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A1 830 accagaggtgatcccaatggttcagcgcctcagcggggcgc

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A3 3753 ctgcaggaatacgcaggactgtttctagacggcatcgacaaggcgcgaagaggaacacgagaaatcca

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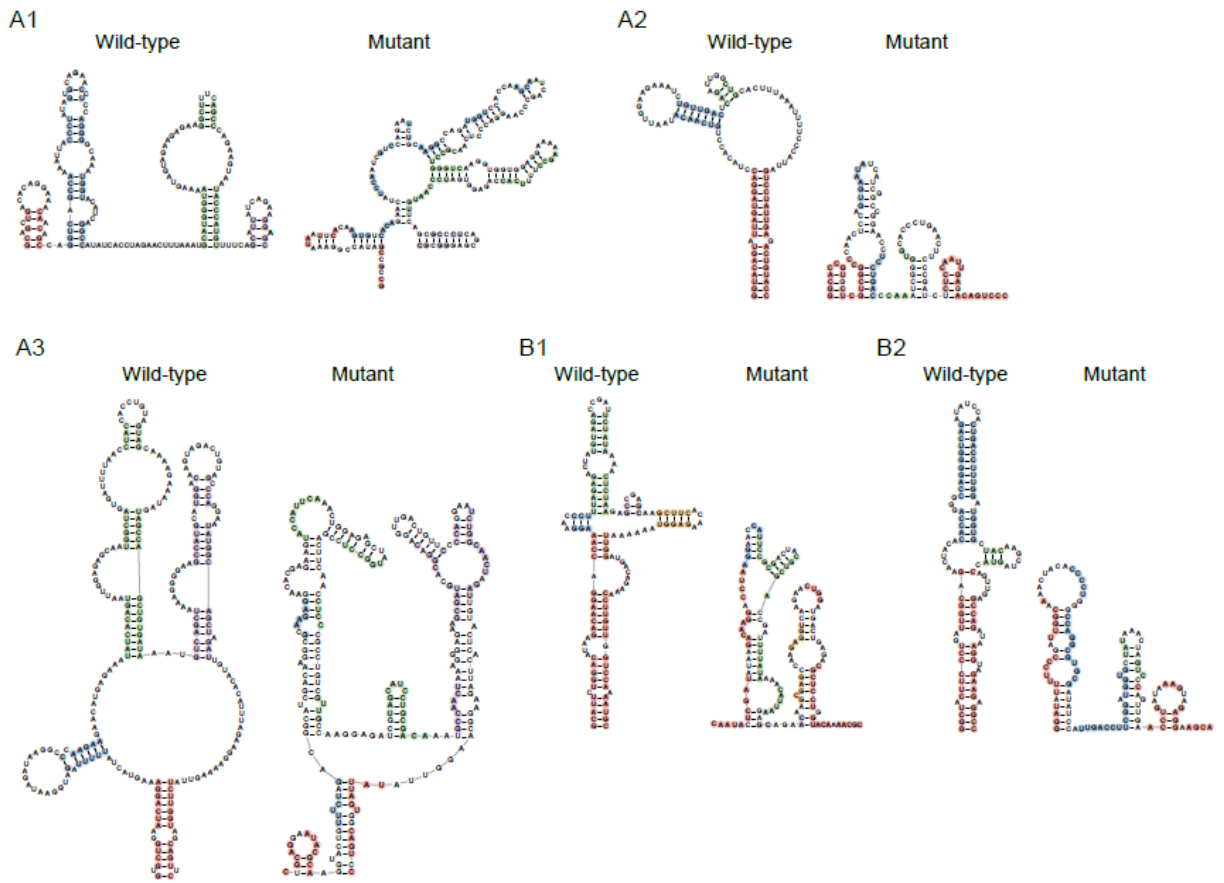
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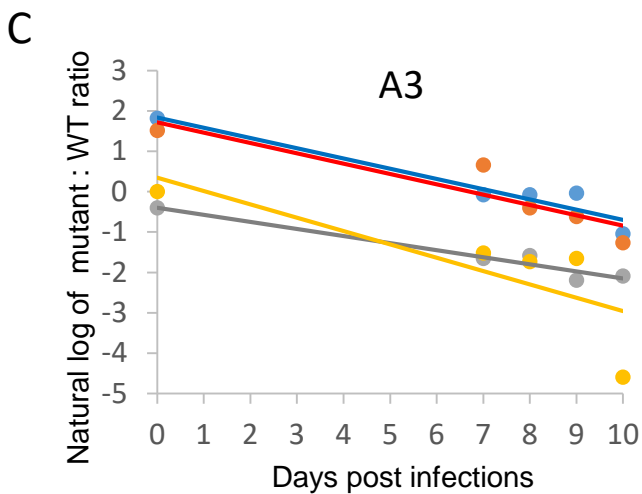
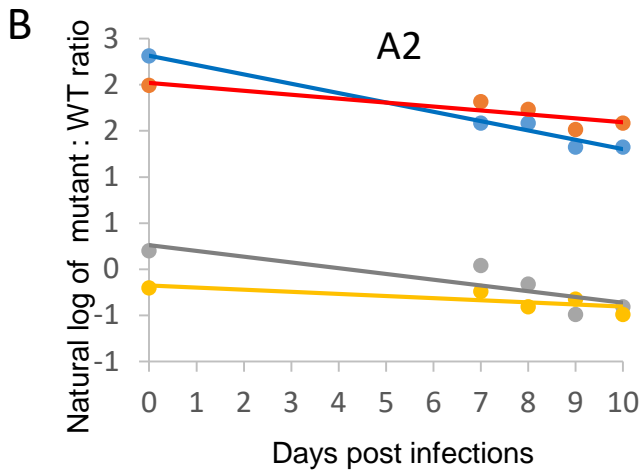
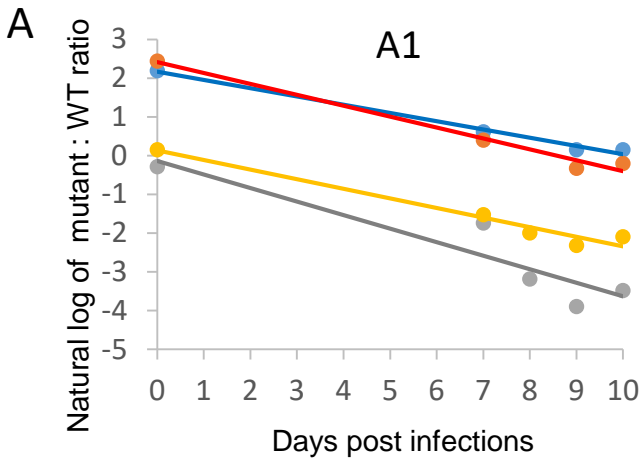
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B2 8747 ttcggatggtgctataaaactagtcaccagttgaacctgataaagtagaggaagca

Supplemental Figure 1. Nucleotide sequence alignments of NL4-3 (wt) and mutants containing synonymous mutations. Sequence identities are marked by grey highlight.



Supplemental Fig. 2. Comparison of the NL4-3 structures (wild-type) and the predicted mutant RNA structures. To illustrate the change of RNA structures, elements in the NL4-3 structures are color-coded and the corresponding mutated sequences are coded in the same colors.



Supplemental Fig. 3. The natural log of the mutant to WT virus ratios were plotted over times and the slope the fitted linear line represents the absolute replication differences of each mutant compared to WT virus. Red, blue, gold and grey lines correspond to Experiment 1A, 2A, 1B, and 2B listed in supplemental Table 1, respectively.

Supplemental Table 1. Mutant and wild-type (NL4-3) virus ratios from various time points of the competition assay. These results were summarized in the CEM portion of Fig. 4B, 4C, and 4D.

		Proportion of mutant virus detected (%)					
Experiments*		Day 0	Day 7	Day 8	Day 9	Day 10	rs
A1	Exp. 1A	90	65	57	54	54	-0.28
	Exp. 2A	92	60	50	42	45	-0.25
	Exp. 1B	43	15	4	2	3	-0.22
	Exp. 2B	54	18	12	9	11	-0.35
A2	Exp. 1A	91	83	83	79	79	-0.1
	Exp. 2A	88	86	85	82	83	-0.04
	Exp. 1B	55	51	46	38	40	-0.06
	Exp. 2B	45	44	40	42	38	-0.02
A3	Exp. 1A	86	48	48	49	26	-0.25
	Exp. 2A	82	66	40	35	22	-0.26
	Exp. 1B	40	16	17	10	11	-0.33
	Exp. 2B	50	18	15	16	1	-0.18

* Mutant virus : wild-type virus ratios are 9:1 and 1:1 in A and B series, respectively.