Supplementary Material

Molecular modelling of capsid protein substitutions in JE-X/5'CprME(S) and JE-X/prME(S) viruses

The serine substitutions at positions 40 and 66 in the capsid protein, which differentiate the JE-X/prME(S) and JE-X/5'CprME(S) viruses, were analyzed using a homology model derived from the solution structure for the dengue-2 capsid protein (Ma et al., 2004). As shown in Figure 5 substitutions at these sites within each capsid monomer flank the central conserved hydrophobic region (residues 46-62 for JE virus), which has been proposed as the site of interaction of the capsid with intracellular membranes during the formation of the flavivirus virion (Ma et al., 2004). Figure S1 illustrates the position of substitution 40 in the monomer with the side chain amino acids important for hydrogen bonding around this position (see also Figure S2). Position 66 is not shown for sake of clarity. The alpha 1, 2, 3, and 4 helices are shown in blue, green, yellow, and magenta respectively. For the JE capsids, position 40 lies within the laterally exposed loop connecting the α1 and α2 helices, whereas position 66 lies within the α3 helix itself (Figure S3). In addition to the proximity of these positions to the conserved hydrophobic region, residue 66 clusters with several adjacent residues (positions 65, 68 and 69), whose side chains contribute to the hydrophobic interior of the 3-helix core of the capsid monomer. The strictly conserved tryptophan residue at position 69 is also implicated as a critical contact with the α4' segment of the opposite capsid monomer, and is thus believed to be important for formation of capsid dimers.

Predicted effects of the substitutions at positions 40 and 66 with respect to local structure were made based on the most favorable energies for the available rotamer configurations generated by the Swissmodel platform tool. Figure S2 illustrates the models for the substitution at position 40. Top panel shows the dengue-2 capsid. Middle panel shows the capsid for JE-X/5'CprME(S), and bottom panel shows the capsid for JE-X/prME(S). For the dengue structure, the white arrow identifies hydrogen bond between the backbone oxygen of methionine 37 (side chain not shown) and the backbone nitrogen of glycine 40. For the JE structures, the white arrows identifies a hydrogen bond between the backbone oxygen of leucine 37 and the backbone nitrogen of serine 40 (JE-X/5'CprME(S) or glycine 40 (JE-X/prME(S). For the JE capsids, orange arrow identifies hydrogen bond between the backbone oxygen of leucine 37 and the backbone oxygen of aspartic acid 39. The serine residue in JE-X/5'CprME(S) is predicted to engage in hydrogen bonding with the backbone oxygen of aspartic acid at position 39, whereas this bond is not predicted in the case of the JE-X/prME(S) capsid. The impact of the serine substitution in the JE-X/5'CprME(S) capsid is predicted to include an increased polar effect on the α1 and α2 interhelical loop relative to the native glycine residue, due to a hydroxyl moiety in the serine and the potential stabilization of the aspartic acid residue at the apex of the loop. These factors may detract from the hydrophobic character of the adjacent conserved region formed by residues 46-62 which are proposed to mediate interaction of the capsid with the membrane surface.

Figure S3 compares the models for substitution at position 66. For clarity, most side chains are not shown. Amino acid 65 is isoleucine in the dengue 2 capsid and leucine in

the JE capsids. Top panel shows the dengue-2 capsid. Middle panel shows the JE-X/5'CprME)S) capsid, and bottom panel shows that for JE-X/prME(S). All hydrogen bonds are between backbone nitrogen and oxygens, except for those of the serine 66 side chain for the JE-X/5'CprME(S) capsid. In the case of the dengue-2 and JE-X/prME(S) capsid, the side chains of leucine 66 and proline 61 likely interact hydrophobically. This interaction is not predicted in the case of the JE-X/5'CprME(S) capsid, where the serine residue is instead accommodated within the helical segment and forms hydrogen bonds between the backbone oxygen of lysine 63 and nitrogen of glycine 67. The impact of the serine substitution in the JE-X/5'CprME(S) capsid is predicted to include a decreased hydrophobic character and less stabilization of the α3 helix segment relative to a leucine residue at the same position as found in the dengue-2 and JE-X/prME(S) structures.

In the context of the overall model for the structure of the JE virus capsid, the two serine substitutions found at positions 40 and 66 of the JE-X/5'CprME(S) virus capsid are predicted to have deleterious effects on the interhelical loop and the α3 helix, largely through loss of hydrophobic character that is believed to be important for membrane association of the capsid, and formation of the monomer core of this protein.

Additional nucleotide sequence analysis of intertypic JE viruses

Given the fact that unexpected nucleotide an amino acid substitutions were observed in the 5' UTR and the structural protein regions of the intertypic viruses, the possibility exists that incompatibility between the JE SA14-14-2 structural proteins and the nonstructural and 3' UTR of the JE Nakayama virus could drive multiple additional

mutations that could affect the virulence properties of the two viruses. To evaluate this possibility, a region of approximatelty 2500 base pairs from within the carboxy-terminus of NS5 through the 3' terminus of the genomes of the two intertypic viruses was subjected to nucleotide sequence analysis. For JE-X/5'CprME(S) virus, 3 silent nucleotide substitutions were detected (nucleotides 9704, 9719, and 9905), and a mixture of two bases was detected at position 9855, resulting in a combination of glycine and arginine at this position. No substitutions were found in the 3' UTR. For the JE-X/prME(S) virus, the same substitutions at nucleotides 9704 and 9719 were found, Only one additional substitution, at position 10,249, encoding a methionine instead of threonine was detected. No substitution was detected in the 3' UTR. Although the remainder of the genomes of these two viruses has not been characterized, these results suggest that there is not a high level of genetic instability as a result of combining the JE-SA14-14-2 and JE Nakayama virus genomes in the context of an intertypic virus.

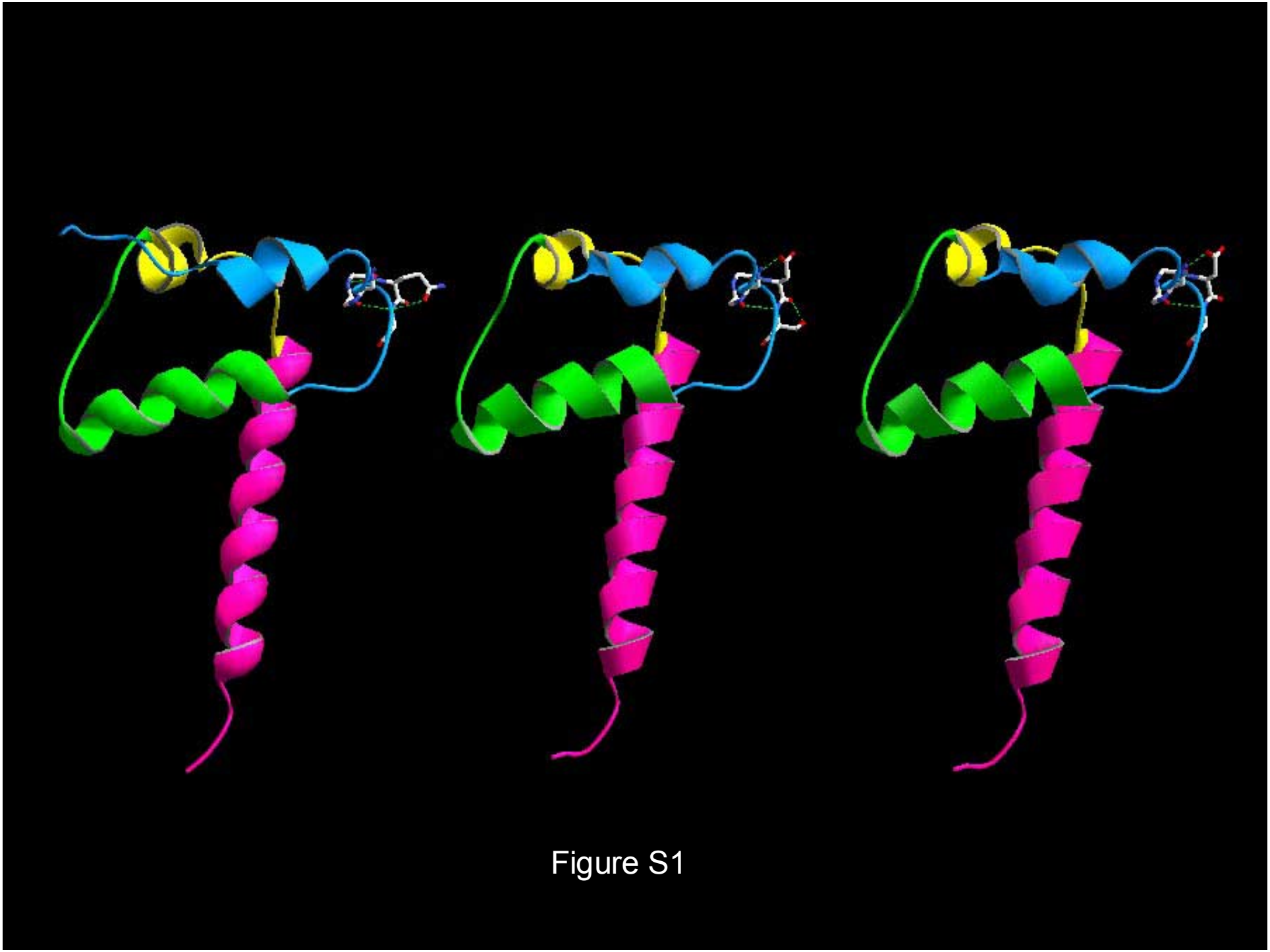


Figure S1

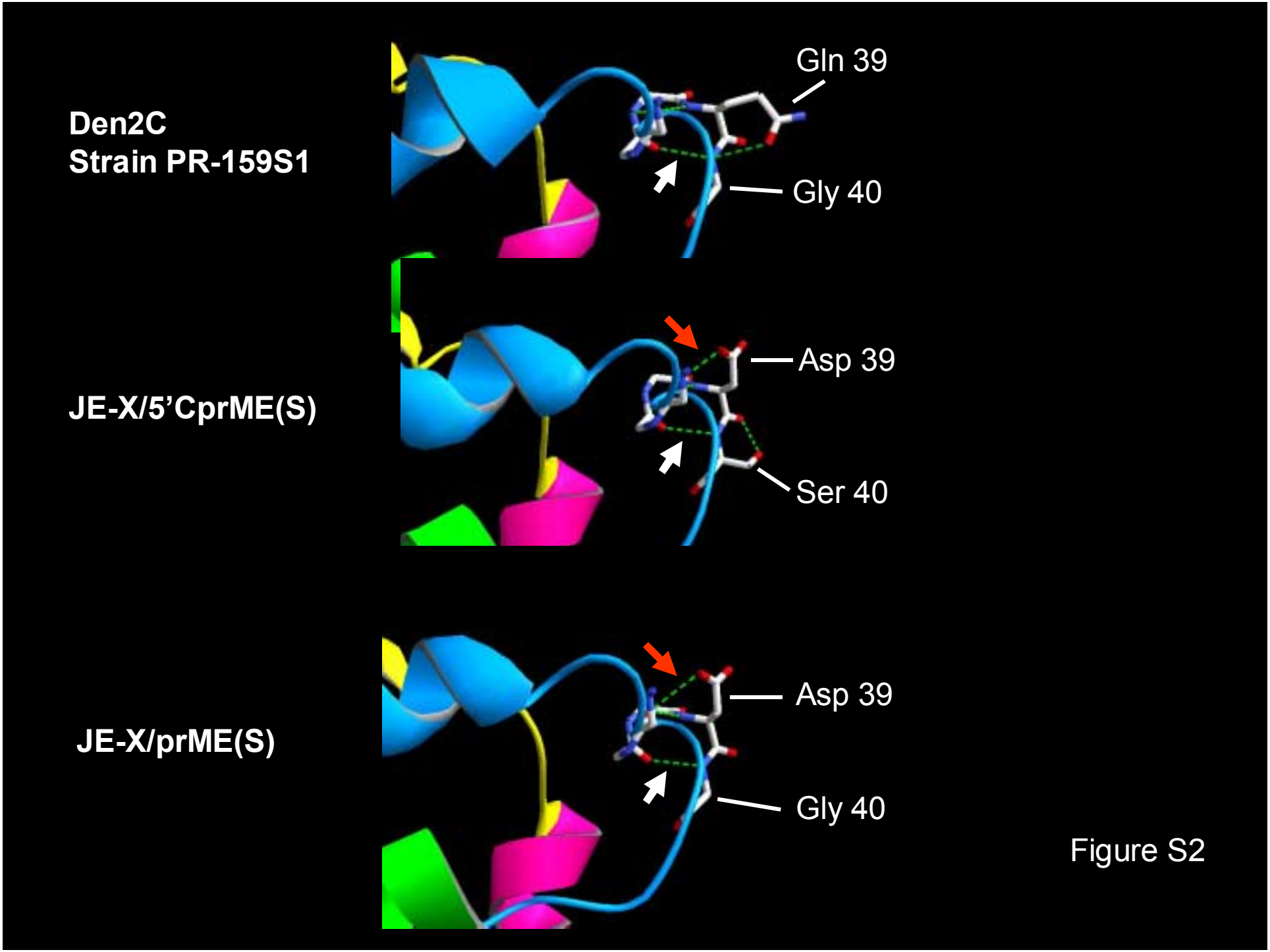
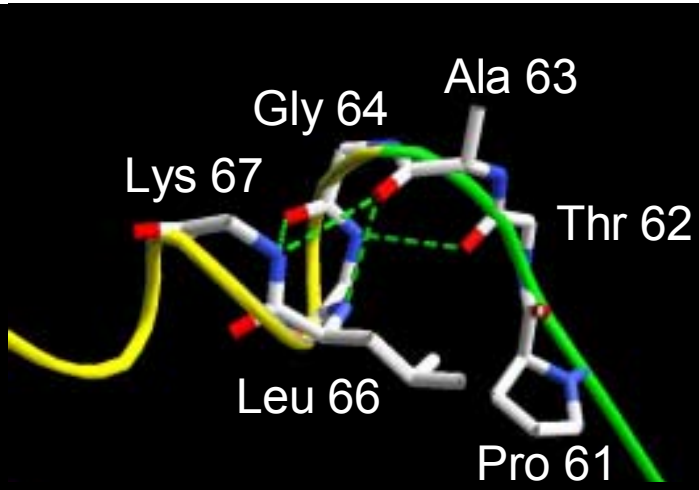
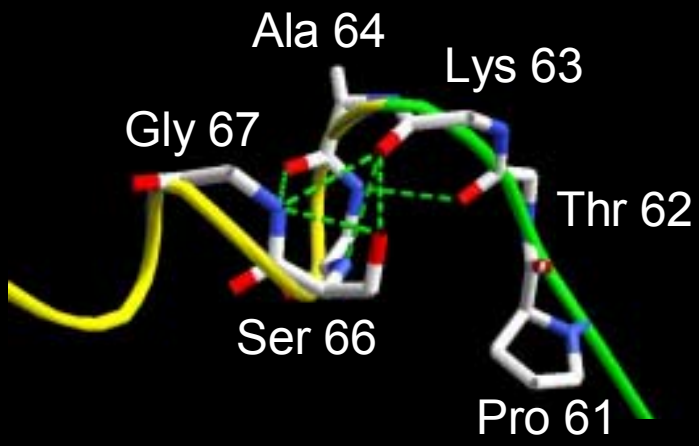

Den2C
Strain PR-159S1
Gln 39
Gly 40
JE-X/5'CprME(S)
Asp 39
Ser 40
JE-X/prME(S)
Asp 39
Gly 40

Figure S2

Den2C
Strain PR-159S1



JE-X/5'CprME(S)



JE-X/prME(S)

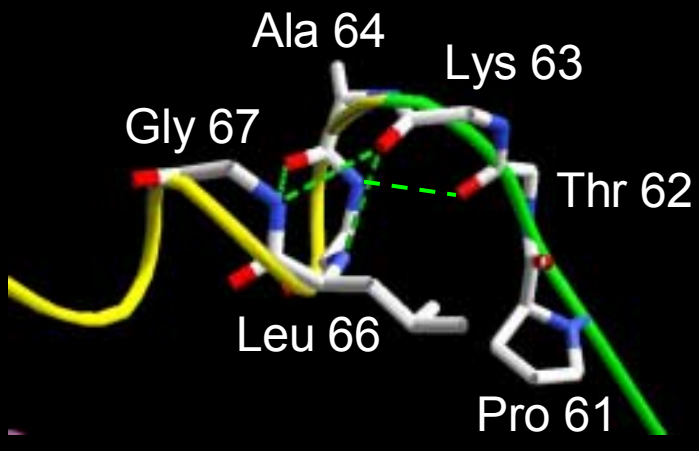


Figure S3