SUPPLEMENTARY ONLINE MATERIAL

Materials and methods

Study design

Inclusion criteria for the selection of HIV-1-infected women from the WITS cohort included: no documented antiretroviral treatment during pregnancy/delivery, detectable plasma virus load during pregnancy (>50 copies/ml), and non-heparin plasma and serum samples available for immune assays collected between 25 weeks of gestation and two months postpartum. Eighty-five HIV-transmitting mothers were eligible. The control, non-transmitting group of HIV-infected mothers were selected at a 1:2 case:control ratio (n = 170) using propensity score matching [1,2] for the following parameters: maternal plasma virus load, peripheral CD4+ T cell count at delivery (or the pregnancy timepoint closest to delivery), mode of delivery (caesarian section vs vaginal delivery), and infant gestational age (based on estimated delivery date) [3,4]. The post-matching clinical characteristics of the continuous variables (plasma viral load, CD4 count, gestational age) were compared by student's t test and birth type was compared by Chi squared test (Table 1). Four non-transmitting and 3 transmitting subjects were removed following matching due to lack of adequate sample, and one non-transmitting subject was removed due to the detection of antiretroviral drug in the plasma. As no breastfeeding occurred in this population, all transmission events were *in utero* or peripartum [5]. Thirteen percent of the infected infants in the HIV-transmitting group were infected *in utero* and 52% were infected peripartum, the remaining 35% did not have a perinatal transmission mode retrospectively defined due to lack of infant birth sample availability.

Selection of humoral immune response variables

The five types of primary humoral immune assays that were selected for the RV144 immune correlate analysis ("clade B modified RV144 model"): Env V1V2 IgG binding, Env IgA binding breadth, Env IgG avidity, ADCC, neutralization [6] were tailored to focus on clade B Env antigens/viruses and analyzed together in an RV144 clade B-modified humoral immune response model. We also selected additional humoral immune assays to assess antibody responses that had previously been implicated to play a role in MTCT ("MTCT model") including gp120 IgG binding, gp41 IgG binding, V3 IgG binding, and gp41 IgA binding [7-16] and analyzed them in parallel.

Statistical analysis

The statistical analysis plan was finalized prior to data analysis. We used multivariable logistic regression with transmission status as the dependent variable [17]. All continuous immune response variables were mean-centered and scaled to one so that the estimated odds ratios were all per one standard deviation increment. All regression analyses were adjusted for delivery mode, log maternal plasma viral load, infant gestational age, and peripheral CD4+ T cell count. In primary analyses, we applied multivariable logistic regression models for both the primary analysis of the six RV144-adapted humoral immune variables and four MTCT-related humoral immune variables in parallel. Combined immune variable scores (Env-binding IgA, neutralization, V3 binding IgG) were defined as a weighted combination of multiple immune response variables [18]. We also used change point models to model the threshold of the association between identified immune correlates of risk and the transmission status[19]. In secondary analyses, each individual humoral immune response measured in this cohort was

studied in logistic regression models. To correct for multiplicity, we applied a false discovery rate (q value) [20] and family-wise error rate[21], setting a significance threshold of $p < 0.05$, and $q < 0.2$ [6]. This approach optimizes the hypothesis-generating discovery of immune correlates at the expense of a 20% false positivity rate. A post-hoc analysis was performed using CD4 blocking assay results for the antigens B.JRFL, B.63521 and B.6240 as predictors in multivariable logistic regression models predicting transmission status adjusting for delivery mode, log maternal plasma viral load, infant gestational age, and peripheral CD4+ T cell count. No multiple comparison adjustments were performed in post-hoc analyeses.

Definition of score variables

Each score was defined similarly as a weighted combination of multiple immune response variables. For example, to define the V3 IgG or Env IgA score, we first scaled each IgG/IgA binding variable to have standard deviation of one. Then we computed a weight designed to maximize the diversity of the signals contained in all the Env-binding IgA variables [6,18].

Missing data

Neutralization score was missing in six subjects due to limited sample volume. Missing clinical variables included: 8 subjects did not have delivery mode, 11 subjects did not have viral load and 1 subject did not have infant gestational age. We took a multiple imputation approach to handle the missing data in the primary analysis. Twenty imputation datasets were carried out [22] and the regression results across the 20 datasets were combined with the help of the R package mitools from the Comprehensive R Archive Network (CRAN) to properly account for the uncertainty in the regression coefficient estimates [23]. Comparing multiple imputation results with single imputation results, we saw that the two results were nearly identical except for the odds ratio and p value for the neutralization score, which needed to be imputed. As such, in the secondary analysis, we used a single dataset with imputed clinical variables and, when studying the impact of the neutralization variables, excluded subjects with missing neutralization variables.

Post-matching cohort characteristics

Analysis of the post-matching transmitting and non-transmitting cohort revealed no significant differences in the peripheral CD4+ T cell count, plasma virus load, mode of delivery or gestational age (Table 1). Moreover, the year in which the case and control subjects were enrolled was equally distributed. While the available plasma sample that was closest to delivery was selected in all subjects, the distribution of the peripartum visit at which maternal plasma was available differed between the transmitting and non-transmitting subjects (Table 1), with 46% of transmitting mothers with plasma available from the delivery time point, and only 10% of non-transmitting women with plasma available from delivery. Thus, we performed a pilot study that assessed Env IgG and IgA binding, Env-specific IgG avidity, ADCC, and neutralization score in 24 HIV-infected pregnant women with two samples available during the study window. The humoral Env-specific antibody responses were then compared between the earlier and later timepoints by student's t test. The overwhelming majority of antibody responses were not statistically different between peripartum timepoints (Table S1). However, we detected slightly higher IgG responses against the clade B scaffolded V1V2 antigens in the 2nd trimester compared to 3rd trimester (fold difference in mean OD: 0.18, $p = 0.04$ for gp70 case A2 V1V2 and 0.15, $p = 0.01$ for gp70 case A2 V1V2 V169K) and more potent neutralization of the tier 1

strain SF162 (fold difference in mean ID_{50}: 1.45, $p < 0.001$ for NAb SF162) between 3rd trimester and delivery, but these differences did not persist when comparing across other visit windows (Table S1 and Fig. S1). Thus, we selected the maternal plasma sample that was closest for delivery for all subjects for the main study.

Humoral Env-specific response assays

All assays were performed blinded to transmission status. Maternal samples were aliquoted for each assay in an observed, quality-controlled manner (Duke CFAR GCLP-Compliant AIDS Program) to avoid aliquoting mistakes.

Env and V1V2 Binding IgG ELISA

Plate-based binding IgG ELISA to scaffolded (gp70) and avi-tagged V1V2 proteins and MN gp41 and gp120 were performed as follows. Direct binding ELISAs were conducted in 384 well ELISA plates (Costar) coated with 2ug/ml antigen in 0.1M sodium bicarbonate and blocked with assay diluent (PBS containing 4% (w/v) whey protein/ 15% Normal Goat Serum/ 0.5% Tween-20/ 0.05% Sodium Azide). Duplicate sera were incubated for 90 min in three fold serial dilutions beginning at 1:33.3 followed by washing with PBS/0.1% Tween-20. 10ul HRP conjugated goat anti-human secondary antibody (Jackson ImmunoResearch C:109-035-008) was diluted to 1:10,000 in assay diluent without azide, incubated room temperature for 1 hour, washed and detected with 20µl SureBlue Reserve (KPL 53-00-03) for 15 minutes. Reaction was stopped with the addition of 20ul HCL stop solution. Plates were read at 450nm. The plasma dilution with the majority of samples in the linear range (optical density (OD) between 0.5 and 3.5) was determined for each antigen in the pilot study (1:100 for V1V2 antigens, 1:72900 for MN gp41, and 1:8100 for MN gp120) and the OD at the selected dilution was analyzed. To improve the precision of the summary measure, a five-parameter logistic curve was fitted to the dilution series for each subject using the R package nCal from the Comprehensive R Archive Network (CRAN)(21), the fitted OD at the selected dilution was taken to be the readout for the subject. The IgG response against the MuLV gp70 scaffold control was determined and the variance of the V1V2 IgG response was similar with and without subtraction of the gp70 response; therefore, the gp70 response was not subtracted from the gp70 V1V2 IgG responses, similar to the analysis that was done for the RV144 immune correlate analysis [6]. A panel of negative control sera was included for each antigen.

Env IgA and V3 IgG binding antibody multiplex assays (BAMA)

BAMA for IgA were performed as previously described [6,24-26] after IgG depletion from maternal plasma/sera. The multiclade panel of consensus and primary Env gp120 and gp140 antigens, MN gp41, consensus C1 and V3 peptides were covalently coupled to carboxylated fluorescent beads and incubated with the plasma/sera at predetermined dilutions of 1:30 (used for analysis of gp120 and C1 antigens), 1:90 (used for analysis of gp41 and gp140 antigens), 1:2500 (V3 peptides), and 1:500 (MLV gp70-scaffolded B. MN V3 protein) and was detected with a PE-conjugated goat anti-human IgA antibody (Jackson Immunoresearch) at 4 µg/ml, or with a PE-conjugated goat anti-human IgG (Southern Biotech) at 2 µg/ml. Antibody measurements were acquired on the Bio-Plex instrument (Bio-Rad) and the readout was expressed in median fluorescent intensity (MFI). Positive control polyclonal (HIVIG) and/or monoclonal antibodies were included in each assay to ensure specificity, consistency and reproducibility between assays. Negative controls included in every assay were blank beads and HIV-1 negative sera. The preset assay criteria for sample reporting were: coefficient of variation (CV) for duplicate

values ≤ 20% for the IgA and ≤ 15% for the V3 assays and >100 beads counted per sample. To control for reproducibility and Env protein performance, we used a preset criteria that the positive control titer (HIVIG) included in each assay had to be +/- three standard deviations of the mean for each antigen, tracked with Levy Jennings plot calculated with a four-parameter logistic equation (Sigma plot, Systat Software). We then assessed the concentration of the V3-specific IgG antibodies by measuring the response against the clade B V3 linear peptide, sequence: Bio-V3.B Bio-KKKNNTRKSIHIGPGRAFYATGDIIGDIRQAHC at an optimized plasma dilution of 1:31,250 to the known concentration of a serial diluted anti-V3 specific monoclonal antibody standard that was isolated from an RV144 vaccine recipient, CH22 mAb [27]. The concentration was calculated by nonlinear calibration using the five parameter logistic model using the R package nCal [23]. The median concentration of linear consensus clade B V3-binding IgG in transmitting mothers' plasma was 23.8 µg/ml (interquartile range: 8.5-55.3 µg/ml) compared to 39.4 µg/ml (interquartile range: 12.5– 72.6 µg/ml) in non-transmitting women.

Surface Plasmon Resonance (SPR) measurements of plasma IgG avidity

Using the multiplex array format, purified plasma IgG avidity was measured on a BIAcore 4000 instrument (BIAcore/GE Healthcare). Using a Series S CM5 chip (BIAcore/GE Healthcare) 6000-16000 RU of gp120 and gp140 envelope proteins were immobilized through amine coupled directly on the chip surface. For biotinylated V1V2 proteins (C.1086.V1V2, B.caseA2.V1V2, B.CaseA2 V1V2N156QN160Q), the Series S CM5 chip first had 1400-2500 RU of Streptavidin immobilized onto the surface, then the biotinylated V1V2 proteins were then immobilized through Streptavidin/ Biotin interaction and immobilized between 1350-1600 RU. All proteins were immobilized in duplicate spots on the chip. Purified plasma IgG at 200ug/mL was flowed over the chip at 30uL/min for 150s and allowed to dissociate for 600s. Regeneration of the surface was performed using Glycine pH 2.0 flowing over the surface for 30s two times. Non-specific interactions were subtracted out using the negative control respiratory syncytial virus (RSV) mAb palivizumab flowed over each surface. Antigen surface activity was monitored by flowing the positive control mAbs CH58 and A32 at 10 µg/mL. Antigen surface decay was monitored by running CH58 and A32 every 20 cycles throughout the entire run and was used to normalize the purified plasma IgG response. Data analysis was performed using BIAcore 4000 evaluation and BIAcore evaluation 4.1 software (BIAcore/GE Helathcare). Data analysis and Avidity score calculation was done as previously described [6]. In order to primarily assess avidity to epitopes of broadly neutralizing antibodies, avidity against the clade B Env B.6420 gp140 was selected as the primary avidity variable based on the ability of the B.6420 gp120 to bind to broadly HIV-neutralizing mAbs, including the V2-specific monoclonal antibody PG9 (32.1 nM), but not the nonbroadly-neutralizing V2-specific monoclonal antibody CH58 [28].

ADCC

HIV-1 reporter viruses used in ADCC assays were replication-competent infectious molecular clones (IMC) designed to encode the SF162.LS (accession number EU123924) or the transmitted/founder WITO.c (accession number JN944948) subtype B *env* genes in *cis* within an isogenic backbone that also expresses the *Renilla* luciferase reporter gene and preserves all viral orfs. The Env-IMC-LucR viruses used were NL-LucR.T2A-SF162.ecto (IMC_{SF162}) and NL-LucR.T2A-WITO.ecto (IMC_{WITO})[29]. IMCs were titrated in order to achieve maximum expression within 72 hours post-infection by detection of Luciferase activity and intra-cellular p24 expression. We infected $CEM.NKR_{CCR5}$ cells (NIH AIDS Research and Reference Reagent Repository) with IMC_{SF162} and IMC_{WITO} by incubation with the appropriate $TCID_{50}$/cell dose of

IMC for 0.5 hour at 37ºC and 5% CO_2 in presence of DEAE-Dextran (7.5 µg/ml). The cells were subsequently resuspended at $0.5x10^6$/ml and cultured for 72 hours in complete medium containing 7.5µg/ml DEAE-Dextran. The infection was monitored by measuring the frequency of cells expressing intracellular p24. Assays performed using the IMC-infected target cells were considered reliable if the percentage of viable $p24^+$ target cells was ≥20% on assay day.

A luciferase-based ADCC assay was performed as previously described [28]. Briefly, HIV-1 IMC_{SF162} and IMC_{WITO} infected $CEM.NKR_{CCR5}$ cells were used as targets. Whole PBMC obtained from a HIV seronegative donor with the F/V Fc-gamma Receptor (FcRγ) IIIa phenotype were used as the source of NK effector cells. After overnight resting, the PBMC were used as effector cells at an effector to target ratio of 30:1. The target and effector cells were incubated in the presence of 5-fold serial concentrations of plasma starting at 1:50 dilution for 6 hours at 37ºC in 5% CO_2. The final read-out was the luminescence intensity generated by the presence of residual intact target cells that have not been lysed by the effector population in presence of ADCC-mediating mAb. The % of killing was calculated using the formula:

$$\% \text{ killing} = \frac{(\text{RLU of Target + Effector well}) - (\text{RLU of test well})}{\text{RLU of Target + Effector well}} \times 100$$

In this analysis, the RLU of the target plus effector wells represents spontaneous lysis in absence of any source of Ab. Plasma samples collected from a HIV-1 seronegative and seropositive donor were used as negative and positive control samples, respectively, in each assay.

Neutralization

Neutralization was performed with heat-inactivated plasma or sera or recombinantly-produced monoclonal antibodies as previously described [30] using Tat-regulated Luc reporter gene expression to quantify reductions in virus infection in TZM-bl cells (NIH AIDS Reagent Program, contributed by John Kappes and Xiaoyn Wu). For the MTCT immune correlate study, two clade B tier 1A (B.MN.3, B.SF162.LS), four tier 1B (B.Bal.26, B.SS1196.1, B.6535.3, B.1012.11TC21), four tier 2 (B.AC10.0.29, B.REJO4541.67, B.RHPA4259.7, B.WITO4160.33) Env pseudovirions produced in 293T cells were selected for the neutralization panel. The tier 1 and 2 variants were selected based on their ability to be differentially neutralized by a panel of HIV-neutralizing sera [31], potentially representing distinct neutralization epitope targets. Twelve three fold dilutions of plasma starting at 1:40 were included, yet target cell toxicity of some of the samples limited detection of neutralization below 1:100, and thus our cut off of detection was set at 1:100 for all plasma samples and viruses. Autologous V3-specific IgG antibodies were tested for neutralization against autologous *env* pseudovirus variants starting at a concentration of 50 µg/ml. The neutralization titer is reported as the dilution or concentration at which the relative luminescence units were reduced by 50% (inhibitory dose or concentration 50%, ID_{50} or IC_{50}) compared to the RLU in virus control wells. Any sample with an ID_{50} <100 was set to 50. Plasma samples that had neutralizing activity against a nonspecific retrovirus (Murine Leukemia Virus, SVA.MLV) were tested for the presence of antiretroviral drugs and if detected, the subject was removed from the study (n = 1 non-transmitter). Inadequate plasma volume was available for the neutralization panel in 6 subjects, so these subjects did not have a neutralization score assigned. All other assays were performed on all study subjects.

Soluble CD4 plasma blocking assays

Similar to the previously-described soluble CD4 (sCD4) blocking assay [32], 384 well ELISA plates (Costar #3700) were coated with 30ng/well of each HIV-1 Env (B.JRFL, B.6240, B.63521) overnight at 4℃ and blocked with assay diluent (PBS containing 4% (w/v) whey protein/ 15% Normal Goat Serum/ 0.5% Tween20/ 0.05% Sodium Azide) for 1 hour at room temperature. All assay steps were conducted in assay diluent (except substrate) and incubated for 1.5 hours at room temperature followed by washing with PBS/0.1% Tween-20. Plasma samples were diluted 1:50 and incubated in triplicate wells. To measure plasma antibody sCD4 binding site blocking, 10µl of a predetermined saturating concentration of sCD4 (Progenics Pharm Inc., 0.64 µg/ml) was added following the plasma incubation step. sCD4 binding was detected by 1 hour incubations with biotinylated anti-CD4 mAb OKT4 (0.015 µg/ml) and streptavidin-HRP at 1:30,000 dilution followed by TMB substrate. Plates were read with a plate reader at 450nm. Triplicate wells were background subtracted and averaged. Percent inhibition was calculated as follows: 100-(plasma triplicate mean/no plasma control mean)*100.

Generation of full length env sequences and env pseudovirion production from maternal plasma

Viral RNA (vRNA) was prepared from plasma samples (500 µL) using the EZ1Virus Mini Kit V2.0 on BIO ROBOT EZ1 (Qiagen; Valencia, CA). Reverse transcription was performed with 20 µL of vRNA and 80 pmol primer 1.R3.B3R (5'-ACTACTTGAAGCACTCAAGGCAAGCTTTATTG-3'; HXB2 nt9611-9642) in 50 µL using Superscript III (Invitrogen; Carlsbad, CA). The complete *env* genome was amplified by single genome amplification (SGA) using OFM19 (5'-GCACTCAAGGCAAGCTTTATTGAGGCTTA-3'; nt9604-9632) and VIF1 (5'-GGGTTTATTACAGGGACAGCAGAG -3'; nt4900-4923) as first round primers, and env1A (5'-GGCTTAGGCATCTCCTATGGCAGGAAGAA-3'; nt5954-5982) and envN (5'-CTGCCAATCAGGGAAGTAGCCTTGTGT -3'; nt9145-9171) as the second round primers. The PCR thermocycling conditions were as follows: one cycle at 94°C for 2 minutes; 35 cycles of a denaturing step at 94°C for 15 seconds, an annealing step at 55°C for 30 second, an extension step at 68°C for 4 minutes; and one cycle of an additional extension at 68°C for 10 minutes. The PCR products were purified with the QiaQuick PCR Purification kit (Qiagen; Valencia, CA). The SGA amplicons were directly sequenced by the cycle sequencing and dye terminator methods on an ABI 3730xl genetic analyzer (Applied Biosystems, Foster City, CA). Individual sequences were assembled and edited using Sequencher 4.7 (Gene Codes, Ann Arbor, MI). The sequences were aligned and the manual adjusted for optimal alignment using Seaview. The Neighbor-joining (NJ) tree was constructed using the Kimura 2-parameter model. The CMV promoter was added to the 5′ end of each *env* gene amplified by SGA using the promoter addition PCR (pPCR) method as described [33]. *Env* mutants containing single or double mutants that were associated with neutralization susceptibility changes were constructed using the Quick Change II Site-Directed Mutagenesis kit (Agilent Technologies, Santa Clara, CA). All final *env* mutants were confirmed by sequencing. The Env pPCR products and mutant were used for generation of pseudoviruses by cotransfecting with the *env*-deficient HIV-1 backbone pSG3Δenv into 293T cells in a 6-well tissue culture plate using FuGENE6 transfection reagent (Roche Diagnostics; Indianapolis, IN). The pPCR product was used for generation of pseudoviruses by cotransfecting with the *env*-deficient HIV-1 backbone pSG3Δenv into 293T cells in a 6-well tissue culture plate using FuGENE6 transfection reagent (Roche Diagnostics; Indianapolis, IN). Transfected cells were maintained in DMEM with 10% FBS at 37°C with 5% CO2. Forty-eight hours after transfection, supernatants were harvested and stored in 20% FBS medium at -80°C. Neutralization sensitivity (tier designation) of the *env* pseudoviruses was determined by assessing neutralization potency by HIVIG and plasma

samples from HIV-infected individuals with established neutralization potency (H243 and S455) against the panel of autologous viruses in the TZM-bl neutralization assay. The heat map demonstrating the hierarchical neutralization sensitivity of the virus variants was produced using tools available from www.lanl.gov/content/sequence/HEATMAP/heatmap.html.

Isolation of B.Con V3 tetramer-specific B cells and monoclonal antibody production

Peripheral Env V3-specific IgG-expressing memory B cell isolation from a non-transmitting mother at six months postpartum was performed as described [34-36] with the following modifications. Thawed PBMCs were stained with a viability marker (Aqua Vital Dye- Life technologies-L34957), SAV-AF647 (Life technologies-S21374), SAV-BV421 (BioLegend-405225) labeled-tetramers and the following antibodies: CD27 PE-Cy7 (eBioscience), anti-IgG FITC (Jackson Immuno Research), IgD PE, CD19 APC-Cy7, CD3 PE-Cy5, CD235a PE-Cy5 (BD Pharmingen), CD14 BV605, CD16 BV570 (Sony/iCyt), CD10 ECD, CD38 APC AF700 (Beckman Coulter) (Table S8). Total B cells were gated as viable (Aqua Vital Dye negative) CD14/CD16 and CD3/CD235a negative, and CD19+. Env V3-specific, IgG-expressing memory B cells were further selected by gating for IgG+ and Con.B V3 peptide tetramer +/+ cells using dual color antigen-specific labeling (SAV-AF647 and SAV-BV421). Flow cytometric data was acquired on a BD FACS AriaII (BD Biosciences) at the time of sorting and the data analyzed using FlowJo (Tree Star Inc). Cell sorting of the double positive tetramer stained cell population was performed using a FACSAria2 (BD Biosciences) as single-cells into 96-well plates precharged with an RNA stabilization cocktail and subjected to immunoglobulin gene RNA amplification, as previously described [37]. Immunoglobulin gene analysis was performed as previously described [38]. Overlapping PCR was used to construct full length IgG1 (for heavy chain) and kappa or lambda (for light chain) cassettes for expression of recombinant antibodies [37]. Transiently-transfected antibodies were tested for Env V3 binding against a panel of consensus V3 peptides and MLVgp70-scaffolded V3 antigens by ELISA [28]. Selected antibodies were cloned into pcDNA 3.3 (Invitrogen) and co-transfected into 293T cells using polyethylenimine [39] for large-scale production of the V3-specific antibodies for neutralization assays.

Abbreviations:

ADCC, antibody-dependent cellular cytotoxicity; CD4bs, CD4 binding site; Env, envelope; mAb, monoclonal antibody; MTCT, mother-to-child transmission; SAV, streptavidin; SGA, single genome amplification; V1V2, first and second variable region; V3, third variable region; Women and Infant Transmission Study, (WITS).

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Authors' contributions:

SP designed the study and provided the oversight of the laboratory work, interpreted data, and wrote the manuscript, YF and NV designed and performed the statistical analysis, GF designed and performed immunologic assays (YF, NV, and GF equally contributed to this manuscript), PG contributed to statistical analysis design and interpretation, RP, FJ, JP, AM, BL, KL, GO, KW, XS, NY developed and performed immunologic assays, HC, JP, ES, EF performed monoclonal antibody production, DM, JW, TG, TV, DM developed, optimized, and performed the flow cytometric B cell isolation, FC, AK performed virus sequencing and production, SX, XL performed monoclonal antibody sequencing, RL, SW performed sample and database management, SD performed data cleaning and quality control, MS performed quality control of sample aliquotting, HL oversaw monoclonal antibody isolation and production, GF, MA, DM, GT designed and provided oversight of immunoassays and interpreted the data, TD led sample acquisition and management and interpreted data, MAM led antigen-specific B cell isolation, FG led virus isolation and signature sequence analysis and BH oversaw study design, data analysis and interpretation and manuscript development.

Conflict of interest statements:

No conflict of interest exists among the authors

Figures S1-5:

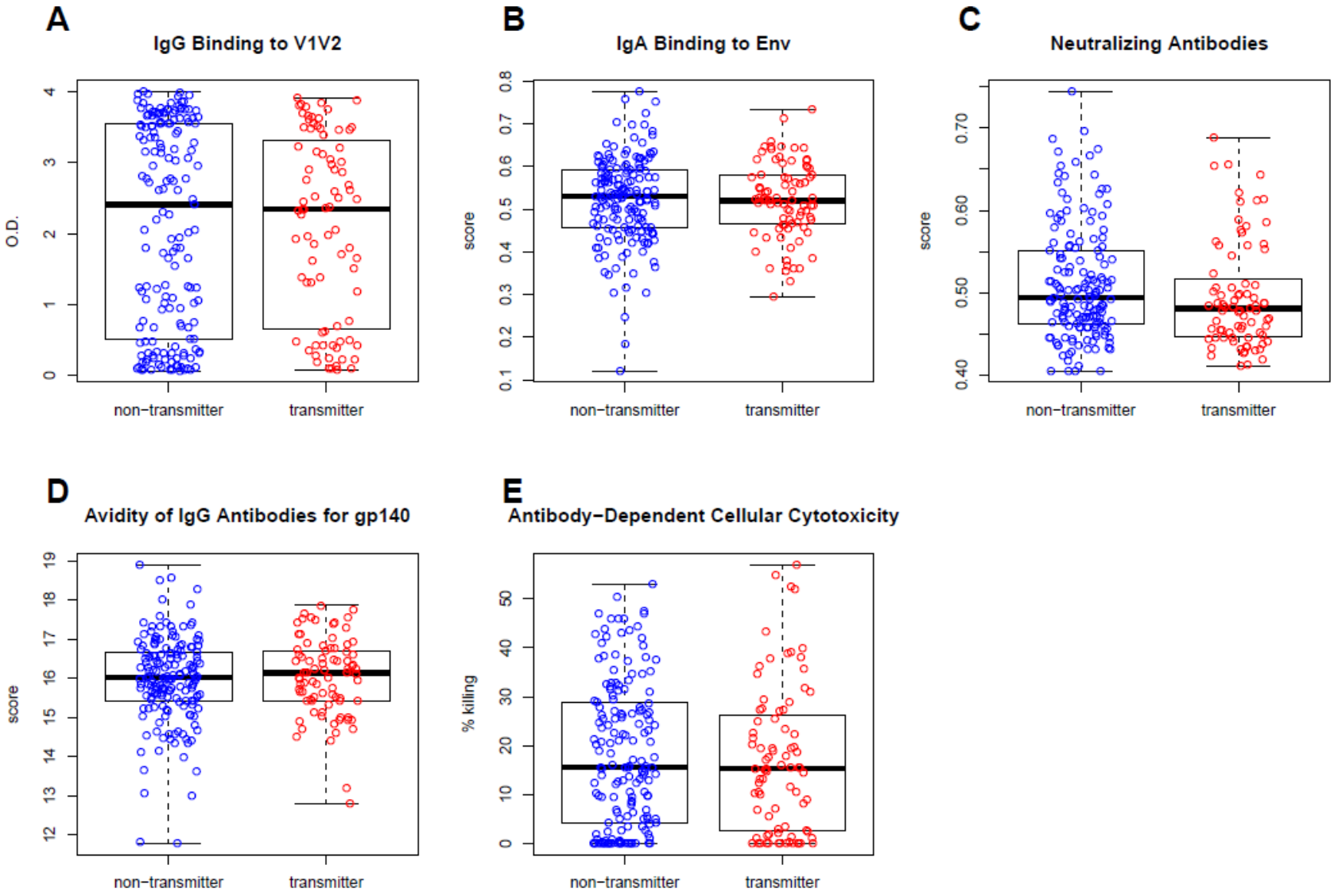

Figure S1. Comparison of the primary RV144 clade B-modified humoral immune responses in HIV-infected transmitting and non-transmitting women. Neither the maternal V1V2-specific IgG response (A), the clade B-modified Env-binding IgA score (B), clade B

neutralizing antibody score (C), clade B gp140 avidity (D) or the ADCC response (E) correlated with the risk of MTCT. Non-transmitting women are shown on the left (blue) and transmitting women are shown on the right (red) of each graph.

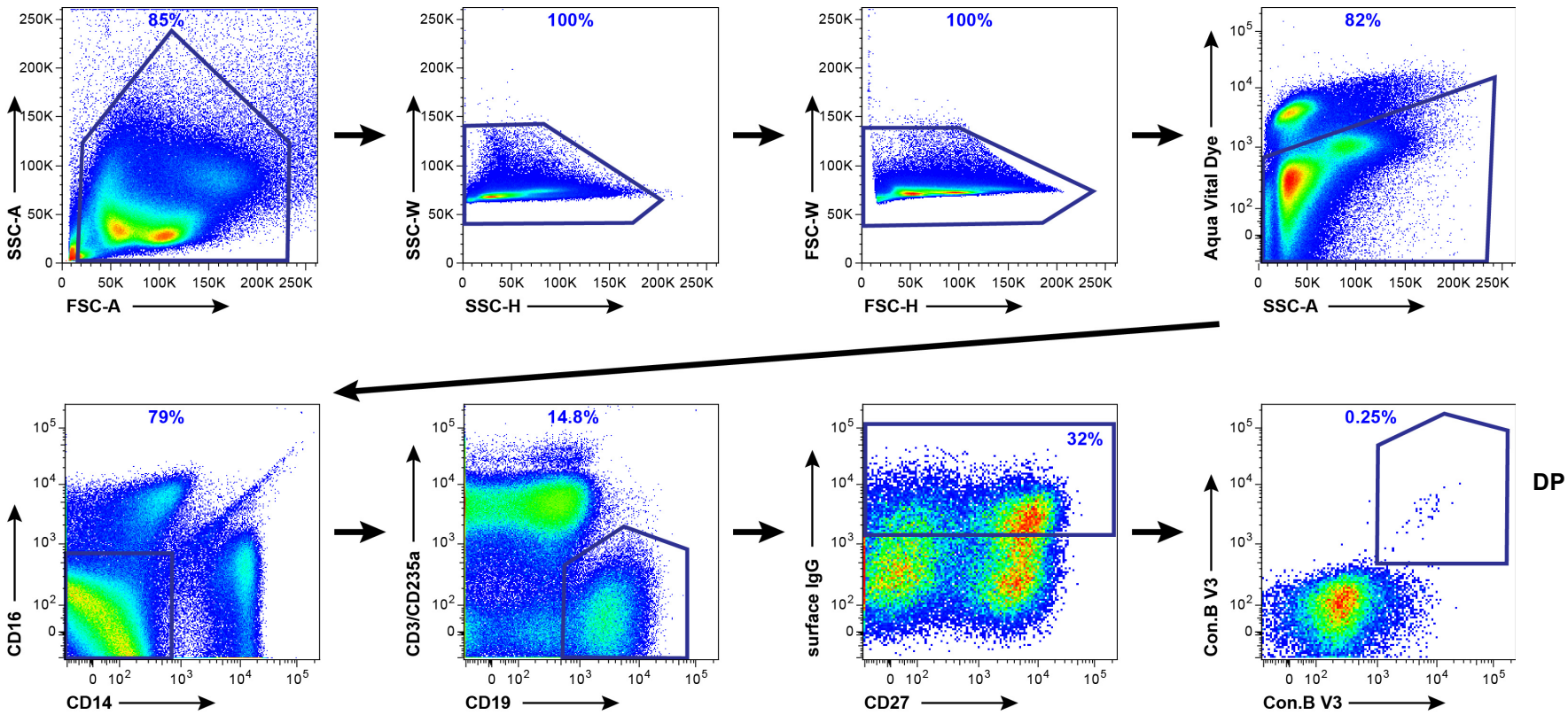

Figure S2. Gating scheme for isolation of Env V3-specific IgG-producing memory B cells for recombinant V3-specific mAb production. PBMCs from a non-transmitting mother were stained for B cell markers and dually labeled Env Con.B V3 peptide tetramer. The gating scheme was as follows: generous lymphocyte gate on forward scatter/side scatter plot, selection for singlet events by side scatter and forward scatter, selection of live, aqua vital-dye negative cells, selection of CD14/CD16 negative cells, selection of CD19 positive, CD3/CD235a negative cells, selection of IgG-expressing cells (memory B cells), then sorting of CD19+IgG+Con.B V3 tetramer (AF647 vs BV421) double positive (DP) cells (diagonal stripe).

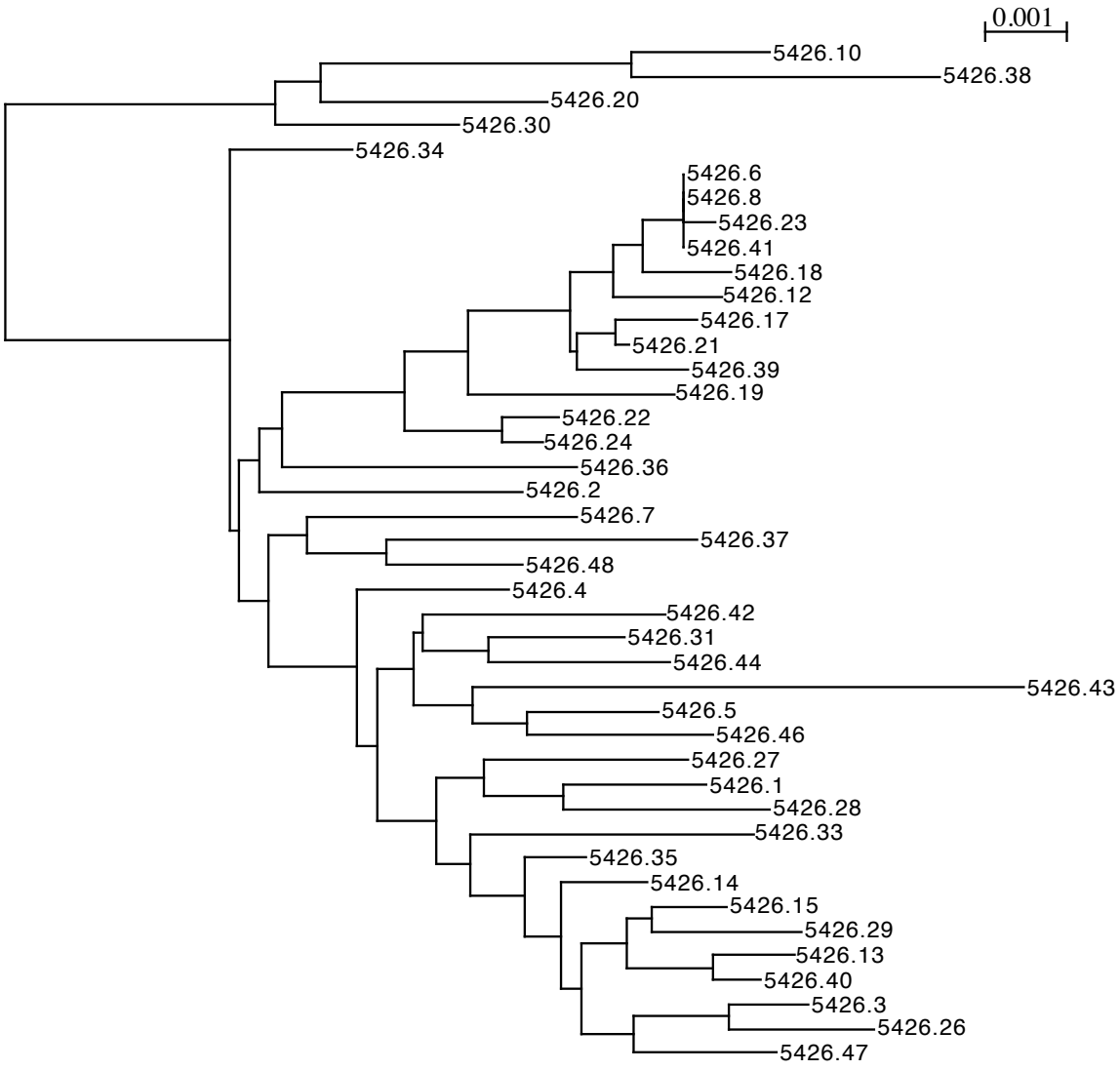

Figure S3. Phylogenetic tree analysis of complete *env* gene sequences isolated fro collected at two months postpartum of a non-transmitting, HIV-1-infected mother and neutralization sensitivity against autologous Env V3-specific monoclonal antibo phylogenetic tree was constructed with the nucleotide sequences of complete *env* gene neighbor-joining method. The tree was midpoint rooted. Horizontal branch lengths are draw (the scale bar represents 0.001 nucleotide substitution per site). Vertical separation is for c All sequenced, full length *env* genes are included, though three *env* variants yielded very low functional virus and were unable to be used in neutralization assays (Fig. 2).

Fig. S4

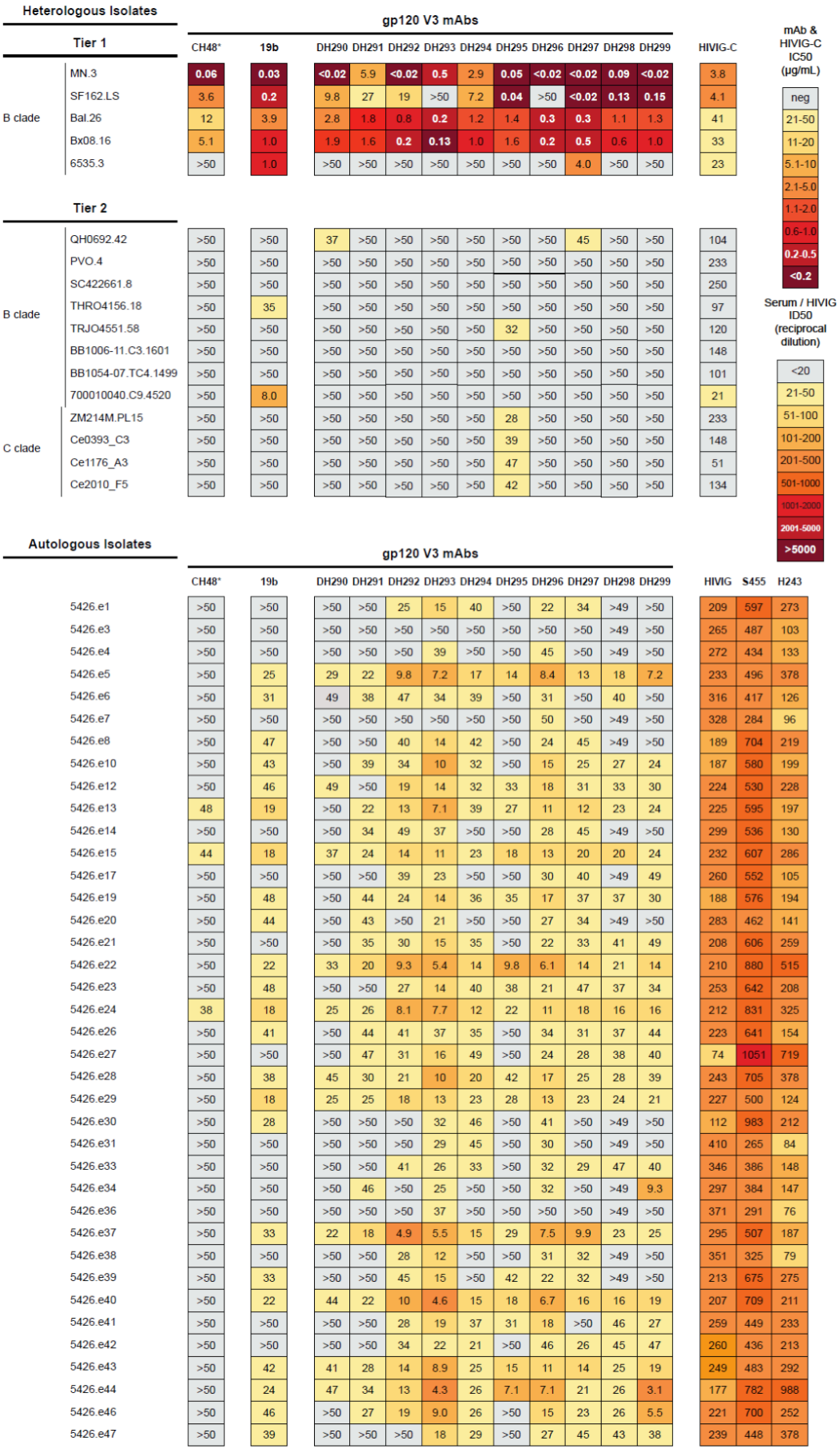
Heterologous Isolates
gp120 V3 mAbs
Tier 1
CH48*
19b
DH290
DH291
DH292
DH293
DH294
DH295
DH296
DH297
DH298
DH299
HIVIG-C
MN.3 0.06 0.03 <0.02 5.9 <0.02 0.5 2.9 0.05 <0.02 <0.02 0.09 <0.02 3.8
SF162.LS 3.6 0.2 9.8 27 19 >50 7.2 0.04 >50 <0.02 0.13 0.15 4.1
B clade
Bal.26 12 3.9 2.8 1.8 0.8 0.2 1.2 1.4 0.3 0.3 1.1 1.3 41
Bx08.16 5.1 1.0 1.9 1.6 0.2 0.13 1.0 1.6 0.2 0.5 0.6 1.0 33
6535.3 >50 1.0 >50 >50 >50 >50 >50 >50 >50 4.0 >50 >50 23
Tier 2
QH0692.42 >50 >50 37 >50 >50 >50 >50 >50 >50 45 >50 >50 104
PVO.4 >50 >50 >50 >50 >50 >50 >50 >50 >50 >50 >50 >50 233
SC422661.8 >50 >50 >50 >50 >50 >50 >50 >50 >50 >50 >50 >50 250
THRO4156.18 >50 35 >50 >50 >50 >50 >50 >50 >50 >50 >50 >50 97
B clade
TRJO4551.58 >50 >50 >50 >50 >50 >50 >50 32 >50 >50 >50 >50 120
BB1006-11.C3.1601 >50 >50 >50 >50 >50 >50 >50 >50 >50 >50 >50 >50 148
BB1054-07.TC4.1499 >50 >50 >50 >50 >50 >50 >50 >50 >50 >50 >50 >50 101
700010040.C9.4520 >50 8.0 >50 >50 >50 >50 >50 >50 >50 >50 >50 >50 21
ZM214M.PL15 >50 >50 >50 >50 >50 >50 >50 28 >50 >50 >50 >50 233
C clade
Ce0393_C3 >50 >50 >50 >50 >50 >50 >50 39 >50 >50 >50 >50 148
Ce1176_A3 >50 >50 >50 >50 >50 >50 >50 47 >50 >50 >50 >50 51
Ce2010_F5 >50 >50 >50 >50 >50 >50 >50 42 >50 >50 >50 >50 134
mAb & HIVIG-C IC50 (µg/mL)
neg
21-50
11-20
5.1-10
2.1-5.0
1.1-2.0
0.6-1.0
0.2-0.5
<0.2
Serum / HIVIG ID50 (reciprocal dilution)
<20
21-50
51-100
101-200
201-500
501-1000
1001-2000
2001-5000
>5000
Autologous Isolates
gp120 V3 mAbs
CH48*
19b
DH290
DH291
DH292
DH293
DH294
DH295
DH296
DH297
DH298
DH299
HIVIG
S455
H243
5426.e1 >50 >50 >50 >50 25 15 40 >50 22 34 >49 >50 209 597 273
5426.e3 >50 >50 >50 >50 >50 >50 >50 >50 >50 >50 >49 >50 265 487 103
5426.e4 >50 >50 >50 >50 >50 39 >50 >50 45 >50 >49 >50 272 434 133
5426.e5 >50 25 29 22 9.8 7.2 17 14 8.4 13 18 7.2 233 496 378
5426.e6 >50 31 49 38 47 34 39 >50 31 >50 40 >50 316 417 126
5426.e7 >50 >50 >50 >50 >50 >50 >50 >50 50 >50 >49 >50 328 284 96
5426.e8 >50 47 >50 >50 40 14 42 >50 24 45 >49 >50 189 704 219
5426.e10 >50 43 >50 39 34 10 32 >50 15 25 27 24 187 580 199
5426.e12 >50 46 49 >50 19 14 32 33 18 31 33 30 224 530 228
5426.e13 48 19 >50 22 13 7.1 39 27 11 12 23 24 225 595 197
5426.e14 >50 >50 >50 34 49 37 >50 >50 28 45 >49 >50 299 536 130
5426.e15 44 18 37 24 14 11 23 18 13 20 20 24 232 607 286
5426.e17 >50 >50 >50 >50 39 23 >50 >50 30 40 >49 49 260 552 105
5426.e19 >50 48 >50 44 24 14 36 35 17 37 37 30 188 576 194
5426.e20 >50 44 >50 43 >50 21 >50 >50 27 34 >49 >50 283 462 141
5426.e21 >50 >50 >50 35 30 15 35 >50 22 33 41 49 208 606 259
5426.e22 >50 22 33 20 9.3 5.4 14 9.8 6.1 14 21 14 210 880 515
5426.e23 >50 48 >50 >50 27 14 40 38 21 47 37 34 253 642 208
5426.e24 38 18 25 26 8.1 7.7 12 22 11 18 16 16 212 831 325
5426.e26 >50 41 >50 44 41 37 35 >50 34 31 37 44 223 641 154
5426.e27 >50 >50 >50 47 31 16 49 >50 24 28 38 40 74 1051 719
5426.e28 >50 38 45 30 21 10 20 42 17 25 28 39 243 705 378
5426.e29 >50 18 25 25 18 13 23 28 13 23 24 21 227 500 124
5426.e30 >50 28 >50 >50 >50 32 46 >50 41 >50 >49 >50 112 983 212
5426.e31 >50 >50 >50 >50 >50 29 45 >50 30 >50 >49 >50 410 265 84
5426.e33 >50 >50 >50 >50 41 26 33 >50 32 29 47 40 346 386 148
5426.e34 >50 >50 >50 46 >50 25 >50 >50 32 >50 >49 9.3 297 384 147
5426.e36 >50 >50 >50 >50 >50 37 >50 >50 >50 >50 >49 >50 371 291 76
5426.e37 >50 33 22 18 4.9 5.5 15 29 7.5 9.9 23 25 295 507 187
5426.e38 >50 >50 >50 >50 28 12 >50 >50 31 32 >49 >50 351 325 79
5426.e39 >50 33 >50 >50 45 15 >50 42 22 32 >49 >50 213 675 275
5426.e40 >50 22 44 22 10 4.6 15 18 6.7 16 16 19 207 709 211
5426.e41 >50 >50 >50 >50 28 19 37 31 18 >50 46 27 259 449 233
5426.e42 >50 >50 >50 >50 34 22 21 >50 46 26 45 47 260 436 213
5426.e43 >50 42 41 28 14 8.9 25 15 11 14 25 19 249 483 292
5426.e44 >50 24 47 34 13 4.3 26 7.1 7.1 21 26 3.1 177 782 988
5426.e46 >50 46 >50 27 19 9.0 26 >50 15 23 26 5.5 221 700 252
5426.e47 >50 39 >50 >50 >50 18 29 >50 27 45 43 38 239 448 378

Figure S4. Env V3-specific, heterologous tier 1 virus-neutralizing antibodies isolated from a non-transmitting woman can neutralize all autologous plasma HIV-1 env variants isolated from maternal plasma. Neutralization potency (IC_{50}) of heterologous V3-specific mAbs (19B and CH48) and 10 autologous maternal V3-specific IgG mAbs (DH290-299) against tier 1 and 2 heterologous and autologous SGA-derived *env* pseudovirus variants. Polyclonal anti-HIV antibody, HIVIG-C, and two serum samples from HIV-infected individuals (S455 and H243) is included to assess the neutralization-sensitivity of the *env* pseudovirus variants.

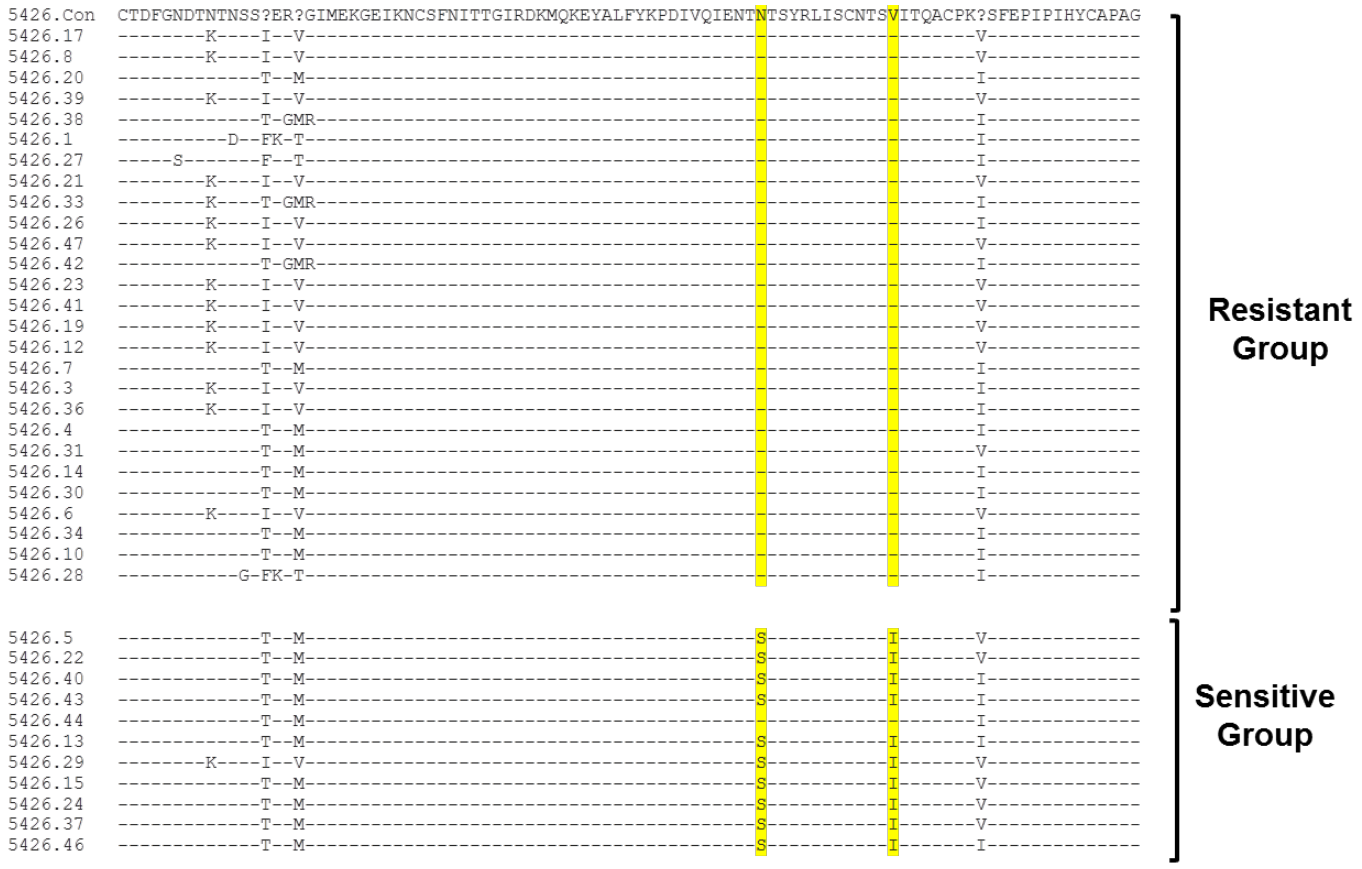

Figure S5. Alignment of variable loop 1 and 2 (V1,V2) and constant region 2 (C2) sequences from Env variants isolated from a non-transmitting mother grouped by neutralization sensitivity to autologous V3-specific mAbs (Fig. S4). While the sequence in the V3 loop was uniform among the Env sequences of the non-transmitting mother, two unique mutations were identified in the V2 loop and C2 region of the Env sequences from the group of the most neutralization-sensitive pseudovirus variants (Sensitive group). The V2 loop mutation (N188S) moves a potential N-linked glycosylation site by three amino acids (www.lanl.gov/content/sequence/GLYCOSITE/glycosite.html).

Tables S1-8:

Table S1. Clinical characteristics of the post-matching cohort of transmitting and nontransmitting HIV-1 infected, untreated mothers

Variable	Transmission status	N[1]	Mean (Range)	SD	P value[2]
Plasma viral load (copies/ml)	Nontransmitters	160	62,906 (167 – 1,031,250)	139,273	
	Transmitters	83	112,235 (699 – 3,101,258)	347,778	0.22
CD4 Count (cells/µl)	Nontransmitters	170	553.3 (32 - 2330)	333.3	
	Transmitters	83	488.5 (16 – 1792)	285.3	0.08
Gestational Age (weeks)	Nontransmitters	170	37.8 (28 – 43)	2.8	
	Transmitters	84	37.3 (26 – 42)	3.2	0.18
Variable	**Transmission status**	**N**	**Percent**	**P value[3]**	
Birth type					
Vaginal birth	Nontransmitters	125/170	74%	0.74	
	Transmitters	64/85	75%		
Cesarean section	Nontransmitters	40/170	24%		
	Transmitters	17/85	20%		
Birth year					
1990	Nontransmitters	37/166	22%		
	Transmitters	14/83	17%		
1991	Nontransmitters	53/166	32%		

	Transmitters	**23/83**	**28%**
1992	**Nontransmitters**	**32/166**	**19%**
	Transmitters	**20/83**	**24%**
1993	**Nontransmitters**	**28/166**	**17%**
	Transmitters	**18/83**	**22%**
1994-1998	**Nontransmitters**	**16/83**	**9%**
	Transmitters	**8/83**	**10%**
Visit			
25 weeks gestation	**Nontransmitters**	**58/166**	**35%**
	Transmitters	**8/83**	**10%**
34 weeks gestation	**Nontransmitters**	**41/166**	**25%**
	Transmitters	**33/166**	**40%**
Delivery	**Nontransmitters**	**16/166**	**10%**
	Transmitters	**38/83**	**46%**
2 months postpartum	**Nontransmitters**	**51/166**	**31%**
	Transmitters	**4/83**	**5%**

1. **Missing data from 85 transmitters and 170 clinically-matched non-transmitters included: plasma viral load of 10 non-transmitters and 2 transmitter, and gestational age of 1 transmitter. Two transmitters and 4 non-transmitters were removed from the study following matching due to lack of adequate sample availability.**

2. **P value calculated by student's t test**

P value calculated by Chi squared test

Table S2. Comparison of maternal plasma Env-specific IgG/IgA responses collected prepartum and peripartum (Pilot study)

Humoral assay type (units)	Env antigen/virus	25 wks vs 34 wks	p	34 wks vs delivery	p	25 wks vs delivery	p
Env-specific IgG (difference in mean OD)	AE.A244 V1V2	0.15	0.09	0.1	0.06	0.14	0.3
	C.1086 V1V2	0.12	0.08	0.14	0.19	0.19	0.1
	B.CaseA V1V2 gp70	**0.18**	**0.04**	0.13	0.09	0.17	0.1
	B.CaseA2V1V2169K gp70	**0.15**	**0.01**	0.15	0.09	0.12	0.3
	B.MN gp120	-0.02	0.48	0.2	0.26	0.18	0.0
	B.MN gp41	-0.03	0.45	0.16	0.16	0.16	0.0
Env-specific IgA (fold change in MFI)	00MSA4076 gp140	0.94	0.58	0.8	0.24	1.11	0.5
	A1con03140CF gp140	0.93	0.37	0.81	0.39	1.33	0.2
	AE.A244gp120	0.75	0.32	1.47	0.73	0.26	0.1
	B.Con gp120	0.93	0.13	0.94	0.42	1.18	0.1
	B.C.C1 peptide IgA	0.92	0.26	0.92	0.6	0.99	0.9
	Con6 gp120	1	0.99	1.06	0.66	1.12	0.5
	ConS gp140	0.9	0.63	0.59	0.46	1	1
	B.MN gp41	0.95	0.57	0.98	0.84	1.1	0.4
	B.MN gp120	0.92	0.35	1.23	0.34	0.87	0.5
	B.VRC gp140	1.36	0.16	0.43	0.41	2.9	0.3
Neutralization (fold change in ID_{50})	B.101211TC21 NAb	1.4	0.43	1.64	0.45	1	NA
	B.65353 NAb	1.04	0.9	1.66	0.13	1.93	0.1
	B.AC10029 NAb	1.13	0.57	1.73	0.16	1	NA
	B.BaL26 NAb	1.02	0.91	1.19	0.15	1.48	0.1
	B.MN3 NAb	1.04	0.89	1.74	0.2	1.21	0.1
	B.REJO454167 NAb	1.39	0.5	1.67	0.38	1.62	0.2
	B.RHPA42597 NAb	1.16	0.24	1.26	0.17	1	NA
	B.SF162LS NAb	0.78	0.08	**1.45**	**<0.01**	0.98	0.8
	B.SS11961 NAb	1.14	0.7	1.52	0.18	1.53	0.1
	B.WITO416033 NAb	1.34	0.35	1.51	0.36	1	NA
IgG avidity (fold change in avidity score)	B.MN gp120	0.97	0.56	1.06	0.35	1.12	0.4
	B.6240 gp140	0.96	0.42	1.01	0.87	0.99	0.8
	AE.A244 V1V2K169V	0.93	0.27	1.22	0.39	0.68	0.3
	B.CaseA2 V1V2K169V	1.05	0.47	1.05	0.67	0.76	0.2
	C.1086 V1V2	1.09	0.46	1.09	0.26	0.79	0.4
	B.63521 V1V2	1.19	0.38	0.92	0.53	0.92	0.7
	AE.A244 V1V2	1.1	0.27	1.11	0.52	0.84	0.5
	B.CaseA2 V1V2	1.06	0.66	0.95	0.47	0.74	0.2
ADCC difference in mean % specific lysis)	B.SF162	-1.14	0.68	5.66	0.07	7.33	0.0

Table S3. Odds ratios of HIV-1 MTCT for each measured Env-specific humoral immune responses (secondary analysis)

	Odds Ratio (95% CI)	P value	Q value
B.SF162 Nab	0.67 (0.50-0.90)	0.006	0.25
B.MN3 Nab	0.71 (0.54-0.95)	0.02	0.4
B.V3 IgG	0.75 (0.58-0.98)	0.035	0.47
B.MN V3 gp70 IgG	0.78 (0.59-1.01)	0.061	0.49
M.V3 IgG	0.78 (0.59-1.01)	0.061	0.49
B.WITO ADCC	1.26 (0.96-1.65)	0.088	0.57
B.BaL26 Nab	0.79 (0.60-1.05)	0.106	0.57
B.RHPA42597 Nab	0.78 (0.57-1.07)	0.117	0.57
B.SS11961 Nab	0.80 (0.60-1.08)	0.141	0.57
B.CaseA2 V1V2 IgG avidity	0.81 (0.61-1.07)	0.142	0.57
B.AC10029 Nab	0.80 (0.59-1.09)	0.158	0.57
B.65353 Nab	0.82 (0.61-1.10)	0.177	0.59
B.REJO454167 Nab	0.83 (0.62-1.11)	0.204	0.6
A.00MSA4076 gp140 IgA	0.84 (0.64-1.10)	0.211	0.6
B.MN gp120 avidity	0.85 (0.65-1.11)	0.23	0.61
Con6 gp120 IgA	0.86 (0.66-1.13)	0.275	0.69
A1con03 gp140 IgA	0.88 (0.67-1.14)	0.329	0.7
AE.A244 gp120K169V IgG avidity	0.89 (0.68-1.16)	0.382	0.7
ConS gp140 IgA	0.89 (0.68-1.17)	0.395	0.7
C.1086 V1V2 IgG avidity	0.89 (0.67-1.18)	0.411	0.7
AE.A244 V1V2 IgG	0.89 (0.68-1.17)	0.413	0.7
B.C.C1 peptide IgA	1.12 (0.85-1.48)	0.415	0.7
B.MN gp120 IgG	0.89 (0.68-1.17)	0.415	0.7
B.CaseA2 V1V2N156QN160Q IgG avidity	1.11 (0.86-1.44)	0.419	0.7
B.6240 gp120mutC IgG avidity	0.90 (0.69-1.17)	0.435	0.7
AE.A244gp120 IgG	0.90 (0.69-1.19)	0.466	0.72
AE.C1peptide IgA	1.10 (0.83-1.44)	0.513	0.76
B.101211TC213257 Nab	1.08 (0.82-1.42)	0.576	0.8
B.SF162 ADCC	0.93 (0.70-1.22)	0.58	0.8
B.con03 gp140 IgA	0.93 (0.71-1.22)	0.6	0.8
B.CaseA2V1V2169K gp70 IgG	0.95 (0.73-1.25)	0.728	0.9
B.WITO416033 Nab	1.05 (0.79-1.38)	0.75	0.9
B.MN gp41 IgA	0.96 (0.73-1.26)	0.761	0.9
AE.A244 gp120 monomer	0.97 (0.73-1.28)	0.817	0.9
B.6240 gp140 IgG avidity	1.03 (0.78-1.36)	0.838	0.9
AE.A244 gp120 IgA	0.97 (0.74-1.28)	0.853	0.9
B.MN gp41 IgG	0.98 (0.74-1.28)	0.858	0.9
B.CaseA V1V2 gp70 IgG	1.02 (0.78-1.34)	0.879	0.9
C.1086 V1V2 IgG	0.98 (0.75-1.29)	0.88	0.9
B.MN gp120 IgG	1.00 (0.76-1.31)	0.999	1

Table S4. Odds ratios of peripartum HIV-1 MTCT for each measured Env-specific humoral immune responses (secondary analysis)

	Odds Ratio (95% CI)	P-value	Q-value
B.MN3 NAb	**0.54 (0.35-0.83)**	**0.005**	**0.1**
B.SF162LS NAb	**0.54 (0.35-0.84)**	**0.005**	**0.1**
B.BaL26 NAb	0.65 (0.43-0.98)	0.037	0.38
B.V3 IgG	0.67 (0.46-0.99)	0.042	0.38
A1con03 gp140 IgA	0.69 (0.47-1.01)	0.057	0.38
M.V3 IgG	0.69 (0.47-1.03)	0.064	0.38
B.SS11961 NAb	0.68 (0.44-1.04)	0.07	0.38
B.MN V3 gp70 IgG	0.71 (0.49-1.05)	0.082	0.38
B.AC10029 NAb	0.68 (0.44-1.06)	0.087	0.38
A.00MSA4076 gp140 IgA	0.74 (0.50-1.09)	0.125	0.5
ConS gp140 IgA	0.78 (0.53-1.13)	0.186	0.64
B.65353 NAb	0.76 (0.50-1.16)	0.193	0.64
B.RHPA42597 NAb	0.77 (0.50-1.19)	0.237	0.71
B.REJO454167 NAb	0.79 (0.52-1.19)	0.256	0.71
B.MN gp120 IgG avidity	0.81 (0.55-1.18)	0.267	0.71
B.MN gp120 IgG	0.81 (0.54-1.20)	0.284	0.71
B.conenv03 gp140 IgA	0.83 (0.57-1.20)	0.318	0.74
Con6 gp120 IgA	0.83 (0.56-1.22)	0.333	0.74
B.MN gp41 IgG	0.84 (0.56-1.24)	0.369	0.74
B.CaseA2 V1V2 IgG	0.84 (0.56-1.24)	0.372	0.74
B.WITO ADCC	1.17 (0.80-1.71)	0.419	0.77
B.6240 gp120mutC IgG avidity	0.85 (0.57-1.27)	0.423	0.77
B.WITO416033 Nab	0.86 (0.57-1.28)	0.45	0.78
B.MN gp41 IgA	0.88 (0.60-1.28)	0.491	0.82
C.1086C V1V2 IgG	1.13 (0.77-1.67)	0.53	0.82
B.CaseA2 V1V2 IgG	1.11 (0.75-1.65)	0.587	0.82
AE.A244 V1V2 IgG	0.90 (0.60-1.34)	0.594	0.82
AE.A244 gp120 IgA	0.90 (0.61-1.34)	0.603	0.82
B.MN gp120 IgA	0.90 (0.61-1.33)	0.609	0.82
B.CaseA2 V1V2N156QN160Q IgG avidity	0.91 (0.61-1.34)	0.618	0.82
B.C.C1 peptide IgA	1.08 (0.73-1.60)	0.696	0.9
AE.A244 gp120K169V IgG avidity	0.93 (0.64-1.36)	0.723	0.9
B.101211TC213257 NAb	1.06 (0.72-1.56)	0.754	0.9
ADCC SF162	1.06 (0.72-1.57)	0.765	0.9
AE.C1 peptide IgA	1.05 (0.71-1.57)	0.798	0.91
B.CaseA2V1V2169K gp70 IgG	1.03 (0.70-1.52)	0.89	0.96
B.6240 gp140C IgG avidity	0.97 (0.66-1.44)	0.89	0.96
C.1086 V1V2 IgG avidity	1.00 (0.67-1.47)	0.983	0.98

AE.A244 gp120 IgG avidity	1.00 (0.69-1.47)	0.983	0.98
AE.A244 gp120 IgA	1.00 (0.67-1.49)	0.985	0.98

Table S5. Interpretation of significant interactions between maternal HIV-1 Env-specific humoral immune responses and their association with MTCT risk

Percentile	Odds Ratio[1]	Lower CI[2]	Upper CI[3]	P value[4]
Effect of increasing IgG MNgp120 at selected percentiles of IgG B.6240 gp140 avidity				
20%	1.085	0.786	1.497	0.62
50%	0.812	0.609	1.085	0.159
80%	0.606	0.4	0.917	**0.018**
Effect of increasing IgG MN gp41 at selected percentiles of IgG B.6240 gp140 avidity				
20%	1.257	0.89	1.775	0.193
50%	0.916	0.686	1.223	0.551
80%	0.663	0.44	1	0.05
Effect of increasing IgG V3 score at selected percentiles of IgG 6240 gp140 avidity				
20%	0.845	0.621	1.151	0.286
50%	**0.646**	**0.474**	**0.88**	**0.006**
80%	**0.492**	**0.317**	**0.761**	**0.001**
Effect of increasing NAb score at selected percentiles of IgG MN gp41				
20%	1.119	0.722	1.735	0.616
50%	0.807	0.581	1.122	0.203
80%	**0.508**	**0.308**	**0.84**	**0.008**
Effect of increasing NAb response against SF162 at selected percentiles of IgG MN gp41				
20%	0.804	0.535	1.207	0.293
50%	**0.552**	**0.381**	**0.8**	**0.002**
80%	**0.324**	**0.179**	**0.583**	**<0.0001**

[1] Odds ratio per increasing one standard deviation for each humoral immune variable

[2] Lower confidence interval

[3] Upper confidence interval

[4] immune variable interactions with $p < 0.05$ are bolded

Table S6. Odds ratios of HIV-1 MTCT in multivariable analyses of the post-hoc analysis of maternal plasma CD4 blocking activity and the risk of MTCT

HIV Envelope proteins	Odds Ratio (95% CI[1])	P[1] value
Entire MTCT cohort		
B.63521 gp120	**0.70 (0.52 – 0.93)**	**0.014**
B.6240 gp120	0.75 (0.56 – 1.01)	0.058
B.JRFL gp120	**0.74 (0.56 – 0.98)**	**0.036**

[1] immune variable ORs at $p < 0.05$ are in bold

Table S7. Correlations of percent sCD4 blocking for B.JFRL Env neutralization potency against B.MN, and IgG binding to B.V3 in plasma of HIV-1-infected mothers

	% CD4 blocking (B.JRFL)	B.MN Nab	B.V3 IgG
Entire MTCT cohort			
% CD4 blocking (B.JRFL gp120)	1		
B.MN NAb	0.72	1	
B.V3 IgG	0.66	0.78	1

Table S8. The variable gene usage and binding-specificity (area under the curve) of the autologous Env V3-specific IgG1 mAbs isolated from a non-transmitting HIV-1-infected, untreated mother at six months postpartum

Ig ID	V_H	V_H Mutation	V_L	Con B.V3	Con C.V3	ConS V3	MN gp120_	gp70 ConAG. V3	gp70 ConA. V3	gp70 ConC. V3	gp70 MN. V3	gp70 A24492TH2 V3
DH290	3~7*01	3.6%	K 1~9*01	14.5[1]	0	1.6	16.2	3.8	3.0	3.1	14.3	0.6
DH291	1~46*02	4.3%	K 4~1*01	15.9	0	1.8	14.3	0	1.7	0.2	3.5	0.3
DH292	1~24*01	3.4%	L 3~1*01	16.4	6.7	9.7	16.5	11.4	11.3	13.1	16.5	0.2
DH293	1~f*01	2.5%	K 4~1*01	15.8	11.1	13	15.4	12.5	16.5	16.9	9.6	0.3
DH294	1~8*01	2.8%	L 1~40*01,02	15.6	0	1.6	12.3	0	0	0	9.4	0.6
DH295	5~51*03	2.3%	L 3~1*01	14.3	14.4	15.3	15.6	16.5	17.4	16.2	15.8	1.7
DH296	3~66*01	3.8%	K 2D~29*01	17	0	1.9	15.6	0	0	0	16	1
DH297	1~f*01	4.8%	L 1~51*01	13.1	12.7	12.6	16.1	15.4	16.9	16	15.7	0.2
DH298	3~30*04	3.2%	K 2D~29*02	16.7	0	2.4	15.5	0	5.8	0	10.4	0.4
DH299	5~51*03	2.0%	L 1~51*01	14	10.3	14.1	16.3	10.8	16.2	16.1	16.2	0.2

[1] Each value indicates area under the curve (AUC) of mAb binding to each V3 peptide, gp120, or gp70-scaffolded V3 antigens antigen, grey shading indicates binding.

Table S9. Flow cytometry antigen-specific B cell sort setup configuration details

Detector Array (Laser)	PMT	LP Mirror	BP Filter	Intended Fluor	Voltage
Octagon					
488nm Blue laser	A	755	780/60		
Coherent Sapphire	B	635	710/50		
100mW	C	600	610/20		
	D	550	575/25		
	E	505	525/50	FITC	550
	F	----	488/10	Side Scatter (SSC)	275
Octagon					
532nm Green laser	A	755	780/60	PE-Cy7	585
Compass	B	685	710/50		
150mW	C	635	660/20	PE-CY5	530
	D	600	610/20	ECD	500
	E	550	575/25	PE	370
Octagon					
406nm Violet laser	A	755	780/60		
Cube	B	685	705/70		
100mW	C	630	670/30		
	D	595	605/40	BV605	490
	E	570	585/42	BV570	485
	F	557	560/40		
	G	505	525/50	Aqua Vital Dye	555
	H	----	450/50	BV421	505
Trigon					
639nm Red laser	A	755	780/60	APC-Cy7	640
40mW	B	690	730/45	APC-AF700	600
	C	----	670/30	AF647	560
					Gain
Diode				Forward Scatter (FSC)	175

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