Supporting Material

Protein-mediated antagonism between HIV reverse transcriptase ligands nevirapine and Mg-ATP

S1. Effects of NVP (200 μM) on the HSQC spectra of I63M, L74M, and L289M RT mutants.

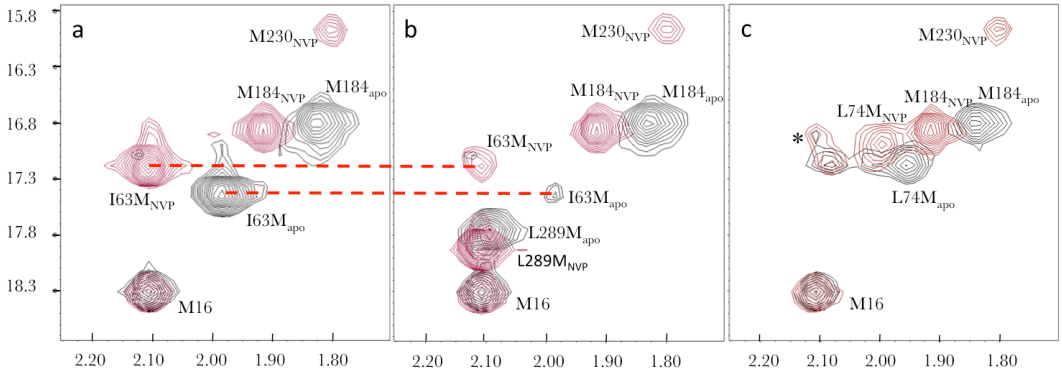

Figure S1. ^{1}H-^{13}C HSQC spectra of a) [$^{13}CH_3$-Met_{66}]RT($I63M_{66}$); b) [$^{13}CH_3$-Met_{66}]RT($L289M_{66}$); c) [$^{13}CH_3$-Met_{66}]RT($L74M_{66}$) in the absence (black) or presence (red) of 200 μM NVP. . Note that spectrum b) has a small level of contamination with the RT($I63M_{66}$). In c) the resonances labeled with an asterisk most probably arise from some unoflded material (Met_{rc} = (2.1,16.9)).

S2: High concentration ^{1}H-^{13}C HSQC spectrum of [$^{13}CH_3$-Met_{66}]RT(I63M_{66}) mutant

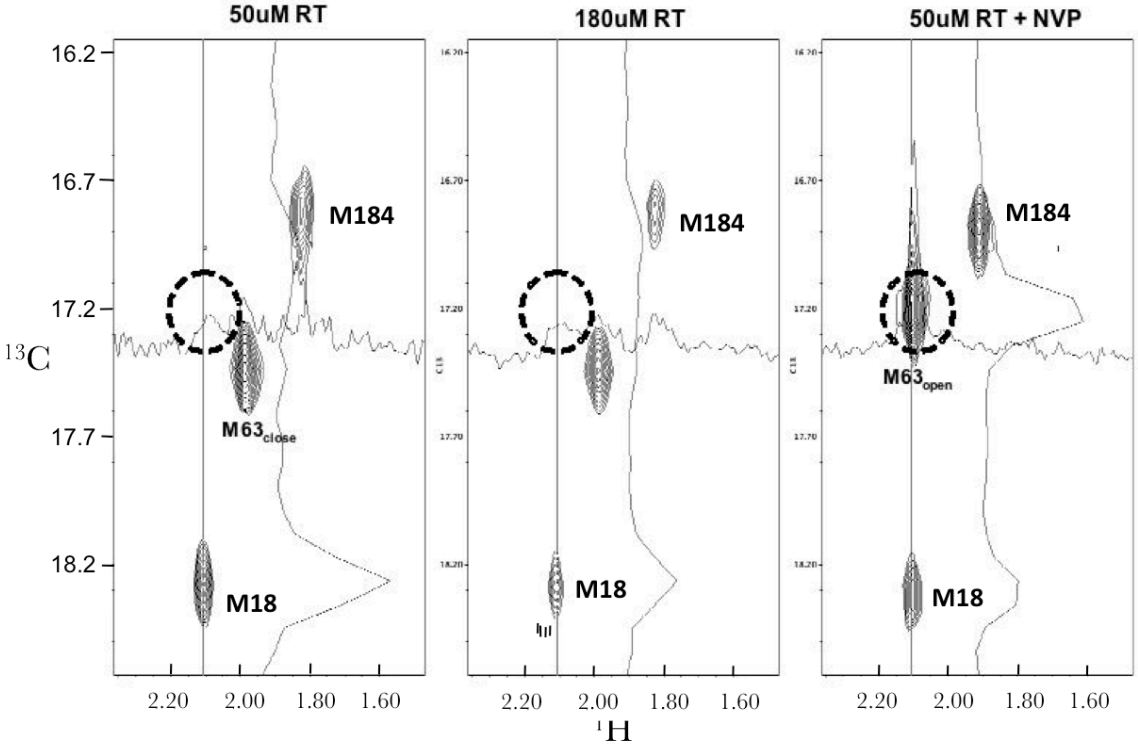

Figure S2. ^{1}H-^{13}C HSQC spectra of [$^{13}CH_3$-Met_{66}]RT(I63M_{66}) illustrating the absence of a resonance at the "open" position observed in the NVP-RT complex, even at high concentration.

S3: Effect of temperature on the spectrum of [$^{13}CH_3$-Met_{66}] RT(I63M_{66})

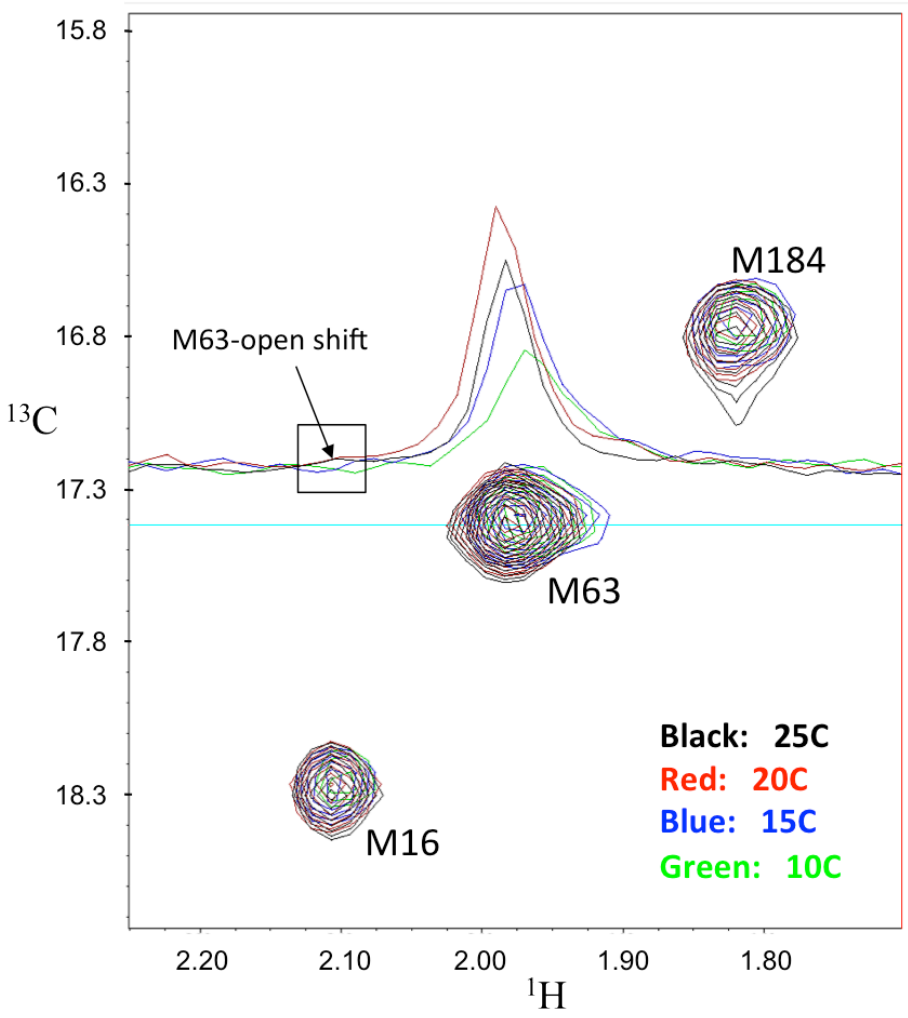

Figure S3: Effect of temperature on the ^{1}H-^{13}C HSQC spectrum of 45 μM [$^{13}CH_3$-Met_{66}]RT(I63M_{66}). Green (10 °C), blue (15 °C), red (20 °C), and black (25 °C). The 1D ^{1}H spectra obtained at the indicated ^{13}C shift value are also shown. Resonance intensities decreased with temperature, but normalized relative to M16, the intensities remained constant. The temperature-dependent variations were insufficient to support interpretation of shifts as an indication of changes in the conformational equilibrium.

S4: ^{1}H-^{13}C HSQC spectrum of nevirapine

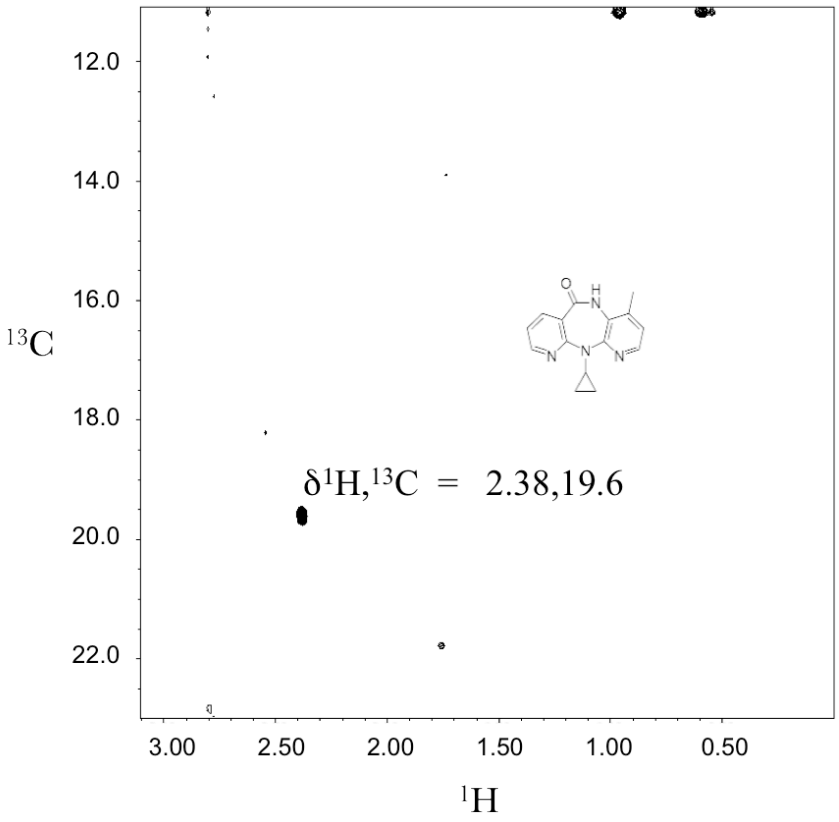

Figure S4. ^{1}H-^{13}C HSQC spectrum of unlabeled nevirapine showing the region of interest.

S5. Structure overlay of RT•MnATP with RT•DNA•MgTTP

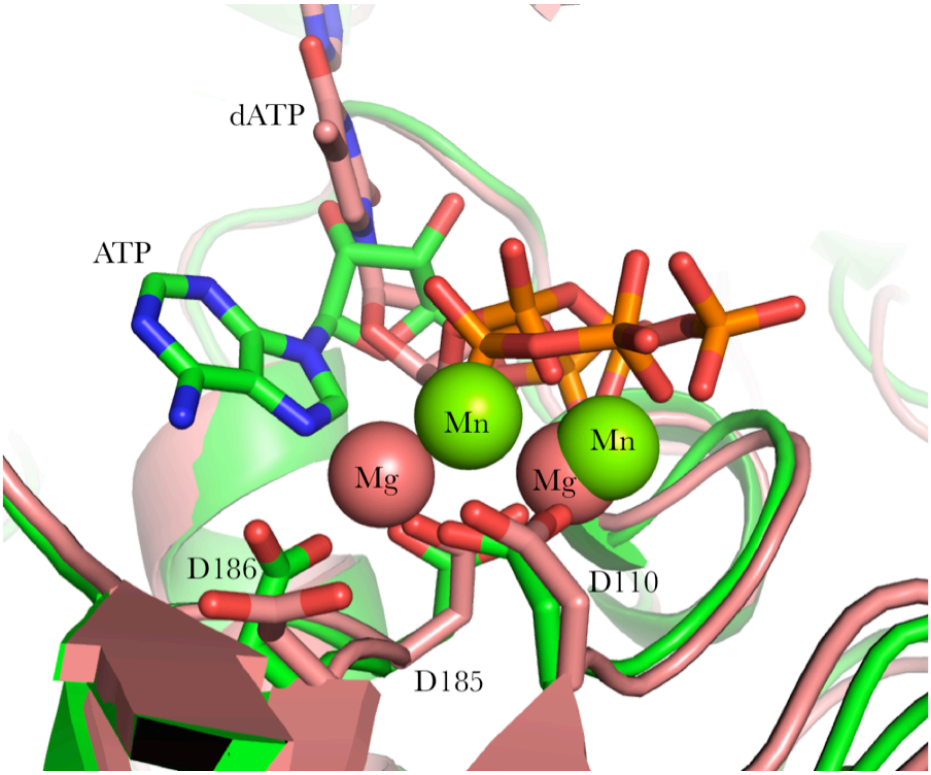

Figure S5. Superposition of the active site regions of the MnATP complex (pdb code 2IAJ, green), with the RT•DNA•MgTTP complex (pdb code: 1RTD, mauve). As can be seen in the figure, the active site carboxylate ligands D110, D185, and D186 and the loop positions superimpose reasonably well, while the ATP and TTP ligands do not superimpose well. This comparison supports the conclusion that the active site structure is similar in both complexes.

S6. Structure overlay of RT•MnATP (Green) with RT•NVP (mauve)

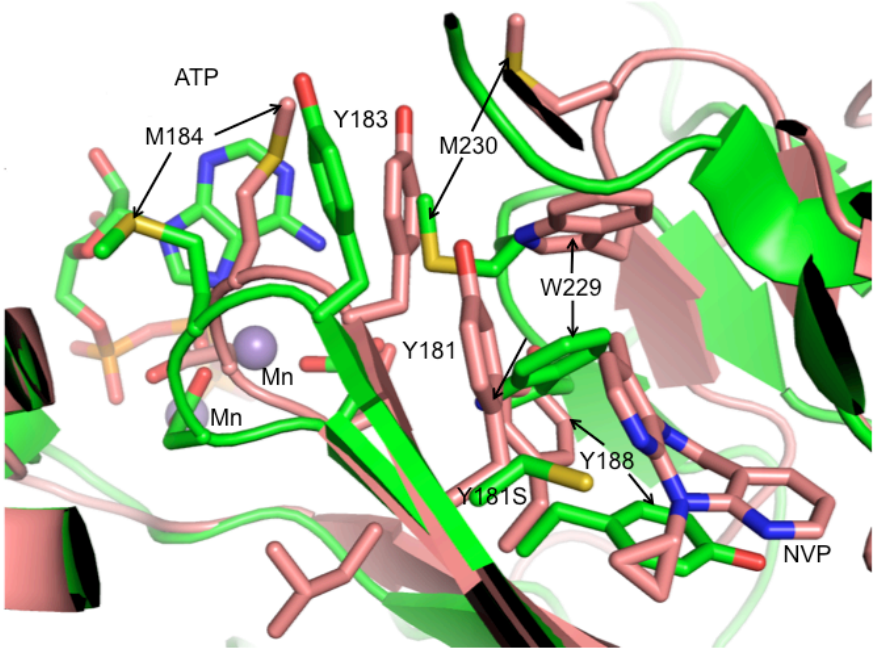

Figure S6. Superposition of the active site and NNRTI binding regions of RT for a structure containing MnATP (pdb code: 2IAJ, green) and NVP (pdb code: 1VRT, mauve). As can be seen in the figure, the MnATP structure also contains a Y181S mutation. Although the ATP and NVP binding sites do not overlap directly, the structural differences are consistent with significant protein-mediated interactions.