

Supplemental Data

for

A plant peroxisome lipase, SSD5, is required for steryl ester accumulation and activation of autoimmunity

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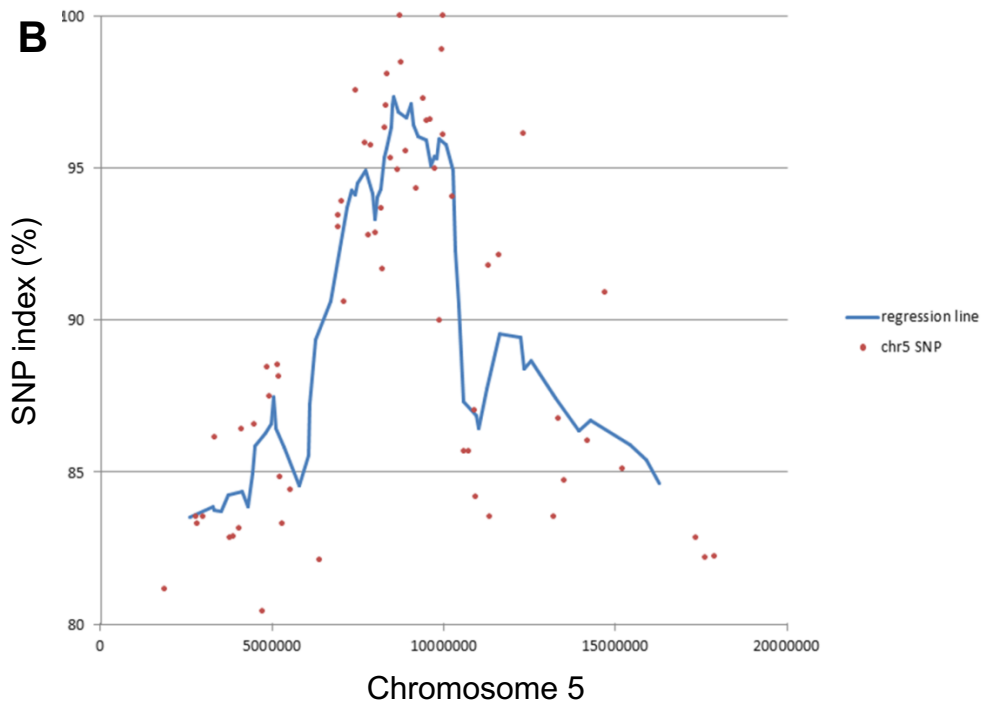


Figure S1. Identification of *ssd5-1* mutation by MutMap. **A**, Examples of segregating F₂ plants from the cross, *pen1 syp122 ssd5-1* x *pen1 syp122*. Partially rescued plants marked with yellow sticks. **B**, Genome sequencing of DNA pooled from 30 partially rescued plants revealed a chromosome interval in which the mutant SNP-index is approaching 100%. Here, At5G22370, At5G24200 and At5G24320 had non-synonymous SNPs in exons. To test which of these mutations were responsible for the rescue of *pen1 syp122*, we crossed a T-DNA insertion mutation in At5G24320 into *pen1 syp122*, and we transformed *pen1 syp122 ssd5-1* with constructs for 35S-driven expression of the At5G22370 and At5G24200-encoded proteins.

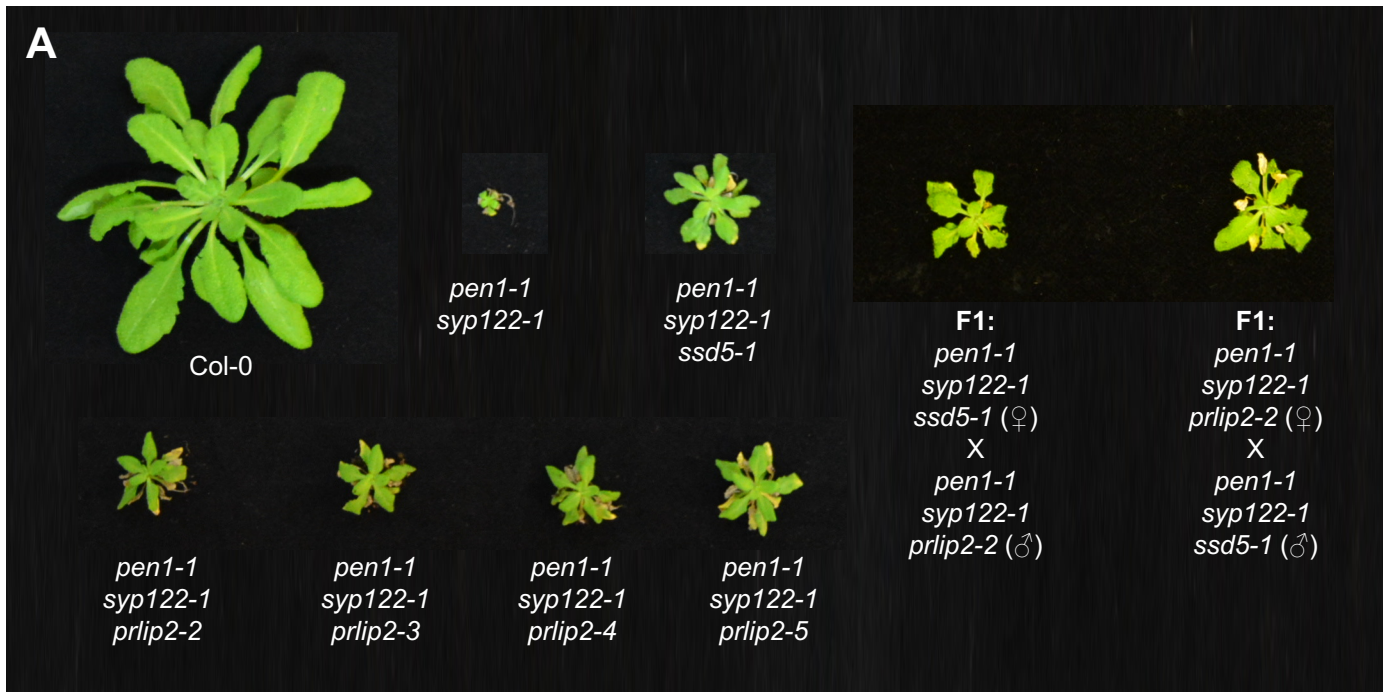


Figure S2. Additional *ssd5* alleles generated by CRISPR/Cas9. A, Four CRISPR/Cas9 *ssd5* indel alleles rescue *pen1 syp122* to the same level as the *ssd5-1* EMS allele. Note the complementary test cross (right) confirming *SSD5* and *PRLIP2* to be the same gene, therefore, mutant alleles of *prlip2-2*, *prlip2-3*, *prlip2-4* and *prlip2-5* are also called *ssd5-2*, *ssd5-3*, *ssd5-4* and *ssd5-5* in this study. **B,** Sequence analysis of four CRISPR/Cas9 *prlip2* indel alleles.

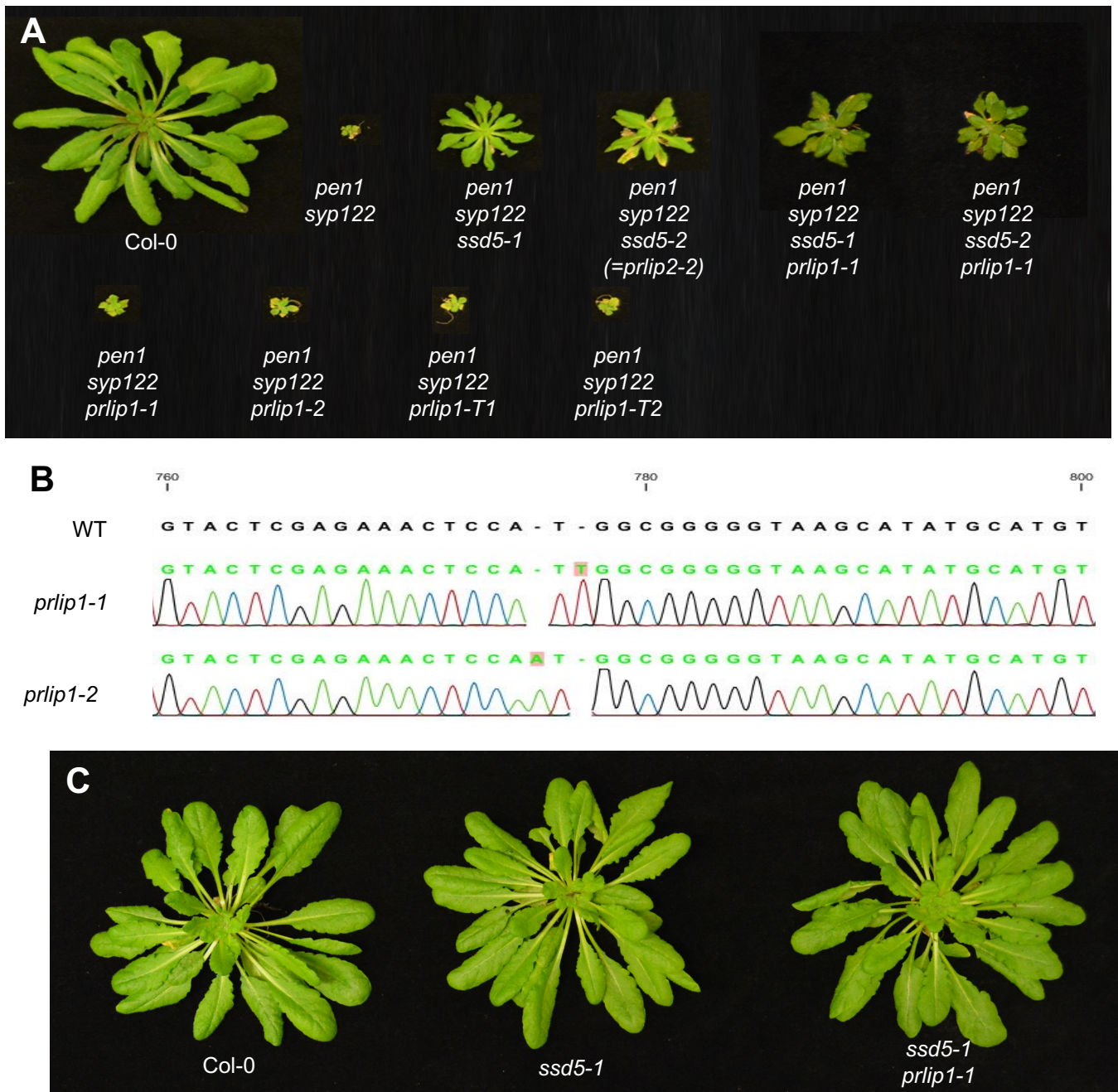


Figure S3. *PRLIP1* is not required for the LMM phenotype of *pen1 syp122*. **A**, Two CRISPR/Cas9 *prlip1* indel alleles and two *prlip1* T-DNA insertion alleles (*prlip1-T1* (SALK_204525C) and *prlip1-T2* (SALK_206804C)) do not rescue *pen1 syp122*, unlike *ssd5* mutant alleles. **B**, Sequence analysis of two CRISPR/Cas9 *prlip1* indel alleles. **C**, No apparent growth phenotype is observed for *ssd5-1 prlip1-1*.

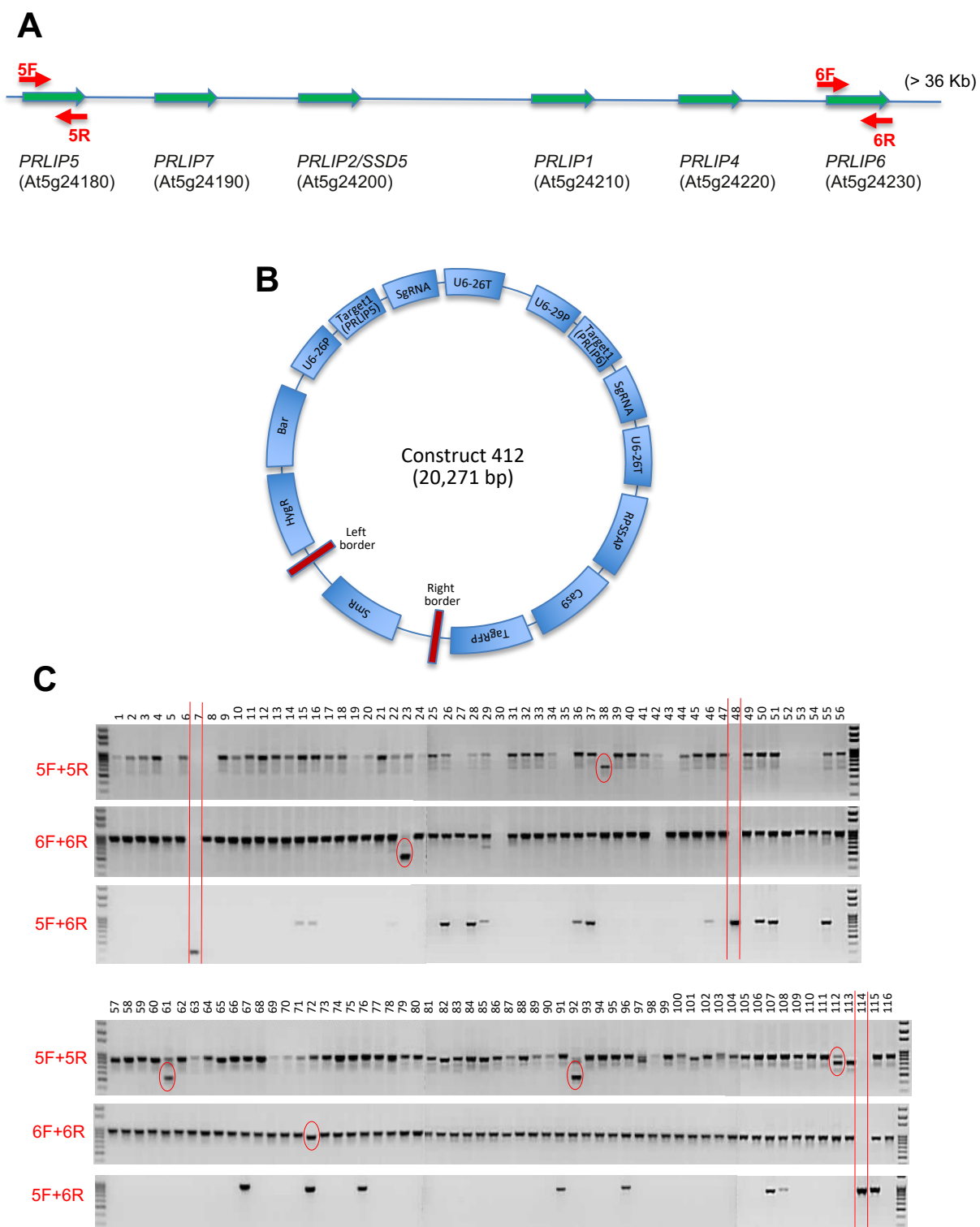


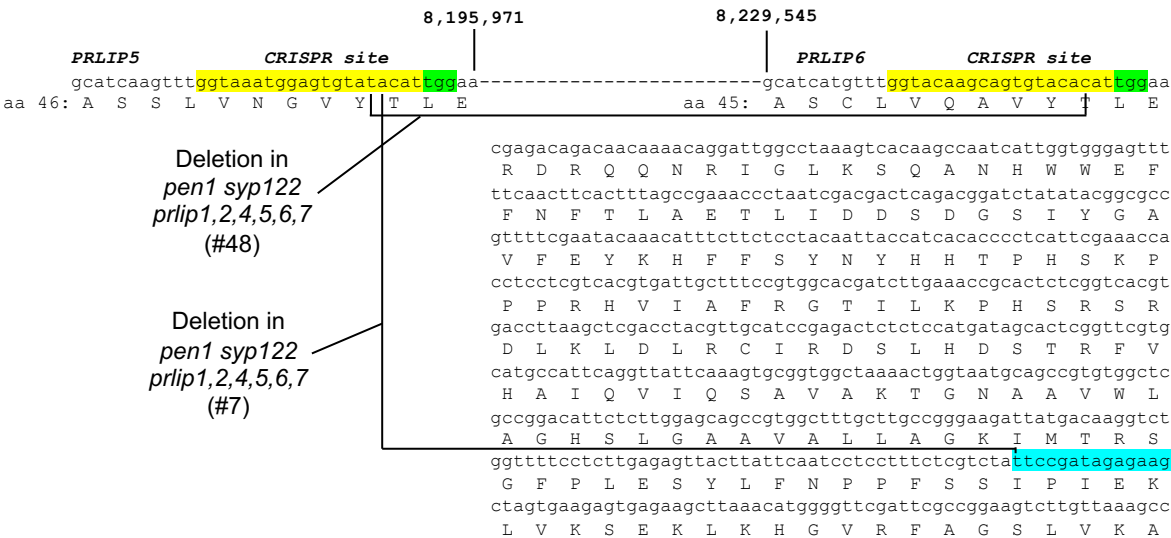
Figure S4. See legend below.

D

Plant #7



Plant #48



E

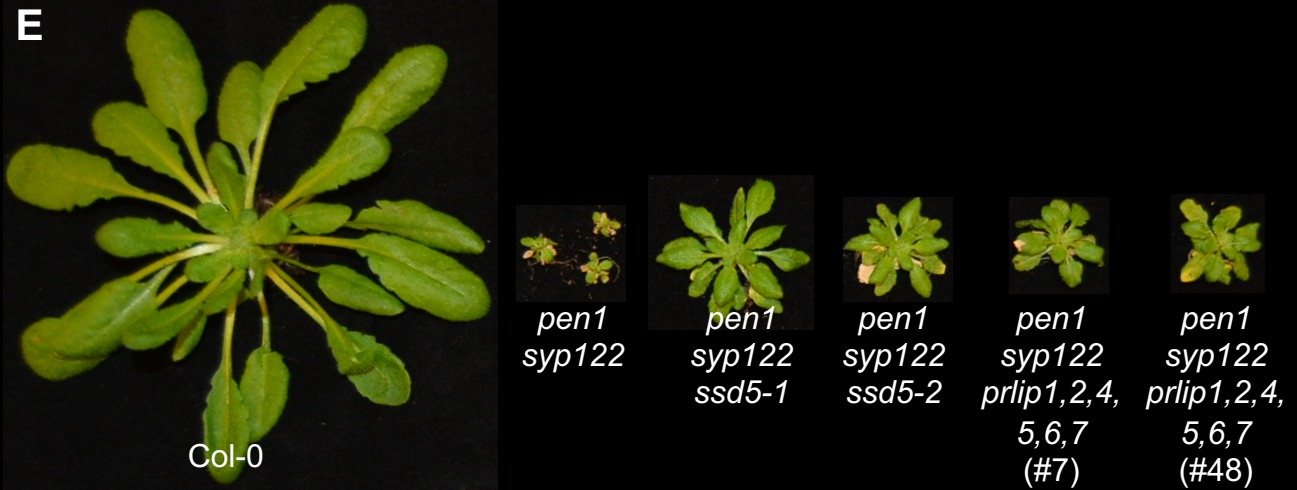


Figure S4. *SSD5* is the only gene in the *PRLIP* gene cluster required for the *pen1 syp122* LMM phenotype. A, The *PRLIP* gene cluster. See also Jakab et al. (2003). B, Double CRISPR/Cas9 T-DNA plasmid construct for targeting both *PRLIP5* and *PRLIP6* (not to scale), developed from pKI1.1R (Tsutsui et al., 2017) and pHEE2E-TRI (Wang et al., 2015). C, PCR-based selection of three *PRLIP* gene cluster deletion lines, #7, #48 and #114. D, Sequencing confirmation of lines #7 and #48. E, *pen1 syp122* LMM rescuing effect of *ssd5* mutations and of *PRLIP* gene cluster deletions.

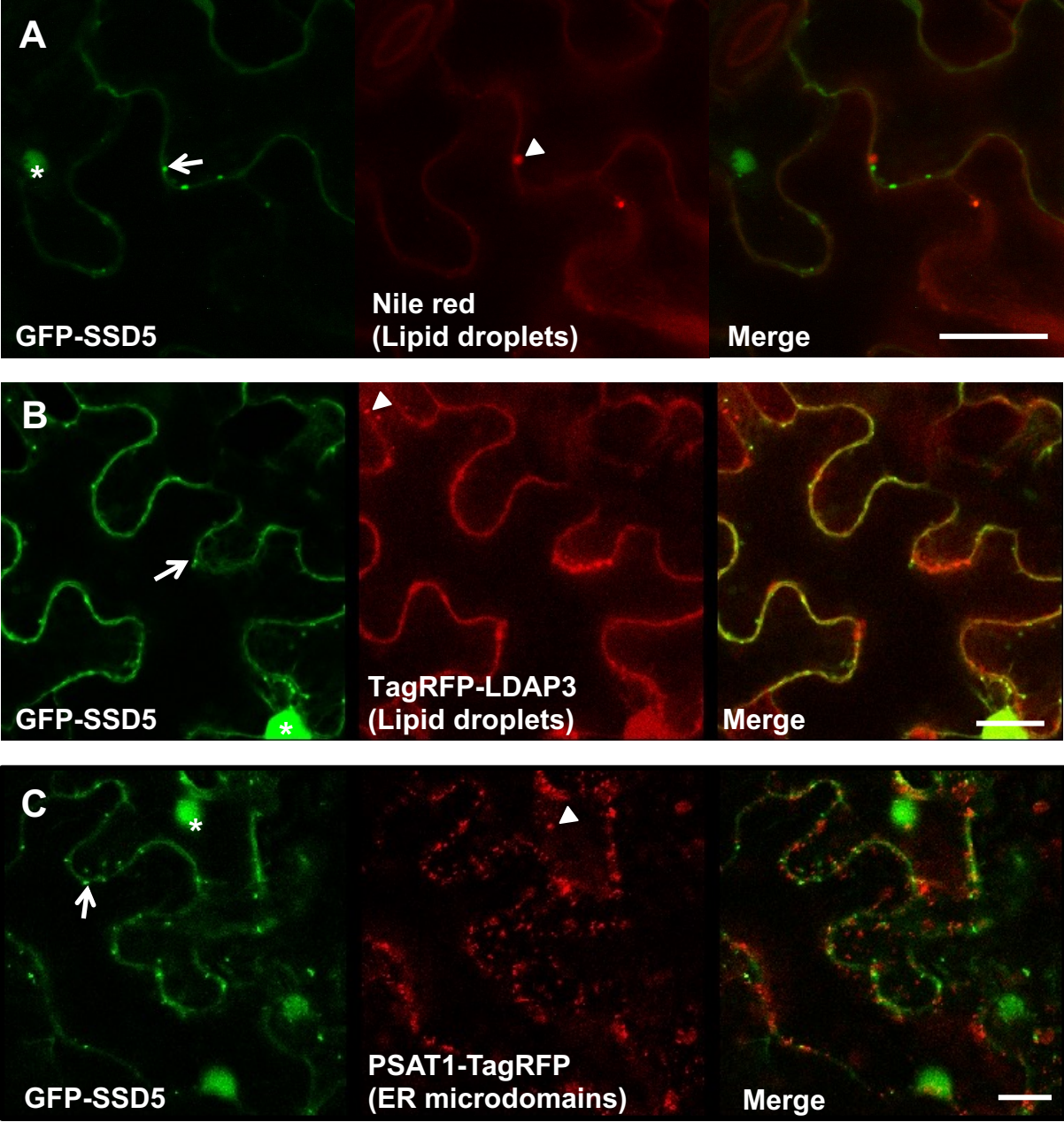


Figure S5. See legend below.

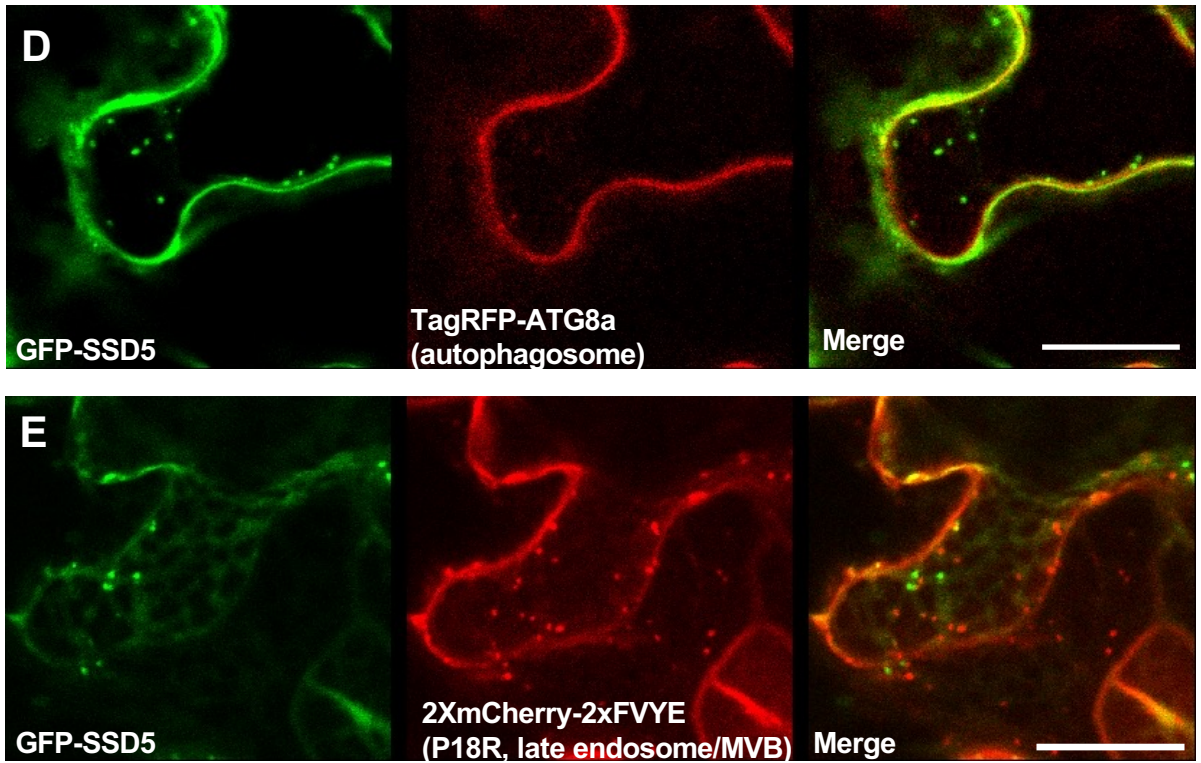


Figure S5. GFP-SSD5 does not co-localize with selected discrete structure markers. **A**, Leaf epidermal cells of two-week-old transgenic *Arabidopsis* seedlings *ssd5-1* expressing GFP-SSD5 were stained by Nile red (specific dye of sterol ester bodies). **B-E**, Leaf epidermal cells of *Nicotiana benthamiana* transformed with a construct for GFP-SSD5 and a constructs for the lipid droplet marker proteins, TagRFP-LDAP3 (Horn et al., 2013) (**B**) and PSAT1-TagRFP (Shimada et al., 2019) (**C**), a construct for the autophagosome marker protein, TagRFP-ATG8a (Dubiel et al., 2020) (**D**) and a construct for the late endosomes/multivesicular bodies, 2XmCherry-2xFYVE (Simon et al., 2014) (**E**). Size bars, 20 μ m.

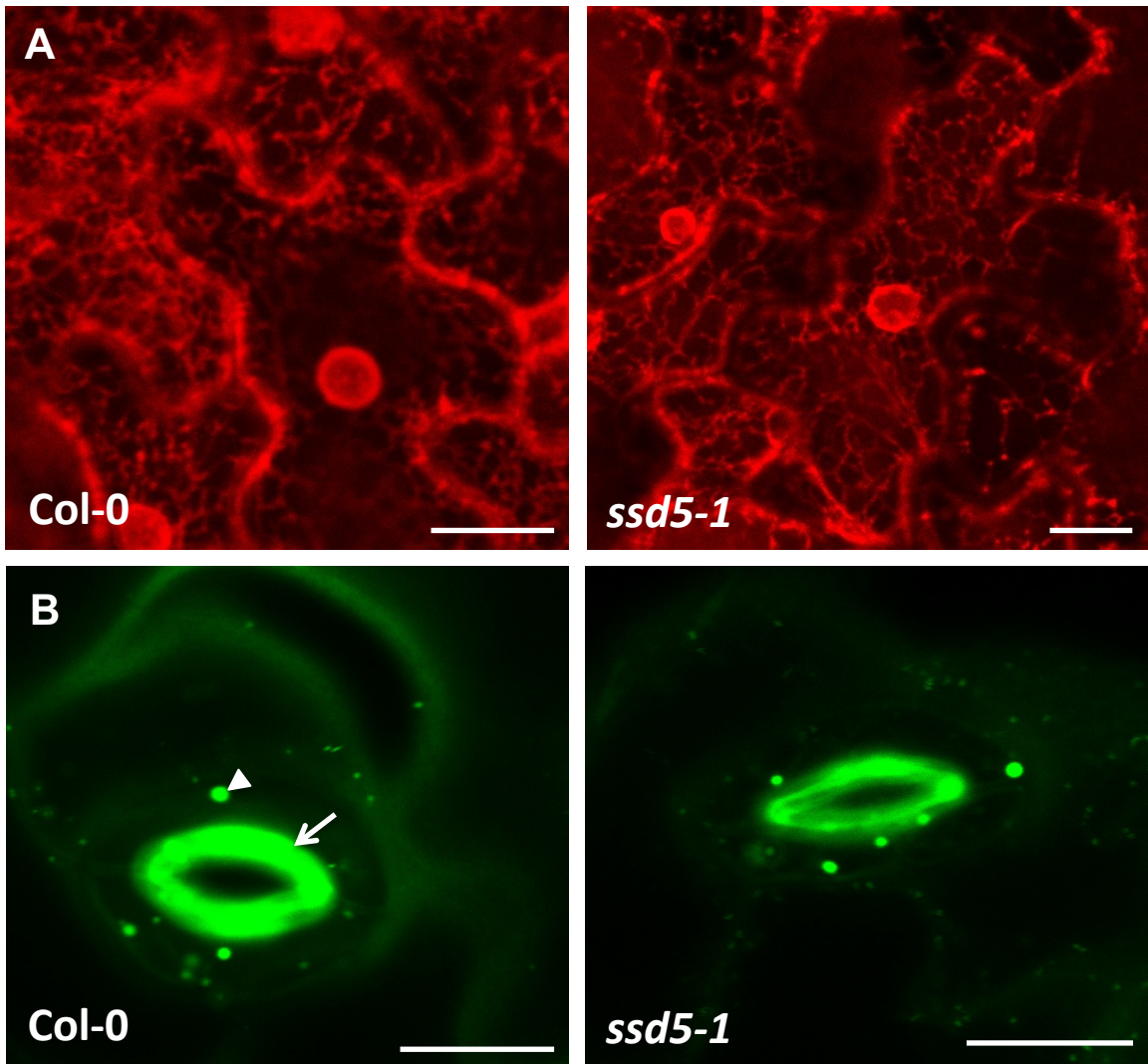


Figure S6. ER and peroxisomes are normal in *ssd5-1*. **A**, ER reticular structure labeled by SP-mCherry-HDEL. Size bars, 20 μm . **B**, N-bodipy labeling of peroxisome (arrowhead) in stomatal cells. Arrow, autofluorescence of cell wall towards stomatal pore. Size bars, 10 μm .

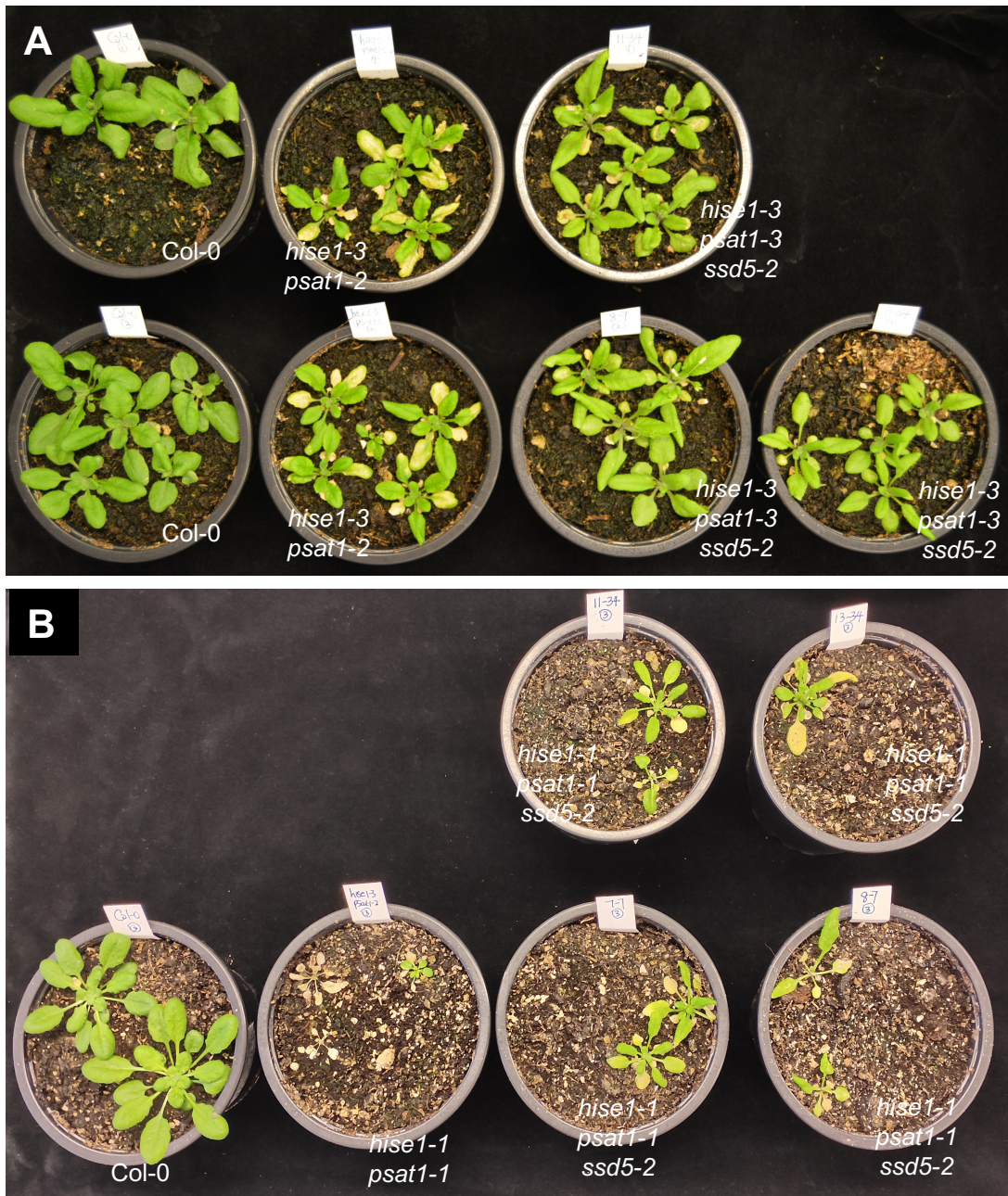


Figure S7. Mutating *SSD5* affects plant sterol homeostasis. Stress phenotype of *hise1-3 psat1-2* partially rescued by *ssd5-2*. **A**, four-week-old plants grown at 16 h day. **B**, seven-week-old plants grown at 8 h day. **A** and **B**, seedlings were grown on ½ MS medium with 1% sucrose for 10 day before transfer to soil.

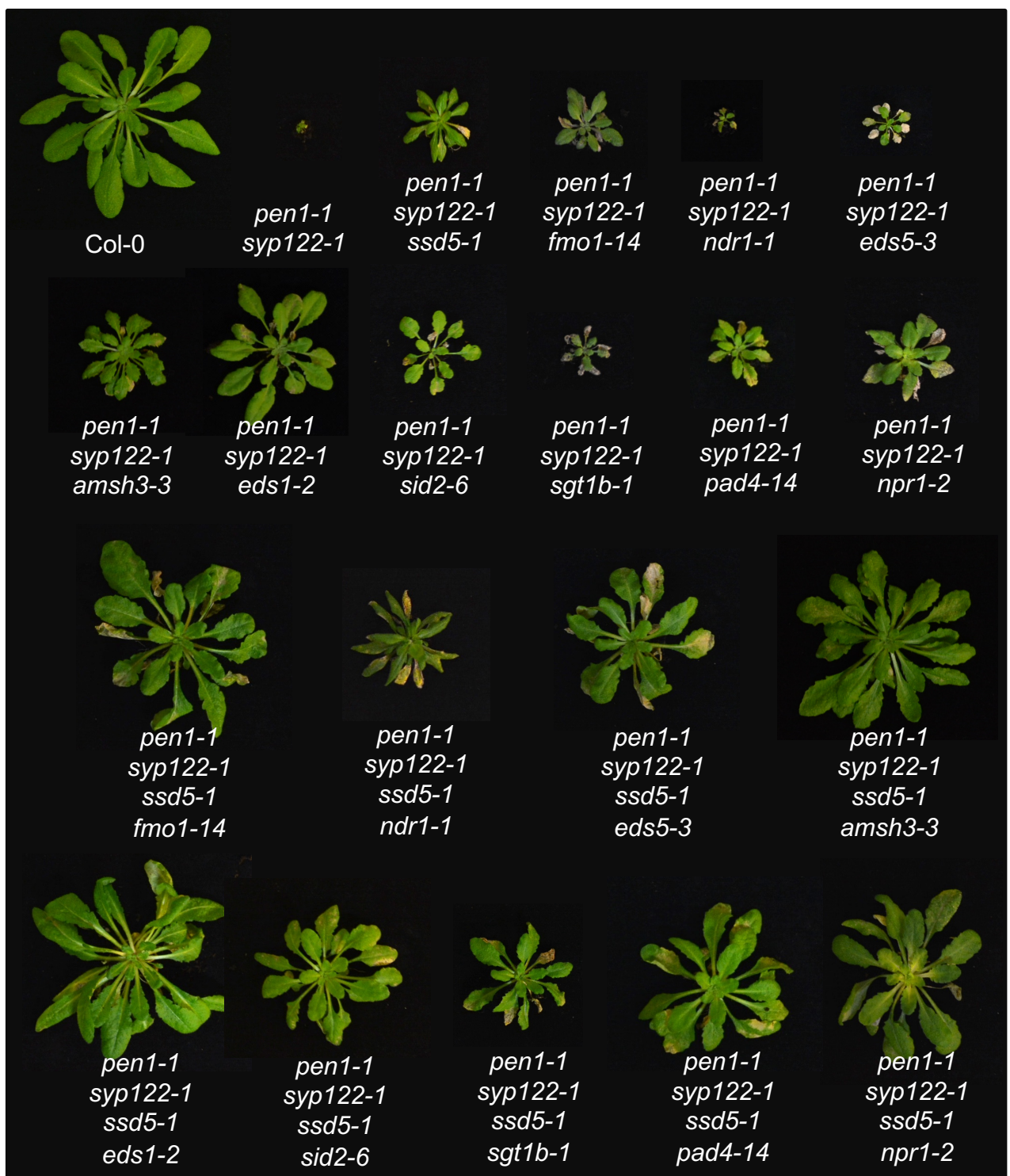


Figure S8. SSD5 contributes to the lesion mimic phenotype of *pen1 syp122* in addition to other signaling components. Combining *ssd5-1* and mutant alleles of known immunity signaling components in each case increase the rescue of the LMM phenotype of *pen1 syp122*. All rescued triple mutants, except *pen1 syp122 ssd5-1*, *pen1 syp122 fmo1-14* and *pen1 syp122 sid2-6* are described in Zhang et al. (2008). Rescued triple mutants with the novel mutant alleles, *fmo1-14* and *sid2-6*, are described in Supplemental Information. For the down-stream signaling roles of the following immunity components, see these references: NDR1 in CNL-activated ETI and EDS1 in TNL-activated ETI (Aarts et al., 1998), NPR1 in salicylic acid signalling (Ding et al., 2018), AMSH3 in CNL-activated ETI (Schultz-Larsen et al., 2018), EDS1 and PAD4 in PTI (Pruitt et al., 2021), EDS1 and PAD4 in TNL-activated ETI (Sun et al., 2021) and FMO1, NPR1, EDS1 and PAD4 in N-hydroxyphenylpyruvic acid and salicylic signalling (Zeier, 2021). See Kadota & Shirasu (2012) for the role of SGT1b in NLR stabilization. Plants grown for 6 weeks in short day conditions.

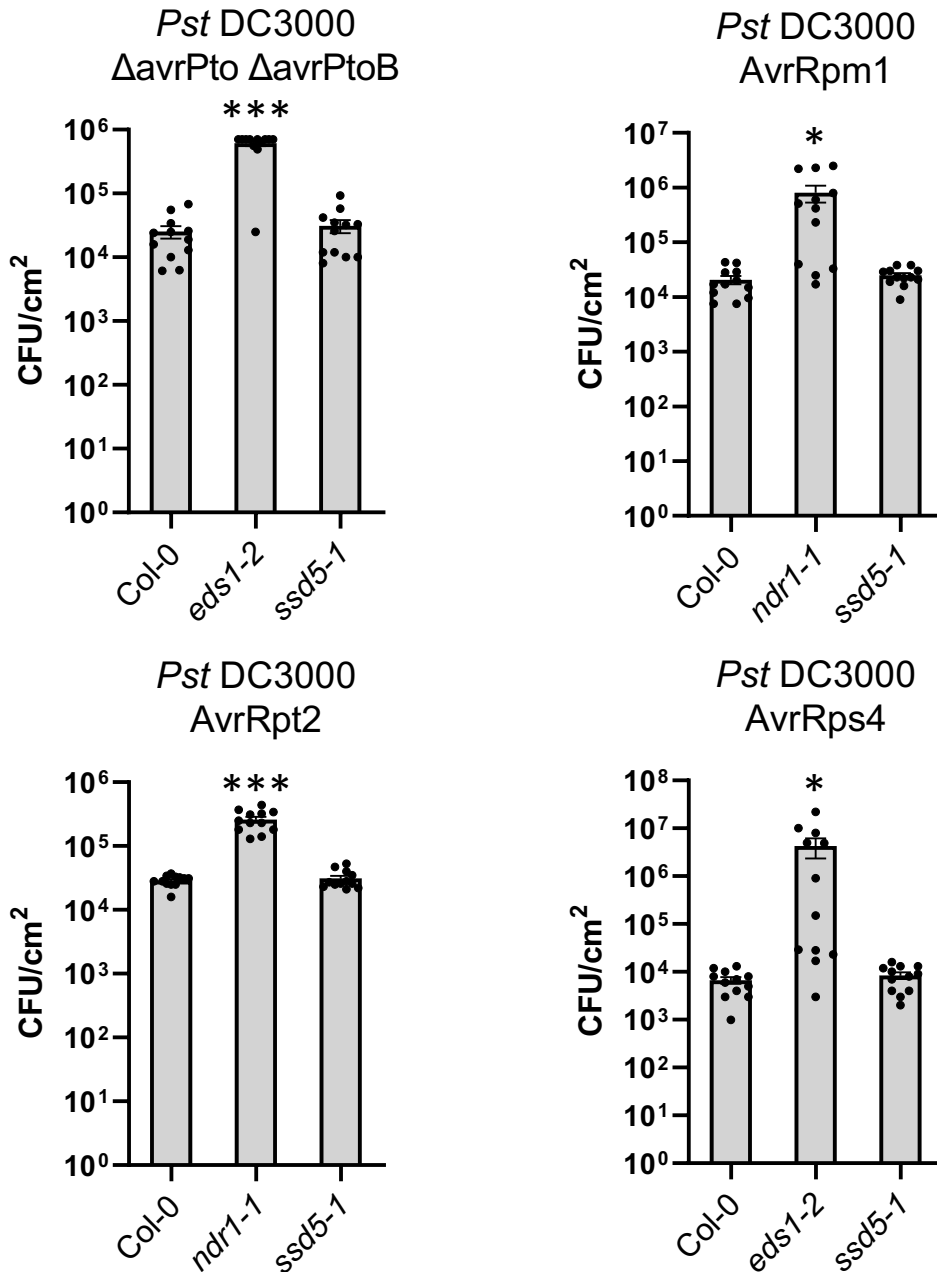
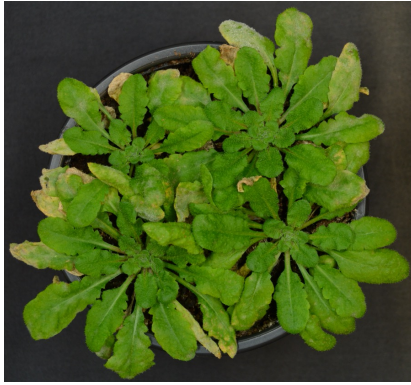


Figure S9. SSD5 is neither required for PTI nor for CNL or TNL-activated ETI. Plants of Col-0, *ssd5-1* and the positive control mutants, *eds1-2* and *ndr1-1* (Aarts et al., 1998), were spray inoculated with different version *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 bacteria, either mutated in or over-expressing specific effector genes. This way *ssd5-1*'s immunity was analysed. *Pst* DC3000 Δ avrPto Δ avrPtoB was used to test PTI (Lin & Martin, 2005), *Pst* DC3000 AvrRpm1 and *Pst* DC3000 AvrRpt2 were used to test CNL-mediated ETI (Grant et al., 1995; Kunkel et al., 1993) while *Pst* DC3000 AvrRps4 was used to test TNL-mediated ETI (Hinsch & Staskawicz, 1996). Differences in bacterial growth (3 days after inoculation) were analyzed by Student's T-test. *, $P < 0.05$; ***, $P < 0.001$; error bars, SE.



Col-0



ssd5-1



eds1-2

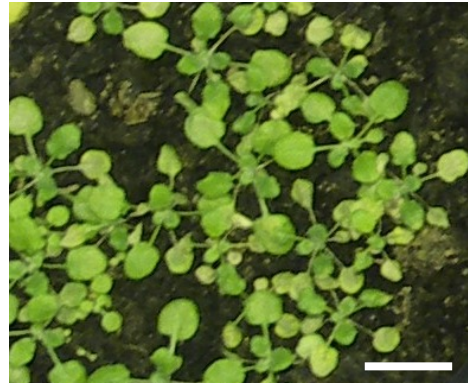
Figure S10. *ssd5-1* has normal susceptibility to powdery mildew. Powdery mildew symptoms 10 days after inoculation with *Golonovinomycetes orontii*. *eds1-2* used as hypersusceptible control.

Supplemental Information

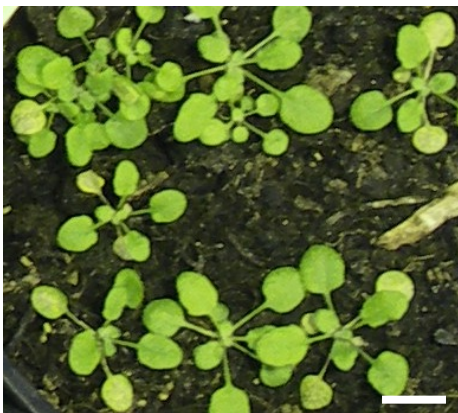
New mutant alleles of *SID2* and *FMO1* rescue *pen1 syp122* LMM phenotype. *sid2-6* and *fmo1-14* were identified in a population of EMS mutants that were partially rescued in the *pen1-1 syp122-1* LMM phenotype. The population is described in Zhang et al. (2008). The allelisms to *SID2* and *FMO1* were demonstrated in complementation crosses to *pen1-1 syp122-1 sid2-1* and *pen1-1 syp122-1 fmo1-1* (see F₁ plants below). The allele namings, *sid2-6* and *fmo1-14*, were made following those in van Wees & Glazebrook (2003) and Zhang et al. (2008), respectively.



Lesion mimic *pen1-1 syp122-1*
FMO1/fmo1-14 EDS5/eds5-3



Lesion mimic *pen1-1 syp122-1*
FMO1/fmo1-14 SID2/sid2-1



Partially rescued lesion mimic
pen1-1 syp122-1 sid2-6/sid2-1



Partially rescued lesion mimic
pen1-1 syp122-1 fmo1-1/fmo1-14

Plants grown for 6 weeks in short day conditions. Size bars, 1 cm.

The two mutants above show lesion mimic phenotype, the two at the below are rescued to different degree.

The *sid2-6* sequence has the G3266T nucleotide substitution in the genomic DNA. This is the last base pair of the last intron, affecting the splicing process.

The *fmo1-14* sequence has the G800A nucleotide substitution causing the R267K amino acid substitution.

Supplemental references

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