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Supplemental Information

RNA Secondary Structure Motifs of the Influenza A Virus as Targets for siRNA-Mediated RNA Interference

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Table S1. Primers and probe used in the experiments.

Primer for gene-specific reverse transcription	
	ATGAGTCTTCTAACCGAGGTCG
Primers for real-time PCR	
forward	AGACCAATCTTGTCACCTCTGAC
reverse	AGGGCATTTTGGACAAAGCGTCTACG
Probe for real-time PCR	
	FAM-TCACCGTGCCCAGTGAGCGAGGACTGC-TAMRA

All oligonucleotides are DNA. FAM – 5’end labelled with 5-FAM; TAMRA – 3’end labelled with 6-TAMRA.

Table S2. Conserved base pairs[1] used as constraints for determination of A/California/04/2009 (H1N1) (+)RNA5 folding in Dynalign mode of RNAstructure 6.1 which were incorporated to guide folding of (+)RNA5 from strain A/Vietnam/1203/2004 (H5N1).

Table S3. A/California/04/2009 (H1N1) sequence conservation among influenza type A strains in siRNA target regions**.**

siRNA name	Target region*	Sequence conservation (%)
1498	1498-1516	96.6
1342	1342-1360	76.7
1312	1312-1330	90.0
1090	1090-1108	72.9
1008	1008-1026	88.4
901	901-919	82.6
682	682-700	79.5
613	613-631	82.7
471	471-489	85.3
448	448-466	92.4
412	412-430	89.9
183	183-201	92.5

* (+)RNA5 nucleotide numbering from 5' end

Table S4. Thermodynamic parameters of duplex formation[a]

	Average of curve fits				**T_M^{-1} vs log C_T plots**					
siRNA	**$-\Delta H^o$ (kcal/mol)**	**$-\Delta S^o$ (eu)**	**$-\Delta G^o_{37}$ (kcal/mol)**	**T_M [b] (°C)**	**$-\Delta H^o$ (kcal/mol)**	**$-\Delta S^o$ (eu)**	**$-\Delta G^o_{37}$ (kcal/mol)**	**T_M[b] (°C)**	**$-\Delta\Delta G^o_{37}$ (kcal/mol)**	**ΔT_M[b] (°C)**
183	143.9 ± 6.08	398.3 ± 18.1	20.38 ± 0.51	70.0	98.8 ± 4.2	265.3 ± 12.5	16.59 ± 0.36	72.1	0	0
183-sF1	86.09 ± 5.891	229.8 ± 17.3	14.80 ± 0.52	69.9	91.6 ± 4.9	246.4 ± 14.7	15.26 ± 0.39	69.6	1.33	-2.5
183-sF2	151.0 ± 18.7	411.4 ± 54.9	23.43 ± 1.72	76.1	99.03 ± 8.9	261.1 ± 25.9	18.04 ± 0.95	77.8	-1.45	5.7
471	161.6 ± 9.4	440.2 ± 27.1	25.08 ± 0.99	77.2	158.12 ± 14.2	430.1 ± 41.0	24.70 ± 1.51	77.3	0	0
471-sMe1	62.3 ± 4.7	161.5 ± 14.5	12.28 ± 0.29	68.5	64.7 ± 14.1	168.4 ± 42.2	12.48 ± 1.30	68.4	12.22	-8.9
471-sF1	80.6 ± 4.9	210.3 ± 14.0	15.46 ± 0.63	75.6	85.6 ± 14.8	224.6 ± 43.1	15.93 ± 1.49	75.2	8.77	-2.1
471-sF2	144.7 ± 9.7	385.8 ± 27.8	25.10 ± 1.18	82.6	143.6 ± 29.6	382.6 ± 83.4	24.98 ± 3.83	82.7	-0.28	5.4
613	156.8 ± 6.1	433.0 ± 17.9	22.58 ± 0.58	72.3	143.7 ± 10.3	394.3 ± 30.4	21.39 ± 0.94	72.8	0	0
613-sMe2	60.3 ± 4.9	159.51 ± 14.7	10.82 ± 0.42	60.8	63.6 ± 7.9	169.5 ± 24.1	11.02 ± 0.51	60.5	10.37	-12.3
613-sF1	163.2 ± 8.0	444.5 ± 23.0	25.34 ± 0.89	77.4	168.3 ± 25.0	459.3 ± 72.0	25.91 ± 2.73	77.3	-4.52	4.5
613-aF1	127.8 ± 14.5	337.86 ± 41.2	23.02 ± 1.76	82.9	126.9 ± 17.7	335.3 ± 50.5	22.96 ± 2.15	83.1	-1.57	10.3
613-asF1	181.5 ±1.1	483.9 ± 3.0	31.49 ± 0.22	86.4	136.8 ± 0.1	357.8 ±0.1	25.87±0.01	88.1	-4.48	15.3
613-sPS	160.9 ± 7.6	445.2 ± 22.6	22.82 ± 0.67	71.9	159.1 ± 24.5	439.9 ± 71.6	22.68 ± 2.40	72.0	-1.29	-0.8
613-aPS	167.8 ± 25.2	465.3 ± 73.5	23.47 ± 2.38	71.8	150.0 ± 15.2	413.1 ± 44.5	21.88 ± 1.40	72.4	-0.49	-0.4
613-asPS	151.8 ± 7.3	418.6 ± 21.6	21.99 ± 0.67	72.1	150.6 ± 19.5	415.1 ± 56.8	21.90 ± 1.89	72.2	-0.51	-0.6

a – solutions: 100 mM NaCl, 20 mM sodium cacodylate, 0.5 mM Na_2EDTA, pH 7, b - calculated for 10^{-4} M oligomer concentration.

REFERENCE

1. Soszynska-Jozwiak, M, Michalak, P, Moss, WN, Kierzek, R, Kesy, J, and Kierzek, E (2017). Influenza virus segment 5 (+) RNA - secondary structure and new targets for antiviral strategies. *Sci Rep* **7:** 15041.