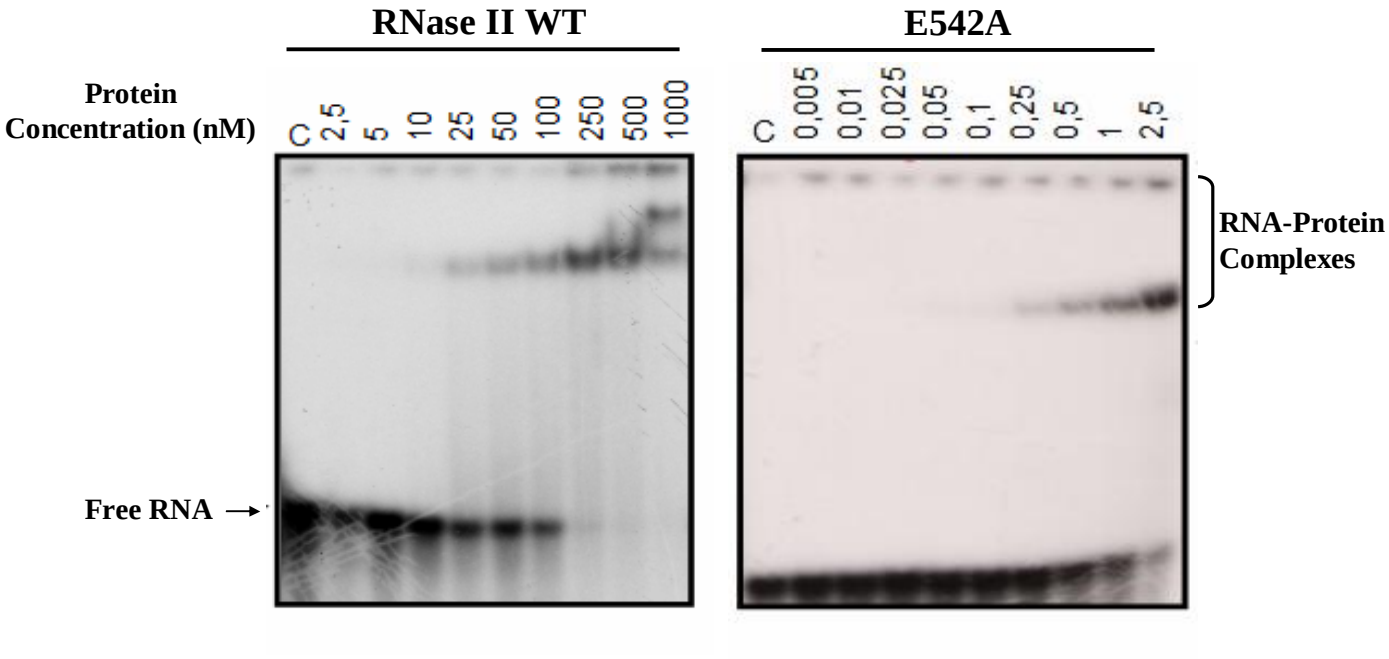

Figure S1. Comparison of RNA-binding ability of RNase II wt and E542A mutant.
A. 10,000 cpms of polyA 35 nt were incubated under the conditions described above. The enzyme concentrations used are indicated in the Figure. A control reaction without enzyme added was performed (C).

Electrophoretic Mobility Shift Assay (EMSA).

EMSAs were performed with a poly(A) chain of 35nt RNA oligomer as described previously (1, 2). Briefly, the substrate was labelled at there 5'-end using [γ-32ATP] and T4 polynucleotide kinase. The substrate was then purified with Microcon YM-3 Centrifugal Filter Devices (Millipore) to remove the non-incorporated nucleotides. Binding reactions were performed in 10 µl of volume containing 20 mM Tris-HCl pH8, 100 mM KCl, 1 mM DTT, 0.5 µg/µl BSA and 10 mM EDTA, 1 fmol of substrate and increasing concentration of enzyme. Mixtures containing increasing concentration of each enzyme were incubated for 10 min at 37 ºC and then subjected to UV crosslinking - 600 mJ, 10 min, 254 nm at 4 ºC. The samples were analyzed in a 10% non-denaturing polyacrylamide gel, and the RNA-protein complexes were detected by using the phosphorImager system from Molecular Dynamics

References:

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