Т

Supplemental material

JCB

Zhou et al., http://www.jcb.org/cgi/content/full/jcb.201108098/DC1

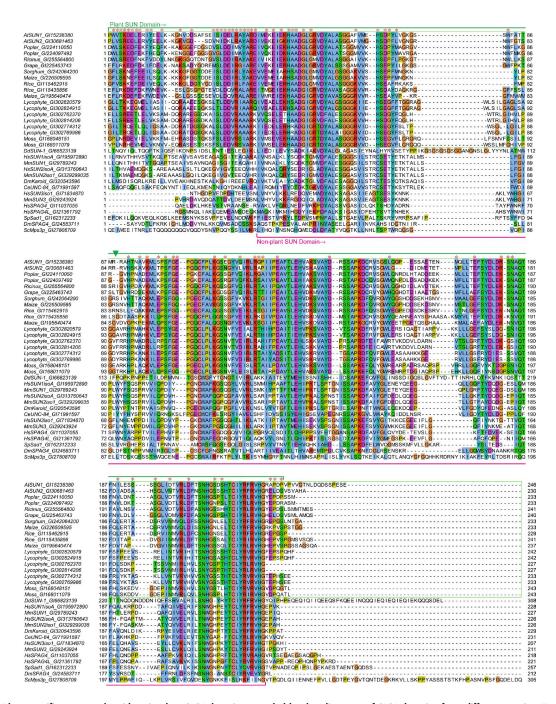


Figure S1. Plant-specific conserved residues in plant SUN domains revealed by the alignment of SUN domains from different species. To identify plant SUN proteins, the protein sequence of AtSUN1 (At5g04990) was used for a BLAST search against the green plant (taxid 33090) nonredundant protein sequence database (National Center for Biotechnology Information). Protein sequences showing high similarities to the SUN domain region and an expect value <0.0001 were selected. Sequences of SUN proteins immediately after the predicted CCD were aligned using the European Molecular Biology Laboratory–European Bioinformatics Institute ClustalW2 program. Plant SUN domains are identified by the species common name followed by the National Center for Biotechnology Information GenInfo Identifier number: Poplar, Populus trichocarpa; Ricinus, Ricinus communis; Grape, Vitis vinifera; Sorghum, Sorghum bicolor; Maize, Zea mays; Rice, Oryza sativa; Lycophyte, Selaginella moellendorffii; Moss, Physcomitrella patens. Nonplant SUN proteins are identified by two-letter abbreviations of species names followed by the National Center for Biotechnology Information GenInfo Identifier number: Dd, Dictyostelium discoideum; Hs, Homo sapiens; Mm, Mus musculus; Dm, Drosophila melanogaster; Ce, Caenorhabditis elegans; Sp, Schizosaccharomyces pombe; Sc, Saccharomyces cerevisiae. The alignment was assigned with the ClustalX color scheme using Jalview. The plant SUN domains were group colored in Jalview (indicated by a light green frame), which highlights the plant-specific residues (denoted by red asterisks on top of the alignment). The proposed range of plant SUN domains is indicated by the bottom red lines. The dark green arrowhead denotes the break point of the Arabidopsis NSUN and CSUN domains used in this study.

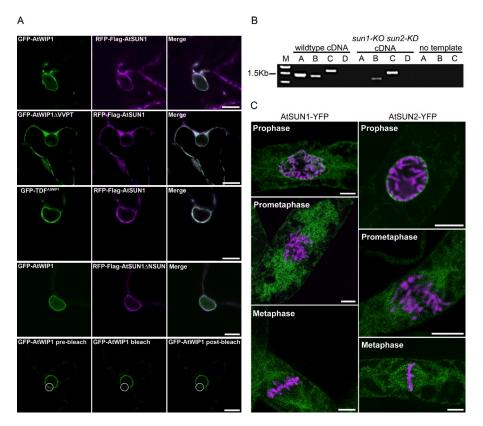


Figure S2. AtWIP1 and AtSUN localization and characterization of sun1-KO sun2-KD. (A) RFP-Flag-AtSUN1 was coexpressed in N. benthamiana leaves with GFP-AtWIP1 (first row), GFP-AtWIP1 \(VVPT \) (second row), and GFP-TDFAtWIP1 (third row). RFP-Flag-AtSUN1 \(\Delta NSUN \) \(\was as coexpressed with GFP-AtWIP1 \) (fourth row). These samples were used for FRAP assay (as illustrated in the bottom row; for results, see Fig. 3). The white circles indicate the region that was photobleached and monitored for recovery. Bars, 10 µm. (B) Semiquantitative RT-PCR showing the mRNA levels of AtSUNs in sun1-KO sun2-KD. Total RNA was extracted from mature (before bolting) plants and used as templates for generating cDNA with M-MulV reverse transcriptase (M) and random hexamer primers (M-MuLV Tag RT-PCR kit [ProtoScript; New England Biolabs, Inc.]). PCR products were obtained using primers amplifying the AtSUN1 coding sequence (lane A, forward primer 5'-ATGTCGGCATCAACGGTGTCG-3' and reverse primer 5'-TTATTCACTTTCAGGTGAAGAGTCCTG-3'), AISUN2 coding sequence (lane B, forward primer 5'-ATGTCGGCGTCAACGGTGTC-3' and reverse primer 5'-TCAAGCATGAGCAACAGAGAC-3'), and PP2A (lane C, forward primer 5'-ATGTCTATGGTTGATGAGCC-3' and reverse primer 5'-GCTAGACATCACATTGTC-3'). The results show that PP2A (lane C) mRNA is present in both wild-type and sun1-KO sun2-KD samples and has similar levels in both samples. AtSUN1 (lane A) mRNA is present in the wild-type sample but not in the sun1-KO sun2-KD sample. AtSUN2 (lane B) mRNA is present in both wild-type and sun1-KO sun2-KD samples but with a lower level in the sun1-KO sun2-KD samples, indicating that sun1-KO sun2-KD is an AISUN2 knockdown line. PCR without templates (last three lanes) or PCR using total RNA as templates (lane D) resulted in no PCR products. (C) AtSUN1 and AtSUN2 mitotic localization in tobacco BY-2 cells. BY-2 cells were transformed and synchronized according to previously described protocols (Graumann and Evans, 2011). AtSUN1-YFP or AtSUN2-YFP (shown in green) was stably expressed in BY-2 cells and chromatin labeled with DRAQ5 (Biostatus Limited; shown in magenta). Different mitotic stages are shown, and AtSUNs were absent from the preprophase band (prophase), cortical division zone (prometaphase/metaphase), or KTs (prometaphase/metaphase), which are three sites of RanGAP localization in mitotic plant cells (Xu et al., 2007, 2008). Bars, 10 µm.

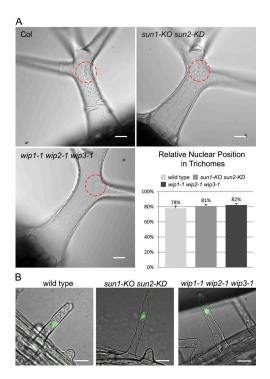
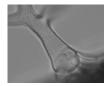
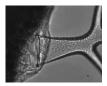


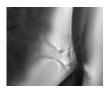
Figure S3. Nuclear position in root hairs and trichomes is not affected in sun1-KO sun2-KD and wip1-1 wip3-1. (A) Trichome nuclear position of wild type, sun1-KO sun2-KD, and wip1-1 wip2-1 wip3-1 was observed from fully expanded young leaves of 25-d-old plants. The nuclei are highlighted by red dotted circles. In wild type, the trichome nuclei are close to the first branch point (Folkers et al., 1997), and this position is not affected in sun1-KO sun2-KD and wip1-1 wip2-1 wip3-1, as shown by the relative nuclear position histogram (calculated by the distance from the nucleus to the baseline of a trichome divided by the distance from the second branch point to the baseline). Student's t test shows no significant differences among wild type, sun1-KO sun2-KD, and wip1-1 wip2-1 wip3-1 (P > 0.05; n = 50). Error bars represent SEM. (B) The nuclear position of root hairs was observed using 11-d-old seedlings. In wild type, the nucleus migrates to the growing root hair and keeps a fixed distance from the tip (Chytilova et al., 2000), which is unchanged in sun1-KO sun2-KD and wip1-1 wip2-1 wip3-1. Bars, 20 μm.



Video 1. Nuclear movement from the trichome baseline to the first branch point in a wild-type trichome. Images were taken from a rosette-leaf trichome (indicated by a red arrow) of a 25-d-old wild-type plant using a digital camera (DS-Qi1Mc) for 40 min at 1 frame per second, and the video is played at 60x speed.



Video 2. Nuclear movement from the trichome baseline to the first branch point in a sun1-KO sun2-KD trichome. Images were taken from a rosette-leaf trichome (indicated by a red arrow) of a 25-d-old sun1-KO sun2-KD plant using a digital camera (DS-Qi1Mc) for 76 min at 1 frame per second, and the video is played at 60× speed.



Video 3. Nuclear movement from the trichome baseline to the first branch point in a wip1-1 wip2-1 wip3-1 trichome. Images were taken from a rosette-leaf trichome (indicated by a red arrow) of a 25-d-old wip1-1 wip2-1 wip3-1 using a digital camera (DS-Qi1Mc) for 29 min at 1 frame per second, and the video is played at 60x speed.

References

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