Supplemental Information

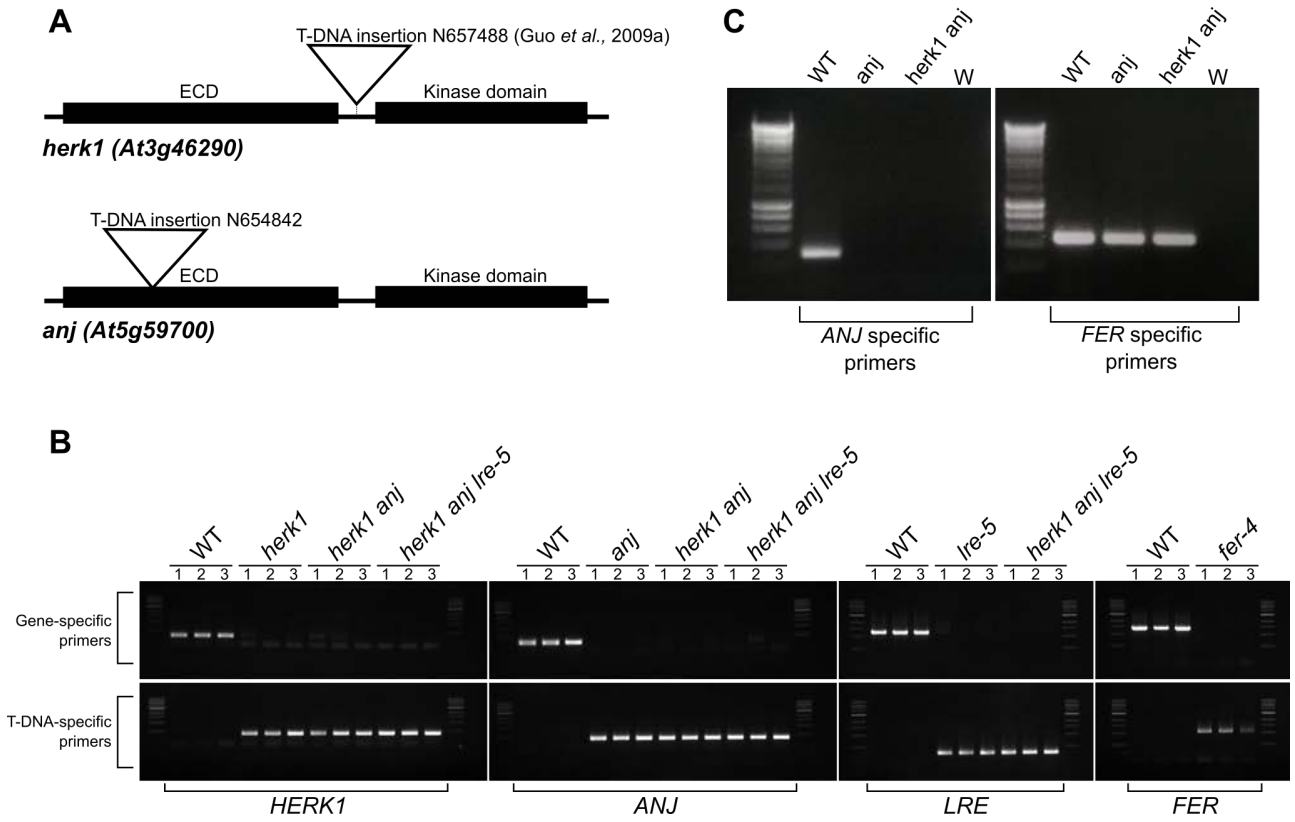

Figure S1. Confirmation of *ANJEA* gene expression knock out and genotyping of T-DNA lines used in this study. (A) Diagram of the domain organisation of *HERK1* and *ANJEA* and T-DNA insertion sites in the lines used in this study, *herk1-1* and *anj-1*. (B) Genotyping PCRs to verify homozygosity in the lines used in this study. DNA from three independent seedlings per line was analysed. (C) RT-PCR analysis of *ANJ* gene expression in wild-type, *anj* and *herk1 anj* plants. RNA was extracted from multiple inflorescences from five plants per line. W indicates a water control with no cDNA added to the RT-PCR reaction.

Figure S2. Growth comparison of WT and *herk1 anj* plants, and polytubey in *herk1 anj* plants.

(A-B) Representative wild-type plants at 10 and 21 days old. (C-D) Representative *herk1 anj* plants at 10 and 21 days old. (E-F) Representative *fer-4* plants at 10 and 21 days old. (G) Representative wild-type and *herk1 anj* plants (left and right, respectively) at 5 weeks old. Scale bars = 1.5 cm.

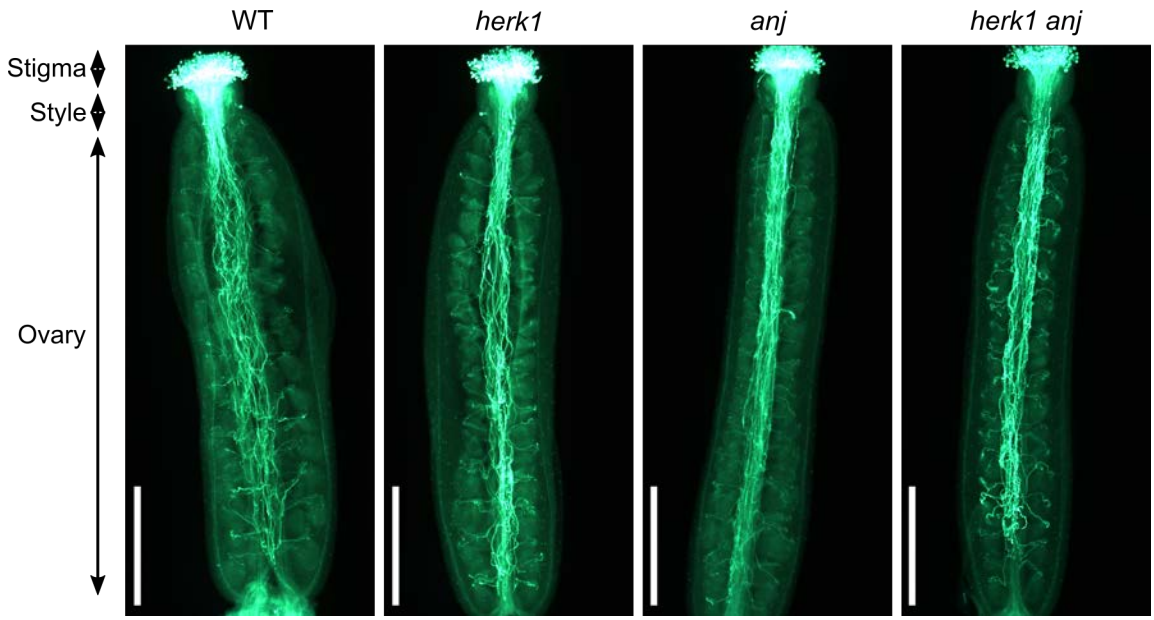

Figure S3. Pollen tube growth and targeting of ovules is not altered in *herk1 anj* plants. Aniline blue staining of pollen tubes in self-pollinated stage 16 flowers in wild-type, *herk1*, *anj* and *herk1 anj* plants. Scale bars = 500 µm.

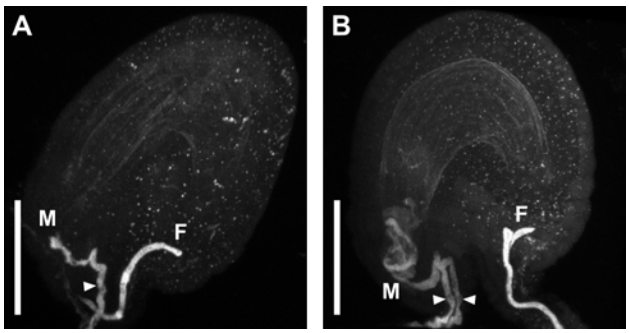

Figure S4. ***herk1 anj*** **ovules attract multiple pollen tubes.** (A) Representative image of a normal pollen tube reception event in a wild-type ovule. (B) Representative image of a *herk1 anj* ovule displaying pollen tube overgrowth and multiple pollen tubes in the micropyle. Images are maximum intensity projections from confocal microscopy images across several z-planes of ovules stained with aniline blue. M, micropyle. F, funiculus. White arrowhead, pollen tube. Scale bars = 50 µm.

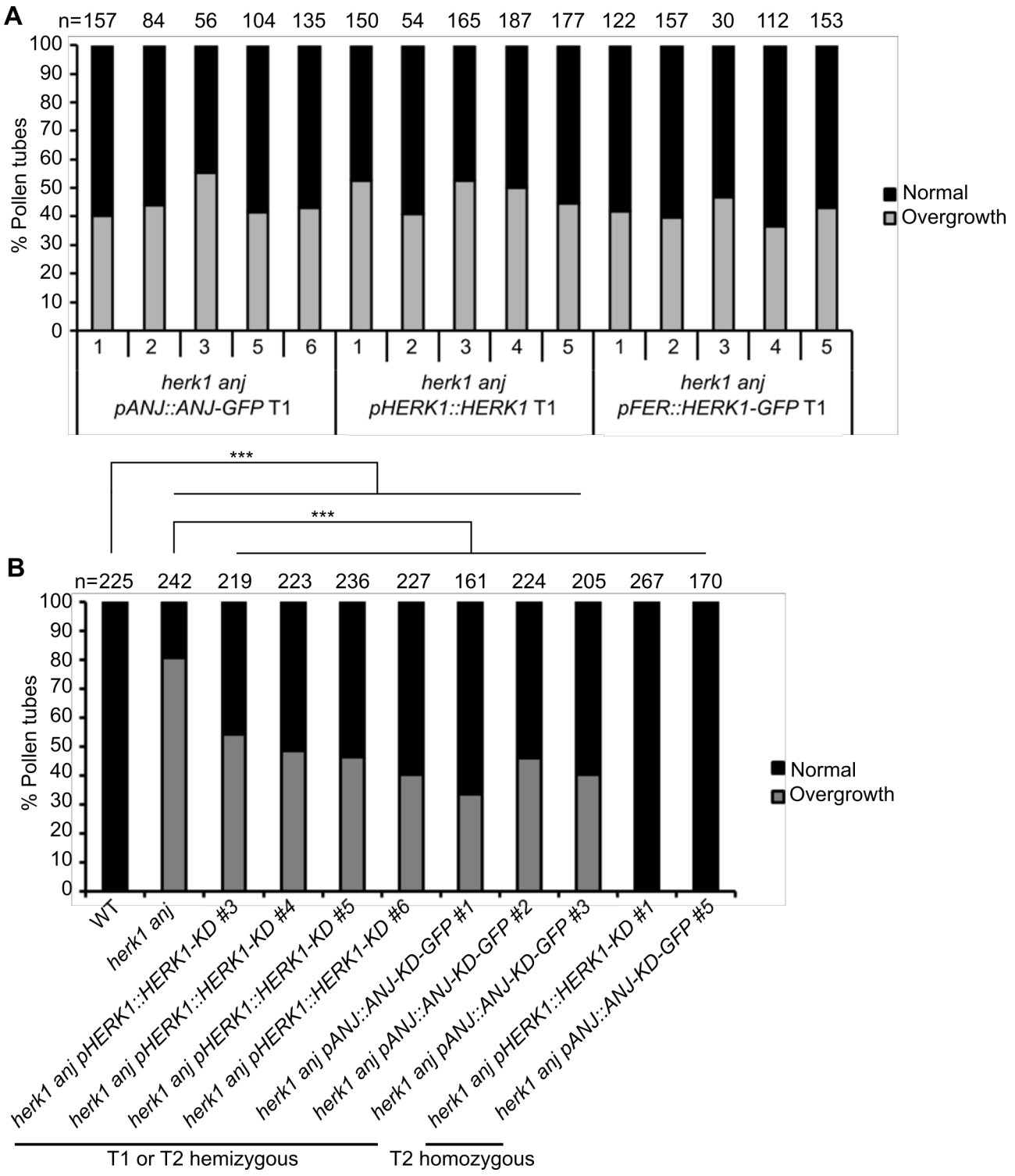

Figure S5. The *herk1 anj* defect in pollen tube reception can be complemented by expression of *HERK1*, *ANJ*, *HERK1-KD* and *ANJ-KD* constructs. (A) Percentage of pollen tubes with normal reception at the female gametophyte (black bars) and displaying overgrowth (grey bars) in siliques of five independent T1 *herk1 anj* plants transformed with *pANJ::ANJ-GFP*, *pHERK1::HERK1* and *pHERK1::HERK1-GFP*. Pollen tube reception was scored for ovules in at least three siliques per

line. (B) Percentage of pollen tubes with normal reception at the female gametophyte (black bars) and displaying overgrowth (grey bars) in WT, *herk1 anj* plants and at least 4 independent lines of *herk1 anj* transformed with *pHERK1::HERK1-KD* or *pANJ::ANJ-KD-GFP* from generations T1 or T2. Pollen tube reception was scored for ovules in at least three siliques per line. *** $p<0.001$ (χ-square tests).

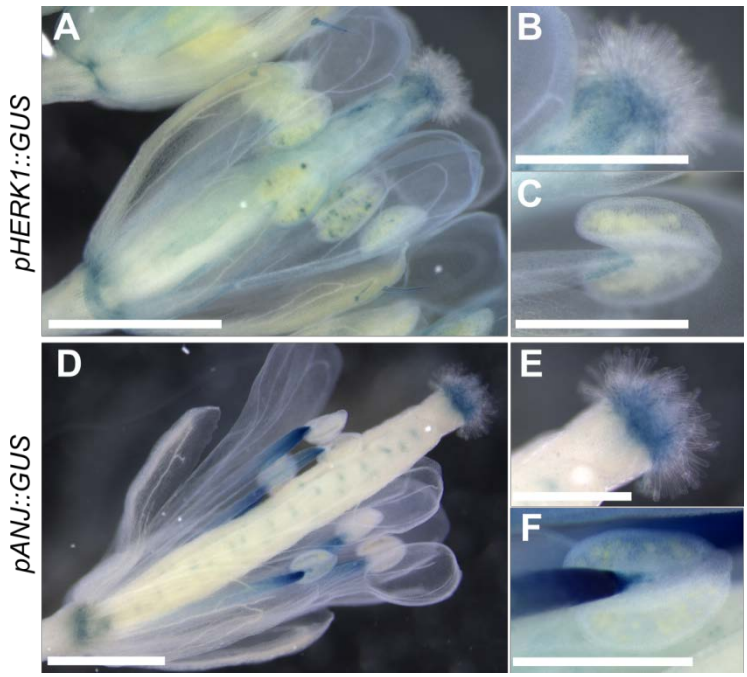

Figure S6. Expression pattern of *HERK1* and *ANJ* in flowers. (A-C) Representative image of the expression pattern in flowers of *HERK1* as shown by *pHERK1::GUS*. Details of a mature stigma and anther are shown in (B) and (C), respectively. GUS activity in at least four T1 lines was examined. (D-F) Representative image of the expression pattern in flowers of *ANJ* as shown by *pANJ::GUS*. Details of a mature stigma and anther are shown in (E) and (F), respectively. GUS activity in at least four T1 lines was examined. Scale bars = 1 mm in (A) and (D); 0.5 mm in (B,C) and (E,F).

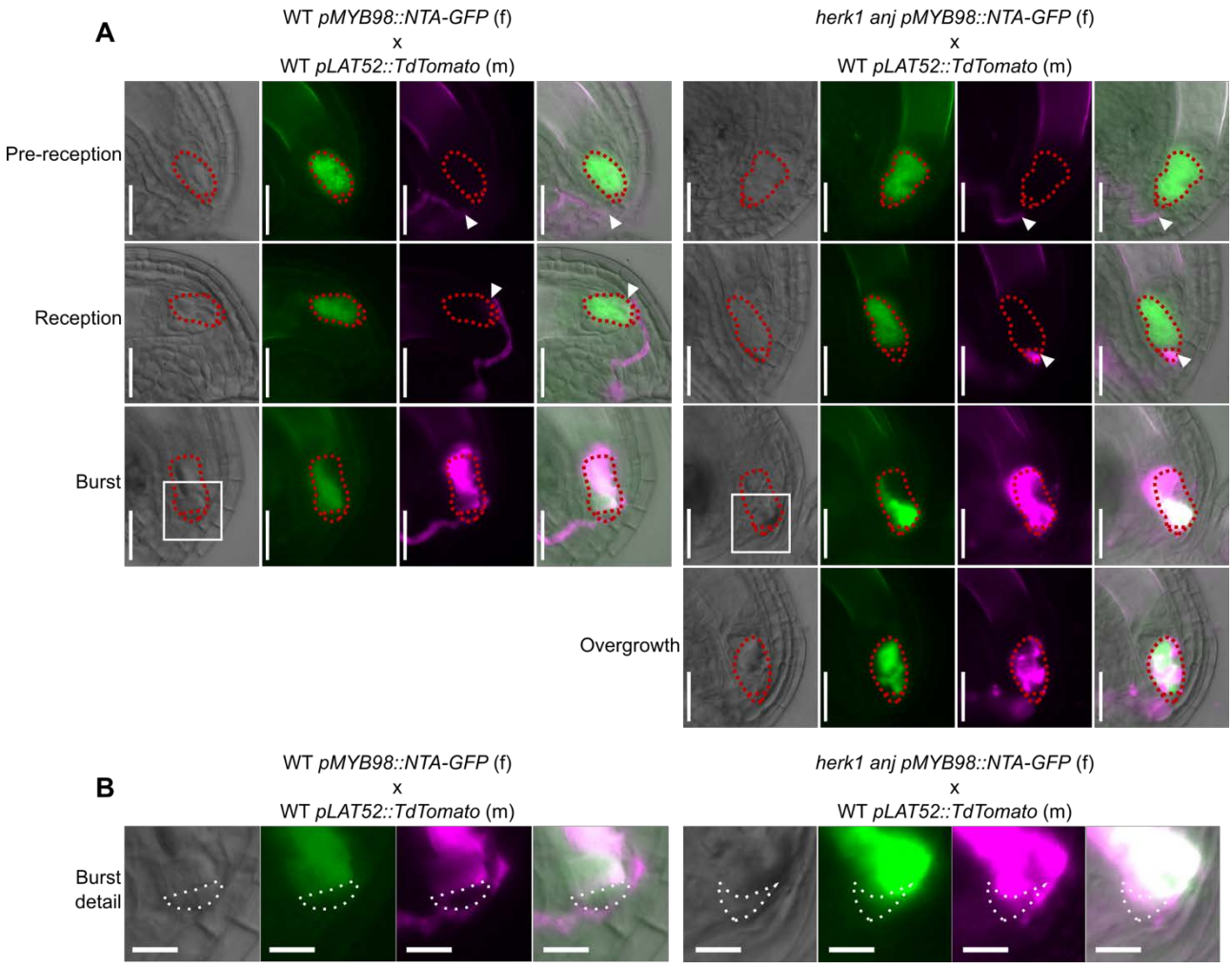

Figure S7. NTA localisation in the synergid cells of WT and *herk1 anj* at different stages of pollen tube reception. (A) DIC images are shown in grey. In green is NTA-GFP fluorescence in ovules expressing *pMYB98::NTA-GFP*. In magenta, TdTomato fluorescence from pollen tubes expressing *pLAT52::TdTomato*. On the right are merged DIC and fluorescence images. Red dotted lines delineate the synergid cells. White arrowheads indicate the pollen tube tip. (B) Detailed images of the filiform apparatus corresponding to the areas highlighted with white squares in (A). White dotted lines delineate the filiform apparatus. Scale bars = 25 µm in (A) and 10 µm in (B).

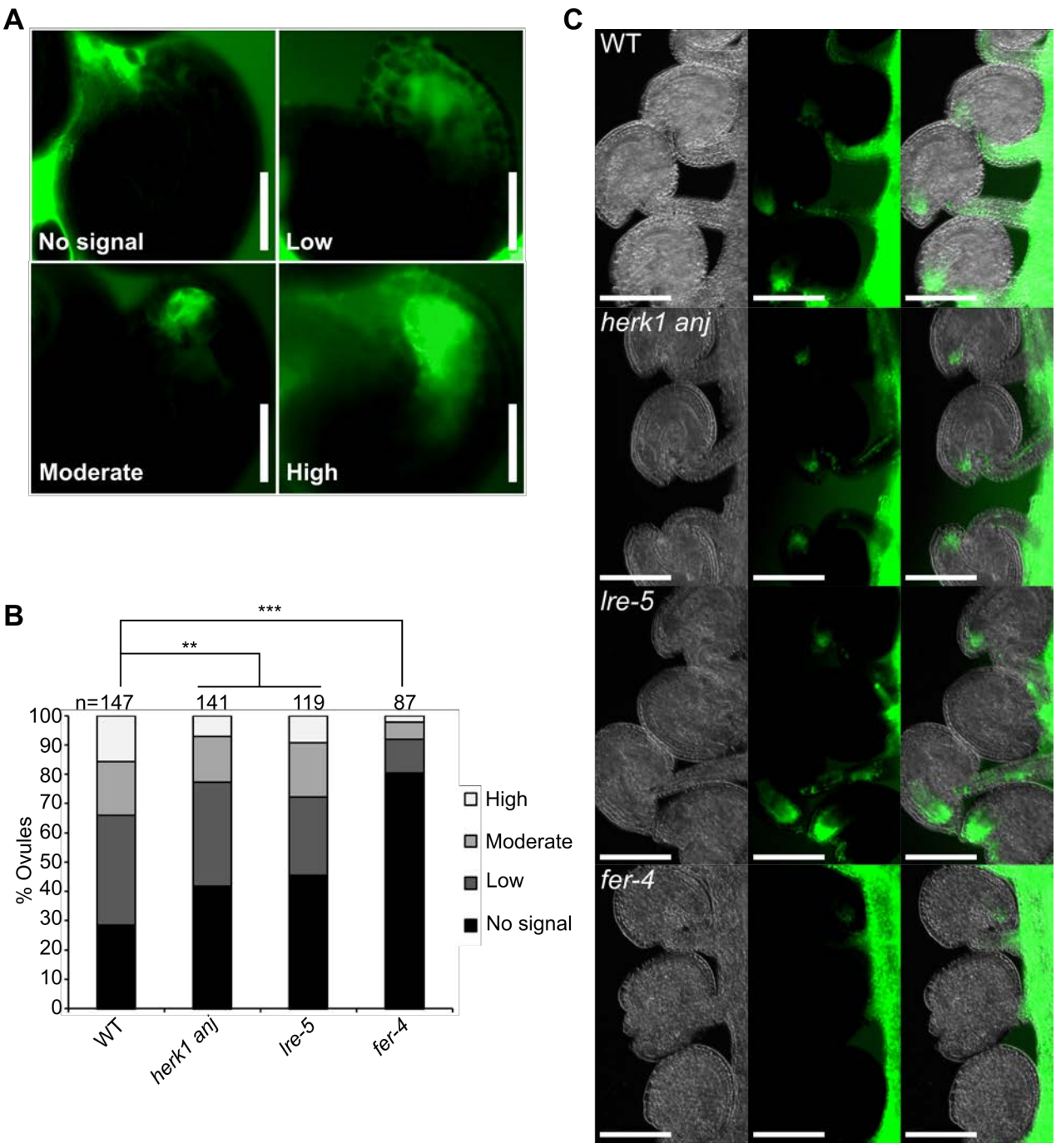

Figure S8. H_2DCF-DA measurements of ROS production in *herk1 anj* ovules. (A) Images of H_2CDF-DA fluorescence in representative ovules corresponding to each category used in the ROS assays presented in this study. Scale bars = 25 µm. (B) Quantification of the H_2CDF-DA staining of ROS in ovules from wild-type, *herk1 anj*, *lre-5*, and *fer-4* plants at 0 HAE. Categories are listed in the legend. Ovules analysed from six siliques per line. ** $p<0.01$; *** $p<0.001$ (χ-square tests). (C) Representative images of H_2CDF-DA staining of ROS in three ovules from wild-type, *herk1 anj*, *lre-5* and *fer-4* plants at 20 hours after emasculation (HAE). Scale bars = 100 µm.

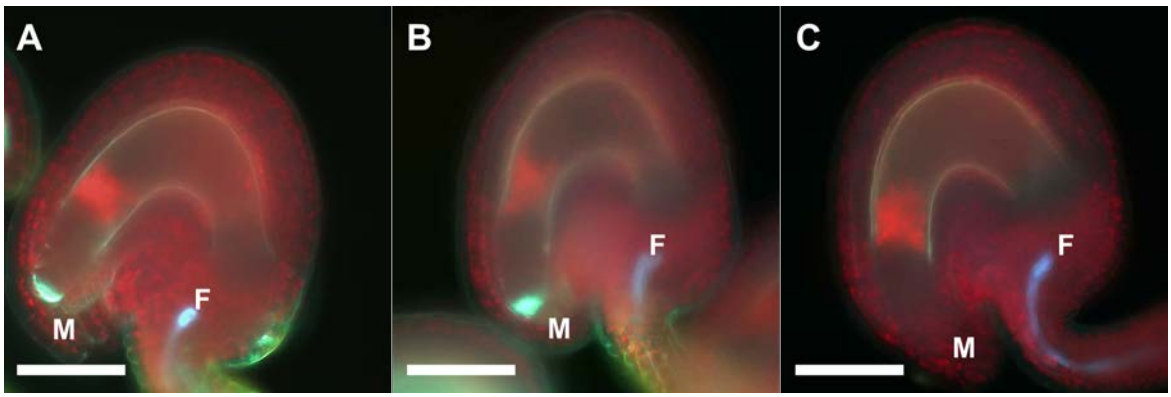

Figure S9. Callose accumulation at the filiform apparatus in *herk1 anj* mutants. (A) Representative image of a mature ovule from a wild-type plant. SR2200 white fluorescence at the filiform apparatus indicates accumulation of callose. (B) Representative image of a mature ovule from a *herk1 anj* plant. SR2200 white fluorescence at the filiform apparatus indicates accumulation of callose. (C) Representative image of the background autofluorescence present in mature ovules. Chlorophyll red autofluorescence can be seen in all cell layers in the ovule. Blue autofluorescence from the xylem lignin within the funiculus can be seen. Scale bars = 25 μm. M, micropyle. F, funiculus.

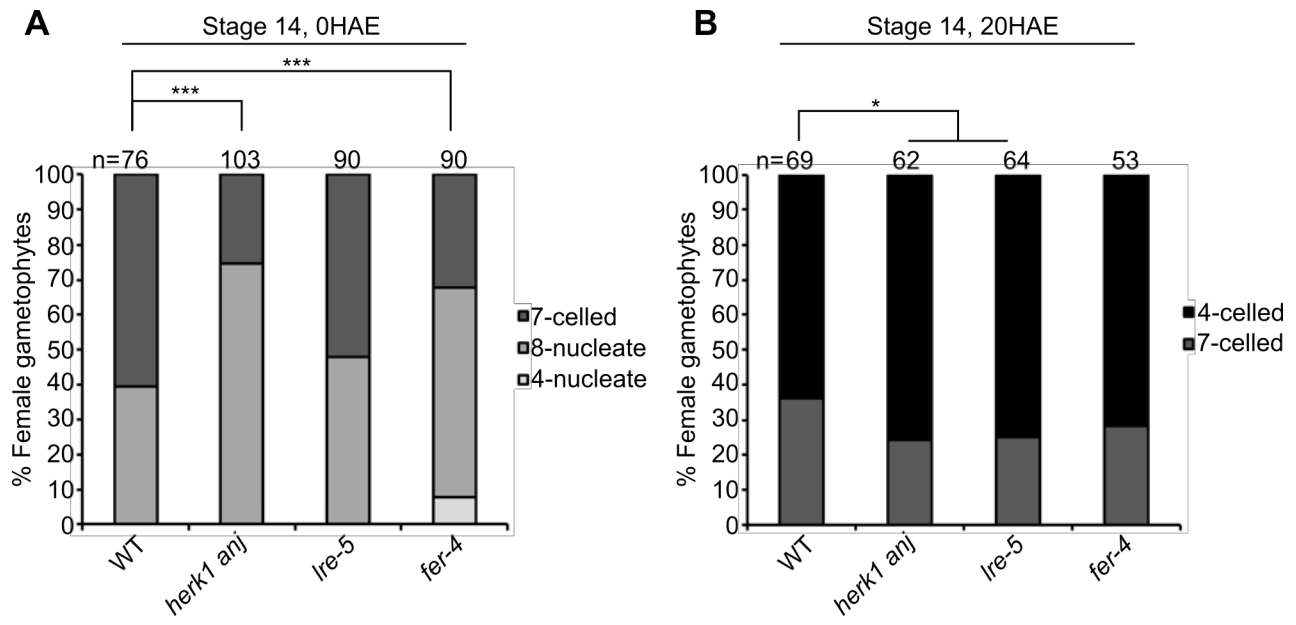

Figure S10. Female gametophyte development at 0 and 20 HAE. (A) Female gametophyte developmental stage in ovules from stage 14 flowers at 0 HAE in wild-type, *herk1 anj*, *lre-5* and *fer-4* as assessed by confocal microscopy as per (61). Ovules analysed from five siliques per line. *** $p<0.001$ (χ-square tests). (B) Female gametophyte development stage in ovules from stage 14 flowers at 20 HAE in wild-type, *herk1 anj*, *lre-5* and *fer-4*. Ovules analysed from five siliques per line. * $p<0.05$ (χ-square tests).

Table S1. List of Arabidopsis lines used in this study. Sources and NASC stock identifiers are listed where relevant.

Experimental Models: Organisms/Strains		
Arabidopsis thaliana: Col-0	NASC	N1092
Arabidopsis thaliana: herk1-1	NASC	N657488
Arabidopsis thaliana: anj-1	NASC	N654842
Arabidopsis thaliana: fer-4	Prof. A. Cheung (16)	NASC ID: N69044
Arabidopsis thaliana: lre-5	Dr. R. Palanivelu (28)	NASC ID: N66102
Arabidopsis thaliana: herk1 anj	This study	N/A
Arabidopsis thaliana: herk1 anj lre-5	This study	N/A
Arabidopsis thaliana: Col-0 *x pHERK1::GUS*	This study	N/A
Arabidopsis thaliana: Col-0 *x pANJ::GUS*	This study	N/A
Arabidopsis thaliana: Col-0 *x pHERK1::HERK1*	This study	N/A
Arabidopsis thaliana: Col-0 *x pANJ::ANJ-GFP*	This study	N/A
Arabidopsis thaliana: Col-0 *x pLRE::LRE-Citrine*	This study	N/A
Arabidopsis thaliana: Col-0 *x pMYB98::NTA-GFP*	This study	N/A
Arabidopsis thaliana: Col-0 *x pFER::FER-GFP*	This study	N/A
Arabidopsis thaliana: herk1 anj x pHERK1::HERK1	This study	N/A
Arabidopsis thaliana: herk1 anj x pANJ::ANJ-GFP	This study	N/A
Arabidopsis thaliana: herk1 anj x pLRE::LRE-Citrine	This study	N/A
Arabidopsis thaliana: herk1 anj x pMYB98::NTA-GFP	This study	N/A

Arabidopsis thaliana: herk1 anj x pFER::FER-GFP	This study	N/A
Arabidopsis thaliana: lre-5 x pHERK1::HERK1	This study	N/A
Arabidopsis thaliana: lre-5 x pANJ::ANJ-GFP	This study	N/A
Arabidopsis thaliana: lre-5 x pLRE::LRE-Citrine	This study	N/A
Arabidopsis thaliana: lre-5 x pMYB98::NTA-GFP	This study	N/A
Arabidopsis thaliana: lre-5 x pFER::FER-GFP	This study	N/A
Arabidopsis thaliana: Col-0 *x pLAT52::TdTomato*	Dr. M. Bayer (unpublished)	N/A

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105 **Table S2. List of primers used for cloning, genotyping PCR or RT-PCR.**

Oligonucleotides (5' - 3')	
HERK1 genotyping fw	GTTGCTCGCGGTAGTCTTCT
HERK1 genotyping rv	CTGTCCTGAATTCCGCAAGC
ANJEA genotyping & RT-PCR fw	CTCCTCTGTAGCAAAACCAGGA
ANJEA genotyping & RT-PCR rv	CTCACGTTTACTCCCTCGGG
LRE genotyping fw	AAGCCAGTTTTAGAGTACGAAGA
LRE genotyping rv	TCAAGTCAACACTAACAAAGCAAAAACAGCGG
FER genotyping & RT-PCR fw	CATTGACGCGATTCATGTTT
FER genotyping & RT-PCR rv	GAGTATTTCAGACGGCAGCA
SALK LB genotyping primer	ATTTTGCCGATTTCGGAAC
GABI LB genotyping primer	GTGGATTGATGTGATATCTCC
pHERK1 fw	TAGGTACCTAGAATGTTTTTCTCAAGTTTTCTTCC
HERK1 rv	TAAGGATCCTCTTCCTTCAGATTTCACCAGTTGTG
pANJ fw	TTAGGTACCTTGTGGAATCATGAAATCGTAGTGT
ANJ rv	TAGGATCCACGTCCCTCAGATTTGATCAGCTGCG
pFER fw	TAGGTACCCGAGTTGTAAAAGGCCTGGC
FER rv	TAAGGATCCACGTCCCTTTGGATTCATGA
HERK1-KD fw	AGAAACGTGAGATCTGCAAACATATTGCTTGACGA
HERK1-KD rv	AGATCTCACGTTTCTGTGAATGACCGGTTTCGAGT
ANJ-KD fw	AGAAACGTCAGATCCGCCAACATATTGCTTGA
ANJ-KD rv	GGATCTGACGTTTCTGTGAATCACGGGTTTCG

pHERK1 pentrdtopo fw	CACCTAGAATGTTTTTCTCAAGTTTTCTTCC
pHERK1 pentrdtopo rv	AACCTGGAAATGGAACAGATC
pANJ pentrdtopo fw	CACCTTGTGGAATCATGAAATCGTAGT
pANJ pentrdtopo rv	TTCACAAAACCTGGAAATTTTAAATAATT
HERK1 pentrdtopo fw	CACCATGGGTATTGAAAAGTTTGAAACTTTCATC
HERK1 pentrdtopo rv	TCTTCCTTCAGATTTCACCAGTTGTG
ANJ pentrdtopo fw	CACCATGGGTGGTGAAAAGTTTGGATTTTTGATTTGG
ANJ pentrdtopo rv	ACGTCCCTCAGATTTGATCAGCTGCG
FER pentrdtopo fw	CACCATGAAGATCACAGAGGGACG
FER pentrdtopo rv	ACGTCCCTTTGGATTCATGA
HERK1exJM fw	GGATATTGATCTTAGCACTCTTGTGG
HERK1exJM rv	AACCCGAGATTACTCTTACTGCT
ANJexJM fw	GCTTGATCTGAGCTCTTATTTATCCA
ANJexJM rv	CCACCAACATTCTTCTTAGTGGTTG
LRE(23-138) fw	GATATCGGATGGTGTGTTTGAATCA
LRE(23-138) rv	CCGGCGTTTAGGTTATGTGAATAGAG

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Table S3. List of plasmids used in the present study and their corresponding sources.

Recombinant DNA	Reference
pHERK1::HERK1 in pGreen-IIS	This study
pANJ::ANJ-GFP in pGreen-IIS	This study
pFER::FER-GFP in pGreen-IIS	This study
pHERK1:HERK1-KD in pGreen-IIS	This study
pANJ::ANJ-KD-GFP in pGreen-IIS	This study
pHERK1::GUS in pGWB433	This study
pANJ::GUS in pGWB433	This study
p35S::HERK1-GFP in pGWB405	This study
p35S::HERK1-MYC in pGWB420	This study
p35S::ANJ-GFP in pGWB405	This study
p35S::ANJ-MYC in pGWB420	This study
pFER::HERK1-GFP in pMDC111	Prof. U. Grossniklaus (26)
pMYB98::NTA-GFP in pMDC83	Dr. S. Kessler (65)
p35S::HA-LRE in pSK	Dr. C. Li (15)
p35S::HA-LRE in pMLBart	This study
pLRE::LRE-Citrine in pMDC99	Prof. U. Grossniklaus (66)
pGreen-IIS – Cterm GFP	(62)
pGWB405	(64)
pGWB420	(64)
pGWB433	(64)

pGADT7	Clontech
pGBKT7	Clontech

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