

RCS1

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AtRIN4      1  MA-RSNVPKFGNWEAEENVPYTAYFDKARKTRAPGSKIMNPNDPEVNSDS--OSOAPPHP
GmRIN4A    1  MAQRSHVPKFGNWDSEGENVPYTAYFDKARKGR-TGARIINPNDPEENADLSLDNPSSDHL
GmRIN4B    1  MAQRSHVPKFGNWDSEGENVPYTAYFDKARKGR-TGTRIINPNDPEENADLSFDNPSSDNL
GmRIN4C    1  MAQRSNVPMLG--KSEENVSDTAHSDKAQKGQ-PGSKMINPNDTKENSDV----VSSAGL
GmRIN4D    1  MAQHSNVPKFGNOESEDNVLDTAHSDKAQKGQ-SGSKMINPNDTKENSDI----VSSADL
  
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AtRIN4      58  PSSRTKPEQVDTVRRSREHMR-----SREESELKQFGDA-----GGSNE-----AA
GmRIN4A    60  PPTRPRANSEDOSGKGSLP-----LED-DPKHFVDSPARHDNVSSRSGSRSHGVGSA
GmRIN4B    60  PPTRPRTNSEDOSGKGSLH-----LED-DPKNFIESPARHDNVSSRSGSRSHGVGSA
GmRIN4C    54  PHSKPRVHSEDPSGKGSVRSIHELOMSREDGDPKQFTDSPARHG--GDSAYRGHGVGSA
GmRIN4D    56  PHSKPRVHSEDPSGKGSVRTTHELOKSREDGDPKQFTDSPARHG--GDSSHRGHGVGSA
  
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AtRIN4      100  NKRQGRASQNNSYDN---KSPLH-----KNSYDGT---GKSRPKPTNLR
GmRIN4A    111  ENRRRHSTQSTGSEYSIERSPLHRQARAPGRDSPOWEPKNSYDNSSQGTPGRSRLRPVN-R
GmRIN4B    111  DNRRRHSTQSTGSEYSIERSPLHRQARAPGRDSPOWEPKNSYDSSGTPGRSRLRPAN-R
GmRIN4C    112  DNRKRPSRQSTGSEHSIDRSPLHRQAKTPGRDSPSWEGKNSYDSSHGTPGRSRLRPN-R
GmRIN4D    114  DNRKRPSRQSTGPEHNIDRSPLHRQAKTPGRDSPSWEGKNSYDSSHGTPGRSRLRPSY-R
  
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V

RCS2

V

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AtRIN4      138  ADESPEKVTVVPKFGDWDENNPSSADGYTHIFNKVREERSSGAN-VSGS-SRTPTHOSSR
GmRIN4A    170  GDETPDKGAAVPKFGDWDVNNPSSSADGFTHIFNKVREERQGVPGOVPGTPNERPQAIRGQ
GmRIN4B    170  GDETPDKGAAVPKFGDWDVNNPASADGFTHIFNKVREERQGGPGOVPGTPNERPOPINGL
GmRIN4C    171  GDETPDKGAAVPKFGEWDESNPASADGYTHIFNKVREERKQVGAGHVPTNGRQYAARNQ
GmRIN4D    173  GDETPDEGAAVPKFGEWDESNPASADGYTHIFNKVREERKQVGAGHVPTNGRQYAARNQ
  
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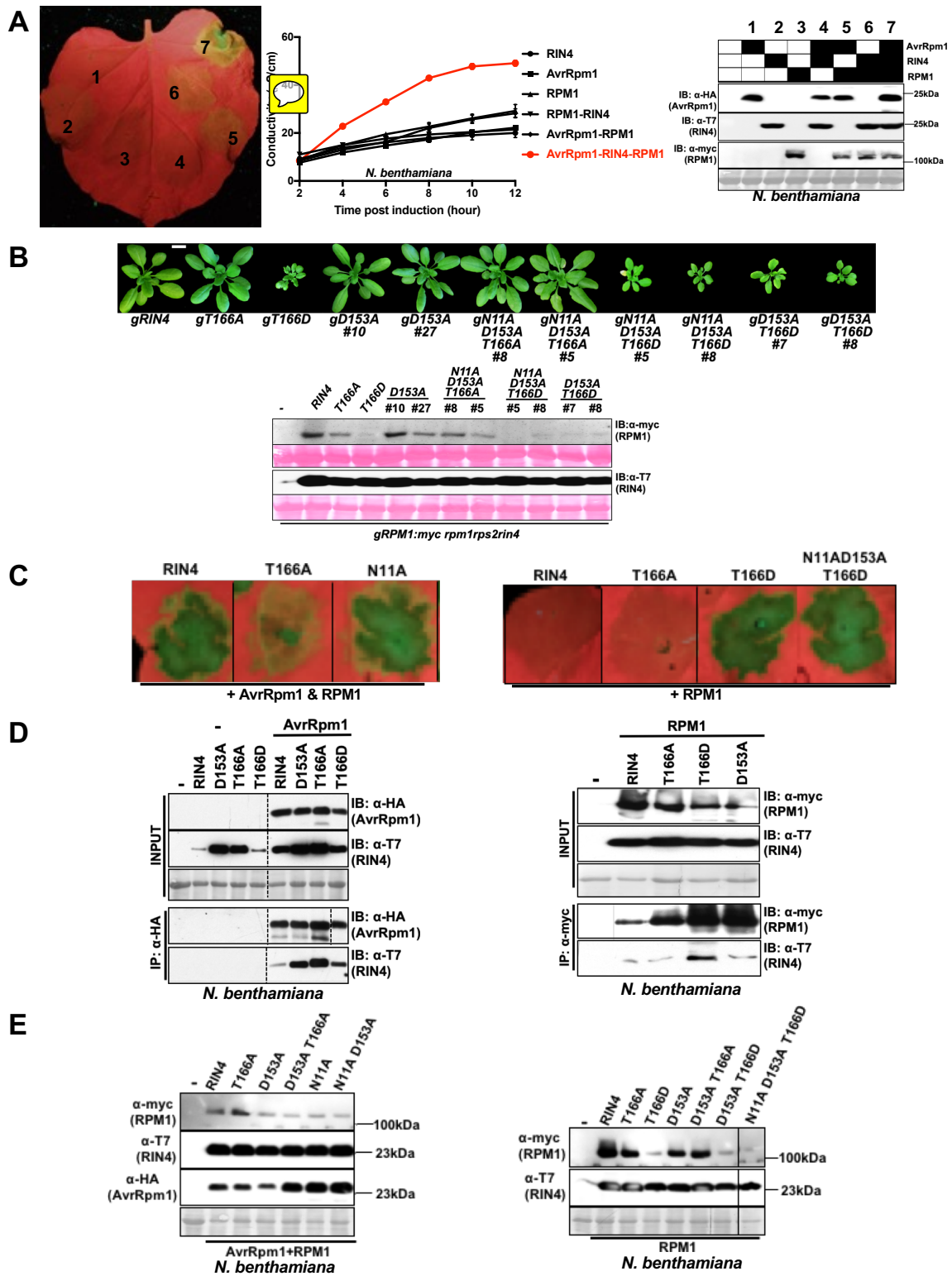
Palmitoylation site

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AtRIN4      196  NPNTSSCCCFGGGK-
GmRIN4A    230  SNDDKVQCCCFAWGGK
GmRIN4B    230  SNDDKVQCCCFAWGGK
GmRIN4C    231  PADDKAQSCCFCWG-KK
GmRIN4D    233  RANDKAQSCCFCWG-KK
  
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Supplemental Figure 1. RIN4 is Highly Conserved Between Species.

A box shade alignment illustrates the level of amino acid identity between soybean and Arabidopsis RIN4 proteins. RCS1 and RCS2 indicate the AvrRpt2 cleavage sites conserved among all RIN4 proteins. The region between the red arrow-heads span the region of RIN4 shown to interact with AvrB.



Supplemental Figure 2. ADP-ribosylation of AtRIN4 on D153 Promotes Phosphorylation on T166.

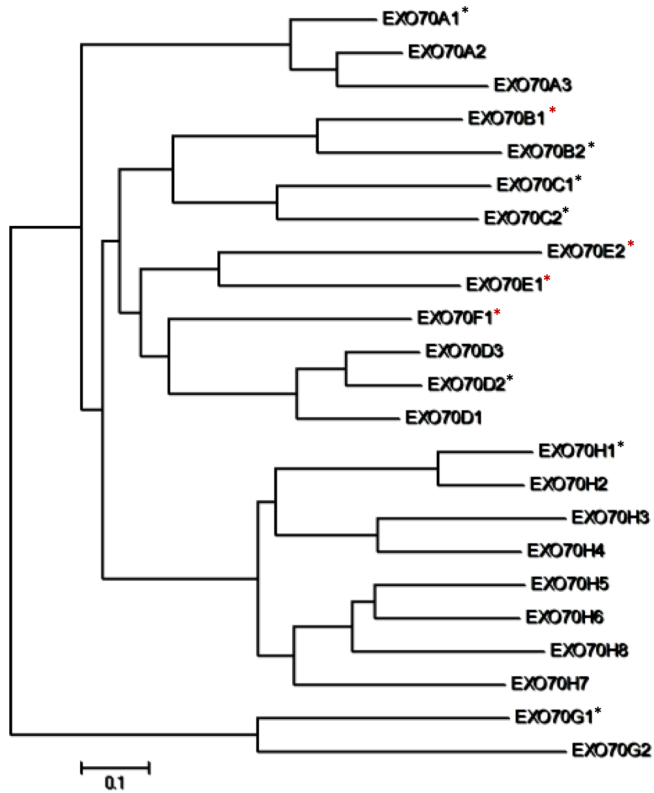
(A) Reconstruction of AvrRpm1-dependent RPM1 activation in *N. benthamiana*. AvrRpm1, AtRIN4 or RPM1 constructs were infiltrated alone or in combination with each other. The picture was taken 12 hours post induction of AvrRpm1 with 20 μ M estradiol treatment. This confirms that RPM1 can only be activated following co-expression of AvrRpm1 and AtRIN4 (left). Conductivity measurements for AvrRpm1-mediated RPM1 activation in this reconstruction system (middle). Expression of each protein was confirmed by immunoblots with α -HA for AvrRpm1, α -T7 for RIN4 and α -myc for RPM1 (right).

(B) Phenotypes of Arabidopsis transgenic lines for AtRIN4^{D153A}, AtRIN4^{N11AD153AT166A}, AtRIN4^{N11AD153AT166D} and AtRIN4^{D153AT166D} confirms that the phospho-mimic AtRIN4^{T166D} is epistatic to loss of ADP ribosylation in triggering effector-independent cell death. Two independent T2 transgenic lines of each AtRIN4 mutant allele were selected and protein expression of RPM1 and AtRIN4 mutant alleles was monitored by immunoblot with α -myc for RPM1 or α -T7 for AtRIN4, respectively.

(C) ADP-ribosylation at AtRIN4^{N11} is not required for AvrRpm1-mediated RPM1 activation (left) and the phospho-mimic AtRIN4^{T166D} is epistatic to loss of ADP ribosylation in triggering effector-independent cell death (right) in *N. benthamiana*. The visible cell death phenotypes are shown 12 hours post-induction of AvrRpm1 with 20 μ M of estradiol treatment (left) and 2.5 days post-inoculation (right).

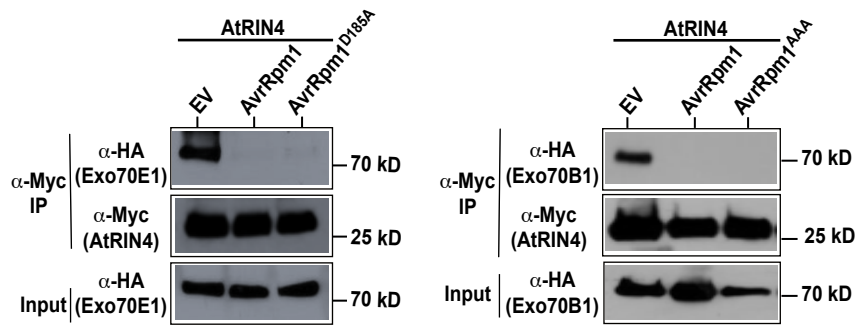
(D) Co-immunoprecipitation (CoIP) of AtRIN4 mutant proteins with AvrRpm1 or RPM1 in *N. benthamiana* was performed by immunoprecipitation with α -HA for AvrRpm1 (left) or α -myc for RPM1 (right) followed by immunoblots with α -T7 for AtRIN4. Samples were taken 12 hours post-estradiol treatment for CoIP of AtRIN4 with AvrRpm1 or 2.5 days post inoculation for CoIP of AtRIN4 alleles with RPM1, respectively.

(E) Expression of AtRIN4 alleles, AvrRpm1 and RPM1 used to test AvrRpm1-mediated or effector-independent RPM1 activation. Total proteins were extracted 6 hours post-estradiol (20 μ M) treatment for AvrRpm1-mediated RPM1 activation and expression was confirmed by immunoblots with α -HA for AvrRpm1, α -T7 for AtRIN4 alleles and α -myc for RPM1 (left). Protein expression of AtRIN4 alleles and RPM1 during effector-independent activation were verified by immunoblots with α -T7 for AtRIN4 alleles and α -myc for RPM1 from the samples taken 2 days post-infiltration in *N. benthamiana* (right). Note that RPM1 activation by the phospho-mimic AtRIN4^{T166D} results in diminution of RPM1 levels.



Supplemental Figure 3. Phylogenetic Tree of Arabidopsis EXO70 Family Members.

Evolutionary relationships among proteins of the Arabidopsis EXO70 family (Synek et al., 2006) were inferred using the Neighbor-joining method (Saitou and Nei, 1987) implemented in MEGA6 (Tamura et al., 2013). The scale bar indicates an evolutionary distance of 0.1 substitutions per amino acid position. The asterisks indicate EXO70 proteins that were tested for interaction with AtRIN4 through a targeted yeast two-hybrid assay, with the red asterisks indicating those shown to interact with AtRIN4 (Figure 7A).



Supplemental Figure 4. AvrRpm1 Blocks Association of AtRIN4 with Arabidopsis EXO70 Proteins. The indicated proteins were transiently expressed in *N. benthamiana* and then *AtRIN4* was immunoprecipitated using anti-myc agarose beads. EXO70B1 and EXO70E1 co-precipitated with *AtRIN4*, but this interaction was blocked in the presence of both wild-type and catalytic site mutant AvrRpm1 proteins.