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Supplemental Information

The PARP1-Siah1 Axis Controls HIV-1 Transcription and Expression of Siah1 Substrates

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Supplemental Figures **Figure S1**

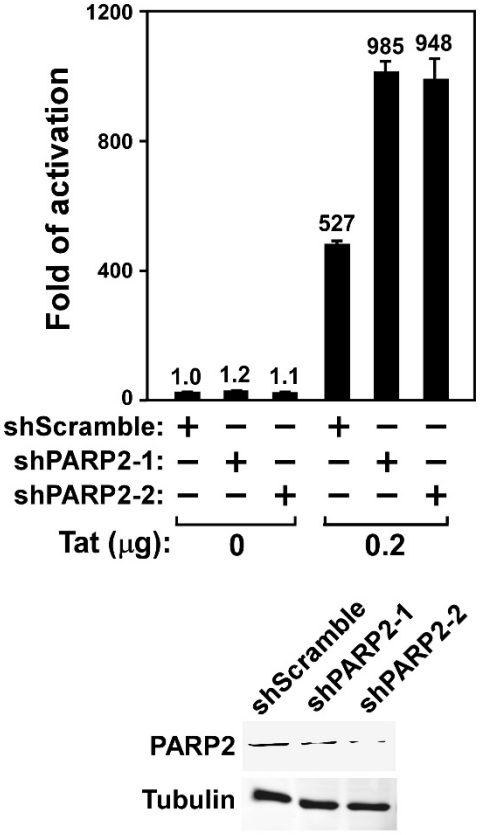

Figure S1. The shRNA-mediated silencing of PARP2 expression promotes Tat-transactivation. Related to Figure 1. NH1 cells containing an integrated HIV-1 LTR-luciferase reporter construct were co-transfected with a plasmid expressing the indicated shRNA and the Tat-expressing construct or an empty vector. Luciferase activities were measured in extracts of the cells and compared to that in the first lane, which was set at 1.0. Error bars represent mean ± SD from three separate measurements. The PARP2 and α-Tubulin levels in the extracts were determined by Western blotting and shown at the bottom.

Figure S2

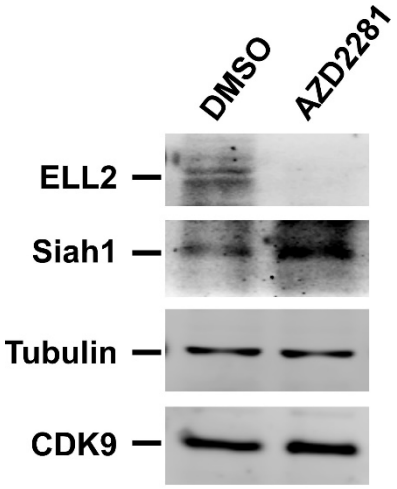

Figure S2. Treatment of Jurkat 1G5 cells with AZD2281 decreased the endogenous protein level of ELL2 but increased the level of Siah1. Related to Figures 2 & 4. 1G5 cells were treated with 20 μM AZD2281 or the DMSO control for 24 hrs. Whole cell extracts were prepared and analyzed by Western blotting to detect the indicated proteins.

Figure S3

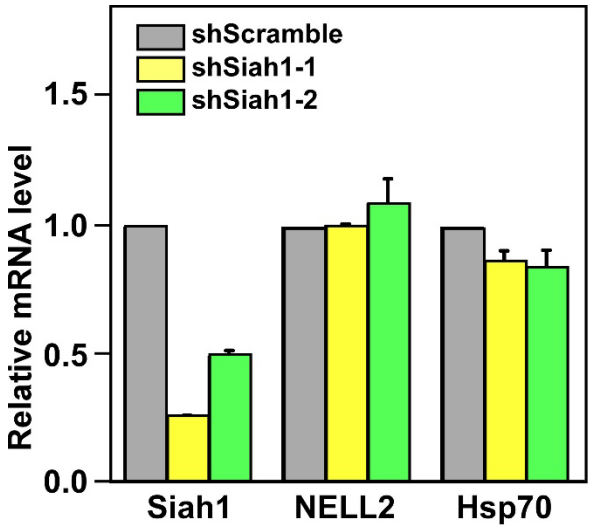

Figure S3. The expression of NELL2 and Hsp70, two known PARP1 targeted genes, was not significantly affected by the KD of Siah1. Related to Figure 4. Total RNAs isolated from HeLa cells, which were transfected with the indicated shRNAs, were analyzed by qRT-PCR for the genes marked at the bottom. The signals were normalized to those of GAPDH and displayed, with the RNA levels in the shScramble-expressing cells set to 1. The error bars represent mean +/- SD from three independent measurements.

Figure S4

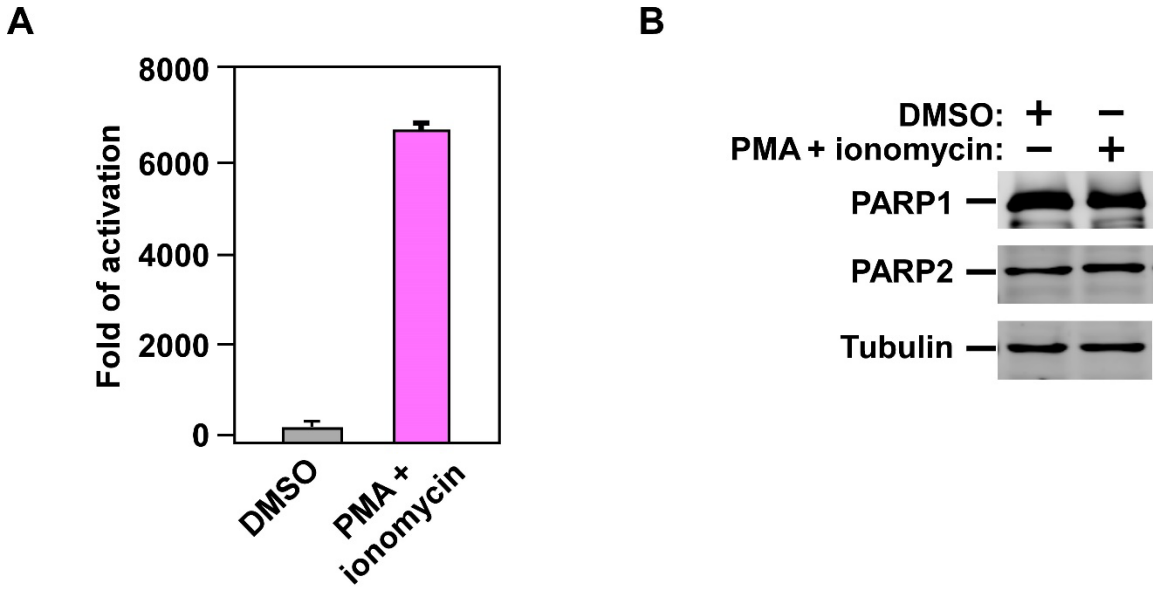

Figure S4. PMA plus ionomycin dramatically elevate HIV-1 LTR activity without significantly altering the PARP1 and 2 protein levels. Related to Figure 4 & Discussion. A. Jurkat 1G5 cells containing an integrated HIV-1 LTR-luciferase reporter construct were treated with the indicated agents. Luciferase activities were measured in extracts of the treated cells and compared to that of the DMSO lane, which was set at 1.0. Error bars represent mean ± SD from three separate measurements. **B.** The indicated proteins present in the extracts were determined by Western blotting.

Supplemental Table

Table S1. Primers used in this study. Related to Experimental Procedures.

Primer name	Sequence
Siah1-RT-F	CTGCTTTGACTATGTGTTACCGC
Siah1-RT-R	ACTGAATTAGCCACTTTCTCCAT
β-Actin-RT-F	CATGTACGTTGCTATCCAGGC
β-Actin-RT-R	CTCCTTAATGTCACGCACGA
GAPDH-RT-F	AGGTGAAGGTCGGAGTCAAC
GAPDH-RT-R	CGCTCCTGGAAGATGGTGAT
AFF1-RT-F	ACAAGAAAGGTGACCGAAGAG
AFF1-RT-R	GAAGAGTTTGCTGGTTGGAATG
AFF4-RT-F	CACACCATAATAGTGAAGGAG
AFF4-RT-R	GGGTTCAGGCTCGGGAGAT
ELL2-RT-F	CACCAGCCGTTCAGAATCTCCT
ELL2-RT-R	GGTGGTACTCTGTTCGTCAGGT
Siah1-Y78A-F	AAGAATGGGCGGTAACACAGCGTCAAAGCAGACTGGACAC
Siah1-Y78A-R	GTGTCCAGTCTGCTTTGACGCTGTGTTACCGCCCATTCTT
Siah1-Q87A-F	CAAACAAGATGGCCACTCGCACATTGAAGAATGGGCGGTAA
Siah1-R97A-F	GTGAGCTTTGGGGCACAGTTGCTACAAACAAGATGGC
Siah1-R97A-R	GCCATCTTGTTTGTAGCAACTGTGCCCCAAAGCTCAC
Siah1-R97A-K99D-F	GCAAGTTGGACAACATGTGAGGTCTGGGGCACAGTTGCTACAAACAAGATG
Siah1-R97A-K99D-R	CATCTTGTTTGTAGCAACTGTGCCCCAGACCTCACATGTTGTCCAACTTGC
Siah1-ChIP-3FN	AGACTTCCAGGCACCTAAGTG
Siah1-ChIP-3RN	CGCTGGATGCTGATATGAGC
shPARP1-F	GATCCTTGGTAGCAAGGCAGAGAATTCAAGAGATTCTCTGCCTTGCTACCAATTTTTTGGAAA
shPARP1-R	AGCTTTTCCAAAAAATTGGTAGCAAGGCAGAGAATCTCTTGAATTCTCTGCCTTGCTACCAAG
shPARP2-F	GATCCAGAGAAAAGGCGATGAGGTTTCAAGAGAACCTCATCGCCTTTTCTCTTTTTTTGGAAA
shPARP2-R	AGCTTTTCCAAAAAAAGAGAAAAGGCGATGAGGTTCTCTTGAAACCTCATCGCCTTTTCTCTG
shPARP2-1-F	CCGGACTATCTGATTCAGCTATTAGCTCGAGCTAATAGCTGAATCAGATAGTTTTTTG
shPARP2-1-R	AATTCAAAAAACTATCTGATTCAGCTATTAGCTCGAGCTAATAGCTGAATCAGATAGT
shPARP2-2-F	CCGGTCTGAATCCAGATGGTTATACCTCGAGGTATAACCATCTGGATTCAGATTTTTG
shPARP2-2-R	AATTCAAAAATCTGAATCCAGATGGTTATACCTCGAGGTATAACCATCTGGATTCAGA
ACK-F	ATGTCATCACCGTCATCGAG
ACK-R	TGTGGATGAAGCTGTTCTGC

CBP-F	ACACAGGGCAATACCAAGAG
CBP-R	TTGCGTCCACAGCAATATCC
β-catenin-F	TGAAGGTGCTATCTGTCTGC
β-catenin-R	CCTTCCTGTTTAGTTGCAGC
IPTR1-F	GCTGAAGACACTATCACTGC
IPTR1-R	TATCAGTTCCTGGGTCACTG
NELL2-F	AGCTGTCTCGAGCTGAACAG
NELL2-R	GACTTAAGTGGGCAGTCAGG
HSP70-HSPA4-F	ACTCTTGAGGCCTACTACAG
HSP70-HSPA4-R	AAGATGCACTGGACACACTG
NcoR-shRNA-1F	CCGGGCCATCAAACACAATGTCAAACTCGAGTTTGACATTGTGTTTGATGGCTTTTTG
NcoR-shRNA-1R	AATTCAAAAAGCCATCAAACACAATGTCAAACTCGAGTTTGACATTGTGTTTGATGGC
NcoR-shRNA-2F	CCGGGCTCTCAAAGTTCAGACTCTTCTCGAGAAGAGTCTGAACTTTGAGAGCTTTTTG
NcoR-shRNA-2R	AATTCAAAAAGCTCTCAAAGTTCAGACTCTTCTCGAGAAGAGTCTGAACTTTGAGAGC
NcoR-F	AGGACAAGTTTATCCAGCATCC
NcoR-R	GCAATTTGCTGGTTTCTGCC

Supplemental Experimental Procedures

Cell culture, Reagents

NH1 cells, HeLa cells and PARP1 KO HeLa cells were all grown in DMEM with 5% FBS. Jurkat-based 1G5 cells (A gift from Dr. Andrew Rice of Baylor College) were maintained in RPMI1640 with 10% FBS. PARP1 inhibitor AZD2281 was purchased from Selleckchem (S1060). Ionomycin (1 μM) and PMA (10 ng/ml) were used to stimulate Jurkat 1G5 cells. Antibodies for Western blotting were listed as follows: Anti-PARP1 (46D11) was purchased from Cell Signaling Technology. Anti-Poly(ADP-ribose) (ALX-210-890A-0100) was purchased from Enzo Life Science. Anti-ELL2 (A302-505A), anti-ELL (A301-645A), anti-ENL (A302-268A), anti-AF9 (A300-595A) and anti-AFF1 (A302-344A) were all from Bethyl Laboratories. Anti-tubulin (ab6046), anti-AFF4 (ab57077) and anti-PARP2 (ab176330) antibodies were from Abcam. Anti-CyclinT1 (sc-10750), anti-CBP (sc-7300), anti-ACK (sc-28336) and anti-β-catenin (sc-133240) were from Santa Cruz Biotechnology. The antibodies against CDK9, Brd4, LARP7 and HEXIM1 were generated in our own laboratory and have been described previously (He et al., 2008).

Plasmids

Plasmids coding for HA-ELL2, Flag-ELL2 and Flag-Siah1 WT (Long form 313 amino acids) were constructed in our previous work (Liu et al., 2012). Mutations in Siah1 were generated by using the KAPA HiFi PCR kit (KR0368, Roche). Primers used for mutagenesis were listed in supplemental Table S1. The Flag-tagged, shPARP1-resistent WT and mutant PARP1 were constructed in pcDNA3 vector. Primers were listed in supplemental Table S1.