Supplementary Figure 1: Gel mobility shift analysis

A. Gel mobility shift analysis of a set of equilibrium binding reactions including variable concentrations of YTH domain protein and 2.6μM of RNA probe containing one YTH-binding motif. Probe RNA: GCAUGC. RNA probe was uniformly labeled with ^{32}P. The concentration of YTH domain protein ranges from 0.86μM to 12.9μM. After electrophoresis, the gel was dried and scanned on a Typhoon Scanner. Bound and free RNA were quantified in each, using ImageQuant software. The K_d calculated from the plot $\ln([RNA_{bound}]/[RNA_{free}])$ vs $\ln[Protein_{free}]$ is 26μM with a standard deviation of 8.5μM.

Measuring equilibrium binding constant (K_d) using gel retardation assay.

When analyzing RNA:Protein complexes, these complexes coexist with free RNA and protein in the binding reagent. This dynamic balance can be described by the equilibrium equation:

$[RNA:Protein_{bound}] \rightleftharpoons [RNA_{free}] + [Protein_{free}]$

The equilibrium binding constant K_d, which describes the RNA and protein interaction is formalized by the equation: $K_d = [RNA_{free}]\,[Protein_{free}]/[RNA:Protein_{bound}]$, as the concentration of RNA:Protein complexes is equal to the concentration of bound RNA, the equation can be rearranged to $(1/K_d)[Protein_{free}] = [RNA_{bound}]/[RNA_{free}]$. After natural logarithm is applied, the equation became linear: $\ln([RNA_{bound}]/[RNA_{free}])=\ln[Protein_{free}] + \ln(1/K_d)$. By plotting $\ln([RNA_{bound}]/[RNA_{free}])$ vs $\ln[Protein_{free}]$, the K_d can be obtained when the concentration of bound RNA is equal to the concentration of free RNA.

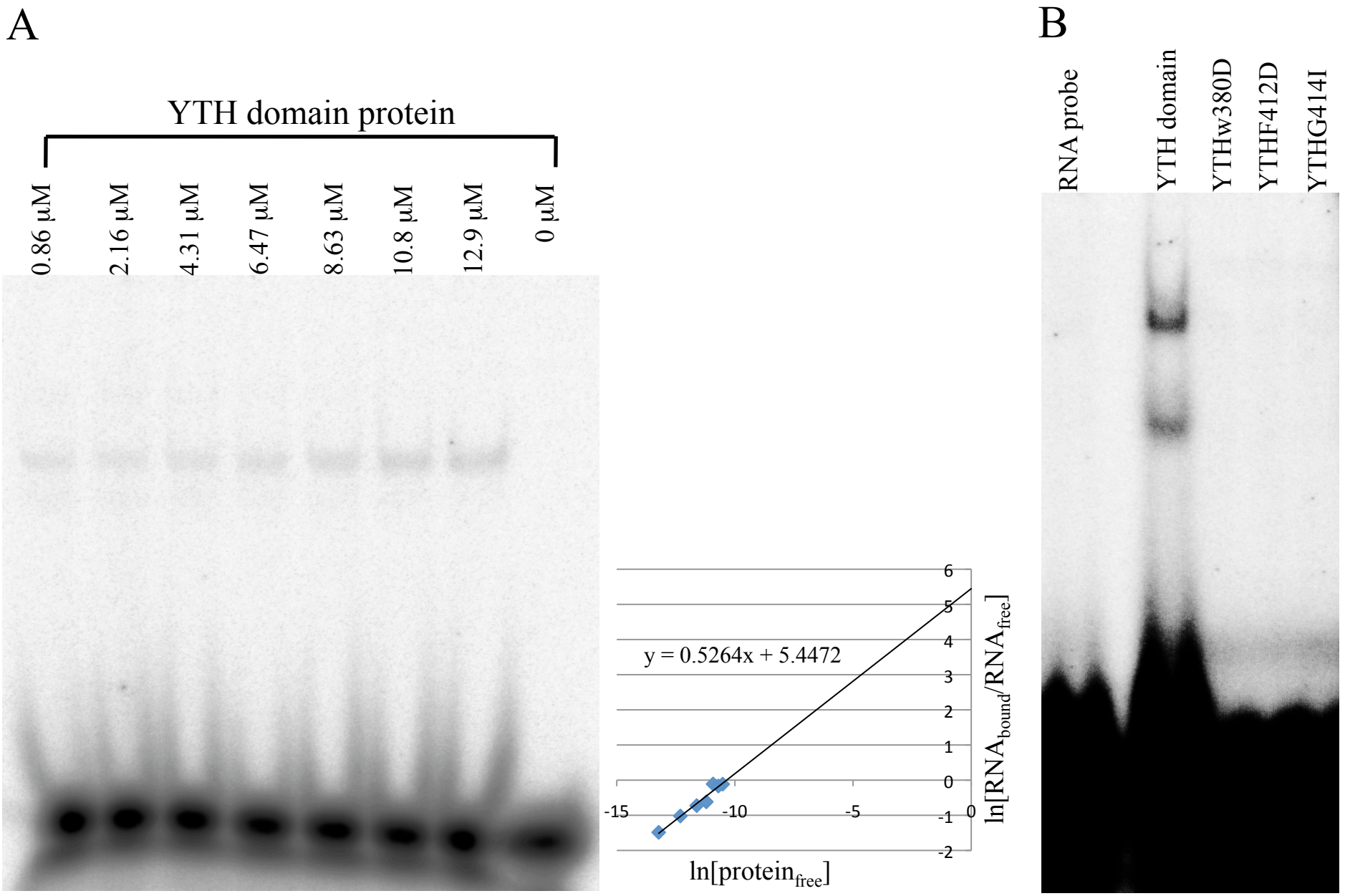
B. Gel mobility shift analysis of YTH domain mutants (W380D, F412D and G414I) using RNA probes containing the YTH-binding motif. 20μM of recombinant, bacteria-expressed YTH domain and YTH domain mutants were incubated with 15μM YTH-binding motif RNA probe. Probe RNA: GCAUGC.

Supplementary figure 2: Comparision between the YTH domain and the PUA/DUF55 domain

A-C. Structural similarities between the YTH, DUF55 and PUA domains. A. YTH domain, (PDB: 2yud), B. DUF55 domain (PDB 2ar1), C. PUA domain PDB: 2ane (N-terminal PUA domain of E. *coli* Lon protease). The secondary structure elements are colored from N-terminus (blue) to C-terminus (red) to emphasize the similarity.

D. Localization of the conserved residues on the YTH domain. Numbers are indicated as in Figure 5A.

Supplemental Fig. 1



Supplemental Fig. 2