SUPPORTING INFORMATION

for

# Biochemical Characterization of Yeast Xrn1

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| [RNA] (μM) | $k_{obs}$ |
|---|---|
| 0.05 | 0.02 ± 0.003 |
| 0.15 | 0.10 ± 0.01 |
| 0.45 | 0.42 ± 0.24 |
| 1.35 | 1.20 ± 0.30 |
| 4.05 | 1.23 ± 0.14 |
| 12.15 | 2.30 ± 0.18 |
| 31.70 | 17.28 ± 0.60 |

**Table S1: $k_{obs}$ of Michaelis-Menten style kinesics of ScXrn1ΔC.** Observed kinetic rate of ScXrn1ΔC obtained using the 5'-monophosphorylated 80HP construct at indicated concentrations. ScXrn1ΔC concentration was kept constant at 0.05 μM. $k_{obs}$ reported as per phosphodiester bond hydrolysis. The relative stoichiometries of ScXrn1ΔC molecules to RNA molecules are as follows: 1:1, 1:3, 1:9, 1:27, 1:81, 1:243, and 1:634. Error reported as 1 standard deviation for n=9 replicates excluding 31.7 μM where n=3.

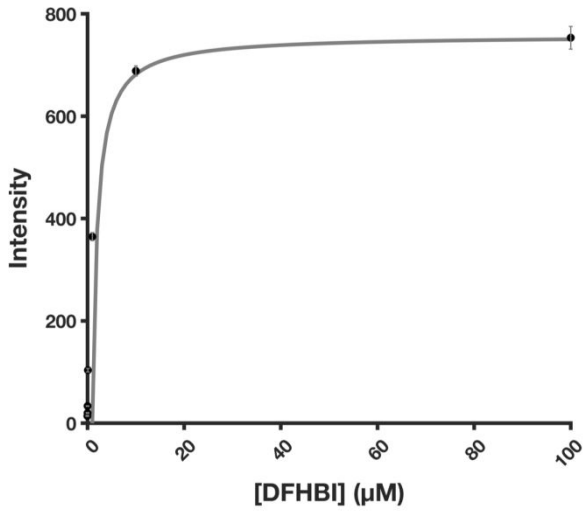


**Figure S1: iSpinach $K_d$.** Binding curve of iSpinach aptamer and DFHBI as a function of fluorescence intensity and DFHBI concentration determined using the 80HP construct. The $K_d$ for the iSpinach aptamer in our system was determined to be 1.01 ± 0.1 μM. Error reported as 1 standard deviation for n=10 replicates.

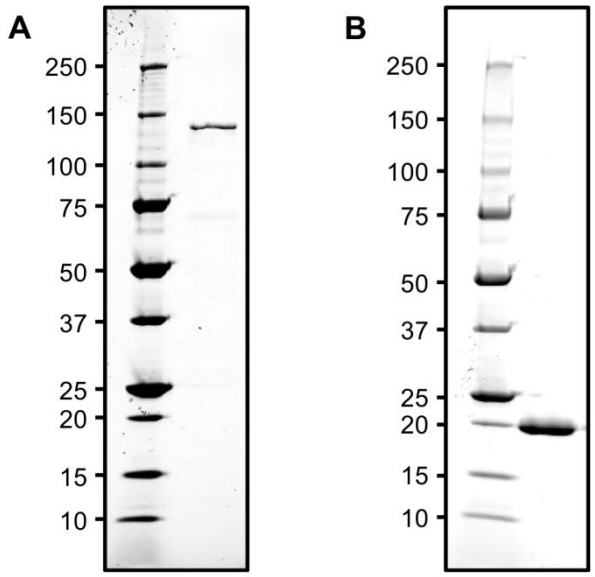


**Figure S2: Purification of ScXrn1ΔC and BdRppH.** A) ScXrn1ΔC present as a single pure band by SDS-PAGE between the 100 and 150 kDa markers corresponding to the expected 145 kDa mass of the protein. B) BdRppH present as a single pure band by SDS-PAGE at the 20 kDa marker corresponding to the expected 19 kDa mass of the protein.

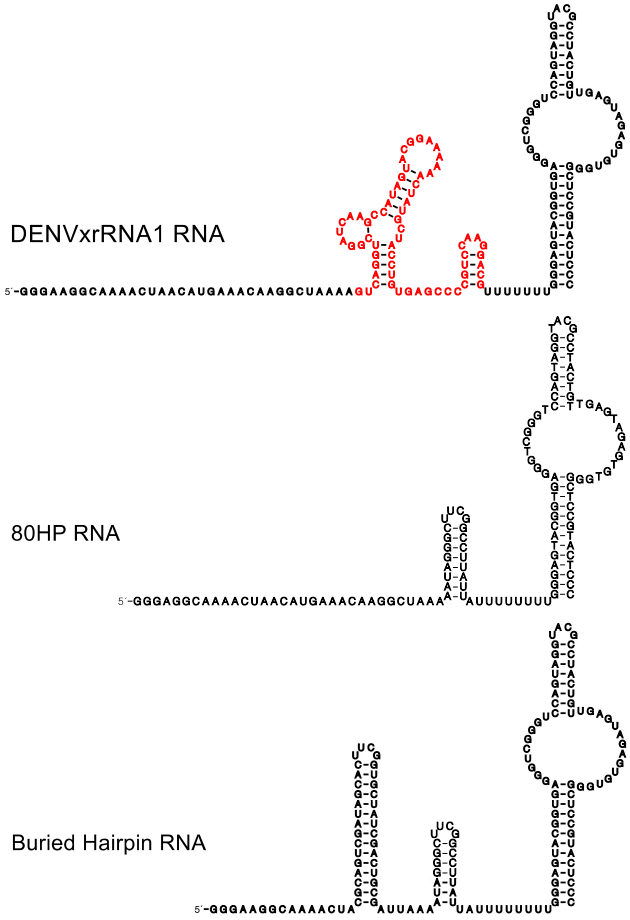


**Figure S3: Secondary Structure of RNA constructs.** Secondary structures of the main RNAs used in this study. Highlighted in red is the Xrn1 resistant structure from the Dengue Virus 3' UTR.

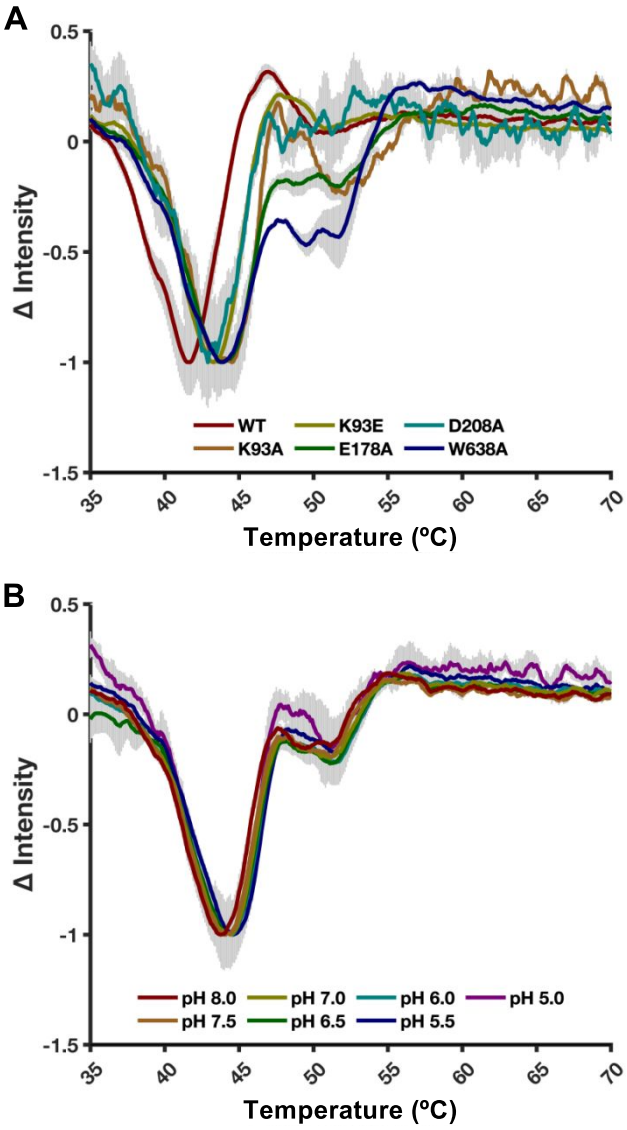


**Figure S4: Mutational and pH-dependent thermal denaturation of ScXrn1ΔC.** A) Thermal denaturation experiments of ScXrn1ΔC and indicated point mutants. B) Thermal denaturation experiments with the wild-type ScXrn1ΔC conducted at varying pH. Error in each experiment is reported as 1 standard deviation for n=3 replicates.