Supplemental Material and Methods

RT-PCR

Total RNA was isolated from the roots and hypocotyls of wild-type seedlings, from the stem of 5-week-old wild-type plants, and from the rosette leaves, buds and siliques from wild-type adult plants using RNeasy plant mini kit (Qiagen). Reverse transcription and cDNA synthesis were performed using cDNA Synthesis kit (Gibco BRL) and according to the manufacture's instruction.

RNA gel-blot analysis

Total RNA from each organ as noted above was isolated using aurintricarboxylic acid (ATA). 100 mg of each organ was homogenized in 800 µL of extraction buffer (50 mM Tris-HCl, pH8.0, 0.3 M NaCl, 0.5 M EDTA, 100 mM ATA, 2% SDS, 2% triisopropylnaphthalenesulfonic acid sodium salt) and 15 µL of 2-mercaptoethanol. 112 µL of 3M KCl was added to the samples and incubated on ice for 5 min. The samples were centrifuged at 5,000*g* for 5 min at 4°C. The supernatants were added to 950 µL of 8M LiCl and incubated for 2 h at 4°C. After centrifugation of 18,000*g* for 20 min at 4°C, the supernatants were removed and 400 µL of distilled water, 40 µL of 3M sodium acetate and 350 µL of phenol chloroform mixture (1:1) were added to the pellets and mixed. The samples were centrifused at 18,000*g* for 5 min at 4°C and the supernatants were moved to 350 µL of phenol chloroform mixture. After centrifugation, the supernatants were moved to 450 µL of chloroform and recentrifuged. The collected supernatants were mixed with 1 mL of ethanol and incubated on ice for 10 min. The samples were centrifuged and the pellets were rinsed with 70% ethanol, and then the dried pellets were dissolved in distilled water. Ten µg of total RNA from each organ was electrophoresed in 1% agarose gel. The gel was then

incubated in 0.05N NaOH for 20 min and the RNA were transferred to a nylon membrane (Hybond N+; Amersham Biosciences). The membrane was hybridized with a dogoxigenin-labeled RNA probe prepared by *in vitro* transcription (templates: SGR6 cDNA digested by SalI).

Antibody production

The middle region of SGR6 (residues~~,~~ Phe618-Asn1241; At2g36810) was amplified by PCR with the primers SGR6-middle F and SGR6-middle R and then cloned into the *Eco*RI site of pET32c**.** The His-SGR6 fusion protein was produced in *Escherichia coli* BL21. The fusion proteins were purified on Ni-Sepharose column and used for immunizing rabbits.

Protein extraction and subfractionation

Protein extraction and subfractionation were carried out as described in Tamura et al. (2005), with minor modifications. 0.5 g of inflorescence stems from 6-week-old plants without fruits was homogenized on ice in 10 mL of extraction buffer (100 mM HEPES-KOH, pH 7.5, 0.3 M sucrose, 5 mM $MgCl_2$, 5 mM EGTA and 5 mM EDTA) with a protease inhibitor cocktail (Sigma, St. Louis, MO)**.** The homogenate was passed through Miracloth (Calbiochem, La Jolla CA) and centrifuged at 1,000*g* for 10 min at 4°C to remove debris. Protein concentration of the supernatant was determined with BCA kit (PIERCE) to use samples including equal protein amount for western

blot analysis or suborganeller fractionation.

To perform western blot analysis, protein extracts from stems of wild-type or *sgr6* alleles were mixed with equal volume of 2×laemmli sample buffer (100 mM Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 0.01% bromphenol blue and 4% 2-mercaptoethanol) and incubated at 25°C overnight. The samples were separated by SDS-PAGE followed by immunoblotting using an anti-SGR6 antibody.

To perform subfractionation, protein extract from six-week-old wild-type stem was ultra-centrifuged at 100,000*g* for 1 h at 4°C to obtain microsomal pellets. The pellets were suspended in 100 µL of each solution buffer as follows: high-salt buffer (100 mM HEPES-KOH, pH 7.5, 0.3 M sucrose, 1 M NaCl and 5 mM EDTA), urea buffer (100 mM HEPES-KOH, pH7.5, 0.3 M sucrose, 8 M urea and 5 mM EDTA), alkaline buffer (100 mM Na_2CO_3, 0.3 M sucrose and 5 mM EDTA), TritonX-100 buffer (1% [v/v] TritonX-100, 100 mM HEPES-KOH, pH7.5, 0.3 M sucrose and 5 mM EDTA), SDS buffer (2% [w/v] SDS, 100 mM HEPES-KOH, pH7.5, 0.3 M sucrose and 5 mM EDTA). The samples were incubated for 30 min at 25°C and ultra-centrifuged at 100,000*g* for 1 h at 4°C. Supernatants were concentrated with Trichloroacetic acid. The pellets (S100) and P100 were resuspended in 20 µL of 1×laemmli sample buffer (50 mM Tris-HCl, pH

6.8, 2% SDS, 10% glycerol, 0.005% bromophenol blue and 2% 2-mercaptoethanol) and incubated at 25°C overnight. The samples were separated by SDS-PAGE followed by immunoblotting using the anti-SGR6 antibody.

Movie legends

Supplemental movie 1 Endodermal cell that shows the dynamic VM structures and amyloplast dynamics during a 2-min time window within 5-10 min after stem cutting.

Supplemental movie 2 Endodermal cell that does not show the dynamic VM structures and amyloplast dynamics during a 2-min time window within 5-10 min after stem cutting.

Supplemental movie 3 Endodermal cell of the wild type during a 2-min time window within 5-10 min after stem cutting.

Supplemental movie 4 Endodermal cell of the *sgr6-1* mutant during a 2-min time window within 5-10 min after stem cutting.

Supplemental movie 5 Endodermal cell of the wild type during a 2-min time window within 25-30 min after stem cutting.

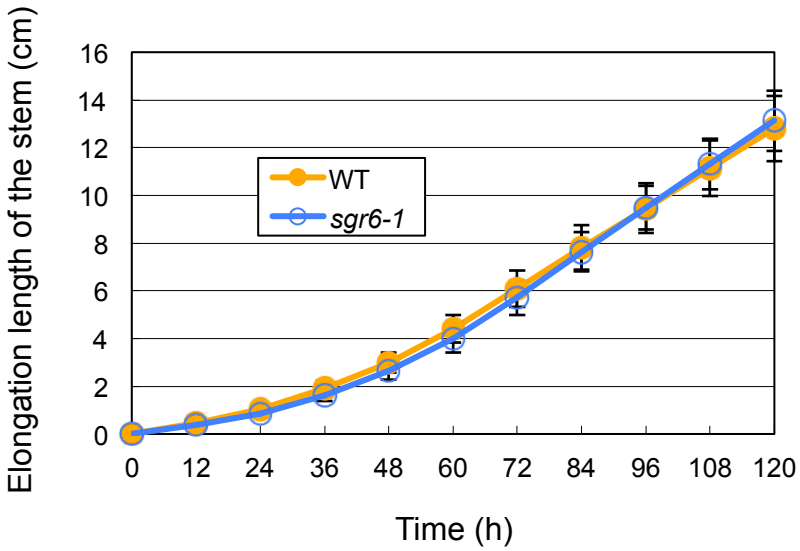
Supplemental movie 6 Endodermal cell of the *sgr6-1* mutant during a 2-min time window within 25-30 min after stem cutting.

Supplemental movie 7 4D imaging of the wild-type endodermal cell within 10-20 min after stem cutting.

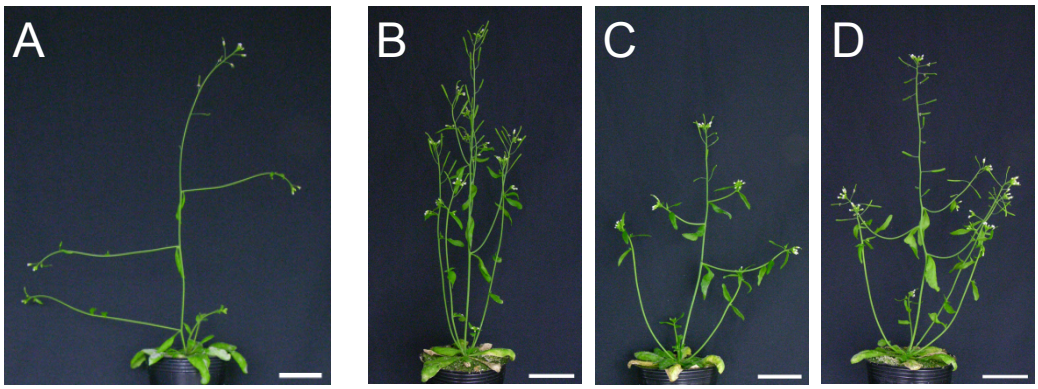
Supplemental movie 8 4D imaging of the *sgr6-1* endodermal cell within 10-20 min after stem cutting.

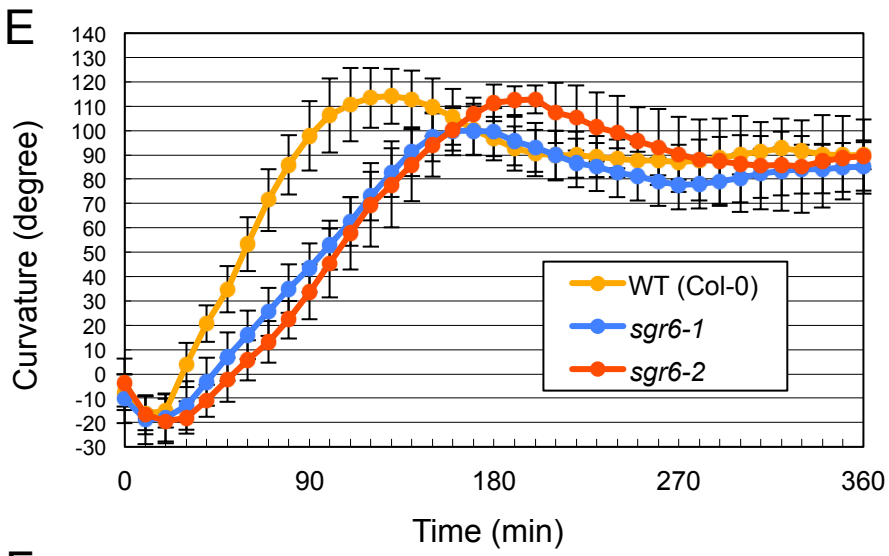
Supplemental movie 9 4D imaging of the *sgr6-1* endodermal cell within 20-30 min after stem cutting.

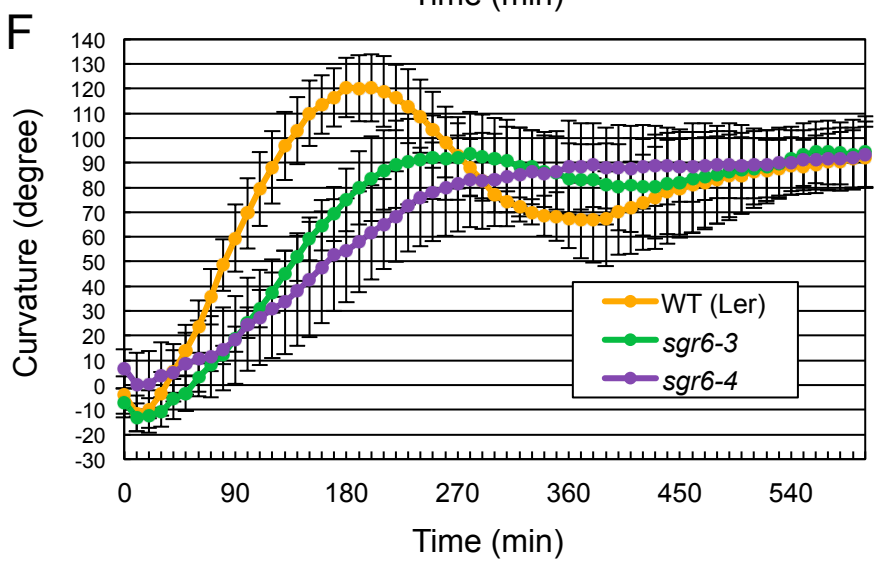
Supplemental movie 10 4D imaging of the *sgr6-1* endodermal cell within 30-40 min after stem cutting.



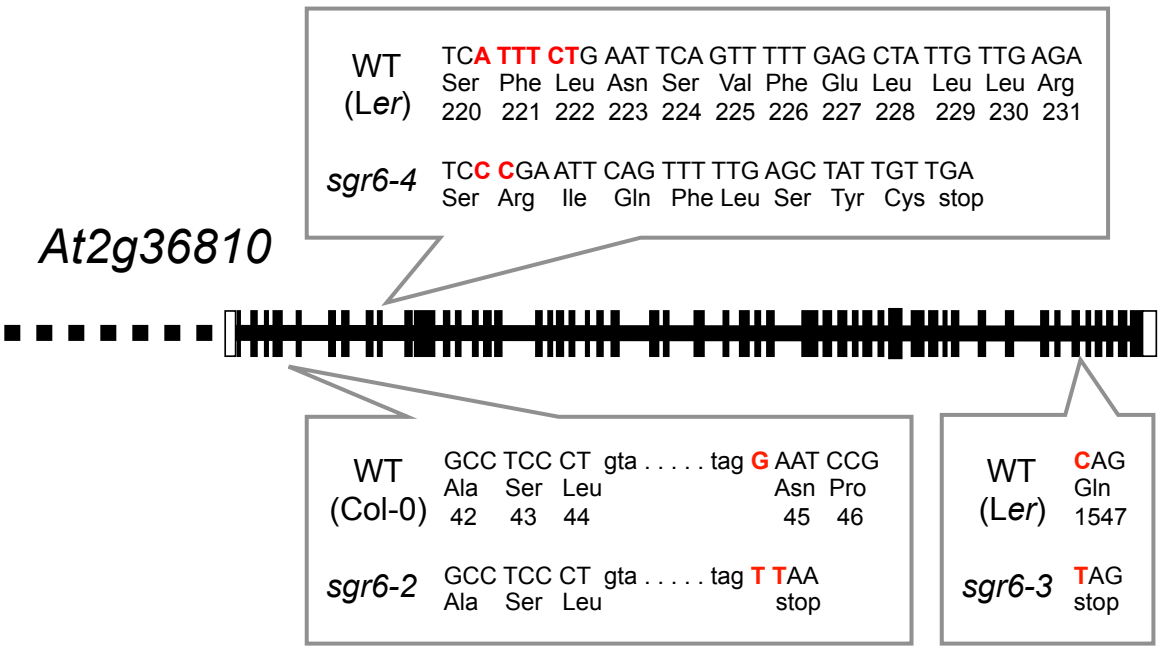
Supplemental Fig. S1 Growth of the primary inflorescence stems of wild type (yellow closed circles) and *sgr6-1* (blue open circles). Elongation length in five days after the length of stems reached approximately 1.5 cm were measured at 12-h intervals. Fifty-five individuals of each genotype were examined. Bars represent SD.



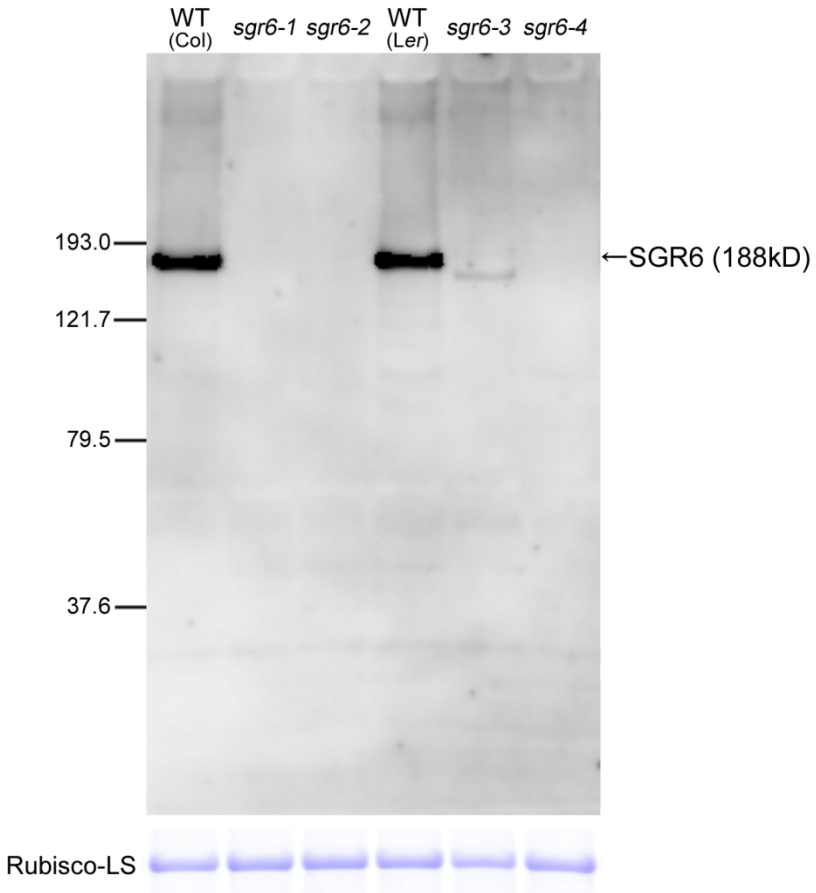




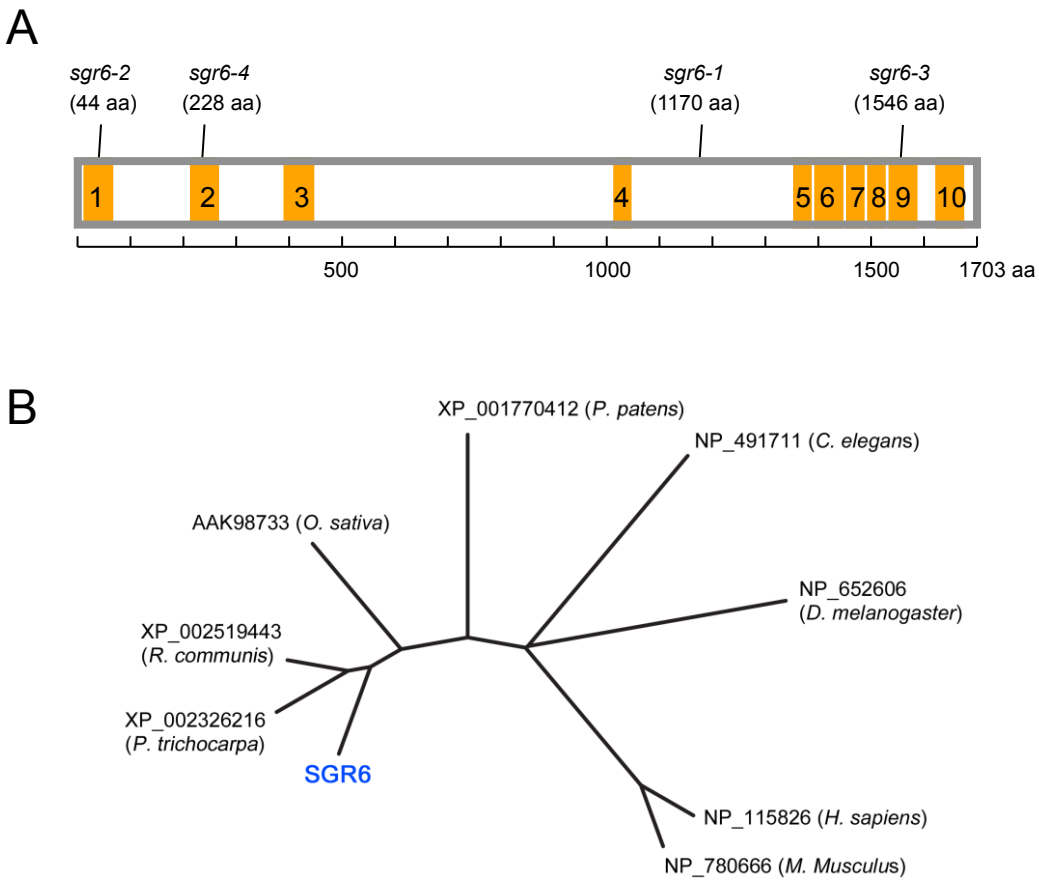
Supplemental Fig. S2 Phenotype of *sgr6* alleles. (A-D) Six-week-old plants of *sgr6-2* (A), L*er* (B), *sgr6-3* (C) and *sgr6-4* (D). Bars = 3 cm (E, F) Gravitropic phenotype of *sgr6* alleles. At least five individuals of each genotype were examined. Bars represent SD.



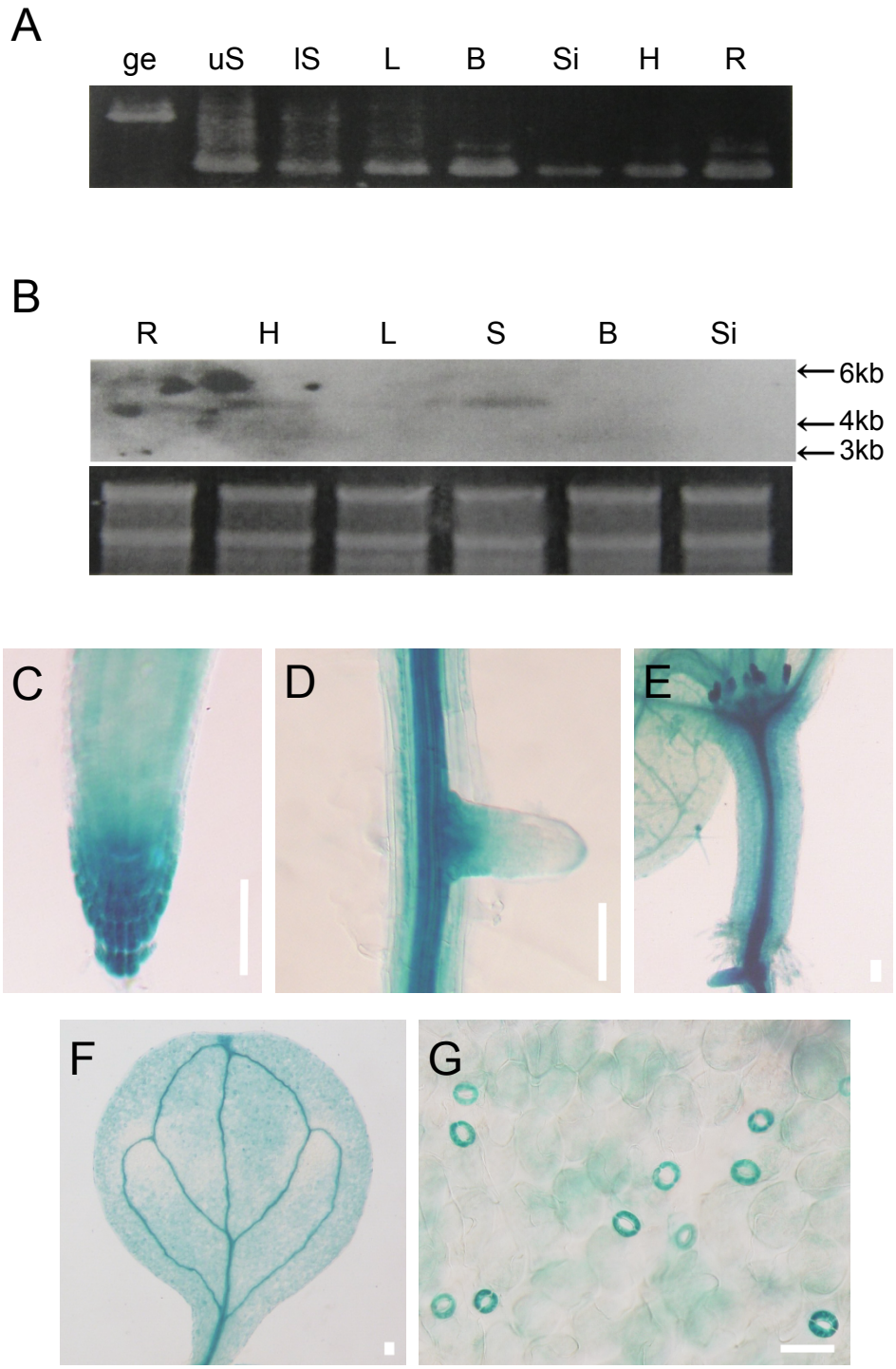
Supplemental Fig. S3 Positions and mutations of *sgr6* alleles. See Fig. 2A for detail of the gene structure of *At2g36810*.



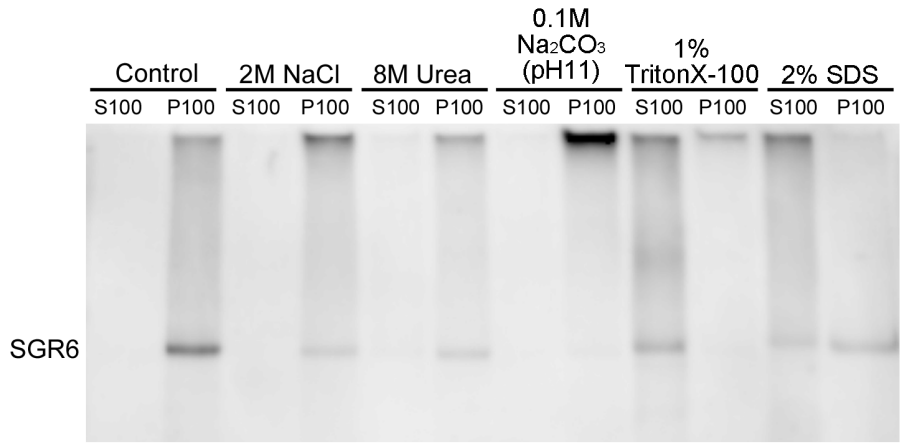
Supplemental Fig. S4 Western blot analysis of SGR6 protein. Proteins were extracted from six-week-old plants of each genotype and subjected to SDS-PAGE, followed by immunoblot analysis using anti-SGR6 antibody. An approximately 190-kDa protein band was detected only in extracts prepared from the wild-type plants (Col-0 and L*er*). Lower panel shows CBB staining to indicate that a similar amount of protein was applied to each lane of the gel.



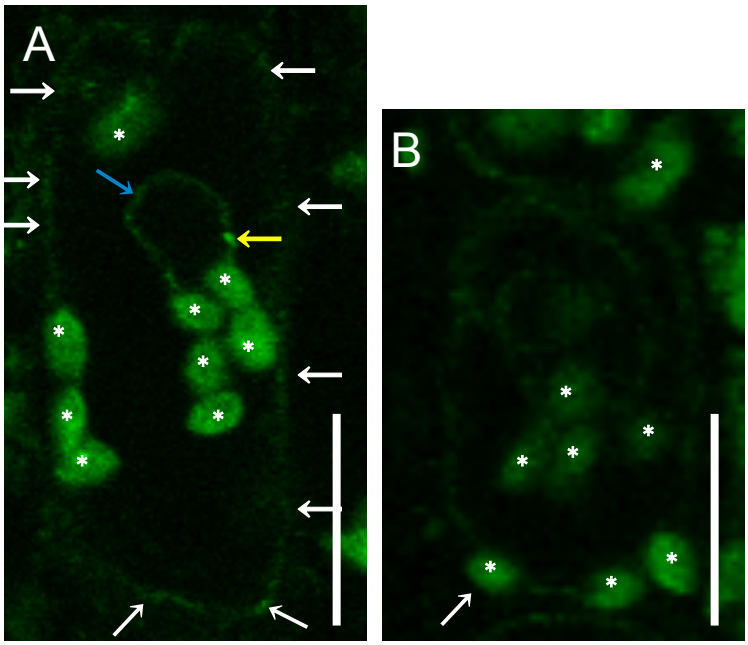
Supplemental Fig. S5 (A) Structure of SGR6 protein. Each orange box indicates a predicted HEAT repeat. Numbers given in parentheses are predicted length of truncated SGR6 result from each *sgr6* alleles. (B) Phylogenetic tree of SGR6 homologs. (C) Structures of SGR6 homologs. Gray lines and orange boxes indicate the full length of homologs and the relative positions of HEAT repeat, respectively.



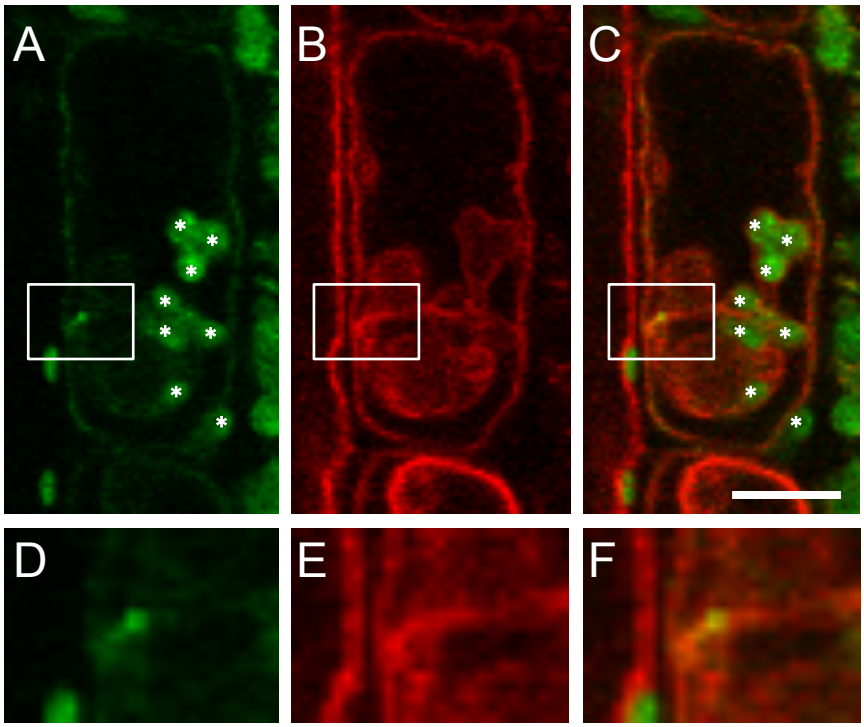
Supplemental Fig. S6 Expression analysis of *SGR6.* (A) RT-PCR analysis. ge, genome; uS, upper stem; lS, lower stem; L, leave; B, bud; Si, silique; H, hypocotyl; R, root. (B) Northern blot analysis. R, root; H, hypocotyl; L, leave; S, stem; B, bud; Si, silique. (C-G) GUS staining of *SGR6pro:GUS*. (C) Root apex, (D) lateral root, (E) hypocotyl, (F) cotyledon, (G) Guard cells in the abaxial side of cotyledon. Bars = 100 μm.



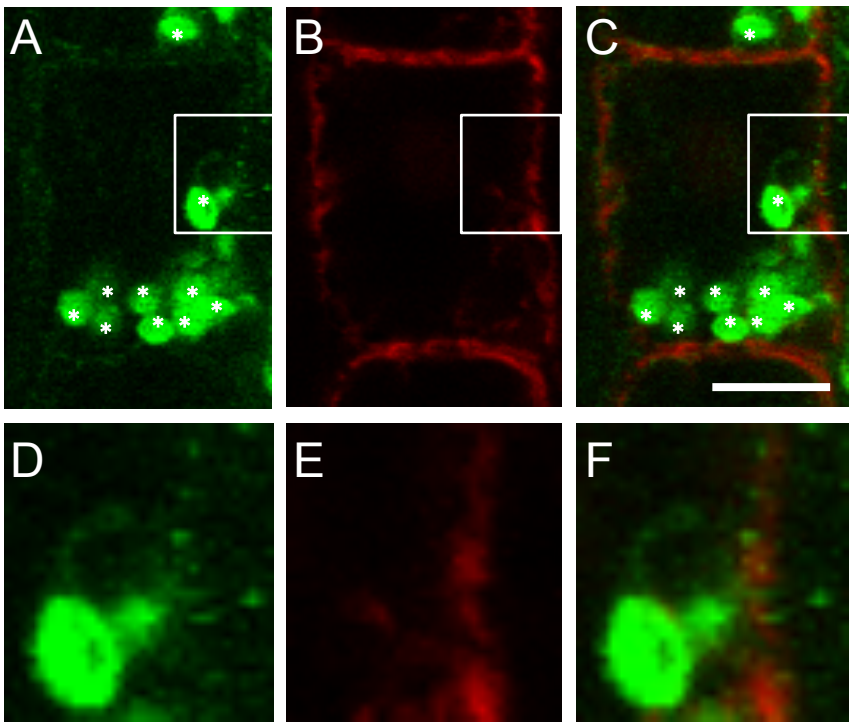
Supplemental Fig. S7 Solubilization of SGR6 protein. Proteins were extracted from six-week-old wild-type stem and ultra-centrifuged. The obtained microsomal pellets were suspended in each solution buffer indicated above and ultra-centrifuged. The S100 concentrated by TCA and P100 were subjected to SDS-PAGE, followed by immunoblot analysis using anti-SGR6 antibody.



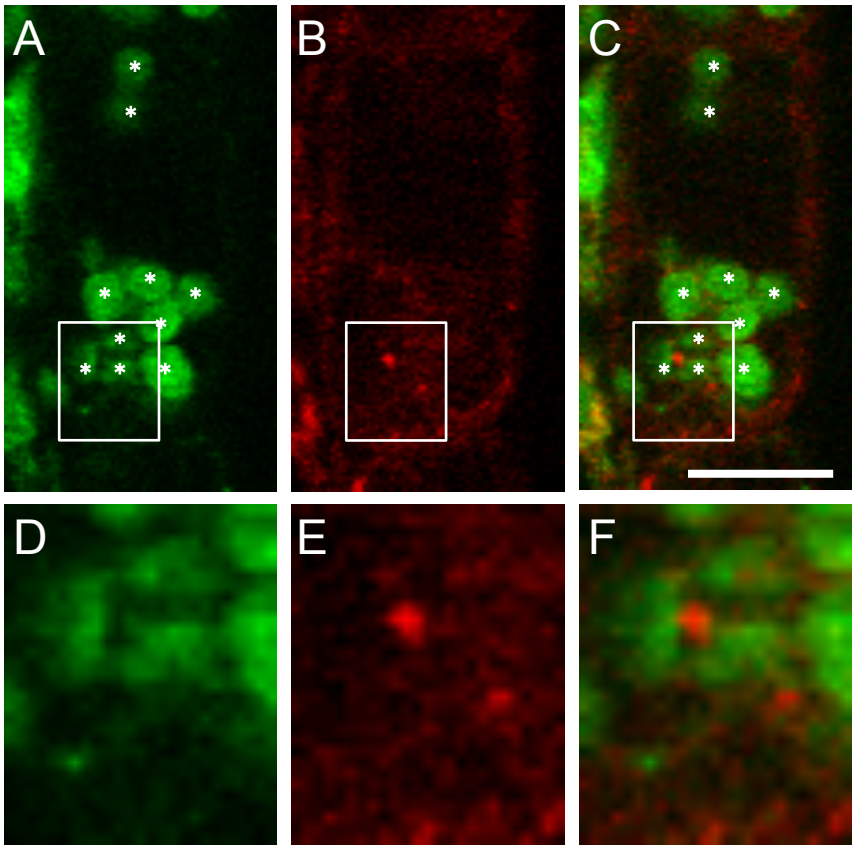
Supplemental Fig. S8 Intracellular localization analysis of SGR6 (1). The endodermal cells in stems of *SGR6pro:GFP-SGR6/sgr6-1* (a line for the complementation test) were observed. Asteriscs indicate the autofluorescence of chlorophyll in amyloplasts. (A) The GFP signals were detected along the periphery of cells (white arrows) and circular forms within the cells (blue arrow). Relative large dot-like signals occasionally emerged during the observation. (B) Autofluorescence of chlorophyll in an amyloplast (white arrow) was detected out side of the peripheral GFP signals. Bars = 10 μm.



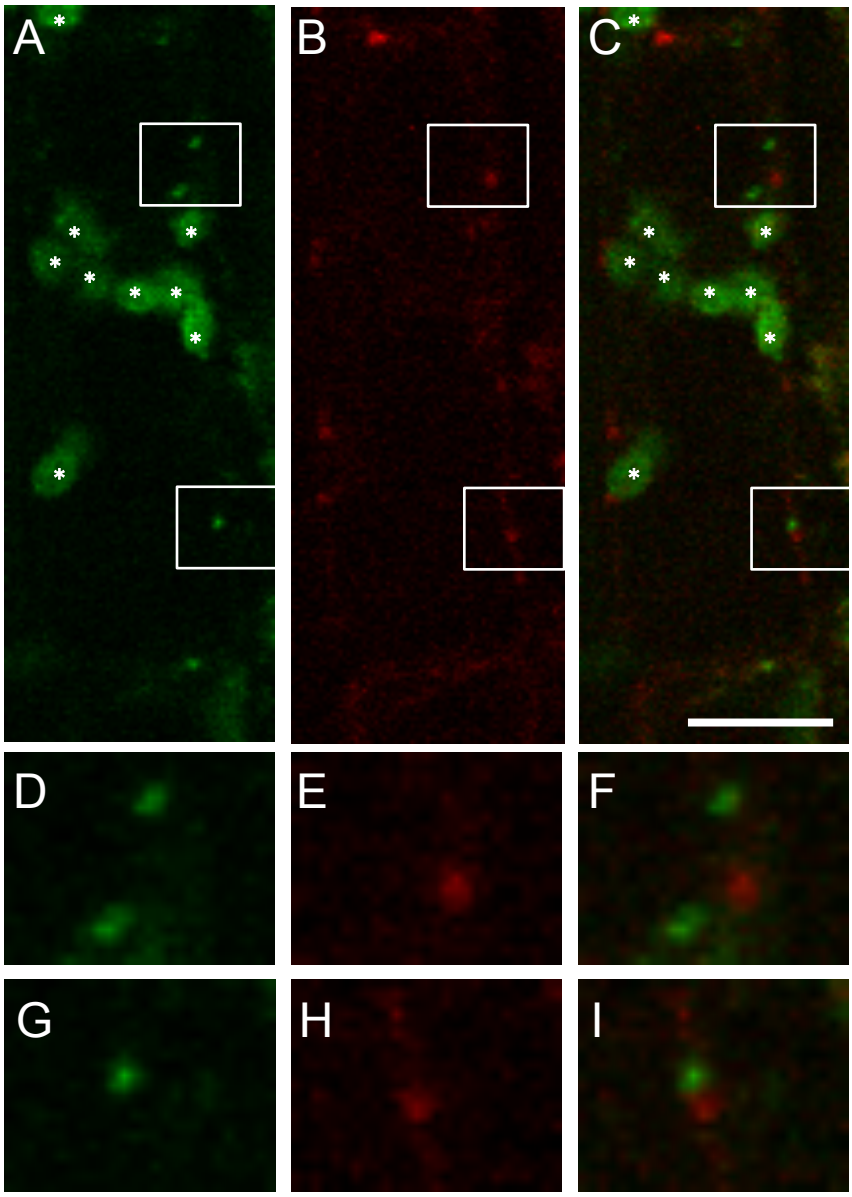
Supplemental Fig. S9 Intracellular localization analysis of SGR6 (2). The endodermal cells in stems of *SGR6pro:GFP-SGR6, VAM3pro:mRFP-VAM3/sgr6-1 vam3-1* were observed. Images 30 sec after those in Fig. 5B are shown. Green, GFP fluorescence; red, RFP fluorescence. Asteriscs indicate the autofluorescence of chlorophyll in amyloplasts. D, E, and F are insets in A, B and C. Bar = 10 μm.



Supplemental Fig. S10 Intracellular localization analysis of SGR6 (3). The endodermal cells in stems of *SGR6pro:GFP-SGR6, SCRpro:mRFP-er/sgr6-1* were observed. Green, GFP fluorescence; red, RFP fluorescence. Asteriscs indicate the autofluorescence of chlorophyll in amyloplasts. D, E, and F are insets in A, B and C. Bar = 10 μm.



Supplemental Fig. S11 Intracellular localization analysis of SGR6 (4). The endodermal cells in stems of *SGR6pro:GFP-SGR6, SCRpro:ARA6-mRFP/sgr6-1* were observed. Green, GFP fluorescence; red, RFP fluorescence. Asteriscs indicate the autofluorescence of chlorophyll in amyloplasts. D, E, and F are insets in A, B and C. Bar = 10 μm.



Supplemental Fig. S12 Intracellular localization analysis of SGR6 (5). The endodermal cells in stems of *SGR6pro:GFP-SGR6, SCRpro:mRFP-ARA7/sgr6-1* were observed. Green, GFP fluorescence; red, RFP fluorescence. Asteriscs indicate the autofluorescence of chlorophyll in amyloplasts. D, E, and F are upper insets in A, B and C. G, H, and I are lower insets in A, B and C. Bar = 10 μm.

Supplemental Table Primer sequences

Primer name	Sequence
SGR6pro_F	AAGCTTTTTGAACCAAGAAAGAATTCACATCAC
SGR6pro_R	AAGCTTTTCAAAAACCGATCAACTATTTCAAAAACCC
cSGR6_F	GCTCTAGAATGGCTTCTTCAAGTTTAGGGAGC
cSGR6_R	TCCCCCGGGTGGAATTCACACAGACCGTAATGCACATG
promoter SGR6 F	GGGGTCGACCGCCCAATCTATTAACCC
promoter SGR6 R	GGGGGATCCGGAAGCTTCTCTAACCACAGAGG
SGR6-middle F	GGGAATTCGAATCATTGGATGTCACCCAGG
SGR6-middle R	GGGAATTCTCTCCCTTATGTGAATCAC