An epidemic Zika virus isolate suppresses antiviral immunity by disrupting antigen presentation pathways

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Zika virus (ZIKV) has emerged as an important global health threat, with the recently acquired capacity to cause severe neurological symptoms and to persist within host tissues. We previously demonstrated that an early Asian lineage ZIKV isolate induces a highly activated CD8 T cell response specific for an immunodominant epitope in the ZIKV envelope protein in wild-type mice. Here we show that a contemporary ZIKV isolate from the Brazilian outbreak severely limits CD8 T cell immunity in mice and blocks generation of the immunodominant CD8 T cell response. This is associated with a more sustained infection that is cleared between 7- and 14-days post-infection. Mechanistically, we demonstrate that infection with the Brazilian ZIKV isolate reduces the cross-presentation capacity of dendritic cells and fails to fully activate the immunoproteasome. Thus, our study provides an isolate-specific mechanism of host immune evasion by one Brazilian ZIKV isolate, which differs from the early Asian lineage isolate and provides potential insight into viral persistence associated with recent ZIKV outbreaks.
Zika virus (ZIKV) is a mosquito-borne pathogen of the *Flaviviridae* family (*Flavivirus* genus), which was first isolated in the Zika Forest region of Uganda in 1947. From this initial isolation through the remainder of the twentieth century, only a small number of isolated infections were reported. However, in April–July 2007 ZIKV was identified as the cause of a major outbreak on Yap Island, Federated States of Micronesia, infecting approximately 73% of the island’s population. This was followed by another major outbreak in French Polynesia, during which ~32,000 people were infected over the course of 6 months in 2013. More recently, ZIKV caused a major epidemic that began in Brazil in 2015, before spreading rapidly throughout South and Central America, with some local transmission in the southern United States. During this epidemic, ZIKV infections were reported in 84 countries worldwide, and over 800,000 suspected and confirmed cases were reported in the Americas alone. Together, these outbreaks represent a striking change in phenotype for ZIKV and suggest that this previously innocuous virus may have acquired a novel epidemic capacity.

The French Polynesian and South and Central American outbreaks also marked the first instances in which ZIKV infection was associated with severe neurological symptoms. ZIKV infection during pregnancy can cause fetal microcephaly, while the most common symptom observed in adults is Guillain–Barré Syndrome, an autoimmune ascending paralysis. However, other neurological symptoms, as well as uveitis and hematomaxoria (blood in the semen) have also been reported in adults, and correlate with the detection of ZIKV RNA in cerebrospinal fluid, aqueous humor, and semen, respectively. It has also been reported that ZIKV RNA may be persistently detected in some tissues. In women, ZIKV RNA may be detected in vaginal secretions until 13 days post-onset of symptoms (POS), while in men, some semen samples remained positive over 100 days POS. Likewise, in both men and women ZIKV RNA has been detected as late as 36 days POS in urine and 15 days POS in serum. Together, the apparent increase in epidemic capacity, novel association with neurological symptoms, and persistence of ZIKV RNA suggest that recent ZIKV isolates may have evolved to counter host immunity and establish more sustained infections.

Several studies have begun to compare epidemic and historical ZIKV isolates in order to understand whether contemporary isolates have an enhanced capacity to evade host immunity. Genetic analyses of ZIKV have identified two main lineages, African and Asian. All of the outbreaks described above were caused by Asian lineage isolates, some of which have been found to possess conserved mutations that correlate with neuroinvasion, evasion of the type I interferon (IFN-I) response, and microcephaly. Comparisons of African and Asian lineage ZIKV infection in A129 mice, which lack the IFN-α/β receptor (IFNAR), and STAT2 KO mice have found that infection with African lineage ZIKV was lethal, while mice survived infection with a Puerto Rican ZIKV isolate (Asian lineage) and with a Puerto Rican ZIKV isolate (Asian lineage). In STAT2 KO mice, mortality correlated with induction of IFN-α and IFN-β mRNA, with the highest levels observed following infection with the Ugandan isolate MR766 (African lineage). Although these studies were undertaken in immunocompromised hosts, which limits their usefulness in interrogating immune responses to infection, they suggest that contemporary ZIKV isolates induce a less inflammatory and less lethal infection than historical ZIKV isolates. However, it is unknown whether some epidemic isolates of ZIKV have improved capacity to evade host immunity compared to earlier, pre-epidemic Asian lineage isolates.

Improving our understanding of the immune response to ZIKV, and how this relates to sustained virus detection and the neurological symptoms associated with infection is a priority for addressing this major public health concern. Although no outbreaks are currently ongoing, 90 countries and territories remain at risk of ZIKV transmission (based on past local transmission), and another 56 have the mosquito vector but have not yet reported transmission. To address whether the immune response to ZIKV has changed, our group previously established an immunocompetent mouse model of ZIKV infection. Our data demonstrated that an early Asian lineage ZIKV isolate (PLCal_ZV, accession number KF993678: referred to herein as ZIKVCDN) establishes an active, albeit rapidly cleared, infection in C57BL/6 wild type (WT) mice. This leads to a robust and prototypical antiviral innate immune response, including dendritic cell (DC) and natural killer cell activation. Furthermore, we have used a previously described surrogate marker approach to identify and track the total antigen-experienced CD8 T cell response to ZIKV infection. Importantly, this approach enabled us to identify an immunodominant CD8 T cell epitope in the ZIKV envelope protein (Env294–302), which has also been independently described by other groups. Thus, our model provides an important baseline and an opportunity for comparisons of the immune response to historical and contemporary ZIKV isolates. In addition, since the mice are immunocompetent and do not succumb to infection, this model allows us to query the impact of epidemic ZIKV isolates on antiviral cytotoxic T cell responses.

Herein, we demonstrate that a Brazilian ZIKV isolate (HS-2015-BA-01, accession number KX520666; referred to herein as ZIKYBR) actively suppresses CD8 T cell immunity following infection. This correlates with reduced IFN-I production, reduced activation of DCs, inhibition of cross-presentation, and failure to induce immunoproteasome activation, which results in the complete abrogation of the immunodominant Env294–302-specific CD8 T cell response. Together, these factors result in sustained, but not chronic, infection with replicative virus detected as late as 7 days following infection. Thus, our findings suggest that this epidemic ZIKV isolate has developed a number of ways to either avoid or actively counter immune responses. Although the current study is limited to one epidemic isolate, our data provide some insight that could potentially explain at least one of the mechanisms behind the increased pathogenesis and a broader scope of transmission associated with ZIKV infection during the 2015 outbreak in the Americas.

**Results**  
**Reduced CD8 T cell response to ZIKYBR infection correlates with virus detection at later time points post-infection.** We previously characterized the T cell response to an early Asian lineage ZIKV isolate PLCal_ZV (accession number KF993678: referred to herein as ZIKVCDN) in immunocompetent mice. This isolate was taken from a Canadian patient who acquired the infection in Thailand in 2013 (prior to the South and Central American outbreak). During this outbreak, ZIKV infections were reported in 84 countries worldwide, and over 800,000 suspected and confirmed cases were reported in the Americas alone. Together, these outbreaks have marked the first instances in which ZIKV infection is associated with severe neurological symptoms.

In the current study, we performed a comparative analysis of the immune response induced by the ZIKVCDN and ZIKYBR isolates. We found that ZIKYBR infection results in a reduced CD8 T cell response compared to ZIKVCDN infection. This is evidenced by the reduced expression of IFN-γ and the reduced number of CD8 T cells in the spleen and lymph nodes of ZIKYBR-infected mice.

**Immune response to ZIKVCDN and ZIKYBR infection:** We analyzed the immune response to ZIKVCDN and ZIKYBR infection by measuring the expression of IFN-γ and the number of CD8 T cells in the spleen and lymph nodes. We found that ZIKYBR infection results in a reduced CD8 T cell response compared to ZIKVCDN infection. This is evidenced by the reduced expression of IFN-γ and the reduced number of CD8 T cells in the spleen and lymph nodes of ZIKYBR-infected mice.

**Mechanisms underlying the reduced CD8 T cell response:** To understand the mechanisms underlying the reduced CD8 T cell response to ZIKYBR infection, we performed a comparative analysis of the immune response induced by the ZIKVCDN and ZIKYBR isolates. We found that ZIKYBR infection results in a reduced CD8 T cell response compared to ZIKVCDN infection. This is evidenced by the reduced expression of IFN-γ and the reduced number of CD8 T cells in the spleen and lymph nodes of ZIKYBR-infected mice.

**Conclusion:** In conclusion, our study provides important insights into the mechanisms underlying the reduced CD8 T cell response to ZIKYBR infection. This is evidenced by the reduced expression of IFN-γ and the reduced number of CD8 T cells in the spleen and lymph nodes of ZIKYBR-infected mice. These findings suggest that ZIKYBR infection results in a reduced CD8 T cell response compared to ZIKVCDN infection. This is evidenced by the reduced expression of IFN-γ and the reduced number of CD8 T cells in the spleen and lymph nodes of ZIKYBR-infected mice.
protein, two each in the premembrane (PrM), NS3, and NS4A proteins, and one each in the capsid and NS2A proteins. These differences have been demonstrated to lead to different phenotypes between these two viruses in cell culture models, with ZIKV BR causing more cytopathic effects and increased replicative efficiency in certain cell types. Despite these studies, whether these isolates induce different immune responses in immunocompetent mice remained unknown.

We first assessed the total CD8 T cell response to infection with each ZIKV isolate in the blood over time using a surrogate marker approach we have previously validated in the context of ZIKV infection. WT C57BL/6 mice were infected with ZIKV CDN or ZIKV BR and the total antigen-experienced (CD8αloCD11ahi) CD8 T cell response was analyzed in the blood at various days post-infection (dpi). We observed that, as previously described, ZIKV CDN induced a robust CD8αloCD11ahi CD8 T cell response that peaked at 7 dpi (Fig. 1a, b). However, both the frequency and number of CD8αloCD11a hi CD8 T cells responding to ZIKV BR infection were severely reduced compared to infection with ZIKV CDN (Fig. 1a, b). In contrast, the CD11a+CD49d+ (also validated as markers of antigen-experienced CD4 T cells during ZIKV infection) CD4 T cell response was comparable between the two infections (Fig. 1c, d). Thus, our data suggest that ZIKV BR infection suppresses CD8 T cell immunity despite inducing a numerically equivalent CD4 T cell response in the blood.

Given the importance of CD8 T cells for viral clearance, we asked whether the reduced CD8 T cell response to ZIKV BR had an impact on the capacity of the host to control ZIKV infection. To address this question, we quantified ZIKV RNA in the spleen at various time points following infection with ZIKV CDN or ZIKV BR. Spleens were harvested at 12 h post-infection (hpi), a time point at which viral load peaks following ZIKV infection22, as well as 3, 5, and 7 dpi, to analyze viral kinetics. As additional controls, mice were mock-infected or injected with an equivalent dose of UV-inactivated ZIKV CDN (UV-ZIKV; 12 hpi only). The viral burden was assessed using RT-qPCR and expressed as PFU equivalents per gram of tissue. As previously described, we did not detect any ZIKV RNA above the limit of detection (LOD) in mice that were injected with UV-ZIKV, confirming that infection with live ZIKV is required for detection of viral RNA (Fig. 1e). Although viral load was significantly lower in the spleen 12 hpi with ZIKV BR compared to ZIKV CDN, viral load declined 3 dpi with ZIKV CDN, while remaining unchanged following ZIKV BR infection (Fig. 1e). Strikingly, and in contrast to declining viral load in ZIKV CDN-infected mice during the time course, we observed increasing viral loads at 5 and 7 dpi with ZIKV BR (Fig. 1e). Thus, our data suggest that dampened CD8 T cell responses to ZIKV BR are associated with more sustained detection of viral RNA in WT mice.

In order to confirm that the viral RNA we detected at this late time point was representative of the replicative virus, and to determine whether this led to increased viral dissemination, we measured the amount of infectious virus in the spleen and kidney by plaque assay 7 dpi with each isolate. We detected no ZIKV CDN above the LOD in either organ (Fig. 1f, g). However, infectious ZIKV BR was detectable in 9/10 mice 7 dpi: 5/10 in both the spleen and kidney, 3/10 in the kidney alone, and 1/10 in the spleen alone (Fig. 1f, g). Although the number of PFU per gram of tissue is lower than the number of PFU equivalents per gram of tissue calculated by RT-qPCR (Fig. 1e), this likely reflects that more copies of the genome are produced than infectious virus particles, as well as the potentially higher sensitivity of RT-qPCR. Since the South and Central American epidemic was associated with neurological symptoms and persistent detection of ZIKV RNA in reproductive tissues, we additionally assessed the brain and ovaries for the presence of infectious virus 7 dpi with ZIKV BR. However, we detected no virus in either of these tissues (Supplementary Fig. 1), suggesting that this virus is unable to widely disseminate in immunocompetent mice despite the reduced CD8 T cell response. Further analysis of the spleen and kidney for infectious virus 14 dpi with ZIKV BR revealed that the virus was cleared from both of these tissues at this time point (Supplementary Fig. 1). Thus, ZIKV BR induces a more sustained, but not chronic, infection in immunocompetent mice with replicative virus detected as late as day 7 post-infection in the spleen and kidney, which is cleared before day 14 post-infection.

CD8 T cells are less activated and do not respond to the Env294–302 epitope following ZIKV BR infection. Since our data demonstrated an overall defect in the CD8 T cell response to ZIKV BR, we next sought to further analyze this phenotype in the spleen 7 dpi. Our data demonstrate an overall decrease in total spleen cellularity following ZIKV BR infection (Fig. 2a). In addition, ZIKV BR induced significantly less total CD8 T cell expansion, as the frequency and number of CD8αloCD11a hi CD8 T cells were severely reduced during ZIKV BR infection, compared to infection with ZIKV CDN (Fig. 2b, d, e). Similar to our observations in the blood (Fig. 1a, b), both the frequency and number of CD8αloCD11a hi CD8 T cells were severely reduced during ZIKV BR infection, compared to infection with ZIKV CDN (Fig. 2c, f, g). Thus, the overall accumulation of cells in the spleen is reduced following ZIKV BR infection, which corresponds to a significantly reduced induction of an antigen-experienced CD8 T cell response.

Previously, we and others identified Env294–302 as an immunodominant CD8 T cell epitope in the ZIKV envelope protein22,24,25. Therefore we tested the functionality of the CD8 T cell response to this epitope following each infection by analyzing the capacity of CD8αloCD11a hi CD8 T cells to produce the effector cytokine IFN-γ following stimulation with Env294–302 peptide. While we observed detectable IFN-γ production by CD8αloCD11a hi CD8 T cells following infection with ZIKV CDN, we observed no IFN-γ response to this peptide following ZIKV BR infection (Supplementary Fig. 2). In addition, we did not detect Env294–302-specific CD8 T cells by H-2Db class I MHC (H-2I b tetramer (H-2Db Env294–302) staining in ZIKV BR-infected mice, despite a robust Env294–302-specific response in ZIKV CDN-infected mice (Fig. 2h–i)). In addition to the immunodominant Env294–302 epitope, several other immunogenic ZIKV peptides have been identified24,25. Thus, we tested a panel of 9 immunogenic peptides from ZIKV structural and NS proteins to determine their capacity to elicit IFN-γ production by CD8αloCD11a hi CD8 T cells. While we observed variable responsiveness to 7 of the epitopes, ZIKV CDN infection elicited a consistent subdominant response above background following restimulation with PrM169–177 and NS52993–3000 (Supplementary Fig. 3). Meanwhile, ZIKV BR infection failed to induce a consistent response against any of the peptides tested (Supplementary Fig. 3). These data indicate that ZIKV BR does not induce a CD8 T cell response against the immunodominant Env294–302 epitope and causes global changes in the capacity of CD8 T cells to recognize ZIKV-derived epitopes, despite the absence of any amino acid differences in these epitopes.

One possible explanation for the decreased CD8 T cell response to ZIKV BR infection could include differences in antigen load following infection. Since we detected less ZIKV BR RNA in the spleen 12 hpi compared to ZIKV CDN (Fig. 1e), this difference could limit antigen availability for activating CD8 T cell responses. To address this question, we conducted a dose-
**Fig. 1 Reduced CD8 T cell response to ZIKVBR is associated with virus detection at later time points following infection.**

**a, b** Frequency (a) and number (b) of CD8αloCD11ahi CD8 T cells in the peripheral blood of mice at indicated dpi with ZIKVCDN or ZIKVBR. Data are representative of two independent experiments with n = 3 mice per group, sampled repeatedly at indicated time points. Data were analyzed by two-tailed, unpaired Student’s t-test at each time point. In a day 5 p = 0.0041, day 7 p = 0.0004, day 10 p = 0.0015, day 14 p = 0.0067, day 20 p = 0.0073, day 30 p = 0.0132, day 40 p = 0.0137, day 50 p = 0.0157. In b day 5 p = 0.0004, day 7 p = 0.0014, day 10 p = 0.0003, day 14 p = 0.0218, day 20 p = 0.0169, day 30 p = 0.0002, day 40 p = 0.0371, day 50 p = 0.0327.

**c, d** Frequency (c) and number (d) of CD11a+CD49d+ CD4 T cells in the peripheral blood of mice at indicated dpi with ZIKVCDN or ZIKVBR. Data are representative of two independent experiments with n = 3 mice per group, sampled repeatedly at indicated time points. Data were analyzed by two-tailed, unpaired Student’s t-test at each time point. In d day 0 p = 0.0407.

**e** Viral RNA was quantified in the spleen using RT-qPCR analysis. Data are presented as PFU equivalents per gram of tissue after comparison to a standard curve of Ct value versus log10(PFU) for each isolate. Mice were sacrificed 12 hpi and 3, 5, and 7 dpi with ZIKVCDN or ZIKVBR, or following mock-infection or injection with UV-ZIKV (12 hpi only). Data are representative of three independent experiments, with n = 3 mice per group at each time point. Data were analyzed by two-tailed, unpaired Student’s t-test at each time point. 12 hpi p = 0.001, 5 dpi p = 0.000079, 7 dpi p = 0.00014, f, g Viral burden in the spleen (f) and kidney (g) were quantified via plaque assay 7 dpi with each ZIKV isolate. N.D. indicates no data recorded above the LOD (dotted line). Data are pooled from two experiments with n = 5 mice per group. All data are shown as mean ± SEM and *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001. Red squares = ZIKVCDN-infected mice, blue triangles = ZIKVBR-infected mice, black squares UV-ZIKV-injected mice. Source data are provided as a Source Data file.
Fig. 2 ZIKV<sup>BR</sup> induces a less robust CD8 T cell response that does not respond to the immunodominant Env<sub>294-302</sub> epitope. 

- **a** Total spleen cellularity 7 dpi with ZIKV<sup>CDN</sup> or ZIKV<sup>BR</sup>, *p* = 0.0146.
- **b-g** Representative flow cytometry plots (**b**), frequency (**d**), and number (**e**) of CD8<sup>+</sup> T cells 7 dpi with ZIKV<sup>CDN</sup> or ZIKV<sup>BR</sup>. In **d** *p* = 0.0047 and in **e**, *p* = 0.0008. Representative flow cytometry plots (**c**), frequency (**f**), and number (**g**) of CD8<sup>+</sup>CD11<sup>hi</sup>CD8<sup>+</sup> T cells in the spleen 7 dpi with ZIKV<sup>CDN</sup> or ZIKV<sup>BR</sup>. In **f** *p* = 0.0002 and in **g**, *p* = 0.0004. 
- **h-j** Representative flow cytometry plots of H-2Db Env<sub>294-302</sub> tetramer-positive CD8<sup>+</sup>CD11<sup>hi</sup>CD8<sup>+</sup> T cells from ZIKV<sup>CDN</sup> and ZIKV<sup>BR</sup>-infected mice (**h**), and frequency (**i**) and number (**j**) of H-2Db Env<sub>294-302</sub> tetramer-positive CD8<sup>+</sup>CD11<sup>hi</sup>CD8<sup>+</sup> T cells in the spleen 7 dpi with ZIKV<sup>CDN</sup> or ZIKV<sup>BR</sup>. In **i** *p* = 0.000019 and in **j**, *p* = 0.000045. 

All data are representative of four independent experiments with *n* = 3 mice per group and are shown as mean ± SEM. All data were analyzed by a two-tailed, unpaired Student’s t-test. *p* < 0.05, **p** < 0.01, ***p** < 0.001, and ****p** < 0.0001. Red squares = ZIKV<sup>CDN</sup>-infected mice, blue triangles = ZIKV<sup>BR</sup>-infected mice.

Source data are provided as a Source Data file.
dependence experiment, in which we infected mice with either 1 × 10^5 PFU or 1 × 10^6 PFU of ZIKV CDN, or 1 × 10^5 PFU or 5 × 10^5 PFU of ZIKV BR (thereby reducing the potential antigen dose for ZIKV CDN while increasing it for ZIKV BR). Between varying doses of the same virus, we observed no impact on either the total CD8α^hiCD11a^hiCD8 T cell response nor the Env^292–302-specific CD8 T cell response (Supplementary Fig. 4). Further, when comparing mice infected with 1 × 10^5 PFU of ZIKV CDN to those infected with 5 × 10^5 PFU of ZIKV BR, with the latter group receiving an inoculation 50 times larger than the former, we still observed the same large reduction in both the total CD8α^hiCD11a^hiCD8 T cell response and the Env^292–302-specific CD8 T cell response (Supplementary Fig. 4). Thus, our data demonstrate that antigen load likely is not responsible for the reduced magnitude of the CD8 T cell response to ZIKV BR infection, nor the lack of an Env^292–302-specific CD8 T cell response.

To assess CD8 T cell activation following ZIKV BR infection, we analyzed the phenotype of total antigen-experienced CD8 T cells in the spleen at the peak of the T cell response 7 dpi. Following infection, effector CD8 T cells can be broadly classified as CD127^-KLRG1^- memory precursor cells (MPCs), which preferentially survive contraction and seed the memory T cell pool, and CD127^-KLRG1^+ terminal effector cells (TECs), which are critical for the acute immune response, and largely die via apoptosis following peak expansion.33. Following infection with ZIKV CDN, the majority of CD8α^loCD11a^hi CD8 T cells expressed a TEC phenotype, while more CD8α^hiCD11a^hi CD8 T cells expressed a MPC phenotype following ZIKV BR infection (Fig. 3a–c). We next examined the expression of CD62L (L-selectin), a lymph node homing receptor that is downregulated following TCR stimulation and is typically expressed by naive and central memory T cells, but not effector T cells.34 A higher frequency and number of CD8α^loCD11a^hi CD8 T cells retained cell surface expression of CD62L during ZIKV BR infection compared to ZIKV CDN infection (Fig. 3d–f), suggesting a less activated phenotype. Finally, a significantly smaller frequency and number of CD8α^loCD11a^hi CD8 T cells responding to ZIKV BR infection were positive for expression of the key cytotoxic molecule granzyme B than those responding to ZIKV CDN infection (Fig. 3g–i), suggesting they have reduced cytolytic capacity. Together, these data demonstrate that the small number of antigen-experienced CD8 T cells induced by ZIKV BR infection present a less activated phenotype than those induced by ZIKV CDN infection.

Reduced dendritic cell activation and IFN-I production following ZIKV BR infection do not impact CD8 T cell proliferation. Inflammatory cytokines such as IFN-I provide a crucial signal during CD8 T cell priming that is important for their activation, accumulation, acquisition of effector functions, and capacity to respond to low levels of antigen.35–37. In contrast, cytokines such as IL-10 have broad immunoregulatory effects that dampen inflammatory responses, including reducing CD8 T cell accumulation and function.38–40. Further, Asian lineage ZIKV infection of human peripheral blood mononuclear cells has been shown to induce more IL-10 production than infection with African lineage isolates.41. We, therefore, hypothesized that the blunted CD8 T cell response to ZIKV BR could be the result of either increased production of anti-inflammatory cytokines such as IL-10, or reduced production of inflammatory cytokines such as IFN-I. To determine whether IL-10 production during ZIKV BR infection contributed to the blunted CD8 T cell response, we infected WT and IL10^-/- mice with ZIKV BR and assessed the impact of IL-10 deficiency on the antigen-experienced CD8 T cell response 7 dpi. We observed no differences in the frequency or number of CD8α^loCD11a^hi CD8 T cells between WT and IL10^-/- mice, indicating that IL-10 does not play a role in blunting the CD8 T cell response to ZIKV BR infection (Supplementary Fig. 5). We next assessed Ifna (IFN-α) and Ifnb1 (IFN-β) mRNA expression in the spleen 12, 24, and 72 hpi with each ZIKV isolate by RT-qPCR. In contrast to robust expression following ZIKV CDN infection, ZIKV BR infection did not induce IFN-α/β mRNA expression above mock-infection levels at 12 hpi (Fig. 4a, b). IFN-α/β mRNA expression remained elevated in ZIKV CDN-infected mice at 24 hpi and returned to baseline 72 hpi, with minimal expression detected at each time point in ZIKV BR-infected mice (Supplementary Fig. 6). Furthermore, analysis of bioactive IFN-I protein levels 12, 24, and 72 hpi, as well as 7 dpi, with each ZIKV isolate revealed the presence of IFN-I in the serum was drastically reduced following infection with ZIKV BR, and remained low at all time points analyzed (Fig. 4c and Supplementary Fig. 6). Following ZIKV CDN infection, IFN-I protein levels mirrored the RT-qPCR results, with robust production 12 and 24 hpi, which returned to baseline 72 hpi and 7 dpi (Fig. 4c and Supplementary Fig. 6). Analysis of the IFN-stimulated genes (ISGs) Ifna1, Ifna17, Ifna15, and Oas1a at 12, 24, and 72 hpi demonstrated a consistent trend for increased expression following ZIKV CDN infection (Supplementary Fig. 6). Although not to the same extent as ZIKV CDN, ZIKV BR did induce ISG expression above mock infection, suggesting some IFN-I signaling occurs following infection, which is consistent with the detection of low levels of bioactive IFN-I following infection at 12 hpi (Fig. 4c and Supplementary Fig. 6). However, even at 7 dpi when viral load is high in the spleen and kidney (Fig. 1e–g), we observed little to no IFN-I production in ZIKV BR-infected mice. These data demonstrate that IFN-I production is poorly induced by ZIKV BR infection and that this is not simply a delay in the induction of the response, but rather a sustained defect despite continued viral replication. Thus, our data suggest that while IL-10 does not contribute to the reduced antigen-experienced CD8 T cell response to ZIKV BR infection, this defect may be due to a reduced IFN-I response.

Since ZIKV BR poorly induces IFN-I and ISG production, we reasoned that providing a source of IFN-I could restore the magnitude antigen-experienced CD8 T cell response. To test this, we infected mice with ZIKV CDN or ZIKV BR and determined the impact of treating ZIKV BR-infected mice on days 2 and 3 post-infection with the toll-like receptor (TLR)-3 agonist polyinosinic-polycytidylic acid (pI:C) or murine rIFN-β on the CD8α^loCD11a^hi CD8 T cell response 7 dpi. Although we observed a small, but significant increase in the frequency of CD8α^loCD11a^hi CD8 T cells in pI:C-treated mice compared to control-treated animals, neither treatment had an impact on the total number of CD8α^loCD11a^hi CD8 T cells in ZIKV BR-infected mice (Fig. 4d–f and Supplementary Fig. 7). Thus, reduced IFN-I production following ZIKV BR infection is not sufficient to explain the abrogated antigen-experienced CD8 T cell response.

The lower accumulation of antigen-experienced CD8 T cells following ZIKV BR infection could be due to a reduction in the proliferative capacity of CD8 T cells. To address this question, mice were infected with either isolate, pulsed with BrdU, and maintained on BrdU drinking water for the duration of the experiment. As cells divide, BrdU becomes incorporated into the DNA in the place of thymidine, and the amount of BrdU incorporation may be used as a read-out of proliferation. We observed an equivalent frequency of BrdU+/CD8α^loCD11a^hi CD8 T cells following either infection, indicating that proliferation is not impacted by ZIKV BR infection (Fig. 4g–i). In addition, intracellular staining for Ki67, a marker of cell cycle entry, in CD8α^loCD11a^hi CD8 T cells was also equivalent between ZIKV CDN and ZIKV BR infection (Supplementary Fig. 8). These data indicate that the decreased magnitude of the CD8α^loCD11a^hi
CD8 T cell response to ZIKVBR is not due to a defect in proliferation and suggest that once CD8 T cells are primed, they are capable of proliferating normally. Since our data suggest that the reduced magnitude of the CD8 T cell response to ZIKVBR is not due to a defect in proliferation, we examined whether there was a defect in T cell priming. Thus, we injected mice with UV-ZIKV or infected with ZIKVCDN or ZIKVBR and analyzed DC activation 2 dpi based on cell surface expression of the co-stimulatory molecules CD80 and CD86. Although both markers were significantly upregulated on DCs following ZIKVCDN infection, we did not observe significant upregulation following ZIKVBR infection, which suggests DCs are poorly activated by ZIKVBR infection (Fig. 4j–m). These data suggest that although the level of DC activation observed following ZIKVBR infection is sufficient to induce comparable CD4 T cell responses (Fig. 1c, d), it is not sufficient to fully

Fig. 3 CD8 T cells present a less activated phenotype following ZIKVBR infection. a–c Representative flow cytometry plots of CD127 and KLRG1 expression by CD8αloCD11ahi CD8 T cells from ZIKVCDN and ZIKVBR-infected mice (a), and frequency (b) and number (c) of CD127loKLRG1hi CD8 T cells (circles; TEC) and CD127hiKLRG1lo CD8 T cells (squares; MPC) in the spleen 7 dpi with ZIKVCDN or ZIKVBR. In b TEC p = 0.0004 and MPC p = 0.0002. In c TEC p = 0.000049 and MPC p = 0.0003. d–f Representative histogram (d), frequency (e) and number (f) of CD62L+ CD8αloCD11ahi CD8 T cells in the spleen 7 dpi with ZIKVCDN or ZIKVBR. Line on histogram indicates gating strategy used to identify CD62L+ cells. In e p = 0.0003 and in f p = 0.01. g–i Representative histogram (g), frequency (h), and number (i) of granzyme B+ CD8αloCD11ahi CD8 T cells in the spleen 7 dpi with ZIKVCDN or ZIKVBR. Shaded histogram indicates isotype control. Line on histogram indicates gating strategy used to identify granzyme B+ cells. In h p = 0.0026 and in i, p = 0.0005. All data are representative of two independent experiments with n = 3 mice per group and are shown as mean ± SEM. All data were analyzed by a two-tailed, unpaired Student’s t-test. * p < 0.05, ** p < 0.01, *** p < 0.001, and **** p < 0.0001. Red symbols = ZIKVCDN-infected mice, blue symbols = ZIKVBR-infected mice. Source data are provided as a Source Data file.

CD8 T cell response to ZIKVBR is not due to a defect in proliferation and suggest that once CD8 T cells are primed, they are capable of proliferating normally. Since our data suggest that the reduced magnitude of the CD8 T cell response to ZIKVBR is not due to a defect in proliferation, we examined whether there was a defect in T cell priming. Thus, we injected mice with UV-ZIKV or infected with ZIKVCDN or ZIKVBR and analyzed DC activation 2 dpi based on cell surface expression of the co-stimulatory molecules CD80 and CD86. Although both markers were significantly upregulated on DCs following ZIKVCDN infection, we did not observe significant upregulation following ZIKVBR infection, which suggests DCs are poorly activated by ZIKVBR infection (Fig. 4j–m). These data suggest that although the level of DC activation observed following ZIKVBR infection is sufficient to induce comparable CD4 T cell responses (Fig. 1c, d), it is not sufficient to fully
activate CD8 T cells. However, reduced DC activation during ZIKV<sup>BR</sup> infection is unlikely to explain the complete lack of a response against the Env<sub>294–302</sub> epitope, suggesting that several mechanisms may be involved in the capacity of ZIKV<sup>BR</sup> to counter immune responses.

**ZIKV<sup>BR</sup> modulates cross-presentation and immunoproteasome activation to subvert antigen-specific CD8 T cell response.** Antigen-experienced CD8 T cells do not respond to the immunodominant Env<sub>294–302</sub> epitope following ZIKV<sup>BR</sup> infection (Fig. 2h–j and Supplementary Fig. 2). As such, we next investigated whether this was reflective of broader changes to the CD8 T
Fig. 4 Reduced IFN-I production and dendritic cell activation following ZIKVBR infection do not impact antigen-experienced CD8 T cell division. a. b. Ifna (a) and Ifnb (b) mRNA expression in the spleen were analyzed by RT-qPCR 12 hpi with ZIKVCDN or ZIKVBR, or mock-infection. Data are expressed as fold change over expression in mock-infected mice 12 hpi. Dotted line indicates a fold change of 1. Data are representative of two independent experiments with n = 3 mice per group, and were analyzed by two-tailed, unpaired Student’s t-test. In a p = 0.0047 and in b p = 0.000004. c. Total bioactive IFN-I were analyzed in the serum 12 hpi with ZIKVCDN or ZIKVBR using the B16-blue reporter cell line. Dotted line indicates average OD in the serum of mice 12 h post-mock infection. Data are representative of two independent experiments with n = 3 mice per group, and were analyzed by two-tailed, unpaired Student’s t-test, p = 0.000011. d-f Representative flow cytometry plots of CD69CD11c+CD8 T cells from ZIKVCDN, or ZIKVBR-infected mice treated with PBS 2 and 3 dpi, and from ZIKVBR-infected mice treated with pC2 and 3 dpi. Frequency (e) and number (f) of CD69CD11c+CD8 T cells in the spleen 7 dpi with ZIKVCDN or ZIKVBR, after treatment with either PBS or pC2 and 3 dpi. Data are representative of two independent experiments with n = 5 mice per group and were analyzed by one-way ANOVA with Tukey’s post-test for multiple comparisons. In e ZIKVCDN + PBS versus ZIKVBR + PBS p = 0.000001, ZIKVCDN + PBS versus ZIKVBR + pC2 p = 0.0000001, ZIKVBR + PBS versus ZIKVBR + pC2 p = 0.0112. In f ZIKVCDN + PBS versus ZIKVBR + PBS p = 0.00000008, ZIKVCDN + PBS versus ZIKVBR + pC2 p = 0.00000059. g-i Representative histogram (g), frequency (h), and number (i) of BrdU+ CD69CD11c+CD8 T cells in the spleen 7 dpi with ZIKVCDN or ZIKVBR. Line on histogram indicates gating strategy used to identify BrdU+ cells. Data are representative of two independent experiments with n = 3 mice per group, and were analyzed by two-tailed, unpaired Student’s t-test. In i p = 0.0013. j-m Representative histograms and geometric mean fluorescence intensity (gMFI) of CD80+CD11c−CD8 T cells. Data are representative of two independent experiments with n = 3 mice per group, and were analyzed by one-way ANOVA with Tukey’s post-test of multiple comparisons. In j ZIKVCDN versus UV-ZIKV p = 0.0002 and ZIKVCDN versus ZIKVBR p = 0.0001. In k ZIKVCDN versus UV-ZIKV p = 0.000002 and ZIKVCDN versus ZIKVBR p = 0.000002. All data are shown as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001. Red squares and red lines = ZIKVCDN-infected mice, blue triangles and blue lines = ZIKVBR-infected mice, black squares and black lines = UV-ZIKV-infected mice, gray lines = isotype controls. Source data are provided as a Source Data file.

cell pool that is activated following ZIKVBR infection. To address this question, we analyzed TCR Vβ subunit usage among antigen-experienced CD8 T cells following each infection. We observed that the frequencies of most Vβ subunits are comparable regardless of the infection (Fig. 5a). However, a higher frequency of CD8αβCD11aβ CD8 T cells use TCR Vβ10b following ZIKVCDN infection, whereas a higher frequency of Vβ6 and Vβ9-expressing CD8αβCD11aβ CD8 T cells were observed following ZIKVBR infection (Fig. 5a). Together, these data suggest that ZIKVBR infection is associated with broad changes to the CD8 T cell repertoire, which could be due to alterations in priming of the CD8 T cell response.

Initial priming of a naive CD8 T cell requires the presentation of cognate antigen in the context of MHC-I, typically by mature DCs. The presentation of exogenously acquired antigen by a DC occurs via a mechanism known as cross-presentation42. To determine whether ZIKVBR is capable of altering cross-presentation to subvert priming of the CD8 T cell response, we analyzed the capacity of each ZIKV isolate to disrupt cross-presentation of ovalbumin (OVA) by bone marrow-derived DCs (BMDCs). Briefly, BMDCs were infected with either ZIKVCDN or ZIKVBR for 6 h (incubation with an equivalent amount of UV-inactivated ZIKVCDN or ZIKVBR was used as controls). Following infection, BMDCs were incubated with LPS, so that their capacity to stimulate CD8 T cell responses would be independent of each virus’ capacity to activate the BMDCs (Supplementary Fig. 9), as well as OVA257–264 peptide or OVA protein. Four hours later, proliferation dye-labeled TCR-transgenic OT-I CD8 T cells (specific for the OVA257–264 epitope) were added to BMDCs from each infection group and OT-I proliferation was assessed 3 days later. We observed no differences in OT-I proliferation following incubation with BMDCs that were pulsed with OVA257–264 peptide, regardless of the virus isolate or UV-inactivated virus (Fig. 5b). However, when BMDCs were pulsed with OVA protein, we observed reduced OT-I proliferation when the BMDCs had been infected with ZIKVBR, and not ZIKVCDN, or either UV-inactivated virus (Fig. 5c). Importantly, these differences were not due to a change in the capacity of BMDCs to uptake OVA protein (Supplementary Fig. 9). Thus, these data suggest that ZIKVBR is able to disrupt cross-presentation, independently of impacting antigen uptake, to evade CD8 T cell responses.

To further elucidate the mechanism by which ZIKVBR subverts CD8 T cell immunity, we asked whether ZIKVBR infection also led to alterations in the generation of immunogenic epitopes. The immunoproteasome is an alternative form of the constitutive proteasome that is induced during immune responses to generate antigens for presentation on MHC-I43. Exposure to inflammatory cytokines such as IFN-γ leads to the production of large molecular protein (LMP)2, LMP10 and LMP7, which substitute for the β1, β2, and β5 subunits of the constitutive proteasome, respectively43. Given the role of IFN-γ in immunoproteasome induction, we first assessed Ifna (IFN-γ) mRNA expression in the spleen 12 hpi with ZIKVCDN or ZIKVBR by RT-qPCR. Although ZIKVBR induced detectable IFN-γ, we observed significantly higher induction of IFN-γ following infection with ZIKVCDN (Fig. 5d). In line with this observation, ZIKVBR infection induced significantly less mRNA expression of Psmb8 (LMP7) in the spleen 12 hpi compared to ZIKVCDN infection (Fig. 5e), suggesting activation of the immunoproteasome is impaired during ZIKVBR infection. To determine whether the immunoproteasome plays a functional role in the generation of the Env294–302-specific CD8 T cell response during ZIKVCDN infection, we treated ZIKVCDN-infected mice with either vehicle control or ONX 0914, an LMP7-selective inhibitor that has previously been used to ameliorate disease progression in mouse models of autoimmune disease44–47. We observed that ONX 0914 treatment caused a significant reduction in both the frequency and number of Env294–302-specific CD8 T cells following ZIKVCDN infection (Fig. 5f–h). Thus, generation of the Env294–302-specific CD8 T cell response is at least partially dependent on the immunoproteasome. This suggests that failure to induce the immunoproteasome during ZIKVBR infection could be responsible for the lack of a response against the Env294–302 epitope.

ZIKVBR actively disrupts the antigen-specific CD8 T cell response. In addition to determining mechanisms of immune evasion by ZIKVBR, we also asked whether ZIKVBR is actively suppressing immune responses or passively avoiding detection. To address this question, we reasoned that if ZIKVBR is actively disrupting a pathway, and doing so in a dominant manner, it would be capable of disrupting the response induced by ZIKVCDN in the context of co-infection. Thus, we infected mice with ZIKVCDN or ZIKVBR alone, or co-infected mice with both isolates, and assessed the impact of co-infection on IFN-I
production (12 hpi) and the Env294–302-specific CD8 T cell response (7 dpi). Given that antigen-experienced CD8 T cells do not respond to this epitope during ZIKVBR infection, this approach enabled us to analyze the impact of infection with ZIKVBR on the ZIKVCDN-specific CD8 T cell response. We observed that co-infection did not reduce IFN-I production, at either the mRNA or protein level, compared to ZIKVCDN infection alone, indicating that ZIKVBR does not block IFN-I production in a dominant manner (Fig. 6a–c). However, we observed a significant reduction in both the frequency and number of Env294–302-specific CD8 T cells following co-infection, suggesting that ZIKVBR actively disrupts the antigen-specific CD8 T cell response to ZIKVCDN (Fig. 6d–f). Thus, these data demonstrate that ZIKVBR modulates the CD8 T cell response through both active and passive mechanisms.

Discussion
Since its initial isolation, ZIKV has emerged as a major global health concern, which retains the capacity to cause severe neurological symptoms in both adults and infants as it encounters naive host populations. As such, developing robust means of studying the immune response to infection, and how it may have changed in recent outbreaks, is key to developing novel
Fig. 5 ZIKVBR infection suppresses antigen cross-presentation and immunoproteasome induction. a TCR Vβ subunit usage among CD8αCD11aCD8 T cells 7 dpi with ZIKVCDN or ZIKVBR. Data are representative of two independent experiments with n = 3 mice per group. b, c Proliferation dye dilution in OT-1 CD8 T cells 3 days following co-culture with BMDCs. Prior to co-culture with labeled OT-1 CD8 T cells, DCs were infected with ZIKVCDN or ZIKVBR, or UV-inactivated ZIKVCDN or ZIKVBR, at an MOI of 5 for 6 h, followed by a 4-h incubation with LPS and either OVA257–264 peptide (b) or OVA protein (c). Data are representative of three independent experiments. d mRNA expression of Ifng in the spleen was analyzed by RT-qPCR 12 hpi with ZIKVCDN or ZIKVBR, or mock-infection. Data are expressed as fold change over expression in mock-infected mice 12 hpi. Dotted line indicates a fold change of 1. Data are provided as a Source Data file. e mRNA expression of Pmmb in the spleen was analyzed by RT-qPCR 12 hpi with ZIKVCDN or ZIKVBR, or mock-infection. Data are expressed as fold change over expression in mock-infected mice 12 hpi. Dotted line indicates a fold change of 1. Data are pooled from two independent experiments with n = 3 mice per group and were analyzed by two-tailed, unpaired Student’s t-test, p = 0.0313. f mRNA expression of Pmmb in the spleen was analyzed by RT-qPCR 12 hpi with ZIKVCDN or ZIKVBR, or mock-infection. Data are expressed as fold change over expression in mock-infected mice 12 hpi. Dotted line indicates a fold change of 1. Data are pooled from two independent experiments with n = 3 mice per group and were analyzed by two-tailed, unpaired Student’s t-test, p = 0.0466. g–i Frequency of H-2Dβ Env302–309 tetramer-positive CD8αCD11ahiCD8 T cells from ZIKVCDN-infected mice following treatment with vehicle control or ONX 0914 (f). Frequency (g) and number (h) of H-2Dβ Env302–309 tetramer-positive CD8αCD11ahiCD8 T cells in the spleen 7 dpi with ZIKVCDN. Mice were treated s.c. with vehicle control or ONX 0914 prior to infection. Data are pooled from two independent experiments with n = 3 (first experiment), n = 4 (ONX 0914 treated, second experiment), or n = 5 mice per group (vehicle-treated, second experiment), and were analyzed by two-tailed, unpaired Student’s t-test. In g p = 0.0034 and in h p = 0.0414. All data are shown as mean ± SEM. *p < 0.05, and **p < 0.01. Red squares and red lines = ZIKVCDN-infected mice or BMDC, blue triangles and blue lines = ZIKVBR-infected mice or BMDC, black lines = UV- ZIKVBR inoculated BMDCs, gray lines = UV-ZIKVCDN inoculated BMDCs. Source data are provided as a Source Data file.

vaccination and treatment strategies. Here, we have used our previously established model of ZIKVCDN infection22 as a baseline to which we compared the immune response induced by one epidemic isolate, ZIKVBR. Although mice are infected i.v. in this model, which is less representative of the natural route of infection, this is not a concern, as depletion of both CD4 and CD8 T cells of future studies.

Persistent detection of ZIKV RNA has been reported in a number of human studies and case reports from recent ZIKV outbreaks11,12,14,52–55. A prospective cohort study from Brazil reported that the median time to clearance of viral RNA was 13 days POS in vaginal secretions, 15 days in serum, 11 days in urine, and 42 days in semen, although in 11% of men ZIKV RNA remained detectable in semen 90 days POS12,14. Since longitudinal studies from previous outbreaks were not conducted, it is not possible to definitively state whether this represents a newly acquired characteristic of ZIKV. However, for some patients, the presence of ZIKV RNA in bodily fluids such as the cerebrospinal fluid, aqueous humor, or semen correlate with previously unreported symptoms such as meningitis or meningoencephalitis, uveitis, and hematopsomnia, respectively11,13,56–58. Although we report sustained detection of ZIKVBR RNA in the spleen, and infectious virus in the spleen and kidney 7 dpi, ZIKVBR was not detected in neurological or reproductive tissues. Thus, while our findings support an important and valuable model to interrogate how ZIKVBR counters innate and adaptive immune responses, and to study ZIKV immunity more broadly, it is less appropriate for studies of viral neuropathogenesis or sexual transmission.

Our data establish that, compared to ZIKVCDN, ZIKVBR induces a severely reduced CD8 T cell response and that the few responding CD8 T cells present are less activated. In contrast to robust DC activation and IFN-I production observed following ZIKVCDN infection, ZIKVBR poorly activated DCs and induced little to no IFN-I at multiple time points post-infection. In spite of this deficit, exogenous induction or addition of IFN-I was not sufficient to restore CD8 T cell numbers. During ZIKV infection, T cell responses are largely considered to play an important and protective role59. Our group and others have demonstrated that ZIKV infection in immunocompetent mice can lead to induction of CD8 T cell immunity, featuring the production of key effector cytokines such as IFN-γ or TNF-α, and the cytolytic molecule granzyme B, suggesting they are important for ZIKV immunity22,25. Further, T cells were shown to protect immunocompetent mice from high viral loads in the central nervous system and severe disease following intracranial infection, as this protection was lost in T cell-deficient mice60. The correlation between reduced CD8 T cell activation and accumulation following ZIKVBR infection and more sustained virus detection suggests CD8 T cells may play a protective role in this context. However, it has also been suggested that T cells are dispensable for ZIKV infection, as depletion of both CD4 and CD8 T cells prior to infection with a Brazilian ZIKV isolate led to only a small but significant weight loss compared to control-treated mice61. It
is worth noting, however, that the IFN response was not analyzed in this study, so it remains possible that this isolate did not induce a robust IFN-I or CD8 T cell response, similar to our observations herein61.

Immunoproteasome activation and cross-presentation of antigens are important steps in the generation of a protective antigen-specific antiviral CD8 T cell response42,43. The importance of the immunoproteasome in generating CD8 T cell responses varies depending on the infection. During murine cytomegalovirus infection, nearly all CD8 T cell epitopes are dependent on LMP7 expression62. Conversely, the generation of CD8 T cell responses against LCMV infection is only partially dependent on the immunoproteasome, and requires deletion of all three subunits of the immunoproteasome in order to observe a
major impact.\(^\text{62}\) Our data establish the importance of immunoproteasome induction in ZIKV immunity, since following ZIKV\(^{\text{CDN}}\) infection, generation of the Env\(_{294-302}\) specific CD8 T cell response is at least partially dependent on LMP7. Further, ZIKV\(^{\text{CDN}}\) also induced subdominant PrM\(_{169}\) and NS5\(_{2993-3000}\) specific responses, which were not observed following ZIKV\(^{\text{BR}}\) infection. These were the only two previously described epitopes that elicited a response in our model, which is likely due to differences between WT mice and the previously described epitopes that elicited a response in our model, with a particularly severe impact on the CD8 T cell response. Reduced CD8 T cell responses may be related to longer-lasting infections with ZIKV, which could contribute to sustained detection of viral RNA and pathogenesis observed during recent outbreaks.

**Methods**

**Cell lines.** Vero cells (African Green Monkey kidney epithelial cells, provided by Steven Varga, University of Iowa) were cultured in DMEM (Wisent) supplemented with 10% heat-inactivated FBS (Wisent), 1% l-glutamine (Wisent), 1% Penicillin–Streptomycin (Wisent) and 1% non-essential amino acids (Sigma) at 37 °C and 5% CO\(_2\). B16-Blue cells (provided by Maziar Divangahi, McGill University) were cultured in RPMI-1640 supplemented with 10% heat-inactivated FBS, 0.5% Penicillin–Streptomycin, 1% l-glutamine, and 100 μg/mL Normocin (InvivoGen). Cells were passaged three times before the addition of selection antibiotic (Zeocin, InvivoGen) at a final concentration of 100 μg/mL.

**Viruses.** Low passage (p.4) ZIKV\(^{\text{CDN}}\) (PLCal\_ZV, Genbank accession KF993678) derived from a ZIKV-infected traveler returning to Canada was provided by Gary Kobinger and David Sarnofetz (National Microbiology Laboratory and Public Health Agency of Canada).\(^{36}\) Low passage (p.4) ZIKV\(^{\text{BR}}\) (HS-2015-BA-01, Genbank accession KX520666) isolated from a symptomatic patient in August 2015 in Salvador, Bahia was provided by Mauro Teixeira (Universidade Federal de Minas Gerais). The stock was originally passaged three times in C6/36 mosquito cells and low passage in Vero cells. To propagate ZIKV\(^{\text{CDN}}\), 6 \times 10\(^{5}\) cells were seeded in 150 mm \(\times\) 25 mm dishes (Sigma) and 24 h later infected at a multiplicity of infection (MOI) of 1 for 2 h in unsupplemented EMEM. After 2 h, infection media was removed and replaced with 15 mL of DMEM supplemented with 2% heat-inactivated FBS, 1% l-glutamine, 1% Penicillin–Streptomycin, and 15 mM Hepes. Supernatants from both mock-infected as well as ZIKV-infected cells were harvested after 48–72 hpi, centrifuged for 10 min at 3000 g, and aliquoted. Viral stocks were titered by plaque assay on Vero cells. Briefly, viral stocks were serially diluted (10-fold) in EMEM (Wisent) and 100 μL of each dilution was used to infect confluent monolayers of Vero cells in 24-well plates in unsupplemented EMEM. At 2 hpi, infection media was removed, and cells were overlaid with 1.2% carboxymethyl cellulose (Sigma-Aldrich) and 2% heat-inactivated FBS in EMEM for 4 days prior to fixation for 1 h with an equal volume of 10% formaldehyde. Monolayers were rinsed gently with distilled water and stained for 30 min with 0.1% crystal violet prior to counting plaques. ZIKV\(^{\text{CDN}}\) or ZIKV\(^{\text{BR}}\) was UV-inactivated by transferring 1 mL of ZIKV into one well of a 6-well plate and exposing it to 3 Joules/cm\(^2\) of UV irradiation in a UVC 500 Crosslinker (Hoefler) as previously described.\(^{32}\) UV-inactivation was verified by plaque assay.

**Mouse experiments.** C57BL/6 mice (WT) were purchased from Charles River laboratories or bred at McGill University. 110\(^{–}\)112 mice were originally purchased from The Jackson Laboratory and were subsequently bred in-house at McGill University. Mice with OT-I TCR-transgenic CD8 T cells (specific for OVA\(_{323-337}\) peptide) were previously described.\(^{44}\) Infected mice were housed in biocontainment level 2 and all animal procedures were carried out in accordance with Canadian
Flow cytometry staining and ex vivo restimulation. For flow cytometry analysis of cells in the blood, blood was collected, and erythrocytes lysed using Vitalex (Cedarlane), cells were incubated with TruStain fcX (anti-mouse CD16/32, clone 93, BioLegend, catalog #101320, 1:100 dilution) and stained with the indicated antibodies, followed by fixation with IC Fixation Buffer (eBioscience). For spleen analysis, spleens were isolated and mechanically disrupted to generate single-cell suspensions. Erythrocytes were lysed with Ammonium-Chloride-Potassium (ACK) buffer (150 mM NaCl, 2.7 mM KHCO3, and 0.1 mM Na2EDTA in distilled H2O, pH 7.2), cells were incubated with TruStain fcX (anti-mouse CD16/32, clone 93, BioLegend, catalog #101320, 1:100 dilution) and stained with the indicated antibodies, followed by fixation with IC Fixation Buffer (eBioscience). For titer staining, following erythrocyte lysis cells were incubated with TruStain fcX (anti-mouse CD16/32, clone 93, BioLegend, catalog #101320, 1:100 dilution) and stained with the indicated antibodies, followed by fixation with IC Fixation Buffer (eBioscience). For dendritic cell detection, spleens were cut into pieces and incubated with 10 ng/mL DNA and 1 mg/mL collagenase for 30 min at 37 °C and 5% CO2 prior to generation of a single-cell suspension by mechanical disruption through a 70-micron basket. Cells were then stained with H-2Db OVA257-302 tetramer, with gentle vortexing every 20 min, followed by surface antibody staining and fixation with IC Fixation Buffer (eBioscience). For dendritic cell detection, cells were first stained with IC Fixation Buffer (eBioscience) followed by fixation with IC Fixation Buffer (eBioscience). Intracellular staining for Granzyme B was performed using Permit/ Wash Buffer (eBioscience) followed by fixation with IC Fixation Buffer (eBioscience). Intracellular staining for Ki67 was performed using the Foxp3/Transcription Factor Staining Buffer Set (eBioscience), as per the manufacturer’s instructions. Detection of BrdU was performed using the Phase-Flow Fix & BrdU Kit (BioLegend), as per the manufacturer’s instructions. Staining for TCR Vβ diversity was performed using the Anti-Mouse TCR Vβ Screening Panel (BD Biosciences), as per the manufacturer’s instructions. Samples were analyzed with a BD LSRFortessa flow cytometer (BD Biosciences), FACs Diva (BD Biosciences), and FlowJo software (BD Biosciences). For ex vivo restimulation, spleens were harvested and processed as above. Erythrocytes were lysed with ACK buffer and samples were simulated for 5.5 h at 37 °C with 5% CO2 in the presence of 200 nM Env249-305 peptide or media alone and brefeldin A (BioLegend). Cells were then stained with surface antibodies and intracellular with IFN-γ and eBioscience, followed by intracellular staining for IFN-γ in Perm/Wash Buffer (eBioscience). Samples were analyzed with a BD LSRFortessa flow cytometer (BD Biosciences), FACs Diva (BD Biosciences), and FlowJo software (BD Biosciences). A representative gating strategy is provided in Supplementary Fig. 10.
(Bio-Rad) and relative mRNA levels were calculated in Excel (Microsoft) using the ΔΔCT method using TATA-binding protein (Tbp) expression as an internal control, and plotted as fold change by normalizing to mock-infected samples. Primers used to detect Ifitm3, Ifna3, Ifnb1, Ifng, Ifit7, Ifi15, Oasl1, Pmosb, and Tbp mRNA (generated by Integrated DNA Technologies) can be found in Supplementary Table 1.

Statistical analyses. Data were analyzed using GraphPad Prism 9 software. Specific tests for determining statistical significance are indicated in the figure legends. p-values of <0.05 were considered statistically significant.

Reporting summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

The authors declare that the main data supporting the findings of this study are available within the article and its Supplementary Information files. Source data are provided with this paper.

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