Appendix Figures:

SARS-CoV-2 nucleocapsid protein undergoes phase separation with RNA and partitions with human hnRNPs

Theodora Myrto Perdikari^{*1}, Anastasia C. Murthy^{*2}, Veronica H. Ryan^{*3}, Scott Watters^{*4}, Mandar T. Naik⁴, Nicolas L. Fawzi^{4,5#}

 ¹Center for Biomedical Engineering
²Molecular Biology, Cell Biology & Biochemistry Graduate Program
³Neuroscience Graduate Program
⁴Department of Molecular Pharmacology, Physiology, and Biotechnology
⁵Robert J. and Nancy D. Carney Institute for Brain Science Brown University, Providence, RI, USA

*These authors contributed equally

[#]Correspondence: <u>nicolas fawzi@brown.edu</u>

Keywords: biomolecular condensates, intrinsically disordered proteins, liquid-liquid phase

separation, heterogeneous nuclear ribonucleoproteins, RNA-binding proteins

Table of Contents	Page Number
Appendix Figure S1	2
Appendix Figure S2	2
Appendix Figure S3	3



Appendix Figure S1: Low pH conditions induce aggregation of N in the presence of RNA. A) DIC micrographs of 50 μ M N in varying pH conditions without and with TEV protease to cleave the MBP tag to initiate phase separation. At lower pH conditions, droplets appear to be non-spherical, consistent with less fluid behavior. Scale bars represent 50 μ m.



Appendix Figure S2: Divalent metal salts do not substantially alter N LLPS.

Addition of 2 mM MgCl₂ or CaCl₂ does not alter LLPS of 50 μ M MBP-N in the presence of 0.5 mg/mL RNA in 50 mM Tris, 70 mM NaCl, pH 7.4 at room temperature. Error bars are standard deviation of three replicates.



