

Supplementary Table S1. The mutations in the *S1ses* mutant

Chr Position	Reference (Micro-Tom)	<i>s1ses</i> mutant	Mutation type	Indel length	DP4 (V,W,X,Y)	Locus	ITAG number	Annotation	Mutatio n	Effect of mutation
62,468,747	T	TC	IN	1 0,0,3,4		IGR				
62,475,310	GT	G	DEL	-1 0,0,17,25		Intragenic region Solyc07g062570	Ubiquitin-conjugating enzyme E2 N			intron
62,482,892	C	CAAAATGACT	IN	9 0,0,11,2		IGR				
62,485,918	AG	AGG	IN	1 0,0,1,1		IGR				
62,501,963	TC	T	DEL	-1 0,0,1,1		IGR				
62,501,964	C	CTTTTTTTTT	IN	1 0,0,5,4		IGR				
62,525,634	GAAAAAAA	GAAAAAAA	DEL	-1 0,0,7,13		IGR				
62,613,928	AT	ATCT	IN	2 0,0,1,2		IGR				
62,614,410	A	AAAAAAAAA	IN	2 0,0,0,10		IGR				
62,618,272	A	CAAAAAAAA	IN	1 0,0,9,8		IGR				
62,621,533	C	CTTTTTTTTT	IN	2 0,0,9,19		IGR				
62,664,178	C	CTTTTTTTTT	IN	1 0,0,5,12		IGR				
63,002,908	A	CAAAAAAAA	DEL	-1 0,0,23,15		IGR				
63,008,548	T	C	SNP	13,21,168,247		IGR				
63,020,867	GAAAA	GAAA	DEL	-1 0,0,0,4		Intragenic region Solyc07g063290	Unknown Protein			intron
63,033,736	G	GATGT	IN	4 1,0,8,15		Intragenic region Solyc07g063320	LanC-like protein 2			intron
63,035,794	C	G	SNP	0,0,2,1		IGR				
63,037,848	GGAG	GAGAG	IN	9 0,0,2,1		IGR				
63,045,924	AGTG	AGAATTACCGTG	IN	9 0,0,11,14		IGR				
63,053,924	A	AAAAC	IN	4 0,0,4,2		IGR				
63,058,609	TT	TTCAT	IN	3 0,0,3,1		IGR				
63,070,286	CA	CAGA	IN	2 0,0,3,0		IGR				
63,070,690	T	TAAC	IN	3 0,0,1,2		IGR				
63,080,600	G	A	SNP	0,0,1,2		IGR				
63,088,782	CAAGAACGAAAG	CAAG	DEL	-10 0,0,2,0		Intragenic region Solyc07g063390	Beta-glucosidase			intron
63,091,155	CAAAAAA	CAAAAAAAA	IN	7 0,0,2,1		IGR				
63,091,163	AGG	A	DEL	-2 0,0,1,1		IGR				
63,091,165	GA	GA	IN	2 0,0,3,4		IGR				
63,091,769	C	CT	IN	1 0,0,1,2		IGR				
63,091,881	C	T	SNP	0,0,2,0		IGR				
63,092,103	A	C	SNP	0,0,4,0		IGR				
63,102,932	AGG	AGGG	IN	1 0,0,2,2		IGR				
63,104,712	TG	TTAATTCAAG	IN	7 0,0,17,13		IGR				
63,105,333	CA	CA	IN	4 0,0,2,3		IGR				
63,105,358	AG	A	DEL	-1 0,0,1,1		IGR				
63,108,880	AAA	AAAACAA	IN	4 0,0,14,14		IGR				
63,113,058	GA	GA	IN	1 0,0,8,11		IGR				
63,126,005	AAAAA	TTAAAAAA	IN	2 0,0,22,18		Intragenic region Solyc07g063420	NAC domain transcription factor			intron
63,129,991	AAAAA	TA	DEL	-1 0,0,6,7		IGR				
63,137,609	GTTT	GTT	DEL	-1 0,0,0,3		IGR				
63,182,669	A	ATATTTTC	IN	7 0,0,5,8		IGR				
63,183,264	TA	TAA	DEL	8 0,0,2,5		IGR				
63,196,959	AAA	AAAAGAAA	IN	4 0,0,6,4		IGR				
63,197,263	T	TCAAAAAACA	IN	9 0,0,3,0		IGR				
63,197,574	AATA	AA	DEL	-2 0,0,0,2		IGR				
63,198,726	AC	A	DEL	-1 0,0,14,20		IGR				
63,207,057	A	AATTAAAGAGCATATGATT	IN	25 0,0,3,1		IGR				
63,211,620	GAAA	AAGGATATT	IN	1 0,0,1,2		Intragenic region Solyc07g063520	Transmembrane protein 34			intron
63,213,768	TTGT	TT	DEL	-2 0,0,2,2		Intragenic region Solyc07g063530	Unknown Protein			intron
63,217,639	T	TG	IN	1 0,0,3,1		Intragenic region Solyc07g063530	Unknown Protein			3'UTR
63,218,656	CT	CTT	IN	1 0,0,0,4		IGR				
63,224,093	CTT	C	DEL	-3 0,2,12,6		Intragenic region Solyc07g063550	Arf-GAP with GTPase, ANK repeat and PH domain-containing protein 1			intron
63,224,569	CTATGGAAATA	C	DEL	-9 0,0,7,1		Intragenic region Solyc07g063550	Arf-GAP with GTPase, ANK repeat and PH domain-containing protein 1			intron
63,230,602	ATTTTT	ATTTTTTT	IN	3 0,0,0,4		IGR				
63,233,770	C	T	SNP	0,0,3,1		Intragenic region Solyc07g063560	Cotton fiber expressed protein 1			3'UTR
63,255,279	T	TA	IN	1 0,0,0,4		Intragenic region Solyc07g063590	Myosin-like protein			intron
63,263,762	C	CG	IN	1 0,0,1,1		Intragenic region Solyc07g063600	Chlorophyll a/b binding protein 13, chloroplast			intron
63,264,622	A	T	SNP	0,0,2,2		IGR				
63,271,176	TCCC	TCC	DEL	-1 0,0,1,1		IGR				
63,276,145	TA	TA	DEL	-1 0,0,5,3		IGR				
63,276,316	TTTT	TTTTTTGTTT	IN	6 0,0,13,6		IGR				
63,279,307	GT	GTAT	IN	2 0,0,0,4		IGR				
63,285,846	T	TA	IN	1 0,0,1,1		Intragenic region Solyc07g063650	Ubiquitin carboxyl-terminal hydrolase			intron
63,286,441	AC	ACACTC	IN	4 0,0,3,0		Intragenic region Solyc07g063650	Ubiquitin carboxyl-terminal hydrolase			intron
63,286,442	CTC	CACTCTAGAAAGCCAAGGC	IN	20 0,0,11,11		Intragenic region Solyc07g063650	Ubiquitin carboxyl-terminal hydrolase			intron
63,297,255	CA	CA	IN	1 0,0,9,3		IGR				
63,299,377	T	C	SNP	0,0,0,6		IGR				
63,301,438	G	GTATT	IN	5 0,0,2,1		IGR				
63,302,667	ATT	A	DEL	-2 0,0,3,0		IGR				
63,303,142	GAAGAAGCAACCACAA	GAA	DEL	-13 0,0,12,13		Intragenic region Solyc07g063670	Unknown Protein			exon
63,304,222	T	A	SNP	0,0,1,3		Intragenic region Solyc07g063670	Unknown Protein			exon
63,309,151	T	TCCTC	IN	4 0,0,25,14		Intragenic region Solyc07g063680	CHP-rich zinc finger protein-like			intron
63,311,339	ATTCACT	A	DEL	-7 0,0,4,0		IGR				
63,313,247	CTTTTTTT	CTTTTTTT	IN	1 0,0,4,4		IGR				
63,437,474	CTTTTTTT	ATTTTTTT	IN	2 0,0,5,12		IGR				
63,444,260	CA	CA	IN	1 0,0,4,18		IGR				
63,473,474	CTTTTTTT	CTTTTTTT	IN	1 0,0,12,8		IGR				
63,508,655	TA	TAAAAA	IN	3 0,0,2,7		IGR				
63,862,271	AAAAA	TAAAAA	IN	2 0,0,10,9		Intragenic region Solyc07g064550	Genomic DNA chromosome 5 P1 clone MBG1			intron
63,948,376	TATATAT	TATATAT	IN	2 0,0,3,2		IGR				
63,977,710	TTTTTTTT	CTTTTTTT	IN	1 0,2,13,17		Intragenic region Solyc07g064720	GDSL esterase/lipase At5g55050			intron
64,027,233	A	AAG	IN	2 0,0,4,1		IGR				
64,036,652	ACT	AAACCT	IN	3 0,0,2,0		IGR				
64,036,653	CTA	CA	DEL	-1 0,0,3,1		IGR				
64,069,862	CA	CA	IN	1 0,0,8,6		IGR				
64,079,800	ACCCCCCCCCCCC	ACCCCCCCCCCCCCCCC	IN	6 0,0,1,2		IGR				
64,082,904	TA	TAAAAA	DEL	-1 0,0,4,1		Intragenic region Solyc07g064880	Small ubiquitin-related modifier			intron
64,094,485	TA	TCAAA	IN	4 0,0,3,5		IGR				
64,136,178	CTTTTTTT	ATTTTTTT	IN	1 0,1,5,9		IGR				
64,265,168	ATATATATATATATATAT	TTATATATATATATATAT	IN	41 0,0,3,0		IGR				
64,298,439	CTTTTTTT	ATATATATATATAT	IN	1 0,0,13,9		IGR				
64,421,532	GATATATATATATATATATA	GATATATATATATATATA	IN	38 0,0,5,3		IGR				
64,593,337	TATATATATAT	TATATATATAT	IN	1 0,0,4,2		Intragenic region Solyc07g065700	SEC14-like protein 1			exon
64,611,647	CTTTTTTT	CTTTTTTT	IN	1 0,2,15,15		IGR				

Chr Position; physical position of mutation on the tomato reference genome (SL2.40). Reference (Micro-Tom); Sequence of WT tomato Micro-Tom genome (SL2.40). DP4; Four numbers correspond to numberd of forward ref alleles, reverse ref, forward non-ref, and reverse non-ref alleles, used in variant calling. Locus, mapped position for *slices* mutation. Mutation position; Exon indicates that homozygous indel or SNPs found in *slices* mutant are located within the exon region, while intron indicates that the indel or SNPs are located within the intron region. IGR, intergenic region. ITAG name, ITAG gene number definceed in SL2.40. Effect of mutation; Missense mutation causes non-synonymous mutation. Frame shift indicates the gap of amino acid transrstration.

Supplementary Table S2. List of primers

Primer name	Purpose	Forward Primer Sequence	Reverse Primer Sequence	Restriction Enzyme
<i>SSES</i> marker-1	DNA marker (<i>SES</i> mutation)	CGAGTAAAAATGGACAGAAGAAGAA	TGATGAAACAGGAGTTAATTAGGG	-
<i>SSES</i> marker-2	DNA marker (<i>SES</i> mutation)	ACACCATACTAGCCAGACCATGT	ACAATGAGCCACCAGTTGAAGCTC	<i>Bsl</i> I
<i>NPT</i> II marker	DNA marker (<i>NPT</i> II)	ATGATTGAACAAGATGGATTGCAC	TCAGAAGAACTCGTCAAGAAGGCG	-
Whole <i>SSES</i> gene	In-Fusion	GATTACGCCAAGCTTCAATTAGCGATGGACGAAGTICTAGGACGCGTCGACCTCACTTCTAGACTGCCATAAAGC		-

Supplementary Table S3. Homology with S1SES

	Protein	Identity	Similarity	Gaps
SPL/NZZ	AT4G27330	26.4%	39.2%	33.7%
TIE1	AT4G28840	12.6%	21.3%	60.0%
TIE2	AT2G20080	12.9%	18.4%	67.9%
TIE3	AT1G29010	21.1%	31.8%	36.3%
TIE4	AT2G34010	17.1%	28.3%	38.0%

Each Homology was calculated with The European Bioinformatics Institute.

SPL/NZZ, SPOROCYTELESS/NOZZLE

TIE, TCP INTERACTOR CONTAINING EAR MOTIF PROTEIN

Supplementary Table S4. *t*-butyl alcohol series

	<i>t</i> -butyl alcohol	Ethanol	DW
1	10 mL	40 mL	50 mL
2	20 mL	50 mL	30 mL
3	35 mL	50 mL	15 mL
4	55 mL	45 mL	0 mL
5	75 mL	25 mL	0 mL
6	100 mL	0 mL	0 mL

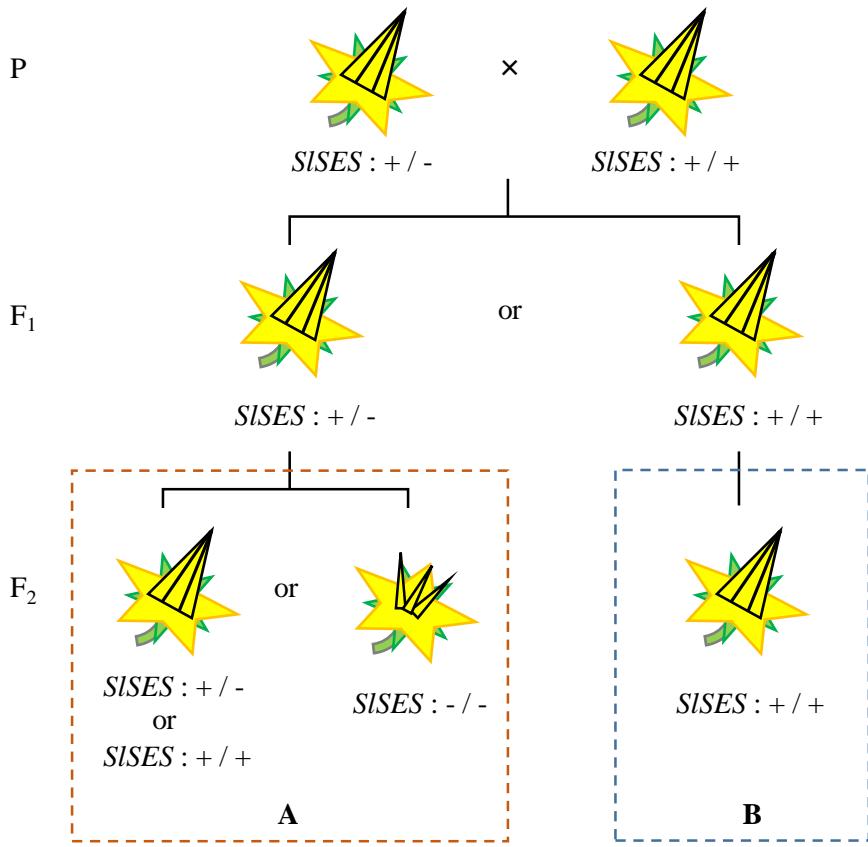
Each solution are composed with *t*-butyl alcohol, ethanol and distilled water (DW).

Supplementary Table S5. List of primers for qRT-PCR

Gene	Solyc ID	Forward Primer Sequence (5'→3')	Reverse Primer Sequence (5'→3')
<i>SISES</i>	<i>Solyc07g063670</i>	AGCCTTGCTTCACTCATACAGTT	ACTCATCATCGTTGCTTCATTCTC
<i>MS10</i> ³⁵	<i>Solyc02g079810</i>	AAATGACACCAGTGCCACTG	AGTAGATTCCGTGGATGATCTCC
<i>BAM1/2-like</i>	<i>Solyc02g091840</i>	AATTCCCCGGGGACACAAAAG	ATAAACCCCCGAGCCTAGAAAG
<i>EMSI/EXS-like</i>	<i>Solyc09g098420</i>	TTTCGCGAAGTGTCAAGCC	ATTGTTGCCGAGTGAGATCCC
<i>TAG1</i>	<i>Solyc02g071730</i>	GCAGAAGAGGGAAGTTGATTACAC	GTCTAGGGTAATGGITGTTGGTTG
<i>SIWUS</i>	<i>Solyc02g083950</i>	AAGAAGAGGCTATTGCTGCTG	ACCCCATGTGAAGATGGTGATG
<i>SIINO</i>	<i>Solyc05g005240</i>	TACCCCAACATGACTCACAAGC	TTGCTATCTCTTGGGACCATGG
<i>ANT-like</i>	<i>Solyc04g077490</i>	CTGCTGCCCTCATTAGTCTTG	AAAGACTGGTAGGTGAGCCATG
<i>PIN1-like</i>	<i>Solyc03g118740</i>	CCCACAACCCATAAACAAAAC	GCACAAACAGCAGTCATAACATGG
<i>BEL1-like</i>	<i>Solyc08g081400</i>	TGATGGAGACTCATCCTGGAG	CCAGTTGGCGAGCTAAATG
<i>SIREV</i>	<i>Solyc11g069470</i>	ACTCGACATGCTGGAGACAAC	AGATACCACCAGGCAAACACG
<i>CNA-like</i>	<i>Solyc03g120910</i>	GTCTAGGCCGTTTCAGTTGG	ACCAGCTCTGTTACTCTGCTTC
<i>PHB/PHV-like</i>	<i>Solyc02g024070</i>	GCTCTCGTTCTCTGGAGGAG	TCTCCTCTGTAAACACAAGGC
<i>GOB</i>	<i>Solyc07g062840</i>	TGCATGAATATGCCCTGATGG	AGTAGCGGCACCATAGAAC

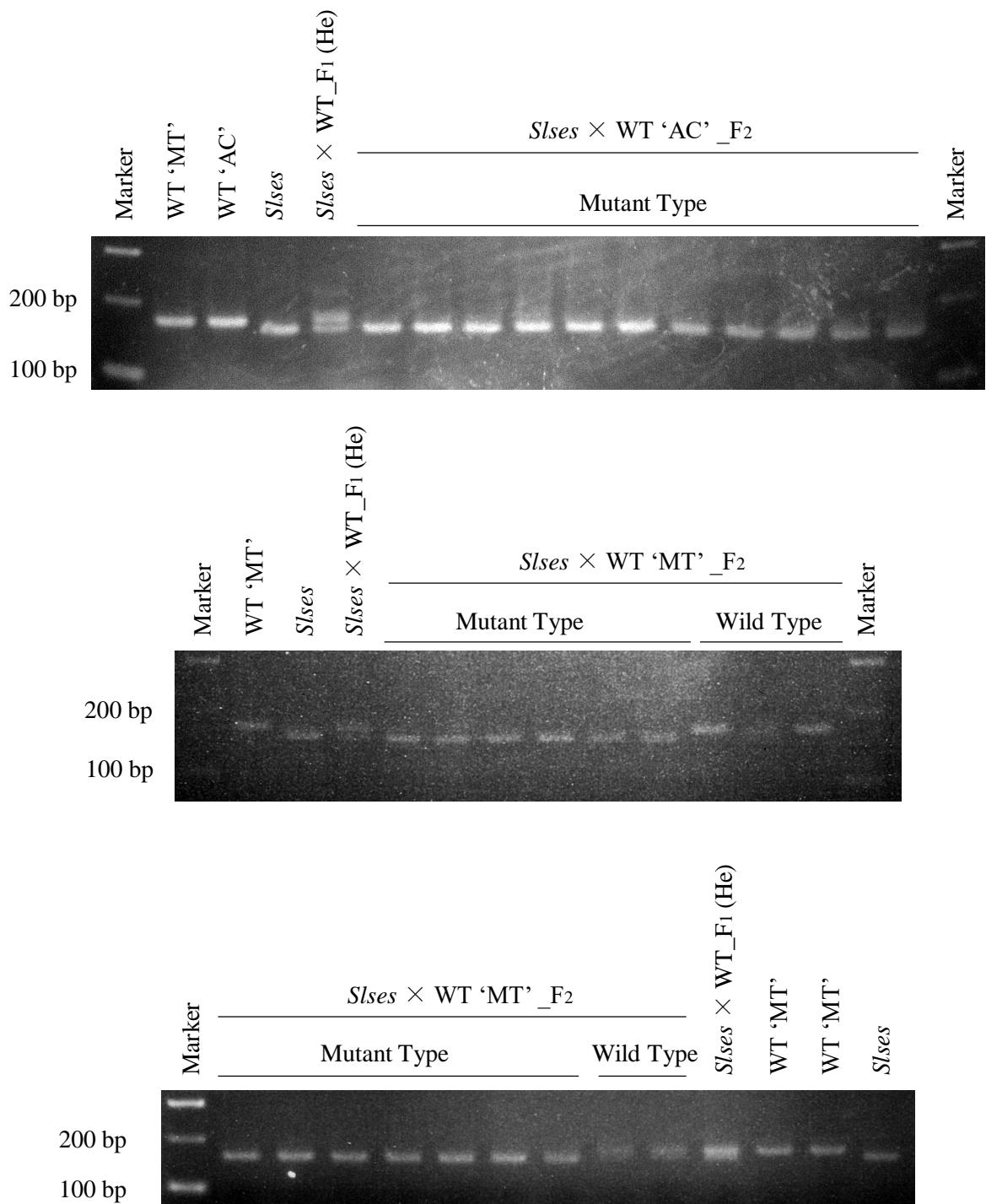
SISES, SEXUAL STERILITY; *MS10*³⁵, MALE STERILE 10³⁵; *BAM1/2*, BARELY ANY MERISTEM1 and 2;

EMSI/EXS, EXCESS MICROSPOROCYTES1/EXTRA SPOROGENEOUS CELLS; *TAG1*, TOMATO AGAMOUS1; *SIWUS*, WUSCHEL; *SIINO*, INNER NO OUTER; *ANT*, AINTEGUMENTA; *PIN1*, PIN-FORMED1; *BEL1*, BELLI; *SIREV*, REVOLUTA; *CNA*, CORONA; *PHB/PHV*, PHABULOSA/PHAVOLUTA; *GOB*, GOBLET



Supplementary Fig. S1. Schematic diagram of crossing analysis.

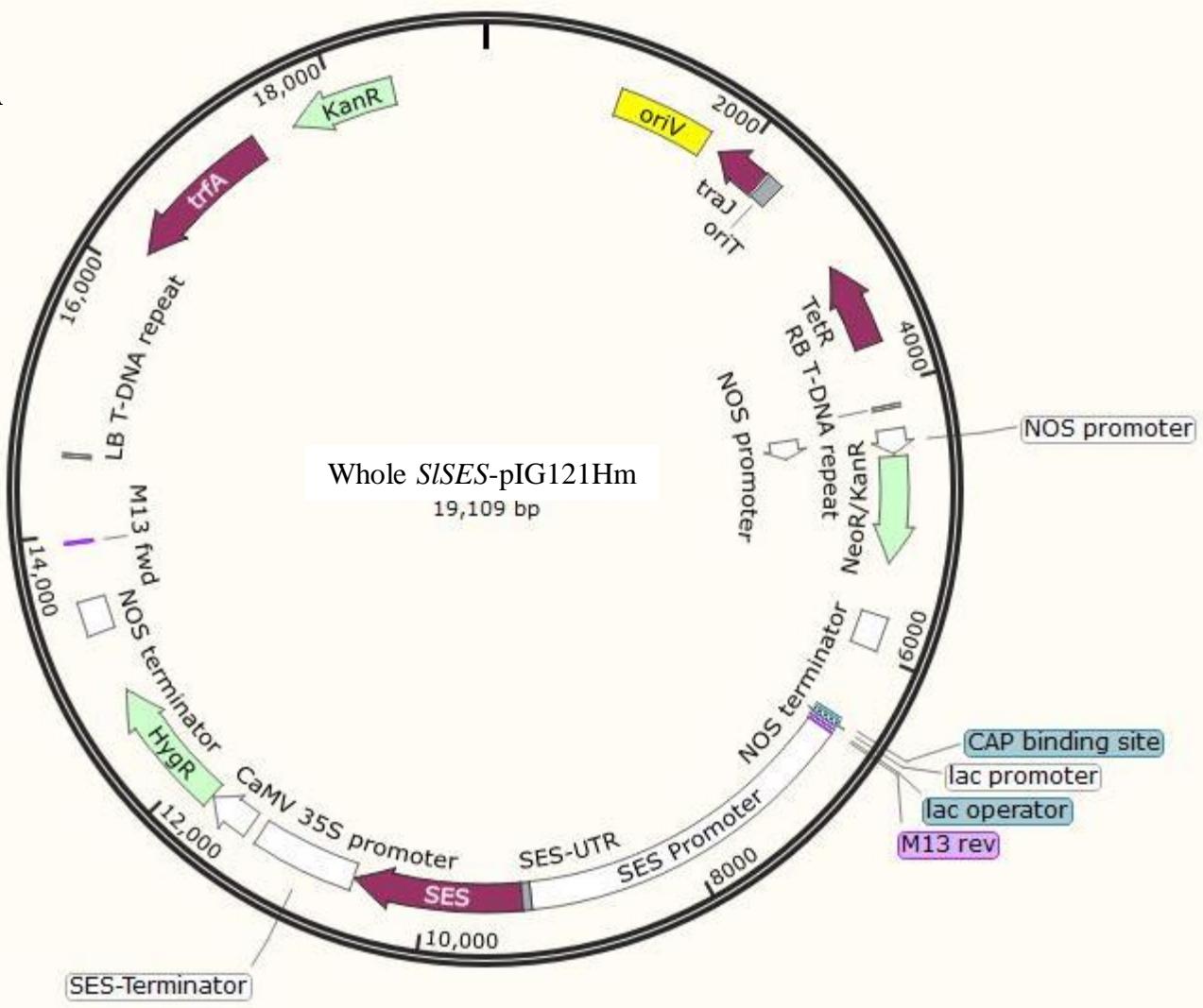
The *SISES* homozygous mutant plants showed sexual sterility, and therefore the *SISES* heterozygous mutant plants were used for crossing. All of F_1 plants showed wild type phenotype, but there were two types of genotypes. One type was the *SISES* heterozygous genotype, and F_2 plants from this genotype were segregated to WT phenotype (left image) and mutant phenotype (right image) (A). Another type was WT genotype, and F_2 plants from this genotype showed WT phenotype (B). + indicates wild type *SISES* and - indicates mutated *SISES*.



Supplementary Fig. S2. Linkage analysis of the 13-bp deletion on *Solyc07g063670* by *SlSES* marker-1.

Micro-Tom WT and Ailsa Craig showed upper band (173-bp), the *slses* mutant showed lower band (160-bp) and the heterozygous showed both bands. All of F₂ plants which showed mutant phenotype had the 13-bp deletion. The F₂ population were constructed from crossed with 'Micro-Tom (MT)' WT or 'Ailsa Craig (AC)'. He, heterozygous.

A

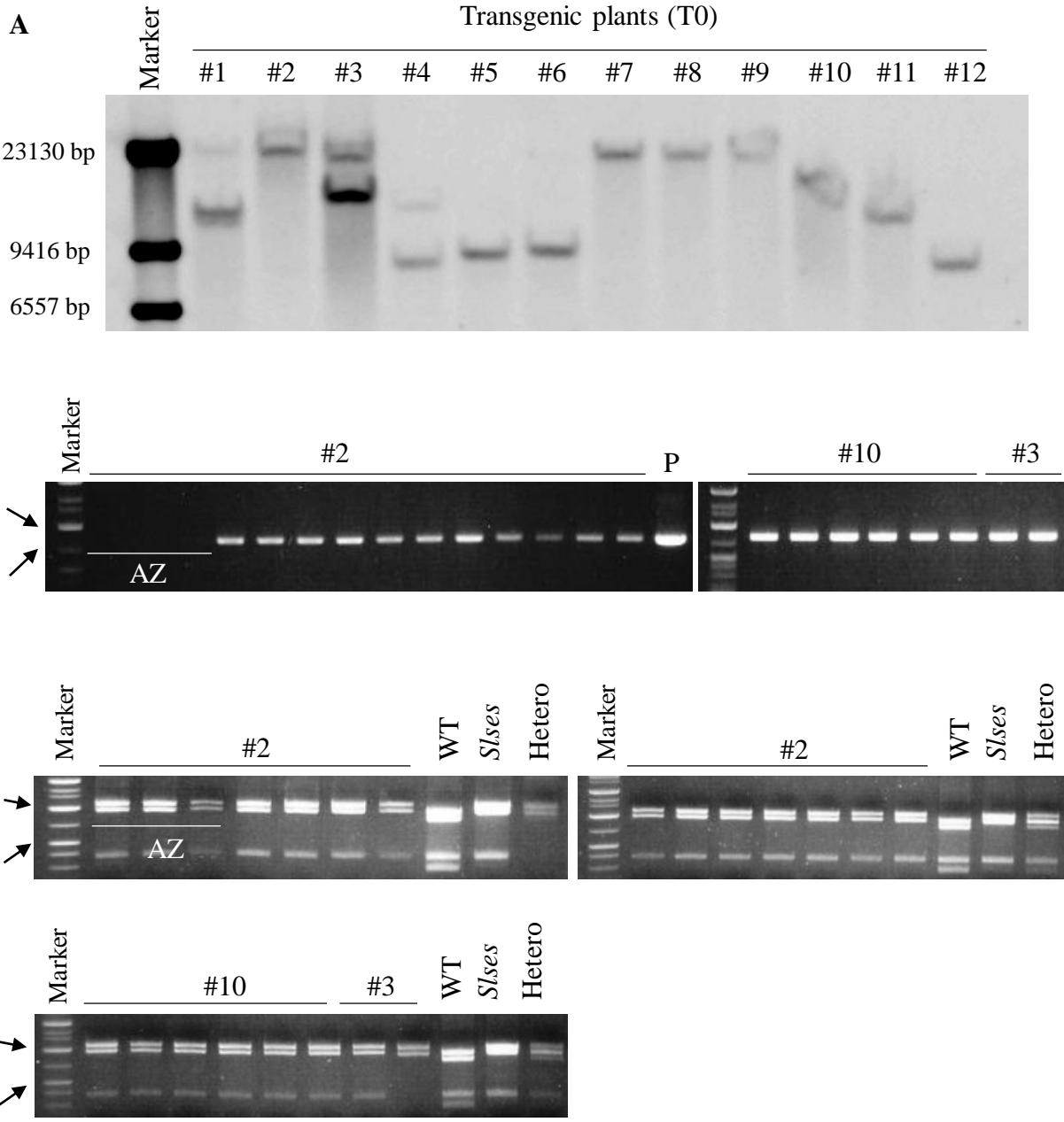


B



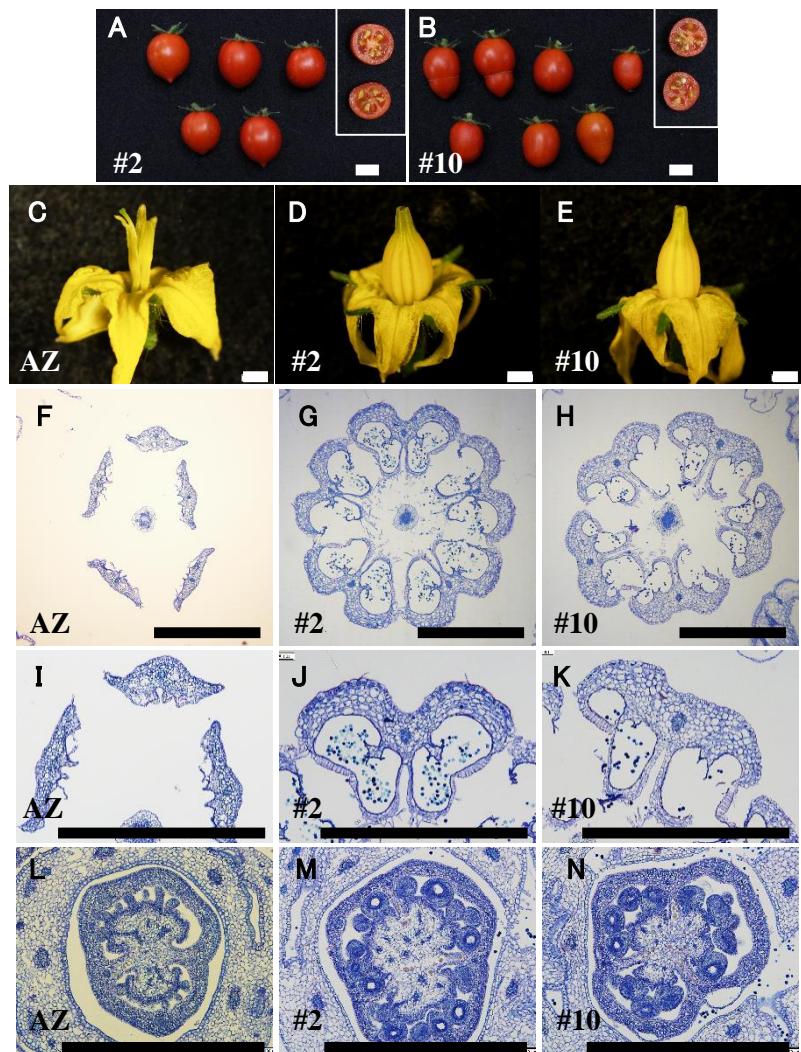
Supplementary Fig. S3. The vector map for the whole *SSES* gene transgenic plant.

(A) Entire vector map constructed from pIG121Hm vector. (B) The transferred region to the plant, from right border (RB) to left border (LB). *NPTII* indicates kanamycin resistance gene. *HPT* indicates hygromycin resistance gene.

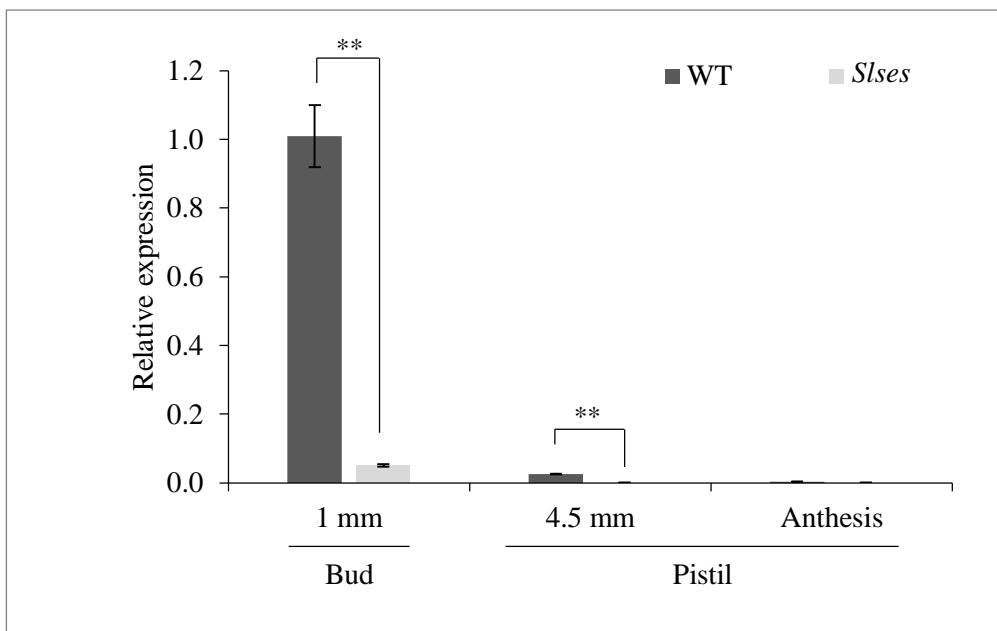


Supplementary Fig. S4. Selection of the plants which not only have transgene but also be the *S1ses* homozygous genotype.

(A) Confirmation of copy numbers of transgene in the T0 plants by southern blotting analysis. The probe recognized *NPTII*. (B) *NPTII* existence confirmation in the T1 plants by *NPTII* marker (See supplementary Table S2). (C) The genotype analysis of the T1 plants by *S1SES* marker-2 (See supplementary Table S2). #2, #3, #10 indicate line number and P indicates used vector as a positive control. AZ, azygous; Hetero, heterozygous.



Supplementary Fig. S5. Complementation experiment verified *Solyc07g063670* was responsible for the *Slses* phenotypes. (A, B) The red ripe fruits of the transgenic *Slses* mutant in which *SlSES* whole gene was induced. Bar = 1 cm. (C-E) The anthesis flower. (F-N) The cross section of the flower at anthesis flower, (F-K) the anther and pollen, (L-N) the ovary and ovules. Bar = 1 mm. (A, D, G, J, M) The transgenic line #2, (B, E, H, K, N) the transgenic line #10, (C, F, I, L) azygous (AZ). AZ plant doesn't have transgenes.



Supplementary Fig. S6. The expression analysis of *S1SES* in WT and the *Slses* mutant by qRT-PCR.

The *S1SES* expression of the *Slses* mutant at 1 mm bud and 2 stages of pistils from 4.5 mm bud and anthesis stages. n = 3, error bar = SE, statistical analysis was realized using the Student's t-test, **P<0.01; *P<0.05.