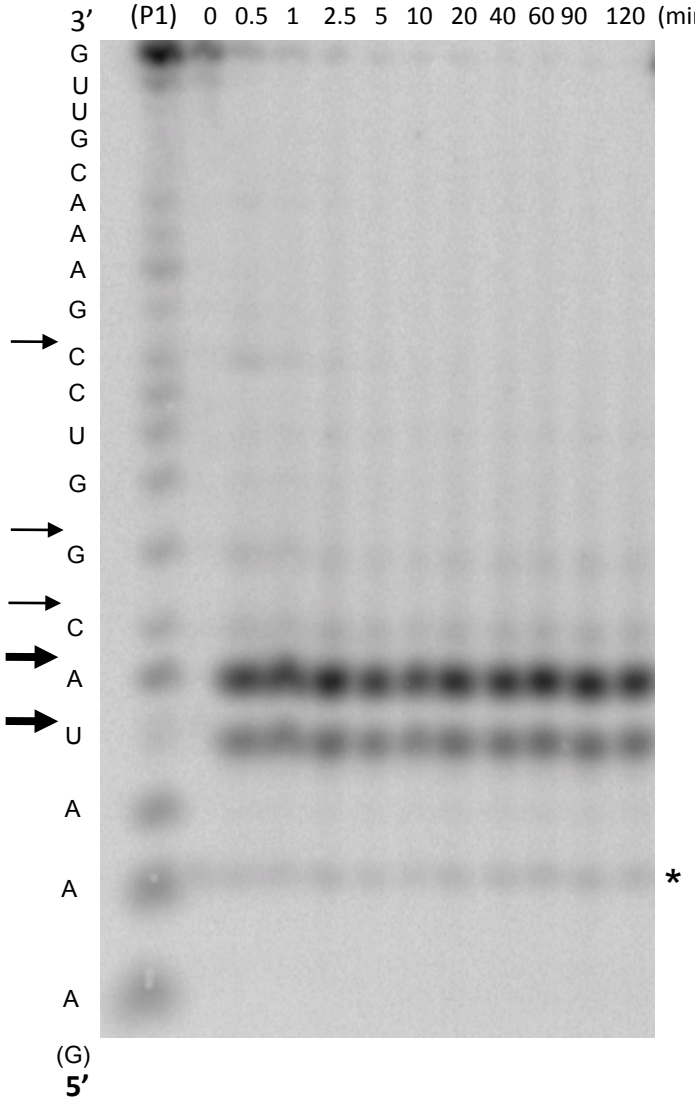
Alla and Nicholson
Supplemental Figure S1



Supplemental Figure S1. Stable products of Hs-RNase H1 cleavage of the 21 bp RNA-DNA heteroduplex substrate. Reactions were carried out essentially as described in *Materials and Methods*, using (preformed) heteroduplex containing 5’-^{32}P-labeled RNA. Reaction times are at the top of the gel image. “P1” refers to P1 nuclease reaction, creating a ladder of labeled products with 3’-hydroxyl termini, similar to RNase H1. Arrows indicate the main cleavage products. The 5’-terminal G (indicated by parentheses) is below the bottom of the gel image. The asterisk on the lower right hand side of the image denotes a nonenzymatic cleavage product that is present in all lanes.

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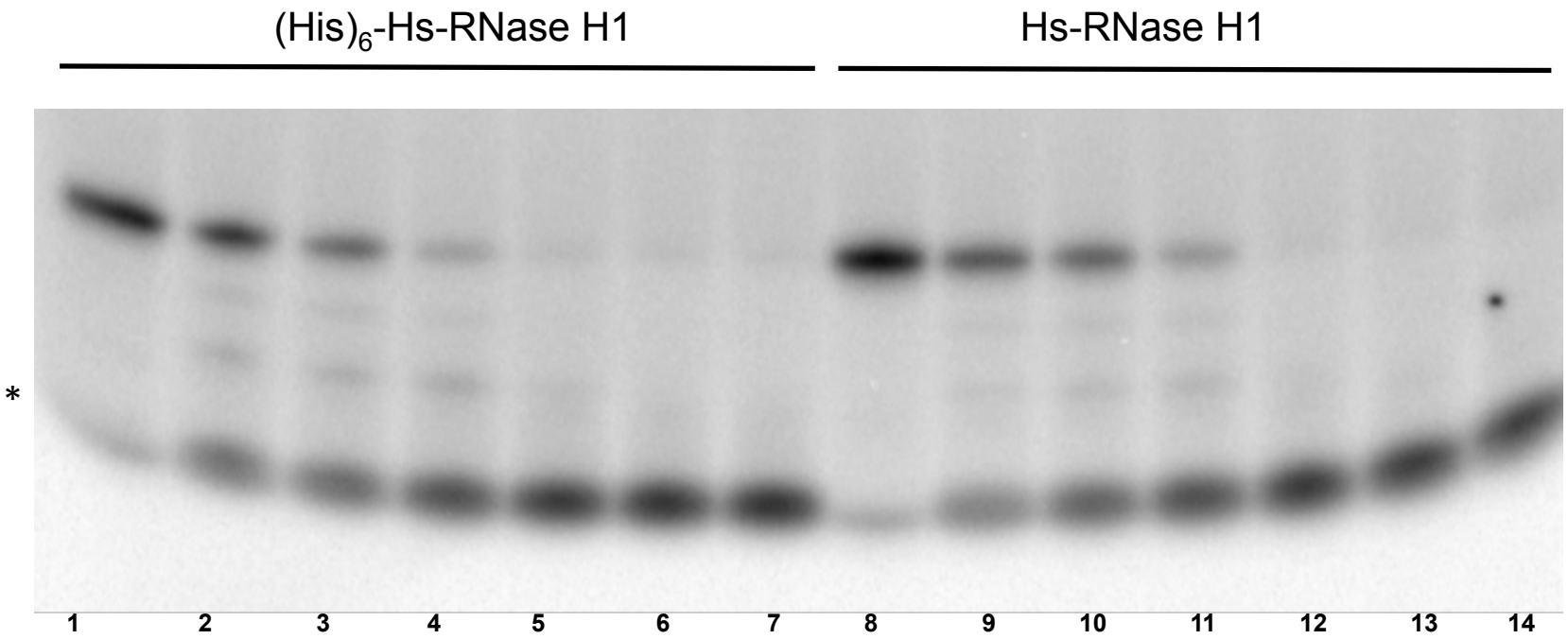
Supplemental Figure S2



Supplemental Figure S2. Comparison of hybrid substrate cleavage by Hs-RNase H1 with or without an N-terminal $(His)_6$ affinity tag. Hs-RNase H1 was purified and the $(His)_6$ tag removed as described in *Materials and Methods*. "$(His)_6$-Hs-RNase H1" refers to enzyme that was mock-treated with thrombin and purified in the same manner as the thrombin-treated enzyme ("Hs-RNase H1"). Cleavage assays used 100 nM (preformed) 5'-^{32}P-labeled hybrid substrate and 30 nM enzyme in the standard assay buffer (see Materials and Methods). Lanes 2-7, and lanes 9-14 represent 0.5, 1, 2.5, 5, 10, and 30 min time points for $(His)_6$-Hs-RNase H1 and Hs-RNase H1 reaction, respectively. Lanes 1 and 8 are control reactions lacking Mg^{2+} that were incubated for 30 min. The asterisk on the left hand side indicates the position of a nonenzymatic cleavage product that is present in lanes 1 and 8. See Supplemental Fig. S1 for identification of the stable products.

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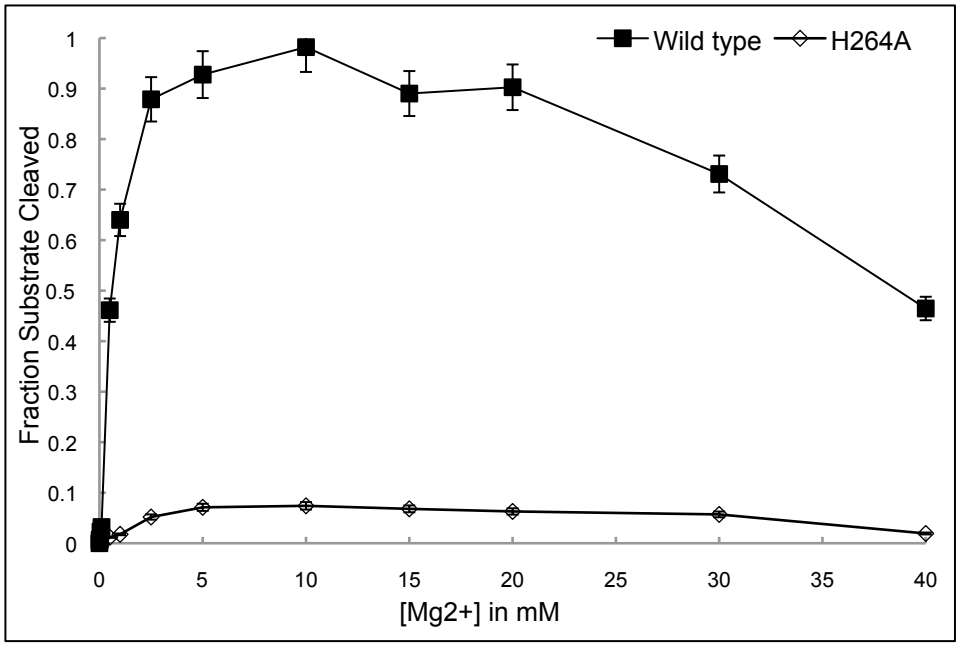
Supplemental Figure S3

A



B

Mg^{2+} concentration ratio (mM/mM)	WT	H264A
15 / 10	0.90	0.92
20 / 10	0.92	0.85
30 / 10	0.74	0.77
40 / 10	0.47	0.26
60 / 10	0.15	0.09

Supplemental Figure S3. Comparison of the Mg^{2+} titration behavior of Hs-RNase H1 and the H264A mutant enzyme. Panel A shows the fraction of heteroduplex substrate cleaved as a function of Mg^{2+} ion concentration, using either WT or H264A mutant Hs-RNase H1. The data for the wt enzyme are from Figure 2, and the experimental conditions are provided in the Figure 2 legend. Panel B provides the ratios of the fraction of substrate cleaved, as determined by dividing the value at each Mg^{2+} concentration (15, 20, 30, 40, 50 mM) by the value obtained at 10 mM Mg^{2+}, for both WT and H264A mutant enzymes. Note that the H264A mutant is inhibited to a greater extent by higher Mg^{2+} concentrations.