**Supplementary information**

# Amino acid residues in HIV-2 reverse transcriptase that restrict the development of nucleoside analogue resistance through the excision pathway

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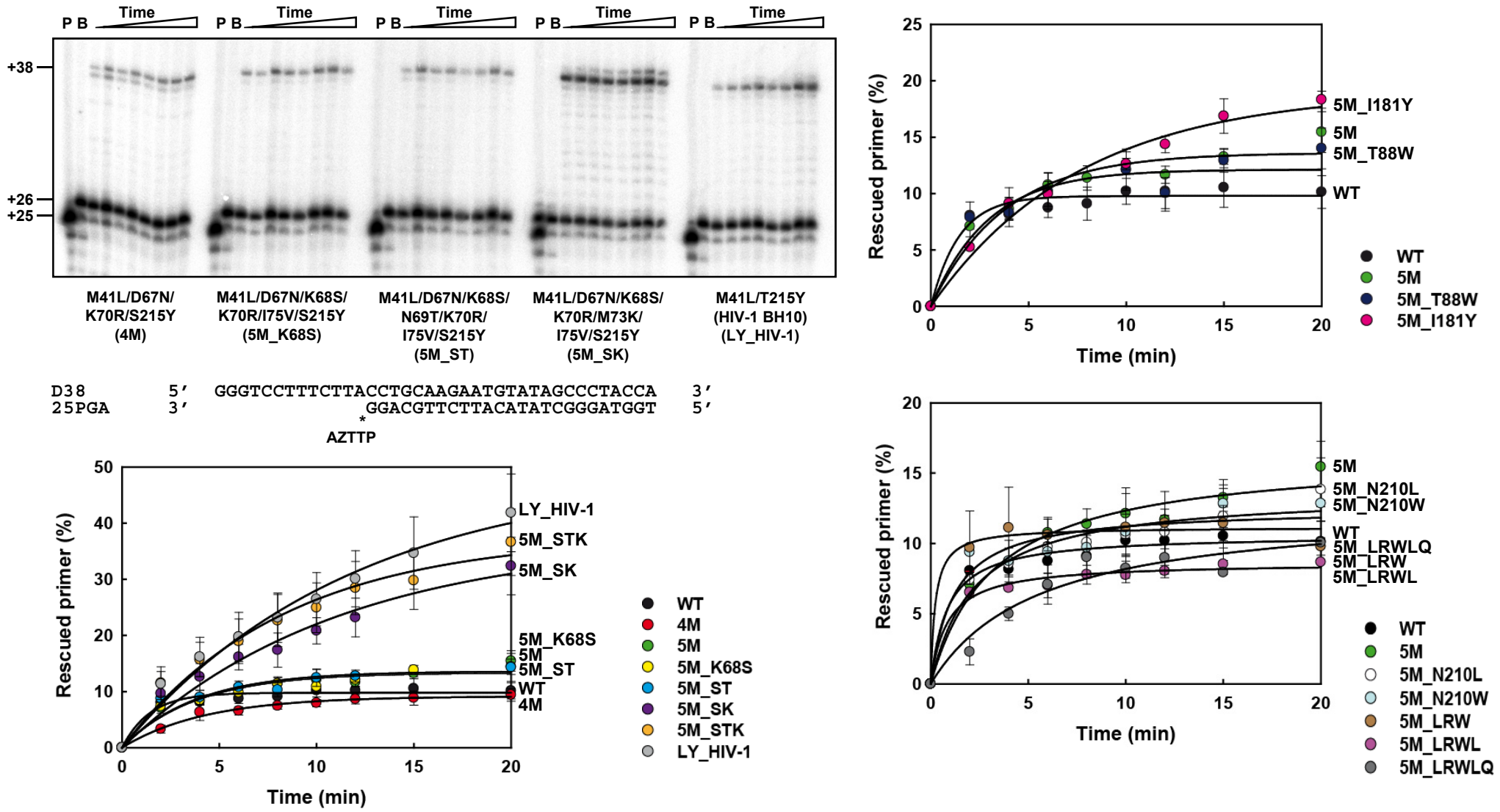


**Fig. S1. ATP-mediated excision of AZTMP from DNA/DNA template/primers by WT and mutant RTs.** Assays were carried out with 38/25-mer DNA/DNA complexes (sequences shown below the upper panel). The inhibitor was first incorporated at position +1 (indicated with an asterisk) of the 25-nucleotide primer (lane P) to generate a 26-nucleotide product (lane B). Excision of the inhibitor and further primer extension in the presence of 3.2 mM ATP and a mixture of dNTPs led to the formation of a fully extended 38-nucleotide product. Aliquots were removed 2, 4, 6, 8, 10, 12, 15 and 20 min after the addition of ATP. Time courses of the excision reactions are shown in three panels, each one containing data from different sets of related RTs. Time courses obtained with WT HIV-2 RT and mutant 5M are included in all panels for comparison. All dNTPs in the assays were supplied at 100 µM, except for dATP whose concentration was 1 µM. Template/primer and active RT concentrations in these assays were 30 nM and 24 nM, respectively. Represented values (means ± standard deviations [error bars]) were obtained from at least three independent experiments.

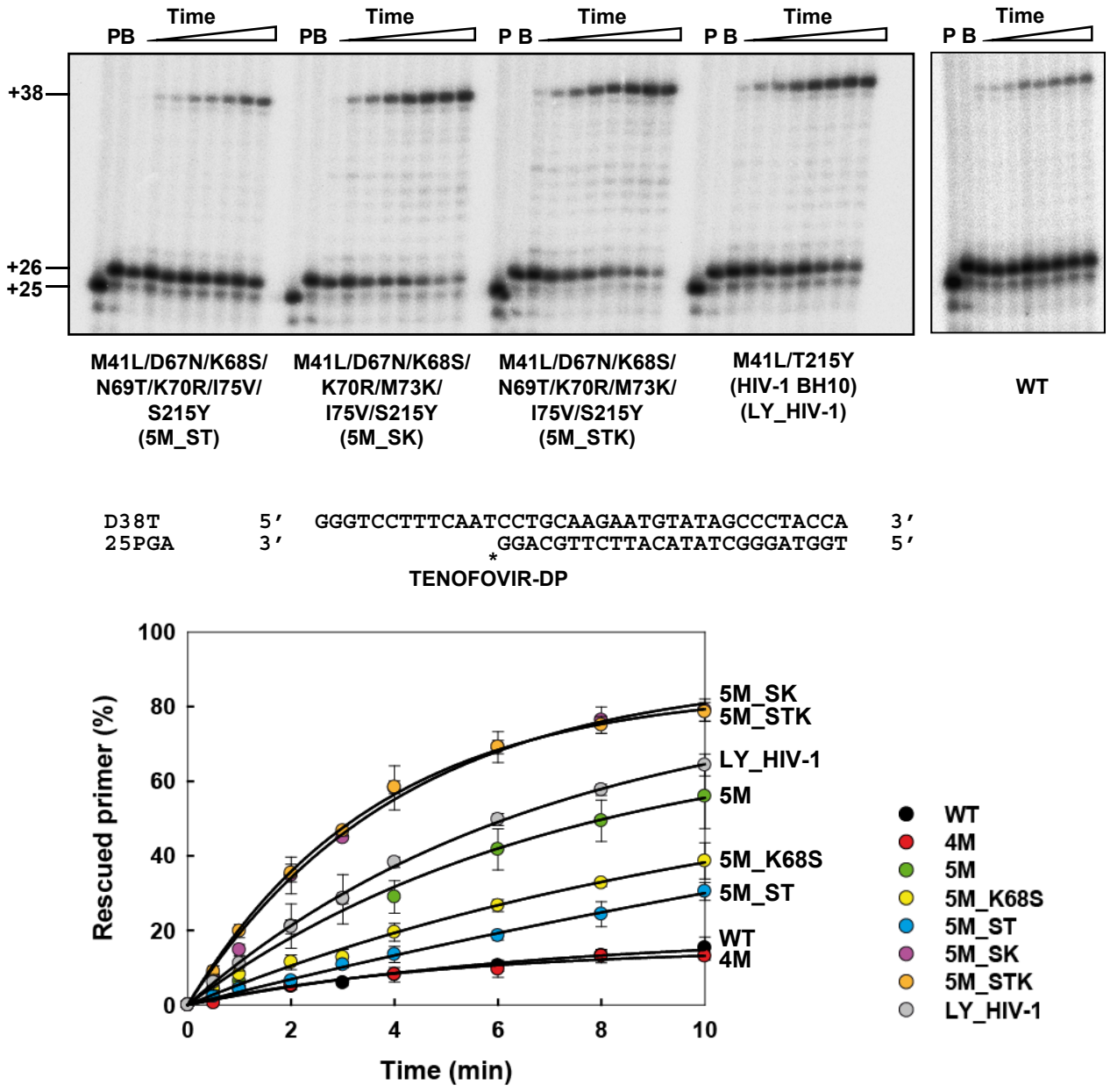


**Fig. S2. ATP-mediated excision of tenofovir from DNA/DNA template/primers by WT and mutant RTs.** Assays were carried out with D38T/25PGA DNA/DNA complexes (sequences shown below the upper panel). Primers (lane P) were blocked with tenofovir diphosphate to generate a 26-nucleotide product (lane B). The excision of the inhibitor, followed by extension of the primer is achieved in the presence of 3.2 mM ATP and the four dNTPs. A fully extended 38-nucleotide product is formed. Aliquots were removed 0.5, 1, 2, 3, 4, 6, 8 and 10 min after the addition of ATP. Time courses of the excision reactions are shown below. All dNTPs in the assays were supplied at 100 µM, except for dTTP whose concentration was 1 µM. Template/primer and active RT concentrations in these assays were 30 nM and 24 nM, respectively. Represented values were obtained from at least three independent experiments.

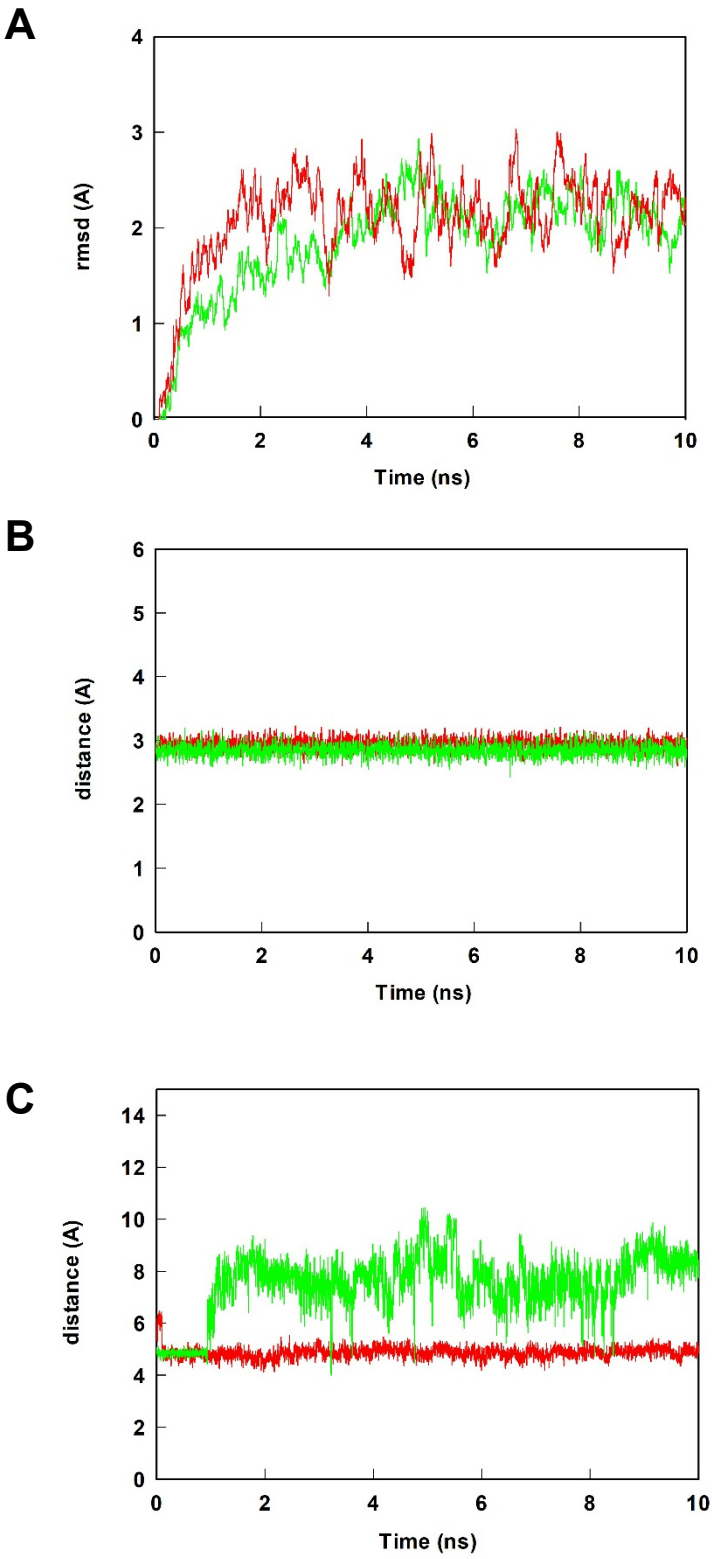


**Fig. S3. Time evolution of conformational changes and relevant interatomic distances in the ternary complexes containing mutant HIV-1 RTs D67N/K70R (red) and D67N/K70R/M73K (green).** (A) Evolution of the root-mean-square deviation (rmsd) of the Cα atoms of the 66-kDa subunit of the RT (residues 1-389). (B) Evolution of the interatomic distance between the 3′ oxygen of the primer and the α phosphorous of the incoming dNTP during molecular dynamics simulation. (C) Evolution of the interatomic distances between the ζ carbon of $Arg^{70}$ and the γ phosphorous of the incoming dNTP in ternary complexes containing HIV-1 RTs D67N/K70R/M73K and D67N/K70R. Data for the D67N/K70R RT model were taken from Kisic *et al*. [32]

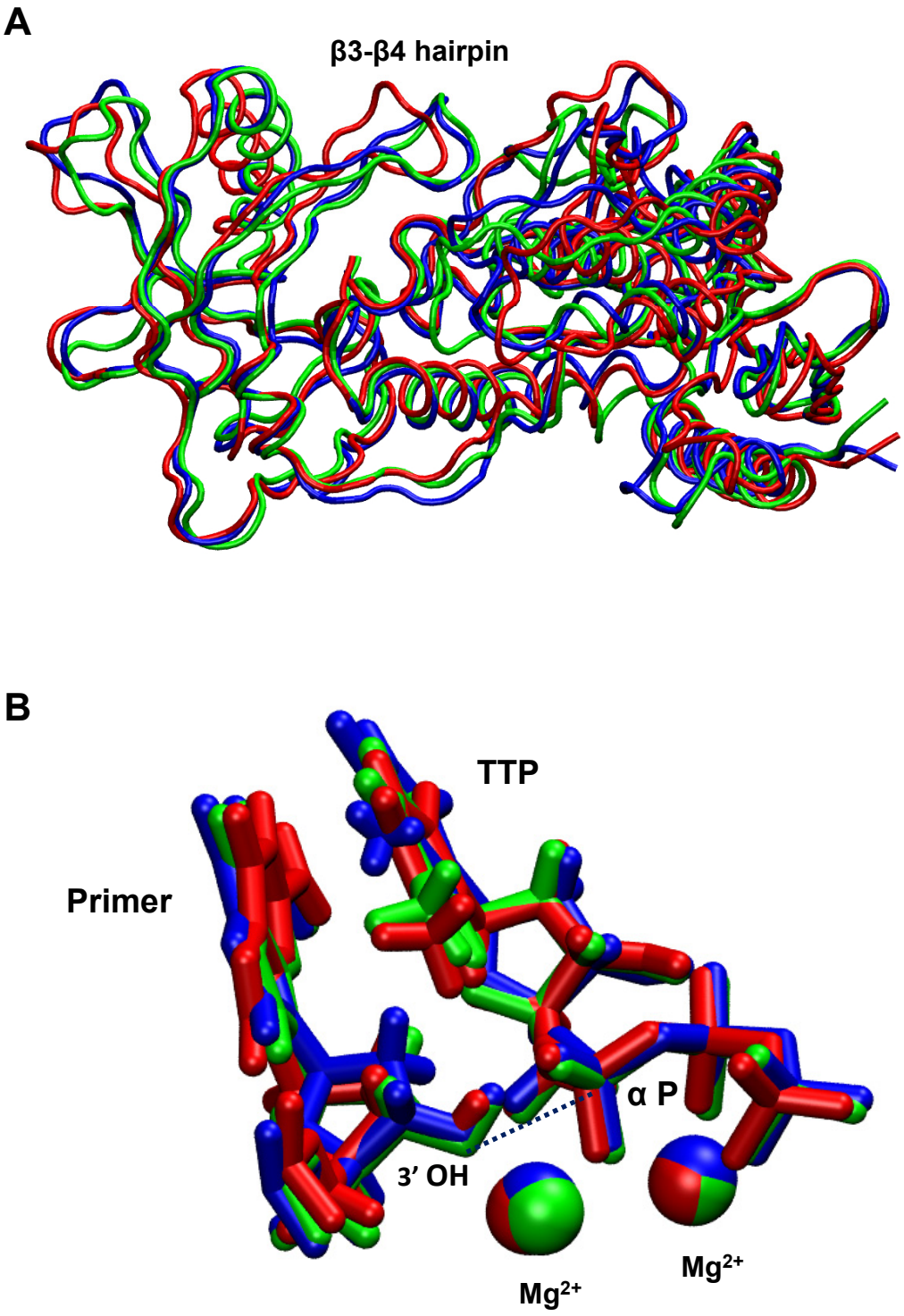


**Fig. S4. Structural alignment of the HIV-1 RTs obtained from the molecular dynamics simulations.** (A) Cα traces of superimposed HIV-1 RT polymerase domains of the catalytically competent WT enzyme (green), and mutants D67N/K70R (blue) and D67N/K70R/K73M (red). (B) View of their catalytic sites showing the location of the incoming dNTP and the two $Mg^{2+}$ ions. The dotted line shows the distance between the 3′ oxygen of the primer and the α phosphorous of the incoming dNTP at the end of the simulation.

**Table S1. DNA oligonucleotides used in mutagenesis reactions.**[a]

| Virus | Amino acid substitutions | Oligonucleotide sequences |
|---|---|---|
| HIV-$2_{ROD}$ | M41L | 5′ GAAATCTGTGAAAAACTGGAAAAAGAAG-3′<br>3′ CTTTAGACACTTTTTGACCTTTTTCTTC-5′ |
| | D67N/K70R | 5′ CAATCAAGAAAAAGAACAAAAACAGATGGAGGATGCT 3′<br>3′ GTTAGTTCTTTTTCTTGTTTTTGTCTACCTCCTACGA 5′ |
| | K68S | 5′ CAAGAAAAAGAACAGCAACAGATGGAGG-3′<br>3′ GTTCTTTTTCTTGTCGTTGTCTACCTCC-3′ |
| | N69T | 5′ AAAAAGAACAGCACCAGATGGAGGAT 3′<br>3′ TTTTTCTTGTCGTGGTCTACCTCCAT 5′ |
| | N69T/M73K | 5′ AGAAAAAGAACAGCACCAGATGGAGGAAGCTAGTAGATTTC 3′<br>3′ TCTTTTTCTTGTCGTGGTCTACCTCCTTCGATCATCTAAAG 5′ |
| | M73K | 5′ AACAGATGGAGGAAGCTAGTAGATTTC 3′<br>3′ TTGTCTACCTCCTTCGATCATCTAAAG 5′ |
| | I75V | 5′ ATGGAGGATGCTAGTAGATTTCAGAG 3′<br>3′ TACCTCCTACGATCATCTAAAGTCTC 5′ |
| | T88W | 5′ TAACTCAAGATTTCTGGGAAATTCAGTTA 3′<br>3′ ATTGAGTTCTAAAGACCCTTTAAGTCAAT 5′ |
| | I181Y | 5′ AGGATGTCATTATCTATCAGTACATGGAT 3′<br>3′ TCCTACAGTAATAGATAGTCATGTACCTA 5′ |
| | N210L | 5′ TCAAGGAACTTCTACTTGGCCTAGGATT 3′<br>3′ AGTTCCTTGAAGATGAACCGGATCCTAA 5′ |
| | N210W | 5′ TCAAGGAACTTCTATGGGGCCTAGGATTT 3′<br>3′ AGTTCCTTGAAGATACCCCGGATCCTAAA 5′ |
| | G211R/L212W | 5′ GGAACTTCTACTTCGCTGGGGATTTTATAC 3′<br>3′ CCTTGAAGATGAAGCGACCCCTAAAATATG 5′ |
| | F214L | 5′ CTTCGCTGGGGACTTTATACACCAGATGAGAAG 3′<br>3′ GAAGCGACCCCTGAAATATGTGGTCTACTCTTC 5 |
| | S215Y | 5′ TGGCCTAGGATTTTATACCCCAGATGA 3′<br>3′ ACCGGATCCTAAAATATGGGGTCTACT 5′ |
| HIV-$1_{BH10}$ | D67N/K70R | 5′ CCATAAAGAAAAAAAACAGTACTAGATGGAGAAAATT 3′<br>3′ GGTATTTCTTTTTTTTGTCATGATCTACCTCTTTTAA 5′ |
| | D67N/K70R/K73M | 5′ CCATAAAGAAAAAAAACAGTACTAGATGGAGAATGTTAGTAGATTTC 3′<br>3′ GGTATTTCTTTTTTTTGTCATGATCTACCTCTTACAATCATCTAAAG 5′ |

[a] Left column indicates the mutation(s) introduced in the RT. Mutagenized codons are shown underlined.