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LETTER TO THE EDITOR

Inhibition of Metalloprotease TRABD2A Facilitates the Study of HIV-1 Replication in Resting CD4$^+$ T Cells

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Although the HIV-1 particle can enter resting CD4$^+$ T cells efficiently *in vitro*, the replication cycle is suppressed in resting CD4$^+$ T cells, unlike in stimulated CD4$^+$ T cells (1–3). Thus, it is difficult to study HIV-1 replication in resting CD4$^+$ T cells without a technique that allows HIV-1 to produce detectable virions in culture supernatants. Recently, we discovered that inhibiting the membrane metalloprotease TRABD2A could allow HIV-1 to complete its replication cycle and produce detectable virions in the cell culture supernatants from resting CD4$^+$ T cells (4, 5). Therefore, we aimed to examine the effectiveness of studying HIV-1 replication in resting CD4$^+$ T cells by inhibiting TRABD2A. We decided to screen cellular proteins potentially involved in HIV-1 replication by employing RNA interference (RNAi) technology. Before screening, we assessed gene expression in activated or resting CD4$^+$ T cells using RNA-seq (SRA accession number PRJNA522052). Among the potential candidates, we observed that only ATF3 and CLC transcripts were not detected in resting CD4$^+$ T cells, whereas all the others were detected (data not shown). We electroporated small interfering RNA (siRNA) for each gene into HIV-1_{NL4-3}-infected resting CD4$^+$ T cells and divided them into two groups based on treatment or nontreatment with 1,10-phenanthroline, which can inhibit TRABD2A protease activity, thereby permitting the release of HIV-1 progeny virions from resting CD4$^+$ T cells. We used TZM-bl reporter cells to measure virion infectivity, as viral progeny infectivity could reflect the amount or integrality of the produced virions. As expected, only in the presence, not in the absence, of 1,10-phenanthroline could we detect the virions produced from resting CD4$^+$ T cells. A knockdown of CycT1 or CDK9 could significantly inhibit HIV-1 progeny infectivity (Fig. 1A), and the decreased infectivity should be due to fewer virions in cell culture, as they are required for Tat-transactivated HIV-1 transcription. To determine if this was the case, we measured the levels of produced virions using p24 enzyme-linked immunosorbent assays (ELISAs). Our results showed that the depletion of CycT or CDK9 did indeed reduce virion production (Fig. 1B). Moreover, both transcript levels were decreased using their specific siRNAs (Fig. 1C).

In contrast, the depletion of either March8 or MX2 resulted in increased HIV-1 progeny infectivity. March8 reportedly inhibited Env gp120 incorporation into HIV-1 virions, thereby causing lower infectivity of the produced virions, and MX2 reportedly inhibited viral cDNA translocation into the nucleus after reverse transcription, thus leading to lower viral production (6–8). Interestingly, we observed that the knockdown of PDS5B, a regulator of sister chromatid cohesion, could result in lower infectivity of HIV-1 virions in culture supernatants, as fewer virions were produced from resting CD4$^+$ T cells. Thus, PDS5B might play a role in HIV-1 replication, suggesting the need for further investigation to elucidate its activity. Importantly, the depletion of other can-

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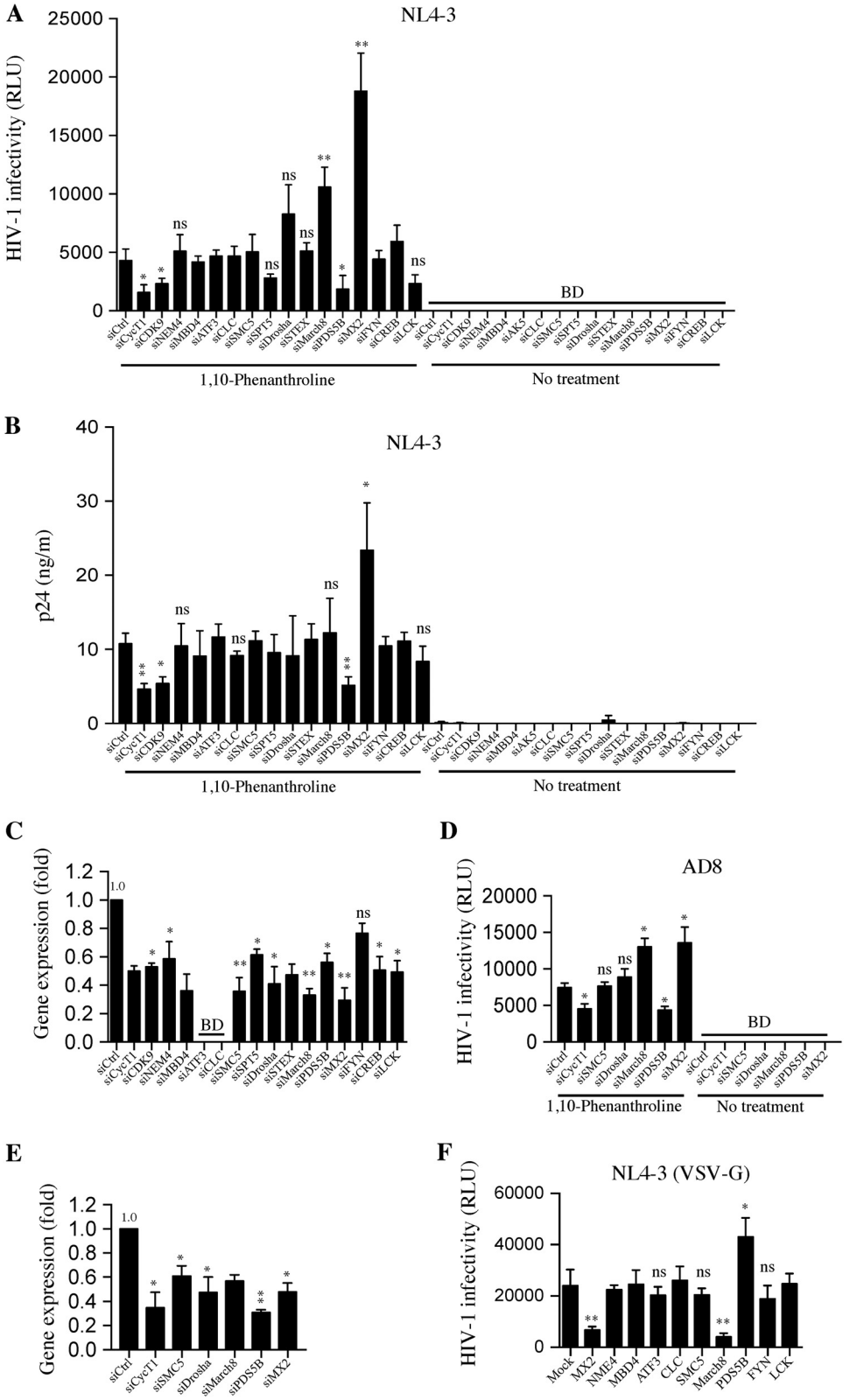

FIG 1 siRNA-based screening to identify cellular factors associated with HIV-1 replication in resting CD4$^+$ T cells. (A to E) Resting CD4$^+$ T cells isolated from independent healthy donors ($n = 3$; the graph shows the mean ± standard error of the mean [SEM]) were treated with VLP-Vpx and spinoculated with HIV-$1_{NL4.3}$ (A to C) or HIV-1_{AD8} (VSV-G) (D and E). Six hours after infection, the infected cells were washed twice to remove the input virus. Twenty-four hours after infection, the infected cells were aliquoted for tandem siRNA electroporation against each gene or

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FIG 1 Legend (Continued)
control, as indicated, in the presence or absence of 1,10-phenanthroline (2.5 μM). After 48 h of treatment, TZM-bl indicator cells or p24 ELISA was used to measure virion infectivity or production in culture supernatants. Total RNA was extracted from 1,10-phenanthroline-treated cells for quantitative PCR (qPCR) to measure each gene transcript level, normalized to GAPDH transcripts by their specific primers. (F) 293T cells were transfected with each gene, as indicated, or mock expression vectors; 24 h later, the cells were infected with HIV-1_{NL4-3} (VSV-G). Twenty-four hours after infection, TZM-bl indicator cells were used to measure produced virion infectivity. BD, below the detection limit; *, $P < 0.05$; **, $P < 0.01$ (two-tailed unpaired *t* test).

didate proteins did not affect the production or infectivity of progeny virions significantly, indicating that none were involved in HIV-1 replication. Because the CCR5 coreceptor is very limited in resting CD4$^+$ T cells, this technique for inhibiting TRABD2A is inefficient for studying CCR5-tropic HIV-1 infection of resting CD4$^+$ T cells. To overcome this barrier, we used vesicular stomatitis virus G protein (VSV-G) combined with replication-competent CCR5-tropic HIV-1_{AD8} to facilitate viral entry into resting CD4$^+$ T cells. Consistently, we obtained similar results by inhibiting TRABD2A and by knocking down the candidate genes (Fig. 1D and E). Next, we confirmed our screening results by overexpressing these candidate genes in 293T cells infected with HIV-1_{NL4-3} (VSV-G) (Fig. 1F). Taken together, our results show that inhibiting TRABD2A could provide an effective method for identifying host cell factors associated with HIV-1 replication in resting CD4$^+$ T cells.

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