**The tomato CONSTANS-LIKE protein SlCOL1 regulates fruit yield by repressing SFT gene expression**

Cui L, Zheng F, Wang J, Zhang C, Zhang D, Gao S, Zhang C, Ye J, Zhang Y, Ouyang B, Wang T, Hong Z, Ye Z, Zhang J

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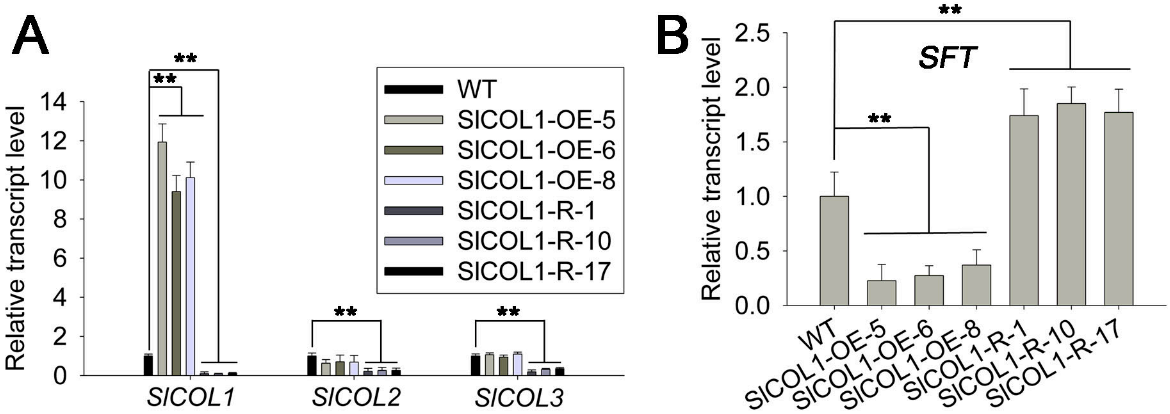

**Fig. S1** Transcript levels of *SlCOL1*, *SlCOL2*, *SlCOL3* and *SFT* in *SlCOL1* transgenic and WT plants. **A-B** Quantitative RT-PCR analysis of *SlCOL1*, *SlCOL2* and *SlCOL3* expression (**A**) and *SFT* expression (**B**) in the young leaves of the WT tomato and three representative lines each of *SlCOL1*-OE and *SlCOL1*-RNAi. Asterisks indicate statistically significant differences. **, $P < 0.01$.

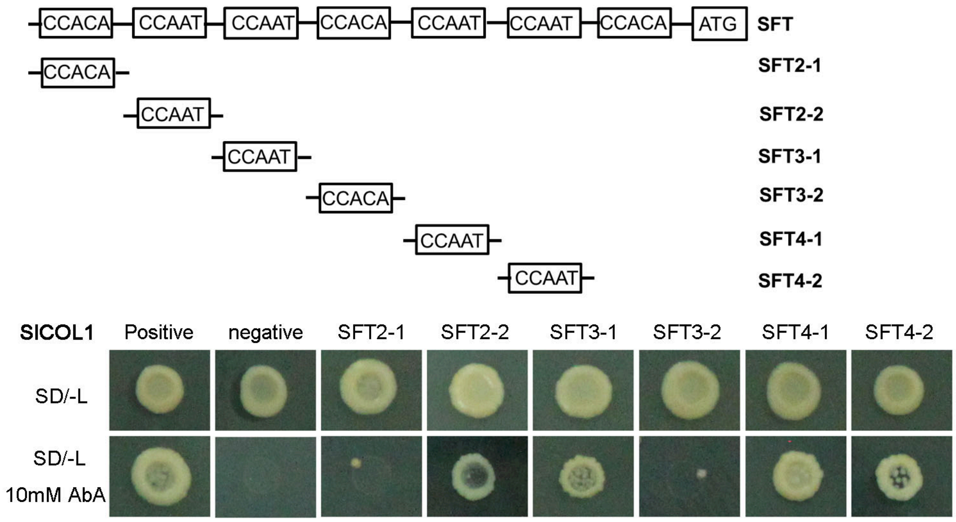

**Fig. S2** Yeast-one hybrid (Y1H) analysis of SlCOL1 binding to the different core sequences of the *SFT* promoter. Six constructs containing six different promoter fragments (SFT2-1 to SFT4-2) were used in Y1H assays. The bait vectors, SFT2-1 to SFT4-2, and the SlCOL1-containing prey vector were introduced into the yeast strain Y1H Gold. The enhanced resistance to antibiotic aureobasidin A (AbA) indicated an interaction between the bait and prey. Co-transformation of the bait vectors, SFT2-1 to SFT4-2, with either pGADT7 or pGADT-Rec2-53 served as negative and positive controls, respectively.

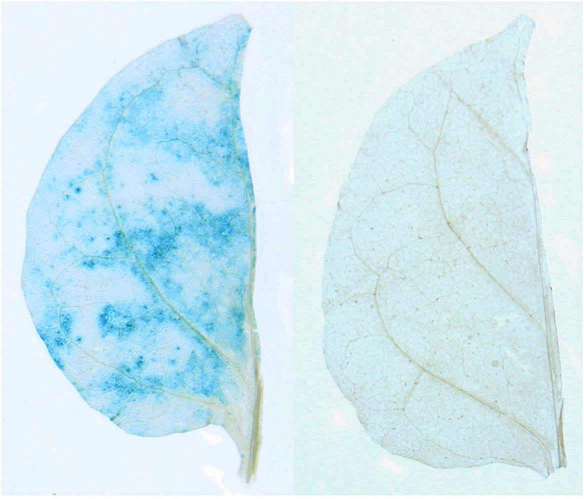
ProSFT untransformed

**Fig. S3** GAL4/UAS-based analysis on ProSFT-GUS.

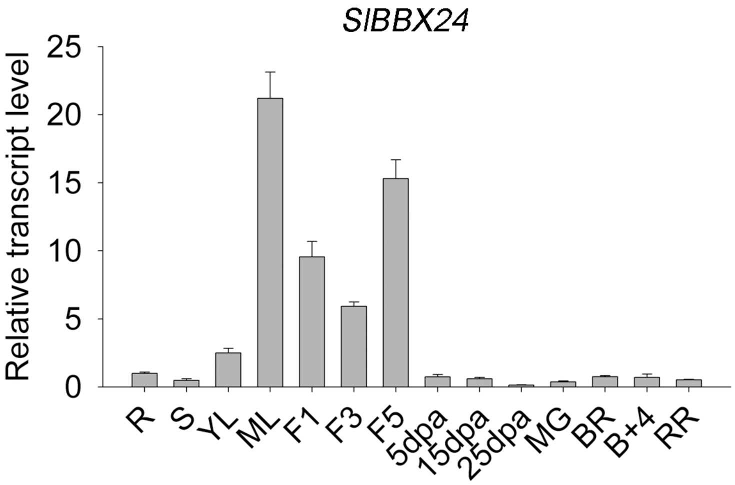

**Fig. S4** Transcript levels of *SlBBX24* in different tomato organs. R, roots; S, stems; Yl, young leaves; Ml, mature leaves; F1, flower buds; F3, unfold flowers; F5, fold flowers; fruits at 5DPA, 15DPA and 25DPA, 5, 15 and 25 days post anthesis, respectively; MG, mature green stage fruits; BR, breaker stage fruits; B+4, four days after breaker stage fruits; RR, red ripe stage fruits. All samples were collected from plants nine weeks after planting.

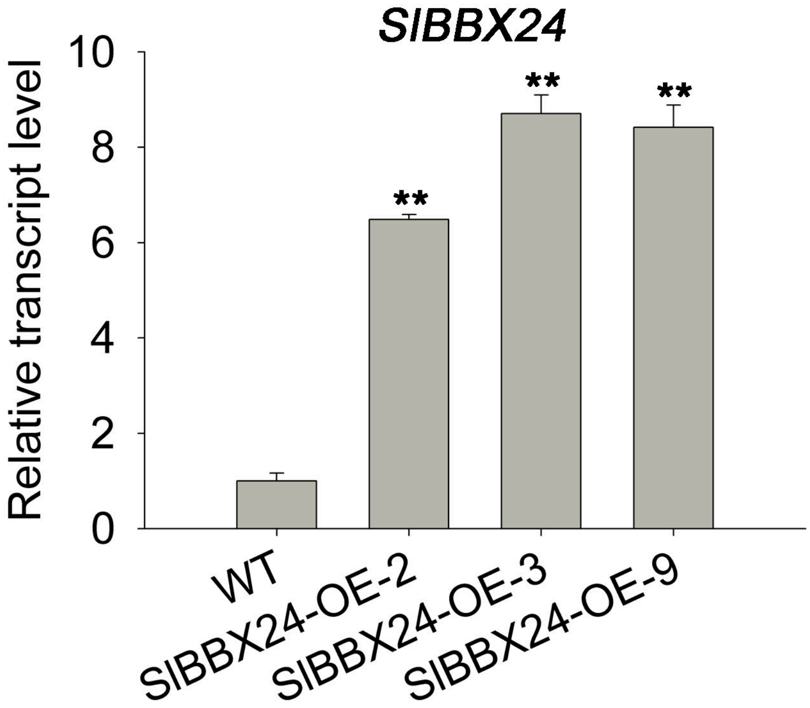

**Fig. S5** Quantitative RT-PCR analysis of *SlBBX24* transcript levels in young leaves of *SlBBX24*-OE lines. WT, wild-type tomato plants; OE-2, OE-3, and OE-9, three representative lines from the *SlBBX24*-overexpression (*SlBBX24*-OE) experiment. Asterisks indicate statistically significant differences. **, $P < 0.01$.

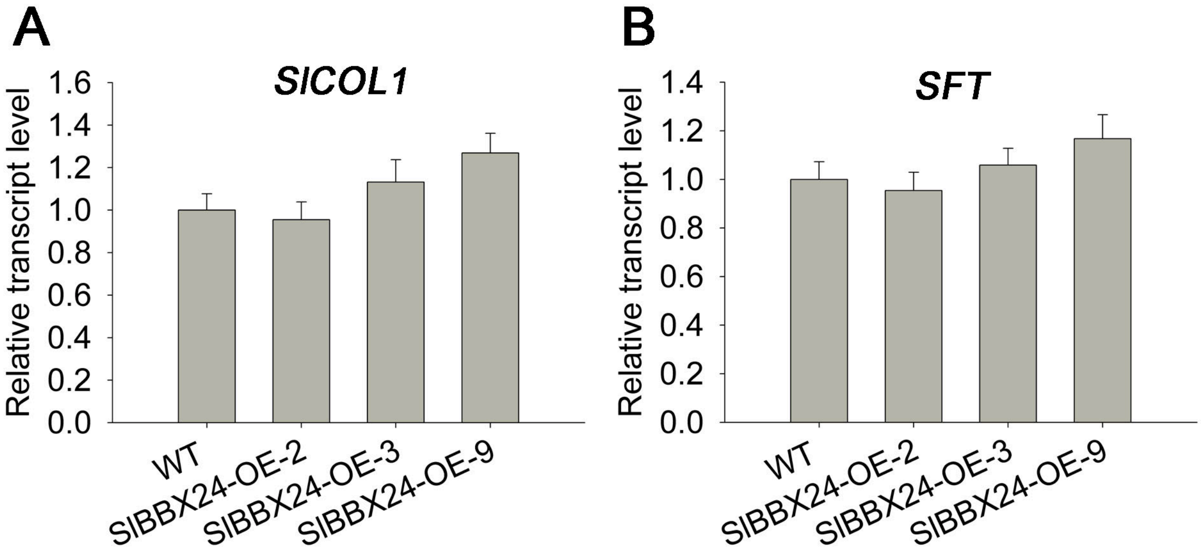

**Fig. S6** Quantitative RT-PCR analysis of *SlCOL1* and *SFT* transcript levels in young leaves of *SlBBX24*-OE lines. **A-B** WT, wild-type tomato plants; OE-2, OE-3, and OE-9, three representative lines from the *SlBBX24*-overexpression (*SlBBX24*-OE) experiment.

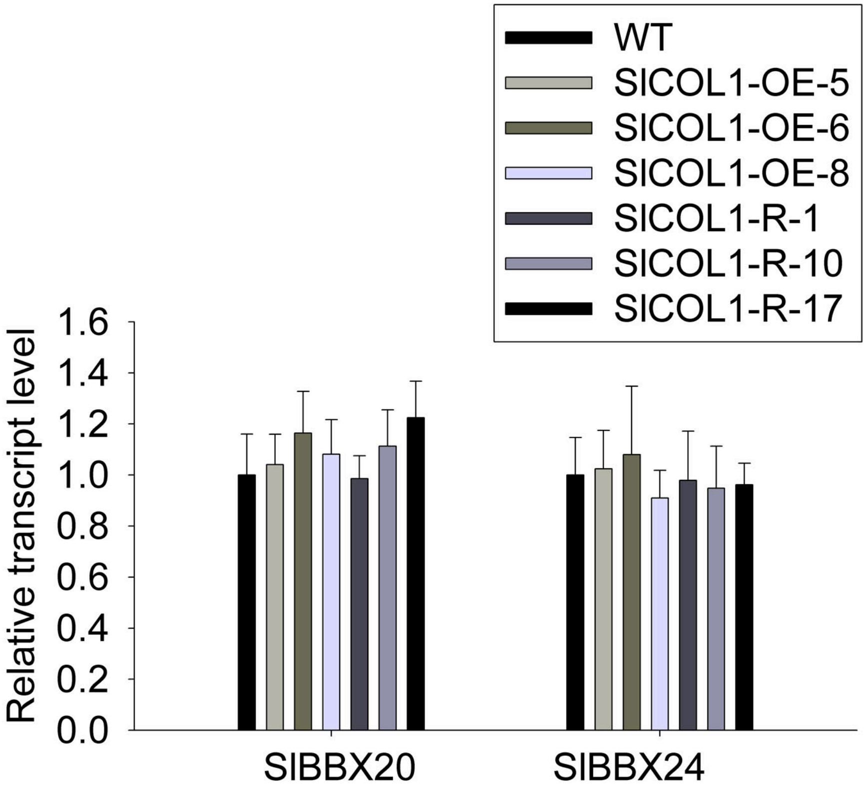

**Fig. S7** Quantitative RT-PCR analysis of *SlBBX20* and *SlBBX24* expression in young leaves of transgenic tomato plants. WT, wild-type tomato plants; OE-5, OE-6, and OE-8, three representative lines from the *SlCOL1*-overexpression (*SlCOL1*-OE) experiment; R-1, R-10 and R-17, three representative lines from the *SlCOL1*-RNAi plants.

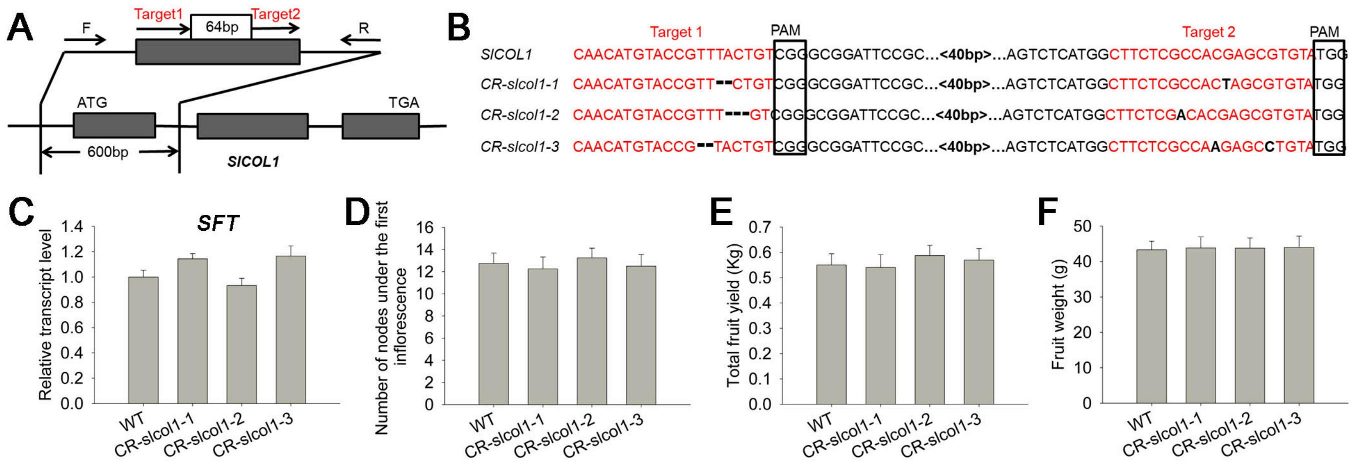

**Fig. S8** Flowering time and fruit yield phenotype of CR-*slcol1* transgenic tomato plants. **A** Schematic illustration of the two sgRNA target sites (red arrows) in *SlCOL1*. Black arrows represent the location of the primers that were used for PCR-based genotyping. **B** Verification of the CR-*slcol1* mutant alleles by DNA sequencing analysis. The red font indicates sgRNA target sequences. The black boxes indicate protospacer-adjacent motif (PAM) sequences. **C** Quantitative RT-PCR analysis of *SFT* expression in the young leaves of the WT tomato and three representative lines of CR-*slcol1*. **D** Number of nodes under the first inflorescence in the WT tomato and three representative lines of CR-*slcol1* eight weeks after planting. **E** Total fruit yield of the WT tomato and three representative lines of CR-*slcol1*. **F** Mean values of fruit weights from the CR-*slcol1* transgenic and WT tomato plants.

**Table S1. Primers used in this study.**

| Primer name | Sequence (5'-3') |
|---|---|
| SlCOL1-OE-FW | AAAAAGCAGGCTAGTTAGTCCAATGTTGAAAAAAGAG |
| SlCOL1-OE-RV | AGAAAGCTGGGTTTTAGGGGCTTGTGGTTGA |
| SlCOL1-RNAi-FW | AAAAAGCAGGCTCGATGGATGTCAGTTTTGTTC |
| SlCOL1-RNAi-RV | AGAAAGCTGGGTTTTAGGGGCTTGTGGTTGA |
| SlCOL1-DT1-FW | GAATCTAACAGTGTAGTTTGCAACATGTACCGTTTACTGTGTTTTAGAGCTAGAAATAG |
| SlCOL1-DT2-RV | GCTATTTCTAGCTCTAAAACTACACGCTCGTGGCGAGAAGCAAACTACACTGTTAGATT |
| SlCOL1-DET-FW | CTCTAGTCTCCCCCAAGAAGGC |
| SlCOL1-DET-RV | ATGTCAGCGTCGCAAGAGG |
| SlBBX24-OE-FW | CATTTGGAGAGGACACGCTCGAGTCTTGTGTGAAACTCAAATGGGTAT |
| SlBBX24-OE-RV | TCTCATTAAAGCAGGACTCTAGATACACACAAGCACACATGCACAT |
| SlBBX24-DT1-FW | GAATCTAACAGTGTAGTTTGTGTGATTTGCTGTGCTGATGGTTTTAGAGCTAGAAATAG |
| SlBBX24-DT2-RV | GCTATTTCTAGCTCTAAAACAAGGCTTCATCTTCAGTGCCCAAACTACACTGTTAGATT |
| SlBBX24-DET-FW | CACACAAATCTCCCTCTTCCACT |
| SlBBX24-DET-RV | GAAAGTCCAAAGCCCAAACC |
| SlCOL1-1302-FW | ACGGGGGACTCTTGACCATGGTAATGTTGAAAAAAGAGAACAGTAACA |
| SlCOL1-1302-RV | AAGTTCTTCTCCTTTACTAGTGAATGAAGGGACAATTCCATAATT |
| SlCOL1-Q-FW | CGCTGACATTCACTCTGCAAAC |
| SlCOL1-Q-RV | CTCAAGAATCCATCATCCTCGG |
| SlBBX24-Q-FW | CTACTGTGATTTGCTGTGCTGATG |
| SlBBX24-Q-RV | GGAGGAAGCTTGTTAGATAGGCAC |
| SlBBX20-Q-FW | ACTGAGACTCTTCCTGGTTGGC |
| SlBBX20-Q-RV | GGAAAAAACTACACATCTGGTCGT |
| SlCOL3-Q-FW | CCATTTACTGCCAGGCGGAT |
| SlCOL3-Q-RV | GTTTCAACAGCTGGAGGACCG |
| SlCOL2-Q-FW | GTTGAAAAACGAGAACAGTGGG |
| SlCOL2-Q-RV | GAATCTGCCCTGCAGTAAACG |
| SlActin-Q-FW | GTCCTCTTCCAGCCATCCA |
| SlActin-Q-RV | ACCACTGAGCACAATGTTACCG |
| β-actin-Fw | ATGGCAGACGGAGAGGATATTCA |
| β-actin-Rv | GCCTTTGCAATCCACATCTGCTG |
| ProSlCOL1-GUS-FW | TGCATCCAACGCGTTGGGAGCTCGTTCAGACAAATGGCAGAGCAG |
| ProSlCOL1-GUS-RV | GCCTTCGCCATTCTAGACTCGAGAGCAATGAGTAGATCAACTATGAGTCA |
| SFT1- PAbai-FW | AGCTTGAATTCGAGCTCGGTACCGTCATGATTCGATTAGAAGAATTCCT |
| SFT1- PAbai-RV | ACATACAGAGCACATGCCTCGAGGACGATGGTTGACGATAAACAAA |
| SFT2- PAbai-FW | AGCTTGAATTCGAGCTCGGTACCGTGATTGTTCATCTATGTGTTCCTGT |

| | |
|---|---|
| SFT2- PAbai-RV | ACATACAGAGCACATGCCTCGAGGGAATGAAACCCCAATCAAGAA |
| SFT3-PAbai-FW | AGCTTGAATTCGAGCTCGGTACCAAATGGTTGATGAGTTAGATTGAAG |
| SFT3-PAbai-RV | ACATACAGAGCACATGCCTCGAGATCCTCTGAATTTTTTACGAGTCCT |
| SFT4-PAbai-FW | AGCTTGAATTCGAGCTCGGTACCTAAACCAGTTTAGGTAGGGGTAAGG |
| SFT4-PAbai-RV | ACATACAGAGCACATGCCTCGAGGAATACACCCCAAAGCGTCC |
| SFT5-PAbai-FW | AGCTTGAATTCGAGCTCGGTACCGACGCTTTGGGGTGTATTCG |
| SFT5-PAbai-RV | ACATACAGAGCACATGCCTCGAGGACGATGGTTGACGATAAACAAA |
| SFT2-1-PAbai-FW | AGCTTGAATTCGAGCTCGGTACCGTGATTGTTCATCTATGTGTTCCTGT |
| SFT2-1-PAbai-RV | ACATACAGAGCACATGCCTCGAGATTACAAAATCAGAATGTGATTGTAGG |
| SFT2-2-PAbai-FW | AGCTTGAATTCGAGCTCGGTACCCCTACAATCACATTCTGATTTTGTAAT |
| SFT2-2-PAbai-RV | ACATACAGAGCACATGCCTCGAGGGAATGAAACCCCAATCAAGAA |
| SFT3-1-PAbai-FW | AGCTTGAATTCGAGCTCGGTACCAGTAAGGCTTTTTGATTCTAGGGAG |
| SFT3-1-PAbai-RV | ACATACAGAGCACATGCCTCGAGATCGGTGGGATAACCAATTACTAAC |
| SFT3-2-PAbai-FW | AGCTTGAATTCGAGCTCGGTACCGTGTGGATGTCACTCACGATGG |
| SFT3-2-PAbai-RV | ACATACAGAGCACATGCCTCGAGATCCTCTGAATTTTTTACGAGTCCT |
| SFT4-1-PAbai-FW | AGCTTGAATTCGAGCTCGGTACCTAAACCAGTTTAGGTAGGGGTAAGG |
| SFT4-1-PAbai-RV | ACATACAGAGCACATGCCTCGAGCAAGCGATATATCGCATAAAGTGA |
| SFT4-2-PAbai-FW | AGCTTGAATTCGAGCTCGGTACCTCACTTTATGCGATATATCGCTTG |
| SFT4-2-PAbai-RV | ACATACAGAGCACATGCCTCGAGGAATACACCCCAAAGCGTCC |
| ProSFT-GUS-FW | TGCATCCAACGCGTTGGGAGCTCGTGATTGTTCATCTATGTGTTCCTGT |
| ProSFT-GUS-RV | GCCTTCGCCATTCTAGACTCGAGGACGATGGTTGACGATAAACAAA |
| SlCOL1-AD-FW | ACGTACCAGATTACGCTCATATGATGTTGAAAAAAGAGAACAGTAACA |
| SlCOL1-AD-RV | TACGATTCATCTGCAGCTCGAGCTCAGAATGAAGGGACAATTCCAT |