Supporting Information

Direct Detection of Conserved Viral Sequences and Other Nucleic Acid Motifs with Solid-State Nanopores

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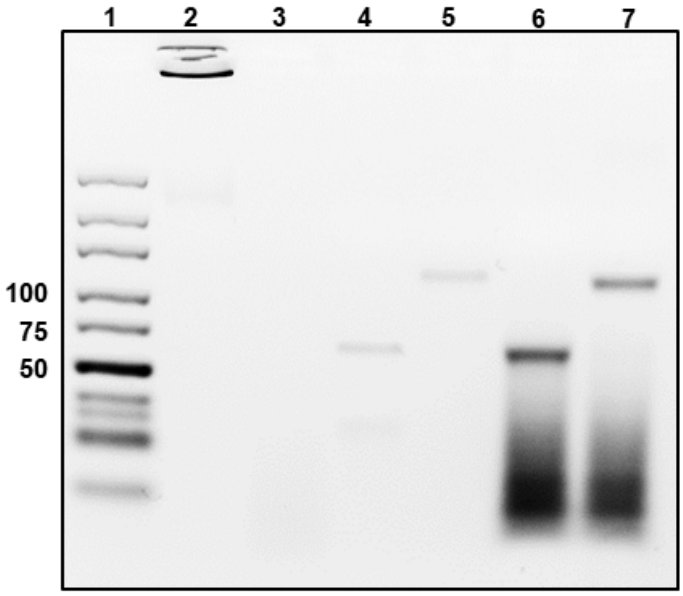

Figure S1 Gel analysis of isolation of 60 bp DNA construct from M13mp18. *Lane 1*: Ladder; *Lane 2*: single-strand M13mp18; *Lane 3*: M13mp18 fully digested by MBN; *Lane 4*: synthetic biotinylated 60 bp duplex DNA construct; *Lane 5*: same as lane 4 bound to MS; *Lane 6*: 60 bp duplex isolated from M13mp18 DNA; and *Lane 7*: same as lane 6 bound to MS.

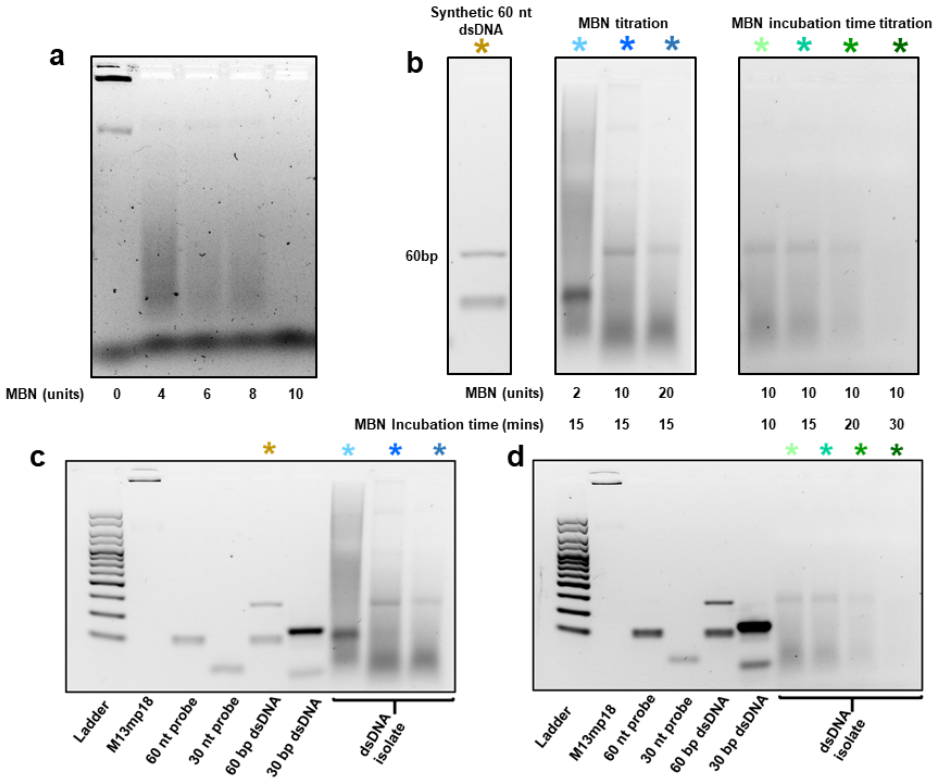

Figure S2 Gel optimization of amount and incubation time for Mung Bean Nuclease (MBN) digestion of single-strand M13mp18 DNA. (a) Digestion of 1.25 µg M13mp18 in the presence of different amounts of MBN as indicated. 10 units of MBN is required to fully digest the material. (b) Isolation of 60 bp dsDNA product from M13mp18 with MBN. Both the amount of MBN and incubation time were varied. Digestion with 2 units of MBN leaves single-strand overhangs attached to the end product, resulting in an undefined or 'smeared' band on gel. 10 units of MBN and 15 mins of incubation at 30 ºC was required to completely digest single-strand regions Full gels are shown in (c) and (d). Asterisk colors indicate lanes shown in (b).

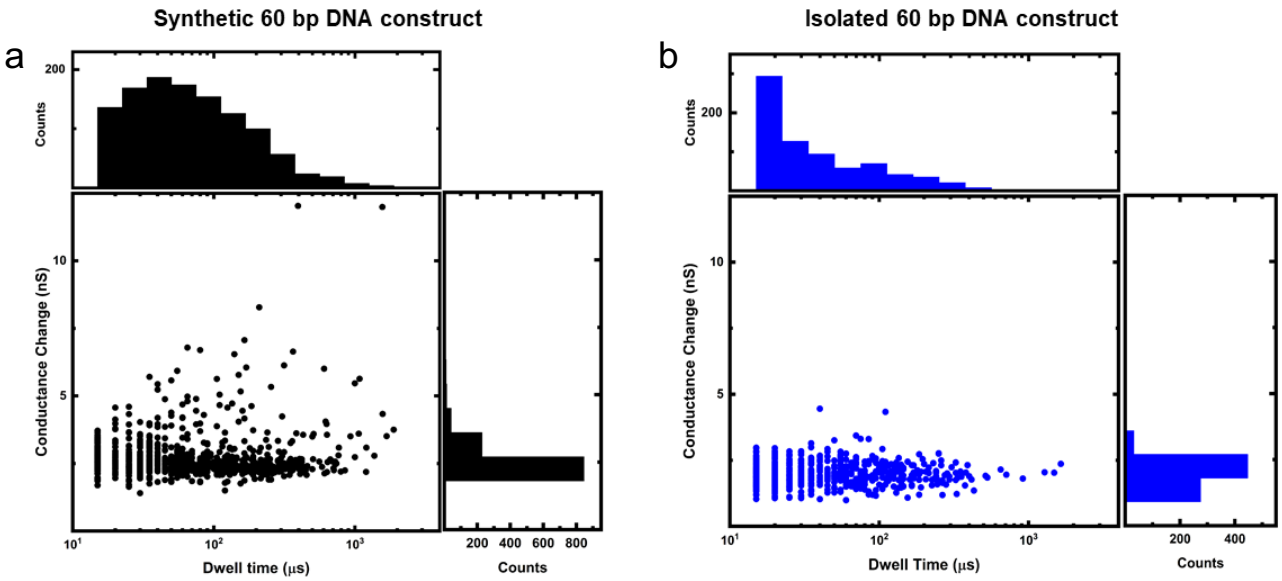

Figure S3 Dwell time *vs.* conductance change scatter plots and accompanying histograms for 100 nM synthetic (a) and isolated (b) 60 nt dsDNA following incubation with MS measured at 300 mV. n = 1159 in (a) and 756 in (b).

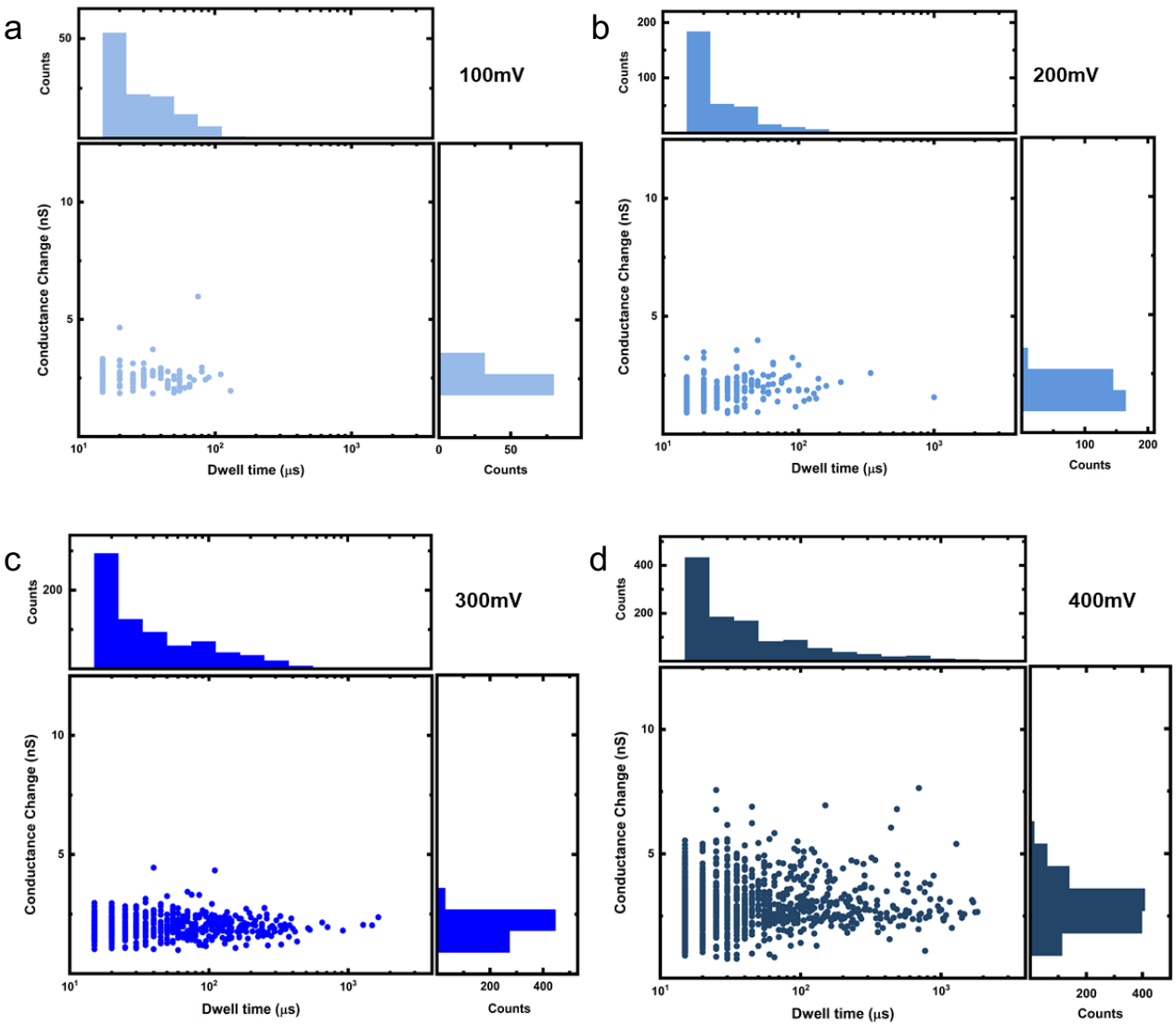

Figure S4 Dwell time *vs.* conductance change scatter plots and accompanying histograms for 60 bp dsDNA constructs isolated from M13mp18 after incubation with MS. Measurements performed at 100 (a), 200 (b), 300 (b), and 400 mV (d) and n = 115, 322, 756, and 1,148, respectively.

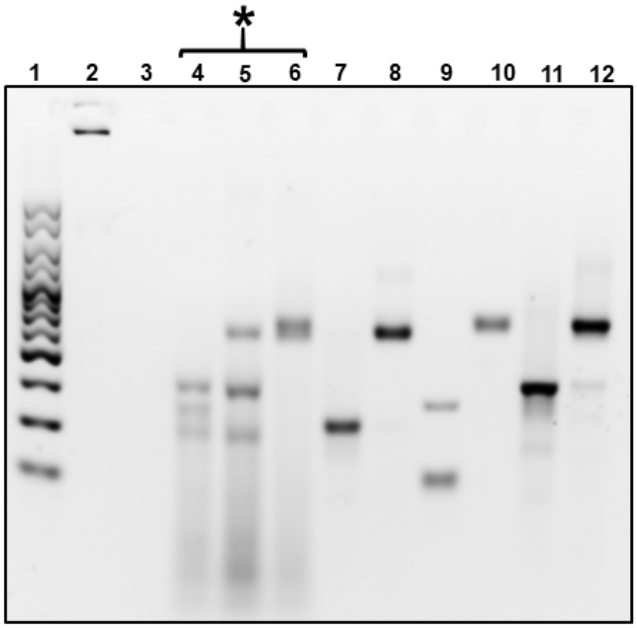

Figure S5 Full gel showing isolation of multiple distinct products from M13mp18. *Lane 1*: ladder; *Lane 2*: single-strand M13mp18; *Lane 3*: M13mp18 fully digested by MBN; *Lane 4*: isolated biotinylated products from M13mp18 using three DNA probes (50, 60, and 75 nt); *Lane 5*: electromobility shift assay in the presence of MS when only the 60 nt probe is biotinylated; *Lane 6*: electromobility shift assay in the presence of MS when all probes are biotinylated. *Lanes 7*, *9*, and *11* show synthetic 50 nt dsDNA, 60 nt dsDNA, and 75 nt dsDNA, respectively. Their electromobility shift assays (MS-bound) are shown in *Lanes 8*, *10*, and *12*, respectively. Lanes marked with an asterisk are shown in Fig. 4a of the main text.

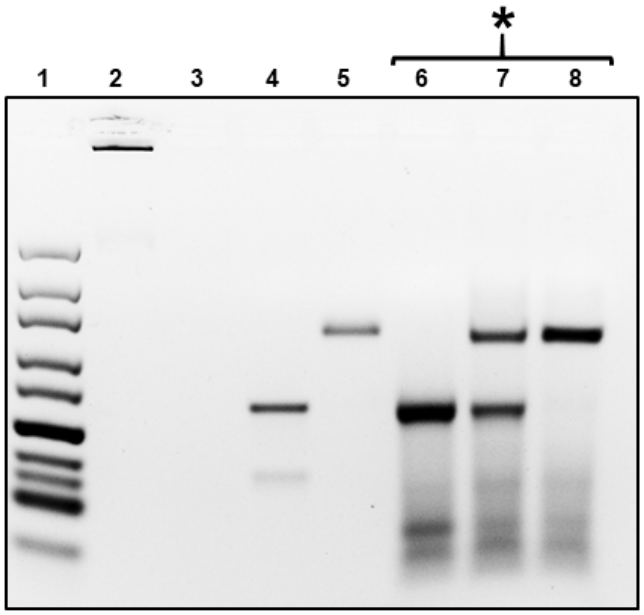

Figure S6 Full gel showing isolation of two products of the same length from M13mp18. *Lane 1*: ladder; *Lane 2*: single-strand M13mp18; *Lane 3*: M13mp18 digested by MBN; *Lane 4*: synthetic monobiotinylated 60 bp duplex construct; *Lane 5*: synthetic monobiotinylated 60 bp duplex construct bound to MS; *Lane 6*: two independent nonbiotinylated 60 bp isolates; *Lane 7*: electromobility shift assay in the presence of MS when only one 60 nt probe is biotinylated; *Lane 8*: electromobility shift assay in the presence of MS when both 60 nt probes are biotinylated. The lanes marked with an asterisk are shown in Fig. 4c, inset in the main text.

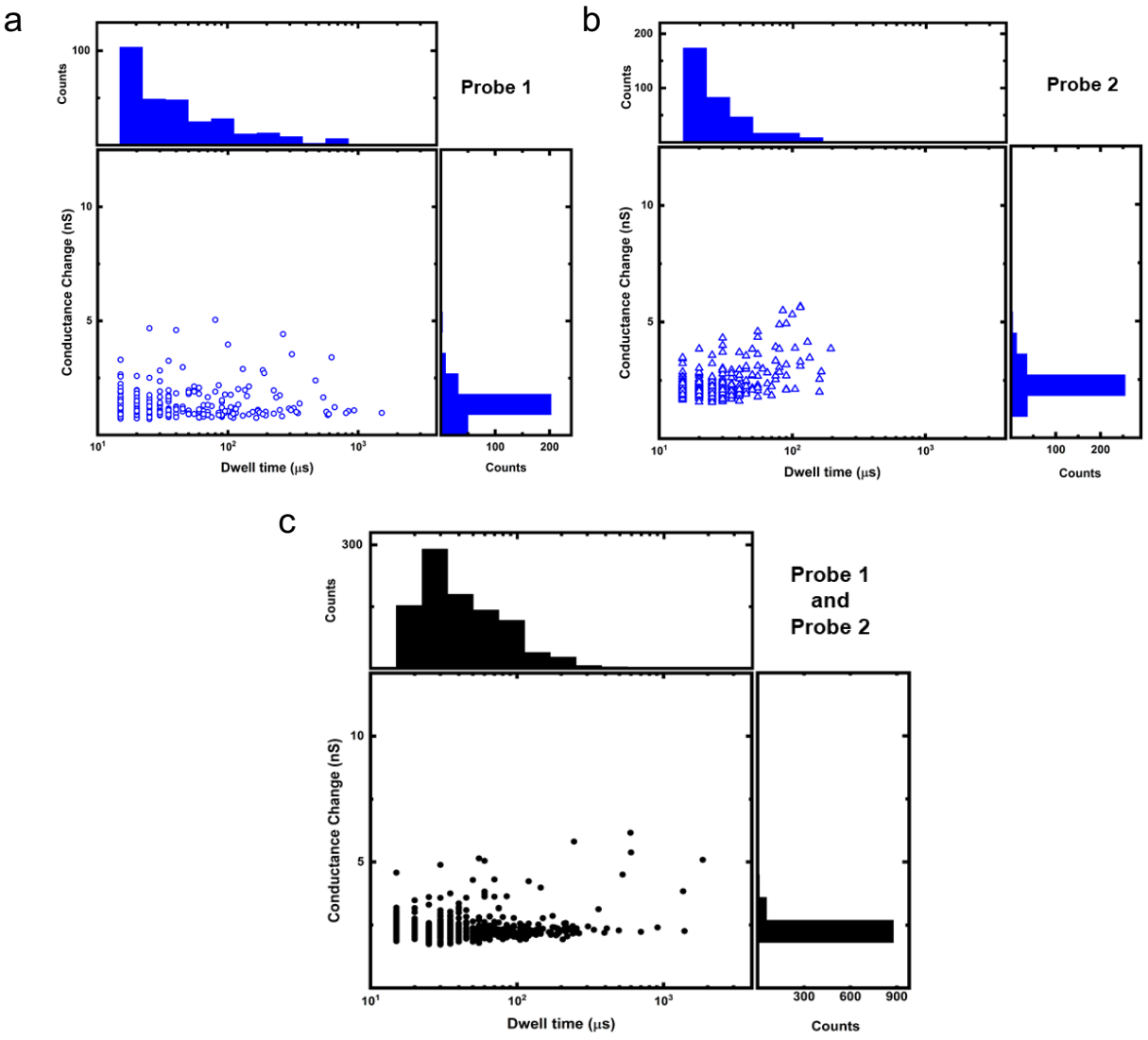

Figure S7 Dwell time *vs.* conductance change scatter plots and accompanying histograms for two different 60 nt dsDNA constructs (50 nM) isolated independently (a and b) and simultaneously (c) from M13mp18 after conjugation with MS. Measurements performed at 300 mV. The total number of events considered were n = 299 (a), 348 (b), and 967 (c).

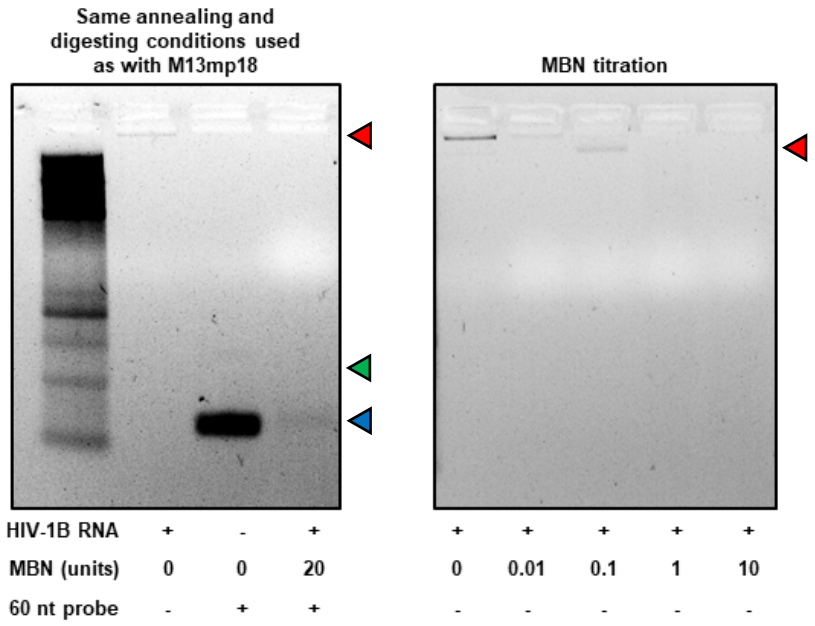

Figure S8 Gel analysis of target isolation from full-length HIV-1B RNA. *Left:* isolation carried out under identical conditions used to isolate 60 bp target from M13mp18. Lane contents indicated at bottom (*Lane 1* shows a ladder). The product (anticipated position marked with green arrow) was digested along with unannealed probe (blue arrow) and single strand HIV-1B (red arrow). *Right:* MBN titration (amounts indicated) carried out against 10 μg HIV-1B RNA. Undigested RNA (red arrow) was observed below 1 U.

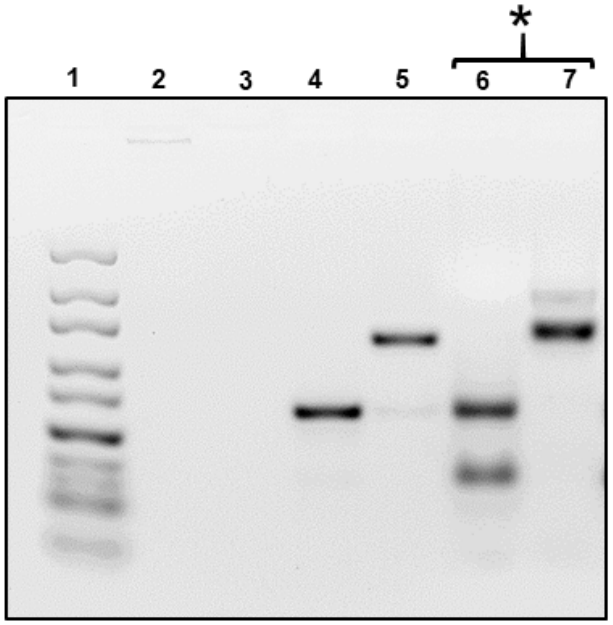

Figure S9 Gel analysis of sequence isolation from full-length HIV-1B RNA. *Lane 1*: ladder; *Lane 2*: HIV-1B alone; *Lane 3*: HIV-1B RNA after MBN digestion; *Lane 4*: synthetic monobiotinylated 60 bp DNA construct, *Lane 5*: synthetic monobiotinylated 60 bp DNA construct bound to MS; *Lane 6*: biotinylated 60 bp RNA/DNA heteroduplex isolated from full length HIV-1B RNA; and *Lane 7*: biotinylated 60 bp RNA/DNA heteroduplex isolated from full length HIV-1B RNA bound to MS**.** The lanes marked with an asterisk are shown in Fig. 5a in the main text.

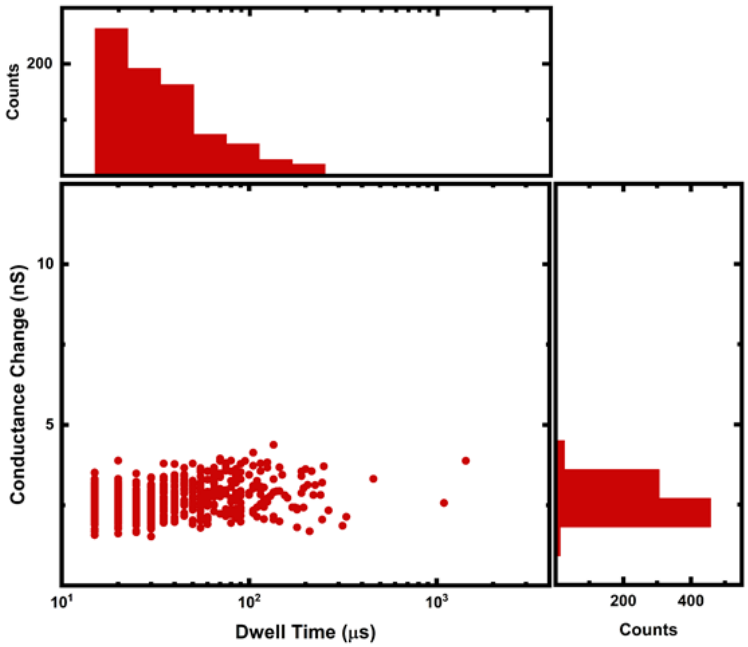

Figure S10 Dwell time *vs.* conductance change scatter plot and accompanying histogram for 100 nM 60 nt RNA/DNA heteroduplex isolated from HIV-1B RNA after incubation with MS, measured at 300 mV. The total number of events considered are n = 805.

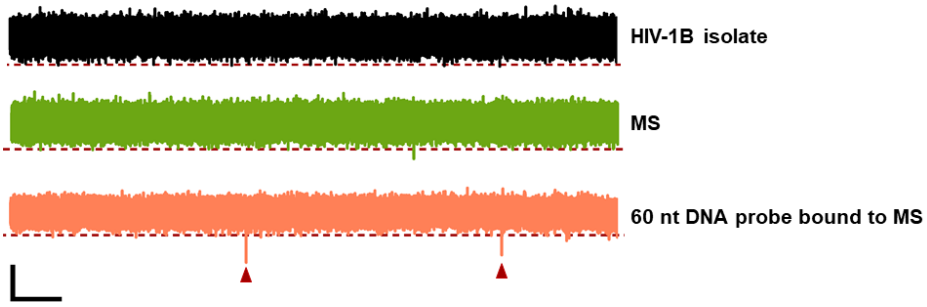

Figure S11 Current traces for 100 nM HIV-1B isolate alone (top), 4 μM MS (middle), and 100 nM 60 nt DNA probe bound to MS (bottom) measured at 300 mV. Red arrows denote identified events and dashed lines indicate threshold conductance level. Scale bars represent 250 ms (horizontal) and 500 pA (vertical).

Probes	Complimentary (M13mp18/HIV-1B)	Sequences (5' ⟶ 3')
50 nt	M13mp18	AAGGTAAAGTAATTCTG**T**CCAGACGACGACAATAAACAACATGTTCAGCT
60 nt -1	M13mp18	CGAACTAACGGAACAACATTATTACAGG**T**AGAAAGATTCATCAGTTGAGATTTAGGAATA
60 nt - 2	M13mp18	ACGCCAGGGTTTTCCCAGTCACGACGTTG**T**AAAACGACGGCCAGTGCCAAGCTTGCATGC
75 nt	M13mp18	TGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTG**T**AAAACGACGGCCAGTGCCAAGCTTGCA TGCCTGCAGGTC
60 nt	HIV-1B	ACAGTCTACTTGTCCATGCATGGCTTC**T**CCTTTTAGCTGACATTTATCACAGCTGGCTAC

Table 1 List of probes. In each sequence, **T** represents biotinylated thymine.

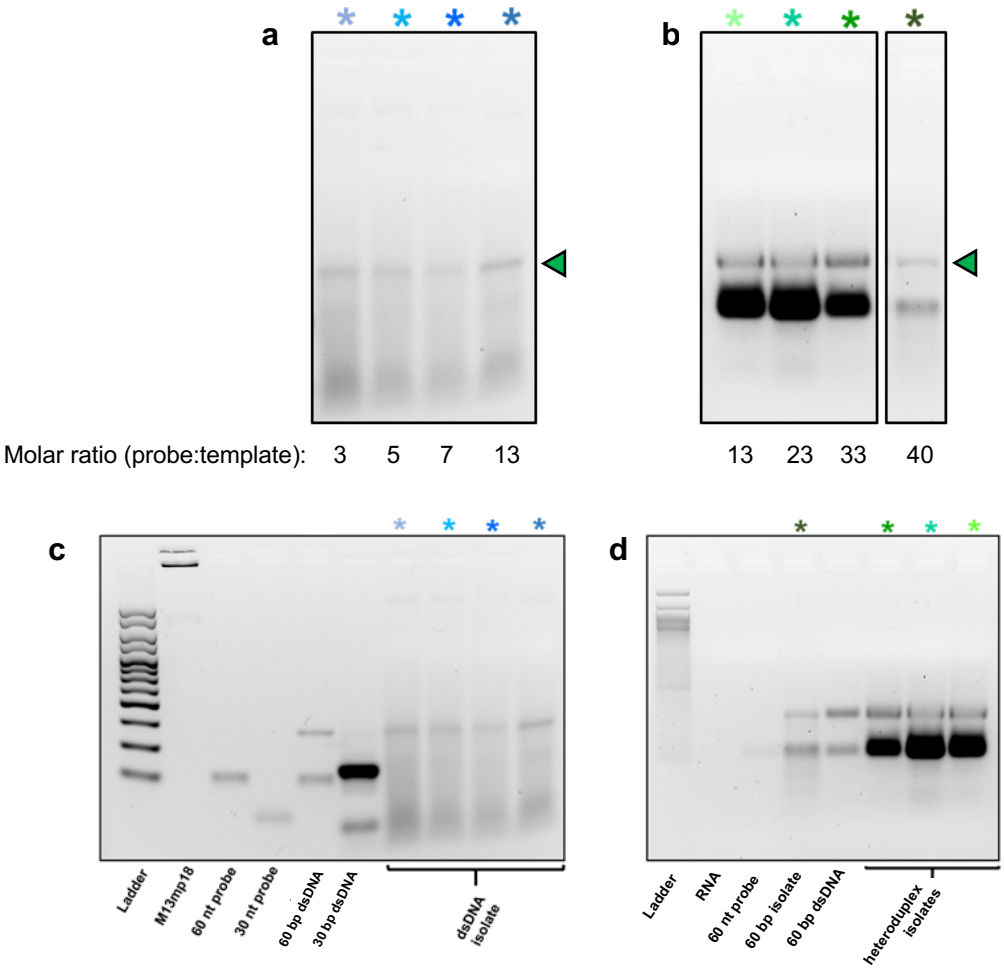

Figure S12 Gel optimization of probe required for efficient annealing to both M13mp18 DNA (a) and HIV-1B RNA (b). Gels are cropped for easy comparison. Molar ratios indicated at bottom. Increasing amount of 60 nt probe yields higher relative intensity of the 60 bp dsDNA or RNA/DNA heteroduplex product (green arrows). The full gels are shown in (c) and (d) and the colors of asterisks indicate lanes shown in (a) and (b).