Supplemental Data

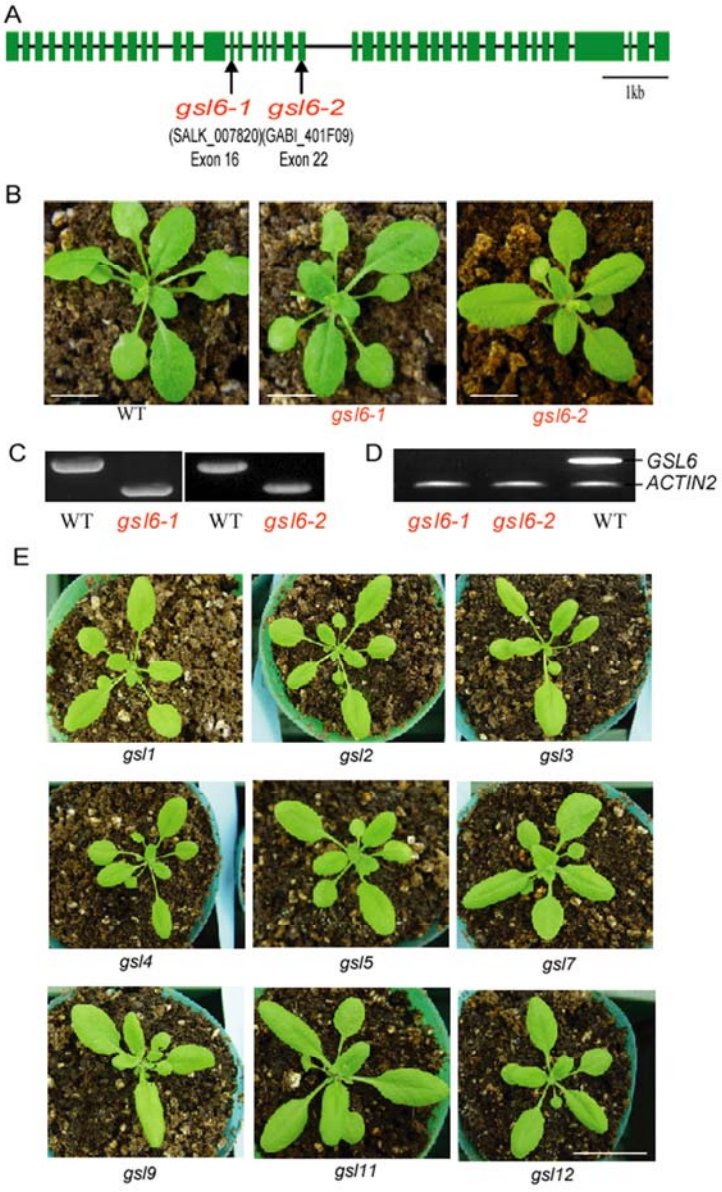

Figure S1. Loss-of-function mutations in the *GSL* gene family. (A to D) Isolation of knock-out mutants for *GSL6*. (A) Schematic representation of T-DNA insertions in the GSL6 gene. (B) The *gsl6* seedling mutant phenotypes resemble the wild-type in vegetative development. (C) Genotyping PCR results of *GSL6* T-DNA alleles. (D) RT-PCR analysis showing likely null mutations. Total RNA was extracted from 20-day-old wild-type and mutant plants. (E) Developmental phenotypes at rosette leaf stage in *gsl* mutants. Not included: *gsl6*, *gsl8*, and *gsl10*. Scale bars = 1 cm.

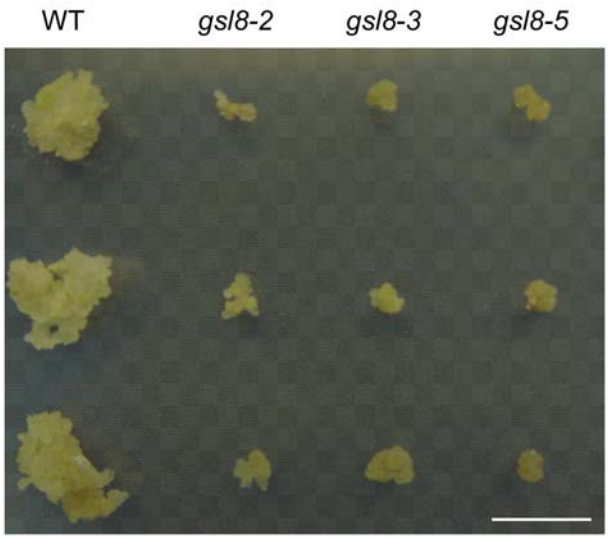

Figure S2. Calli induction from *gsl8* mutant seedlings. Three replicates are shown. Cotyledons from 7-day old WT, *gsl8-2, gsl8-3 and gsl8-5* mutant seedlings were grown on calli-inducing medium for three weeks. Scale bar = 1 cm.

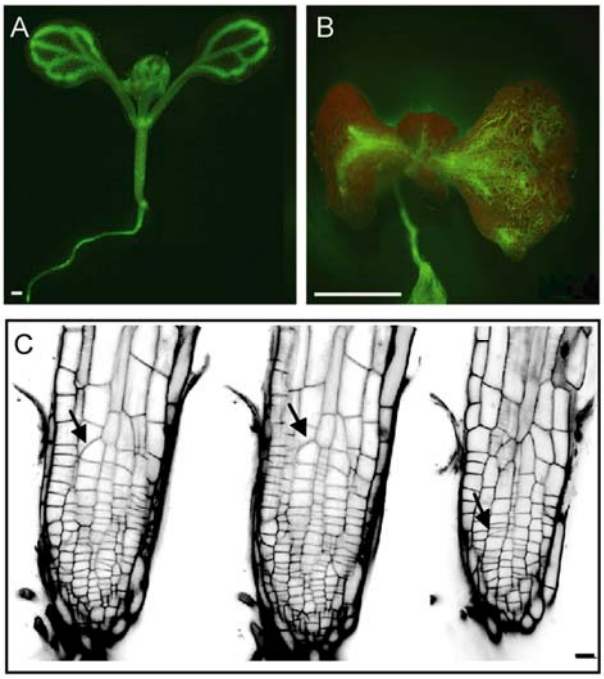

Figure S3. Patterning phenotypes of *gsl8* mutants. (A and B) Comparison of vascular patterns in wild-type (A) and *gsl8-4* (B) cotyledons after the incubation of root tips in the membrane-impermeable HPTS dye (8-hydroxypyrene-1,3,6-trisulphonic acid). Root tips from wild-type and homozygous *gsl8-4* mutant seedlings were cut to allow dye penetration, and a 1.0-2.0 µl drop of HPTS-acetate (5 mg/ml) was applied. The seedlings were imaged with a fluorescent dissection microscope (SZX12, Olympus) after translocation had proceeded for 30-60 min. Some veins in *gsl8-4* appeared to end abruptly. Other five *gsl8* mutant alleles showed similar results to those of *gsl8-4*. (C) Propidium iodide stained root tips (*gsl8-4*). All PI images have inverted grayscales. Arrows indicate aberrant division planes. Scale bars: 1 mm in (A, B), 10 µm in (C).

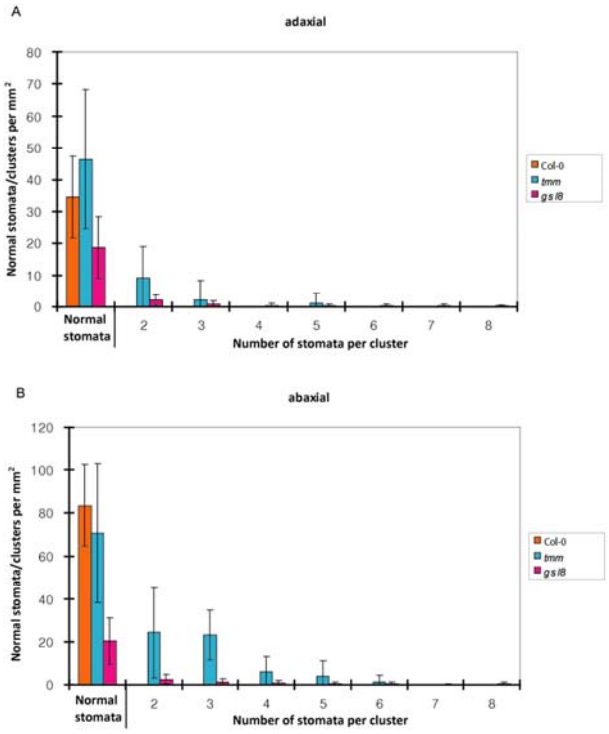

Figure S4. Distribution of normal stomata and of clustered stomata in the epidermis of cotyledons in wild-type, *tmm*, and *gsl8-4*. (A) Adaxial epidmermis. **(**B) Abaxial epidermis. Bars are SD. The stomatal density and stomatal index of 10-day-old cotyledons of *gsl8-4* and *tmm* mutants (a representative mutant of stomatal clustering) were measured using light microscopy. The area of the microscope field was calculated using the ImageJ program. The number of fields and plants scored were 78 and 25 for *gsl8*, 20 and 15 for *tmm*, and 10 and 3 for Col-0.

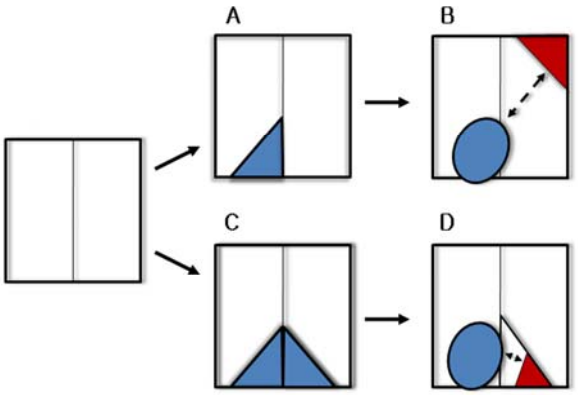

Figure S5. Primary and secondary stomatal patterning mechanisms in wild-type Arabidopsis. Both mechanisms involve oriented asymmetric divisions. Stomatal precursor cells are shown as triangles (meristemoids) and ovals (guard mother cells, GMCs). Meristemoids develop into GMCs, but often first divide asymmetrically (amplifying divisions). (A and B) Primary mechanism: Intercellular signaling (double-headed arrow) might orient the new asymmetric division in a neighbor cell (cell at right) away from a previously formed meristemoid (now the blue oval GMC at left). (C and D) Secondary mechanism: simultaneous entry divisions in both cells can cause meristemoids to form in contact (C). Intercellular signaling (double headed arrow) might orient the asymmetric division of one meristemoid so that the regenerated meristemoid (red) no longer contacts the other precursor cell, here the GMC at the left in (D).

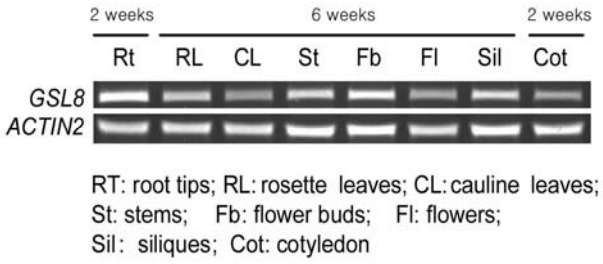

Figure S6. *GSL8* is expressed in all organs tested using RT-PCR. Organs included were from 2-week-old or 6 week-old seedlings grown on MS medium and included root tips (Rt), cotyledons (Cot), rosette leaves (RL), cauline leaves (CL), stems (St), floral buds (Fb), flowers (Fl), and siliques (Sil) .

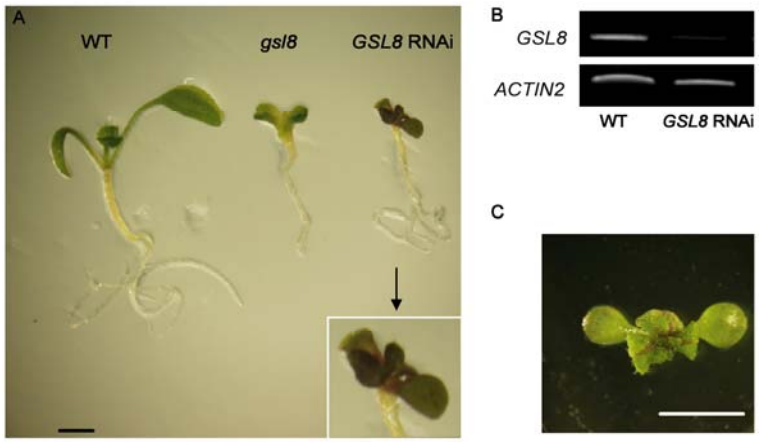

Figure S7. Dexamethasone-inducible RNAi line of *GSL8* phenocopies T-DNA insertional mutants. (A) 10-day-old seedlings of wild-type, *gsl8-4* and RNAi line. (B) *GSL8* expression is reduced in an RNAi background. ACTIN2 was used as an internal control. (C) 3-week-old *GSL8* RNAi seedling. Dexamethasone was treated at 4 days after germination.

Supplemental Tables

Table S1. Identity of T-DNA insertion lines in *GSL* family genes (excluding *GSL8* and *GSL10*).

GSL	Cals	AGI code	Mutant ID	Background
GSL1	*CalS11*	At4g04970	GABI_236G11	Col
GSL2-2	*CalS5-2*	At2g13680	SALK_026354 (1)	Col
GSL3	*CalS2*	At2g31960	SALK_011560	Col
GSL4	*CalS8*	At3g14570	SALK_047978	Col
GSL5	*CalS12*	At4g03550	GABI_089H05 (2)	Col
GSL6-1	*CalS1-1*	At1g05570	SALK_007820	Col
GSL6-2	*CalS1-2*	At1g05570	GABI_401F09	Col
GSL7	*CalS7*	At1g06490	SALK_048921	Col
GSL9	*CalS4*	At5g36870	SALK_009569	Col
GSL11	*CalS6*	At3g59100	SALK_019534	Col
GSL12	*CalS3*	At5g13000	SALK_003469	Col

Table S2. Segregation analysis of *GSL8/gsl8.*

	No Sucrose				3% Sucrose			
Line	Total	Germinated	Homo	Homo%	Total	Germinated	Homo	Homo%
gsl8-2/+	500	458	11	2.3	427	420	46	9.9
gsl8-3/+	nt	nt	nt	nt	200	200	36	15.3
gsl8-5/+	500	463	13	2.7	400	382	36	8.6
Total	1000	921	24	2.5	1027	1002	118	10.5

nt: non-tested

Table S3. Primers used to isolate homozygous *gsl* family mutants.

Primer name	Primer sequence
GSL1 LP	5'- GTATACCTCTAGTGACGGCGC -3'
GSL1 RP	5'-TCCTAATTGTCACGAGTTCCAG -3'
GSL2-2 LP	5'- ACCTCTCCCAAACGATCAAAG -3'
GSL2-2 RP	5'- TGGATCGAGAATTCAGACTGG -3'
GSL3 LP	5'- GTGCCTCGCTGTAGATTATCG -3'
GSL3 RP	5'- ACAGCAGCAGTTTGATATGCC -3'
GSL4 LP	5'- ACTCCATGCAATTCATATGCC -3'
GSL4 RP	5'- CTTCCTCTCGATAAAGGAGGG -3'
GSL5 LP	5'- GGAAATCCCATCCTCAGTCTC -3'
GSL5 RP	5'- TTTGTTAACGCAGATGCTGC -3'
GSL6-1 LP	5'- CTGTTCTTGCAAAACCAATGG -3'
GSL6-1 RP	5'- TCCTCCTCCCTGTTTTCTAGC -3'
GSL6-2 LP	5'- ATCAAACAGTGGAGATCGGTG -3'
GSL6-2 RP	5'- ACAAAGCGCTCTCATGCATAC -3'
GSL7 LP	5'- CGATAGAGGTTCCAAAACGTG -3'
GSL7 RP	5'- ATGGATGGTTTTCCTATTGGC -3'
GSL9 LP	5'- GACAATAGCCGAGGTTGTTTG -3'
GSL9 RP	5'- ATATAAGCCCGCGGTATACCG -3'
GSL11 LP	5'- TATTTGACACATGCCTGCTTG -3'
GSL11 RP	5'- CAATCCCCAGTCCAAGTACTG -3'
GSL12 LP	5'- GGGCAATCTCAACAAGAGATG -3'
GSL12 RP	5'- CTTTGATAAAATGGTTGGCCAG -3'

Table S4. Primers used to isolate homozygous gsl8 mutants.

Primer name	Primer sequence
GSL8-1 LP	5'-TAGTTCCGCAGACAAAGTTGC-3'
GSL8-1 RP	5'-TCACATGCATATAGCTGTGGG-3'
GSL8-2 LP	5'-TTCAGAAGTTGCATCTGCATG-3'
GSL8-2 RP	5'-ACACTCTGGAAGAAAGCGGAC-3'
GSL8-3 LP	5'-CTTATGTATGGCGCCTACAGC-3'
GSL8-3 RP	5'-CACCACAGGTGAATTCAGATATG-3'
GSL8-4 LP	5'-GCATCACACCAGCCTAAAATC-3'
GSL8-4 RP	5'-ATATGCTGCGATGTTTTCACC-3'
GSL8-5 LP	5'-CCCAAGCCTCGTCTTTCTAAG-3'
GSL8-5 RP	5'-GGTTTACTTGCCCTCACAAATC-3'
GSL8-6 LP	5'-GGGACTGTGCATTTGAAAGTG-3'
GSL8-6 RP	5'-TTTCATTCGGCAGTGATTTTC-3'

T-DNA (LB1) for SALK mutants:	5'-TGGTTCACGTAGTGGGCCATCG-3'
T-DNA (LB1) for GABI mutants:	5'-CCCATTTGGACGTGAATGTAGACAC-3'
T-DNA (LB3) for SAIL mutants	5'-TAGCATCTGAATTTCATAACCAATCTCGATACAC-3'

Supplemental References

(1) Dong, X., Hong, Z., Sivaramakrishnan, M., Mahfouz, M. and Verma, D.P.S. (2005) Callose synthase (CalS5) is required for exine formation during microgametogenesis and for pollen viability in Arabidopsis. *Plant J.* **42,** 315-328.

(2) Jacobs, A.K., Lipka, V., Burton, R.A., Panstruga, R., Strizhov, N., Schulze-Lefert, P. and Fincher, G.B.J. (2003) An Arabidopsis Callose Synthase, GSL5, Is Required for

Wound and Papillary Callose Formation. *Plant Cell,* **15,** 2503-2513