Expanded View Figures

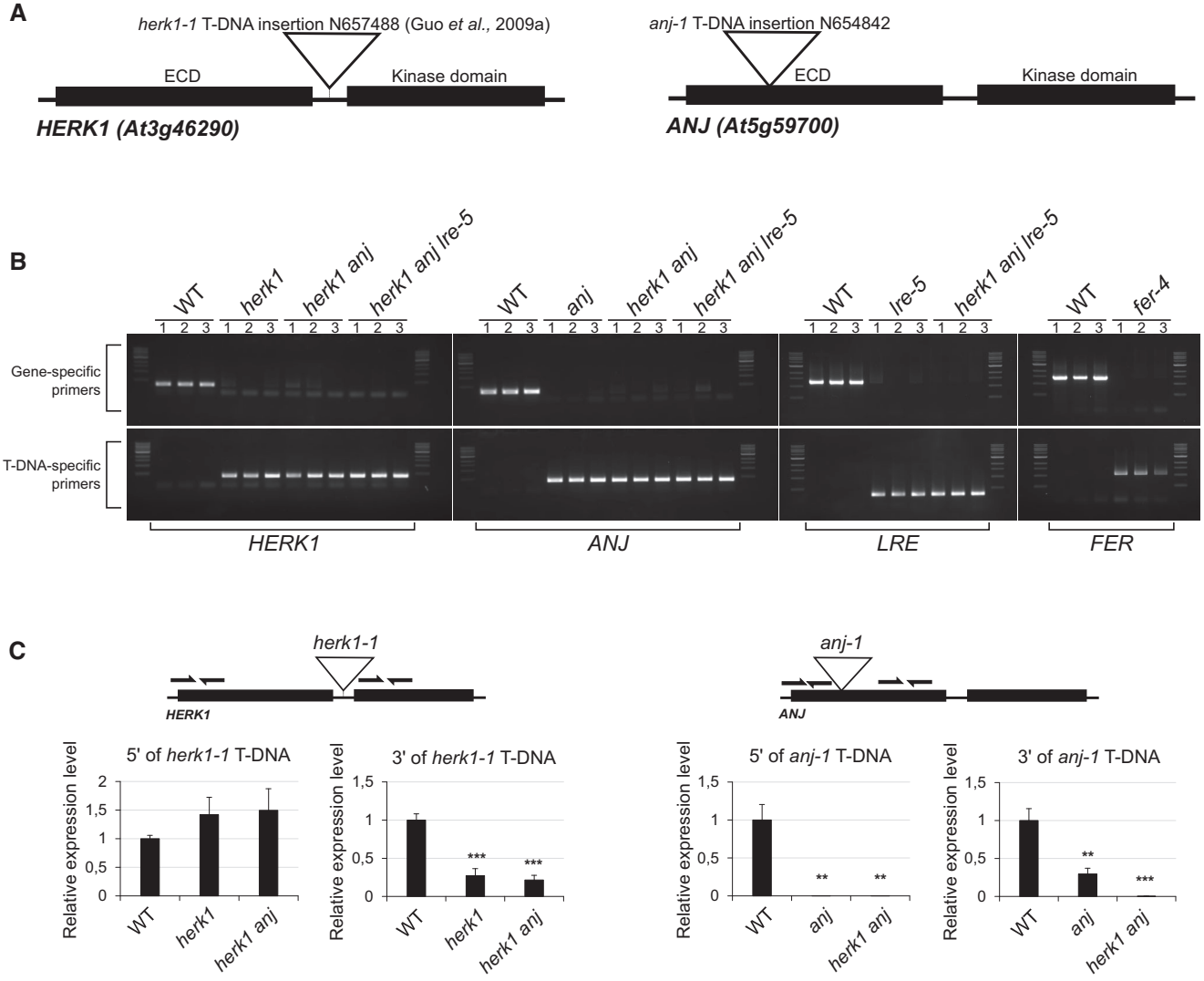


Figure EV1. Confirmation of *ANJEA* gene expression knock out and genotyping of T-DNA lines used in this study.

A 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–qPCR analysis of HERK1 gene expression in wild-type, *herk1* and *herk1 anj* plants, and *ANJ* gene expression in wild-type, *anj* and *herk1 anj* plants. RNA was extracted from multiple inflorescences from three plants per line. Data presented are means ± SD. $^{**}P < 0.01$; $^{***}P < 0.001$ (Student's *t*-test).

Source data are available online for this figure.

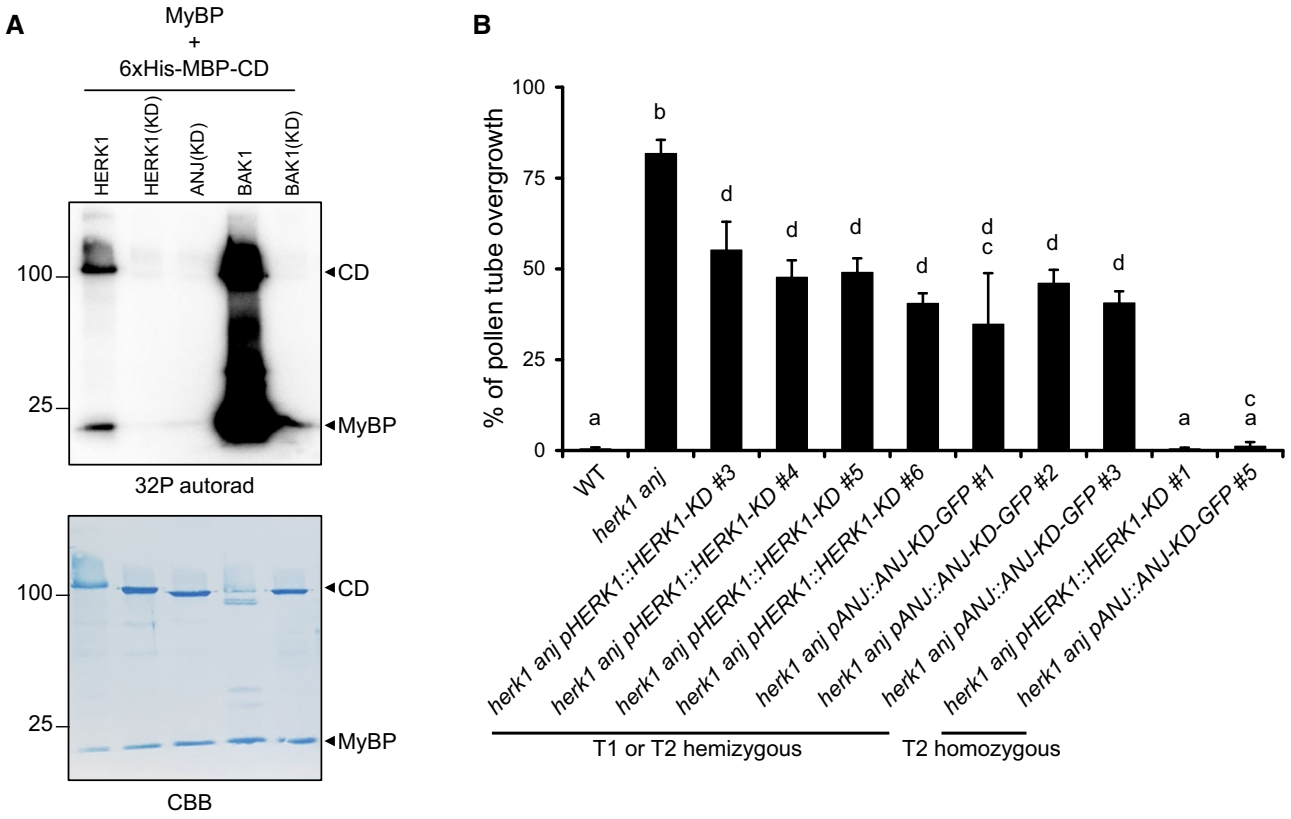


Figure EV2. Kinase activity of HERK1 and ANJ is not required for complementation of the pollen tube overgrowth phenotype.

A Kinase activity was assayed for wild-type HERK1, wild-type BAK1 (positive control) and kinase-dead versions (KD) of HERK1, ANJ and BAK1 using ^{32}P incorporation into myelin basic protein (MyBP; trans-phosphorylation) and the cytosolic domains of the receptor kinases (CD; auto-phosphorylation). Coomassie Brilliant Blue (CBB) staining of the membrane is shown below as a loading control.

B Percentage of pollen tubes displaying overgrowth at the female gametophyte in WT, *herk1 anj* plants and at least four 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 ($n \geq 3$). Data presented are means ± SD (one-way ANOVA followed by Bonferroni's *post hoc* test; letters mark statistically significant differences between samples, $P < 0.05$). *pANJ::ANJ-KD-GFP* T1 line 4 was excluded from the figure as it likely had multiple T-DNA insertions.

Source data are available online for this figure.

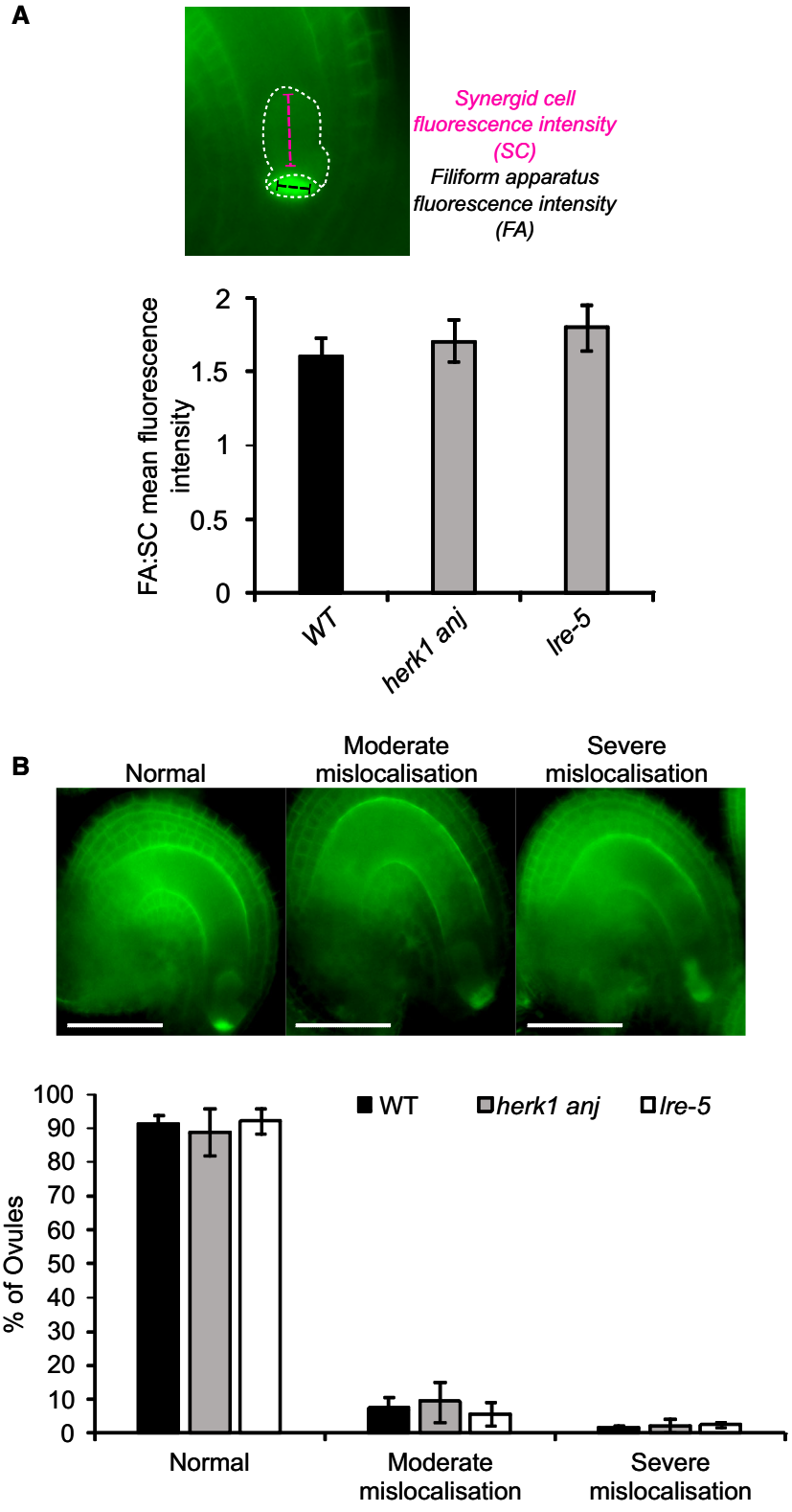


Figure EV3. Quantification of FER-GFP mislocalisation in the synergid cells of *herk1 anj* and *lre-5* ovules.

A Ratio between fluorescence intensities at the filiform apparatus (FA) and the synergid cell cytoplasmic region (SC) in mature ovules from wild-type (Col-0), *herk1 anj* and *lre-5* emasculated flowers expressing *pFER::FER-GFP*. Fluorescence profiles for each region of the synergid cells were recorded as exemplified in the upper panel and averaged prior to the ratio calculation. At least 23 ovules obtained from three siliques per plant were scored for three plants per line, with means per plant (n = 3) used for the Student's t-tests ($P > 0.05$). Data presented are means ± SD.

B Quantification of moderate and severe mislocalisation defects in the accumulation of FER-GFP at the filiform apparatus in mature ovules from wild-type (Col-0), *herk1 anj* and *lre-5* emasculated flowers expressing *pFER::FER-GFP*. Ovules with clear FER-GFP expression were assigned to one of the three categories presented in the upper panel, as per Ref. [76]. No statistically significant differences were detected in Student's t-test comparisons with wild type. At least 23 ovules obtained from three siliques per plant were scored for three plants per line, with means per plant (n = 3) used for the Student's t-tests ($P > 0.05$). Data presented are means ± SD.

Source data are available online for this figure.

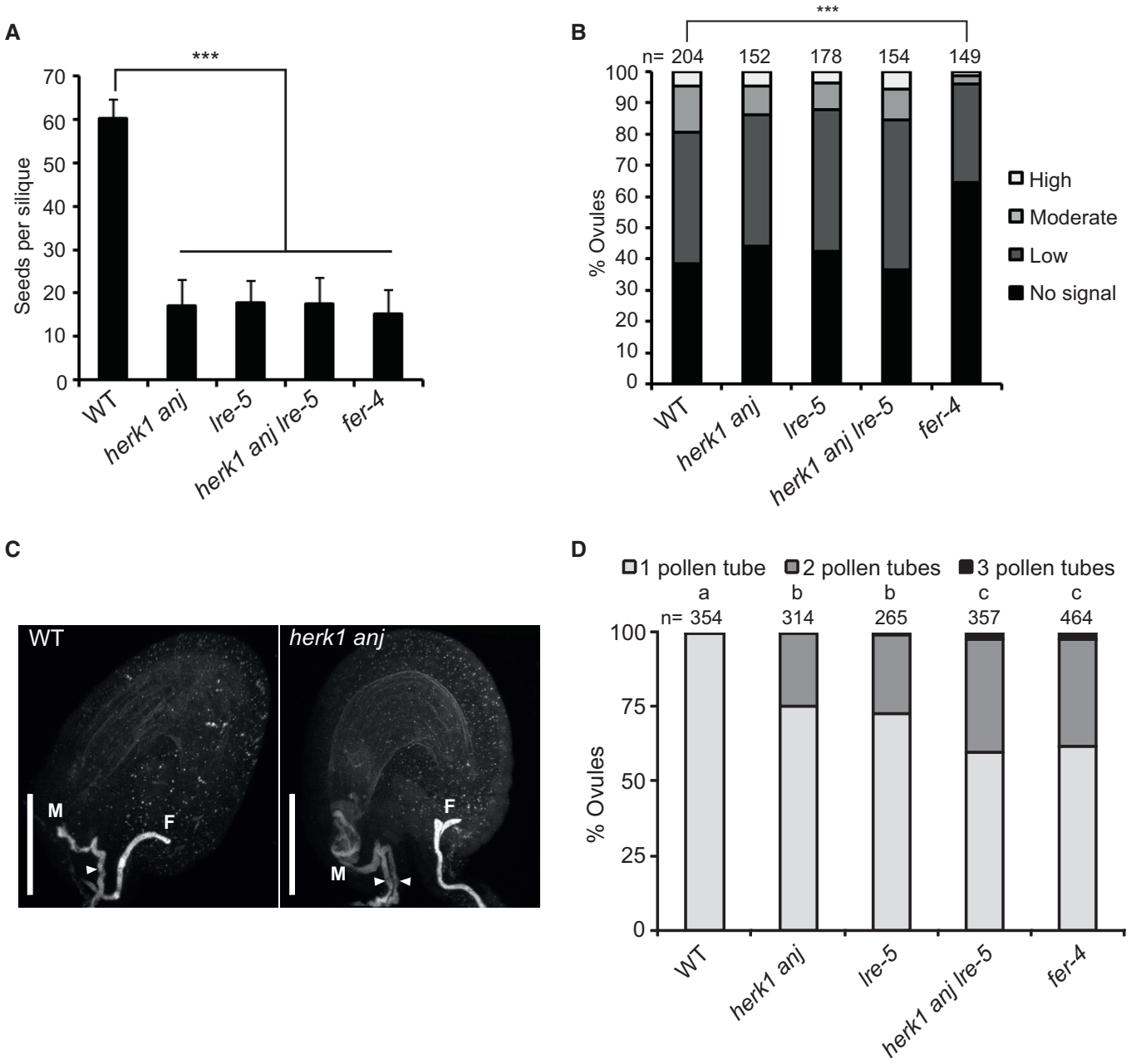


Figure EV4. HERK1, ANJ and LRE do not act additively in seed set or ROS production, but mutants attract multiple pollen tubes.

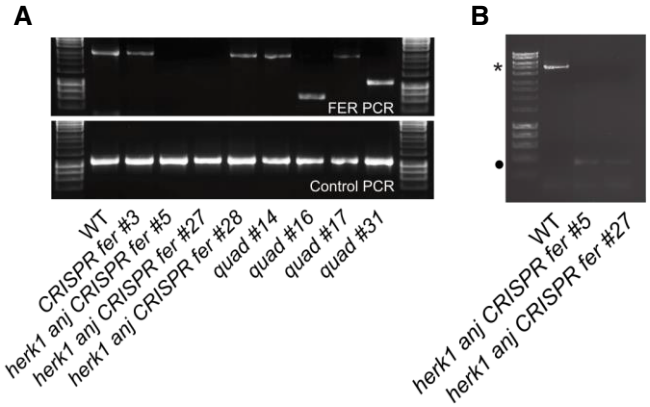
A Quantification of developing seeds per silique in wild-type, *herk1 anj*, *lre-5*, *herk1 anj lre-5* and *fer-4* plants. Fully expanded siliques were dissected and photographed under a stereomicroscope. $n = 25$. Data presented are means ± SD. ***$P < 0.001$ (Student's *t*-test).

B Quantification of the H_2DCF-DA staining of ROS in ovules from wild-type, *herk1 anj*, *lre-5*, *herk1 anj lre-5* and *fer-4* plants at 20 HAE. Categories are listed in the legend (see also Appendix Fig S7A). Ovules dissected from at least five siliques per line. ***$P < 0.001$ (χ^2 tests).

C Representative image of a normal pollen tube reception event in a wild-type ovule by confocal microscopy on the left and a *herk1 anj* ovule displaying pollen tube overgrowth and multiple pollen tubes in the micropyle on the right. 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.

D Polytubey quantification in wild-type (Col-0), *herk1 anj*, *lre-5*, *herk1 anj lre-5* and *fer-4* ovules by epifluorescence microscopy following hand pollination at 24 h after emasculation. Ovules from 10 to 13 siliques per line were scored for the number of pollen tubes present at the micropyle if fertilised (total fertilised ovules analysed per line > 265). Letters (a, b, c) mark statistically significant differences between samples in multiple Fisher's exact test pairwise comparisons ($P < 0.001$).

Source data are available online for this figure.



C

fer CRISPR line	Change in FER mRNA
fer #3	1 nt insertion (A) at 570 nt (Y120*)
herk1 anj fer #5	Large deletion/rearrangement (see panel B)
herk1 anj fer #27	Large deletion/rearrangement (see panel B)
herk1 anj fer #28	Inversion 714-2480 nt (FER 1-239 + 57 aa then *)
herk1 anj lre fer #14	1 nt insertion (T) at 570 nt (FER 1-120 + 45 aa frame shift then *)
herk1 anj lre fer #16	Δ570-2781 nt
herk1 anj lre fer #17	1 nt insertion (C) at 570 (FER 1-120 + 45 aa frame shift then *)
herk1 anj lre fer #31	Δ925-2690 nt

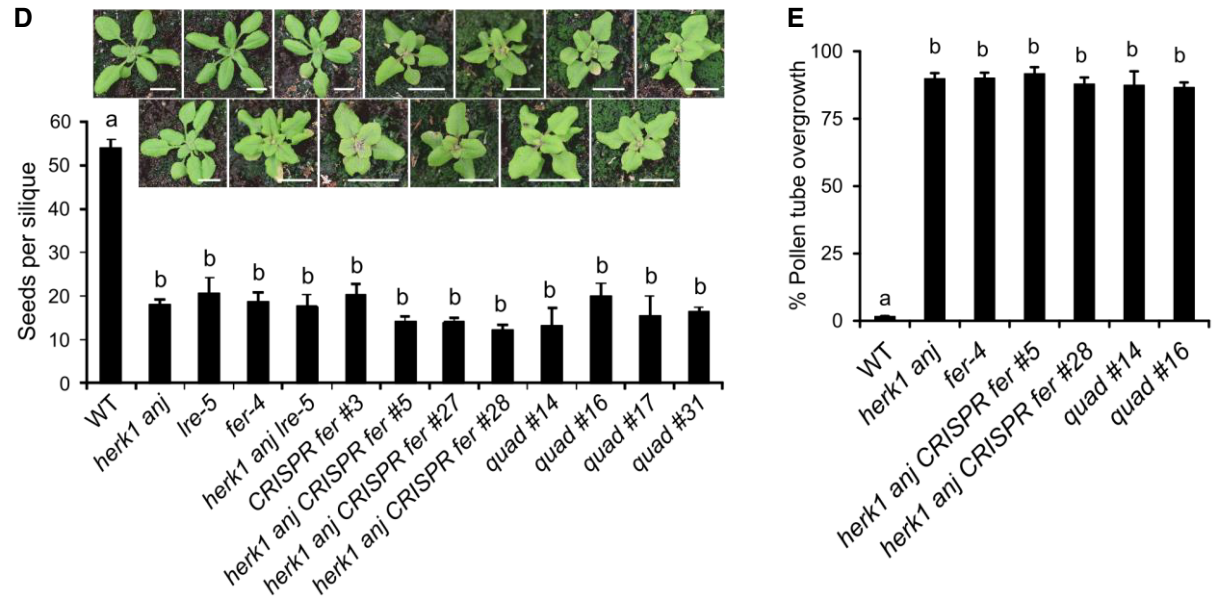


Figure EV5. Quantification of seed set in CRISPR-Cas9 *fer* mutants.

A PCR amplification of *FER* and control genomic DNA from wild-type and CRISPR-Cas9 *fer* mutants.

B For *herk1 anj CRISPR fer* lines 5 and 27, PCR of the *FER* locus using primers 1.7 kb upstream and 1.1 kb downstream of the *CRISPR* target sites (CRISPR-Cas9 *fer* mutant genotyping outer primers) was also performed. The expected 5.1 kb band from the wild-type Col-0 plant is indicated by an asterisk. The band indicated by a black dot was cloned and sequenced but does not contain *FER* DNA and is therefore an artefact, leading to the conclusion that *herk1 anj CRISPR fer* lines #5 and #27 contain large deletions or rearrangements that extend beyond the targeted region.

C Molecular characterisation of the *CRISPR* lines.

D, E Developing seeds per silique (D) and pollen tube overgrowth (E) in wild-type, single, double, triple and quadruple mutants as listed. Quad = *herk1 anj lre-5* CRISPR *fer*. Fully expanded siliques were dissected and photographed using an SLR camera. Three plants per line and five siliques per plant were analysed. Data presented are means per plant (n = 3) ± SD. Letters (a, b) mark statistically significant differences between samples in one-way ANOVA analysis followed by Bonferroni's *post hoc* comparison of means ($P < 0.05$). Pictures above (D) are of plants at 21 days after sowing. Scale bars = 1 cm.

Source data are available online for this figure.