1 Research Article
2 **Cocain** ²**Cocaine-induced DNA-PK relieves RNAP II pausing by promoting** ³
TRIM28 phosphorylation.

5 Adhikarimayum Lakhikumar Sharma¹, Priya Tyagi¹, Meenata Khumallambam¹, Mudit Tyagi^{1*}
⁶

¹ Center for Translational Medicine, Thomas Jefferson University, 1020 Locust Street, Philadelphia, PA
20107 - 19107 - 1910 ⁸19107, USA

¹⁰**Abstract**

4

6
7

12 12 Drug abuse continues to pose a significant challenge in HIV control efforts. In our investigation, we
13 discovered that cocaine not only uprequlates the expression of DNA-dependent protein kinase 13 discovered that cocaine not only upregulates the expression of DNA-dependent protein kinase
14 (DNA-PK) but also augments DNA-PK activation by enhancing its phosphorylation at S2056. 14 (DNA-PK) but also augments DNA-PK activation by enhancing its phosphorylation at S2056.
15 Moreover, DNA-PK phosphorylation triggers the translocation of DNA-PK into the nucleus. The 15 Moreover, DNA-PK phosphorylation triggers the translocation of DNA-PK into the nucleus. The
16 finding that cocaine promotes nuclear translocation of DNA-PK further validates our observation of 16 finding that cocaine promotes nuclear translocation of DNA-PK further validates our observation of
17 enhanced DNA-PK recruitment at the HIV long terminal repeat (LTR) following cocaine exposure. 17 enhanced DNA-PK recruitment at the HIV long terminal repeat (LTR) following cocaine exposure.
18 By activating and facilitating the nuclear translocation of DNA-PK, cocaine effectively orchestrates 18 By activating and facilitating the nuclear translocation of DNA-PK, cocaine effectively orchestrates
19 multiple stages of HIV transcription, thereby promoting HIV replication. Additionally, our study 19 multiple stages of HIV transcription, thereby promoting HIV replication. Additionally, our study
20 indicates that cocaine-induced DNA-PK promotes hyper-phosphorylation of RNA polymerase II 20 indicates that cocaine-induced DNA-PK promotes hyper-phosphorylation of RNA polymerase II
21 (RNAP II) carboxyl-terminal domain (CTD) at Ser5 and Ser2 sites, enhancing both initiation and 21 (RNAP II) carboxyl-terminal domain (CTD) at Ser5 and Ser2 sites, enhancing both initiation and
22 elongation phases, respectively, of HIV transcription. Cocaine's enhancement of transcription 22 elongation phases, respectively, of HIV transcription. Cocaine's enhancement of transcription
23 initiation and elongation is further supported by its activation of cvclin-dependent kinase 7 (CDK7) 23 initiation and elongation is further supported by its activation of cyclin-dependent kinase 7 (CDK7)
24 and subsequent phosphorylation of CDK9, thereby promoting positive transcriptional elongation 24 and subsequent phosphorylation of CDK9, thereby promoting positive transcriptional elongation
25 factor b (P-TEFb) activity. We demonstrate for the first time that cocaine, through DNA-PK 25 factor b (P-TEFb) activity. We demonstrate for the first time that cocaine, through DNA-PK
26 activation, promotes the specific phosphorylation of TRIM28 at Serine 824 (p-TRIM28, S824). This 26 activation, promotes the specific phosphorylation of TRIM28 at Serine 824 (p-TRIM28, S824). This
27 modification converts TRIM28 from a transcriptional inhibitor to a transactivator for HIV 27 modification converts TRIM28 from a transcriptional inhibitor to a transactivator for HIV
28 transcription. Additionally, we observe-that-phosphorylation-of-TRIM28 (p-TRIM28, S824) promotes 28 transcription. Additionally, we observe that phosphorylation of TRIM28 (p-TRIM28, S824) promotes
29 the transition from the pausing phase to the elongation phase of HIV transcription. thereby 29 the transition from the pausing phase to the elongation phase of HIV transcription, thereby
30 facilitating the production of full-length HIV genomic transcripts. This finding corroborates the 30 facilitating the production of full-length HIV genomic transcripts. This finding corroborates the
31 observed enhanced RNAP II CTD phosphorylation at Ser2, a marker of transcriptional elongation, 31 observed enhanced RNAP II CTD phosphorylation at Ser2, a marker of transcriptional elongation,
32 following cocaine exposure. Accordingly, upon cocaine treatment, we observed elevated 32 following cocaine exposure. Accordingly, upon cocaine treatment, we observed elevated
33 recruitment of p-TRIM28-(S824) at the HIV LTR. Overall, our results have unraveled the intricate 33 recruitment of p-TRIM28-(S824) at the HIV LTR. Overall, our results have unraveled the intricate
34 molecular mechanisms underlving cocaine-induced HIV transcription and gene expression. These 34 molecular mechanisms underlying cocaine-induced HIV transcription and gene expression. These
35 findings hold promise for the development of highly targeted therapeutics aimed at mitigating the 35 findings hold promise for the development of highly targeted therapeutics aimed at mitigating the detrimental effects of cocaine in individuals living with HIV. 36 detrimental effects of cocaine in individuals living with HIV.
37

38 38 **Keywords**
39 Cocaine DNA

39 Cocaine, DNA-PK, HIV Transcription, HIV gene expression, replication, RNA polymerase, TRIM28,
40 RNAP II Pause release, Elongation. 40 RNAP II Pause release, Elongation.
41

41 42 **Subject Areas**
43 HIV, Transcription, c

43 HIV, Transcription, gene regulation, Virology, Immunology, epigenetics
44

⁴⁵**Highlights of the study**

-
- 47 a. Cocaine upregulates both the expression and activity of DNA-PK.
48 b. Cocaine augments the phosphorylation of DNA-PK selectively 48 b. Cocaine augments the phosphorylation of DNA-PK selectively at S2056, a post-translational modification that marks functionally active form of DNA-PK.
- 49 modification that marks functionally active form of DNA-PK.
50 c. Cocaine enhances the nuclear translocation of DNA-PK. 50 c. Cocaine enhances the nuclear translocation of DNA-PK.
51 d. The DNA-PK inhibition severely impairs HIV transcription
- 51 d. The DNA-PK inhibition severely impairs HIV transcription, replication, and latency reactivation.
52 e. Cocaine facilitates the initiation and elongation phases of HIV by enhancing RNAPII CT
- ⁵²e. Cocaine facilitates the initiation and elongation phases of HIV by enhancing RNAPII CTD
- 53 phosphorylation at Ser5 and Ser2, respectively, by stimulating DNA-PK.
54 f. Cocaine also supports initiation and elongation phases of HIV transcript 54 f. Cocaine also supports initiation and elongation phases of HIV transcription by stimulating CDK7
55 (the kinase of TFIIH) and CDK9 (the kinase subunit of P-TEFb), respectively. 55 (the kinase of TFIIH) and CDK9 (the kinase subunit of P-TEFb), respectively.
56 g. Cocaine-mediated activation of DNA-PK relieves RNAP II pausing by reve
- ⁵⁶g. Cocaine-mediated activation of DNA-PK relieves RNAP II pausing by reversing the inhibitory ⁵⁷effect of pausing factor TRIM28 and converting it into a transactivator by catalyzing its
- 58 bhosphorylation at S824 site.
59 bh. Thus, cocaine, by activatino 59 h. Thus, cocaine, by activating DNA-PK, facilitates the multiple phases of HIV transcription,
60 mamely, initiation, RNAP II pause-release, and elongation. 60 namely, initiation, RNAP II pause-release, and elongation.
61
- 61

⁶²**1. Introduction**

63 The onset of acquired immunodeficiency syndrome (AIDS), triggered by Human Immunodeficiency
64 Virus type 1 (HIV), is one of the most profoundly impactful diseases humanity has faced. Since the 64 Virus type 1 (HIV), is one of the most profoundly impactful diseases humanity has faced. Since the
65 identification of HIV in 1981, extensive endeavors have been undertaken to combat HIV infection. 65 identification of HIV in 1981, extensive endeavors have been undertaken to combat HIV infection.
66 These efforts have catalyzed significant progress in the realms of immunology and HIV virology, 66 These efforts have catalyzed significant progress in the realms of immunology and HIV virology,
67 marking notable advancements along the way [1-4]. However, HIV eradication or a preventive 67 marking notable advancements along the way [1-4]. However, HIV eradication or a preventive
68 vaccine is vet to be developed [5]. The current anti-HIV drug regimens (anti-retroviral therapy. ART) 68 vaccine is yet to be developed [5]. The current anti-HIV drug regimens (anti-retroviral therapy, ART)
69 have been highly successful in lowering HIV/AIDS-related mortality and improving the quality of life 69 have been highly successful in lowering HIV/AIDS-related mortality and improving the quality of life
70 for people living with HIV (PLWH) [1, 6]. As ART can effectively diminish the viral load to 70 for people living with HIV (PLWH) [1, 6]. As ART can effectively diminish the viral load to
71 undetectable levels through standard methodologies, the substantially decreased levels of HIV 71 undetectable levels through standard methodologies, the substantially decreased levels of HIV
72 while on ART facilitates the restoration and sustenance of a robust immune system. This restoration 72 while on ART facilitates the restoration and sustenance of a robust immune system. This restoration
73 enables the body to effectively defend against opportunistic infections and illnesses [7-9]. In 73 enables the body to effectively defend against opportunistic infections and illnesses [7-9]. In
74 addition. ART greatly reduces the risk of HIV transmission [7]. On other hand. dangerous behavior. 74 addition, ART greatly reduces the risk of HIV transmission [7]. On other hand, dangerous behavior,
75 such as unprotected sex and needle sharing by illicit drug users, significantly increases HIV 75 such as unprotected sex and needle sharing by illicit drug users, significantly increases HIV
76 transmission risk [10, 11]. Although there has been remarkable achievement in controlling HIV, the 76 transmission risk [10, 11]. Although there has been remarkable achievement in controlling HIV, the
77 prevalence of illicit drug usage remains a significant contributor to new HIV infection due to their 77 prevalence of illicit drug usage remains a significant contributor to new HIV infection due to their
78 perilous behavior [12-17]. Cocaine (Coc), a powerfully addictive stimulant drug has a high potential 78 Perilous behavior [12-17]. Cocaine (Coc), a powerfully addictive stimulant drug has a high potential
79 for abusing tendency [18-21]. Cocaine is primarily used orally, intranasal, intravenously, or by 79 for abusing tendency [18-21]. Cocaine is primarily used orally, intranasal, intravenously, or by
80 inhalation [22]. Continuous use of cocaine interferes with normal brain function; thus, it 80 inhalation [22]. Continuous use of cocaine interferes with normal brain function; thus, it
81 compromises judgment and decision-making capability, leading to risky behavior such as needle 81 compromises judgment and decision-making capability, leading to risky behavior such as needle
82 sharing and sexual behavior, including trading sex for drugs [23, 24]. Once infected, cocaine further 82 sharing and sexual behavior, including trading sex for drugs [23, 24]. Once infected, cocaine further 83 increases the severity of the HIV infection; stimulates HIV replication, including in the central 83 increases the severity of the HIV infection; stimulates HIV replication, including in the central 84 nervous system (CNS); and accelerates the occurrence of neurocognitive impairments [25-28]. 84 nervous system (CNS); and accelerates the occurrence of neurocognitive impairments [25-28].
85 Studies have also documented that cocaine use accelerates CD4+ T cell loss, even in ART-treated 85 Studies have also documented that cocaine use accelerates CD4+ T cell loss, even in ART-treated
86 individuals [29, 30]. However, the precise mechanisms by which cocaine and HIV synergize to 86 individuals [29, 30]. However, the precise mechanisms by which cocaine and HIV synergize to compromise the health of individuals living with HIV (PLWH) remain unclear. compromise the health of individuals living with HIV (PLWH) remain unclear.

88 Similar to host cell gene transcription, RNA polymerase II (RNAP II) is required for HIV
89 transcription. RNAP II is regulated by specific phosphorylation events in the carboxyl-terminal 89 transcription. RNAP II is regulated by specific phosphorylation events in the carboxyl-terminal
80 domain (CTD) of RNAP II large subunit [31]. The human RNAP II CTD consists of 52 tandem 90 domain (CTD) of RNAP II large subunit [31]. The human RNAP II CTD consists of 52 tandem
91 repeats of a consensus sequence Tvr1-Ser2-Pro3-Thr4-Ser5-Pro6-Ser7 [32-35]. Many known repeats of a consensus sequence Tyr1-Ser2-Pro3-Thr4-Ser5-Pro6-Ser7 [32-35]. Many known

92 kinases can phosphorylate RNAP II CTD. However, most notable kinases that phosphorylates
93 RNAP II are cyclin-dependent kinase 7 (CDK7) that phosphorylate RNAP II at Ser5 and CDK9 that 93 NRNAP II are cyclin-dependent kinase 7 (CDK7) that phosphorylate RNAP II at Ser5 and CDK9 that 94 phosphorylate RNAP II at Ser2 [31, 34, 36]. Our previous studies documented that DNA-PK can 94 phosphorylate RNAP II at Ser2 [31, 34, 36]. Our previous studies documented that DNA-PK can
95 phosphorylate RNAP II CTD in all three serine residues (Ser2, Ser5, and Ser7) [37]. We have also 95 phosphorylate RNAP II CTD in all three serine residues (Ser2, Ser5, and Ser7) [37]. We have also
96 shown that transactivator of transcription (Tat) protein, which is vital for HIV transcription, is a 96 shown that transactivator of transcription (Tat) protein, which is vital for HIV transcription, is a
97 potential substrate of DNA-PK [37]. Data generated from our previous study also suggested that 97 potential substrate of DNA-PK [37]. Data generated from our previous study also suggested that
98 cellular activation augments both the nuclear translocation and HIV LTR recruitment of DNA-PK 98 cellular activation augments both the nuclear translocation and HIV LTR recruitment of DNA-PK
99 [37-39]. $[37-39]$.

100 DNA-PK, a protein kinase, requires association with DNA to become catalytically active. DNA-PK
101 holoenzyme consisting of two components: a 450 kDa catalytic subunit (DNA-PKcs), which is a 101 holoenzyme consisting of two components: a 450 kDa catalytic subunit (DNA-PKcs), which is a
102 serine/threonine kinase, and a regulatory component known as Ku, a heterodimer of Ku70 and 102 serine/threonine kinase, and a regulatory component known as Ku, a heterodimer of Ku70 and
103 Ku80 [40-42]. DNA-PK is well studied for its role in repairing DNA damage and maintaining the 103 Ku80 [40-42]. DNA-PK is well studied for its role in repairing DNA damage and maintaining the
104 stability of the genome, including during V(D)J recombination [43-45]. DNA-PK especially plays a 104 stability of the genome, including during V(D)J recombination [43-45]. DNA-PK especially plays a
105 crucial role in the non-homologous end joining (NHEJ) DNA repair pathway [46]. While multiple 105 crucial role in the non-homologous end joining (NHEJ) DNA repair pathway [46]. While multiple
106 recent studies, including our own, have suggested a potential involvement of DNA-PK in 106 recent studies, including our own, have suggested a potential involvement of DNA-PK in
107 transcriptional regulation [37, 47], the precise role of DNA-PK in the transcription process was 107 transcriptional regulation [37, 47], the precise role of DNA-PK in the transcription process was
108 delineated by our research [37]. It has been documented that DNA-PK interacts with various 108 delineated by our research [37]. It has been documented that DNA-PK interacts with various
109 transcription factors and components of the transcription machinery [47]. Notably, DNA-PK not only 109 transcription factors and components of the transcription machinery [47]. Notably, DNA-PK not only
110 engages with numerous transcription factors, such as TFIIH, P-TEFb, p53, NF-kB, and SP1, but 110 engages with numerous transcription factors, such as TFIIH, P-TEFb, p53, NF-κB, and SP1, but
111 also modulates their activity through phosphorylation. These interactions typically amplify the 111 also modulates their activity through phosphorylation. These interactions typically amplify the 112 expression of genes regulated by these transcription factors. expression of genes regulated by these transcription factors.

113 During HIV transcription, phosphorylation of RNAP II CTD at the position Ser5 is associated with
114 the early stages of transcription, particularly transcription initiation. This modification recruits 114 the early stages of transcription, particularly transcription initiation. This modification recruits
115 capping enzyme complexes that add a 7-methylguanosine cap to the nascent RNA molecule, which 115 capping enzyme complexes that add a 7-methylguanosine cap to the nascent RNA molecule, which
116 protects RNA from degradation and later facilitates its processing. However, phosphorylation of 116 protects RNA from degradation and later facilitates its processing. However, phosphorylation of 117 RNAP II CTD at Ser2 is linked to the elongation phase of transcription, as this post-translational 117 RNAP II CTD at Ser2 is linked to the elongation phase of transcription, as this post-translational
118 modification of RNAP II makes it processive or elongation-proficient, as it reduces the slipping of 118 modification of RNAP II makes it processive or elongation-proficient, as it reduces the slipping of 119 RNAP II from DNA template. This modification also facilitates the recruitment of transcription factors 119 RNAP II from DNA template. This modification also facilitates the recruitment of transcription factors
120 involved in mRNA maturation and processing, including splicing and polyadenylation. For efficient 120 involved in mRNA maturation and processing, including splicing and polyadenylation. For efficient
121 transcription elongation, not only processive RNAP II is required, but also the removal of negative 121 transcription elongation, not only processive RNAP II is required, but also the removal of negative
122 transcription factors (NFs) that promote promoter-proximal pausing of RNAP II is essential [48-52]. 122 transcription factors (NFs) that promote promoter-proximal pausing of RNAP II is essential [48-52].
123 Analogous to cellular gene expression, HIV Transcriptional initiation also halts after generating 123 Analogous to cellular gene expression, HIV Transcriptional initiation also halts after generating
124 short nascent mRNA of around 60 nucleotides due to the binding of negative transcription factors 124 short nascent mRNA of around 60 nucleotides due to the binding of negative transcription factors
125 (NFs) at the HIV LTR [53-55]. Some notable NFs are the negative elongation factor (NELF) and the 125 (NFs) at the HIV LTR [53-55]. Some notable NFs are the negative elongation factor (NELF) and the 126 57.
126 5.6-dicholoro-1-β-d-ribofuranoxylbenzimidazole (DRB) sensitivity-inducing factor (DSIF) [56, 57]. 126 5,6-dicholoro-1-β-d-ribofuranoxylbenzimidazole (DRB) sensitivity-inducing factor (DSIF) [56, 57].
127 Recently, in addition to DSIF and NELF, another inhibitory factor is the tripartite motif-containing 28 127 Recently, in addition to DSIF and NELF, another inhibitory factor is the tripartite motif-containing 28
128 (known as TRIM28, KAP1, TIF1β), which has been shown to promote promoter-proximal pausing at 128 (known as TRIM28, KAP1, TIF1β), which has been shown to promote promoter-proximal pausing at
129 cellular gene promoters [39, 58-60]. TRIM28 was initially identified as a transcriptional corepressor 129 cellular gene promoters [39, 58-60]. TRIM28 was initially identified as a transcriptional corepressor
130 due to its interaction with members of the Kruppel transcription factor family (KRAB) and its 130 due to its interaction with members of the Kruppel transcription factor family (KRAB) and its
131 potential direct binding to specific DNA sequences [58, 59]. These transcription factors often 131 potential direct binding to specific DNA sequences [58, 59]. These transcription factors often
132 function as transcriptional repressors. When TRIM28 binds to KRAB-containing transcription 132 function as transcriptional repressors. When TRIM28 binds to KRAB-containing transcription
133 factors, it facilitates the recruitment of co-repressors, histone deacetylases (HDACs), and chromatin 133 factors, it facilitates the recruitment of co-repressors, histone deacetylases (HDACs), and chromatin
134 remodeling complexes. This results in the compaction of chromatin structure and inhibition of gene 134 remodeling complexes. This results in the compaction of chromatin structure and inhibition of gene
135 transcription. In many inactivated genes, TRIM28 stabilizes the pausing of RNAP II near the 135 transcription. In many inactivated genes, TRIM28 stabilizes the pausing of RNAP II near the
136 transcriptional start site (TSS), which promotes promoter-proximal pausing and accumulation of transcriptional start site (TSS), which promotes promoter-proximal pausing and accumulation of

137 RNAP II near gene promoter [58]. The modulation of RNAP II pausing depends on phosphorylation
138 of TRIM28 at the specific site, Ser824. Similar to the SPT5 subunit of DSIF, the phosphorylation of 138 of TRIM28 at the specific site, Ser824. Similar to the SPT5 subunit of DSIF, the phosphorylation of 139 TRIM28 is crucial in converting it from a pausing or negative elongation factor to a positive 139 TRIM28 is crucial in converting it from a pausing or negative elongation factor to a positive
140 elongation factor [39, 58, 59, 61]. DNA-PK is the principal kinase that directly interacts with TRIM28 140 elongation factor [39, 58, 59, 61]. DNA-PK is the principal kinase that directly interacts with TRIM28
141 and catalyzes the phosphorylation of TRIM28 at serine 824 residue (p-TRIM28, S824), converting it 141 and catalyzes the phosphorylation of TRIM28 at serine 824 residue (p-TRIM28, S824), converting it
142 to an elongation factor [39, 60]. However, pertaining to HIV transcription, the role of TRIM28 is still 142 to an elongation factor [39, 60]. However, pertaining to HIV transcription, the role of TRIM28 is still 143 not clear. Nevertheless. TRIM28 is known to play a complex role in the control of HIV and other 143 not clear. Nevertheless, TRIM28 is known to play a complex role in the control of HIV and other
144 DNA/RNA viruses, influencing both positive and negative regulatory pathways. Specifically 144 DNA/RNA viruses, influencing both positive and negative regulatory pathways. Specifically
145 concerning HIV-1, TRIM28 is implicated in the regulation of viral latency and reactivation. However, 145 concerning HIV-1, TRIM28 is implicated in the regulation of viral latency and reactivation. However, 146 further investigation is required to delineate its direct or indirect impact on HIV proviral gene 146 further investigation is required to delineate its direct or indirect impact on HIV proviral gene
147 expression. Initially, TRIM28 was identified as a restrictor of HIV through its interaction with 147 expression. Initially, TRIM28 was identified as a restrictor of HIV through its interaction with
148 Integrase, hindering viral integration into the host chromatin [62]. This discovery suggests that 148 Integrase, hindering viral integration into the host chromatin [62]. This discovery suggests that 149 TRIM28 may functionally link integration and transcription processes. Subsequently, Randolph et al. 149 TRIM28 may functionally link integration and transcription processes. Subsequently, Randolph et al.
150 [63] proposed a paradigm wherein TRIM28 governs a switch from repression to activation. Viruses 150 [63] proposed a paradigm wherein TRIM28 governs a switch from repression to activation. Viruses
151 could exploit a transcriptional repressor like TRIM28 for their activation by promoting site-specific 151 could exploit a transcriptional repressor like TRIM28 for their activation by promoting site-specific
152 phosphorylation (pS473 and/or pS824), thereby enhancing viral gene expression for infection and 152 phosphorylation (pS473 and/or pS824), thereby enhancing viral gene expression for infection and
153 modulating immune gene expression for precise cell fate responses. Reports also suggested that 153 modulating immune gene expression for precise cell fate responses. Reports also suggested that
154 TRIM28 also contribute to HIV-1 transcriptional inhibition by depleting Tat in myeloid lineage with 154 TRIM28 also contribute to HIV-1 transcriptional inhibition by depleting Tat in myeloid lineage with 155 the help of CTIP2 [64]. Consequently, targeting TRIM28 presents a promising therapeutic avenue 155 the help of CTIP2 [64]. Consequently, targeting TRIM28 presents a promising therapeutic avenue
156 during viral infection or latency by addressing upstream TRIM28 regulators, modulating TRIM28 156 during viral infection or latency by addressing upstream TRIM28 regulators, modulating TRIM28
157 enzymatic activities, and disrupting TRIM28 protein-protein interactions [63]. 157 enzymatic activities, and disrupting TRIM28 protein-protein interactions [63].
158

158 159 The elongation phase of HIV transcription is greatly enhanced by the Tat protein of HIV, as Tat
160 enhances the recruitment of host cell elongation factor positive transcriptional elongation factor b 160 enhances the recruitment of host cell elongation factor positive transcriptional elongation factor b
161 (P-TEFb) to the HIV LTR. Subsequently, the CDK9 subunit of P-TEFb catalyze the hyper 161 (P-TEFb) to the HIV LTR. Subsequently, the CDK9 subunit of P-TEFb catalyze the hyper
162 phosphorylation of RNAP II CTD at Ser2 and make RNAP II processive [49, 65]. In addition, CDK9 162 phosphorylation of RNAP II CTD at Ser2 and make RNAP II processive [49, 65]. In addition, CDK9
163 also catalyze the phosphorylation of negative factors, namely DSIF and NELF, and relieve their 163 also catalyze the phosphorylation of negative factors, namely DSIF and NELF, and relieve their
164 negative impact on HIV transcription [66, 67]. Our previous studies have shown that the lack of P-164 negative impact on HIV transcription [66, 67]. Our previous studies have shown that the lack of P-
165 TEFb in quiescent primary T cells is responsible for HIV latency, even in the presence of adequate 165 TEFb in quiescent primary T cells is responsible for HIV latency, even in the presence of adequate
166 NF-kB activation [68]. P-TEFb complex consists of other subunits, mainly ELL2, ENL, AFF4, AF9; 166 NF-kB activation [68]. P-TEFb complex consists of other subunits, mainly ELL2, ENL, AFF4, AF9;
167 together, it is called super elongation complex (SEC) [69-71]. Thus, the HIV Tat protein plays a 167 together, it is called super elongation complex (SEC) [69-71]. Thus, the HIV Tat protein plays a
168 significant role in augmenting the elongation phase of HIV transcription and generating full-length 168 significant role in augmenting the elongation phase of HIV transcription and generating full-length
169 genomic transcripts of HIV [1, 72-74]. In the absence of Tat, the elongation or completion of HIV 169 genomic transcripts of HIV [1, 72-74]. In the absence of Tat, the elongation or completion of HIV
170 transcripts is inefficient. Once HIV Tat is available, it positively regulates HIV transcription. Tat 170 transcripts is inefficient. Once HIV Tat is available, it positively regulates HIV transcription. Tat 171 binding to trans-activation response (TAR) element, an RNA stem loop structure of HIV transcript, 171 binding to trans-activation response (TAR) element, an RNA stem loop structure of HIV transcript,
172 brings an essential transcriptional component, positive transcription elongation factor b (P-TEFb), 172 brings an essential transcriptional component, positive transcription elongation factor b (P-TEFb), 173 thereby enhancing the efficiency of viral transcription. HIV transcription auto accelerates its 173 thereby enhancing the efficiency of viral transcription. HIV transcription auto accelerates its
174 transcription by generating more Tat protein [75, 76]. Thus, the enhanced rate of HIV transcriptional 174 transcription by generating more Tat protein [75, 76]. Thus, the enhanced rate of HIV transcriptional 175 elongation results in a higher number of complete genomic HIV transcriptional 175 175 elongation results in a higher number of complete genomic HIV transcripts and generation of more 176 viral 176 viral particles.
177

178 178 In our previous publication, we clarified the important role of DNA-PK during HIV transcription and
179 documented the continuous presence and gliding of DNA-PK with RNAP II along the HIV genome 179 documented the continuous presence and gliding of DNA-PK with RNAP II along the HIV genome
180 during transcription [37, 39]. Additionally, we identified the impact of cocaine use on promoting HIV 180 during transcription [37, 39]. Additionally, we identified the impact of cocaine use on promoting HIV
181 transcription and replication [16, 17, 27, 28]. Later, we endeavored to define the underlying transcription and replication [16, 17, 27, 28]. Later, we endeavored to define the underlying

182 molecular mechanism through which cocaine augments HIV transcription and found that cocaine
183 promoted HIV transcription by inducing different mechanisms [27, 28]. To expand upon this 183 promoted HIV transcription by inducing different mechanisms [27, 28]. To expand upon this 184 subject, in the present study, we focus on understanding the role of cocaine-stimulated DNA-PK in 184 subject, in the present study, we focus on understanding the role of cocaine-stimulated DNA-PK in
185 relieving RNAP II pausing during HIV transcription by catalyzing TRIM28 phosphorylation 185 relieving RNAP II pausing during HIV transcription by catalyzing TRIM28 phosphorylation
186 selectively at S824 residue. We found that cocaine further enhanced the nuclear localization of 186 selectively at S824 residue. We found that cocaine further enhanced the nuclear localization of 187 DNA-PK, where DNA-PK facilitates HIV transcription. We noted that cocaine exposure not only 187 DNA-PK, where DNA-PK facilitates HIV transcription. We noted that cocaine exposure not only
188 augmented the nuclear translocation but also enhanced its functional activity by increasing its 188 augmented the nuclear translocation but also enhanced its functional activity by increasing its
189 phosphorylation at specific residue, Ser2056. Subsequently, we substantiated increased HIV 189 phosphorylation at specific residue, Ser2056. Subsequently, we substantiated increased HIV
190 transcription following cocaine exposure by examining the effect of cocaine-induced DNA-PK on 190 transcription following cocaine exposure by examining the effect of cocaine-induced DNA-PK on
191 the phosphorylation of specific sites on RNAP II CTD, namely Ser2 and Ser5. To further 191 the phosphorylation of specific sites on RNAP II CTD, namely Ser2 and Ser5. To further
192 authenticate the precise role of cocaine-induced DNA-PK in CTD phosphorylation, we investigated 192 authenticate the precise role of cocaine-induced DNA-PK in CTD phosphorylation, we investigated
193 the inhibitory potential of clinically evaluated DNA-PK inhibitors in reversing the influence of DNA-193 the inhibitory potential of clinically evaluated DNA-PK inhibitors in reversing the influence of DNA-
194 PK. These findings were further validated by conducting DNA-PK knockdown experiments in the 194 PK. These findings were further validated by conducting DNA-PK knockdown experiments in the
195 presence or absence of cocaine, demonstrating the specific impact of cocaine-induced DNA-PK 195 presence or absence of cocaine, demonstrating the specific impact of cocaine-induced DNA-PK
196 stimulation. Overall, our data demonstrate the crucial role of cocaine-mediated DNA-PK stimulation 196 stimulation. Overall, our data demonstrate the crucial role of cocaine-mediated DNA-PK stimulation
197 in relieving RNAP II pausing by converting TRIM28 from a transcriptional inhibitor to transcriptional 197 in relieving RNAP II pausing by converting TRIM28 from a transcriptional inhibitor to transcriptional
198 activator protein. These findings are validated across diverse cell types belonging to both lymphoid 198 activator protein. These findings are validated across diverse cell types belonging to both lymphoid
199 and myeloid lineages, including microglia, the macrophages that reside in the CNS. This 199 and myeloid lineages, including microglia, the macrophages that reside in the CNS. This
100 comprehensive study expands our understanding of the complex interplay among cocaine, DNA-200 comprehensive study expands our understanding of the complex interplay among cocaine, DNA-
201 PK, and TRIM28 and their influence on HIV transcription. Consequently, it illuminates potential 201 PK, and TRIM28 and their influence on HIV transcription. Consequently, it illuminates potential
202 therapeutic strategies for addressing HIV replication and/or mitigating the toxicities associated with 202 therapeutic strategies for addressing HIV replication and/or mitigating the toxicities associated with
203 drug abuse. Additionally, given that ART is unable to restrict HIV transcription or latency-203 drug abuse. Additionally, given that ART is unable to restrict HIV transcription or latency-
204 reactivation, defining all factors and mechanisms that regulate HIV transcription will help open new 204 reactivation, defining all factors and mechanisms that regulate HIV transcription will help open new and a
205 avenues for better translational interventions. avenues for better translational interventions.

²⁰⁷**2. Materials and Methods**

²⁰⁸*2.1. Plasmid construction, gene transfer, transfection, and VSV-G pseudotyped virus* ²⁰⁹*generation*

210 The pHR'p-Luc plasmid was constructed by inserting the EcoRI and XhoI fragment of HIV pNL4-3
211 into the pHR' plasmid, as detailed previously [77, 78]. The procedure to knockdown the DNA-PK 211 into the pHR' plasmid, as detailed previously [77, 78]. The procedure to knockdown the DNA-PK
212 was also described previously [39]. The short-lived variant of green fluorescent protein (d2EGFP) 212 was also described previously [39]. The short-lived variant of green fluorescent protein (d2EGFP)
213 was inserted at the nef position using the Mlul and Xhol sites. Site-directed mutagenesis was 213 was inserted at the nef position using the MluI and XhoI sites. Site-directed mutagenesis was
214 conducted to substitute histidine at position 13 with leucine (H13L) (CAT to TTA), following 214 conducted to substitute histidine at position 13 with leucine (H13L) (CAT to TTA), following
215 established procedures [79, 80]. Human Embryonic Kidney 293 cells (HEK 293T) were cultured in 215 established procedures [79, 80]. Human Embryonic Kidney 293 cells (HEK 293T) were cultured in
216 Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2.05 ml-glutamine (Hyclone, 216 Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2.05 ml-glutamine (Hyclone, 217 ThermoScientific), 10% fetal bovine serum (Gemini), and 1 U/mL penicillin/streptomycin. Cells were 217 ThermoScientific), 10% fetal bovine serum (Gemini), and 1 U/mL penicillin/streptomycin. Cells were
218 seeded, grown to 70% confluency, and rinsed with Opti-MEM I (1X) + GlutaMAX-I Reduced Serum 218 seeded, grown to 70% confluency, and rinsed with Opti-MEM I (1X) + GlutaMAX-I Reduced Serum
219 Medium (Gibco) before transfection. Transfection was done by using Lipofectamine 2000 219 Medium (Gibco) before transfection. Transfection was done by using Lipofectamine 2000
220 (Invitrogen) as per the manufacturer's instructions. Briefly, 35 µL of Lipofectamine 2000 reagent 220 (Invitrogen) as per the manufacturer's instructions. Briefly, 35 μ L of Lipofectamine 2000 reagent 221 was mixed with 500 μ L Opti-MEM. Separately, 18 μ g of plasmid DNA mixture (3 μ g pCMV Δ 8.9.1, 4 221 was mixed with 500 µL Opti-MEM. Separately, 18 µg of plasmid DNA mixture (3 µg pCMVΔ8.9.1, 4
222 µg pMD.G, 3 µg pMDL-q/p-RRE, 1 µg pRSV-Rev, and 7 µg of either pHR'P-Luc or pNL4-3-ΔE-222 µg pMD.G, 3 µg pMDL-g/p-RRE, 1 µg pRSV-Rev, and 7 µg of either pHR'P-Luc or pNL4-3-ΔE-
223 EGFP for generating pNL4-3-ΔE-EGFP and pHR'p-P-Luc pseudotyped viruses, respectively) was ²²³EGFP for generating pNL4-3-ΔE-EGFP and pHR'p-P-Luc pseudotyped viruses, respectively) was 224 prepared [28]. The two solutions were combined and incubated at room temperature (RT) for 30
225 minutes (min) to form the lipid-DNA complex, which was then introduced into the cells. Five hours minutes (min) to form the lipid-DNA complex, which was then introduced into the cells. Five hours

226 after transfection, the culture medium was replaced with fresh DMEM. The cell supernatant 227 containing the virus was collected at 48 hours (h) and 72 h post-transfection. 227 containing the virus was collected at 48 hours (h) and 72 h post-transfection.
228

²²⁹*2.2. Generation of Luciferase cell line and latently infected Jurkat T-cell clones*

231 231 The pHR'p-Luc virus was transduced into the Jurkat cell line via spinoculation in the presence of 8
232 yg/ml polybrene. Successful infection was subsequently confirmed by Luciferase assay [78]. The 232 µg/ml polybrene. Successful infection was subsequently confirmed by Luciferase assay [78]. The 233 isolation of Clone 2D10 cells, characterized by the H13L Tat mutation, was detailed in our previous 233 isolation of Clone 2D10 cells, characterized by the H13L Tat mutation, was detailed in our previous
234 study [80]. Specifically, Vesicular Stomatitis Virus Protein G (VSV-G)-pseudotyped HIV particles 234 study [80]. Specifically, Vesicular Stomatitis Virus Protein G (VSV-G)-pseudotyped HIV particles
235 were generated through triple transfection of 293T cells using Lipofectamine 2000 reagent 235 were generated through triple transfection of 293T cells using Lipofectamine 2000 reagent
236 (Invitrogen, Waltham, MA, USA). Virus titers were determined by infecting 2x10⁶ Jurkat T-cells with 236 (Invitrogen, Waltham, MA, USA). Virus titers were determined by infecting 2x10⁶ Jurkat T-cells with 237 serial dilutions of concentrated virus preparation obtained from harvested medium supernatant. Six 237 serial dilutions of concentrated virus preparation obtained from harvested medium supernatant. Six
238 hours post-infection, cells were rinsed with phosphate-buffered saline (PBS), and RPMI 1640 238 hours post-infection, cells were rinsed with phosphate-buffered saline (PBS), and RPMI 1640
239 medium was replenished. Expression of d2EGFP was assessed by fluorescently activated cell 239 medium was replenished. Expression of d2EGFP was assessed by fluorescently activated cell
240 sorting analysis (FACS Calibur) 72 h post-infection, and d2EGFP expression was subsequently 240 sorting analysis (FACS Calibur) 72 h post-infection, and d2EGFP expression was subsequently
241 analyzed every week until cells were fully shut down without detectable d2EGFP expression before 241 analyzed every week until cells were fully shut down without detectable d2EGFP expression before
242 reactivation experiments. reactivation experiments.

243

²⁴⁴*2.3. Cell culture and cell experiments*

245 246 Microglial, THP-1, MT-4, peripheral blood mononuclear cells (PBMC), Jurkat, and derivatives of 247 Jurkat cells (Clone 2D10 and Jurkat-pHR'P-Luc) were cultured in either DMEM or RPMI 1640 247 Jurkat cells (Clone 2D10 and Jurkat-pHR'P-Luc) were cultured in either DMEM or RPMI 1640
248 medium. The culture medium was supplemented with 10% fetal bovine serum (FBS), penicillin (100 248 medium. The culture medium was supplemented with 10% fetal bovine serum (FBS), penicillin (100 249 IU/ml), streptomycin (100 IU/ml), and 25 mM HEPES. Cells were maintained at 37°C in a 5% $CO₂$ 249 IU/ml), streptomycin (100 IU/ml), and 25 mM HEPES. Cells were maintained at 37°C in a 5% CO₂
250 environment. Fresh medium was replenished every 2-3 days, and cell density was kept at 2x10⁶ 250 environment. Fresh medium was replenished every 2-3 days, and cell density was kept at 2x10⁶
251 cells/ml. cells/ml.

252

²⁵³*2.4. HIV replication-competent Virus*

255 255 The Human Immunodeficiency Virus Type 1 (strain 93/TH/051) was obtained from the National
256 Institute of Health AIDS reagent program. Primary HIV isolates were cultured following the 256 Institute of Health AIDS reagent program. Primary HIV isolates were cultured following the
257 instructions provided in the datasheet obtained through the UNAIDS Network for HIV Isolation and 257 instructions provided in the datasheet obtained through the UNAIDS Network for HIV Isolation and
258 Characterization. Briefly, 4 X 10⁶ stimulated Jurkat cells (cells previously stimulated with PHA for 4 258 Characterization. Briefly, 4 X 10⁶ stimulated Jurkat cells (cells previously stimulated with PHA for 4
259 days and treated with polybrene) were collected and exposed to HIV (strain 93/TH/051) for 30 min 259 days and treated with polybrene) were collected and exposed to HIV (strain 93/TH/051) for 30 min
260 at 37°C. Following this, fresh media was added, and the cells were incubated for 5 days. Cell free 260 at 37°C. Following this, fresh media was added, and the cells were incubated for 5 days. Cell free 261 units vas recovered, aliquoted in multiple stock, and stored at -80°C till use. 261 virus was recovered, aliquoted in multiple stock, and stored at -80°C till use.
262

262 ²⁶³*2.5. Cocaine treatment and Inhibitor treatment*

265 265 Cocaine hydrochloride was obtained from the National Institute on Drug Abuse (NIDA) Drug Supply
266 Program. In this study, various cocaine concentrations were employed. Nonetheless, the maximum 266 Program. In this study, various cocaine concentrations were employed. Nonetheless, the maximum
267 concentration utilized was 30 µM cocaine, which falls below the levels typically observed in the 267 concentration utilized was 30 μ M cocaine, which falls below the levels typically observed in the 268 plasma of human drug users. All cocaine treatments were conducted at a concentration of 10 μ M 268 plasma of human drug users. All cocaine treatments were conducted at a concentration of 10 μM
269 unless otherwise specified. Acute treatment involved exposing the cells to cocaine for 3 h, whereas unless otherwise specified. Acute treatment involved exposing the cells to cocaine for 3 h, whereas

270 chronic treatment entailed exposing the cells to cocaine twice daily for two consecutive days, with 271 an additional 3h exposure prior to cell harvesting. Inhibitors (M3814 and NU7441) were treated for 271 an additional 3h exposure prior to cell harvesting. Inhibitors (M3814 and NU7441) were treated for 222
272 overnight (24 h) prior exposing to cocaine. 272 overnight (24 h) prior exposing to cocaine.
273

273 ²⁷⁴*2.6. Infection of cells with replication-competent virus*

275 276 Cells (5x10⁶ cells) were either untreated or exposed to cocaine for 3 h in the presence or absence
277 of M3814 and, subsequently, were either uninfected or infected with replication-competent virus (1 277 of M3814 and, subsequently, were either uninfected or infected with replication-competent virus (1
278 mL) for 24 h and 48 h to assess HIV gene expression. Inhibitors were administered 24 h before HIV 278 mL) for 24 h and 48 h to assess HIV gene expression. Inhibitors were administered 24 h before HIV
279 infection, with the specific doses mentioned in the figure legends. 279 infection, with the specific doses mentioned in the figure legends.
280

281 ²⁸¹*2.7. Western blot analysis of total cell lysate*

282 283 Cells $(1x10^6 \text{ or } 5x10^6 \text{ cells approx.})$ were treated with cocaine in the presence or absence of M3814 284 (DNA-PK inhibitor) and/or infected with a replication-competent virus for 24 h and 48 h.
285 Subsequently, samples were collected and washed with 1 mL of ice-cold PBS, and 100 µL of 1X 285 Subsequently, samples were collected and washed with 1 mL of ice-cold PBS, and 100 µL of 1X
286 passive lysis buffer (Promega, Madison, WI, USA) was added to the cells. The cell lysate with the 286 passive lysis buffer (Promega, Madison, WI, USA) was added to the cells. The cell lysate with the
287 lysis buffer was then incubated on ice for 30 min. During the incubation, cells were vortexed for 30 287 lysis buffer was then incubated on ice for 30 min. During the incubation, cells were vortexed for 30
288 seconds (sec) for complete lysis after every 10 min. Following incubation, the cell lysate was 288 seconds (sec) for complete lysis after every 10 min. Following incubation, the cell lysate was
289 centrifuged at the highest speed for 30 min, and the supernatant was analyzed for protein 289 centrifuged at the highest speed for 30 min, and the supernatant was analyzed for protein
290 concentration using the Pierce™ BCA Protein Assay Kit. Protein concentration was normalized, and 290 concentration using the Pierce™ BCA Protein Assay Kit. Protein concentration was normalized, and
291 an equal amount of protein was mixed with 5X Laemmle Sample buffer, heated to 95°C for 10 min, 291 an equal amount of protein was mixed with 5X Laemmle Sample buffer, heated to 95°C for 10 min,
292 and then resolved by SDS-PAGE on a 6% or 12% gel at 120 volts until the dye reached the bottom. 292 and then resolved by SDS-PAGE on a 6% or 12% gel at 120 volts until the dye reached the bottom.
293 The resolved proteins were then transferred to a nitrocellulose membrane. The membranes were 293 The resolved proteins were then transferred to a nitrocellulose membrane. The membranes were 294 blocked with 3% Bovine serum albumin (BSA) for 1 h and incubated with primary antibodies at 4 °C 294 blocked with 3% Bovine serum albumin (BSA) for 1 h and incubated with primary antibodies at 4°C
295 overnight and then with secondary antibody (1:15000 dilution) for 1 h at room temperature. After 295 overnight and then with secondary antibody (1:15000 dilution) for 1 h at room temperature. After
296 three washes with 1X TBST, the blot was detected using the Odyssey infrared imaging system 296 three washes with 1X TBST, the blot was detected using the Odyssey infrared imaging system
297 application software version 3.0 (Li-cor Bioscience). 297 application software version 3.0 (Li-cor Bioscience).
298

²⁹⁹*2.8. Western blot analysis of cytoplasmic and nuclear extracts*

300
301 301 Cells $(5x10^6$ or $1X 10^7$ cells approx.) were exposed to cocaine at various doses and time points, 302 with or without the inhibitor. Subsequently, cells were collected and washed with 1 ml of ice-cold
303 PBS. Following our established protocol, we fractionated cytosolic and nuclear proteins. Initially, 303 PBS. Following our established protocol, we fractionated cytosolic and nuclear proteins. Initially, 304 cells were allowed to swell in 200 µl - 500 µl of cytoplasmic extract (CE) buffer (1 mM Hepes KOH 304 cells were allowed to swell in 200 µl - 500 µl of cytoplasmic extract (CE) buffer (1 mM Hepes KOH
305 pH 7.9, 60 mM KCl, 1 mM EDTA, 0.5% NP-40, 1 mM DTT, and 1 mM PMSF) for 10 min on ice, 305 pH 7.9, 60 mM KCl, 1 mM EDTA, 0.5% NP-40, 1 mM DTT, and 1 mM PMSF) for 10 min on ice,
306 during which cells were vortexed for lysis. Nuclei were then pelleted at 4000 r.p.m for 10 min. The 306 during which cells were vortexed for lysis. Nuclei were then pelleted at 4000 r.p.m for 10 min. The 307 cytoplasmic lysates were transferred to new Eppendorf tubes for analysis of cytoplasmic proteins. 307 cytoplasmic lysates were transferred to new Eppendorf tubes for analysis of cytoplasmic proteins.
308 The nuclei were washed twice with 1 ml of CE buffer, pelleted at high-speed centrifugation for 2 308 The nuclei were washed twice with 1 ml of CE buffer, pelleted at high-speed centrifugation for 2
309 min, and subsequently resuspended in 80 µl of nuclear extract (NE) buffer (250 mM Tris pH 7.8, 60 309 min, and subsequently resuspended in 80 µl of nuclear extract (NE) buffer (250 mM Tris pH 7.8, 60
310 mM HCl, 1 mM EDTA, 1 mM DTT, and 1 mM PMSF). The nuclei were lysed by 8 freeze-thaw 310 mM HCl, 1 mM EDTA, 1 mM DTT, and 1 mM PMSF). The nuclei were lysed by 8 freeze-thaw
311 cycles in liquid nitrogen. The nuclear lysate was cleared by centrifugation at high speed for 1 min, 311 cycles in liquid nitrogen. The nuclear lysate was cleared by centrifugation at high speed for 1 min,
312 and the supernatant was transferred into a new microfuge tube. Total nuclear protein concentration 312 and the supernatant was transferred into a new microfuge tube. Total nuclear protein concentration
313 in the samples was normalized using a standard BCA assay. An equal amount of total nuclear in the samples was normalized using a standard BCA assay. An equal amount of total nuclear

314 samples was loaded and resolved by 6% or 10% or 12% SDS-PAGE gel for electrophoresis. The
315 proteins on the gels were transferred onto nitrocellulose membranes; blocked with 3% BSA for an 315 proteins on the gels were transferred onto nitrocellulose membranes; blocked with 3% BSA for an
316 hour; incubated with primary antibodies overnight and with secondary antibodies for an hour; and 316 hour; incubated with primary antibodies overnight and with secondary antibodies for an hour; and
317 finally detected using the Odyssey infrared imaging system application software version 3.0 (Li-cor 317 finally detected using the Odyssey infrared imaging system application software version 3.0 (Li-cor
318 Bioscience). 318 Bioscience).
319

³²⁰*2.9. Chromatin Immunoprecipitation (ChIP) assay*

322 322 The ChIP assays were performed using our well-established protocol [81]. Briefly 1 x 10⁸ cells
323 underwent fixation in 0.5% formaldehyde for 10 min with rotation at room temperature, facilitating 323 underwent fixation in 0.5% formaldehyde for 10 min with rotation at room temperature, facilitating
324 the crosslinking of proteins to DNA. Subsequently, glycine was added to reverse the crosslinking 324 the crosslinking of proteins to DNA. Subsequently, glycine was added to reverse the crosslinking
325 process. Cells were harvested, washed twice with ice-cold PBS, and allowed to swell for 10 min in 5 325 process. Cells were harvested, washed twice with ice-cold PBS, and allowed to swell for 10 min in 5
326 ml CE Buffer. Nuclei were pelleted after centrifugation at 4000 rpm for 10 min and resuspended in 1 326 ml CE Buffer. Nuclei were pelleted after centrifugation at 4000 rpm for 10 min and resuspended in 1
327 ml of SDS Lysis buffer (50 mM Tris-HCl, 1% SDS, 10 mM EDTA, 1 mM PMSF, 1 µq/ml aprotinin, 1 327 ml of SDS Lysis buffer (50 mM Tris-HCl, 1% SDS, 10 mM EDTA, 1 mM PMSF, 1 µg/ml aprotinin, 1
328 yug/ml pepstatin A). Genomic DNA was fragmented to lengths less than 800 bp by sonication 328 µg/ml pepstatin A). Genomic DNA was fragmented to lengths less than 800 bp by sonication
329 (Misonex 3000) under the following conditions: Output 2.5 for 20 sec, repeated eight times. For 329 (Misonex 3000) under the following conditions: Output 2.5 for 20 sec, repeated eight times. For
330 each sample, 200 µl of sonicated samples were mixed with 800 µl of ChIP dilution buffer (0.01% 330 each sample, 200 µl of sonicated samples were mixed with 800 µl of ChIP dilution buffer (0.01%
331 SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl pH 8.1, 167 mM NaCl). Samples were 331 SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl pH 8.1, 167 mM NaCl). Samples were
332 incubated with specific antibodies including IgG, DNA-PKcs, RNAP II, CDK7, CDK9, pTRIM28 332 incubated with specific antibodies including IgG, DNA-PKcs, RNAP II, CDK7, CDK9, pTRIM28
333 (S824), and H3K27me3 at +4°C overnight. Protein A/G Sepharose was pre-saturated with salmon 333 (S824), and H3K27me3 at +4°C overnight. Protein A/G Sepharose was pre-saturated with salmon
334 sperm DNA and 1% BSA, and 100 µl of 25% Protein A-Sepharose were utilized in DNA-protein 334 sperm DNA and 1% BSA, and 100 µl of 25% Protein A-Sepharose were utilized in DNA-protein
335 immunoprecipitation. Following 3 h of incubation, Antibody-DNA-protein complexes were washed 335 immunoprecipitation. Following 3 h of incubation, Antibody-DNA-protein complexes were washed
336 with 1 ml of each washing buffer. The first wash occurred with low salt immune complex wash 336 with 1 ml of each washing buffer. The first wash occurred with low salt immune complex wash 337 buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8.1, 150 mM NaCl), followed 337 buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8.1, 150 mM NaCl), followed
338 by high salt immune complex wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-338 by high salt immune complex wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-
339 HCl pH 8.1, 500 mM NaCl). The complexes underwent further washing with lithium chloride buffer 339 HCI pH 8.1, 500 mM NaCI). The complexes underwent further washing with lithium chloride buffer
340 (0.25 M LiCI, 1% NP-40, 1% sodium deoxycholate, 1 mM EDTA, and 10 mM Tris HCI pH 8.0) and 340 (0.25 M LiCl, 1% NP-40, 1% sodium deoxycholate, 1 mM EDTA, and 10 mM Tris HCl pH 8.0) and
341 twice with TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0). Protein DNA complexes were 341 twice with TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0). Protein DNA complexes were
342 eluted from protein A/G Sepharose twice using 250 µl of freshly prepared elution buffer (1% SDS 342 eluted from protein A/G Sepharose twice using 250 µl of freshly prepared elution buffer (1% SDS
343 and 0.1 mM NaHCO3). Twenty microliters of 5 M NaCl were added to the total eluate, and Protein-343 and 0.1 mM NaHCO3). Twenty microliters of 5 M NaCl were added to the total eluate, and Protein-
344 DNA complexes were reversed-cross-linked at 65°C overnight. Ten microliters of 0.5 M EDTA. 10 ul 344 DNA complexes were reversed-cross-linked at 65°C overnight. Ten microliters of 0.5 M EDTA, 10 µl
345 of 2 M Tris-HCl pH 6.5, and 2 µl of 10 ng/ml proteinase-K were added, and samples were incubated 345 of 2 M Tris-HCl pH 6.5, and 2 µl of 10 ng/ml proteinase-K were added, and samples were incubated
346 at 50°C for 2 h followed by phenol extraction and ethanol precipitation. Precipitated DNA samples 346 at 50°C for 2 h followed by phenol extraction and ethanol precipitation. Precipitated DNA samples
347 were dissolved in 100 µl of TE buffer, and 2 µl of the sample was utilized in real-time PCR using 347 were dissolved in 100 µl of TE buffer, and 2 µl of the sample was utilized in real-time PCR using
348 SYBR green PCR master mix (Thermo Scientific), following the method described previously by 348 SYBR green PCR master mix (Thermo Scientific), following the method described previously by
349 Kim et al [51]. No-antibody control values were subtracted from each sample value to eliminate 349 Kim et al [51]. No-antibody control values were subtracted from each sample value to eliminate
350 non-specific background signal. The primer sets utilized in real-time PCR amplification are listed in 350 non-specific background signal. The primer sets utilized in real-time PCR amplification are listed in 351 Supplementary Table S1. 351 Supplementary Table S1.
352

³⁵³*2.10. RNA extraction and real-time quantitative PCR (qPCR)*

355 355 Total RNAs were extracted from 5x10⁵ cultured cells using an RNA isolation kit (Qiagen, Hilden, 356 Germany) according to the manufacturer's instructions. The isolated RNAs were meticulously 356 Germany) according to the manufacturer's instructions. The isolated RNAs were meticulously
357 assessed for their integrity, purity, and yield. Subsequently, using the isolated RNAs as the assessed for their integrity, purity, and yield. Subsequently, using the isolated RNAs as the

358 template, first-strand complementary DNA (cDNA) was synthesized utilizing M-MLV Reverse
359 Transcriptase (Thermo Scientific, Waltham, MA). In brief, approximately 3 µg of extracted RNA was 359 Transcriptase (Thermo Scientific, Waltham, MA). In brief, approximately 3 µg of extracted RNA was
360 Freverse transcribed in a total volume of 20 µl with 350 µM dNTP, 50 µM oligo (dT), 5X M-MuLV 360 reverse transcribed in a total volume of 20 µl with 350 µM dNTP, 50 µM oligo (dT), 5X M-MuLV
361 buffer, 200 U RNase inhibitors, and 200 U M-MuLV reverse transcriptase. The RNA, oligo (dT), and 361 buffer, 200 U RNase inhibitors, and 200 U M-MuLV reverse transcriptase. The RNA, oligo (dT), and
362 dNTPs were mixed and incubated at 65°C for 5 min, followed by 37°C for 50 min and 70°C for 10 362 dNTPs were mixed and incubated at 65°C for 5 min, followed by 37°C for 50 min and 70°C for 10
363 min. The cDNA was subsequently diluted and subjected to real-time PCR using the Real-Time PCR 363 min. The cDNA was subsequently diluted and subjected to real-time PCR using the Real-Time PCR
364 svstem 7500TH (Life Technologies, Carlsbad, CA, USA), For all samples, Actin/GAPDH was 364 system 7500TH (Life Technologies, Carlsbad, CA, USA). For all samples, Actin/GAPDH was
365 measured as the internal control and utilized for data normalization. The primer sets utilized for the 365 measured as the internal control and utilized for data normalization. The primer sets utilized for the 366 amplification are listed in Supplementary Table S1. amplification are listed in Supplementary Table S1.

367

³⁶⁸*2.11. Luciferase assay*

370 370 1x10⁴ or 5x10⁵ cells harboring pHR'P-Luc were plated in 12-well plates with complete RPMI media
371 (supplemented with 10% FBS, penicillin, and streptavidin). The cells were incubated with cocaine 371 (supplemented with 10% FBS, penicillin, and streptavidin). The cells were incubated with cocaine
372 (chronically, treating twice per day with cocaine) for 48 h in presence and absence of M3814. 372 (chronically, treating twice per day with cocaine) for 48 h in presence and absence of M3814.
373 Luciferase levels in the cells were assessed using a Luciferase Assay System kit (Promega, 373 Luciferase levels in the cells were assessed using a Luciferase Assay System kit (Promega,
374 Madison, WI, USA). Briefly, the cells were harvested, washed, and lysed with 1 X passive lysis 374 Madison, WI, USA). Briefly, the cells were harvested, washed, and lysed with 1 X passive lysis
375 buffer. After incubating 30 min at RT, cells were centrifuged at high speed for 2 min, and 375 buffer. After incubating 30 min at RT, cells were centrifuged at high speed for 2 min, and
376 supernatant were transferred to a new Eppendorf tube. 10 µl of each sample lysate was added 376 supernatant were transferred to a new Eppendorf tube. 10 µl of each sample lysate was added
377 followed by 50 µl of luciferase substrate/assay buffer to individual wells of white plates to reflect 377 followed by 50 µl of luciferase substrate/assay buffer to individual wells of white plates to reflect
378 light and maximize light output signal. Each sample was tested in triplicate. Luminescence was read 378 light and maximize light output signal. Each sample was tested in triplicate. Luminescence was read 379 in a Veritas Microplate Luminometer (Turner Biosystems). 379 in a Veritas Microplate Luminometer (Turner Biosystems).
380

381 ³⁸¹*2.12. Flow cytometry (FACS) analysis*

382 383 FACS analyses were performed on 2D10 cells (Jurkat cells infected with VSV-G pseudotyped HIV
384 virus carrying the GFP gene under the control of the HIV LTR promoter). Briefly, 2D10 cells were 384 virus carrying the GFP gene under the control of the HIV LTR promoter). Briefly, 2D10 cells were
385 treated with inhibitor M3814 for 24 h. The next day, cells were activated/stimulated with 10 ng/ml 385 treated with inhibitor M3814 for 24 h. The next day, cells were activated/stimulated with 10 ng/ml
386 Tumor Necrosis Factor alpha (TNF-a) for another 48 h. Cells were then harvested, washed, re-386 Tumor Necrosis Factor alpha (TNF-α) for another 48 h. Cells were then harvested, washed, re-
387 suspended with PBS, and analyzed with a FACS Calibur (BD Biosciences) using FlowJo software 387 suspended with PBS, and analyzed with a FACS Calibur (BD Biosciences) using FlowJo software 388 (Treestar Inc.). ³⁸⁸(Treestar Inc.).

389 ³⁹⁰*2.13. Quantification and statistical analysis*

391 392 Data are expressed as the mean standard deviation (mean \pm SD). Comparisons between two stand two quoups were 393 groups were performed using Student's t-test. Comparisons between more than two groups were
394 carried out by one-way or two-way analysis of variance (ANOVA). If the p-value obtained from 394 carried out by one-way or two-way analysis of variance (ANOVA). If the p-value obtained from
395 ANOVA was under 0.05 (p < 0.05), it was considered statistically significant. All statistical ³⁹⁵ANOVA was under 0.05 (p < 0.05), it was considered statistically significant. All statistical 396 calculations were carried out using a GraphPad prism. All the statistical details of experiments can as 397 be found in the figure legends. 397 be found in the figure legends.
398

³⁹⁹**3. Results**

-
- 401 ⁴⁰¹*3.1. Cocaine enhances both the catalytic activity and nuclear translocation of DNA-PK.*

402
403 403 The crucial role of DNA-PK during DNA double strand break repair is well established [43-45].
404 However, for the first time, we documented the vital role of DNA-PK in supporting gene 404 However, for the first time, we documented the vital role of DNA-PK in supporting gene
405 transcription [37]. To define the underlying molecular mechanism through which DNA-PK 405 transcription [37]. To define the underlying molecular mechanism through which DNA-PK
406 augments HIV transcription, we confirmed that DNA-PK augment HIV transcription by supporting 406 augments HIV transcription, we confirmed that DNA-PK augment HIV transcription by supporting
407 both the initiation and elongation phases of transcription [46]. Later, the crucial role of DNA-PK in 407 both the initiation and elongation phases of transcription [46]. Later, the crucial role of DNA-PK in
408 supporting other cellular genes by enhancing RNAP II CTD phosphorvlation was confirmed by us 408 supporting other cellular genes by enhancing RNAP II CTD phosphorylation was confirmed by us
409 and others [39, 58-60]. Previously, we identified the significant impact of cocaine on enhancing HIV 409 and others [39, 58-60]. Previously, we identified the significant impact of cocaine on enhancing HIV
410 transcription and replication [27, 28]. However, to develop therapeutic strategies aimed at 410 transcription and replication [27, 28]. However, to develop therapeutic strategies aimed at 411 mitigating the toxic effects resulting from HIV replication and cocaine exposure, it is imperative to 411 mitigating the toxic effects resulting from HIV replication and cocaine exposure, it is imperative to 412
412 elucidate all the factors and mechanisms through which HIV and cocaine collaborate to induce cell 412 elucidate all the factors and mechanisms through which HIV and cocaine collaborate to induce cell
413 toxicity via heightened HIV transcription. To investigate the role of cocaine in enhancing HIV 413 toxicity via heightened HIV transcription. To investigate the role of cocaine in enhancing HIV
414 transcription, we assessed the expression and nuclear level of DNA-PKcs, the catalytic subunit of 414 transcription, we assessed the expression and nuclear level of DNA-PKcs, the catalytic subunit of 415 DNA-PK. The impact of cocaine on the functional/catalytic activity of DNA-PK was evaluated by 415 DNA-PK. The impact of cocaine on the functional/catalytic activity of DNA-PK was evaluated by
416 examining phosphorylation of p-DNA-PKcs at serine 2056 (p-DNA-PK S2056), a post translational 416 examining phosphorylation of p-DNA-PKcs at serine 2056 (p-DNA-PK S2056), a post translational
417 modification that marks functionally active form of DNA-PK. We treated Jurkat cells, a T cell line, 417 modification that marks functionally active form of DNA-PK. We treated Jurkat cells, a T cell line, 418 with increasing doses of cocaine for a duration of 3 h. Later, cells were harvested, and nuclear 418 with increasing doses of cocaine for a duration of 3 h. Later, cells were harvested, and nuclear
419 lysates were subjected to immunoblotting using antibodies specific for either total DNA-PKcs or 419 lysates were subjected to immunoblotting using antibodies specific for either total DNA-PKcs or
420 phosphorylated form of DNA-PKcs (pDNA-PKcs S2056) to evaluate cocaine impact. We found 420 phosphorylated form of DNA-PKcs (pDNA-PKcs S2056) to evaluate cocaine impact. We found
421 higher levels of both DNA-PKcs and pDNA-PKcs S2056 in the nucleus upon cocaine exposure 421 higher levels of both DNA-PKcs and pDNA-PKcs S2056 in the nucleus upon cocaine exposure
422 compared to the untreated cell control (Ctrl) (Figures 1A & 1B). The densitometric analyses of the 422 compared to the untreated cell control (Ctrl) (**Figures 1A & 1B**). The densitometric analyses of the 423 protein bands validated a significant increase in the expression and nuclear level of both DNA-423 protein bands validated a significant increase in the expression and nuclear level of both DNA-
424 PKcs and pDNA-PKcs S2056 following cocaine-mediated cell stimulation. We further confirmed the 424 PKcs and pDNA-PKcs S2056 following cocaine-mediated cell stimulation. We further confirmed the
425 effect of cocaine in upregulating and activating the DNA-PK in a dose-dependent manner using 425 effect of cocaine in upregulating and activating the DNA-PK in a dose-dependent manner using
426 varying cell types belonging to different lineages, namely, microglial cells, a primary immune cell 426 varying cell types belonging to different lineages, namely, microglial cells, a primary immune cell
427 found in CNS and MT-4 (Figures 1C, 1D, 1E & 1F). These findings confirmed significant 427 found in CNS and MT-4 **(Figures 1C, 1D, 1E & 1F).** These findings confirmed significant
428 upregulation of DNA-PK expression and functional activation of DNA-PK (pDNA-PKcs S2056) by 428 upregulation of DNA-PK expression and functional activation of DNA-PK (pDNA-PKcs S2056) by
429 cocaine and in a cell lineage independent manner. cocaine and in a cell lineage independent manner.

430 Subsequently, we examined the impact of cocaine on DNA-PK levels and activation in a time-
431 dependent manner (**Figures 1G & 1H**) by treating the Jurkat cells infected with pHR'P-Luc with a 431 dependent manner (**Figures 1G & 1H**) by treating the Jurkat cells infected with pHR'P-Luc with a
432 fixed dose of cocaine (10 uM) for 30 min and 3 h. with untreated cells as a control (Figures 1G & ⁴³²fixed dose of cocaine (10 μM) for 30 min and 3 h, with untreated cells as a control (**Figures 1G &** 433 **1H**). Upon analyzing the nuclear extract, we found upregulation of nuclear DNA-PK level within 30
434 min, which remained higher even after 3 h. min, which remained higher even after 3 h.

435 Furthermore, to establish the ubiquity of the phenomenon, MT-4 cells were treated with increasing
436 doses of cocaine for 3 h, and the translocation of DNA-PKcs from cytoplasm to nucleus was 436 doses of cocaine for 3 h, and the translocation of DNA-PKcs from cytoplasm to nucleus was
437 evaluated by immunoblotting, analyzing both cytoplasmic and nuclear protein fractions on the 437 evaluated by immunoblotting, analyzing both cytoplasmic and nuclear protein fractions on the
438 same blot. As a control, we evaluated HDAC-1 levels, a protein that predominantly exists in the 438 same blot. As a control, we evaluated HDAC-1 levels, a protein that predominantly exists in the
439 nucleus, and only a small portion was present in the cell cytoplasm. Accordingly, we found 439 nucleus, and only a small portion was present in the cell cytoplasm. Accordingly, we found
440 abundant presence of HDAC-1 in the nuclear extract of the cell, validating the purity of our nuclear 440 abundant presence of HDAC-1 in the nuclear extract of the cell, validating the purity of our nuclear
441 fraction and our assay conditions. As loading control, we examined the presence of Beta-actin 441 fraction and our assay conditions. As loading control, we examined the presence of Beta-actin
442 protein, which is constitutively expressed in the cell and can be detected in both cytoplasmic and 442 protein, which is constitutively expressed in the cell and can be detected in both cytoplasmic and
443 nuclear fractions. Interestingly, we noted significantly enhanced translocation of both DNA-PKcs nuclear fractions. Interestingly, we noted significantly enhanced translocation of both DNA-PKcs
444 and (pDNA-PKcs 2056) into the nucleus following cocaine treatments (Figures 1I, 1J & 1K). The ⁴⁴⁴and (pDNA-PKcs 2056) into the nucleus following cocaine treatments (**Figures 1I, 1J & 1K**). The 445 enhanced nuclear localization of DNA-PK following cell stimulation was also observed previously
446 [38]. These results suggest that cocaine augments DNA-PK function both by enhancing its 446 [38]. These results suggest that cocaine augments DNA-PK function both by enhancing its
447 upregulation and nuclear translocation, besides augmenting the catalytic activity of DNA-PK by 447 upregulation and nuclear translocation, besides augmenting the catalytic activity of DNA-PK by
448 specifically increasing its phosphorylation at S2056. Moreover, higher nuclear translocation of specifically increasing its phosphorylation at S2056. Moreover, higher nuclear translocation of

449 DNA-PK following cocaine exposure clearly suggests a role for DNA-PK in DNA transections,
450 including transcription. Altogether, these results confirm that cocaine intake promotes activation of 450 including transcription. Altogether, these results confirm that cocaine intake promotes activation of 451 DNA-PK by enhancing both the nuclear translocation and functional activity of DNA-PK. DNA-PK by enhancing both the nuclear translocation and functional activity of DNA-PK.

452
453

⁴⁵³*3.2. Cocaine-induced HIV transcription augments overall HIV replication.*

455 455 To evaluate the impact of cocaine on HIV transcription and subsequently to HIV gene expression,
456 we freshly infected Jurkat cells with non-replicating attenuated HIV, pHR'P-Luc, to generate the 456 we freshly infected Jurkat cells with non-replicating attenuated HIV, pHR'P-Luc, to generate the
457 Jurkat-pHR'P-Luc cell line [78]. The pHR'P-Luc is an HIV-based lentivirus that expresses luciferase 457 Jurkat-pHR'P-Luc cell line [78]. The pHR'P-Luc is an HIV-based lentivirus that expresses luciferase
458 reporter gene under the control of the HIV LTR promoter (Figure 2A). Therefore, expression of 458 reporter gene under the control of the HIV LTR promoter (Figure 2A). Therefore, expression of 459 luciferase indicates ongoing HIV transcription and gene expression. Figures 2B and 2D depict the 459 luciferase indicates ongoing HIV transcription and gene expression. **Figures 2B and 2D** depict the 460 schematic overview of the cell treatment procedures. As anticipated from our previous studies [27, 460 schematic overview of the cell treatment procedures. As anticipated from our previous studies [27, 461 28], a significant increase in luciferase counts was observed in a dose-dependent manner, 461 28], a significant increase in luciferase counts was observed in a dose-dependent manner,
462 validating cocaine-mediated upregulation of HIV gene expression (**Figure 2C**). To further confirm 462 validating cocaine-mediated upregulation of HIV gene expression (**Figure 2C**). To further confirm
463 the impact of cocaine-mediated cell stimulation on HIV gene expression and replication, PBMC 463 the impact of cocaine-mediated cell stimulation on HIV gene expression and replication, PBMC
464 cells were chronically treated with cocaine prior to being infected with a replication-competent dual 464 cells were chronically treated with cocaine prior to being infected with a replication-competent dual
465 tropic HIV Type 1 strain 93/TH/051 for a period of 24 h. The HIV transcripts were quantified via real-465 tropic HIV Type 1 strain 93/TH/051 for a period of 24 h. The HIV transcripts were quantified via real-
466 time qPCR using primer sets that amplify the Envelope (*Env*) region of the HIV genome. A 466 time qPCR using primer sets that amplify the Envelope (*Env*) region of the HIV genome. A
467 significant upregulation of HIV gene expression was confirmed in the presence of cocaine (**Figure** 467 significant upregulation of HIV gene expression was confirmed in the presence of cocaine (**Figure** 468 2E). Next, The HIV protein expression was evaluated via immunoblotting using antibodies against ⁴⁶⁸**2E**). Next, The HIV protein expression was evaluated via immunoblotting using antibodies against 469 Gag subunits (p24) of HIV by comparing the cell lysates of cocaine treated or untreated HIV
470 infected cells (Figures 2F & 2G). The upregulation of p24 confirms enhanced HIV gene expression 470 infected cells (**Figures 2F & 2G**). The upregulation of p24 confirms enhanced HIV gene expression
471 and replication in the presence of cocaine. Together, these results suggest that cocaine induced 471 and replication in the presence of cocaine. Together, these results suggest that cocaine induced
472 signaling pathways promote activation of both cell status and transcription machinery, including 472 signaling pathways promote activation of both cell status and transcription machinery, including
473 DNA-PK stimulation (p-DNA-PK S2056) (Figure 1), resulting in enhanced HIV transcription and 473 DNA-PK stimulation (p-DNA-PK S2056) (**Figure 1**), resulting in enhanced HIV transcription and 474 consequently higher HIV replication. 474 consequently higher HIV replication.
475

475 ⁴⁷⁶*3.3. Partial DNA-PK inhibition is sufficient to restrict HIV transcription, replication, and* ⁴⁷⁷*latency reactivation.*

478 479 We have shown that DNA-PK plays an important role during HIV transcription [37, 39]. To extend 480 further on those findings and establish the translational potential of DNA-PK inhibition in restricting 480 further on those findings and establish the translational potential of DNA-PK inhibition in restricting
481 HIV transcription and replication, we evaluated the role of a clinically evaluated DNA-PK inhibitor 481 HIV transcription and replication, we evaluated the role of a clinically evaluated DNA-PK inhibitor
482 (DNA-PKi), M3814. Interestingly, in a recent clinical study, DNA-PK inhibitors, including M3814 at 482 (DNA-PKi), M3814. Interestingly, in a recent clinical study, DNA-PK inhibitors, including M3814 at 483 dosages from 110 µM to 320 µM were found safe and highly effective as potential anti-cancer drugs 483 dosages from 110 µM to 320 µM were found safe and highly effective as potential anti-cancer drugs
484 [82-92], validating the safety of these agents for human use [84]. Notably, we found that partial 484 [82-92], validating the safety of these agents for human use [84]. Notably, we found that partial
485 DNA-PK inhibition by only 20 µM (less than 1/5) is sufficient to restrict HIV transcription, replication, 485 DNA-PK inhibition by only 20 μM (less than 1/5) is sufficient to restrict HIV transcription, replication,
486 and latency reactivation without any cell toxicity.

486 and latency reactivation without any cell toxicity.
487 We assessed the effect of M3814 on HIV transc 487 We assessed the effect of M3814 on HIV transcription and latency reactivation. The infected Jurkat
488 cells that harbor latent HIV provirus (pHR'P-Luc) in their genome, which expresses luciferase 488 cells that harbor latent HIV provirus (pHR'P-Luc) in their genome, which expresses luciferase
489 reporter gene under the control of HIV LTR promoter (Figure 2A). These cells, Jurkat-pHR'P-Luc, 489 reporter gene under the control of HIV LTR promoter (**Figure 2A**). These cells, Jurkat-pHR'P-Luc, 490 were incubated overnight (24 h) with increasing concentrations (5 µM, 10 µM, 15 µM and 20 µM) of 490 were incubated overnight (24 h) with increasing concentrations (5 μ M, 10 μ M, 15 μ M and 20 μ M) of 491 M3814. The next day, the cells were stimulated with 10 ng/ml Tumor Necrosis Factor alpha (TNF- α) 491 M3814. The next day, the cells were stimulated with 10 ng/ml Tumor Necrosis Factor alpha (TNF-α)
492 for another 48 h (Fiqure 3A). A strong M3814-mediated dose-dependent inhibition of HIV ⁴⁹²for another 48 h (**Figure 3A**). A strong M3814-mediated dose-dependent inhibition of HIV

493 transcription was observed, indicated by highly reduced luciferase counts, marking restricted HIV
494 qene expression when DNA-PK was selectively inhibited using highly specific and clinically 494 gene expression when DNA-PK was selectively inhibited using highly specific and clinically
495 evaluated DNA-PKi (**Figure 3B**). As controls, cells were either treated with TNF-a alone (positive 495 evaluated DNA-PKi (**Figure 3B**). As controls, cells were either treated with TNF-α alone (positive 496 control) or left untreated (negative control) or left untreated (negative control). The inverse correlation betwee 496 control) or left untreated (negative control). The inverse correlation between luciferase counts and latency
497 M3814 concentration confirms a direct role DNA-PK in supporting HIV transcription and latency 497 M3814 concentration confirms a direct role DNA-PK in supporting HIV transcription and latency
498 reactivation (Fiqure 3B). These findings were further validated by examining the presence of 498 reactivation (**Figure 3B**). These findings were further validated by examining the presence of 499 luciferase protein in the cell extracts by performing immunoblotting using antibody specific to 499 luciferase protein in the cell extracts by performing immunoblotting using antibody specific to
500 Luciferase protein (Luciferase antibody: sc-74548) (Figure 3C). The strong dose-dependent ⁵⁰⁰Luciferase protein (Luciferase antibody: sc-74548) (**Figure 3C**). The strong dose-dependent 501 inhibition of luciferase by M3814 established a vital role of DNA-PK during HIV transcription.
502 Overall, these findings demonstrate a pivotal role of DNA-PK in supporting HIV transcription and 502 Overall, these findings demonstrate a pivotal role of DNA-PK in supporting HIV transcription and
503 Iatency reactivation. Moreover, the data obtained confirm our previous findings where we used 503 latency reactivation. Moreover, the data obtained confirm our previous findings where we used
504 another highly specific clinically tested DNA-PKi (Nu7441) [39]. 504 another highly specific clinically tested DNA-PKi (Nu7441) [39].
505

505 506 To exclude the possibility that the reduced luciferase activity upon M3814 treatment was not due to
507 cell loss, we performed cell viability assay. The Jurkat-pHR'P-Luc cells were cultured with different 507 cell loss, we performed cell viability assay. The Jurkat-pHR'P-Luc cells were cultured with different 508 concentrations (2 μ M-40 μ M) for M3814 for 48–72 h, and cell cytotoxicity was determined by MTS-508 concentrations (2 μM-40 μM) for M3814 for 48–72 h, and cell cytotoxicity was determined by MTS-509 PMS cell proliferation assay (Promega, Madison, WI, USA). We did not observe any significant cell
510 cytotoxicity even at 40 µM of M3814 treatment (Figure 3D). 510 cytotoxicity even at 40 μM of M3814 treatment (**Figure 3D**).
511

512 512 The impact of M3814 in restricting the reactivation of latent HIV was further confirmed using another
513 Iatently infected cell line, 2D10 cells. The 2D10-cell line is a latently infected Jurkat T-cell line, which 513 latently infected cell line, 2D10 cells. The 2D10-cell line is a latently infected Jurkat T-cell line, which
514 harbors a latent HIV provirus in their genome that expresses a reporter short-lived green 514 harbors a latent HIV provirus in their genome that expresses a reporter short-lived green
515 fluorescent protein (d2EGFP) from HIV LTR promoter [78, 80]. Thus, GFP expression marks 515 fluorescent protein (d2EGFP) from HIV LTR promoter [78, 80]. Thus, GFP expression marks
516 ongoing HIV gene expression. The 2D10 cells were treated for 24 h with different doses of M3814. 516 ongoing HIV gene expression. The 2D10 cells were treated for 24 h with different doses of M3814.
517 Next day, cells were activated with 10 ng/ml TNF-a for another 48 h. Later, we quantified GFP 517 Next day, cells were activated with 10 ng/ml TNF-α for another 48 h. Later, we quantified GFP
518 expression through flow cytometric analysis. The TNF-α, which we used as a positive control, was 518 expression through flow cytometric analysis. The TNF-α, which we used as a positive control, was 519 able to stimulate latent HIV in more than 90% of cells compared to the control (unstimulated cells), 519 able to stimulate latent HIV in more than 90% of cells compared to the control (unstimulated cells), 520 marked by GFP expression in most (90%) cells. As anticipated, we observed a clear dose-520 marked by GFP expression in most (90%) cells. As anticipated, we observed a clear dose-
521 dependent inhibition of HIV proviral reactivation upon DNA-PK inhibition, indicated by the reduced 521 dependent inhibition of HIV proviral reactivation upon DNA-PK inhibition, indicated by the reduced
522 GFP expression in cells treated with the M3814 in a dose-dependent manner compared to the 522 GFP expression in cells treated with the M3814 in a dose-dependent manner compared to the 523 positive control (TNF- α treated) (Figure 3E & 3F). Overall, these data suggested that DNA-PK-523 positive control (TNF-α treated) (**Figure 3E & 3F**). Overall, these data suggested that DNA-PK-524 mediated stimulation of HIV transcription is required for the reactivation of latent HIV provirus.
525

525 526 To assess the impact of different highly specific and clinically evaluated DNA-PK inhibitors on HIV
527 replication, Jurkat cells were treated with the increasing doses of different DNA-PK inhibitors, 527 replication, Jurkat cells were treated with the increasing doses of different DNA-PK inhibitors,
528 M3814, and NU7441 for 24 h. The next day, cells were activated with 10 ng/ml TNF-α for 3 h. Later, 528 M3814, and NU7441 for 24 h. The next day, cells were activated with 10 ng/ml TNF-α for 3 h. Later, 529 cells were infected with a replication-competent dual tropic HIV (Type 1 strain 93/TH/051). The cell 529 cells were infected with a replication-competent dual tropic HIV (Type 1 strain 93/TH/051). The cell
530 lysates were prepared after either 4 h post infection (4hpi) or 6 h post infection (6hpi), as shown in 530 lysates were prepared after either 4 h post infection (4hpi) or 6 h post infection (6hpi), as shown in
531 the figure (**Figure 4A**). The lysates were analyzed by immunoblotting with HIV cocktail antibodies 531 the figure (**Figure 4A**). The lysates were analyzed by immunoblotting with HIV cocktail antibodies
532 p55, p24, and p17. The results show a clear inhibition of all HIV protein (HIV p55, HIV p24, and HIV 532 p55, p24, and p17. The results show a clear inhibition of all HIV protein (HIV p55, HIV p24, and HIV sasa
533 p17) with increasing doses of DNA-PK inhibitors M3814 (Figures 4B & 4C) and NU7441 (Figures 533 p17) with increasing doses of DNA-PK inhibitors M3814 (**Figures 4B & 4C**) and NU7441 (**Figures** 534 **4D & 4E**). The stronger suppression of HIV replication was noted with the increasing doses of DNA-534 **4D & 4E**). The stronger suppression of HIV replication was noted with the increasing doses of DNA-
535 PK inhibitors, indicating the target-specific inhibition and confirming the vital role of DNA-PK-535 PK inhibitors, indicating the target-specific inhibition and confirming the vital role of DNA-PK-
536 induced HIV transcription in supporting overall HIV replication. Additionally, the data confirm that in 536 induced HIV transcription in supporting overall HIV replication. Additionally, the data confirm that in
537 the presence of DNA-PKi, TNF-a mediated strong cell stimulation and NF-kB activation is the presence of DNA-PKi, TNF- α mediated strong cell stimulation and NF-kB activation is

538 ineffective in inducing HIV transcription, which suggests that not only cocaine but also TNF-α/NF-539 kB-mediated HIV transcription requires functional DNA-PK.
540

540 ⁵⁴¹*3.4. DNA-PK inhibition strongly suppresses cocaine induced HIV transcription in primary* ⁵⁴²*cells, as well.*

543 544 The above data and our previous publication suggested that cocaine plays a significant role in
545 enhancing HIV transcription and replication [27, 28]. In order to understand the molecular 545 enhancing HIV transcription and replication [27, 28]. In order to understand the molecular
546 mechanisms by which cocaine controls HIV transcription and gene regulation, we investigated 546 mechanisms by which cocaine controls HIV transcription and gene regulation, we investigated
547 whether cocaine promotes HIV transcription and replication by enhancing both the catalytic activity 547 whether cocaine promotes HIV transcription and replication by enhancing both the catalytic activity
548 and nuclear translocation of DNA-PK. To test this hypothesis, we treated the cells infected with 548 and nuclear translocation of DNA-PK. To test this hypothesis, we treated the cells infected with
549 pHR'P-Luc, which carry proviral HIV and expresses luciferase reporter under HIV LTR promoter, 549 pHR'P-Luc, which carry proviral HIV and expresses luciferase reporter under HIV LTR promoter,
550 with 10 µM M3814 for 24 h. The next day, cells were treated with cocaine chronically for two days 550 with 10 µM M3814 for 24 h. The next day, cells were treated with cocaine chronically for two days
551 (10 µM cocaine twice a day for 3 days). Later, the cell extracts were prepared, and the level of 551 (10 µM cocaine twice a day for 3 days). Later, the cell extracts were prepared, and the level of 552 Iuciferase reporter protein expression was determined via luciferase assays. As anticipated from the 552 luciferase reporter protein expression was determined via luciferase assays. As anticipated from the 553 above analysis (**Figure 2B**), we noticed significant uprequistion of luciferase counts, indicating 553 above analysis (**Figure 2B**), we noticed significant upregulation of luciferase counts, indicating
554 enhanced HIV transcription in cocaine-treated samples. However, in the presence of M3814, HIV 554 enhanced HIV transcription in cocaine-treated samples. However, in the presence of M3814, HIV
555 transcription is strongly restricted both in the presence and absence of cocaine (Figure 5A). These 555 transcription is strongly restricted both in the presence and absence of cocaine (**Figure 5A**). These
556 results confirmed the specific role of cocaine-stimulated DNA-PK in promoting HIV transcription and 556 results confirmed the specific role of cocaine-stimulated DNA-PK in promoting HIV transcription and
557 gene expression. Subsequently, to assess the effect of cocaine-mediated DNA-PK stimulation on 557 gene expression. Subsequently, to assess the effect of cocaine-mediated DNA-PK stimulation on
558 HIV transcription and replication, we treated the Jurkat T cells (Figure 5B & 5C) and PBMC (Figure 558 HIV transcription and replication, we treated the Jurkat T cells (**Figure 5B & 5C**) and PBMC (**Figure 559 5D, 5E & Supplementary Figure 31**) with M3814 overnight (24 h). The next day, fresh media was ⁵⁵⁹**5D, 5E & Supplementary Figure S1**) with M3814 overnight (24 h). The next day, fresh media was 560 provided with cocaine for 3 h. After 3 h of cocaine exposure, cells were infected with replication
561 competent virus (93/TH/051) for 24 h. The HIV transcripts were quantified using real-time qPCR 561 competent virus (93/TH/051) for 24 h. The HIV transcripts were quantified using real-time qPCR
562 using primer sets that amplify the Nuc-2 (**Figure 5B & 5D**) and *Env* (**Figure 5C & 5E**) region of the 562 using primer sets that amplify the Nuc-2 (**Figure 5B & 5D**) and *Env* (**Figure 5C & 5E**) region of the 563 HIV genome. A significant uprequisition of HIV transcript was observed in the presence of cocaine, 563 HIV genome. A significant upregulation of HIV transcript was observed in the presence of cocaine,
564 but, as anticipated, the presence of M3814 strongly restricted HIV gene expression in a dose-564 but, as anticipated, the presence of M3814 strongly restricted HIV gene expression in a dose-
565 dependent manner (Figure 5B, 5C, 5D & 5E). These results were further validated by examining 565 dependent manner (**Figure 5B, 5C, 5D & 5E**). These results were further validated by examining
566 the expression of HIV protein in the absence or presence of M3814. The Jurkat cells were treated 566 the expression of HIV protein in the absence or presence of M3814. The Jurkat cells were treated
567 with M3814 for 24 h. The next day, cells were treated with cocaine for 3 h. Later, we infected the 567 with M3814 for 24 h. The next day, cells were treated with cocaine for 3 h. Later, we infected the
568 cells with replication competent HIV (93/TH/051) for another 24 h. The cell lysates were then 568 cells with replication competent HIV (93/TH/051) for another 24 h. The cell lysates were then
569 subjected to immunoblotting to detect HIV proteins p24 and p17. Again, we noted higher levels of 569 subjected to immunoblotting to detect HIV proteins p24 and p17. Again, we noted higher levels of
570 HIV proteins, p24 and p17, following cocaine exposure. However, in the presence of inhibitor, the 570 HIV proteins, p24 and p17, following cocaine exposure. However, in the presence of inhibitor, the
571 level of HIV proteins dropped sharply, further demonstrating that cocaine-induced DNA-PK plays a 571 Level of HIV proteins dropped sharply, further demonstrating that cocaine-induced DNA-PK plays a
572 Lorucial role in HIV transcription and replication (Figure 5F & 5G). Overall, these results confirm that 572 crucial role in HIV transcription and replication (**Figure 5F & 5G**). Overall, these results confirm that 573 cocaine-mediated DNA-PK stimulation is required for HIV gene expression and consequently for 573 cocaine-mediated DNA-PK stimulation is required for HIV gene expression and consequently for
574 HIV replication. 574 HIV replication.
575

575 576 3.5. Cocaine promotes HIV transcription by enhancing the phosphorylation of the C-
577 **commini terminal domain (CTD) of RNA polymerase II (RNAP II)** ⁵⁷⁷*terminal domain (CTD) of RNA polymerase II (RNAP II)*

578 579 RNAP II is the main enzyme that transcribes eukaryotic DNA into mRNA. The C-terminal domain
580 (CTD) of RNAP II consists of a repeating sequence of 7 amino acids (heptapeptide) with the 580 (CTD) of RNAP II consists of a repeating sequence of 7 amino acids (heptapeptide) with the
581 consensus sequence Tyr1-Ser2-Pro3-Thr4-Ser5-Pro6-Ser7 (YSPTSPS) around 52 times [32-35]. 581 consensus sequence Tyr1-Ser2-Pro3-Thr4-Ser5-Pro6-Ser7 (YSPTSPS) around 52 times [32-35].
582 All residues within the CTD heptad repeat can be post-translationally modified by phosphorylation All residues within the CTD heptad repeat can be post-translationally modified by phosphorylation

583 (tyrosine, threonine, serine, and proline). However, in RNAP II CTD, Serine 5 and Serine 2
584 phosphorylation (Ser5-P and Ser2-P) are the best studied and the most established indicators of 584 phosphorylation (Ser5-P and Ser2-P) are the best studied and the most established indicators of
585 ongoing transcription. Specifically, the phosphorylation of RNAP II CTD at Ser5 is linked to the 585 ongoing transcription. Specifically, the phosphorylation of RNAP II CTD at Ser5 is linked to the
586 initiation phase of transcription, marking initial movement of RNAP II from the gene promoter, 586 initiation phase of transcription, marking initial movement of RNAP II from the gene promoter,
587 whereas phosphorylation of Ser2 is found to be correlated with the elongation phase of 587 whereas phosphorylation of Ser2 is found to be correlated with the elongation phase of
588 transcription. Notably, to generate a full-length HIV transcript, both initiation and elongation phases 588 transcription. Notably, to generate a full-length HIV transcript, both initiation and elongation phases
589 are required. Therefore, we evaluated if cocaine enhances HIV transcription by hyper-589 are required. Therefore, we evaluated if cocaine enhances HIV transcription by hyper-
590 phosphorylating RNAP II CTD, we analyzed phosphorylation of RNAP II CTD at Ser2 and Ser5 590 phosphorylating RNAP II CTD, we analyzed phosphorylation of RNAP II CTD at Ser2 and Ser5
591 upon cocaine exposure. THP-1 cells were treated with increasing concentrations of cocaine for 3 h. 591 upon cocaine exposure. THP-1 cells were treated with increasing concentrations of cocaine for 3 h.
592 Later, nuclear lysate was subjected to immunoblotting to probe with RNAP II Ser2-P, RNAP II Ser5-592 Later, nuclear lysate was subjected to immunoblotting to probe with RNAP II Ser2-P, RNAP II Ser5-
593 P, and RNAP II Total. The activation of p65, a subunit of NF-kB, was analyzed as a positive control 593 P, and RNAP II Total. The activation of p65, a subunit of NF-kB, was analyzed as a positive control
594 to confirm cocaine-mediated cell stimulation. As anticipated, we observed stimulation of p65, 594 to confirm cocaine-mediated cell stimulation. As anticipated, we observed stimulation of p65,
595 marked by enhanced level of p65 in the nucleus compared to untreated cells (Ctrl). Notably, we 595 marked by enhanced level of p65 in the nucleus compared to untreated cells (Ctrl). Notably, we
596 also found hyper-phosphorylates RNAP II CTD at both Ser2 and Ser5 residues following cocaine 596 also found hyper-phosphorylates RNAP II CTD at both Ser2 and Ser5 residues following cocaine
597 treatment (Figures 6A & 6B). The dose-dependent upregulation of RNAP II CTD phosphorylation 597 treatment (**Figures 6A & 6B**). The dose-dependent upregulation of RNAP II CTD phosphorylation
598 further confirmed the direct impact of cocaine in enhancing the phosphorylation of RNAP II CTD. 598 further confirmed the direct impact of cocaine in enhancing the phosphorylation of RNAP II CTD.
599

600 600 To further validate the ubiquity of our findings, results were confirmed in MT-4 cells. The cells were
601 treated with different doses of cocaine for 3 h before being infected with a dual tropic HIV 601 treated with different doses of cocaine for 3 h before being infected with a dual tropic HIV
602 (93/TH/051). After 3 h, nuclear extracts were examined for RNAP II at Ser2 and Ser5. The hyper-602 (93/TH/051). After 3 h, nuclear extracts were examined for RNAP II at Ser2 and Ser5. The hyper-
603 phosphorylation of RNAP II at both the Ser2 and Ser5 positions of RNAP II CTD upon cocaine 603 phosphorylation of RNAP II at both the Ser2 and Ser5 positions of RNAP II CTD upon cocaine
604 treatment was evaluated (Figures 6C & 6D). The dose-dependent hyper-phosphorylation of RNAP 604 treatment was evaluated (**Figures 6C & 6D**). The dose-dependent hyper-phosphorylation of RNAP
605 II CTD was clearly evident. 605 II CTD was clearly evident.
606

607 607 Subsequently, we examined if DNA-PK is involved in the cocaine-induced RNAP II CTD
608 phosphorylation. We hypothesized that if cocaine-induced DNA-PK catalyzes the RNAP II CTD 608 phosphorylation. We hypothesized that if cocaine-induced DNA-PK catalyzes the RNAP II CTD
609 phosphorylation, then inhibition of DNA-PK should impair the cocaine stimulated RNAP II CTD 609 phosphorylation, then inhibition of DNA-PK should impair the cocaine stimulated RNAP II CTD
610 hyper-phosphorylation. To test this hypothesis, the THP-1 cells were treated with increasing 610 hyper-phosphorylation. To test this hypothesis, the THP-1 cells were treated with increasing
611 concentrations of M3814 for 24 h. Next day, cells were exposed to cocaine for 3 h. Later, nuclear 611 concentrations of M3814 for 24 h. Next day, cells were exposed to cocaine for 3 h. Later, nuclear
612 protein lysates were analyzed by immunoblotting to examine RNAP II CTD phosphorylation at the 612 protein lysates were analyzed by immunoblotting to examine RNAP II CTD phosphorylation at the
613 sites Ser2 and Ser5. As shown in the figure (Figure 6E), cocaine treatment significantly upregulates 613 sites Ser2 and Ser5. As shown in the figure (**Figure 6E**), cocaine treatment significantly upregulates 614 RNAP II CTD phosphorylation at Ser2 and Ser5, validating the above results. We noted a 614 RNAP II CTD phosphorylation at Ser2 and Ser5, validating the above results. We noted a
615 significant reduction of CTD phosphorylation at both Ser2 and Ser5 in the presence of M3814 when 615 significant reduction of CTD phosphorylation at both Ser2 and Ser5 in the presence of M3814 when
616 compared to cocaine alone samples. The dose-dependent inhibition of RNAP II CTD 616 compared to cocaine alone samples. The dose-dependent inhibition of RNAP II CTD
617 phosphorylation at both Ser5 and Ser2 with M3814 confirmed our hypothesis and validated that 617 phosphorylation at both Ser5 and Ser2 with M3814 confirmed our hypothesis and validated that
618 cocaine-stimulated DNA-PK plays a vital role in promoting both the initiation and elongation phases 618 cocaine-stimulated DNA-PK plays a vital role in promoting both the initiation and elongation phases
619 of HIV transcription by catalyzing both Ser5 and Ser 2, respectively (Figures 6E & 6F). Overall, the 619 of HIV transcription by catalyzing both Ser5 and Ser 2, respectively (**Figures 6E & 6F**). Overall, the 620 results demonstrate that by activating DNA-PK, cocaine promotes different stages of HIV 620 results demonstrate that by activating DNA-PK, cocaine promotes different stages of HIV
621 transcription, a necessity to produce complete HIV genomic transcripts or new HIV progeny. 621 transcription, a necessity to produce complete HIV genomic transcripts or new HIV progeny.
622

622 ⁶²³*3.6. Cocaine enhances the elongation phase of HIV transcription not only by stimulating* ⁶²⁴*DNA-PK but also via P-TEFb activation.*

625 626 The above results demonstrate that cocaine promotes both the initiation and elongation phases of 627 HIV transcription by enhancing RNAP II CTD phosphorylation at Ser5 and Ser2. We further HIV transcription by enhancing RNAP II CTD phosphorylation at Ser5 and Ser2. We further

628 investigated if cocaine promotes the elongation phase by stimulating P-TEFb. The CDK9 is the 629 kinase subunit of P-TEFb complex, which plays a crucial role in catalyzing the phosphorylation of 629 kinase subunit of P-TEFb complex, which plays a crucial role in catalyzing the phosphorylation of 630 RNAP II processive or 630 RNAP II CTD at position Ser2, a post-translational modification that makes RNAP II processive or
631 elongation proficient. We examined the stimulation of P-TEFb following cocaine exposure. Jurkat-631 elongation proficient. We examined the stimulation of P-TEFb following cocaine exposure. Jurkat-
632 pHR'P-Luc cells were exposed to increasing doses of cocaine for 3 h. Subsequently, nuclear 632 pHR'P-Luc cells were exposed to increasing doses of cocaine for 3 h. Subsequently, nuclear
633 lysates were subjected to immunoblotting using specific antibodies against CDK7 (TFIIH), p-CDK9 633 lysates were subjected to immunoblotting using specific antibodies against CDK7 (TFIIH), p-CDK9
634 (thr186), and total CDK9. The data indicated that cocaine enhances CDK7, thereby facilitating the 634 (thr186), and total CDK9. The data indicated that cocaine enhances CDK7, thereby facilitating the
635 initiation of HIV transcription. Additionally, the data shows an increase in the phosphorylation of 635 initiation of HIV transcription. Additionally, the data shows an increase in the phosphorylation of 636 CDK9 at threonine residue 186, which marks functionally active CDK9. However, cocaine did not 636 CDK9 at threonine residue 186, which marks functionally active CDK9. However, cocaine did not
637 affect the level of total CDK9 (Figures 7A & 7B). To further validate these findings, Jurkat-pHR'P-637 affect the level of total CDK9 (**Figures 7A & 7B**). To further validate these findings, Jurkat-pHR'P-638 Luc cells were treated with escalating doses of cocaine for 2 h and subsequently infected with 639 replication-competent HIV (strain 93/TH/051) for an additional hour. As shown in the Fiqure 7C, 639 replication-competent HIV (strain 93/TH/051) for an additional hour. As shown in the Figure 7C, 640
640 Jurkat-pHR'P-Luc cells were treated as follows: untreated and uninfected (Lane 1), infected with 640 Jurkat-pHR'P-Luc cells were treated as follows: untreated and uninfected (Lane 1), infected with
641 HIV (93/TH/051) in the absence of cocaine (Lane 2), treated with cocaine without HIV infection 641 HIV (93/TH/051) in the absence of cocaine (Lane 2), treated with cocaine without HIV infection
642 (Lane 3), or pre-treatment with different concentrations of cocaine before infecting with HIV (Lane 642 (Lane 3), or pre-treatment with different concentrations of cocaine before infecting with HIV (Lane
643 4–6). The nuclear lysates were analyzed via immunoblotting using specific antibodies against main 643 4–6). The nuclear lysates were analyzed via immunoblotting using specific antibodies against main
644 P-TEFb subunits, CDK9 and Cyclin T1. The obtained data clearly shows the enhanced 644 P-TEFb subunits, CDK9 and Cyclin T1. The obtained data clearly shows the enhanced
645 phosphorylation of CDK9 and also upregulation of Cyclin T1 upon cocaine treatment, demonstrating 645 phosphorylation of CDK9 and also upregulation of Cyclin T1 upon cocaine treatment, demonstrating
646 that cocaine further supports the ongoing elongation phase of HIV transcription by stimulating 646 that cocaine further supports the ongoing elongation phase of HIV transcription by stimulating
647 CDK9. Nevertheless, it does not affect the level of CDK9. Actin was used as a loading control, while 647 CDK9. Nevertheless, it does not affect the level of CDK9. Actin was used as a loading control, while
648 P24 was probed to mark the ongoing HIV replication. Densitometric analysis of protein bands 648 P24 was probed to mark the ongoing HIV replication. Densitometric analysis of protein bands
649 validated a significant increase to p-CDK9 (thr186) and Cyclin T1 but not CDK9 total levels 649 validated a significant increase to p-CDK9 (thr186) and Cyclin T1 but not CDK9 total levels
650 compared to untreated cells (control) (**Figures 7C & D)**. We also evaluated the impact of cocaine 650 compared to untreated cells (control) (**Figures 7C & D)**. We also evaluated the impact of cocaine 651 on another kinase, CDK7, which is a component of TFIIH complex that is mainly responsible for 651 on another kinase, CDK7, which is a component of TFIIH complex that is mainly responsible for
652 Ser5 phosphorylation, another RNAP II CTD modification required for the initiation phase of 652 Ser5 phosphorylation, another RNAP II CTD modification required for the initiation phase of 653 transcription. As expected, we noted uprequistion of CDK7 upon cocaine treatment. The results 653 transcription. As expected, we noted upregulation of CDK7 upon cocaine treatment. The results
654 again demonstrate that by enhancing CDK7, cocaine facilitates the initiation phase of HIV 654 again demonstrate that by enhancing CDK7, cocaine facilitates the initiation phase of HIV
655 transcription. 655 transcription.
656

657 657 To further substantiate that cocaine-induced phosphorylation of CDK9 and activation of CDK7 are
658 due to DNA-PK activation, we conducted experiments using DNA-PK knockdown cells. The wild 658 due to DNA-PK activation, we conducted experiments using DNA-PK knockdown cells. The wild
659 type (WT) and DNA-PK knockdown (DNA-PK KD) cells were treated with cocaine for 3 h. 659 type (WT) and DNA-PK knockdown (DNA-PK KD) cells were treated with cocaine for 3 h.
660 Subsequently, we analyzed phosphorylation of CDK9 and activation of CDK7, We found a 660 Subsequently, we analyzed phosphorylation of CDK9 and activation of CDK7. We found a
661 significant reduction to p-CDK9 (Thr186) levels, as well as in total CDK9 and CDK7 following DNA-661 significant reduction to p-CDK9 (Thr186) levels, as well as in total CDK9 and CDK7 following DNA-
662 PK depletion (**Figures 7E & 7F**). Our findings in wild type cells confirmed our above results, ⁶⁶²PK depletion (**Figures 7E & 7F**). Our findings in wild type cells confirmed our above results, 663 indicating that cocaine exposure led to an increase in pCDK9 phosphorylation and activation of 664 CDK7. However, in DNA-PK KD cells, we observed a persistent reduction in pCDK9 (thr186) 664 CDK7. However, in DNA-PK KD cells, we observed a persistent reduction in pCDK9 (thr186)
665 phosphorylation and CDK7 activation upon cocaine exposure, suggesting that cocaine-induced 665 phosphorylation and CDK7 activation upon cocaine exposure, suggesting that cocaine-induced
666 CDK9 phosphorylation and activation of CDK7 are DNA-PK specific. Together, these findings 666 CDK9 phosphorylation and activation of CDK7 are DNA-PK specific. Together, these findings
667 confirmed our hypothesis by validating that cocaine-induced DNA-PK facilitates the initiation and 667 confirmed our hypothesis by validating that cocaine-induced DNA-PK facilitates the initiation and
668 elongation phases of HIV transcription by stimulating CDK7 (THIIH) and CDK9 (P-TEFb), 668 elongation phases of HIV transcription by stimulating CDK7 (THIIH) and CDK9 (P-TEFb), 669 respectively. 669 respectively.
670

- $\frac{1}{6}$
- 671

⁶⁷²*3.7. Cocaine-induced DNA-PK relieves the RNAP II pausing by phosphorylating TRIM28 at* ⁶⁷³*S824*

675 675 Later, we examined the impact of cocaine-induced TRIM28 activation (p-TRIM28-(S824) in
676 Frelieving RNAP II pausing. TRIM28 is one of the RNAP II pausing factors, which restricts the flow 676 relieving RNAP II pausing. TRIM28 is one of the RNAP II pausing factors, which restricts the flow
677 of RNAP II on DNA template after transcription of the first 50 to 60 nucleotides. Additionally, it has 677 of RNAP II on DNA template after transcription of the first 50 to 60 nucleotides. Additionally, it has 678
678 been recently documented that TRIM28 potently suppresses HIV expression by utilizing both 678 been recently documented that TRIM28 potently suppresses HIV expression by utilizing both 679 SUMO E3 ligase activity and epigenetic adaptor function I631. However, phosphorylation of 679 SUMO E3 ligase activity and epigenetic adaptor function [63]. However, phosphorylation of 680
680 TRIM28 at its Ser824 converts TRIM28 from a pausing factor to transcription-supporting factor [39, 680 TRIM28 at its Ser824 converts TRIM28 from a pausing factor to transcription-supporting factor [39, 681 Ser
681 59, 601. To further extend on our previous findings [39], in this investigation, for the first time, we 681 59, 60]. To further extend on our previous findings [39], in this investigation, for the first time, we 682 provide the evidence that DNA-PK is the main kinase that catalyzes the phosphorylation of 682 provide the evidence that DNA-PK is the main kinase that catalyzes the phosphorylation of 683 TRIM28 at Ser824 (p-TRIM28-(S824) and reverses the inhibitory effect of TRIM28 on gene 683 TRIM28 at Ser824 (p-TRIM28-(S824) and reverses the inhibitory effect of TRIM28 on gene
684 transcription. We hypothesized that if cocaine stimulates DNA-PK and plays a major role in 684 transcription. We hypothesized that if cocaine stimulates DNA-PK and plays a major role in
685 supporting not only initiation but also the elongation phase of HIV transcription, then cocaine-685 supporting not only initiation but also the elongation phase of HIV transcription, then cocaine-
686 induced DNA-PK should be able to relieve RNAP II pausing, a prerequisite for the elongation 686 induced DNA-PK should be able to relieve RNAP II pausing, a prerequisite for the elongation
687 phase of transcription. To test this hypothesis, we examined the neutralization of RNAP II pausing 687 phase of transcription. To test this hypothesis, we examined the neutralization of RNAP II pausing
688 through the conversion of TRIM28 from a transcriptionally repressive factor (TRIM28) to a 688 through the conversion of TRIM28 from a transcriptionally repressive factor (TRIM28) to a
689 transcriptionally active factor (p-TRIM28 S824) by cocaine-induced DNA-PK-mediated 689 transcriptionally active factor (p-TRIM28 S824) by cocaine-induced DNA-PK-mediated
690 phosphorylation of TRIM28. The THP-1 cells were treated with increasing doses of cocaine for 3 h. 690 phosphorylation of TRIM28. The THP-1 cells were treated with increasing doses of cocaine for 3 h.
691 The nuclear lysates were analyzed by immunoblotting to detect the phosphorylated form of 691 The nuclear lysates were analyzed by immunoblotting to detect the phosphorylated form of 692 TRIM28 (p-TRIM28-(S824) and total TRIM28. The expression of Actin protein among samples was 692 TRIM28 (p-TRIM28-(S824) and total TRIM28. The expression of Actin protein among samples was
693 evaluated as loading control. As expected, following cocaine exposure, we found enhanced 693 evaluated as loading control. As expected, following cocaine exposure, we found enhanced
694 TRIM28 phosphorylation at the position S824 (p-TRIM28-(S824) in a dose-dependent manner 694 TRIM28 phosphorylation at the position S824 (p-TRIM28-(S824) in a dose-dependent manner 695 (Figures 8A & 8B). The densitometric analyses of protein bands further establish the significant ⁶⁹⁵(**Figures 8A & 8B**). The densitometric analyses of protein bands further establish the significant 696 dose-dependent increase to p-TRIM28-(S824) levels upon cocaine treatment compared to the
697 untreated cell control. Thus, showing that cocaine by enhancing phosphorylation of TRIM28 697 untreated cell control. Thus, showing that cocaine by enhancing phosphorylation of TRIM28
698 relieves the RNAP II pausing during HIV transcription. These results were further confirmed in 698 relieves the RNAP II pausing during HIV transcription. These results were further confirmed in 699 Jurkat cells (Fiqures 8C & 8D). ⁶⁹⁹Jurkat cells (**Figures 8C & 8D**).

700 For examining the kinetics of TRIM28 phosphorylation upon cocaine exposure, we treated the
701 Jurkat-pHR'P-Luc cells with a fixed dose of cocaine (10 µM) for different durations: 30 min, 3 h, and 701 Jurkat-pHR'P-Luc cells with a fixed dose of cocaine (10 μM) for different durations: 30 min, 3 h, and 702 6 h (Figures 8E & 8F). Then, we analyzed the nuclear lysates to assess the levels of p-TRIM28 702 6 h (**Figures 8E & 8F**). Then, we analyzed the nuclear lysates to assess the levels of p-TRIM28
703 (S824) and TRIM28 total: we noted significant phosphorylation of TRIM28 at 3 h upon cocaine 703 (S824) and TRIM28 total; we noted significant phosphorylation of TRIM28 at 3 h upon cocaine
704 exposure. As anticipated, densitometry analyses revealed a significant increase of TRIM28 704 exposure. As anticipated, densitometry analyses revealed a significant increase of TRIM28
705 phosphorylation following cocaine treatment in a unique kinetics (**Figures 8E & 8F**). Together, our 705 phosphorylation following cocaine treatment in a unique kinetics (**Figures 8E & 8F**). Together, our 706 data establish that cocaine-mediated enhanced TRIM28 phosphorylation (p-TRIM28-(S824) plays a 706 data establish that cocaine-mediated enhanced TRIM28 phosphorylation (p-TRIM28-(S824) plays a
707 crucial role in transitioning HIV transcription from pausing to the elongating phase by antagonizing 707 crucial role in transitioning HIV transcription from pausing to the elongating phase by antagonizing 708 the pausing effect of TRIM28, and thus, relieving RNAP II pausing. 708 the pausing effect of TRIM28, and thus, relieving RNAP II pausing.
709

710 710 The results were also reproduced in Jurkat cells infected with replication competent virus
711 (93/TH/051). Jurkat-pHR'P-Luc cells were treated with increasing concentrations of cocaine for 3 h 711 (93/TH/051). Jurkat-pHR'P-Luc cells were treated with increasing concentrations of cocaine for 3 h
712 before being infected with 93/TH/051. After 3 h. nuclear extracts were examined for p-TRIM28 712 before being infected with 93/TH/051. After 3 h, nuclear extracts were examined for p-TRIM28
713 (S824) and TRIM28 total. The enhanced phosphorylation of TRIM28 at S824 (p-TRIM28-(S824) 713 (S824) and TRIM28 total. The enhanced phosphorylation of TRIM28 at S824 (p-TRIM28-(S824)
714 upon cocaine treatment was confirmed (Figures 8G & 8H). 714 upon cocaine treatment was confirmed (Figures 8G & 8H).
715

716 Subsequently, to determine if the cocaine-induced DNA-PK is responsible for TRIM28
717 phosphorylation (p-TRIM28 S824), we examined the impact of DNA-PK inhibition on TRIM28 phosphorylation (p-TRIM28 S824), we examined the impact of DNA-PK inhibition on TRIM28

718 phosphorylation. We found a dose-dependent inhibition of TRIM28 phosphorylation and almost
719 complete elimination of TRIM28 phosphorylation (p-TRIM28 S824) in cells treated with 10 µM 719 complete elimination of TRIM28 phosphorylation (p-TRIM28 S824) in cells treated with 10 μ M
720 M3814 (**Figures 8I and 8J**). Together, these findings confirm that cocaine-induced DNA-PK plays a 720 M3814 (**Figures 8I and 8J**). Together, these findings confirm that cocaine-induced DNA-PK plays a
721 vital role in RNAP II pause release by enhancing TRIM28 phosphorylation at a specific site (p-721 vital role in RNAP II pause release by enhancing TRIM28 phosphorylation at a specific site (p-
722 TRIM28-(S824), which converts TRIM28 from an inhibitory factor to a transactivator (Fiqures 8). ⁷²²TRIM28-(S824), which converts TRIM28 from an inhibitory factor to a transactivator (**Figures 8**).

723 We further confirmed the specific role of cocaine-stimulated DNA-PK in catalyzing phosphorylation
724 of TRIM28 and reversing its inhibitory effect during HIV transcription by performing experiments 724 of TRIM28 and reversing its inhibitory effect during HIV transcription by performing experiments
725 using DNA-PK knock down (KD) cells. Cells were infected with lentiviral vectors expressing shRNA 725 using DNA-PK knock down (KD) cells. Cells were infected with lentiviral vectors expressing shRNA
726 either against catalytic subunit of DNA-PK (DNA-PKcs) or scrambled shRNA, which do not target 726 either against catalytic subunit of DNA-PK (DNA-PKcs) or scrambled shRNA, which do not target
727 any cellular gene. These cells were treated with cocaine for 30 min and 3 h. Later, phosphorylation 727 any cellular gene. These cells were treated with cocaine for 30 min and 3 h. Later, phosphorylation
728 of p-TRIM28 at S824 and total TRIM28 was analyzed. In DNA-PK knockdown cells, we observed a 728 of p-TRIM28 at S824 and total TRIM28 was analyzed. In DNA-PK knockdown cells, we observed a
729 clear reduction in the levels of p-TRIM28-(S824) but not TRIM28 (Figures 8K & 8L). However, in 729 clear reduction in the levels of p-TRIM28-(S824) but not TRIM28 (**Figures 8K & 8L**). However, in
730 cells harboring scrambled shRNA, which express normal levels of DNA-PK, we noted enhanced 730 cells harboring scrambled shRNA, which express normal levels of DNA-PK, we noted enhanced
731 phosphorylation level of p-TRIM28 upon the cocaine exposure, validating our previous results. We 731 phosphorylation level of p-TRIM28 upon the cocaine exposure, validating our previous results. We
732 also noted the level of phosphorylated TRIM28 remains reduced in the DNA-PK KD cells upon 732 also noted the level of phosphorylated TRIM28 remains reduced in the DNA-PK KD cells upon
733 exposure to cocaine, confirming that cocaine induces TRIM28 phosphorylation is DNA-PK specific. 733 exposure to cocaine, confirming that cocaine induces TRIM28 phosphorylation is DNA-PK specific.
734 Thus, the results demonstrated that the enhanced phosphorylation of TRIM28 induced by cocaine 734 Thus, the results demonstrated that the enhanced phosphorylation of TRIM28 induced by cocaine
735 is directly associated with the stimulation of DNA-PK triggered by cocaine (Figure 8K). 735 is directly associated with the stimulation of DNA-PK triggered by cocaine (Figure 8K).
736

737 737 To understand the cellular kinetics of TRIM28, we analyzed the cytosolic and nuclear levels of p-
738 TRIM28 (S824) and TRIM28 upon cocaine exposure. We also analyzed the impact of cocaine on 738 TRIM28 (S824) and TRIM28 upon cocaine exposure. We also analyzed the impact of cocaine on
739 two main RNAP II pausing factors, namely DSIF (SPT-5) and NELF (NELF-E). Interestingly, we did 739 two main RNAP II pausing factors, namely DSIF (SPT-5) and NELF (NELF-E). Interestingly, we did
740 not observe any significant changes in DSIF and NELF upon cocaine exposure (**Figure 9A, 9B &** 740 not observe any significant changes in DSIF and NELF upon cocaine exposure (**Figure 9A, 9B &** 741 9C). These results clearly document that cocaine primarily relieves RNAP II pausing by inducing 741 **9C**). These results clearly document that cocaine primarily relieves RNAP II pausing by inducing
742 DNA-PK mediated phosphorylation of TRIM28 (p-TRIM28-(S824). Altogether, our data validate that 742 DNA-PK mediated phosphorylation of TRIM28 (p-TRIM28-(S824). Altogether, our data validate that
743 cocaine-stimulated DNA-PK relives RNAP II pausing by antagonizing the effect of negative/pausing 743 cocaine-stimulated DNA-PK relives RNAP II pausing by antagonizing the effect of negative/pausing
744 factors, mainly TRIM28, via its phosphorvlation at ser824 (p-TRIM28-(S824), during HIV 744 factors, mainly TRIM28, via its phosphorylation at ser824 (p-TRIM28-(S824), during HIV
745 transcription. 745 transcription.
746

747 ⁷⁴⁷*3.8. Cocaine boosts HIV transcription by enhancing the recruitment of DNA-PK and* ⁷⁴⁸*pTRIM28 at HIV LTR promoter.*

750 750 Previously, we documented the parallel presence of DNA-PK along with RNAP II throughout the
751 HIV proviral genome during HIV transcription [37, 39]. Additionally, we have shown the recruitment 751 HIV proviral genome during HIV transcription [37, 39]. Additionally, we have shown the recruitment
752 of TRIM28 at HIV long terminal repeat (LTR) during HIV transcription [39]. We also found that cell 752 of TRIM28 at HIV long terminal repeat (LTR) during HIV transcription [39]. We also found that cell
753 activation enhances both the nuclear translocation and LTR recruitment of DNA-PK [39]. Given that 753 activation enhances both the nuclear translocation and LTR recruitment of DNA-PK [39]. Given that
754 cocaine further augments the nuclear translocation of DNA-PK, we hypothesize that enhanced 754 cocaine further augments the nuclear translocation of DNA-PK, we hypothesize that enhanced
755 nuclear translocation of DNA-PK should translate into higher recruitment of DNA-PK and TRIM28 at 755 nuclear translocation of DNA-PK should translate into higher recruitment of DNA-PK and TRIM28 at
756 HIV LTR. To test this hypothesis, we evaluated the recruitment of DNA-PK and p-TRIM28-(S824) at 756 HIV LTR. To test this hypothesis, we evaluated the recruitment of DNA-PK and p-TRIM28-(S824) at
757 HIV LTR in the presence and absence of cocaine by chromatin Immunoprecipitation (ChIP) assay 757 HIV LTR in the presence and absence of cocaine by chromatin Immunoprecipitation (ChIP) assay
758 using our standard methodology [28, 37, 68, 93]. The ChIP assays were performed using 758 using our standard methodology [28, 37, 68, 93]. The ChIP assays were performed using
759 antibodies, namely IgG (control), DNA-PKcs, RNAP II, CDK7, CDK9, pTRIM28 (S824), and 759 antibodies, namely IgG (control), DNA-PKcs, RNAP II, CDK7, CDK9, pTRIM28 (S824), and
760 H3K27me3. The analysis was done in Jurkat cells freshly infected with p-HR'P-Luc (Figure 2A). 760 H3K27me3. The analysis was done in Jurkat cells freshly infected with p-HR'P-Luc (**Figure 2A**).
761 The recruitment of RNAP II at HIV LTR was assessed as positive control to mark ongoing HIV The recruitment of RNAP II at HIV LTR was assessed as positive control to mark ongoing HIV

762 transcription. We examined CDK7 as a marker of transcriptional initiation, as CDK7 (TFIIH) plays a
763 trole during the initiation phase of HIV transcription. The recruitment of CDK9 (P-TEFb) at HIV LTR 763 role during the initiation phase of HIV transcription. The recruitment of CDK9 (P-TEFb) at HIV LTR
764 was evaluated to indicate the elongation phase of HIV transcription, as recruitment of P-TEFb is 764 was evaluated to indicate the elongation phase of HIV transcription, as recruitment of P-TEFb is
765 crucial to support HIV transcriptional elongation. The immunoprecipitated DNA was analyzed using 765 crucial to support HIV transcriptional elongation. The immunoprecipitated DNA was analyzed using
766 four primer sets targeting different regions of HIV LTR. The first primer set amplifies the promoter 766 four primer sets targeting different regions of HIV LTR. The first primer set amplifies the promoter
767 region of the LTR (-116 to +4 with respect to the transcription start site. Figures 10A & 10E). The 767 region of the LTR (-116 to +4 with respect to the transcription start site, **Figures 10A & 10E**). The 768 second primer set amplifies the Nuc-1 region of the LTR (+30 to +134 with respect to the 768 second primer set amplifies the Nuc-1 region of the LTR (+30 to +134 with respect to the 769 transcription start site, **Figures 10B & 10F**). The factors that mainly bind at the promoter and Nuc-1 769 transcription start site, **Figures 10B & 10F**). The factors that mainly bind at the promoter and Nuc-1
770 region usually mark factors involved in the initiation phase of HIV transcription. The third primer set 770 region usually mark factors involved in the initiation phase of HIV transcription. The third primer set
771 amplifies the downstream Nuc-2 region of the LTR (+283 to +390 with respect to the transcription 771 amplifies the downstream Nuc-2 region of the LTR (+283 to +390 with respect to the transcription
772 start site, Figure 10C & 10G). The fourth primer set amplifies further downstream ENV region of 772 start site, **Figure 10C & 10G**). The fourth primer set amplifies further downstream ENV region of 773 HIV (+2599 to +2697, Figure 10D & 10H). The factors that bind around Nuc-2 region and 773 HIV (+2599 to +2697, **Figure 10D & 10H**). The factors that bind around Nuc-2 region and
774 downstream primarily represent those involved in the elongation phase of transcription. Following 774 downstream primarily represent those involved in the elongation phase of transcription. Following
775 cocaine treatment, as anticipated, we found higher recruitment of RNAP II showing upregulation of 775 cocaine treatment, as anticipated, we found higher recruitment of RNAP II showing upregulation of 776 HIV transcription. Moreover, enhanced RNAP II levels at promoter, Nuc-1, Nuc-2, and Env region of 776 HIV transcription. Moreover, enhanced RNAP II levels at promoter, Nuc-1, Nuc-2, and Env region of
777 provirus in cocaine treated cells indicate enhanced ongoing HIV gene expression upon cocaine 777 provirus in cocaine treated cells indicate enhanced ongoing HIV gene expression upon cocaine
778 treatment. Interestingly, in parallel to the recruitment of RNAP II, we observed significantly 778 treatment. Interestingly, in parallel to the recruitment of RNAP II, we observed significantly
779 enhanced recruitment of DNA-PKcs at the promoter, Nuc-1, Nuc-2, and the Env regions of LTR 779 enhanced recruitment of DNA-PKcs at the promoter, Nuc-1, Nuc-2, and the Env regions of LTR
780 following cocaine treatment (**Figures 10A, 10B, 10C & 10D**). These results corroborate our 780 following cocaine treatment (**Figures 10A, 10B, 10C & 10D**). These results corroborate our
781 previous data, where we showed the continuous presence and gliding of DNA-PKcs with RNAP II 781 previous data, where we showed the continuous presence and gliding of DNA-PKcs with RNAP II
782 along the HIV genome during transcription [37]. Notably, we also found enrichment of p-TRIM28-782 along the HIV genome during transcription [37]. Notably, we also found enrichment of p-TRIM28-
783 (S824) at the promoter and Nuc-1 region (Figures 10E & 10F). However, we did not observe 783 (S824) at the promoter and Nuc-1 region (**Figures 10E & 10F**). However, we did not observe
784 significant changes in the Nuc-2 and Env region of HIV LTR (**Figures 10G & 10H**). Meanwhile, we 784 significant changes in the Nuc-2 and Env region of HIV LTR (**Figures 10G & 10H**). Meanwhile, we
785 noted substantially higher recruitment of CDK7 (kinase subunit of TFIIH) at promoter and Nuc-1 of 785 noted substantially higher recruitment of CDK7 (kinase subunit of TFIIH) at promoter and Nuc-1 of 786 the LTR region. However, we observed a decrease of CDK7 recruitment at Nuc-2 but no significant 786 the LTR region. However, we observed a decrease of CDK7 recruitment at Nuc-2 but no significant
787 changes in the Env region (**Figures 10E, 10F, 10G & 10H**). The finding that after cocaine 787 changes in the Env region (**Figures 10E, 10F, 10G & 10H**). The finding that after cocaine
788 stimulation, CDK7 was enriched at the LTR promoter but not at the Nuc-2 region validates its 788 stimulation, CDK7 was enriched at the LTR promoter but not at the Nuc-2 region validates its
789 involvement specifically during the initiation phase of HIV transcription. Interestingly, the loss of 789 involvement specifically during the initiation phase of HIV transcription. Interestingly, the loss of 790 H3K27Me3 from HIV LTR following cocaine treatment demonstrates the removal of transcriptionally 790 H3K27Me3 from HIV LTR following cocaine treatment demonstrates the removal of transcriptionally
791 Frepressive (heterochromatin) structure and establishment of transcriptionally active (euchromatin) 791 repressive (heterochromatin) structure and establishment of transcriptionally active (euchromatin)
792 structure at HIV LTR following cocaine treatment. These data further validate our previous findings, 792 structure at HIV LTR following cocaine treatment. These data further validate our previous findings,
793 vhere we showed that cocaine enhances HIV transcription by promoting euchromatin structure at 793 where we showed that cocaine enhances HIV transcription by promoting euchromatin structure at
794 HIV LTR [28]. As anticipated, following cocaine exposure, we also found enhanced recruitment of 794 HIV LTR [28]. As anticipated, following cocaine exposure, we also found enhanced recruitment of 795 CDK9 (kinase subunit of P-TEFB) specifically at the downstream region of LTR but not much at the 795 CDK9 (kinase subunit of P-TEFB) specifically at the downstream region of LTR but not much at the 796 September 796 September 796 September 706 September 706 September 796 September 706 September 706 September 706 Septe 796 promoter region, validating its role during the elongation phase of transcription (**Figure 10G & 10H**).
797 Following cocaine exposure, the specific enrichment of CDK9 (P-TEFb) at the downstream region 797 Following cocaine exposure, the specific enrichment of CDK9 (P-TEFb) at the downstream region
798 of LTR and CDK7 (TFIIH) at the promoter region validates the authenticity of our assay system and 798 of LTR and CDK7 (TFIIH) at the promoter region validates the authenticity of our assay system and
799 ChIP analysis. 799 ChIP analysis.
800 Overall

⁸⁰⁰Overall, our results demonstrate that cocaine stimulates and enhances the nuclear 801 translocation and catalytic activity of DNA-PK (p-DNA-PK S2056), which leads to its higher
802 recruitment at HIV LTR. DNA-PK subsequently catalyzes the phosphorylation of TRIM28 (p-802 recruitment at HIV LTR. DNA-PK subsequently catalyzes the phosphorylation of TRIM28 (p-
803 TRIM28 S824) and converts TRIM28 from a pausing factor to a transcription activator. Overall, 803 TRIM28 S824) and converts TRIM28 from a pausing factor to a transcription activator. Overall, 804 these modifications relieve RNAP II pausing and promote HIV transcriptional elongation, a 804 these modifications relieve RNAP II pausing and promote HIV transcriptional elongation, a
805 necessity to make complete HIV genomic transcripts, which are required for generating viral 805 necessity to make complete HIV genomic transcripts, which are required for generating viral 806 progeny. progeny.

807

808 ⁸⁰⁸*3.9. Cocaine induced DNA-PK activation promotes HIV transcription by supporting several aspects of* ⁸⁰⁹*HIV transcription.*

810 To summarize our findings from current and previous investigations, we present the following model
811 for DNA-PK role during HIV transcription (Figure 11) [37, 39]. In our previous studies, we have 811 for DNA-PK role during HIV transcription (**Figure 11**) [37, 39]. In our previous studies, we have 812 established the association of DNA-PK and RNAP II along HIV proviral DNA template throughout 812 established the association of DNA-PK and RNAP II along HIV proviral DNA template throughout
813 HIV gene expression. In this study, we found that cocaine exposure augments the nuclear 813 HIV gene expression. In this study, we found that cocaine exposure augments the nuclear
814 translocation and functional activation of DNA-PK (p-DNA-PK S2056). DNA-PK subsequently 814 translocation and functional activation of DNA-PK (p-DNA-PK S2056). DNA-PK subsequently
815 facilitates the multiple critical phases of HIV transcription, namely initiation, RNAP II pause release, 815 facilitates the multiple critical phases of HIV transcription, namely initiation, RNAP II pause release, 816 and elongation. Cocaine-induced DNA-PK promotes the initiation phase of transcription by 816 and elongation. Cocaine-induced DNA-PK promotes the initiation phase of transcription by
817 catalvzing the phosphorvlation of RNAP II CTD at Ser5. In addition, cocaine-stimulated DNA-PK 817 catalyzing the phosphorylation of RNAP II CTD at Ser5. In addition, cocaine-stimulated DNA-PK
818 facilitates the elongation phase of HIV transcription by both directly catalyzing and promoting the 818 facilitates the elongation phase of HIV transcription by both directly catalyzing and promoting the
819 recruitment of P-TEFb for the phosphorvlation of Ser2 within the RNAP II CTD. The 819 recruitment of P-TEFb for the phosphorylation of Ser2 within the RNAP II CTD. The 820 hyperphosphorylation of RNAP II CTD at Ser2 makes RNAP II processive or elongation proficient. 820 hyperphosphorylation of RNAP II CTD at Ser2 makes RNAP II processive or elongation proficient.
821 Another noteworthy finding is that cocaine-stimulated DNA-PK relieves the RNAP II pausing 821 Another noteworthy finding is that cocaine-stimulated DNA-PK relieves the RNAP II pausing
822 selectively through TRIM28 by catalyzing TRIM28 phosphorylation at Ser824 (p-TRIM28 S824). 822 selectively through TRIM28 by catalyzing TRIM28 phosphorylation at Ser824 (p-TRIM28 S824).
823 This modification transforms TRIM28 from a transcription pausing factor to a transcription-823 This modification transforms TRIM28 from a transcription pausing factor to a transcription-
824 supporting factor. Thus, phosphorylation of TRIM28 at Ser824 relieves RNAP II pausing and allows 824 supporting factor. Thus, phosphorylation of TRIM28 at Ser824 relieves RNAP II pausing and allows
825 RNAP II to proceed along DNA template or transcriptional elongation. Our findings collectively 825 RNAP II to proceed along DNA template or transcriptional elongation. Our findings collectively
826 underscore the profound impact of cocaine-induced DNA-PK activation on various facets of HIV 826 underscore the profound impact of cocaine-induced DNA-PK activation on various facets of HIV
827 transcription, ultimately culminating in the potent promotion of viral gene expression. Therefore, 827 transcription, ultimately culminating in the potent promotion of viral gene expression. Therefore, 828 DNA-PK inhibitors profoundly inhibit HIV transcription. replication. DNA-PK inhibitors profoundly inhibit HIV transcription, replication, and latency-reactivation.

⁸²⁹**4. Discussion**

831 831 HIV/AIDS remain a dreadful disease, as an effective vaccine or cure is yet to develop [5, 94-98].
832 Nevertheless, with the introduction of ART, the quality of PLWH significantly increases [1, 6]. 832 Nevertheless, with the introduction of ART, the quality of PLWH significantly increases [1, 6].
833 However, one has to rely on medication for the rest of one's life to keep control of HIV disease 833 However, one has to rely on medication for the rest of one's life to keep control of HIV disease
834 progression. The anti-HIV therapy, ART, is highly effective in suppressing viral replication, 834 progression. The anti-HIV therapy, ART, is highly effective in suppressing viral replication,
835 maintaining healthy immune system, and reducing risk of HIV transmission. Unfortunately, cocaine, 835 maintaining healthy immune system, and reducing risk of HIV transmission. Unfortunately, cocaine, 836 one of the most abused drugs by HIV patients, can disrupt reqular activities potentially leading to 836 one of the most abused drugs by HIV patients, can disrupt regular activities potentially leading to 837 inconsistent or missed doses of ART. Poor adherence usually leads to treatment failure. 837 inconsistent or missed doses of ART. Poor adherence usually leads to treatment failure,
838 development of drug resistant HIV strain and compromised immune functions [99, 100]. Cocaine 838 development of drug resistant HIV strain and compromised immune functions [99, 100]. Cocaine
839 further affects the normal functioning of immune cells, suppressing the immune system and 839 further affects the normal functioning of immune cells, suppressing the immune system and
840 exacerbating the effect of HIV infection leading to faster disease progression, specially making HIV 840 exacerbating the effect of HIV infection leading to faster disease progression, specially making HIV
841 patients vulnerable to opportunistic infections. Furthermore, given that cocaine strongly impacts 841 patients vulnerable to opportunistic infections. Furthermore, given that cocaine strongly impacts
842 brain functioning, cocaine use by HIV patients not only accelerates HIV replication in the CNS but 842 brain functioning, cocaine use by HIV patients not only accelerates HIV replication in the CNS but
843 also exacerbates normal brain functioning. The interaction between cocaine and HIV is a 843 also exacerbates normal brain functioning. The interaction between cocaine and HIV is a
844 multifaceted and concerning issue. Therefore, understanding the molecular mechanisms that 844 multifaceted and concerning issue. Therefore, understanding the molecular mechanisms that
845 aovern HIV life cycle, especially transcription and replication, are crucial for relieving from HIV and 845 govern HIV life cycle, especially transcription and replication, are crucial for relieving from HIV and
846 cocaine induced neurotoxicity in addition to HIV cure and eradication [16, 17, 27, 39]. In this study, 846 cocaine induced neurotoxicity in addition to HIV cure and eradication [16, 17, 27, 39]. In this study, 847 we showed the pivotal role played by cocaine-induced activation of DNA-PK in bolstering various 847 we showed the pivotal role played by cocaine-induced activation of DNA-PK in bolstering various
848 stages of HIV transcription, consequently augmenting HIV replication. Our investigation has 848 stages of HIV transcription, consequently augmenting HIV replication. Our investigation has
849 unveiled that cocaine significantly upregulates the expression of DNA-PK, prompts its translocation unveiled that cocaine significantly upregulates the expression of DNA-PK, prompts its translocation

850 into the nucleus, and enhances the functional activity of DNA-PK by enhancing its phosphorylation
851 at S2056. Subsequently, the cocaine-induced DNA-PK facilitates transcriptional initiation by 851 at S2056. Subsequently, the cocaine-induced DNA-PK facilitates transcriptional initiation by
852 augmenting the phosphorylation of CTD at Ser5, relieves RNAP II pausing through TRIM28 852 augmenting the phosphorylation of CTD at Ser5, relieves RNAP II pausing through TRIM28
853 phosphorylation at S824, and promotes transcriptional elongation both by directly catalyzing the 853 phosphorylation at S824, and promotes transcriptional elongation both by directly catalyzing the
854 phosphorylation of CTD at Ser2 and through P-TEFb stimulation and recruitment. Accordingly, upon 854 phosphorylation of CTD at Ser2 and through P-TEFb stimulation and recruitment. Accordingly, upon
855 specific inhibition or depletion of DNA-PK using specific inhibitors or knockdown, respectively, we 855 specific inhibition or depletion of DNA-PK using specific inhibitors or knockdown, respectively, we
856 found profound restriction to cocaine-induced HIV transcription and replication. These collective 856 found profound restriction to cocaine-induced HIV transcription and replication. These collective
857 results unveil the underlying molecular mechanisms through which cocaine-induced DNA-PK 857 results unveil the underlying molecular mechanisms through which cocaine-induced DNA-PK
858 stimulation augments HIV transcription and replication. 858 stimulation augments HIV transcription and replication.
859

860 860 DNA-PK is a serine/threonine protein kinase complex composed of a heterodimer of Ku proteins
861 (Ku70/Ku80) and a catalytic subunit DNA-PKcs [40, 41]. DNA-PK is a critical component of the 861 (Ku70/Ku80) and a catalytic subunit DNA-PKcs [40, 41]. DNA-PK is a critical component of the
862 cellular response following DNA damage [40, 41]. DNA-PK is one of the main components of DNA 862 cellular response following DNA damage [40, 41]. DNA-PK is one of the main components of DNA
863 repair pathway upon double-strand breaks, especially in the NHEJ DNA double-strand break repair 863 repair pathway upon double-strand breaks, especially in the NHEJ DNA double-strand break repair
864 pathway [43, 44], Therefore, DNA-PK is extensively studied in DNA double strand break repair, The 864 pathway [43, 44]. Therefore, DNA-PK is extensively studied in DNA double strand break repair. The 865 DNA-PK role in HIV transcription was first identified as a complex that phosphorylates the 865 DNA-PK role in HIV transcription was first identified as a complex that phosphorylates the
866 transcription factor SP1 [101] and as a interacting component of RNAP II [47]. Nevertheless, its role 866 transcription factor SP1 [101] and as a interacting component of RNAP II [47]. Nevertheless, its role
867 in transcription was understudied. For the first time, we demonstrated the precise role of DNA-PK 867 in transcription was understudied. For the first time, we demonstrated the precise role of DNA-PK
868 during any transcription process by defining the mechanism through which DNA-PK promotes HIV 868 during any transcription process by defining the mechanism through which DNA-PK promotes HIV
869 transcription and involved mechanisms [37, 39]. Later, several studies emerged that further 869 transcription and involved mechanisms [37, 39]. Later, several studies emerged that further
870 strengthened the link between DNA-PK and transcriptional regulation [102]. Given that, HIV 870 strengthened the link between DNA-PK and transcriptional regulation [102]. Given that, HIV
871 transcription is the fundamental step that plays a crucial role in regulating HIV replication and 871 transcription is the fundamental step that plays a crucial role in regulating HIV replication and
872 latency-reactivation. In our previous studies we have documented the underlying molecular 872 latency-reactivation. In our previous studies we have documented the underlying molecular
873 mechanism through which DNA-PK promotes HIV transcription [39]. Moreover, we found that 873 mechanism through which DNA-PK promotes HIV transcription [39]. Moreover, we found that
874 cocaine also enhances HIV transcription and replication [16, 27, 28]. These facts prompted us to 874 cocaine also enhances HIV transcription and replication [16, 27, 28]. These facts prompted us to
875 study if the cocaine-enhanced HIV transcription and replication is due to the activation of DNA-PK. 875 study if the cocaine-enhanced HIV transcription and replication is due to the activation of DNA-PK.
876 In this investigation, we demonstrated that cocaine significantly upregulates nuclear level of DNA-876 In this investigation, we demonstrated that cocaine significantly upregulates nuclear level of DNA-
877 PK and augments its activity by enhancing its phosphorylation at Serine 2056 residues. We 877 PK and augments its activity by enhancing its phosphorylation at Serine 2056 residues. We
878 reproduced these findings in cells of different lineages, including both lymphoid and myeloid 878 reproduced these findings in cells of different lineages, including both lymphoid and myeloid
879 lineages. Given that in our previous findings we noted higher recruitment of DNA-PK at HIV LTR 879 lineages. Given that in our previous findings we noted higher recruitment of DNA-PK at HIV LTR
880 following cell stimulation [37, 39], we evaluated if cell stimulation by cocaine also results in 880 following cell stimulation [37, 39], we evaluated if cell stimulation by cocaine also results in
881 enhanced nuclear translocation of DNA-PK. We found that cocaine-induced cell stimulation was 881 enhanced nuclear translocation of DNA-PK. We found that cocaine-induced cell stimulation was
882 sufficient and promoted the nuclear translocation of DNA-PK (Figure 1). Interestingly, the nuclear 882 sufficient and promoted the nuclear translocation of DNA-PK (Figure 1). Interestingly, the nuclear
883 levels of the DNA-PK significantly increased following cocaine treatment with the corresponding 883 levels of the DNA-PK significantly increased following cocaine treatment with the corresponding
884 decrease in the cytoplasmic levels, indicating the translocation of DNA-PK towards the nucleus 884 decrease in the cytoplasmic levels, indicating the translocation of DNA-PK towards the nucleus
885 (Figures 11 & 1J). Subsequently, we analyzed the corresponding uprequlation of DNA-PK 885 (**Figures 1I & 1J)**. Subsequently, we analyzed the corresponding upregulation of DNA-PK
886 recruitment of DNA-PK due to higher availability of DNA-PK in the nucleus by ChIP assay. As 886 recruitment of DNA-PK due to higher availability of DNA-PK in the nucleus by ChIP assay. As
887 expected, upon cocaine exposure, we found a notable increase in the recruitment of DNA-PK. 887 expected, upon cocaine exposure, we found a notable increase in the recruitment of DNA-PK.
888 Additionally, along with DNA-PK, we found the corresponding higher recruitment of RNAP II at HIV 888 Additionally, along with DNA-PK, we found the corresponding higher recruitment of RNAP II at HIV
889 LTR following cocaine treatment (Figure 10A, 10B, 10C & 10D). This finding reaffirmed our prior 889 LTR following cocaine treatment (**Figure 10A, 10B, 10C & 10D**). This finding reaffirmed our prior
890 findings where we established DNA-PK interaction with RNAP II and showed parallel-enhanced 890 findings where we established DNA-PK interaction with RNAP II and showed parallel-enhanced
891 recruitment of both DNA-PK and RNAP II following cell stimulation [37]. Interestingly, paralleling the 891 recruitment of both DNA-PK and RNAP II following cell stimulation [37]. Interestingly, paralleling the 892 recruitment of RNAP II, we also noted an augmented recruitment of pNA-PK not only at the 892 recruitment of RNAP II, we also noted an augmented recruitment of DNA-PK not only at the
893 promoter and Nuc-1 regions but also at the downstream regions of the HIV genome (Figure 10A, 893 promoter and Nuc-1 regions but also at the downstream regions of the HIV genome (**Figure 10A,** *10B*, **10C & 10D**). This validates the role of DNA-PK in different phases of HIV transcription, **10B, 10C & 10D**). This validates the role of DNA-PK in different phases of HIV transcription,

895 including initiation, RNAP II pause release, and elongation phases. Accordingly, we found higher
896 levels of RNAP II at the promoter, Nuc-1, and Env region of the provirus, signifying enhanced 896 levels of RNAP II at the promoter, Nuc-1, and Env region of the provirus, signifying enhanced
897 ongoing HIV gene expression following cocaine exposure. This observation further strengthens our 897 ongoing HIV gene expression following cocaine exposure. This observation further strengthens our 898 previous results proposing that DNA-PK and RNAP II are part of a larger transcription complex [37, 898 previous results proposing that DNA-PK and RNAP II are part of a larger transcription complex [37, 899 39]. Later, we assessed if HIV infection promotes cell stimulation and consequently DNA-PK 899 39]. Later, we assessed if HIV infection promotes cell stimulation and consequently DNA-PK
800 activation. Notably, we found significant upregulation of DNA-PK and its activation (p-DNA-PK 900 activation. Notably, we found significant upregulation of DNA-PK and its activation (p-DNA-PK
901 S2056), suggesting crucial role of DNA-PK during HIV transcription. Together, these findings 901 S2056), suggesting crucial role of DNA-PK during HIV transcription. Together, these findings
902 underscore the intricate relationship between cocaine exposure and the LTR recruitment of DNA-902 underscore the intricate relationship between cocaine exposure and the LTR recruitment of DNA-
903 PK, shedding light on the potential mechanism through which cocaine augments HIV transcription. 903 PK, shedding light on the potential mechanism through which cocaine augments HIV transcription.
904 Our previous findings, where we establish the vital role of DNA-PK during HIV transcriptio

904 Our previous findings, where we establish the vital role of DNA-PK during HIV transcription
905 [37, 39], has been extended by others. The role of DNA-PK in general cell transcription has also 905 [37, 39], has been extended by others. The role of DNA-PK in general cell transcription has also
906 been documented [60], validating the important role of DNA-PK during basic transcriptional 906 been documented [60], validating the important role of DNA-PK during basic transcriptional
907 process. To further validate our findings and establish the crucial role of cocaine in stimulating 907 process. To further validate our findings and establish the crucial role of cocaine in stimulating
908 DNA-PK during HIV transcription, we employed a highly specific DNA-PK inhibitor, M3814. The 908 DNA-PK during HIV transcription, we employed a highly specific DNA-PK inhibitor, M3814. The
909 dose-dependent inhibition of HIV transcription by M3814. indicated by reduced luciferase gene 909 dose-dependent inhibition of HIV transcription by M3814, indicated by reduced luciferase gene
910 expression from LTR promoter (Figure 3B & 3C), confirmed the direct role of DNA-PK in promoting 910 expression from LTR promoter (**Figure 3B & 3C**), confirmed the direct role of DNA-PK in promoting
911 HIV transcription. Given that, TNF-a was unable to reactivate the latent HIV in the presence of 911 HIV transcription. Given that, TNF-α was unable to reactivate the latent HIV in the presence of 912 M3814, demonstrating that DNA-PK inhibitors could be useful in restricting the reactivation of latent 912 M3814, demonstrating that DNA-PK inhibitors could be useful in restricting the reactivation of latent 913 HIV provirus as well (Figure 3E & 3F). Interestingly, we did not observe any noticeable cell toxicity 913 HIV provirus as well (**Figure 3E & 3F**). Interestingly, we did not observe any noticeable cell toxicity
914 with the used concentrations of M3814 (Figure 3D), establishing the physiological significance of 914 with the used concentrations of M3814 (Figure 3D), establishing the physiological significance of 915
915 the findings. Subsequently, we also evaluated the effect of two different DNA-PKcs inhibitors, 915 the findings. Subsequently, we also evaluated the effect of two different DNA-PKcs inhibitors,
916 M3814, and NU7441 on HIV replication. We found that the more specific DNA-PK inhibitors (DNA-916 M3814, and NU7441 on HIV replication. We found that the more specific DNA-PK inhibitors (DNA-
917 PKi) were better at repressing HIV gene expression and replication (Figure 4). This observation 917 PKi) were better at repressing HIV gene expression and replication (**Figure 4**). This observation
918 again confirmed the target-specific impact of DNA-PKi. Moreover, cell viability analysis validated the 918 again confirmed the target-specific impact of DNA-PKi. Moreover, cell viability analysis validated the 919
919 physiological viability of the pre-clinically and clinically tested DNA-PK inhibitors as potential HIV 919 physiological viability of the pre-clinically and clinically tested DNA-PK inhibitors as potential HIV
920 therapeutics. 920 therapeutics.
921

921 922 Previously, we identified both the presence of DNA-PK at HIV LTR and direct catalyzation of RNAP
923 II CTD phosphorylation by DNA-PK [37, 39]. We investigated whether cocaine induced HIV 923 II CTD phosphorylation by DNA-PK [37, 39]. We investigated whether cocaine induced HIV
924 transcription and replication is also due to DNA-PK stimulation and subsequently RNAP II CTD 924 transcription and replication is also due to DNA-PK stimulation and subsequently RNAP II CTD
925 phosphorylation, we examined the state of RNAP II CTD phosphorylation following cocaine 925 phosphorylation, we examined the state of RNAP II CTD phosphorylation following cocaine
926 exposure. The significant upregulation of Ser2 and Ser5 phosphorylation following cocaine 926 exposure. The significant upregulation of Ser2 and Ser5 phosphorylation following cocaine
927 treatment in a dose-dependent manner confirmed that cocaine augments HIV transcription by 927 treatment in a dose-dependent manner confirmed that cocaine augments HIV transcription by
928 supporting RNAP II CTD phosphorylation (Figure 6A & 6B). Given that, Ser5 phosphorylation is the 928 supporting RNAP II CTD phosphorylation (**Figure 6A & 6B**). Given that, Ser5 phosphorylation is the 929 marker of transcriptional initiation, and Ser2 phosphorylation is the 900gation phase of 929 marker of transcriptional initiation, and Ser2 phosphorylation is linked to the elongation phase of 930 transcription, including HIV transcription. The data obtained showed that cocaine facilitates both the 930 transcription, including HIV transcription. The data obtained showed that cocaine facilitates both the
931 initiation and elongation phases of transcription. The results were reproduced in the cells of multiple 931 initiation and elongation phases of transcription. The results were reproduced in the cells of multiple 932 lineages to show the ubiquitous prevalence of the observed phenomenon (Figure 6C, 6D, 6E & 932 lineages to show the ubiquitous prevalence of the observed phenomenon (**Figure 6C, 6D, 6E &** 933 **6F**). Subsequently, we explored whether cocaine-enhanced RNAP II phosphorylation is a result of 933 **6F**). Subsequently, we explored whether cocaine-enhanced RNAP II phosphorylation is a result of 934 DNA-PK activation using a clinically evaluated highly specific DNA-PK inhibitor (M3814) in the 934 DNA-PK activation using a clinically evaluated highly specific DNA-PK inhibitor (M3814) in the
935 presence of cocaine (Figures 6E & 6F). The dose-dependent inhibition of RNAP II CTD 935 presence of cocaine (**Figures 6E & 6F)**. The dose-dependent inhibition of RNAP II CTD
936 phosphorylation at both Ser2 and Ser5 sites by M3814 validated the specific role of DNA-PK in 936 phosphorylation at both Ser2 and Ser5 sites by M3814 validated the specific role of DNA-PK in
937 catalyzing CTD phosphorylation. Altogether, our findings confirmed our hypothesis that cocaine, 937 catalyzing CTD phosphorylation. Altogether, our findings confirmed our hypothesis that cocaine,
938 through activation of DNA-PK, significantly influences both the initiation and elongation phases of through activation of DNA-PK, significantly influences both the initiation and elongation phases of

939 HIV transcription, contributing to a more comprehensive understanding of the molecular mechanism
940 behind cocaine's impact on HIV gene expression. 940 behind cocaine's impact on HIV gene expression.
941

942 942 The previous studies have established the CDK9 subunit of P-TEFb as the main player that
943 promotes RNAP II processivity by catalyzing RNAP II CTD phosphorylation at Ser2 position; thus, 943 promotes RNAP II processivity by catalyzing RNAP II CTD phosphorylation at Ser2 position; thus,
944 supports the elongation phase of transcription [103, 104]. Therefore, we sought to investigate the 944 supports the elongation phase of transcription [103, 104]. Therefore, we sought to investigate the
945 nuclear level of P-TEFb. Analyzing the nuclear level, our results suggested that cocaine significantly 945 nuclear level of P-TEFb. Analyzing the nuclear level, our results suggested that cocaine significantly
946 enhances the phosphorylation of CDK9 and Cyclin T1, indicating that cocaine further supports the 946 enhances the phosphorylation of CDK9 and Cyclin T1, indicating that cocaine further supports the
947 ongoing elongation phase of HIV transcription through P-TEFb stimulation. Nevertheless, cocaine 947 ongoing elongation phase of HIV transcription through P-TEFb stimulation. Nevertheless, cocaine
948 does not affect the Total CDK9 level. Later, we examined the impact of cocaine on the initiation 948 does not affect the Total CDK9 level. Later, we examined the impact of cocaine on the initiation
949 phase of HIV transcription, and, as anticipated, we found significant upregulation of CDK7, a 949 phase of HIV transcription, and, as anticipated, we found significant upregulation of CDK7, a
950 subunit of TFIIH that is well known to support the initiation phase of transcription, including HIV 950 subunit of TFIIH that is well known to support the initiation phase of transcription, including HIV
951 transcription. These findings were validated in different cell types, both myeloid and lymphoid cells 951 transcription. These findings were validated in different cell types, both myeloid and lymphoid cells
952 (Figures 7A, 7B, 7C, 7D, 7E & 7F). To further validate that cocaine-induced phosphorylation of 952 (**Figures 7A, 7B, 7C, 7D, 7E & 7F**). To further validate that cocaine-induced phosphorylation of 953 CDK9 and activation of total CDK7 are indeed reliant on the specific activation of DNA-PKcs, we 953 CDK9 and activation of total CDK7 are indeed reliant on the specific activation of DNA-PKcs, we
954 conducted experiments using a DNA-PKcs knockdown cell line exposed to cocaine. In the absence 954 conducted experiments using a DNA-PKcs knockdown cell line exposed to cocaine. In the absence
955 of DNA-PKcs, we observed a marked decrease in p-CDK9 (Thr186) levels, as well as a reduction in 955 of DNA-PKcs, we observed a marked decrease in p-CDK9 (Thr186) levels, as well as a reduction in
956 total CDK9 and CDK7. Notably, in wild-type cells, exposure to cocaine resulted in the anticipated 956 total CDK9 and CDK7. Notably, in wild-type cells, exposure to cocaine resulted in the anticipated
957 enhancement of CDK7 and CDK9 phosphorylation, consistent with our previous findings. However, 957 enhancement of CDK7 and CDK9 phosphorylation, consistent with our previous findings. However,
958 in DNA-PKcs knockdown cells, the levels of pCDK9 (Thr186) and CDK7 remained reduced 958 in DNA-PKcs knockdown cells, the levels of pCDK9 (Thr186) and CDK7 remained reduced
959 following cocaine exposure, providing strong evidence that cocaine-induced CDK9 phosphorylation 959 following cocaine exposure, providing strong evidence that cocaine-induced CDK9 phosphorylation
960 and CDK7 activation are specifically mediated by DNA-PKcs. The impact of cocaine on both the 960 and CDK7 activation are specifically mediated by DNA-PKcs. The impact of cocaine on both the
961 initiation and elongation phases of HIV transcription was further validated by showing the presence 961 initiation and elongation phases of HIV transcription was further validated by showing the presence
962 of TFIIH (CDK7) and P-TEFb (CDK9 and CyclinT1), respectively, at HIV LTR (Fiqure 10) through 962 of TFIIH (CDK7) and P-TEFb (CDK9 and CyclinT1), respectively, at HIV LTR (**Figure 10**) through
963 ChIP assays, upon cocaine treatment. Given that P-TEFb plays a crucial role during the elongation 963 ChIP assays, upon cocaine treatment. Given that P-TEFb plays a crucial role during the elongation
964 phase, accordingly we found specific enrichment of CDK9 at the downstream region, namely, Nuc-2 964 phase, accordingly we found specific enrichment of CDK9 at the downstream region, namely, Nuc-2
965 and Env region of HIV, but highly reduced recruitment at promoter and the Nuc-1 region in cocaine 965 and Env region of HIV, but highly reduced recruitment at promoter and the Nuc-1 region in cocaine
966 treated cells (Figure 10). Similarly, after cocaine stimulation, CDK7 was enriched as expected at 966 treated cells (**Figure 10**). Similarly, after cocaine stimulation, CDK7 was enriched as expected at 967 the LTR promoter but not at the Nuc-2 region, again validating its requirement especially during the 967 the LTR promoter but not at the Nuc-2 region, again validating its requirement especially during the 968 initiation phase of HIV transcription. Our previous findings showed not only the direct interaction 968 initiation phase of HIV transcription. Our previous findings showed not only the direct interaction
969 between DNA-PK and RNAP II, but also parallel recruitment of DNA-PK along RNAP II at HIV LTR 969 between DNA-PK and RNAP II, but also parallel recruitment of DNA-PK along RNAP II at HIV LTR
970 upon cell stimulation [37, 39], In addition, we have shown cell stimulation following cocaine 970 upon cell stimulation [37, 39]. In addition, we have shown cell stimulation following cocaine
971 exposure [27]. These findings prompted us to investigate whether cocaine-mediated cell stimulation 971 exposure [27]. These findings prompted us to investigate whether cocaine-mediated cell stimulation
972 and induced DNA-PK activation enhances RNAP II CTD phosphorylation, both via directly 972 and induced DNA-PK activation enhances RNAP II CTD phosphorylation, both via directly
973 catalyzing and through promoting P-TEFb recruitment at HIV LTR. As expected, we found parallel 973 catalyzing and through promoting P-TEFb recruitment at HIV LTR. As expected, we found parallel
974 recruitment of DNA-PK and RNAP II along HIV genome following cocaine treatment, confirming that 974 recruitment of DNA-PK and RNAP II along HIV genome following cocaine treatment, confirming that 975 cocaine-induced cell stimulation is sufficient not only to activate DNA-PK (Figure 1), but also to 975 cocaine-induced cell stimulation is sufficient not only to activate DNA-PK (**Figure 1**), but also to 976 enrich DNA-PK at HIV LTR proportional to RNAP II recruitment at LTR (Figure 10). Interestingly, 976 enrich DNA-PK at HIV LTR proportional to RNAP II recruitment at LTR (**Figure 10**). Interestingly,
977 the decrease in the recruitment of H3K27Me3 at HIV LTR following cocaine treatment demonstrates 977 the decrease in the recruitment of H3K27Me3 at HIV LTR following cocaine treatment demonstrates
978 the loss of repressive epigenetic structure, and establishment of transcription-supporting 978 the loss of repressive epigenetic structure, and establishment of transcription-supporting
979 euchromatin structure, aligning with our previous findings [39]. Altogether, these findings 979 euchromatin structure, aligning with our previous findings [39]. Altogether, these findings
980 demonstrate that cocaine-induced DNA-PK facilitates transcriptional initiation by catalyzing the 980 demonstrate that cocaine-induced DNA-PK facilitates transcriptional initiation by catalyzing the
981 RNAP II CTD at Ser5. Furthermore, cocaine-mediated DNA-PK stimulation augments the 981 RNAP II CTD at Ser5. Furthermore, cocaine-mediated DNA-PK stimulation augments the
982 elongation phase of HIV transcription by enhancing the phosphorylation of RNAP II CTD at Ser2 982 elongation phase of HIV transcription by enhancing the phosphorylation of RNAP II CTD at Ser2
983 both via directly catalyzing and promoting the recruitment of P-TEFb. both via directly catalyzing and promoting the recruitment of P-TEFb.

984
985 985 We also explored whether cocaine can facilitate HIV transcription by promoting RNAP II pause
986 release. We found that cocaine profoundly enhances TRIM28 phosphorylation at its serine 824 986 release. We found that cocaine profoundly enhances TRIM28 phosphorylation at its serine 824
987 residue. This specific phosphorylation event relieves the TRIM28-mediated pausing to RNAP II and 987 residue. This specific phosphorylation event relieves the TRIM28-mediated pausing to RNAP II and
988 even converts TRIM28 into a transcription-supporting factor [58, 60]. The established interaction 988 even converts TRIM28 into a transcription-supporting factor [58, 60]. The established interaction
989 between TRIM28 and RNAP II underscores the significant role of TRIM28 in regulating HIV 989 between TRIM28 and RNAP II underscores the significant role of TRIM28 in regulating HIV
990 transcription. Additionally, our studies, in line with previous research, have elucidated that DNA-PK 990 transcription. Additionally, our studies, in line with previous research, have elucidated that DNA-PK
991 interacts with TRIM28 and catalyzes its phosphorylation at serine 824, resulting in the formation of 991 interacts with TRIM28 and catalyzes its phosphorylation at serine 824, resulting in the formation of 992
992 p-TRIM28-(S824) [39, 60]. This phosphorylation event has been associated with positive elongation 992 p-TRIM28-(S824) [39, 60]. This phosphorylation event has been associated with positive elongation
993 factors, suggesting its potential role in facilitating the transition from transcriptional pausing to 993 factors, suggesting its potential role in facilitating the transition from transcriptional pausing to
994 elongation. Consequently, this modification transforms TRIM28 from a transcriptionally repressive 994 elongation. Consequently, this modification transforms TRIM28 from a transcriptionally repressive
995 factor into a transcriptionally active one. Therefore, we investigated whether cocaine can convert 995 factor into a transcriptionally active one. Therefore, we investigated whether cocaine can convert
996 TRIM28 from a transcriptionally repressive factor to a transcriptionally active one by examining the 996 TRIM28 from a transcriptionally repressive factor to a transcriptionally active one by examining the 997 phosphorylation of TRIM28 at S824. We observed that, upon cocaine exposure, the 997 phosphorylation of TRIM28 at S824. We observed that, upon cocaine exposure, the
998 phosphorylation of TRIM28 at S824 significantly increases in a dose-dependent-manner (Fiqure 8A 998 phosphorylation of TRIM28 at S824 significantly increases in a dose dependent manner (**Figure 8A**
999 **& 8B**). These findings were confirmed in cells of different lineages, validating the uniformity of the 999 **& 8B**). These findings were confirmed in cells of different lineages, validating the uniformity of the 000 findings (**Figure 8C, 8D, 8E, 8F, 8G, 8H, 8I & 8J**). Later, we analyzed both cytosolic and nuclear 000 findings (**Figure 8C, 8D, 8E, 8F, 8G, 8H, 8I & 8J**). Later, we analyzed both cytosolic and nuclear
001 levels of p-TRIM28 (S824) and TRIM28 upon cocaine exposure. We noted a significant increase in 001 levels of p-TRIM28 (S824) and TRIM28 upon cocaine exposure. We noted a significant increase in
002 nuclear levels of p-TRIM28 (S824) in cocaine treated cells, but TRIM28 total did not change 002 nuclear levels of p-TRIM28 (S824) in cocaine treated cells, but TRIM28 total did not change
003 significantly (**Figure 9A**). These findings further validated the cocaine-induced activation and 003 significantly (**Figure 9A**). These findings further validated the cocaine-induced activation and
004 phosphorvlation of TRIM28 at S824. Subsequently, we analyzed the recruitment of p-TRIM28-004 phosphorylation of TRIM28 at S824. Subsequently, we analyzed the recruitment of p-TRIM28-
005 (S824) at HIV LTR using ChIP assavs. As anticipated. we noted enhanced recruitment of 005 (S824) at HIV LTR using ChIP assays. As anticipated, we noted enhanced recruitment of
006 phosphorylated TRIM28 (S824) in parallel to DNA-PK recruitment along HIV genome after cocaine 006 phosphorylated TRIM28 (S824) in parallel to DNA-PK recruitment along HIV genome after cocaine
007 treatment (Figure 10E & 10F). The accumulation of p-TRIM28 (S824) marks the presence of the 007 treatment (**Figure 10E & 10F**). The accumulation of p-TRIM28 (S824) marks the presence of the 008 transcription-supporting form of TRIM28 and thus indicates the transformation of paused RNAP II 008 transcription-supporting form of TRIM28 and thus indicates the transformation of paused RNAP II
009 into a processive elongating RNAP II. This observation strongly suggests that by enhancing the 009 into a processive elongating RNAP II. This observation strongly suggests that by enhancing the
010 phosphorylation of TRIM28, cocaine effectively alleviates RNAP II pausing, thereby providing 010 phosphorylation of TRIM28, cocaine effectively alleviates RNAP II pausing, thereby providing
011 essential support to the process of HIV transcription. This is another molecular mechanism through 011 essential support to the process of HIV transcription. This is another molecular mechanism through
012 which cocaine influences the regulation of transcriptional processes, specifically within the context 012 which cocaine influences the regulation of transcriptional processes, specifically within the context
013 of HIV gene expression. We further investigated whether cocaine-induced phosphorylation of 013 of HIV gene expression. We further investigated whether cocaine-induced phosphorylation of
014 TRIM28 at S824 is a result of cocaine induced-DNA-PK activation. Upon treating cells with a 014 TRIM28 at S824 is a result of cocaine induced-DNA-PK activation. Upon treating cells with a
015 specific DNA-PK inhibitor. we observed dose-dependent inhibition of cocaine-induced 015 specific DNA-PK inhibitor, we observed dose-dependent inhibition of cocaine-induced
016 phosphorylation of TRIM28 at S824 (Figure 8I & 8J). This finding confirms the critical role played 016 phosphorylation of TRIM28 at S824 (**Figure 8I & 8J**). This finding confirms the critical role played 017 by DNA-PK in promoting RNAP II pause release by selectively catalyzing TRIM28 phosphorylation 017 by DNA-PK in promoting RNAP II pause release by selectively catalyzing TRIM28 phosphorylation
018 at S824 and subsequently promoting HIV transcription following cocaine exposure. 018 at S824 and subsequently promoting HIV transcription following cocaine exposure.
019

020 020 Overall, our findings presented here provide compelling and robust evidence affirming the pivotal
021 vrole plaved by cocaine on HIV transcription and gene expression. Our investigations have revealed 021 role played by cocaine on HIV transcription and gene expression. Our investigations have revealed
022 that cocaine significantly upregulates the nuclear levels of DNA-PK, augments its catalytic activity 022 that cocaine significantly upregulates the nuclear levels of DNA-PK, augments its catalytic activity
023 through specific phosphorvlation at S2056, besides enhancing its nuclear translocation. We found 023 through specific phosphorylation at S2056, besides enhancing its nuclear translocation. We found
024 that cocaine-induced activation of DNA-PK significantly contributes to various stages of HIV 024 that cocaine-induced activation of DNA-PK significantly contributes to various stages of HIV
025 transcription, subsequently bolstering the process of HIV replication. Specifically, the activation of 025 transcription, subsequently bolstering the process of HIV replication. Specifically, the activation of 026 cocaine-induced DNA-PK assumes a critical role in facilitating transcriptional initiation by 026 cocaine-induced DNA-PK assumes a critical role in facilitating transcriptional initiation by
027 augmenting the phosphorylation of RNAP II CTD at Ser5, alleviating RNAP II pausing through the 027 augmenting the phosphorylation of RNAP II CTD at Ser5, alleviating RNAP II pausing through the
028 phosphorylation of TRIM28 at S824 and promoting transcriptional elongation through both the ⁰²⁸phosphorylation of TRIM28 at S824 and promoting transcriptional elongation through both the

029 catalysis of CTD phosphorylation at Ser2 and the enhancement of P-TEFb activity. It is noteworthy
030 that our observations have distinctly demonstrated that inhibition or depletion of DNA-PK results in 030 that our observations have distinctly demonstrated that inhibition or depletion of DNA-PK results in
031 a substantial impediment to cocaine-induced HIV transcription and replication. The overall findings 031 a substantial impediment to cocaine-induced HIV transcription and replication. The overall findings
032 suggest a comprehensive insight into the underlying molecular mechanisms by which cocaine-032 suggest a comprehensive insight into the underlying molecular mechanisms by which cocaine-
033 induced DNA-PK effectively elevates HIV transcription and gene expression (Figure 11). 033 induced DNA-PK effectively elevates HIV transcription and gene expression (Figure 11).
034

035 035 Additionally, we have established the translational potential of DNA-PK inhibitors in curtailing HIV
036 gene expression, replication, and the reactivation of latent provirus. These outcomes advocate for 036 gene expression, replication, and the reactivation of latent provirus. These outcomes advocate for 037 the potential therapeutic application of specific DNA-PK inhibitors as adjuncts in ART regimens, 037 the potential therapeutic application of specific DNA-PK inhibitors as adjuncts in ART regimens,
038 thereby augmenting the efficacy of anti-HIV therapy and potentially curbing the incidence of HIV-038 thereby augmenting the efficacy of anti-HIV therapy and potentially curbing the incidence of HIV-
039 associated cancers, given that DNA-PK inhibitors are currently under investigation for cancer 039 associated cancers, given that DNA-PK inhibitors are currently under investigation for cancer
040 treatment. It is noteworthy that while anti-retroviral therapy (ART) treatment effectively controls HIV 040 treatment. It is noteworthy that while anti-retroviral therapy (ART) treatment effectively controls HIV
041 treplication, it is ineffective in regulating HIV gene expression from reactivated latent provirus. These 041 replication, it is ineffective in regulating HIV gene expression from reactivated latent provirus. These
042 findings strongly advocate for the inclusion of transcriptional inhibitors, such as DNA-PK inhibitors. 042 findings strongly advocate for the inclusion of transcriptional inhibitors, such as DNA-PK inhibitors, 043
043 to supplement ART regimens to mitigate the transient reactivation of latent proviruses, confirmed 043 to supplement ART regimens to mitigate the transient reactivation of latent proviruses, confirmed
044 also by our previous findings involving HIV patients' samples [39]. also by our previous findings involving HIV patients' samples [39].

045
046 **Limitation of the study**

047
048 048 Our study has a limitation. We did not consistently quantify the precise amount of replication-
049 competent viruses used for cell infection. However, we maintained an equal viral load in both the 049 competent viruses used for cell infection. However, we maintained an equal viral load in both the 050
050 control (mock) and test samples. 050 control (mock) and test samples.
051

⁰⁵²**Conclusion**

053 054 Understanding the molecular mechanisms that control the HIV life cycle, particularly in transcription
055 and replication, is crucial for HIV cure and eradication. Our research findings presented herein 055 and replication, is crucial for HIV cure and eradication. Our research findings presented herein
056 provide strong and compelling evidence for the important role of cocaine-induced activation of DNA-056 provide strong and compelling evidence for the important role of cocaine-induced activation of DNA-
057 PK in supporting various phases of HIV transcription, subsequently bolstering HIV replication. Our 057 PK in supporting various phases of HIV transcription, subsequently bolstering HIV replication. Our
058 investigations have revealed that cocaine significantly upregulates the levels of DNA-PK 058 investigations have revealed that cocaine significantly upregulates the levels of DNA-PK
059 expression triggers the activation of DNA-PK through enhanced phosphorvlation at S2056 and 059 expression, triggers the activation of DNA-PK through enhanced phosphorylation at S2056, and
060 induces its translocation into the nucleus. The activation of cocaine-induced DNA-PK plays a crucial 060 induces its translocation into the nucleus. The activation of cocaine-induced DNA-PK plays a crucial
061 role in promoting transcriptional initiation by enhancing the phosphorylation of CTD at Ser5, 061 role in promoting transcriptional initiation by enhancing the phosphorylation of CTD at Ser5,
062 alleviating RNAP II pausing by phosphorylating TRIM28 at S824 and facilitating transcriptional 062 alleviating RNAP II pausing by phosphorylating TRIM28 at S824 and facilitating transcriptional
063 elongation by both catalyzing the phosphorylation of CTD at Ser2 and enhancing the P-TEFb 063 elongation by both catalyzing the phosphorylation of CTD at Ser2 and enhancing the P-TEFb
064 recruitment. Notably, our data demonstrate that inhibiting or depleting DNA-PK severely impedes 064 recruitment. Notably, our data demonstrate that inhibiting or depleting DNA-PK severely impedes
065 cocaine-induced HIV transcription and replication. These results collectively unveil the underlying 065 cocaine-induced HIV transcription and replication. These results collectively unveil the underlying
066 molecular mechanisms through which cocaine-induced DNA-PK enhances HIV transcription and 066 molecular mechanisms through which cocaine-induced DNA-PK enhances HIV transcription and
067 dene expression. gene expression.

⁰⁶⁸**Acknowledgements**

069 We are grateful to the National institute of Health for the research grant to M.T. We are also
070 thankful to the Flow Cytometry core facility of Thomas Jefferson University. We would like to thank

thankful to the Flow Cytometry core facility of Thomas Jefferson University. We would like to thank

071 Dr. Kartik Krishnamurthy for the support and assistance. We also want to thank the reviewers for
072 providing constructive criticisms prior to publication. MT-4 cells were obtained through the NIH 072 providing constructive criticisms prior to publication. MT-4 cells were obtained through the NIH
073 AIDS Reagent Program, Division of AIDS, NIAID, NIH: MT-4 from Dr. Douglas Richman. Moreover, 073 AIDS Reagent Program, Division of AIDS, NIAID, NIH: MT-4 from Dr. Douglas Richman. Moreover,
074 we would like to thank the Center for Translational Medicine, Thomas Jefferson University. 074 we would like to thank the Center for Translational Medicine, Thomas Jefferson University, 075 including all staff members for their technical support and assistance in conducting the experiments 075 including all staff members for their technical support and assistance in conducting the experiments
076 for this study. We express our gratitude to Liz Declan for her assistance in editing the manuscript 076 for this study. We express our gratitude to Liz Declan for her assistance in editing the manuscript 077 for English language clarity. for English language clarity.

078

⁰⁷⁹**Funding**

080 M.T. was supported by research grants (Grant Nos.: R01DA041746 and 1R21MH126998-01A1)
081 from the National Institute on Drug Abuse (NIDA) and the National Institute on Mental Health 081 from the National Institute on Drug Abuse (NIDA) and the National Institute on Mental Health
082 (NIMH) of the National Institute of Health. The funders had no role in study design, data collection, 082 (NIMH) of the National Institute of Health. The funders had no role in study design, data collection, 083 and analysis, decision to publish, or preparation of the manuscript. and analysis, decision to publish, or preparation of the manuscript.

⁰⁸⁴**Author information**

⁰⁸⁵*Authors and Affiliations*

086 Center for Translational Medicine, Thomas Jefferson University, 1020 Locust Street, Philadelphia, 087 PA 19107, USA PA 19107, USA

⁰⁸⁸*Adhikarimayum Lakhikumar Sharma, Priya Tyagi, Meenata Khumallambam, Mudit Tyagi*

⁰⁸⁹**Contributions**

090
091 091 The research was conceptualized by MT and planned by ALS and MT. ALS, PT, and MK
092 conducted the experiments. ALS and MT carried out data analysis and prepared the initial 092 conducted the experiments. ALS and MT carried out data analysis and prepared the initial
093 manuscript draft. Both ALS and MT contributed to manuscript revisions. MT oversaw the project 093 manuscript draft. Both ALS and MT contributed to manuscript revisions. MT oversaw the project
094 and secured funding. All authors participated in reviewing and approving the final manuscript 094 and secured funding. All authors participated in reviewing and approving the final manuscript
095 version. version.

⁰⁹⁶**Corresponding author**

097 Correspondence to Mudit Tyagi.

⁰⁹⁸**Ethics declarations**

- ⁰⁹⁹Ethics approval and consent to participate: Not applicable.
- ¹⁰⁰**Consent for publication.**

101 Not applicable

¹⁰²**Competing interests**

- 103 The authors declare no competing interests.
104
-

104 ¹⁰⁵**Data Availability**

107 107 The datasets generated from this study are included in this manuscript.
108

¹⁰⁹**Key resources table**

HIV replication-competent virus (HIV strain 93/TH/051; R5- and X4-tropic virus isolated from a seropositive individual in Thailand)

Chemicals, peptides, and recombinant protein

NIH AIDS reagent ARP-2165

Triton X-100 Sigma-Aldrich (Burlington, MA, USA) Cat# T928

RPMI1640 Invitrogen (Waltham, MA, USA) Cat# 11835–030

- 110
111
-

112 ¹¹²**References**

113 114 1. Tyagi M, Bukrinsky M: **Human immunodeficiency virus (HIV) latency: the major hurdle in HIV eradication**. *Mol Med* 2012, **18**(1):1096-1108. 115 **eradication**. *Mol Med* 2012, **18**(1):1096-1108.
116 2. Mbonye U, Karn J: **Control of HIV latency l**

¹¹⁶2. Mbonye U, Karn J: **Control of HIV latency by epigenetic and non-epigenetic mechanisms**. *Curr* ¹¹⁷*HIV Res* 2011, **9**(8):554-567.

- 118 3. Nguyen K, Dobrowolski C, Shukla M, Cho WK, Luttge B, Karn J: **Inhibition of the H3K27**
119 **demethylase UTX enhances the epigenetic silencing of HIV proviruses and induces HIV-1 DNA demethylase UTX enhances the epigenetic silencing of HIV proviruses and induces HIV-1 DNA**
120 **hypermethylation but fails to permanently block HIV reactivation**. *PLoS Pathog* 2021, ¹²⁰**hypermethylation but fails to permanently block HIV reactivation**. *PLoS Pathog* 2021,
- 121 **17**(10):e1010014.
122 4. Choudhary SK, M ¹²²4. Choudhary SK, Margolis DM: **Curing HIV: Pharmacologic approaches to target HIV-1 latency**. ¹²³*Annu Rev Pharmacol Toxicol* 2011, **51**:397-418.
- ¹²⁴5. Hokello J, Sharma AL, Tyagi M: **An Update on the HIV DNA Vaccine Strategy**. *Vaccines (Basel)*
- 125 2021, **9**(6).
126 6. Walensky I 126 6. Walensky RP, Paltiel AD, Losina E, Mercincavage LM, Schackman BR, Sax PE, Weinstein MC,
127 Freedberg KA: The survival benefits of AIDS treatment in the United States. *J Infect Dis* 2006, 127 Freedberg KA: **The survival benefits of AIDS treatment in the United States**. *J Infect Dis* 2006,
128 **194**(1):11-19.
- 128 **194**(1):11-19.
129 7. Saag MS: **HI** ¹²⁹7. Saag MS: **HIV 101: fundamentals of antiretroviral therapy**. *Top Antivir Med* 2019, **27**(3):123- 130 127.
131 8. Wils
- 131 8. Wilson EM, Sereti I: **Immune restoration after antiretroviral therapy: the pitfalls of hasty or** *incomplete repairs. Immunol Rev* **2013, 254(1):343-354.** ¹³²**incomplete repairs**. *Immunol Rev* 2013, **254**(1):343-354.
- ¹³³9. Gay CL, Cohen MS: **Antiretrovirals to prevent HIV infection: pre- and postexposure** ¹³⁴**prophylaxis**. *Curr Infect Dis Rep* 2008, **10**(4):323-331.
- 135 10. Bertoni N, Singer M, Silva CM, Clair S, Malta M, Bastos FI: **Knowledge of AIDS and HIV transmission among drug users in Rio de Janeiro, Brazil**. *Harm Reduct J* 2011, **8**:5. **transmission among drug users in Rio de Janeiro, Brazil**. *Harm Reduct J* 2011, **8**:5.
137 11. Iskandar S, Basar D, Hidayat T, Siregar IM, Pinxten L, van Crevel R, Van der Ven AJ
- 137 11. Iskandar S, Basar D, Hidayat T, Siregar IM, Pinxten L, van Crevel R, Van der Ven AJ, De Jong CA:
138 **High risk behavior for HIV transmission among former injecting drug users: a survey from High risk behavior for HIV transmission among former injecting drug users: a survey from Indonesia**. *BMC Public Health* 2010, **10**:472. ¹³⁹**Indonesia**. *BMC Public Health* 2010, **10**:472.
- 140 12. Baggaley RF, Boily MC, White RG, Alary M: **Risk of HIV-1 transmission for parenteral exposure and blood transfusion: a systematic review and meta-analysis.** *AIDS* **2006, 20(6):805-**¹⁴¹**exposure and blood transfusion: a systematic review and meta-analysis**. *AIDS* 2006, **20**(6):805- 142 812.
143 13. Huds
- ¹⁴³13. Hudgins R, McCusker J, Stoddard A: **Cocaine use and risky injection and sexual behaviors**. *Drug* ¹⁴⁴*Alcohol Depend* 1995, **37**(1):7-14.
- 145 14. Purcell DW, Parsons JT, Halkitis PN, Mizuno Y, Woods WJ: **Substance use and sexual transmission risk behavior of HIV-positive men who have sex with men**. *J Subst Abuse* 2001, 146 **transmission risk behavior of HIV-positive men who have sex with men**. *J Subst Abuse* 2001,
147 **13**(1-2):185-200. 147 **13**(1-2):185-200.
148 15. Molitor F, Truax
- 148 15. Molitor F, Truax SR, Ruiz JD, Sun RK: Association of methamphetamine use during sex with risky sexual behaviors and HIV infection among non-injection drug users. *West J Med* 1998, ¹⁴⁹**risky sexual behaviors and HIV infection among non-injection drug users**. *West J Med* 1998, 150 **168**(2):93-97.
151 16. Tyagi M, We
- 151 16. Tyagi M, Weber J, Bukrinsky M, Simon GL: **The effects of cocaine on HIV transcription**. *J*
152 *Neurovirol* 2016, **22**(3):261-274. 152 *Neurovirol* 2016, **22**(3):261-274.
153 17. Tyagi M, Bukrinsky M, Simon C
- 153 17. Tyagi M, Bukrinsky M, Simon GL: **Mechanisms of HIV Transcriptional Regulation by Drugs of Abuse**. *Curr HIV Res* 2016, **14**(5):442-454. 154 **Abuse**. *Curr HIV Res* 2016, 14(5):442-454.
155 18. Ciccarone D: **Stimulant abuse: pharmaco**
- 155 18. Ciccarone D: **Stimulant abuse: pharmacology, cocaine, methamphetamine, treatment, attempts at pharmacotherapy**. *Prim Care* 2011, **38**(1):41-58. ¹⁵⁶**at pharmacotherapy**. *Prim Care* 2011, **38**(1):41-58.
- 157 19. Pomara C, Cassano T, D'Errico S, Bello S, Romano AD, Riezzo I, Serviddio G: **Data available on the extent of cocaine use and dependence: biochemistry, pharmacologic effects and global** the extent of cocaine use and dependence: biochemistry, pharmacologic effects and global
159 **burden of disease of cocaine abusers**. *Curr Med Chem* 2012, **19**(33):5647-5657. **burden of disease of cocaine abusers**. *Curr Med Chem* 2012, **19**(33):5647-5657.
160 20. Calatayud J, Gonzalez A: **History of the development and evolution of local an**
- 160 20. Calatayud J, Gonzalez A: **History of the development and evolution of local anesthesia since the cocal leaf**. *Anesthesiology* 2003, **98**(6):1503-1508. 161 **coca leaf**. *Anesthesiology* 2003, **98**(6):1503-1508.
162 21. Goldstein RA, DesLauriers C, Burda AM: Coca
- 162 21. Goldstein RA, DesLauriers C, Burda AM: **Cocaine: history, social implications, and toxicity--a review**. *Dis Mon* 2009, 55(1):6-38. 163 **review**. *Dis Mon* 2009, **55**(1):6-38.
164 22. **Jeffcoat AR, Perez-Reves M, Hill J**
- ¹⁶⁴22. Jeffcoat AR, Perez-Reyes M, Hill JM, Sadler BM, Cook CE: **Cocaine disposition in humans after** ¹⁶⁵**intravenous injection, nasal insufflation (snorting), or smoking**. *Drug Metab Dispos* 1989, 166 **17**(2):153-159.
167 23. Klinkenberg W
- ¹⁶⁷23. Klinkenberg WD, Sacks S, Hiv/Aids Treatment Adherence HO, Cost Study G: **Mental disorders** ¹⁶⁸**and drug abuse in persons living with HIV/AIDS**. *AIDS Care* 2004, **16 Suppl 1**:S22-42.

- 169 24. Khalsa JH, Elkashef A: **Interventions for HIV and hepatitis C virus infections in recreational drug users**. *Clin Infect Dis* 2010, **50**(11):1505-1511.
- 170 **drug users**. *Clin Infect Dis* 2010, **50**(11):1505-1511.
171 25. Buch S, Yao H, Guo M, Mori T, Mathias-Costa B, 171 25. Buch S, Yao H, Guo M, Mori T, Mathias-Costa B, Singh V, Seth P, Wang J, Su TP: **Cocaine and HIV-1 interplay in CNS: cellular and molecular mechanisms**. *Curr HIV Res* 2012, **10**(5):425-428. ¹⁷²**HIV-1 interplay in CNS: cellular and molecular mechanisms**. *Curr HIV Res* 2012, **10**(5):425-428.
- 173 26. Parikh N, Nonnemacher MR, Pirrone V, Block T, Mehta A, Wigdahl B: **Substance abuse, HIV-1 and hepatitis**. *Curr HIV Res* 2012, **10**(7):557-571. ¹⁷⁴**and hepatitis**. *Curr HIV Res* 2012, **10**(7):557-571.
- 175 27. Sharma AL, Shafer D, Netting D, Tyagi M: **Cocaine sensitizes the CD4(+) T cells for HIV infection by co-stimulating NFAT and AP-1**. *iScience* 2022, 25(12):105651.
- 176 **infection by co-stimulating NFAT and AP-1**. *iScience* 2022, 25(12):105651.
177 28. Sahu G, Farley K, El-Hage N, Aiamkitsumrit B, Fassnacht R, Kashanchi F, 177 28. Sahu G, Farley K, El-Hage N, Aiamkitsumrit B, Fassnacht R, Kashanchi F, Ochem A, Simon GL,
178 Karn J, Hauser KF et al: Cocaine promotes both initiation and elongation phase of HIV-1 178 Karn J, Hauser KF *et al*: **Cocaine promotes both initiation and elongation phase of HIV-1**
179 **transcription by activating NF-kappaB and MSK1 and inducing selective epigenetic** 179 **transcription by activating NF-kappaB and MSK1 and inducing selective epigenetic modifications at HIV-1 LTR**. *Virology* 2015, 483:185-202. 180 **modifications at HIV-1 LTR**. *Virology* 2015, 483:185-202.
181 29. Dash S, Balasubramaniam M, Villalta F, Dash C, Pandhar
- 181 29. Dash S, Balasubramaniam M, Villalta F, Dash C, Pandhare J: **Impact of cocaine abuse on HIV pathogenesis**. *Front Microbiol* 2015, **6**:1111. 182 **pathogenesis**. *Front Microbiol* 2015, **6**:1111.
183 30. Sonti S, Tyagi K, Pande A, Daniel R, Sharma
- 183 30. Sonti S, Tyagi K, Pande A, Daniel R, Sharma AL, Tyagi M: **Crossroads of Drug Abuse and HIV Infection: Neurotoxicity and CNS Reservoir**. *Vaccines (Basel)* 2022, **10**(2). 184 **Infection: Neurotoxicity and CNS Reservoir**. *Vaccines (Basel)* 2022, 10(2).
185 31. Riedl T, Egly JM: **Phosphorylation in transcription: the CTD and more**.
- ¹⁸⁵31. Riedl T, Egly JM: **Phosphorylation in transcription: the CTD and more**. *Gene Expr* 2000, **9**(1- 186 2):3-13.
187 32. Brookes
- 187 32. Brookes E, Pombo A: **Modifications of RNA polymerase II are pivotal in regulating gene** expression states. *EMBO Rep* 2009, **10**(11):1213-1219. **expression states**. *EMBO Rep* 2009, **10**(11):1213-1219.
189 33. Hsin JP, Manley JL: **The RNA polymerase II** C
- 189 33. Hsin JP, Manley JL: **The RNA polymerase II CTD coordinates transcription and RNA processing**. *Genes Dev* 2012, **26**(19):2119-2137. 190 **processing**. *Genes Dev* 2012, **26**(19):2119-2137.
191 34. Dahmus ME: **Phosphorylation of the C-termina**
- ¹⁹¹34. Dahmus ME: **Phosphorylation of the C-terminal domain of RNA polymerase II**. *Biochim Biophys* 192 *Acta* 1995, **1261**(2):171-182.
193 35. Orphanides G, Lagrange T, R
- 193 35. Orphanides G, Lagrange T, Reinberg D: **The general transcription factors of RNA polymerase II**.
194 *Genes Dev* 1996, **10**(21):2657-2683. 194 *Genes Dev* 1996, **10**(21):2657-2683.
195 36. Isel C, Karn J: **Direct evidence that**
- 195 36. Isel C, Karn J: **Direct evidence that HIV-1 Tat stimulates RNA polymerase II carboxyl-terminal**
196 **and all domain hyperphosphorylation during transcriptional elongation**. *J Mol Biol* 1999, 290(5):929-¹⁹⁶**domain hyperphosphorylation during transcriptional elongation**. *J Mol Biol* 1999, **290**(5):929- 197 941.
198 37. Tyag
- 198 37. Tyagi S, Ochem A, Tyagi M: **DNA-dependent protein kinase interacts functionally with the RNA polymerase II complex recruited at the human immunodeficiency virus (HIV) long terminal** 199 **polymerase II complex recruited at the human immunodeficiency virus (HIV) long terminal**

199 **polymerase II complex recruited at the human immunodeficiency virus (HIV) long terminal**

199 **polymerase II complex recr** ²⁰⁰**repeat and plays an important role in HIV gene expression**. *J Gen Virol* 2011, **92**(Pt 7):1710- 201 1720.
202 38. Nagas
- 202 38. Nagasawa M, Watanabe F, Suwa A, Yamamoto K, Tsukada K, Teraoka H: **Nuclear translocation of the catalytic component of DNA-dependent protein kinase upon growth stimulation in normal** 203 **the catalytic component of DNA-dependent protein kinase upon growth stimulation in normal human T lymphocytes**. *Cell Struct Funct* 1997, 22(6):585-594. 204 **human T lymphocytes**. *Cell Struct Funct* 1997, 22(6):585-594.
205 39. Zicari S, Sharma AL, Sahu G, Dubrovsky L, Sun L, Yue H, Jac
- 205 39. Zicari S, Sharma AL, Sahu G, Dubrovsky L, Sun L, Yue H, Jada T, Ochem A, Simon G, Bukrinsky
206 Metal: **DNA dependent protein kinase (DNA-PK) enhances HIV transcription by promoting** 206 Met al: **DNA dependent protein kinase (DNA-PK) enhances HIV transcription by promoting 207 RNA polymerase II activity and recruitment of transcription machinery at HIV LTR.** 207 **RNA polymerase II activity and recruitment of transcription machinery at HIV LTR.**
208 *Oncotarget* 2020, 11(7):699-726. ²⁰⁸*Oncotarget* 2020, **11**(7):699-726.
- 209 40. Hartley KO, Gell D, Smith GC, Zhang H, Divecha N, Connelly MA, Admon A, Lees-Miller SP,
210 Anderson CW, Jackson SP: **DNA-dependent protein kinase catalytic subunit: a relative of** 210 Anderson CW, Jackson SP: **DNA-dependent protein kinase catalytic subunit: a relative of phosphatidylinositol 3-kinase and the ataxia telangiectasia gene product. Cell 1995, 82(5):849-**²¹¹**phosphatidylinositol 3-kinase and the ataxia telangiectasia gene product**. *Cell* 1995, **82**(5):849-
- 212 856.
213 41. Gottl 213 41. Gottlieb TM, Jackson SP: **The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen**. *Cell* 1993, **72**(1):131-142. 214 **association with Ku antigen**. *Cell* 1993, **72**(1):131-142.
215 42. Reeves WH, Sthoeger ZM: **Molecular cloning of cDNA**
- 215 42. Reeves WH, Sthoeger ZM: **Molecular cloning of cDNA encoding the p70 (Ku) lupus autoantigen**.
216 *J Biol Chem* 1989, **264**(9):5047-5052. ²¹⁶*J Biol Chem* 1989, **264**(9):5047-5052.
- 217 43. Lees-Miller SP, Godbout R, Chan DW, Weinfeld M, Day RS, 3rd, Barron GM, Allalunis-Turner J:
218 **Absence of p350 subunit of DNA-activated protein kinase from a radiosensitive human cell line**. ²¹⁸**Absence of p350 subunit of DNA-activated protein kinase from a radiosensitive human cell line**. ²¹⁹*Science* 1995, **267**(5201):1183-1185.

- 220 44. Taccioli GE, Gottlieb TM, Blunt T, Priestley A, Demengeot J, Mizuta R, Lehmann AR, Alt FW,
221 Jackson SP, Jeggo PA: Ku80: product of the XRCC5 gene and its role in DNA repair and V(D)J 221 Jackson SP, Jeggo PA: **Ku80: product of the XRCC5 gene and its role in DNA repair and V(D)J recombination**. *Science* 1994, **265**(5177):1442-1445.
- ²²²**recombination**. *Science* 1994, **265**(5177):1442-1445. 223 45. Blackford AN, Jackson SP: **ATM, ATR, and DNA-PK: The Trinity at the Heart of the DNA Damage Response**. *Mol Cell* 2017, **66**(6):801-817. **224 Damage Response**. *Mol Cell* 2017, **66**(6):801-817.
225 46. Dylgieri E. Knudsen KE: **DNA-PKcs: A Targetal**
- ²²⁵46. Dylgjeri E, Knudsen KE: **DNA-PKcs: A Targetable Protumorigenic Protein Kinase**. *Cancer Res*
- 226 2022, **82**(4):523-533.
227 47. Dvir A, Stein LY, 0 227 47. Dvir A, Stein LY, Calore BL, Dynan WS: **Purification and characterization of a template-**²²⁸**associated protein kinase that phosphorylates RNA polymerase II**. *J Biol Chem* 1993, 229 **268**(14):10440-10447.
230 48. Peterlin BM, Price DH
- ²³⁰48. Peterlin BM, Price DH: **Controlling the elongation phase of transcription with P-TEFb**. *Mol Cell* 231 2006, **23**(3):297-305.
232 49. Kim YK, Bourgeois 0
- 232 49. Kim YK, Bourgeois CF, Isel C, Churcher MJ, Karn J: **Phosphorylation of the RNA polymerase II**
233 **and Carlo and Stephan and Stephan by CDK9** is directly responsible for human immunodeficiency virus 233 carboxyl-terminal domain by CDK9 is directly responsible for human immunodeficiency virus **type 1 Tat-activated transcriptional elongation**. *Mol Cell Biol* 2002, 22(13):4622-4637.
- ²³⁴**type 1 Tat-activated transcriptional elongation**. *Mol Cell Biol* 2002, **22**(13):4622-4637. 235 50. Yamamoto S, Watanabe Y, van der Spek PJ, Watanabe T, Fujimoto H, Hanaoka F, Ohkuma Y:
236 **Studies of nematode TFIIE function reveal a link between Ser-5 phosphorylation of RNA** 236 **Studies of nematode TFIIE function reveal a link between Ser-5 phosphorylation of RNA**
237 **polymerase II and the transition from transcription initiation to elongation**. *Mol Cell Biol* 2001, **polymerase II and the transition from transcription initiation to elongation**. *Mol Cell Biol* 2001, 238 **21**(1):1-15.
- 238 **21**(1):1-15.
239 51. Kim YK, B 239 51. Kim YK, Bourgeois CF, Pearson R, Tyagi M, West MJ, Wong J, Wu SY, Chiang CM, Karn J:
240 **Recruitment of TFIIH to the HIV LTR is a rate-limiting step in the emergence of HIV from** 240 **Recruitment of TFIIH to the HIV LTR is a rate-limiting step in the emergence of HIV from** 241 **latency**. *EMBO J* 2006, 25(15):3596-3604. 241 **latency**. *EMBO J* 2006, **25**(15):3596-3604.
242 52. Saunders A, Core LJ, Lis JT: **Breaking ba**
- ²⁴²52. Saunders A, Core LJ, Lis JT: **Breaking barriers to transcription elongation**. *Nat Rev Mol Cell Biol* 243 2006, **7**(8):557-567.
244 53. Kao SY, Calman AI
- ²⁴⁴53. Kao SY, Calman AF, Luciw PA, Peterlin BM: **Anti-termination of transcription within the long terminal repeat of HIV-1 by tat gene product**. *Nature* 1987, **330**(6147):489-493.
246 54. Guenther MG, Levine SS, Boyer LA, Jaenisch R, Young RA: A chromatin
- 246 54. Guenther MG, Levine SS, Boyer LA, Jaenisch R, Young RA: **A chromatin landmark and transcription initiation at most promoters in human cells**. *Cell* 2007, 130(1):77-88. **transcription initiation at most promoters in human cells**. *Cell* 2007, **130**(1):77-88.
248 55. Muse GW, Gilchrist DA, Nechaev S, Shah R, Parker JS, Grissom SF, Zeitlinger J, Ade
- 248 55. Muse GW, Gilchrist DA, Nechaev S, Shah R, Parker JS, Grissom SF, Zeitlinger J, Adelman K: **RNA polymerase is poised for activation across the genome**. *Nat Genet* 2007, **39**(12):1507-1511. 249 **polymerase is poised for activation across the genome**. *Nat Genet* 2007, **39**(12):1507-1511.
250 56. Wu CH, Yamaguchi Y, Benjamin LR, Horvat-Gordon M, Washinsky J, Enerly E, Lar
- 250 56. Wu CH, Yamaguchi Y, Benjamin LR, Horvat-Gordon M, Washinsky J, Enerly E, Larsson J, Lambertsson A, Handa H, Gilmour D: **NELF and DSIF cause promoter proximal pausing on the** 251 Lambertsson A, Handa H, Gilmour D: **NELF and DSIF cause promoter proximal pausing on the hsp70 promoter in Drosophila**. *Genes Dev* 2003, 17(11):1402-1414. **hsp70 promoter in Drosophila**. *Genes Dev* 2003, 17(11):1402-1414.
253 57. Yamaguchi Y, Shibata H, Handa H: **Transcription elongation factor**
- 253 57. Yamaguchi Y, Shibata H, Handa H: **Transcription elongation factors DSIF and NELF: promoter-**²⁵⁴**proximal pausing and beyond**. *Biochim Biophys Acta* 2013, **1829**(1):98-104.
- ²⁵⁵58. Bunch H, Calderwood SK: **TRIM28 as a novel transcriptional elongation factor**. *BMC Mol Biol* 256 2015, **16**:14.
257 59. Bunch H, La
- 257 59. Bunch H, Lawney BP, Lin YF, Asaithamby A, Murshid A, Wang YE, Chen BP, Calderwood SK:
258 **Transcriptional elongation requires DNA break-induced signalling**. *Nat Commun* 2015, 6:10191. 258 **Transcriptional elongation requires DNA break-induced signalling**. *Nat Commun* 2015, **6**:10191.
259 **60.** Bunch H, Zheng X, Burkholder A, Dillon ST, Motola S, Birrane G, Ebmeier CC, Levine S, Fargo D,
- 259 60. Bunch H, Zheng X, Burkholder A, Dillon ST, Motola S, Birrane G, Ebmeier CC, Levine S, Fargo D,
260 Hu G et al: **TRIM28 regulates RNA polymerase II promoter-proximal pausing and pause** ²⁶⁰Hu G *et al*: **TRIM28 regulates RNA polymerase II promoter-proximal pausing and pause** ²⁶¹**release**. *Nat Struct Mol Biol* 2014, **21**(10):876-883.
- 262 61. Czerwinska P, Mazurek S, Wiznerowicz M: **The complexity of TRIM28 contribution to cancer**. *J* Biomed Sci 2017, 24(1):63. ²⁶³*Biomed Sci* 2017, **24**(1):63.
- ²⁶⁴62. Allouch A, Di Primio C, Alpi E, Lusic M, Arosio D, Giacca M, Cereseto A: **The TRIM family** ²⁶⁵**protein KAP1 inhibits HIV-1 integration**. *Cell Host Microbe* 2011, **9**(6):484-495.
- ²⁶⁶63. Randolph K, Hyder U, D'Orso I: **KAP1/TRIM28: Transcriptional Activator and/or Repressor of** ²⁶⁷**Viral and Cellular Programs?** *Front Cell Infect Microbiol* 2022, **12**:834636.
- 268 64. Ait-Ammar A, Bellefroid M, Daouad F, Martinelli V, Van Assche J, Wallet C, Rodari A, De Rovere
269 M, Fahrenkrog B, Schwartz C *et al*: **Inhibition of HIV-1 gene transcription by KAP1 in myeloid** ²⁶⁹M, Fahrenkrog B, Schwartz C *et al*: **Inhibition of HIV-1 gene transcription by KAP1 in myeloid** ²⁷⁰**lineage**. *Sci Rep* 2021, **11**(1):2692.

- 271 65. Parada CA, Roeder RG: **Enhanced processivity of RNA polymerase II triggered by Tat-induced phosphorylation of its carboxy-terminal domain**. *Nature* 1996, 384(6607):375-378.
- **phosphorylation of its carboxy-terminal domain**. *Nature* 1996, **384**(6607):375-378.
273 66. Ivanov D, Kwak YT, Guo J, Gaynor RB: **Domains in the SPT5 protein that** ²⁷³66. Ivanov D, Kwak YT, Guo J, Gaynor RB: **Domains in the SPT5 protein that modulate its transcriptional regulatory properties**. *Mol Cell Biol* 2000, **20**(9):2970-2983.
275 67. Fujinaga K, Irwin D, Huang Y, Taube R, Kurosu T, Peterlin BM: I
- ²⁷⁵67. Fujinaga K, Irwin D, Huang Y, Taube R, Kurosu T, Peterlin BM: **Dynamics of human** 276 **immunodeficiency virus transcription: P-TEFb phosphorylates RD and dissociates negative effectors from the transactivation response element**. *Mol Cell Biol* 2004, **24**(2):787-795. **effectors from the transactivation response element**. *Mol Cell Biol* 2004, **24**(2):787-795.
278 68. Tyagi M, Pearson RJ, Karn J: **Establishment of HIV latency in primary CD4+ cells**
- 278 68. Tyagi M, Pearson RJ, Karn J: **Establishment of HIV latency in primary CD4+ cells is due to epigenetic transcriptional silencing and P-TEFb restriction**. *J Virol* 2010, **84**(13):6425-6437. ²⁷⁹**epigenetic transcriptional silencing and P-TEFb restriction**. *J Virol* 2010, **84**(13):6425-6437.
- ²⁸⁰69. Sobhian B, Laguette N, Yatim A, Nakamura M, Levy Y, Kiernan R, Benkirane M: **HIV-1 Tat** 281 **assembles a multifunctional transcription elongation complex and stably associates with the
282 7SK snRNP**. *Mol Cell* 2010, **38**(3):439-451. ²⁸²**7SK snRNP**. *Mol Cell* 2010, **38**(3):439-451.
- ²⁸³70. He N, Chan CK, Sobhian B, Chou S, Xue Y, Liu M, Alber T, Benkirane M, Zhou Q: **Human** 284 **Polymerase-Associated Factor complex (PAFc) connects the Super Elongation Complex (SEC)**
285 **to RNA polymerase II on chromatin**. *Proc Natl Acad Sci U S A* 2011, **108**(36):E636-645. ²⁸⁵**to RNA polymerase II on chromatin**. *Proc Natl Acad Sci U S A* 2011, **108**(36):E636-645.
- 286 71. Chou S, Upton H, Bao K, Schulze-Gahmen U, Samelson AJ, He N, Nowak A, Lu H, Krogan NJ, 287 2hou Q et al: HIV-1 Tat recruits transcription elongation factors dispersed along a flexible 287 Zhou Q *et al*: **HIV-1 Tat recruits transcription elongation factors dispersed along a flexible AFF4 scaffold**. *Proc Natl Acad Sci U S A* 2013, **110**(2):E123-131.
- 288 **AFF4 scaffold**. *Proc Natl Acad Sci U S A* 2013, 110(2):E123-131.
289 72. Tyagi M, Rusnati M, Presta M, Giacca M: **Internalization of** 289 72. Tyagi M, Rusnati M, Presta M, Giacca M: **Internalization of HIV-1 tat requires cell surface heparan sulfate proteoglycans**. *J Biol Chem* 2001, **276**(5):3254-3261. 290 **heparan sulfate proteoglycans**. *J Biol Chem* 2001, **276**(5):3254-3261.
291 73. Barboric M, Peterlin BM: A new paradigm in eukarvotic biology:
- 291 73. Barboric M, Peterlin BM: **A new paradigm in eukaryotic biology: HIV Tat and the control of transcriptional elongation**. *PLoS Biol* 2005, 3(2):e76. 292 **transcriptional elongation**. *PLoS Biol* 2005, 3(2):e76.
293 74. Tahirov TH, Babayeva ND, Varzavand K, Cooper JJ,
- 293 74. Tahirov TH, Babayeva ND, Varzavand K, Cooper JJ, Sedore SC, Price DH: **Crystal structure of HIV-1 Tat complexed with human P-TEFb**. Nature 2010, **465**(7299):747-751. 294 **HIV-1 Tat complexed with human P-TEFb**. *Nature* 2010, **465**(7299):747-751.
295 75. Karn J: **Tackling Tat**. *J Mol Biol* 1999, **293**(2):235-254.
- ²⁹⁵75. Karn J: **Tackling Tat**. *J Mol Biol* 1999, **293**(2):235-254.
- 296 76. Taube R, Fujinaga K, Wimmer J, Barboric M, Peterlin BM: **Tat transactivation: a model for the regulation of eukaryotic transcriptional elongation**. *Virology* 1999, **264**(2):245-253. ²⁹⁷**regulation of eukaryotic transcriptional elongation**. *Virology* 1999, **264**(2):245-253.
- ²⁹⁸77. Dull T, Zufferey R, Kelly M, Mandel RJ, Nguyen M, Trono D, Naldini L: **A third-generation** 299 **lentivirus vector with a conditional packaging system**. *J Virol* 1998, **72**(11):8463-8471.
200 78. Tyagi M. Karn J: **CBF-1 promotes transcriptional silencing during the establishment**
- 300 78. Tyagi M, Karn J: **CBF-1 promotes transcriptional silencing during the establishment of HIV-1 latency**. *EMBO J* 2007, **26**(24):4985-4995. 301 **latency**. *EMBO J* 2007, **26**(24):4985-4995.
302 79. Hokello J, Lakhikumar Sharma A, Tyagi M
- 302 79. Hokello J, Lakhikumar Sharma A, Tyagi M: **AP-1 and NF-kappaB synergize to transcriptionally activate latent HIV upon T-cell receptor activation**. *FEBS Lett* 2021, **595**(5):577-594. 303 **activate latent HIV upon T-cell receptor activation**. *FEBS Lett* 2021, **595**(5):577-594.
304 80. Pearson R, Kim YK, Hokello J, Lassen K, Friedman J, Tyagi M, Karn J: **Epigenetic**
- ³⁰⁴80. Pearson R, Kim YK, Hokello J, Lassen K, Friedman J, Tyagi M, Karn J: **Epigenetic silencing of** 305 **human immunodeficiency virus (HIV) transcription by formation of restrictive chromatin**
306 **https://web/2016.com/integration in the structures at the viral long terminal repeat drives the progressive entry of HIV int** 306 **structures at the viral long terminal repeat drives the progressive entry of HIV into latency.** *J***
307** *Virol* **2008, 82**(24):12291-12303. ³⁰⁷*Virol* 2008, **82**(24):12291-12303.
- ³⁰⁸81. Tyagi M, Karn J: **CBF-1 promotes transcriptional silencing during the establishment of HIV-1** 309 **latency**. *EMBO J* 2007, **26**(24):4985-4995.
310 82. Carr MI, Zimmermann A, Chiu LY, Zer
- ³¹⁰82. Carr MI, Zimmermann A, Chiu LY, Zenke FT, Blaukat A, Vassilev LT: **DNA-PK Inhibitor,** ³¹¹**M3814, as a New Combination Partner of Mylotarg in the Treatment of Acute Myeloid** 312 **Leukemia**. *Front Oncol* 2020, **10**:127.
313 83. Sun Q, Guo Y, Liu X, Czauderna F,
- ³¹³83. Sun Q, Guo Y, Liu X, Czauderna F, Carr MI, Zenke FT, Blaukat A, Vassilev LT: **Therapeutic Implications of p53 Status on Cancer Cell Fate Following Exposure to Ionizing Radiation and

215 https://www.frag.org/Exposure to Ionizing Radiation and

2019, 17(12):2457-2468. the DNA-PK Inhibitor M3814**. *Mol Cancer Res* 2019, 17(12):2457-2468.
316 84. van Bussel MTJ, Awada A, de Jonge MJA, Mau-Sorensen M, Nielsen
- 316 84. van Bussel MTJ, Awada A, de Jonge MJA, Mau-Sorensen M, Nielsen D, Schoffski P, Verheul
317 HMW, Sarholz B, Berghoff K, El Bawab S et al: A first-in-man phase 1 study of the DNA-317 HMW, Sarholz B, Berghoff K, El Bawab S *et al*: **A first-in-man phase 1 study of the DNAdependent protein kinase inhibitor peposertib (formerly M3814) in patients with advanced solid tumours**. *Br J Cancer* 2021, **124**(4):728-735. ³¹⁹**solid tumours**. *Br J Cancer* 2021, **124**(4):728-735.
- ³²⁰85. Wise HC, Iyer GV, Moore K, Temkin SM, Gordon S, Aghajanian C, Grisham RN: **Activity of M3814, an Oral DNA-PK Inhibitor, In Combination with Topoisomerase II Inhibitors in Ovarian Cancer Models**. Sci Rep 2019, 9(1):18882. ³²²**Ovarian Cancer Models**. *Sci Rep* 2019, **9**(1):18882.

- 323 86. Zenke FT, Zimmermann A, Sirrenberg C, Dahmen H, Kirkin V, Pehl U, Grombacher T, Wilm C, 324 Fuchss T, Amendt C et al: **Pharmacologic Inhibitor of DNA-PK, M3814, Potentiates** 324 Fuchss T, Amendt C *et al*: **Pharmacologic Inhibitor of DNA-PK, M3814, Potentiates Radiotherapy and Regresses Human Tumors in Mouse Models**. *Mol Cancer Ther* 2020, ³²⁵**Radiotherapy and Regresses Human Tumors in Mouse Models**. *Mol Cancer Ther* 2020, 326 **19**(5):1091-1101.
327 87. Hardcastle IR, Co
- 327 87. Hardcastle IR, Cockcroft X, Curtin NJ, El-Murr MD, Leahy JJJ, Stockley M, Golding BT, Rigoreau
328 L. Richardson C. Smith GCM *et al*: Discovery of potent chromen-4-one inhibitors of the DNA-328 L, Richardson C, Smith GCM *et al*: **Discovery of potent chromen-4-one inhibitors of the DNA-**
329 **dependent protein kinase (DNA-PK) using a small-molecule library approach**. Journal of ³²⁹**dependent protein kinase (DNA-PK) using a small-molecule library approach**. *Journal of*
- ³³⁰*Medicinal Chemistry* 2005, **48**(24):7829-7846. 331 88. Zhao Y, Thomas HD, Batey MA, Cowell IG, Richardson CJ, Griffin RJ, Calvert AH, Newell DR,
332 Smith GC, Curtin NJ: Preclinical evaluation of a potent novel DNA-dependent protein kinase 332 Smith GC, Curtin NJ: **Preclinical evaluation of a potent novel DNA-dependent protein kinase inhibitor NU7441**. *Cancer Res* 2006, **66**(10):5354-5362.
- ³³³**inhibitor NU7441**. *Cancer Res* 2006, **66**(10):5354-5362. ³³⁴89. Davidson D, Amrein L, Panasci L, Aloyz R: **Small Molecules, Inhibitors of DNA-PK, Targeting DNA Repair, and Beyond**. *Front Pharmacol* 2013, 4:5.
336 90. Cornell L, Munck J, Curtin N, Reeves H: **DNA-Pk or**
- ³³⁶90. Cornell L, Munck J, Curtin N, Reeves H: **DNA-Pk or Atm Inhibition Inhibits Non-Homologous End Joining and Enhances Chemo- and Radio Sensitivity in Hepatocellular Cancer Cell Lines.**
338 *Gut* 2012, **61**:A201-A201. ³³⁸*Gut* 2012, **61**:A201-A201.
- ³³⁹91. Feng FY, Brenner JC, Hussain M, Chinnaiyan AM: **Molecular pathways: targeting ETS gene** ³⁴⁰**fusions in cancer**. *Clinical cancer research : an official journal of the American Association for* ³⁴¹*Cancer Research* 2014, **20**(17):4442-4448.
- ³⁴²92. Jekimovs C, Bolderson E, Suraweera A, Adams M, O'Byrne KJ, Richard DJ: **Chemotherapeutic compounds targeting the DNA double-strand break repair pathways: the good, the bad, and the promising**. *Front Oncol* 2014, 4:86. **the promising**. *Front Oncol* 2014, **4**:86.
 345 93. Sharma AL, Hokello J, Sonti S, Zicari S
- 345 93. Sharma AL, Hokello J, Sonti S, Zicari S, Sun L, Alqatawni A, Bukrinsky M, Simon G, Chauhan A, Daniel R et al: CBF-1 Promotes the Establishment and Maintenance of HIV Latency by 346 Daniel R *et al*: **CBF-1 Promotes the Establishment and Maintenance of HIV Latency by Recruiting Polycomb Repressive Complexes, PRC1 and PRC2, at HIV LTR**. *Viruses* 2020, **Recruiting Polycomb Repressive Complexes, PRC1 and PRC2, at HIV LTR**. *Viruses* 2020, 12(9). ³⁴⁸**12**(9).
- 349 94. Hokello J, Tyagi P, Dimri S, Sharma AL, Tyagi M: **Comparison of the Biological Basis for Non-**350 **HIV Transmission to HIV-Exposed Seronegative Individuals, Disease Non-Progression in HIV**
351 **Long-Term Non-Progressors and Elite Controllers**. *Viruses* 2023, 15(6). ³⁵¹**Long-Term Non-Progressors and Elite Controllers**. *Viruses* 2023, **15**(6).
- 352 95. Sharma AL, Hokello J, Tyagi M: **Circumcision as an Intervening Strategy against HIV Acquisition in the Male Genital Tract**. *Pathogens* 2021, **10**(7). 353 **Acquisition in the Male Genital Tract**. *Pathogens* 2021, **10**(7).
354 96. Hokello J, Sharma AL, Dimri M, Tyagi M: **Insights into the Property**
- ³⁵⁴96. Hokello J, Sharma AL, Dimri M, Tyagi M: **Insights into the HIV Latency and the Role of** ³⁵⁵**Cytokines**. *Pathogens* 2019, **8**(3).
- ³⁵⁶97. Sharma AL, Singh TR, Singh LS: **Antiretroviral resistance, genotypic characterization and** 357 **origin of Human Immunodeficiency Virus among the infected wives of Intravenous drug users in Manipur**. *Sci Rep* 2018, **8**(1):15183. ³⁵⁸**in Manipur**. *Sci Rep* 2018, **8**(1):15183.
- 359 98. Hokello J, Sharma AL, Tyagi M: **Efficient Non-Epigenetic Activation of HIV Latency through** *the T-Cell Receptor Signalosome. Viruses* **2020, 12(8).** 360 **the T-Cell Receptor Signalosome**. *Viruses* 2020, 12(8).
361 99. Liu H, Miller LG, Hays RD, Golin CE, Wu T, Wen
- ³⁶¹99. Liu H, Miller LG, Hays RD, Golin CE, Wu T, Wenger NS, Kaplan AH: **Repeated measures longitudinal analyses of HIV virologic response as a function of percent adherence, dose timing,**
363 **eenotypic sensitivity, and other factors**. *J Acquir Immune Defic Syndr* 2006, 41(3):315-322. ³⁶³**genotypic sensitivity, and other factors**. *J Acquir Immune Defic Syndr* 2006, **41**(3):315-322.
- 364 100. Parruti G, Manzoli L, Toro PM, D'Amico G, Rotolo S, Graziani V, Schioppa F, Consorte A, Alterio L, Toro GM *et al*: Long-term adherence to first-line highly active antiretroviral therapy in a ³⁶⁵L, Toro GM *et al*: **Long-term adherence to first-line highly active antiretroviral therapy in a** ³⁶⁶**hospital-based cohort: predictors and impact on virologic response and relapse**. *AIDS Patient* ³⁶⁷*Care STDS* 2006, **20**(1):48-56.
- 368 101. Jackson SP, MacDonald JJ, Lees-Miller S, Tjian R: **GC box binding induces phosphorylation of Sp1 by a DNA-dependent protein kinase**. *Cell* 1990, **63**(1):155-165. **Sp1 by a DNA-dependent protein kinase.** *Cell* 1990, **63**(1):155-165.
370 102. Goodwin JF, Knudsen KE: **Beyond DNA repair: DNA-PK functi**
- ³⁷⁰102. Goodwin JF, Knudsen KE: **Beyond DNA repair: DNA-PK function in cancer**. *Cancer Discov* 371 2014, **4**(10):1126-1139.
372 103. Marshall NF, Peng J, X
- 372 103. Marshall NF, Peng J, Xie Z, Price DH: **Control of RNA polymerase II elongation potential by a novel carboxyl-terminal domain kinase**. *J Biol Chem* 1996, 271(43):27176-27183. ³⁷³**novel carboxyl-terminal domain kinase**. *J Biol Chem* 1996, **271**(43):27176-27183.

- 374 104. Marshall NF, Price DH: **Purification of P-TEFb, a transcription factor required for the transition into productive elongation**. *J Biol Chem* 1995, 270(21):12335-12338. ³⁷⁵**transition into productive elongation**. *J Biol Chem* 1995, **270**(21):12335-12338.
- 376
- 377
- 378

378 ³⁷⁹**Figure Legends**

380
381

381 **Figure 1: Cocaine enhances both the catalytic activity and nuclear translocation of DNA-PK.** Jurkat 382 cells harboring the pHR'-P-Luc provirus (A), microglial cells (C), and MT-4 cells (E) were treated with different 382 cells harboring the pHR'-P-Luc provirus (A), microglial cells (C), and MT-4 cells (E) were treated with different
383 concentrations of cocaine (Coc: 5, 10, and 20 µM) for 3 h (Lanes 2 to 4). Jurkat-pHR'-P'-Luc cells w 383 concentrations of cocaine (Coc: 5, 10, and 20 μ M) for 3 h (Lanes 2 to 4). Jurkat-pHR'-P'-Luc cells were
384 treated with 10 uM cocaine (Coc) in replicates for 30 min and 3 h (Lanes 3 to 6) (G). Cells were harvested 384 treated with 10 µM cocaine (Coc) in replicates for 30 min and 3 h (Lanes 3 to 6) (G). Cells were harvested,
385 and nuclear lysates were analyzed by immunoblotting using specific antibodies, pDNA-PKcs (S2056) and 385 and nuclear lysates were analyzed by immunoblotting using specific antibodies, pDNA-PKcs (S2056) and 386 DNA-PKcs, as indicated. Actin, a constitutively expressed protein, was used as a loading control. 386 DNA-PKcs, as indicated. Actin, a constitutively expressed protein, was used as a loading control.
387 Densitometric analysis of protein bands (normalized to actin) confirmed the significant upregulation of total 387 Densitometric analysis of protein bands (normalized to actin) confirmed the significant upregulation of total
388 DNA-PKcs and its phosphorylated form, pDNA-PKcs S2056 (pDNA-PKcs), following cocaine treatment (B, D, 388 DNA-PKcs and its phosphorylated form, pDNA-PKcs S2056 (pDNA-PKcs), following cocaine treatment (**B, D,** 389 **F, & H**). MT-4 cells were treated with increasing doses of cocaine for 3 h. Cells were harvested and lysed, 389 **F, & H**). MT-4 cells were treated with increasing doses of cocaine for 3 h. Cells were harvested and lysed, 390 and both cellular and nuclear lysates were analyzed by immunoblotting with antibodies against pDNA-PKcs 390 and both cellular and nuclear lysates were analyzed by immunoblotting with antibodies against pDNA-PKcs
391 (S2056), DNA-PKcs, HDAC1, and Actin (I). Densitometric analysis of protein bands, normalized to actin, 391 (S2056), DNA-PKcs, HDAC1, and Actin (**I**). Densitometric analysis of protein bands, normalized to actin, 392 validated the enhancement in both the catalytic activity and nuclear translocation of DNA-PK (**J & K**). 392 validated the enhancement in both the catalytic activity and nuclear translocation of DNA-PK (J & K).
393 Immunoblots are representative of at least three independent experiments. The results are expressed as 393 Immunoblots are representative of at least three independent experiments. The results are expressed as 394 mean \pm SD and analyzed by one- or two-way ANOVA, followed by Tukey's multiple comparison test. 394 mean \pm SD and analyzed by one- or two-way ANOVA, followed by Tukey's multiple comparison test.
395 Asterisks over the bars indicate significant differences: $*p < 0.05$ for the comparison of cocaine-treated cells ³⁹⁵Asterisks over the bars indicate significant differences: ∗p < 0.05 for the comparison of cocaine-treated cells 396 vs. untreated cells (Ctrl).

397 **Figure 2: Cocaine-induced HIV transcription augments overall HIV replication.** Structure of the lentiviral 398 vector (pHR'-PNL-Luc) carrying the reporter luciferase gene under the HIV LTR promoter (A). Schematic 398 vector (pHR'-PNL-Luc) carrying the reporter luciferase gene under the HIV LTR promoter (A). Schematic
399 representation of the cocaine (Coc) treatment for the luciferase reporter assay (B). Jurkat-pHR'-P-Luc cells 399 representation of the cocaine (Coc) treatment for the luciferase reporter assay (**B**). Jurkat-pHR'-P-Luc cells
400 were chronically treated with 5 uM – 20 uM of cocaine. The cells were lysed, and luciferase reporter pr 400 were chronically treated with 5 μ M – 20 μ M of cocaine. The cells were lysed, and luciferase reporter protein
401 expression levels were assessed using luciferase assays (C). Schematic depiction of the cocaine tr 401 expression levels were assessed using luciferase assays (C). Schematic depiction of the cocaine treatment
402 and subsequent infection of PBMC cells with replication-competent HIV (D). HIV transcripts were quantified 402 and subsequent infection of PBMC cells with replication-competent HIV (D). HIV transcripts were quantified
403 by real-time PCR using primer sets that amplify the Envelope (Env) region of the HIV genome (E). The level 403 by real-time PCR using primer sets that amplify the Envelope (Env) region of the HIV genome (**E**). The level
404 of Gag/p24 protein was analyzed by immunoblotting with specific antibodies against HIV p24 (**F**). Actin, 404 of Gag/p24 protein was analyzed by immunoblotting with specific antibodies against HIV p24 (F). Actin, a
405 constitutively expressed protein, was used as a loading control in the same blot. Densitometric analysis of 405 constitutively expressed protein, was used as a loading control in the same blot. Densitometric analysis of
406 protein bands (normalized to actin) confirmed a significant increase in p24 levels compared to untreated c 406 protein bands (normalized to actin) confirmed a significant increase in p24 levels compared to untreated cells
407 (Ctrl) (G). Immunoblots are representative of at least three independent experiments. The results are 407 (Ctrl) (G). Immunoblots are representative of at least three independent experiments. The results are 408 expressed as mean \pm SD, analyzed by one-way ANOVA followed by Tukey's multiple comparison test (C & 408 expressed as mean \pm SD, analyzed by one-way ANOVA followed by Tukey's multiple comparison test (C & 409 E) or unpaired t-test (G). Asterisks over the bars indicate significant differences: $*$ p < ⁴⁰⁹**E**) or unpaired t-test (**G**). Asterisks over the bars indicate significant differences: ∗p < 0.05 for the comparison of cocaine-treated cells vs. untreated cells.

⁴¹¹**Figure 3: Partial DNA-PK inhibition severely impairs HIV transcription and latency reactivation.** 412 Schematic representation of protocol for M3814 inhibitor and TNF- α treatment in the luciferase reporter assay
413 (A). Jurkat-pHR'-P-Luc cells were treated with 5, 10, 15, and 20 µM of M3814 for 24 h, followed by a 413 (A). Jurkat-pHR'-P-Luc cells were treated with 5, 10, 15, and 20 μM of M3814 for 24 h, followed by activation
414 with TNF-α (10 ng/ml) for another 48 h. Cells were lysed, and the level of reporter protein expression 414 with TNF- α (10 ng/ml) for another 48 h. Cells were lysed, and the level of reporter protein expression was
415 determined by a luciferase assay (B). The same lysates were analyzed by immunoblotting using specific 415 determined by a luciferase assay (**B**). The same lysates were analyzed by immunoblotting using specific
416 antibodies against the luciferase protein (sc-74548) (C). Jurkat-pHR'-P-Luc cells were cultured with different 416 antibodies against the luciferase protein (sc-74548) (C). Jurkat-pHR'-P-Luc cells were cultured with different
417 concentrations (2 μM to 40 μM) of M3814 for 48–72 h, and cell cytotoxicity was determined via MTS-PMS 417 concentrations (2 μM to 40 μM) of M3814 for 48–72 h, and cell cytotoxicity was determined via MTS-PMS cell
418 proliferation assay (Promega, Madison, WI, USA) (D). Latently HIV-infected 2D10 cells, which express the 418 proliferation assay (Promega, Madison, WI, USA) (D). Latently HIV-infected 2D10 cells, which express the
419 reporter short-lived green fluorescent protein (d2EGFP) from the HIV LTR promoter, were treated with 5 uM reporter short-lived green fluorescent protein (d2EGFP) from the HIV LTR promoter, were treated with 5 μ M

420 or 10 μM of M3814 for 24 h and then stimulated with TNF-α for another 48 h. Cells were subjected to GFP
421 expression analysis via flow cytometry (**E & F**). Immunoblots are representative of at least three independe 421 expression analysis via flow cytometry (**E & F**). Immunoblots are representative of at least three independent
422 experiments The results are expressed as mean ± SD and analyzed by one- or two-way ANOVA followed by 422 experiments The results are expressed as mean \pm SD and analyzed by one- or two-way ANOVA followed by 423 Tukey's multiple comparison test. Asterisks over the bars indicate significant differences: **p < 0.01 and ** 423 Tukey's multiple comparison test. Asterisks over the bars indicate significant differences: **p < 0.01 and ***p
424 < 0.001 for the comparison of inactive vs. activated cells (TNF- α) and activated cells (TNF- α) 424 < 0.001 for the comparison of inactive vs. activated cells (TNF-α) and activated cells (TNF-α) vs. activated 425 cells (TNF-α) in the presence of the DNA-PK inhibitor, M3814. cells (TNF-α) in the presence of the DNA-PK inhibitor, M3814.

426 **Figure 4: Partial DNA-PK inhibition restricts HIV replication.** Schematic timeline for the treatment with 427 M3814, NU7441 inhibitors, TNF-α, and replication-competent HIV (A). Jurkat cells were treated overnight wi 427 M3814, NU7441 inhibitors, TNF-α, and replication-competent HIV (A). Jurkat cells were treated overnight with
428 different concentrations of M3814 (5, 10, 15, and 20 μM) (**B**) and NU7441 (5, 10, 15, and 20 μM) (**D**) f 428 different concentrations of M3814 (5, 10, 15, and 20 μM) (**B**) and NU7441 (5, 10, 15, and 20 μM) (**D**) for 24 h
429 (Lanes 5-12). The next day, cells were activated with 10 ng/ml TNF-α for 3 h (Lanes 3, 4, 6, 8, 10, & 429 (Lanes 5-12). The next day, cells were activated with 10 ng/ml TNF-α for 3 h (Lanes 3, 4, 6, 8, 10, & 12).
430 Subsequently, cells were infected with a replication-competent dual-tropic HIV (Type 1 strain 93/TH/051) 430 Subsequently, cells were infected with a replication-competent dual-tropic HIV (Type 1 strain 93/TH/051)
431 (Lanes 1-12). Cell Ivsates were prepared 4 h (NU7441) or 6 h (M3814) post-infection (hpi). Total cell Ivsates 431 (Lanes 1-12). Cell lysates were prepared 4 h (NU7441) or 6 h (M3814) post-infection (hpi). Total cell lysates
432 were analyzed by SDS-PAGE, transferred to a nitrocellulose membrane, and detected with specific HIV 432 were analyzed by SDS-PAGE, transferred to a nitrocellulose membrane, and detected with specific HIV
433 antibodies as indicated. Immunoreactive proteins were detected using appropriately labeled secondary 433 antibodies as indicated. Immunoreactive proteins were detected using appropriately labeled secondary
434 antibodies with Licor. Actin was used as a loading control. Densitometric analysis of protein bands relative to 434 antibodies with Licor. Actin was used as a loading control. Densitometric analysis of protein bands relative to
435 actin (C & E). Immunoblots are representative of at least three independent experiments. The results a 435 actin (C & E). Immunoblots are representative of at least three independent experiments. The results are 436 expressed as mean \pm SD and analyzed by one-way ANOVA followed by Tukey's multiple comparison test. 436 expressed as mean \pm SD and analyzed by one-way ANOVA followed by Tukey's multiple comparison test.
437 Asterisks over the bars indicate significant differences: **p < 0.01 for the comparison of inactive vs. activat 437 Asterisks over the bars indicate significant differences: $*p < 0.01$ for the comparison of inactive vs. activated
438 cells (TNF-a) and activated cells (TNF-a) vs. activated cells (TNF-a) in the presence of DNA-PK inh 438 cells (TNF-α) and activated cells (TNF-α) vs. activated cells (TNF-α) in the presence of DNA-PK inhibitors,
439 NU7441 or M3814. ⁴³⁹NU7441 or M3814.

440 Figure 5: Cocaine-mediated DNA-PK activation promotes HIV transcription and replication in both cell
441 lines and primary cells. Jurkat-pHR'-P-Luc cells were treated with 10 µM of M3814 for 24 h. The next day, ⁴⁴¹**lines and primary cells.** Jurkat-pHR'-P-Luc cells were treated with 10 µM of M3814 for 24 h. The next day, 442 cells were treated with cocaine twice daily for 48 h and again 3 h before harvesting. Cells were lysed, and the
443 level of reporter protein expression was determined using a luciferase reporter assay (A). Jurkat cel ⁴⁴³level of reporter protein expression was determined using a luciferase reporter assay (**A**). Jurkat cells (**B & C**) ⁴⁴⁴and PBMCs (**D & E**) were treated with 10 µM of M3814 for 24 h, then treated with cocaine for 3 h, and 445 subsequently infected with replication-competent HIV for another 3 to 6 h. HIV transcripts were quantified by
446 real-time PCR using primer sets that amplify the Nuc-2 (**B & D**) and Env (**C & E**) regions of the HIV g 446 real-time PCR using primer sets that amplify the Nuc-2 (**B & D**) and Env (C & E) regions of the HIV genome.
447 Jurkat cells were treated with 10 uM of M3814 for 24 h (Lanes 7 to 12), then treated with cocaine for 3 h 447 Jurkat cells were treated with 10 µM of M3814 for 24 h (Lanes 7 to 12), then treated with cocaine for 3 h
448 (Lanes 3-6 & 10-12) and infected with replication-competent HIV across all lanes (Lanes 1-12) for another 5 448 (Lanes 3-6 & 10-12) and infected with replication-competent HIV across all lanes (Lanes 1-12) for another 5
449 h. The levels of HIV p24 and p17 proteins were analyzed via immunoblotting using antibodies against these 449 h. The levels of HIV p24 and p17 proteins were analyzed via immunoblotting using antibodies against these
450 HIV proteins (F). Actin, a constitutively expressed protein, was used as a loading control. Densitometric 450 HIV proteins (F). Actin, a constitutively expressed protein, was used as a loading control. Densitometric
451 analysis of protein bands (normalized to actin) was performed (G). Immunoblots are representative of at leas 451 analysis of protein bands (normalized to actin) was performed (G). Immunoblots are representative of at least
452 three independent experiments. The results are expressed as mean ± SD and analyzed by two-way ANOVA 452 three independent experiments. The results are expressed as mean \pm SD and analyzed by two-way ANOVA 453 followed by Tukey's multiple comparisons test. Asterisks over the bars indicate significant differences: *p < 453 followed by Tukey's multiple comparisons test. Asterisks over the bars indicate significant differences: ∗p <
454 0.05 for the comparison of cocaine-treated samples vs. untreated (Ctrl) and the comparison of cocaine p 454 0.05 for the comparison of cocaine-treated samples vs. untreated (Ctrl) and the comparison of cocaine plus
455 inhibitor-treated samples vs. cocaine alone-treated samples. inhibitor-treated samples vs. cocaine alone-treated samples.

456 Figure 6: Cocaine promotes HIV transcription by enhancing the phosphorylation of the C-terminal
457 domain (CTD) of RNA polymerase II (RNAP II). THP-1 cells were treated with increasing doses of cocaine 457 **domain (CTD) of RNA polymerase II (RNAP II).** THP-1 cells were treated with increasing doses of cocaine
458 (5, 10, 15, and 20 µM) for 3 h (A). MT-4 cells were treated as follows: untreated and uninfected (Lane 1), ⁴⁵⁸(5, 10, 15, and 20 µM) for 3 h (**A**). MT-4 cells were treated as follows: untreated and uninfected (Lane 1), 459 infected with HIV (93/TH/051) without cocaine treatment (Lane 2), treated with cocaine without HIV infection
460 (Lane 3), or pre-treated with different concentrations of cocaine before HIV infection (Lanes 4 to 6) (C) ⁴⁶⁰(Lane 3), or pre-treated with different concentrations of cocaine before HIV infection (Lanes 4 to 6) (**C**). Cells 461 were harvested, and nuclear lysates were analyzed by immunoblotting with specific antibodies against
462 phosphorylated RNAP II, RNAP II Ser2, and RNAP II Ser5. Actin, a constitutively expressed protein, was 462 phosphorylated RNAP II, RNAP II Ser2, and RNAP II Ser5. Actin, a constitutively expressed protein, was
463 used as a loading control. Densitometric analysis of protein bands (normalized to actin) confirmed significant 463 used as a loading control. Densitometric analysis of protein bands (normalized to actin) confirmed significant
464 hyper-phosphorylation of RNAP II CTD at both Ser2 and Ser5 residues following cocaine treatment (B & D) 464 hyper-phosphorylation of RNAP II CTD at both Ser2 and Ser5 residues following cocaine treatment (**B & D**).
465 THP-1 cells were treated with cocaine in the absence or presence of different concentration of M3814 (0.5, THP-1 cells were treated with cocaine in the absence or presence of different concentration of M3814 (0.5, 5,

466 and 10 μM) (**E**). Cells were harvested, and nuclear extracts were evaluated via immunoblotting using specific
467 antibodies against RNAP II Ser2. RNAP II Ser5. and total RNAP II. Densitometric analvsis of protein ban 467 antibodies against RNAP II Ser2, RNAP II Ser5, and total RNAP II. Densitometric analysis of protein bands
468 (normalized to actin) confirmed a significant increase in RNAP II CTD phosphorylation at both Ser2 and Ser5 468 (normalized to actin) confirmed a significant increase in RNAP II CTD phosphorylation at both Ser2 and Ser5
469 upon cocaine treatment. However, a significant reduction in CTD phosphorylation at both Ser2 and Ser5 was 469 upon cocaine treatment. However, a significant reduction in CTD phosphorylation at both Ser2 and Ser5 was
470 observed upon DNA-PK inhibition with M3814 compared to cocaine-alone samples (F). Immunoblots are 470 observed upon DNA-PK inhibition with M3814 compared to cocaine-alone samples (F). Immunoblots are 471 representative of at least three independent experiments. The results are expressed as mean \pm SD and 471 representative of at least three independent experiments. The results are expressed as mean \pm SD and 472 analyzed by two-way ANOVA followed by Tukey's multiple comparison test. Asterisks over the bars indicate 472 analyzed by two-way ANOVA followed by Tukey's multiple comparison test. Asterisks over the bars indicate
473 significant differences. $* p < 0.05$ is for the comparison of cocaine-treated samples against untreated (Ctrl 473 significant differences. $*$ p < 0.05 is for the comparison of cocaine-treated samples against untreated (Ctrl)
474 and the comparison of cocaine plus inhibitors treated against cocaine alone-treated samples and the comparison of cocaine plus inhibitors treated against cocaine alone-treated samples

475 Figure 7: Cocaine enhances the elongation phase of HIV transcription not only by stimulating DNA-PK
476 but also via P-TEFb activation. Jurkat-pHR'P-Luc cells were treated with increasing doses of cocaine (5, 10, 476 **but also via P-TEFb activation.** Jurkat-pHR'P-Luc cells were treated with increasing doses of cocaine (5, 10,
477 15, 20, and 25 μM) for 3 h (A). Jurkat-pHR'P-Luc cells were treated as follows: untreated and uninfect ⁴⁷⁷15, 20, and 25 µM) for 3 h (**A**). Jurkat-pHR'P-Luc cells were treated as follows: untreated and uninfected 478 (Lane 1), infected with HIV (93/TH/051) without cocaine treatment (Lane 2), treated with cocaine without HIV
479 infection (Lane 3), or pre-treated with different concentrations of cocaine before HIV infection (Lanes 4 479 infection (Lane 3), or pre-treated with different concentrations of cocaine before HIV infection (Lanes 4 to 6)
480 (C). Cells were harvested, and nuclear lysates were analyzed by immunoblotting with specific antibodie 480 (C). Cells were harvested, and nuclear lysates were analyzed by immunoblotting with specific antibodies 481 against P-TEFb subunits CDK9 and Cyclin T1, as well as CDK7 (TFIIH). Actin was used as a loading control. 481 against P-TEFb subunits CDK9 and Cyclin T1, as well as CDK7 (TFIIH). Actin was used as a loading control.
482 Densitometric analysis of protein bands (normalized to actin) confirmed a significant increase in CDK7, Cycl 482 Densitometric analysis of protein bands (normalized to actin) confirmed a significant increase in CDK7, Cyclin
483 T1. and p-CDK9 (Thr186) compared to untreated (Ctrl) cells (**B & D**). Wild type (WT) and DNA-PK knockdo ⁴⁸³T1, and p-CDK9 (Thr186) compared to untreated (Ctrl) cells (**B & D**). Wild type (WT) and DNA-PK knockdown 484 (DNA-PK KD) cells were treated with cocaine for 30 min and 3 h, and nuclear extracts were subjected to 485 immunoblotting (E). Densitometric analysis of protein bands (normalized to actin) showed increased p-CDK9 485 immunoblotting (E). Densitometric analysis of protein bands (normalized to actin) showed increased p-CDK9
486 phosphorylation and CDK7 activation in WT cells upon cocaine exposure (F). However, in DNA-PK KD cells, 486 phosphorylation and CDK7 activation in WT cells upon cocaine exposure (F). However, in DNA-PK KD cells, 487 the lack of p-CDK9 (Thr186) phosphorylation and CDK7 activation upon cocaine treatment demonstrated that 487 the lack of p-CDK9 (Thr186) phosphorylation and CDK7 activation upon cocaine treatment demonstrated that
488 cocaine-induced activations are DNA-PK specific (F). Immunoblots are representative of at least three 488 cocaine-induced activations are DNA-PK specific (F). Immunoblots are representative of at least three
489 independent experiments. The results are expressed as mean ± SD for three independent experiments, 489 independent experiments. The results are expressed as mean \pm SD for three independent experiments, 490 analyzed by two-way ANOVA followed by Tukey's multiple comparisons test. Asterisks over the bars indicate 490 analyzed by two-way ANOVA followed by Tukey's multiple comparisons test. Asterisks over the bars indicate 491 significant differences: $*p < 0.05$ compared to untreated cells (Ctrl). significant differences: $*D < 0.05$ compared to untreated cells (Ctrl).

⁴⁹²**Figure 8: Cocaine-induced DNA-PK relieves RNAP II pausing by phosphorylating TRIM28 at S824.** ⁴⁹³THP-1 (**A & B**) and Jurkat cells (**C & D**) were treated with increasing doses of cocaine, and the nuclear 494 lysates were analyzed via immunoblotting using specific antibodies against pTRIM28 (S824) and total 495 TRIM28. Densitometric analysis confirmed a significant increase in pTRIM28 (S824) levels compared to 495 TRIM28. Densitometric analysis confirmed a significant increase in pTRIM28 (S824) levels compared to 496 untreated cells (Ctrl) (A, B, C & D). Jurkat-pHR'P-Luc cells were treated with cocaine (10 µM) for varying 496 untreated cells (Ctrl) (**A, B, C & D**). Jurkat-pHR'P-Luc cells were treated with cocaine (10 μM) for varying
497 durations (30 min, 3 h, and 6 h), and the nuclear lysates were analyzed via immunoblotting using specifi 497 durations (30 min, 3 h, and 6 h), and the nuclear lysates were analyzed via immunoblotting using specific
498 antibodies against pTRIM28 (S824) and total TRIM28. Densitometric analysis of protein bands (normalized to 498 antibodies against pTRIM28 (S824) and total TRIM28. Densitometric analysis of protein bands (normalized to
499 actin) confirmed a significant increase in pTRIM28 (S824) levels compared to untreated cells (Ctrl) (E & F) 499 actin) confirmed a significant increase in pTRIM28 (S824) levels compared to untreated cells (Ctrl) (**E & F**).
500 THP-1 cells were treated as follows: untreated and uninfected (Lane 1), infected with HIV (93/TH/051) w 500 THP-1 cells were treated as follows: untreated and uninfected (Lane 1), infected with HIV (93/TH/051) without
501 cocaine (Lane 2), treated with cocaine without HIV infection (Lane 3), or pre-treated with different 501 cocaine (Lane 2), treated with cocaine without HIV infection (Lane 3), or pre-treated with different
502 concentrations of cocaine before HIV infection (Lanes 4 to 6). Nuclear lysates were analyzed via 502 concentrations of cocaine before HIV infection (Lanes 4 to 6). Nuclear lysates were analyzed via
503 immunoblotting using specific antibodies against pTRIM28 (S824) and total TRIM28 (G). Densitometric 503 immunoblotting using specific antibodies against pTRIM28 (S824) and total TRIM28 (G). Densitometric
504 analysis of protein bands (normalized to actin) confirmed a significant increase in pTRIM28 (S824) levels 504 analysis of protein bands (normalized to actin) confirmed a significant increase in pTRIM28 (S824) levels
505 compared to untreated cells (Ctrl) (H). THP-1 cells were treated with different concentrations of M3814 in t 505 compared to untreated cells (Ctrl) (H). THP-1 cells were treated with different concentrations of M3814 in the
506 presence and absence of cocaine (10 µM), and the nuclear lysates were analyzed via immunoblotting using 506 presence and absence of cocaine (10 µM), and the nuclear lysates were analyzed via immunoblotting using
507 specific antibodies against pTRIM28 (S824) and total TRIM28 (I). Densitometric analysis of protein bands 507 specific antibodies against pTRIM28 (S824) and total TRIM28 (**I**). Densitometric analysis of protein bands
508 (normalized to actin) confirmed a significant increase in pTRIM28 (S824) levels compared to untreated cells 508 (normalized to actin) confirmed a significant increase in pTRIM28 (S824) levels compared to untreated cells
509 (Ctrl). However, the presence of the inhibitor (M3814) severely impaired pTRIM28 (S824) compared to the 509 (Ctrl). However, the presence of the inhibitor (M3814) severely impaired pTRIM28 (S824) compared to the 510 cocaine-treated sample (J). WT and DNA-PK KD cells were treated with cocaine for 30 min and 3 h, and 510 cocaine-treated sample (**J**). WT and DNA-PK KD cells were treated with cocaine for 30 min and 3 h, and 51 and
511 nuclear lysates were subjected to immunoblotting (**K**). Densitometric analysis of protein bands (normali 511 nuclear lysates were subjected to immunoblotting (K). Densitometric analysis of protein bands (normalized to
512 actin) confirmed enhanced phosphorylation of p-TRIM28 in WT cells upon cocaine exposure. However, in actin) confirmed enhanced phosphorylation of p-TRIM28 in WT cells upon cocaine exposure. However, in

513 DNA-PK KD cells, phosphorylated TRIM28 levels remained reduced upon cocaine exposure, confirming that 514
514 cocaine-induced TRIM28 phosphorylation is DNA-PK specific (L). Immunoblots are representative of at least 514 cocaine-induced TRIM28 phosphorylation is DNA-PK specific (L). Immunoblots are representative of at least
515 three independent experiments. The results are expressed as mean ± SD for three independent experiments, 515 three independent experiments. The results are expressed as mean \pm SD for three independent experiments, 516 analyzed by two-way ANOVA followed by two-way followed by two-way ANOVA followed by Tukey's multiple comp 516 analyzed by two-way ANOVA followed by Tukey's multiple comparisons test. Asterisks over the bars indicate
517 significant differences. *p < 0.05 is for the comparison of cocaine-treated samples against untreated (Ctrl) 517 significant differences. $*p < 0.05$ is for the comparison of cocaine-treated samples against untreated (Ctrl) and
518 the comparison of cocaine plus inhibitors treated against cocaine alone-treated samples the comparison of cocaine plus inhibitors treated against cocaine alone-treated samples

⁵¹⁹**Figure 9: Cocaine promotes RNAP II pause-release by phosphorylating TRIM28 at S824.** Jurkat cells 520 were exposed to increasing concentrations of cocaine, and both cytoplasmic and nuclear extracts were
521 subjected to immunoblotting using specific antibodies against pTRIM28 (S824), total TRIM28, DSIF (SPT-5), 521 subjected to immunoblotting using specific antibodies against pTRIM28 (S824), total TRIM28, DSIF (SPT-5),
522 NELF-E, and HDAC1. Densitometric analysis of protein bands (normalized to actin) confirmed a significant 522 NELF-E, and HDAC1. Densitometric analysis of protein bands (normalized to actin) confirmed a significant
523 increase in nuclear pTRIM28 (S824) levels following cocaine exposure compared to untreated cells (Ctrl) (A 523 increase in nuclear pTRIM28 (S824) levels following cocaine exposure compared to untreated cells (Ctrl) (A,
524 B & C). Immunoblots are representative of at least three independent experiments. The results are express 524 **B & C**). Immunoblots are representative of at least three independent experiments. The results are expressed
525 as mean ± SD for three independent experiments, analyzed by two-way ANOVA followed by Tukey's multiple 525 as mean \pm SD for three independent experiments, analyzed by two-way ANOVA followed by Tukey's multiple
526 comparisons test. Asterisks over the bars indicate significant differences. Statistical significance is set 526 comparisons test. Asterisks over the bars indicate significant differences. Statistical significance is set as p <
527 0.05 (*) compared to untreated cells (Ctrl). 0.05 ($*$) compared to untreated cells (Ctrl).

528 Figure 10: Cocaine enhances HIV transcription by promoting recruitment of DNA-PKcs and pTRIM28
529 (S824) at HIV LTR promoter. Jurkat cells freshly infected with replication-incompetent HIV, pHR'P-Luc, were 529 (S824) at HIV LTR promoter. Jurkat cells freshly infected with replication-incompetent HIV, pHR'P-Luc, were
530 exposed to cocaine. Chromatin immunoprecipitation (ChIP) assays were conducted to assess the recruitment 530 exposed to cocaine. Chromatin immunoprecipitation (ChIP) assays were conducted to assess the recruitment
531 kinetics of DNA-PKcs, RNAP II, CDK7 (TFIIH), pTRIM28 (S824), total CDK9, and H3K27me3 at the promoter 531 kinetics of DNA-PKcs, RNAP II, CDK7 (TFIIH), pTRIM28 (S824), total CDK9, and H3K27me3 at the promoter
532 (A & E), Nucleosome-1 (B & F), Nucleosome-2 (C & G), and downstream Envelope regions (D & H) of HIV ⁵³²(**A & E**), Nucleosome-1 (**B & F**), Nucleosome-2 (**C & G**), and downstream Envelope regions (**D & H**) of HIV 533 LTR, using specific primer sets. The results are presented as mean \pm SD for three independent experiments, 534 analyzed by two-way ANOVA followed by Tukey's multiple comparisons test. Asterisks above the bars 534 analyzed by two-way ANOVA followed by Tukey's multiple comparisons test. Asterisks above the bars
535 indicate significant differences. Statistical significance is set as $p < 0.05 (*)$ compared to untreated cells (Ctrl). indicate significant differences. Statistical significance is set as $p < 0.05 (*)$ compared to untreated cells (Ctrl).

536 **Figure 11: Cocaine-mediated DNA-PK activation enhances multiple aspects of HIV transcription.**
537 Cocaine-induced DNA-PK activation facilitates various stages of HIV transcription. Firstly, it enhances the 537 Cocaine-induced DNA-PK activation facilitates various stages of HIV transcription. Firstly, it enhances the
538 initiation phase by phosphorylating the C-terminal domain (CTD) of RNA polymerase II (RNAP II) at Ser5. 538 initiation phase by phosphorylating the C-terminal domain (CTD) of RNA polymerase II (RNAP II) at Ser5.
539 Secondly, cocaine-stimulated DNA-PK promotes the elongation phase by both directly catalyzing and 539 Secondly, cocaine-stimulated DNA-PK promotes the elongation phase by both directly catalyzing and
540 facilitating the recruitment of positive transcription elongation factor b (P-TEFb), leading to the 540 facilitating the recruitment of positive transcription elongation factor b (P-TEFb), leading to the
541 phosphorylation of Ser2 within the RNAP II CTD. This posttranslational modification renders RNAP II 541 phosphorylation of Ser2 within the RNAP II CTD. This posttranslational modification renders RNAP II
542 processive, ensuring efficient elongation. Finally, cocaine-induced DNA-PK activity also alleviates RNAP II 542 processive, ensuring efficient elongation. Finally, cocaine-induced DNA-PK activity also alleviates RNAP II
543 pausing by phosphorylating TRIM28 at Ser824 (p-TRIM28 S824). This modification transforms TRIM28 from 543 pausing by phosphorylating TRIM28 at Ser824 (p-TRIM28 S824). This modification transforms TRIM28 from
544 a transcriptional pausing factor to a facilitator (transactivator), thereby supporting HIV transcription. a transcriptional pausing factor to a facilitator (transactivator), thereby supporting HIV transcription.

Lane 1 2 3 4

