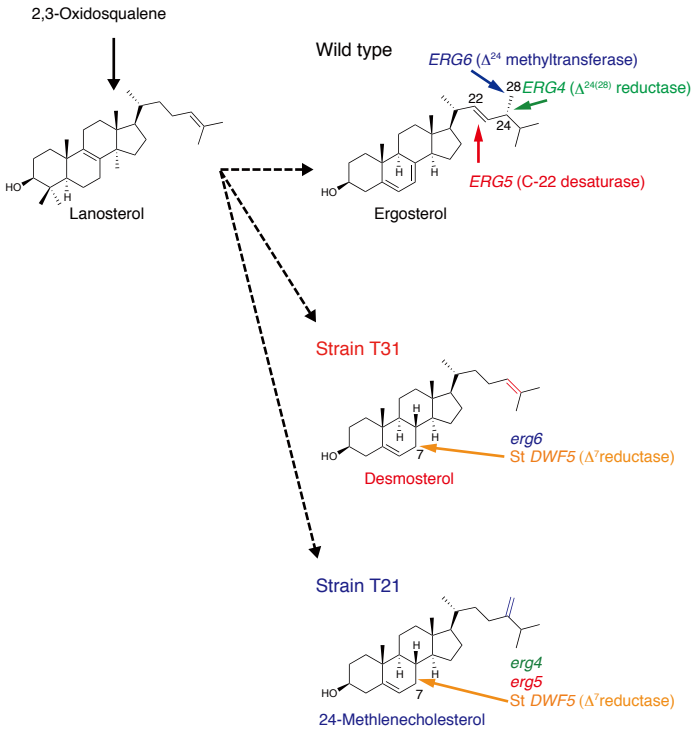
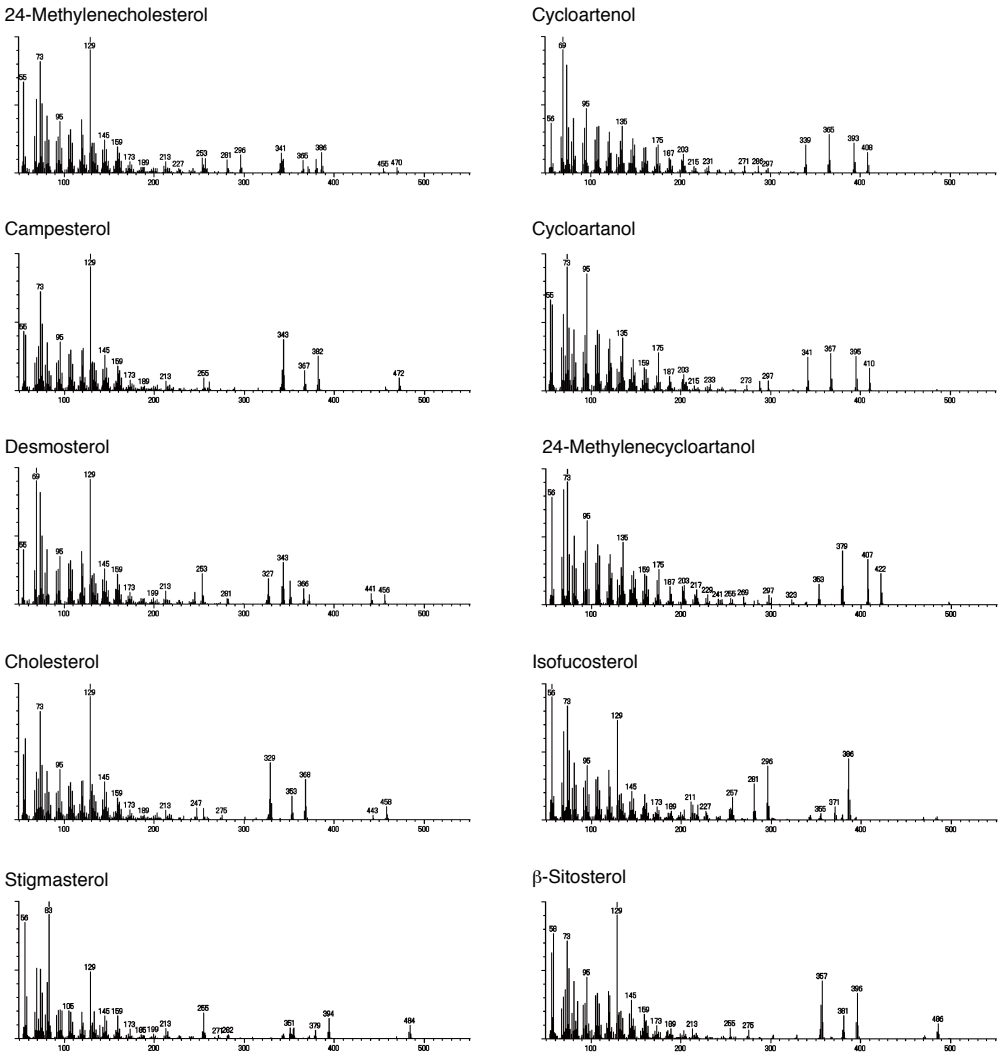
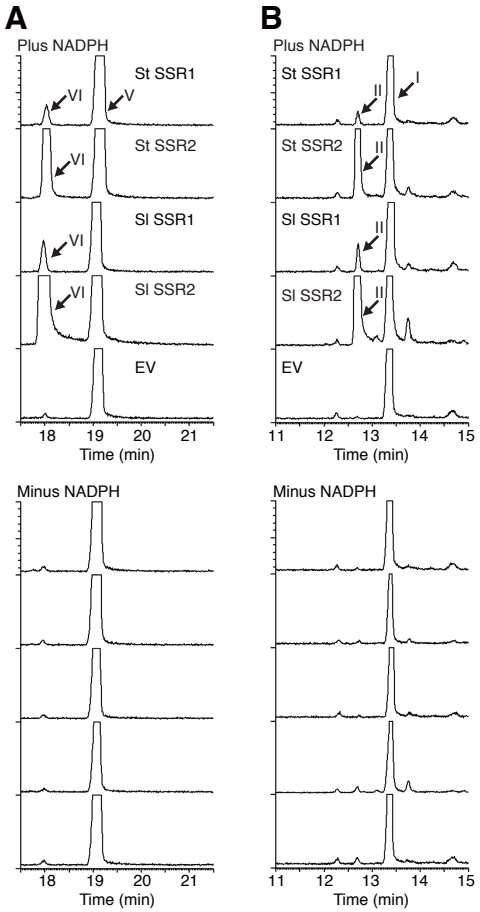


Supplemental Figure 1. Modified Ergosterol Biosynthesis in Yeast Strains T21 and T31.

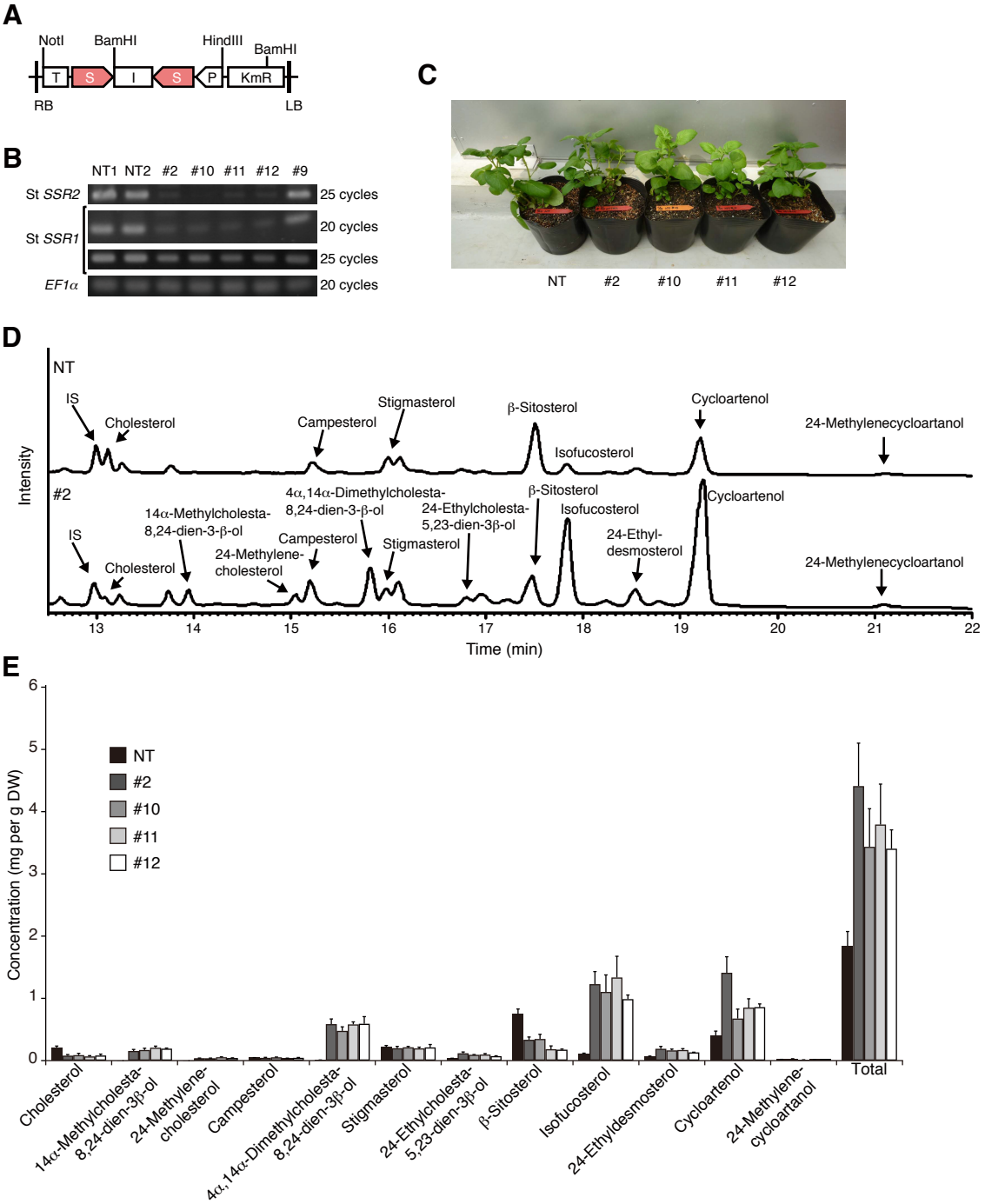
Ergosterol, a yeast sterol, is biosynthesized from lanosterol. In the pathway, ERG4, ERG5 and ERG6 mediate $\Delta^{24(28)}$ reduction, C-22 desaturation and Δ^{24} transmethylation, respectively. Strain T21 is *S. cerevisiae* BY4742 *erg4 erg5* expressing *St DWF5* and accumulates 24-methylenecholesterol. Strain T31 is *S. cerevisiae* BY4742 *erg6* expressing St *DWF5* and accumulates desmosterol.



Supplemental Figure 2. MS Spectra of Authentic Sterol Standards.
The MS spectra of the trimethylsilylated authentic standards were obtained by GC-MS.

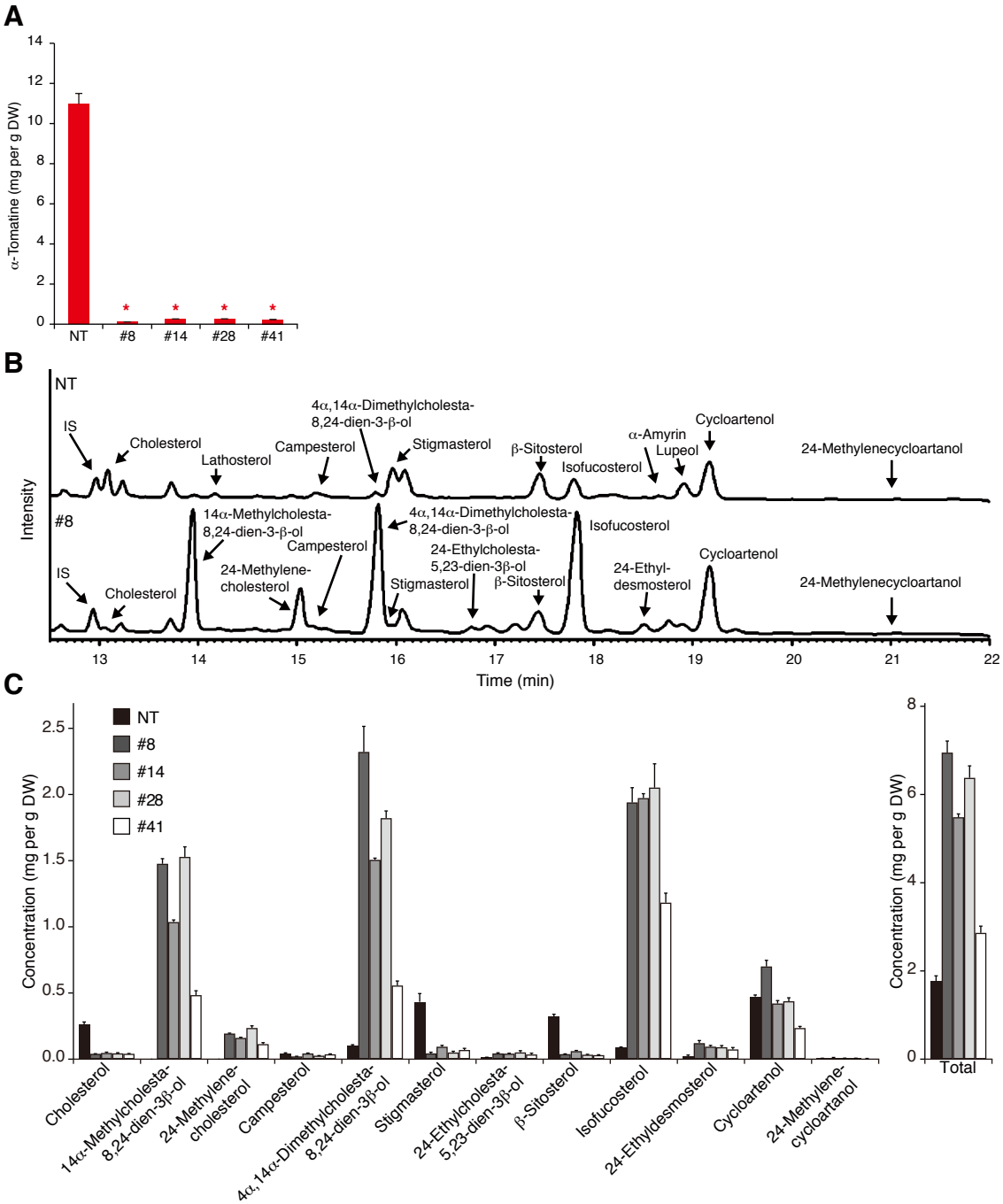


Supplemental Figure 3. In vitro Enzymatic Assays of SSRs.
(**A** and **B**) Enlargements of the chromatograms shown in Figures 4B and 4C, respectively. Peak labels refer to Figures 3A and 4A.



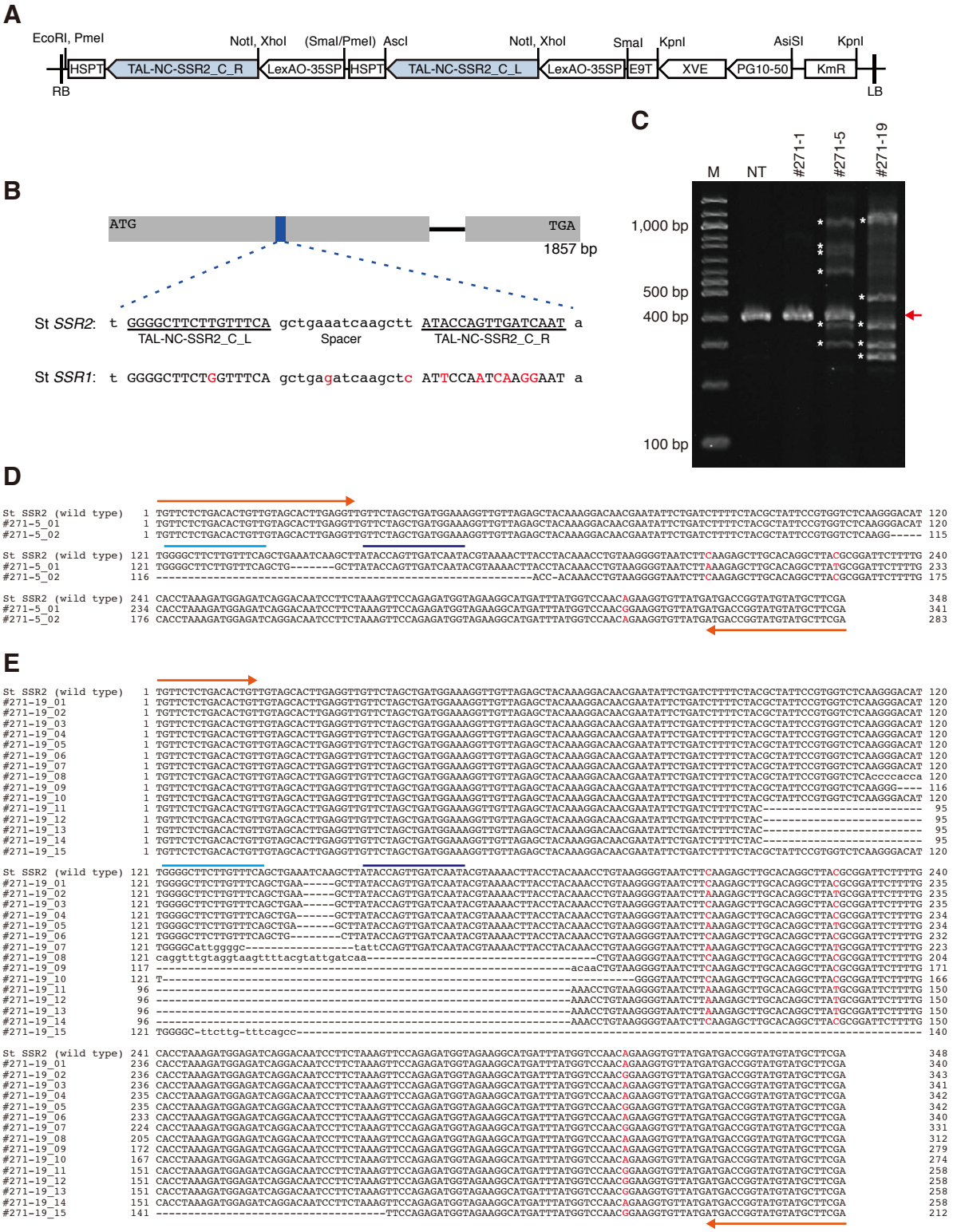
Supplemental Figure 4. St *SSR2*-Silenced Potatoes.
(**A**) T-DNA region of the St *SSR2* RNAi binary vector pKT251. RB, right border; T, *Cauliflower mosaic virus 35S* terminator; S, cDNA fragment of St *SSR2*; I, third intron of *At4g14210* from *A. thaliana*; P, *Cauliflower mosaic virus 35S* promoter;

KmR, *kanamycin resistance gene*; LB, left border. (**B**) RT-PCR analysis of St *SSR1* and St *SSR2* expression levels in non-transformants (NT) and five independent St *SSR2*-RNAi potato lines (#2, #10, #11, #12 and #9). Expression of potato *elongation factor 1*α (*EF1*α) was used as the control. *Note*: In the 4 RNAi lines (#2, #10, #11 and #12), the reduction of mRNA level was observed; whereas in the line #9, no apparent such reduction was observed suggesting failure of RNAi in the line #9. (**C**) St *SSR2*-silenced (lines #2, #10, #11 #12) and non-transformed (NT) potato plants. (**D**) Analysis of sterol fractions from stems of non-transformed and St *SSR2*-silenced potatoes by GC-MS. Total ion chromatograms of sterol fractions from stems of in vitro cultured non-transformed potato (NT, upper chromatogram) and St *SSR2*-silenced potato plant line #2 (#2, lower chromatogram). (25,26,26,26,27,27,27-$^{2}H_{7}$)Cholesterol was included as an internal standard (IS). (**E**) Sterol composition in stems of in vitro cultured non-transformed (NT) and St *SSR2*-silenced (#2, #10, #11 and #12) potato plants (mean and s.d., $n = 3$). Total sterol levels of the four St *SSR2*-silenced lines were significantly different from that of the non-transformant (Dunnett's test, $P < 0.05$). DW, dry weight.



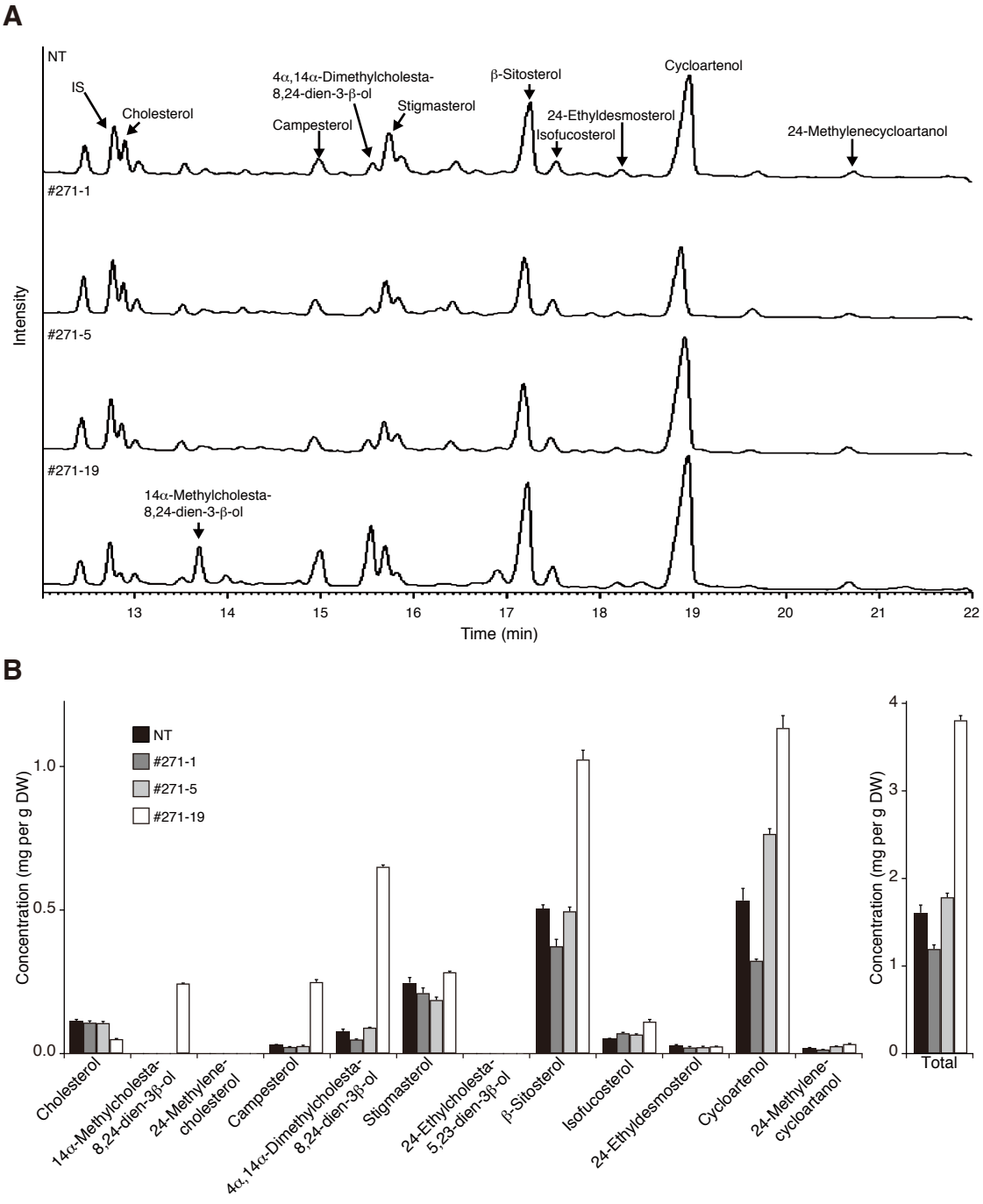
Supplemental Figure 5. SI *SSR2*-Silenced Tomatoes.
(**A**) LC-MS analysis of the predominant SGA (α-tomatine) levels in leaves from non-transformed (NT) and SI *SSR2*-silenced (#8, #14, #28 and #41) tomatoes (mean and s.d., *n* = 3). Asterisks indicate significant differences from the non-transformant (Dunnett's test, $P < 0.05$). DW, dry weight. (**B**) Analysis of sterol

fractions from stems of non-transformed and Sl *SSR2*-silenced tomatoes by GC-MS. Total ion chromatograms of sterol fractions from leaves of non-transformed tomato (NT, upper chromatogram) and Sl *SSR2*-silenced tomato line #8 (#8, lower chromatogram). (25,26,26,26,27,27,27-$^{2}H_{7}$)Cholesterol was included as an internal standard (IS). (**C**) Sterol composition of leaves from non-transformed (NT) and Sl *SSR2*-silenced (#8, #14, #28 and #41) tomatoes (mean and s.d., *n* = 3). DW, dry weight.



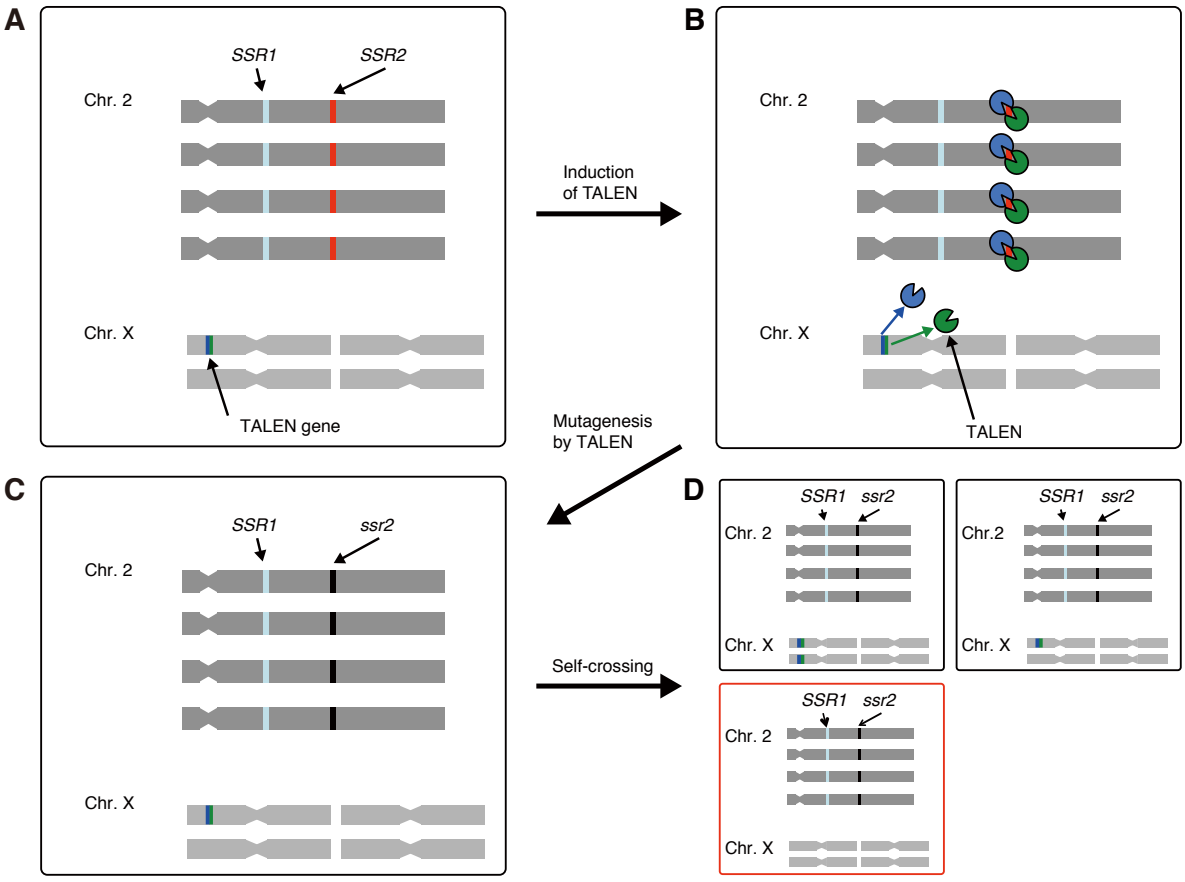
Supplemental Figure 6. TALEN Expression Vector Targeting St *SSR2*.

(**A**) Schematic representation of the T-DNA region of the TALEN expression construct. RB and LB, T-DNA right and left borders respectively; HSPT, *heat shock protein* terminator from *A. thaliana*; TAL-NC-SSR2_C_R and TAL-NC-SSR2_C_L, TALEN regions targeting St *SSR2*; LexAO-35SP, *LexA* operator fused to *Cauliflower mosaic virus 35S* minimal promoter; E9T, *rbcS E9* poly(A) addition sequence, XVE, *chimeric transcription factor*; PG10-50, *PG10-50* synthetic promoter. (**B**) TALEN target site in the St *SSR2* gene. The structure of the St *SSR2* gene is depicted. Gray boxes and a black line represent exons and an intron of the St *SSR2* gene, respectively. The location of the TALEN target site (site C) is indicated with a blue box. Sequences below the gene model are the TALEN target site in St *SSR2* and a potential off-target site in St *SSR1*. Recognition sequences by the TALEN are underlined. The spacer sequences are in lowercase letters. Mismatches between the target St *SSR2* sequence and the potential off-target site in St *SSR1* are shown in red letters. (**C**) Amplification of DNA fragments spanning the TALEN target site. TALEN target sites were amplified from genomic DNA extracted from either non-transformed (NT) or TALEN-transformed potato plants after estradiol treatment. M, Marker (Quick-load DNA Ladders (100 bp), NEB); NT, non-transformant; #271-1, #271-5 and #271-19, TALEN-transformed lines #271-1, #271-5 and #271-19, respectively. The red arrow indicates the size of PCR products from the intact alleles of St *SSR2*. Asterisks indicate extra bands probably due to heteroduplex formation. (**D** and **E**) A PCR product spanning the TALEN target site was amplified from TALEN-transformed lines #271-5 (**D**) and #271-19 (**E**) and sequenced to reveal TALEN-induced mutations. Light and dark blue lines indicate left and right TALEN recognition sequences, respectively. Primer sequences for amplification of the target site are indicated by orange arrows. Dashes and lowercase letters in the sequence alignments indicate deletions and insertions, respectively. Red letters represent natural polymorphisms between multiple St *SSR2* alleles.



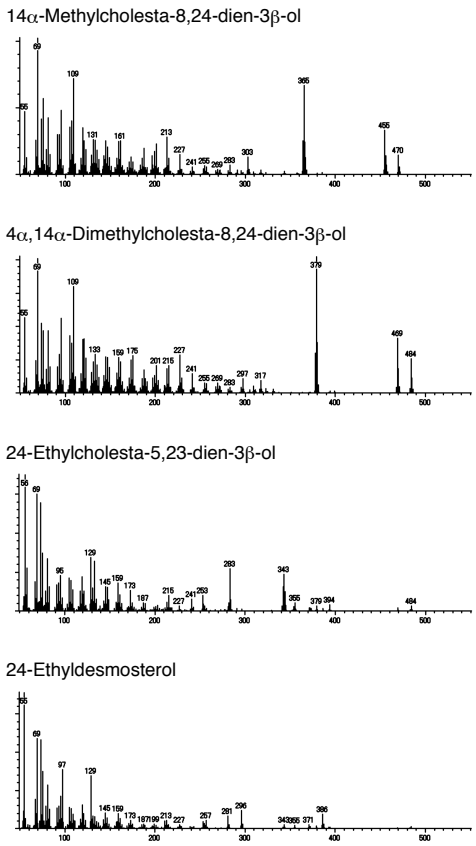
Supplemental Figure 7. St *SSR2*-Disrupted Potato.
(**A**) Analysis of sterol fractions from leaves of non-transformed and St *SSR2*-TALEN transformed potatoes by GC-MS. Total ion chromatograms of leaf sterol fractions from a non-transformed potato plant (NT), transformants with some intact alleles of St *SSR2* (#271-1 and #271-5) and a transformant without an intact allele of St

SSR2 (#271-19). (25,26,26,26,27,27,27-$^{2}H_{7}$)Cholesterol was included as an internal standard (IS). (**B**) Sterol composition in leaves of NT, #271-1, #271-5 and #271-19 (mean and s.d., $n = 3$). The cholesterol levels of NT, #271-1 and #271-5 were significantly different from that of #271-19 (Dunnett's test, $P < 0.05$). DW, dry weight.



Supplemental Figure 8. Flow Diagram of TALEN-Induced *SSR2* Knockout in Potato.

(**A**) A TALEN expression cassette is inserted into the region unlinked to the St *SSR2* target site. (**B** and **C**) TALEN targeting St *SSR2* is induced and generates St *SSR2* alleles with mutations at all four loci in the potato tetraploid genome without modifying the St *SSR1* loci (**C**). (**D**) An St *SSR2*-knockout potato without the transgenes will be obtained by a segregation after self-crossing the transformant.



Supplemental Figure 9. MS Spectra of Sterols in Potato and Tomato.
MS spectra of sterols tentatively identified as 14α-methylcholesta-8,24-dien-3β-ol, 4α,14α-dimethylcholesta-8,24-dien-3β-ol, 24-ethylcholesta-5,23-dien-3β-ol, and 24-ethyldesmosterol in potato and tomato.

Supplemental Table 1. Levels of Brassinosteroids in Leaves from Non-transformed and St *SSR2*-RNAi Transformed Potatoes.

Brassinosteroids	Brassinosteroid content (mean ± s.d., pmol/g dry weight, *n* = 4)		
	NT	#11	#12
Brassinolide	ND	ND	ND
Castasterone	18.89 ± 0.58	21.75 ± 4.67	18.31 ± 3.40

Levels of biologically active brassinosteroids in the leaves of non-transformed (NT) and St *SSR2*-RNAi transformed (#11 and #12) potatoes. ND, not detected.

Supplemental Table 2. TALEN Pairs Designed to Target St *SSR2*.

TALEN ID	RVDs	Target sequence (5′ to 3′)
SSR2_A_L	NN NI NN NI NI NG NN NG NG NI NI NI NN NI NN NN NG	GAGAATGTTAAAGAGGT tgtgaagcgtcttggc CAGAGGAATGCAGAAA
SSR2_A_R	NG NG NG HD NG NN HD NI NG NG HD HD NG HD NG NN	
SSR2_B_L	NN NN NN HD HD NI NI NI NG NN NG HD NI NI NN NN	GGGCCAAATGTCAAGG gtcactatcccaatga ATCTTTCCCTTGC
SSR2_B_R	NN HD NI NI NN NN NN NI NI NI NN NI NG	
SSR2_C_L	NN NN NN NN HD NG NG HD NG NG NN NG NG NG HD NI	GGGGCTTCTTGTTTCA gctgaaatcaagctt ATACCAGTTGATCAAT
SSR2_C_R	NI NG NG NN NI NG HD NI NI HD NG NN NN NG NI NG	

The amino acid sequences of the repeat variable di-residues (RVDs) responsible for nucleotide recognition of three TALEN pairs (A, B and C) are shown. Target sequences of each TALEN pair are indicated; uppercase letters and lowercase letters indicate the TAL effector binding sequence and spacer sequence, respectively.

Supplemental Table 3. Functional Evaluation of Engineered TALENs Targeting the SSR2 gene by the SSA Assay.

Firefly luciferase reporter	Renilla luciferase reporter	TALEN expression vector	Luc	Rluc	Luc/Rluc	Fold activation
pGL4-SSA-ZFA36	pRL-CMV	pcDNA-SSA-HPRT1_B_L+R	21672	38966	0.5561772	1
pGL4-SSA-HPRT1_B-15	pRL-CMV	pcDNA-SSA-HPRT1_B_L+R	286418	52914	5.4128964	9.732323784
pGL4-SSA-ZFA36	pRL-CMV	pcDNA-SSA-SSR2_A_L+R	23204	55163	0.4206443	1
pGL4-SSA-SSR2_A	pRL-CMV	pcDNA-SSA-SSR2_A_L+R	215667	74736	2.8857177	6.860233018
pGL4-SSA-ZFA36	pRL-CMV	pcDNA-SSA-SSR2_B_L+R	18474	46619	0.3962762	1
pGL4-SSA-SSR2_B	pRL-CMV	pcDNA-SSA-SSR2_B_L+R	49796	57442	0.8668918	2.187594989
pGL4-SSA-ZFA36	pRL-CMV	pcDNA-SSA-SSR2_C_L+R	18561	43144	0.4302105	1
pGL4-SSA-SSR2_C	pRL-CMV	pcDNA-SSA-SSR2_C_L+R	357102	54916	6.502695	15.11514865

Activities of three custom TALENs against the SSR2 gene were evaluated by a human cell (HEK293T)-based SSA assay. Luc, Firefly luciferase activity. Rluc, Renilla luciferase activity. Fold activation is the ratio of calculated Luc/Rluc value of each sample to that of each negative control.

Supplemental Table 4. Nucleotide Sequences of Oligonucleotides Used for Preparation of SSA Reporter Vectors.

TALEN	Oligonucleotide	Orientation	Nucleotide sequence (5′ to 3′)
SSR2_A	SSA-SSR2_A-s	Sense	GTCGGATGAGAATGTTAAAGAGGTtgtgaagcgtcttggcCAGAGGAATGCAGAAAAGGT
	SSA-SSR2_A-as	Antisense	CGGTACCTTTTCTGCATTCCTCTGgccaagacgcttcacaACCTCTTTAACATTCTCATC
SSR2_B	SSA-SSR2_B-s	Sense	GTCGGATGGGCCAAATGTCAAGGgtcactatcccaatgaATCTTTCCCTTGCAGGT
	SSA-SSR2_B-as	Antisense	CGGTACCTGCAAGGGAAAGATtcattgggatagtgacCCTTGACATTTGGCCCATC
SSR2_C	SSA-SSR2_C-s	Sense	GTCGGATGGGGCTTCTTGTTTCAgctgaaatcaagcttATACCAGTTGATCAATAGGT
	SSA-SSR2_C-as	Antisense	CGGTACCTATTGATCAACTGGTATaagcttgatttcagcTGAAACAAGAAGCCCCATC

Supplemental References

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Sigrist, C.J.A., Cerutti, L., Hulo, N., Gattiker, A., Falquet, L., Pagni, M., Bairoch, A., and Bucher, P. (2002). PROSITE: a documented database using patterns and profiles as motif descriptors. Brief Bioinform. **3:** 265–274.