

Figure S1. Alignment of the DNA sequences of Q and Q^{ε5} alleles. The miRNA172-binding site is boxed with black lines, and the SNP flanking the miRNA172-binding site is indicated with black arrow.

Q	ATGGTGTGGATCTCAATGTGGAGTCGCCGCGGACTCGGGACATGCCAGTCTCCGTGCTCAACTCCGCGGACGCCGGTGGCGGCGCTTCGGGTTTCGGCTGCTCGGGAGCC	115
Q ¹	ATGGTGTGGATCTCAATGTGGAGTCGCCGCGGACTCGGGACATGCCAGTCTCCGTGCTCAACTCCGCGGACGCCGGTGGCGGCGCTTCGGGTTTCGGCTGCTCGGGAGCC	115
Q ²	ATGGTGTGGATCTCAATGTGGAGTCGCCGCGGACTCGGGACATGCCAGTCTCCGTGCTCAACTCCGCGGACGCCGGTGGCGGCGCTTCGGGTTTCGGCTGCTCGGGAGCC	115
Q ³	ATGGTGTGGATCTCAATGTGGAGTCGCCGCGGACTCGGGACATGCCAGTCTCCGTGCTCAACTCCGCGGACGCCGGTGGCGGCGCTTCGGGTTTCGGCTGCTCGGGAGCC	115
Q ⁴	ATGGTGTGGATCTCAATGTGGAGTCGCCGCGGACTCGGGACATGCCAGTCTCCGTGCTCAACTCCGCGGACGCCGGTGGCGGCGCTTCGGGTTTCGGCTGCTCGGGAGCC	115
Q ⁵	ATGGTGTGGATCTCAATGTGGAGTCGCCGCGGACTCGGGACATGCCAGTCTCCGTGCTCAACTCCGCGGACGCCGGTGGCGGCGCTTCGGGTTTCGGCTGCTCGGGAGCC	115
Q	CTGATGATGACGACTCTCCGCGGAGCCGCGCGGCTCGGGCCGGGTTGCTCAGGAGGAGCTCTTCCCGCGCTCGCGCGCGGACGCGGGGCGCGCGGGGTTGACATGGG	230
Q ¹	CTGATGATGACGACTCTCCGCGGAGCCGCGCGGCTCGGGCCGGGTTGCTCAGGAGGAGCTCTTCCCGCGCTCGCGCGCGGACGCGGGGCGCGCGGGGTTGACATGGG	230
Q ²	CTGATGATGACGACTCTCCGCGGAGCCGCGCGGCTCGGGCCGGGTTGCTCAGGAGGAGCTCTTCCCGCGCTCGCGCGCGGACGCGGGGCGCGCGGGGTTGACATGGG	230
Q ³	CTGATGATGACGACTCTCCGCGGAGCCGCGCGGCTCGGGCCGGGTTGCTCAGGAGGAGCTCTTCCCGCGCTCGCGCGCGGACGCGGGGCGCGCGGGGTTGACATGGG	230
Q ⁴	CTGATGATGACGACTCTCCGCGGAGCCGCGCGGCTCGGGCCGGGTTGCTCAGGAGGAGCTCTTCCCGCGCTCGCGCGCGGACGCGGGGCGCGCGGGGTTGACATGGG	230
Q ⁵	CTGATGATGACGACTCTCCGCGGAGCCGCGCGGCTCGGGCCGGGTTGCTCAGGAGGAGCTCTTCCCGCGCTCGCGCGCGGACGCGGGGCGCGCGGGGTTGACATGGG	230
Q	SCAGCAGGCCCCGGCGCTGCGCCGATGCGGCCGCTGTGGCAGCGCGGCGCGCGGAGGAGTCTCTGCTGGCGCAGCGGATGGCGCCCGCGAAGAGACGCGCGGGGCGCGAGG	345
Q ¹	SCAGCAGGCCCCGGCGCTGCGCCGATGCGGCCGCTGTGGCAGCGCGGCGCGCGGAGGAGTCTCTGCTGGCGCAGCGGATGGCGCCCGCGAAGAGACGCGCGGGGCGCGAGG	345
Q ²	SCAGCAGGCCCCGGCGCTGCGCCGATGCGGCCGCTGTGGCAGCGCGGCGCGCGGAGGAGTCTCTGCTGGCGCAGCGGATGGCGCCCGCGAAGAGACGCGCGGGGCGCGAGG	345
Q ³	SCAGCAGGCCCCGGCGCTGCGCCGATGCGGCCGCTGTGGCAGCGCGGCGCGCGGAGGAGTCTCTGCTGGCGCAGCGGATGGCGCCCGCGAAGAGACGCGCGGGGCGCGAGG	345
Q ⁴	SCAGCAGGCCCCGGCGCTGCGCCGATGCGGCCGCTGTGGCAGCGCGGCGCGCGGAGGAGTCTCTGCTGGCGCAGCGGATGGCGCCCGCGAAGAGACGCGCGGGGCGCGAGG	345
Q ⁵	SCAGCAGGCCCCGGCGCTGCGCCGATGCGGCCGCTGTGGCAGCGCGGCGCGCGGAGGAGTCTCTGCTGGCGCAGCGGATGGCGCCCGCGAAGAGACGCGCGGGGCGCGAGG	345
Q	TCGCGCAGCTCGCAGTACAGGGGCGTCACTTCTACCGCAGGACCGGGCGGTGGGAGTCGCACATCTGGGATTGCGGGAAGCAGGTCTACTTGGTGGTTTGCACACTGCGCACG	460
Q ¹	TCGCGCAGCTCGCAGTACAGGGGCGTCACTTCTACCGCAGGACCGGGCGGTGGGAGTCGCACATCTGGGATTGCGGGAAGCAGGTCTACTTGGTGGTTTGCACACTGCGCACG	460
Q ²	TCGCGCAGCTCGCAGTACAGGGGCGTCACTTCTACCGCAGGACCGGGCGGTGGGAGTCGCACATCTGGGATTGCGGGAAGCAGGTCTACTTGGTGGTTTGCACACTGCGCACG	460
Q ³	TCGCGCAGCTCGCAGTACAGGGGCGTCACTTCTACCGCAGGACCGGGCGGTGGGAGTCGCACATCTGGGATTGCGGGAAGCAGGTCTACTTGGTGGTTTGCACACTGCGCACG	460
Q ⁴	TCGCGCAGCTCGCAGTACAGGGGCGTCACTTCTACCGCAGGACCGGGCGGTGGGAGTCGCACATCTGGGATTGCGGGAAGCAGGTCTACTTGGTGGTTTGCACACTGCGCACG	460
Q ⁵	TCGCGCAGCTCGCAGTACAGGGGCGTCACTTCTACCGCAGGACCGGGCGGTGGGAGTCGCACATCTGGGATTGCGGGAAGCAGGTCTACTTGGTGGTTTGCACACTGCGCACG	460
Q	CGCGCGCAGGGGCTACGATCGCGCGGATCAAGTTTCGGGGGCTGGAGGCCGACATCAACTTCAATCTGAGCGACTACGAGGAGGATTGAGCAGATGAGGAACGTGGACAA	575
Q ¹	CGCGCGCAGGGGCTACGATCGCGCGGATCAAGTTTCGGGGGCTGGAGGCCGACATCAACTTCAATCTGAGCGACTACGAGGAGGATTGAGCAGATGAGGAACGTGGACAA	575
Q ²	CGCGCGCAGGGGCTACGATCGCGCGGATCAAGTTTCGGGGGCTGGAGGCCGACATCAACTTCAATCTGAGCGACTACGAGGAGGATTGAGCAGATGAGGAACGTGGACAA	575
Q ³	CGCGCGCAGGGGCTACGATCGCGCGGATCAAGTTTCGGGGGCTGGAGGCCGACATCAACTTCAATCTGAGCGACTACGAGGAGGATTGAGCAGATGAGGAACGTGGACAA	575
Q ⁴	CGCGCGCAGGGGCTACGATCGCGCGGATCAAGTTTCGGGGGCTGGAGGCCGACATCAACTTCAATCTGAGCGACTACGAGGAGGATTGAGCAGATGAGGAACGTGGACAA	575
Q ⁵	CGCGCGCAGGGGCTACGATCGCGCGGATCAAGTTTCGGGGGCTGGAGGCCGACATCAACTTCAATCTGAGCGACTACGAGGAGGATTGAGCAGATGAGGAACGTGGACAA	575
Q	GGAGGAGTTCGTGCACATCTCCGCGCCGACGAGCAGGGGTTTCGCGAGGGGGAGCTCCAAGTACCGGGCGTCAAGTCCCAAGTGCAGCGCTGGGAGGCAAGGATGGGCCAG	690
Q ¹	GGAGGAGTTCGTGCACATCTCCGCGCCGACGAGCAGGGGTTTCGCGAGGGGGAGCTCCAAGTACCGGGCGTCAAGTCCCAAGTGCAGCGCTGGGAGGCAAGGATGGGCCAG	690
Q ²	GGAGGAGTTCGTGCACATCTCCGCGCCGACGAGCAGGGGTTTCGCGAGGGGGAGCTCCAAGTACCGGGCGTCAAGTCCCAAGTGCAGCGCTGGGAGGCAAGGATGGGCCAG	690
Q ³	GGAGGAGTTCGTGCACATCTCCGCGCCGACGAGCAGGGGTTTCGCGAGGGGGAGCTCCAAGTACCGGGCGTCAAGTCCCAAGTGCAGCGCTGGGAGGCAAGGATGGGCCAG	690
Q ⁴	GGAGGAGTTCGTGCACATCTCCGCGCCGACGAGCAGGGGTTTCGCGAGGGGGAGCTCCAAGTACCGGGCGTCAAGTCCCAAGTGCAGCGCTGGGAGGCAAGGATGGGCCAG	690
Q ⁵	GGAGGAGTTCGTGCACATCTCCGCGCCGACGAGCAGGGGTTTCGCGAGGGGGAGCTCCAAGTACCGGGCGTCAAGTCCCAAGTGCAGCGCTGGGAGGCAAGGATGGGCCAG	690
Q	CTGCTCGGCAAGAAGTACATATATCTGGGCTCTTTGACAGCGAAGTTGAAGCTGCAAGGGCGTACGACAGGGCGGCGATTGCTTCAATGGGAGGGAAGCTGTGACTAACTTTG	805
Q ¹	CTGCTCGGCAAGAAGTACATATATCTGGGCTCTTTGACAGCGAAGTTGAAGCTGCAAGGGCGTACGACAGGGCGGCGATTGCTTCAATGGGAGGGAAGCTGTGACTAACTTTG	805
Q ²	CTGCTCGGCAAGAAGTACATATATCTGGGCTCTTTGACAGCGAAGTTGAAGCTGCAAGGGCGTACGACAGGGCGGCGATTGCTTCAATGGGAGGGAAGCTGTGACTAACTTTG	805
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Q ⁴	CTGCTCGGCAAGAAGTACATATATCTGGGCTCTTTGACAGCGAAGTTGAAGCTGCAAGGGCGTACGACAGGGCGGCGATTGCTTCAATGGGAGGGAAGCTGTGACTAACTTTG	805
Q ⁵	CTGCTCGGCAAGAAGTACATATATCTGGGCTCTTTGACAGCGAAGTTGAAGCTGCAAGGGCGTACGACAGGGCGGCGATTGCTTCAATGGGAGGGAAGCTGTGACTAACTTTG	805
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Q ¹	AGAGCAGCTCTACAAATGGGATGCTCCACCGGACGCCGAAATGAGGCAATTGTTGATGCTGATGCTCTTGACTTGGATCTCGGATGTCGCAACCCACCGCGCAGATCCCAA	920
Q ²	AGAGCAGCTCTACAAATGGGATGCTCCACCGGACGCCGAAATGAGGCAATTGTTGATGCTGATGCTCTTGACTTGGATCTCGGATGTCGCAACCCACCGCGCAGATCCCAA	920
Q ³	AGAGCAGCTCTACAAATGGGATGCTCCACCGGACGCCGAAATGAGGCAATTGTTGATGCTGATGCTCTTGACTTGGATCTCGGATGTCGCAACCCACCGCGCAGATCCCAA	920
Q ⁴	AGAGCAGCTCTACAAATGGGATGCTCCACCGGACGCCGAAATGAGGCAATTGTTGATGCTGATGCTCTTGACTTGGATCTCGGATGTCGCAACCCACCGCGCAGATCCCAA	920
Q ⁵	AGAGCAGCTCTACAAATGGGATGCTCCACCGGACGCCGAAATGAGGCAATTGTTGATGCTGATGCTCTTGACTTGGATCTCGGATGTCGCAACCCACCGCGCAGATCCCAA	920
Q	EAGGGACACATCATCGCGGCTTCAGTTAACTTTTATTGCCCTGAATCGTCAACCAATGATCTCTTCTCAGCCAATGAGCTCATCTTCTGCCAGTGGGCTGTGCATCAA	1035
Q ¹	EAGGGACACATCATCGCGGCTTCAGTTAACTTTTATTGCCCTGAATCGTCAACCAATGATCTCTTCTCAGCCAATGAGCTCATCTTCTGCCAGTGGGCTGTGCATCAA	1035
Q ²	EAGGGACACATCATCGCGGCTTCAGTTAACTTTTATTGCCCTGAATCGTCAACCAATGATCTCTTCTCAGCCAATGAGCTCATCTTCTGCCAGTGGGCTGTGCATCAA	1035
Q ³	EAGGGACACATCATCGCGGCTTCAGTTAACTTTTATTGCCCTGAATCGTCAACCAATGATCTCTTCTCAGCCAATGAGCTCATCTTCTGCCAGTGGGCTGTGCATCAA	1035
Q ⁴	EAGGGACACATCATCGCGGCTTCAGTTAACTTTTATTGCCCTGAATCGTCAACCAATGATCTCTTCTCAGCCAATGAGCTCATCTTCTGCCAGTGGGCTGTGCATCAA	1035
Q ⁵	EAGGGACACATCATCGCGGCTTCAGTTAACTTTTATTGCCCTGAATCGTCAACCAATGATCTCTTCTCAGCCAATGAGCTCATCTTCTGCCAGTGGGCTGTGCATCAA	1035
Q	CATGGCACGGCAGTAGCACTCAGCAGCACCAGCGTTTGTACCCATCTGCTTGTGATGGTTCTACCCGAACTACAGGTGCAAGTGCAGGAGGAGGCCCATGGAGGCAAGGCCCC	1150
Q ¹	CATGGCACGGCAGTAGCACTCAGCAGCACCAGCGTTTGTACCCATCTGCTTGTGATGGTTCTACCCGAACTACAGGTGCAAGTGCAGGAGGAGGCCCATGGAGGCAAGGCCCC	1150
Q ²	CATGGCACGGCAGTAGCACTCAGCAGCACCAGCGTTTGTACCCATCTGCTTGTGATGGTTCTACCCGAACTACAGGTGCAAGTGCAGGAGGAGGCCCATGGAGGCAAGGCCCC	1150
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Q ⁴	CATGGCACGGCAGTAGCACTCAGCAGCACCAGCGTTTGTACCCATCTGCTTGTGATGGTTCTACCCGAACTACAGGTGCAAGTGCAGGAGGAGGCCCATGGAGGCAAGGCCCC	1150
Q ⁵	CATGGCACGGCAGTAGCACTCAGCAGCACCAGCGTTTGTACCCATCTGCTTGTGATGGTTCTACCCGAACTACAGGTGCAAGTGCAGGAGGAGGCCCATGGAGGCAAGGCCCC	1150
Q	CTGAGCAGCCGCTGCTCTTCCCGGCTGGGGGTGGCAAGCGCAAGCATGCCGCGGGCTCTCCCACTCGGTTGCTTTACGCTGCAGGATATCAGATTTTCTACCGCCG	1265
Q ¹	CTGAGCAGCCGCTGCTCTTCCCGGCTGGGGGTGGCAAGCGCAAGCATGCCGCGGGCTCTCCCACTCGGTTGCTTTACGCTGCAGGATATCAGATTTTCTACCGCCG	1265
Q ²	CTGAGCAGCCGCTGCTCTTCCCGGCTGGGGGTGGCAAGCGCAAGCATGCCGCGGGCTCTCCCACTCGGTTGCTTTACGCTGCAGGATATCAGATTTTCTACCGCCG	1265
Q ³	CTGAGCAGCCGCTGCTCTTCCCGGCTGGGGGTGGCAAGCGCAAGCATGCCGCGGGCTCTCCCACTCGGTTGCTTTACGCTGCAGGATATCAGATTTTCTACCGCCG	1265
Q ⁴	CTGAGCAGCCGCTGCTCTTCCCGGCTGGGGGTGGCAAGCGCAAGCATGCCGCGGGCTCTCCCACTCGGTTGCTTTACGCTGCAGGATATCAGATTTTCTACCGCCG	1265
Q ⁵	CTGAGCAGCCGCTGCTCTTCCCGGCTGGGGGTGGCAAGCGCAAGCATGCCGCGGGCTCTCCCACTCGGTTGCTTTACGCTGCAGGATATCAGATTTTCTACCGCCG	1265
miR172-binding sites		
Q	CGCGGCGGCAACTCGCCCGCGCGCGGCTACCGGACCAACACGGTTCTACTTCCCGCGCCGCGGCAACTGA	1344
Q ¹	CGCGGCGGCAACTCGCCCGCGCGCGGCTACCGGACCAACACGGTTCTACTTCCCGCGCCGCGGCAACTGA	1344
Q ²	CGCGGCGGCAACTCGCCCGCGCGCGGCTACCGGACCAACACGGTTCTACTTCCCGCGCCGCGGCAACTGA	1344
Q ³	CGCGGCGGCAACTCGCCCGCGCGCGGCTACCGGACCAACACGGTTCTACTTCCCGCGCCGCGGCAACTGA	1344
Q ⁴	CGCGGCGGCAACTCGCCCGCGCGCGGCTACCGGACCAACACGGTTCTACTTCCCGCGCCGCGGCAACTGA	1344
Q ⁵	CGCGGCGGCAACTCGCCCGCGCGCGGCTACCGGACCAACACGGTTCTACTTCCCGCGCCGCGGCAACTGA	1344

Figure S2. Alignment of the cDNA sequences of Q and five Q^c alleles. The miRNA172-binding site is boxed with black lines.

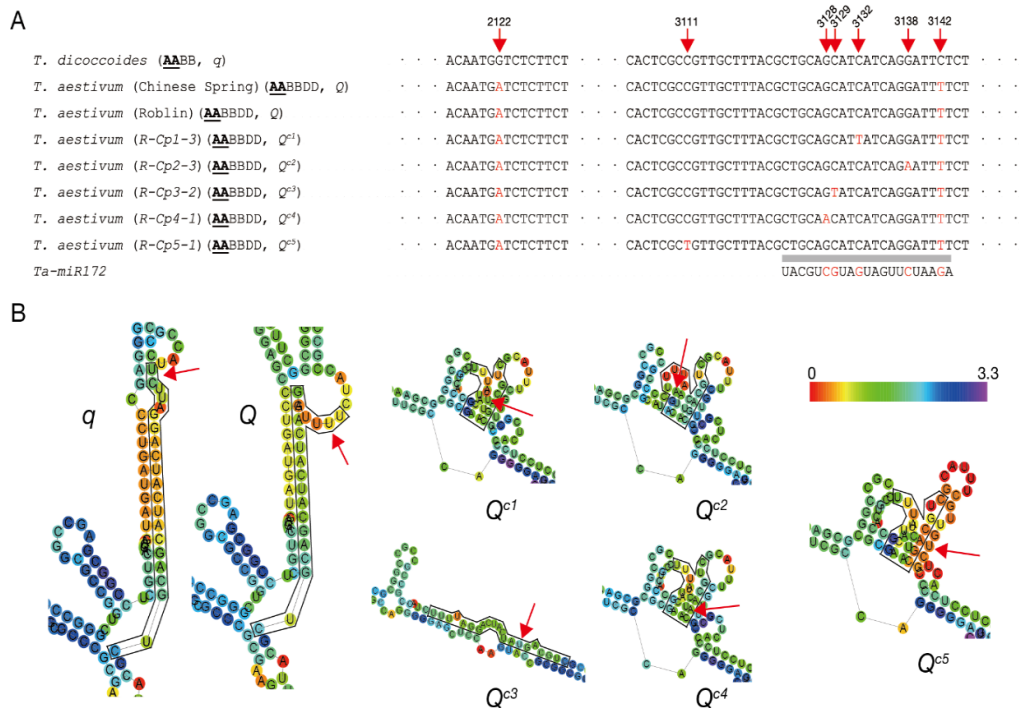


Figure S3. Effect of point mutations on the RNA secondary structure around the miRNA172-binding site. (A) Seven point-mutations in the DNA sequences of *q/Q/Q^{c1}/Q^{c2}/Q^{c3}/Q^{c4}/Q^{c5}* alleles. The underlined is the miRNA172-binding site. The Genebank numbers are in order of AY702957.1, KX620763.1, KX620765-KX620768, and MW419115. (B) Comparison of the predicted RNA secondary structures of *q/Q/Q^{c1}/Q^{c2}/Q^{c3}/Q^{c4}/Q^{c5}* alleles around the miRNA172-binding site, which drawing encoding positional entropy. The heat maps indicate the positional entropy, from low (red) to high (purple). The miRNA172-binding site is boxed with black lines. The red arrows represent the point mutations.

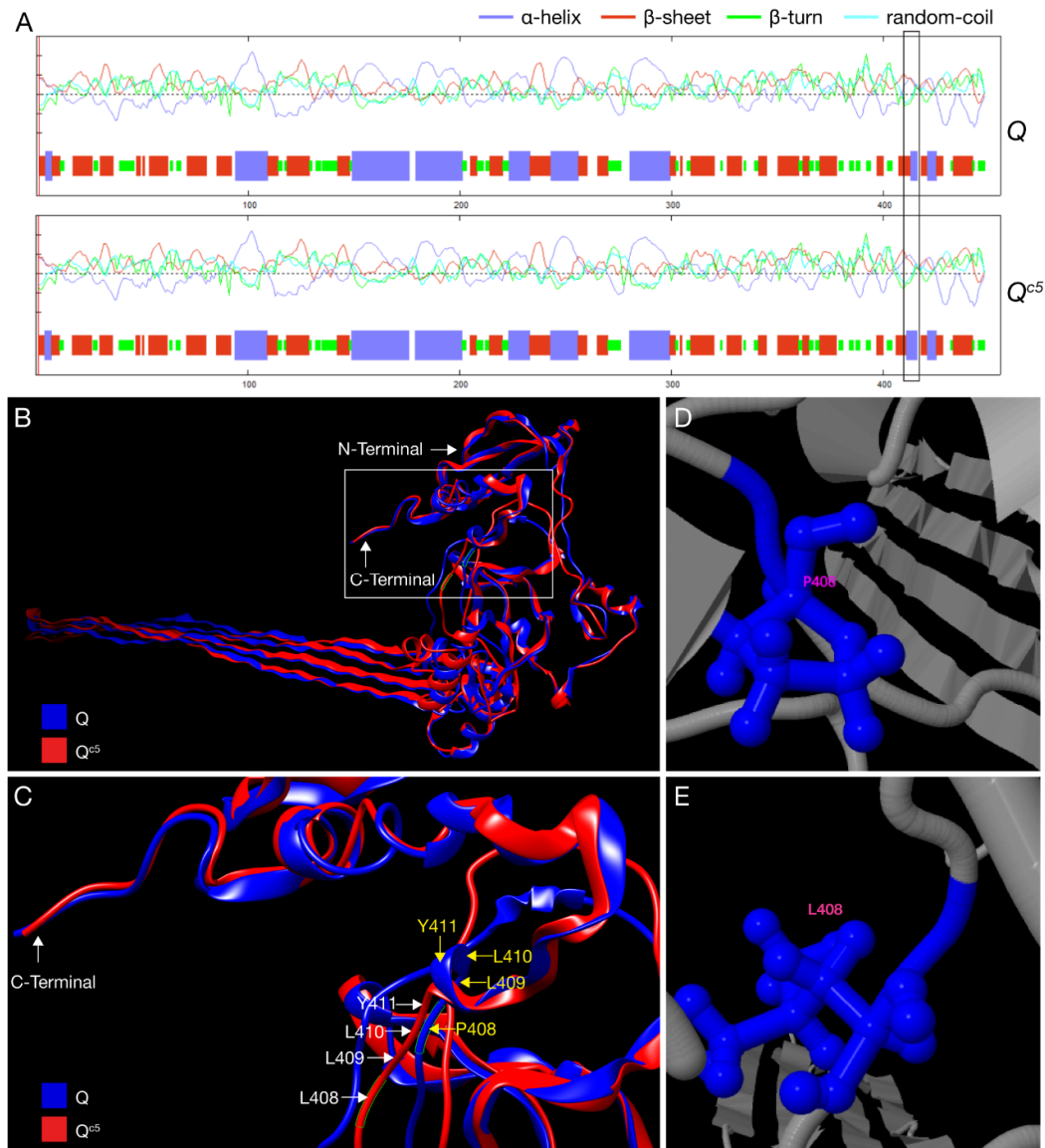


Figure S4. The number of amino acid residues for forming α -helix is more in Q^{c5} than in Q around the changed residue. (A) Comparison of predicted secondary structures of Q and Q^{c5} . The difference between Q and Q^{c5} is boxed by black line. (B-E) Comparison of 3D structure model of Q and Q^{c5} . (B) The superposition of full-length Q and Q^{c5} was compared by TM-align. (C) Magnification of the white box in (B). The yellow arrows show the position and type of amino acid residues in Q and the white arrows show that in Q^{c5} . The α -helix in the is ended in 409th residue of Q protein and 411th residue in Q^{c5} . (D) and (E) show the structure of 408th residue, in which (D) present proline in Q and (E) present leucine in Q^{c5} .

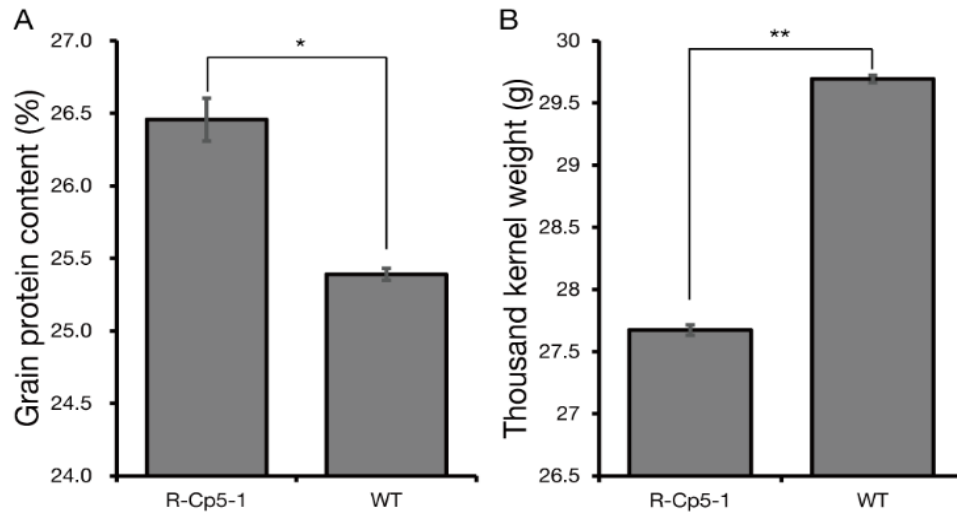


Figure S5. Comparison of the grain protein content (A) and thousand kernel weight (B) between *R-Cp5-1* and its WT in the glasshouse experiment.

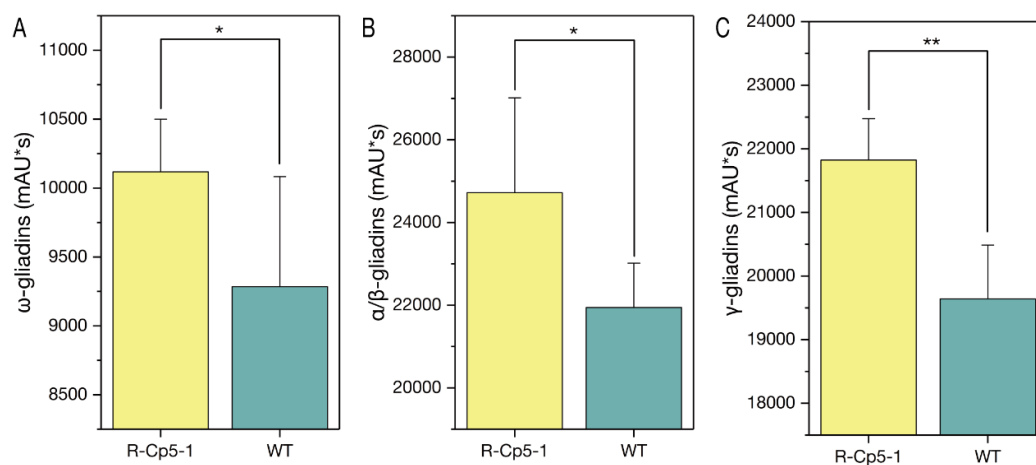


Figure S6. Comparison of the contents of α - (α/β -) (A), γ - (B) and ω -gliadins (C) of *R-Cp5-1* and its WT.

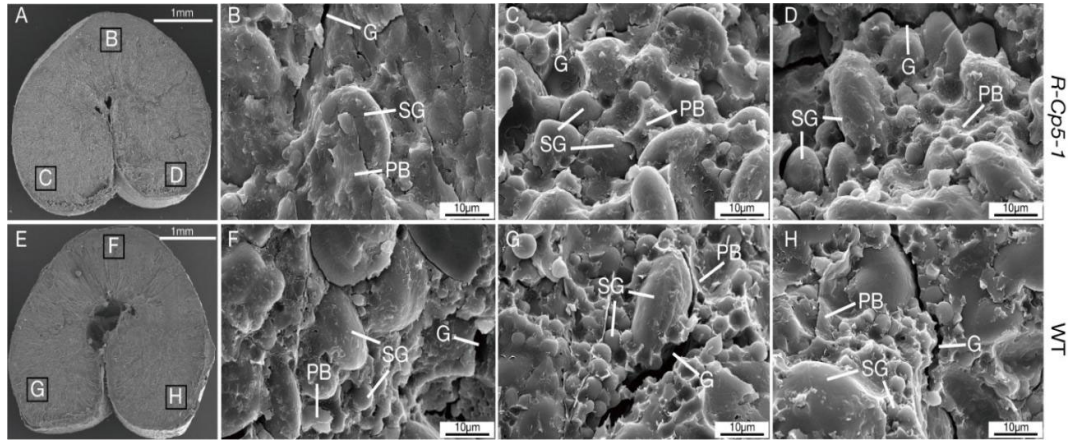


Figure S7. Scanning electron microscopic observation of three representative areas of the endosperm of mature seeds. (A-D) and (E-H) are images of cross-sections of *R-Cp5-1* and its WT, respectively. (B-D, E-H) Magnifications of the black boxes in (A) and (E), respectively. PB, protein body; SG, starch granule; G, gap between PB and SG. Scale bars, 1 mm in (A) and (E), and 10 μm in others.

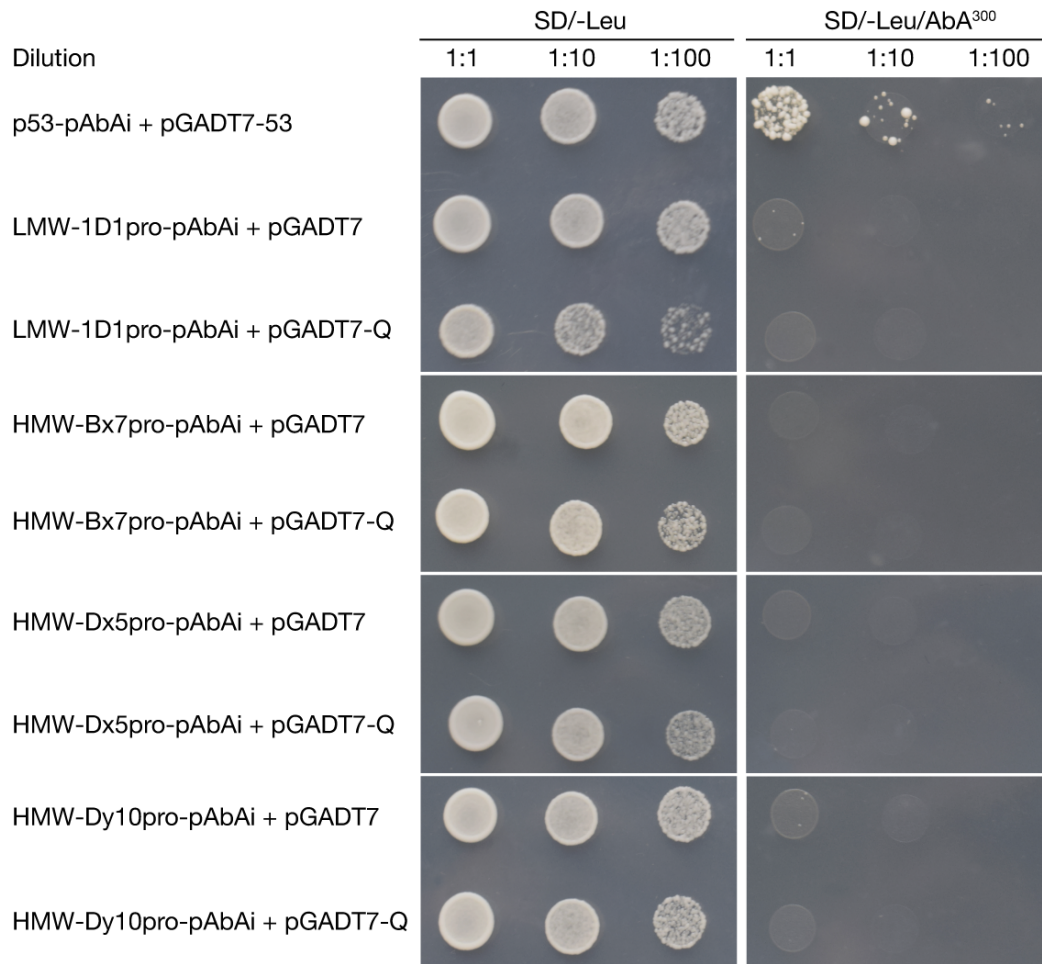


Figure S8. Yeast one-hybrid analysis showing that Q cannot bind the promoter of SSPs. Yeast cells were co-transformed with each combination. Cells were grown in liquid medium to an OD₆₀₀ of 0.7, and a dilution series was prepared. For each dilution, cells were spotted onto medium (synthetic dropout, -Leu) supplemented with 300 ng/mL Aureobasidin A (AbA) to suppress background growth. The combination of p53-pAbAi and pGADT7-53 was used as positive control; each SSP promoter was co-transformed with pGADT7 as its negative control.

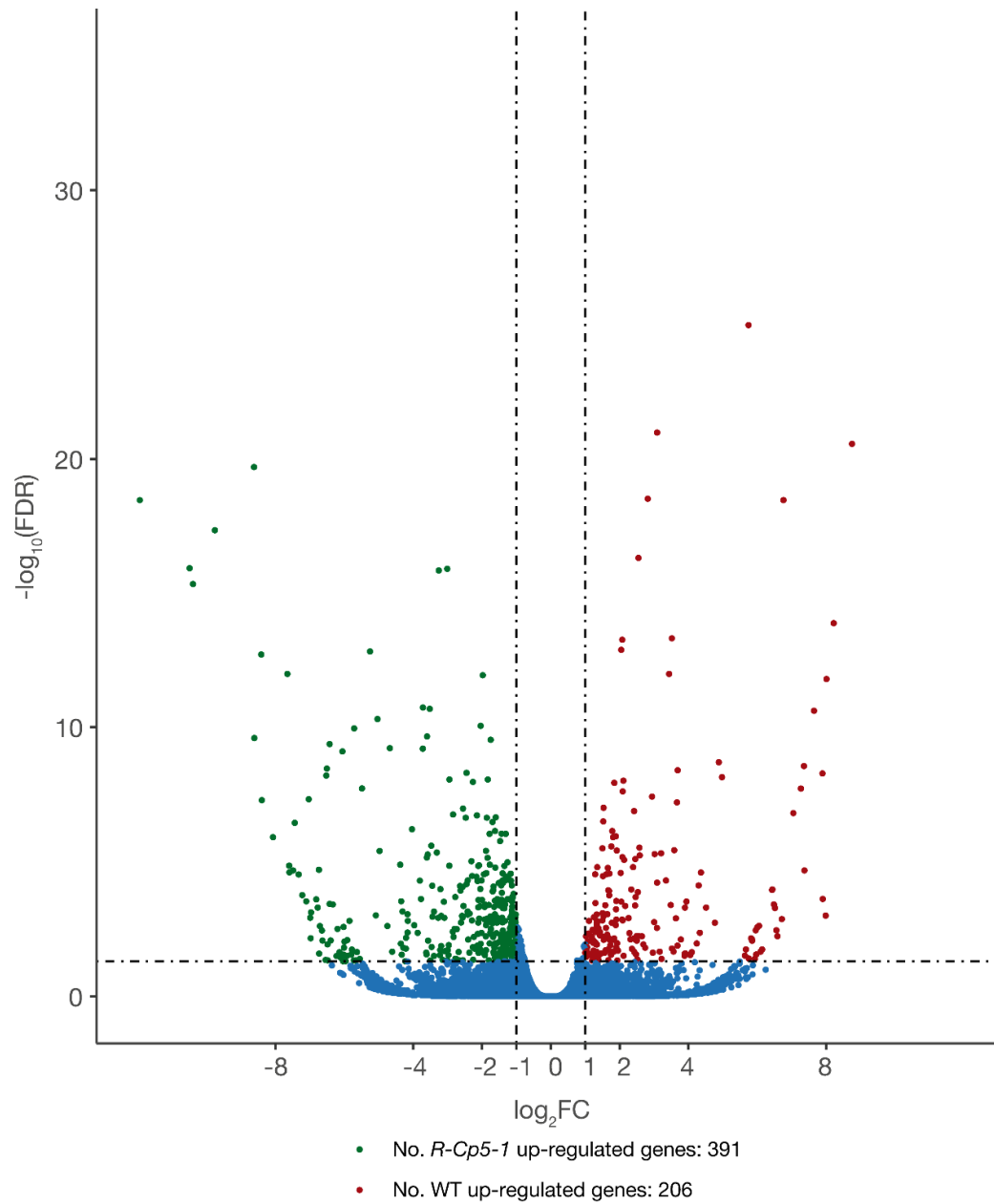


Figure S9. Volcano plot shows the DEGs in RNA-seq data.

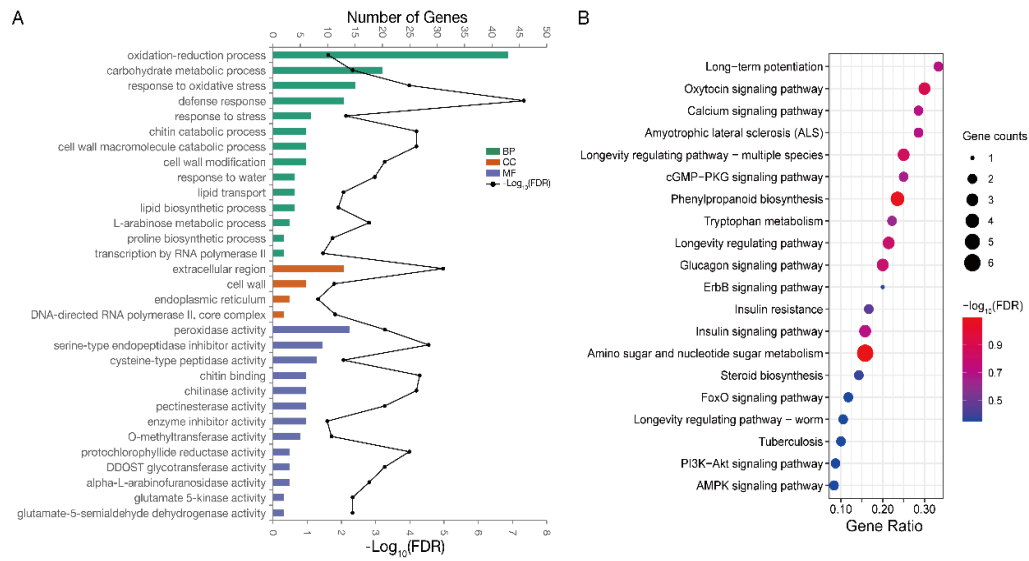


Figure S10. GO items (A) and top 20 KEGG pathways (B) enriched in DEGs between *R-Cp5-1* and its WT.

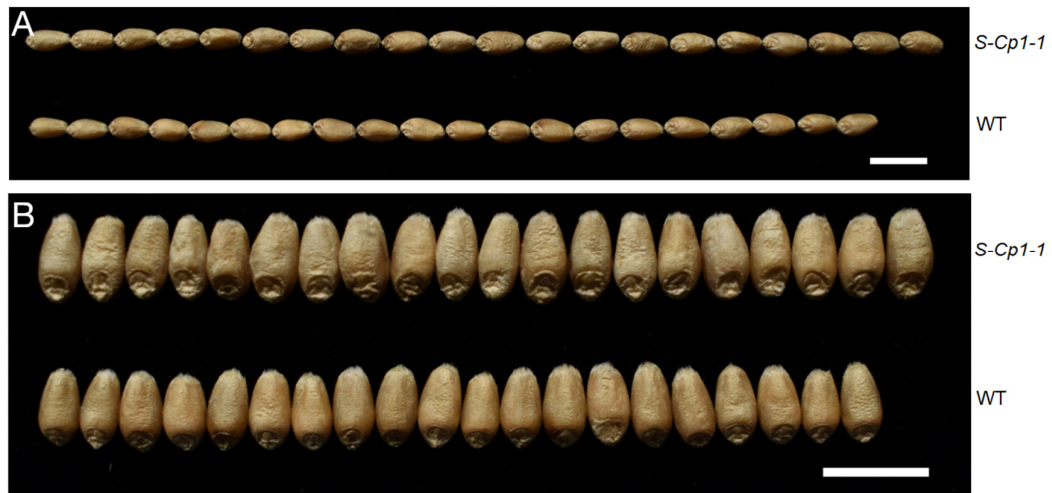


Figure S11. Comparison of seed length and seed width between *S-Cp1-1* (with Q^{cl} allele) and its WT (with Q allele). Length (A) and width (B) of 20 seeds of *S-Cp1-1* (up) and its WT (down). Scale bar, 1 cm.

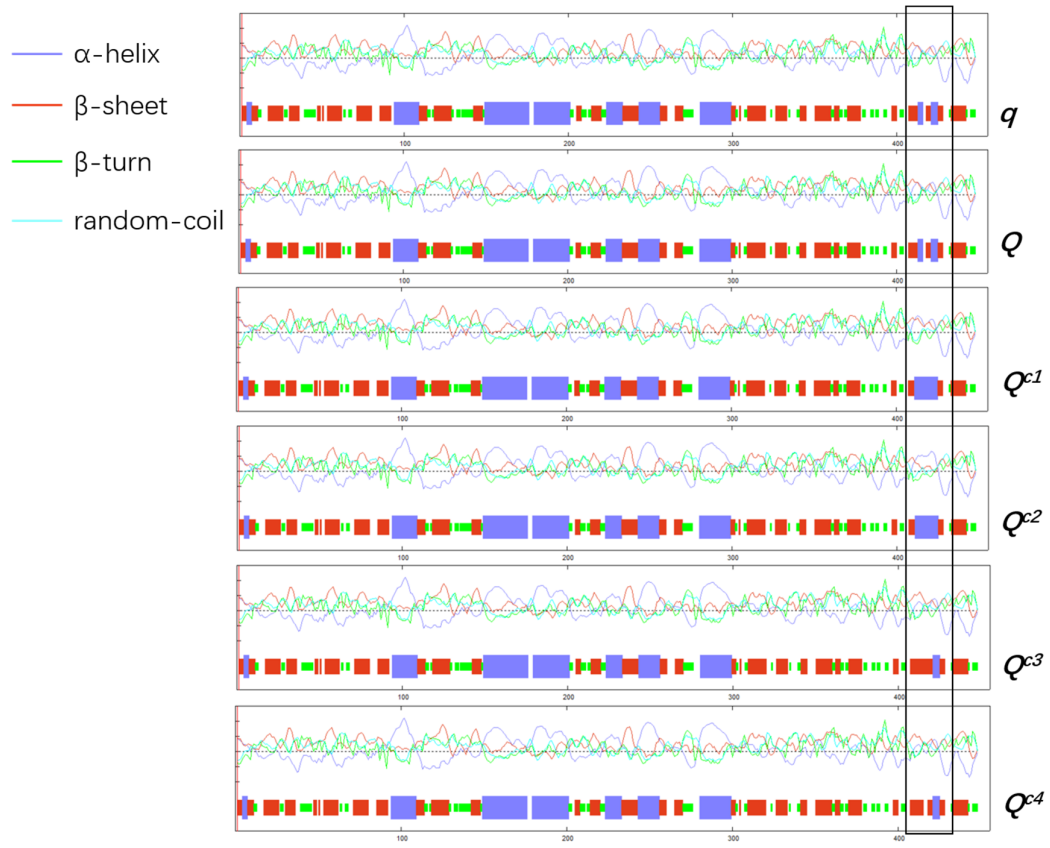


Figure S13. Comparison of the predicted secondary structures of q , Q , and Q^{c1} - Q^{c4} . The black frame highlights the amino acid substitutions.

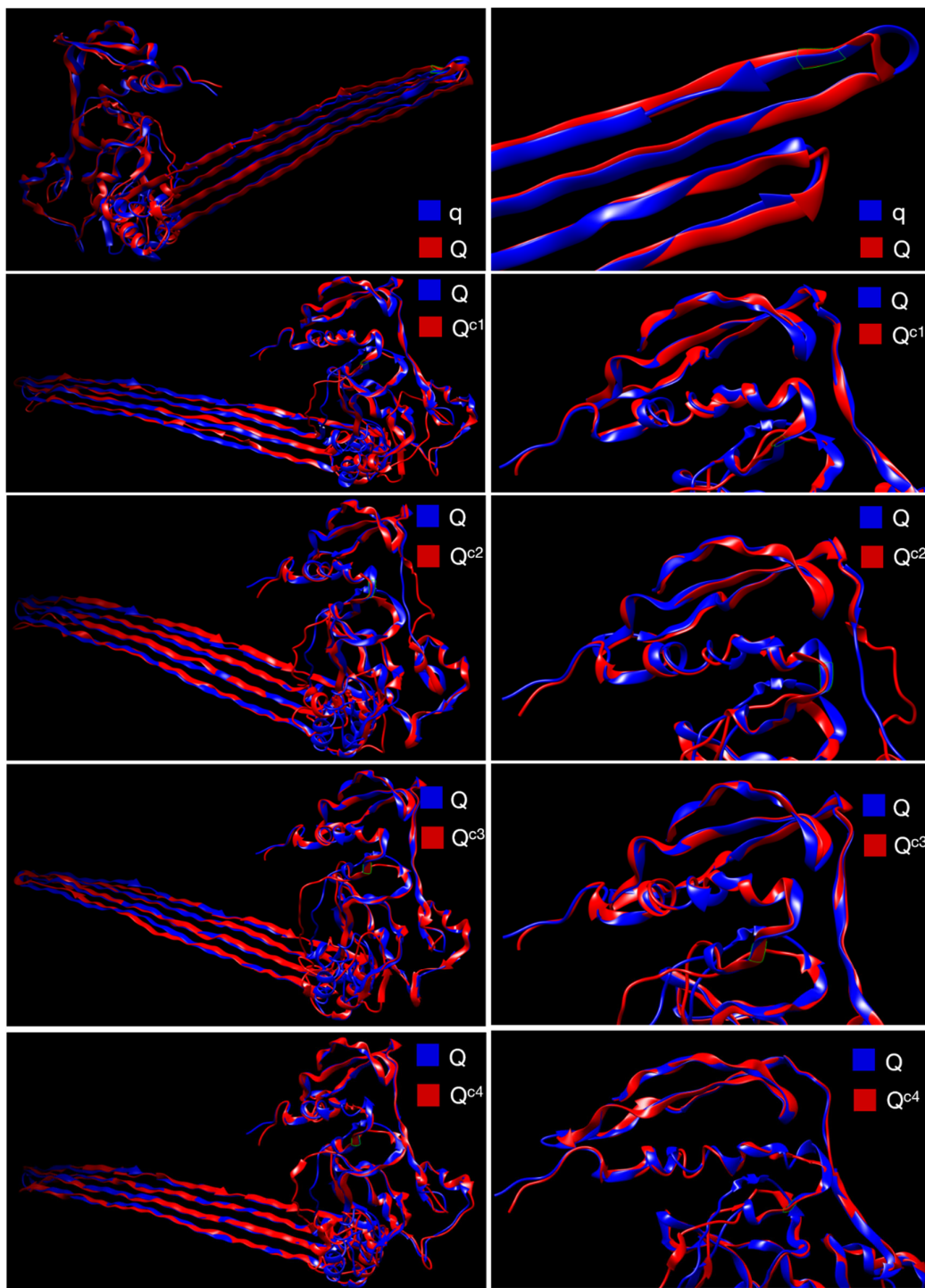


Figure S14. Comparison of the predicted 3D protein structures of q , Q , and Q^{c1} - Q^{c4} .