Supplemental Figure 1. Primer extension of HIV-1 RT polymorphisms of 172R and K in the presence of AZT with or without ATP.

Primer extension activities of HIV-1 RT TAM-2 with 172R or 172K were determined by drug inhibition assay in the absence or presence of AZT and ATP. The presence of an arginine rather than a lysine at codon 172 resulted in significantly higher primer extension only when the assays were carried out in the presence of ATP. A nucleic acid template/primer comprising a 100 nt DNA template annealed to a Cy3-5’-labeled 18 nt DNA primer was used. The reactions were run for 40 min, and fractionated on a 6% polyacrylamide gel.
Supplemental Figure 2. RNase H cleavage activity of HIV-1 RT with polymorphisms at codon 172.
RNase H activity of RTs 172R, 172K, TAM-2/172R and TAM-2/172K were evaluated using 50 nM T\textsubscript{RNA}/P\textsubscript{DNA} and 20 nM each HIV-1 RT in a buffer containing 50 mM Tris-HCl, pH 7.8 and 50 mM NaCl. Reactions were initiated by the addition of 6 mM MgCl\textsubscript{2}. The reaction times were 2, 5 and 10 min.

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
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</tbody>
</table>

![Image of gel electrophoresis showing cleavage products for different RTs at different timepoints]
Supplemental Table 1. X-ray data collection and refinement statistics

<table>
<thead>
<tr>
<th>Data collection</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Wavelength (Å)</td>
<td>1.0</td>
</tr>
<tr>
<td>Resolution (Å)</td>
<td>2.15</td>
</tr>
<tr>
<td>Resolution (Å)</td>
<td>2.15 (2.27-2.15)</td>
</tr>
<tr>
<td>Space group</td>
<td>C2</td>
</tr>
<tr>
<td>Cell dimensions</td>
<td></td>
</tr>
<tr>
<td>$a$ (Å)</td>
<td>159.2</td>
</tr>
<tr>
<td>$b$ (Å)</td>
<td>72.2</td>
</tr>
<tr>
<td>$c$ (Å)</td>
<td>109.3</td>
</tr>
<tr>
<td>$\beta$ (°)</td>
<td>97.6</td>
</tr>
<tr>
<td>Observed reflections</td>
<td>200916</td>
</tr>
<tr>
<td>Unique reflections</td>
<td>65991</td>
</tr>
<tr>
<td>Redundancy</td>
<td>3.0 (2.9)</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>98.8 (99.7)</td>
</tr>
<tr>
<td>$R_{\text{sym}}$</td>
<td>0.051 (0.424)</td>
</tr>
<tr>
<td>Avg I/σ</td>
<td>8.8 (1.4)</td>
</tr>
</tbody>
</table>

| Refinement statistics                                 |       |
| Resolution (Å)                                       | 30-2.15|
| No. of reflections (working)                         | 62131 |
| No. of reflections (test)                            | 3315  |
| $R_{\text{work}}$                                    | 0.225 |
| $R_{\text{free}}$                                    | 0.258 |
| Overall $B$ value (Å²)                               | 58.0  |
| Wilson $B$ value (Å²)                                | 49.1  |
| RMSD bond length (Å)                                 | 0.010 |
| RMSD Angle (°)                                       | 1.4   |

| Ramachandran plot (%)                                 |       |
| Favored                                              | 91.6  |
| Allowed                                              | 96.5  |
| Disallowed                                           | 3.5   |

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*a* Values given in parentheses are for the highest resolution shell.

*b* $R_{\text{sym}} = \Sigma_{hkl} |I| - <|I|> / \Sigma_{hkl} |I|.$

*c* $R_{\text{cryst}} = \Sigma_{hkl} |F_{\text{obs}} - F_{\text{calc}}| / \Sigma_{hkl} |F_{\text{obs}}|.$

*d* $R_{\text{free}} = R_{\text{cryst}}$, except 5% of the data excluded from the refinement.

**Supplemental Table 2. Antiviral activities of NRTIs against HIV-1s with NRTI resistance mutations in the background of 172K or 172R**

<table>
<thead>
<tr>
<th>Mutation(s)</th>
<th>AZT EC₅₀ (fold increase)a</th>
<th>ddI EC₅₀ (fold increase)a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>172R</td>
<td>172K</td>
</tr>
<tr>
<td><strong>Excision</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>0.033 ± 0.01</td>
<td>0.054 ± 0.02 (1.6)</td>
</tr>
<tr>
<td>N348I</td>
<td>0.22 ± 0.03 (6.7)</td>
<td>0.06 ± 0.01 (1.8)c</td>
</tr>
<tr>
<td>TAM-1b</td>
<td>0.28 ± 0.06 (8.5)</td>
<td>0.16 ± 0.05 (4.8)</td>
</tr>
<tr>
<td>69ins complexb</td>
<td>2.50 ± 0.17 (76)</td>
<td>0.22 ± 0.04 (6.7)c</td>
</tr>
<tr>
<td>N348I/TAM-1</td>
<td>1.37 ± 0.04 (42)</td>
<td>0.30 ± 0.03 (9.1)c</td>
</tr>
<tr>
<td>TAM-2b</td>
<td>0.55 ± 0.07 (17)</td>
<td>0.03 ± 0.007 (0.9)c</td>
</tr>
<tr>
<td><strong>Discrimination</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K65R</td>
<td>0.02 ± 0.00 (0.6)</td>
<td>0.02 ± 0.00 (0.6)</td>
</tr>
<tr>
<td>L74V</td>
<td>0.06 ± 0.03 (1.8)</td>
<td>0.05 ± 0.01 (1.5)</td>
</tr>
<tr>
<td>M184V</td>
<td>0.02 ± 0.01 (0.6)</td>
<td>0.03 ± 0.01 (0.9)</td>
</tr>
<tr>
<td>Q151M complexb</td>
<td>3.40 ± 0.50 (103)</td>
<td>3.33 ± 0.40 (101)</td>
</tr>
<tr>
<td><strong>Combination</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L74V/N348I</td>
<td>0.15 ± 0.01 (4.5)</td>
<td>0.06 ± 0.01 (1.8)c</td>
</tr>
<tr>
<td>L74V/TAM-1</td>
<td>0.29 ± 0.12 (8.8)</td>
<td>0.08 ± 0.02 (2.4)c</td>
</tr>
<tr>
<td>L74V/N348I/TAM-1</td>
<td>0.81 ± 0.17 (25)</td>
<td>0.22 ± 0.07 (6.7)c</td>
</tr>
<tr>
<td>M184V/N348I</td>
<td>0.15 ± 0.04 (4.5)</td>
<td>0.05 ± 0.01 (1.5)c</td>
</tr>
<tr>
<td>M184V/TAM-1</td>
<td>0.31 ± 0.08 (9.4)</td>
<td>0.10 ± 0.04 (3.0)c</td>
</tr>
<tr>
<td>M184V/ N348I/TAM-1</td>
<td>0.34 ± 0.12 (10)</td>
<td>0.20 ± 0.09 (6.1)</td>
</tr>
</tbody>
</table>

a Mean values ± standard deviations from at least three independent experiments. The relative increase in EC₅₀ for recombinant viruses compared with none/172R are shown in parentheses.

b “TAM-1” and “TAM-2” carry the “M41L and T215Y”, and “D67N, K70R, T215F and K219Q” mutations, respectively. 69ins complex carries 69 insertion and TAM1. Q151M complex carries Q151M, A62V, V75I, F77L and F116V.
EC₅₀ value of NRTI resistant mutations with 172K is significantly different from that with 172R (P<0.05 by t-test).
### Supplemental Table 3. Antiviral activities of NNRTIs against HIV-1s with NNRTI resistance mutations in the background of 172K or 172R

<table>
<thead>
<tr>
<th>Mutation</th>
<th>EC$_{50}$ (fold increase)$^a$</th>
<th>NVP</th>
<th>EFV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>172R</td>
<td>172K</td>
<td>172R</td>
</tr>
<tr>
<td>None</td>
<td>0.05 ± 0.01</td>
<td>0.03 ± 0.01 (0.6)</td>
<td>0.002 ± 0.0006</td>
</tr>
<tr>
<td>K103N</td>
<td>5.4 ± 0.53 (108)</td>
<td>3.20 ± 1.04 (64)$^b$</td>
<td>0.056 ± 0.012 (28)</td>
</tr>
<tr>
<td>V106M</td>
<td>4.1 ± 0.76 (82)</td>
<td>0.35 ± 0.19 (7)$^b$</td>
<td>0.029 ± 0.005 (15)</td>
</tr>
<tr>
<td>V108I</td>
<td>0.8 ± 0.07 (16)</td>
<td>0.14 ± 0.06 (2.8)$^b$</td>
<td>0.004 ± 0.001 (2)</td>
</tr>
<tr>
<td>Y181C</td>
<td>&gt;10 ± 0 (&gt;200)</td>
<td>&gt;10 ± 0 (&gt;200)</td>
<td>0.004 ± 0.001 (2)</td>
</tr>
<tr>
<td>Y188L</td>
<td>&gt;10 ± 0 (&gt;200)</td>
<td>&gt;10 ± 0 (&gt;200)</td>
<td>0.31 ± 0.104 (155)</td>
</tr>
<tr>
<td>N348I</td>
<td>1.1 ± 0.06 (22)</td>
<td>0.18 ± 0.07 (3.6)$^b$</td>
<td>0.005 ± 0.001 (2.5)</td>
</tr>
</tbody>
</table>

$^a$ Mean values ± standard deviations from at least three independent experiments (triplicates in each experiment). The relative increase in EC$_{50}$ for recombinant viruses compared with none/172R are shown in parentheses.

$^b$ EC$_{50}$ value of NNRTI resistant mutation with 172K is significantly different from that with 172R ($P<0.05$ by $t$-test).
Supplemental Table 4. Frequency of 172K in clinical samples

<table>
<thead>
<tr>
<th>treatment group / mutation</th>
<th>Total (n)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>172K n (%)</th>
<th>P-value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>No RTIs (untreated)</td>
<td>9569</td>
<td>68 (0.7)</td>
<td></td>
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<tr>
<td>NRTI-treated</td>
<td>4373</td>
<td>32 (0.7)</td>
<td>0.9140</td>
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<tr>
<td>NNRTI-treated</td>
<td>3786</td>
<td>45 (1.2)</td>
<td>0.0085</td>
</tr>
</tbody>
</table>

NRTI-associated resistance mutation

<table>
<thead>
<tr>
<th>mutation</th>
<th>Total (n)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>172K n (%)</th>
<th>P-value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>M41L</td>
<td>1526</td>
<td>12 (0.8)</td>
<td>0.7440</td>
</tr>
<tr>
<td>K65R</td>
<td>125</td>
<td>6 (4.8)</td>
<td>0.0004</td>
</tr>
<tr>
<td>D67N</td>
<td>1198</td>
<td>12 (1)</td>
<td>0.2814</td>
</tr>
<tr>
<td>69ins</td>
<td>34</td>
<td>1 (2.9)</td>
<td>0.2178</td>
</tr>
<tr>
<td>K70R</td>
<td>999</td>
<td>6 (0.6)</td>
<td>0.8429</td>
</tr>
<tr>
<td>L74V</td>
<td>296</td>
<td>4 (1.4)</td>
<td>0.1698</td>
</tr>
<tr>
<td>Q151M</td>
<td>111</td>
<td>11 (9.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>M184V</td>
<td>1868</td>
<td>8 (0.4)</td>
<td>0.2121</td>
</tr>
<tr>
<td>L210W</td>
<td>984</td>
<td>8 (0.8)</td>
<td>0.6908</td>
</tr>
<tr>
<td>T215Y</td>
<td>1461</td>
<td>7 (0.5)</td>
<td>0.3936</td>
</tr>
</tbody>
</table>

NNRTI associated resistant mutation

<table>
<thead>
<tr>
<th>mutation</th>
<th>Total (n)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>172K n (%)</th>
<th>P-value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>K103N</td>
<td>767</td>
<td>7 (0.9)</td>
<td>0.5038</td>
</tr>
<tr>
<td>V106M</td>
<td>44</td>
<td>0 (0)</td>
<td>&gt;0.9999</td>
</tr>
<tr>
<td>V108I</td>
<td>111</td>
<td>1 (0.9)</td>
<td>0.5500</td>
</tr>
<tr>
<td>Y181C</td>
<td>343</td>
<td>8 (2.3)</td>
<td>0.0046</td>
</tr>
<tr>
<td>Y188L</td>
<td>72</td>
<td>3 (4.2)</td>
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<td>G190A</td>
<td>196</td>
<td>3 (1.5)</td>
<td>0.1706</td>
</tr>
</tbody>
</table>

<sup>a</sup> The Stanford HIV Drug Resistance database was accessed on 23 May 2008.

<sup>b</sup> The P-value was determined by the Fisher's exact test. The P-values for isolates deposited in the Stanford HIV Drug Resistance Database were determined based on a comparison with the no RTIs (untreated) group. Numbers in bold letters indicate statistically significant values (<0.05).