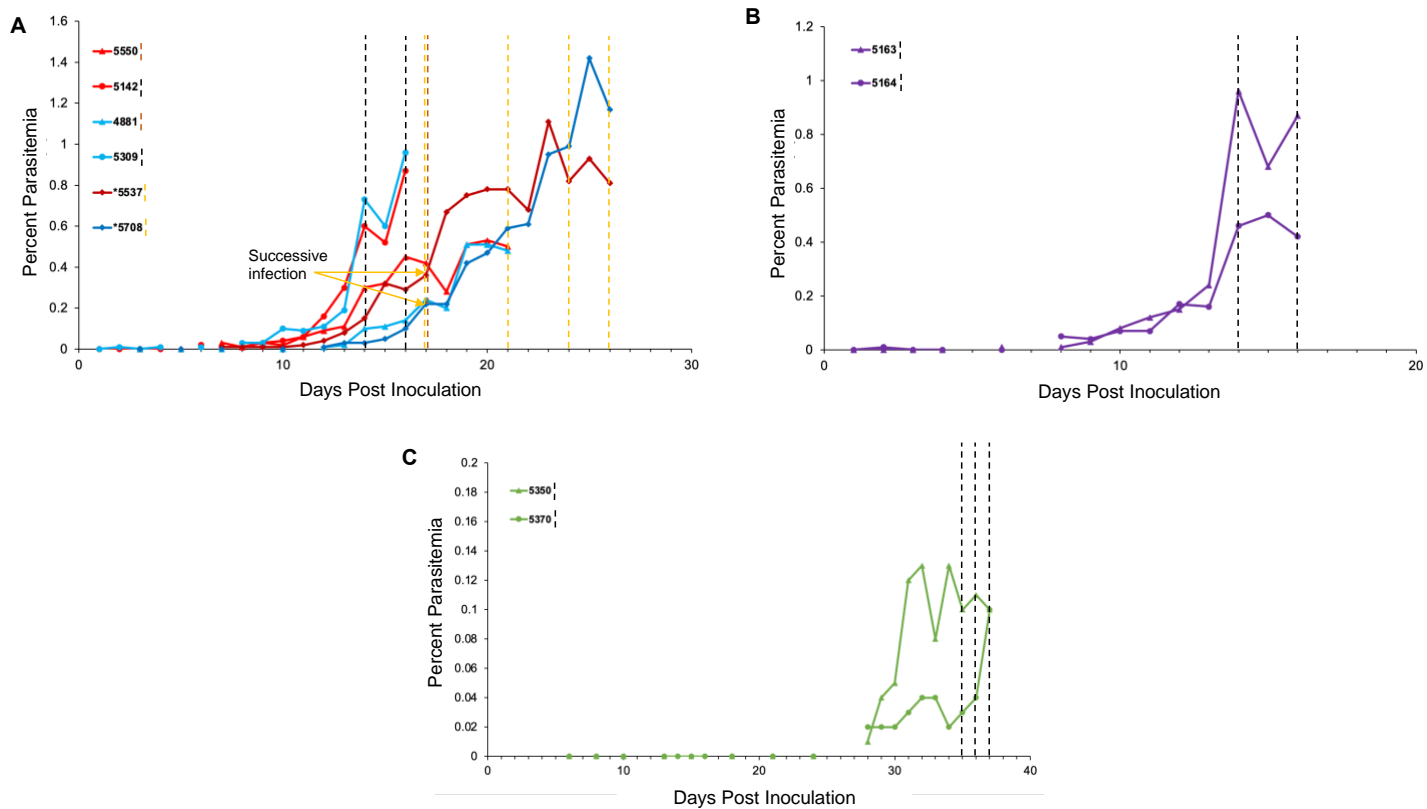
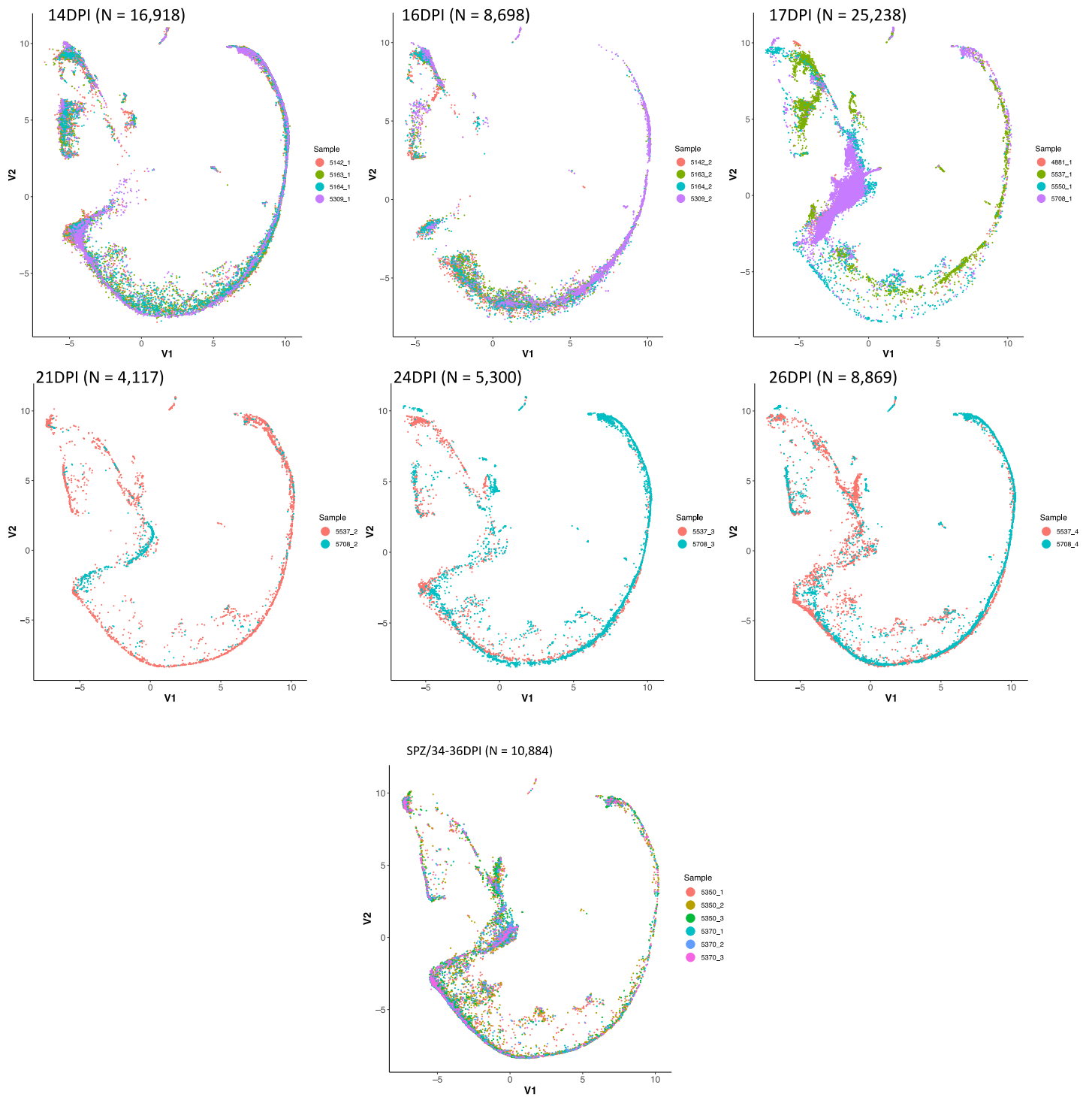


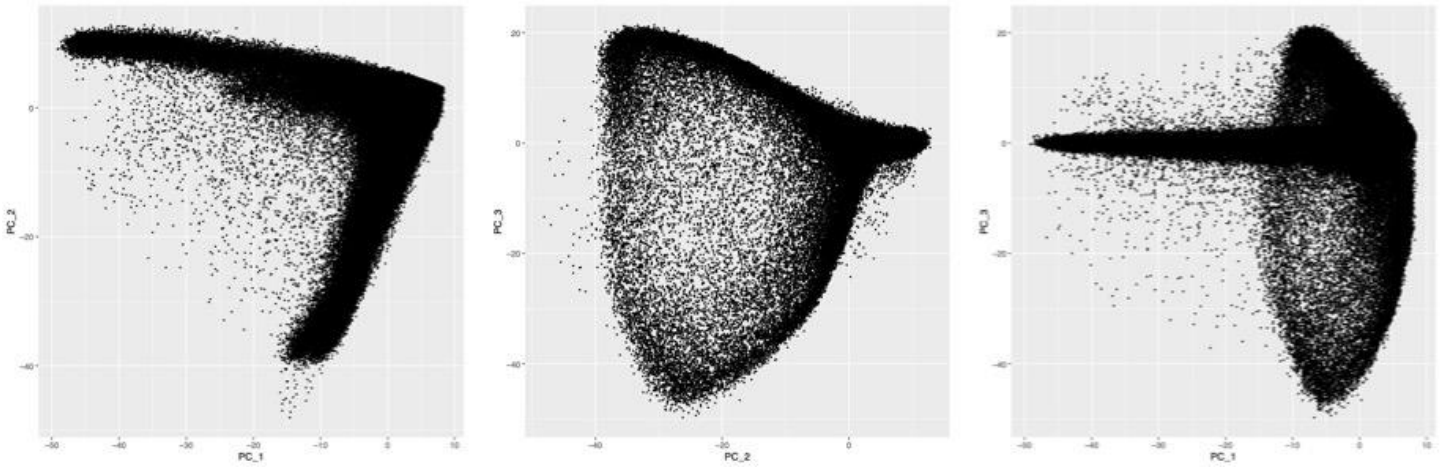
Supplemental Figure 1: NIH-1993-F3 generation. Origin of the NIH-1993-F3 strain of *P. vivax* through self-cross of NIH-1993 and passages through Saimiri monkeys and *Anopheles freeborni* mosquitoes. Serial passages of recombinant progeny from a genetic cross of the NIH-1993-RxS line (F0) through a chimpanzee (Pt8A006) were made by parasitized RBC injection (pRBC) or sporozoite inoculation (SPZ) through naïve, splenectomized Saimiri monkeys over three generations (F1-F3). Pt, *Pan troglodytes* (chimpanzee); Sb, *Saimiri boliviensis boliviensis*. Blue and black lines indicate inoculation with cryopreserved and fresh sporozoites, respectively. Solid and dashed red lines represent cryopreserved and fresh pRBCs, respectively.



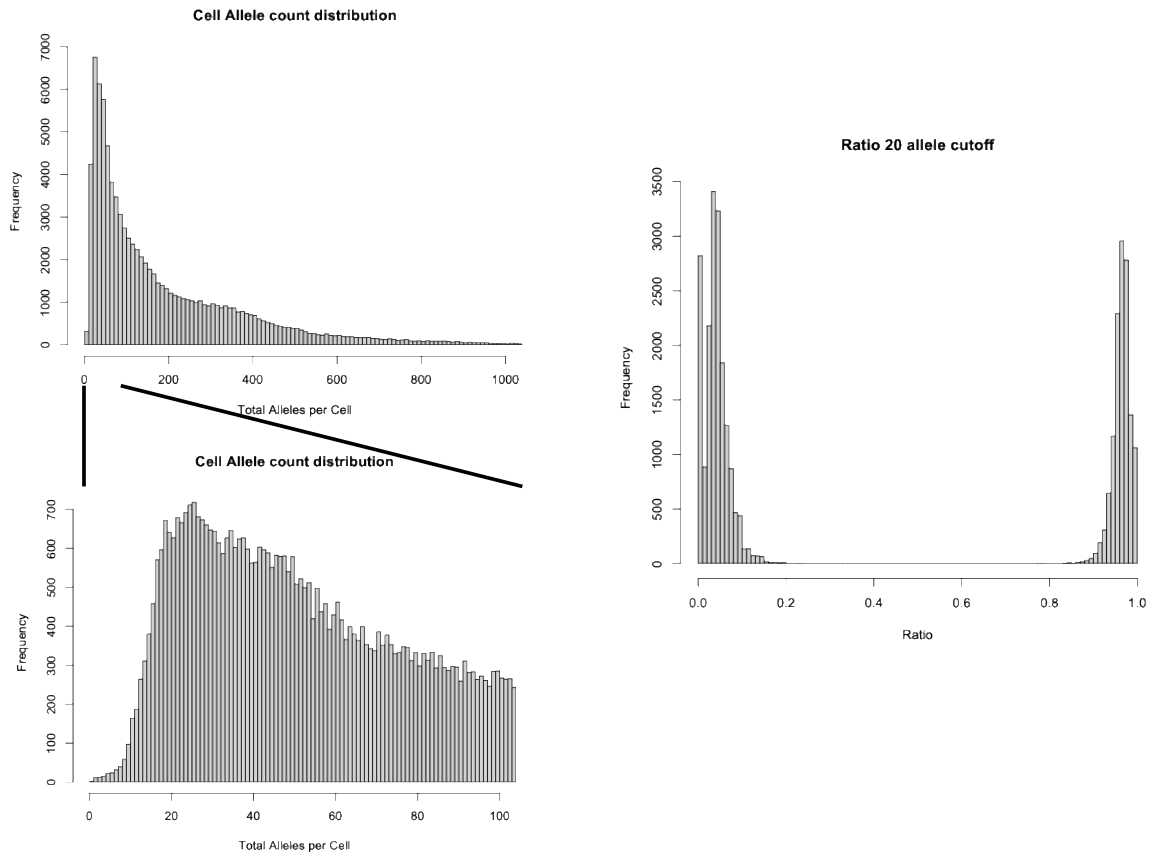
Supplemental Figure 2: Timeline of the blood collections and parasitemia for each animal A) mono and consecutive infections (note the arrows indicating the second inoculation at day 17), B) simultaneous coinfections, C) sporozoite infections. Each curve corresponds to the parasitemia in a specific animal (see also Supplemental Data 1 for more details on each animal).



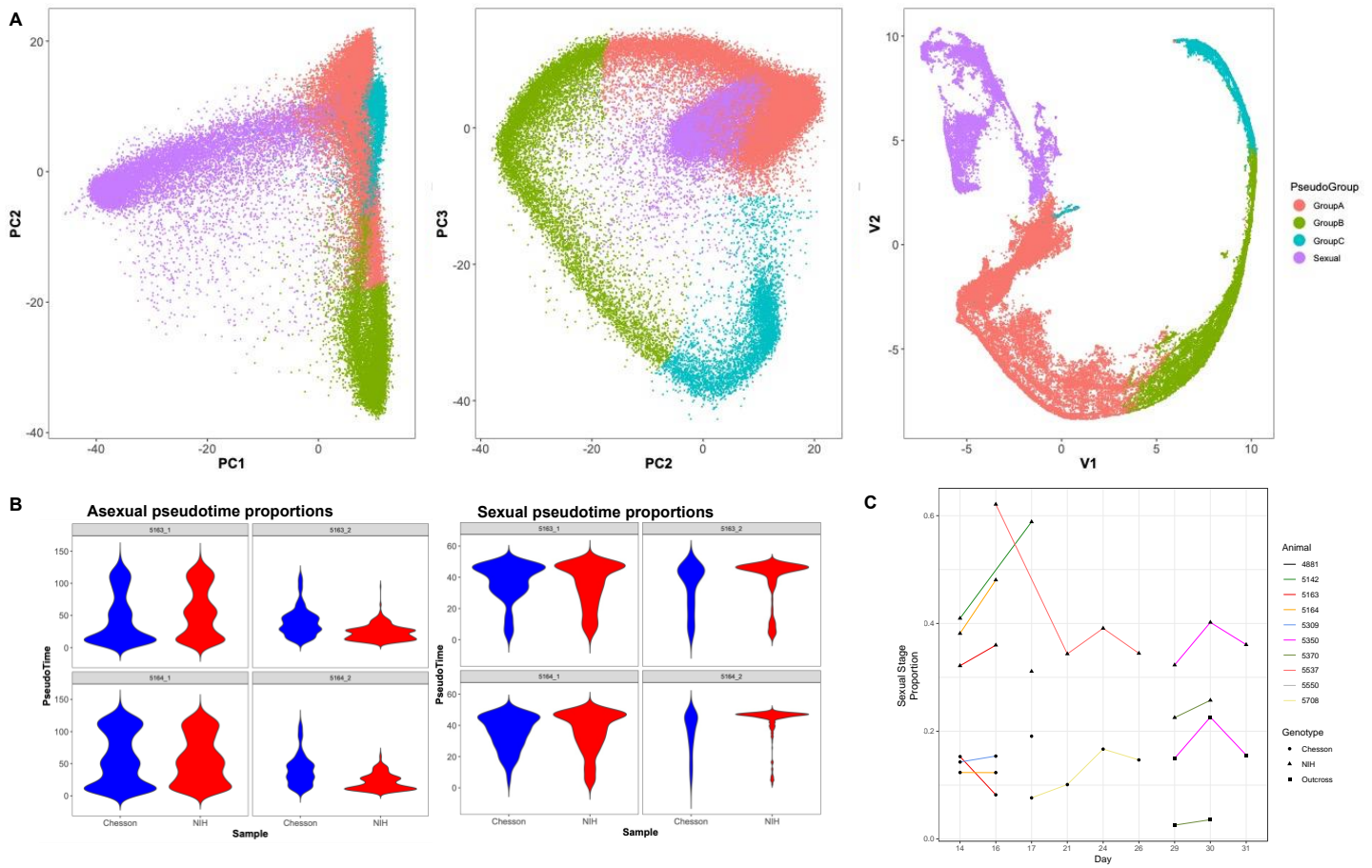
Supplemental Figure 3: UMAP by Time Point. UMAP of all blood stage parasites split by time since inoculation (dpi) and colored by animal.



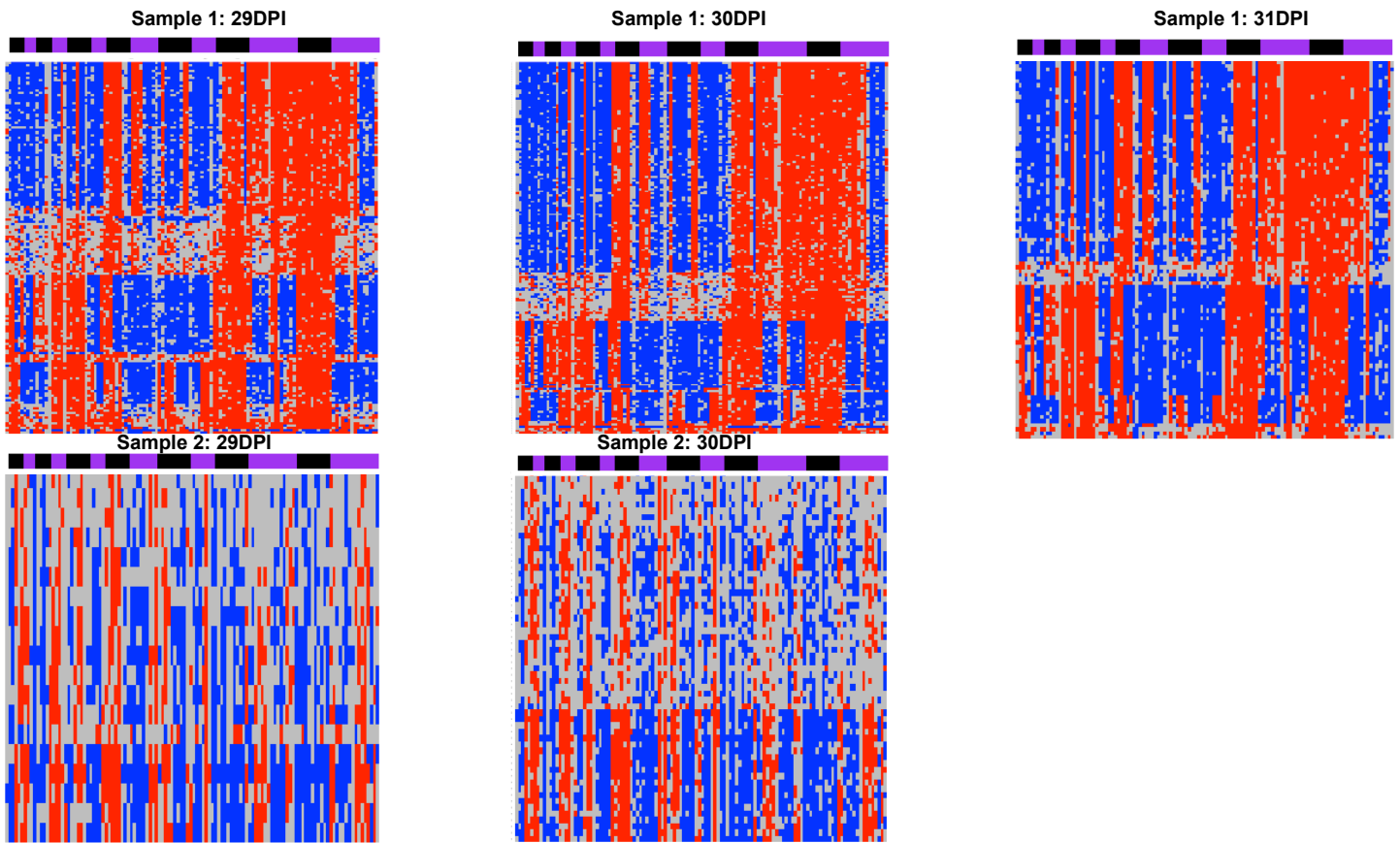
Supplemental Figure 4: Expanded PCA. PCA with all *P. vivax* blood stage parasites characterized by more than 250 UMIs.



Supplemental Figure 5: Detailed Genotyping Analysis. The left panels show the distribution of the number of differentiating alleles successfully characterized per single cell (top: all cells, bottom: zoom in on the cells with 0-100 alleles). The right panel shows the ratio of NIH/Chesson alleles for all cells from the mono-infections (only cells with at least 20 alleles are included in this analysis).



Supplemental Figure 6: Pseudobulk Analysis. A) PCA and UMAP showing the cells pooled together for pseudobulk analysis: red – asexual A, green – asexual B, blue – asexual C, purple – female gametocyte S. B) Distribution of pseudotimes for all asexual (left) and sexual (right) parasites from the coinfections. The pseudotimes are separated by genotypes (Red: NIH-1993-F3, Blue: Chesson) and each box shows the data from one blood sample. C) Variations in the proportion of sexual stages (y-axis) according to the sampling time (x-axis, in dpi). Data points from the same animals are linked by a line.



Supplemental Figure 7: Extended outcross analysis by sample. Reconstruction of the haplotypes across the entire genome for outcrossed parasites from two monkeys (top and bottom panels) and over time.

See Figure 5 for details.