Supplementary Materials:

Supplementary Methods:

Immunostaining frozen tissue sections

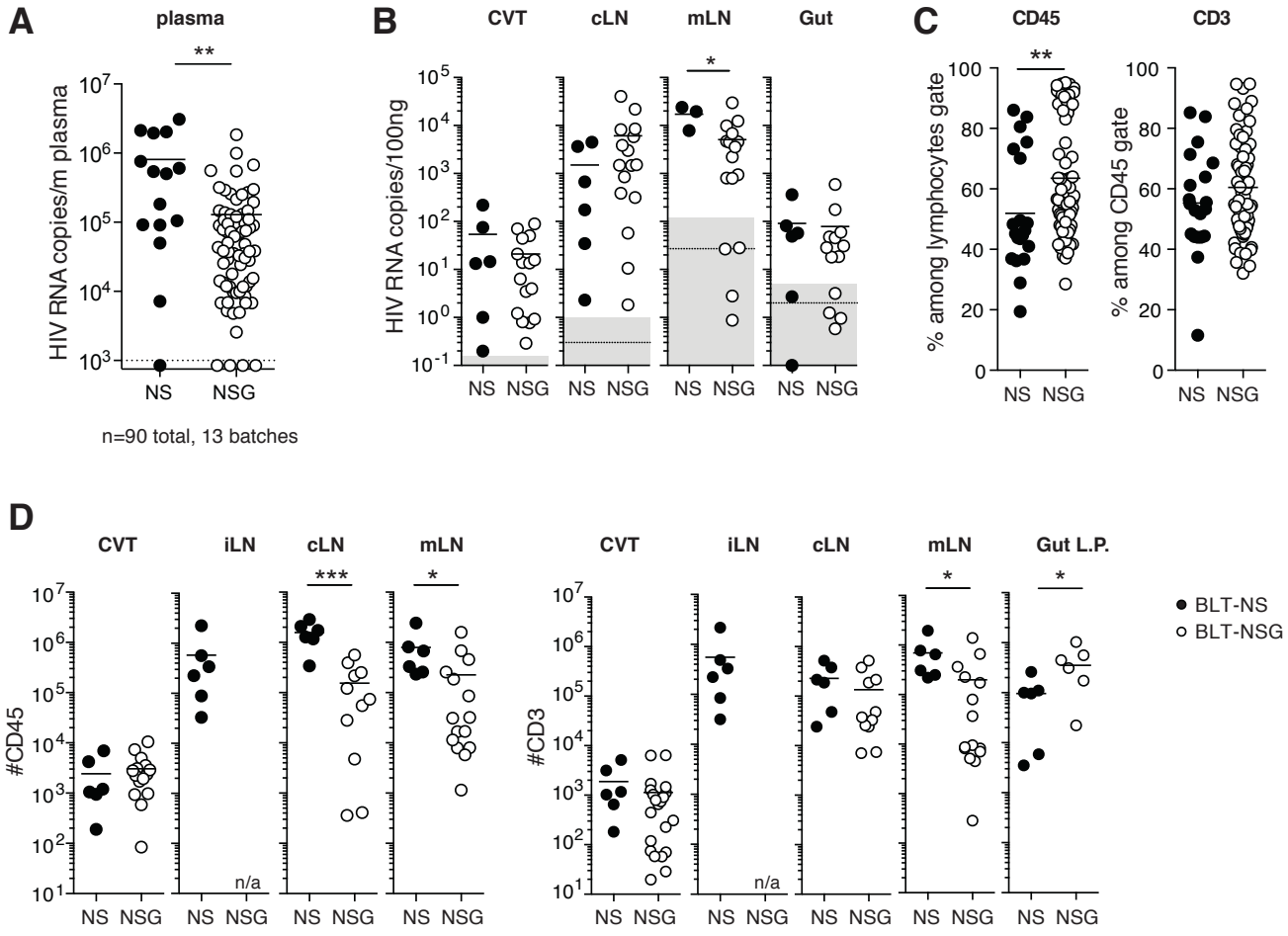
Eight μm frozen sections of CVT from BLT mice previously fixed with paraformaldehyde were processed using standard techniques. Sections were blocked with 2% BSA, 2% secondary antibody serum and Fc Receptor Blocker (Innovex), and stained using anti-human CD8 or anti-human CD4 rabbit IgG antibodies. Anti-rabbit IgG was used as secondary antibody and detection signal was improved using tyramide Signal Amplification kit (Molecular Probes). Nuclei were stained using Hoechst. Sections were mounted with VectaShield Hard set (Vector H-1400) and analyzed using confocal microscopy (Zeiss 710).

In vitro activation of $CD4^+$ T cell

Murine $CD4^+$ T cells were isolated from spleen and LNs by negative magnetic labeling (CD4 T cell separation kit, Miltenyi Biotech). $CD4^+$ T cells were then seeded at 10^6 cells/mL in C10 medium in anti-CD3/CD28 mAbs coated plates and cultured for 2 days at 37°C 5% CO_2. Activated cells were then washed and expanded in C10 medium containing 6 ng/mL recombinant murine IL-2 (Peprotech) at 37°C 5% CO_2 for 3 days. Activated $CD4^+$ T cells were then separated from dead cells and debris by Ficoll gradient (GE Healthcare) centrifugation. After culture, 70-80% of $CD4^+$ T cells were of central memory like phenotype expressing both CD44 and CCR7.

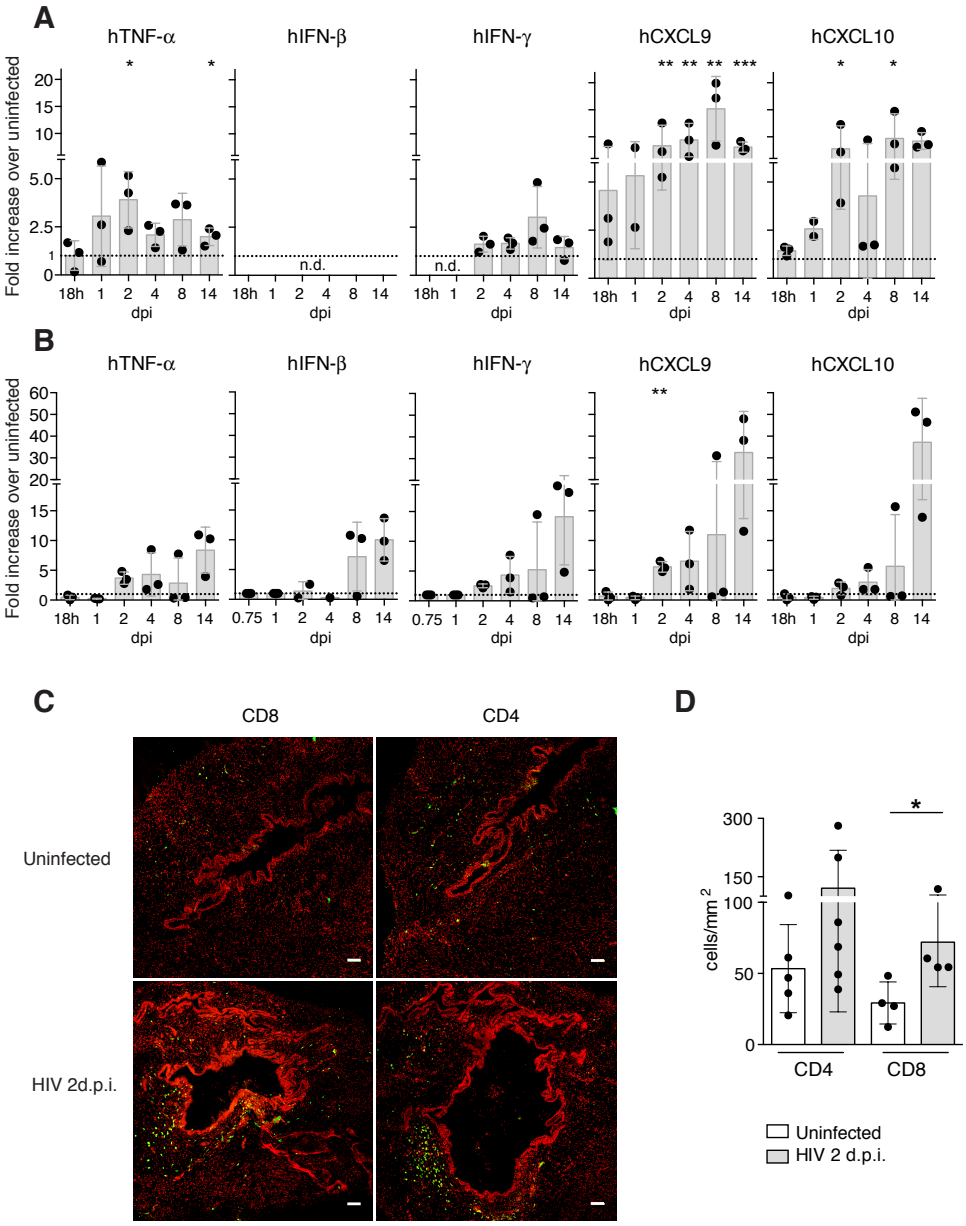
Human naïve $CD4^+$ T cells were isolated from human peripheral blood by negative magnetic selection (Myltenyi Biotech). Cells were then activated with anti-CD3/CD8 antibody coated beads (dynabeads, ThermoFisher) for 2 days and then expanded in C10 medium supplemented with 4 ng/mL recombinant human IL-2. 70-80% of activated $CD4^+$ T cells were CD45RA negative and CCR5 positive after culture.

Figure S1



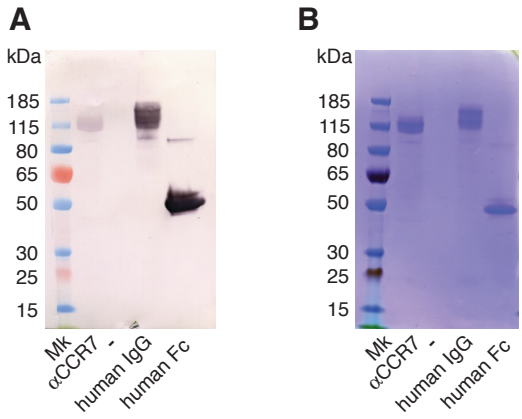
Comparison of viral loads and human immune cells reconstitution in tissues of BLT-NS and BLT-NSG mice. BLT-NOD-*scid* (NS, closed circles) and BLT-NOD-*scid* IL2Rγ$^{-/-}$ (NSG, open circles) were infected by atraumatic intravaginal application of 1 x10^5 $TCID_{50}$ $HIV\text{-}1_{JRCSF}$. HIV RNA in plasma (A) or tissues (B) was detected by RT-qPCR at 14 dpi. [A) BLT-NS: n=15; -NSG: n=75; B) BLT-NS: n=6; -NSG: n=16] C) Percentages of human $CD45^+$ (left) and human $CD3^+$ (right) cells in peripheral blood at the time of infection. (BLT-NS: n=19; -NSG: n=19) D) Numbers of human $CD45^+$ (left panels) or $CD3^+$ (right panels) cells in tissues at 14 dpi (BLT-NS: n=6; -NSG: n=15). * $p<0.05$, ** $p<0.01$, *** $p<0.001$. n/a: not available. Iliac LN from BLT-NSG were often too small to be analyzed. Data for $CD45^+$ cell counts in the gut lamina propria (L.P.) was not obtained as anti-CD45 mAb was not added to the staining panel in these samples.

Figure S2



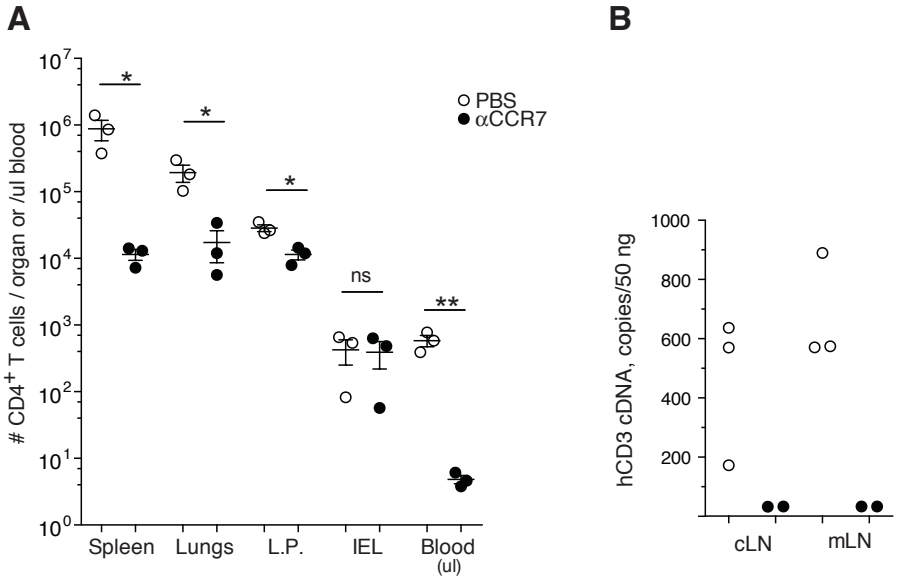
Human immune response following HIV infection. Human cytokine and chemokine expression in CVT (**A**) and iLN (**B**) at different days post IVAG infection (dpi) of BLT-NS mice as measured by qPCR (n=3 per time point). Results are represented as fold over the mean value obtained with 3 uninfected animals. Each dot represents one mouse, bars represent mean and STDEV; Dashed line represent a ratio of 1 compared to uninfected. n.d.: not detected. **C**) Immunostaining of human $CD4^+$ or $CD8^+$ T cells (green) in the CVT of uninfected or infected BLT mice 2 dpi. Red: Nuclei stain Hoescht. Bar: 50 um. Pictures of one representative animal per group (anti-CD4 staining: uninfected n=5, infected n=6; anti-CD8 staining: uninfected n=4, infected n=4) . **D**) Quantification of human $CD4^+$ or $CD8^+$ T cells present in uninfected (white bars) CVT or 2 dpi (gray bars) as represented in C. Each dot represents the mean number of cells in 10 fields of view from the same tissue. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Figure S3



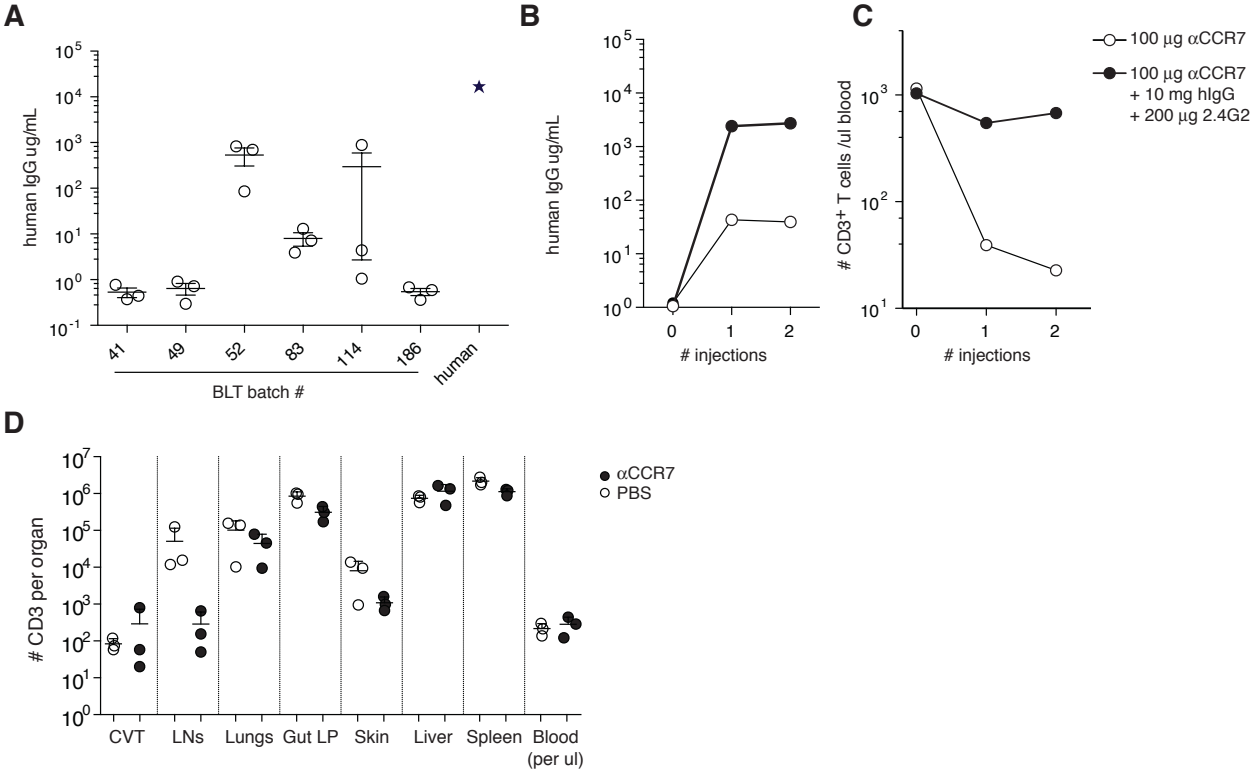
The human CCR7 mAb is a non-glycosylated IgG1 mAb. **A**) Glycan blotting using the mannose specific lectin *Lens culinaris* agglutinin (LCA) of CCR7 mAb (αCCR7)(10 ug). Human IgG and the Fc fragment of human IgG were used as positive controls. **B**) Corresponding Commassie stained non-reducing SDS-PAGE. Mk: molecular weight markers.

Figure S4



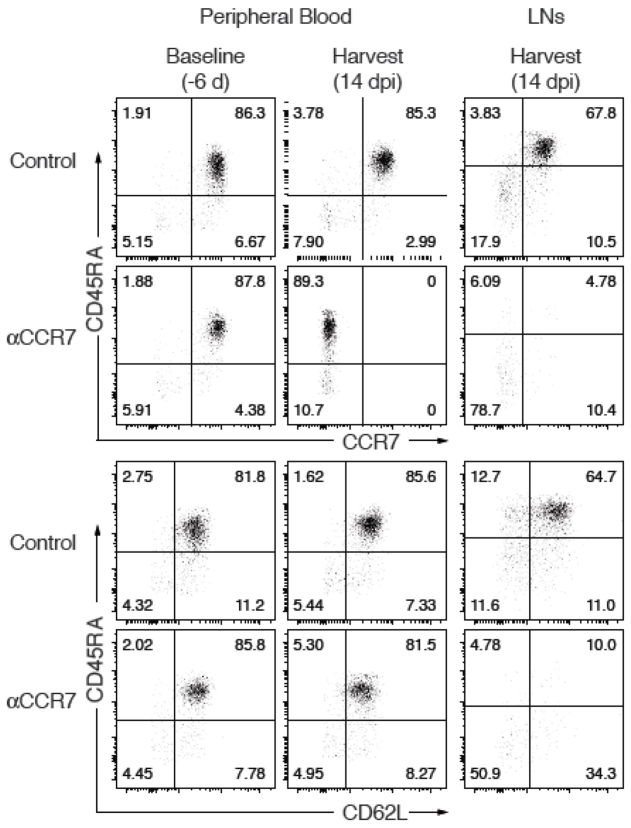
CCR7 mAb treatment induced T cell depletion in blood and tissues. A) Number of $CD4^+$ T cells in tissues of CCR7 mAb- (αCCR7, closed circles, n=3) or PBS-treated (open circles, n=3) infected BLT-NSG mice after 14 days of treatment. $CD4^+$ T cell depletion was observed in spleen, lung, gut lamina propria (L.P.) and blood of CCR7 mAb-treated mice compared to PBS-treated mice. However, no depletion was observed in gut intra-epithelial lymphocytes (IEL), which is expected as T cells from IEL do not recirculate and therefore likely do not express CCR7 *(32)*. **B**) Number of human CD3 copies per 50 ng of cDNA of cervical (c) or mesenteric (m) lymph nodes (LNs) of CCR7 mAb- (closed circles, n=2) or PBS-treated (open circles, n=3) mice. The presence of human T cells was analyzed by qPCR due to the small size of the LNs in CCR7-mAb treated mice (n=2-3 mice/group). * $p<0.05$, ** $p<0.01$.

Figure S5



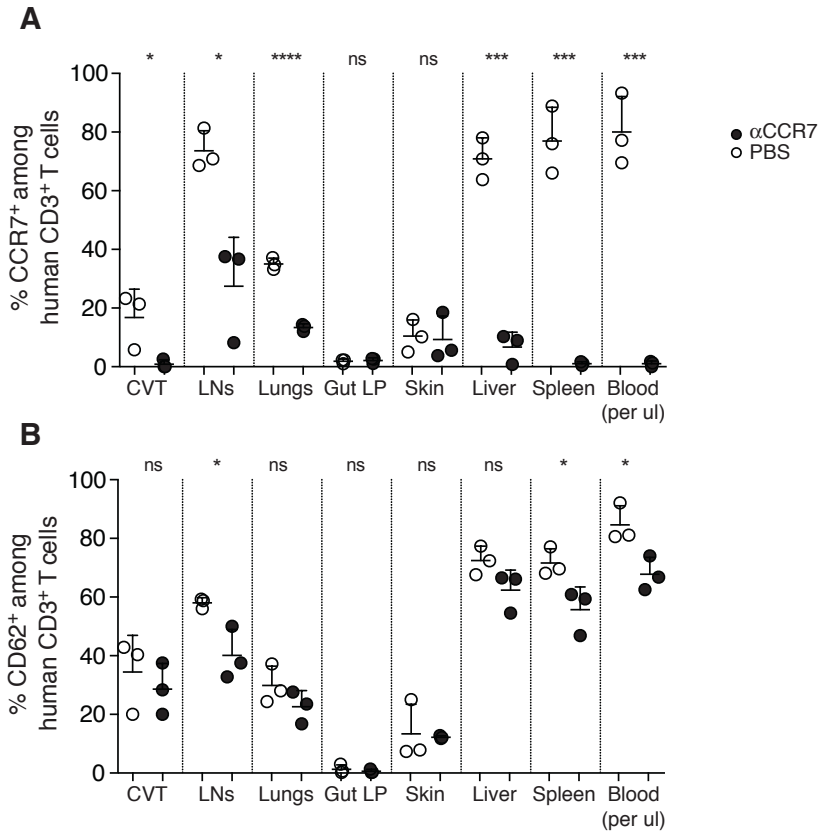
Treatment with human IgG inhibits T cell depletion induced by CCR7 mAb in BLT humanized mice. **A**) Level of endogenous human IgG in plasma of different batches of uninfected BLT-NSG mice (circles, n=3 per batch) compared to human plasma (star, n=1). **B, C**) BLT-NSG mice were injected i.p. with CCR7 mAb (αCCR7) alone (open circles) or with human IgG (hIgG)(10 mg) and 2.4G2 (200 ug) (closed circles). The level of human IgG in plasma (**B**) and the number of human $CD3^+$ T cells (**C**) were measured 24h after 0, 1 or 2 injections applied 2 days apart. **D**) BLT-NSG mice were treated with human IgG (hIgG)(10 mg), 2.4G2 (200 ug) and CCR7 mAb (green circles, n=3) or PBS (open circles, n=3) i.p. and CCR7 mAb or PBS IVAG. The number of human $CD3^+$ T cells was analyzed in tissues after 14 days of treatment. LP: lamina propria; LNs: axillary and cervical lymph nodes, CVT: cervico-vaginal tissue.

Figure S6



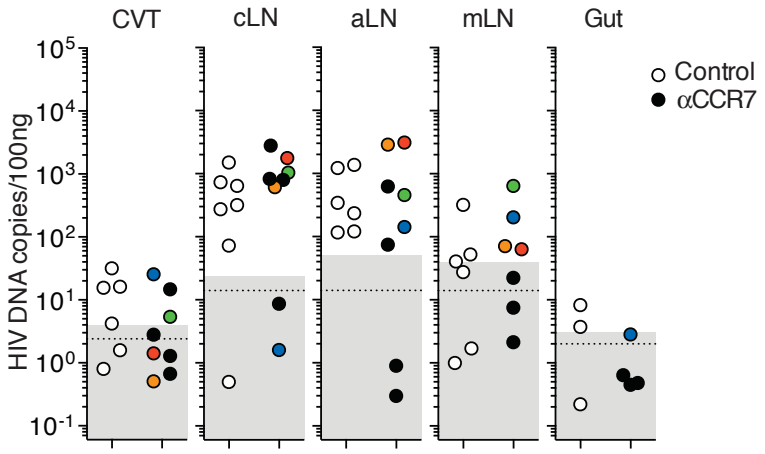
T cell subsets in peripheral blood and LNs of CCR7 mAb treated and control mice. Representative flow cytometry plots showing the percentages of CD45RA and CCR7 (top panels) or CD45RA and CD62L (bottom panels) expression on human $CD3^+$ T cells from blood and LNs of control (n=7) or CCR7 mAb (αCCR7, n=8)-treated BLT-NSG humanized mice. Since anti-human CCR7 mAb blocked subsequent CCR7 staining, expression of CD62L was used as a surrogate marker to detect the presence of $CCR7^+$ T cells.

Figure S7



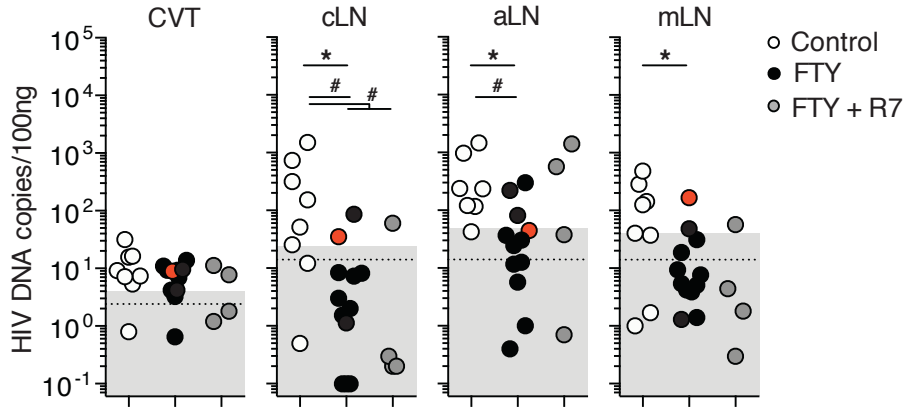
CCR7 mAb treatment of BLT mice blocked subsequent CCR7 staining in tissue. Percentage of human $CD3^+$ T cell expressing **A**) CCR7 or **B**) CD62L in tissues of infected control (PBS, grey, n=3) or CCR7 mAb (αCCR7, green, n=3) treated mice after 14 days of treatment assessed by flow cytometry. CCR7 mAb treatment in vivo blocked subsequent CCR7 staining of human T cells recovered from tissues of treated mice (A) but did not block the staining of CD62L (B), which is co-expressed with CCR7 on Tcm found in tissue. All mice also received hIgG + 2.4G2 treatment. CVT: cervico-vaginal tissue; LNs: axillary and cervical lymph nodes; LP: lamina propria. T-test: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p< 0.0001$.

Figure S8



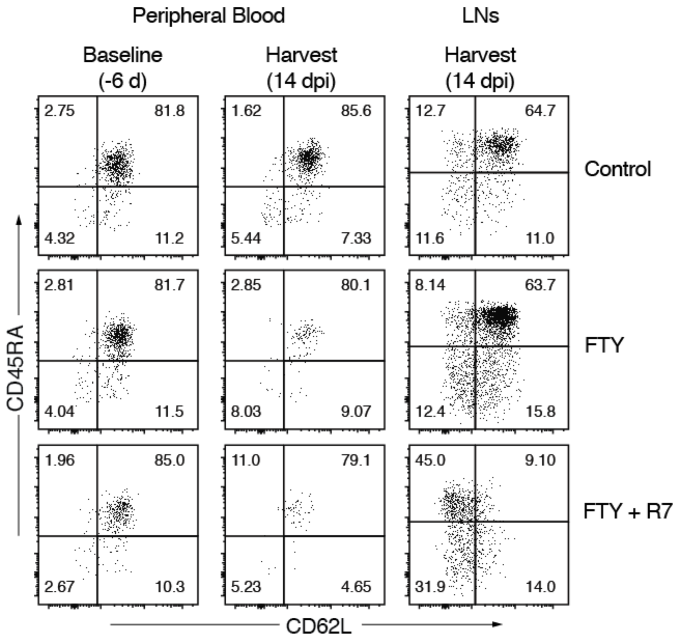
Effect of CCR7 mAb treatment on the presence of HIV DNA in tissues at 14 dpi. BLT-NSG mice were treated with PBS (control, open symbols, n=7) or CCR7 mAb + hIgG+ 2.4G2- (αCCR7, black symbols, n=8). Each circle represents one mouse. Mice with positive viremia are shown with colors corresponding to the colors in Figure 2. Dotted line: limit of sensitivity of the assay corresponding to the mean value of 3-5 uninfected animals; grey box: mean of uninfected animals ± 2 SD. CVT: Cervico-vaginal tissue; LN: lymph node; c: cervical; a: axillary; m: mesenteric.

Figure S9



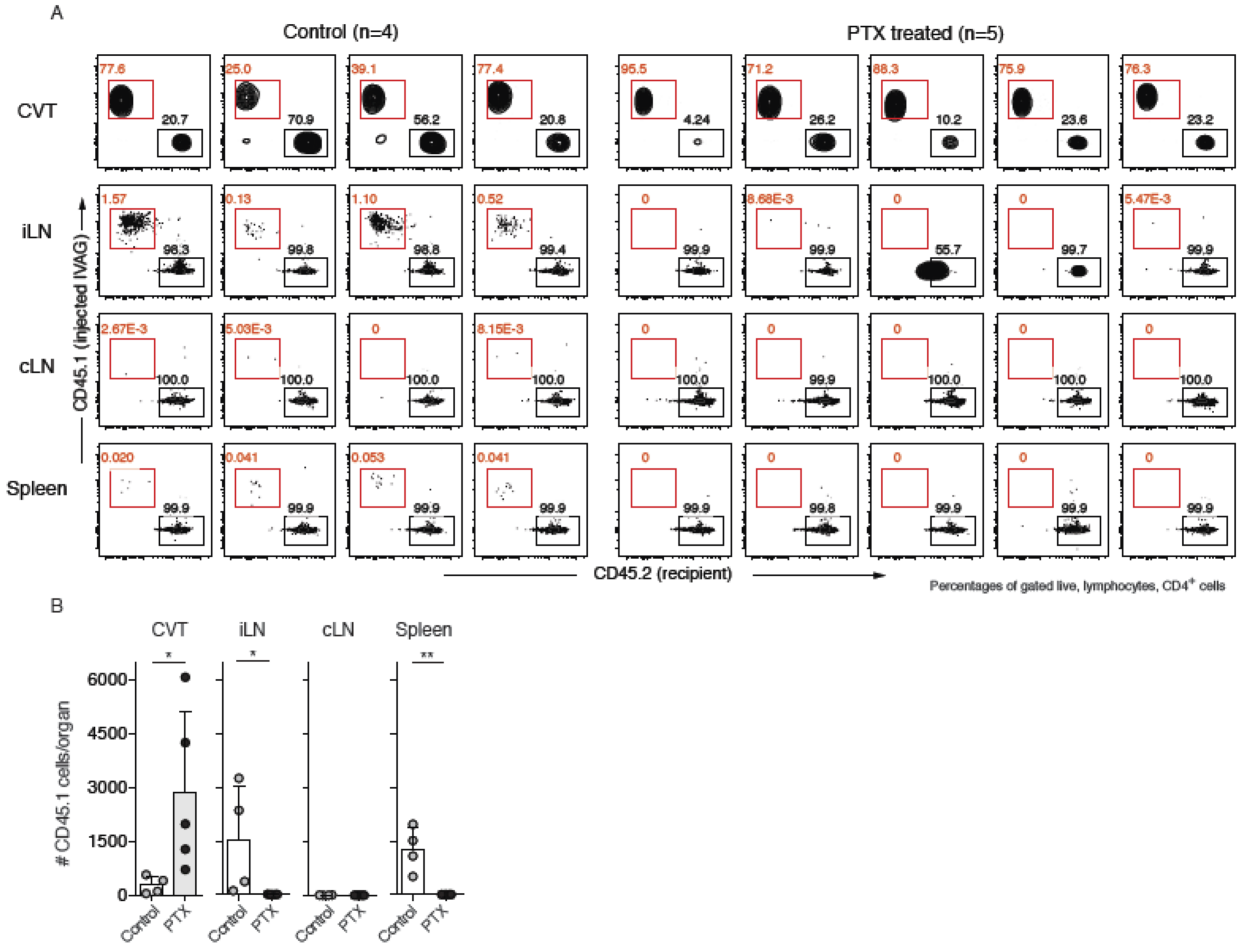
Effect of FTY720 and FTY720 + CCR7 mAb treatments on the presence of HIV DNA in tissues at 14 dpi. BLT-NSG mice were treated with PBS (control, open circles, n=8), FTY720 (FTY, closed circles, n=12) or FTY720 + CCR7 mAb (FTY + R7, grey circles, n=4). Each dot represents one mouse. The FTY720-treated mouse with borderline viremia is shown in red as in Figure 3. Dotted line: limit of sensitivity of the assay corresponding to the mean result of 3-5 uninfected animals; grey box: mean of uninfected animals ± 2 SD. CVT: Cervico-vaginal tissue; LN: lymph nodes; c: cervical; a: axillary; m: mesenteric. Kruskal-Wallis test was used to perform statistical comparisons of mean percentages of viral load (# $p<0.05$), and Fisher exact test was used to perform statistical comparisons of the number of viremic and non-viremic tissues in treated versus untreated mice (* $p<0.05$).

Figure S10



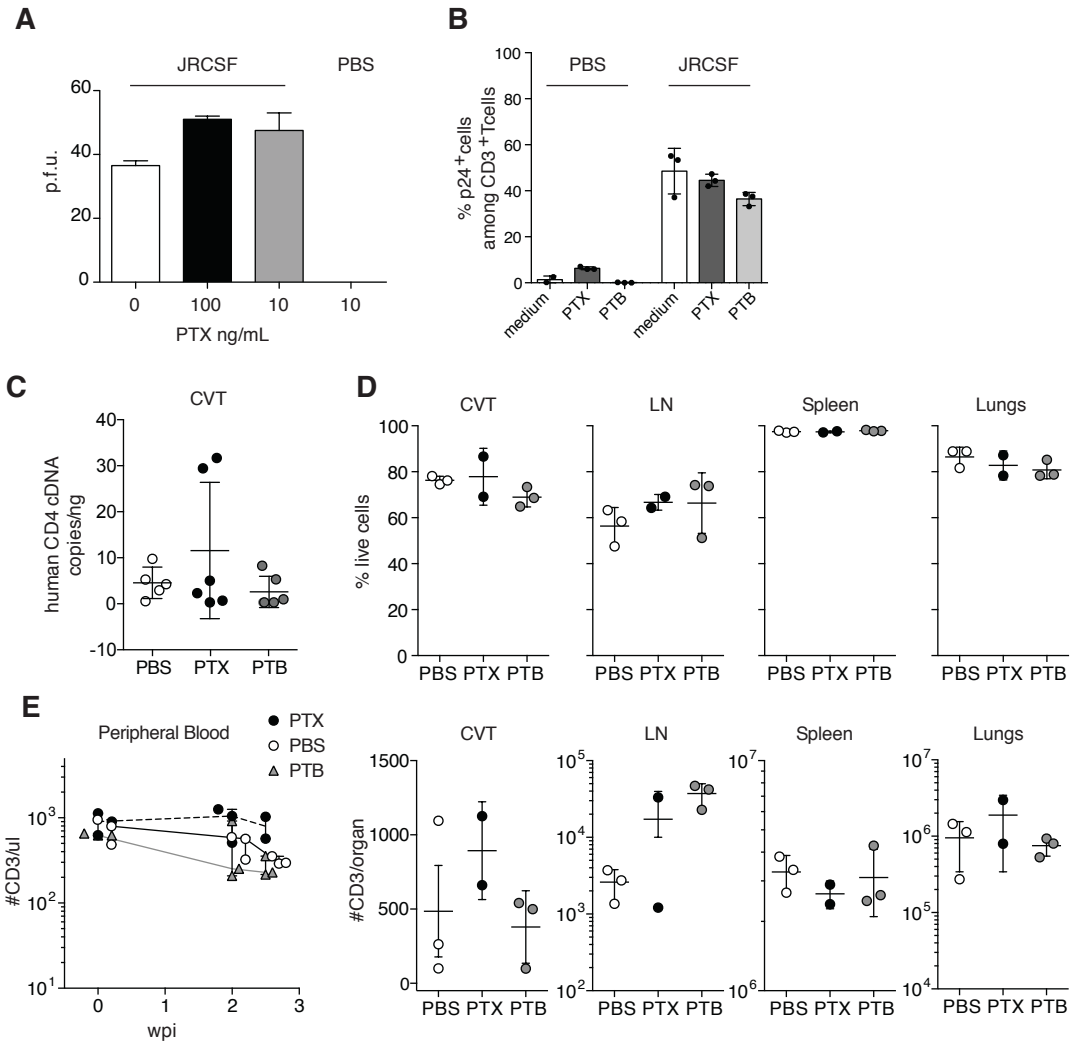
T cell subsets in peripheral blood and LNs of PBS-, FTY720- and FTY720 + CCR7 mAb-treated BLT-NSG mice. Representative flow cytometry plots showing the percentages of CD45RA and CD62L expressing human $CD3^+$ T cells in blood or LNs of control (top panels, n=8), FTY720- (FTY; middle panels, n=12) or FTY720 + CCR7 mAb (FTY + R7; bottom panels, n=4) treated mice.

Figure S11



PTX treatment inhibits CD4$^+$ T cell trafficking from the CVT to LNs. In vitro generated central memory like CD4$^+$ T cells from CD45.1 mice were incubated with PTX (200 ng/mL) or medium alone (Control) for 2 hours at 37°C, washed and resuspended in PBS. 10^6 CD45.1 CD4$^+$ T cells were adoptively transferred into CD45.2 mice by intra-vaginal injection. CVT, iliac (i) and cervical (c) LNs and spleen were harvested 24 hours post injection and analyzed for the presence of CD45.1$^+$ (red gates) and CD45.2$^+$ (black gates) CD4$^+$ T cells by flow cytometry. **A**) Contour plot showing the percentages of injected CD45.1$^+$ (red gate) and endogenous CD45.2$^+$ (black gate) CD4$^+$ T cells after gating on live singlet lymphocytes. **B**) Dot plots summarizing the numbers of CD45$^+$ CD4$^+$ T cells per organ shown in A. * $p<0.05$, ** $p<0.01$.

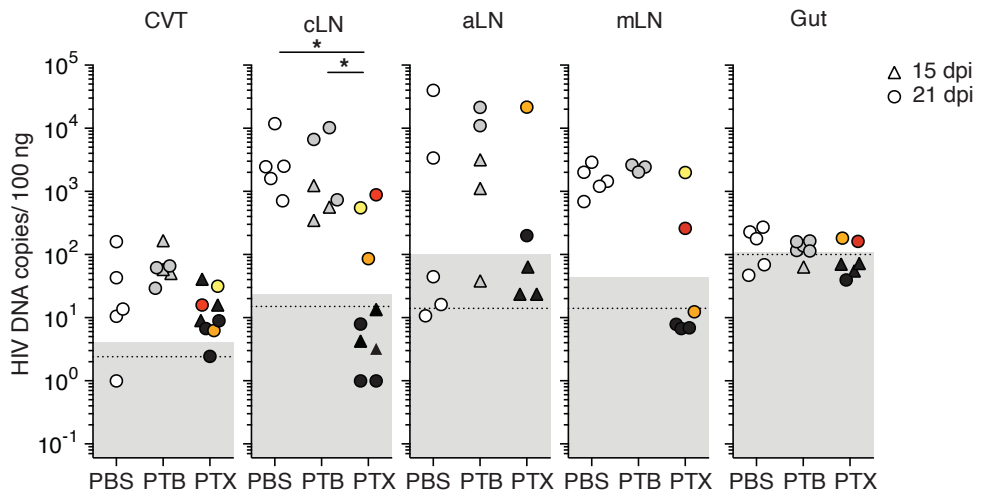
Figure S12



PTX treatment does not affect HIV infection in vitro or cell viability in vivo.

A) MAGI.CCR5 cells reporter assay. PTX (10 ng/ml and 100 ng/ml) or vehicle was added to MAGI cells 3h post infection with 14,000 $TCID_{50}$/well of HIV_{JRCSF}. Plaque forming units (pfu) were quantified 48h post infection. **B**) In vitro infection of human $CD4^+$ T cells. 10^5 in vitro generated central memory like human $CD4^+$ T cells were infected with MOI 10 of HIV_{JRCSF}. 10 ng/mL of PTX, PTB or equivalent volume of medium were added 3h post infection and every 24 hours thereafter. The percentages of infected cells were analyzed by flow cytometry for intracellular HIV p24 72h post infection. **C**) qPCR analysis of human CD4 cDNA in CVT of PBS (white, n=3), PTX (black, n=2)- or PTB (grey, n=3)-treated BLT-NSG mice at 3 weeks post infection (wpi). Viability of cells extracted (**D**) and numbers of human $CD3^+$ T cells in different tissues (**E**) were analyzed by flow cytometry 3 weeks post infection (wpi). Bars represent mean and STDEV. CVT: Cervico-vaginal tissue; LN: lymph nodes.

Figure S13



Effect of PTX treatment on the presence of HIV DNA in tissues. BLT-NSG mice were treated with PBS (open symbols, n=7), PTB (grey symbols, n=6) or PTX (black symbols, n=9) IVAG 3h post HIV infection and tissues were harvested after 14 (triangles) and 21 (circles) days post infection (dpi). Each symbol represents one mouse, three mice with positive plasma viremia are shown with different colors as in Fig 4. Dotted line: limit of sensitivity of the assay corresponding to the mean result of 3-5 uninfected animals; grey box: mean of uninfected animals ± 2 SD. CVT: Cervico-vaginal tissue; LN: lymph nodes; c: cervical; a: axillary; m: mesenteric. Fisher exact tests were used to perform statistical comparisons of the number of viremic and non-viremic tissues in treated versus untreated mice. * $p<0.05$.

Table S1

BLT batch	41	49	55	83	127	122	137	241	200	218	247
HLA-A1	24;02	11;01	03;01	11;05	02;01	25;01	03;01		02:01	02:01	01:01
HLA-A2	32;01	24;02	68;01	34;02	03;01	74;00	11;01		11:01	11:01	32:01
HLA-B1	18;01	15;01	07;02	40;01	44;02	15;03	27;06		15:01	15:01	13:02
HLA-B2	44;02	48;01	44;03	44;03	51;01	18;01	45;01		18:01	35:01	51:01
HLA-C1	07;01	07;02	04;01	03;04	05;01	02;10	03;04		01:02	03:03	06:02
HLA-C2	07;04	08;01	07;07	07;01	15;02	12;03	15;05		07:04	04:01	16:02
Exp.	**Dissemination (Fig. 1)**					**PTX experiment (Fig. 2)**			**CCR7/FTY720 experiment (Figs. 3 and 4)**		

HLA type of BLT batches.