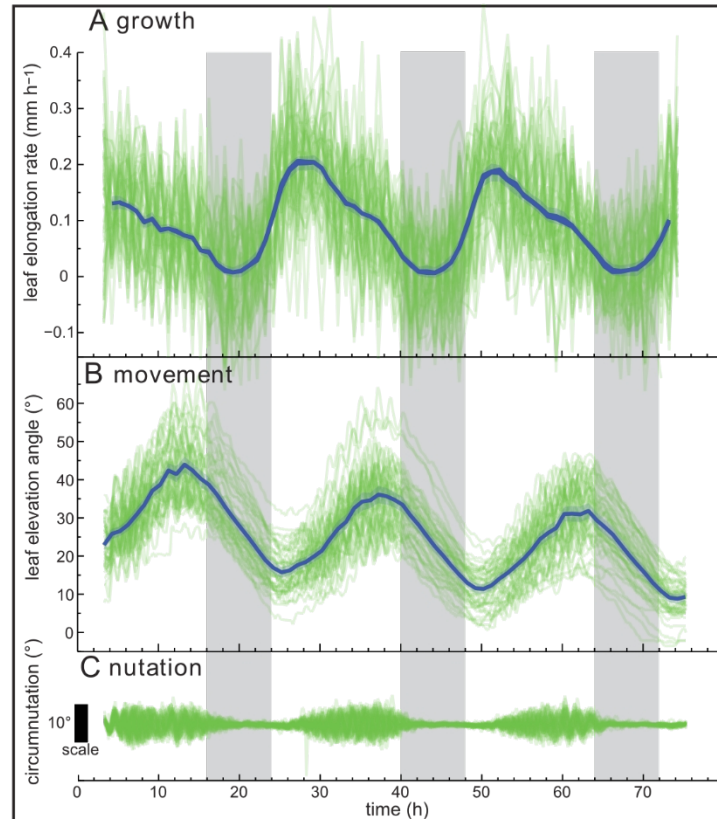


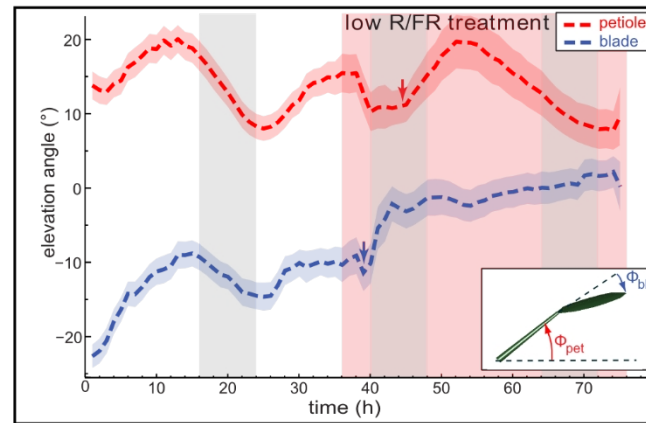
**Supplemental Figure 1. Image analysis algorithm to compute  $P_P$  and  $P_T$  from time-lapse images.**

Flow chart illustrating one time step  $i$  of the image analysis algorithm to compute the leaf tip point  $P_T$  and the petiole-blade intersection point  $P_P$ . I: Height-scaled image of a plant obtained with the laser scanner; II: point cloud representing the plant surface after 3D transformation;  $P_0$  is manually selected each 24 h at zeitgeber time 3 (ZT3) or linearly interpolated for intermediate  $i$ ; if  $i=1$ , the approximate leaf tip point  $P_T'(1)$  is manually selected; if  $i > 1$  the leaf tip point of the previous time step is used to enter the calculation:  $P_T'(i) = P_T(i-1)$ ; III: filtering of points (in green) within a defined area around  $P_T'$ ; IV: computation of  $P_T$  as the median of 10-20 leaf points with the largest distance to  $P_0$ ; using  $P_0$  and  $P_T$ , points are related to a leaf as highlighted by the dashed rectangle; V: selected points are rotated to the x-y plane and normalized such that  $P_0 = (0,0,0)$  and  $P_T = (0,1,0)$ ; approximated leaf width is computed using the highlighted points (in yellow) close to  $P_T$ ; VI: highlighted points (in yellow) are filtered using the previously computed value for leaf width; VII: leaf width as a function of normalized axis position; the maximum of the first-order derivative is the approximate the position of  $P_P$  highlighted with a dashed rectangle; VIII: computation of  $P_P$  as is the centroid of selected points inside the dashed rectangle; in the subsequent iteration step  $i+1$  the image of the same plant taken at the subsequent time step is processed and  $P_T$  and  $P_P$  computed for each leaf; the algorithm is automated and only needs user input at the first iteration step  $i=1$ .



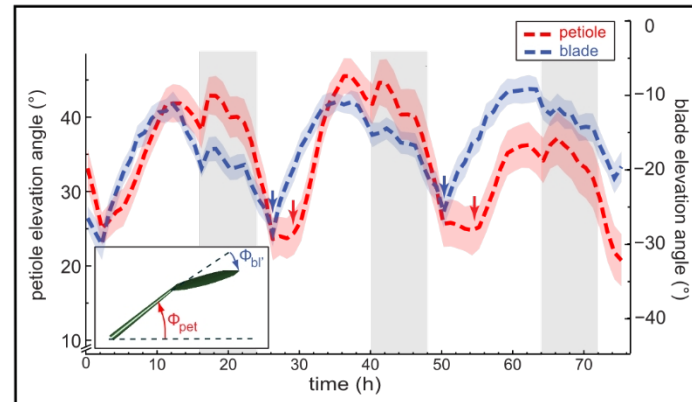
**Supplemental Figure 2. Definition of principal output.**

(A) Leaf elongation rate (growth), (B) leaf elevation angle (movement) and (C) circumnutations (nutations) of leaves 1 and 2 grown in continuous day (L/L) measured on 53 leaves (30 plants). Col-0 plants were grown for 14 d in standard L/D (16/8). At time  $t=0\text{h}$  (ZT0) lights were switched on for imaging and kept on in L/L; vertical gray bars represent subjective night periods. Opaque green lines represent data of 53 individual leaves. The solid blue line of leaf elongation rate is mean moving average (3 h) of individual curves. The blue line of leaf elevation angle represent mean value of data points each 60 min (conversely to raw data sampled each 10 min). The blue opaque band around the mean lines is the 95% confidence interval of mean estimate. Circumnutations are computed by detrending individual curves of leaf elevation angle. The trend line was computed using piecewise linear regression (regression parameter  $\tau=0.7$ ).  $I_{\text{tip}}$  and  $\Phi_{\text{tip}}$  were used to compute the graphs.



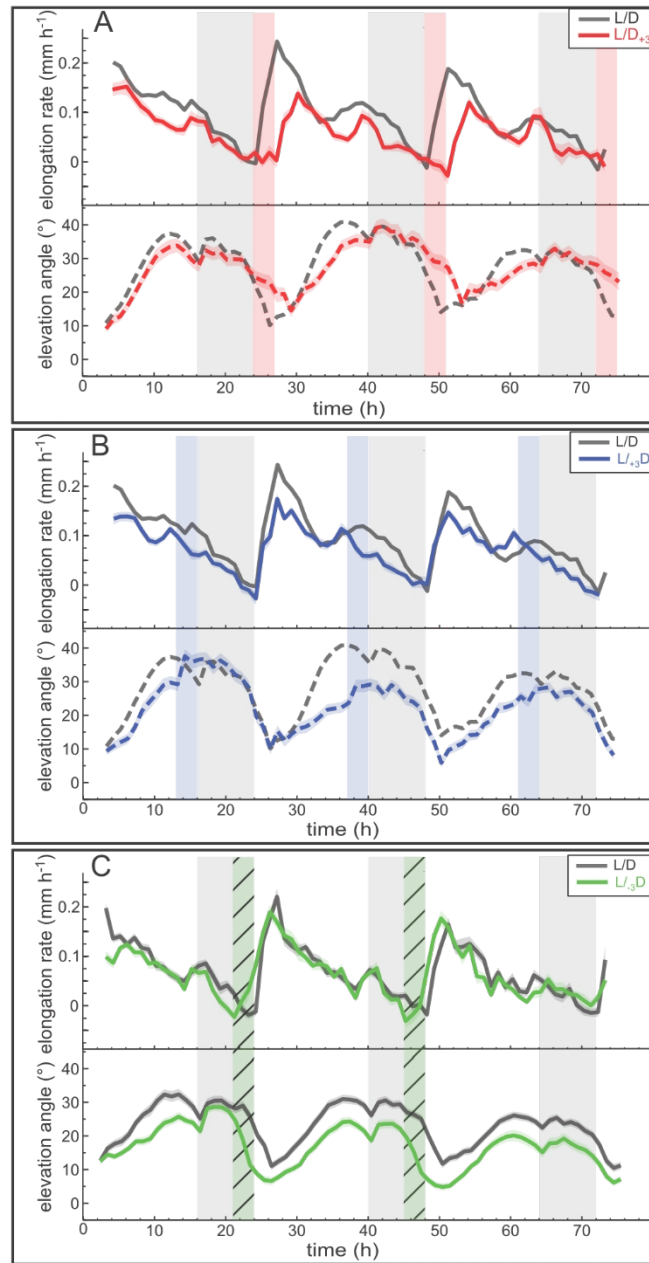
**Supplemental Figure 3. In response to a low R/FR treatment the blade upward movement precedes the petiole upward movement.**

Elevation angle of petioles (in red) and blades (in blue) of leaves 1 and 2 in continuous day measured on 28 leaves; Col-0 plants were grown for 14 d in standard long day (16/8) followed by 2 d continuous light (L/L). At time  $t = 0$  plants were imaged in L/L (subjective nights are darkened); after 36 h the R/FR ratio was decreased to simulate shade (highlighted by the red rectangle). Leaf elevation angle are mean values. The opaque band around the mean lines is the 95% confidence interval of mean estimate. Arrows indicate the beginning of rapid upward movement.



**Supplemental Figure 4. In L/D conditions the blade upward movement precedes the petiole upward movement.**

Elevation angle of petioles (in red; leaf scale) and blades (in blue, right scale) of leaves 1 and 2 in long day conditions (L/D; 16/8) measured on 19 leaves (30 plants); Col-0 plants were grown for 14 d in standard L/D conditions. Beginning from time  $t=0$  plants were imaged in L/D. Vertical gray bars represent true night periods. Elevation angle are mean values. The opaque band around the mean lines is the 95% confidence interval of mean estimate. Arrows indicate the beginning of rapid upward movement.

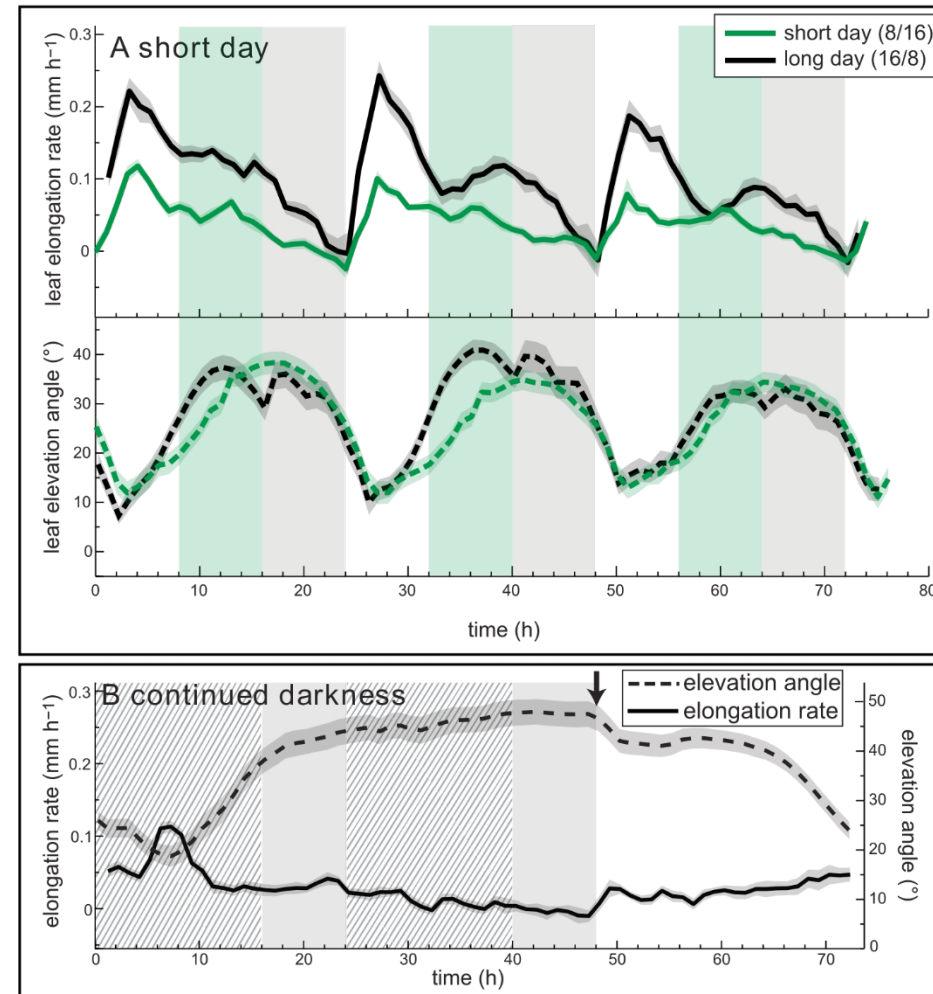


### Supplemental Figure 5: Light is required at dawn to trigger leaf growth.

(A) This is the same growth data as plotted on Figure 7A, in addition we included leaf movement for those plants, night was prolonged after dawn by 3 h (L/D<sub>+3</sub>; red line  $n_{\text{leaf}}=27$ ), (B) This is the same growth data as plotted on Figure 7B, in addition we included leaf movement for those plants, night was prolonged before dusk by 3 h (L<sub>+3</sub>D; blue line,  $n_{\text{leaf}}=54$ ).

(C) This is the same growth data as plotted on Figure 7C, in addition we included leaf movement for those plants, night was shortened before dawn by 3 h (L/D<sub>-3</sub>; green line  $n_{\text{leaf}}=35$ )

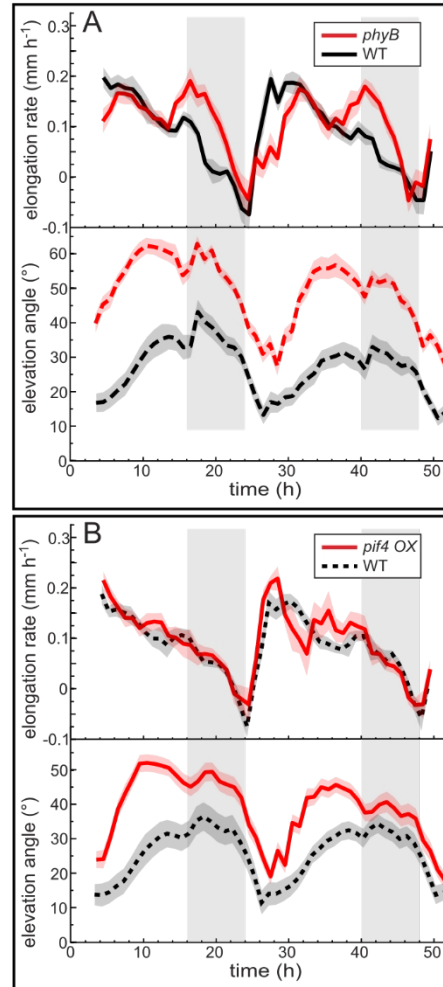
Col-0 plants were grown for 14 d in standard L/D (16/8) conditions before measurement; vertical gray bars represent true night periods; vertical red/blue bars indicate prolonged night periods (A,B) and vertical hatched green bar shortened night period (C). Leaf elongation rate was computed as mean moving average (3 h) of individual curves. Leaf elevation angles are mean values. The opaque band around the mean lines is the 95% confidence interval of mean estimate,  $n_{\text{leaf}}$  = number of leaves. Day 1 of the experiment represents the 1<sup>st</sup> day when the plants were subjected to an abrupt change in night length.  $l_{\text{tip}}$  and  $\Phi_{\text{tip}}$  were used to compute the graphs.



**Supplemental Figure 6. Growth and movements are altered by shortening day length or in continuous darkness.**

(A) Leaf elongation rate and leaf elevation angle of leaf 1, 2 in long day (black line,  $n_{\text{leaf}}=27$ ) and short day (green line,  $n_{\text{leaf}}=47$ ). Col-0 plants were grown for 14 days in standard long day (16/8h) or for 18 days under short day (8/16h) condition before measurement under the same conditions.

(B) Leaf elongation rate and leaf elevation angle of leaf 1, 2 in prolonged darkness (D/D,  $n_{\text{leaf}}=41$ ). Col-0 plants were grown for 14 days in standard L/D (16/8) conditions before measurement. At time  $t=0$  lights were not switched on and plants were imaged for 48h in darkness followed by 24h of light; vertical gray bars represent subjective night periods and the hatched part the subjective day; The arrow marks the time when light was switched on. Solid lines of leaf elongation rate are the mean moving average (3h) of individual curves. Solid lines of leaf elevation angle are mean values. The opaque band around the mean lines is the 95% confidence interval of mean estimate.  $n_{\text{leaf}}$  = number of measured leaves.  $l_{\text{tip}}$  and  $\Phi_{\text{tip}}$  were used to compute the graphs.



**Supplemental Figure 7: Plants with elevated levels of PIF4 maintain leaf growth rhythms robust in amplitude.**

**(A)** Leaf elongation rate and leaf elevation angle of leaves 1 and 2 in the *phyB* mutant ( $n_{\text{leaf}}=29$ ) and Col-0 ( $n_{\text{leaf}}=30$ ) grown in standard long-day conditions. **(B)** Leaf elongation rate and leaf elevation angle of leaves 1 and 2 in the *PIF4* overexpressor line ( $n_{\text{leaf}}=30$ ) and Col-0 ( $n_{\text{leaf}}=30$ ) grown in L/D. Col-0, *phyB* and *PIF4* OX plants were grown for 14 d in L/D prior to imaging in the same conditions. Vertical gray bars represent night periods. Solid lines of leaf elongation rate are the mean moving average (3 h) of individual curves. Leaf elevation angle are mean values. The opaque band around the mean lines is the 95% confidence interval of mean estimate.  $n_{\text{leaf}}$  = number of measured leaves.  $l_{\text{tip}}$  and  $\Phi_{\text{tip}}$  were used to compute the graphs.



## Supplemental Methods

### *Geometric definition of leaf length and elevation angle*

In *Arabidopsis*, the basal end of leaves is located in the center of the plant, which we geometrically define as the point  $P_0$  (Figure 1A). The distal end of leaves –the leaf tip– is defined by the point  $P_T$ . Hence the vector  $P_0P_T$  delineates length ( $l_{tip}$ ) and elevation angle ( $\Phi_{tip}$ ) of a leaf (Figure 1A). A leaf is further subdivided into petiole and blade. The point  $P_P$  defines the junction between them (Figure 1A). Thus  $P_0P_P$  delineates length ( $l_{pet}$ ) and elevation angle ( $\Phi_{pet}$ ) of the petiole, and  $P_PP_T$  length ( $l_{bl}$ ) and elevation angle ( $\Phi_{bl}$ ) of the blade. In reality, the proximodistal axis of an *Arabidopsis* leaf is slightly curved. We approximate this curve by  $P_0$ ,  $P_P$  and  $P_T$  (Figure 1A). In many cases petioles cannot be scanned and  $P_P$  not reliably be estimated. This is attributed to the measurement geometry of the laser and plant architecture (e.g. steep leaf angle). However, the vector  $P_0P_T$  is available in most cases and represent a simplified yet robust description of the leaf axis (Figure 1A). Geometrically,  $\Phi_{tip}$  is a good estimate for mean leaf elevation angle of a slightly curved line, but  $l_{tip}$  is smaller than actual leaf length  $l_{leaf}=l_{pet}+l_{bl}$  (Figure 1A). To estimate the degree of leaf curvature, we computed the elevation angle of the leaf blade relative to the petiole ( $\Phi_{bl}'$ ) (Figure 1A). Realistic values for  $\Phi_{bl}'$  for leaf 1 and 2 and in our experimental conditions were  $-15^\circ > \Phi_{bl}' > -30^\circ$  (Figure 4C). Using these values (assuming  $l_{pet}=l_{bl}$ ) and applying the law of cosines,  $l_{tip}$  is underestimated compared to  $l_{leaf}$  by 0.9% to 3.4%. The diel elongation rate computed from  $l_{tip}$  did not differ much from the one computed from  $l_{leaf}$  (Figure 1B). The elongation rate using  $l_{tip}$  is amplified compared to  $l_{leaf}$  around the night/day transition (Figure 1B, indent), which can mainly be attributed to relatively fast down- and upward movements of the leaf blade relative to the petiole (Supplemental Figure 4). Nevertheless the vector  $P_0P_T$  yields a simplified yet robust estimate of the diurnal pattern of leaf elongation rate (growth), and leaf elevation angle (movements) and nutations (Supplemental Figure 2).

### *Image processing*

The goal of our image analysis algorithm is to compute  $P_0$ ,  $P_P$  and  $P_T$  for each leaf and each time step with minimal user interaction. The algorithm is implemented in Matlab (MathWorks Inc., Natick, MA, USA) and illustrated in Supplemental Figure 1 online. Each 24h, at ZT3 (time of lowest leaf position),  $P_0$  is manually selected for each plant in the image (e.g. at hourly time steps  $i=1,25,49,73$ ) and linearly interpolated for intermediate  $i$ . At  $i=1$ , the approximate position of the tip  $P_T'$  for each leaf is manually selected. The algorithm autonomously computes  $P_T$  and  $P_P$  for each selected leaf (Supplemental Figure 1 online). At



following time steps  $i = i + 1$ , we set  $P_T'(i) = P_T(i-1)$  and compute  $P_T$  and  $P_P$ . Note that in our experiments *Arabidopsis* leaves were between 1.0 and 2.0 cm long. Between successive time steps, the leaf tip did usually not move more than 2 mm away from the previous positions. Hence the Euclidian distance  $|P_T(i)-P_T(i-1)|$  was usually smaller than 2 mm, which facilitated automated leaf tracking over time. Apart from manual selection of  $P_0$  for each plant (each 24h) and  $P_T'$  for each leaf at  $i=1$ , the algorithm is fully automated. To assess the precision of the semi-automated computation of  $P_P$  and  $P_T$ , we plotted time-courses of  $l_{pet}$ ,  $l_{tip}$  and  $\Phi_{pet}$ ,  $\Phi_{tip}$  (spherical coordinates of  $P_P$  and  $P_T$ ) for each analyzed leaf. This yields smooth and continuous curves if the algorithm worked properly. Wrongly computed values for  $P_P$  or  $P_T$  led to discontinuities (e.g. missing values or sharp peaks) in plotted curves. Leaves showing such discontinuities were not considered in further analyses. The whole analysis pipeline can be run on a regular notebook running Matlab (min. 8 GB RAM) and requires 1-2 minutes of user interaction per plant including post-processing quality assessment (Dornbusch et al., 2012).

### *Data processing*

Leaf elongation rate was computed as the difference in leaf length  $l_{tip}$  between successive time steps  $i$  for each leaf 1 and 2 (and each plant) in one experiment (Supplemental Figure 2A). Owing to the variance in the data, elongation curves for each individual leaf were smoothed using a moving average over 3h. We then computed mean values for each time point (Supplemental Figure 2A online, blue line) and 95% confidence interval of mean estimate (Supplemental Figure 2A online, opaque blue band around mean line).

Leaf elevation angle  $\Phi_{tip}$  follows a characteristic diurnal pattern frequently overlaid with short-period ultradian circumnutations (Supplemental Figure 2B online). Leaf movements as rate of change in  $^\circ h^{-1}$  were computed as difference in  $\Phi_{tip}$  between successive hourly time-steps (Figure 3C). Diurnal leaf movements were visualized using computed hourly mean values for  $\Phi_{tip}$  and 95% confidence interval of mean estimate (Supplemental Figure 2B online, blue curve). This compensated for the nutation effects. These circumnutations are visualized in a separate plot as nutations around the diurnal trendline of  $\Phi_{tip}$  (Supplemental Figure 2C online). This trendline is computed for each individual leaf using piecewise linear regression (regression parameter  $\tau=0.7$ ). Data for  $\Phi_{tip}$  for each leaf is subtracted from this trend line and plot as opaque line (Supplemental Figure 2C online). Diel elongation rates and leaf movements (absolute changes in leaf elevation angle) were computed summing corresponding hourly values over a period of 24h starting from ZT2.25 (Figure 1B, Figure 5).

## Reference

**Dornbusch, T., Lorrain, S., Kuznetsov, D., Fortier, A., Liehti, R., Xenarios, I., and Fankhauser, C.** (2012). Measuring the diurnal pattern of leaf hyponasty and growth in *Arabidopsis* - a novel phenotyping approach using laser scanning. *Funct Plant Biol.* 39: 860-869.