

Supplementary Information

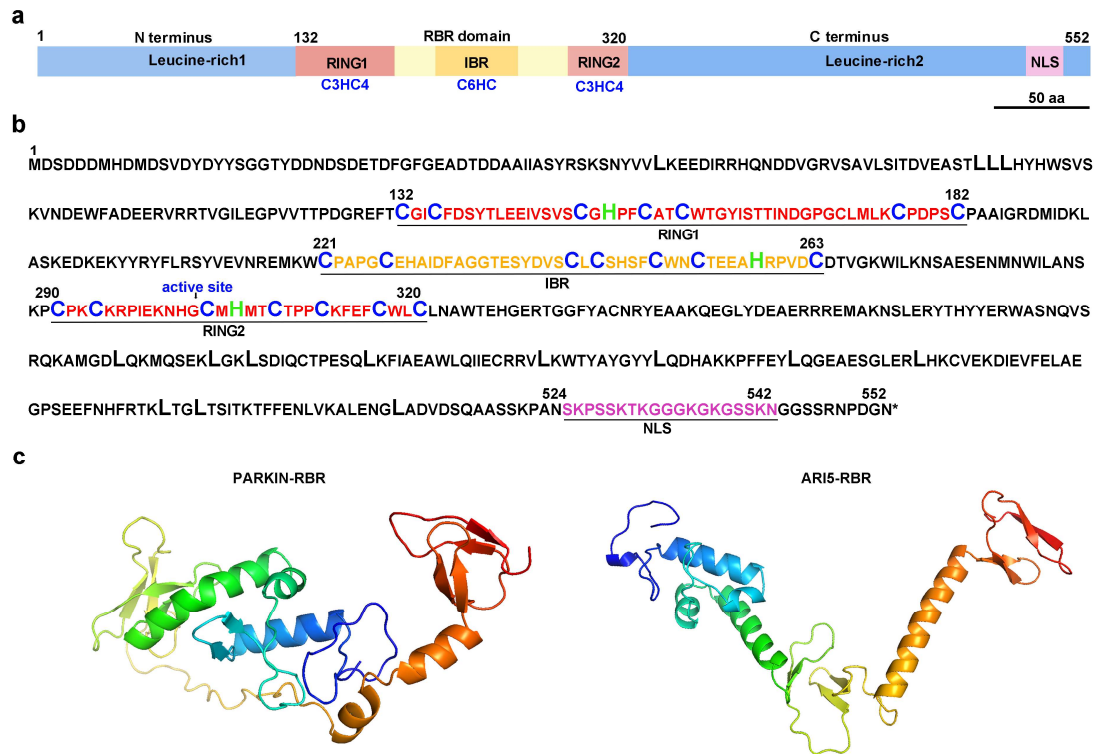
The Arabidopsis PARKIN-like E3 ligase ARIADNE5 regulates plant development by targeting TCP4 for degradation

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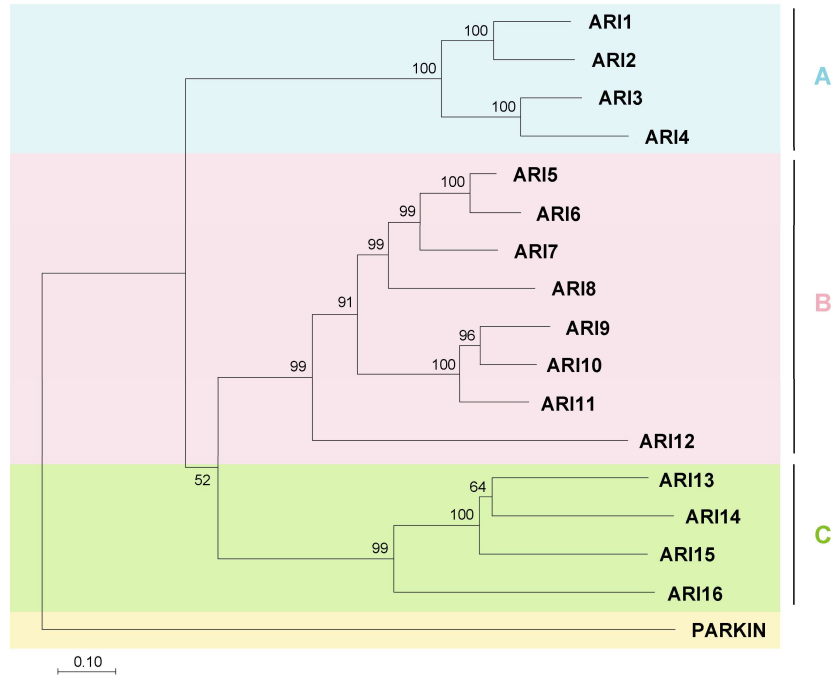
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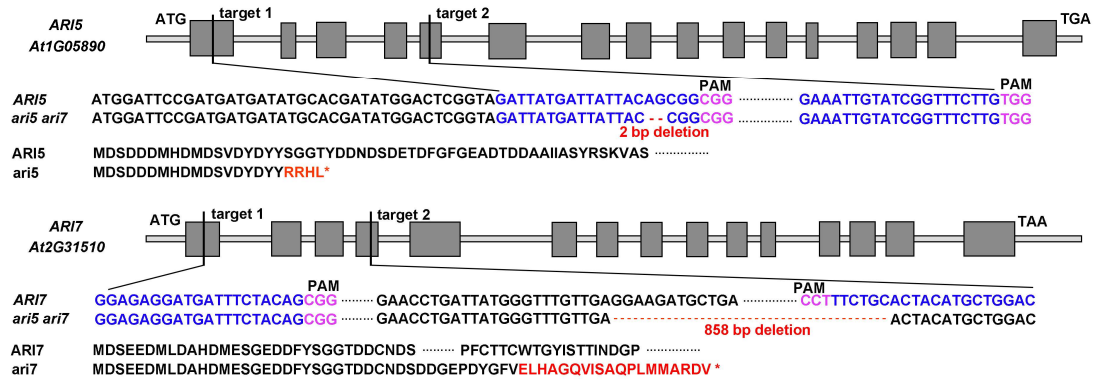
Supplementary Fig. 1. Conserved RBR domain in Arabidopsis ARI5 and human PARKIN.

a Domain organization of ARI5 showing RBR domain (residues 132-320) with C3HC4-type RING1, C6HC-type IBR, and C3HC4-type RING2 motifs. Leucine-rich regions span both N-terminal and C-terminal segments, with the latter containing a nuclear localization signal (NLS). **b** *ARI5* (*At1g05890*) encodes a 552-residue protein containing two leucine-rich regions, RBR domain (cysteine in blue; histidine in green), and C-terminal NLS (purple). **c** AlphaFold2-predicted RBR structures of PARKIN (left) and ARI5 (right).



Supplementary Fig. 2. Phylogenetic clustering of Arabidopsis ARI with PARKIN homolog.

Neighbor-joining analysis of 16 ARI proteins (Arabidopsis) and PARKIN (human) based on full-length sequences. Bootstrap values (1000 replicates) shown at nodes. Phylogenetic reconstruction and statistical validation were performed in MEGA11. Subgroups are manually shaded with colored backgrounds: A (blue), B (pink; containing ARI5/6/7), C (green), and PARKIN (yellow). *ARI4* and *ARI6* are pseudogenes.

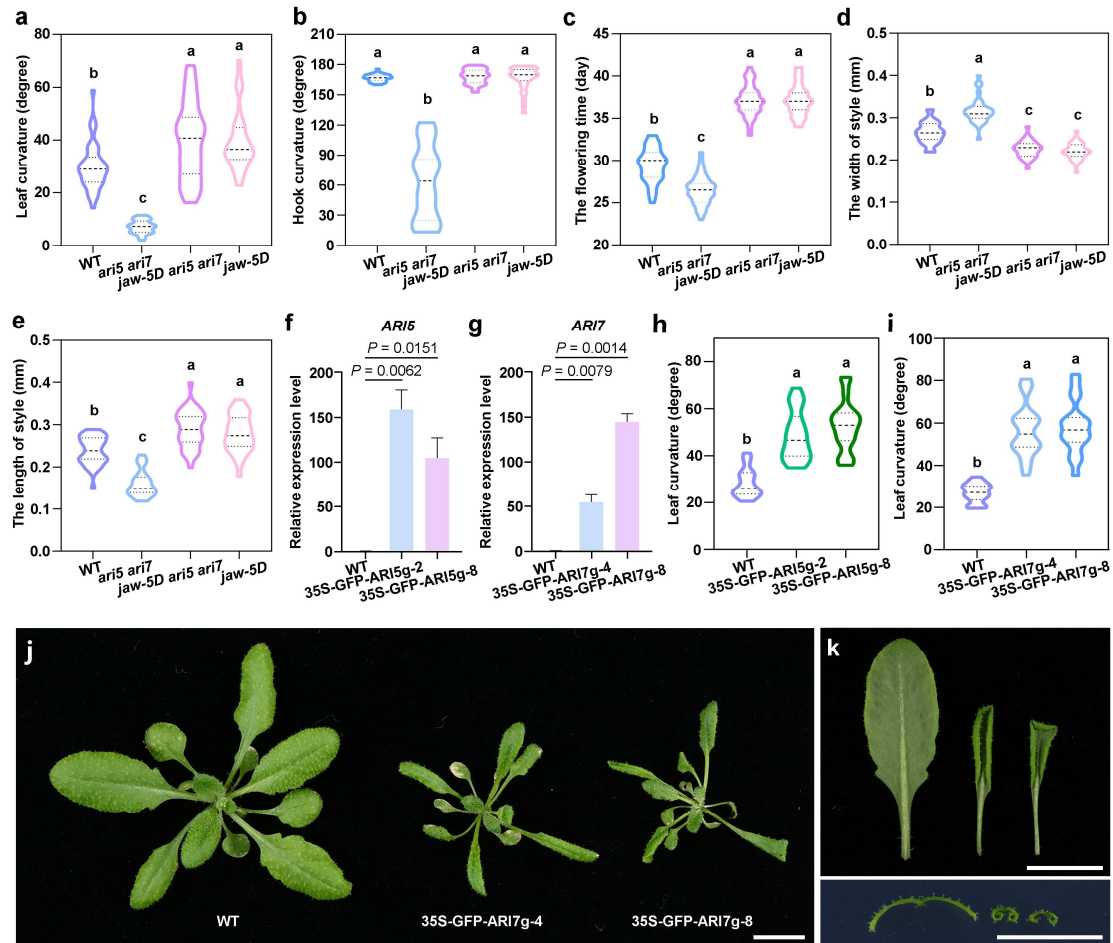


Supplementary Fig. 3. The mutations in *ari5 ari7* mutant.

A 2-bp frameshift deletion in exon 1 of *ARI5* introduces a premature termination codon in *ari5*. A 858-bp frameshift deletion spanning exon 1 and exon 4 of *ARI7* leads to premature termination in *ari7*.



Supplementary Fig. 4. Leaf rosette phenotype of 35S-GFP-ARI5 *ari5 ari7* plant.
a 21-d-old WT, *ari5 ari7* and 35S-GFP-ARI5 *ari5 ari7* plants. Bar = 1 cm. **b** Leaf Rosette diameter quantification in 21-d-old plants. Data are presented as mean $\pm$ SD (n = 40). Distinct lowercase letters indicate statistically significant differences (one-way ANOVA, $P < 0.05$). Violin plots display data distributions, with medians (bold lines) and quartiles (thin lines).



Supplementary Fig. 5. Statistical results and overexpression analysis of *ARI7*.

a The statistical analysis of leaf curvature in Fig. 7b. **b** The statistical analysis of apical hook curvature in Fig. 7c-f. **c** The statistical analysis of flowering time in Fig. 7k. **d,e** The statistical analysis of style width (**d**) and length (**e**) in Fig. 7g-j. **f,g** qRT-PCR quantification of *ARI5* (**f**) and *ARI7* (**g**) transcript levels in 35S-GFP-*ARI5g-2*, 35S-GFP-*ARI5g-8*, 35S-GFP-*ARI7g-4*, and 35S-GFP-*ARI7g-8*. The values were normalized to that in wild-type control (mean $\pm$ SD, $n=3$ biological replicates). One-way ANOVA analysis of variance was performed with LSD/Duncan pairwise comparison testing. **h** The statistical analysis of leaf curvature in Fig. 7m. **i** The statistical analysis of leaf curvature in (**k**). Distinct lowercase letters indicate statistically significant differences (one-way ANOVA, $P < 0.05$). Sample sizes: $n=40$ (**a**); $n=31$ (**b**); $n=30-34$ (**c**); $n=32$ (**d,e**); $n=20$ (**h**); $n=20$ (**i**). **j** 21-d-old WT, 35S-GFP-*ARI7g-4* and 35S-GFP-*ARI7g-8* are shown. **k** Top, from left to right, the sixth leaf of WT, 35S-GFP-*ARI7g-4* and 35S-GFP-*ARI7g-8*. Bottom, transverse sections from these leaves. Bars = 1 cm.

Supplementary Table 1. Candidate proteins interacting with TCP4 by IP-MS

AT number	Name	TCP4 PSMs	WT PSMs*
AT1G15750	TOPLESS (TPL)	14	0
AT1G80490	TOPLESS-RELATED 1 (TPR1)	14	1
AT3G16830	TOPLESS-RELATED 2 (TPR2)	7	0
AT5G27030	TOPLESS-RELATED 3 (TPR3)	2	0
AT3G18520	HISTONE DEACETYLASE 15 (HDA15)	13	2
AT5G60970	TCP5	2	0
AT5G25220	KNOTTED1-LIKE HOMEODOMAIN GENE 3 (KNAT3)	5	0
AT1G05890	ARIADNE 5 (ARI5)	2	0
AT2G20580	26S PROTEASOME REGULATORY SUBUNIT S2 1A (RPN1A)	15	1
AT4G28470	RPN1B	12	0
AT2G32730	RPN2A	8	0
AT1G04810	RPN2B	6	0

*PSMs (Peptide Spectrum Matches) values reflect the confidence level of peptide identifications from mass spectrometry data.

Supplementary Table 2. Primers used in this study.

Primers	Sequences 5'→3'
CRISPR/Cas9 sgRNA clone primers	
ARI5-sgRNA-v1-F	GATTG ATTATGATTATTACAGCGG
ARI5-sgRNA-v1-R	AAAC CCGCTGTAATAATCATAAT C
ARI5-sgRNA-v2-F	GATTG AAATTGTATCGGTTTCTT G
ARI5-sgRNA-v2-R	AAAC CAAGAAACCGATACAATT T C
ARI7-sgRNA-v1-F	GATTG GAGAGGATGATTTCTACAG
ARI7-sgRNA-v1-R	AAAC CTGTAGAAATCATCCTCT C C
ARI7-sgRNA-v2-F	GATTG TCCAGCATGTAGTGCAGAA
ARI7-sgRNA-v2-R	AAAC TTCTGCACTACATGCTGG A C
Genotype analysis	
CR-ARI5-F	ACCCTACTTTGTCCAGCGAG
CR-ARI5-R	GCATCTAACGATAATGGAGGG
CR-ARI7-F	TAATTTTGCAAGAAGAGGGG
CR-ARI7-R	TAAACGTAAGCTACCACTC
jaw-5D-F	TTCATCAGCCGTTACTGGAGACT
jaw-5D-R	GGATTTGGAAAGGTGGGTGTATT
DL3	GCTTTCGCCTATAAATACGACGG
Entry vector primers	
Linker1	GCCGCCCCCTTCACC
Linker2	GGCGCGCCCACCCTT
FC-ARI5-F	Linker1+ATGGATTCCGATGATGATATGCACG
FC-ARI5-Rsc	Linker2+TCAATTGCCATCTGGGTTTCTGCTGG
FC-ARI7-F	Linker1+ATGGATTCTGAAGAAGACATGCTCG
FC-ARI7-Rsc	Linker2+TTATAAGTTGTCATCTGGGTTTCTGC
ARI5-deR1-F	GAGAATTTACACCTGCTGCTATTGGTCGAGATATG
ARI5-deR1-R	CCAATAGCAGCAGGTGTAAATTCTCTGCCATCAG
ARI5-deR2-F	GCCAATTCAAAGCCTCTTAACGCATGGACAGAACAC
ARI5-deR2-R	CATGCGTTAAGAGGCTTTGAATTGGCAAGTATCC
Topo-M-F	GATGCATGGTTACTCACCAC
Topo-M-R	GTGGTGAGTAACCATGCATC

FC-ARI5pro-F	Linker1+CTAGGTTTCAAACATTAATATATTATAC
FC-ARI5pro-R	Linker2+GATCTTTAGATAAAGACTAGAGATTTAGCGG
FC-ARI7pro-F	Linker1+TTAATATAATTGTTTTGACGTAAAAAAAAAAAAACC
FC-ARI7pro-R	Linker2+GATCAGAGAAAACGAAATGGC
TOPO-F	AAGGGTGGGCGCGCCGACCCAGCTTTCTTGTAACA
TOPO-R	GGTGAAGGGGGCGGCCGCGGAGCCTGCTTTTTTTGT
Ubiquitination cascade in <i>E. coli</i> primers	
ACYCDuet-ARI5-F	GCCAGGGATCCATGGATTCCGATGATGATATGC
ACYCDuet-ARI5-R	GGCCTAACGTACATATTGCCATCTGGGTTTCTGC
ACYCDuet-F	GGCAATATGTACGTTAGGCCTGAGCA
ACYCDuet-R	ATCGGAATCCATGGATCCCTGGCTTGGTGATG
CDFDuet-TCP4-F	CAGAATTCGGAATGTCTGACGACCAATTCCA
CDFDuet-TCP4-R	GGCCTTTTAAACAGTATGGCGAGAAATAGAGGAAGC
CDFDuet-F	CTCGCCATACTGTAAAAGGCCTTACCC
CDFDuet-R	GGTCGTCAGACATTCCGAATTCTGAAATCCTTCCC