Supplementary Material

Title: Contributions of the N-terminal intrinsically disordered region of the SARS-CoV-2 nucleocapsid protein to RNA-associated phase separation

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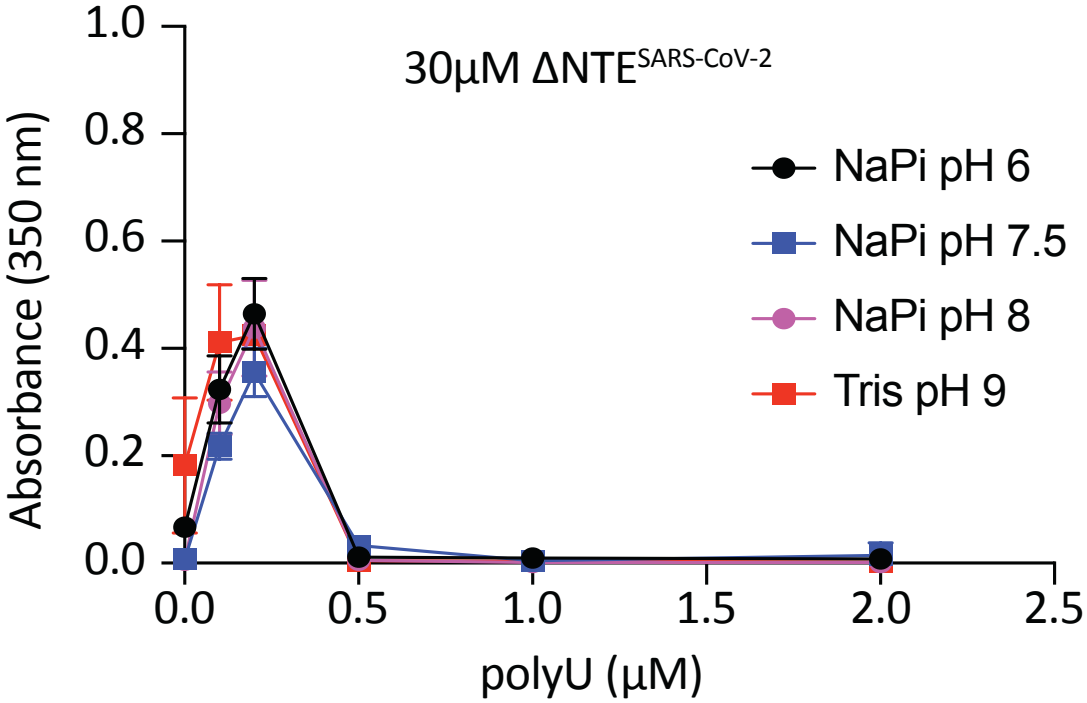

Figure S1. Absorbance at 350 nm of 30 µM ΔNTE$^{SARS\text{-}CoV\text{-}2}$ without or with 0.1, 0.2, 0.5, 1, 2 µM polyU RNA in 20 mM sodium phosphate buffer at the pH values 6 (black), 7.5 (blue), and 8 (pink) and in 20 mM Tris buffer at pH 9 (red).

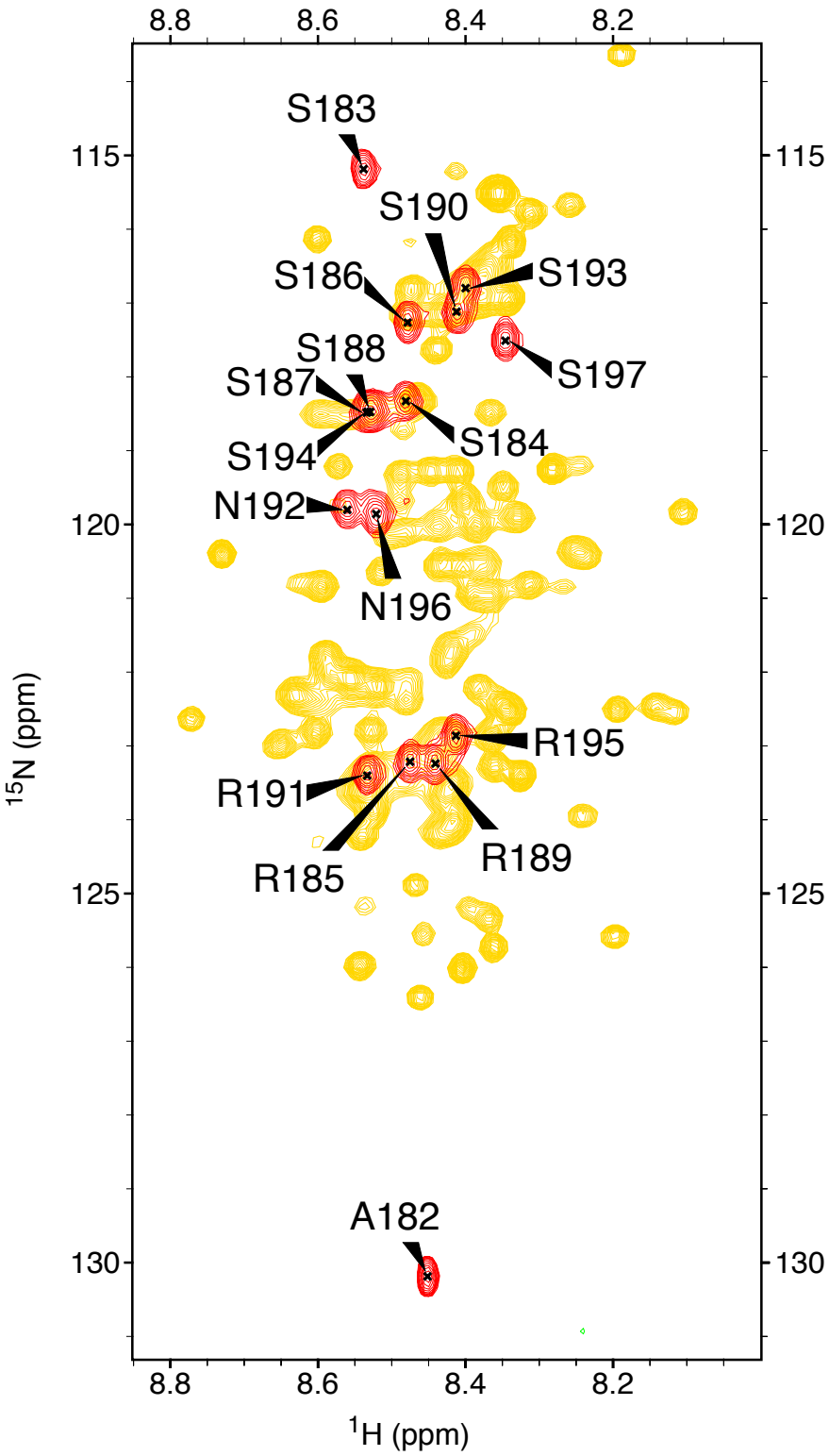

Figure S2. Superposition of ^{1}H-^{15}N HSQC spectra of full-length $N^{SARS\text{-}CoV\text{-}2}$ in yellow and the SR region of $N^{SARS\text{-}CoV\text{-}2}$ in red measured at 5 °C with amino-acid assignments.

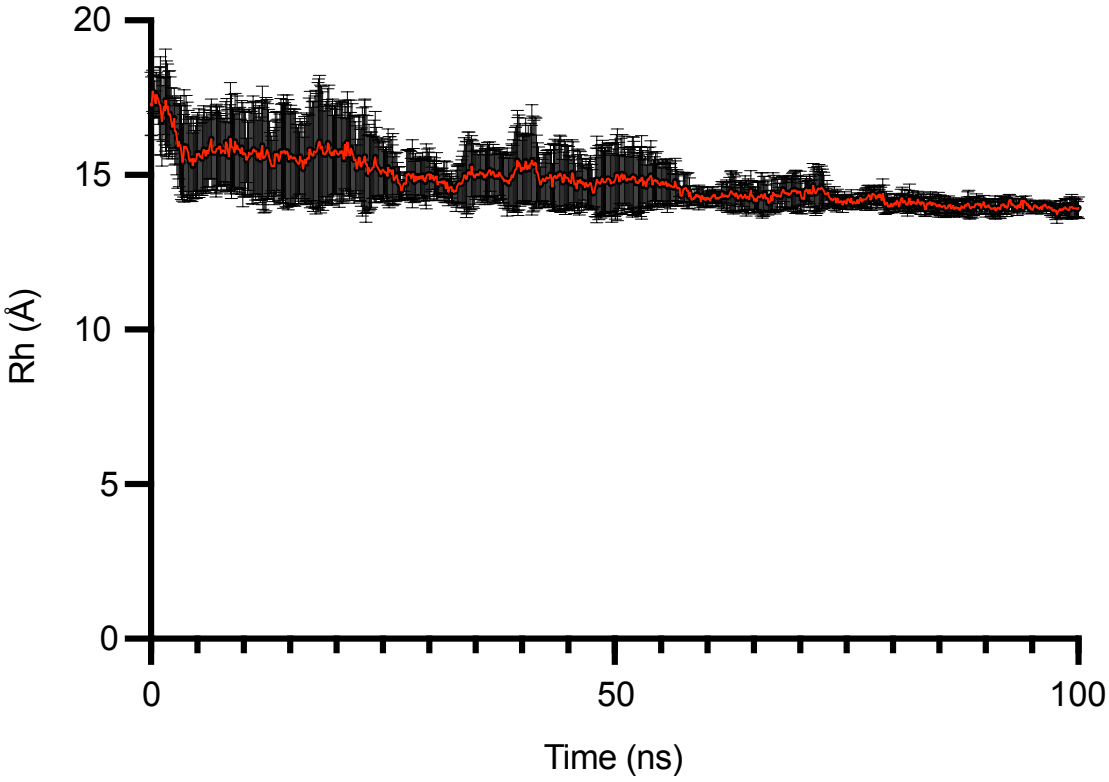

Figure S3. Hydrodynamic radius (Rh) of the NTE measured by 100 ms long molecular dynamics simulations for residues 1-50. An average value of Rh from 5 independent simulations and the standard deviation is represented by red line and black error bars, respectively.

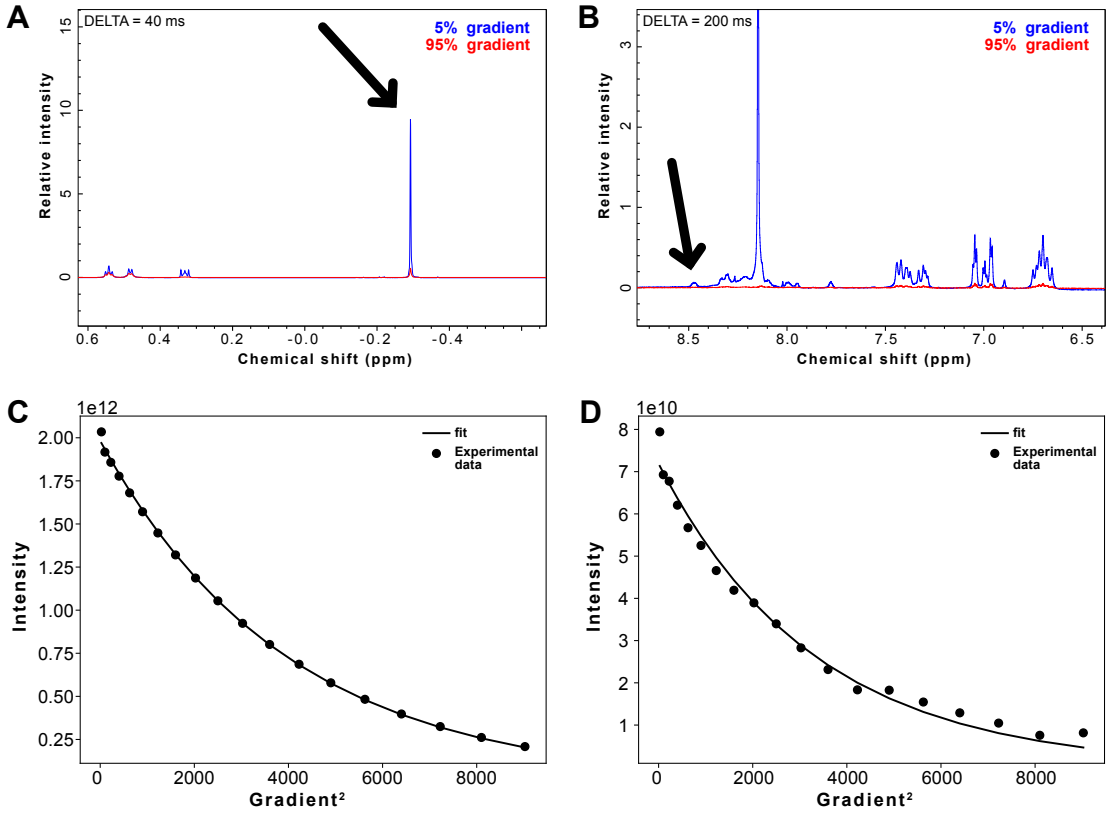

Figure S4. NMR diffusion of the NTE using pulse-field-gradient experiments. A-B Overlay of 1D experiments measured with 5 % (blue) and 95 % (red) gradient strength with diffusion delays optimized for DSS (40 ms) and the NTE (200 ms). Black arrows indicate peaks that were used for analysis. C-D Fit of intensities for DSS signal (C) and a selected peak from amide region of the NTE (D).

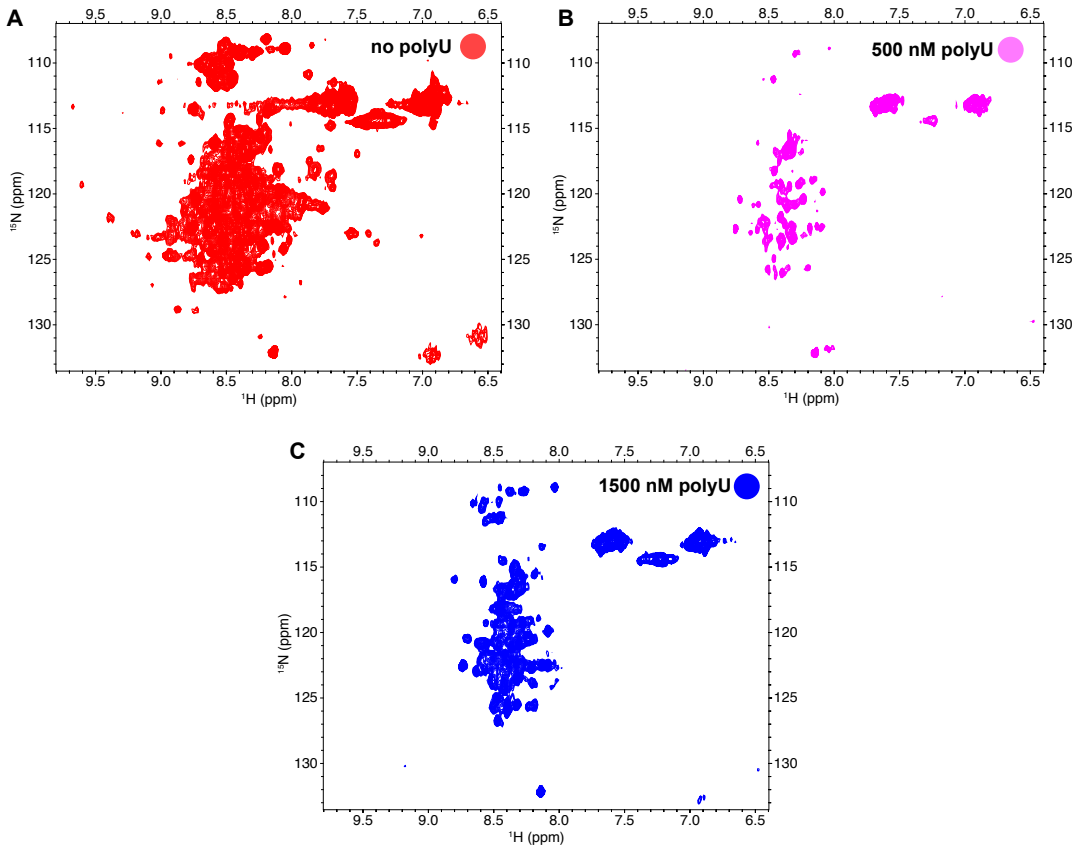

Figure S5. PolyU titration of $N^{SARS\text{-}CoV\text{-}2}$. ^{1}H-^{15}N HSQC spectra of the full-length $N^{SARS\text{-}CoV\text{-}2}$ with 0 nM (A), 500 nM (B), and 1500 nM (C) concentration of polyU. All spectra were measured at 5 °C.

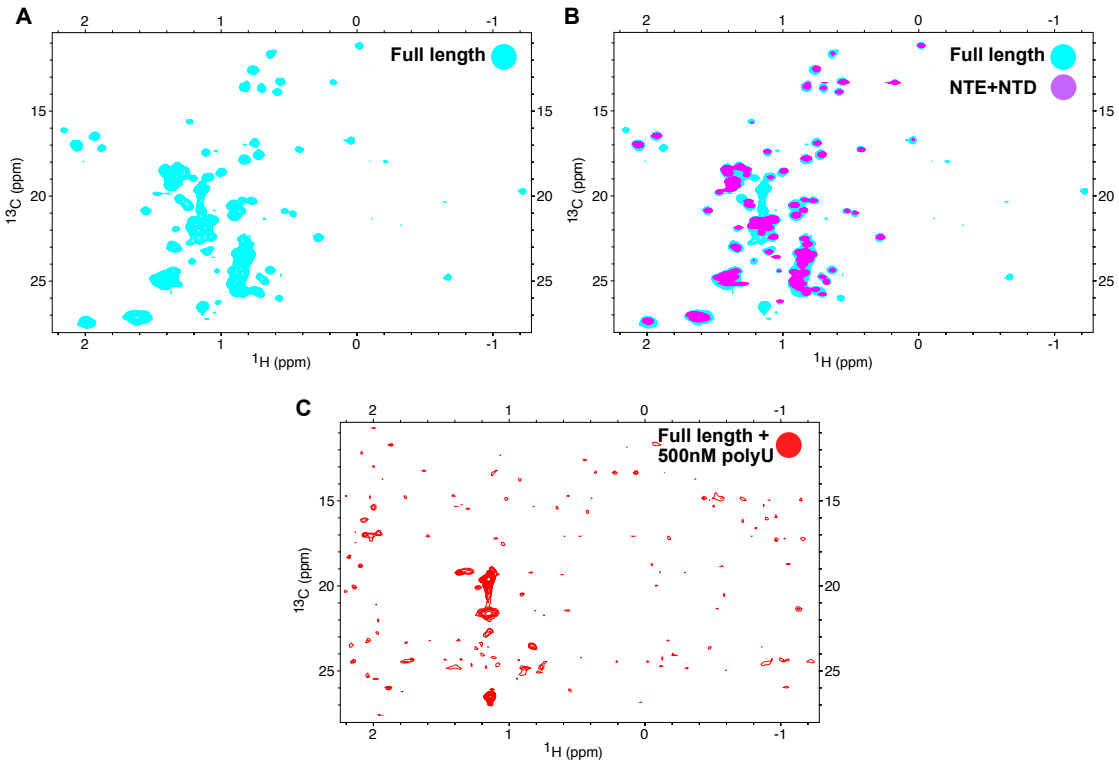

Figure S6. ^{13}C natural abundance NMR XL-ALSOFAST [1] spectra of methyl region. A) Full-length $N^{SARS\text{-}CoV\text{-}2}$. B) Overlay of full-length $N^{SARS\text{-}CoV\text{-}2}$ with a construct missing the dimerization domain and the C-terminal extension. C) Spectrum of full-length $N^{SARS\text{-}CoV\text{-}2}$ upon addition of 500nM polyU. All spectra were measured at 25 °C on 800 MHz spectrometer.

1. Rößler P, Mathieu D, Gossert AD (2020) Enabling NMR Studies of High Molecular Weight Systems Without the Need for Deuteration: The XL-ALSOFAST Experiment with Delayed Decoupling. Angewandte Chemie International Edition 59:19329–19337.

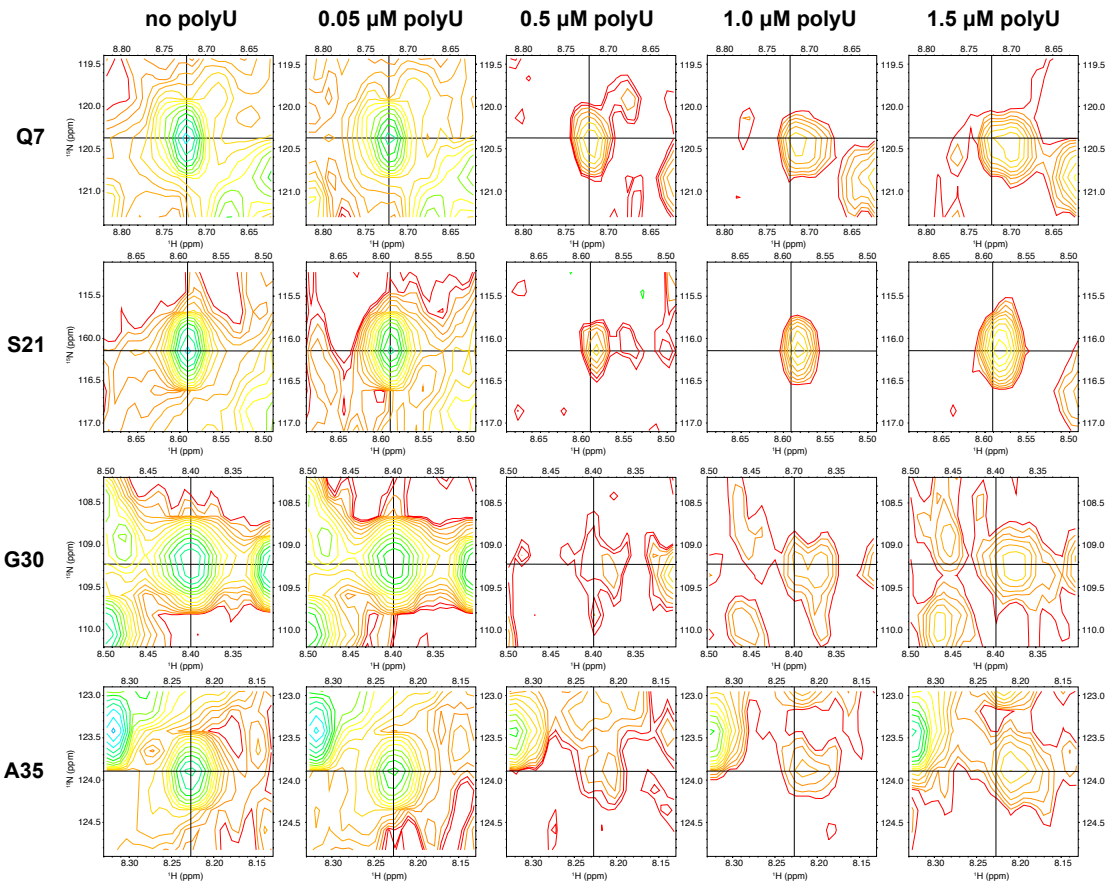

Figure S7. Titration of $N^{SARS\text{-}CoV\text{-}2}$ with polyU. ^{1}H-^{15}N HSQC spectra of Q7, S21, G30, and A35 in the presence of 0, 0.05, 0.5, 1.0 and 1.5 µM polyU RNA. Spectra were measured at 5 °C at 700MHz.

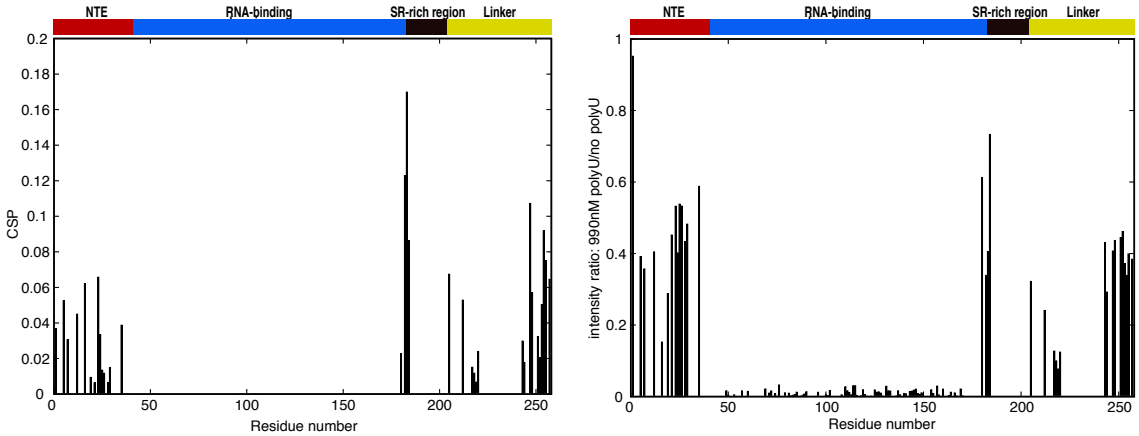

Figure S8. Titration of N(1-257) with polyU measured at 25 °C at 700 MHz. Chemical shift perturbation between protein without polyU and 990 nM polyU concentration (left). PolyU concentration was adjusted to compensate for protein charge change resulting from deletion of the C-terminal part. Intensity ratio between the spectra with 990 nM polyU and without polyU (right). RNA-binding domain signals are indicated with small bars estimated from spectral noise due to the fact that the signals were broadened beyond detection upon addition of polyU.