SUPPLEMENTAL INFORMATION

Solution structure of mouse hepatitis virus (MHV) nsp3a and determinants of the interaction with MHV nucleocapsid (N) protein[¶]

Sarah C. Keane[1] and David P. Giedroc[1]*

[1]Department of Chemistry, Indiana University, Bloomington, IN 47405-7102 USA

Contains Figures S1-S7 and Supplemental References

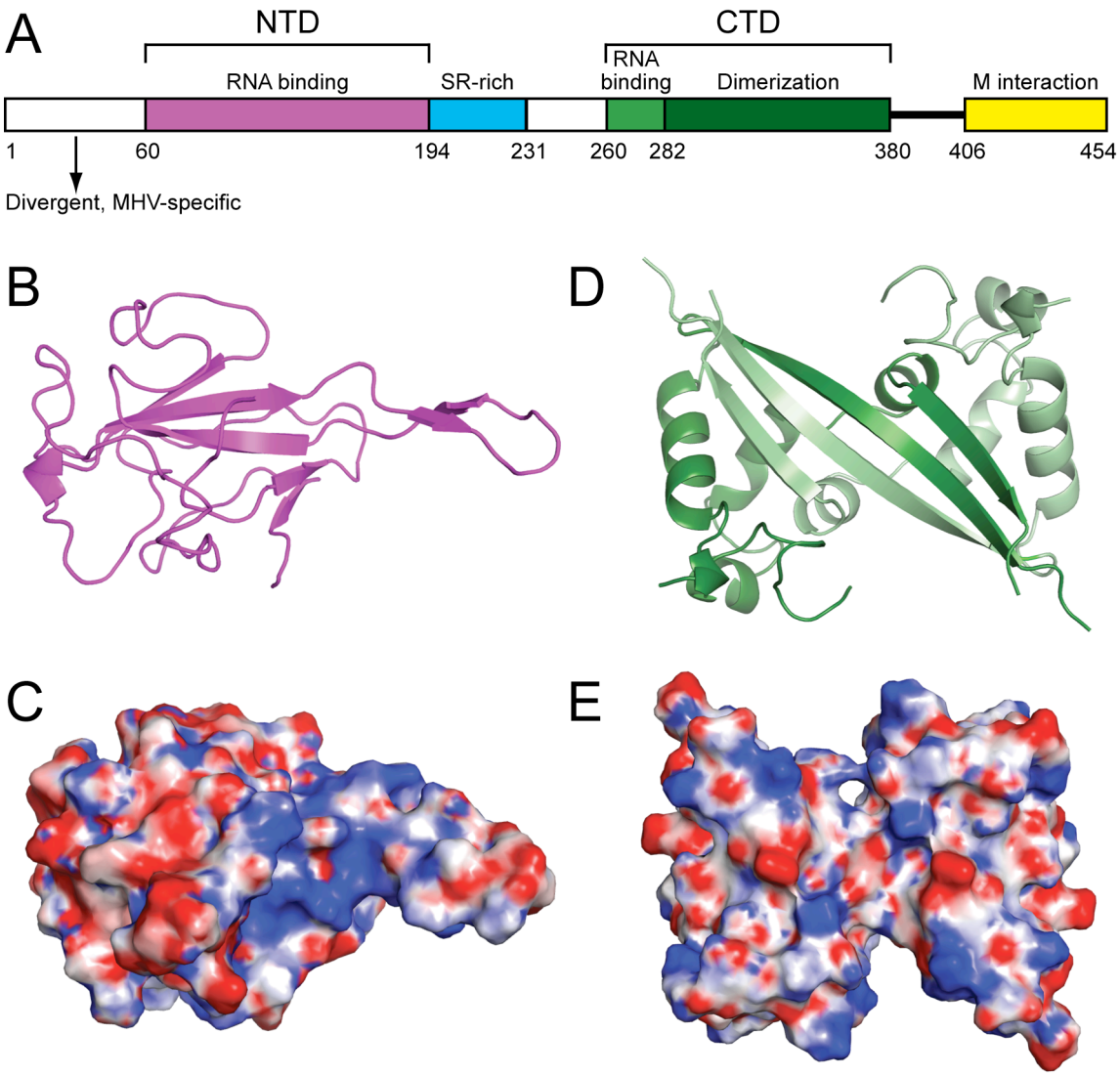

Figure S1. (A) CoV N protein domain organization. (B) Crystal structure of MHV NTD, residues 64-194 (PDB 3HD4) (1). (C) Electrostatic surface potential of MHV NTD, same view as (B). (D) Crystal structure of MHV CTD dimer, residues 287-381 (2). (E) Electrostatic surface potential of MHV CTD, same view as (D).

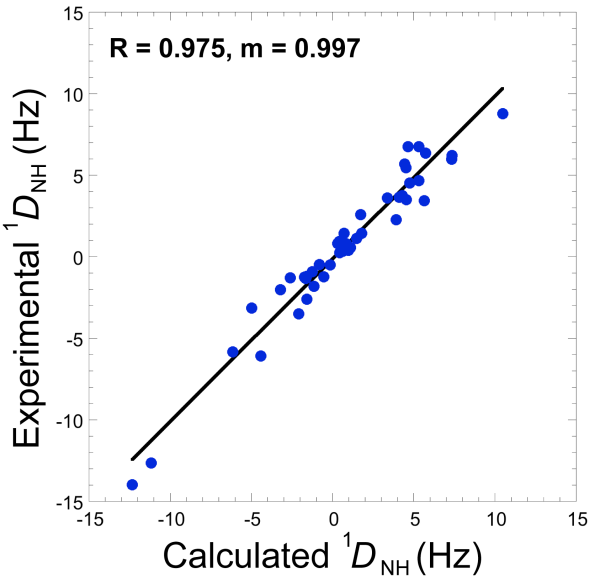

Figure S2. Correlation of experimental backbone N-H ($^1D_{NH}$) RDC constraints with those calculated from the NMR solution structure. *R*, correlation coefficient; *m*, slope.

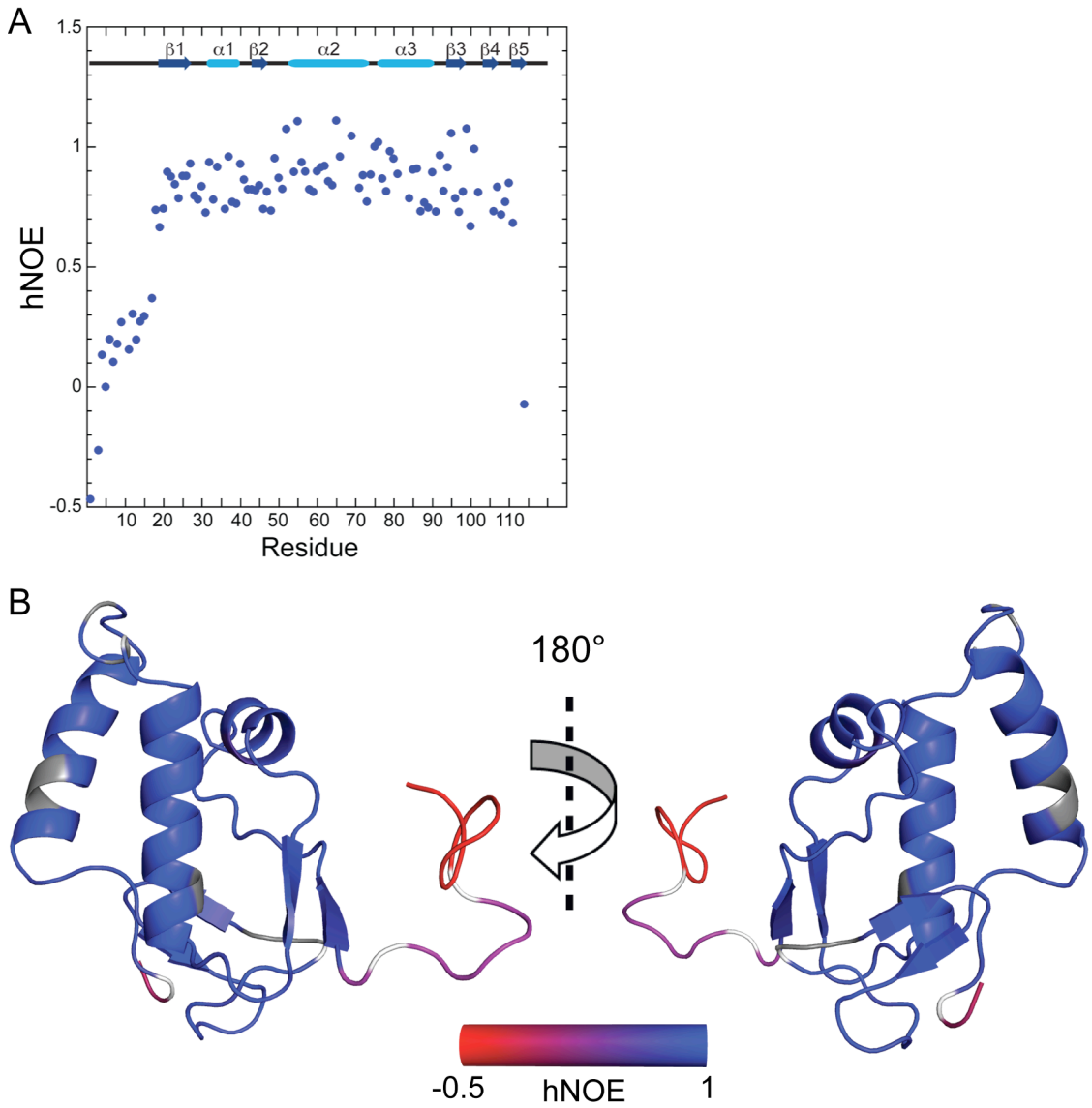

Figure S3. Fast timescale dynamics of MHV nsp3a. (A) ^{1}H-^{15}N steady-state heteronuclear NOE (hNOE) of MHV nsp3a. Regions of significant N-H internal motions (ps-ns) are identifiable by low hNOE values. (B) Value of the hNOE painted on the average solution structure of MHV nsp3a from *red* (low hNOE, dynamic) to *blue* (high hNOE, rigid). Proline residues are colored *white* while residues with no information are shaded *grey*. Two views of the structure are shown.

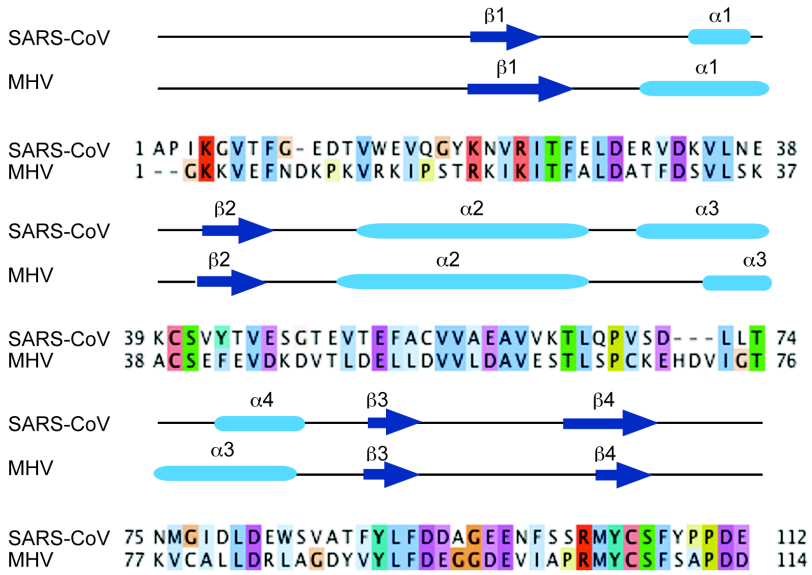

Figure S4. Sequence alignment of nsp3a from SARS-CoV and MHV. The secondary structure of MHV and SARS-CoV nsp3a, as defined from the TALOS+ prediction and the solution structure (2IDY) (3), respectively, is shown for comparison. The TALOS+ prediction shows good agreement with the solution structure (Fig. 2, main text).

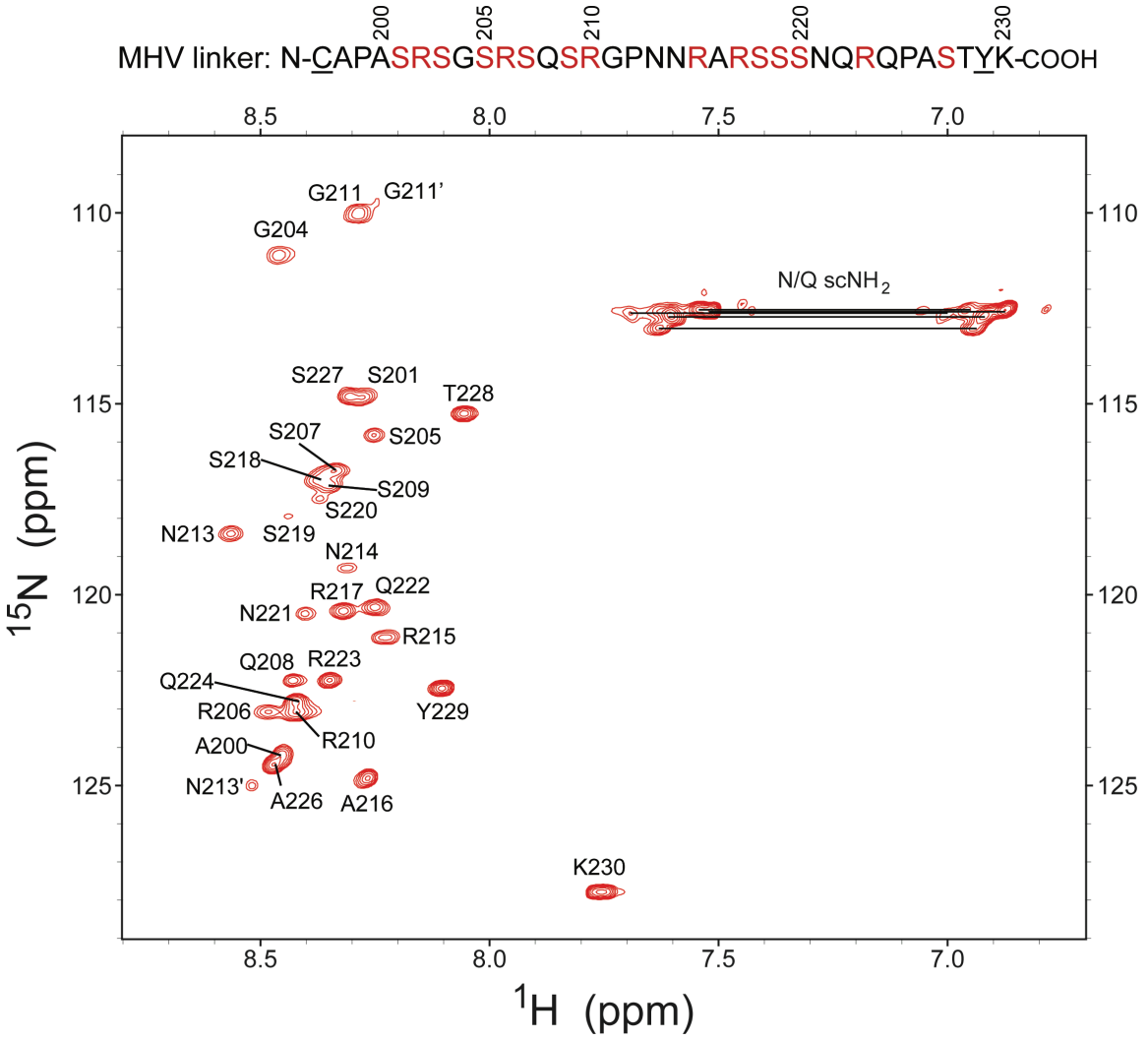

Figure S5. ^{1}H,^{15}N HSQC spectrum of the MHV SR-linker peptide. Backbone amide resonance assignments are labeled using one-letter amino acid code. Resonances that are part of the minor conformation observed are labeled G211' and N213'. The six carboxamide NH_2 groups from three N and three Q side chains were not assigned, but are labeled N/Q scNH$_2$. The sequence of the peptide is shown at the *top*, with the Ser/Arg residues shaded *red*, and underlined residues non-native to the MHV sequence.

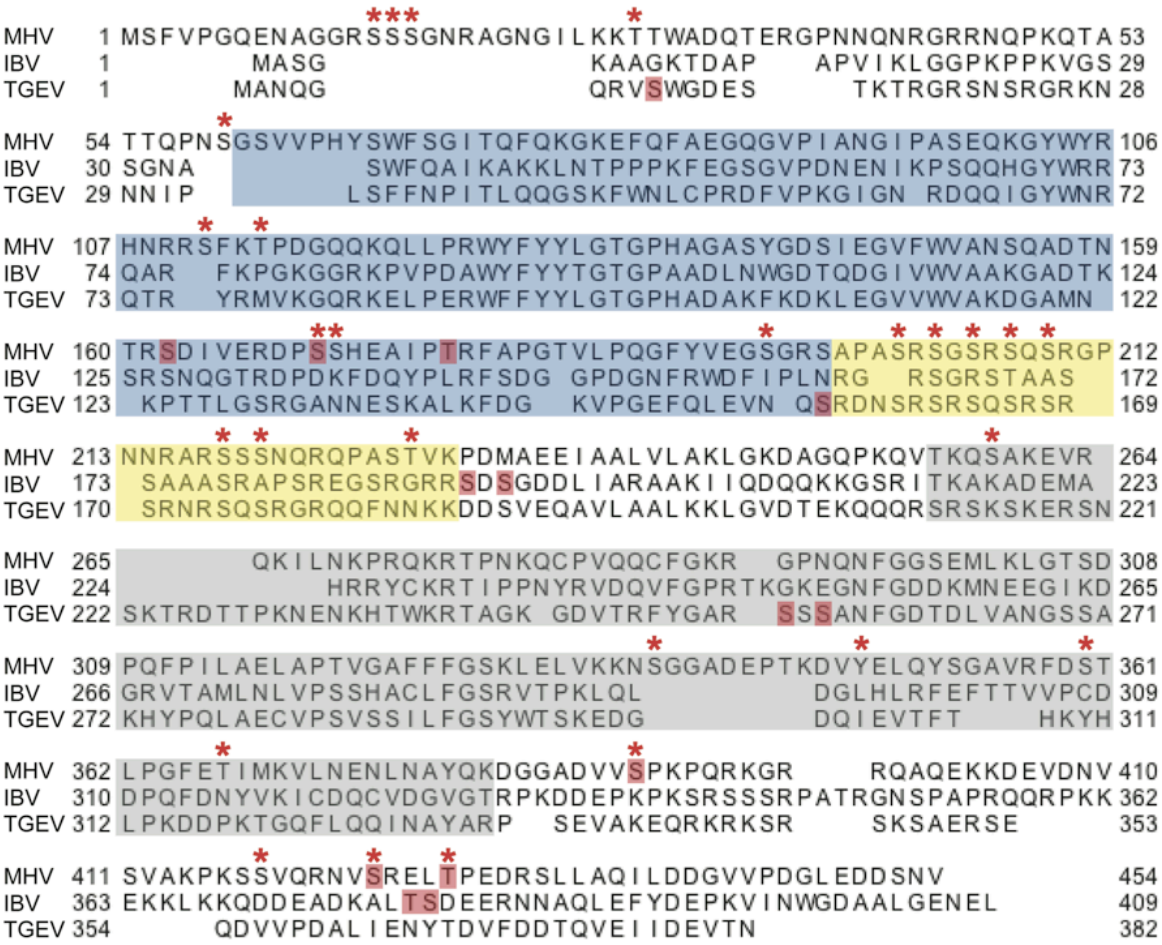

Figure S6. Multiple sequence alignment of MHV, IBV, and TGEV N proteins highlighting predicted and identified sites of phosphorylation. Individual functional domains of interest were shaded for clarity. The NTD is shaded in a *blue* box, the SR-rich linker is shaded in *yellow*, and the CTD in *grey*. Domain boundaries are indicated based on the MHV globular domains. Sites of phosphorylation that have been identified by mass spectrometric techniques are highlighted in red boxes for all three viruses. Sites in MHV that are strongly predicted (those receiving a score of ≥ 0.7 by NetPhos 2.0) (4) to be phosphorylated are marked with a red *.

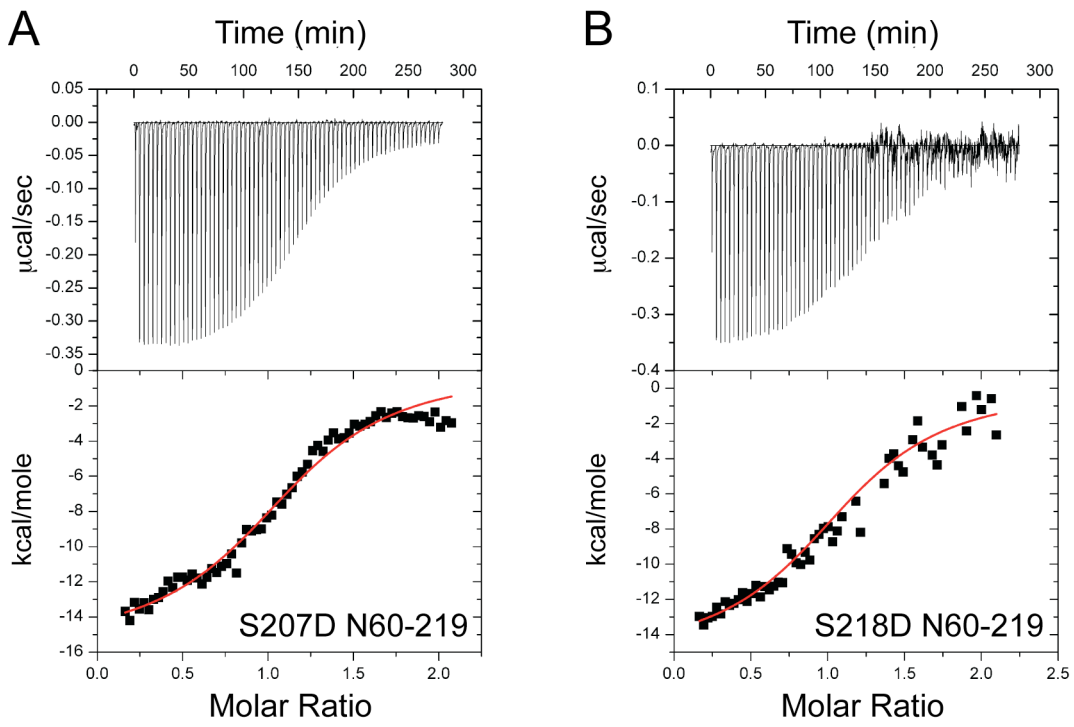

Figure S7. Representative ITC titrations of MHV nsp3a (200 µM) into (A) MHV S207D N60-219 (20 µM) and (B) MHV S218D N60-219 (20 µM) in 0.15 M KP_i, 5 mM TCEP, pH 6.0. The red line indicates the best fit according to a single-site binding model (see Table 2, main text for fitted parameters).

References

1. **Grossoehme, N. E., L. Li, S. C. Keane, P. Liu, C. E. Dann, 3rd, J. L. Leibowitz, and D. P. Giedroc.** 2009. Coronavirus N protein N-terminal domain (NTD) specifically binds the transcriptional regulatory sequence (TRS) and melts TRS-cTRS RNA duplexes. J. Mol. Biol. **394:**544-557.
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3. **Serrano, P., M. A. Johnson, M. S. Almeida, R. Horst, T. Herrmann, J. S. Joseph, B. W. Neuman, V. Subramanian, K. S. Saikatendu, M. J. Buchmeier, R. C. Stevens, P. Kuhn, and K. Wuthrich.** 2007. Nuclear magnetic resonance structure of the N-terminal domain of nonstructural protein 3 from the severe acute respiratory syndrome coronavirus. J. Virol. **81:**12049-12060.
4. **Blom, N., S. Gammeltoft, and S. Brunak.** 1999. Sequence and structure-based prediction of eukaryotic protein phosphorylation sites. J. Mol. Biol. **294:**1351-1362.