Supplemental Figure 1: Images of representative hydroxyl radical footprinting gels. They are from left to right: the first observation of RT dependent hypersensitive cleavage along with the 18nt primer used in our DDDP assays serving as a size marker; the gel used to generate the representative profiles of R1T and RT1ZM shown in figures 5 and 6; the gel used to generate the representative profiles of Newtop in figure 5; a gel that shows RT dependent hypersensitive cleavage of the stems of R1T variants Zam1 and Acut. The secondary structure of Acut, which does not appear in the main text is also show.

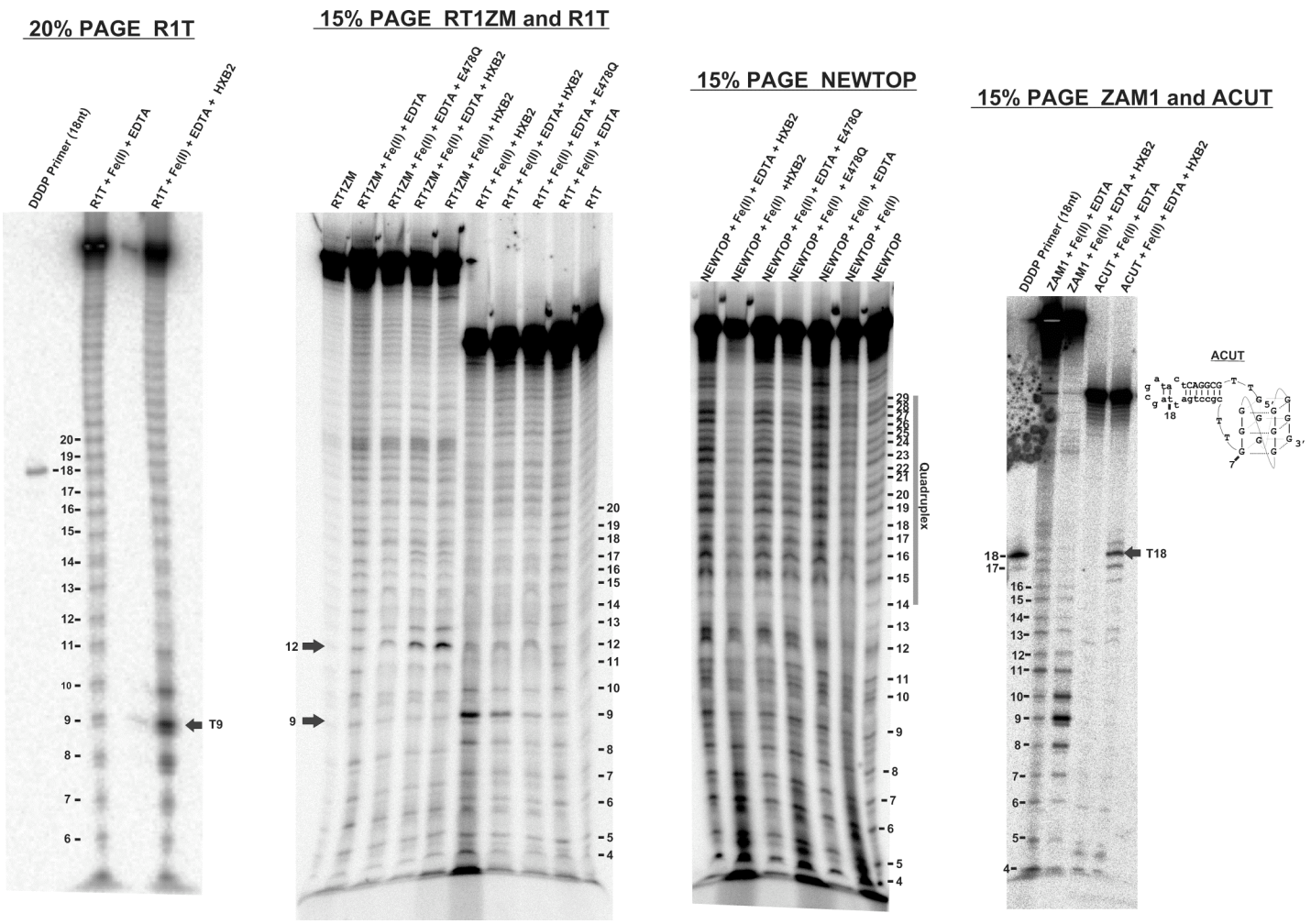
Supplemental Figure 2: Hydroxyl radical cleavage of R1TZM bound to RT in the presence of relatively high Fe(II) concentrations (200 μM Fe(II), 400 μM EDTA) ,obscuring the RNase H active site dependent cleavage observed at lower Fe(II) concentrations. The RT1t49(-5) variant RT1ZM displays hypersensitive cleavage by hydroxyl radicals at position T12, and the hypersensitive cleavage is not significantly diminished by the E478Q RNase H mutation.

Supplemental Figure 3: In order to facilitate visualization of how the quadruplex module of R1T could bind to RT we used the PatchDock server (http://bioinfo3d.cs.tau.ac.il/PatchDock/) (1,2). PatchDock is an efficient algorithm that has been successfully applied to predicting protein-protein and protein-DNA interfaces. Scoring of docked complexes is determined based on shape complementarity with a penalty for steric clash that allows for some overlap between the two surfaces that can implicitly account for structural accommodation. An overlay of the 10 highest scoring docking interactions of RT with the myc promoter-derived quadruplex is shown. The 10 docking sites occupy multiple positions between the fingers and thumb subdomains. While these docking solutions are unlikely to reflect the exact mode of interaction, it is clear that the dimensions of the quadruplex are compatible with accommodation by the cleft between the thumb and fingers subdomains upon minor adjustments to residues in the RT. The quadruplex used for docking is derived from the myc promoter sequence (PDB 1XAV) and it was docked into HIV-1 RT (PDB 3KK2). The waters, substrate, and ions were removed from the protein structure prior to docking, and the lowest energy NMR structure from the myc promoter quadruplex was used for docking.

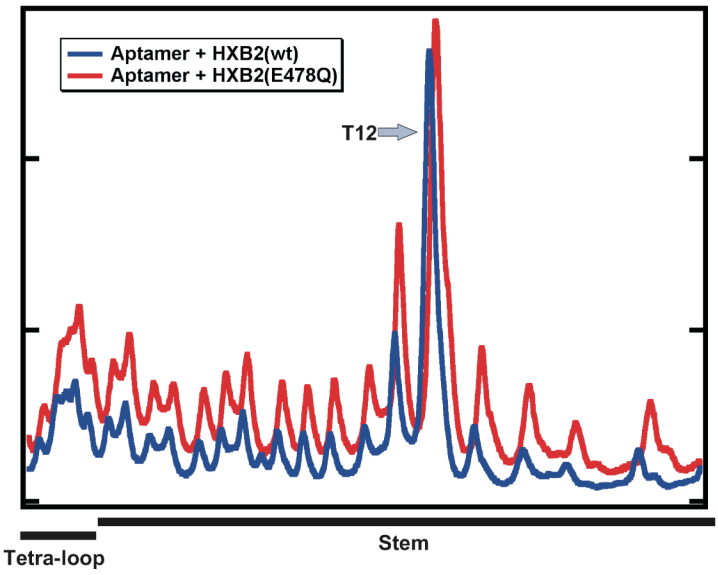
Supplemental References

1. Schneidman-Duhovny, D., Inbar, Y., Nussinov, R. and Wolfson, H.J. (2005) PatchDock and SymmDock: servers for rigid and symmetric docking. *Nucleic Acids Res*, 33, W363-367.
2. Duhovny, D., Nussinov, R. and Wolfson, H. (2002), *Proceedings of the 2nd Workshop on Algorithms in Bioinformatics(WABI) Lecture Notes in Computer Science*. Springer Verlag, Rome, Italy, Vol. 2452, pp. 185-200.

Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure S3