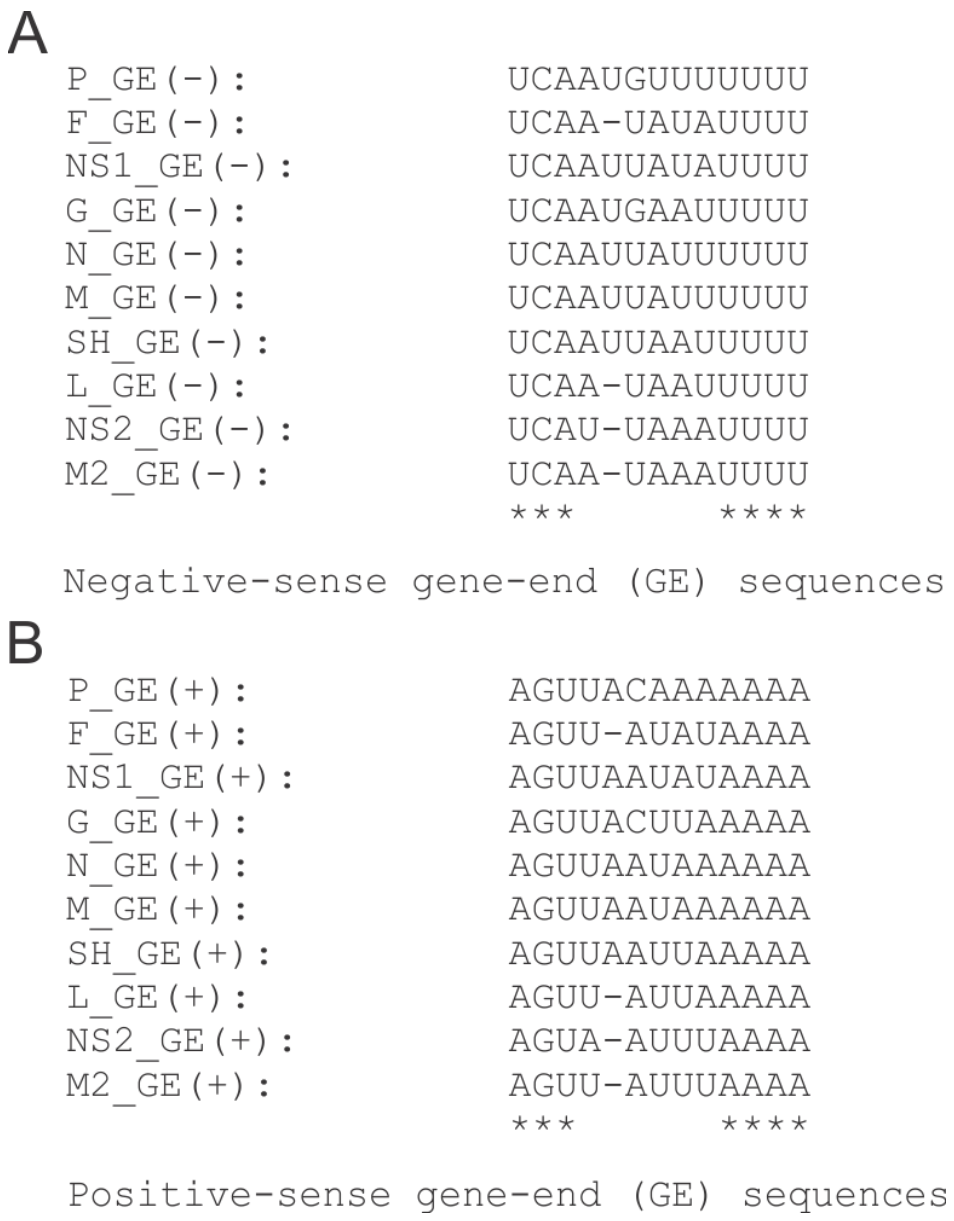

Figure S1: The sequence alignments of negative-sense and positive-sense gene-end (GE) of the HRSV genes. Related to STAR Methods.

A. The sequence alignment of negative sense GE of HRSV genes, namely, P, F, NS1, G, N, M, SH, L, NS2, and M2. * denotes the conserved positions among GE sequences.

B. The sequence alignment of positive sense GE of HRSV genes: P, F, NS1, G, N, M, SH, L, NS2, and M2.

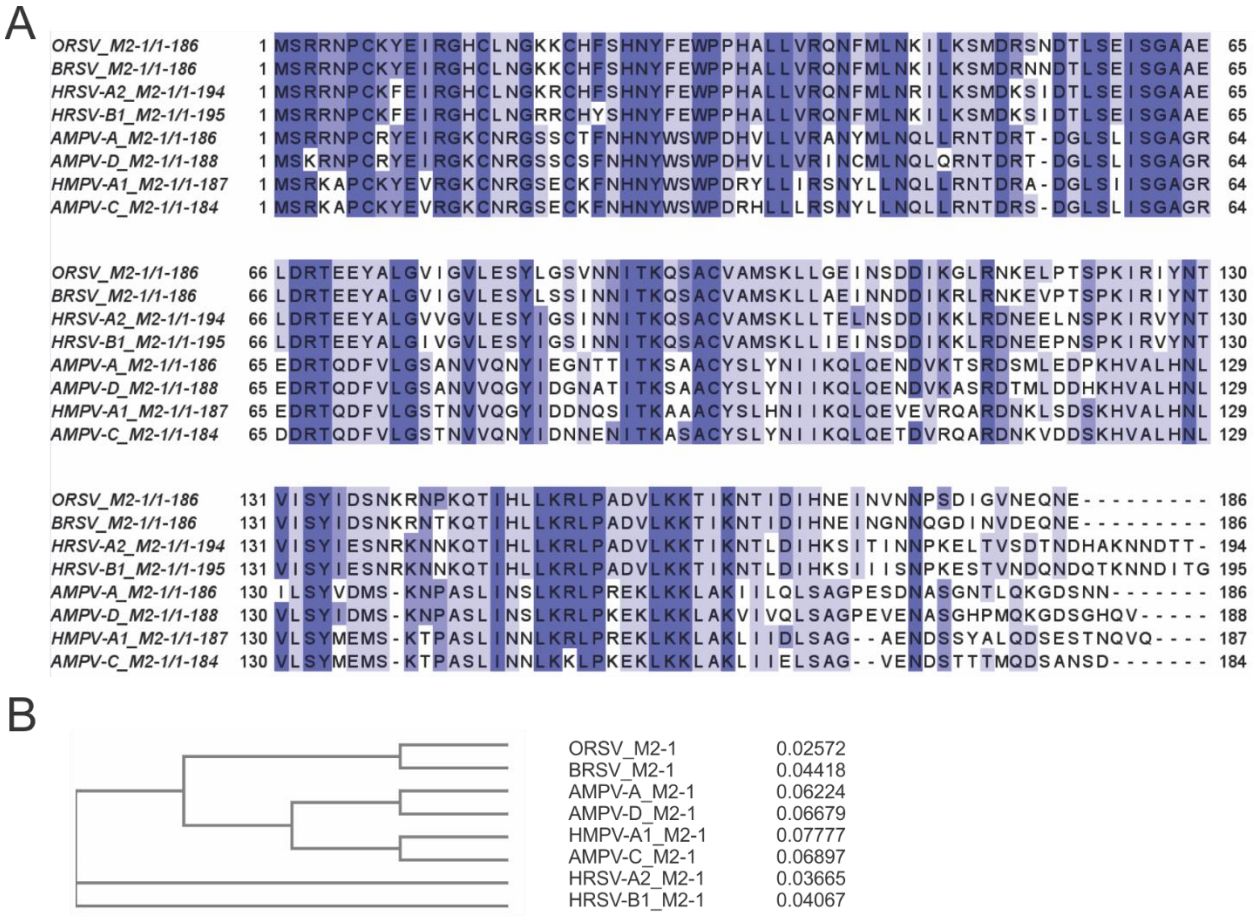

Figure S2: Multiple sequence alignments of the M2-1 proteins from *Pneumoviridae*. Related to Figures 2 and 4.

A. Multiple sequence alignments of the M2-1 proteins from RSV (ORSV, BRSV, A2 and B1 strains of HRSV) and MPV (A, C, and D strains of AMPV and HMPV). The conservation is highlighted in the degree of the blue color, most conservation with dark blue and least conservation with light blue.

B. The Phylogenetic tree of M2-1 from RSV and MPV families. The scores are shown next to each subfamily. A2 and B1 strains of HRSV are distal from the rest of families, including ORSV and BRSV.

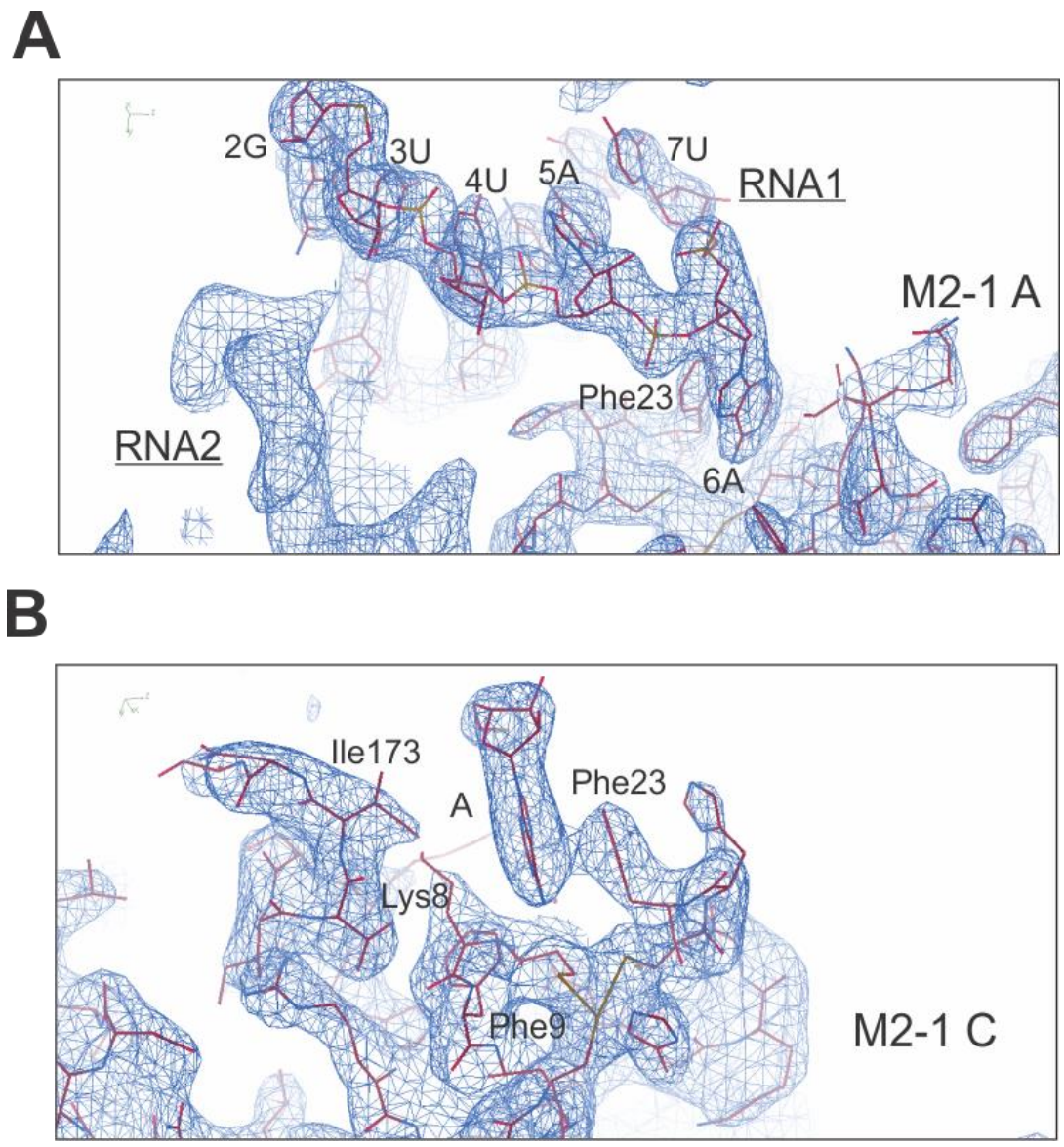

Figure S3: The representative density maps used for building RNA. Related to Figures 1 and 2.

A. The representative electron density $2F_{obs}$ - F_{calc} map (contoured at 1.5σ) shows the electron density of the RNA molecule adjacent to M2-1 protomer A. The meshes show the electron density of the modeled RNA and M2-1.

B. The representative electron density $2F_{obs}$ - F_{calc} map (contoured at 1.5σ) shows the electron density of the molecule adjacent to M2-1 protomer C. The meshes show the electron density of the modeled RNA and M2-1.

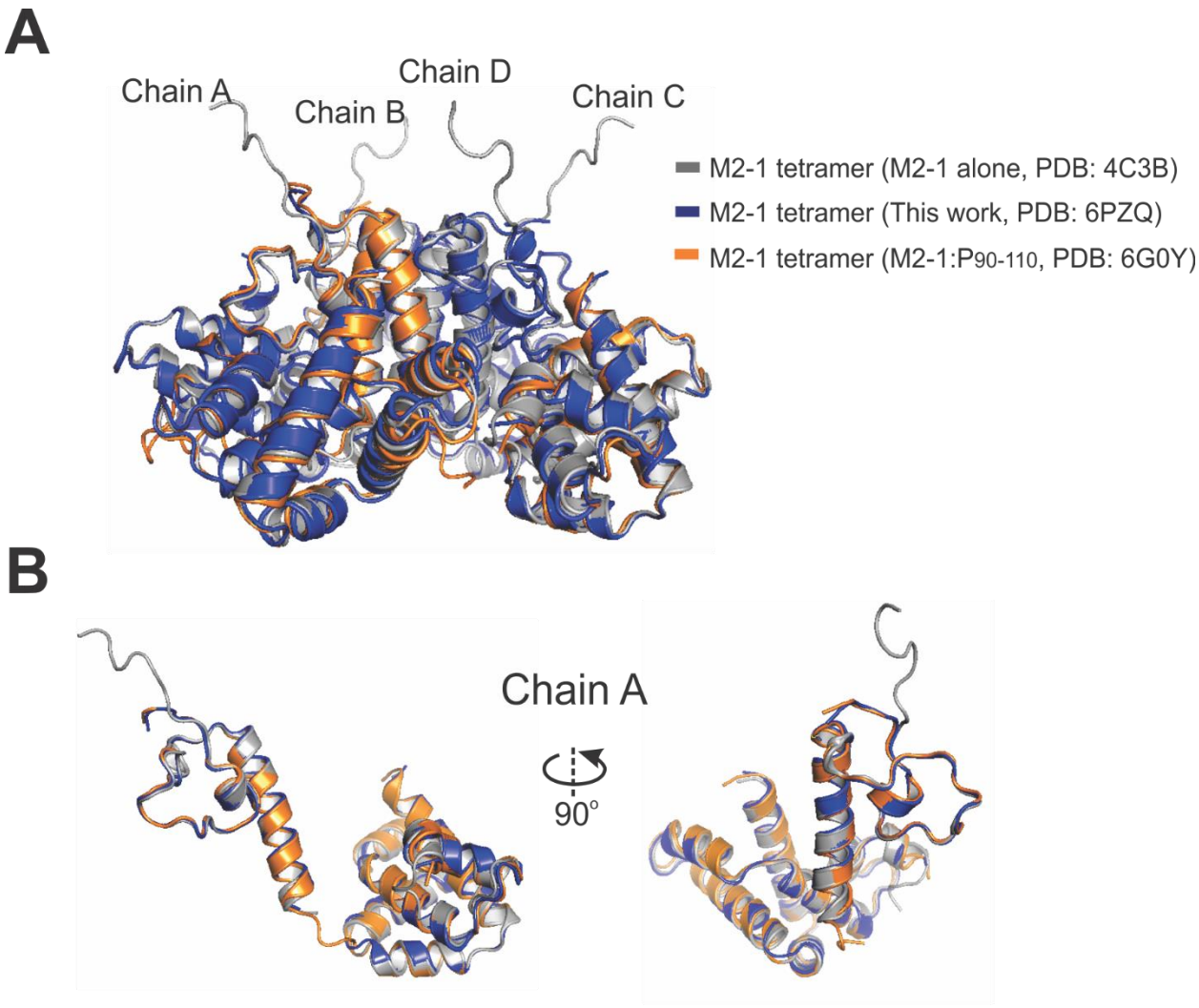

Figure S4: Superimposition of M2-1 of M2-1:SH7, M2-1 alone, and M2-1:P_{90-110}. Related to Figure 1.

A. Superimposition of M2-1 of M2-1:SH7 (blue), M2-1 alone (gray, PDB: 4C3B) and M2-1:P90-110 (orange, PDB: 6G0Y).
B. Superimposition of the chain A of M2-1 from M2-1:SH7, M2-1 alone, and M2-1:P_{90-110}. The RMSD are < 0.5 Å.

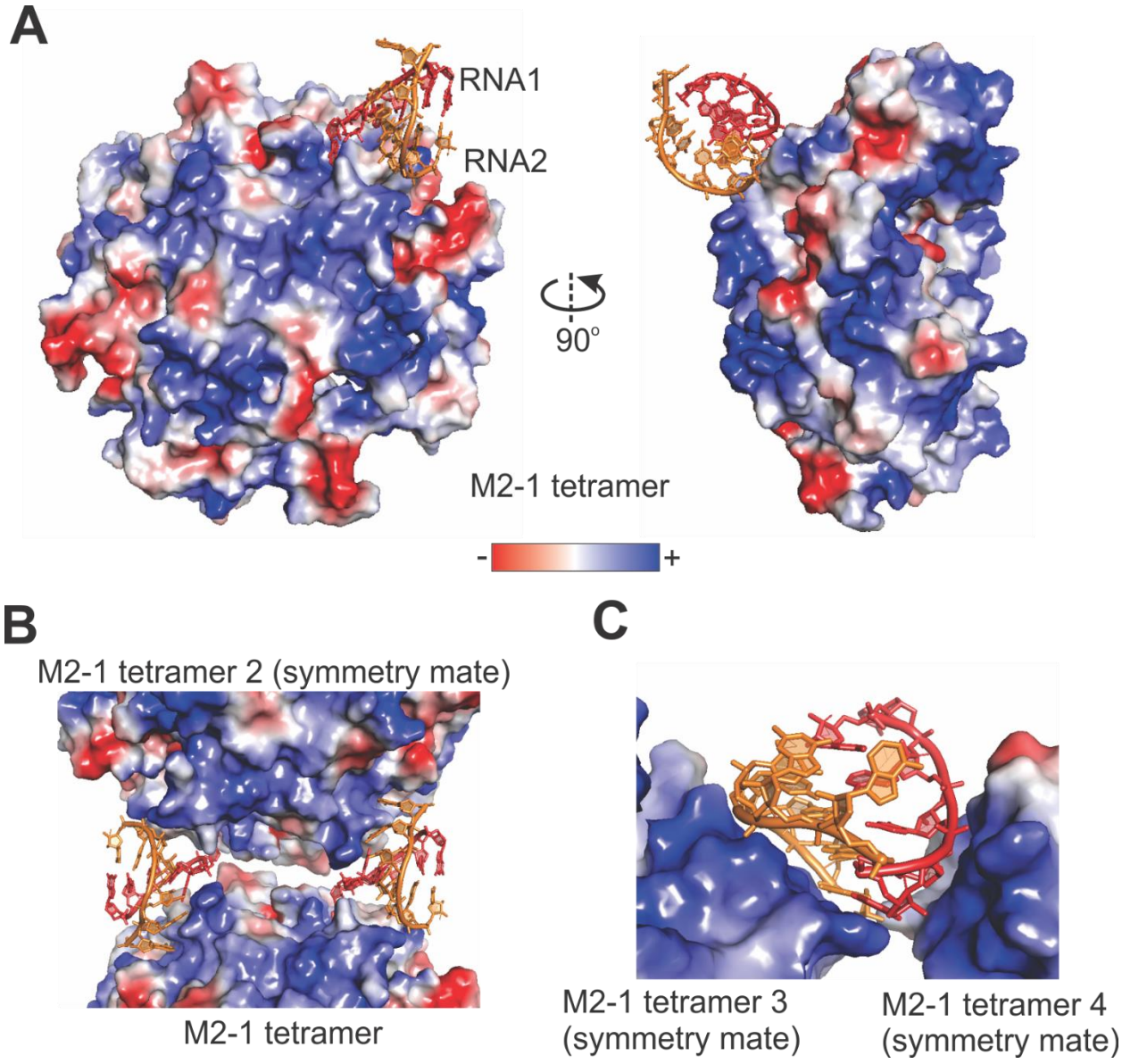

Figure S5: The electrostatic surface of the M2-1:SH7 complex. Related to Figures 1, 3, and 4.

A. Surface representation of the electrostatic potential of the M2-1 in the front view and side view. The negative charge is in red, and the positive charge is in blue (calculated using APBS).

B. The zoom-in view of the interactions between the zinc-binding domain (ZBD) of two M2-1 tetramers (the upper is the symmetry mate 2) and RNAs.

C. The zoom-in view of the interactions between the core domain (CD) of two M2-1 tetramers (both are symmetry mates 3 and 4) and RNAs.

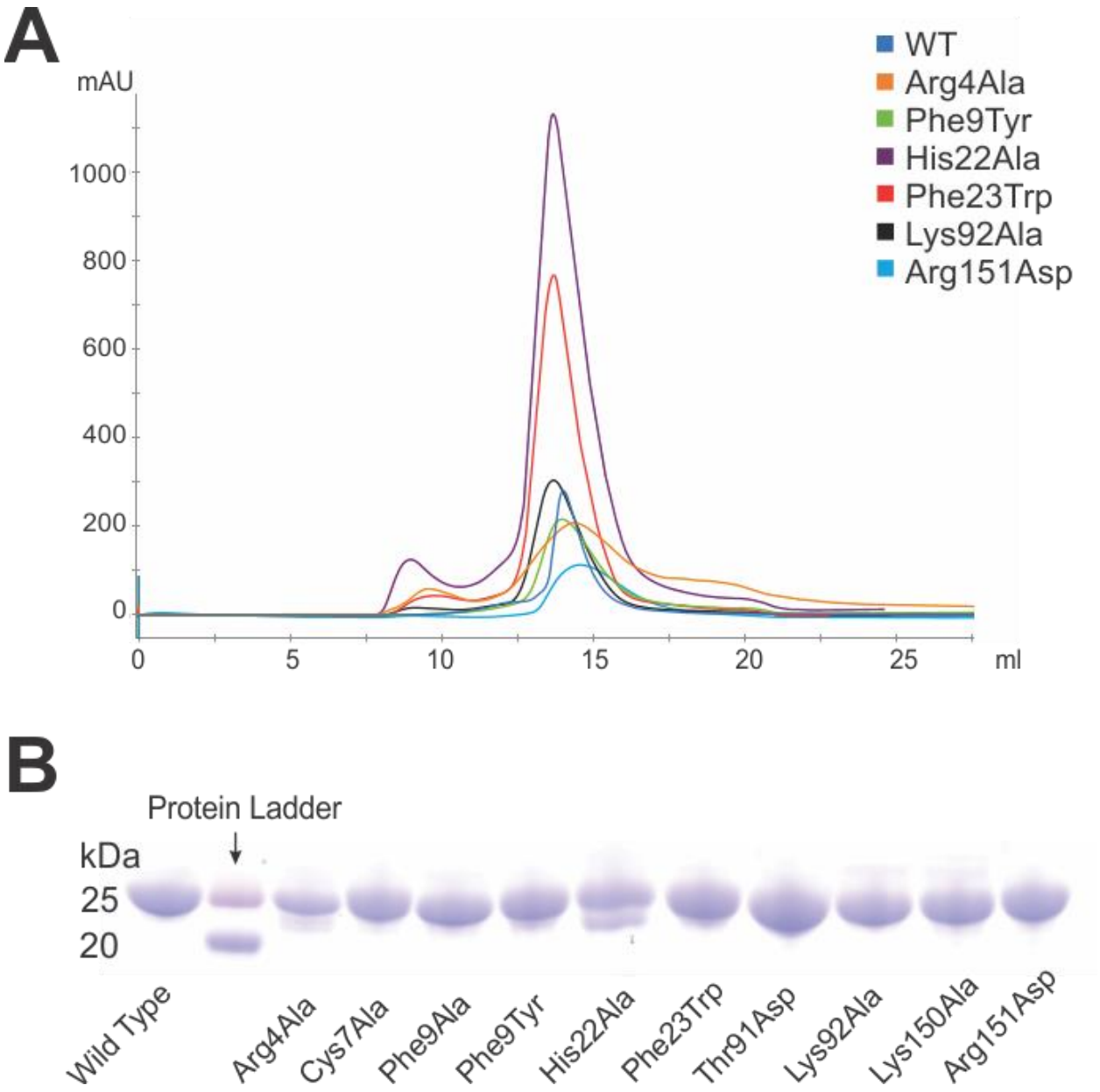

Figure S6: The wild-type and mutant HRSV M2-1 proteins. Related to Figures 2 and 4.

A. The representative size exclusion chromatography profiles of wild-type (WT) and mutant HRSV M2-1 proteins, including Arg4Ala, Phe9Tyr, His22Ala, Phe23Trp, Lys92Ala, and Arg151Asp.

B. The SDS-PAGE gel of WT and mutant HRSV M2-1 proteins.

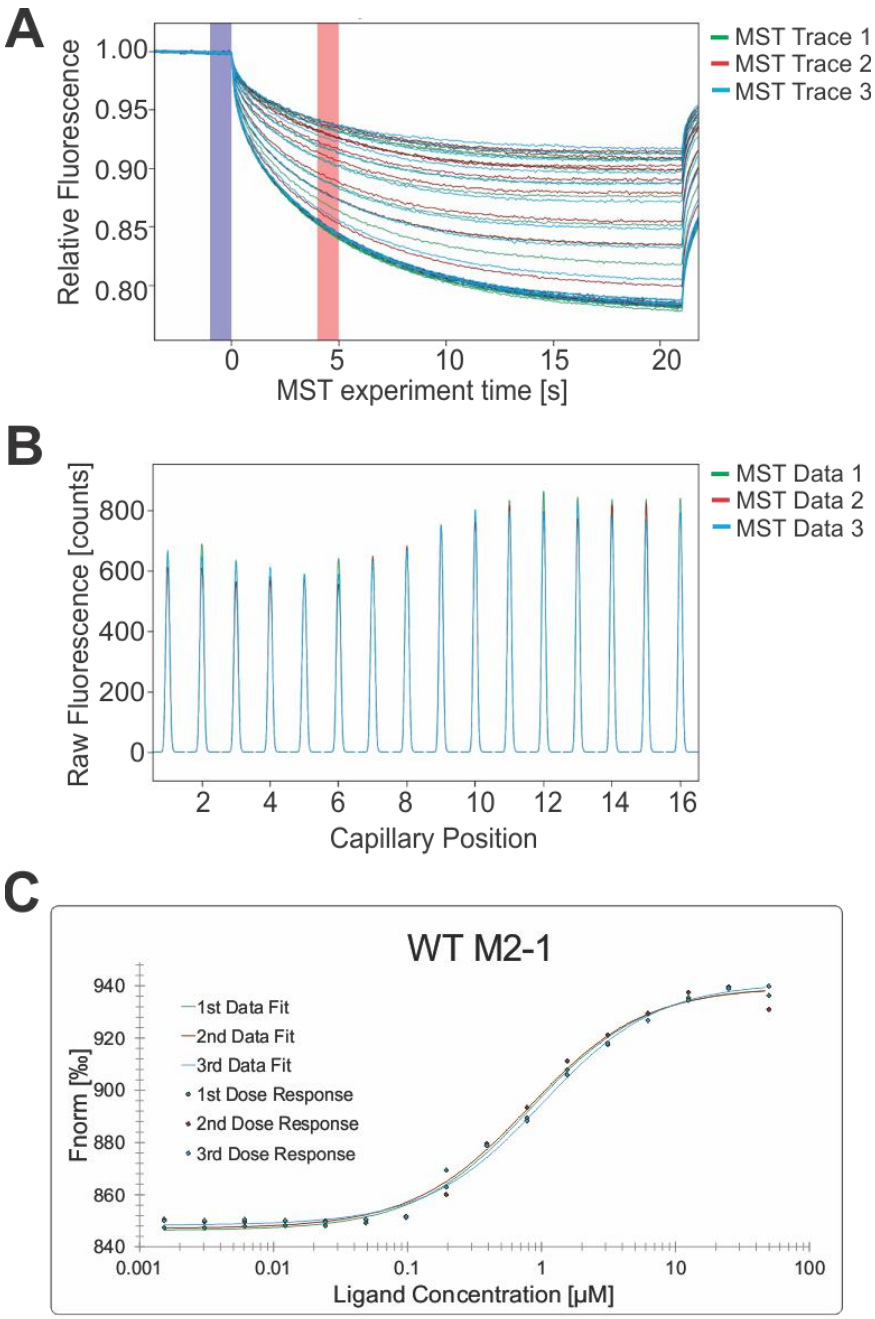

Figure S7: Affinity analysis of HRSV M2-1 to RNA using MicroScale Thermophoresis (MST) assay. Related to Figures 2 and 4. The data were acquired with a triplicate.

A. The representative MicroScale Thermophoresis (MST) experiments show the traces for 16 multiple scans compared using relative fluorescence for WT M2-1 proteins.

B. The MST data show the raw fluorescence counts for the individual capillary position (a total of 16 capillaries).

C. The Kd fitting of the MST data measurements.

M2-1 Proteins	Kd (μM)	Kd (μM) Error	Average (μM)
Wild Type	0.819	0.070	0.867±0.090
	0.789	0.095	
	0.993	0.075	
Arg4Ala	2.026	0.149	1.760±0.208
	1.520	0.119	
	1.732	0.131	
Cys7Ala	9.602	1.234	8.56±1.018
	7.180	1.013	
	8.907	1.132	
Phe9Ala	5.085	0.383	5.693±0.511
	6.337	0.369	
	5.659	0.363	
Phe9Tyr	0.739	0.011	0.813±0.087
	0.766	0.113	
	0.936	0.123	
His22Ala	1.737	0.333	1.592±0.104
	1.542	0.232	
	1.497	0.215	
Phe23Trp	0.726	1.043	0.711±0.019
	0.72	1.058	
	0.68	9.132	
Thr91Asp	1.54	0.164	1.716±0.210
	2.01	0.137	
	1.60	0.158	
Lys92Ala	5.01	0.596	5.175±0.810
	6.24	0.801	
	4.27	0.917	
Lys150Ala	0.79	0.092	1.111±0.238
	1.37	0.098	
	1.17	0.096	
Arg151Asp	1.03	0.067	1.212±0.331
	0.93	0.098	
	1.68	0.125	

Table S1: The MST measurements of the interactions between M2-1 and SH7 RNA. Related to Figures 2 and 4. All data were measured as triplicates through NT.115 MST instrument at room temperature.

M2-1_Arg4Ala_F:	TCCAATGCAATGAGCAGGgcGAACCCCT
M2-1_Arg4Ala_R:	CTCGAACTTACAGGGGTTCgcCCTGCT
M2-1_Cys7Ala_F:	ATGAGCAGGAGGAACCCCGCTAAGTTC
M2-1_Cys7Ala_R:	GCCCCTGATCTCGAACTTAGCGGGGTT
M2-1_Phe9Ala_F:	AGGAGGAACCCCTGTAAGgcCGAGATC
M2-1_Phe9Ala_R:	GCAGTGGCCCCTGATCTCGgcCTTACA
M2-1_Phe9Tyr_F:	AACCCCTGTAAGTaCGAGATCAGGGGCCAC
M2-1_Phe9Tyr_R:	GCCCCTGATCTCGtACTTACAGGGGTTCCT
M2-1_His22Ala_F:	CTGAACGGCAAGAGGTGCGCCTTCAGC
M2-1_His22Ala_R:	GAAGTAGTTGTGGCTGAAGGCGCACCT
M2-1_Phe23Trp_F:	AAGAGGTGCCACtggAGCCACAACTACTTC
M2-1_Phe23Trp_R:	GTAGTTGTGGCTccaGTGGCACCTCTTGCC
M2-1_Thr91Asp_F:	GGCAGCATCAACAACATCgaCAAGCAG
M2-1_Thr91Asp_R:	CACACAGGCGCTCTGCTTGtcGATGTT
M2-1_Lys92Ala_F:	AACAACATCACCgcGCAGAGCGCCTGTGTG
M2-1_Lys92Ala_R:	ACAGGCGCTCTGCgcGGTGATGTTGTTGAT
M2-1_Lys150Ala_F:	CAGACCATCCACCTGCTGgcGAGGCTG
M2-1_Lys150Ala_R:	CACGTCGGCGGGCAGCCTCgcCAGCAG
M2-1_Arg151Asp_F:	CACCTGCTGAAGgacCTGCCCGCCGACGTG
M2-1_Arg151Asp_R:	GTCGGCGGGCAGgtcCTTCAGCAGGTGGAT

Table S2: The primers used in the mutagenesis of the M2-1 proteins. Related to STAR Methods. The primers are synthesized by Eurofins Genomics.