Supplementary data

EDL3 is an F-box protein involved in regulation of abscisic acid signalling in *Arabidopsis thaliana*

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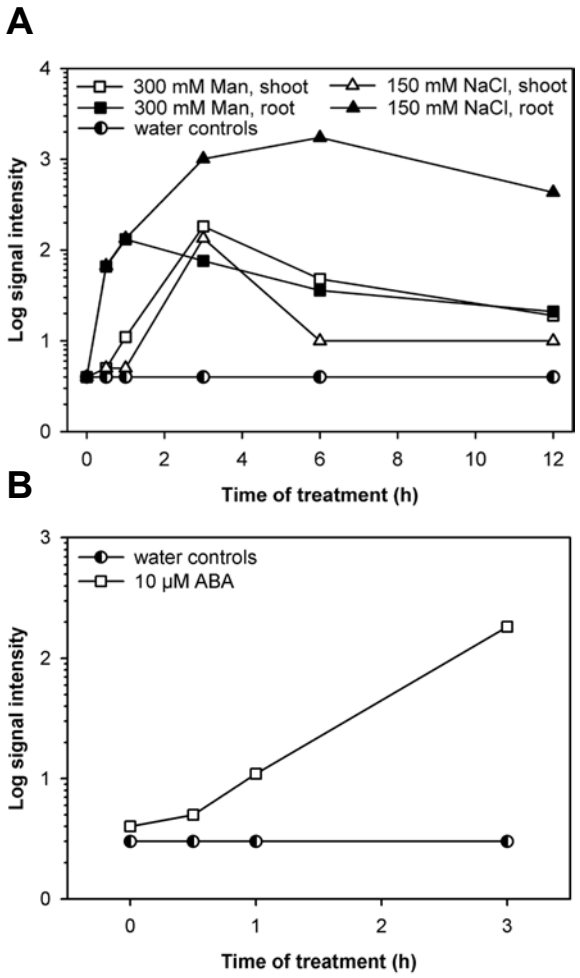

Fig. S1. Microarray data of *EDL3* transcript accumulation under osmotic stress, high salinity, and in the presence of exogenous ABA. (A) Increases in signal intensity under osmotic and salt stress conditions. Plants were grown on liquid medium and high concentrations of mannitol or salt were added to adjust osmotic or salt stress conditions, respectively. (B) Increases in signal intensity upon application of ABA. Plants were grown on liquid medium and ABA was added to a concentration of 10 µM. The AtGenExpress Visualization Tool (AVT; http://jsp.weigelworld.org/expviz/ expviz.jsp) was used to detect physiological conditions that induce strong increases in *EDL3* transcript levels. Absolute normalized values for signal intensities were downloaded from AVT to plot graphs. Data was extracted from AtGenExpress Abiostress experiments (A) and from AtGenExpress Hormone experiments (B).

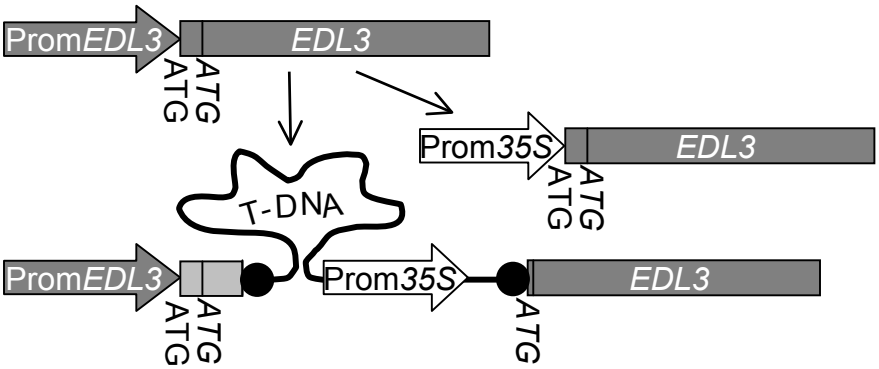

Fig. S2. Schematic overview of the *EDL3* wild type gene, the construct used to create overexpressor lines, and the T-DNA integration site in the *edl3-1* line. T-DNA integration created a duplication of a small part of the *EDL3* reading frame (light gray bar). Prom*EDL3*, endogenous promoter of *EDL3*; Prom*35S*, 35S promoter of the Cauliflower Mosaik virus.

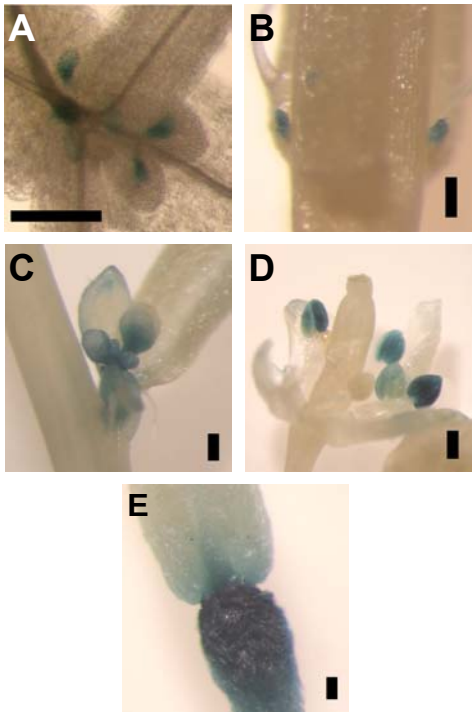

Fig. S3. Analysis of *EDL3* promoter activity during *Arabidopsis* development. Plants were grown in a phytochamber under 16 h light / 8 h dark cycles and harvested for histochemical GUS analysis. (A) Histochemical analysis of Pro_{EDL3}-GUS activity in stipules of developing leaves in one-week-old seedlings and at the base of bracts in three-week-old plants (B). Pro_{EDL3}-GUS activity in young floral buds (C), flowers close to anthesis (D), and flowers after abscission of sepals and petals (E). Bars, 0.1 mm in supplemental figure 2A and 0.5 mm in all other sub-figures.

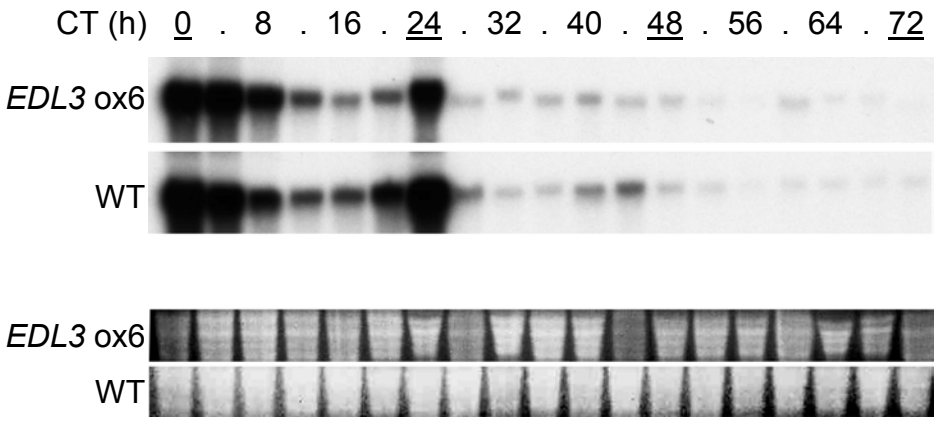

Fig. S4. Circadian rhythm of *LHCB1* transcript accumulation after transfer to continuous darkness. *EDL3* ox6 and wild type seedlings were grown under 12 h light / dark cycles for four days and were then transferred to continuous darkness. Seedlings were harvested every four hours for 72 h and total RNA was isolated. *LHCB1* transcript levels were measured by hybridization of the corresponding DNA probe to blots of 5 µg of total RNA. Fluorescent signals of rRNA stained with ethidium bromide are shown as a loading control.

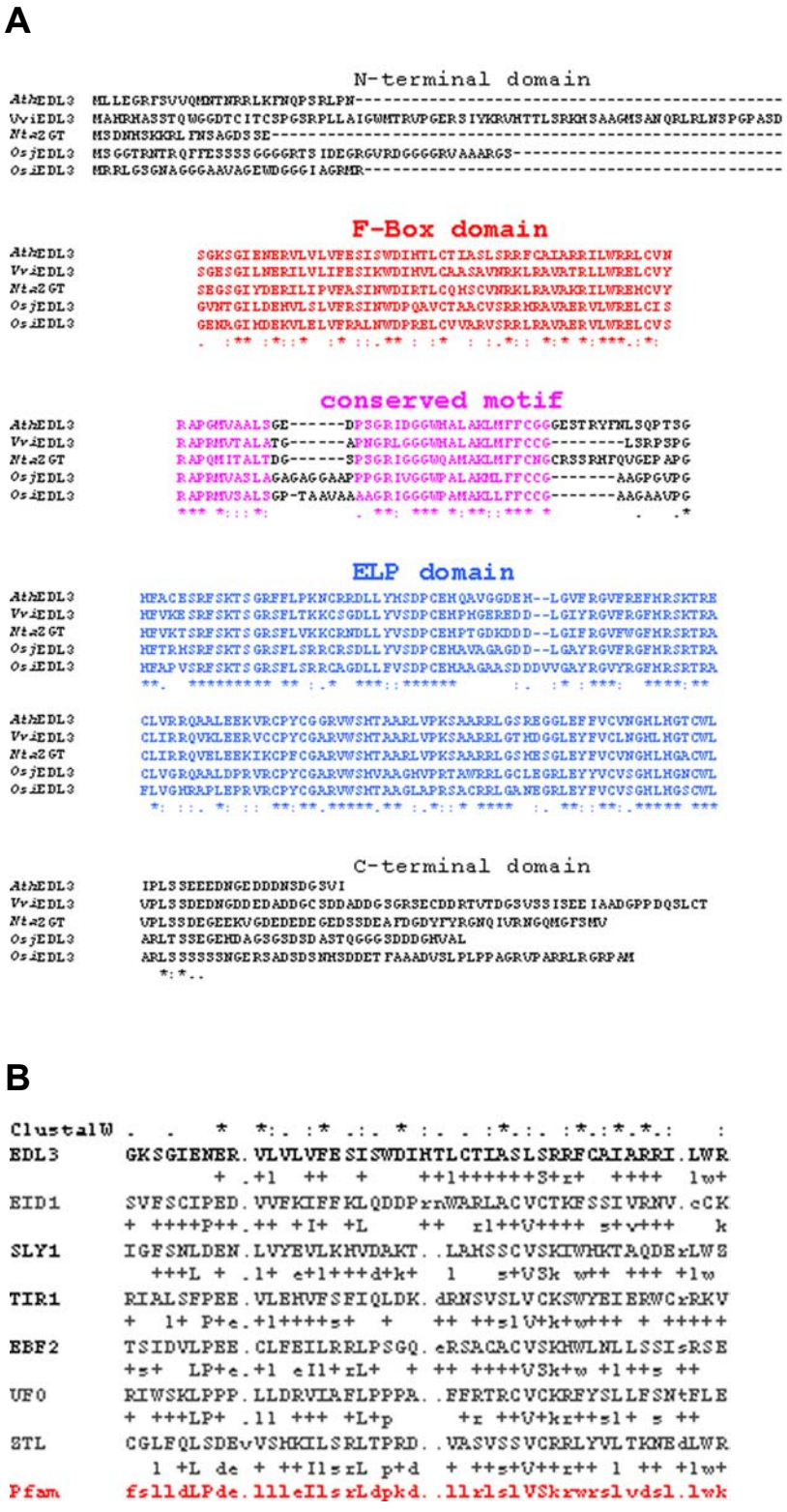

Fig. S5. Domain structure of EDL3 proteins. (A) Alignment of EDL3 proteins from different plants species. Conserved domains are visualized by different colours. ELP domain, conserved domain in EID1-like Proteins. (B) Sequence comparison of F-box domains. The putative EDL3 F-box domain was compared to the respective domain of different approved Arabidopsis F-box proteins and the Pfam consensus sequence. Sequences homologous to *Arabidopsis* EDL3 were isolated from databases using the protein blast program tool at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/). The following protein sequences were selected for further analyses: *Vitis vinifera*, CAO22496; *Nicotiana tabacum*, AAK56924; *Oryza sativa* cv. Japonica, BAF06461; *Oryza sativa* cv. ndica, EAY98541. Alignments were done using ClustalW version 1.82 at the European Bioinformatics Institute (http://www.ebi.ac.uk/clustalw/index.html#) and were manually adjusted as needed. Pfam (http://pfam.janelia.org/) was utilized to search for protein motifs. Default values were used to run all programs. *, identical residue; :, highly conserved residues; ., conserved residues; *Ath*, *Arabidopsis thaliana*; *Vvi*, *Vitis vinifera*; *Nta*, *Nicotiana tabacum*; *Osj*, *Oryza sativa* cv. Japonica; *Osi*, *Oryza sativa* cv. Indica.

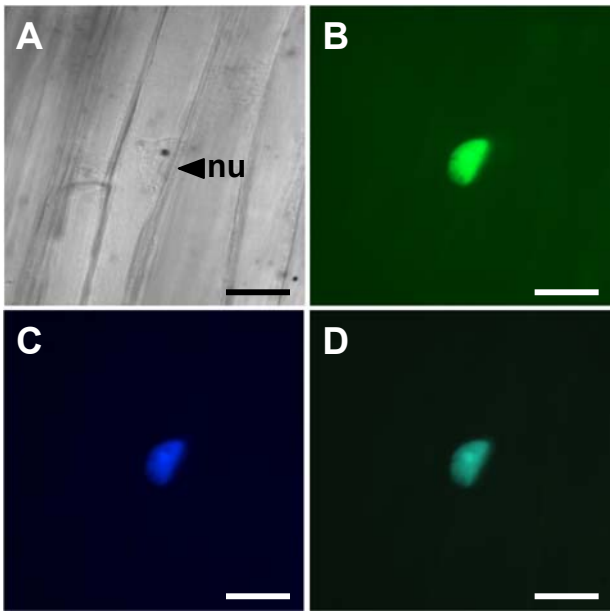

Fig. S6. Sub-cellular localisation of EDL3-YFP in cells of transfected mustard seedlings. (A) DIC (differential interference contrast) image of the nucleus in the co-transfected mustard hypocotyl cell shown in figures 5B to C. (B) YFP signals obtained from the co-transfected cell. The YFP-specific filter set shows the signal from the introduced EDL3-YFP fusion protein. (C) CFP signals obtained from the co-transfected cell. The CFP-specific filter set shows the signal from the CPRF2-CFP transfection control that is localized to the nucleus of transfected mustard cells. (D) Merged picture of YFP and CFP signals. Images of the YFP- and CFP-specific filter sets were combined to visualize co-localisation of EDL3-YFP and CPRF2-CFP fusion proteins. Bar, 40 µm.

Table S1. Primers used in this study

Name	Sequence 5' 3'	Purpose
EDL3-F	AAG GAT CCA AAT GCT TCT AGA GGG AAG ATT CTC	cloning of cDNA fragment
EDL3-R	CAC CTC GAG ATT AGA TCA CAC TTC CGT CAC	cloning of cDNA fragment
EDL3DT	AAG AGC TCG GAT CAC ACT TCC GTC ACT GTT GTC GTC	cloning of cDNA fragment, removel of stop codon
PEDL3-F	GCA GTG CCT AGG GTT TTC GGA G	cloning of promoter fragment
PEDL3-R	GTG GAT CCT CTG GAC GAC GGA GAA TCT TCC	cloning of promoter fragment
LBb1	GCG TGG ACC GCT TGC TGC ACC T	analysis of T-DNA insertion
ROK2_RB1	TCC AGA AAC CCG CGG CTG AG	analysis of T-DNA insertion
EDL3_SALK_5'	TGG ACG GAC CTT TTG GGA GCT T	analysis of T-DNA insertion
EDL3_SALK_3'	GCT CGC ACG GAT CAC TCA TGT	analysis of T-DNA insertion
ACTIN1_Realt_F	GGC TCC AAG CAG CAT GAA G	qRT-PCR, *ACTIN1*
ACTIN1_Realt_R	ACC CTC CAA TCC AGA CAG AGT ATT	qRT-PCR, *ACTIN1*
ACTIN1_Probe	*JOE*-CAA AGT CGT TGC CCC TCC AGA GAG G-*BHQ1*	qRT-PCR, TaqMan probe, *ACTIN1*
EDL3_Realt_F2	AGA GTT CTT CGT GTG CGT GAA C	qRT-PCR, *EDL3*
EDL3_Realt_R2	CCT CCG ATG AAA CGG GAA T	qRT-PCR, *EDL3*
EDL3_Probe	*FAM*-CAC TTG CAC GGC ACT TGC TGG C-*BHQ1*	qRT-PCR, TaqMan probe, *EDL3*
ABI1qPCR-F	CGG TGG TTG CCG TTG TTT	qRT-PCR, *ABI1*
ABI1qPCR-R	GGC TCT AGA GTC ACC GCA GTT AG	qRT-PCR, *ABI1*
ABI1_Probe	*FAM-CGT CTC ACA TCT TCG TC-BHQ1*	qRT-PCR, TaqMan probe, *ABI1*
ACTIN-F	CAT CAG GAA GGA CTT GTA CGG	semi-quantitative RT-PCR, *ACTIN1*
ACTIN-R	GAT GGA CTT GAC TCG TCA TAC	semi-quantitative RT-PCR, *ACTIN1*
EDL3RT-F	CCA TTA GCT GGG ACA TCC ACA CGC	semi-quantitative RT-PCR, *EDL3*
EDL3RT-R	TCG CAC GGA TCG CTC ATG TAT AGG	semi-quantitative RT-PCR, *EDL3*
CAB4-F	CCT GAT TAT CTC CCG GCA AGT CTT GC	semi-quantitative RT-PCR, *CAB4*
CAB4-R	ACA TCG CCA ACC TCC CGT TTG C	semi-quantitative RT-PCR, *CAB4*
CO-F	GTC AAC ACC AAC AAA ACT GCA GCG	semi-quantitative RT-PCR, *CONSTANS*
CO-R	CTT GCT CCT CGG CTT CGA TTT CTC	semi-quantitative RT-PCR, *CONSTANS*