

**Supplementary Figure 1.** Reduced irradiance increases the number of small phyB-NBs in different parts of the leaf and in the hypocotyl of young seedlings.

Key: 1) Abaxial surface of the basal portion of the petiole of expanding leaves (2 week old plants), 2) Adaxial surface of the basal portion of the petiole of expanding leaves, 3) Adaxial surface of the leaf blade in expanding leaves, 4) Abaxial surface of the basal portion of the petiole of fully expanded leaves (3.5 week old plants), 5) Hypocotyl cells of 3 d old seedlings.

Arabidopsis plants were grown under high irradiance white light and transferred to low irradiance 4 h after the beginning of the photoperiod and the number of phyB-NBs was measured 2 h later. Data are means  $\pm$  SE of at least 5 plants, two leaves per plant. Factorial ANOVA indicates that the effect of irradiance on the number of small phyB-NBs was significant (P<0.0001), while the interaction between cell type and irradiance and the effect of cell type were not significant (P>0.05).



**Supplementary Figure 2.** Small phyB-NBs are not an artefact caused by sample irradiation during confocal microscopy.

A. Small phyB-NBs are present in plants grown under low irradiance for 2 h even if the confocal plane is selected by irradiating a leaf area distant from the site of observation to prevent previous exposure (not focused photographs). The arrows show small phyB-NBs. Then the same nucleus was focused to confirm the small phyB-NBs.

B. The number of small phyB-NBs does not increase if exposure to confocal light is repeated. The same nucleus was recorded on repeated occasions. Representative nucleus and quantitative data are shown.



Supplementary Figure 3. Dynamic leaf position in response to changes in irradiance.

A. Long-term kinetics. Leaf angle of plants transferred from high to low irradiance on day 0 and control plants maintained under high irradiance during 7 days.

B. Reversal of the leaf angle response. Leaf angle of plants transferred from high to low irradiance on day 0 and returned to high irradiance on day 1. Control plants remained under high irradiance.

C. Rapid reversal of the leaf angle response. Leaf angle of plants transferred from high to low irradiance 4 h after the beginning of the photoperiod and returned to high irradiance 4 h later. Control plants remained under high irradiance.

D. Response to diurnal changes in irradiance: Leaf angle in plants exposed to high irradiance, low irradiance, or simulated fluctuations in irradiance typical of a sunny day. Irradiance ( $\mu$ mol.m<sup>-2</sup>.s<sup>-1</sup>) was 50, 100, 150, 200 (midday), 150, 100 and 50.

The protocols are indicated for each experimental setting. White bars= high irradiance, grey bars= reduced irradiance, black bars= darkness. Arrows indicate time of measurements. Data represent mean  $\pm$  SE of at least 10 plants. Different letters denote significant differences among means (*P* < 0.05) in ANOVA followed by Bonferroni posttests.



**Supplementary Figure 4.** Reduced hyponastic response correlates with reduced auxin signalling status in the *phyA* mutant.

A. Reduced hyponastic response to low irradiance in different phyA null alleles.

B. Reduced GUS activity driven by DR5 in fully expanded leaves of the *phyA* mutant background grown under high irradiance. Staining of representative fully expanded leaves (left) and quantitative data (right).

C. The wild type grown at low irradiance phenocopies the reduced GUS activity driven by DR5 observed in the *phyA* mutant. Plants were grown under high irradiance for two weeks, transferred to low irradiance or left as high irradiance controls and new leaves of approximately 1 cm length were harvested 7 d later.

Data are means  $\pm$  SE of at least 10 plants. Different letters denote significant differences among means (P < 0.05) in ANOVA followed by Bonferroni post-tests when more than two conditions are compared (A, C).

## GUS activity

For quantitative analysis of GUS activity, rosette leaves were harvested in liquid nitrogen, homogenized in 50 µl ice-cooled extraction buffer, and microcentrifuged at 4°C. The supernatant was stored at  $-80^{\circ}$ C (for less than 1 week). GUS activity was measured by using 4-methylumbelliferyl- $\beta$ - d-glucuronide (MUG from Sigma, St Louis, MO, USA) as substrate (Jefferson et al., 1987) and expressed per unit protein (Lowry et al., 1951). Standard curves were prepared with 4-methylumbelliferone (4-MU from Sigma). For GUS staining, rosette leaves were soaked in 90% cold acetone for 20 min (prefixation) and rinsed with water. Cold staining solution (2 mM 5-bromo-4-chloro-3-indolyl  $\beta$ -d-glucuronide, 2 mM ferrocyanide, and 50 mM sodium phosphate buffer) was infiltrated on ice and then incubated overnight at 37°C. Stained leaves were fixed for 30 min in each of the following solutions: 20% ethanol, 35% ethanol, FAA (50% ethanol, 5% formaldehyde, and 10% acetic acid). To remove residual chlorophyll, leaves were subjected to 3-4 consecutive washes of 2 hours each in 70% ethanol (Blázquez et al., 1997). Leaves were visualized with a binocular loop (Zeiss Stemi 2000-C, Carl Zeiss Jena GmbH) and photographs were taken with a digital camera.

- Blázquez MA, Soowal LN, Lee I, Weigel D (1997) LEAFY expression and flower initiation in Arabidopsis. Development **124:** 3835-3844
- Lowry OH, Rosenbrough NJ, A.L. F, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem **193**: 265-275

**Table S1.** Primers used in the analysis of gene expression by qPCR.

Gene name	Locus	Sequence	Amplicon length (bp)
IAA6	At1g52830	TACAGGAGAAAGAAGAACAATGAG	159
		GCCAAGACAGCCGAAGAG	
IAA19	At3g15540	GGCAGAGAAGATGATGAAGAAGAG	111
		TCAGCGTCACCACCAGATG	
GH3.3	At2g23170	TTCCGCTCCACAGTTCAAG	178
		GCCAGGTATAGTCTTCGTCTC	
SAUR68	At1g29510	AGGGTTGTTTCGTGGTCTAC	135
		TGATTGGTCCTTCCGTTGG	
YUC2	At4g13260	CAACTTCAATGCTCTTCCTTC	161
		GAACCAACCGAGACATACG	
YUC8	At4g28720	GATTGTATTGCTTCTCTATGG	193
		CACACTCGTTGAACTTAGG	
YUC9	At1g04180	TGATGATGATGAAGTGGCTAC	166
		GCACCGATGTCAAGAACC	
XTH19	At4g30290	TGACCCAACCGCTAACTTTC	177
		GCCCAATGCTCTGCTTCC	
XTH22	At5g57560	CAAGAACAAACCAATGAGAATG	200
		ACGAGCCAGTAGTAGTCC	
YLS8*	At5g08290	TGGATGAGGTGCTTGCGTCTG	101
		CTCGTACATGGTGTTGAAGTCTGG	

\*Czechowski, T., Stitt, ., Altmann, T., Udvardi, M.K. and Scheible, W.-R. (2005) Genome-wide identification and testing of superior reference genes for transcript normalization in Arabidopsis. Plant Physiol **139**, 5-17