Table S3. Qβ point mutants obtained by site-directed mutagenesis. For each mutant, the genomic position of the nucleotide substitution, the mutated gene, the amino acid change, and the relative fitness effect ± SEM are shown. Assays were done in triplicate and fitness values were corrected to account for the presence of additional mutations and other sources of experimental error (see Methods). Notice that some mutations fall within regions with overlapping genes (the respective amino acid substitutions are indicated in these cases).

Mutation	Gene	Amino acid substitution	Relative fitness effect
A161C [1]	Maturation	Thr35Pro	-0.007 ± 0.007
A328G	Maturation	None	0.003 ± 0.029
C665G	Maturation	Leu203Val	-0.440 ± 0.016
G733U	Maturation	Try225Cys	-1 (lethal)
U772G	Maturation	Phe238Leu	-0.365 ± 0.078
A891U	Maturation	Tyr278Phe	-0.095 ± 0.056
C939G	Maturation	Pro294Arg	-0.072 ± 0.023
C1096U	Maturation	None	-0.026 ± 0.045
G1149C	Maturation	Arg364Pro	-1 (lethal)
U1165A	Maturation	None	-0.039 ± 0.017
G1283U	Maturation	Asp409Tyr	-1 (lethal)
U1288G	Maturation	Ser410Arg	-1 (lethal)
G1329A	Intergenic	Intergenic	-1 (lethal)
C1442A	Read-through/ Coat	Ala34Asp/Ala34Asp	-1 (lethal)
U1485A	Read-through/ Coat	None/none	-0.043 ± 0.052
A1525U	Read-through/Coat	Asn62Tyr /Asn62Tyr	0.035 ± 0.027
U1809C	Read-through	None	-0.027 ± 0.061
C1814U	Read-through	Pro158Leu	-0.014 ± 0.041
G1895U	Read-through	Arg185Leu	-0.059 ± 0.012
A2090U	Read-through	Gln250Leu	-0.230 ± 0.011
C2360G	Replicase	Thr4Arg	-1 (lethal)
U2379A	Replicase	None	-1 (lethal)
G2787U	Replicase	Glu146Asp	-0.098 ± 0.010
G2793U	Replicase	Met148Ile	-0.093 ± 0.031
C2797A	Replicase	Arg150Ser	-0.081 ± 0.011
U3027C	Replicase	None	0.025 ± 0.032
U3075C	Replicase	None	0.000 ± 0.044
A3094G	Replicase	Ile249Val	0.007 ± 0.003
U3101G	Replicase	Leu251Arg	-1 (lethal)
U3210G [2]	Replicase	Cys287Try	-1 (lethal)
G3213U	Replicase	Glu288Asp	-0.047 ± 0.038
G3397C	Replicase	Asp350His	-0.260 ± 0.034
G3415U	Replicase	Val356Phe	-1 (lethal)
U3452C	Replicase	Val368Ala	-0.329 ± 0.019
U3483G	Replicase	None	0.016 ± 0.014
G3661U	Replicase	Val438Leu	-0.223 ± 0.02
U3686A	Replicase	Val446Glu	-1 (lethal)
G3815C	Replicase	Arg489Pro	0.032 ± 0.041
G3916C	Replicase	Asp523His	-0.228 ± 0.038
U3951G	Replicase	Asp534Glu	-0.004 ± 0.041
G4066U	Replicase	Ala573Ser	-0.069 ± 0.091
U4082A [1]	Replicase	Phe578Tyr	-0.352 ± 0.019

[1] The presence of the mutation could not be confirmed by sequencing.

[2] Despite the mutation was classified as lethal (Fig. S1A), mutant viruses with apparently neutral fitness were successfully recovered from a single plaque. A likely explanation is that the mutation was quasi-lethal and had partially reverted or been compensated by a secondary mutation in the isolated plaque.