SUPPLEMENTARY MATERIAL

DHX9/RHA Binding to the PBS-Segment of the Genomic RNA during HIV-1 Assembly Bolsters Virion Infectivity

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RHA incorporation to virions does not involve RRE.

HIV-1 molecular clone pNL43Rev(-)R(-).S [1] lacks the Rev-responsive element (RRE) and the Rev open reading frame and contains simian retrovirus constitutive transport element (CTE) that recruits cellular nuclear transport proteins, substituting for RRE/Rev activity. Virions were produced by transfection of pNL43Rev(-)R(-)S(+) to HEK293 and cell-free supernatant was harvested 48 h later. Virions were collected by ultracentrifugation over a 20% sucrose cushion in an SW41 rotor at 40,000 rpm for 2 hours. Virions were resuspended in RIPA buffer (50 mM Tris, 150 mM NaCl, 1% NP40, 0.25% deoxycholic acid, 1 mM EDTA). Equivalent amounts of virions were determined by HIV Gag p24 ELISA (Zeptometrix) and separated by SDS-PAGE and the level of RHA in virions was determined by Western blot.

***In vitro* transcription to make H8 deuterated RNA.**

In vitro transcription was performed on template DNAs 5´-$L^{344\text{-}DISm}$ and 5´-$L^{344\text{-}\Delta PBS}$ [2]. The template DNA samples were amplified in DH5α cells and isolated using plasmid Mega protocol (Plasmid Mega Kit, Qiagen). Plasmids were linearized with BciVi (70 U enzyme per milligram of DNA at 37°C) subjected to *in vitro* T7 transcriptions with H8-deuterated purines and regular pyrimidines. The RNA samples were then purified by polyacrylamide gel electrophoresis and Elutrap (Whatman) prior to NMR data collection.

SUPPLEMENTARY FIGURES

Figure S1. Residues of the Rev responsive element (RRE) are dispensable for RHA recruitment to HIV-1 virions. WT, CES and NL43Rev(-)R(-).S virions were produced in HEK293 cells, collected by centrifugation and quantified by ELISA. Equivalent Gag p24 was subjected to immunoblotting with RHA antiserum (top gel) and Gag p24 antiserum (bottom gel). Although the virion loading for NL43Rev(-)R(-).S was slightly less efficient than WT and CES, RHA was

readily detectable in NL43Rev(-)R(-)S(+) virions, demonstrating RHA accumulates in virions independently of RRE.

Figure S2. Deuteration of purine H8 improves spectral sensitivity and resolution. Top panel, 2D ^{1}H-^{1}H NOESY spectrum collected for the fully protonated 5´-L$^{344\text{-DISm}}$ RNA. This 339-nt RNA was prepared with the DIS palindrome loop (AAGCGCGCA) substituted by a GAGA tetraloop on the frame of 5´-L^{344} to maintain the RNA folding but eliminate dimerization at DIS [3]. Thus, the RNA remains monomeric in solution and gives rise to NMR signals with narrow line width due to the lower molecular weight and enhanced tumbling rate. Bottom panel, 2D ^{1}H-^{1}H NOESY spectrum collected for H8-deuterated [5´-L^{344}]$_2$, a dimeric RNA of 688 nt. Due to the dilution of magnetic environment by the site-specific H8-editing strategy, sensitivity and resolution improvement were achieved compared with the fully protonated 5´-L$^{344\text{-DISm}}$ RNA (top spectrum), especially for the adenosine H2 finger print region (6.4-7.3ppm), despite the fact that [5´-L^{344}]$_2$ is twice the size of 5´-L$^{344\text{-DISm}}$.

Figure S3. PBS residues in the dimeric 5´-L^{344} gave rise to site-specific chemical shift changes when in complex with RHA^{N300}. Superposition of the 2D NOESY spectra collected for [5´-L^{344}]$_2$ (black) and [5´-L^{344}:RHA^{N300}]$_2$ (orange) on the top panel, and [5´-L^{344}]$_2$ (black) and [5´-L$^{344\text{-}\Delta PBS}$]$_2$ (cyan) on the bottom panel. Green arrows on the top panel denote cross peaks shifted upon RHA^{N300} titration. The peak marked with asterisk shows under low contour levels. On the bottom panel, the peaks shown in the [5´-L^{344}]$_2$ spectrum but absent in the [5´-L$^{344\text{-}\Delta PBS}$]$_2$ spectrum were assigned to PBS, and some of these peaks exhibited chemical shift changes upon RHA binding, as shown by comparison with the [5´-L^{344}:RHA^{N300}]$_2$ spectrum (top panel). (b-d) Zoom-in views of (a). The site-specific chemical shift perturbations observed upon RHA^{N300} titration were absent in the [5´-L$^{344\text{-}\Delta PBS}$]$_2$ spectrum.

Figure S4. Chemical shift perturbations were observed when titrating RHA^{N300} into the TAR-Poly(A) segment RNA. The adenosine fingerprint region of TAR-Poly(A) (black) and TAR-Poly(A) in complex with RHA^{N300} (green) is shown. Many residues shifted or broadened upon RHA^{N300} titration, but these spectral changes were not detected in the $[5'\text{-}L^{344}:RHA^{N300}]_2$ spectrum as shown in Figure 3b.

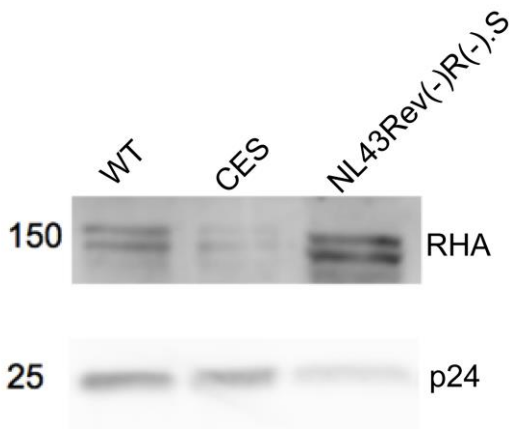

Figure S1

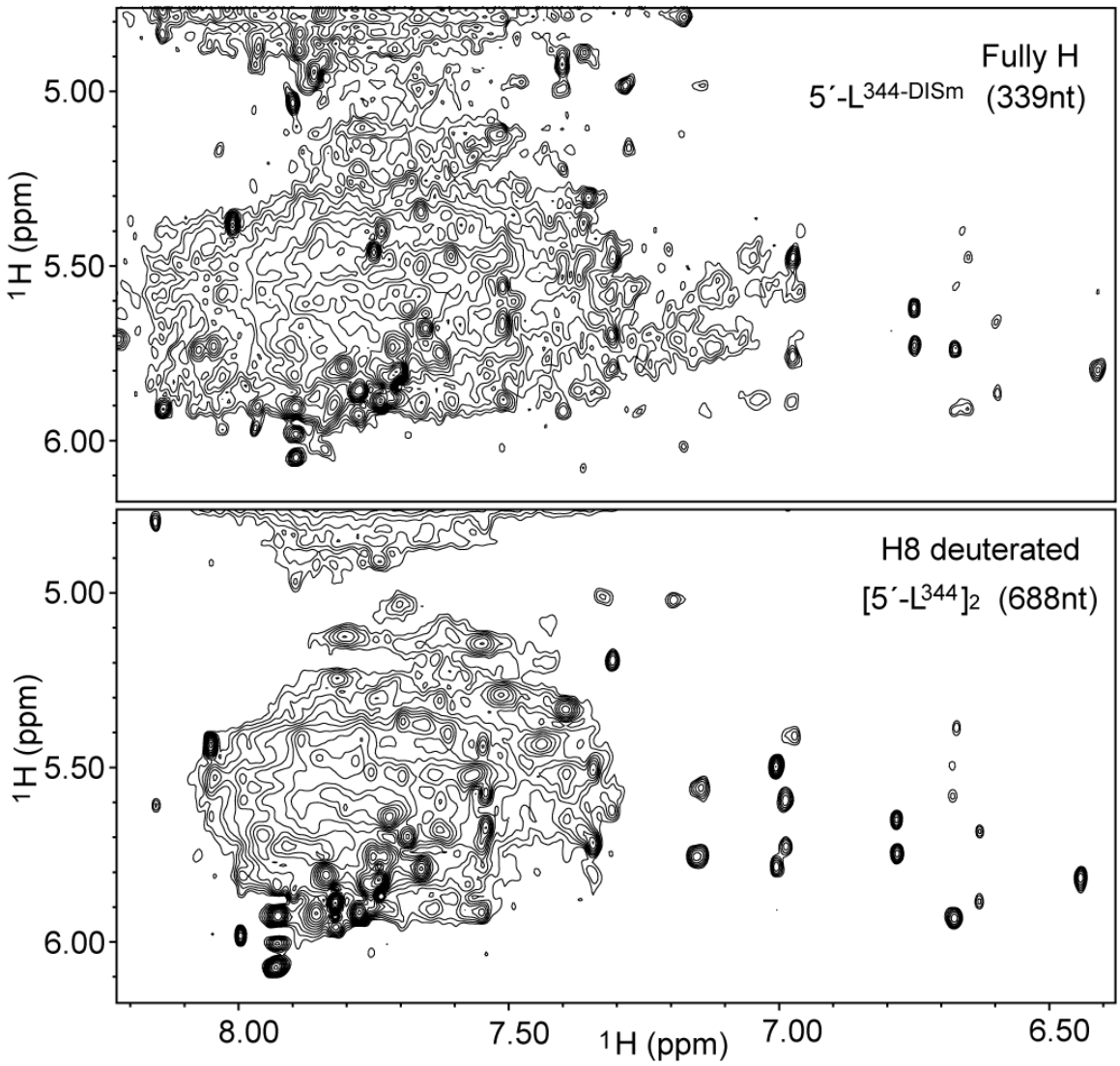
Fully H
5´-L344-DISm (339nt)
H8 deuterated
[5´-L344]2 (688nt)
1H (ppm)
5.00
5.50
6.00
8.00
7.50
7.00
6.50

Figure S2

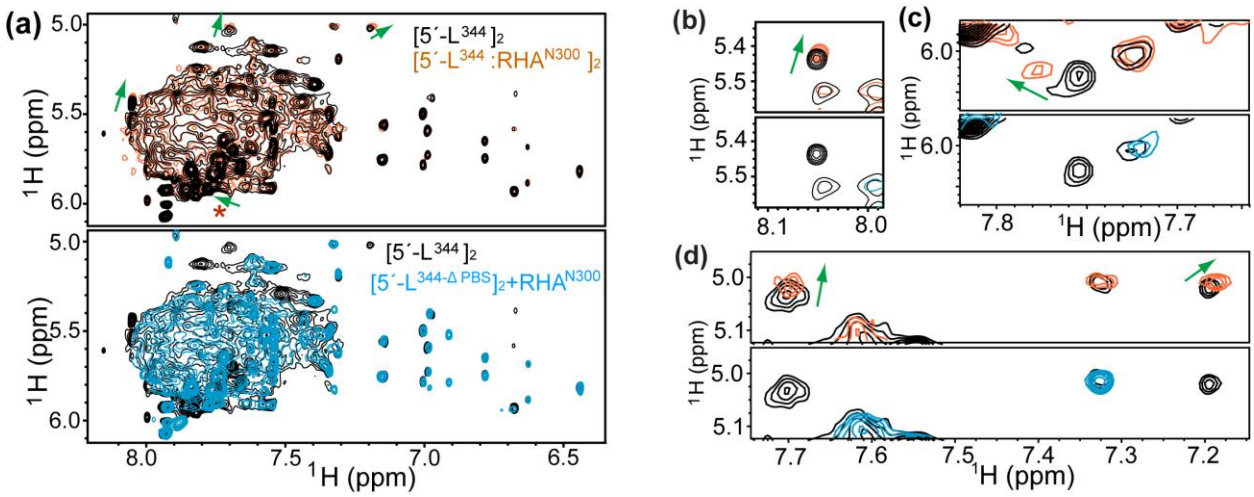
(a)
[5′-L344]2
[5′-L344:RHAN300]2
[5′-L344]2
[5′-L344-ΔPBS]2+RHAN300
1H (ppm)
(b)
(c)
(d)

Figure S3

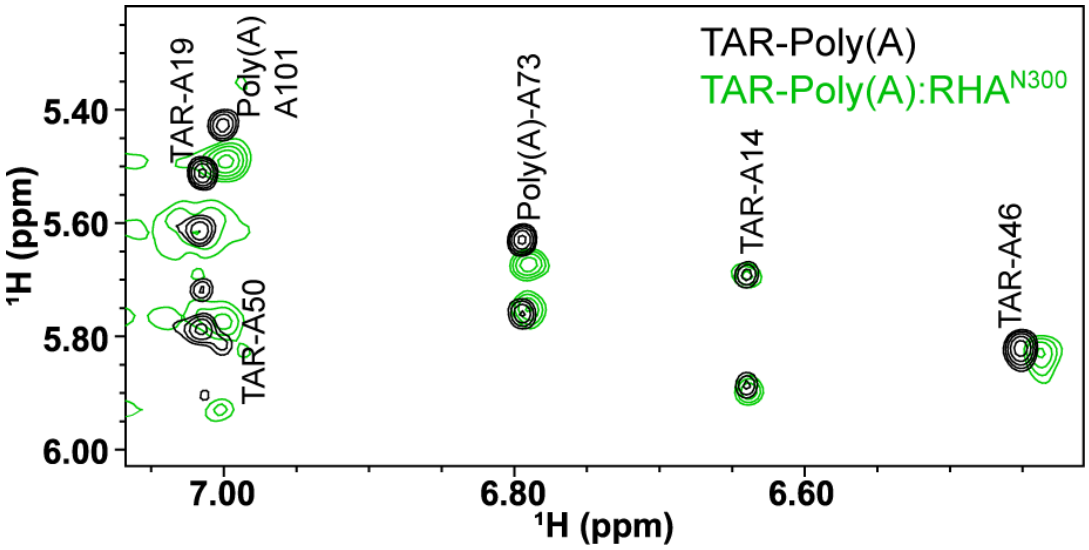

Figure S4

REFERENCE

[1] Zolotukhin AS, Valentin A, Pavlakis GN, Felber BK. Continuous propagation of RRE(-) and Rev(-)RRE(-) human immunodeficiency virus type 1 molecular clones containing a cis-acting element of simian retrovirus type 1 in human peripheral blood lymphocytes. J Virol. 1994;68:7944-52.

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[3] Lu K, Heng X, Garyu L, Monti S, Garcia EL, Kharytonchyk S, et al. NMR detection of structures in the HIV-1 5'-leader RNA that regulate genome packaging. Science. 2011;334:242-5.