Cell Reports Medicine, Volume *1*

Supplemental Information

Neutralizing Antibodies Protect against Oral Transmission of Lymphocryptovirus

Swati Singh, Leah J. Homad, Nicholas R. Akins, Claire M. Stoffers, Stefan Lackhar, Harman Malhi, Yu-Hsin Wan, David J. Rawlings, and Andrew T. McGuire

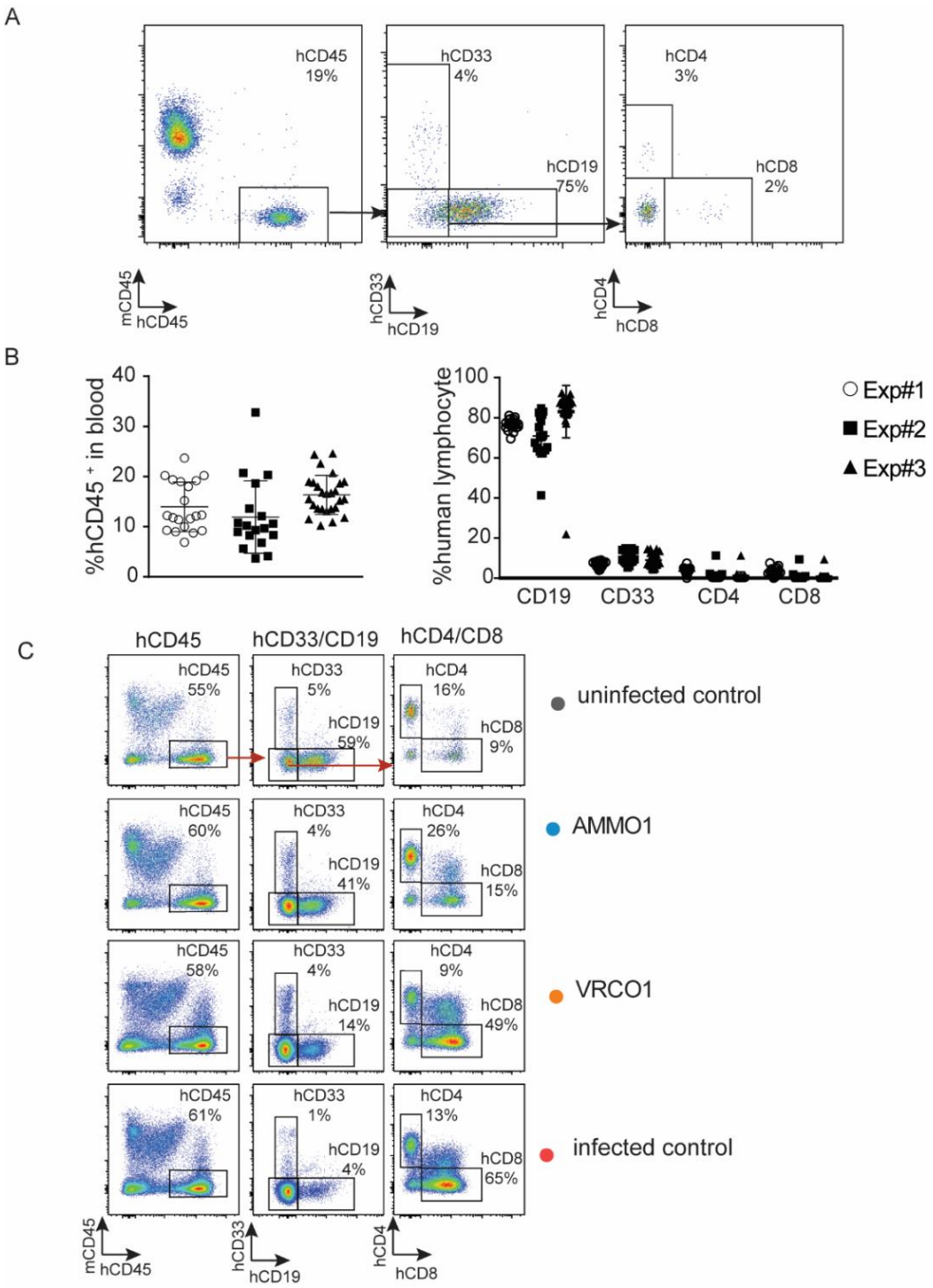

Figure S1. Human cell engraftment in NSG recipient mice prior to EBV infection, **related to Figure 1.** The frequency of human-derived cells in peripheral blood mononuclear cells from NSG mice were measured 12-14 weeks following HSC engraftment but prior to viral challenge. (**A**) Representative flow plot showing the gating strategy used to analyze peripheral blood and splenocytes. (**B**) Frequency of human (h) hCD45$^+$ (left) and, hCD45$^+$hCD19$^+$hCD45$^+$hCD33$^+$, hCD45$^+$hCD4$^+$ and hCD45$^+$hCD8$^+$ cells (right) from 3 independent experiments. (**C**) Representative flow plot showing the gating and frequency of hCD45$^+$ hCD19$^+$, hCD45$^+$hCD33$^+$, hCD45$^+$hCD4$^+$ and hCD45$^+$hCD8$^+$ cells in the peripheral blood of mice from the indicated groups following viral challenge.

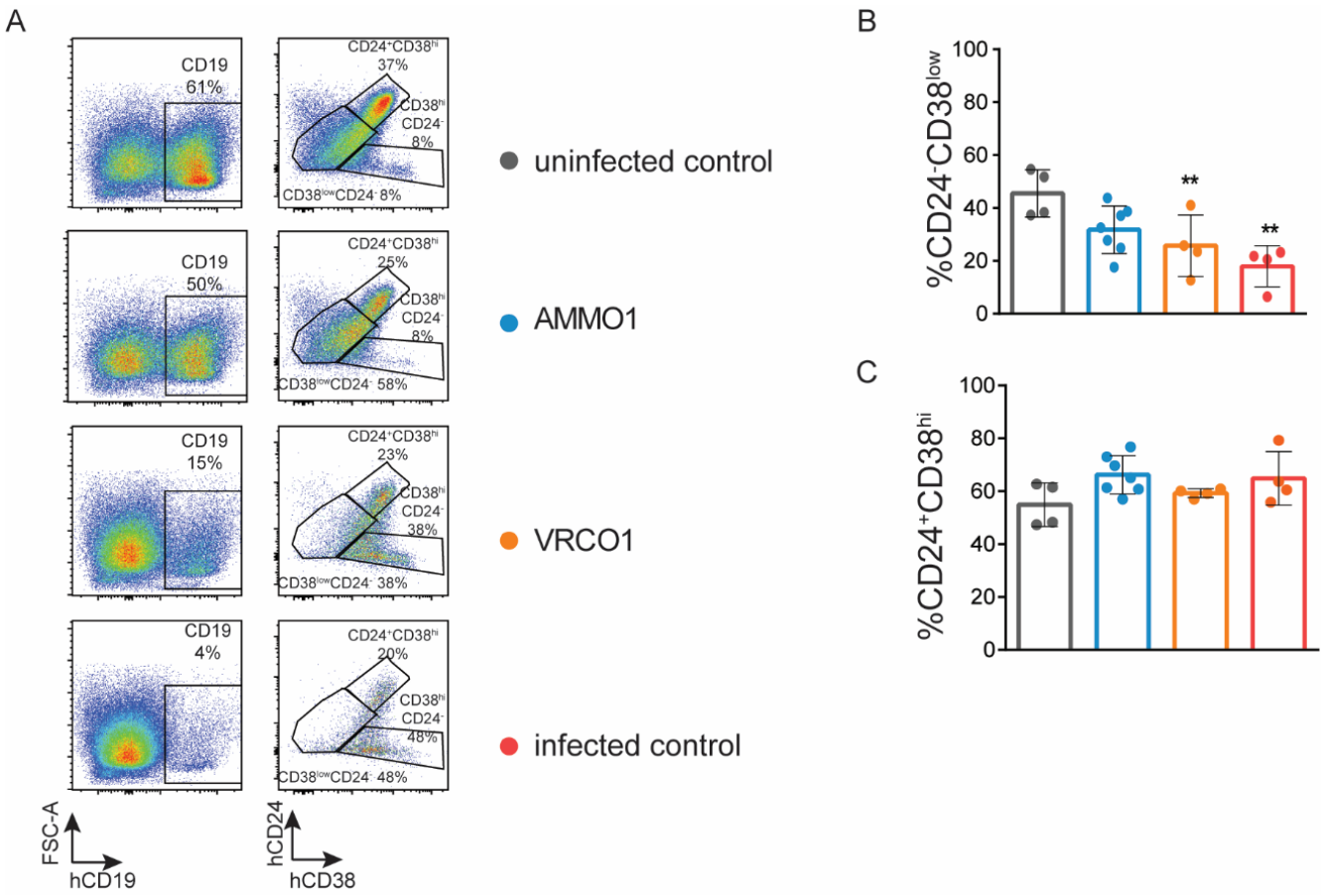

Figure S2. Human B cell phenotype in humanized mice following viral challenge, related to Figure 1. (**A**) Representative flow plot showing the staining and gating strategy to identify B cell subsets in splenocytes based upon staining with hCD19, hCD38 and hCD24 (pre-gated on the $mCD45^{-}hCD45^{+}$ human cell population). Frequency of (**B**) $hCD45^{+}hCD19^{+}hCD24^{-}hCD38^{low}$ and (**C**) $hCD45^{+}hCD19^{+}hCD24^{+}hCD38^{hi}$ cells from the animals from Figure 1. Viral infection is associated with a decrease in the overall proportion of hCD19+ cells and a greater proportion of $hCD45^{+}hCD19^{+}hCD24^{-}hCD38^{hi}$ cells. The frequency of $hCD45^{+}hCD19^{+}hCD24^{-}hCD38^{hi}$ cells is shown in Figure 1K. Asterisks represent groups that are statistically different from the uninfected controls determined by One Way ANOVA, Sidak multiple comparisons test **, $p \leq 0.002$.

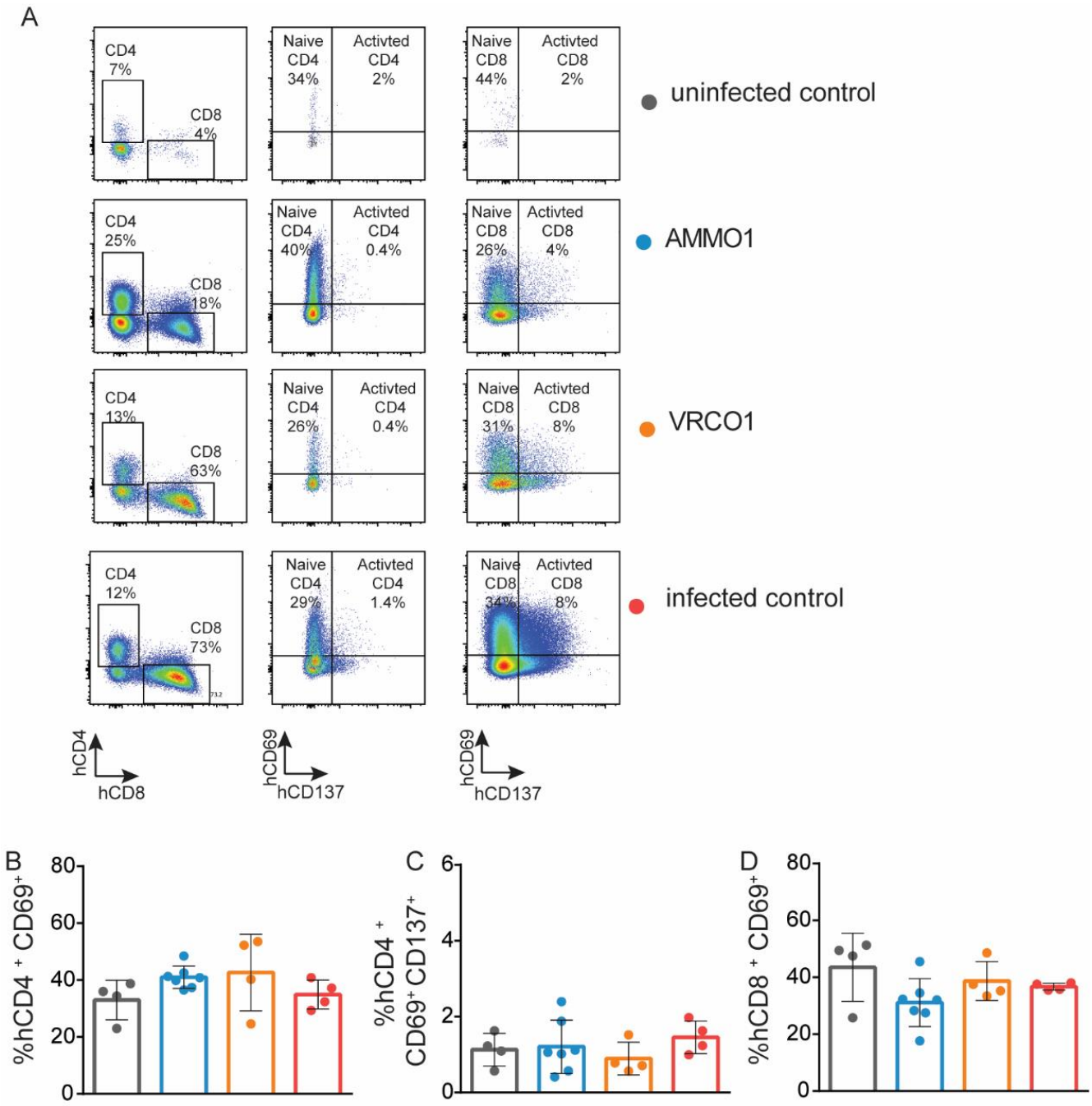

Figure S3. Splenic T cell phenotype in humanized mice following viral challenge, related to Figure 1. (**A**) Representative flow plot showing the staining and gating strategy of $hCD4^+$, $hCD8^+$, $hCD69^+$ and $hCD137^+$ T cells at necropsy (pre-gated on engrafted human cells based upon $mCD45^-hCD45^+$ staining). Frequency of (**B**) naïve $hCD45^+hCD69^+hCD137^-$ and (**C**) activated $hCD45^+hCD69^+hCD137^+$ $hCD4^+$ T cells, and the frequency of (**D**) naïve $hCD45^+hCD69^+hCD137^-$ $hCD8^+$ T cells in the splenocytes of the animals from Figure 1. The frequency of activated $hCD45^+hCD69^+hCD137^+$ $hCD8^+$ T cells is shown in Figure 1M.

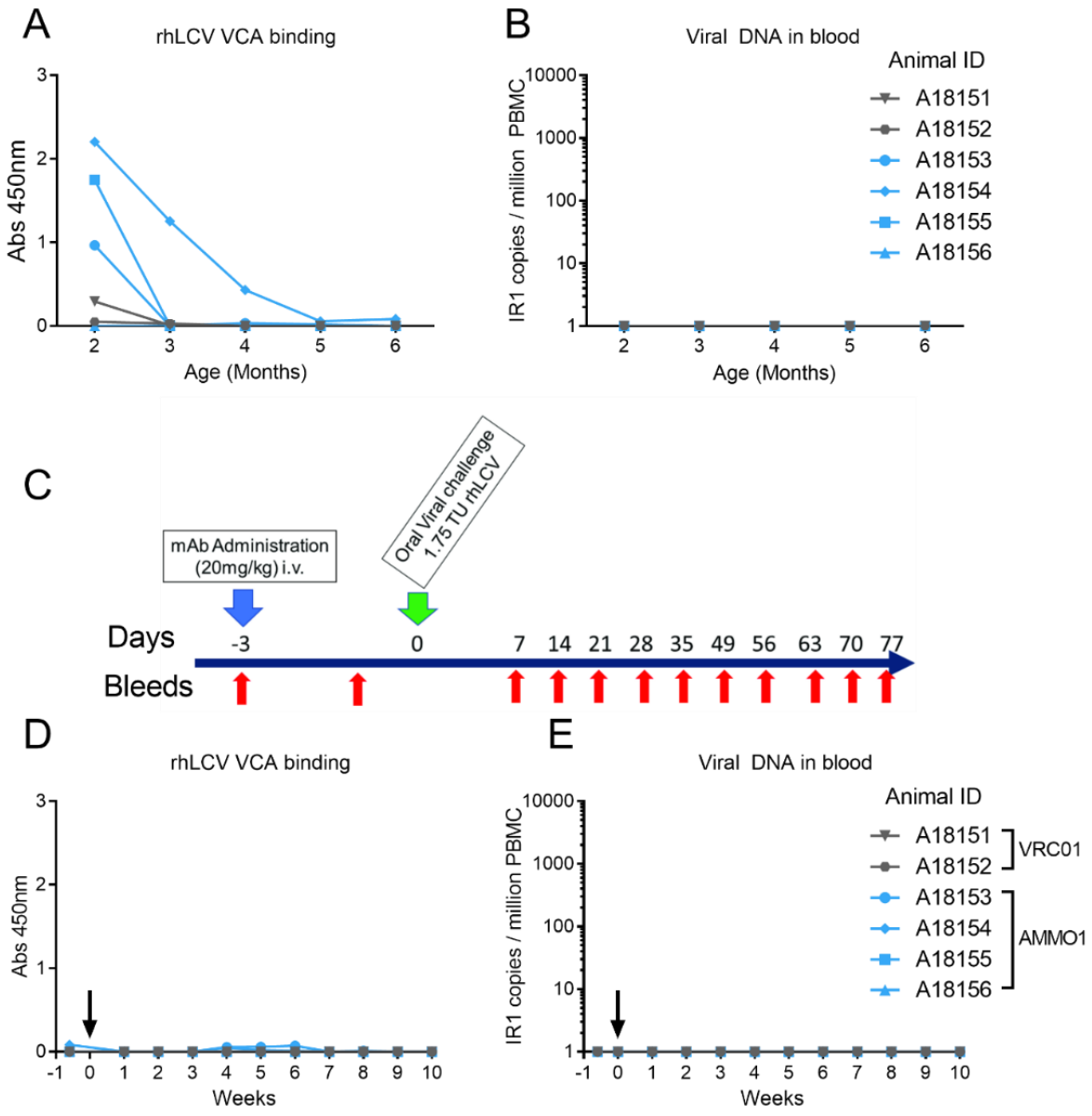

Figure S4. Monitoring for RhLCV infection in study macaques prior to and following an initial low-dose challenge, related to Figure 4. Rhesus macaques used in this study were obtained within 24-48 hours of birth. Beginning at 2 months of age, blood was collected monthly. (**A**) Plasma reactivity to pooled peptides derived from the rhesus lymphocryptovirus small viral capsid antigen (rhLCV VCA) was measured by ELISA. (**B**) Viral DNA was quantified in PMBC with a digital droplet PCR assay using primers against the internal repeat region 1 (IR1). Data from macaques that went on to receive infusions of AMMO1 or VRC01 are shown in blue and gray, respectively. (**C**) Timeline of initial challenge study. At ~6 months of age rhesus macaques were infused iv with 20 mg/kg of VRC01 (n=2, gray symbols) or AMMO1 (n=4, blue symbols). Three days later the macaques were challenged orally with 1.75 transforming units of rhLCV. Following challenge, blood was collected weekly for 10 weeks. (**D**) Plasma reactivity to pooled peptides derived from rhLCV VCA was measured by ELISA. (**E**) Viral DNA was quantified in PMBC using a digital droplet PCR assay with primers and probes against the internal repeat region 1 (IR1) of rhLCV. Black arrows indicate the time of viral challenge in **D** and **E**.