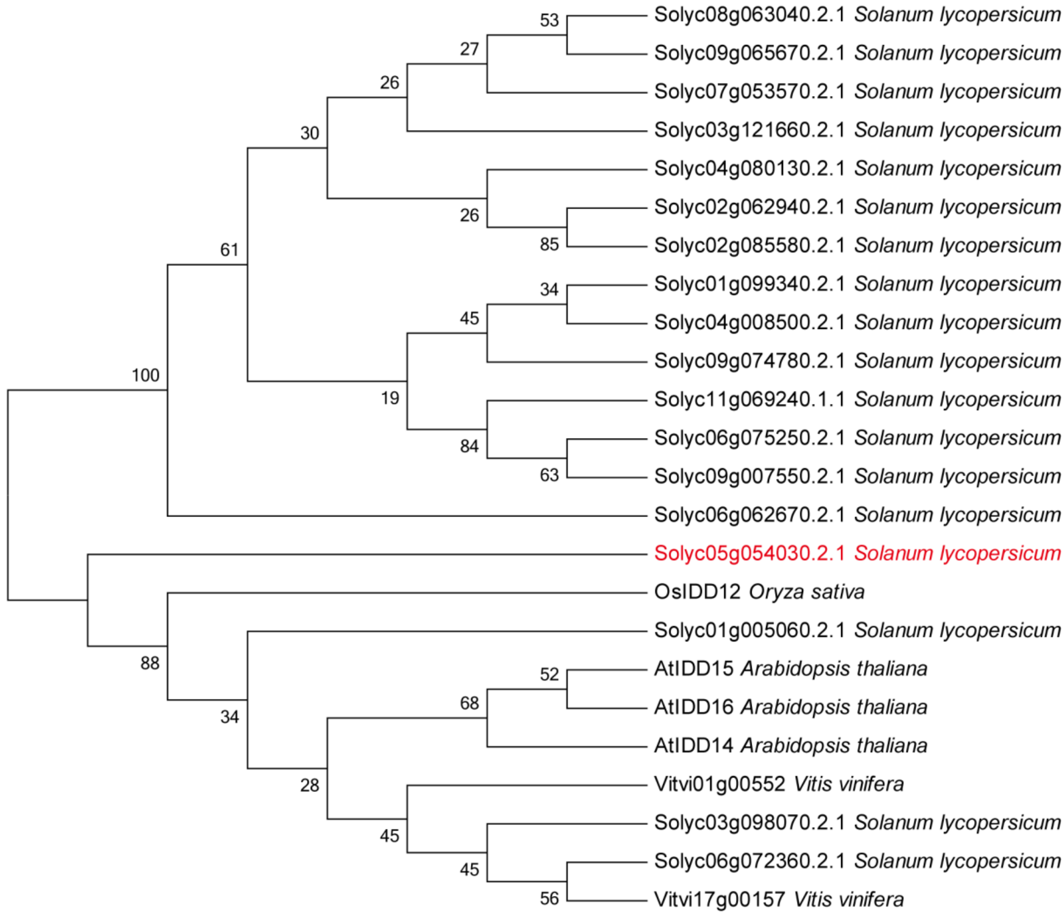
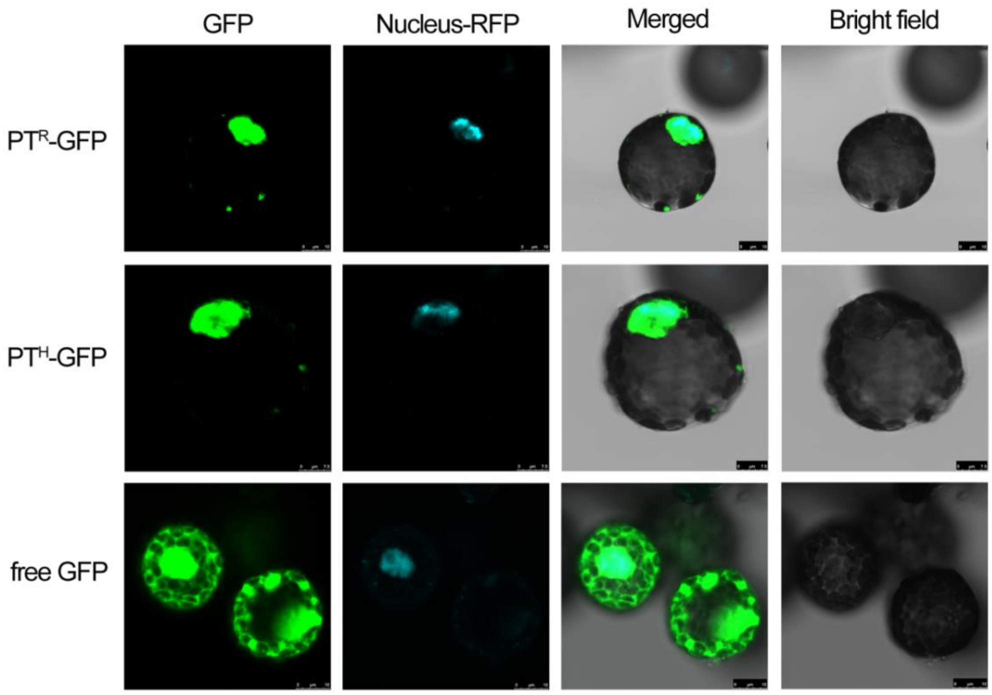
Variation in the fruit development gene *POINTED TIP* regulates protuberance of tomato fruit tip

Song *et al.*



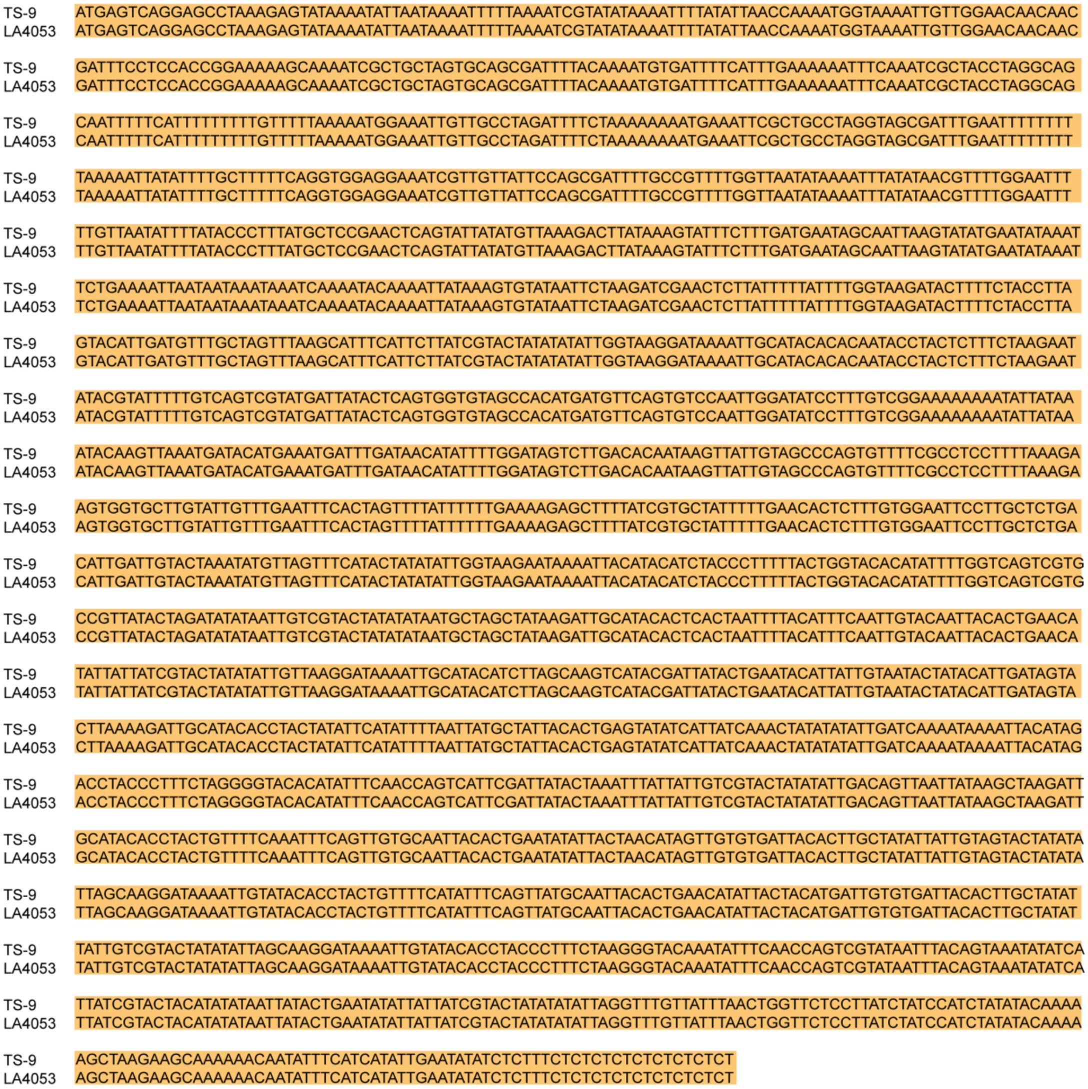
Supplementary Fig. 1. Phylogenetic tree analysis of PT.

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.



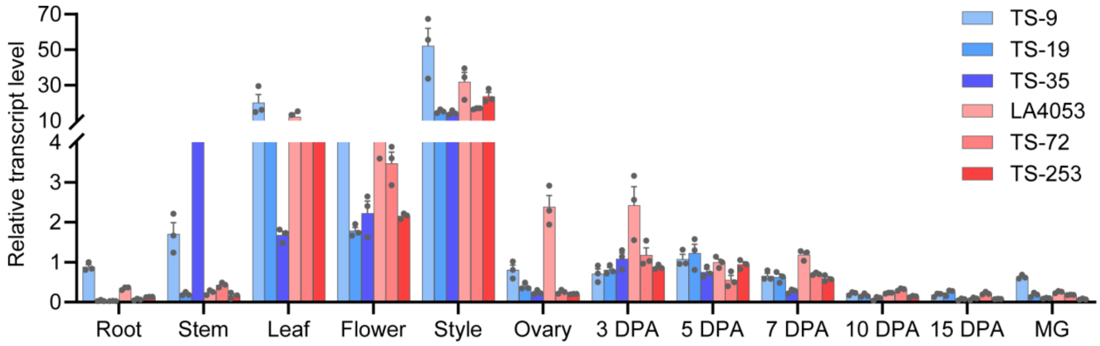
Supplementary Fig. 2. Subcellular localization of PT^R and PT^H proteins.

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.



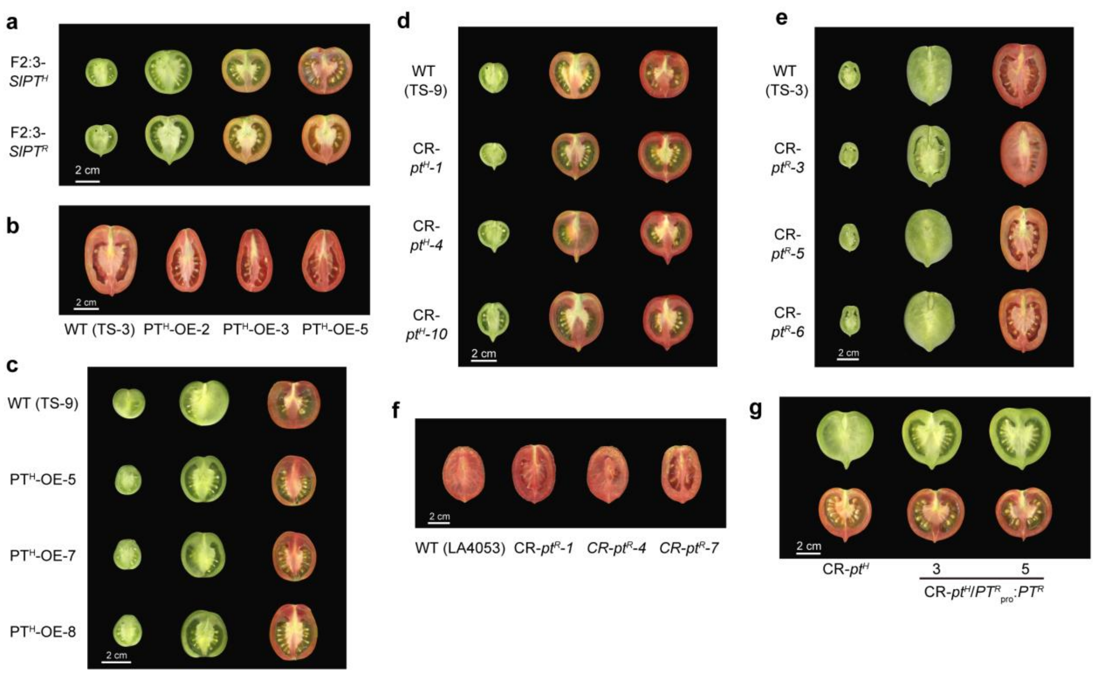
Supplementary Fig. 3. Alignment of the *PT* promoter sequences.

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



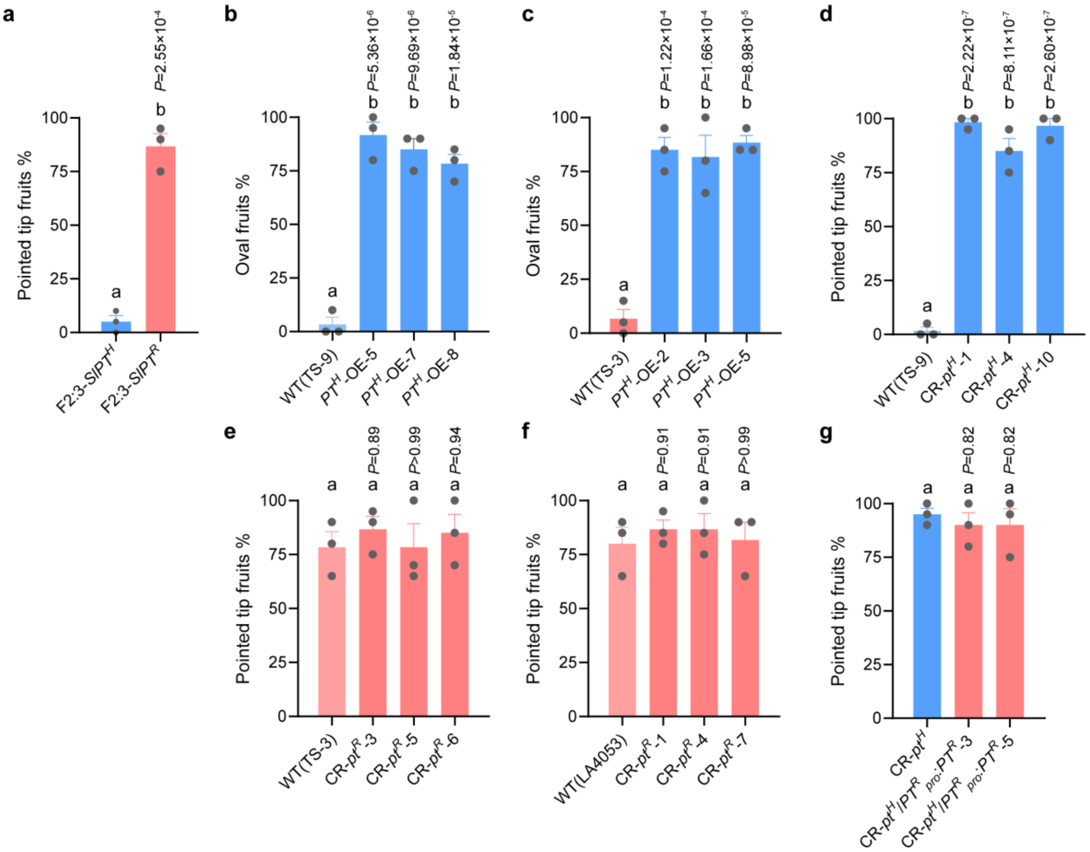
Supplementary Fig. 4. Expression patterns of *PT*.

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 ± SE. *n*=three biological replicates. Source data are provided as a Source Data file.



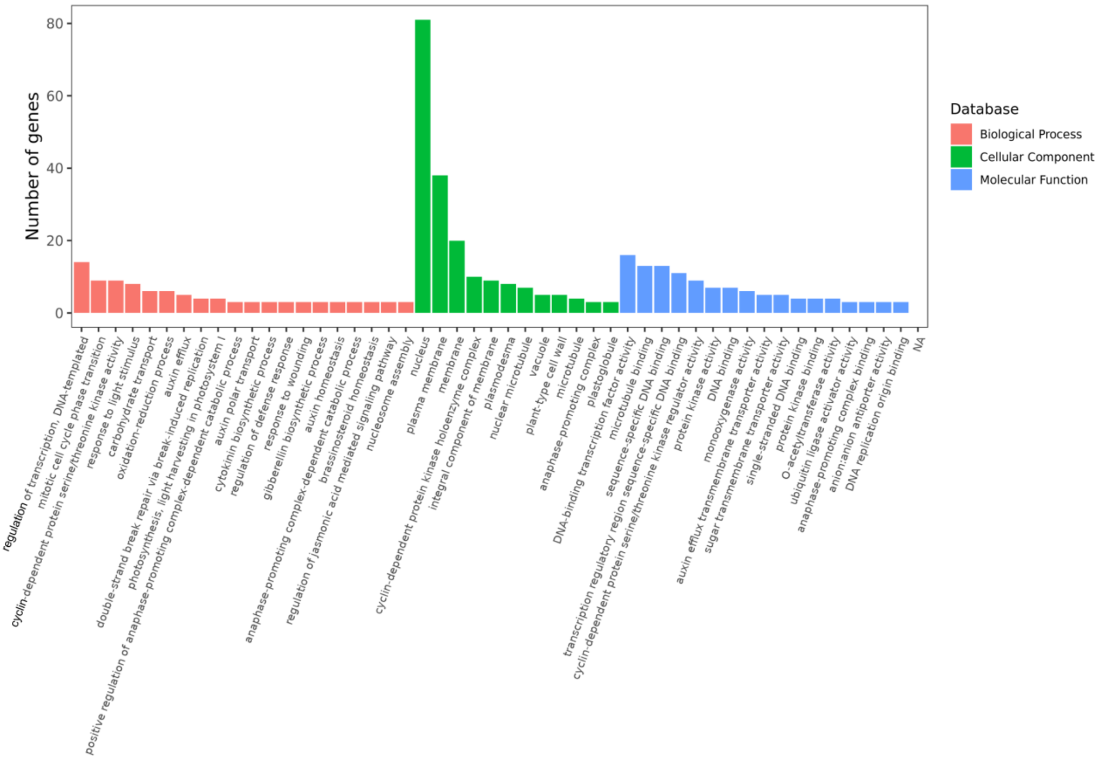
Supplementary Fig. 5. Longitudinal sections from fruit produced by different transgenic lines and pertinent wild-type plants.

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 (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).



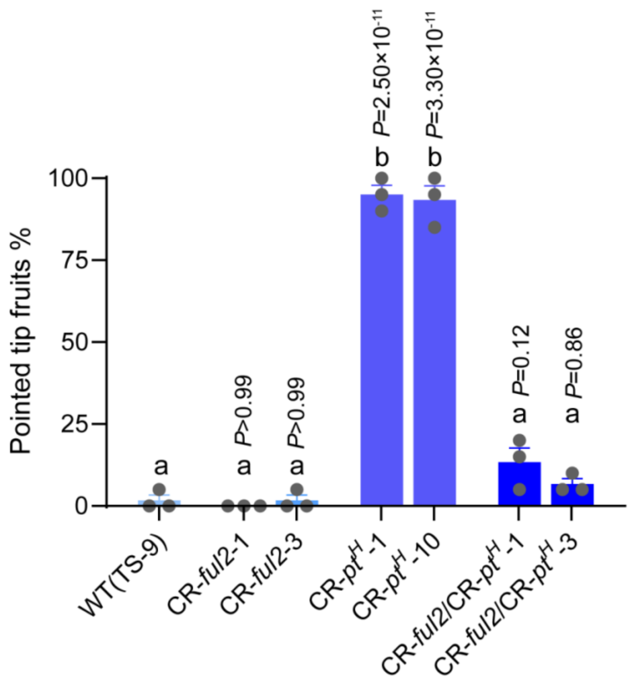
Supplementary Fig. 6. Percentage of different fruit morphology produced by different transgenic lines and their pertinent wild-type plants.

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 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 ± 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.



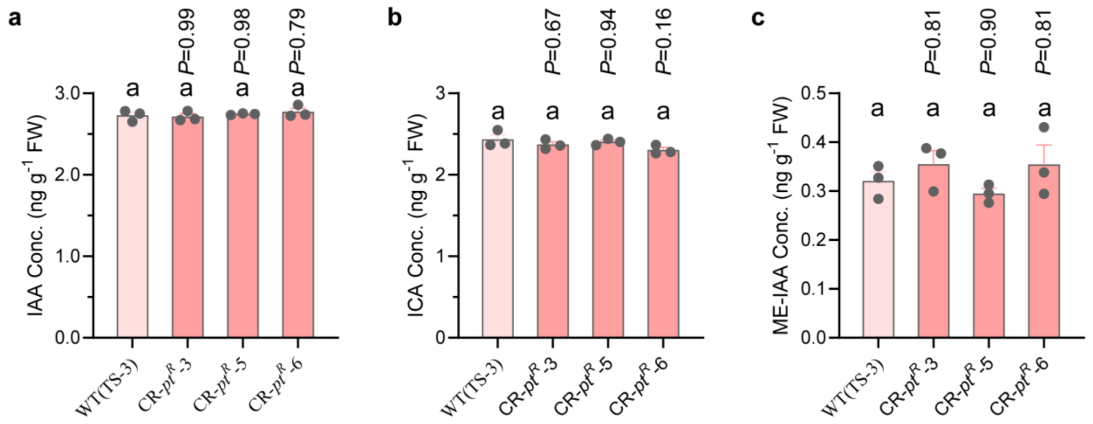
Supplementary Fig. 7. GO enrichment analysis of differentially expressed genes in the CR-*pt*^{H} and wild-type TS-9 lines.

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 ± 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 ± 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 Table 1. List of primers used for genotyping individuals from the F2:3 population.

Marker type	Maker name	Enzyme	Annealing temp (°C)	Primer sequence(5'-3')	
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