SUPPLEMENTAL DATA

SKIP interacts with c-Myc and Menin to promote HIV-1 Tat Transactivation

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SUPPLEMENTAL METHODS

Antisera for ChIP

Polyclonal antisera to SKIP was described previously (Bres et al., 2005). The other antisera were obtained commercially from the following sources: Santa Cruz: c-Myc (N-262), TRRAP (T-17), RNAPII (N-20), CyclinT1 (H-245 for ChIP and T-18 for Western Blot), CDK7 (C-4); Millipore: H3K4Me3 (07-473), H4Ac (06-598), H3Ac (06-599); Abcam: H3K4Me1 (ab8895), GST (ab9085); Medimabs: H2Bub; Covance: Ser2-P RNAPII (H5), Ser5-P RNAPII (H14); Research Diagnostics inc.: GADPH (6C5); Bethyl Laboratories: Menin (A300-105A), Ash2L (A300-112A), RBBP5 (A300-109A), Set1A (A300-289A), MLL1 (A300-087A).

ChIP Protocol

Proteins were cross-linked with 1.5 mM of Ethylene glycol bis[succinimidylsuccinate] in PBS at room temperature for 20 min prior to be fixed in 1% formaldehyde for 10 min. The cross-linking was stopped by addition of glycine to final concentration of 0.125 M for 5 min. The cells were extensively washed in cold PBS and harvested in Cell lysis buffer (50 mM Tris-HCl pH 8.0, 85 mM KCl, 0.5% NP-40 and protease inhibitors). Cells were incubated 10 min on ice prior to be centrifuged at 5,000 rpm for 10 min. The nuclei were resuspended in Nuclear Lysis Buffer (50 mM Tris-HCl pH 8.0, 10 mM EDTA, 1% SDS and protease inhibitor), sonicated, yielding genomic DNA fragments with a bulk size of 300-500 bp. Soluble chromatin was collected after centrifugation at 14,000 rpm. DNA/protein complexes were reverse cross-linked overnight in 1% SDS, 50 mM NaHCO3, 0.3 M NaCl and 10 µg of RNAse A. DNA was isolated by phenol-chloroform extraction, quantified and analyzed by agarose gel. 25 to 50 µg of DNA was used for each immunoprecipitation. Lysates were incubated with the appropriate antibody overnight and immunocomplexes were recovered by adding 20 µl of proteinA / proteinG blocked beads (Millipore). Beads were washed twice in 50 mM Tris-HCl pH 8.0 and 2 mM EDTA and four times

in 100 mM Tris-HCl pH 8, 500 mM LiCl, 1% NP-40 and 1% deoxycholic acid, prior to be reverse cross-linked. Recovered DNA was resuspended in 100 µl of TE. Each PCR reaction was performed in triplicate and the comparative Ct method was used to calculate relative expression, as compared to input. Each experiment was performed at least in triplicate.

Protein GST pull-down interaction assays, direct binding and co-immunoprecipitation experiments

After 3 h incubation at 4°C, the GST-protein coupled beads were washed 3 times for 10 min in Washing buffer (20 mM Hepes, pH 8.0, 10% glycerol, 0.3 M KCl, 0.1% NP-40, 0.2 mM EDTA, 2 mM DTT, 0.1 mM PMSF and protease inhibitors) and once in Washing buffer containing 0.1 M KCl. For binding to recombinant His-Menin, 100 nM GST fusions proteins were mixed in 500 µl of buffer B (50 mM HEPES, pH 7.9, 150 mM NaCl, 0.2 mM EDTA, 12.5 mM MgCl2, 40 µM ZnSO4, 10% Glycerol, 0.5% Tween20, 1 mM DTT and 0.2 mM PMSF) containing 100 ng/µl of BSA and incubated for 4 h at 4°C with rocking. Beads were then washed five times with buffer B, prior to analysis by SDS-PAGE and immunoblot. Co-immunoprecipitation experiments were performed by incubating a HeLa nuclear extract with the indicated antibodies for 3 hr in buffer B, followed by a 1 hr incubation with protein A beads. Beads were washed 3 times in Washing buffer prior to analysis by SDS-PAGE and immunoblot.

Histone extraction procedure

siRNAs transfected cells were collected and washed twice in cold PBS prior to be resuspended in Lysis buffer (10 mM Hepes, pH7.9, 1.5 mM MgCl2, 10 mM KCl, 0.5 mM DTT and 1.5 mM PMSF). Hydrochloric acid was added to final concentration of 0.2N and cells were incubated on ice for 10 min prior to be centrifuged at 11,000 x g. Supernatants were then dialyzed twice in 0.1N HCl and overnight in water. Proteins were quantified prior to be resolved on SDS-PAGE and then Coomassie stained or transferred to PVDF for Western blotting analysis.

Small-scale chromatin fractionation

Cellular fractionation was performed as described previously (Wysocka et al. 2001). After two washes in 1X PBS, cells were resuspended in buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 1.5 mM MgCl2, 0.34 M sucrose, 10% glycerol, 1 mM DTT, protease inhibitor cocktail, and 0.1% Triton X-100) and incubated on ice for 8 min. Nuclei (fraction P1) were collected by centrifugation (5 min., 1,300 × g, 4°C), the supernatant (fraction S1) was clarified by high-speed centrifugation (5 min, 20,000 × g, 4°C), and the supernatant (fraction S2) was collected. The P1 nuclei were washed once in buffer A and lysed for 30 min in buffer B (3 mM EDTA, 0.2 mM EGTA, 1 mM DTT, and protease inhibitor cocktail). The insoluble chromatin (fraction P3) and soluble (fraction S3) fractions were separated by centrifugation (5 min, 1,700 × g, 4°C). The P3 fraction was washed with buffer B prior to immunoblot.

Sequences for siRNA

Control/GFP (Invitrogen)	sense, 5'-GCCCUCGCCGGACACGCUGdTdT-3'
	antisense, 5'-CAGCUGUCCGGCGAGGCdTdT-3'
SKIP (Invitrogen)	sense, 5'-AUGUCGAAUGCGCUGGCCAdTdT-3'
	antisense, 5'-UGGCCAGCGCAUUCGACAUdTdT-3'
SKIP (Ambion)	sense, 5'-GUAUGGUGAUGACCUAGAAdTdT-3'
	antisense, 5'-UUCUAGGUCAUCACCAUACdAdT-3'.
CyclinT1 (Invitrogen)	sense, 5'- CUACCACAAGUGCAGUGCCdTdT-3'
	antisense, 5'-GGCACUGCACUUGUGGUAGdTdT-3'
c-Myc (Ambion)	sense, 5'-AGACCUUCAUCAAAAACAUdTdT-3'
	antisense, 5'-AUGUUUUUGAUGAAGGUCUdCdG-3'
Ash2L (Ambion)	sense, 5'-GCCUGGUAUUUUGAAAUCAdTdT-3'
	antisense, 5'-UGAUUUCAAAAUACCAGGAdAdC-3'

Menin (Ambion)	sense, 5’-GACCUACUACUAUCGGGAUGAAdTdT-3’
	antisense, 5’-UUCAUCCCGAUAGUAGGUCdTdT-3’
MLL1 (Ambion)	sense, 5’- GGUUGCUAUAUGUUCCGAAdTdT-3’
	antisense, 5’-UUCGGAACAUAUAGCAACCdAdA -3’
RNF20 (Ambion)	sense, 5’-GGUCCGCAAGGAGUAUGAAdTdT-3’
	antisense, 5’-UUCAUACUCCUUGCGGACCdTdG-3’

Primer sequences for qPCR

HIV-1 promoter	Fow	5’-CCTGTACTGGGTCTCTCTGGTTAGA-3’
	Rev	5’- AGCTCCCAGGCTCAGATCTG-3’
Luciferase	Fow	5’- TATGGGCTCACTGAGACTACATCAG-3’
	Rev	5’- CATCCCCCTCGGGTGTAATC-3’
c-Myc E2	Fow	5’- CCCTCAACGTTAGCTTCACCA-3’
	Rev	5’- ACCGAGTCGTAGTCGAGGTCA-3’
TRRAP	Fow	5’- TCCAGCAGAGACTGCACACC-3’
	Rev	5’- TGTTTGGCAGCAATCAGAAGC-3’
GADPH	Fow	5’- TCGTCATGGGTGTGAACCAT-3’
	Rev	5’- TGATGATCTTGAGGCTGTTGTCA-3’
beta-actin	Fow	5’- ATCGTCCACCGCAAATGCTTCTA-3’
	Rev	5’- AGCCATGCCAATCTCATCTTGTT-3’
SKIP	Fow	5’- AGAGATCCATGTGGCCCAGTA-3’

	Rev	5’- GCGCATTCGACATTTTTTTCT-3’
RNF20	Fow	5’- GGCCAGCAGTTCCAGTGAAG-3’
	Rev	5’- TCCACACGTTCCTGCAGCT-3’
PABPC1 (ChIP)	Fow	5’- CAGCGGCAGTGGATCGA-3’
	Rev	5’- GGACAAAAATCAACCGGAATTG-3’
PABPC1 (Expression)	Fow	5’- CATGGGTGGAGCTGGTCAAT-3’
	Rev	5’- TCCTAGCAGAGATCCATGCAGAT-3’

SUPPLEMENTAL FIGURES

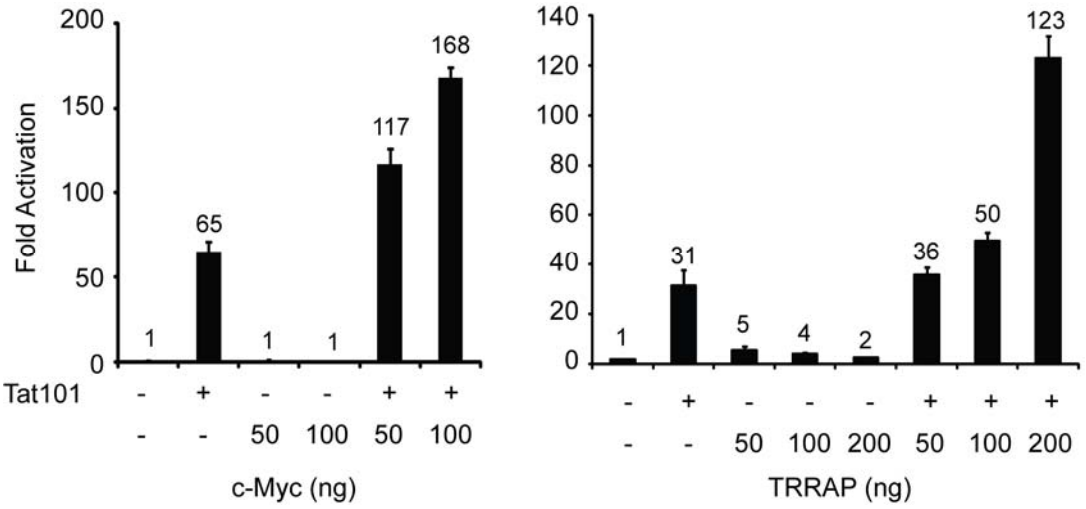

Figure S1: Transient expression of c-Myc and TRRAP enhances Tat activation of an integrated HIV-1 promoter. Hela cells containing an HIV-1 LTR-luciferase reporter gene integrated, were transfected alone or together with 5 ng (left panel) or 2 ng (right panel) of HIV-1 Tat101 and c-Myc- or TRRAP-expression vectors, as indicated. For normalization, cells were transfected with the control pRL-TK vector, and luciferase and renilla activities were analyzed 48 h after transfection. Fold activation refers to the HIV-1 LTR activity relative to mock-transfected cells. All graphs represent mean and standard error obtained from three independent experiments.

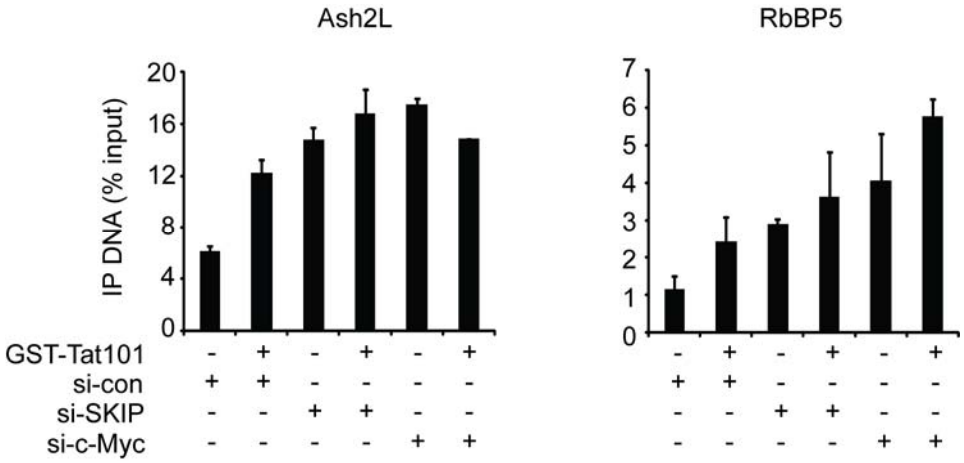

Figure S2: Neither SKIP nor c-Myc are required to load Ash2L and RbBP5 to HIV-1 promoter. ChIP analysis of the Ash2L and RbBP5 at the HIV-1 LTR promoter in si-con, si-SKIP, and si-c-Myc transfected cells. All graphs represent mean and standard error obtained from three independent experiments.

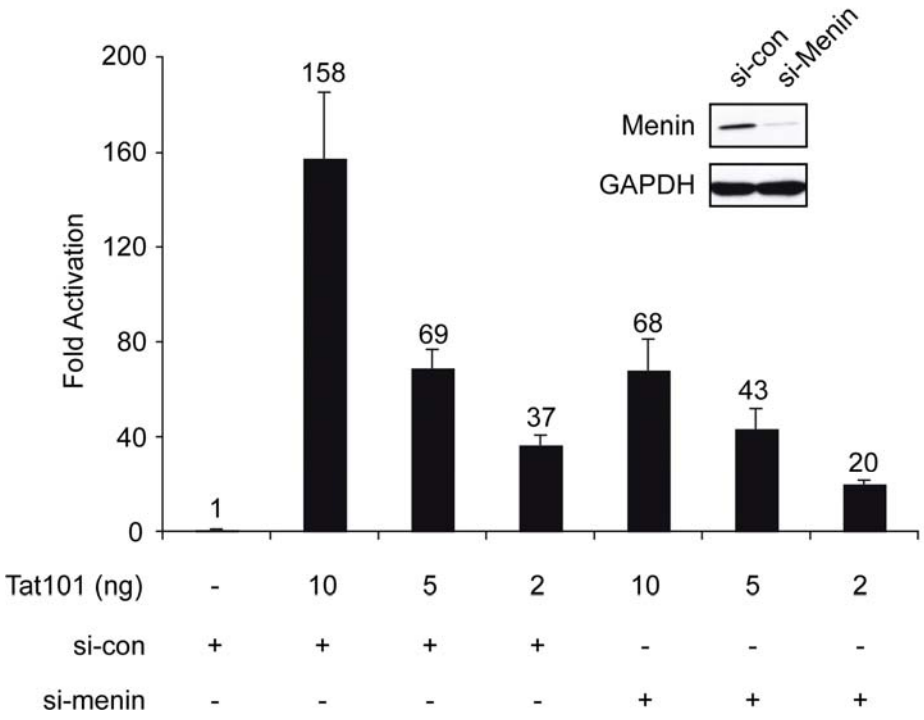

Figure S3: Menin is required for Tat-induced HIV-1 transcription. HeLa HIV-1 LTR:Luc (luciferase) activity induced with the indicated amount of Tat101 plasmid in si-control and si-Menin transfected cells. All graphs represent mean and standard error obtained from three independent experiments. Inset, analysis of knockdown efficiency by Immunoblot.

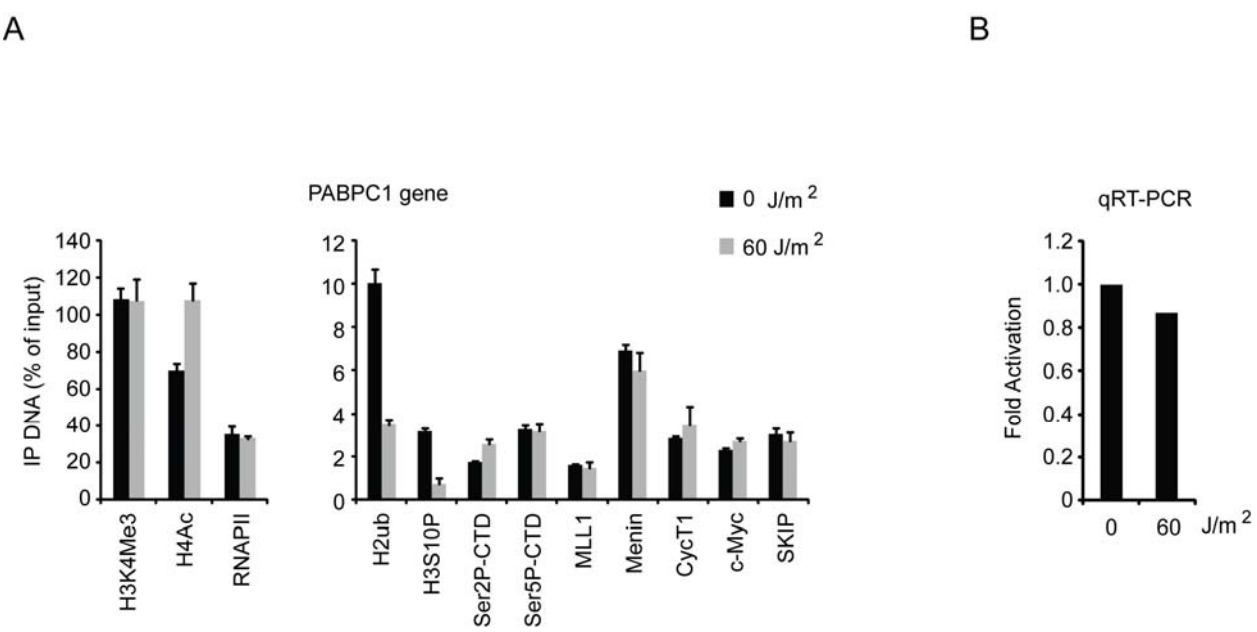

Figure S4: UV-induction causes increase of H4Ac and decrease of H2Bub and H3S10P at the PABPC1 gene without affecting its transcription. (A) ChIP analysis of the indicated factors and histone modifications in absence (black) or upon UVC treatment (grey) as indicated. (B) qRT-PCR analysis of the PABPC1 gene in mock or UV-treated cells. All graphs represent mean and standard error obtained from three independent experiments.

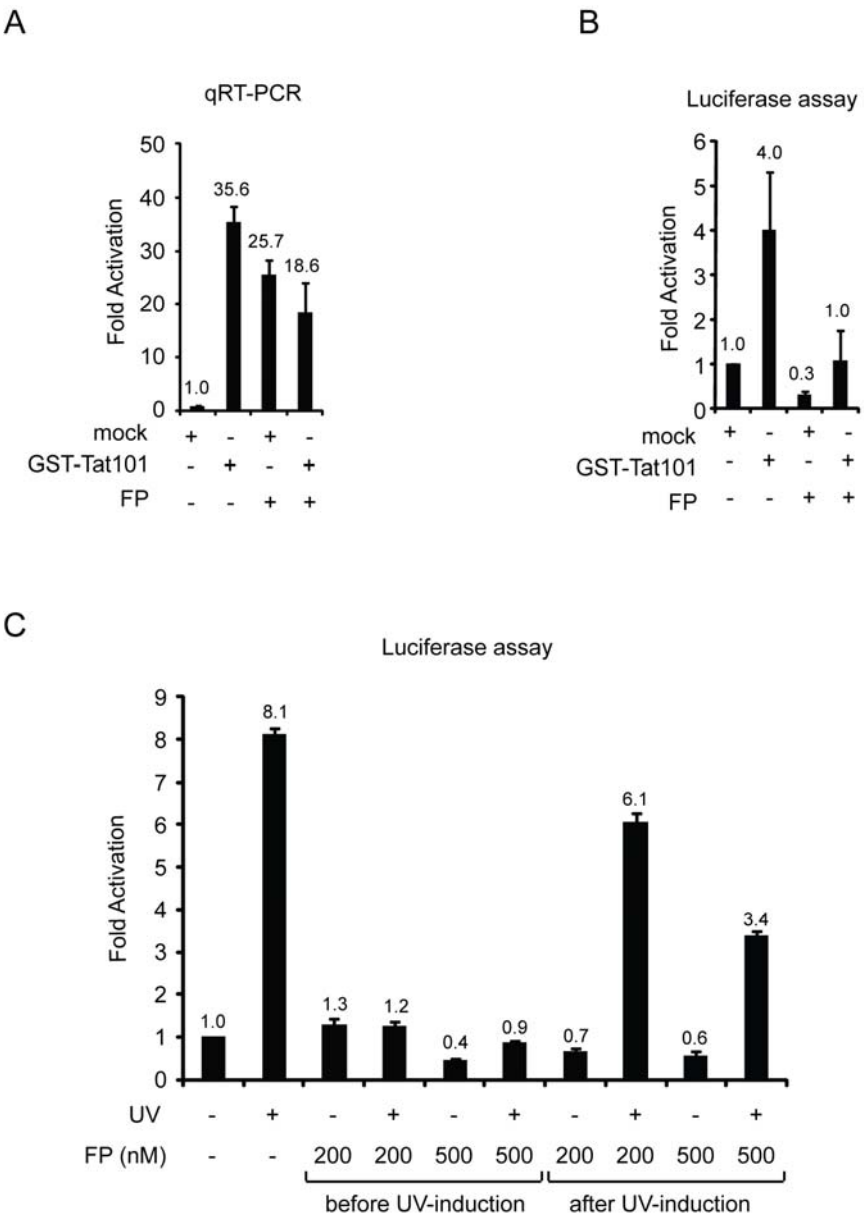

Figure S5: Flavopiridol blocks Tat-induced HIV-1 transcription. (A) qRT-PCR analysis of the luciferase RNA, normalized to b-actin RNA, in HeLa LTR:Luc cells that were mock-treated or treated with 500nM Flavopiridol (FP) prior to be either transduced with GST (mock) or Gst-Tat101. (B) Luciferase assay performed in HeLa LTR:Luc cells treated as described in (A). (C) Luciferase assay performed in HeLa LTR:Luc cells treated with indicated amount of FP either 1 hr prior UV-induction or 18 hr post UV-induction. All graphs represent mean and standard error obtained from three independent experiments.