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Supplemental Information

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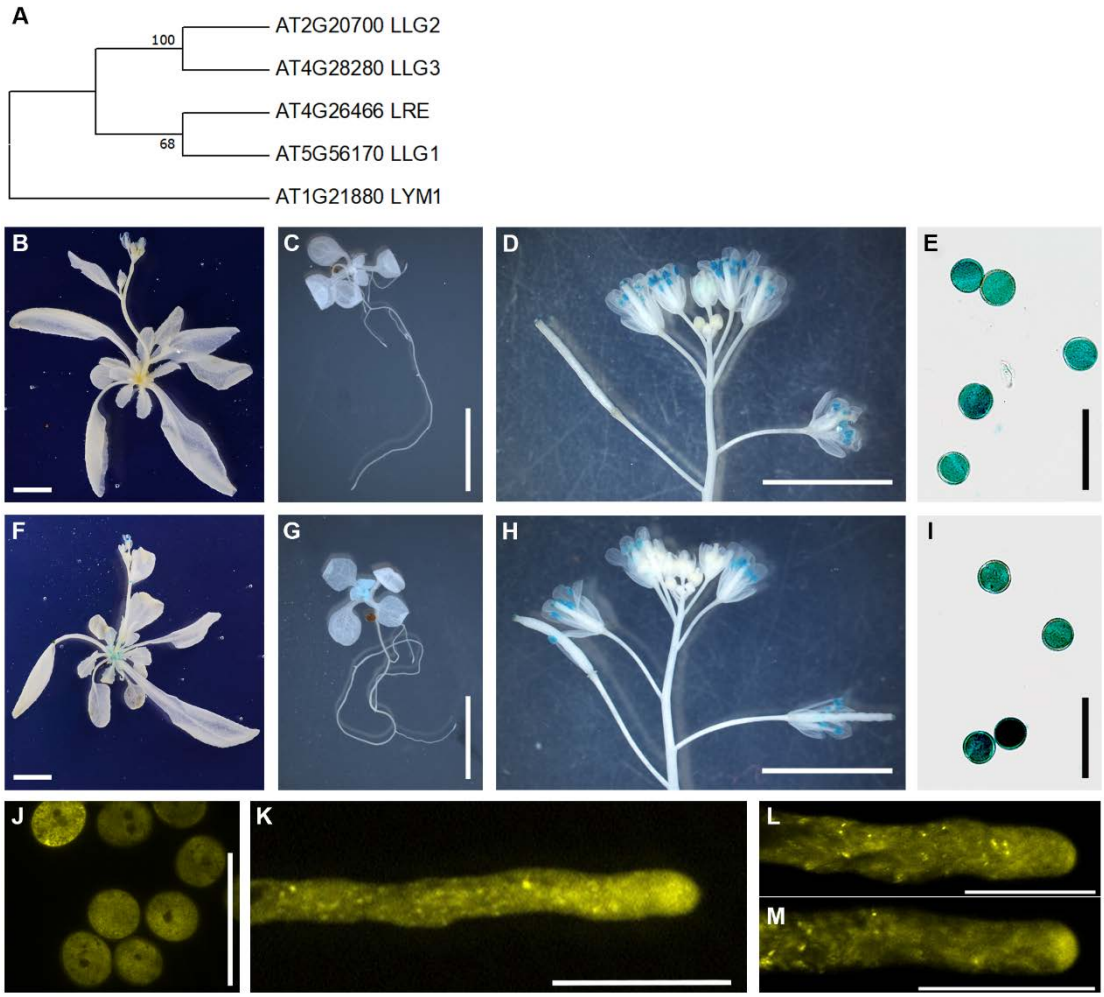

Figure S1. Expression pattern of *LLG2 and LLG3*. Related to Figure 1.

(A) Phylogenetic analysis was conducted using Neighbor-joining method by MEGA6. Values on the tree are bootstrap values.

(B-I) Promoter activities of *LLG2* **(B-E)** and *LLG3* **(F-I)** in mature plants, inflorescences, mature pollen grains and young seedlings. Images are representative for more than 10 plants analyzed each except for (**B**) and (**F**), where only three plants were observed in each group.

(J-M) Localization of YFP-LLG3 protein in Arabidopsis pollen grain (**J**) and tube (**K**) using their endogenous promoters, and mCitrine-LLG2 and mCitrine-LLG3 in tobacco pollen tubes (**L-M**) using the *LAT52* promoter.

Scale bars: 1cm **(B and F)**, 5 mm **(C-D and G-H)**, 50 μm **(E-J)**, 20 μm **(K-M).**

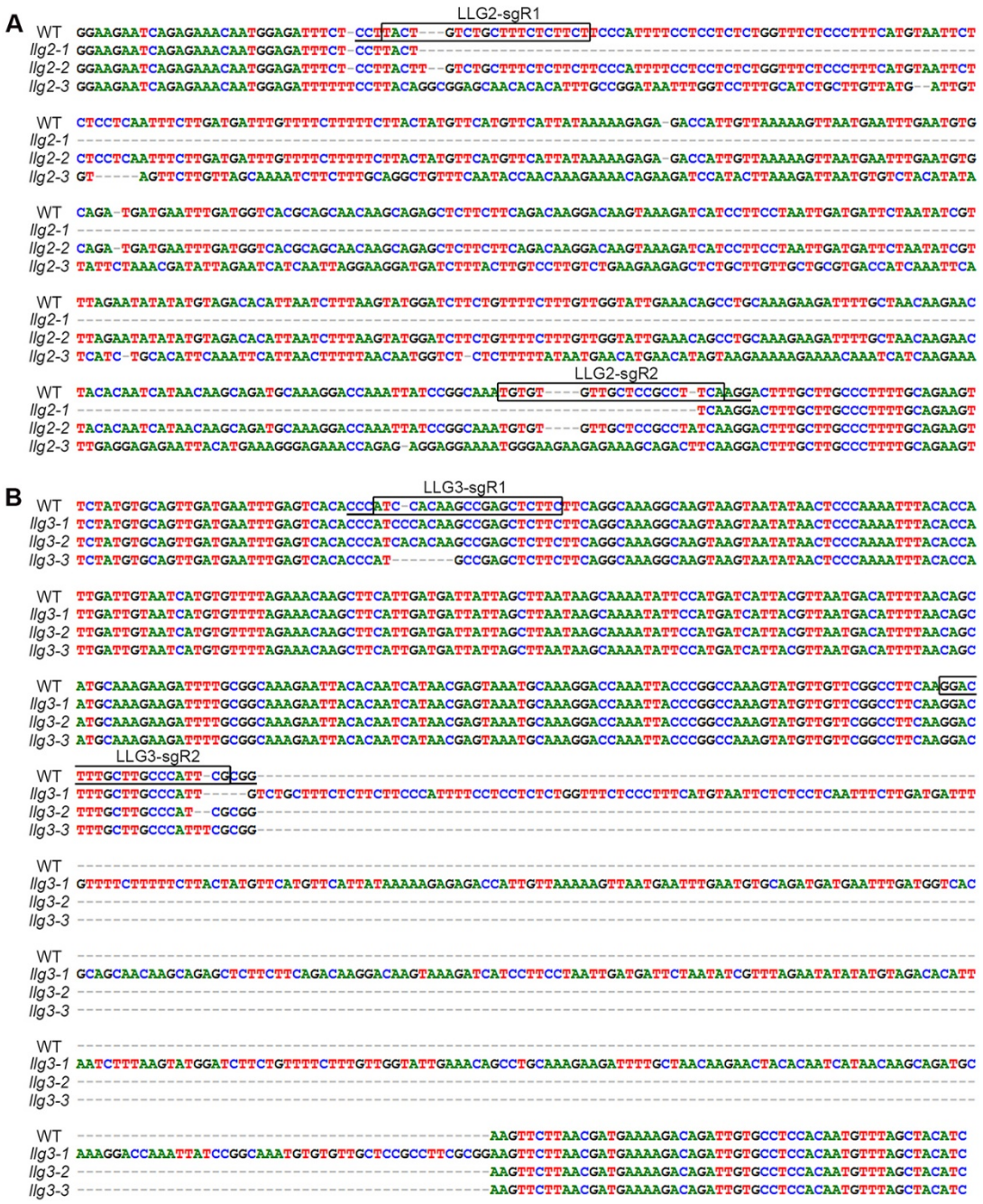

Figure S2. Sequences alignment of WT and *llg2llg3* mutants. Related to Figure 1.

PCR products containing the CRISPR/Cas9 targets in *LLG2* **(A)** and *LLG3* **(B)** were amplified and sequenced to detect mutations. Sequences framed in black are sgRNAs within gene loci. The sequence alignment for each group was performed by MEGA6.

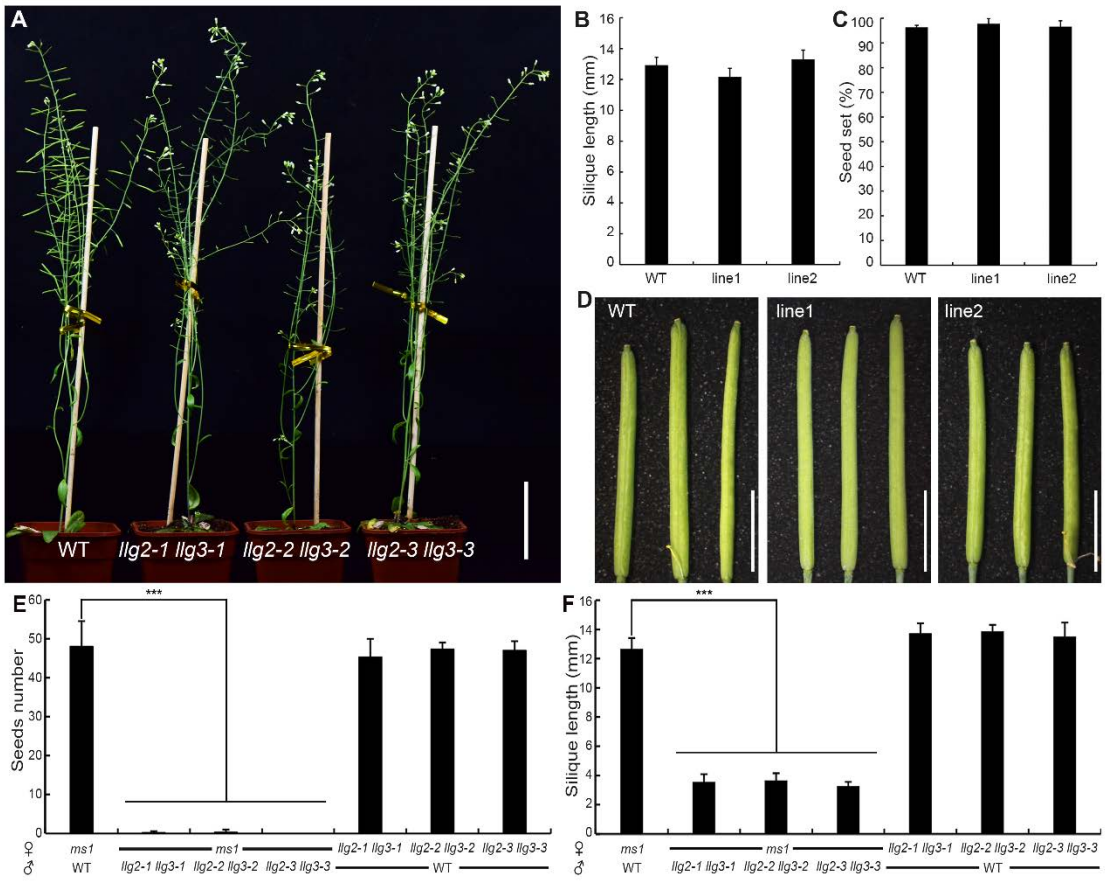

Figure S3. LLG2/3 contribute to plant fertility. Related to Figures 1 and 2.

(A) *llg2 llg3* double mutants exhibit normal vegetative growth but show fertility defects. More than 20 plants were observed in each group.

(B-D) Complementary assay of *llg2 llg3* double mutant with exogenous LLG2. Silique length **(B)** and seed set in the silique **(C)** of wild-type plants and *LLG2p*::*SP-GFP-LLG2* transgenic lines in the background of the *llg2 llg3* double mutant. Data are mean +/- SD of 5 siliques in each group. **(D)** Siliques of wild-type plants and transgenic lines.

(E-F) Fertility defects of *llg2 llg3* double mutants are male-specific. Silique length **(E)** and seed number per silique **(F)** of crossed plants shown in Figure 2A. Data are mean +/- SD of 9 siliques in each group. *** P< 0.001 (Student's test).

Scale bars: 5.0 cm **(A)**, 5.0 mm **(D)**.

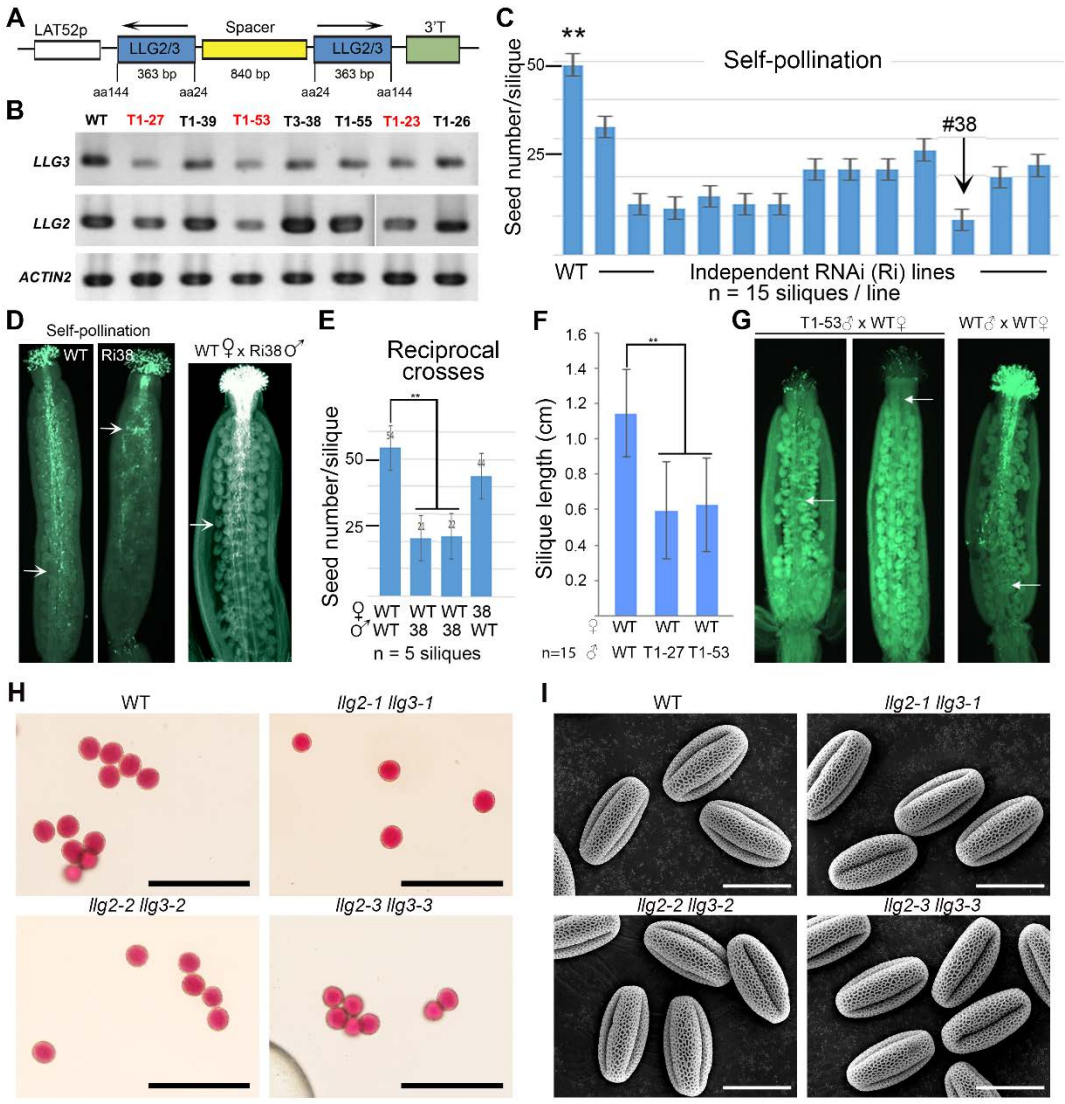

Figure S4. *LLG2/3* mutants show reduced seed yield, but normal pollen grains. Related to Figure 2.

(A) Schematic diagram of the *LLG2/3* RNAi construct. LAT52p was used as a pollen-specific promoter; 3'T indicates the transcription termination site; DNA sequence spanning amino acid residues 24-144 was used.

(B) Semi-quantitative RT-qPCR of *LLG2/3* RNAi mutants.

(C) Average seed yield per silique form independent T1 *LLG2/3* RNAi (Ri) lines. More than sixty T1 lines were generated. Seed yields are from lines that produced noticeably shorter siliques compared to wild type plants after self-pollination. **, significantly different ($p<0.01$) from Ri lines.

(D-E) Pollen tube growth phenotype of *LLG2/3* RNAi line Ri-38. T3 plants was used. **(D)** Pollen tube growth was reduced in self-pollinated Ri-38 pistil and also in a Ri-38 pollen to wild type pistil cross-pollination. (**E**) In reciprocal crosses, seed yields were reduced when Ri38 was used as pollen donor. **, significantly different ($p<0.01$) from wild type self-pollination and crosses where Ri38 was the female donor.

(F-G) Reproductive phenotype of *LLG2/3* RNAi line Ri-53 and Ri-27. Ri-lines (T3 plants) were used as pollen donor to hand-pollinate WT pistils. **(F)** Silique lengths. **(G)** Pollen tube growth was reduced when Ri-53 was used as pollen donor on wild type pistil relative to using wild type as pollen donor. **, significantly different ($p<0.01$). Arrows in **(D)** and **(F)** locations of the longest pollen tubes.

(H) Alexander staining of *llg2 llg3* pollen grains. More than 500 pollen grains were observed in each group.

(I) Scanning electron microscope (SEM) of pollen grains of *llg2 llg3* double mutant. More than 100 pollen grains were observed in each group.

Scale bars, 100 μm **(H)**, 20 μm **(I).**

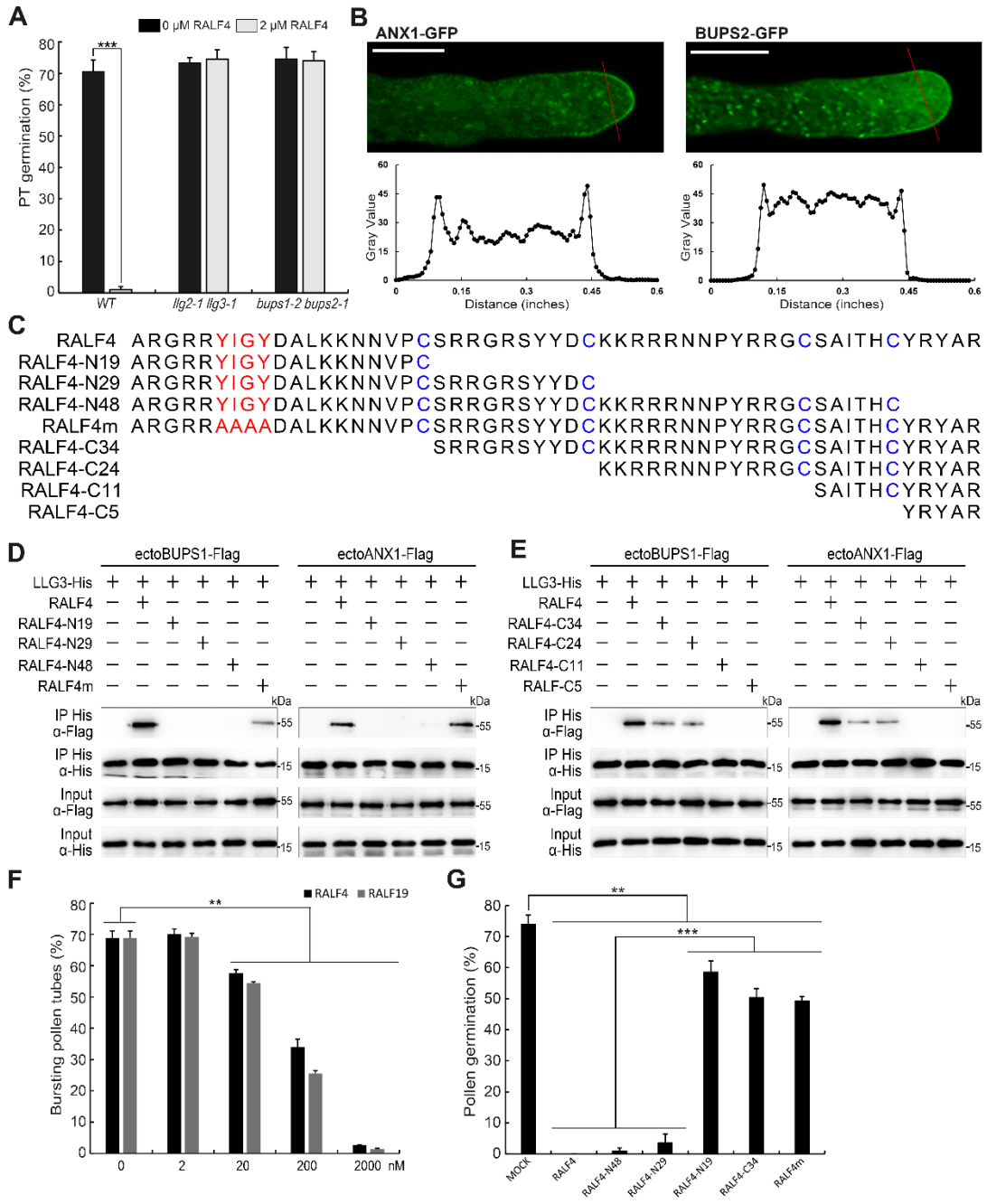

Figure S5. *in vitro* RALF4 treatment of WT, *llg2 llg3*, *bups1 bups2* and *ralf4 ralf19* pollen tubes and the induction of RALF4 variants on association of LLG3 with BUPS1/ANX1. Related to Figure 5.

(A) Pollen germination rate of WT, *llg2 llg3* and *bups1 bups2* with/without RALF4 treatment. Pollen grains were spread on pollen germination medium containing 2 μM RALF4 peptide. Quantitative analysis was conducted 7 hours after germination.

(**B**) Localization of ANX1-GFP and BUPS2-GFP using the *LAT52* promoter. Plot by

Image J at the bottom indicates the GFP signal along the red line. Scale bars, 10 μm.

(C) Peptide sequence of RALF4 and RALF4 variants in Figure 5A and Figure S5.

(D-E) Pull-down assays with LLG3-His and Flag-tagged BUPS1/ANX1 ectodomains with and without the addition of WT or C-terminal **(D)** and N-terminal **(E)** mutated RALF4 variants (each 100 nM) as indicated. The ectoBUPS1/ANX1-Flag proteins purified from transgenic tobacco leaves and His-tagged LLG2/3 generated in *E. coli* were subjected to binding assays with Ni Sepharose. Western blots were probed with α-Flag and α-His antibodies, respectively.

Similar results were obtained in three independent experiments.

(**F-G**) Pollen tube burst effect of RALF4/19 on *ralf4 ralf19* and RALF4 variants on WT pollen tubes. (**F**) Pollen tube burst of *ralf4 ralf19* tubes depending on RALF4/19 peptide concentration. (**G**) Pollen germination of WT tubes in medium containing each 2 μM of the RALF4 variants. Pollen tube bursting phenotypes (%) and pollen germination among the overall pollen on the medium were analyzed 7 hours after incubation. Data are mean +/- SD. More than 1500 pollen grains/tubes were analyzed in each group. ** $P < 0.01$; *** $P < 0.001$ (Student's test).

Purpose	Primer name	Sequence (5'-3')
Promoter cloning	LLG2-Pro-F	CACCTTGGTGGAGGATGAAGATGC
	LLG2-Pro-R	CATTGTTTCTCTGATTCTTCCTCT
	LLG3-Pro-F	CACCAAGGAGTGGTTGATTAAACA
	LLG3-Pro-R	CATGTTTTTGTTTCCTCCTCTATT
RT-PCR analysis of *LLG2/3* RNAi lines	LLG2-RT-F	ATGGAGATTTCTCCTTACTGTCTG
	LLG2-RT-R	TCAAGACGATAAGAACAAAAG
	LLG3-RT-F	ATGAAGATTACTCATCATTGTTTG
	LLG3-RT-R	TTAGAAGAGGTGAAACAAGATGGA
CRISPR/Cas9 mutant genotyping	LLG2-SR-F	ACTGACACGGCGTCGTTTAGGCTAT
	LLG2-SR-R	GGTAGAAAGAACCGCGAGAGATGCAGT
	LLG2-CX	GGACTGGGTGACATCAGTGCAGTCA
	LLG3-SR-F	TATGATCCGCTATATCCGCAGCA
	LLG3-SR-R	AGGTCGACTGGTAGAGTTATCGACACC
	LLG3-CX	AAACGGTGATTAGCAAAACGTGTG
Protein expression in *E. coli*	LLG2-mat-F	CACCATGTATGATGAATTTGATGGTCACGC
	LLG2-mat-R.SC	TCAAGAAGTTGCGGATGCGGACT
	LLG3-mat-F	CACCATGCATCACATCTCTCTTGATGA
	LLG3-mat-R.SC	TTATGATGTAGGGGTGACGTCGG
	LLG1-mat-F	CCCAAGCTTGGGATGAGTTTCATTTCAGATGGGGTC
	LLG1-mat-R.SC	CGGACTAGTCCGTCACGAGGTAGTTGCTGCGTTTA
Complementary assays	LLG2-C-F	CGGGGTACCCCGTGAAAGGGAGAAACCAGAGAG
	LLG2-C-R	GGCTGCTGCTGCTGCTGCTGCTTGTAATTCTCTCCTCAATTTC
	GFP-C-F	GATCTGGACGGGGTACCCCGATGGTGAGCAAGGGCGAGGA
	GFP-C-R	GCAGCAGCCCGCTCGAGCGGCTTGTACAGCTCGTCCATGC
Protein localization in tobacco PT	LLG2-F	CACCATGGAGATTTCTCCTTACTGTCTGCTTTCT
	LLG2-R	TCAAGACGATAAGAACAAAAGACACAGAACG
	LLG3-F	CACCATGGAGATTTCTCCTTACTGTCTGCTTTCT
	LLG3-R	TTAGAAGAGGTGAAACAAGATGGAAACG
	ANX1-F	CACCATGAGCGGGAAAACTCGGAT
	ANX1-R	TCGTCCTTTGGGATTTACAATCTG
	BUPS2-F	CACCATGGAGATAAGAAAGAAACCAAACATACC
	BUPS2-R	TCTTCCGTTAAGGCTAGCAAACTG
Protein localization in Arabidopsis	LLG3-V-F	GGCTGCTGCTGCTGCTGCTGCTCATCACATCTCTCTTGATGAATTTG
	LLG3-V-R	CGGGGTACCCCGAGAGAAAGCGAATCCAGACAAAAGG
	YFP-tag-F	GCTTTCTCTCGGGGTACCCCGATGGTGAGCAAGGGCGAGGAG
	YFP-tag-R	CAGCAGCAGCAGCAGCCCGCTCGAGCGGCTTGTACAGCTCGTCCATGC

Table S1. Primers used in the work. Related to STAR Methods.