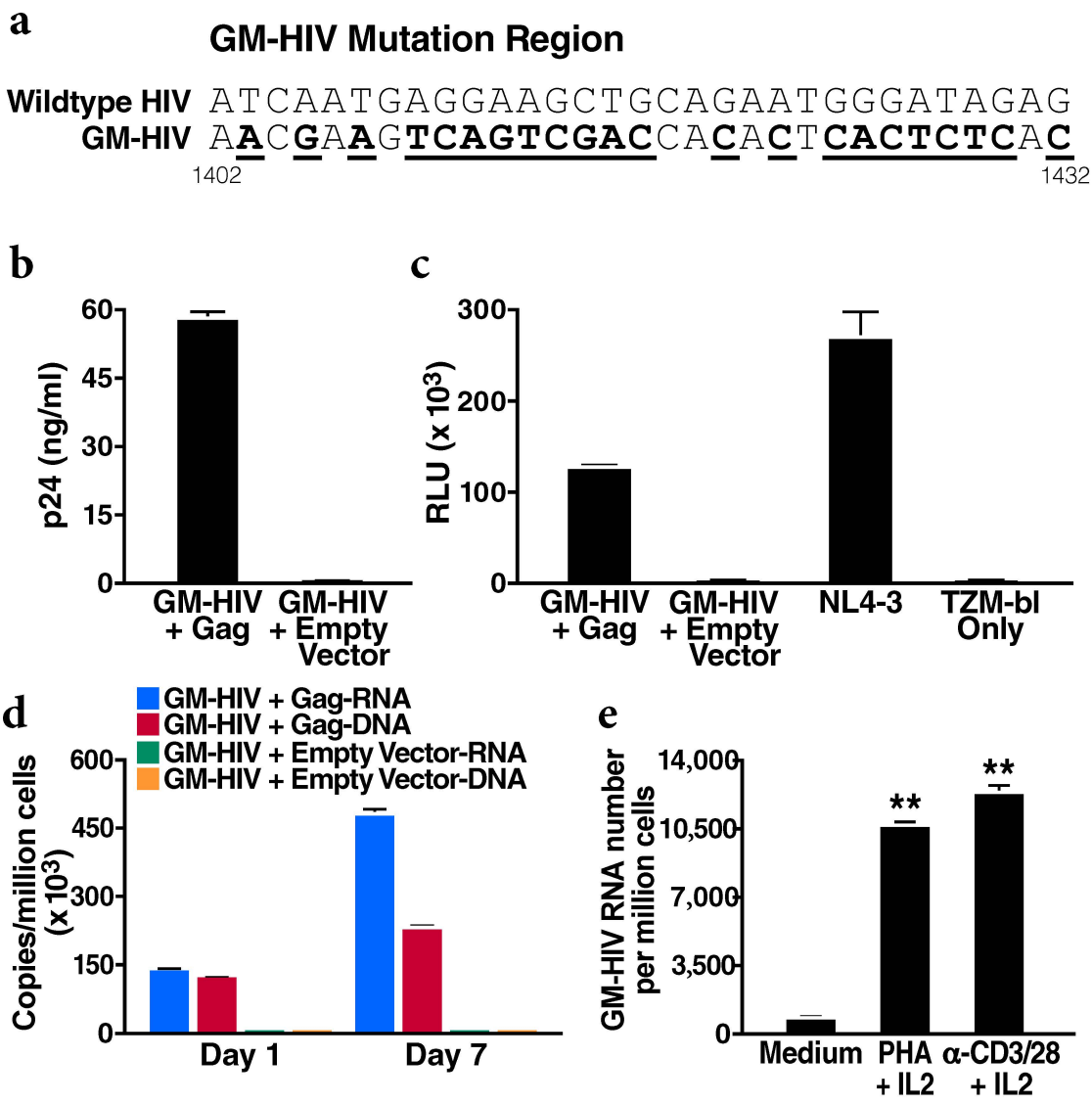
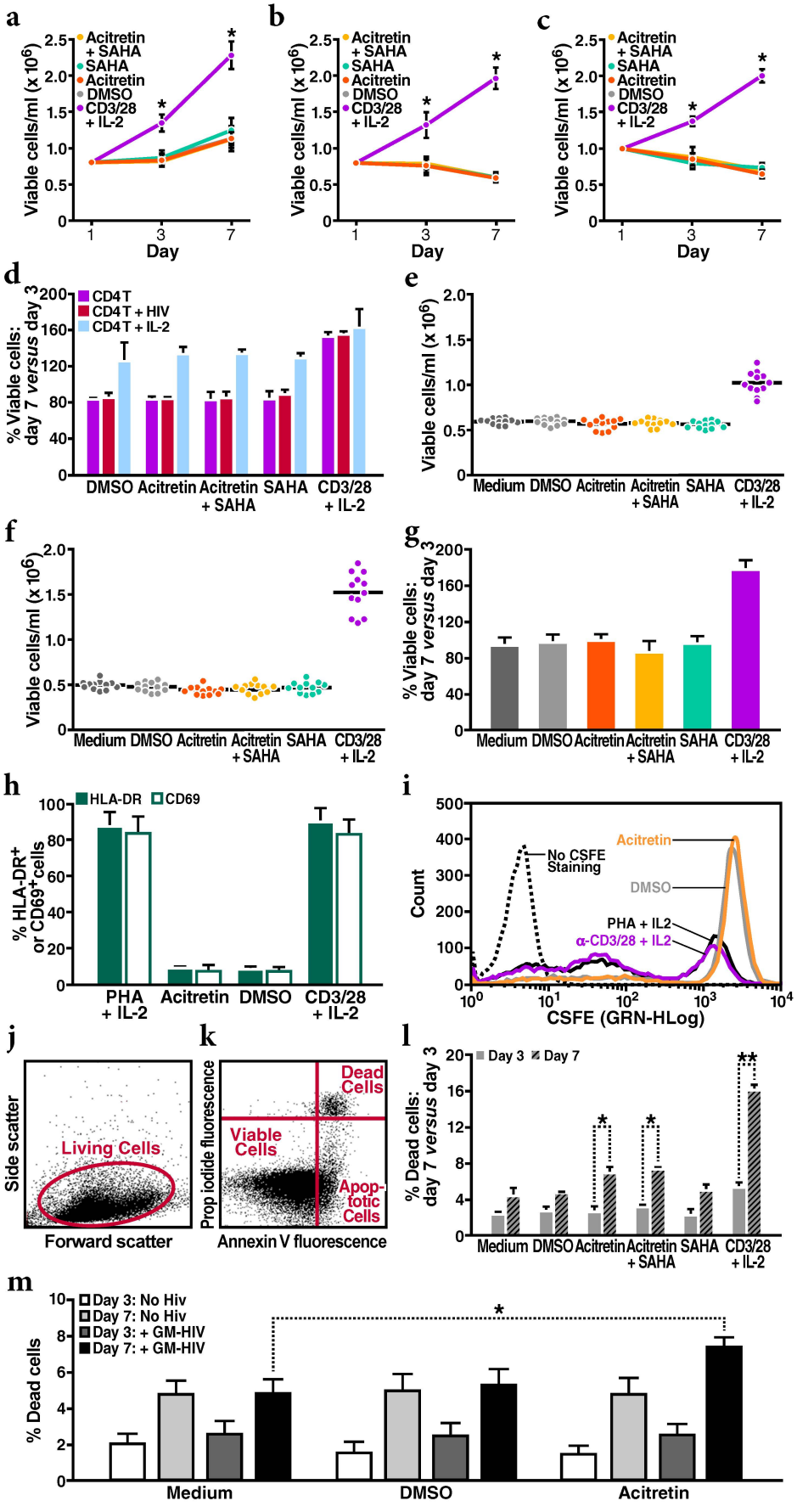
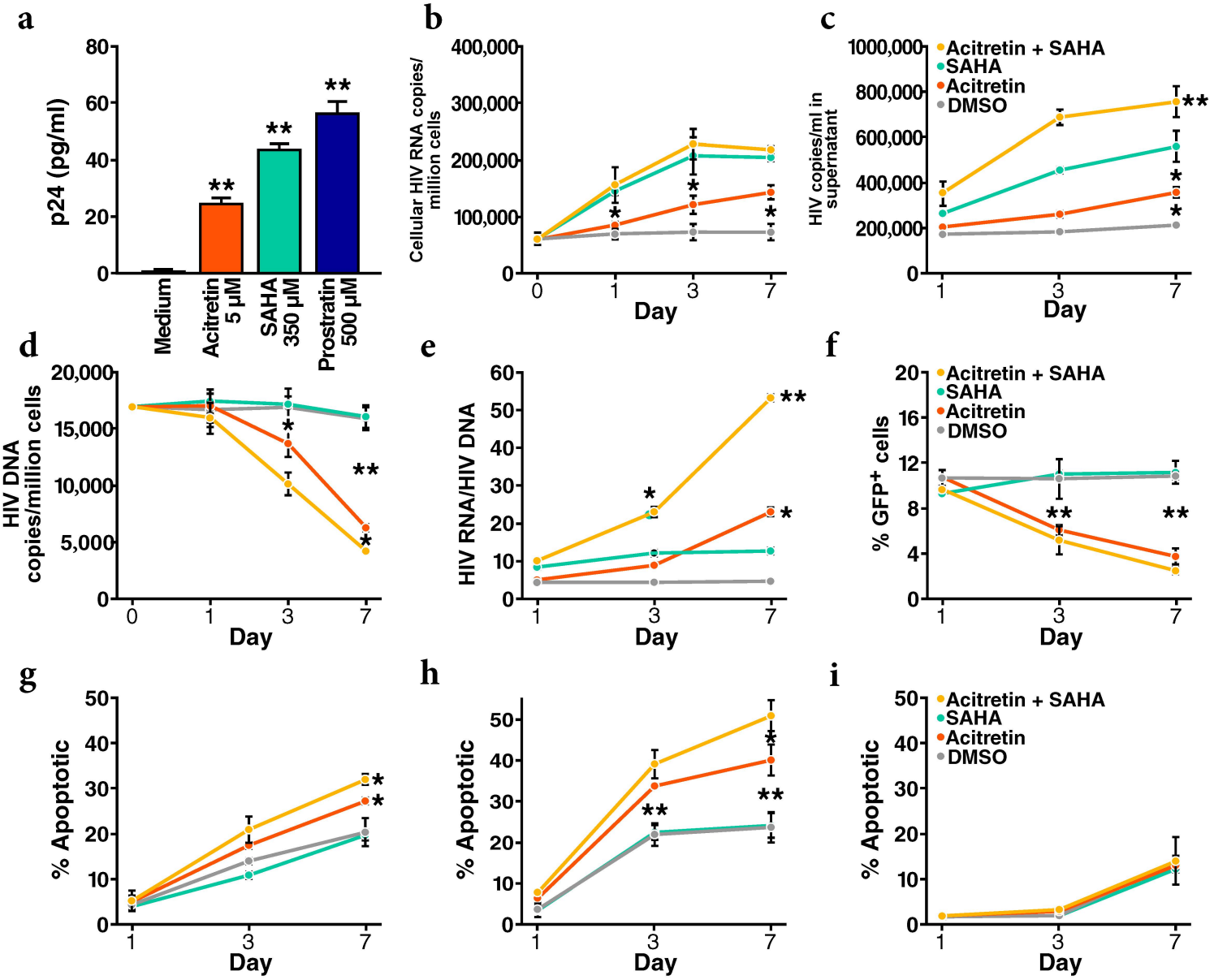
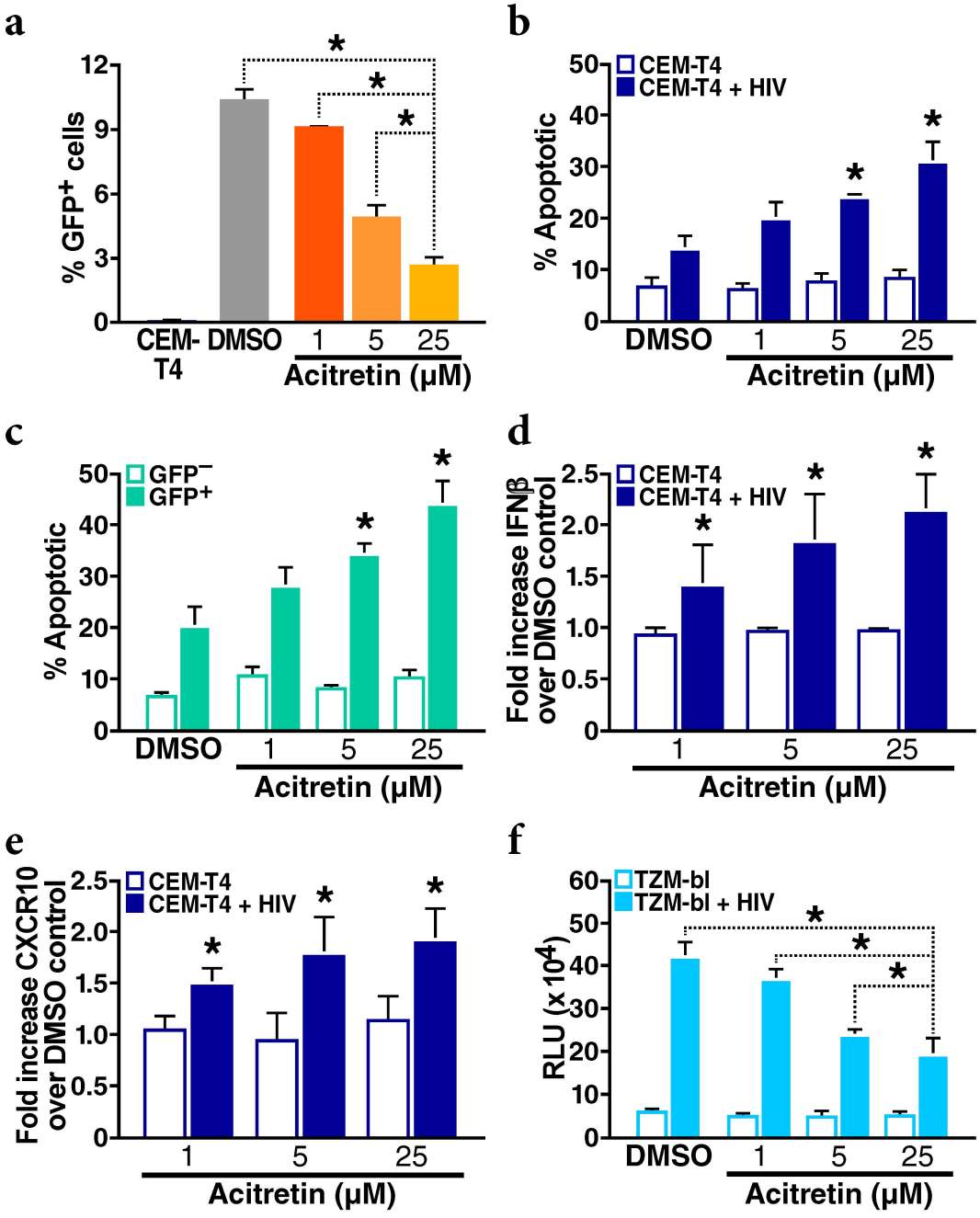

a
GM-HIV Mutation Region
Wildtype HIV ATCAATGAGGAAGCTGCAGAATGGGATAGAG
GM-HIV AACGAAGTCAGTCGACCACACTCACTCTCAC
1402
1432
b
p24 (ng/ml)
60
45
30
15
0
GM-HIV + Gag
GM-HIV + Empty Vector
c
RLU (x 10³)
300
200
100
0
GM-HIV + Gag
GM-HIV + Empty Vector
NL4-3
TZM-bl Only
d
GM-HIV + Gag-RNA
GM-HIV + Gag-DNA
GM-HIV + Empty Vector-RNA
GM-HIV + Empty Vector-DNA
Copies/million cells (x 10³)
600
450
300
150
0
Day 1
Day 7
e
GM-HIV RNA number per million cells
14,000
10,500
7,000
3,500
0
**
**
Medium
PHA + IL2
α-CD3/28 + IL2

Li *et al*, Supplementary Figure 2



Li *et al*, Supplementary Figure 3




a
% GFP+ cells
12
9
6
3
0
CEM-T4
DMSO
1
5
25
Acitretin (μM)
b
% Apoptotic
50
40
30
20
10
0
CEM-T4
CEM-T4 + HIV
DMSO
1
5
25
Acitretin (μM)
c
% Apoptotic
GFP−
GFP+
DMSO
1
5
25
Acitretin (μM)
d
Fold increase IFNβ over DMSO control
2.5
2.0
1.5
1.0
0.5
0
CEM-T4
CEM-T4 + HIV
1
5
25
Acitretin (μM)
e
Fold increase CXCR10 over DMSO control
CEM-T4
CEM-T4 + HIV
1
5
25
Acitretin (μM)
f
RLU (x10^4)
60
50
40
30
20
10
0
TZM-bl
TZM-bl + HIV
DMSO
1
5
25
Acitretin (μM)

Supplementary Figure legends

Supplementary Figure 1 (S-Fig.1)

Mutagenesis of p24 in *gag* and construction of GM-HIV:

(a) Mutagenesis of p24-*gag*. Nucleic acids in the p24 of HIV *gag* that were substituted are underlined and shown in comparison to the HXB2 reference (wild-type). The coordinates are numbered according to the convention for the laboratory strain HXB2. The region from the BssHII (711) restriction site to the SpeI (1507) restriction site from pNL4-3 (pNL4-3 was obtained through the NIH AIDS Reagent Program, division of AIDS, NIAID, NIH (courtesy of Dr. Malcolm Martin), was excised by restriction digestion and subcloned into pcDNA3.1 TOPO TA vector (Invitrogen, USA). The Quikchange II XL Site-Directed Mutagenesis Kit (Stratagene, USA) was employed to mutate positions from 1404 to 1432 as shown. The sequence-verified mutated fragment was religated into the pNL4-3 backbone to create the Gag-mutated-NL4-3 plasmid (pGM-HIV).

(b) Producing GM-HIV. The pGM-HIV requires trans-complementation by a gag expression vector (wildtype *gag* gene was cloned from pNL4-3 into pcDNA3.1 expression vector (Invitrogen, USA) for production of GM-HIV viral particles (including p24) in supernatants of 293T cells after transfection. Otherwise supernatants of 293T cells transfected with pGM-HIV with empty vector (pcDNA3.1) are devoid of p24.

(c). Only supernatants of 293T cells transfected with both pGM-HIV and the gag expression vector, but not supernatants of 293T cells transfected with pGM-HIV and empty vector can infect TZM-bl cells[1] (obtained from the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH courtesy of Dr. John C Kappes, Dr. Xiaoyun Wu, and Tranzyme Inc.). Wildtype HIV NL4-3 was used as a positive control. TZM-bl infection was detected using the Beta-Glo® assay kit (Promega, Madison WI) and measured with a microplate reader (VICTOR3) (PerkinElmer) three days post-infection. Luciferase activity is presented as relative light units (RLU).

(d). GM-HIV viruses are able to infect CD4 T cells. Primary CD4+ T-cells from healthy control donors stimulated with PHA+IL2 were incubated with supernatant from 293T cells transfected with either pGM-HIV+ gag expression plasmid (GM-HIV+Gag) or pGM-HIV+ empty vector (GM-HIV+empty vector) for seven days. At day 1 and day 7,

replicate wells were harvested for cellular RNA and DNA extraction. Real-time PCR was performed to measure cellular GM-HIV DNA, and RT-real time PCR was performed to measure cellular GM-HIV RNA. In CD4+ T-cells incubated with GM-HIV, both cellular GM-HIV DNA and RNA were detectable and both increased between day 1 to day 7. However in CD4+ T-cells incubated with supernatants harvested from 293T cells transfected with pGM-HIV+ empty vector (GM-HIV+empty vector) neither GM-HIV DNA nor RNA were measurable. These results indicate that the GM-HIV can infect primary CD4+ T-cells.

(e). Primary CD4 T cells latently infected with GM-HIV respond to PHA and anti-CD3/28 stimulation. To assess whether the cells infected using the spinoculation protocol for establishing latent infection in unstimulated primary CD4+ Tcells indeed contain inducible, latent proviruses, 10 days post-GM-HIV infection, cells were stimulated with either PHA (3µg/ml) plus IL-2 (10 unit/ml) or anti-CD3/28-beads plus IL-2(10 unit/ml). 48 hours after stimulation, cellular GM-HIV RNA was measured and both PHA plus IL-2 and anti-CD3/28 plus IL-2 conditions resulted in a significant increase in cellular GM-HIV RNA compared to medium control (**p<0.001).

All data are mean ±s.e.m. from triplicate samples and representative of three experiments. A student's t-Test was used to compare experimental conditions(**s-Fig.1b,c,d,e**); $^{**}P < 0.01$, $^{*}P < 0.05$.

Supplemental Figure-2 (S-Fig.2)

Acitretin alone does not activate CD4 T cells and does not impact total viable CD4 Tcell numbers.

Healthy donor CD4 T cells were isolated from peripheral blood mononuclear cells by negative selection with the EasySep Human CD4 T-cell enrichment kit (Stemcell, Vancouver, BC). After overnight culture, the cells were infected with HIV NL4-3 (1ng p24/1x106 cells) by spinoculation at 2000 g for 2 hours. Uninfected controls underwent mock spinoculation. The cells were washed with RPMI three times immediately after

infection, and once the next day to remove all residual inoculum. Subsequently, the cells were cultured in RPMI with saquinavir (5 μM) for 10 days to allow attrition of productively infected cells, and cellular HIV RNA and DNA were measured by real-time PCR assay before additional use. Next, the infected or mock infected CD4 T cells were cultured in medium with antiretroviral drugs: 1 μM indinavir (IDV), 10 μM nevirapine (NVP), and 600 nM raltegravir (RAL) (NIH AIDS Reagent Program). Both infected and uninfected cells were treated with: Acitretin (5 μM), equivalent amount of DMSO, medium only, SAHA (335 nM) , acitretin(5 μM) plus SAHA(335 nM)(A+S), Anti-CD3/28 beads (used at 1 bead per cell plus IL-2 at 10U/ml)(CD3/28+IL2).

(a) 5 μM acitretin neither significantly increases nor reduces viable CD4 T cell numbers during 7 days of in vitro culture with IL-2. Absolute viable cell numbers in culture wells were measured using the Guava ViaCount assay (Millipore, Billerica, MA) with the EasyCyte6HT-2L flow cytometer (Millipore, Billerica, MA) according to the manufacturer's instructions. Viable CD4 T cell numbers were measured at days 0, 3 and 7. Acitretin or acitretin plus SAHA did not significantly alter viable cell numbers with IL-2 present in culture compared to DMSO, Medium and SAHA control although, there was a trend for higher cell counts at day 7 for all conditions when IL-2 was included. No differences were seen between acitretin, acitretin + SAHA or medium and DMSO controls. Only treatment with anti-CD3/28 beads significantly increased viable cell number compare to all other treatments at day3 and day7 ($P<0.05$) with a greater increase between days 3 and 7 than between 0 and 3.

(**b**) **In the absence of IL 2, a perceptible, but non-significant decline in viable cell number of uninfected cells occurs from Day3 to Day7**. Acitretin and acitretin plus SAHA did not significantly reduce viable cell number compare to DMSO, medium, and SAHA control. Only anti-CD3/28 beads +IL2 treatment significantly increased viable cell number compared to all other treatment at day3 and day7 ($P<0.05$).

(**c) Viable CD4 cell numbers of HIV infected CD4 T cells also declined in the absence of IL2 to a degree similar to that observed for CD4 T cells without HIV infection (b).** Acitretin and acitretin plus SAHA did not significantly reduce viable cell numbers compared to DMSO, medium, and SAHA control. Only anti-CD3/28 bead

treatment significantly increased viable cell numbers compared to all other treatment conditions at day3 and day7 ($P<0.05$).

(**d) Summary of viable cell number change during 7 days of culture displayed as % of viable day 3 cell numbers at day 7**. These patterns were not different between acitretin, acitretin+ SAHA, SAHA, medium or DMSO conditions regardless of infection status. Only antiCD3/antiCD28 +IL2 beads resulted in increases in viable cell numbers compared with day 3 numbers.

(**e,f, g**) **Acitretin treatment does not significantly reduce viability of CD4 T cells from 12 HIV+ study participants on ART**. All 12 HIV-positive participants (*n* = 12) were on ART and had suppressed plasma viral loads (<50 copies mL−1) for at least 1 year. Peripheral blood mononuclear cells were purified from whole blood by density gradient centrifugation. CD4 T lymphocytes were enriched by negative selection as described above for healthy donor cells. The purity of CD4 T cells was assessed by flow cytometry and was typically >95%. Cells were rested overnight before additional use. The CD4 T cell culture plated at 0.6x10^6/ml in complete RPMI with antiretroviral drugs:1 µM indinavir, 10 µM nevirapine, and 600 nM raltegravir. CD4 T cells were treated with: Acitretin (5 µM), an equivalent amount of DMSO, medium only, SAHA (350 nM) , acitretin(5 µM) plus SAHA(350 nM)(A+S), Anti-CD3/28 beads at 1 bead per cell plus IL-2 at 10U/ml(CD3/28+IL2). Viable cell numbers were measured with the Guava ViaCount assay (Millipore, Billerica, MA) using an EasyCyte6HT-2L flow cytometer (Millipore, Billerica, MA) according to the manufacturer's instructions at day 3 (e) and day7 (f). Acitretin and acitretin plus SAHA did not significantly reduce viable cell numbers compared to DMSO, medium, or SAHA conditions while anti-CD3/28 bead treatment significantly increased viable cell numbers compared to all other conditions at day3 (e) and day7 ($P<0.05$)(f). Viable cells from day7 were on average 10-18% lower compared to day 3 but there were no differences between acitretin treatment conditions and controls while anti-CD3/anti-CD28/IL2 conditions resulted in significant increases (g).

(**h**) **Acitretin does not significantly activate CD4 T cells in vitro**. Healthy donor CD4 T cells were isolated from peripheral blood mononuclear cells by negative selection with

the EasySep Human CD4 T-cell enrichment kit (Stemcell, Vancouver, BC). The cells were cultured in completed RPMI with 10% FBS, Penicillin, streptomycin and glutamine, and treated with: Acitretin (5 μM), equivalent concentration of DMSO, 3μg/ml of PHA plusIL-2 at 20u/ml, anti-CD3/28 beads at 1 bead per cell plus IL-2 at 10U/ml (CD3/28+IL2). After 7 days, cells were harvested, washed twice in cold PBS, and once with cold staining buffer (PBS +2% FCS, 0.1% sodium azide). 1x10^6 cells were resuspended in 100μl of cold staining buffer and 15μl of 1mg/ml of Normal Human IgG Control (R&D System, Minneapolis, MN) for 15 minutes. Cell samples were then stained with either 10μl of Human CD69 APC‐conjugated Antibody (R&D System, Minneapolis, MN) or 10μl of Human HLA‐DR PE‐conjugated Antibody (R&D System, Minneapolis, MN) and incubated on ice for 30 minutes, and washed three times with cold staining buffer. The cells were analyzed with an EasyCyte6HT-2L flow cytometer (Millipore, Billerica, MA) according to the manufacturer's instructions. Acitretin did not increase HLA-DR positive cells compared to DMSO control. However both PHA plus IL2 and anti-CD3/28 plus IL2 significantly increased % HLA-DR and CD69 positive cells (h).

(**i**). **Acitretin does not increase CD4 T cell proliferation in vitro**. To measure cell proliferation in CD4 T cells. Healthy donor CD4 T cells were isolated from peripheral blood mononuclear cells by negative selection with the EasySep Human CD4 T-cell enrichment kit (Stemcell, Vancouver, BC). The cells were stained with CellTrace™ CFSE Cell Proliferation Kit (Life Technologies, Grand Island, NY), then cultured in completed RPMI with 10% FBS, Penicillin, streptomycin and glutamine, and treated with: Acitretin (5 μM), equivalent concentration of DMSO, 3μg/ml of PHA plusIL-2 at 20u/ml, anti-CD3/28 beads at 1 bead per cell plus IL-2 at 20U/ml (CD3/28+IL2). After 7 days, CFSE dilution by cell division was analyzed by flow cytometry: acitretin (green) did not increase cell division compared to DMSO(blue) but both PHA(black) and anti-CD3/38 (red) increased cell division. The dashed line shows cells without CFSE staining.

(**j**) Example of of "Living cell" gate of representative CD4 +Tcells from HIV subjects analyzed by EasyCyte6HT-2L flow cytometer (Millipore, Billerica, MA) according to the manufacturer's instructions.

(**k**) Gate for dead cells and apoptotic cells according the manufacturer's instructions of Alexa Fluor 488 annexin V/Dead Cell Apoptosis Kit (Life Technologies, Grand Island, NY) and two publications[3-4] , Y axis is Propidium iodide(PI) staining, and X axis is Annexin V staining.

(**l**) Average % dead cells from 12 HIV patients (*n* =12) CD4+ T cells at Day3 and Day7 for the cultures with medium, DMSO, acitretin, acitretin plus SAHA, SAHA, and anti-CD3/28-bead plus IL-2. "Dead cells" correspond to cells gated to the right up quadrant in S2-k.

(**m**). Average % dead cells from healthy donor CD4 Tcells with or without GM-HIV infection at Day 3 and Day7 and treated with medium, DMSO, and acitretin. "Dead cells" correspond to cells gated to the right up quadrant in S2-k.

Values represent mean ± s.e.m of duplicate samples from 12 HIV patients and from four healthy Donor CD4+ T cells. A student's t-Test was used to compare experimental conditions(**s-Fig.2a, b, c, l, m**); $**P < 0.01$, $*P < 0.05$.

Supplemental Figure-3 (S-Fig. 3)

Timecourse of acitretin effects on virologic and apoptotic outcomes using the latent model of CEM T4 infection and GFP-HIV.

CEM-T4 cells were infected with 1000 pg of p24 of GFP-HIV/1x10^6 cells for 5 hours, and unbound virus was removed by washing the cells three times with RPMI. Subsequently, the cells were cultured in medium for 10 days to permit clearance of productively infected cells. All cells were maintained in the presence of antiretroviral drugs: 1 µM indinavir (IDV), 10 µM nevirapine (NVP), and 600 nM raltegravir (RAL) (NIH AIDS Reagent Program) to prevent viral spread. Infected or uninfected cells were placed into 24 well tissue culture plates at 0.25x10^6 cells per well. Both infected and

uninfected cells were treated with acitretin (5μM), an equivalent concentration of DMSO, SAHA (350 nM), or acitretin (5 μM) plus SAHA (350 nM) (Acitretin +SAHA)).

(**a**) **GFP-HIV infected CEM-T4 cells established using the latent infection protocol respond to LRAs stimulation**. 10 days after infection in medium with Saquinavir (5 μM), the cells were stimulated with 5μM acitretin, 350nM SAHA, or 500nM prostratin in the presence of antiretroviral drugs (indinavir, nevirapine, raltegravir). 72 hours after stimulation, p24 production in the supernatant was measured with the HIV-1 p24 ELISA kit (PerkinElmer). Acitretin, SAHA and prostratin each significantly increased p24 production (**P<0.001) in comparison with medium control. The inducibility of viral production is compatible with latent infection of CEM-T4 cells by GFP-HIV prepared using this procedure. The inclusion of the integrase inhibitor raltegravir prevents confounding from unintegrated viral DNA in assessments using this latent model.

(**b**) **Timecourse of induction of cell-associated HIV RNA**. Cells from replicate wells were sampled at day1, day 3 and day7 post treatment with study agents. HIV RNA copy number was measured using a locked nucleic acid (LNA) Taqman assay for HIV-1. Results were normalized to RNA mass measured by nanodrop as million cell equivalents (similar results are obtained normalized to GAPDH RNA). Cell associated HIV RNA continued to increase to day 7 for the acitretin only condition while maximal RNA induction was already apparent by day 3 for those conditions including SAHA. HIV RNA induction by acitretin was significantly higher compared to DMSO at day3 and day7, but significantly less than induction by SAHA, at day 1, day 3 and day7 (*P<0.05). Acitretin plus SAHA increased HIV RNA copy numbers more than either SAHA or acitretin alone (A). While HIV RNA expression was induced by acitretin treatment, the magnitude of induction was lower than with SAHA. In the available data from cell line models, the kinetics of induction of HIV RNA was also different than what has been described for SAHA which typically produces a rapid rise in HIV RNA expression (within 1 day). These results suggest distinct mechanisms of reversal of viral latency by these drugs.

(**c**) **Timecourse of HIV RNA released into culture supernatant during acitretin treatment**. Supernatants were harvested at day 1, day 3 and day7, HIV RNA was

extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA) and HIV copy number was measured with a locked nucleic acid (LNA) Taqman assay for HIV-1. Both acitretin, and SAHA increased supernatant HIV RNA at day3 and day7 compared to DMSO, but the greatest increases were seen with acitretin plus SAHA (*P<0.05, **p<0.01) .

(**d**), **Timecourse of reduction of cellular HIV DNA copy concentrations in a latent infection model**. CEM T4 cells infected with GFP- HIV were maintained with antiretroviral drugs (1 µM indinavir (IDV), 10 µM nevirapine (NVP), and 600 nM raltegravir (RAL). Cultures were sampled at day1, day 3 and day7. HIV copy number was measured in total extracted DNA with a locked nucleic acid (LNA) Taqman assay for HIV-1. Acitretin and acitretin plus SAHA significantly reduced HIV DNA concentrations compared to DMSO, and SAHA, at day 3 and day7 (*P<0.05, **P<0.001).

(**e**), Timecourse of change in **HIV RNA normalized to HIV DNA**. Data from (S3-b) and (S3-d) presented as change in HIV RNA/ HIV DNA over time. Only Acitretin plus SAHA significantly increased HIV RNA/DNA ratio at day3, but both acitretin and acitretin plus SAHA resulted in significantly increased HIV RNA/DNA ratios at day7 compared with DMSO and SAHA conditions. (*p<0.05, ** p<0.001).

(**f**) **Timecourse of reduction of GFP-HIV infected cells in CEM-T4 cells by acitretin**. To determine effects of acitretin treatment on the infected cell population over time, GFP positive cells were measured with the EasyCyte6HT-2L flow cytometer (Millipore, Billerica, MA) at days 1, 3 and 7 of acitretin or comparator treatments according to the manufacturer's instructions. 80,000 cells were measured for each sample. A significant reduction of %GFP positive cells (GFP-HIV infected cells) was noted at day 3 and continued at day 7 for conditions with acitretin and acitretin plus SAHA (Acitretin +SAHA)) compared to SAHA and DMSO controls (**P<0.001).

(**g**) **Timecourse of induction of apoptosis by acitretin in GFP-HIV infected CEM-T4 cells**. To study apoptosis in GFP-HIV infected CEM-T4 cells, Alexa Fluor 647 Annexin V (Life Technologies, Grand Island, NY) was used to detect apoptosis in GFP-HIV-

infected CEM-T4 cells to avoid possible interference with Alexa Fluor 488 annexin V apoptosis and caspase 3/7-green flow cytometry assays by GFP. Measurements were made for all cells (regardless of GFP expression). At Day7, 5µM acitretin or acitretin (5 µM) plus SAHA (350 nM) (Acitretin +SAHA)) resulted in significantly greater levels of apoptotic cells compared to SAHA and DMSO controls in the total cell population (*P<0.05) .

(**h,i**) **Acitretin preferentially induces apoptosis in HIV-infected cells**. Analysis of cells gating positive for GFP (**h**) Acitretin and acitretin (5 µM) plus SAHA (350 nM) (Acitretin +SAHA) resulted in robust increases in apoptosis compared to DMSO or SAHA (** P<0.001), while acitretin plus SAHA further increased apoptosis (*P<0.05). However no differences in % apoptotic cells was seen among conditions for cells that gated GFP negative (**i**).

For (**S-Fig. 3b-i**)

yellow data plots ( ) correspond to acitretin+SAHA treatment;

green data plots ( ) correspond to SAHA treatment;

orange data plots ( ) correspond to acitretin treatment,

grey data plots ( )correspond to DMSO treatment.

All data are mean ±s.e.m. from triplicate samples and representative of three experiments. A student's t-Test was used to compare experimental conditions (**s-Fig.3a-h**); ***P* < 0.01, **P* < 0.05.

Supplemental Figure- 4 (S-Fig. 4)

Dose response of latently infected CEM-T4 cells and infected TZM-bl cells to varying acitretin concentrations for apoptotic and virologic outcomes.

(**a**), **Demonstration of dose response to acitretin in reduction of GFP-HIV infected CEM-T4 cells**. CEM-T4 cells were left uninfected (control) or infected with 1000 pg of p24 of GFP-HIV/1x10^6 cells for 5 hours, and unbound virus was removed by washing

the cells three times with RPMI. Subsequently, the cells were cultured in medium with Saquinavir (5 μM) for 7 days to prevent spreading infection and to allow clearance of productively infected cells. Next, both infected and uninfected cells were aliquoted into separate 24 well plates at 0.25x10^6 cells per well. All cells were cultured with antiretroviral drugs: 1 μM indinavir, 10 μM nevirapine, and 600 nM raltegravir (NIH AIDS Reagent Program) to prevent spreading infection and both infected and uninfected cells were treated with acitretin at 1μM, 5μM or 25μM, or a concentration of DMSO equivalent to the 25μM acitretin condition. At day 7, GFP positive cells were measured using the EasyCyte6HT-2L flow cytometer (Millipore, Billerica, MA) according to the manufacturer's instructions. 80,000 cells were analyzed for each sample. There was a dose response in the reduction of GFP positive cells (GFP-HIV infected cells) to acitretin treatments; 5μM and 25 μM of concentration of acitretin was associated with significantly lower % of GFP+ cells than 1μM acitretin, and DMSO control conditions (*$P<0.05$).

(**b, c), Demonstration of dose response to acitretin in apoptosis of GFP-HIV infected CEM-T4 cells**. To study dose response to acitretin in CEM-T4 cells with or without GFP-HIV (latent) infection, Alexa Fluor 647 Annexin V (Life Technologies, Grand Island, NY) was used to detect apoptosis in GFP-HIV-infected CEM-T4 cells as prepared in "a" since GFP interferes with Alexa Fluor 488 annexin V apoptosis and caspase 3/7-green flow cytometry assays. The study was performed using the EasyCyte6HT-2L flow cytometer (Millipore, Billerica, MA) according to the manufacturer's instructions, 100,000 cells per sample were measured for each condition. Over the range of concentrations tested, acitretin did not increase apoptosis in CEM-T4 cells without GFP-HIV infection. However a dose response to acitretin was observed in the % apoptotic, GFP-HIV infected CEM-T4 cells. Both 5μM and 25μM acitretin treatments resulted in a significantly higher % apoptotic cells ($P<0.05$) (b). Moreover, the dose response with increasing apoptosis was observed exclusively in cells gating GFP positive (with GFP-HIV) ($P<0.05$), but not with GFP negative cells(c).

(**d, e**) **Demonstration of dose response to acitretin in IFN-β and CXCL-10 released by GFP-HIV infected CEM-T4 cells**. Supernatants from GFP-HIV-infected and

uninfected CEM-T4 cell cultures prepared and treated in “a” were collected at day 7. The supernatants were analyzed with the following ELISAs: (1) Verikine Human IFN-β kit (BPL Assay Science, Piscataway, NJ), and (2) Human CXCL10/IP-10 kit (R&D Systems, Minneapolis, MN). Assays were performed according to the manufacturers’ protocols. Compared to uninfected CEM-T4 cells, IFN-β (d) and CXCL-10 (e) in supernatant from GFP-HIV infected CEM-T4 cells are significantly higher ($P<0.05$) and demonstrated a dose response to acitretin (d, e).

(**f)Dose response to acitretin in reduction of HIV infectivity in TZM-bl -cells**. The TZM-bl cell line provides a sensitive and quantitative detection of HIV infection (1). TZM-bl cells were cultured in DMEM medium with 10% FBS, penicillin/streptomycin and supplemented with in a T75 flask. Cells at about 75% confluence were infected with 5ng of p24 of a viral stock of HIV NL43 in T75 flask overnight. The inoculum and medium were removed the next day, and the cells were washed once with DMEM and cells cultured for three more days with 5 μM Saquinavir Next, cells were aliquoted into wells of a 96 well plate at 30,000 cells per well, cells were maintained in medium with antiretroviral drugs: 1 μM indinavir (IDV), 10 μM nevirapine (NVP), and 600 nM raltegravir (RAL) (NIH AIDS Reagent Program) to prevent spreading HIV infection. Control TZM-bl cells without HIV infection were also aliquoted into a separate 96 well plate at the same density as the HIV infected cells. On the next day, both infected and uninfected cells were treated with: 1μM, 5μM, 25μM of Acitretin, or an amount of DMSO to yield the same DMSO concentration as the 25μM acitretin condition. The cells were cultured for an additional 72 hours. The TZM-bl cells contain a HIV LTR controlled β-galactosidase gene, and HIV production was assayed with the Beta-Glo assay (Promega, Madison WI) using a VICTOR3 plate reader (PerkinElmer). β-galactosidase expression is presented as relative light units (RLU). Acitretin had no impact on beta galactosidase expression from uninfected TZM bl cells. In contrast, infected TZM-bl cells showed a dose-response to acitretin with greater reductions in RLU (betagalactosidase expression) with increasing acitretin dose. RLU with acitretin at 5μM and 25 μM were significantly lower than 1μM acitretin, and DMSO (*$P<0.05$).

All data are mean ±s.e.m. from triplicate samples and representative of three experiments. A student's t-Test was used to compare experimental conditions(**s-Fig.4a-i**); $**P < 0.01$, $*P < 0.05$.

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HIV Patient ID	Age	CD4 Count (Cells/mm^3)	ART	Years at <50 Copies/ml
A01	**68**	**1222**	**ABC/DLV**	**>5**
A02	**67**	**572**	**TDF/ZDV/LPV**	**>5**
A03	**51**	**857**	**TDF/FTC/ATV**	**>5**
A04	**51**	**774**	**TDF/FTC/ EFV**	**>5**
A05	**44**	**817**	**TDF/FTC/RTV**	**>4**
A06	**48**	**511**	**TDF/FTC/NVP**	**>5**
A07	**47**	**1012**	**ATZ/TDF/ZDV/RTV**	**>4**
A08	**53**	**322**	**TDF/FTC/DLV/LPV**	**>5**
A09	**57**	**282**	**TDF/FTC/RAL**	**>2**
A10	**51**	**872**	**ELV/COB/TDF/FTC**	**>1**
A11	**52**	**589**	**TDF/FTC/EFV**	**>9**
A12	**56**	**728**	**TDF/FTC/DLG**	**>4**
A13	**57**	**282**	**TDF/FTC/RTV**	**2**
A14	**51**	**874**	**TDF/FTC/ELV/COB**	**2**
A15	**53**	**589**	**TDF/FTC/EFV**	**9**
A16	**56**	**728**	**ABC/3TC/DLG**	**7**

Supplemental Table 1 legend.

ABC abacavir; TDF tenofovir; FTC emcitritabine; DRV darunavir; RTV ritonavir; MVC maraviroc; ZDV zidovudine; 3TC lamivudine; LPV lopinavir; ATV atazanavir; EFV efavirenz; RLP rilpivirine; NVP nevirapine; DLG dolutegravir; ELV elvitegravir; COB cobicistat; ETR etravirine

All participants are male. HIV positive subjects were recruited from the San Francisco VAMC ID Clinic and provided informed consent under an approved IRB protocol. All participants had achieved sustained undetectable plasma viral loads (<50 copies/ml) for a median of 5 years. Their median age was 54.5 years, and median CD4 T cell count was 599 cells/mm3. Samples from A01-A12 were included in studies of apoptosis, cytokine expression and cellular HIV DNA levels. Samples from A13-A16 were studied for acitretin increased HIV transcription, and HIV release (supernatant HIV RNA).