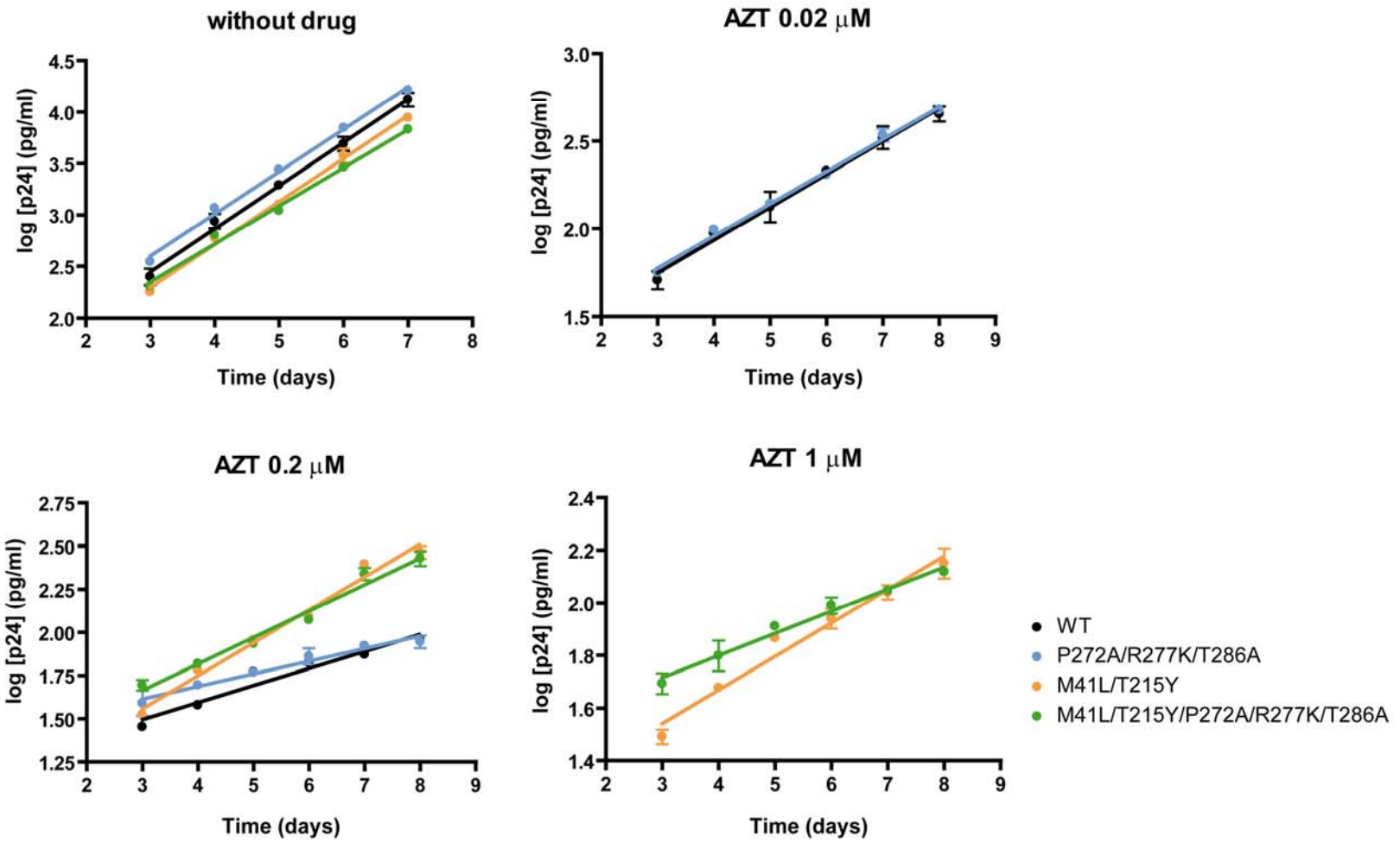
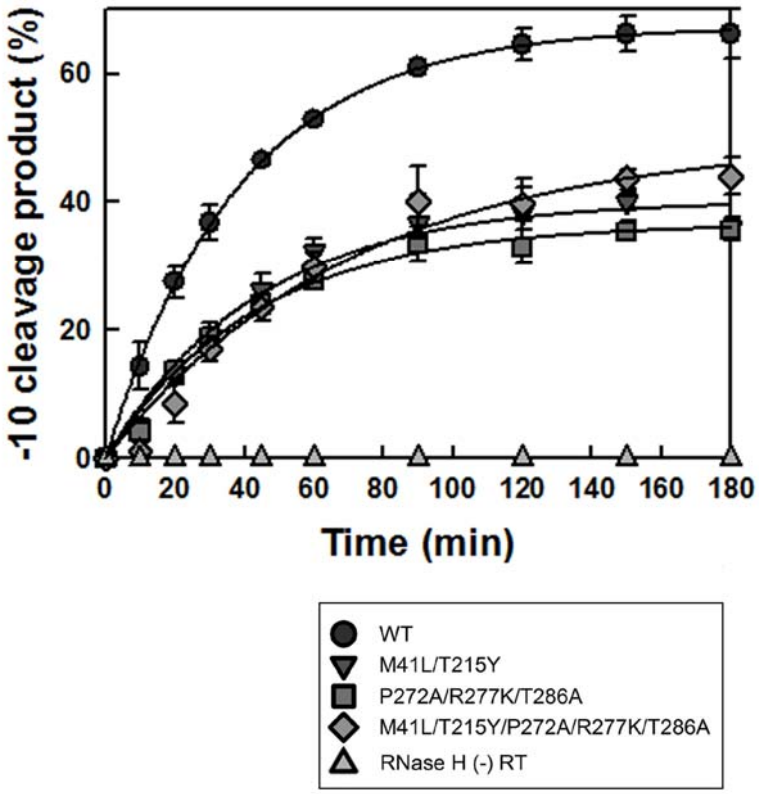
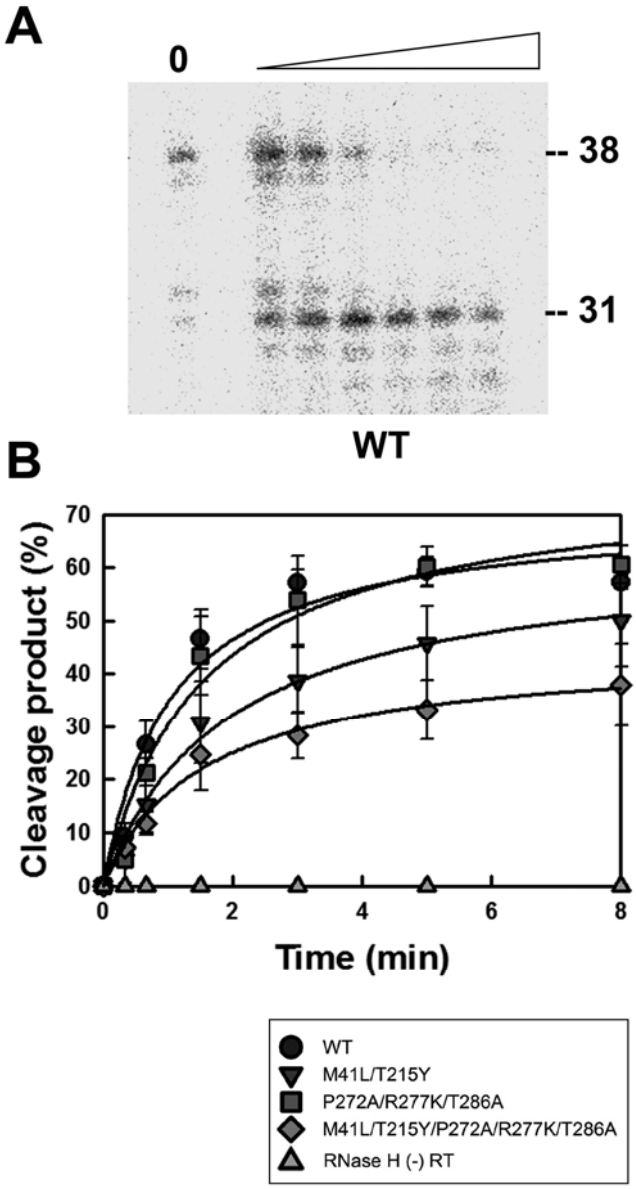


Supplementary Figure S1. Effects of the AZT concentration on the replication kinetics of recombinant HIV-1 clones bearing mutant or WT RTs. The production of antigen HIV-1 p24 in culture supernatants was determined at days 3, 4, 5, 6, 7 and 8. For each virus and AZT concentration, the slope of the plot provides an estimate of the viral replication capacity.



Supplementary Figure S2. Kinetics of formation of the -10 RNase H cleavage product generated by WT and mutant RTs during ATP mediated AZTMP excision. The intensity of the RNase H cleavage product was determined by densitometric analyses from at least three independent experiments (a representative example is shown in Fig. 5C). The apparent rates for RNase H cleavage obtained from these experiments were 0.0101 ± 0.0016 min^{-1} for WT RT, 0.0056 ± 0.0006 min^{-1} for mutant P272A/R277K/T286A RT, 0.0060 ± 0.0003 min^{-1} for mutant M41L/T215Y RT, and 0.0057 ± 0.0009 min^{-1} for mutant M41L/T215Y/P272A/R277K/T286A RT. An RNase H-deficient RT (HIV-1 group O RT with mutations V75I/E478Q) was included as a control.



Supplementary Figure S3. RNase H activity of WT and mutant RTs under limiting concentrations of template-primer. The [^{32}P]RNA/DNA substrate D38rna/25PGA (1.5 nM) was cleaved at 37ºC in the presence of the corresponding RT at 3 nM (active enzyme concentration). Time points were obtained after incubating the samples for 0.33, 0.66, 1.5, 3, 5 and 8 min, respectively. A representative autoradiogram of the RNase H cleavage activity of WT is shown in panel (A). Panel (B) shows the time courses of the RNase H cleavage reactions carried out with WT and mutant RTs. Represented values (averages ± standard deviations) were obtained from three independent experiments. An RNase H-deficient RT (HIV-1 group O RT with mutations V75I/E478Q) was included as a control.

SUPPLEMENTARY TABLE 1

Ability of the next complementary dNTP to inhibit ATP-dependent rescue of primers terminated with d4T in RNA-DNA duplexes, by mutant RTs

RTs	IC_{50} (μM)
M41L/T215Y	4.4 ± 1.6
M41L/T215Y/P272A/R277K/T286A	3.9 ± 1.0
MAK_SSSY	10.6 ± 4.8 (7.8 ± 0.3) [a]

Assays were carried out in the presence of 3.2 mM ATP. All dNTPs in these assays were supplied at 100 μM, except for dATP whose concentration ranged from 1 to 200 μM. Active enzyme concentrations in these assays were in the range of 10-20 nM, and the concentration of D38RNA/25PGA template-primer was 30 nM. Samples were incubated for 0-15 min depending on the assay. In all cases, incubation times were within the linear range of the corresponding time-course. The percent inhibition was plotted against the concentration of dATP, and the data were fitted to a hyperbola to obtain the IC_{50} for each enzyme. Reported values were obtained from 2-3 experiments and are given in means ± standard deviations.

[a] Value obtained under the same conditions, but with the heteropolymeric DNA/DNA substrate D38/25PGA