

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									
		5'-400		1	Figure 10		1	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	0
2	735	728	/33.3 ± 7.8				1410	1015	1313.3 ± 137.	5
		14	Figu	ire 1E						
peak	rep 1	-XL ren 2	mean + SD	rep 1	+XL rep 2	mean + SD				
1	95	98	96.5 ± 2.2	724	714	719 ± 7.1				
2	202	211	206.5 ± 6.4							
3	/61	770	765.5 ± 6.4				l			
					Fi	gure 2				
	-	-SL8 / -XL		I	+SL8 / -XI	uie 20		+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	96 196	98 202	97 ± 1.4	110	105 218	107.5 ± 3.5 225 + 9.9	732	728	749	736.5 ± 11.2
3	150	202	100 1 112	361	358	359.5 ± 2.1	010	025	052	010.0 1 11.
4				487	448	467.5 ± 27.6				
5				622 755	578 717	600 ± 31.1 736 ± 26 9				
0				,55	, 1,	750 1 20.5				1
					r:	aura 3				
	Figure 3									
		WT (fro	m Fig S1B)			ΔΝΤΕ			ΔSR	
peak	rep 1	rep 2	rep 3	mean ± SD	rep 1	rep 2	mean ± SD	rep 1	rep 2	mean ± SD
2	840	829	852	730.5 ± 11.2 840.3 ± 11.5	701	804	701.5 ± 1 789.5 ± 20.5	357	324	230 ± 8.5 340.5 ± 23.3
3								472	432	452 ± 28.3
4								589	552	570.5 ± 26.2
6								706 844	831	697.5 ± 12 837.5 ± 9.2
		ALH		Fig	ure 3D (cont	inued)	1	ACTE		
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 ± 11.3 340 + 7.1	200	215	207.5 ± 10.6				
4	555	545	540 1 7.1	428	436	432 ± 5.7				
5				562	568	565 ± 4.2				
6										_
					Fi	gure 4				
		WT (fro	m Fig S1B)		116	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 ± 0.7 347 + 1.4
2	840	829	852				340 5 + 23 3	346	348	21/
3	840	829	852	640.5 ± 11.5	472	432	340.5 ± 23.3 452 ± 28.3	346 456	348 452	454 ± 2.8
4	840	829	852	640.5 ± 11.5	472 589	432	340.5 ± 23.3 452 ± 28.3 570.5 ± 26.2	346 456 569	348 452 540	454 ± 2.8 554.5 ± 20.5
5 6	840	829	852	840.3 ± 11.5	472 589 706 844	432 552 689 831	340.5 ± 23.3 452 ± 28.3 570.5 ± 26.2 697.5 ± 12 837 5 ± 9 2	456 569	348 452 540	454 ± 2.8 554.5 ± 20.5
3 4 5 6	840	829	852	840.3 ± 11.3	472 589 706 844	432 552 689 831	340.5 ± 23.3 452 ± 28.3 570.5 ± 26.2 697.5 ± 12 837.5 ± 9.2	346 456 569	348 452 540	454 ± 2.8 554.5 ± 20.5
5 6	840	829	852	640.3 ± 11.3	472 589 706 844	432 552 689 831	340.5 ± 23.3 452 ± 28.3 570.5 ± 26.2 697.5 ± 12 837.5 ± 9.2	346 456 569	348 452 540	454 ± 2.8 554.5 ± 20.9
3 4 5 6	840	829	852 Fig Figu	ure 5 re 5D	472 589 706 844	432 552 689 831	340.5 ± 23.3 452 ± 28.3 570.5 ± 26.2 697.5 ± 12 837.5 ± 9.2	346 456 569	348 452 540	454 ± 2.8 554.5 ± 20.5
5 6	840	829 WT (+kinase	852 Figu Figu	ure 5 re 5D \$188/	472 589 706 844	432 552 689 831 kinases)	340.5 ± 23.3 452 ± 28.3 570.5 ± 26.2 697.5 ± 12 837.5 ± 9.2	346 456 569	348 452 540	454 ± 2.8 554.5 ± 20.9
s 4 5 6 peak	840	829 WT (+kinase rep 2 207	852 Figu Figu (rs) 221 + 10 8	ure 5 re 5D re 1 736	472 589 706 844 A + S206A (+ rep 2 730	432 552 689 831 kinases) mean ± SD 733 + 4 2	340.5 ± 23.3 452 ± 28.3 570.5 ± 26.2 697.5 ± 12 837.5 ± 9.2	346 456 569	348 452 540	454 ± 2.8 554.5 ± 20.9
5 6 peak 1 2	840 rep 1 235 335	829 WT (+kinase rep 2 207 308	Figure 5D 221±19.8 321.5±19.1	ure 5 re 5D rep 1 736	472 589 706 844 A + S206A (+ rep 2 730	432 552 689 831 kinases) mean ± SD 733 ± 4.2	340.5 ± 23.3 452 ± 28.3 570.5 ± 26.2 697.5 ± 12 837.5 ± 9.2	346 456 569	348 452 540	454 ± 2.8 554.5 ± 20.5
5 6 peak 1 2 3	840 rep 1 235 335 445	829 WT (+kinase rep 2 207 308 447	Figures Figures rs) 221 ± 19.8 321.5 ± 19.1 446 ± 1.4	ure 5 re 5D S188, rep 1 736	472 589 706 844 A + S206A (+ rep 2 730	432 552 689 831 kinases) mean ± SD 733 ± 4.2	340.5 ± 23.3 452 ± 28.3 570.5 ± 26.2 697.5 ± 12 837.5 ± 9.2	346 456 569	348 452 540	454 ± 2.8 554.5 ± 20.5
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s 4 5 6 1 2 3 ypeak 1	840 rep 1 235 335 445 rep 1 528	829 WT (+kinase rep 2 207 308 447 SL4a rep 2 501	852 Figure Figure State	040.3 ± 11.3 re 50 50 rep 1 736 rep 1 736	472 589 706 844 A + S206A (+ rep 2 730 Fig Fig SL7 rep 2 484	432 552 689 831 kinases) mean ± SD 733 ± 4.2 cure S1 ure S1 mean ± SD 502 ± 25.5	340.5 ± 23.3 452 ± 28.3 570.5 ± 26.2 697.5 ± 12 837.5 ± 9.2	340 456 569 	348 452 540 <u>540</u> <u>518</u> <u>rep 3</u> 749	454 ± 2.8 554.5 ± 20.9 mean ± 5D 736.5 ± 11.7
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s 4 5 6 1 2 3 peak 1 2 3 peak 1 2 3 peak 1 2 3	840 rep 1 235 335 445 rep 1 528 742 876 rep 1 743 rep 1 743	829 WT (+kinase rep 2 207 308 447 SL4a rep 2 501 735 S14 735 S15 Figure S18 SL8 (GRAFI) rep 2 736 Figure S18 Figure S18 F	Fig Fig Figure S1 mean ± SD 221 ± 19.8 321.5 ± 19.1 446 ± 1.4 mean ± SD 514.5 ± 19.1 738.5 ± 4.9 875.5 ± 0.7 739.5 ± 4.9 X mean ± SD 739.5 ± 4.9 XA AAFIX) mean ± SD 730 ± 7.1 823 ± 8.5 AFIX	040.3 ± 11.3 ree 5 ree 50 rep 1 736 rep 1 520 630 722	472 589 706 844 A + \$206A (+ rep 2 730 Fig Fig SL7 rep 2 484 599 703	432 552 689 831 kinases) 733 ± 4.2 733 ± 4.2 733 ± 4.2 502 ± 25.5 614.5 ± 21.9 712.5 ± 13.4	340.5 ± 23.3 452 ± 28.3 570.5 ± 26.2 697.5 ± 12 837.5 ± 9.2	346 455 569 rep 2 728 829	348 452 540 <u>518</u> <u>rep 3</u> 749 852	454 ± 2.8 554.5 ± 20.9 765.5 ± 11.1 840.3 ± 11.9
s 4 5 6 1 2 3 3 peak 1 2 3 peak 1 2 3 peak	840 rep 1 235 335 445 rep 1 528 742 876 rep 1 743 100 rep 1 735 829 11 rep 1	829 WT (+kinase rep 2 207 308 447 447 501 735 875 Figure S10 518 (GRAFD rep 2 736 Figure S13 Figure S13 Figure S13 Figure S13 Figure S14 Figure S15 Figure S15 Figure S16 Figure	852 Fig mean ± SD 221 ± 19.8 221 ± 19.4 446 ± 1.4 mean ± SD 514.5 ± 19.1 738.5 ± 4.9 735.5 ± 0.7 XX mean ± SD 739.5 ± 4.9 730.5 ± 7.1 823 ± 8.5 AFIX) mean ± SD 730.5 ± 7.1 87.5 ± 5.5 AFIX)	040.3 ± 11.3 re 50 5188/ rep 1 736 736 722	472 589 706 844 A + S206A (+ rep 2 730 Fig Fig SL7 rep 2 484 599 703	432 552 689 831 kinases) 733 ± 4.2 733 ± 4.2 yure S1B mean ± SD 502 ± 25.5 614.5 ± 25.1 712.5 ± 13.4	340.5 ± 23.3 452 ± 28.3 570.5 ± 26.2 697.5 ± 12 837.5 ± 9.2 rep 1 732 840	346 456 569 rep 2 728 829	348 452 540 <u>540</u> <u>518</u> <u>rep 3</u> 749 852	454 ± 2.8 554.5 ± 20.9 736.5 ± 11.2 840.3 ± 11.9
s 4 5 6 1 2 3 9 peak 1 2 3 9 peak 1 2 3 9 peak 1 2 3 9 peak 1 2 3 9 peak 1 2 3 9 peak 1 2 3 9 peak	840 rep 1 235 335 345 rep 1 528 742 876 rep 1 743 743 100 rep 1 735 829 10 rep 1 248	829 WT (+kinase rep 2 207 308 447 447 514 rep 2 503 518 (GRAFI) rep 2 736 Figure S18 518 (GRAFI) rep 2 736 Figure S38 Pigure S38 0+ 5-600 (GG rep 2 725 817 Figure S38 00 + 5L8 (GRAFI) rep 2 725 817	852 Fig. rean ± SD 221 ± 198. 321.5 ± 19.1 446 ± 1.4 mean ± SD 514.5 ± 19.1 738.5 ± 4.9 773.5 ± 0.7 738.5 ± 4.9 739.5 ± 4.9 730.5 ± 4.9 AKFIX mean ± SD 730.5 ± 7.1 823 ± 8.5 AFIX mean ± SD 730.5 ± 1.9 730.5 ± 1.9 730.5 ± 3.9 730.5 ± 4.9 XAFIX mean ± SD 730.5 ± 4.9 XAFIX mean ± SD 730.5 ± 7.1 823.5 ± 0.7 734.5 ± 0.7 748.5 ± 0.7 734.5 ± 0.7 734.5 ± 0.7 734.5 ± 0.7 734.5 ± 0.7 734.5 ± 0.7 734.5 ± 0.7 734.5 ± 0.7 734.5 ± 0.7 734.5 ± 0.7 734.5 ± 0.7 734.5 ± 0.7 734.5 ± 0.7 <td>040.3 ± 11.3 re 50 5188/ rep 1 736 722</td> <td>472 589 706 844 A + S206A (+ rep 2 730 Fig Fig 517 rep 2 484 599 703</td> <td>432 552 689 831 mean ± SD 733 ± 4.2 mean ± SD 502 ± 25.5 614.5 ± 25.5 614.5 ± 25.5</td> <td>340.5 ± 23.3 452 ± 28.3 570.5 ± 26.2 697.5 ± 12 837.5 ± 9.2 rep 1 732 840</td> <td>346 455 569 rep 2 728 829</td> <td>348 452 540 <u>540</u> <u>540</u> <u>749</u> 852</td> <td>454 ± 2.8 554.5 ± 20.9 736.5 ± 11.2 840.3 ± 11.9</td>	040.3 ± 11.3 re 50 5188/ rep 1 736 722	472 589 706 844 A + S206A (+ rep 2 730 Fig Fig 517 rep 2 484 599 703	432 552 689 831 mean ± SD 733 ± 4.2 mean ± SD 502 ± 25.5 614.5 ± 25.5 614.5 ± 25.5	340.5 ± 23.3 452 ± 28.3 570.5 ± 26.2 697.5 ± 12 837.5 ± 9.2 rep 1 732 840	346 455 569 rep 2 728 829	348 452 540 <u>540</u> <u>540</u> <u>749</u> 852	454 ± 2.8 554.5 ± 20.9 736.5 ± 11.2 840.3 ± 11.9

Table S2. RNA sequences used in this study.

Table S2	. RNA sequences used in this study.
5'-400	auuaaagguuuauaccuucccagguaacaaaccaaccaac
5'-600	auuaaagguuuauaccuucccagguaacaaaccaaccaac
5'-800	auuaaagguuuauaccuucccagguaacaaaccaaccaac
Nsp3	uuaugaaagacauucucucucucauuuuguuaacuuagacaaccugagagcuaauaacacu aaagguucauugccuauuaauguuauaguuuuugaugguaaaucaaaaugugaagaauca ucugcaaaaucagcgucuguuuacuacagucagcuuaugugucaaccuauacuguuacuag aucaggcauuagugucugauguuggugauagugcggaaguugcaguuaaaauguuugaugc uuacguuaauacguuuucaucaacuuuuaacguaccaauggaaaaacucaaaaacacuaguu gcaacugcagaagcugaacuugcaaagaauguguccuuagacaaugucuuaucuacuuuua uuucagcagcucggcaaggguuuguugauucagauguagaaacuaaagauguuguugaaug ucuuaaauugucacaucaaucugacauagaaguuacuggcgauaguuguaauaacuauaug gugcgcgucauauuaaugcgcagguagcaaaagucacaacuuguuguugau gugcgcgucauauuaaugcgcagguagcaaaagucacaacauugcuuugau

Nsp8/9	gccaugcaacguaaguuggaaaagauggcugaucaagcuaugacccaaauguauaaacagg cuagaucugaggacaagagggcaaaaguuacuagugcuaugcagacaaugcuuuucacuau gcuuagaaaguuggauaaugaugcacucaacaacauuaucaacaaugcaagagaugguugu guucccuugaacauaauaccucuuacaacagcagccaaacuaaugguugucauaccagacua uaacacauauaaaaauacgugugaugguacaacauuuacuuaugcaucagcauugugggaa auccaacagguuguagaugcagauaguaaaauuguucaacuuagugaaauuaguauggaca auucaccuaauuuagcauggccucuuauuguaacagcuuuaagggccaauucugcugucaa auuacagaauaaugagcuuaguccuguugcacuacgacagaugucuugugugac acacaaacugcuugcacugaugacaaugcguuagcuuacuacaacaacaaaagggaggua gguuuguacuugcacugaugacaaugcguuagcuuacuacaacaacaaaagggaggua
Nsp10	ggaagccaauauggaucaagaauccuuugguggugcaucguguugucuguacugccguugc cacauagaucauccaaauccuaaaggauuuugugacuuaaaagguaaguauguacaaauac cuacaacuugugcuaaugacccuguggguuuuacacuuaaaaacacagucuguaccgucugc gguauguggaaagguuauggcuguaguugugaucaacuccgcgaacccaugcuucagucag
SL4a	uuaaaaucuguguggcugucacucggcugcaugcuuagugcacucacgcaguauaa
SL7	acguggcuuuggagacuccguggaggaggucuuaucagaggcacgu
SL8	gauggcacuuguggcuuaguagaaguugaaaaaggcguuuugccucaacuugaacagcccu auguguucauc
mSL8	gauAgcaAuuguAAcuuaguagUCACGgaaaaagUcguuuugccucaacuugaacagc ccuauAugAucauc