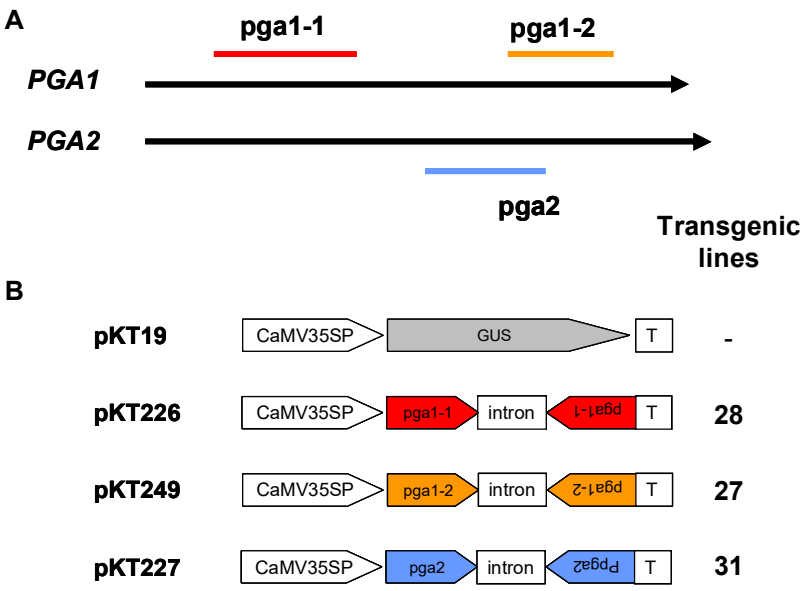
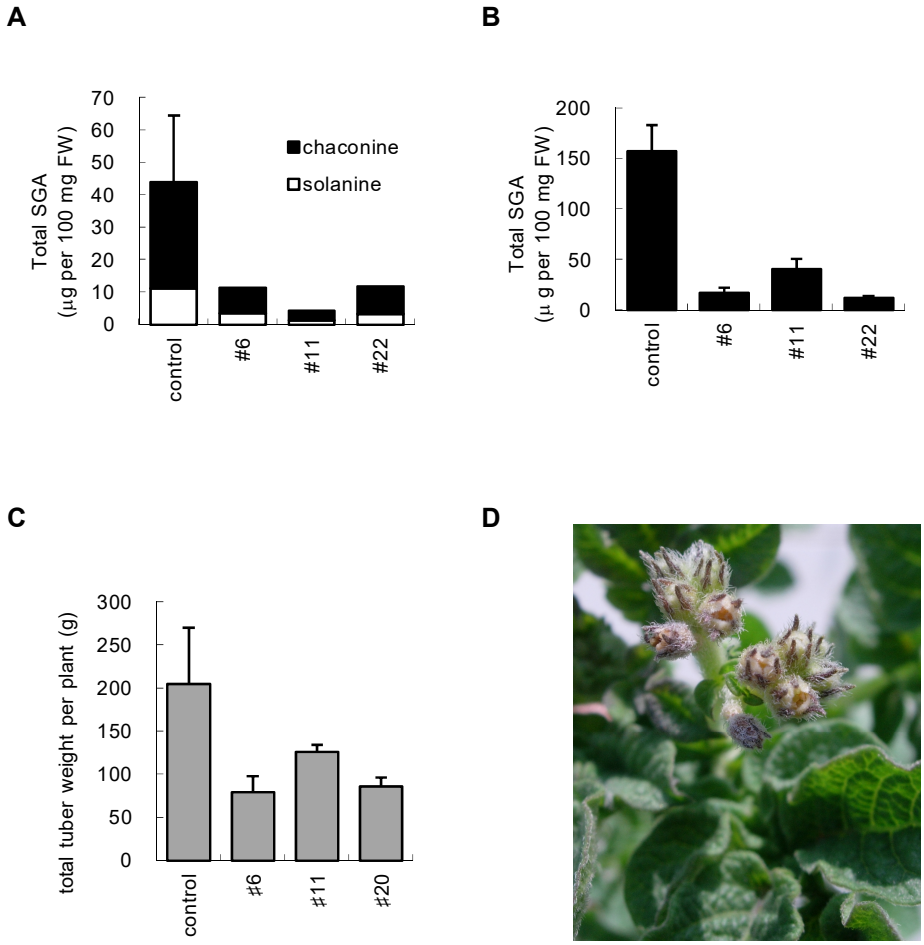
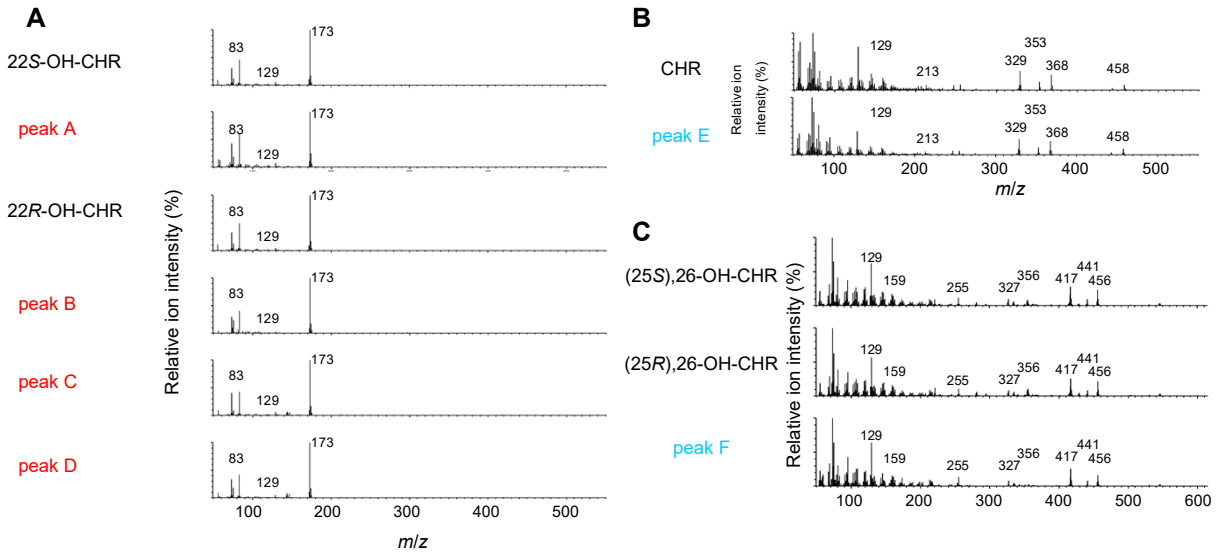


Supplementary Figure S1. *PGA1*- and *PGA2*-knockdown vectors. A, Regions used in constructing the knockdown vectors (pKT226: red line, pKT249: orange line and pKT227: blue line). B, Structure of control (pKT19) and knockdown vectors and the number of transgenic lines obtained. CaMV35SP, Cauliflower mosaic virus 35S promoter; GUS, β-glucuronidase gene; intron, intron of the *Arabidopsis* phytoene desaturase gene (PDS, At4g14210); T, terminator.

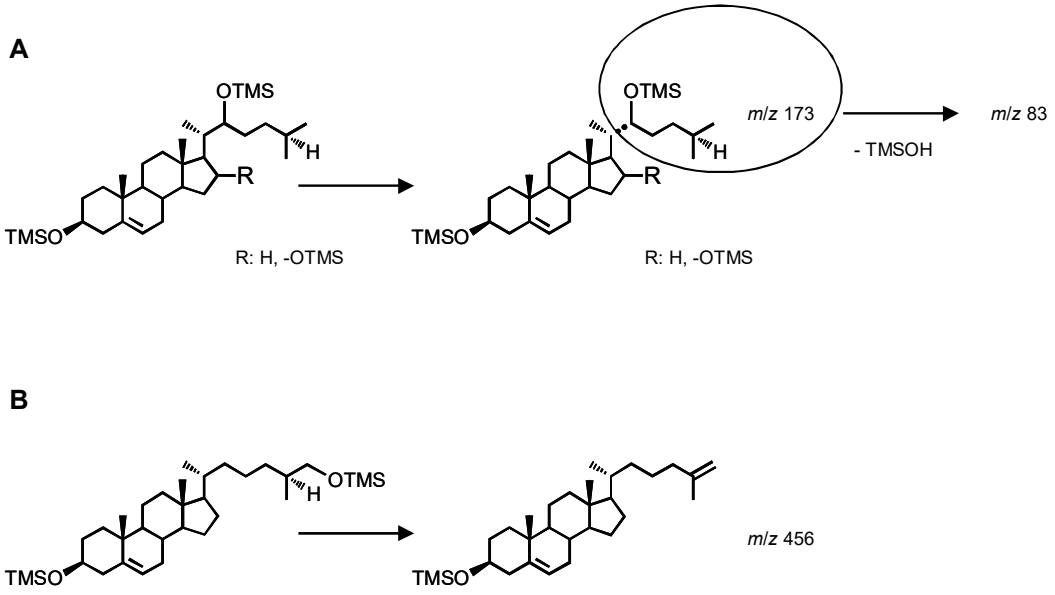
Supplementary Figure S2. Tubers of *PGA1*-knockdown plants with light exposure. A and B, Partly peeled tubers of control (A) and the *PGA1*-knockdown plant pKT226-67 (B) after dark exposure (left) and after light exposure (right). Tubers of both the control and *PGA1*-knockdown plants accumulated chlorophyll and anthocyanin after light exposure and their peels darkened.



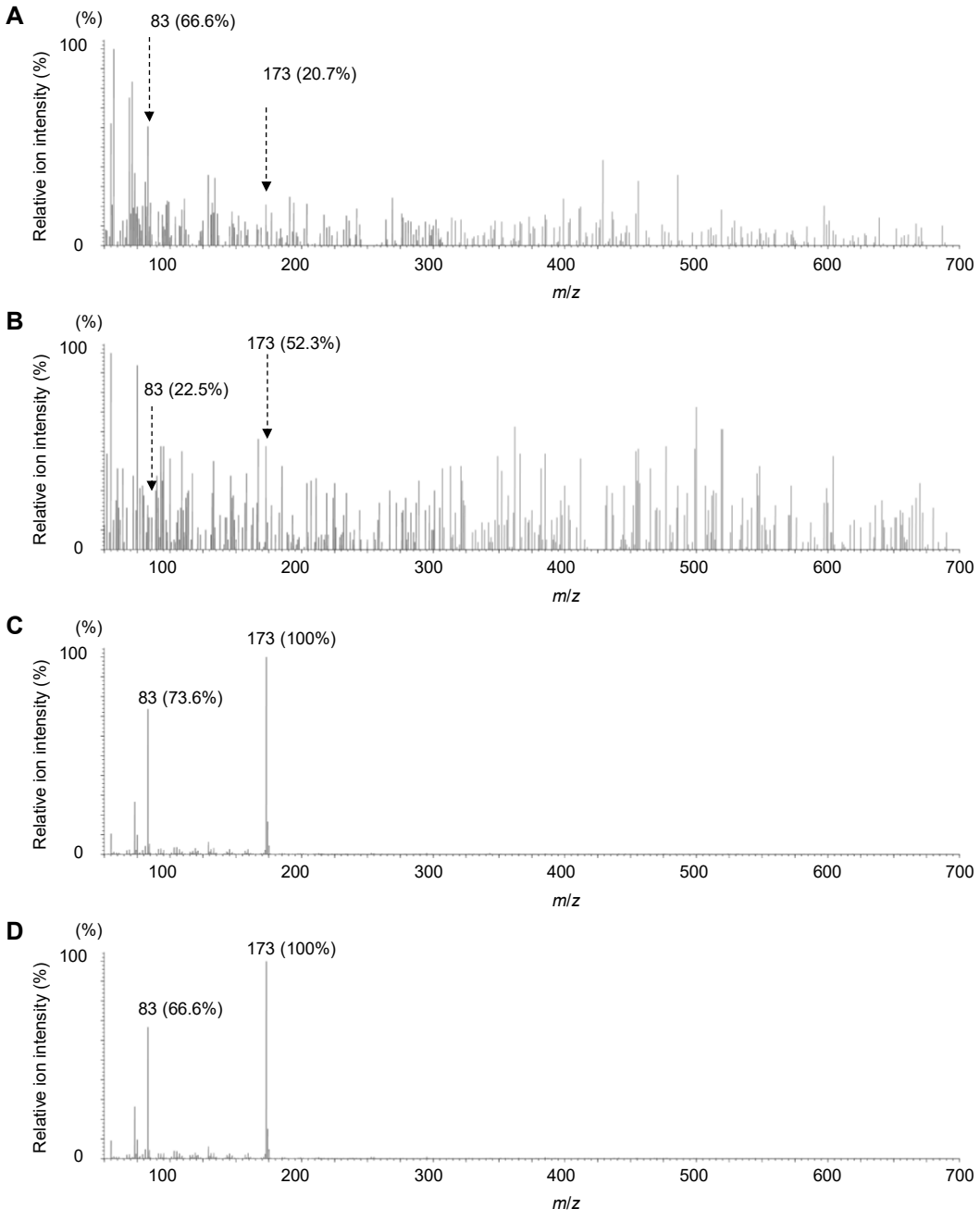
Supplementary Figure S3. SGA content, yield and phenotype of the *PGA1*-knockdown plants (pKT249). A, LC-MS analysis of SGA (α-solanine and α-chaconine) levels in the stems of in vitro grown *PGA1*-knockdown plants (pKT249) (mean and s.d., n = 4). B, LC-MS analysis of the SGA levels (total SGA = α-solanine + α-chaconine) of the *PGA1*-knockdown plants (pKT249) in the peel of harvested tubers without light exposure (mean and s.d., n = 9). C, Yields of the tubers from *PGA1*-knockdown plants (pKT249) (mean and s.d., n = 3). D, Flowers of the *PGA1*-knockdown plant (pKT249-6).



Supplementary Figure S4. Mass spectra of accumulated compounds in *PGA1*- and *PGA2*-knockdown plants. A, The mass spectra of peaks A-F in Figure 2A and authentic standards, 22*S*-hydroxycholesterol (22*S*-OH-CHR) and 22*R*-hydroxycholesterol (22*R*-OH-CHR). B, The mass spectra of peak E in Figure. 2A and authentic standards, cholesterol (CHR). C, The mass spectra of peak F in Figure 2B and authentic standards, (25*S*),26-hydroxycholesterol ((25*S*),26-OH-CHR) and (25*R*),26-hydroxycholesterol ((25*R*),26-OH-CHR) .The mass spectra of peaks C and D also match those of authentic 22*S*-OH-CHR and 22*R*-OH-CHR. Peaks C and D were expected to be 22-OH-CHR derivatives as shown in Supplementary Figure S5. Peak E is coincident with that of cholesterol. Peak F is coincident with that of 26-OH-CHR. The enantiomer form of Peak F was not determined.

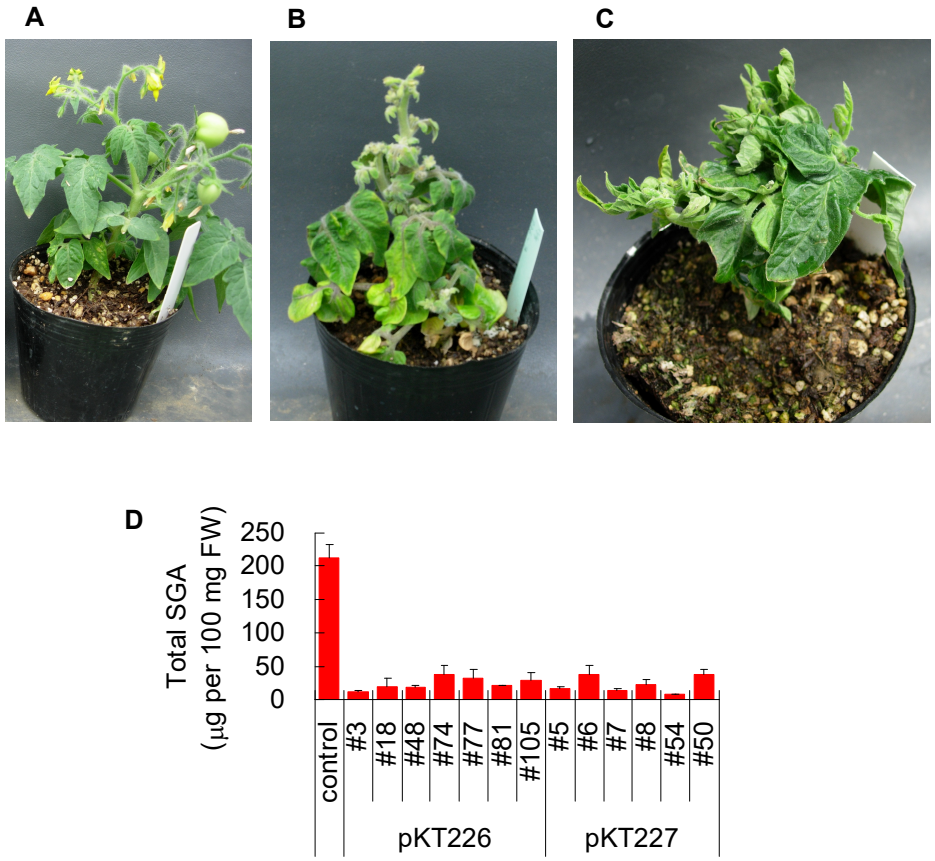


Supplementary Figure S5. Proposed assignment of fragment ions of hydroxycholesterol derivatives. A, The mass spectrum of trimethylsilylated 22-OH-CHR and 22-OH-CHR derivatives such as 16,22*S*-diOH-CHR and 16,22*R*-diOH-CHR show characteristic fragment ions at *m*/*z* 173 and 83, although their authentic standards were not available. B, The mass spectrum of trimethylsilylated 26-OH-CHRs showed a characteristic fragment ion at *m*/*z* 456.



Supplementary Figure S6. Mass spectra of PGA2 metabolites and authentic standards of sterols. A, PGA2 metabolites (Rt = 20.9 min). B, PGA2 metabolites (Rt = 21.2 min). C, Authentic standard, 22*R*-hydroxycholesterol (Rt = 21.2 min). D, Authentic standard, 22*S*-hydroxycholesterol (Rt = 20.9 min). Characteristic ions (*m*/*z* = 83 and 173 in Supplementary Figure S5) and their relative ion intensity are indicated.

Supplementary Figure S7. In vitro growth of sprout tips on tissue culture media. A to C, Sprout tips cut from tubers of the *PGA1*-knockdown plants pKT226-67 were placed on tissue culture media without plant hormones. Plantlets one week (A), two weeks (B) and six weeks (C) after transfer to media. The *in vitro* plants grew normally.



Supplementary Figure S8. Phenotype and SGA content of the *PGA* ortholog-knockdown tomato plants. A, Control. B, The PGA1 homolog-knockdown tomato plant (pKT226-3). C, The PGA2 homolog-knockdown tomato plant (pKT227-6). D, LC-MS analysis of SGA (α-tomatine) levels (mean and s.d., *n* = 3) in the leaves of the PGA1 homolog (pKT226)- and PGA2 homolog (pKT227)-knockdown tomato plants grown in the greenhouse. FW, fresh weight.