Title:

Origin of HIV-1 envelope gp41 antibodies from terminal ileum B cells that share cross-reactivity with commensal bacteria

Authors:

Ashley M. Trama[1*], M. Anthony Moody[1], S. Munir Alam[1], Frederick H. Jaeger[1], Bradley Lockwood[1], Robert Parks[1], Krissey E. Lloyd[1], Christina Stolarchuk[1], Richard Scearce[1], Andrew Foulger[1], Dawn J. Marshall[1], John F. Whitesides[1], Thomas L. Jeffries Jr.[1], Kevin Wiehe[1], Lynn Morris[2], Bronwen Lambson[2], Kelly Soderberg[1], Kwan-Ki Hwang[1], Georgia D. Tomaras[1], Nathan Vandergrift[1], Katherine J. L. Jackson[3], Krishna M. Roskin[3], Scott D. Boyd[3], Thomas B. Kepler[4], Hua-Xin Liao[1], and Barton F. Haynes[1]

Affiliation:

[1]Duke Human Vaccine Institute, Departments of Medicine, Pediatrics, Surgery, Molecular Genetics and Microbiology, Immunology, Duke University School of Medicine and Duke and Duke Global Health Institute, Durham, NC; [2] Centre for HIV and STIs, National Institute for Communicable Diseases, Johannesburg, South Africa, [3]Stanford University, Stanford, CA; [4] Boston University, Boston, MA

To whom correspondence should be addressed: Ashley M. Trama, Duke Human Vaccine Institute, Duke University Medical Center, 2 Genome Court, Durham, NC 27710, USA. Email address: ashley.trama@dm.duke.edu Tel: +919-668-3605, Fax: 919-684-5230

Abbreviations used:

AHI, acute HIV-1 infection; BAMA, binding antibody multiplex assay; CHI, chronic HIV-1 infection; CON-S, Consensus-S gp140; EHI, early HIV-1 infection; Env, HIV-1 envelope; mAb, monoclonal antibody; UCA, unmutated common ancestor; WCL, whole cell lysate

Running title:

HIV-1 antibodies cross-react with human microbiota

Abstract

A subset of B cells in the intestine reacts with intestinal microbiota. Here we have studied in HIV-1 infection the antibody response of ileum B cells to HIV-1 envelope (Env) to determine the breadth of antibody response to HIV-1, and to probe the relationship of intestinal B cell reactivity with commensal bacteria to HIV-1 infection-induced antibody responses. Using recombinant antibody technology, we found that the dominant ileum B cell response was to Env gp41, and 82% of gp41 antibodies cross-reacted with commensal bacteria. Pyrosequencing of blood B cells revealed HIV-1 antibody clonal lineages shared between ileum and blood. Mutated IgG antibodies cross-reactive with both Env gp41 and commensal bacteria could also be isolated from the ileum of HIV-1 uninfected individuals. Thus, the antibody response to HIV-1 may be shaped by intestinal B cells stimulated by commensal bacteria to develop a pre-infection pool of memory B cells cross-reactive with HIV-1 gp41.

Highlights

- HIV-1-reactive B cells in the terminal ileum primarily target Env gp41.
- HIV-1 gp41-reactive antibodies cross- react with commensal bacteria.
- B cell clonal lineages are shared between then terminal ileum and blood.

Introduction

The plasma cell and memory B cell pools in intestine contain a normal subset of B cells reactive with intestinal commensal bacteria (Benckert et al., 2011). In acute HIV-1 infection (AHI), virus replication is prominent in the gastrointestinal tract with early depletion of CD4+ T cells (Brenchley et al., 2004; Guadalupe et al., 2003; Mehandru et al., 2006; Pope and Haase, 2003; Veazey et al., 1998; 2001) as well as early destruction of B cell germinal centers (Levesque et al., 2009). Initial plasma (Tomaras et al., 2008) and mucosal fluid (Yates et al., 2013) antibody response in AHI is targeted to HIV-1 Env gp41. The AHI gp41 antibody response is non-neutralizing and does not select viral escape mutants (Tomaras et al., 2008). Rather it is the initial autologous gp120

neutralizing antibody response that is the first Env antibody shown to select viral escape mutants (Moore et al., 2009; Richman et al., 2003; Wei et al., 2003).

Recombinant monoclonal antibodies (mAbs) isolated from blood plasmablasts and/or plasma cells (hereafter termed plasma cells) of individuals with AHI were predominantly targeted to Env gp41, and were polyreactive with both host and environmental antigens including commensal bacteria (Liao et al., 2011). These observations raised the hypothesis that a component of the peripheral blood HIV-1 Env gp41 response in blood originates from polyreactive memory B cells activated prior to transmission by environmental antigens (Liao et al., 2011).

Here we have used single B cell sorting and recombinant antibody technology to probe the plasma cell and memory B cell repertoire of the terminal ileum in early and chronic HIV-1 infection. We found that the terminal ileum plasma cell and memory B cell repertoire was comprised of predominantly polyclonally activated, non-HIV-1 reactive antibodies, and the dominant early HIV-1 B cell response in the terminal ileum was targeted to Env gp41. Remarkably, 82% of HIV-1 gp41-reactive terminal ileum antibodies cross-reacted with intestinal commensal bacterial antigens, and mutated antibodies cross-reactive with Env gp41 and intestinal commensal bacteria were isolated from HIV-1 uninfected individuals. These data demonstrated that gp41-commensal bacterial antigen cross-reactive antibodies originate in intestine, and provide evidence that the gp41 Env response in HIV-1 infection can be derived from a pre-infection memory B cell pool triggered by commensal bacteria that cross-reacts with Env.

Results

HIV-1 gp41 reactive antibodies in terminal ileum in early and chronic HIV-1 infection individuals.

We investigated the plasma cell response to HIV-1 infection within the terminal ileum of 6 early HIV-1 infection (EHI) individuals (**Table S1**). We expressed 114 mAbs from plasma cells and 140 mAbs from memory B cells recovered from terminal ileum. Of the 254 total mAbs isolated from EHI

individuals, only 5 (2.0%) reacted with gp41 and none (0.0%) with gp120 (**Fig. 1 and Table S2**). HIV-1-reactive mAbs primarily utilized heavy chain variable gene segments from V_H family 3. V_H mutation frequencies ranged from 0.0% to 10.4%, and HCDR3 lengths ranged from 11 to 25 amino acids. There were no statistical differences between the mean V_H mutation frequencies and HCDR3 lengths of the HIV-1 reactive antibodies compared to non HIV-1 reactive antibodies isolated from terminal ileum plasma cells from EHI individuals **(Fig. 1B-C**). All recombinant HIV-1 mAbs were expressed with an IgG1 backbone; their original isotypes were IgA1, IgA2 and IgG3 (**Table S2**). IgA2 and IgG3 only made up 6.7% and 5.1% of total terminal ileum mAbs isolated from EHI, respectively (**Table S3**). Four of the 5 gp41-reactive mAbs were low affinity with EC50s of >100μg/mL. DH300 had the highest apparent affinity with an EC50 of only >25μg/mL (**Fig. 1D and Table S2**). With a V_H mutation frequency of 10.4%, the heavy chain of DH300 was also the most mutated of the EHI terminal ileum HIV-1-reactive mAbs isolated (**Table S2**). These HIV-1-reactive mAbs were tested for neutralization against the easy-to-neutralize (tier 1) viruses, ADA, MN, and SF162 and the difficult-to-neutralize virus (tier 2) DU156 and all were non-neutralizing when assayed in the TZM-bl pseudovirus infection assay. Thus, the plasma cell and memory B cell response in EHI was polyclonal, and the HIV-1-reactive mAbs were targeted to Env gp41 and were non-neutralizing.

We next characterized the plasma cell response in the terminal ileum of 3 chronically HIV-1 infected (CHI) individuals, 038-7, 004-0, and 071-8 (**Table S1**). From these individuals we expressed 158 mAbs from terminal ileum plasma cells; 14 (8.8%) of these mAbs reacted with HIV-1 antigens, 9 (5.7%) with Env gp41, 4 (2.5%) with HIV-1 capsid protein (p24), and 1 (0.6%) with Env gp120 (**Fig. 2 and Table S2**). Similar to the gp41 antibodies from EHI individuals, the HIV-1-reactive mAbs isolated from CHI individual 071-8 predominantly used V_H family 3 gene segments; mutation frequencies ranged from 1.3% - 7.0%, and the majority of the original mAbs were of IgA isotype (**Table S2**). In contrast, 8 of the 9 (89%) HIV-1-reactive mAbs from plasma cells of CHI individual 004-0 used V_H1-69; all these V_H1-69 antibodies were originally IgG1 (**Table S2**). Both individuals, 071-8 and 004-0, have 3 genomic copies of V_H1-69 as determined by digital PCR (**Table S4**). The number of B cells

utilizing the gene segment V_H1-69 has been reported to be proportional to the gene copy number of certain V_H1-69 alleles (Sasso et al., 1996). However, this was not seen at the terminal ileum single B cell level in our study, where both 071-8 and 004-0 had three genomic copies of V_H1-69, yet 004-0 predominately used V_H1-69 to respond HIV-1 infection (**Table S2**).

The V_H mutation frequencies of antibodies from individual 004-0 ranged from 3.3% to 11.9%, and HCDR3 lengths ranged from 12 to 23 amino acids (**Table S2**). There were no statistical differences between the mean V_H mutation frequencies and HCDR3 lengths of the HIV-1-reactive mAbs compared to non-HIV-1-reactive mAbs isolated from terminal ileum plasma cells from CHI individuals (**Fig. 2B-C**). The estimated EC50s for gp41 binding of these antibodies ranged from <0.1µg/mL to >100µg/mL (**Table S2).** DH306 and DH309 had high apparent affinities to gp41 (EC50s of <0.1µg/mL). DH310, DH311, DH312 and DH314 had high affinities to Gag p24 (EC50s of <1µg/mL -<0.1) (**Fig. 2D and Table S2**). These HIV-1-reactive mAbs were also tested for neutralization against viruses, ADA, MN, SF162, and DU156 in TZM-bl assays and were non-neutralizing.

Because the HIV-1 antigen-specific terminal ileum mAbs account for such a small proportion of the plasma cell and memory B cell response as measured by single cell sorting, we next quantified the Env-specific memory B cell pool by an alternative method. We assayed paired PBMC and terminal ileum samples from four CHI individuals (078-2, 067-8, 072-3 and 076-4) (**Table S1**) by flow cytometry analysis of HIV-1 Env-specific memory B cells with a fluorescent-labeled consensus group M gp140 Env, consensus-S (CON-S) previously shown to bind to clade B-reactive antibodies (Liao et al., 2006; Tomaras et al., 2008). We found means of 0.04 ± 0.02%, 0.26 ± 0.24%, and 0.20 ± 0.29% IgM, IgG and IgA CON-S gp140-reactive memory B cells, respectively, in blood (**Table S5**). The mean percentage of IgM, IgG and IgA CON-S gp140 reactive memory B cells in terminal ileum were 0.01 ± 0.02%, 0.05 ± 0.1%, and 0.03 ± 0.06%, respectively (**Table S5**). Thus, by flow cytometry with a fluorophor-labeled Env, there was also a relative dearth of HIV-1 Env-reactive memory B cells in terminal ileum compared to blood in CHI.

Terminal Ileum HIV-1-reactive antibodies were cross-reactive with commensal bacterial antigens.

We tested HIV-1-reactive mAbs isolated from terminal ileum of EHI for reactivity to antigens in anaerobic commensal bacteria whole cell lysates (WCL) by surface plasmon resonance (SPR), and to both anaerobic and aerobic commensal bacteria WCLs by western blot analysis. Of the 6 gp41-reactive antibodies from EHI, all were reactive to anaerobic intestinal commensal bacteria by both SPR and western blot (**Fig. 3A-C, S1, and Table S6**). Similarly, 11 of the 16 HIV-1-reactive mAbs isolated from the terminal ileum of CHI cross-reacted with anaerobic commensal bacteria by SPR and western blot (**Fig. 3A-B, 3E, S1 and Table S6**). Antibody reactivity to aerobic and anaerobic commensal bacteria was also tested in Luminex-based binding antibody multiplex assays (BAMA) (**Fig. S2A**). Fourteen of 17 antibodies positive in western blot and SPR also could be confirmed in BAMA (**Fig. 3D, 3F, S1, S2, and Table S6**).

To determine if HIV-1 and commensal bacteria cross-reactive mAbs are polyreactive/ autotreactive, we tested the HIV-1-reactive mAbs in Luminex AtheNA ANA II and HEp-2 immunofluorescence ANA assays. Four of the 6 gp41- commensal bacteria cross-reactive mAbs from EHI terminal ileum were not reactive with additional antigens by these assays (**Fig. S1B and Table S6**). Six the 11 HIV-1 and commensal bacteria cross-reactive mAbs isolated from CHI terminal ileum plasma cells were not polyreactive/ autoreactive by AtheNA ANA II assays and HEp-2 ANA staining (**Fig. S2B and Table S6**).

In addition to HIV-reactive mAbs, we produced and purified 19 terminal ileum mAbs that did not bind HIV-1 epitopes by ELISA or BAMA (**Table S2**). Of these, 4 antibodies (21%) were reactive with intestinal commensal bacterial WCLs by both western blot and BAMA (**Fig. S1A, S3, and Table S6**). Three of these 4 antibodies were not reactive in AtheNA ANA II or HEp-2 ANA assays (**Table S6).** Therefore, not all commensal bacteria-reactive antibodies from intestine were cross-reactive with gp41.

Affinity maturation of commensal bacteria cross-reactive antibodies to autologous envelope

To determine if HIV-1 gp41-reactive antibodies that were cross-reactive with commensal bacteria underwent affinity maturation to gp41, we inferred the heavy and light chain unmutated common ancestors (UCA) of 5 gp41-reactive mAbs, DH306, DH309, DH308, DH305, and DH319 and produced their UCAs, termed DH306 UCA, DH309 UCA, DH308 UCA, DH305 UCA, and DH319 UCA, respectively. For mAbs isolated from 004-0, DH306, DH309, and DH308, we determined UCA and mature antibody affinities to autologous HIV-1 004-0 gp140 and heterologous HIV-1 MN gp41, as well as relative binding to commensal bacterial antigens. Affinity for the autologous Env increased from undetectable binding to 0.62 nM when comparing the UCA DH306 UCA and the mature antibody DH306, and similarly increased from 4.44 nM to 0.34 nM for DH309 UCA and DH309 (**Fig. 4A,B and D**). The mature antibody DH306 also had a greater reactivity to commensal bacteria compared to its UCA (**Fig. 4A**). Binding to the 004-0 T/F gp140 was undetectable for DH308 UCA and DH308, however affinity to MN gp41 increased from 9.97nM to 0.41nM (**Fig 4C-D**). In contrast, DH305 UCA and DH319 UCA had high affinities of 3.55nM and 0.41nM to MN gp41, respectively, and affinity did not increase upon accumulation of mutations in the mature mAbs, DH305 and DH319 (**Fig. 4E-F**). Therefore, in three commensal bacterial antigen cross-reactive gp41 clonal lineages, affinity maturation to gp41 could be demonstrated from UCAs to mature antibodies.

HIV-1 gp41 commensal bacterial cross-reactive antibodies isolated from the terminal ileum of uninfected individuals.

If pre-infection terminal ileum antibodies cross-reactive with intestinal commensal bacteria and gp41 are responsible for the initial antibody response to HIV-1 Env gp41 following HIV-1 infection, mutated gp41 and gut flora cross-reactive antibodies should exist in the terminal ileum of uninfected individuals. To investigate this hypothesis, we sorted single plasma cells and memory B cells from 3 HIV-1 uninfected individuals (**Table S1**), and identified two low affinity gp41-reactive antibodies, DH366 and DH367, both of which also reacted with intestinal commensal bacteria (**Fig. 5, S1B, and**

Table S2). Both antibodies used V_H gene segments from family 3 and were class-switched to IgG; the V_H mutation frequencies of these antibodies were 5.2% and 9.7% (**Table S2**). Therefore, commensal bacteria-reactive mutated B cells that are cross-reactive with Env gp41 can be found in the intestinal B cell repertoire of HIV-1 uninfected individuals, supporting the notion that the initial gp41 antibody response to HIV-1 derived from preexisting commensal bacterial cross-reactive memory B cells.

E.coli **RNA polymerase is one intestinal bacterial antigen cross-reactive with HIV-1 gp41 antibodies**

To identify antigens in commensal bacteria cross-reactive with gp41 mAbs, we used the AHI blood-derived HIV-1 gp41, gut bacterial WCL-reactive antibody 558_2 previously reported to bind to a ~520kDa band of both aerobic and anaerobic commensal bacteria WCLs (Liao et al., 2011) (**Fig. 6A**). The large molecular weight fraction of bacterial WCL was isolated by size exclusion chromatography (SEC) (**Fig. 6B**), and isoelectric zoom fractionation showed that the protein reactive with mAb558_2 migrated to the gel compartment with pH7-10 (**Fig. S4A**). *E.coli* RNA polymerase subunits β, β', and α were identified by liquid chromatography-tandem mass spectrometry (LC/MS/MS) of the 520kDa excised bands from two lanes of the SEC-enriched >500kD fraction analyzed on a NativePAGE gel (**Fig. 6B-C and S4B-D**). We determined that mAb558_2 binding was specific for the core enzyme of *E.coli* RNA polymerase (**Fig. 6D and S4E-F**). By western blot, we mapped the specificity of mAb558_2 to the 37kDa α subunit of recombinant *E.coli* RNA polymerase (**Fig. 6D**).

When Env gp41and intestinal bacteria cross-reactive mAbs isolated from EHI and CHI terminal ileum were evaluated in BAMA for binding to recombinant *E.coli* RNA polymerase, we found that 2 of 14 (14.3%) Env gp41 and intestinal commensal bacteria cross-reactive EHI and CHI antibodies also reacted with *E.coli* RNA polymerase (**Fig. 6E-G**) Moreover, the gp41-commensal bacterial cross-reactive antibody DH367 isolated from the terminal ileum of an uninfected individual also reacted with recombinant *E.coli* RNA polymerase by BAMA (**Fig. 6H**).

Terminal ileum HIV-1-reactive antibody clonal lineage members shared by terminal ileum and peripheral blood compartments.

We next asked if HIV-1 and commensal bacteria cross-reactive B cells re-circulate in the terminal ileum and peripheral blood. We studied paired blood samples of three of the individuals (042-8, 004-0, and 071-8) from whom we had isolated terminal ileum plasma cell and memory B cell mAbs (**Table S1**). We sorted single plasma cells and memory B cells from PBMC and identified 13 antibodies with HIV-1 reactivity (**Fig. S5, Table S2**). By single cell PCR, we were able to identify 4 clonal lineages within the terminal ileum, and 7 clonal lineages within the blood (**Table S7**). However, by these methods we were unable to identify any clonal lineages with members shared between the terminal ileum and blood.

We next conducted pyrosequencing of genomic DNA isolated from PBMCs taken at the same time as terminal ileum samples and searched the sequences for V_H members clonally-related to the 149 terminal ileum $V_HD_HJ_H$ sequences isolated from these same two individuals by single cell sorting. By this method we identified a total of 18 clonal lineages that had members in both terminal ileum and blood compartments from chronically infected individuals 004-0 and 071-8 (**Fig. 7 and S6, and Table S7**). Thus, 12% of terminal ileum B cells isolated by single cell PCR had cross-compartment clonal lineage members in the blood. Of these 18 cross-compartment clonal lineages, we determined that two clonal lineages were cross-reactive with Env gp41 and intestinal commensal bacteria, and one lineage was cross-reactive with HIV-1 Gag p24 and commensal bacteria (**Fig. 1D, 2D, 7, and S6, and Table S2**).

To determine if the cross-compartmentalization of B cell clonal lineages identified in 004-0 and 071-8 was due to contamination of the terminal ileum tissue biopsies with blood B cells trafficking through the ileum vasculature without entering the tissue, we performed quantitative image analysis of B cells in the terminal ileum of HIV-1 infected individuals and found that of the 12 terminal ileum biopsies studied, only 0.2% of the CD20+ cells within the tissue samples were found within blood

vessels (**Fig. S7**). Thus, blood contamination of the biopsy could not explain the 12% of terminal ileum B cells isolated by single cell PCR as contaminating B cells from the blood compartment.

Discussion

In this study we have demonstrated that the dominant plasma cell antibody population to HIV-1 in both EHI and CHI in the terminal ileum was non-neutralizing, directed to Env gp41 and was cross-reactive with intestinal commensal bacterial antigens. One such bacterial antigen identified was the 37,000 MW subunit of *E.coli* RNA polymerase. Similar specificities of gp41-commensal bacteria cross-reactive mutated antibodies could be isolated from HIV-1 uninfected individuals. Moreover, we demonstrated sharing of terminal ileum clonal lineage members with the blood compartment, providing support for the hypothesis that blood B cells cross-reactive with intestinal bacteria and gp41 are derived from the intestinal tract.

The preponderance of gp41 antibodies in terminal ileum plasma cell and memory B cell pools now potentially explains the mechanism of induction of gp41 antibody immunodominance in plasma and mucosal fluid studies (Tomaras et al., 2008; Yates et al., 2013). Liao et al. showed both a predominance of blood gp41 antibodies from HIV-1 plasma cell-derived mAbs from AHI, and found them to be a minority of the plasma cell pool 17-46 days after HIV-1 transmission (Liao et al., 2011). The polyclonal pool of non-HIV-1-reactive B cells is likely due to the massive cytokine storm that occurs early on after HIV-1 transmission (Stacey et al., 2009), and prompted us to ask if the plasma cell and memory B cell pools in terminal ileum would be a location of a more robust HIV-1 Env antibody response. Instead, we found in both EHI and CHI that terminal ileum contained primarily non-HIV-1-reactive polyclonal plasma and memory B cells, and the few HIV-1-reactive B cells that were present were targeted to Env gp41.

Host-specific bacterial colonization of the gastrointestinal tract is required for normal development of the intestinal immune system (Chung et al., 2012; Erturk-Hasdemir and Kasper, 2013; Hooper et al., 2012). Germ-free mice have numerous immunological deficiencies including; small

Peyer's patches and mesenteric lymph nodes, reduced secretory IgA, fewer plasma cells, CD4+ T cells and CD8+ T cells, and diminished antimicrobial peptide production (Erturk-Hasdemir and Kasper, 2013; Hooper et al., 2012; Round and Mazmanian, 2009). Re-colonization of germ-free mice with host-specific commensal bacteria ameliorates these defects (Chung et al., 2012; Smith et al., 2007). The presence of intestinal commensal bacteria induces immune maturation that is not only required for gut homeostasis, but helps generate a pool of mature adaptive immune cells prepared to protect the host from infections. The preHIV-1 infection presence of B cells within the intestine cross-reactive with both bacterial antigens and HIV-1 gp41 is evidence of molecular mimicry between HIV-1 antigens and bacteria antigens, and suggests an explanation for why the initial antibody response to HIV-1 acute infection in the plasma and mucosal fluids is to gp41 (Fujinami et al., 1983; Liao et al., 2013; Oldstone, 1998; Srinivasappa et al., 1986; Tomaras et al., 2008; Yates et al., 2013).

Isolation of mutated gp41-gut flora cross-reactive antibodies from terminal ileum HIV-1-uninfected individuals directly suggests that commensal or pathogenic bacteria or other cross-reactive environmental antigens can trigger gp41 cross-reactive responses before HIV-1 infection. These data provide evidence in support of the hypothesis that the dominant HIV-1 gp41 antibody response after HIV-1 transmission is mediated by previously- activated memory B cells that are present before HIV-1 infection and cross-reactive with intestinal bacteria. Once HIV-1 infection occurs, then gp41 would begin to trigger the previously activated commensal bacterial-driven lineages toward affinity maturation to gp41-specific antibodies. A critical test of this notion would be to demonstrate that reactivity in commensal bacteria-gp41 lineage begins with a gut flora-reactive UCA followed by acquisition of gp41 reactivity upon affinity maturation. In the present study, we provide three examples of gp41- reactive antibodies, DH306, DH308, and DH309, that showed affinity maturation to autologous and/or heterologous Env (**Fig. 4**). In the case of antibody DH306, reactivity of the UCA with gp41 but not the T/F Env gp140 may well be an example of cross-reactive stimulation of the UCA by an environmental gp41- cross-reactive antigen before transmission, that gave rise to the affinity mature antibody that, after infection, reacted with the autologous T/F Env. DH308 mature and UCA

bound to MN gp41 with nanomolar affinity, but did not bind to the autologous T/F gp140 (**Fig. 4**). It is important to note that antibody DH308 was isolated from 004-0 individual 3 years into infection. Thus it is likely that a T/F Env variant selected by antibodies over time initiated the DH308 lineage, give the high level of affinity mature to gp41 from ~10nM in DH308 UCA to 0.4M in the mature antibody DH308 (**Fig. 4D**). DH305 UCA and DH319 UCA are examples of naturally paired, unmutated, $V_HD_HJ_H$ and V_LJ_L with high affinities for viral antigens, and B cell clonal lineages reaching an affinity ceiling prior to accumulation of the mutations found in the mature mAbs as previously described (Batista and Neuberger, 1998; Poulsen et al., 2007; Roost et al., 1995). We have also previously shown that in a reconstructed blood gp41 clonal lineage, the UCA and the first intermediate antibody in the lineage were commensal bacteria-reactive but *not* gp41-reactive (Liao et al., 2011). Instead, gp41 reactivity only occurred later in clonal lineage development, and after gp41 reactivity occurred, there was affinity maturation to HIV-1 env gp41 in the clonal lineage. The presence of CD4+ memory T cells cross-reactive with both HIV-1 antigens and microbial peptides in uninfected adults (Su et al., 2013, Campion et al.,2014 in press) and the 5.2% and 11.9% V_H mutation frequencies of DH306 and DH309, suggested that the affinity maturation of gp41-commensal bacteria cross-reactive B cells to gp41 is T cell- dependent.

We now directly demonstrate the intestinal tract origin for commensal bacteria-gp41 cross-reactive antibodies found in the blood. Moreover, we demonstrated that 21% of commensal bacteria-reactive B cells were not gp41-reactive, adding additional support that, in HIV-1-infected individuals, the gp41-reactive plasma cells and memory B cells represented a response to HIV-1. The proportion of these control non-HIV-1 reactive antibodies isolated from the terminal ileum of HIV-1 infected individuals that reacted with gut flora (21%) is greater than the ~12% of plasma cells from the terminal ileum of normal individuals determined to be reactive with specific gut flora by Beckert et al. (2011). Microbial translocation that occurs in HIV-1 infection may account for this higher level of commensal bacteria reactive terminal ileum B cells in our study (Brenchley et al., 2006).

A critical test of the hypothesis that blood gp41-commensal bacteria- reactive B cells arise in the intestine was to determine if commensal bacteria-gp41 clonal lineages shared members with blood B cells. Indeed we have now found evidence for three such intestinal commensal bacteria-gp41 clonal lineages shared both by terminal ileum and peripheral blood compartments (**Fig. 7 and S6, Table S7**).

In summary, these data provide evidence for the hypothesis that the post-infection B cell response to HIV-1 is shaped by the pre-infection B cell repertoire to environmental antigens. Env gp41 antibodies cross-react with human intestinal commensal bacteria suggesting commensal bacteria play critical roles in shaping the pre-infection response to HIV-1, and demonstrate a major role for the memory B cell pool in contributing to the initial antibody response to HIV-1. These data also raise the hypothesis that the human B cell response to a wide variety of other infectious agents may similarly be affected by cross-reactivity to environmental antigens.

Experimental Procedures

Study subjects*.* Terminal ileum, blood and bone marrow samples were collected from 6 EHI individuals 47 to 200 days after transmission, 10 CHI individuals greater than 200 days after transmission as estimated from patient history and Fiebig classification (Fiebig et al., 2003), and 3 HIV-1 uninfected individuals (**Table S1**). All individuals studied were from the United States. Table S1 shows the clinical characteristics of the individuals studied. All work related to human subjects was with trial participant's informed consent and in compliance with Institutional Review Board protocols approved by Duke University Medical Center and the University of North Carolina Medical Center.

Flow Cytometry Analysis of Terminal Ileum and Blood B cells*.* Terminal ileum mononuclear cells were isolated from gut tissues and a single cell suspension was formed by passing cells through 100 μM cell strainer (Fisher Scientific) and labeled with a panel of fluorochrome conjugated mAbs to label distinct B cell subsets in blood and terminal ileum. A detailed protocol is included in extended experimental procedures.

PCR amplification of plasma cell and memory B cell immunoglobulin V_H and V_L genes. The Ig $V_HD_HJ_H$ and V_LJ_L genes of the sorted plasma cell and memory B cells were amplified by RT and nested PCR using the method as reported (Liao et al., 2009; Tiller et al., 2008; Wardemann et al., 2003; Wrammert et al., 2008). The PCR products amplified by this method contain sufficient coding region sequences for the constant regions of either heavy- or light-chain genes to allow for the identification of the IgH subclass and types of light chains (Liao et al., 2009).

Sequencing, sequence annotation, quality control, and data management of Ig $V_HD_HJ_H$ and V_LJ_L sequences was completed as previously described (Liao et al., 2011)**.** A detailed explanation is included in extended experimental procedures.

High-throughput DNA sequencing of Ig V(D)J gene segments. Using the QIAamp DNA mini kit, genomic DNA samples were isolated from aliquots of PBMCs of individuals from two of the individuals that we also had terminal ileum samples from. As described by Boyd et al. (2009), heavy chain V gene segment family specific primers and a consensus J segment primer were multiplexed and used to amplify the rearranged $V_HD_HJ_H$ Ig heavy chain sequences. Six barcoded V(D)J libraries from independent aliquots of DNA template from each sample were amplified, pooled and sequenced using the 454 platform with Titanium chemistry (Roche); (Boyd et al., 2009).

Identification of clone members and inference of UCA. Clonal relatedness of $V_HD_HJ_H$ and V_LJ_L sequences was determined as described (Kepler; 2013; Liao et al., 2013).

Expression of $V_HD_HJ_H$ and V_LJ_L as full-length IgG1 recombinant mAb. PCR was used to assemble linear full-length Ig heavy- and light-chain gene expression cassettes using the Ig $V_HD_HJ_H$ and V_L gene pairs as previously described (Liao et al., 2009; 2011; 2013). We determined that in most cases, isolation of one heavy chain and two light chains is an artifact of sorting two B cells into one well and the heavy chain and light chain pairing could not be precisely determined. These antibodies were not included in statistical analysis of frequencies of total antibodies, but such heavy chain sequences were used for analysis of clonal relationships and $V_HD_HJ_H$ and V_L pairs that reacted with

HIV-1 antigens were included in the total list of HIV-1 antibodies. Additional explanation is included in extended experimental procedures.

Assays for antibody reactivity. The recombinant mAb expressed in small-scale and large-scale transfections were assayed for antibody reactivity to HIV-1 antigens and a panel of non-HIV-1 antigens by ELISA and BAMA as previously described (Liao et al., 2009; 2011). Antibodies produced in large scale and protein A purified were titrated at concentrations ranging from 100μg/mL to 0.046 μg/mL at threefold dilutions for ELISA assays. Positivity cutoffs for reactivity were set at threefold above background and an OD of 0.130 at 100 μg/mL. An antigen list is included in extended experimental procedures.

The apparent affinity of HIV-1 reactive antibodies was calculated in molar concentration from EC50 values using a four parametric sigmoid curve fitting analysis. Antibodies were titrated by three-fold dilutions at concentrations ranging from 100μg/mL to 0.046 μg/mL titrations. Antibodies were considered to have high affinity if the EC50 was less than <1μg/mL, mid-range affinity if the EC50 ranged between 1-50μg/mL and low affinity if the EC50 was >50μg/mL.

As previously described (Tomaras et al., 2008), BAMA assays are conducted with carboxylated fluorescent beads (Luminex Corp, Austin, TX) covalently coupled to small quantities (25μg) of antigen and are incubated with antibody from small-scale transfection or after column purification and binding is detected with biotin labeled mouse anti-human IgG (Southern biotech, Birmingham, AL). An antigen list is included in extended experimental procedures. Due to limited supply of commensal bacteria WCL from each preparation, we used BAMA to determine commensal bacteria reactivity for non-HIV-1 reactive terminal ileum antibodies and HIV-1 reactive blood antibodies. We utilized an operational definition of aerobic and anaerobic commensal bacteria reactivity by taking the average plus 3*STDEV and at least 100 MFI of the lowest tertile of antibodies that we tested. Antibodies were screened in duplicate for reactivity at 100μg/mL and antibodies with positive reactivity were titrated by twofold dilutions ranging from 100μg to 3.1μg/mL.

For indirect immunofluorescence on HEp-2 cells, all antibodies grown in large scale were assayed for reactivity to HEp-2 cells at 50 μg/ml and 25 μg/mL (Inverness Medical Professional Diagnostics) by indirect immunofluorescence staining (Haynes et al., 2005). Antibody reactivity to autoantigens was also determined by antibody multiplex AtheNA Multi-Lyte ANA II test (Wampole Laboratories, Princeton, NJ) (Haynes et al., 2005). Antibodies were studied in a dose dilution starting at 50 μg/ml and determined to be reactive when binding antibody multiplex assay scores were 225 MFI units or greater (Haynes et al., 2005).

For Western blot analysis of commensal bacteria reactivity 100 μg of both aerobic and anaerobic lysates were run on 4-12% Tris-Bis SDS-PAGE (Life Technologies, Carlesbad, CA) for 1 hour 29 min at 150 volts in both reduced and non-reduced conditions. NuPage sample reducing agent at 1X was used for reducing conditions (Life Technologies, Carlsbad, CA). Antigens were transferred to nitrocellulose using Life Technologies iBlot Gel Transfer system (Carlesbad, CA). Antibody binding was tested at 20 μg/ml for all antibodies and the anti-Human IgG (whole molecule)-Alkaline Phosphatase, antibody produced in goat (Sigma, St. Louis, MO) was used at a 1:5000 dilution. Detection occurred directly on the nitrocellulose using Western Blue (Promega, Madison, WI).

Surface Plasmon Resonance (SPR). To confirm the reactivity to gp41 of the antibodies isolated from the terminal ileum of uninfected individuals SPR binding assays were performed on a BIAcore 3000 (GE Healthcare) maintained at 25˚C. Recombinant catalase (GE Healthcare) and HIV-1 gp41 MN were immobilized on a CM5 sensor chip by standard amine coupling, as previously described (Alam et al., 2009; 2008).

To determine the reactivity of terminal ileum HIV-1-reactive mAbs to anaerobic intestinal commensal bacteria, SPR binding assays were performed on a Biacore 4000 (BIAcore, Inc.). HIV-1 reactive mAbs were immobilized on CM5 sensor chips by standard amine coupling and reactivity was determined by double reference subtraction. Response generated by nonspecific binding of anaerobic commensal bacteria WCL to control antibody palivizumab (anti-RSV IgG1 mAb) (Johnson et al., 1997)

was subtracted from signal generated by antigen binding to HIV-1-reactive mAbs. The positivity cutoff was calculated as three times the response generated by antigen binding to a second negative control antibody Ab1248 (anti-Influenza/ Hemagglutinin). Rate constants were measured using 1:1 Langmuir equation. Glycine-HCL pH2.0 was used as the regeneration buffer. The commensal bacteria whole cell lysate is heterogeneous and individual proteins are in unknown concentrations. Therefore, accurate on-rates (ka) and apparent affinities (Kd) could not be calculated. The log avidity score (response unit/ kd) provides a method for measuring qualitative differences in antibody binding to WCL antigens and compare the relative affinity of the antibodies (greater or lower affinity) in the absence of Kd calculations (Flynn et al., 2011; Haynes et al., 2012; Lynch et al., 2014).

To determine the affinity of mature mAbs and their respective UCAs to autoglogous Env, 040 gp140 (Bar et al., 2012; Liao et al., 2013), from individual 004-0 (**Table S1**) and MN gp41, SPR binding titrations were performed using a BIAcore 3000 maintained at 25˚C. Antibodies were captured on a CM5 sensor chip coupled with anti-human Fc antibody. Rate constants were measured using global curve fitting to binding curves obtained from Env titrations.

Preparation of Intestinal Anaerobic and Aerobic Commensal Bacteria Lysates. Two separate preparations of bacteria were inoculated from stool specimens from 4-5 individuals and grown on agar plates under anaerobic and aerobic conditions at 30˚C. For each preparation the individual stool samples were pooled, but aerobic and anaerobic extracts were prepared separately. A detailed protocol is included in extended experimental procedures.

Identification of gp41 mAb reactive protein in intestinal bacterial lysate. Anaerobe gut lysate (total protein ~4mg) was fractionated on a Superdex S200 (GE Healthcare) size exclusion column and protein fractions with molecular size >500 kDa were pooled and concentrated. SPR binding and western blot analysis (~520 kDa band) confirmed that the high molecular weight fraction from size exclusion chromatography was gp41 mAb276-reactive. The size enriched and mAb276-reactive fraction comprised about 10-20% of the total gut lysate proteins. About 180 µg of size fractionated lysate protein was loaded on two adjacent lanes of a blue native gel and ~520 kDa bands that blotted

with Mab 276 was cut out of the coomassie stained blue native gel. The cut out protein band was subjected to trypsin and chymotrypsin digestion and protein identification performed by LC-MS/MS.

IgG V_H1-69 copy number assay. Primers and a probe designed for V_H1-69 gene segments were used in the QX100 Droplet Digital PCR system by Bio-Rad. A more detailed protocol is included in extended experimental procedures.

Statistical Analysis. All analysis datasets were compiled and completed with SAS v9.2 (SAS Institute Inc., Cary, NC). To compare V_H mutation frequency and HCDR3 lengths, a Mixed Model was performed to account for multiple observations taken from individual patients.

Quantitative Image Analysis. Quantitative image analysis was performed on terminal ileum tissue B cells and blood vessels as described (Levesque et al., 2009).

Supplemental Information

Supplemental information includes extended experimental procedures, 7 figures, and 7 tables.

References

Alam, S.M., Morelli, M., Dennison, S.M., Liao, H.X., Zhang, R., Xia, S.M., Rits-Volloch, S., Sun, L., Harrison, S.C., Haynes, B.F., et al. (2009). Role of HIV membrane in neutralization by two broadly neutralizing antibodies. Proc. Natl. Acad. Sci. U.S.a. *106*, 20234–20239.

Alam, S.M., Scearce, R.M., Parks, R.J., Plonk, K., Plonk, S.G., Sutherland, L.L., Gorny, M.K., Zolla-Pazner, S., Vanleeuwen, S., Moody, M.A., et al. (2008). Human immunodeficiency virus type 1 gp41 antibodies that mask membrane proximal region epitopes: antibody binding kinetics, induction, and potential for regulation in acute infection. J. Virol. *82*, 115–125.

Bar, K.J., Tsao, C.-Y., Iyer, S.S., Decker, J.M., Yang, Y., Bonsignori, M., Chen, X., Hwang, K.-K., Montefiori, D.C., Liao, H.-X., et al. (2012). Early low-titer neutralizing antibodies impede HIV-1 replication and select for virus escape. PLoS Pathog. *8*, e1002721.

Batista, F.D., and Neuberger, M.S. (1998). Affinity dependence of the B cell response to antigen: a threshold, a ceiling, and the importance of off-rate. Immunity *8*, 751–759.

Benckert, J., Schmolka, N., Kreschel, C., Zoller, M.J., Sturm, A., Wiedenmann, B., and Wardemann, H. (2011). The majority of intestinal IgA+ and IgG+ plasmablasts in the human gut are antigen-specific. J. Clin. Invest. *121*, 1946–1955.

Boyd, S.D., Marshall, E.L., Merker, J.D., Maniar, J.M., Zhang, L.N., Sahaf, B., Jones, C.D., Simen, B.B., Hanczaruk, B., Nguyen, K.D., et al. (2009). Measurement and clinical monitoring of human

lymphocyte clonality by massively parallel VDJ pyrosequencing. Sci Transl Med *1*, 12ra23.

Brenchley, J.M., Price, D.A., Schacker, T.W., Asher, T.E., Silvestri, G., Rao, S., Kazzaz, Z., Bornstein, E., Lambotte, O., Altmann, D., et al. (2006). Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med *12*, 1365–1371.

Brenchley, J.M., Schacker, T.W., Ruff, L.E., Price, D.A., Taylor, J.H., Beilman, G.J., Nguyen, P.L., Khoruts, A., Larson, M., Haase, A.T., et al. (2004). CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J. Exp. Med. *200*, 749–759.

Campion, S.L., Brodie, T.M., Fischer, W., Korber, B.T., Rossetti, A., Goonetilleke, N., McMichael, A.J., and Sallusto, F. (2014). Proteome wide analysis of HIV specific naïve and memory CD4+ T cells in unexposed blood donors. J. Exp. Med. In press

Chung, H., Pamp, S.J., Hill, J.A., Surana, N.K., Edelman, S.M., Troy, E.B., Reading, N.C., Villablanca, E.J., Wang, S., Mora, J.R., et al. (2012). Gut immune maturation depends on colonization with a host-specific microbiota. Cell *149*, 1578–1593.

Erturk-Hasdemir, D., and Kasper, D.L. (2013). Resident commensals shaping immunity. Curr. Opin. Immunol. *25*, 450–455.

Fiebig, E.W., Wright, D.J., Rawal, B.D., Garrett, P.E., Schumacher, R.T., Peddada, L., Heldebrant, C., Smith, R., Conrad, A., Kleinman, S.H., et al. (2003). Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. Aids *17*, 1871–1879.

Flynn, B.J., Kastenmüller, K., Wille-Reece, U., Tomaras, G.D., Alam, M., Lindsay, R.W., Salazar, A.M., Perdiguero, B., Gomez, C.E., Wagner, R., et al. (2011). Immunization with HIV Gag targeted to dendritic cells followed by recombinant New York vaccinia virus induces robust T-cell immunity in nonhuman primates. Proc. Natl. Acad. Sci. U.S.a. *108*, 7131–7136.

Fujinami, R.S., Oldstone, M.B., Wroblewska, Z., Frankel, M.E., and Koprowski, H. (1983). Molecular mimicry in virus infection: crossreaction of measles virus phosphoprotein or of herpes simplex virus protein with human intermediate filaments. Proc. Natl. Acad. Sci. U.S.a. *80*, 2346–2350.

Guadalupe, M., Reay, E., Sankaran, S., Prindiville, T., Flamm, J., McNeil, A., and Dandekar, S. (2003). Severe CD4+ T-Cell Depletion in Gut Lymphoid Tissue during Primary Human Immunodeficiency Virus Type 1 Infection and Substantial Delay in Restoration following Highly Active Antiretroviral Therapy. J. Virol. *77*, 11708–11717.

Haynes, B.F., Fleming, J., St Clair, E.W., Katinger, H., Stiegler, G., Kunert, R., Robinson, J., Scearce, R.M., Plonk, K., Staats, H.F., et al. (2005). Cardiolipin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies. Science *308*, 1906–1908.

Haynes, B.F., Gilbert, P.B., McElrath, M.J., Zolla-Pazner, S., Tomaras, G.D., Alam, S.M., Evans, D.T., Montefiori, D.C., Karnasuta, C., Sutthent, R., et al. (2012). Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N. Engl. J. Med. *366*, 1275–1286.

Hooper, L.V., Littman, D.R., and Macpherson, A.J. (2012). Interactions between the microbiota and the immune system. Science *336*, 1268–1273.

Johnson, S., Oliver, C., Prince, G.A., Hemming, V.G., Pfarr, D.S., Wang, S.C., Dormitzer, M., O'Grady, J., Koenig, S., Tamura, J.K., et al. (1997). Development of a humanized monoclonal antibody (MEDI-493) with potent in vitro and in vivo activity against respiratory syncytial virus. J.

Infect. Dis. *176*, 1215–1224.

Kepler, T.B. Statistical methods for the analysis of B-cell repertoire dynamics and affinity maturation. Front Immunol.

Kepler, T.B. (2013). Reconstructing a B-cell clonal lineage. I. Statistical inference of unobserved ancestors. F1000Res *2*, 103.

Levesque, M.C., Moody, M.A., Hwang, K.-K., Marshall, D.J., Whitesides, J.F., Amos, J.D., Gurley, T.C., Allgood, S., Haynes, B.B., Vandergrift, N.A., et al. (2009). Polyclonal B cell differentiation and loss of gastrointestinal tract germinal centers in the earliest stages of HIV-1 infection. PLoS Med. *6*, e1000107.

Liao, H.-X., Chen, X., Munshaw, S., Zhang, R., Marshall, D.J., Vandergrift, N., Whitesides, J.F., Lu, X., Yu, J.-S., Hwang, K.-K., et al. (2011). Initial antibodies binding to HIV-1 gp41 in acutely infected subjects are polyreactive and highly mutated. Journal of Experimental Medicine *208*, 2237–2249.

Liao, H.-X., Levesque, M.C., Nagel, A., Dixon, A., Zhang, R., Walter, E., Parks, R., Whitesides, J., Marshall, D.J., Hwang, K.-K., et al. (2009). High-throughput isolation of immunoglobulin genes from single human B cells and expression as monoclonal antibodies. J. Virol. Methods *158*, 171–179.

Liao, H.-X., Lynch, R., Zhou, T., Gao, F., Alam, S.M., Boyd, S.D., Fire, A.Z., Roskin, K.M., Schramm, C.A., Zhang, Z., et al. (2013). Co-evolution of a broadly neutralizing HIV-1 antibody and founder virus. Nature *496*, 469–476.

Liao, H.-X., Sutherland, L.L., Xia, S.-M., Brock, M.E., Scearce, R.M., Vanleeuwen, S., Alam, S.M., McAdams, M., Weaver, E.A., Camacho, Z., et al. (2006). A group M consensus envelope glycoprotein induces antibodies that neutralize subsets of subtype B and C HIV-1 primary viruses. Virology *353*, 268–282.

Lynch, H.E., Stewart, S.M., Kepler, T.B., Sempowski, G.D., and Alam, S.M. (2014). Surface plasmon resonance measurements of plasma antibody avidity during primary and secondary responses to anthrax protective antigen. J. Immunol. Methods *404*, 1–12.

Mehandru, S., Poles, M.A., Tenner-Racz, K., Jean-Pierre, P., Manuelli, V., Lopez, P., Shet, A., Low, A., Mohri, H., Boden, D., et al. (2006). Lack of mucosal immune reconstitution during prolonged treatment of acute and early HIV-1 infection. PLoS Med. *3*, e484.

Moore, P.L., Gray, E.S., and Morris, L. (2009). Specificity of the autologous neutralizing antibody response. Curr Opin HIV AIDS *4*, 358–363.

Oldstone, M.B. (1998). Molecular mimicry and immune-mediated diseases. Faseb J. *12*, 1255–1265.

Pope, M., and Haase, A.T. (2003). Transmission, acute HIV-1 infection and the quest for strategies to prevent infection. Nat Med *9*, 847–852.

Poulsen, T.R., Meijer, P.-J., Jensen, A., Nielsen, L.S., and Andersen, P.S. (2007). Kinetic, affinity, and diversity limits of human polyclonal antibody responses against tetanus toxoid. J. Immunol. *179*, 3841–3850.

Richman, D.D., Wrin, T., Little, S.J., and Petropoulos, C.J. (2003). Rapid evolution of the neutralizing antibody response to HIV type 1 infection. Proc. Natl. Acad. Sci. U.S.a. *100*, 4144–4149.

Roost, H.P., Bachmann, M.F., Haag, A., Kalinke, U., Pliska, V., Hengartner, H., and Zinkernagel, R.M.

(1995). Early high-affinity neutralizing anti-viral IgG responses without further overall improvements of affinity. Proc. Natl. Acad. Sci. U.S.a. *92*, 1257–1261.

Round, J.L., and Mazmanian, S.K. (2009). The gut microbiota shapes intestinal immune responses during health and disease. Nat. Rev. Immunol. *9*, 313–323.

Sasso, E.H., Johnson, T., and Kipps, T.J. (1996). Expression of the immunoglobulin VH gene 51p1 is proportional to its germline gene copy number. J. Clin. Invest. *97*, 2074–2080.

Smith, K., McCoy, K.D., and Macpherson, A.J. (2007). Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. Semin. Immunol. *19*, 59–69.

Srinivasappa, J., Saegusa, J., Prabhakar, B.S., Gentry, M.K., Buchmeier, M.J., Wiktor, T.J., Koprowski, H., Oldstone, M.B., and Notkins, A.L. (1986). Molecular mimicry: frequency of reactivity of monoclonal antiviral antibodies with normal tissues. J. Virol. *57*, 397–401.

Stacey, A.R., Norris, P.J., Qin, L., Haygreen, E.A., Taylor, E., Heitman, J., Lebedeva, M., DeCamp, A., Li, D., Grove, D., et al. (2009). Induction of a striking systemic cytokine cascade prior to peak viremia in acute human immunodeficiency virus type 1 infection, in contrast to more modest and delayed responses in acute hepatitis B and C virus infections. J. Virol. *83*, 3719–3733.

Su, L.F., Kidd, B.A., Han, A., Kotzin, J.J., and Davis, M.M. (2013). Virus-Specific CD4+ Memory-Phenotype T Cells Are Abundant in Unexposed Adults. Immunity *38*, 373–383.

Tiller, T., Meffre, E., Yurasov, S., Tsuiji, M., Nussenzweig, M.C., and Wardemann, H. (2008). Efficient generation of monoclonal antibodies from single human B cells by single cell RT-PCR and expression vector cloning. J. Immunol. Methods *329*, 112–124.

Tomaras, G.D., Yates, N.L., Liu, P., QIN, L.I., Fouda, G.G., Chavez, L.L., Decamp, A.C., Parks, R.J., Ashley, V.C., and Lucas, J.T. (2008). Initial B cell responses to transmitted HIV-1: virion-binding IgM and IgG antibodies followed by plasma anti-gp41 antibodies with ineffective control of initial viremia. J. Virol.

Veazey, R.S., DeMaria, M., Chalifoux, L.V., Shvetz, D.E., Pauley, D.R., Knight, H.L., Rosenzweig, M., Johnson, R.P., Desrosiers, R.C., and Lackner, A.A. (1998). Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. Science *280*, 427–431.

Veazey, R.S., Marx, P.A., and Lackner, A.A. (2001). The mucosal immune system: primary target for HIV infection and AIDS. Trends in Immunology *22*, 626–633.

Wardemann, H., Yurasov, S., Schaefer, A., Young, J.W., Meffre, E., and Nussenzweig, M.C. (2003). Predominant autoantibody production by early human B cell precursors. Science *301*, 1374–1377.

Wei, X., Decker, J.M., Wang, S., Hui, H., Kappes, J.C., Wu, X., Salazar-Gonzalez, J.F., Salazar, M.G., Kilby, J.M., Saag, M.S., et al. (2003). Antibody neutralization and escape by HIV-1. Nature *422*, 307–312.

Wrammert, J., Smith, K., Miller, J., Langley, W.A., Kokko, K., Larsen, C., Zheng, N.-Y., Mays, I., Garman, L., Helms, C., et al. (2008). Rapid cloning of high-affinity human monoclonal antibodies against influenza virus. Nature *453*, 667–671.

Yates, N.L., Stacey, A.R., Nolen, T.L., Vandergrift, N.A., Moody, M.A., Montefiori, D.C., Weinhold, K.J., Blattner, W.A., Borrow, P., Shattock, R., et al. (2013). HIV-1 gp41 envelope IgA is frequently elicited after transmission but has an initial short response half-life. Mucosal Immunol *6*, 692–703.

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Figure Legends

Figure 1

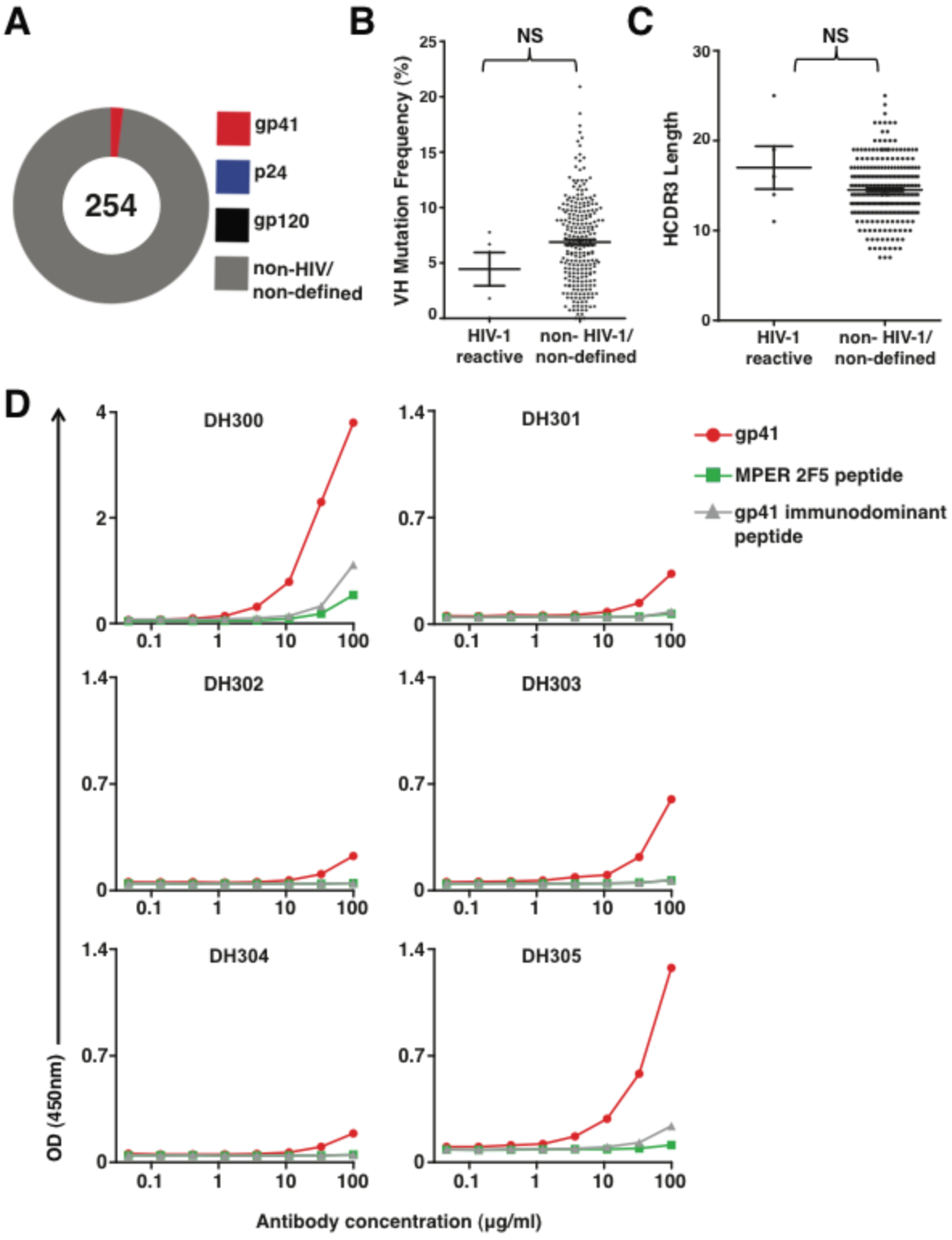


Fig. 1. Characteristics of antibodies isolated from terminal ileum plasma cells and memory B cells of EHI individuals. (A) The total number of mAbs generated from wells with one $V_HD_HJ_H$ and one V_LJ_L gene isolated is indicated in the center of the pie chart. The percentage of mAbs binding to gp41, gp120, p24 and non-HIV-1 antigens are indicated by colors. (B) Frequency of somatic mutations in V_H gene segments of HIV-1-reactive antibodies compared to non-HIV-1-reactive or non-defined mAbs from terminal ileum plasma cells and memory B cells of 6 EHI individuals. (C) The HCDR3 lengths of HIV-1-reactive mAbs compared to non-HIV-1-reactive or non-defined antibodies isolated from terminal ileum B cells, with plasma cells and memory B cells pooled. (D) Six recombinant mAbs (DH300, DH301, DH302, DH303, DH304, and DH305) in threefold dilutions ranging from 100 to 0.05 μg/ml (X Axis) were evaluated for reactivity with HIV-1 rgp41, SP62= 2F5 MPER gp41 epitope peptide (QQEKNEQELLELDKWASLWN) and sp400= gp41 immunodominant peptide (RVLAVERYLRDQQLLGIWGCSGKLICTTAVPWNASWSNKSLNK) by ELISA. (See also **Table S1-S3**)

Figure 2

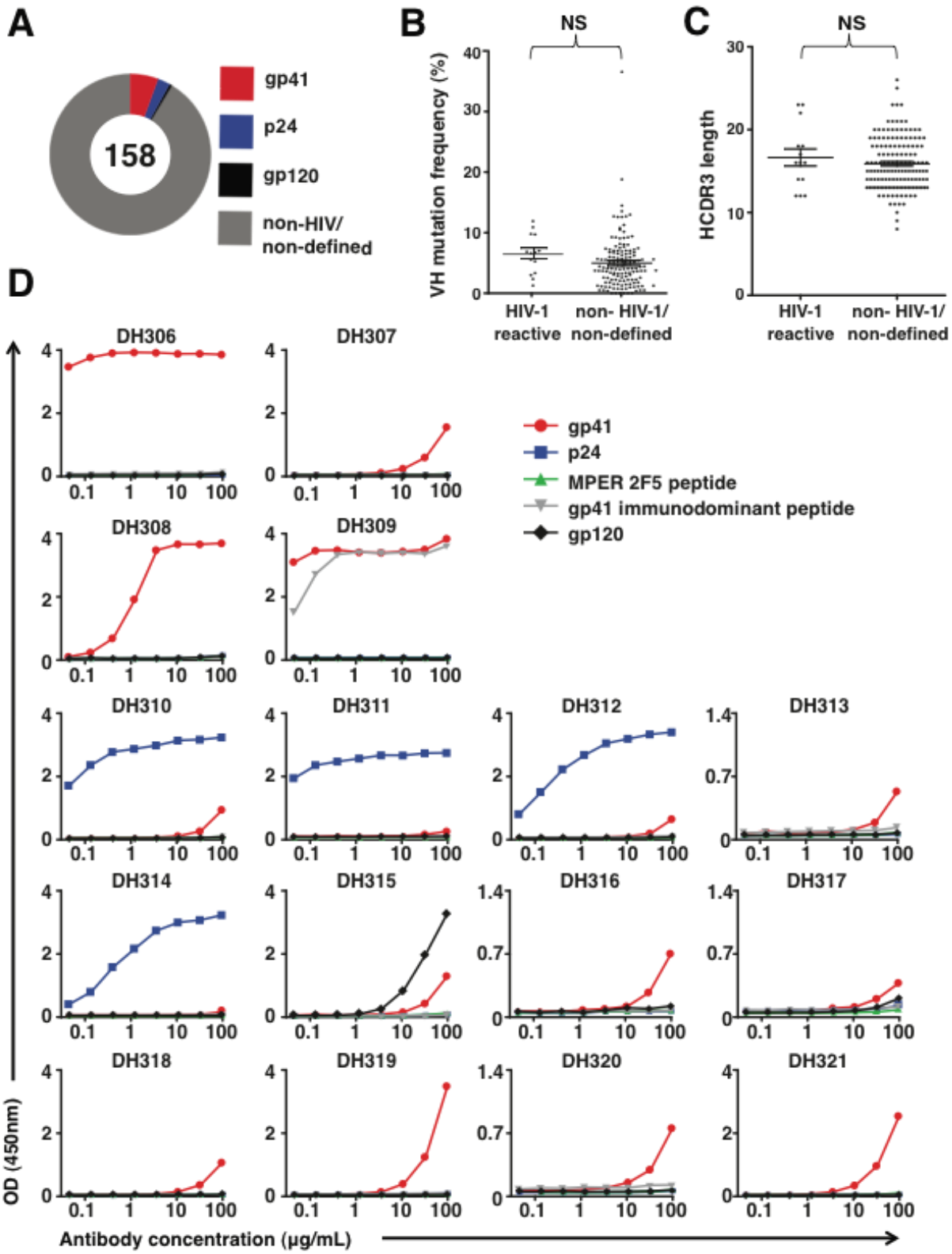

A
158
gp41
p24
gp120
non-HIV/
non-defined
B
NS
VH mutation frequency (%)
HIV-1
reactive
non- HIV-1/
non-defined
C
NS
HCDR3 length
HIV-1
reactive
non- HIV-1/
non-defined
D
DH306
DH307
DH308
DH309
DH310
DH311
DH312
DH313
DH314
DH315
DH316
DH317
DH318
DH319
DH320
DH321
gp41
p24
MPER 2F5 peptide
gp41 immunodominant peptide
gp120
OD (450nm)
Antibody concentration (µg/mL)

Fig. 2. Characteristics of antibodies isolated from terminal ileum plasma cells of CHI individuals. (A) The total number of mAbs generated is indicated in the center of the pie chart. The percentage of mAbs binding to gp41, gp120, p24 and non-HIV-1/ non-defined antigens are indicated by colors. (B) Frequency of somatic mutations in V_H gene segments of HIV-1-reactive antibodies compared to non-HIV-1 or non-defined reactive antibodies from terminal ileum plasma cells and memory B cells of 3 CHI subjects. (C) The HCDR3 lengths of HIV-1 reactive antibodies compared to non-HIV-1 or non-defined reactive antibodies isolated from terminal plasma cells of 3 CHI individuals. (D) Sixteen recombinant mAbs (DH306, DH307, DH308, DH309, DH310, DH311, DH312, DH313, DH314, DH315, DH316, DH317, DH318, DH319, DH320, and DH321) produced in a rIgG1 backbone were evaluated for reactivity with HIV-1 rgp41, p24, sp62= 2F5 MPER gp41 epitope peptide (QQEKNEQELLELDKWASLWN) and sp400= gp41 immunodominant peptide (RVLAVERYLRD-QQLLGIWGCSGKLICTTAVPWNASWSNKSLNK) and MN gp120 gD- by ELISA in threefold dilutions ranging from 100 to at least 0.05 μg/ml (X Axis). (See also **Tables S1-S2, and S4-S5**)

Figure 3

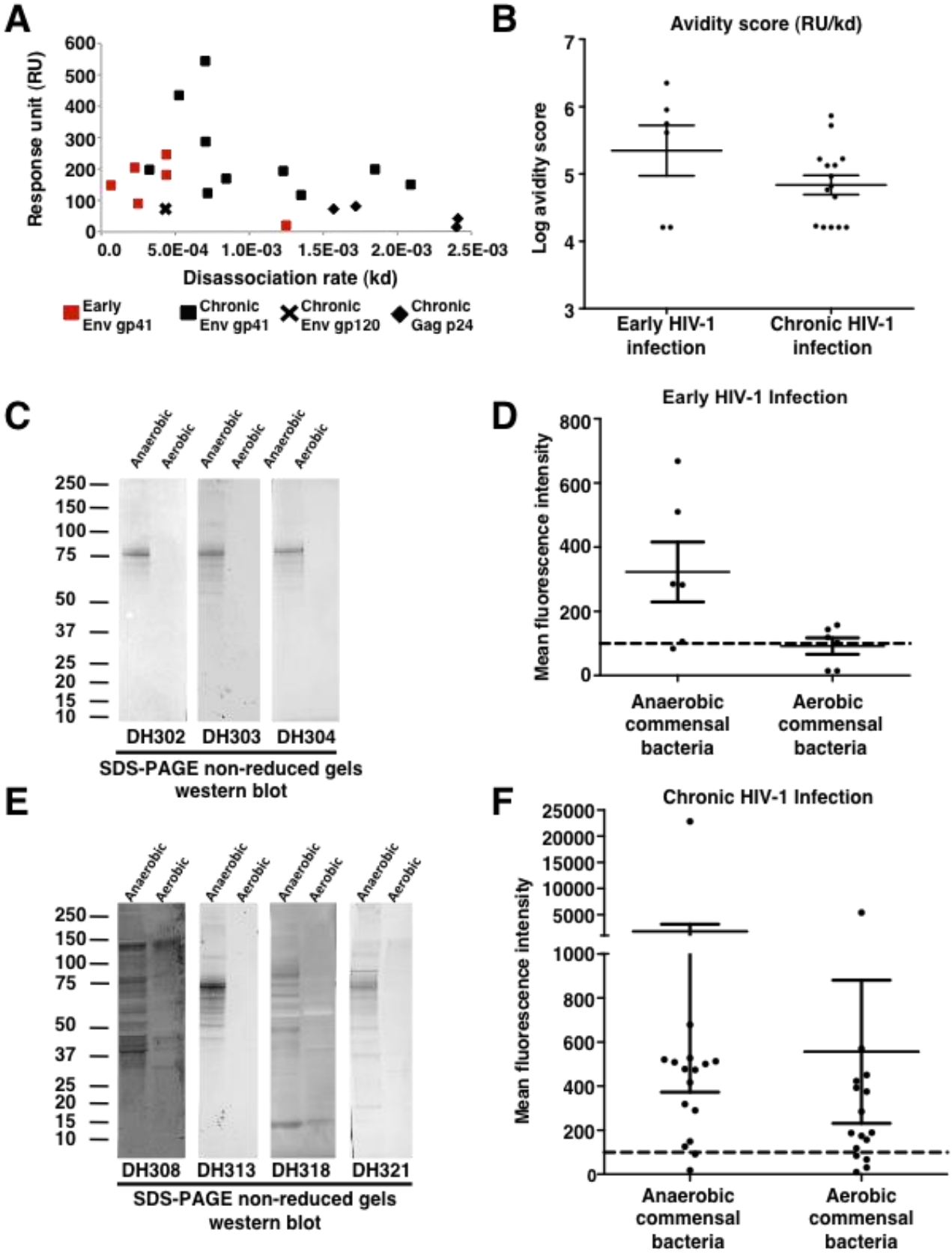
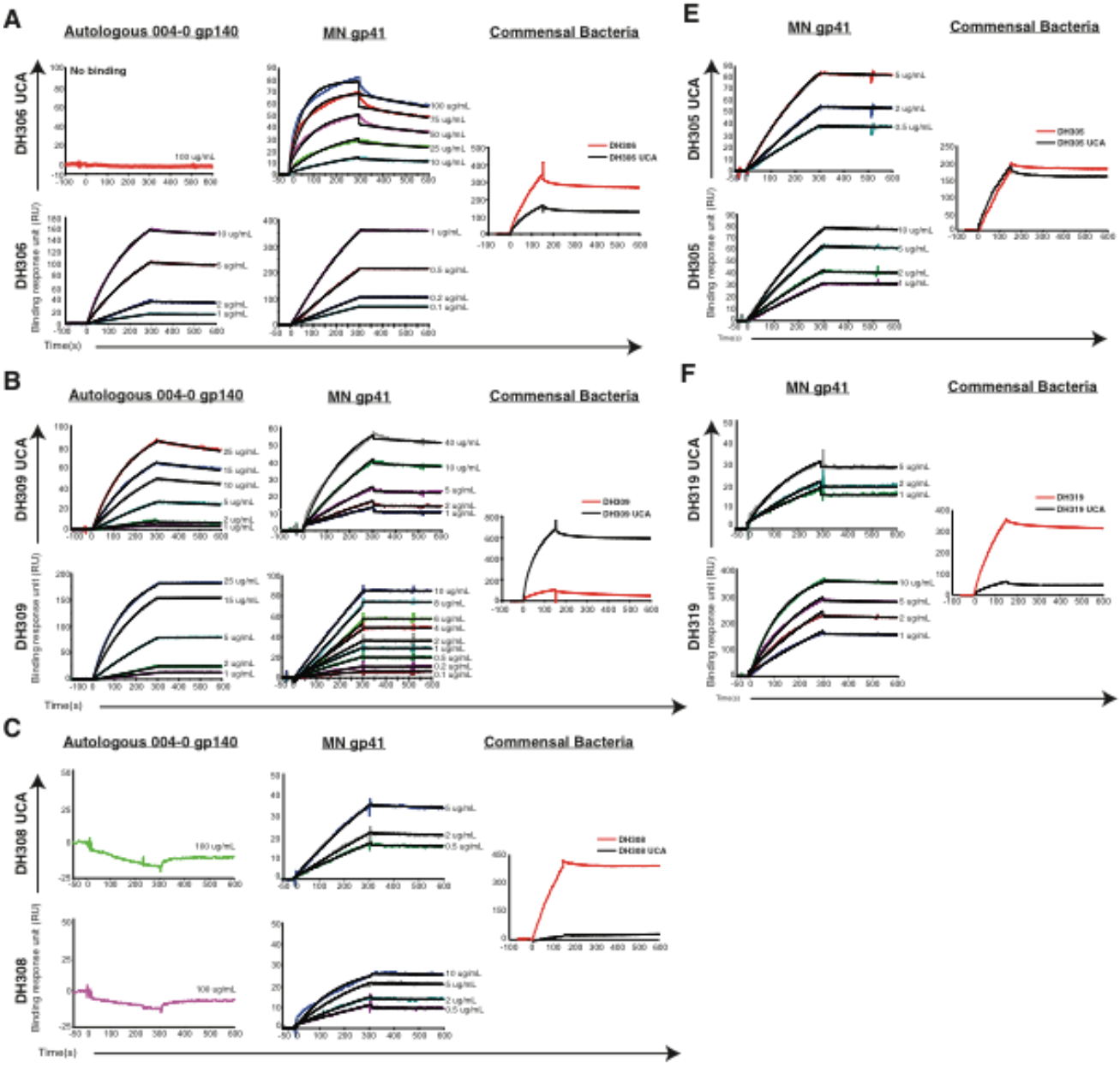

A
Response unit (RU)
600
500
400
300
200
100
0
0.0
5.0E-04
1.0E-03
1.5E-03
2.0E-03
2.5E-03
Disassociation rate (kd)
Early Env gp41
Chronic Env gp41
Chronic Env gp120
Chronic Gag p24
B
Avidity score (RU/kd)
Log avidity score
7
6
5
4
3
Early HIV-1 infection
Chronic HIV-1 infection
C
Anaerobic
Aerobic
250
150
100
75
50
37
25
20
15
10
DH302
DH303
DH304
SDS-PAGE non-reduced gels western blot
D
Early HIV-1 Infection
Mean fluorescence intensity
800
600
400
200
0
Anaerobic commensal bacteria
Aerobic commensal bacteria
E
Anaerobic
Aerobic
250
150
100
75
50
37
25
20
15
10
DH308
DH313
DH318
DH321
SDS-PAGE non-reduced gels western blot
F
Chronic HIV-1 Infection
Mean fluorescence intensity
25000
20000
15000
10000
5000
1000
800
600
400
200
0
Anaerobic commensal bacteria
Aerobic commensal bacteria

Fig. 3. Commensal bacteria cross-reactivity of HIV-1-reactive antibodies isolated from terminal ileum plasma cells and memory B cells of EHI and CHI individuals.

(A) HIV-1-reactive mAbs isolated from terminal ileum plasma cells and memory B cells of EHI and CHI individuals were tested for reactivity to anaerobic commensal bacteria by SPR. The response unit and off-rate for each antibody that reacted with anaerobic commensal bacteria in this assay is plotted. Antibodies isolated from EHI individuals are indicated in red, and antibodies isolated from CHI individuals in black. The HIV-1-reactivity of the antibodies is indicated by shapes. (B) The avidity score (RU/ kd) of the terminal ileum HIV-1 reactive antibodies binding to anaerobic WCL by SPR. (C) HIV-1-reactive mAbs isolated from EHI terminal ileum B cells were also tested for reactivity to anaerobic and aerobic commensal bacteria WCL by SDS-PAGE western blot. 100µg of each anaerobic and aerobic WCL was loaded in individual lanes and mAbs were tested at 20 μg/mL in both non-reducing and reducing conditions. Three representative westerns under non-reducing conditions are shown. (D) HIV-1-reactive mAbs isolated from terminal ileum plasma cells and memory B cells of EHI individuals were tested for reactivity to anaerobic and aerobic commensal bacteria by BAMA at 100 μg/mL. (E) HIV-1-reactive mAbs isolated from CHI terminal ileum B cells were also tested for reactivity to anaerobic and aerobic commensal bacteria WCLs by SDS-PAGE western blot. 100µg of anaerobic and aerobic WCLs was loaded in individual lanes and mAbs were tested at 20 μg/ml in both non-reducing and reducing conditions. Four representative westerns under non-reducing conditions are shown. (F) HIV-1-reactive mAbs isolated from terminal ileum plasma cells and memory B cells of CHI individuals were also tested for reactivity to anaerobic and aerobic commensal by BAMA at 100 μg/mL (See also **Fig. S1-S3, and Tables S2 and S6**).

Figure 4



D

	004-0 gp140			MN gp41			Anaerobic Bacteria WCL	
Ab ID	On rate (ka)	Off rate (kd)	Kd	On rate (ka)	Off rate (kd)	Kd	Response Unit	Off rate (kd)
DH306 UCA		no binding		5.82×10^{3}	5.84×10^{-4}	172nM	553	7.73×10^{-4}
DH306	2.07×10^{5}	1.29×10^{-4}	0.62nM	2.33×10^{5}	5.80×10^{-5}	0.25nM	260	7.05×10^{-4}
DH309 UCA	9.16×10^{4}	4.06×10^{-5}	4.44nM	4.21×10^{3}	1.54×10^{-4}	36.6nM	1030	6.81×10^{-4}
DH309	9.41×10^{4}	3.21×10^{-5}	0.34nM	1.12×10^{3}	1.77×10^{-5}	15.8nM	171	1.85×10^{-3}
DH308 UCA		no binding		9.98×10^{3}	9.95×10^{-5}	9.97nM	24	1.31×10^{-3}
DH308		no binding		3.49×10^{4}	1.43x10-^{5}	0.41nM	408	5.26×10^{-4}
DH305 UCA	N/A	N/A	N/A	1.81×10^{4}	6.41×10^{-5}	3.55nM	159	8.96×10^{-4}
DH305	N/A	N/A	N/A	7.95×10^{3}	6.70×10^{-5}	8.42nM	181	4.40×10^{-4}
DH319UCA	N/A	N/A	N/A	3.49×10^{4}	1.43×10^{-5}	0.41nM	71.3	2.13×10^{-3}
DH319	N/A	N/A	N/A	2.82×10^{4}	5.36×10^{-5}	1.90nM	517	7.04×10^{-4}

Fig. 4. HIV-1 gp41 and commensal bacteria cross-reactive antibodies from the terminal ileum affinity mature to autologous and heterologous HIV-1 envelope. (A-C) SPR binding curves of UCA and mature mAbs immobilized with an anti-Fc receptor antibody binding to titrations of autologous HIV-1 gp140 from individual 004-0 and to MN gp41. Relative binding to commensal bacteria was also determined by SPR. (A) DH306 UCA and DH306, (B) DH309 UCA and DH309, and (C) DH308 UCA and DH308. (D) Table of the on rates, off rates and Kd for each mature mAb and UCA pair binding to the HIV-1 Envs tested and the response unit and off rate of mAb binding to anaerobic bacteria WCL. (E-F) SPR binding curves of UCA and mature mAbs immobilized with an anti-Fc receptor antibody binding to titrations of MN gp41. Autologous Env was not available for these two mAbs. Relative binding to commensal bacteria was also determined by SPR. (E) DH305 UCA and DH305. (F) DH319 UCA and DH319.

Figure 5

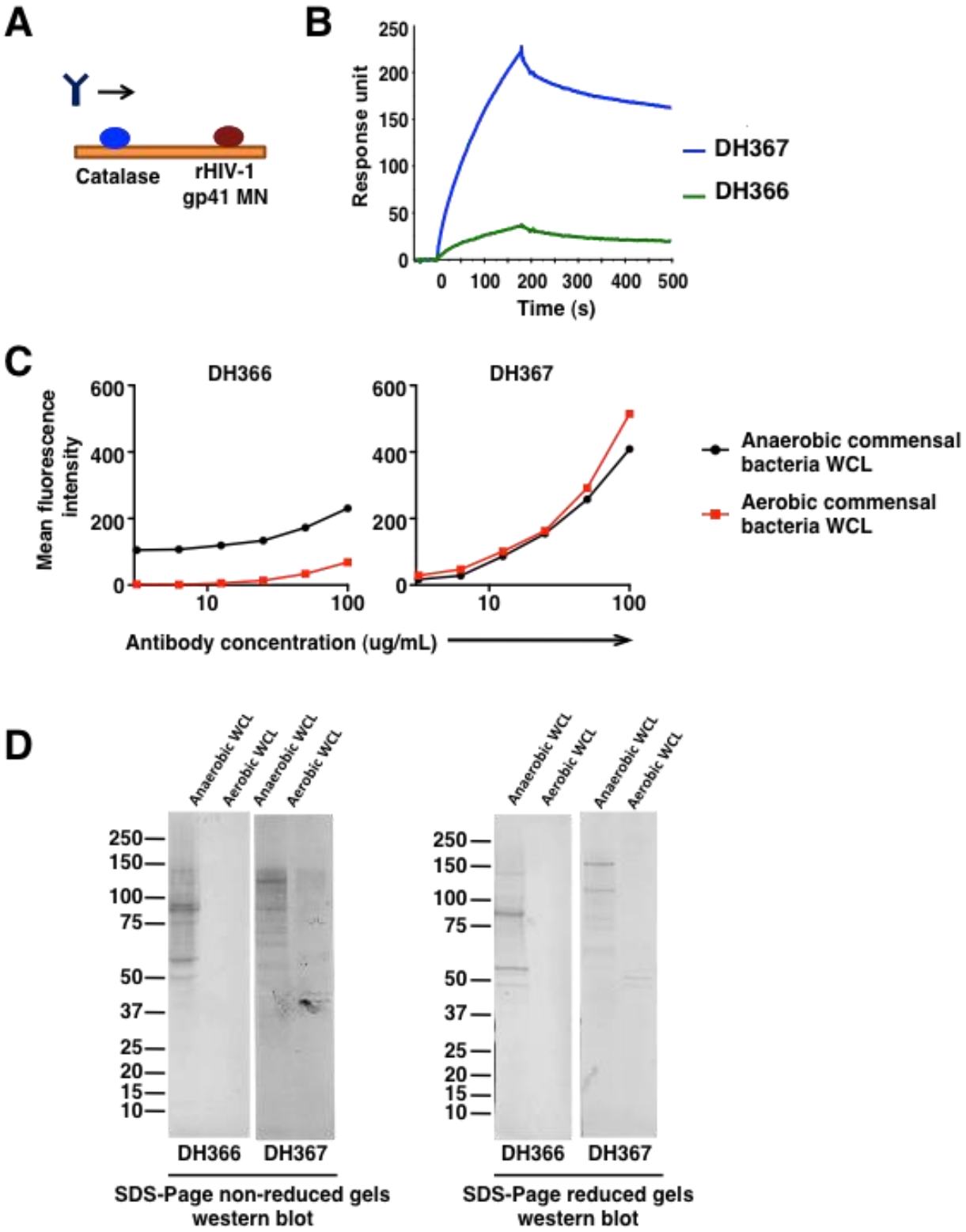

A
Catalase
rHIV-1
gp41 MN
B
Response unit
250
200
150
100
50
0
0
100
200
300
400
500
Time (s)
DH367
DH366
C
DH366
DH367
Mean fluorescence intensity
600
400
200
0
10
100
Antibody concentration (ug/mL)
Anaerobic commensal bacteria WCL
Aerobic commensal bacteria WCL
D
Anaerobic WCL
Aerobic WCL
Anaerobic WCL
Aerobic WCL
250
150
100
75
50
37
25
20
15
10
DH366
DH367
SDS-Page non-reduced gels western blot
SDS-Page reduced gels western blot

Fig. 5. HIV-1-reactive antibodies isolated from terminal ileum plasma cells and memory B cells of uninfected individuals react to both HIV-1 gp41 and commensal bacteria. (A) SPR strategy used to determine HIV-1-reactivity of mAbs isolated from the terminal ileum of uninfected individuals. Signal generated by antibody binding to catalase was subtracted from signal generated by antibody binding to recombinant MN gp41. (B) Antibodies DH366 and DH367 isolated from terminal ileum plasma cells that were natural IgG1 and IgG3 antibodies, respectively, were produced in a rIgG1 backbone were evaluated for reactivity with HIV-1 MN gp41 by SPR. The V_H of DH366 and DH367 were mutated 6.6% and 11.8% respectively. (C) DH366 and DH367 were tested for reactivity to anaerobic and aerobic commensal bacteria by BAMA. Dilutions were twofold ranging from 100μg to 3.1μg/mL (X axis). (D) Reactivity to anaerobic and aerobic commensal bacteria under non-reducing and reducing conditions by SDS-PAGE western blot.

Figure 6

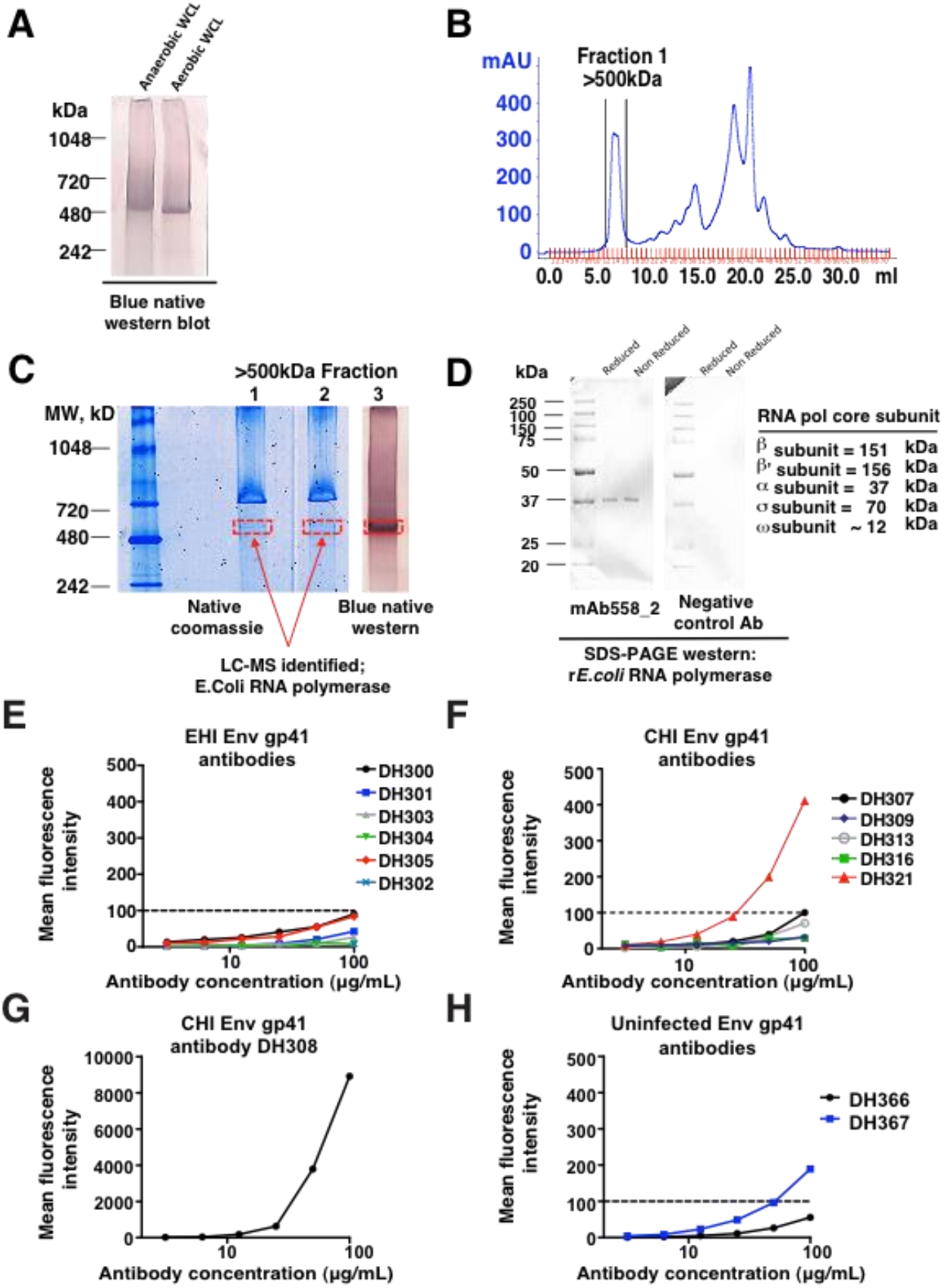

A
Anaerobic WCL
Aerobic WCL
kDa
1048
720
480
242
Blue native western blot
B
mAU
Fraction 1 >500kDa
400
300
200
100
0
0.0 5.0 10.0 15.0 20.0 25.0 30.0 ml
C
>500kDa Fraction
1 2 3
MW, kD
1048
720
480
242
Native coomassie
Blue native western
LC-MS identified; E.Coli RNA polymerase
D
kDa
Reduced
Non Reduced
Reduced
Non Reduced
250
100
150
75
50
37
25
20
RNA pol core subunit
β subunit = 151 kDa
β' subunit = 156 kDa
α subunit = 37 kDa
σ subunit = 70 kDa
ω subunit ~ 12 kDa
mAb558_2
Negative control Ab
SDS-PAGE western: rE.coli RNA polymerase
E
EHI Env gp41 antibodies
Mean fluorescence intensity
Antibody concentration (µg/mL)
DH300
DH301
DH303
DH304
DH305
DH302
F
CHI Env gp41 antibodies
Mean fluorescence intensity
Antibody concentration (µg/mL)
DH307
DH309
DH313
DH316
DH321
G
CHI Env gp41 antibody DH308
Mean fluorescence intensity
Antibody concentration (µg/mL)
H
Uninfected Env gp41 antibodies
Mean fluorescence intensity
Antibody concentration (µg/mL)
DH366
DH367

Fig. 6. Identification of *E.coli* RNA polymerase as one cross-reactive commensal bacterial antigen recognized by HIV-1 gp41 antibodies. (A) Western blot analysis following Native-PAGE gel run showing that mAb 558_2 (Liao et al., 2011) binds to a ~520kDa protein band in anaerobic and aerobic intestinal bacterial WCL. (B). Protein fractions from bacterial WCL with molecular weight ~500kDa were collected following size exclusion chromatography (SEC). (C) The ~500kDa fraction shows enrichment of the 520 kDa protein by 1D Native coomassie blue (lanes1 and 2) and Blue-Native western blotting with mAb558_2 (lane 3). The ~520kDa bands from two gels, identified in two red boxes, were excised from the gel and determined to be *E.coli* RNA polymerase by liquid chromatography-mass spectrometry. (D) Recombinant *E.coli* RNA polymerase core protein was run on a denaturing SDS-PAGE gel under both reducing and non-reducing conditions and blotted with mAb558_2 and a HA flu reactive antibody Ab1248 as a negative control. (E-H) Reactivity of terminal ileum Env gp41- commensal bacteria cross- reactive antibodies with r*E.coli* RNA polymerase was determined by BAMA. Dilutions were twofold ranging from 100μg/mL to 3.1μg/mL (x axis). (E) HIV-1 gp41-commensal bacteria cross- reactive mAbs isolated from the terminal ileum of EHI individuals. (F) HIV-1 gp41- commensal bacteria cross-reactive mAbs isolated from the terminal ileum of CHI individuals. DH309 and DH316 are representative of the other lowest binders, DH317 and DH318. (G) DH308, a gp41 mAbs isolated from a terminal ileum plasma cell from CHI individual 004-0, is the strongest r*E.coli* RNA polymerase binder. DH308 used the V_H gene segment 1-69, which was 8.6% mutated and was naturally IgG1. (H) HIV-1 gp41-commensal bacteria cross-reactive mAbs isolated from the terminal ileum of uninfected individuals. (See also **Fig. S4**)

Figure 7

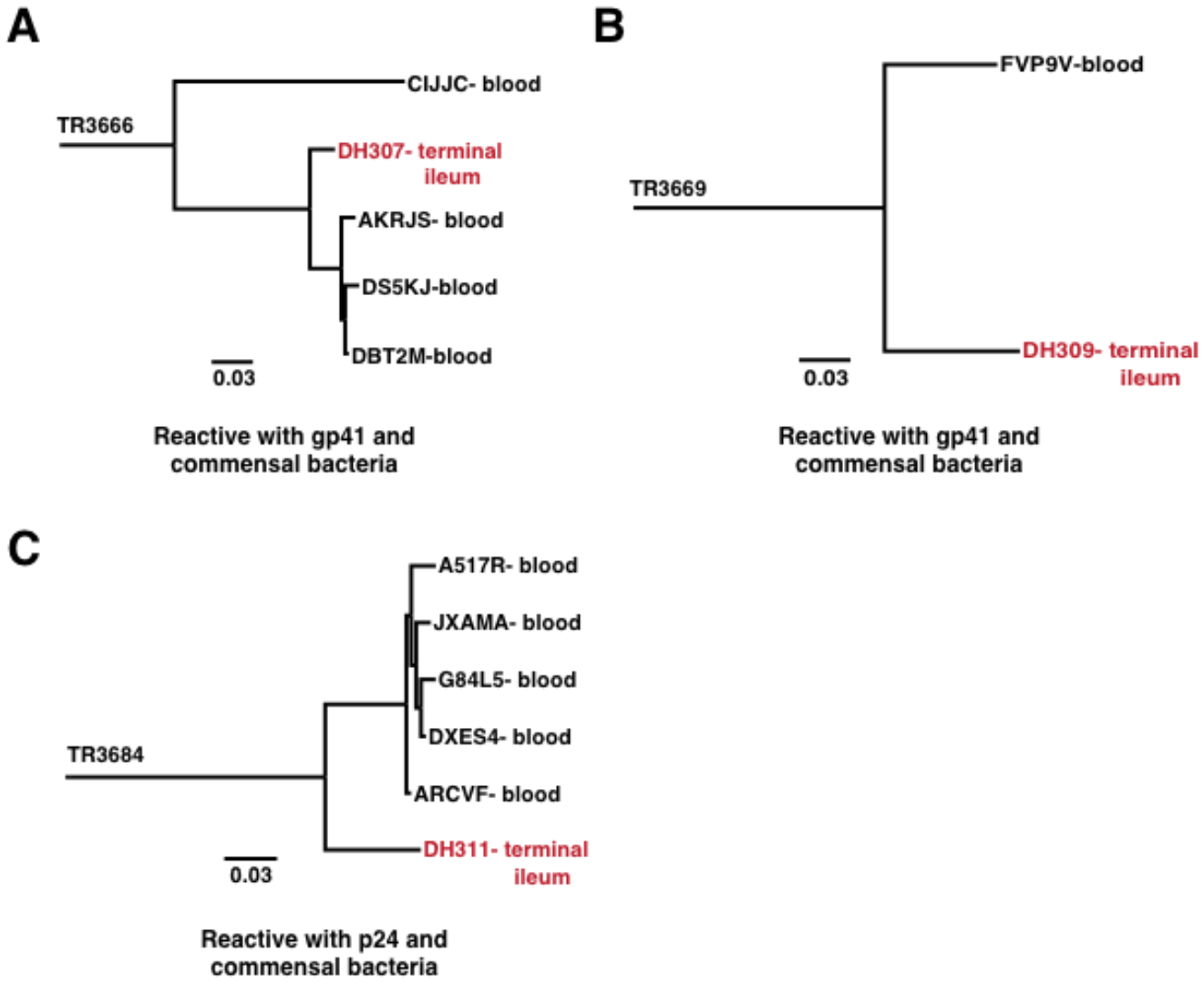

A
TR3666
CIJJC- blood
DH307- terminal ileum
AKRJS- blood
DS5KJ-blood
DBT2M-blood
0.03
Reactive with gp41 and commensal bacteria
B
TR3669
FVP9V-blood
DH309- terminal ileum
0.03
Reactive with gp41 and commensal bacteria
C
TR3684
A517R- blood
JXAMA- blood
G84L5- blood
DXES4- blood
ARCVF- blood
DH311- terminal ileum
0.03
Reactive with p24 and commensal bacteria

Fig. 7. Phylogenetic trees of Ig heavy chain clonal lineages with members derived from blood B cells and terminal ileum B cells, with known antibody reactivity. All trees are rooted on the inferred UCA. Nodes are labeled with the antibody or sequence ID and sample they were isolated from. Red nodes indicate B cells isolated by single cell PCR from terminal ileum B cells that were produced in large scale, and screened for reactivity to HIV-1 antigens and commensal bacteria WCLs. The reactivity of the terminal ileum mAb is noted below each tree. Nodes labeled in black indicate $V_HD_HJ_H$ sequences identified by pyrosequencing of time matched peripheral PBMCs. Tree IDs are located on the left side of each tree. (A) TR3666 (B) TR3669, (C) TR3684. (See also **Fig. S5-S7 and Table S7**)

Supplemental References

Ewing, B., and Green, P. (1998). Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Res. *8*, 186–194.

Gao, F., Weaver, E.A., Lu, Z., Li, Y., Liao, H.-X., Ma, B., Alam, S.M., Scearce, R.M., Sutherland, L.L., Yu, J.-S., et al. (2005). Antigenicity and immunogenicity of a synthetic human immunodeficiency virus type 1 group m consensus envelope glycoprotein. J. Virol. *79*, 1154–1163.

Hindson, B.J., Ness, K.D., Masquelier, D.A., Belgrader, P., Heredia, N.J., Makarewicz, A.J., Bright, I.J., Lucero, M.Y., Hiddessen, A.L., Legler, T.C., et al. (2011). High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. Anal. Chem. *83*, 8604–8610.

Kawatsu, K., Kumeda, Y., Taguchi, M., Yamazaki-Matsune, W., Kanki, M., and Inoue, K. (2008). Development and Evaluation of Immunochromatographic Assay for Simple and Rapid Detection of Campylobacter jejuni and Campylobacter coli in Human Stool Specimens. Journal of Clinical Microbiology *46*, 1226–1231.

Luftig, M.A., Mattu, M., Di Giovine, P., Geleziunas, R., Hrin, R., Barbato, G., Bianchi, E., Miller, M.D., Pessi, A., and Carfí, A. (2006). Structural basis for HIV-1 neutralization by a gp41 fusion intermediate-directed antibody. Nat. Struct. Mol. Biol. *13*, 740–747.

Munshaw, S., and Kepler, T.B. (2010). SoDA2: a Hidden Markov Model approach for identification of immunoglobulin rearrangements. Bioinformatics *26*, 867–872.

Rossio, J.L., Esser, M.T., Suryanarayana, K., Schneider, D.K., Bess, J.W., Vasquez, G.M., Wiltrout, T.A., Chertova, E., Grimes, M.K., Sattentau, Q., et al. (1998). Inactivation of human immunodeficiency virus type 1 infectivity with preservation of conformational and functional integrity of virion surface proteins. J. Virol. *72*, 7992–8001.

Scheid, J.F., Mouquet, H., Feldhahn, N., Walker, B.D., Pereyra, F., Cutrell, E., Seaman, M.S., Mascola, J.R., Wyatt, R.T., Wardemann, H., et al. (2009). A method for identification of HIV gp140 binding memory B cells in human blood. J. Immunol. Methods *343*, 65–67.

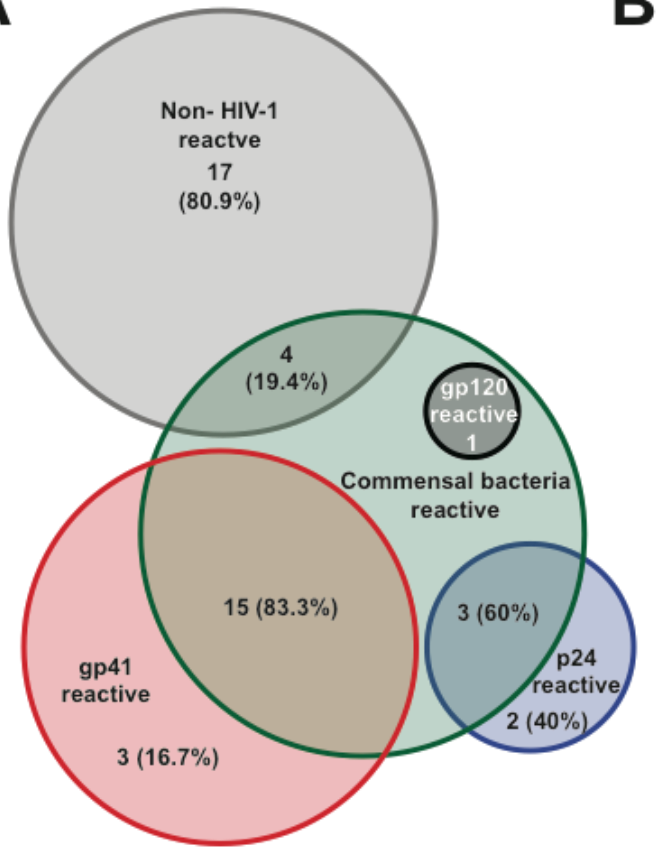
Smith, T.F., and Waterman, M.S. (1981). Identification of common molecular subsequences. J. Mol. Biol. *147*, 195–197.

Weaver, E.A., Lu, Z., Camacho, Z.T., Moukdar, F., Liao, H.-X., Ma, B.-J., Muldoon, M., Theiler, J., Nabel, G.J., Letvin, N.L., et al. (2006). Cross-subtype T-cell immune responses induced by a human immunodeficiency virus type 1 group m consensus env immunogen. J. Virol. *80*, 6745–6756.

Supplemental Figures

Figure S1

A



B

Summary of binding characteristics of HIV-1 reactive antibodies isolated from terminal ileum plasma cells and memory B cells

Infection type	Ab ID	HIV-1 reactivity	Comensal bacteria reactive[a]	Autoreactive or polyreactive[b]
EHI	DH300	gp41	Yes	Yes
EHI	DH301	gp41	Yes	No
EHI	DH302	gp41	Yes	No
EHI	DH303	gp41	Yes	No
EHI	DH304	gp41	Yes	No
EHI	DH305	gp41	Yes	No
CHI	DH306	gp41	No	Yes
CHI	DH307	gp41	Yes	Yes
CHI	DH308	gp41	Yes	Yes
CHI	DH309	gp41	Yes	No
CHI	DH310	p24	Yes	No
CHI	DH311	p24	Yes	No
CHI	DH312	p24	No	No
CHI	DH313	gp41	Yes	Yes
CHI	DH314	p24	No	No
CHI	DH315	gp120	Yes	Yes
CHI	DH316	gp41	Yes	No
CHI	DH317	p24	Yes	No
CHI	DH318	gp41	Yes	No
CHI	DH319	gp41	No	No
CHI	DH320	gp41	No	Yes
CHI	DH321	gp41	Yes	Yes
Uninfected	DH366	gp41	Yes	No
Uninfected	DH367	gp41	Yes	No

[a] Antibodies that were positive for commensal bacgteria reactivty by SPR and western blot assays are indicated by Yes

[b] Antibodies that were reactive to any antigens tested in AtheNA ANA or in Hep-2 staining are indicated by Yes.

Figure S1, Related to Figure 3. Summary of terminal ileum antibody binding characteristics to HIV-1 antigens, commensal bacteria and autoantigens. (A) A Venn diagram showing the HIV-1 antigen and commensal bacteria cross-reactivity of all antibodies isolated from terminal ileum plasma cells and memory B cells. Different colored circles represent antibody reactivities: red= Env gp41-reactive, blue= Gag p24, black= Env gp120, green= commensal bacteria, and gray= non-HIV reactive. (B) A summary table of HIV-1-reactive mAbs isolated from terminal ileum plasma cells and memory B cells and their cross-reactivity with with commensal bacteria by SPR and western blot, and autoantigens by AtheNA and Hep-2 staining assays.

Fig. S2

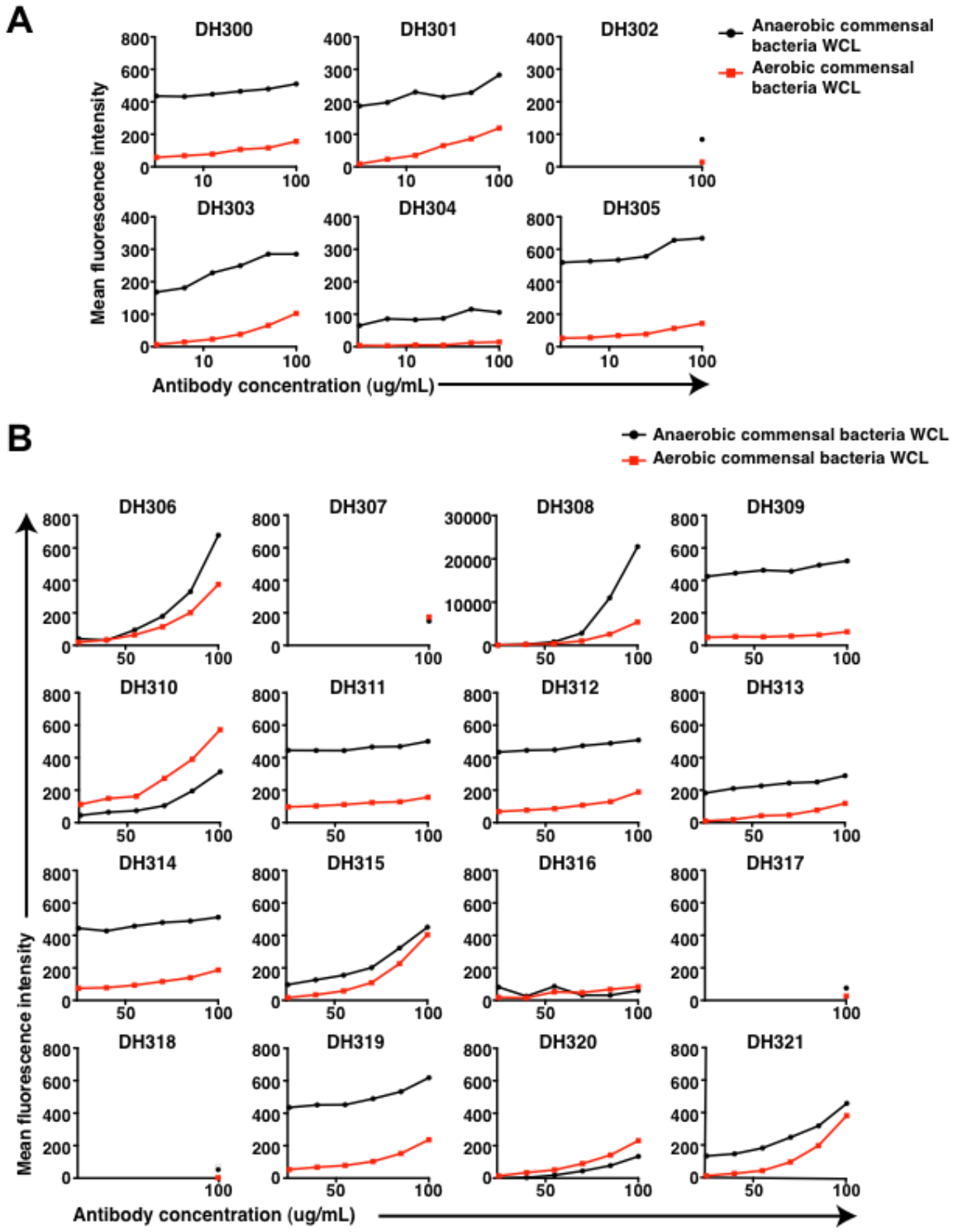

A
DH300
DH301
DH302
DH303
DH304
DH305
Anaerobic commensal bacteria WCL
Aerobic commensal bacteria WCL
Mean fluorescence intensity
Antibody concentration (ug/mL)
B
DH306
DH307
DH308
DH309
DH310
DH311
DH312
DH313
DH314
DH315
DH316
DH317
DH318
DH319
DH320
DH321
Anaerobic commensal bacteria WCL
Aerobic commensal bacteria WCL
Mean fluorescence intensity
Antibody concentration (ug/mL)

Figure S2, Related to Figure 3. Commensal bacteria cross-reactivity of HIV-1-reactive antibodies isolated from terminal ileum plasma cells and memory B cells of EHI and CHI individuals. HIV-1 reactive antibodies isolated from the terminal ileum that had an MFI of 100 or greater at 100µg/mL by an initial screen in binding antibody multiplex assays were titrated by twofold dilutions ranging from 100ug to 3.1µg/mL (X axis) to test for reactivity to two different preparations of anaerobic and aerobic commensal bacteria whole cell lysate. Whichever prep the antibody bound strongest is displayed. (A) HIV-1-reactive antibodies isolated from EHI terminal ileum (DH300, DH301, DH302, DH303, and DH304). Ab3118 had an MFI less than 100 when tested and was not titrated. (B.) HIV-1-reactive antibodies isolated from CHI terminal ileum (DH306, DH307, DH309, DH310, DH311, DH312, DH313, DH314, DH315, DH316, DH319, DH320, and DH321). DH306, DH317, and DH318 had and MFI less than 100 when tested at 100µg/mL and were not titrated.

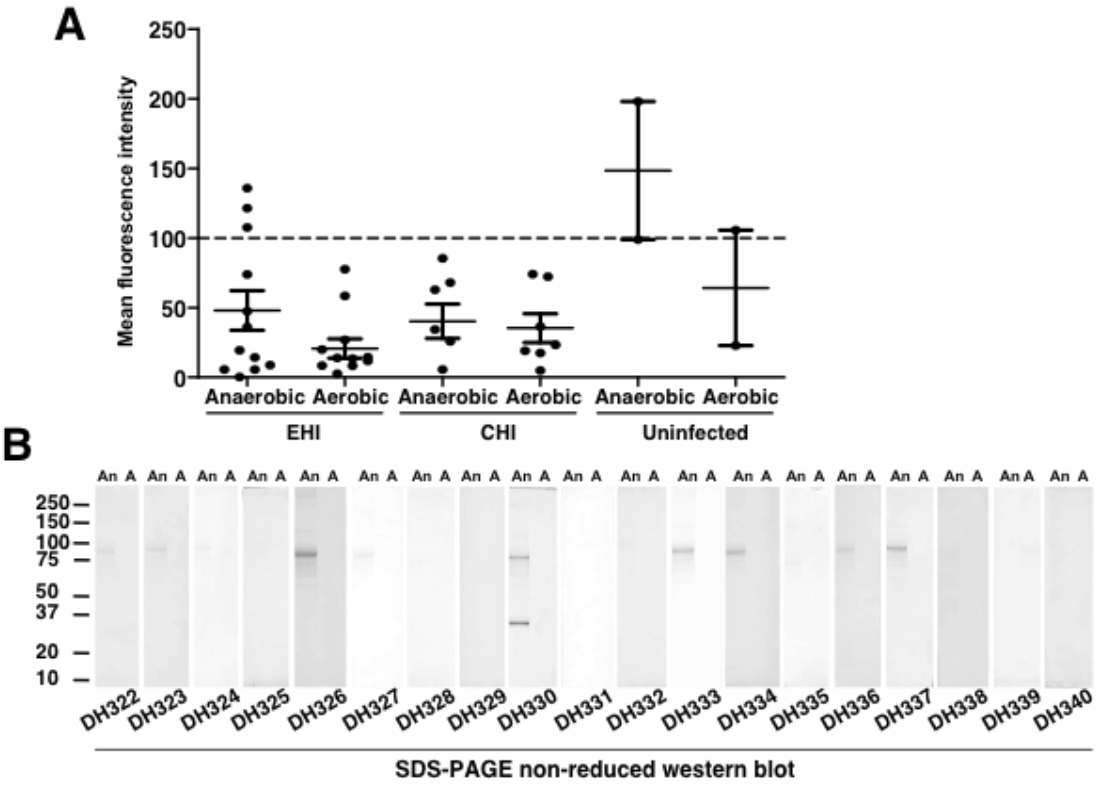
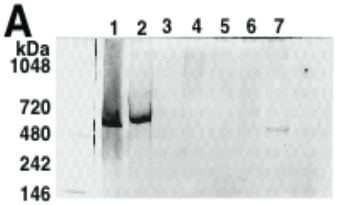


Fig. S3

Figure S3, Related to Figure 3. Commensal Bacteria reactivity of non-HIV-1 reactive antibodies isolated from terminal ileum plasma cells and memory B cells of EHI, CHI and uninfected indivdiuals (A) Antibodies isolated from terminal ileum plasma cells and memory B cells of EHI, CHI and uninfected individuals that were not reactive with HIV-1 antigens were tested for reactivity to anaerobic and aerobic commensal bacteria at 100µg/mL by binding antibody multiplex assays. The positivity cut off of 100 MFI units is shown with a dotted line. (B) Non-HIV reactive antibodies were tested for reactivity to Anaerobic {An} and Aerobic {A} commensal bacteria by western blot in both non-reducing and reducing conditions.

Fig. S4

A



1: Anaerobic WCL
2: Fraction 1, >500kDa
3: pH 3-4.6
4: pH 4.6-5.4
5: pH 5.4-6.2
6: pH 6.2-7
7: pH 7-10

Blue native western

B

RPOA_CITK8 (100%), 36,512.1Da
DNA-directed RNA polymerse subunit alpha
4 unique peptides, 4 unique spectra, 4 total spetra, 34/329 amino acids (10% coverge)

MQGSVTEFLKPRLVDIEQVSSTHAKVTLEPLERGFGHTLGNALRRILLSSMPGCAVTEVEIDGVLHE
YSTKEGVQEDILEILLNLKGLAVRVQGKDEVILTLNKSGIGPVTAADITHDGDVEIVKPQHVICHLTDE
NASISMRIKVQRGRGYVPASTRIHSEEDERPIGRLLVDACYSPVERIAYNVEAARVEQRTDLDKLVI
EMETNGTIDPEEAIRRAATILAEQLEAFVDLRDVRQPEVKEEKPEFDPILLRPVDDLELTVRSANCLK
AEAIHYIGDLVQRTEVELLKTPNLGKKSLTEIKDVLASRGLSLGMRLENWPPASIADE

C

RPOB_EC024 (100%), 150,635.9Da
DNA-directed RNA polymerase subunit beta
22 unique peptides, 22 unique spectra, 24 total spectra, 232/1342 amino acids (17% coverage)

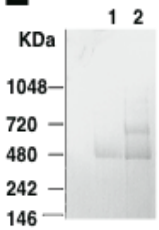
MVYSYTEKKRIRKDFGKRPQVLDVPYLLSIQLDSFQKFIEQDPEGQYGLEAAFRSVFPIQSYSGNSELQYVSYRLGEPVFDVQECQIRGVTYSAPLRVKLRLVIYEREAPEGTVKDIKEQEVYMGEIPL
MTDNGTFVINGTERVIVSQLHRSPGVFFDSDKGKTHSSGKVLYNARIIPYRGSWLDFEFDPKDNLFVRIDRRRKLPATIILRALNYTTEQILDLFFEKVIFEIRDNKLQMELVPERLRGETASFDIEANGKV
YVEKGRRITARHIRQLEKDDVKLIEVPVEYIAGKVVAKDYIDESTGELICAANMELSLDLLAKLSQSGHKRIETLFTNDLDHGPYISETLRVDPTNDRLSALVEIYRMMRPGEPPTREAAESLFENLFFSED
RYDLSAVGRMKFNRSLLREEIEGSGILSKDDIIDVMKKLIDIRNGKGEVDDIDHLGNRRIRSVGEMAENQFRVGLVRVERAVKERLSLGDLDTLMPQDMINAKPISAAVKEFFGSSQLSQFMDQNNPLSE
ITHKRRISALGPGGLTRERAGFEVRDVHPTHYGRVCPIETPEGPNIGLINSLSVYAQTNEYGFLETPYRKVTDGVVTDEIHYLSAIEEGNYVIAQANSNLDEEGHFVEDLVTCRSKGESSLFSRDQVDYM
DVSTQQVVSVGASLIPFLEHDDANRALMGANMQRQAVPTLRADKPLVGTGMERAVAVDSGVTAVAKRGGVVQYVDASRIVIKVNEDEMYPGEAGIDIYNLTKYTRSNQNTCINQMPCVSLGEPVERG
DVLADGPSTDLGELALGQNMRVAFMPWNGYNFEDSILVSERVVQEDRFTTIHIQELACVSRDTKLGPEEITADIPNVGEAALSKLDESGIVYIGAEVTGGDILVGKVTPKGETQLTPEEKLLRAIFGEKAS
DVKDSSLRVPNGVSGTVIDVQVFTRDGVEKDKRALEIEEMQLKQAKKDLSEELQILEAGLFSRIRAVLVAGGVEAEKLDKLPRDRWLELGLTDEEKQNQLEQLAEQYDELKHEFEKKLEAKRRKITQGD
DLAPGVLKIVKVYLAVKRRIQPGDKMAGRHGNKGVISKINPIEDMPYDENGTPVDIVLNPLGVPSRMNIGQILETHLGMAAKGIGDKINAMLKQQQEVAKLREFIQRAYDLGADVRQKVDLSTFSDEEVM
RLAENLRKGMPIATPVFDGAKEAEIKELLKLGDLPTSGQIRLYDGRTGEQFERPVTVGYMYMLKLNHLVDDKMHARSTGSYSLVTQQPLGGKAQFGGQRFGEMEVWALEAYGAAYTLQEMLTVKSDD
VNGRTKMYKNIVDGNHQMEPGMPESFNVLLKEIRSLGINIELEDE

D

RPOC_ECO24 (100%), 155,164.1 Da
DNA-directed RNA polymerse subunit beta'
19 unique peptide, 19 unique spectra, 22 total spectra, 196/1407 amino acids (14% coverage)

MKDLLKFLKAQTKTEEFDAIKIALASPDMIRSWSFGEVKKPETINYRTFKPERDGLFCARIFGPVKDYECLCGKYKRLKHRGVICEKCGVEVTQTKVRRERMGHIELASPTAHIWFLKSLPSRIGLLL
DMPLRDIERVLYFESYVVIEGGMTNLERQQILTEEQYLDALEEFGDEFDAKMGAEAIQALLKSMDLEQECEQLREELNETNSETKRKKLTKRIKLLEAFVQSGNKPEWMILTVLPVLPPDLRPLVPLD
GGRFATSDLNDLYRRVINRNNRLKRLLDLAAPDIIVRNEKRMLQEAVDALLDNGRRGRAITGSNKRPLKSLADMIKGKQGRFRQNLLKRVDYSGRSVITVGPYLRLHQCGLPKKMALELFKPFIYGK
LELRGAKKMVEREEAVVWDILDEVIREHPVLLNRAPTLHRLGIQAFEPVLIEGKAIQLHPLVCAAYNADFDGDQMAVHVPLTLEAQLEARALMMSTNNILSPANGEPIIVPSQDVVLGLYYMTRDCVN
AKGEGMVLTGPKEAERLYRSGLASLHARVKVRITEYEKDANGELVAKTSLKDTTVGRAILWMIVPKGLPYSIVNQALGKKAISKMLNTCYRILGLKPTVIFADQIMYTGFAYAARSGASVGIDDMVIPE
KKHEIISEAEAEVAEIQEQFQSGLVTAGERYNKVIDIWAAANDRVSKAMMDNLQTETVINRDGQEEKQVSFNSIYMMADSGARGSAAQIRQLAGMRGLMAKPDGSIIETPITANFREGLNVLQYFIS
THGARKGLADTALKTANSGYLTRRLVDVAQDLVVTEDDCGTHEGIMMTPVIEGGDVKEPLRDRVLGRVTAEDVLKPGTADILVPRNTLLHEQWCDLLEENSVDAVKVRSVVSCDTDFGVCAHCYG
RDLARGHIINKGEAIGVIAAQSIGEPGTQLTMRTFHIGGAASRAAAESSIQVKNKGSIKLSNVKSVVNSSGKLVITSRNTELKLIDEFGRTKESYKVPYGAVLAKGDGEQVAGGETVANWDPHTMPVI
TEVSGFVRFTDMIDGQTITRQTDELTGLSSLVVLDSAERTAGGKDLRPALKIVDAQGNDVLIPGTDMPAQYFLPGKAIVQLEDGVQISSGDTLARIPQESGGTKDITGGLPRVADLFEARRPKEPAILA
EISGIVSFGKETKGKRRLVITPVDGSDPYEEMIPKWRQLNVFEGERVERGDVISDGPEAPHDILRLRGVHAVTRYIVNEVQDVYRLQGVKINDKHIEVIVRQMLRKATIVNAGSSDFLEGEQVEYSR
VKIANRELEANGKVGATYSRDLLGITKASLATESFI SAASFQETTRVLTEAAVAGKRDELRGLKENVIVGRLIPAGTGYAYHQDRMRRRAAGEAPAAPQVTAEDASASLAELLNAGLGGSDNE

E



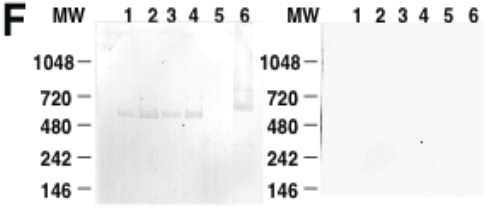
Blue native western

1: E coli RNA Polymerase Coreenzyme (5 ug/lane)

2: E coli RNA Polymerase Holoenzyme (5 ug/lane)

Primary Ab: mAb558_2 (10 ug/ml)

F



1: E coli RNA Polymerase Coreenzyme (5 ug/lane)
2: E coli RNA Polymerase Holoenzyme (5 ug/lane)
3: E coli RNA Polymerase Coreenzyme+GroEL (5 ug/lane)
4: E coli RNA Polymerase Holoenzyme+GroEL (5 ug/lane)
5: GroEL (5ug/lane)
6: Aerobe whole lysate (100 ug/lane)

Primary Ab: mAb558_2 (10 ug/ml)

Negative control mAb (10 ug/ml)

Blue native western

Figure S4, Related to Figure 6. Identification of *E.coli* RNA polymerase as an antigen in intestinal bacterial lysate reactive to Ab558_2. (A) Isoelectric zoom fractionation shows migration of mAb reactive protein to gel compartment A4 with pH6.2 -7 by Blue Native western. (B-D) LC-MS identification of *E.coli* RNA polymerase subunit peptides. (B) α subunit peptides. 4 total spectra- 4 unique spectra resulting in identification of 4 unique peptides. (C) β subunit peptides**.** 24 total spectra – 22 unique spectra resulting in identification of 22 unique peptides. (D) β' subunit peptides. 22 total spectra-19 unique spectra resulting in identification of 19 unique peptides. (E) Recombinant *E.coli* RNA polymerase coreenzyme and holoenzyme (Core protein+ s subunit) (Epicentre Biotechnologies, Madison, WI) were run on a NativePAGE gel, and reactivity of mAb 558_2 detected by NativePAGE western blotting. Reactivity to both core and holoenzyme was detected and thus, suggesting that mAb 558_2 binds to RNA polymerase coreenzyme. (F) NativePage western blot of E.coli RNA polymerase, GroEL and aerobic commensal bacteria. Recombinant *E.coli* RNA polymerase Coreenzyme alone(1), Recombinant *E.coli* RNA polymerase holoeenzyme alone(2), Recombinant *E.coli* RNA polymerase coreenzyme and recombinant GroEL(3), Recombinant *E.coli* RNA polymerase holoeenzyme and recombinant GroEL(4), GroEL alone (5), and aerobic commensal bacteria whole cell lysage (6). Left panel primary antibody is HIV-1 and commensal bacteria WCL cross-reactive mAb 558_2. The primary antibody on the right panel is Flu-reactive antibody that does not react with commensal bacteria or HIV-1 antigens.

Fig. S5

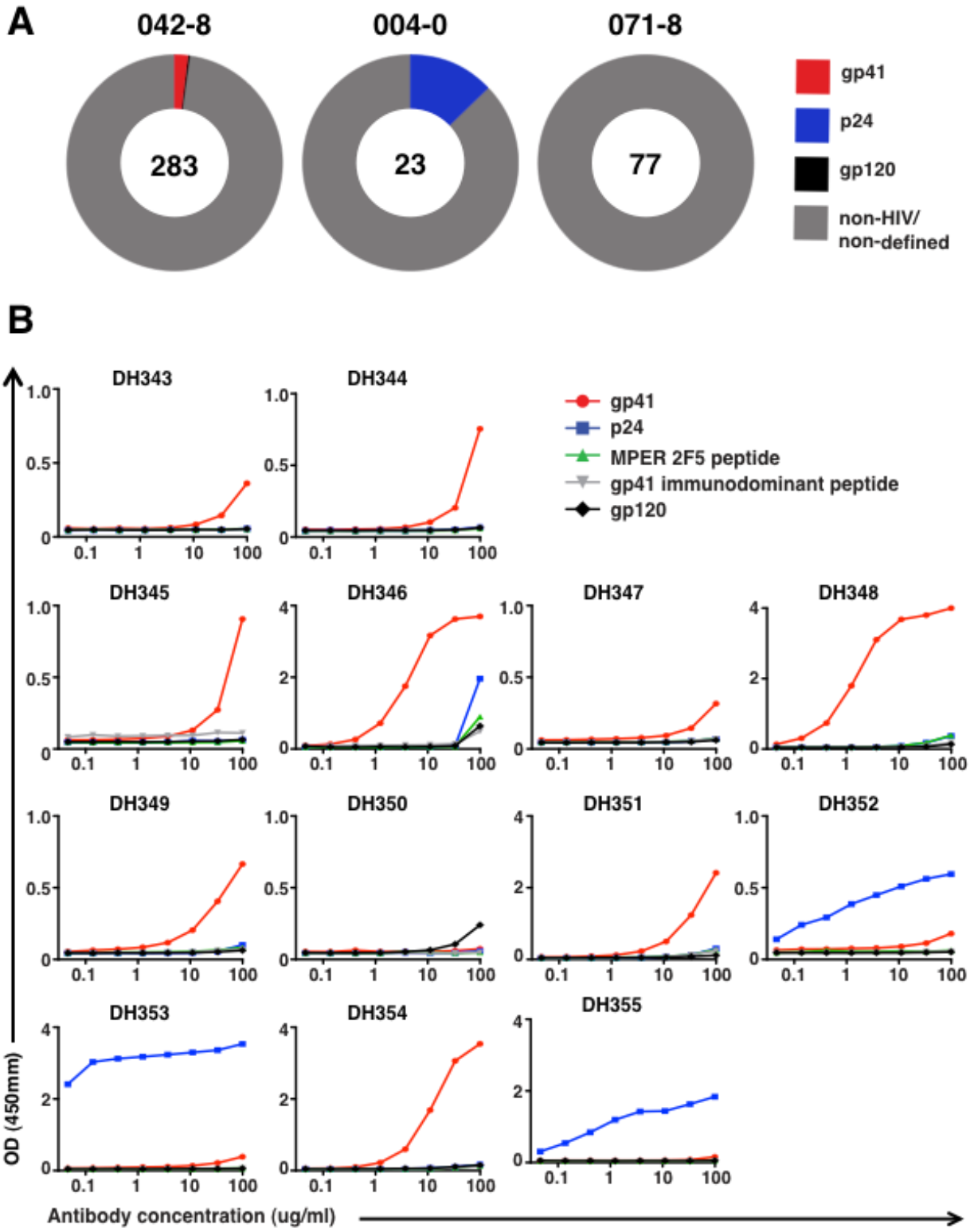

A
042-8
004-0
071-8
283
23
77
gp41
p24
gp120
non-HIV/
non-defined
B
DH343
DH344
DH345
DH346
DH347
DH348
DH349
DH350
DH351
DH352
DH353
DH354
DH355
gp41
p24
MPER 2F5 peptide
gp41 immunodominant peptide
gp120
OD (450mm)
Antibody concentration (ug/ml)

Figure S5. Related to Figure 7. Binding of HIV-1-reactive antibodies isolated from peripheral blood plasma cells of EHI and CHI individuals. (A) HIV-1-reactivity of antibodies isolated from plasma cells and memory B cells from the peripheral blood of 3 individuals (042-8, 004-0 and 071-8). The total number of antibodies generated from wells with one $V_HD_HJ_H$ and one V_LJ_L gene isolated is indicated in the center of the pie chart. The percentage of antibodies binding to gp41, gp120, p24 and non HIV-1 antigens are indicated by colors. (B) Thirteen recombinant antibodies (DH343, DH344, DH345, DH346, DH347, DH348, DH349, DH350, DH351, DH352, DH353, DH354 and DH355) produced in a rIgG1 backbone were evaluated for reactivity with HIV-1 rgp41, p55, sp62, sp400 and gp120 by ELISA in threefold dilutions ranging from 100 to at least 0.05 μg/mL (X Axis).

Fig. S6

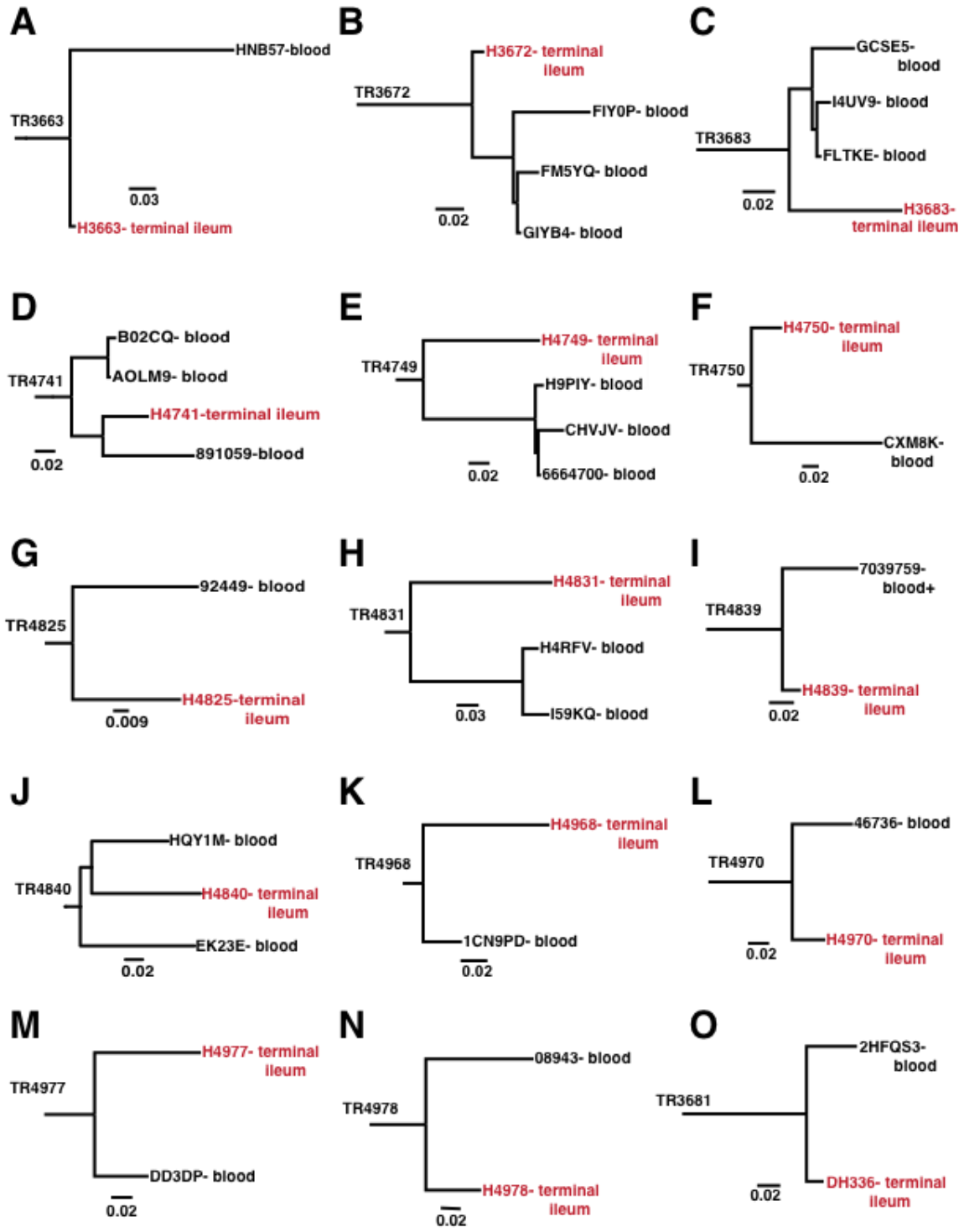
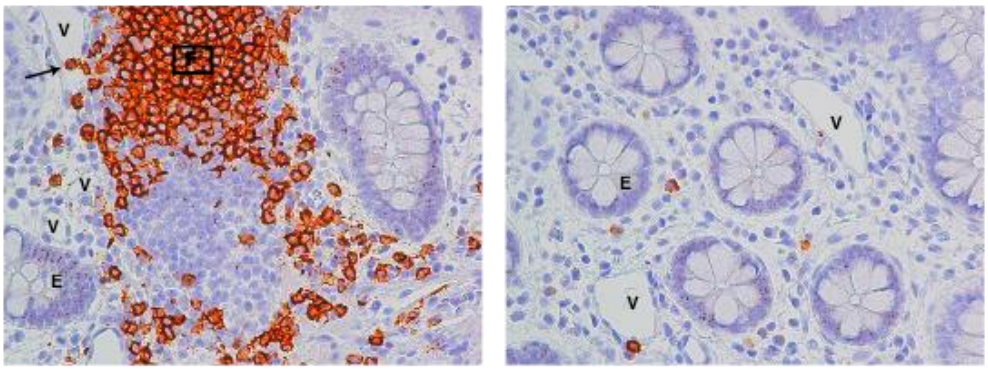

A
HNB57-blood
TR3663
0.03
H3663- terminal ileum
B
H3672- terminal ileum
TR3672
FIY0P- blood
FM5YQ- blood
0.02
GIYB4- blood
C
GCSE5- blood
I4UV9- blood
TR3683
FLTKE- blood
0.02
H3683- terminal ileum
D
B02CQ- blood
TR4741
AOLM9- blood
H4741-terminal ileum
0.02
891059-blood
E
H4749- terminal ileum
TR4749
H9PIY- blood
CHVJV- blood
0.02
6664700- blood
F
H4750- terminal ileum
TR4750
CXM8K- blood
0.02
G
92449- blood
TR4825
H4825-terminal ileum
0.009
H
H4831- terminal ileum
TR4831
H4RFV- blood
0.03
I59KQ- blood
I
7039759- blood+
TR4839
H4839- terminal ileum
0.02
J
HQY1M- blood
TR4840
H4840- terminal ileum
EK23E- blood
0.02
K
H4968- terminal ileum
TR4968
1CN9PD- blood
0.02
L
46736- blood
TR4970
H4970- terminal ileum
0.02
M
H4977- terminal ileum
TR4977
DD3DP- blood
0.02
N
08943- blood
TR4978
H4978- terminal ileum
0.02
O
2HFQS3- blood
TR3681
DH336- terminal ileum
0.02

Figure S6, Related to Figure 7. Phylogenetic trees of Ig heavy chain clonal lineages with members derived from blood B cells and terminal ileum B cells, with unknown antibody reactivity. All trees are rooted on the inferred UCA. Nodes are labeled with antibody or sequence ID and sample they were isolated from. Red indicates B cells isolated by single cell PCR from terminal ileum B cells. Nodes labeled in black indicate V_H sequences identified by pyrosequencing of time matched peripheral PBMCs (A) TR3663, (B) TR3672, (C)TR3683, (D) TR4741, (E)TR4749, (F) TR4750, (G)TR4825, (H)TR4831, (I) TR4839, (J)TR4840, (K) TR4968, (L) TR4970, (M) TR4977, (N) TR4978, (O) TR3681.

Fig. S7

A



B

Summary of quantitative image analysis of CD20+ cells in blood vessels within termianl ileum tissue biopsy

PTID	cells/mm3			# Images
	Average # CD20+ cells in vessels	Average total # CD20+ cells	Average % CD20+ cells in vessels	
019-2	2857.7	1441504.6	0.2%	2
036-0	470.9	110470.4	0.4%	6
037-1	0.0	15088.65	0.0%	5
049-7	0.0	46478.4	0.0%	4
042-8	565.1	17244.2	3.3%	5
071-8	0.0	10238.7	0.0%	5
078-2	0.0	49038.1	0.0%	5
067-8	1883.8	234412.9	0.8%	3
072-3	0.0	528102.6	0.0%	1
076-4	0.0	91609.6	0.0%	1
034-6	0.0	265847.6	0.0%	3
040-6	0.0	74096.0	0.0%	4
Average	478.8	240344.3	0.2%	3.8

Figure S7. Related to Figure 7. Quantitative image analysis of B cells in the terminal ileum of HIV-1 Infected individuals. (A) Two representative images at 40x magnification of terminal ileum tissue sections stained with CD20 to differentiate B cell, used to quantitate the relative number of intravascular B cells in terminal ileum tissue. E= gut epithelium, F= lymphoid follicle, V= blood vessel and arrows indicate B cells that are within blood vessels. (B) Summary of quantitative image analysis of CD20+ B cells in terminal ileum tissue biopsies.

Supplementary Tables

Table S1. Related to Figures 1 and 2. Clinical data for 12 HIV-1 infected and 3 uninfected individuals in this study

Patient ID	Age (gender)	Estimated Days Since Infection[a]	Time on ART	Viral Load copes/ml	CD4 count cells/ul
019-2	18 (M)	47	n/a[c]	24,545	973
052-2	19 (M)	141	n/a[c]	4,888	n/a[d]
036-0	24 (M)	105	79 days	974	1102
037-1	21 (M)	180-200	65 days	187	980
049-7	47 (M)	100	31 days	142	386
042-8	32 (M)	180-200	7 days	253,751	457
038-7	23 (M)	Chronic[b]	786 days	<50	919
004-0	59 (M)	Chronic[b]	0[c]	16,800	957
071-8	21 (M)	Chronic[b]	3 years	<40	528
078-2	31 (M)	Chronic[b]	n/a[c]	4,628	608
067-8	21 (M)	Chronic[b]	n/a[c]	26,376	607
072-3	29 (M)	Chronic[b]	n/a[c]	7,780	915
076-4	20 (M)	Chronic[b]	n/a[c]	6,988	669
034-6	25 (M)	Uninfected	n/a[c]	n/a[d]	n/a[d]
045-0	34 (M)	Uninfected	n/a[c]	n/a[d]	n/a[d]
040-6	49 (F)	Uninfected	n/a[c]	n/a[d]	n/a[d]

[a] Days since infection was determined by clinical information of exposure, onset of AHI symptoms or Fiebig stage at enrollment.

[b] Chronic represents infection time greater than 200 days. Subject 004-0 was infected for 1198 days at the time of terminal ileum biopsy, but accurate time of transmission could not be determined for other chronically infected individuals.

[c] n/a= not applicable. Individuals were not on retroviral therapy at the time of terminal ileum biopsy.

[d] n/a= not applicable

Table S2, Related to Figures 1-2. Characteristics of purified antibodies isolated from the terminal ileum and peripheral blood plasma cells and memory B cells of HIV-1 infected and uninfected individuals. **(Separate File)**

Table S3, Related to Figure 1. Isotypes of antibodies isolated from memory B cells and plasma cells of EHI, CHI and uninfected individuals

Ig Isotype and Class	EHI		CHI		Uninfected	
	N	%	N	%	N	%
IgM	111	43.7%	12	7.6%	54	54.5%
IgD	1	0.4%	0	0.0%	0	0.0%
IgA1	91	35.8%	98	62.0%	33	33.3%
IgA2	17	6.7%	6	3.8%	3	3.0%
IgG1	20	7.9%	32	20.3%	3	3.0%
IgG2	0	0.0%	0	0.0%	0	0.0%
IgG3	13	5.1%	10	6.3%	6	6.1%
IgG4	1	0.4%	0	0.0%	0	0.0%
Total	254		158		99	

Table S4, Related to Figure 2. VH1-69 copy number assay determined by digital PCR

Subject ID	Droplet Counts			V_H1-69 Copy Number
	V_H1-69	RPP30	Total Count	
004-0	781	527	11,183	3
042-8	924	960	10,237	2
071-8	1120	797	10,268	3

The number of droplets displayed is the average number from one experiment run in triplicate. Similar results were confirmed in a second experiment, also run in triplicate. Diploid genomes contain two copies of ribonuclease P protein subunit P30 (RPP30), which was used as a reference gene to determine the number of VH1-69 copies for these three individuals.

Table S5, Related to Figure 2. Percentage of HIV-1 CON-S gp140 envelope-reactive B cells as a subset of IgM, IgG, ad IgA memory B cells isolated from paired blood and terminal ileum samples

PTID	% IgM Memory B cells		% IgG Memory B cells		% IgA Memory B cells	
	Blood	Terminal Ileum	Blood	Terminal Ileum	Blood	Terminal Ileum
072-3	0.08%	0.00%	0.56%	0.00%	0.00%	0.00%
078-2	0.06%	0.05%	0.09%	0.20%	0.00%	0.11%
067-8	0.00%	0.00%	0.36%	0.00%	0.62%	0.00%
076-4	0.00%	0.00%	0.04%	0.00%	0.18%	0.00%
Mean	0.04%	0.01%	0.26%	0.05%	0.20%	0.03%

CON-S gp140 labeled AF647 and Pacific Blue reactive IgD-, IgM+, IgG+ or IgA+ memory B cells were gated with the following strategy: singlet, viable CD45+, CD3/14/16/235a-, CD19+, surface IgD, surface immununoglubulin (IgM, IgG or IgA)+, CD27+.

Table S6, Related to Figure 6. Binding characteristics of purified antibodies isolated from the terminal ileum and peripheral blood plasma cells and memory B cells of HIV-1 infected and uninfected individuals. **(separate file)**

Table S7, Related to Figure 7. Clonal lineages identified by a combination of single cell PCR of terminal ileum B cells and pyrosequencing of blood B cells from individuals 004-0 and 071-8

Subject ID	Clone ID	VH	JH	HCDR3 Length	Isotype and Class	# of INT Clone Members	# BLD Clone Members
				aa			
004-0	TR3686	1~69	6	18	G1	2	0
004-0	TR3665	3~11	6	25	A1	2	0
004-0	TR3668	3~72	4	15	M	2	0
004-0	TR3672	4~39	5	13	G1	2	0
004-0	TR3510	3~33	4	17	M	0	2
004-0	TR3663	4~59	5	17	G3	1	1
004-0	TR3666	1~69	6	16	G1	1	4
004-0	TR3669	1~69	6	23	G1	1	1
004-0	TR3672	4~39	5	13	G1	1	3
004-0	TR3681	3~9	3	20	G1	1	1
004-0	TR3683	3~30	4	8	G1	1	3
004-0	TR3684	1~69	3	12	G1	1	5
071-8	TR4741	4~31	6	16	A1	1	3
071-8	TR4749	1~46	5	16	A1	1	3
071-8	TR4750	5~51	4	15	M	1	1
071-8	TR4825	1~46	6	15	G1	1	1
071-8	TR4831	3~33	5	19	A1	1	2
071-8	TR4839	3~73	5	11	M	1	1
071-8	TR4840	4~31	3	14	G1	1	2
071-8	TR4968	3~7	3	15	A1	1	1
071-8	TR4970	4~34	3	20	A1	1	1
071-8	TR4977	3~23	3	14	A1	1	1
071-8	TR4978	3~20	3	16	A1	1	1

Extended Experimental Procedures

Flow Cytometry Analysis of Terminal Ileum and Blood B cells. Multicolor flow cytometry analysis and sorting are performed-using a four laser (488nm, 532nm, 640nm, and 405nm) BD FACSAria II-SORP (BD Biosciences, Mountain View, CA) for nine-color flow cytometry. Combinations of the following antibodies were used: for human IgG (PE), CD3 (PE-Cy5), CD16 (PE-Cy5), CD19 (APC-Cy7), CD20 (PE-Cy7), CD27 (Pacific Blue), CD235a (PE-Cy5), IgD (PE), IgM (FITC; BD Biosciences), and CD14 (PE-Cy5) and CD38 (APC-Cy5.5; both from Invitrogen, Carlsbad, CA). To characterize single B cells from the blood (PBMCs) and isolated terminal ileum cells, we sorted single plasmablasts/ plasma cells (CD19+CD3-CD14-CD16-CD235a-CD27hiCD38hiCD20-IgD-) or memory B cells (CD19+CD3-CD14-CD16-CD235a-IgD-), as previously described (Levesque et al., 2009; Liao et al., 2011), into individual wells of 96-well plates, and stored at -80°C until PCR was performed as described (Liao et al., 2011; 2009; 2013). HIV-1 Env gp140-reactive memory B cell phenotyping was performed on PBMC and terminal ileum samples from CHI individuals using AF647 and Pacific blue dual color labeled- CON-S gp140 known to react with all clades of HIV positive sera (Tomaras et al., 2008), using the methods previously described (Scheid et al., 2009).

Sequencing, sequence annotation, quality control, and data management of Ig $V_HD_HJ_H$ and V_LJ_L sequences was completed as previously described (Liao et al., 2011)**.** A PCR purification kit (Qiagen, Valencia, CA) was used to purify all PCR products of Ig $V_HD_HJ_H$ and V_L genes, and PCR products were sequenced in forward and reverse directions using an ABI 3700 instrument and BigDye sequencing kit (Applied Biosystems). Base calling for each sample was performed using Phred (Ewing and Green, 1998). The forward and reverse strand of the immunoglobulin genes were assembled into one final nucleotide sequence using an assembly algorithm based on the quality scores at each base position (Munshaw and Kepler, 2010). The isotype and subclasses of the Ig heavy-chain and types of light-chain were determined by comparing the constant region sequences of the isolated Ig $V_HD_HJ_H$ and V_L PCR products with the constant region sequences of the known Ig isotypes and subclass, kappa and lambda genes using a Smith-Waterman alignment algorithm (Smith

and Waterman, 1981). Heavy, Kappa, and Lambda sequences were assigned unique H, K and L identification numbers. Genetic information inferred by using SoDA, such as gene segment usage, somatic mutations, n-nucleotides, and CDR3 length is stored in a relational database to facilitate subsequent statistical analysis (Munshaw and Kepler, 2010).

Expression of $V_HD_HJ_H$ and V_LJ_L as full-length IgG1 recombinant mAb. Transfection supernatants were screened for expressed IgG quantities and for antigen reactivity using ELISA and binding antibody multiplex assays (Liao et al., 2009). The initial screen was used to identify antibodies with relatively high affinity. Those antibodies isolated from terminal ileum plasma cells or memory B cells, or blood plasma cells with an ELISA reading three times above background and an OD of greater than OD 0.130, or a mean fluorescence intensity (MFI) of three times or greater above background and an MFI of 20 for HIV-1 antigens, were produced on a large scale and purified to confirm antibody reactivity in replicate. Purified antibody was produced in bulk cultures by transient transfection of 293 Freestyle cells with 1 mg of each Ig heavy- and light-chain genes either cloned in pcDNA plasmids or synthesized (GeneScript, Piscataway, NJ) per 1 L transfection as previously described (Liao et al., 2009; 2011).

Assay antigens for antibody reactivity. HIV-1 antigens for ELISA assays included HIV-1 MN recombinant gp41 (Immunodiagnostics), gp41 5-helix bundle protein (a gift from Michael Root, Thomas Jefferson University) (Luftig et al., 2006). HIV-1 group M consensus gp120 (Liao et al., 2006) , 040 gp140 (Bar et al., 2012; Liao et al., 2013), gp120 CON-6 (Gao et al., 2005; Weaver et al., 2006), AT 2-inactivated HIV-1 ADA virions (Rossio et al., 1998) (a gift from J. Lifson, National Cancer Institute, Frederick, MD), the Env peptides immunodominant region peptide sp400 (RVLAVERYLRD-QQLLGIWGCSG-KLICTTAVPWN-ASWSNKSLNK), gp41 MPER region peptide SP62 (QQEKNEQELLELDKWASLWN).

Antigens for BAMA included HIV-1 MN recombinant gp41 (Immunodiagnostics), HIV-1 group M consensus gp120 (Liao et al., 2006) (Liao et al., 2006), 040 gp140 (Bar et al., 2012; Liao et al., 2013) (Bar et al., 2012; Liao et al., 2013), gp120 CON-6 (Gao et al., 2005; Weaver et al., 2006), HIV-1

reverse transcriptase, p66 (Worthington Biochemical), HIV-1 Gag precursor, p55 (Protein Sciences), Non-HIV-1 antigens Hepatitis E2 (Immunodiagnostics), *E.coli* RNA polymerase (Sigma, St. Louis, MO), tetanus toxoid (EMD Millipore, Darmstadt, Germany), and whole-cell lysates of anaerobic and aerobic intestinal commensal bacterial extracts (Kawatsu et al., 2008; Liao et al., 2011). Whole cell lysates of anaerobic and aerobic commensal bacteria, respectively, from stool cultures pooled from multiple (4-5) individuals were tested (Liao et al., 2011).

Preparation of intestinal anaerobic and aerobic commensal bacteria lysates. Aerobic and anaerobic bacteria cultures were harvested by adding 5mL PBS to the plates and then scraped with a cell scraper (Thermo Fisher Scientific), and rinsed with an additional 5mL PBS. The harvested bacteria was then treated with 100 μl of bacterial protease inhibitor cocktail (Sigma-Aldrich), and then sonicated with a Misonix 3000 Sonicator at maximum voltage for 15-s pulses, followed by 15-s rests on ice for 3 min. After sonication, 1.5g of acid-washed glass beads (Sigma- Aldrich) was added to the extract. The extracts were vortexed for 1 min at maximum setting and then chilled for 1 min on ice. This process was repeated four times for a total of five vortex-chill cycles. The resulting extracts were spun for 20 min at 3,000 g. The supernatants were harvested and measured for protein concentration by Nanodrop. Samples were aliquoted and frozen at -80 ˚C until they were used.

IgG VH1-69 copy number assay. Genomic DNA was digested with *XbaI* (New England Biolabs) and *BamHI* (Promega) before being heated at 65˚C for 20 minutes to inactivate the enzymes 75ng of digested DNA was added to the Bio-Rad 2x ddPCR supermix with Taqman probes and primers at full concentrations of 100nM and 500nM respectively. Samples were converted into droplets before being amplified by PCR with the following cycling conditions: 95 ˚C for 10 minutes followed by 40 cycles of 94˚C for 30 seconds and 60˚C for 1 minute with a final extension at 98˚C for 10 minutes. Positive droplets were counted and the number of V_H1-69 droplets was compared to the number of positive droplets produced by the amplification of a control gene, ribonuclease P protein (RPP30) (Hindson et al., 2011) that has two copies per diploid genome. Experiments were run twice in triplicate.

VH1-69 Forward primers: CCCTATCYTTGGTAYAGCAAACTACG

VH1-69 Reverse primer: GGATGTGGGTTTTCACACTGTG

VH1-69 Probe: AATCCACGAGCACAGCCTACATGGA