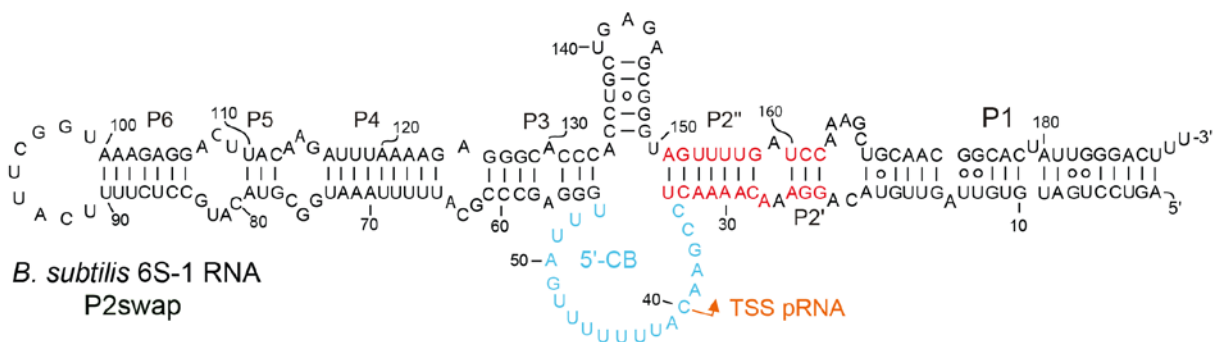
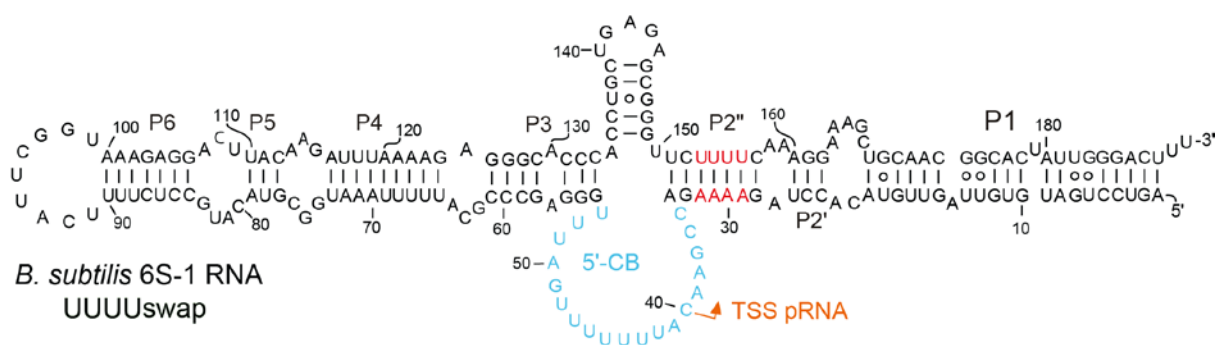
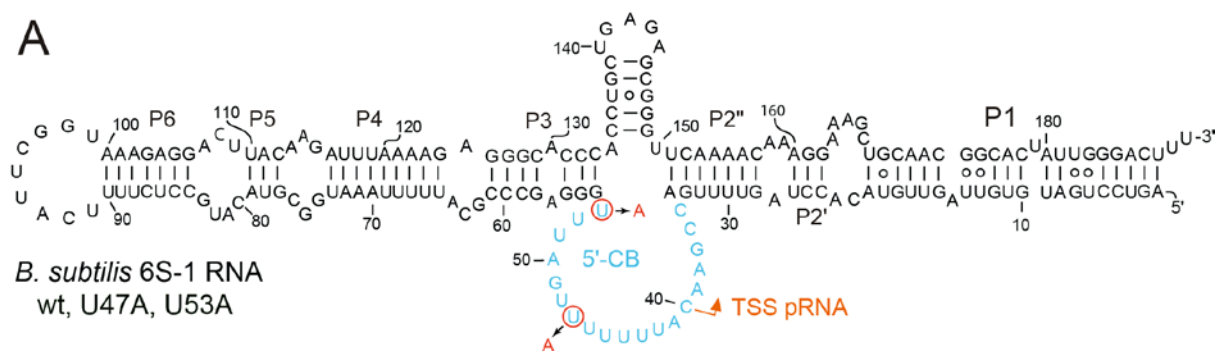


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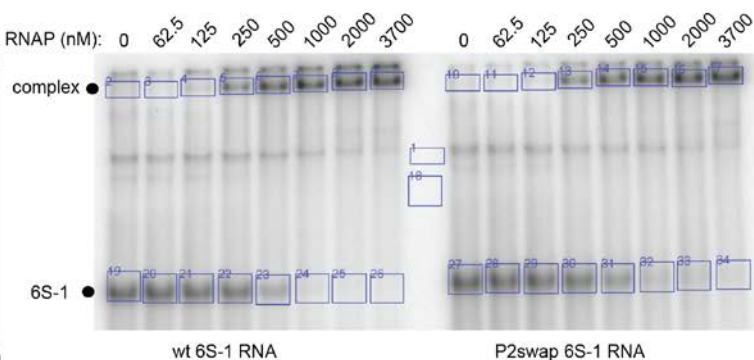
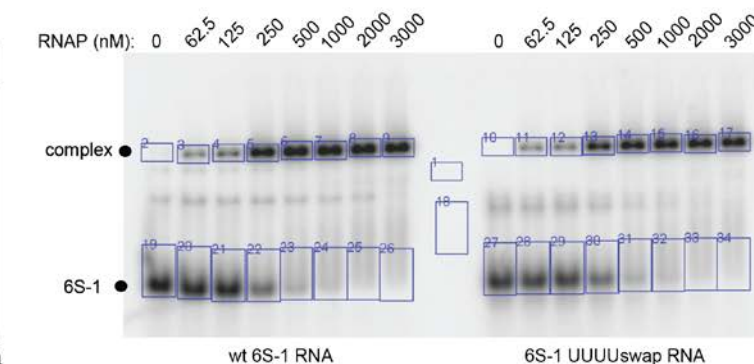
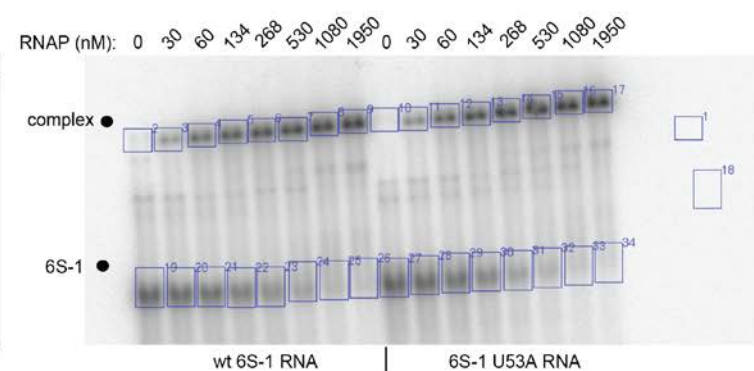
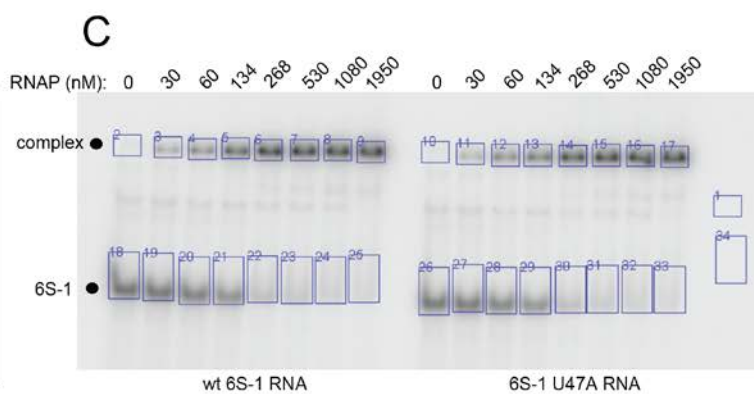
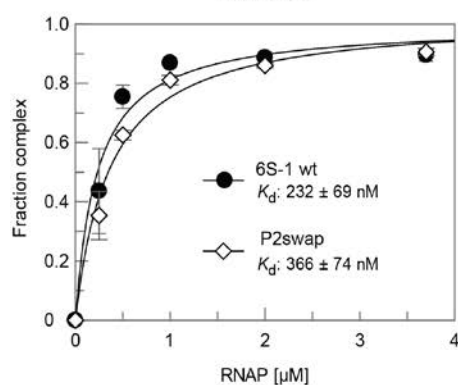
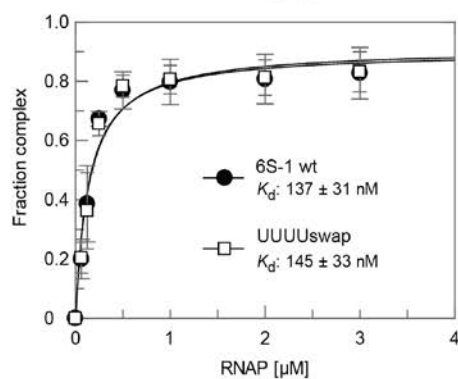
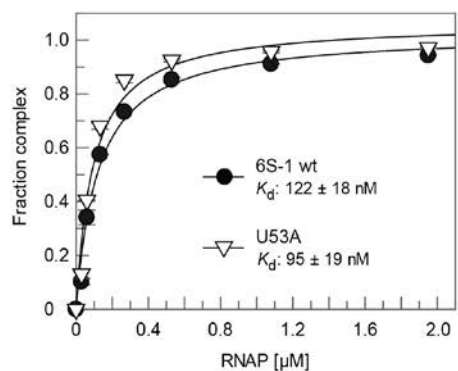
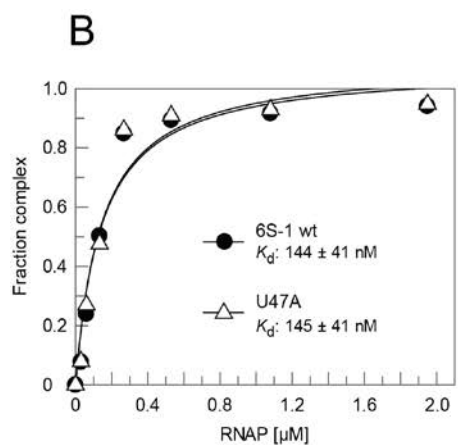


Figure S2: Binding affinity of σ^A -RNAP to 6S-1 RNA wt and other mutant RNAs. (A) Secondary structure presentation of wt 6S-1 RNA and mutants U47A and U53A thereof (top), mutant RNA UUUUswap (middle) and RNA P2swap (bottom), with mutations highlighted in red letters. (B) 6S-1 RNA: σ^A -RNAP binding affinity plotted as the fraction of complex as a function of σ^A -RNAP concentration. The data was fit to an equation for a single ligand binding site using Grafit version 5.0.13; data point errors are standard errors of the mean (SEM) and calculated K_d values are depicted within the graphs. Data for the U47A and U53A mutants are based on two and those for mutants UUUUswap and P2 swap on three individual experiments. (C) Phosphorimages of exemplary gels with quantification boxes used for K_d determination; for the example at the top, box 1 pixels were subtracted as background from boxes 2 to 17, and box 34 was subtracted from boxes 18 to 33; for the wt 6S-1 RNA panel, the calculated value for $(\text{box 2} - \text{box 1}) / [(\text{box 2} - \text{box 1}) + (\text{box 18} - \text{box 34})]$ was defined as 0 complex formation and subtracted from the "Fraction complex" values for the corresponding lanes with boxes 3/19, 4/20, 5/21, 6/22, 7/23, 8/24 and 9/25. For more details, see Materials and Methods of the main text.