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

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## 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 $\mathbf{P T}^{\mathbf{R}}$ and $\mathbf{P T}^{\mathbf{H}}$ proteins.
Tobacco protoplasts were co-transformed with plasmids that express either $\mathrm{PT}^{\mathrm{R}}$-GFP or $\mathrm{PT}^{\mathrm{H}}$-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.

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AGCTAAGAAGCAAAAAACAATATTTCATCATATTGAATATATCTCTTTCTCTCTCTCTCTCTCTCT

## 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 $P T$ 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 $P T^{R}$ and $P T^{H}$ alleles from the $\mathrm{F} 2: 3$ population. b, c Fruit from $P T^{H}$-overexpressing lines (b: $P T^{H}$-OE-2, $P T^{H}$-OE-3, and $P T^{H}$-OE-5) and its pertinent wild type control (TS-3 $P T^{R}+/+$ ), (c: $P T^{H}-\mathrm{OE}-5, P T^{H}-\mathrm{OE}-7$, and $P T^{H}-\mathrm{OE}-8$ ) and the pertinent wild-type line (TS-9 $P T^{H}+/+$ ). d-f Fruit from CR-pt ${ }^{H}$ mutants (CR-pt $t^{H}-1$, CR-pt $t^{H}-4$, and CR-pt ${ }^{H}-10$ ) and pertinent wild type control (TS-9) that produces non-pointed tip fruit (d), CR-pt $t^{R}$ mutants (CR-pt $t^{R}-3, \mathrm{CR}-p t^{R}-5$, and CR-pt $t^{R}-6$ ) and its pertinent wild type control (TS-3) that produces fruit with pointed tips (e), $\mathrm{CR}-p t^{R}$ mutants ( $\mathrm{CR}-p t^{R}-1, \mathrm{CR}-p t^{R}-4$, and $\mathrm{CR}-p t^{R}-7$ ) and the pertinent wild type control (LA4053) that produces fruit with a pointed tip (f). g Fruit from CR-pt $t^{H} / P T^{R}{ }_{\text {pro }}: P T^{R}$ lines (CR-pt $/ P T^{R}{ }_{\text {pro }}: P T^{R}-3$ and CR-pt $\left./ P T^{R}{ }_{\text {pro }}: P T^{R}-5\right)$ 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 $P T^{R}$ and $P T^{H}$ alleles from the F2:3 population. b, c Percentage of oval fruits from $P T^{H}$-overexpressing lines $\left(P T^{H}-\mathrm{OE}-5, P T^{H}-\mathrm{OE}-7\right.$, and $\left.P T^{H}-\mathrm{OE}-8\right)$ and pertinent wild-type (TS-9, $P T^{H}$ allele, $\mathbf{b}$ ), $P T^{H}$-overexpressing lines $\left(P T^{H}\right.$-OE-2, $P T^{H}$-OE-3, and $P T^{H}$-OE-5) and pertinent wild-type (TS-3, $P T^{R}$ allele, c). d-f Percentage of pointed tip fruits from CR-pt ${ }^{H}$ mutants (CR-pt ${ }^{H}-1, \mathrm{CR}-p t^{H}-4$, and CR-pt $t^{H}-10$ ) and pertinent wild type control (TS-9, d), CR-pt $t^{R}$ mutants (CR $-p t^{R}-3, \mathrm{CR}-p t^{R}-5$, and $\left.\mathrm{CR}-p t^{R}-6\right)$ and its pertinent wild type control (TS-3, e), CR-pt mutants (CR-pt $t^{R}-1, \mathrm{CR}-p t^{R}-4$, and CR-pt $t^{R}-7$ ) and the pertinent wild type control (LA4053, f). g Percentage of pointed tip fruits from CR-pt ${ }^{H} / P T^{R}{ }_{\text {pro }}: P T^{R}$ lines (CR-pt $t^{H} / P T^{R}{ }_{\mathrm{pro}}: P T^{R}-3$ and $\mathrm{CR}-p t^{H} / P T^{R}{ }_{\mathrm{pro}}: P T^{R}-5$ ) and its pertinent control (CR-pt $t^{H}$. 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 $t^{H}$ 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.

b



Supplementary Fig. 10. Auxin content of pointed tips in CR-pt $\boldsymbol{t}^{R}$ 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, $P T^{R}$ 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.

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

## F2:3 population.

| Marker <br> type | Maker <br> name | Enzyme | Annealing <br> temp $\left({ }^{\circ} \mathrm{C}\right)$ | 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 | $V s p I$ | 55 | F | TCACCGTTACCAATTTCACCAT |
|  |  |  |  | R | GCTCTTAGTTCCTACATCTCCAAGTT |

