Supplemental Digital Content 1

Extended Materials and Methods:

Cell isolation and cell culture conditions

$CD4^+$ T-cells were isolated from the PBMC's of healthy human donors by Ficoll gradient (GE healthcare, Buckinghamshire, UK), and enriched with the EasySep negative selection human $CD4^+$ T-cell kit (StemCell Technologies, Vancouver, Canada). Informed written consent was obtained from all subjects (Canadian Blood Services Research Ethics Board Committee Protocol Reference #2005-003). Isolated $CD4^+$ T-cells were grown in complete RPMI-1640 media (Sigma-Aldrich, Oakville, Canada) containing 10% Fetal Bovine Serum (FBS), 1% penicillin, 1% streptomycin and 0.1% gentamycin. $CD4^+$ T-cells were activated with the addition of 10 μg/mL anti-CD3 (UCHT-1, Antibody Core Facility, Sunnybrook Research Institute, Toronto, Canada), 2 μg/mL anti-CD28 (10R-CD28bHU, Fitzgerald Industries International, Acton, USA) and 10 units/mL recombinant human Interleukin-2 (IL-2, Sigma-Aldrich). In experiments requiring PBMCs from an HIV-1^+ donor, cells were depleted of $CD8^+$ cells with Dynabeads CD8 (Invitrogen, Lafayette, USA), followed by growth in complete RPMI media supplemented with activators (anti-CD28, anti-CD3 and IL-2).

Western blot and antibodies

One million $CD4^+$ T-cells were lysed with RIPA buffer (50 mM HEPES, pH 7.3, 1% Nonidet P-40, 0.1% SDS, 0.1% Na deoxycholate, 150 mM NaCl, 1 mM Na_3VO_4, 50 M $ZnCl_2$, 2 mM EDTA and 2 mM PMSF) and run on an 8% reducing SDS-PAGE gel, and subsequently transferred to an Immobilon-P membrane for Western blot detection. Blots were blocked with

1x Tris-buffer saline (TBS), 5% skimmed-milk powder and 0.5% Nonidet P-40 (BioShop, Burlington, Canada). In other experiments, samples were run on a TGX Stain-Free FastCast Acrylamide gel to visualise total protein (Bio-Rad, Mississauga, Canada). Anti-ABL1/ARG (Ab-3 clone 24-21, Calbiochem, Oakville, Canada), anti-SAMHD1 (Proteintech, Rosemont, USA) or anti-RNA polymerase II (Bethyl Laboratories, Montgomery, USA) were used to probe the blots. To measure phospho-SAMHD1 (Thr592, clone D702M, Cell Signaling Technology), phospho-RNA polymerase II CTD (Tyr1, Active Motif, Carlsbad, USA) or phospho-RNA polymerase II (S2, Bethyl Laboratories), blots were stripped and then blocked with 1x TBS, 3% w/v BSA and 0.1% Tween-20. After washing, secondary goat anti-mouse HRP (Bio-Rad), goat anti-rabbit HRP (Bio-Rad) or goat anti-rat (Sigma-Aldrich) antibody was added, followed by enhanced chemiluminescent (ECL) detection (GE Healthcare). Protein phosphorylation of SAMHD1 or RNA polymerase II was normalized to total unstained protein and quantified with the Molecular Imager GelDoc XR+ system using Image Lab software (Bio-Rad).

siRNA knockdown experiments

Pools of four siRNA duplexes (siGENOME SMARTpool) targeting *ABL1* (cat # M-003100-02-0005) or *ARG* (cat # M-003101-02-0005, Dharmacon RNAi technologies, Lafayette, USA) were electroporated into 1.0 x 10^7 enriched $CD4^+$ T-cells with the Amaxa® Human T Cell Nucleofector® Kit (Lonza, Cologne, Germany), program V-024. Non-targeting (*NT*) siRNA (cat # D-001206-13-05) served as a negative control for off-target knockdown. Cells were mixed with 900 nM of siRNA and 2 μL of siGuard RNase inhibitor® (Genlantis, San Diego, USA). Twelve hours post-transfection, media was changed with 2 mL of RPMI with (anti-CD28, anti-CD3 and IL-2) or without activators. $CD4^+$ T-cells were then prepared for quantification of

mRNA (qRT-PCR) and cell viability assessment at 24-hours post-siRNA knockdown, or HIV-1 infection at 48-hours post-siRNA knockdown.

qRT-PCR and qPCR

Twenty-four hours after siRNA electroporation, total RNA was extracted from 1.5 million $CD4^+$ T-cells from each siRNA treatment using the Trizol method (Invitrogen). Total RNA was redisolved in 10 mM Tris-HCl pH 8.0 and quantified by NanoDrop. 1 μg of cDNA was then synthesized using the iScript Reverse Transcriptase kit, following the manufacturer's instructions (BioRad). The primer sets used to detect ABL1 or ARG cDNA (synthesized by the Center for Applied Genomics, The Hospital for Sick Children, Toronto, Canada) were as follows: *ABL1* forward, 5′- TTCAGCGGCCAGTAGCATCTGACTTT -3′; ABL1 reverse, 5′- TGTGATTATAGCCTAAGACCCGGAG -3′; ARG forward, 5′- CTTTTAGGTGTGTGTACT -3′; ARG reverse, 5′- CCAAAAGCCCAGACGTCA -3′; GAPDH forward, 5′- CCACATCGCTCAGACACCAT -3′; GAPDH reverse, 5′- ACATGTAAACCATGTAGTTGAGGTC -3′ [19,20]. For each qRT-PCR run, 25 ng template cDNA (with the exception of the no-template control, NTC) was added to PCR tubes containing: 12.5 μL 2x SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK), 300 nM of each forward and reverse primers, and PCR grade H_2O (Roche Diagnostics, Indianapolis, USA) up to a final volume of 25 μL. PCR cycling parameters were as follows: initial denaturation at 95°C for 10 min; 40-50 cycles of amplification of 95°C for 15 sec, 54°C for 30 sec (ABL, 58°C for ARG and GAPDH) and 60°C for 30 sec. Duplicate reactions were analyzed using the Rotor-Gene RG-3000 thermocycler (Corbett Research, Montreal, Canada). Three biological replicates in the ABL1 and ARG siRNA-treated groups were compared to the non-targeting siRNA-treated group by the $2^{-\Delta CT}$ comparative C_T method [21].

In HIV-1 infection experiments of $CD4^+$ T-cells pre-treated with siRNA, cells were infected with an R5 virus (JR-FL or Ba-L) or an X4 virus (HXB2 or IIIB). Viral cDNA was isolated 24 hours post-infection in 5 x 10^5 cells with the QIAamp DNA Blood Mini Kit (Qiagen). Details of these methods have been described previously [16]. Briefly, four viral cDNA targets were amplified by SYBR Green qPCR on the Rotor-Gene RG-3000 thermocycler (Corbett Research) or on the CFX96 Touch Real-Time PCR Detection System (BioRad): early reverse transcripts (early RT), late reverse transcripts (late RT), 2-LTR circles or integrated virus by *Alu-gag* nested PCR. Human *β-globin* was measured to normalize the data by the $2^{-\Delta\Delta CT}$ comparative C_T method [21].

Infection with HIV-1 luciferase reporter viruses

We infected 2 x 10^5 $CD4^+$ T-cells electroporated with siRNA with 1.0 ng *nef*-deficient HXB2 (X4) or 26 ng JR-FL (R5) luciferase reporter viruses, in 200 µL complete RPMI media. Viruses were made as previously described [16]. We then measured luciferase activity three days post-infection (Promega, Madison, USA). Other $CD4^+$ T-cells were infected with HXB2 or JR-FL for a shorter time, to collect cDNA at 24-hours post-infection and quantify early infection by qPCR.

Ex vivo dasatinib experiments and p24 ELISA

Enriched $CD4^+$ T-cells from a healthy human donor were grown in RPMI with or without activators. Cells were administered 100 nM dasatinib (BMS-354825, Selleckchem, Houston, USA) or an equivalent volume of DMSO vehicle at the same time as T-cell activation. After 48hrs incubation, cells were lysed for Western blot detection of SAMHD1 phosphorylation.

Written informed consent was received from an HIV^+ blood donor in accordance with the University of Toronto Research Ethics Board. Their PBMCs were isolated and depleted of $CD8^+$ T-cells using Dynabeads CD8, according to the manufacturer's instructions (Invitrogen,

#111.47D). Cells were grown in complete RPMI media with activators in triplicate wells of 0.9 million cells per 450 μL. They were also initially dosed with 50 μL of dasatinib (10 nM, 50 nM or 100 nM), or an equivalent volume of DMSO pre-mixed with complete RPMI media. Media and fresh drugs were replenished twice a week at 250 μL. Every seven days, virus production in the supernatant was quantified by $p24^{gag}$ antigen ELISA, following the manufacturer's instructions (ZeptoMetrix, Franklin, USA). Cell viability was also quantified on days 14 and 21, by the XTT Cell Viability Assay (Invitrogen). The experiment ended after 21 days.

Human leukocyte reconstitution of NSG mice

All animal procedures were approved by the Animal Ethics Review Committee at the University of Toronto (AUP 20010744, 20010745). These experiments occurred at the CCBR animal care facility at U of T, a pathogen free facility that regulates temperature, humidity and light/dark cycles. Mice were administered standard chow and facility-filtered water *ad libitum*. Mice were anesthetized with isoflurane for all procedures. Following myeloablation and engraftment, mice were monitored daily for any clinical signs of sickness (weight loss, inactivity, graft-versus-host disease). Some mice were euthanized due to graft-versus-host complications prior to any infection. At the experimental endpoint, all animals were anesthetized and humanely euthanized by exsanguination, with every effort to minimize suffering.

We characterized the leukocyte subpopulations in a published humanized mouse model suitable for HIV-1 infection studies [18,22]. Briefly, four-week old male and female NOD/*LtsZ-scidIL2Rγ*^{null} (NSG) mice were purchased from Jackson Labs, Bar Harbor, USA. To deplete hematopoietic stem cells, mice were injected with 50 mg/kg myeloablative busulfan by intraperitoneal (i.p.) injection, given at two 25 mg/kg doses 12 hours apart (Sigma-Aldrich). This was done to deplete

mouse bone marrow cells and permit engraftment of human cells. Twenty-four hours thereafter, we isolated $CD34^+$ cells from pooled cord blood hematopoietic progenitor cells (CB-HPCs) thawed from frozen (Stem Cell Technologies, #70008). 250,000 $CD34^+$ cells were transplanted into each mouse via tail-vein injection. Multi-lineage reconstitution of human leucocytes was confirmed by flow cytometry 19 weeks after human $CD34^+$ cell engraftment.

Ba-L infection of humanized NSG mice

HIV-1 Ba-L ($TCID_{50}$ = 10,000) was injected into 19 humanized mice by i.p. injection. Starting at week 5, blood samples were taken weekly via saphenous bleed in EDTA-coated tubes and 20 µL of plasma was collected. Each sample was diluted to 1 mL with deionized distilled water and analyzed for viral RNA using the m2000 RealTime HIV-1 Viral Load Assay (Abbott Molecular). Between weeks 5 and 7 of infection, mice were sub-Q injected daily with 25 mg/kg T-20 peptide fusion inhibitor (NIH AIDS Reagent Program, Division of AIDS, NIAD, USA), 25-30 mg/kg dasatinib, or vehicle diluent (PBS containing 5.1% PEG-400 + 5.1% Tween-80). No mice died or were euthanized during Ba-L infection or exposure to the drugs tested.

Flow cytometry and data acquisition

At 19 weeks post-engraftment, we collected mouse peripheral blood from saphenous vein for flow cytometry study. PBMCs were stained with anti-mouse CD45 (clone 30-F11) conjugated to PerCP-Cy5.5 (eBiosciences), anti-human CD45 (clone 2D1) conjugated to APC-Cy7 (BD Biosciences), anti-human CD33 (clone WM53) conjugated to Alexa Fluor 700 (BD Biosciences), anti-human CD3 (clone UCHT1) conjugated to PE-Cy7 (eBiosciences), anti-human CD4 (RPA-T4) conjugated to APC (BD Biosciences), anti-human CD8β (clone 2ST8.5H7) conjugated to ECD (Beckman Coulter), anti-human TCRαβ (clone IP26) conjugated

to FITC (eBioscience), anti-human CD19 (clone HIB19) conjugated to PE (eBioscience), anti-human CCR5 (clone 2D7/CCR5) conjugated to Brilliant Violet 421 (BD Biosciences), and live/dead fixable aqua (Molecular Probes). Cells were acquired using a LSRFortessa (BD Biosciences).

Statistical analysis

Means were compared using a two-tailed, unpaired Student's *t* test and corrected for multiple comparisons when more than two means were considered in an experiment. FACS data shows the percent positive cells collected and was analyzed by FlowJo version10 software (Treestar). A two-way ANOVA was performed to analyze data in Figure 5b. For all figures, an asterisk (*) denotes a p value < 0.05, two asterisks (**) denotes a p value < 0.01 and three asterisks (***) denotes a p value < 0.001. Error bars shown are the standard error around the mean (SEM).

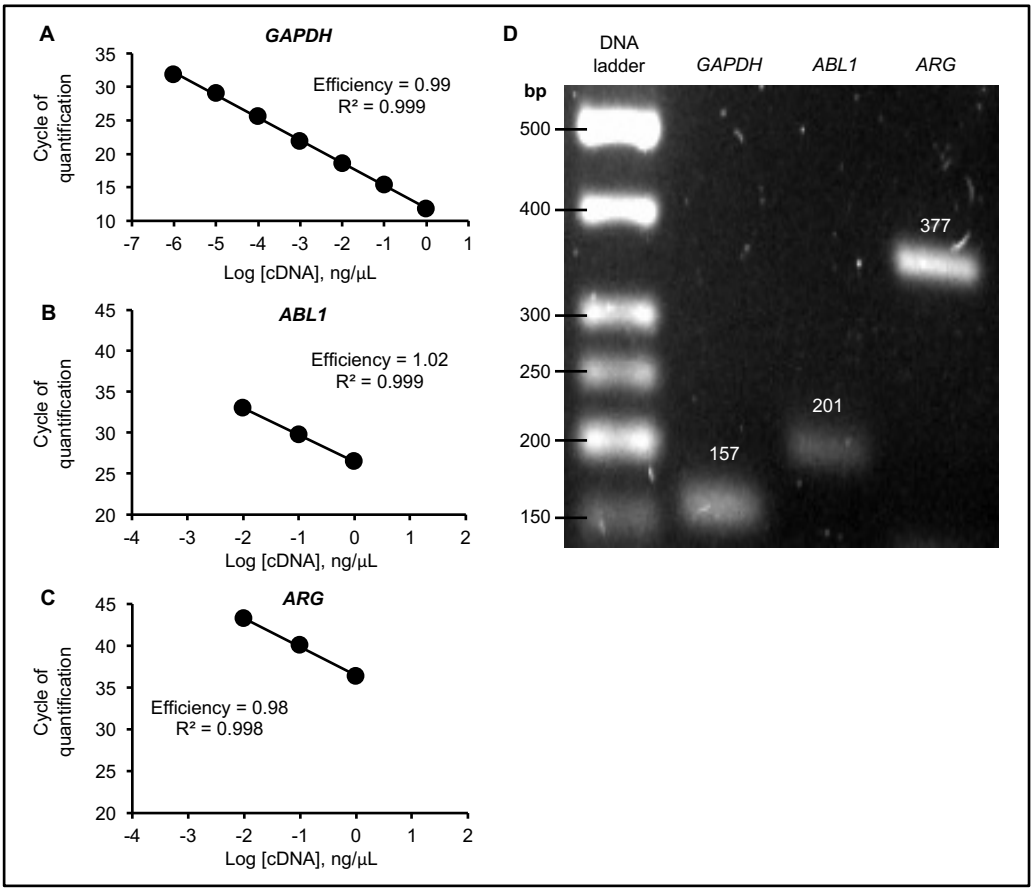

Figure S1: qRT-PCR standard curves and DNA amplicon products.

To satisfy assumptions of the $2^{-\Delta CT}$ comparative C_T method, primer efficiency curves were performed on serial dilutions of cDNA reverse transcribed from $CD4^+$ T-cell lysates. Primer sets tested were targeting (**A**) *GAPDH*, (**B**) *ABL1* or (**C**) *ARG*. Each data point is the mean of 4 technical replicate PCR reactions. (**D**), Representative cDNA amplicons after 40 qRT-PCR cycles were run on a 2% agarose gel. The predicted amplicon size for each primer set is displayed.

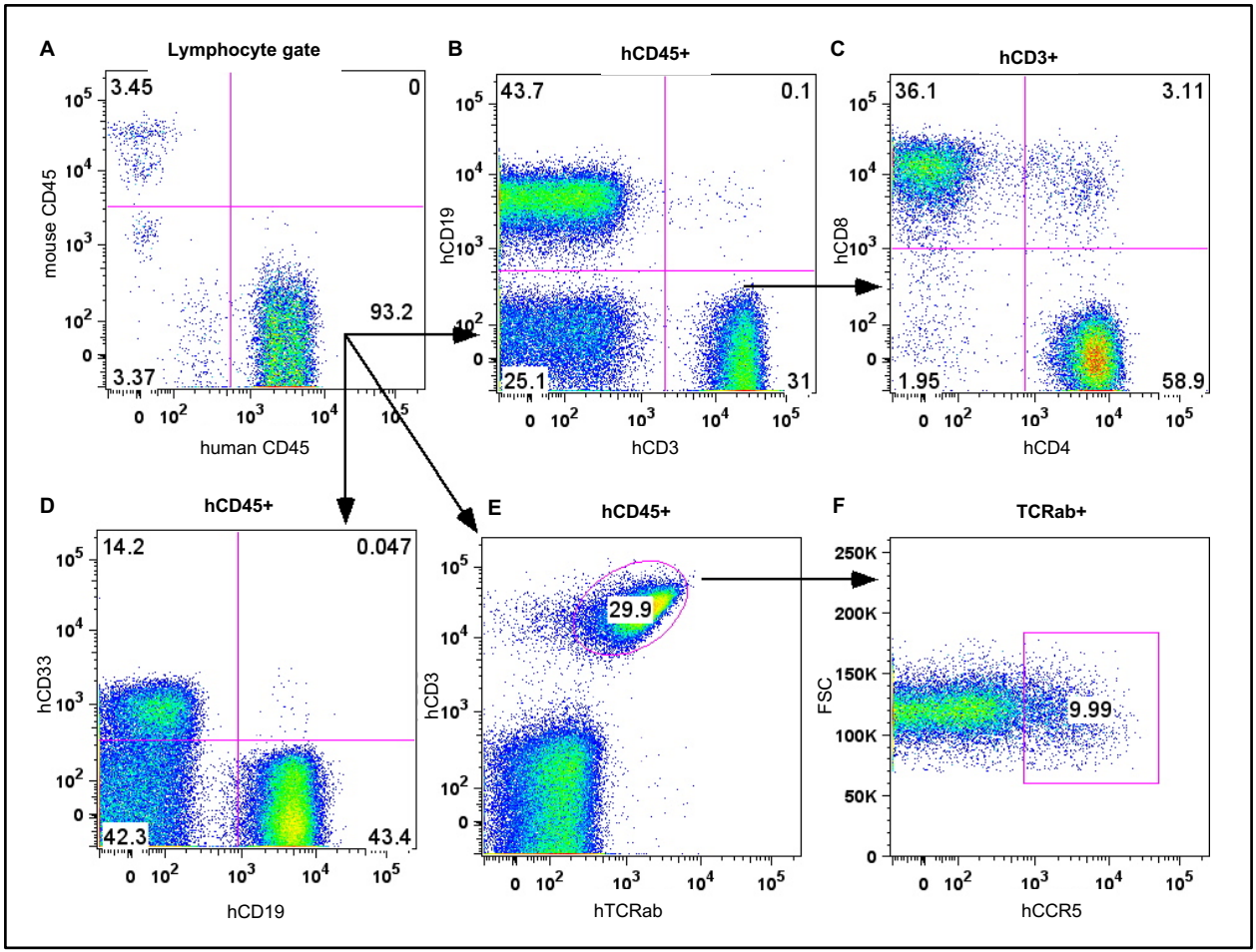

Figure S2: Human leukocyte multilineage reconstitution in NSG mice.

Flow cytometry was performed on the PBMCs collected from a representative mouse 19-weeks post-transplant of CD34$^+$ cells derived from CB-HPCs. Cells were analyzed via doublet-exclusion, viability staining and lymphocyte-gating. (**A**), One million PBMCs were prepared for FACS and stained with mouse CD45 and human CD45. (**B**), hCD45$^+$ cell population stained with human CD19 and human CD3. (**C**), Subset of hCD45$^+$ hCD3$^+$ cells that were stained with human CD8 and human CD4. (**D**), hCD45$^+$ cell population stained with human CD33. (**E**), hCD45$^+$ cells stained with human CD3 and human TCR antibody. (**F**), Subset of hCD45$^+$ hTCRab$^+$ cells that were stained with human CCR5. This figure is representative of three independent experiments.