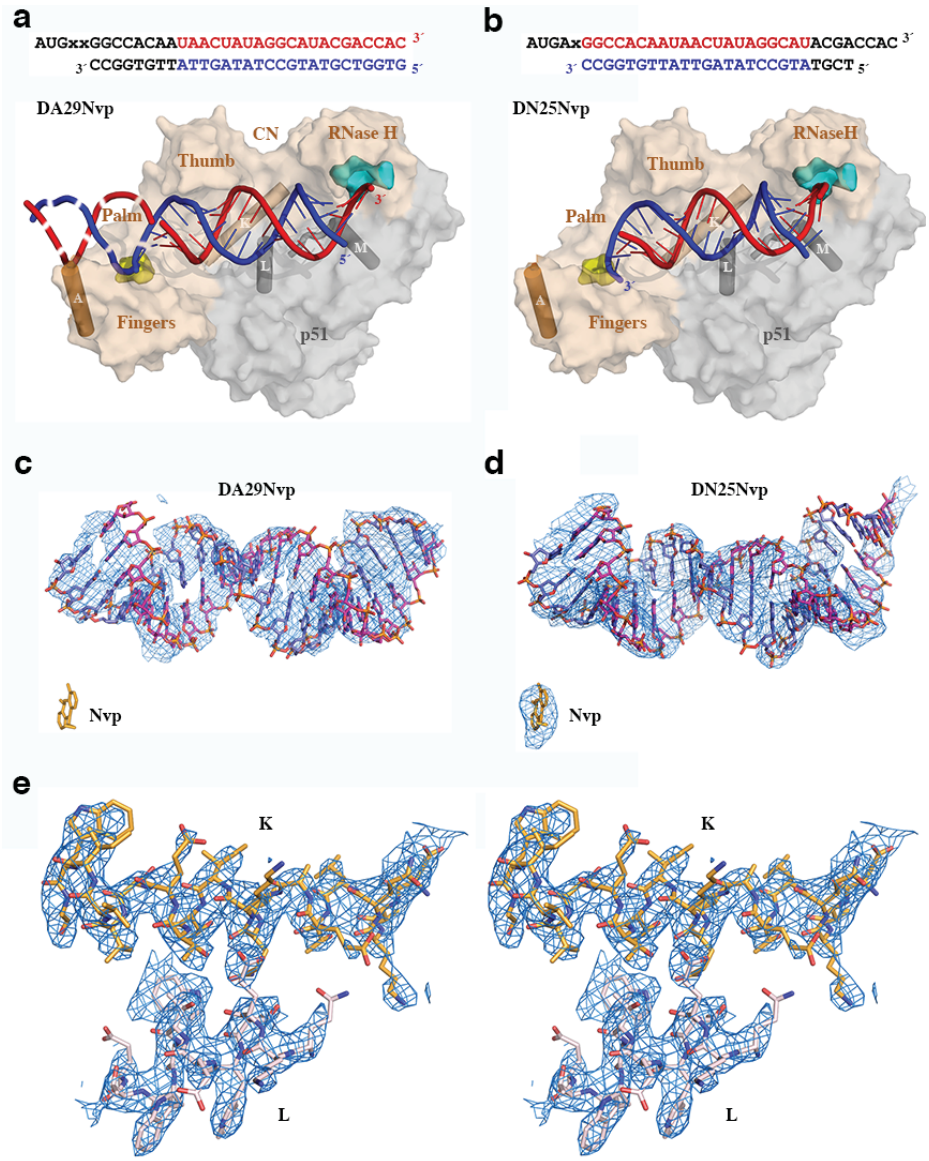

Figure S1 Structures of HIV-1 RT complexed with RNA/DNA and NNRTI. (**a**) The DA29Nvp and (**b**) the DN25Nvp structure. RT is shown as molecular surface with p66 in wheat and p51 in silver. Domains and subdomains in p66 are marked (CN standing for connection), and the polymerase and RNase H active site are highlighted in yellow and cyan, respectively. The four helices in RT are shown as cylinders and labeled as landmarks. The hybrid is shown as tube-and-ladder with RNA in red and DNA in blue. The nucleic acid sequence is shown in the same color scheme. The "x" denotes the abasic tetrahydrofuran substitution, and black letters indicate bases that are not traceable. (**c**, **d**) The RNA (red) and DNA (blue) hybrid and Nevirapine in the DA29Nvp and DN25Nvp structure are shown with the simulated annealing omit map contoured at 2.5 σ and 3.0 σ, respectively. **(e)** A stereo view of α-helices at the subunit interface in WT22Efv with the simulated annealing omit map. The helix K in p66 and helix L in p51 are superimposed with the simulated annealing omit map contoured at 0.8σ.

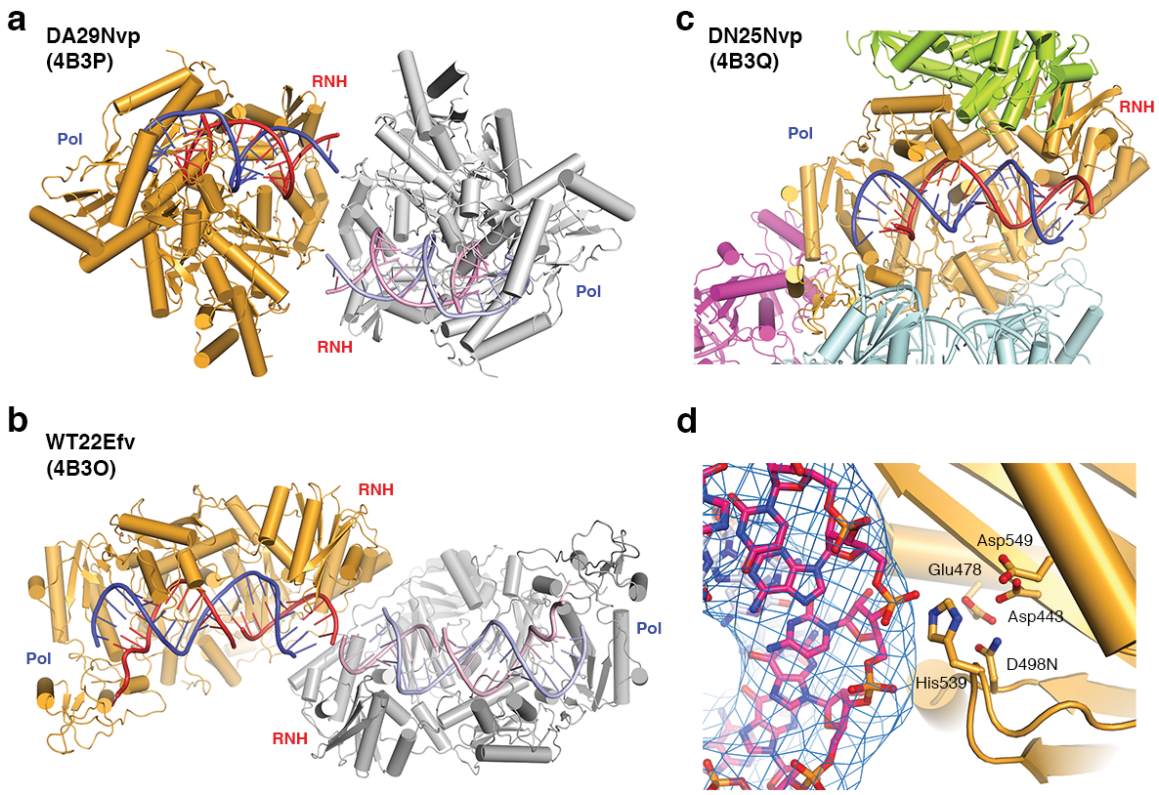

Figure S2 Crystal lattice contacts. (**a**) The end of the RNA/DNA hybrid near the RNase H domain (labeled as RNH in red) in the DA29Nvp crystal (PDB: 4B3P) contacts the RT of a neighboring molecule (silver, pink and light blue). The polymerase domain (including palm, thumb and fingers subdomains) is labeled as Pol in blue. (**b**) The end-to-end contact of RNA/DNA hybrid in the WT22Efv crystal (PDB: 4B3O). The symmetry mate is shown in silver (RT), pink (RNA) and light blue (DNA). (**c**) The hybrid in the DN25Nvp crystal (PDB: 4B3Q) is not in lattice contact. All symmetry mates (green, magenta and pale blue) contact the protein only. (**d**) Close-up of the RNase H active site in the DN25Nvp structure. The RNA (pink) and DNA (light blue) are shown with the 2Fo-Fc map contoured at 1σ.

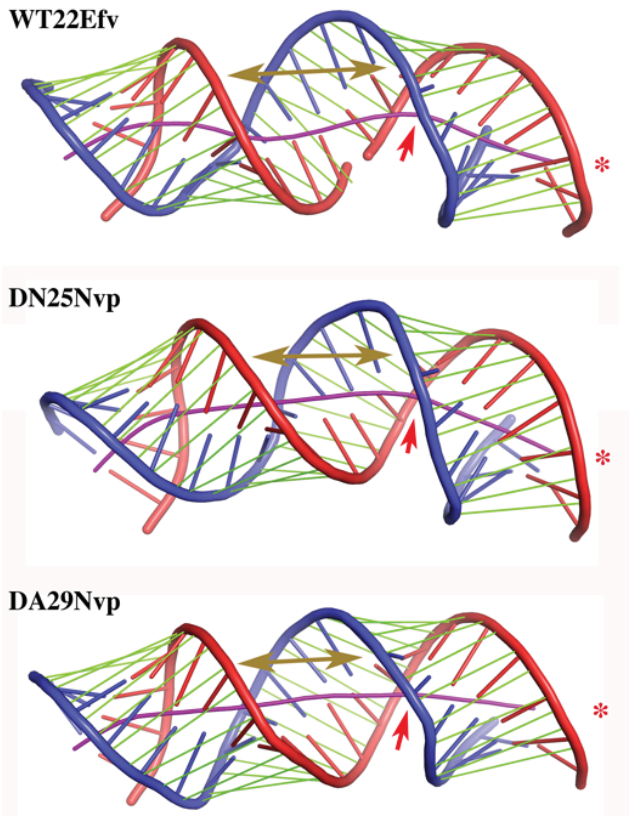

Figure S3 The three RNA/DNA structures observed in this study. RNA (red) and DNA (blue) are shown as tube-and-ladder. The width of the minor groove is indicated by the green lines [63]. The helical axis is shown in purple. The brown double arrowhead marks the widened major groove. The first bend (on the left) in each duplex is adjacent to the p66 thumb and is universally observed in all RT-nucleic acid complexes. The red arrowhead points at the second bend unique in the three RT-hybrid complexes, where the hybrid interacts with the C-terminus of p51. The second bend is most prominent in the DN25Nvp structure. Location of the RNase H active site is mark by the red asterisk.

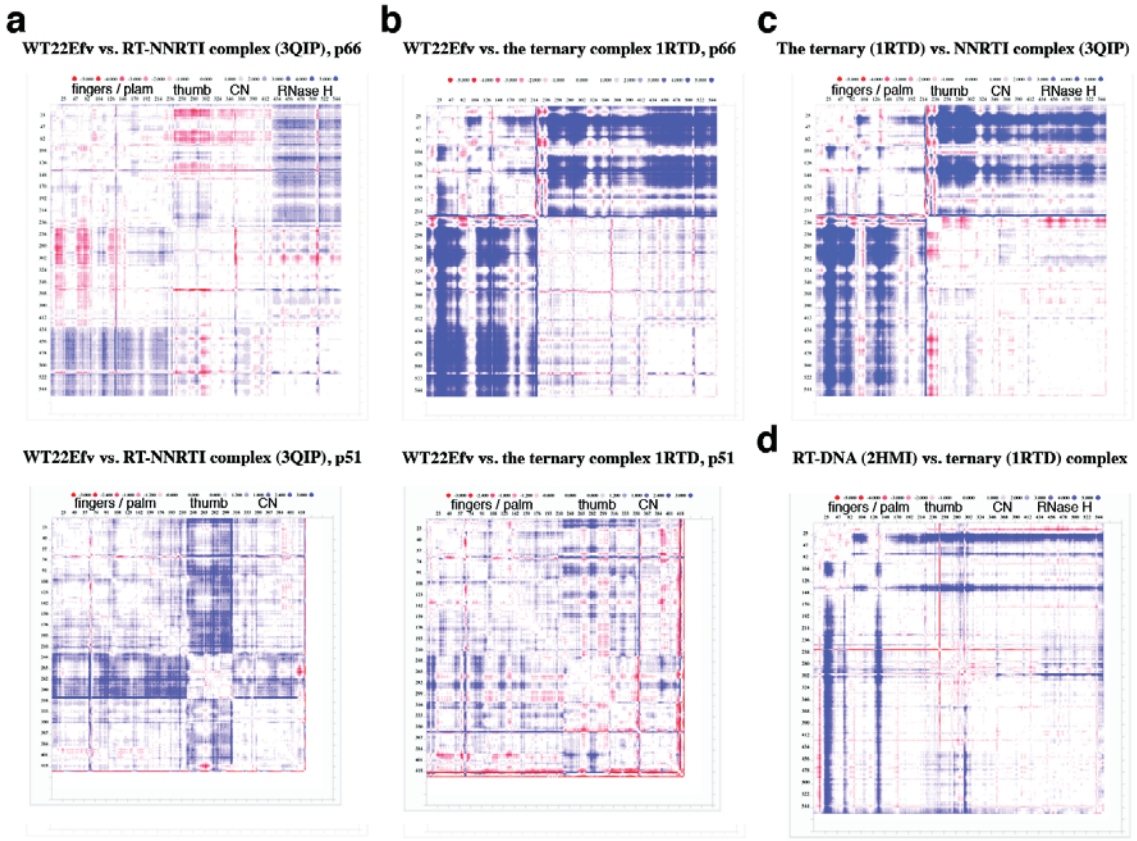

Figure S4 Structural comparison of HIV-1 RT. (**a**) Difference distance matrix plot (DDMP) [60] of RT between the WT22Efv (4B3O) structure and 3QIP [14], which contains a nevirapine and a pyrimidinol carboxylic acid (RNase H inhibitor). The plotting scale (Red to blue) is -5Å to +5Å for the p66 (top) and -3Å to 3Å for the p51 subunit (bottom). (**b**) DDMP of RT between the WT22Efv (4B3O) structure and the ternary complex 1RTD [26]. (**c**) DDMP of p66 between RT bound to inhibitors (3QIP) and to DNA substrate (1RTD). (**d**) DDMP of p66 in an RT-DNA binary (2HMI [38]) versus a ternary complex (1RTD) shows that they are similar except for the fingers subdomain.

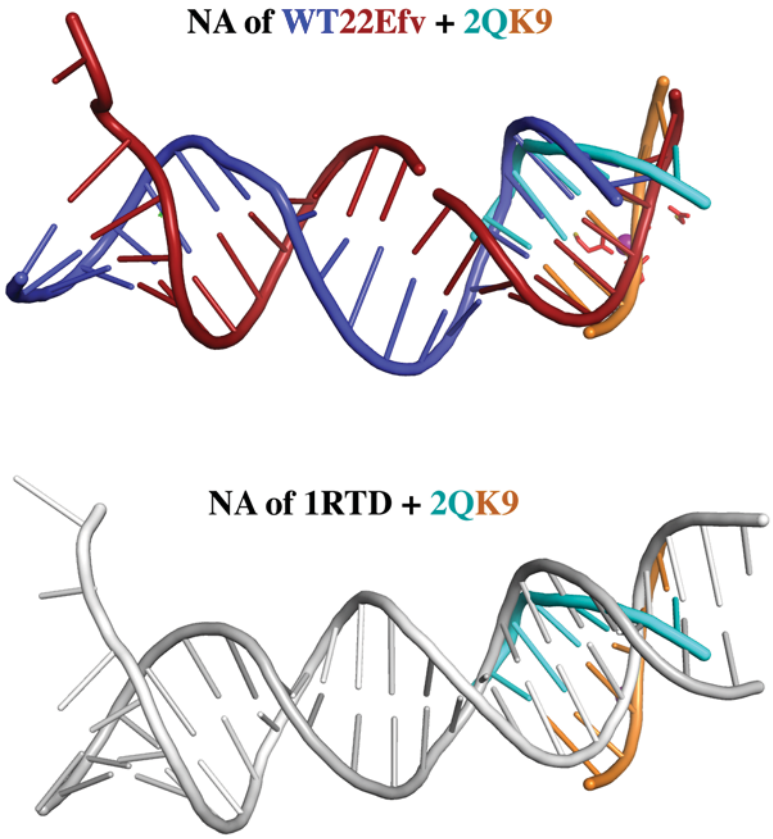

Figure S5 The RNA/DNA hybrid structure compatible with RNA degradation. The hybrid in the WT22Efv structure (red RNA and blue DNA) can be easily connected with the RNA/DNA hybrid (orange RNA and cyan DNA) complexed with human RNase H1 (PDB: 2QK9 [10]) after superposition of the human enzyme with the RT RNase H domain. (**b**) The DNA duplex (silver) in the 1RTD structure [26], however, cannot be linked with the RNA/DNA hybrid (orange RNA and cyan DNA) complexed with human RNase H1 after superposition of the human enzyme with the RT RNase H domain.

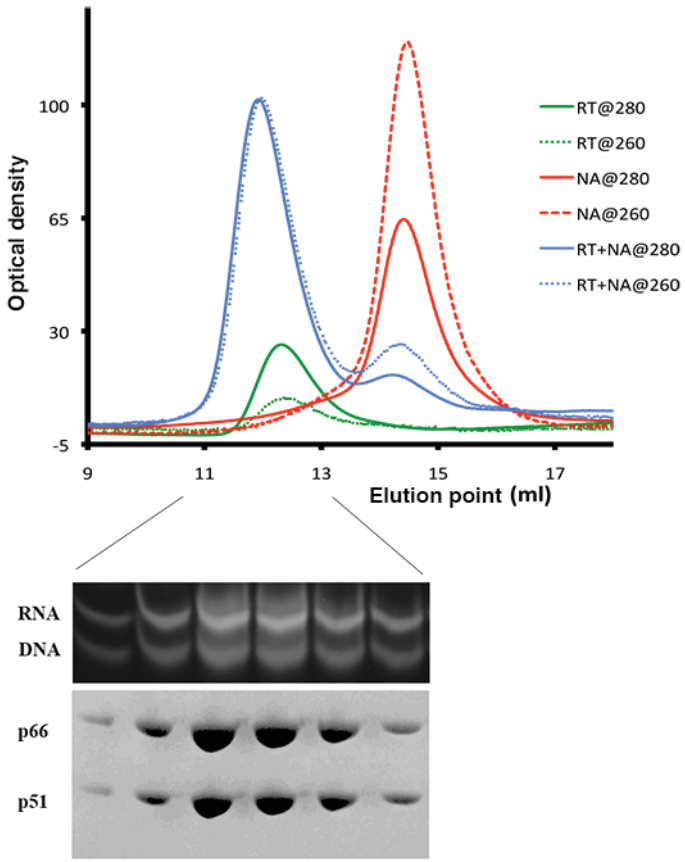

Figure S6 Preparation of the HIV-1 RT and RNA/DNA hybrid complexes. (**a**) Gel filtration profiles of the complex (RT+NA, blue), apo-protein (RT, green) and RNA/DNA hybrid (NA, red) alone. (**b**) TBE urea and SDS/polyacrylamide gel analyses of peak fractions of the complex from gel filtration chromatography confirm the 1:1:1 molar ratio of the p66, p51 and RNA/DNA hybrid.

Supplementary Video Legends

Video 1 Structural differences between RT–NNRTI and the RT–RNA/DNA–NNRTI complex. The small molecule inhibitors, nevirapene (Nvp) and pyrimidinol carboxylic acid (N4P), and Mg^{2+} (colored green) in the RT–NNRTI complex (3QIP [14]) are shown as spheres and labeled. The RNase H active site is marked by the conserved carboxylates shown as red sticks. The five structural subdomains of p66 are color coded as red palm, blue fingers, green thumb, yellow connection and cyan RNase H. The p51 subunit is shown in pale green. The RNA/DNA hybrid in the RT–RNA/DNA–NNRTI complex (WT22Efv of this study, 4B3O) is shown as red and blue tube-and-ladder, and efavirenz (Efv) as purple spheres. Four helices at the subunit interface are labeled for reference. Only the RT structure is shown in the interpolated states.

Video 2 Structural differences between RT–DNA–dATP and the RT–RNA/DNA–NNRTI complex. The DNA in RT–DNA–dATP complex (3KK2 [26]) is shown as yellow tube-and-ladder, the dATP as sticks, and the Mg^{2+} as a green sphere. The RNase H active site is marked by the conserved carboxylates shown as red sticks. The p66 subunit is shown in pale pink, and the p51 subunit in pale green. The RNA/DNA hybrid in the RT–RNA/DNA–NNRTI structure (WT22Efv, 4B3O) is shown as yellow (DNA) and orange (RNA) tube-and-ladder. Four helices at the subunit interface are colored in pink (p66) and blue (p51) reference. Locations of drug resistant mutations (Gly333, Asn348, Ala360, and Gln509) are marked. Three loops containing Gly333, Ala360 and Gln509 that contact the widened major groove are highlighted in pea green, and the loop containing Asn348 and the adjacent p51 thumb region are highlighted in teal. Only the RT structure is shown in the interpolated states.

Video 3 Morphing of nucleic acid complexed with HIV-1 RT from the DNA polymerization to RNA degradation-compatible state. The DNA in the polymerization mode (1RTD [26]) and RNA/DNA compatible with RNA degradation (4B3O, this study) were used in this morphing analysis after superposition of the p51 subunits. The template strand (RNA in the RNA/DNA hybrid) is shown in red and the primer strand (DNA) is shown in blue. The nick in the RNA strand of 4B3O is shown as a break in the backbone.