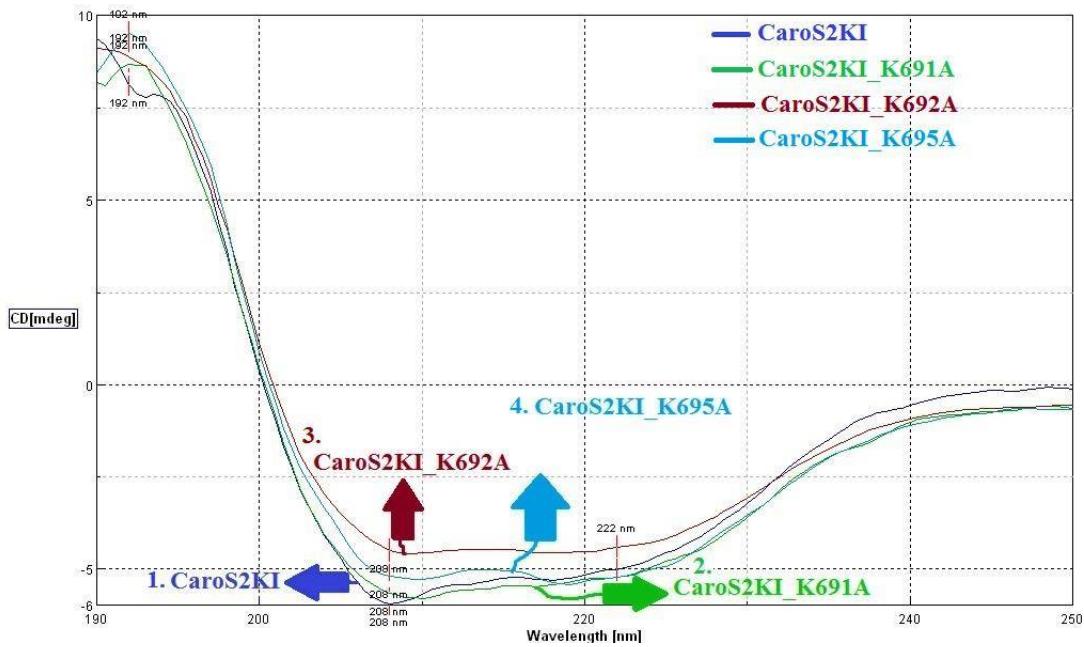
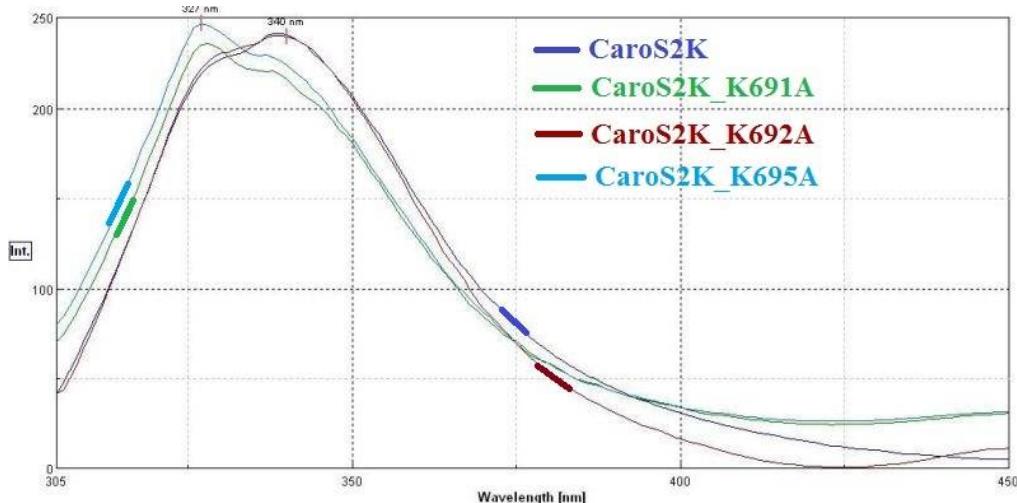


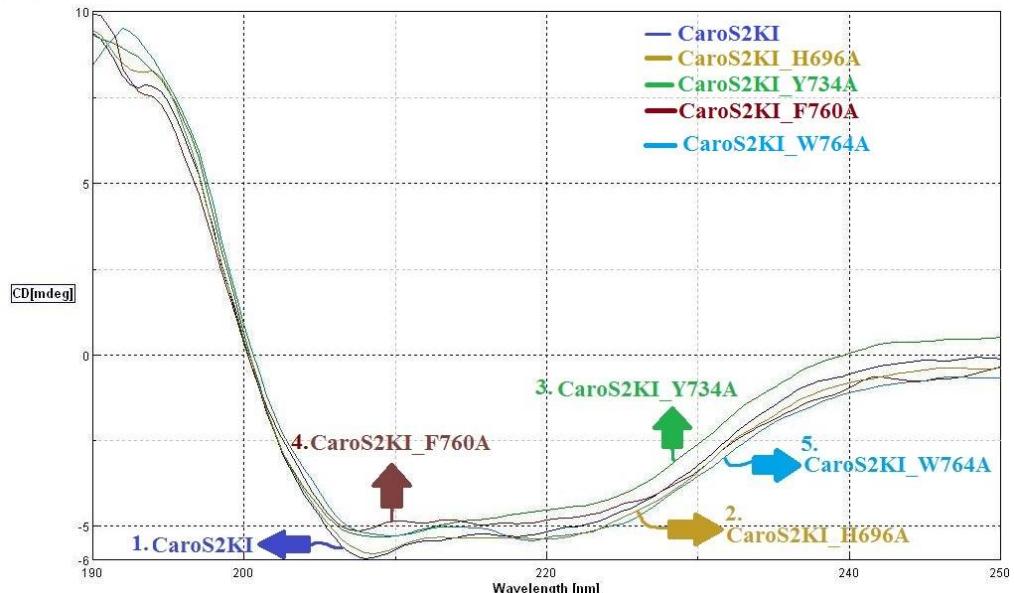
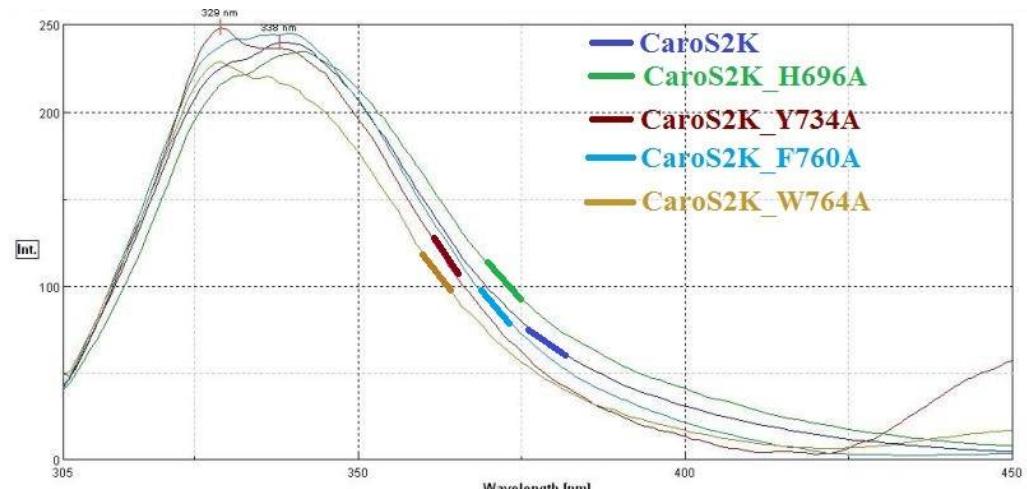
A



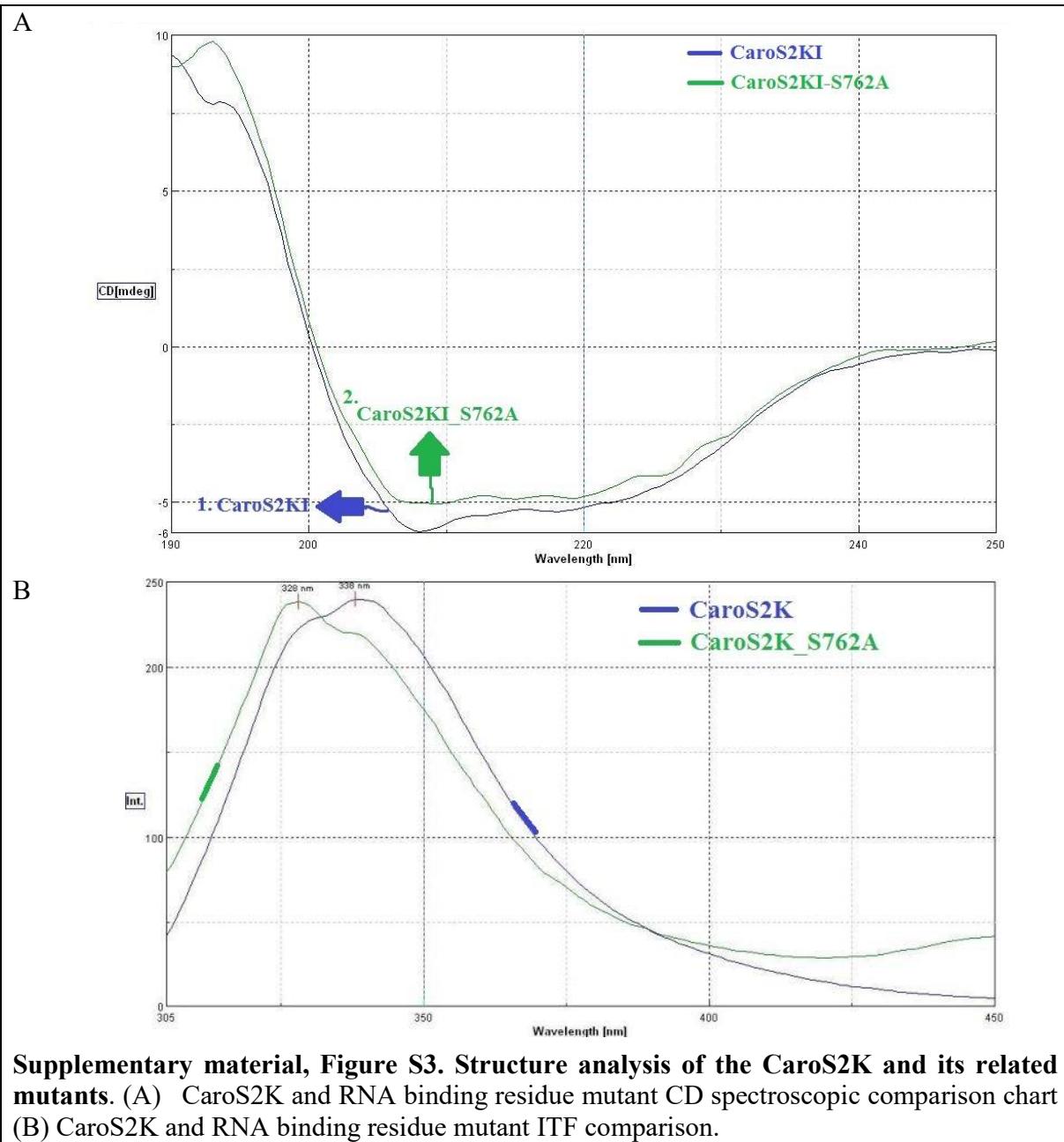
B



**Supplementary material, Figure S1. Structure analysis of the CaroS2K and its related mutants.** (A) CD spectroscopy comparison chart of CaroS2K and Lysine residue mutants (B) ITF comparison of CaroS2K with Lysine residues.

**A****B**

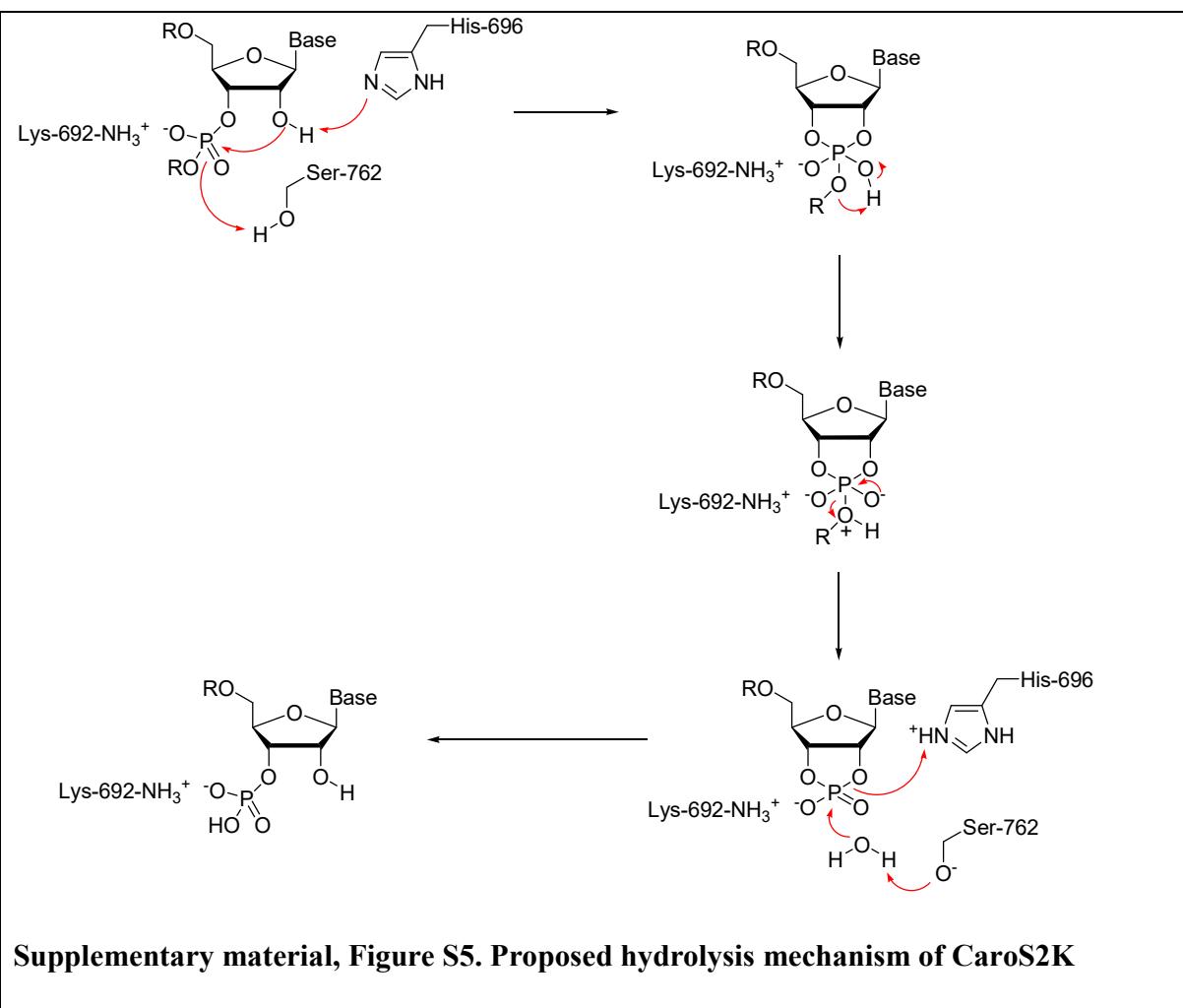
**Supplementary material, Figure S2. Structure analysis of the CaroS2K and its related mutants.** (A) CaroS2K, the Histidine residue mutant, and hydrophobic residue mutants CD spectroscopic comparison chart (B) ITF comparison of CaroS2K, the Histidine residue mutant, and hydrophobic residue mutants



**Supplementary material, Figure S3. Structure analysis of the CaroS2K and its related mutants.** (A) CaroS2K and RNA binding residue mutant CD spectroscopic comparison chart (B) CaroS2K and RNA binding residue mutant ITF comparison.

	651	660	670	680	690	700	710	720	730	740	750	760	770	780
CaroS2K	MEFDQYIIWPTADGS <span style="color:red">G</span> VEPI <span style="color:blue">Y</span> VFN <span style="color:blue">D</span> PLDSDFTRK <span style="color:blue">Q</span> LOKKYL <span style="color:blue">K</span> HAKDFGIV <span style="color:blue">D</span> TRK <span style="color:blue">N</span> SET <span style="color:blue">I</span> TKF <span style="color:blue">R</span> D <span style="color:blue">A</span> I <span style="color:blue">I</span> T <span style="color:blue">T</span> LEEKET <span style="color:blue">E</span> KGTLY <span style="color:blue">K</span> D <span style="color:blue">S</span> KVFFNP <span style="color:blue">K</span> T <span style="color:blue">T</span> N <span style="color:blue">V</span> V <span style="color:blue">V</span> W <span style="color:blue">D</span> K <span style="color:blue">D</span> K <span style="color:blue">N</span> K <span style="color:blue">F</span> IS <span style="color:blue">G</span> W <span style="color:blue">K</span> L <span style="color:blue">D</span> V <span style="color:blue">D</span> S <span style="color:blue">Q</span> Y <span style="color:blue">K</span> N <span style="color:blue">Y</span> V <span style="color:blue">N</span> G													
ColicinD	GGLRDFI <span style="color:blue">Y</span> W <span style="color:blue">P</span> R <span style="color:blue">D</span> ARGT <span style="color:blue">G</span> VE <span style="color:blue">V</span> V <span style="color:blue">M</span> LNDPLDSGRFSRK <span style="color:blue">Q</span> LOKKY <span style="color:blue">K</span> HAGDFG <span style="color:blue">I</span> S <span style="color:blue">D</span> TK <span style="color:blue">K</span> NRET <span style="color:blue">I</span> TKF <span style="color:blue">R</span> D <span style="color:blue">A</span> I <span style="color:blue">I</span> E <span style="color:blue">H</span> LS <span style="color:blue">D</span> O <span style="color:blue">T</span> E <span style="color:blue">K</span> G <span style="color:blue">T</span> Y <span style="color:blue">R</span> E <span style="color:blue">K</span> G <span style="color:blue">S</span> K <span style="color:blue">V</span> Y <span style="color:blue">F</span> N <span style="color:blue">P</span> H <span style="color:blue">T</span> M <span style="color:blue">N</span> V <span style="color:blue">V</span> I <span style="color:blue">K</span> S <span style="color:blue">H</span> E <span style="color:blue">F</span> L <span style="color:blue">S</span> G <span style="color:blue">W</span> K <span style="color:blue">I</span> H <span style="color:blue">P</span> RD <span style="color:blue">D</span> NGR <span style="color:blue">I</span> Y <span style="color:blue">L</span> ETG													
KlebicinB	T <span style="color:blue">G</span> VEPI <span style="color:blue">Y</span> V <span style="color:blue">M</span> LSP <span style="color:blue">D</span> SG <span style="color:blue">K</span> Y <span style="color:blue">T</span> RR <span style="color:blue">Q</span> LY <span style="color:blue">K</span> Y <span style="color:blue">K</span> -KA <span style="color:blue">D</span> F <span style="color:blue">G</span> I <span style="color:blue">D</span> TK <span style="color:blue">I</span> NG <span style="color:blue">E</span> T <span style="color:blue">I</span> TKF <span style="color:blue">R</span> D <span style="color:blue">A</span> I <span style="color:blue">E</span> AN <span style="color:blue">H</span> LS <span style="color:blue">S</span> U <span style="color:blue">D</span> T <span style="color:blue">F</span> E <span style="color:blue">K</span> G <span style="color:blue">T</span> Y <span style="color:blue">R</span> R <span style="color:blue">D</span> K <span style="color:blue">G</span> S <span style="color:blue">K</span> V <span style="color:blue">Y</span> F <span style="color:blue">N</span> P <span style="color:blue">K</span> T <span style="color:blue">T</span> N <span style="color:blue">M</span> V <span style="color:blue">V</span> I <span style="color:blue">Q</span> W <span style="color:blue">D</span> F <span style="color:blue">F</span> LS <span style="color:blue">G</span> W <span style="color:blue">K</span> I <span style="color:blue">H</span> P <span style="color:blue">A</span> RD <span style="color:blue">M</span> G <span style="color:blue">R</span> Y <span style="color:blue">L</span> ETG													
Pyocin53	LSLD <span style="color:blue">D</span> LI <span style="color:blue">Y</span> IFP--ADSGW <span style="color:blue">K</span> P <span style="color:blue">V</span> V <span style="color:blue">M</span> LSSP <span style="color:blue">D</span> SG <span style="color:blue">I</span> FTRK <span style="color:blue">Q</span> LLKY <span style="color:blue">Q</span> SHADDFG <span style="color:blue">F</span> GR <span style="color:blue">R</span> NA <span style="color:blue">N</span> IT <span style="color:blue">I</span> LT <span style="color:blue">E</span> FR <span style="color:blue">D</span> K <span style="color:blue">I</span> LE <span style="color:blue">H</span> LS <span style="color:blue">S</span> P <span style="color:blue">T</span> I <span style="color:blue">E</span> K <span style="color:blue">G</span> V <span style="color:blue">Y</span> HY <span style="color:blue">R</span> Y <span style="color:blue">Q</span> G <span style="color:blue">S</span> K <span style="color:blue">V</span> Y <span style="color:blue">F</span> N <span style="color:blue">P</span> D <span style="color:blue">T</span> K <span style="color:blue">I</span> Y <span style="color:blue">V</span> W <span style="color:blue">L</span> K <span style="color:blue">E</span> D <span style="color:blue">G</span> M <span style="color:blue">F</span> LS <span style="color:blue">G</span> W <span style="color:blue">Y</span> LE <span style="color:blue">P</span> G <span style="color:blue">S</span> K <span style="color:blue">Q</span> E <span style="color:blue">K</span> N <span style="color:blue">Y</span> LENE													
Pyocin52	IFP--ADSGW <span style="color:blue">K</span> P <span style="color:blue">V</span> V <span style="color:blue">M</span> LSSP <span style="color:blue">D</span> SG <span style="color:blue">I</span> FTR <span style="color:blue">Q</span> LLQK <span style="color:blue">F</span> DSH <span style="color:blue">K</span> Y <span style="color:blue">D</span> G <span style="color:blue">L</span> GE <span style="color:blue">K</span> E <span style="color:blue">S</span> A <span style="color:blue">H</span> NT <span style="color:blue">I</span> LA <span style="color:blue">E</span> FR <span style="color:blue">D</span> K <span style="color:blue">I</span> LE <span style="color:blue">H</span> LD <span style="color:blue">D</span> P <span style="color:blue">A</span> RT <span style="color:blue">V</span> E <span style="color:blue">K</span> G <span style="color:blue">T</span> Y <span style="color:blue">H</span> SE <span style="color:blue">V</span> K <span style="color:blue">S</span> K <span style="color:blue">V</span> Y <span style="color:blue">H</span> Y <span style="color:blue">N</span> R <span style="color:blue">A</span> R <span style="color:blue">T</span> H <span style="color:blue">I</span> V <span style="color:blue">V</span> I <span style="color:blue">G</span> E <span style="color:blue">D</span> G <span style="color:blue">M</span> F <span style="color:blue">V</span> S <span style="color:blue">G</span> W <span style="color:blue">R</span> I <span style="color:blue">E</span> PG <span style="color:blue">T</span> Q <span style="color:blue">Y</span> N <span style="color:blue">F</span> Y <span style="color:blue">H</span> K <span style="color:blue">N</span> E													
Consensus	....d,i...p.,a,s <span style="color:red">G</span> ep <span style="color:blue">I</span> Y <span style="color:blue">V</span> mlsdPLDsg,xtRk <span style="color:blue">Q</span> L,KK <span style="color:blue">K</span> ,K <span style="color:blue">H</span> a,DFgi,dt.,N,eLt <span style="color:blue">t</span> kFRD <span style="color:blue">A</span> T,eH <span style="color:blue">s</span> dk,T,EKG <span style="color:blue">t</span> Y,r,kg <span style="color:blue">S</span> K <span style="color:blue">V</span> ,Xn <span style="color:blue">p</span> .T, nv <span style="color:blue">V</span> !i..tg, FIS <span style="color:blue">G</span> W <span style="color:blue">K</span> i#p.,d#...,Yleng													

**Supplementary material, Figure S4. The multiple sequence alignment of CaroS2K with other bacteriocins.**



Supplementary material, Figure S5. Proposed hydrolysis mechanism of CaroS2K

**Supplementary material, Table S1.** Plasmids used in this study

Strain or Plasmid	Relevant characteristics	Source of reference
pES2TKD677_Q688A	Derived from pES2TKD677 with Ala substitution from position Q688	This study
pES2TKD677_K691A	Derived from pES2TKD677 with Ala substitution from position K691	This study
pES2TKD677_K692A	Derived from pES2TKD677 with Ala substitution from position K692	This study
pES2TKD677_K695A	Derived from pES2TKD677 with Ala substitution from position K695	This study
pES2TKD677_H696A	Derived from pES2TKD677 with Ala substitution from position H696	This study
pES2TKD677_Y734A	Derived from pES2TKD677 with Ala substitution from position Y734	This study
pES2TKD677_F760A	Derived from pES2TKD677 with Ala substitution from position F760	This study
pES2TKD677_S762A	Derived from pES2TKD677 with Ala substitution from position S762	This study
pES2TKD677_W764A	Derived from pES2TKD677 with Ala substitution from position Q688	This study

**Supplementary material, Table S2.** Primers used in this study.

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S2_H696A_forT	GTATCTAAAGCTGCCAAGATTGGTATTGTTGATACC
S2_H696A_forS	TTTTGGTATTGTTGATACC
S2_H696A_revT	TCTTGGCAGCTTAAGATACTTTGTCCAGTGCTTACG
S2_H696A_revS	TTTTGTCCAGTTGCTTACG
S2_K698A_forT	AACATGCCGCAGATTGGTATTGTTGATACCAGAA
S2_K698A_forS	TATTGTTGATACCAGAA
S2_K698A_revT	CCAAAATCTGCGGCATTTAAGATACTTTGTCCAG
S2_K698A_revS	TAAGATACTTTGTCCAG
S2_R320A_forT	GCTGCGGAGGCAAAATTAACTGAAATCCACCAAGTGATC
S2_R320A_forS	TGAAATCCACCAAGTGATC
S2_R320A_revT	GTAAATTTGCCTCCGCAGCGTAATTGGATGTCTAAC
S2_R320A_revS	GGTAATTGGATGTCTAAC
S2_K321A_forT	GCGGAGCGAGCATTAACTGAAATCCACCAAGTGATCTC
S2_K321A_forS	AATCCACCAAGTGATCTC
S2_K321A_revT	TCAGTTAATGCTCGCTCCGCAGCGTAATTGGATGTCTC
S2_K321A_revS	AGCGGTATTGGATGTCTC
S2_T323A_forT	CGAAAATTAGCTGAAATCCACCAAGTGATCTCTTTGC
S2_T323A_forS	ACCAAGTGATCTCTTTGC
S2_T323A_revT	GGATTTCAGCTAATTTCGCTCCGCAGCGTAATTGG
S2_T323A_revS	CTCCGCAGCGTAATTGG
S2_K346A_forT	CCATTTCAGGAGCAACTAAAATTGGCGGTTACCAAG
S2_K346A_forS	TGTTGCGGTTACCAAG
S2_K346A_revT	GTTTTAGTTGCTCTGAAATGGTGCAGAGCTTCTT
S2_K346A_revS	TTGCGGCAGAGCTTCTT
S2_T347A_forT	TCAGGAAAAGCTAAAATTGGCGGTTACCAAGAACAG
S2_T347A_forS	TGCGGTTTACCAAGAACAG
S2_T347A_revT	ACAGTTTAGCTTCTGAAATGGTGCAGAGCT
S2_T347A_revS	AATGGTTGCGGCAGAGCT
S2_R550A_forT	CTTCCCATTGCTCTGGCATTCTGATGAGAATGGAG
S2_R550A_forS	TTTCTGATGAGAATGGAG
S2_R550A_revT	ATGCCAGAGCAATGGAACATCCAGACTCCATTTC
S2_R550A_revS	ATCCAGACTCCATTTC
S2_T685A_forT	GATCGGTTGCTCGTAAGCAACTGGACAAAAAGTATCT
S2_T685A_forS	AACTGGACAAAAAGTATCT
S2_T685A_revT	GCTTACGAGCAAACCGATCTGAATCCAAGGGATCGTT
S2_T685A_revS	TGAATCCAAGGGATCGTT
S2_K687A_forT	GTTTACTCGTGCACGACTGGACAAAAAGTATCTAAAC
S2_K687A_forS	ACAAAAAAGTATCTAAAC
S2_K687A_revT	CCAGTTGCGCACGACTAAACCGATCTGAATCCAAGGGAA
S2_K687A_revS	CGATCTGAATCCAAGGGAA
S2_Q688A_forT	ACTCGTAAGGCACGGACAAAAAGTATCTAAACATGC
S2_Q688A_forS	AAAGTATCTAAACATGC
S2_Q688A_revT	TTGTCCAGTGCCTACGAGTAAACCGATCTGAATCCAAGG
S2_Q688A_revS	AAACCGATCTGAATCCAAGG

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S2_K691A_forT	CAACTGGACGCAAAGTATCTAACATGCCAAAGATTG
S2_K691A_forS	TAAACATGCCAAAGATTG
S2_K691A_revT	AGATACTTGCCTCAGTTGCTTACGAGTAACCGATC
S2_K691A_revS	CTTACGAGTAAACCGATC
S2_K692A_forT	CTGGACAAAGCGTATCTAACATGCCAAAGATTG
S2_K692A_forS	ATGCCAAAGATTG
S2_K692A_revT	GTITAAGATACGCTTGTCCAGTTGCTTACGAGTAACCG
S2_K692A_revS	TTGCTTACGAGTAAACCG
S2_D699A_forT	CATGCCAAAGCTTGTATTGTTGATACCAGAAAAAATAG
S2_D699A_forS	TTGATACCAAGAAAAAATAG
S2_D699A_revT	CAATACCAAAAGCTTGGCATGTTAAGATACTTTGTC
S2_D699A_revS	TTAAGATACTTTGTC
S2_R706A_forT	GTTGATACCGAAAAAATAGTGAAACACTGACTAAATTAG
S2_R706A_forS	AAACACTGACTAAATTAG
S2_R706A_revT	CACTATTTTGCCTGTTGATCAACAATACCAAAATCTTGG
S2_R706A_revS	AATACCAAAATCTTGG
S2_K707A_forT	GATACCAGAGCAAATAGTGAAACACTGACTAAATTAG
S2_K707A_forS	AAACACTGACTAAATTAG
S2_K707A_revT	TCACTATTGCTCTGGTATCAACAATACCAAAATCTTGG
S2_K707A_revS	AACAATACCAAAATCTTGG
S2_S709A_forT	CAGAAAAAAATGCTGAAACACTGACTAAATTAGAGACGC
S2_S709A_forS	TGACTAAATTAGAGACGC
S2_S709A_revT	GTGTTTCAGCATTCTGGTATCAACAATACCAAA
S2_Y734A_forT	AAGGGACAGCTACTTGTGAAGGATTCAAAGGTTTC
S2_Y734A_forS	AAGGATTCAAAGGTTTC
S2_Y734A_revT	CACAAGTAGAGCTGCCCCCTTCAAAAGTTCTTTCC
S2_Y734A_revS	TTTCAAAAGTTCTTTCC
S2_S740A_forT	GTGAAGGATGCAAAGGTTCTTAACCGAAGACGAA
S2_S740A_forS	TTAACCCGAAGACGAA
S2_S740A_revT	AAAAACCTTGCATCCTCACAAAGTAGATATGCCCT
S2_S740A_revS	AAAGTAGATATGCCCT
S2_D755A_forT	GTTGTCATGGCTAAGGATAATAAATTATTCCGGTTGG
S2_D755A_forS	AAATTATTCCGGTTGG
S2_D755A_revT	ATTATCCTAGCCATGACAACACATTATTCTCTTCCG
S2_D755A_revS	AACATTATCGTCTCGG
S2_D757A_forT	CATGGATAAGGCTAATAAATTATTCCGGTTGGAAGTTGG
S2_D757A_forS	ATTCCGGTTGGAAGTTGG
S2_D757A_revT	AAATTATTAGCCTATCCATGACAACACATTATCGT
S2_D757A_revS	ACAACAAACATTATCGT
S2_F760A_forT	GATAATAAAGCTATTCCGGTTGGAAGTTGGATGTTGATT
S2_F760A_forS	TGGAAGTTGGATGTTGATT
S2_F760A_revT	ACCGGAAATAGCTTATTATCCTATCCATGACAACAC
S2_F760A_revS	CTTATCCATGACAACAC
S2_S762A_forT	AAATTATTGCCGGTTGGAAGTTGGATGTTGATTCTCAG
S2_S762A_forS	TTGGATGTTGATTCTCAG
S2_S762A_revT	CTTCCAACCGGAATAAATTATTATCCTTATCCATGAC
S2_S762A_revS	ATTATCCTTATCCATGAC

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S2_W764A_forT	ATTCGGGTGCGAAGTTGGATGTTGATTCTCAGCAGTAT
S2_W764A_forS	GTTGATTCTCAGCACTAT
S2_W764A_revT	ATCCAACCTCGCACCGAAATAAATTATTATCCTTATCC
S2_W764A_revS	AAATTATTATCCTTATCC
S2_D767A_forT	TGGAAGTTGGCTGTTGATTCTCAGCAGTATAAAAAC TACG
S2_D767A_forS	AGCAGTATAAAAAC TACG
S2_D767A_revT	GAGAATCACAGCCAACITCCAACCGGAAATAAATTATTATC
S2_D767A_revS	ACCGGAAATAAATTATTATC

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