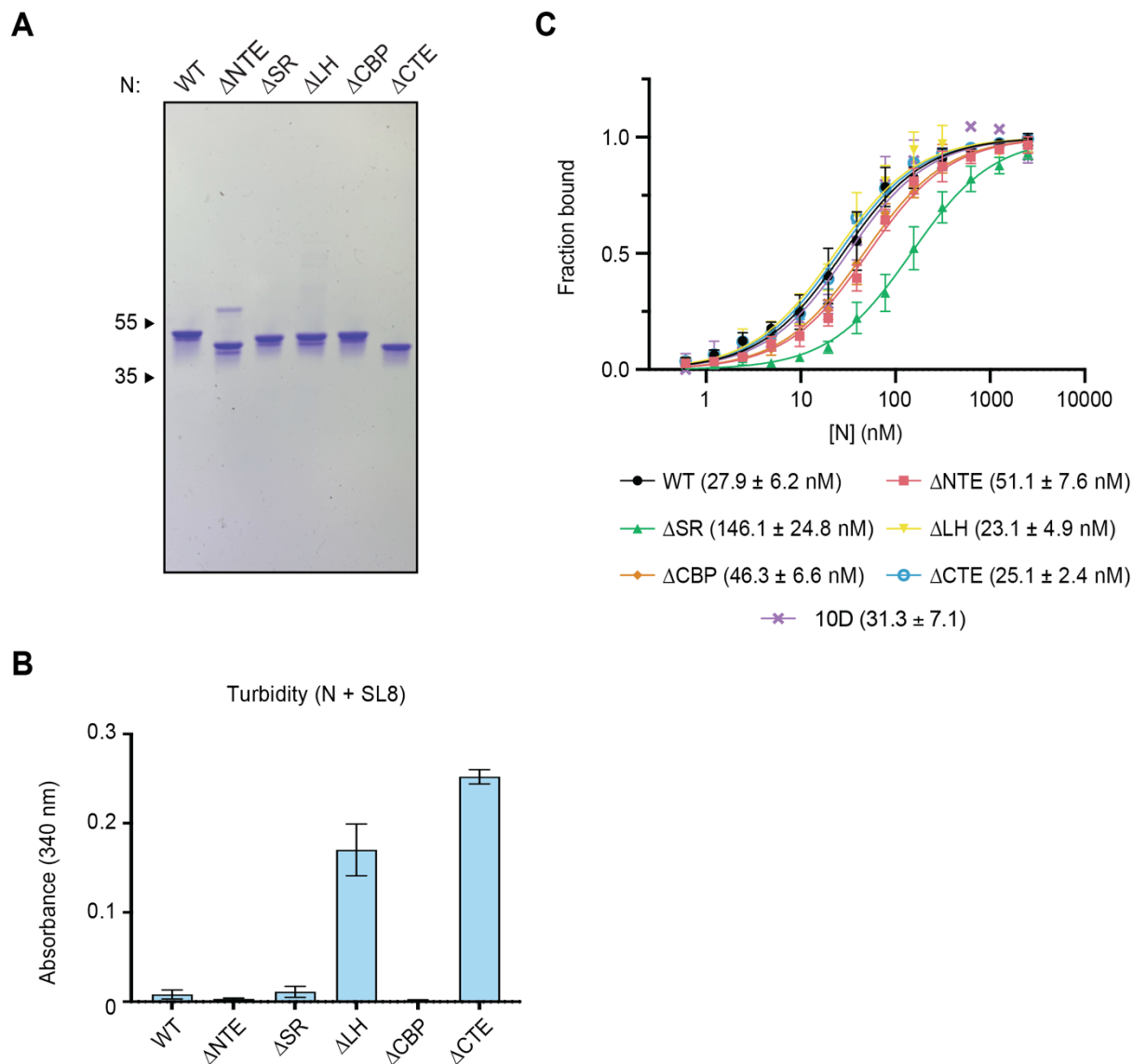
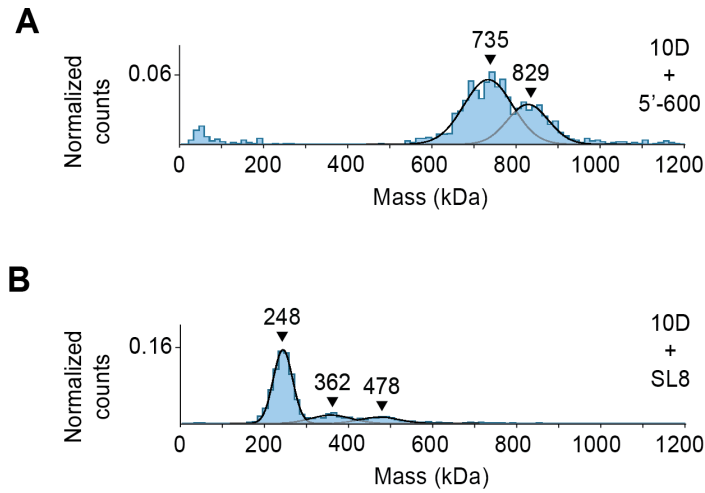


**Fig S1. vRNP formation with stem-loop RNAs.** (A) Denaturing gel electrophoresis of N protein mixed with indicated RNAs, related to Fig 2b. (B) Mass photometry analyses of crosslinked N protein complexes with indicated RNAs. Results with SL8 are reproduced in Fig. 2D. Representative of two independent experiments (table S1). (C) N protein in complex with SL8 RNA was separated by glycerol gradient centrifugation in the presence of crosslinker (GraFix) and analyzed by native gel electrophoresis. (D) Fractions 7 and 8 of GraFix-purified N-SL8 vRNPs (from C) were combined and analyzed by mass photometry. Representative of two independent experiments (table S1). (E) N protein was combined with SL8 RNA or mutant SL8 RNA (mSL8), crosslinked, and analyzed by native (top) and denaturing (bottom) gel electrophoresis. Predicted secondary structures are shown below. See table S2 for sequences.



**Fig S2. Analysis of N protein deletion mutants.** (A) SDS-PAGE of N protein constructs used in this study, stained with Coomassie Blue. Molecular weight markers at left (kDa). (B) Absorbance at 340 nm was used to quantify the turbidity of wild-type and mutant N proteins mixed with SL8 RNA. All values are normalized to absorbance at 260 nm. (C) The indicated concentrations of N protein were incubated with 10 nM RNA (an entirely degenerate 10-nt RNA oligo with a 3'-FAM modification) and fluorescence anisotropy was measured. Data points reflect mean  $\pm$  SEM of three independent experiments.  $K_D$  of each mutant is shown below.



**Fig S3. Analysis of complex formation by 10D mutant.** Mass photometry analysis of GraFix-purified **(A)** fractions 7 + 8 of N protein in complex with 5'-600 RNA and **(B)** fractions 19 + 20 of N protein in complex with SL8 RNA. Representative of two independent experiments (table S1).

**Table S1. Summary of mass photometry results (kDa).**

Figure 1									
Figure 1C									
5'-400			5'-600			5'-800			
peak	rep 1	rep 2	mean ± SD	rep 1	rep 2	mean ± SD	rep 1	rep 2	mean ± SD
1	630	631	630.5 ± 0.7	802	853	827.5 ± 36	756	721	738.5 ± 24.7
2	739	728	733.5 ± 7.8				1418	1613	1515.5 ± 137.9

Figure 1E						
-XL			+XL			
peak	rep 1	rep 2	mean ± SD	rep 1	rep 2	mean ± SD
1	95	98	96.5 ± 2.2	724	714	719 ± 7.1
2	202	211	206.5 ± 6.4			
3	761	770	765.5 ± 6.4			

Figure 2										
Figure 2D										
-SL8 / -XL			+SL8 / -XL			+SL8 / +XL (from Fig S1B)				
peak	rep 1	rep 2	mean ± SD	rep 1	rep 2	mean ± SD	rep 1	rep 2	rep 3	mean ± SD
1	96	98	97 ± 1.4	110	105	107.5 ± 3.5	732	728	749	736.5 ± 11.2
2	196	202	199 ± 4.2	232	218	225 ± 9.9	840	829	852	840.3 ± 11.5
3				361	358	359.5 ± 2.1				
4				487	448	467.5 ± 27.6				
5				622	578	600 ± 31.1				
6				755	717	736 ± 26.9				

Figure 3										
Figure 3D										
WT (from Fig S1B)				ΔNTE			ΔSR			
peak	rep 1	rep 2	rep 3	mean ± SD	rep 1	rep 2	mean ± SD	rep 1	rep 2	mean ± SD
1	732	728	749	736.5 ± 11.2	701	702	701.5 ± 1	236	224	230 ± 8.5
2	840	829	852	840.3 ± 11.5	775	804	789.5 ± 20.5	357	324	340.5 ± 23.3
3								472	432	452 ± 28.3
4								589	552	570.5 ± 26.2
5								706	689	697.5 ± 12
6								844	831	837.5 ± 9.2

Figure 3D (continued)									
ΔLH			ΔCBP			ΔCTE			
peak	rep 1	rep 2	mean ± SD	rep 1	rep 2	mean ± SD	rep 1	rep 2	mean ± SD
1	100	113	106.5 ± 9.2	92	107	99.5 ± 10.6	n/a	n/a	n/a
2	214	230	222 ± 11.3	200	215	207.5 ± 10.6			
3	335	345	340 ± 7.1	303	332	317.5 ± 20.5			
4				428	436	432 ± 5.7			
5				562	568	565 ± 4.2			
6									

Figure 4										
Figure 4D										
WT (from Fig S1B)				ΔSR (from fig 3)			10D			
peak	rep 1	rep 2	rep 3	mean ± SD	rep 1	rep 2	mean ± SD	rep 1	rep 2	mean ± SD
1	732	728	749	736.5 ± 11.2	236	224	230 ± 8.5	231	230	230.5 ± 0.7
2	840	829	852	840.3 ± 11.5	357	324	340.5 ± 23.3	346	348	347 ± 1.4
3					472	432	452 ± 28.3	456	452	454 ± 2.8
4					589	552	570.5 ± 26.2	569	540	554.5 ± 20.5
5					706	689	697.5 ± 12			
6					844	831	837.5 ± 9.2			

Figure 5						
Figure 5D						
WT (+kinases)			S188A + S206A (+kinases)			
peak	rep 1	rep 2	mean ± SD	rep 1	rep 2	mean ± SD
1	235	207	221 ± 19.8	736	730	733 ± 4.2
2	335	308	321.5 ± 19.1			
3	445	447	446 ± 1.4			

Figure S1										
Figure S1B										
SL4a			SL7			SL8				
peak	rep 1	rep 2	mean ± SD	rep 1	rep 2	mean ± SD	rep 1	rep 2	rep 3	mean ± SD
1	528	501	514.5 ± 19.1	520	484	502 ± 25.5	732	728	749	736.5 ± 11.2
2	742	735	738.5 ± 4.9	630	599	614.5 ± 21.9	840	829	852	840.3 ± 11.5
3	876	875	875.5 ± 0.7	722	703	712.5 ± 13.4				

Figure S1D			
SL8 (GRAFIX)			
peak	rep 1	rep 2	mean ± SD
1	743	736	739.5 ± 4.9

Figure S3			
Figure S3A			
10D + 5'-600 (GRAFIX)			
peak	rep 1	rep 2	mean ± SD
1	735	725	730 ± 7.1
2	829	817	823 ± 8.5

Figure S3B			
10D + SL8 (GRAFIX)			
peak	rep 1	rep 2	mean ± SD
1	248	249	248.5 ± 0.7
2	362	367	364.5 ± 3.5
3	478	487	482.5 ± 6.4

**Table S2. RNA sequences used in this study.**

5'-400	<p>                     auuaaagguuuauaccuucccagguaacaaaccaaccaacuucgaucucuuguagaucug                      uucucuaaacgaacuuuaaaaucuguguggcugucacucggcugcaugcuuagugcacucac                      gcaguauaauuaaauaacuaauuacugucguugacaggacacgaguaacucgucuaucuuuc                      gcaggcugcuuacgguuucguccguguugcagccgaucaucagcacaucuaagguuucguccg                      ggugugaccgaaagguaaagauggagagccuugucccugguuucaacgagaaaacacacguc                      caacucaguuugccuguuuuacagguucgagcugcugcguacguggcuuuggagacuccgu                      ggaggaggucuuauacagaggcacgucaacu                 </p>
5'-600	<p>                     auuaaagguuuauaccuucccagguaacaaaccaaccaacuucgaucucuuguagaucug                      uucucuaaacgaacuuuaaaaucuguguggcugucacucggcugcaugcuuagugcacucac                      gcaguauaauuaaauaacuaauuacugucguugacaggacacgaguaacucgucuaucuuuc                      gcaggcugcuuacgguuucguccguguugcagccgaucaucagcacaucuaagguuucguccg                      ggugugaccgaaagguaaagauggagagccuugucccugguuucaacgagaaaacacacguc                      caacucaguuugccuguuuuacagguucgagcugcugcguacguggcuuuggagacuccgu                      ggaggaggucuuauacagaggcacgucaacuuaaagauggcacuuguggcuuaguagaa                      guugaaaaaggcguuuugccucaacuugaacagcccuauguguucaucaaacguucggau                      cucgaacugcaccucauggucauguuauugguugagcugguagcagaacucgaaggcauuc                      guacggucguaguggugagacacuugguguccuuguccucauguggg                 </p>
5'-800	<p>                     auuaaagguuuauaccuucccagguaacaaaccaaccaacuucgaucucuuguagaucug                      uucucuaaacgaacuuuaaaaucuguguggcugucacucggcugcaugcuuagugcacucac                      gcaguauaauuaaauaacuaauuacugucguugacaggacacgaguaacucgucuaucuuuc                      gcaggcugcuuacgguuucguccguguugcagccgaucaucagcacaucuaagguuucguccg                      ggugugaccgaaagguaaagauggagagccuugucccugguuucaacgagaaaacacacguc                      caacucaguuugccuguuuuacagguucgagcugcugcguacguggcuuuggagacuccgu                      ggaggaggucuuauacagaggcacgucaacuuaaagauggcacuuguggcuuaguagaa                      guugaaaaaggcguuuugccucaacuugaacagcccuauguguucaucaaacguucggau                      cucgaacugcaccucauggucauguuauugguugagcugguagcagaacucgaaggcauuc                      guacggucguaguggugagacacuugguguccuuguccucaugugggcaauaccagug                      gcuuaccgcaagguucuuucguuagaacgguaauaaaggagcugguggcauaguuaacg                      gcgccgaucuaaagucuuugacuuaggcagcagcugcugcugaucuuuugaaguuuu                      ucaagaaaacuggaacacuaaacaauagcagugguguuacccgugaacucaugcugagcuu                      aacg                 </p>
Nsp3	<p>                     uuaugaaagacauucucucucucuuuuuguuuacuuagacaaccugagagcuaauaacacu                      aaagguucauugccuauuaauguuauaguuuuuugaugguaaaucuaaaaugugaagaauca                      ucugcaaaaucagcugcuguuuacuacagucagcuuauugugucaaccuauacuguuacuag                      aucaggcauuagugucugauguuggugauagugcgggaaguugcaguuaaaauguuugauc                      uuacguuaauacguuuuaucauacuuuuacguaccaauaggaaaaacuaaaacacuaguu                      gcaacugcagaagcugaacuugcaagaauuguccuuaagacaauugcuuauacuacuuuu                      uuucagcagcucggcaaggguuugauucagauagaaacuaaagauguuuguugaug                      ucuuaaaauugucacaucauacugacauagaaguuaucuggcgauaguuguaauaacuauaug                      cucaccuuaaacaaguuugaaaacaugacaccccugaccuuggugcuuguauugacugua                      gugcgcgucuuuuauugcgcagguagcaaaaagucacaacauugcuuugau                 </p>

Nsp8/9	gccaugcaacguaaguuggaaaagauggcugaucaagcuauagacccaaauguauaaacagg cuagaucugaggacaagagggcaaaaguacuagugcuauagcagacaauugcuuuucacuau gcuuagaaaaguuggauaaugaugcacucaacaacauuaucaacaauugcaagagaugguugu guucccuugaacauaaauaccucuuaacaacagcagccaaacuaaugguugucacuaccagacua uaacacauauaaaaauacgugugaugguacaacauuuacuuaugcaucagcauugugggaa auccaacagguuguagauagcagauaguaaaaauugucaacuagugaaaauaguaggaca auucaccuaauuuagcauggccucuuauguaacagcuuaagggccaaauucugcugucua auuacagaauaaugagcuuaguccuguugcacuacgacagaugucuugugcugccgguacu acacaaacugcuugcacugaugacaauugcguuagcuuacuacaacaacaagggaggua gguuuguacuugcacuguuauccgauuuacaggauuugaaaugggcuaga
Nsp10	ggaagccaauauggaucaagaauccuuugguggugcaucguguugucuguacugccguugc cacauagaucauccaaauccuaaaggauuuugugacuuaaaagguaaguauguacaaaauac cuacaacuugugcuauaugacccugugguuuuacacuuaaaaacacagucuguaccgucugc gguauguggaaagguuauggcuguaguugugaucaacuaccggaacccaugcuucagucag cugaugcacaaucguuuuuaaacgguuugcgguguaagugcagcccgucuuacaccgugc ggcacaggcacuaguacugaugucguauacagggcuuuugacaucuacaaugauaaaaguag cugguuuugcuaaaauccuaaaaaacuaauuguugucgcuuccaagaaaaggacgaagauga caauuuauuugauucuuacuauuaguuaagagacacacuuucucuaacuaccaacaugaa gaaacaauuuauuuuuacuuaaggauuuguccagcuguugcuuaacaugacuucuuuagu uuagaauagacggugacaugguaccacauauaucacgucaacgucuuacu
SL4a	uuaaaaucuguguggcugucacucggcugcaugcuuagugcacucacgcaguauaa
SL7	acguggcuuuggagacuccguggaggaggucuuaucaagaggcacgu
SL8	gauggcacuuguggcuuaguagaaguugaaaaaggcguuuugccucaacuugaacagcccu auguguuacuc
mSL8	gauAgcaAuuguAAcuuaguagUCACGgaaaaagUcguuuugccucaacuugaacagc ccuauAugAucauc