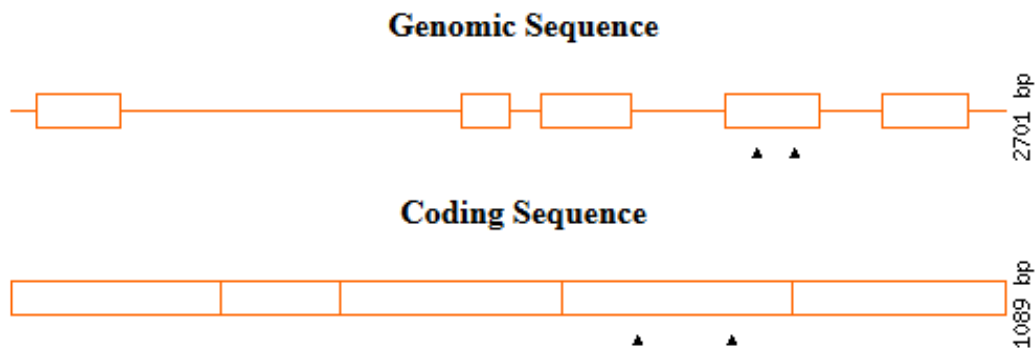


Supplemental Figure 1. Missense mutations in *SICUS1* (gene model: SLSBM_L54139_01; http://www.kazusa.or.jp/tomato_sbm/cgi-bin/genemodel.cgi) detected by TILLING (Targeting Induced Local Lesions IN Genomes) in 6912 M2 families of an EMS -mutagenized Micro-Tom tomato population (Just *et al.*, 2013).

A, Position of the mutations in *SICUS1* and predicted effect. SIFT (<http://sift.jcvi.org/>) predicts whether an amino acid substitution affects protein function.

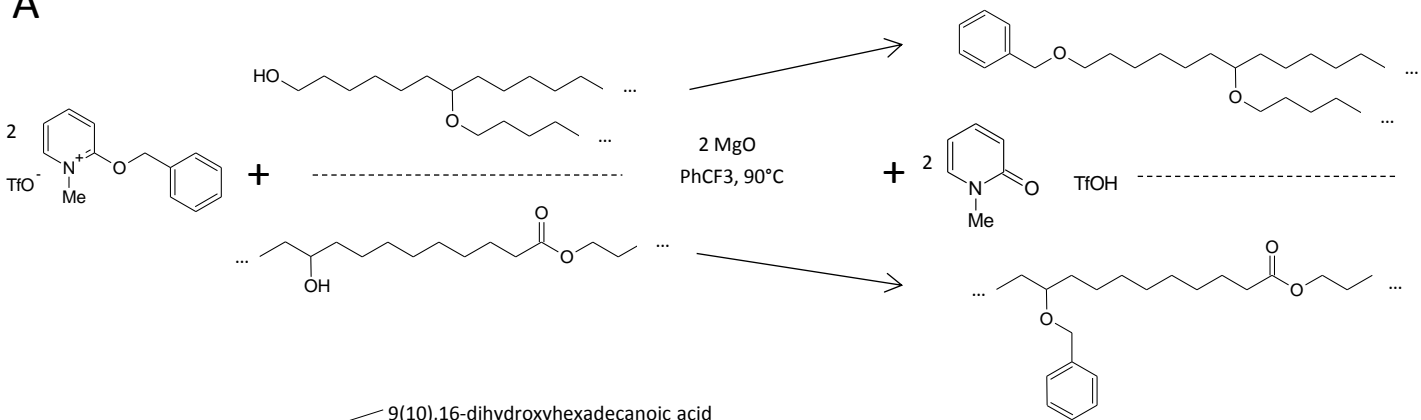
M2 family	Mutation nt (ATG)	Mutation aa (ATG)	Mutation	SIFT prediction
<i>cus1-b</i>	G1955T	R229L	Missense	TOLERATED with a score of 0.53
<i>cus1-c</i>	G2059A	D264N	Missense	TOLERATED with a score of 0.57

B, Graphic representation of mutations identified in *SICUS1* obtained with PARS ESNP program (Taylor and Greene, 2003). Black triangles represent the two missense mutations .

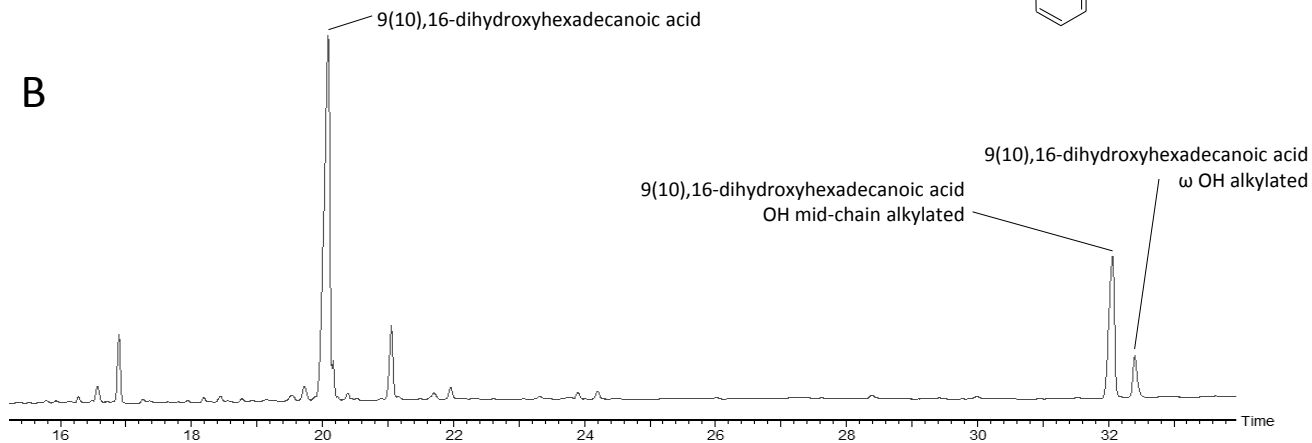


Supplemental Figure 1. Missense mutations in *SICUS1* (gene model: SLSBM_L54139_01; http://www.kazusa.or.jp/tomato_sbm/cgi-bin/genemodel.cgi) detected by TILLING (Targeting Induced Local Lesions IN Genomes) in 6912 M2 families of an EMS-mutagenized Micro-Tom tomato population (Just *et al.*, 2013). A, Position of the mutations in *SICUS1* and predicted effect. SIFT (<http://sift.jcvi.org/>) predicts whether an amino acid substitution affects protein function. B, Graphic representation of mutations identified in *SICUS1* obtained with PARSESNP program (Taylor and Greene, 2003). Black triangles represent the two missense mutations.

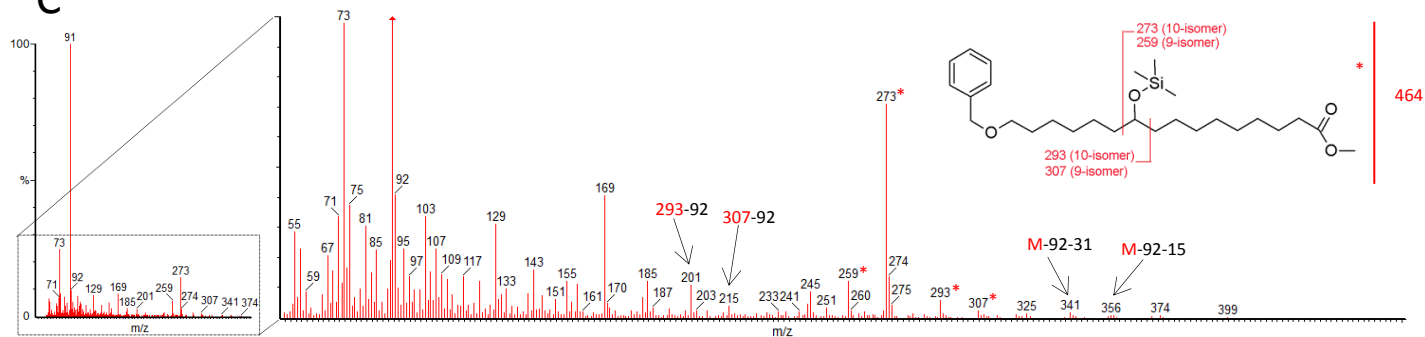
A



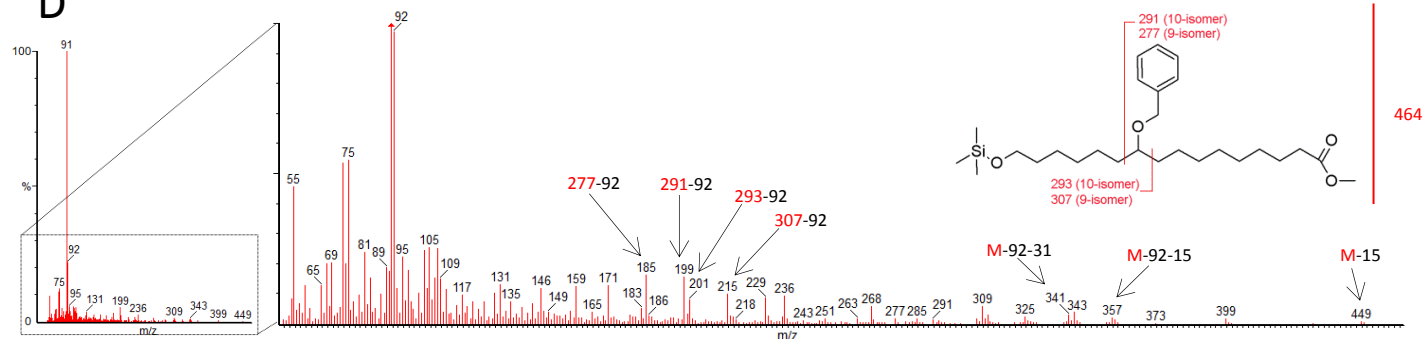
B



C

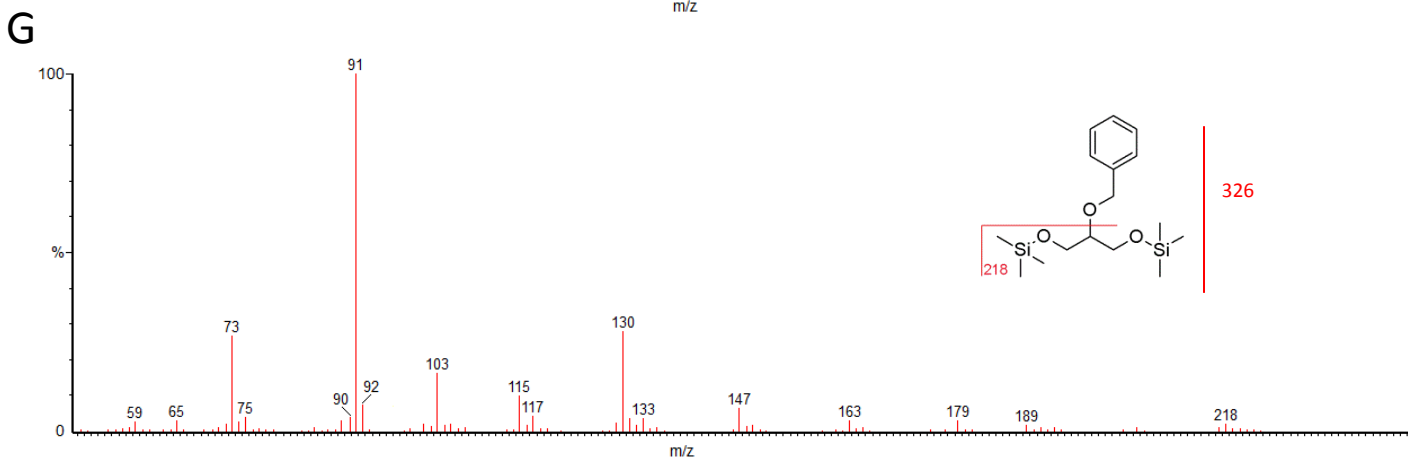
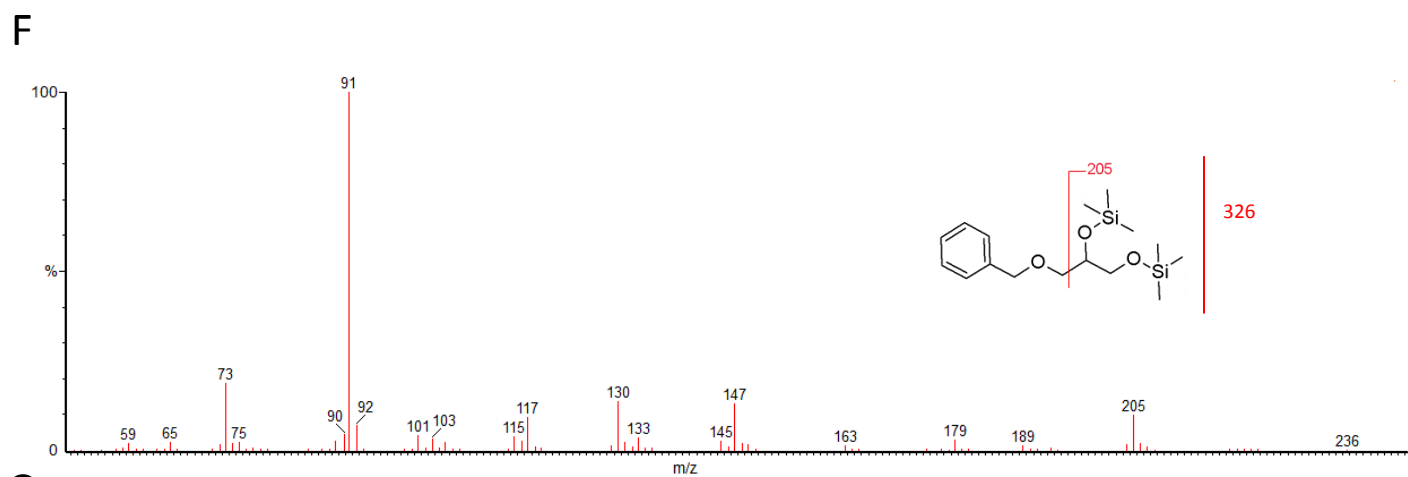
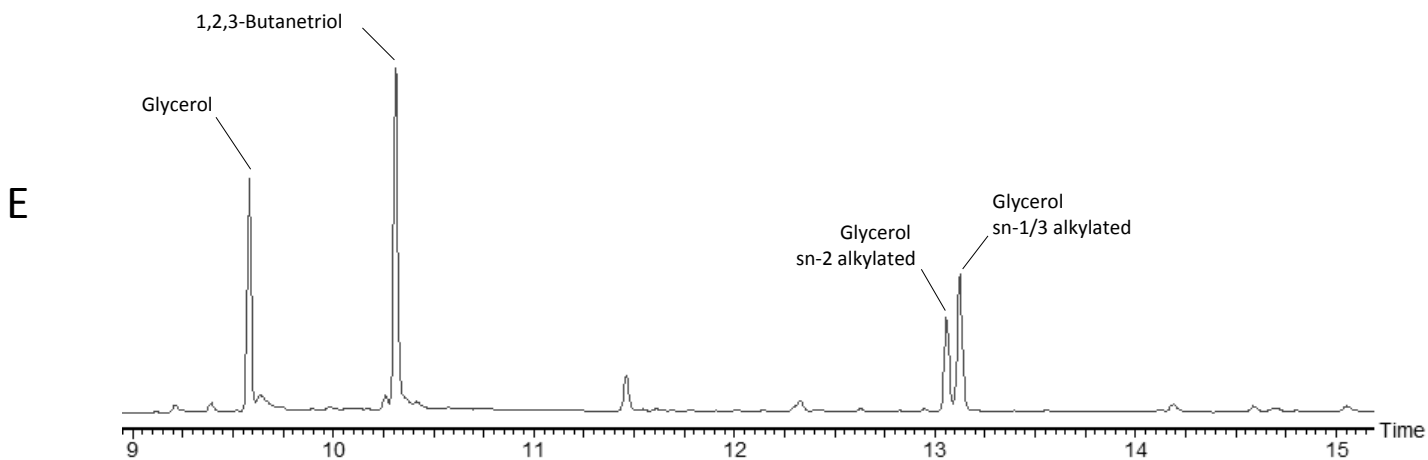


D



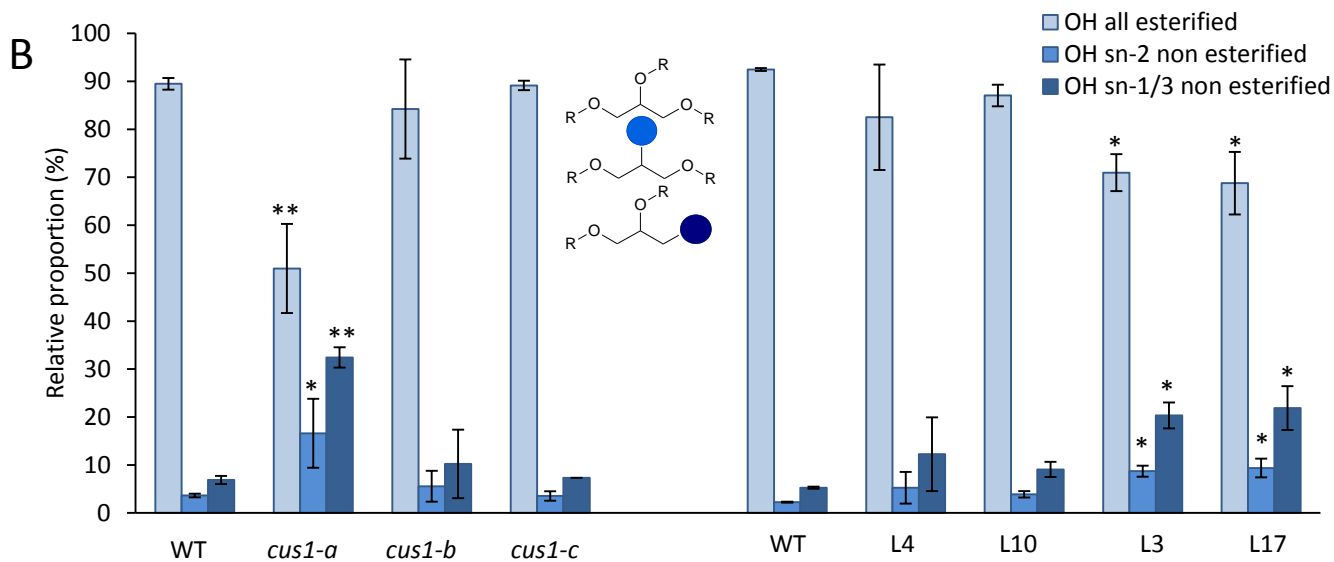
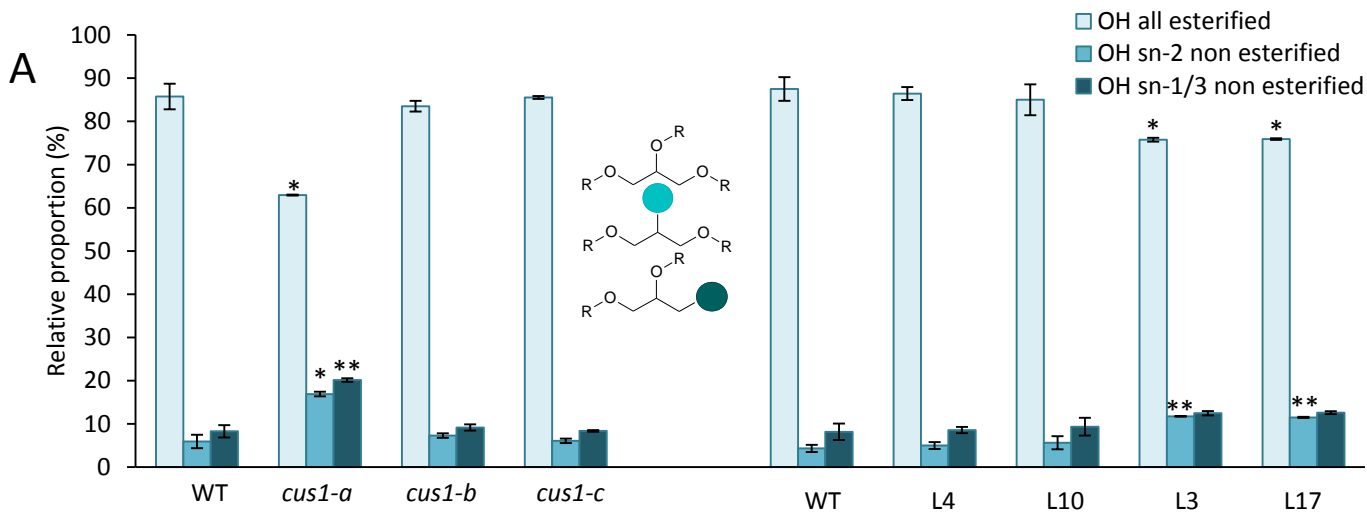
Supplemental Figure 2. Benzyl etherification of non esterified cutin monomers.

A, Reaction scheme with primary or secondary OH groups of 9(10)16-dihydroxyhexadecanoic acid . B and E, GC-Chromatogram; C,D,F and G, mass spectra of O-alkylated and silylated derivatized cutin monomers (TMS). C, ω-O-alkylated-dihydroxyhexadecanoic acid. D, mid-chain O-alkylated-dihydroxyhexadecanoic acid. F, glycerol alkylated in *sn*-1/3 position . G, glycerol alkylated in *sn*-2 position .



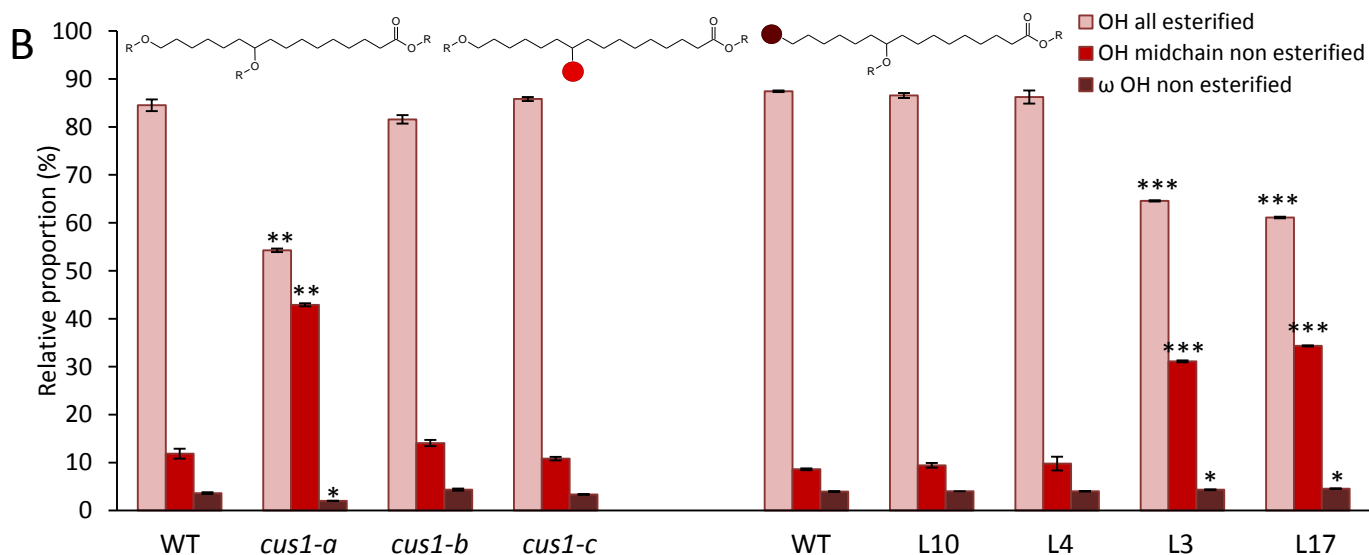
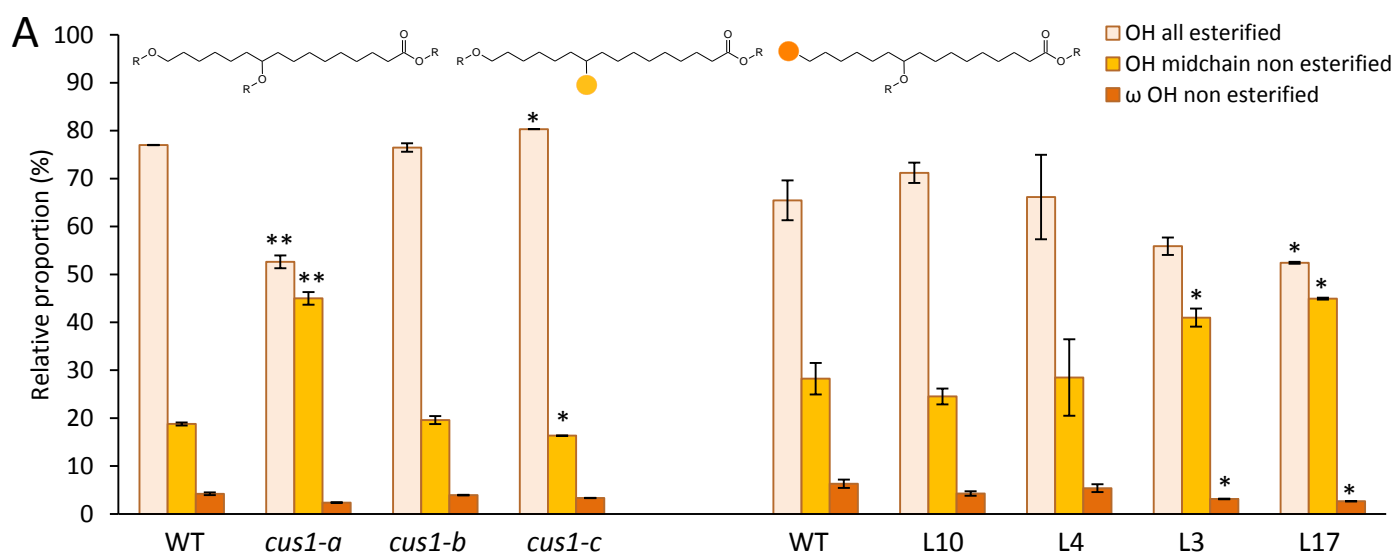
Supplemental Figure 2 (continue). Benzyl etherification of non esterified cutin monomers.

A, Reaction scheme with primary or secondary OH groups of 9(10)16-dihydroxyhexadecanoic acid . B and E, GC-Chromatogram; C,D,F and G, mass spectra of O-alkylated and silylated derivatized cutin monomers (TMS). C, ω -O-alkylated-dihydroxyhexadecanoic acid. D, mid-chain O-alkylated-dihydroxyhexadecanoic acid. F, glycerol alkylated in *sn*-1/3 position . G, glycerol alkylated in *sn*-2 position .



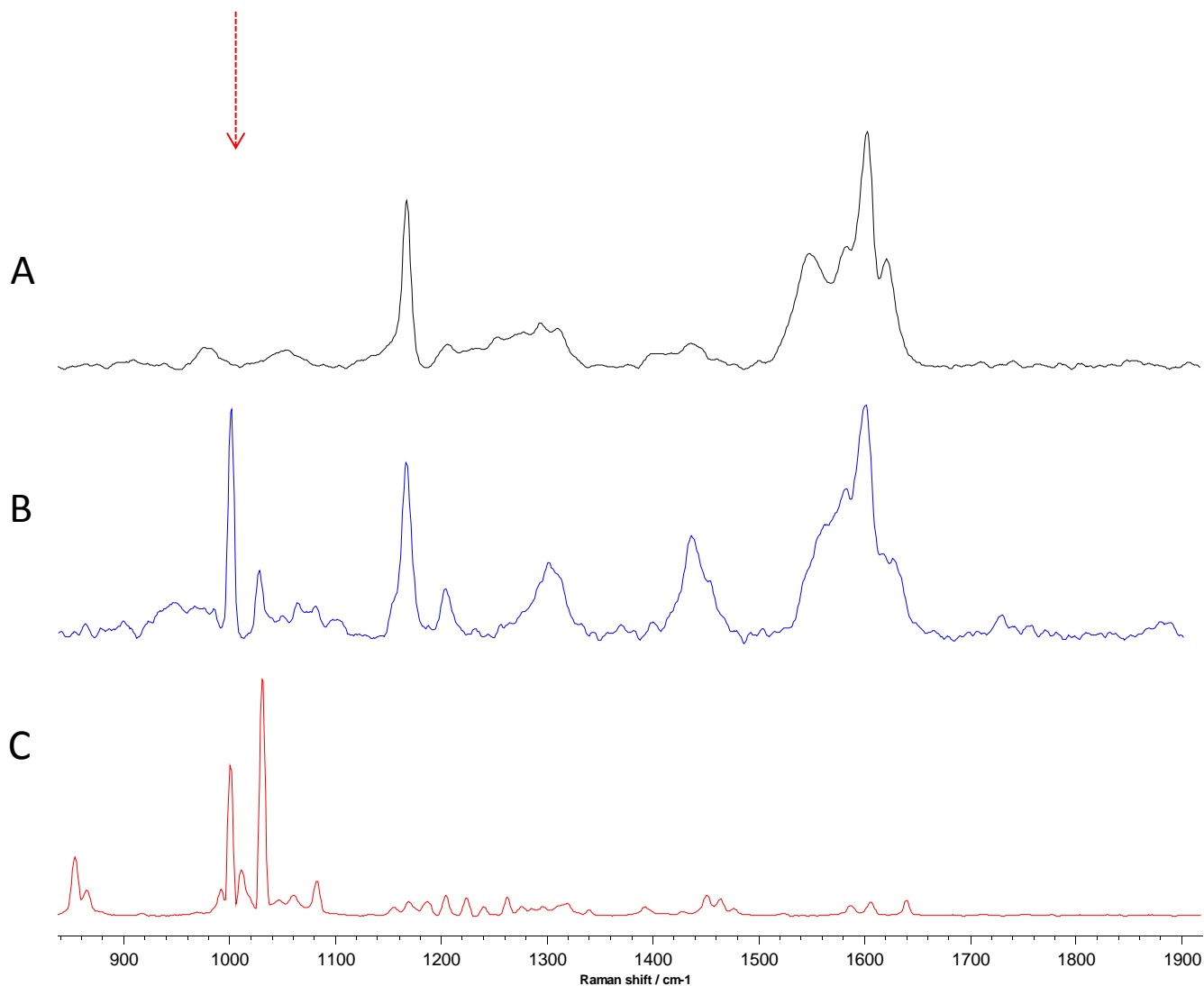
Supplemental Figure 3. Esterification levels of glycerol OH groups of tomato fruit cutin isolated from *cus1* mutants and *Pro35S:SiCUS1^{RNAi}* lines.

A, 20 Days Post Anthesis (DPA) fruits. B, red ripe (RR) fruits. Left panel, *cus1* mutants. Right panel, *Pro35S:SiCUS1^{RNAi}* lines. WT, wild type. Mean values (\pm S.D.) are calculated from 9 independent measurements (3 fruits, 3 technical replicates each). Significant differences from the corresponding WT are indicated by asterisks (t-test; *, $P < 0.05$; **, $P < 0.01$).



Supplemental Figure 4. Esterification levels of 9(10),16-dihydroxyhexadecanoic acid OH groups of tomato fruit cutin from *cus1* mutants and *Pro35S:SICUS1^{RNAi}* lines.

A, 20 Days Post Anthesis (DPA) fruits. B, red ripe (RR) fruits. Left panel, *cus1* mutants. Right panel, *Pro35S:SICUS1^{RNAi}* lines. WT, wild type. Mean values (\pm S.D.) are calculated from 9 independent measurements (3 fruits, 3 technical replicates each). Significant differences from the corresponding WT are indicated by asterisks (t-test; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$)



Supplemental Figure 5. Typical Raman spectra of non-alkylated (A) and alkylated (B) cutin and benzyloxymethylpyridinium triflate (BPT) reagent, (C) over the range 820 – 1900 cm^{-1} . The spectrum of labelled cutin shows characteristic peak at 1002 cm^{-1} (red arrow) that stand for BPT aromatic groups.