The Lysine Methyltransferase SMYD5 Amplifies HIV-1 Transcription and is Post-transcriptionally Upregulated by Tat and USP11

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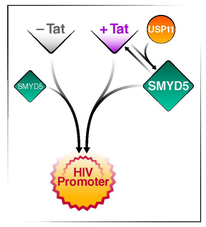
Abstract

A successful HIV-1 cure strategy may require enhancing HIV-1 latency to silence HIV-1 transcription. Epigenetic modulators show promise as latency-promoting agents *in vitro* and *in vivo*. Here, we identify SET and MYND domain-containing protein 5 (SMYD5) as an epigenetic host factor required for HIV-1 transcription. SMYD5 is expressed in CD4$^+$ T cells and activates the HIV-1 promoter with or without the viral Tat protein, while knockdown of SMYD5 decreases HIV transcription in cell lines and primary T cells. SMYD5 associates *in vivo* with the HIV promoter and binds both TAR RNA and Tat. Tat is methylated by SMYD5 *in vitro*, and in cells expressing Tat, SMYD5 protein levels are increased. The latter requires expression of the Tat cofactor and ubiquitin-specific protease 11 (USP11). We propose that SMYD5 is a new host activator of HIV-1 transcription stabilized by Tat and USP11 and together with USP11 a possible target for latency-promoting therapy.



SMYD5 Knockdown Inhibits HIV-1 Reactivation from Latency in J-Lat Cells

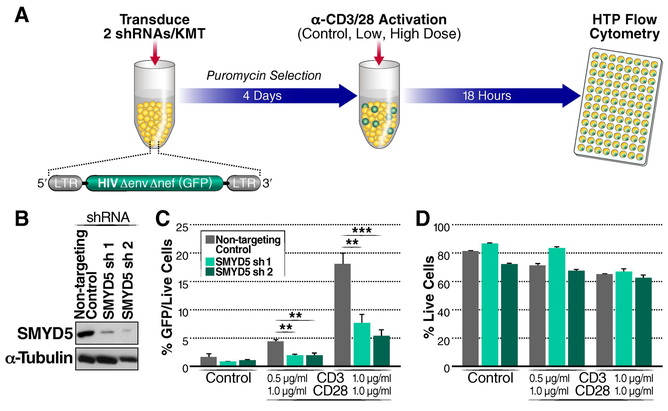


Figure 1: SMYD5 knockdown inhibits HIV-1 reactivation from latency in J-Lat cells. **(A)** Schematic of the workflow. J-Lat 5A8 cells were transduced with SMYD5 shRNA, puromycin selected, activated with CD3/CD28 antibodies for 18h and GFP+ cells analyzed by flow cytometry. **(B)** SMYD5 protein levels in J-Lat 5A8 cells after targeting its RNA with two different lentiviral shRNAs or non-targeting control. **(C)** Reactivation of the latent HIV reporter, as measured by GFP flow cytometry. Both SMYD5 shRNAs suppress HIV-1 reactivation under basal conditions and upon activation with CD3/CD28. **(D)** Cell viability (% live cells), as monitored by forward and side scatter analysis and viability stain, under the same conditions as **(C)**. Average (mean +/- SD) from triplicate experiments is shown.

Acknowledgements

We thank members of the Ott, Marson, Krogan and Greene laboratories for helpful discussions, reagents and expertise. We thank J. Srivastava, N. Raman and the Gladstone Flow Cytometry Core for assistance with Flow Cytometry, J. Carroll for graphics, F. Chanut for editorial assistance, and V. Fonseca and L. Weiser for administrative assistance. This work was supported by the National Institutes of Health/National Institute of Allergy and Infectious Diseases (NIH/NIAID, grant nos. DP1DA038043 and R37AI083139 to M.O.), the NIH/National Institute on Drug Abuse (grant no. R01DA043142), and the NIH (grant no. P30 AI027763 to Flow Cytometry Core). The work was also supported by the UCSF Aids Research Institute with funding from amfAR (grant no. 109301-59-RGRL). D.B. was also funded by the Gilead HIV Cure Mentored Scientist Award from the UCSF Aids Research Institute. Further, we gratefully acknowledge support from the HOPE Collaboratory (UM1AI164559) to M.O. and the James B. Pendleton Charitable Trust.

SMYD5 is Expressed and Upregulated upon Activation in Primary CD4+ T Cells

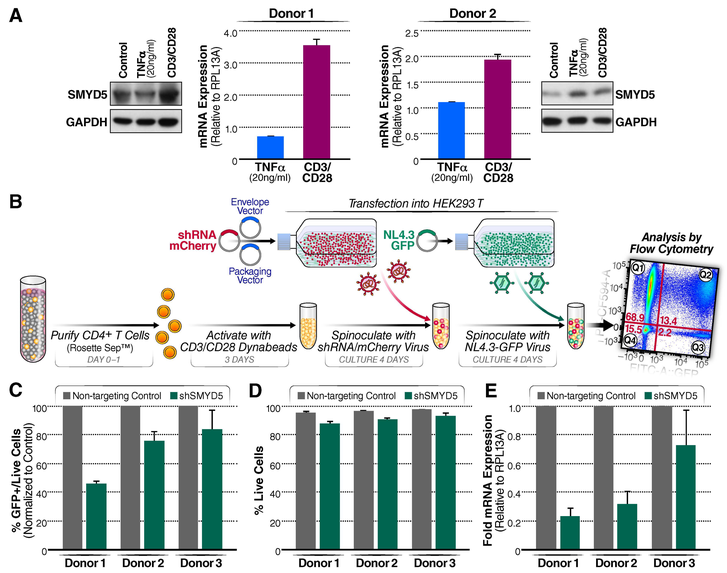


Figure 2: SMYD5 is expressed and upregulated upon activation in primary CD4$^+$ T cells and its knockdown inhibits HIV-1 expression. (A) SMYD5 protein and mRNA expression in CD4+ T cells from the blood of two independent human donors. The cells were treated with 20ng/ml TNFα or CD3/CD28 Dynabeads (1 bead per 2 cells) for 24h, then lysed in Flag-IP buffer for western blot analysis or in RLT+ buffer to isolate mRNA (Qiagen). RNA levels were analyzed by RT-qPCR and normalized to RPL13A RNA. **(B)** ShRNA-mediated knockdown of SMYD5 in CD4$^+$ T cells. Schematic of the workflow. Primary CD4$^+$ T cells isolated from uninfected blood donors were activated with αCD3/CD28 Dynabeads for 3 days, infected first with lentiviral particles encoding shRNA against SMYD5 or non-targeting control, and second with a GFP-tagged reporter virus in the background of HIV_{NL4-3}. **(C-E)** Impact of scrambled or SMYD5-targeting shRNAs on the percentage of HIV-expressing (GFP+) cells (C), the percentage of live cells as measured by forward scatter analysis and viability stain (D), and the level of SMYD5 mRNA, as determined by RT-PCR normalized to RPL13A mRNA (E), among activated CD4 + T cells from three independent donors. Average (mean ± SD) from triplicate experiments performed with three different donors is shown.

SMYD5 is Recruited to the HIV-1 LTR and Activates HIV-1 Transcription

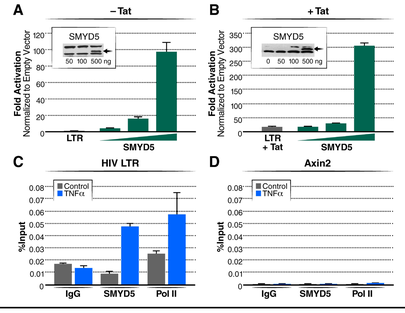


Figure 3: SMYD5 is recruited to the HIV-1 LTR and activates HIV-1 transcription. (A/B) Luciferase assays in HeLa cells transfected with an HIV-1 LTR luciferase construct and increasing amounts of an expression vector for SMYD5 (0; 5; 50 and 500 ng) in **(A)** the absence of Tat or **(B)** the presence of Tat. Expression of SMYD5 strongly activates the HIV-1 LTR and increases in the presence of Tat. Results of three biological replicates (±SEM) are shown. **(C/D)** ChIP assays with antibodies against SMYD5, Pol II, and IgG control at the HIV-1 LTR, followed by qPCR using primers specific for **(C)** HIV-1 LTR or **(D)** the Axin2 control. Chromatin was prepared from J-Lat 5A8 cells treated with TNFα to stimulate the LTR or untreated. All ChIPs and qPCRs were repeated at least three times, and representative results of three technical replicates are shown.

SMYD5 Methylates Histones and Tat *in vitro*

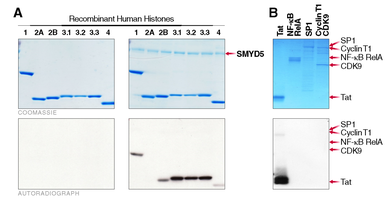


Figure 4: SMYD5 methylates histones and Tat *in vitro*. *In vitro* methylation of **(A)** recombinant histones and **(B)** synthetic Tat peptide (aa 1–72), recombinant NFκB RelA, Sp1, Cyclin T1 or CDK9 incubated with recombinant full-length SMYD5 enzyme and radiolabeled H^3-S-adenosyl-L-methionine (SAM). All *in vitro* methylation assays on isolated histones, Tat peptides, NF-κB, SP1 and pTEFb were repeated at least three times, and representative Coomassie stain (top) and corresponding autoradiographs (bottom) are shown.

Tat Associates with SMYD5 and Stabilizes SMYD5

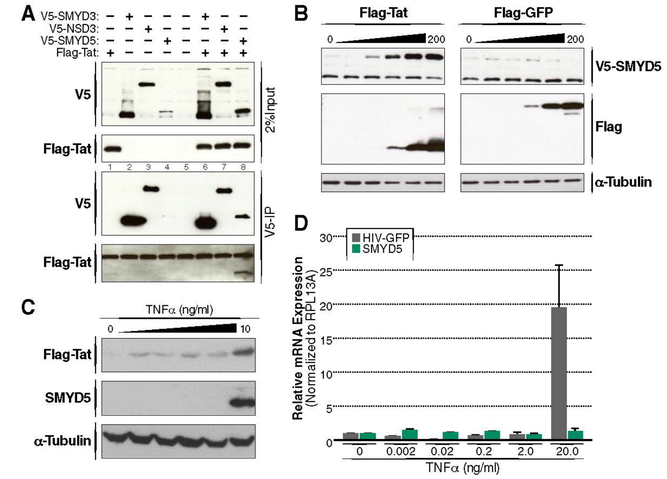


Figure 5: Tat physically associates with SMYD5 and stabilizes SMYD5. (A) Tat co-immunoprecipitates with SMYD5. Cellular extracts from HEK293T cells expressing V5-SMYD3, V5-NSD3, V5-SMYD5 or empty vector, alone or together with Flag-Tat, were prepared 48h after transfection and subjected to purification with anti-V5 affinity gel. The eluates were resolved by SDS-PAGE and subjected to western blotting. **(B)** Tat increases SMYD5 protein expression. HEK293T cells were transfected with 500ng V5-SMYD5 and 0-200ng of Flag-Tat or 0-200ng Flag-GFP plasmids. After 48h cells were lysed and subjected to western blot analysis. **(C, D)** TNFα increases SMYD5-Tat co-immunoprecipitation but not SMYD5 RNA production. J-Lat 5A8 cells were treated with increasing amounts of TNFα. After 48h cells were lysed in Flag-IP buffer and subjected to western blot analysis (C) or lysed in RLT+ buffer to isolate mRNA (Qiagen) (D). RNA levels were analyzed by RT-qPCR and normalized to RPL13A RNA. All IPs, western blots and qPCRs were repeated at least three times. Representative results of IPs and western blots are shown. qPCR results of three biological replicates (±SEM) are shown.

SMYD5 and Tat Interact with USP11

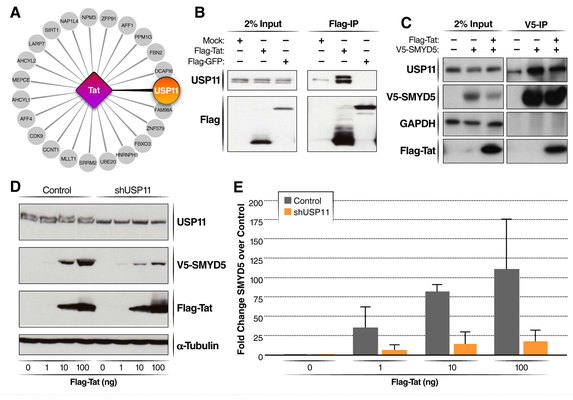


Figure 6: SMYD5 and Tat interact with USP11. (A) Network representation of the interactions of HIV-1 Tat with human proteins (Jager et al., 2011), highlighting USP11. **(B)** Endogenous USP11 co-immunoprecipitates with Flag-Tat. HEK293T cells were transfected with 200ng of Flag-Tat WT, Flag-GFP or empty vector. After 48h cells were lysed in Flag-IP buffer and subjected to Flag-immunoprecipitation and western blot analysis of endogenous USP11. Note that we consider the lower band in the blot as unspecific as it is not downregulated in Figure 6D. **(C)** Tat and endogenous USP11 co-immunoprecipitate with V5-SMYD5. HEK293T cells were transfected with plasmids expressing V5-SMYD5 or empty vector, alone or together with Flag-Tat. After 48h cells were lysed in Flag-IP buffer and subjected to V5-immunoprecipitation and western blot analysis. All IPs and western blots were repeated at least three times. Representative results of IPs and western blots are shown. **(D)** USP11 knockdown inhibits Tat-mediated SMYD5 stabilization. HeLa cells were transduced with lentiviral vectors expressing shRNA targeting USP11 or a non-targeting control and selected by puromycin treatment. Successfully transduced cells were transfected with increasing amounts of Tat and after 48h subjected to western blot analysis. Note that we consider the lower band in the blot as unspecific. **(E)** Quantification of 3 independent experiments/ western blots of SMYD5 bands in (D) by densitometric analyses using ImageJ. Each V5-SMYD5 band from shUSP11 was compared to its corresponding control treated band (for example: 1ng Tat control vs. 1ng Tat shUSP11). Data are represented as means plus SEM.