

Fig. S1 Stomatal pattern in Ler, *yda1*, $\Delta Nyda1$ and mpk6-4+MPK6AEF. (a) Stomata (arrows) in Ler (wild type) formed according to one-cell spacing rule, (b) stomatal clusters (ellipses) visible in *yda1*, whereas only epidermal cells with no stomata observed in $\Delta Nyda1$ (c). (d) Stomatal phenotype of mpk6-4+MPK6AEF exhibiting stomatal clusters very similar to *yda1*. Bars, 20µm.



Fig. S2 Phenotype of 14 days old plants of Ler, *yda1* and $\Delta Nyda1$ used for IAA content analysis. (a) Representative phenotype of Ler, *yda1* and $\Delta Nyda1$ plants. (b) Graph depicting mean (± S.D.) of primary root length of Ler (N=18), *yda1* (N=13) and $\Delta Nyda1$ (N=15). (c) Graph depiction of lateral root density (number of lateral roots per 1cm of root branching zone) of Ler (N=18), *yda1* (N=13) and $\Delta Nyda1$ (N=13) and $\Delta Nyda1$ (N=13) and $\Delta Nyda1$ (N=13). (c) Graph depiction of lateral root density (number of lateral roots per 1cm of root branching zone) of Ler (N=18), *yda1* (N=13) and $\Delta Nyda1$ (N=15). *, p < 0.05; ***, p < 0.001. Bar, 1cm.



Fig. S3 Root hair emergence with respect to root tip in Ler (a), *yda1* (b) and $\Delta Nyda1$ (c). Note earlier root hair emergence in *yda1* (b) and $\Delta Nyda1$ (c). Brackets roughly denote the distance between root tip and root hair appearance which was used for measurements of 8 days old plants depicted in (d) comparing distance from root tip to root hair zone among Ler (N=30), *yda1* (N=30) and $\Delta Nyda1$ (N=30). ***, p<0.001. Bar, (a,b) 500 µm; (c) 200 µm.



- Carbon fixation in photosynthetic organisms (61 proteins)
- Glyoxylate and dicarboxylate metabolism (29 proteins)
- Glycolysis / Gluconeogenesis (18 proteins)
- Metabolic pathways (13 proteins)
- Citrate cycle (TCA cycle) (11 proteins)
- Photosynthesis (10 proteins)
- Pyruvate metabolism (10 proteins)
- Nitrogen metabolism (8 proteins)
- Cyanoamino acid metabolism (7 proteins)
- Fructose and mannose metabolism (7 proteins)
- Pentose phosphate pathway (6 proteins)
- Biosynthesis of secondary metabolites (5 proteins)
- Glutathione metabolism (5 proteins)
- Tyrosine metabolism (5 proteins)
- Cysteine and methionine metabolism (5 proteins)
- Oxidative phosphorylation (5 proteins)

Fig. S4 Classification of differentially regulated proteins detected by comparative proteomic analysis of Arabidopsis *yda1* mutant and wild type (Ler) according to KEGG annotations.



Metabolic pathways (56 proteins)

Fig. S5. Classification of differentially regulated proteins detected by comparative proteomic analysis of Arabidopsis $\Delta Nyda1$ mutant and wild type (Ler) according to KEGG annotations.



- Carbon fixation in photosynthetic organisms (54 proteins)
- Glycolysis / Gluconeogenesis (32 proteins)
- Metabolic pathways (17 proteins)
- Glyoxylate and dicarboxylate metabolism (14 proteins)
- Biosynthesis of secondary metabolites (10 proteins)
- Citrate cycle (TCA cycle) (8 proteins)
- Cyanoamino acid metabolism (7 proteins)
- Fructose and mannose metabolism (6 proteins)
- Pentose phosphate pathway (6 proteins)
- Pyruvate metabolism (6 proteins)
- Photosynthesis (6 proteins)
- One carbon pool by folate (5 proteins)
- Protein processing in endoplasmic reticulum (5 proteins)

Fig. S6 Classification of differentially regulated proteins detected by comparative proteomic analysis of Arabidopsis *yda1* and $\Delta Nyda1$ mutants according to KEGG annotations.



Fig. S7 Semithin sections stained with toluidine blue showing root tips of wild type Ler (a, b), *yda1* (c, d) and $\Delta Nyda1$ (e, f) mutant roots. Note, that regular alignments of cell files are disturbed in mutant roots showing disoriented and ectopic cell divisions, especially in the area of stem cell niche (circles in a, c, e and details in b, d, f). Bars, (a,c,e) 30 µm, (b,d,f) 10µm.



Fig. S8 Phenotype of 4 days old *mpk6-4+MPK6AEF* seedlings. (a, b) Development of primary roots in Col-0 (a) and *mpk6-4+MPK6AEF* (b). *mpk6-4+MPK6AEF* show thick and short primary roots in comparison to Col-0 plants. (c-f) Root cell architecture in *mpk6-4+MPK6AEF* seedlings stained with FM4-64 styryl dye. Surface (c, e) and central (d, f) optical sections of *mpk6-4+MPK6AEF* primary roots. Boxed area (c) and higher magnification (e) show disturbances in the cell division planes. Oblique cell division planes are marked with arrow (e). Root tips of *mpk6-4+MPK6AEF* showed defect organization of the stem cell niche (d, f). Bars, (a) 1 mm; (b) 500 µm; (c, d) 50 µm and (e, f) 20 µm.



Fig. S9 Identification of multinucleate and binucleate cells in *yda1* main root epidermis after staining of plasma membrane with fixable FM 4-64 FX styryl dye and DNA counterstaining with DAPI (a). Asterisks mark the nuclei and arrowheads show the incomplete cell walls. Two adjacent binucleate main root epidermal cells of *yda1* immunostained against tubulin (MT, green) and counterstained for DNA (DAPI, red) as visualized by CLSM (b) and DIC (c). One of them has residual phragmoplast (arrowhead) while in none is a cell plate discernible. Nuclei are marked with asterisks. (d, e) Visualization of incomplete cytokineses in living lateral root epidermal cells of *yda1* with DIC optics. In (d) a single nucleus (asterisk) is protruding between two compartments defined by incomplete cell wall stubs (arrowheads). In (e) arrowhead points to incomplete cell wall stub in binucleate cell (asterisks mark nuclei). Bars, 10 μm.



Fig. S10 Overview of mitotic and cytokinetic microtubule arrays of *yda1* primary root wholemounts immunostained for tubulin (green) and counterstained with the DNA stain DAPI (blue). (a, b) Clustering of mitotic stages in primary roots of *yda1* mutant as shown by the occurrence of multiple phragmoplasts (arrows in a and b) together with preprophase bands (PPBs, outlined arrowheads in b) and a mitotic spindle (full arrowhead in b). Bars, 10 μ m.



Fig. S11 Quantitative analysis of transcript levels of *MPK3* and *MPK6* genes in extracts from Ler, *yda1* and *ΔNyda1* plants. Graph depiction (mean \pm S.D.) of *MPK3* and *MPK6* transcript levels quantified by qPCR and normalized against *EF1* α transcripts. *, p < 0.05; ***, p < 0.001.



Fig. S12 Quantitative analysis of transcript level of *YODA* gene in extracts from 14 days old plants of Ler, *yda1* and $\Delta Nyda1$. Graph depiction (mean \pm S.D.) of *YODA* transcript level quantified by qPCR and normalized against *EF1* α transcripts. qPCR was performed with three independent biological samples in technical triplicates. *, p < 0.05; ***, p < 0.001.



Fig. S13 Quantitative analysis of MPK3 and MPK6 protein level of 14 days old Ler plants. (a) Representative western blot from Ler extract probed with antibodies against MPK3 and MPK6 with respective loading control by using of stain-free gel (b). (c) Bar chart represented band optical densities (mean \pm S.D.; averaged from 3 biological repeats) from three independent western blots. *, p<0.05.



Fig. S14 Phenotypes of Col-0, *mpk6-2* mutant and *mpk6-2* mutant rescued with *proMPK6::GFP:MPK6* construct. Arrested development of primary root in *mpk6-2* mutant was rescued by expression of GFP-tagged MPK6 protein driven by its own promoter. All seedlings were 5 days old. Bar, 1 cm.



Fig. S15 Immunofluorescent colocalization of MPK3 and microtubules in preprophase bands (PPBs) and phragmoplasts of Ler, *yda1* and $\Delta Nyda1$ cells. (a-c) PPBs (brackets in both panels) are devoid of MPK3 immunofluorescence in all three cases. (d) Accumulation of MPK3 signal within a phragmoplast of a Ler cytokinetic cell (arrows in both panels) and in the vicinity of the nuclei (arrowheads in MPK3 panel). (e) Faint enrichment of MPK3 localization on phragmoplast structure (arrows in both panels) in *yda1* cytokinetic cell. (f) Weak MPK3 labeling of the phragmoplast in epidermal cytokinetic cells of $\Delta Nyda1$ (arrow in both panels). Bars, 10µm.



Fig. S16 Scatter plot (a) demonstrating colocalization between cortical microtubules and MAP65-1 in a root epidermal cell of Ler outlined in (b). Horizontal axis represents green channel (tubulin) and vertical axis represents red channel (MAP65-1). Crossed hairline (a) corresponds to threshold setting according to Costes *et al.*, 2004 as explained in Materials and Methods section. For red channel, threshold values coincided with y-axis so a hairline is not visible. Therefore colocalization occurs above horizontal hairline and right to the y-axis. Bar, 10 μ m.



Fig. S17 Scatter plot (a) demonstrating colocalization between PPB and MAP65-1 in a root epidermal preprophase cell of Ler outlined in (b) as shown in Figure 9b. Horizontal axis represents green channel (tubulin) and vertical axis represents red channel (MAP65-1). Crossed hairlines represent threshold setting according to Costes *et al.*, 2004 as explained in Materials and Methods sections. Colocalization occurs in the top right quadrant. Bar, 10 μm.



Fig. S18 Scatter plot (a) demonstrating colocalization between microtubules and MAP65-1 in the phragmoplast of a root epidermal cytokinetic cell of Ler outlined in (b). Horizontal axis represents green channel (tubulin) and vertical axis represents red channel (MAP65-1). Crossed hairline (a) corresponds to threshold setting according to Costes *et al.*, 2004 as explained in Materials and Methods section. For red channel, threshold values coincided with y-axis so a hairline is not visible. Therefore colocalization occurs above horizontal hairline and right to the y-axis. Bar, 10 µm.



Fig. S19 Scatter plot (a) demonstrating colocalization between cortical microtubules and MAP65-1 in the outlined root epidermal cell of *yda1* (b) presented in Figure 9d. Horizontal axis represents green channel (tubulin) and vertical axis represents red channel (MAP65-1). Crossed hairline (a) corresponds to threshold setting according to Costes *et al.*, 2004 as explained in Materials and Methods section. For red channel, threshold values coincided with y-axis so a hairline is not visible. Therefore colocalization occurs above horizontal hairline and right to the y-axis. Bar, 10 μ m.



Fig. S20 Scatter plot (a) demonstrating colocalization between microtubules and MAP65-1 in the PPB of a root epidermal preprophase cell of *yda1* outlined in (b). Horizontal axis represents green channel (tubulin) and vertical axis represents red channel (MAP65-1). Crossed hairlines represent threshold setting according to Costes *et al.*, 2004 as explained in Materials and Methods sections. Colocalization occurs in the top right quadrant. Bar, 20 μ m.



Fig. S21 Scatter plot (a) demonstrating colocalization between microtubules and MAP65-1 in the phragmoplast of a root epidermal cytokinetic cell of *yda1* outlined in (b) selected from Figure 9f. Horizontal axis represents green channel (tubulin) and vertical axis represents red channel (MAP65-1). Crossed hairline (a) corresponds to threshold setting according to Costes *et al.*, 2004 as explained in Materials and Methods section. For red channel, threshold values coincided with y-axis so a hairline is not visible. Therefore colocalization occurs above horizontal hairline and right to the y-axis. Bar, 20 µm.



Fig. S22 Scatter plot (a) demonstrating colocalization between cortical microtubules and MAP65-1 in a root epidermal cell of $\Delta Nyda1$ outlined in (b). Horizontal axis represents green channel (tubulin) and vertical axis represents red channel (MAP65-1). Crossed hairlines represent threshold setting according to Costes *et al.*, 2004 as explained in Materials and Methods sections. Colocalization occurs in the top right quadrant. Bar, 10 µm.



Fig. S23 Scatter plot (a) demonstrating colocalization between microtubules and MAP65-1 in the PPB of a root epidermal preprophase cell of $\Delta Nyda1$ outlined in (b). Horizontal axis represents green channel (tubulin) and vertical axis represents red channel (MAP65-1). Crossed hairlines represent threshold setting according to Costes *et al.*, 2004 as explained in Materials and Methods sections. Colocalization occurs in the top right quadrant. Bar, 10 µm.



Fig. S24 Scatter plot (a) demonstrating colocalization between microtubules and MAP65-1 in the phragmoplast of a root epidermal cell of $\Delta Nyda1$ outlined in (b) and depicted in Figure 9i. Horizontal axis represents green channel (tubulin) and vertical axis represents red channel (MAP65-1). Crossed hairlines represent threshold setting according to Costes *et al.*, 2004 as explained in Materials and Methods sections. Colocalization occurs in the top right quadrant. Bar, 10 µm.