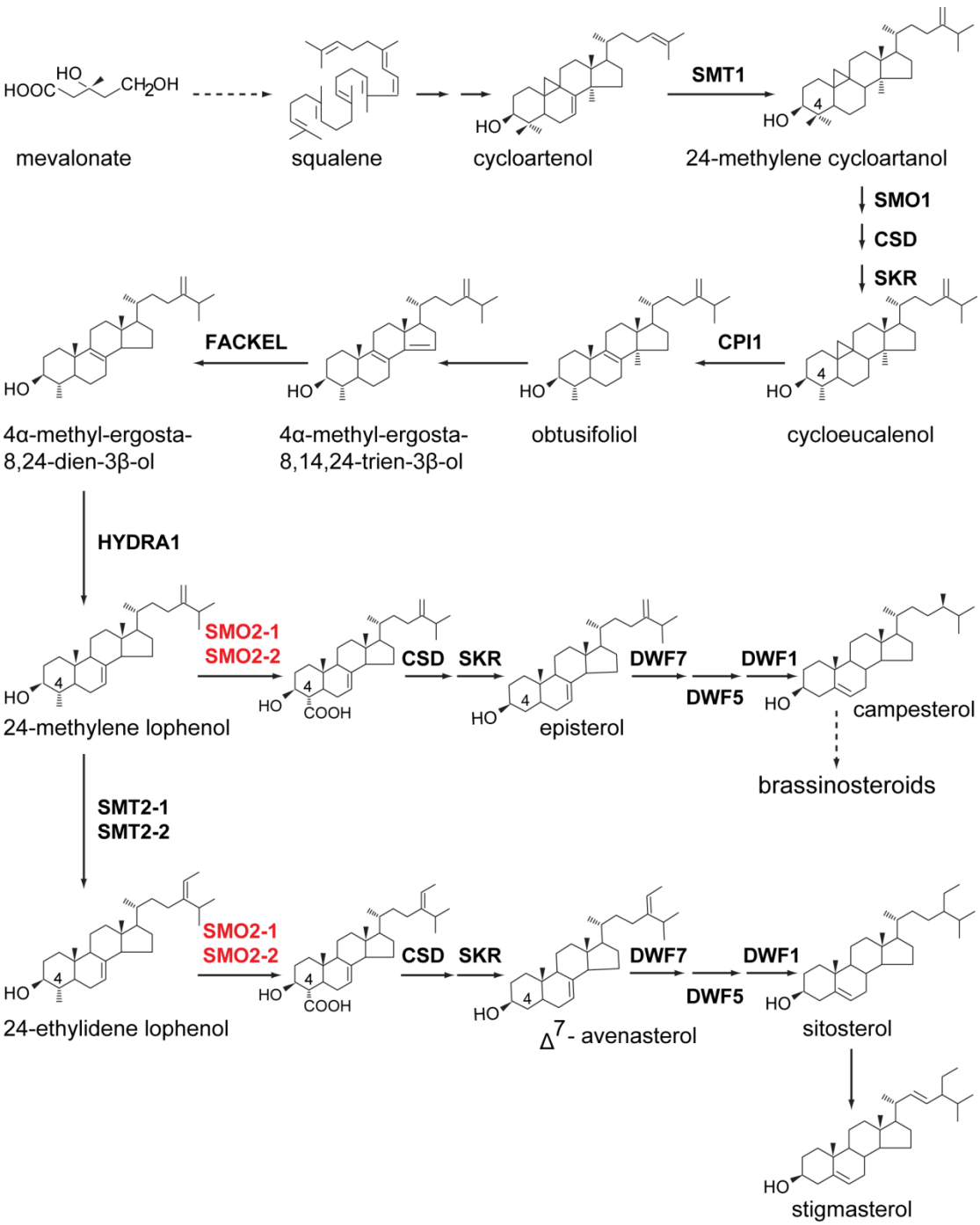

Supplemental Figure S1. Sterol biosynthetic pathway in higher plants. Arrows with dashed lines represent several biosynthetic steps. Abbreviations: SMT, sterol methyltransferase; SMO, sterol 4α-methyl oxidase; CPI1, cyclopropylsterol isomerase1; CSD, 4α-carboxysterol-C-3-dehydrogenase/C-4-decarboxylase; SKR, sterone ketoreductase; FACKEL, $\Delta^{8,14}$-sterol C-14-reductase; HYDRA1, Δ^{8}-sterol 8,7-isomerase; DWF7, Δ^{7}-sterol C-5 desaturase; DWF5, Δ^{7}-sterol C-7 reductase; DWF1, C-24 reductase.

A

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SMO2-1  MASFVESGWQYLVTHFSDFQLACIGSFLLHESVFFLSGLPFIFLERQGFLSKYKIQTKNN 60
SMO2-2  MDSLVESGWKYLVTHFSDFQLACIGSFILHESVFFLSGLPYIFLERTGFLSNYKIQTKSN 60
        * *:*****:*****************:************:***** ****:******.*
SMO2-1  TPAAQGKCITRLLLYHFSVNLPLMLASYPVFRAMGMRSSFPLPSWKEVSAQILFYFIIED 120
SMO2-2  TPEAQGKCIARLLLYHCCVNLPLMMASYPVFRFMGMESSFPLPSWKVVSAQILFYFIIED 120
        ** ******:****** .******:******* ***.********* **************
SMO2-1  FVFYWGHRILHSKWLYKNVHSVHHEYATPFGLTSEYAHPAEILFLGFATIVGPALTGPHL 180
SMO2-2  FVFYWGHRILHTKWLYKNVHSVHHEYATPFGLTSEYAHPAEILFLGFATIVGPALTGPHL 180
        ************:************************************************
SMO2-1  ITLWLWMVLRVLETVEAHCGYHFPWSLSNFLPLYGG----------ADFHDYHHRLLY 228
SMO2-2  ITLWLWMMLRVIETVEAHCGYHFPWSPSNFLPLYGGSLILMWESFAYSADFHDYHHRLLY 240
        *******:***:*************** *********          ************
SMO2-1  TKSGNYSSTFVYMDWIFGTDKGYRRLKTLKENGDMKQT 266
SMO2-2  TKSGNYSSTFVYMDWIFGTDKGYRKLKALKET------ 272
        *************************:**:***.
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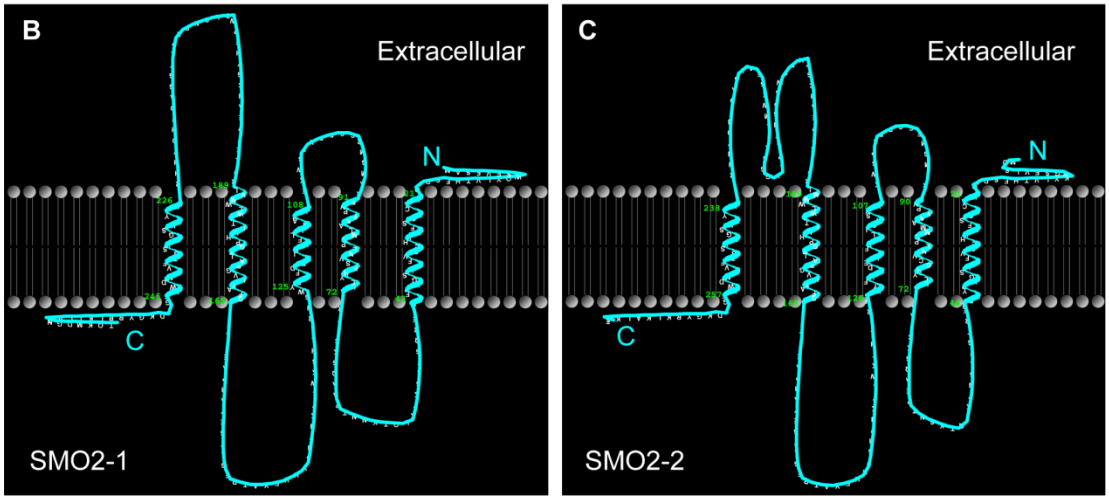
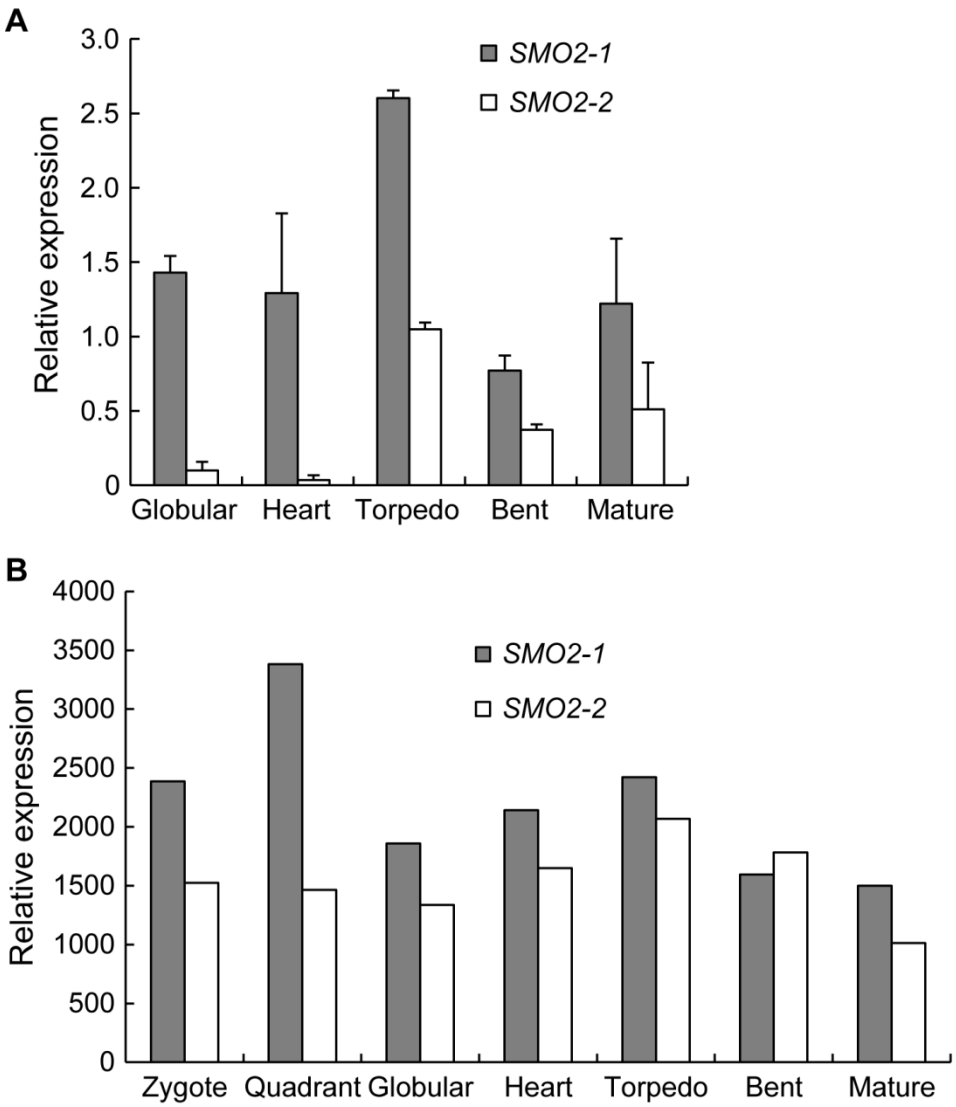
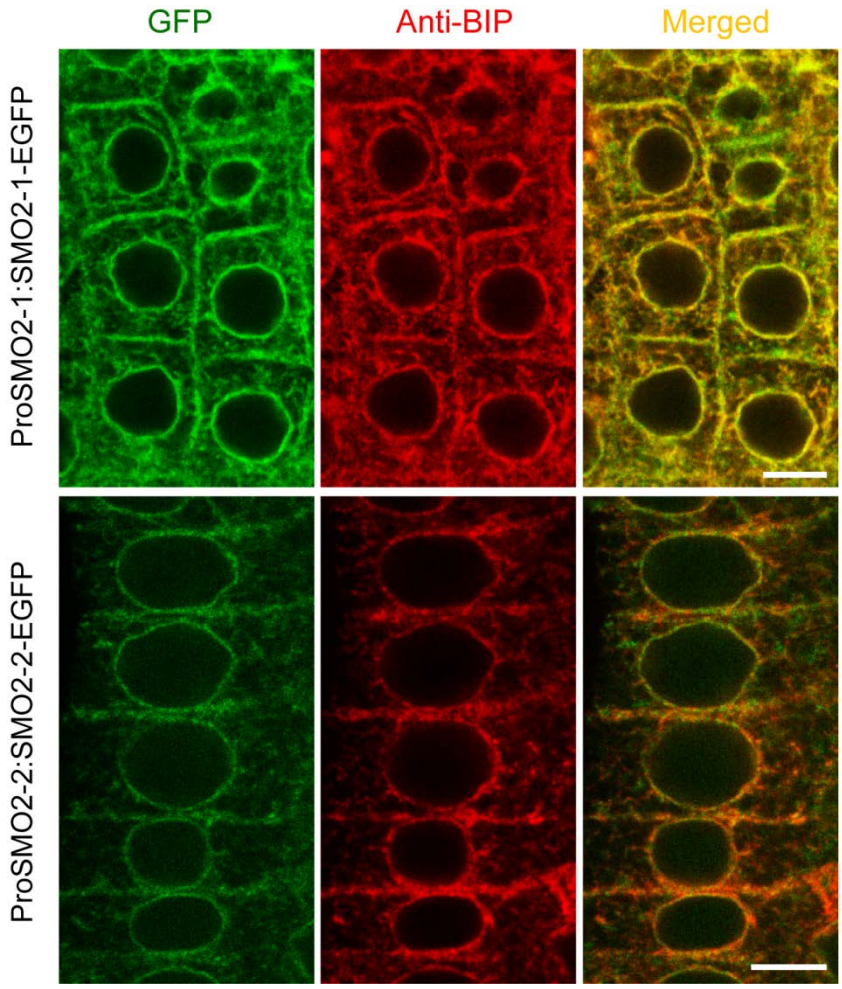


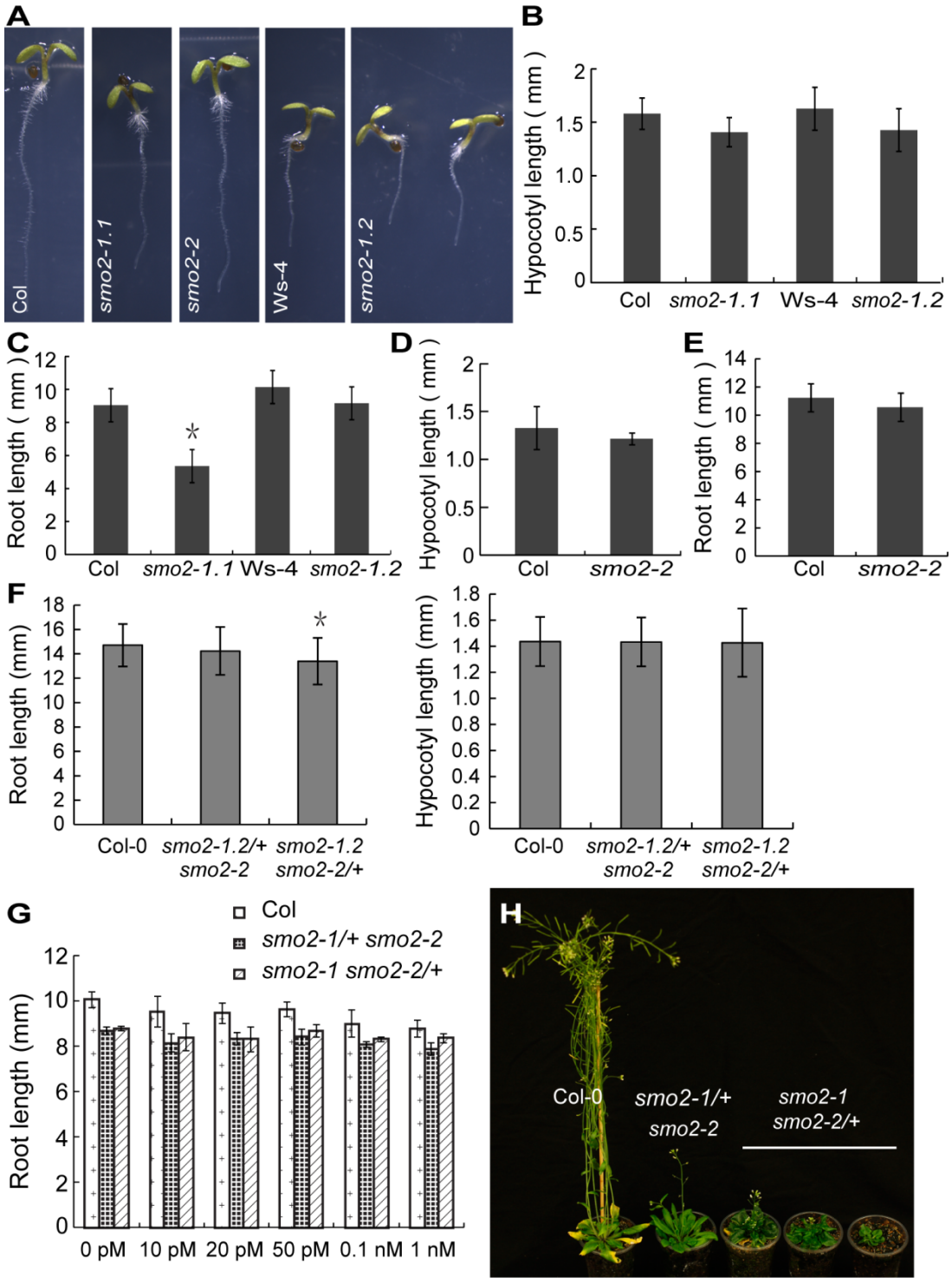
Supplemental Figure S2. Sequence alignment and protein topology of SMO2 proteins. A, Amino acid sequence alignment of the SMO2-1 and SMO2-2 proteins. The alignment was performed online at http://www.ebi.ac.uk/Tools/msa/clustalw2/. B and C, Predicted topology of the Arabidopsis SMO2-1 and SMO2-2 proteins. The prediction was performed using HMMTOP 2.0 and visualized by TMRPres2D [Feraru E, Vosolsobě S, Feraru MI, Petrášek J, Kleine-Vehn J (2012) Evolution and structural diversification of PILS putative auxin carriers in plants. Front Plant Sci 3: 227].



Supplemental Figure S3. Relative transcript levels of the *SMO2-1* and *SMO2-2* genes in developing Arabidopsis seeds and embryos. A. Relative transcript levels of the *SMO2-1* and *SMO2-2* genes in developing seeds. Arabidopsis seeds containing embryos at the globular, heart, torpedo, bent and mature stages were used for total RNA extraction. Transcript levels were determined by qRT-PCR. The average results of three experiments are shown. B. Relative transcript levels of the *SMO2-1* and *SMO2-2* genes in developing embryos. Gene expression data were downloaded from the "Gene Expression Map of *Arabidopsis* Embryo Development" database (http://www2.bri.nrc.ca/plantembryo). A relative expression value less than 777.6 indicates expression at very low levels or represents background; the data represent the normalized values of four replicates.

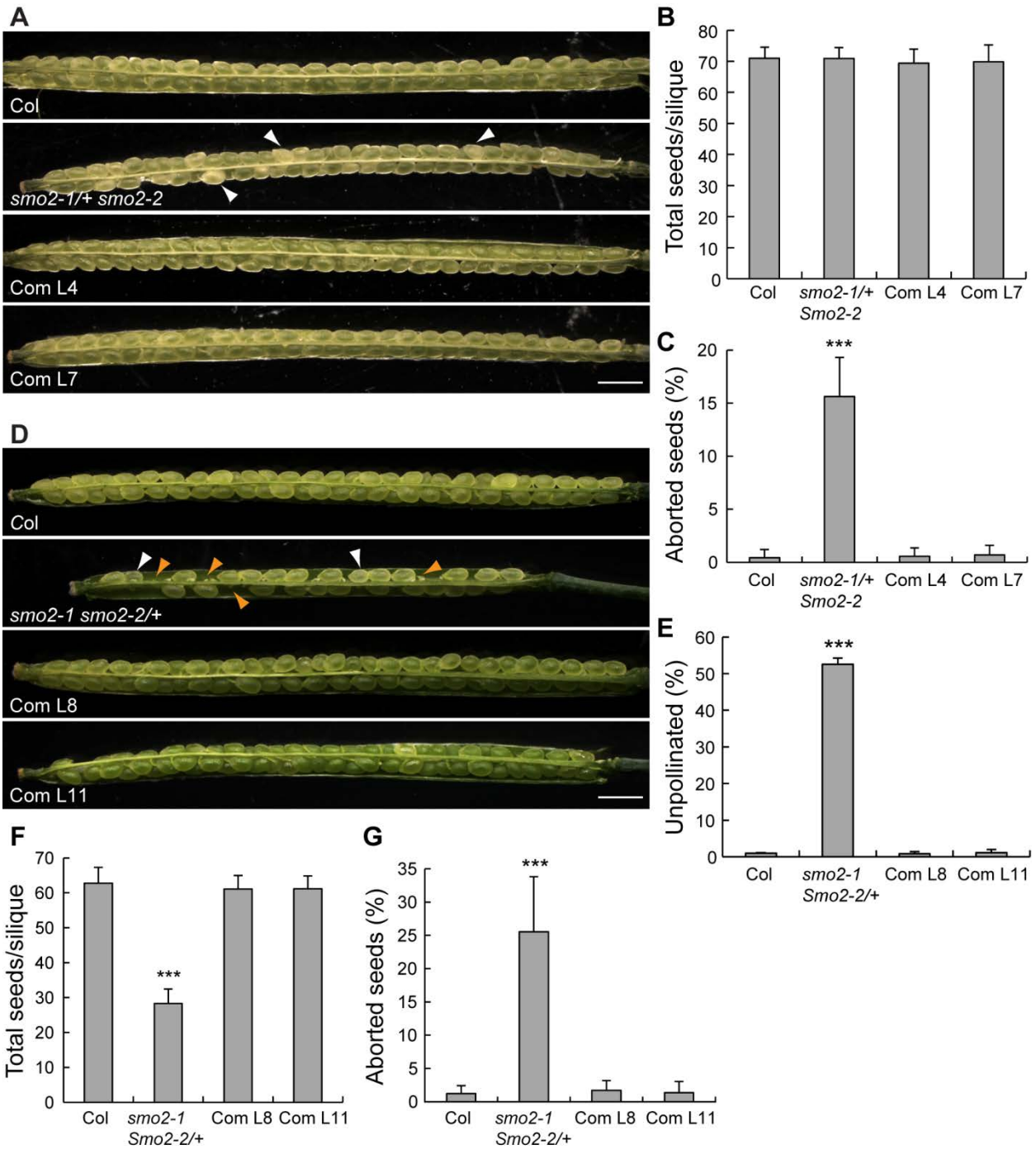


Supplemental Figure S4. ER localization of the SMO2-1 and SMO2-2 proteins. The *ProSMO2-1:SMO2-1-EGFP* and *ProSMO2-2:SMO2-2-EGFP* constructs were transformed into wild-type plants, and immunolocalization was performed on roots of transgenic plants using an anti-BiP (an ER-intrinsic chaperone used as a positive ER marker) antibody. Root cells were viewed under confocal microscopy. Bars = 10 μm.

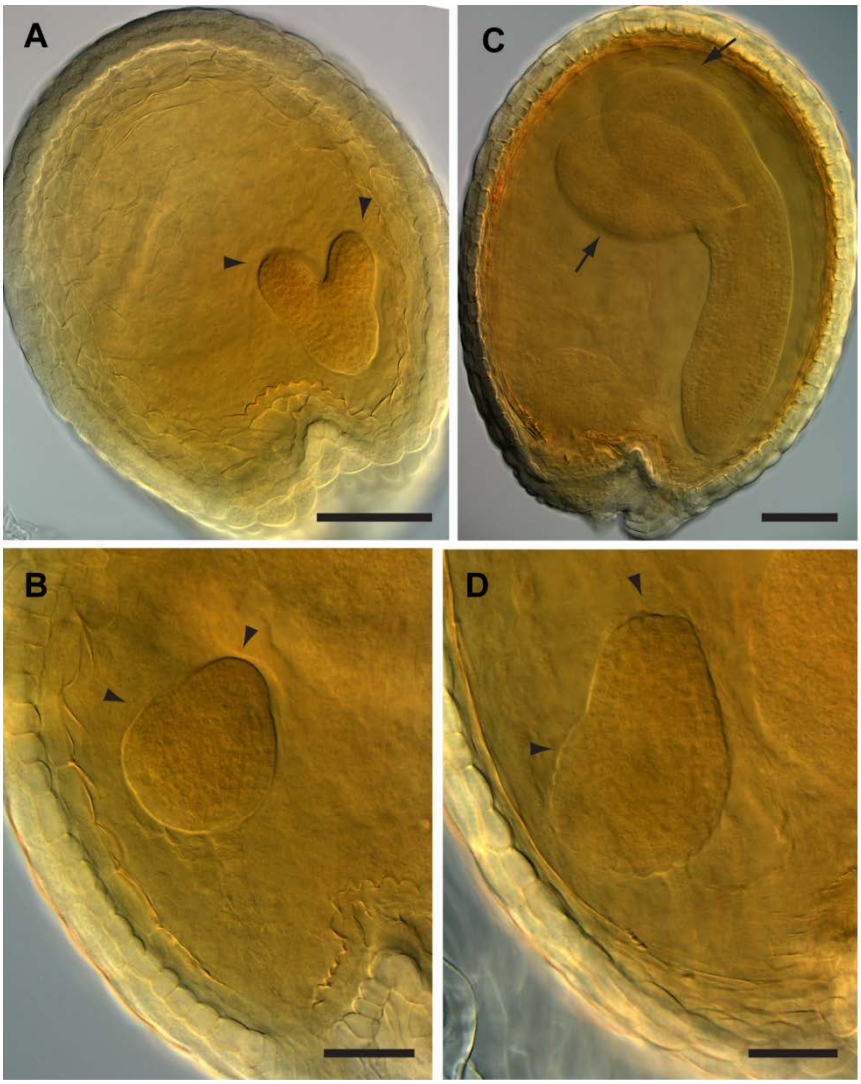


Supplemental Figure S5. Phenotypes of *smo2* mutants. A, Five-day-old seedling phenotypes of *smo2-1* and *smo2-2* single mutants grown under 16 h-light and 8 h-dark growth conditions. B to E, Hypocotyl and root lengths of 5-day-old wild-type (Col and Ws-4), *smo2-1*, and *smo2-2* seedlings. The data from three independent experiments are presented as the means ± SD (n = 20 for each experiment). Asterisk indicates a P value < 0.05 compared with wild type. F, Hypocotyl and root lengths of 5-day-old wild-type (Col), *smo2-1.2/+ smo2-2*, and *smo2-1.2 smo2-2/+* seedlings. N = 21. Asterisk indicates

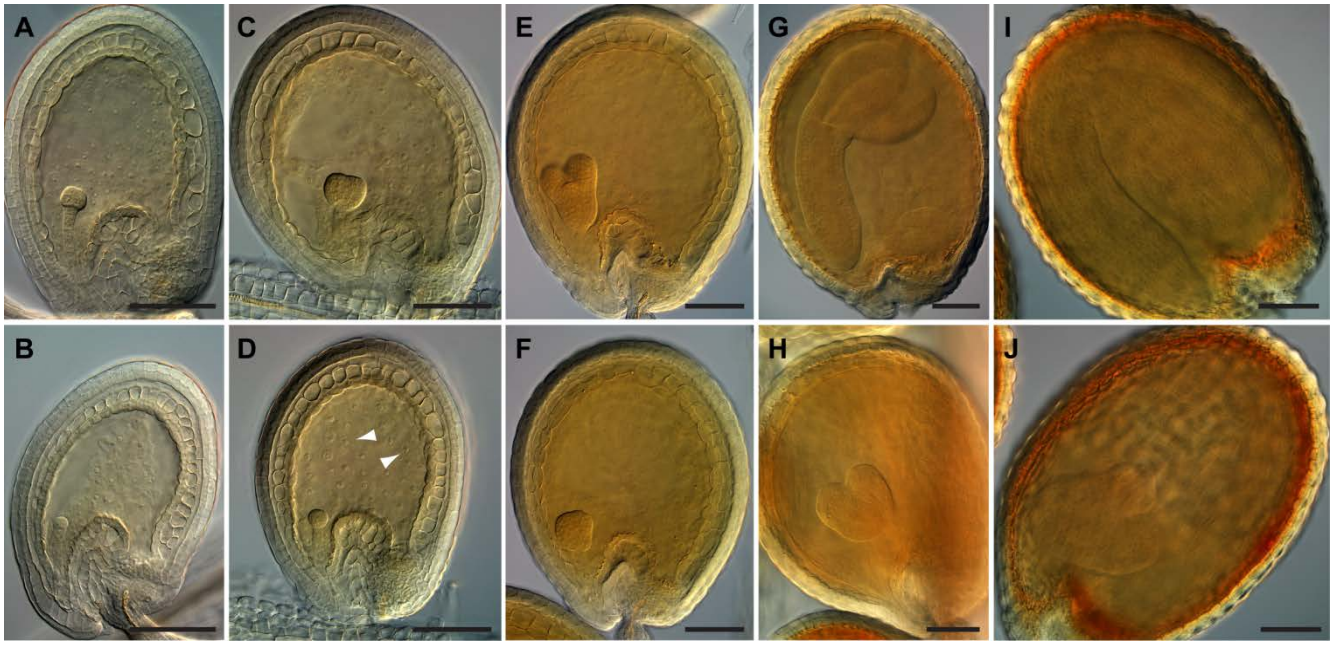
a P value < 0.05 compared with wild type. G, Root lengths of wild-type, *smo2-1/+ smo2-2* and *smo2-1 smo2-2/+* seedlings grown on MS plates with the addition of 0 pM, 10 pM, 20 pM, 50 pM, 0.1 nM and 1 nM 24-epibrassinolide (BL) for 5 days. The data from three independent experiments are presented as the means ± SD (n = 10 to 27 for each experiment). H, Phenotypes of 8-week-old plants. Note the phenotypic variability within the *smo2-1 smo2-2/+* mutant plants.



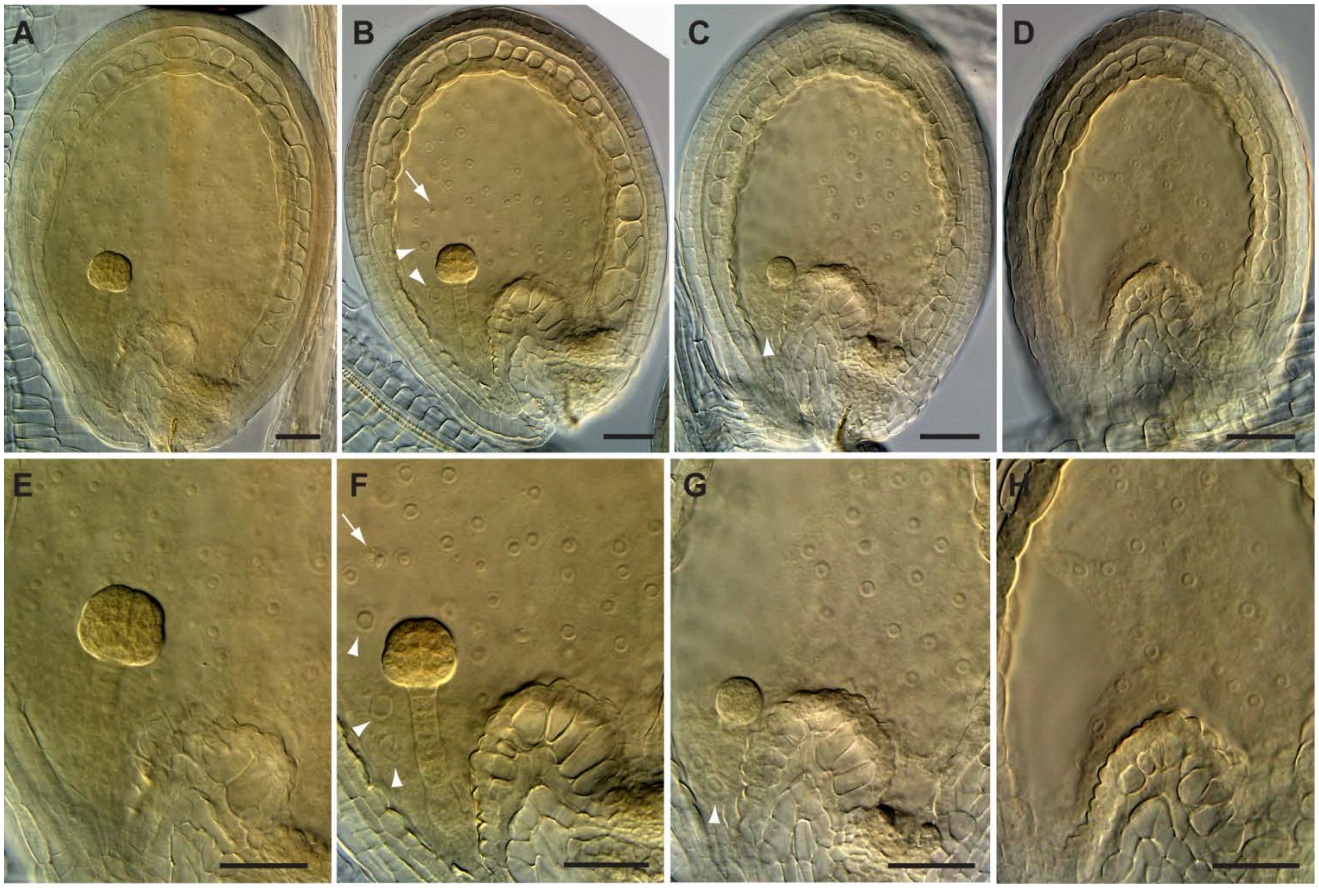
Supplemental Figure S6. *smo2-1/+ smo2-2* and *smo2-1 smo2-2/+* complementation experiments. A, Siliques 6-8 days after pollination from wild-type, *smo2-1/+ smo2-2*, and *ProSMO2-2:SMO2-2-EGFP* transgenic lines 4 (Com L4) and 7 (Com L7) in a *smo2-1 smo2-2* homozygous background. B and C, Quantification of total and aborted seeds in siliques from wild-type, *smo2-1/+ smo2-2*, and complemented lines 4 and 7. D, Siliques from wild-type, *smo2-1 smo2-2/+*, and *ProSMO2-1:SMO2-1-EGFP* transgenic lines 8 (Com L8) and 11 (Com L11) in a *smo2-1 smo2-2* homozygous background. E to G, Quantification of unpollinated ovules and total and aborted seeds in siliques from wild-type, *smo2-1 smo2-2/+*, and complemented lines 8 and 11 The data were derived from three experiments and are presented as the means ± SD. For each experiment, 10 siliques from 5 plants were examined. Significant differences were analyzed using Student's t-test (*** $P < 0.001$). White arrowheads indicate aborted seeds, and orange arrowheads indicate undeveloped ovules. Bars = 1 mm.



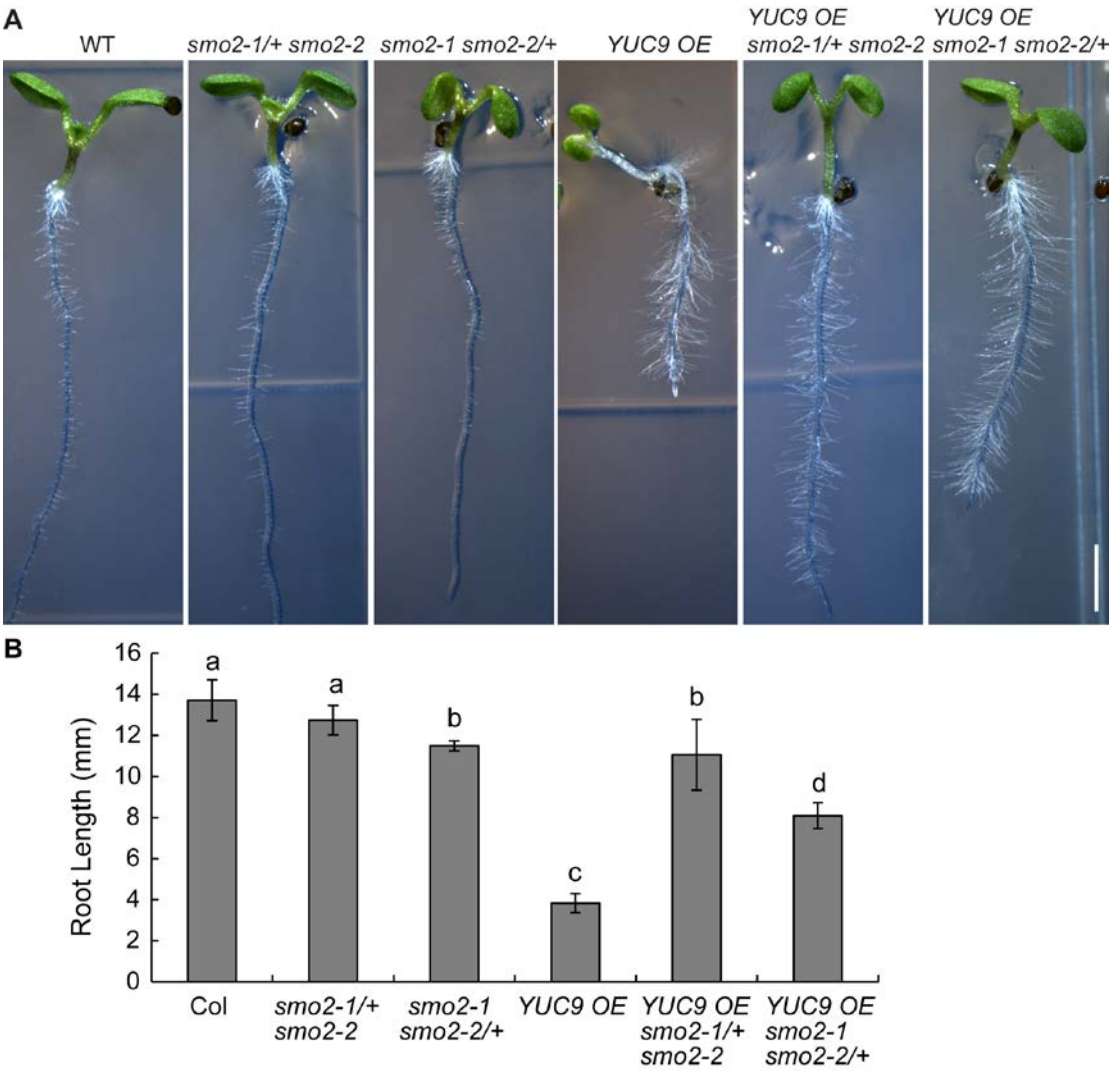
Supplemental Figure S7. Embryos of *smo2-1 smo2-2/+* plants have different cotyledon sizes. A, Seed with an embryo at the heart stage from the same silique as the seed shown in (B). B, Delayed double-mutant embryo at the early heart stage. Note that one cotyledon primordium is larger than the other. C, Seed with an embryo at the walking stick stage from the same silique as the seed shown in (D). D, Delayed double-mutant embryo at the heart-like stage. Note that one cotyledon tip is much larger than the other. Arrowheads indicate cotyledon primordia, and arrows indicate cotyledons. Bars = 50 μm.



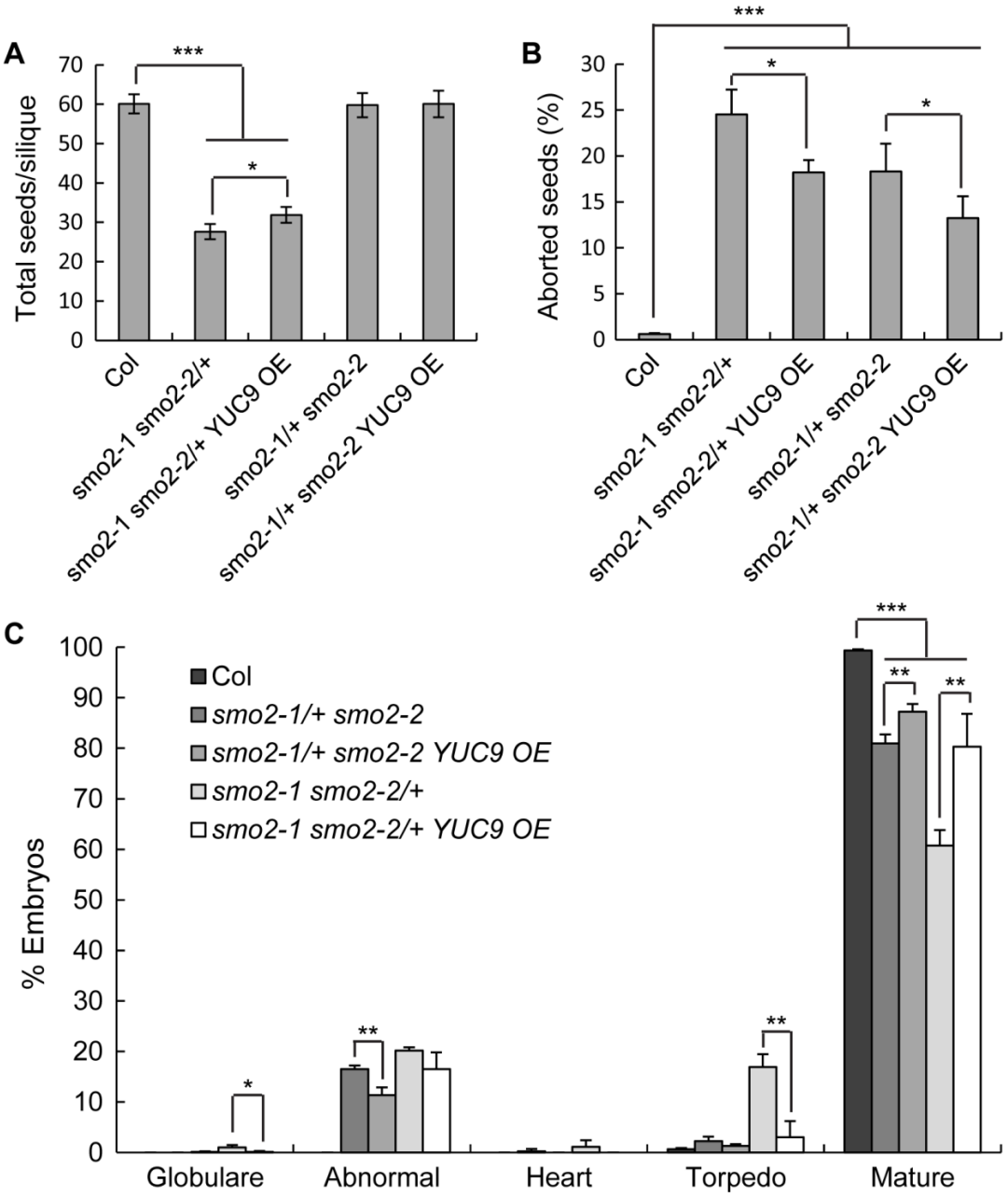
Supplemental Figure S8. Embryonic development of *smo2-1 smo2-2/+* mutants. A, Seed containing an embryo at the early globular stage from the same silique as the seed shown in (B). B, Delayed mutant embryo at the two-cell stage. C, Seed with an embryo at the triangular stage from the same silique as the seed shown in (D). D, Delayed mutant embryo at the eight-cell stage with a short suspensor. Arrowheads indicate degenerating endosperm nuclei. E, Seed with an embryo at the heart stage from the same silique as the seed shown in (F). F, Delayed mutant embryo at the triangular stage. G, Seed with an embryo at the walking stick stage from the same silique as the seed shown in (H). H, Abnormal embryo that could be classified as the heart-like stage. I, Seed containing a mature embryo from the same silique as the seed shown in (J). J, Abnormal embryo that could be classified as the heart-like stage. Bars = 100 μm.



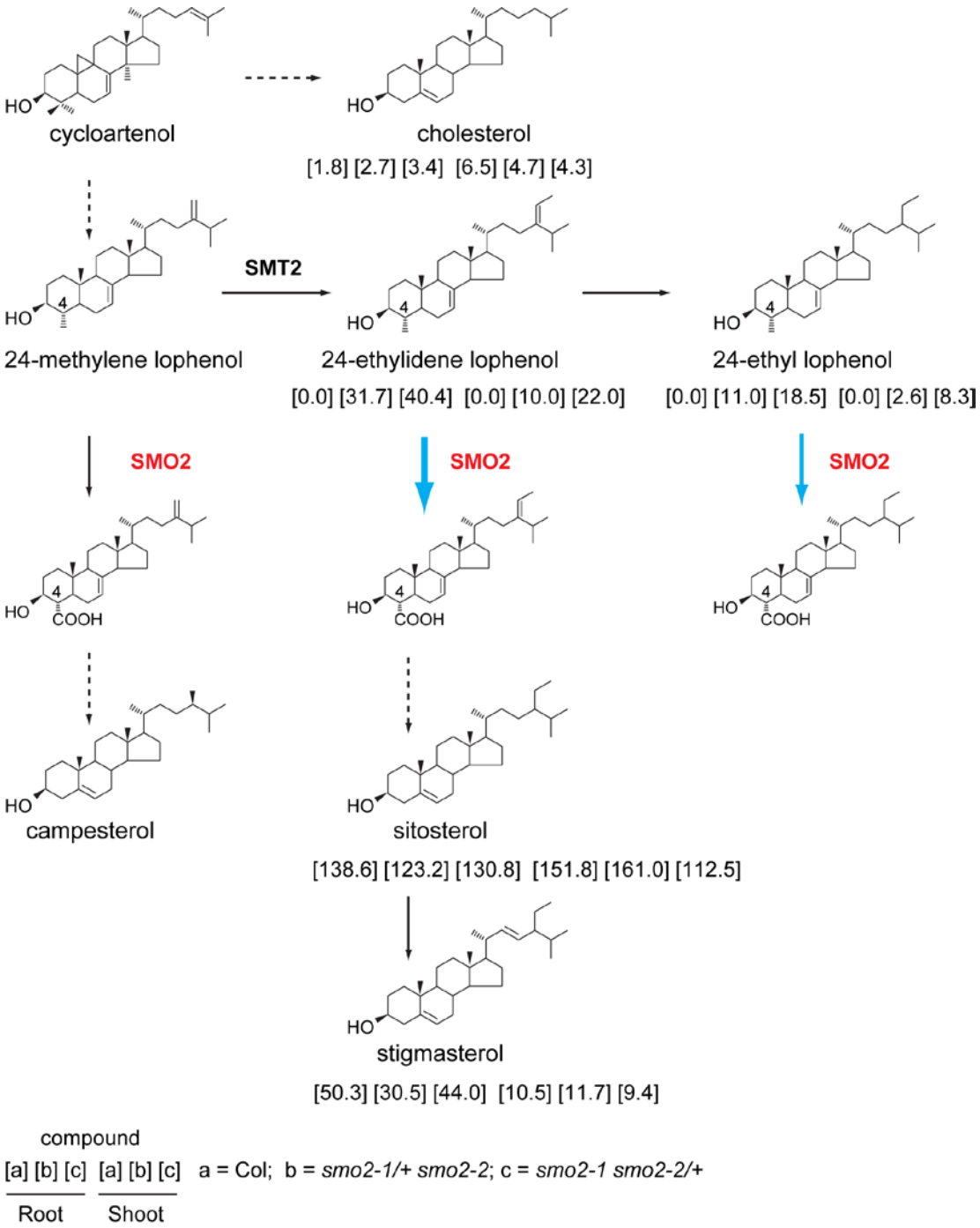
Supplemental Figure S9. Embryonic and endosperm defects of *smo2-1 smo2-2/+* seeds. Cleared seeds from a silique 3 days post-anthesis from the *smo2-1 smo2-2/+* mutant are shown. A, Seed containing a normal globular-stage embryo and properly proliferating endosperm nuclei. B, Seed containing a normal globular-stage embryo but delayed endosperm proliferation and enlarged endosperm nuclei. C, Seed with delayed embryonic and endosperm development. D, Seed with only endosperm nuclei but no embryo. E to H, Enlarged images of the embryos and endosperm nuclei shown in (A-D). Arrowheads indicate large endosperm nuclei, and arrows indicate dividing endosperm nuclei. Bars = 50 μm.



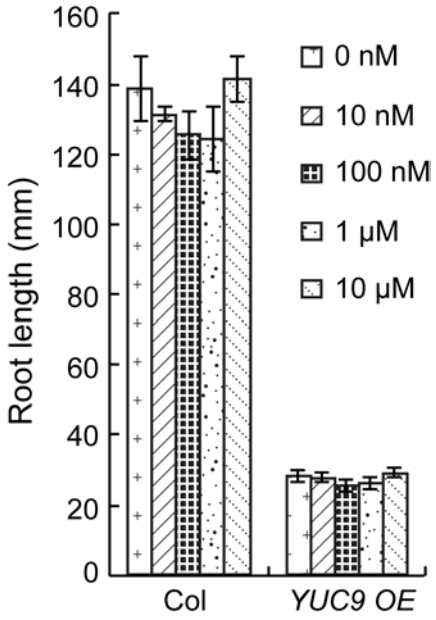
Supplemental Figure S10. *smo2-1/+ smo2-2* and *smo2-1 smo2-2/+* mutants suppressed the short root phenotype of *YUC9* OE seedlings. A, Phenotypes of 6-day-old seedlings. Bar = 0.1 mm. B, Root length of 6-day-old seedlings. The data were derived from three experiments and are presented as the means ± SD (n = 15 for each experiment). Bars in each column with different letters differ significantly (Student's t-test, $P < 0.05$).



Supplemental Figure S11. *YUC9* OE partially rescues *smo2-1 smo2-2* embryonic lethality. A and B, Quantification of total and aborted seeds in siliques of the indicated genotypes. C, Embryonic developmental stage quantification of seeds from siliques 10 days after pollination. The average results of 3 experiments are shown. Seeds from 15 siliques were examined for each experiment in (A) and (B), and seeds from 10 siliques were examined for each experiment in (C). *P < 0.05, ** P < 0.01, ***P < 0.001, Student's t-test.



Supplemental Figure S12. Accumulation of SMO2 substrates in *smo2-1/+ smo2-2* and *smo2-1 smo2-2/+* mutants. Values shown are the content of each compound (ng/mg fresh weight) in roots and shoots of wild-type (Col), *smo2-1/+ smo2-2* and *smo2-1 smo2-2/+* mutants. Arrows with dashed lines represent several biosynthetic steps. Blue arrows indicate reactions that were evidenced by the accumulation of precursor compounds in *smo2-1/+ smo2-2* and *smo2-1 smo2-2/+* mutants. The thickness of the arrow represents substrate preference as revealed by the level of accumulation of precursors.



Supplemental Figure S13. The SMO2 substrate 24-ethylidene lophenol has no effects on root growth of either wild-type or *YUC9* OE seedlings. The root lengths of 5-day-old wild-type and *YUC9* OE seedlings grown on MS medium supplemented with 0 nM, 10 nM, 100 nM, 1 µM and 10 µM 24-ethylidene lophenol are shown. The values represent averages of three biological replicates (n = 9 to 19 for each experiment).

Supplemental Table S1. Primers used in this study.

Purpose	Primer name	Primer sequence 5'→ 3'
Genotyping	LBa1	TGGTTCACGTAGTGGGCCATC
	FLAG_RB4	TCACGGGTTGGGGTTTCTAC
	SMO2-1_F1(1270bp)	GCATCATGAAAGAACCTGAGC
	SMO2-1_R1(2256bp)	AATAGGCCACACGAGCAGCA
	SMO2-1(SAK-105017)-F2	GATGTGTTAGCAATAGCATTCA
	SMO2-1(SAK-105017)-R2	ACTCCCAATACATGCCAGTTG
	SMO2-2_F1(-61)	CACACTCTCTGCCTATCTCCG
	SMO2-2_R1(1134)	CCAGGACGGCAGAGGAAAACT
SMO2-1 promoter cloning	SMO2-1_pF(-742)XhoI	GACGCTCGAGACTATAGTGATTACTAATCAATCA
	SMO2-1_pR(-21)HandIII	GACGAAGCTTGGATGGATCTATGAGAGACAG
SMO2-1 terminator cloning	SMO2-1_tF(2124)NotI	GACGGCGGCCGCAATTGCCACTTTGGATTGCAG
	SMO2-1_tR(3221)SacI	GAGAGCTCCACTAATCTCCTCATCACTC
SMO2-1 genomic DNA cloning	SMO2-1_F2_-742_KpnI	GCGGGTACCACTATAGTGATTACTAATCAATCA
	SMO2-1_R2_2121_XhoI	CGGCCTCGAGCGTTTGTTTCATGTCACCGTT
SMO2-1 RT-PCR	SMO2-1_F5_-103	ATTTTGGTGACAGTCTGTTGTT
	SMO2-1_R5_2388	TACAGAAAAATGAGACAAGGATA
SMO2-2 promoter cloning	SMO2-2_F3_-992_XhoI	GCTGCTCGAGTGACACCGATTATCCAGTGAC
	SMO2-2_R3_-1_HindIII	GCGAAGCTTGGATACCAACAGAAGTAGAAAAC
SMO2-2 terminator cloning	SMO2-2_F4_2036_NotI	AGCGGCGGCCGCACCTGACAACAAACAAACGTGA
	SMO2-2_R4_2678_SacI	GCGGAGCTCTAAGGACACGTAAGTGGACAC
SMO2-2 genomic DNA cloning	SMO2-2_F5_-992_KpnI	GCGGGTACCTGACACCGATTATCCAGTGAC
	SMO2-2_R5_2036_XhoI	CGGCCTCGAGTTCTTTTAGGGCCTTAAGTTTTC
SMO2-2 RT-PCR	SMO2-2_F2_-95	GTCACACAGTTTTCGTTTCTCT
	SMO2-2_R2_2257	AGTAGTAACAACAAGTCCAATAG
ACTIN2 RT-PCR reference	ACTIN2_F1_266	GAGCATGGTGTTGTTAGCAAC
	ACTIN2_R1_866	CAGCACCAATCGTGATGACTT
SMO2-1 qRT-PCR	SMO2-1_qRT-F	TGGAATCTGGTTGGCAGTACC

	SMO2-1_qRT-R	AGGGTAGGAGGCCAACATCAG
SMO2-2 qRT-PCR	SMO2-2_qRT-F	CGTTGAATCCGGTTGGAAGT
	SMO2-2_qRT-R	GGGCAAGTTTACGCAGCAA
TIP41 qRT-PCR referenc	TIP41_qRT-F	GTATGAAGATGAACTGGCTGACAAT
	TIP41_qRT-R	ATCAACTCTCAGCCAAAATCGCAAG

Introduced restriction enzyme site are underlined.