Single-nucleotide identification of ultra-conserved m6A positions on HIV-1 RNA

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BACKGROUND

N6-methyladenosine (m6A) is the most abundant internal modification of eukaryotic messenger RNA and regulates multiple RNA-related biological processes. M6A modifications of HIV-1 RNA have been reported, but precise location and functional effects of m6A variations in HIV-1 transcripts remain poorly characterized, particularly in people living with HIV (PLWH).

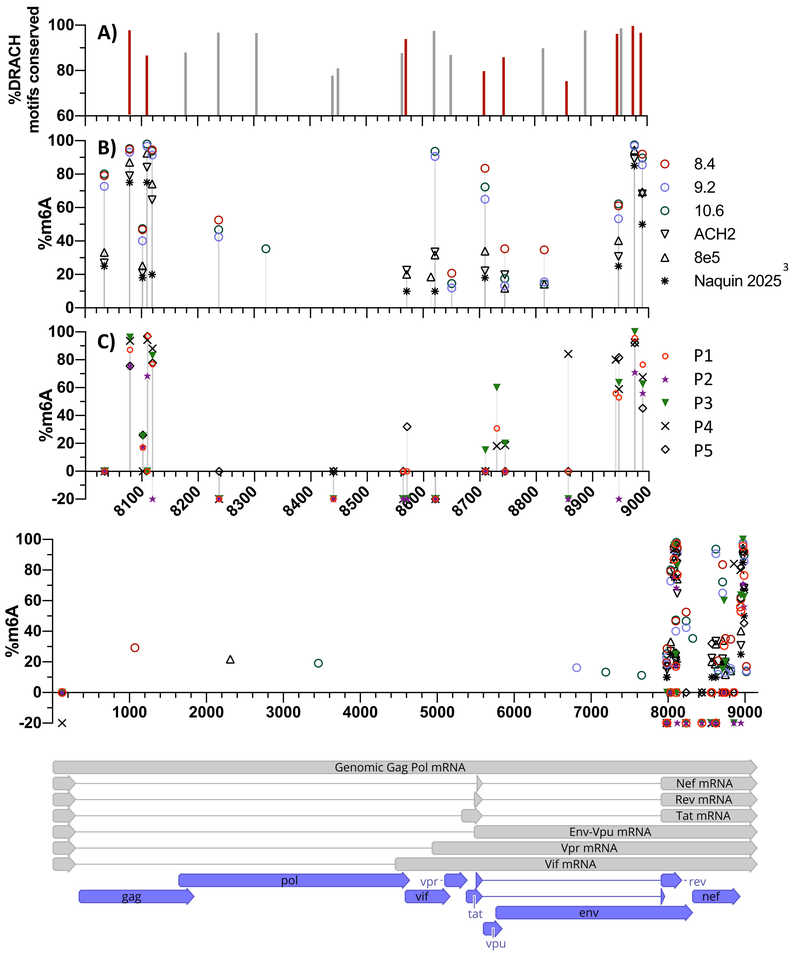


Fig. 1: The HIV-1 m6A profile is highly conserved at the 3′ end. **(A)** Percentage of DRACH motifs conserved across 615 unique intact proviral genomes from 236 PLWH. **(B, C)** m6A profiles obtained by GLORI-seq using 100–300 ng of mRNA extracted from **(B)** HIV-1–infected cell lines stimulated with PMA/ionomycin (48h; *n* = 2). Only sites common to both replicates, showing >10% m6A and a >50% reduction upon treatment with STM3006 (48h, 2 µM), are shown. **(C)** mCD4⁺ T cells from 5 PLWH, activated with CD3/CD28 antibodies (48h) and electroporated with Tat mRNA (48 h; *n* = 1). A total of 100–800 million reads were aligned to either the HXB2 reference genome or autologous proviral sequences obtained by FLIP-seq[2]. Negative values indicate coverage below 15 reads.

Ultra-conserved m6A sites are enriched at the 3'end of HIV-1 RNA *in vivo*

RESULTS

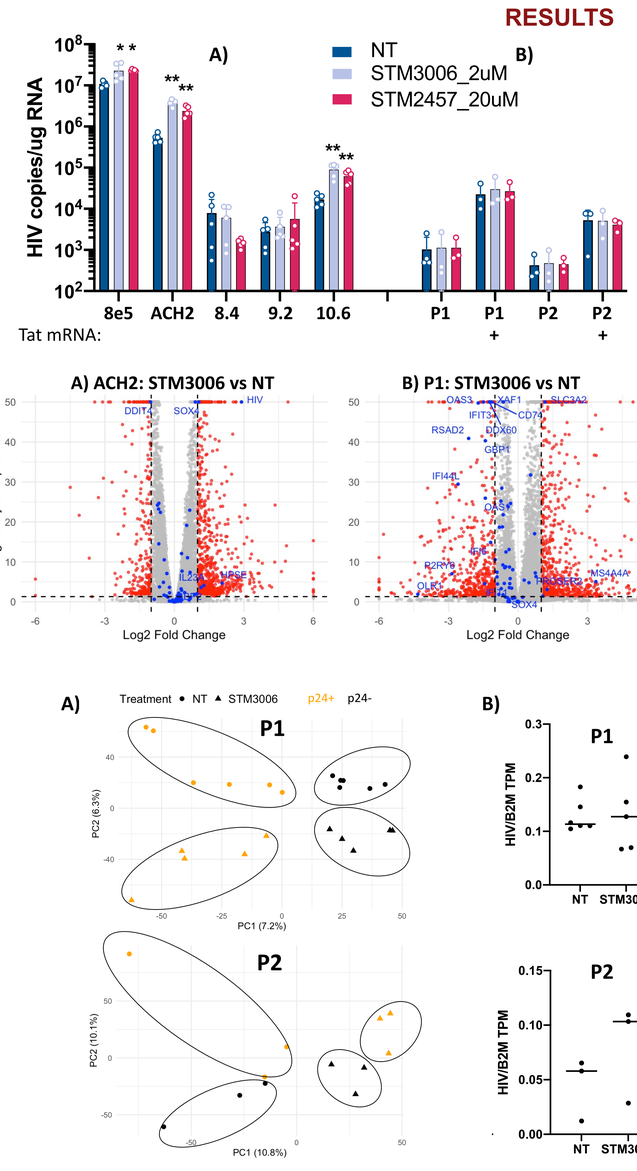


Fig. 2: METTL3 inhibition increases HIV-1 expression in certain HIV-1–infected cell lines but not in primary CD4⁺ T cells from PLWH. **(A)** HIV-1–infected cell lines or **(B)** resting mCD4⁺ T cells from PLWH were electroporated (EH-100) with Tat mRNA and treated with METTL3 inhibitors for 48h at the indicated concentrations. 250ng of total RNA was used for RT-ddPCR with primers and a probe targeting the 5′ LTR region[4].

Fig. 3: METTL3 inhibition does not affect ISG expression in HIV-1–infected cell lines but appears to reduce it in primary CD4⁺ T cells from PLWH. **(A)** An HIV-1–infected cell line (ACH2, n=3) or **(B)** resting mCD4⁺ T cells from PLWH were treated with STM3006 (48h, 2uM, n=2). 100 ng of total RNA was used for mRNA sequencing. A panel of 86 interferon-stimulated genes (ISGs) is highlighted in blue[5], and gene names are shown when *padj* < 0.05, $|\log_2 FC| > 1$ and baseMean > 10.

Fig. 4: METTL3-inhibited p24⁺ cells exhibit a distinct transcriptomic profile but do not display increased HIV-1 expression. Resting mCD4⁺ T cells from PLWH were electroporated (EH-100) with Tat mRNA and treated with STM3006 (48h, 2µM). 30-60 p24⁺ or p24⁻ cells were sorted as previously described in Pardons et *al.*[6] prior to RNA-seq library preparation. **(A)** Only protein-coding genes with TPM > 0 were included in the principal component analysis (PCA). P1: *n* = 6; P2: *n* = 3. **(B)** Quantification of reads mapping to autologous proviral sequence.

METHODS

- GLORI-seq[1] in combination with the METTL3 inhibitor STM3006 to accurately identify m6A sites on HIV-1 in infected cell lines (Jlat 8.4, Jlat 9.2, Jlat 10.6, 8e5, ACH2) and mCD4+ T cells from 5 PLWH under cART .
- RT-ddPCR
- Bulk and pseudo-bulk RNA-seq in HIV-1 infected cell lines and/or mCD4+ T cells from PLWH

CONCLUSIONS

- HIV-1 RNA is m6A-methylated at highly conserved positions, predominantly at the 3′ end.
- Beyond these conserved sites, participant-specific loss or gain of certain m6A sites is observed, suggesting an important biological function.
- Global m6A removal via small-molecule inhibition of METTL3 does not consistently enhance latency reversal in HIV-1–infected cell lines or in mCD4+ T cells from PLWH.

ADDITIONAL KEY INFORMATION

References:
[1]Sun et *al.* (2025), Nat. Methods
[2]Lee and Lichterfeld (2022), Methods and Mol. Bio.
[3]Naquin et *al.* (2025), NAR Genom. Bioinform.
[4]Yukl et *al.* (2018), Sci. Trans. Med.
[5]Guirguis et *al.* (2023), Cancer Discov.
[6]Pardons et *al.* (2023), Nat. Commun.

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We identified a novel chemical signature on HIV-1 RNA that appears to be highly conserved between participants. These findings pave the way for a detailed characterization of their functional relevance.