

Humoral and Cellular Immune Responses Following a Secondary Zika Virus Challenge in Non-Human Primates

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ABSTRACT

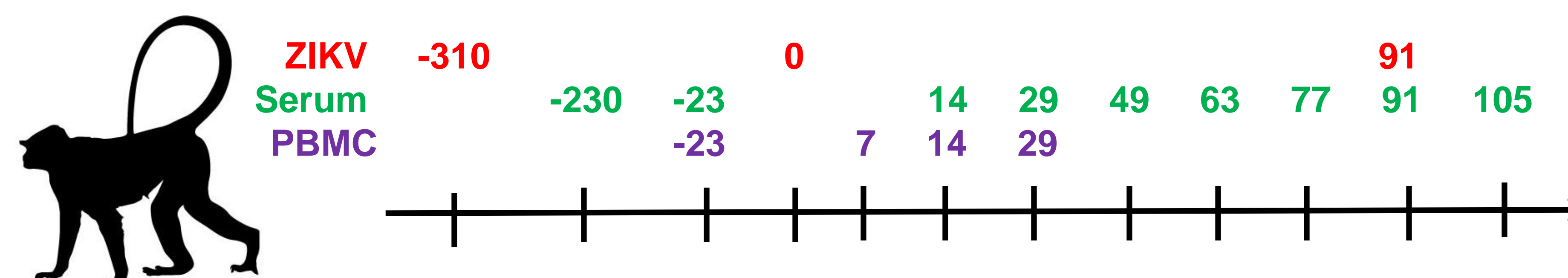
Zika virus recently emerged into the Western hemisphere leading to more than 580,000 suspected cases in South, Central, and North America from 2015 to the present. The current outbreak has been linked to neurological disorders such as Guillain-Barré syndrome and defects in the developing fetus characterized by placental insufficiency, microcephaly, ocular abnormalities, and increased risk of miscarriages. Mouse models of Zika virus disease, while useful, have limitations in the study of immune responses as these animals are refractory to a natural Zika virus infection. On the other hand, non-human primates (NHPs) are the natural reservoir of Zika virus and represent the model that most accurately reflects human pathogenesis and immunity. Assessment of the immune response dynamics following a Zika virus infection will influence our understanding of natural immunity. Furthermore, characterizing the immune response to a secondary Zika virus challenge will help to define the parameters of a protective immune response and gauge vaccine efficacy. In this study, we challenged 6 rhesus macaques that had previously been infected with Zika virus. Serum and peripheral blood monocytes (PBMCs) were collected at pre-challenge and days 7, 14, 29, 49, 63, 77, 91, and 105 post challenge. Humoral immune responses were analyzed by serum neutralization assays and were found to peak at day 14 after secondary challenge. Antiviral T cell responses against the viral envelope protein were evaluated by intracellular cytokine staining and flow cytometry. Antiviral T cell dynamics were highly variable across individual animals, with levels generally highest at day 14 after secondary challenge. A majority of the antiviral CD8⁺ and CD4⁺ T cells only secreted a single cytokine. This study highlights the memory recall response against a secondary Zika challenge. Further studies will determine how these findings compare to novel vaccination strategies which elicit immunologic memory.

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CONCLUSIONS

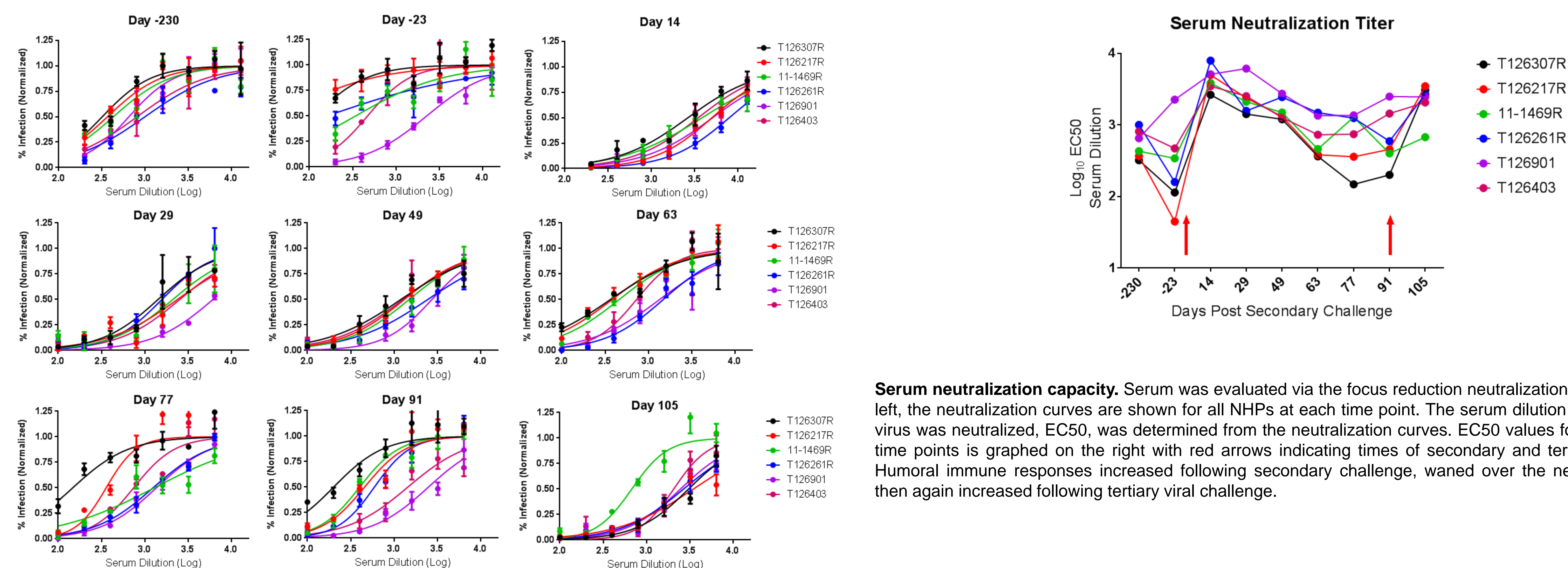
- Previous Zika virus exposure protects NHPs from 2^o challenge
- Humoral immune responses elevated post 2^o and 3^o challenge.
- Antiviral T cell responses elevated post 2^o challenge

STUDY OUTLINE



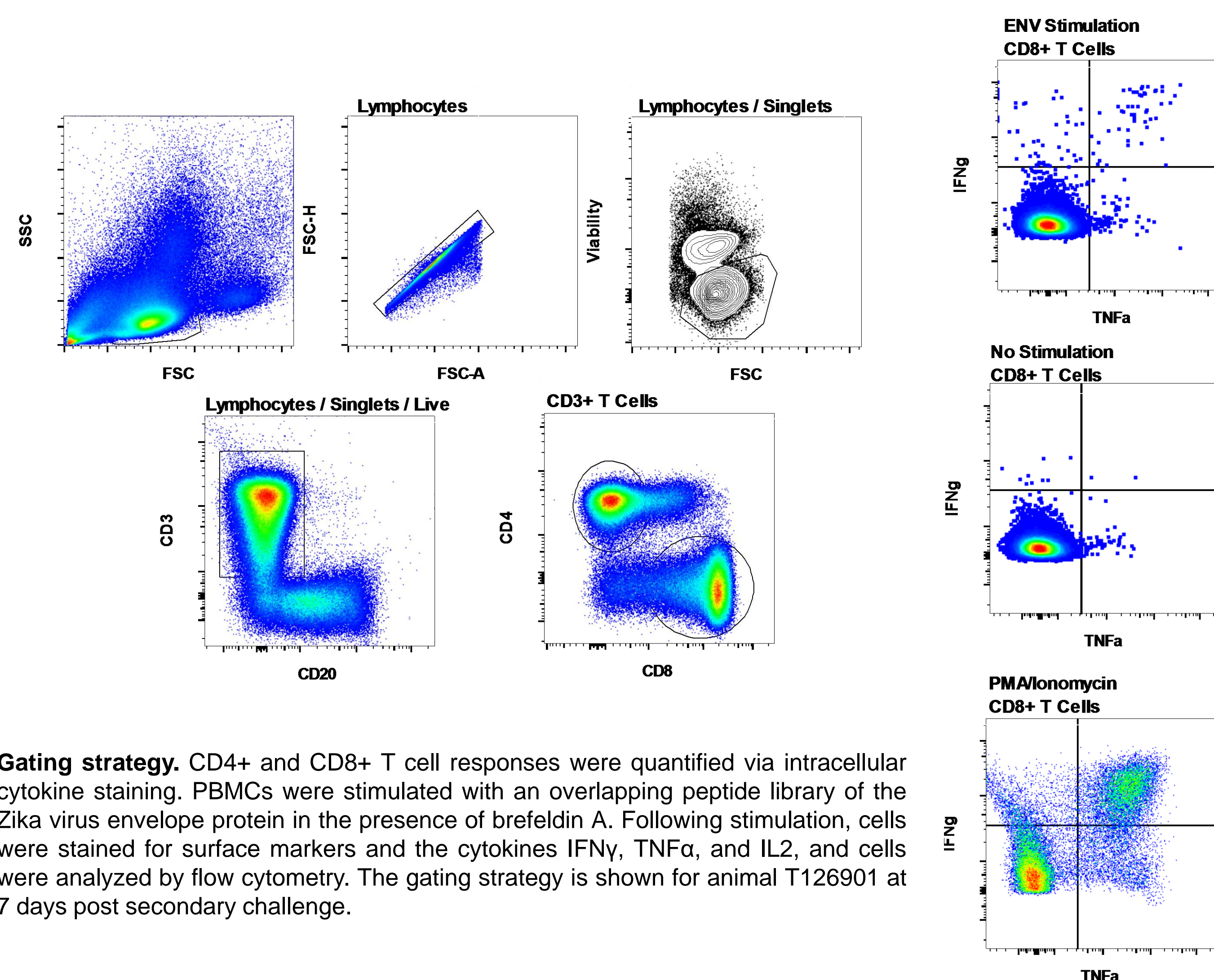
Six Chinese-origin rhesus macaques were challenged with 1x10⁶ PFU (plaque forming units) of Zika virus strain PRVABC59 on study day -310, 0, and 91. All time points are relative to the secondary challenge date. Serum and PBMC were collected at the indicated times days for downstream analysis. No virus was detected in the serum following secondary or tertiary challenge.

HUMORAL IMMUNITY

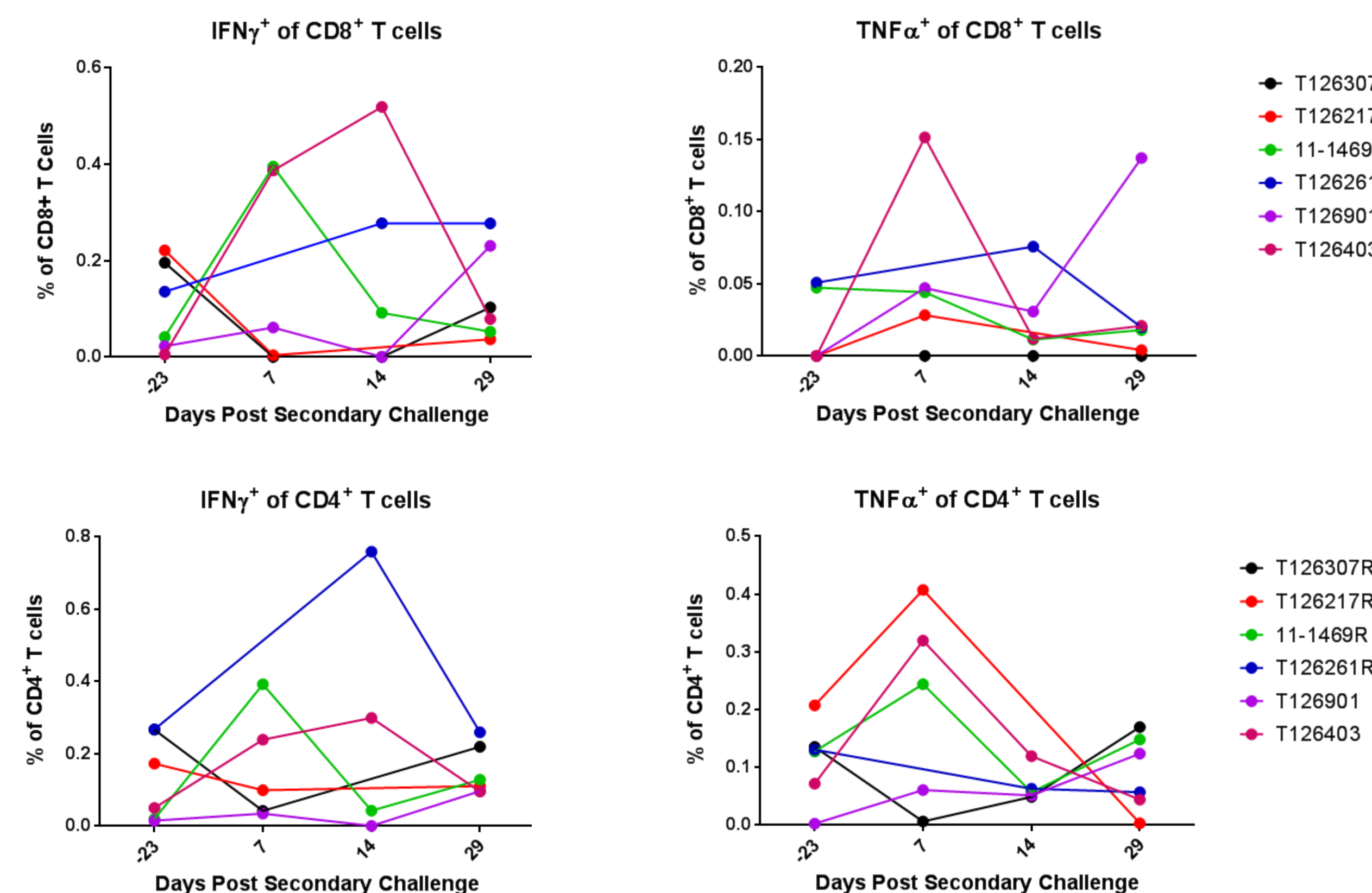


Serum neutralization capacity. Serum was evaluated via the focus reduction neutralization test (FRNT). On the left, the neutralization curves are shown for all NHPs at each time point. The serum dilution at which 50% of the virus was neutralized, EC50, was determined from the neutralization curves. EC50 values for all animals and all time points is graphed on the right with red arrows indicating times of secondary and tertiary viral challenge. Humoral immune responses increased following secondary challenge, waned over the next two months, and then again increased following tertiary viral challenge.

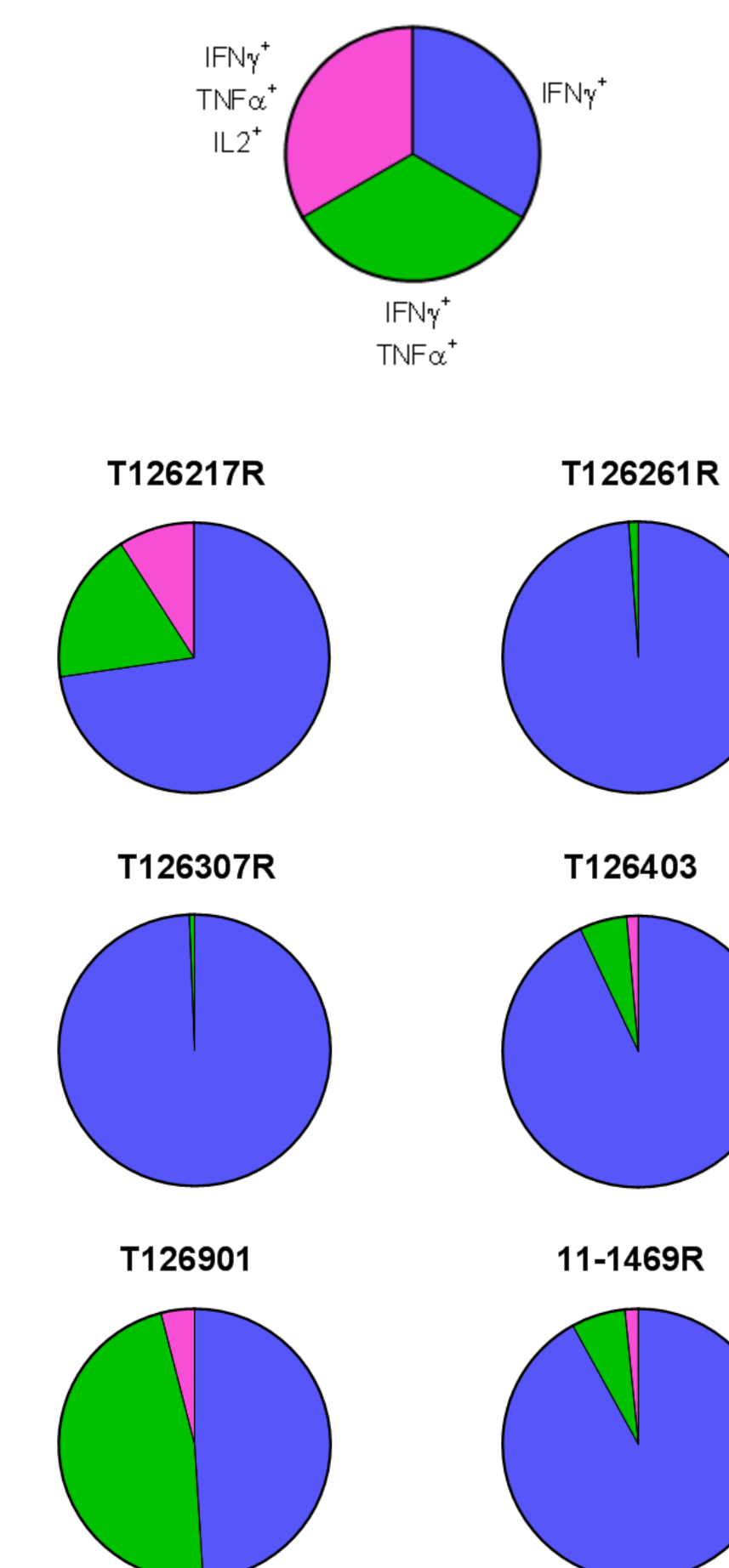
CELLULAR IMMUNITY



Gating strategy. CD4⁺ and CD8⁺ T cell responses were quantified via intracellular cytokine staining. PBMCs were stimulated with an overlapping peptide library of the Zika virus envelope protein in the presence of brefeldin A. Following stimulation, cells were stained for surface markers and the cytokines IFN γ , TNF α , and IL2, and cells were analyzed by flow cytometry. The gating strategy is shown for animal T126901 at 7 days post secondary challenge.



Antiviral T cell frequency increased post secondary challenge. IFN γ ⁺ and TNF α ⁺ secreting T cells was determined by intracellular cytokine staining post peptide library stimulation and flow cytometry. The frequency of IFN γ ⁺ and TNF α ⁺, CD4⁺ and CD8⁺ cells are graphed for all animals at all time points pre and post secondary viral challenge. Background staining resulting from no peptide stimulation was subtracted.



Antiviral T cells are largely monofunctional. Cytokine secreting CD8⁺ T cells were analyzed for cytokine secretion and categorized into single, bi-functional, or polyfunctional. Shown is a representative plot from each NHP at a single time point post secondary challenge.