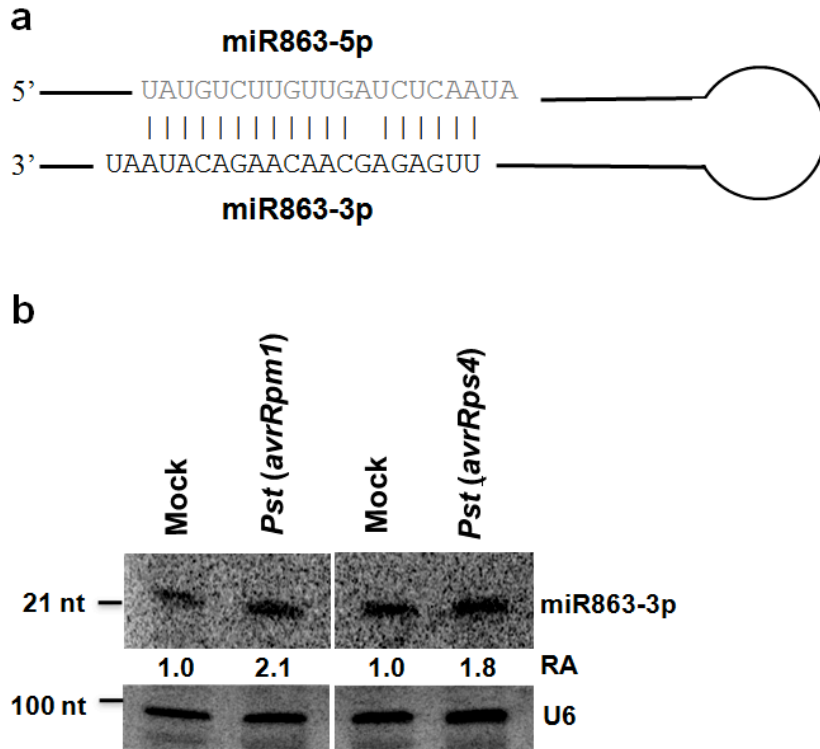


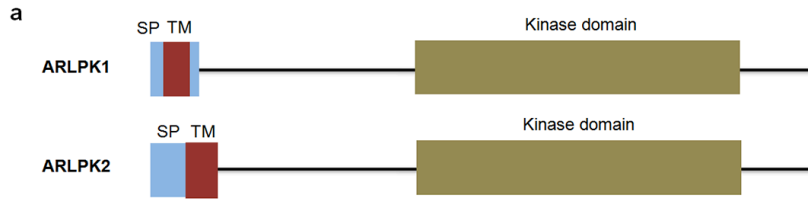
## Supplementary Figures and Legends (JIN)



**Supplementary Figure 1. miR863-3p is induced by bacterial infection.**

(a) miR863 precursor fold-back structure containing miR863-3p and -5p with 2' overhang.

(b) miR863-3p levels in mock-, *Pst (avrRpm1)*- and *Pst (avrRps4)*-infected Col-0 WT plants were detected by Northern blot. Bacterial inoculum concentration:  $1 \times 10^7$  CFU/ml. *Pst (avrRpm1)*- *Pst (avrRps4)*-infected leaf tissue was collected at 8 hpi and 24 hpi, respectively. U6 was used as a loading control. Relative abundance (RA) levels are indicated.



**b**

**ARLPK1**

Signal peptide Transmembrane domain

MRKSHLVKLLAVSLPTTFLIMAIHIFCRRTTETNEVQYDVESPYEKQEFSDNGSETEEEL  
 IIFNGGEDLTICDILDAPGEVIGKSSYGTLYKATLQKSGKVRVLRFLRPLCAVNSDSKEFNGVI  
 ESLGFVRHDNLVPLLGFYVGNRGEKLMIHPPFGSSGNLSAFIKFLAGGDVDAHKWSNILSITI  
 GIAKALDHLHTGMQKPIVHGNLKSKNVLLDKSFRPRVSDFGLHLLNLAAGQEVLEASAA  
 EGYKAPELIKMKKEVSKESDVYSFGVIMLELVSGKEPTNKNPTGSVLDNRNRLSDLYRPEIIRR  
 CLKDGNVTEECVLEYFQLAMSCCSPSPTLRPSFKQVLRKLEEIRK

Kinase domain

**ARLPK2**

Signal peptide Transmembrane domain

MRKSLLTLLGVSLSAFLIVIFHIFLRRKESSTESDQYDVESLDHNKQGFSSETEELVIFQG  
 GEDLTICDILDAPGEVIGKSSYGTLYKASLQKSGKIRVLRFLRPVCTVRSDSKEFNGHIEITLVR  
 HENLVPLLGFYAGNRGEKLMVHPFGSGNLSDFIRSGDDESARKWINLRITIGISKALDHLHT  
 GMQKPIVHGNLKSKNVLLSSSFEPRIKSDVSAAGQEVLEASAAEGYKAPELIKMKD  
 VSKESDVYSFGVIMLELVSGKEPINENATGDDEFYLPDFMRNAVLDHRLSDLYRPEILGSDD  
 NLSEECVLKYFQLAMSCCSPSPSLRPNVKQVLRKLEEIGKF

Kinase domain

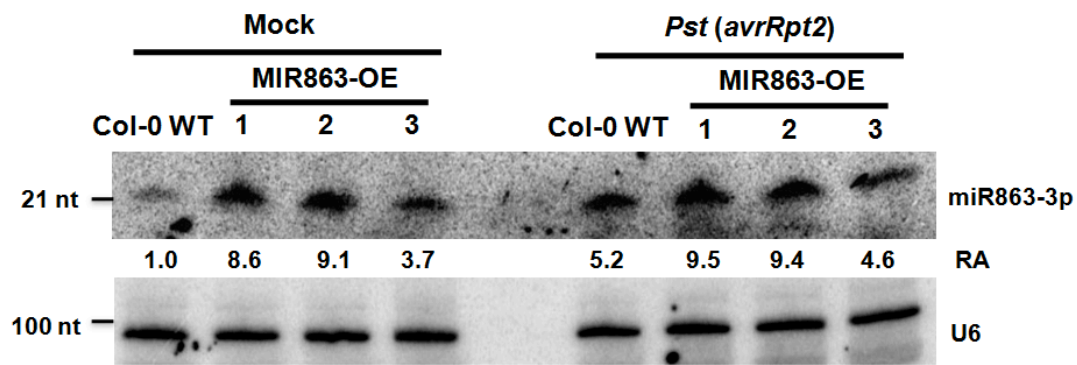
**Supplementary Figure 2. ARLPK1 and ARLPK2 are atypical receptor-like kinases lacking extracellular domains.**

- (a) Diagrams of ARLPK1 and ARLPK2 with important predicted domains indicated. SP (blue box): signal peptide; TM (red box): transmembrane domain; kinase domain (brown box)
- (b) Amino acid sequences of ARLPK1 and ARLPK2 with signal peptide (blue), transmembrane domain (highlighted in yellow), and kinase domain (brown) indicated.

	I		II		III		
ARLPK1	ILDAPGEVI	<b>G</b> <span style="border: 1px solid black;">K</span> <b>S</b> <span style="border: 1px solid black;">S<b>Y</b><b>G</b>TLYKATLQ</span>	RSGK	<b>V</b> <span style="border: 1px solid black;">R<b>V</b><b>L</b><b>R</b><b>F</b></span>	LRPLCAVNSDSK-	<b>E</b> FNGVIESLG 134	
ARLPK2	ILDAPGEVI	<b>G</b> <span style="border: 1px solid black;">K<b>S</b><span style="border: 1px solid black;">S<b>Y</b><b>G</b>TLYKASLQ</span></span>	RSGK	<b>I</b> <span style="border: 1px solid black;">R<b>V</b><b>L</b><b>R</b><b>F</b></span>	LRPVCTVRSDSK-	<b>E</b> FNGI IETLG 128	
AKIK1	MTNNFQRV	<b>V</b> <b>G</b> <b>G</b> <b>G</b> <b>F</b> <b>G</b> VVCHGTINGSE	Q	<b>V</b> <b>A</b> <b>V</b> <b>K</b> <b>V</b> <b>L</b> <b>S</b>	QSSSQGYKHFKA	<b>E</b> VDLLL---- 636	
		GxGxxG		VAIKxL		E	
	IV		V				
ARLPK1	FVRHDN	<b>L</b> <span style="border: 1px solid black;">VPLLGFYVGNRGEKLM</span>	IHPFFGSSGNLSAFIKCGD	VDAHKWSN	ILSITI 190		
ARLPK2	FVRHEN	<b>L</b> <span style="border: 1px solid black;">VPLLGFYAGNRGEKLM</span>	VHPFFGS-GNLSDFIRSGD	DES	SRKWINILRITI 183		
AKIK1	RVHHTN	<b>L</b> <span style="border: 1px solid black;">VSLVGYCDE</span>	RDHLALIEFLPKGDLRQ	HLSGKSGGS	FIN-WGNRLRIAL 691		
	L						
	VI		VII				
ARLPK1	----	<b>G</b> <span style="border: 1px solid black;">I<span style="border: 1px solid black;">A<span style="border: 1px solid black;">K<span style="border: 1px solid black;">A</span></span></span></span>	L	DHLHTGMQKPIV	<b>H</b> <span style="border: 1px solid black;">G<span style="border: 1px solid black;">N<b>L</b><b>K</b><b>S</b><b>K</b><b>N</b>VLLDKSFRPRV</span></span>	<b>S</b> <b>D</b> <b>F</b> <b>G</b> <b>L</b> HLLLNLAAGQ 242	
ARLPK2	----	<b>G</b> <span style="border: 1px solid black;">I<span style="border: 1px solid black;">S<span style="border: 1px solid black;">K<span style="border: 1px solid black;">A</span></span></span></span>	L	DHLHTGMQKPIV	<b>H</b> <span style="border: 1px solid black;">G<span style="border: 1px solid black;">N<b>L</b><b>K</b><b>S</b><b>K</b><b>N</b>VLLSSSFEPRI</span></span>	<b>S</b> <b>D</b> <b>F</b> <b>G</b> <b>L</b> HLLLNLSAGQ 235	
AKIK1	EAAL	<b>G</b> <b>L</b> <b>E</b> <b>Y</b> <b>L</b> HSGCTPP	I	----	<b>H</b> <b>R</b> <b>D</b> <b>I</b> <b>K</b> <b>T</b> <b>T</b> <b>N</b> ILLDEQLKAKL	<b>A</b> <b>D</b> <b>F</b> <b>G</b> L	
	G--YL		HRDLKxxN		DFG		
	VIII		IX				
ARLPK1	EVLEAS	----	A	E-GYK	<b>A</b> <b>P</b> <b>E</b> <b>L</b> <b>I</b> <b>K</b> <b>M</b> <b>K</b> <b>E</b> <b>V</b> <b>S</b> <b>K</b> <b>E</b> <b>S</b> <b>D</b> <b>V</b> <b>Y</b> <b>S</b> <b>F</b> <b>G</b> VIMLELVSGKEPTNKNPTG-	293	
ARLPK2	EILDVS	----	A	E-GYK	<b>A</b> <b>P</b> <b>E</b> <b>L</b> <b>I</b> <b>K</b> <b>M</b> <b>K</b> <b>D</b> <b>V</b> <b>S</b> <b>K</b> <b>E</b> <b>S</b> <b>D</b> <b>V</b> <b>Y</b> <b>S</b> <b>L</b> <b>G</b> VIMLELVSGKEPINENATGD	287	
AKIK1	ETHI-	STVVAGT	P	G	<b>I</b> <b>D</b> <b>E</b> <b>P</b> <b>E</b> <b>Y</b> <b>Y</b> <b>Q</b> TTRLG	<b>E</b> <b>K</b> <b>S</b> <b>D</b> <b>V</b> <b>Y</b> <b>S</b> <b>F</b> <b>G</b> I	
		APE		D-YS-G		795	
			X				
ARLPK1	KEPTNKNPTG	-----	S	VLDNRN	LS	DLYRPEI	IRRCLKDGNG-VTEECVLE 328
ARLPK2	KEPINENATGD	DEFYLP	D	FMRNAV	LD	HRLS	DLYRPEILG---SDDN--LSEECVLK 327
AKIK1	-----	-----	S	HISQ	W	FELTRG	DITKIMDPNLNGDYESRSVWR 830
	XI						
ARLPK1	YFQL	<b>A</b> <b>M</b> <b>S</b> <b>C</b> <b>C</b> <b>S</b> <b>P</b> <b>S</b> <b>P</b> <b>T</b> <b>L</b>	<b>R</b> <b>P</b> <b>S</b> <b>F</b> <b>K</b> <b>Q</b> <b>V</b> <b>L</b> <b>R</b> <b>K</b> <b>L</b> <b>E</b> <b>E</b> <b>I</b> <b>R</b> <b>K</b>			358	
ARLPK2	YFQL	<b>A</b> <b>M</b> <b>S</b> <b>C</b> <b>C</b> <b>S</b> <b>P</b> <b>S</b> <b>P</b> <b>S</b> <b>L</b>	<b>R</b> <b>P</b> <b>N</b> <b>V</b> <b>K</b> <b>Q</b> <b>V</b> <b>L</b> <b>R</b> <b>K</b> <b>L</b> <b>E</b> <b>E</b> <b>I</b> <b>G</b> <b>K</b> <b>F</b>			359	
AKIK1	VLEL	<b>A</b> <b>M</b> <b>S</b> <b>C</b> <b>A</b> <b>N</b> <b>P</b> <b>S</b> <b>S</b> <b>V</b> <b>N</b> <b>R</b>	<b>P</b> <b>N</b> <b>M</b> <b>S</b> <b>Q</b> <b>V</b> <b>A</b> <b>N</b> <b>E</b> <b>L</b> <b>K</b> <b>E</b> <b>C</b> <b>L</b> <b>V</b> <b>S</b>			880	
	M-----R						

### Supplementary Figure 3. Amino acid alignments of ARLPK1, ARLPK2, and AKIK1 kinase domains.

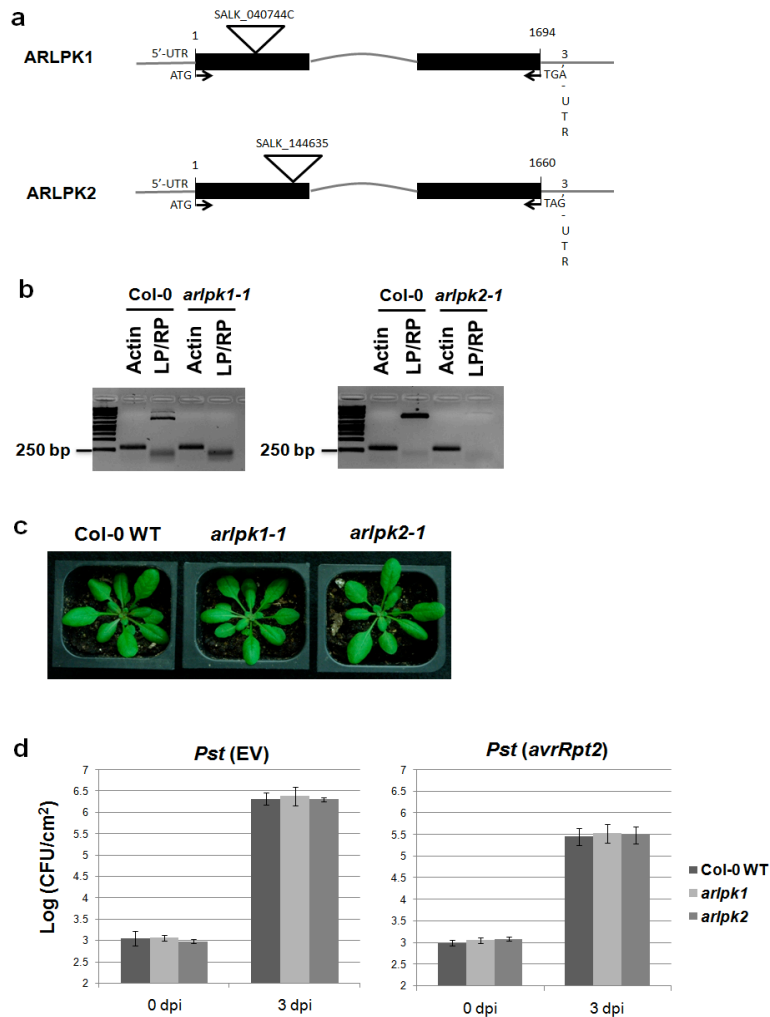
Alignments were made using Clustal Omega (1.2.1)<sup>1</sup>. Consensus sequences of active receptor-like kinases are indicated below the alignments<sup>2-6</sup> and include: the glycine rich loop (GxGxxG), the Val-Ala-x-Lys-x-Leu (VA-K-L), glutamate (E), leucine (L), Gly-x-x-Tyr-Leu (GxxYL), His-Arg-Asp-Leu-Lys-x-x-Asn (HRDLKxxN) loop, Asp-Phe-Gly (DFG) motif, Ala-Pro-Glu (APE), Asp-x-Tyr-Ser-x-Gly (DxYSxG), and Met-x-x-x-x-x-x-x-x-Arg (MxxxxxxxxR). Features and amino acids that are conserved in ARLPK1, ARLPK2, and AKIK1 are in red, while those that deviate from the consensus are boxed. Subdomains are indicated by Roman numerals.



**Supplementary Figure 4. Expression of miR863-3p in MIR863-OE lines after mock- or *Pst (avrRpt2)* treatment.**

miR863-3p levels in mock- and *Pst (avrRpt2)*-infected Col-0 WT and MIR863-OE plants were detected by Northern blot. Bacterial inoculum concentration:  $1 \times 10^7$  CFU/ml. Leaf tissue was collected at 14 hpi. U6 was used as a loading control. Relative abundance (RA) levels are indicated.





**Supplementary Figure 5. Analysis *arlpk1-1* and *arlpk2-1* single mutants.**

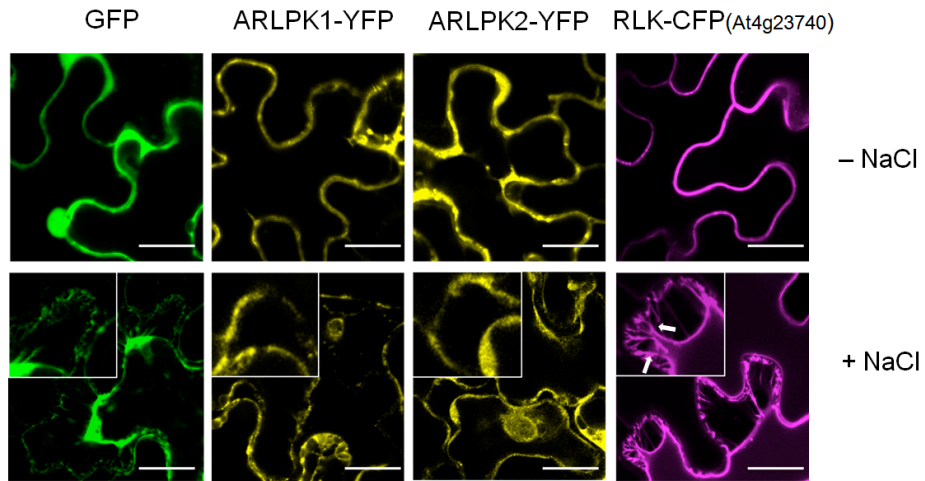
(a) Gene structure of ARLPK1 and ARLPK2 with *arlpk1-1* (SALK\_040744C) and *arlpk2-1* (SALK\_144635)

T-DNA insertion sites indicated, respectively. Black boxes: exons, curved lines: introns, lines: UTRs, triangles: T-DNA insertion sites. Nucleotide positions are indicated by the numbers.

(b) T-DNA insertions in *arlpk1-1* (SALK\_144635) and *arlpk2-1* (SALK\_040744) were confirmed by real-time RT-PCR. *Actin* was used as control.

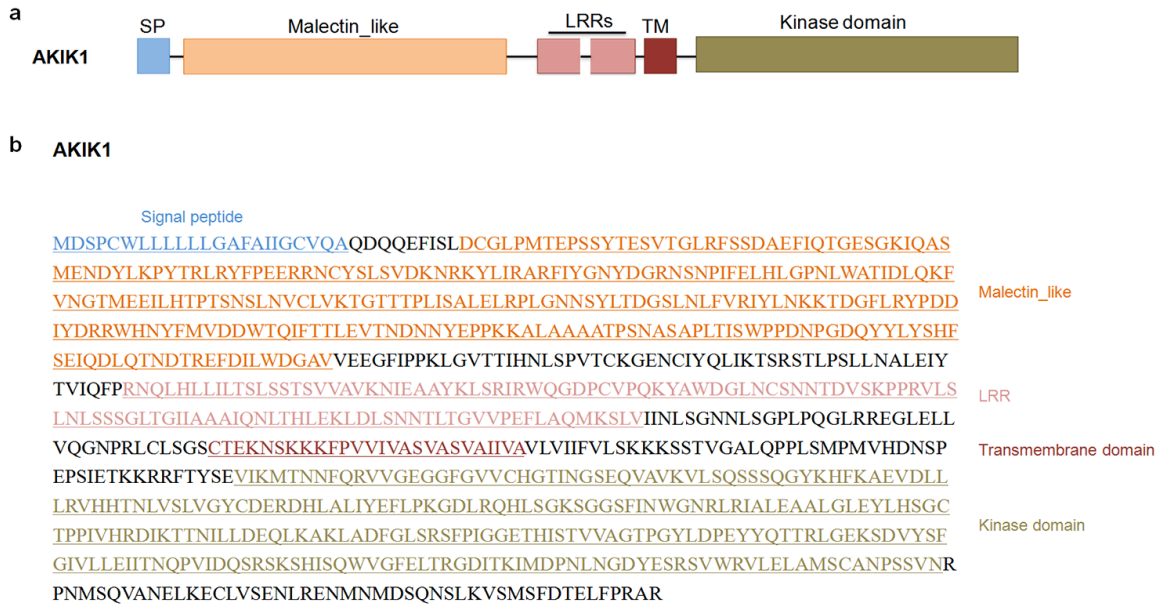
(c) Phenotypes of 4-week-old *arlpk1-1* and *arlpk2-1* mutant plants compared with Col-0 WT plants.

(d) Bacteria growth in *Pst* (EV)- and *Pst* (*avrRpt2*)-infected Col-0 WT, *arlpk1-1*, and *arlpk2-1* mutants. Bacterial inoculum concentration:  $5 \times 10^5$  CFU/ml. Bacterial growth was measured 3 dpi. Error bars represent standard deviation for at least 15 leaf discs.



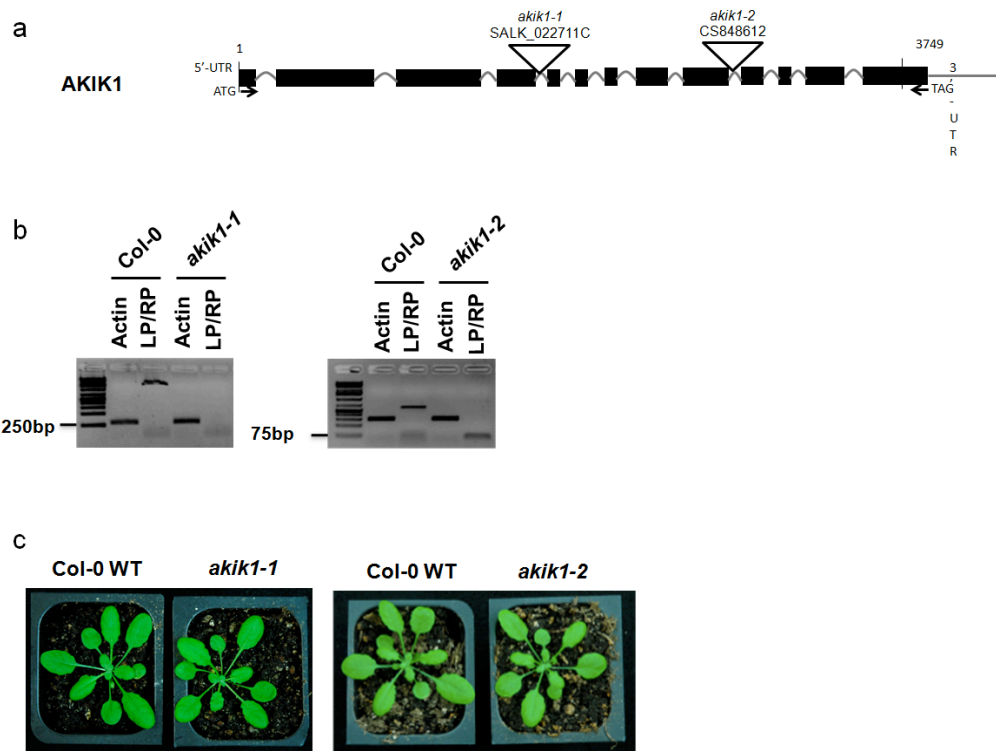
**Supplementary Figure 6. ARLPK1 and ARLPK2 localize in the endoplasmic reticulum.**

Subcellular localization of ARLPK1-YFP and ARLPK2-YFP in *N. benthamiana* was observed at 48 hpi with confocal microscopy. For the plasmolysis treatment, tissue was treated with 5% NaCl for 5-10 min before visualization (+ NaCl), or with water (– NaCl) as a control. GFP empty vector was used as control. RLK-CFP, a known plasma membrane (PM)-localized RLK (At4g23740) was fused to CFP at the C-terminus, was used as a PM-localization control. Arrows indicate Hechtian strands on the bottom panel. Fluorescent images were taken of the middle of the cell. Scale bars: 20  $\mu$ m.



**Supplementary Figure 7. AKIK1 is a receptor-like kinase.**

- (a) Diagram of AKIK1 with important predicted domains indicated. SP (blue box): signal peptide; malectin-like (peach box); LRR (pink box): Leucine-rich repeat; TM (red box): transmembrane domain; kinase domain (brown box).
- (b) Amino acid sequence of AKIK1 with signal peptide (blue), malectin-like domain (peach); LRR domains (pink), transmembrane domain (red), and kinase domains (brown) indicated.

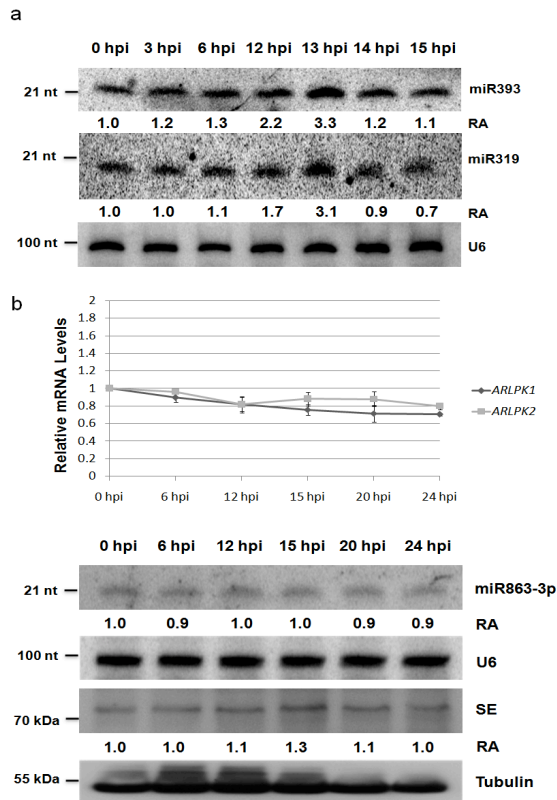


**Supplementary Figure 8. Analysis of *akik1-1* and *akik1-2* mutants.**

(a) Gene structure of AKIK1 with *akik1-1* (SALK\_022711C) and *akik1-2* (CS848612) T-DNA insertion sites indicated. Black boxes: exons, curved lines: introns, lines: UTRs, triangles: T-DNA insertion sites. Nucleotide positions are indicated by the numbers.

(b) T-DNA insertions in *akik1-1* and *akik1-2* were confirmed by real-time RT-PCR. Actin was used as control.

(c) Phenotypes of 4-week-old *akik1-1* and *akik1-2* mutants compared with Col-0 WT plants.



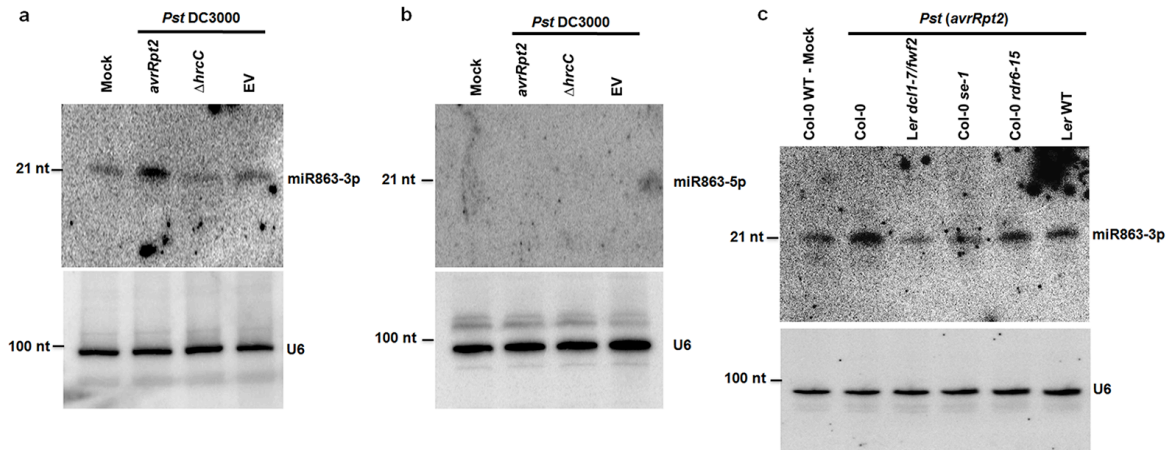
**Supplementary Figure 9. Expression levels of miR393 and miR319 and *ARLPK1*, *ARLPK2*, and SE in Col-0 WT plants infected with various strains of *Pst*.**

(a) Time course of miR393 and miR319 levels in *Pst* (*avrRpt2*)-infected Col-0 WT plants were detected by Northern blot analysis. U6 was used as loading control. Relative abundance (RA) levels are indicated. Bacterial inoculum concentration:  $5 \times 10^6$  CFU/ml.

(b) Top: Time course of relative expression levels of *ARLPK1* and *ARLPK2* transcripts in *Pst* (EV)-infected Col-0 WT plants were detected by real-time RT-PCR. Bacterial inoculum concentration:  $5 \times 10^6$  CFU/ml. Error bars indicate standard deviation from three technical replicates. Bottom: Time course of miR863-3p levels in *Pst* (EV)-infected Col-0 WT plants were detected by Northern blot. U6 was used as loading control. SE protein levels in *Pst* (EV)-inoculated Col-0 WT plants were detected by Western blot using an anti-SE antibody.  $\alpha$ -Tubulin was used as a loading control. Relative abundance (RA) levels are indicated. Bacterial inoculum concentration:  $5 \times 10^6$  CFU/ml.

Supplementary Figure 10. Uncropped images of each gel and blot presented in the manuscript with corresponding Figure numbers indicated.

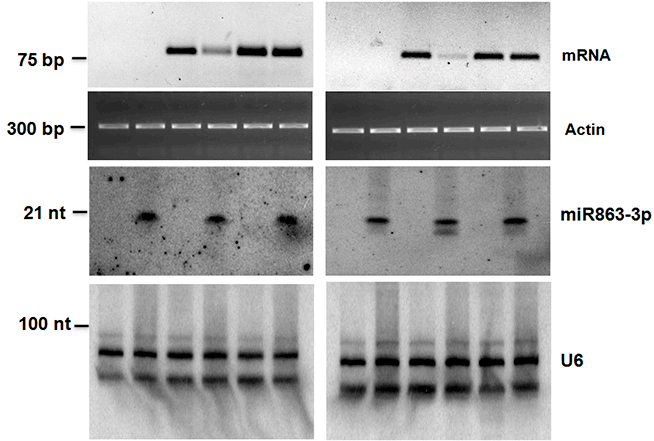
For Figure 1a–c



For Figure 2c,e,f

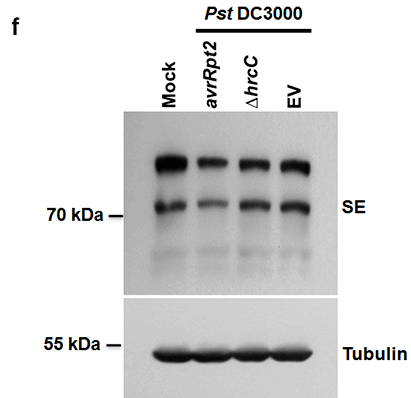
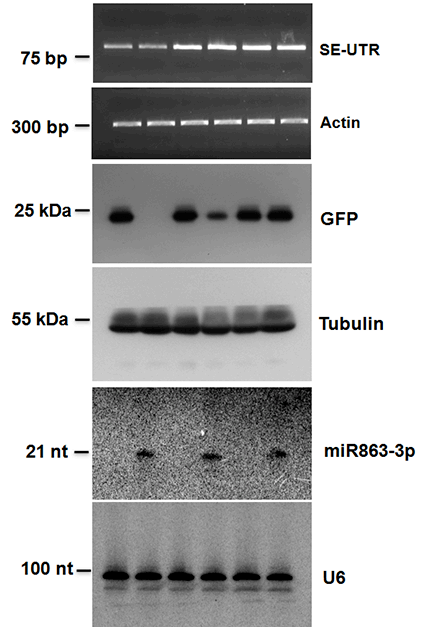
**C**

Vector	+	-	-	-	-	-	+	-	-	-	-	-
MIR863	-	+	-	+	-	+	-	+	-	+	-	+
wtARLPK1	-	-	+	+	-	-	-	-	-	-	-	-
mARLPK1	-	-	-	-	+	+	-	-	-	-	-	-
wtARLPK2	-	-	-	-	-	-	-	-	+	+	-	-
mARLPK2	-	-	-	-	-	-	-	-	-	-	+	+

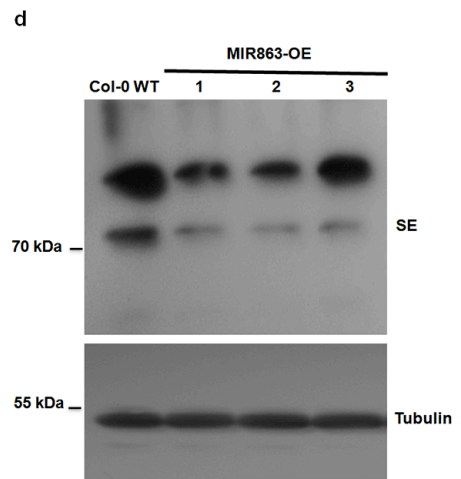
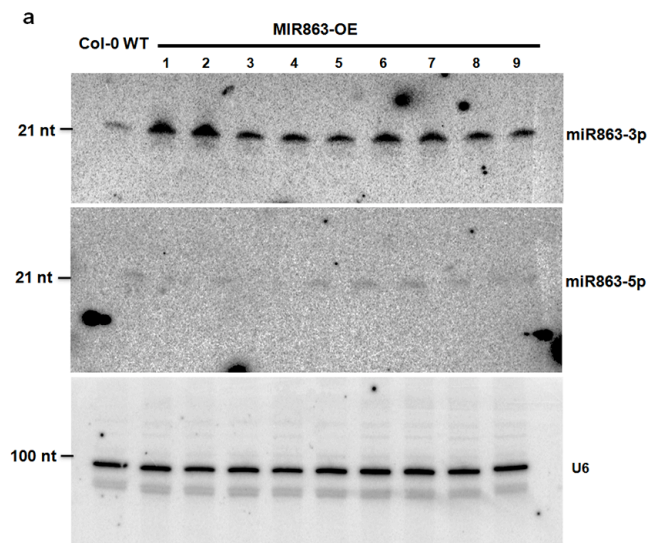


**e**

GFP Vector	+	-	-	-	-	-
MIR863	-	+	-	+	-	+
GFP-wtSE-3'UTR	-	-	+	+	-	-
GFP-mSE-3'UTR	-	-	-	-	+	+

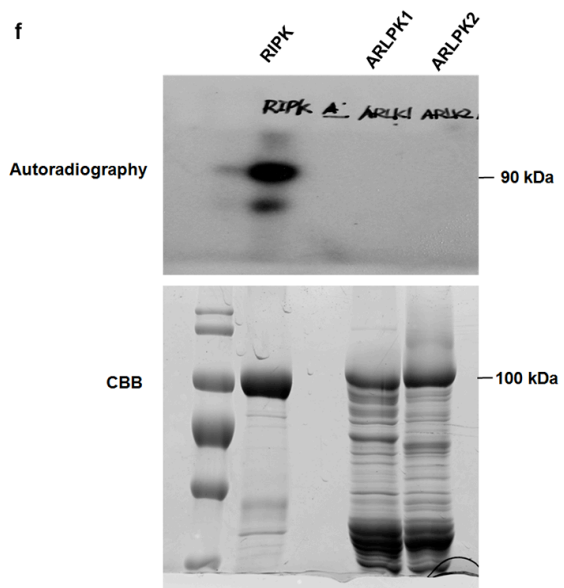
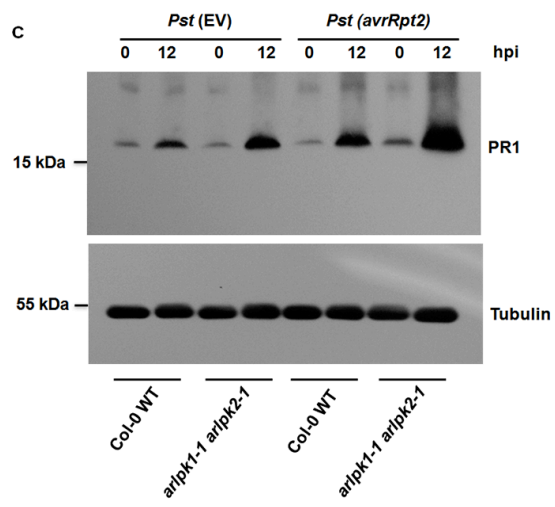


**For Figure 3a–b**

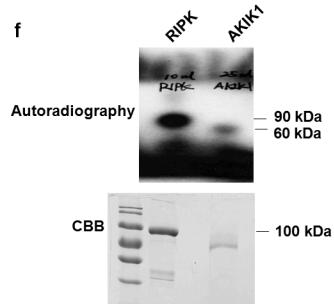
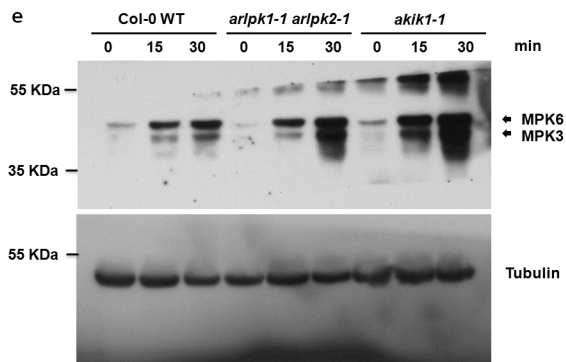
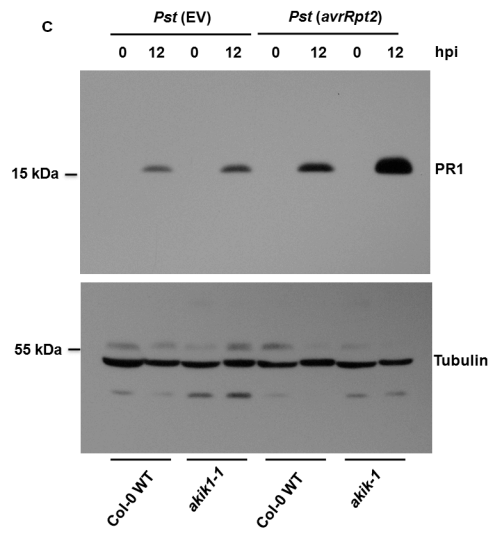
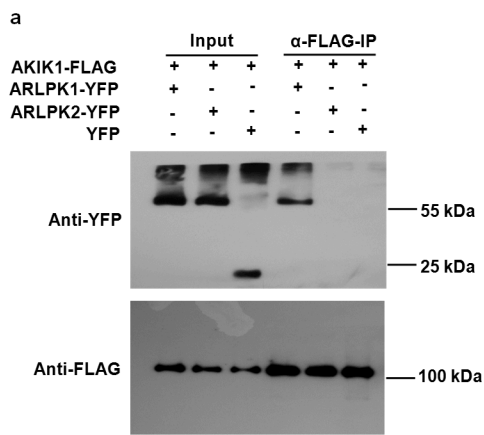




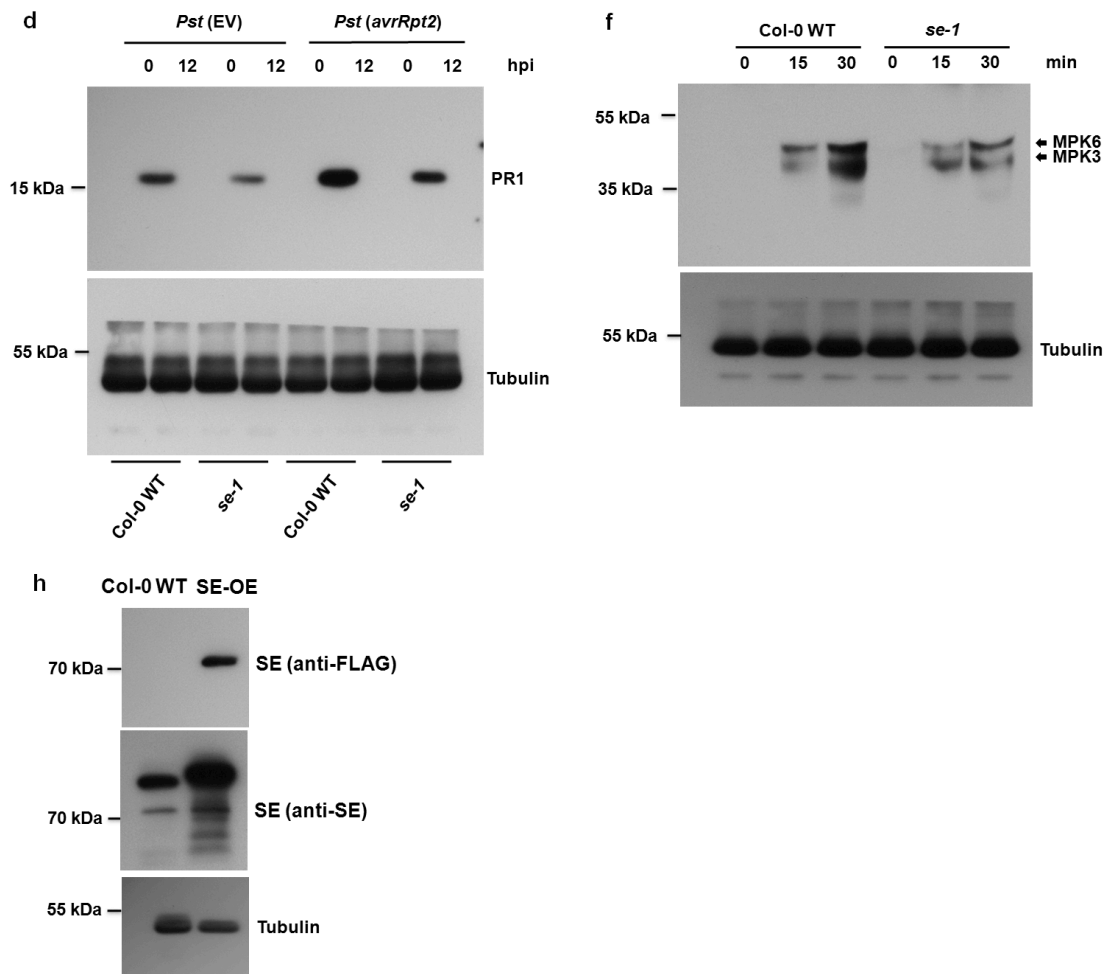
For Figure 4c,f



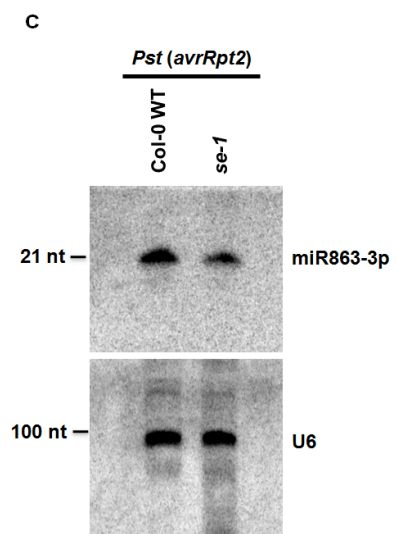
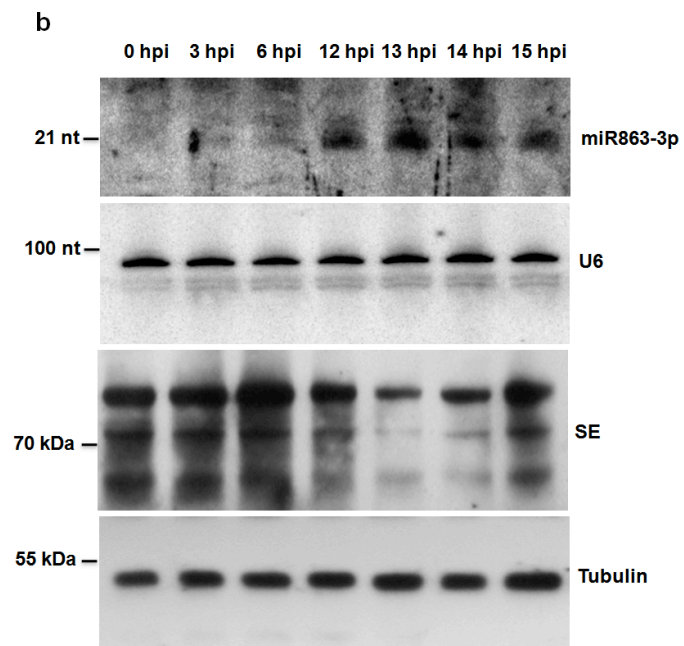
For Figure 6a,c,e,f



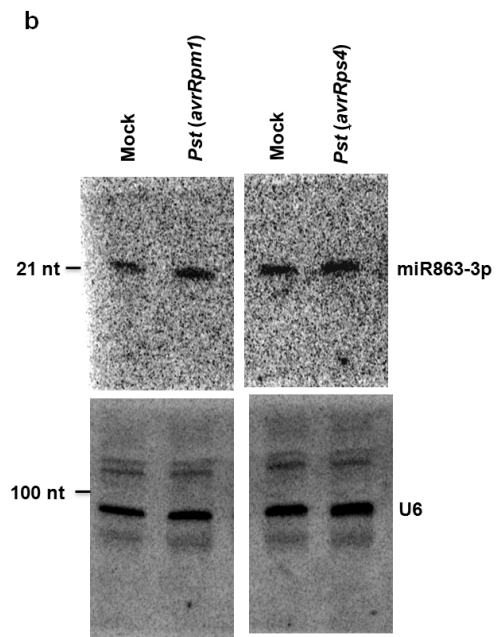
For Figure 7d,f,h



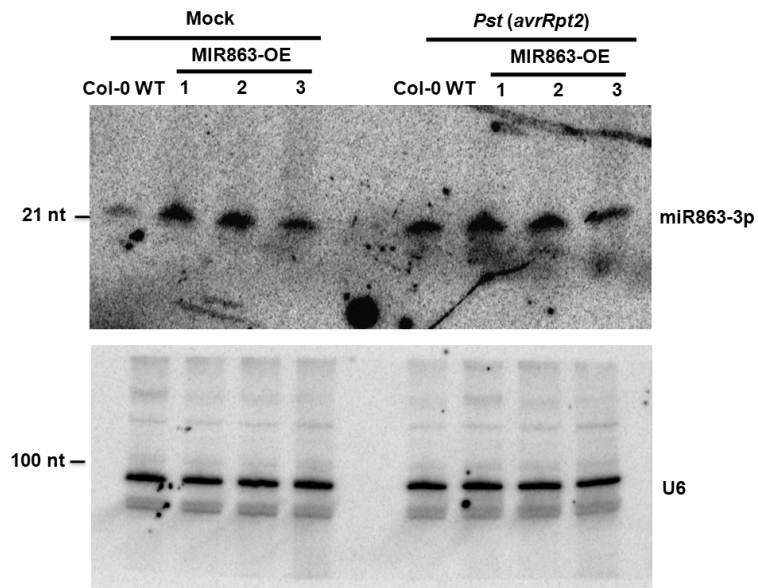
For Figure 8b,c



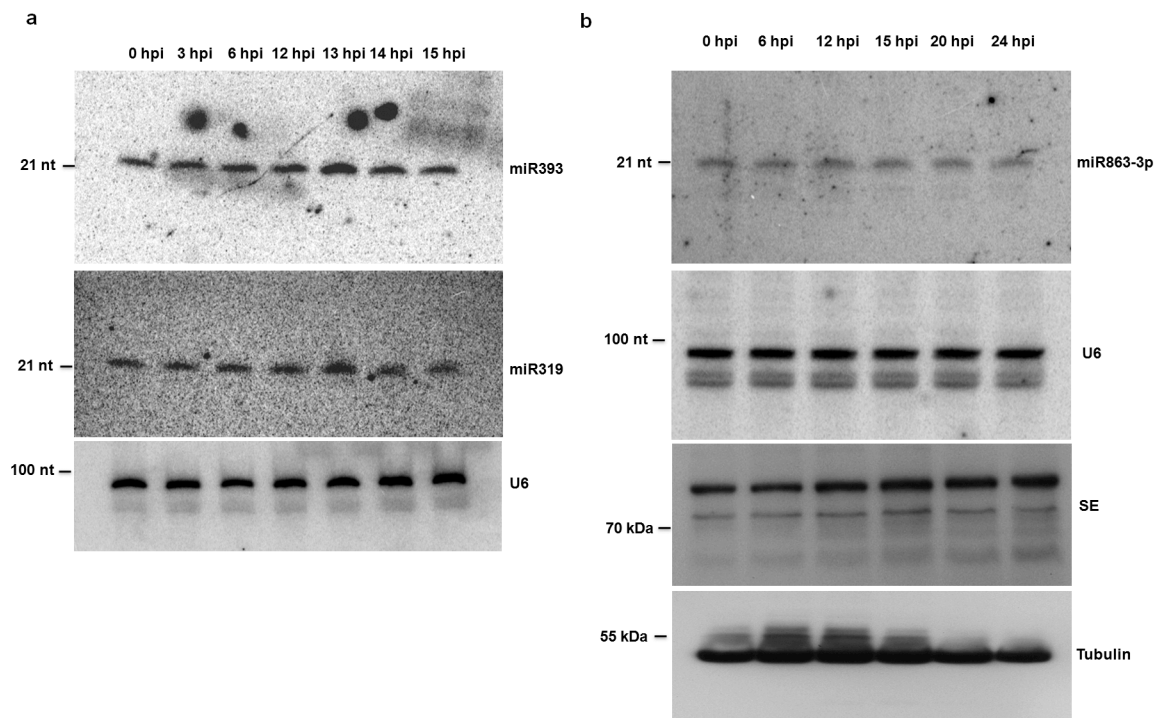
For Supplementary Figure 1b



For Supplementary Figure 4



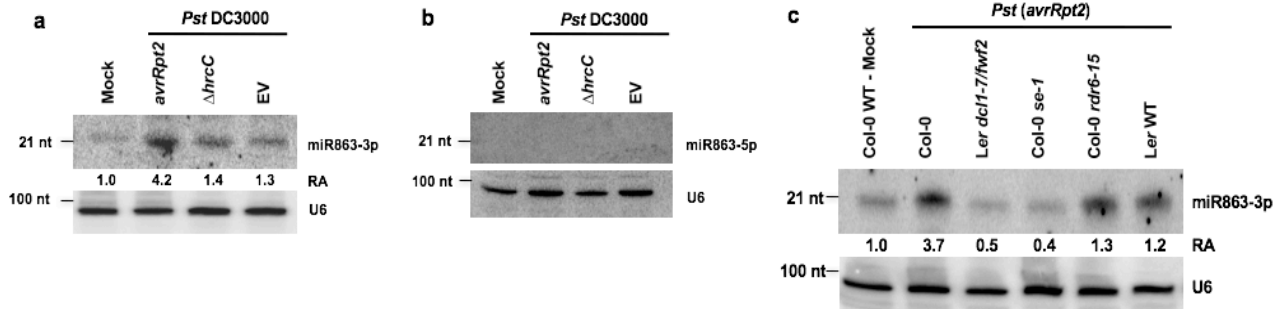
For Supplementary Figure 9a,b



Supplementary Figure 11. Results for biological replicates conducted in this study with corresponding figure numbers indicated.

Figure 1a–c

Replicate 2



Replicate 3

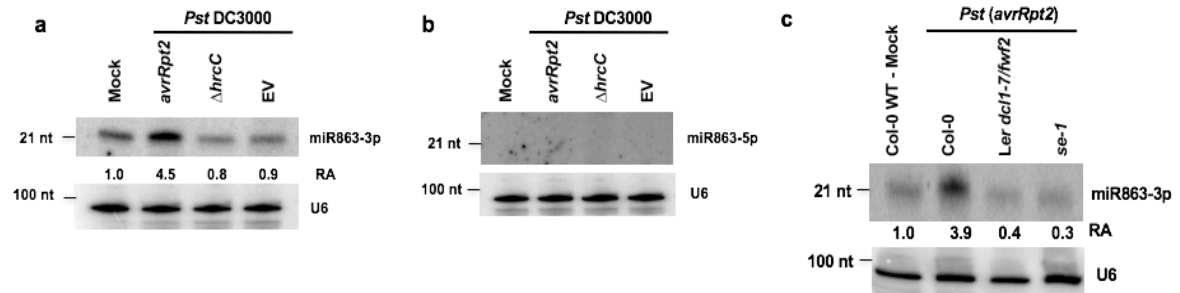




Figure 2b

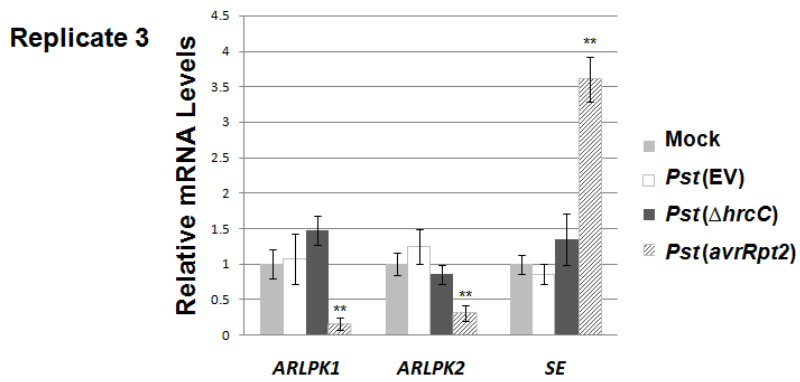
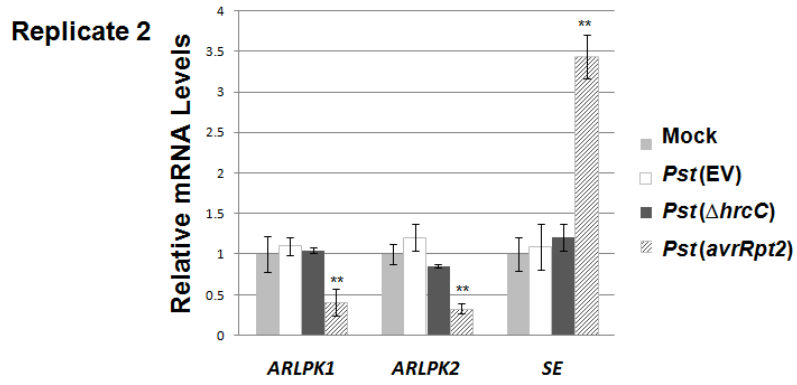


Figure 2c, 2e

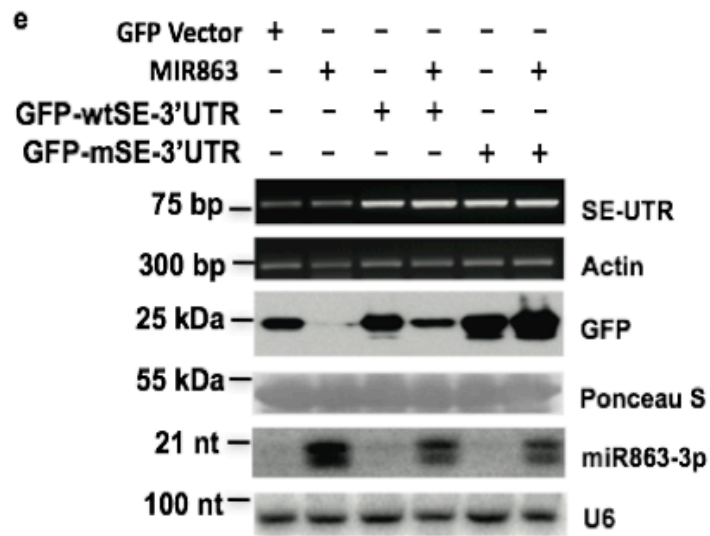
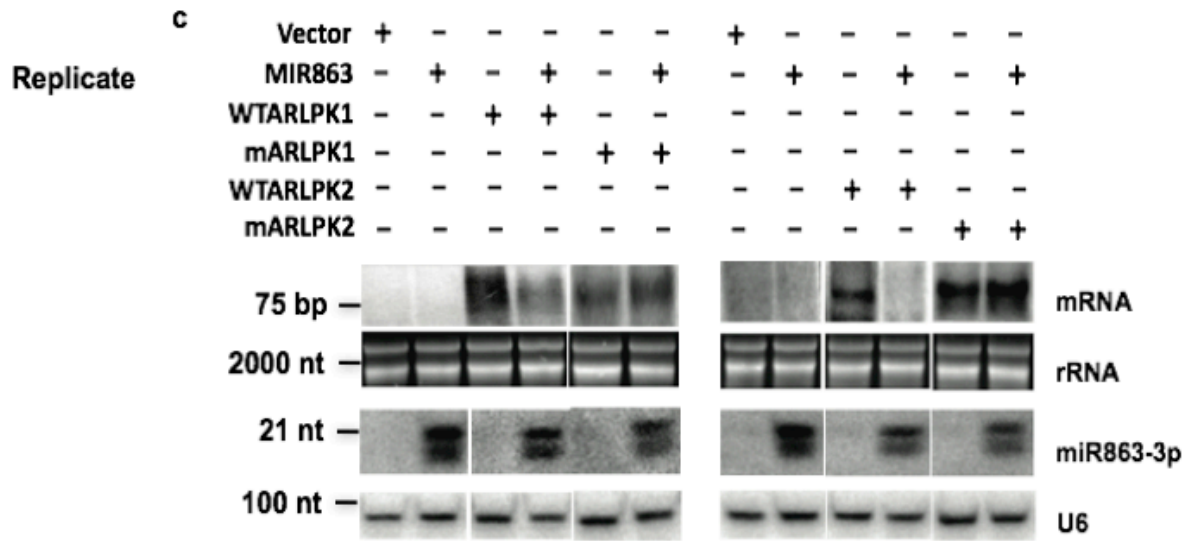
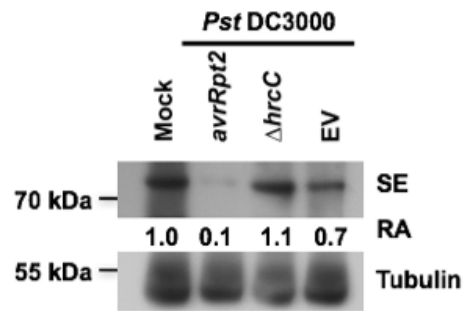


Figure 2f

Replicate 2



Replicate 3

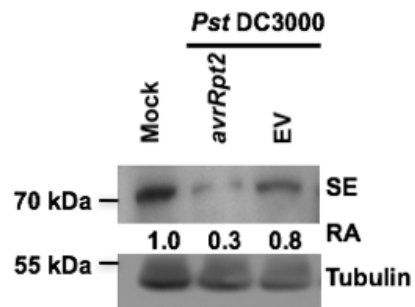


Figure 3a

Replicate

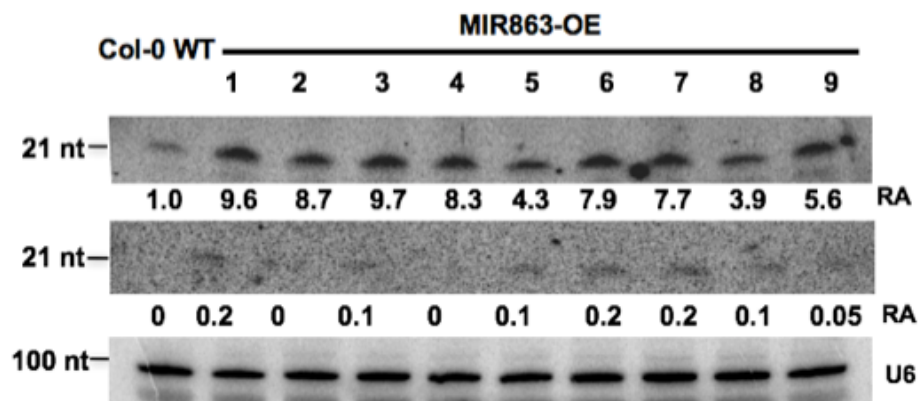
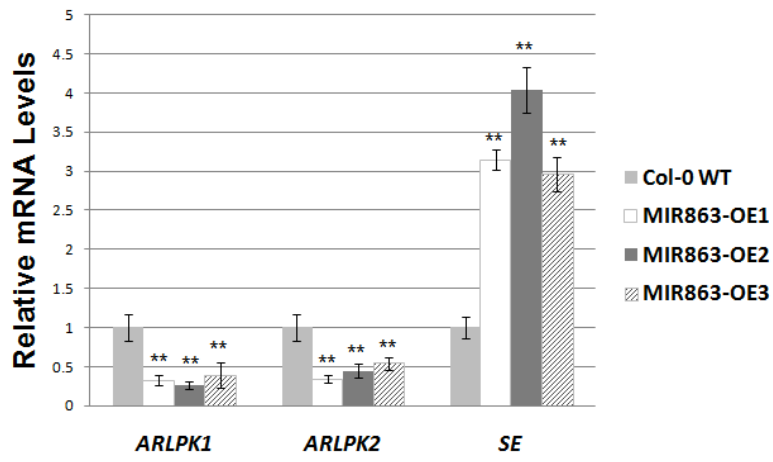


Figure 3c

Replicate 2



Replicate 3

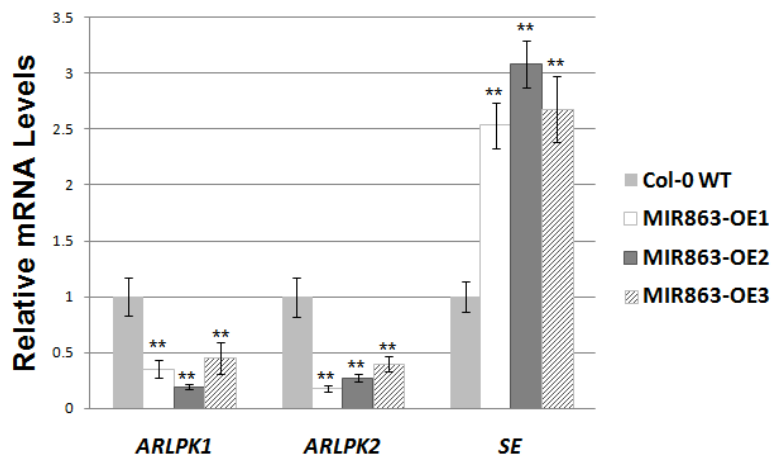


Figure 3d

Replicate

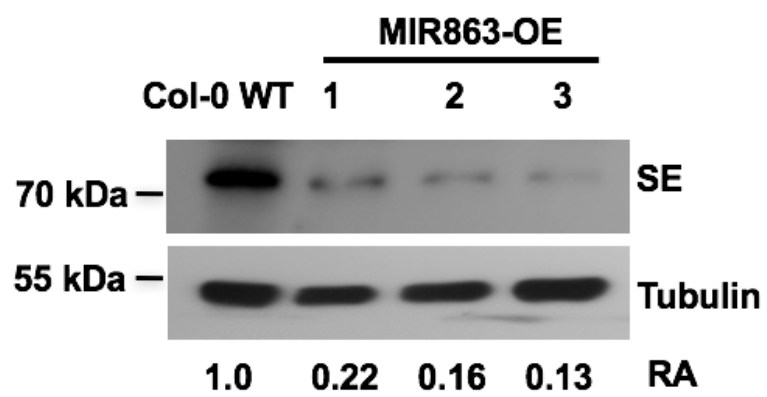


Figure 3e

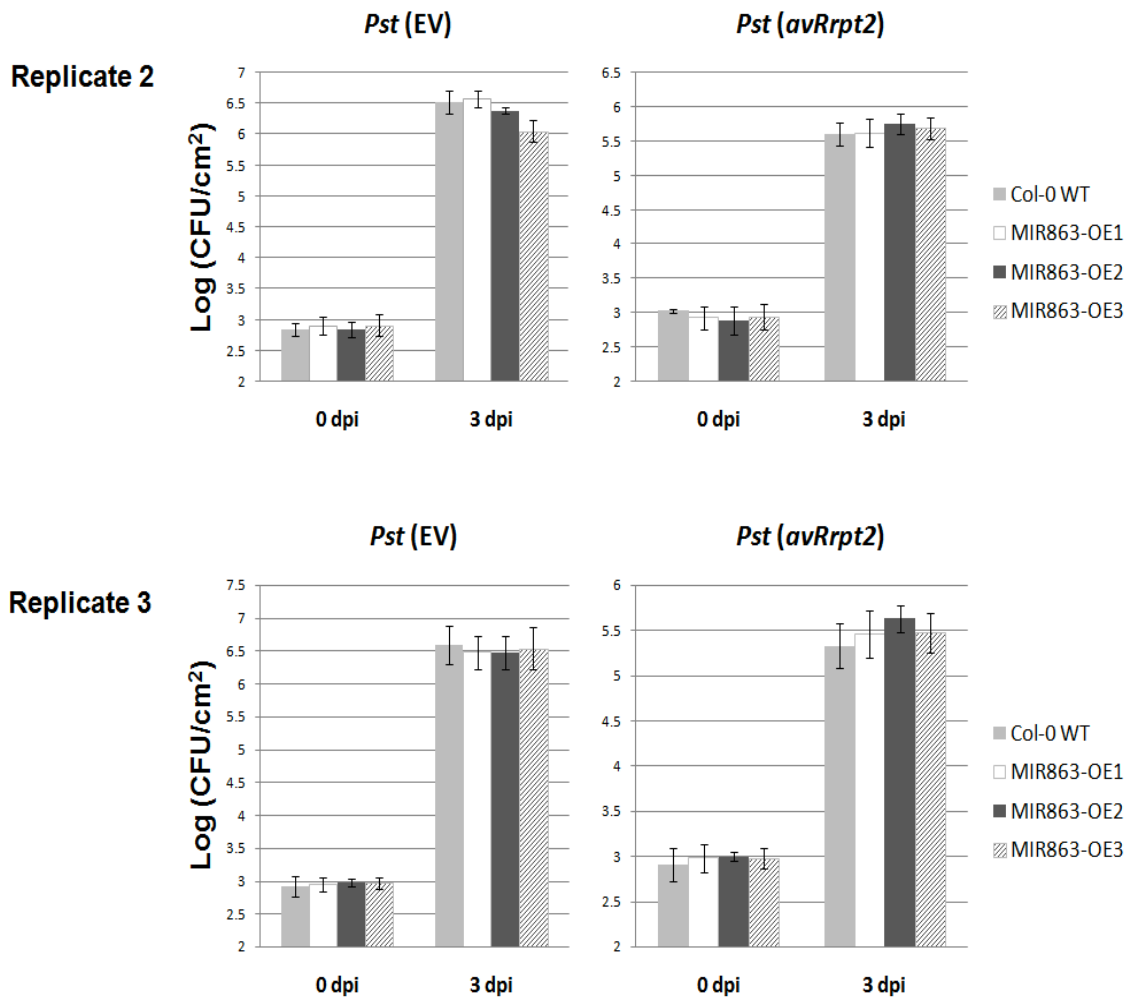


Figure 4b

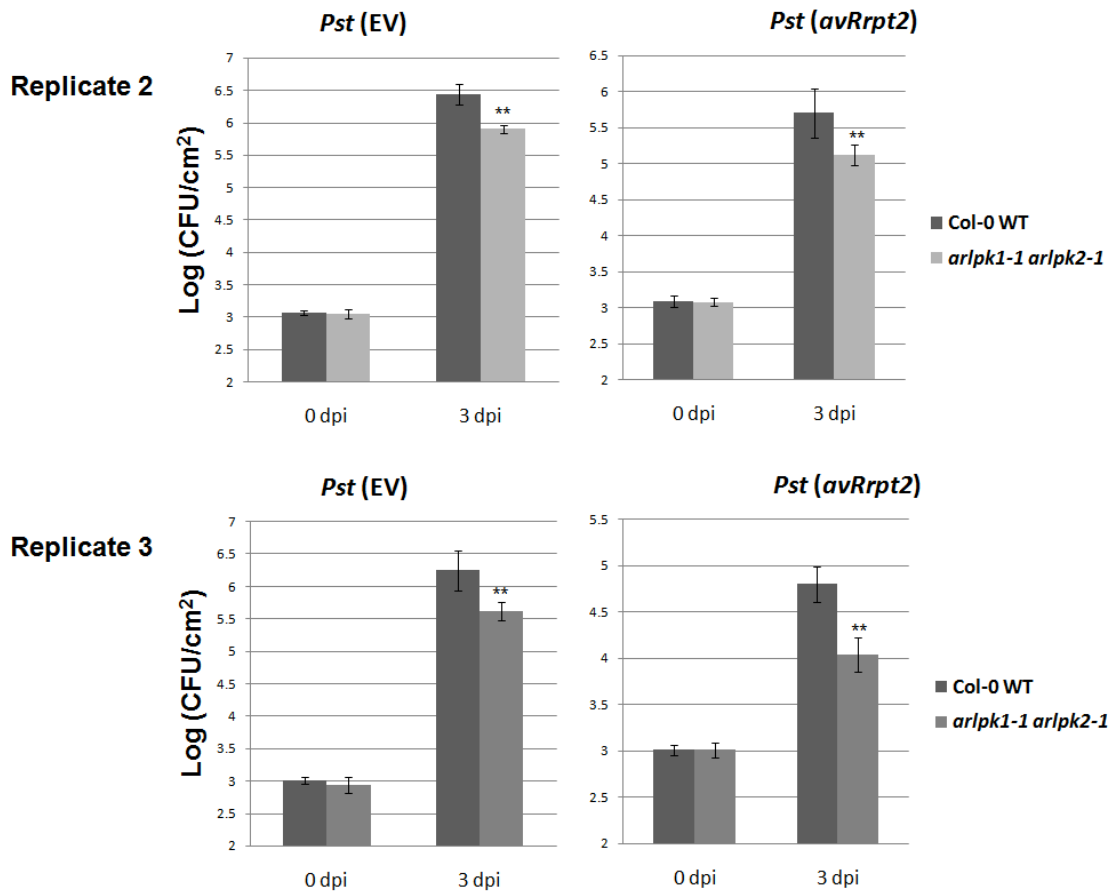
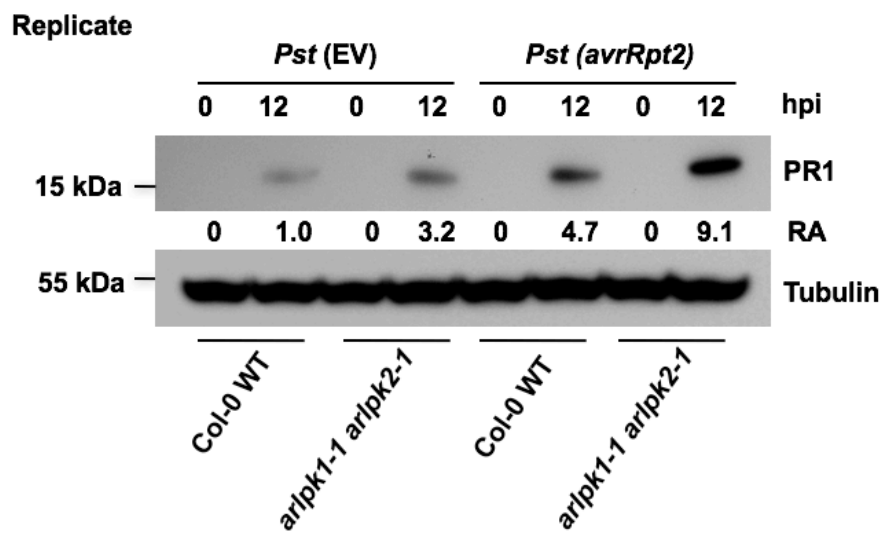


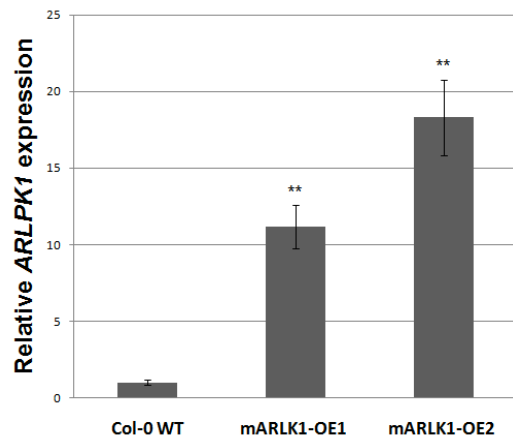


Figure 4c



**Figure 4d**

**Replicate 2**



**Replicate 3**

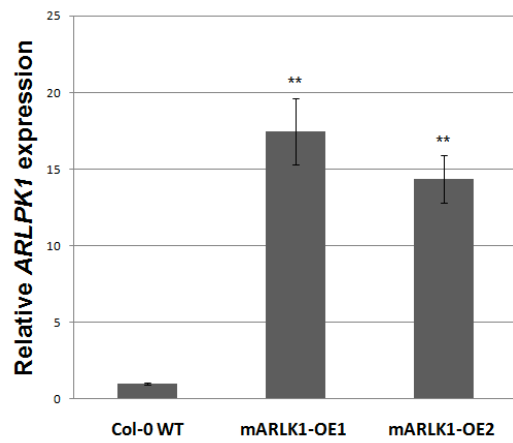


Figure 4e

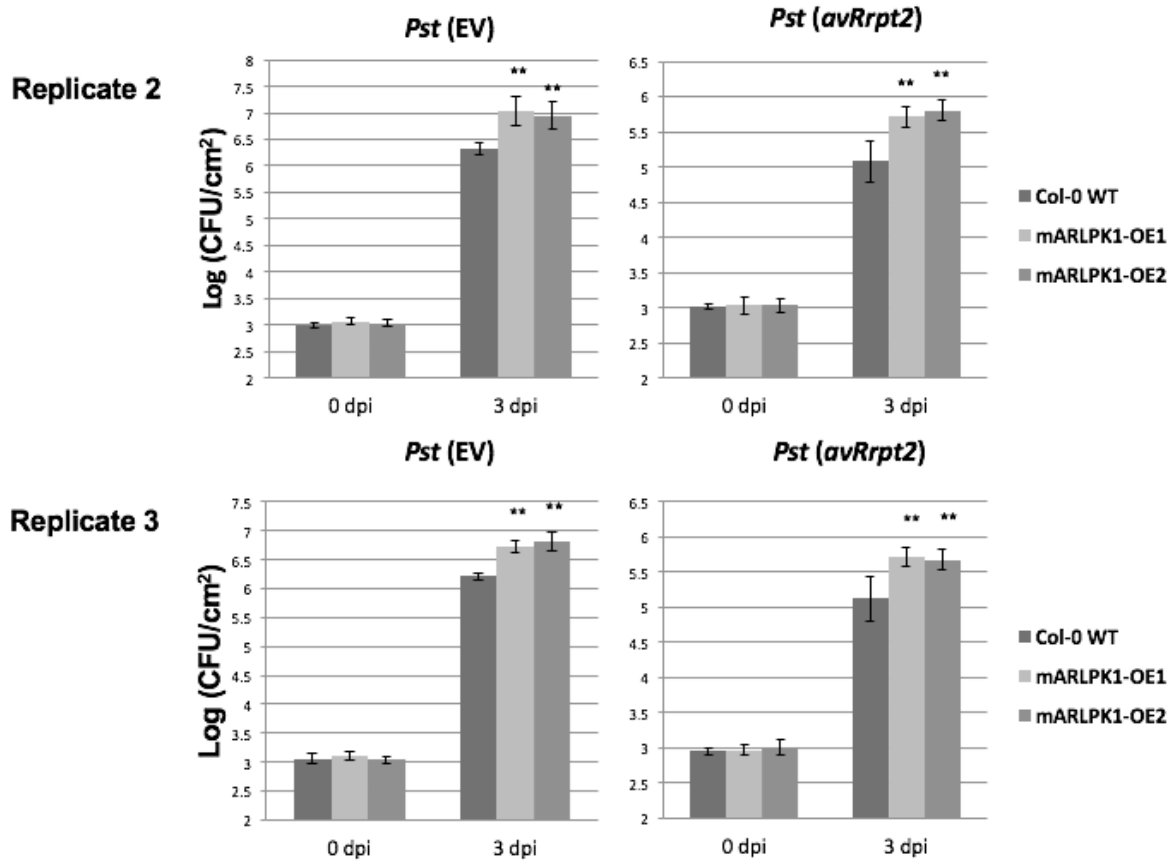
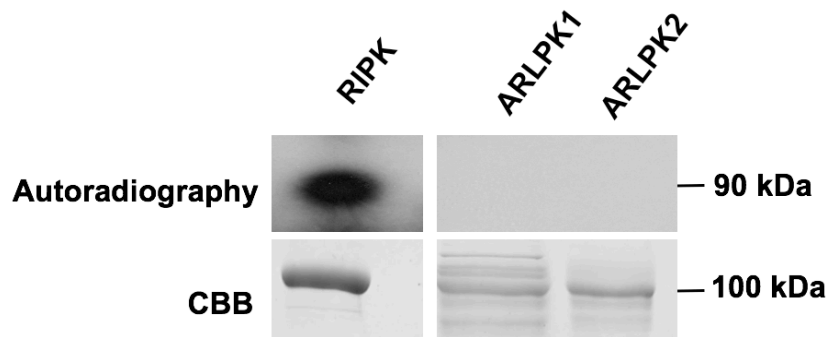


Figure 4f

Replicate



**Figure 6a**

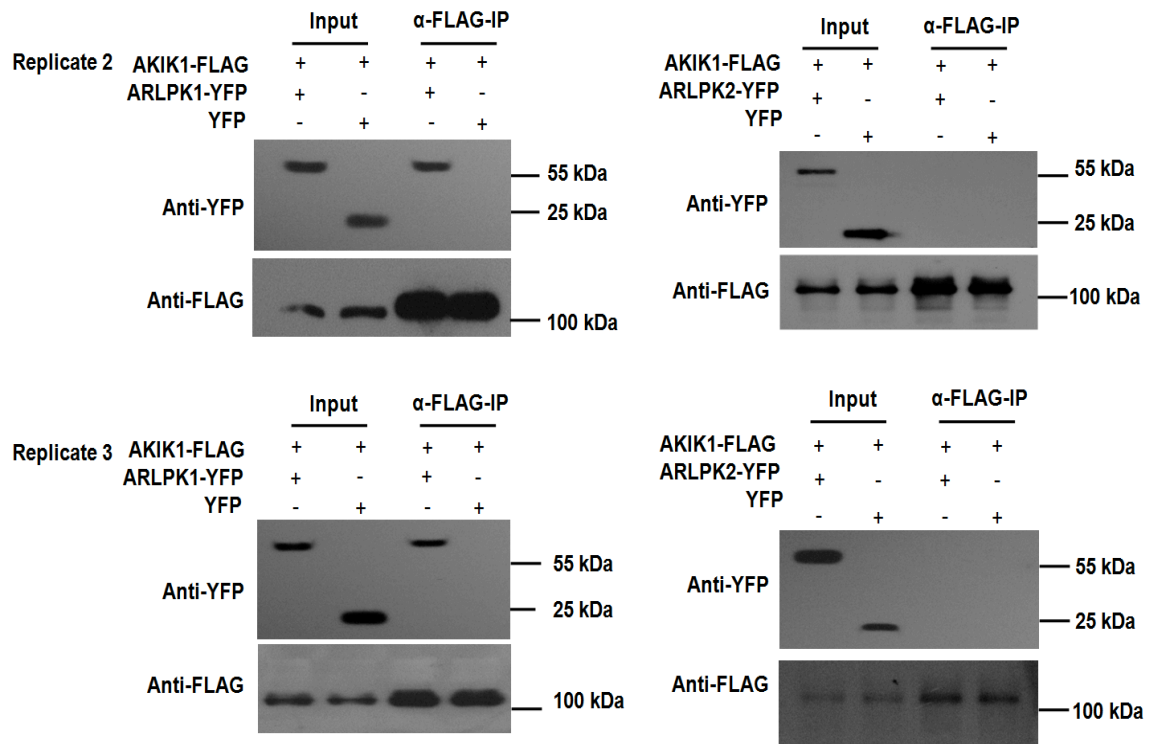


Figure 6b

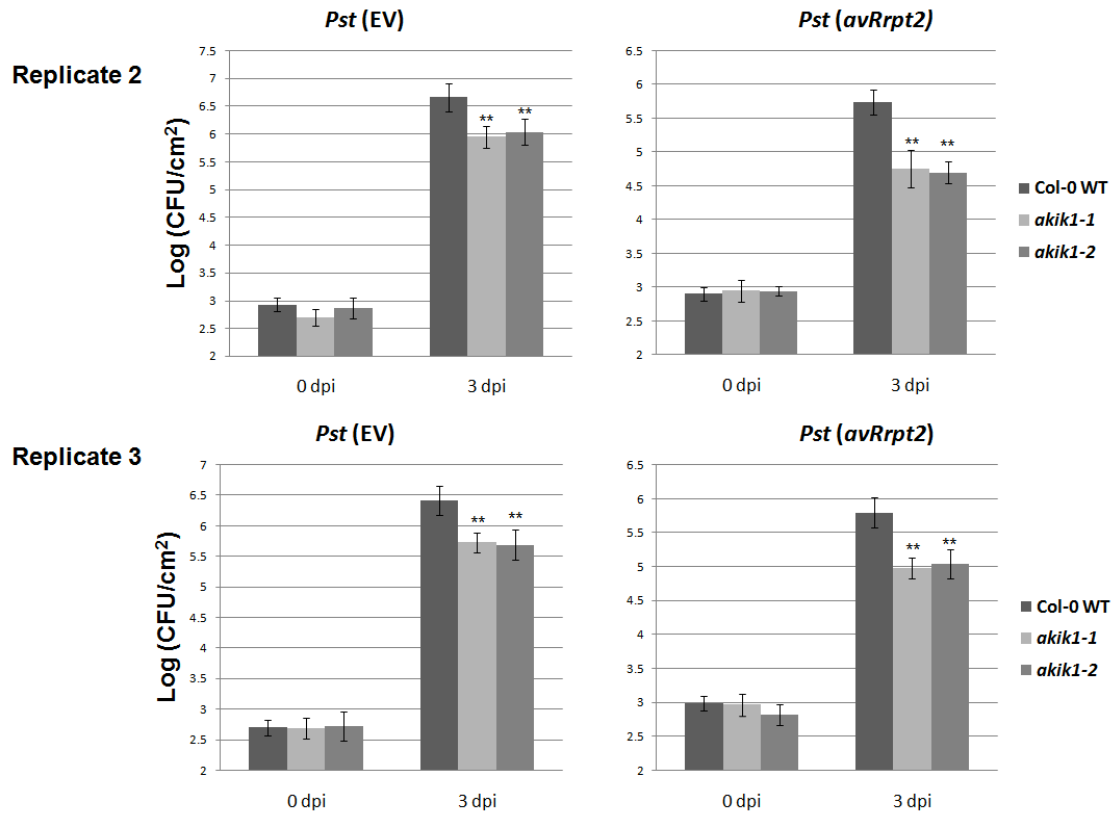


Figure 6c

Replicate

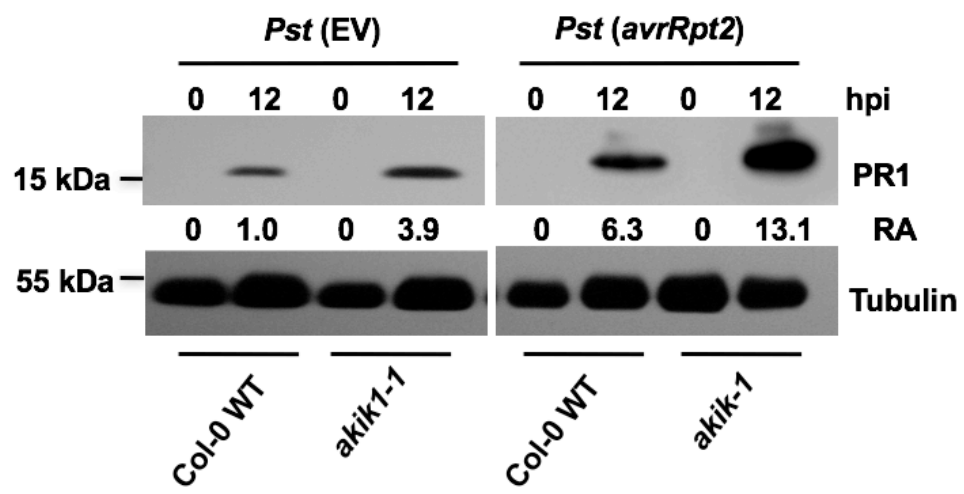


Figure 6d

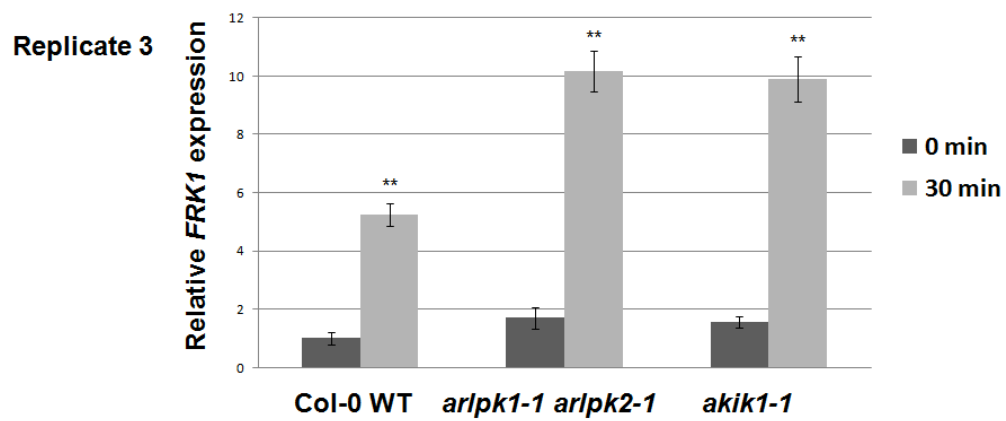
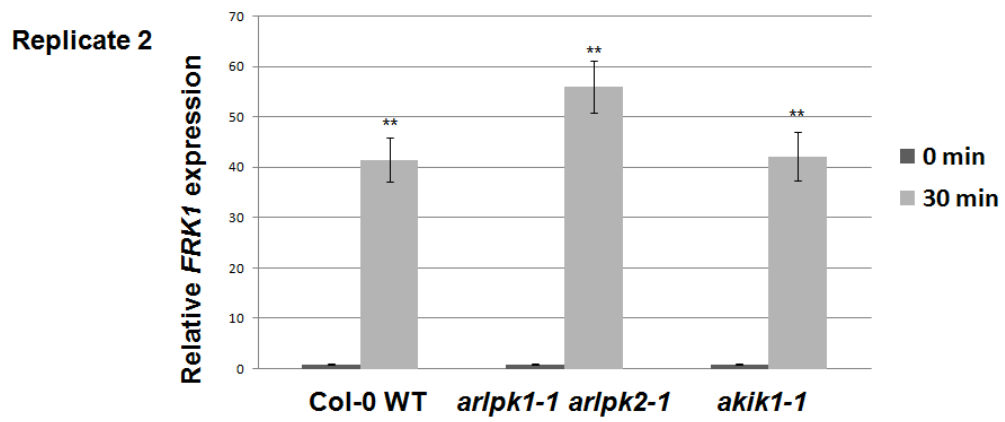
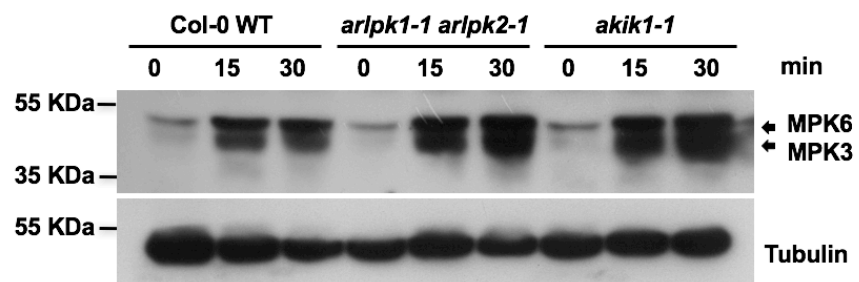


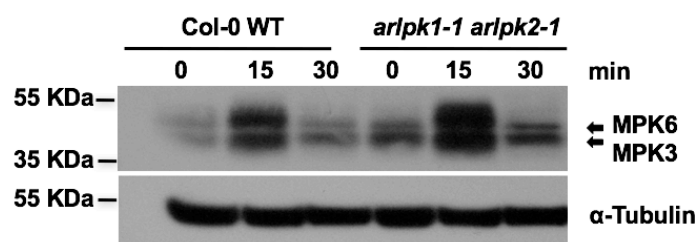


Figure 6e

Replicate 2



Replicate 3



**Figure 6f**

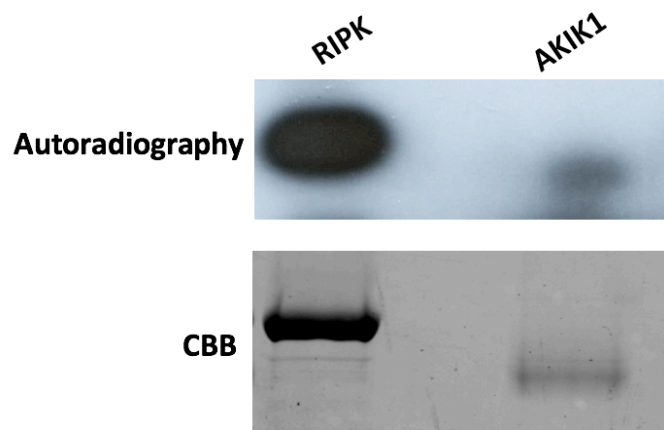


Figure 7c

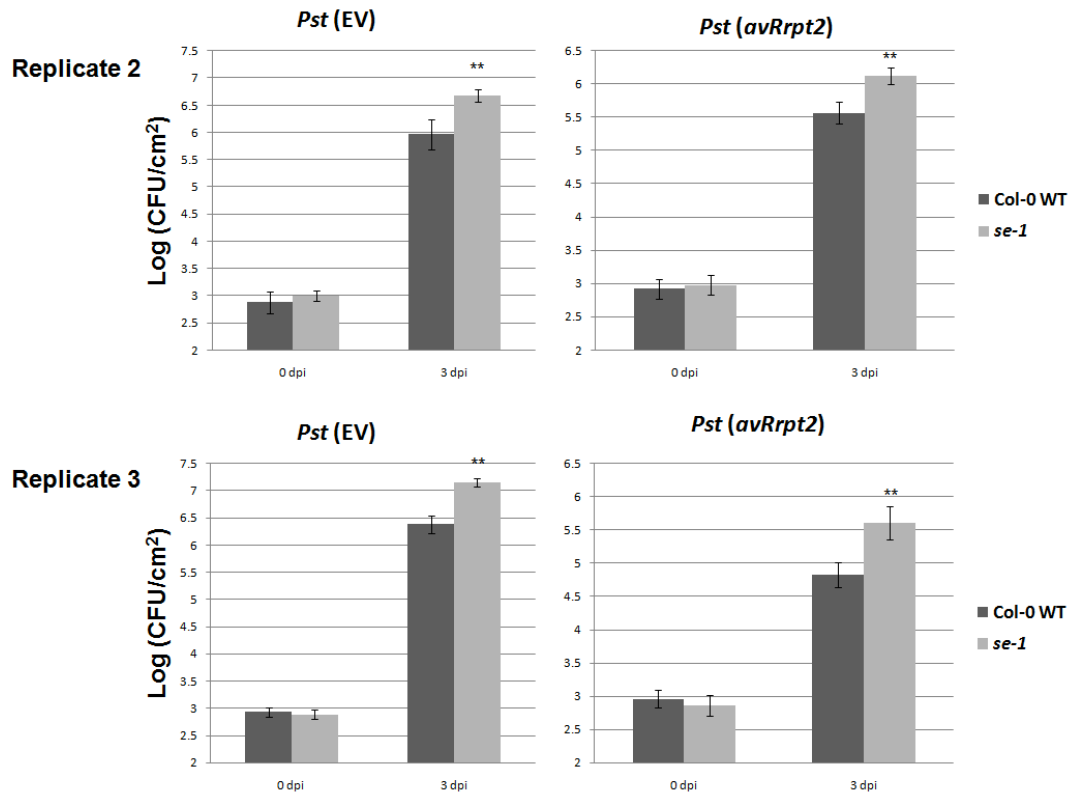


Figure 7d

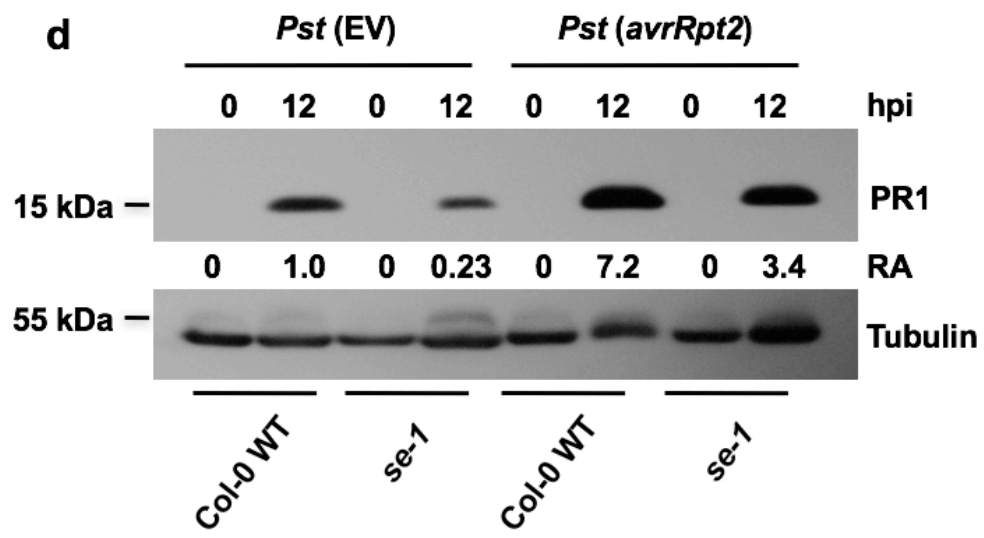
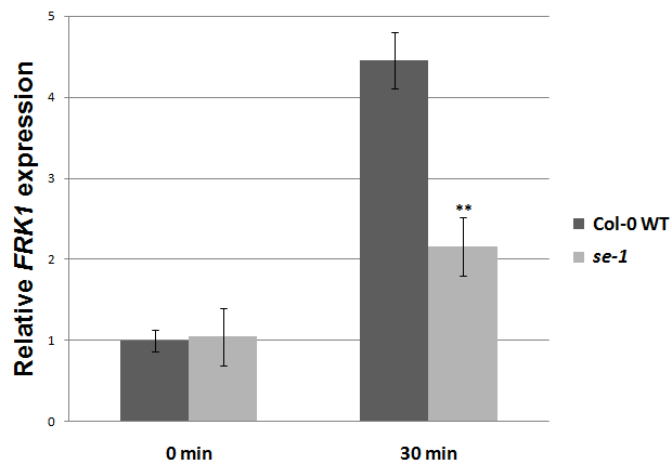


Figure 7e

Replicate 2



Replicate 3

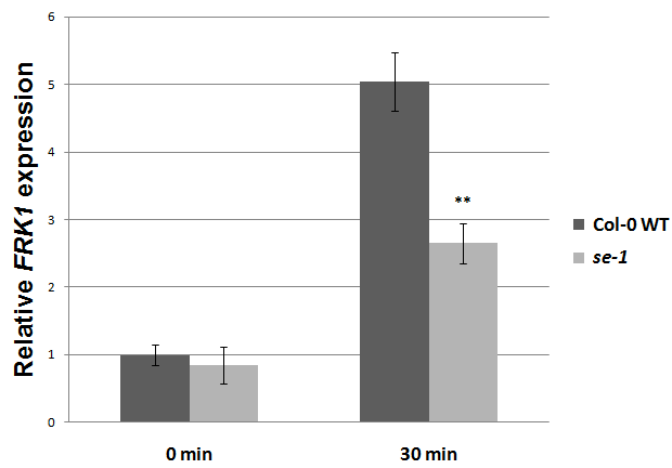


Figure 7f

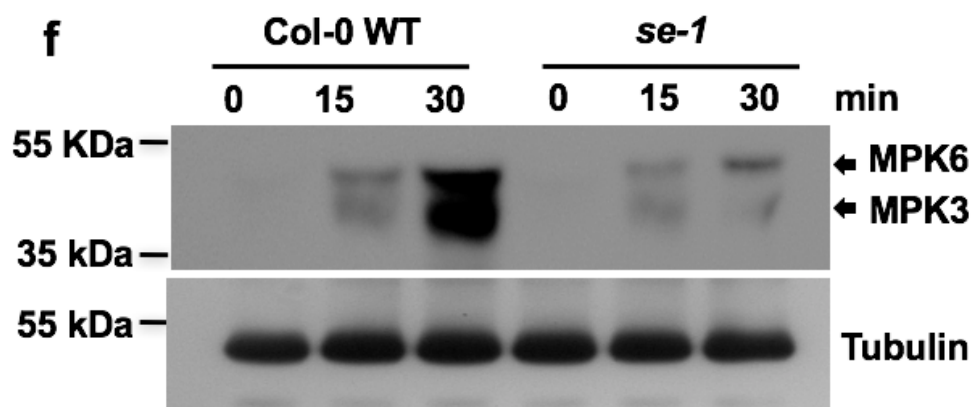


Figure 7h

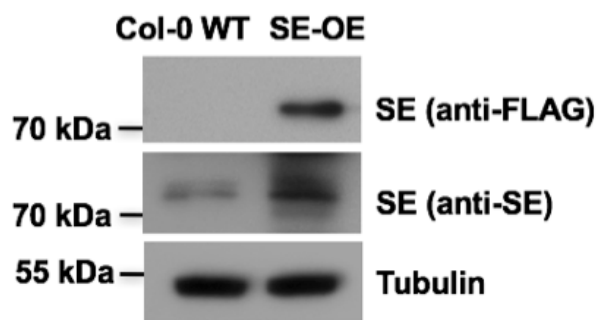


Figure 7i

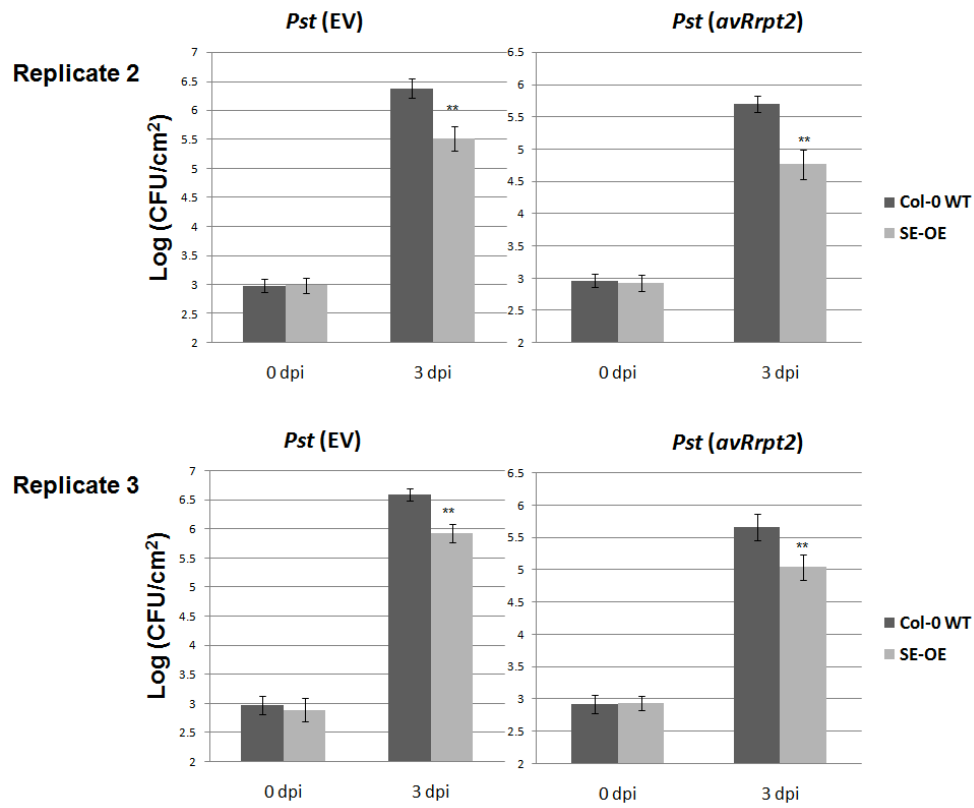
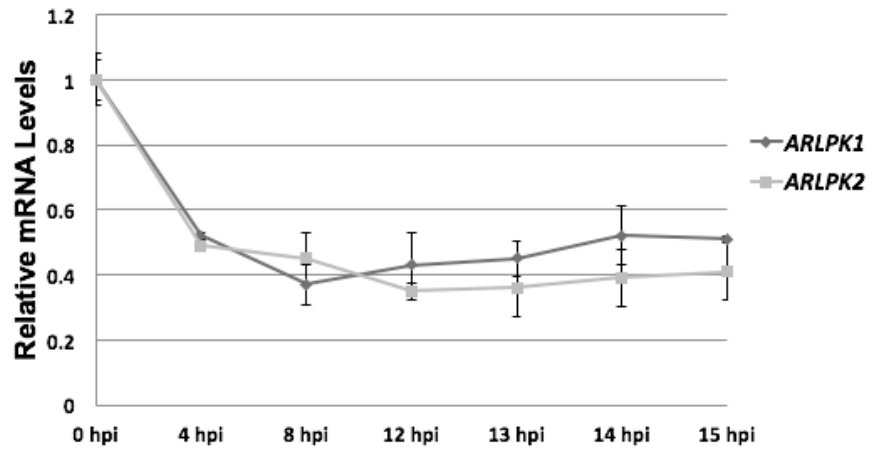




Figure 8a

Replicate 2



Replicate 3

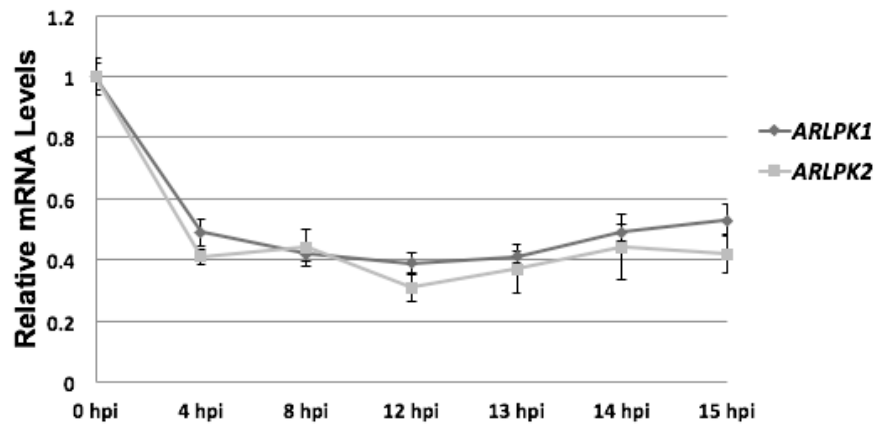


Figure 8b

Replicate

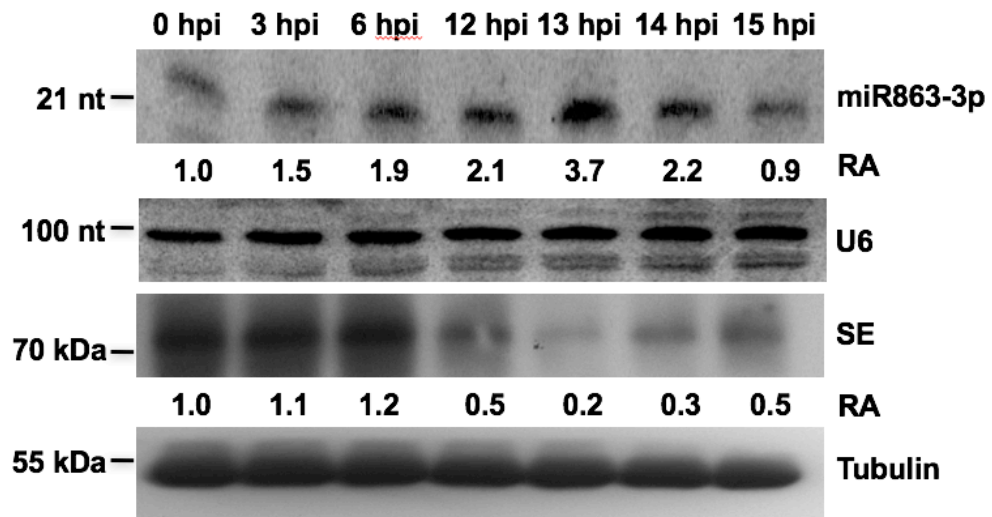
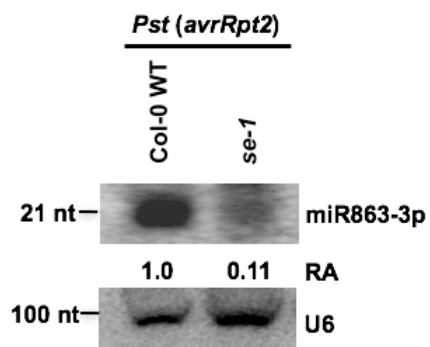
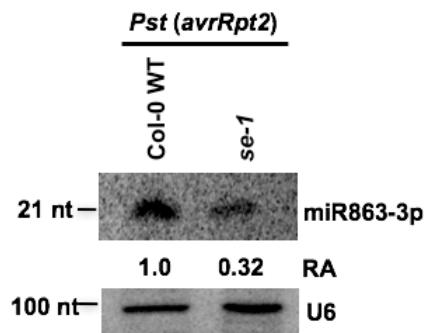


Figure 8c

Replicate 2



Replicate 3



**Supplementary Table 1. Primers used in this study.**

Primer Name	Sequence
mARLPK1-F	5'-CTTTCAGCTTGCAATGAGCTGCTGTAGCCCTTCGCCTACTCTTAGG-3'
mARLPK1-R	5'-GAG TAGGCCGAAGGGCTACAGCAGCTCATTGCAAGCTGAAAGTACTCG-3'
mARLPK2-F	5'-TTTTCAGCTTGCAATGAGCTGCTGTAGCCCTTCGCCTTCTCTTAGG-3'
mARLPK2-R	5'-GAGAAGGCCGAAGGGCTACAGCAGCTCATTGCAAGCTGAAAATACTT-3'
mSE-3'UTR-F	5'-CTTTTGATGTCTGCGGGACTTCCCATACACCTTTTCACTCACTAA-3'
mSE-3'UTR-R	5'-AAGTATTGATAACAACAATGTGGATAAAAACGCATTATTCAATACAAG-3'
ARLPK1-R 5'RACE	5' TCATTTCTGATCTCTTCAAGCTTCC 3'
ARLPK2-R 5'RACE	5' CTAAAATTTCCCAATCTCTTCAAG 3'
ACTIN2-F	5'-AGTGGTCGTACAACCGGTATTGT-3'
ACTIN2-R	5'-CTTGCCCATCGGGTAATTCATAG-3'
ARLPK1_OE-F	5'-CACCATGAGAAAATCCCATTTGGTAAA-3'
ARLPK1_OE-R	5'- TCATTTCTGATCTCTTCAAGCTTC-3'
ARLPK2-OE-F	5'-CACCATGAGAAAATCTCTACTTCTAAC-3'
ARLPK2_OE-R	5'-CTAAAATTTCCCAATCTCTTCAAGTTT-3'
ARLPK1-qPCR-F	5'-TTATTAGGAGATGTCTGAAGGAT-3'
ARLPK1-qPCR-R	5'-TCATTTCTGATCTCTTCAAGCT-3'
ARLPK2-qPCR-F	5'-TGAGGAATGCTGTTCTTGATC-3'
ARLPK2-qPCR-R	5'-CTAAAATTTCCCAATCTCTTCAA-3'
AT5G59650-OE-F	5'-CACCATGGATAGTCCTTGTGGCTTTTGCTG-3'
AT5G59650-OE-R	5'-CTACCTTGCCCTCGAAACAA-3'
FRK1-F	5'-TGCACTTACCCTCCTTCG-3'
FRK1-R	5'-GACAGTAGAAGCCGGTTGGT-3'
LBb1.3	5'-ATTTTGCCGATTTGCGAAC-3'
SALK_022711-LP	5'-TTTGTTCCACATAGGTACCCG-3'
SALK_022711-RP	5'-GGAAGGGGACCACTAAGATTG-3'
CS848612-LP	5'-ACCTCCACTGAGTATGCCAATG-3'
CS848612-RP	5'-ATCCAGGAGTACCAGCAACCAC-3'
MIR863-3P-OE-F	5'-CACCAGAACCCGATGGAGACCAACA-3'
MIR863-3P-OE-R	5'-CAAACCCGTGAGCTTCCAGA-3'
MIR863-3P-probe	5'-ATTATGTCTGTTGCTCTCAA-3'
MIR863-5P-probe	5'-TATTGAGATCAACAAGACATA-3'
SALK_144635-LP	5'-CCCTTACAAGGCCAAGAAATC-3'
SALK_144635-RP	5'-AGCTGTGTGGTTCTCTTTGG-3'

SALK_040744C-LP	5'-TTTTGGGAGGTGATTTCGTAC-3'
SALK_040744C-RP	5'-AATCTTTTCAGCTTCCTCGC-3'
SERRATE-qPCR-F	5'-ATGATCCTAATGCTCCAGGAG-3'
SERRATE-qPCR-R	5'-CGTCTAGGATCTTGCTAAAG-3'

## Supplementary References

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