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**RESEARCH ARTICLE**

# FUNCTIONAL GAIN OF FRUIT NETTED- CRACKING IN AN INTROGRESSION LINE OF TOMATO WITH HIGHER EXPRESSION OF THE *FNC* GENE

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## SUPPLEMENTARY MATERIALS

**Table S1** Indel marker primers used for fine mapping of the *FNC* gene

Primer name	Primer sequence
M1	Fw: GGGCAAGGCAATAATTTACTAC Rv: AATGGTTGTCATCCAAACTCTGC
M2	Fw: TTGCAATTCGTTTTTCTCATGTC Rv: CAAGTTGCATCAAATAAATACGAAA
M3	Fw: TTGCAATTCGTTTTTCTCATGTC Rv: CATCAAGTTGCATCAAATAAATACG
M4	Fw: TCATGAACTTTCAAGCAAAAACAAT Rv: TGCTAATACTATTGCCCTATCCCA
M5	Fw: TGACCAAATTCAAACACTTAGTAGCA Rv: GTGAAGTTTGAGTCTTGGCCATT
M6	Fw: TATTGCCAGTCAGTTCGCTTTTAT Rv: GTAAGGGTGGAGTTCAATAGATGC
M7	Fw: GCATAGCAATAACGTCATGACTCA Rv: AAATTAAGTGTGCGAAAAACATGTGG
M8	Fw: TAACAGACGTGTAAAACATCCTTGC Rv: ATTGGCCTTGCTTATGAATCAGA
M9	Fw: ATTCATTGTTTGAATATGAGCTTCG Rv: GATAAGCCTTTTAGCACCGTCAA
M10	Fw: GATCTTACTAGGTTCAATTTGTGGGA Rv: AAATTTTGGAGATTGCCTAGAGATG
M11	Fw: GTGCTCCAATGGTTCCTCTAAGA Rv: TTGGTGCTGATGAGAAAAGTAGGT
M12	Fw: TTCGAACATTGATAAGCTACGTAGTG Rv: TAGGCAATTTTTTATGAGTTAGGCA
M13	Fw: ACGGACTATCTTCTCCACTTGGT Rv: GACATGTATTGGAATATTGTGGCAG
M14	Fw: ACTGGCGGCACTACTTTGAACTA Rv: ATGAGCATTGGGCTTATGAACTT
M16	Fw: GTCTCTTCTATTGCTCTTTTGG Rv: ATTTCTCGAAGATGAGTCAAACCTGA
M17	Fw: CTGTATCTGTAAATCACATTCGCTT Rv: CGTTCTGATGACTTTACTTCCAATAG
M19	Fw: AACCGTCCGTGTCTTTTCTTATT Rv: CTTTACTCATCTCTCCTGGAATCTTAC
M20	Fw: TTACCACAACGCTCAACAAGATTTA Rv: TTGCCTACTGGTCAAGGATAGATTT
M21	Fw: AGTCTCTTTGATGCTTTATGTCCAG Rv: TCTCTGTCTGTGCTTGTGTTGATAA
M22	Fw: TCCTGAAATATGACAGTAGAACCCA Rv: GGATCAGTGCTATTCCCTGTACATT

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M23	Fw: GCTCTGATACTCAACTTTTGGCATA Rv: CTGGCTATTAACGCTACAACAAGA
M24	Fw: TGCGGCTTCATTTTCATCTGTAA Rv: ATCCTCAAATGTATGACGCCCT
M25	Fw: AGCTTGAATGATTGCAACTGAC Rv: TTGTGGTGAGTTGCTAAAGCTGTAT
M26	Fw: ACTTTGTGGTGTCTATTTGGATTGA Rv: TGAATTGCATCCACCTGTAAAGTT
M27	Fw: TGCATACTCCTCTTGTTATTTGACTT Rv: TCTCAATCTTCACCTCAGAACAGTAC
M28	Fw: TAACTCCTGCTCAGAGAGGCTAAC Rv: TAGCTATATTGACTCATGGACAGGC
M29	Fw: ATGGTTGGATTATGGTGAAGGAGT Rv: GTCATGCTTCTCAGTTTGTCTACCA
M30	Fw: GAACCAGACACCATTAACTTTAGGC Rv: GAGAACTGTTTCGAGGATCTATCATG
M31	Fw: CCTGTTGCCATTGAACTATCACTAA Rv: GCACTTGATAGAGGTTCAAGTCGATA
M32	Fw: ACTTTGTGGTGCCAATGTTTTCT Rv: TCTGCTTTTCTGCAACTTTGACTAC
M33	Fw: CGTCCAAGTCTAAAAGAGTCTATGATG Rv: CTTCTGATTTAGCCTTTTCATCTTCT
M34	Fw: ATTCATTTGGTAGGTAGGTTTTAGCT Rv: CCAAGCATCACCATCCATTATATTA
M35	Fw: AAGTACCTGAAGTCCAGATTTAGCT Rv: TGGTGTCTGTTCTTCCACATCAT
M41	Fw: TGCCAATATTGCATAATTTCTCACT Rv: CTCTAATTCGTGTTTATGTCCGTT
M42	Fw: CCATATGTCACTTTGCATGTAATTG Rv: TCAATCCCTAGCGTTTTCCCTATC
M46	Fw: ATCGTTTTGATTGTGGTATGTGAAG Rv: AATTGTTGTTATACACAGGAGCACC
M47	Fw: CATGATCACTAACAAGGGATGAAAA Rv: GATGTTGTTCCCTCCACCTCTAATA
M48	Fw: AAACGCATTATTTTGTATGTGGAG Rv: TTCCCTCGTTTTAGCTAATGTGAT
M49	Fw: GCACCTTCAACTCAACTCATAACA Rv: TAACAATTTTTCTCATTTCCAGGAC
M50	Fw: CATCAGACCATACTCCTCCAAAT Rv: TTGTAAGGAGGATGAGTTAAAGGGA
M51	Fw: TTGGACTCGTCAAAGGCTAATCA Rv: CCATCTCTACCTTCAATTCAACTGTC
M52	Fw: TGCGATAACAACAACATAACCCT Rv: ATGACCACTAATAACACAGACAACCTC

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**Table S2** Primers used in this work

Primer name	Gene ID	Primer sequence
FNC-Q		Fw: TCCAACCAGGTATTCATGTGCTGTC RV: CGCAGATTTTCTCGTCATTCAC TTC
FNC-101YFP		Fw: ATATGGGATCTACTAGTGAATTCATGTGTATAGTAGTGT TTTATTGG RV: TCGAGCCCGGGGTACCGTCGACTTGCATTTGGAGTTTTTCAATCCG
FNC-OE-FW		Fw: CATTTGGAGAGGACACGCTCGAGATGTGTATAGTAGTGT TTTATTGGGAAGC RV: TCTCATTAAGCAGGACTCTAGATTATTGCATTTGGAGTTTTTCAATC
4CL-Q	Solyc06g068650.2.1	Fw: TGTATTTGTACGTTAAACCACCTGC RV: ATACATACATTTTTCTCCTTTTGCC
PAL-Q	Solyc10g086180.1.1	Fw: CACCTGCCTCTATACGGTAAAATT RV: ACACCAGCCATGTCCCTTACAGTCA
NLTP9-Q	Solyc09g082270.2.1	Fw: AACTCCACCTCTTAGCCAATGCAAT RV: GAACTCTGTTAGATCCACGTCCTGA
LTP-Q	Solyc09g065430.2.1	Fw: CAAACTCCTCCACTTAGTCGATGCA RV: AGATGAACCAGTTGGAGCAGGGGAA
Laccase13-Q	Solyc06g082240.2.1	Fw: CCTATGTGTGAGCCATCAGGGTTTA RV: TCTTTCTTGAGAGGTA CTTCACAT
PG-Q	Solyc08g060970.2.1	Fw: ATGGAGGGCCAAGCACTTATCTT RV: TACCAGCAACACTTCTTTCAATAAGAAA
Desaturase-Q	Solyc12g044950.1.1	Fw: ATGGGAGGTGGTGGTAATATGTCTG RV: ACTCTTTTCGAGAGGATTTTTCTTCTG
EXPA11-Q	Solyc04g081870.2.1	Fw: TTCGGGATTTGAAGTTTATGAGTTT RV: AATAAAAGAAGACAGAGGAAGGGGG
SIGPAT5-Q	Solyc04g011600.2.1	Fw: GTTATTGTCAAGGGCAAACCACCAC RV: ATCGAGAGATTGAGTACGTCACGGC
SIASFT-Q	Solyc03g097500.2.1	Fw: TGGCTCAGGTGACAAAAGTTCAAATG RV: AAACCTCTCGCAATTTACCCCAAG
GDSL-Q	Solyc02g090210.2.1	Fw: ATTGAAGGAGAACGAGTGTGTGGAG RV: AAAAGTTGCTCCGTCGAGTTCTTTG
FAR-Q	Solyc06g074390.2.1	Fw: CTGCATATGTATCAGGGGAAAAAAG RV: CTCTCAGCTGTTTCAATGTTTCCTC
KCS-Q	Solyc03g005320.2.1	Fw: TCTTGCTAAACA ACTATTGCAGGTG RV: TCCATACTCACCACAAGTGCATAAG

**Table S3** Genes ID of the candidate region

Gene ID	Annotated Function
Solyc04g082500.2.1	ATP binding / serine-threonine kinase
Solyc04g082510.2.1	Serine/threonine-protein kinase
Solyc04g082520.1.1	Ring zinc finger protein (Fragment)
Solyc04g082530.2.1	Bromodomain-containing protein
Solyc04g082540.1.1	Ser/Thr-rich protein T10 in DGCR region
Solyc04g082550.1.1	Trehalose-6-phosphate phosphatase
Solyc04g082560.2.1	Actin cytoskeleton-regulatory complex protein PAN1
Solyc04g082570.2.1	U-box domain-containing protein
Solyc04g082580.2.1	Kelch repeat-containing F-box family protein
Solyc04g082590.2.1	Canopy homolog 2
Solyc04g082600.2.1	Serine/threonine phosphatase family protein
Solyc04g082610.2.1	Glutamate-gated kainate-type ion channel receptor subunit GluR5
Solyc04g082620.2.1	Serine threonine-protein kinase
Solyc04g082630.2.1	Glyceraldehyde-3-phosphate dehydrogenase B
Solyc04g082640.2.1	Unknown Protein
Solyc04g082650.2.1	Palmitoyltransferase erf2
Solyc04g082660.2.1	Peptidyl-prolyl cis-trans isomerase
Solyc04g082670.2.1	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1
Solyc04g082680.2.1	Chloroplast channel forming outer membrane protein
Solyc04g082690.1.1	Ring finger protein
Solyc04g082700.2.1	Solute carrier family 2%2C facilitated glucose transporter member 12
Solyc04g082710.2.1	Cathepsin B-like cysteine proteinase 3
Solyc04g082720.2.1	Small heat shock protein
Solyc04g082730.1.1	Unknown Protein
Solyc04g082740.2.1	Heat shock protein-like protein
Solyc04g082750.2.1	3-beta hydroxysteroid dehydrogenase/isomerase family protein
Solyc04g082760.2.1	SWI/SNF complex subunit SMARCC1
Solyc04g082770.2.1	RNA-binding protein 68390-68829
Solyc04g082780.2.1	Cinnamoyl CoA reductase-like 1
Solyc04g082790.2.1	Atp-dependent RNA helicase
Solyc04g082800.2.1	Unknown Protein
Solyc04g082810.2.1	AT-hook DNA-binding protein (Fragment)
Solyc04g082820.2.1	ARID/BRIGHT DNA-binding domain-containing protein
Solyc04g082830.2.1	Auxin efflux carrier family protein
Solyc04g082840.2.1	Cell division protein kinase 2
Solyc04g082860.1.1	UDP-glucose glucosyltransferase
Solyc04g082870.1.1	Pentatricopeptide repeat-containing protein
Solyc04g082880.2.1	Diphosphate--fructose-6-phosphate 1-phosphotransferase

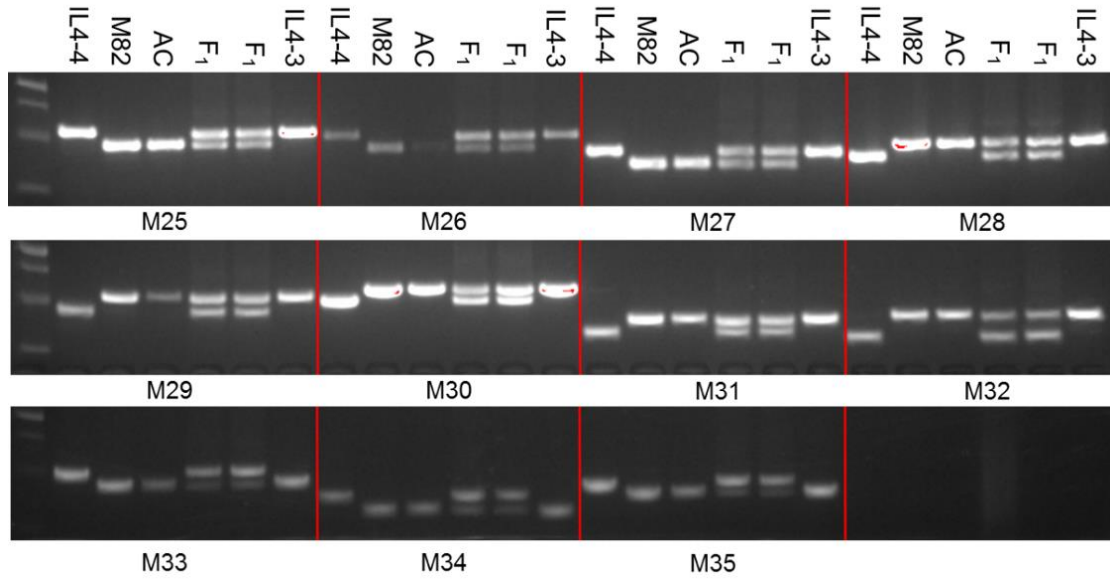


Fig. S1 Screening indel markers for the *FNC* gene fine mapping.

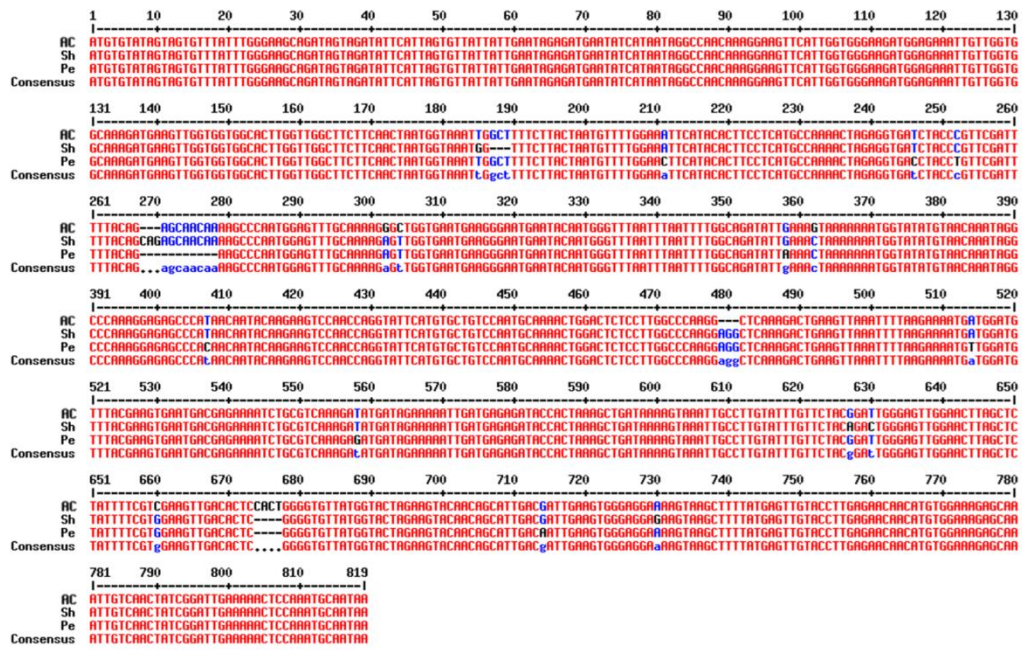
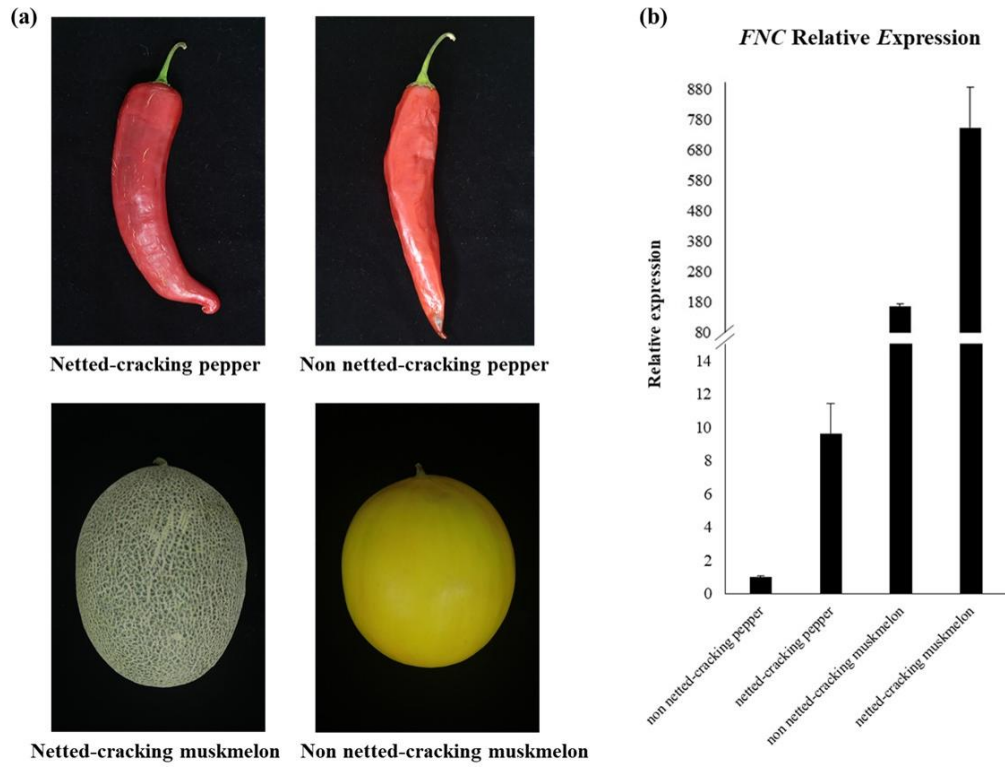
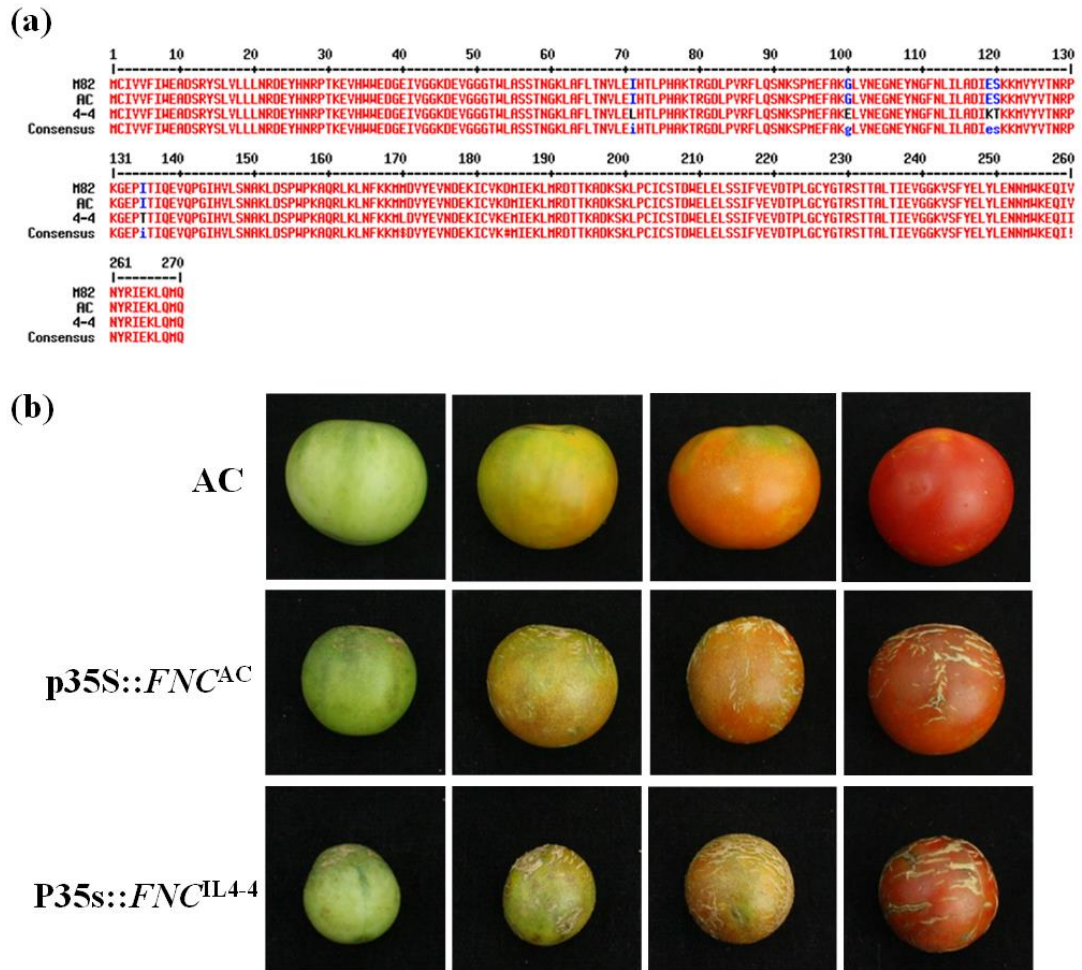


Fig. S2 Alignment of *FNC* gene sequences from the tomato cultivar Ailsa Craig (AC), wild species *Solanum habrochaites* (Sh) LA1777 and *S. pennellii* (Pe) LA0716, showing the differences in *FNC* alleles.



**Fig. S3** Expressions of homologous *FNC* in netted-cracking/non-netted-cracking pepper and muskmelon. (a) Mature fruit of peppers and muskmelons with netted-cracking and non-netted-cracking phenotypes. (b) Expression of *FNC* in netted-cracking phenotypes was higher than that in non-netted-cracking ones.



**Fig. S4** The amino acid sequence alignment and overexpressing phenotype of FNC in different development stages of fruit. (a) Amino acid sequence alignment of FNC protein from AC, M82 and IL4-4. (b) Overexpression of  $FNC^{IL4-4}$  and  $FNC^{AC}$  lead to the appearance of the netted-cracking in tomato during different stages of fruit.

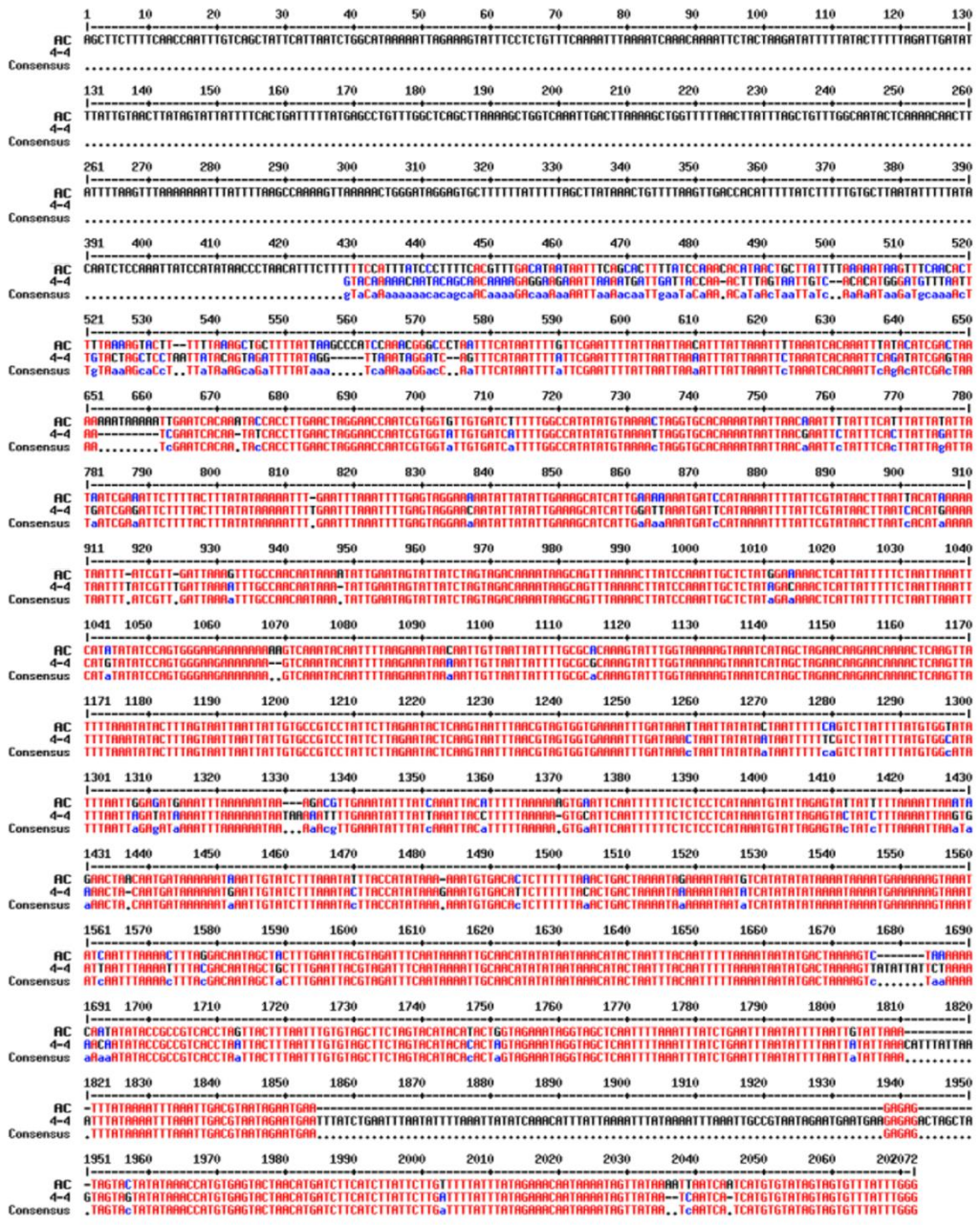
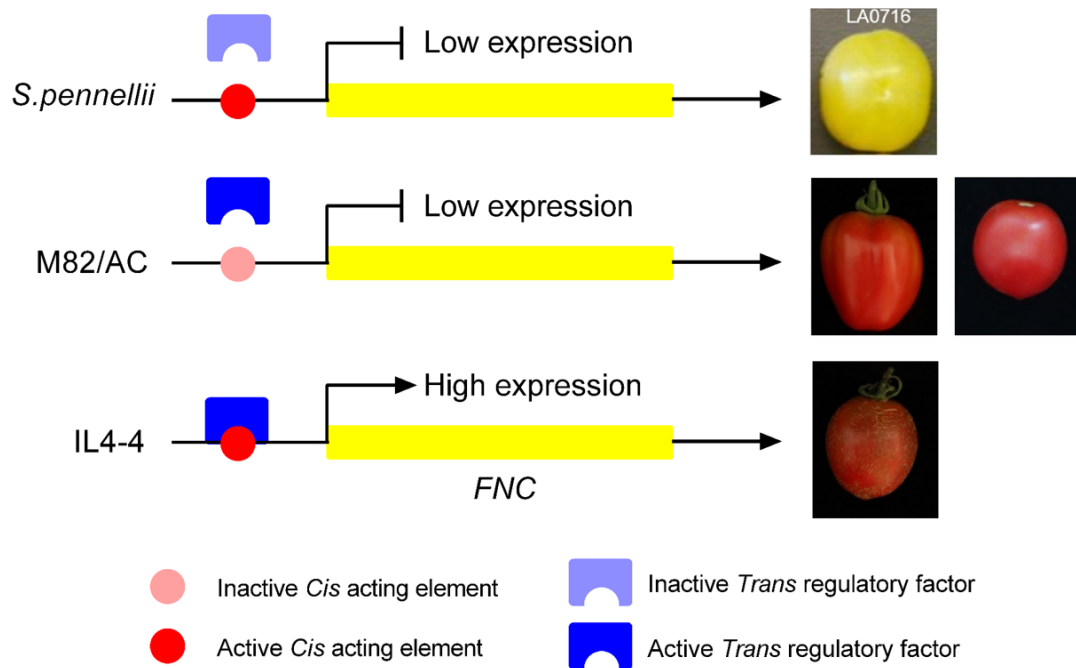


Fig. S5 Sequence alignment of *FNC* promoter regions from the netted-cracking IL4-4 and the non-cracking AC.



**Fig. S6** The proposed mechanism of netted-cracking. The netted-cracks on tomato fruit are postulated to be a result of the synergism (in *cis-trans* acting) of two loci (*FNC* and another *trans*-regulatory factor) at different locations in the genomes. In *S. pennellii*, the inactive *trans*-regulatory was unable to bind to active *cis*-acting elements on *FNC* promoters, and the active *trans*-regulatory was also unable to bind to inactive *cis*-acting elements on *FNC* promoters in M82 and AC. However, in IL4-4, an active *trans*-regulatory was able to bind to active *cis*-acting elements on *FNC* promoters to increase its expression and cause the formation of netted-cracking.