Supplemental Material

Supplemental Table S1 List of NSP5-interacting proteins identified by mass spectrometry. Pull-down complexes NSP5 from control cell extracts were analysed by mass spectrometry.NSP5C, NSP5-bound beads not incubated with extract; NSP51, NSP52 and NSP53 represent the three regions of the SDS-PAGE gel from top to bottom. Proteins that are undetectable in the extract-incubated control beads are

											NSP5C				NSP51				NSP52				NSP53						
Accession	Description	ΣCoverage	Σ# Proteins	Σ# Unique Peptides	Σ# Peptides	Σ# PSMs	NSP5C: Area	NSP51: Area	NSP52: Area	NSP53: Area	Score A4	Coverage A4	# Peptides A4	# PSM A4	Score B4	Coverage B4	# Peptides B4	# PSM B4	Score C4	Coverage C4	# Peptides C4	# PSM C4	Score D4	Coverage D4	# Peptides D4	# PSM D4	# AAs	MW [kDa]	calc. pI
Q5E9A3	Poly(rC)-binding protein 1 OS=Bos taurus GN=PCBP1 PE=2 SV=1 - [PCBP1_BOVIN]	29.78	1	5	6	6	0.000E0	0.000E0	0.000E0	1.739E8													317.10	29.78	6	6	356	37.5	7.09
Q61990	Poly(rC)-binding protein 2 OS=Mus musculus GN=Pcbp2 PE=1 SV=1 - [PCBP2_MOUSE]	15.47	1	0	3	3	0.000E0	0.000E0	0.000E0	2.096E8													155.01	15.47	3	3	362	38.2	6.79
Q3SZF3	Heterogeneous nuclear ribonucleoprotein H2 OS=Bos taurus GN=HNRNPH2 PE=2 SV=1 - [HNRH2_BOVIN]	22.27	1	0	6	8	0.000E0	0.000E0	0.000E0	6.944E8													366.53	22.27	6	8	449	49.2	6.30
P31942	Heterogeneous nuclear ribonucleoprotein H3 OS=Homo sapiens GN=HNRNPH3 PE=1 SV=2 - [HNRH3_HUMAN]	14.45	1	2	3	3	0.000E0	0.000E0	0.000E0	1.078E8													123.35	14.45	3	3	346	36.9	6.87
P52597	Heterogeneous nuclear ribonucleoprotein F OS=Homo sapiens GN=HNRNPF PE=1 SV=3 - [HNRPF_HUMAN]	26.99	1	4	6	9	0.000E0	0.000E0	0.000E0	7.374E8													390.84	26.99	6	9	415	45.6	5.58
P61978	Heterogeneous nuclear ribonucleoprotein K OS=Homo sapiens GN=HNRNPK PE=1 SV=1 - [HNRPK_HUMAN]	26.13	1	0	8	9	0.000E0	0.000E0	1.007E8	0.000E0									329.25	26.13	8	9					463	50.9	5.54
P51991	Heterogeneous nuclear ribonucleoprotein A3 OS=Homo sapiens GN=HNRNPA3 PE=1 SV=2 - [ROA3_HUMAN]	17.46	1	0	5	6	0.000E0	0.000E0	0.000E0	6.884E7													262.45	17.46	5	6	378	39.6	9.01
Q14103	Heterogeneous nuclear ribonucleoprotein D0 OS=Homo sapiens GN=HNRNPD PE=1 SV=1 - [HNRPD_HUMAN]	6.20	1	0	2	2	0.000E0	0.000E0	0.000E0	1.075E8													86.56	6.20	2	2	355	38.4	7.81
Q8VEK3	Heterogeneous nuclear ribonucleoprotein U OS=Mus musculus GN=Hnrnpu PE=1 SV=1 - [HNRPU_MOUSE]	3.38	1	0	2	2	0.000E0	0.000E0	1.608E7	0.000E0									77.49	3.38	2	2					800	87.9	6.24
Q00839	Heterogeneous nuclear ribonucleoprotein U OS=Homo sapiens GN=HNRNPU PE=1 SV=6 - [HNRPU_HUMAN]	9.09	1	3	5	7	0.000E0	5.288E7	1.608E7	0.000E0					222.63	9.09	5	5	77.49	3.27	2	2					825	90.5	6.00
P52272	Heterogeneous nuclear ribonucleoprotein M OS=Homo sapiens GN=HNRNPM PE=1 SV=3 - [HNRPM_HUMAN]	13.29	1	7	7	7	0.000E0	0.000E0	4.894E7	0.000E0									252.63	13.29	7	7					730	77.5	8.70
P14866	Heterogeneous nuclear ribonucleoprotein L OS=Homo sapiens GN=HNRNPL PE=1 SV=2 - [HNRPL_HUMAN]	10.70	1	4	4	5	0.000E0	0.000E0	4.169E7	0.000E0									230.85	10.70	4	5					589	64.1	8.22
P26599	Polypyrimidine tract-binding protein 1 OS=Homo sapiens GN=PTBP1 PE=1 SV=1 - [PTBP1_HUMAN]	16.76	1	5	5	6	0.000E0	0.000E0	0.000E0	9.062E7													307.35	16.76	5	6	531	57.2	
Q16630	Cleavage and polyadenylation specificity factor subunit 6 OS=Homo sapiens GN=CPSF6 PE=1 SV=2 - [CPSF6_HUMAN]	13.97	1	0	5	5	0.000E0	0.000E0	3.730E7	0.000E0									307.11	13.97	5	5					551	59.2	7.15
Q3T169	40S ribosomal protein S3 OS=Bos taurus GN=RPS3 PE=2 SV=1 - [RS3_BOVIN]	13.99	1	0	3	3	0.000E0	1.324E7	0.000E0	0.000E0					108.37	13.99	3	3									243	26.7	9.66
Q4R8K5	Eukaryotic initiation factor 4A-I OS=Macaca fascicularis GN=EIF4A1 PE=2 SV=1 - [IF4A1_MACFA]	9.85	1	0	3	3	0.000E0	0.000E0	0.000E0	7.317E7													170.56	9.85	3	3	406	46.1	5.48
P33176	Kinesin-1 heavy chain OS=Homo sapiens GN=KIF5B PE=1 SV=1 - [KINH_HUMAN]	2.60	1	0	2	2	0.000E0	1.014E7	0.000E0	0.000E0					81.75	2.60	2	2									963	109.6	6.51
P11021	78 kDa glucose-regulated protein OS=Homo sapiens GN=HSPA5 PE=1 SV=2 - [GRP78_HUMAN]	34.71	1	0	18	21	0.000E0	0.000E0	1.501E8	2.672E7									797.35	34.71	18	19	75.33	4.43	2	2	654	72.3	5.16
P08238	Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4 - [HS90B_HUMAN]	28.04	1	0	18	26	0.000E0	2.597E7	1.634E8	6.164E7					249.04	8.43	5	5	557.48	26.10	17	17	145.24	7.46	4	4	724	83.2	5.03
P63017	Heat shock cognate 71 kDa protein OS=Mus musculus GN=Hspa8 PE=1 SV=1 - [HSP7C_MOUSE]	15.63	1	0	9	10	0.000E0	0.000E0	1.324E8	0.000E0									351.94	15.63	9	10					646	70.8	5.52
P08107	Heat shock 70 kDa protein 1A/1B OS=Homo sapiens GN=HSPA1A PE=1 SV=5 - [HSP71_HUMAN]	10.76	1	4	5	5	0.000E0	0.000E0	7.805E7	0.000E0									179.87	10.76	5	5					641	70.0	5.66
P46109	Crk-like protein OS=Homo sapiens GN=CRKL PE=1 SV=1 - [CRKL_HUMAN]	9.57	1	0	2	2	0.000E0	0.000E0	0.000E0	3.360E7													106.88	9.57	2	2	303	33.8	6.74
P10809	60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2 - [CH60_HUMAN]	5.76	1	0	3	3	0.000E0	0.000E0	2.163E7	0.000E0									107.27	5.76	3	3					573	61.0	5.87
Q96QK1	Vacuolar protein sorting-associated protein 35 OS=Homo sapiens GN=VPS35 PE=1 SV=2 - [VPS35_HUMAN]	5.03	1	0	3	3	0.000E0	0.000E0	1.629E7	0.000E0									96.89	5.03	3	3					796	91.6	5.49
Q92597	Protein NDRG1 OS=Homo sapiens GN=NDRG1 PE=1 SV=1 - [NDRG1_HUMAN]	15.74	1	0	4	4	0.000E0	0.000E0	0.000E0	1.425E8													236.85	15.74	4	4	394	42.8	5.82
Q5R4F4	Protein transport protein Sec31A OS=Pongo abelii GN=SEC31A PE=2 SV=1 - [SC31A_PONAB]	6.60	1	0	6	6	0.000E0	5.844E7	0.000E0	0.000E0					195.92	6.60	6	6									1106	121.6	6.77

Supplemental Table S2 List of cellular proteins interacting with NSP2 identified by mass spectrometry. Pull-down complexes of NSP2 from control cell extracts were analysed by LC-MS/MS. Only those proteins that were detected in bead-bound NSP2 lane but not detected in the control His-tagged-beads are shown. NSP2C represents NSP2-bound beads not incubated with cell extract. NSP21, NSP22 and NSP23 represent three different regions of the SDS-PAGE gel.

											NSP2C				NSP21				NSP22				NSP23						
Accession	Description	ΣCoverage	Σ# Proteins	Σ# Unique Peptides	Σ# Peptides	Σ# PSMs	NSP2C: Area	NSP21: Area	NSP22: Area	NSP23: Area	Score A4	Coverage A4	# Peptides A4	# PSM A4	Score B4	Coverage B4	# Peptides B4	# PSM B4	Score C4	Coverage C4	# Peptides C4	# PSM C4	Score D4	Coverage D4	# Peptides D4	# PSM D4	# AAs	MW [kDa]	calc. pI
H9FXJ8	Polypyrimidine tract-binding protein 1 isoform c OS=Macaca mulatta GN=PTBP1 PE=2 SV=1 - [H9FXJ8_MACMU]	22.26	1	0	6	6	0.000E0	0.000E0	0.000E0	2.217E8													384.20	22.26	6	6	530	57.1	9.17
Q5T6W5	Heterogeneous nuclear ribonucleoprotein K OS=Homo sapiens GN=HNRNPK PE=2 SV=1 - [Q5T6W5_HUMAN]	11.68	1	0	3	4	0.000E0	0.000E0	6.509E7	0.000E0									145.95	11.68	3	4					428	47.5	5.63
E9PCY7	Heterogeneous nuclear ribonucleoprotein H, N-terminally processed OS=Homo sapiens GN=HNRNPH1 PE=4 SV=1 - [E9PCY7_HUMAN]	11.66	1	0	3	3	0.000E0	0.000E0	0.000E0	3.660E8													178.16	11.66	3	3	429	47.1	6.34
H3BKW0	Cleavage and polyadenylation-specificity factor subunit 6 (Fragment) OS=Mus musculus GN=Cpsf6 PE=4 SV=1 - [H3BKW0_MOUSE]	21.43	1	0	2	3	0.000E0	0.000E0	2.535E7	0.000E0									199.40	21.43	2	3					182	21.2	9.73
E9PNE6	Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=3 SV=1 - [E9PNE6_HUMAN]	9.80	1	0	4	4	0.000E0	0.000E0	5.765E7	0.000E0									114.49	9.80	4	4					500	54.9	5.68
B7Z446	Protein NDRG1 OS=Homo sapiens GN=NDRG1 PE=2 SV=1 - [B7Z446_HUMAN]	9.15	1	0	2	2	0.000E0	0.000E0	0.000E0	1.760E8													103.42	9.15	2	2	328	35.5	6.39
A6NIT8	Heterogeneous nuclear ribonucleoprotein L OS=Homo sapiens GN=HNRNPL PE=2 SV=1 - [A6NIT8_HUMAN]	8.11	1	0	2	4	0.000E0	0.000E0	5.387E7	1.227E8									120.59	8.11	2	2	150.94	8.11	2	2	456	50.5	7.50
G5AM31	Heterogeneous nuclear ribonucleoprotein U (Fragment) OS=Heterocephalus glaber GN=GW7_16292 PE=4 SV=1 - [G5AM31_HETGA]	8.02	1	0	4	4	0.000E0	1.351E8	0.000E0	0.000E0					154.01	8.02	4	4									611	69.0	9.17
Q96BA7	HNRPU protein OS=Homo sapiens PE=2 SV=1 - [Q96BA7_HUMAN]	6.79	1	0	4	4	0.000E0	1.351E8	0.000E0	0.000E0					154.01	6.79	4	4									722	79.7	7.87
H9EN93	78 kDa glucose-regulated protein OS=Macaca mulatta GN=HSPA5 PE=2 SV=1 - [H9EN93_MACMU]	6.73	1	0	3	3	0.000E0	0.000E0	3.078E7	0.000E0									102.15	6.73	3	3					654	72.3	5.16
B7Z446	Protein NDRG1 OS=Homo sapiens GN=NDRG1 PE=2 SV=1 - [B7Z446_HUMAN]	9.15	1	0	2	2	0.000E0	0.000E0	0.000E0	1.760E8													103.42	9.15	2	2	328	35.5	6.39
H9FRF8	Protein transport protein Sec31A isoform 2 OS=Macaca mulatta GN=SEC31A PE=2 SV=1 - [H9FRF8_MACMU]	6.70	1	0	4	4	0.000E0	4.757E7	0.000E0	0.000E0					162.79	6.70	4	4									1165	127.4	7.40
Q5JP53	Tubulin beta chain OS=Homo sapiens GN=TUBB PE=3 SV=1 - [Q5JP53_HUMAN]	36.85	1	0	9	14	0.000E0	0.000E0	0.000E0	2.409E9													482.96	36.85	9	14	426	47.7	4.81
F8VVB9	Tubulin alpha-1B chain (Fragment) OS=Homo sapiens GN=TUBA1B PE=4 SV=1 - [F8VVB9_HUMAN]	36.84	1	0	5	11	0.000E0	0.000E0	0.000E0	2.600E9													538.79	36.84	5	11	247	27.5	5.20
Q9JJ54	Heterogeneous nuclear ribonucleoprotein D0 OS=Rattus norvegicus GN=Hnrnpd PE=1 SV=1 - [HNRPD_RAT]	6.23	1	0	2	2	0.000E0	0.000E0	0.000E0	1.075E8													86.56	6.23	2	2	353	38.2	7.81
G2HEQ8	Heat shock protein HSP 90-beta OS=Pan troglodytes PE=2 SV=1 - [G2HEQ8_PANTR]	13.40	1	0	8	9	0.000E0	0.000E0	2.651E8	0.000E0									240.96	13.40	8	9					724	83.1	5.06
E9PNE6	Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=3 SV=1 - [E9PNE6_HUMAN]	9.80	1	0	4	4	0.000E0	0.000E0	5.765E7	0.000E0									114.49	9.80	4	4					500	54.9	5.68
E9PKE3	Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=3 SV=1 - [E9PKE3_HUMAN]	7.81	1	0	4	4	0.000E0	0.000E0	5.765E7	0.000E0									114.49	7.81	4	4					627	68.8	5.52
Q53EU7	N-myc downstream regulated gene 1 variant (Fragment) OS=Homo sapiens PE=2 SV=1 - [Q53EU7_HUMAN]	7.61	1	0	2	2	0.000E0	0.000E0	0.000E0	1.760E8													103.42	7.61	2	2	394	42.8	5.82
H9EN93	78 kDa glucose-regulated protein OS=Macaca mulatta GN=HSPA5 PE=2 SV=1 - [H9EN93_MACMU]	6.73	1	0	3	3	0.000E0	0.000E0	3.078E7	0.000E0									102.15	6.73	3	3					654	72.3	5.16

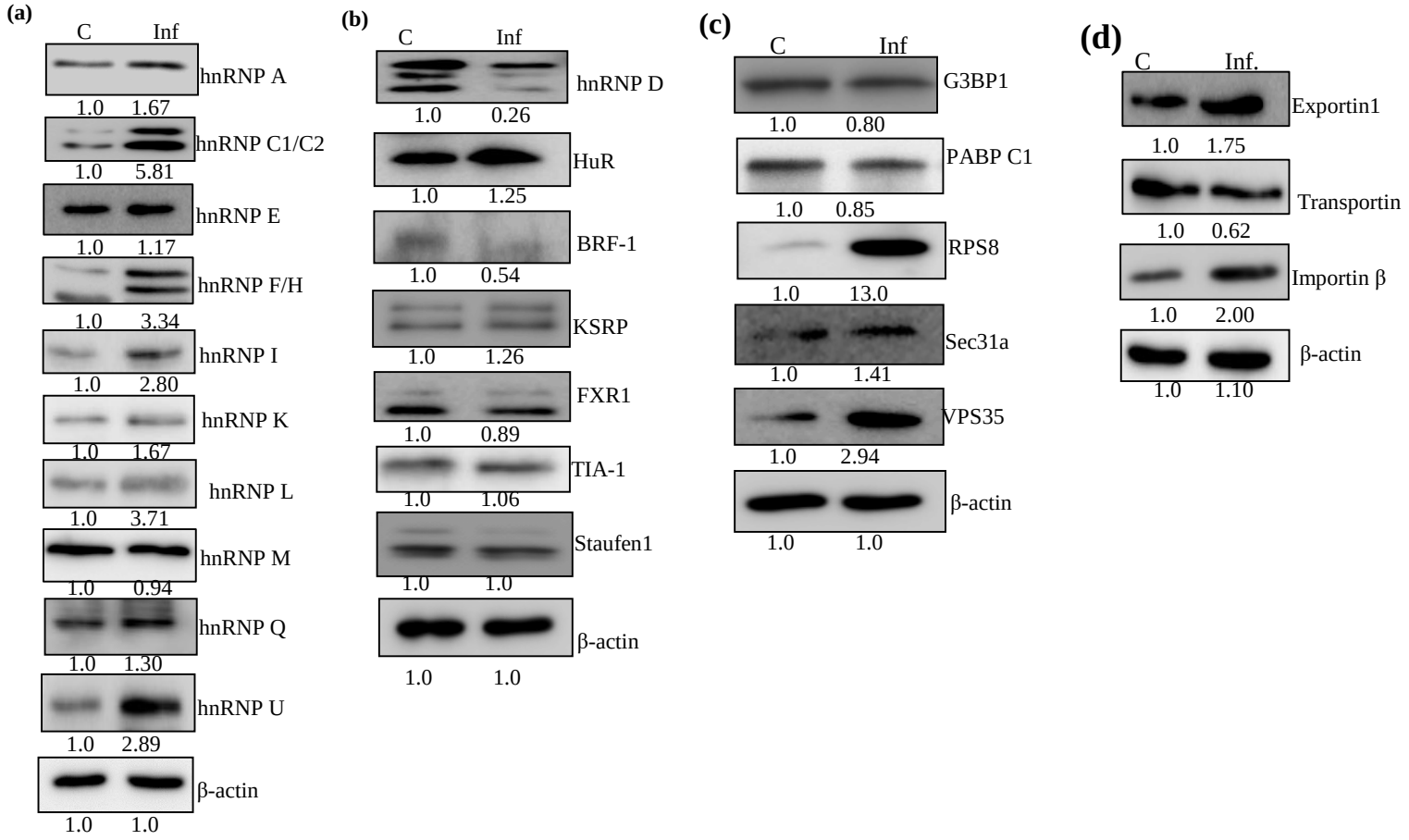
Supplemental Table S3 Time-dependent fold change in the level of cellular proteins in the cytoplasm and the nucleus during the course of rotavirus infection. The fold change in the cytoplasm or the nucleus was calculated by normalizing the values at different time points with reference to that in the respective compartments in the serum-grown control cells (Figs. 2, 3, 4 and 6). The fold changes in the cytoplasm and nucleus of host protein levels in each figure are normalized with respect to the levels of β-actin and PCNA, respectively, shown in the same figure. For proteins hnRNP A1, D, F/H, K/J, L, Q and U, HuR, KSRP and BRF1 which were undetectable in the cytoplasm of control cells, the fold change in the cytoplasm with reference to that in serum-control control cells could not be calculated. So it was calculated by normalizing the values with reference to that of their first appearance in the respective compartments during the course mock infection or virus infection. For example, the fold change for hnRNP L in cytoplasm of mock-infected cells was calculated with reference to that at 2 hr post serum starvation. Quantification of band intensities in western blots were done using Image J software. S.C., serum-grown control cells. The significant fold changes in the protein levels at 8 hpi are highlighted.

S.No.	Cellular protein	Cytoplasm	Mock-infected Cytoplasm (hpi)				Infected Cytoplasm (hpi)				Nucleus	Mock-infected Nucleus (hpi)				Infected Nucleus (hpi)			
		S.C	2	4	8	10	2	4	8	10	S.C.	2	4	8	10	2	4	8	10
1	Importin-β	1.0	1.1	1.2	0.9	1.0	1.0	1.6	2.0	2.0	1	1.4	0.9	1.4	1.4	1.3	1.3	1.4	1.4
2	Exportin1	1.0	0.8	0.9	0.4	0.4	1.2	1.9	2.8	3.0	1	1.4	1.3	1.3	1.4	1.4	1.3	1.4	1.4
3	Transportin	1.0	1.1	0.9	0.9	1.0	0.8	0.8	1.0	1.3	1	0.9	1.0	1.0	0.7	0.6	0.8	0.2	0.3
4	Ran	1.0	0.9	0.9	0.8	0.9	1.3	1.7	2.7	1.9	1	1.5	0.9	1.0	0.8	1.2	1.3	1.3	1.3
5	KSRP	1.0	1.2	0.5	1.2	0.2	1.8	1.2	3.0	6.0	1	0.6	1.5	1.4	1.8	1.3	1.9	1.7	2.2
6	TTP	1.0	0.6	0.9	1.3	1.6	1.1	2.0	1.2	1.1	1	1.3	1.4	1.2	1.2	1.0	1.1	0.9	1.2
7	FXR1	1.0	1.2	1.3	1.1	1.4	1.4	1.9	1.4	1.1	1	0.5	0.4	0.3	0.2	0.5	0.4	0.4	0.3
8	hnRNP D	--	---	---	---	---	---	1.0	1.4	1.1	1	0.8	0.9	1.0	0.7	1.0	0.1	---	---
9	HuR	--	---	---	---	---	---	1.0	11.4	7.8	1	0.7	0.8	0.3	0.5	0.8	0.4	0.2	---
10	BRF1	--	---	---	---	---	1.0	1.2	2.3	3.1	1	0.6	0.8	1.2	1.2	1.5	0.2	---	---
11	TIAL-1	1.0	2.2	3.3	2.5	3.3	2.5	3.1	2.2	1.6	1	0.8	0.9	0.8	0.8	0.8	0.5	0.4	0.2
12	TIA-1	1.0	2.0	1.3	0.4	0.2	0.3	1.8	1.4	1.6	1	2.5	0.6	2.0	2.7	1.9	0.8	1.8	1.6
13	hnRNP A1	1.0	0.6	2.9	0.6	0.8	2.2	9.5	21.0	21.1	1	0.8	0.7	0.4	0.4	0.6	0.5	0.4	0.06
14	hnRNP C1/C2	1.0	1.6	2.6	1.9	3.0	1.4	2.0	2.3	4.8	1	0.8	0.6	0.3	0.4	0.2	0.6	0.8	0.7
15	hnRNP E (PCBP1)	1.0	1.3	1.1	2.2	2.0	1.6	1.6	2.8	2.1	1	2.0	2.0	1.7	0.8	1.1	2.2	2.5	2.1
16	hnRNP F/H	---	1.0	0.5	2.5	0.9	3.1	5.8	7.9	5.6	1	2.0	0.8	2.2	2.6	2.0	2.0	2.2	2.7
17	hnRNP I (PTBP1)	1.0	1.3	0.9	0.9	1.0	1.9	2.6	5.4	9.6	1	1.4	1.1	1.4	1.4	1.1	1.0	0.8	0.4
18	hnRNP K/J	---	---	---	---	---	1.0	1.6	5.4	2.5	1	1.0	1.0	1.0	1.0	1.0	0.9	0.6	0.2
19	hnRNP L	---	1.0	2.3	6.8	7.6	4.2	9.3	9.8	28.0	1	0.9	0.9	0.4	0.6	0.5	0.7	0.5	0.1
20	hnRNP M	1.0	0.7	1.4	1.3	1.7	1.1	2.5	4.3	2.0	1	0.6	0.9	0.7	0.8	1.0	0.8	0.6	0.6
21	hnRNP Q	---	---	---	---	---	---	---	---	---	1	1.8	1.1	1.6	1.7	1.5	1.3	1.6	1.5
22	hnRNP U	---	---	---	---	---	1.0	1.2	1.8	1.6	1	2.3	1.6	2.7	2.3	2.2	2.4	2.0	1.5
23	Staufen-1	1.0	1.0	1.1	0.7	0.8	1.0	1.1	1.3	1.3	1	1.7	1.1	1.6	1.6	1.6	1.4	1.9	1.8
24	PABP C1	1.0	1.2	3.5	0.7	0.5	0.4	0.5	0.1	0.1	1.0	3.1	4.0	5.5	2.5	3.1	5.8	9.8	2.5
25	Sec31A	1.0	0.9	0.7	0.8	0.06	0.6	0.7	3.7	3.0	---	---	---	---	---	---	---	----	----
26	VPS35	1.0	2..1	3.2	4.4	2.5	2.7	5.6	6.8	7.5	---	---	---	---	---	---	---	---	---
27	RPS8	1.0	0.8	1.0	0.6	0.8	0.5	0.5	2.3	0.8	1.0	1.8	10.8	9.0	2.6	2.0	7.2	10.6	2.0
28	β-actin (for FIG. 2c, 2d)	1.0	1.1	1.2	1.0	1.1	1.1	1.0	1.0	1.2	--	---	---	---	---	---	---	---	---
29	β-actin (for FIG. 3c)	1.0	0.7	1.0	0.9	1.0	1.0	1.0	1.0	1.0	--	---	---	---	---	---	---	---	---
30	β-actin (for FIG. 6a)	1.0	1.2	1.1	1.0	1.2	1.0	1.1	1.0	0.9	--	---	---	---	---	---	---	---	---
31	β-actin (for FIG. 3d-e)	1.0	0.9	0.7	0.8	0.7	1.0	0.8	0.9	1.0	--	---	---	---	---	---	---	---	---
32	PCNA (for FIG. 2c,2d)	--	---	---	---	---	---	---	---	---	1	1.4	1.4	1.6	1.8	1.4	1.5	1.7	1.4
33	PCNA (for FIG. 3c)	--	---	---	---	---	---	---	---	---	1	0.8	1.2	0.8	0.8	1.5	1.2	0.9	1.0
34	PCNA (for FIG. 6a)	--	---	---	---	---	---	---	---	---	1	1.2	1.0	1.1	1.2	1.0	1.0	1.2	1.0
35	PCNA (for FIG. 3d-e)	--	---	---	---	---	---	---	---	---	1.0	1.0	1.0	0.9	0.9	0.8	1.0	1.0	0.7

Supplemental Table S4 Details including source, host, dilution, and catalogue numbers of different antibodies. MAbs against the cellular proteins were primarily used in confocal imaging experiments. PAbs were used only when MAbs were not available. Lower and higher dilutions were used in confocal imaging and immunoblotting experiments, respectively.

Antibody	Source	Dilution	Antibody	Source	Dilution
HuR Millipore: 07-468 Santacruz: Sc-374285 Mouse	Rabbit MAb	1:5000 *1:500*	hnRNP E1/PCBP1 Santacruz Biotech:sc-137249- mouse	MAb	1:2000 *1:100*
hnRNP D Abnova: H00003184-BO1P Mouse	MAb	1:1000 *1:100*	Importin-β Millipore: 05-1530 - Mouse	MAb	1:2000 *1:200*
BRF-1 Abnova: H00000677-MO2 Mouse	MAb	1:1000 *1:200*	Exportin-1 BD: 611832 - Mouse	Mab	1:2000 *1:200*
TIAR Santacruz Biotech:sc-398373 Mouse	MAb	1:500 *1:200*	Transportin-1 Millipore: 0501515- Mouse	MAb	1:2000 *1:200*
TIA-1 Santacruz Biotech:sc-365349 Mouse	MAb	1:500 *1:100*	Ran Upstate: 07-517	Rabbit	1:2000 *1:200*
hnRNP F Snatacruz Biotech: sc32309 Mouse & Thermo-pierce:MA5-18024	MAb	1:500 *1:100*	CPSF6 Santacruz: sc-376228 -Mouse	MAb	1:2000 *1:200*
hnRNP I (PTB1) Santacruz Biotech: 73391 – Mouse	MAb	1:500 *1:100*	SG I VP6 (Dr. H. B. Greenberg) SGII VP6 (Dr. H. B. Greenberg)	MAb Mab	*1:200* *1:200*
FXR-1 Millipore: 05-1529 Mouse	MAb	1:2000	NSP5 Lab generated NSP5: (Dr. O. Burrone)	Rabbit Guina pig	1:2000 *1:200*
hnRNP A1 Millipore: 05-1521 Mouse	MAb	1:3000 *1:200*	NSP2 Lab generated NSP2: (Dr. O. Burrone)	Rabbit Guinea pig	1:2000 *1:1000*
hnRNP C1/C2 Millipore: 05-1520 Mouse	MAb	1:3000 *1:200*	DLP-VP6 Lab generated	Rabbit	1:1000 *1:1000*
hnRNP K/J Millipore: 05-1519 Snatacruz Biotech: sc-28380 - mouse	MAb	1:2000 *1:200*	SC-35 Millipore: 04-1550 - Mouse	MAb	1:1000
hnRNP L Millipore: 05-1518- Mouse ThermPierce: PA5-19599 - Rabbit	MAb	1:3000 *1:200*	β-tubulin Thermo-Pierce: MA16308 - Mouse	MAb	1:2000
hnRNP M Thermo-Pierce: PA5-30247 & MA1-91607 - Mouse	Rabbit	1:3000 *1:200*	PCNA Millipore: 1742353 - Mouse	MAb	1:1000
hnRNP Q Millipore: 05-1517 - Mouse	MAb	1:2000	TTP Millipore: ABE-285 & MABE65- Mouse	MAb	1:2000 1:200
KSRP Millipore: 611286 - Mouse	MAb	1:3000 1:200	Staufen1-Thremo-Pierce: sc-390992-Mouse Thermo-Pierce: PA5-28479 -Rabbit	MAb	1:2000 *1:200*
G3BP1 BD: 611126 - Mouse	Mab	1:2000 *1:200*	Sec31A BD: 612351- Mouse	MAb	1:2000 *1:200*
hnRNP U Millipore: 05-1516 – mouse	Mab	1:2000 *1:200*	VPS35 Thermo-Pierce: PA5-21898- Rabbit Santacruz: sc-374372- Mouse	MAb	1:2000 *1:100*
β-Actin Thermo-Pierce: MA5-15739- Mouse	MAb	1:2000	RPS8 Thermo-Pierce: PA5-31676	Rabbit	1:1000 *1:100*
ZBP1 Thermo-Pierce: PA5-20455	Rabbit	*1:200*	HSP70 Thermo=Pierce: PA5-28003	Rabbit	1:2000 *1:200*
mCherry Clontech; 632543- Mouse	MAb	*1:200*	Hsc70 BD: 51-6892GR- Mouse	MAb	1:2000 *1:200*
Anti-6X-His Clontech:894-1 – Mouse	MAb	1:2000 *1:200*	anti- Myc Clontech: 51826 –Mouse	Mab	1:2000 *1:200*
Anti-GFP Chemicon: A33080	Rabbit	1:2000 *1:200*	Anti-rabbit secondary IgG- Alexa488- Invitr –ogen); -Cy5 –GEHealthcare	goat anti-rabbit	1:200
Anti-guinea pig secondary IgG-Alex633 Invitrogen		1:200	Ati-mouse secondary IgG-Cy3: GE Healthcare	Goat anti-mouse	1:200

Fig. S1 Comparative analysis of the total steady-state levels of cellular proteins in virus-infected and uninfected MA104 cells. (a) hnRNPs, (b) ARE-BPs (c) cytoplasmic proteins and (d) Nuclear transport proteins. 50 µg of the control uninfected and infected cell extract (8 hpi) was analysed by immunoblotting using specific antibodies. C, unifected serum-grown control cells; Inf, RRV-infected cells. Fold change in the level of each protein in virus-infected cells compared to that in the control cells is indicated.



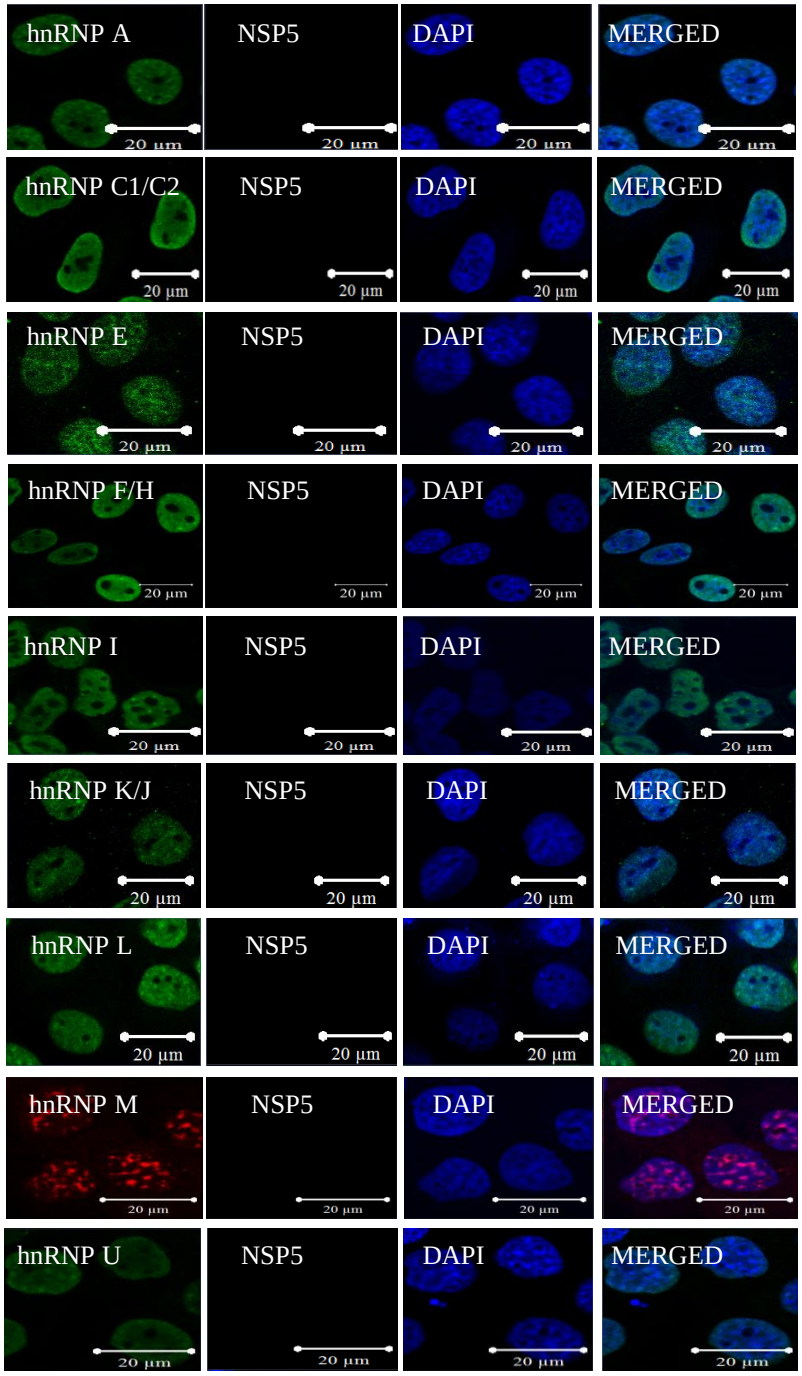


Fig. S2 Predominant nuclear localization of hnRNPs and ARE-BPs in serum-grown control MA104 cells. MA104 cells grown in the presence of 10% FBS were processed for confocal imaging as described in Methods and Material section. The host proteins were detected using specific primary antibodies (MAbs) and fluorescent Cy3-tagged secondary antibodies. NSP5 and VP6 were detected using rabbit PAbs and Cy5-tagged rabbit secondary antibody. Since the control cells do not express viral proteins, NSP5 is undetectable, further indicating that the rabbit polyclonal antibodies are specific to NSP5 and do not cross react with cellular proteins. Note the significant presence of TTP and TIAL-1 in both compartments, correlating with the results in Fig. 2b. Scale bar: 20µm.

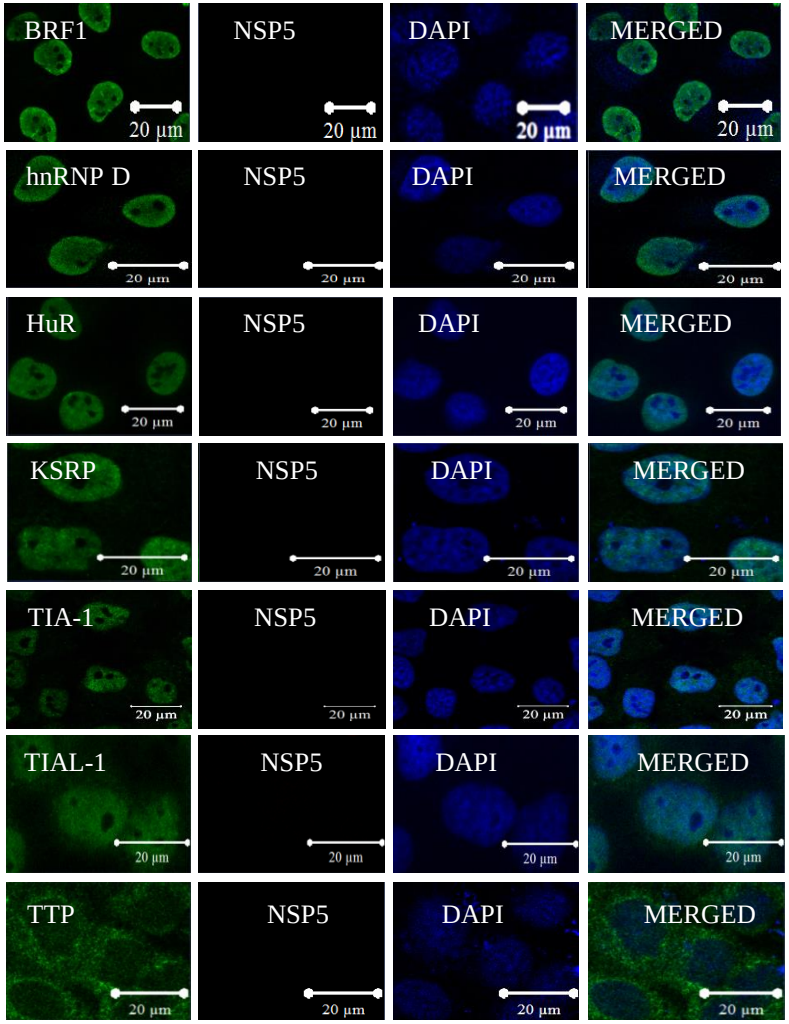


Fig. S3 Time-dependent detection of viroplasm formation after infection of MA104 cell with RRV. Note the initiation of formation of punctate structures of NSP5 and TIA1, and those of VP6 and RPS8 and their colocalization by 4 hpi, suggesting that the colocalization of the nuclear proteins as well as the abundant cytoplasmic proteins with VS is specific, but is not due to cell lysis or abundance of the host protein. See Fig. 2 legend for experimental details. Scale bar: 10μm.

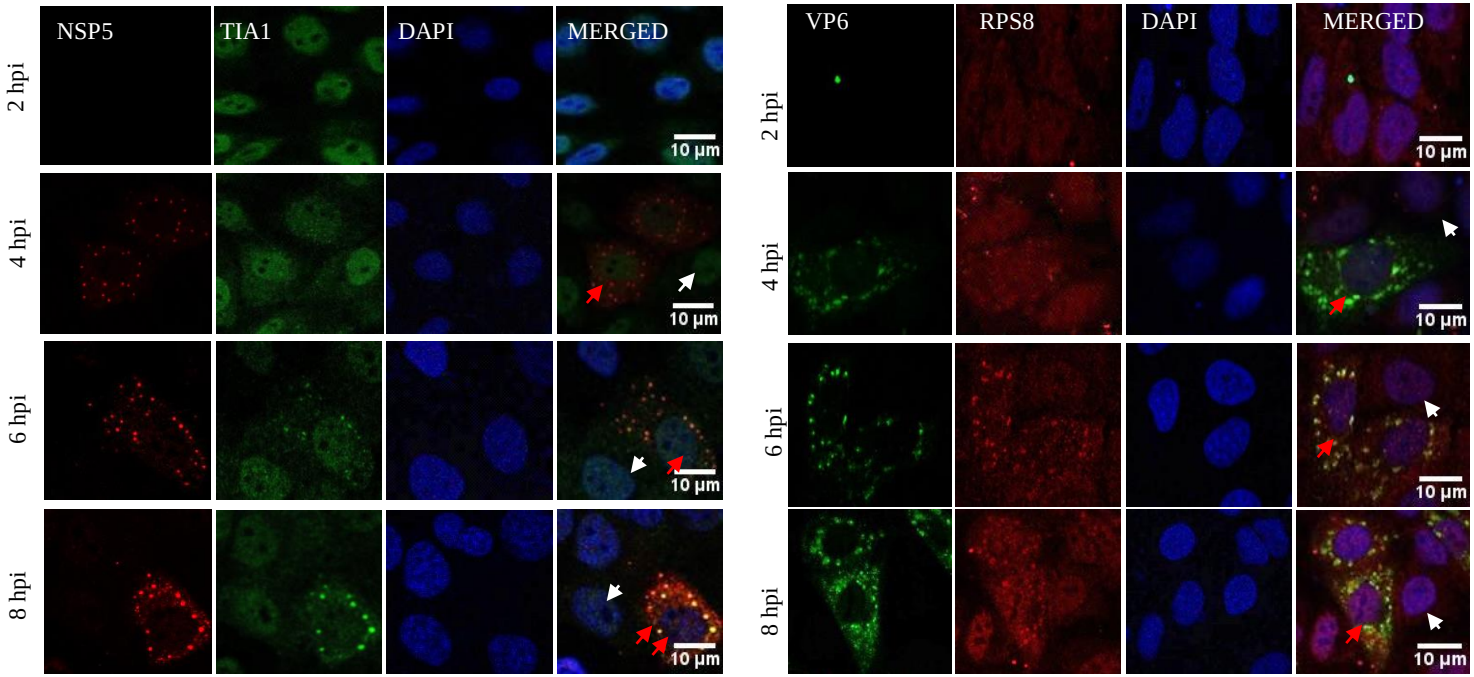


Fig. S4 Z-stack analysis of colocalization of viral and nuclear proteins in the VS. **(a)** The viroplasmic viral protein NSP2 and the host protein hnRNP D, and (**b**) NSP5 and the host protein hnRNP L in the punctate VSs in RRV-infected MA104 cells (at 6 hpi) were visualized by immunofluorescence using rabbit anti-NSP2 and anti-NSP5, and mouse anti-hnRNP D and hnRNP L primary antibodies, and Cy5-tagged (Red) anti-rabbit and Cy3-tagged (Green) anti-mouse secondary antibodies. Z-stacks of images, collected at 0.35 µm intervals ranging from 0 to 2.10 µm at 4x zoom using 63x objective under Zeiss LSM 880 microscope, indicate that both the viral and host proteins are enriched in the same punctate structures in the cytoplasm and that the colocalization is not due to nonspecific fluorescence or due to overlapping diffuse distribution of the two proteins. Z-stack analyses for several host proteins also revealed similar results. Note the perfect localization of the punctate structures of the viral and host proteins at the same site in different sections in the cytoplasm of the infected MA104 cells. Scale bar: 20µm.

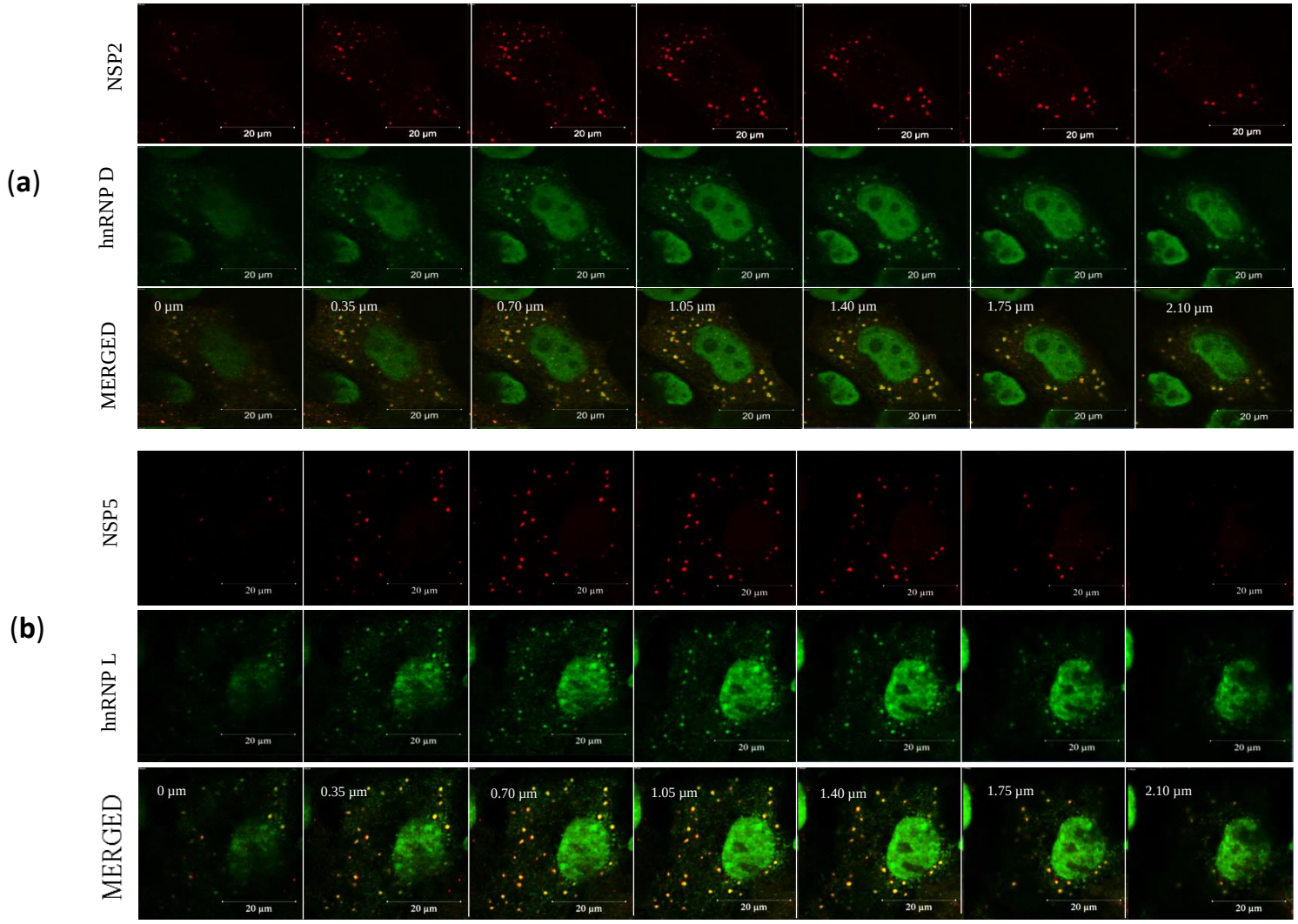


Fig. S5 Colocalization of fluorescent protein-tagged host proteins with viroplasms. HEK293T cells were transfected with plasmid vectors expressing ECFP-NSP5, mCH-VPS35, ECFP-hnRNPC1, ECFP-hnRNP D, ECFP-hnRNP K or ECFP. 36 hrs posttransfection, the cells were infected with RRV. At 8 hpi, NSP2 and NSP5 expressed from the virus were detected by specific PAbs and fluorescent dye-tagged secondary antibodies. The proteins expressed from the vectors were detected by their fluorescence. Note the colocalization of the ectopically-expressed tagged NSP5 and host proteins with viroplasms. The transfected and infected cells are indicated by orange arrow heads. The sites of quantification of colocalization are indicated by white broken line. Scale bar: 20µm.

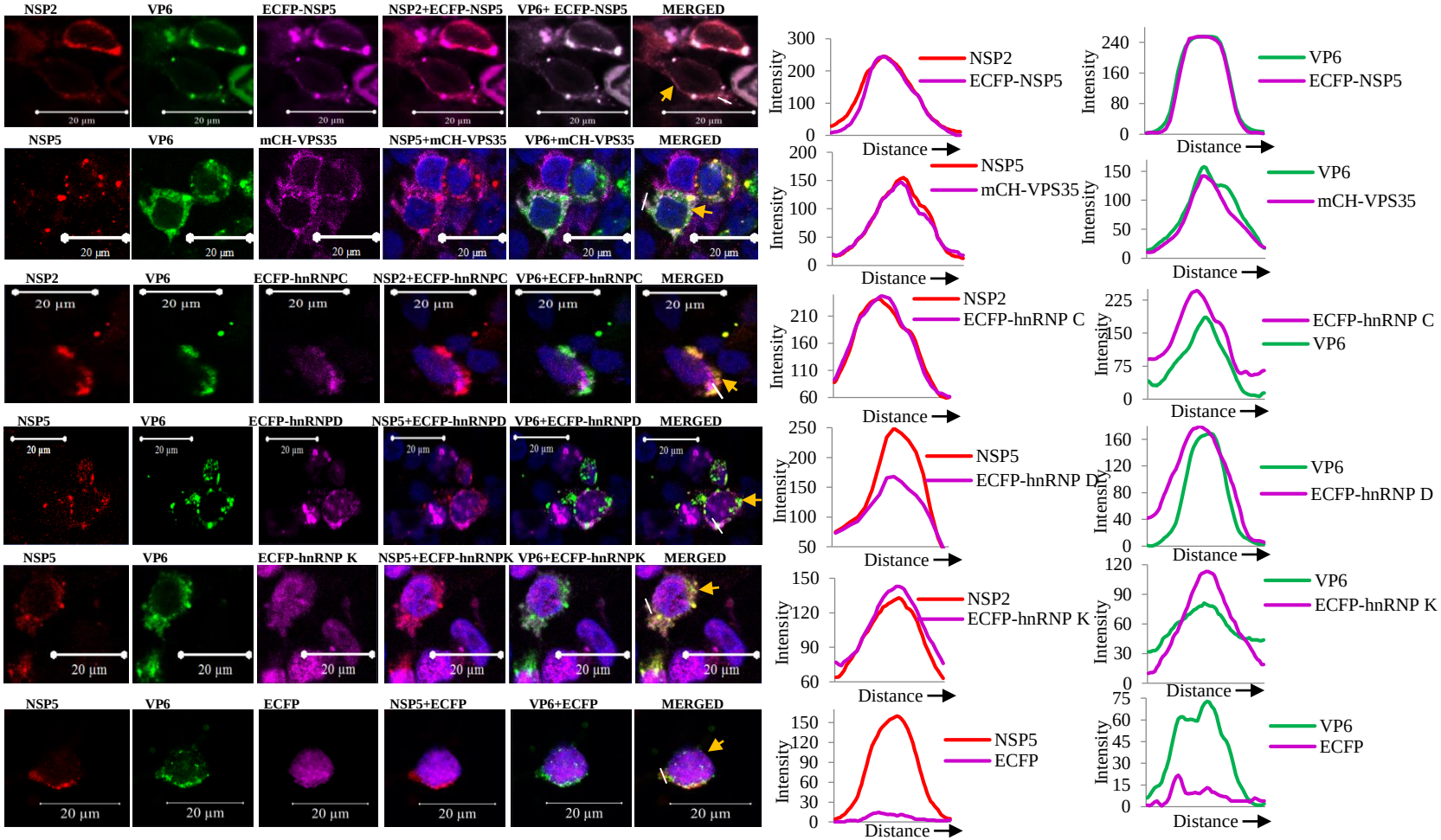


Fig. S6 Analysis of the influence of ectopic expression of (**a**) IS2-NSP5, (**b**)SA11-NSP2, and (**c**) IS2-VP6 in HEK293T cells on the cytoplasmic relocalization of hnRNPs and ARE-BPs. Transfected and untransfected cells are indicated by orange and white arrows, respectively. Scale bar: 10μm.