Cell

EXTENDED EXPERIMENTAL PROCEDURES

siRNAs, q-PCR Oligonucleotides, and RNA Oligonucleotides

Double stranded RNA oligonucleotides directed against target sequences in Drosha (5′-gag tat tta ctt gct cag u-3′), Dgcr8 (5′-cat cgg aca aga gtg tga u-3′), Rck/p54 (5′-gca gaa acc cta tga gat t-3′), Lsm-1 (5′-gtg aca tcc tgc cac ctc act t-3′), Xrn1 (5′-aga tga act tac cgt aga a-3′), GW182 (5′-tag cgg acc aga cat ttc t-3′), TRBP (5′-cac gtc agg ctt acc tgg ata-3′) (Lee et al., 2006), Setx (5′-aga gat ctc ttc ata tac a-3′), Xrn2 (5′-aca ctg tag tca gta tta a-3′), Rrp6 (5′-aaa atg gcg cca ccc agt a-3′ and 5′-tgc tgg tgc tgc ttt tgt a-3′), and a control siRNA (Scrambled; 5′-gcg cgc ttt gta gga ttc g-3′) were used.

The following oligonucleotide pairs were used for quantitative PCR: Tex14 ChIP (Forward 5′ CAA AAG CGT GTG TGG AGA AA 3′; Reverse 5′ CCC CTG ATC TGG CTT GTT TA), Tex14 RT-PCR (Forward 5′ ACA GGA AGA GCG CAT GTC TT 3′; Reverse 5′ CAC TGG TGT CTG GCT TCT GA 3′), PTP4A (Forward 5′ ACA TTT AAT CAC ATC CCC CTT C 3′; Reverse 5′ TCT CTC TTA ATC ATG GCA ACG 3′), DLGAP1 ChIP (Forward 5′ AAC ACC ATC ACC AAC ATC AC 3′; Reverse 5′ TGA GAA GCT AGA AGC AGA GAG 3′), DLGAP1 RT-PCR (Forward 5′ CCC AAC CTC AGA AAT CAC AC 3′; Reverse 5′ TCT CCC ATC CTT GCT ACA C 3′), PDE4B ChIP (Forward 5′ TCC CCT TTC TCT CTC ATC TTC 3′; Reverse 5′ CAT CTG TTT TGC CAC TTC ATC 3′), PDE4B RT-PCR (Forward 5′ ACT TCC TTC CCC TGT TGT C 3′; Reverse 5′ ACC ACT GCT CCT TTC TAC C 3′), YIF1B ChIP (Forward 5′ CCC ACC AGC TTT TCG ATG AC 3′; Reverse 5′ TGC AGG ATA ACT CAG GCC AC 3′), YIF1B RT-PCR (Forward 5′ TCT CCT TCT TGC CCT TCT CC 3′; Reverse 5′ ACC ATC TCT TCT CTC CCA TCC 3′), HERV-H (Forward 5′ TCT CCC TTC GCT GAC TCT CT 3′; Reverse 5′ CAT CCA TGT GAC GAG ACC AC 3′).

The sequence of TAR RNA used is as follows: 5′GGGUCUCUCUGGUUAGACCAGAUCUGAGCCUGGGAGCUCUCUGGCUAACU AGGGAACCC3′).

The sequences of NS, Sp1 and TAR 1, TAR2 and TAR3 RNA oligonucleotides are as follows: NS 5′ GCGCGCUUAGUAGGAUUC GUUGCG 3′; Sp1 5′ UGGGCGGGACUGGGGAGUGGC 3′; TAR1 (11-28) 5′ GUUAGACCAGAUCUGAGC 3′; TAR2 (40-58) 5′ CUGG CUAACUAGGGAACCC 3′; TAR3 (15-35) 5′ GACCAGAUCUGAGCCUGGGAG 3′.

SUPPLEMENTAL REFERENCE

Lee, Y., Hur, I., Park, S.Y., Kim, Y.K., Suh, M.R., and Kim, V.N. (2006). The role of PACT in the RNA silencing pathway. EMBO J. *25*, 522–532.

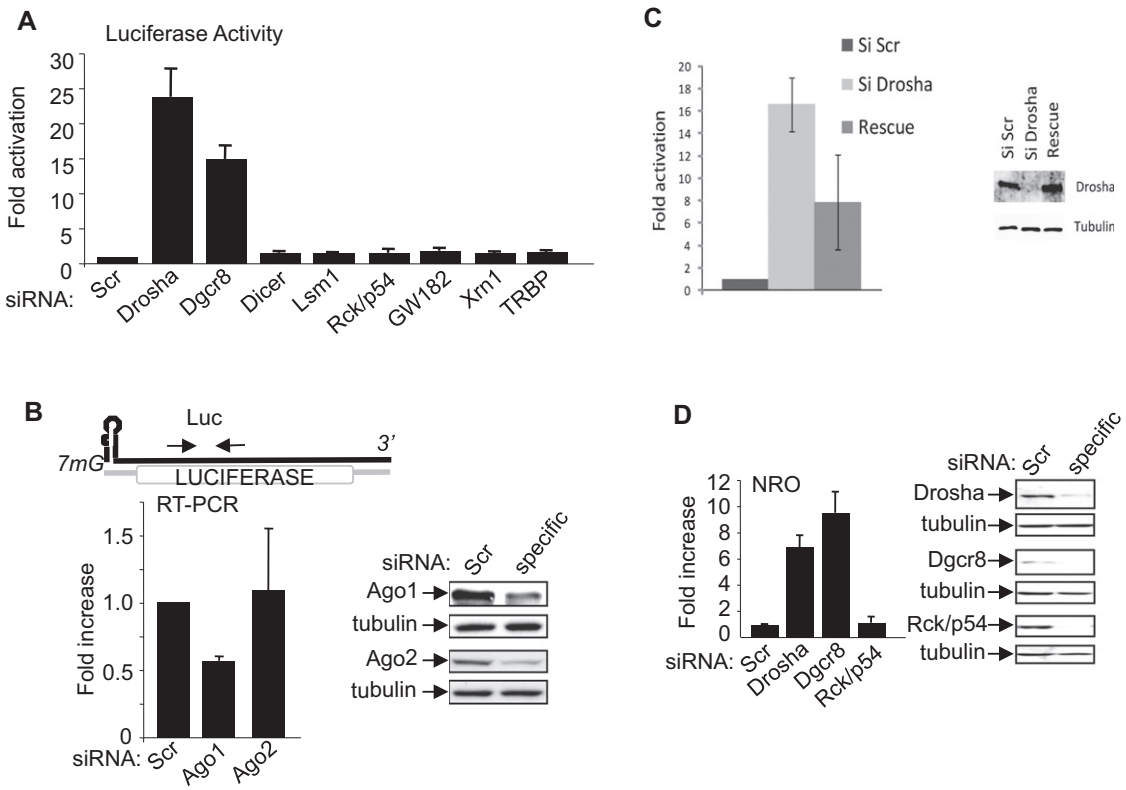


Figure S1. Drosha and Dgcr8 Repress Transcription from the Integrated HIV-1 Promoter Independently of RNAi, Related to Figure 1

(A) Extracts of HeLa LTR-luc cells transfected with the indicated siRNAs were analyzed for Luciferase activity. Results are presented as fold activation relative to control (Scr) cells.

(B) RNA isolated from HeLa LTR-Luc cells transfected with siRNA targeting Ago1, Ago2 or a control was analyzed by RT-q-PCR using the primers indicated on the schematic above the graph. Values were normalized to that of GAPDH in the same samples. The result for Scr-treated cells was attributed a value of 1. The knockdown of specific factors was validated by immunoblot (right).

(C) Overexpression of Drosha siRNA-resistant mutant rescues HIV-1 LTR activity in Drosha knock-down cells. HeLa LTR-luc cells were transfected with siRNA directed against the 3′ UTR of Drosha, or a control, as indicated. Cells were re-transfected 24 hr later with a plasmid expressing Drosha lacking its 3′ UTR. Extracts were harvested 48 hr later and analyzed for Luciferase activity and western blot using the antibodies indicated. Results are presented as fold activation relative to control siRNA-transfected cells.

(D) RNA isolated from HeLa cells infected with HIV-1 Tat(−) virus and transfected with the indicated siRNA was analyzed by NRO using primers in Gag. Values were normalized to that of GAPDH in the same samples. The result for Scr-treated cells was attributed a value of 1The knockdown of specific factors was validated by immunoblot (right). All graphs show mean ± SE from 3 independent experiments.

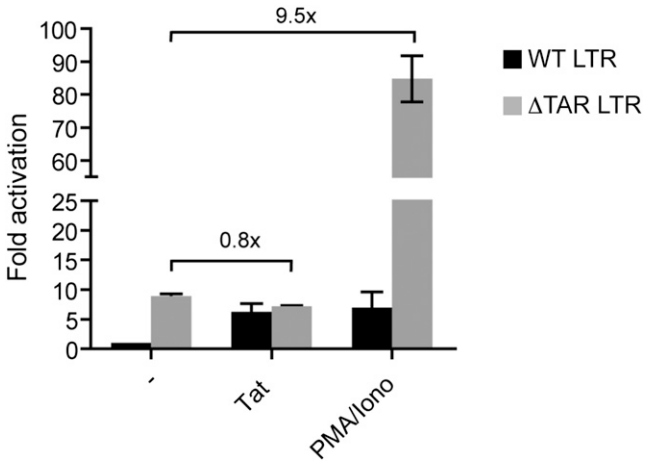


Figure S2. Deletion of TAR Sequence Enhances Basal LTR Activity, Abolishes Tat-Mediated Transactivation but Does Not Diminish Activation by PMA, Related to Figure 2

HeLa LTR-Luc and HeLa LTRΔTAR-Luc cells were treated as indicated. Luciferase activity in cell extracts was measured 16 hr later. Fold activation is the ratio between values obtained for each sample and HeLa LTR-Luc mock-treated cells. The numbers indicated on the top of the bars are the ratios between the indicated samples. Graph shows mean ± SE from 3 independent experiments.

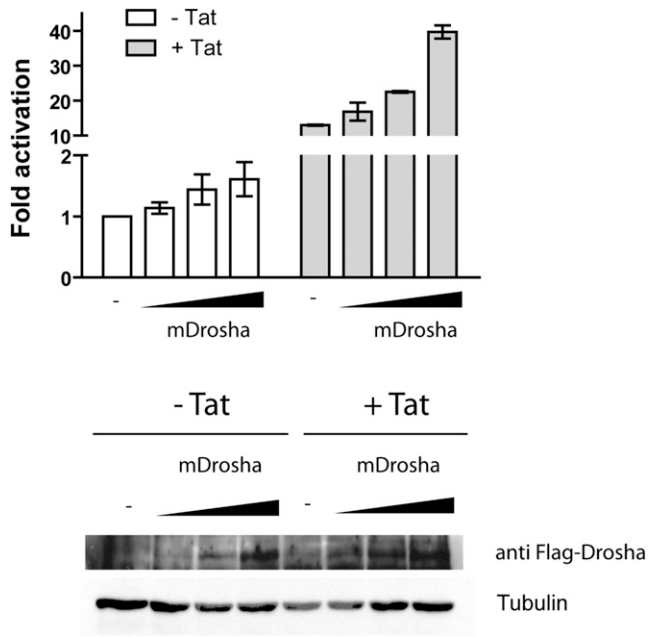
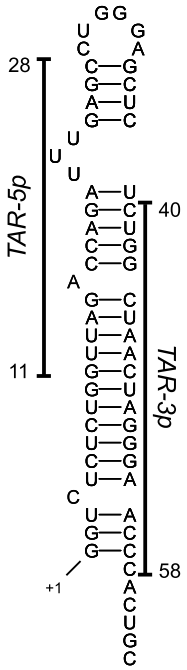


Figure S3. Drosha Endonucleolytic Activity Is Required for Repression of the HIV-1 Promoter, Related to Figure 3

HeLa LTR-Luc cells were mock-transfected or transfected with increasing concentrations of a plasmid encoding a Flag-Drosha mutant lacking its endonucleolytic activity, and transduced with Tat, as indicated. Luciferase activity in cell extracts was measured 16 hr later. Fold activation is the ratio between values obtained for each sample and HeLa LTR-Luc mock-treated cells. Graph shows mean ± SE from 3 independent experiments. Extracts were immunoprobed with Flag or tubulin antibodies.



TAR-5p

		reads
11-28	GUUAGACCAGAUUUGAGC	8
10-28	GGUUAGACCAGAUUUGAGC	5
11-27	GUUAGACCAGAUUUGAG	3

TAR-3p

		reads
40-58	CUGGCUAACUAGGGAACCC	120
40-59	CUGGCUAACUAGGGAACCCA	84
42-60	GGCUAACUAGGGAACCCAC	64
42-58	GGCUAACUAGGGAACCC	63
39-59	UCUGGCUAACUAGGGAACCCA	48
40-58	CUGGCUAACUAGGG	34
39-55	UCUGGCUAACUAGGGAA	34
42-62	GGCUAACUAGGGAACCCACUG	31

Figure S4. Small TAR-Derived RNA Sequences Identified in HIV-1-Infected Cells, Related to Figure 4

Number of reads corresponding to TAR RNA sequence identified from HIV-1 infected cells analyzed by RNA-seq. Locations of the most abundant reads from TAR-5p and TAR-3p are shown on the schematic drawing of TAR at left. Sequences transfected into HeLa-LTR-luc cells (TAR1 nt 11-28 and TAR2 nt 40-58) are shown in red.