Supporting Information

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SI Results

Nucleocapsid Binding Competition Assay. To analyze the nucleocapsid (NC) binding characteristics of the mumps virus (MuV) phosphoprotein, a competitive binding assay was developed. First, His-tagged MuV P clone C286-391 or N1-194 was bound to a small Ni affinity column in saturating amounts. Both clones have been shown to be capable of binding MuV NLP in the experiments previously discussed in this report. Any remaining unbound C286-391 or N1-194 was then washed away. Next, MuV NLP was added to the loaded column in a 1:1 molar ratio. Any unbound NLP was then washed away with several washes. The NLP:N1-194 or NLP:C286-391 complex was then incubated for 30 min with thrombin-cleaved C286-391 or N1-194, respectively, in a 1:1 molar ratio of N1-194:C286-391. Any unbound thrombin-cleaved P fragment was then allowed to flow through the column. The loaded column was then washed again to remove any remaining unbound sample. The column was then eluted and analyzed on SDS/PAGE. Neither the N1-194:NLP complex or C286-391:NLP complex was able to coelute with their respective thrombin-cleaved counterpart (Fig. S8 *A* and *B*, lane 9). Several control experiments were conducted to determine if the two nucleocapsid binding P clones can interact with each other. Fig. S8 details the results from this experiment. Neither C286-391 nor N1-194 were shown to interact with each other (Fig. S8 *F* and *G*), or interact with itself (Fig. S8 *C* and *D*).

SI Methods

Virus and Cells. Vero cells were grown in complete media [CM; DMEM supplemented with 10% (wt/vol) FBS, 1× glutamine, and 1% (vol/vol) penicillin-streptomycin (P/S)]. BSRT7 cells were maintained in DMEM supplemented with 10% FBS, 1% P/S, 10% tryptose phosphate broth (TPB), and 400 μg/mL G418 sulfate antibiotic (Mediatech). Cells were cultured at 37 °C with 5% CO_2. The MuVIowa/US/06 strain (MuV-IA) was used in our studies.

Purification of Authentic NC from Virions. Released MuV-IA in the supernatant of infected cells was harvested by centrifugation over a 20% sucrose cushion in a SW28 rotor at 28,000 rpm for 1.5 h. The pellet was then resuspended in TNE (10 mM Tris; 200 mM NaCl; 1 mM EDTA; pH 7.4) with 1% Triton X-100 to disrupt the virions. The preparation was then centrifuged in a SW 41 rotor on a continuous 20–40% CsCl gradient for 18 h at 32,000 rpm. Fractions containing purified nucleocapsids were analyzed by SDS/PAGE.

Recombinant Protein Expression and Purification. Expression vectors were transformed into *Escherichia coli* strain BL21(DE3). The P fragments P_{CTD} (residues 286–391) and P_{NTD} (residues 1–194) were expressed and purified using Ni-affinity chromatography as reported (1). Proteins were further purified by size-exclusion chromatography (Sephacryl S-75 or S-200; GE Healthcare) in TNE.

RNase A Treatment. Purified MuV NC was incubated with and without RNase A (0.5 mg/mL) at 37 °C overnight. Absorbance measurements were carried out at 260 nm and 280 nm using a NanoDrop 2000 (ThermoScientific).

Virus Propagation. For infections, MuV-IA stock was diluted in PBS with 10% CM. Cells of ∼70% confluence were infected with MuV-IA at an MOI = 0.01. At 96 h postinfection, or when maximum syncytia formation occurred, the supernatant containing MuV virions was collected.

Plaque Assays. Vero cells were grown to ∼70% confluent. Media was removed and cells were inoculated with serial dilutions of MuV. After incubation at 37 °C for 1 h, media was removed and a 1:1:1 mixture of 2× DMEM, CM, and 1.5% agarose was added to monolayers and allowed to solidify. Six days later, cells were fixed with 4% formaldehyde and stained with 1% crystal violet.

Plasmids and Transfections. The plasmids used in this work were constructed using standard molecular cloning techniques. Plasmid construction details and sequence files are available upon request. MuV-P domains were cloned into the pCAGGS expression vector. P_{NTD} had amino acid residues 1–194 and P_{CTD} had residues 286–391 of P. Cells were transfected using JetPRIME (Polyplus-transfection) following the manufacturer's protocols.

Image Analysis. Micrographs were selected for image processing based on optimal defocus, ice thickness and astigmatism. CTF correction and 3D reconstructions were performed using the iterative helical real space reconstruction approach (IHRSR) using the SPARX/EMAN2 package (2–4). For both the NC only and NC-P_{CTD} reconstructions, resolution was determined by splitting the data into two independent sets and generating reconstructions from each unique set. A Fourier shell correlation of 0.5 was determined by comparing unsymmetrized reconstructions (Figs. S1 and S4).

Nucleocapsid Binding Competition Assay. To analyze the nucleocapsid binding characteristics of the MuV phosphoprotein, a competitive binding assay was developed. A small column containing 50(l of charged Chelating Sepharose Fast Flow beads was first saturated with C286-391 or N1-194. After allowing the sample to flow through the column, the beads were washed with 10 column volumes (CV) of binding Buffer A, which contained 20 mM Tris (pH 7.9), 50 mM NaCl, 5 mM Imidazole. NLP, or truncated NLP, in Buffer A was added to the loaded column in 1:1 molar ratio of N:P and allowed to incubate at room temperature for 15 min. The NLP solution was then allowed to flow through. Next, the loaded column was washed with 10 CV of Buffer A, then again with 5 CV of binding Buffer B [20 mM Tris (pH 7.9), 50 mM NaCl, 50 mM Imidazole]. The NLP:N1-194 or NLP:C286-391 complex was then incubated for 30 min with thrombin-cleaved C286-391 or N1-194, respectively, in a 1:1 molar ratio of N1-194:C286-391. Thrombin treatment was done as described (5). Next, the loaded column was washed with 10 CV of binding Buffer B [20 mM Tris (pH 7.9), 50 mM NaCl, 50 mM Imidazole]. The column was then eluted with 5 CV of elution buffer containing 20 mM Tris (pH 7.9), 50 mM NaCl, 500 mM Imidazole. The eluate was then denatured in SDS/PAGE sample buffer and electrophoresed on a 12% SDS/PAGE gel. Gels were stained with 1% Coomasie Brilliant blue dye. Several control experiments were conducted to determine whether the two nucleocapsid binding P clones can interact with each other. Pulldown assays were conducted with the following: C286-391 plus thrombin treated N1-194 or thrombin treated C286-391; N1-194 plus thrombin treated C286-391 or thrombin treated N1-194. A small column containing 50μl of charged Chelating Sepharose Fast Flow beads was first saturated with his-tagged C286-391 or N1-194. After allowing the sample to flow through the column, the beads were washed with 10 CV of binding Buffer A, which contained 20 mM Tris (pH 7.9), 50 mM NaCl, 5 mM Imidazole. Thrombin cleaved N1-194 or thrombin cleaved C286-391 in Buffer A was added to the loaded column in 1:1 molar ratio of N1-194:C286-391, and allowed to incubate at room temperature

for 30 min. The thrombin-treated sample was then allowed to flow through. Next, the loaded column was washed with 10 CV of Buffer B [20 mM Tris (pH 7.9), 50 mM NaCl, 50 mM Imidazole]. The column was then eluted with 5 CV of elution buffer containing 20 mM Tris (pH 7.9), 50 mM NaCl, 500 mM Imidazole. The eluate was then denatured in SDS/PAGE sample buffer and electrophoresed on a 12% SDS/PAGE gel. Gels were stained with 1% Coomasie Brilliant blue dye.

1. Cox R, et al. (2013) Structural and functional characterization of the mumps virus phosphoprotein. *J Virol* 87(13):7558–7568.
2. Behrmann E, et al. (2012) Real-space processing of helical filaments in SPARX. *J Struct Biol* 177(2):302–313.
3. Hohn M, et al. (2007) SPARX, a new environment for Cryo-EM image processing. *J Struct Biol* 157(1):47–55.
4. Egelman EH (2007) Single-particle reconstruction from EM images of helical filaments. *Curr Opin Struct Biol* 17(5):556–561.
5. Ding H, Green TJ, Lu S, Luo M (2006) Crystal structure of the oligomerization domain of the phosphoprotein of vesicular stomatitis virus. *J Virol* 80(6):2808–2814.

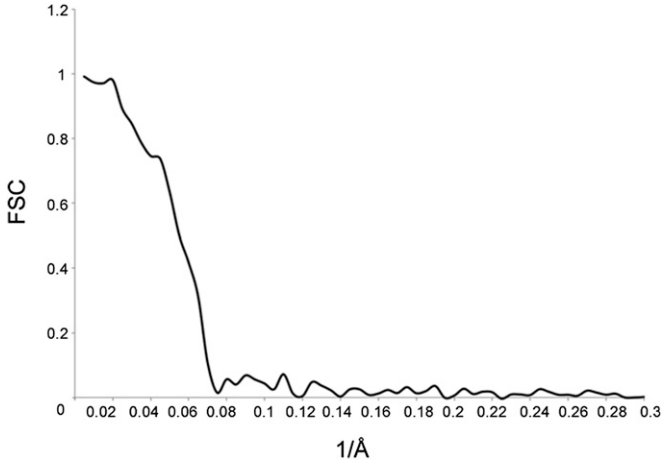

Fig. S1. FSC correlation for cryo-EM reconstruction of the authentic MuV NC. At FSC = 0.5, the resolution of the MuV NC reconstruction was determined to be 18 Å. Resolution was determined by splitting the data into two independent sets and generating reconstructions from each unique set.

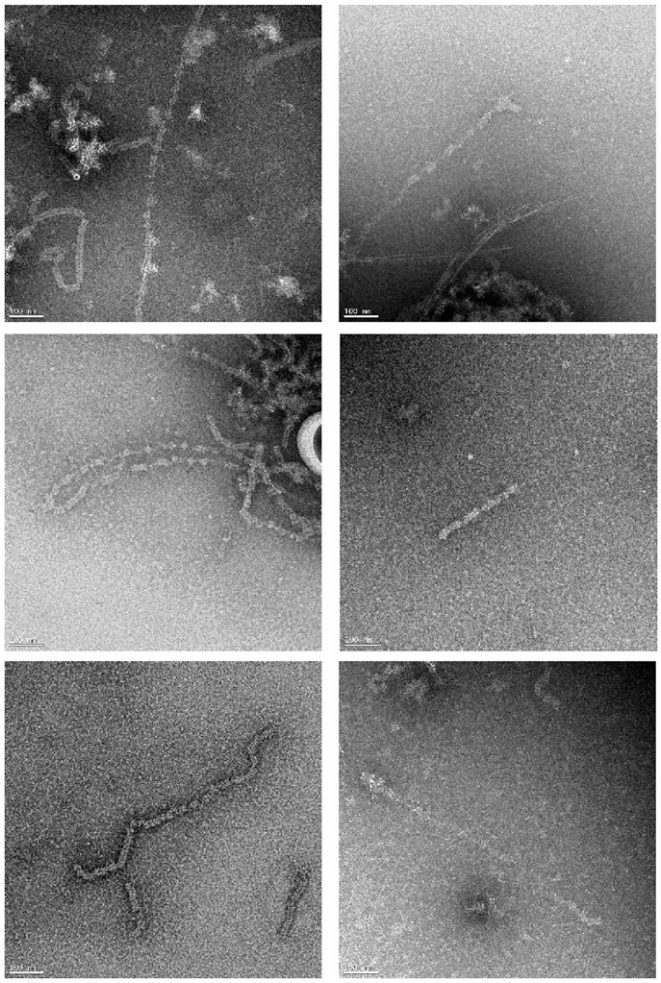

Fig. S2. Images of uncoiled NC in the presence of P_{NTD}. A large number of unwound NC segments were seen in the NC-P_{NTD} images. Unwound NC segments were not observed in other samples, including NC only, NC-P_{CTD}, and empty NC.

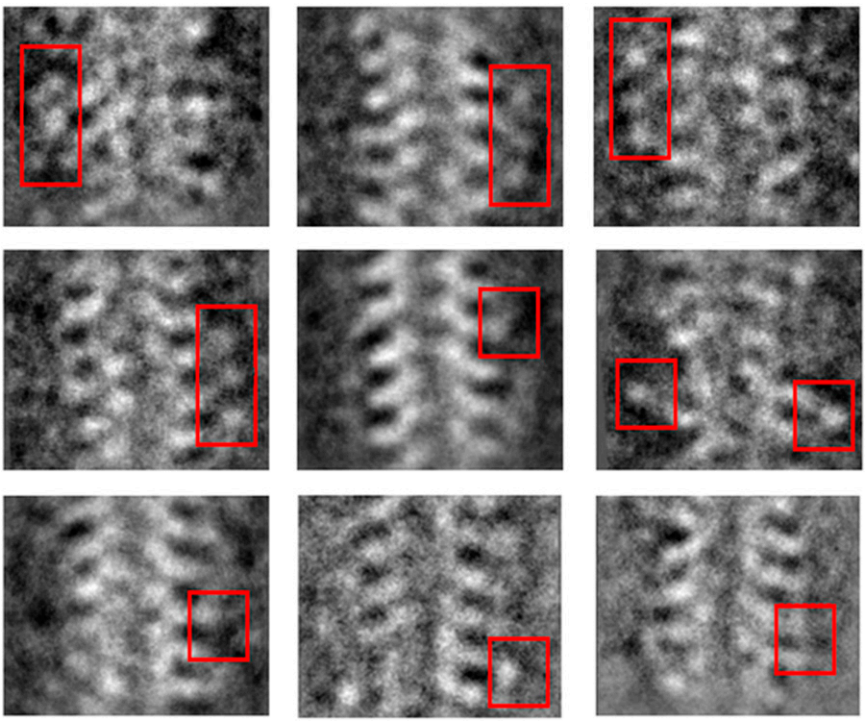

Fig. S3. Negatively stained NC-P_{CTD} complexes. Examples of negatively stained NC-P_{CTD} displaying a decorated appearance. The additional densities are boxed out in red.

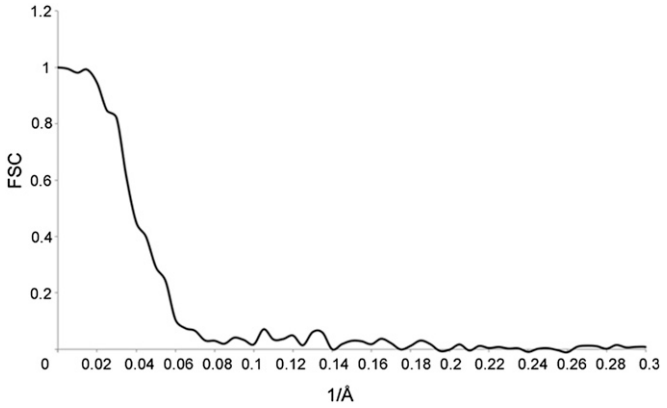

Fig. S4. FSC correlation for cryo-EM reconstruction of the MuV NC-P_{CTD} complex. At FSC = 0.5, the resolution of the MuV NC-P_{CTD} complex reconstruction was determined to be 25 Å. Resolution was determined by splitting the data into two independent sets and generating reconstructions from each unique set.

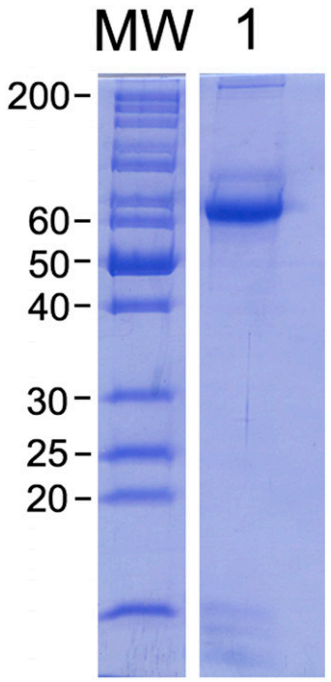

Fig. S5. SDS/PAGE of MuV NC preparation. SDS/PAGE analysis of MuV virion derived NC is shown in lane 1. The molecular weight ladder is shown in the MW lane.

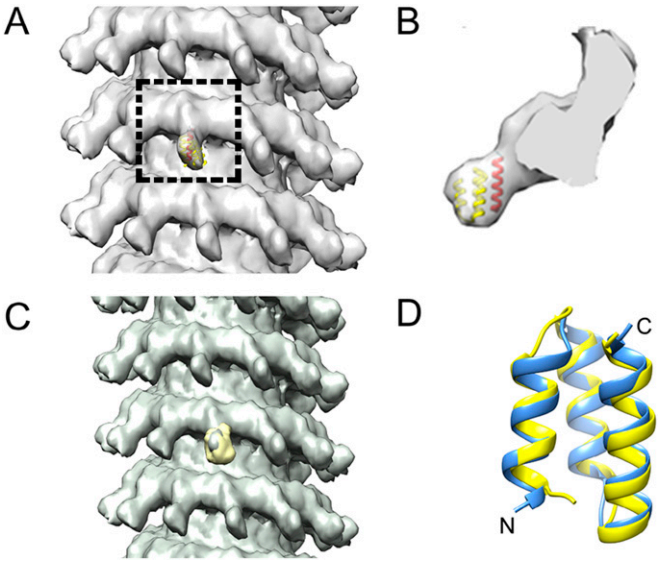

Fig. S6. Overlay of the MuV and MeV C-terminal NBD structures. (*A* and *B*) View of the MeV PXD (yellow)-N MoRe (red) structure fitted into the NC-P_{CTD} reconstruction at a sigma level of 2.0. (*A*) The MeV PXD-N MoRe structure is boxed out. (*B*) A magnified side view of the MeV PXD-N MoRe structure fitted into the MuV NC- P_{CTD} reconstruction. (*C*) A simulated density map of the MeV PXD-N MoRe structure fitted into the additional density observed in the MuV NC-P_{CTD} reconstruction. (*D*) The structure of the MeV PXD (yellow) in its complex with the MoRe of N (PDB ID code 1T6O) is closely superimposable with that of the MuV PXD (blue) (PDB ID code 3BBZ) by Chimera (1). The additional density seen in the MuV NC-P_{CTD} reconstruction is the approximate size of the MeV PXD-N MoRe structure. The MeV PXD-N MoRe structure fit into the additional density in the MuV NC-P_{CTD} map with a correlation of 0.9195.

1. Pettersen EF, et al. (2004) UCSF Chimera—a visualization system for exploratory research and analysis. *J Comput Chem* 25(13):1605–1612.

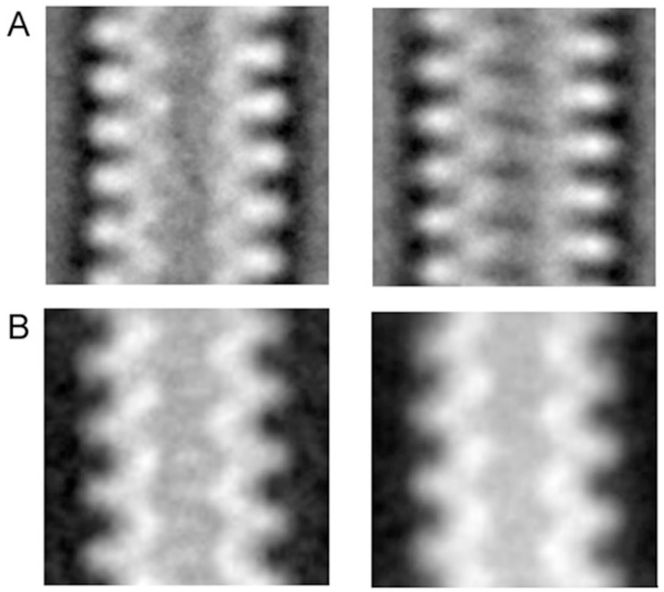

Fig. S7. Effect of stain on the NC structure. (*A*) Class averages of purified NC imaged using 1% uranyl acetate. (*B*) Class averages of purified NC using 2% phosphotungstic acid. The choice of stain appeared to have no effect on NC uncoiling. Class averages were made using EMAN2 (1–3).

1. Behrmann E, et al. (2012) Real-space processing of helical filaments in SPARX. *J Struct Biol* 177(2):302–313.
2. Hohn M, et al. (2007) SPARX, a new environment for Cryo-EM image processing. *J Struct Biol* 157(1):47–55.
3. Egelman EH (2007) Single-particle reconstruction from EM images of helical filaments. *Curr Opin Struct Biol* 17(5):556–561.

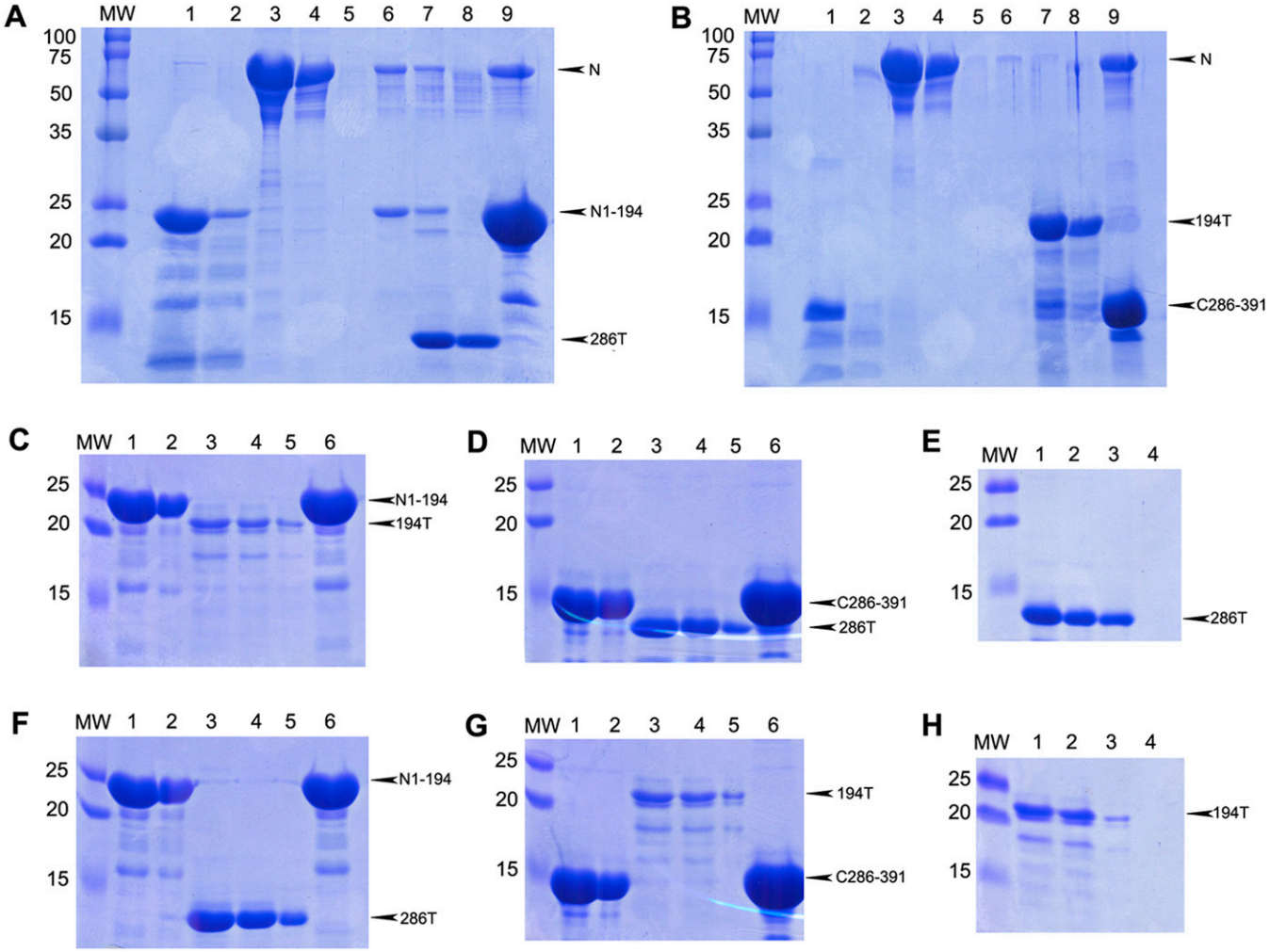

Fig. S8. Competitive Binding Assay. (*A*) His-tagged N1-194 was loaded onto the column and incubated with NLP. After washing the complex, thrombin-cleaved C286-391 (286T) was added and incubated for 30 min. After washing away any unbound sample (*A*, lane 8), the complex was eluted (*A*, lane 9). Lanes 1–9 correspond to N1-194 flow through, buffer A wash, NLP flow through, buffer A wash, buffer A wash, buffer B wash, 286T flow through, buffer B wash, and the elution, respectively. (*B*) His-tagged C286-391 was loaded onto the column and incubated with NLP. After washing the complex, thrombin-cleaved N1-194 (194T) was added. After washing away any unbound sample (*B*, lane 8), the complex was eluted (*B*, lane 9). Lanes 1–9 correspond to C286-391 flow through, buffer A wash, NLP flow through, buffer A wash, buffer A wash, buffer B wash, 194T flow through, buffer B wash, and the elution, respectively. (*C* and *F*) A pulldown assay was used to test if N1-194 interacts with 194T (*C*) or 286T (*F*). Lanes 1–6 correspond to C286-391 flow through, buffer A wash, flow through of 194T (*C*) or C286-391 (*F*), buffer A wash, buffer B wash and elution, respectively. (*D* and *G*) A pulldown assay was used to test if C286-391 interacts with 286T (*D*) or 194T (*G*). Lanes 1–6 correspond to C286-391 flow through, buffer A wash, flow through of 286T (*D*) or 194T, buffer A wash, buffer B wash and elution, respectively. (*E* and *H*) Control to determine if 286T (*E*) or 194T (*H*) can nonspecifically bind the column. Lanes 1–4 correspond to flow through, buffer A wash, buffer B wash and elution, respectively.

Table S1. 260/280 OD ratios

Sample ID	260/280
No RNase A; O/N incubation 37 °C	1.17
No RNase A on ice (just purified NC)	1.6
RNase A Treated at time = 0	1.37
RNase A treated at time = O/N incubation at 37 °C	0.73

Table S2. Observations of nucleocapsid uncoiling

Sample ID	Stain	No. of micrographs	Coiled NC	Uncoiled NC	% uncoiling
NC alone	UA	104	144	15	9.43
NC alone	PTA	10	41	2	4.65
Empty NC	UA	14	24	1	4.0
NC-P_{NTD}	PTA	71	165	110	40.0
NC-P_{CTD}	PTA	35	66	5	7.04