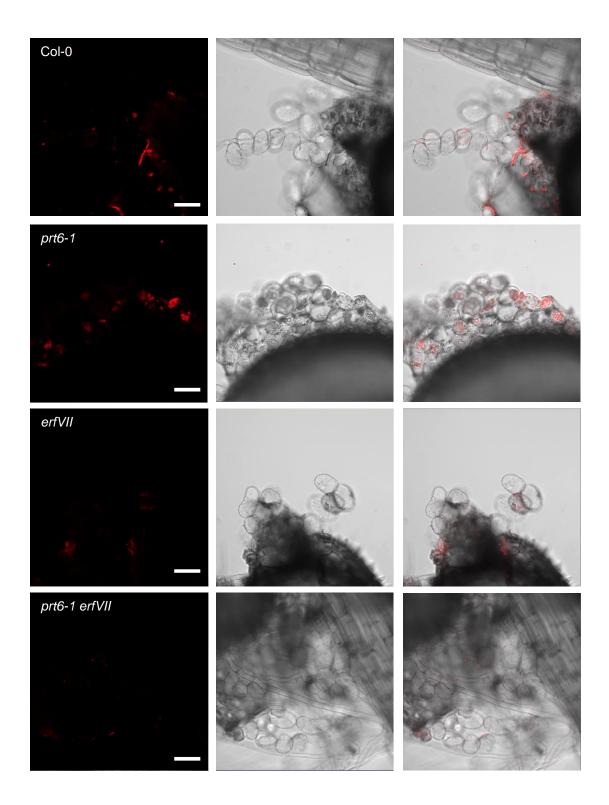
Supplementary Information

Genetic interactions between ABA signalling and the Arg/N-end rule pathway during Arabidopsis seedling establishment.

Hongtao Zhang¹, Lucy Gannon¹, Peter D. Jones^{2,3}, Chelsea A. Rundle¹, Kirsty L. Hassall⁴, Daniel J. Gibbs⁵, Michael J. Holdsworth², Frederica L. Theodoulou^{1,*}

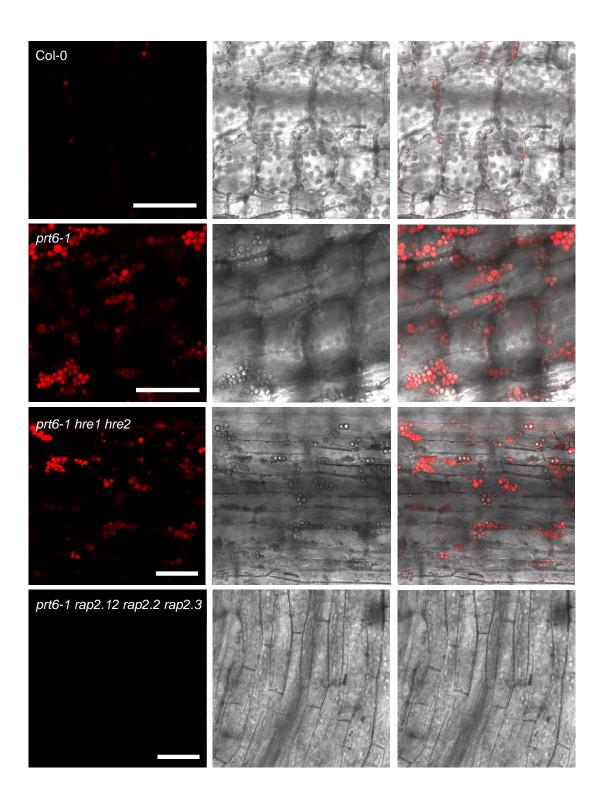
- 1. Plant Sciences Department, Rothamsted Research, Harpenden, AL5 2JQ, UK
- 2. School of Biosciences, University of Nottingham, Loughborough, LE12 5RD, UK
- Current address: Department of Cardiovascular Sciences, University of Leicester, LE3 7QP, UK
- Computational and Analytical Sciences Department, Rothamsted Research, Harpenden, AL5 2JQ, UK
- 5. School of Biosciences, University of Birmingham, Edgbaston, B15 2TT UK

* Corresponding author. Email: freddie.theodoulou@rothamsted.ac.uk



Supplementary Figure S1 Oil bodies are retained in the endosperm of *prt6* seedlings

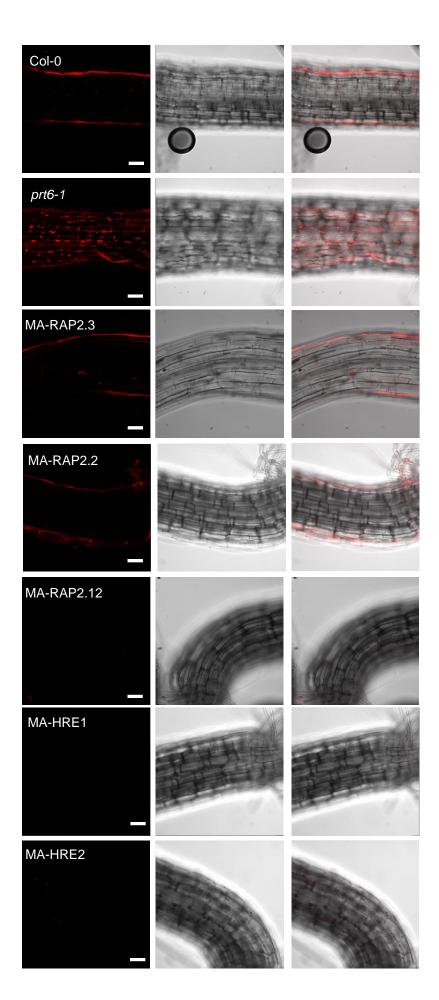
Endosperms and seed coats were dissected from 4 d old seedlings of *prt6-1* and *erfVII* combination mutants, stained with Nile Red and visualised by confocal microscopy. Left panel: Nile Red; middle panel, bright field; right panel, merge. Bar = $50 \mu m$.



Supplementary Figure S2. Oil body retention in light-grown *prt6* seedlings is dependent on RAP-type ERFVII transcription factors

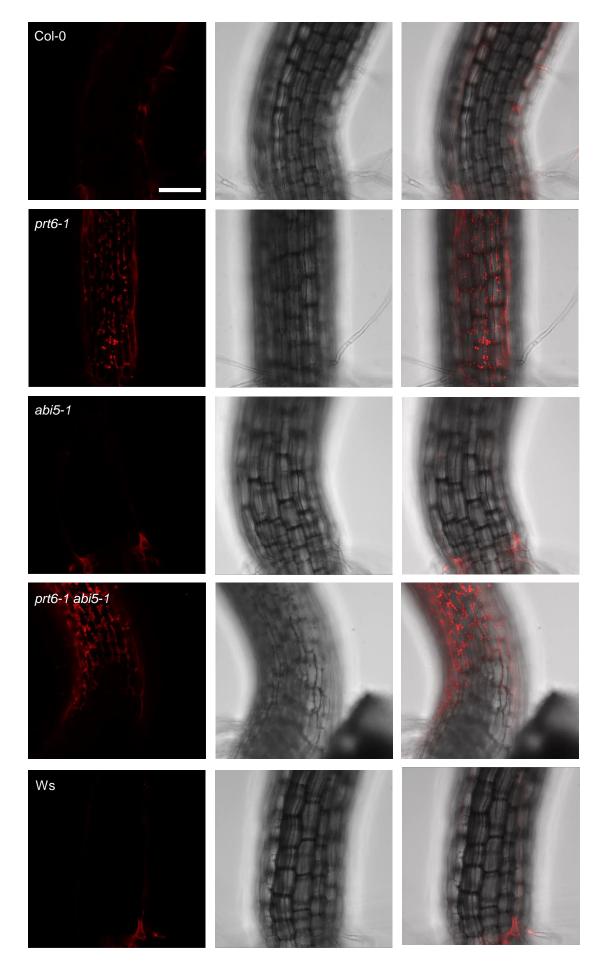
Hypocotyls of 5 d old, light-grown seedlings were stained with Nile Red and visualised by confocal microscopy. Left panel: Nile Red; middle panel, bright field, right panel, merge. Bar = $50 \mu m$.

Figure S3



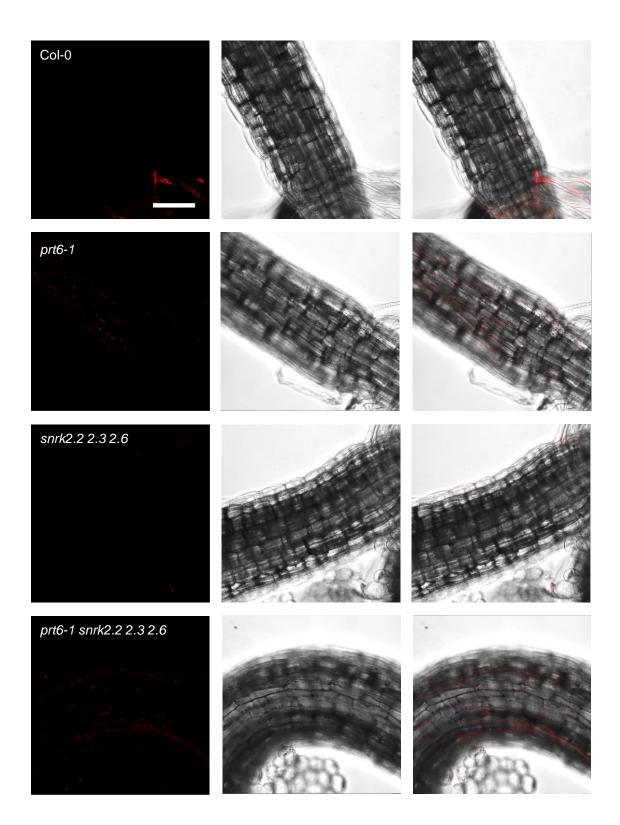
Supplementary Figure S3. Oil body phenotype of seedlings ectopically expressing stabilised ERFVII transcription factors

Hypocotyls of 5 d old, light-grown seedlings were stained with Nile Red and visualised by confocal microscopy. Results are representative of two independent transgenic lines for each construct. Left panel: Nile Red; middle panel, bright field, right panel, merge. Bar = $50 \mu m$.



Supplementary Figure S4. Oil body phenotype of *prt6-1 abi5-1* seedlings

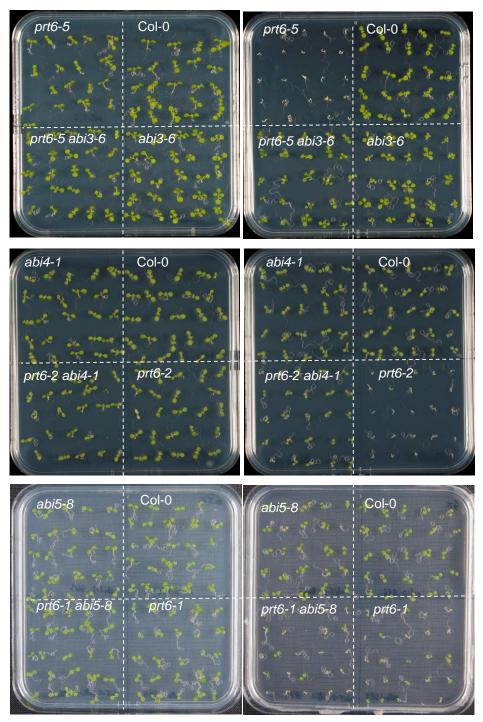
Hypocotyls of 5 d old, light-grown seedlings were stained with Nile Red and visualised by confocal microscopy. Left panel: Nile Red; middle panel, bright field, right panel, merge. Bar = $100 \mu m$.



Supplementary Figure S5. Oil body phenotype of *prt6-1 snrk2.2 2.3 2.6* seedlings

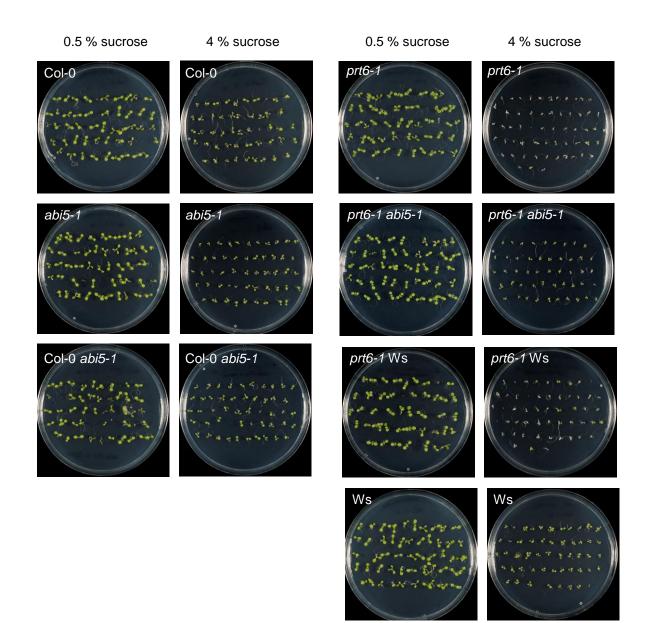
Hypocotyls of 5 d old, light-grown seedlings were stained with Nile Red and visualised by confocal microscopy. Left panel: Nile Red; middle panel, bright field, right panel, merge. Bar = $100 \mu m$.

0.5 x MS + 0.5 % sucrose



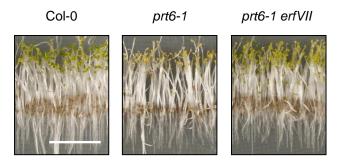
Supplementary Figure S6. Sugar sensitivity of *prt6* seedling establishment requires ABA signalling

Seeds of *prt6* and ABA signalling combination mutants were germinated on 0.5 x MS medium containing 0.5 % or 4 % sucrose. Seedlings were photographed after 5 d growth under long days.



Supplementary Figure S7. Sugar sensitivity of *prt6* seedling establishment requires *ABI5*

Seeds of *prt6-1* and *abi5-1* combination mutants were germinated on 0.5 x MS medium containing 0.5 % or 4 % sucrose. Seedlings were photographed after 5 d growth under long days.

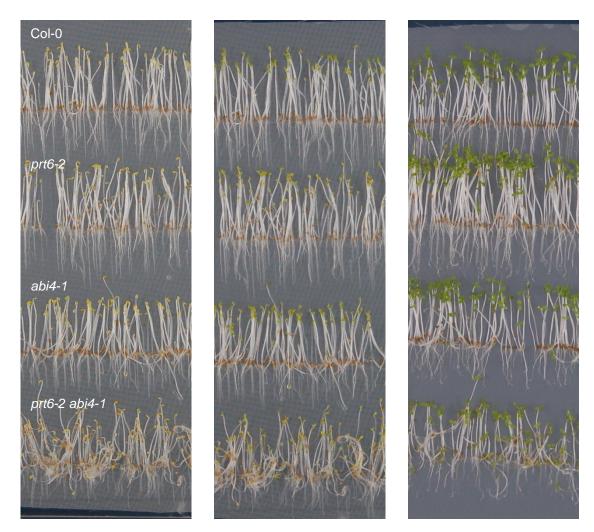


В

0 h

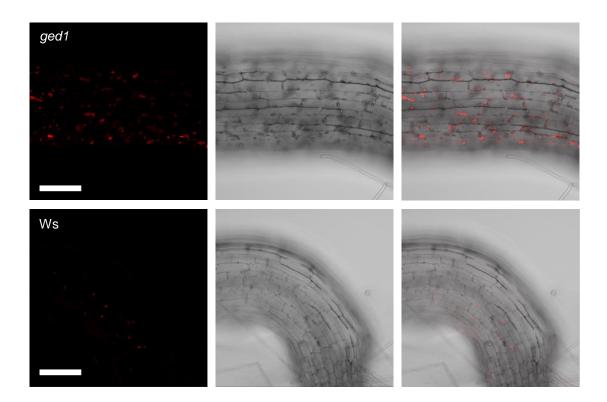
5 h

24 h



Supplementary Figure S8. Regreening of etiolated seedlings

A. Images of Col-0, *prt6-1*, and *prt6-1 erfVII* 6 h after transfer to light. Bar = 1 cm. B. Images of Col-0, *prt6-2*, *abi4-1* and *prt6-2 abi4-1* seedlings 0, 5 and 24 h after transfer to illumination.



Supplementary Figure S9. Oil body phenotype of *ged1* seedlings

Hypocotyls of 5 d old, light-grown seedlings were stained with Nile Red and visualised by confocal microscopy. Left panel: Nile Red; middle panel, bright field, right panel, merge.. Bar = $100 \ \mu m$

Table S1. Arabidopsis thaliana mutants discussed in this study

Allele	Accession	Mutagen	Comments	Ref
prt6-1	Col-0	T-DNA; SAIL_1278 _H11	Null	19
prt6-2	Col-0	T-DNA; GK270G04	Null	19
prt6-4	Ler	Fast neutron	Null. The <i>prt6-4</i> mutation is an insertion 497178 kb from the top of chromosome 5 into the <i>PRT6</i> (AT5G02310) gene.	19
prt6-5	Col-0	T-DNA; SALK_0510 88	Null	24
ged1	Ws	EMS	Null <i>prt6</i> allele. Chromosome exchange between two syntenic regions on chromosomes 3 and 5 containing 108 anchors (chromosome 3: At3g09300– At3g12320 and chromosome 5: At5g02100– At5g06980)	26
abi3-6	Col-0	Fast neutron	Null. The <i>abi3-6</i> mutation carries a 0.75 kb deletion within the region between position 1.078 and 1.944 of the <i>ABI3</i> gene. About one-third of the coding region of the <i>ABI3</i> gene is deleted.	61
abi4-1	Col-0	γ-irradiation	1 bp deletion (Δ G) in codon 157 leading to a frameshift. The resulting protein contains the presumed DNA binding and dimersiation domains but lacks the presumed activation domain and is inactive as a transcriptional activator in yeast [37].	33, 35
abi5-1	Ws	T-DNA	<i>abi5-1</i> contains a G-to-T substitution resulting in an early translation termination such that the mutant protein lacked 81 C-terminal amino acids, including the conserved basic and leucine zipper domains required for DNA binding and dimerization. The mutation does not co-segregate with the T-DNA.	36
abi5-8	Col-0	T-DNA; SALK_0131 63	Incomplete loss of function (less severe than <i>abi5-1</i>)	20

Table S2 Protein groups with increased abundance in the N-terminome of prt6etiolated seedlings that are known to be regulated by ABI4

Proteomics datasets from [21] were interrogated with a list of *ABI4* targets compiled from [39].

AGI code	Description
AT1G48130.1	1-cysteine peroxiredoxin 1
AT1G54870.1	NAD(P)-binding Rossmann-fold superfamily protein
AT2G28490.1	RmIC-like cupins superfamily protein
AT3G58450.1	Adenine nucleotide alpha hydrolases-like superfamily protein
AT3G15670.1	Late embryogenesis abundant protein (LEA) family protein

Table S3 Primers used in this study

Primer	Sequence 5'-3'
MA-RAP2.12_F	AAAAACGCGTATGGCTGGAGGAGCTATAATATC
RAP2.12_R	AAAGGATCCATGTGCGGAGGAGCTGTAAT
MA-RAP2.2_F	AAAAACGCGTATGGCTGGAGGAGCTATAATC
RAP2.2_R	AAATCTAGATCAAAAGTCTCCTTCCAGCAT
ABI3F	GCTGGCTCAGCTTCTGCTAT
ABI3R	AAATTCTTTTCTGGTTTCCATCC
ABI4F	CCGCTTCTTCTCCTTCCAC
ABI4R	GAGGGAGGAGGGTCTTAGGG
ABI5F	AACATGCATTGGCGGAGT
ABI5R	TTGTGCCCTTGACTTCAAACT
ACT2F	CCGCTCTTTCTTTCCAAGC
ACT2R	CCGGTACCATTGTCACACAC
TUB4F	GCTGAGAACAGCGATTGTCTT
TUB4R	AGTTCCCATTCCAGATCCAGT
abi3-6 F3	CTTGGGCTATATTGAGATCAG
abi3-6 R6	GGCCAATACATCCAGGTCCCT
abi4-1F1	CGAGATCCGAGAGCCACGTA
abi4-1R1	GGTCCCACCATTAAGGAAGA
abi5-1 LP avall	ACAGCTTTATGGTGTGTTTCAAGG
abi5-1 RP avall	CCATCTGAAGACACCGGGCTTAAC
abi5-8LP3	TCACCAGCTAGAAGCTCAACG
abi5-8RP3	GCTTGGGTCATCATTATATCCG
ged1 V2 prt6 LP	TTGACATGGAACCGGTAGCC
ged1 V2 prt6 RP	TGACCATTCCCTATAAGCCGG
ged1 V2 myb3r3 LP	CGTTTGTTTCCGTTTTGTTCCG
ged1 V2 myb3r3 RP	AGTCTCAACTCCAATGACGC