Supplemental Material to:

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Specific temperature-induced perturbations of secondary mRNA structures are associated with the cold-adapted temperature-sensitive phenotype of influenza A virus

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Supplementary Material

Supplementary Files: Comparisons of significantly changing positions between mRNAs. Each file presents the comparison of significantly changing positions between wild-type mRNA and the correspondent mutant. The upper seven rows correspond to a wt mRNA while the lower seven rows correspond to a ts/ca counterpart. Each row corresponds to a difference vector v_{32-33} , ..., v_{32-39} containing changes of base pairing probabilities between 32 °C and a particular higher temperature. Positions in which base paring probabilities significantly change with temperature elevation in both sequences and those where these changes only affect one of the phenotypes are marked blue and orange, respectively. Position numbers of the coding sequence are indicated at the top of the alignment.

Also, corrected sequences used in the research are presented.

All supplementary files can be accessed via the following Web page: <u>http://frishman.wzw.tum.de/fileadmin/ts-ca-influenza/</u> And <u>http://www.landesbioscience.com/journals/rnabiology/article/22081/</u>

Supplementary Figures 1-19: Distributions of significantly changing positions along the mRNAs of wild type (wt) and ca/ts strains. A sliding window of size 20 was moved in steps of 1 position over the vectors v_{32-39} and the percentage of significantly changing positions in the window was calculated for each possible starting position. Panels A and C depict resulting density plots for the mRNAs of wild type (wt) and ca/ts strains, respectively. The location of clusters of significantly changing positions identified by the DBSCAN algorithm are depicted in panels B and D with grey color. Synonymous and non-synonymous mutations are depicted in panels B and D with red and blue vertical lines, respectively.

Supplementary figure 1:



Supplementary figure 2:



Supplementary figure 3:



Supplementary figure 4:



Supplementary figure 5:



Supplementary figure 6:



Supplementary figure 7:



Supplementary figure 8:



Supplementary figure 9:



Supplementary figure 10:



Supplementary figure 11:



Supplementary figure 12:



Supplementary figure 13:



Supplementary figure 14:



Supplementary figure 15:



Supplementary figure 16:



Supplementary figure 17:



Supplementary figure 18:



Supplementary figure 19:



Supplementary figure 20: Optimal mRNA secondary structures for NS2 gene predicted by RNAfold tool. a. Len/wt at 32 °C; b. Len/wt at 39 °C; c. Len/47/ca at 32 °C; d. Len/47/ca at 39 °C. Nucleotides that belong to identified statistically significant cluster (positions 290–308) are highlighted with blue color. Single nucleotide substitution (marked as red) constitutes difference between NS2 genes of Len/wt and Len/47/ca.

