Fragile X Mental Retardation Protein Recognizes a G quadruplex Structure Within the Survival Motor Neuron Domain Containing 1 mRNA 5'-UTR

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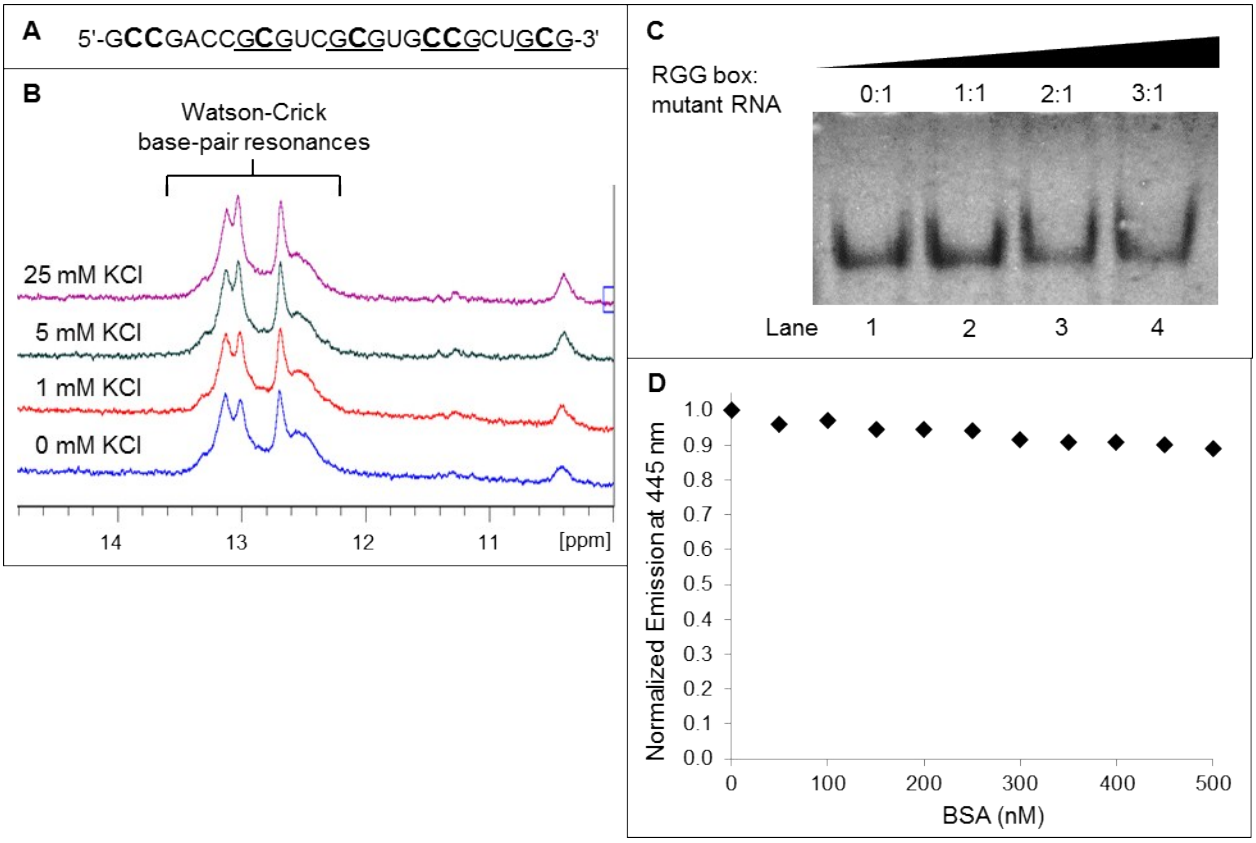
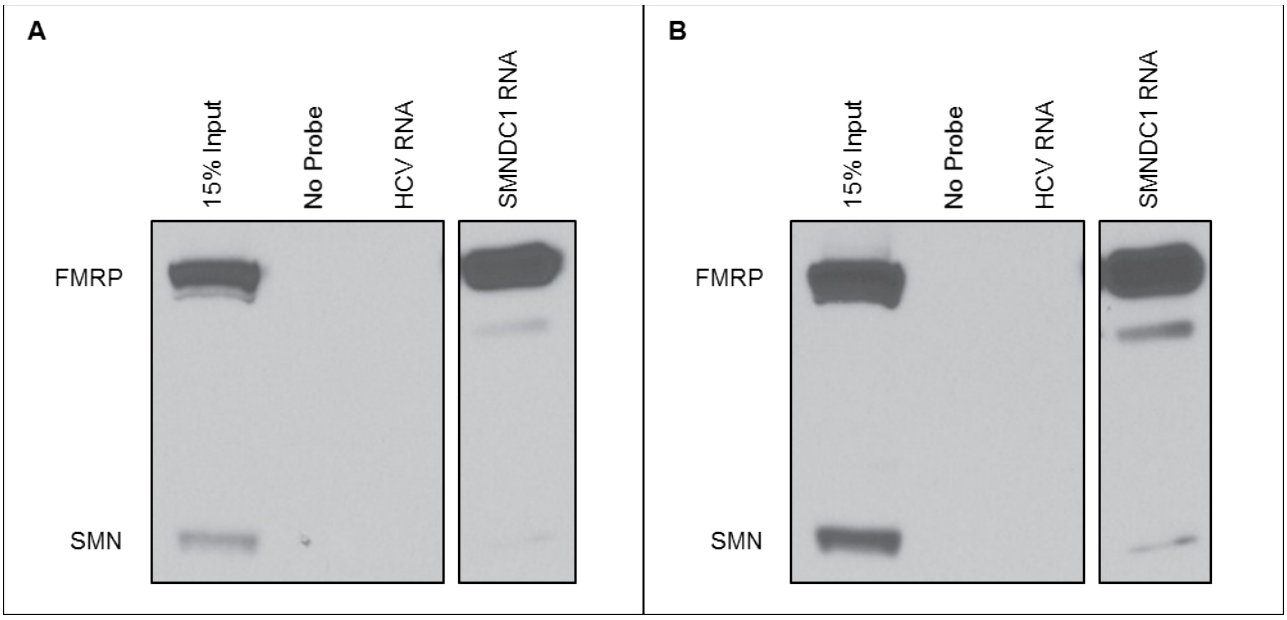
ELECTRONIC SUPPLEMENTAL INFORMATION



Supplemental Figure 1. (A) Sequence of the mutant 25 nt segment from the SMNDC1 mRNA 5'-UTR. Critical G-to-C substitutions are shown in the large, bold font, abolishing G quadruplex structure formation. For illustrative purposes, the underlines from Figure 1A remain to indicate the locations of G tracts predicted to be involved in the G quadruplex structure. **(B)** Characteristic Watson-Crick base-pairing resonances of the 1D ^{1}H NMR spectra of 230 μM mutant SMNDC1

mRNA in 10 mM cacodylic acid, pH 6.5, 40°C, in the presence of increasing KCl concentrations. Absence of characteristic G quadruplex structure imino proton resonances indicates that the mutant version of SMNDC1 RNA does not form the G quadruplex structure. **(C)** EMSA using 20% native PAGE of 10 μM mutant SMNDC1 mRNA in the presence of 5 mM KCl, with ratios of FMRP RGG box peptide:mutant SMNDC1 mRNA from 0:1 (lane 1) to 3:1 (lane 4). Gels were visualized by UV shadowing at 254 nm. The free mRNA band is minimally, if at all, decreased indicating a lack of FMRP RGG box peptide binding mutant SMNDC1 RNA. The mobility of the mutant SMNDC1 RNA in the 3:1 ratio lane may be minimally shifted, likely due to non-specific electrostatic interactions between the highly positively charged RGG box peptide with the negative RNA backbone. This mobility shift is obviously minimal compared to RGG box peptide binding the wild-type SMNDC1 RNA at the 1:1, 2:1, and 3:1 ratios (Figure 3A), which demonstrates that the RGG box peptide in the presence of the G quadruplex structure utilizes a specific binding mechanism. This shift for the mutant SMDNC1 RNA at the 3:1 ratio lane could also be due to simply not migrating perfectly consistent through the gel. **(D)** Fluorescence spectroscopy curve of SMNDC1_12PC RNA while titrating binding negative control BSA in the presence of 150 mM KCl, 10 mM cacodylic acid, pH 6.5, at 25°C. Results indicate specific binding of SMNDC1 RNA by FMRP ISO1 and the RGG box peptide. The lack of PC reporter quenching upon titrating BSA indicates the quenching from separately titrating FMRP ISO1 and FMRP RGG box peptide (Figures 3B,C) are not due to non-specific protein aggregation and are instead a result of specific binding.



Supplemental Figure 2. Binding of 5' biotinylated SMNDC1 mRNA by FMRP after incubation with E17 mouse brain lysates. RNA probes were precipitated and potential co-purified FMRP and SMN proteins were assessed by immunoblot at **(A)** the medium exposure and **(B)** the longest exposure. Precipitated SMNDC1 mRNA was simultaneously exposed to immunoblot by FMRP and SMN with all other samples. However, the gel contained mRNA targets unrelated to this study, thus the SMNDC1 samples are shown in a separate window.