Supplemental Information for

Phosphorylation Toggles the SARS-CoV-2 Nucleocapsid Protein Between Two Membrane-Associated Condensate States

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Supporting Table Supplemental Figures S1 to S13 Supplemental Methods Bibliography

Supporting Table 1

Name Sequence SARS-COV-2 ATGCATCACCACCATCATCATGAGAATTTATATTTCCAGGGCAGCGATAACC Nucleocapsid GCCCGCAGAATCAGCGCAACGCGCCGCGCGCATTACCTTTGGCGGCCCCGAC (N) protein CGATAGCACCGGCAGCAACCAAAACGGAGAGCGCAGCGC
SARS-COV-2 ATGCATCACCACCATCATCATGAGAATTTATATTTCCAGGGCAGCGATAACC Nucleocapsid GCCCGCAGAATCAGCGCAACGCGCGCGCGCGCATTACCTTTGGCGGCCCGAA (N) protein CGATAGCACCGGCAGCAGCAACCAAAACGGAGAGCGCAGCGGCG
Nucleocapsid GCCCGCAGAATCAGCGCGCACGCGCGCGCATTACCTTTGGCGGCCCGAG (N) protein CGATAGCACCGGCAGCAACCAAAACGGAGAGCGCGCGCGC
(N) protein CGATAGCACCGGCAGCAACCAAAACGGAGAGCGCAGCGC
CAAACAACGCCGCCCCCAAGGCCTGCCGAACAACACCGCGAGCTGGTT ACCGCGCTGACGCAGCATGGCAAAGAAGATCTGAAATTTCCGCGCGGCC
AAGGCGTGCCGATTAACACCAACAGCAGCCCGGATGATCAGATTGGCTAT
TATCGCCGCGCGACCCGCCGCATTCGCGGCGGCGATGGCAAAATGAAAG
ATCTGAGCCCGCGCTGGTATTTTTATTATCTGGGCACCGGCCCGGAAGCG
GGCCTGCCGTATGGCGCGAACAAAGATGGCATTATTTGGGTGGCGACCG
AAGGCGCGCTGAACACCCCGAAAGATCATATTGGCACCCGCAACCCGGC
GAACAACGCGGCGATTGTGCTGCAGCTGCCGCAAGGCACCACCCTGCC
GAAAGGCTTCTATGCCGAAGGTTCGCGCGGAGGGAGCCAAGCGTCATCA
GACCGAACCGAAAAAAGAIAAAAAAAAAAAAGCGGAIGAAACCCAAGCGC
TGCCGCAGCGTCAGAAAAACAGCAGACCGTTACACTGCTGCCGGCGGC
GGATCTGGATGATTTTAGCAAACAGCTGCAGCAGAGCATGAGCAGCGCG
GATAGCACCCAAGCG
SL4 RNA FAM – 5'
FAM labeled CUGUGUGGCUGUCACUCGGCUGCAUGCUUAGUGCACUCACGCAG – 3'
polyA RNA FAM – 5' AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
FAM labeled
1-1000 RNA GGGTTAAAGGTTTATACCTTCCCAGGTAACAAACCAACCA
CTTGTAGATCTGTTCTCTAAACGAACTTTAAAATCTGTGTGGCTGTCACTC
GGCTGCATGCTTAGTGCACTCACGCAGTATAATTAATAACTAATTACTGTCG
TTGACAGGACACGAGTAACTCGTCTATCTTCTGCAGGCTGCTTACGGTTT
GTCCGTGTTGCAGCCGATCATCAGCACATCTAGGTTTCGTCCGGGTGTGA
CGTCCAACTCAGTTTGCCTGTTTTACAGGTTCGCGACGTGCTCGTACGTG
GCTTTGGAGACTCCGTGGAGGAGGTCTTATCAGAGGCACGTCAACATCT
AAAGATGGCACTTGTGGCTTAGTAGAAGTTGAAAAAGGCGTTTTGCCTCA
ACTTGAACAGCCCTATGTGTTCATCAAACGTTCGGATGCTCGAACTGCAC
CTCATGGTCATGTTATGGTTGAGCTGGTAGCAGAACTCGAAGGCATTCAG
AATACCAGTGGCTTACCGCAAGGTTCTTCTTCGTAAGAACGGTAATAAAGG
AGCTGGTGGCCATAGTTACGGCGCCGATCTAAAGTCATTTGACTTAGGCG
AACATAGCAGTGGTGTTACCCGTGAACTCATGCGTGAGCTTAACGGAGGG
GCATACACTCGCTATGTCGATAACAACTTCTGTGGCCCTGATGGCTACCCT
CTTGAGTGCATTAAAGACCTTCTAGCACGTGCTGGTAAAGCTTCATGCAC
TTGTCCGAACAACTGGACTTTATTGACACTAAGAGGGGTGTATACTGCTGC

GA N RNA GGGTTAAAGGTTTATACCTTCCCAGGTAACAAACCAAAC		CGTGAACATGAGCATGAAATTGCTTGGTACACGGAACGTTCTGGGCCCTC
N RNA GGGTTAAAGGTTTATACCTTCCCAGGTAACAAACCAAAC		GA
CTTGTACATCTGTTCTCTAAACCAAACTAAACTAAATGTCTGATAATGACC CAAAATCAGCGAAATGCACCCCGCATTACGTTTGGTGGCCCCCAGATT AACTGGCAGTAACCAGAATGCACCCCGCATTACGTTTGGTGGCGCCGATCAAAACAA CGTCGGCCCCAAGGTTACCCAATAATACTGCGTCTTGGTGCACGGCCGAGAACAAA CGTCGGCCCCAAGGTTACCCAAGGAGACCCTTAAATTCCTCCTGGGGGCCAGAAGCG CAATTAACACCAATAGCAGTCCAGGTGAGCGCAAAATGGACAAAGGAGCTGCACTACCGAAGAG CTACCAGACGAATTCGTGGTGGTGGTGGCGGCAAAATGGAAGACTTGCGGACTTCCCTAT GGTGCTAACAAAGACGGCCACAATTGGGCTGCAACCAGGGACCTCGCCTAA AGATGGTATTTCTACTACCATGGGCACCCGCAAAATGGACGACCTTGAC CGTGCTACAAAGACGGCACTCAAGTGGGCCGCAGAAGCCTGGACTTCCCCTAT GGTGCTACAAAGACCGCCGCAAACTCCAGGGCAGCAATGGCTGCAAT CGTGCTACAAAGATGCCTGCAAACCCCAGGCAACCAAAGGCCTCTACCGGA AAGGGAGGCAGAGGGCGGCAGTCAAGCCCCGCAAAATGCTGGGGGACTTCTC CTGCTAGAATGGCTGCGCAATGGCGGTGATGCTGCTCTTGCTTTGCGTGG CTTGCAAGATTGCCTGCAAAACCCAACGGCAGCAAAAATGCTGGTGGTAAAGGCCAACA ACAACAAGGCCAAACTGTCACAACCCAAGGGAAATTCTGCTGCTG CTTGCACAACTGCCCAGAACCGACAAACCTAAAGGCCTAAAGGCCTAAAG ACCCCCGGCAAAAACGTCCACAACTGCACACAAAATGCTGGAGCAACAAC ACACAAAGGCCAAAACTGCCACACTGACAAACCTAAAGGCCTTAAGA AGCCTCGGCAAAAACGTGCACAACCCAAAAACCACAAGGCTT TCGCCAGACGTGGTCCAGAACAACCCAAAACAACACCAAGGACTT TCGCCAGACGTGGTCCAGAACAACCCAAAACGCAAAACCCACAAG ACTAATCAGACAAGGAACTGATACAAACATTGGCCGCATGAAACAACGAA ACTATCCAGACCTCCACGACTGACCAACAGGCAAAATTGGCATGGAA ACTATCAGACAAGGAACTCAAACCCCAACAGAGGCAAAACGAA ACTATCCAGACCTCCACCACAAAACGCCAAACGAAA ACTATCCAGACCTCCACCACAACAGCCTAAAACGAACAACAACAACAACAACAACAACAACAACACCAAAA ACCACC	N RNA	GGGTTAAAGGTTTATACCTTCCCAGGTAACAAACCAACCA
CAAAATCAGCGAAATGCACCCGGGATTAGGTTTGGTGGACCCTCAAAACAA CGTCGGCCCAAGGTAACCAGAATGGAGAACGCAGCGGGGGCGGATCAAAACAA CGTCGGCCCCAAGGTTTACCCCATAAATCGCGTCTGGTCACCGCGTCT CACTCAACATGGCAAGGAAGACCTTAAATTCCCTCGAGGACCAGGGGTC CACTCAACATGGCAAGGAAGACCTTAAATTCCCTCGAGGACAGGGGTC CACTAACACGAATTGGTGGTGGTGACGGAAAATGGCTACTACCGAGAA AGATGGTATTTCTACTACCTAGGAACTGGGCCACAAATTGGCTACTACCGAGA AGATGGTATTTCTACTACCTAGGAACTGGGCCACAAATTGGCTGCACTGAGAA AGATGGTATTTCTACTACCTAGGAACTGGGCCACGAAAGCTGGACTTCACCGAG AGATGGTAACAAAGACGGCATCATATGGGTGTCAACTGAGGGGAGCTTGAA AGATGGTAACAAAGACGGCATCATGGCGGCAGTCAGGCGTCTAACAATGGTGCTGCAT CGTGCTACAAATTCCTCCTAAGGAACACATTGCCAACAGGGGAACTTCT CGTGCAACAGTTCAAGAAATTCAACTCCAGGCAGTAGGGGGAACTTCT CGTGCAACAGTTCAAGAAATTGACCTCAGGCGGTGATGGGGGTCATAGCG TCGCAACAGTTCAAGAAATGGCGGTGATGCTGCTCTTGCTGCTGC CTGCTAGAATGGCGGGCCAGCAAAACCTAGGCGGTAAATGGCGGTACATGCGCGTTGAAG ACAACAACAACGTCAAACCTAAGGCGGAACAAACTGGCGCTTCTAAGA ACAACAACAACGTGCACATAGCGCGATGAGGAACTTGCACAAGGC CTTGGCAAGGTGGTCCAGAAAACCTAAGGCAAAATGTCACACAGCTT TCGGCAGACGTGGTCCAGAAAACCTAAGCACACACGAAGTTGCACCAAGGA ACTAATCAGACAAGGAACAAACCTAAGCCAAAACCCAAGGAAATTGCCAGGAAC ACTACAACAATCCAAACGTTCACCACAAGGCACATTGGCAGCATTGGACACGCATG GTCACAAGATCCAAACTTCCACCAACACCACAGGACAAAACGAAGAA GTCACACAGTCCAACATTCCACCAACAGCCTTAAAGGACAAAACGAA GAAGGCTGATGAAACTCAAACCCAACAGCCTAAAAGGACAAAACAGA GAAGGCTGATGAAACTCAAACCCAACAGCCTAAAAGGACAAAACAGA GAAGGCTGATGAAACTCAACGCCTTAACGCCTTAAAGGACAAAACAGA GAAGGCTGATGAAACTCAACCCAACAGCCCTAAAAGGACAAAACAGA GAAGGCTGATGAAACTCAACCCAACAGCCCTAAAAGGACAAAACAGA GAAGGCTGATGAAACTCAACCCAACC		CTTGTAGATCTGTTCTCTAAACGAACAAACTAAAATGTCTGATAATGGACCC
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CACTCAACATGGCAAGGAAGACTTAAATTCCCTCGAGGACAAGGCGTTC CAATTAACACCAATGCAGTCCAGATGACCAAATTGGCTACTACCGAAGG CTACCAACGAGCAATTCGTGGTGGTGCACGTAAAATGAAAGGACTCCAGTCCA AGATGGTATTTCTACTACCTAGGAACTGGGCCGGAAGCTGGACCTTACCAT GGTGCTAACAAAGACGGCACCATATGGGCCAGAAGCTGGGCCTTGAC TACCACCAAAAGATCACCATTGGCACCGCAATCCTGCTAACAATGCTGCAAT GGTGCTAACAACTCCTCAGGGACCAACATTGCCAAAAGGGGGACCTCTAC AAGGGAGGAGGAGGGCGGCAGTCAAGCCCTGTCCTGCTTACCAATGCTGCAAT CGTGCTACAACTTCCTCAAGGAACAACATTGCCGAAAGGGGGACATCTC CTGCACAACTTGCCAAGGACAACCTCAGGCAGCAGCAGTGAAGGGCACACA ACGCAGAGTGCAAGAGCCGCGCAGCGCA		CGTCGGCCCCAAGGTTTACCCCAATAATACTGCGTCTTGGTTCACCGCTCT
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IACACCAAAAGAICACATIGGCACCCGCAAAAGGCTTCTCAAAAGGCTGCTACGCAG CGTGCTACAACTTCCTCAAGAAATTCAACTCCAGGCAGCAGTGGGGAACTTCTC CTGCAACAGTTCAAGAAATTCAACTCCAGGCAGCAGTAGGGGAACTTCTC CTGCAACAGTTGAACAAGCTACGCGGCGATGCTGCTCTTCTTCTTCTTC CTTGACAGATTGAACCAGCTTGAGAGCAAAATGTCGGGTAAAGGCCAACA ACAACAAGGCCAAACTGTCACTAAGAAATGTCGGGTGCTAGGGCTTTCAAGA AGCCTCGGCAAAAACGTACTGCCACTAAGAAATGTACAATGTAACAAGAGCAACAAGCATTGCCGCCAAAATTGCGCCGCAAACTGTCACAAGGATACAATGTAACAACGATCAAGGACAAAGCAACAAGCATCGGCACTAGAAAGGACCAAGGACAAAACGTACCAAGGATTGGCACTAAAGCATTGGCGCGCAATGGCAAGGA AGCCCTCGGCGAAAAACGTACCACGGATGGCAGGAAGGACAGAAATTTGGGGACCAGGA AGCACCTCGGGAACGTGGTTGACCAACAAGGACTAAAAGAACGACAAATT TGCCCCCAGCGCTCAGCGGTTGACCCAACAAGGGCCATAAAAAGAA GTCACACCTTCGGGAACGTGGTTGACCAACAGGGCCATAAAAAGAAAG		GGTGCTAACAAAGACGGCATCATATGGGTTGCAACTGAGGGAGCCTTGAA
CGIGGTACAACTICCICAAGGAACAACAATGCCAAAAAGGTCICTACGCAG AAGGGAGCAAGAGGCGGCAGTCAAGCCTCTTCTCGTTCCTCATCACGTAG TCGCAACAGTTCAAGAATGCCGGCGTGATGCGCTCTTGCTGCTACGCTG CTTGACAAATGGCTGGCAATGGCGGGGAGCAGCAGGGCCAACA ACAACAAGGCCAAAACGTCGCACTAAGACAATGCTGGTGAGGCCAACA ACAACAAGGCCAAAACGTCTGCCACTAAGACATCTGGTGGAGGCCAACA ACAACAAGGCCAAACGGTCCCGCCACTAAAGCATTGGGCGCCAACA ACAACAAGGCCAAACGGACCTGCTCACCAAGGAATTTTGGGGACCAGGA ACTAATCAGACAAGGAACGAACTACCCAAGGAATTTTGGGGACCAGGA ACTAATCAGACAAGGAACGAGCTGGTTGACCAACGAGTGCCATCAAATGGAA GTCACACCTTCGGGAACGTGGTTGACCTACACAGGTGCCATCAAATTGGA GCACAAGATCCAAATTTCCAACGAGCCTTACACAGGTGCCATCAAATTGGA GACGAATACAAAACTCAAGCCTTACCACAGGGCCCAACAAGGAA GAAGGCTGATGAAACTCAAGCCTTACCACAGGGCCAAAACAGCAAA CTGTGACTCTTCTTCCTGCTGCAAGAGCCTAACAGGACAGAAAGAA		TACACCAAAAGATCACATTGGCACCCGCAATCCTGCTAACAATGCTGCAAT
AAGGGAGCAGAGGCGGCAGTCAAGCCTTCTCTCTTCTCT		CGIGCIACAACIICCICAAGGAACAACAIIGCCAAAAGGCIICIACGCAG
TCGCAACAGTTCAAGAAATTCAACTCCAGGCAGCAGTAGGGGAACTTCTC CTGCTAGAATGGCTGGCAGCGGCGATGCTGCTCTGCTTGCT		AAGGGAGCAGAGGCGGCAGTCAAGCCTCTTCTCGTTCCTCATCACGTAG
CTGCTAGAATGGCTGGCAATGGCGGTGATGCTGCTGTGCTTGCT		TCGCAACAGTTCAAGAAATTCAACTCCAGGCAGCAGTAGGGGAACTTCTC
CTTGACAGATTGAACCAGCTTGAGAGCAAAATGTCTGGTGAAAGGCCAACA ACAACAAGGCCAAAACGTACTGACTAAGAAATCTGCTGCTGAGGATTCTAAGA AGCCTCGGCAAAAACGTACTGCCACTAAAGCATACAATGTAACACAAGGTT TCGGCAGACGTGGTCCAGAACAAACCCAAGGAAATTTGGGGACCAGGA ACTAATCAGACAAGGACTGATTACAAAACATTGGCCGCAAATTGGACCAGT TGCCCCCAGCGCTTCAGCGTTGTCGGAATTCGCCACAAATTGGACAAT GCCCCCAGCGCTTCAGCGTTGTCGGAATGCGCCACAAATTGGACAAT GCACAACCTTCGGGAACGTGGTTGACCTAACACAGGTGCCATCAAATTGGA GCACAACGTCCAAATTTCCAAGAGCCTACACAGGGCCAAAAAAGAA GAAGGCTGATGAAACATTCCCACCAACAGAGCCTAAAAAGGACAAAAAGAA GAAGGCTGATGAAACATTCCCACCAACAGAGCCTAAAAAGGACAAAAAGAA CTGTGACTCTTCTTCCTGCTGCAGCATTGGATGAATTCCCAAACAACAGCAAA CTGTGACTCTTCTTCCTGCTGCAGATTTGGATGATTTCTCCAAACAATTGC AACAATCCATGAGCAGTGCTGACTCAACTCA		CTGCTAGAATGGCTGGCAATGGCGGTGATGCTGCTCTTGCTTG
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TCGGCAGACGTGGTCCAGAACAAACCCAAGGAAATTTTGGGGACCAGGA ACTAATCAGACAAGGAACTGATTACAAACATTGGCCGCAAATTGGCAGAATT TGCCCCAGCGCTTCAGCGTTCTCCGGAATGCGCAATTGGCATGGAA GTCACACCTTCGGGAACCTGGTTGACCTACACAGGTGCCATCAAATTGGA GGCACACCCCCCCCCCCCCAGACGTCAACAAGGACATTTGGACAGAAGAACAAGCACAAAAGGACAAAAGGACAAAAGGACAAAAAGAA GAAGGCTGATGAAACTCCAACCCACAGAGCCTAAAAAGGACAAAAAGGACAAAAAGGACAAAACGCAAAA GAAGGCTGATGAAACTCCAGCCCACAGAGCACAGAAGGAACAAGCACAAA CTGTGACTCTTCTTCCTGCTGCAGATTTGGATGATTTCCCCAAACAAA		AGCCTCGGCAAAAACGTACTGCCACTAAAGCATACAATGTAACACAAGCTT
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TGCCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGCATTGGCATGGAA GTCACACCTTCGGGAACGTGGTTGACCTACACAGGTCCCAAATTGGA TGACAAAGATCCAAAGTTCAAAGATCAAAGTCAATTTGCTGAATAAGCATATT GACGCTATCAAAACATTCCCACCAACAGAGCCTAAAAAGGACAAAAAGAA GAAGGCTGATGAAAACTCAAGCCTTACCGCAGAGACAGAAGAAACAGCAAA CTGTGACTCTTCTTCCTGCTGCAGATTTGGATGATTTCTCCAAACAATGC AACAATCCATGAGCAGTGCTGACTCAACTCAGG Primer 1- CCATCCGGCGTAATACGACTCACTATAGGG 1000 Forward Primer N GTGTGATGGATATCTGCAGATTCGC Forward Primer N GTGTGATGGATATCTGCAGATTCGC Forward Primer N GTGTGATGGATATCTGCAGATTCGC Goxactagagagagagagagagagagagagagagagagagaga		ACTAATCAGACAAGGAACTGATTACAAACATTGGCCGCAAATTGCACAATT
GTCACACCTTCGGGAACGTGGTTGACCTACACAGGTGCCATCAAATTGGA TGACAAAGATCCAAATTTCAAAGATCAAGTCATTTGCTGAATAAGCATATT GACGCATACAAAACATTCCCACCAACAGAGCCTAAAAAGGACAAAAAGAA GAAGGCTGATGAAACTCCCACCAACGAGGCCAAAAAGGAACAGCAAA CTGTGACTCTTCTTCCTGCTGCAGCATCACGAGGACAAAAAGGA CTGTGACTCTTCTTCCTGCTGCAGATTTGGATGATTTCTCCAAACAATTGC AACAATCCATGAGCAGTGCTGACTCAACTCAGG 1000 Porward Primer 1- CTAGAAAGATAGGACGTTCCGTGTACCAAG 1000 Reverse Primer N GTGTGATGGATATCTGCAGAATTCGC Forward Primer N GTGTGAGGATATCTGCAGAATTCGC 6xHis-GFP- M(104-222) CGAGGAGCTGTTCACCATCACCATCACCATGGTTCTTCTGTGAGCAAGGG GTGCCCGTGCCCTGGCCCACCCTGGAGCTGCAGCGGAGGCGAAGGCGAAGGCGAAGGCTGCCACCCTGGACCTGACCTGACCACCACCGGCACAGGCGAAGGCGAAGCTGCTTCAAGG CGCCCGTGCCCTGGCCCACCCTCGTGACCCTGAAGCACCACCACCACCGGCACA CCTGCGGTAACCGCCACAGCTGCACGCCGCGAGGCGAAGGCGAACA CCTGCTGGCCCTGGCCCACCCCTGGTGACCCTGACGACACCACCACCGCGACA ACGACGGCACCTACAAAGACCGCGCCCGGGCAAGCACCACCACCACCACCAC		TGCCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGCATTGGCATGGAA
TGACAAAGATCCAAATTTCAAAGATCAAGTCATTTGCTGAATAAGCATATT GACGCATACAAAACATTCCCACCAACAGAGCCTAAAAAGGACAAAAAGAA GAAGGCTGATGAAACTCTAAGCCTTACCGCCGAGAGAACAAAAGAA CTGTGACTCTTCTTCTGCTGCAGATTACGACGACAGAAGAAACAGCAAA CTGTGACTCTTCTTCTGCTGCAGATTACGACTCAACTCA		GTCACACCTTCGGGAACGTGGTTGACCTACACGGTGCCATCAAATTGGA
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ForwardPrimer N ReverseCATGAGTTTAGGCCTGAGTTGAGTCAG6xHis-GFP- M(104-222)ATGGGTTCTTCTCACCATCACCATCACCATGGTTCTTCTGTGAGCAAGGG CGACGTAAACGGCCACAAGTTCAGCGTGCGCGGCGAGGGCGAGGGCGA GCCACCAACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAG CTGCCCGTGCCCTGGCCCACCCTGGACCACCCTGACCTACGGCGTGC AGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACCATCTCTTCAAG TCCGCCATGCCCGAAGGCTACGTCCAGGAGGCGAAGTTCAAGGAGCACCACCTTCAAGG ACGACGGCACCTACAAGACCGCGCGAGGGCAACACCCGCGCCGAGGTGAAGTTCGAGGGCGACA CCCTGGTGAACCGCATCGACGACGACACTTCAAGGAGGACGG CAACATCCTGGGGCACAAGCTGGAGTACAACTTCAACAGCCACAACGTCT ATATCACGGCCGACAAGCAGGACGGCAGCGTGCAGCTCGACGCCACACTACAAGATC CGCCACAACGTCGAGGACGGCAGCGGCAGCGTGCAGCTCGACCACCACTACAAG CCCTGGTGAACCCCCATCGACGACGGCACCACTACAAGGCGAACTTCAAGATC CGCCACAACGTCGAGGACGGCACGGCAGCGTGCAGCTCGACCACAACCACT ACCTGAGCACCCCATCGAGGCGACGGCACCACGGCACCACCACCACT ACCTGAGCACCCCAGTCCAAGCTGAAGCAGCAAGACCCCCAACGACAACCACT ACCTGAGCACCCCAGTCCAAGCTGAAGCAGCAAGACCCCAACGACAACCACT ACCTGAGCACCCCAGTCCAAGCTGAGCAAAGACCCCCAACGAAAGACCCCAACGAAAGCGCGA TCACATGGTCCTGCTGACGCGCGCCGCGCGCGCGGGATCACTCTCGGC	Primer N	GTGTGATGGATATCTGCAGAATTCGC
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Reverse 6xHis-GFP- M(104-222) ATGGGTTCTTCTCACCATCACCATCACCATGGTTCTTCTGTGAGCAAGGG CGACGTAAACGGCCACAAGTTCAGCGTGCGCGGCGAGGGCGAGGGCGA TGCCACCAACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAG CTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGC AGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAG TCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCACTTCTTCAAG TCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTCCTTCAAGG ACGACGGCACCTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACA CCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGG CAACATCCTGGGGCACAAGCTGGAGTACAACTTCAACAGCCACAACGTCT ATATCACGGCCGACAAGCAGAAGAACGGCATCAACGCCACACTACAAGATC CGCCACAACGTCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG CAGAACACCCCCATCGGCGACGGCAGCGTGCAGCTCGCCGACCACTACCAG CAGAACACCCCCATCGGCGACGGCAGCGTGCAGCTCGCCGACCACTACCAG CAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACT ACCTGAGCACCCCATCGGCGACGGCACCGCGCGCGGGATCAACCACCACACGTC ACCTGAGCACCCCATCGGCGACGGCACCGCCGCGGGATCAACCCCACACGACGA	Primer N	CATGAGTTTAGGCCTGAGTTGAGTCAG
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CGACGTAAACGGCCACAAGTTCAGCGTGCGCGGCGAGGGCGAGGGCGA CGACGTAAACGGCCACAAGTTCAGCGTGCGCGGCGAGGGCGAGGGCGA CTGCCCGTGCCCTGGCCCACCCTGAAGTTCATCTGCACCACCGGCAAG CTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGC AGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAG TCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTCCTTCAAGG ACGACGGCACCTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGGCGACA CCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGG CAACATCCTGGGGCACAAGCTGGAGTACAACTTCAACAGCCACAACGTCT ATATCACGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATC CGCCACAACGTCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG CAGAACACCCCCATCGGCGACGGCAGCGTGCAGCTCGCCGACCACCACCAC CCTGAGCACCCCATCGGCGACGGCCCCGTGCTGCCGCCGACAACCACT ACCTGAGCACCCCAGTCCAAGCTGAGCAAAGACCCCCAACGAGAAGCGCGA TCACATGGTCCTGCTGGAGTTCGTGACCGCCGCGGGATCACTCTCGGC	$M(104_222)$	
TGCCACCAACGGCCACAAGTTCAGCGTGCGCGGCGAGGGCGAGGGCGAAG TGCCACCAACGGCCAAGCTGACCCTGAAGTTCATCTGCACCACCGGGCAAG CTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGC AGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAG TCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTCCTTCAAGG ACGACGGCACCTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGGCGACA CCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGG CAACATCCTGGGGCACAAGCTGGAGTACAACTTCAACAGCCACAACGTCT ATATCACGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATC CGCCACAACGTCGAGGACGACAGCAGCAGCAGCAGCGCGCGC	M(104-222)	
CTGCCACCAACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGGCAAG CTGCCCGTGCCCTGGCCCACCCTCGTGACCACCTGACCTACGGCGTGC AGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAG TCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTCCTTCAAGG ACGACGGCACCTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGGCGACA CCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGGAGGACGG CAACATCCTGGGGCACAAGCTGGAGTACAACTTCAACAGCCACAACGTCT ATATCACGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATC CGCCACAACGTCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG CAGAACACCCCCATCGGCGACGGCAGCGTGCAGCTCGCCGACCACCACGT ACCTGAGCACCCCATCGGCGACGGCCCCGTGCTGCCGCCGACAACCACT ACCTGAGCACCCAGTCCAAGCTGAGCAAAGACCCCCAACGAGAAGCGCGA TCACATGGTCCTGCTGGAGTTCGTGACCGCCGCGGGATCACTCTCGGC		
AGTGCTTCAGCCGCTACCCCGACCACCACGACGCACCACGACCTACGGCGTGC AGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACGACTTCTTCAAG TCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTCCTTCAAGG ACGACGGCACCTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGGCGACA CCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGGGGACGG CAACATCCTGGGGGCACAAGCTGGAGTACAACTTCAACAGCCACAACGTCT ATATCACGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATC CGCCACAACGTCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG CAGAACACCCCCATCGGCGACGGCCGCGTGCTGCTGCCCGACAACCACT ACCTGAGCACCCAGTCCAAGCTGAGCAAAGACCCCCAACGAGAAGCGCGA TCACATGGTCCTGCTGGCGAGTTCGTGACCGCCGCGGATCACTCTCGGC		
AGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAG TCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTCCTTCAAGG ACGACGGCACCTACAAGACCCGCGCGCGAGGTGAAGTTCGAGGGGCGACA CCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGGGGACGG CAACATCCTGGGGGCACAAGCTGGAGTACAACTTCAACAGCCACAACGTCT ATATCACGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATC CGCCACAACGTCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG CAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACCAACCA		
ACGACGGCACCTACAAGGCTACGTCCAGGAGCGCACCATCTCCTTCAAGG ACGACGGCACCTACAAGACCCGCGCGCGAGGTGAAGTTCGAGGGGCGACA CCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGGGGACGG CAACATCCTGGGGGCACAAGCTGGAGTACAACTTCAACAGCCACAACGTCT ATATCACGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATC CGCCACAACGTCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG CAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACT ACCTGAGCACCCAGTCCAAGCTGAGCAAAGACCCCAACGAGAAGCGCGA TCACATGGTCCTGCTGGAGTTCGTGACCGCCGCGGGATCACTCTCGGC		
ACGACGGCACCTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACA CCCTGGTGAACCGCATCGAGCTGAAGGCCACGACTTCAAGGAGGACGG CAACATCCTGGGGCACAAGCTGGAGTACAACTTCAACAGCCACAACGTCT ATATCACGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATC CGCCACAACGTCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG CAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACT ACCTGAGCACCCAGTCCAAGCTGAGCAAAGACCCCAACGAGAAGCGCGA TCACATGGTCCTGCTGGAGTTCGTGACCGCCGCGGGATCACTCTCGGC		
CCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGG CAACATCCTGGGGCACAAGCTGGAGTACAACTTCAACAGCCACAACGTCT ATATCACGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATC CGCCACAACGTCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG CAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACT ACCTGAGCACCCAGTCCAAGCTGAGCAAAGACCCCAACGAGAAGCGCGA TCACATGGTCCTGCTGGAGTTCGTGACCGCCGCGGGATCACTCTCGGC		ACGACGGCACCIACAAGACCCGCGCCGAGGIGAAGIICGAGGGCGACA
CAACATCCTGGGGCACAAGCTGGAGTACAACTTCAACAGCCACAACGTCT ATATCACGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATC CGCCACAACGTCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG CAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACT ACCTGAGCACCCAGTCCAAGCTGAGCAAAGACCCCCAACGAGAAGCGCGA TCACATGGTCCTGCTGGAGTTCGTGACCGCCGCGGGATCACTCTCGGC		CCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGG
ATATCACGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATC CGCCACAACGTCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG CAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACT ACCTGAGCACCCAGTCCAAGCTGAGCAAAGACCCCCAACGAGAAGCGCGA TCACATGGTCCTGCTGGAGTTCGTGACCGCCGCGGGATCACTCTCGGC		CAACATCCTGGGGCACAAGCTGGAGTACAACTTCAACAGCCACAACGTCT
CGCCACAACGTCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG CAGAACACCCCCATCGGCGACGGCCCCGTGCTGCCGCGACAACCACT ACCTGAGCACCCAGTCCAAGCTGAGCAAAGACCCCCAACGAGAAGCGCGA TCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGC		ATATCACGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATC
CAGAACACCCCCATCGGCGACGGCCCCGTGCTGCCGCCGACAACCACT ACCTGAGCACCCAGTCCAAGCTGAGCAAAGACCCCCAACGAGAAGCGCGA TCACATGGTCCTGCTGGAGTTCGTGACCGCCGCGGGATCACTCTCGGC		CGCCACAACGTCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG
ACCTGAGCACCCAGTCCAAGCTGAGCAAAGACCCCAACGAGAAGCGCGA TCACATGGTCCTGCTGGAGTTCGTGACCGCCGCGGGATCACTCTCGGC		CAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACT
TCACATGGTCCTGCTGGAGTTCGTGACCGCCGCGGGATCACTCTCGGC		ACCTGAGCACCCAGTCCAAGCTGAGCAAAGACCCCAACGAGAAGCGCGA
		TCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGC
		ATGGACGAGCTGTACAAGGGGGATCGAGGAAAACCTGTACTTCCAATCCAA
TGCAGCTCGCACACGCAGTATGTGGGTCCTTTAACCCGGAGACCAATATTCT		

	TCTGAACGTCCCCTTGCATGGTACTATCCTTACCCGCCCCCTTCTGGAGA
	GTGAACTGGTGATCGGTGCCGTCATCTTACGTGGGCATTTACGCATCGCG
	GGGCACCACTTAGGGCGCTGTGACATTAAAGACTTACCCAAGGAAATTAC
	TGTAGCTACTTCGCGTACTCTTTCCTATTATAAGTTAGGCGCATCACAGCGC
	GTGGCGGGCGATTCTGGCTTTGCAGCATATTCACGCTACCGCATTGGGAA
	TTATAAATTAAATACAGATCACTCAAGTTCCTCCGATAACATCGCCCTGTTG
	GTACAG
6xHis-GFP-	ATGGGTTCTTCTCACCATCACCATCACCATGGTTCTTCTGTGAGCAAGGG
Nsp3Ubl1	CGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGG
•	CGACGTAAACGGCCACAAGTTCAGCGTGCGCGGCGAGGGCGAGGGCGA
	TGCCACCAACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAG
	CTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGC
	AGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAG
	TCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTCCTTCAAGG
	ACGACGGCACCTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACA
	CCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGG
	CAACATCCTGGGGCACAAGCTGGAGTACAACTTCAACAGCCACAACGTCT
	ATATCACGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATC
	CGCCACAACGTCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG
	CAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACT
	ACCTGAGCACCCAGTCCAAGCTGAGCAAAGACCCCAACGAGAAGCGCGA
	TCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGC
	ATGGACGAGCTGTACAAGGGGATCGAGGAAAACCTGTACTTCCAATCCAA
	TGCATCTTCTAATGGCGCACCGACAAAAGTTACATTTGGAGACGATACCGT
	GATCGAAGTTCAGGGCTACAAAAGCGTGAACATCACCTTCGAGCTGGATG
	AACGTATCGATAAAGTGCTGAACGAGAAATGCAGCGCATATACCGTGGAAC
	TGGGTACCGAAGTGAACGAATTTGCCTGTGTTGTTGCAGATGCAGTGATC
	AAAACCTTACAGCCGGTTAGCGAACTGCTGACACCTTTAGGCATTGATCTG
	GATGAATGGAGCATGGCAACCTATTATCTGTTCGACGAAAGCGGCGAGTT
	CAAACTGGCATCACACATGTATTGCAGCTTCTATCCGCCTGATGAA

Supplemental Figures

A. Unmodified N, theoretical mass: 47300.46 Da





Figure S1. Confirmation of N protein Phosphorylation. A) LC-MS profile for N protein prior to phosphorylation and following phosphorylation showing a mass increase of the main peak from 47.3 kDa to 48.0 kDa. This mass increase likely represents the addition of 9 (+ 720 Da) phosphate groups. B) SuperSep Phos-tag SDS-PAGE of N protein sample before, after, and during phosphorylation reaction at the timepoints indicated.

12. Rxn mixture at 120 min



Figure S2. Characterization of morphology of N condensate samples. A) Representative images from the formation of condensates over time with N or pN protein – without RNA, with a crowding agent (5% PEG8000), with unstructured polyA RNA or with viral RNA fragments. Droplet morphology depends on protein and RNA combination. Scale bar = 5 µm. B) Quantification of droplet morphology from A. Circularity of droplets at 60 minutes was measured as $C = (4 \pi * area/perimeter^2) * (1 - 0.5/r)^2$ where $r = perimeter/2\pi$. All protein and RNA combinations have a circularity ~ 1 except for condensates formed from N protein and N RNA. (n = 5 independent trials).



Figure S3. Recovery half times quantified from fluorescence recovery after photobleaching (FRAP) experiments. FRAP recovery curves following protein bleaching were fit to a simple exponential model. Phosphorylation reduces the recovery half time of N protein in condensates, indicating an increase in the diffusion of N. Data represents 3 independent trials.



Figure S4. Noise floor for the MSD data shown in Figure 4D. Ensemble MSD versus lag time for the protein and RNA combinations tested in this study, including the noise floor in black. The noise floor was calculated as the average of 12 videos from 3 independent trials. The experimental setup includes a microfluidic temperature controller (Cherry Biotech) that causes vibrations resulting in noise.



Figure S5. The viscosity of N condensates as a function of frequency as obtained from the microrheology experiments. These results were used to calculate the zero-shear viscosities plotted in Figure 4 and S9.



Figure S6. Frequency-dependent viscous and elastic moduli for N vs. pN condensates not shown in Figure 4. Plot with the average frequency-dependent viscous modulus (G', red) and elastic modulus (G', blue) of N/pN + 5% PEG or N/pN + 1 mg/mL polyA RNA or N/pN + 300 nM N RNA condensates. Data is calculated from the MSDs in Figure S5 (from $n \ge 10$ videos over 3 independent samples).



Figure S7. Micropipette aspiration (MPA) of N/pN protein condensates. A) The relationship between aspiration pressure and shear rate S, defined as $S = d(Lp/Rp)^2/dt$, where Lp is the aspiration length, Rp is the radius of the pipette, and t is time, for N or pN condensates with viral RNA. The viscosity is calculated as the slope of the best fit line divided by 4. Data is plotted in Figure 4I. B) Aspiration pressure vs. shear rate S for N or pN condensates with 5% PEG-8000 and 1 mg/mL polyA RNA. C) Comparison of viscosities measured for each N and pN condensate via MPA and microrheology. The trend in viscosities for the condensate compositions tested is consistent between the two methods, but MPA measurements were consistently lower than microrheology measurements. Plotted against each other, we obtain a slope of 0.72 and an R² fit of 0.99.



Figure S8. Repetition of material property experiments with N RNA. A) These experiments assess how condensates formed from N or pN protein plus N RNA interact with membranes. Using optical tweezers, condensates are trapped and brought to the surface of GUVs. GUVs have either no protein fragment or GFP-Nsp3¹⁻¹¹¹ or GFP-M¹⁰⁴⁻²²² displayed at their surface. Representative images show N or pN condensates with N RNA do not interact with naked membranes, but do bind to and may wet the surface of GUVs with Nsp3 depending on the N protein phosphorylation status. Condensates with N but not pN interact with GUVs with the M

protein fragment. B) Interaction between N and membrane protein fragments was confirmed using a partitioning experiment. C) Fluorescence recovery after photobleaching with N/pN and N RNA compared to N/pN and 1-1000 RNA. Unmodified N has lower mobility when condensed with N RNA (recovery half-life = 3.5 ± 0.1 min), compared to with 1-1000 RNA (recovery half-life = 2.6 ± 0.1 min). pN recovery curves are similar across RNA samples (pN + 1-1000 RNA recovery half-life = 1.6 ± 0.1 min vs. 1.4 ± 0.1 for N RNA. D) Ensemble MSD versus lag time for N or pN and N RNA vs. 1-1000 RNA. E) The zero-shear viscosity of the protein and RNA condensates studied, calculated from the particle-tracking results after noise correction. Data from n ≥ 10 videos from 3 independent trials. F) Quantification of the timescales at which the elastic modulus dominates (color) versus the viscous modulus dominates (grey) in protein and RNA condensates. Error bars represent one standard deviation (± 1 s.d.).



Figure S9. Dimerization and oligomerization analysis for N vs. pN. A) SDS-PAGE gel shows N vs. pN protein (at increasing concentrations) following chemical crosslinking. Crosslinking retains dimer / oligomer state in denaturing conditions of SDS-PAGE. N or pN protein is crosslinked using Bissulfosuccinimidyl suberate (BS3). SDS-PAGE separates oligomers by size. Increasing concentrations of protein results in a greater degree of oligomerization, but phosphorylation has little effect on oligomerization. B) Analysis of the dimerization domain conformation using smFRET. A full-length construct of N was labeled at positions 245 and 363 flanking the dimerization domain. Transfer efficiencies are similar for unmodified and phosphorylated N protein at low concentration (100 pm labeled protein, monomer regime) and high concentration (100 pm labeled + 1 μ M unlabeled protein, dimer regime), indicating no shift in conformation of the dimerization domain occurs upon phosphorylation. C) Using a full-length construct that is single labeled at position 363, we measured the dimerization constant for N vs.

pN protein. Binding isotherms of dimerization for N vs. pN indicate a small shift in dissociation constant (quantified to the right). Binding experiments have been analyzed accounting for the possibility of forming dimers of labeled molecules with other labeled molecules, labeled molecules with other unlabeled molecules, as well as between unlabeled molecules, according to the equation developed in Cubuk et al, 2024¹. Error bars represent one standard deviation (±1 s.d.).



Figure S10. Measuring the dynamic nature of the linker domain by assessing the dependence of fluorescence lifetime on transfer efficiency. Comparison of fluorescence lifetimes for the full-length N in its unmodified and phosphorylated states with fluorophores flanking the central linker at positions 172 and 245. Two concentrations were tested, (1) 100 pM labeled protein as the monomer regime, (2) 100 pM labeled protein + 1 μ M unlabeled protein where dimerization is expected. Grey line: linear dependence is expected for a rigid molecule. Green line: the donor lifetime (normalized by the donor lifetime in absence of acceptor: τ_{DA}/τ_{D0}) in the limit of dynamics much faster than the burst duration but slower than the fluorophore lifetime. In all cases, the populations sit near the dynamic line (green) as opposed to falling on the static line (gray), indicating the linker domain remains dynamic in both concentrations and regardless of phosphorylation status.







Figure S12. Extended data for Small Angle X-Ray Scattering experiments for

phosphorylated N protein at different concentrations. First column, raw data in the form of scattering profiles. Second column, the Guinier peak analysis plot indicates the validity of doing the Guinier analysis in the third column². Fourth column, analysis that the normalized residuals are randomly distributed about zero.

	Ν	pN
0.5 mg/mL		~~ \
1 mg/mL		
1.5 mg/mL		
3 mg/mL	~\$\$ [*]	
4 mg/mL		

Figure S13. Bead model representations for N and pN from SAXS data at varying protein concentrations.

Supporting Methods

LC-MS: A sample with protein at a concentration of 10 µM was dissolved in 50% ammonium acetate solution and diluted 1:10 fold. The sample was then transferred into an LC vial. Reverse phase liquid chromatography (RPLC) was performed on an Acquity column BEH -C4 -300A°, 1.7 micron (300 µm x 100 mm, Waters Corporation). Column temperature was set at 40°C. The following mobile phases were used A: Water with 0.1% formic acid; B: acetonitrile with 0.1% formic acid. The M-Class micro flow LC (Waters Instruments) was operated at a flow rate of 8 μ L/min, and 5 μ L of sample was injected with an autosampler after equilibration of the column. For the separation of proteins, the following concentration gradients were used: starting with 5 – 90% B for 55 minutes and then back to 5% B for minutes 55 - 62. Prior to injection on the RPLC column, a blank run was performed. ESI-MS experiments were performed using a quadrupole-TWIMS-TOF hybrid mass spectrometer (Synapt G2 HDMS; Waters Corporation) in positive ionization mode with m/z scan range 400 - 4000. The applied experimental parameters were capillary voltage, 3.0 kV; sampling cone voltage, 40 V; source offset 80°C; source temperature, 100°C; desolvation temperature 250°C; cone gas 50 L/Hr and desolvation gas 600 L/Hr. Data analysis was performed using MassLynx 4.1 and protein deconvolution was performed using Mas Ent 1.

BS3 Cross-linking Experiment: Protein (2-10 μ M in PBS at pH 7.5) was chemically cross-linked with bis(sulfosuccinimidyl) suberate (BS3) (Thermo Fisher) (50 μ M) at room temperature for 30 min and quenched by adding Tris (pH 7.5) to a 20 mM final concentration. Reaction mixtures were then analyzed by SDS-PAGE.

smFRET lifetime analysis: Analysis of fluorescence lifetime from mean FRET transfer efficiencies was conducted as described in Cubuk et al. 2024¹.

smFRET dimerization constant: Modeling of dimerization was conducted as described in Cubuk et al. 2024¹.

Bibliography

- 1. Cubuk, J. *et al.* The dimerization domain of SARS CoV 2 Nucleocapsid protein is partially disordered as a monomer and forms a high affinity dynamic complex. *bioRxiv* (2024).
- 2. Putnam, C. D. Guinier peak analysis for visual and automated inspection of small-Angle X-ray scattering data. *J. Appl. Crystallogr.* **49**, 1412–1419 (2016).