

Corrigendum: Plant cholesterol biosynthetic pathway overlaps with phytosterol metabolism

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Two of the Supplementary Information files originally published contained errors. In Supplementary Table 1a, the SolycIDs for SMO1 and SMO2 were interchanged. In Supplementary Data 1, the SolycIDs for SMO3 and SMO4 were interchanged. Both errors have now been corrected.

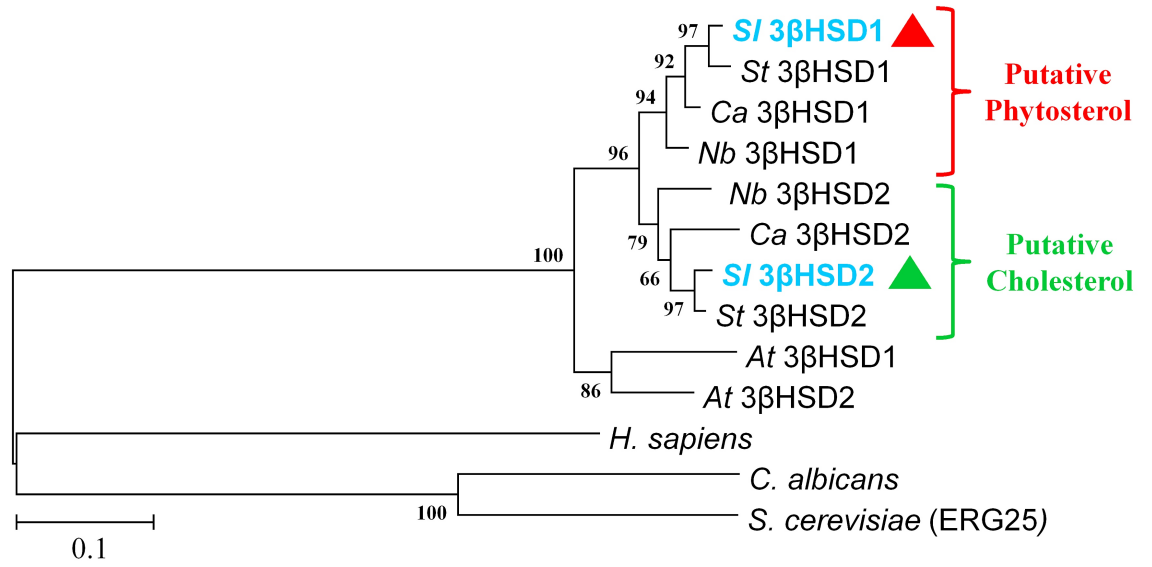
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Plant cholesterol biosynthetic pathway overlaps with phytosterol metabolism

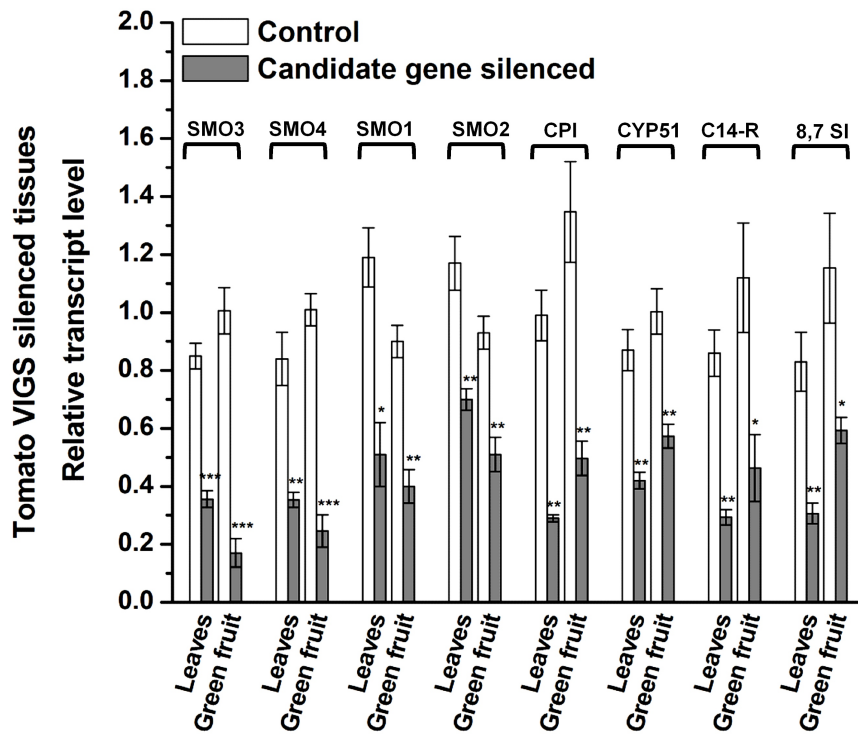
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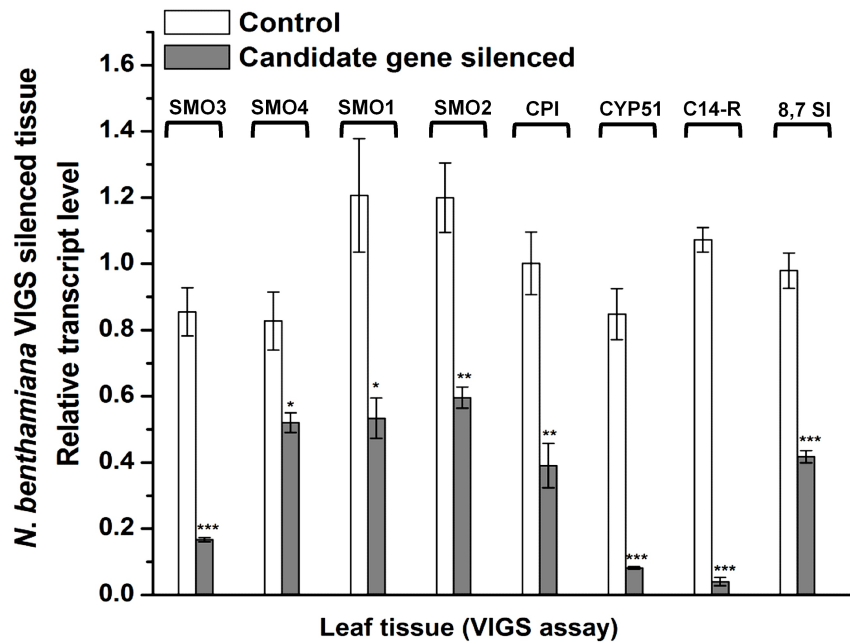
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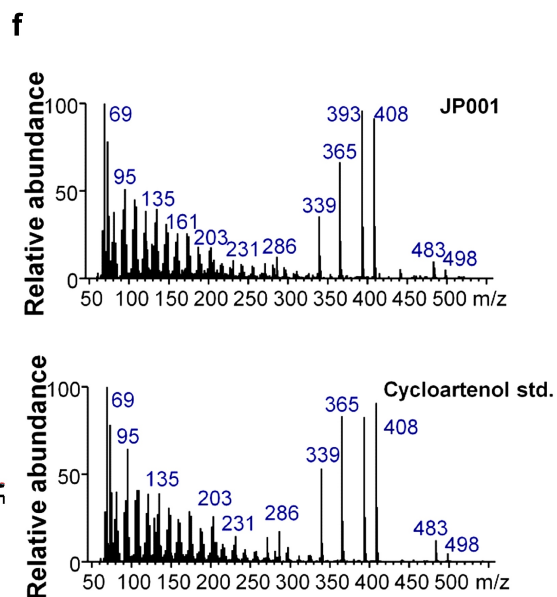
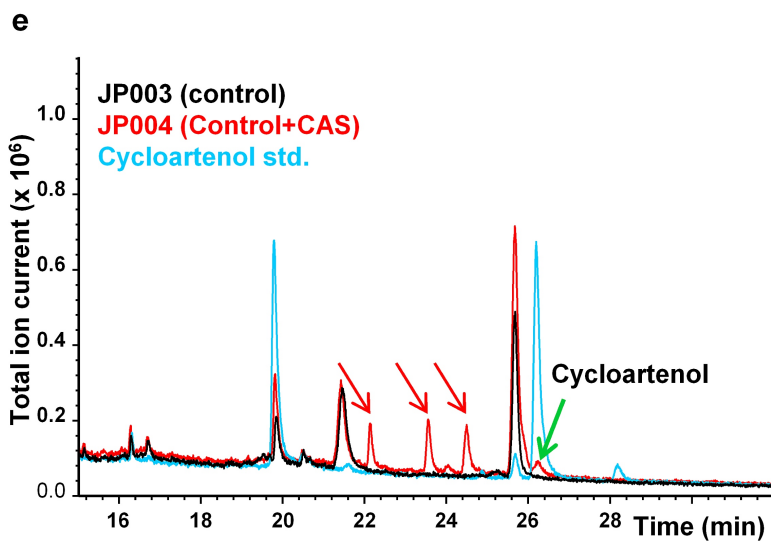
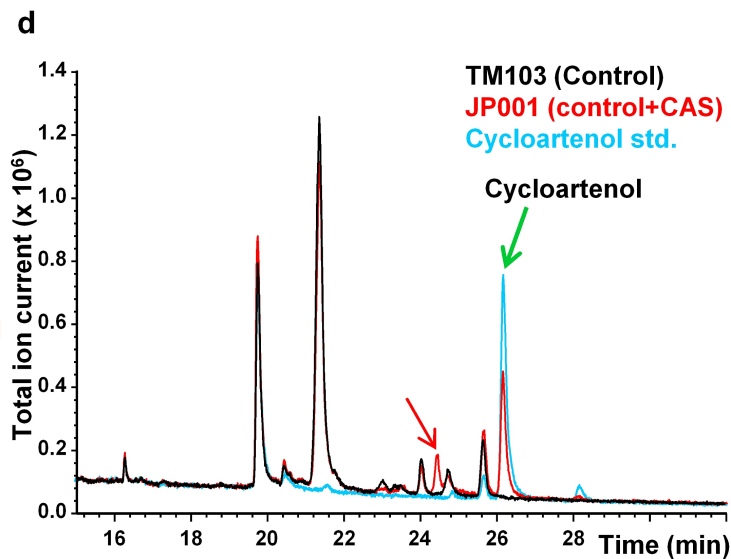
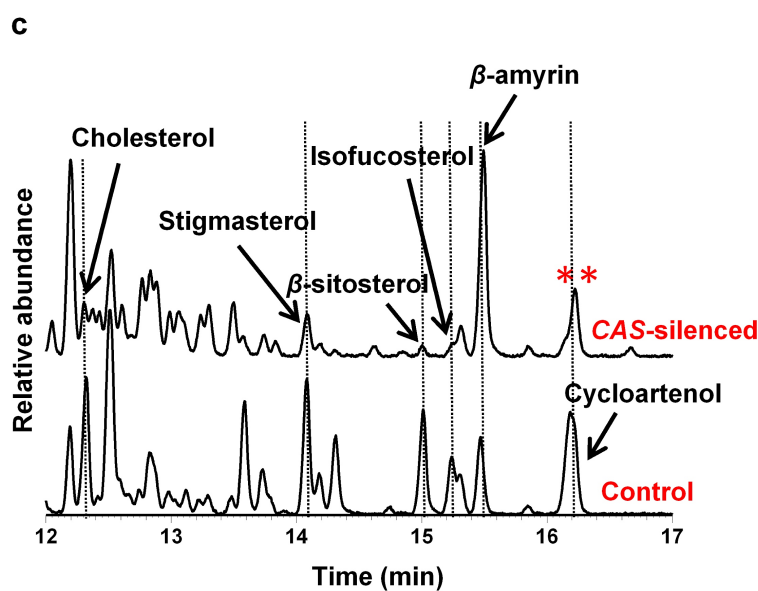
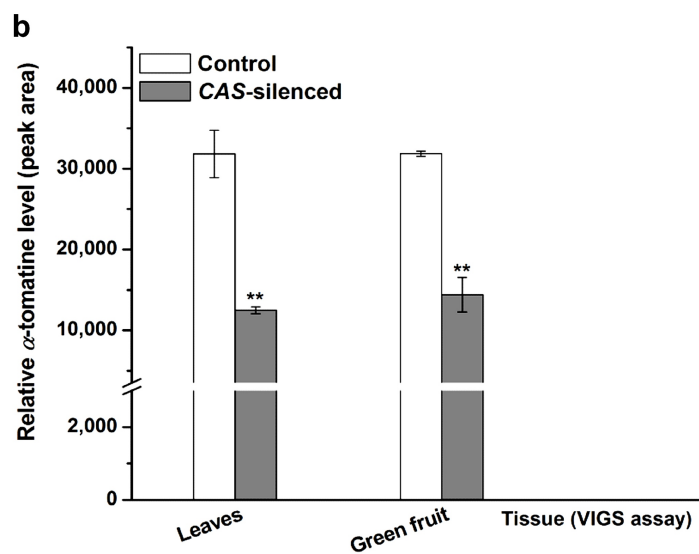
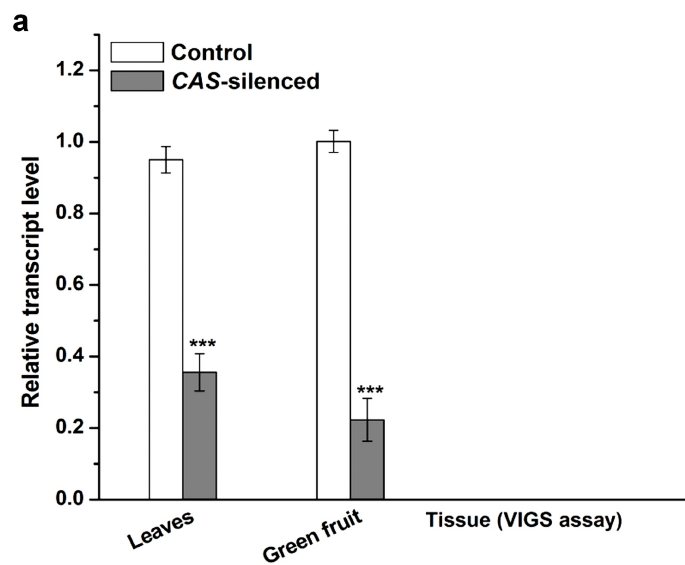
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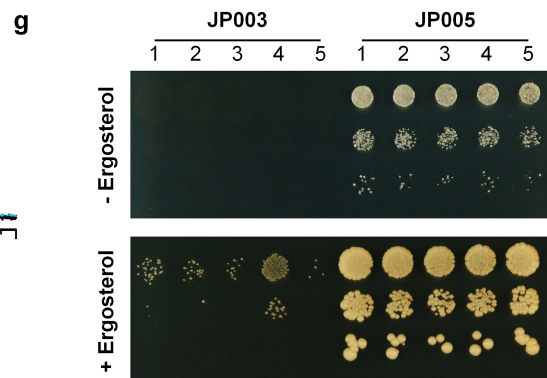
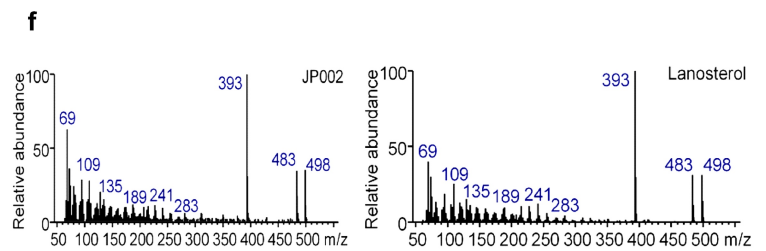
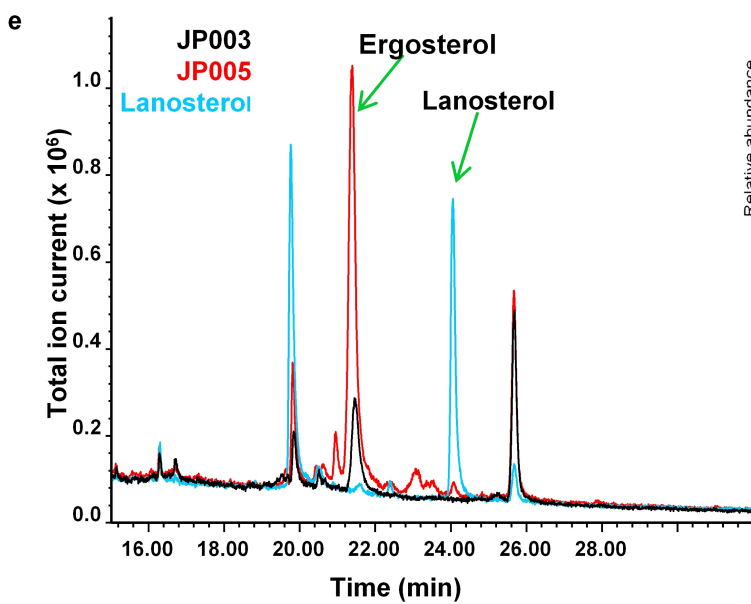
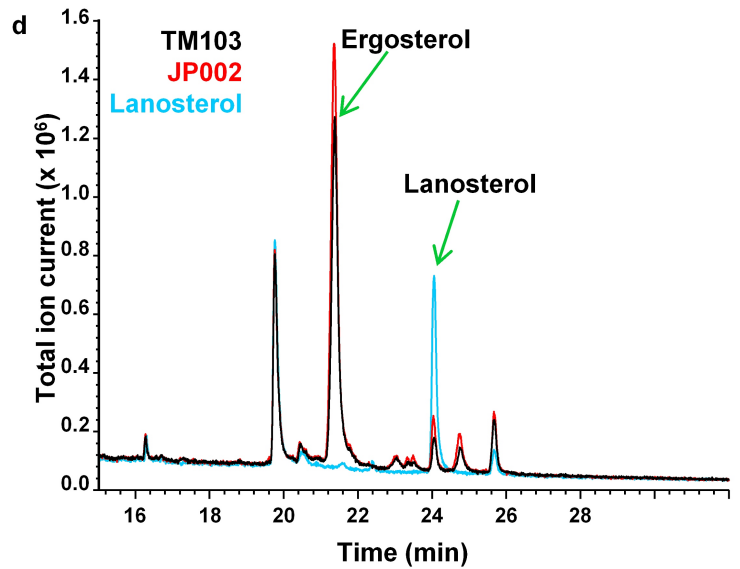
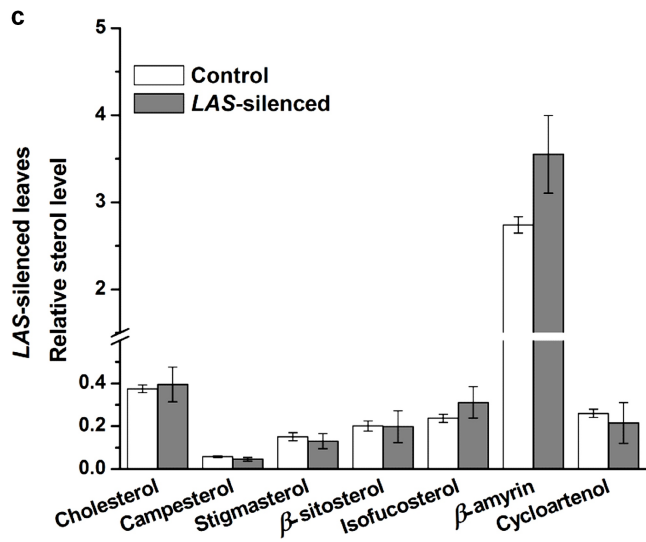
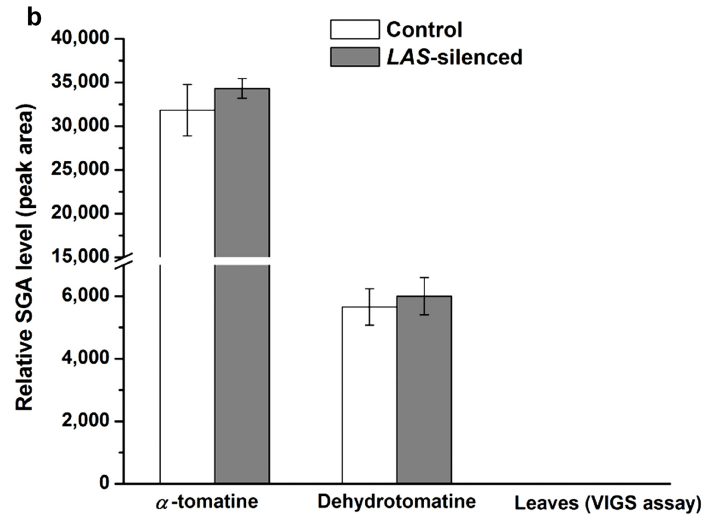
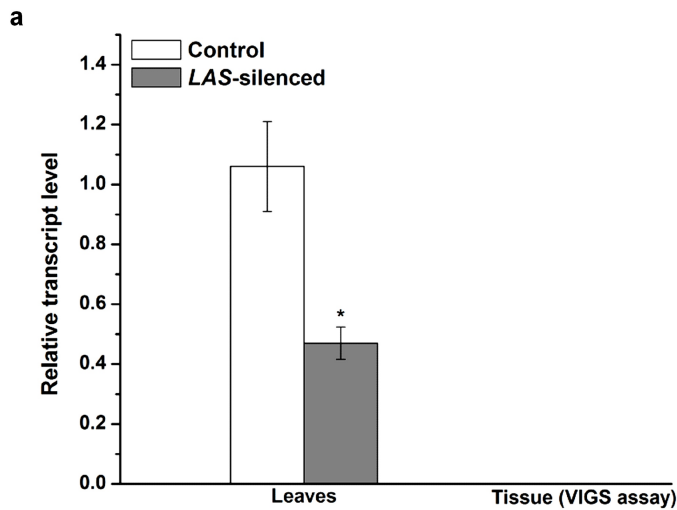
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Supplementary Figure 1: The tomato 3 β -HYDROXYSTEROID DEHYDROGENASE/C4-DECARBOXYLASE proteins and Virus Induced Gene Silencing experiments. **a**, Phylogenetic relationship among plant 3 β -hydroxysteroid dehydrogenase/c4-decarboxylases (3 β HSD) proteins. The following species were covered: tomato (Sl), potato (St), tobacco (Nt), *N. benthamiana* (Nb), *Capsicum annuum* (Ca), and *Arabidopsis thaliana* (At). *H. sapiens*, *C. albicans* and *S. cerevisiae* 3 β HSD proteins served as outgroups. The evolutionary history was inferred using the Neighbor-Joining method in MEGA6⁴⁴ and percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown in branches. Sequence information used for the phylogenetic study is given in Supplementary Data 2. **b, c** Expression of VIGS-silenced candidate tomato cholesterol genes (grey bars) in leaves and green fruit (**b**) and *N. benthamiana* leaves (**c**) as compared to control plants (white bars) determined by quantitative real-time PCR (qRT-PCR). For tomato and *N. benthamiana*, the pTRV2 vector harboring *Del/Ros* and the pTRV2 empty vector were used for control transformations, respectively. Values (panel **b** and **c**) represent mean $\pm$ standard error (n=3). Asterisks indicate significant changes from control plants as calculated by a Student's *t*-test (*P-value < 0.05; **P-value < 0.01; ***P-value < 0.001).



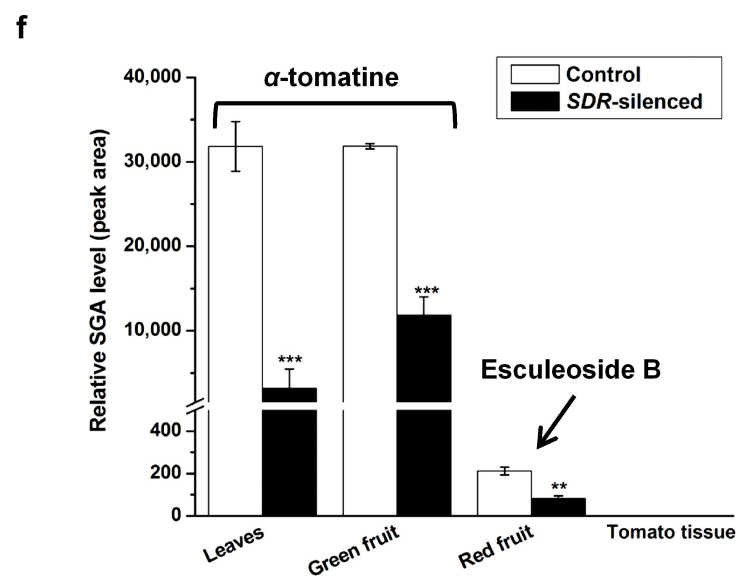
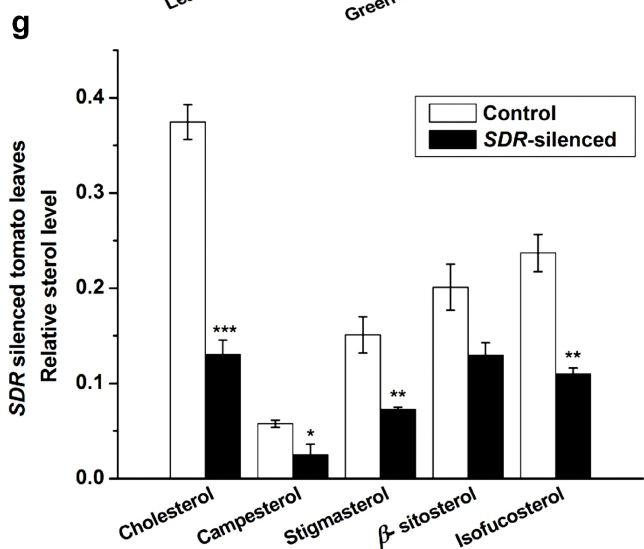
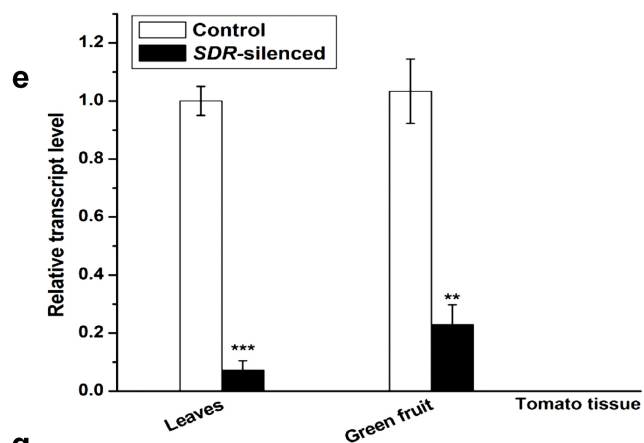
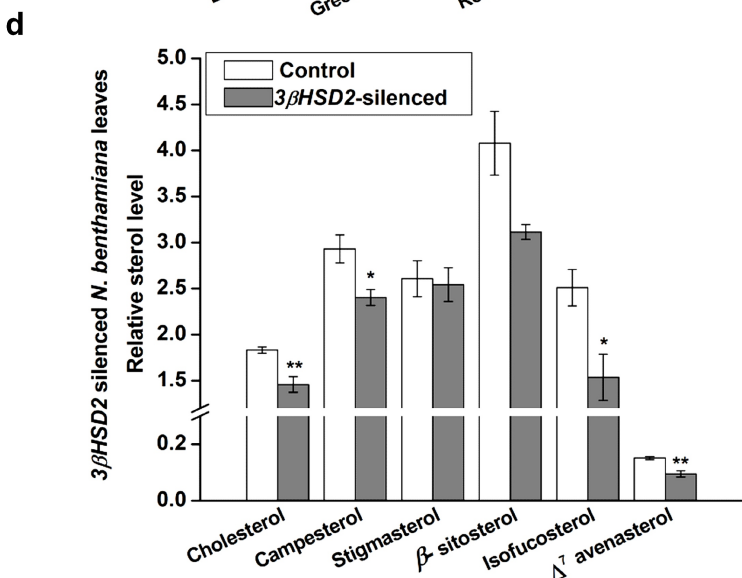
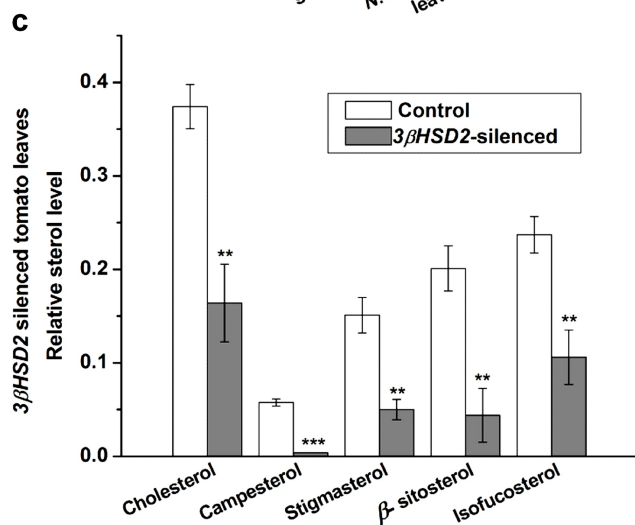
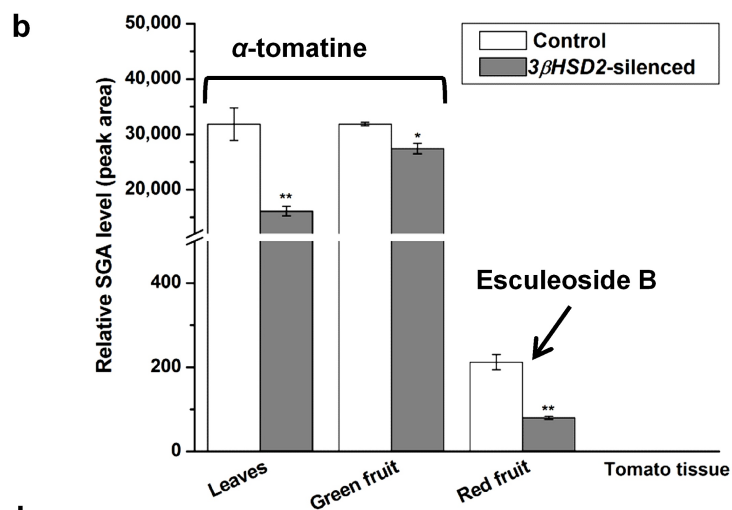
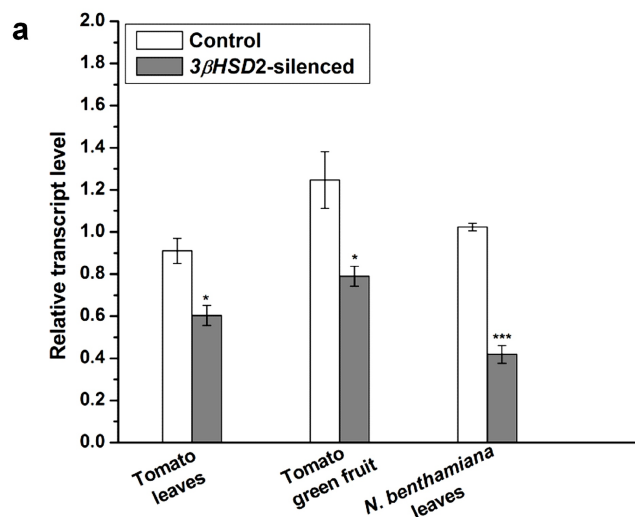
Supplementary Figure 2: The tomato CYCLOARTENOL SYNTHASE is required for both cholesterol and phytosterol biosynthesis. a, *CYCLOARTENOL SYNTHASE* (CAS) expression in leaves and green fruit of CAS-silenced and control plants determined by qRT-PCR. b, CAS-silenced plants exhibit reduced α -tomatine amounts in leaves and green fruit compared to control. c, Sterol profiling of CAS-silenced and control tomato leaves by GC-MS shows significant reduction in cholesterol and phytosterols compared to control leaves. **indicates presence of a different sterol compound at the same retention time instead of cycloartenol. The GC-MS spectra of ** is provided in Supplementary Figure 11 (panel e). d-f, Functional characterization of tomato CAS in *S. cerevisiae*. d, Overlay of GC chromatograms showing the accumulation of cycloartenol in strain JP001 (red) expressing CAS but not in the control strain TM103 (black). An authentic cycloartenol standard (light blue) was included in the analysis. A second new peak (red arrow) likely corresponds to a cycloartenol derivative generated by endogenous yeast protein(s). e, Overlay of GC chromatograms of lanosterol synthase-deficient GIL77 strains expressing a control construct (JP003, black) or CAS (JP004, red) showing the accumulation of low amounts of cycloartenol in strain JP004. Several metabolites likely derived from cycloartenol accumulate in this strain. An authentic cycloartenol standard is shown in light blue. f, GC-MS spectra of strain JP001 produced cycloartenol and an authentic cycloartenol standard. Values in panels a and b represent mean $\pm$ standard error (n=3). Asterisks indicate significant changes from control plants as calculated by a Student's *t*-test (*P-value < 0.05; **P-value < 0.01; *P-value < 0.001). Yeast strains used in this study are listed in Supplementary Data Table 1b.**



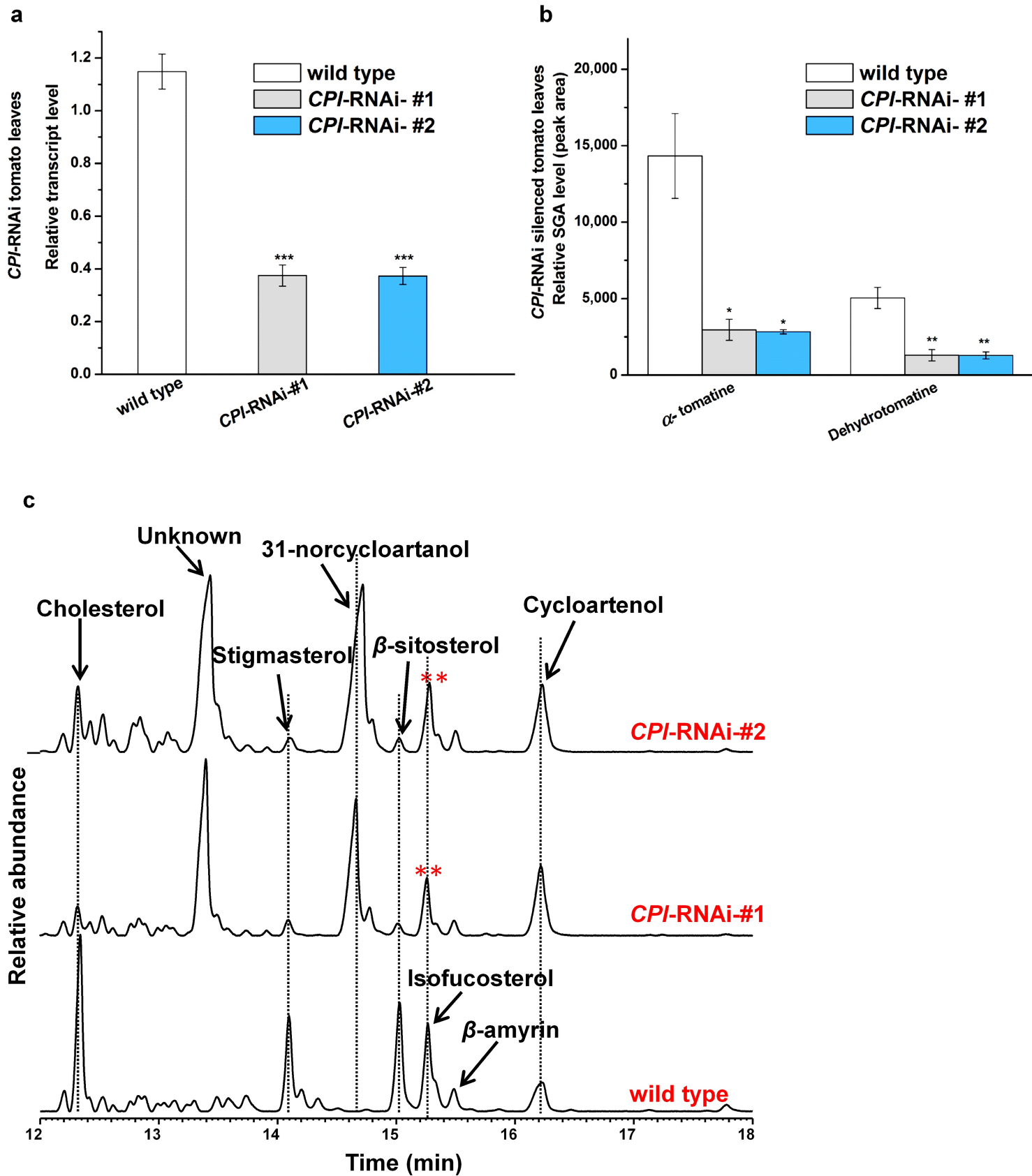
Supplementary Figure 3: Functional characterization of LAS suggests its minimal role in sterol biosynthesis in tomato. **a**, Relative expression level of *LAS* gene in VIGS-silenced leaves compared to control plants. **b**, Level of α -tomatine and dehydrotomatine in leaves of *LAS*-silenced plants. **c**, Relative abundance of cholesterol and other sterols levels in leaves of silenced *LAS* tomato as compared to control plants measured using GC-MS. Relative metabolite levels are expressed as ratios of peak areas compared to internal standard (epicholesterol). **d-g**, Functional characterization of *LAS* in *S. cerevisiae*. **d**, Overlay of GC chromatograms of the control strain TM103 (black) and strain JP002 (red) that expresses *LAS*. No unique peaks were observed in extracts of strain JP002, though slightly enhanced levels of ergosterol and lanosterol (indicated with green arrows) were observed. An authentic lanosterol standard (light blue) was included in the analysis. **e**, Overlay of GC chromatograms of lanosterol synthase-deficient GIL77 strains expressing a control construct (JP003, black) or *LAS* (JP005, red) showing the accumulation of low amounts of lanosterol in strain JP005. Strain JP005 also accumulates high amounts of ergosterol, indicating the produced lanosterol is further metabolized by the endogenous sterol synthesis enzymes. An authentic lanosterol (light blue) standard was included in the analysis. **f**, Comparison of the EI-MS spectra of the lanosterol produced in strain JP002 (left) and an authentic lanosterol standard (right). **g**, Functional complementation of the ergosterol auxotrophy of the GIL77 strain by *LAS*. Five colonies of strains JP003 and JP005 were dropped on plates with and without ergosterol. Growth on plates without ergosterol was observed only for strain JP005. Values (panel a-c) indicate mean $\pm$ standard error (n=3). Significant changes compared to control plants are

calculated by student's t-test: *P value < 0.05; **P value < 0.01; ***P value < 0.001.

Yeast strains used in this study are listed in Supplementary Data Table 1b.

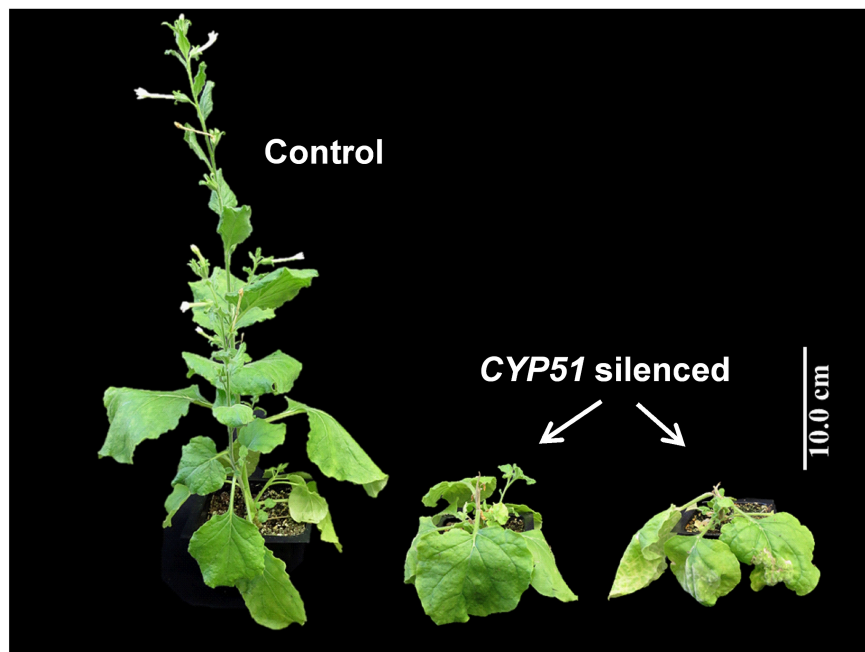


Supplementary Figure 4: 3 β -HYDROXYSTEROID DEHYDROGENASE/C4-DECARBOXYLASE 2 and a SIDE CHAIN OXIDOREDUCTASE act in both the cholesterol and phytosterol pathways. **a**, Expression of *3 β -HYDROXYSTEROID DEHYDROGENASE/C4-DECARBOXYLASE 2* (*3 β HSD2*) in *3 β HSD2* silenced tomato leaves and green fruit and *N. benthamiana* (leaves) in comparison to control by qRT-PCR assays. **b, f** Reduced levels of α -tomatine (in leaves and green fruit) and esculeoside B (in red, ripe tomato fruit) in *3 β HSD2* (**b**) and *SDR* (**f**) silenced plants. **c, d** The sterol profile of *3 β HSD2*-silenced tomato (**c**) and *N. benthamiana* (**d**) leaves. **e**, *SDR* expression in VIGS-silenced tomato leaves and green fruit tissues analyzed by qRT PCR. **g**, The sterol profile of *SDR*-silenced tomato leaves. Values represent mean $\pm$ standard error (n=3). Asterisks indicate significant changes from control samples as calculated by a Student's *t*-test (*P-value < 0.05; **P-value < 0.01; ***P-value < 0.001).

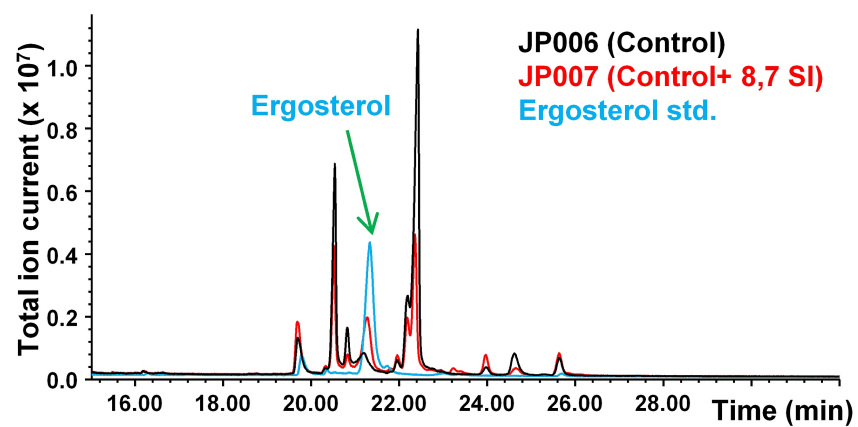


Supplementary Figure 5: *CYCLOPROPYL STEROL ISOMERASE* silenced transgenic tomato plants show altered levels of cholesterol and phytosterols. a, *CYCLOPROPYL STEROL ISOMERASE* (*CPI*) expression in leaves of *CPI*-RNAi-#1 and *CPI*-RNAi-#2 transgenic tomato lines (qRT-PCR assays). **b,** SGAs levels in leaves of wild type (WT; non-transformed) and *CPI*-silenced plants. **c,** GC-MS analysis of the sterol profiles of WT and *CPI*-silenced tomato leaves. **Presence of an unknown compound different than isofucosterol at the same retention time (see also Figure 5c). For GC-spectra, see Supplementary Fig. 11, panel **f**. Values indicate means of three biological replicates $\pm$ standard error. Asterisks indicate significant changes from wild-type samples as calculated by a Student's *t*-test (*P-value < 0.05; **P-value < 0.01; ***P-value < 0.001).

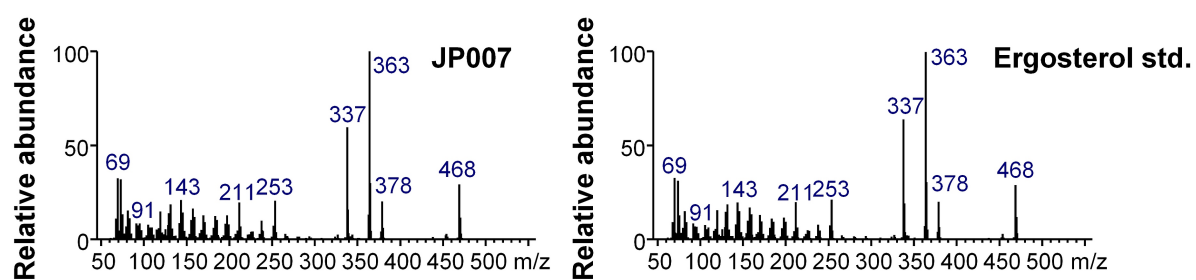
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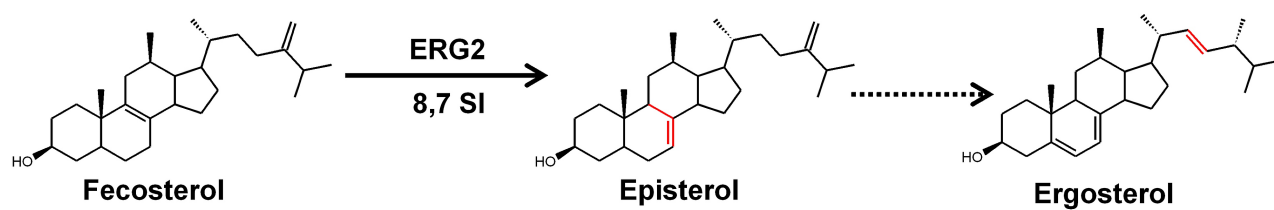
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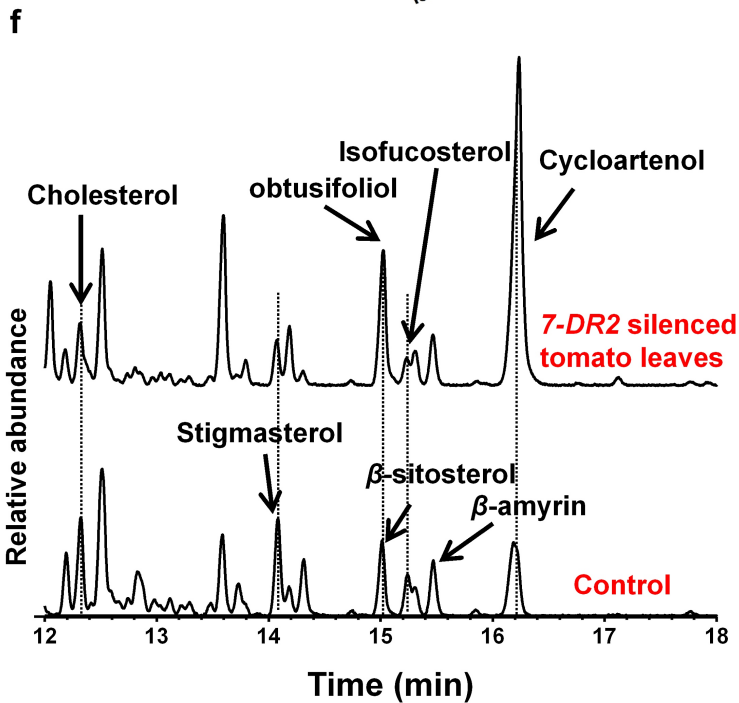
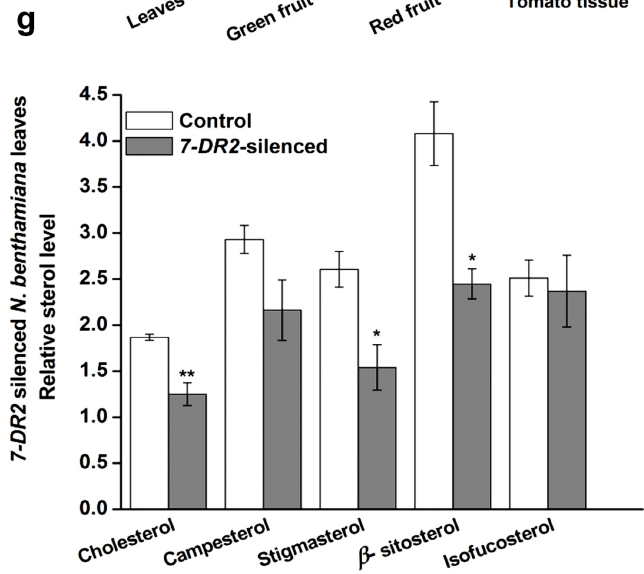
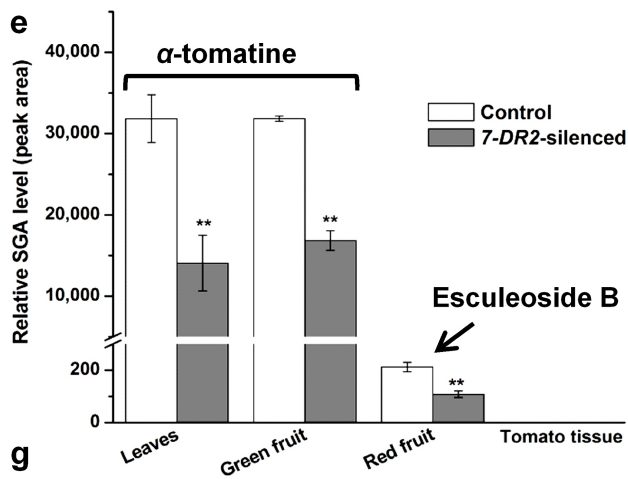
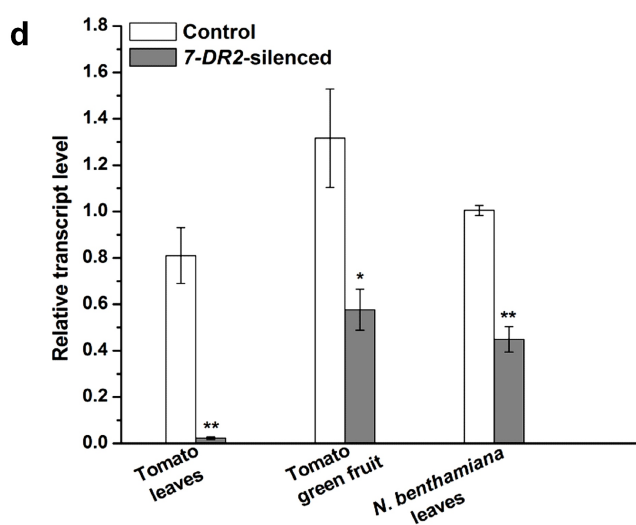
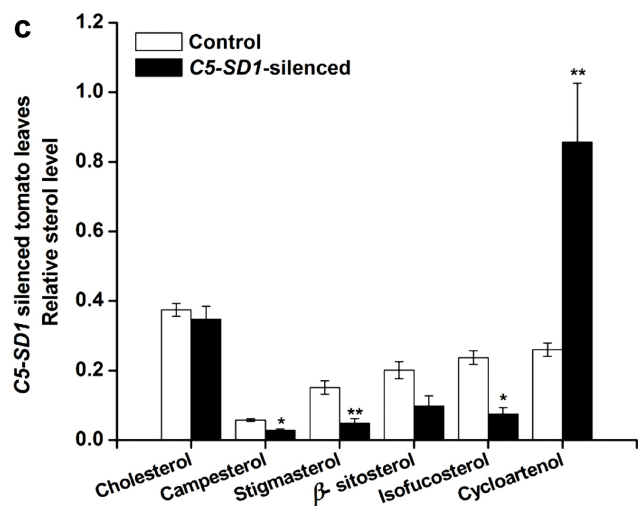
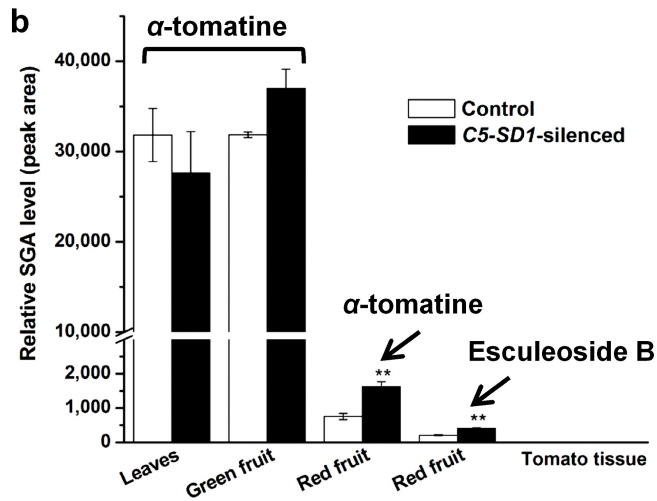
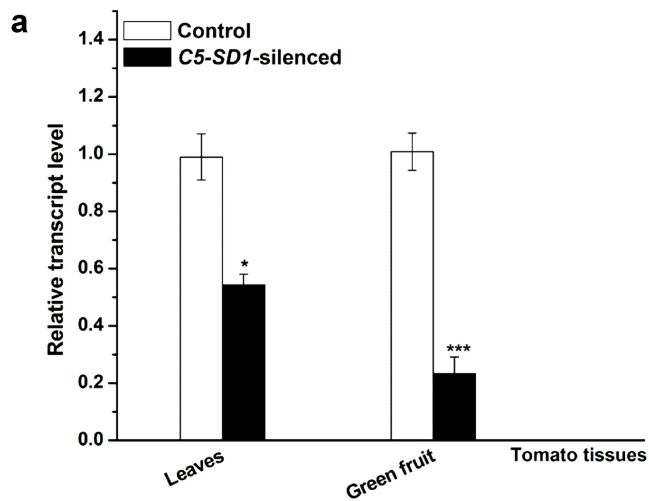
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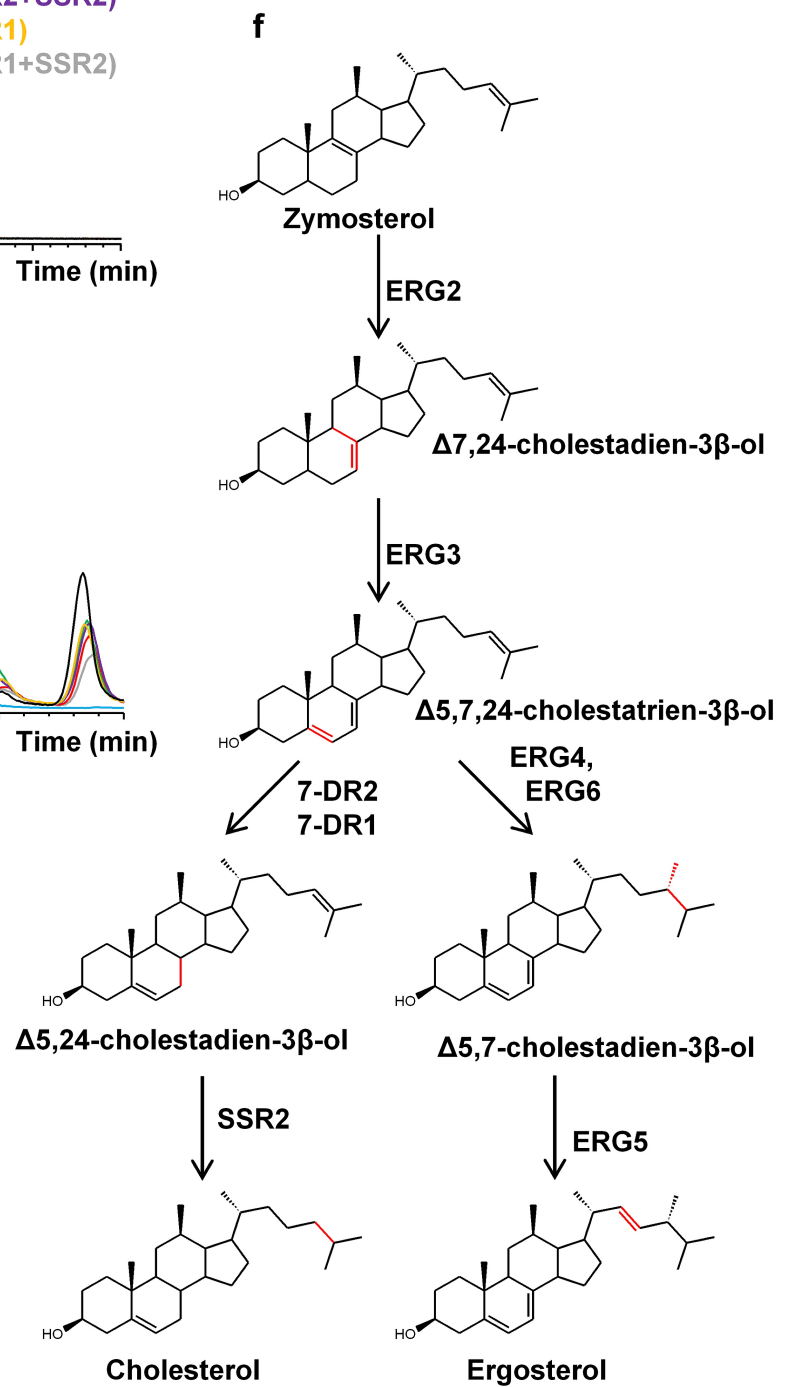
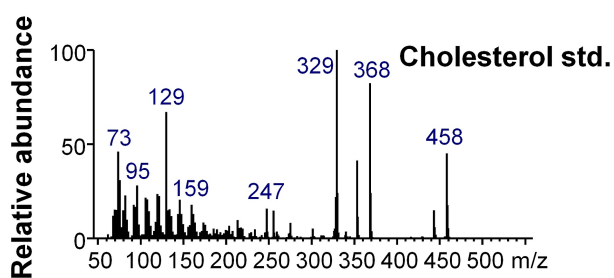
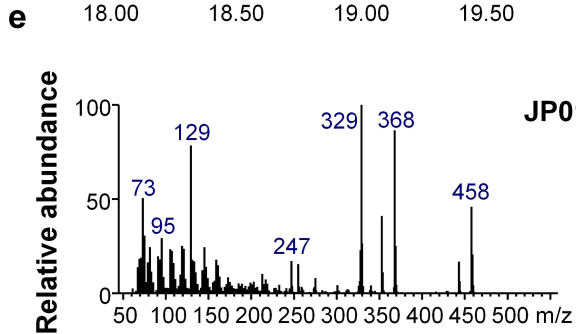
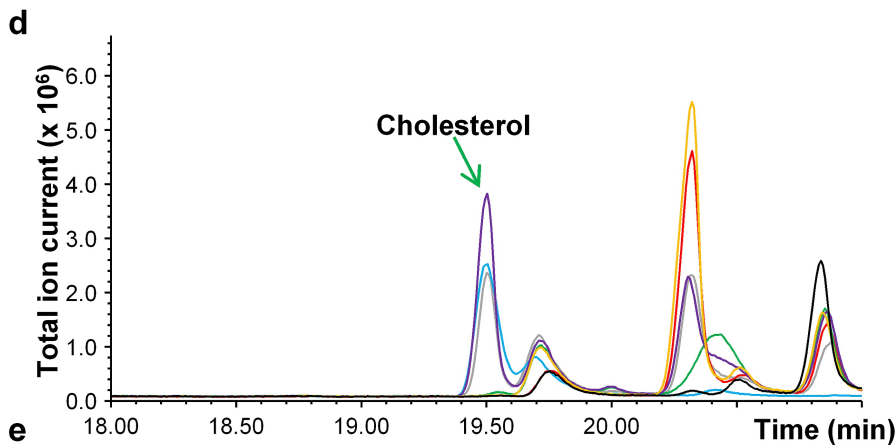
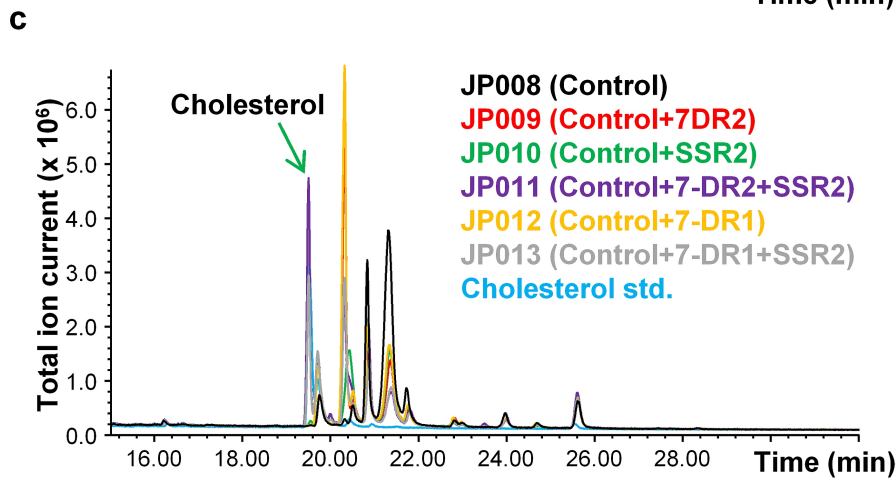
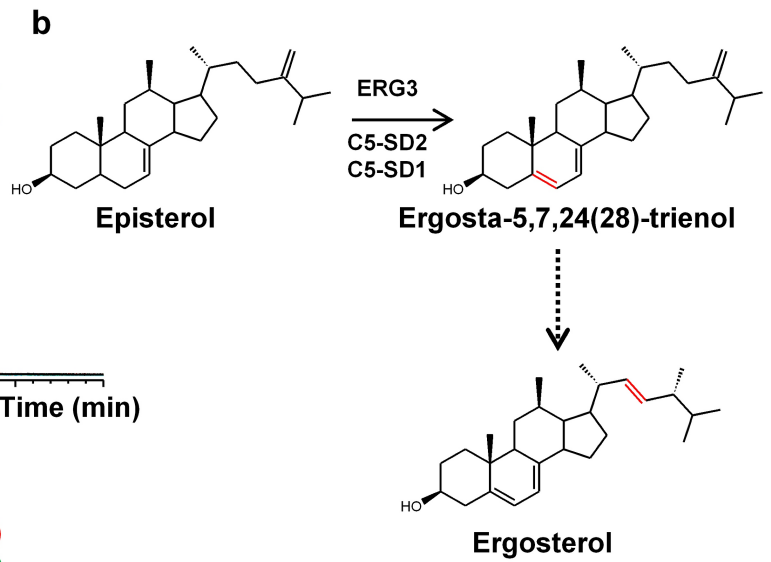
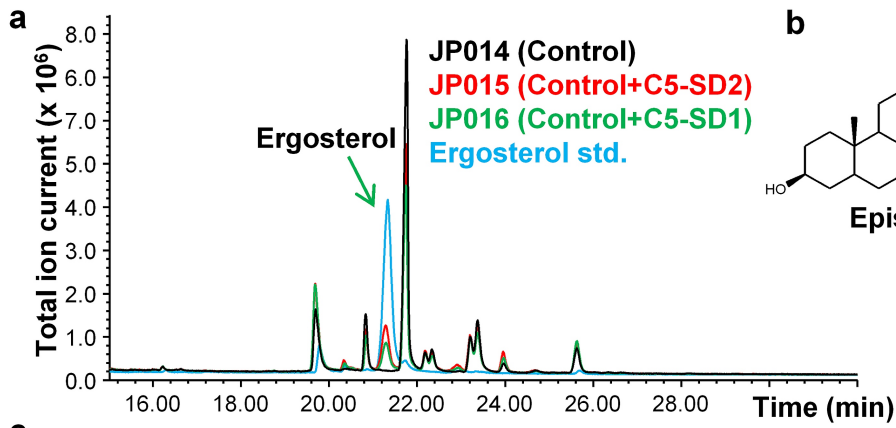
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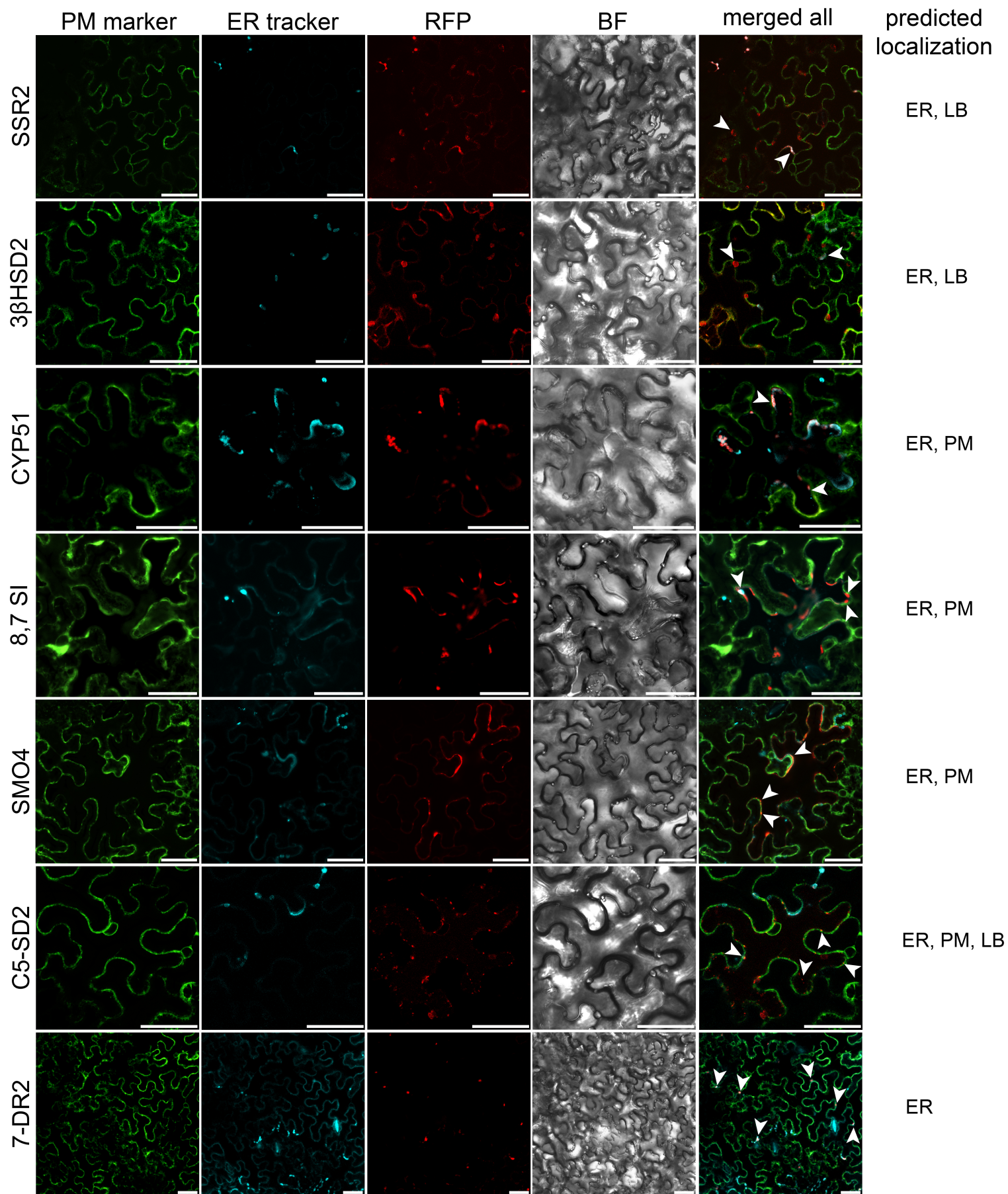
Supplementary Figure 6: Silencing of the tomato *CYP51* in *N. benthamiana* and functional assay of 8,7 SI in *S. cerevisiae*. **a**, A growth retarded phenotype (right side) observed in *CYP51*-VIGS silenced *N. benthamiana* plants 4 weeks after infection. Control plants are infected with pTRV2 empty vector. **b**, Overlay of GC chromatograms showing the accumulation of ergosterol (green arrow) in strain JP007 (red) expressing 8,7 SI, but not in the control yeast strain JP006 (black). An authentic ergosterol standard is shown in light blue. **c**, Comparison of the GC-MS spectra of the ergosterol accumulating in strain JP007 (left) and the ergosterol standard (right). **d**, Reaction catalyzed by ERG2 and 8,7 SI in *S. cerevisiae*. Yeast strains used in this study are listed in Supplementary Table 1b.



Supplementary Figure 7: *In planta* functional characterization of the *STEROL C-5(6) DESATURASE1* and *7-DEHYDROCHOLESTEROL REDUCTASE2* genes. **a**, Relative expression of *STEROL C-5(6) DESATURASE 1 (C5-SD1)* in leaves and green fruit of VIGS-silenced tomato plants measured by qRT PCR. **b**, Levels of α -tomatine and esculeoside B in *C5-SD1* VIGS-silenced tomato leaves and fruit tissues. **c**, Relative abundance of major sterols in *C5-SD1* VIGS silenced tomato leaves. **d**, Differential expression analysis of *7-DEHYDROCHOLESTEROL REDUCTASE 2 (7-DR2)* silenced tomato leaves and green fruit and *N. benthamiana* leaves, analyzed by qRT PCR. **e**, Altered levels of α -tomatine and esculeoside B in *7-DR2*-silenced tomato green tissues and red fruit, respectively. **f**, Sterols in leaves of *7-DR2* VIGS silenced and control tomato plants as determined by GC-MS. **g**, Sterol levels in *7-DR2* silenced and control *N. benthamiana* leaves. Values represent mean $\pm$ standard error (n=3). Asterisks indicate significant changes from control plants as calculated by a Student's *t*-test (*P-value < 0.05; **P-value < 0.01; ***P-value < 0.001).



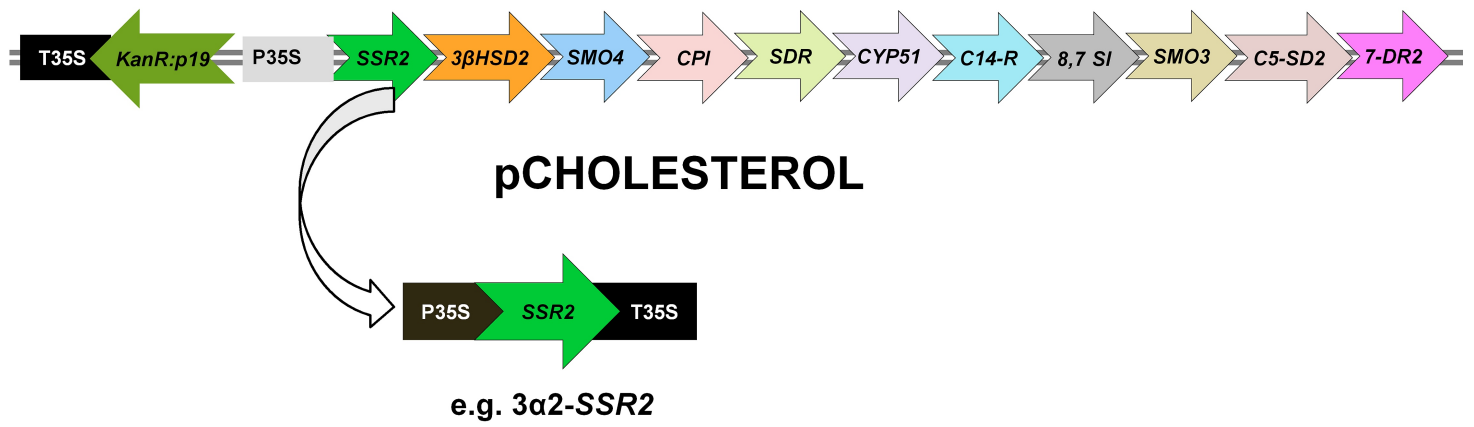
Supplementary Figure 8: Characterization of the tomato STEROL C-5(6) DESATURASE and 7-DEHYDROCHOLESTEROL REDUCTASE enzymes in *S. cerevisiae*. **a**, Overlay of GC chromatograms showing the accumulation of ergosterol (green arrow) in strains JP015 (red) and JP016 (green), expressing the tomato *C5-SD2* and *C5-SD1* in the *erg3* background respectively, but not in the control strain JP014 (black). An authentic ergosterol standard is shown in light blue. **b**, The reaction catalyzed by the ERG3 and the heterologously expressed tomato *C5-SD1* and *C5-SD2* in *S. cerevisiae* (as presented in 'a'). **c**, Overlay of GC chromatograms showing cholesterol accumulation (green arrow) in strains JP011 (purple) expressing the tomato *7-DR2* and JP013 (grey) expressing *7-DRI*, but not in the control strains. An authentic cholesterol standard is shown in light blue. **d**, Zoom in on the 18 to 21 min. region of the chromatogram presented in panel **c**. **e**, Comparison of the GC-MS spectra of the cholesterol accumulating in strain JP013 and an authentic cholesterol standard. **f**, The scheme depicts the native and altered sterol biosynthetic pathway that resulted in cholesterol accumulation in the *erg5erg6* double knock-out yeast strain. Starting from zymosterol, the endogenous yeast ERG2 and ERG3 proteins and the heterologous tomato *7-DR1/2* and SSR2 proteins catalyze cholesterol accumulation. In wild-type yeast, the pathway leads to ergosterol by the combined action of ERG4, ERG5 and ERG6. Yeast strains used in this study are listed in Supplementary Table 1b.



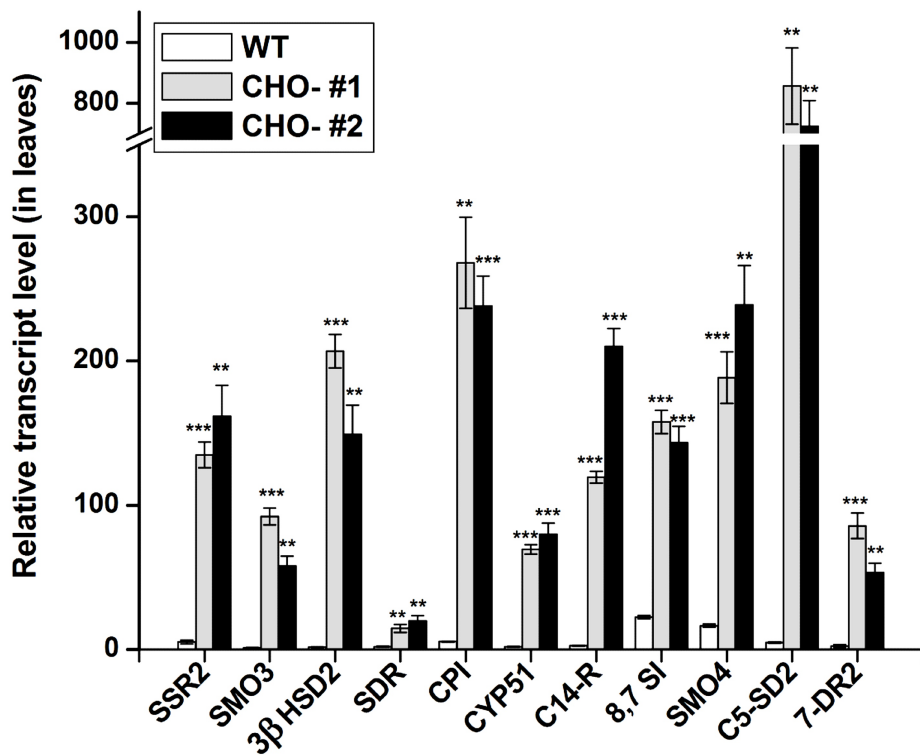
Supplementary Figure 9: Subcellular localization of the cholesterologenesis proteins.

N. benthamiana leaves were infiltrated with two *A. tumefaciens* cultures harboring (i) a vector expressing the cholesterologenesis protein fused to the Red Fluorescent Protein (RFP) and (ii) a vector with a plasma membrane (PM) organelle marker fused to the Green Fluorescent Protein (GFP). Confocal images were taken from infiltrated cell discs stained with the endoplasmic reticulum (ER) organelle specific dye (ER-tracker). All cholesterol proteins examined were found to localize to the ER, some also in the plasma membrane (PM) and/or in lipid body like structures (LB). Scale bars = 50 μ M. PM, plasma membrane marker; BF, bright field.

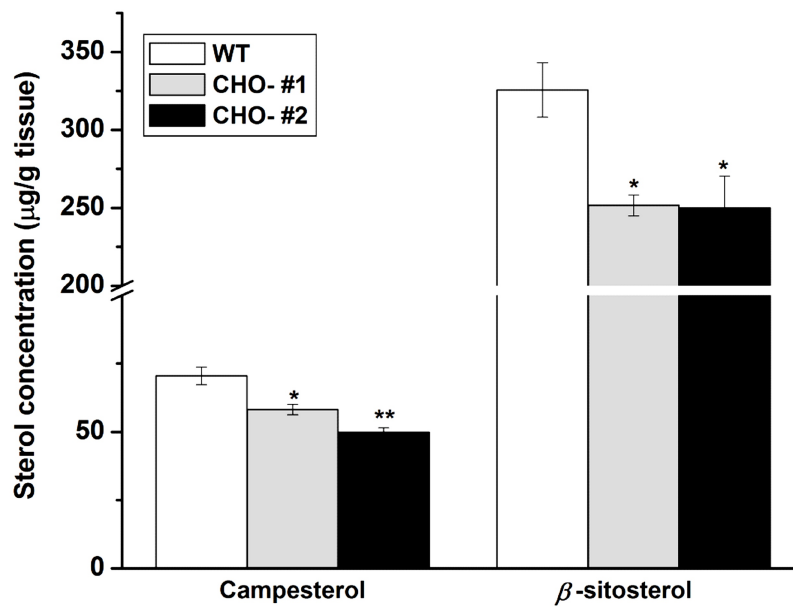
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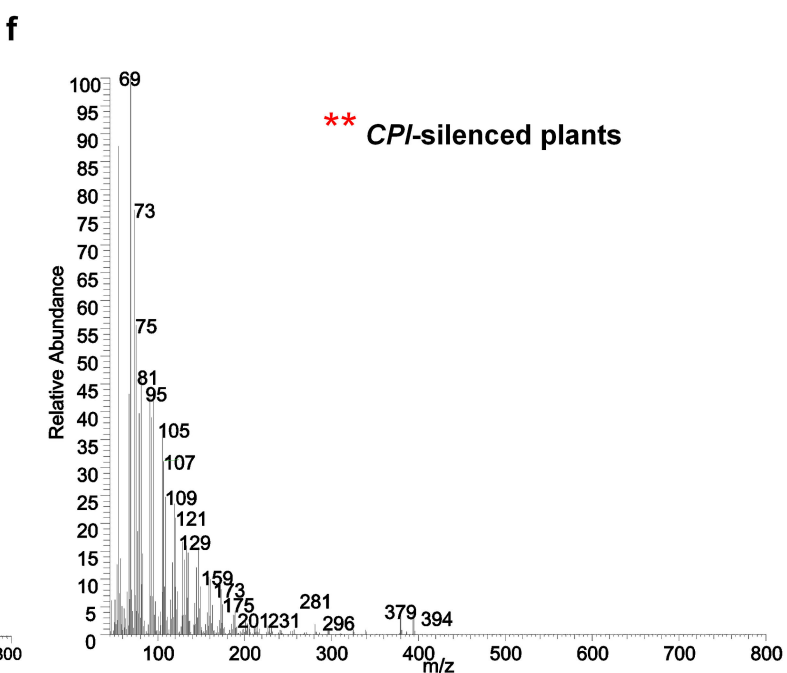
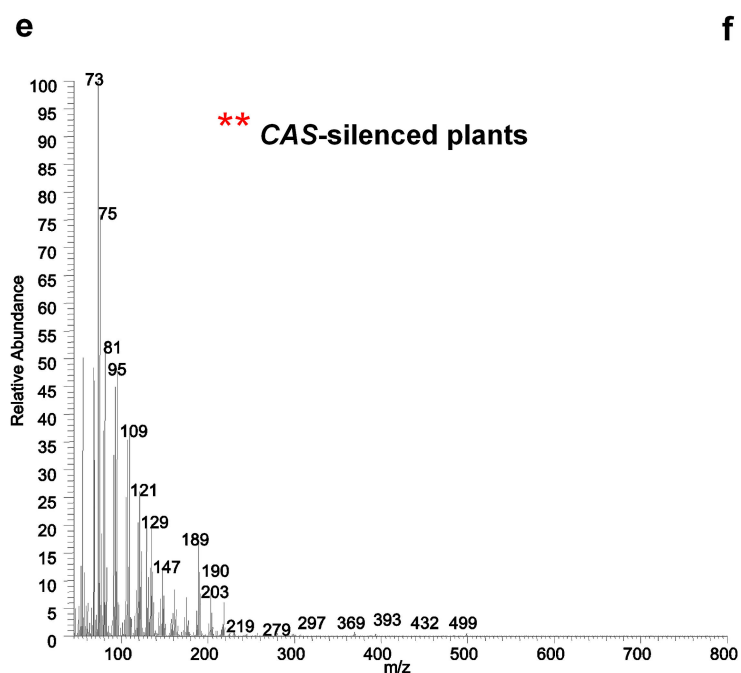
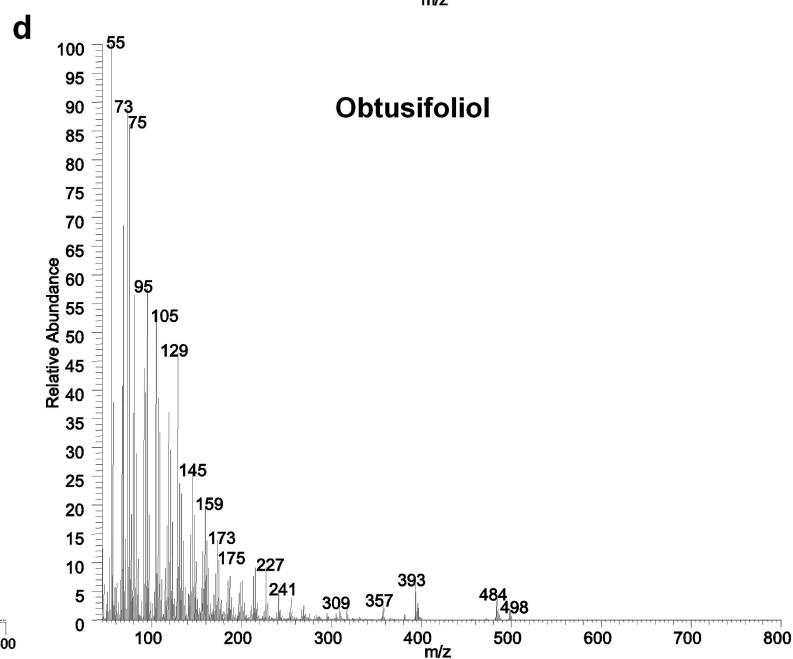
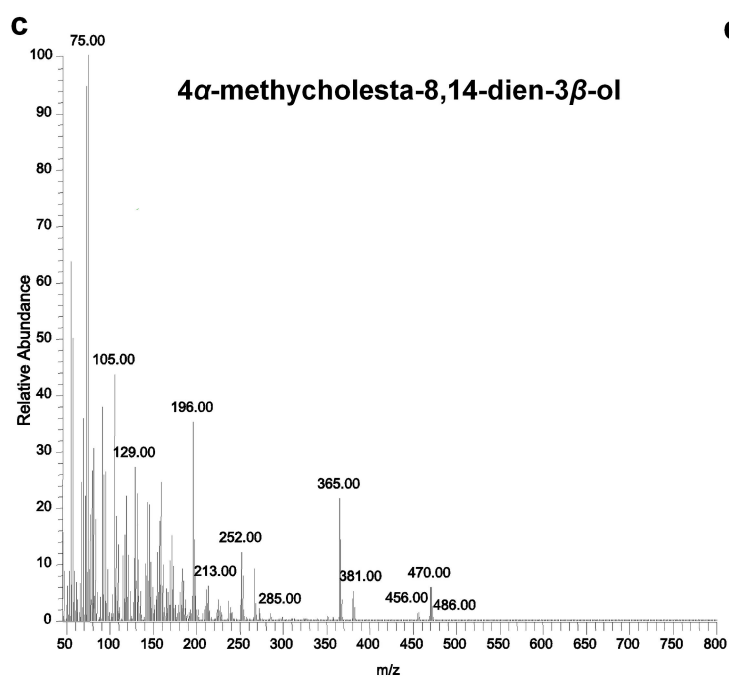
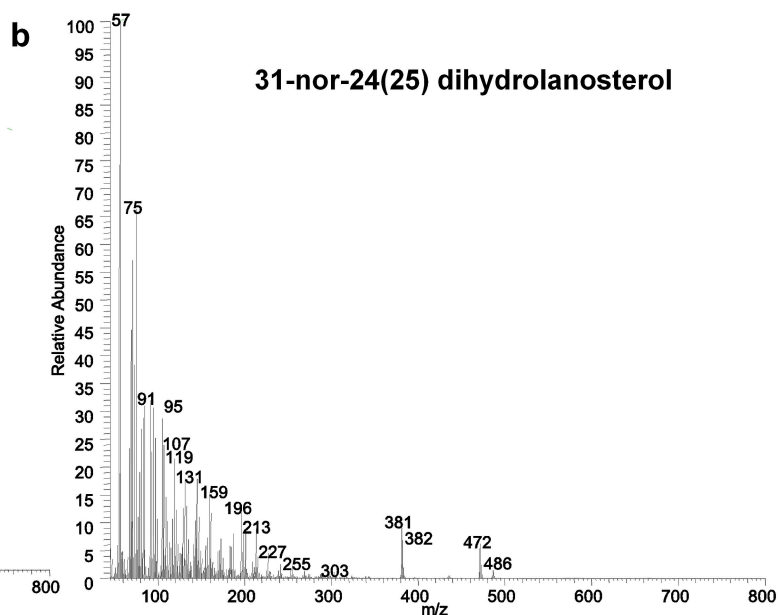
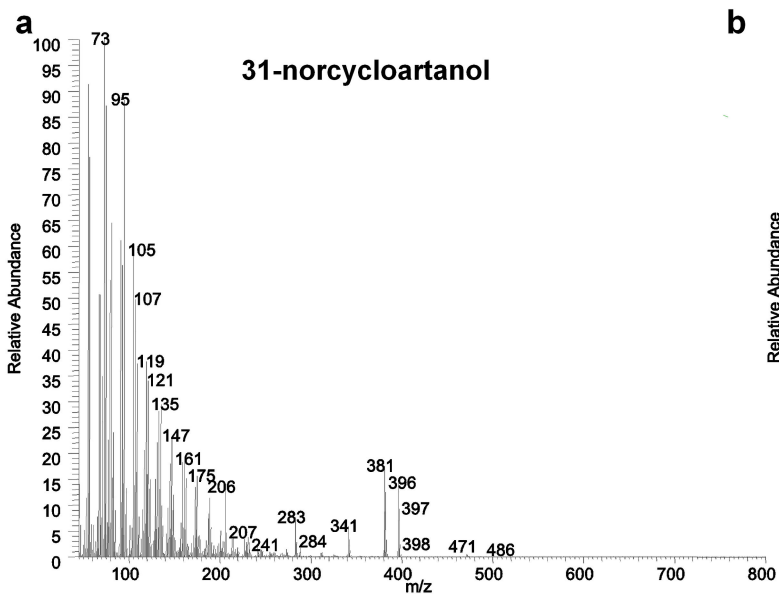
b



c



Supplementary Figure 10: Engineering the tomato cholesterologenesis pathway in Arabidopsis. **a.** A multi-gene plant transformation vector was constructed by combining multiple transcription units; e.g. for the *SSR2* gene [promoter (p) CaMV35S-*SSR2*-CaMV35S terminator (t)]. *KanR*, kanamycin resistance; p19, p19 suppressor of gene silencing; 35S, CaMV35S promoter/terminator. See Materials and Methods for details on vector construction. **b,** Heterologous expression of the 11 tomato cholesterol pathway genes in two independent Arabidopsis transformants lines (CHO-#1 and CHO-#2) analyzed by qRT-PCR and compared to wild-type plants (WT). Values represent mean $\pm$ standard error (n=3). Asterisks indicate significant changes from WT plants as calculated by the Student's *t*-test (*P-value < 0.05; **P-value < 0.01; ***P-value < 0.001). **c,** Absolute concentrations ($\mu\text{g/g}$ leaf tissue) of campesterol and β -sitosterol determined with GC-MS in the CHO- #1 and CHO- #2 pCHOLESTEROL expressing Arabidopsis lines (compared to WT). Values represent mean $\pm$ standard error (n=7 for WT and n=3 for individual transgenic lines). Asterisks indicate significant changes from wild type plants as calculated by the Student's *t*-test (*P-value < 0.05; **P-value < 0.01; ***P-value < 0.001).



Supplementary Figure 11: MS spectra of sterols putatively identified in the present study. **a, b, c** The MS spectra of the trimethylsilylated (TMS) intermediates of the cholesterol biosynthetic pathway in plants obtained by GC-MS (31-norcycloartanol, 31-nor-24(25) dihydrolanosterol and 4 α -methycholesta-8,14-dien-3 β -ol respectively). **d**, TMS spectra of obtusifoliol, a known sterol precursor in the plant phytosterol pathway that was tentatively identified by comparison of its MS spectra with published spectra and the relative retention time^{45,46,47,48}. **e, f** GC-MS spectra of unidentified compounds. In **(e)**, the compound eluted at the same retention time as cycloartenol in *CAS*-silenced plants and in **(f)** the presence of a compound at the same retention time as that of isofucosterol in *CPI*-silenced plants is seen (see also Figure 5c).

Supplementary Table 1a The homologous tomato and *N. benthamiana* genes characterized in this study.

Gene details	Tomato (SolyC Number)	<i>N. benthamiana</i> (Gene Id)	% homology (amino acid)
C14-R (Sterol C-14 reductase)	Solyc09g009040	Niben101Scf05392g01019	89 %
7-DR2 (7-dehydrocholesterol reductase 2)	Solyc06g074090	Niben101Scf03031g02018	88 %
SSR2 (Sterol side chain Reductase 2)	Solyc02g069490	Niben101Scf00271g04029	91 %
SMO4 (Sterol methyl oxidase 4)	Solyc06g005750	Niben101Scf05278g00007	86 %
SMO3 (Sterol methyl oxidase 3)	Solyc01g091320	Niben101Scf03486g02010	87 %
3 β HSD2 (3 β -hydroxysteroid dehydrogenase/C4-decarboxylase 2)	Solyc02g081730	Niben101Scf04940g00004	88 %
SDR (Uncharacterized oxidoreductase)	Solyc01g073640	Niben101Scf09360g02004	83 %
C5-SD2 (Sterol C-5(6) desaturase 2)	Solyc02g086180	Niben101Scf01991g00010	95 %
CPI (Cyclopropylsterol isomerase)	Solyc12g098640	Niben101Scf08006g01023	92 %
CYP51 (Sterol C14- demethylase)	Solyc01g008110	Niben101Scf01808g01002	93 %
SMO1 (Sterol methyl oxidase 1)	Solyc08g079570	Niben101Scf06533g00012	90 %
SMO2 (Sterol methyl oxidase 2)	Solyc06g076410	Niben101Scf05372g02001	94 %
8,7 SI (Sterol 8,7 isomerase)	Solyc06g082980	Niben101Scf06919g00004	91 %
C5-SD1 (Sterol C-5(6) desaturase 1)	Solyc02g063240	Niben101Scf07072g01010	89 %
7-DR1 (7-dehydrocholesterol reductase 1)	Solyc01g009310	Niben101Scf06967g03015***	80 %
CAS (Cycloartenol synthase)	Solyc04g070980	NbS00021029g0013	90 %
LAS (Lanosterol synthase)	Solyc05g047580	NbS00053226g0006	70 %

*** Putative identification

Supplementary Table 1b The yeast strains used in this study.

Strain	Genotype	Reference
<i>S288c</i> BY4742	MAT α <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	Euroscarf
TM1	S288c BY4742; P _{erg7} ::P _{MET3} - <i>ERG7</i>	Moses et al., 2014
TM103	TM1; pESC-URA-DEST[<i>GAL10/tHMG1</i> ; <i>GAL1/ccdB</i>]	This study
JP001	TM1; pESC-URA-DEST[<i>GAL10/tHMG1</i> ; <i>GAL1/CAS</i> (<i>Solyc04g070980</i>)]	This study
JP002	TM1; pESC-URA-DEST[<i>GAL10/tHMG1</i> ; <i>GAL1/LAS</i> (<i>Solyc05g047580</i>)]	This study
GIL77	<i>gal2, hem3-6, erg7, ura3-167</i>	Kushiro et al., 1998
JP003	GIL77; pAG426[<i>GPD/ccdB</i>]	This study
JP004	GIL77; pAG426[<i>GPD/CAS</i> (<i>Solyc04g070980</i>)]	This study
JP005	GIL77; pAG426[<i>GPD/LAS</i> (<i>Solyc05g047580</i>)]	This study
PA059	S288c BY4742; <i>trp1</i>	This study
PA103	PA059; p414[<i>TEF1/Cas9</i>]	This study
PA110	PA103; <i>erg2</i>	This study
JP006	PA110; pAG423[<i>GPD/ccdB</i>]	This study
JP007	PA110; pAG423[<i>GPD/8, 7 SI</i> (<i>Solyc06g082980</i>)]	This study
PA003	S288c BY4742; <i>erg5</i>	This study
PA004	S288c BY4742; <i>erg6</i>	This study
PA016	S288c BY4742; <i>erg5, erg6</i>	This study
JP008	PA016; pAG423[<i>GPD/ccdB</i>]; pAG425[<i>GPD/ccdB</i>]	This study
JP009	PA016; pAG423[<i>GPD/7-DR2</i> (<i>Solyc06g074090</i>)]; pAG425[<i>GPD/ccdB</i>]	This study
JP010	PA016; pAG423[<i>GPD/ccdB</i>]; pAG425[<i>GPD/SSR2</i> (<i>Solyc02g069490</i>)]	This study
JP011	PA016; pAG423[<i>GPD/7-DR2</i> (<i>Solyc06g074090</i>)]; pAG425[<i>GPD/ SSR2</i> (<i>Solyc02g069490</i>)]	This study
JP012	PA016; pAG423[<i>GPD/7-DR1</i> (<i>Solyc01g009310</i>)]; pAG425[<i>GPD/ccdB</i>]	This study
JP013	PA016; pAG423[<i>GPD/7-DR1</i> (<i>Solyc01g009310</i>)]; pAG425[<i>GPD/ SSR2</i> (<i>Solyc02g069490</i>)]	This study
Y12667	BY4742; MAT α <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i> ; YMR056w::kanMX4	Euroscarf
JP014	Y12667; pAG423[<i>GPD/ccdB</i>]	Cárdenas et al., 2016
JP015	Y12667; pAG423[<i>GPD/C5-SD2</i> (<i>Solyc02g086180</i>)]	Cárdenas et al., 2016
JP016	Y12667; pAG423[<i>GPD/C5-SD1</i> (<i>Solyc02g063240</i>)]	This study

Supplementary Table 2. Oligonucleotides used in generation of plasmids for CRISPR/Cas9 in yeast

Oligo	Sequence	Description
combi1715	TAATACGACTCACTATAGGG	T7 sequencing primer
combi2287	GGAATAAGGGCGACACGG	bla internal Rv
combi3244	<u>GTTAAC</u> CGGCCGCAAAATTAAAGCC	HpaI-CYC1t Rv
combi3245	ggggacaagttgtacaaaaagcaggcttaAAGGGAACAAAAGCTGGAGC	attB1-SNR52p Fw
combi3246	ggggaccactttgtacaagaagctgggtaAAAGCCTTCGAGCGTCCC	attB2-CYC1t Rv
combi3247	<u>GTTAAC</u> <u>GCTAGC</u> GAGGGAACAAAAGCTGGAGC	HpaI-NheI-TEFp Fw
combi3480	CGCTATTGAAGAGAGCTCATG	ERG5 RFLP Fw
combi3481	AAAATGGACCGATGATGGGC	ERG5 RFLP Rv
combi3482	AGTAGGCAGCATAAGATGAGTG	ERG6 RFLP Fw
combi3483	GCCTTGGAATTTGGTAATCG	ERG6 RFLP Rv
crispr001	GGATACTGAAGGCAGTAGTGATCATTTATCTTTCACTGCGGAGAAG	ERG5 gRNA left Rv
crispr002	TTTGGGCAGAGTTGTTCTTCGATCATTTATCTTTCACTGCGGAGAAG	ERG6 gRNA left Rv
crispr014	AGAGTTCCTCGGTTTGCCGATCATTTATCTTTCACTGCGGAGAAG	TRP1 gRNA left Rv
crispr018	ACTACTGCCTTCAGTATCCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG	ERG5 gRNA right fw
crispr019	GAAGAACAACCTGCCCCAAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG	ERG6 gRNA right fw
crispr031	GGCAAACCGAGGAACCTCTGTTTATAGAGCTAGAAATAGCAAGTTAAAATAAGG	TRP1 gRNA right fw
crispr037	TGTAGGCGTCACTACTGCCTTCAGTTAACTGTAACACTTAAGTCTATGTCATATTTGA	ERG5 HR donor Fw
crispr038	TCAAATATGACATAGACTTAAGTGTTACAGTTAACTGAAGGCAGTAGTGACGCCTACA	ERG5 HR donor Rv
crispr039	GATGTGCAAGAACAACCTCTGCCAAAAGTAACCGTTTCAGAAGTACTTGAGAAATTGGG	ERG6 HR donor Fw
crispr040	CCCAATTTCTCAAGTACTTCTGAACGGTTACTTTTGGGCAGAGTTGTTCTTCGACATC	ERG6 HR donor Rv
crispr059	AACTGCATGGAGATGAGTCGTGGCATTAAACAGAGTTCCTCGGTTTGCCAGTTATT	TRP1 HR donor Fw
crispr060	AATAACTGGCAAACCGAGGAACCTCTGTTATTAATGCCACGACTCATCTCCATGCAGTT	TRP1 HR donor Rv
crispr119	AGGTAGTTCTGGTCCATTGG	TRP1 RFLP fw
crispr120	ACACCATTGTCTCCACACC	TRP1 RFLP rev
crispr181	CTCATTACGGGGACGAATACGATCATTTATCTTTCACTGCGGAGAAG	ERG2 gRNA left Rv
crispr182	GTATTCGTCCCCGTAATGAGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG	ERG2 gRNA right Fw
crispr195	CCCACTCCTTTTGTTGATTGG	ERG2 RFLP Fw
crispr196	GTGTCCAAAACCCGAATGG	ERG2 RFLP Rv

Restriction sites are underlined; Gateway attB sites are in lower case.