2 Materials and Methods

2.1 Animal Study Protocol

Animal study protocol VRC-12-417 (Tracking SIV Infection of T cells and its Impact on B Cell Responses in Early Infection), was approved by the Animal Care and Use Committee (ACUC) at the Vaccine Research Center, NIAID/NIH. Six rhesus macaques (*macaca mulatta*, of Indian origin) were infected intravenously with a 1:3000 dilution of SIVmac251. This dose had been shown in previous studies to be infectious with a single IV injection. Each animal was sampled four times (up to three biopsies and euthanasia) in early infection. Blood and tissue samples (inguinal lymph node, bone marrow, and gut) were taken at each biopsy, with additional tissues taken at necropsy (mesenteric and axillary lymph nodes, jejunum and ilium, and spleen). Each animal was sampled on a different schedule to obtain an n=3 or n=4 at each time point while maintaining an appropriate interval between biopsies of individual animals.

Table 2.1: Bleed Schedule

Day/Week/ Month	**Procedure**						**Bleed Volume**
	RM-1	**RM-2**	**RM-3**	**RM-4**	**RM-5**	**RM-6**	
Day 0	Infection						
Week 1	Biopsy Bleed	Biopsy Bleed	Biopsy Bleed				5-10ml
Week 2	Euthanasia	Biopsy Bleed		Biopsy Bleed			5-10ml
Week 3		Euthanasia			Biopsy Bleed	Biopsy Bleed	5-10ml
Week 4			Biopsy Bleed	Biopsy Bleed	Biopsy Bleed	Biopsy Bleed	5-10ml
Month 2			Biopsy Bleed	Biopsy Bleed	Biopsy Bleed	Biopsy Bleed	5-10ml
Month 6			Euthanasia	Euthanasia	Euthanasia	Euthanasia	5-10ml

Additional samples were from previously completed studies (approved by the NIAID/VRC ACUC) of natural infection, vaccines, and healthy controls and are listed in a table at the end of this chapter.

2.2 Sample Processing

Blood draws were received in 15 mL EDTA anticoagulation tubes and spun at 1800 rpm for 15 minutes with braking and acceleration set to the lowest level. Plasma was removed from the surface and stored at -80C. Lymphocytes were removed and diluted in PBS, layered on top of Ficoll-Paque PLUS (GE Healthcare Life Sciences), and spun for 25 min at 1800 rpm with no braking or acceleration. The buffy coat containing lymphocytes was removed, washed 2x with 10 mL R10, counted and cryopreserved using sterile filtered foetal bovine serum (FBS) with 10% DMSO. R10 was prepared by sterile filtration of RPMI with 10% FBS and 1X Penicillin-Streptomycin-Glutamine (100x from Thermo Fisher).

Bone marrow biopsies were diluted twofold with PBS and layered over Ficoll-Paque PLUS and centrifuged fro 25 minute at 1800 RPM with no braking or acceleration.. The buffy coat containing lymphocytes was removed, washed 2x with 10 mL R10 by re-suspending cells and centrifuging at 1500 rpm for 7 minutes, counted and either immediately stained for FACS or cryopreserved using sterile filtered FBS with 10% DMSO.

For all tissues, fat and connective tissues were removed using surgical scalpels before tissues were minced and strained into single cell suspensions using a 70uM filter. Gut tissues were incubated with Collagenase-D (1mg/mL in RPMI+ PSG) shaking for 30 min at 37°C before washing twice in R10 and commencing fat/connective tissue removal and cell dissociation. After dissociation, cells were washed twice in R10 and counted before cryopreservation.

Single cell suspensions of splenocytes were incubated in 10 mL ACK Lysing Buffer (Thermo Fisher) for 7 minutes at room temperature, diluted with 40 mL PBS, washed (1500 rpm 5 minutes) and resuspended in 30 mL PBS. Diluted splenocytes were layered on 15 mL Ficoll-Paque and spun for 25 min at 1800 rpm with no braking or acceleration.. The buffy coat containing lymphocytes was removed, washed 2x with 10 mL R10, counted and cryopreserved using sterile filtered foetal bovine serum (FBS) with 10% DMSO.

2.3 Flow cytometry and fluorescence-activated cell sorting

For conventional flow cytometry, live cells were washed in 1mL PBS, centrifuged at 1500rpm for 5 minutes and resuspended in the residual volume after removing the supernatant. Samples were stained with 5ul of Aqua (1:40 dilution) for 5 minutes at room temperature in the dark and subsequently with an antibody cocktail (Table 2.6-Table 2.12 for different experiments) for 25 minutes at room temperature in the dark. Samples were washed with PBS and incubated with 200 uL of 2% paraformaldehyde for 30 minutes at 4°C in the dark before acquisition on a custom-built LSR II (BD Biosciences). For intracellular staining (ICS), cells were washed with 1 mL Perm/Wash Buffer (BD Biosciences) after surface staining, and incubated with CytoFix/CytoPerm Solution (BD Biosciences) for 15 min in the dark at room temperature. Cells were washed again in 1 mL Perm/Wash and intracellularly stained for 20 min at room temperature, then washed with 2 mL PBS and resuspended in 2% PFA. Alternatively, cells were fixed with FACSjuice (1X BD FACS Lysing solution, Tween 20 in PBS) on ice for 10 minutes, and washed with 1mL FACSwash (2% FBS in PBS) before ICS.

For fluorescence-activated cell sorting (FACS), cells were either sorted immediately after isolation or thawed from cryopreservation, washed in 10 mL R10, and resuspended in R10 at <10 million cells/mL. Cells were rested at 37℃ for 30-60 minutes before washing with PBS. Cells were gently resuspended and 5uL of 1:40 dilution of Aqua live/dead cell dye was added for 5 minutes at the dark at room temperature. The appropriate antibody cocktail was added and cells were stained for 25 minutes at room temperature in the dark. Cells were then washed in R10, filtered using 70uM sterile filters and sorted on a custom-built FACSAria II (BD Biosciences).

2.4 DNA and RNA Extraction

Quantitative polymerase chain reaction (qPCR) was used to measure SIV infection in sorted T cell subsets. Live cells were sorted into 300 uL R10 in 1.5 mL Eppendorf tubes, pelleted at maximum speed for 1 minute before supernatant was aspirated from the pellet. The pellet was resuspended in Proteinase K (Boehringer) at 100ul/mL in Tris-HCL (10mM, pH8)[324]. The volume of lysis solution varied by experiment but was a minimum of 10ul per 100,000 cells sorted. The lysis solution was incubated for a minimum of one hour at 56°C before inactivation at 95℃ for 10 minutes and storage at -20°C.

SIV RNA was measured using quantitative reverse transcription polymerase chain reaction (qRT-PCR). Two methods were used to extract RNA for qRT-PCR. In the first, cells were sorted into 200-300 uL R10, and resuspended in 1 mL RNAlater (Thermo Fisher) and frozen at -80C until RNA extraction. RNA was extracted using RNAqueous-Micro Total RNA isolation kits (Thermo Fisher) according to the manufacturer's instructions with the addition of an internal standard derived from a replication competent avian retrovirus (RCAS). Each sample had 5 ul of titred RCAS stock added at the lysis step, and recovery of RCAS was measured alongside SIV RNA to quantify loss of RNA during the extraction process [325].

In the second method, live cells were sorted into 300 uL FBS, and pelleted by centrifugation at 400rpm for 10 minutes. Supernatants were removed and cell pellets were resuspended in 300uL cold RNAzol (Molecular Research Center) before cryopreservation at -80℃. For RNA extraction, RNA lysates were thawed, and sterile water (0.4 volume of lysate) was added before shaking and incubating for 15 min at room temperature. Samples were centrifuged at 16,000 rpm for 15 minutes, and RNA was extracted from the top (liquid) phase. DNA in the bottom phase was preserved for later extraction and stored at -80℃. For RNA extraction, 1 uL glycogen (20 ug/ul) was added, and RNA was precipitated by adding an equal volume of isopropanol to the RNA lysate/water mixture and vortexing, followed by centrifugation at full

speed for 15 minutes. Supernatant was removed and the pellet was washed 2x with 750 ul 75% ethanol before drying the pellet and dissolving the RNA in sterile water. DNA was extracted from the bottom phase after thawing, solubilizing the pelleted with 900 ul DNAzol (Molecular Research Center) and precipitation with 2 ul glycogen (20 ug/ul) and 500 ul 100% ethanol. The solution was vortexed, incubated for 10 minutes at room temperature and centrifuged for 15 at full speed in a bench top microcentrifuge. The supernatant was discarded and the DNA pellet was washed 2x with 75% ethanol before drying and dissolution in 300 uL NaOH (8 mM) and neutralization by 24 uL HEPES (0.1M).

2.5 In Vitro infections of cell lines and primary cells

Virus stocks were obtained from the NIH AIDS Reagent Program (SIVmac251 32H) and from existing lab stocks of culture supernatants (SIVmac251, SIVsmE660). Two cell lines were used for SIV propagation and virus expansion: H9 and CEMx174, obtained from the NIH AIDS Reagent Program. Both cell lines were maintained at 37°C with 5% CO_2 and cultured in R10 at a density of $1x10^5$-$1x10^6$ cells/mL and split at 1:2 twice weekly. For infection of cell lines, 1x106 cells were pelleted (5 minutes 1500 rpm), resuspended in 1 mL viral supernatant, and spinoculated for 1 hour at 1200g at 32°C. Cells were then washed with 10mL R10 and resuspended in 10 mL R10 in a 25 mL culture flask at 37°C. After 2-3 days based on confluence of cells and observation of syncytia, 5 mL fresh R10 was added to the culture. Infection was confirmed by intracellular staining with p24 (Beckman Coulter), and supernatants from peak infection (3-5 days) were stored at -80°C.

Infection of primary cells was done using fresh PBMC. After isolation of lymphocytes using Ficoll-Paque, CD4 T cells were positively selected using the CD4+ T cell Isolation kit for nonhuman primates (Miltenyi Biotec), and washed in R10. Cells at $1x10^5$ cells/mL were activated using either Concanavalin A at 25ul/mL (Sigma-Aldrich) or phytohemaglutainin at 10 ug/mL (Fisher Scientific) in R10 overnight. The next morning cells were washed and resuspended in R10 + IL2 (50U/mL) and incubated for 24-48 hours. Frozen virus stocks were concentrated using 0.5 mL 100,000 MWCO filters and spun for 10 minutes at 14,000 rpm. Cells were gently pelleted and had supernatant was removed before re-suspension in the residual volume. Concentrated virus was layered on top of the cells and incubated for 2 hours at 37°C. Cells were washed with R10 and cultured in R10+IL2. Cultures were checked daily for syncytia, and Infection was measured by intracellular p24 staining.

2.6 Construction of absolute standards for qRT-PCR

RNA standards for qRT-PCR were created to measure absolute quantities of spliced and unspliced SIV RNA in infected cells. For gag RNA, previously published primers and probes were used [326] and for tat/rev RNA, primers and probes were designed across the D4/A7 splice site to uniquely amplify multiply spliced SIV RNA.

PBMCs were isolated from fresh blood draws and infected with SIVmac251 as described above, and total RNA was extracted and purified after 5 days of infection using the RNAqueous-Micro Total RNA isolation kits (Thermo Fisher) according to the manufacturer's instructions. cDNA synthesis was performed on the purified RNA by first incubating 1 uL of reverse primers for tat/rev (2 uM), 1 ug total RNA, 1 uL dNTP (10 mM) and 8 uL PCR-grade water at 65°C for 5 minutes followed by 1 minute on ice. Next, 4 ul 5x First Strand buffer (Invitrogen), 1 uL 0.1M DTT, and 1 uL RNAseout (Invitrogen) were added to the reaction mix and incubated at 42°C for 2 minutes. Next, 1 uL Superscript III (Invitrogen) was added along with 3uL PCR-grade water for a total volume of 20 uL, and the reaction mixture was incubated at 45°C for 50 minutes followed by 70°C for 15 minutes. The resulting cDNA was amplified by adding 5 uL 10X PCR Buffer (TRIS pH 8.4, KCL, Thermo Fisher), 1.5 uL 50 mM MgCl2, 1 uL 10 mM dNTPs, 1 uL each of the forward and reverse primers (10uM), 0.4 uL Platinum *Taq* DNA Polymerase (Thermo Fisher), and 38.1 uL PCR-grade water to 2 uL cDNA, and amplifying using the following thermocycling program: 94°C for 5 minutes, 35 cycles [94°C 30 seconds, 55°C 30 seconds, 72°C 45 seconds], 72°C 10 minutes, 4°C hold. Two microliters of the reaction mixture were run on a 2% agarose gel with a 100bp ladder in 1xTAE at 70 volts, and stained with 10uL SYBRGold to confirm amplification. The remaining reaction mixture was purified using the Wizard SV Gel and PCR Clean-up System (Promega) according manufacturer's instructions. DNA was quantified using a Nanodrop 1000 (Nanodrop).

Ligation was performed using p-Gem-T Easy Vector (Promega) according to manufacturer's instructions, including positive and negative controls. The ligation mixture (7.5 uL) was incubated with 50ul of DH5-a cells for 30 minutes on ice before each tube was heat shocked for 50 seconds at 42°C. 950 uL of S.O.C. media (Thermo Fisher) was added to each tube and incubated shaking at 37°C for 2 hours. LB-agar plates with ampicillin (100ug/mL), X-gal (100ug/mL) and IPTG (100 ug/mL) were prepared (Invitrogen) and 100 ul or 200 uL of transformed cells were spread over plates before an overnight incubation. The following day, colonies were picked and amplified with 2.5 uL HiFi buffer (Fisher Scientific), 1 uL 50 mM

$MgSO_4$, 0.5 uL 10 mM dNTPs, 1 uL M13 forward and reverse primers (20 mM), and 0.14 uL HiFi Taq DNA polymerase (Thermo Fisher) with the following thermocycling conditions: 94°C for 5 minutes, 35 cycles [94°C 30 seconds, 57 30 seconds], 68°C 3 minutes, 4°C hold. Samples were sent to Agencourt Bioscience for Sanger sequencing, and plasmids with the appropriate amplicons were grown to 100mL cultures in LB. Plasmids were purified using Plasmid Midiprep Kits (Qiagen) according to the manufacturer's instructions, linearized with restriction enzyme PstI (NEB), run on a 1% agarose gel and purified using the Wizard SV Gel and PCR Clean-up System (Promega). RNA synthesis was performed using the MEGAscript T7 Transcription Kit (Thermo Fisher) according to the manufacturer's instructions, and purified RNA was quantified on a Bioanalyzer High Sensitivity RNA chip. RNA was diluted in PCR-grade water to create standards of 2 copies to $2x10^7$ copies/uL and stored at -89°C.

2.7 PrimeFlow

All RNA flow cytometry experiments were done using a modified version of the eBiosciences PrimeFlow protocol. All wash steps were centrifuged for 800g for 5 minutes in a swinging bucket centrifuge unless otherwise noted. Cryopreserved cells were thawed in a 37°C water bath, resuspended in R10, washed, and rested for 30 min at 37C. In cases where the viability of the cells was below 40%, a dead cell removal was performed using the Dead Cell Removal Kit (Miltenyi Biotec). After centrifugation of cells at 300g for 5 minutes and incubation for 15 minutes at room temperature with Dead Cell Removal Microbeads at a ratio of 100 uL beads per 10^7 total cells, cells were added to an LS MACS column and eluted using 3 x 3 mL Binding Buffer. After elution, cells were washed in R10 and re-counted. All samples were split into three aliquots, with up to 5 million cells per aliquot: surface stain only, RNA probe, and no probe control. Cells were stained with 10 uL of a 1:40 dilution of LIVE/DEAD Fixable Aqua (Thermo Fisher) for five minutes and subsequently with the surface antibody panel for 25 minutes at 2-8°C (Table 2). Surface stain only samples were additionally stained with CD3 at this time before washing in PBS and fixation with 2% paraformaldehyde (PFA) and storage at 2-8°C. Probe samples were washed in 1 mL PBS and resuspended in Fixation Buffer 1 for 30 minutes at 2-8°C, washed two times in 1X Permeabilization Buffer and stained with CD3 for 25 minutes at 2-8°C. Following CD3 staining, samples were washed with Permeabilization Buffer, fixed using 1mL Fixation Buffer 2, and incubated at room temperature in the dark for 60 minutes. Cells were washed in RNA Wash buffer and transferred to low-bind 1.5 mL Eppendorf tubes included in the kit.

Target probes were thawed and diluted 20x in RNA Target Probe diluent pre-warmed to 40°C and 100 uL were added to cell suspensions and incubated for 2 hours at 40°C. No-probe

controls had only 100 uL Target Probe Diluent added. Samples were washed 2x with RNA Wash Buffer and gently resuspended in 100 uL residual buffer before addition of 100 uL RNA PreAmp mix pre-warmed to 40°C. Cells were briefly vortexed and incubated at 40°C for 1.5 hours, washed three times with RNA Wash Buffer, and resuspended in 100uL residual buffer before adding 100 uL RNA Amp Mix. Each sample was briefly vortexed and incubated at 40°C for 1.5 hours and subsequently washed twice with RNA Wash Buffer. RNA Label probes were diluted 100x in RNA Label Probe Diluent and 100uL of the probe-diluent mix was added to each sample before incubation for 1 hour at 40°C. Samples were then washed twice with RNA wash buffer and washed and resuspended in 1mL PBS before acquiring on the flow cytometer.

For all RNA flow samples, the surface-only stain was used to confirm gating and frequencies of T cell subsets in the full probe staining protocol samples. No-probe controls that underwent the full RNAFlow staining protocol were used to set the FMO gates for the probe-positive samples. Compensation on the flow cytometer was performed using antibody-bound beads and the PrimeFlow Compensation Kit (eBiosciences)

To compare the staining of the ACD RNAscope probes and amplifiers with the eBiosciences PrimeFlow kit, some samples were incubated ACD reagents for the target probe and amplification steps (all samples underwent identical surface and ICS staining and fixation/permeabilization). ACD samples were incubated with ACD target probes (2 drops) for 2 hours at 40°C, washed, and subsequently incubated with 2 drops each of Amp1 (30 minutes), Amp2 (15 minutes), Amp3 (30 minutes), and Amp4 (15 minutes) with two washes in between each incubation. Samples were washed, resuspended in PBS, and acquired on the flow cytometer.

2.8 SIV sequencing

Full length SIV genomes were sequenced from plasma RNA and from DNA and RNA extracted from sorted lymph node T cell subsets. Lymph node samples were stained with the panel in Table 2.8, and seven populations were sorted: live singlet CD14- CD16- CD20- CD3+ CD4+ lymphocytes were divided into naïve (CCR7+ CD45RA+ CD28+ CD95-) and central memory (CD45RA- CD28+ CD95+), while two populations were sorted from CD3+ CD4- cells: CXCR5+ and CXCR5-. Central memory T cells were further divided based on expression of CXCR5 and PD1, with four populations (CXCR5++PD1++, CXCR5+PD1+, CXCR5+PD1-, and CXCR5-PD1-). Each of the seven populations was sorted from lymph

node biopsies at 2 weeks 4 weeks, 10 weeks, and 24 weeks post infection and both DNA and RNA were extracted using protocols described above.

Plasma samples were diluted to 10 mL with PBS and clarified by centrifugation at 200g for 10 minutes with no braking. Clarified plasma was overlaid onto 25 mL of a 1:10 dilution of OptiPrep (Sigma Aldrich) in PBS and centrifuged at 20,000 rpm for 3 hours at 4°C with no braking. Plasma and Optiprep were removed and the viral pellet was resuspended in 400 uL cold RNAzol RT (Molecular Research Center) and frozen at -80°C. For RNA extraction, lysates were thawed, and sterile water (0.4 volume of lysate) was added before shaking and incubating for 15 min at room temperature. Samples were centrifuged at 16,000 rpm for 15 minutes, and 85% of the RNA-containing supernatant was removed to a fresh tube. 0.5% volumes of BAN (Molecular Research Center) was added to the supernatant, mixed, and incubated for 5 minutes at room temperature. The mixture was centrifuged at 12,000g for 10 min at room temperature. 90% of this supernatant was added to a fresh tube (containing 1ul glycogen and 4 uL water), and RNA was precipitated from this mixture by the addition of 1 volume of isopropanol, vortexing, and incubation for 10 minutes at room temperature. RNA was pelleted by centrifugation at full speed for 15 minutes, and the pellet was washed twice with 750 ul 75% ethanol. The pellet was dried and dissolved in sterile water and frozen at -80°C.

The protocol for amplification, sequencing, and assembly was adapted from Gall et al[327], with primers designed to generate four overlapping amplicons covering nearly the full SIV genome, ranging from 1791 bases to 2859 bases. RNA was amplified using Superscript III One-Step RT-PCR with Platinum Taq High Fidelity kit (Invitrogen), with 12.5 uL 2X Reaction Mix, 0.5 ul SuperScriptIII/HiFI Platinum Taq, 1 ul primer mix (5 uM), 1.5 uL 5 mM $MgSO_4$, 4 uL template RNA, and 5.5 uL PCR-grade water, and thermocycling conditions of 50°C 60 minutes, 94°C 2 minutes, 2 cycles [94°C 15 seconds, 60°C 60 seconds, 68°C 4 minutes], 2 cycles [94°C 15 seconds, 58°C 60 seconds, 68°C 4 minutes], 41 cycles [94°C 15 seconds, 55°C 60 seconds, 68°C 4 minutes], 68°C 10 minutes, 4°C hold. DNA was amplified using Platinum Taq High Fidelity kit (Invitrogen), with 2.5 ul 10x HiFI PCR buffer, 0.5 ul 10 mM dNTP, 0.1 ul HiFi Platinum Taq, 1 ul primer mix (5 uM), 1 ul 5 mM $MgSO_4$, 5 ul template DNA, and 14.9 uL PCR-grade water, and thermocycling conditions of 94°C 2 minutes, 30 cycles [94°C 15 seconds, 55°C 60 seconds, 68°C 4 minutes], 10°C hold. After amplification, samples were run on a 1% agarose gel for 60 minutes at 100V and stained with SYBRGOld to visualise bands. Samples were multiplexed and sequenced using Illumina

HiSeq 2500 Rapid Run with 250 base pair paired end reads by the Sequencing Core at the Wellcome Trust Sanger Institute.

2.9 B cell sorting and 5' RACE sequencing

A SIV mac239 gp140 foldon trimer probe (molecular weight 140,000) containing an Avitag (Avidity) and a His tag was provided by Rosie Mason and Mario Roederer[328] and was conjugated to Streptavidin-APC (Invitrogen). After diluting 10 ug of the probe into PBS and protease inhibitor to a final volume of 117 uL, 8 uL of Streptavidin-APC was added in 5 aliquots, incubating the probe-SA-APC mix for 10 minutes shaking at 4C between each addition. Conjugated protein was stored at 4C until use.

For immunoglobulin heavy chain repertoire sequencing, serial biopsies of three animals (ZF61, ZF76, and ZG13) were used to analyse the evolution of the humoral response. PBMC, bone marrow, inguinal lymph node biopsies from 4 weeks, 10 weeks, and 24 weeks post infection were sorted and sequenced. For each tissue, samples were thawed (with the exception of bone marrow, which was sorted fresh), stained with the B cell sorting panel, and five populations of B cells were sorted into 200ul fresh R10., After excluding T cells and monocytes, 19+20+ B cells were sorted on expression of IgM, IgD, IgG, CD27, and gp140 to obtain naïve, IgG memory, IgM memory, antigen-specific IgG memory, and antigen-specific IgM memory B cells. Cells were pelleted, resuspended and stored in RNAlater until RNA extraction. RNA was extracted using µMACS mRNA Isolation Kit (Miltenyi Biotec) and cDNA was synthesized by first incubating 8 ul RNA with 1uL 5' CDS Oligo dT primers (12 uM) for 1 minute at 72°C and cooling on ice, before the addition of 1uL Superscript II (Invitrogen), 1uL SMARTer oligo (12uM), ul dNTP (1mM), 1 ul DTT (20 mM), 1 ul RNAseOUT (Invitrogen), and 3.5 ul 5X RT Buffer (Clontech) and incubating at 42°C for 120 minutes followed by 70°C for 120 minutes. cDNA synthesis was cleaned up with using Agencourt AMPure XP beads (Beckman Coulter) according to the manufacturer's instructions. PCR amplification of BCR heavy chains was performed using Kapa HiFi Real-Time PCR Library Amplification kits (Kapa) with Ig constant region primers (5 uL of 2uM) and 5PIIA primer (0.7 ul of 12uM). Samples were sequenced on the Illumina MiSeq platform with paired end reads of 300 base pairs.

2.10 Single cell B cell sequencing

The protocol for single cell sorts for B cell heavy chain sequencing was adapted from Tiller *et al* [329]. Individual cells were sorted into 96 well plates, either dry or containing 0.5 uL RNAse Out (Thermo Fisher), 5 ul 5X buffer (Invitrogen, for Superscript III), 1.25 ul DTT (Thermo Fisher), 0.0625 uL Igepal (Sigma), 0.26 uL *S. cerevisiae* carrier RNA (Sigma) and 13 uL PCR

qualified water (Thermo Fisher); and immediately frozen at -80. For cDNA synthesis, dry plates had the above mixture added to each well after thawing on ice immediately before cDNA synthesis. Reverse transcription was performed after adding 0.3 uL Superscript III (Invitrogen), 3ul random hexamers (GeneLink, 150 ng/uL), and 2 uL dNTP (10mM, Thermo Fisher) to each well, with a PCR program of 10 minutes at 42°C, 10 minutes at 25°C, 60 minutes at 50°C, 5 minutes at 94°C, and a hold at 4°C. 26 uL PCR-grade water was added to each well.

Nested PCR was used to amplify the resulting cDNA, using 4uL cDNA, 2.5 ul 10X Buffer (Qiagen), 0.5 uL dNTP (10mM, Thermo Fisher), 0.5 uL $MgCl_2$ (25 mM, Qiagen), 5 uL Q-solution (Qiagen), 0.5 uL Forward Outer Primer mixture (25 mM), 0.5 uL Reverse Outer Primer mixture (20 mM), 0.2 uL HotStart Taq Plus (Qiagen), and 11.3 uL PCR-grade water (Qiagen) per well. Thermocycling conditions were: 94°C for 5 minutes, 50 cycles of [94°C for 30 seconds, 55°C for 30 seconds, 70°C for 60 seconds], 70°C for 10 minutes, and hold at 4°C. The second PCR used 3 ul of the first round PCR with a mastermix of 2.5 ul 10X Buffer (Qiagen), 0.5 uL dNTP (10mM, Thermo Fisher), 5 uL Q-solution (Qiagen), 0.5 uL Forward Inner Primer mixture (25 mM), 0.5 uL Reverse Inner Primer mixture (20 mM), 0.2 uL HotStart Taq Plus (Qiagen), and 12.8 uL PCR-grade water (Qiagen) per well. Thermocycling conditions were: 94°C for 5 minutes, 50 cycles of [94°C for 30 seconds, 60°C for 30 seconds, 70°C for 60 seconds], 70°C for 10 minutes, and hold at 4°C. Amplification of heavy chains was verified using gel electrophoresis, with 5 uL of second-round PCR product diluted with 1uL 6X Orange Gel Loading Dye (NEB) run on a 2% agarose gel alongside a 100 bp ladder (NEB) at 100 volts for 20-30 minutes. Gels were visualised using SYBR Gold Gel Stain (Thermo Fisher) diluted 10,000X in TAE for 30 minutes and positive wells were sent for Sanger sequencing to GATC.

2.11 Bioinformatics analysis

For each sequencing run of pooled immunoglobulin heavy chain samples, sequences were demultiplexed into sample groups. FASTQ files were generated from raw BAM files and quality controlled using QUASR7.01[330] with a median sequence quality cutoff of 34. Paired reads that passed the quality control filtering and overlapped by at least 30 base pairs were joined, and primers were trimmed from the full-length sequences. Each sequence was aligned using BLAST[331] against a database of IgJ genes and removed if it did not match a reference J sequence with an e-value of 0.1 or less. Open reading frames were calculated for each sequence and sequences passed if the length was above a given threshold and had a V gene

match to the reference database with an e-value of 1. Sequences were clustered based on sequence similarity with CD-HIT with a sequence identity threshold of 0.85. Diversity statistics including Gini Index and Renyi index for the clusters and vertices were calculated as in Bashford-Rogers *et al* [332], as were network plots.

Virus sequences were assembled *de novo* using IVA [333] and underwent IVA's quality control pipeline using SIV reference genomes (accession numbers KC522232, M33262 and M76764). All contigs mapping to reference genomes underwent hypermutation analysis using Hypermut [334], and contigs that were significantly hypermutated ($p<0.01$) were removed from the assemblies. In cases where overlapping contigs mapped to the genome, sequences were manually merged to create a single consensus sequence for each sample. All sequences were first aligned using MAFFT [335] and manually edited. Pairwise distances were calculated in MEGA [336]. Maximum likelihood trees were generated using FastTree [337] with a generalized time-reversible (GTR) model of nucleotide evolution and rapid bootstrapping with 1,000 resamples, and trees were rooted on reference sequence KC522232.

2.12 Statistical Analysis

All statistics were calculated in GraphPad Prism. In Chapter 3, the Wilcoxon matched-pairs test was used to evaluate differences in population proportions between paired samples from individual animals. Multiple T-tests were used to analyze differences between germline divergence, CDR3 length, diversity measures (Gini and Renyi index, vertices in largest cluster), and proportion of shared sequences between animals and populations. Chi-squared tests were used to calculated differences in V and J gene distribution proportions. T-tests were used to analyze differences in computational derived isoelectric point, hydrophobicity, aliphatic index, and charge in CDR3 regions. In chapter 4, one-way Anova tests were used to analyze expression patterns of surface receptors on T cell subpopulations, copy numbers of SIV DNA and RNA in T cell subpopulations, and the proportion of SIV RNA probe-positive cells. In chapter 5, one-way Anova tests were used to analyze pairwise distances between sets of sequences from different animals, T cell subpopulations, and nucleic acid origins (DNA, RNA or plasma).

2.13 PCR Primers

Table 2.2: SIV DNA/RNA quantification primers

Primers	Sequence
M13 Forward	GTAAAACGACGGCCAG
M13 Reverse	CAGGAAACAGCTATGAC
SIV tatrev_F04a	GAACTCCGAAAAAGGCTAAGGCTAATACA
SIV tatrev_F04c	GAACTCCGAARAAGRCTAAGRCTAATMCA
SIV tatrev Left D4A7	TCCGAAAAAGGCTAAGGCTA
SIV tatrev_R04d	CCGTCTCRTTCTTTGCCTTCTCTGGTT
SIV tatrev_R04c	CCKTCTCCTTCTTCTCCTTCTTTG GTT
SIV tatrev Right D4A7	CTCCACCGTCTCCTTCTTTG
Gag Forward	GTCTGCGTCATPTGGTGCATTC
Gag Reverse	CACTAGKTGTCTCTGCACTATPTGTTTTG
Probes (for qRT-PCR)	5' FAM 3' BHQ-1
SIV tatrev_P04a	CTGCATCAAACAAACCCATATCCAACAGGACC
SIV tatrev_P04c	CTGCATCAAACAAATCCCTATCCACAAGGRCC
Gag Probe	CTTCPTCAGTKTGTTTCACTTTCTCTTCTGCG

Table 2.3: SIV full genome sequencing primers

Primer Mixes	Forward	Reverse
Amp1	TGTCTTTTATCCAGGAAGGGGTA	CTCTAATTAACCTACAGAGATGTTTGT, CTCTAGTTAACCTACAGAGATGTTTGT
Amp2	AAAATTGAAGCAGTGGCCATTAT	TTCTTATGAGCTCTCGGGAACCT
Amp3	GCTTTACAGCGGGAGAAGTG	ATTGCAGAACCTGCCGTTG
Amp4	CAGTCACCATTATGTCTGGATTG	GAATACAGAGCGAAATGCAGTG

Table 2.4: 5' RACE BCR sequencing primers

CDS Oligo dT	TTTTTTTTTTTTTTTTTTTTTTTTVN
SMARTER IIA	AAGCAGTGGTATCAACGCAGAGTACATrGrGrG
5PIIA	AAGCAGTGGTATCAACGCAGAGT
RhHu IgM	GAGCGAGGGGGAAAAGGGTTGGGGCGGATGCA
RhHu IgG	GCCAGGGGGAAGACCGATGGGCCCTTGGTGGA
RhHu IgD	CTGATATGATGGGGAACACATCCGGAGCCTTGG

Table 2.5 B cell single cell sorting/sequencing primers

Outer Forward L1 Primers - Mix @ equal volumes to 25uM		Inner Forward SE Primers - Mix @ equal volumes to 25uM	
5'VH1.L1	ATGGACTKGACCTGGAGG	5'VH1A.SE	TGGCAGCAGCTACAGGTGC
5'VH2.L1	ATGGACACGCTTTGCTCC	5'VH1B.SE	TGACAGCAGCTACAGGCGC
5'VH3A.L1	ATGGAGTTKGGGCTGAGC TG	5'VH1C.SE	TGGCAGCAGCAACAGGCAC
5'VH3B.L1	ATGGAGTTTGKRCTGAGC TGG	5'VH2.SE	GTCCCGTCCTGGGTCTTGTC
5'VH3C.L1	ATGGAGTCRTGGCTGAGC TGG	5'VH3A.SE	GCTGTTTGGAGAGGTGTCCAGT GTG
5'VH3D.L1	ATGGAGTTTGTGCTGAGTT TGG	5'VH3B.SE	GCCATATTAGAAGGTGTCCAGT GTG
5'VH4.L1	ATGAAGCACCTGTGGTTC	5'VH3C.SE	GCTCTTTTGAAAGGTGTCCAGT GTG
5'VH5A.L1	ATGGGGTCAACTGCCATC	5'VH3D.SE	GCTATTTTAAGAGGTGTCCAGT GTG
5'VH5B.L1	ATGGGGTCCACCGTCACC	5'VH3E.SE	GCTATTTTAAAAGGTGTCCAGT GTG
5'VH6.L1	ATGTCTGTCTCCTTCCTCA	5'VH4.SE	AGCTCCCAGATGGGTCYTGTCC
5'VH7.L1	ATGGACCTCACCTGGAGC	5'VH5.SE	GCTGTTCTCCARGGAGTCTGTG
5'VH2.L1	ATGGACACGCTTTGCTCC	5'VH6.SE	GGCCTCCCATGGGGTGTC
		5'VH7A.SE	GCAGCAACAGGTGCCCACTC
		5'VH7B.SE	GCAGCAACAGGCACCCACTC
Reverse Outer Primer - Use @ 20uM		Reverse Inner Primer - Use @ 20uM	
3'IgG Outer	GGAAGGTGTGCACGCCGCT GGTC	3'IgG Inner	GTTCAGGGAAGTAGTCCTTGA C

2.14 Flow Cytometry Panels

Table 2.6: In vitro infection panel

Surface marker	Fluorophore	µl per test	Clone	Company	Notes
Live/Dead	Aqua	5		Thermo Fisher	
CD4	FITC	2.5	L200	BD Biosciences	
CD3	Cy7APC	3	SP34-2	BD Biosciences	
CD8	BV785	2	RPA-T8	BioLegend	
p24	PE	0.5	KC57	BioLegend	intracellular

Table 2.7: RNA Flow panel and probes

Surface marker	*Fluorophore*	*µl per test*	*Clone*	*Company*	*Notes*
CD4	BV605	4	L200	BD Biosciences	
CD8	BV785	2	RPA-T8	BioLegend	
PD1	BV711	2	EH12.2H7	BioLegend	
CXCR5	PE	5	MU5UBEE	eBiosciences	
CD28	TRPE	7	CD28.2	Beckman Coulter	
CD95	Cy5PE	7	DX2	BD Biosciences	
CD3	Cy7APC	3	SP34-2	BD Biosciences	ICS in probe samples
RNA Probe		*Channel*	*Probe Type*	*Company*	
SIVmac239 gag, pol, env		Ax647	Type 1	eBiosciences	
B2M macaca Mulatta		Ax488	Type 4	eBiosciences	
SIVmac239 SENSE C1		Ax488	C1	ACD	
DAPB		Ax647	C2	ACD	

Table 2.8: SIV full genome sequencing panel

Surface marker	Fluorophore	µl per test	Clone	Company	
CCR7	Ax700	2	150503	BD Biosciences	
CD14	BV510	1.5	M5E2	BioLegend	
CD16	BV510	1	3G8	BioLegend	
CD8	BV510	2	RPA-T8	BioLegend	
CD20	BV570	2.5	2H7	BD Biosciences	
CD45RA	Qd655	1	MEM-56	Invitrogen	
PD-1	BV785	2	EH12.2H7	BioLegend	
gdT	APC	2	B1	BioLegend	
CD3	Cy7APC	2	SP34-2	BD Biosciences	
CD4	FITC	4	L200	BD Biosciences	
CXCR5	PE	5	MU5UBEE	eBiosciences	
CD28	TRPE	7	CD28.2	Beckman Coulter	
CD95	Cy5PE	7	DX2	BD Biosciences	

Table 2.9: Lymph node Tscm staining panel

Surface marker	Fluorophore	µl per test	Clone	Company	
CCR7	BV421		150503	BD Biosciences	
CD14	BV510	1.5	M5E2	BioLegend	
CD16	BV510	1	3G8	BioLegend	
CD4	BV605		L200	BD Biosciences	
CD8	BV785	2.5	RPA-T8	BioLegend	
gdT	APC	2	B1	BioLegend	
CXCR3	Ax700	3	1C6	BD Biosciences	
CD3	Cy7APC	2	SP34-2	BD Biosciences	
CD45RA	FITC		MEM-56	Invitrogen	
CXCR5	PE	5	MU5UBEE	eBiosciences	
CD127	Cy7PE	5	R34.34	Beckman Coulter	
CD28	TRPE	7	CD28.2	Beckman Coulter	
CD95	Cy5PE	7	DX2	BD Biosciences	

Table 2.10 Lymph node Chemokine staining panel

Surface marker	Fluorophore	µl per test	Clone	Company	
ICOS	PacBlue	2.5	C398.4A	BioLegend	
CD14	BV510	1.5	M5E2	BioLegend	
CD16	BV510	1	3G8	BioLegend	
CD8	Qd565	0.5	3B5	Invitrogen	
CD4	BV605	2.5	L200	BD Biosciences	
PD-1	Biotin	0.25	EH12.2H7	eBioscience	20 min 37c
Streptavidin	BV650	0.5		BD Biosciences	
CCR6	Ax647	2.5	G034E3	BioLegend	
CXCR3	Ax700	3	1C6	BD Biosciences	
CD3	Cy7APC	2	SP34-2	BD Biosciences	
SLAM	FITC	5	A12 (7D4)	BioLegend	
CXCR5	PE	5	MU5UBEE	eBiosciences	
CCR4	Cy7PE	3	TG6	BioLegend	
CD28	TRPE	7	CD28.2	Beckman Coulter	
CD95	Cy5PE	7	DX2	BD Biosciences	

Table 2.11: Lymph node Activation staining panel

Surface marker	Fluorophore	µl per test	Clone	Company	
ICOS	PacBlue	2.5	C398.4A	BioLegend	
CD14	BV510	1.5	M5E2	BioLegend	
CD16	BV510	1	3G8	BioLegend	
CD20	BV570	2.5	2H7	BioLegend	
CD25	BV605	5	BC96	BioLegend	
PD-1	Biotin	0.25	EH12.2H7	eBioscience	20 min 37c
Streptavidin	BV650	0.5		BD Biosciences	
CD8	BV785	1.5	RPA-T8	BioLegend	
gdT	APC	2	B1	BioLegend	
CCR7	Ax700	2	150503	BD Biosciences	20 min 37c
CD3	Cy7APC	2	SP34-2	BD Biosciences	
CD4	FITC	4	L200	BD Biosciences	
CXCR5	eFluor710	5	MU5UBEE	eBiosciences	
SLAM	PE	2	A12 (7D4)	BioLegend	
CD127	Cy7PE	5	R34.34	Beckman Coulter	
CD28	TRPE	7	CD28.2	Beckman Coulter	
CD95	Cy5PE	7	DX2	BD Biosciences	

Table 2.12: B cell sorting Panel

Surface marker	Fluorophore	µl per test	Clone	Company
CD3	Cy7APC	2	SP34-2	BD Biosciences
CD14	Qd800	1	Tuk4	Invitrogen
CD19	ECD	10	J3-119	Beckman Coulter
CD20	BV570	2.5	2H7	BD Biosciences
CD27	Cy5PE	3	CLB 27/1	Invitrogen
CD138	FITC	20	MI15	BD Biosciences
IgD	PE	0.5	polyclonal	Southern Biotech
IgM	V450	2.5	G20-127	BD Biosciences
IgG	Ax680	1.5	G18-145	VRC
Gp140	APC	1		VRC

2.15 Animal Specimens

Table 2.13: Animal Study Protocols and Samples

ASP	Animals	Tissues	Infection time	Virus
238.2	8-23, 08D213, 8-46, ZG08, ZG32, ZG72	Axillary LN, PBMC	6 months	SIVsmE660
206	8E-9, DBME, 7F-8, AV33X	Inguinal LN, PBMC	2 years	SIVsmE660
238.3	8-40, 8-76, 8-118, 8-130, 08D0236, 8-8, 8C-8, 8-160, 8-88, ZC26, ZG38, 8-28, 8-160	Axillary LN, Inguinal LN, PBMC	Various	SIVmac251
274	4140, FLI, CK3P, 4131, IAC	PBMC	Uninfected	N/A
417	ZF76, ZG13, ZF61, ZH29, ZF32, ZF60	Mesenteric, Axillary, Inguinal lymph nodes; ilium/jejenum, bone marrow, PBMC	5 days – 6 months	SIVmac251
	6049, 4975. 4939, 4755, 4875, 4931	Axillary LN, PBMC	acute	SIVmac251
	7-20, FA4L, 4359, 08D272, 0G-4, 4359,	Axillary LN, Inguinal LN, PBMC	uninfected	N/A

2.15 IGH Sequencing Results – cells sorted and sequencing reads retained

Table 2.14: IgG cells sorted, reads retained in initial QC

Sample ID	cells sorted	% reads retained	N raw reads (1)	N raw reads (2)	N joined reads	N reads primer matched	N reads w/t ORF
ZF61_4_BM_gp140_IgG_A	400	0.001884037	119977	106155	66358	681	2
ZF61_4_BM_gp140_IgG_B	400	1.232868655	110509	85005	8965	1232	1048
ZF61_4_BM_gp140_IgG_0	400	58.01973777	741355	709300	413969	412846	411534
ZF61_4_BM_mem_IgG_0	35000	45.65305531	683304	656070	316233	312567	299516
ZF61_4_INGLN_gp140_IgG_0	660	39.4418844	702418	672298	294667	290508	265167
ZF61_4_INGLN_mem_IgG_0	46200	42.26670078	492954	472987	204526	203691	199916
ZF61_4_LN_gp140_IgG_A	660	0.04477126	100976	73708	40712	5626	33
ZF61_4_PBMC_gp140_IgG_A	925	3.501358608	109804	93478	39263	3532	3273
ZF61_4_PBMC_gp140_IgG_0	925	0.007790975	144337	141189	124166	34752	11
ZF61_4_PBMC_gp140_IgG_B	925	0.003057668	112359	98114	73251	72090	3
ZF61_4_PBMC_mem_IgG_0	81000	45.36098407	974316	938227	795788	529735	425589
ZF61_10_BM_gp140_IgG_A	800	0.56186009	137670	99135	5976	2733	557
ZF61_10_BM_gp140_IgG_B	800	0.892450991	118893	92330	15752	3404	824
ZF61_10_BM_gp140_IgG_0	800	2.025189468	701671	675838	57448	14476	13687
ZF61_10_BM_mem_IgG_0	32000	61.08007179	773744	759968	479731	475077	464189
ZF61_10_INGLN_gp140_IgG_0	810	56.51454317	855255	815675	494905	474658	460975
ZF61_10_INGLN_mem_IgG_0	50000	51.4240943	807108	775370	411241	409566	398727
ZF61_10_LN_gp140_IgG_A	810	1.740061571	132649	104594	12140	4464	1820
ZF61_10_PBMC_gp140_IgG_A	9500	0.370091304	64622	45124	6969	2950	167
ZF61_10_PBMC_gp140_IgG_0	9500	0.021945028	201659	200501	197998	19299	44
ZF61_10_PBMC_gp140_IgG_B	9500	0.769555715	125848	108894	65890	41921	838
ZF61_10_PBMC_mem_IgG_0	188000	41.67404232	1348768	1309489	1062072	733686	545717
ZF61_24_BM_gp140_IgG_A	136	3.254085305	108154	84755	5294	3692	2758
ZF61_24_BM_gp140_IgG_B	136	0.006732258	114908	103977	89585	15920	7
ZF61_24_INGLN_gp140_IgG_0	14100	68.18606818	641237	626738	434448	429513	427348
ZF61_24_INGLN_mem_IgG_0	65000	56.85390583	601522	579633	342120	339165	329544
ZF61_24_LN_gp140_IgG_0	14100	11.76154276	118432	94843	26441	18238	11155
ZF61_24_PBMC_gp140_IgG_A	32000	16.45421771	118508	108732	81016	61264	17891
ZF61_24_PBMC_gp140_IgG_B	32000	0.002799186	115235	107174	95853	84959	3
ZF61_24_PBMC_gp140_IgG_0	32000	47.18404473	471801	459986	230870	227254	217040
ZF61_24_PBMC_mem_IgG_0	100000	53.63241337	414706	405956	231709	225740	217724
ZF76_4_BM_gp140_IgG_A	500	9.389990734	107048	85261	10998	9038	8006
ZF76_4_BM_gp140_IgG_B	500	16.6828574	104763	88528	38613	37921	14769
ZF76_4_BM_gp140_IgG_0	500	70.45376107	423952	414337	299380	295839	291916

ZF76_4_BM_mem_IgG_0	4000	32.91392335	491113	478132	179278	177828	157372
ZF76_4_INGLN_gp140_IgG_0	450	29.11383823	500083	486717	243324	242354	141702
ZF76_4_INGLN_mem_IgG_0	50000	68.79591918	537592	527149	373652	371491	362657
ZF76_4_LN_gp140_IgG_A	450	13.23685527	102042	81953	14548	12249	10848
ZF76_4_PBMC_gp140_IgG_A	2100	5.297656646	123582	96571	14271	9855	5116
ZF76_4_PBMC_gp140_IgG_B	2100	6.571236926	173712	135667	27772	18750	8915
ZF76_4_PBMC_gp140_IgG_0	2100	47.20007315	112920	109360	93108	64564	51618
ZF76_4_PBMC_mem_IgG_0	55600	48.48522424	513786	500008	410303	289601	242430
ZF76_10_BM_gp140_IgG_A	184	0.043593563	46236	27527	5230	205	12
ZF76_10_BM_gp140_IgG_B	184	3.912032593	115539	93762	28397	10159	3668
ZF76_10_BM_mem_IgG_0	6000	53.04104973	672534	658835	365650	361358	349453
ZF76_10_INGLN_gp140_IgG_0	725	57.06777349	529120	517518	298854	296230	295336
ZF76_10_INGLN_mem_IgG_0	50000	67.62548418	505729	495680	348797	346978	335206
ZF76_10_LN_gp140_IgG_A	725	8.961356972	118561	95153	17448	10635	8527
ZF76_10_PBMC_gp140_IgG_A	12000	0.005880969	117750	102024	58592	20385	6
ZF76_10_PBMC_gp140_IgG_0	12000	0.064155594	60458	59231	55209	26905	38
ZF76_10_PBMC_gp140_IgG_B	12000	2.01740865	129938	110290	56517	40234	2225
ZF76_24_BM_gp140_IgG_B	70	4.909163605	102127	80034	5900	4796	3929
ZF76_24_BM_gp140_IgG_A	70	3.521197307	126854	99824	10010	6151	3515
ZF76_24_BM_gp140_IgG_0	70	63.62772105	441900	431268	282637	280395	274406
ZF76_24_BM_mem_IgG_0	800	59.1310805	474951	464600	278631	276939	274723
ZF76_24_INGLN_gp140_IgG_0	17000	31.65349207	164618	52247	17065	16608	16538
ZF76_24_INGLN_gp140_IgG_1	1254	7.731235338	99975	97617	81093	48864	7547
ZF76_24_INGLN_mem_IgG_0	55000	46.32120699	1105623	1063800	512170	505928	492765
ZF76_24_LN_gp140_IgG_A	1254	1.960541954	136024	105175	12858	3355	2062
ZF76_24_LN_gp140_IgG_0	1254	11.53451181	123547	100299	15820	13040	11569
ZF76_24_PBMC_gp140_IgG_A	1974	0.009918077	152339	100826	20087	39	10
ZF76_24_PBMC_gp140_IgG_C	1974	9.330800764	119346	93186	19483	13869	8695
ZF76_24_PBMC_gp140_IgG_B	1974	1.405850211	125156	106697	54250	38232	1500
ZF76_24_PBMC_gp140_IgG_2	1974	0.045345348	106041	103649	89705	62083	47
ZF76_24_PBMC_gp140_IgG_1	1974	44.28984793	100019	97458	84068	64396	43164
ZF76_24_PBMC_gp140_IgG_0	1974	58.87757475	992251	958781	581353	571875	564507
ZF76_24_PBMC_mem_IgG_1	54700	0.043983647	403441	393328	331640	242733	173
ZF76_24_PBMC_mem_IgG_2	54700	52.32840263	544841	520142	414829	308589	272182
ZF76_24_PBMC_mem_IgG_0	54700	44.5326107	1179843	1140270	528254	522679	507792
ZG13_4_BM_gp140_IgG_B	1700	3.702078003	113751	93407	14999	12618	3458
ZG13_4_BM_gp140_IgG_A	1700	0.108841945	99347	85445	54853	39434	93
ZG13_4_BM_gp140_IgG_0	1700	45.47953232	570796	546105	257202	249367	248366
ZG13_4_BM_mem_IgG_0	5000	30.75053452	599593	571073	186402	179925	175608
ZG13_4_INGLN_gp140_IgG_0	970	47.6935442	825715	790065	387750	380081	376810
ZG13_4_INGLN_mem_IgG_0	60000	41.96192362	535690	512181	217656	216167	214921
ZG13_4_LN_gp140_IgG_A	970	0.461759885	66946	50026	2904	247	231
ZG13_4_PBMC_gp140_IgG_A	2900	1.985192417	132811	98328	6254	5451	1952
ZG13_4_PBMC_gp140_IgG_B	2900	5.460049944	136495	103717	18933	14086	5663
ZG13_4_PBMC_gp140_IgG_0	2900	51.5819399	101041	98139	86358	55491	50622
ZG13_4_PBMC_mem_IgG_0	67000	48.45189811	747244	717976	598099	414839	347873
ZG13_10_BM_gp140_IgG_A	5500	6.484749617	126118	101145	11887	7272	6559
ZG13_10_BM_gp140_IgG_B	5500	7.579800647	135972	109053	18256	14768	8266
ZG13_10_BM_gp140_IgG_0	5500	47.81176324	1322477	1287315	721398	696443	615488
ZG13_10_BM_mem_IgG_0	212000	43.55508872	1278845	1248132	740165	725606	543625
ZG13_10_INGLN_gp140_IgG_B	1000	58.75039813	96930	94190	78574	57497	55337
ZG13_10_INGLN_gp140_IgG_0	1000	54.64746791	1209590	1191466	727343	720974	651106
ZG13_10_INGLN_mem_IgG_0	100000	54.94394739	1210253	1186653	738513	730876	651994
ZG13_10_LN_gp140_IgG_A	1000	2.087242521	122367	94862	7928	2249	1980
ZG13_10_PBMC_gp140_IgG_B	37000	0.004169272	34951	23985	6333	21	1
ZG13_10_PBMC_gp140_IgG_A	37000	6.017580879	121510	94307	13108	12335	5675
ZG13_10_PBMC_gp140_IgG_0	37000	45.73507917	269423	262033	210227	141328	119841
ZG13_10_PBMC_mem_IgG_0	200000	46.34787112	1333097	1272477	1047959	774583	589766
ZG13_10_PBMC_mem_IgG_B	200000	38.41656485	1704079	1643528	1406317	928315	631387
ZG13_24_AXLN_mem_IgG_10KA	10000	50.55769213	228356	218938	176574	120548	110690
ZG13_24_BM_gp140_IgG_B	300	7.569688464	121254	97613	11543	7530	7389
ZG13_24_BM_gp140_IgG_A	300	7.064758725	133472	106642	12728	10116	7534
ZG13_24_BM_gp140_IgG_0	300	46.99070108	1332411	1301549	685296	678571	611607
ZG13_24_BM_mem_IgG_0	4150	51.13825587	1326928	1280951	745934	723801	655056
ZG13_24_INGLN_gp140_IgG_0	7750	38.25261325	671930	639721	264991	246063	244710
ZG13_24_INGLN_mem_IgG_0	100000	41.73335327	553406	528467	227611	225031	220547
ZG13_24_LN_gp140_IgG_0	7750	3.802326647	137393	109170	11861	6690	4151
ZG13_24_PBMC_gp140_IgG_B	70000	0.022375432	99974	71507	13399	45	16
ZG13_24_PBMC_gp140_IgG_A	70000	2.383507186	64100	56849	22005	15901	1355
ZG13_24_PBMC_gp140_IgG_0	70000	26.66268896	456347	434086	123347	119083	115739
ZG13_24_PBMC_mem_IgG_0	100000	25.76331385	415257	390796	124791	112353	100682

Table 2.15: IgM cells sorted, reads retained in initial QC

Sample ID	cells sorted	% reads retained	N raw reads (1)	N raw reads (2)	N joined reads	N reads primer matched	N reads w/t ORF
ZF61_4_INGLN_gp140_IgM_0	34	0.01807764	129439	127229	122091	104517	23
ZF61_4_LN_gp140_IgM_A	34	0.430322322	119015	107129	84101	39556	461
ZF61_4_LN_mem_IgM_A	15000	4.529062949	118067	91233	19254	17181	4132
ZF61_4_PBMC_gp140_IgM_A	1000	2.450521227	130296	99285	10284	3720	2433
ZF61_4_PBMC_gp140_IgM_0	1000	52.83587416	103975	100886	85005	61428	53304
ZF61_4_PBMC_mem_IgM_A	40000	1.075792788	131251	106712	40715	37959	1148
ZF61_4_PBMC_mem_IgM_0	40000	53.63846837	416788	400828	332064	239733	214998
ZF61_10_INGLN_gp140_IgM_0	1870	22.521391	79147	76434	39339	17258	17214
ZF61_10_LN_gp140_IgM_A	1870	7.00964865	140725	109549	24439	19136	7679
ZF61_10_LN_mem_IgM_A	24000	3.636905522	150122	117435	25889	21557	4271
ZF61_10_PBMC_gp140_IgM_A	335	0.011114591	105516	98969	87842	82842	11
ZF61_10_PBMC_mem_IgM_A	100000	5.939079987	130843	104629	31663	20279	6214
ZF61_10_PBMC_mem_IgM_0	100000	52.42990417	855963	827831	681478	508541	434031
ZF61_24_LN_gp140_IgM_B	5600	10.08725974	145989	116434	32254	25899	11745
ZF61_24_PBMC_gp140_IgM_A	8800	0.527262223	131716	122899	103828	30932	648
ZF61_24_PBMC_gp140_IgM_0	8800	44.2447732	205874	200218	172336	116366	88586
ZF61_24_PBMC_mem_IgM_A	100000	0.970246542	107099	86679	20855	14042	841
ZF76_4_INGLN_gp140_IgM_0	600	0.059412912	138400	136334	126834	33071	81
ZF76_4_LN_gp140_IgM_A	600	2.148417754	98172	79128	30224	25305	1700
ZF76_4_LN_mem_IgM_A	22000	9.167259958	105737	82871	24241	18152	7597
ZF76_4_PBMC_gp140_IgM_A	1900	6.037209651	132807	106854	16932	7413	6451
ZF76_4_PBMC_gp140_IgM_0	1900	53.51379949	93472	90728	77759	57463	48552
ZF76_4_PBMC_mem_IgM_A	26000	5.014683505	135048	103177	16019	11979	5174
ZF76_4_PBMC_mem_IgM_0	26000	0.116352867	109081	103994	84859	51047	121
ZF76_10_INGLN_gp140_IgM_0	4400	2.009672339	279470	268601	215527	96834	5398
ZF76_10_LN_gp140_IgM_A	4400	0.107061605	123046	112085	94350	88911	120
ZF76_10_LN_mem_IgM_A	22000	7.807399843	144139	109678	21660	17777	8563
ZF76_10_PBMC_gp140_IgM_A	280	2.471986236	147233	112177	17548	3322	2773
ZF76_10_PBMC_gp140_IgM_0	280	55.50623645	224393	215828	182209	132002	119798
ZF76_10_PBMC_mem_IgM_A	30000	0.998697351	144503	85211	46932	16607	851
ZF76_24_LN_gp140_IgM_B	17000	4.553755523	115376	84875	11227	9541	3865
ZF76_24_LN_gp140_IgM_A	17000	0.005794462	135291	120805	97818	71493	7
ZF76_24_MESLN_gp140_IgM_0	17000	36.27283463	384022	375143	338785	235368	136075
ZF76_24_PBMC_gp140_IgM_A	22000	1.496638351	144045	99356	9487	3970	1487
ZF76_24_PBMC_mem_IgM_A	100000	0.056174515	146926	106810	47140	813	60
ZG13_4_INGLN_gp140_IgM_0	500	0.042529256	159022	145782	120777	30500	62
ZG13_4_LN_gp140_IgM_A	500	2.686193768	129151	109523	60092	46481	2942
ZG13_4_LN_mem_IgM_A	32400	0.008619649	81546	58007	15905	29	5
ZG13_4_PBMC_gp140_IgM_A	1080	6.64446936	110843	88299	12344	6415	5867
ZG13_4_PBMC_gp140_IgM_0	1080	37.02056217	119161	115552	97176	53926	42778
ZG13_4_PBMC_mem_IgM_A	59200	7.055342406	142352	110512	22040	17719	7797
ZG13_4_PBMC_mem_IgM_0	59200	52.7107682	537816	516883	418865	304753	272453
ZG13_10_LN_gp140_IgM_A	3600	6.238246198	149277	111666	27174	20842	6966
ZG13_10_LN_mem_IgM_A	50000	5.619294705	134564	104070	18982	15012	5848
ZG13_10_PBMC_gp140_IgM_A	1150	2.227893923	127588	98344	21526	11018	2191
ZG13_10_PBMC_gp140_IgM_0	1150	39.57855669	456437	437022	350074	238211	172967
ZG13_10_PBMC_mem_IgM_A	200000	7.717391304	113517	90160	22737	16991	6958
ZG13_10_PBMC_mem_IgM_0	200000	51.39466952	1454324	1405315	1158894	871178	722257
ZG13_24_LN_gp140_IgM_B	1620	5.350380433	110683	86349	17148	11779	4620
ZG13_24_LN_gp140_IgM_A	1620	8.686488893	130737	101065	21311	17139	8779
ZG13_24_LN_mem_IgM_0	100000	7.576042162	118001	90509	18602	14203	6857
ZG13_24_MESLN_gp140_IgM_0	1620	47.49855615	210381	202583	166904	118045	96224
ZG13_24_PBMC_gp140_IgM_A	1500	6.065806512	245762	192291	45280	31622	11664
ZG13_24_PBMC_gp140_IgM_0	1500	49.55568653	91203	88226	72143	53090	43721
ZG13_24_PBMC_mem_IgM_A	100000	9.070920457	142108	115975	40517	14620	10520