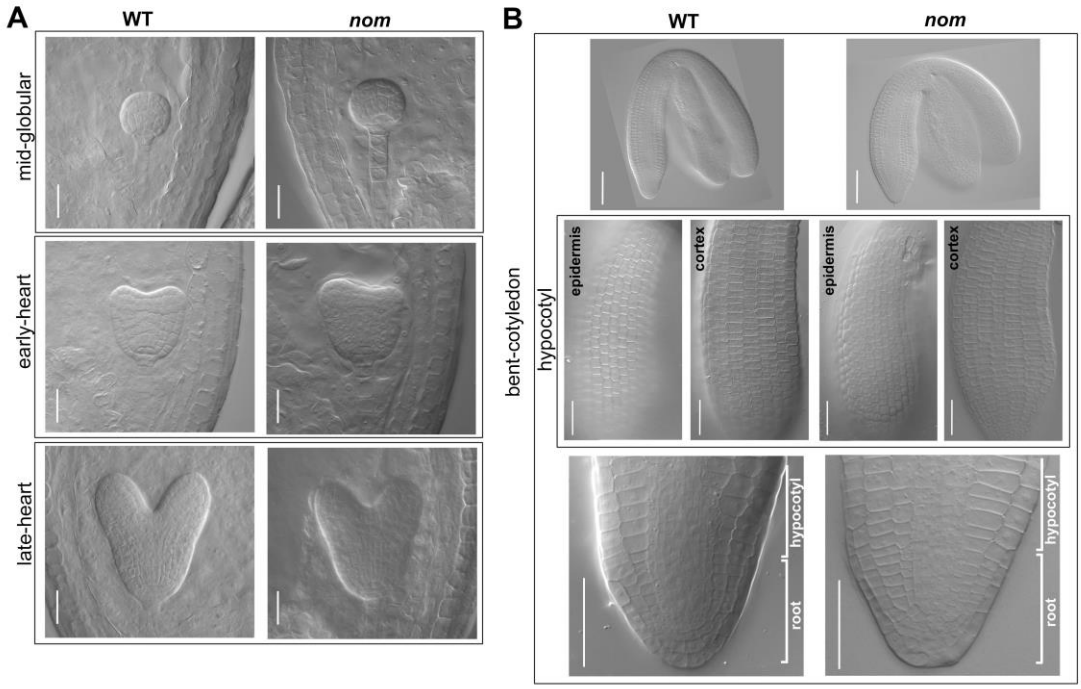

Figure S1. Division patterns along the root-hypocotyl axis are normal in *nom* embryos. Differential interference contrast (DIC) images of WT and *nom* embryos at different stages of development, stages were classified according to Jurgens, G. and Mayer, U. (Jurgens and Mayer, 1994), in (A) scale bars, 25 µm; in (B) at the top, whole seedlings, scale bars, 100 µm; in the centre, longitudinal view of the hypocotyl epidermis and cortex, note epidermal division patterns are unaltered in *nom*, scale bars, 50 µm; at the bottom, close ups of the root tips; scale bars, 50 µm.

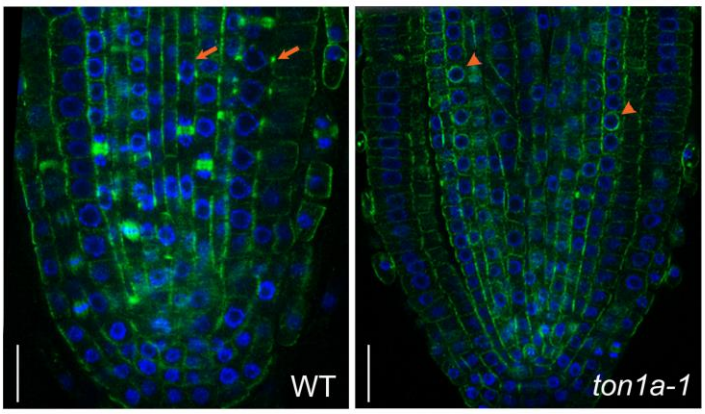

Figure S2. In the *ton1a-1* mutant, cells do not form PPBs. Confocal longitudinal, single sections in the meristematic root tips of 4 dpg WT and *ton1a-1* seedlings immunostained with α-tubulin (green) and counterstained with DAPI (blue). PPB in the cortex and vasculature of WT (orange arrows); abundant MT surrounding the nucleus in the endodermis and pericycle of *ton1a-1* (orange arrowheads). Scale bars, 25 μm.

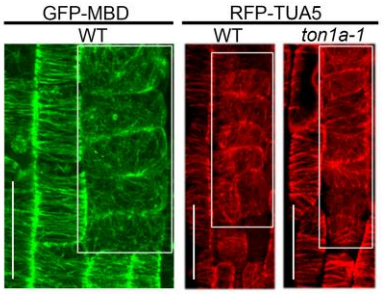

Figure S3. In root meristematic epidermal cells, interphase microtubules are not organised. Representative images of maximum intensity projections of confocal optical sections in the meristematic root epidermis of the microtubule marker line GFP-MBD (microtubule binding domain of MAP4) expressed in WT and of the microtubule marker line RFP-TUA5 expressed in WT and *ton1a-1* seeedlings. Note that epidermal cells (framed within white outlines) are present in the same projections as lateral root cap cells, which surround the epidermal tissue in the meristematic zone. In epidermal cells, microtubules are not organised, whilst in lateral root cap, microtubules have a prevalent transverse orientation; scale bars, 25 μm (for RFP-TUA5 WT n= 12 seedlings, *ton1a-1* n= 10 seedlings).

A

position (bp)	name	type	forward primer	reverse primer	in-del	TAIR accession
18001597	ALS	CAPS	GGCAACACATGTTCTTGGTG	ATCACAGGACAAGTCCCTCG		1945563
20017437	dc2	dCAPS	GTGCCGGAATCTCTAACCTG	GTGGTCTACACTAATTTATCACGAAT		
20358561	ind 1	INDEL	CGTATCACGAACCCCAAGAG	TGAATCACGGAAACATTCCA	TAGTAGAC/-	
20376570	ind 2	INDEL	CTTTCCGATCTTGATCTTCGTTAT	TCTCTCTCTCTCTCGTTTTTGACA	GAGAGAGG/-	
20387455	PUR5	CAPS	AAACCTTTCACTCCTCCTTTTTC	GATGTAGACCTTGCTGAAAA		1945637
20494897	ind 5	INDEL	TCACATGAAAAGTGTCAAACAACAC	GTCGTATGCAGTAGTTTTATTTCCA	CTAAA/-	
20762869	CIW20	SSLP	CATCGGCCTGAGTCAACT	CACCATAGCTTCTTCCTTTCTT		3433031
23168372	NGA112	SSLP	CTCTCCACCTCCTCCAGTACC	TAATCACGTGTATGCAGCTGC		1945516

B

ex1to8f	5'-ATGGATCTCAAGACTTTGGTCACT-3'
ex1to8r	5'-TCAATCTCTCCCTTCTTCTTCATT-3'
05ex1to7f	5'-TGATGGATCTCAAGACCTTAGTCA-3'
05ex1to7r	5'-CTTTCCCTTCTTCTTCTTCACTTG-3'
ex2-3to7f	5'-GCTTCTCCTTCAGGAAGGTTACTA-3'
ex2-3to7r	5'-TAGACAACCCGGCTCTTCTATC-3'
EF1a-f	5'-ATGCCCCAGGACATCGTGATT-3'
EF1a-r	5'-TTGGCGGCACCCTTAGCTGGA-3'
CL001f	5'-ATTGGTGAAGAAAAGGAGTTTCAA-3'
CL001r	5'-TGACGGATCAGATCAAAATAACAG-3'
Site2-1f	5'-AAGCTTCATGCTTCTCCTCCAG-3'
Site2-1r	5'-TCCACGAATCTTTTGGCTTT-3'
8474	5'-ATAATAACGCTGCGGACATCTACATTTT-3'
NF09	5'-GAAGCTGAAATGTTTATCCAAAGC-3'
3144	5'-GTGGATTGATGTGATATCTCC-3'
DC48	5'-CAACTTGCTTTGTTTTCATTTCAA-3'

Supplementary Table S1. Sequences of primer pairs. (A) Primer pairs used for mapping and their genomic position on chromosome III. (B) Primer pairs used in cloning, genotyping and RT-PCR experiments.

Supplementary Materials and Methods

Mutagenesis

The *nom* mutant was generated by ethyl methanesulfonate (EMS) mutagenesis of the *GL2::GUS* line (WS ecotype). 0.48 g of dried seeds were imbibed and vernalised in water for four days and treated in 0.3% (v/v) EMS for 8 hours, thoroughly rinsed with water and sown on soil. Pools of 4 day-post-germination (dpg) M2 seedlings were pre-screened for those seedlings with normal root growth that, under the compound microscope, displayed defects in the orientation of cell division in the root meristematic epidermis. Candidate pools were re-screened by cutting off the primary root tip and analysing the phenotype of the meristematic epidermis, once identified the tips with mutant phenotypes, their corresponding seedlings were traced back and moved onto soil.

Segregation analysis

Upon outcrossing *nom* to Col-0, all F1 seedlings were normal and displayed a wild type (WT) organisation of the root meristematic epidermis. The F1 plants were left to self–fertilise and the meristematic epidermis of 539 F2 seedlings was analysed, 430 displayed a WT phenotype and 109 a *nom* phenotype, so 20.22% displayed the mutant phenotype. These values are slightly lower than the 25% expected for a recessive mutation, as the chi test on our sample gives a χ^2=6.31, which exceeds for a p=0.05 that of χ^2 =3.84 and they are likely due to mis-scoring under the compound microscope as the mutant phenotype is subtle. In fact, we did not observe embryo lethality or failure of the seedlings to germinate and in the subsequent phenotypic analyses with the confocal microscope of genotyped, homozygous *nom/ton1a-1* mutant meristems we always observed the *nom* phenotype behaving as fully penetrant and we can confidently conclude that the *nom* phenotype results from a recessive mutation.

Mapping and complementation

Homozygous *nom* plants in the WS background were crossed to ecotype Col-0. In the F2 *nom* homozygous mutant seedlings were selected for their meristematic epidermal phenotype under the compound microscope and their DNA was isolated. We initially mapped *nom* to chromosome 3, between the markers ALS and NGA112; the other markers tested, which were evenly distributed through the five chromosomes, gave recombinant frequencies close to 50%, indicating independent segregation. We then fine mapped the mutation to a 10,885bp interval by screening 884 F2 seedlings

displaying the *nom* mutant phenotype using a PCR-based approach with SSLP, dCAPS and Indel markers selected from the TAIR collection and designed using TAIR (http://www.arabidopsis.org) and the EnsemblPlants (http://plants.ensembl.org) for essential genome information. Primer pairs sequences can be found in Supplementary Table S1.We identified one recombinant for the ind2 marker and one recombinant for the PUR5 marker and we then comparatively sequenced candidate genes in this interval from the parental transgenic *GL2::GUS* line and the *nom* allele. Two point mutations were identified within the sequence of the *TON1a* gene in *nom*, one inside intron 1 and one in the canonical GT acceptor splice site of intron 2 (Fig. 2A).

We confirmed the mapping results by complementation and expressed, in the *nom* mutant background, a 5.6Kb genomic construct encompassing the entire *TON1a* gene and including 3Kb upstream the start and 1Kb downstream the stop codons of *TON1a*. About 120 T2 seedling roots for each of 12 independent BASTA resistant T1 transformants were examined and found to segregate WT roots with no defects in cell division orientation in the root meristematic epidermis. Root tips of T2 populations were phenotyped under the compound microscope to detect and score plants with *nom* and WT phenotype meristematic epidermis. To further verify the complementation, seedlings from a chosen segregating T2 line where subjected to confocal analysis (Fig. 2B, C) and genotyped to ascertain that those root tips with a WT phenotype were *nom* homozygous mutant at the endogenous locus.

Plant vectors and transformation

The construct to complement the *nom* mutation was generated by amplifying 5.6Kb of genomic DNA (Col-0 ecotype) from JAty 76F24 clone with primers CL001F+R using Phusion Taq (Thermo Scientific). The genomic fragment was subcloned into pGEM-T (Promega), released with NotI and cloned into the binary vector pMLBART/pW3. The pMLBART/pW3 carries the bialophos resistance gene (BAR) that encodes the phosphinotricin acetyl transferase and confers to transformed plants resistance to the herbicide ammonium-glufosinate, commercially known as BASTA (Bayer). Constructs were confirmed by sequencing. The Agrobacterium strain GV3101 was used to transform homozygous *nom* mutant plants by the floral dip method (Clough and Bent, 1998).

Microscopy

Z-series of root meristems stained with Schiff-PI method were collected at intervals of 0.4-0.5 µm with an objective HC PL APO 20x/0.70 IMM CORR CS at a 400Hz scan speed on Bidirectional mode. Seedlings from GFP expressing lines were counterstained with 0.1-0.5 mg/ml propidium iodide (PI) solution (Sigma-Aldrich). Confocal images of seedlings expressing RFP-TUA5 were collected at intervals of 0.3-0.5 µm with HCX PL APO 40x/1.25-0.75 oil CS or HCK PL APO 63x/1.20 W CORR CS objectives, Airy set on 1 and scan speed between 200-400Hz. For double-stained samples, z-series were always collected in sequential mode.

Images of resin embedded and sectioned root meristem, prepared according to Dolan et al. (Dolan et al., 1993), were captured with a Leica DM 6000 microscope.

Differential interference contrast (DIC) images of embryos cleared with a chloral hydrate solution (glycerol, chloral hydrate and water in a ratio of 1:8:3), were captured with a Leica DM 6000 microscope.

Marker gene analysis and PCR based genotyping

The T-DNA insertion lines were confirmed by PCR-genotyping and sequencing. Transgenic marker lines in the *ton1a-1* mutant background were generated by genetic crossing. Homozygous seedlings for *ton1a-1* and expressing the transgene markers were identified from segregating F2 population based on marker fluorescence, phenotype and genotype, then reconfirmed in the F3 generation and analysed. WT siblings were used as controls in all the experiments.

The *ton1a-1* mutant allele was genotyped using primers Site2-1F and Site2-1R that generate a dCAP marker with BstNI enzyme (CCWGG) that cuts the WT but not the *ton1a-1* allele. The *ton1a-2* allele was genotyped with the primer pair 3144 and DC48 and the *ton1a-3* allele with the primer pair 8474 and NF09. Primer sequences can be found in Supplementary Table S1B.

Expression analysis

RNA was extracted using the RNeasy Plant mini kit (Qiagen), and treated with DNnase I (Roche) to remove DNA contamination. cDNA was obtained and amplified with the different primer pairs with the One Step RT-PCR kit (Qiagen). For each sample, 350 ng of total RNA were used for 35 cycles in a single step RT-PCR reaction.

Immunolabelling

Seedlings were fixed for 1 hour in 4% (w/v) paraformaldehyde prepared in PEM (50mM Pipes, 10mM EGTA, 5mM MgSo4, pH 7) supplemented with 0.05% Triton X-100. For the first 30 minutes seedlings were fixed under mild vacuum. After fixation seedlings were rinsed four times in PEM for 30 minutes, transferred onto slides with wide shallow wells and digested for 15 minutes at room temperature in a cell wall enzyme mix of 1% driselase from *Basidiomycetes* sp. (Sigma-Aldrich), 2% cellulase from *Trichoderma viride* (Sigma-Aldrich), 0.1% pectolyase from *Aspergillus japonicus* (Sigma-Aldrich) in PEM, rinsed again four times in PEM for 30 minutes. Samples were further permeabilized for 40 minutes in PEM containing 3% IGEPAL (Sigma-Aldrich) and 10% DMSO, rinsed for 15 minutes in PEM and 15 minutes in PBS (137mM NaCl, 2.7mM KCl, 7mM Na_2HPO_4, 3mM NaH_2PO_4). An incubation of one hour in a blocking solution of 3%BSA, 0.05% Trition X100 in PBS, was followed by O/N incubation at 4°C with monoclonal anti-α-tubulin antibody produced in mouse, clone B-5-1-2 (Sigma-Aldrich Cat. No. T5168), and diluted 1:150 in blocking solution. Seedlings were rinsed six times in freshly made blocking solution for 1 hour and incubated for 2 hours with a secondary antibody Alexa Fluor 488 goat anti-mouse IgG (Invitrogen Cat. N. A-11001) diluted 1:300 in blocking solution, then rinsed three times in blocking solution and three times in PBS for a total of 1 hour. Samples were counterstained with 1 µg/ml of 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) in H_2O, washed twice in H_2O and mounted in Vectashield (Vector Laboratories), after carefully removing excess water.

References

Clough, S. J. and Bent, A. F. (1998). Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. *Plant J* **16**, 735-43.

Dolan, L., Janmaat, K., Willemsen, V., Linstead, P., Poethig, S., Roberts, K. and Scheres, B. (1993). Cellular organisation of the Arabidopsis thaliana root. *Development* **119**, 71-84.

Jurgens, G. and Mayer, U. (1994). Arabidopsis. *In EMBRYOS. Colour atlas of development (ed. J. Brand). London: Wolfe publications.*, 7-21.