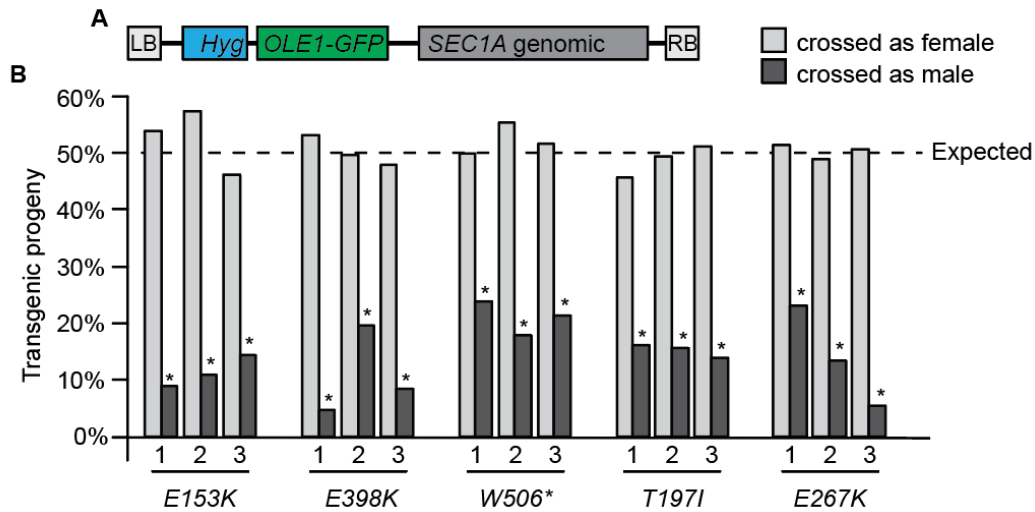
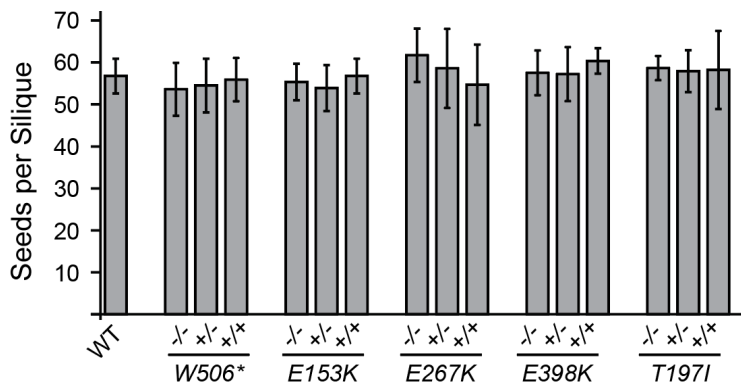


Supplemental Material



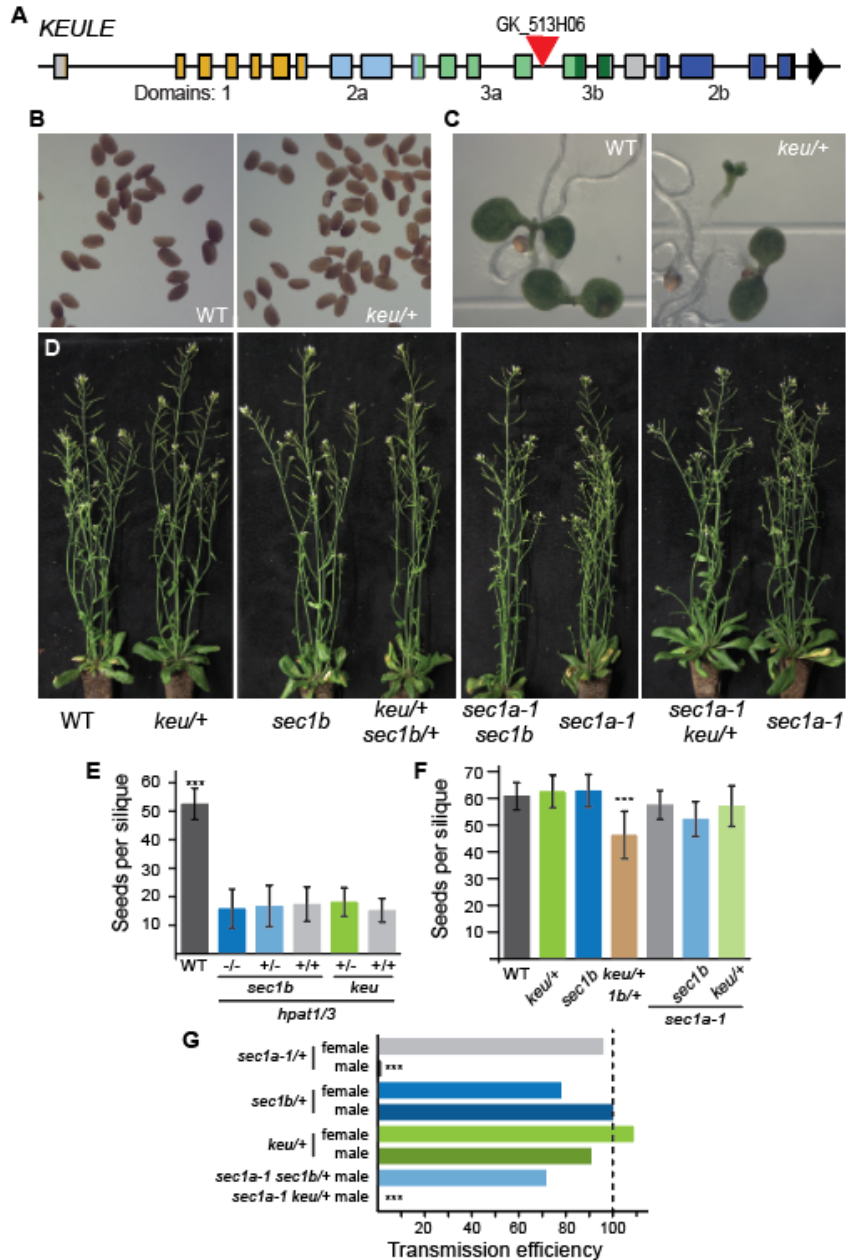
Supplemental Fig. 1 Rescue of suppression of the *hpat1/3* phenotype by the WT *SEC1A* genomic sequence.

A) In order to assay for rescue, we generated a transgene containing the WT genomic *SEC1A* sequence (5.2 kb) with a reporter construct including the *OLE1-GFP* reporter (Shimada et al., 2010) which confers green fluorescence to seeds carrying the transgene. LB and RB are left and right border, Hyg is hygromycin resistance. **B**) The *SEC1A* transgene construct was transformed into each of the five suppressor lines (fully homozygous *hpat1/3 sec1a* plants). In the T1 generation, three transgenic lines per family were reciprocally crossed to WT and the inheritance of the transgene was scored in the cross progeny based on seed fluorescence. In all cases, the transgene transmitted as expected through the female (N=47 to 164), but had significantly reduced transmission through the male (N=28 to 148), as expected for a transgene rescuing an improved pollen fertility suppressor mutant. The dashed line at 50% marks the expected value for a transgene with no impact on transmission. ** marks statistically significant deviation from the expected 50% transgene transmission, χ^2 P-value<0.005.



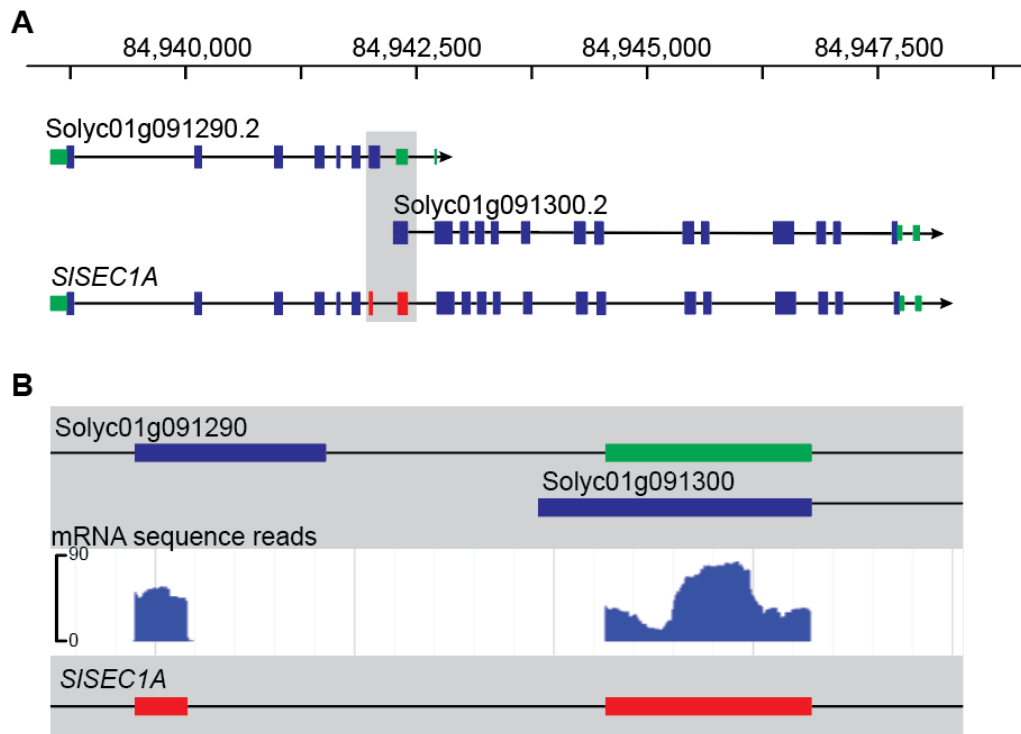
Supplemental Fig. 2 Seed set is maintained in *sec1a* mutants in the WT background

The suppressor lines were outcrossed to WT to remove the *hpat1* and *hpat3* mutations, plants were grouped based on *sec1a* genotype and the number of seeds per silique counted (mean \pm SD, N \geq 10 per genotype). No statistically significant differences were found between WT and any other sample by T-test.



Supplemental Fig. 3 The impact of *keule* and *sec1b* on seed set and fertility

A) The structure of the *KEULE* locus with the position of the insertion in the GABI-Kat line marked by red arrow. **B)** Seeds segregating from *keule/+* plants are often malformed and germinate into malformed seedlings (**C**). **D)** Whole plant pictures of the indicated genotypes. WT, *keule/+*, *sec1b* and *keule/+ sec1b/+* plants are siblings from a segregating population as are the two following pairs of plants. **E** and **F)** Mean number of seeds per silique (\pm SD) for the indicated genotypes. Within the *hpat1/3* background, neither the *sec1b* nor *keule* mutation significantly altered seed set compared to *hpat1/3* sibling plants (**E**). In the WT background, the only statistically significant difference in seed set was a reduction in *sec1b/+ keule/+* plants, consistent with their redundant function in ovule viability (**F**; Karnahl et al., 2018). **G)** Transmission efficiency of SM mutants determined by crossing the indicated genotypes and genotyping the resulting progeny. No single SM mutant had a statistically significant change in transmission through the female. *sec1a* male transmission was significantly reduced relative to the expected value (100%) and the transmission of the *sec1a keule* double mutant was abolished. *** marks χ^2 P-value <0.0005.



Supplemental Fig. 4 The modified tomato *SEC1A* gene model

A) Gene model diagrams for *SISEC1A* based on the tomato ITAG 2.4 genome release. Coding exons are in blue, untranslated regions are in green and the modified exons are in red. Zoom of the gray box region is shown in **B** along with mRNA sequence reads supporting the alterations to the exons.