SUPPLEMENTARY MATERIAL

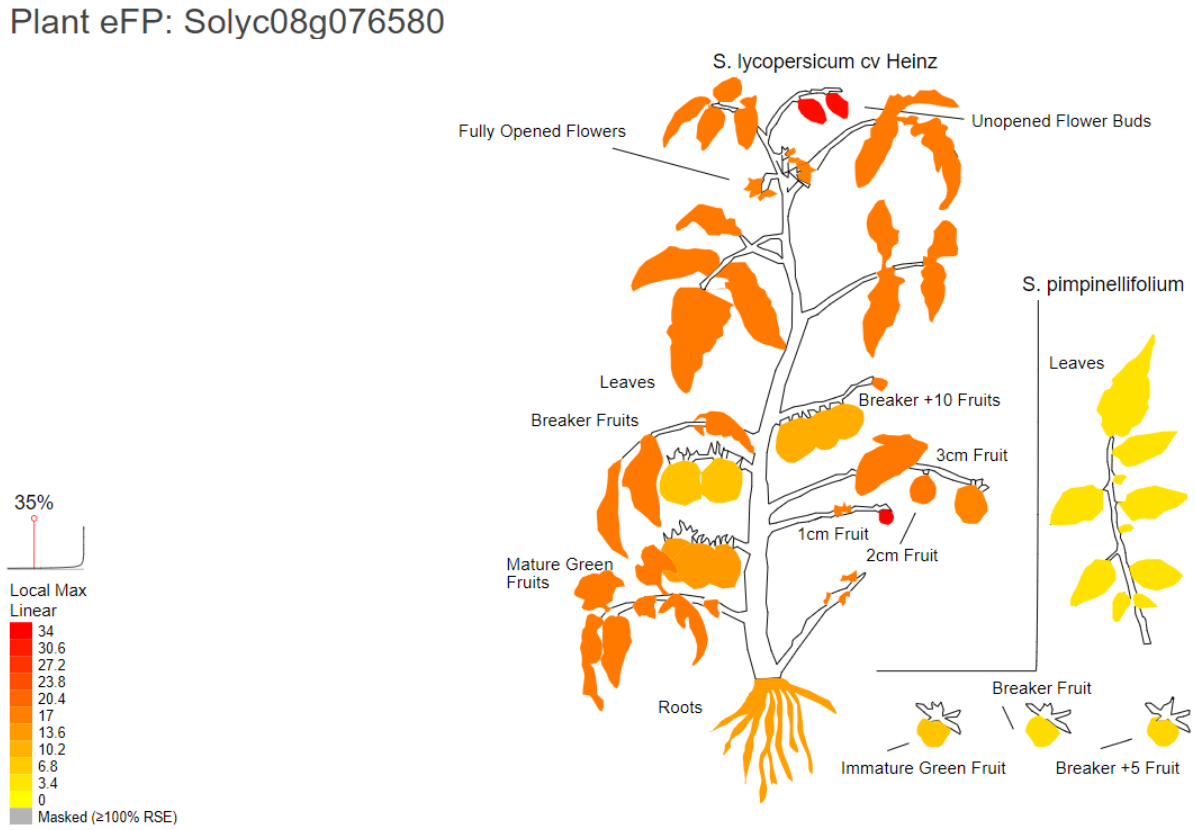


Figure S1 Plant BAR eFP browser showing the expression of *SAMBA* (*Solyc08g076580*) in distinct tomato tissues. Image extracted from the BAR ePlant tomato browser (https://bar.utoronto.ca/eplant_tomato/).

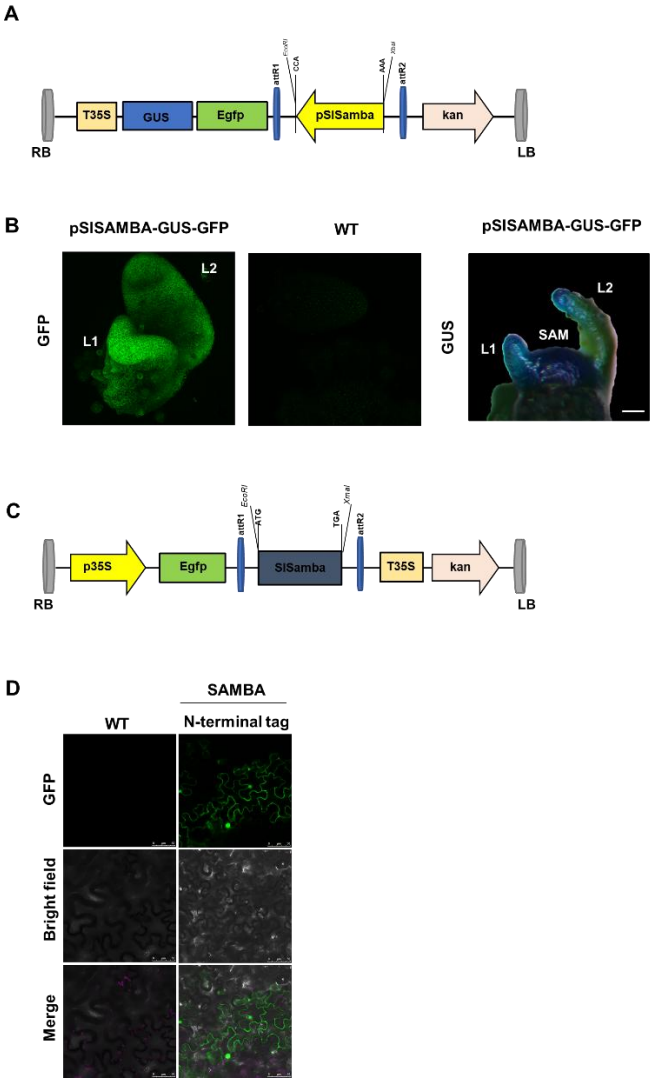


Figure S2 Expression of pSISAMBA-GUS-GFP and SAMBA:EGFP vectors used for subcellular localization analysis. (A) To study the dynamic expression of *SlSAMBA*, a 1.6-kb fragment upstream of the ATG codon of the *SlSAMBA* gene was inserted in frame into EcoRI and XmaI sites of pKGWFS7 upstream EGFP and GUS coding region and introduced into tomato plants. The pKGWFS7 contains the selective marker *neomycin phosphotransferase II* (*nptII*) that gives resistance to kanamycin. (B) *SlSAMBA*-promoter-directed expression of GFP and GUS in the early vegetative meristem (4-day-old) of tomato transformed plants (T3). Images of tomato apices showing vegetative meristem observed under fluorescence excitation (GFP) with a confocal microscope. Leaf primordium (L) and shoot apical meristem (SAM). The right lower panel shows the WT vegetative meristem without almost fluorescence. *SAMBA*-promoter-directed expression of GUS in tomato transformed plants (T3). (C) Vector used in the subcellular localization of SAMBA:EGFP (N-terminal) fusion in epidermal cells of *Nicotiana benthamiana*. (D) SISAMBA:EGFP (N-terminal) tagged protein is localized in the nucleus and the cytoplasm. The upper panel shows the GFP signal field, the middle panel shows the bright field, and the lower panel shows the merged fields. No fluorescence is observed in the wild-type (WT) leaf tissues (control). Scale bar = 50 μm.

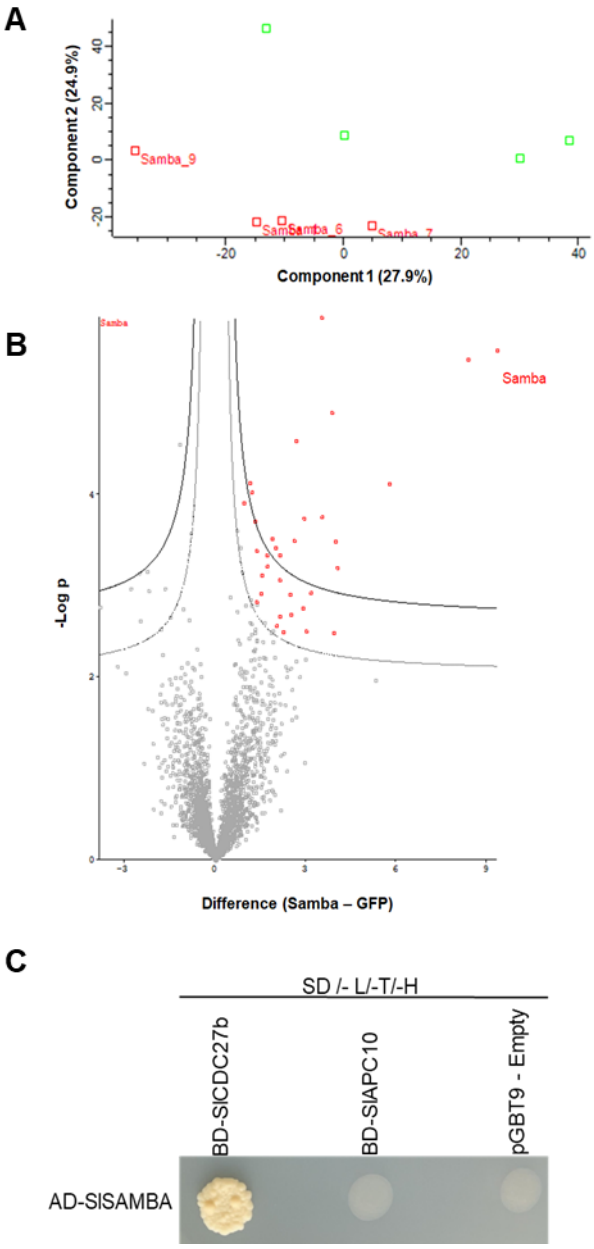


Figure S3 Statistical analysis on SlSAMBA, eGFP TurboID samples, and SlSAMBA interacts with CDC27b in the yeast two-hybrid (Y2H) assay. *35S::GFP-TurboID* and *XVE::samba-TurboID* expression constructs were used for the rhizogenic *Agrobacterium*-mediated transformation of tomato. Transformed hairy roots were treated with β-estradiol (100 µM) for 24 h and biotin (50 µM) for 2 h. Proteins were extracted from the hairy root tissue, enriched with streptavidin beads, digested with trypsin, and identified by mass spectrometry. The MaxQuant software was used for peptide and protein identification on the acquired raw files and the Perseus software for statistical data analysis (Cox and Mann, 2008; Tyanova et al., 2016). (A) Sample variability represented by a principal component analysis (PCA) plot. Red circles and green squares are SAMBA and eGFP samples, respectively. (B) Volcano plot of pairwise comparison between SAMBA and eGFP samples. A two-sample Student's t test was done to identify enriched proteins in the Samba samples. The full line indicates the cut-off at false discovery rate (FDR)=0.01 and S0=0.1 (i.e., artificial within-group variance; which defines the relative importance of the P value and difference between means). The t test difference was plotted against the t test –log (*P* value). (C) Yeast two-hybrid interactions between SlSAMBA and APC/C subunits. SlSAMBA was fused to the activation domain, while SlCDC27b and SlAPC10 were fused to the binding domain. Empty vector (pGBT9) was used as a control for autoactivation.

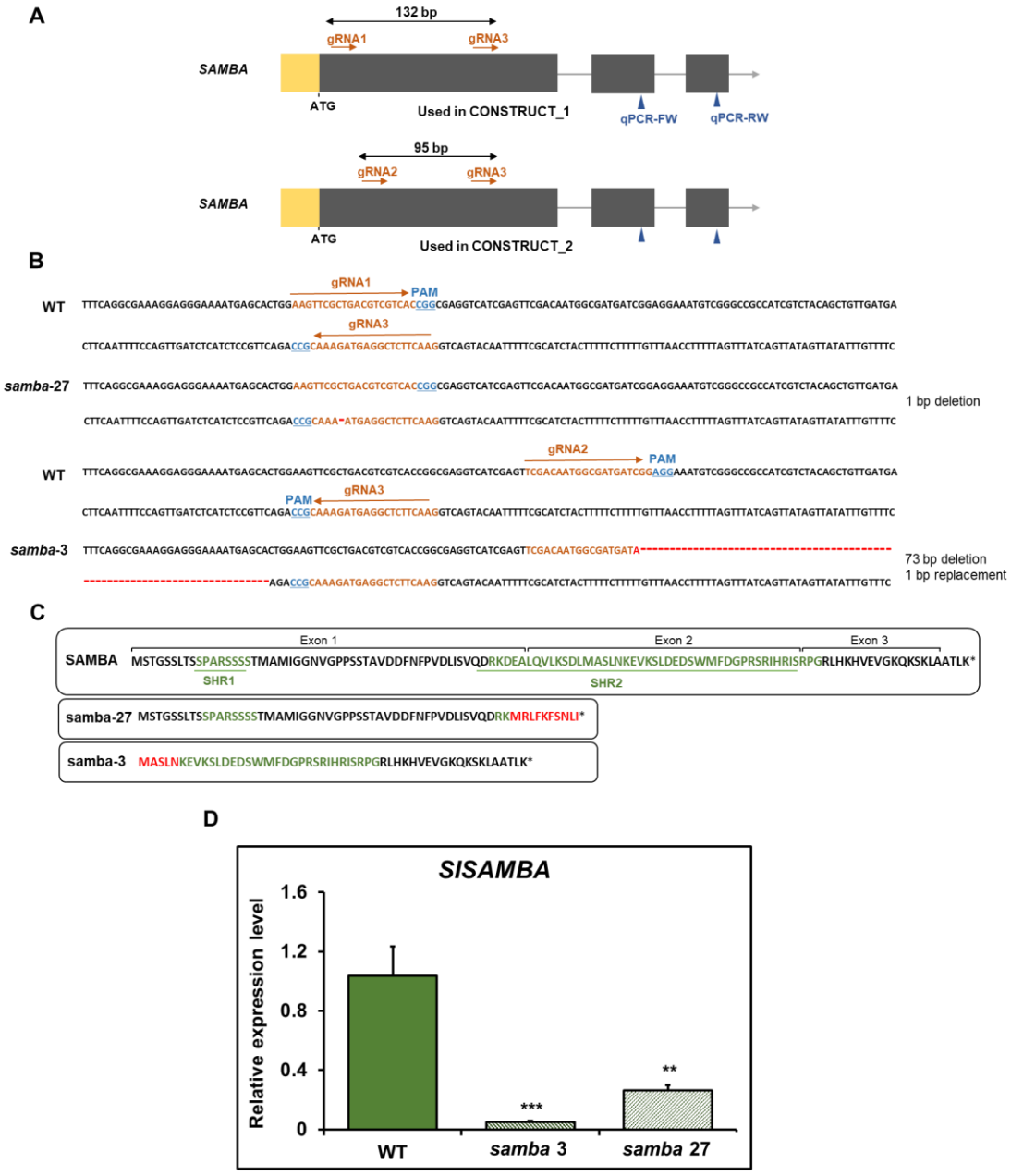


Figure S4 Generation of *slsamba* mutants by CRISPR/Cas9. (A) Structural representation of the *SISAMBA* gene, showing the different target sites of the two gRNAs used per construct. UTR, exons, and introns are indicated by yellow, black boxes, and grey bars, respectively. qPCR-FW and qPCR-RW indicate the position of the forward and reverse primers used for *SISAMBA* quantitative RT-PCR (qRT-PCR). (B) The wild-type (WT) sequence is shown with the gRNA sequences highlighted in orange and the PAM sequence in blue. #3 and #27 are the mutants obtained in this study. The mutation sites are shown in red. (C) Amino acid sequences of SISAMBA in WT and *slsamba* mutant isoforms. SAMBA homology region 1 (SHR1) and SHR2, as defined by Eloy et al. (2012), are marked in green and the missense amino acids are indicated in red. Asterisks represent a stop codon. (D) qRT-PCR transcript analysis of wild-type (WT) and *slsamba* plants (#3 and #27). Total RNA was prepared from whole seedlings harvested 30 days after sowing (DAS) and amplified by qRT-PCR. All values were normalized against the expression level of the *β-ACTIN*. Data are means ± SD (n = 3). Significant differences (ANOVA followed by Dunnett's test) are indicated by asterisks (*P < 0.05 and **P < 0.01).

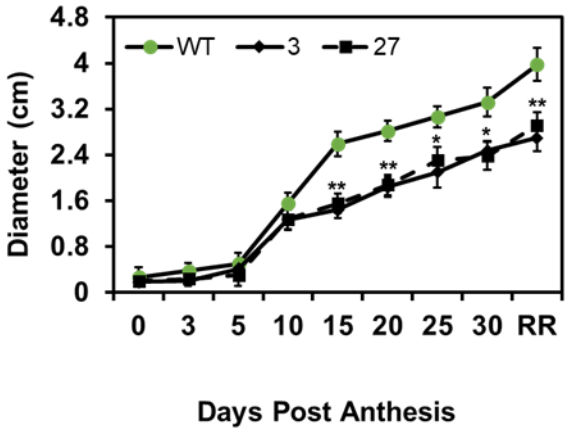


Figure S5 Phenotypic effect of sl*samba* gene editing on tomato fruit diameter. Diameter of the third fruit per inflorescence for WT and *slsamba* (#3 and #27) at different stages. Data are means ± SEM (*n* = 24). Significant differences (ANOVA followed by Dunnett's test) are indicated by asterisks (*p< 0.05 and **p< 0.01).

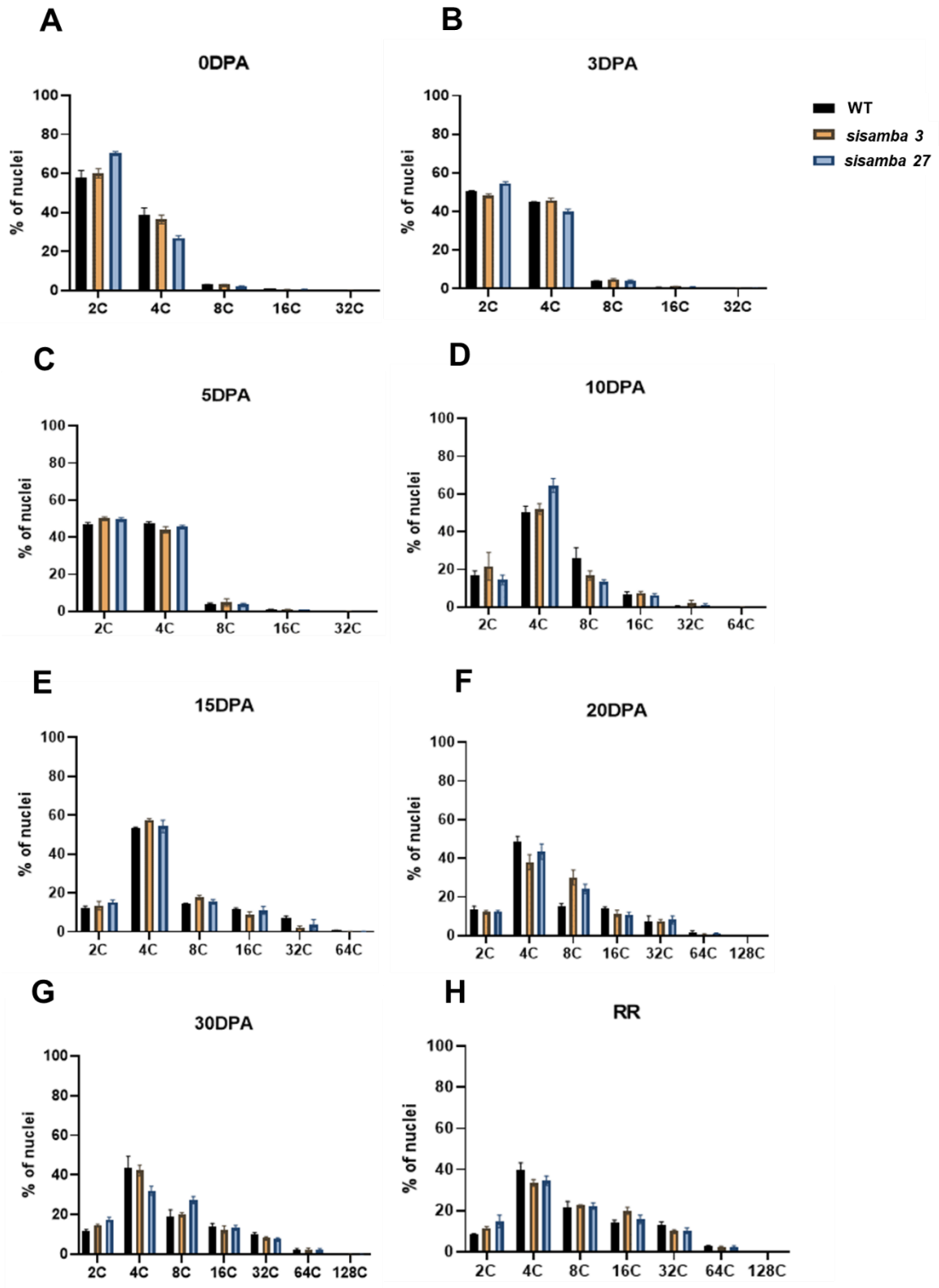


Figure S6 Flow cytometry analysis of nuclear DNA ploidy distribution in pericarps of wild-type (WT) and *slsamba* fruits (#3 and #27). Nuclei were isolated from pericarps of 0, 3, 5, 10, 15, 20, and 30 days after pollination (DPA) green (A -G) and 58 DPA red ripe tomato fruit (H) using chilled CyStain UV Precise P Nuclei Extraction buffer. Nuclei were stained by DAPI (4, 6-diamidino-2-phenylindole) at the final concentration of 50 μg/ml for flow cytometric analysis.

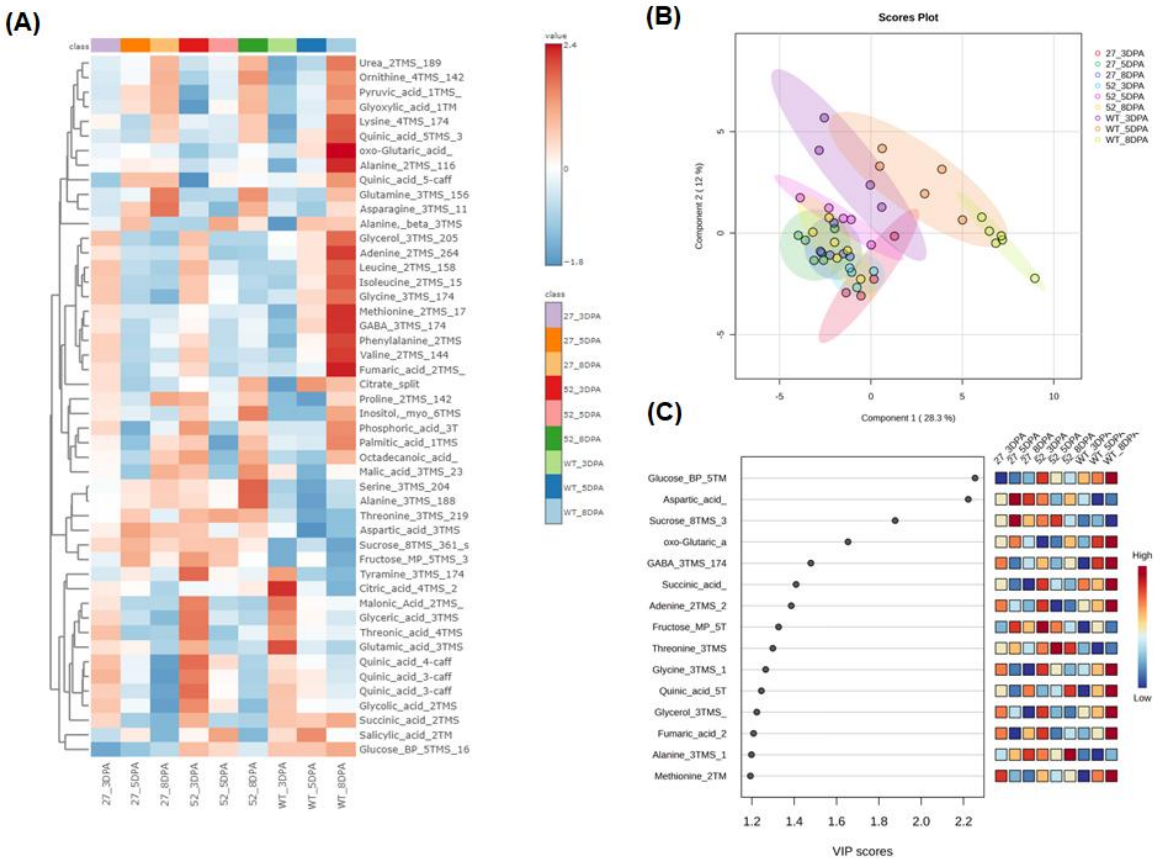


Figure S7 Metabolic characterization of tomato fruit at three developmental stages -3, -5, and 8 days post anthesis (DPA) collected and analyzed by GC–MS. (A) Heat map of the metabolites identified in this study. (B) Partial least square-discriminant analysis (PLS-DA). (C) Variable importance in projection (VIP) scores of the PLS-DA model. Metabolites included in this VIP score list have scores higher than 1, which indicates those that mostly contributed to the separation observed in the PLS-DA model. PLS-DA was carried out by combining data from all stages of development. The data were normalized by using Log and Auto-scaling transformations on the MetaboAnalyst platform 6.0 (n = 4-6).

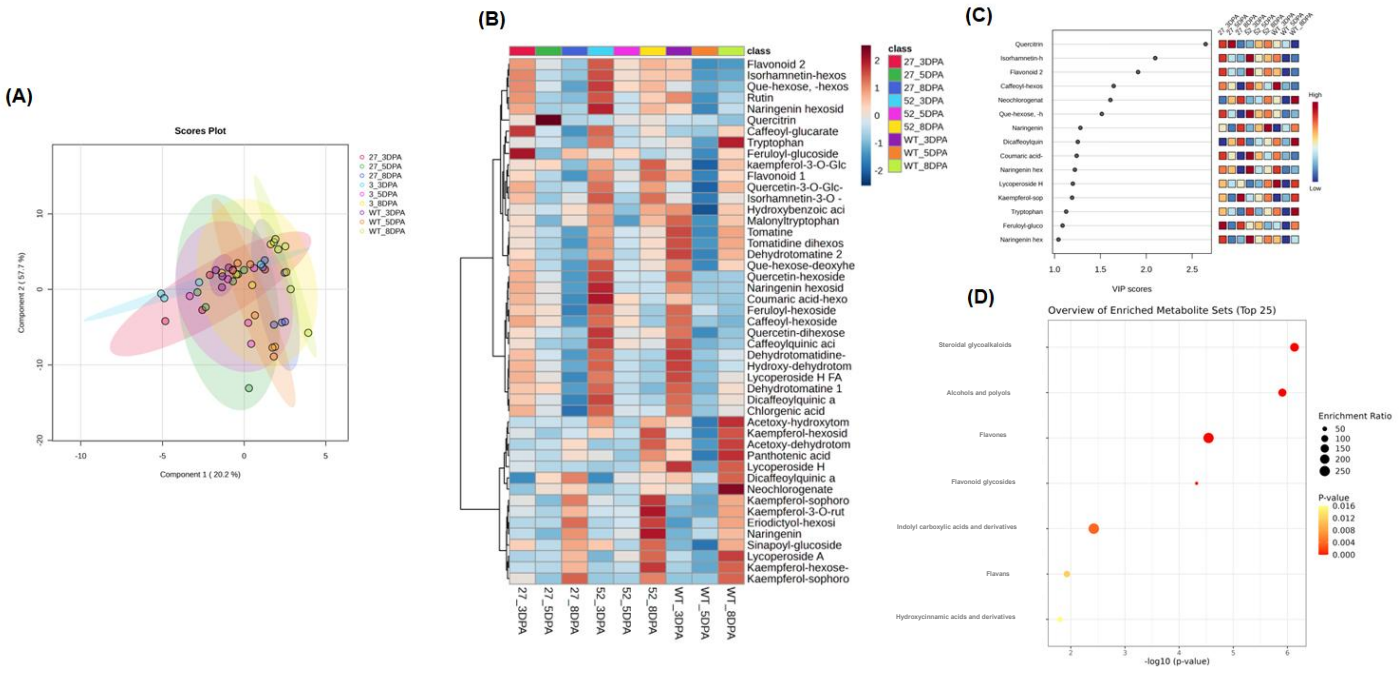


Figure S8 Metabolic characterization of tomato fruit at three developmental stages -3, -5, and 8 days post anthesis (DPA) collected and analyzed by LC–MS. (A) Partial least square-discriminant analysis (PLS-DA). (B) Heat map representation of the metabolite contents identified in this study. (C) The panel shows the top 15 variable importance in projection (VIP) identified by PLS-DA. The colored boxes on the right indicate the relative concentrations of each metabolite at each stage of development. (D) Quantitative enrichment analysis (QEA) overview presenting the top 25 related metabolic pathways ranked according to the P value. The data were normalized by using Log and Auto-scaling transformations on the MetaboAnalyst platform 6.0 (n = 4-6).

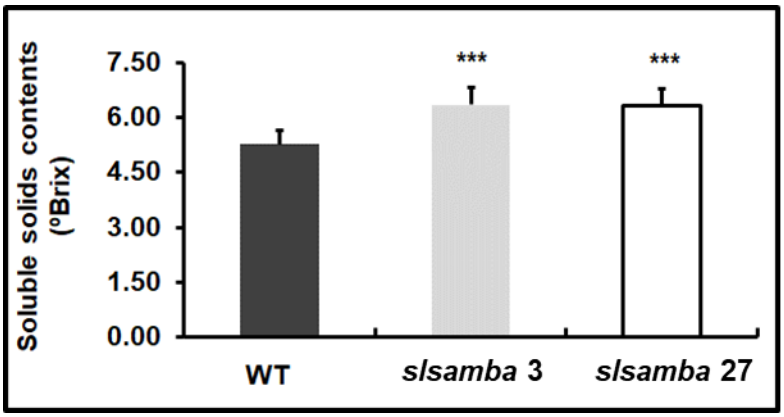
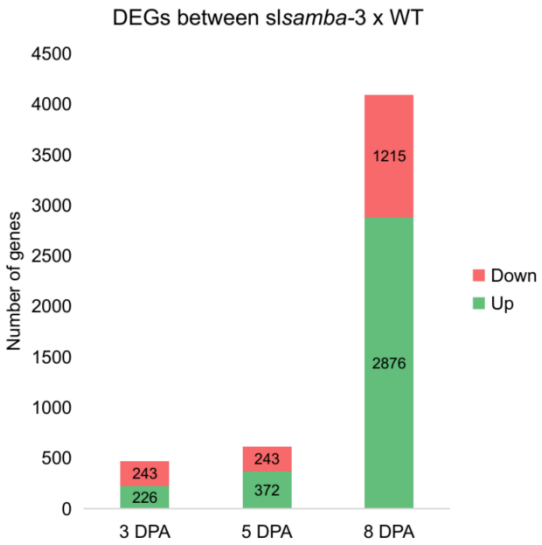


Figure S9 Soluble solids (°Brix) of red ripe tomato fruits from *slsamba* lines and wild-type plants. Values means ± SE.

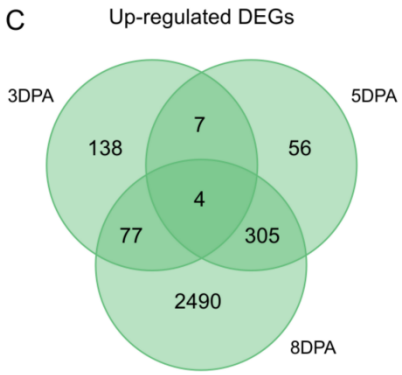
A

	3 DPA	5 DPA	8 DPA
Expressed genes (CPM >1 in all 3 samples)	13101	18859	17969
Numbrer of DEGs	469	615	4091
% of total	3.58	3.26	22.77

B



C



D

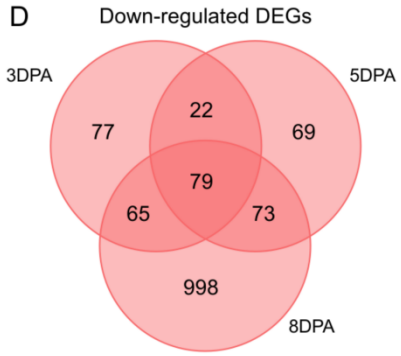


Figure S10 Results of differential expression analysis between *slsamba-3* and WT fruits. (A) Number of DEGs compared to the total expressed genes at each time point. (B) Bar plot of the number of DEGs at 3 DPA, 5 DPA, and 8 DPA, indicating up-regulated (green) and down-regulated (red) genes. (C) Venn diagram of up-regulated genes at all three time points. (D) Venn diagram of down-regulated genes at all three time points.

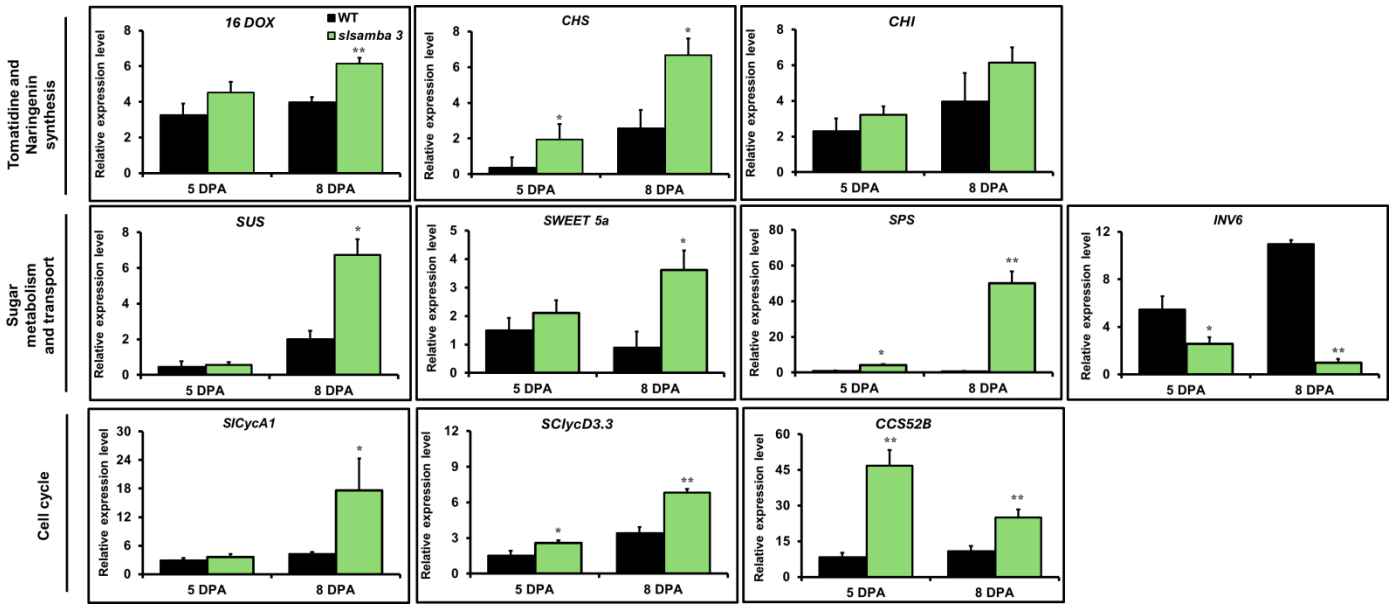


Figure S11 Relative expression by qRT-PCR in independent samples of *Solyc10g0181901* (*16alpha,22,26-Trihydroxycholesterol – 16DOX*), *Solyc01g090600* (*Chalcone synthase – CHS*), *Solyc05g010310* (*Chalcone-flavone isomerase - CHI*), *Solyc03g098290* (*Sucrose synthase - SUS*), *Solyc03g114200* (*SWEET 5a*), *Solyc08g042000* (*Sucrose phosphate synthase - SPS*), *Solyc10g083290* (*Invertase 6 – INV6*), *SlCycA1* (*Solyc11g005090*), *SlCycD3.3* (*Solyc04g078470*), *Solyc12g056490* (*CCS52B*) in line 3 and WT fruits harvested at three developmental stages (5-, 8- and 52-days post anthesis). Bars represent standard errors (SEs) of three biological replicates and two technical replicates. Significant differences (ANOVA followed by Dunnett's t test) are shown by asterisks (*P < 0.05 and **P < 0.01).

Table S1. Prediction of SISAMBA subcellular localization by Cello.

SeqID: Solyc08g076580.2.1

Analysis Report:

SVM	LOCALIZATION	RELIABILITY
Amino Acid Comp.	Cytoplasmic	0.463
N-peptide Comp.	Nuclear	0.718
Partitioned seq. Comp.	Nuclear	0.734
Physico-chemical Comp.	Chloroplast	0.419
Neighboring seq. Comp.	Chloroplast	0.442

CELLO Prediction:

Nuclear	**1.696** *
Cytoplasmic	**1.514** *
Mitochondrial	0.923
Chloroplast	0.488
Extracellular	0.212
Plasma Membrane	0.057
Golgi	0.023
Peroxisomal	0.022
Cytoskeletal	0.021
Vacuole	0.020
ER	0.016
Lysosomal	0.008

Table S2. Transmembrane prediction of the Solyc08g076580.2 (SlSAMBA) protein through the HMMTOP.

Protein: Solyc08g076580.2
Length: 118
N-terminus: IN
Number of transmembrane helices: 0
Transmembrane helices:

Total entropy of the model: 17.0075
Entropy of the best path: 17.0075

The best path:

```
seq  SLYCGMSTGS SLTSSPARSS SSTMAMIGGN VGPPSSTAVD DFNFPVDLIS    50
pred IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII

seq  VQDRKDEALQ VLKSDLMASL NKEVKSLDED SWMFDGPRSR IHRISRPGRL   100
pred IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII

seq  HKHVEVGKQK SKLAATLK  118
pred IIIIIIIIII IIIIIIII
```

Table S3. List of the 41 metabolites identified by GC–MS of fruits at -3, -5, or -8 days post anthesis (DPA) from *slamba* and WT lines.

Amino acids	Organic acids	Sugars	Fatty acids	Sugar alcohols	Others
Alanine	Citric acid	Fructose	Palmitic acid	Glycerol	Adenine
Asparagine	Fumaric acid	Glucose	Octadecanoic_acid	Myo-inositol	Salicylic acid
Aspartic acid	Glyceric acid	Sucrose			Tyramine
GABA	Glycolic acid				Urea
Glutamic acid	Glyoxylic acid				
Glutamine	Malic acid				
Glycine	Malonic acid				
Isoleucine	Oxo-glutaric acid				
Leucine	Phosphoric acid				
Lysine	Pyruvic acid				
Methionine	Quinic acid				
Ornithine	Succinic acid				
Phenylalanine	Threonic_acid				
Proline					
Serine					
Threonine					
Valine					

Table S4. Oligonucleotide sequences used in this work.

Primers	Sequence	Amplicon	Gene	References
SlSAMBA_FW	GATTTCTAGGCCAGGACGCC	80 bp	*Solyc08g076580.2.1*	This work
SlSAMBA_RW	AATGTGGCCGCCAACTTAGAT			
Sl β -ACTIN_FW	GGTCCCTCTATTGTCCACAG	130 bp	*Solyc04g081490*	Ferreira Silva et al. 2014
Sl β -ACTIN_RW	TGCATCTCTGGTCCAGTAGGA			
Chalcone synthase_FW	ATTGGCAAGGCTCTTCCTCC	107 bp	*Solyc01g090600.5*	This work
Chalcone synthase_RW	CGCTCCAATTTCTCCTTAATTC			
Chalcone-flavone isomerase_FW	CACCTGGTGCTTCCATCCTT	135 bp	*Solyc05g010310.3.1*	This work
Chalcone-flavone isomerase_RW	ATTCCAGCACAGCCTCTGAC			
Sucrose synthase_FW	TGGTTTGCCCGATACTGGAG	157 bp	*Solyc03g098290.4.1*	This work
Sucrose synthase_RW	GAGGCGAGTGACCACAAGAA			
SWEET_FW	CTCACTGTCATGCGTCGAGT	153 bp	*Solyc03g114200.4.1*	This work
SWEET_RW	AATGTTCCCAGACCATTCGG			
Sucrose phosphate synthase_FW	AGAGCAAACTGGTAGTGGGC	149 bp	*Solyc08g042000.3.1*	This work
Sucrose phosphate synthase_RW	GTTCCAGCTTGTCTCGTCCA			
Invertase 6_FW	CAACAACAGCTTGGATGGGC	142 bp	*Solyc10g083290.4.1*	This work
Invertase 6_RW	GCTGAGTGGAGTGGGTGTTT			
2-oxoglutarate_FW	TTGCCGCTCAACAACTTGTG	101 bp	*Solyc10g018190.2.1*	This work
2-oxoglutarate_RW	GACTTCCATTGCCTCGTCCA			
SlCycA1_FW	CTGCTCGGAACTCGGTTTCT	122 bp	*Solyc11g005090.1.1*	This work
SlCycA1_RW	CAGAACAGCAGTCCCTGAGG			
SlCycD3.3_FW	GCATCTGCCACAATGTTGCA	121 bp	*Solyc04g078470.3.1*	This work
SlCycD3.3_RW	GCCTGTAACATCCTTCCACCT			
CCS52B_FW	TGTGGGCTCAAATGGTCTCC	134 bp	*Solyc12g056490.1.1*	This work
CCS52B_RW	ATCGCCTTTACAGCAGCAGT			