Supplementary information

Template-primer binding affinity and RNase H cleavage specificity contribute to the strand transfer efficiency of HIV-1 reverse transcriptase

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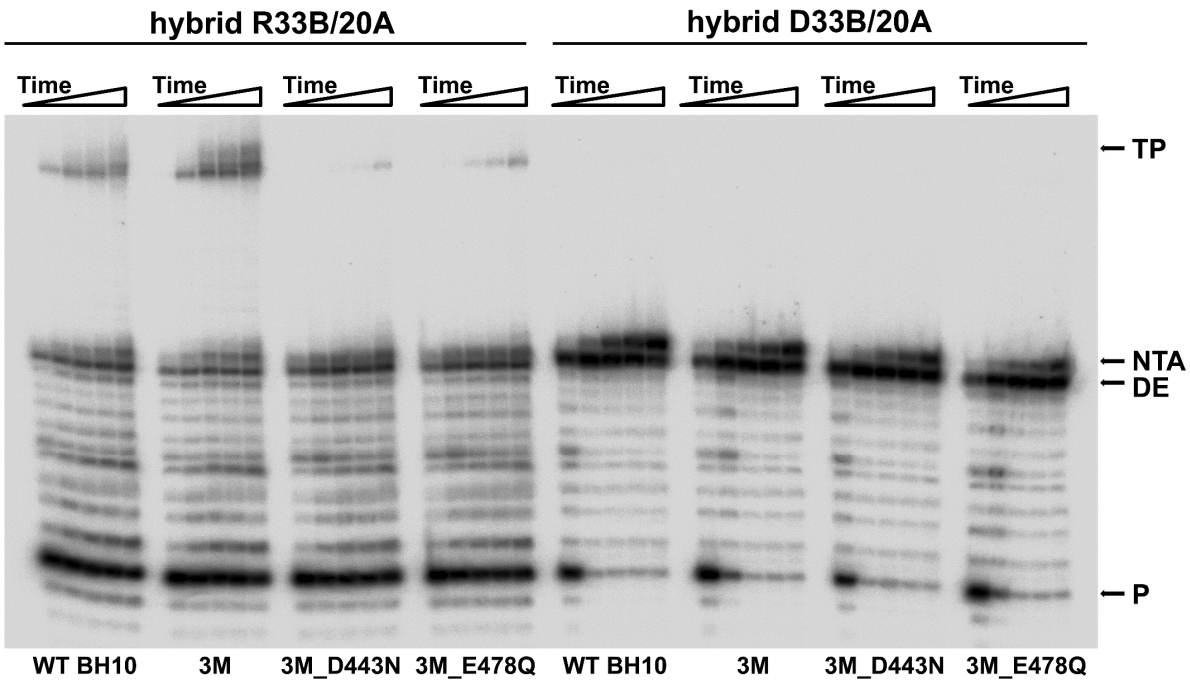
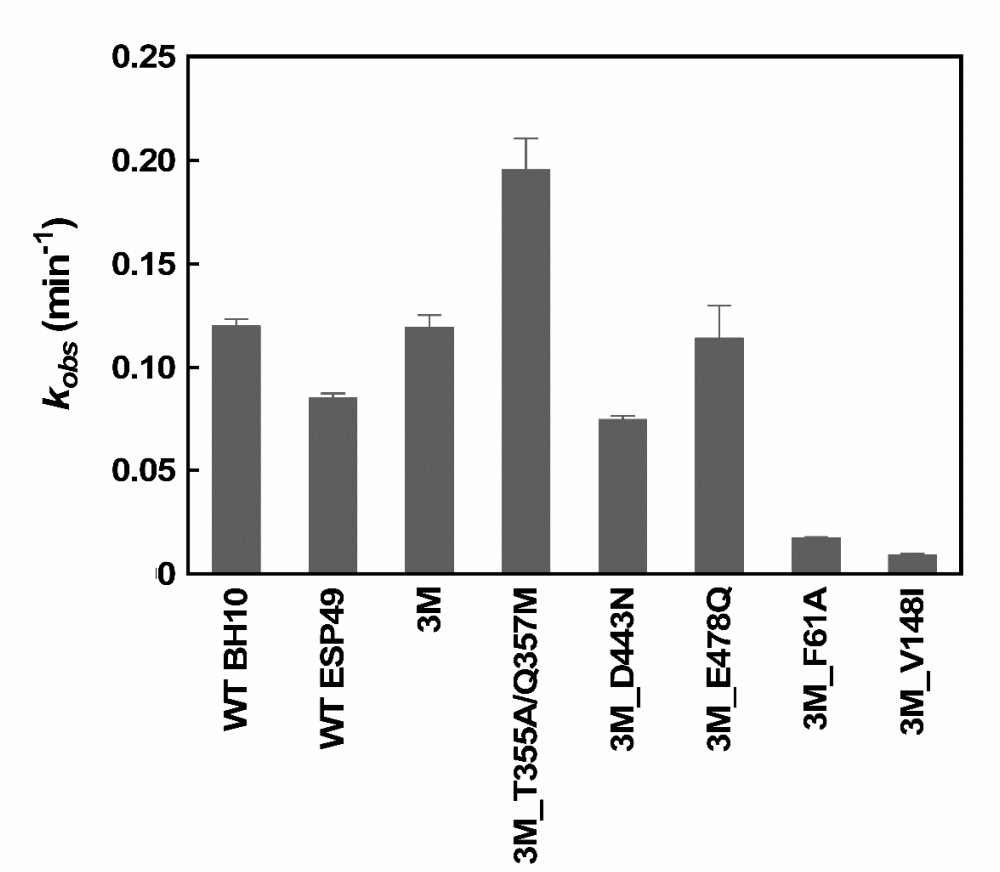
Supplementary Table S1. Rates of nontemplated nucleotide addition of HIV-1 RTs using blunt-ended DNA/DNA substrates.

	k_{obs} (min^{-1})		
dNTPs	**WT BH10**	**3M_T355A/Q357M**	**3M_V148I**
A	0.313 ± 0.012	0.601 ± 0.034	(1.98 ± 0.02) x 10^{-2}
G	(2.03 ± 0.16) x 10^{-2}	(3.53 ± 0.52) x 10^{-2}	(4.70 ± 0.04) x 10^{-3}
C	(4.60 ± 0.15) x 10^{-3}	(2.76 ± 0.38) x 10^{-2}	(5.10 ± 0.11) x 10^{-3}
T	(4.20 ± 0.05) x 10^{-3}	(7.80 ± 0.37) x 10^{-3}	(2.40 ± 0.07) x 10^{-3}
A+G	0.217 ± 0.008	0.419 ± 0.017	(1.53 ± 0.03) x 10^{-2}
A+C	0.222 ± 0.004	0.478 ± 0.027	(1.85 ± 0.03) x 10^{-2}
A+T	0.358 ± 0.020	0.816 ± 0.050	(2.35 ± 0.03) x 10^{-2}
G+C	0.173 ± 0.009	0.329 ± 0.021	(1.12 ± 0.05) x 10^{-2}
G+T	(2.51 ± 0.03) x 10^{-2}	(3.47 ± 0.17) x 10^{-2}	(6.40 ± 0.21) x 10^{-3}
C+T	(9.60 ± 0.44) x 10^{-3}	(4.48 ± 0.36) x 10^{-2}	(4.70 ± 0.43) x 10^{-3}
A+G+C	0.227 ± 0.009	0.492 ± 0.019	(1.47 ± 0.03) x 10^{-2}
A+G+T	0.269 ± 0.012	0.496 ± 0.026	(1.66 ± 0.03) x 10^{-2}
A+C+T	0.247 ± 0.013	0.595 ± 0.039	(1.88 ± 0.04) x 10^{-2}
G+C+T	0.120 ± 0.003	0.195 ± 0.015	(9.30 ± 0.57) x 10^{-3}
A+G+C+T	0.225 ± 0.007	0.442 ± 0.024	(1.35 ± 0.02) x 10^{-2}

Reactions were carried out at 37ºC with DNA/DNA hybrid D28B/25B (30 nM), using an RT concentration of 60 nM. Each dNTP was supplied at 500 µM each. Reported incorporation rates (k_{off}) represent the average ± SD, of at least three independent experiments.



Supplementary Figure S1. Strand transfer activity of WT and mutant HIV-1 RTs. Assays were carried out with two different template-primers containing: 1) a 5' ^{32}P-radiolabeled DNA primer (20A), an RNA donor (R33B), and DNA acceptors containing 3'-end sequences that overlap with the 5' nucleotide sequence of R33B; and 2) a 5' ^{32}P-radiolabeled DNA primer (20A), an DNA donor (D33B), and DNA acceptors containing 3'-end sequences that overlap with the 5' nucleotide sequence of D33B. Reactions were carried out in the presence of 6-7.5 nM RTs, 30 nM donor-primer (R33B/20A, D33B/20A) and 60 nM acceptor (35D). Aliquots were removed at 1, 5, 15, 30 and 60 min. TP, transfer product; DE, donor extension product; NTA, nontemplated nucleotide addition product, and P, un-extended primer.



Supplementary Figure S2. Rates of nontemplated nucleotide addition of HIV-1 RTs using blunt-ended DNA/DNA substrates, in the presence of a mixture of dGTP, dCTP and dTTP. Reactions were carried out at 37ºC with DNA/DNA hybrid D28B/25B (30 nM), using an RT concentration of 60 nM. Each dNTP was supplied at 500 μM each. Reported incorporation rates (k_{off}) represent the average ± SD, of at least three independent experiments.

Supplementary Table S2. Nucleotide sequences of mutagenic primers.

Amino acid substitutions	Primers 5' – 3'
L92P (HIV-1_{BH10} RT)	5'-CTTCTGGGAAGTTCAACCAGGAATACCACATCC-3' 5'-GGATGTGGTATTCCTGGTTGAACTTCCCAGAAG-3'
L92P (HIV-1_{ESP49} RT)	5'-TTTTGGGAGGTACAGCCAGGTATCCCACATCCG-3' 5'-CGGATGTGGGATACCTGGCTGTACCTCCCAAAA-3'
D443N (HIV-1_{ESP49} RT)	5'- GAAACCTATTATGTAAATGGAGCAGCTA-3' 5'-TAGCTGCTCCATTTACATAATAGGTTTC-3'
E478Q (HIV-1_{ESP49} RT)	5'-CCAATCAAAAGGCTCAATTAATGGCAG-3' 5'-CTGCCATTAATTGAGCCTTTTGATTGG-3'