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Supplementary Materials for

RALF peptide signaling controls the polytubey block in *Arabidopsis*

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Materials and Methods

Plant material and growth conditions

Arabidopsis thaliana (Arabidopsis, Columbia-0 ecotype) was used as wild type (WT) plant. The T-DNA insertion mutants *fer-4* [GK-106A06, GABI-Kat, (*12*)] and *myb97 myb101 myb120* [*myb97-1 myb101-1 myb120-3*, (*19*)] were gifted from De Ye (College of Biological Sciences, China Agricultural University). The *anj herk1* and *fer anj herk1* [*herk1 anj* and *herk1 anj* CRISPR *fer* #27, respectively, (*13*)] were gifted from Lisa Smith (Department of Animal and Plant Sciences, University of Sheffield). *fer*+/- was gifted from Chao Li (East China Normal University) and Qiaohong Duan (Shandong Agricultural University). *aca9* [*aca9-1*, ssJH no. 108, (*27*)] was gifted by Jeffrey F. Harper (College of Agriculture, Biotechnology and Natural Resources, University of Nevada). The *anj*-/- *herk1*+/- and *anj*+/- *herk1*-/- were screened from the F2 generation of cross between WT and *anj herk1* mutant. These mutants were confirmed by polymerase chain reaction (PCR) and DNA sequencing as described previously with primers listed in Table S2.

Plants were grown in a growth chamber with LED lights (GPL production modules DR/W and DR/B/FR, Philips) under long-day conditions (16 hr light/ 8 hr dark) at 22 ± 2°C.

Phylogenic analysis

Protein sequences of the CrRLK1L family and the recently re-annotated RALF family (*20*) of Arabidopsis (Table S1) were downloaded from the Arabidopsis Information Resource (http://www.arabidopsis.org/). Phylogenetic analysis was performed by using MEGA X (http://www.megasoftware.net/). Full-length amino acid sequences were used for ClustalW sequence alignment. Based on conserved residues, Neighbor-Joining trees were constructed with 500 replicates of bootstrap.

Plasmid construction and plant transformation

To generate the GFP reporters for RALF genes, genomic sequence of each *RALF* gene containing the promoter region and the coding sequence was cloned into pDONR221 and finally cloned into the vector pK7FWG0, which was developed from pK7FWG2 (Department of Plant Systems Biology, VIB-Ghent University), by using BP and LR reaction following the protocol of the manufacturer (Invitrogen). The promoter region of each gene is indicated by the primers listed in Table S2. The obtained construct was transformed into WT plants.

For promoter-GUS reporter assays, promoters of each *RALF* gene were cloned into pDONR221, and then pB7GUSWG0 (*33*) by using BP and subsequent LR reaction. The obtained construct was transformed into WT plants.

For receptor reporter lines, a nuclear localized mCitrine under the control of *FER*, *ANJ* or *HERK1* promoter regions together with a ubiquitous expressed plasma membrane marker were cloned via Golden Gate assembly using the GreenGate plasmid set (*34*). Therefore, the 5' regions of *FER* (1,420 bp), *ANJ* (2,235bp) or *HERK1* (2,388bp) were amplified by PCR using Col-0 genomic DNA as template and cloned into the pGGA000 plasmid to generate the pGGA_FER, pGGA_ANJ and pGGA_HERK1 constructs. In the following assembly of the M-intermediate plasmids, pGGA_FER, pGGA_ANJ and pGGA_HERK1 were combined with pGGB005 (SV40-NLS), pGGC_GSAGAG-mCitrine, pGGD_SGAGAG-mCitrine, pGGE_HSP18.2 and pGGG001 (F-H) into pGGM000 to generate pBLAM065 (FERpro:NLS-mCit-mCit_HSP18.2_FH), pBLAM067 (ANJpro:NLS-mCit-mCit_HSP18.2_FH) and pBLAM066 (HERK1pro:NLS-mCit-mCit_HSP18.2_FH) constructs. Furthermore, the N-intermediate pBLAN038 (HA_P16pro:mScatlet-Remorin-Anker_HSP18.2_Basta) was cloned by assembly of pGGG002

(H-A), pGGA005 (P16), pGGB002 (Omega element), pGGC_GSAGAG-mScarlet, pGGD_Remorin-Anker, pGGE_HSP 18.2, and pGGF009 (BASTA selection marker) into pGGN000. In a final Golden Gate assembly reaction, different M-intermediates were combined with pBLAN038 into pGGZ003 to create final expression plasmids pBLAZ179 (FERpro:NLS-mCit-mCit_HSP18.2_FH_HA_P16pro:mScarlet-Remorin-Anker_HSP18.2_Basta), pBLAZ181 (ANJpro:NLS-mCit-mCit_FH_HA_P16pro:mScarlet-Remorin-Anker_HSP18.2_Basta) and pBLAZ180 (HERK1pro:NLS-mCit-mCit_FH_HA_P16pro:mScarlet-Remorin-Anker_HSP18.2_Basta), respectively. FER gene reporter line was created by cloning the FER gene into pGGAE00 to create pGGAE_FERpro:FER. The M intermediate pBLAM068 (FERpro:FER-mCit_HSP18.2_FH) was assembled by the combination of pGGAE_FERpro:FER, pGGD_SGAGAG-mCitrine, pGGE_HSP 18.2 and pGGG001 (F-H) into pGGM000. The finale expression plasmid was generated by the assembly of pBLAM068 and pBLAN038 into pGGZ003 resulting in pBLAZ182 (FERpro:FER-mCit_HSP18.2_FH_HA_P16pro:mScartlet-Remorin-Anker_HSP18.2_BASTA).

Arabidopsis plants were transformed through the floral dip method with agrobacteria GV3101 strain (*35*).

RNA-seq analysis

For RNA-seq of transmitting tract and septum tissues, WT flowers were emasculated and excised 30 hours later. After removing the stigma and style, pistils were longitudinally cut and opened along both sides of the septum. Septum tissues were collected after carefully removing funiculus and ovules with a syringe needle and transferred immediately into liquid nitrogen. Total RNA was extracted from septum tissues of 30 flowers by using the Plant Total RNA Purification Kit (GeneMark). RNA was sequenced with a Hi-seq PE150 sequencer by Novogene.

For RNA-seq of WT and *myb97 myb101 myb120* plants (3 replicates for each group), semi-*in vivo* germinated pollen tubes were used to extract RNA for sequencing with an Illumina Hi-seq 2500 sequencer in the Biodynamic Optical Imaging Center (BIOPIC) of Peking University followed by bioinformatic analysis on the High Performance Computing Platform of the Center for Life Science.

To analyze RNA-seq data, Tophat2 (*36*) was used to align clean reads with the Arabidopsis genome version in TAIR10 with default parameters. Cufflinks (*37*) was used to calculate and normalize the differential expression levels of RNA from these two samples using FPKM (Fragments Per Kilobase of transcript per Million fragments mapped) values.

Real-time RT-qPCR

Transmitting tract and septum tissues from 3 emasculated pistils were manually collected under a stereomicroscope at 30 HAE (hours after emasculation). Total RNA was isolated using Dynabeads^{TM} mRNA DIRECT^{TM} Micro Purification Kit (Thermo Scientific). Eluted RNA was reverse-transcribed using Superscript^{TM} II reverse transcriptase Kit (Thermo Scientific). Each cDNA was amplified by KAPA HiFi HotStart ReadyMix (Roche) for 19 cycles and purified by Agencourt AMPure XP beads (Beckman Coulter). The reverse transcription and amplification steps were adapted from a published procedure (*38*). For real-time RT-qPCR, three biological replicates were performed using UltraSYBR Mixture (CWbiotech) on a QuantStudio 3 Real-Time PCR System (Applied Biosystems). *ACTIN8* (At1g49240) was used as internal control to calculate relative expression levels of each gene. Primers used for RT-qPCR are listed in Table S2.

Plasmid construction and plant transformation for CRISPR/Cas9-mediated mutagenesis

All *ralf* mutants were obtained by previously reported egg cell-specific promoter-controlled CRISPR/Cas9 system (*39*, *40*). For the *ralf6 ralf7 ralf1*6 mutant, two fragments containing four sgRNAs or three fragments containing six sgRNAs were amplified from pCBC-DT1T2 by using the primers RALF6_BsF/F0-1 and RALF6_BsR/R0-1, RALF7_BsF/F0-1 and RALF16_BsR/R0-1; RALF6_BsF/F0-2 and RALF6_BsR/R0-2, RALF7_BsF/F0-2 and RALF7_BsR/R0-2, RALF16_BsF/F0-2 and RALF16_BsR/R0-2, then cloned into pENTR-MSR respectively (*33*) to produce pENTR-RALF6 and pENTR- RALF7-16. The two dual-spaces were finally constructed into the vector pENTR-RALF6-7-16 by digestion and ligation using *Spe* I/*Hin*d III and *Xba* I/*Hin*d III, respectively. The fragment amplified from pENTR-RALF6-7-16 with primers of RALF6_BsF and RALF16_BsR, was cloned into pHEE401E by Golden-Gate Cloning (*41*), producing the destination vector pHEE401E-RALF6-7-16. To obtain *ralf36 ralf37* mutants, RALF36_sgRNA1_U6_26t-U6_29p-RALF37_sgRNA2 was amplified from pCBC-DT1T2 by using the primers RALF36-BsF/F0 and RALF37-BsR/R0. The dual-spacers were cloned into pHEE401E binary vector to produce the plasmid pHEE401E-RALF36-37. Constructs pHEE401E-RALF6-7-16 and pHEE401E-RALF36-37 were transformed into WT plants by *Agrobacterium*-mediated floral dip method (*35*). To obtain the *ralf6 ralf7 ralf1*6 *ralf36 ralf37* quintuple mutants, pHEE401E-RALF36-37 was transformed into the *ralf6 ralf7 ralf16* triple mutant and screened for quintuple mutants. The *hap2*-/- mutants were obtained by using the similar strategy as for *ralf36 ralf37* mutants with the primer pairs HAP2-BsF/F0 and HAP2-BsR/R0.

Transgenic plants screening for *ralf* mutants by CRISPR/Cas9 technology

T_0 generation seeds were screened on the resistant plate with 37.5 μg/mL hygromycin. In the T_1 generation, plants with effective mutations were identified by direct sequencing of PCR products by using identifier-primers (RALF6-CRI-F/R, RALF7-CRI-F/R, RALF16-CRI-F/R, RALF36-CRI-F/R and RALF37-CRI-F/R). The mutation pattern was reconfirmed in the T_2 generation. Cas9-free plants were identified by Hyg-IDF/ Hyg-IDR. Cas9-free seeds were confirmed by growing on resistant plates.

Semi-*in vivo* ovule targeting assay

The semi-*in vivo* ovule targeting assay was conducted as described previously (*10*, *42*). Emasculated WT stigmas were hand cut and placed onto solid pollen germination medium (SPGM: 0.01% H_3BO_3, 5 mM $CaCl_2$, 5 mM KCl, 1 mM $MgSO_4$, 15% w/v sucrose, pH adjusted to 7.5 with KOH in 1.2% w/v low gelling temperature agarose) in a small culture dish with a 2-mm-thick, 14-mm-diametral cover glass in the bottom center. Appropriate numbers of pollen grains were placed on excised stigma and each three ovules positioned with complete funiculus at appropriate distances below cut stigmata. Then, culture dishes were placed into a 22℃ incubator allowing pollen germination, growth, and attraction. Each assay was conducted with three biological repeats and 3-5 samples for each repeat.

Pollination assay, aniline blue staining, and microscopic observation

For aniline blue staining, pollen grains were collected from freshly opened flowers, and pollinated to pistils that had been emasculated 24-36 hours earlier. At certain hours after pollination, siliques were harvested and fixed in acetic acid/ethanol 1:3 for more than two hours in vacuum, then rehydrated through a series of ethanol (70%, 50%, 30%) and ddH_2O for 15 min each time. After treatment with 8 M NaOH overnight, samples were rinsed with ddH_2O twice and

stained with aniline blue solution (0.3% decolorized aniline blue, in 108 mM K_3PO_4) for more than 2 hours in the dark. Stained samples were observed under a fluorescence microscope (Olympus BX51 and Zeiss Axio Imager D2) equipped with an ultraviolet filter set. Pollen tube length was measured and processed with Fiji (Image J).

Histochemical GUS staining was performed as previously reported (*5*). Briefly, inflorescences or pistils were incubated in 90% acetone on ice for 30 minutes. Then samples were rinsed by phosphate buffer [50 mM Na_2HPO_4/NaH_2PO_4 (pH7.0), 2 mM $K_3Fe(CN)_6$, 2 mM $K_4Fe(CN)_6$] twice for 15 minutes each time, transferred into a staining solution containing 2 mM X-gluc (Sigma), infiltrated under the vacuum for 2 hours and incubated in 37°C. 70% ethanol was used to terminate staining. Samples were rehydrated by 50%, 30%, 10% of ethanol and finally ddH_2O.

For SEM, flowers at floral stage 12 were emasculated. Excessive pollen grains were placed on the emasculated stigma 36 hours later. After 5 hours of pollination, the pistils were fixed in FAA (100 mL: 50 mL ethyl alcohol, 5 mL glacial acetic acid, 5 mL formaldehyde and 40 mL ddH_2O). Fixed pistils were dehydrated in a graded ethanol series of 50%, 60%, 70%, 80%, 95% and 100% (30 min each time). Pistils were subsequently CO_2-critical point-dried (Hitachi critical point dryer, HCP-2; Hitachi Koki) and mounted for further dissection. After sputter coating with gold, samples were examined with a Scanning Electron Microscope (Hitachi S3000N, JOEL JSM-6610).

For confocal microscopy of the receptor reporter lines, hand cross-sections of pistils were imaged using a Zeiss LSM980/Airyscan 2 microscope with GaAsP-PMT detectors. mCitrine (displayed in magenta) was excited using the 488 nm diode laser and emission was captured from 499-552 nm. mScarlet (displayed in gray) was excited using a 561 nm diode laser and emission was captured from 570-632 nm in a two-track process. Images of the septum were acquired with a VisiScope Spinning Disc system from Visitron using a 40× objective, and processed using Zeiss ZEN 3.1, ZEN blue 3.4.91 and Fiji (Image J).

Detection of RALF36 and RALF37 in pollen tubes by immunostaining

Pollen grains were placed on solidified SPGM and germinated in a humid box at 22℃ for 4 hours. Detection of RALF36 and RALF37 by immunostaining was conducted according to a previously reported protocol (*43*). Pollen tubes were fixed in 4% paraformaldehyde in PEM (50 mM PIPES, 5 mM EGTA, 5 mM $MgCl_2$, pH6.9) with 18% sucrose for 90 min. Subsequently, pollen tubes were washed with PEM once and PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.0) twice. Pollen tubes were incubated overnight at 4°C in a solution of PBS containing 3% BSA (Bovine Serum Albumin) with RALF36 or RALF37 primary antibody (diluted at 1:100) followed by three washes in PBS with 3% BSA. Subsequently, samples were incubated with Alexa Fluor 488 goat anti-rabbit IgG (Catalog # A11008, Invitrogen) for RALF36 and RALF37 (diluted at 1:800 in PBS with 3% BSA) for 2 hours at room temperature, then washed three times with PBS containing 3% BSA. Pollen tube rupture treatment was done with 8% D (−) Mannitol (Catalog # M9647, Sigma) for 2 minutes or 15 minutes before fixation.

Primary antibodies against RALF36 and RALF37 were raised and produced by ABclonal Biotechnology. Pollen tubes were observed under a confocal laser scanning microscope (Nikon A1R) using a 40× objective. Samples were excited under 488 nm laser with emitted light measured at 500-550 nm for GFP observation. Fluorescence pixel intensities of images were measured using ImageJ software.

Recombinant protein expression, purification and peptide synthesis

C-terminus His-tagged ectoFER, ectoANJ and ectoHERK1 were expressed and purified according to a previously described protocol (*44*). Extracellular domain sequences of FER (residues 27-447), ANJ (residues 25-405) and HERK1 (residues 25-405) were cloned into a modified pFastBac 1 vector (Invitrogen) with an N-terminal hemolin signal peptide. Recombinant baculoviruses with sf21 insect cells (cultured in the SF-900 medium at 28℃) were generated and amplified according to the Bac-to-Bac baculovirus expression system (Invitrogen). Both proteins were expressed in High Five insect cells (Invitrogen) at 22°C. One litre of cells (2.0×10^6 cells/mL) was infected with 30 ml recombinant baculovirus. Supernatant was collected via centrifugation after infection for 60 hours. Fusion proteins were purified using Ni-NTA (Novagen) from the supernatant. Purified proteins were eluted by elution buffer containing 25 mM Tris-HCl pH8.0, 150 mM NaCl, 250 mM imidazole and further purified by size-exclusion chromatography (Hiload 16/60 Superdex 200 prep grade, GE Healthcare) in buffer containing 10 mM Bis-Tris pH 6.0, 100 mM NaCl. C-terminal His-tagged ectoCVY1 (residues 22-398) was expressed and purified through a similar procedure as described above for ectoFER, ectoANJ and ectoHERK1. C-terminal HA-tagged FER (residues 27-447) or ANJ/HERK1 (residues 25-405) was generated by reconstructing the expression vector. Briefly, the sequence of 3×HA tags followed by a prescission protease cleavage site was inserted between ectoFER (or ectoANJ or ectoHERK1) and C-terminal 6×His tag. HA-tagged protein was expressed in High Five insect cells as described above. Initial purification of HA-tagged ectoFER (or ectoANJ or ectoHERK1) protein was performed using Ni-NTA (Novagen) with buffer containing 25 mM Tris-HCl, pH8.0, 150 mM NaCl. The His-tag was then removed using prescission protease (purified in our own lab). Obtained proteins were further purified through size-exclusion chromatography (Hiload 16/60 Superdex 200 prep grade, GE Healthcare) in buffer containing 10 mM Bis-Tris pH6.0, 100 mM NaCl.

MBP-tagged ectodomains were expressed in *E. coli* and extracted as previously described (*45*). Sequences of ectodomain were cloned into the pMALGW vector (*46*), and then transformed into the BL21 *E. coli* strain obtained from TransGen Biotech. 1/1000 1M IPTG was added to induce protein expression at 18℃ overnight when bacteria were grown at 37℃ to an OD_{600} of 0.5. Induced bacteria were collected at room temperature and centrifuged. Pellets were re-suspended with MBP lysis buffer (25 mM Tris, pH8.0, 100 mM NaCl, 5% Glycerol), and subsequently sonicated at intervals of 10 sec-work and 20 sec-rest for up to 10 minutes. Insoluble pellets were removed and suspensions transferred into a new tube after centrifugation. Protein extracts were incubated with MBP-beads (Amylose resin, NEB) at 4℃. MBP wash buffer made by Tris, NaCl and Glycerol at the same concentration as in MBP lysis buffer was used to clean beads for 5 times and MBP elution buffer (MBP lysis buffer and 10 mM maltose) was added to elute MBP-tagged proteins from beads.

Biotinylated RALF and elf24 peptides used in this study (Table S3) were synthesized by Scilight Biotechnology LLC with a purity higher than 95%. All peptides were diluted in sterile ddH_2O.

Pull-down assays between RALF peptides and RLK ectodomains

Purified tagged RLK ectodomains were mixed with biotinylated RALF peptides in 500 μL pull-down buffer (20 mM Tris-HCl, pH7.5, 1% IGEPAL) and incubated at 4°C for 1 hour. RLK ectodomain samples and RALF peptides were used at a final concentration of 50 nM in one tube. Then 50 μL Streptavidin Magnetic Beads (New England Biolabs) were added in each sample for three hours incubation at 4°C and washed with 1.0 mL pull-down buffer for 5-6 times. Bound proteins were eluted with 100 μL SDS loading buffer from beads at 100 °C for 10 min. SDS-PAGE

and western blot analysis were used to exam the interaction. Mouse anti-His antibody (TransGen Biotech, 1:5,000 for detection), mouse anti-HA antibody (Sigma-Aldrich, H9658, 1:5,000 for detection), mouse anti-MBP antibody (ABclonal, AE016, 1:5,000 for detection), goat anti-mouse IgG HRP conjugated secondary antibody (Cwbio, CW0102S, 1:3,000 for detection) were used in western blots. All experiments were repeated for at least three times.

The dose gradient assay was conducted in the same condition while the concentrations of the peptides were adjusted to the range of 0 to 20 μM. For the interaction enhancement assay, 5 nM His-tagged ectoFER and 25 nM HA-tagged ectoANJ or ectoHERK1 were incubated together with 125 nM peptide in TBST buffer (50 mM Tris-HCl, pH7.5, 150 mM NaCl, 0.1% Tween-20) at 4°C for 1 hour. Then, 30 μL Chelating Sepharose Fast Flow (GE Healthcare, GE17-0575-01) was added to incubate at 4°C for another 1 hour. All samples were washed for 5-6 times with TBST buffer. After elution, protein samples were subjected to SDS-PAGE and western blot analysis to exam interactions.

Microscale thermophoresis (MST) assay

MST assays were applied to determine the binding affinities. 200 nM purified His-tagged ectoFER, ectoANJ, ectoHERK1 or ectoCVY1 was labelled with 100 nM His-labeling dye solution introduced in the kit (Monolith™ His-Tag Labeling Kit RED-tris-NTA, MO-L008) for 30 min at room temperature in HEPES buffer (10 mM HEPES, 150 mM NaCl, 0.05% P20). Labelled proteins were mixed with a prepared gradient-diluted RALF peptide concentrations ranging from 0.0076 to 250 μM in the HEPES buffer (the final concentration of protein samples is 50 nM). Then samples were centrifuged at 13,000 rpm and 4°C for 10 min and loaded into the capillaries (Monolith™ NT.115 Standard Treated Capillaries, MO-K022). All measurements were performed using a Monolish NT.115 device (NanoTemper Technologies) at medium MST power and 80% LED power. Raw data was analyzed by MO Affinity Analysis software (V2.2.4). All experiments were repeated for at least three times.

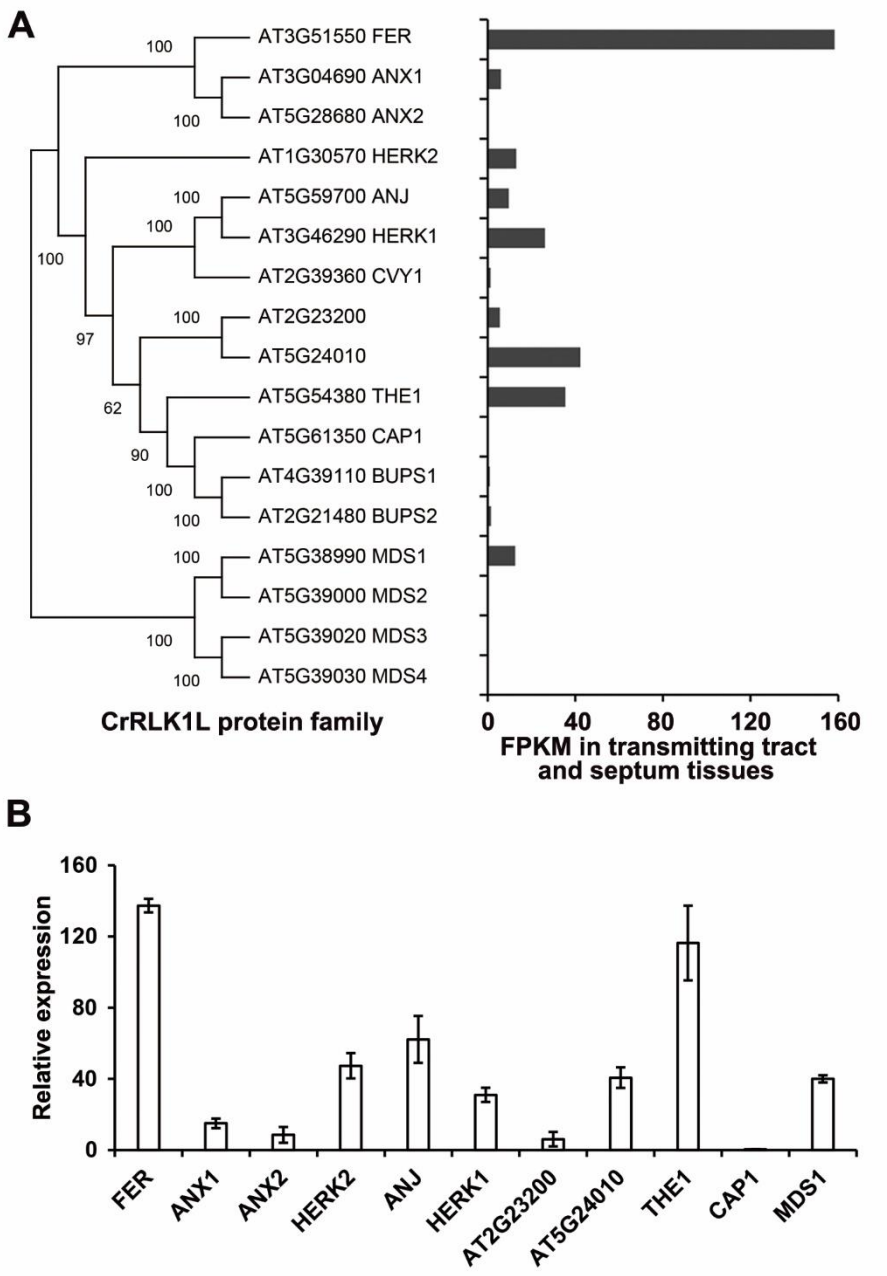

Fig. S1. Gene expression analysis of *MLD-RLK* (*CrRLK1L*) family members in transmitting tract and septum tissues.

Relative expression levels of the *MLD-RLK* (*CrRLK1L*) family in wild type transmitting tract and septum tissues from RNA-seq data. Phylogenetic tree based on full-length protein sequences to indicate the relationship of the 17 members. Sequence alignment and bootstrap tree construction were conducted by MEGA-X, using ClustalW and Neighbor-Joining strategy, respectively. FPKM: Fragments Per Kilobase per Million fragments mapped. (**B**) Real-time RT-qPCR analysis of transcript levels of *CrRLK1L* family members in the septum tissues. *ACTIN8* was used as an internal control for quantification. n=3, data are mean values ± SD.

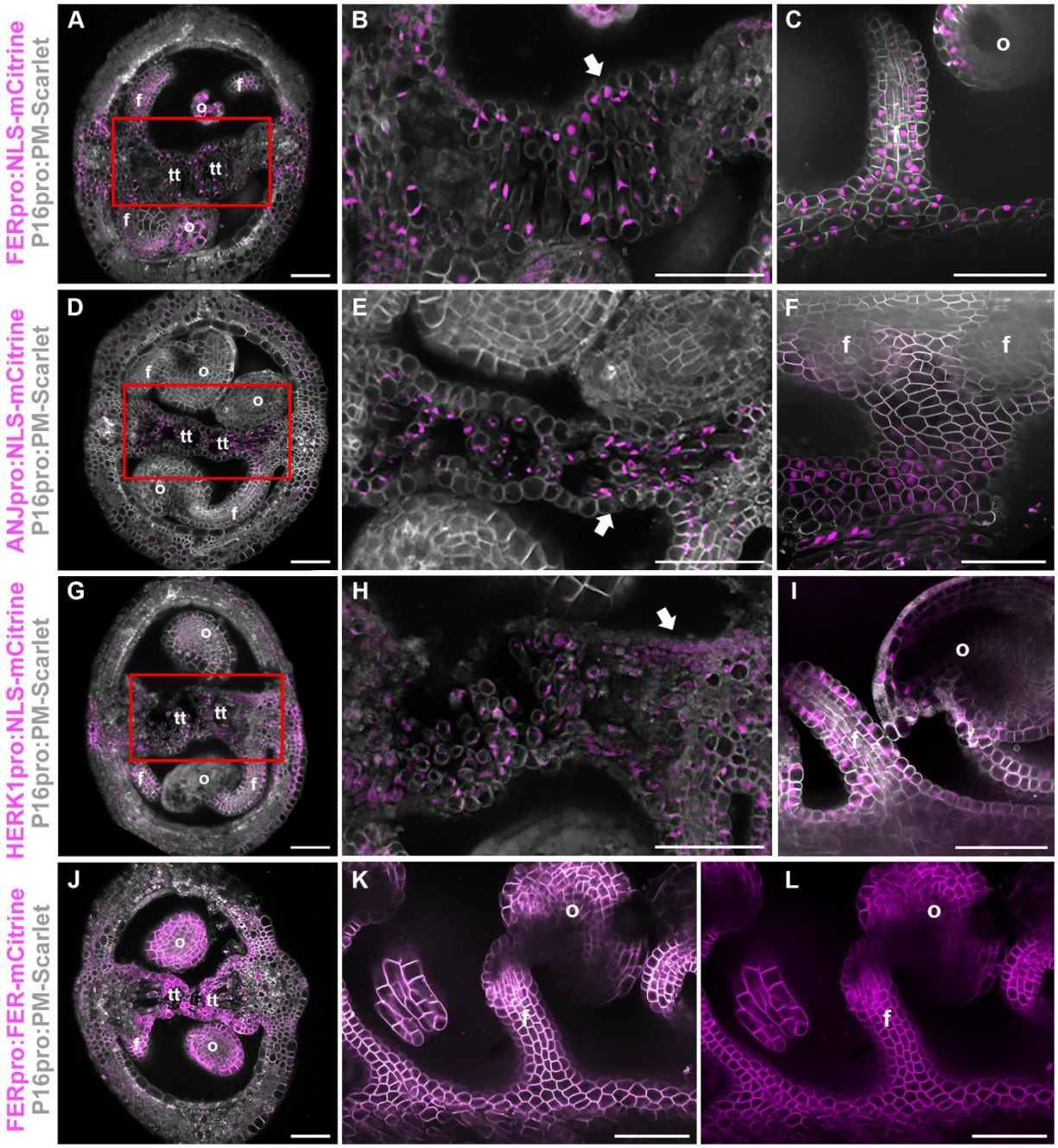

Fig S2. Expression pattern of FER, ANJ and HERK1 in the pistil of *Arabidopsis thaliana.* Cross-sections (**A**, **D** and **G**) and longitudinal sections (**C**, **F** and **I**) showing expression pattern of NLS-2×mCitrine reporter (magenta) under control of the FER (**A** and **B**), ANJ (**D** and **E**) and HERK1 (**G** and **H**) promoter regions together with an mScarlet-labeled plasma membrane marker driven by the P16 promoter. (**B**, **E**, **H**) Magnification of the transmitting track region indicated in the red box in A, D and G. White arrows indicate signals in epidermal layer of the septum. (**J** and **K**) A gene reporter line co-expressing FERpro:FER-mCitrine with an mScarlet-labeled plasma membrane marker under the control of the P16 promoter confirms the broad expression pattern of FER and its general plasma membrane localization in epidermal cells of the septum (J) and the

whole funiculus (K). **L** shows the FER-mCitrine signal alone in K. f: funiculus; tt: transmitting track; o: ovule. Scale bars: 50 µm.

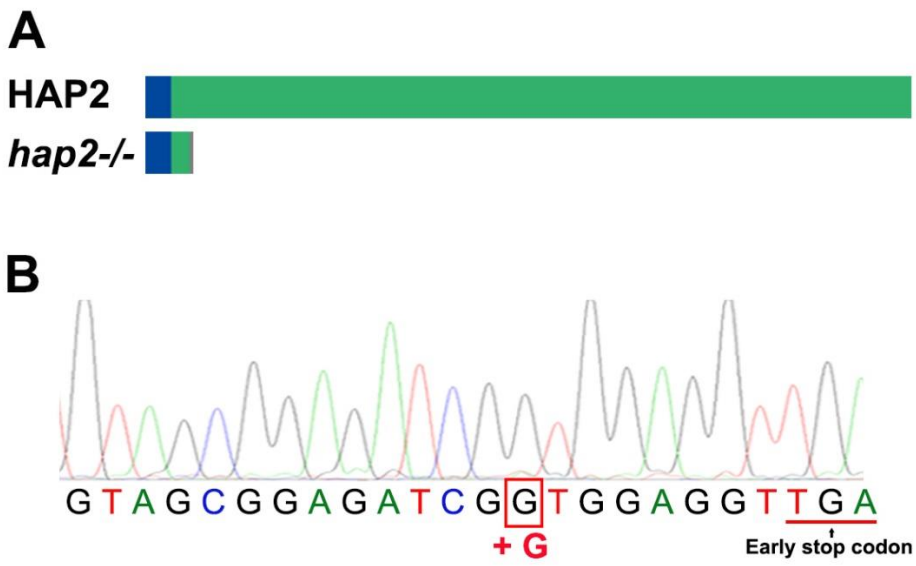

Fig. S3. Mutation site leads to a loss-of-function *hap2-/-* mutation.

(**A**) Schematic diagram of protein structure of wild-type HAP2 and that in mutated *hap2-/-*. Blue box, signal peptide; gray box, missense sequence due to frame shift mutation. (**B**) Sequencing result of the mutation site of *HAP2* in the *hap2-/-* mutant. Red box indicates inserted base. Arrow indicates an early stop codon generated by an additional G.

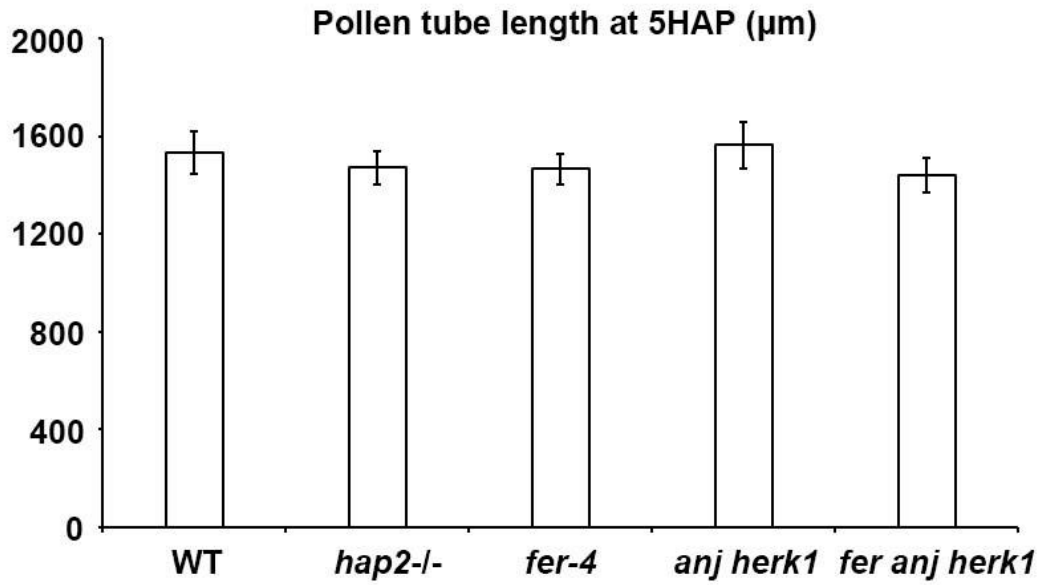

Fig. S4. Pollen tube length in WT, *hap2*-/-, *fer-4*, *anj herk1* and *fer anj herk1* mutants.
Statistical analysis of pollen tube length in WT pistils pollinated with WT or *hap2*-/- pollen, *fer-4*, *anj herk1* and *fer anj herk1* pistils pollinated with WT pollen at 5 HAP. The analysis was repeated for at least three times for this experiment. Data are mean values ± SD.

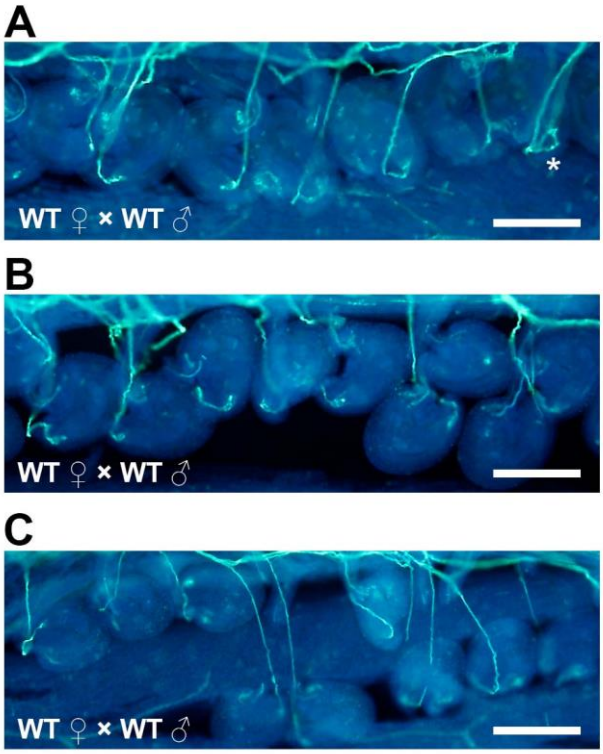

Fig. S5. Aniline blue staining of pollen tube emergence in WT pistils at 5 HAP.

(**A**). Representative image of pollen tube emergence in WT pistils. Image related to Figure 1C and 1D. (**B**). Representative image of pollen tube emergence in WT pistils. Image related to Figure 2D and 2E. (**C**). Representative image of pollen tube emergence in WT pistils. Image related to Figure 4F and 4G. Scale bars, 100 μm.

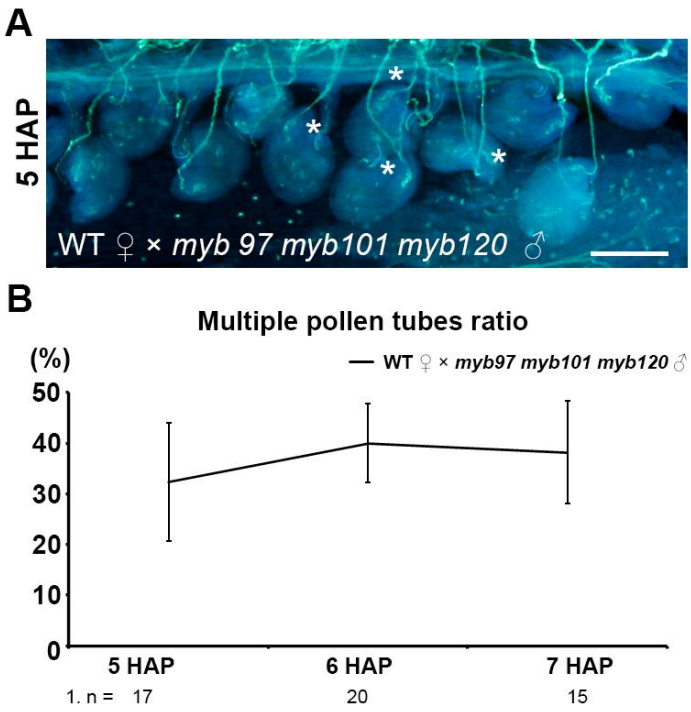

Fig. S6. Analysis of multiple pollen tube emergence in the *myb97 myb101 myb120* triple mutant.

(**A**) Aniline blue staining of multiple pollen tubes in the *myb97 myb101 myb120* mutant at 5 HAP. The analysis was repeated for at least three times for this experiment. (**B**) Statistical analysis of (**A**). White asterisks indicate ovules targeted by multiple pollen tubes. 'n' refers to the number of pistils. Data are mean values ± SD. Scale bar, 100 μm.

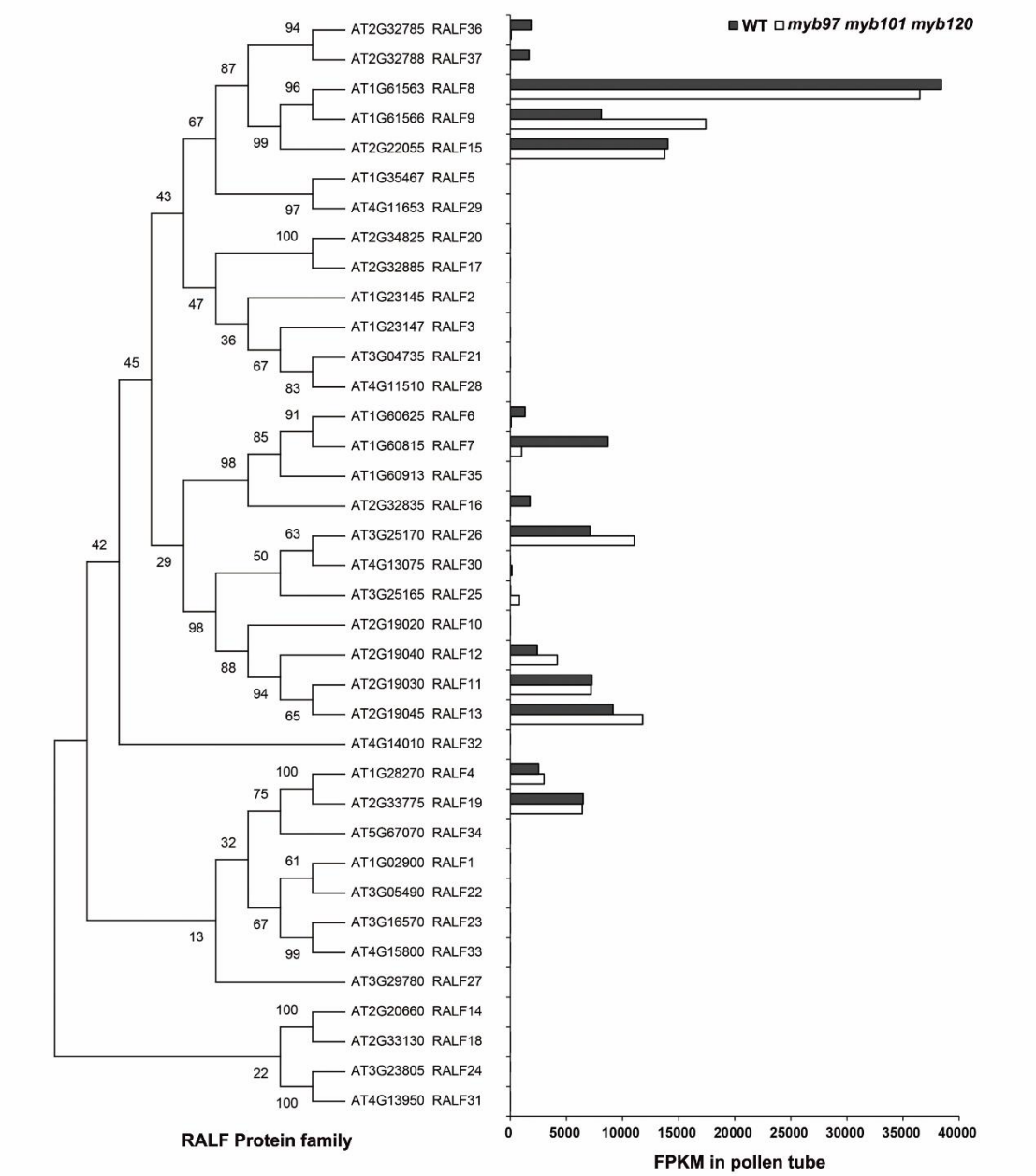

Fig. S7. Gene expression analysis of *RALF* family members in the pollen tube.

Relative expression levels of *RALF*s in semi-*in vivo* geminated pollen tube of wild type (WT) and *myb97 myb101 myb120* pollen tubes based on RNA-seq data. Phylogenetic tree based on full-length protein sequences indicates the relationship of the 37 members. Sequence alignment and bootstrap tree construction were conducted by MEGA-X, using ClustalW and Neighbor-Joining strategy respectively. FPKM: Fragments Per Kilobase per Million fragments mapped.

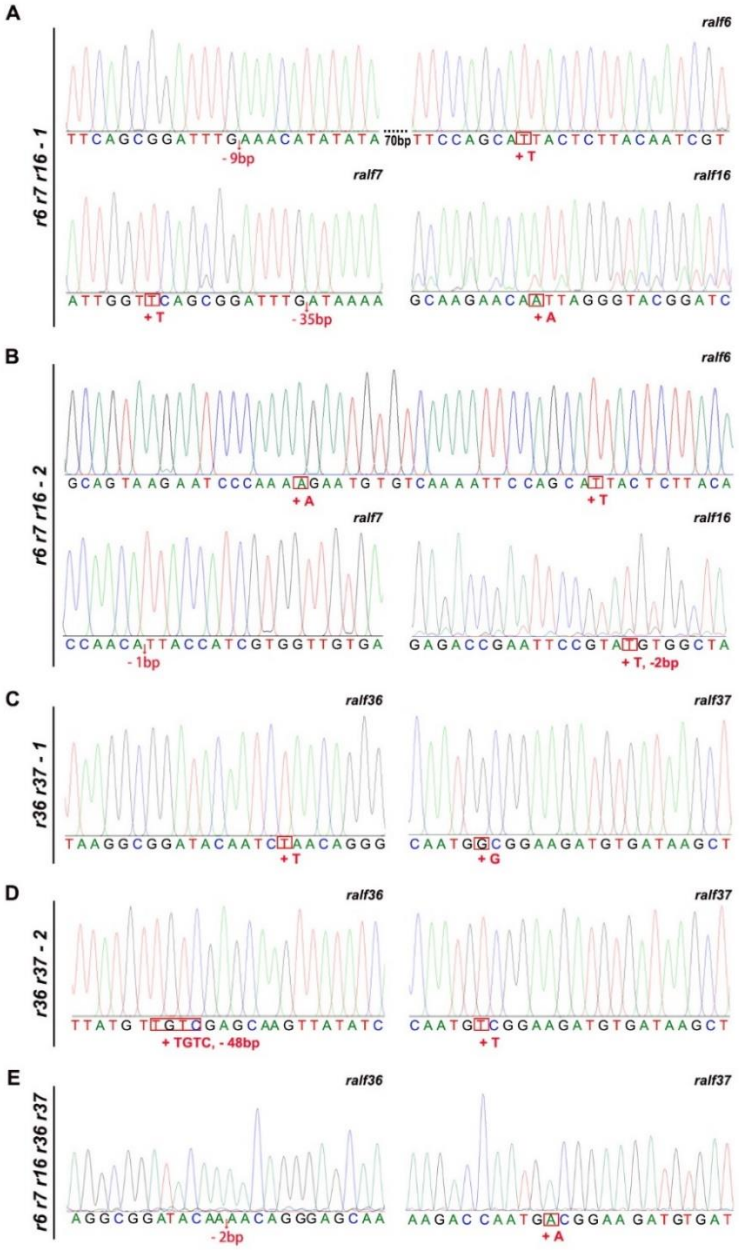

Fig. S8. Mutation sites of *RALF6*, *RALF7*, *RALF16, RALF36* and *RALF37* in the *r6 r7 r16-1/-2* triple, *r36 r37-1/-2* double and *r6 r7 r16 r36 r37* quintuple mutants.

(**A** and **B**) Sequencing result of the mutation sites of *RALF6* (two mutation sites), *RALF7* and *RALF16* in the *r6 r7 r16-1* (A) and *r6 r7 r16-2* (B) mutants, respectively. (**C** and **D**) Sequencing result of the mutation sites of *RALF36* and *RALF37* in the *r36 r37-1* (C) and *r36 r37-2* (D) mutants, respectively. (**E**) Sequencing result of the mutation sites of *RALF36* and *RALF37* in the *r6 r7 r16 r36 r37* mutant. The mutation sites of *RALF6*, *RALF7* and *RALF16* in the quintuple mutant are the same with those in the *r6 r7 r16-1* mutant. Red boxes indicate inserted bases and arrows deleted bases.

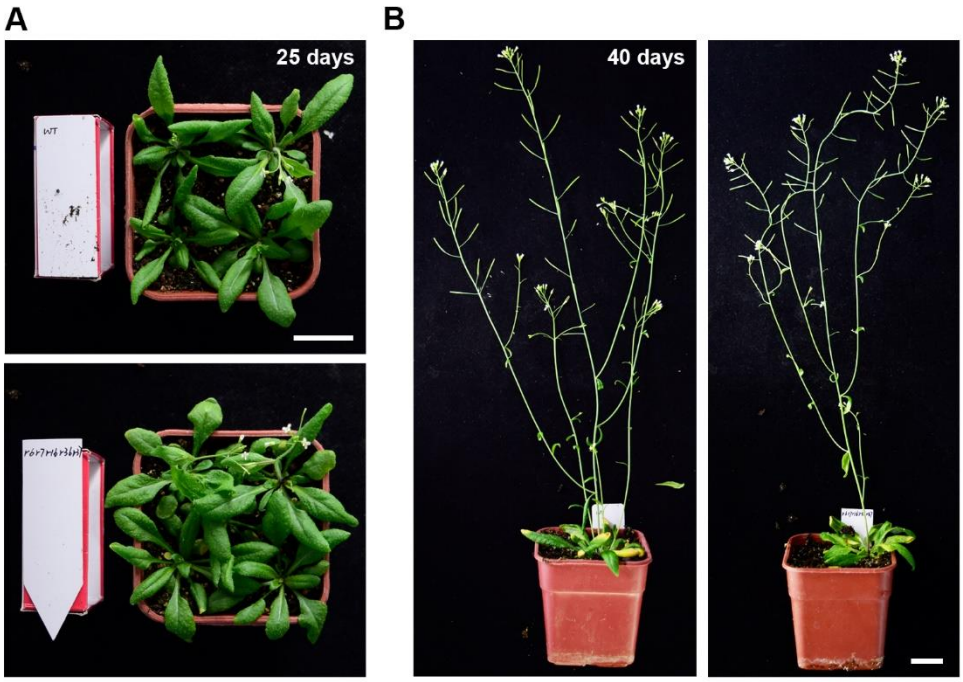

Fig. S9. Images of whole WT plants and *r6 r7 r16 r36 r37* mutants.

(**A**). 25-days-old grown wild-type (up) and *r6 r7 r16 r36 r37* (bottom) plants. Scale bar, 2.5 cm.
(**B**). 40-days-old grown wild-type (left) and *r6 r7 r16 r36 r37* (right) plants. Scale bar, 2.5 cm.

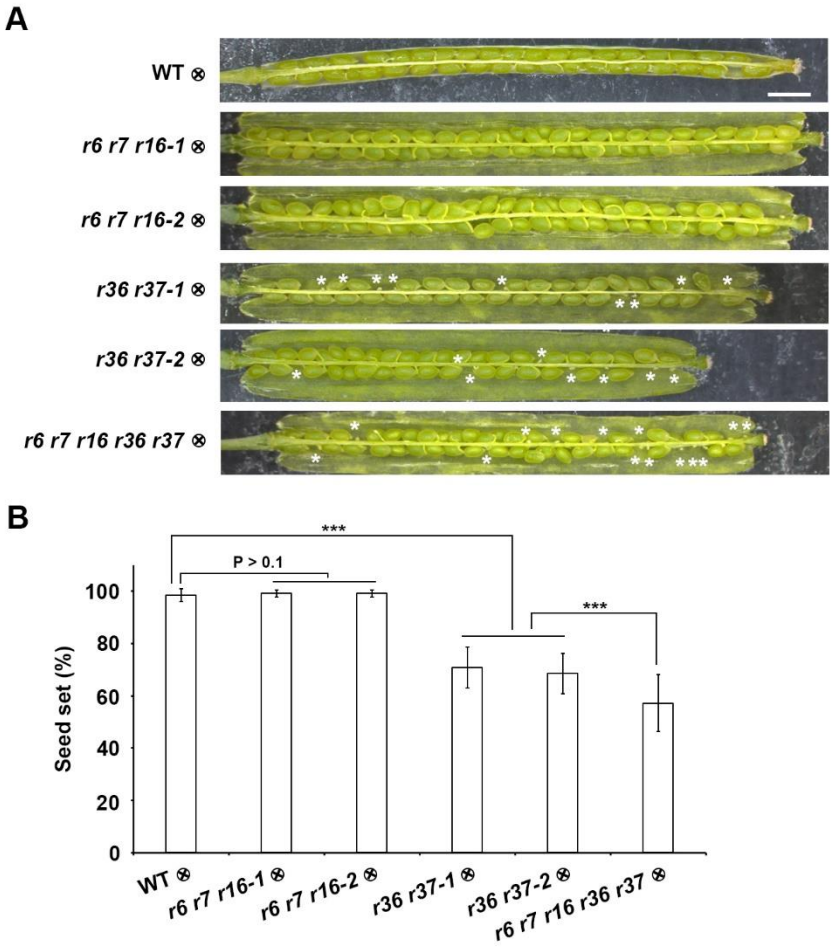

Fig. S10. Fertility of WT, *r6 r7 r16-1/-2*, *r36 r37-1/-2* and *r6 r7 r16 r36 r37* mutant plants.
(**A**) Silique of WT, *r6 r7 r16-1/-2*, *r36 r37-1/-2* and *r6 r7 r16 r36 r37* mutant plants. (**B**) Statistical analysis of (A). White asterisks indicate unfertilized ovules. Data are mean values ± SD; *** shows $P<0.01$ (Student's *t* test). Scale bar, 1 mm.

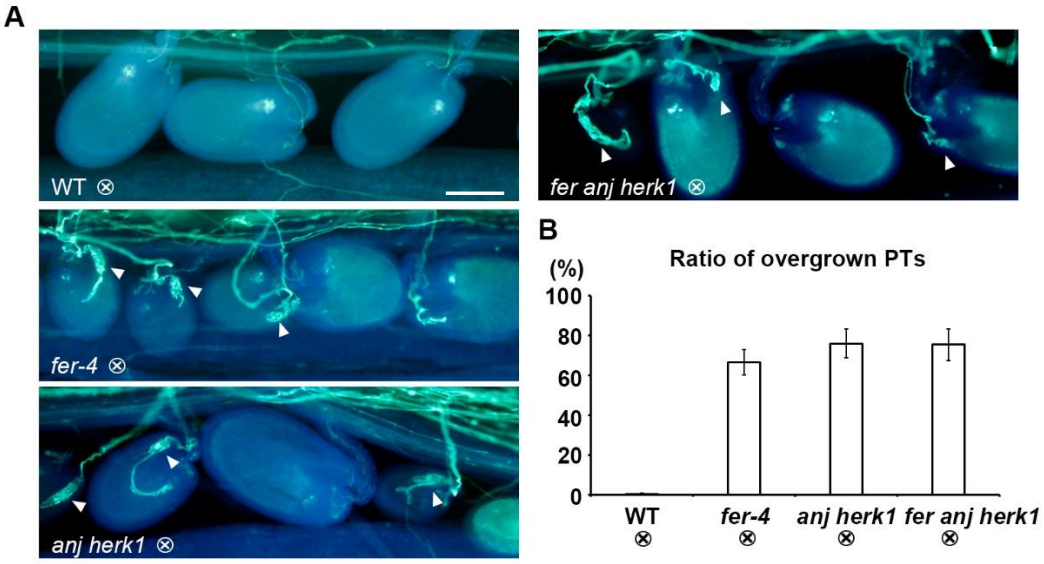

Fig. S11. Analysis of overgrown pollen tubes in selfed WT, *fer-4*, *anj herk1* and *fer anj herk1* mutant ovules.

(**A**) Aniline blue staining of overgrown pollen tubes in self-crossed WT, *fer-4*, *anj herk1* and *fer anj herk1* mutants at two days after anthesis. The analysis was repeated for at least three times for this experiment. Scale bar, 100 μm. (**B**) Statistical analysis of (A). White arrowheads indicate ovules with overgrown pollen tubes. Data are mean values ± SD.

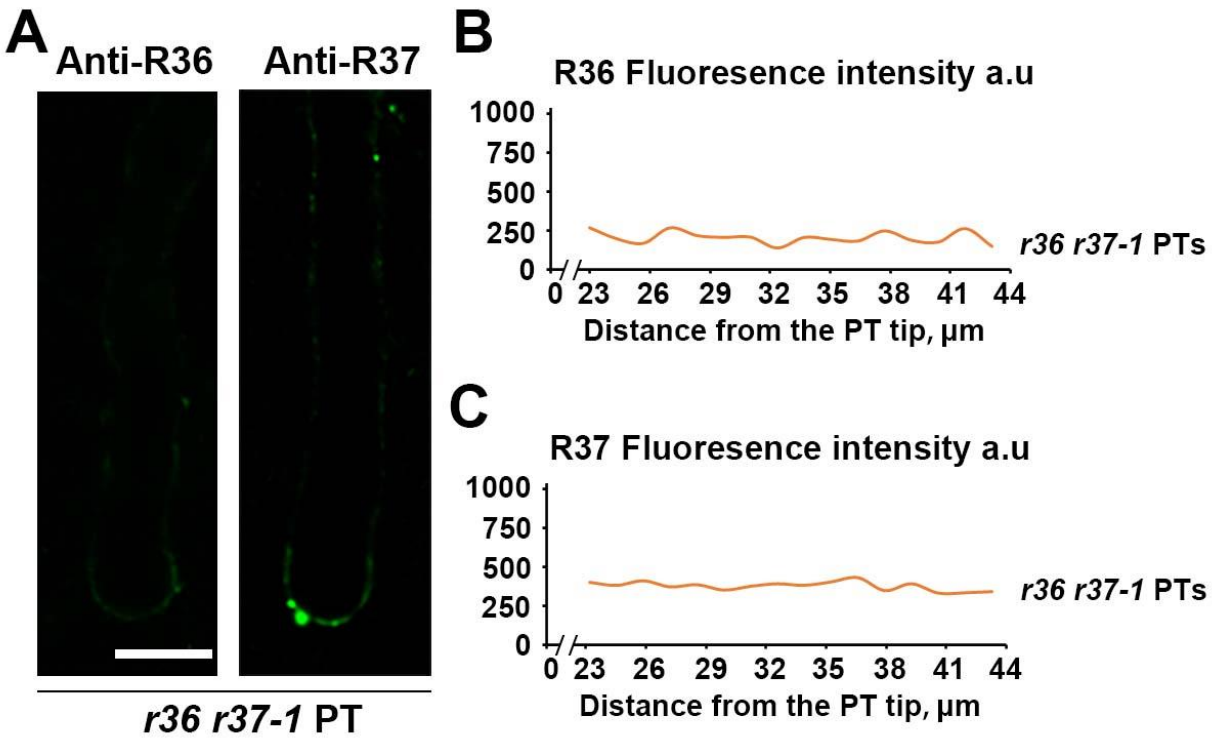

Fig. S12. Immunofluorescence of RALF36 and RALF37 in *r36 r37-1* mutant pollen tubes.
(**A**) Immunofluorescence images of RALF36 and RALF37 in the *r36 r37-1* mutant. Scale bar, 10 μm. (**B** and **C**) Statistical analysis of (A) (n=12 for anti-R36 and 15 for anti-R37). Data are mean values ± SD.

Table S1. Sequences used to build phylogenetic tree

CrRLK1Ls		
Name	**Gene ID**	**Sequence**
FER	**AT3G51550**	MKITEGRFRLSLLLLLLLISAATLISAADYSPTEKILLNCGGGASNLTDTDNRI WISDVKSKFLSSSSEDSKTSPALTQDPSVPEVPYMTARVFRSPFTYTFPVASG RKFVRLYFYPNSYDGLNATNSLFSVSFGPYTLLKNFSASQTAEALTYAFIIKE FVVNVEGGTLNMTFTPESAPSNAYAFVNGIEVTSMPDMYSSTDGTLTMVGS SGSVTIDNSTALENVYRLNVGGNDISPSADTGLYRSWYDDQPYIFGAGLGIPE TADPNMTIKYPTGTPTYVAPVDVYSTARSMGPTAQINLNYNLTWIFSIDSGF TYLVRLHFCEVSSNITKINQRVFTIYLNNQTAEPEADVIAWTSSNGVPFHKDY VVNPPEGNGQQDLWLALHPNPVNKPEYYDSLLNGVEIFKMNTSDGNLAGT NPIPGPQVTADPSKVLRPTTRKSKSNTAIIAGAASGAVVLALIIGFCVFGAYRR RKRGDYQPASDATSGWLPLSLYGNSHSAGSAKTNTTGSYASSLPSNLCRHFS FAEIKAATKNFDESRVLGVGGFGKVYRGEIDGGTTKVAIKRGNPMSEQGVH EFQTEIEMLSKLRHRHLVSLIGYCEENCEMILVYDYMAHGTMREHLYKTQN PSLPWKQRLEICIGAARGLHYLHTGAKHTIIHRDVKTTNILLDEKWVAKVSD FGLSKTGPTLDHTHVSTVVKGSFGYLDPEYFRRQQLTEKSDVYSFGVVLFEA LCARPALNPTLAKEQVSLAEWAPYCYKKGMLDQIVDPYLKGKITPECFKKF AETAMKCVLDQGIERPSMGDVLWNLEFALQLQESAEENGKGVCGDMDMD EIKYDDGNCKGKNDKSSDVYEGNVTDSRSSGIDMSIGGRSLASEDSDGLTPS AVFSQIMNPKGR
THE1	**AT5G54380**	MVFTKSLLVLLWFLSCYTTTTSSALFNPPDNYLISCGSSQNITFQNRIFVPDSL HSSLVLKIGNSSVATSTTSNNSTNSIYQTARVFSSLASYRFKITSLGRHWIRLH FSPINNSTWNLTSASITVVTEDFVLLNNFSFNNFNGSYIFKEYTVNVTSEFLTL SFIPSNNSVVFVNAIEVVSVPDNLIPDQALALNPSTPFSGLSLLAFETVYRLNM GGPLLTSQNDTLGRQWDNDAEYLHVNSSVLVVTANPSSIKYSPSVTQETAP NMVYATADTMGDANVASPSFNVTWVLPVDPDFRYFVRVHFCDIVSQALNT LVFNLYVNDDLALGSLDLSTLTNGLKVPYFKDFISNGSVESSGVLTVSVGPD SQADITNATMNGLEVLKISNEAKSLSGVSSVVKSLLPGGSGSKSKKKAVIIGSL VGAVTLILLIAVCCYCCLVASRKQRSTSPQEGGNGHPWLPLPLYGLSQTLTK STASHKSATASCISLASTHLGRCFMFQEIMDATNKFDESSLLGVGGFGRVYK GTLEDGTKVAVKRGNPRSEQGMAEFRTEIEMLSKLRHRHLVSLIGYCDERSE MILVYEYMANGPLRSHLYGADLPPLSWKQRLEICIGAARGLHYLHTGASQSI IHRDVKTTNILLDENLVAKVADFGLSKTGPSLDQTHVSTAVKGSFGYLDPEY FRRQQLTEKSDVYSFGVVLMEVLCCRPALNPVLPREQVNIAEWAMAWQKK GLLDQIMDSNLTGKVNPASLKKFGETAEKCLAEYGVDRPSMGDVLWNLEY ALQLEETSSALMEPDDNSTNHIPGIPMAPMEPFDNSMSIIDRGGVNSGTGTDD DAEDATTSAVFSQLVHPRGR

	AT5G24010	MAFPINLTQTLLFFFCPLLHLSFAAFTPTDNYLINSGSNTNTSFFTTRSFLSDSS EPGSSFLSTDRSISISDTNPSPDSPVLYNTARVFPVGGSYKFQVTTKGTHFIRL HFAPFKASRFNLRSAKFRVLINGFSVINSFSTSSVVVKEFILKIDDPVLEISFLPF KASGFGFVNAVEVFSAPKDYIMDQGTKLVIPNSAQIFSNLSSQVLETVHRINV GGSKLTPFNDTLWRTWVVDDNYLLLRAAARRAWTTHSPNYQNGGATREIA PDNVYMTAQEMDRDNQELQARFNISWGFQVDEKRVLHLVRLHFCDIVSSSL NQLYFNVFINEYLAFKDVDLSTLTFHVLASPLYIDFVAESDRSGMLRISVGPS DLSNPARVNALLNGVEIMRILSPVSSEVVSGKRNVVWIVVGSVLGGFVFLSL FFLSVLCLCRRKNNKTRSSESTGWTPLRRFRGSSNSRTTERTVSSSGYHTLRI SFAELQSGTNNFDRSLVIGVGGFGMVFRGSLKDNTKVAVKRGSPGSRQGLP EFLSEITILSKIRHRHLVSLVGYCEEQSEMILVYEYMDKGPLKSHLYGSTNPPL SWKQRLEVCIGAARGLHYLHTGSSQGIIHRDIKSTNILLDNNYVAKVADFGL SRSGPCIDETHVSTGVKGSFGYLDPEYFRRQQLTDKSDVYSFGVVLFEVLCA RPAVDPLLVREQVNLAEWAIEWQRKGMLDQIVDPNIADEIKPCSLKKFAETA EKCCADYGVDRPTIGDVLWNLEHVLQLQESGPLNIPEEDYGDVTDPRTARQ GLSNGSNIERDYGDGTSGIISSTQVFSQLMTNAGR
BUPS1	**AT4G39110**	MEIRKKPNIFTVLVIDFSSKPSMALLLAILLFLSGPSASAVAAAAVGPATGFKP ADDILIDCGSKSSSKTPDGRVFKSDQETIQYIEAKEDIQVSAPPSDKVASPIYL TARIFREEATYKFHLTRPGWHWVRLHFLAFPNDKFDLQQATFSVLTEKYVL LHNFKISNNNNDSQAAVQKEYLVNMTDAQFALRFRPMKSSAAFINAIEVVS APDELISDSGTALFPVIGFSGLSDYAYQSVYRVNVGGPLIMPQNDTLGRTWIP DKEFLKDENLAKDVKTTPSAIKYPPEVTPLIAPQTVYATAVEMANSLTIDPNF NVSWNFPSNPSFNYLIRLHFCDIVSKSLNDLYFNVYINGKTAISGLDLSTVAG NLAAPYYKDIVVNATLMGPELQVQIGPMGEDTGTKNAILNGVEVLKMSNSV NSLDGEFGVDGRTTGMGKHGMVATAGFVMMFGAFIGLGAMVYKWKKRP QDWQKRNSFSSWLLPIHAGDSTFMTSKGGSQKSNFYNSTLGLGRYFSLSELQ EATKNFEASQIIGVGGFGNVYIGTLDDGTKVAVKRGNPQSEQGITEFQTEIQ MLSKLRHRHLVSLIGYCDENSEMILVYEFMSNGPFRDHLYGKNLAPLTWKQ RLEICIGSARGLHYLHTGTAQGIIHRDVKSTNILLDEALVAKVADFGLSKDVA FGQNHVSTAVKGSFGYLDPEYFRRQQLTDKSDVYSFGVVLLEALCARPAINP QLPREQVNLAEWAMQWKRKGLLEKIIDPHLAGTINPESMKKFAEAAEKCLE DYGVDRPTMGDVLWNLEYALQLQEAFTQGKAEETENAKPDVVTPGSVPVS DPSPITPSVTTNEAATVPVPAKVEENSGTAVDEHSGTAMFTQFANLNGR

CVY1	**AT2G39360**	MINLKLFLELKLCFLITLLCSSHISSVSDTFFINCGSPTNVTVNNRTFVSDNNL VQGFSVGTTDSNSGDESTLFQTARVFSDESSSTYRFPIEEHGWFLIRIYFLPLV SASQDLTTARFSVSAQNFTLIREYKPSTTSVVREYILNVTTDSLLLQFLPRTGS VSFINALEVLRLPETLIPEDAKLIGTQKDLKLSSHAMETVSRVNMGNLSVSRD QDKLWRQWDSDSAYKAHFGTPVMNLKAVNFSAGGITDDIAPVYVYGTATR LNSDLDPNTNANLTWTFKVEPGFDYFVRFHFCNIIVDPFGFERQIRFDIFVNSE KVRTIDMTEVLNGTFGAPFFVDAVMRKAKSREGFLNLSIGLVMDVSSYPVS FINGFEISKLSNDKRSLDAFDAILPDGSSSNKSSNTSVGLIAGLSAALCVALVF GVVVSWWCIRKRRRRNRQMQTVHSRGDDHQIKKNETGESLIFSSSKIGYRY PLALIKEATDDFDESLVIGVGGFGKVYKGVLRDKTEVAVKRGAPQSRQGLA EFKTEVEMLTQFRHRHLVSLIGYCDENSEMIIVYEYMEKGTLKDHLYDLDD KPRLSWRQRLEICVGAARGLHYLHTGSTRAIIHRDVKSANILLDDNFMAKVA DFGLSKTGPDLDQTHVSTAVKGSFGYLDPEYLTRQQLTEKSDVYSFGVVML EVVCGRPVIDPSLPREKVNLIEWAMKLVKKGKLEDIIDPFLVGKVKLEEVKK YCEVTEKCLSQNGIERPAMGDLLWNLEFMLQVQAKDEKAAMVDDKPEASV VGSTMQFSVNGVGDIAGVSMSKVFAQMVREETR
ANX2	**AT5G28680**	MNEKLRILFSFLCFFYVLLVSPSQSNGQDISLSCGASEPAVDQDKKKWEPDT KFLKTPNTVHAPATYQDPSLLSTVPYMTSRIFTAPATYEIPVKGDKRHMLRL HFYPSTYTGLNILDSYFSVAANDLTLLSNFSAAITCQALTQAYLVREYSLAPS EKDVLSIIFTPSDKHPKAFAFINGIEVIPMPELFDTASLVGFSDQTSDTKTANL QTMFRLNVGGQDIPGSQDSGGLTRTWYNDAPYIFSAGLGVTLQASNNFRID YQKMPVSTAPADVYKTARSQGPNGDINMKSNLTWMFQVDTNFTYIMRLHF CEFQLAKINQKVFNIFINNRTAQGDTNPADILGWTGGKGIPTYKDYAIYVDA NTGGGGEEISLQMTPSTFGQPEYYDSQLNGLEIFKIDTMKNLAGPNPKPSPM QANEDVKKDFQGDKRITAFVIGSAGGVAAVLFCALCFTMYQRKRKFSGSDS HTSSWLPIYGNSHTSATKSTISGKSNNGSHLSNLAAGLCRRFSLSEIKHGTHN FDESNVIGVGGFGKVYKGVIDGGTKVAIKKSNPNSEQGLNEFETEIELLSRLR HKHLVSLIGYCDEGGEMCLIYDYMSLGTLREHLYNTKRPQLTWKRRLEIAIG AARGLHYLHTGAKYTIIHRDVKTTNILLDENWVAKVSDFGLSKTGPNMNGG HVTTVVKGSFGYLDPEYFRRQQLTEKSDVYSFGVVLFEVLCARPALNPSLSK EQVSLGDWAMNCKRKGTLEDIIDPNLKGKINPECLKKFADTAEKCLSDSGL DRPTMGDVLWNLEFALQLQETADGSRHRTPSNGGGSVDLGGGGGGVTVNI SAGESDLGDDLSSEENSGIFSQIVNPKGR

HERK1	**AT3G46290**	MGIEKFETFILISTISILLCICHGFTPVDNYLINCGSPTNGTLMGRIFLSDKLSSK LLTSSKEILASVGGNSGSDIYHTARVFTEVSSYKFSVTRGRHWVRLYFNPFD YQNFKMGSAKFAVSSQSHVLLSDFTVTSSKVVKEYSLNVTTNDLVLTFTPSS GSFAFVNAIEVISIPDTLITGSPRFVGNPAQFPDMSMQGLETIHRVNMGGPLV ASNNDTLTRTWVPDSEFLLEKNLAKSMSKFSTVNFVPGYATEDSAPRTVYG SCTEMNSADNPNSIFNVTWEFDVDPGFQYYFRFHFCDIVSLSLNQLYFNLYV DSMVAATDIDLSTLVDNTLAGAYSMDFVTQTPKGSNKVRVSIGPSTVHTDY PNAIVNGLEIMKMNNSKGQLSTGTFVPGSSSSSKSNLGLIVGSAIGSLLAVVF LGSCFVLYKKRKRGQDGHSKTWMPFSINGTSMGSKYSNGTTLTSITTNANY RIPFAAVKDATNNFDESRNIGVGGFGKVYKGELNDGTKVAVKRGNPKSQQ GLAEFRTEIEMLSQFRHRHLVSLIGYCDENNEMILIYEYMENGTVKSHLYGS GLPSLTWKQRLEICIGAARGLHYLHTGDSKPVIHRDVKSANILLDENFMAKV ADFGLSKTGPELDQTHVSTAVKGSFGYLDPEYFRRQQLTDKSDVYSFGVVL FEVLCARPVIDPTLPREMVNLAEWAMKWQKKGQLDQIIDQSLRGNIRPDSLR KFAETGEKCLADYGVDRPSMGDVLWNLEYALQLQEAVIDGEPEDNSTNMI GELPPQINNFSQGDTSVNVPGTAGRFEESSIDDLSGVSMSKVFSQLVKSEGR
ANJEA	**AT5G59700**	MGGEKFGFLIWILSIPCLIFLCYGYVPVDNYLINCGSSTNVTVTSRVFISDNLA SNFLTSPNEILAASNRNSNSDIYQTARIFTGISKYRFSVARGRHWIRLHFNPFQ YQNFQMVSAKFSVSSETHVLLSDFTVSSRVMKEYSLNVATDHLELTFTPSGD SFAFLNALEVVSVPDTLFSGDPSFAGSPGKFQGLSWQALETVYRVNMGGPR VTPSNDTLSRIWEPDSEFLVEKNLVKSVSKIASVDYVPGFATEETAPRTVYGT CTEMNSADNPSSNFNVTWDFDVDPGFQYFLRFHFCDIVSKALNQLYFNLYV DSMDVVENLDLSSYLSNTLSGAYAMDFVTGSAKLTKRIRVSIGRSSVHTDYP TAILNGLEIMKMNNSKSQLSIGTFLPSGSSSTTKKNVGMIIGLTIGSLLALVVL GGFFVLYKKRGRDQDGNSKTWIPLSSNGTTSSSNGTTLASIASNSSYRIPLVA VKEATNSFDENRAIGVGGFGKVYKGELHDGTKVAVKRANPKSQQGLAEFR TEIEMLSQFRHRHLVSLIGYCDENNEMILVYEYMENGTLKSHLYGSGLLSLS WKQRLEICIGSARGLHYLHTGDAKPVIHRDVKSANILLDENLMAKVADFGL SKTGPEIDQTHVSTAVKGSFGYLDPEYFRRQQLTEKSDVYSFGVVMFEVLC ARPVIDPTLTREMVNLAEWAMKWQKKGQLEHIIDPSLRGKIRPDSLRKFGET GEKCLADYGVDRPSMGDVLWNLEYALQLQEAVVDGDPEDSTNMIGELPLR FNDYNHGDTSVNFSVAKEGRFDEEESSVDDSSGVSMSKVFSQLIKSEGR

	AT2G23200	MENFCFQDSVSLFITIMVLVLLPRLSLSDTSTYTRPENFYVNCGSDSNVFYGG QTFVGDTNSSTNSVSFTNKGTEVINDQSSVAPEIYRTVRIFRHPSSYKFKLDSL GLHFVRLHFSVVFSRADLLTARFTVSATSGSNHHLKSFSPQNLTNTPRVEEFL LMMNSLEFEIRFVPDHSSLALINAIEVFSAPDDLEIPSASDKNLHTIYRLNVGG EKITPDNDTLGRTWLPDDDDFLYRKDSARNINSTQTPNYVGGLSSATDSTAP DFVYKTAKAMNRSSNEQVGMLMNVTWSFKVKSNHRHFIRIHFSDILSNLSN SDSDFYLFVNGYWRVDVKPSEQPRLASPFFKDVVNVSDGSGLLNISIGTKEA NKDAGFLNGLEMMEVLSKSGSDYSNRSSSRVHIITGCAVAAAAASALVFSLL FMVFLKRRRSKKTKPEVEGTVWSPLPLHRGGSSDNRPISQYHNSPLRNLHLG LTIPFTDILSATNNFDEQLLIGKGGFGYVYKAILPDGTKAAIKRGKTGSGQGIL EFQTEIQVLSRIRHRHLVSLTGYCEENSEMILVYEFMEKGTLKEHLYGSNLPS LTWKQRLEICIGAARGLDYLHSSGSEGAIIHRDVKSTNILLDEHNIAKVADFG LSKIHNQDESNISINIKGTFGYLDPEYLQTHKLTEKSDVYAFGVVLLEVLFAR PAIDPYLPHEEVNLSEWVMFCKSKGTIDEILDPSLIGQIETNSLKKFMEIAEKC LKEYGDERPSMRDVIWDLEYVLQLQMMTNRREAHEEDSTAINSGGSLVAPR LMVSDSFSTNSIFQNGDESKNRFGFTDSSETRVFSQLKISDAR
BUPS2	**AT2G21480**	MEIRKKPNIPMCLVLDSSSRPFMTLLFTILLFLTGLASAVGAVGGSPTAGFKP ADDILIDCGSKSSTKTPEGRVFKSDSETVQYIEAKDDIQVSAPPSDKLPSPIYL TAKIFREEAIYKFHLTRPGWHWVRLHFFAFPNDKFDLQQATFSVLTEKYVLL HNFKLSNDNNDSQATVQKEYLLNMTDAQFALRFKPMKGSAAFINGIELVSA PDELISDAGTSLFPVNGFSGLSDYAYQSVYRVNVGGPLITPQNDTLGRTWTP DKEYLKDENLAKDVKTNPTAIIYPPGVTPLIAPQTVYATGAEMADSQTIDPN FNVTWNFPSNPSFHYFIRLHFCDIISKSLNDLYFNVYINGKTAISGLDLSTVAG DLSAPYYKDIVVNSTLMTSELQVQIGPMGEDTGKKNAILNGVEVLKMSNSV NSLDGEFGVDGQRASMGKQGMVATAGFVMMFGAFVGLGAMVYKWKKRP QDWQKRNSFSSWLLPIHAGDSTFMTSKTGSHKSNLYNSALGLGRYFSLSELQ EVTKNFDASEIIGVGGFGNVYIGTIDDGTQVAIKRGNPQSEQGITEFHTEIQML SKLRHRHLVSLIGYCDENAEMILVYEYMSNGPFRDHLYGKNLSPLTWKQRL EICIGAARGLHYLHTGTAQGIIHRDVKSTNILLDEALVAKVADFGLSKDVAF GQNHVSTAVKGSFGYLDPEYFRRQQLTDKSDVYSFGVVLLEALCARPAINP QLPREQVNLAEWAMLWKQKGLLEKIIDPHLVGAVNPESMKKFAEAAEKCL ADYGVDRPTMGDVLWNLEYALQLQEAFSQGKAEAEEVETPKPVAVPAAAP TSPAATTAAASERPVSQTEEKDDSTVDQHSGTTMFTQFASLNGR

HERK2	**AT1G30570**	MSKLRKKYLEHLLCVLIFFTYVIGYGEAQSKSFLVDCGSNATTEVDGRTWV GDLSPNKSVTLQGFDAITASTSKGSSVYAEIYKTARVFDAVLNYTFEGITQG NYFVRLHFSPFAIENHNVNESSFSVFADGLRLMLDINIAGEIAHKNLILESTGH NATASSLVKEFLLPTGPGKLVLSFIPEKGSFGFVNAIEIVSVDDKLFKESVTKV GGSEVELGLGGRGIETMYRLNVGGPKLGPSKDLKLYRTWETDLSYMVIENA GVEVKNSSNITYALADDSPVAPLLVYETARMMSNTEVLEKRFNISWKFEVD PNFDYLVRLHFCELLVDKQNQRIFRIYINNQTAAGNFDIFAHAGGKNKGIYQ DYLDPVSSKNDVLWIQLGPDSSVGASGDALLSGLEIFKLSKNGNLAHLIRFDS TGHSVSDSKMRIIWISVGAGIAIIIFFVFLGILVVCLCKKRRSKSDESKNNPPG WRPLFLHVNNSTANAKATGGSLRLNTLAASTMGRKFTLAEIRAATKNFDDG LAIGVGGFGKVYRGELEDGTLIAIKRATPHSQQGLAEFETEIVMLSRLRHRHL VSLIGFCDEHNEMILVYEYMANGTLRSHLFGSNLPPLSWKQRLEACIGSARG LHYLHTGSERGIIHRDVKTTNILLDENFVAKMSDFGLSKAGPSMDHTHVSTA VKGSFGYLDPEYFRRQQLTEKSDVYSFGVVLFEAVCARAVINPTLPKDQINL AEWALSWQKQRNLESIIDSNLRGNYSPESLEKYGEIAEKCLADEGKNRPMM GEVLWSLEYVLQIHEAWLRKQNGENSFSSSQAVEEAPESFTLPACSNQDSSE TEQSQTGSALHNSA
ANX1	**AT3G04690**	MSGKTRILFFLTCLSFLLVFPTRSNGQDLALSCGTSEASADQDKKKWEPDTK FLKTGNSIHATATYQDPSLLSTVPYMTARIFTAPATYEIPIKGDKRHLLRLYF YPSTYTGLNISNSYFTVEANDVTLLSNFSAAITCQALTQAYLVKEYSLAPTDK DVLSIKFTPSDKYRDAFAFINGIEVIQMPELFDTAALVGFTDQTMDAKTANL QSMFRLNVGGQDIPGSQDSGGLTRTWYNDAPYIFSAGLGVTLQASNNFRINY QNMPVSIAPADIYKTARSQGPNGDINLKSNLTWMFQIDKNFTYILRLHFCEFQ LSKINQKVFNIYINNRTAQADTTPADIIGWTGEKGIPMYKDYAIYVDANNGG EEITLQMTPSTFGQPEYYDSSLNGLEIFKMDTMKNLAGPNPEPSPMQAEEEV KKEFKNEKRHAFIIGSAGGVLAVLIGALCFTAYKKKQGYQGGDSHTSSWLPI YGNSTTSGTKSTISGKSNNGSHLSNLAAGLCRRFSLPEIKHGTQNFDDSNVIG VGGFGKVYKGVIDGTTKVAVKKSNPNSEQGLNEFETEIELLSRLRHKHLVSL IGYCDEGGEMCLVYDYMAFGTLREHLYNTKKPQLTWKRRLEIAIGAARGLH YLHTGAKYTIIHRDVKTTNILVDENWVAKVSDFGLSKTGPNMNGGHVTTV VKGSFGYLDPEYFRRQQLTEKSDVYSFGVVLFEILCARPALNPSLPKEQVSL GDWAMNCKRKGNLEDIIDPNLKGKINAECLKKFADTAEKCLNDSGLERPTM GDVLWNLEFALQLQETADGTRHRTPNNGGSSEDLGRGGMAVNVAGRDDV SDLSSEDNTEIFSQIVNPKGR

CAP1	**AT5G61350**	MGGDFRHFSSHVSLLLLFLLIVKSSSSFTPADNYLIDCGSSDETKLSDGRNFKS DQQSVAFLQTDEDIKTSVDSIPITDSNASTLPLYLTARIFAGKSTYSFYISRPGR HWIRLHFYPLNHPLYNLTNSVFSVTTDTTVLLHDFSAGDTSSIVFKEYLIYAA EKLSLYFKPHKGSTAFINAVEIVSVPDELVPDSASSVPQAPDFKGLSSFSLEIL HRINIGGDLISPKIDPLSRTWLSDKPYNTFPEGSRNVTVDPSTITYPDGGATAL IAPNPVYATAEEMADAQTSQPNFNLSWRMSVDFGHDYFIRLHFCDIVSKSLN DLIFNVFINKLSAISALDLSSLTSALGTAYYADFVLNASTITNGSILVQVGPTP NLQSGKPNAILNGLEIMKLNNAAGSLDGLFGVDGKYKGPIGGMSSKKLAIA GIGFVMALTAFLGVVVLLVRWQRRPKDWQKQNSFSSWLLPLHASHSSYISS KGGSTSRRMSIFGSKKSKSNGFSSFFSNQGLGRYFPFTELQTATQNFDENAVC GVGGFGKVYIGEIDGGTQVAIKRGSQSSEQGINEFQTEIQMLSKLRHRHLVSL IGFCDENKEMILVYEYMSNGPLRDHLYGSKENDPNPIPTLSWKQRLEICIGSA RGLHYLHTGAAQGIIHRDVKTTNILLDENLVAKVSDFGLSKDAPMDEGHVS TAVKGSFGYLDPEYFRRQQLTDKSDVYSFGVVLFEVLCARPVINPQLPREQV NLAEYAMNLHRKGMLEKIIDPKIVGTISKGSLRKFVEAAEKCLAEYGVDRPG MGDVLWNLEYALQLQEASAQVDLSEDKTTMNIEMDLIPGEEMQSPSHSIP
MDS1	**AT5G38990**	MICHVLVIFTILVSAVVDATASYEPTDVFLINCGDTSNNMDYSGRNWTTENP KFMSSNAVDDASFTSSASYQESGIPQVPYLKARIFRYDFTYSFPVSPGWKFLR LYFYPTRYGSDFDAVKSFFSVNVNRFTLLHNFSVKASIPESSSLIKEFIVPVNQ TLDLTFTPSPNSLAFVNGIEIISMPDRFYSKGGFDDVVRNVGRDVDFEIDNST AFETVYRVNVGGKVVGDVGDSGMFRRWLSDEGFLLGINSGAIPNITGVKIN YTDKTPAYVAPEDVYTTCRLMGNKDSPELNLNFNLTWLFEVDAGFAYIVRL HFCETQPEVNKTGDRVFSIFFGYQLAMREMDVFRLSGGFRLPMYLDFKVLV DADGTSQRPSLRVDLTPYKEDYPTYYDAILSGVEILKLSNSDGNLAGLNPIPQ LSPPPQSITPLKGKGKSSHVLPIIIAVVGSAVALAFFVLVVVLVVMKRKKKSN ESSVDTTNKPSTNSSWGPLLHGTGSTNTKSASSLPSDLCRRFSIYEIKSATNDF EEKLIIGVGGFGSVYKGRIDGGATLVAVKRLEITSNQGAKEFDTELEMLSKL RHVHLVSLIGYCDDDNEMVLVYEYMPHGTLKDHLFRRDKASDPPLSWKRR LEICIGAARGLQYLHTGAKYTIIHRDIKTTNILLDENFVAKVSDFGLSRVGPTS ASQTHVSTVVKGTFGYLDPEYYRRQILTEKSDVYSFGVVLLEVLCCRPIRMQ SVPPEQADLIRWVKSNFNKRTVDQIIDSDLTADITSTSMEKFCEIAIRCVQDR GMERPPMNDVVWALEFALQLHETAKKKNDNVESLDLMPSGEVGTTTDGE DDLFSRTTGHVGKSTTTDDSVLVVGDERSGSSWGVFSEINEPKAR

MDS2	**AT5G39000**	MIRHALLIFSILVSTPIVGEGATSTYEPTDVFLFNCGDTSNNVDVSGRNWTAE NQKILSSNLVNASFTAQASYQESGVSQIPYMTARIFRSEFTYSFPVTPGSNFLR LYFYPTRYGSQFNAVKSFFSVKVNGFTLLNNFSADLTVKASKPQTEFIIKEFII PVYQTLNLTFTPSLDSLAFVNGIEIVSIPNRFYSKGGFDDVITNVGSSVDFHIE NSTAFETVYRLNVGGKTVGDSGMFRRWVSDDEIILSESSGISPIVPDIKINYTE KTPSYVAPDDVYATSRSMGNADHPEQNLNFNLTWLFTVDAGFSYLVRLHFC ETLSEVNKEGQRVFSIFIENQTATLEMDVFRMSGGSWIPMYLDYTVIAGSGS GRRHDLRLDLHPLVSINPKYYDAILNGVEILKMNDPDGNLAGPNPDPLVSPD LIPNRATPRIRKNKSHILPITLAVVGSLVVLAMFVVGVLVIMKKKKKSKPSTN SSWCPLPHGTDSTNTKPAKSLPADLCRRFSIFEIKSATNDFEDKLIIGVGGFGS VYKGQIDGGATLVAVKRLEITSNQGAKEFETELEMLSKLRHVHLVSLIGYCD EDNEMVLVYEYMPHGTLKDHLFRRDKTSDPPLSWKRRLEICIGAARGLQYL HTGAKYTIIHRDIKTTNILLDENFVTKVSDFGLSRVGPTSASQTHVSTVVKGT FGYLDPEYYRRQVLTEKSDVYSFGVVLLEVLCCRPIRMQSVPPEQADLIRWV KSNYRRGTVDQIIDSDLSADITSTSLEKFCEIAVRCVQDRGMERPPMNDVVW ALEFALQLHETAKKKNDNVESLDLMPSGEVGTTTDGEDDLFSRTTGHVGKS TTTDDSVLVVGDERSGSSWGVFSEINEPKAR
MDS3	**AT5G39020**	MNCNVLFLLSVLVSVTAGVTAAYHPTDVFLFNCGDTSNNVDNSGRNWTVE SRQILSSNLVNASFTSEASYQKAGVSRIPYMKARIFRSEFTYSFPVTPGSIFLRL YFYPTQYKSGFDAVNSFFSVKVNGFTLLRNFNADSTVQASIPLSNSLIKEFIIP VHQTLNLTFTPSKNLLAFVNGIEIVSMPDRFYSKGGFDNVLRNVSSDVDFQI DNSTAFESVHRLNVGGQIVNEVDDSGMFRRWLSDDSFGNSGSIVNVPGVKI NYTEKTPAYVAPYDVYATSRLMGNSSNLMFNLTGMFLTVDAGYNYLVRLH FCETLPQVTKAGQRVFSIFVEDKMAKKETDVIRLSGGPRIPMYLDFSVYVGF ESGMIQPELRLDLVPLKDTNQTYYDAILSGVEILKLNDSDGNLARPNPELLVS TDSTPDDSNVTPPIKGKPHVLVIILIVVGSVIGLATFIVIIMLLIRQMKRKKNKK ENSVIMFKLLLKQYIYAELKKITKSFSHTVGKGGFGTVYRGNLSNGRTVAVK VLKDLKGNGDDFINEVTSMSQTSHVNIVSLLGFCYEGSKRAIISEFLEHGSLD QFISRNKSLTPNVTTLYGIALGIARGLEYLHYGCKTRIVHFDIKPQNILLDDNF CPKVADFGLAKLCEKRESILSLIDTRGTIGYIAPEVVSRMYGGISHKSDVYSY GMLVLDMIGARNKVETTTCNGSTAYFPDWIYKDLENGDQTWIIGDEINEED NKIVKKMILVSLWCIRPCPSDRPPMNKVVEMIEGSLDALELPPKPSRHISTEL VLESSSLSDGQEAEKQTQTLDSTII

MDS4	**AT5G39030**	MICFILFVFSFLVSVSATAPYKPDDVFLINCGETDVPFDNHGRTWTQEEKNIL PKNSDNASFSSVVSYKEESGIPQVPYMTARIFRSDFTYSFPVSPGWKFLRLYF YPTSYKSGFDAVNSFVSVTVNDFTLLQNFSADLTVKASIPESKSLIKEFIVPVY LTLNLTFRPSNNSLAFVNGIEIVSMPDRFYSKGGFDDLITNVGSLIDFEIDNST ASETVHRLNVGGHMVDEVNDSGMFRRWLSDDYEFLIGGVSPYMPDVNISY TEKTPAYVAPAYVYSTCRMMGNAQDTYLNLNFNLTWLFTVDAGFSYLVRL HFFEKYLNKANQRVFSIFLGNQMAREEMDVIRLSGGPRIPIYLDFRIYVGSES GPRPDLRLDLHPLVKDNPEYYEAILNGVEILKLNNSGNLAIIQDNELKPNPPL SSNLTPNHVTQQIKGKSSHLLVKIFIAVGPGTGLATFVVVLMLWMRQMKRK NRKEERVVMFKKLLNMYTYAELKKITKSFSYIIGKGGFGTVYGGNLSNGRK VAVKVLKDLKGSAEDFINEVASMSQTSHVNIVSLLGFCFEGSKRAIVYEFLE NGSLDQFMSRNKSLTQDVTTLYGIALGIARGLEYLHYGCKTRIVHFDIKPQNI LLDGNLCPKVSDFGLAKLCEKRESVLSLMDTRGTIGYIAPEVFSRMYGRVSH KSDVYSFGMLVIDMIGARSKEIVETVDSAASSTYFPDWIYKDLEDGEQTWIF GDEITKEEKEIAKKMIVVGLWCIQPCPSDRPSMNRVVEMMEGSLDALEIPPK PSMHISTEVITESSSLSDGGEDV

RALFs		
Name	**Gene ID**	**Sequence**
RALF1	**AT1G02900**	MDKSFTLFLTLTILVVFIISSPPVQAGFANDLGGVAWATTGDNGSGCHGSIAE CIGAEEEEMDSEINRRILATTKYISYQSLKRNSVPCSRRGASYYNCQNGAQA NPYSRGCSKIARCRS
RALF2	**AT1G23145**	MEARHMLVTILLLSFVFMNIMKVEAQKVIGYPAIGRDGARGCSPKDPSCPQQ PEKPYKRGCEKITRCERDRRKQAHLRNPRKVLDVVAVMAKAKQLY
RALF3	**AT1G23147**	MSNLRGTNRFILVAVLVSFVFLSIMNAEARKEIGYPKQRFGEDRTNPYEEITP PLIGGCDPKNPQTCLPKQPANPYRRGCLKITRCQRDV
RALF4	**AT1G28270**	MGVKMLLIFGLLILAMVAKSVNATYPLTKSCINGQGCIGEDDELESLMDSET NRRQLARGRRYIGYDALKKNNVPCSRRGRSYYDCKKRRRNNPYRRGCSAIT HCYRYAR
RALF5	**AT1G35467**	MLKAQVFMFVTVLVFVCVFINSNDAKRYIEYPPWQKHPCNPRFPTPDCYKR TPANPYRRGCTCISRCRRDCGGLSTWKKLLDTILKIPV
RALF6	**AT1G60625**	MAAHKKSHIRIFFVSVMIILSLFSGFGEGQTYINYNGMKGDIIPGCSSKNPKEC VKIPAYSYNRGCEISTRCQRQQHSSSS
RALF7	**AT1G60815**	MSARKKNRIHVFFVSIMIIISLVSGFGEGIKQINYKDLIKDTIPGCTSKNPKECV KVPANTYHRGCEISTRCHREQHSSSG
RALF8	**AT1G61563**	MGMSKSIKVILSLALVVFLALAGTKVEASVRYITYPAIDRGDHAVHCDKAHP NTCKKKQANPYRRGCGVLEGCHRETGPKPT
RALF9	**AT1G61566**	MGMSKSIKVILSLALVVFLALAATKVEATRYITYPAIDRGDHAVHCDKAHPN TCKKKEANPYQRGCEKINRCRGG
RALF10	**AT2G19020**	MKALVICLLVIFAAVIAVPVESRRKHLDYGVITKCAGPNPPPGCYPPGAQQK NPTPANEYRRGCSKITRCKRD
RALF11	**AT2G19030**	MKAWLICLLVICAAVIAEPVESRNYIEYGAINKCAGPNPPPGCNPPGAEQKNP TPVNEYSRGCSKIHRCRRD

RALF12	**AT2G19040**	MKAWVIGLLVICAVVIAEPVESRNYIEYGAINKCAGPNPPPGCNPPGTEQKN PTPVNEYSRGCSKIHRCRRD
RALF13	**AT2G19045**	MKAWVICLLVICAAVIAEPVESRNYIEYGAINKCAGPNPPPGCNPPGAEQKN PNPVNEYSRGCSKIHRCRRD
RALF14	**AT2G20660**	MKLLIFAVIISVVLFPVLVSSRTIKCDQLSGKCINGEEKEIMNMRLGLDVSSRR ILQASRYISYEALKKNLPDNRRGEPDQRDNPYRRSCDVHSHCYRFT
RALF15	**AT2G22055**	MGMSKSIKVIVSLALILFLALAATKVEATRYISYRGMNHGDHAIHCDKAHPN TCKKQVANPYRRGCGTIERCRRDTGRK
RALF16	**AT2G32835**	MVAYEKSPIVFLFATMMLVMFLFCGSGEARTLGYGSIKGDRIPACGYKNPNS CVKQPVNHYHRGCEKITRCARDAARYTESFNVDDDESPIINLH
RALF17	**AT2G32885**	MGISKKTVVQSFALIIIISIVMSTTEANSIGAPAMREDLPKGCAPGSSAGCKMQ PANPYKPGCEASQRCRGG
RALF18	**AT2G33130**	MMNNMKLLIIAVMIISAALLPALVVGSRPVKCDNCMDGGEKEEIMKMSSGV DVSHRILQAKRFIDYEALKKNLPAKPDGKPDKPDNKYRRGCSAATGCYRFT N
RALF19	**AT2G33775**	MGIKILLILGLLTLAVVAESANATWTLTKSCVNGQGCIGEDGELDYLMDSET NRRQLAARRSYISYGALRKNNVPCSRRGRSYYDCKKRKRANPYRRGCSVIT HCYRQTS
RALF20	**AT2G34825**	MVLSKKTIMQSFALMIILSIVMSTTEAKTIGNPAMREDEPKGCPPGSPASCKM QPANPYKPGCEASQRCRGT
RALF21	**AT3G04735**	MSNMKITNRFMLVATFIACVFISSMNMTVGKVIGYPGLKPDLPCDHHRYPSA CAPSEQPVNPYRRGCSKIHRCRRDSPPAPISRKMLIRGQLIYNNAYNAYIQYP
RALF22	**AT3G05490**	MTNTRAIYAVIAILAIVISAVESTGDFGDSLDFVRAGSSSLFSGCTGSIAECIAE EEEMEFDSDISRRILAQKKYISYGAMRRNSVPCSRRGASYYNCQRGAQANPY SRGCSTITRCRR
RALF23	**AT3G16570**	MRGLSRNSGAAAIFAILLILAVHNWSVAVSSQSTEFAGDFPPFETECRGTIAE CSVSAALGDGGDLFYGGGEMGEEFEMDSEINRRILATRRYISYGALRRNTIPC SRRGASYYNCRRGAQANPYSRGCSAITRCRRS
RALF24	**AT3G23805**	MSRSLALVYLSLLCLQTHLSISVTVPIPSVNGEIDAMLNRNGVIGEEEGEEMM PSEISRRVMMMRKQYISYETLRRDMVPCQKPGASYYACRSGQANAYNRGC SVITRCARDTNDIKT
RALF25	**AT3G25165**	MKTFMIILLVICSILIVGRVEANDNKRKYLLLDPCLRPNAPPGCHRQPYKPRT PVNVYSRGCTTINRCRRVQNP
RALF26	**AT3G25170**	MKAWMIILLVICVAVVVEQSEARKGRKYLNPGVLDRCRGPNPPAGCHPHNS HHKPRVPVHNYSRGCSRITRCRRDA
RALF27	**AT3G29780**	MTKTFFSFSFFFTSSLLLLLAATSATASTGNVTSGLRYDGCAPGDTVGECITA TVEEEDEEGVEAVVRRILQQRKYLSYKTLQKQPTCDGRIAGNCIGTVNPKGA TCTYYQRCKRAA
RALF28	**AT4G11510**	MSILKETKRFMVVAMFIACVFISNNMNVAVANEIGYPGMGRGDRQPGCDH GNCPPDQPANPYHRGCEKSKRCRGPDPPALPRKMI
RALF29	**AT4G11653**	MIKTKEVTFVTILIVLCVFISTIHAKRYIEYPIRLDLGKGCDPRFPTAACYKRTP ANPYRRPCTTANRCRRSTSSTRVPSLKTFVEIPPM
RALF30	**AT4G13075**	MKAWVICLMVISIFMMIEPTLAAGGGKFLNPGVLDPCLRPNPPPECQAPGSA GKPRERVNEYKVGCSKLTRCDRVG

RALF31	**AT4G13950**	MFNSTALVIFAILFLLISADAFPIPSPNGEIDAMLIRNSIIGEDEDLMPTEISRRV LMAQKRYIGYETLRRDMVPCQKPGASYYDCRSGQANSYSRGCDTITRCARD TNDINT
RALF32	**AT4G14010**	MEIKPSRIFSTITIFFLCLLLAHVTSKASSSSLCNGSVAECSSMVETEEMSVIME SWSSQRLTEEQAHKLSYGALRRNQPACDGGKRGESYSTQCLPPPSNPYSRGC SKHYRCGRDS
RALF33	**AT4G15800**	MRGLSTKPVAIIIAILTVHFLFAAVTSQSSGDFVPIESKCNGTIAECSLSTAEEE FEMDSEINRRILATTKYISYGALRRNTVPCSRRGASYYNCRRGAQANPYSRG CSAITRCRR
RALF34	**AT5G67070**	MAASSLNLLLILSLLTFISLQRSESLSDNPSLTLLPDGFDWPISHSDEFDIIDGEE SFEVTEEDDGVTDRRSLYWRRTKYYISYGALSANRVPCPPRSGRSYYTHNCF RARGPVHPYSRGCSSITRCRR
RALF35	**AT1G60913**	MAAHKMSLTSLFFVSIVIVLSLFSGFGEGRYIKYRAIAKDRVPDCTQDPKNCV RVPVNQYHLPPGCQNTTHCYREKYHI
RALF36	**AT2G32785**	MAMLKAISVLCVALLIIFVVKADTINREQVISYESMRVNHAWGCSQKYPQFC QKTRANPYTKPPPKNSEAS
RALF37	**AT2G32788**	MGISKKSTKAIIIYMALIMVFFTFATLKTNAEDVISYEVLLQDHAWGCSPKFP RLSCLKQKANP

Table S2. Primers

Purpose	Primers Name	Sequences(5'-3')
Spacers cloning (for *hap2* mutant)	HAP2-BsF	ATATATGGTCTCGATTGattgtagcggagatcgtggGTT
	HAP2-F0	TGattgtagcggagatcgtggGTTTTAGAGCTAGAAATAGC
	HAP2-R0	AACctgtctttccacgtgcacaCAATCTCTTAGTCGACTCTAC
	HAP2-BsR	ATTATTGGTCTCGAAACctgtctttccacgtgcacaC
Spacers cloning (for *ralf 6 ralf7 ralf16* mutant)	RALF6-BsF-1	ATATATGGTCTCGATTGtgttcagcggatttggagaGTT
	RALF6-F0-1	TGtgttcagcggatttggagaGTTTTAGAGCTAGAAATAGC
	RALF6-R0-1	AACgcatactcttacaatcgtgCAATCTCTTAGTCGACTCTAC
	RALF6-BsR-1	ATTATTGGTCTCGAAACgcatactcttacaatcgtgC
	RALF7-BsF-1	ATATATGGTCTCGATTGttataatatcattggtcagGTT
	RALF7-F0-1	TGttataatatcattggtcagGTTTTAGAGCTAGAAATAGC
	RALF16-R0-1	AACtaatgttcttgcttctccaCAATCTCTTAGTCGACTCTAC
	RALF16-BsR-1	ATTATTGGTCTCGAAACtaatgttcttgcttctccaC
	RALF6-BsF-2	ATATATGGTCTCGATTGgaattttgacacattctttGTT
	RALF6-F0-2	TGgaattttgacacattctttGTTTTAGAGCTAGAAATAGC
	RALF6-R0-2	AACcgattgtaagagtatgctgCAATCTCTTAGTCGACTCTAC
	RALF6-BsR-2	ATTATTGGTCTCGAAACcgattgtaagagtatgctgC
	RALF7-BsF-2	ATATATGGTCTCGATTGcaaccacgatggtaagtgtGTT
	RALF7-F0-2	TGcaaccacgatggtaagtgtGTTTTAGAGCTAGAAATAGC
	RALF7-R0-2	AACaaagaatgcgtcaaagttcCAATCTCTTAGTCGACTCTAC
	RALF7-BsR-2	ATTATTGGTCTCGAAACaaagaatgcgtcaaagttcC
	RALF16-BsF-2	ATATATGGTCTCGATTGgagaccgaattccggcatgGTT
	RALF16-F0-2	TGgagaccgaattccggcatgGTTTTAGAGCTAGAAATAGC
	RALF16-R0-2	AACggtgtgctcgtgatgctgcCAATCTCTTAGTCGACTCTAC
	RALF16-BsR-2	ATTATTGGTCTCGAAACggtgtgctcgtgatgctgcC
Spacers cloning (for *ralf36 ralf37* mutant)	RALF36-BsF	ATATATGGTCTCGATTGtaaggcggatacaatcaacGTT
	RALF36-F0	TGtaaggcggatacaatcaacGTTTTAGAGCTAGAAATAGC
	RALF37-R0	AACatgcggaagatgtgataagCAATCTCTTAGTCGACTCTAC
	RALF37-BsR	ATTATTGGTCTCGAAACatgcggaagatgtgataagC
Genotyping for CRISPR mutants and T-DNA mutants	RALF6-CRI-F	ACTCTATCAGCACATACATTATGCACT
	RALF6-CRI-R	GGGCTTTAATCGTAGGGGTAAAGA
	RALF7-CRI-F	TGAACAGTTTAACTGTGCTGCCA
	RALF7-CRI-R	ATAAACAATTCAAGGTTACAAAAACAAATGGAG
	RALF16-CRI-F	ATATGTATTATTCCCTGGCATATGTAACAACA
	RALF16-CRI-R	AAATAATCTCAGGAAAAGCATAGAATACA
	RALF36-CRI-F	ATAAAGCATCAACGCAAAATTAGAAAT
	RALF36-CRI-R	GAATACATTTGCCGCCCTCTT
	RALF37-CRI-F	TAATCCCTTCATGTTTCTCCACA
	RALF37-CRI-R	CCGCAGAAAATTTAGATATATGAGT
	HAP2-CRI-F	CGTCTTGAATCTCGCTGTTCCC
	HAP2-CRI-R	ATGTGAACTACGTTGGCTATTTGGTG
	ANJ-LP	TTCCAGGTTTTGTGAAGATGG
	ANJ-RP	AATCCGTGTGAACACTCGATC
	FER-genotyping-F	*Reference 13*
	FER-genotyping-R	*Reference 13*

	HERK1-genotyoing-F	*Reference 13*
	HERK1-genotyping-R	*Reference 13*
	CRISPR-Cas9 fer mutant genotyping inner primer F	*Reference 13*
	CRISPR-Cas9 fer mutant genotyping inner primer R	*Reference 13*
	CRISPR-Cas9 fer mutant genotyping outer primer F	*Reference 13*
	CRISPR-Cas9 fer mutant genotyping outer primer R	*Reference 13*
	MYB97-1-LP	*Reference 19*
	MYB97-1-RP	*Reference 19*
	MYB101-1-LP	*Reference 19*
	MYB101-1-RP	*Reference 19*
	MYB120-3-LP	*Reference 19*
	MYB120-3-RP	*Reference 19*
	ACA9-1-LP	TCTCAATCTTGCTCTTAATCCATT
	ACA9-1-RP	AGGACAACAAGAGCCACTGAG
	LBb1.3	ATTTTGCCGATTTCGGAAC
CAS9 identification (for CRISPR mutants)	Hyg-IDF	CAAAGATCGTTATGTTTATCGGCACT
	Hyg-IDR	AAGAAGATGTTGGCGACCTCGTATT
Subcellular localization observation	RALF6-NSC-pDONR221-R	GGGGACCACTTTGTACAAGAAAGCTGGGTAACTCGAACTCGAATGCTGTTGAC
	RALF7-NSC-pDONR221-R	GGGGACCACTTTGTACAAGAAAGCTGGGTAACCCGAACTCGAATGCTGTTC
	RALF16-NSC-pDONR221-R	GGGGACCACTTTGTACAAGAAAGCTGGGTAATGGAGATTAATAATGGGACTTTCATC
	RALF36-NSC-pDONR221-R	GGGGACCACTTTGTACAAGAAAGCTGGGTATGAAGCTTCACTGTTTTTTGGGG
	RALF37-NSC-pDONR221-R	GGGGACCACTTTGTACAAGAAAGCTGGGTAGGGATTGGCCTTTTGTTTGAGA
Expressiom pattern analysis	pRALF6-pDONR221-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAGGTTCTTTTCAACGAAGCAGTGA
	pRALF6-pDONR221-R	GGGGACCACTTTGTACAAGAAAGCTGGGTATGTTTTTTGAGAAAACAAAAACAATGT
	pRALF7-pDONR221-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTACGTTGAGAGAGGAGGTAGCTACAGC
	pRALF7-pDONR221-R	GGGGACCACTTTGTACAAGAAAGCTGGGTATGTTTTTTTTTAAAAACAATGTATTTGAATC
	pRALF16-pDONR221-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAGCAGACAATGGTAACTAGTAACTGGACA
	pRALF16-pDONR221-R	GGGGACCACTTTGTACAAGAAAGCTGGGTAATTTTTTTTTGTTAATTTTCTTCTTAACTATTTT

	pRALF36-pDONR221-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAGCATAACCATCTCTTGCTTCTATACCA
	pRALF36-pDONR221-R	GGGGACCACTTTGTACAAGAAAGCTGGGTAATTTTCAAAACGAACAAAGTAAACTAAATG
	pRALF37-pDONR221-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAGGGTTGGAATTTTTGTGATA
	pRALF37-pDONR221-R	GGGGACCACTTTGTACAAGAAAGCTGGGTAATCTTTTTCTTTCTTTTTTTTTTGAATGAA
	GG_FER_A_F	AACAGGTCTCAACCTAATGTGATGTTTGTGTTATA
	GG_FER_B_R	AACAGGTCTCTTGTTCGATCAAGAGCACTTCTCCGG
	GG_FER_E_R	AACAGGTCTCTCTGAACGTCCCTTTGGATTCATGAT
	GG_ANJ_A_F	AACAGGTCTCAACCTCAAAATATAAATATTAGTTT
	GG_ANJ_B_R	AACAGGTCTCTTGTTCTTCACAAAACCTGGAAATT
	GG_HERK1_A_F	AACAGGTCTCAACCTGAGTAAACGGCTCACGTTTGT
	GG_HERK1_B_R	AACAGGTCTCTTGTTTTTGACCCAATAGAGAATAA
Modules for GreenGate cloning	GG_E_HSP18.2_F	AACAGGTCTCACTGCTGAATATGAAGATGAAGATG
	GG_E_HSP18.2_R	AACAGGTCTCATAGTTATGAGCCCATCTTATCTTT
	P16	*Reference 34*
	Omega element	*Reference 34*
	SV40-NLS	*Reference 34*
	BASTA selection marker	*Reference 34*
	F-H adapter	*Reference 34*
	H-A adapter	*Reference 34*
	GSAGAG-mCitrine	*Reference 47*
	GSAGAG-mScarlet	*Reference 47*
	SGAGAG-mCitrine	*Reference 47*
	Remorin-Anker	*Reference 47*
Ectodomains expression	FER-ED-TOPO-F	caccGCTGATTACTCTCCAACAGAGAA
	FER-ED-TOPO-R(SC)	TTACGTATTGCTTTTCGATTTCCTAGT
	ANJ-ED-TOPO-F	caccAATTACCTCATCAACTGTGGAT
	ANJ-ED-TOPO-R(SC)	TTACATACCAACATTCTTCTTAGTGG
	HERK1-ED-TOPO-F	caccAATTACTTGATCAACTGTGGATC
	FER-*Bam* H I-27-5’	GAAATTGGATCCATGGCTGCTGATTACTCTCCAACA
	FER-447-His-T-*Xho* I-3’	GAAATTCTCGAGTCAGTGATGGTGATGGTGATGAGCCGTATTGCTTTTCGATTT
	FER-447-*Xho* I-3’	GAAATTCTCGAG AGCCGTATTGCTTTTCGATTT
	HERK1-*Bam* H I-25-5’	GAAATTGGATCCATGTTCACACCTGTGGATAATTAC
	HERK1-405-His-T-*Xho* I-3’	GAAATTCTCGAGTCAGTGATGGTGATGGTGATGCCCGAGATTACTCTTACTGCT

	HERK1-405-*Xho* I-3’	GAAATTCTCGAG CCCGAGATTACTCTTACTGCT
	ANJ-*Bam* H I-25-5’	GAAATTGGATCCATG TACGTACCAGTGGATAATTAC
	ANJ-405-His-T-*Xho* I-3’	GAAATTCTCGAGTCAGTGATGGTGATGGTGATG ACCAACATTCTTCTTAGTGGT
	ANJ-405-*Xho* I-3’	GAAATTCTCGAG ACCAACATTCTTCTTAGTGGT
	CVY1-*Bam* H I-22-5’	GAAATTGGATCCATG TCACACATCTCCTCTGTTTCT
	CVY1-398-His-T-*Xho* I-3’	GAAATTCTCGAGTCAGTGATGGTGATGGTGATG AGAACCATCAGGTAAAATGGC
qRT-PCR analysis	FER-RT-F	TCTCCGTCTCCTTTGGTCCT
	FER-RT-R	GTTGGACCCATAGACCTCGC
	HERK1-RT-F	GTAGGCGGTAACTCTGGCTC
	HERK1-RT-R	GTTAGACGCAACAAGCGGAC
	HERK2-RT-F	AAAACGAGCCACCCCACATT
	HERK2-RT-R	TTCCGCAAGGTTGATCTGGT
	ANJ-RT-F	TTCCGTCATCGCCATTTGGT
	ANJ-RT-R	GTGACGGGTCGATGATGTGT
	ANX1-RT-F	TACCGAGACGCATTTGCCTT
	ANX1-RT-R	CCTTTCTCTCCTGTCCACCC
	ANX2-RT-F	CAGCTCGATCACAAGGACCA
	ANX2-RT-R	ATGGCTTAGGGTTTGGACCG
	AT2G23200-RT-F	AGTGGCTCAGAAGGAGCAATC
	AT2G23200-RT-R	ACTCGGTCTCTCATCTCCATAC
	AT5G24010-RT-F	TGTGGAGGACTTGGGTTGTT
	AT5G24010-RT-R	GCCAACCACGATCCAGACAA
	MDS1-RT-F	TAGGTGGAAAAGTGGTGGGC
	MDS1-RT-R	AAGCCCCGCAAGATTACCAT
	THE1-RT-F	ACCGTTAGCGTTGGACCTG
	THE1-RT-R	CCCCAAGCAACGAACTCTCA
	CAP1-RT-F	CGCCGTTGAAATCGTCTCTG
	CAP1-RT-R	CGCTGGTTAGTGAGGAGAGG
	ACTIN8-RT-F	ACTTTCCAGCAGATGTGGATC
	ACTIN8-RT-R	CGGGTTTTCAAACCTGCTCC

Table S3. Ectodomains of Receptor-like kinases (RLKs) for protein expression

RLKs	Sequence
FER	AADYSPTEKILLNCGGGASNLTDTDNRIWISDVKSKFLSSSSEDSKTSPALTQDPS VPEVPYMTARVFRSPFTYTFPVASGRKFVRLYFYPNSYDGLNATNSLFSVSFGPY TLLKNFSASQTAEALTYAFIIKEFVVNVEGGTLNMTFTPESAPSNAYAFVNGIEVT SMPDMYSSTDGTLTMVGSSGSVTIDNSTALENVYRLNVGGNDISPSADTGLYRS WYDDQPYIFGAGLGIPETADPNMTIKYPTGTPTYVAPVDVYSTARSMGPTAQINL NYNLTWIFSIDSGFTYLVRLHFCEVSSNITKINQRVFTIYLNNQTAEPEADVIAWTS SNGVPFHKDYVVNPPEGNGQQDLWLALHPNPVNKPEYYDSLLNGVEIFKMNTS DGNLAGTNPIPGPQVTADPSKVLRPTTRKSKSNTA
ANJ	YVPVDNYLINCGSSTNVTVTSRVFISDNLASNFLTSPNEILAASNRNSNSDIYQTAR IFTGISKYRFSVARGRHWIRLHFNPFQYQNFQMVSAKFSVSSETHVLLSDFTVSSR VMKEYSLNVATDHLELTFTPSGDSFAFLNALEVVSVPDTLFSGDPSFAGSPGKFQ GLSWQALETVYRVNMGGPRVTPSNDTLSRIWEPDSEFLVEKNLVKSVSKIASVD YVPGFATEETAPRTVYGTCTEMNSADNPSSNFNVTWDFDVDPGFQYFLRFHFCDI VSKALNQLYFNLYVDSMDVVENLDLSSYLSNTLSGAYAMDFVTGSAKLTKRIRV SIGRSSVHTDYPTAILNGLEIMKMNNSKSQLSIGTFLPSGSSSTTKKNVG
HERK1	FTPVDNYLINCGSPTNGTLMGRIFLSDKLSSKLLTSSKEILASVGGNSGSDIYHTAR VFTEVSSYKFSVTRGRHWVRLYFNPFDYQNFKMGSAKFAVSSQSHVLLSDFTVT SSKVVKEYSLNVTTNDLVLTFTPSSGSFAFVNAIEVISIPDTLITGSPRFVGNPAQFP DMSMQGLETIHRVNMGGPLVASNNDTLTRTWVPDSEFLLEKNLAKSMSKFSTV NFVPGYATEDSAPRTVYGSCTEMNSADNPNSIFNVTWEFDVDPGFQYYFRFHFC DIVSLSLNQLYFNLYVDSMVAATDIDLSTLVDNTLAGAYSMDFVTQTPKGSNKV RVSIGPSTVHTDYPNAIVNGLEIMKMNNSKGQLSTGTFVPGSSSSSKSNLG
CVY1	SHISSVSDTFFINCGSPTNVTVNNRTFVSDNNLVQGFSVGTTDSNSGDESTLFQTA RVFSDESSSTYRFPIEEHGWFLIRIYFLPLVSASQDLTTARFSVSAQNFTLIREYKPS TTSVVREYILNVTTDSLLLQFLPRTGSVSFINALEVLRLPETLIPEDAKLIGTQKDL KLSSHAMETVSRVNMGNLSVSRDQDKLWRQWDSDSAYKAHFGTPVMNLKAV NFSAGGITDDIAPVYVYGTATRLNSDLDPNTNANLTWTFKVEPGFDYFVRFHFCN IIVDPFGFERQIRFDIFVNSEKVRTIDMTEVLNGTFGAPFFVDAVMRKAKSREGFL NLSIGLVMDVSSYPVSFINGFEISKLSNDKRSLDAFDAILPDGS

Peptide sequences used for synthesis

Peptide	Sequence
Biotin-RALF6	Biotin-QTYINYNGMKGDIIPGCSSKNPKECVKIPAYSYNRGCEISTRCQRQQHSSSS
Biotin-RALF7	Biotin-EGIKQINYKDLIKDTIPGCTSKNPKECVKVPANTYHRGCEISTRCHREQHSSSG
Biotin-RALF16	Biotin-RTLGYGSIKGDRIPACGYKNPNSCVKQPVNHYHRGCEKITRCARDAARYTESFN VDDDESPIINLH
Biotin-RALF36	Biotin-REQVISYESMRVNHAWGCSQKYPQFCQKTRANPYTKPPPKNSEAS
Biotin-RALF37	Biotin-AEDVISYEVLLQDHAWGCSPKFPRLSCLKQKANP
Biotin-elf24	Acetyl-SKEKFERTKPHVNVGTIGHVDHGK-biotin

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