Supporting Information: Methods S1–S6 and supporting References

Methods S1 Quantitative colocalizations

For colocalization purposes pinhole sizes for green (488/505 nm excitation/emission) and red (561/572 nm excitation/emission) channels were matched, and the optical section thickness in cases of axial acquisitions of defined imaging depths, was optimized according to Nyquist criteria as automatically set by Zeiss Zen 2012 software (Carl Zeiss, Jena, Germany). Such images were exported as 16-bit, uncompressed TIF files without adjustments. Cropping and reorientations were done with Microsoft Powerpoint with no intensity or contrast manipulation. Photomultiplier (PMT) gain/offset adjustments for separate channels were made during live imaging using the range indicator mode, in order to avoid over- or under-exposures. For achieving quantitative interpretations of colocalizations, preparations of Ler, *yda1* and *ΔNyda1* were conducted simultaneously using the same solutions for fixations, washings, aldehyde quenching, enzyme digestions, permeabilization, blocking and antibody incubations. During microscopic documentation of such simultaneously prepared samples, PMT settings and emission spectrum cut-offs for Alexa Fluor 488 and Alexa Fluor 546 were adjusted only once to ensure comparable CLSM image acquisitions of similarly prepared root wholemounts. Colocalization analyses between MAP65-1 and microtubules was done strictly on single plane confocal sections using either the colocalization tool of Zen 2012 software or the JACoP plugin (Bolte & Cordelières, 2006) of Image J (http://rsbweb.nih.gov/ij/). Both approaches yield Pearson's and Mander's coefficients and intensity-corrected scatter plots and for most cases Zen 2012 was sufficient to deem colocalization. In both cases the iterative Costes approach (Costes *et al.*, 2004) was automatically implemented for background thresholds.

Methods S2 Chemicals

Common chemicals were all purchased from Sigma, Merck and Karl Roth. Styryl dyes (FM4-64 and FM 4-64 FX) were from Invitrogen (Life Technologies Czech Republic s.r.o., Prague, Czech Republic). Primary antibodies used for whole mount immunofluorescence localizations and western blot analyses included: rat anti-alpha tubulin monoclonal antibody (mAb, clone YOL1/34, Abcam, Cambridge, United Kingdom), mouse anti-alpha tubulin mAb (clone DM1-a, Sigma or Abcam), mouse monoclonal anti-actin antibody (clone 10-B3), rabbit

polyclonal sera against MPK3 and MPK6 (all from Sigma, Sigma-Aldrich spol. s.r.o., Prague, Czech Republic). Rabbit polyclonal affinity purified serum against the phosphorylated TEY motif of mammalian ERK1 and ERK2 (anti-pTEpY) was from Cell Signaling (Cell Signaling Technology, Biotech A.S., Prague, Czech Republic). Rabbit polyclonal anti-MAP65-1 serum was a kind gift from Dr. Andrei Smertenko (Durham University). Alexa Fluor 488 and 546 secondary anti-rat and anti-rabbit antibodies were from Invitrogen, while horseradish peroxidase conjugated anti-rabbit and anti-mouse IgGs were from Santa Cruz Biotechnology (Exbio, Vestec, Czech Republic). Indole-3-acetic acid (IAA) was from Duchefa Biochemie (BioTech a.s., Prague, Czech Republic) and auxinole (α-[2,4-dimethylphenylethyl-2-oxo]-IAA) was kindly provided by Prof. Ken-ichiro Hayashi (Okayama University of Science, Okayama, Japan).

Methods S3 Root morphometry and phenotyping

Phenotyping of Ler, *yda* and *ΔNyda* roots was carried out under a Leica MZ FLIII stereo microscope operated by Leica software (Leica Microsystems, Wetzlar, Germany) and under a light microscope Zeiss Axio Imager M2 equipped with DIC optics (Carl Zeiss, Jena, Germany). For root tissue patterning studies, roots of Ler, *yda1* and *ΔNyda1* were labeled with 4 μM aqueous solutions of the red fluorescing styryl dye FM4-64, or alternatively, roots were fixed, embedded in epoxy resin, semi-thin sectioned and stained with 1% w⁄v toluidine blue in 1% w⁄v aqueous borax as described by Beck *et al.* (2010). Semithin sections were examined and documented with a Leica DFC290 camera coupled to a Leica DMRBE upright microscope (Leica Microsystems, Wetzlar, Germany) operated through the IRFANVIEW freeware (http://www.irfanview.de). Length measurements used thereon were carried out with freeware image analysis software Image J (http://rsbweb.nih.gov/ij/).

Methods S4 Visualization of stomata

Chlorophyll from leaves of Ler, *yda1* and *ΔNyda1* plants was extracted with a mixture of ethanol and acetic acid (1:1) and subsequently, leaves were fixed on slides with clearing solution (Weijers et al. 2001) containing chloral hydrate, water and glycerol (8:3:1). Samples were observed under a light microscope Zeiss Axio Imager M2 equipped with DIC optics (Carl Zeiss, Jena, Germany).

Methods S5 Quantitative analysis of transcript levels by quantitative PCR

Total RNA from 14-day old seedlings of Ler, *yda1* and *ΔNyda1* was isolated from liquid nitrogen powders of the respective tissues using TRI Reagent® RNA Isolation Reagent (Sigma-Aldrich, spol. s.r.o., Prague, Czech Republic) according to manufacturer´s protocol. RNA concentration and purity was determined before DNase I digestion with NanoDrop Lite (Thermo Scientific, Waltham, USA). Template-primer mix for reverse transcription was composed of 0.5 µl oligo-dT primers (0.25 µg per reaction), 125 ng RNA and H_2O (PCR-grade) in total volume of 10 µl. The mixture was denatured at 70°C for 10 min. Following components were added: 4 µl M-MLV Reverse Transcriptase 5x reaction buffer (Promega, Madison WI, USA), 1 µl deoxynucleotide mix (10 mM each), 0.4 µl (16 units) RNasin® Plus RNase inhibitor (Promega, spol. s.r.o., Prague, Czech Republic), 0.4 µl (40 units) M-MLV Reverse Transcriptase (Promega) and H_2O (PCR-grade) in total volume of 20 µl. PCR reactions were performed under the following conditions: 42°C for 4 h and finally 70°C for 10 min for inactivation of reverse transcriptase. qRT-PCR reactions were performed in 96-well plate with StepOnePlus Real-Time PCR system (Applied Biosystems, Foster City, USA) using SYBR® Green to monitor dsDNA synthesis. Reaction contained 5 µl *Power* SYBR® Green PCR Master Mix (Life Technologies, Carlsbad, USA), 2.5 µl cDNA and 2.5 µl gene-specific primers (0.5 µM). The following standard thermal profile was used for all PCR reactions: 95°C for 10 min, 40 cycles of 95°C for 15 s, and 60°C for 1 min. Experiments were run in three biological replicates and the intra-assay variability was determined with technical triplicates. The expression data were normalized to the expression of *EF1α* (elongation factor 1-alpha) as a reference gene and relative gene expression was calculated by $2^{(\text{-delta delta Cq})}$ method. To avoid amplification of genomic DNA targets, primers (Supplementary Table S1) were designed to span exon-exon junction. Specificity of the target amplification was further verified by melting curve analysis of reaction products.

Methods S6 Proteomic analysis

Fourteen days old Ler, *yda1* and *ΔNyda1* seedlings (in three independent biological replicates) were used for proteomic analysis. The preparation of trypsin-digested extracts was performed as described by Takáč *et al*. (2011) with some modifications. Briefly, proteins were extracted by phenol extraction followed by successive methanolic ammonium acetate and acetone precipitation. The precipitates were dissolved in 6 M urea and in total 10 µg of protein was

reduced and alkylated with dithiothreitol and iodoacetamide, respectively. Proteins were digested with 5 µl of trypsin (0.1 µg $µl^{-1}$) at 37 °C overnight. Digestion was stopped by the addition of 4 µl of 1% (v/v) formic acid. The peptides were desalted using C18 extraction disks (3MEmpore) as described by Rappsilber *et al*. (2007).

The 2-D LC-MS/MS analysis was conducted on a nanoAcquity 2D-UPLC system directly coupled to Xevo G2-S Q-TOF tandem mass spectrometer (Waters, Milford, MA, USA). Peptides were first separated on an XBridge PST C18 NanoEase Column (5 µm particles, 300 µm X 50 mm) using ACN steps of 10.8%, 20.4% and 65% ACN in 20 mM Ammonium Formate (pH 10). Each fraction was eluted onto a Symmetry C18 nanoACQUITY Trap Column (5 µm particles, 180 µm X 20 mm) at a flow rate of 2 µl min^{-1}. Peptides in each ACN step were then separated on an ACQUITY UPLC PST C18 nanoACQUITY Column (1.7 µm particles, 75 µm X 100 mm) with a 70 minutes long gradient from 3% to 40% of ACN (in 0.1% v/v formic acid) at a flow rate of 0.45 µl min^{-1}. MS and MS/MS data were acquired in MSE mode (low collision energy, 6 eV; high collision energy ramping from 14 eV to 40 eV), with Glu-fibrinopeptide (1 pmol $µl^{-1}$, flow rate 0.3 µl min^{-1}, 785.8425 Da [M+2H]2+) infused every 60s as a lock mass.

Three independent biological samples were processed and analyzed in triplicates with ProteinLynx Global Server (PLGS, version 2.5.2, Waters). Mass accuracy was corrected with the lock mass, and fragment ions were assigned to precursors based on similar retention times. The processed data were searched against the NCBI Arabidopsis genus taxonomy referenced protein database (31,856 entries as of December 2011) along with the reversed decoy database, using the following settings: FDR 1%; maximum of 2 trypsin missed cleavages; precursor tolerance 10 ppm; fragment tolerance 20 ppm. Quantitative analysis of identified proteins (confidence > 95%) was based on ion peak intensities observed at low collision energy mode, carried out with the Expression Analysis function in PLGS. Auto-normalization in regards to total ion intensities was performed. Only the proteins with ANOVA $p \leq 0.05$ were reported as differentially expressed.

Supporting References

Bolte S, Cordelières FP. 2006. A guided tour into subcellular colocalization analysis in light microscopy. *Journal of Microscopy* **224:** 213-232.

Costes SV, Daelemans D, Cho EH, Dobbin Z, Pavlakis G, Lockett S. 2004. Automatic and quantitative measurement of protein-protein colocalization in live cells. *Biophysical Journal* **86:** 3993-4003.

Weijers D, Franke-van Dijk M, Vencken RJ, Quint A, Hooykaas P, Offringa R. 2001. An Arabidopsis Minute-like phenotype caused by a semi-dominant mutation in a RIBOSOMAL PROTEIN S5 gene. *Development* **128:** 4289-4299.

Rappsilber J, Mann M, Ishihama Y. 2007. Protocol for micro-purification, enrichment, pre-fractionation and storage of peptides for proteomics using StageTips. *Nature Protocols* **2:** 1896–1906.

Takač T, Pechan T, Šamaj J. 2011. Differential proteomics of plant development. *Journal of Proteomics* **74:** 577–588.