

N-myristoylation site

AtAIRP3 : **MCNLS**SSGGEGRRRRRRNHTAAPPPPP**PFSSSLFPP**ELFTEIQANPIVFAAVT**PYPNPN**-----**ENPVYQYP** : 68
 At5g03200 : **MCNLS**SLIFCCGRQRSN-----**IP**AMETAP**LELEP**-----**PNRFVFAAVP**PYL**PNPN**-----**PNYVDQYP** : 55
 At3g53410 : **MGNVIS**-----**GGSR**-----**PNHRDRTSP**PYP**PNPN**-----**AQYQGNYP** : 34
 At5g19080 : **MG**--**ISLSK**--**RFRDNNNNHHHPHN**--**PF**YYYS**DPP**Q**QP**---**PPQNG**SYSHNY**FVSTPQLSL**PP**PPA**CP**PE** : 64
 At3g06140 : **MG**--**ISFSNNRRRRDNNRRRLH**HYPP**PE**YY**YLDPP**EP**PPF**PP**PHYD**NY**SNYHLS**-----**P**PL**PP**CP**PE** : 62

AtAIRP3 : ASYYH**PPGAMP**LPY**YH**-HLQH**PPHP**Y**HNH**SWAPV-----**AMARY**PYAGHM**ACPT**PY**VEH**CAV**TI** : 132
 At5g03200 : GNCLP**PPVTEP**MLPY**YFNH**LHH**YPPNS**Y**CL**-LPH**PLF**-----**HGGRYP**-----**IL**PP**ETVY**HC**AVTI** : 112
 At3g53410 : S-----**DCARY**PY**G**--**EMAS**PV**Q**VEH**CAVTI** : 63
 At5g19080 : **SSSQPP**ESQISYR**PYGQ**NY**HQ**QY**Y**Q**Q**APP**YF**GYHHNGFN**PMMP**PV**YFG**TP**VAV**ME**EP**P**AV**VEH**CAV**TI : 137
 At3g06140 : -----**QIN**SCSY**GH**-**YH**-----**HP**Q**EP**CY**F**TAQ**PN**W**W**GM**MR**PA**Y**Y**CP**-P**Q**P**Q**T**Q**F**EP**K**P**Y**LE**CA**NAK**VI : 121

DAR2 domain

AtAIRP3 : **RNDVNLKKE**SLRLE**PD**NPGR**FLV**S**TF**DA**TVS**GRIS**V**IFFAKE**SEDC**R**IT**AT**KED**IL**PP**IT**DFE**H**GLG**QK : 205
 At5g03200 : **RNDVNLKKE**TLRLE**PD**EN**EP**FL**LV**S**TF**DA**SM**GRIT**V**VFFA**TE**DA**ECN**FR**AT**K**ED**IL**PP**IT**DFE**H**GLG**QK : 185
 At3g53410 : **RNDINLKKE**TLRLE**PD**EQ**NP**GR**FL**L**S**TF**DA**SV**GS**IT**V**VMFFA**KEG**K**DCN**L**AT**K**ED**IL**PP**IT**DFE**H**GLG**QK : 136
 At5g19080 : **RNDVMVNRK**TVR**LV**AD**DL**NP**CH**Y**LV**SE**VD**AL**DF**GS**FT**IL**FF**GE**ESK**CT**IV**PH**L**PE**AF**PP**IK**VP**FOR**G**AG**QK : 210
 At3g06140 : **RNDVMVNRK**TVR**LV**ED**DL**V**PC**H**H**LVSE**VD**AL**DF**GS**FT**IL**FF**FA**KE**EP**NC**IT**IP**Q**FP**EV**Y**SE**TR**F**H**FOR**G**EG**Q**K : 194

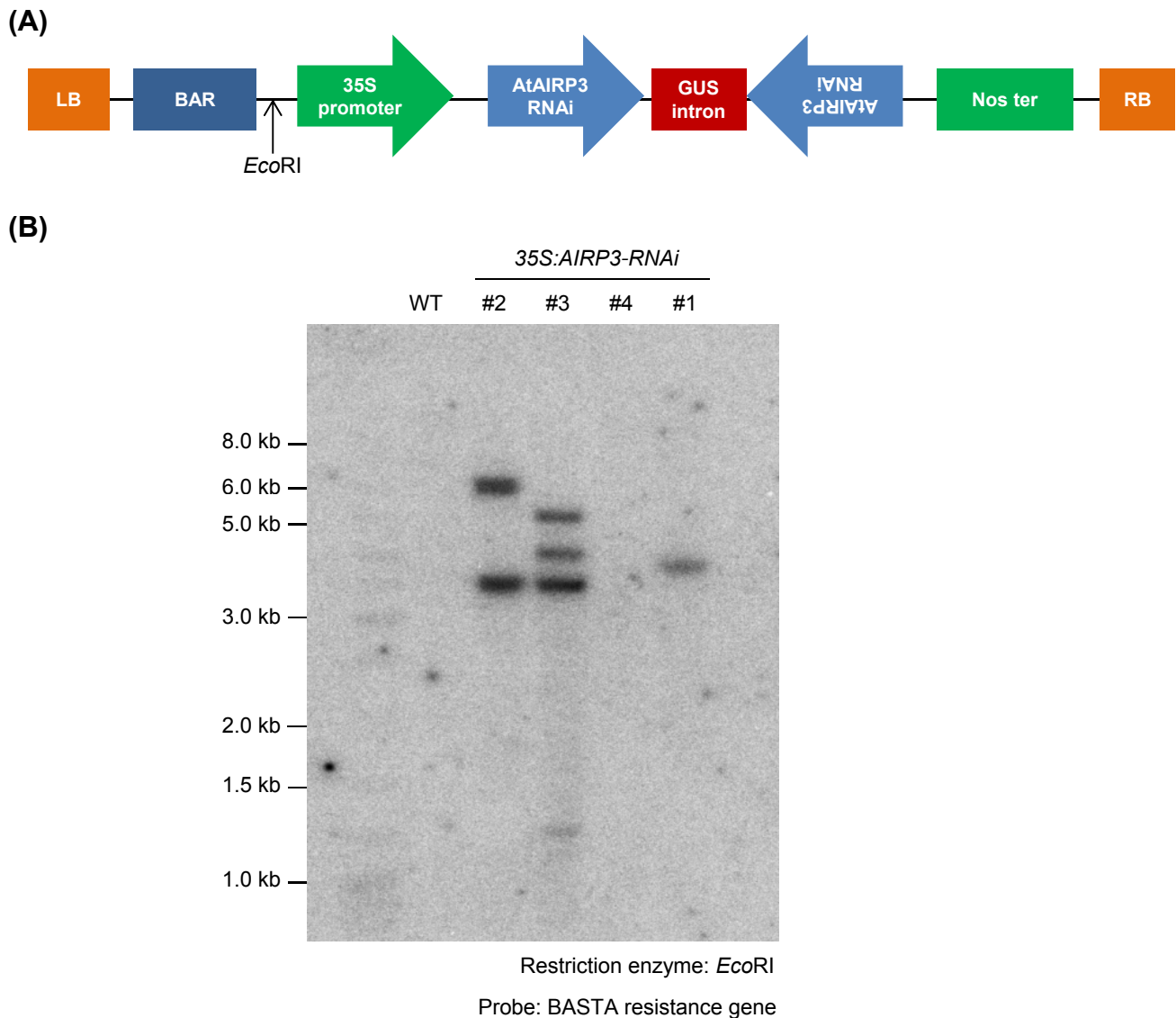
AtAIRP3 : **FKCS**SGSG**IDE**SV**ED**VEL**FAA**AD**TE**Y**EL**AV**KA**E**AA**FS**GG**ENE**EE**ERS**SG**SK**NA**Q**IT**Q**AV**Y**E**KD**KGE**IK**IR** : 277
 At5g03200 : **FKCS**SGTG**IDL**TA**E**KD**SEL**FA**EV**LD**DF**EL**AV**KA**E**AT**P**----**A**EE**GK**SG**ST**N**VO**IT**Q**V**Y**Y**TK**E**KGE**IK**IE** : 251
 At3g53410 : **FKQ**AC**GT**G**IDE**SD**M**SE**AD**LV**E**-**AN**E**ED**V**Y**EV**AV**KA**E**V**S**---**E**DD**H**PE**SG**TP**NR**Q**IT**H**V**LV**E**K**D**H**GE**Y**K**AR : 204
 At5g19080 : **FLQ**AB**GT**G**IDL**GF**SL**DD**LS**R**PS**PE**EV**Y**EL**VI**SA**ET**VI**S---**P**SS**V**SE**EP**LV**H**Q**IT**Q**AV**LE**KT**NI**GS**FK**V**K : 279
 At3g06140 : **FLQ**PS**GT**G**IDL**S**EV**LD**DL**SR**PL**ED**VY**EL**VI**SA**ET**VI**S**---**P**NS**IS**EQ**SS**V**H**Q**VT**Q**AV**LE**KD**NI**GS**FK**V**K : 263

RING domain (C3HC4 type)

AtAIRP3 : **VVKQILWV**NG**TRY**EL**Q**E**Y**IG**G**-NT**VE**G**DD**S**AD**AN**DF**G**KE**CV**IC**LS**EP**RD**TT**VL**PC**R**H**MC**MS**GC**AR**VL**RE** : 349
 At5g03200 : **VVKQILWV**NR**RY**EL**Q**E**Y**IG**E**-NT**VD**G**S**DE-----**G**KE**CV**V**CL**SE**PR**DT**TV**LP**PC**R**H**MC**MS**GC**AR**AL**RE** : 315
 At3g53410 : **VVKQILWV**NG**NR**Y**EL**Q**E**Y**IG**G-NT**VD**D**N**GE---**I**AN**ER**G**KE**CV**IC**LS**EP**RD**TT**VL**PC**R**H**MC**MS**GC**AR**IL**RE** : 273
 At5g19080 : **VMKQILW**IE**GER**Y**EL**Q**E**Y**IG**D**NS**IT**OG**T**A**AS**G**LE**DT**G**G**KE**CV**IC**LE**PK**DT**AV**MP**CR**HL**CL**CS**LC**AE**EL**RE** : 351
 At3g06140 : **VVKQILW**IE**GV**RY**EL**Q**E**Y**IG**---**S**IT**OG**-**A**AS**G**LE**DT**G**ES**GS**G**TE**CV**IC**MT**EA**KD**TA**VL**PC**R**HL**CM**CS**DC**AE**EL**RE : 332

AtAIRP3 : **OTNR**CP**IC**ROP**VE**EL**LE**IK**Y**H**GN**SG**SN**NT**G**Q**ET**VE**QE** : 388
 At5g03200 : **OTNR**CP**V**CROP**VE**EL**LE**IK**Y**NG----- : 337
 At3g53410 : **OTNR**CP**IC**ROP**VD**EL**LE**IT**V**NN**DR**N----- : 299
 At5g19080 : **OTNR**CP**IC**ROP**I**HE**LV**IK**Y**ESS**DE**Q**H**----- : 378
 At3g06140 : **OSNR**CP**IC**ROP**I**EL**LE**IK**Y**SS**DE**Q**H**----- : 359

Supplemental Figure S1. Sequence analysis of AtAIRP3/LOG2. Deduced amino-acid sequences of Arabidopsis AtAIRP3/LOG2 and four homologous RING E3 Ub ligases are aligned. The Gly residue at the +2 position was predicted as a putative N-myristoylation site. The DAR2 domain in the central region and a single C3HC4-type RING motif in the C-terminal region are indicated. Closed circles in the RING motif are putative Zn²⁺-interacting amino-acid residues. Amino-acid sequences identical in all five proteins are presented in black. Amino-acid residues conserved in at least three of the five sequences are presented in gray.



Supplemental Figure S3. Construction of *35S:AtAIRP3-RNAi* transgenic plants. A, Schematic structure of *AtAIRP3 RNAi* binary vector construct. LB, left border; BAR, BASTA resistance gene; Nos ter, nopaline synthase terminator; RB, right border. B, Genomic Southern blot analysis of *35S:AtAIRP3-RNAi* T4 transgenic plants. Genomic DNAs were extracted from 4-week-old rosette leaves of independent T4 *RNAi* transgenic lines #1, #2, #3, and #4. Isolated DNAs were digested by *EcoRI* restriction enzyme and probed with ³²P-labeled BASTA resistant gene. Genomic DNA from wild-type plants was used as a negative control.

Supplemental Table S1. PCR primer sequences used for this article

Name	DNA Sequence	Purpose
LP1	ATCCGGTTTACCAGTACCCTG	genotyping PCR
RP1	TCAATACCTTAGCACATCCGC	genotyping PCR
SAIL_729_A08 LBP3	TAGCATCTGAATTTTCATAACCAATCTCGATACAC	genotyping PCR
SP1	ATGACACTAATCCTTCCGGAGA	RT-PCR
SP2	ATGTCGACATATGTGTATGTGTAGC	RT-PCR
SP3	CGTAATCTCTATATTATGAATTGACAA	RT-PCR
AtRAB18 FW	ATCGATCAAACCTCATCAAAGTCTAA	RT-PCR
AtRAB18 RV	CGAGCTAGAGCTGGATCCAGA	RT-PCR
AtUBC10 FW	TGGATATGGCGTCGAAGC	RT-PCR
AtUBC10 RV	GTGGGATTTTCCATTTAGCC	RT-PCR
AtRD29A FW	CAGGTGAATCAGGAGTTGTTTT	RT-PCR
AtRD29A RV	GCCGGAATTTATCCTCTCT	RT-PCR
AtAIRP3 qRT FW	CATGTCGACATATGTGTATGTGTAGC	qRT-PCR
AtAIRP3 qRT RV	AAAGCCTTCAACAGGTTGCC	qRT-PCR
AtRAB18 qRT FW	GGCTTGGGAGGAATGCTT	qRT-PCR
AtRAB18 qRT RV	TTGATCTTTTGTGTATTCCCTTCT	qRT-PCR
GAPDHc qRT FW	TGAAATCAAAAAGCTATCAAGG	qRT-PCR
GAPDHc qRT RV	CATCATCCTCGGTGTATCCAA	qRT-PCR
BASTA prove FW	ATGAGCCCAGAACGACGCC	Southern blot
BASTA prove RV	ATATCCGAGCGCCTCGTG	Southern blot
AtAIRP3promoter FW <i>Bam</i> HI	GAATTCGTGGTTAGGTATGCGATTCTATTATG	GUS staining
AtAIRP3promoter RV <i>Nco</i> I	CCATGGATGTTATTGGCGTTAAACCCAG	GUS staining
AtAIRP3 CDS <i>Bam</i> HI FW	GGATCCATGGGAAACATTAGCAGCAG	AtAIRP3-flag
AtAIRP3 CDS <i>Bam</i> HI RV	GGATCCCTCTGTTCACCTGTTTCTCC	AtAIRP3-flag
AtAIRP3 RNAi1 FW <i>Bam</i> HI	GGATCCGAAGAATGCGCAGATTACTCAAG	35S:AtAIRP3 RNAi
AtAIRP3 RNAi1 RV <i>Eco</i> RI	GAATTCGATGGCATTTCATCCATAC	35S:AtAIRP3 RNAi
AtAIRP3 RNAi FW <i>Xho</i> I	CTCGAGGAAGAATGCGCAGATTACTCAAG	35S:AtAIRP3 RNAi
AtAIRP3 RNAi FW <i>Pst</i> I	CTGCAGCGATGGCATTTCATCCATAC	35S:AtAIRP3 RNAi
MBP-AtAIRP3 ΔN FW <i>Eco</i> RI	GAATTCATGTCTTGGGCTCCCGT	MBP-AtAIRP3
MBP-AtAIRP3 ΔN RV <i>Eco</i> RI	GAATTCCTACTCTGTTCACCTGTTTCTCC	MBP-AtAIRP3
35S:AtAIRP3 FW <i>Bam</i> HI	GGATCCATCTTAACCTCTCTGCTTTTGCTT	35S:AtAIRP3
35S:AtAIRP3 RV <i>Bam</i> HI	GGATCCCTCTGTTCACCTGTTTCTCCCT	35S:AtAIRP3
AtAIRP3 RING mutagenesis FW	AATGTGTTATATCTTTGTCTGAACC	AtAIRP3 RING mutagenesis

AtAIPR3 RING mutagenesis RV	GGTTCAGACAAAGATATAACACATT	AtAIPR3 RING mutagenesis
AtRD21 CDS FW <i>Bam</i> HI	GGATCCATGGGGTTCCTTAAGCCA	Y2H & RD21 recombinant protein
AtRD21 CDS RV <i>Hind</i> III	AAGCTTTTAGGCAATGTTCTTTCTGCC	Y2H & RD21 recombinant protein
AtRD21 ΔN FW	ATGGGTGAGAGAAGGACTAGCCTAC	Y2H & RD21 recombinant protein
AtRD21 C FW	ATGCCGGGACCTTCACCTCCA	Y2H & RD21 recombinant protein
AtRD21 m RV	GTTTGGCGGGTTTTCGC	Y2H & RD21 recombinant protein
AtRGA1 FW <i>Bam</i> HI	GGATCCATGAAGAGAGATCATCACCAATT	RGA1-flag
AtRGA1 RV <i>Bam</i> HI	GGATCCGTACGCCCGTCGAGAG	RGA1-flag
