**Supplemental Methods**

***Generation of b12-IgA2 or b12-IgG1 antibody producing mice and measurement of human IgA and IgG***

Lentiviruses encoding b12-IgA2 and b12-IgG1 genes were used to create 293T cells that stably produced b12-IgA2 and b12-IgG1, respectively. Ten weeks old Rag2-/-γC-/- mice were injected (s.c.) with 1-5x10^6 cells of b12-IgA2- or b12-IgG1-transduced cells. Peripheral blood was collected from the mice periodically. The plasma concentrations of human IgA or human IgG in mouse blood were measured by ELISA (Bethyl lab).

***Generation of Human Immune System (HIS) mice***

Human Immune System (HIS) mice were generated as described (*30*). The one day old neonatal Balb/c $Rag2^{-/-}\gamma c^{-/-}$mice were irradiated (200rads) and injected intrahepatically with 1.25-2 x10^5 human cord blood $CD34^+$ HSPCs (AllCells or Lonza) in 50 μl PBS per mouse. Transplanted mice were tested for human cell engraftment 9-12 weeks later. For transduction with transgene, the mice were injected with cord blood $CD34^+$ HSPCs infected with lentivirus vector *in vitro*. Hemagglutinin (HA) peptide tag was used to detect b12-IgA transgene.

***Collections of mucosal secretions and saliva from mice and measurement of human IgA***

**Genital tract secretions collection**: Female mice were anesthetized and an aseptic surgical sponge (ear wicks, 2 by 5 mm, DeRoyal ear wicks; Powell, Ind.) was gently inserted into the vagina. The sponge was retrieved 30 min later and transferred to a microcentrifuge tube. Then the samples were immediately frozen on dry ice and stored in

a -80 °C freezer until needed. Spin-X microcentrifuge tubes containing a 0.2 cellulose acetate filter (Fisher Scientific) were pre-blocked with 0.5 ml of sterile PBS with 2% bovine serum albumin (BSA) and 0.05% Tween 20 for 30 min at room temperature and centrifuged at 4,500 rpm for 5 min. The filter was washed twice with 0.05 ml of sterile PBS. Each frozen sponge was placed in a pre-blocked Spin-X microcentrifuge tube and incubated in 400 µl of ice-cold sterile reconstitution buffer (0.5% bovine serum albumin and 0.05% Tween 20 in PBS in the presence of proteinase inhibitor cocktail (Roche)) for 1h on ice. The reconstituted genital secretion samples were collected by centrifugation at 4,500 rpm for 5 min and promptly used for ELISA.

**Bronchoalveolar lavage (BAL) collection**: The trachea and thoracic cavity was surgically exposed and the end of the trachea was closed just below the larynx using a hemostatic clamp. Using a 30-G needle, 0.5 ml ice-cold PBS was gently and slowly injected into the trachea at just below the closing. The lavage was pulled back into the syringe from the lungs inflated with the liquid. Average recovery volume ranged from 0.25 to 0.4 ml.

**Intestinal secretions collection**: The intestines were isolated from the mice and the fragment from just below the gastroduodenal junction through the cecum was removed and clamped on each end with hemostatic clamps. The intestines were rinsed externally twice in cold PBS. The intestines were then injected via a 27-G needle at two or three sites with a total volume of 2ml of lavage solution (25 mM NaCl, 40 mM $Na_2SO_4$, 10 mM KCl, 20 mM $NaHCO_3$, 50 mM EDTA, 0.1 mg/ml Soybean trypsin inhibitor, 162 mg/ml Polyethylen glycol, molecular weight 3350, Sigma). After 10 min of incubation at room temperature, the contents of the intestines were gently squeezed into a 15 ml conical tube and vortexed vigorously. The samples including fecal materials were clarified at 4 °C by centrifugation at 700xG for 10 min. Phenylmethylsulfonyl fluoride (PMSF) was added to the supernatant (1 mM) and the supernatant was centrifuged 15 min at 4 °C at 13,000 rpm in a microcentrifuge. The supernatant was incubated on ice for 15 min and 50 µl of 3.5% globulin free BSA per ml. Samples were stored at -20 °C for later antibody measurement by ELISA.

**Saliva collection**: Mice were injected intraperitoneally with 100 µl Pilocarpine (0.1 mg/ml, Sigma) to induce salivary flow. An aseptic surgical sponge (ear wicks, 2 by 5 mm) was inserted to the cheek pouch of the mouse under mild anesthesia to absorb the saliva. Five min later, the sponge was removed from the mice and transferred to a microcentrifuge tube and stored at -80 °C until needed. Saliva was recovered using Spin-X microcentrifuge tubes with filter as described above in genital secretion collection section except with 0.3 ml of reconstitution buffer instead of 0.4 ml.

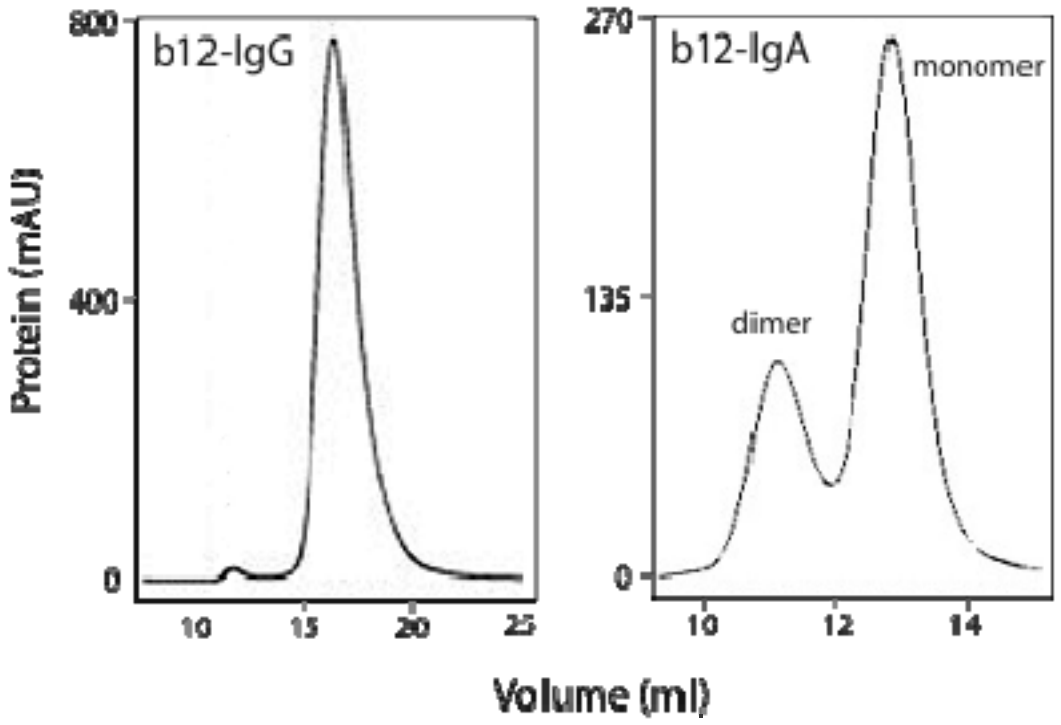


**Figure S1. Purification of b12-IgG, monomeric b12-IgA and polymeric b12-IgA.** Size fractionation of antibody on a gel filtration column (Superdex 16/60). IgA was pooled in polymeric IgA (mostly dimer) and monomeric IgA.

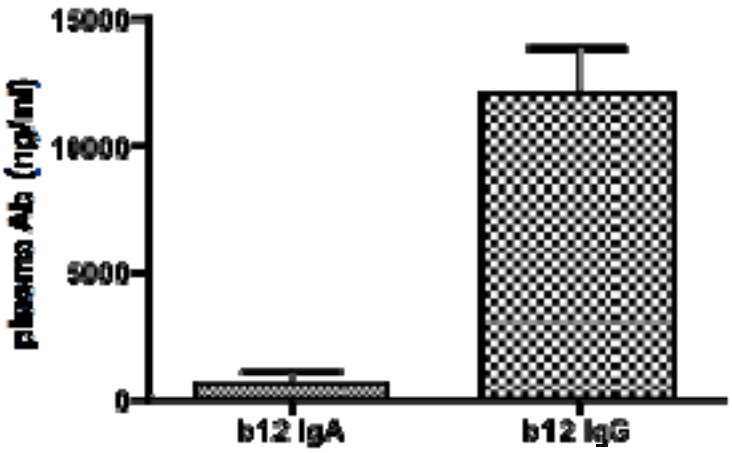


**Figure S2. Plasma antibody concentrations of b12-IgA and b12-IgG in mice with genetically engineered human cells transduced with b12-IgA or b12-IgG gene.** Human IgA (for b12IgA-transduced) or human IgG (for b12-IgG-transduced) concentration was measured in blood of Rag2-/-γC-/- mice injected (s.c.) with gene-transduced 293T cells. Data are mean ± s.e.m. when the tumor sizes were 1.0 $cm^2$ (n=3-5)

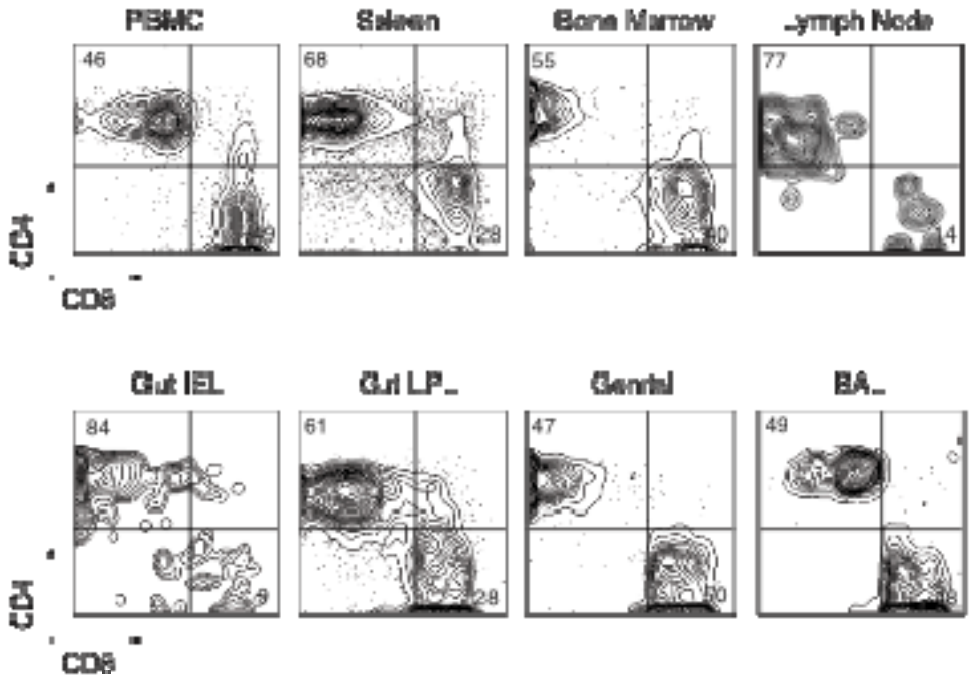


**Figure S3. T cell development in Hu-BLT mice expressing b12-IgA**. Lymphocytes were isolated from the peripheral blood, spleen, bone marrow, lymph node, gut, genital tract and bronchoalveolar lavage (BAL) of hu-BLT mice after 10-16 weeks of transplantation of HSCs transduced with pHAGE6-EEK-b12-IgA lentivirus. Intraepithelial lymphocytes (IEL) and lamina propria lymphocytes (LPL) were isolated from the entire gut.

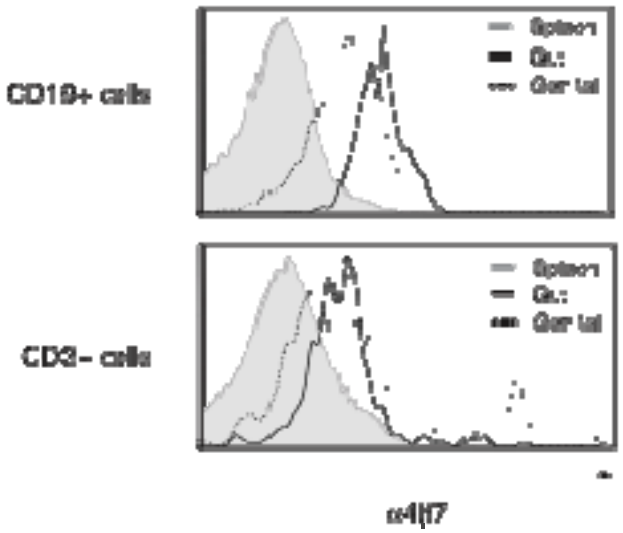


**Figure S4. Expression of mucosal homing receptor on B and T-cells of hu-BLT-b12a mice.** Histogram shows homing receptor α4β7 integrin expression on $CD19^+$ B-cells and $CD3^+$ T cells isolated from the spleen (filled histogram), gut (solid line) and genital tract (dotted line) of hu-BLT-b12a mice.

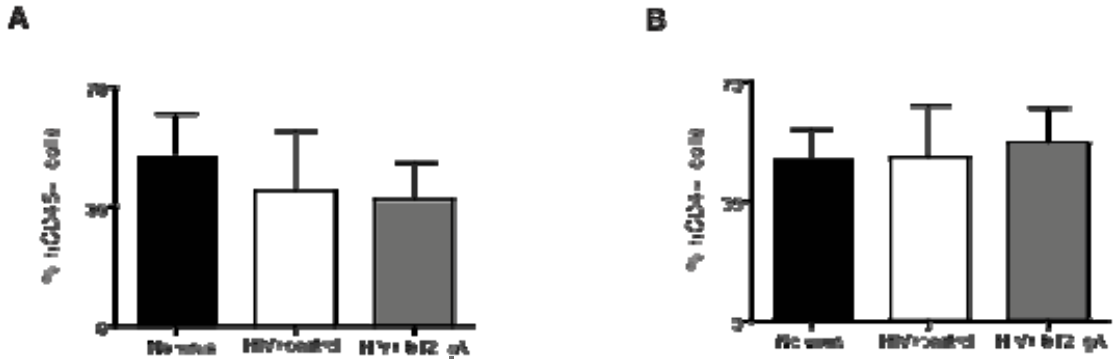


**Figure S5. Human cell engraftment in hu-BLT mice before challenge.** Bar graphs represents percent human $CD45^+$ cells (A) in PBMC and percent human $CD4^+$ T cells (B) among human cells of hu-BLT mice before HIV challenge.

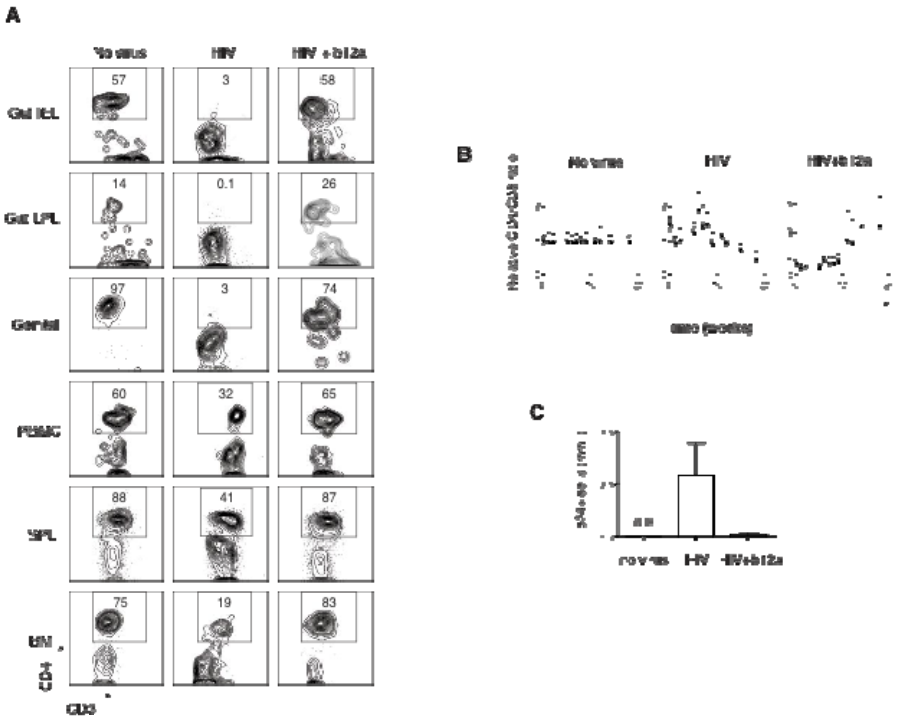


**Figure S6. Inhibition of mucosal transmission of HIV-1 in HIS mice transduced with b12-IgA.** HIS mice were challenged with R5 tropic HIV-1 (JR-CSF, 170 ng of p24) through intravaginal route at 14-20 weeks post-transplantation or left unchallenged (No virus). Peripheral blood of the mice was collected periodically. Peripheral blood of the mice was collected periodically. Mice were sacrificed after 8-10 weeks of HIV-1 challenge and lymphocytes from various tissues were isolated and analyzed by flow cytometry. (A) Protection of mucosal and peripheral $CD4^+$ T-cells in HIS mice transduced with b12-IgA from HIV-1 mucosal challenge. Flow cytometry of lymphocytes isolated from various tissues; intestinal intraepithelium (Gut IEL); intestinal lamina propria (Gut LPL); female genital tract (Genital); peripheral blood mononuclear cells (PBMC); spleen (SPL); bone marrow (BM) of HIS mice transduced with b12-IgA gene (HIV+b12a) or HIS mice with control vector (HIV). Cells were pre-gated on $CD45^+CD3^+$ cells. (B) Changes of CD4/CD8 ratio in PBMCs of HIS mice after HIV-1 mucosal challenge. Data are mean ± s.e.m. at each time points (weeks after challenge, n=2-7); no challenge, HIV-1 challenge in control gene-transduced HIS mice, HIV-1 challenge in b12-IgA-transduced HIS mice. (C) $P24^+$ cells were determined by counting

immunohistochemically stained cells from genital and intestinal tract tissue sections of HIS mice. (n=2-5, mean ± s.e.m.)

Table S1. Mucosally secreted b12-IgA in transduced hu-BLT mice

| | [a]Bronchoalveolar lavage (ng/ml) | [b]Saliva (ng/ml) | [c]Intestinal secretion (ng/ml) | [d]Fecal material (ng/g) |
|---|---|---|---|---|
| b12-IgA | 3.14 | 6.60 | 4.61 | 4.80 |

B12-IgA was measured by gp120 ELISA. Numbers are max concentration of antibody in each mucosal secretion. a. Bronchoalveolar lavage was collected in 0.5 ml PBS; b. saliva, undiluted; c. intestinal secretion was collected in 2 ml lavage solution; d. fecal material was collected and resuspended in ~1ml of lavage solution

Table S2. Human immune cell reconstitution and b12-IgA expression in HIS mice#

| group | human cell engraftment (CD45+) | % B cells | % T cells | % CD4 T cells | % HA+ CD19+ cells |
|---|---|---|---|---|---|
| No HIV | 29.4 (2.0) | 90.0 (1.7) | 8.7 (1.5) | 49.0 (3.2) | 9.2 (0.92) |
| HIV | 33.3 (4.8) | 92.0 (2.0) | 8.0 (2.0) | 51.4 (7.4) | N/A |
| HIV in b12 IgA | 34.8 (4.1) | 90.0 (5.9) | 10.0 (5.9) | 50.2 (4.5) | 10.8 (1.6) |