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## Supplemental information

# Characterization of SARS-CoV-2 nucleocapsid protein reveals multiple functional consequences of the C-terminal domain

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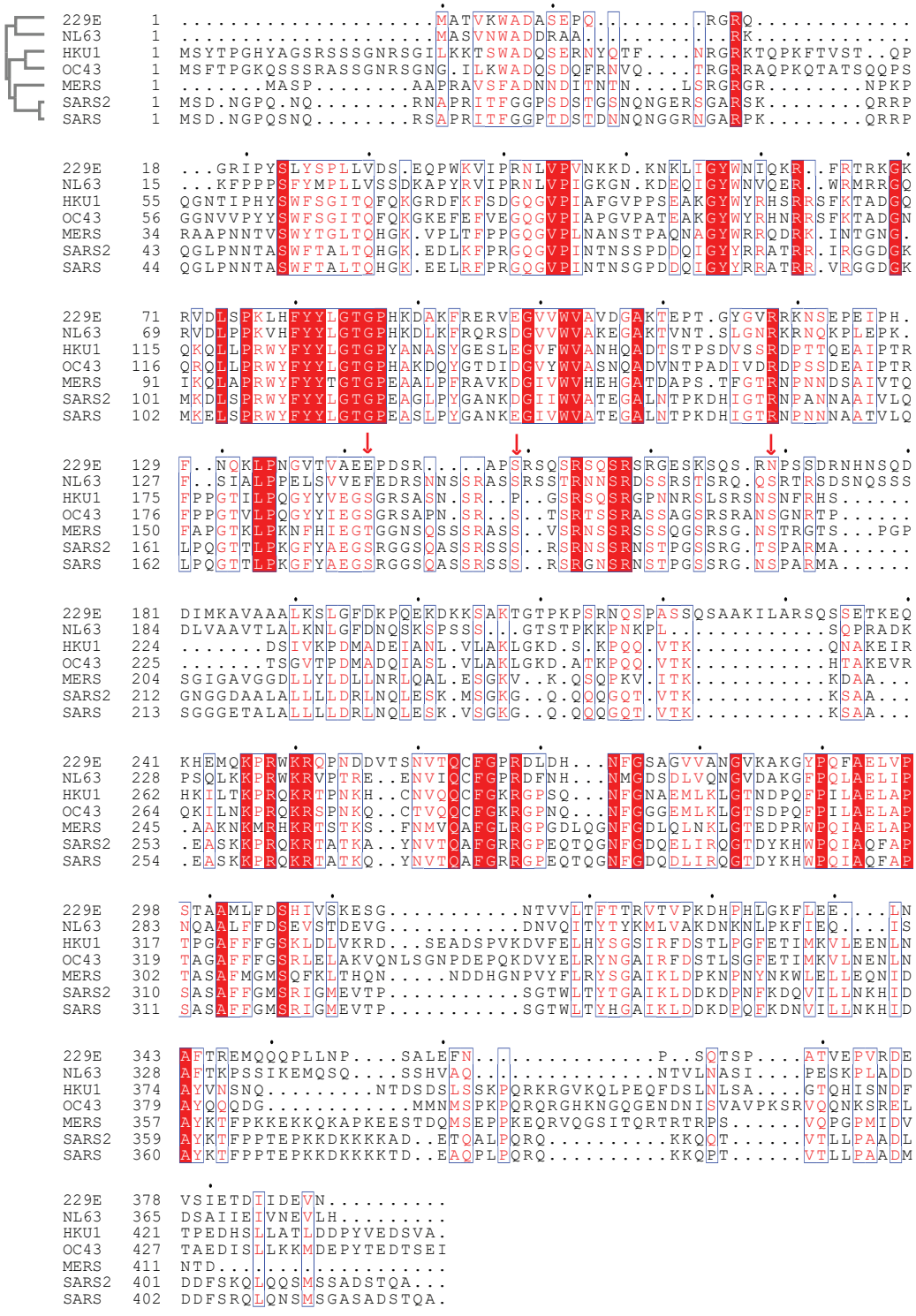
A



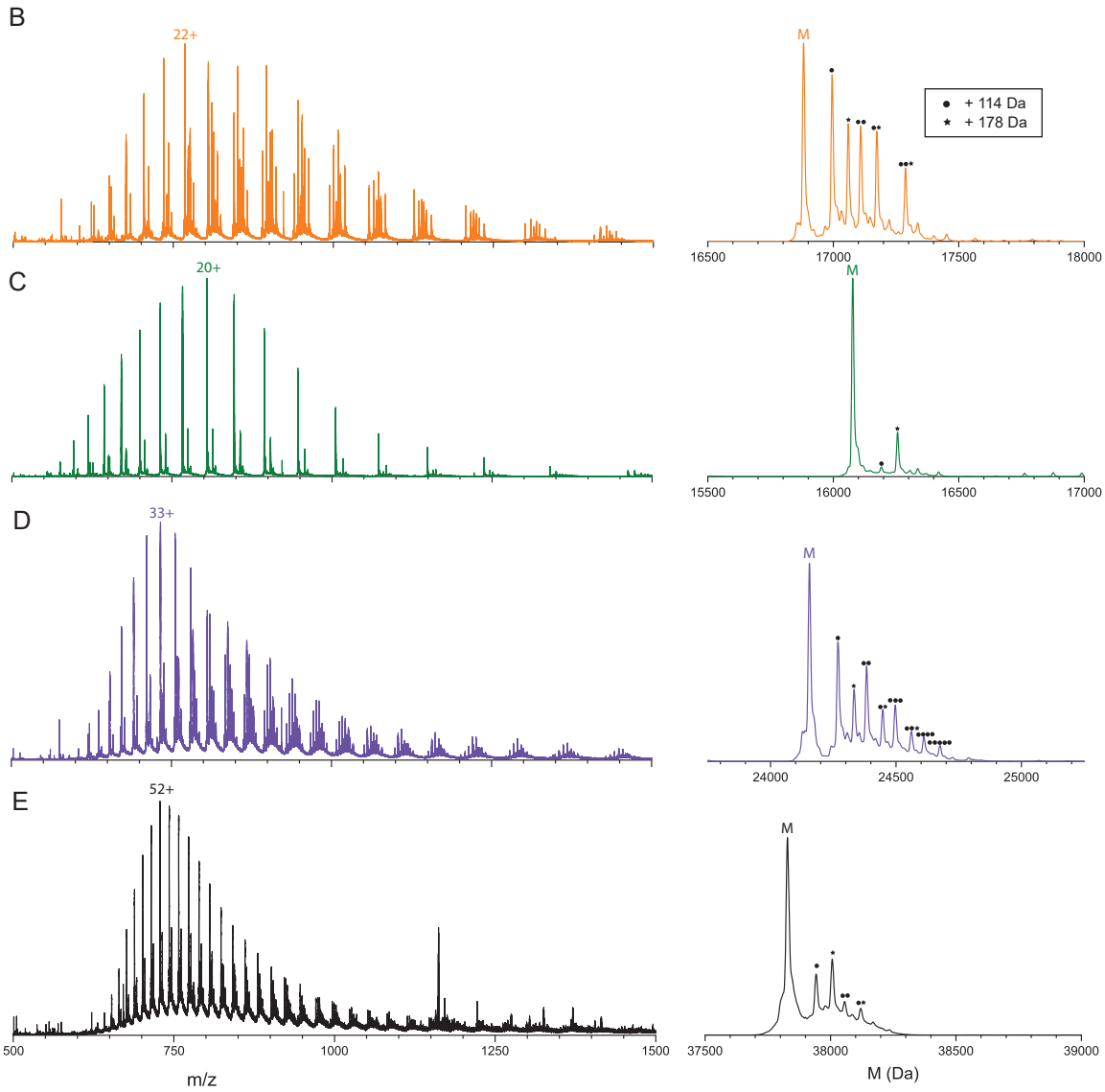

B

| | 229E | NL63 | HK1 | OC43 | MERS | SARS |
|---|---|---|---|---|---|---|
| FL | 26 | 28 | 34 | 36 | 49 | 90 |
| Narm | 12 | 0 | 17 | 29 | 22 | 84 |
| NTD | 32 | 30 | 44 | 43 | 60 | 92 |
| LKR | 26 | 36 | 31 | 34 | 46 | 89 |
| CTD | 30 | 28 | 36 | 37 | 54 | 96 |
| Carm | 10 | 21 | 15 | 18 | 14 | 74 |

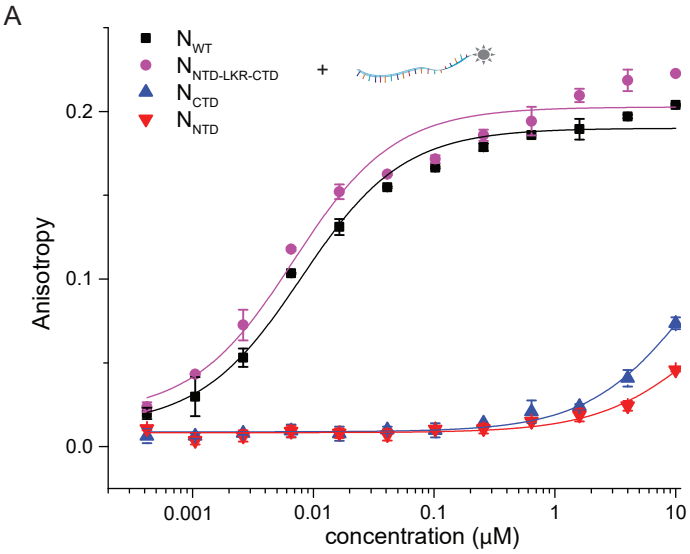
**Supplementary Figure 1, related to Figure 1. Multiple sequence alignment of coronavirus nucleocapsids. A.** Multiple sequence alignment of coronavirus nucleocapsids. Sequences were aligned using Clustal Omega. Accession numbers used are 229E (APT69891.1), NL63 (YP_003771.1), HK1 (AAT98585.1), OC43 (AAR01019.1), MERS (AKL80590.1), SARS (AAP30037.1), SARS2 (YP_009724397.2). Alignments were analyzed using ESPript3. The three serines (176, 188, and 206) are labeled with red arrows. **B**. Sequence identity between SARS-CoV-2 N and that of common cold coronaviruses and MERS and SARS. FL, full length. All units are in %. Percent identity matrixes for corresponding domains of N are generated using Clustal2.1.

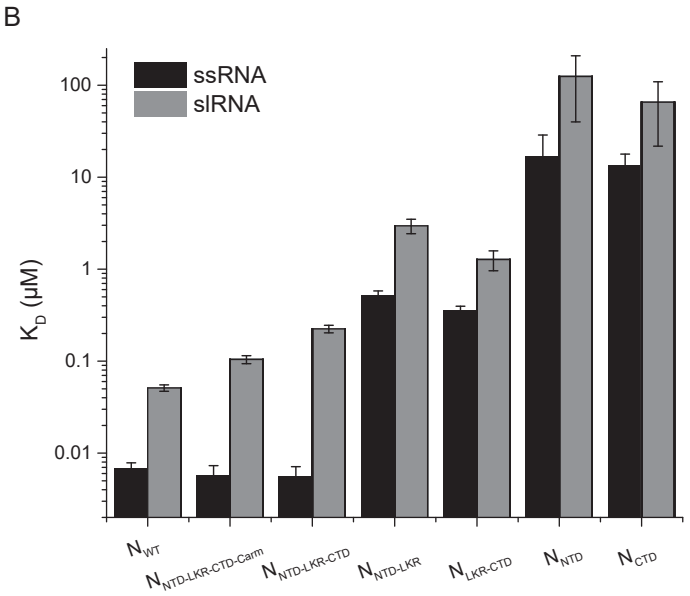
A

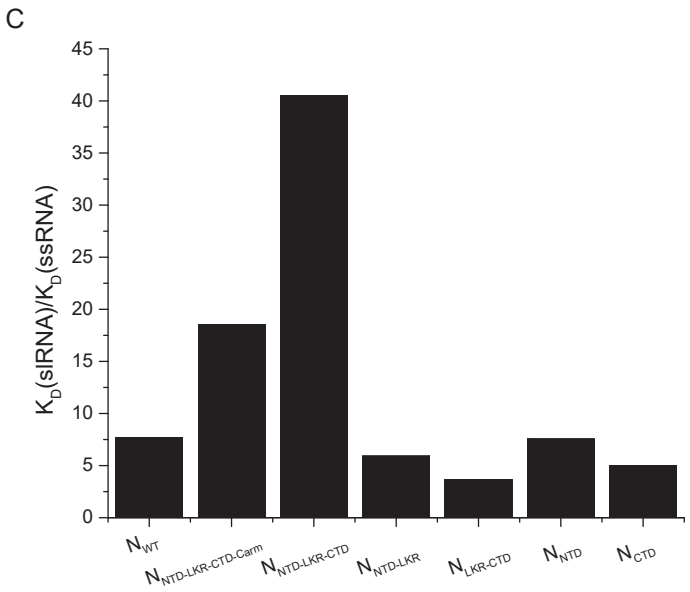
| | MW (kDa) | polydispersity (%) | |
|---|---|---|---|
| | | peak 1 | peak 2 |
| $N_{WT}$ | 46 | 20 ± 1 | 40 ± 20 |
| $N_{NTD-LKR-CTD}$ | 35 | 17 ± 7 | 30 ± 20 |
| $N_{NTD-LKR}$ | 22 | 23 ± 3 | |
| $N_{NTD}$ | 14 | 7 ± 2 | |
| $N_{CTD}$ | 14 | 15 ± 1 | |



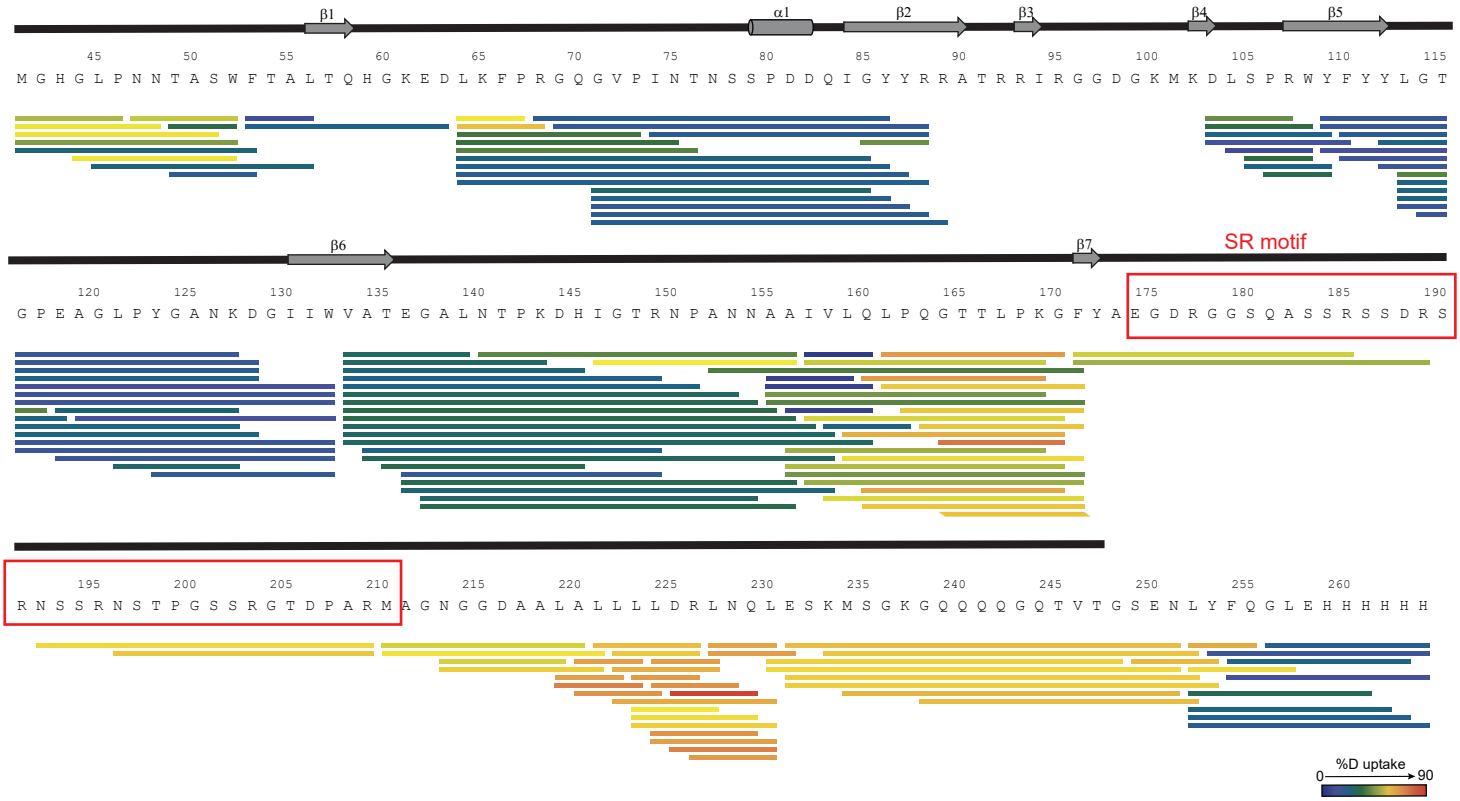
**Supplementary Figure 2, related to Figure 1. Denaturing mass spectra of N protein truncations $N_{NTD}$, $N_{CTD}$, $N_{NTD-LKR}$, and $N_{NTD-LKR-CTD}$. A.** DLS polydispersity table for N constructs. Higher values indicate broader size distributions. Numbers are reported as average and standard deviation of three experiments. Deconvolution yields experimental M values of 16,881 Da, 16,078 Da, 24,155 Da, and 37,829 Da for (**B**) $N_{NTD}$, (**C**) $N_{CTD}$, (**D**) $N_{NTD-LKR}$, and (**E**) $N_{NTD-LKR-CTD}$ respectively, matching theoretical values within 1 Da, based on protein sequence. Deconvoluted mass spectra (right) and adduct series corresponding to pervasive trifluoroacetic adducts (delta mass 114 Da, circle) and a-N-gluconoylation (delta mass 178 Da, star). TFA adducts are introduced by the ion pairing reagent in solvent, while α-N-gluconoylation is a common modification occurring on His-tagged proteins. Native spray of NTD (not pictured) yielded no peaks with delta mass 114 Da, but retained a single delta mass 178 Da, confirming transient TFA adducts are an artifact of the denaturing experiment, but the α-N-gluconoylation of the His-tag is covalent.


A
N_WT
N_NTD-LKR-CTD
N_CTD
N_NTD
+
Anisotropy
0.0
0.1
0.2
0.001
0.01
0.1
1
10
concentration (μM)

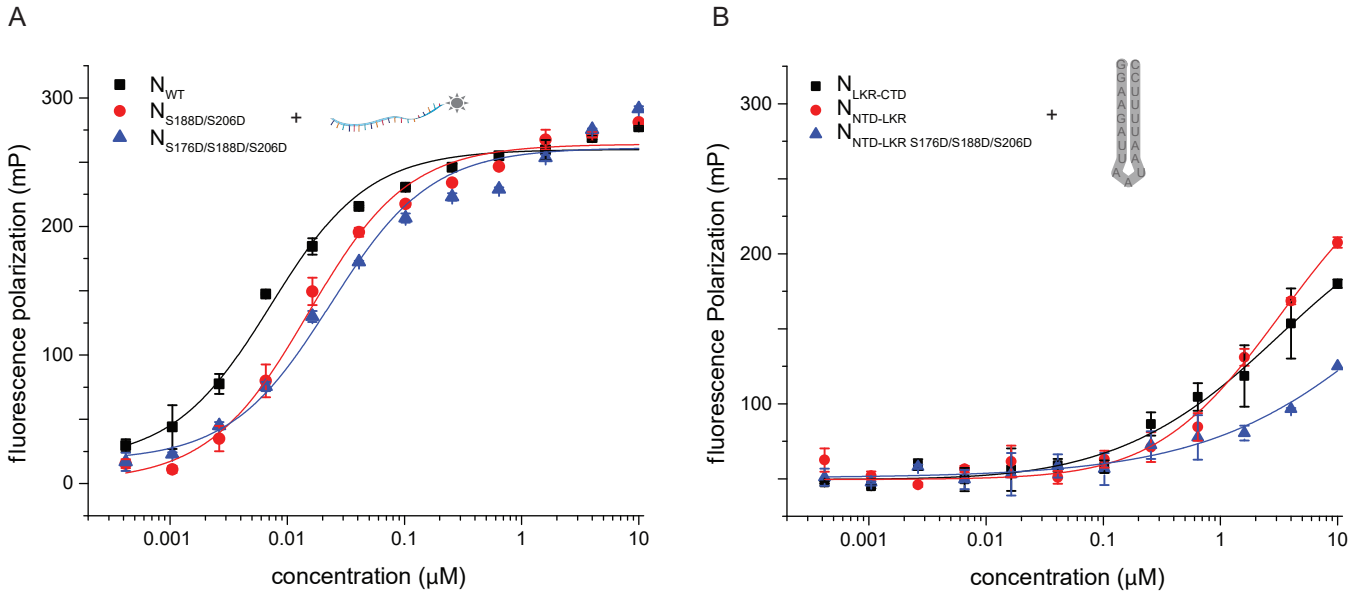

B
ssRNA
slRNA
K_D (μM)
100
10
1
0.1
0.01
N_WT
N_NTD-LKR-CTD-Carm
N_NTD-LKR-CTD
N_NTD-LKR
N_LKR-CTD
N_NTD
N_CTD


C
K_D(slRNA)/K_D(ssRNA)
0
5
10
15
20
25
30
35
40
45
N_WT
N_NTD-LKR-CTD-Carm
N_NTD-LKR-CTD
N_NTD-LKR
N_LKR-CTD
N_NTD
N_CTD

**Supplementary Figure 3, related to Figure 2. Nucleocapsid binds stem-loop RNA with reduced affinity. A.** Fluorescence anisotropy binding curves of N constructs to a 20-nt ssRNA. Anisotropy values were converted from polarization according to previous research (Kozlov et al., 2012). The fitted $K_D$ values are 0.007 ± 0.001 μM ($N_{WT}$, black square), 0.006 ± 0.002 μM ($N_{NTD\text{-}LKR\text{-}CTD}$, magenta circle), 14 ± 5 μM ($N_{CTD}$, blue up triangle) and 18 ± 14 μM ($N_{NTD}$, red down triangle). These values are very close to those of polarization. In this system, binding monitored by anisotropy is similar to that of polarization. **B.** Fitted $K_D$ values for N constructs binding to ssRNA (black) and slRNA (grey). **C.** Ratio of $K_D$ of slRNA over that of ssRNA for N constructs. The reduced binding to slRNA is around 5-fold for most N constructs. The reduction is higher for those of $N_{NTD\text{-}LKR\text{-}CTD\text{-}Carm}$ and $N_{NTD\text{-}LKR\text{-}CTD}$, suggesting Narm and Carm are more involved in slRNA binding.


β1
α1
β2
β3
β4
β5
β6
β7
SR motif
41 M G H G L P N N T A S W F T A L T Q H G K E D L K F P R G Q G V P I N T N S S P D D Q I G Y Y R R A T R R I R G G D G K M K D L S P R W Y F Y Y L G T
116 G P E A G L P Y G A N K D G I I W V A T E G A L N T P K D H I G T R N P A N N A A I V L Q L P Q G T T L P K G F Y A E G D R G G S Q A S S R S S D R S
191 R N S S R N S T P G S S R G T D P A R M A G N G G D A A L A L L L L D R L N Q L E S K M S G K G Q Q Q Q G Q T V T G S E N L Y F Q G L E H H H H H H
%D uptake
0
90

**Supplementary Figure 4, related to Figure 3. Sequence coverage of $N_{NTD\text{-}LKR\ S176D/S188D/S206D}$ in HDX-MS and HDX of the unbound state.** Protein coverage map of unbound state $N_{NTD\text{-}LKR\ S176D/S188D/S206D}$ HDX yielding 152 peptides with 93.3% sequence coverage. Peptide bars are colored according to their average %HDX relative to the color bar, where cooler colors depict low average %HDX and warmer colors depict high average %HDX. The secondary structure reported by PDB 6M3M is shown above the sequence. Overall, the HDX of the unbound state is largely consistent with the reported secondary structure and a well-ordered tertiary structure; regions outside of the reported structure undergo relatively rapid HDX, consistent with a lack of backbone hydrogen bonding. Interestingly, despite a lack of reported secondary structure in the region of 155-160, relatively low HDX was observed, consistent with either hydrogen bonding of secondary/tertiary structure or a hydrophobic pocket. SR-motif in LKR are boxed in red.


A
B
N WT
N S188D/S206D
N S176D/S188D/S206D
fluorescence polarization (mP)
concentration (μM)
N LKR-CTD
N NTD-LKR
N NTD-LKR S176D/S188D/S206D
fluorescence Polarization (mP)
concentration (μM)

**Supplementary Figure 5, related to Figure 4. Phosphomimics of N reduce RNA binding. A.** Fluorescence polarization binding curves of N constructs to a 20-nt ssRNA. The fitted $K_D$ values are 0.007 ± 0.001 μM for $N_{WT}$, 0.015 ± 0.002 μM for $N_{S188D/S206D}$, and 0.023 ± 0.006 μM for $N_{S176D/S188D/S188D}$. **B.** Fluorescence polarization binding curves of N constructs to a 19-nt slRNA. The fitted $K_D$ values are 1.3 ± 0.3 μM ($N_{LKR-CTD}$, black square), 3.0 ± 0.5 μM ($N_{NTD-LKR}$, red circle), and 2.9 ± 1.4 μM ($N_{NTD-LKR\ S176D/S188D/S206D}$, blue up triangle).