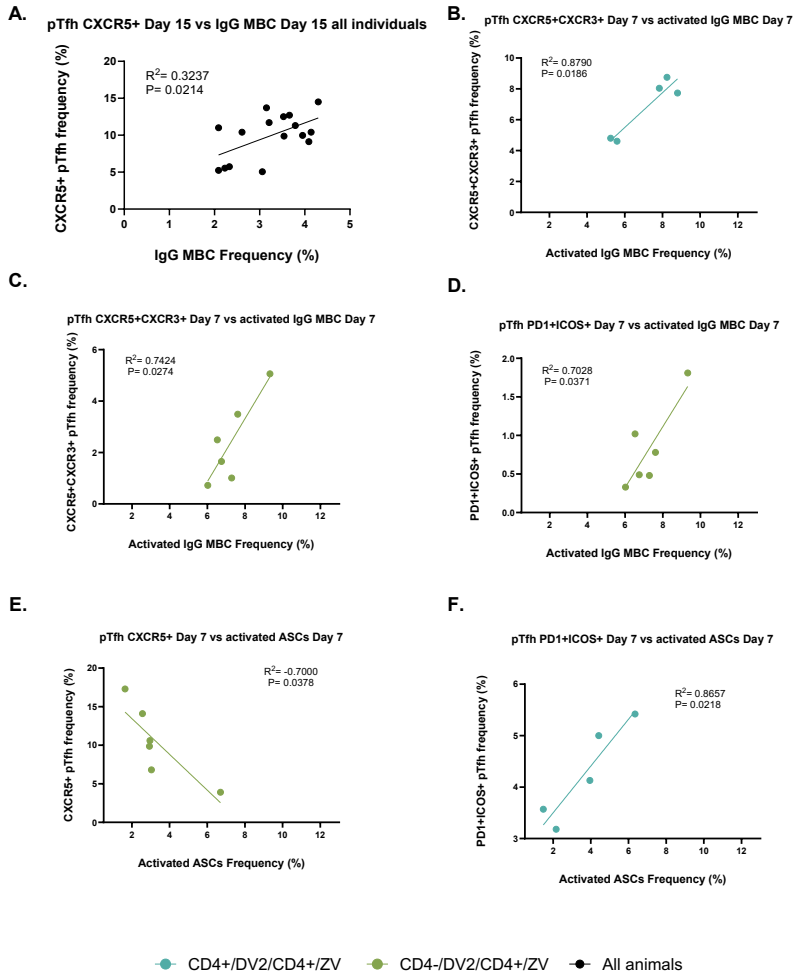
1446
1447
1448 **SFig 3. DENV NS1 levels are affected by absence of CD4+ T cells after DENV-2 infection.**
1449 DENV-NS1 levels after DENV-2 infection were measured using a commercial ELISA test. CD4-
1450 depleted animals are depicted in purple and undepleted animals are depicted in turquoise. Dotted
1451 lines indicate the limit of detection for each test. Statistically significant differences among and
1452 within groups were calculated by two-way ANOVA using Tukey's multiple comparisons test and
1453 unpaired t-tests.
1454



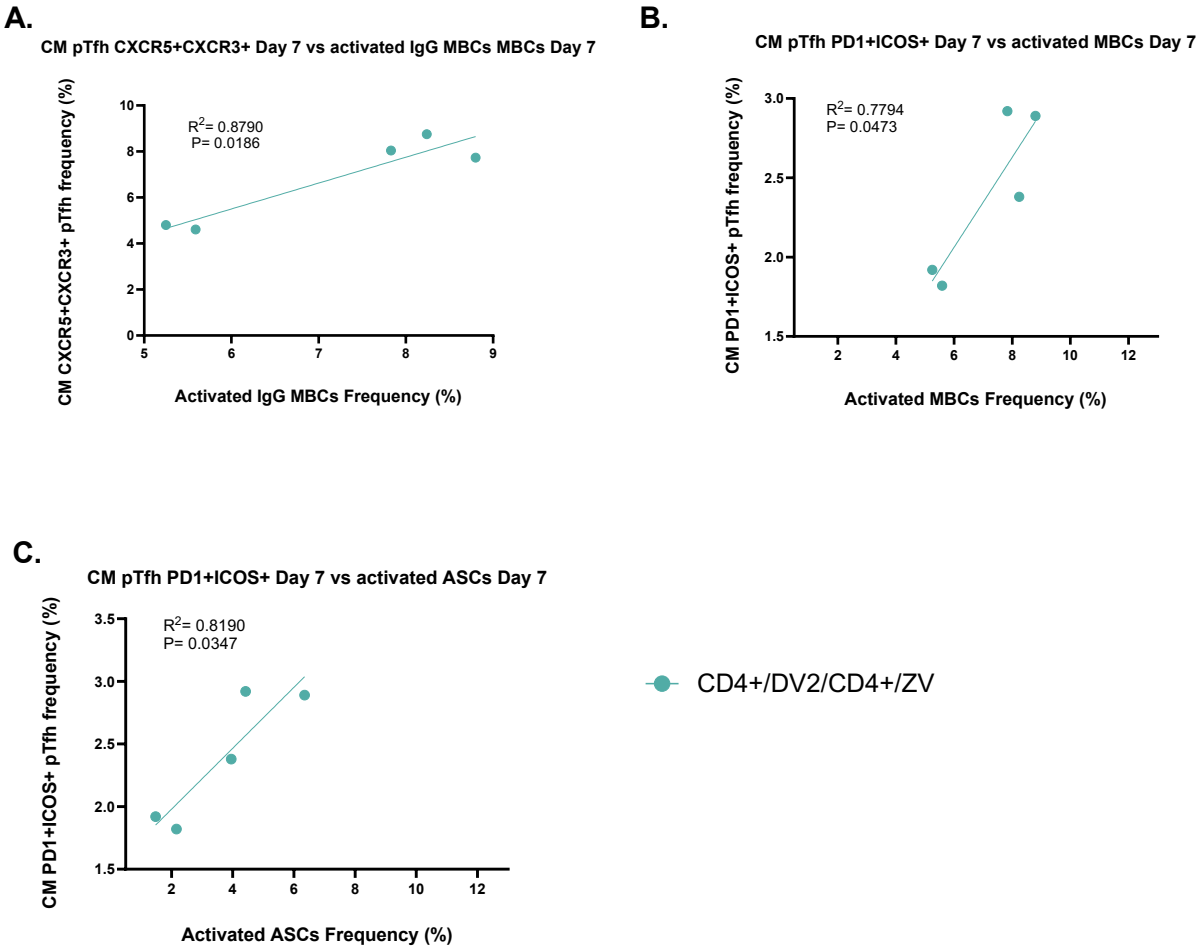
SFig 4. Depletion of CD4+ T cells before DENV infection increases DENV NS1 anti-IgG levels. DENV-NS1 anti-IgG levels were measured using commercial ELISA tests. **(A)** CD4-depleted animals are depicted in purple and undepleted animals are depicted in turquoise. DENV NS1 anti-IgG levels after DENV-2 infection. **(B)** Animal cohorts are depicted as follows based on treatment regime: CD4+/DV2/CD4+/ZV (shown in turquoise), CD4-/DV2/CD4+/ZV (shown in green), CD4+/DV2/CD4-/ZV (shown in yellow), CD4-/DV2/CD4-/ZV (shown in purple), and CD4+/DV2/CD8-/ZV (shown in red). DENV NS1 anti-IgG levels after ZIKV infection. No limit of detection or cut-off value is provided because this value will vary depending on flavivirus disease prevalence in the geographical location where the test is performed. Statistically significant differences among and within groups were calculated by two-way ANOVA using Tukey's multiple comparisons test and multiple t-tests.



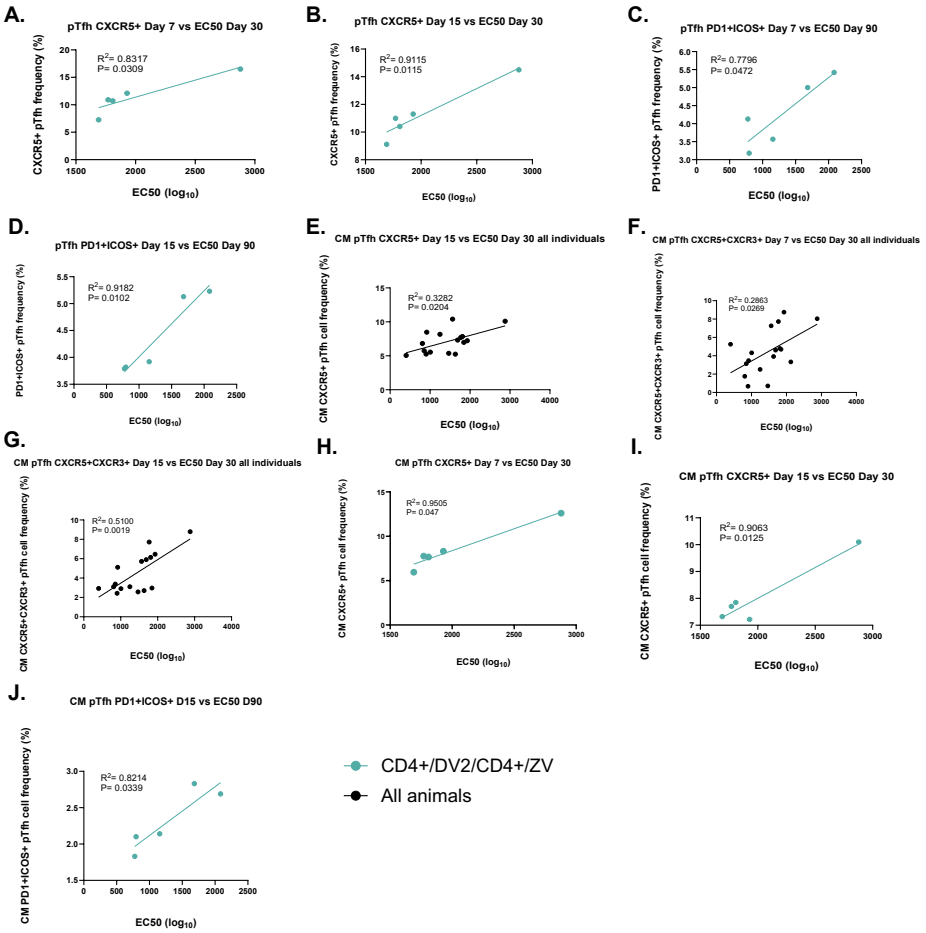
SFig 5. CD4+ T cell depletion status prior to DENV infection has no impact in production of ZIKV NS1-binding IgG after ZIKV infection. ZIKV NS1-IgG response after ZIKV infection in the presence of DENV immunity was assessed using a commercial ELISA test. Animal cohorts are depicted as follows based on treatment regime: Animal cohorts are depicted as follows based on treatment regime: CD4+/DV2/CD4+/ZV (shown in turquoise), CD4-/DV2/CD4+/ZV (shown in green), CD4+/DV2/CD4-/ZV (shown in yellow), CD4-/DV2/CD4-/ZV (shown in purple), and CD4+/DV2/CD8-/ZV (shown in red). Dotted lines indicate the limit of detection for each test. Binding capacity of ZIKV NS1-IgG antibodies from CD4-depleted and undepleted animals after ZIKV infection. Statistically significant differences among and within groups were calculated by two-way ANOVA using Tukey's multiple comparisons test and unpaired t-tests.



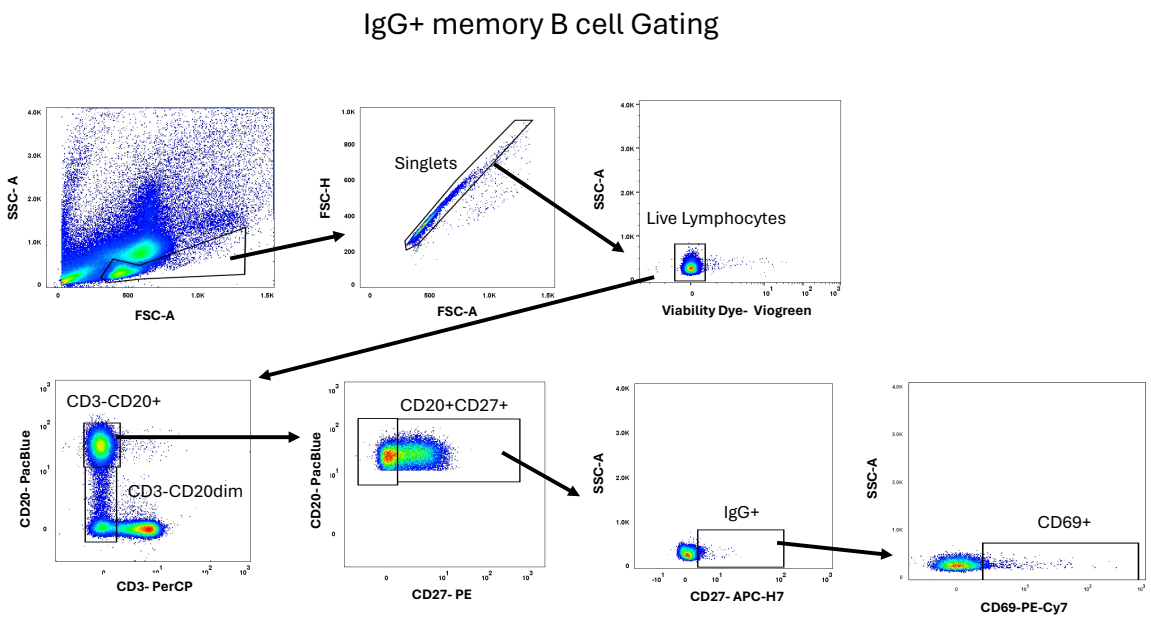
Sfig 6. **Frequencies of pTfh cells correlate with B cell response cell subsets such as total IgG$^+$ MBCs, activated IgG$^+$ MBCs, and activated ASCs during the secondary ZIKV infection.** Pearson correlations and simple linear regressions were performed on data from pTfh and B cell subsets. All experimental animals are shown in black. Animal cohorts are depicted as follows based on treatment regime: CD4+/DV2/CD4+/ZV (shown in turquoise) and CD4-/DV2/CD4+/ZV (shown in green). **(A)** The levels of total pTfh cells of the CXCR5$^+$ phenotype (shown as X5+ in graphs), measured via Flow Cytometry from PBMCs, isolated at day 15 p.c., correlated with frequencies of total IgG$^+$ MBCs, measured via Flow Cytometry from isolated whole blood at day 15 p.c.. **(B-C)** Correlations were performed comparing CXCR5$^+$CXCR3$^+$ pTfh cells (shown as X5+X3+) from day 7 p.c. with **(B)** activated IgG$^+$ MBCs from day 7 p.c., in the CD4+/DV2/CD4+/ZV group, and in the **(C)** CD4-/DV2/CD4+/ZV group. Correlations were performed comparing PD1+ICOS+ pTfh cells, from day 7 p.c., with **(D)** activated IgG$^+$ MBCs in the CD4-/DV2/CD4+/ZV group. A correlation was performed comparing CXCR5$^+$ (X5+) pTfh cells, from day 7 p.c., with **(E)** activated ASCs in the CD4-/DV2/CD4+/ZV group. A correlation was performed comparing PD1+ICOS+ pTfh cells, from day 7 p.c., with **(F)** activated ASCs in the CD4+/DV2/CD4+/ZV group (+/+). Pearson's rank correlation coefficients were used to calculate correlations, and R^2- and P-values are indicated. Linear regression is shown as lines and each dot represents one animal.



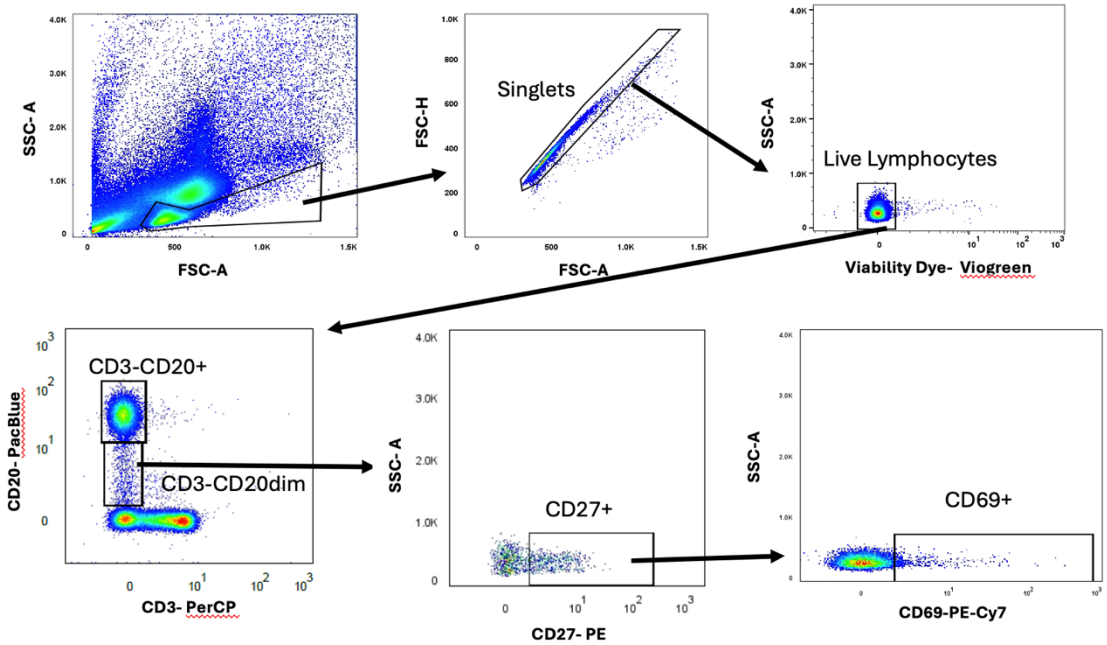
SFig 7. Frequencies of CM pTfh cell subsets correlate with B cell response cell subsets. Pearson correlations and simple linear regressions were performed using data from B cell subsets such as activated IgG^+ MBCs and ASCs during the secondary ZIKV infection**.** Animal cohorts are depicted as follows based on treatment regime: CD4+/DV2/CD4+/ZV (shown in turquoise). All significant correlations with CM pTfh cells were only observed in the CD4+/DV2/CD4+/ZV group. Correlations were performed and observed for the CM $CXCR5^+CXCR3^+$ pTfh cells (shown as X5+X3+), from day 7 p.c., with **(A)** activated IgG^+ MBCs, from day 7 p.c. in the CD4+/DV2/CD4+/ZV group. Correlations were performed for comparing CM PD1+ICOS+ pTfh cells, from day 7 p.c., with **(B)** activated IgG^+ MBCs in the CD4+/DV2/CD4+/ZV group. Correlations were performed for CM PD1+ICOS+ pTfh cells with **(C)** activated ASCs, from day 7 p.c., in the CD4+/DV2/CD4+/ZV group. Pearson's rank correlation coefficients were used to calculate correlations, and R^2- and P-values are indicated. Linear regression is shown as lines and each dot represents one animal.



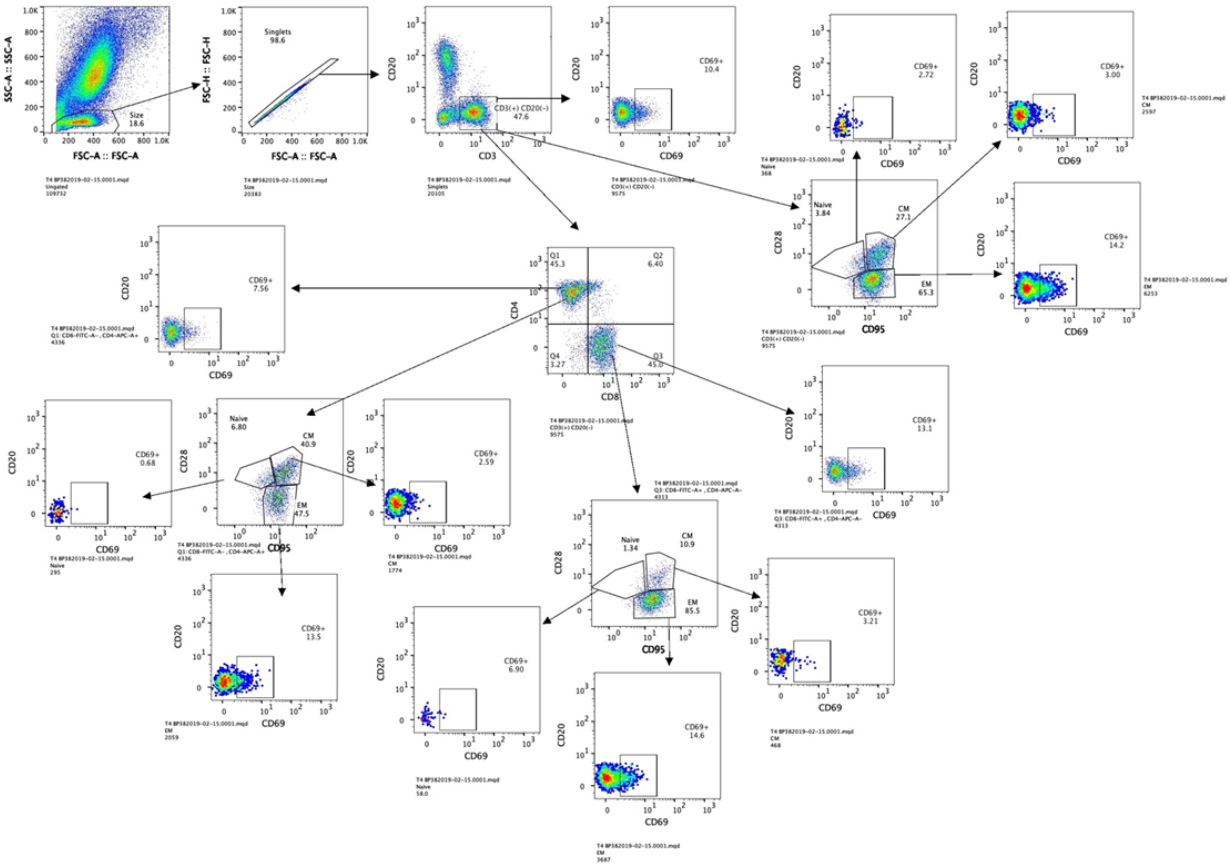
SFig 8. Frequencies of pTfh cell subsets correlate with EC50 (log_{10}) values. Data obtained from Focus/Plaque Reduction Neutralization Tests on collected sera from days 30 and 90 p.c. during secondary ZIKV infection was analyzed using Pearson and simple linear regression with pTfh cells. All experimental animals are shown in black. Animal cohorts are depicted as follows based on treatment regime: CD4+/DV2/CD4+/ZV (shown in turquoise). **(A-B)** Correlations were performed comparing $CXCR5^+$ pTfh cells (shown as X5+), from **(A)** days 7 and **(B)** days 15 p.c. with EC50 values from day 30 p.c., in the CD4+/DV2/CD4+/ZV group. **(C-D)** Correlations were performed comparing PD1+ICOS+ pTfh cells from **(C)** day 7 and **(D)** day 15 p.c. with EC50 values from day 90 p.c., in the CD4+/DV2/CD4+/ZV group. **(E-I)** Frequencies of CM pTfh cell subsets correlate with EC50 values from days 30 and 90 p.c.. **(E-G)** Correlations were performed using all experimental comparing **(E)** CM $CXCR5^+$ (X5+) pTfh cells, from day 15 p.c., with EC50 values from day 30 p.c., comparing **(F)** CM $CXCR5^+CXCR3^+$ (shown as X5+X3+) pTfh cells, from day 7 p.c., with EC50 values from day 30 p.c., and comparing **(G)** CM $CXCR5^+CXCR3^+$ (X5+X3+) pTfh cells, from day 15 p.c.. Correlations were performed comparing CM $CXCR5^+$ (X5+) pTfh cells, from **(H)** day 7 and **(I)** day 15 p.c., with EC50 values from day 30 p.c.. Correlations were performed comparing CM PD1+ICOS+ pTfh cells, from day 15 p.c., with **(J)** EC50 values from day 90 p.c.. Pearson's rank correlation coefficients were used to calculate correlations, and R^2- and P-values are indicated. Linear regression is shown as lines and each dot represents one animal.



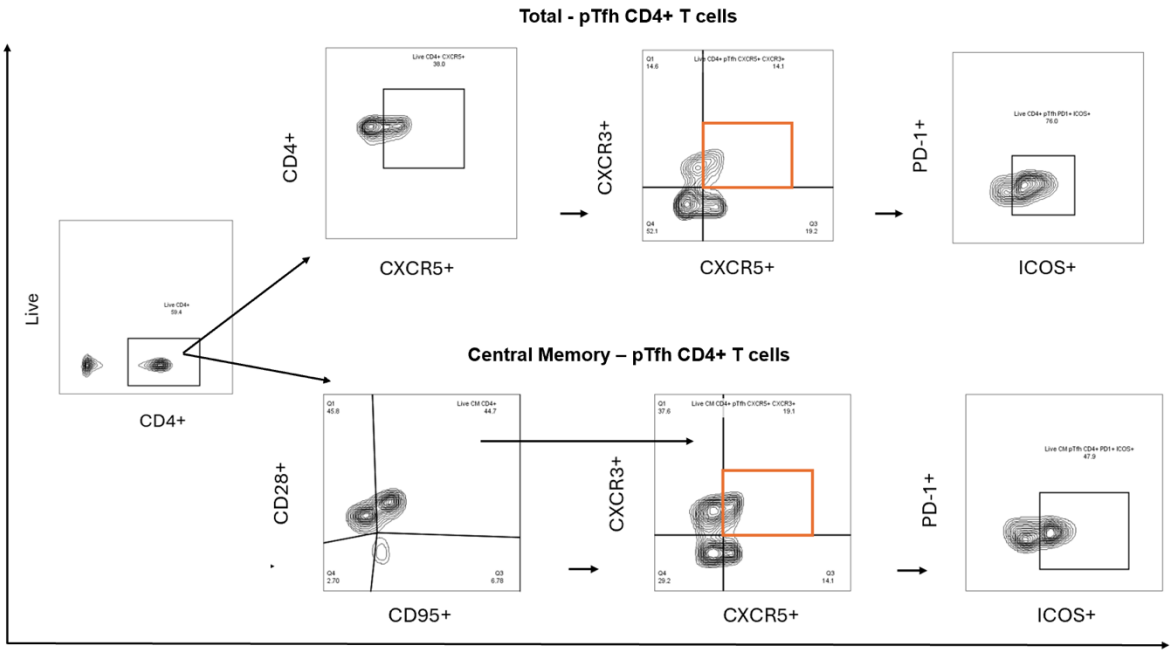
1538



1539
1540
1541 **SFig 9. Gating strategy for B cells.** Gating strategy used to define B cells and subsets.
1542 Lymphocytes were gated based on their characteristic forward and side scatter pattern (FSC, SSC).
1543 B cells were defined as CD20+CD3-.
1544



SFig 10. Gating strategy for T cells. Gating strategy used to define T cells and subsets. Lymphocytes were gated based on their characteristic forward and side scatter pattern (FSC, SSC). T cells were defined as CD3+CD20+. CD4+ and CD8+ T cells were defined as CD3+CD4+ and CD3+CD8+, respectively.



SFig 11. Gating strategy for pTfh CD4+ T cells. Gating strategy used to define pTfh CD4+ T and total central memory (CM- pTfh) CD4+ T cells based on CD28+, CD95+, CXCR5+, CXCR3+, PD1+, and ICOS+ surface markers expression.

Parameter	Day of measurement for PCA
TFH_CD4_ ICOS_PD1	Day 7, Day 15
zikav_od_igm	Day 15, Day 30
zikav_od_igg	Day 15, Day 30, Day 90
denv_od_igm	Day 15
denv_od_igg	Day 15, Day, 30, Day 90
neutralization vs. ZikaV	Day 15, Day 30, Day 90
neutralization vs. DengueV	Day 15, Day 30, Day 90
CD4_T_cells	Day 15, Day 30, Day 90
CD8_T_cells	Day 15, Day 30, Day 90
T4_CM	Day 15, Day 30, Day 90
T4_CM_CD69	Day 15, Day 30, Day 90
T8_CM	Day 15, Day 30, Day 90
T8_CM_CD69	Day 15, Day 30, Day 90
T4_EM	Day 15, Day 30, Day 90
T4_EM_CD69	Day 15, Day 30, Day 90
T8_EM	Day 15, Day 30, Day 90
T8_EM_CD69	Day 15, Day 30, Day 90
CD4_Naive	Day 15, Day 30, Day 90
CD4_Naive_CD69	Day 15, Day 30, Day 90
CD8_Naive	Day 15, Day 30, Day 90
CD8_Naive_CD69	Day 15, Day 30, Day 90
B_Cells	Day 15, Day 30, Day 90
B_Cells_CD69	Day 15, Day 30, Day 90
CD27_CD69	Day 15, Day 30, Day 90
CD20_CD27	Day 15, Day 30, Day 90
EDIII_CRI_D1	DAY 90
EDIII_CRI_D2	DAY 90
EDIII_CRI_D3	DAY 90
EDIII_CRI_D4	DAY 90
EDIII_CRI_Z	DAY 90
NS1_CRI_D1	DAY 90
NS1_CRI_D2	DAY 90
NS1_CRI_D3	DAY 90
NS1_CRI_D4	DAY 90
NS1_CRI_Z	DAY 90
OD = Optical Density	
CM = Central Memory	
tfh = T Follicular Helper	
CRI = Cross Reactivity Index	

1557 Table S3**. Parameters used to perform PCA analysis.** PCA analysis was performed using
1558 parameters from days 7, 15, 30 and 90 post ZIKV infection.

1559 **References**
1560
1561 Abbink, P., Larocca, R.A., Visitsunthorn, K., Boyd, M., De La Barrera, R.A., Gromowski, G.D.,
1562 Kirilova, M., Peterson, R., Li, Z., Nanayakkara, O.*, et al.* (2017). Durability and correlates of
1563 vaccine protection against Zika virus in rhesus monkeys. Science translational medicine *9*.
1564 Aberle, J.H., Koblischke, M., and Stiasny, K. (2018). CD4 T cell responses to flaviviruses. J Clin
1565 Virol *108*, 126-131.
1566 Adams, C., Jadi, R., Segovia-Chumbez, B., Daag, J., Ylade, M., Medina, F.A., Sharp, T.M.,
1567 Munoz-Jordan, J.L., Yoon, I.K., Deen, J.*, et al.* (2021). Novel Assay to Measure Seroprevalence
1568 of Zika Virus in the Philippines. Emerg Infect Dis *27*, 3073-3081.
1569 Aliota, M.T., Dudley, D.M., Newman, C.M., Mohr, E.L., Gellerup, D.D., Breitbach, M.E.,
1570 Buechler, C.R., Rasheed, M.N., Mohns, M.S., Weiler, A.M.*, et al.* (2016). Heterologous
1571 Protection against Asian Zika Virus Challenge in Rhesus Macaques. PLoS neglected tropical
1572 diseases *10*, e0005168.
1573 Amorim, J.H., dos Santos Alves, R.P., Bizerra, R., Araujo Pereira, S., Ramos Pereira, L.,
1574 Nascimento Fabris, D.L., Santos, R.A., Romano, C.M., and de Souza Ferreira, L.C. (2016).
1575 Antibodies are not required to a protective immune response against dengue virus elicited in a
1576 mouse encephalitis model. Virology *487*, 41-49.
1577 Ana Carolina Bernardes Terzian, A.S.S., Mânlio Tasso de Oliveira Mota, Rafael Alves da Silva,
1578 Cássia Fernanda Estofolete, Tatiana Elias Colombo, Paula Rahal, Kathryn A. Hanley, Nikos
1579 Vasilakis, Jorge Kalil, Maurício Lacerda Nogueira (2017). Viral load and cytokine response
1580 profile does not support antibody-dependent enhancement in dengue-primed Zika-infected
1581 patients. Clin Infect Dis *DOI 10.1093/cid/cix558*.
1582 Andrade, P., Narvekar, P., Montoya, M., Michlmayr, D., Balmaseda, A., Coloma, J., and Harris,
1583 E. (2020). Primary and Secondary Dengue Virus Infections Elicit Similar Memory B-Cell
1584 Responses, but Breadth to Other Serotypes and Cross-Reactivity to Zika Virus Is Higher in
1585 Secondary Dengue. J Infect Dis *222*, 590-600.
1586 Angelo, M.A., Grifoni, A., O'Rourke, P.H., Sidney, J., Paul, S., Peters, B., de Silva, A.D.,
1587 Phillips, E., Mallal, S., Diehl, S.A.*, et al.* (2017). Human CD4(+) T Cell Responses to an
1588 Attenuated Tetravalent Dengue Vaccine Parallel Those Induced by Natural Infection in
1589 Magnitude, HLA Restriction, and Antigen Specificity. J Virol *91*.
1590 Appaiahgari, M.B., Saini, M., Rauthan, M., Jyoti, and Vrati, S. (2006). Immunization with
1591 recombinant adenovirus synthesizing the secretory form of Japanese encephalitis virus envelope
1592 protein protects adenovirus-exposed mice against lethal encephalitis. Microbes Infect *8*, 92-104.
1593 Aquino, V.H., Fumagalli, M.J., Silva, A., de Moura Negrini, B.V., Rojas, A., Guillen, Y., Bernal,
1594 C., and Figueiredo, L.T.M. (2023). Linear epitope mapping in the E and NS1 proteins of dengue
1595 and Zika viruses: Prospection of peptides for vaccines and diagnostics. PLoS One *18*, e0292451.
1596 Bardina, S.V., Bunduc, P., Tripathi, S., Duehr, J., Frere, J.J., Brown, J.A., Nachbagauer, R.,
1597 Foster, G.A., Krysztof, D., Tortorella, D.*, et al.* (2017). Enhancement of Zika virus pathogenesis
1598 by preexisting antiflavivirus immunity. Science (New York, NY) *DOI 10.1126/science.aal4365*.
1599 Beicht, J., Kubinski, M., Zdora, I., Puff, C., Biermann, J., Gerlach, T., Baumgärtner, W., Sutter,
1600 G., Osterhaus, A., Prajeeth, C.K.*, et al.* (2023). Induction of humoral and cell-mediated
1601 immunity to the NS1 protein of TBEV with recombinant Influenza virus and MVA affords
1602 partial protection against lethal TBEV infection in mice. Frontiers in immunology *14*, 1177324.
1603 Beltramello, M., Williams, K.L., Simmons, C.P., Macagno, A., Simonelli, L., Quyen, N.T.,
1604 Sukupolvi-Petty, S., Navarro-Sanchez, E., Young, P.R., de Silva, A.M.*, et al.* (2010). The human

1605 immune response to Dengue virus is dominated by highly cross-reactive antibodies endowed
1606 with neutralizing and enhancing activity. Cell host & microbe *8*, 271-283.
1607 Bentebibel, S.E., Lopez, S., Obermoser, G., Schmitt, N., Mueller, C., Harrod, C., Flano, E.,
1608 Mejias, A., Albrecht, R.A., Blankenship, D.*, et al.* (2013). Induction of
1609 ICOS+CXCR3+CXCR5+ TH cells correlates with antibody responses to influenza vaccination.
1610 Sci Transl Med *5*, 176ra132.
1611 Beran, J., Lattanzi, M., Xie, F., Moraschini, L., and Galgani, I. (2019). Second five-year follow-
1612 up after a booster vaccination against tick-borne encephalitis following different primary
1613 vaccination schedules demonstrates at least 10 years antibody persistence. Vaccine *37*, 4623-
1614 4629.
1615 Best, K., Guedj, J., Madelain, V., de Lamballerie, X., Lim, S.Y., Osuna, C.E., Whitney, J.B., and
1616 Perelson, A.S. (2017). Zika plasma viral dynamics in nonhuman primates provides insights into
1617 early infection and antiviral strategies. Proceedings of the National Academy of Sciences of the
1618 United States of America *114*, 8847-8852.
1619 Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L., Drake, J.M.,
1620 Brownstein, J.S., Hoen, A.G., Sankoh, O.*, et al.* (2013). The global distribution and burden of
1621 dengue. Nature *496*, 504-507.
1622 Biswal, S., Borja-Tabora, C., Martinez Vargas, L., Velásquez, H., Theresa Alera, M., Sierra, V.,
1623 Johana Rodriguez-Arenales, E., Yu, D., Wickramasinghe, V.P., Duarte Moreira, E., Jr.*, et al.*
1624 (2020). Efficacy of a tetravalent dengue vaccine in healthy children aged 4-16 years: a
1625 randomised, placebo-controlled, phase 3 trial. Lancet *395*, 1423-1433.
1626 Blaney, J.E., Jr., Durbin, A.P., Murphy, B.R., and Whitehead, S.S. (2006). Development of a live
1627 attenuated dengue virus vaccine using reverse genetics. Viral Immunol *19*, 10-32.
1628 Blaney, J.E., Jr., Sathe, N.S., Goddard, L., Hanson, C.T., Romero, T.A., Hanley, K.A., Murphy,
1629 B.R., and Whitehead, S.S. (2008). Dengue virus type 3 vaccine candidates generated by
1630 introduction of deletions in the 3' untranslated region (3'-UTR) or by exchange of the DENV-3
1631 3'-UTR with that of DENV-4. Vaccine *26*, 817-828.
1632 Borges, M.B., Marchevsky, R.S., Carvalho Pereira, R., da Silva Mendes, Y., Almeida Mendes,
1633 L.G., Diniz-Mendes, L., Cruz, M.A., Tahmaoui, O., Baudart, S., and Freire, M. (2019). Detection
1634 of post-vaccination enhanced dengue virus infection in macaques: an improved model for early
1635 assessment of dengue vaccines. PLoS pathogens *15*, e1007721.
1636 Braga, J.U., Bressan, C., Dalvi, A.P.R., Calvet, G.A., Daumas, R.P., Rodrigues, N., Wakimoto,
1637 M., Nogueira, R.M.R., Nielsen-Saines, K., Brito, C.*, et al.* (2017). Accuracy of Zika virus
1638 disease case definition during simultaneous Dengue and Chikungunya epidemics. PLoS One *12*,
1639 e0179725.
1640 Brasil, P., Pereira, J.P., Jr., Moreira, M.E., Ribeiro Nogueira, R.M., Damasceno, L., Wakimoto,
1641 M., Rabello, R.S., Valderramos, S.G., Halai, U.A., Salles, T.S.*, et al.* (2016). Zika Virus
1642 Infection in Pregnant Women in Rio de Janeiro. The New England journal of medicine *375*,
1643 2321-2334.
1644 Breitbach, M.E., Newman, C.M., Dudley, D.M., Stewart, L.M., Aliota, M.T., Koenig, M.R.,
1645 Shepherd, P.M., Yamamoto, K., Crooks, C.M., Young, G.*, et al.* (2019). Primary infection with
1646 dengue or Zika virus does not affect the severity of heterologous secondary infection in
1647 macaques. PLoS pathogens *15*, e1007766.
1648 Brien, J.D., Uhrlaub, J.L., and Nikolich-Zugich, J. (2008). West Nile virus-specific CD4 T cells
1649 exhibit direct antiviral cytokine secretion and cytotoxicity and are sufficient for antiviral
1650 protection. J Immunol *181*, 8568-8575.

1651 Campos, G.C., Sardi, S.I., Sarno, M., and Brites, C. (2016). Zika virus infection, a new public
1652 health challenge. The Brazilian journal of infectious diseases : an official publication of the
1653 Brazilian Society of Infectious Diseases *20*, 227-228.
1654 Cao-Lomeau, V.M., Blake, A., Mons, S., Lastere, S., Roche, C., Vanhomwegen, J., Dub, T.,
1655 Baudouin, L., Teissier, A., Larre, P.*, et al.* (2016). Guillain-Barre Syndrome outbreak associated
1656 with Zika virus infection in French Polynesia: a case-control study. Lancet (London, England)
1657 *10.1016/S0140-6736(16)00562-6*.
1658 Carroll, T., Lo, M., Lanteri, M., Dutra, J., Zarbock, K., Silveira, P., Rourke, T., Ma, Z.M., Fritts,
1659 L., O'Connor, S.*, et al.* (2017). Zika virus preferentially replicates in the female reproductive
1660 tract after vaginal inoculation of rhesus macaques. PLoS pathogens *13*, e1006537.
1661 Carteaux, G., Maquart, M., Bedet, A., Contou, D., Brugieres, P., Fourati, S., Cleret de
1662 Langavant, L., de Broucker, T., Brun-Buisson, C., Leparc-Goffart, I.*, et al.* (2016). Zika Virus
1663 Associated with Meningoencephalitis. The New England journal of medicine *374*, 1595-1596.
1664 Castro-Trujillo, S., Segura, K., Bolívar-Marín, S., Salgado, D.M., Bosch, I., Vega, R., Rojas,
1665 M.C., and Narváez, C.F. (2023). NS1-Specific Antibody Response Facilitates the Identification
1666 of Children With Dengue and Zika in Hyperendemic Areas. Pediatr Infect Dis J.
1667 Chen, M., Venturi, V., and Munier, C.M.L. (2023). Dissecting the Protective Effect of CD8(+) T
1668 Cells in Response to SARS-CoV-2 mRNA Vaccination and the Potential Link with Lymph Node
1669 CD8(+) T Cells. Biology (Basel) *12*.
1670 Churdboonchart, V., Bhamarapravati, N., Peampramprecha, S., and Sirinavin, S. (1991).
1671 Antibodies against dengue viral proteins in primary and secondary dengue hemorrhagic fever.
1672 Am J Trop Med Hyg *44*, 481-493.
1673 Cimini, E., Castilletti, C., Sacchi, A., Casetti, R., Bordoni, V., Romanelli, A., Turchi, F., Martini,
1674 F., Tumino, N., Nicastri, E.*, et al.* (2017). Human Zika infection induces a reduction of IFN-
1675 gamma producing CD4 T-cells and a parallel expansion of effector Vdelta2 T-cells. Scientific
1676 reports *7*, 6313.
1677 Coffey, L.L., Keesler, R.I., Pesavento, P.A., Woolard, K., Singapuri, A., Watanabe, J., Cruzen,
1678 C., Christe, K.L., Usachenko, J., Yee, J.*, et al.* (2018). Intraamniotic Zika virus inoculation of
1679 pregnant rhesus macaques produces fetal neurologic disease. Nature communications *9*, 2414.
1680 Coffey, L.L., Pesavento, P.A., Keesler, R.I., Singapuri, A., Watanabe, J., Watanabe, R., Yee, J.,
1681 Bliss-Moreau, E., Cruzen, C., Christe, K.L.*, et al.* (2017). Zika Virus Tissue and Blood
1682 Compartmentalization in Acute Infection of Rhesus Macaques. PLoS One *12*, e0171148.
1683 Collins, M.H., McGowan, E., Jadi, R., Young, E., Lopez, C.A., Baric, R.S., Lazear, H.M., and de
1684 Silva, A.M. (2017). Lack of Durable Cross-Neutralizing Antibodies Against Zika Virus from
1685 Dengue Virus Infection. Emerging infectious diseases *23*, 773-781.
1686 Costa, S.M., Azevedo, A.S., Paes, M.V., Sarges, F.S., Freire, M.S., and Alves, A.M. (2007).
1687 DNA vaccines against dengue virus based on the ns1 gene: the influence of different signal
1688 sequences on the protein expression and its correlation to the immune response elicited in mice.
1689 Virology *358*, 413-423.
1690 Costa, S.M., Paes, M.V., Barreto, D.F., Pinhão, A.T., Barth, O.M., Queiroz, J.L., Armôa, G.R.,
1691 Freire, M.S., and Alves, A.M. (2006). Protection against dengue type 2 virus induced in mice
1692 immunized with a DNA plasmid encoding the non-structural 1 (NS1) gene fused to the tissue
1693 plasminogen activator signal sequence. Vaccine *24*, 195-205.
1694 Crooks, C.M., Weiler, A.M., Rybarczyk, S.L., Bliss, M.I., Jaeger, A.S., Murphy, M.E.,
1695 Simmons, H.A., Mejia, A., Fritsch, M.K., Hayes, J.M.*, et al.* (2021). Previous exposure to

1696 dengue virus is associated with increased Zika virus burden at the maternal-fetal interface in
1697 rhesus macaques. PLoS Negl Trop Dis *15*, e0009641.
1698 Crotty, S. (2014). T follicular helper cell differentiation, function, and roles in disease. Immunity
1699 *41*, 529-542.
1700 Crotty, S. (2015). A brief history of T cell help to B cells. Nat Rev Immunol *15*, 185-189.
1701 de Alwis, R., Beltramello, M., Messer, W.B., Sukupolvi-Petty, S., Wahala, W.M., Kraus, A.,
1702 Olivarez, N.P., Pham, Q., Brien, J.D., Tsai, W.Y*., et al.* (2011). In-depth analysis of the antibody
1703 response of individuals exposed to primary dengue virus infection. PLoS Negl Trop Dis *5*,
1704 e1188.
1705 Deauvieau, F., Sanchez, V., Balas, C., Kennel, A., A, D.E.M., Lang, J., and Guy, B. (2007).
1706 Innate immune responses in human dendritic cells upon infection by chimeric yellow-fever
1707 dengue vaccine serotypes 1-4. Am J Trop Med Hyg *76*, 144-154.
1708 Dejnirattisai, W., Jumnainsong, A., Onsirisakul, N., Fitton, P., Vasanawathana, S., Limpitikul,
1709 W., Puttikhunt, C., Edwards, C., Duangchinda, T., Supasa, S*., et al.* (2010). Cross-reacting
1710 antibodies enhance dengue virus infection in humans. Science *328*, 745-748.
1711 Duangchinda, T., Dejnirattisai, W., Vasanawathana, S., Limpitikul, W., Tangthawornchaikul, N.,
1712 Malasit, P., Mongkolsapaya, J., and Screaton, G. (2010). Immunodominant T-cell responses to
1713 dengue virus NS3 are associated with DHF. Proc Natl Acad Sci U S A *107*, 16922-16927.
1714 Dudley, D.M., Aliota, M.T., Mohr, E.L., Newman, C.M., Golos, T.G., Friedrich, T.C., and
1715 O'Connor, D.H. (2019). Using Macaques to Address Critical Questions in Zika Virus Research.
1716 Annual review of virology *6*, 481-500.
1717 Dudley, D.M., Aliota, M.T., Mohr, E.L., Weiler, A.M., Lehrer-Brey, G., Weisgrau, K.L., Mohns,
1718 M.S., Breitbach, M.E., Rasheed, M.N., Newman, C.M*., et al.* (2016). A rhesus macaque model
1719 of Asian-lineage Zika virus infection. Nature communications *7*, 12204.
1720 Duffy, M.R., Chen, T.H., Hancock, W.T., Powers, A.M., Kool, J.L., Lanciotti, R.S., Pretrick, M.,
1721 Marfel, M., Holzbauer, S., Dubray, C*., et al.* (2009). Zika virus outbreak on Yap Island,
1722 Federated States of Micronesia. The New England journal of medicine *360*, 2536-2543.
1723 Durbin, A.P., Kirkpatrick, B.D., Pierce, K.K., Elwood, D., Larsson, C.J., Lindow, J.C., Tibery,
1724 C., Sabundayo, B.P., Shaffer, D., Talaat, K.R*., et al.* (2013). A single dose of any of four
1725 different live attenuated tetravalent dengue vaccines is safe and immunogenic in flavivirus-naive
1726 adults: a randomized, double blind clinical trial. J Infect Dis.
1727 Durbin, A.P., Kirkpatrick, B.D., Pierce, K.K., Schmidt, A.C., and Whitehead, S.S. (2011).
1728 Development and clinical evaluation of multiple investigational monovalent DENV vaccines to
1729 identify components for inclusion in a live attenuated tetravalent DENV vaccine. Vaccine *29*,
1730 7242-7250.
1731 Elong Ngono, A., and Shresta, S. (2019). Cross-Reactive T Cell Immunity to Dengue and Zika
1732 Viruses: New Insights Into Vaccine Development. Frontiers in immunology *10*, 1316.
1733 Elong Ngono, A., Young, M.P., Bunz, M., Xu, Z., Hattakam, S., Vizcarra, E., Regla-Nava, J.A.,
1734 Tang, W.W., Yamabhai, M., Wen, J*., et al.* (2019). CD4+ T cells promote humoral immunity
1735 and viral control during Zika virus infection. PLoS pathogens *15*, e1007474.
1736 Elsner, R.A., and Shlomchik, M.J. (2020). Germinal Center and Extrafollicular B Cell Responses
1737 in Vaccination, Immunity, and Autoimmunity. Immunity *53*, 1136-1150.
1738 Fink, K. (2012). Origin and Function of Circulating Plasmablasts during Acute Viral Infections.
1739 Frontiers in immunology *3*, 78.

1740 Fink, K., Manjarrez-Orduño, N., Schildknecht, A., Weber, J., Senn, B.M., Zinkernagel, R.M.,
1741 and Hengartner, H. (2007). B Cell Activation State-Governed Formation of Germinal Centers
1742 following Viral Infection. The Journal of Immunology *179*, 5877-5885.
1743 Furtado, J.M., Esposito, D.L., Klein, T.M., Teixeira-Pinto, T., and da Fonseca, B.A. (2016).
1744 Uveitis Associated with Zika Virus Infection. The New England journal of medicine *375*, 394-
1745 396.
1746 George, J., Valiant, W.G., Mattapallil, M.J., Walker, M., Huang, Y.S., Vanlandingham, D.L.,
1747 Misamore, J., Greenhouse, J., Weiss, D.E., Verthelyi, D.*, et al.* (2017). Prior Exposure to Zika
1748 Virus Significantly Enhances Peak Dengue-2 Viremia in Rhesus Macaques. Scientific reports *7*,
1749 10498.
1750 Godoy-Lozano, E.E., Téllez-Sosa, J., Sánchez-González, G., Sámano-Sánchez, H., Aguilar-
1751 Salgado, A., Salinas-Rodríguez, A., Cortina-Ceballos, B., Vivanco-Cid, H., Hernández-Flores,
1752 K., Pfaff, J.M.*, et al.* (2016). Lower IgG somatic hypermutation rates during acute dengue virus
1753 infection is compatible with a germinal center-independent B cell response. Genome Med *8*, 23.
1754 Goncalves, A.J., Oliveira, E.R., Costa, S.M., Paes, M.V., Silva, J.F., Azevedo, A.S., Mantuano-
1755 Barradas, M., Nogueira, A.C., Almeida, C.J., and Alves, A.M. (2015). Cooperation between
1756 CD4+ T Cells and Humoral Immunity Is Critical for Protection against Dengue Using a DNA
1757 Vaccine Based on the NS1 Antigen. PLoS Negl Trop Dis *9*, e0004277.
1758 Goncalvez, A.P., Chien, C.H., Tubthong, K., Gorshkova, I., Roll, C., Donau, O., Schuck, P.,
1759 Yoksan, S., Wang, S.D., Purcell, R.H.*, et al.* (2008). Humanized monoclonal antibodies derived
1760 from chimpanzee Fabs protect against Japanese encephalitis virus in vitro and in vivo. J Virol *82*,
1761 7009-7021.
1762 Graham, N., Eisenhauer, P., Diehl, S.A., Pierce, K.K., Whitehead, S.S., Durbin, A.P.,
1763 Kirkpatrick, B.D., Sette, A., Weiskopf, D., Boyson, J.E.*, et al.* (2020). Rapid Induction and
1764 Maintenance of Virus-Specific CD8(+) T(EMRA) and CD4(+) T(EM) Cells Following
1765 Protective Vaccination Against Dengue Virus Challenge in Humans. Frontiers in immunology
1766 *11*, 479.
1767 Grifoni, A., Angelo, M.A., Lopez, B., O'Rourke, P.H., Sidney, J., Cerpas, C., Balmaseda, A.,
1768 Silveira, C.G.T., Maestri, A., Costa, P.R.*, et al.* (2017a). Global Assessment of Dengue Virus-
1769 Specific CD4(+) T Cell Responses in Dengue-Endemic Areas. Frontiers in immunology *8*, 1309.
1770 Grifoni, A., Angelo, M.A., Lopez, B., O'Rourke, P.H., Sidney, J., Cerpas, C., Balmaseda, A.,
1771 Silveira, C.G.T., Maestri, A., Costa, P.R.*, et al.* (2017b). Global Assessment of Dengue Virus-
1772 Specific CD4(+) T Cell Responses in Dengue-Endemic Areas. Frontiers in immunology *8*, 1309-
1773 1309.
1774 Grifoni, A., Pham, J., Sidney, J., O'Rourke, P.H., Paul, S., Peters, B., Martini, S.R., de Silva,
1775 A.D., Ricciardi, M.J., Magnani, D.M.*, et al.* (2017c). Prior Dengue Virus Exposure Shapes T
1776 Cell Immunity to Zika Virus in Humans. J Virol *91*.
1777 Grifoni, A., Voic, H., Dhanda, S.K., Kidd, C.K., Brien, J.D., Buus, S., Stryhn, A., Durbin, A.P.,
1778 Whitehead, S., Diehl, S.A.*, et al.* (2020). T Cell Responses Induced by Attenuated Flavivirus
1779 Vaccination Are Specific and Show Limited Cross-Reactivity with Other Flavivirus Species. J
1780 Virol *94*.
1781 Guirakhoo, F., Arroyo, J., Pugachev, K.V., Miller, C., Zhang, Z.X., Weltzin, R.,
1782 Georgakopoulos, K., Catalan, J., Ocran, S., Soike, K.*, et al.* (2001). Construction, safety, and
1783 immunogenicity in nonhuman primates of a chimeric yellow fever-dengue virus tetravalent
1784 vaccine. J Virol *75*, 7290-7304.

1785 Guirakhoo, F., Pugachev, K., Arroyo, J., Miller, C., Zhang, Z.X., Weltzin, R., Georgakopoulos,
1786 K., Catalan, J., Ocran, S., Draper, K.*, et al.* (2002). Viremia and immunogenicity in nonhuman
1787 primates of a tetravalent yellow fever-dengue chimeric vaccine: genetic reconstructions, dose
1788 adjustment, and antibody responses against wild-type dengue virus isolates. Virology *298*, 146-
1789 159.
1790 Gupta, A.K., Lad, V.J., and Koshy, A.A. (2008). Survival of mice immunized with monoclonal
1791 antibodies against glycoprotein E of Japanese encephalitis virus before or after infection with
1792 Japanese encephalitis, West Nile, and Dengue viruses. Acta Virol *52*, 219-224.
1793 Guy, B., Barban, V., Mantel, N., Aguirre, M., Gulia, S., Pontvianne, J., Jourdier, T.M., Ramirez,
1794 L., Gregoire, V., Charnay, C.*, et al.* (2009). Evaluation of interferences between dengue vaccine
1795 serotypes in a monkey model. Am J Trop Med Hyg *80*, 302-311.
1796 Guy, B., Noriega, F., Ochiai, R.L., L'Azou, M., Delore, V., Skipetrova, A., Verdier, F.,
1797 Coudeville, L., Savarino, S., and Jackson, N. (2017). A recombinant live attenuated tetravalent
1798 vaccine for the prevention of dengue. Expert Rev Vaccines *16*, 1-13.
1799 Guy, B., Nougarede, N., Begue, S., Sanchez, V., Souag, N., Carre, M., Chambonneau, L.,
1800 Morrisson, D.N., Shaw, D., Qiao, M.*, et al.* (2008). Cell-mediated immunity induced by chimeric
1801 tetravalent dengue vaccine in naive or flavivirus-primed subjects. Vaccine *26*, 5712-5721.
1802 Guy, B., Ooi, E.E., Ramos-Castañeda, J., and Thomas, S.J. (2020). When Can One Vaccinate
1803 with a Live Vaccine after Wild-Type Dengue Infection? Vaccines *8*.
1804 Guzman, M.G., Alvarez, M., and Halstead, S.B. (2013). Secondary infection as a risk factor for
1805 dengue hemorrhagic fever/dengue shock syndrome: an historical perspective and role of
1806 antibody-dependent enhancement of infection. Arch Virol *158*, 1445-1459.
1807 Guzman, M.G., Jaenisch, T., Gaczkowski, R., Ty Hang, V.T., Sekaran, S.D., Kroeger, A.,
1808 Vazquez, S., Ruiz, D., Martinez, E., Mercado, J.C.*, et al.* (2010). Multi-country evaluation of the
1809 sensitivity and specificity of two commercially-available NS1 ELISA assays for dengue
1810 diagnosis. PLoS Negl Trop Dis *4*.
1811 Haddow, A.D., Nalca, A., Rossi, F.D., Miller, L.J., Wiley, M.R., Perez-Sautu, U., Washington,
1812 S.C., Norris, S.L., Wollen-Roberts, S.E., Shamblin, J.D.*, et al.* (2017). High Infection Rates for
1813 Adult Macaques after Intravaginal or Intrarectal Inoculation with Zika Virus. Emerging
1814 infectious diseases *DOI 10.3201/eid2308.170036*.
1815 Hadinegoro, S.R., Arredondo-Garcia, J.L., Capeding, M.R., Deseda, C., Chotpitayasunondh, T.,
1816 Dietze, R., Muhammad Ismail, H.I., Reynales, H., Limkittikul, K., Rivera-Medina, D.M.*, et al.*
1817 (2015). Efficacy and Long-Term Safety of a Dengue Vaccine in Regions of Endemic Disease.
1818 The New England journal of medicine *373*, 1195-1206.
1819 Halstead, S. (2003). Neutralization and Antibody-Dependent Enhancement of Dengue Viruses.
1820 Advances in Viruses Research *60*.
1821 Halstead, S.B. (2018). Safety issues from a Phase 3 clinical trial of a live-attenuated chimeric
1822 yellow fever tetravalent dengue vaccine. Hum Vaccin Immunother *14*, 2158-2162.
1823 Halstead, S.B., Rojanasuphot, S., and Sangkawibha, N. (1983). Original antigenic sin in dengue.
1824 Am J Trop Med Hyg *32*, 154-156.
1825 Haltaufderhyde, K., Srikiatkhachorn, A., Green, S., Macareo, L., Park, S., Kalayanarooj, S.,
1826 Rothman, A.L., and Mathew, A. (2018). Activation of Peripheral T Follicular Helper Cells
1827 During Acute Dengue Virus Infection. J Infect Dis *218*, 1675-1685.
1828 Hassert, M., Wolf, K.J., Schwetye, K.E., DiPaolo, R.J., Brien, J.D., and Pinto, A.K. (2018).
1829 CD4+T cells mediate protection against Zika associated severe disease in a mouse model of
1830 infection. PLoS pathogens *14*, e1007237.

1831 Henchal, E.A., Henchal, L.S., and Schlesinger, J.J. (1988). Synergistic interactions of anti-NS1
1832 monoclonal antibodies protect passively immunized mice from lethal challenge with dengue 2
1833 virus. J Gen Virol *69 (Pt 8)*, 2101-2107.
1834 Henein, S., Adams, C., Bonaparte, M., Moser, J.M., Munteanu, A., Baric, R., and de Silva, A.M.
1835 (2021). Dengue vaccine breakthrough infections reveal properties of neutralizing antibodies
1836 linked to protection. J Clin Invest *131*.
1837 Huang, C.Y., Kinney, R.M., Livengood, J.A., Bolling, B., Arguello, J.J., Luy, B.E., Silengo, S.J.,
1838 Boroughs, K.L., Stovall, J.L., Kalanidhi, A.P.*, et al.* (2013). Genetic and phenotypic
1839 characterization of manufacturing seeds for a tetravalent dengue vaccine (DENVax). PLoS Negl
1840 Trop Dis *7*, e2243.
1841 Izmirly, A.M., Pelletier, A.N., Connors, J., Taramangalam, B., Alturki, S.O., Gordon, E.A.,
1842 Alturki, S.O., Mell, J.C., Swaminathan, G., Karthik, V.*, et al.* (2022). Pre-vaccination frequency
1843 of circulatory Tfh is associated with robust immune response to TV003 dengue vaccine. PLoS
1844 Pathog *18*, e1009903.
1845 Kallás, E.G., Cintra, M.A.T., Moreira, J.A., Patiño, E.G., Braga, P.E., Tenório, J.C.V., Infante,
1846 V., Palacios, R., de Lacerda, M.V.G., Batista Pereira, D.*, et al.* (2024). Live, Attenuated,
1847 Tetravalent Butantan-Dengue Vaccine in Children and Adults. The New England journal of
1848 medicine *390*, 397-408.
1849 Katzelnick, L.C., Bos, S., and Harris, E. (2020a). Protective and enhancing interactions among
1850 dengue viruses 1-4 and Zika virus. Curr Opin Virol *43*, 59-70.
1851 Katzelnick, L.C., Gresh, L., Halloran, M.E., Mercado, J.C., Kuan, G., Gordon, A., Balmaseda,
1852 A., and Harris, E. (2017). Antibody-dependent enhancement of severe dengue disease in humans.
1853 Science *358*, 929-932.
1854 Katzelnick, L.C., and Harris, E. (2017). Immune correlates of protection for dengue: State of the
1855 art and research agenda. Vaccine *35*, 4659-4669.
1856 Katzelnick, L.C., Narvaez, C., Arguello, S., Lopez Mercado, B., Collado, D., Ampie, O.,
1857 Elizondo, D., Miranda, T., Bustos Carillo, F., Mercado, J.C.*, et al.* (2020b). Zika virus infection
1858 enhances future risk of severe dengue disease. Science *369*, 1123-1128.
1859 Katzelnick, L.C., Zambrana, J.V., Elizondo, D., Collado, D., Garcia, N., Arguello, S., Mercado,
1860 J.C., Miranda, T., Ampie, O., Mercado, B.L.*, et al.* (2021). Dengue and Zika virus infections in
1861 children elicit cross-reactive protective and enhancing antibodies that persist long term. Science
1862 translational medicine *13*, eabg9478.
1863 Kinney, R.M., Butrapet, S., Chang, G.-J.J., Tsuchiya, K.R., Roehrig, J.T., Bhamarapravati, N.,
1864 and Gubler, D.J. (1997). Construction of Infectious cDNA Clones for Dengue 2 Virus: Strain
1865 16681 and Its Attenuated Vaccine Derivative, Strain PDK-53. Virology *230*, 300-308.
1866 Kirkpatrick, B.D., Whitehead, S.S., Pierce, K.K., Tibery, C.M., Grier, P.L., Hynes, N.A.,
1867 Larsson, C.J., Sabundayo, B.P., Talaat, K.R., Janiak, A.*, et al.* (2016). The live attenuated
1868 dengue vaccine TV003 elicits complete protection against dengue in a human challenge model.
1869 Science translational medicine *8*, 330ra336.
1870 Koblischke, M., Mackroth, M.S., Schwaiger, J., Fae, I., Fischer, G., Stiasny, K., Heinz, F.X., and
1871 Aberle, J.H. (2017). Protein structure shapes immunodominance in the CD4 T cell response to
1872 yellow fever vaccination. Scientific reports *7*, 8907.
1873 Koblischke, M., Stiasny, K., Aberle, S.W., Malafa, S., Tsouchnikas, G., Schwaiger, J., Kundi,
1874 M., Heinz, F.X., and Aberle, J.H. (2018). Structural Influence on the Dominance of Virus-
1875 Specific CD4 T Cell Epitopes in Zika Virus Infection. Frontiers in immunology *9*, 1196.

1876 Landry, S.J., Moss, D.L., Cui, D., Ferrie, R.P., Fullerton, M.L., Wells, E.A., Yang, L., Zhou, N.,
1877 Dougherty, T., and Mettu, R.R. (2017). Structural Basis for CD4+ T Cell Epitope Dominance in
1878 Arbo-Flavivirus Envelope Proteins: A Meta-Analysis. Viral Immunol *30*, 479-489.
1879 Larocca, R.A., Abbink, P., Ventura, J.D., Chandrashekar, A., Mercado, N., Li, Z., Borducchi, E.,
1880 De La Barrera, R.A., Eckels, K.H., Modjarrad, K.*, et al.* (2021). Impact of prior Dengue
1881 immunity on Zika vaccine protection in rhesus macaques and mice. PLoS Pathog *17*, e1009673.
1882 Lei, C., Yu, Q., Wang, H., Liu, J., Chen, S., Zhao, Z., and Qiu, L. (2021). Responses of CD27(+)
1883 CD38(+) plasmablasts, and CD24(hi) CD27(hi) and CD24(hi) CD38(hi) regulatory B cells
1884 during primary dengue virus 2 infection. J Clin Lab Anal *35*, e24035.
1885 Leonova, G.N., and Pavlenko, E.V. (2009). Characterization of neutralizing antibodies to Far
1886 Eastern of tick-borne encephalitis virus subtype and the antibody avidity for four tick-borne
1887 encephalitis vaccines in human. Vaccine *27*, 2899-2904.
1888 Li, N., Zhang, Z.R., Zhang, Y.N., Liu, J., Deng, C.L., Shi, P.Y., Yuan, Z.M., Ye, H.Q., and
1889 Zhang, B. (2020). A replication-defective Japanese encephalitis virus (JEV) vaccine candidate
1890 with NS1 deletion confers dual protection against JEV and West Nile virus in mice. NPJ
1891 Vaccines *5*, 73.
1892 Li, Z., Wang, J., Cheng, X., Hu, H., Guo, C., Huang, J., Chen, Z., and Lu, J. (2021). The
1893 worldwide seroprevalence of DENV, CHIKV and ZIKV infection: A systematic review and
1894 meta-analysis. PLoS Negl Trop Dis *15*, e0009337.
1895 Liang, H., Tang, J., Liu, Z., Liu, Y., Huang, Y., Xu, Y., Hao, P., Yin, Z., Zhong, J., Ye, L.*, et al.*
1896 (2019). ZIKV infection induces robust Th1-like Tfh cell and long-term protective antibody
1897 responses in immunocompetent mice. Nature communications *10*, 3859.
1898 Lima, N.S., Moon, D., Darko, S., De La Barrera, R.A., Lin, L., Koren, M.A., Jarman, R.G.,
1899 Eckels, K.H., Thomas, S.J., Michael, N.L.*, et al.* (2021). Pre-existing Immunity to Japanese
1900 Encephalitis Virus Alters CD4 T Cell Responses to Zika Virus Inactivated Vaccine. Front
1901 Immunol *12*, 640190.
1902 Lucas, C.G.O., Kitoko, J.Z., Ferreira, F.M., Suzart, V.G., Papa, M.P., Coelho, S.V.A.,
1903 Cavazzoni, C.B., Paula-Neto, H.A., Olsen, P.C., Iwasaki, A.*, et al.* (2018). Critical role of
1904 CD4(+) T cells and IFNgamma signaling in antibody-mediated resistance to Zika virus infection.
1905 Nature communications *9*, 3136.
1906 Maikova, G.B., Chernokhaeva, L.L., Vorovitch, M.F., Rogova, Y.V., and Karganova, G.G.
1907 (2016). [Vaccines based on the Far-Eastern and European strains induce the neutralizing
1908 antibodies against all known tick-borne encephalitis virus subtypes]. Vopr Virusol *61*, 135-139.
1909 Mangada, M.M., and Rothman, A.L. (2005). Altered cytokine responses of dengue-specific
1910 CD4+ T cells to heterologous serotypes. J Immunol *175*, 2676-2683.
1911 Manh, D.H., Weiss, L.N., Thuong, N.V., Mizukami, S., Dumre, S.P., Luong, Q.C., Thanh, L.C.,
1912 Thang, C.M., Huu, P.T., Phuc, L.H.*, et al.* (2020). Kinetics of CD4(+) T Helper and CD8(+)
1913 Effector T Cell Responses in Acute Dengue Patients. Frontiers in immunology *11*, 1980-1980.
1914 Martinez-Murillo, P., Pramanik, L., Sundling, C., Hultenby, K., Wretenberg, P., Spångberg, M.,
1915 and Karlsson Hedestam, G.B. (2016). CD138 and CD31 Double-Positive Cells Comprise the
1916 Functional Antibody-Secreting Plasma Cell Compartment in Primate Bone Marrow. Front
1917 Immunol *7*, 242.
1918 Marzan-Rivera, N., Serrano-Collazo, C., Cruz, L., Pantoja, P., Ortiz-Rosa, A., Arana, T.,
1919 Martinez, M.I., Burgos, A.G., Roman, C., Mendez, L.B.*, et al.* (2022). Infection order outweighs
1920 the role of CD4(+) T cells in tertiary flavivirus exposure. iScience *25*, 104764.

1921 McCracken, M.K., Gromowski, G.D., Friberg, H.L., Lin, X., Abbink, P., De La Barrera, R.,
1922 Eckles, K.H., Garver, L.S., Boyd, M., Jetton, D.*, et al.* (2017). Impact of prior flavivirus
1923 immunity on Zika virus infection in rhesus macaques. PLoS Pathog *13*, e1006487.
1924 Mecharles, S., Herrmann, C., Poullain, P., Tran, T.H., Deschamps, N., Mathon, G., Landais, A.,
1925 Breurec, S., and Lannuzel, A. (2016). Acute myelitis due to Zika virus infection. Lancet
1926 (London, England) *387*, 1481.
1927 Messina, J.P., Brady, O.J., Golding, N., Kraemer, M.U.G., Wint, G.R.W., Ray, S.E., Pigott,
1928 D.M., Shearer, F.M., Johnson, K., Earl, L.*, et al.* (2019). The current and future global
1929 distribution and population at risk of dengue. Nat Microbiol *4*, 1508-1515.
1930 Mladinich, K.M., Piaskowski, S.M., Rudersdorf, R., Eernisse, C.M., Weisgrau, K.L., Martins,
1931 M.A., Furlott, J.R., Partidos, C.D., Brewoo, J.N., Osorio, J.E.*, et al.* (2012). Dengue virus-
1932 specific CD4+ and CD8+ T lymphocytes target NS1, NS3 and NS5 in infected Indian rhesus
1933 macaques. Immunogenetics *64*, 111-121.
1934 Morita, R., Schmitt, N., Bentebibel, S.E., Ranganathan, R., Bourdery, L., Zurawski, G., Foucat,
1935 E., Dullaers, M., Oh, S., Sabzghabaei, N.*, et al.* (2011). Human blood CXCR5(+)CD4(+) T cells
1936 are counterparts of T follicular cells and contain specific subsets that differentially support
1937 antibody secretion. Immunity *34*, 108-121.
1938 Nath, B., Vandna, Saini, H.M., Prasad, M., and Kumar, S. (2020). Evaluation of Japanese
1939 encephalitis virus E and NS1 proteins immunogenicity using a recombinant Newcastle disease
1940 virus in mice. Vaccine *38*, 1860-1868.
1941 Nivarthi, U.K., Swanstrom, J., Delacruz, M.J., Patel, B., Durbin, A.P., Whitehead, S.S.,
1942 Kirkpatrick, B.D., Pierce, K.K., Diehl, S.A., Katzelnick, L.*, et al.* (2021). A tetravalent live
1943 attenuated dengue virus vaccine stimulates balanced immunity to multiple serotypes in humans.
1944 Nature communications *12*, 1102.
1945 Onlamoon, N., Noisakran, S., Hsiao, H.M., Duncan, A., Villinger, F., Ansari, A.A., and Perng,
1946 G.C. (2010). Dengue virus-induced hemorrhage in a nonhuman primate model. Blood *115*, 1823-
1947 1834.
1948 Osorio, J.E., Brewoo, J.N., Silengo, S.J., Arguello, J., Moldovan, I.R., Tary-Lehmann, M.,
1949 Powell, T.D., Livengood, J.A., Kinney, R.M., Huang, C.Y.*, et al.* (2011a). Efficacy of a
1950 tetravalent chimeric dengue vaccine (DENVax) in Cynomolgus macaques. Am J Trop Med Hyg
1951 *84*, 978-987.
1952 Osorio, J.E., Huang, C.Y., Kinney, R.M., and Stinchcomb, D.T. (2011b). Development of
1953 DENVax: a chimeric dengue-2 PDK-53-based tetravalent vaccine for protection against dengue
1954 fever. Vaccine *29*, 7251-7260.
1955 Osorio, J.E., Velez, I.D., Thomson, C., Lopez, L., Jimenez, A., Haller, A.A., Silengo, S., Scott,
1956 J., Boroughs, K.L., Stovall, J.L.*, et al.* (2014). Safety and immunogenicity of a recombinant live
1957 attenuated tetravalent dengue vaccine (DENVax) in flavivirus-naive healthy adults in Colombia:
1958 a randomised, placebo-controlled, phase 1 study. Lancet Infect Dis *14*, 830-838.
1959 Paixao, E.S., Cardim, L.L., Costa, M.C.N., Brickley, E.B., de Carvalho-Sauer, R.C.O., Carmo,
1960 E.H., Andrade, R.F.S., Rodrigues, M.S., Veiga, R.V., Costa, L.C.*, et al.* (2022). Mortality from
1961 Congenital Zika Syndrome - Nationwide Cohort Study in Brazil. The New England journal of
1962 medicine *386*, 757-767.
1963 Pantoja, P., Perez-Guzman, E.X., Rodriguez, I.V., White, L.J., Gonzalez, O., Serrano, C.,
1964 Giavedoni, L., Hodara, V., Cruz, L., Arana, T.*, et al.* (2017). Zika virus pathogenesis in rhesus
1965 macaques is unaffected by pre-existing immunity to dengue virus. Nature communications *8*,
1966 15674.

1967 Pardy, R.D., Gentile, M.E., Carter, A.M., Condotta, S.A., King, I.L., and Richer, M.J. (2022). An
1968 Epidemic Zika Virus Isolate Drives Enhanced T Follicular Helper Cell and B Cell-Mediated
1969 Immunity. J Immunol *208*, 1719-1728.
1970 Patel, B., Longo, P., Miley, M.J., Montoya, M., Harris, E., and de Silva, A.M. (2017). Dissecting
1971 the human serum antibody response to secondary dengue virus infections. PLoS Negl Trop Dis
1972 *11*, e0005554.
1973 Perera, D.R., Ranadeva, N.D., Sirisena, K., and Wijesinghe, K.J. (2024). Roles of NS1 Protein in
1974 Flavivirus Pathogenesis. ACS Infect Dis *10*, 20-56.
1975 Perez-Guzman, E.X., Pantoja, P., Serrano-Collazo, C., Hassert, M.A., Ortiz-Rosa, A., Rodriguez,
1976 I.V., Giavedoni, L., Hodara, V., Parodi, L., Cruz, L.*, et al.* (2019). Time elapsed between Zika
1977 and dengue virus infections affects antibody and T cell responses. Nature communications *10*,
1978 4316.
1979 Petphong, V., Kosoltanapiwat, N., Limkittikul, K., Maneekan, P., Chatchen, S., Jittmittraphap,
1980 A., Sriburin, P., Chattanadee, S., and Leaungwutiwong, P. (2023). Detection of Anti-ZIKV NS1
1981 IgA, IgM, and Combined IgA/IgM and Identification of IL-4 and IL-10 as Potential Biomarkers
1982 for Early ZIKV and DENV Infections in Hyperendemic Regions, Thailand. Trop Med Infect Dis
1983 *8*.
1984 Pinheiro, J.R., Camilo Dos Reis, E., Souza, R., Rocha, A.L.S., Suesdek, L., Azevedo, V., Tiwari,
1985 S., Rocha, B.G.S., Birbrair, A., Méndez, E.C.*, et al.* (2021). Comparison of Neutralizing Dengue
1986 Virus B Cell Epitopes and Protective T Cell Epitopes With Those in Three Main Dengue Virus
1987 Vaccines. Frontiers in immunology *12*, 715136.
1988 Qiao, L., Martelli, C.M.T., Raja, A.I., Sanchez Clemente, N., de Araùjo, T.V.B., Ximenes,
1989 R.A.A., Miranda-Filho, D.B., Ramond, A., and Brickley, E.B. (2021). Epidemic preparedness:
1990 Prenatal Zika virus screening during the next epidemic. BMJ Glob Health *6*.
1991 Quigley, M.F., Gonzalez, V.D., Granath, A., Andersson, J., and Sandberg, J.K. (2007). CXCR5+
1992 CCR7- CD8 T cells are early effector memory cells that infiltrate tonsil B cell follicles. Eur J
1993 Immunol *37*, 3352-3362.
1994 Raengsakulrach, B., Nisalak, A., Gettayacamin, M., Thirawuth, V., Young, G.D., Myint, K.S.,
1995 Ferguson, L.M., Hoke, C.H., Jr., Innis, B.L., and Vaughn, D.W. (1999). Safety, immunogenicity,
1996 and protective efficacy of NYVAC-JEV and ALVAC-JEV recombinant Japanese encephalitis
1997 vaccines in rhesus monkeys. Am J Trop Med Hyg *60*, 343-349.
1998 Raut, R., Corbett, K.S., Tennekoon, R.N., Premawansa, S., Wijewickrama, A., Premawansa, G.,
1999 Mieczkowski, P., Rückert, C., Ebel, G.D., De Silva, A.D.*, et al.* (2019). Dengue type 1 viruses
2000 circulating in humans are highly infectious and poorly neutralized by human antibodies. Proc
2001 Natl Acad Sci U S A *116*, 227-232.
2002 Regla-Nava, J.A., Elong Ngono, A., Viramontes, K.M., Huynh, A.T., Wang, Y.T., Nguyen,
2003 A.T., Salgado, R., Mamidi, A., Kim, K., Diamond, M.S.*, et al.* (2018). Cross-reactive Dengue
2004 virus-specific CD8(+) T cells protect against Zika virus during pregnancy. Nature
2005 communications *9*, 3042.
2006 Rivino, L., Kumaran, E.A., Jovanovic, V., Nadua, K., Teo, E.W., Pang, S.W., Teo, G.H., Gan,
2007 V.C., Lye, D.C., Leo, Y.S.*, et al.* (2013). Differential targeting of viral components by CD4+
2008 versus CD8+ T lymphocytes in dengue virus infection. J Virol *87*, 2693-2706.
2009 Rivino, L., and Lim, M.Q. (2017). CD4(+) and CD8(+) T-cell immunity to Dengue - lessons for
2010 the study of Zika virus. Immunology *150*, 146-154.

2011 Rodriguez-Morales, A.J., Villamil-Gómez, W.E., and Franco-Paredes, C. (2016). The arboviral
2012 burden of disease caused by co-circulation and co-infection of dengue, chikungunya and Zika in
2013 the Americas. Travel medicine and infectious disease *14*, 177-179.
2014 Rogers, T.F., Goodwin, E.C., Briney, B., Sok, D., Beutler, N., Strubel, A., Nedellec, R., Le, K.,
2015 Brown, M.E., Burton, D.R.*, et al.* (2017). Zika virus activates de novo and cross-reactive
2016 memory B cell responses in dengue-experienced donors. Science immunology *2*.
2017 Roth, C., Delgado, F.G., Simon-Lorière, E., and Sakuntabhai, A. (2018). Immune Responses to
2018 Dengue and Zika Viruses-Guidance for T Cell Vaccine Development. Int J Environ Res Public
2019 Health *15*.
2020 Rothman, A.L. (2010). Cellular immunology of sequential dengue virus infection and its role in
2021 disease pathogenesis. Curr Top Microbiol Immunol *338*, 83-98.
2022 Rouers, A., Chng, M.H.Y., Lee, B., Rajapakse, M.P., Kaur, K., Toh, Y.X., Sathiakumar, D., Loy,
2023 T., Thein, T.L., Lim, V.W.X.*, et al.* (2021). Immune cell phenotypes associated with disease
2024 severity and long-term neutralizing antibody titers after natural dengue virus infection. Cell Rep
2025 Med *2*, 100278.
2026 Roze, B., Najioullah, F., Signate, A., Apetse, K., Brouste, Y., Gourgoudou, S., Fagour, L., Abel,
2027 S., Hochedez, P., Cesaire, R.*, et al.* (2016). Zika virus detection in cerebrospinal fluid from two
2028 patients with encephalopathy, Martinique, February 2016. Euro surveillance : bulletin Europeen
2029 sur les maladies transmissibles = European communicable disease bulletin *21*, 10.2807/1560-
2030 7917.ES.2016.2821.2816.30205.
2031 Rupp, R., Luckasen, G.J., Kirstein, J.L., Osorio, J.E., Santangelo, J.D., Raanan, M., Smith, M.K.,
2032 Wallace, D., Gordon, G.S., and Stinchcomb, D.T. (2015). Safety and immunogenicity of
2033 different doses and schedules of a live attenuated tetravalent dengue vaccine (TDV) in healthy
2034 adults: A Phase 1b randomized study. Vaccine *33*, 6351-6359.
2035 Sabchareon, A., Wallace, D., Sirivichayakul, C., Limkittikul, K., Chanthavanich, P.,
2036 Suvannadabba, S., Jiwariyavej, V., Dulyachai, W., Pengsaa, K., Wartel, T.A.*, et al.* (2012).
2037 Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai
2038 schoolchildren: a randomised, controlled phase 2b trial. Lancet *380*, 1559-1567.
2039 Saini, M., and Vrati, S. (2003). A Japanese encephalitis virus peptide present on Johnson grass
2040 mosaic virus-like particles induces virus-neutralizing antibodies and protects mice against lethal
2041 challenge. J Virol *77*, 3487-3494.
2042 Sánchez-Vargas, L.A., and Mathew, A. (2019). Peripheral follicular helper T cells in acute viral
2043 diseases: a perspective on dengue. Future virology *14*, 161-169.
2044 Sandberg, J.T., Ols, S., Löfling, M., Varnaitė, R., Lindgren, G., Nilsson, O., Rombo, L., Kalén,
2045 M., Loré, K., Blom, K.*, et al.* (2021). Activation and Kinetics of Circulating T Follicular Helper
2046 Cells, Specific Plasmablast Response, and Development of Neutralizing Antibodies following
2047 Yellow Fever Virus Vaccination. J Immunol *207*, 1033-1043.
2048 Sariol, C.A., Martinez, M.I., Rivera, F., Rodriguez, I.V., Pantoja, P., Abel, K., Arana, T.,
2049 Giavedoni, L., Hodara, V., White, L.J.*, et al.* (2011). Decreased Dengue Replication and an
2050 Increased Anti-viral Humoral Response with the use of Combined Toll-Like Receptor 3 and 7/8
2051 Agonists in Macaques. PLoS One *6*, e19323.
2052 Sariol, C.A., Munoz-Jordan, J.L., Abel, K., Rosado, L.C., Pantoja, P., Giavedoni, L., Rodriguez,
2053 I.V., White, L.J., Martinez, M., Arana, T.*, et al.* (2007). Transcriptional activation of interferon-
2054 stimulated genes but not of cytokine genes after primary infection of rhesus macaques with
2055 dengue virus type 1. Clin Vaccine Immunol *14*, 756-766.

2056 Sariol, C.A., and White, L.J. (2014). Utility, limitations, and future of non-human primates for
2057 dengue research and vaccine development. Frontiers in immunology *5*, 452.
2058 Schwaiger, J., Aberle, J.H., Stiasny, K., Knapp, B., Schreiner, W., Fae, I., Fischer, G., Scheinost,
2059 O., Chmelik, V., and Heinz, F.X. (2014). Specificities of human CD4+ T cell responses to an
2060 inactivated flavivirus vaccine and infection: correlation with structure and epitope prediction. J
2061 Virol *88*, 7828-7842.
2062 Serrano-Collazo, C., Pérez-Guzmán, E.X., Pantoja, P., Hassert, M.A., Rodríguez, I.V.,
2063 Giavedoni, L., Hodara, V., Parodi, L., Cruz, L., Arana, T.*, et al.* (2020). Effective control of
2064 early Zika virus replication by Dengue immunity is associated to the length of time between the
2065 2 infections but not mediated by antibodies. PLoS Negl Trop Dis *14*, e0008285.
2066 Sharp, T.M., Munoz-Jordan, J., Perez-Padilla, J., Bello-Pagan, M.I., Rivera, A., Pastula, D.M.,
2067 Salinas, J.L., Martinez Mendez, J.H., Mendez, M., Powers, A.M.*, et al.* (2016). Zika Virus
2068 Infection Associated With Severe Thrombocytopenia. Clinical infectious diseases : an official
2069 publication of the Infectious Diseases Society of America *DOI 10.1093/cid/ciw476*.
2070 Shi, J., Hou, S., Fang, Q., Liu, X., Liu, X., and Qi, H. (2018). PD-1 Controls Follicular T Helper
2071 Cell Positioning and Function. Immunity *49*, 264-274.e264.
2072 Shu, P.Y., Chen, L.K., Chang, S.F., Yueh, Y.Y., Chow, L., Chien, L.J., Chin, C., Lin, T.H., and
2073 Huang, J.H. (2000). Dengue NS1-specific antibody responses: isotype distribution and
2074 serotyping in patients with Dengue fever and Dengue hemorrhagic fever. J Med Virol *62*, 224-
2075 232.
2076 Sierra, B., Alegre, R., Perez, A.B., Garcia, G., Sturn-Ramirez, K., Obasanjo, O., Aguirre, E.,
2077 Alvarez, M., Rodriguez-Roche, R., Valdes, L.*, et al.* (2007). HLA-A, -B, -C, and -DRB1 allele
2078 frequencies in Cuban individuals with antecedents of dengue 2 disease: advantages of the Cuban
2079 population for HLA studies of dengue virus infection. Hum Immunol *68*, 531-540.
2080 Silveira, E.L., Kasturi, S.P., Kovalenkov, Y., Rasheed, A.U., Yeiser, P., Jinnah, Z.S., Legere,
2081 T.H., Pulendran, B., Villinger, F., and Wrammert, J. (2015). Vaccine-induced plasmablast
2082 responses in rhesus macaques: phenotypic characterization and a source for generating antigen-
2083 specific monoclonal antibodies. J Immunol Methods *416*, 69-83.
2084 Singh, T., Miller, I.G., Venkatayogi, S., Webster, H., Heimsath, H.J., Eudailey, J.A., Dudley,
2085 D.M., Kumar, A., Mangan, R.J., Thein, A.*, et al.* (2024). Prior dengue virus serotype 3 infection
2086 modulates subsequent plasmablast responses to Zika virus infection in rhesus macaques. mBio,
2087 e0316023.
2088 Stebegg, M., Kumar, S.D., Silva-Cayetano, A., Fonseca, V.R., Linterman, M.A., and Graca, L.
2089 (2018). Regulation of the Germinal Center Response. Frontiers in immunology *9*.
2090 Swain, S.L., McKinstry, K.K., and Strutt, T.M. (2012). Expanding roles for $CD4^+$ T cells in
2091 immunity to viruses. Nat Rev Immunol *12*, 136-148.
2092 Team, R.C., Team, M.R.C., Suggests, M., and Matrix, S. (2018). Package stats. The R Stats
2093 Package.
2094 Team, R.D.C. (2010). R: A language and environment for statistical computing. (No Title).
2095 Thomas, S.J., Hombach, J., and Barrett, A. (2009). Scientific consultation on cell mediated
2096 immunity (CMI) in dengue and dengue vaccine development. Vaccine *27*, 355-368.
2097 Tian, N., Zheng, J.X., Guo, Z.Y., Li, L.H., Xia, S., Lv, S., and Zhou, X.N. (2022). Dengue
2098 Incidence Trends and Its Burden in Major Endemic Regions from 1990 to 2019. Trop Med Infect
2099 Dis *7*.
2100 Tian, Y., Grifoni, A., Sette, A., and Weiskopf, D. (2019). Human T Cell Response to Dengue
2101 Virus Infection. Frontiers in immunology *10*, 2125.

2102 Tricou, V., Gottardo, R., Egan, M.A., Clement, F., Leroux-Roels, G., Sáez-Llorens, X.,
2103 Borkowski, A., Wallace, D., and Dean, H.J. (2022). Characterization of the cell-mediated
2104 immune response to Takeda's live-attenuated tetravalent dengue vaccine in adolescents
2105 participating in a phase 2 randomized controlled trial conducted in a dengue-endemic setting.
2106 Vaccine *40*, 1143-1151.
2107 Tricou, V., Low, J.G., Oh, H.M., Leo, Y.-S., Kalimuddin, S., Wijaya, L., Pang, J., Ling, L.M.,
2108 Lee, T.H., Brose, M.*, et al.* (2020). Safety and immunogenicity of a single dose of a tetravalent
2109 dengue vaccine with two different serotype-2 potencies in adults in Singapore: A phase 2,
2110 double-blind, randomised, controlled trial. Vaccine *38*, 1513-1519.
2111 Valiant, W.G., Huang, Y.S., Vanlandingham, D.L., Higgs, S., Lewis, M.G., and Mattapallil, J.J.
2112 (2018). Zika convalescent macaques display delayed induction of anamnestic cross-neutralizing
2113 antibody responses after dengue infection. Emerging microbes & infections *7*, 130.
2114 Valiant, W.G., Mattapallil, M.J., Higgs, S., Huang, Y.S., Vanlandingham, D.L., Lewis, M.G.,
2115 and Mattapallil, J.J. (2019). Simultaneous Coinfection of Macaques with Zika and Dengue
2116 Viruses Does not Enhance Acute Plasma Viremia but Leads to Activation of Monocyte Subsets
2117 and Biphasic Release of Pro-inflammatory Cytokines. Scientific reports *9*, 7877.
2118 Vazquez, S., Pupo, M., Pelegrino, J.L., Morier, L., Castillo, A., Otero, A., and Guzman, M.G.
2119 (1995). [An immunoenzyme assay for the detection of monoclonal antibodies against the E and
2120 NS1 proteins of the dengue virus]. Rev Cubana Med Trop *47*, 122-126.
2121 Venturi, G., Martelli, P., Mazzolini, E., Fiorentini, C., Benedetti, E., Todone, D., Villalta, D.,
2122 Fortuna, C., Marchi, A., Minelli, G.*, et al.* (2009). Humoral immunity in natural infection by
2123 tick-borne encephalitis virus. J Med Virol *81*, 665-671.
2124 Victora, G.D., and Nussenzweig, M.C. (2012). Germinal centers. Annu Rev Immunol *30*, 429-
2125 457.
2126 Vorovitch, M.F., Maikova, G.B., Chernokhaeva, L.L., Romanenko, V.V., Karganova, G.G., and
2127 Ishmukhametov, A.A. (2019). Comparison of the Immunogenicity and Safety of Two Pediatric
2128 TBE Vaccines Based on the Far Eastern and European Virus Subtypes. Adv Virol *2019*,
2129 5323428.
2130 Wahala, W.M., and Silva, A.M. (2011). The human antibody response to dengue virus infection.
2131 Viruses *3*, 2374-2395.
2132 Waickman, A.T., Friberg, H., Gargulak, M., Kong, A., Polhemus, M., Endy, T., Thomas, S.J.,
2133 Jarman, R.G., and Currier, J.R. (2019a). Assessing the Diversity and Stability of Cellular
2134 Immunity Generated in Response to the Candidate Live-Attenuated Dengue Virus Vaccine
2135 TAK-003. Frontiers in immunology *10*, 1778.
2136 Waickman, A.T., Victor, K., Li, T., Hatch, K., Rutvisuttinunt, W., Medin, C., Gabriel, B.,
2137 Jarman, R.G., Friberg, H., and Currier, J.R. (2019b). Dissecting the heterogeneity of DENV
2138 vaccine-elicited cellular immunity using single-cell RNA sequencing and metabolic profiling.
2139 Nature communications *10*, 3666.
2140 Wan, S.W., Lu, Y.T., Huang, C.H., Lin, C.F., Anderson, R., Liu, H.S., Yeh, T.M., Yen, Y.T.,
2141 Wu-Hsieh, B.A., and Lin, Y.S. (2014). Protection against dengue virus infection in mice by
2142 administration of antibodies against modified nonstructural protein 1. PLoS One *9*, e92495.
2143 Weiskopf, D., Angelo, M.A., Bangs, D.J., Sidney, J., Paul, S., Peters, B., de Silva, A.D., Lindow,
2144 J.C., Diehl, S.A., Whitehead, S.*, et al.* (2015a). The human CD8+ T cell responses induced by a
2145 live attenuated tetravalent dengue vaccine are directed against highly conserved epitopes. J Virol
2146 *89*, 120-128.

2147 Weiskopf, D., Angelo, M.A., Grifoni, A., O'Rourke, P.H., Sidney, J., Paul, S., De Silva, A.D.,
2148 Phillips, E., Mallal, S., Premawansa, S.*, et al.* (2016). HLA-DRB1 Alleles Are Associated With
2149 Different Magnitudes of Dengue Virus-Specific CD4+ T-Cell Responses. J Infect Dis *214*, 1117-
2150 1124.
2151 Weiskopf, D., Bangs, D.J., Sidney, J., Kolla, R.V., De Silva, A.D., de Silva, A.M., Crotty, S.,
2152 Peters, B., and Sette, A. (2015b). Dengue virus infection elicits highly polarized CX3CR1+
2153 cytotoxic CD4+ T cells associated with protective immunity. Proc Natl Acad Sci U S A *112*,
2154 E4256-4263.
2155 Weiskopf, D., and Sette, A. (2014). T-cell immunity to infection with dengue virus in humans.
2156 Frontiers in immunology *5*, 93.
2157 Weiskopf, D., Yauch, L.E., Angelo, M.A., John, D.V., Greenbaum, J.A., Sidney, J., Kolla, R.V.,
2158 De Silva, A.D., de Silva, A.M., Grey, H.*, et al.* (2011). Insights into HLA-restricted T cell
2159 responses in a novel mouse model of dengue virus infection point toward new implications for
2160 vaccine design. J Immunol *187*, 4268-4279.
2161 Wen, J., Duan, Z., and Jiang, L. (2010). Identification of a dengue virus-specific HLA-A*0201-
2162 restricted CD8+ T cell epitope. J Med Virol *82*, 642-648.
2163 Wen, J., Elong Ngono, A., Regla-Nava, J.A., Kim, K., Gorman, M.J., Diamond, M.S., and
2164 Shresta, S. (2017). Dengue virus-reactive CD8(+) T cells mediate cross-protection against
2165 subsequent Zika virus challenge. Nature communications *8*, 1459.
2166 Wen, J., and Shresta, S. (2017). T Cell Immunity to Zika and Dengue Viral Infections. J
2167 Interferon Cytokine Res *37*, 475-479.
2168 Wen, J., Wang, Y.T., Valentine, K.M., Dos Santos Alves, R.P., Xu, Z., Regla-Nava, J.A.,
2169 Ngono, A.E., Young, M.P., Ferreira, L.C.S., and Shresta, S. (2020). CD4(+) T Cells Cross-
2170 Reactive with Dengue and Zika Viruses Protect against Zika Virus Infection. Cell reports *31*,
2171 107566.
2172 White, L.J., Young, E.F., Stoops, M.J., Henein, S.R., Adams, E.C., Baric, R.S., and de Silva,
2173 A.M. (2021). Defining levels of dengue virus serotype-specific neutralizing antibodies induced
2174 by a live attenuated tetravalent dengue vaccine (TAK-003). PLoS Negl Trop Dis *15*, e0009258.
2175 Whitehead, S.S. (2016). Development of TV003/TV005, a single dose, highly immunogenic live
2176 attenuated dengue vaccine; what makes this vaccine different from the Sanofi-Pasteur CYD™
2177 vaccine? Expert Rev Vaccines *15*, 509-517.
2178 Whitehead, S.S., Falgout, B., Hanley, K.A., Blaney Jr, J.E., Jr., Markoff, L., and Murphy, B.R.
2179 (2003a). A live, attenuated dengue virus type 1 vaccine candidate with a 30-nucleotide deletion
2180 in the 3' untranslated region is highly attenuated and immunogenic in monkeys. J Virol *77*, 1653-
2181 1657.
2182 Whitehead, S.S., Hanley, K.A., Blaney, J.E., Jr., Gilmore, L.E., Elkins, W.R., and Murphy, B.R.
2183 (2003b). Substitution of the structural genes of dengue virus type 4 with those of type 2 results in
2184 chimeric vaccine candidates which are attenuated for mosquitoes, mice, and rhesus monkeys.
2185 Vaccine *21*, 4307-4316.
2186 WHO/TDR (2009). Dengue guidelines for diagnosis, treatment,
2187 prevention and control. (Geneva).
2188 Wijesinghe, A., Gamage, J., Goonewardena, H., Gomes, L., Jayathilaka, D., Wijeratne, D.T., de
2189 Alwis, R., Jeewandara, C., Wijewickrama, A., Ogg, G.S.*, et al.* (2020). Phenotype and
2190 functionality of follicular helper T cells in patients with acute dengue infection. J Biomed Sci *27*,
2191 50.

Wong, J.M., Adams, L.E., Durbin, A.P., Muñoz-Jordán, J.L., Poehling, K.A., Sánchez-González, L.M., Volkman, H.R., and Paz-Bailey, G. (2022). Dengue: A Growing Problem With New Interventions. Pediatrics *149*.

Wong, R., Belk, J.A., Govero, J., Uhrlaub, J.L., Reinartz, D., Zhao, H., Errico, J.M., D'Souza, L., Ripperger, T.J., Nikolich-Zugich, J.*, et al.* (2020). Affinity-Restricted Memory B Cells Dominate Recall Responses to Heterologous Flaviviruses. Immunity *53*, 1078-1094.e1077.

Xu, M., Hadinoto, V., Appanna, R., Joensson, K., Toh, Y.X., Balakrishnan, T., Ong, S.H., Warter, L., Leo, Y.S., Wang, C.I.*, et al.* (2012). Plasmablasts generated during repeated dengue infection are virus glycoprotein-specific and bind to multiple virus serotypes. J Immunol *189*, 5877-5885.

Yauch, L.E., Prestwood, T.R., May, M.M., Morar, M.M., Zellweger, R.M., Peters, B., Sette, A., and Shresta, S. (2010). CD4+ T cells are not required for the induction of dengue virus-specific CD8+ T cell or antibody responses but contribute to protection after vaccination. J Immunol *185*, 5405-5416.

Yauch, L.E., and Shresta, S. (2008). Mouse models of dengue virus infection and disease. Antiviral Res *80*, 87-93.

Yauch, L.E., Zellweger, R.M., Kotturi, M.F., Qutubuddin, A., Sidney, J., Peters, B., Prestwood, T.R., Sette, A., and Shresta, S. (2009). A protective role for dengue virus-specific CD8+ T cells. J Immunol *182*, 4865-4873.

Young, E., Yount, B., Pantoja, P., Henein, S., Meganck, R.M., McBride, J., Munt, J.E., Baric, T.J., Zhu, D., Scobey, T.*, et al.* (2023). A live dengue virus vaccine carrying a chimeric envelope glycoprotein elicits dual DENV2-DENV4 serotype-specific immunity. Nature communications *14*, 1371.

Yu, E.D., Wang, H., da Silva Antunes, R., Tian, Y., Tippalagama, R., Alahakoon, S.U., Premawansa, G., Wijewickrama, A., Premawansa, S., De Silva, A.D.*, et al.* (2022). A Population of CD4(+)CD8(+) Double-Positive T Cells Associated with Risk of Plasma Leakage in Dengue Viral Infection. Viruses *14*.

Zhang, F., Wang, L., Niu, X., Li, J., Luo, J., Feng, Y., Yang, Y., He, P., Fan, W., Liang, R.*, et al.* (2019). Phenotypic Characterization of Chinese Rhesus Macaque Plasmablasts for Cloning Antigen-Specific Monoclonal Antibodies. Frontiers in immunology *10*, 2426.

Zhang, S., He, Y., Wu, Z., Wang, M., Jia, R., Zhu, D., Liu, M., Zhao, X., Yang, Q., Wu, Y.*, et al.* (2023). Secretory pathways and multiple functions of nonstructural protein 1 in flavivirus infection. Frontiers in immunology *14*, 1205002.

Zhou, D., Pei, C., Liu, Z., Yang, K., Li, Q., Chen, H., Cao, S., and Song, Y. (2020). Identification of a protective epitope in Japanese encephalitis virus NS1 protein. Antiviral Res *182*, 104930.

Zivna, I., Green, S., Vaughn, D.W., Kalayanarooj, S., Stephens, H.A., Chandanayingyong, D., Nisalak, A., Ennis, F.A., and Rothman, A.L. (2002). T cell responses to an HLA-B*07-restricted epitope on the dengue NS3 protein correlate with disease severity. J Immunol *168*, 5959-5965.

Zompi, S., and Harris, E. (2012). Animal models of dengue virus infection. Viruses *4*, 62-82.