Supplemental Materials

Supplemental Methods

Study Design

Twenty infant rhesus macaques (RM; 1-2 weeks old) were IV infused with either 10mg/kg anti-HIV Env gp120 monoclonal antibody (mAb) DH378 (n=6), 10mg/kg anti-influenza HA mAb CH65 (n=6), 30mg/kg α-HA mAb CH65 (n=2), or 30mg/kg of a tri-mAb cocktail composed of stoichiometric equivalents of 3 anti-HIV Env gp120 mAbs - DH377, DH378, and DH382 (n=6) - one hour prior to the first oral SHIV challenge. Power calculations were employed to determine that with these animal numbers, this study would have >65% power to detect a significant difference in rate of virus acquisition between control mAb-treated and HIV Env-specific mAb-treated groups when correcting for two comparison groups of breast milk mAb-infused RMs (p=0.025, 1 sided test). This power calculation assumed a 100% infection rate in control mAb-treated animals and a <30% infection rate in HIV Env-specific mAb-treated animals. All infant RMs enrolled in this study were prescreened via ELISA to exclude animals demonstrating preexisting immunity to the human mAbs selected for infusion. Animals were subjected to 3 oral challenges per day for 3 consecutive days consisting of 5,000 $TCID_{50}$ SHIV-1157ipd3N4 (NIH AIDS Reagent Program) incubated in 1mL of RPMI containing 1μg/mL DH378, 1μg/mL CH65 (n=6), 3μg/mL CH65 (n=2), or 3μg/mL of the tri-mAb cocktail for 15 minutes, followed by dilution in ~10mL formula feed to simulate oral acquisition via breastfeeding. Animals were re-infused with their respective antibody infusions one week after the initial infusion. Blood and saliva (via weck cell sponges) were collected before each infusion, 1 hour after each infusion, 1 day after each infusion, and at weeks 2, 4, 6, and 8 of the study. All animals were necropsied at week 8 of the study and tissues were collected. The study endpoint was set at 8 weeks post

challenge following an infusion kinetics pilot study in 2 control mAb-treated animals, LD65 and LD77. Tissues were processed either fresh or after overnight shipping at 4°C to isolate mononuclear cells for sorting, or tissues were fixed in formalin for in situ hybridization. Mononuclear cells were isolated by density gradient centrifugation with Ficoll-Paque (GE Healthcare, Waukesha, WI) for lymph nodes, spleen, blood, and tonsils and with Percoll (Sigma-Aldrich) for intestinal tissues, as previously described [1]. This study was unblinded and not randomized. Of note, DH378-treated animal LI68 died due to unrelated causes (choking event) prior to the 4-week time point and CH65-treated animal LH07 necropsy sample shipment was delayed resulting in delayed sample processing.

Ethics Statement

RMs were housed at the Tulane National Primate Research Center (TNPRC) in Tulane, LA in accordance with the standards of the American Association for Accreditation of Laboratory Animal Care. The protocol was approved by the TNPRC's Institutional Animal Care and Use Committee under OLAW Assurance Number P0212 and additionally reviewed by Duke University's Institutional Animal Care and Use Committee under OLAW Assurance Number A060-16-03. TNPRC is AAALAC accredited. This study was carried out in strict accordance with the recommendations in the *Guide for the Care and Use of Laboratory Animals* [2] of the National Institutes of Health (NIH) and the Weatherall report on *The use of non-human primates in research* [3]. All procedures were performed under anesthesia using ketamine hydrochloride and scheduled to limit the number of times an animal was under anesthesia. All efforts were made to minimize stress, improve housing conditions, and provide enrichment opportunities (e.g., social housing when possible, objects to manipulate in cage, varied food supplements, foraging and

task-oriented feeding methods, interaction with caregivers and research staff). Animals were euthanized by Telazol and Buprenorphine IM injection with a secondary method to include vital organ harvest in accordance with the recommendations of the panel on Euthanasia of the American Veterinary Medical Association. Human donor PBMCs used to assess mAb and serum ADCC functionality were from a previously existing collection [4]. Briefly, blood samples from HIV-1 seronegative donors providing written consent were collected between June 2007 and July 2013 at Duke University, Durham, NC according to protocols approved by the Duke University Institutional Review Board. Samples were anonymized prior to processing and cryopreservation.

Production of infusion mAbs

MAbs DH377, DH378, and DH382 were obtained through antigen-specific B cell sorting and Ig variable gene amplification, as previously described [5]. Of note, the IgG1 4A backbone was used containing alanine substitutions designed to enhance binding to Fcγ-receptor IIIa (S298A, E333A, K334A) and Fc-receptor neonatal (N434A). MAbs were produced through transient transfection either by the manufacturer Catalent (DH378; Catalent, Somerset, NJ) or at the Duke Human Vaccine Institute (DH377, DH382, CH65), as previously described [6]. Briefly, appropriate heavy and light chain pairs for each mAb were transiently transfected in Expi293 cells using the ExpiFectamine 293 transfection reagents (ThermoFisher Scientific), according to manufacturer instructions. Transfected cultures were incubated at 37°C, 8% CO_2 for 4 days prior to supernatant harvest and mAb purification with Pierce Protein A agarose beads (ThermoFisher Scientific). Purified mAbs were tested via SDS-Page coomassie and western blot, and quantified via Nanodrop. Infusion mAb endotoxin levels were measured using the Pyrogen-5000 LAL Assay (Lonza), according to manufacturer instructions.

Prescreening for pre-existing anti-idiotype responses and MHC typing of selected infants

Preexisting immunity to the human mAbs selected for infusion was measured by ELISA on pre-infusion serum prior to assigning the infant to the study. Plates were coated with 3μg/mL of either DH377, DH378, DH382, or positive controls goat anti-monkey IgG (Rockland, Limerick, PA) or goat anti-monkey IgA (Rockland, Limerick, PA). Coated plates were then blocked and incubated with a serial dilution series of pre-infusion/pre-challenge infant serum. To measure serum IgG and IgA binding, samples were subsequently incubated with HRP-conjugated mouse anti-monkey IgG (Southern Biotech, Birmingham, AL) or mouse anti-monkey IgA (Southern Biotech, Birmingham, AL), respectively. Of note, both secondary antibodies were not cross reactive to human mAbs. Absorbance was detected with SureBlue Reserve substrate as described above. Samples with absorbance three standard deviations above the mean absorbance of negative control wells at sample dilutions greater or equal to 1:100 were considered positive and ruled out for inclusion in the study.

MHC typing was performed subsequent to assigning an animal to the study on mononuclear cells or isolated genomic DNA by the Research Services division of the Wisconsin National Primate Research Center. Briefly, exon 2 of MHC class I, class I DRB, class II DPA/B, and the class I DQA/B loci were amplified in a Fluidigm Access Array assay. The resultant amplicons were sequenced with the Illumina MiSeq platform. Sequences were then compared to a database of RM alleles such that haplotype determinations could be made for each animal.

HIV-1 Neutralization in TZM-bl Cells

Neutralizing antibody titers were measured by the reduction in Tat-regulated Luc reporter gene expression in a TZM-bl (NIH AIDS Reagent Program) reporter cell assay, as previously described [7]. Briefly, a dose of virus containing ~150,000 relative luminescence unit (RLU) equavalents was incubated with 3-fold serial dilutions of mAb or serum in duplicate for one hour at 37°C in 96-well flat-bottom culture plates. TZM-bl cells were added at 10^4 cells per well in a 100-µl volume. Eight wells of TZM-bl cells alone and TZM-bl cells with virus alone were run on each plate to contextualize maximum and minimum RLUs. After 48 hour incubation at 37°C, samples were incubated in Bright-Glo luciferase reagent (Promega, Madison, WI) for 2 minutes at room temperature. Luminescence was measured from the resulting cell lysate in a 96-well black solid plate on the Victor X3 Light Plate Reader. The 50% inhibitory dose (ID_{50}) titer was calculated as the serum dilution that caused a 50% reduction in RLUs compared to virus-only controls after subtraction of the TZM-bl-only control RLUs. Alternatively, the 50% inhibitory concentration (IC_{50}) titer was calculated as the reagent concentration that caused a 50% reduction in RLU relative to virus-only controls.

Tissue Mononuclear Cell Viral Coculture

Tissue-associated infectious virus titer was assessed through Tat-regulated Luc-F reporter gene expression to quantify infection of TZM-bl reporter cells after coculture with tissue mononuclear cells isolated from RMs. Isolated mononuclear cells from lymphoid and GI tissues were serially diluted in a 96 well format with 10^4 TZM-bl cells/well added in coculture. This coculture was incubated for 72 hours at 37°C and 5% CO_2 prior to luminescence quantification with the Brightglow luminescence detection system (Promega, Madison, WI). For quantitative comparison, tissues were tested with a minimum of 2 replicates to employ the Reed-Meunch

method to estimate the tissue-associated infectious virus titer in units of viable mononuclear cells. Tissue-associated infectious virus titer was calculated as the number of viable cells required to sustain detectable infection in 50% of the replicates, with the detection threshold established as 3 standard deviations above the mean luminescence output of PBMCs from 3 naive RMs (3,019 RLU). Of note, cell number availability was variable between samples, resulting in a range of detection limits (range=2×10^5-4×10^6 mononuclear cells) and number of replicates (2-8 replicates) for the assay.

Plasma Viral RNA Load Quantification

Reverse transcriptase (RT) PCR was performed to determine the SHIV-1157ipd3N4 RM plasma RNA load, as previously described [8]. Briefly, viral RNA was isolated from plasma viral pellets using the QIAsymphony Virus/Bacteria Midi kit on the QIAsymphony SP automated sample preparation platform (Qiagen, Hilden, Germany). A standard curve was generated from serial dilutions of an RNA transcript of the first 731bp of SIVmac239 gag. cDNA was produced from the resulting sample and standard RNA suspensions in a reverse transcriptase reaction using SuperScript III RT enzyme (Invitrogen), PCR nucleotides (Roche, Pleasanton, CA), and RNAse Out (Invitrogen) with the specific reverse primer 5'- CAC TAG GTG TCT CTG CAC TAT CTG TTT TG -3' using an optimized version of the manufacturer's protocol. cDNA was used in quantitative real-time PCR using Gene Expression Mastermix (Applied Biosystems, Foster City, CA) and target specific labeled probe 5'- /56-FAM/CTT CCT CAG TGT GTT TCA CTT TCT CTT CTG CG/3BHQ_1/ -3' and forward 5'- GTC TGC GTC ATC TGG TGC ATT C -3' and reverse primers 5'- CAC TAG GTG TCT CTG CAC TAT CTG TTT TG -3' (Integrated DNA

Technologies, Coralville, IA). RNA copies per milliliter were determined by dividing the copy number by the volume of plasma from which SHIV RNA was extracted.

Mononuclear Cell Provirus Quantification

RM CD4+ T cell-associated genomic DNA (gDNA) was isolated from various GI and lymphoid tissues with the QIAaMP DNA kit (Qiagen, Hilden, Germany) and quantified using the Biorad QX200 droplet digital PCR System according to the manufacturer instructions (Biorad, Hercules, CA). SHIV *gag* specific primers (SIVmac F 5'- GTC TGC GTC ATC TGG TGC ATT C-3' and SIVmac R 5'- CAG TAG GTG TCT CTG CAC TAT CTG TTT TG -3') and probe [FAM]-CTT CCT CAG TGT GTT TCA CTT TCT CTT CTG CG-[TAMRA] were used to amplify and quantify total SHIV provirus copies. The cell count per sample was estimated using the genomic reference gene TERT, which was quantified in multiplex with the *gag* quantification using a human TERT-specific reference assay (Biorad, Hercules, CA). This gene was utilized as it was found to have high homology with RMs. The SHIV proviral load in SHIV copies/million CD4+ T cells was calculated by dividing the SHIV DNA copy number by the TERT copy number divided by 2 multiplied by 10^6 cells.

Some of the ddPCR results were validated through comparison to TaqMan Real-Time qPCR, as previously described [9]. Standard curves from serial dilutions starting at 1 X 10^8 copies/ml of SHIV *gag* plasmid and from the genomic reference gene albumin were used to relate cycle threshold (Ct) values to gene copy numbers. All reactions were performed on the StepOnePlus Real-Time PCR System (ThermoFisher Scientific).

Saliva Weck-Cel sample collection and extraction

RM saliva was collected with Weck-Cel ophthalmic sponges (Beaver-Visitec, Waltham, MA), as previously described [10]. The Weck-Cel sponges were then transferred to Spin-X (Corning Life Sciences, Corning, NY) centrifuge tubes without filters and incubated twice with 300 µl of an extraction buffer per sample (PBS containing 0.25% BSA and 1X protease inhibitor cocktail (Sigma)). Samples were incubated for five minutes and ten minutes in the first and second incubations, respectively. After each incubation, antibodies were extracted from the Weck-Cel sponges by centrifugation and filtered on Spin-X centrifuge tubes with 0.22µm filters. The volume of sample extract was determined by the mass of the sample tubes compared to the average mass of extraction tubes from blank Weck-Cel sponges that underwent mock extractions with an assumed density of saliva of 1kg/L.

Measurement of virus-specific IgG levels in plasma and saliva

Enzyme-linked immunosorbent assays (ELISA) were performed as previously described [11]. Briefly, ELISA plates (384 wells; Corning Life Sciences, Corning, NY) were coated overnight at 4ºC or for one hour at 20ºC with 30 µg/well gp140 1086c, MN.gp41, Bio-V3.C, or Hemagglutinin (HA Solomon Islands; Protein Sciences Corporation, Meriden, CT) in 0.1 M sodium bicarbonate. Plates were blocked with Superblock (PBS containing 4% whey protein, 15% normal goat serum, and 0.5% Tween 20). RM plasma or saliva Weck-Cel extracts were diluted in Superblock and incubated on the plate for one hour at 20ºC. Plasma or saliva IgG was bound with 10µl/well horseradish peroxidase (HRP)-conjugated goat anti-human IgG (Jackson ImmunoResearch, West Grove, PA). Binding was quantified by incubation with room temperature SureBlue Reserve Microwell Substrate (VWR, Radnor, PA) followed by addition of TMB (3,3′,5,5′-tetramethylbenzidine) Stop Solution (VWR, Radnor, PA). Absorbance of the

183 wells was read at 450 nm using a Spectramax Plus spectrophotometer (Molecular Devices,
184 Sunnyvale, CA) immediately after addition of the stop solution. Antibody concentrations in
185 serum and saliva were identified using purified monoclonal antibody 4-parameter standard
186 curves of DH378, CH65, 7B2 (anti-gp41 IgG1 mAb), or the tri-antibody cocktail ranging from 0
187 to 300ng/mL with 3-fold dilutions.
188 To quantify A32 blocking, plates coated with 1086c gp120 were blocked and incubated
189 with sample as described above. Plates were then incubated with 200-ng/ml biotinylated A32
190 mAb for one hour. Inhibition of biotin-A32 binding was detected with streptavidin-HRP at
191 1:30,000, followed by incubation with SureBlue Reserve substrate and subsequent application of
192 TMB Stop Solution. Absorbance of the wells was read at 450 nm using a Spectramax Plus
193 spectrophotometer immediately after addition of the stop solution. The concentration of A32-
194 blocking antibodies in serum was determined using a 4-parameter standard curve of the tri-
195 antibody cocktail ranging from 0 to 60 µg/ml with 3-fold dilutions.
196
197 *Antibody Dependent Cellular-Cytotoxicity*
198 ADCC activity of the purified mAbs and peripheral serum samples was determined by our
199 luciferase-based cell killing assay as previously described [12, 13]. Briefly, CEM.NKR$_{CCR5}$ target
200 cells (NIH AIDS Reagent Program) were infected with SHIV1157-ipd3N4-IMC encoding a
201 *Renilla* luciferase reporter gene [14]. PBMC from a healthy HIV-seronegative donor with the
202 heterozygous 158F/V and 131H/R genotypes for Fcγ-receptor IIIa and Fcγ-receptor IIa,
203 respectively, were used as a source of effector cells. PBMC were added to SHIV-infected target
204 cells in wells of a 96-well plate at a ratio of 30:1. DH382 and CH65 were included as positive
205 and negative controls, respectively. Assay plates were incubated with serial dilutions of RM

serum for 6h at 37°C and 5% CO_2. ADCC activity (percent specific killing) was calculated from the change in Relative Light Units (RLU) (ViviRen luciferase assay; Promega, Madison, WI) resulting from the loss of intact target cells in wells containing effector cells, target cells, and serum or mAb samples compared to amounts in control wells containing target cells and effector cells alone. ADCC activity is reported as either the maximum percent killing observed for each sample or the ADCC endpoint titer, which is defined as the last dilution of serum that intersects the positive cutoff (20% specific killing) after subtraction of the average non-specific background activity observed for plasma collected prior to infusion of mAb from all available animals (n=15).

Flow Cytometry

RM PBMCs or tissue mononuclear cells were stained with a panel of fluorochrome-conjugated antibodies containing CD3 fluorescein isothiocyanate (FITC), CD28 phycoerythrin (PE), CD45 peridinin chlorophyll protein (PerCP), CD8 V500, CD95 PE-Cy7, CD4 allophycocyanin (APC), CD20 APC-H7, and CCR7 Brilliant Violet 421 (BV421) (all from BD Biosciences, San Jose, CA). Yellow Vital dye was added to the panel as a live/dead cell discriminator for some samples. PBMCs from the week 2 timepoint were stained with a modified panel with Aqua Vital Dye and CD8-APC-R700 (BD Biosciences, San Jose, CA) replacing Yellow Vital Dye and CD8 V500, respectively. Additionally, the modified panel added CD69 BV605 (BD Biosciences, San Jose, CA), CD25 BV711 (Biolegend, San Diego, CA), and HLA-DR PE-CF594 (Texas Red; BD Biosciences, San Jose, CA). After 30 minutes of antibody incubation at 4 °C, cells were washed in PBS and incubated with vital dye for 30 minutes at 4°C. Cells for phenotyping were then fixed with 2% formaldehyde solution. Stained cells were acquired on an LSRII flow cytometer (BD

Biosciences, San Jose, CA). Unfixed cells for sorting were resuspended in PBS with 2% FBS. CD4+ and CD8+ cells were bulk sorted and data was acquired on a FACS Aria II cytometer (BD Biosciences, San Jose, CA). CD4+ and CD8+ T cells were positively selected from isolated tissue mononuclear cells by sequential selection of lymphocytes, FSC and SSC singlets, viable cells, CD45+ leukocytes, CD3+ T cells, and CD4+ versus CD8+ T cells. Central memory, effector memory, and naïve CD4+ and CD8+ T cell populations were characterized as CD28+/CD95+, CD28-/CD95+, and CD28+/CD95-, respectively. Transitional memory T cells were identified as CD28+/CCR7-. Analysis of all acquired data was performed using FlowJo software (TreeStar, Ashland, OR).

In Situ Hybridization

In situ hybridization for the detection and quantification of SHIV gag RNA was performed using the Affymetrix protocol according to manufacturer instruction (Affymetrix, Santa Clara, CA). Briefly, fresh dissected RM tissue samples were fixed in Zinc buffered formalin (Anatech Ltd, Battle Creek, MI) at room temperature for 24-48 hours. Tissue specimens were then paraffin-embedded, sectioned at 5µm, and mounted on positively-charged glass slides. Slide-mounted tissue sections were dried at room temperature overnight and then incubated at 60°C for 1 hour to immobilize tissue sections. Slides were deparaffinized in xylene and washed twice in 100% ethanol. The sections then underwent the recommended heat induced epitope retrieval and were digested with 1:100 Protease QF solution (Affymetrix, Santa Clara, CA), followed by hybridization with SHIV *gag*-specific probe, JN560961.1 Simian-Human Immunodeficiency Virus clone SHIV_AD8 (Affymetrix, Santa Clara, CA). SHIV *gag*-specific signal was amplified in subsequent hybridizations with 1:100 PreAmp1 QF and 1:100 Amp1QF (Affymetrix, Santa

Clara, CA). Following these hybridizations, the targeted virus was labeled with Fast Red chromogen (Affymetrix, Santa Clara, CA). After 3 washes in PBS, slides were blocked in a solution of PBS with 0.02% fish skin gelatin (Sigma) and 10% normal goat serum for 40 minutes. Rabbit anti-human CD3 primary antibody (Dako, Glostrup Municipality, Denmark) was diluted 1:100 and applied to slides for 60 minutes at room temperature. Slides were washed three times with phosphate buffered saline containing 0.02% fish skin gelatin and 0.01% TritonX100 (Fisher). The secondary antibody, Alexa Fluor 488-conjugated goat anti-rabbit IgG, was applied to slides for 40 minutes. Following a series of washes, Topro3 DAPI nuclear stain (Invitrogen) was added to each slide. All slides were imaged with a Leica TCS SP8 confocal microscope. Average numbers of CD3+ cells and SHIV gag RNA+ cells were obtained from10 0.5-0.75 mm fields of each stained tissue. The number of SHIV RNA producing cells was reported as the number of SHIV *gag* RNA+ cells per 1,000 CD3+ T cells.

Transmitted/Founder Analysis

Transmitted/Founder (T/F) viral sequences were obtained by single genome amplification (SGA) with subsequent direct amplicon sequencing, as previously described [15]. Briefly, viral RNA was isolated from plasma 2 weeks post infection from each animal by the QiaAmp viral RNA mini kit (Qiagen, Hilden, Germany). cDNA was generated using SuperScript III reverse transcriptase mix (Invitrogen) and antisense primer SHIVEnvR3-out 5' –CTA ATT CCT GGT CCT GAG GTG TAA TCC TG -3'located in the nef reading frame (nt 9250-9278 SHIV1157ipd3N4). The resulting cDNA suspension was diluted and PCR amplified in Platinum Taq DNA polymerase High Fidelity (Invitrogen) such that 30% of reactions were positive to maximize the likelihood of single genome amplification. A first round of PCR amplification was conducted using

SHIVEnvR3-in and SHIVEnvF4-out 5'- TCA TAT CTA TAA TCG TCA CGG AGA CAC TC -3' (nt 5768-5796 in SHIV1157ipd3N4) as primers. A second round of PCR amplification was conducted using 2µl of first round PCR product as template and SHIVEnvF2-in 5'- GTG TTG CTT TCA TTG CCA AGT TTG T -3' (nt 6040-6064) and SHIVEnvR2-in 5'-TGG TAT GAT GCC TTC TTC CTT TTC T-3' (nt 9219-9243) as primers. Amplification conditions for round 1 and round 2 PCR were 1 cycle of 94°C for 2 minutes, 35 cycles of 94°C for 15 seconds, 55°C for 30 seconds, and 68°C for 4 minutes, followed by 1 cycle of 68°C for 10 minutes. Round 2 PCR amplicons were visualized by agarose gel electrophoresis and directly sequenced using an ABI3730xl genetic analyzer (Applied Biosystems, Foster City, CA). Full sequences were constructed from overlapping sequences from each amplicon in Sequencher (Gene Codes, Inc., Ann Arbor, MI). Sequences with two or more double peaks were discarded, as this indicates amplification from multiple templates. Sequences with one double peak were retained with the double peak attributed to Taq polymerase error. Ambiguities in these sequences were read as the consensus sequence. Sequence alignments and phylogenetic trees were constructed using clustalW and Highlighter plots were created using the tool at http://www.lanl.gov.

To identify and enumerate T/F variants, the following conditions were applied. Clusters of related sequences were visually analyzed using phylogenetic trees (Figtree v1.4) and sequences containing <2 mutations were considered a single variant. Variants containing ≥2 mutations were considered as progeny of distinct T/F genomes. Potential G-A hymermutations caused by APOBEC 3G/3F were identified using Hypermut algorithm 2.0 and were reverted for analysis if there were ≤ 2 present. Sequences that had >3 potential APOBEC 3G/3F mutations were not considered for T/F analysis (Hypermut, http://www.hiv.lanl.gov) [16]. Sequence clusters of ≥ 2 sequences with ≥2 shared mutations were considered as distinct T/F variants.

298

299 *Provirus env cloning and pseudovirus preparation*

300 Genomic DNA (gDNA) was isolated from CD4+ T cells to study proviral *env* populations from
301 animals negative for plasma viral load by Qiagen Allprep DNA/RNA mini kit (Qiagen, Hilden,
302 Germany). SHIV *env* amplification through bulk nested PCR was done using this gDNA as
303 template, as described above. Bulk PCR products of envelope gene from animals LG19 and
304 LH73 were cloned in pcDNA3.1/V5-His-Topo (Invitrogen) using shotgun cloning methods. To
305 generate pseudoviruses capable of one round of infection, plasmids containing SHIV envelopes
306 were cotransfected with a second plasmid containing a subtype B *env* deficient HIV genome
307 (SG3Δ*env*) in 293T cells (Invitrogen), as previously described [7]. The infectivity of pseudotyped
308 viruses was screened by single round infection of TZM-bl cells followed by detection of Tat-
309 regulated luminescence with the Bright-Glo luciferase reagent (Promega, Madison, WI), as
310 described above. Infectivity was reported as relative luminescence units (RLUs) and compared
311 to that of a mock infection of TZM-bl cells (~500 RLU).

312

313 *Statistical Analysis*

314 Statistical tests were performed with SAS v9.4 (SAS Institute, Cary NC). Comparisons of viral
315 load, proviral load (copies/million cells), and the number of T/F variants in infants from each
316 mAb treatment group were performed using the exact Wilcoxon test. The proviral load (binary
317 designation), and the number of infants with detectable SHIV in each mAb treatment group were
318 compared with Fisher's exact test. False discovery rate (FDR) p-value correction was used to
319 correct for multiple comparisons. A p-value of <0.05 (two-tailed) was considered as significant
320 for all analyses.

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Supplemental Figures

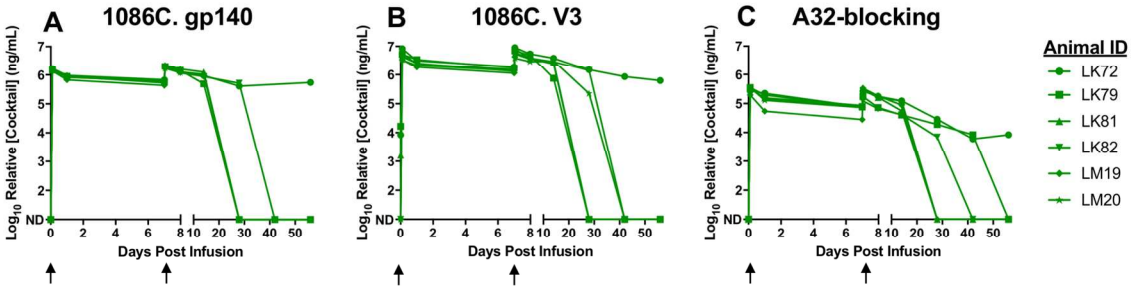


Figure S1. Decay of passively infused mAbs in serum of tri-mAb cocktail-treated infant RMs. Concentrations of infused mAbs in serum from pre infusion to 8 weeks post infusion are depicted for tri-mAb cocktail-treated animals. **A)** 1086C. gp140, **B)** 1086C. V3 peptide, and **C)** A32-blocking ELISAs were employed to estimate the relative concentrations of DH377 and DH382 within the tri-mAb cocktail-treated animals over the course of the study. Black arrows indicate systemic mAb infusions at days 0 and 7. ND indicates not detectable.

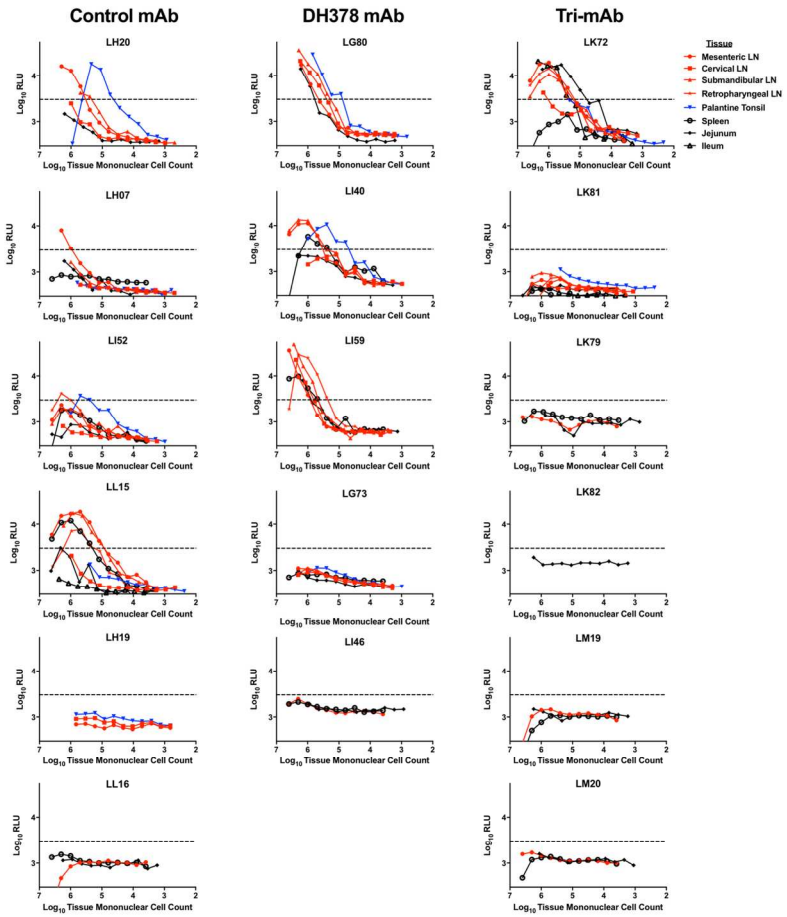
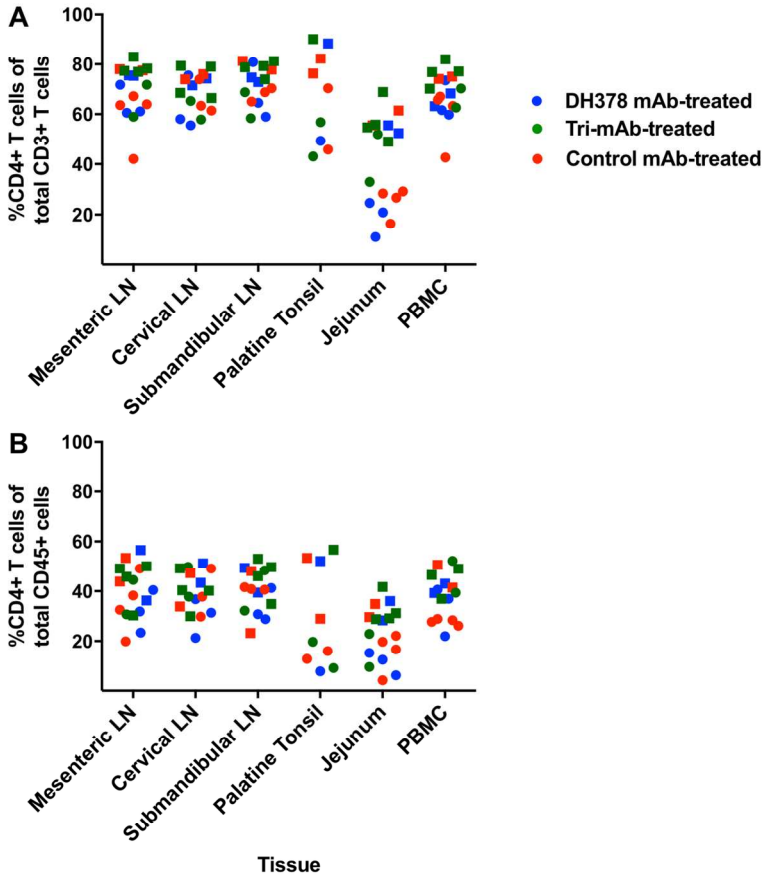


Figure S2. Tissue-associated infectious SHIV levels measured through tissue mononuclear cell coculture with TZM-bl reporter cells. Mononuclear cells isolated from tissues 8 weeks after oral SHIV-1157ipd3N4-challenge of control mAb-, DH378 mAb-, and tri-mAb-treated infant RMs were serially diluted and cocultured with TZM-bl reporter cells for 72hrs, followed by luminescent detection of tissue-associated SHIV infectivity in relative luminscence units (RLU). The RLU limit of detection for positive tissue-associated SHIV infection (dashed line) was defined as 3 standard deviations above the mean maximum RLUs elicited from PBMCs of unchallenged control RMs (n=3) in the coculture assay.



408

409 **Figure S3. CD4+ T cell proportions in tissues of mAb-treated, SHIV-1157ipd3N4**

410 **challenged infant RMs. A)** Proportion of CD4+ T cells of total T cells and **B)** total CD45+

411 mononuclear cells in various lymphoid and GI tissues of mAb-treated and orally SHIV-

412 challenged infant RMs at necropsy (8 weeks post infusion). Red, blue, and green symbols

413 indicate control mAb-, DH378-mAb, and tri-mAb-treated animals, respectively. Squares

414 indicate non-viremic animals, while circles indicate viremic animals.

415

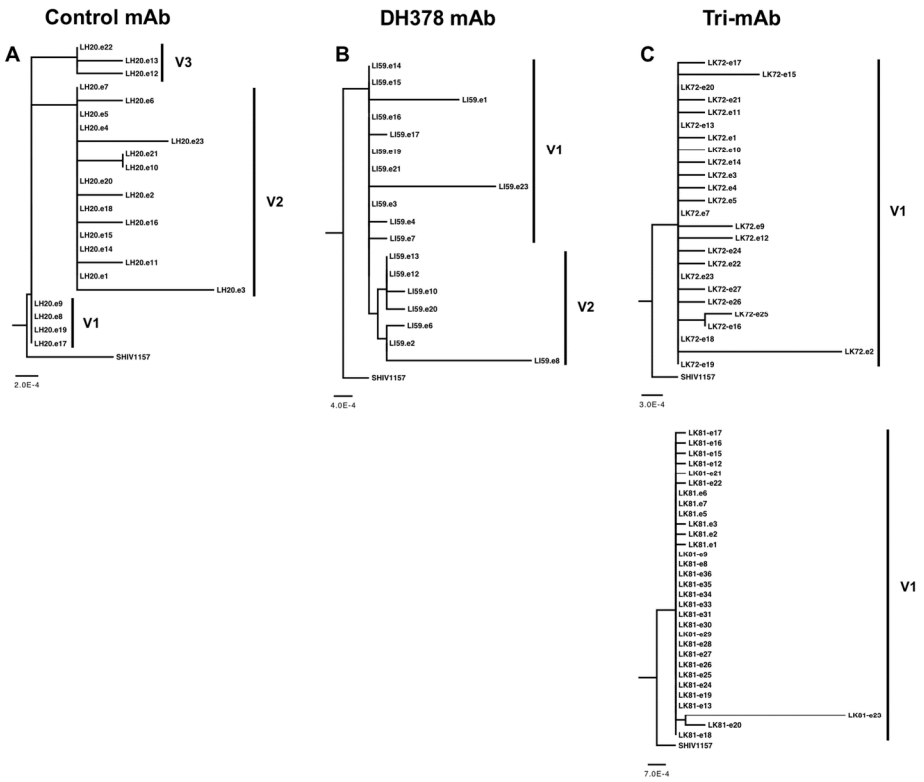
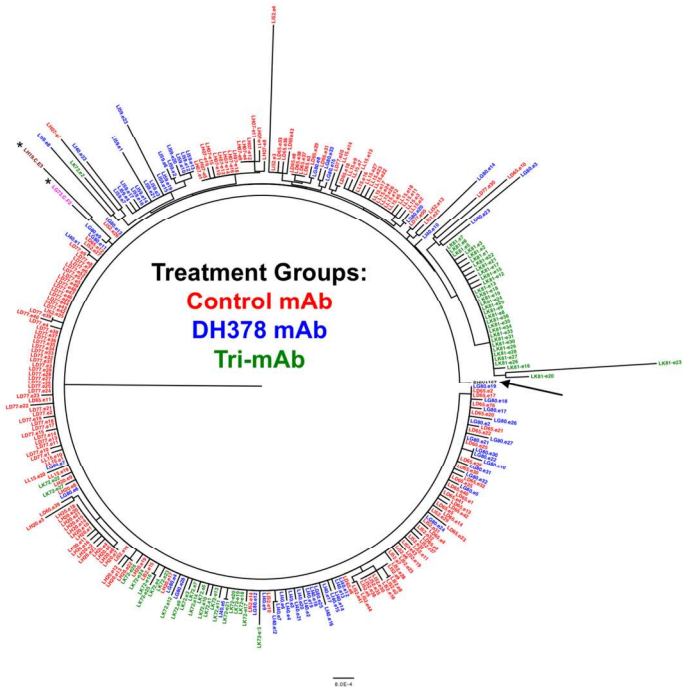


Figure S4. Representative phylogenetic trees of SHIV *env* variants isolated from passively mAb-infused, SHIV-1157ipd3N4- challenged infant RMs. Standard SGA techniques were used to isolate the SHIV envelope gene from plasma of **A)** control mAb-treated, **B)** DH378 mAb-treated, and **C)** tri-mAb-treated infant RMs, and amplicons were sequenced. Clustalw and the Kimura 2 Parameter (K2P) method were used to align amplicons and construct neighbor joining phylogenetic trees rooted at the SHIV-1157ipd3N4 envelope gene (Acc. No. DQ779174). Distinct variant clusters (V; indicated by bold lines) within each animal were defined as having ≥2 unique mutations that were observed in ≥2 amplicons.



426

427 **Figure S5. Phylogenetic tree analysis of SHIV *env* isolated from passively breastmilk mAb-**
428 **infused infant RMs 2 weeks after oral SHIV challenge.** Standard SGA techniques were used
429 to isolate SHIV *env* genes from each animal 2 weeks following oral SHIV challenge and
430 amplicons were sequenced. Clustalw and Kimura 2 Parameter (K2P) method were used to align
431 amplicons and construct a neighbor joining phylogenetic tree rooted at the SHIV-1157ipd3N4
432 stock *env*. Amplicons from control mAb-treated, DH378 mAb-treated and tri-mAb-treated
433 animals are colored red, blue, and green, respectively. The scale bar represents 0.0008
434 nucleotide mutations per site. The black arrow indicates the SHIV-1157ipd3N4 challenge *env*
435 sequence. SHIV proviral *env* sequences isolated from CD4+ T cells in LH19 and LG73
436 submandibular LNs at 8 weeks post infection were included in the tree and are indicated by *.

437