Supplementary Information for

A novel approach to finding conserved features in low-variability gene alignments characterises RNA motifs in SARS-CoV and SARS-CoV-2

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This PDF file includes:

Supporting Information Text

1. Additional discussion of rationale for novel methodology, and results of benchmarking

A. Step 1: Effect of weighting by information content: helps but may be insufficient alone. Our first key idea is to mitigate the effect of loci with artefactually high apparent normalised variability (caused when a high degree of amino acid-level conservation means that there is little possible nucleotide-only conservation), by taking into account the amount of information each locus provides. We do this by weighting each locus by Shannon information (see the Methods for details).

To illustrate possible effects of such a procedure, we apply the procedure to two well-characterised housekeeping genes from *Escherichia coli*, which are highly conserved but have variable regions used in one Multilocus Sequence Typing (MLST) scheme(1). These genes – adenylosuccinate synthetase (purA) and fumarate hydratase C (fumC) – represent the genes used in the scheme with, respectively, the lowest and the highest polymorphism levels per nucleotide. These genes were originally selected for use in typing because their presence and function is highly conserved. They were chosen to allow phylogenetic analysis, but the precise regions within the genes used for such analysis were not chosen to extremise per-locus variability. This means that we might expect to see a mixture of relatively more- and less-conserved regions within what are overall comparatively highly-conserved genetic features, and so these genes are suitable for real-world benchmarking of our algorithm. (We briefly discuss in Section 2 of this Supplementary text known features of these genes, reproduced during our benchmarking.)

In all cases, a slight inverse correlation between the measure of locus variability and the information content of the locus (Fig. S1) simply reflects that the highest Shannon information only occurs in the loci with lowest variability (see Methods). In these low variability genes, any high variability locus may be regarded as an outlier, and many such loci have low information content.

In genes known to have low sequence variability, such weighting indeed reduces the number of outlier loci with apparent high variability (Fig. S2). Mitigating the effects of such loci can reduce identification of regions around these loci identified as conserved. However, paradoxically sometimes this procedure can in fact increase the chances of a region being determined to have low variability (possibly artefactually, possibly by improving signal-to-noise). This is because any remaining high-variability loci are consequently even more outlying than previously, meaning the majority of loci, which are low-variability, is more likely to contain a longer subsequence deemed to be statistically significantly conserved. In such situations, it is important not only to remove artefact, but also to ensure the overall analysis is not skewed by a small number of truly highly variable loci. To accomplish this goal, it is necessary to combine the weighting procedure with the ranking procedure described in the following subsection.

This step is a useful step regardless of the amount of underlying variability in the sequence, because in all cases it would be expected to increase signal-to-noise ratio, and so even in sequences with high variability where there is good delineation between signal and background, one would expect better definition of the boundaries of low variability (conserved) regions.

B. Step 2: Ranking variability. In sequences with low overall variability, single loci with high variability may cause the entirety of the rest of the sequence artefactually to appear highly conserved (Fig. S3). Although weighting by information content reduces this effect (since in general more variable codon usage for an amino acid is less informative than less variable codon usage), it is still possible for this effect to occur when using weighting. The mathematical explanation for the effect is straightforward: the algorithm determining conserved regions appeals to the central limit theorem to argue that the mean variability measure of a sequence tends to a normal distribution. However, when all but a few points in a sequence have very low variability, the distribution is so skewed that this normal limit is not sufficiently approached.

We can address this problem by replacing highly skewed measures of sequence variability with rank values – essentially a non-linear, order-preserving transformation to cause the data to tend faster to the normal limit. As this step results in information loss, it is possible to cause a reduction in sensitivity to detect conserved regions. This technique is therefore mainly appropriate for low-variability datasets that are far from a central limit. Indeed, although weighting will improve the signal-to-noise ratio, it may move the distribution further from the central limit.

We have benchmarked this technique by applying it to two different simulated distributions:

1. A standard normal variable.

2. An exponential distribution with mean 1.

We chose the exponential distribution to illustrate the effect of the technique on a skewed distribution, and the normal distribution to illustrate the effect of the technique on a distribution without skew (where, in fact, ranking positions simply causes information loss). In each case we simulated a "gene" consisting of 500 real numbers, with a "signal" of length 100 in the centre. We varied the "strength" of the signal region (the amount by which the mean of the distribution from which the signal was sampled was reduced).

The results of our benchmarking are shown in Figs. S4 and S5. As expected, we see that when the underlying distribution is not normal, it is more common to identify substantial non-signal regions as signal. This effect can be mitigated by replacing the values at individual loci with their respective ranks; however, the information loss this causes reduces our ability to identify signal regions correctly. It is therefore important to consider whether it is reasonable to analyse any given sequence using the parametric assumption that the central limit is closely approached. If it is reasonable, then using raw values in the analysis will be more sensitive for identifying signals.

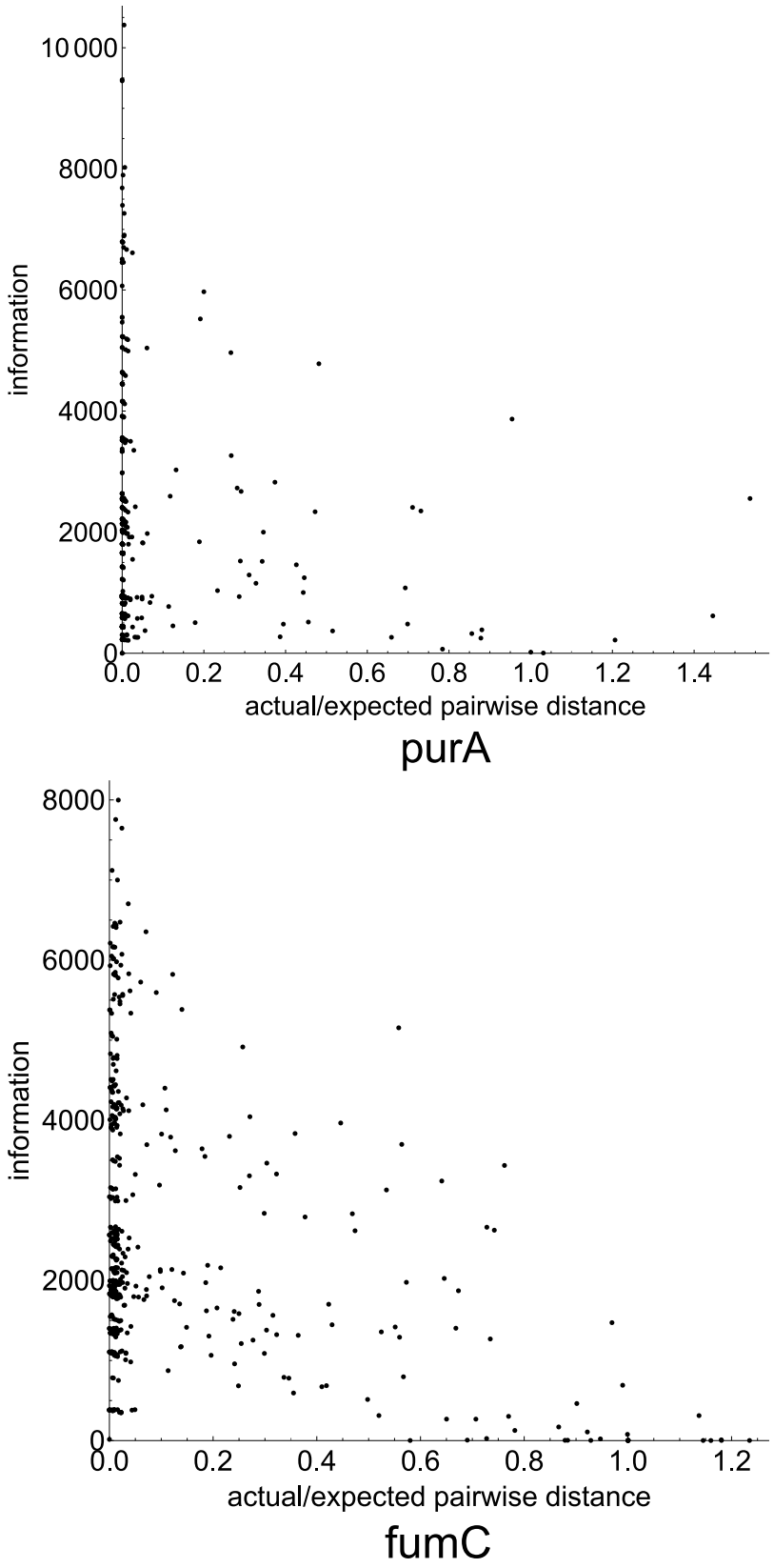

Fig. S1. Shannon information at locus versus measure of locus variability (actual/expected pairwise distance: see Methods for details), for two conserved housekeeping genes of *E. coli*, purA (relatively low polymorphism rate, top) and fumC (relatively high polymorphism rate, bottom). Throughout this work, each locus is a codon within a gene, although in principle different loci could be used with different measures of variability. Each plotted point represents the comparison of information/variability at a single locus. In both cases, most loci have low variability, although the information gained from each low variability locus can vary. High variability loci are rare and many give low information. The absence of high information, high variability loci reflects the high number of combinations of loci producing high variability, meaning the Shannon information cannot be high.

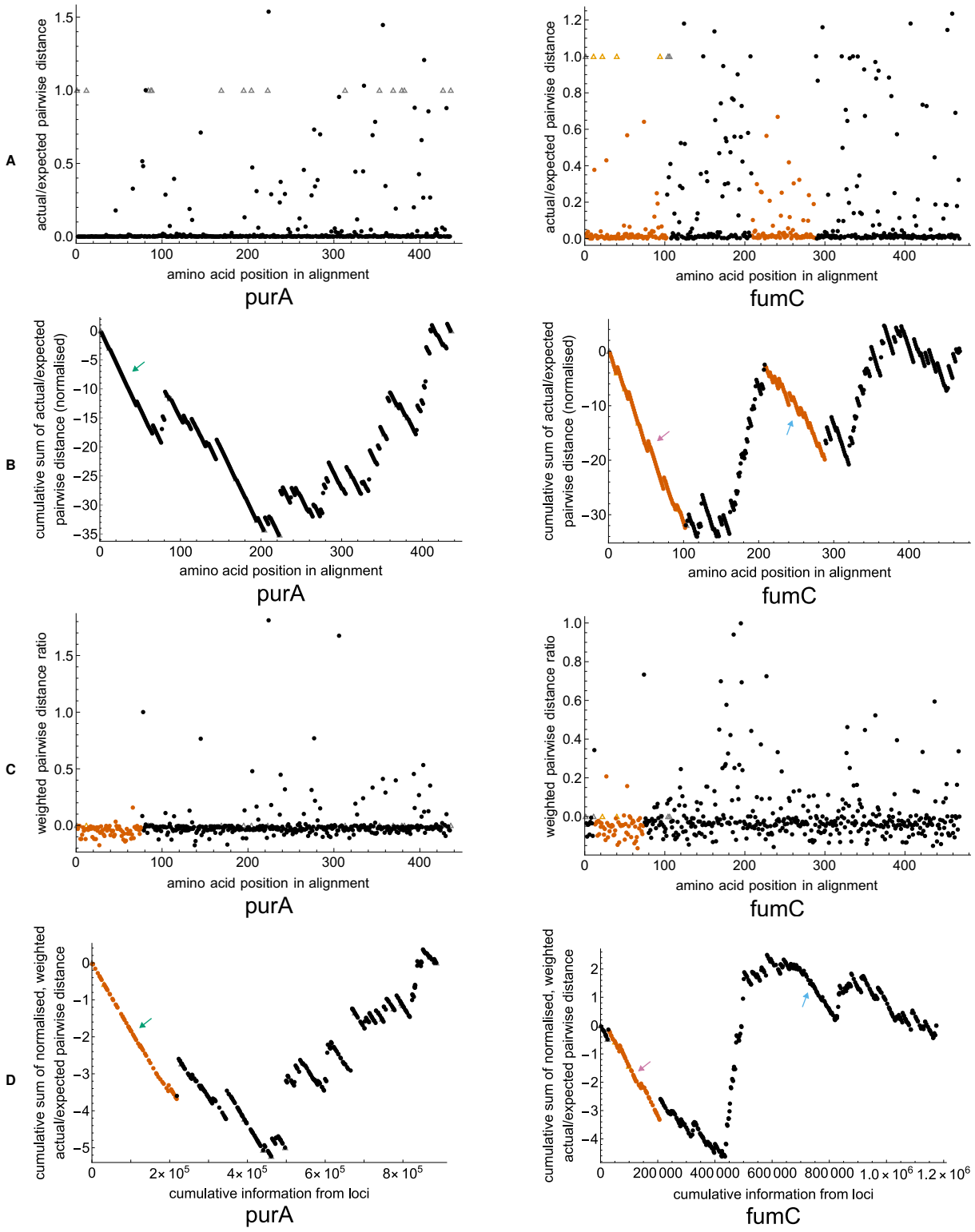

Fig. S2. Effect of weighting loci for information content in *E. coli* housekeeping genes purA (left) and fumC (right). In each case, variability measures are normalised to have mean 0 and standard deviation 1. (**A**) Raw variability measures (actual/expected pairwise distance: see Methods for details). (**B**) Cumulative sums of raw variability measures. (**C**) Weighted variability measures (see Methods for details). (**D**) Cumulative sums of weighted variability measures. Each plotted point represents a single locus (codon within the gene). Orange loci correspond to regions deemed to be significantly conserved by our algorithm. Grey and light orange loci plotted with open triangles yield no information on nucleotide conservation(2). Where raw variability measures are used, such loci are plotted with actual/expected pairwise distance=1 (since actual=expected pairwise distance), but are excluded from analysis. Where weighted variability measures are used, such loci are plotted with weight=0. It can be seen in both cases that weighting reduces the number of outlying, high variability loci contributing to the analysis. In the case of fumC, the effect is that one of the identified conserved regions is delabelled (blue arrows), and the other identified conserved region is circumscribed in extent (pink arrows). In contrast, for purA the reduction in contributions from high variability loci results in a longer low variability region being deemed significantly conserved (green arrows).

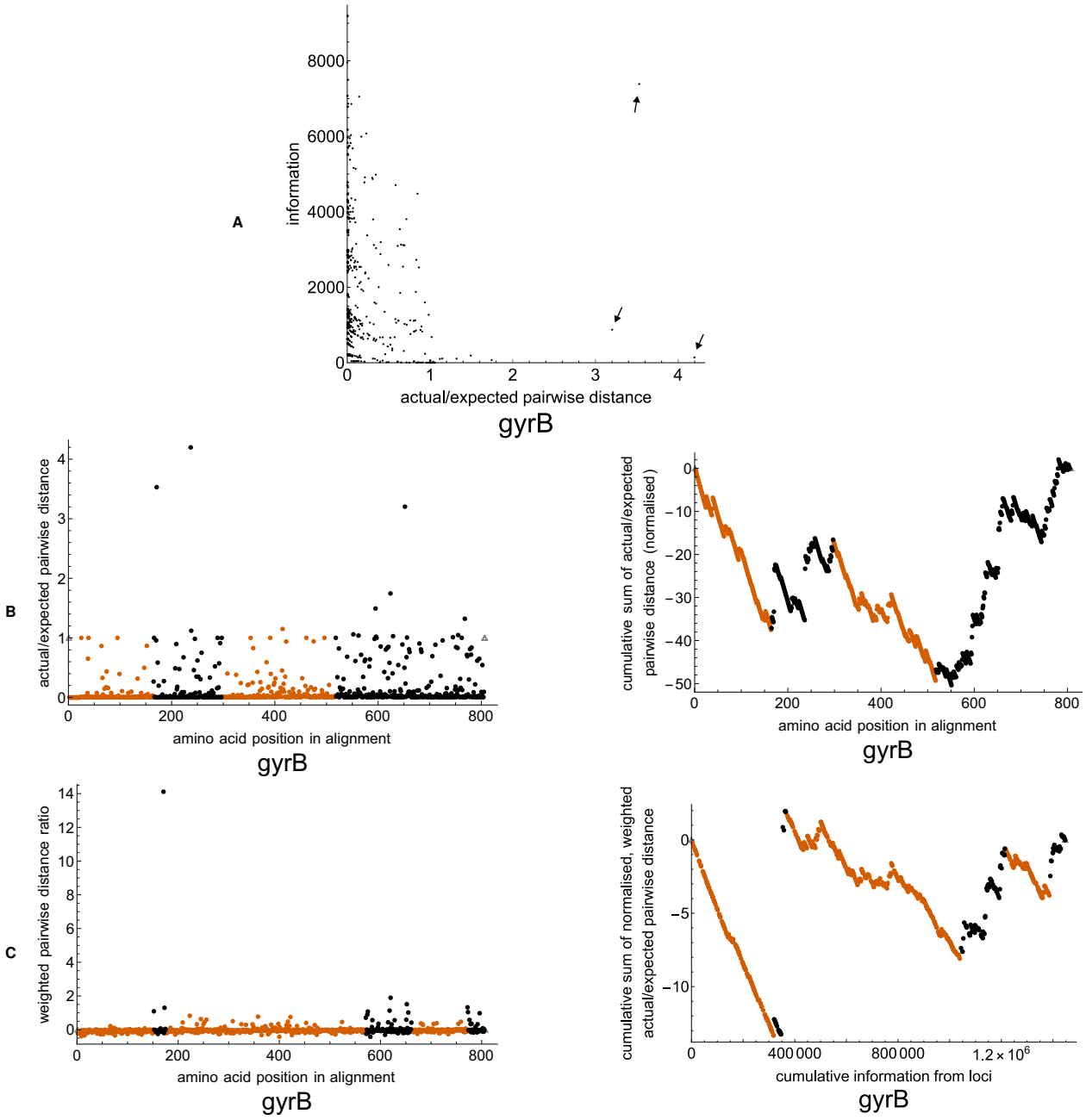

Fig. S3. (**A**) Information content for each codon in the *E. coli* gyrB housekeeping gene, versus measured variability at the codon (see text for explanation). Without weighting by information content, a small number of loci, most with low information, dominate the analysis (arrows). (**B**) Analysis output for *E. coli* gyrB (raw values left, summed values right) with loci equally weighted and raw variability measure, showing output that does distinguish low and high variability regions, but the high variability "ascents" are dominated by a few loci. (**C**) Analysis output for *E. coli* gyrB (raw values left, summed values right) with loci weighted by information content and raw variability measure. Although there is more granularity than with equal weighting, a single high-information locus now dominates the analysis. In both analyses, a consequence of the domination by a small number of high variability points is that a large portion of the gene is determined by the algorithm to be conserved. Weighting reduces the number of loci causing the problem, but because it does not eliminate such loci entirely, the problem persists.

It is possible to combine ranking measured variability with weighting loci for information content (Fig. S6). However, as the ranking of a locus represents an increment dependent on the rank of every locus with lower variability, the weighting of all lower rank loci needs to be taken into account when calculating a weighted rank. The appropriate approach is discussed more fully in the Methods.

C. Step 3: Multiple interfering signals. In our previous work(3), we demonstrated that when looking for contiguous signal and comparing an identified putative signal against background, the presence of an additional signal in the background region unsurprisingly interfered with signal identification, reducing the algorithm's sensitivity. We demonstrated that the effect was worst near where the two signals had similar signal strength. This is appropriate and expected behaviour for an algorithm that aims to find regions of higher conservation in a gene than can be found elsewhere in the gene. However, the aim of the algorithm should be to compare conservation between an identified region and *background* conservation, not the level of conservation in another contiguous, conserved region.

A straightforward adaptation to our algorithm where a region's conservation is deemed non-significant is to check that this is not caused by the presence of another contiguous conserved region. This can be accomplished by repeating the significance analysis in the absence of the next most significant region. We discuss the procedure in detail in the Methods.

We have benchmarked this approach, and results are displayed in Fig. S7. As might be expected, the approach yields an increase in sensitivity for finding signal regions, at the expense of a small decrease in specificity. The trade-off is usually acceptable for two key reasons. First, the initially rejected signal is boosted most when there is a second, long signal – in the absence of such a signal, any boost will come from a small region identified randomly, and so will be much smaller. Second, it is possible to add labels highlighting regions identified only after removal of another region, meaning interpretation can be undertaken with additional caution. In any case, this is a modification to a procedure whose purpose is to identify regions that merit further analysis and/or investigation.

The application of this procedure to the *E. coli* examples shown earlier is displayed in Fig. S8.

D. Lessons from benchmarking. So far, we have considered three possible algorithmic refinements for finding regions of high conservation in genetic data. Given we can choose whether or not to apply each of these refinements, we now have eight possible ways of analysing a given dataset. We therefore consider when each refinement should be applied.

It is important to recognise that from a biological perspective, any combination of these algorithms serves the same purpose: namely, to highlight regions within a genome that merit further biological investigation. Deciding which combination to use is therefore not only a matter of the mathematical properties of an algorithm, but also of how well they serve this biological purpose.

Weighting loci by information content (section A) is the most difficult refinement to benchmark clearly, but appears to improve the signal-to-noise ratio in the data and has a clear mathematical justification. We therefore recommend always including this refinement.

Ranking loci by their variability (section B) appears likely from benchmarking to have a substantial benefit in avoiding false positive results in a minority of cases with non-normal data. There is only a small reduction in identification of true positives. We therefore recommend using the ranking refinement, unless it is clear that the data should approach the (Gaussian) central limit – ideally by formally testing this, but biological context would also be a reasonable way of justifying such a choice. (For example, our earlier work on HIV-1(2) used sequences with high inter-sequence variability, and ranking loci there would be less important than it would be for SARS-CoV-2.)

The procedure for identifying conserved regions that are obscured by other, similarly conserved regions (section C) can always be run, as it is possible to distinguish between regions that are identified using this additional procedure and regions that do not require it, with only a modest increase in computational time. The identification of a region subject to a modestly higher false positive rate can therefore be flagged and taken into account when considering biological significance.

For our application to SARS-CoV and SARS-CoV-2, we therefore analyse data using all the procedural refinements we have described above.

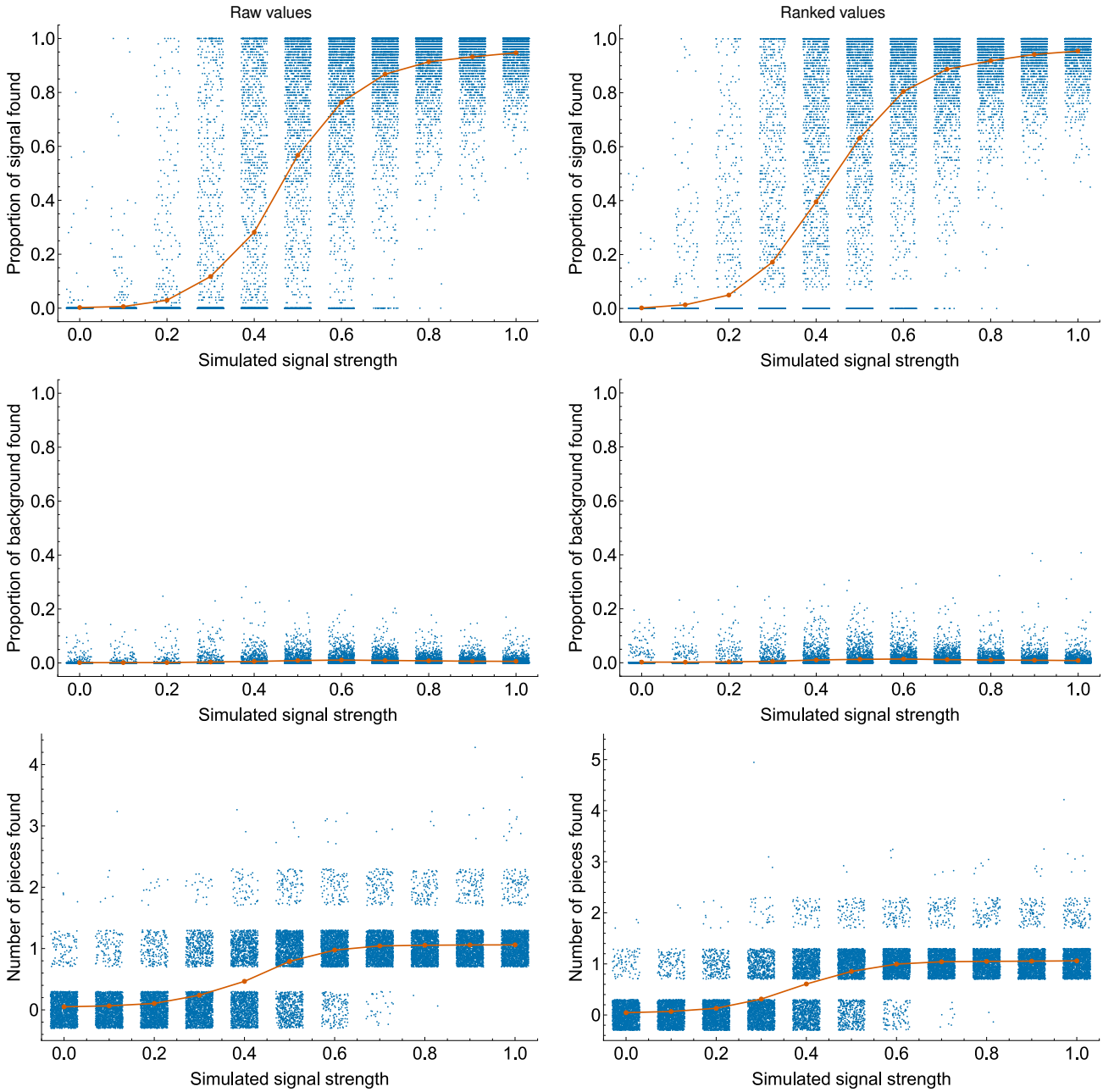

Fig. S4. Effect of analysing Gaussian signal and background using ranking of values. For different signal strengths, 2,000 sequences were simulated with each value in the sequence a standard normal variable, and the middle 100 of 500 positions were offset by the signal strength to yield a signal region. Sequences were generated independently (i.e. not reused for different signal strengths). The algorithm was then applied to attempt to find the signal. The left column shows the effect of running the algorithm on the raw values generated and the right column shows the effect of running the algorithm with the values ranked. The dots show individual data points, dithered horizontally in all plots and vertically as well in the lowest pair of plots. The lines show averages. The upper plots give the proportion of the signal (middle 100 of 500 positions) found by the algorithm. The middle plots give the proportion of the background (other 400 of 500 positions) identified as signal. The bottom plots give the number of separate pieces identified as signal. The results are very similar between the raw and ranked data, with a small increase in proportion of signal found associated with ranking, most probably because in this simulated dataset the smoothing effect of ranking reduces the chances of identifying a smaller piece of signal and missing the remainder.

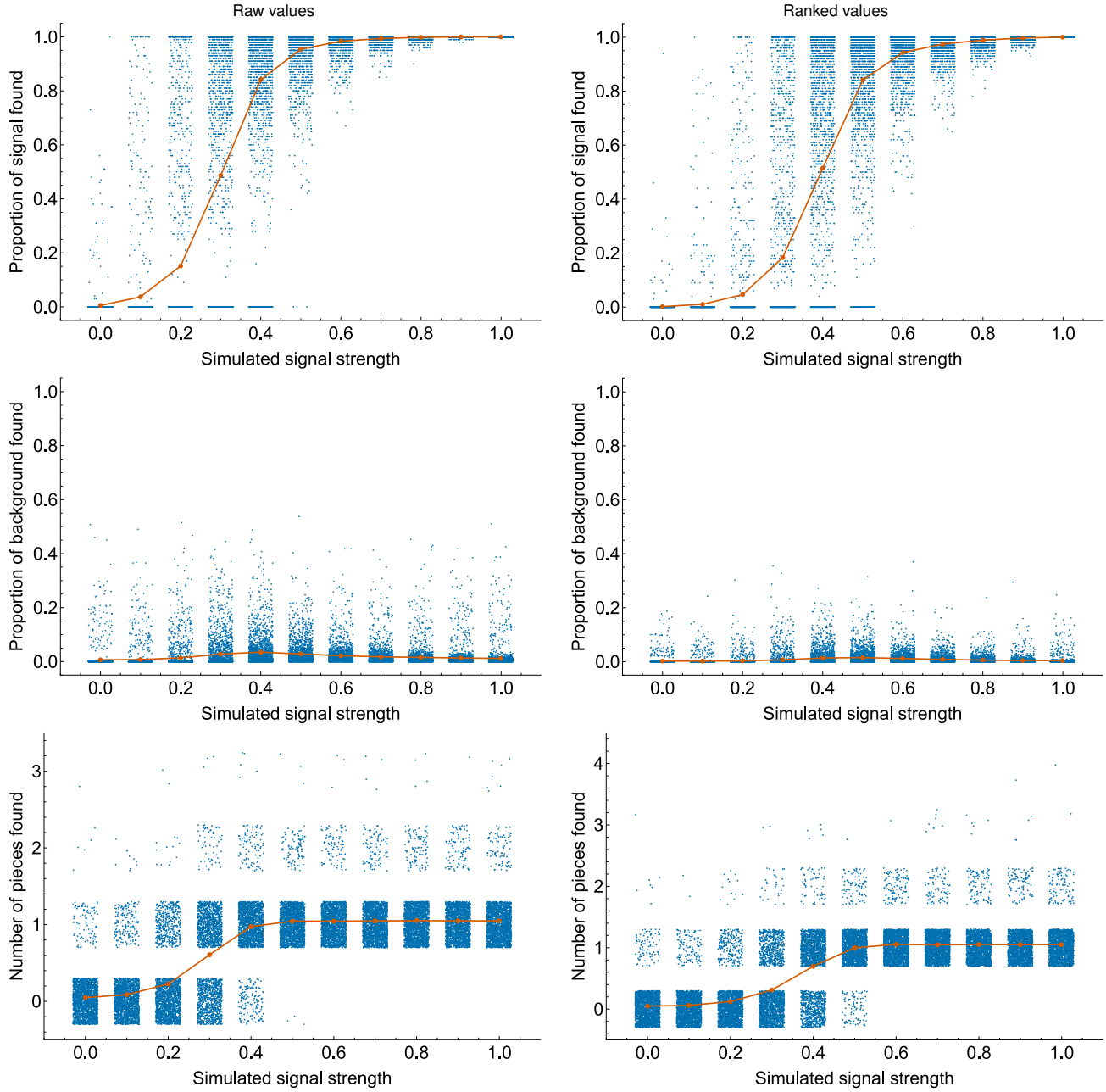

Fig. S5. Effect of analysing exponential signal and background using ranking of values. For different signal strengths, 2,000 sequences were simulated with the middle 100 of 500 positions exponentially distributed with mean $(1 + (\text{simulated signal strength}))$ and the remaining positions exponentially distributed with mean 1. The algorithm was then applied to attempt to find the signal. The left column shows the effect of running the algorithm on the raw values generated and the right column shows the effect of running the algorithm with the values ranked. The dots show individual data points, dithered horizontally in all plots and vertically as well in the lowest pair of plots. The lines show averages. The upper plots give the proportion of the signal (middle 100 of 500 positions) found by the algorithm. The middle plots give the proportion of the background (other 400 of 500 positions) identified as signal. The bottom plots give the number of separate pieces identified as signal. With this skewed distribution, ranking values mitigates a number of instances of miscalling background as signal, at the expense of reducing the sensitivity for finding signal. Note that the algorithm efficiency for finding signal is not directly comparable with the Gaussian signal and background because the standard deviation of signal values is smaller here. We note that although the distribution benchmarked here is skewed, it is still parametric and genetic data with low variability might generate worse algorithm behaviour in misidentifying background as signal.

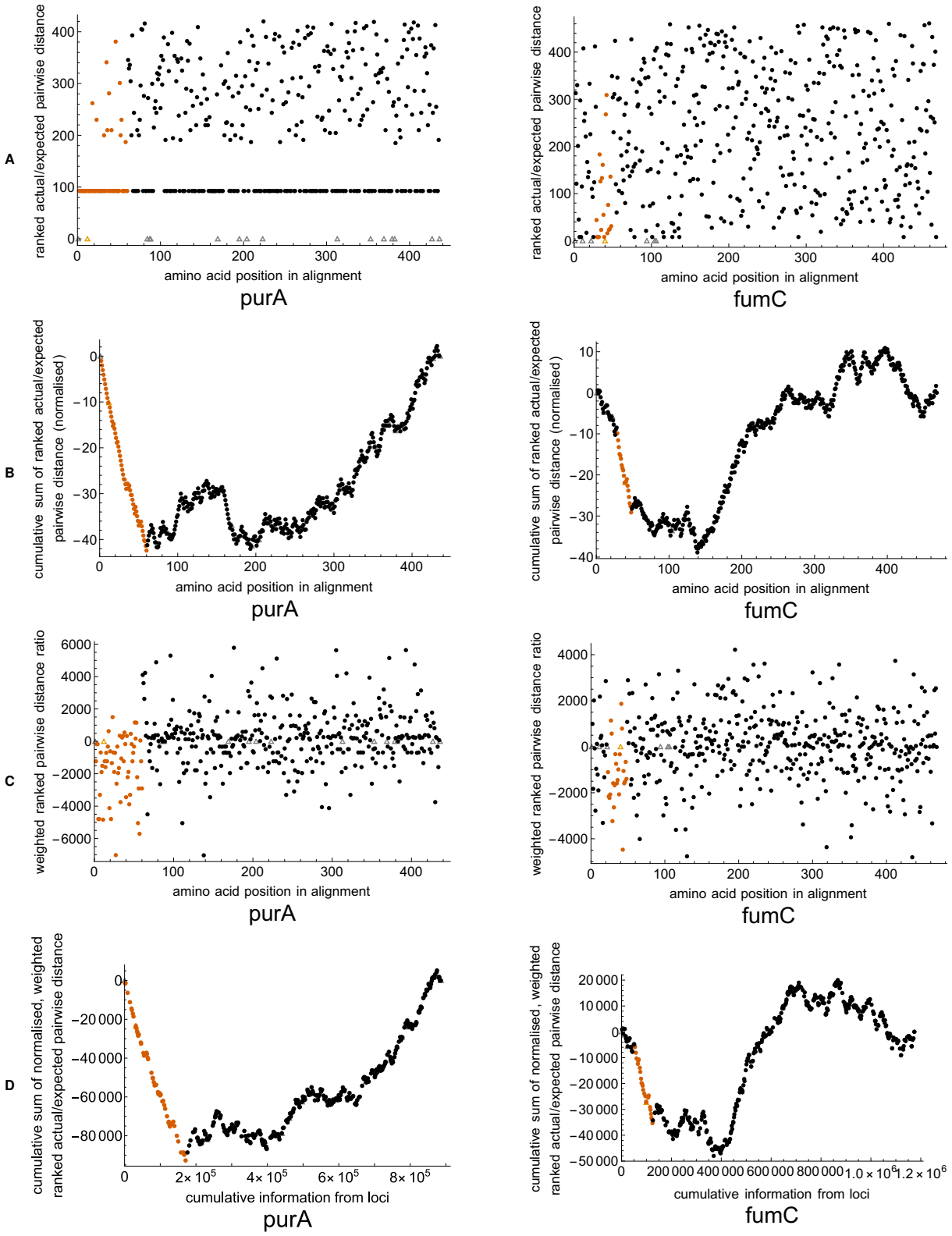

Fig. S6. Effect of ranking information content of loci in *E. coli* housekeeping genes purA (left) and fumC (right). In cumulative sums and where weighting is applied, ranked variability measures are normalised to have mean 0 and standard deviation 1. (**A**) Ranked raw variability measures. (**B**) Cumulative sums of ranked raw variability measures. (**C**) Weighted ranked variability measures (see Methods for details). (**D**) Cumulative sums of weighted ranked variability measures. Orange loci correspond to regions deemed to be significantly conserved by our algorithm. Grey and light orange loci plotted with open triangles yield no information on nucleotide conservation and were not included in analyses(2). Ranking mitigates the effect of outlying, high variability loci. In both cases seen here, ranking alone leads to identification of similar conserved regions to those seen with weighting, and the combined effect is similar, but regions are slightly differently circumscribed.

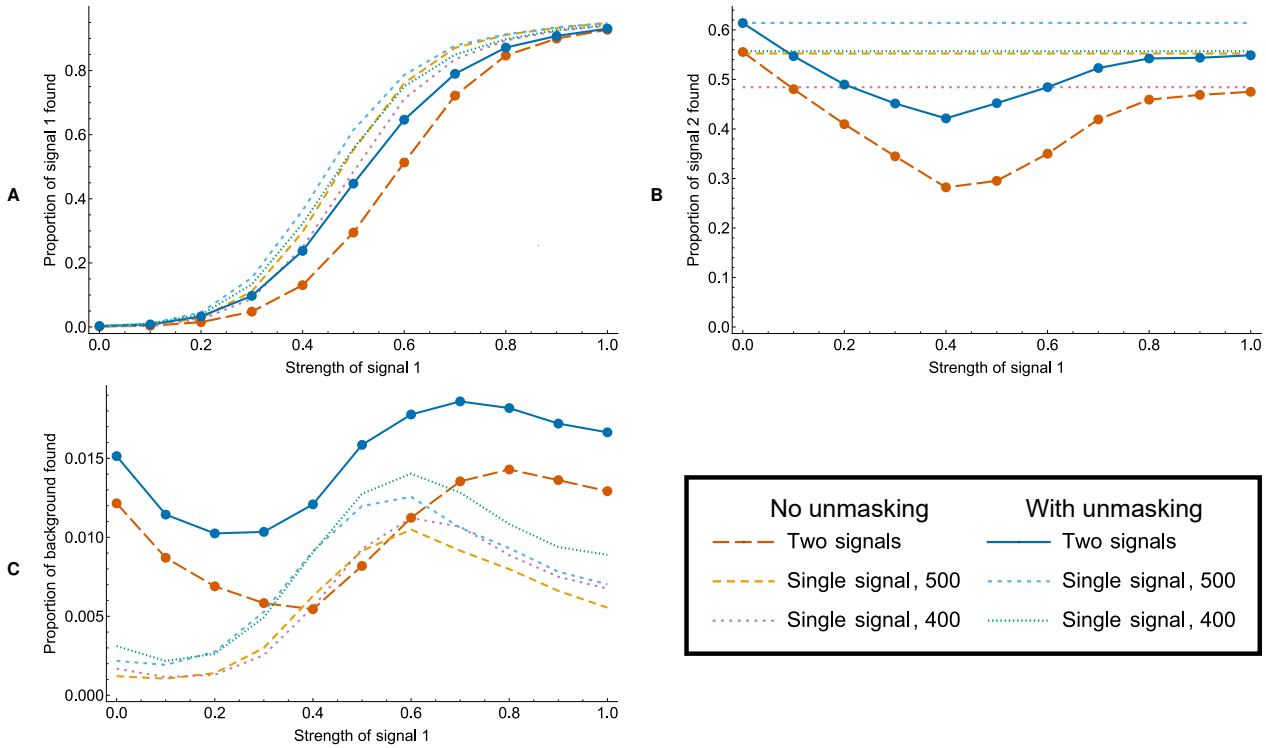

Fig. S7. Effect of implementing a procedure to unmask a signal obscured by the presence of a second signal, by repeating analysis with the next most significant region excluded in the event the first region identified by our algorithm is deemed non-significant. In each case two signals of length 100 are generated in a sequence of length 500. The background points in the sequence are sampled from a standard normal distribution. The signal points are sampled from a normal distribution with mean offset by the plotted "strength" for signal 1, and always offset by 0.5 for signal 2. For each average, 10,000 simulations were run, with the decision on whether to accept/reject a proposed signal region made following 1,000 bootstrap resamples of the simulated data. Results are plotted for the same simulations with and without the signal unmasking procedure used. Also plotted are equivalent results for finding a single signal in a sequence of length 500 and of length 400 (a single signal in a sequence of length 500 is the appropriate comparator when the algorithm searches for the first signal it can find; if this signal is found and excised then the appropriate comparator for finding the second signal is in a sequence of the remaining length of 400). (**A**) Proportion of first signal found as the signal strength varies. (**B**) Proportion of second signal found as the first signal strength varies. (**C**) Proportion of background (non-signal region) found as the first signal strength varies. Note that the average proportion of background found represents at most just over 5 positions in the sequence. Overall, the effectiveness of the algorithm in finding two signals in a sequence differs most from the effectiveness at finding one signal when the two signals are of similar strengths (so that thre is an obscuring effect from the second signal being counted in the background when evaluating the significance of the first signal). The issue can be partially rescued by implementing the unmasking procedure (and this procedure also slightly increases the algorithm's ability to find single signals), at a small cost in increased misidentification of background sequence as signal.

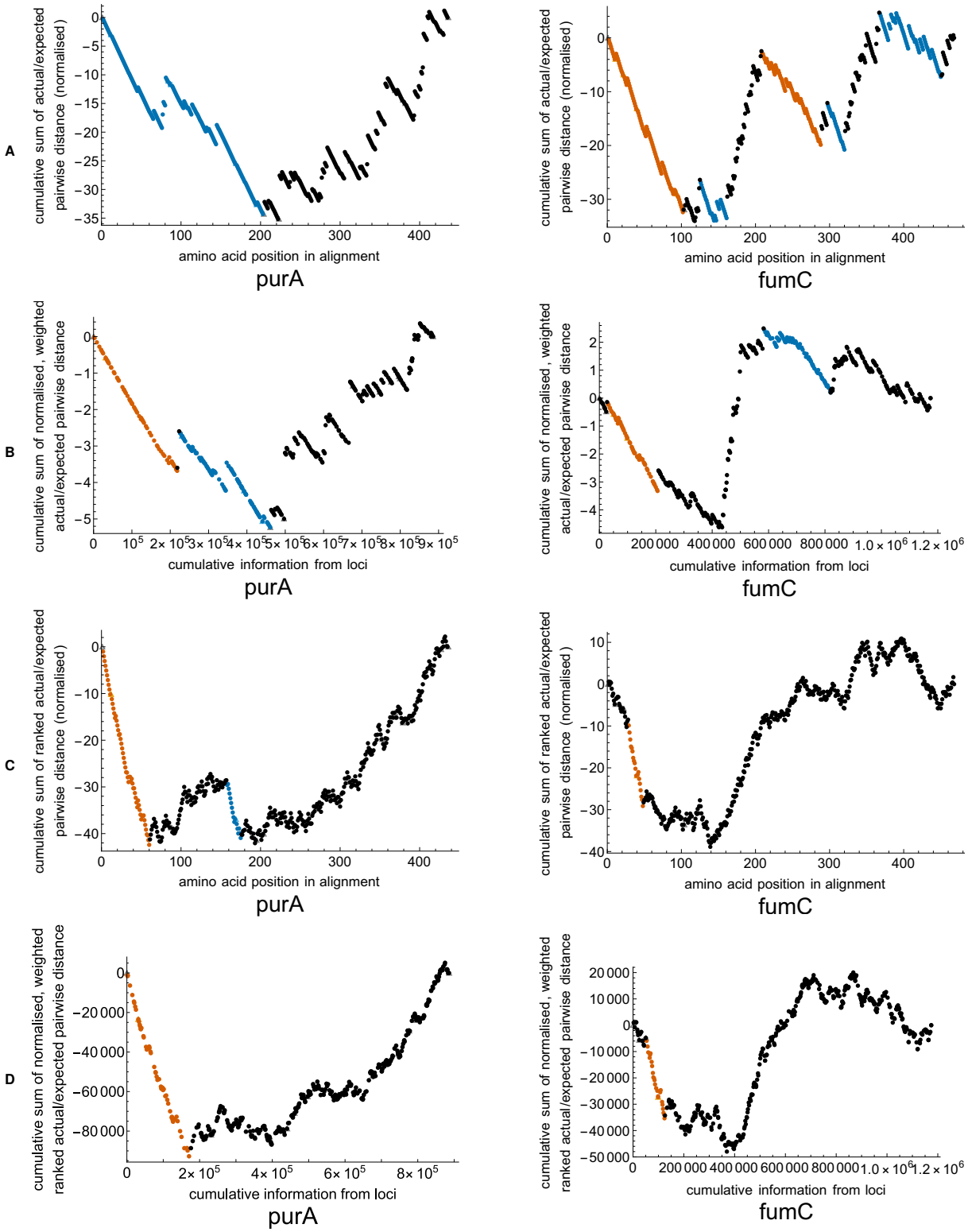

Fig. S8. Effect of including screen for possible interfering signals in *E. coli* housekeeping genes purA (left) and fumC (right). The analyses performed are as in Figs. S2 and S6, save that the analysis for possible interfering signals described in the text is performed. Regions additionally deemed significant after this analysis are highlighted in blue. (**A**) Cumulative sums of raw variability measures. (**B**) Cumulative sums of weighted raw variability measures. (**C**) Cumulative sums of ranked variability measures. (**D**) Cumulative sums of weighted ranked variability measures. Orange loci correspond to regions deemed to be significantly conserved by our algorithm prior to the interfering signals analysis. Grey, light orange and light blue loci plotted with open triangles yield no information on nucleotide conservation and were not included in analyses(2).

2. Notes on conserved regions in *Escherichia coli*

In all of the housekeeping genes we analyse, our algorithm finds regions with relative conservation at the start of the gene. This is a previously described phenomenon (4–11), and serves as a useful positive control for our algorithm. For two genes, we note below additional evidence that corroborates our findings.

Isocitrate dehydrogenase (*icd*) gene There is a region of high diversity at the 3′ end of the gene, visible as a steep ascent in the cumulative sum graphs of Fig. S13. This phenomenon is known to arise from the presence in some strains of *E. coli* of one of two bacteriophages (12). This region of high diversity appropriately results in regions in the middle of the gene being called as relatively conserved by our algorithm in some cases – although the phenomenon being observed could be regarded less as conservation in one region, and more as increased diversity in another.

Malate dehydrogenase (*mdh*) gene Our finding of a conserved region towards the 5′ end of the gene is consistent with the locations of polymorphic loci found in earlier works (13, 14).

3. Additional notes on conserved regions in SARS-CoV-2

Below, we note two regions called as conserved by our algorithm, together with possible biological explanations. In each case, these are not described in the main text because either the evidence for the advanced possible explanation is weak or there is evidence that casts doubt upon the explanation. In each case, we discuss the evidence. We include these discussions as methodological examples of the types of conserved regions that might be found and investigations that should be undertaken to corroborate different putative explanations. We also wish to describe the evidence we have found relating to these regions, in case it later corroborates experimental evidence that should arise.

Candidate negative-sense open reading frame in SARS-CoV-2 nsp7 region Our algorithm identifies two mutually interfering signal regions within the nsp7 region of SARS-CoV-2, each of which is only identified as significant in the absence of the other. The first region identified (NC_045512.2 nucleotide reference 11843–11914) begins with AUG in the +1 negative-sense frame, and ends UAG in that frame. There is not a strong Kozak sequence (UAGAUG**AUG**A) in the reference sequence and there is a UGA in the fifth codon position. If translation were initiated in this frame, then a termination codon close to the initiation complex may simply be read through (with the UGA translated as cysteine or tryptophan), especially in the presence of eIF3 (15); however for the reference sequence there are few other markers of high frequency translational readthrough of termination codons (16). We were therefore motivated to consider for the entire dataset (i) how conserved the putative initiation and termination sites are, (ii) the frequency of Kozak nucleotides near the putative initiation codon, and (iii) frequency of a termination codon in the putative +5 codon position and markers of translational readthrough. Fig S9 shows output of analysing the region for conserved nucleotides in the key positions: there is a high level of conservation.

The region of interest is a long distance from the 5′ terminus of negative-sense RNAs, raising the possibility that if translated, translation initiation may occur via an internal ribosome entry site (IRES) rather than in a cap-dependent fashion.

We undertook searches for known similar peptides with the translated sequence as input (searching with each of cysteine and tryptophan in position 5 of the sequence) using BLASTP 2.13.0+ (17) on the non-redundant proteins database. There were no strong hits: the strongest hit in both cases was a region within a *Pseudomonas* protein (accession WP_019581496.1) with E scores of 0.49 and 1.2 for the tryptophan- and cysteine-containing versions respectively. We also undertook searches for known similar peptides using InterProScan 5 with InterPro 89.0 (18, 19): there were no hits. We note (see the Introduction in the main text) that whilst the absence of known analogues may decrease our confidence that this region represents a translated ORF, the absence of analogues if it were translated would make the resultant protein a more specific drug target.

Overall, the evidence for this region representing a biologically significant ORF is weak: the region of interest is not identified except after removal of a potentially interfering region, it is unclear how translation would be initiated on the negative-sense strand, there is an early termination codon without strong markers of translational readthrough, and the putative product does not have any clearly analogous proteins.

Candidate additional frameshift motif spanning SARS-CoV-2 nsp9 and nsp10 regions Our algorithm identifies a small conserved region within the nsp9 region of SARS-CoV-2. Inspection of the region and surrounding nucleotides demonstrates an A_UUA_AAC motif just 5′ to the conserved nucleotides, NC_045512.2 nucleotide location 12964–12970, as well as several UGGUA motifs within and 3′ to the region (NC_045512.2 nucleotide references 12987–12991, 12994–12998 and 13027–13031) that could pair with the UGCCA motif at NC_045512.2 nucleotide location 13048–13052 to form a pseudoknot (Fig. S10). This is reminiscent of known coronavirus frameshift motifs with a slippery site followed by a pseudoknot (20–22), and manual inspection confirms the possibility of a two-loop motif with pseudoknot. The failure of our algorithm to idenfify the entire region can be partially explained by its spanning nsp9 and nsp10; however, the region that is identified is only called conserved by our algorithm after removal of a potentially interfering signal that is not then itself identified as conserved. This raises the possibility that the identified region is in fact a false positive.

The putative slippery site is similar to, but does not match, known coronavirus frameshift motifs (21). If a −1 frameshift occurred at the putative slippery site, the ribosome would find two UAGs in-frame shortly after frameshifting (NC_045512.2 nucleotide references 12979–12981 and 12997–12999). The putative frameshift would therefore either lead to a premature termination and truncated protein, or there would need to be readthrough of the termination codons (which would likely lead to termination at a UAA at the 5′ end of the nsp10 region, NC_045512.2 nucleotide reference 13030–13032). Premature

termination would not in itself exclude the possibility of a frameshift: indeed, the absence of ribosomal frameshifting at the canonical site, leading to termination and generation of the 1a polyprotein, may be viewed as a premature termination that serves to modulate relative abundances of protein cleavage products. Overall, however, the weak algorithmic conservation call, coupled with the weak slippery site, coupled with the premature termination codons in the −1 frame, mean that further corroboration would be required before there could be considered adequate evidence of a frameshift in this region.

4. Note on additional tables and figures relating to *E. coli* data

Table S1 summarizes locations of conserved regions found by our algorithm in the analysed *E. coli* housekeeping genes, using weighted and ranked codon variability values (see main text for the justification for using this analysis procedure). Fig. S11 contain plots relating per-locus variability measures versus per-locus information, for the analysed housekeeping genes for which plots are not found in the main text. Figs. S12 to S18 contain plots showing variability values and conserved regions applying the different options for analysis procedures.

5. Note on additional tables and figures relating to SARS-CoV-2 and to SARS-CoV data

Tables summarizing locations of conserved regions found by our algorithm in SARS-CoV-2 may be found in the main text. The corresponding tables for SARS-CoV are Tables S2 and S3. Table S12 lists GenBank accession numbers of the sequences used for the SARS-CoV analysis. Figs. S19 and S20 contain plots relating per-locus variability measures versus per-locus information, for the analysed genes and 1ab regions of SARS-CoV-2. The corresponding figures for SARS-CoV are Figs. S61 and S62. Figs. S21 to S28 contain plots showing weighted ranked variability values and conserved regions in SARS-CoV-2 genes and 1ab regions. The corresponding figures for SARS-CoV are Figs. S63 to S71.

6. Note on RNAalifold figures

Figs. S29–S60 and Figs. S72–S96 give predicted folds using RNAalifold of the regions determined to be conserved by our algorithm in SARS-CoV-2 and SARS-CoV, respectively. As noted in the Methods, folds are only attempted for regions ≤ 5500 nucleotides in length – this is motivated primarily by memory constraints when running RNAalifold, but a secondary motivation for avoiding folds of longer regions is that the chances our algorithm has identified the dominant length scale for secondary structure formation would seem lower when there are multiple possible smaller length scales. Figures are not included for SARS-CoV-2 E regions of interest, 7b regions, and the nsp6 region of interest, because RNAalifold does not predict any base pairings in these regions. The fold of the forward sense RNA in the region of nsp16 thought to contain a packaging signal may be found in the main text. Whilst the SARS-CoV folds appear to reflect better the folding potential of the underlying alignments than the SARS-CoV-2 folds (more deep red pairings, indicating all sequences in alignment can make the displayed pairing), it must be remembered that there are far fewer SARS-CoV than SARS-CoV-2 sequences in the respective datasets, and the SARS-CoV sequences represent less evolutionary time than the SARS-CoV-2 sequences used.

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Table S1. Summary of regions of significant conservation found in *E. coli* genes, using weighted and ranked codon variability values. [*]Denotes a region only found by excluding a potentially interfering signal. Z- and p-values in parentheses denote values prior to removal of the next most significant signal. If parenthetical values are absent, then such a signal was removed in an earlier step only. [†]Denotes a gene expressed from the reverse DNA strand.

Gene	Order found	NC_000913 nt location	Z	p
adk	1	497178–497225	3.02	0.0001
icd	1	1195126–1195377	3.05	0.0058
	2*	1195534–1195554	2.42	0.0425
			(2.38)	(0.0803)
fumC	1	1686522–1686445†	2.25	0.0151
recA	1	2823766–2823692†	3.23	0.0321
mdh	1	3384247–3384080†	3.56	0.0005
gyrB	2	3879735–3879082†	3.03	<0.0001
	1	3880116–3879739†	6.27	<0.0001
purA	1	4404690–4404866	4.96	<0.0001

Table S2. Summary of regions of significant conservation found in SARS-CoV genes. [*]Denotes a region only found by excluding a potentially interfering signal. Z- and p-values in parentheses denote values prior to removal of the next most significant signal. If parenthetical values are absent, then such a signal was removed in an earlier step only. sgRNA=subgenomic RNA, TRS=transcription-regulatory sequence (-B=body).

Gene	Order found	AY274119.3 nt location	Z	p	Comment
1ab	1	14211–16889	3.69	0.0227	Reason for conservation unclear.
S	2	21732–22421	2.86	0.0001	Reason for conservation unclear.
	1	24099–24869	3.12	0.0177	Region contains analogues of the 1st and 4th regions identified in SARS-CoV-2 S. Reason for conservation unclear.
E	1	26162–26200	2.64	<0.0001	5′ end of region has identical sequence to region in SARS-CoV-2 that is a possible TRS-B for forming alternate M sgRNA.
7b	1*	27650–27760	1.51	0.0001	Result dominated by two high variability loci and appears artefactual; although
			(1.42)	(0.0792)	region contains both regions identified in SARS-CoV-2, most of 7b is called conserved here by the algorithm.
8b	1*	27867–28019	1.03	0.0373	Result dominated by two high variability loci and appears artefactual.
			(0.84)	(0.5361)	
	2*	28023–28100	1.06	0.0106	Result dominated by two high variability loci and appears artefactual.
			(0.97)	(0.0852)	

Table S3. Summary of regions of significant conservation found in individual proteins of SARS-CoV 1a/1ab. *Denotes a region only found by excluding a potentially interfering signal. Z- and p-values in parentheses denote values prior to removal of the next most significant signal. If parenthetical values are absent, then such a signal was removed in an earlier step only.

Region	Order found	AY274119.3 nt location	Z	p	Comment
NSP2	1*	2017–2547	2.65 (2.47)	0.0343 (0.0941)	Result dominated by high variability loci, even in the weighted ranked setting. Whilst may contain real conservation, there is a risk the result is artefactual.
NSP3	1	6613–6987	2.48	0.0055	Reason for conservation unclear.
NSP6	1*	10978–11412	2.28 (1.99)	0.015 (0.1297)	Reason for conservation unclear.
NSP7	1	11791–11970	1.45	0.0422	Reason for conservation unclear.
	2*	11977–12021	1.47 (1.34)	0.0048 (0.0783)	Reason for conservation unclear.
NSP10	2*	12985–13095	1.74 (1.57)	0.0276 0.1137	Reason for conservation unclear.
	1*	13153–13344	1.72 (1.45)	0.0408 0.3846	Reason for conservation unclear.
NSP12	2*	13372–14037	2.30 (2.04)	0.0479 0.2772	Reason for conservation unclear.
	1*	14208–15852	2.34 (2.02)	0.0421 0.9977	Reason for conservation unclear.
NSP15	1*	19920–20195	2.38 (2.15)	0.0245 0.1014	Putative packaging signal(23, 24); see main text.

Table S4. GenBank accession numbers of sequences used for *E. coli* adk analysis.

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Table S5. GenBank accession numbers of sequences used for *E. coli* icd analysis.

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Table S6. GenBank accession numbers of sequences used for *E. coli* fumC analysis.

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Table S7. GenBank accession numbers of sequences used for *E. coli* recA analysis.

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Table S8. GenBank accession numbers of sequences used for *E. coli* mdh analysis.

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Table S9. GenBank accession numbers of sequences used for *E. coli* gyrB analysis.

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Table S10. GenBank accession numbers of sequences used for *E. coli* purA analysis.

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Table S11. Manual curation steps applied to *E. coli* housekeeping gene sequence collections

adk	Removed because of frameshift mutations: CP055158, CP059988, CP082330. Edited to remove extra nucleotides at 5′ end: CP003034, BA000007, AP018488, NC_002695.
icd	Removed because of frameshift mutations: CP054325, CP054335, CP054457, CP059988, CP063983, CP073609, CP082330, CP025520, CP035841. Removed owing to poor sequence identity (likely wrong gene labelled in database): LN832404, LT615379, LT615374, LT615372.
fumC	Removed because of frameshift mutations: CP054317, CP054319, CP055518, CP048304, CABWHO010000003, UFZW01000006, CP023749, CP027340, SEVU01000007, CP005930, CP006698, CP085517, CP058571, CP077063, CP054457, CP054328, CP054325, CP042627, CP042638, CP042606, CADDWW010000001. Removed as 5′ region not sequenced: CP054314, HE572566, UGFP01000001. Removed owing to poor sequence identity (likely wrong gene labelled in database): UGEE01000003.
recA	Removed because of frameshift mutations: CP042606, AP054335, CP054457, CP059922, CP082330, CNFQ01000001, CP059281. Removed as 3′ region not sequenced: CP054325.
mdh	Removed because of frameshift mutations: VMRI01000001, CP042599, CP042638, CP042627, CP042645, CP042615, CP042982, CP054314, CP054828, CP059925, CP048935, CP077063, CP082330. Removed as 5′ region not sequenced: CP044298.
gyrB	Removed because of frameshift mutations: CP054457, CP082330, NHYO01000001, CP029240, CP088534, CP048935, CP042606, CP042599, CP042638, CP042627, CP042645, CP042615, CP055158, CP051692, CP054828, CP046676. Removed as 5′ region not sequenced: AP024561, CP050214, CP050216, CP050208. Removed as 3′ region not sequenced: CP050194. Edited to remove extra nucleotides at 5′ end: AP025214, CP011342, CP011343, AP024126. Edited by adding gaps to align sequence: CP025703.
purA	Removed because of frameshift mutations: CP042599, CP042638, CP042645, CP054449, CP054454, CP046676, CP082330, CADDWT010000001, CP054457, CP054319, CP044298, CP042627, CP042626. Removed as 5′ region not sequenced: CP088551, CP088570. Removed as sequence contains premature termination codon: CP054328.

Table S12. GenBank accession numbers of sequences used in the SARS-CoV analysis.

AP006557.1	AP006558.1	AP006559.1	AP006560.1	AP006561.1	AY274119.3	AY278487.3	AY278488.2	AY278489.2	AY278490.3
AY278491.2	AY278554.2	AY278741.1	AY279354.2	AY282752.2	AY283794.1	AY283795.1	AY283796.1	AY283797.1	AY283798.2
AY291315.1	AY291451.1	AY297028.1	AY304495.1	AY310120.1	AY313906.1	AY321118.1	AY323977.2	AY338174.1	AY338175.1
AY345986.1	AY345987.1	AY345988.1	AY348314.1	AY350750.1	AY351680.1	AY357075.1	AY357076.1	AY362698.1	AY362699.1
AY390556.1	AY394850.2	AY394977.1	AY394978.1	AY394979.1	AY394980.1	AY394981.1	AY394982.1	AY394983.1	AY394984.1
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AY394995.1	AY394996.1	AY394997.1	AY394998.1	AY394999.1	AY395000.1	AY395001.1	AY395002.1	AY395003.1	AY395004.1
AY427439.1	AY461660.1	AY463059.1	AY463060.1	AY485277.1	AY485278.1	AY502923.1	AY502924.1	AY502925.1	AY502926.1
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AY568539.1	AY595412.1	AY613947.1	AY686864.1	AY714217.1	AY772062.1	AY864805.1	AY864806.1	DQ182595.1	DQ640652.1
DQ898174.1	EU371559.1	EU371560.1	EU371561.1	EU371562.1	EU371563.1	EU371564.1	FJ882926.1	FJ882930.1	FJ882931.1
FJ882938.1	FJ882963.1	GU553363.1	GU553364.1	JF292921.1	JF292922.1	JN854286.1	JQ316196.1	JX162087.1	

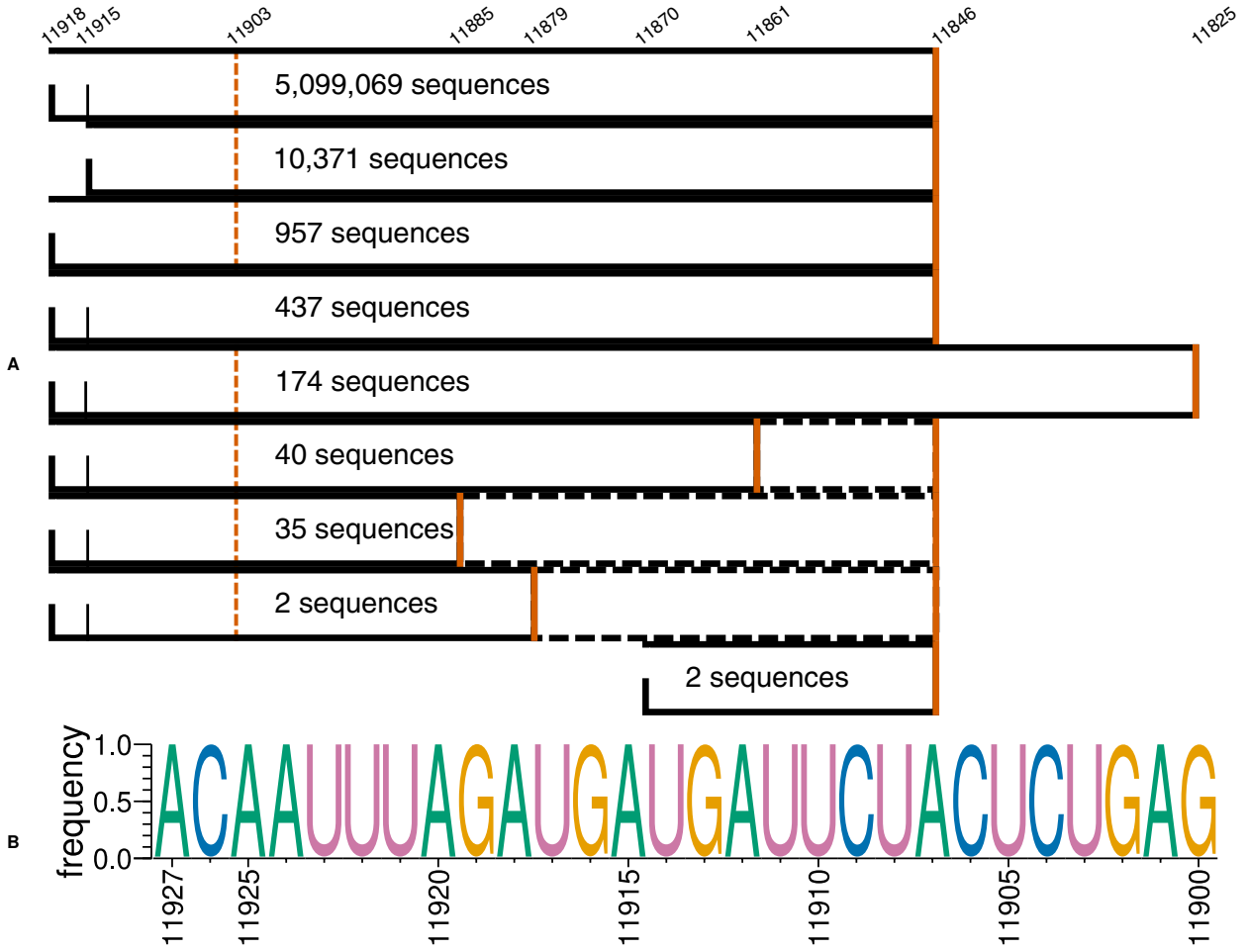

Fig. S9. (A) Schematic of the open reading frame potential of the first region of interest identified within the nsp7 region. Nucleotide references for the $5'$ end of codons in frame for the putative ORF are given at the top of the schematic. Black vertical half-bars represent postulated initiation codons. Orange vertical bars represent termination codons, with the termination codon that would require translational readthrough marked with a dashed line. Regions following apparent premature termination codons are outlined with dashed lines. There is commonly a termination codon in the putative +5 codon position, so that translational readthrough would almost always be required. If such readthrough is possible, then by the crude measures of the presence of initiation and termination codons, almost all sequences contain candidates for this putative ORF. **(B)** Sequence of the putative initiation region and early termination codon in the region of interest, generated using WebLogo3 (25). Although the sequence is highly conserved, it does not yield a strong Kozak sequence and would require translational readthrough of the early termination codon.

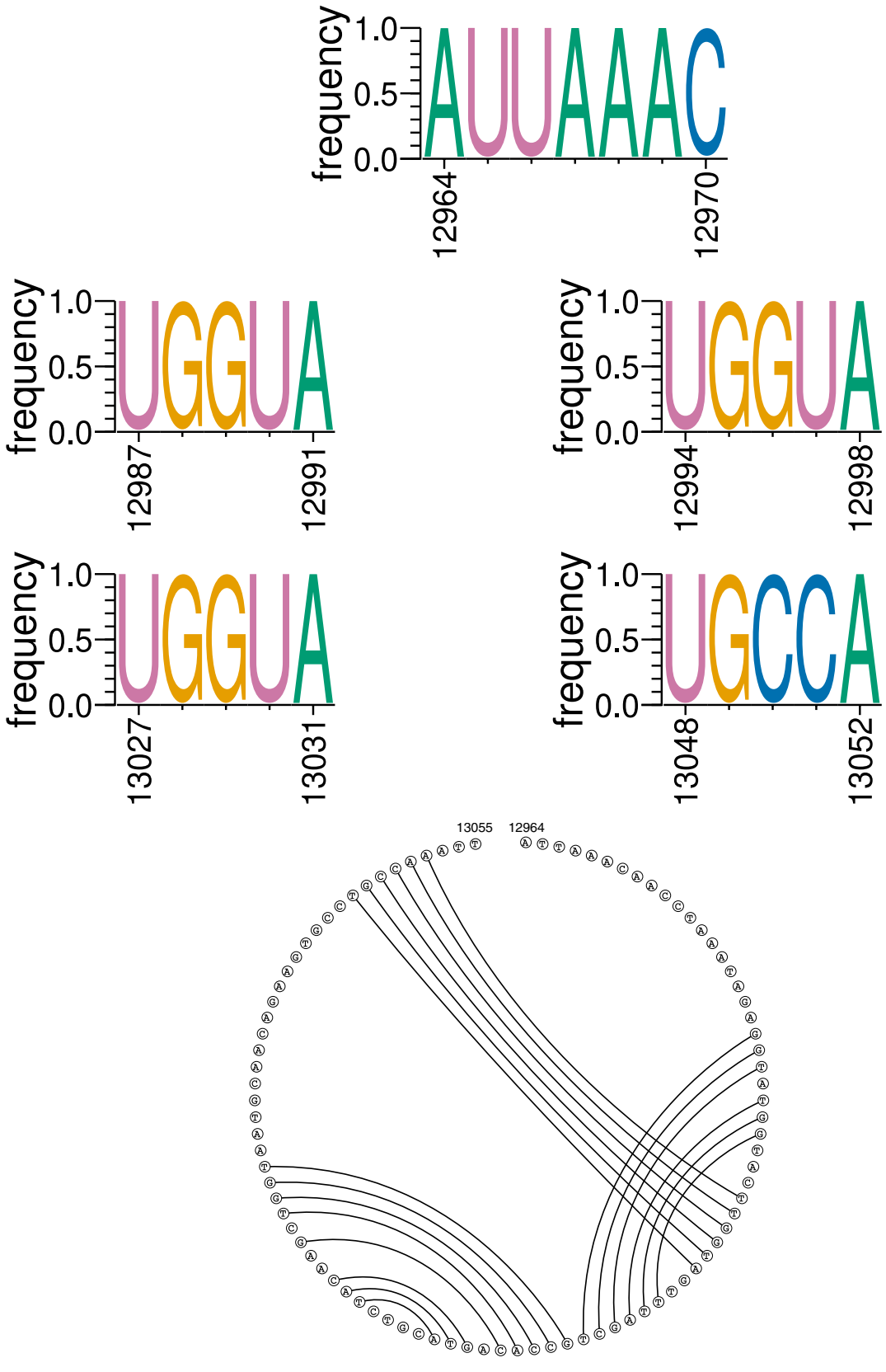

Fig. S10. Top rows: nucleotide frequencies in our dataset for the putative slippery site for a -1 frameshift within the nsp9 region, and for possible pseudoknot motifs, generated using WebLogo3 (25). Bottom: possible secondary structure consisting of two stem-loops with a pseudoknot, drawn in circularised form using the RNAstructure package (26). Note that this particular structure is generated by manual inspection, rather than running free-energy minimisation algorithms.

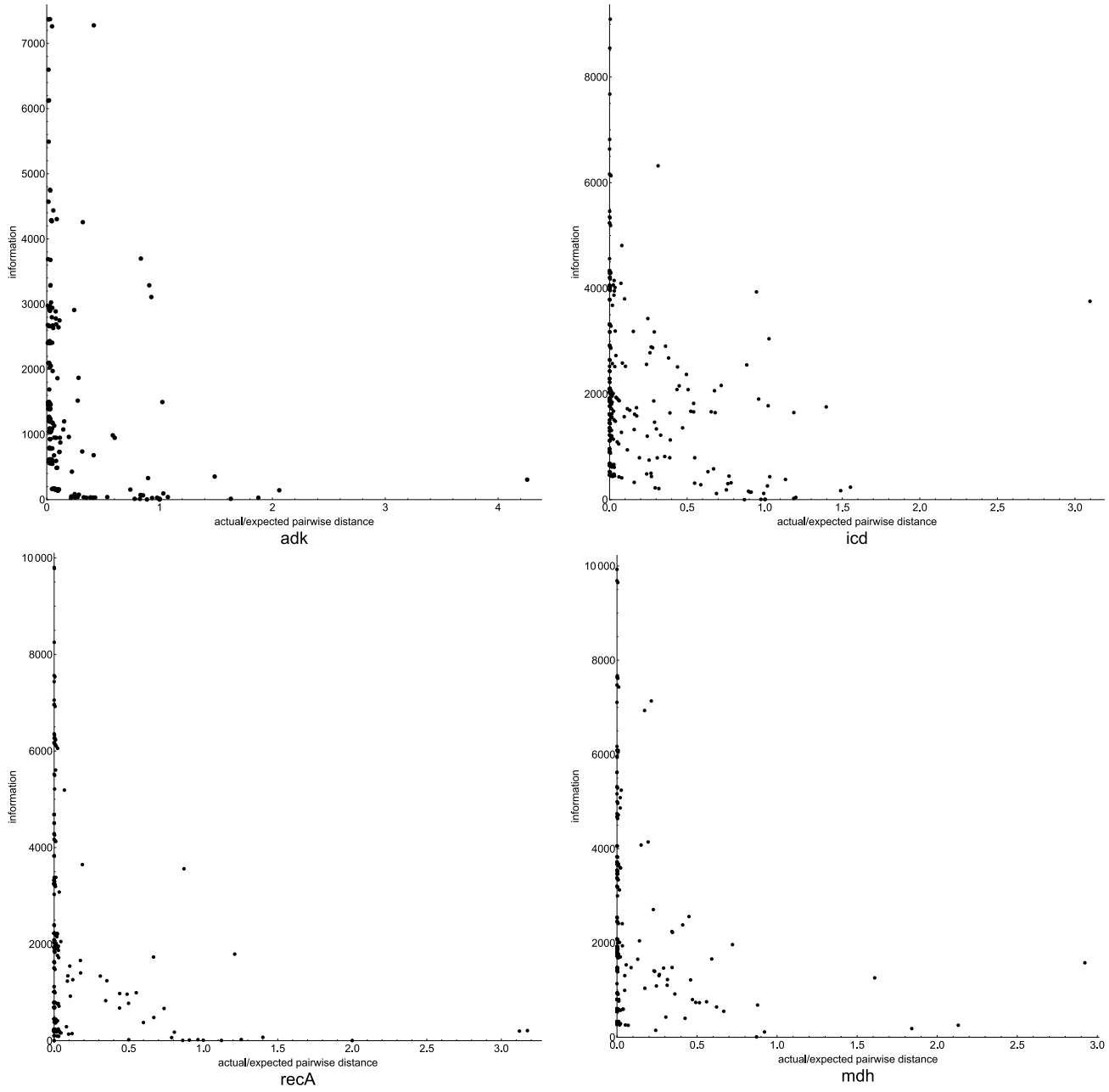

Fig. S11. Shannon information at locus versus measures of variability (actual/expected pairwise distance: see Methods for details) for housekeeping genes adk, icd, recA and mdh of *E. coli*. The plot for gyrB may be found in the main text. Throughout this work, each locus is a codon within a gene, although in principle different loci could be used with different measures of variability. Each plotted point represents the comparison of information/variability at a single locus. In each case, most loci have low variability, although the information gained from each low variability locus can vary. High variability loci are rare and many give low information. The absence of high information, high variability loci reflects the high number of combinations of loci producing high variability, meaning the Shannon information cannot be high.

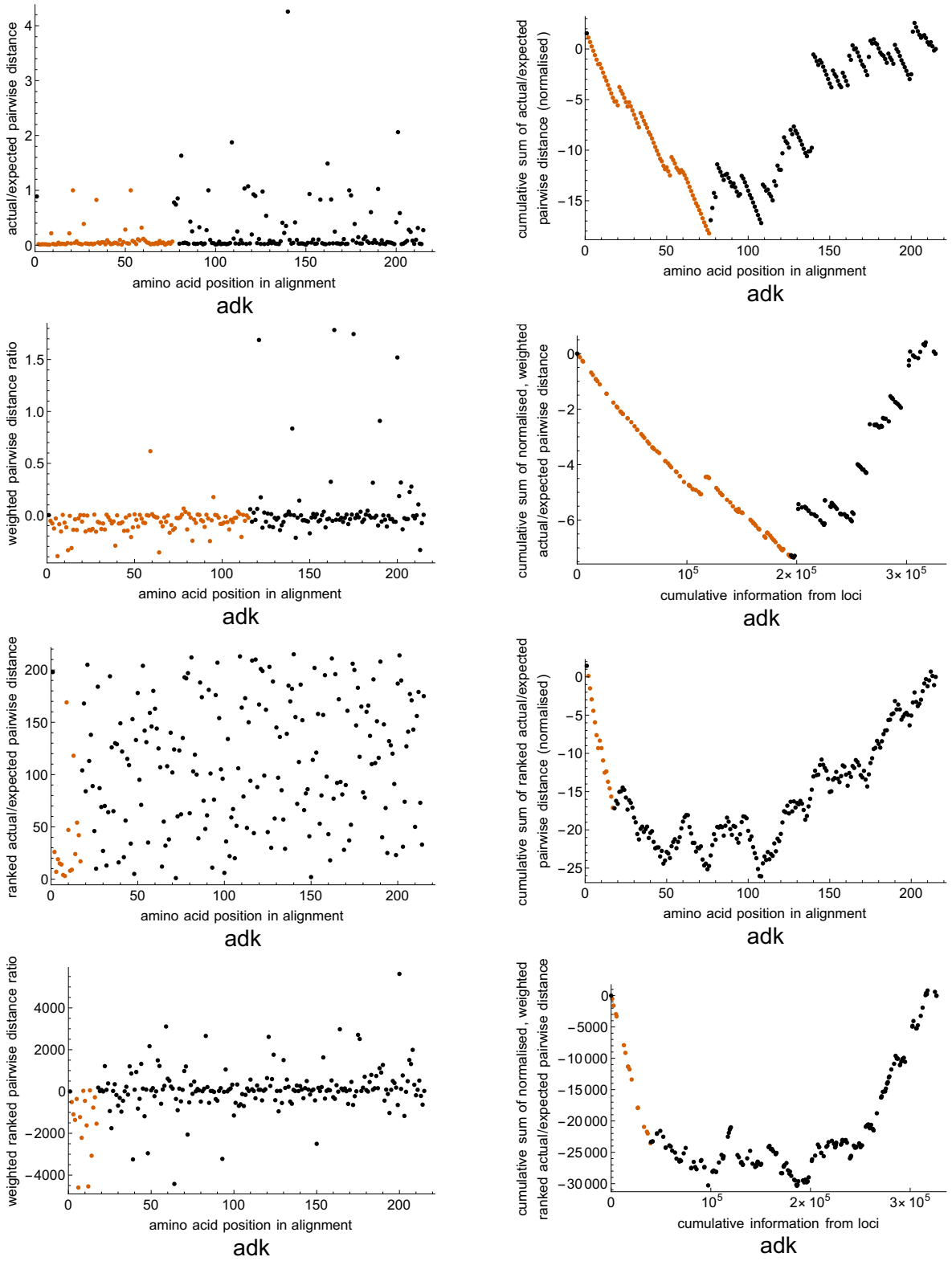

Fig. S12. Output from analyses of *E. coli* adk gene, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. In each case, regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Top row: using raw (unweighted/unranked) pairwise distance ratios. Second row: using weighted, unranked ratios. Third row: using unweighted, ranked ratios. Bottom row: using weighted, ranked ratios.

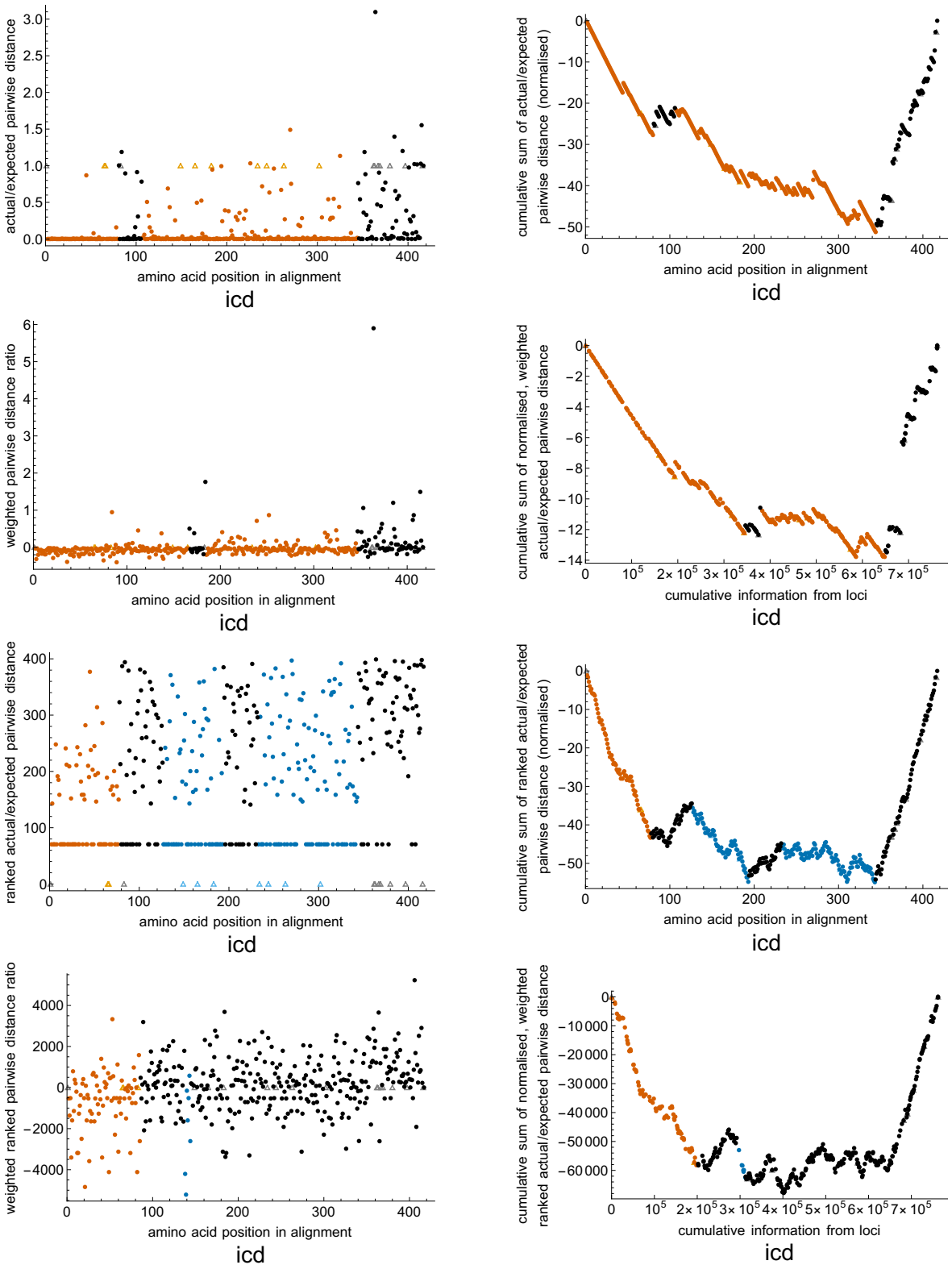

Fig. S13. Output from analyses of *E. coli* icd gene, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. In each case, regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Top row: using raw (unweighted/unranked) pairwise distance ratios. Second row: using weighted, unranked ratios. Third row: using unweighted, ranked ratios. Bottom row: using weighted, ranked ratios.

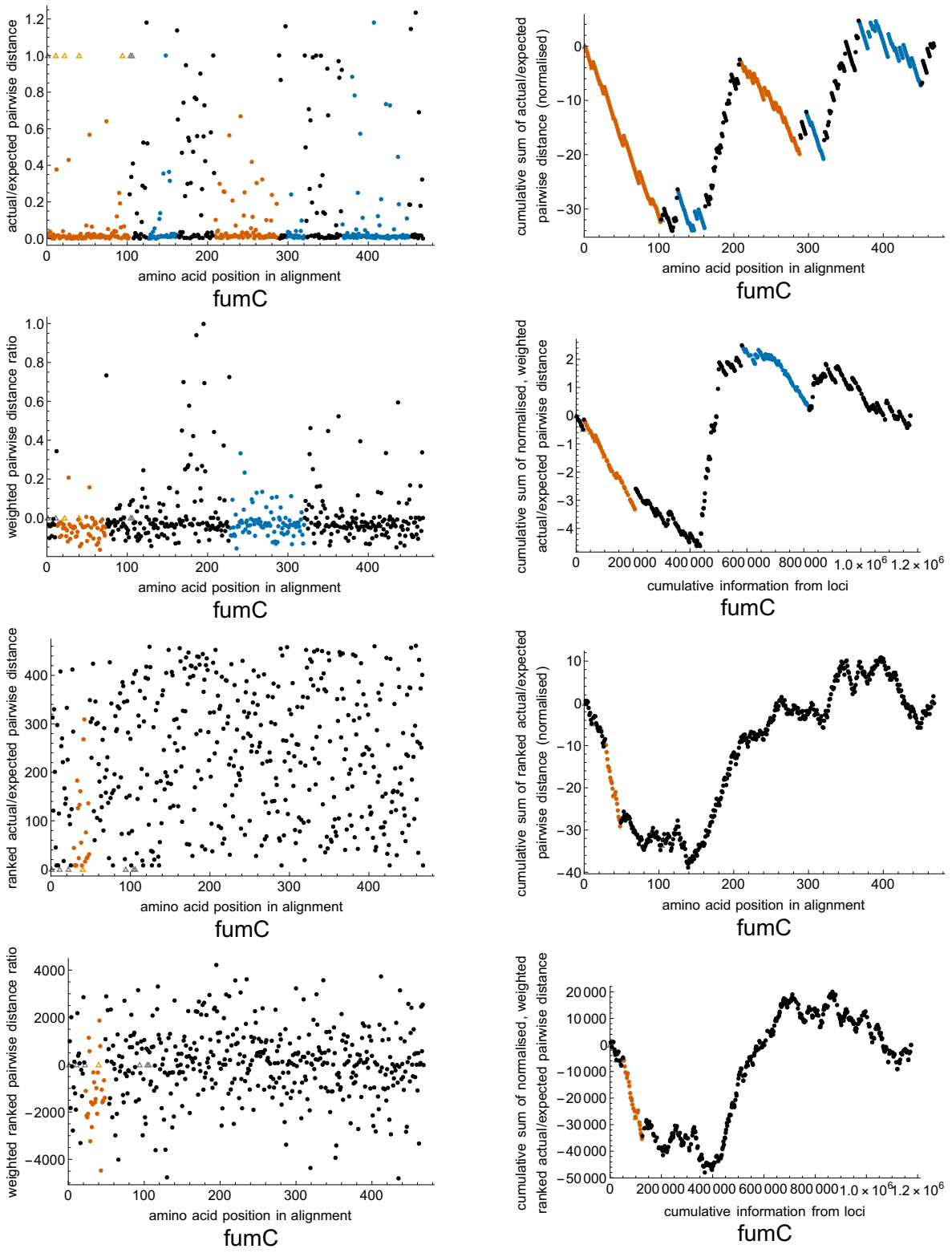

Fig. S14. Output from analyses of *E. coli* fumC gene, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. In each case, regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Top row: using raw (unweighted/unranked) pairwise distance ratios. Second row: using weighted, unranked ratios. Third row: using unweighted, ranked ratios. Bottom row: using weighted, ranked ratios.

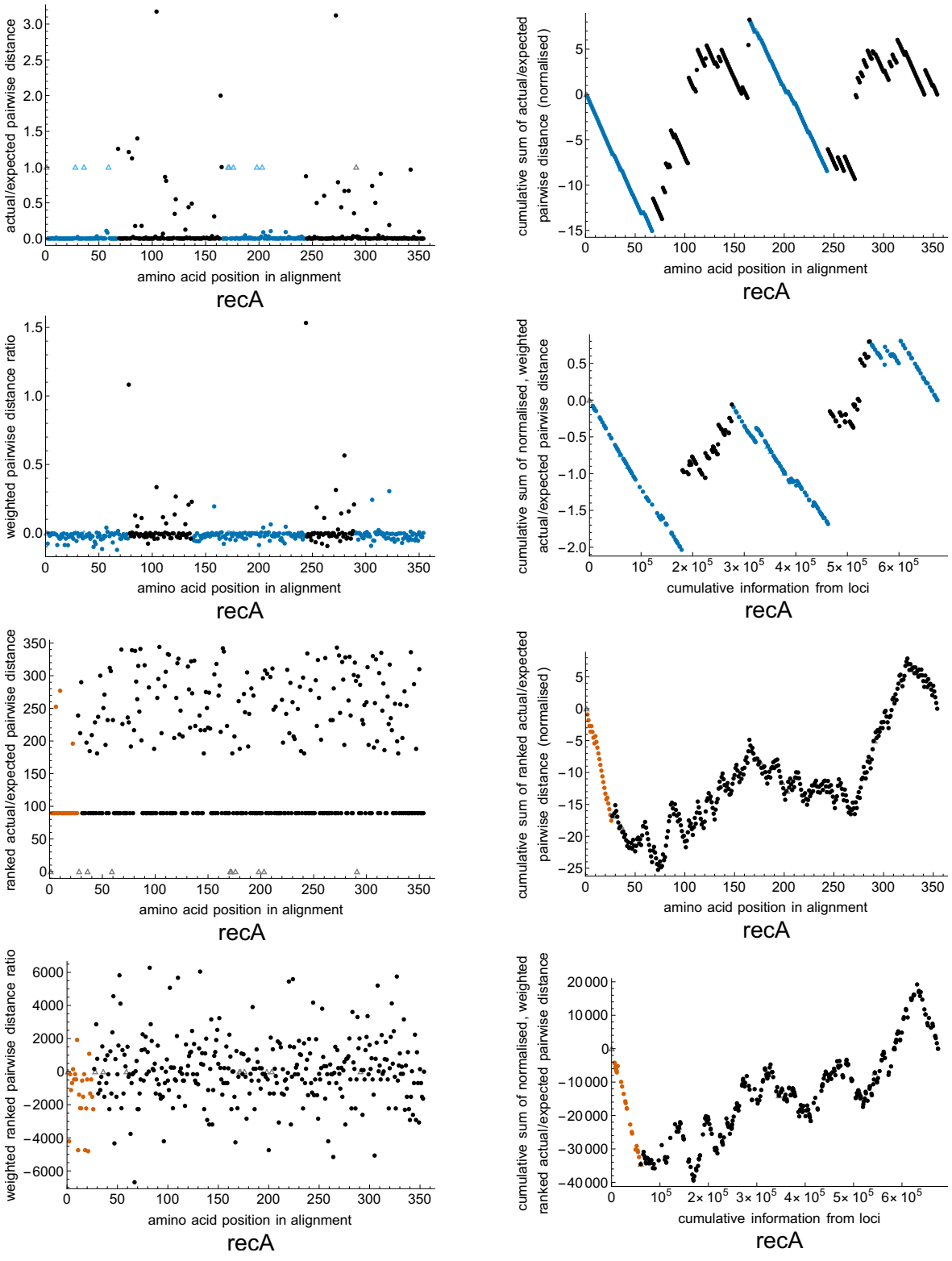

Fig. S15. Output from analyses of *E. coli* recA gene, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. In each case, regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Top row: using raw (unweighted/unranked) pairwise distance ratios. Second row: using weighted, unranked ratios. Third row: using unweighted, ranked ratios. Bottom row: using weighted, ranked ratios.

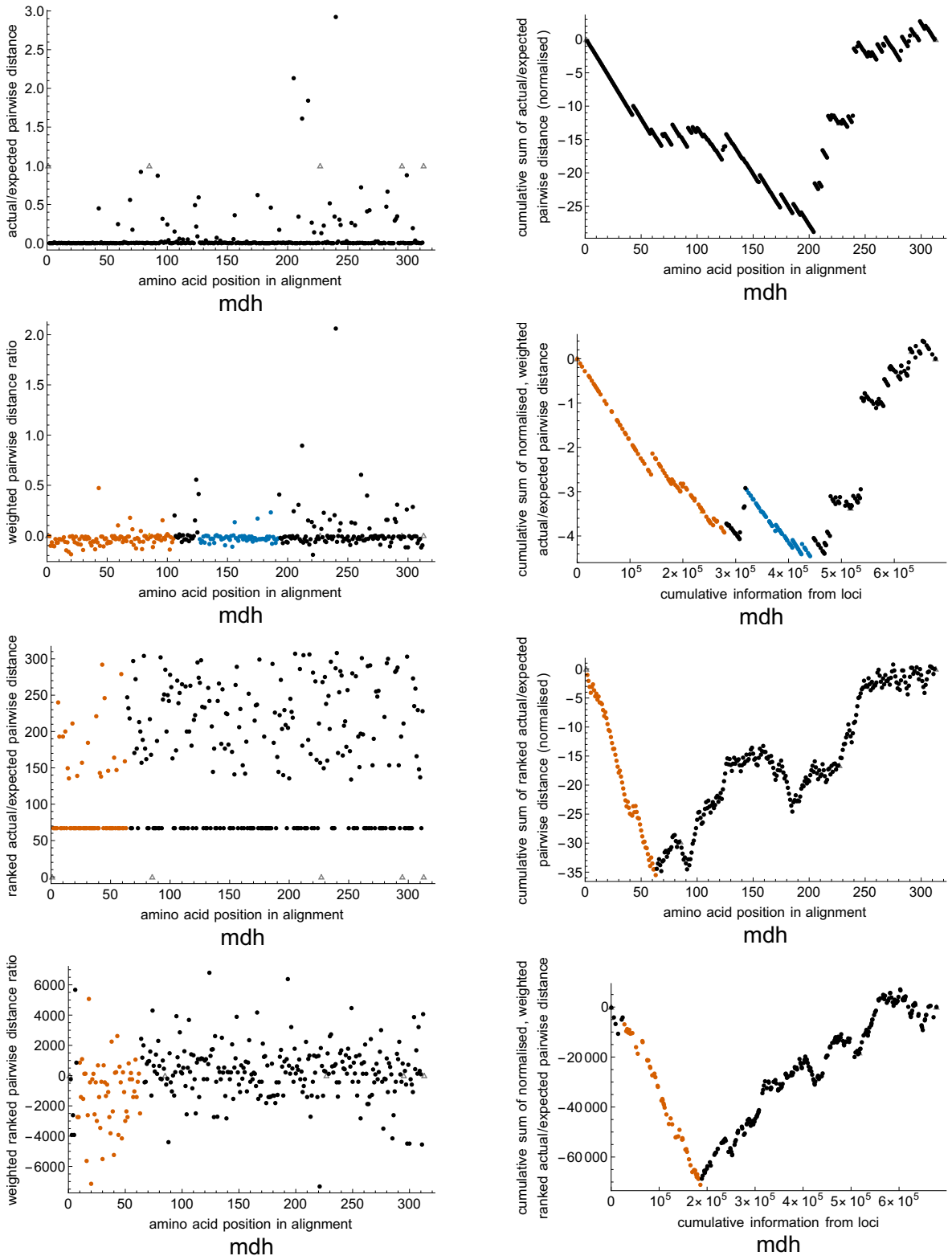

Fig. S16. Output from analyses of *E. coli* mdh gene, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. In each case, regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Top row: using raw (unweighted/unranked) pairwise distance ratios. Second row: using weighted, unranked ratios. Third row: using unweighted, ranked ratios. Bottom row: using weighted, ranked ratios.

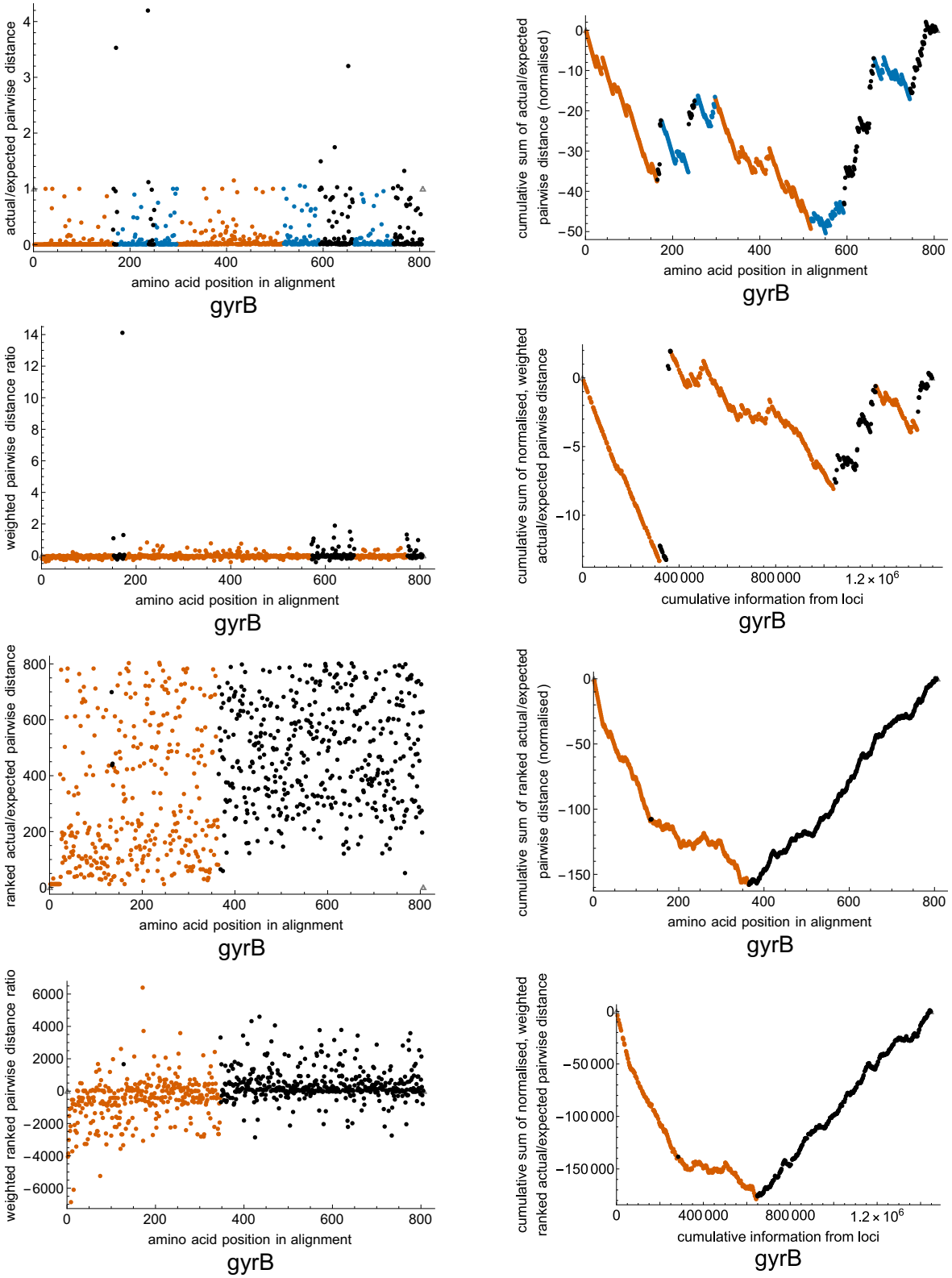

Fig. S17. Output from analyses of *E. coli* gyrB gene, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. In each case, regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Top row: using raw (unweighted/unranked) pairwise distance ratios. Second row: using weighted, unranked ratios. Third row: using unweighted, ranked ratios. Bottom row: using weighted, ranked ratios.

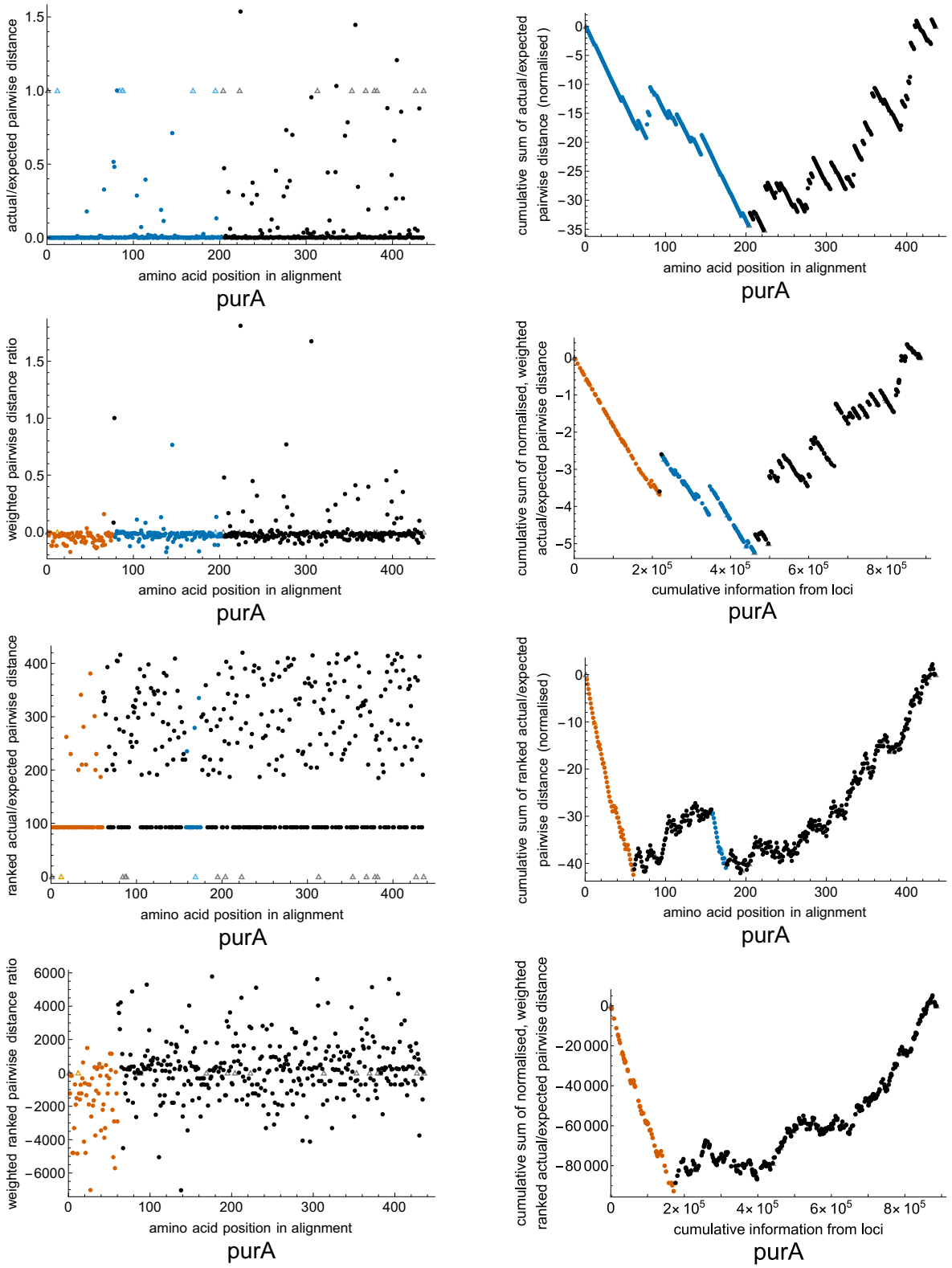

Fig. S18. Output from analyses of *E. coli* purA gene, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. In each case, regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Top row: using raw (unweighted/unranked) pairwise distance ratios. Second row: using weighted, unranked ratios. Third row: using unweighted, ranked ratios. Bottom row: using weighted, ranked ratios.

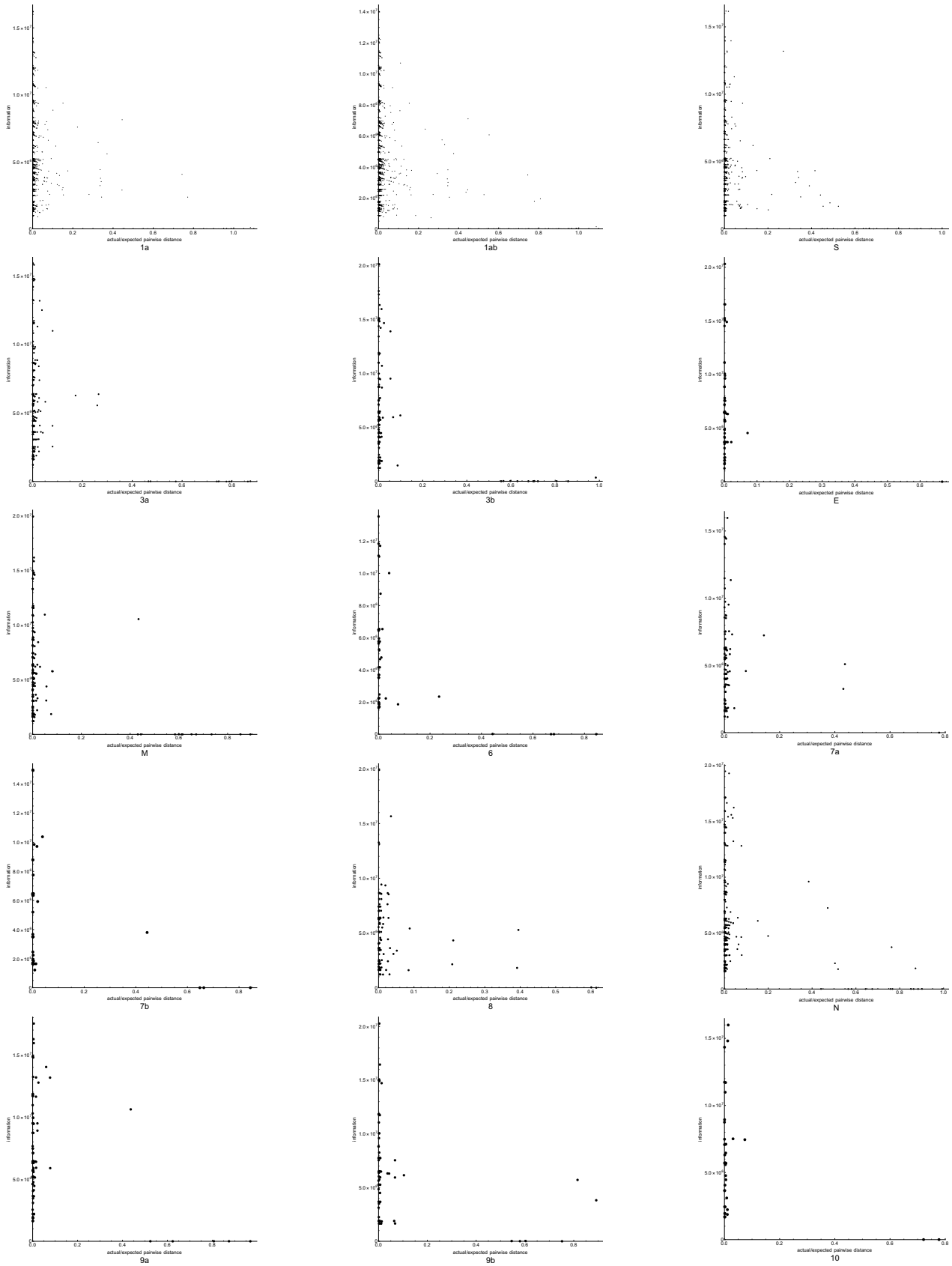

Fig. S19. Locus information versus variability for genes of SARS-CoV-2.

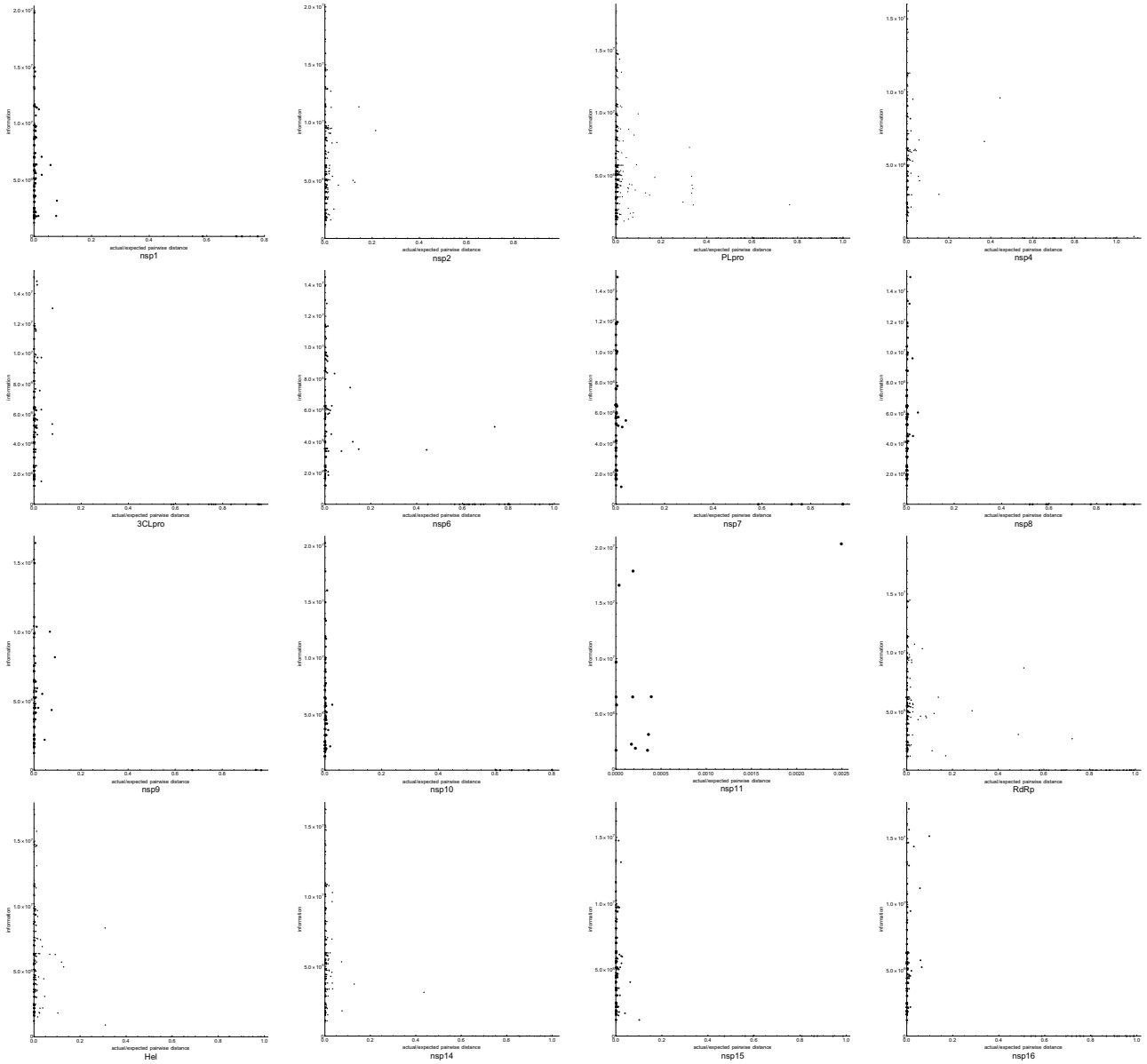

Fig. S20. Locus information versus variability for individual non-structural protein regions in the SARS-CoV-2 1ab region.

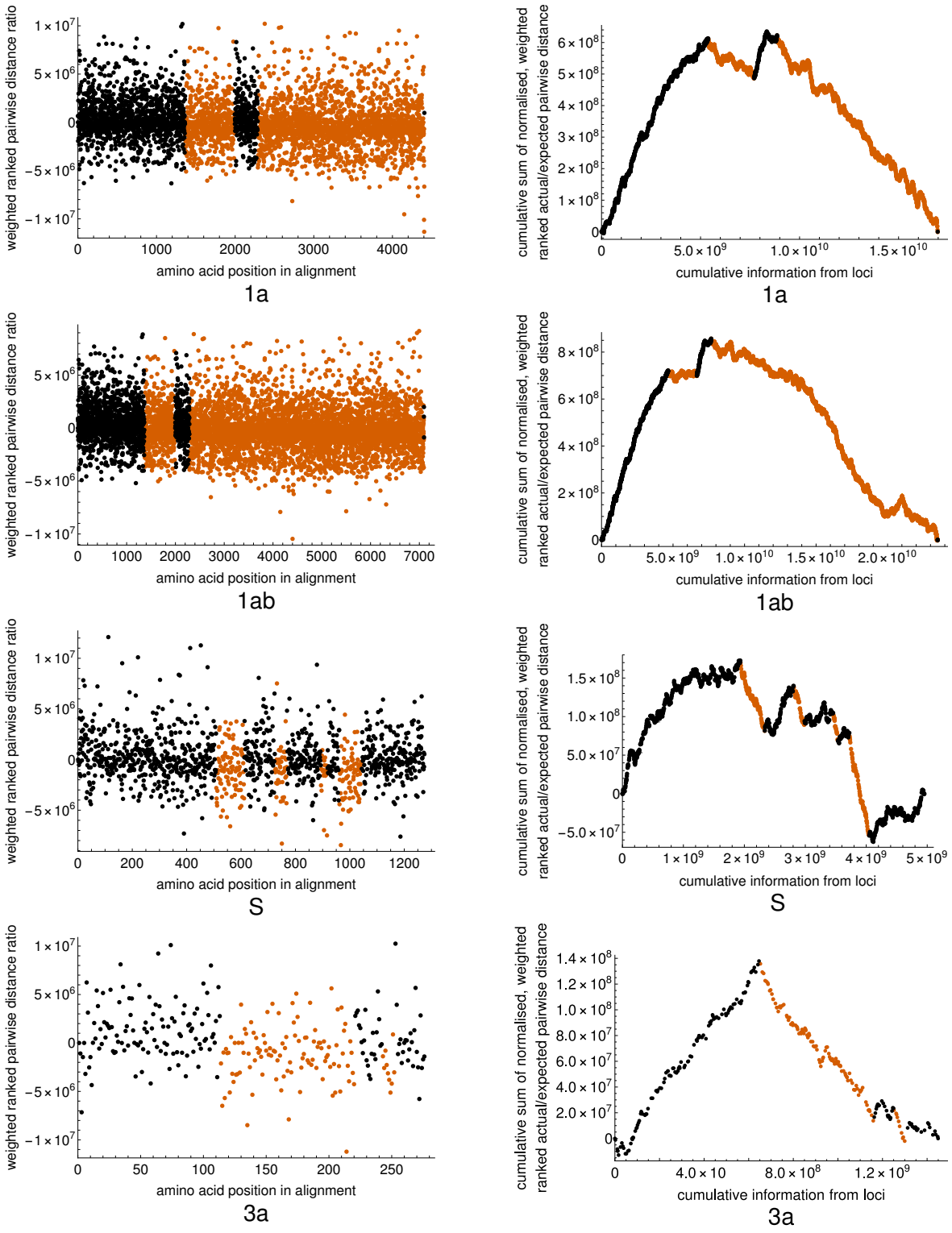

Fig. S21. Output from analyses of SARS-CoV-2 1a, 1ab, S and 3a genes, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. Regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Plots use weighted, ranked pairwise distance ratios. More detail of significant regions found is given in Table 1.

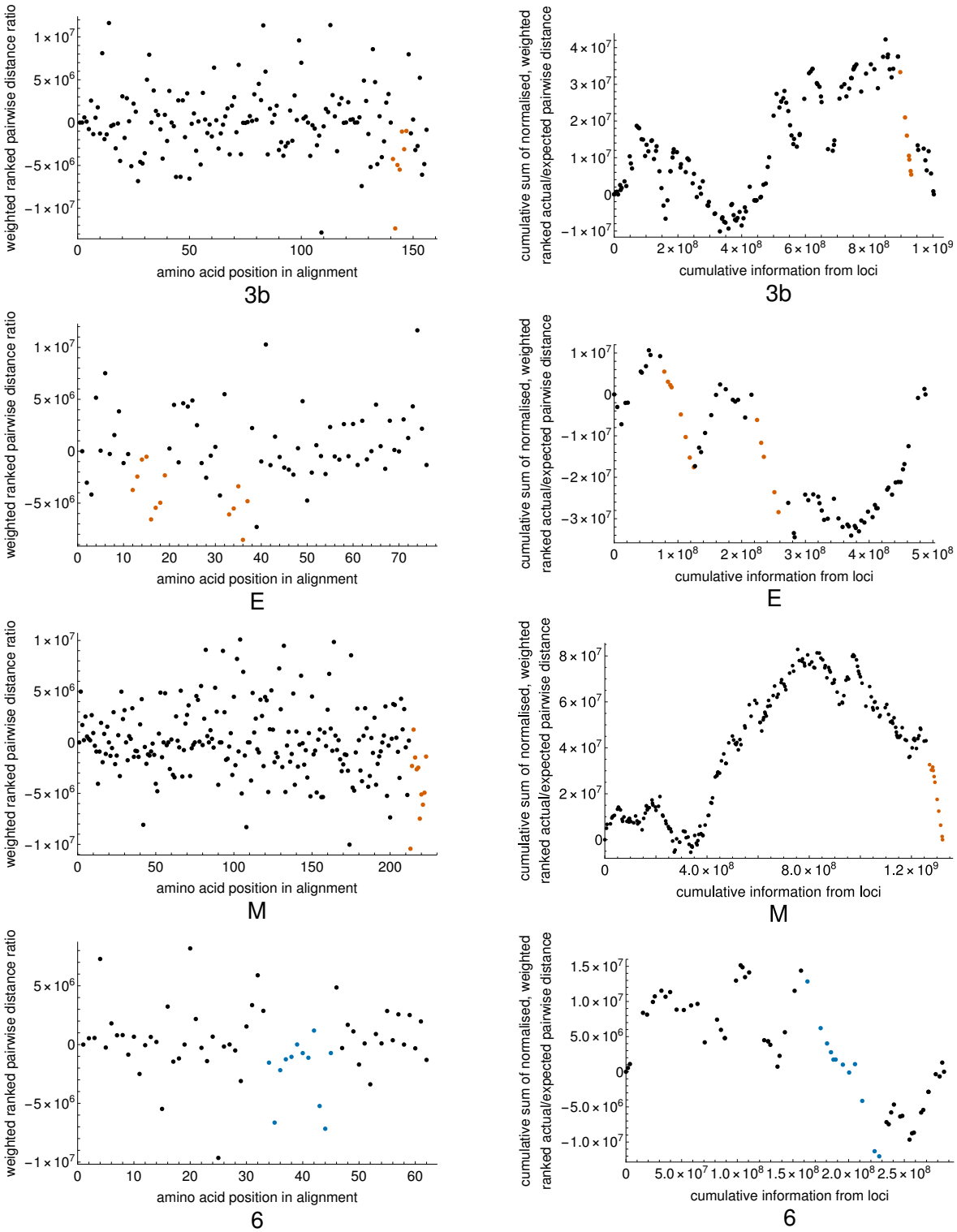

Fig. S22. Output from analyses of SARS-CoV-2 3b, E, M and 6 genes, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. Regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Plots use weighted, ranked pairwise distance ratios. More detail of significant regions found is given in Table 1.

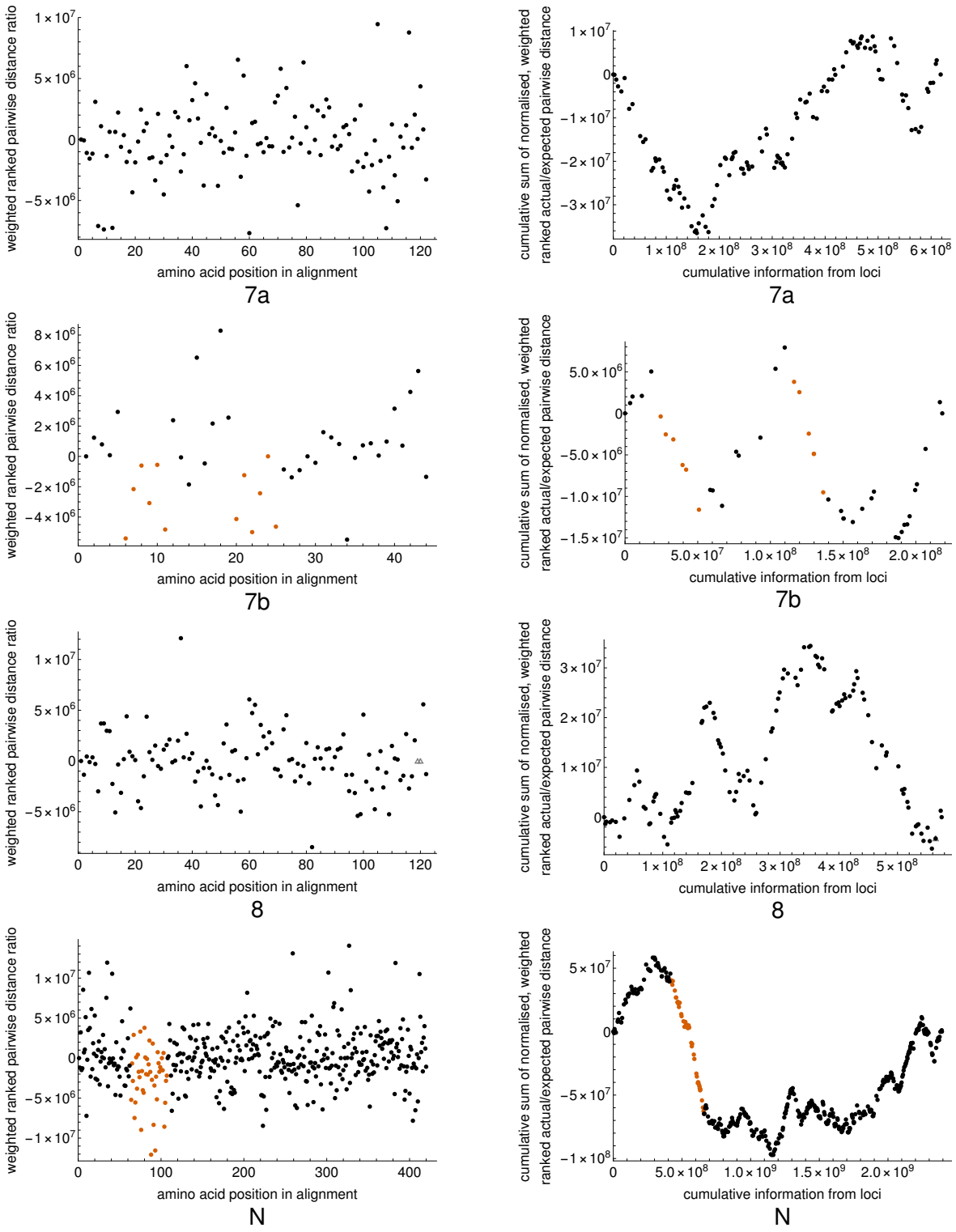

Fig. S23. Output from analyses of SARS-CoV-2 7a, 7b,8 and N genes, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. Regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Plots use weighted, ranked pairwise distance ratios. More detail of significant regions found is given in Table 1.

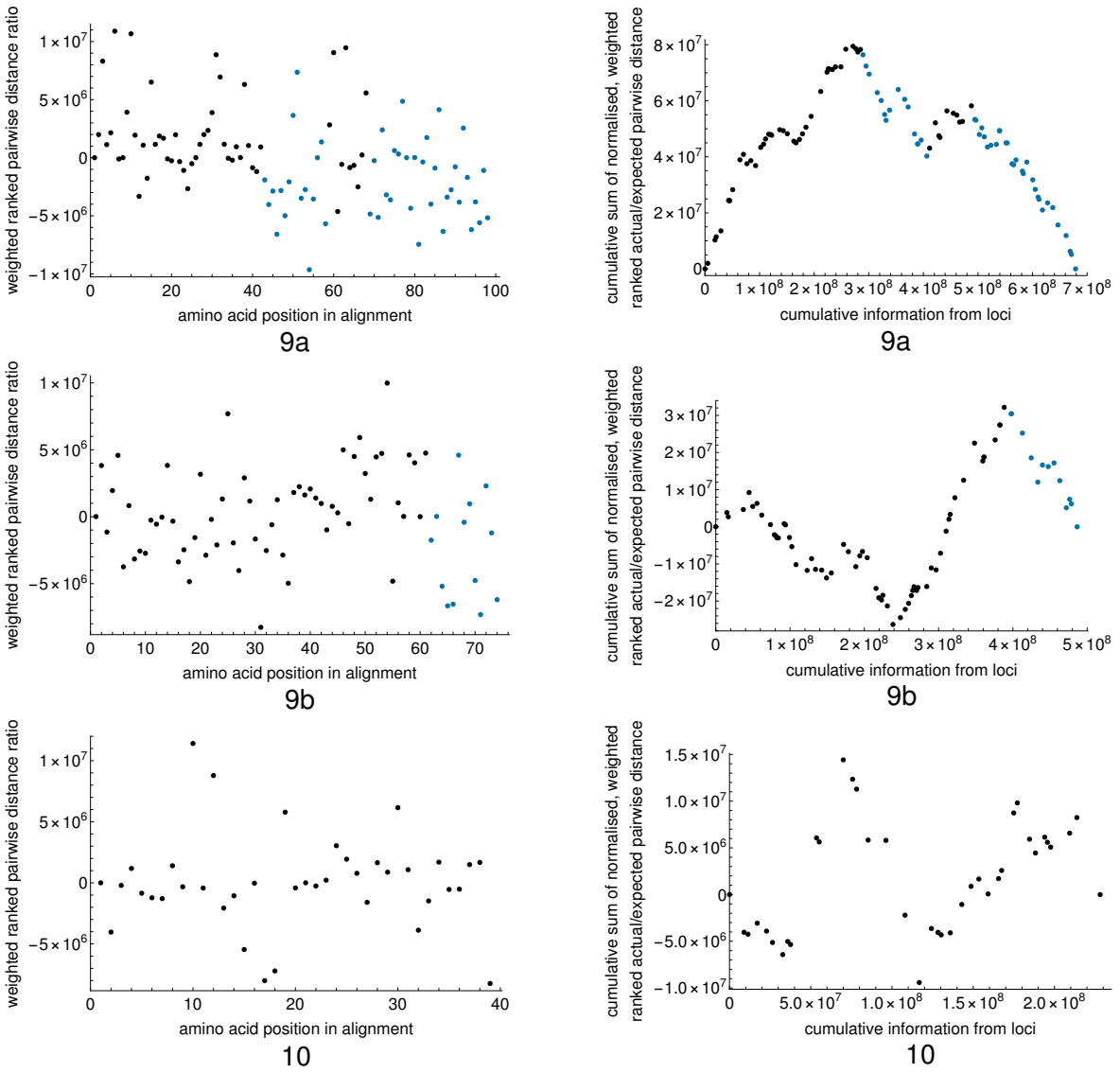

Fig. S24. Output from analyses of SARS-CoV-2 9a, 9b, and 10 genes, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. Regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Plots use weighted, ranked pairwise distance ratios. More detail of significant regions found is given in Table 1.

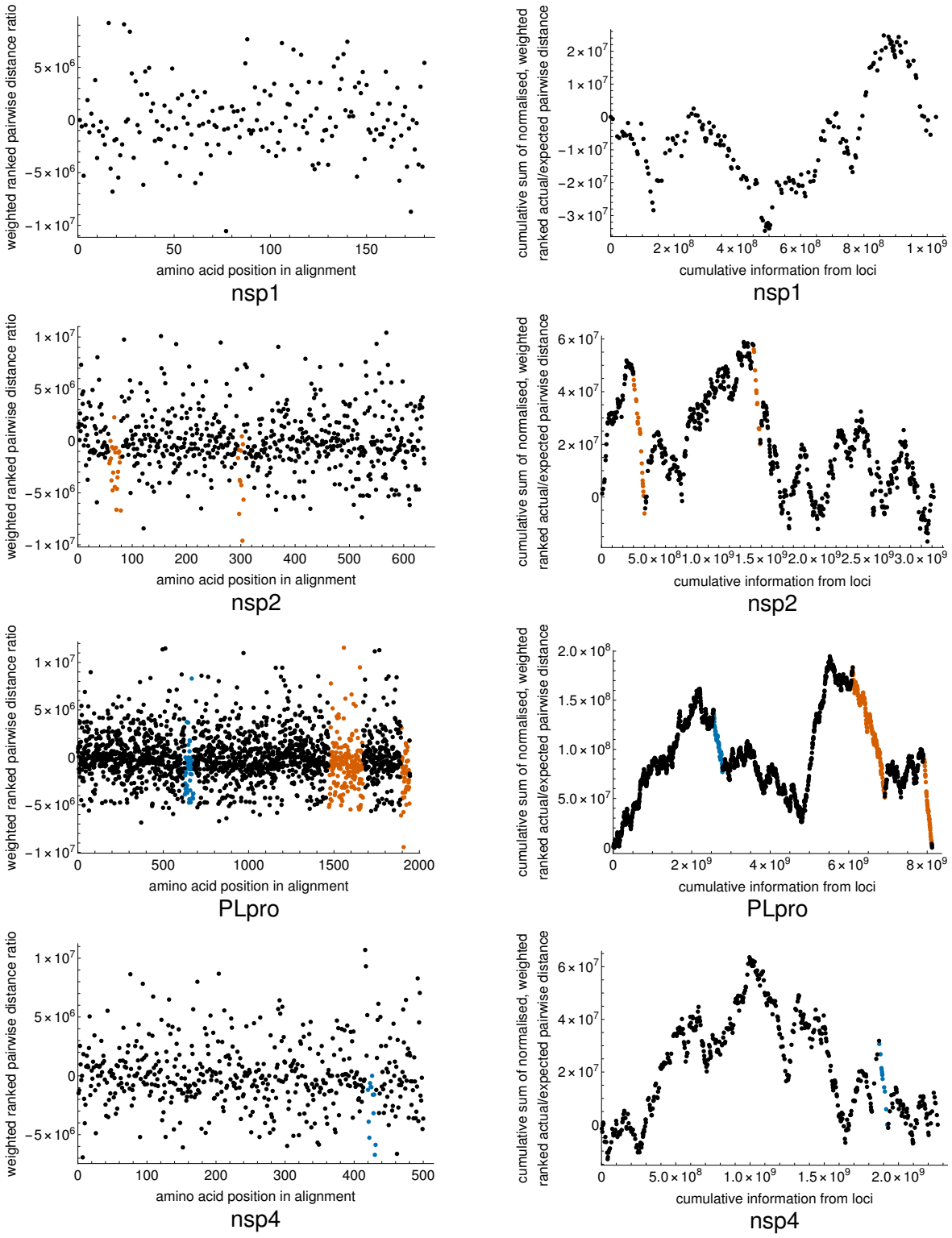

Fig. S25. Output from analyses of SARS-CoV-2 nsp1, nsp2, nsp3 and nsp4 regions, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. Regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Plots use weighted, ranked pairwise distance ratios. More detail of significant regions found is given in Table 2.

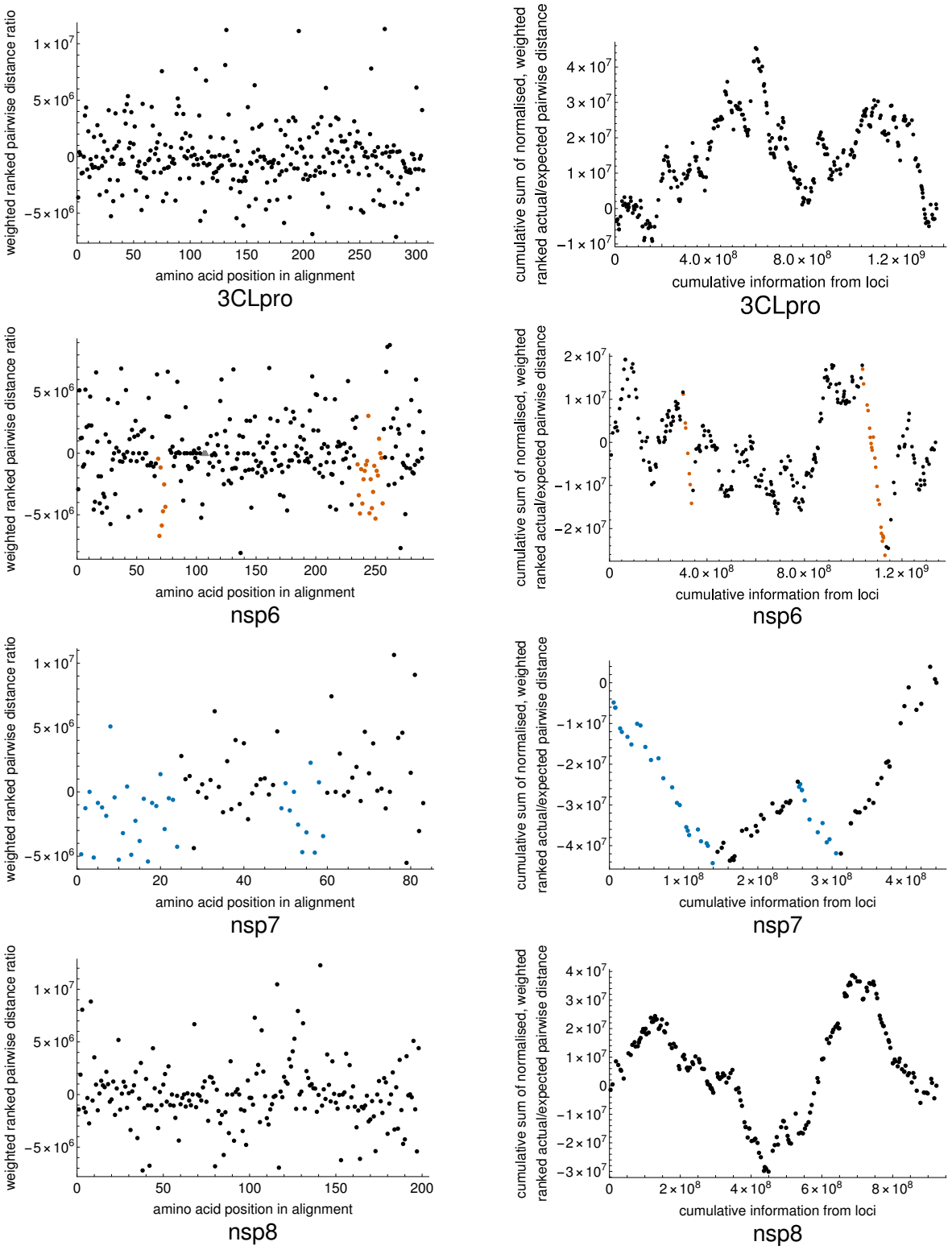

Fig. S26. Output from analyses of SARS-CoV-2 nsp5, nsp6, nsp7 and nsp8 regions, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. Regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Plots use weighted, ranked pairwise distance ratios. More detail of significant regions found is given in Table 2.

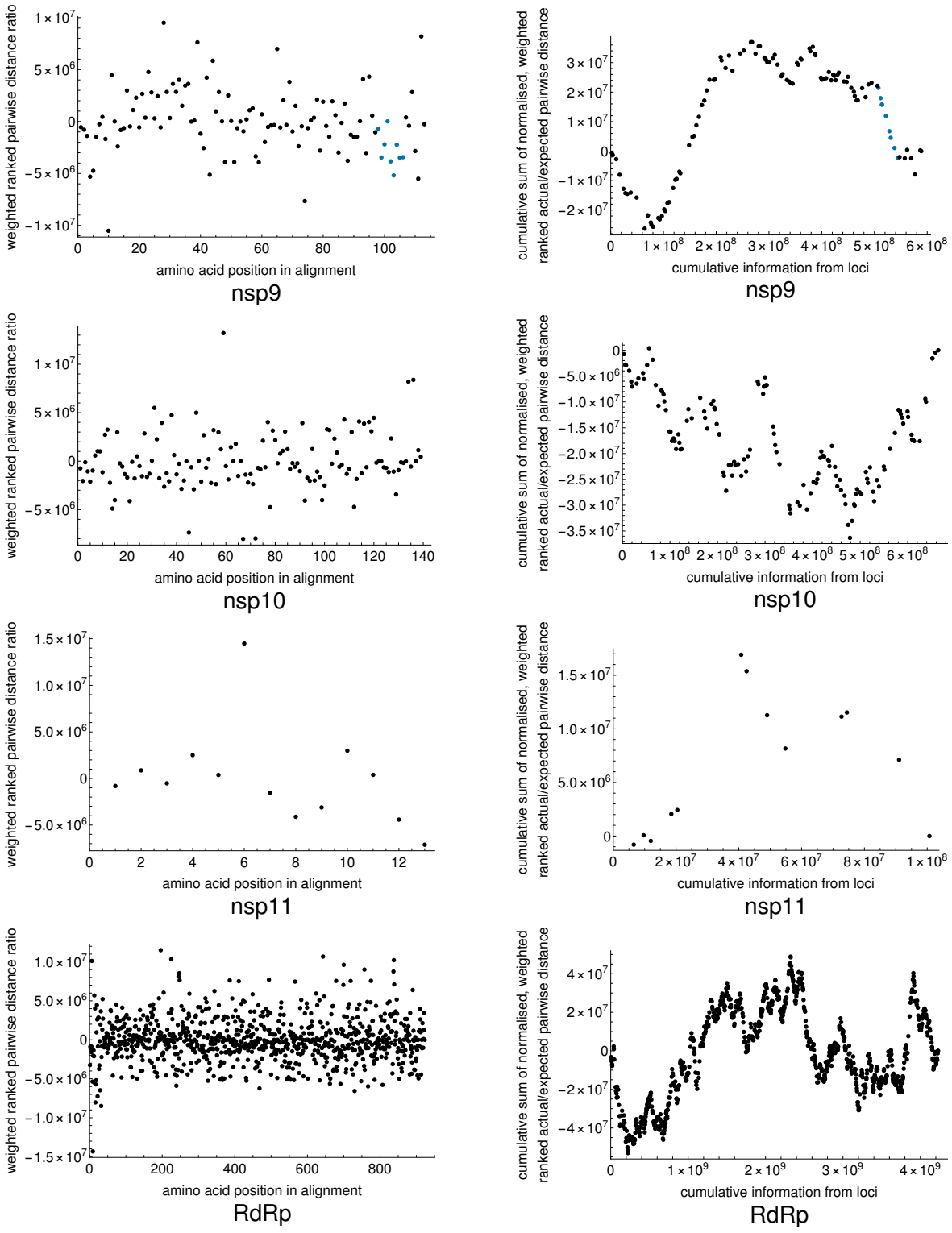

Fig. S27. Output from analyses of SARS-CoV-2 nsp9, nsp10, nsp11 and nsp12 regions, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. Regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Plots use weighted, ranked pairwise distance ratios. More detail of significant regions found is given in Table 2.

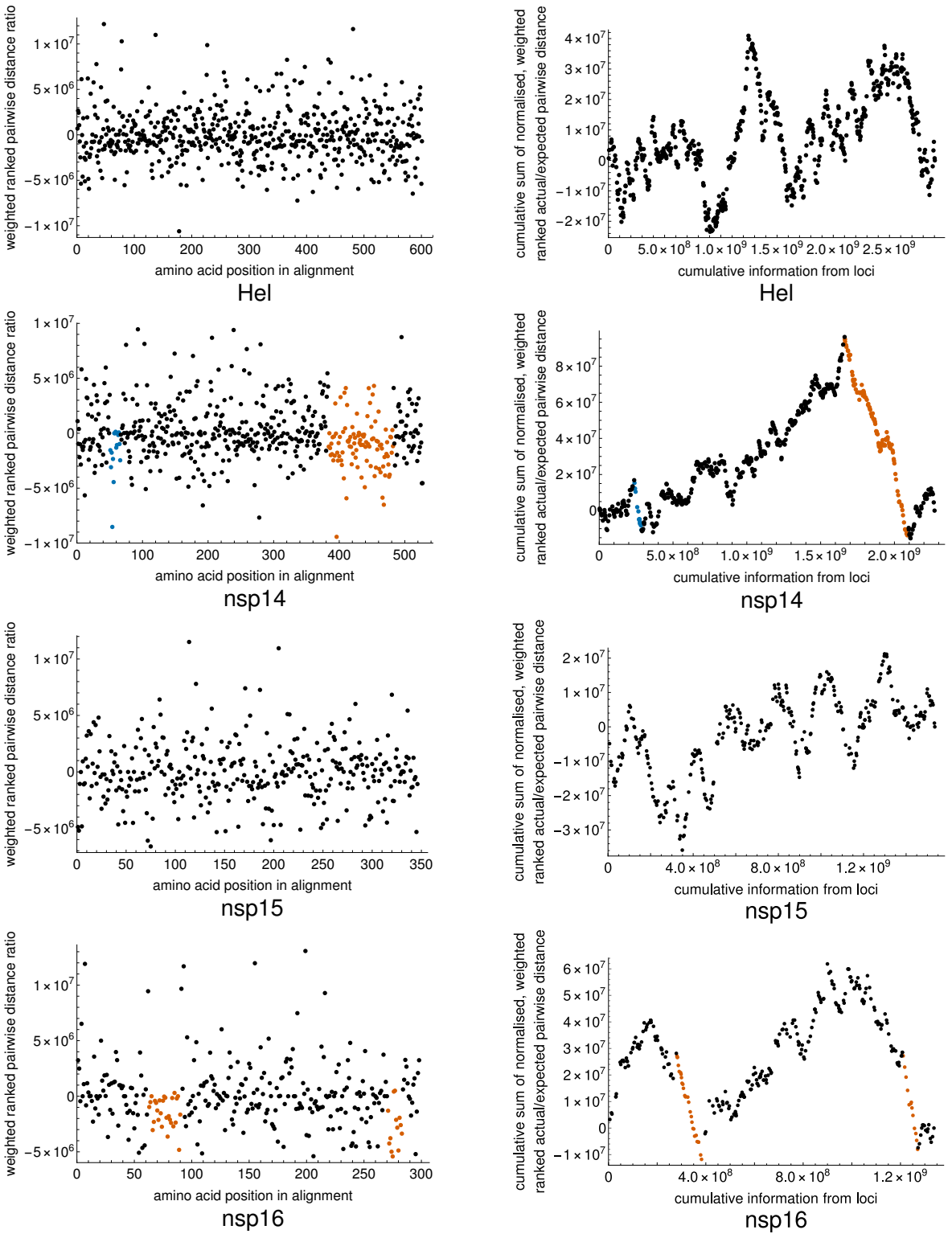

Fig. S28. Output from analyses of SARS-CoV-2 nsp13, nsp14, nsp15 and nsp16 regions, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. Regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Plots use weighted, ranked pairwise distance ratios. More detail of significant regions found is given in Table 2.

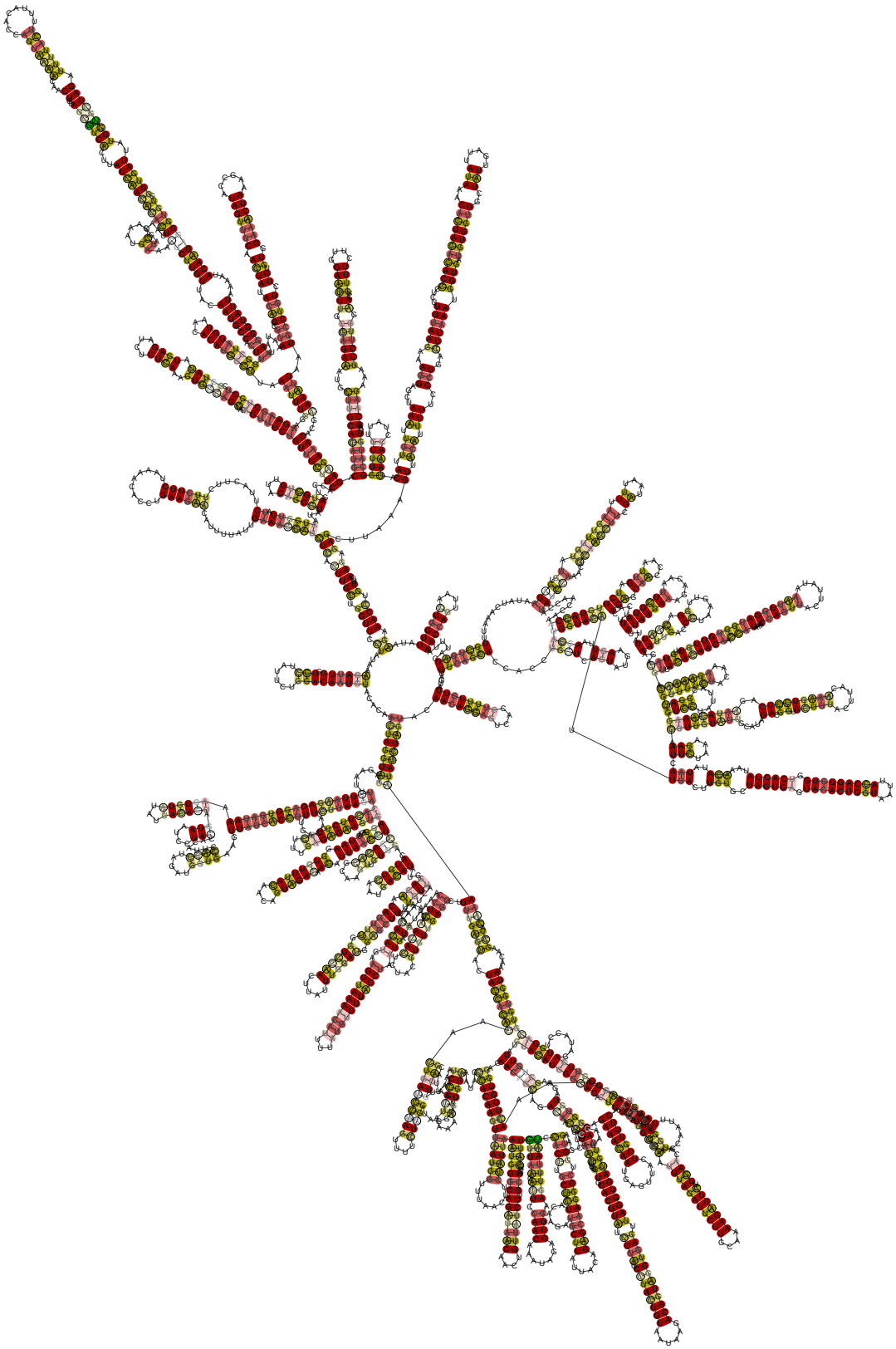

Fig. S29. Output of RNAalifold analysis of the conserved region found 2nd in analysis of the 1a region and 3rd in analysis of the 1ab region of SARS-CoV-2, NC_045512.2 nucleotide location 4335–6247. Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. (Results are displayed using sequences from the 1a analysis, from which fewer sequences were excluded owing to ambiguous nucleotides etc.) Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

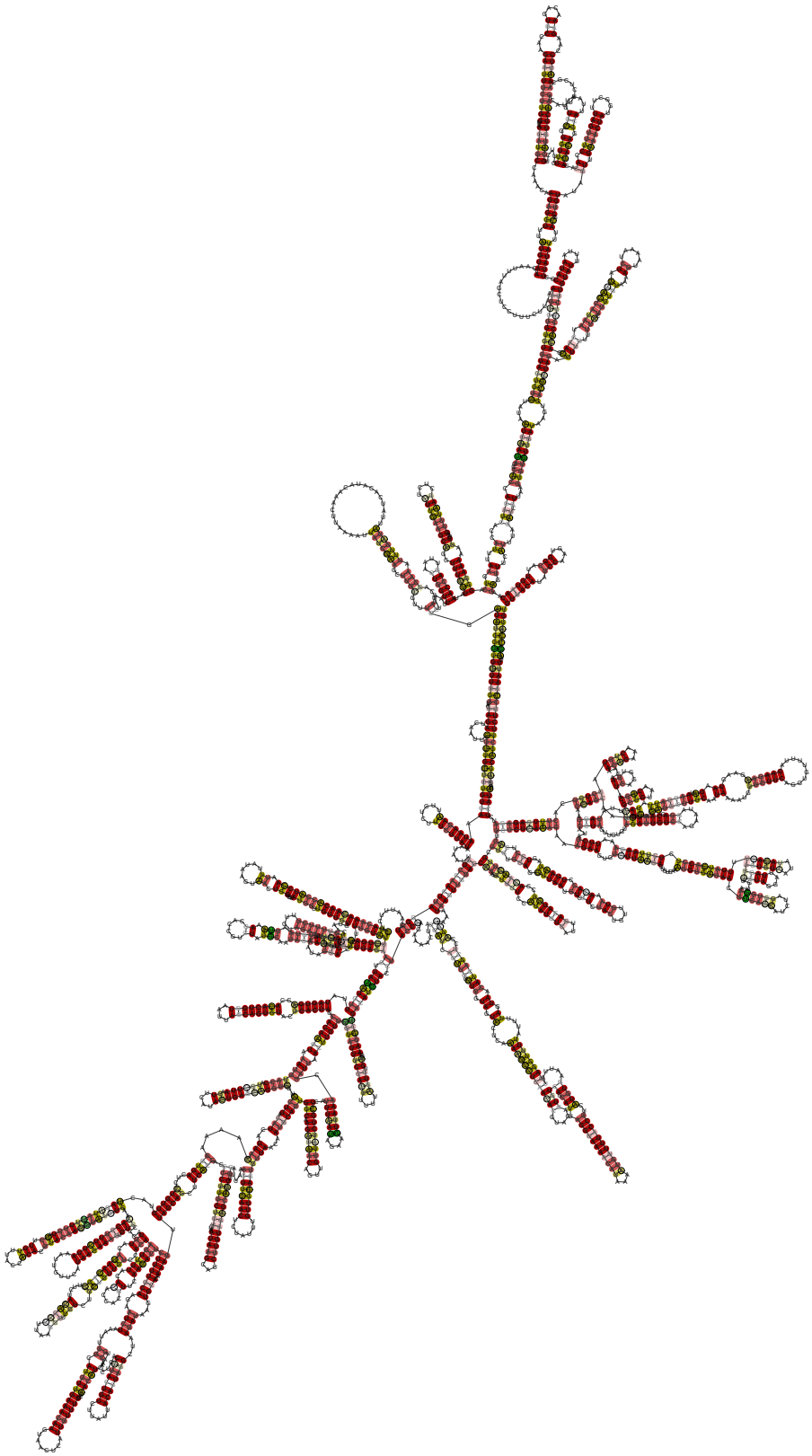

Fig. S30. Output of RNAalifold analysis of the reverse complement of the conserved region found 2nd in analysis of the 1a region and 3rd in analysis of the 1ab region of SARS-CoV-2, NC_045512.2 nucleotide location 4335–6247. Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. (Results are displayed using sequences from the 1a analysis, from which fewer sequences were excluded owing to ambiguous nucleotides etc.) Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

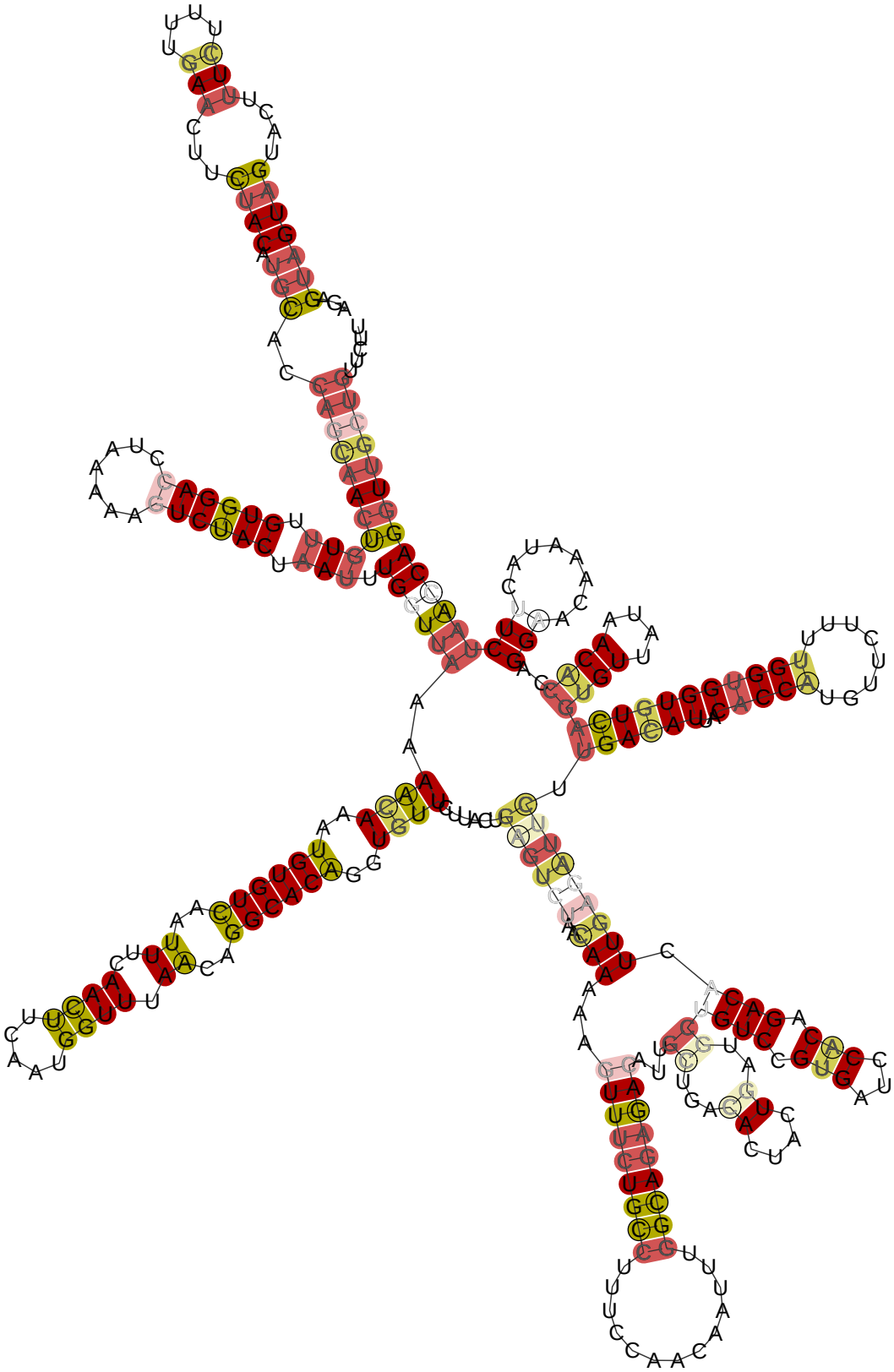

Fig. S31. Output of RNAalifold analysis of the conserved region found 2nd in analysis of the S region of SARS-CoV-2, NC_045512.2 nucleotide location 23087–23395. Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

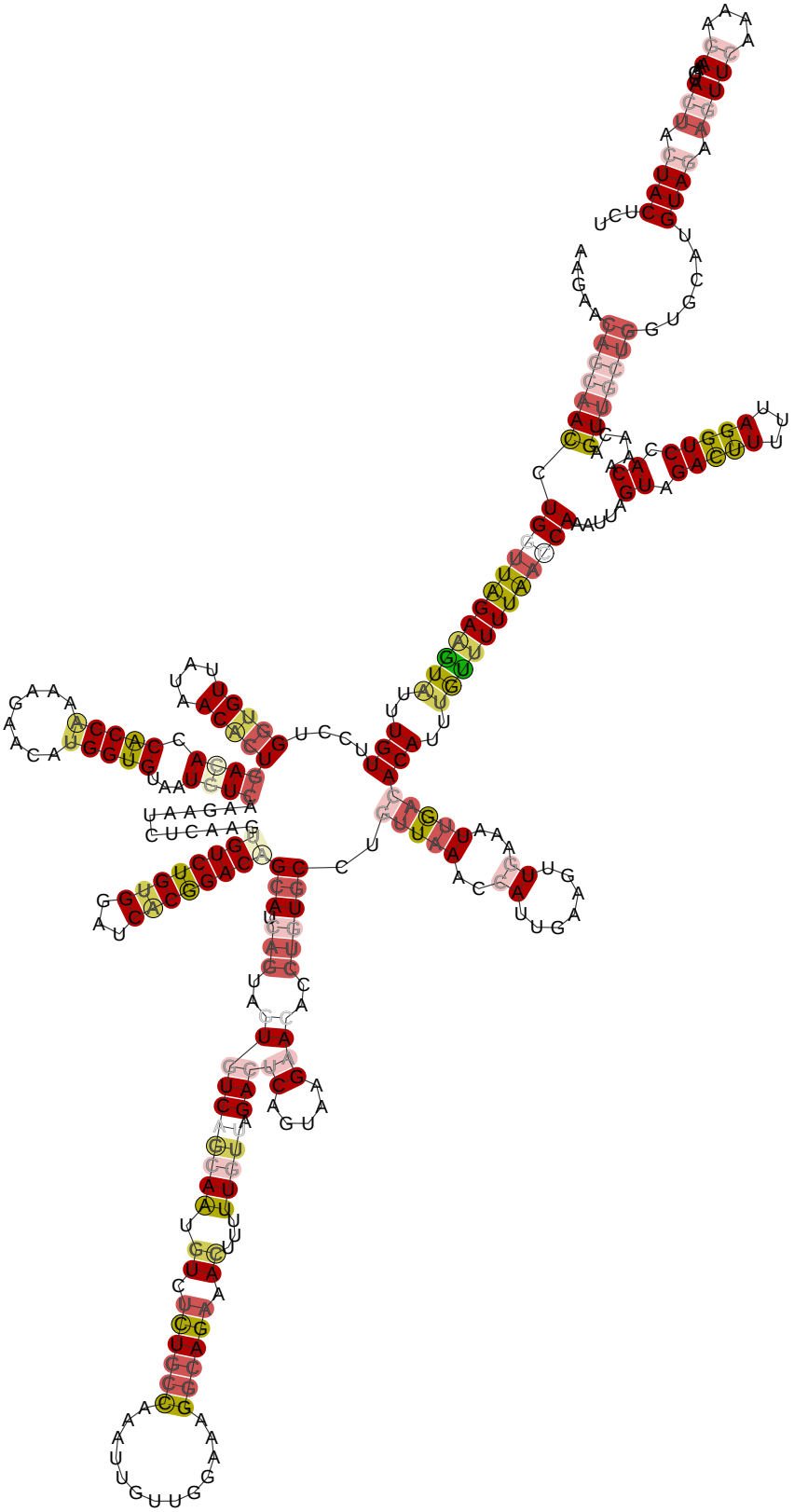

Fig. S32. Output of RNAalifold analysis of the reverse complement of the conserved region found 2nd in analysis of the S region of SARS-CoV-2, NC_045512.2 nucleotide location 23087–23395. Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

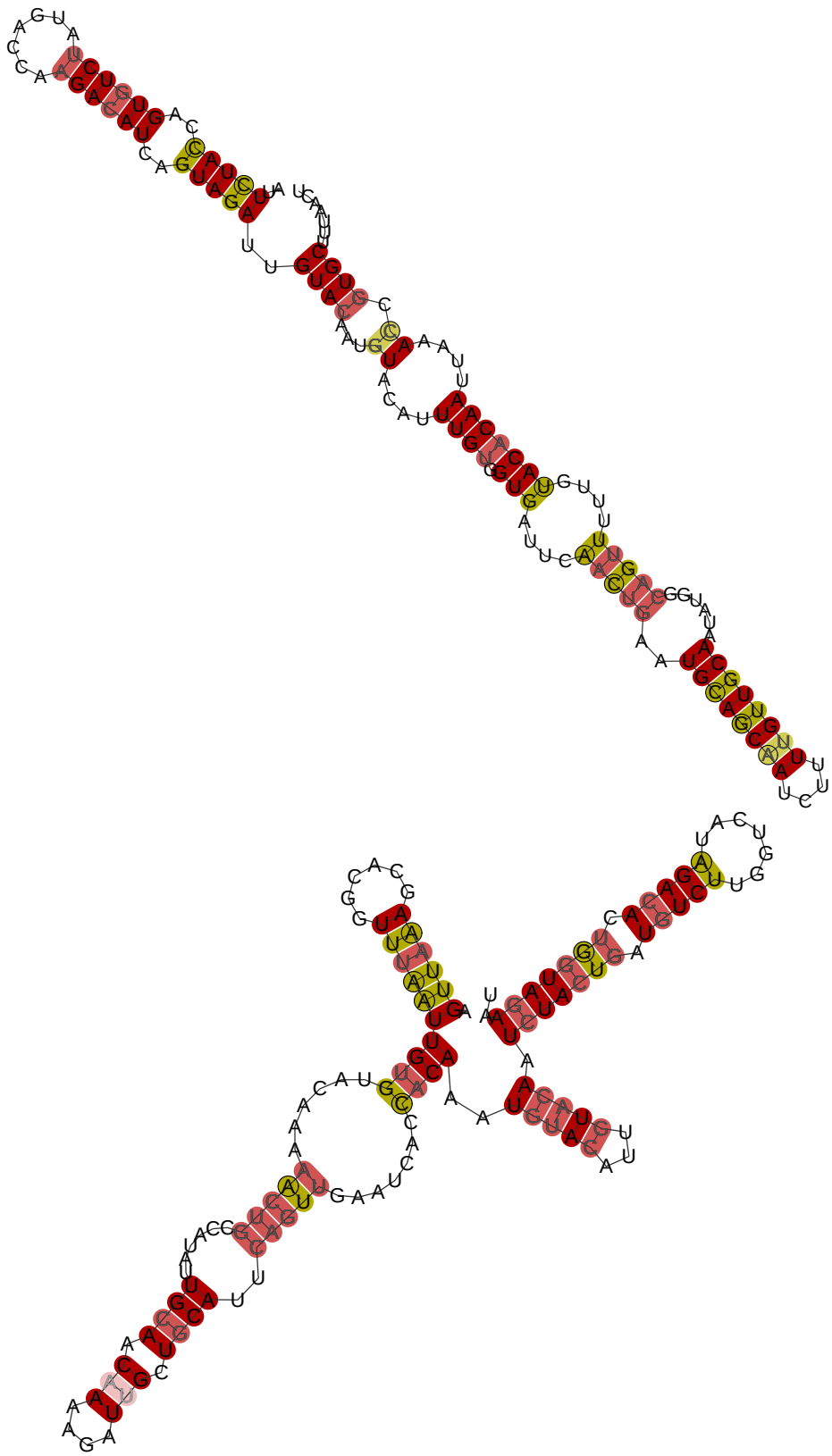

Fig. S33. Output of RNAalifold analysis of the conserved region found 3rd in analysis of the S region of SARS-CoV-2, NC_045512.2 nucleotide location 23735–26052 (top: forward sense; bottom: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

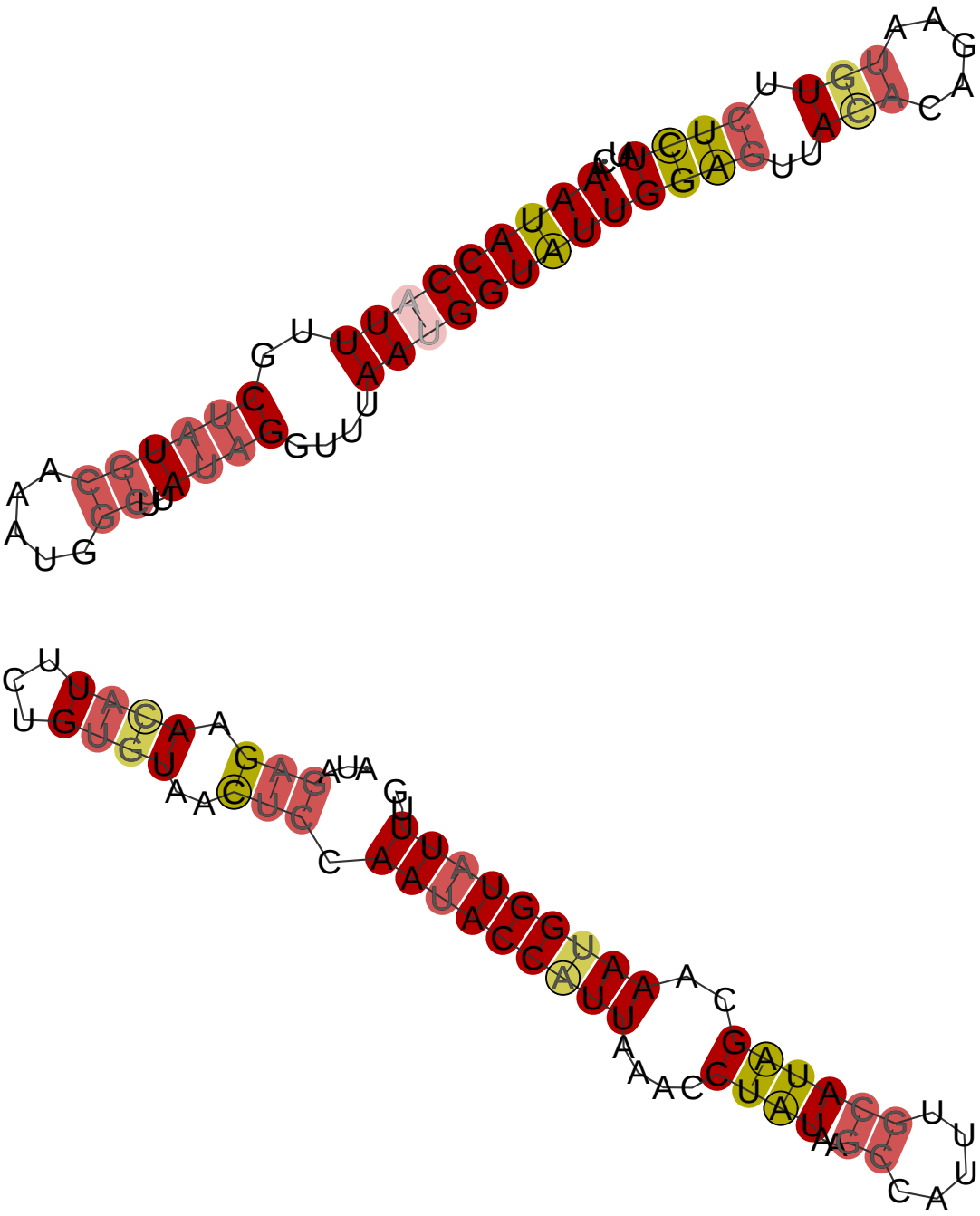

Fig. S34. Output of RNAalifold analysis of the conserved region found 4th in analysis of the S region of SARS-CoV-2, NC_045512.2 nucleotide location 24242–24313 (top: forward sense; bottom: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

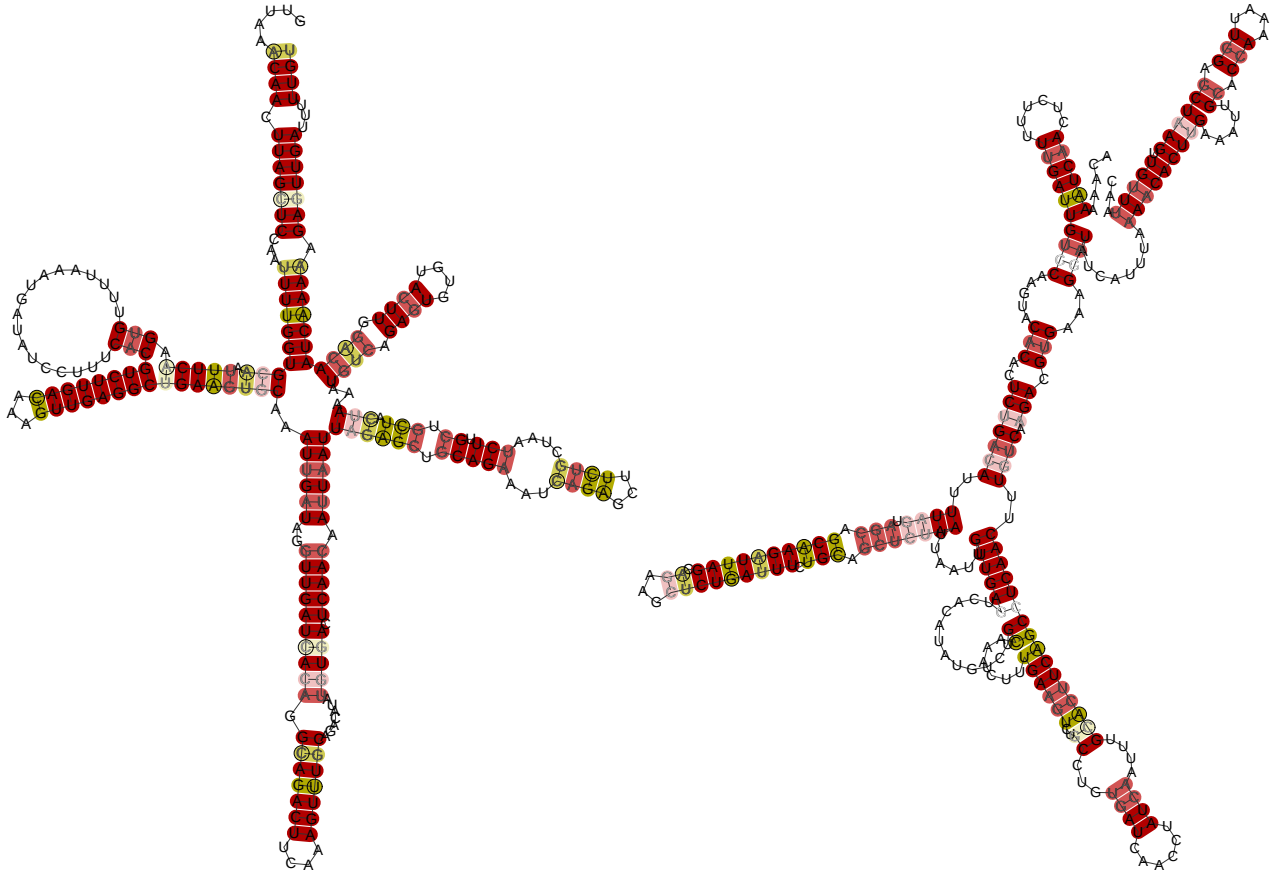

Fig. S35. Output of RNAalifold analysis of the conserved region found 1st in analysis of the S region of SARS-CoV-2, NC_045512.2 nucleotide location 24446–24690 (left: forward sense; right: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

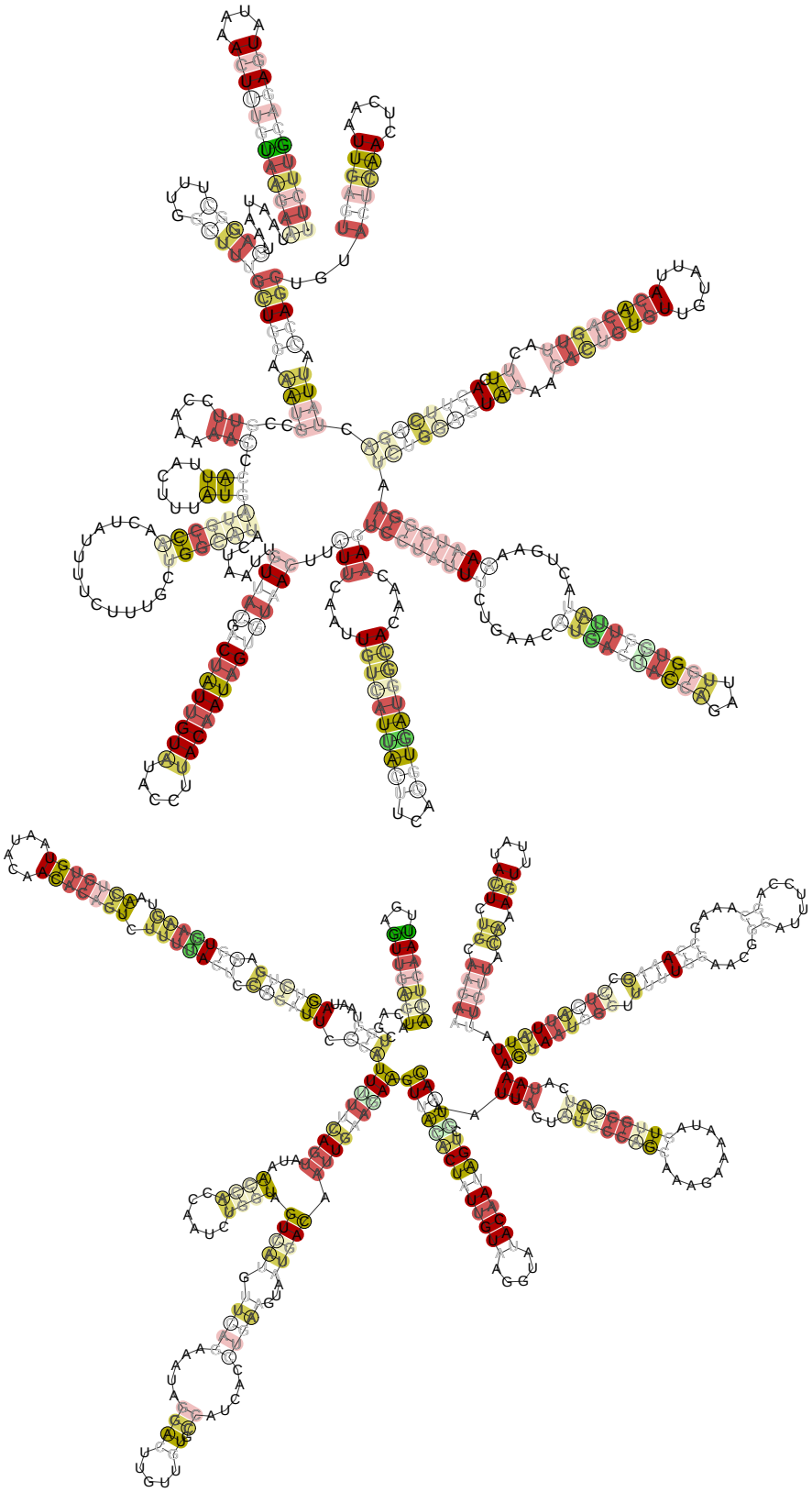

Fig. S36. Output of RNAalifold analysis of the conserved region found 1st in analysis of the 3a region of SARS-CoV-2, NC_045512.2 nucleotide location 25732–26052 (top: forward sense; bottom: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

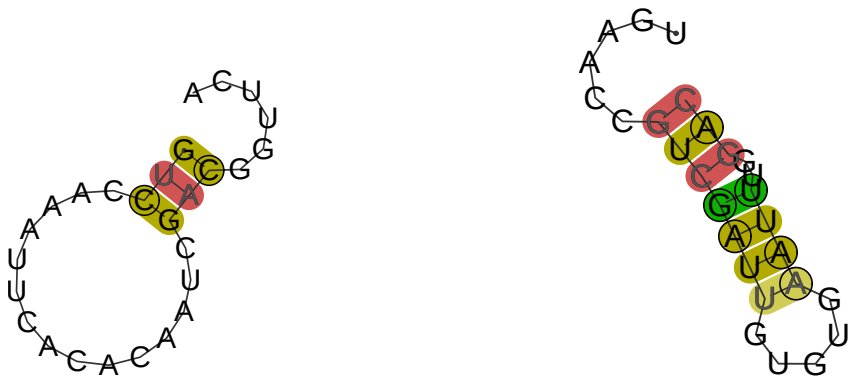

Fig. S37. Output of RNAalifold analysis of the conserved region found 2nd in analysis of the 3a region of SARS-CoV-2, NC_045512.2 nucleotide location 26119–26148 (left: forward sense; right: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

Fig. S38. Output of RNAalifold analysis of the conserved region found 1st in analysis of the 3b region of SARS-CoV-2, NC_045512.2 nucleotide location 26234–26254 (left: forward sense; right: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

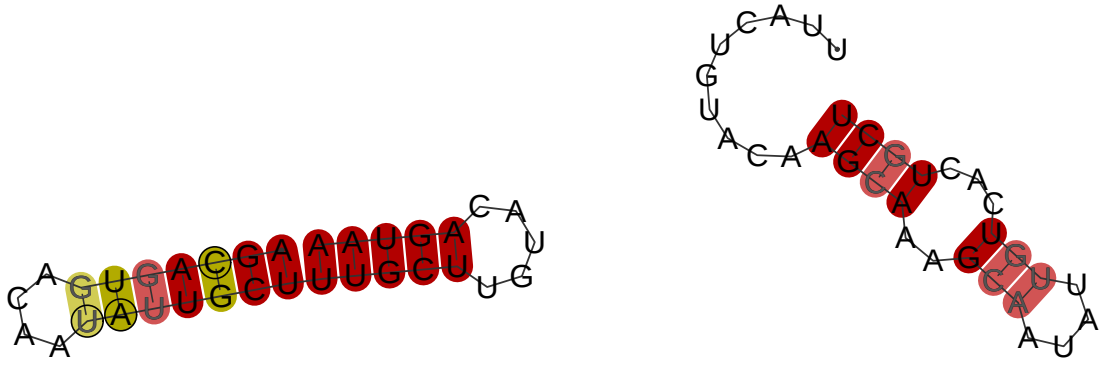

Fig. S39. Output of RNAalifold analysis of the conserved region found 1st in analysis of the M region of SARS-CoV-2, NC_045512.2 nucleotide location 27159–27191 (left: forward sense; right: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

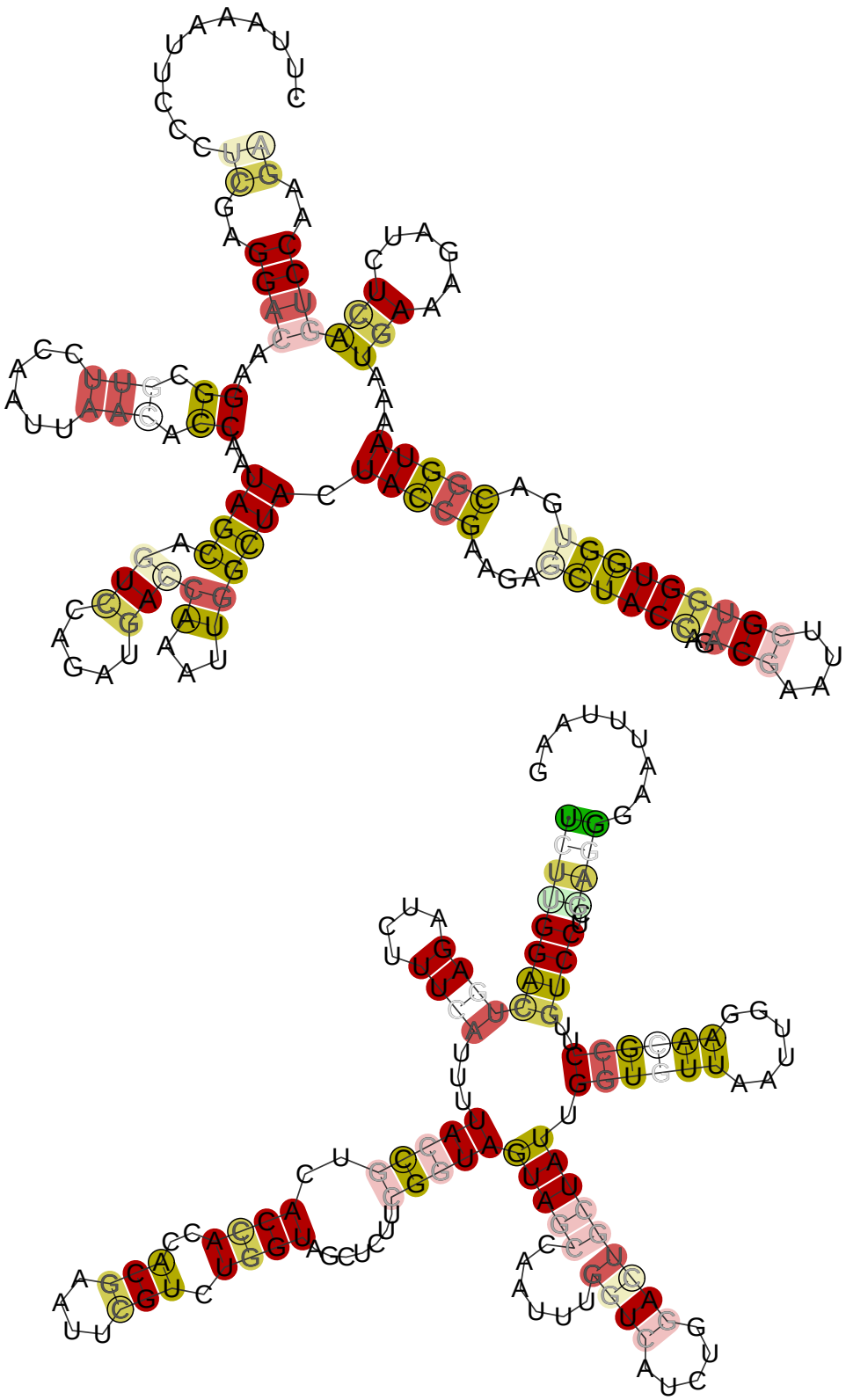

Fig. S40. Output of RNAalifold analysis of the conserved region found 1st in analysis of the N region of SARS-CoV-2, NC_045512.2 nucleotide location 28463–28594 (top: forward sense; bottom: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

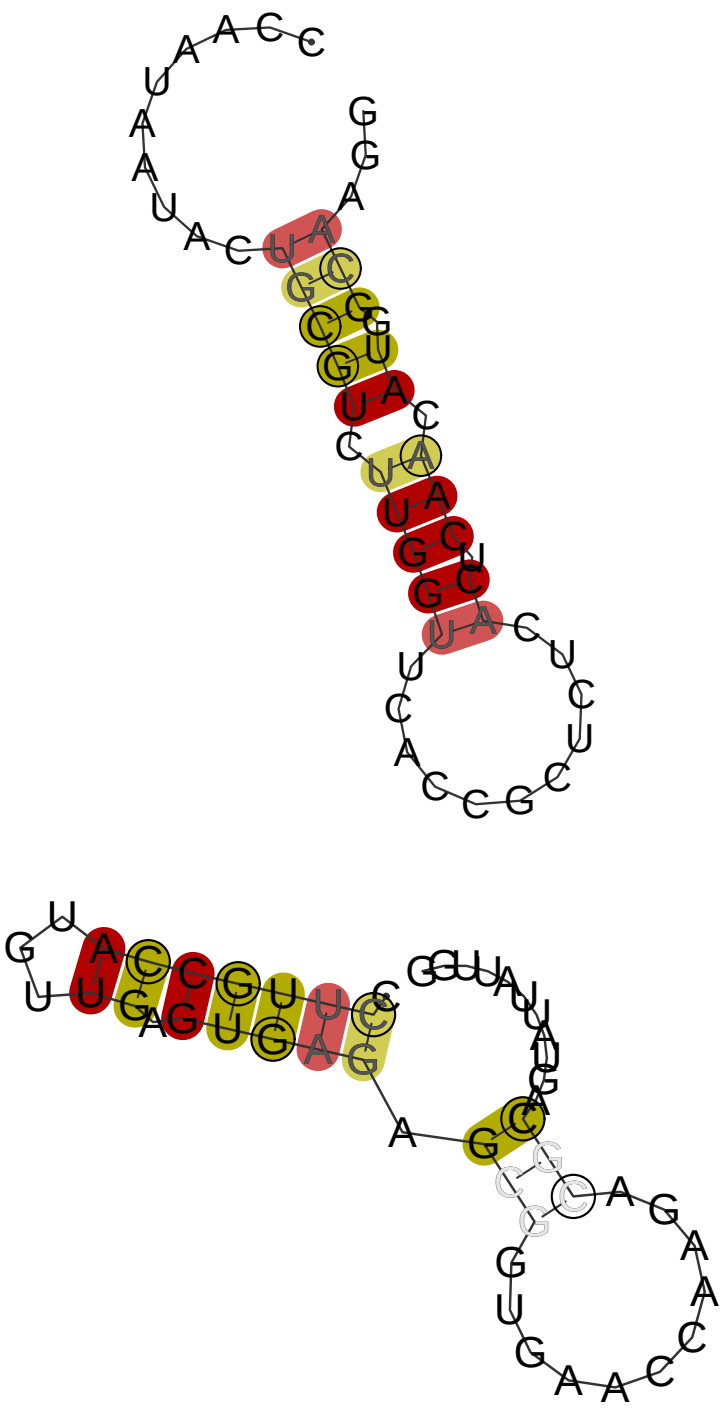

Fig. S41. Output of RNAalifold analysis of the conserved region found 2nd in analysis of the 9a region of SARS-CoV-2, NC_045512.2 nucleotide location 28410–28457 (top: forward sense; bottom: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

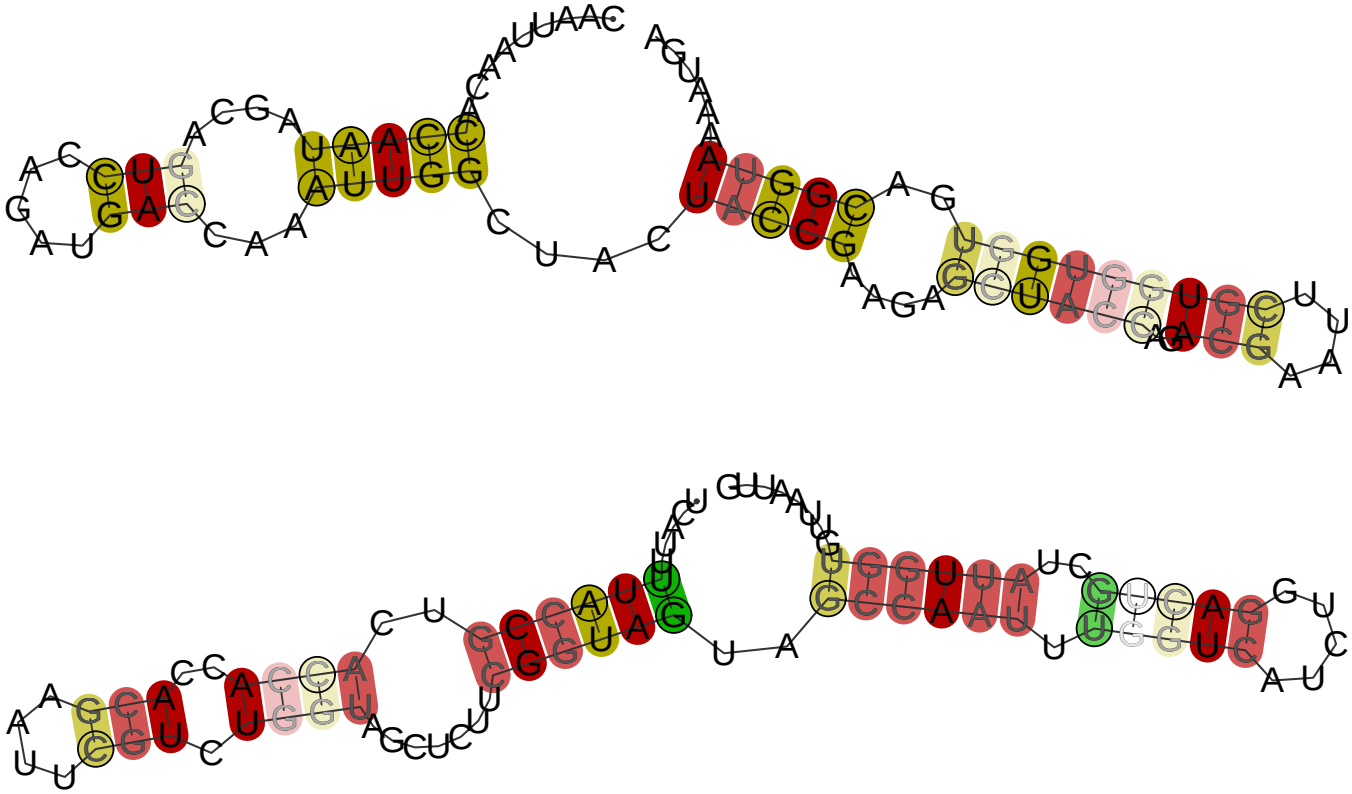

Fig. S42. Output of RNAalifold analysis of the conserved region found 1st in analysis of the 9a region of SARS-CoV-2, NC_045512.2 nucleotide location 28488–28577 (top: forward sense; bottom: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

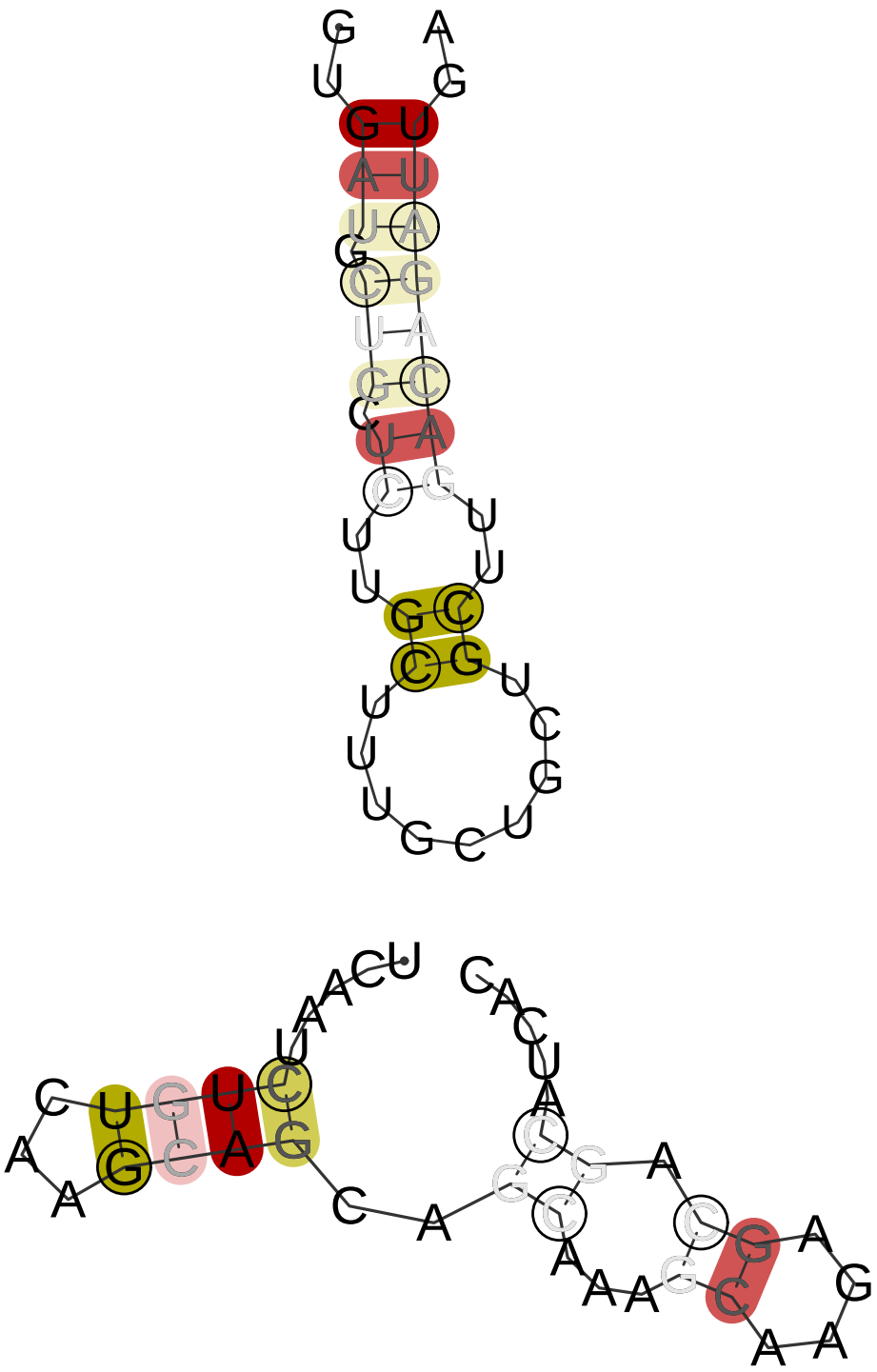

Fig. S43. Output of RNAalifold analysis of the conserved region found 1st in analysis of the 9b region of SARS-CoV-2, NC_045512.2 nucleotide location 28917–28955 (top: forward sense; bottom: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

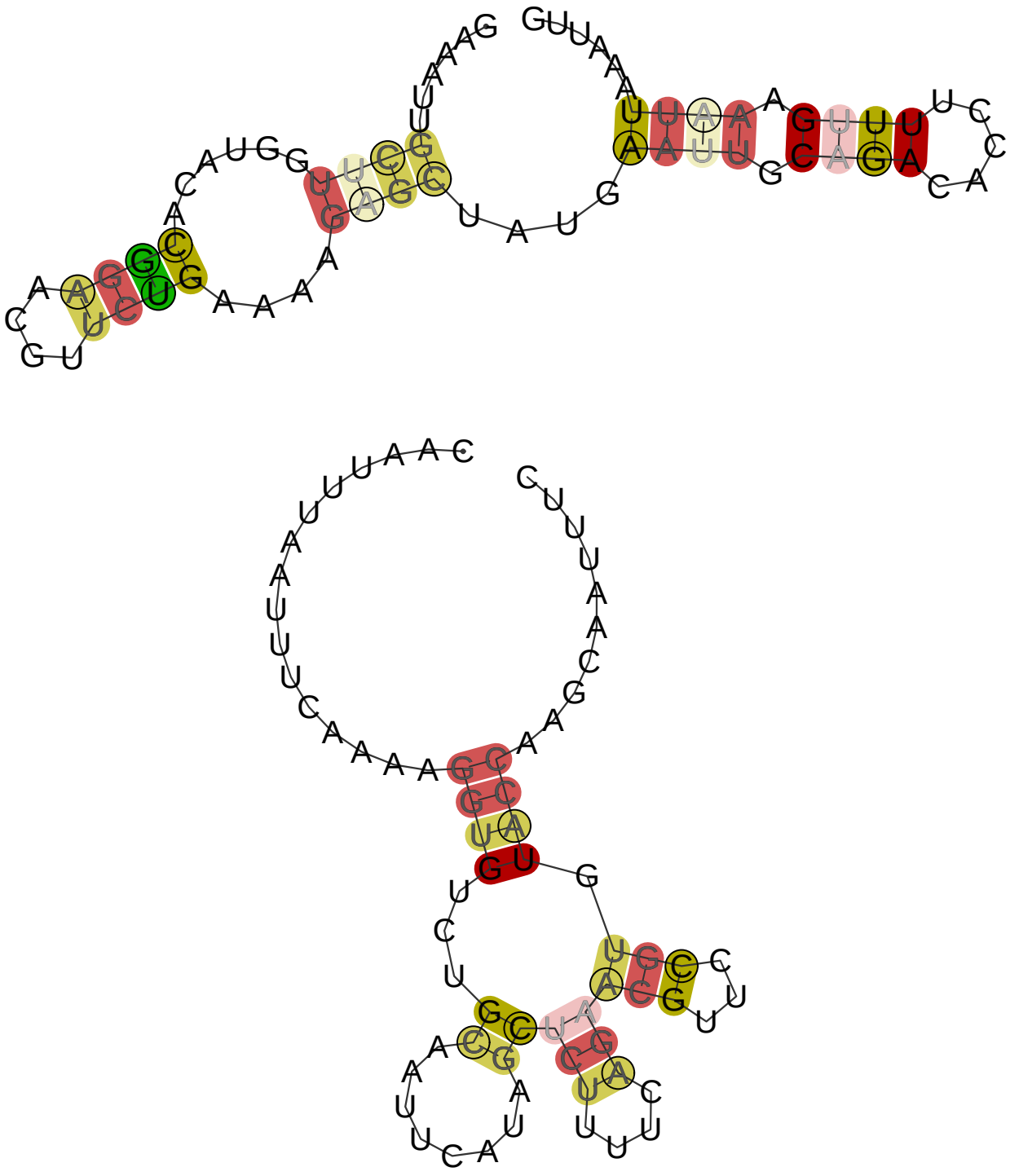

Fig. S44. Output of RNAalifold analysis of the conserved region found 1st in analysis of the nsp2 region of SARS-CoV-2, NC_045512.2 nucleotide location 974–1042 (top: forward sense; bottom: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

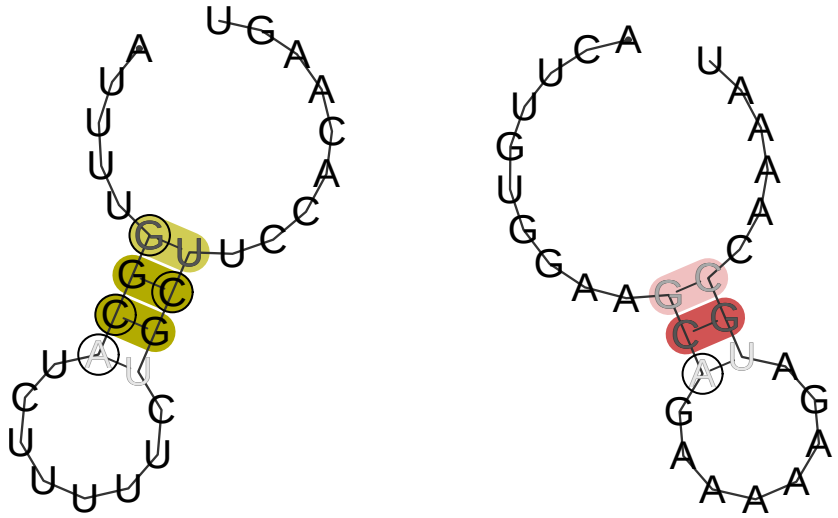

Fig. S45. Output of RNAalifold analysis of the conserved region found 2nd in analysis of the nsp2 region of SARS-CoV-2, NC_045512.2 nucleotide location 1688–1720 (left: forward sense; right: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

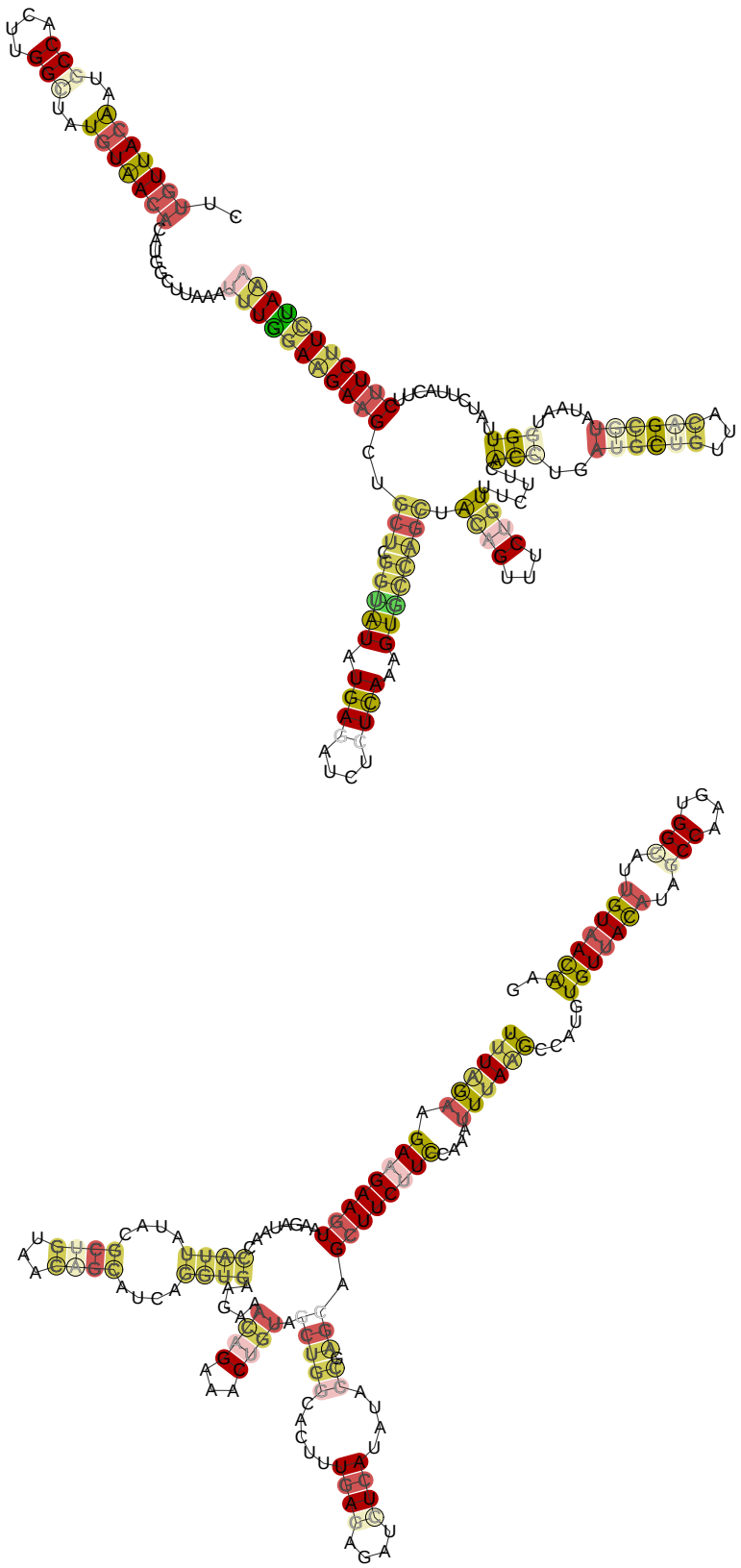

Fig. S46. Output of RNAalifold analysis of the conserved region found 3rd in analysis of the nsp3/PLpro region of SARS-CoV-2, NC_045512.2 nucleotide location 4598–4750 (top: forward sense; bottom: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

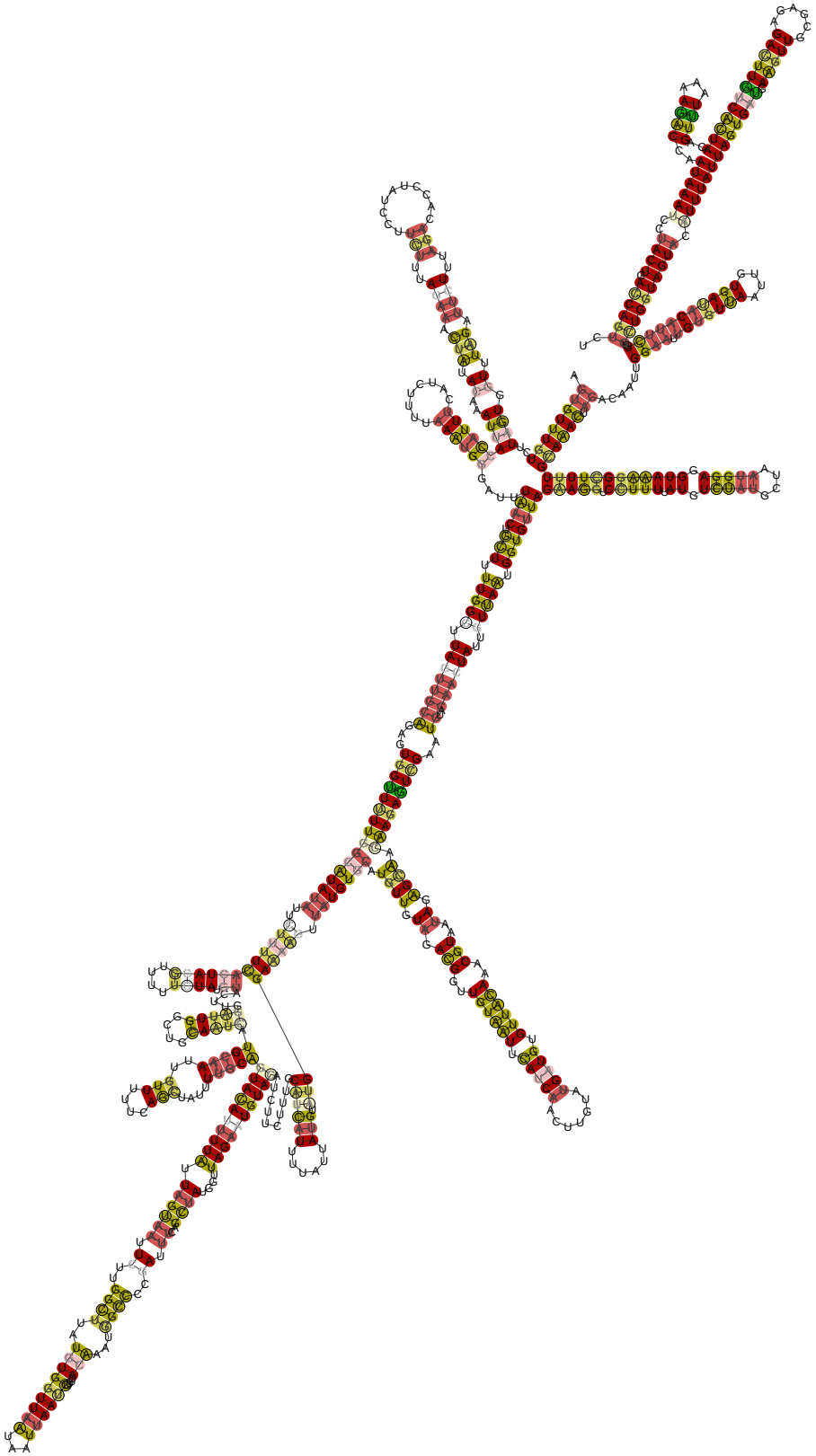

Fig. S47. Output of RNAalifold analysis of the conserved region found 2nd in analysis of the nsp3/PLpro region of SARS-CoV-2, NC_045512.2 nucleotide location 7127–7726. Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

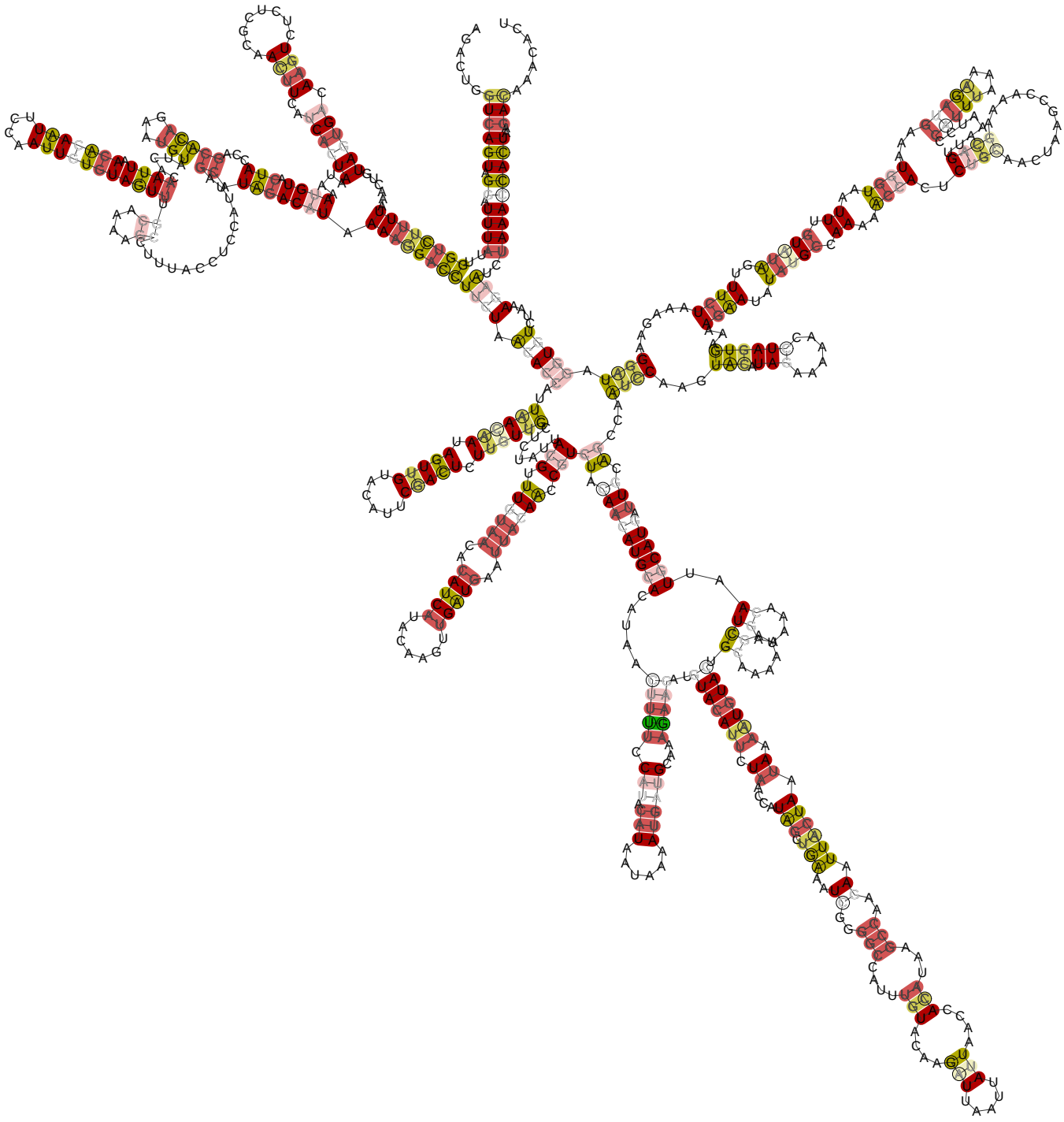

Fig. S48. Output of RNAalifold analysis of the reverse complement of the conserved region found 2nd in analysis of the nsp3/PLpro region of SARS-CoV-2, NC_045512.2 nucleotide location 7127–7726. Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

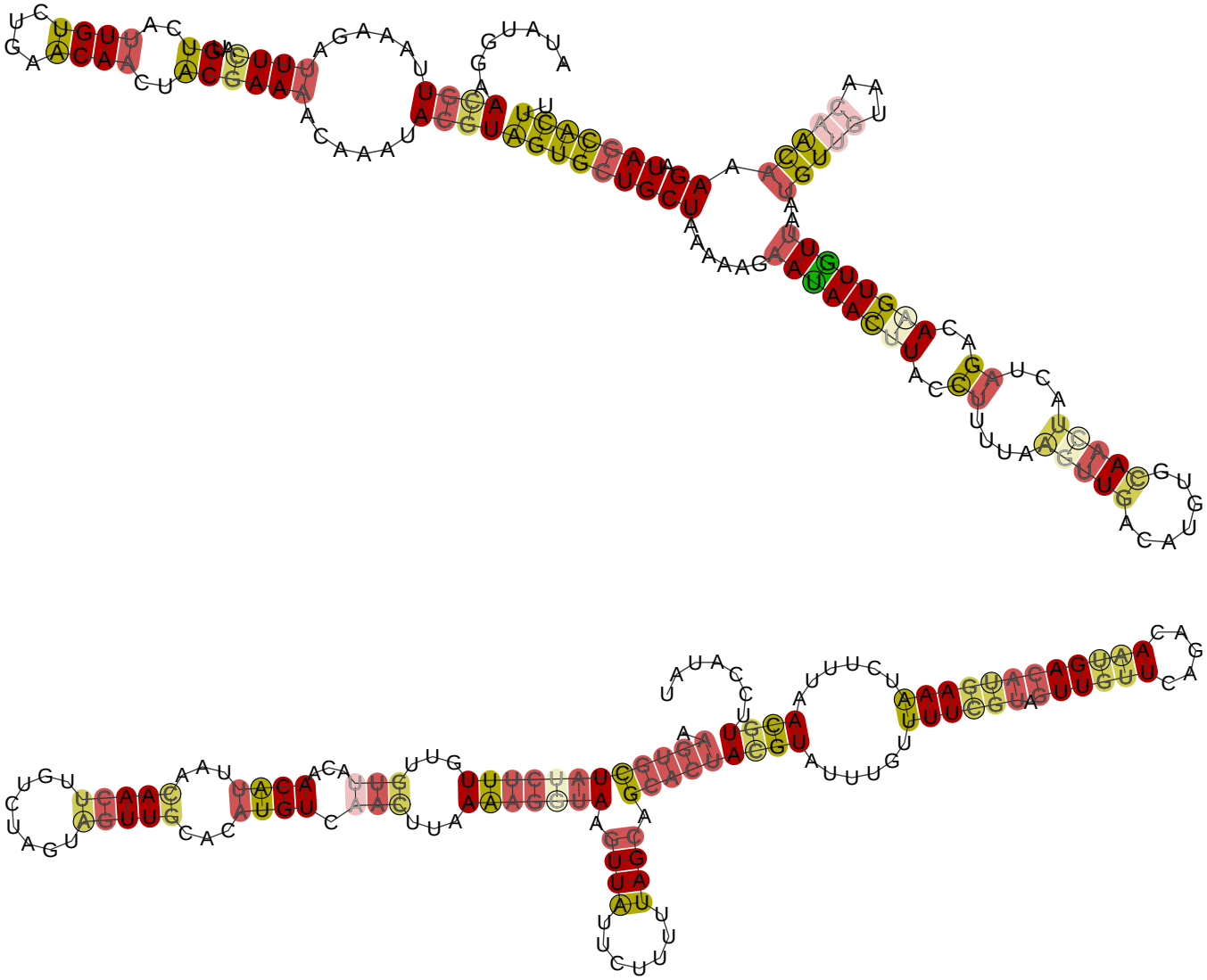

Fig. S49. Output of RNAalifold analysis of the conserved region found 1st in analysis of the nsp3/PLpro region of SARS-CoV-2, NC_045512.2 nucleotide location 8396–8545 (top: forward sense; bottom: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

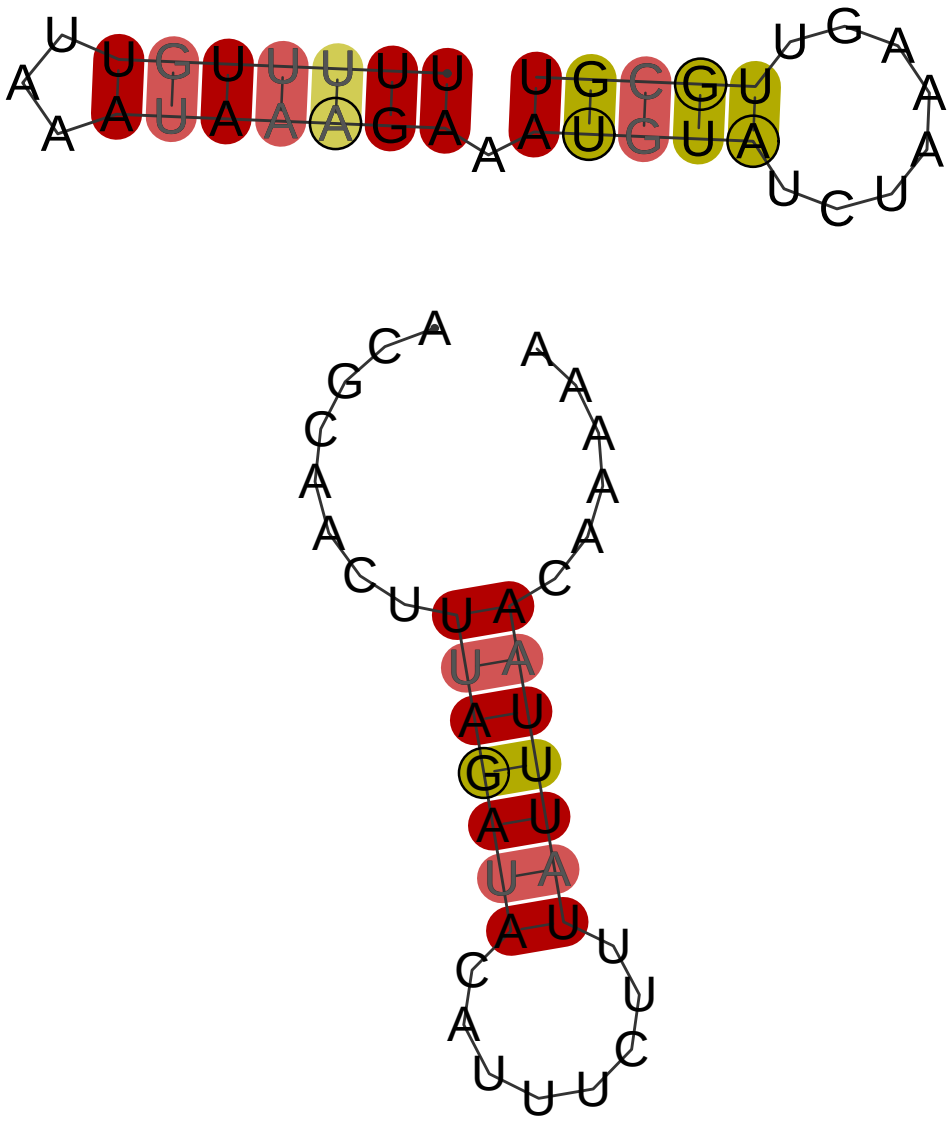

Fig. S50. Output of RNAalifold analysis of the conserved region found 1st in analysis of the nsp4 region of SARS-CoV-2, NC_045512.2 nucleotide location 9812–9847 (top: forward sense; bottom: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

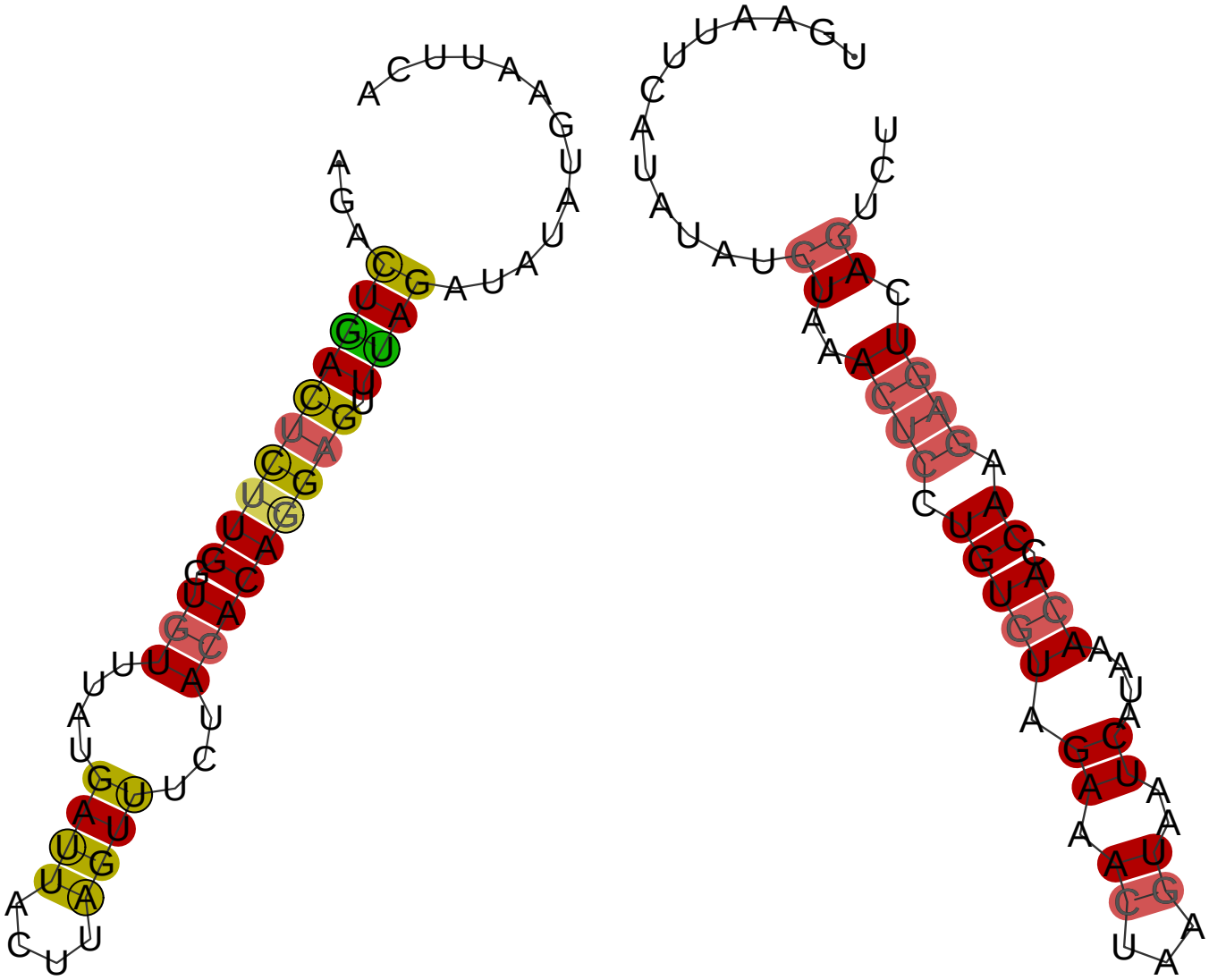

Fig. S51. Output of RNAalifold analysis of the conserved region found 1st in analysis of the nsp6 region of SARS-CoV-2, NC_045512.2 nucleotide location 11675–11740 (left: forward sense; right: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

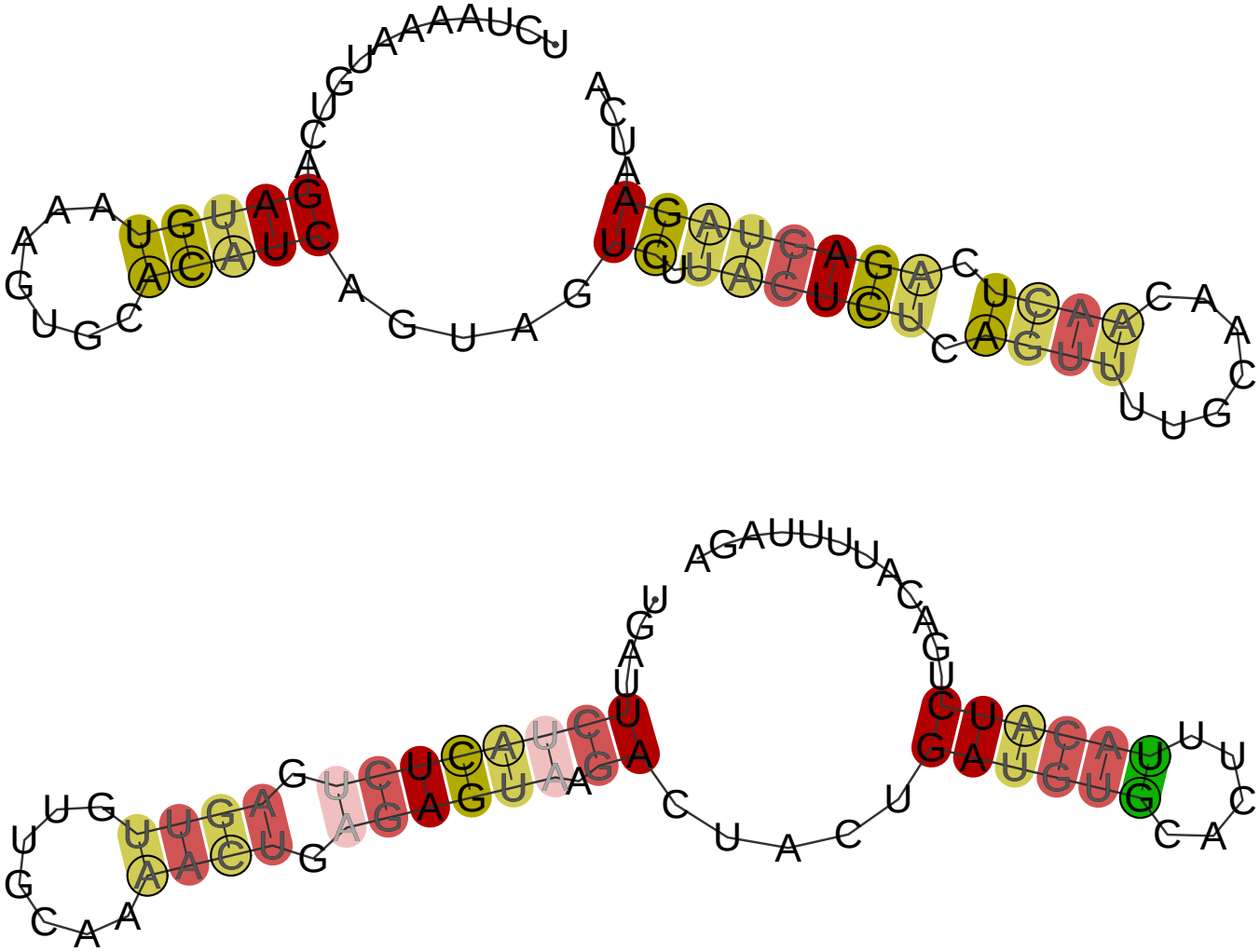

Fig. S52. Output of RNAalifold analysis of the conserved region found 1st in analysis of the nsp7 region of SARS-CoV-2, NC_045512.2 nucleotide location 11843–11914 (top: forward sense; bottom: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

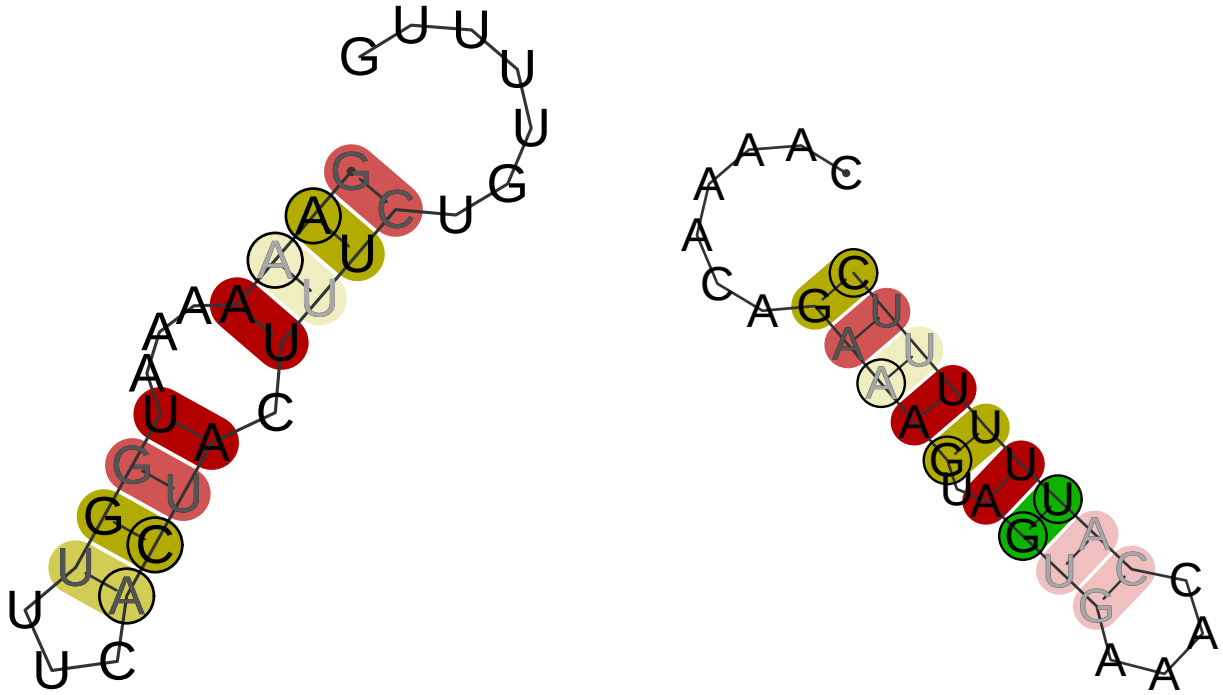

Fig. S53. Output of RNAalifold analysis of the conserved region found 2nd in analysis of the nsp7 region of SARS-CoV-2, NC_045512.2 nucleotide location 11987–12019 (left: forward sense; right: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

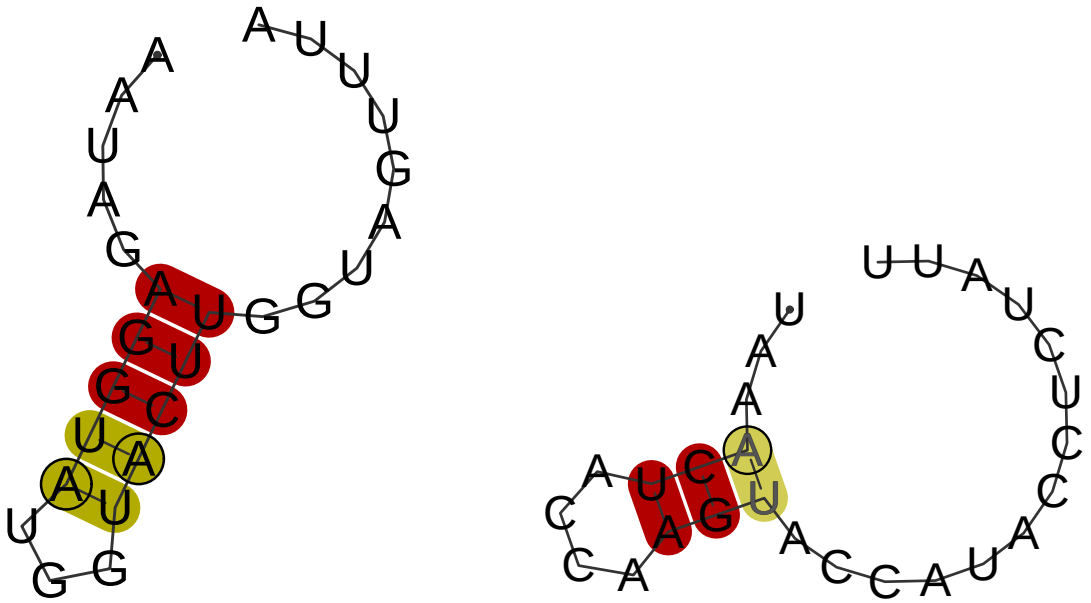

Fig. S54. Output of RNAalifold analysis of the conserved region found 1st in analysis of the nsp9 region of SARS-CoV-2, NC_045512.2 nucleotide location 12977–13003 (left: forward sense; right: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

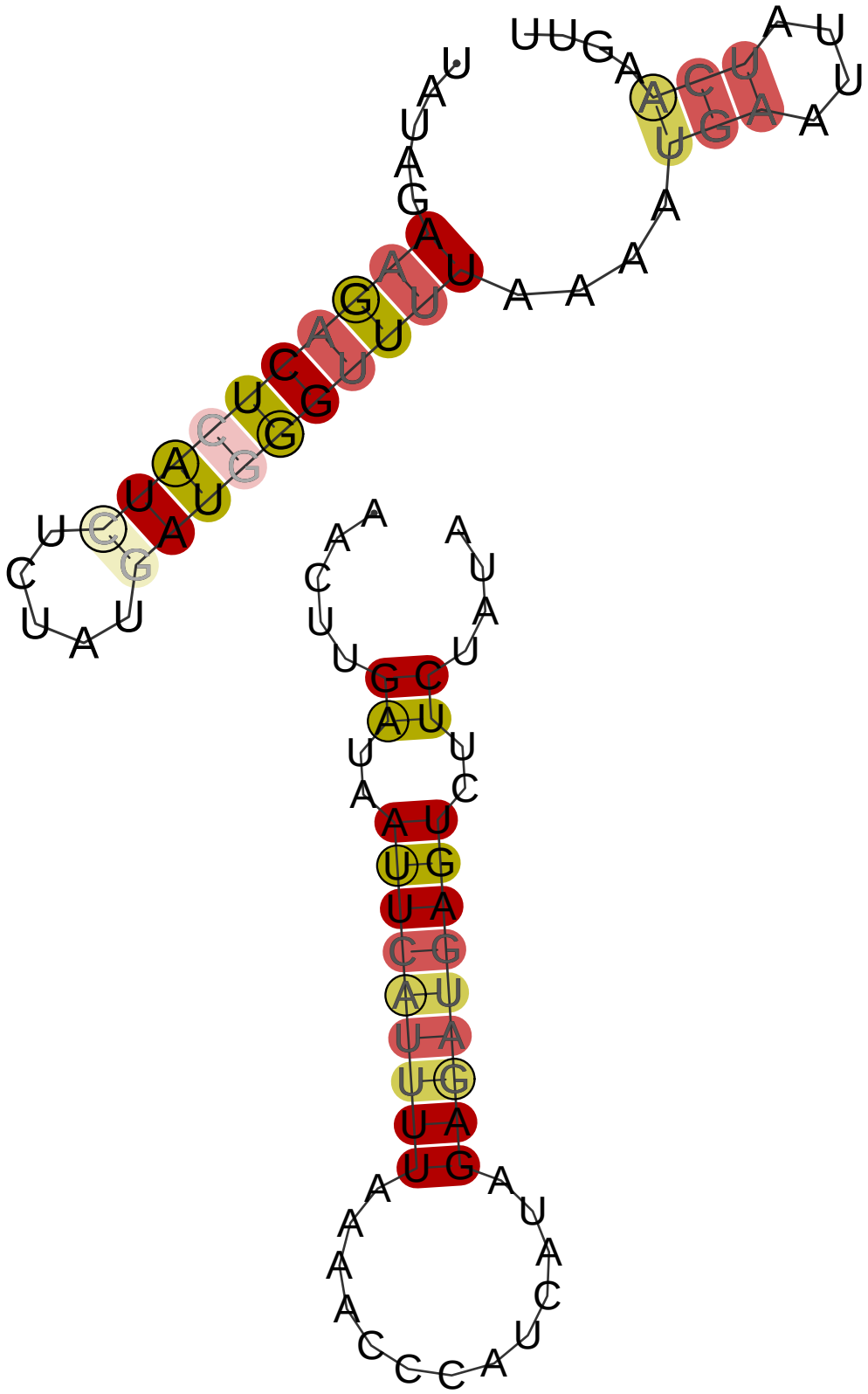

Fig. S55. Output of RNAalifold analysis of the conserved region found 2nd in analysis of the nsp14 region of SARS-CoV-2, NC_045512.2 nucleotide location 18190–18237 (top: forward sense; bottom: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

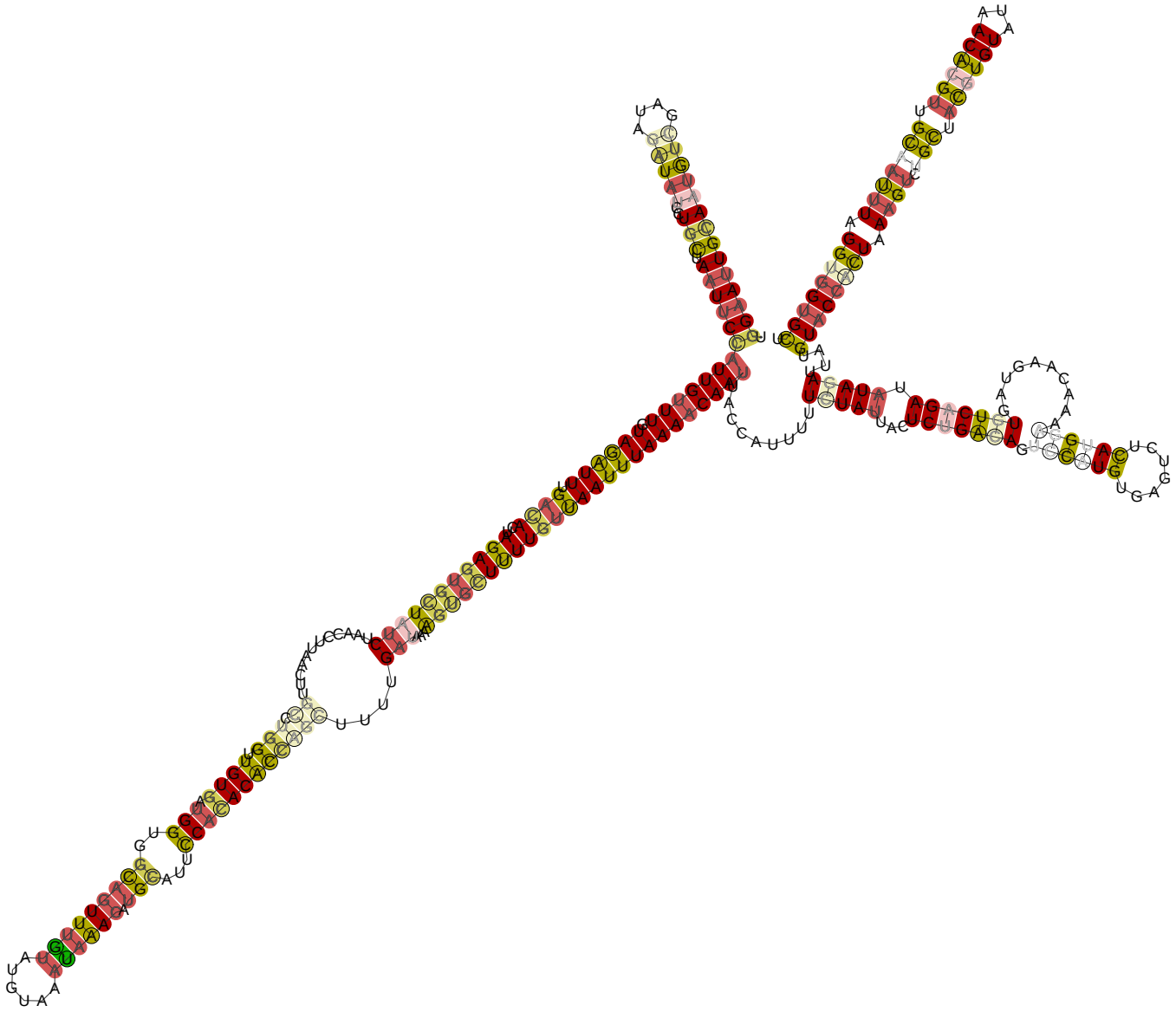

Fig. S56. Output of RNAalifold analysis of the conserved region found 1st in analysis of the nsp14 region of SARS-CoV-2, NC_045512.2 nucleotide location 19189–19485. Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

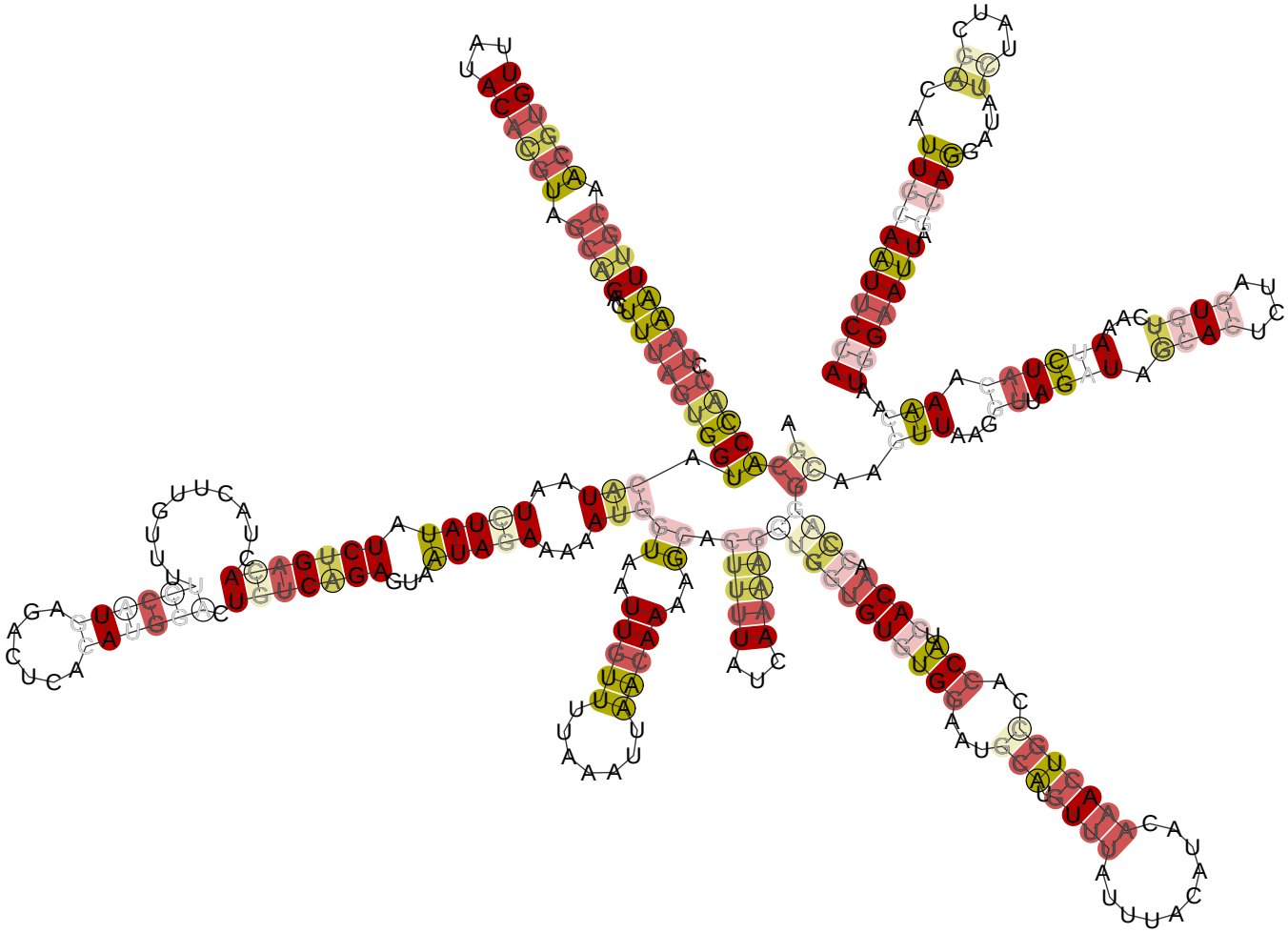

Fig. S57. Output of RNAalifold analysis of the reverse complement of the conserved region found 1st in analysis of the nsp14 region of SARS-CoV-2, NC_045512.2 nucleotide location 19189–19485. Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

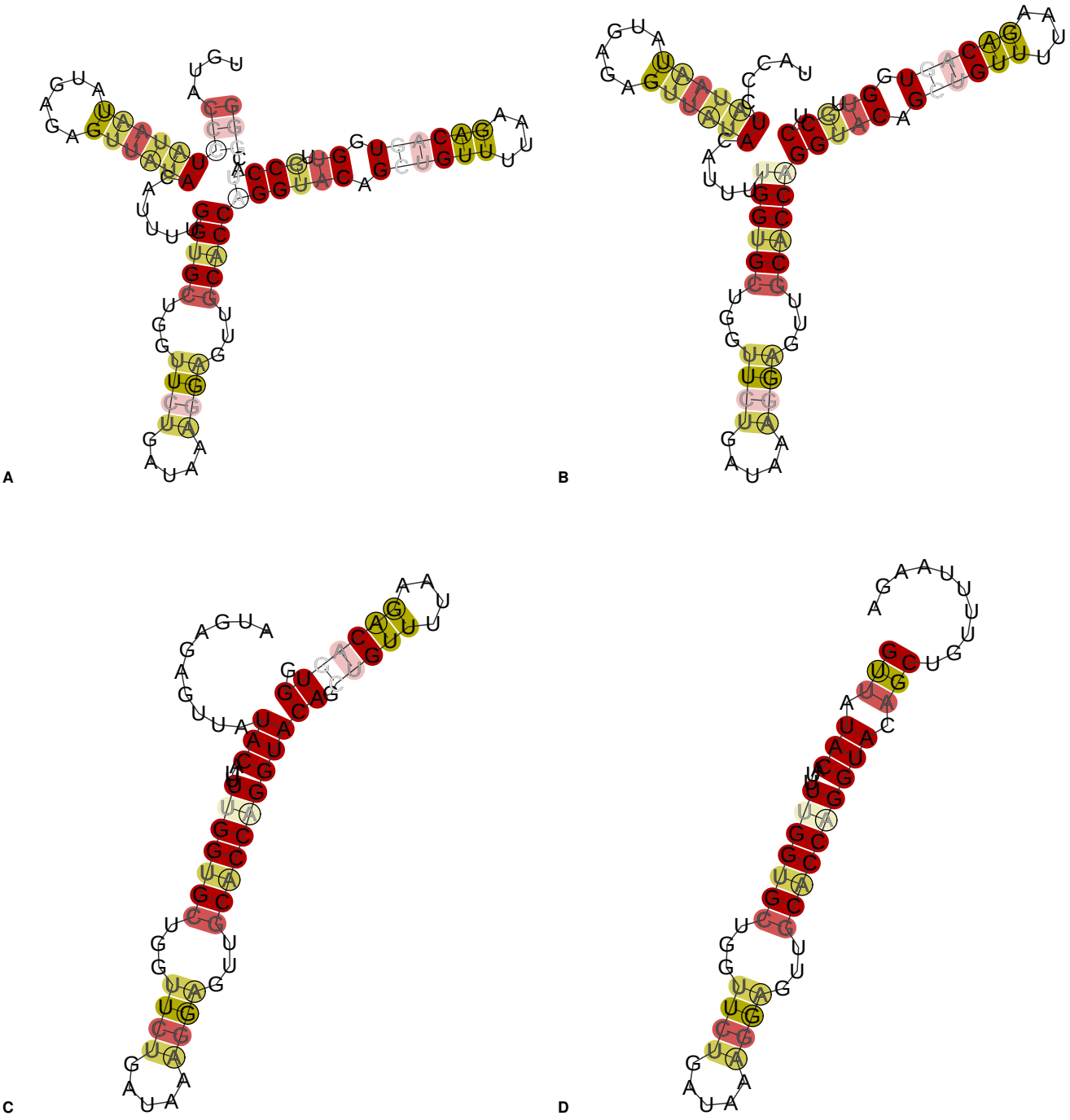

Fig. S58. Sensitivity analysis of predicted structures from the conserved region found 2nd in analysis of the nsp16 region of SARS-CoV-2, NC_045512.2 nucleotide location 20845–20928. (Note that the fold of the region is shown in Fig. 9 in the main text.) Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled. (**A**) Region 20838–20933. (**B**) Region 20840–20928. (**C**) Region 20851–20922. (**D**) Region 20857–20916. Addition of small numbers of nucleotides 5′ and 3′ of the conserved region does not disrupt the predicted structures; removal of nucleotides only disrupts the predicted structures when nucleotides in the structures themselves are removed, and the predicted structure that is left remains stable.

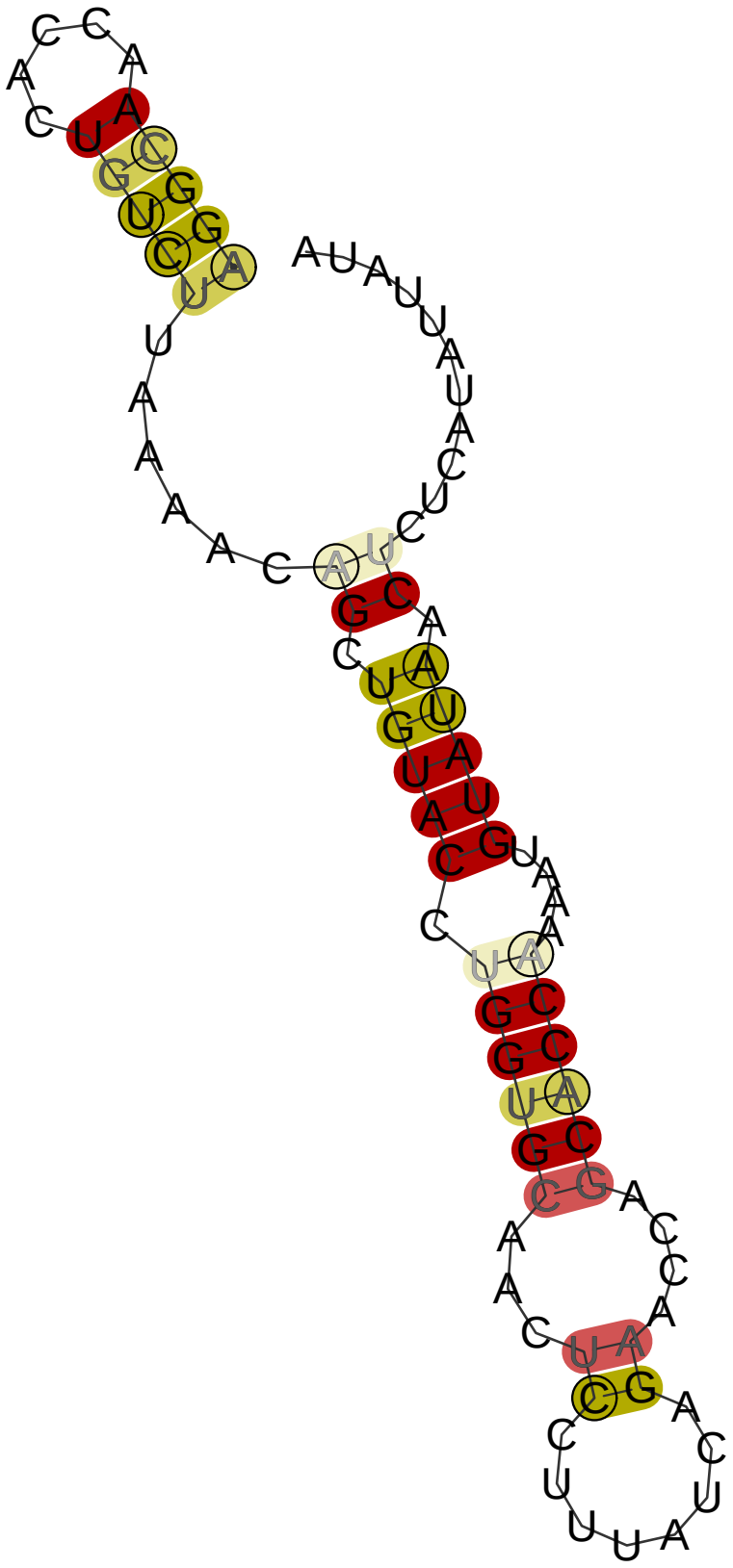

Fig. S59. Output of RNAalifold analysis of the reverse complement of the conserved region found 2nd in analysis of the nsp16 region of SARS-CoV-2, NC_045512.2 nucleotide location 20845–20928. (Note that the fold of the forward sense RNA is shown in Fig. 9 in the main text.) Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

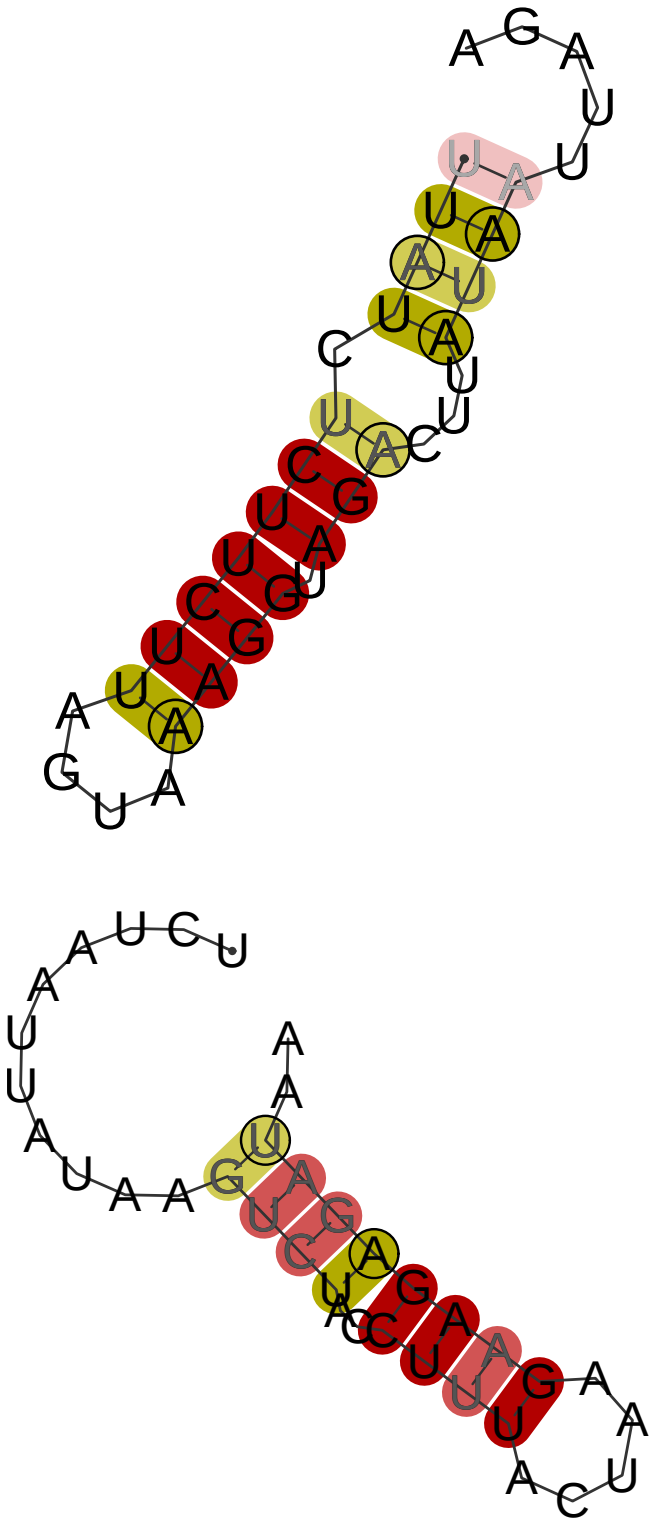

Fig. S60. Output of RNAalifold analysis of the conserved region found 1st in analysis of the nsp14 region of SARS-CoV-2, NC_045512.2 nucleotide location 21469–21507 (top: forward sense; bottom: reverse complement). Prior to folding, aligned sequences used in the main analysis were deduplicated so that one example of each observed primary structure for the conserved region was used as input to the algorithm. Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

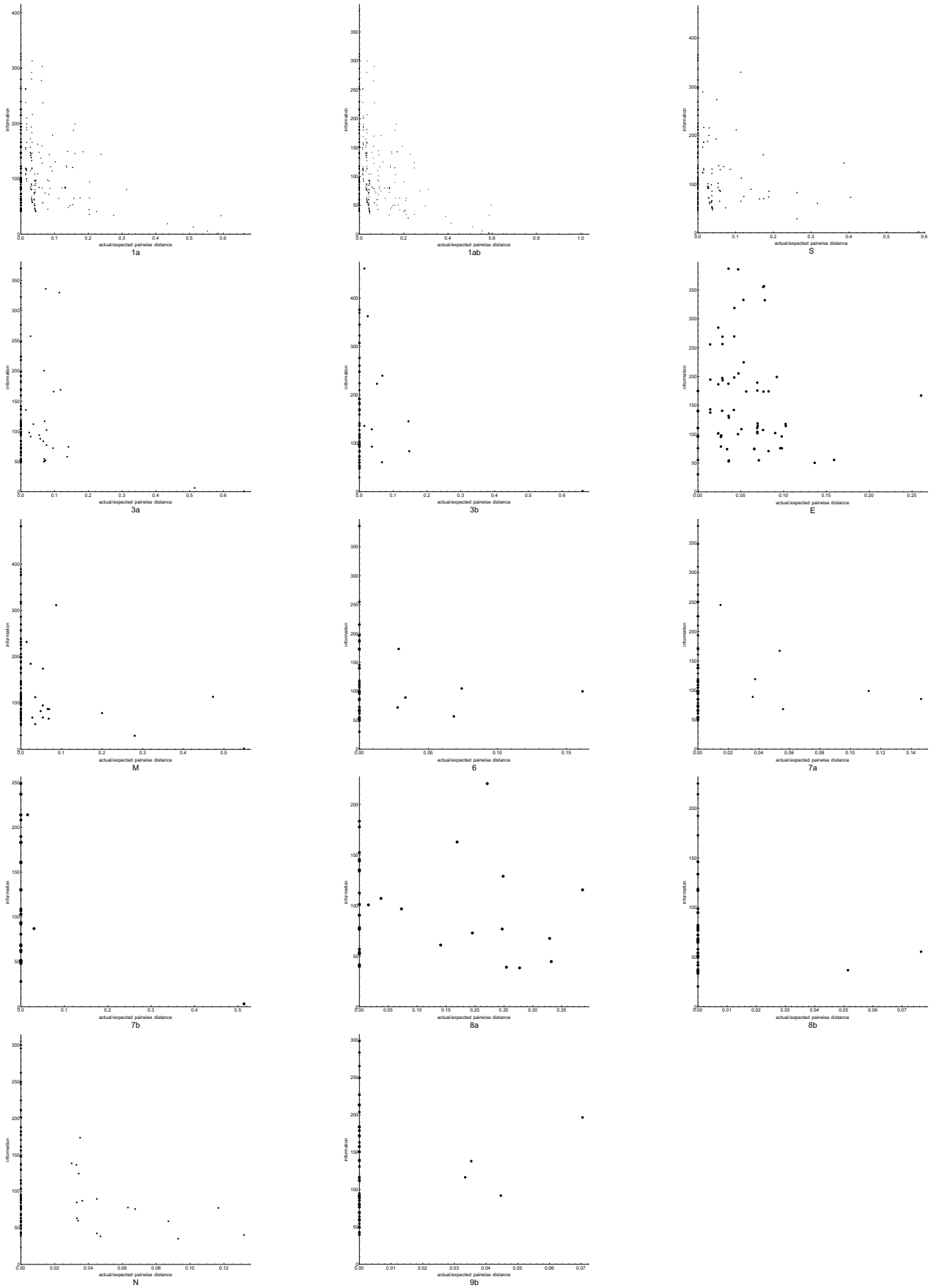

Fig. S61. Locus information versus variability for genes of SARS-CoV.

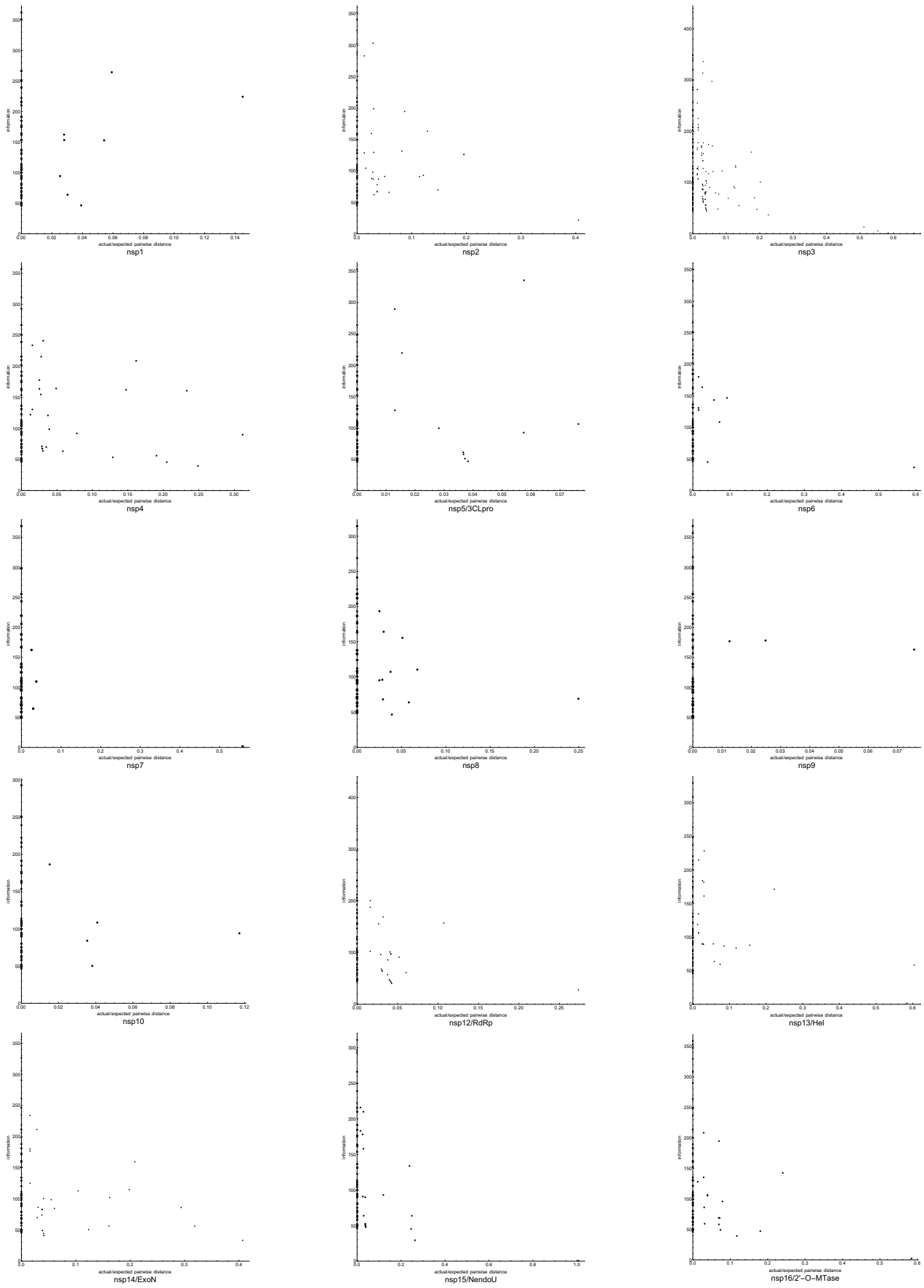

Fig. S62. Locus information versus variability for individual non-structural protein regions in the SARS-CoV 1ab region. The nsp11 plot is omitted as all loci evaluated had zero variability.

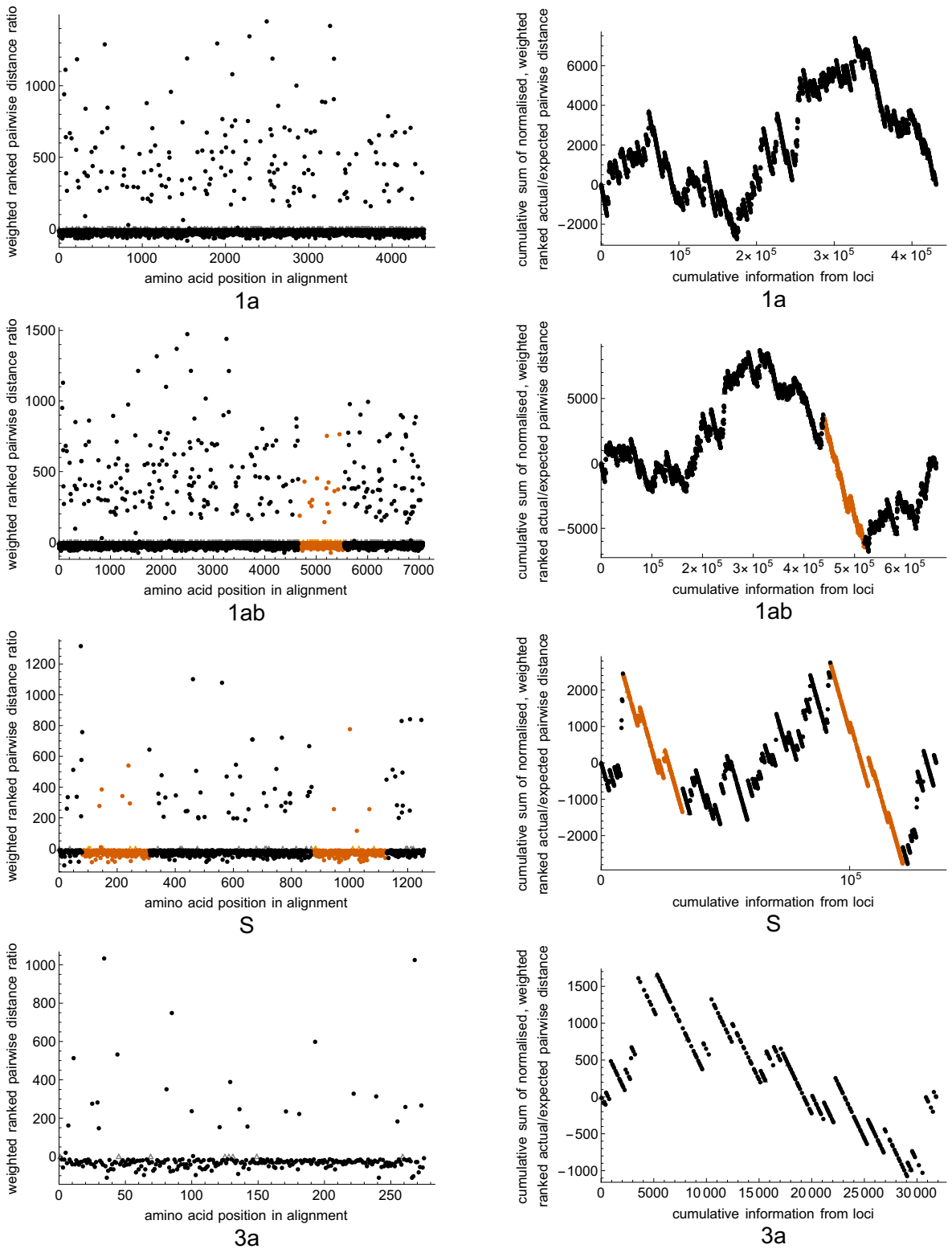

Fig. S63. Output from analyses of SARS-CoV 1a, 1ab, S and 3a genes, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. Regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Plots use weighted, ranked pairwise distance ratios. More detail of significant regions found is given in Table S2.

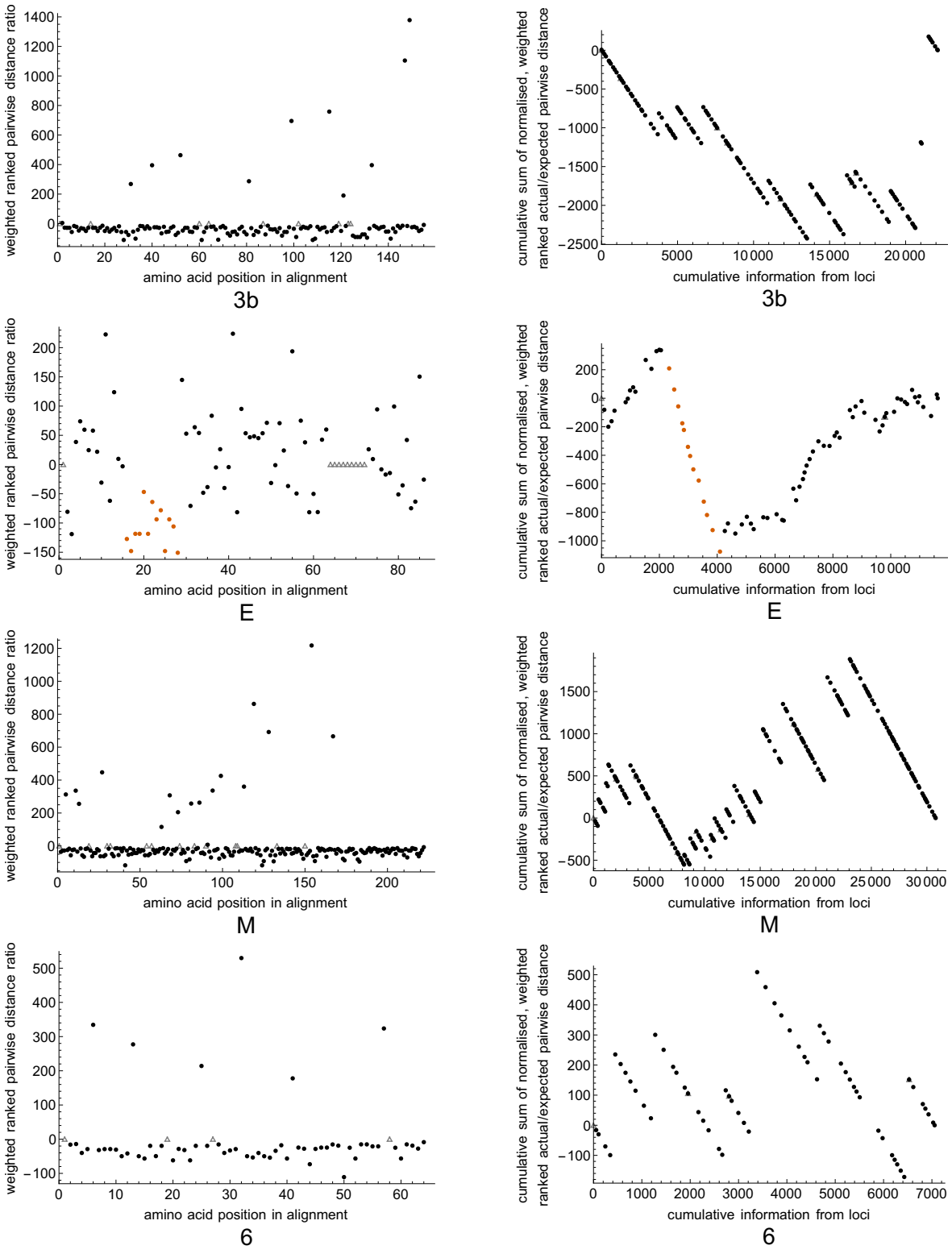

Fig. S64. Output from analyses of SARS-CoV 3b, E, M and 6 genes, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. Regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Plots use weighted, ranked pairwise distance ratios. More detail of significant regions found is given in Table S2.

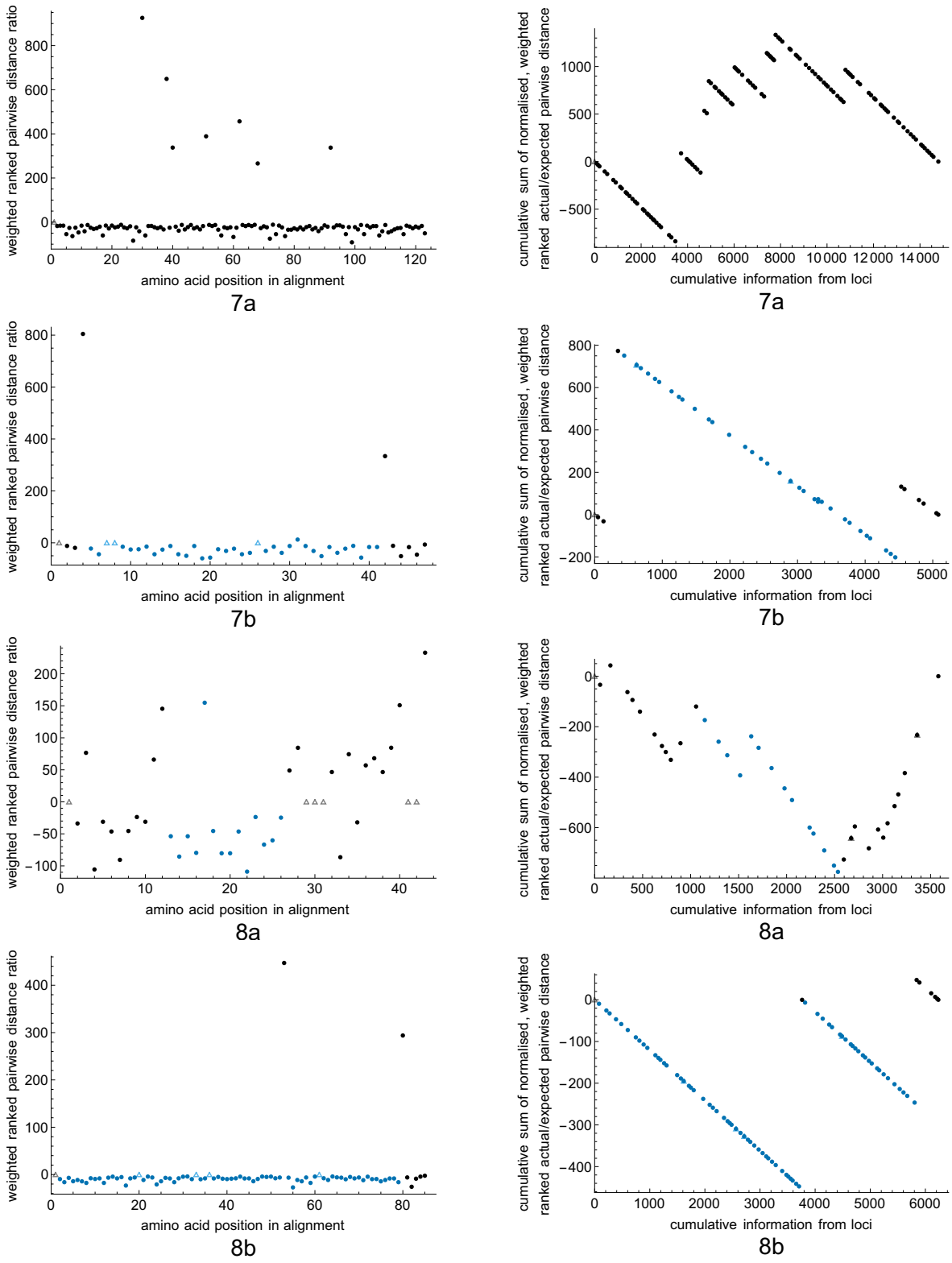

Fig. S65. Output from analyses of SARS-CoV 7a, 7b, 8a and 8b genes, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. Regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Plots use weighted, ranked pairwise distance ratios. More detail of significant regions found is given in Table S2.

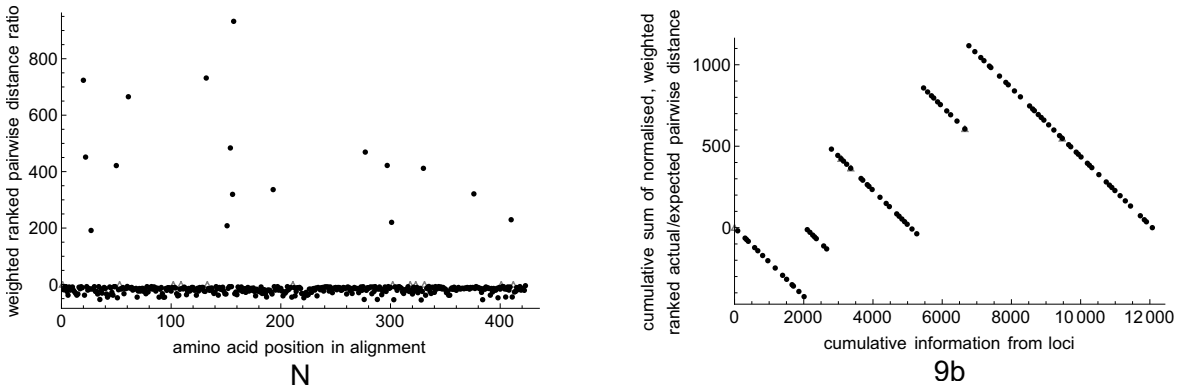

Fig. S66. Output from analyses of SARS-CoV N and 9b genes, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. Regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Plots use weighted, ranked pairwise distance ratios. More detail of significant regions found is given in Table S2.

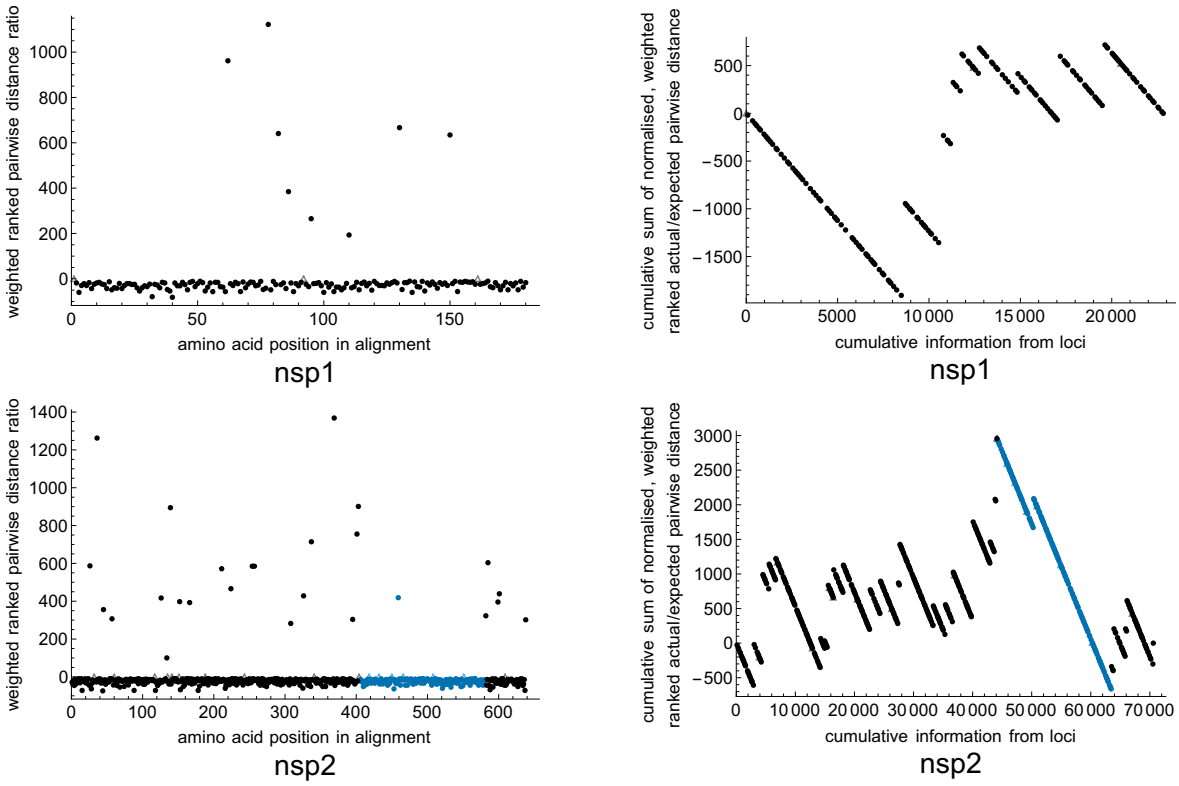

Fig. S67. Output from analyses of SARS-CoV nsp1 and nsp2 regions, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. Regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Plots use weighted, ranked pairwise distance ratios. More detail of significant regions found is given in Table S3.

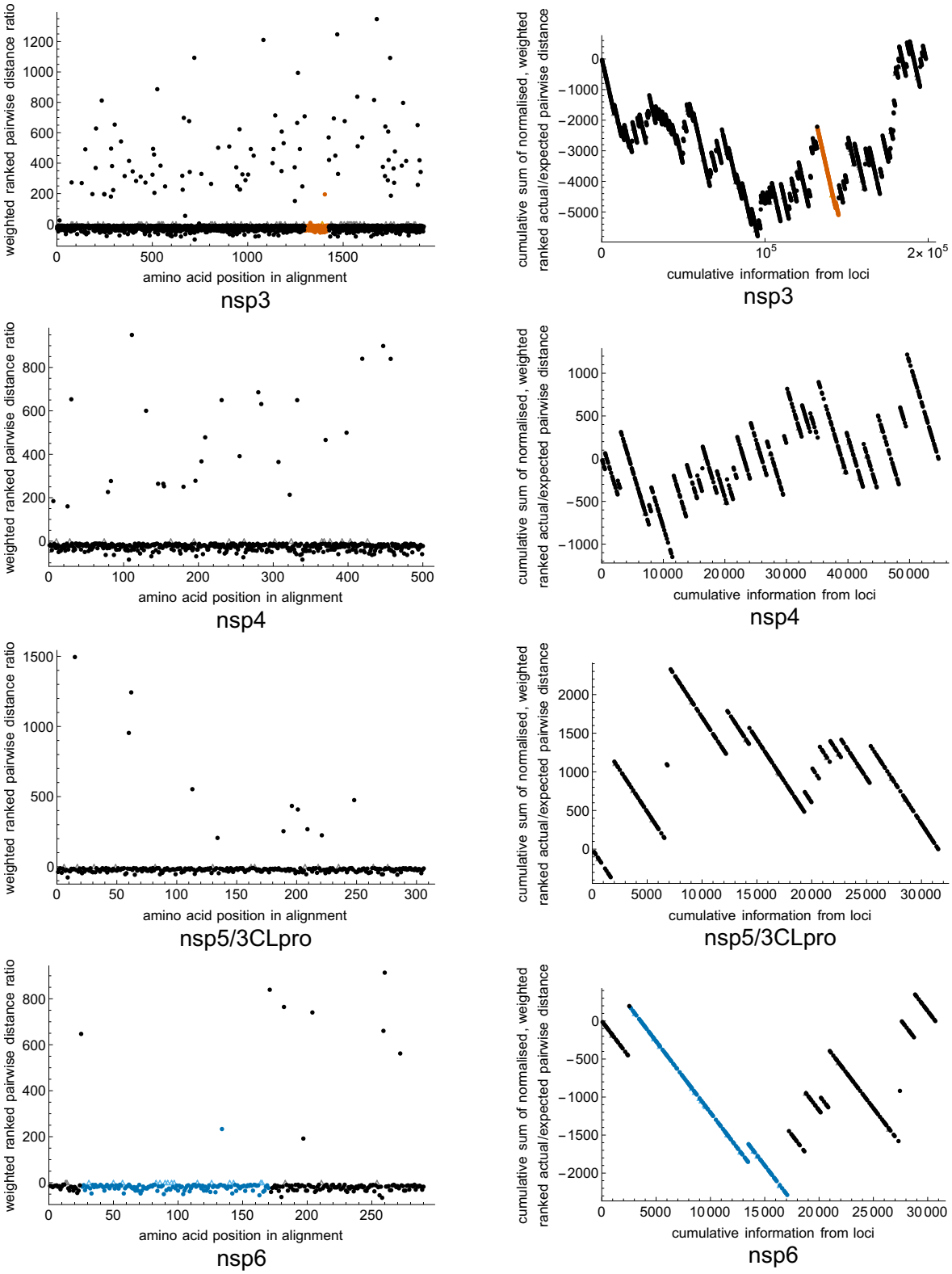

Fig. S68. Output from analyses of SARS-CoV nsp3, nsp4, nsp5 and nsp6 regions, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. Regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Plots use weighted, ranked pairwise distance ratios. More detail of significant regions found is given in Table S3.

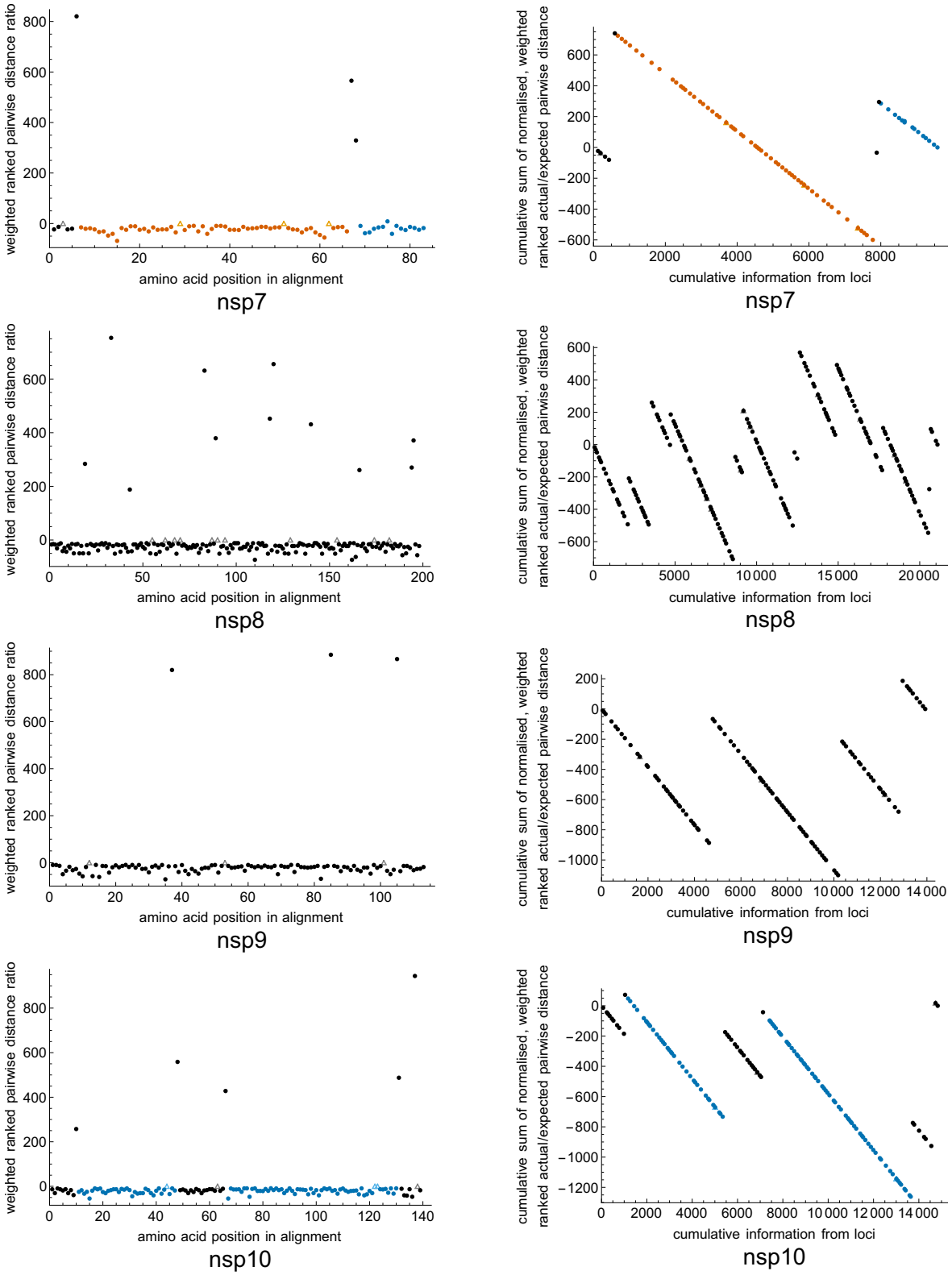

Fig. S69. Output from analyses of SARS-CoV nsp7, nsp8, nsp9 and nsp10 regions, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. Regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Plots use weighted, ranked pairwise distance ratios. More detail of significant regions found is given in Table S3.

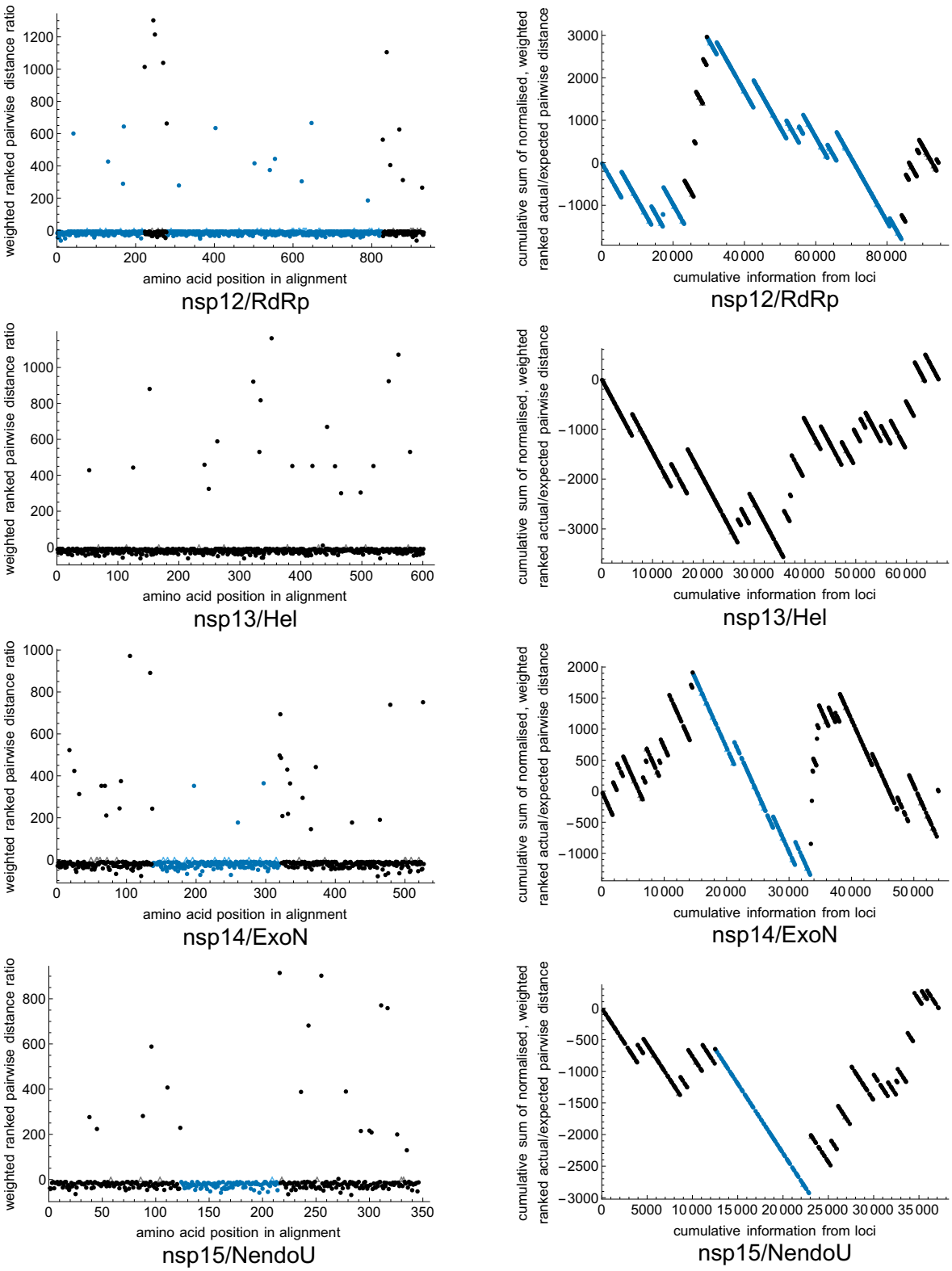

Fig. S70. Output from analyses of SARS-CoV nsp12, nsp13, nsp14 and nsp15 regions, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. Regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Plots use weighted, ranked pairwise distance ratios. More detail of significant regions found is given in Table S3. Note that output from analysis of nsp11 is not included in the Supplementary Information because no variability is seen in any codon locus in the region.

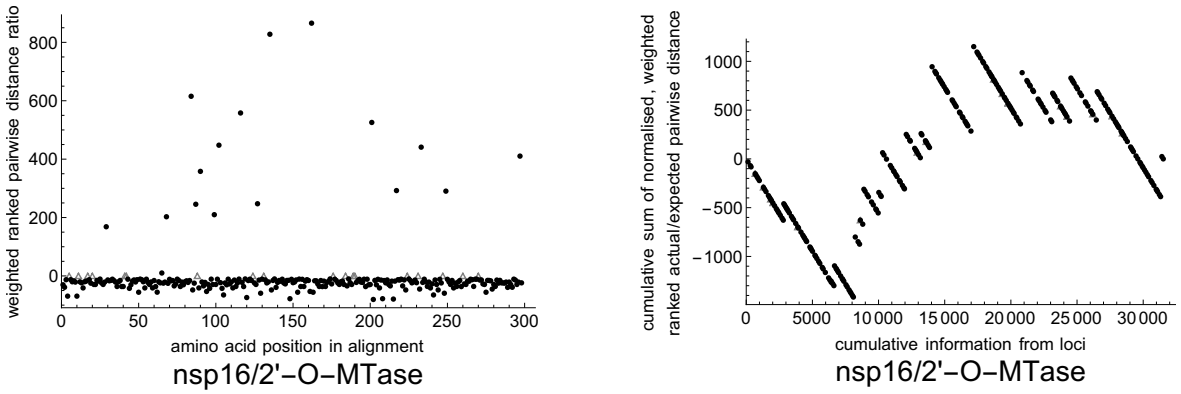

Fig. S71. Output from analyses of SARS-CoV nsp16 region, with raw analysis output on left and cumulative sums on right. Each dot represents one codon. Regions deemed significant by the algorithm are highlighted in orange and regions deemed significant after removal of a potentially interfering region are highlighted in blue. Codons contributing no information to the analysis are in a lighter colour and plotted with open triangles. Plots use weighted, ranked pairwise distance ratios. More detail of significant regions found is given in Table S3.

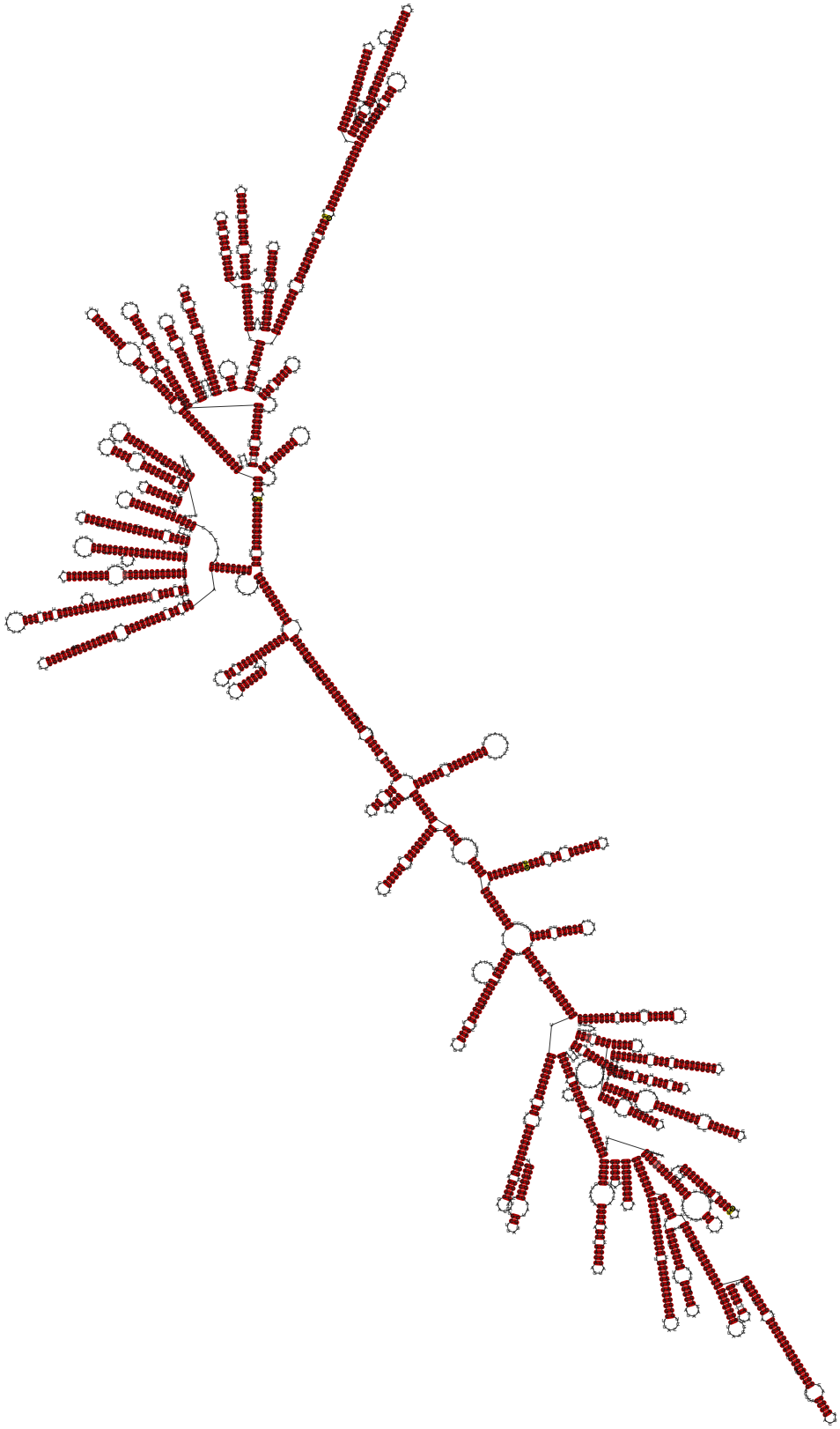

Fig. S72. Output of RNAalifold analysis of the conserved region found 1st in analysis of the 1ab region of SARS-CoV, AY274119.3 nucleotide location 14211–16889. All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

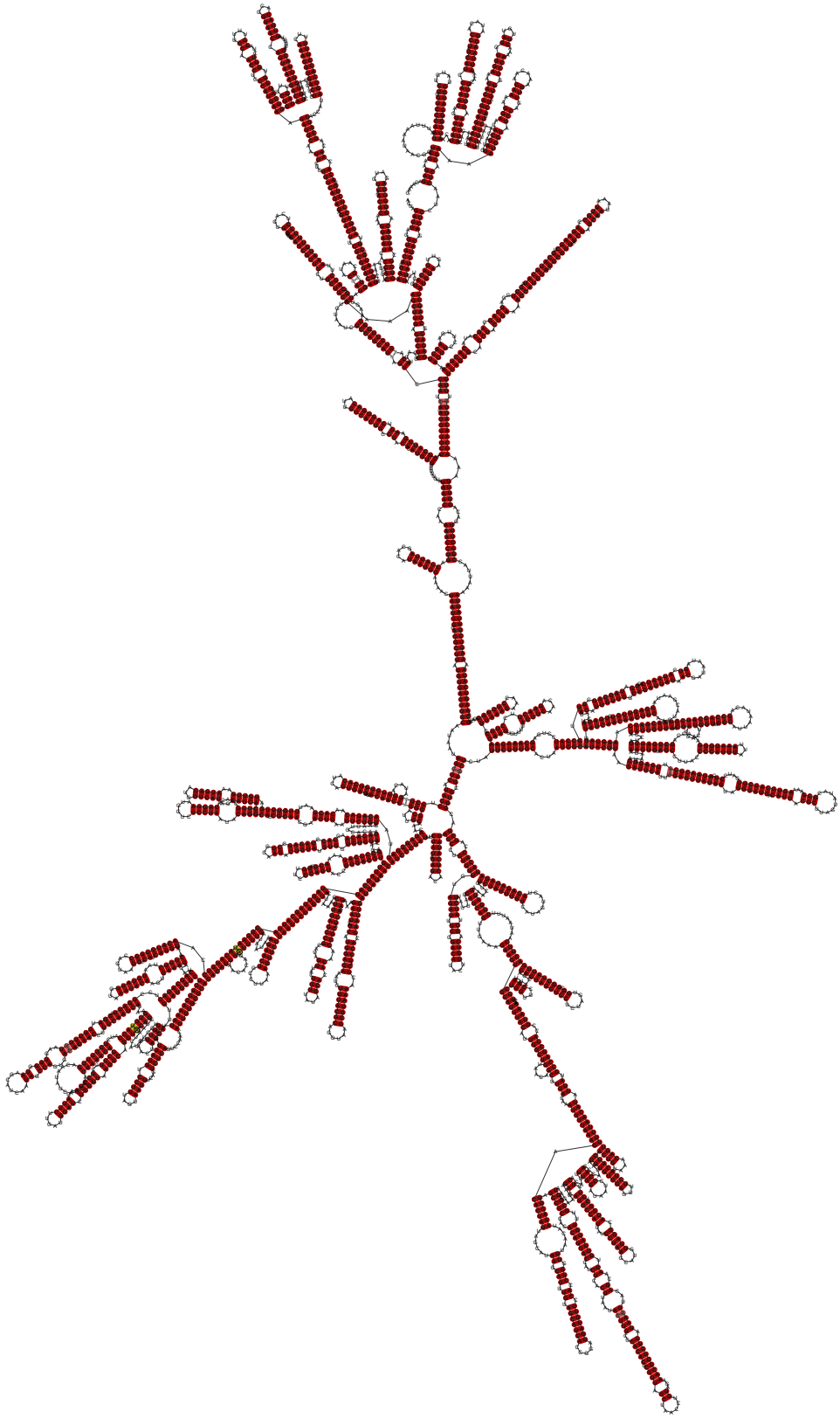

Fig. S73. Output of RNAalifold analysis of the reverse complement of the conserved region found 1st in analysis of the 1ab region of SARS-CoV, AY274119.3 nucleotide location 14211–16889. All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

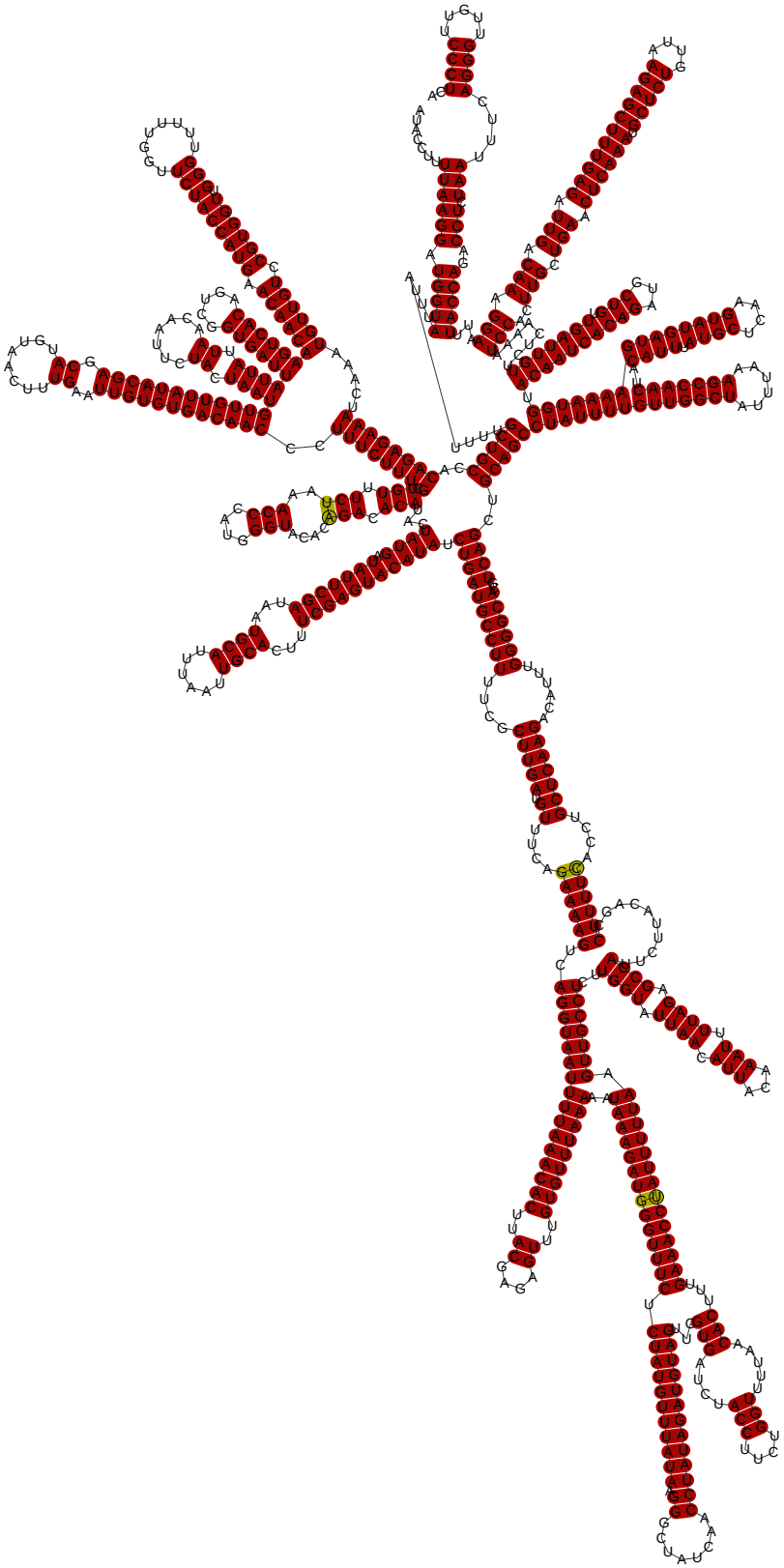

Fig. S74. Output of RNAalifold analysis of the conserved region found 2nd in analysis of the S region of SARS-CoV, AY274119.3 nucleotide location 21732–22421. All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

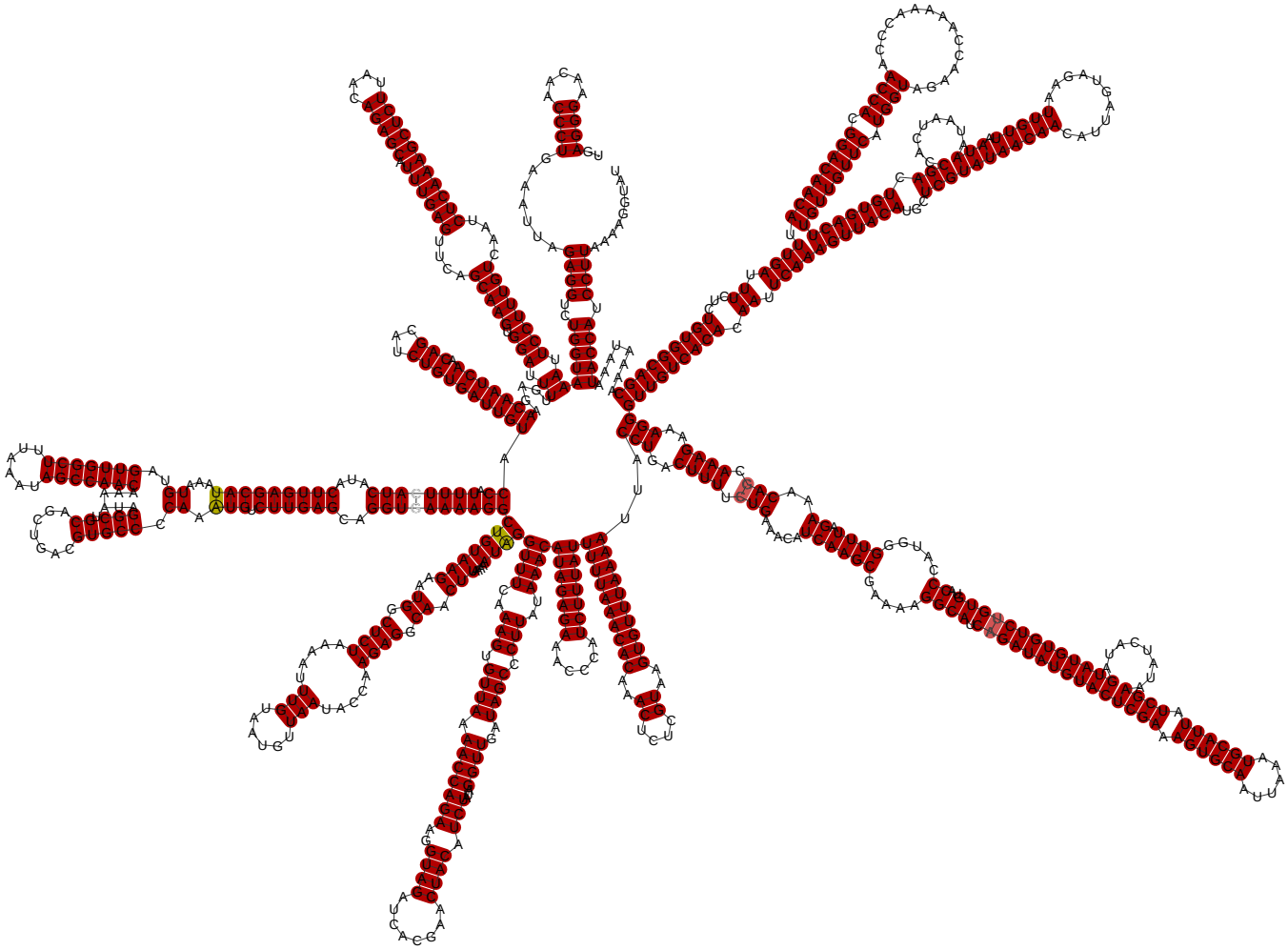

Fig. S75. Output of RNAalifold analysis of the reverse complement of the conserved region found 2nd in analysis of the S region of SARS-CoV, AY274119.3 nucleotide location 21732–22421. All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

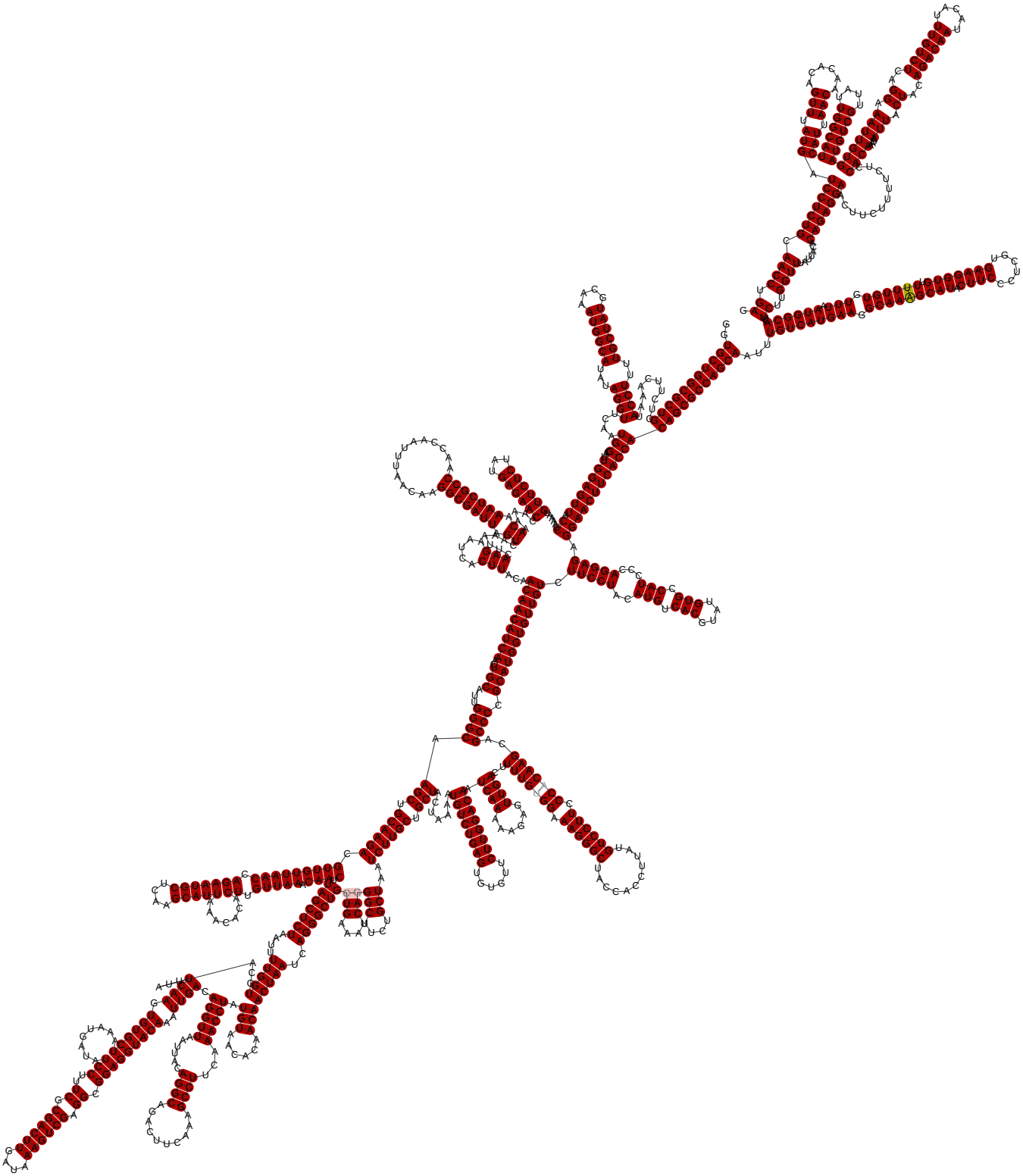

Fig. S76. Output of RNAalifold analysis of the conserved region found 1st in analysis of the S region of SARS-CoV, AY274119.3 nucleotide location 24099–24869. All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

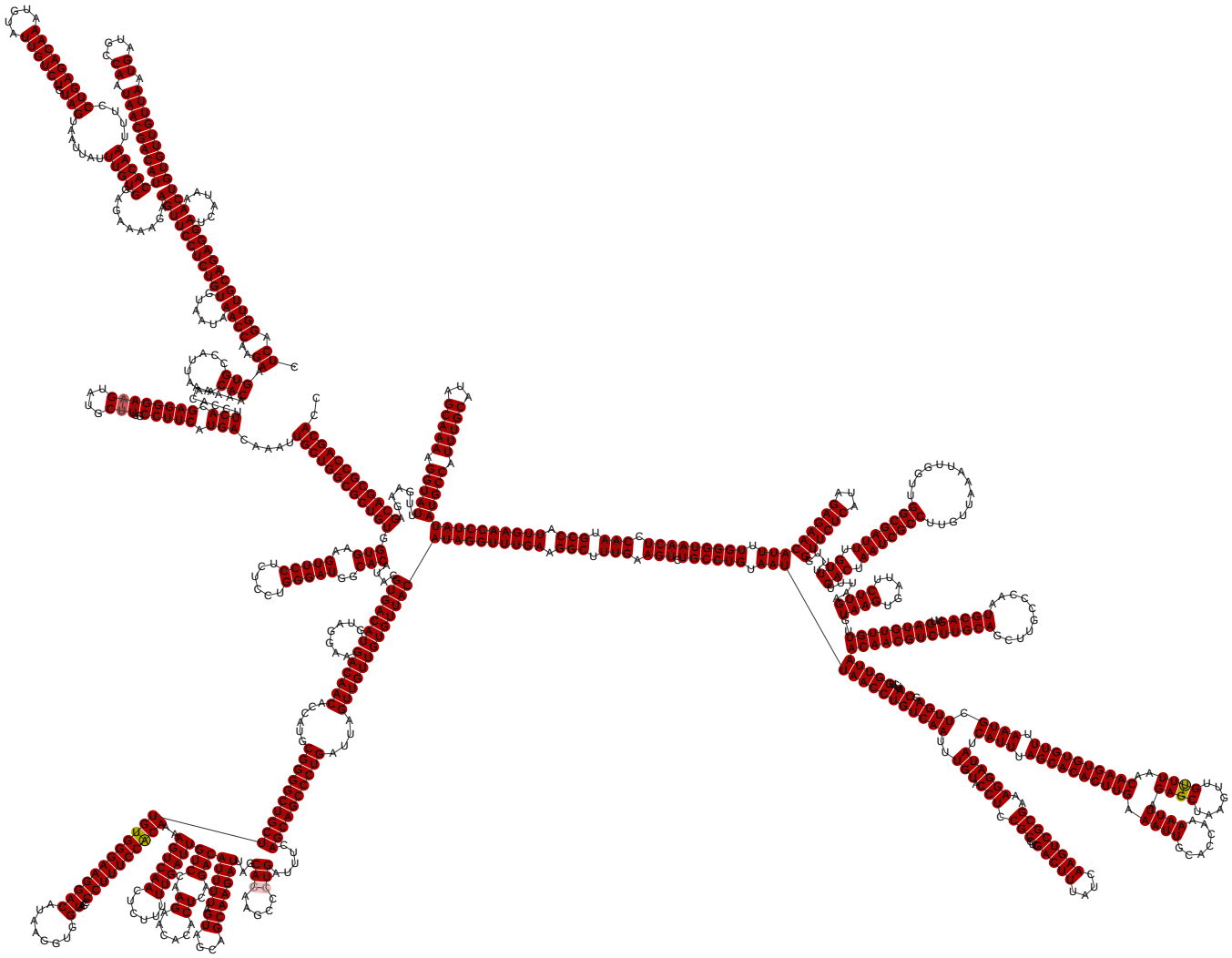

Fig. S77. Output of RNAalifold analysis of the reverse complement of the conserved region found 1st in analysis of the S region of SARS-CoV, AY274119.3 nucleotide location 24099–24869. All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

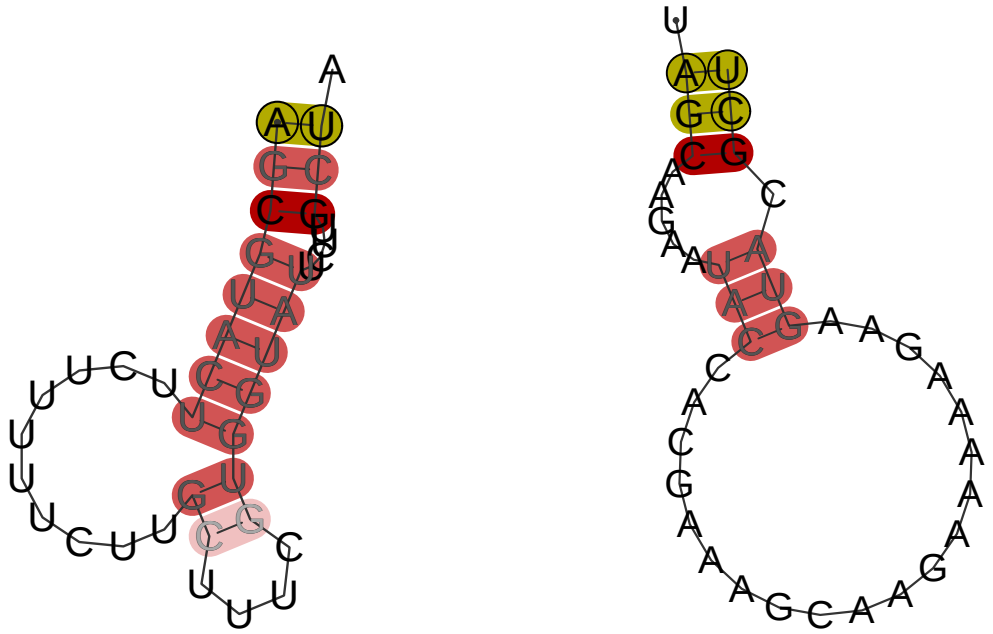

Fig. S78. Output of RNAalifold analysis of the conserved region found 1st in analysis of the E region of SARS-CoV, AY274119.3 nucleotide location 24099–24869 (left: forward sense; right: reverse complement). All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

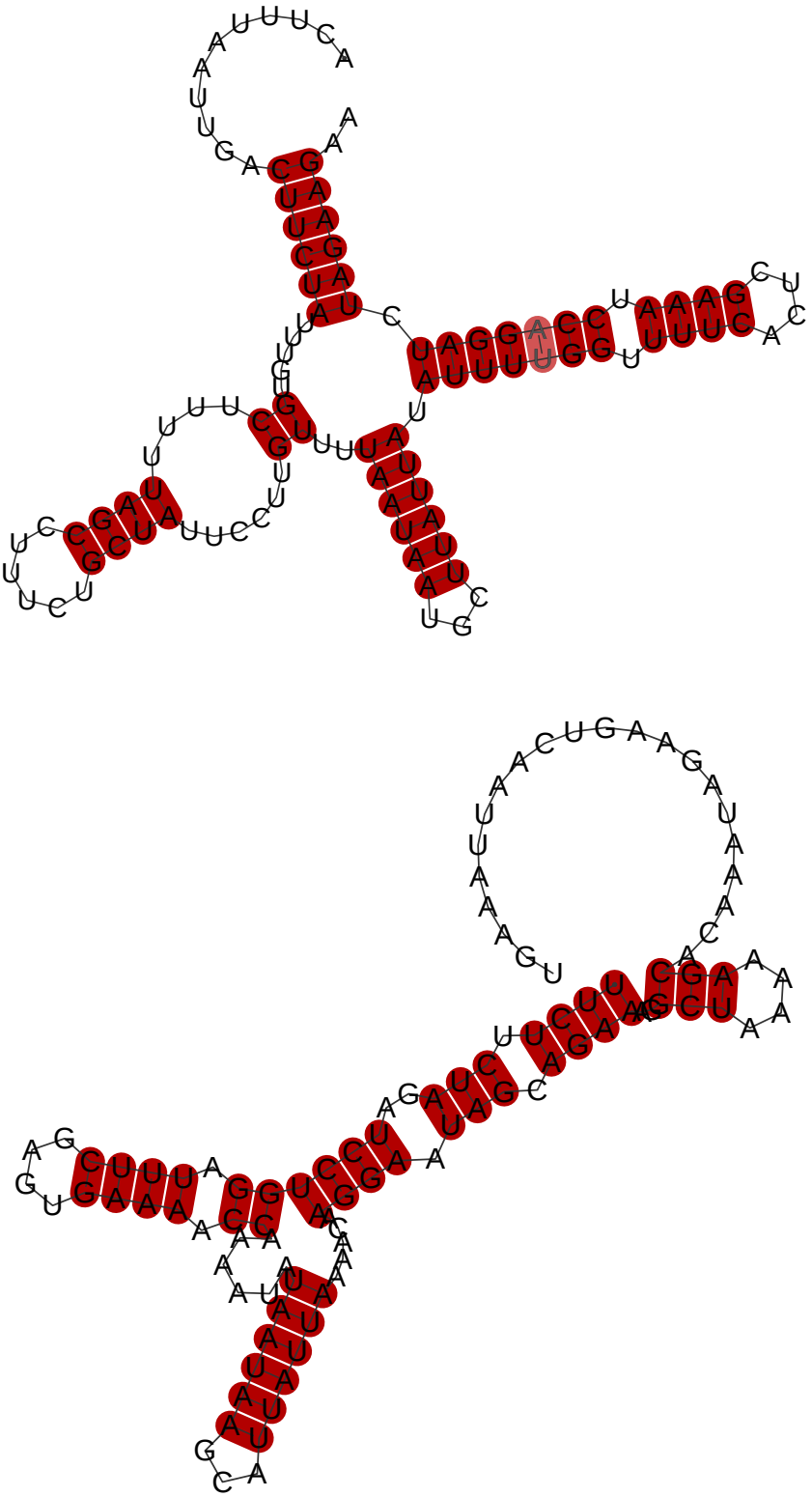

Fig. S79. Output of RNAalifold analysis of the conserved region found 1st in analysis of the 7b region of SARS-CoV, AY274119.3 nucleotide location 27650–27760 (top: forward sense; bottom: reverse complement). All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

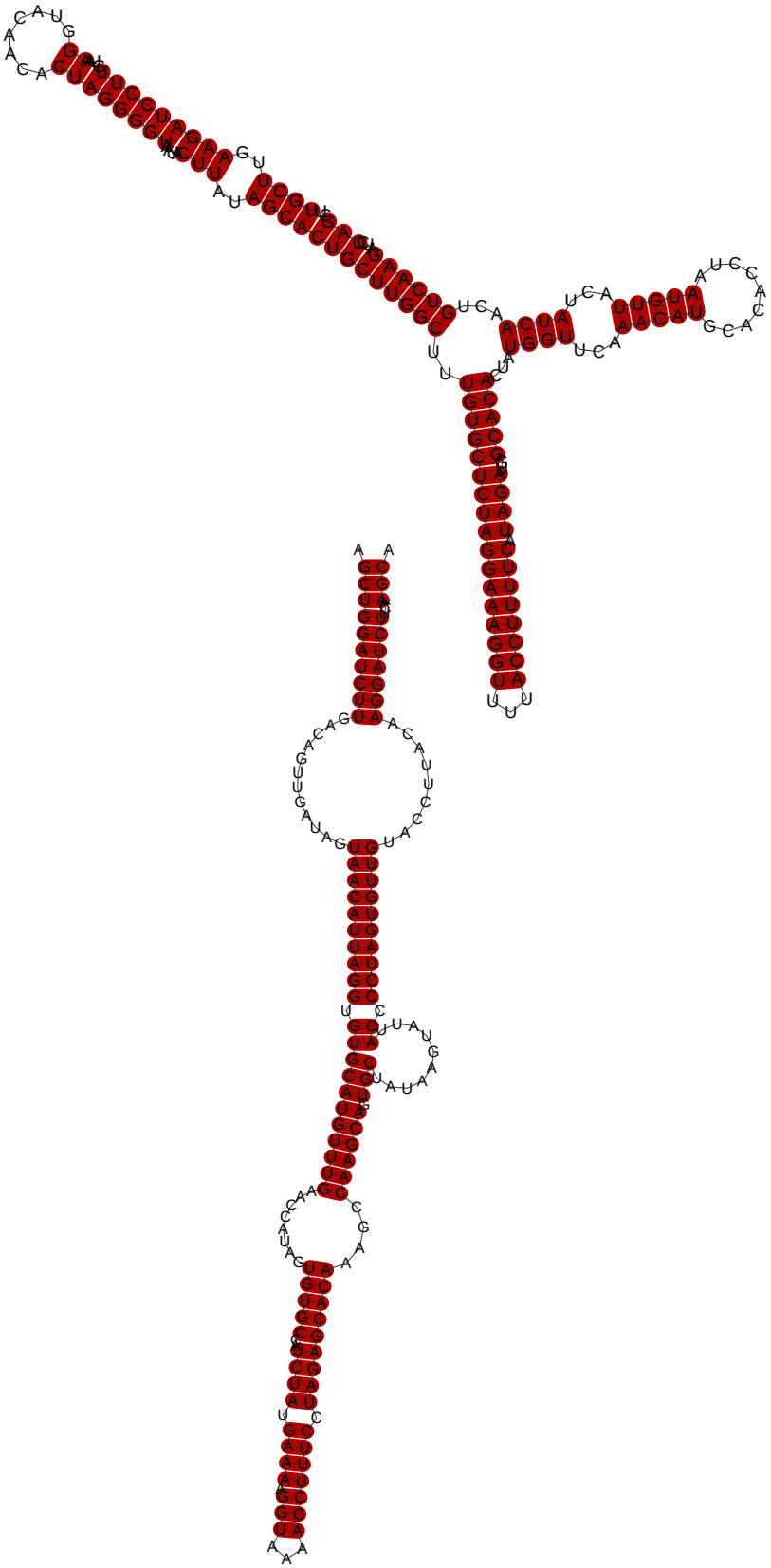

Fig. S80. Output of RNAalifold analysis of the conserved region found 1st in analysis of the 8b region of SARS-CoV, AY274119.3 nucleotide location 27867–28019 (top: forward sense; bottom: reverse complement). All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

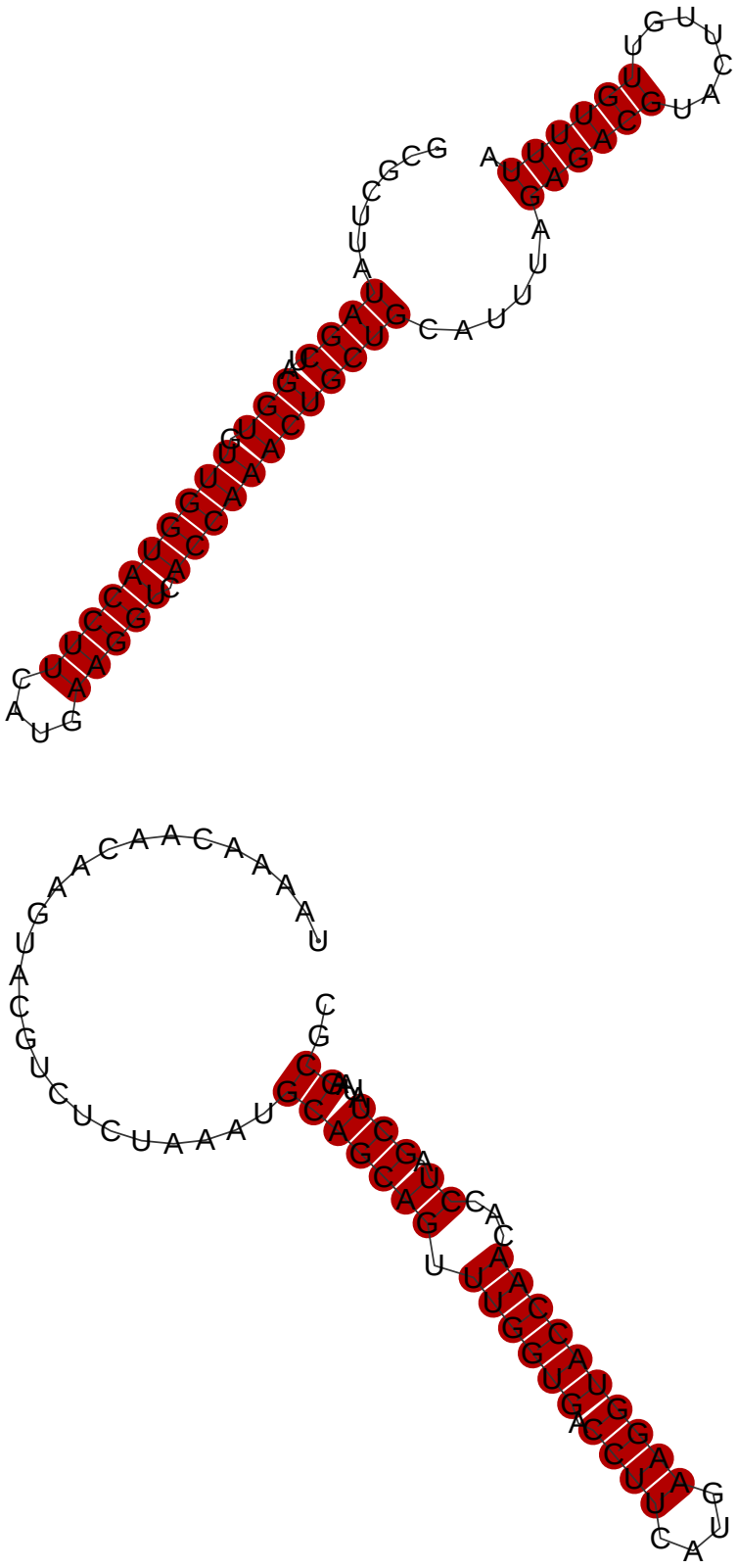

Fig. S81. Output of RNAalifold analysis of the conserved region found 2nd in analysis of the 8b region of SARS-CoV, AY274119.3 nucleotide location 28023–28100 (top: forward sense; bottom: reverse complement). All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

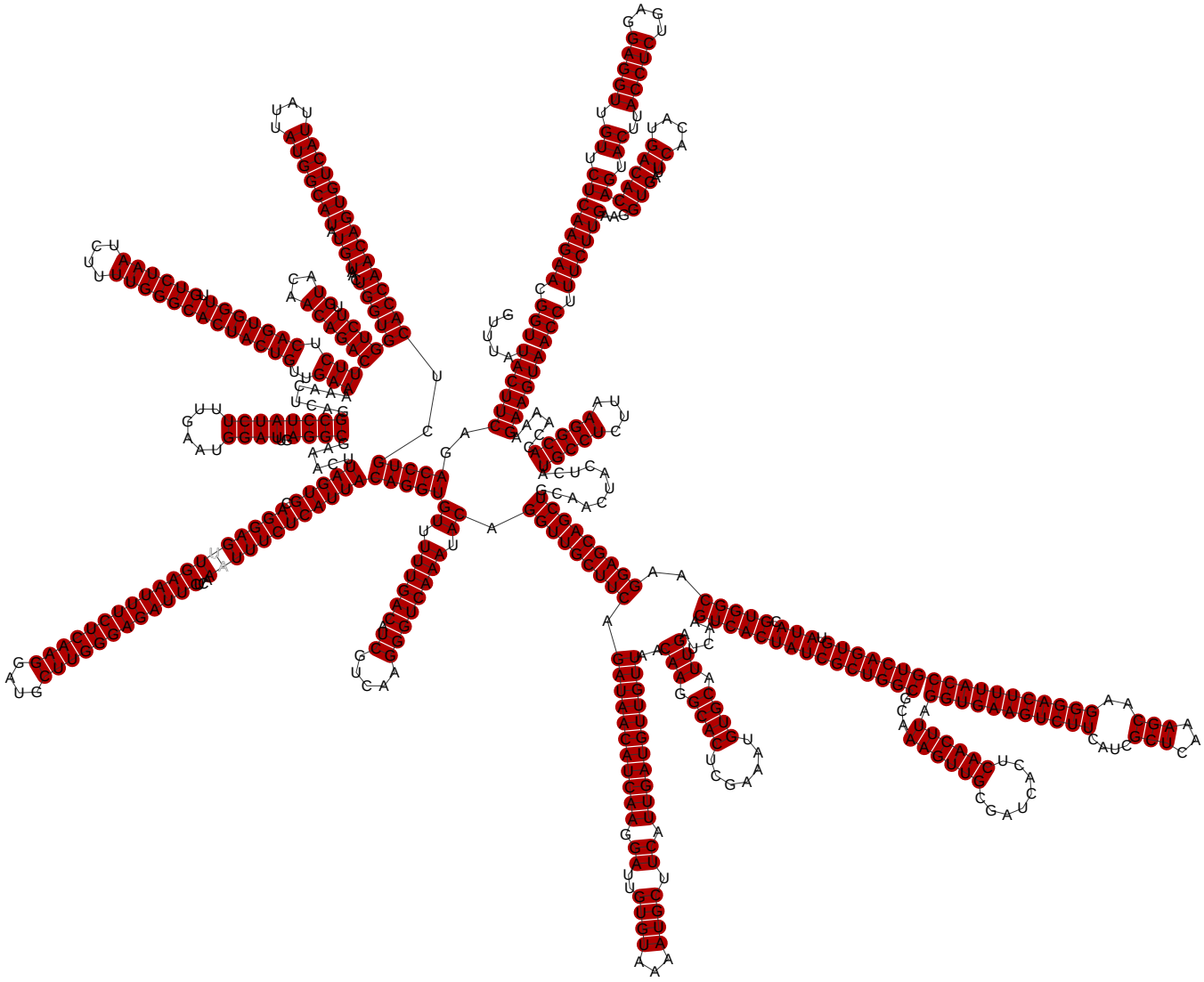

Fig. S82. Output of RNAalifold analysis of the conserved region found 1st in analysis of the nsp2 region of SARS-CoV, AY274119.3 nucleotide location 2017–2547. All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

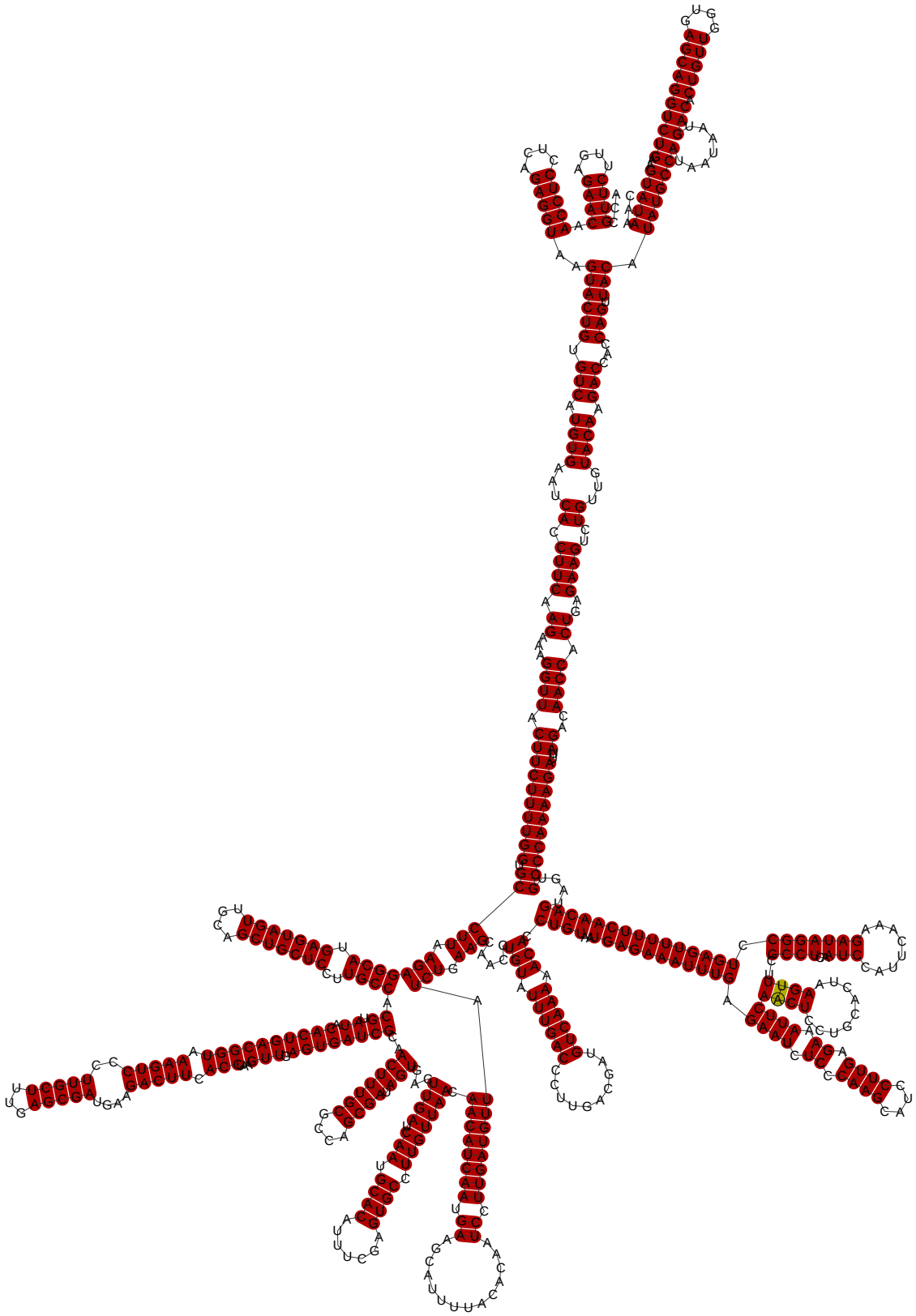

Fig. S83. Output of RNAalifold analysis of the reverse complement conserved region found 1st in analysis of the nsp2 region of SARS-CoV, AY274119.3 nucleotide location 2017–2547. All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

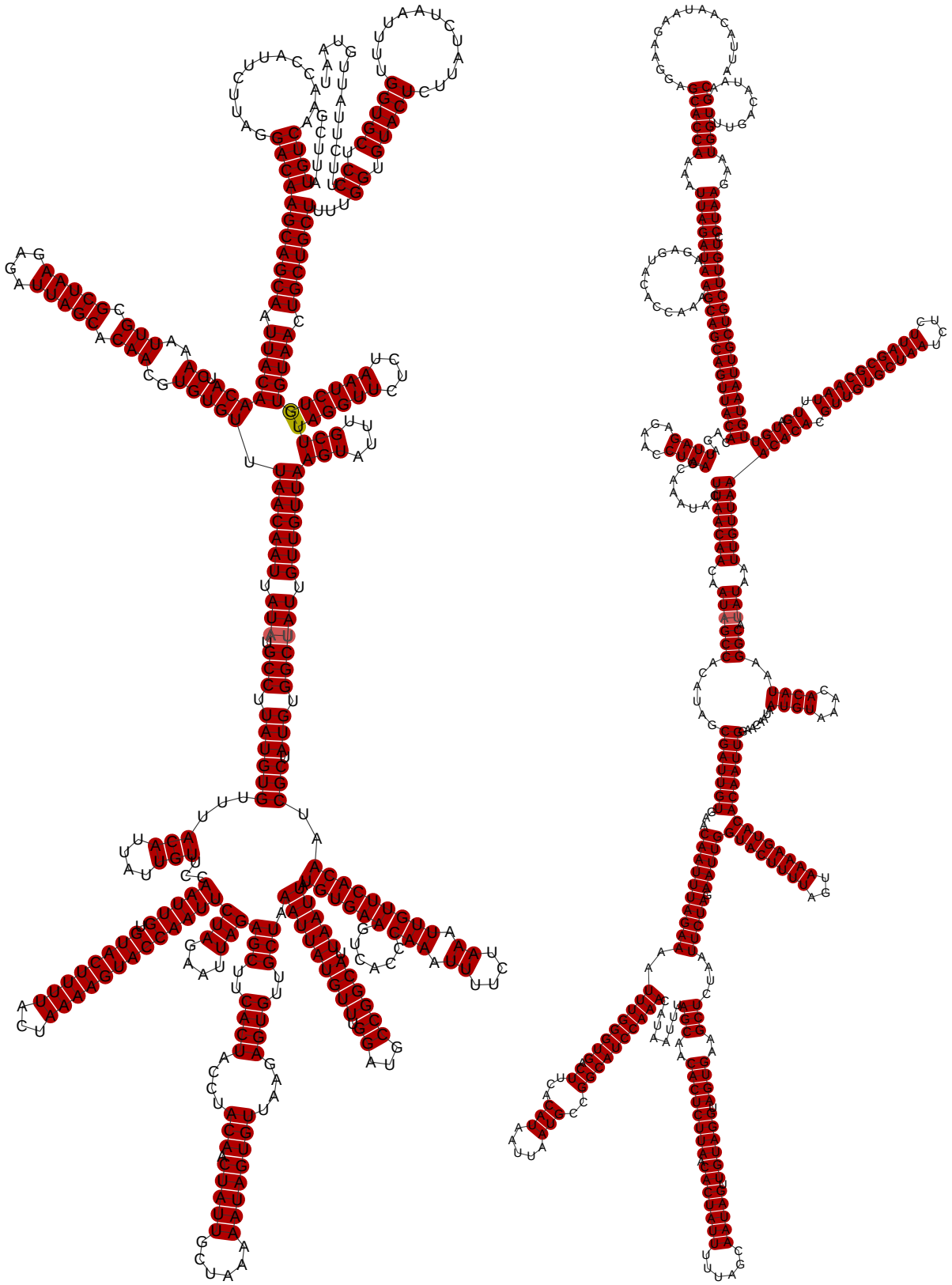

Fig. S84. Output of RNAalifold analysis of the conserved region found 1st in analysis of the nsp3 region of SARS-CoV, AY274119.3 nucleotide location 6613–6987 (left: forward sense; right: reverse complement). All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

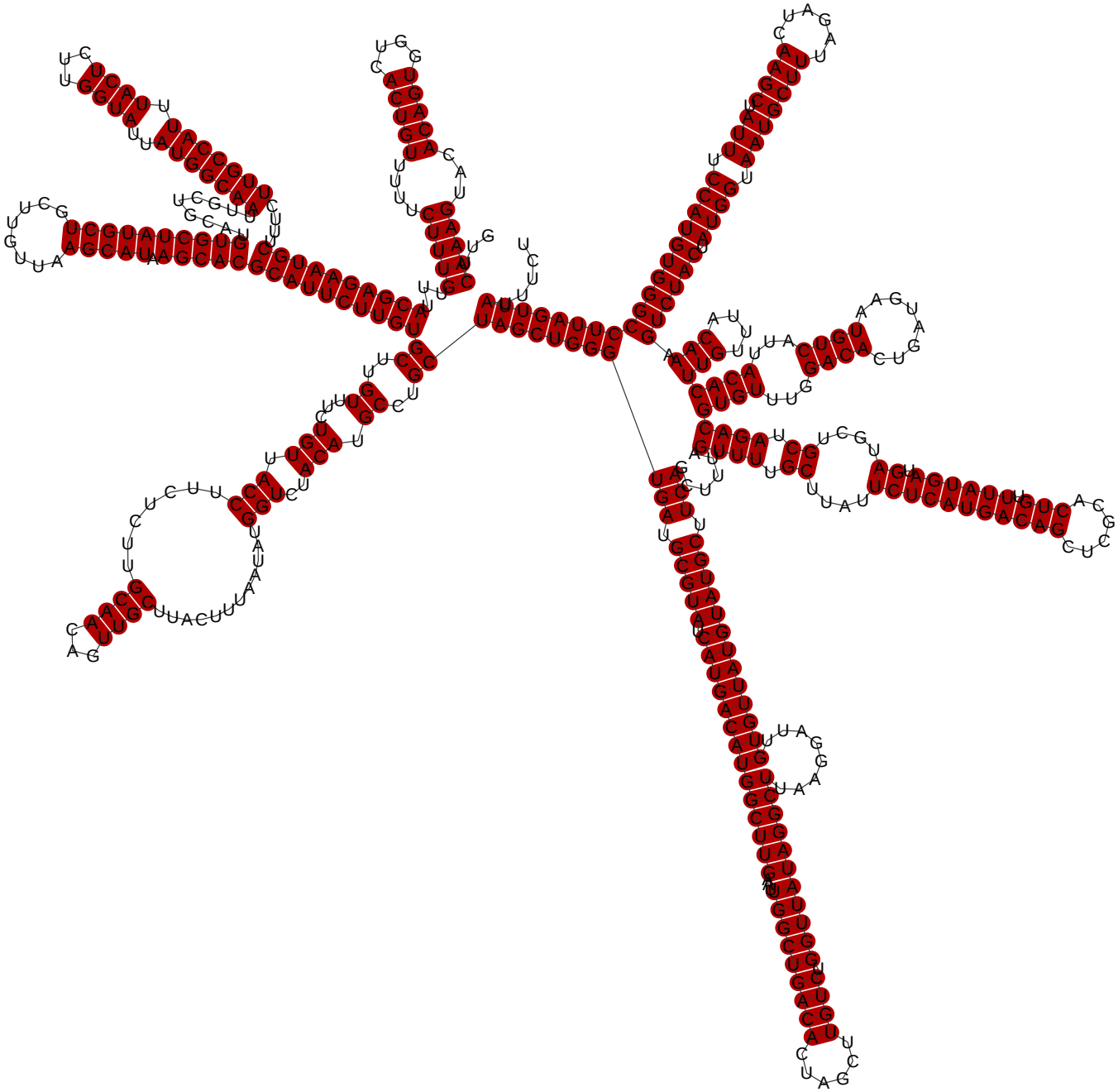

Fig. S85. Output of RNAalifold analysis of the conserved region found 1st in analysis of the nsp6 region of SARS-CoV, AY274119.3 nucleotide location 10978–11412. All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

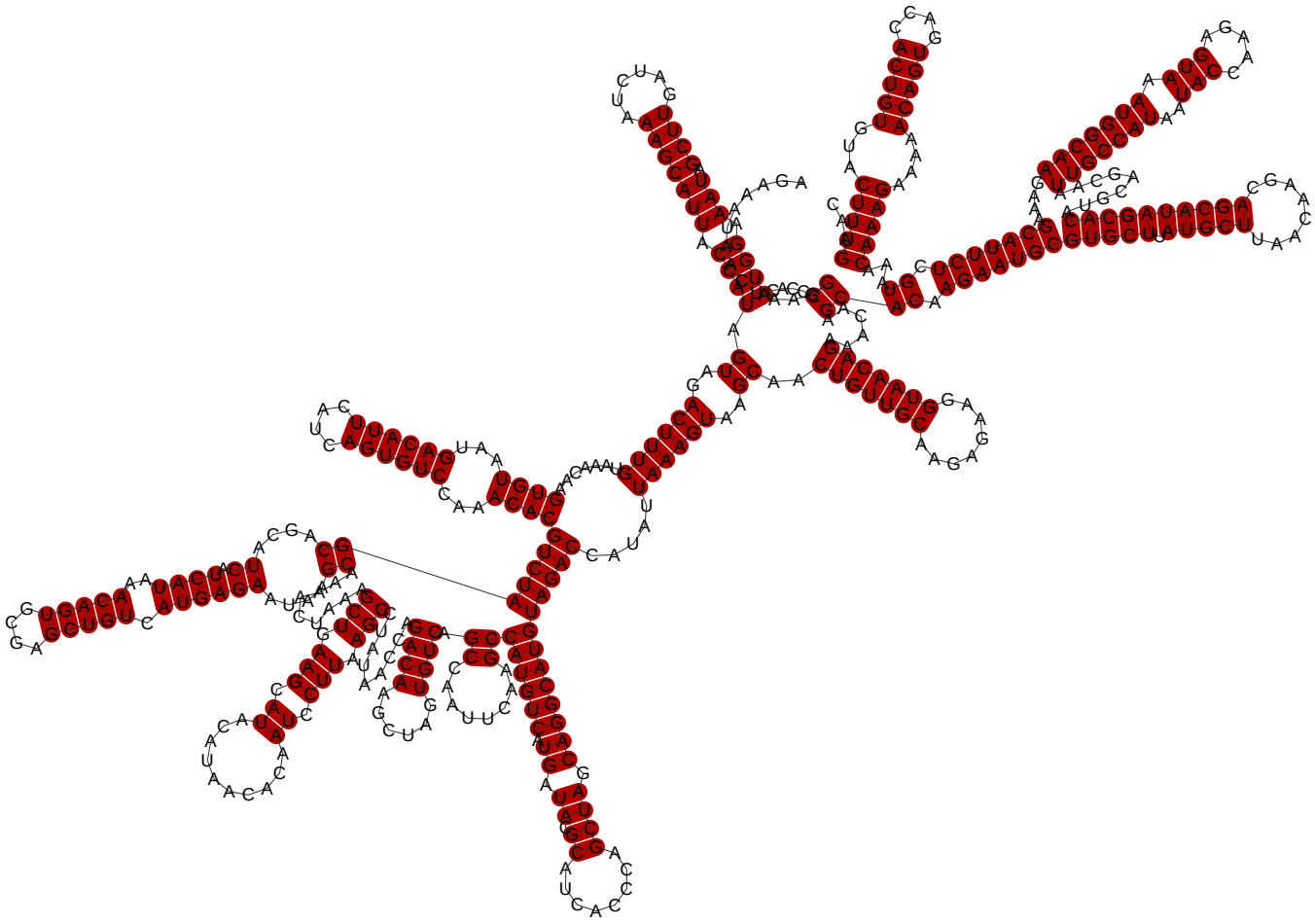

Fig. S86. Output of RNAalifold analysis of the reverse complement of the conserved region found 1st in analysis of the nsp6 region of SARS-CoV, AY274119.3 nucleotide location 10978–11412. All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

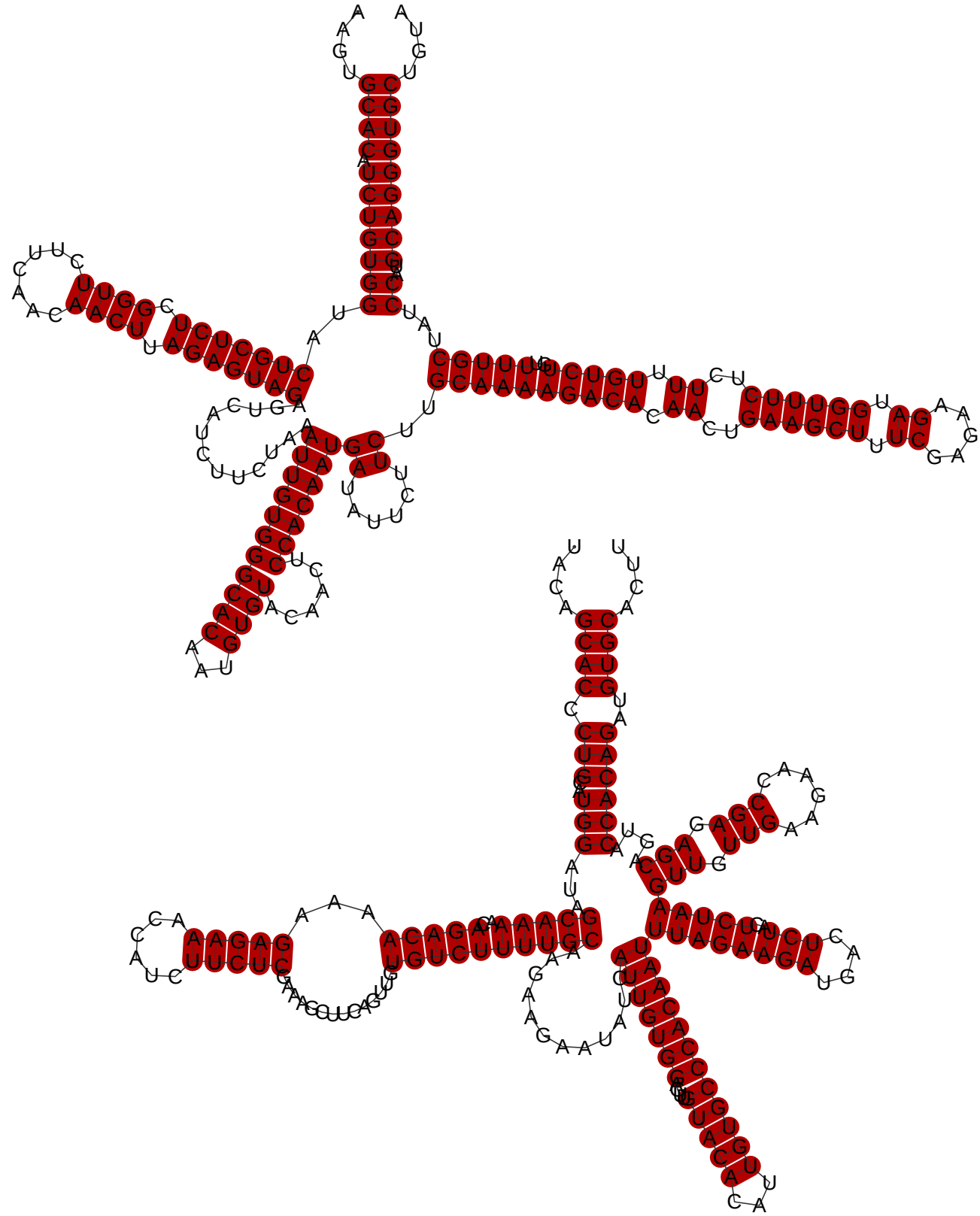

Fig. S87. Output of RNAalifold analysis of the conserved region found 1st in analysis of the nsp7 region of SARS-CoV, AY274119.3 nucleotide location 11791–11970 (top: forward sense; bottom: reverse complement). All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

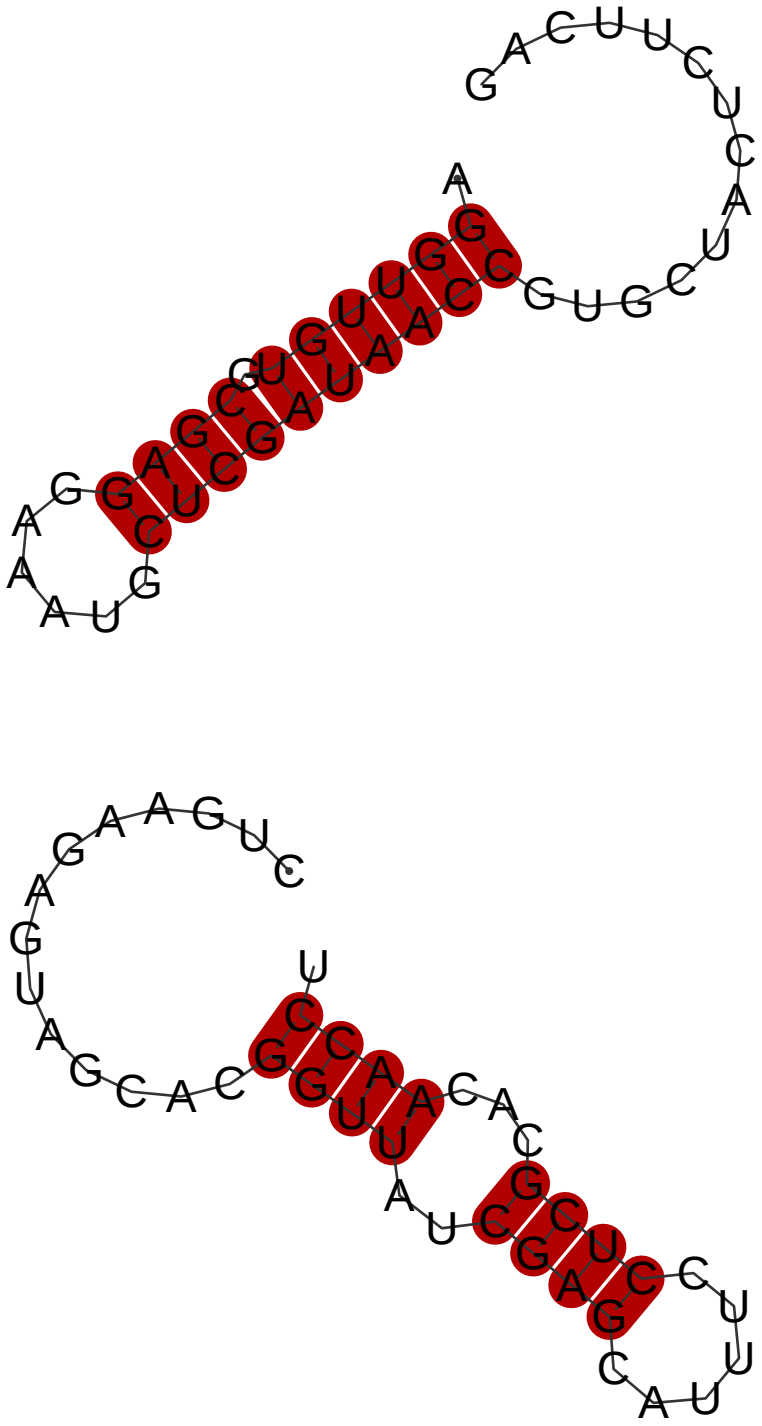

Fig. S88. Output of RNAalifold analysis of the conserved region found 2nd in analysis of the nsp7 region of SARS-CoV, AY274119.3 nucleotide location 11977–12021 (top: forward sense; bottom: reverse complement). All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

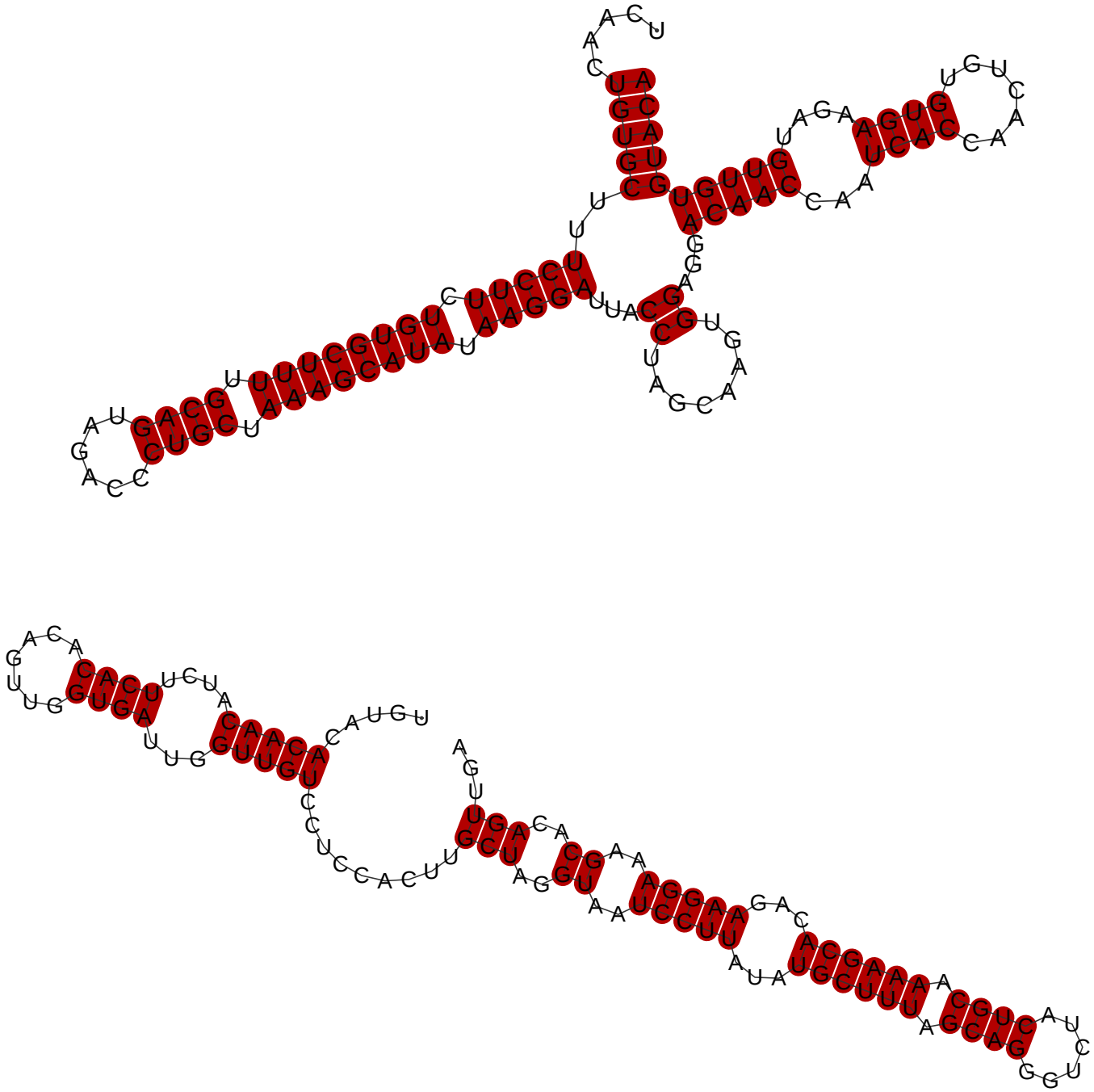

Fig. S89. Output of RNAalifold analysis of the conserved region found 2nd in analysis of the nsp10 region of SARS-CoV, AY274119.3 nucleotide location 12985–13095 (top: forward sense; bottom: reverse complement). All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

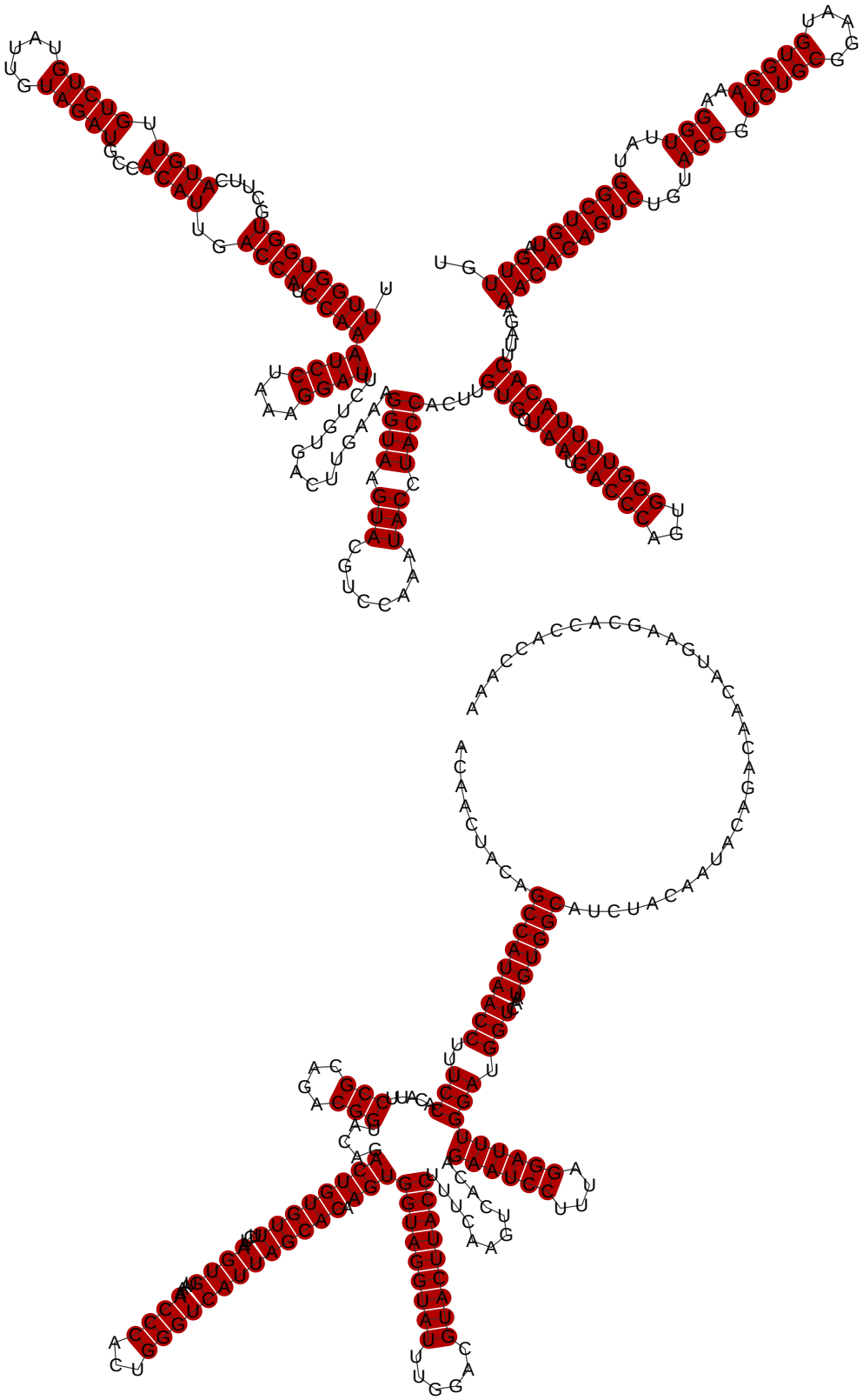

Fig. S90. Output of RNAalifold analysis of the conserved region found 1st in analysis of the nsp10 region of SARS-CoV, AY274119.3 nucleotide location 13153–13344 (top: forward sense; bottom: reverse complement). All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

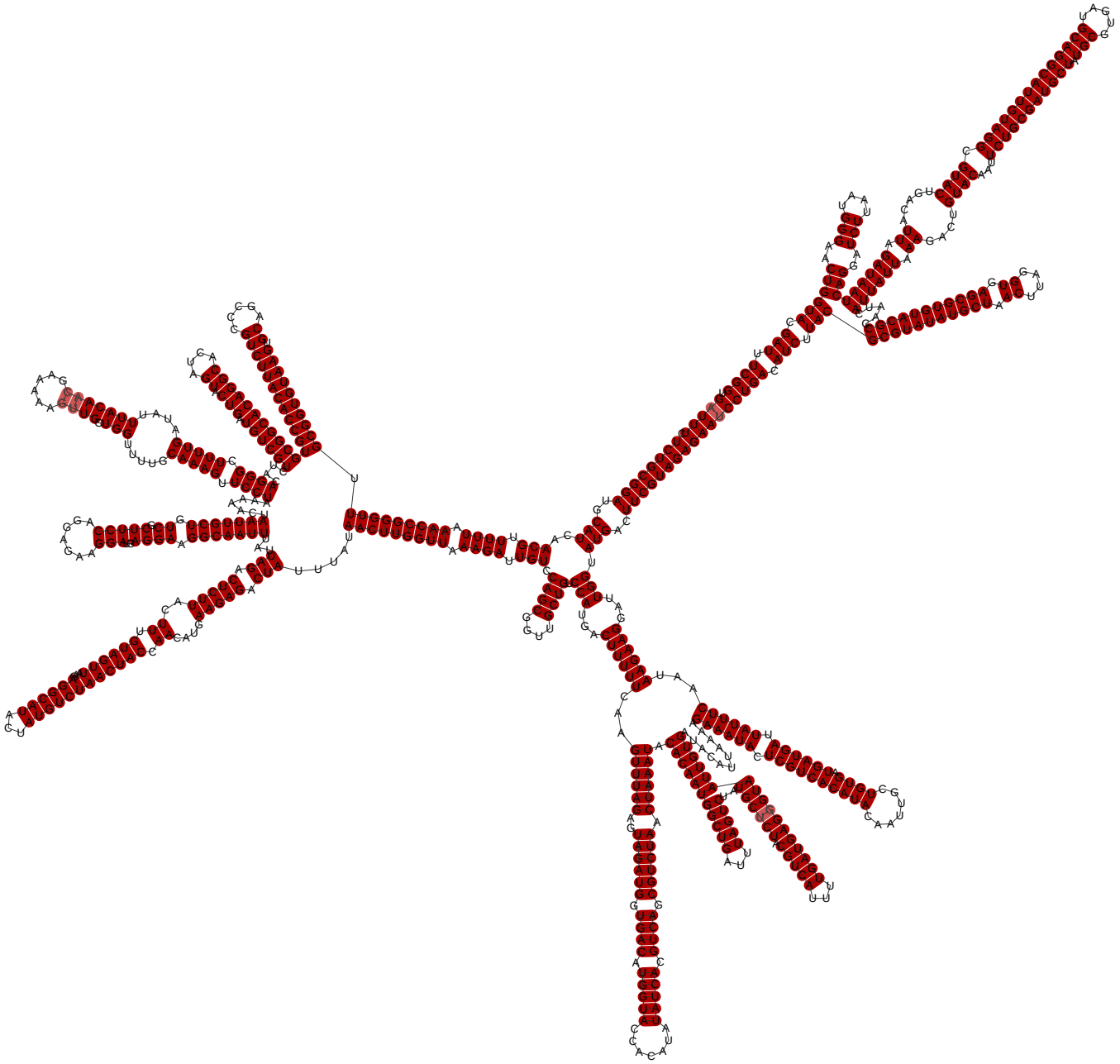

Fig. S91. Output of RNAalifold analysis of the conserved region found 2nd in analysis of the nsp12 region of SARS-CoV, AY274119.3 nucleotide location 13372–14037. All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

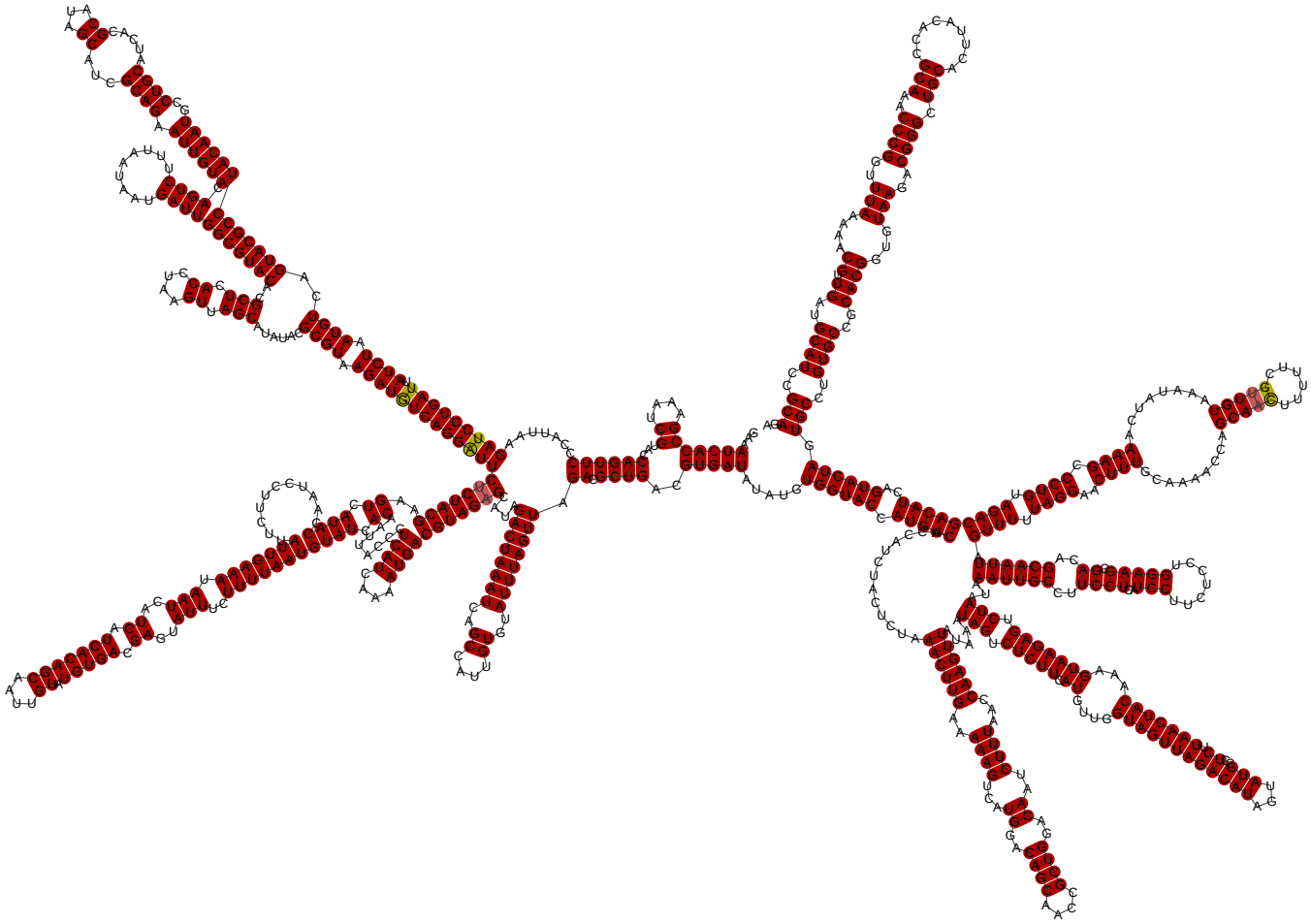

Fig. S92. Output of RNAalifold analysis of the reverse complement of the conserved region found 2nd in analysis of the nsp12 region of SARS-CoV, AY274119.3 nucleotide location 13372–14037. All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

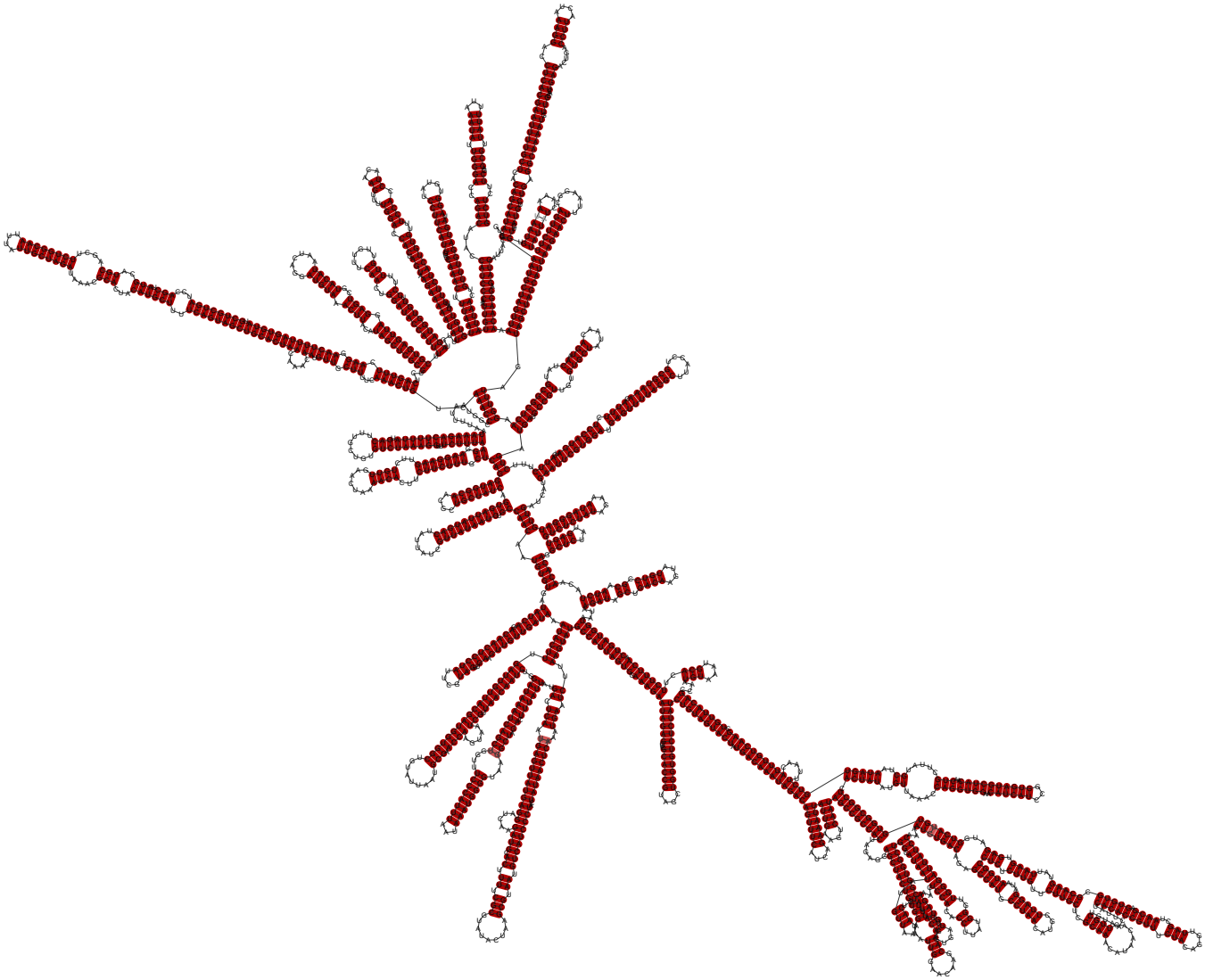

Fig. S93. Output of RNAalifold analysis of the conserved region found 1st in analysis of the nsp12 region of SARS-CoV, AY274119.3 nucleotide location 14208–15852. All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

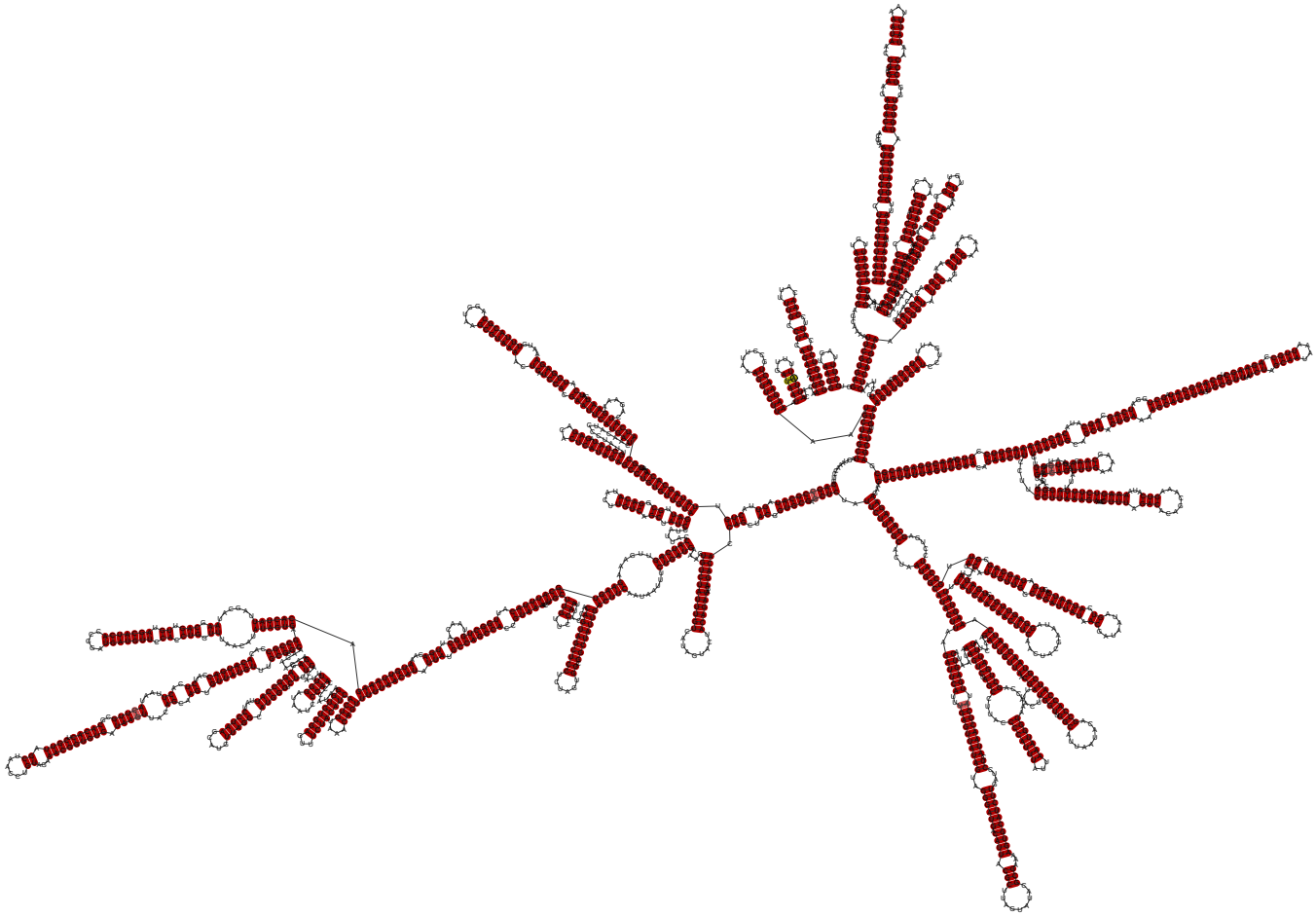

Fig. S94. Output of RNAalifold analysis of the reverse complement of the conserved region found 1st in analysis of the nsp12 region of SARS-CoV, AY274119.3 nucleotide location 14208–15852. All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

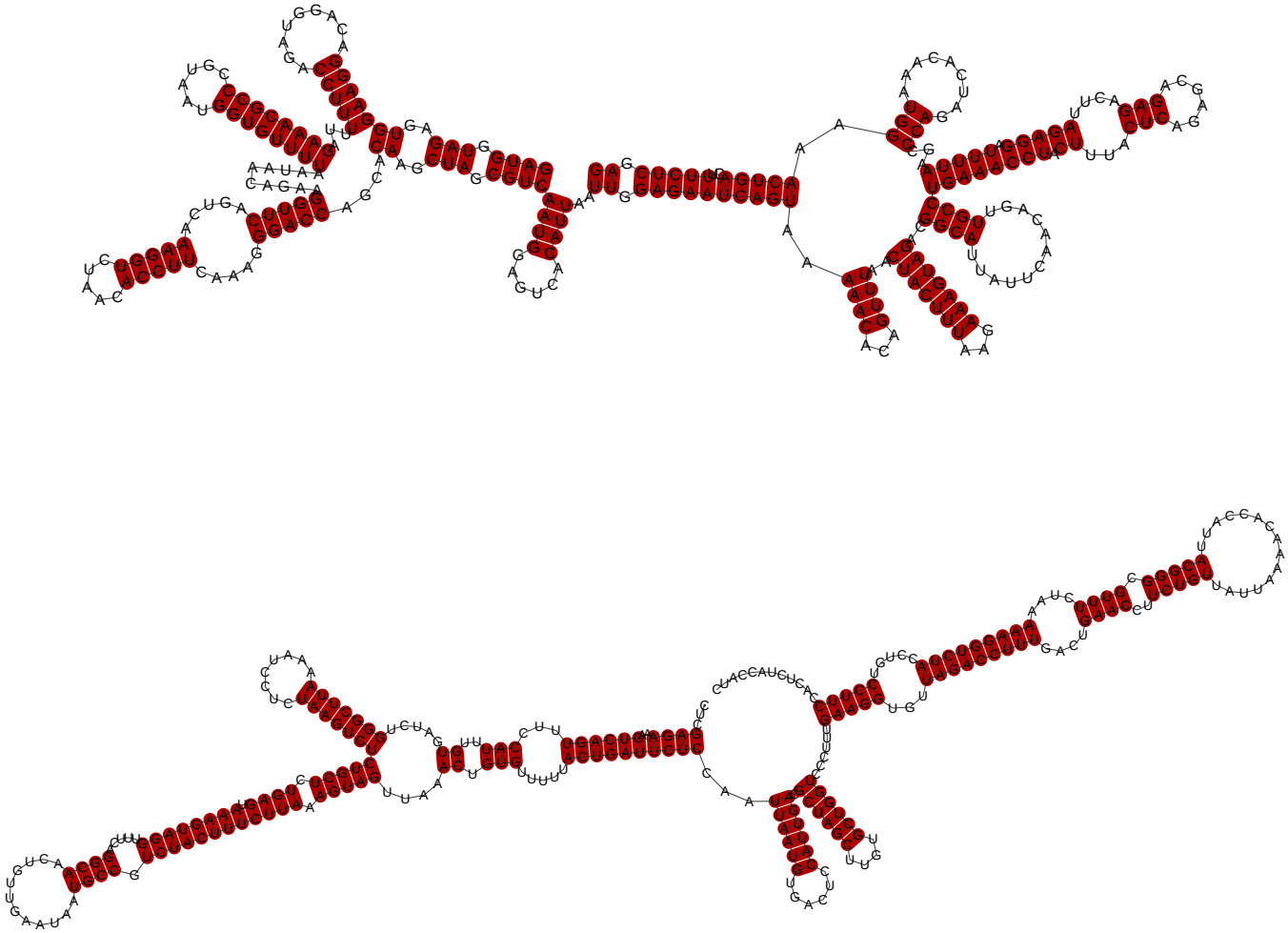

Fig. S95. Output of RNAalifold analysis of the conserved region found 1st in analysis of the nsp15 region of SARS-CoV, AY274119.3 nucleotide location 19920–20195 (top: forward sense; bottom: reverse complement). All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.

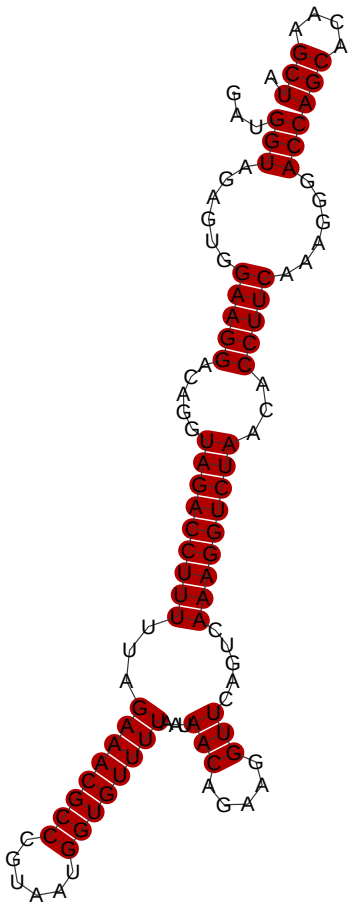

Fig. S96. Output of RNAalifold analysis if a fold is attempted if a positive-sense truncation of the conserved region of Fig. S95, AY274119.3 nucleotide location 19920–20031, is attempted. The central stem-loop at the left of the upper image in the preceding figure is maintained, but the two predicted stem-loops adjacent to it are lost. The change in prediction reflects both low confidence in the exact identification of the appropriate region to fold, because of lower granularity in determining the boundaries of conserved regions when there are few sequences to analyse, and the folding algorithm not being able to use data on covarying base pairs to make better predictions, because no covarying base pairs are seen in the alignment of the region. All sequences used in conservation analysis of the region are included in the RNAalifold analysis (differently from the SARS-CoV-2 RNAalifold analysis, because of the much smaller number of SARS-CoV sequences). Base pairs are highlighted in deep/mid/light red as all/all but one/all but two sequences are capable of forming the pairs shown. Base pairs are highlighted in deep/mid/light yellow as all/all but one/all but two sequences are capable of forming the pair shown or one other base pair (including GU pairs). Base pairs are highlighted in deep/mid/light green as all/all but one/all but two sequences are capable of forming the pair shown or either of another two base pairs (including GU pairs). RNAalifold was used with input options disallowing lonely pairs, allowing G-quadruplexes, and with the ribosum scoring matrix enabled.