File S1

Supporting Information

General strategy to create mutation in ORF of *CSU53*, *ASU53*, and *ASU51*.

Mutations of *CSU53* ORF were generated within an entire region 135, which is carried on plasmid pCA135. Mutations of *ASU53* ORF or *ASU51* ORF were generated within, respectively, the portion pEA104 from region 135, Figure 3B, or the portion pEA243 from region A, Figure 6. For the plasmids and their inserts, see also Table S1. Region 135, the portion pEA104 from region 135 or the portion pEA243 from region A were PCR amplified and each amplicon was individually sub-cloned in the plasmid pUC19 or pCR-Blunt (Invitrogen Corp., Carlsbad, CA). See Table S2 for the primers. The resulting plasmids were used as templates to create different frame-shift or stop-codon mutations with Stratagene's QuickChange Site Directed Mutatagenesis Kit (La Jolla, CA) according to the manufacturer recommendations. Mutations were confirmed by sequencing and corresponding portions finally sub-cloned into the low copy number replicative plasmid pCA88 for further examinations.

Frame-shift mutations in *CSU53* ORF. Plasmids pEA201, pEA158, and pEA227.

Plasmid pEA227 carries the mutation, which was created by two adding an extra G between +65 and +66 with primers CSU53-1MF and CSU53-1MR, respectively. Plasmid pEA201 carries the mutation, which was created by adding two extra G between +232 and +233 with primers CSU53F and CSU53R. Finally, plasmid pEA158 carries the mutation, which was created by replacing T of +112 by G, as well as by introducing an extra G between +111 and +112 with primers AF52 and AF53.

Frame-shift mutation in *ASU53* ORF. Plasmid pEA232.

A frame-shift mutation was created in *ASU53* ORF by adding one extra C between +3 and +4 and another extra C between +6 and +7 using primers ASU53-FM1 and ASU53-FM2, thus, resulting in plasmid pEA232.

Stop-codon mutation in *ASU53* ORF. Plasmid pEA205.

A stop-codon was created in *ASU53* ORF by replacing the T with A at position +12 using primers ASU53-MF and ASU53-MR, thus, resulting in plasmid pEA205.

Frame-shift mutation in *ASU51* ORF. Plasmid pEA243.

A frame-shift mutation was created in *ASU51* ORF by adding an extra T and an extra G between +38 and +39 using primers A51AGE1F and A51AGE1R, thus, resulting in plasmid pEA243.

Opposite orientation of *ASU51*. Plasmids pEA209 and pEA236.

A PCR product of approximately 400 bp was amplified from the plasmid pAK65 in two ways with two pairs of primers. The pair of primers AF217 and AF224 contained, respectively, a *Bam*H1 and a *Pst*1 restriction site in their sequences. The amplicon was sub-cloned into pCA88, which was digested with *Bam*H1 and *Pst*1, resulting in plasmid pEA209 (←*ASU51*, ←*SOU1*). Similarly, the pair of primers AF228 and AF229 containing, respectively, a *Pst*1and a *Bam*H1 restriction site produced an amplicon, which, upon sub-cloning into pCA88, resulted in plasmid pEA236 (→*ASU51*, ←*SOU1*). See Table S2 for the primers and plasmids.

Opposite orientation of *ASU53*. Plasmids pEA104 and pEA234.

A PCR product of approximately 2.1 kb was amplified from the plasmid pCA135 using two pairs of primers AF29 and AF30, as well as AF226 and AF227. Similarly to above, the presence of a *Bam*H1 and *Pst*1 restriction site in the primer sequences determined the amplicon orientation in plasmid pEA104 (←*ASU51*, ←*SOU1*), as well as plasmid pEA236 (→*ASU53*, ←*SOU1*). See Table S2 for the primers and plasmids.

***CSU51* and *ASU51* combined together on the plasmid pEA238.**

A PCR product of approximately 400 bp encompassing *ASU51* ORF, as well as 256 bp of upstream region and 44 bp of downstream region, was amplified from the plasmid pAK65 by using primers AF217 and AF229 as *Bam*H1 – *Bam*H1 fragment and sub-cloned into pAK65 at *Bam*H1 site as *Bam*H1 – *Bam*H1fragment. This insert is similar to the insert of pEA209, Figure 6 thus, resulting in plasmid pEA238.

***CSU53* and *ASU53* combined together on the plasmid pEA162.**

A PCR product of approximately 1.7 kb portion of region 135 encompassing *ASU53* ORF with 1505 bp of 5'-end and 102 bp of 3'-end was PCR amplified from the plasmid pCA135 with primers AF128 and AF129 as *Bam*H1 – *Bam*H1 and sub-cloned into pCA88 at the site *Bam*H1 as *Bam*H1 – *Bam*H1, thus, creating plasmid pEA160. A PCR product of approximately 2.5kb portion of region 135 encompassing *CSU53* ORF with 1400 bp of 5'-end and 100 bp of 3'-end and also having *ASU53* in natural configuration, was similarly amplified with primers AF32 and AF134 as *Pst*1 – *Pst*1 fragment and sub-cloned into pEA160 at the *Pst*1 site as *Pst*1 – *Pst*1, thus, creating plasmid pEA162.