

Re-evaluation of transcription factor function in tomato fruit development and ripening with CRISPR/Cas9-mutagenesis

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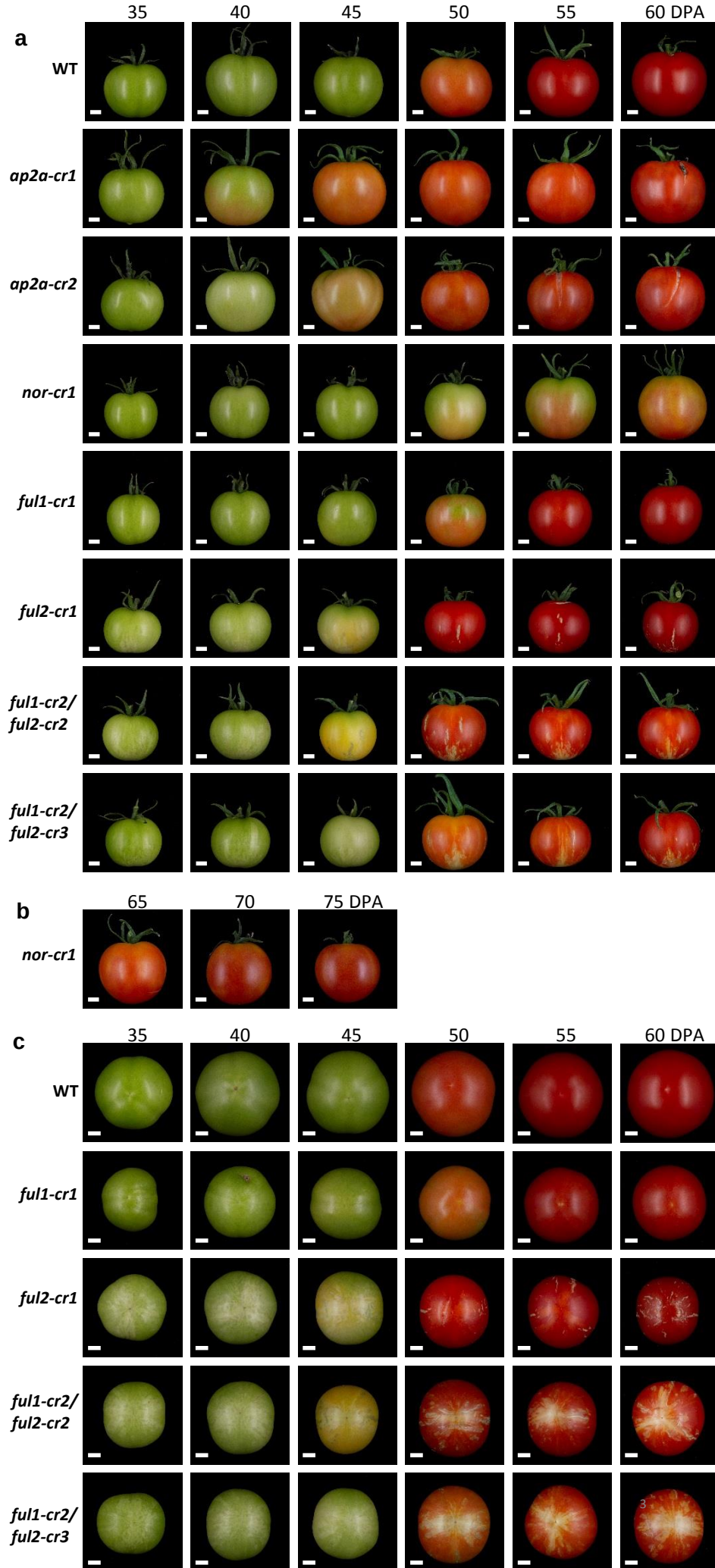
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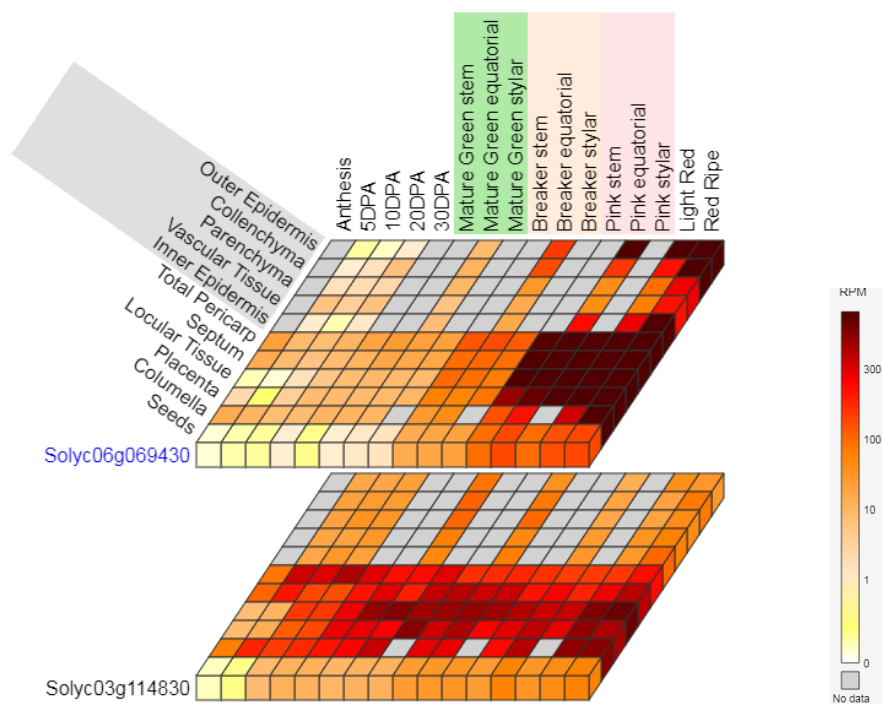
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a		-69	-60	-5	1		t1		t2		74
	WT	ACATCAATAT		AAGAAATG	TGGAATTAAA	TGAT	CCCC	TGATCAGACAA	TGGAGTATGAATCCGATGAAGGTATAACGGT	TAGAT	CGGA
	ap2a-cr1	ACATCAATAT		AAGAAATG	TGGAATTAAA	TGAT	CCCC	TGATCAGA	-----	AGAT	CGGA
	ap2a-cr2	AC-----							-----	AGAT	CGGA
	ap2a-cr3	ACATCAATAT		AAGAAATG	TGGAATTAAA	TGAT	CCCC	TGATCAGA	-----	GAT	CGGA
	ap2a-cr4	ACATCAATAT		AAGAAATG	TGGAATTAAA	TGAT	CCCC	TGATCAGACAA	TGGAGTATGAATCCGATGAAGGTATAACGGT	AGAT	CGGA
b		43			t1		69	540		560	
	WT	CAACCGGGACACGTC	ATCAGCCTCAACTCCC	-ACCGGGG	TTTCGATTCCAC			TAACACACAAAGGTCCATAGA			
	nor-cr1	CAACCGGGACACGTC	ATCAGCCTCAACTCCC	-CCGGGG	TTTCGATTCCAC			TAACACACAAAGGTCCATAGA			
	nor-cr2	CAACCGGGACACGTC	ATCAGCCTCAACTCCC	ACCGGGG	TTTCGATTCCAC			TAACACACAAAGGTCCATAGA			
	nor-cr3	CAACCGGGACACGTC	ATCAGCCTCAACTC	---ACCGGG	TTTCGATTCCAC			TAACACACAAAGGTCCATAGA			
	nor-s	CAACCGGGACACGTC	ATCAGCCTCAACTCCC	-ACCGGGG	TTTCGATTCCAC			TAACACACA--GGTCCATAGA			
	nor-scr1	CAACCGGGACACGTC	ATCAGCCTCAACTCCC	-CCGGGG	TTTCGATTCCAC			TAACACACA--GGTCCATAGA			
	nor-scr2	CAACCGGGACACGTC	ATCAGCCTCAACTCCC	ACCGGGG	TTTCGATTCCAC			TAACACACA--GGTCCATAGA			
c		186	195	234		t2	264	291		t3	324
	WT	ATGTATGTAATTAGCATGGAGAGG		ACAGCTTGT	TCCTACTGATCATACCTCCC	CGGTAT		ACTTAAGGCCAGACTTGAGGTTCTG	-CAGAGGAAC		
	ful1-cr1	ATG-----		-----	-----	TAT		ACTTAAGGCCAGACTTGAGGTTCTG	-CAGAGGAAC		
	ful1-cr2	ATGTATGTAATTAGCATGGAGAGG		ACAGCTTGT	TCCTACTGATCATACCTCCC	CGGTAT		ACTTAAGGCCAGACTTGAGGTTCTG	--AGAGGAAC		
	ful1-cr3	ATGTATGTAATTAGCATGGAGAGG		ACAGCTTGT	TCCTACTGATCATACCTCCC	CGGTAT		ACTTAAGGCCAGACTTGAGGTTCTG	---CAGAGGAAC		
	ful1-cr4	ATGTATGTAATTAGCATGGAGAGG		ACAGCTTGT	TCCTACTGATCATAC	---CCC	CGGTAT	ACTTAAGGCCAGACTTGAGGTTCTG	-CAGAGGAAC		
	ful1-cr5	ATGTATGTAATTAGCATGGAGAGG		ACAGCTTGT	TCCTACTGAT	-----CCC	CGGTAT	ACTTAAGGCCAGACTTGAGGTTCTG	-CAGAGGAAC		
	ful1-cr6	ATGTATGTAATTAGCATGGAGAGG		ACAGCTTGT	TCCTACTGATCA	-----CCC	CGGTAT	ACTTAAGGCCAGACTTGAGGTTCTG	-CAGAGGAAC		
	ful1-cr7	ATGTATGTAATTAGCATGGAGAGG		ACAGCTTGT	TCCTACTGATCATACCTCCC	CGGTAT		ACTTAAGGCCAGACTTGAGGTTCTG	--CAGAGGAAC		
	ful1-cr8	ATGTATGTAATTAGCATGGAGAGG		ACAGCTTGT	TCCTACTGATCATACCTCCC	CGGTAT		ACTTAAGGCCAGACTTGAGGTTCTG	-CAGAGGAAC		
	ful1-cr9	ATGTATGTAATTAGCATGGAGAGG		ACAGCTTGT	TCCTACTGATCATACCTCCC	CGGTAT		ACTTAAGGCCAGACTTGAGGTTCTG	---CAGAGGAAC		
	ful1-cr10	ATGTATGTAATTAGCATGGAGAGG		ACAGCTTGT	TCCTACTGATCATA	---CCC	CGGTAT	ACTTAAGGCCAGACTTGAGGTTCTG	-CAGAGGAAC		
	ful1-cr11	ATGTATGTAATTAGCATGGAGAGG		ACAGCTTGT	TCCTACTGATC	-----CCC	CGGTAT	ACTTAAGGCCAGACTTGAGGTTCTG	-CAGAGGAAC		
	ful1-cr12	ATGTATGTAATTAGCATGGAGAGG		ACAGCTTGT	TCCTACTGAT	-----AT		ACTTAAGGCCAGACTTGAGGTTCTG	-CAGAGGAAC		
	ful1-cr13	ATGTATGTAATTAGCATGGAGAGG		ACAGCTTGT	TCCTACTGATCATACCTCCC	CGGTAT		ACTTAAGGCCAGACTTGAGGTTCTG	-CAGAGGAAC		
	ful1-cr14	ATGTATGTAATTAGCATGGAGAGG		ACAGCTTGT	TCCTACTGATCATA	---CCC	CGGTAT	ACTTAAGGCCAGACTTGAGGTTCTG	--CAGAGGAAC		
	ful1-cr15	ATGTATGTAATTAGCATGGAGAGG		ACAGCTTGT	TCCTACTGATCATACCTCCC	CGGTAT		ACTTAAGGCCAGACTTGAGGTTCTG	-CAGAGGAAC		
	FUL2	TCTTGTCAATGTTAGCATGGAAGG		GCAGCTTAATGCTACTGATATTATA	ACCCCGGTAT			GCTTAAGGCCAGACTTGAGGTTCTG	-CAAGGAAC		
d		35		t1		t2		93			
	WT	AGAACAAAATTAATCGTCAAGTT	ACTTTTTC	AAGAGGCG	-ATC	TGGTTTGCTTAAAAA					
	ful2-cr1	AGAACAAAATTAATCGTCAAGTT	ACTTTTTC	AAGAGGCG	ATC	TGGTTTGCTTAAAAA					
	ful2-cr2	AGAACAAAATTAATCGTCAAGTT	ACTTTTTC	AAGAG	---ATC	TGGTTTGCTTAAAAA					
	ful2-cr3	AGAACAAAATTAATCGTCAAGTT	ACTTTTTC	AAGAGGCG	ATC	TGGTTTGCTTAAAAA					
	ful2-cr4	AGAACAAAATTAATCGTCAAGTT	ACTTTTTC	AAGAGGCG	-ATC	TGGTTTGCTTAAAAA					
	ful2-cr5	AGAACAAAATTAATCGTCAAGTT	ACTTTTTC	AAGAGG	--ATC	TGGTTTGCTTAAAAA					
	FUL1	AGAACAAAATTAACCGTCAAGTT	ACTTCTCG	-AAAGT	TCG	-ATCTGGTTTGCTGAAGAA					

Supplementary Figure 1. All alleles obtained by using CRISPR/CAS9 mutagenesis. **(a)** All mutant alleles in *AP2a*. **(b)** All alleles obtained in *NAC-NOR*. **(c)** 15 alleles obtained in *FUL1*. Alignment differences between *FUL1* and the corresponding region in *FUL2* are shown in green. **(d)** Mutant alleles in *FUL2*. Alignment differences between *FUL2* and the corresponding region in *FUL1* are shown in green. Letters in red indicate spacer sequences and underlined are protospacer adjacent motifs (PAM). The start codon is indicated with red boxes. Numbers represent the location of the nucleotide in coding sequence.



Supplementary Figure 2. Development and ripening of homozygous mutants in the T_1 generation. **(a)** Pictures taken every 5 days from 35 DPA show the difference in developmental and ripening processes among all lines in this study. **(b)** Ripening in fruits of *nor-cr1* is slower than other mutants and its pericarp still stays in dark orange until 75 DPA. **(c)** Development of stripes at the bottom of all *ful* mutants. Light coloured-stripes are visible from the early stages of fruit development and only in mutants with *ful2* alleles. Scale bar, 1 cm.



Supplementary Figure 3. Expression of *FUL1* (Soly06g069430) and *FUL2* (Soly03g114830) in different tissues and stages of tomato fruit development and ripening from the TEA.

Primer name	Sequence (5'-3')	Description
AP2a-t1-F	TGTGGTCTCAATTGGATTCCCCTGATCAGACAAGTTT TAGAG CTAGAAATAGCAAG	Forward primer for target 1 in <i>AP2a</i>
AP2a-t2-F	TGTGGTCTCAATTGGAAGGTATAACGGTTAGATGTTT TAGAG CTAGAAATAGCAAG	Forward primer for target 2 in <i>AP2a</i>
NOR-t1-F	TGTGGTCTCAATTGATCAGCCTCAACTCCCACCGGTTT TAGA GCTAGAAATAGCAAG	Forward primer for target 1 in <i>NOR</i>
NOR-t6-F	TGTGGTCTCAATTGGAAAATATCCTAACGGGGCGGTTT TAGA GCTAGAAATAGCAAG	Forward primer for target 6 in <i>NOR</i>
FUL1-t2-F	TGTGGTCTCAATTGCCTACTGATCATACTCCCGTTT TAGAGC TAGAAATAGCAAG	Forward primer for target 2 in <i>FUL1</i>
FUL1-t3-F	TGTGGTCTCAATTGCAGACTTGAGGTTCTGCAGGTTT TAGAG CTAGAAATAGCAAG	Forward primer for target 3 in <i>FUL1</i>
FUL2-t1-F	TGTGGTCTCAATTGTCAAGTTACTTTTTCAAAGGTTT TAGAGC TAGAAATAGCAAG	Forward primer for target 1 in <i>FUL2</i>
FUL2-t2-F	TGTGGTCTCAATTGCCTTTTCAAAGAGGCGATCGTTT TAGAGC TAGAAATAGCAAG	Forward primer for target 2 in <i>FUL2</i>
CRISPR Universal R	TGTGGTCTCAAGCGTAATGCCAACTTTGTAC	Universal Reverse primer for all gRNAs
NPT2-F	AGACAATCGGCTGCTCTGAT	Genotyping transgenic plants for NPT2
NPT2-R	AGCCAACGCTATGTCCTGAT	Genotyping transgenic plants for NPT2
CAS9-F	CTTTGGCAATATCGTGACG	Genotyping transgenic plants for CAS9
CAS9-R	CGTTCTTCTTCTCCCAGGG	Genotyping transgenic plants for CAS9
AP2a-F	CGTGGGGGTGTATTTAACG	Genotyping <i>ap2a</i> mutants
AP2a-R	GGCGATTCCAAATTGTGG	Genotyping <i>ap2a</i> mutants
NOR-F	CGAATTATCAACCTCGTA	Genotyping <i>nor</i> mutants
NOR-R	TTATCACAACCAAGTGGC	Genotyping <i>nor</i> mutants
FUL1-F	GACCTTCGCTTATAGCTCTATCCC	Genotyping <i>ful1</i> mutants
FUL1-R	CTTCTCCCACATAATGCCTGC	Genotyping <i>ful1</i> mutants
FUL2IN1-F	TTTCTTCCGTCTGTCTCCA	Check <i>FUL2</i> targets region in <i>ful1</i> mutants
FUL2IN1-R	GAATCAGGGCGGCTCAATA	Check <i>FUL2</i> targets region in <i>ful1</i> mutants
FUL2-F	CTGGGGAGATCCTTCC	Genotyping <i>ful2</i> mutants
FUL2-R	TGAGTCCAACTTCAGCATCG	Genotyping <i>ful2</i> mutants
FUL1IN2-F	CGCTGGCTTAGTGCAAGTA	Check <i>FUL1</i> targets region in <i>ful2</i> mutants
FUL1IN2-R	AGCAGAATCGAGCTGGTGT	Check <i>FUL1</i> targets region in <i>ful2</i> mutants
Actin-F	TGAGAGGTGCCTGATGCATTGC	qPCR for <i>Actin</i>
Actin-R	ACGCTTCGACCAAGGGATGG	qPCR for <i>Actin</i>
qAP2a-F	AACGGACCACAATCTTGAC	qPCR for <i>AP2a</i>
qAP2a-R	CTGCTCGGAGTCTGAACC	qPCR for <i>AP2a</i>

Supplementary Table 1. Primers used in this study.