A

Ssu72 peak distribution

- Promoter: 8%
- 5UTR: 1%
- Exon: 1%
- 3UTR: 1%
- TTS: 1%
- ncRNA: 0%
- pseudo: 0%
- Intergenic: 50%
- Intron: 38%

B

Number of genes with Ssu72 peaks on:

|       | TSS | TTS | TSS and TTS |
|-------|-----|-----|-------------|
| Ssu72 | 5950| 1729| 704         |

C

Chen_Fig. S4
Supplemental figure legends

**Figure S1.** The association between Tat and Ssu72. (A) Silver staining of anti-HA immunoprecipitates from HA-Tat HeLa cell whole cell extracts. (B) qRT-PCR analysis of provirus mRNA expression from 2D10 cells after TNF-α treatment for the indicated times. (C) FACS analysis of d2EGFP expression of 2D10 cells after TNF-α treatment for the indicated times. (D) Glycerol gradient fractionation from 2D10 whole cell extracts before and after TNF-α treatment for 16 hours. Aliquots of each fraction were subjected to immunoblot with the indicated antibodies.

**Figure S2.** The synergistic effects on HIV-1 transcription between Tat and Ssu72 require TAR region. (A) Luciferase activities were measured in extracts of HeLa cells transfected with Ssu72 or Pin1 expression constructs and the indicated reporter genes. (B) HIV-1:Luc cells were transfected with CycT1 or Pin1 siRNAs, the luciferase activities were measured as in (A). (C) HIV-1:Luc ΔTAR HeLa cells were transfected with the indicated amounts of Ssu72 expression constructs in the presence or absence of Tat101. Luciferase activities were measured as in (A). (D) HIV-1:Luc cells were transfected with the indicated vector control or Tat expression constructs. Either UTP or Bio-16-UTP were added to initiate the nuclear run-on reaction. Synthesized HIV-1 provirus and P21 RNAs were purified and analyzed by qRT-PCR. Error bars represent the standard deviation obtained from three independent experiments.

**Figure S3.** Ssu72 is required for the reactivation of the latent provirus in 2D10 cells. (A) 2D10 cells were stimulated with TNF-α for the indicated times. Protein lysates were subjected to immunoblot with the indicated antibodies. (B) Immunoblots analysis from cytoplasmic and nuclear fractionation of 2D10 cells treated with TNF-α for the indicated times. (C) qRT-PCR analysis of provirus mRNA expression of 2D10 cells transfected with Ssu72 or CycT1 siRNAs in the presence or absence of TNF-α treatment. Error bars represent the standard deviation obtained from three independent experiments. (D) FACS analysis of d2EGFP expression of 2D10 cells transfected with Ssu72 or CycT1 siRNAs. (E) 2D10 cells were transfected with control or Tat siRNAs for 48 hrs, then treated with TNF-α for another 6 hrs. The lysates were subjected to ChIP analysis with the indicated antibodies. Schematic representation of the genomic organization of the lentiviral vector and the relative locations of the primers used for ChIP are shown at the top of the panel. The knocking down efficiency was monitored by immunoblot analysis. (F) ChIP analysis of the recruitment of the indicated proteins to the provirus in 2B2D (Tat C22G) cells upon TNF-α treatment. Immunoblots analysis monitored Tat protein expression.

**Figure S4.** Ssu72 regulates transcription termination. (A) Normalized genomic distribution of Ssu72 ChIPseq peaks. (B) Table shows the number of genes that contain at least one Ssu72 peak on the TSS (±1Kb), downstream the TTS (0 to +2Kb) or both. (C) Upper images show GROseq profiles from hESCs transfected with Ctrl or Ssu72 siRNAs. Lower panels display Ssu72 and S5P-RNAPII binding sites on MYL12B, TGF2 and NANOG genes. Captures show WIG files visualization using IGV software. Scale bars and gene models are shown above and below the images, respectively.
Supplemental materials and methods

Plasmids, siRNAs, drugs and antibodies
Mammalian expression constructs of HIV-1 Tat101, human Ssu72 and Pin1 were generated by subcloning Tat101, Ssu72 and Pin1 cDNA into pcDNA5 (Invitrogen) vector, respectively. The pGEX-6P-1 and pET-30a vectors were used for generating GST and S-Tag fusion proteins. The point and truncation mutations were generated by site-directed mutagenesis (Stratagene). All the mutations were confirmed by sequence analysis. Synthetic double-stranded RNA (dsRNA) oligonucleotides targeting Ssu72, Pin1, Cyclin T1, AFF1, RPAP2 and SCP1 were purchased from Ambion and the sequences are listed below. Recombinant human TNF-α were ordered from R&D Systems. The antibodies used for Western blots and ChIP assays were against Ssu72 (Cell Signaling [12816], GeneTex [GTX16436]), Cyclin T1 (Santa Cruz [SC-10750]), CDK9 (Santa Cruz [SC-8338]), Tat (Abcam [ab4041] lot# GR65428), NELF-A (Santa Cruz [SC-32911]), NEFL-E (Protein Tech Group [10705-1-AP]), Spt5 (Santa Cruz [SC-28678]), Spt4 (kind gift from Dr. Hiroshi Handa) AFF4 (Bethyl [A302-538A]), AFF1 (Bethyl [A302-344A]) RPAP2 (Protein Tech Group [17401-1-AP]), SCP1 (Abcam [ab136038]) Pcf11 (Bethyl [A303-706A]), NF-kB (Santa Cruz [SC-372]), TBP (Santa Cruz [SC-273]) Spt6 (Bethyl [A300-801A]), IWS1 (Protein Tech Group [16943-1-AP]) RNAPII (Santa Cruz [SC-899], Covance [MMS-126R]), Ser2P (Covance [MMS-129R], Millipore [04-1571]), Ser5P (Covance [MMS-134R], Millipore [04-1572]), Ser7P (Millipore [04-1570]), FLAG (Sigma [A8592]), HA (Cell Signaling [3724], Sigma [E-6779]), GST (Santa Cruz [SC-138]) and HRP conjugated S-Protein (Millipore [69047]).

Cell lines and cell culture
The inducible Tet-OFF, Tat-ON HA-Tat HeLa cells, the HIV-1:Luc wild-type and ΔTAR HeLa cells were were maintained in DMEM supplemented with 10% FBS. The 2D10 Jurkat cells were cultured in RPMI1640 with 10% FBS.

Affinity purification of HA-tagged Tat protein complexes for MudPIT analysis
Fifteen 150 mm dishes of HA-Tat HeLa cells (~90% cell density) were collected and extracted by IP buffer (50 mM Heps-NaOH pH 7.9, 300 mM NaCl, 1% NP-40 (v/v), 10 mM MgCl2, 15% glycerol) with protease inhibitors to a final volume of 15 ml, and homogenized with Type B dounce homogenizer. Extracts were clarified by centrifugation (14,000 x g for 15 min at 4 °C). EZview™ Red Anti-HA Affinity Gel (Sigma) were incubated with the supernatant in a ratio of 80 μl slurry (50% beads in slurry) to 6 mg of total protein for 4 hours on a roller at 4°C. The beads were washed in IP-wash buffer (25 mM Heps-NaOH pH 7.9, 300 mM NaCl, 0.2% NP-40 (v/v) four times for 3 min at 4°C and then twice in HA-elution buffer (25 mM Tris-HCl, pH 7.5, 50 mM NaCl). Finally, HA peptide (200 μg/ml; Sigma) in elution buffer was incubated with the sample for 30 min at room temperature with rotation. Protein identification method is available upon request.

Glutathione S-transferase (GST) and S-tag pull-down experiments
GST and S-Tag fusion expression constructs were expressed in BL21 Escherichia coli cells. Protein expression was induced with 0.2 mM IPTG for 8 hours at 30°C. Crude bacterial lysates were prepared by sonication in lysis buffer (25 mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA, protease inhibitor). About 2 μg of the bait proteins were first bound to S-beads (Novus) or GSH-4B (GE Healthcare) in 1 ml of NP-40 buffer (25mM Tris-HCl pH 7.4, 150mM NaCl, 1mM EDTA, 1% NP-40) for one hour at 4°C. Beads were washed three times with NP-40 buffer, and then 2 μg of the prey proteins were added for another 2 hours. Beads were wash four times with high salt NP40 buffer (500 mM of NaCl). The recovered products were analyzed by immunoblot.

Co-immunoprecipitation
For HA-Tat HeLa cells, cells were transfected with 10 μg of expression constructs for 24 hours. Then, whole cell lysates were prepared with cold NP-40 lysis buffer. 500 μg of cell extracts were incubated with 20 μl of anti-HA affinity gel on a rotator at 4°C overnight. Beads were then washed four times using the lysis buffer. The immune complexes were subjected to SDS-PAGE followed by immunoblotting with the secondary antibody. For 2D10 Jurkat cells, cells were treated with 10 ng/ml of TNF-α and whole cell lysates were prepared with cold NP-40 lysis buffer. 500 μg of cell extracts were incubated with 5 μg of anti-Tat antibody on a rotator at 4°C overnight, followed by addition of protein A/G Sepharose CL-4B beads for 2h at 4°C. Beads were then washed four times using the lysis buffer. The immune complexes were subjected to SDS-PAGE followed by immunoblotting with the secondary antibody.
Nuclear run-on and quantitative RT-PCR
Run-on experiment was performed as described in Patrone et al. 2000 with minor modifications. Briefly, 1 X 10⁷ cells were collected by trypsinization and washed twice with cold PBS. The pellet was resuspended in 1 ml of lysis buffer (10 mM Tris-HCl, pH 7.4, 3 mM MgCl₂, 10 mM NaCl, 150 mM sucrose and 0.5% NP40) and incubated for 5 min on ice. Nuclei were then collected by centrifugation and washed once with lysis buffer devoid of NP40. The pellet was resuspended in 50 µl of freezing buffer (50 mM Tris-HCl, pH 8.3, 40% glycerol, 5 mM MgCl₂ and 0.1 mM EDTA) and stored at -80°C. The nuclei were mixed with 50 µl of 2X transcription buffer (200 mM KCl, 20 mM Tris-HCl, pH 8.0, 5 mM MgCl₂, 200 mM sucrose, 20% glycerol and 4 mM DTT). ATP, GTP and CTP were added to the final concentration of 2 mM for each. Biotin-16-UTP was then added to the final concentration of 0.4 mM to start the reaction. The reaction was incubated for 30 min at 30°C. 6 µl of 250 mM CaCl₂ and 6 µl of RNase-free DNase I (10 U/µl) were added to the reaction and incubated for 10 min at 30°C to stop the reaction. The RNA was purified with RNeasy Kit (Qiagen) and eluted with 50 µl of nuclease-free water. Dynabeads M-280 Streptavidin (Invitrogen) resuspended in 2X binding buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA and 2 M NaCl) were mixed with equal volume of purified RNA and incubated for 2 hours at room temperature with rotation. Beads were washed for three times in 1 ml of 1X binding buffer and resuspended in 20 µl of nuclease-free water and stored at -20°C. 5 µl of run-on RNA was used for RT reaction with Superscript III 1st cDNA synthesis system (Invitrogen) with random hexamer primers. The sequences of the primers for qPCR are: forward: 5'-AAGAGATACGCCCTGGTTCCTG-3' and Reverse: 5'-CGGTAGGCTGCAAAATGTCTCA-3'.

Chromatin immunoprecipitation (ChIP)
ChIP assays were performed essentially the same as described previously (Chen et al. 2011). Briefly, 2 X 10⁷ of 2D10 or 2B2D cells (for four ChIPs) were treated with 10 ng/ml of TNF-α for the indicated times. Cells were fixed with 1% formaldehyde, and then whole-cell lysates were prepared. Protein lysate was subjected to ChIP with the indicated antibodies, followed by DNA purification. ChIP-enriched DNA was analyzed with qPCR. The the primer sequences are listed below.

GRO-seq
hESCs clumps were disaggregated into single cells using Accutase and plated in 10cm2 dishes. The next day, scramble or ssu72 siRNA were transfected using Lipofectamine RNAiMAX Reagent (13778), following manufacturer directions. 48h later cells were collected to proceed with the nuclear run on experiment. Nuclei isolation, run-on, RNA fragmentation and enzymatic TAP and PKN treatments were performed according to Core et al, 2008. In brief, 10⁷ isolated nuclei were used for each nuclear run-on reaction. Samples were resuspended in 100ul of freezing buffer (40% glycerol, 5mM MgCl₂, 0.1mM EDTA, 50mM Tris pH7.5, 2U/ml SUPERase-In) and then 50ul of run-on buffer (1.5mM Tris-Cl pH 8.0, 7.5 mM MgCl₂, 1.5 mM DTT, 450 mM KCl, 0.6U/µl of SUPERase-In, 1.5% sarkosyl, 750µM ATP, GTP, and Br-UPT and 4.5 µM CTP) was added. Run-on reactions were incubated during 5' at 30°C. For RNA hydrolysis, samples were treated with 1M NaOH on ice for 30min. BrU immunoprecipitation was done using anti BrDU beads from Santa Cruz (sc-32323AC). Decapping, 3' de-phosphorylation and 5' phosphorylation were performed using sequential incubations with (1) 1.5 µl tobacco acid pyrophosphatase (TAP, 5 U, Epicentre), at 37°C for 1.5 h, (2) 1 µl PNK at 37°C for 30', and (3) 2ul PNK and 20ul ATP at 37°C for 30'. Required RNA purifications were done using Trizol LS. Glycoblue co-precipitant (Life Technologies, AM9516) was added to facilitate RNA precipitation. BrU-RNA was barcoded, retro-transcribed and amplified using NEBNext Multiplex Small RNA Library Prep Set for Illumina (NEB#E7300S/L). Final libraries were sent to the Next Generation Sequencing core at Salk Institute and sequenced in Illumina HiSeq 2500 high-throughput sequencing system.
## Primers used for ChIP assays

| Primer Name | Forward | Reverse |
|-------------|---------|---------|
| 2D10-A      | GGCTAATTCACTCCCAACGA | GGAAGTAGCCTTGTTGTGTTGAGA |
| 2D10-B      | AGGTTTCTACAAGGAAGTTTCC | ACCCAGTACAAGCAAACAGAGAG |
| 2D10-C      | CTGGGAGCTCTCTGGCTAATCT | TTACCAGAGTCACACAAACAGAG |
| 2D10-D      | GACTGGTGAAGTGCGCAGAAAAT | TTTCACATCGAGATCTAATTC |
| 2D10-E      | AGCAACTCTCTATGTGTGCTGAT | TGCGGTGCTTTATTATTATTTT |
| 2D10-F      | GCAGTGGGAATAGGAGCTTGTGAG | GCTGCGGGCATAGTGCTT |
| 2D10-G      | GTCCGCCCTCAGAAAGAGAA | TCCAGCAGGACCATGTGATC |
| 2D10-H      | ATGGCGGTCTCTCTAATGAC | AACTGTTGGCACGTCCATCC |
| HeLa-Promoter | CCTGACTGGGTCTCTGTGTAGA | AGCTCAGGGCTCAGATCTG |
| Hlea-Coding | TATGGCTCACGAGCAGTACATCAG | CATCCCCCTCGGTTGTAATC |

## siRNA information

| Target Gene | siRNA ID | Sense | Antisense |
|-------------|----------|-------|-----------|
| Ssu72#1     | s26488   | GAACUGCAAGACACCUGUUU tgt | AAACAGGGUCUUUGCAGUUC |
| Ssu72#2     | s26489   | GGAUUUCAUAUGCGGAtt | UCCAGCAUAUGUAAAAC |
| Pin1#1      | s10545   | GGCCGAGUGJACUCUUCAAtt | UGAAGUAGUACACCUCCG |
| Pin1#2      | s10546   | UGCAGAAAGUGAACACUCAAtt | UGAAGUUCACCUCUCG |
| CyclinT1    | s2541    | CGACCCAGACAGAGACUAAtt | UAGUCUAAUGCGGUGG |
| Tat         | Custom   | AAGUUGUUUCUAACAAAtt | UUUGUUUAUGAAAC |
| SCP1#1      | s33804   | GCCCAGGACUCAGACAAAtt | UCUUUGUCUGAGUGC |
| SCP1#2      | s33806   | GCCCGUGUUGGACCAACAtt | UGUGUCUAAACCGAG |
| RPAP2#1     | s36534   | GAAACGCAUGUACUGUAAAtt | UCAAGUACGAGCUG |
| RPAP2#2     | s36535   | GUAACUAAACAGACAGUAAAtt | UACUUGUCUGUUAAG |
| AFF1        | s8823    | GGCGAUGUUCGUGUAAAtt | UUUCAACAGAAACAC |


Supplemental references

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