
Supplementary information

Systemic HIV and SIV latency reversal via non-canonical NF- κ B signalling in vivo

In the format provided by the authors and unedited

Christopher C. Nixon, Maud Mavigner, Gavin C. Sampey, Alyssa D. Brooks, Rae Ann Spagnuolo, David M. Irlbeck, Cameron Mattingly, Phong T. Ho, Nils Schoof, Corinne G. Cammon, Greg K. Tharp, Matthew Kanke, Zhang Wang, Rachel A. Cleary, Amit A. Upadhyay, Chandrav De, Saintedym R. Wills, Shane D. Falcinelli, Cristin Galardi, Hasse Walum, Nathaniel J. Schramm, Jennifer Deutsch, Jeffrey D. Lifson, Christine M. Fennessey, Brandon F. Keele, Sherrie Jean, Sean Maguire, Baolin Liao, Edward P. Browne, Robert G. Ferris, Jessica H. Brehm, David Favre, Thomas H. Vanderford, Steven E. Bosinger, Corbin D. Jones, Jean-Pierre Routy, Nancie M. Archin, David M. Margolis, Angela Wahl, Richard M. Dunham, Guido Silvestri, Ann Chahroudi & J. Victor Garcia

Fig. 1a: Top panel. First stained for p100/p52 then stripped and reprobed for cIAP1 and then stripped and reprobed for β -actin. For p100, p52 and b-actin the molecular weight ladder was acquired in visible light and overlaid in red over the enhanced chemiluminescence (ECL) signal in green.

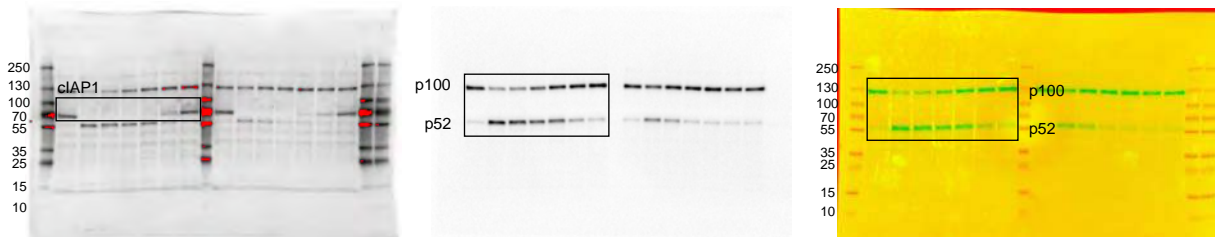


Fig. 1a: Middle panel. First stained for cIAP1 or cIAP2 then stripped and reprobed for β -actin. The molecular weight ladder was acquired in visible light and overlaid in red over the ECL signal in green. β -actin controls were not included in the figure but shown here.

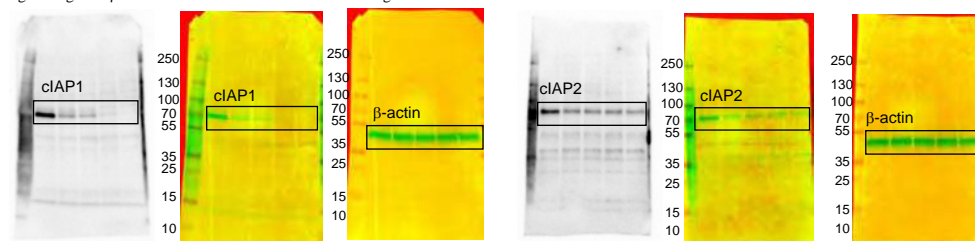
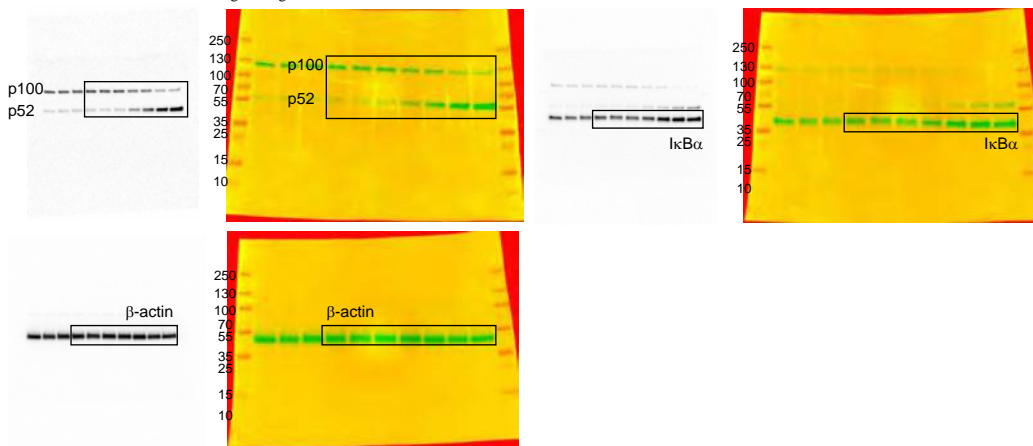
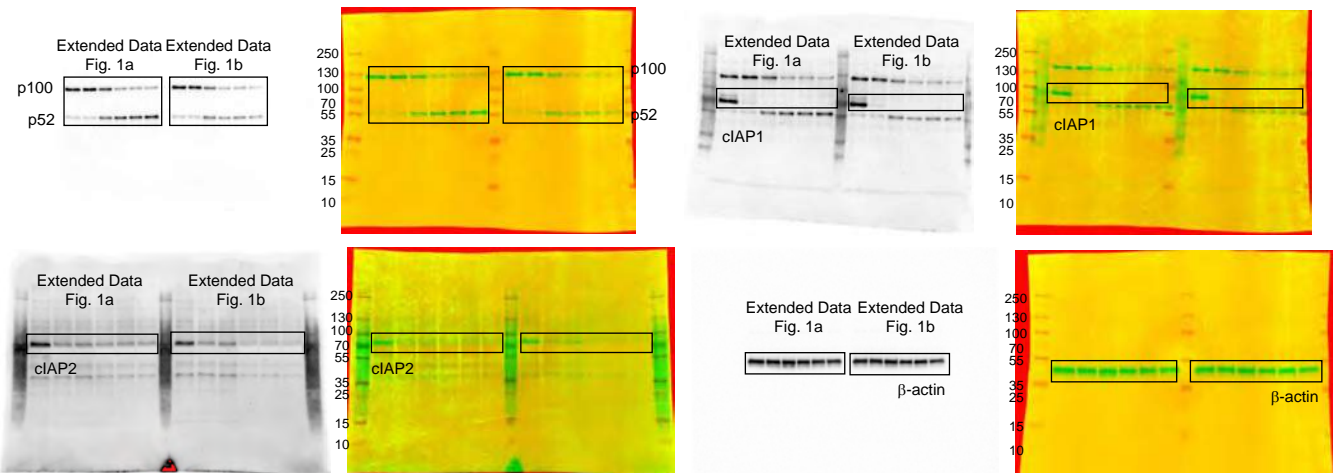


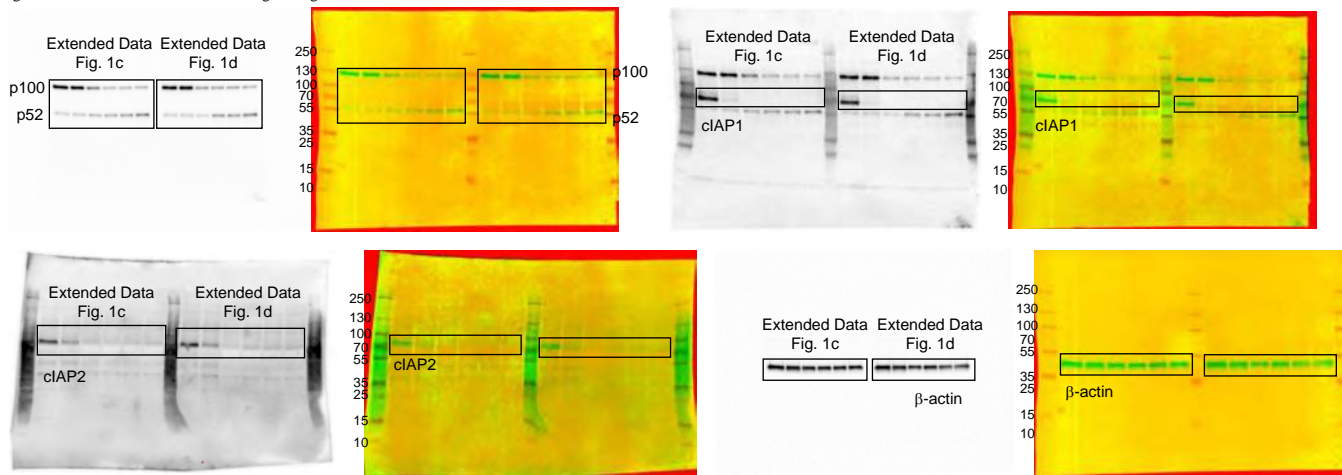
Fig. 1a: Bottom panel. First stained for p100 and p52, then stripped and reprobed for $I\kappa B\alpha$, then stripped and reprobed for β -actin. The molecular weight ladder was acquired in visible light and overlaid in red over the ECL signal in green.



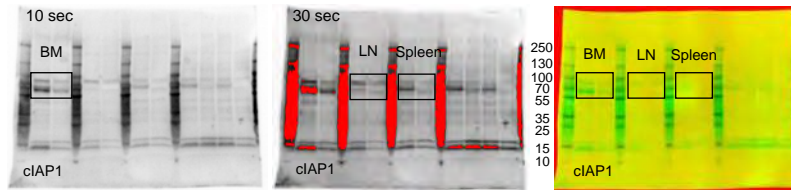
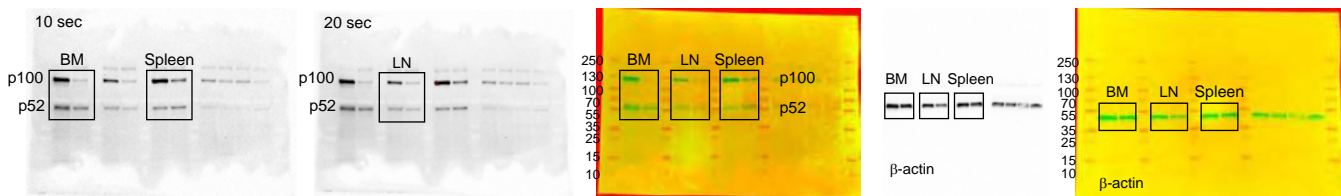
Extended Data Fig. 1a,b. First stained for p100/p52 then stripped and reprobed for cIAP1 and then stripped and reprobed for β -actin. The molecular weight ladder was acquired in visible light and overlaid in red over the ECL signal in green.



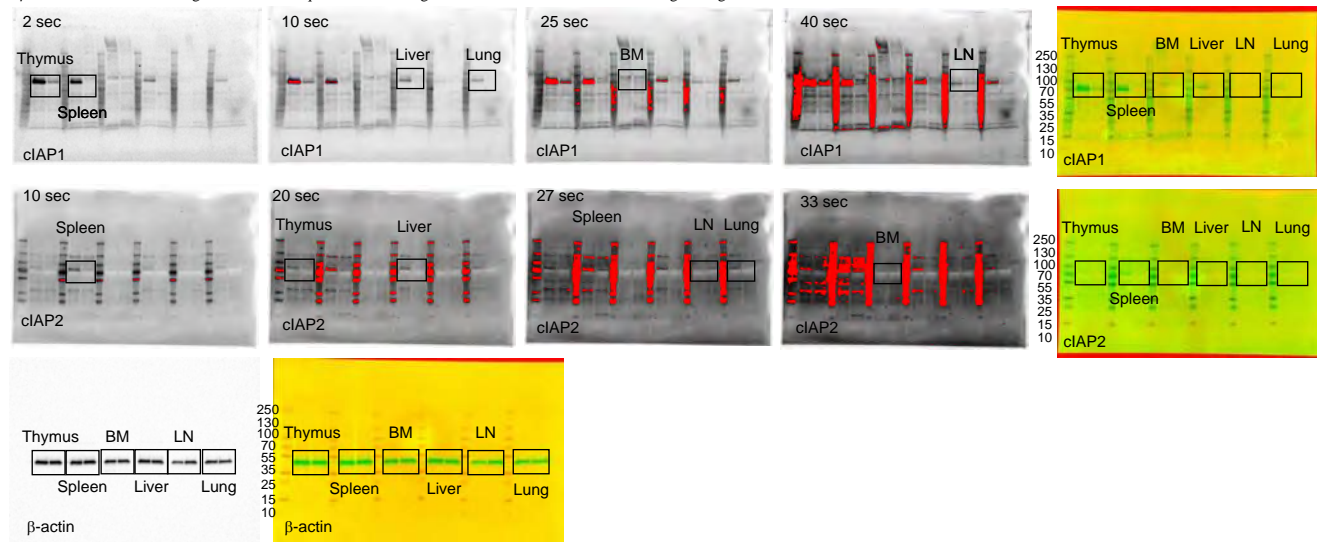
Extended Data Fig. 1c,d. First stained for p100/p52 then stripped and reprobed for cIAP1 and then stripped and reprobed for β -actin. The molecular weight ladder was acquired in visible light and overlaid in red over the ECL signal in green.



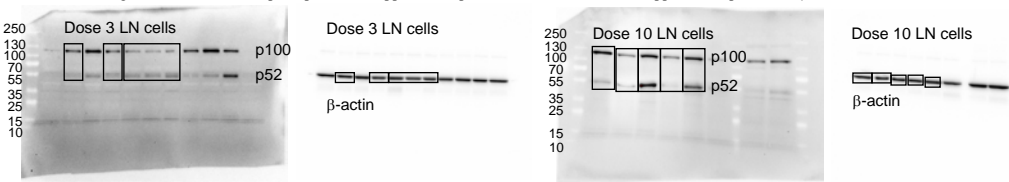
Extended Data Fig. 3a. The same membrane was acquired for different lengths of time to obtain optimal images of the target bands for each tissue. The cIAP1 blot was stripped and reprobed for β -actin. The molecular weight ladder was acquired in visible light and overlaid in red over the ECL signal in green.



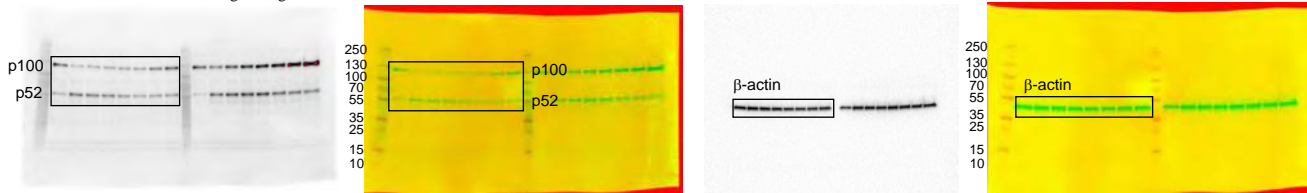
Extended Data Fig. 3b The same membrane was acquired for different lengths of time to obtain optimal images of the target bands for each tissue. The cIAP1 blot was stripped and reprobed for β -actin. The molecular weight ladder was acquired in visible light and overlaid in red over the ECL signal in green.



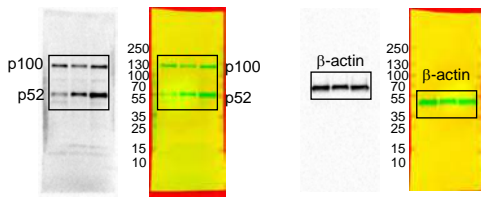
Extended Data Fig. 4c. First stained for p100/p52 then stripped and reprobed for cIAP1 and then stripped and reprobed for β -actin.



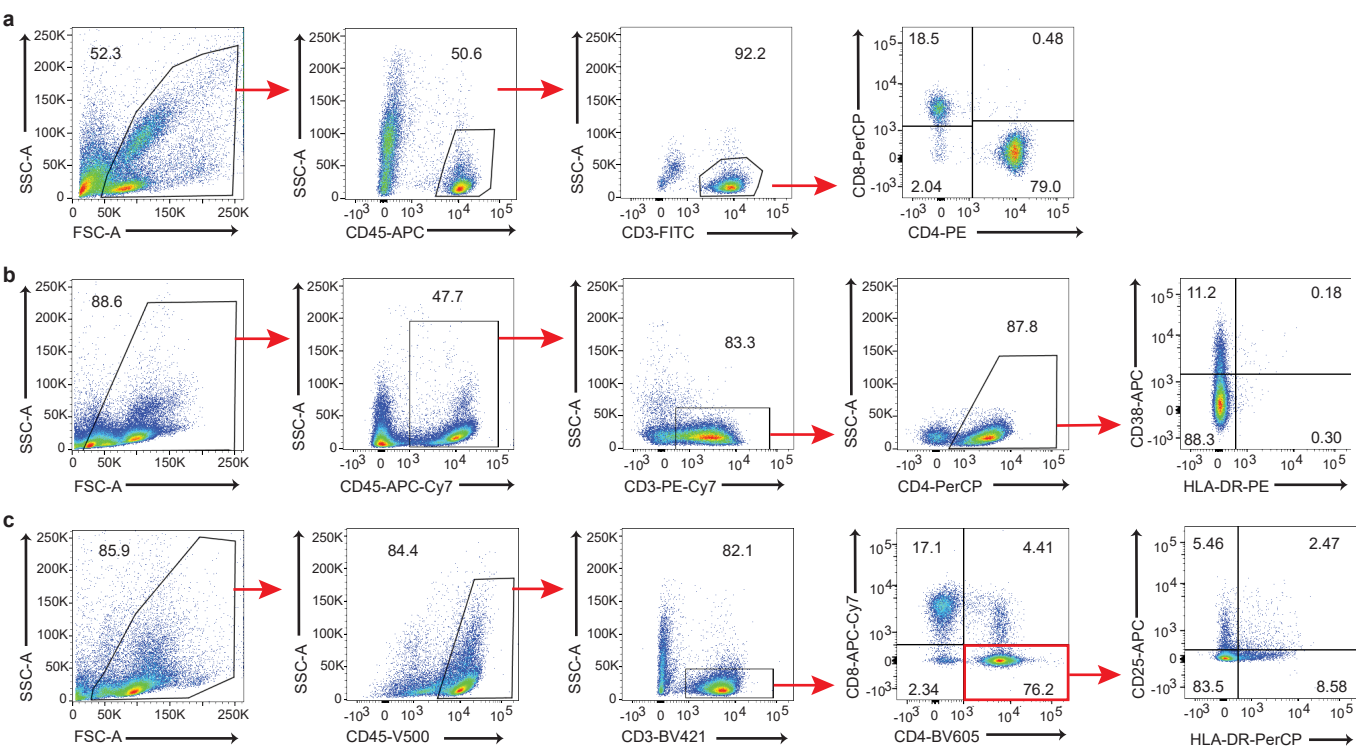
Extended Data Fig. 4d. First stained for p100/p52 then stripped and reprobed for cIAP1 and then stripped and reprobed for β -actin. The molecular weight ladder was acquired in visible light and overlaid in red over the ECL signal in green.



Extended Data Fig. 4f. First stained for p100/p52 then stripped and reprobed for cIAP1 and then stripped and reprobed for β -actin. The molecular weight ladder was acquired in visible light and overlaid in red over the ECL signal in green.



Supplementary Figure 1: Uncropped gel scans



Supplementary Figure 2: Flow cytometric gating strategies in BLT mice. Gating strategies for the identification of (a) human CD4⁺ and CD8⁺ T cells, (b) activated (CD38⁺HLA-DR⁺) T cells, and (c) resting (CD25^{neg} HLA-DR^{neg}) CD4⁺ T cells.

| Experiment | Treatment group | Mouse ID | At time of HIV exposure | |
|------------|-----------------|--------------|-------------------------------|-------------------|
| | | | Peripheral blood humanization | |
| | | | %CD45 ⁺ | %CD4 ⁺ |
| #1 | Control vehicle | 1 | 57.4 | 76.2 |
| | | 2 | 40.8 | 64.8 |
| | | 3 | 49.7 | 70.4 |
| | | 4 | 51.8 | 51.4 |
| | | 5 | 54 | 68 |
| | | 6 | 54.7 | 67.2 |
| | | Mean ± s.e.m | 51.4% ± 2.4% | 66.3% ± 3.4% |
| | AZD5582 | 7 | 55.7 | 77.5 |
| | | 8 | 54.4 | 68.5 |
| | | 9 | 57.2 | 76.9 |
| | | 10 | 51.9 | 70.8 |
| | | 11 | 41.8 | 73.1 |
| 12 | | 47.9 | 50.4 | |
| | Mean ± s.e.m | 51.5% ± 2.3% | 69.5% ± 4.1% | |
| #2 | Control vehicle | 13 | 72.5 | 88.1 |
| | | 14 | 49.1 | 90.1 |
| | | 15 | 41.3 | 86.1 |
| | | 16 | 31.3 | 74.0 |
| | | Mean ± s.e.m | 48.6% ± 8.8% | 84.6% ± 3.62% |
| | AZD5582 | 17 | 74.9 | 72.8 |
| | | 18 | 42.2 | 79.5 |
| | | 19 | 38.3 | 78.6 |
| | | 20 | 37.2 | 82.8 |
| | | Mean ± s.e.m | 48.15 ± 10% | 78.43 ± 2.1% |

Supplementary Table 1: Levels of human cells in BLT mice at the time of HIV-1_{JR-CSF}

exposure as determined by flow cytometry analysis of peripheral blood. BLT mice in

experiment 1 are shown in Fig. 2b,c left panels and BLT mice in experiment 2 are shown in Fig.

2b,c right panels. s.e.m: standard error mean. Gating strategy=Live→human CD45

(%CD45⁺)→human CD3→human CD4 (%CD4⁺).

| Experiment | Treatment group | Mouse ID | Viral load (copies/mL plasma) | | |
|------------|-----------------|----------|-------------------------------|-------|-------|
| | | | 0 h | 24 h | 48 h |
| #1 | Control vehicle | 1 | < LOQ | < LOQ | < LOQ |
| | | 2 | < LOQ | < LOQ | < LOQ |
| | | 3 | < LOQ | < LOQ | < LOQ |
| | | 4 | < LOQ | < LOQ | < LOQ |
| | | 5 | < LOQ | < LOQ | < LOQ |
| | | 6 | < LOQ | < LOQ | < LOQ |
| | AZD5582 | 7 | < LOQ | < LOQ | < LOQ |
| | | 8 | < LOQ | < LOQ | < LOQ |
| | | 9 | < LOQ | < LOQ | < LOQ |
| | | 10 | < LOQ | < LOQ | 553 |
| | | 11 | < LOQ | < LOQ | 773 |
| | | 12 | < LOQ | < LOQ | 876 |
| #2 | Control vehicle | 13 | < LOQ | < LOQ | < LOQ |
| | | 14 | < LOQ | < LOQ | < LOQ |
| | | 15 | < LOQ | < LOQ | < LOQ |
| | | 16 | < LOQ | < LOQ | < LOQ |
| | AZD5582 | 17 | < LOQ | < LOQ | < LOQ |
| | | 18 | < LOQ | < LOQ | 573 |
| | | 19 | < LOQ | < LOQ | 953 |
| | | 20 | < LOQ | < LOQ | 1,574 |

Supplementary Table 2: Detectable plasma viremia in HIV-infected, ART-suppressed BLT mice following a single dose of AZD5582 (3 mg/kg). The limit of quantification (LOQ) for this assay is 350 copies/mL.

| Treatment group | Tissue | Copies HIV-DNA/ 10 ⁵ resting CD4+ T cells | Fold difference between control and AZD5582 |
|-----------------|-----------------|---|--|
| Control vehicle | Lung | 369.303 | 1.3 |
| | Bone marrow | 49.988 | -0.59 |
| | Thymic organoid | 17.58 | -1.56 |
| AZD5582 | Lung | 272.822 | |
| | Bone marrow | 84.33 | |
| | Thymic organoid | 27.43 | |

Supplementary Table 3: Cell-associated HIV-DNA levels in the tissues of ART-suppressed BLT mice following a single dose of AZD5582 (3 mg/kg).

| Analyte | Serum concentration | | | Comparison of serum concentrations | | |
|----------------------------|---------------------|--------------|---------------|------------------------------------|------------|-------------|
| | 0 h | 24 h | 5 d | 0 h vs 24 h | 0 h vs 5 d | 24 h vs 5 d |
| Albumin | 3.2 ± 0.18 | 3.85 ± 0.55 | 2.63 ± 0.10 | p=0.5000 | p=0.1250 | p=0.2500 |
| Alkaline phosphatase | 61.33 ± 30.02 | 58.5 ± 3.1 | 17.75 ± 3.17 | p=0.7500 | p=0.5000 | p=0.1250 |
| Alanine aminotransferase | 118.50 ± 57.40 | 702 ± 170.71 | 33.50 ± 12.87 | p=0.1250 | p=0.2500 | p=0.1250 |
| Amylase | 593.50 ± 21.85 | 607 ± 38.54 | 397 ± 101.04 | p>0.9999 | p=0.2500 | p=0.2500 |
| Aspartate aminotransferase | 235.67 ± 106.18 | 709 ± 216.11 | 92 ± 10.03 | p=0.2500 | p=0.2500 | p=0.1250 |
| Blood urea nitrogen | 23 ± 1.78 | 11.5 ± 0.96 | 15 ± 1 | p=0.1250 | p=0.1250 | p=0.2500 |
| Calcium | 6.38 ± 1.57 | 10.75 ± 0.22 | 11.7 ± 0.26 | p=0.2500 | p=0.1250 | p=0.2500 |
| Creatinine | 0.29 ± 0.06 | 0.11 ± 0.02 | 0.46 ± 0.04 | p=0.1250 | p=0.2500 | p=0.1250 |
| Phosphorus | 4.45 ± 1.4 | 5.45 ± 0.25 | 4.90 ± 0.44 | p>0.9999 | p=0.8750 | p=0.3750 |
| Total bilirubin | 2.15 ± 0.95 | 1.15 ± 0.22 | 0.80 ± 0.14 | p=0.5000 | p=0.2500 | p=0.3750 |
| Total protein | 5.27 ± 0.24 | 5.90 ± 0.97 | 6.45 ± 0.33 | p>0.9999 | p=0.2500 | p=0.8750 |

Supplementary Table 4: Levels of serum indicators for organ drug toxicity. Serum was collected from BALB/c mice (n=4) immediately prior to, 24 h after, and five days after administration of a single dose of AZD5582. Shown are the mean concentrations (\pm standard error mean) of serum albumin (g/dL), alkaline phosphatase (U/L), alanine aminotransferase (U/L), amylase (U/L), aspartate aminotransferase (U/L), blood urea nitrogen (mg/dL), calcium (mg/dL), creatinine (mg/dL), phosphorus (mg/dL), total bilirubin (mg/dL) and total protein (g/dL). A two-sided Wilcoxon matched-pairs signed ranks T test was used to evaluate statistical significance.

| | Tissue | Treatment group | | p value |
|-------------------------------|-----------------|-----------------|-------------|---------|
| | | Control vehicle | AZD5582 | |
| %CD38+HLA-DR+ of CD4+ T cells | Bone marrow | 0.85 ± 0.35 | 1.15 ± 0.68 | 0.8593 |
| | Thymic organoid | 2.7 ± 1.13 | 2.78 ± 1.03 | 0.6991 |
| | Lymph nodes | 1.23 ± 0.50 | 1.40 ± 0.57 | 0.9372 |
| | Spleen | 0.71 ± 0.31 | 0.63 ± 0.21 | 0.8593 |
| | Liver | 1.08 ± 0.38 | 1.04 ± 0.36 | 0.8939 |
| | Lung | 0.56 ± 0.23 | 0.89 ± 0.40 | 0.8139 |
| %CD38+HLA-DR+ of CD8+ T cells | Bone marrow | 1.8 ± 0.43 | 1.69 ± 0.99 | 0.3874 |
| | Thymic organoid | 0.30 ± 0.11 | 0.23 ± 0.08 | 0.8030 |
| | Lymph nodes | 0.35 ± 0.25 | 0.53 ± 0.24 | 0.6991 |
| | Spleen | 1.87 ± 0.50 | 0.62 ± 0.36 | 0.0260 |
| | Liver | 0.23 ± 0.08 | 0.34 ± 0.21 | 0.4827 |
| | Lung | 0.35 ± 0.19 | 0.86 ± 0.47 | 0.4177 |

Supplementary Table 5: AZD5582 does not activate T cells in the tissues of HIV-infected, ART-suppressed BLT mice. Shown is the mean percentage (\pm standard error mean) of CD4⁺ and CD8⁺ T cells co-expressing CD38 and HLA-DR from the bone marrow, thymic organoid, lymph nodes, spleen, liver and lung of HIV-infected, ART-suppressed BLT mice (n=6/group) 24 h after treatment with control vehicle or AZD5582. A two-sided Mann-Whitney test was used to evaluate statistical significance.

| Cytokine/chemokine | Control (pg/mL) | AZD5582 (pg/mL) | Difference between means | p value |
|--------------------|-----------------|-----------------|--------------------------|---------|
| EGF | < LOQ | < LOQ | NA | NA |
| FGF-2 | 616.1 ± 192 | 626.6 ± 218 | 10.5 ± 26 | 0.7835 |
| Eotaxin | < LOQ | < LOQ | NA | NA |
| TGFα | < LOQ | < LOQ | NA | NA |
| G-CSF | < LOQ | < LOQ | NA | NA |
| Flt-3L | 3.205 ± 1.605 | < LOQ | NA | NA |
| GM-CSF | 34.53 ± 16.31 | 32.30 ± 16.1 | -2.23 ± 0.21 | 0.9221 |
| Fractalkine | 26.82 ± 25.22 | 18.67 ± 17.07 | -8.15 ± 8.15 | >0.9999 |
| IFNα2 | 6.038 ± 3.193 | 4.069 ± 2.469 | -1.969 ± 0.724 | 0.7273 |
| IFNγ | 130.1 ± 41.68 | 130.1 ± 41.34 | 0 ± 0.34 | >0.9999 |
| GRO | 55.12 ± 18.23 | 68.16 ± 19.85 | 13.04 ± 1.62 | 0.6234 |
| IL-10 | 40.39 ± 11.51 | 40.37 ± 11.05 | -0.02 ± 0.46 | 0.9372 |
| MCP-3 | < LOQ | < LOQ | NA | NA |
| IL-12p40 | 21.64 ± 6.422 | 22.23 ± 9.533 | 0.59 ± 3.111 | 0.974 |
| MDC | 166.6 ± 12.84 | 145.9 ± 16.25 | -20.73 ± 3.41 | 0.3939 |
| IL-12p70 | < LOQ | < LOQ | NA | NA |
| PDGF-AA | 1,325 ± 505.5 | 1,303 ± 463.8 | -22 ± 41.70 | >0.9999 |
| IL-13 | 4.649 ± 2.096 | 5.444 ± 2.569 | 0.795 ± 0.473 | 0.8485 |
| PDGF-AB/BB | 201 ± 32.67 | 169.7 ± 36.58 | -31.30 ± 3.91 | 0.4848 |
| IL-15 | 2.825 ± 1.225 | < LOQ | NA | NA |
| sCD40L | 65.34 ± 8.32 | 63.31 ± 9.24 | -2.03 ± 0.92 | >0.9999 |
| IL-17A | 15.32 ± 4.241 | 14.75 ± 4.141 | -0.57 ± 0.100 | 0.9372 |
| IL-1RA | 2.170 ± 0.5698 | 3.619 ± 2.019 | 1.449 ± 1.4492 | >0.9999 |
| IL-1α | 33.93 ± 15.72 | 38.85 ± 21 | 4.92 ± 5.280 | 0.8139 |
| IL-9 | < LOQ | < LOQ | NA | NA |
| IL-1β | < LOQ | < LOQ | NA | NA |
| IL-2 | 1.907 ± 0.3074 | 2.066 ± 0.4656 | 0.159 ± 0.1582 | >0.9999 |
| IL-3 | < LOQ | < LOQ | NA | NA |
| IL-4 | 24.58 ± 9.935 | 24.64 ± 8.402 | 0.06 ± 1.533 | 0.8939 |
| IL-5 | 10.10 ± 7.984 | 9.210 ± 7.268 | -0.8900 ± 0.716 | 0.8485 |
| IL-6 | 9.847 ± 5.244 | 10.43 ± 5.650 | 0.5830 ± 0.4060 | 0.8485 |
| IL-7 | < LOQ | < LOQ | NA | NA |
| IL-8 | 60.04 ± 27.66 | 66.50 ± 31.76 | 6.46 ± 4.1 | 0.7381 |
| IP-10 | 1,071 ± 448.9 | 1,076 ± 434.3 | 5.000 ± 14.60 | >0.9999 |
| MCP-1 | 461.5 ± 114.8 | 465.4 ± 120.3 | 3.9 ± 5.500 | >0.9999 |
| MIP-1α | < LOQ | < LOQ | NA | NA |
| MIP-1β | 9.593 ± 3.152 | 10.22 ± 3.037 | 0.6270 ± 0.1150 | 0.7792 |
| RANTES | 840.3 ± 364.8 | 764.3 ± 329.8 | -76 ± 35.00 | 0.5887 |
| TNFα | 21.41 ± 10.45 | 22.6 ± 9.11 | 1.190 ± 1.340 | 0.9805 |
| TNFβ | < LOQ | < LOQ | NA | NA |
| VEGF | 9.014 ± 7.414 | 9.655 ± 5.33 | 0.641 ± 2.084 | 0.5455 |

Supplementary Table 6: Plasma cytokine analysis from mice treated with vehicle control or AZD5582. BLT mice were administered vehicle control (n=6) or AZD5582 (n=6) and peripheral blood plasma collected 24 h later to analyze the levels of 41 human cytokines/chemokines that are commonly associated with cell activation. Shown is the mean concentration (pg/mL) ± standard error mean of each cytokine/chemokine analyzed, the difference between the mean concentrations of each cytokine/chemokine between groups and the corresponding p value as

determined with a two-sided Mann-Whitney U test. Assay range: $3.2-1 \times 10^4$ pg/mL. LOQ: limit of quantification. NA: not applicable. For statistical analysis, values <LOQ were set at 1.6 pg/ml.

| RM ID | Group | Sex | Date of birth | Age at first dose (years) |
|-------|---------|--------|---------------|---------------------------|
| RDm16 | AZD5582 | Male | 4/19/2014 | 3.8 |
| RDl16 | AZD5582 | Male | 4/12/2014 | 3.8 |
| RKn16 | AZD5582 | Male | 4/26/2014 | 3.8 |
| RFk16 | AZD5582 | Male | 4/08/2014 | 3.8 |
| RNp16 | AZD5582 | Male | 4/08/2014 | 3.8 |
| RLu16 | AZD5582 | Male | 6/10/2014 | 3.6 |
| RQs16 | AZD5582 | Male | 5/28/2014 | 4.0 |
| RAr16 | AZD5582 | Male | 5/17/2014 | 4.0 |
| RKp16 | AZD5582 | Male | 5/06/2014 | 4.1 |
| RLy15 | AZD5582 | Male | 5/02/2013 | 5.1 |
| RKl16 | AZD5582 | Male | 4/14/2014 | 4.1 |
| RYs16 | AZD5582 | Male | 5/30/2014 | 4.0 |
| RAj16 | Control | Male | 3/29/2014 | 3.9 |
| RDF16 | Control | Male | 6/27/2013 | 4.6 |
| RNq16 | Control | Male | 5/15/2014 | 3.8 |
| RQd16 | Control | Male | 6/11/2013 | 4.7 |
| RFv15 | Control | Male | 4/20/2013 | 5.2 |
| RKw16 | Control | Male | 7/07/2014 | 3.9 |
| RU16 | Control | Male | 5/29/2014 | 4.0 |
| RKz15 | Control | Male | 5/06/2013 | 5.1 |
| RZe15 | Control | Female | 4/22/2012 | 6.2 |

Supplementary Table 7: Sex and age of AZD5582-treated and control SIV-infected, ART-suppressed RMs.