Variation in the fruit development gene POINTED TIP regulates

protuberance of tomato fruit tip

Song *et al*.



Supplementary Fig. 1. Phylogenetic tree analysis of PT.

Phylogenetic relationships of PT proteins from *Solanum lycopersicum*, *Arabidopsis thaliana*, *Oryza sativa*, and *Vitis vinifera*. The full-length amino acid sequences of PT paralogous and orthologous were downloaded from EnsemblPlants and aligned using Clustal W2. The phylogenetic tree was constructed using the neighbor-joining algorithm in MEGA 7. The red ID indicates PT.



Supplementary Fig. 2. Subcellular localization of PT^R and PT^H proteins.

Tobacco protoplasts were co-transformed with plasmids that express either PT^R-GFP or PT^H-GFP and a nuclear marker Ghd7-CFP. Free GFP served as a control. Green and cyan signals indicate fluorescence from GFP and the nuclear marker, respectively. Three independent experiments were performed.

TS-9	ATGAGTCAGGAGCCTAAAGAGTATAAAATATTAATAAAATTTTTAAAATCGTATATAAAATTTTATATTAACCAAAATGGTAAAATTGTTGGAACAACAAC
LA4053	ATGAGTCAGGAGCCTAAAGAGTATAAAATATTAATAAAATTTTTAAAATCGTATATAAAATTTTATATTAACCAAAATGGTAAAATTGTTGGAACAACAAC
TS-9	GATTTCCTCCACCGGAAAAAGCAAAATCGCTGCTAGTGCAGCGATTTTACAAAATGTGATTTTCATTTGAAAAAATTTCAAATCGCTACCTAGGCAG
LA4053	GATTTCCTCCACCGGAAAAAGCAAAATCGCTGCTAGTGCAGCGATTTTACAAAATGTGATTTTCATTTGAAAAAAATTTCAAATCGCTACCTAGGCAG
TS-9	CAATTTTTCATTTTTTTGTTTTTAAAAATGGAAATTGTTGCCTAGATTTTCTAAAAAAATGAAATTCGCTGCCTAGGTAGCGATTTGAATTTTTTT
LA4053	CAATTTTTCATTTTTTTTGTTTTTAAAAATGGAAATTGTTGCCTAGATTTTCTAAAAAAATGAAATTCGCTGCCTAGGTAGCGATTTGAATTTTTTT
TS-9	TAAAAATTATATTTTGCTTTTTCAGGTGGAGGAAATCGTTGTTATTCCAGCGATTTTGCCGTTTTGGTTAATATAAAATTTATATAACGTTTTGGAATTT
LA4053	TAAAAATTATATTTTGCTTTTTCAGGTGGAGGAAATCGTTGTTATTCCAGCGATTTTGCCGTTTTGGTTAATATAAAATTTATATAACGTTTTGGAATTT
TS-9	TTGTTAATATTTTATACCCTTTATGCTCCGAACTCAGTATTATATGTTAAAGACTTATAAAGTATTTCTTTGATGAATAGCAATTAAGTATTAGAATATAAAT
LA4053	TTGTTAATATTTTATACCCTTTATGCTCCGAACTCAGTATTATATGTTAAAGACTTATAAAGTATTTCTTTGATGAATAGCAATTAAGTATTAAATAAT
TS-9	TCTGAAAATTAAATAAATAAATAAAATAAAATAAAAATTATAAAGTGTATAATTCTAAGATCGAACTCTTATTTTATTTTGGTAAGATACTTTTCTACCTTA
LA4053	TCTGAAAATTAATAAATAAATAAATAAATAAAATA
TS-9 LA4053	GTACATTGATGTTTGCTAGTTTAAGCATTTCATTCTTATCGTACTATATATTGGTAAGGATAAAATTGCATACACACAC
TS-9 LA4053	ATACGTATTTTTGTCAGTCGTATGATTATACTCAGTGGTGTAGCCACATGATGTTCAGTGTCCAATTGGATATCCTTTGTCGGAAAAAAAA
TS-9	ATACAAGTTAAATGATACATGAAATGATTTGATAACATATTTTGGATAGTCTTGACACAATAAGTTATTGTAGCCCAGTGTTTTCGCCTCCTTTTAAAGA
LA4053	ATACAAGTTAAATGATACATGAAATGATTTGATAACATATTTTGGATAGTCTTGACACAATAAGTTATTGTAGCCCAGTGTTTTCGCCTCCTTTTAAAGA
TS-9	AGTGGTGCTTGTATTGTTTGAATTTCACTAGTTTTATTTTTGAAAAGAGCGCTTTTATCGTGCTATTTTTGAACACTCCTTGTGGAATTCCTTGCTCTGA
LA4053	AGTGGTGCTTGTATTGTTTGAATTTCACTAGTTTTATTTTTTGAAAAGAGCTTTTATCGTGCTATTTTTGAACACTCTTTGTGGAATTCCTTGCTCTGA
TS-9 LA4053	CATTGATTGTACTAAATATGTTAGTTTCATACTATATTGGTAAGAATAAAATTACATACA
TS-9 LA4053	CCGTTATACTAGATATATAATTGTCGTACTATATATAATGCTAGCTA
TS-9	TATTATTATCGTACTATATATTGTTAAGGATAAAATTGCATACATCTTAGCAAGTCATACGATTATACTGAATACATTATTGTAATACTATACATTGATAGTA
LA4053	TATTATTATCGTACTATATATTGTTAAGGATAAAATTGCATACATCTTAGCAAGTCATACGATTATACTGAATACATTATTGTAATACTATACATTGATAGTA
TS-9 LA4053	CTTAAAAGATTGCATACACCTACTATATTCATATTTAATTATGCTATTACACTGAGTATATCATTATCAAACTATATATA
TS-9	ACCTACCCTTTCTAGGGGTACACATATTTCAACCAGTCATTCGATTATACTAAATTTATTATTGTCGTACTATATATTGACAGTTAATTATAAGCTAAGATT
LA4053	ACCTACCCTTTCTAGGGGTACACATATTTCAACCAGTCATTCGATTATACTAAATTTATTGTCGTACTATATATTGACAGTTAATTATAAGCTAAGATT
TS-9	GCATACACCTACTGTTTTCAAATTTCAGTTGTGCAATTACACTGAATATATTACTAACATAGTTGTGTGATTACACTTGCTATATTATTGTAGTACTATATA
LA4053	GCATACACCTACTGTTTTCAAATTTCAGTTGTGCAATTACACTGAATATATTACTAACATAGTTGTGTGATTACACTTGCTATATTATTGTAGTACTATATA
TS-9	TTAGCAAGGATAAAATTGTATACACCTACTGTTTTCATATTTCAGTTATGCAATTACACTGAACATATTACTACATGATTGTGTGATTACACTTGCTATAT
LA4053	TTAGCAAGGATAAAATTGTATACACCTACTGTTTTCATATTTCAGTTATGCAATTACACTGAACATATTACTACATGATTGTGTGATTACACTTGCTATAT
TS-9	TATTGTCGTACTATATATTAGCAAGGATAAAATTGTATACACCTACCCTTTCTAAGGGTACAAATATTTCAACCAGTCGTATAATTTACAGTAAATATATCA
LA4053	TATTGTCGTACTATATATTAGCAAGGATAAAATTGTATACACCTACCCTTTCTAAGGGTACAAATATTTCAACCAGTCGTATAATTTACAGTAAATATATCA
TS-9 LA4053	TTATCGTACTACATATAATTATACTGAATATATTATTATCGTACTATATATA
TS-9 LA4053	AGCTAAGAAGCAAAAAACAATATTTCATCATATTGAATATATCTCTTTTCTCTCTC

Supplementary Fig. 3. Alignment of the *PT* promoter sequences.

TS-9 is an accession that produces non-pointed tip fruit. LA4053 is an accession that produces fruit with pointed tips.



Supplementary Fig. 4. Expression patterns of PT.

Relative transcript levels of *PT* in different tissues from pointed tip accessions and non-pointed tip accessions. TS-9, TS-19, and TS-35 produced fruit with non-pointed tips. LA4053, TS-72, and TS-253 developed pointed tip fruit. DPA, day post anthesis; IG, immature green. Error bars indicate mean \pm SE. *n*=three biological replicates. Source data are provided as a Source Data file.



Supplementary Fig. 5. Longitudinal sections from fruit produced by different transgenic lines and pertinent wild-type plants.

a Fruit from plants harboring the PT^R and PT^H alleles from the F2:3 population. **b**, **c** Fruit from PT^H -overexpressing lines (**b**: PT^H -OE-2, PT^H -OE-3, and PT^H -OE-5) and its pertinent wild type control (TS-3 PT^R +/+), (**c**: PT^H -OE-5, PT^H -OE-7, and PT^H -OE-8) and the pertinent wild-type line (TS-9 PT^H +/+). **d-f** Fruit from CR- pt^H mutants (CR- pt^H -1, CR- pt^H -4, and CR- pt^H -10) and pertinent wild type control (TS-9) that produces non-pointed tip fruit (**d**), CR- pt^R mutants (CR- pt^R -3, CR- pt^R -5, and CR- pt^R -6) and its pertinent wild type control (TS-3) that produces fruit with pointed tips (**e**), CR- pt^R mutants (CR- pt^R -1, CR- pt^R -4, and CR- pt^R -7) and the pertinent wild type control (TS-3) that produces fruit with pointed tips (**e**), CR- pt^R mutants (CR- pt^R -1, CR- pt^R -4, and CR- pt^R -7) and the pertinent wild type control (LA4053) that produces fruit with a pointed tip (**f**). **g** Fruit from CR- $pt^H/PT^R_{pro}:PT^R$ lines (CR- $pt^H/PT^R_{pro}:PT^R$ -3 and CR- $pt^H/PT^R_{pro}:PT^R$ -5) and its pertinent control (CR- pt^H).



Supplementary Fig. 6. Percentage of different fruit morphology produced by different transgenic lines and their pertinent wild-type plants.

a Percentage of pointed tip fruits from plants harboring PT^{R} and PT^{H} alleles from the F2:3 population. **b**, **c** Percentage of oval fruits from PT^{H} -overexpressing lines $(PT^{H}-OE-5, PT^{H}-OE-7, and PT^{H}-OE-8)$ and pertinent wild-type (TS-9, PT^{H} allele, **b**), PT^{H} -overexpressing lines (PT^{H} -OE-2, PT^{H} -OE-3, and PT^{H} -OE-5) and pertinent wild-type (TS-3, PT^{R} allele, c). d-f Percentage of pointed tip fruits from CR- pt^{H} mutants (CR- pt^{H} -1, CR- pt^{H} -4, and CR- pt^{H} -10) and pertinent wild type control (TS-9, **d**), $CR-pt^{R}$ mutants ($CR-pt^{R}$ -3, $CR-pt^{R}$ -5, and $CR-pt^{R}$ -6) and its pertinent wild type control (TS-3, e), $CR-pt^R$ mutants ($CR-pt^R-1$, $CR-pt^R-4$, and $CR-pt^R-7$) and the pertinent wild type control (LA4053, f). g Percentage of pointed tip fruits from $CR-pt^{H}/PT^{R}_{pro}:PT^{R}$ lines $(CR-pt^{H}/PT^{R}_{pro}:PT^{R}-3 \text{ and } CR-pt^{H}/PT^{R}_{pro}:PT^{R}-5)$ and its pertinent control (CR-pt^H). 20 fruits from each replicate were harvested and recorded for the morphologies (pointed tip, non-pointed tip or oval). Error bars indicate mean \pm SE. *n*=three biological replicates. Statistically significant differences were determined using a two-tailed t test (a) and one-way ANOVA with Tukey's post-hoc test (b-g). Different letters indicate statistically significant differences (P < 0.05). Source data are provided as a Source Data file.



Supplementary Fig. 7. GO enrichment analysis of differentially expressed genes in the CR-*pt*^H and wild-type TS-9 lines.



Supplementary Fig. 8. Fruit phenotypes of *FUL2* overexpression lines (*FUL2*-OE) and the wild-type control (TS-9).



Supplementary Fig. 9. Percentage of pointed tip fruit produced by different mutants and wild-type TS-9.

20 fruits from each replicate were harvested and recorded for the morphologies (pointed tip or non-pointed tip). Error bars indicate mean \pm SE. *n*=three biological replicates. Statistically significant differences were determined using a one-way ANOVA with Tukey's post-hoc test. Different letters indicate statistically significant differences (P < 0.05). Source data are provided as a Source Data file.



Supplementary Fig. 10. Auxin content of pointed tips in CR-*pt^R* and wild-type TS-3 lines.

Indole-3-acetic acid (IAA, **a**), indole-3-carboxaldehyde (ICA, **b**) and methyl indole-3-acetate (ME-IAA, **c**) content in the distal end of fruit from CR- pt^R and wild-type (TS-3, PT^R allele) were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The fruit was harvested at 14 DPA. Error bars indicate mean \pm SE. *n*=three biological replicates. Statistically significant differences were determined using a one-way ANOVA with Tukey's post-hoc test. Different letters indicate statistically significant differences (P < 0.05). Source data are provided as a Source Data file.

F2:3 population.							
Marker	Maker	Enzyme	Annealing	Pri	mer sequence(5'-3')		
type	name		temp (°C)				
CAPS	BK2	Bsp119I	55	F	TTTTATTGGTCCACGAGCCG		
				R	AAAAATCTATGTCCAAACGAGCC		
CAPS	BK72	SspI	55	F	TATTATGTTGCTGAGCAAAAGGC		
				R	TTGTGGGGTTAAAGTGGAGAAGT		
CAPS	CK9	NcoI	55	F	AGCTTGACTTGGTGATAGAGACC		
				R	GGCGTTCTGTGCTGAAAACA		
CAPS	CK20	SacI	55	F	CCGAAGAGCTTGCTCCTGTA		
				R	AGGGCGGGAAAACTTGTCTT		
CAPS	EK6	PstI	55	F	CGAGACCACGTGCTTAACCA		
				R	CCAGTGCCTTTGTGTTTGCC		
CAPS	EK12	VspI	55	F	TCACCGTTACCAATTTCACCAT		
				R	GCTCTTAGTTCCTACATCTCCAAGTT		

Supplementary Table 1. List of primers used for genotyping individuals from the