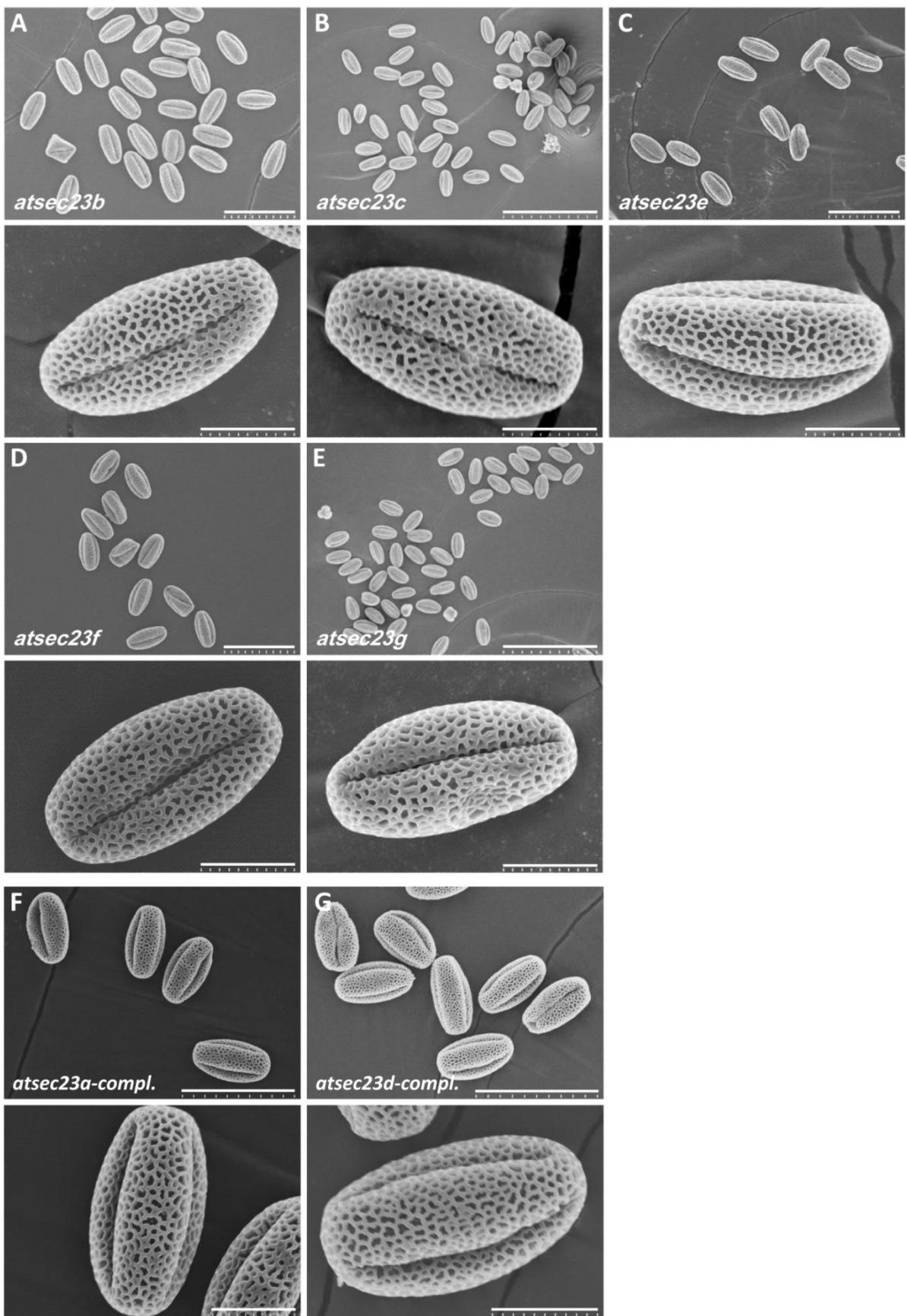


Table S1. Oligonucleotides used in this study.

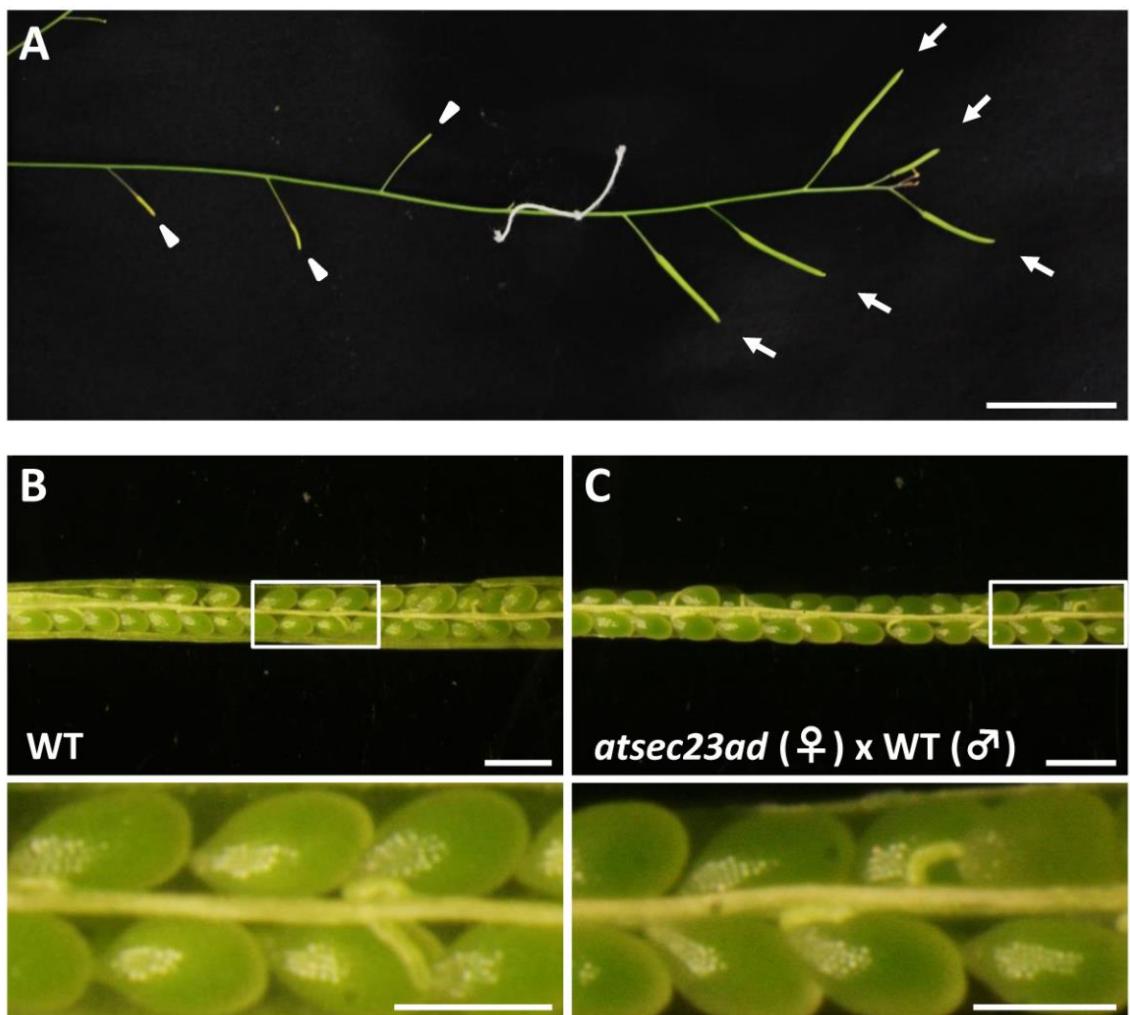
Oligos	Sequence*
<i>attB1</i> adaptor	5'- <u>GGGGACAAGTTGTACAAAAAAGCAGGCT</u> -3'
<i>attB2</i> adaptor	5'- <u>GGGGACCACCTTGACAAGAAAGCTGGGT</u> -3'
<i>P_{nos}-attB4</i>	5'- <u>GGGGACAACCTTGATAGAAAAGTTGGCTGAGACACTATCATGAGCGGAGAATTAAAGG</u> -3'
<i>P_{nos}-attB1r</i>	5'- <u>GGGACTGCTTTTGACAAACTTGTGACACTAGATCCGGTGCAGATTATTG</u> -3'
<i>P_{AtSEC23A-attB1}</i>	5'- <u>GGGGACAAGTTGTACAAAAAAGCAGGCTCCGACACTCGGGATTATTGATGGAAATC</u> -3'
<i>P_{AtSEC23A-attB2}</i>	5'- <u>GGGGACCACCTTGACAAGAAAGCTGGTAGACACTCGGATTCCGAAGTTCTACTT</u> -3'
<i>P_{AtSEC23A-attB4}</i>	5'- <u>GGGGACAACCTTGATAGAAAAGTTGGCTGAGACACTCGGGATTATTGATGGAAATC</u> -3'
<i>P_{AtSEC23A-attB1r}</i>	5'- <u>GGGACTGCTTTTGACAAACTTGTGACACTCGGATTCCGAAGTTCTACTT</u> -3'
<i>P_{AtSEC23D-attB1}</i>	5'- <u>GGGGACAAGTTGTACAAAAAAGCAGGCTCCGACACTCTGGAAACCTATTCAAGCCA</u> -3'
<i>P_{AtSEC23D-attB2}</i>	5'- <u>GGGGACCACCTTGACAAGAAAGCTGGTAGACACTTGTTCAGATCAGATCCTCC</u> -3'
<i>P_{AtSEC23D-attB4}</i>	5'- <u>GGGGACAACCTTGATAGAAAAGTTGGCTGAGACACTCTGGAAACCTATTCAAGCCA</u> -3'
<i>P_{AtSEC23D-attB1r}</i>	5'- <u>GGGACTGCTTTTGACAAACTTGTGACACTTGTTCAGATCAGATCCTCC</u> -3'
<i>AtSEC23A-attB1</i>	5'- <u>AAAAAGCAGGCTCCGACACTATGGCTAACCTACCGAAATC</u> -3'
<i>AtSEC23A-attB2</i>	5'- <u>AGAAAGCTGGTAGACACTCCTGGCTCAGGAGGCAC</u> -3'
<i>AtSEC23D-attB1</i>	5'- <u>AAAAAGCAGGCTCCGACACTATGGCAGTGAGAGCAACGGT</u> -3'
<i>AtSEC23D-attB2</i>	5'- <u>AGAAAGCTGGTAGACACTCTTCATGTATTCAAGTGACAC</u> -3'
RT- <i>AtSEC23A-F</i>	5'-CACATTCAAACTCACGAG-3'
RT- <i>AtSEC23A-R</i>	5'-CCTGGCTCAGGAGGCAC-3'
RT- <i>AtSEC23D-F</i>	5'-GCCTCTCTGGAAAGATGGAGT-3'
RT- <i>AtSEC23D-R</i>	5'-CTTCATGTATTCAAGTGACAC-3'
<i>ACT2-F</i>	5'-CATCTTCTCCGCTCTTCTTCCA-3'
<i>ACT2-R</i>	5'-CTCTTACAATTCCCGCTCTGCTGT-3'
<i>GN-AtSEC23A-F</i>	5'-CATAAGGTGAGTCTGCAGCT-3'
<i>GN-AtSEC23A-R</i>	5'-GACGTCGGTTAACACCACGT-3'
<i>GN-AtSEC23D-R</i>	5'-GAGATTAGCTTGTAAAGCTT-3'
T-DNA-LB	5'-GCAATCAGCTGTTGCCGTCACTGGAG-3'

*Solid and dotted underlines indicate regions corresponding to full-length *attB* sequences (*attB1* and *attB2*; 25 bp, *attB4* and *attB1r*; 22 bp), and regions corresponding to partial *attB1* and *attB2* sequences (12 bp), respectively.

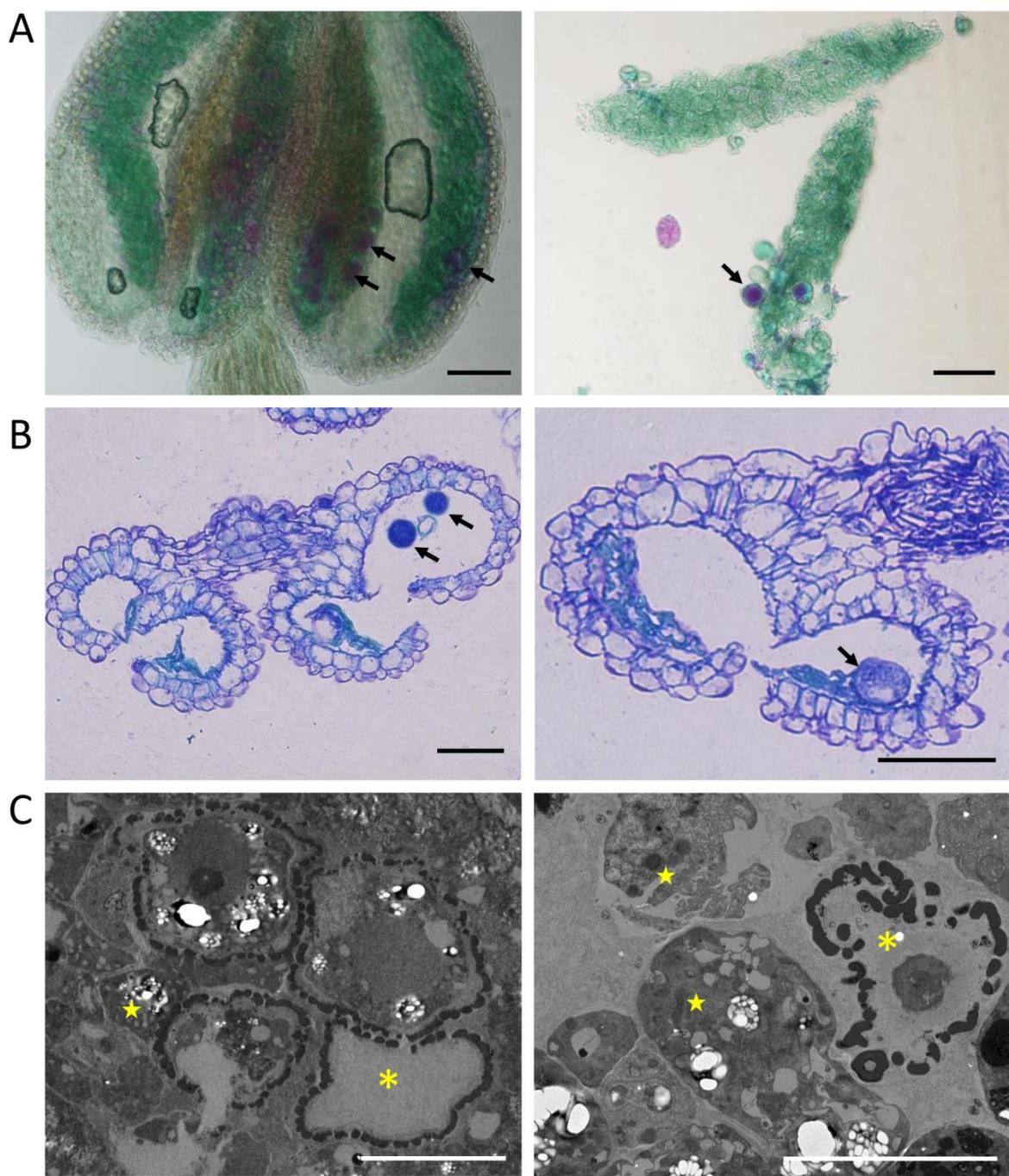
Supplementary Fig. S1. Multiple sequence alignment of SEC23 family proteins in yeast and *A. thaliana*. The yeast and *A. thaliana* amino acid sequences identical to those used in Fig. 1A were re-aligned by the ClustalW ver. 1.83 program (<http://clustalw.ddbj.nig.ac.jp/>). The five domains shown in Fig. 1 are indicated by colored letters: zinc finger (orange), trunk (green), β -barrel (blue), all-helical (red), and gelsolin-like (purple). The previously-reported conserved amino acid residues are highlighted by yellow.



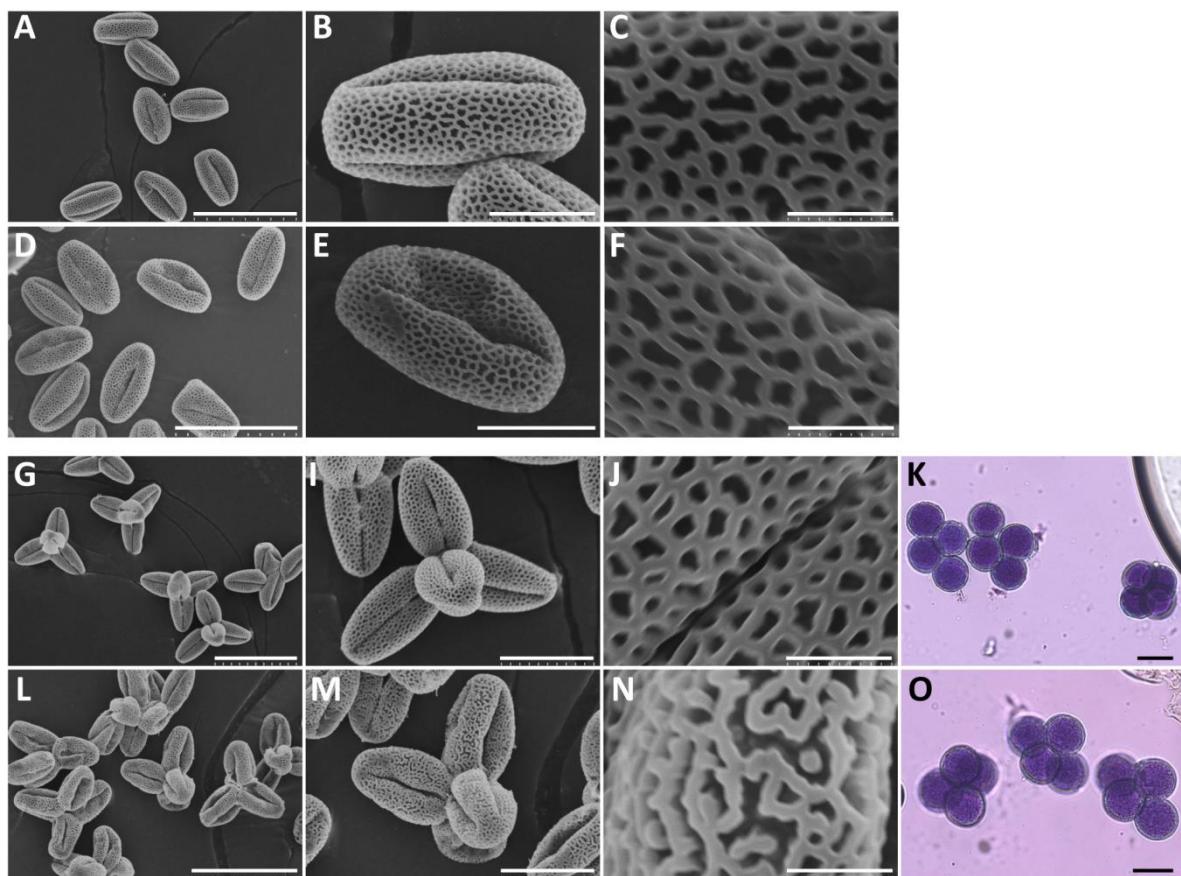
Supplementary Fig. S2. Pollen morphology in T-DNA insertion lines for the remaining *AtSEC23s* and in complemented *atsec23a* and *atsec23d* lines. (A-E) SEM micrographs of pollen grains in T-DNA insertion lines for the *AtSEC23B*, *AtSEC23C*, *AtSEC23E*, *AtSEC23F* and *AtSEC23G*. The lines, SALK_051290 (*atsec23b*), SALK_075252 (*atsec23c*), SALK_080595 (*atsec23e*), SALK_104305 (*atsec23f*), and SALK_027036 (*atsec23g*) were used. (F-G) SEM micrographs of pollen grains in complemented *atsec23a* (F) and *atsec23d* (G) lines. Scale bars = 50 μm in (A, C, D, F and G; upper panels), 100 μm in (B, E; upper panels), and 10 μm in the lower panels.



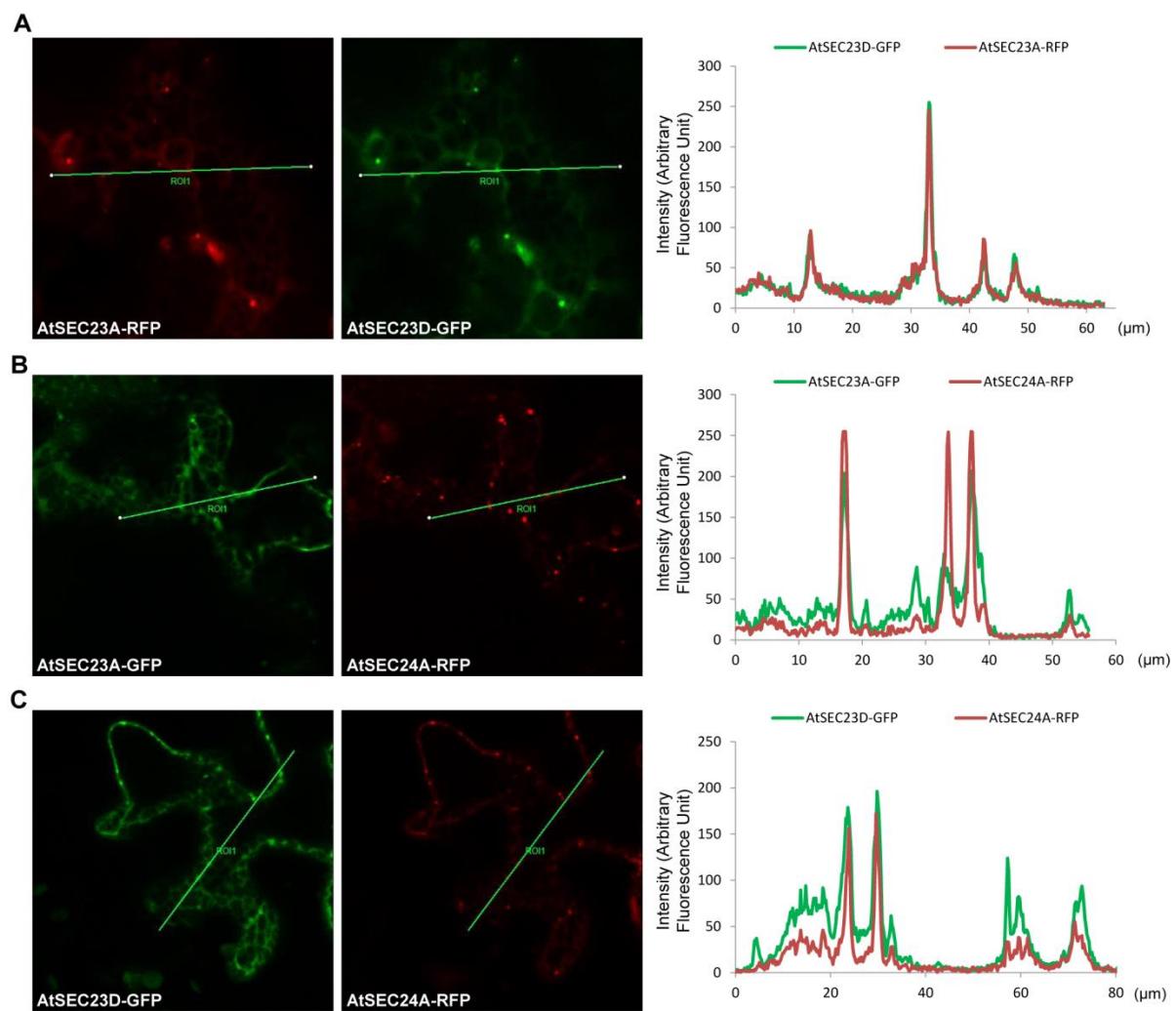
Supplementary Fig. S3. Normal functionality of the female gametophyte of *atsec23ad*. (A) An *atsec23ad* branch with normal elongated siliques after pollination with WT pollen grains. Arrows and arrowheads indicate crossed and self-pollinated siliques, respectively. (B, C) Seed development in siliques of WT and *atsec23ad* pollinated by WT pollen grains. Lower panels are magnifications of the boxed areas in upper panels. Scale bars = 2 cm in (A), 1 mm in (B, C; upper panels), and 0.5 mm in (B, C; lower panels).



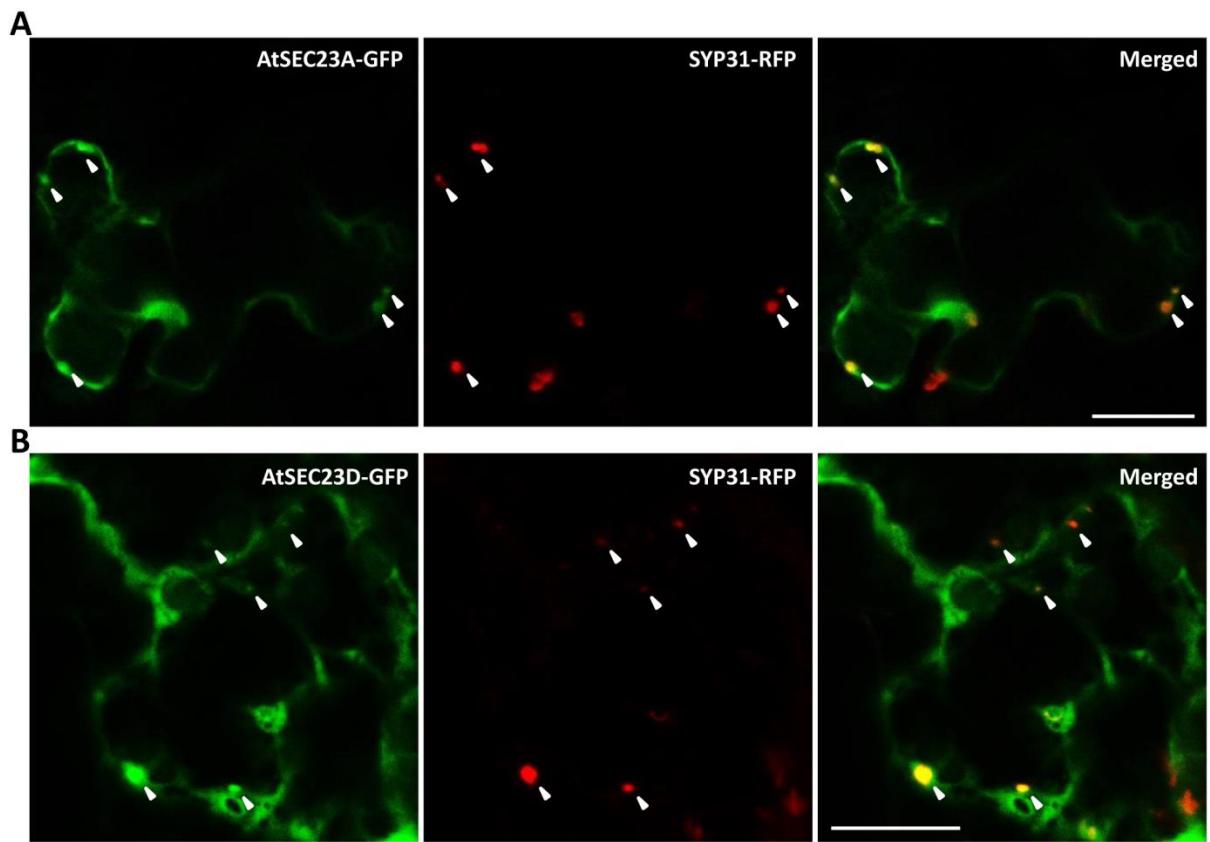
Supplementary Fig. S4. Phenotypes in the *atsec23ad* double mutant. (A) Alexander's staining of *atsec23ad* anthers showing a few positively stained pollen grains (arrows). (B) Technovit semi-thin sections of *atsec23ad* anthers showing a few intact pollen grains indicated by arrows. (C) TEM micrographs of *atsec23ad* microspores at the bicellular stage showing the severe defects. Stars and asterisks show naked microspores with no walls and empty walls without microspore cells, respectively. Scale bars = 50 µm in (A, B) and 10 µm in (C).



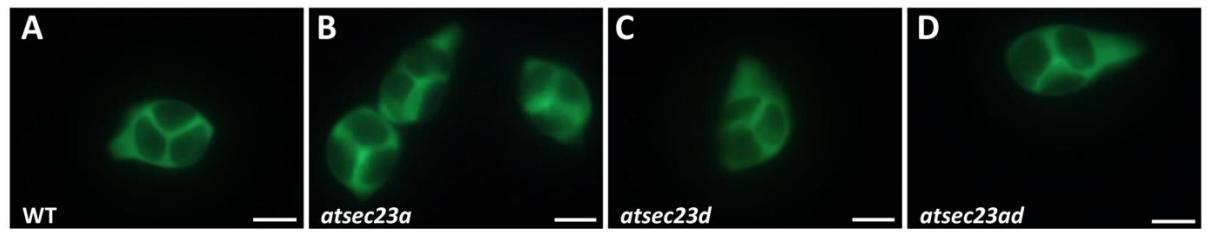
Supplementary Fig. S5. Sporophytic control of *AtSEC23A* and *AtSEC23D*. (A-F) SEM micrographs of pollen grains and their surface structure in heterozygous lines of *atsec23a* (+/*atsec23a*) (A-C) and *atsec23d* (+/*atsec23d*) (D-F). (G-O) Tetrad analysis of heterozygous lines of *atsec23a* in the background of *atsec23d* and *qrt1-2*. SEM micrographs of pollen-tetrads of *qrt1-2/qrt1-2* (G-J) and heterozygous line of *atsec23a* in the *atsec23d* and *qrt1-2* background (+/*atsec23a*, *atsec23d/atsec23d*, *qrt1-2/qrt1-2*) (L-N). Alexander's staining of pollen-tetrads of *qrt1-2/qrt1-2* (K) and +/*atsec23a*, *atsec23d/atsec23d*, *qrt1-2/qrt1-2* (O). Scale bars = 50 µm in (A, D, G, and L), 10 µm in (B, E, I, and M), 3 µm in (C, F, J, and N), and 20 µm in (K and O).



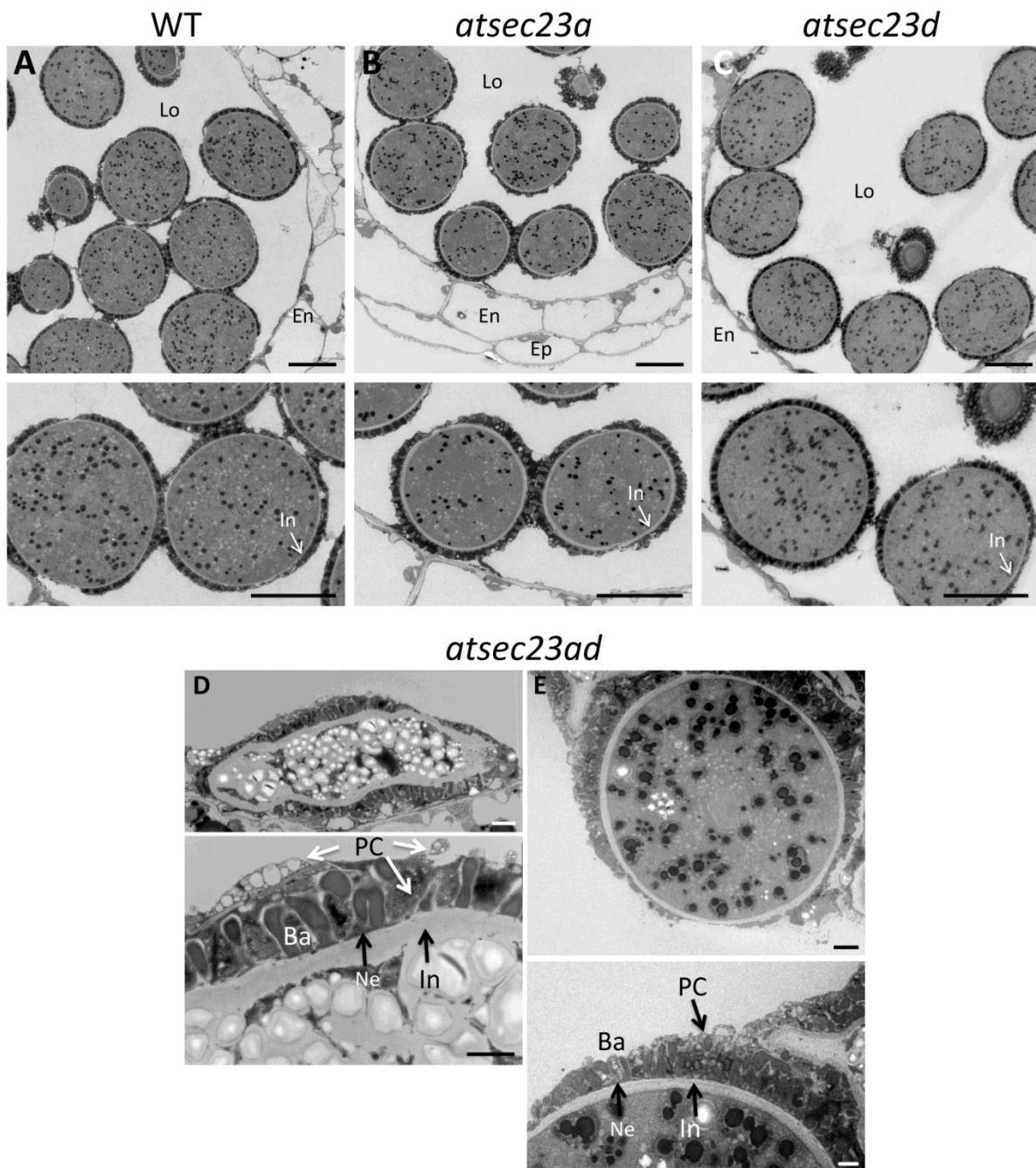
Supplementary Fig. S6. Quantitative co-localization analyses of AtSEC23A and AtSEC23D. (A) Fluorescent images of an epidermal cell co-expressing AtSEC23D-G3GFP and AtSEC23A-TagRFP. (B, C) Fluorescent images of epidermal cells co-expressing AtSEC23A-G3GFP (B) or AtSEC23D-G3GFP (C) with the ERES marker AtSEC24A-TagRFP. Fluorescence intensity on regions of interest (ROIs) indicated in each image was quantified by the software, LAS AF (Leica Microsystems, Wetzlar, Germany) and was plotted on right graphs.



Supplementary Fig. S7. Co-localization analyses of AtSEC23A and AtSEC23D in *N. benthamiana* leaf epidermal cells. Confocal images of *N. benthamiana* leaf epidermal cells co-expressing AtSEC23A-G3GFP (A) or AtSEC23D-G3GFP (B) with the *cis*-Golgi marker SYP31-TagRFP. Arrowheads label the ERESs. Scale bars = 20 μ m.



Supplementary Fig. S8. Aniline blue staining of microspores at the tetrad stage. Aniline blue staining at the tetrad stage of WT, *atsec23a*, *atsec23d*, and *atsec23ad*. Scale bars = 10 μm .



Supplementary Fig. S9. Abnormal thickening of the intine in *atsec23a* pollen grains and defective wall development in *atsec23ad* pollen grains. (A-C) SEM micrographs comparing intine of WT (A), *atsec23a* (B), and *atsec23d* (C) mature pollen grains at the tricellular stage. (D, E) SEM micrographs showing defective walls of *atsec23ad* pollen grains at the tricellular stage. Lower panels are magnifications of pollen-surface structures in the upper panels. Ba, baculum; En, endodermis Ep, epidermis; In, intine; Lo, locule; Ne, nexine; PC, pollen coat. Scale bars = 10 μ m in (A-C), 2 μ m in (D, E: upper panels), and 1 μ m in (D, E: lower panels).

Supplementary Movie S1. Time-lapse confocal imaging of *N. benthamiana* leaf epidermal cells co-expressing AtSEC23A-G3GFP and the ERES marker AtSEC24A-TagRFP. Scale bar = 25 μ m.

Supplementary Movie S2. Time-lapse confocal imaging of *N. benthamiana* leaf epidermal cells co-expressing AtSEC23D-G3GFP and the ERES marker AtSEC24A-TagRFP. Scale bar = 25 μ m.