**Supplementary Tables**

**Supplementary Table 1**. ZIKV NS1-specific antibody measured by enzyme-linked immunosorbent assay (ELISA)

| NHP ID | Primary infection | | | | | |
|---|---|---|---|---|---|---|
| | IgM[1] | | | IgG[1] | | |
| | Day 1 | Day 3 | Day 7 | Day 1 | Day 3 | Day 7 |
| **5305** | ND | ND | 0.153 | ND | ND | 0.110 |
| **5687** | ND | ND | 0.121 | ND | 0.548 | 1.683 |
| **5831** | ND | ND | 0.125 | 0.092 | 0.092 | 1.378 |
| **5825** | ND | ND | 0.142 | ND | ND | ND |
| **5169** | ND | 0.051 | 0.183 | ND | ND | ND |
| **5690** | 0.063 | 0.069 | 0.132 | ND | ND | ND |
| **5818** | 0.053 | 0.051 | 0.267 | ND | ND | ND |
| **5829** | ND | 0.051 | 0.404 | 0.073 | ND | 0.082 |
| **5939** | ND | ND | 0.085 | ND | ND | ND |
| **5963** | ND | ND | 0.077 | ND | ND | ND |

**[1]Mean optical density (405 nm) is shown.**

**ND: not detected**

**Supplementary Table 2**. Neutralizing antibody titers measured by 90% plaque reduction neutralization test ($PRNT_{90}$)

| NHP ID | Primary infection | | Re-challenge | |
|---|---|---|---|---|
| | Day 7 | Day 28 | [1]Day 0 | Day 7 |
| **5169** | 2 | 160 | 160 | 320 |
| **5690** | 2 | 320 | 1280 | 1280 |
| **5818** | 20 | 1280 | 320 | 1280 |
| **5829** | 20 | 160 | 80 | 80 |
| **5939** | 40 | 1280 | 640 | 1280 |
| **5963** | 20 | 160 | 160 | 640 |

**[1]Re-challenge (Day 0) is equivalent to Day 45 post primary infection**



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**Supplementary Figure Legends**

**Supplementary Figure 1. Viral dynamics and shedding in rhesus macaques infected with a Puerto Rican (PR) isolate.** ZIKV RNA was extracted from each specimen and quantitated by qRT-PCR in (**a**) plasma, (**b**) urine, (**c**) saliva and (**d**) CSF.

**Supplementary Figure 2. Blood chemistries during primary ZIKV infection.** Blood chemistries were analyzed on days 0, 3, 7, and 10 during primary ZIKV infection for male (blue, n=5) and female (red, n=5) rhesus monkeys. Black line indicates median.

**Supplementary Figure 3. Body temperature during primary ZIKV infection.** Body temperature was monitored via subcutaneously implanted transponders that recorded peripheral temperature every 30 minutes during the first 14 days following ZIKV infection. Shown are the mean temperatures for male (blue, n=5) and female (red, n=5) monkeys. Temperature fluctuations on days 7 and 14 occurred during anesthesia for biopsies. Mean trough on day of infection indicated by dashed line. Trough temperatures (averaged within the shaded gray regions) on each day post infection were compared to those on day 0 using a repeated measured ANOVA followed by a Dunnett's multiple comparison. Days 1 through 8 were significantly different from baseline (day 0): Days 1 and 2 $p < 0.01$, days 3-7 $p < 0.001$, and day 8 $p < 0.05$.

**Supplementary Figure 4. Complete blood counts during ZIKV infection.** Compete blood counts were analyzed on a) days 0, 1, 2, 3, 4, 5, 7, 10, and 14 during primary infection for 5 male (blue) and 5 female (red) rhesus monkeys and on b) days 0, 1, 2, 3, 4, 5, and 7 during re-infection for 3 male (blue) and 3 female (red) rhesus monkeys. Black line indicates median.



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**Supplementary Figure 5. Peripheral blood mononuclear cell activation and frequencies during primary ZIKV infection.** Activation and absolute numbers of T cells, B cells, NK cells and monocytes were measured by flow cytometry on days 0, 1, 2, 3, 4, 5, 7, 10, and 14 following ZIKV infection of 5 male and 5 female monkeys infected with a Thai ZIKV isolate. (**a**) Percent activated (CD16+) and total numbers per μL whole blood of monocytes. Black line indicates median. Individual values are shown for males (blue) and females (red). (**b**) Comparison of percent CD16+ monocytes on day 2 of infection between males and females by a Mann-Whitney test. Lines indicate median and interquartile range. Percent-activated (CD69+) and total numbers per μL whole blood of naïve (CD28+CD95-), central memory (CD28+CD95+) and effector/effector memory (CD28-CD95+) CD4+ (**c**) and CD8+ (**d**) T cells. (**e**) Percent activated (CD69+) and total numbers per μL whole blood of CD16+, CD16-CD56+, and CD16-CD56- NK cells. (**f**) CD38 expression (geometric mean fluorescence of CD38) and total numbers per μL whole blood of naïve (CD27-) and memory (CD27+) B cells.

**Supplementary Figure 6. ZIKV-specific T cells during primary ZIKV infection.** Degranulation and cytokine production of T cells in PBMC (**a**) and lymph nodes (**b**) were measured by flow cytometry following stimulation of cells from days 0, 7, 14, 21 (PBMC only), and 28 with overlapping peptide pools covering the entire capsid and envelope proteins of ZIKV. Percent of cells expressing CD107a, IFNγ, TNFα, and IL-2 after a 6hr peptide stimulation is shown. A threshold for determining positive values, after background subtraction of un-stimulated corresponding samples, was applied as previously described [18]. Values below this threshold were set to 0. Values greater than 1.5-fold above day of challenge were considered anamnestic. Each monkey indicated by a different color. Triangles are males, circles females.



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**Supplementary Figure 7. Cytokine modulation in rhesus macaques following ZIKV infection.** The levels of various cytokines were measured in plasma on days 0, 1, 3, and 7 post infection. (**a**) Heatmap of cytokine levels at 1, 3, and 7 days post-infection (dpi). Each cytokine level is summarized as the $\log_{10}$ of the ratio relative to baseline (0 dpi), arctangent transformed to reduce influence of extreme values. Rows (cytokines) are clustered using Ward's method and squared Euclidean distance. Columns are grouped by time point sampled (d1, d3, d7), and ordered by animal within time point, as labeled below the heatmap. Animal gender is indicated by text color (blue, male; red, female). (**b**) Cytokines that exhibited significant changes from baseline are indicated. Experiments were performed in duplicate (error bars represent SEM). The comparison of the values from baseline was determined using a Kruskal-Wallis test with multiple comparisons.

**Supplementary Figure 8. ZIKV dynamics modeling. (a)** Representative model fits (solid line) to observed viral load in the plasma (filled circles) using a standard viral dynamic model with an eclipse phase. The parameters fit were the rate of infection ($\beta$), rate of clearance of infected cells ($\delta$) and rate of production of virus from productively infected cells ($p$). Initial conditions, time spent in the eclipse phase ($1/k$) and clearance of free virus ($c$) were fixed. **(b)** Correlation between the observed plasma VL (log10) downslope (measured from peak observation to final detectable observation) and CD16- CD56+ CD69+ NK cells (i) concentration at time of peak VL and (ii) peak concentration but not (iii) concentration at time of infection. R and p values shown are from the Pearson correlation, and p values are shown without correction for multiple testing. **(c)** The half-life (mean 3.1 h) of a productively infected cell estimated from model fits to each monkey (indicated by color) as in (**b**).

**Supplementary Figure 9. ZIKV infectivity from the bodily fluids of infected macaques.** Vero cells were infected (at Day 0) using diluted semen, saliva or CSF. ZIKV RNA in culture supernatants was assessed at various time points after infection. ZIKV-infected blood plasma served as a positive control, mock infection as a negative control.

**Supplementary Figure 10. Detection of ZIKV RNA in lymph node myeloid cells but not in T or B cells.** Axillary lymph nodes were stained for ZIKV RNA (red) by RNAscope and then immunofluorescently stained for (left) CD163 and CD68 (green), DC-SIGN (blue), and DAPI on day 5; (middle) CD163 and CD68 (green), CD20 (blue), and DAPI on day 7; and (right) CD163 and CD68 (green), CD3 (blue), and DAPI on day 5. Left image shows ZIKV RNA in CD163/CD68+ (myeloid) cells, some of which also express DC-SIGN. Middle image shows absence of ZIKV RNA in CD20+ (B) cells. Right image shows ZIKV RNA in CD163/CD68+ (myeloid) cells and absence of ZIKV RNA in CD3+ (T) cells. Images are representative of (n=2) per time point. All images are shown at 60X magnification. Scale bar =200um.



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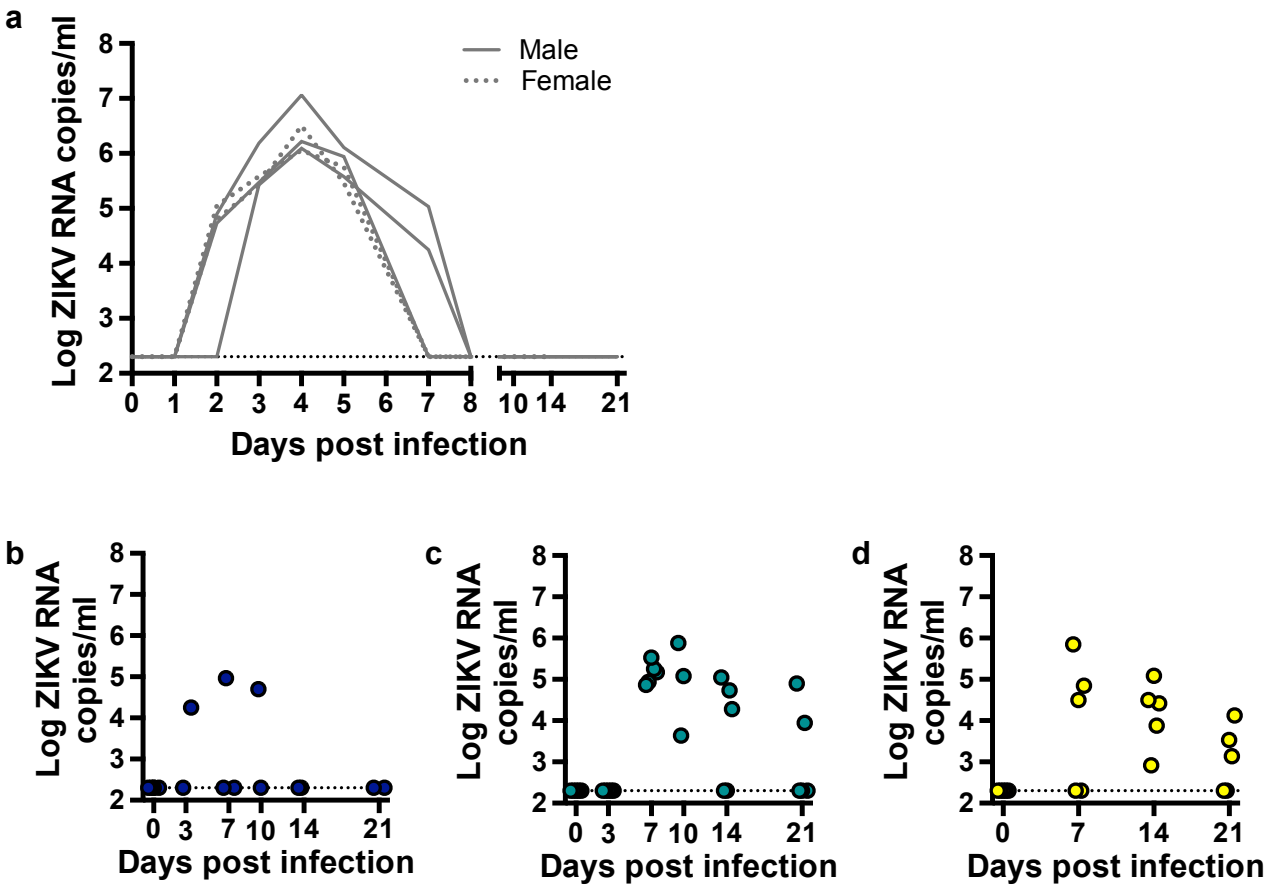


**Supplementary figure 1**

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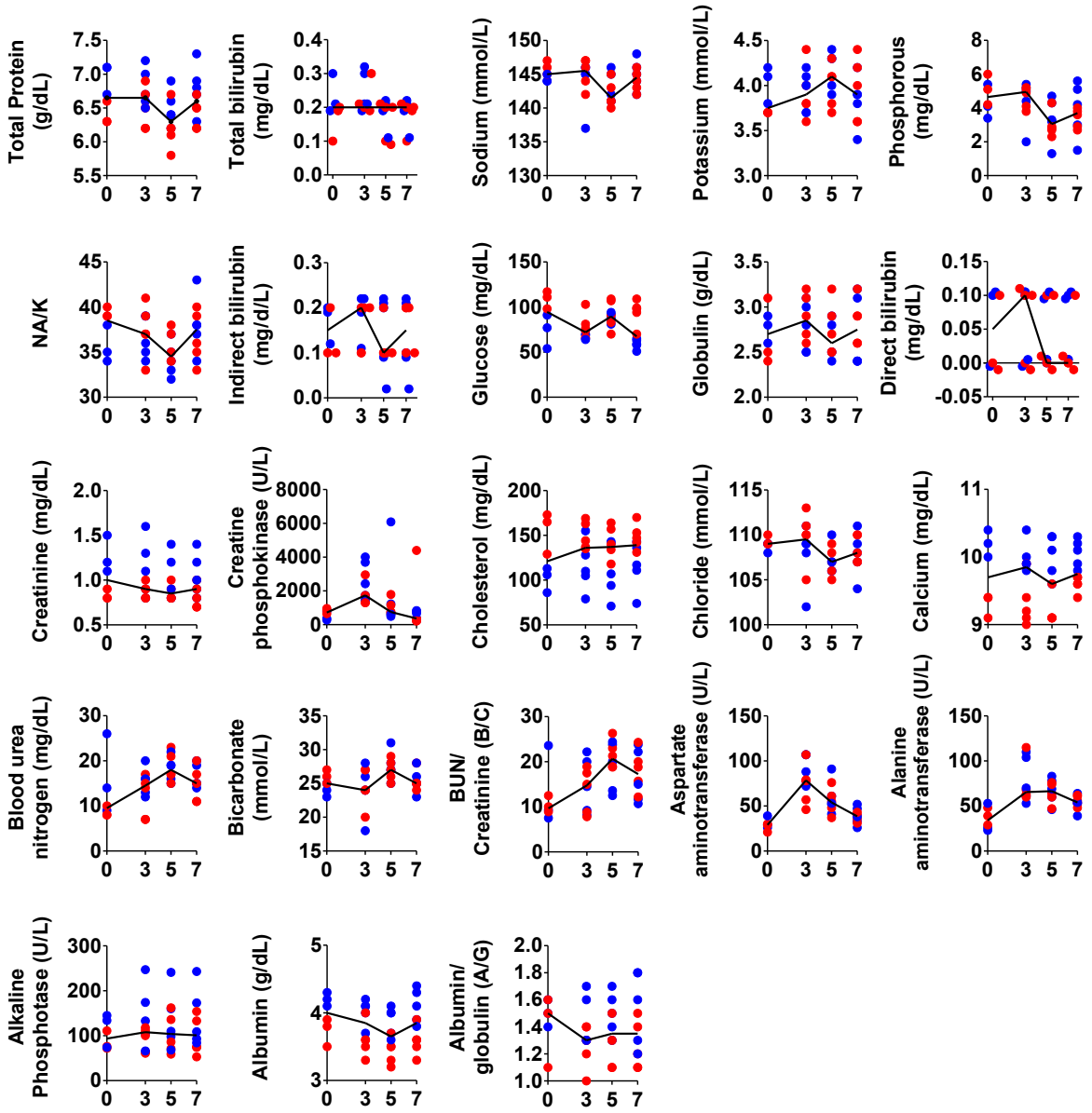


**Supplementary figure 2**

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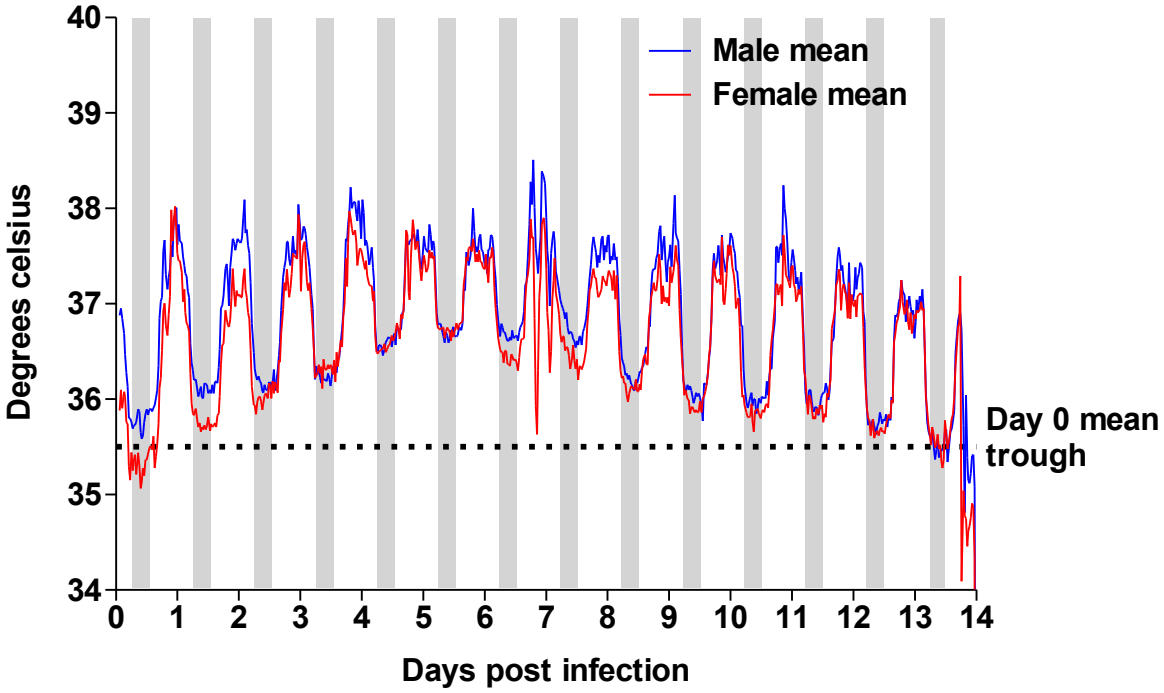


**Supplementary figure 3**

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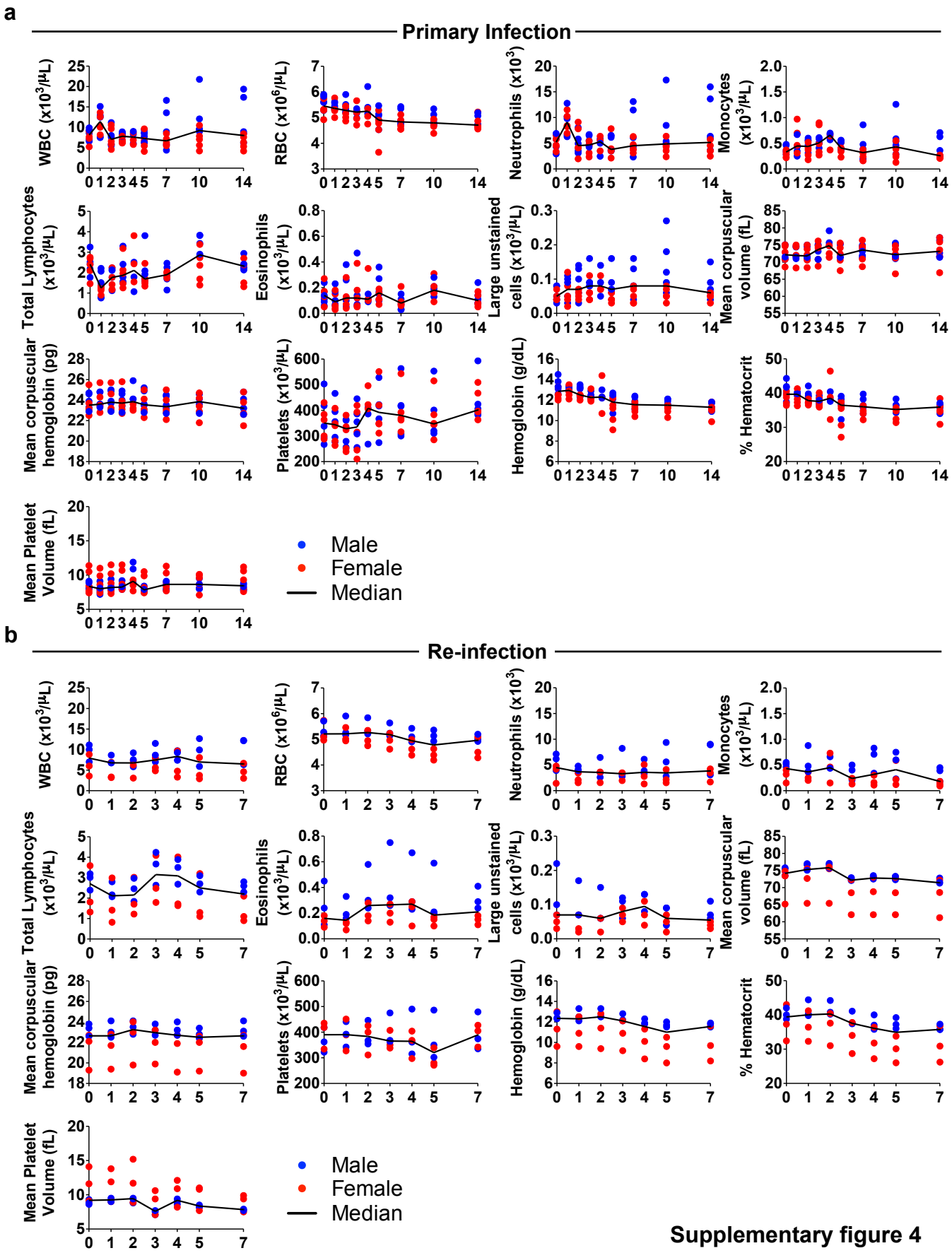


**Supplementary figure 4**

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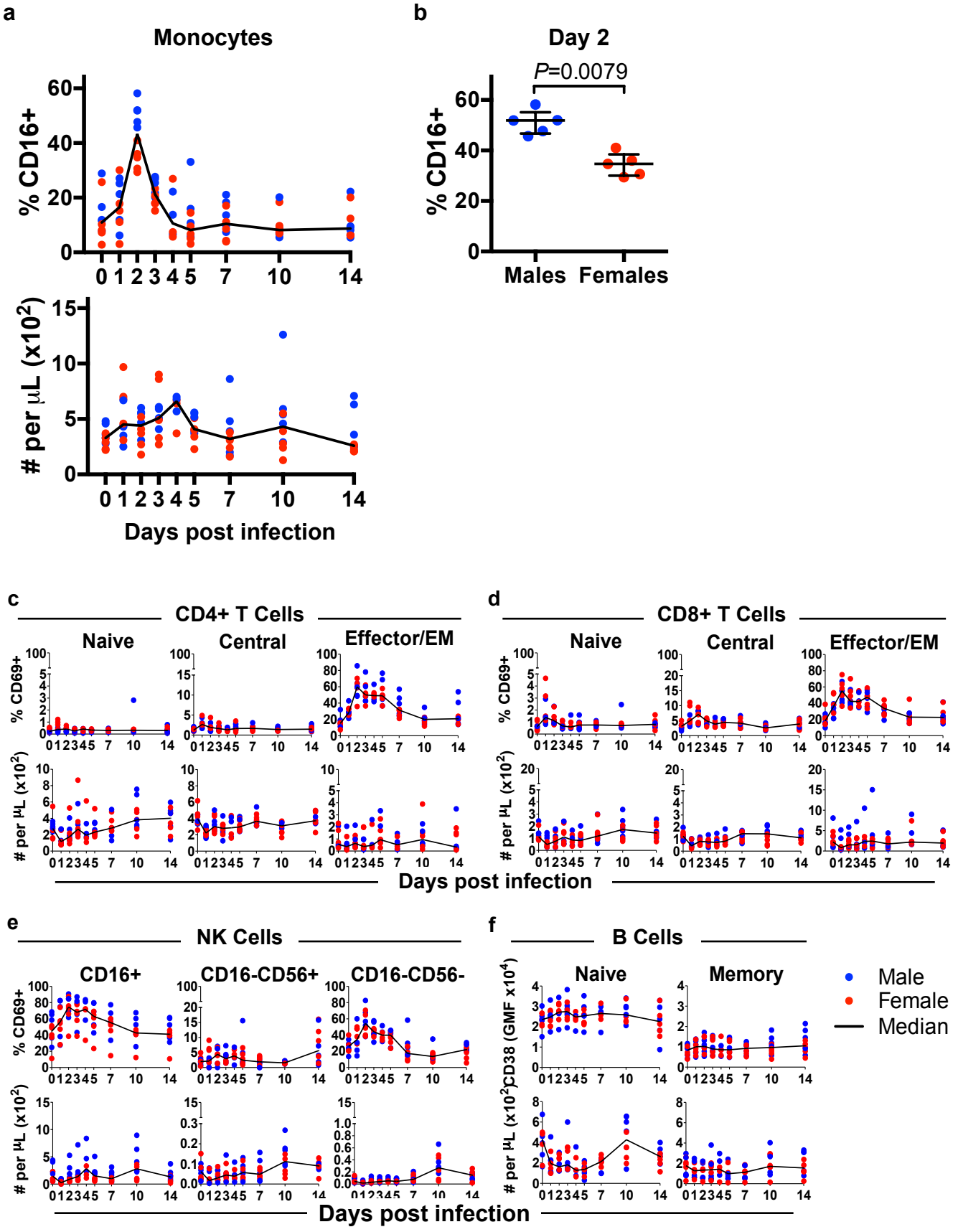


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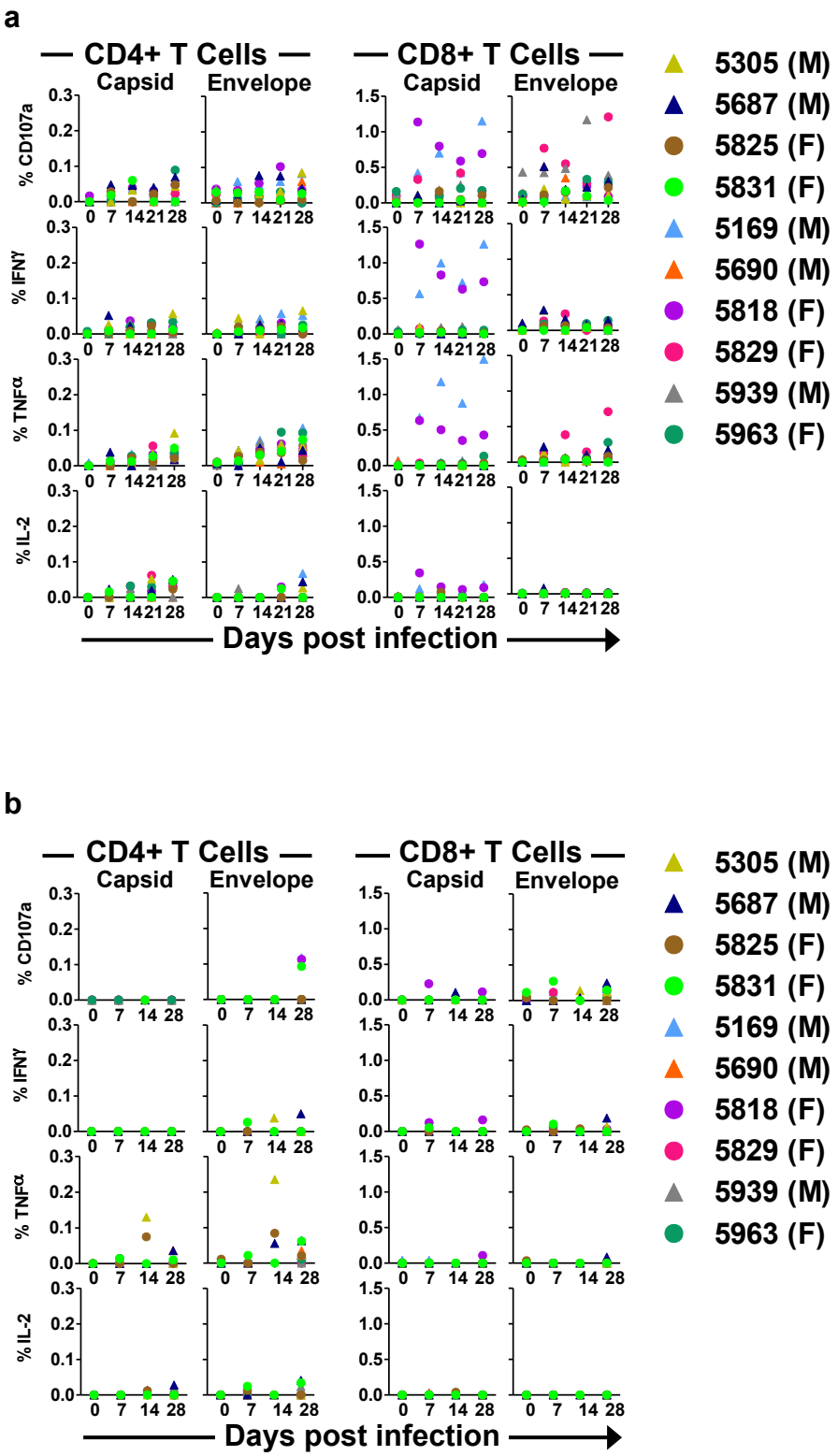


**Supplementary figure 6**

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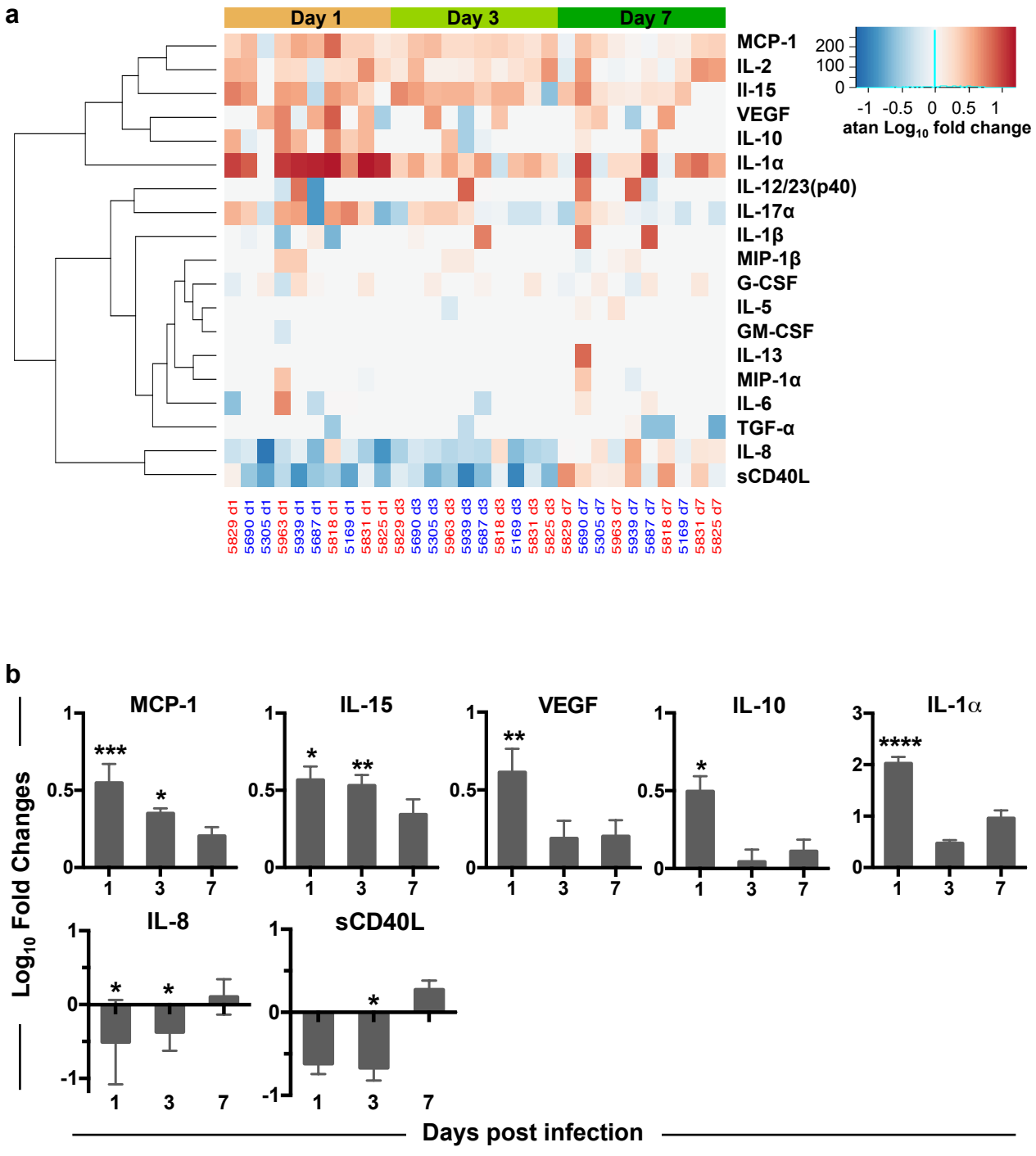


**Supplementary figure 7**

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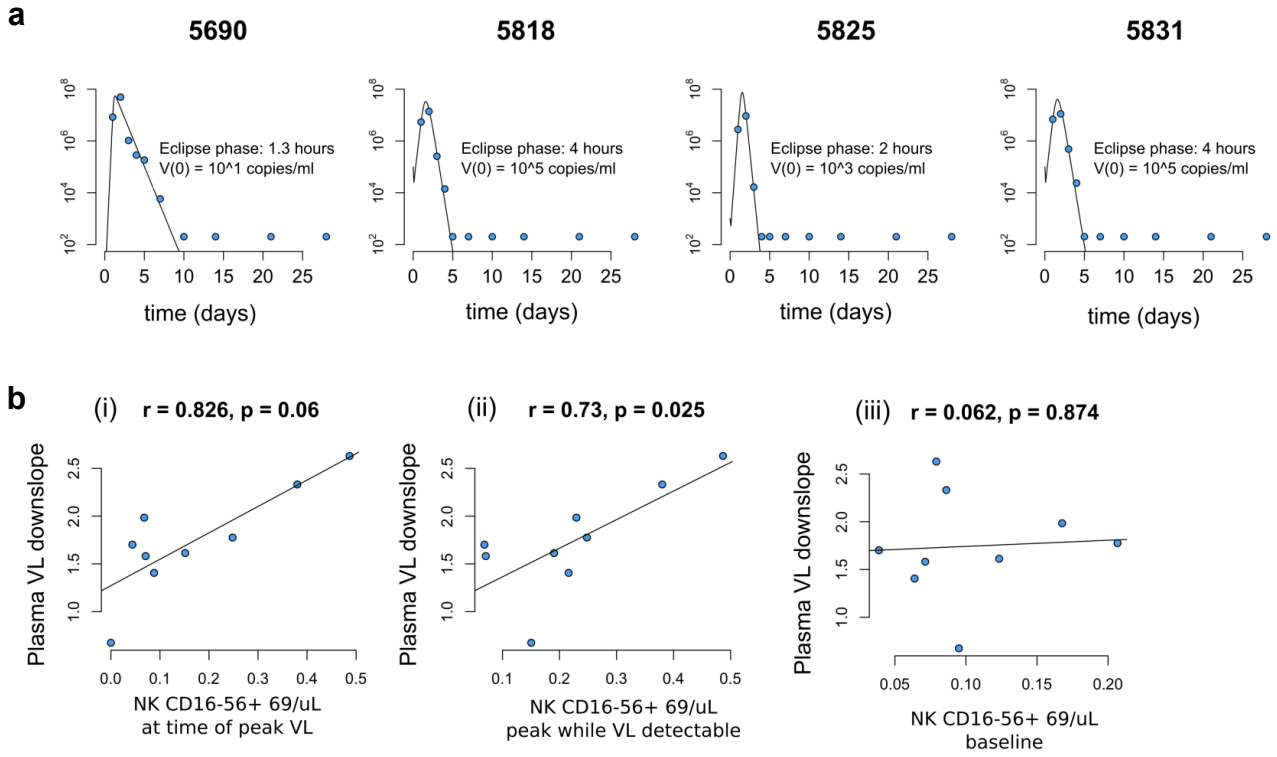
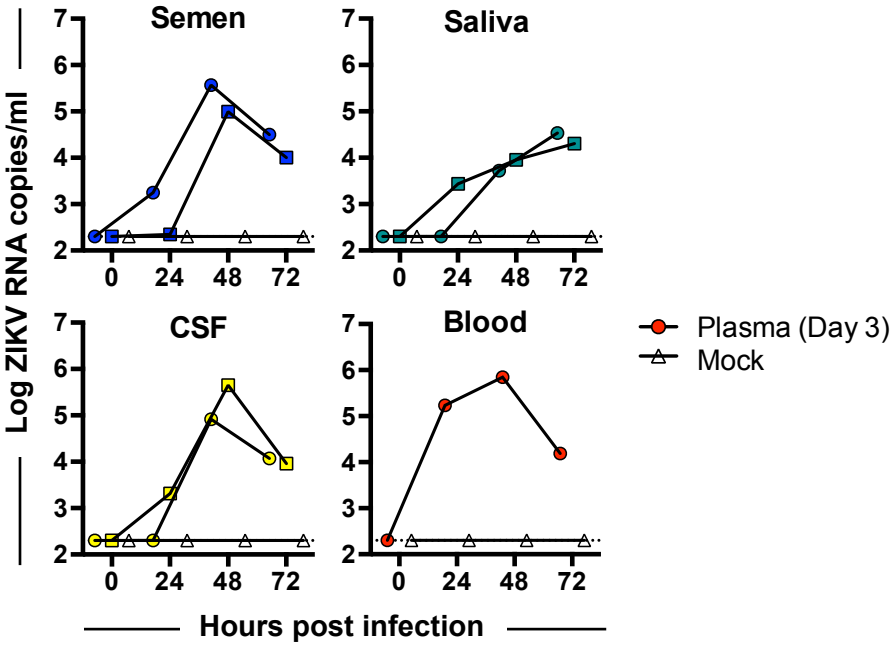



**Supplementary figure 8**




**Supplementary figure 9**

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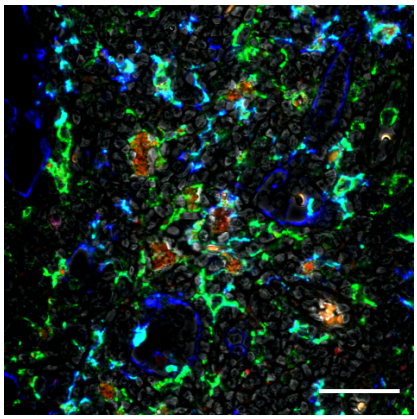
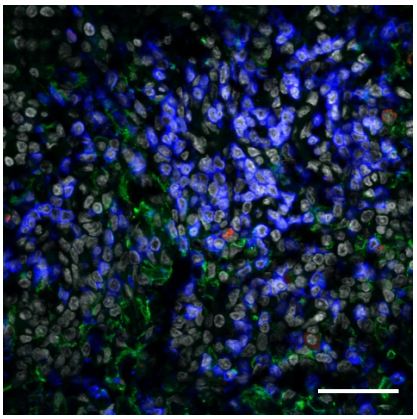
## Lymph Node



vRNA / CD163/CD68 / DC-SIGN / DAPI



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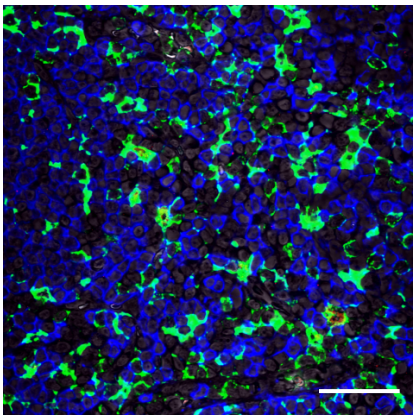

vRNA / CD163/CD68 / CD3 / DAPI

**Supplementary figure 10**

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