1 **Short title**

2 The role of ABA in neighbor proximity-induced hyponasty.

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Title

Abscisic Acid modulates neighbor proximity-induced leaf hyponasty in Arabidopsis.

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Author contributions

O.M. and C.F conceived the original research plan; O.M., J.K., F.G., M.L., Y.C.I., V.C.G., S.C.Z and C.F. conceived experiments; O.M., J.K., F.G., M.L., M.T., Y.C.I., V.C.G., S.C.Z. and C.F. performed experiments and/or analyzed the data; J.K., P.D. and J.M.H. provided unpublished material; C.F. wrote the article with contributions of all the authors; C.F. agrees to serve as the author responsible for contact and ensures communication.

One sentence summary

Abscisic acid biosynthesis and signaling are required to promote leaf hyponasty in response to a drop in the red to far-red ratio.

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52

Abstract

Leaves of shade avoiding plants like *Arabidopsis thaliana* change their growth pattern and position in response to a low red to far-red ratio (LRFR) that is encountered in dense plant communities. In LRFR, transcription factors of the phytochrome interacting family (PIFs) are de-repressed. PIFs induce auxin production, which is required to promote leaf hyponasty thereby favoring access to unfiltered sunlight. Absciscic acid (ABA) has also been implicated in the control of leaf hyponasty. In addition, gene expression patterns suggest that LRFR regulates the ABA response. Here we show that, in leaves, LRFR leads to a rapid increase in ABA levels. Changes in ABA levels depend on PIFs, which regulate the expression of genes encoding isoforms of the enzyme catalyzing a rate-limiting step in ABA biosynthesis. Interestingly, ABA biosynthesis and signaling mutants have more erect leaves in white light but respond less to LRFR. Consistent with this, ABA application decreases the leaf angle in white light but this response is inhibited in LRFR. Tissue-specific interference with ABA signaling indicates that an ABA response is required in different cell types for LRFR-induced hyponasty. Collectively, our data indicate LRFR triggers rapid PIF-mediated ABA production. ABA plays a different role in controlling hyponasty in white light compared to LRFR. Moreover, ABA exerts its activity in multiple cell types to control leaf position.

70 Introduction

71

72 Leaf movements are controlled by the interplay of circadian and light signals (McClung 2013; Hopkins et al.
73 2008; Dornbusch et al. 2014). The physiological significance of these movements is not fully understood,
74 however, leaf position influences both photosynthesis and water loss (Hopkins et al. 2008). In open
75 environments, light reaches intensities higher than the photosynthesis saturation point. Having more erect
76 leaves diminishes light interception and favors cooling which is proposed to be beneficial (Hopkins et al.
77 2008). Consistent with this idea, Arabidopsis accessions from lower latitudes have more erect leaf angles
78 (Hopkins et al. 2008). In addition to these diel leaf movements, several environmental cues including flooding,
79 high temperature and shade lead to rapid upwards leaf repositioning (hyponasty) (Voesenek et al. 2006;
80 Casal and Balasubramanian 2019). This movement is thought to help leaves acclimate to such unfavorable
81 conditions for example by enhancing cooling capacity in warm environments (Crawford et al. 2012; Voesenek
82 et al. 2006; Casal and Balasubramanian 2019).

83 At the molecular level the mechanisms underlying hyponasty are probably best understood in response to a
84 decrease of the red to far-red ratio (low red/far-red, abbreviated LRFR here). LRFR occurs in dense
85 vegetational communities and is a modification of the light environment experienced by plants prior to actual
86 shading (Casal 2013; Ballare and Pierik 2017). Leaf hyponasty is induced by different elements of canopy
87 shade including both low light and LRFR (Mullen et al. 2006; Millenaar et al. 2009; Moreno et al. 2009; Casal
88 2013; Ballare and Pierik 2017). The primary photoreceptor controlling LRFR-regulated responses is
89 phytochrome B (phyB) (Casal 2013; Ballare and Pierik 2017). LRFR inactivates phyB leading to the de-
90 repression of several Phytochrome Interacting Factors, particularly, PIF4, PIF5 and PIF7 (Casal 2013; Ballare
91 and Pierik 2017; Cheng et al. 2021). These PIFs promote shade-induced elongation of the hypocotyl, leaf
92 petioles, and leaf hyponasty (Keller et al. 2011; Kohnen et al. 2016; de Wit et al. 2016; Michaud et al. 2017;
93 Pantazopoulou et al. 2017). phyB inactivation at the leaf rim leads to PIF-mediated auxin production in the
94 blade, followed by auxin transport and redistribution in the petiole where it promotes upwards leaf
95 repositioning (Michaud et al. 2017; Pantazopoulou et al. 2017; Gao et al. 2020).

96 Beside auxin, other hormones also contribute to environmentally-controlled leaf repositioning in
 97 Arabidopsis. Ethylene is particularly important to promote upwards leaf repositioning in response to
 98 waterlogging (Millenaar et al. 2009; Rauf et al. 2013). In contrast, ethylene is not required for low light-
 99 induced hyponasty and it negatively regulates high temperature-induced hyponasty (Millenaar et al. 2009;
 100 van Zanten et al. 2009). The role of abscisic acid (ABA) in controlling leaf position is also complex and poorly
 101 understood. ABA inhibits ethylene-induced hyponasty and in white-light grown plants genetic and
 102 pharmacological experiments indicate that ABA promotes downwards leaf repositioning (Mullen et al. 2006;
 103 Benschop et al. 2007). In contrast, ABA can also positively regulates hyponasty when plants are treated with
 104 an elevated temperature (van Zanten et al. 2009). Access to water is essential for growth and reversible
 105 cellular expansion, suggesting that ABA might be an important regulator of leaf positioning (Tardieu et al.
 106 2015; Yoshida et al. 2019). Indeed, differential petiole growth is a key contributor to upward repositioning of
 107 the leaf (Polko et al. 2012; Rauf et al. 2013). Intriguingly, shade treatments lead to higher ABA levels in tomato
 108 and based on the LRFR-induced gene expression pattern this may also be the case in Arabidopsis (Cagnola et
 109 al. 2012; Kohnen et al. 2016). We found that LRFR leads to a rapid increase in ABA content in leaves and
 110 therefore decided to study how LRFR controls leaf ABA levels and how ABA controls hyponasty.
 111

Results

In LRFR PIFs enhance *NCED* expression and ABA production

Before testing the potential role of ABA in LRFR-induced hyponasty, we first determined whether a reduction of the R/FR ratio (LRFR) rapidly changes ABA levels in Arabidopsis rosettes. 14-day-old-plants grown in long days (LD, 16 hours light, 8 hours darkness), where either kept in standard conditions (white light, WL) or transferred to LRFR (WL supplemented with FR light) at ZT3. Rosettes were collected at the time of transfer to LRFR (ZT3) and 2 hours later (ZT5) and ABA was quantified. This experiment showed that a 2-hour LRFR treatment led to a significant increase in ABA content (Fig. 1A). Since ABA levels fluctuate during the day (Lee et al. 2006; Adams et al. 2018), we also tested the effect of the LRFR treatment later in the day and found that ABA levels increase both in WL and LRFR, but at each time point there was more ABA in LRFR than in WL (Fig. S1A). LRFR led to higher ABA levels both in entire rosettes and in dissected leaves 1 and 2, which we used to analyze the hyponastic response (Fig. S1B).

The rate-limiting step in ABA biosynthesis is catalyzed by 9-cis-epoxycarotenoid dioxygenase (*NCED*) enzymes (Qin and Zeevaert 2002). LRFR triggers a rapid increase in *NCED3* and *NCED5* expression in the cotyledons of young seedlings (Kohnen et al. 2016). We therefore tested whether this is also the case in the leaf blades and the petioles of young Arabidopsis rosettes treated with LRFR. *NCED3* and *NCED5* were both rapidly induced in the petiole, while in the leaf blade only *NCED3* expression was induced (Fig. 1B, S1C). Measuring ABA levels in 2-week-old Arabidopsis rosettes showed that ABA levels were strongly reduced in *nced3nced5* double mutants and LRFR did not lead to enhanced ABA accumulation (Fig. 1C). Given the prevalent role of PIF transcription factors for shade-induced transcriptional reprogramming (de Wit et al. 2016), in particular PIF4, PIF5 and PIF7, we compared ABA levels in the wild type (Col-0) and the *pif4pif5pif7* triple mutant. This experiment showed that ABA levels did not increase in *pif4pif5pif7* in response to LRFR (Fig. 1D). To determine whether this is due to PIF-mediated *NCED* expression, we compared the rapid LRFR-induction of *NCED3* in Col-0 and *pif4pif5pif7*. This experiment showed that in *pif4pif5pif7* *NCED3* expression was reduced in WL and the rapid LRFR-induction was absent (Fig. 1E).

139

140 Genome-wide analysis of PIF4 binding sites identified several PIF4-binding peaks in the *NCED3* promoter of
141 low-blue light grown seedlings (Pedmale et al. 2016). We designed a series of amplicons along the large
142 *NCED3* promoter and found significantly enhanced PIF4 binding in LRFR-grown plants particularly in the
143 region corresponding to peaks 3 and 4 identified in (Pedmale et al. 2016) (Fig. S1D, E). To determine whether
144 the rapid LRFR-induced *NCED3* expression correlates with PIF4 binding, we performed ChIP experiments in
145 plants either maintained in WL or transferred to LRFR for 2 hours and used *HFR1*, a well-known PIF target
146 gene, as a control (Fig. 1F). Our experiments showed enhanced PIF4-HA binding following a LRFR treatment
147 on the promoters of *HFR1* and *NCED3* (Fig. 1G). Collectively these results indicate that PIF transcription
148 factors directly control *NCED3* expression in response to LRFR potentially explaining the observed increase in
149 ABA content upon transfer to LRFR.

150

151 ABA biosynthesis and signaling are required for a normal LRFR-induced hyponastic response

152 To determine the role of ABA biosynthesis during LRFR-induced hyponasty, we compared the hyponastic
153 response of the wild type with the *nced3nced5* double mutants over 2 days in plants either remaining in WL
154 or being transferred to LRFR at ZT3 of day 1. We focused on leaves 1 and 2, which possess a similar hyponastic
155 response compared to other leaves at the same developmental stage (Dornbusch et al. 2014; Michaud et al.
156 2017). *nced3nced5* double mutants had more erect leaves than Col-0 throughout the experiment in WL (Fig.
157 2A). However, this double mutant showed a limited response to a LRFR treatment (Fig. 2A). The *nced3* and
158 *nced5* single mutants had modest phenotypes (Fig. S2A-C). We also tested the *aba2* mutant which is known
159 to have low ABA levels (Gonzalez-Guzman et al. 2002). The phenotype of *aba2* was very similar to that of
160 *nced3nced5* (Fig. 2A, B), consistent with the importance of ABA for normal leaf positioning in WL and a robust
161 hyponastic response to LRFR. We note that despite having more erect leaves *aba2* and *nced3nced5* mutants
162 displayed a normal amplitude of leaf movement in WL, while their ability to respond to LRFR was severely
163 impaired (Fig. 2C).

164

165 To further investigate the role of ABA in the modulation of leaf hyponasty we analyzed higher order mutants
 166 lacking members of the major ABA receptor from the RCAR/PYR1/PYL family (Weiner et al. 2010;
 167 Raghavendra et al. 2010). While leaf movements in the quadruple mutant *pyr1pyl1pyl2pyl4* were very similar
 168 to Col-0, the *pyr1pyl1pyl2pyl4pyl5pyl8* septuple mutant had an interesting phenotype (Fig. 3A, S3A, B). As
 169 observed for ABA biosynthesis mutants, *pyr1pyl1pyl2pyl4pyl5pyl8* had constitutively more erect leaves, a leaf
 170 movement amplitude in WL that was at least as robust as in Col-0 but a reduced response to LRFR (Fig. 3A,
 171 S3A, B). Higher order mutants lacking protein phosphatases from the ABI1 family of ABA co-receptors
 172 (Weiner et al. 2010; Raghavendra et al. 2010) also showed altered leaf movements. This was most striking in
 173 the *abi1abi2hab1pp2ca* quadruple (*Qabi2*) mutant, which showed altered leaf movements both in WL and
 174 LRFR (Fig. 3B, S3C). In contrast to the other mutants tested, *Qabi2* also showed a strong increase in the leaf
 175 elevation angle at night (Fig. S3C). The *hab1abi1pp2ca* triple mutant had wild-type leaf movements in WL but
 176 a reduced response to LRFR (Fig. S3D, E). We also analyzed mutants lacking the SnRK2 kinases acting directly
 177 downstream of the ABA receptor (Weiner et al. 2010; Raghavendra et al. 2010) and found that the
 178 *snrk2.2snrk2.3snrk2.6* triple mutant showed a very severe phenotype (Fig. 3C, S3F). This triple mutant
 179 showed constitutively erect leaves with no response to LRFR (Fig. 3C, S3F). This phenotype was only observed
 180 in the triple mutant and not in the *snrk2.6* or *snrk2.2snrk2.3* mutants (Fig. S3G). Collectively our data indicate
 181 that mutants affecting the positive regulators of ABA signaling (PYR1/PYL and Snrk2 kinases) have very similar
 182 phenotypes to ABA biosynthesis mutants. In contrast, mutants affecting members of the ABI1 protein
 183 phosphatase family also alter leaf movements and the ability to respond to LRFR but the diel leaf movement
 184 patterns were different (Fig. S3).

185
 186 Exogenous auxin (IAA) application to the leaf tip leads to a similar leaf hyponastic response as transferring
 187 plants into LRFR (Michaud et al. 2017; Pantazopoulou et al. 2017). We therefore tested whether ABA
 188 biosynthesis mutants can respond to auxin application. These experiments showed that *nced3nced5* and
 189 *aba2* mutants showed modest responses to IAA application (Fig. 4A, S4A, B). The IAA and LRFR response of
 190 these mutants were very similar (Fig. 2-4, S4A, B) suggesting that normal ABA levels are required to respond
 191 to the LRFR-induced IAA production required to induce hyponasty. Collectively our data indicate that ABA

and ABA signaling modulate diel leaf hyponastic movement and are required for a robust LRFR-induced hyponastic response acting downstream of IAA production.

To determine whether ABA application alters leaf hyponasty we sprayed rosettes with different concentrations of ABA and followed diel leaf movements over 2 days either in WL or LRFR. Consistent with the constitutively high leaf position of WL-grown ABA biosynthesis mutants (Fig. 2), ABA application reduced the leaf angle during the first day after the application in WL in a dose dependent manner (compare Fig. 4B and Fig. 4C). The effect of ABA application was transient as leaf movements of treated and untreated plants were very similar the second day (Fig. 4B, C). Interestingly, in LRFR the effect of exogenous ABA application was largely canceled (Fig. 4B, C). We observed no significant effect following a treatment with 1 μ M ABA and a delay in the hyponastic response when we applied 10 μ M ABA (Fig. 4B, C). Our data show that ABA application inhibits leaf elevation happening during diel leaf movements in WL while in LRFR the effect of exogenous ABA was largely canceled suggesting a reduced sensitivity to ABA in shade-mimicking conditions.

ABA signaling in multiple tissues is required to control hyponasty in LRFR

Given the established importance of ABA in controlling stomata aperture, we first tested whether neighbor-proximity induced changes in leaf transpiration. Young rosettes were transferred from WL to LRFR at ZT3 and a slight increase in transpiration was observed late in the day but the difference between WL and LRFR was not significant (Fig. 5A). We also compared stomata aperture at ZT8 from plants that either remained in WL or were transferred to LRFR at ZT3 and observed no change (Fig. 5B). In contrast, treating plants with ABA or comparing plants during the day with plants that remained in darkness (night extension) validated our stomata opening measuring method as both treatments led to previously documented stomatal closure (Fig. 5A, B). To determine the importance of stomata for diel leaf movements and the response to LRFR we used *epf1epf2* double mutants and EPF2 over-expressing (*EPF2-OX*) plants which maintain a normal stomata morphology but have respectively higher and lower stomata density correlating with higher and lower transpiration (Hepworth et al. 2015). While the *epf1epf2* double mutant had more erect leaves in WL, it responded normally to LRFR (Fig. 5C). *EPF2-OX* plants were very similar to Col-0 (Fig. 5C). Collectively, these

219 data suggest that LRFR-regulated changes in transpiration and stomata aperture are not a major control point
 220 for LRFR-induced leaf hyponasty.
 221
 222 To determine in which cell types ABA signaling is required for normal LRFR-induced leaf hyponasty we
 223 generated lines expressing the dominant negative allele *abi1-1* in stomata (*CYP86A2* promoter), mesophyll
 224 (*CAB3* promoter) and bundle sheath cells (*SCR* and *MYB76* promoters) (Dickinson et al. 2020). We compared
 225 the leaf movements of these plants over 1 long day in WL and then transferred them into LRFR at ZT3 on day
 226 2 and continued imaging for 48 hours. All the lines had a reduced capacity to elevate their leaves in response
 227 to LRFR (Fig. 6A-D, S6A-D). The leaf movement pattern of the previously described *pCOR:abi1-1:RFP* line
 228 (Duan et al. 2013) was similar to our *pCAB3:abi1-1* lines (Fig. 6A, S6E), consistent with a role of ABA in
 229 mesophyll cells to control leaf movements. We also tested the ability of these lines to respond to exogenously
 230 applied ABA and found that disrupting ABA signaling in the stomata, mesophyll or bundle sheath cells largely
 231 prevented ABA-induced leaf repositioning (Fig. 6E-G, S6F, G). Collectively, our results indicate that different
 232 cell types are required for shade-regulated leaf hyponasty and for ABA-induced downward positioning of
 233 leaves grown in WL.
 234

Discussion

We showed that upon transfer to LRFR ABA content rapidly increases in leaves (Fig. 1, S1). Higher ABA levels were also reported in shade treated tomato and in Arabidopsis *phyB* mutants, which display a constitutive shade avoidance response (Cagnola et al. 2012; Gonzalez et al. 2012). Moreover, LRFR leads to increased ABA levels in Arabidopsis buds to prevent lateral shoot development (Gonzalez-Grandio et al. 2013; Holalu and Finlayson 2017). In buds PIFs rapidly induce *BRANCHED1* expression, which was proposed to enhance *NCED3* expression, thereby increasing ABA production (Gonzalez-Grandio et al. 2013; Holalu et al. 2020). In contrast to most previous studies, we looked at a rapid effect of LRFR and detect an increase in *NCED3* and *NCED5* expression within 1-2 hours and higher ABA levels within 2 hours of the shade-simulating treatment (Fig. 1). LRFR promotion of *NCED3* expression and ABA levels depend on PIF4, PIF5 and/or PIF7 and LRFR-induced ABA levels is lost in the *nced3nced5* double mutant (Fig. 1). Consistent with a direct role of PIFs in controlling ABA biosynthesis, we find increased PIF4 binding on the *NCED3* promoter in response to LRFR (Fig. 1, S1). Collectively, our data indicates that in response to LRFR PIFs enhance ABA levels in leaves. In response to LRFR PIFs also mediate rapid IAA production, which, upon asymmetric redistribution in the petiole, controls leaf repositioning (Michaud et al. 2017; Pantazopoulou et al. 2017). Based on IAA application experiments it appears that ABA acts downstream of IAA (Fig. 4, S4). ABA plays a major role in the regulation of water movements (Tardieu et al. 2015; Yoshida et al. 2019). The response to LRFR or temperature elevation triggers rapid IAA production (Quint et al. 2016; Casal and Questa 2018), which promotes growth and repositioning of leaves (van Zanten et al. 2009; Michaud et al. 2017; Pantazopoulou et al. 2017). Whether these responses are due to reversible or irreversible cellular expansion (growth) is not fully understood but in both cases sufficient water supply is required. We propose that in this context, PIF-regulated ABA levels contributes to a normal hyponastic response.

The function of ABA is best understood as a stress hormone. Various abiotic stresses (e.g. drought, salinity) enhance ABA biosynthesis, which limits gas exchange and growth to promote tolerance to unfavorable environments (Yoshida et al. 2019; Kinoshita et al. 2021). For example, when a shade treatment occurs in the

262 presence of high sodium chloride, hypocotyl elongation is inhibited in an ABA-dependent manner (Hayes et
263 al. 2019). However, the relationship between ABA and growth in non-stressful conditions is complex and ABA
264 acts both as an activator or a suppressor of growth (Yoshida et al. 2019). One study on the role of ABA in
265 LRFR-induced hypocotyl elongation concluded that ABA rather prevents growth (Ortiz-Alcaide et al. 2019).
266 The role of ABA in leaf positioning also appears to be complex. In standard light conditions application of ABA
267 prevents hyponasty, while decreasing ABA levels genetically or pharmacologically leads to plants with more
268 erect leaves (Mullen et al. 2006; Benschop et al. 2007) (Fig. 2, 4). In contrast, during high temperature-
269 induced hyponasty ABA was proposed to promote leaf hyponasty (van Zanten et al. 2009). Similarly, we find
270 that in WL conditions mutants with reduced ABA levels or reduced ABA signaling have more erect leaves but
271 maintain robust diel leaf movements (Fig. 2, 3). Applying ABA leads to transient downwards leaf repositioning
272 lasting about a day (Fig. 4). These data indicate that in unstressed conditions ABA limits leaf hyponasty.
273 Having more erect leaves in WL largely correlates with high transpiration. This is true for mutants affecting
274 the PYR/PYL ABA receptors (Gonzalez-Guzman et al. 2012), SnRK2 protein kinases (Fujii and Zhu 2009), ABA
275 biosynthesis mutants (Merlot et al. 2002) or mutants with more stomata (Hepworth et al. 2015). Moreover,
276 PP2C ABA-co-receptor mutants have lower amplitude leaf oscillations during the day in WL (Fig. 3B) and
277 reduced transpiration (Rubio et al. 2009). Collectively this suggests that in WL transpiration, which is
278 controlled by ABA, contributes to leaf position. The role of ABA appears to be different for the LRFR response.
279 Indeed, ABA biosynthesis and the response to ABA are required for full repositioning of the leaves suggesting
280 that in these conditions ABA promotes hyponasty (Fig. 2, 3). Interestingly, the high stomata density mutant
281 *epf1epf2* which, like ABA biosynthesis or ABA response mutants, has more erect leaves in WL and transpires
282 more than the wild type (Hepworth et al. 2015), was able to respond to LRFR (Fig. 5). This suggests that the
283 inability to respond to LRFR in ABA biosynthesis or signaling mutants is not due to their constitutively erect
284 leaves. Moreover, it suggests that having a normal stomata response despite a high or a low transpiration
285 baseline (*epf1epf2* and *EPF2-OX* respectively) (Hepworth et al. 2015), does not prevent LRFR-induced
286 hyponasty (Fig. 5). The different involvement of ABA in controlling hyponasty in high versus low R/FR is also
287 illustrated by the strongly reduced response to applied ABA in LRFR-grown plants (Fig. 4). Similarly, when
288 ABA was applied to heat-treated plants to trigger hyponasty, it did not inhibit upwards repositioning of leaves

(van Zanten et al. 2009). Collectively, these experiments show a differential role of ABA in controlling hyponasty during unstressed conditions and in response to changes in light or temperature triggering a strong hyponastic response.

The mechanisms underlying the control of hyponasty by ABA remain poorly understood. In day-night conditions, stomata open rapidly at dawn leading to CO₂ fixation and enhanced transpiration. However, leaves only start to move upwards several hours later correlating with fast leaf growth (Dornbusch et al. 2014). Hence, stomata opening at dawn does not trigger a rapid leaf elevation in WL conditions. Similarly, when transferred to LRFR, we did not observe significant changes in transpiration or stomatal opening correlating with leaf elevation (Fig. 5, S5). However, based on ABA application experiments (Fig. 4) (Mullen et al. 2006; Benschop et al. 2007) it is reasonable to conclude that regulated stomata opening is required for leaf hyponasty. This is also supported by our finding that *abi1-1* expression from a stomata-specific promoter alters diel leaf movements (Fig. 6, S6). Tissue-specific *abi1-1* expression indicates that ABA signaling is required in multiple tissues for normal leaf positioning (Fig. 6, S6). By controlling transpiration, stomata are a well-known control point of water movements. However, water fluxes are also controlled in other cells including bundle sheath and mesophyll (Negin et al. 2019; Grunwald et al. 2021). In long days, leaf hydraulic conductivity peaks before mid-day (Prado et al. 2019). This pattern in hydraulic conductivity does not directly correlate with leaf position in long-days (see e.g. Fig. 2). In WL, all the *abi1-1* expressing lines were largely unresponsive to ABA application indicating that ABA signaling is required in stomata, mesophyll and bundle sheath to control leaf hyponasty (Fig. S6). Expression of *abi1-1* in mesophyll and stomata clearly altered leaf position in WL, while this was not obvious in the bundle sheath expressing lines (Fig. 6, S6). However, the hyponastic response in plants transferred to LRFR was reduced in all *abi1-1* expressing lines indicating that ABA signaling is required in stomata, mesophyll and bundle sheath for shade-induced leaf hyponasty (Fig. 6, S6). This might be due to altered regulation of the leaf water potential as ABA inhibits H⁺ ATPases in bundle sheath cells similarly to what happens in stomata (Grunwald et al. 2021). It is noteworthy that LRFR also leads to IAA production and a strong auxin response (Michaud et al. 2017; Pantazopoulou et al. 2017). While ABA inhibits H⁺ ATPases, auxin activates these proton pumps (Miao et al. 2022). Hence, we hypothesize that in

316 LRFR the correct balance between IAA and ABA-regulated water movements is required to control leaf
 317 positioning. Another striking feature of the LRFR response is the reduced sensitivity to applied ABA (Fig. 4).
 318 It is noteworthy that reduced ABA sensitivity in LRFR is predicted based on gene expression in seedlings
 319 (Kohnen et al. 2016). Indeed, in cotyledons multiple members of the positively acting *PYR/PYL* and *SNRK2*
 320 genes are downregulated, while some of the negatively acting *PP2C* ABA co-receptors genes are upregulated
 321 (Kohnen et al. 2016). This could be due to higher ABA levels given that a similar gene expression signature
 322 was observed in high light which also triggers ABA production (Huang et al. 2019). That said, high light leads
 323 to 4-5 times higher ABA levels typical of a stress response (Huang et al. 2019), while LRFR only led to a 30%
 324 increase (Fig. 1). We propose that the mechanisms by which ABA controls leaf position depends on the co-
 325 occurrence of an IAA response as observed in LRFR or following an increased temperature. However, the
 326 cellular mechanisms by which ABA controls environmentally regulated leaf positioning require future studies.
 327

328 **Material and methods**

329

330 **Plant material and plasmid construction**

331 All Arabidopsis lines used in this study are in the Col-0 background, *nced3nced5*, *nced3*, *nced5* (Frey et al.
332 2012), *aba2* (Leon-Kloosterziel et al. 1996), *pyr1pyl1pyl2pyl4* (Park et al. 2009), *pyr1pyl1pyl2pyl4pyl5pyl8*
333 (Gonzalez-Guzman et al. 2012) , *hab1abi1pp2ca* (Rubio et al. 2009) , *hab1abi1abi2pp2ca* (named *Qabi2*)
334 (Antoni et al. 2013), *snrk2.2snrk2.3*, *snrk2.6*, *snrk2.2snrk2.3snrk2.6* (Fujii and Zhu 2009) , *pif4pif5pif7* (de Wit
335 et al. 2015), *yuc2yuc5yuc8yuc9* (Nozue et al. 2015), *pPIF4::PIF4-3HA (pif4-101)* (Zhang et al. 2017), *epf1epf2*,
336 *EPF2-OX* (Hepworth et al. 2015) , *pCOR:abi1-1:RFP* (Duan et al. 2013) were previously described.

337 All constructs were obtained with the In-Fusion HD cloning kit (Takara) and sequence verified. *pCAB3:abi1-*
338 *1-citrine* (pMT36) was obtained by replacing the *PHOT1* CDS from *pCAB3:PHOT1-citrine* (Preuten et al. 2013)
339 with *abi1-1* which was amplified with oligonucleotides MT49 and MT50 using plasmid *pEN-1-abi1-1-2* as a
340 template (Barberon et al. 2016). *pSCR:abi1-1citrine* (pMT37) was obtained by replacing the *PHOT1* CDS from
341 *pSCR:PHOT1-citrine* (Preuten et al. 2013) with *abi1-1* which was amplified with oligonucleotides MT49 and
342 MT51 using plasmid *pEN-1-abi1-1-2* as a template (Barberon et al. 2016). *pCYP86A2:abi1-1citrine* (pMT39)
343 was obtained by replacing *pSCR* promoter from pMT37 with the *CYP86A2* promoter which was amplified with
344 oligonucleotides MT57 and MT58 using plasmid *proCYP86A2:GFP-PYR1MANDI* as a template (Park et al.
345 2015). *pMYB76:abi1-1citrine* (pMT41, 2x $DH-35S$ promoter) was obtained by replacing *pSCR* promoter from
346 pMT37 with the 2x $DH-35S$ promoter which was amplified with oligonucleotides MT61 and MT62 using
347 plasmid *pMYB76-2x $DH-35S$ min:GUS* as a template (Dickinson et al. 2020). Transgenic plants were generated
348 by introduction of the plant expression constructs in an *Agrobacterium tumefaciens* strain GV3101/2.
349 Transformation of *A. thaliana* Columbia (Col-0) accession was done by floral dipping. Plasmids contained
350 Basta resistance for plant selection. Oligonucleotides sequences are listed in Table S1.

351

352 **Growth conditions and phenotyping analyses**

353 Seeds were stratified at 4°C for 3 d in darkness and then sown on soil saturated with deionized water in a
354 Percival CU-36L4 incubator (Percival Scientific) at 21 °C, 85% relative humidity, and PAR =175 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$

under LD (16:8) conditions. After 13 days plants were transferred to the Scanalyzer HTS (LemnaTec) for acclimation (with day–night cycles and light conditions as in the incubator) 24 h before scanning. Experiments were performed under long-day conditions (LD, 16h day/8h night). For shade treatments, the R/FR was decreased from 4.2 to 0.2 using FR-emitting diodes positioned on the ceiling of the Scanalyzer HTS. Further experimental details, spectral composition of light, computation of the R/FR ratio, and technical specifications of the phenotyping device are described in detail in Dornbusch *et al.* (2012). For gene expression experiments in Fig. 1B and S1C, Col-0 plants were grown as described in de Wit *et al.* (2015). In brief, seeds were directly sown on soil and stratified at 4°C for 3 d in darkness. Plants were then grown for 14 days at 20 °C, 70% relative humidity, and PAR =220 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ under LD (16:8) conditions. Afterwards, plants were divided over two Percival I-66L incubators (Percival Scientific) at PAR =130 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 24 h before the start of the experiment. Experiment was performed the following day (day 16). At ZT3 on day 16, R/FR was decreased in one of the incubators from 1.4 to 0.2 using FR-emitting diodes positioned on the ceiling.

Pharmacological treatments

Indole-3-acetic acid (IAA, 10 μM ; Sigma-Aldrich) and (+/-)-abscisic acid (ABA, 1-10 μM ; Sigma-Aldrich) solutions were freshly prepared from concentrated dimethylsulfoxide (DMSO) and ethanol (EtOH) stocks, respectively, before each application. Mock solutions were similarly prepared to contain 0.15% (v/v) Tween-20, 0.1% (v/v) DMSO and 0.1% (v/v) EtOH, respectively.

Hormone quantification

ABA measurements were performed as previously described in Glauser *et al.* (2014). In brief, fresh frozen samples were ground to a fine powder using mortars and pestles under liquid nitrogen and about 40 mg of powder was weighed in 2.0 mL Eppendorf tubes. To the tubes were added 5-6 glass beads (2mm diameter), 990 μL of extraction solvent (ethylacetate/formic acid, 99.5 : 0.5, v/v) and 10 μL of internal standard solution containing D6-ABA at 100 ng/ml. The tubes were shaken for 4 min at 30 Hz in a tissue lyser, centrifuged for 3 min, the supernatant was recovered and the pellet re-extracted with 500 μL of extraction solvent. Both

solutions were then combined, evaporated and reconstituted in 100 μ L of methanol 70%. The final extracts were analyzed by UHPLC-MS/MS using an Ultimate 3000 RSLC (Thermo Scientific Dionex) coupled to a 4000 QTRAP (AB Sciex). To quantify ABA in plant samples, a calibration curve based on calibration points at 0.2, 2, 10, 50 and 200 ng/mL, all containing D6-ABA at a fixed concentration of 10 ng/mL, and weighted by $1/x$ was used (x refers to the concentration of the corresponding calibration point).

Stomata opening

Stomatal aperture was measured in leaves 1 and 2 of 2-week-old rosettes at ZT8, either in high R/FR or after 5h in LRFR, using dental paste imprints. We applied dental paste (Take 1 Advanced Light Body Wash, Kerr™, 34149) to the lower leaf epidermis. After solidification, imprints were removed from the leaf and covered with nail polish (60 Seconds Super Shine 740 Clear, Rimmel, France) which was left to dry and peeled off for visualization by light microscopy. For quantification, ellipses were fit into the opening in ImageJ and the length of the minor axis was used as a measure of stomatal aperture.

Leaf transpiration

Col-0 plants were grown for 21 d in hydroponics system in equinoctial (12:12) conditions at PAR=150 μ mol photons $m^{-2} s^{-1}$, 20°C and 60% relative humidity. Growth medium contained 1.5mM KNO₃, 0.75 mM Ca(NO₃)₂, 1 mM MgSO₄, 1 mM KH₂PO₄, 0.5 mM K₂SO₄, 0.35 mM CaCl₂, 12.25 μ M MnSO₄, 61.25 μ M H₃BO₃, 875 nM ZnSO₄, 437.5 nM CuSO₄, 175 nM (NH₄)₆Mo₇O₂₄ and 9 mg/L iron-EDTA (adapted from (Orsel et al. 2004)). Plants were mounted into custom-built gas exchange chambers (Kolling et al. 2015). Three plants were measured in a growth cabinet compartment with a high red to far-red light ratio, i.e. normal growth conditions. Four plants were measured in a growth cabinet compartment with far-red light supplementation by FR-emitting diodes (λ = 740nm). Far-red supplementation was started 3 h after onset of light on the second day. Gas exchange of the aerial parts of the plants was measured continuously for 48 h at 380ppm CO₂ using an infrared gas analyzer (LI-7000 CO₂/H₂O Gas Analyzer, LI-COR Biosciences GmbH). After measurement, leaf area size was extracted from photographs of individual plants and used to normalize the transpiration data. Data was processed, analyzed and plotted using custom-built software (George et al. 2018).

409

410 **Analysis of leaf position**

411 For time-lapse experiments, plants were scanned at intervals of 60 minutes with the Scanalyzer HTS
412 (Lemnatec). As output, we obtained time-lapse images in which the distance of measured plant surface
413 points from a reference plane was color-coded. These images were then transformed into 3D point clouds
414 that yield a precise representation of plant surfaces over time as previously described in Dornbusch *et al.*
415 (2012). Leaf elevation angle (tip elevation angle) was delineated by the vector taking as origin the position of
416 the basal end of the petiole organ and as extremity the position of the tip of the blade organ. A detailed
417 description of the geometric definitions of leaf elevation angle (ϕ_{tip}) as well as image and data processing are
418 available in Dornbusch *et al.* (2012, 2014).

419

420 **RT-qPCR**

421 RNA extraction, cDNA reverse transcription and quantitative RT-PCR were performed as previously described
422 (de Wit *et al.* 2015). In brief, entire leaves 1 and 2 (Fig. 1E) or petioles/lamina of leaves 3 (Fig. 1B and S1C,
423 samples from de Wit *et al.* , 2015) were separately pooled into three biological replicates and frozen in liquid
424 nitrogen. After consecutive RNA extraction and reverse transcription, RT-qPCR was performed in three
425 technical replicates for each sample using QuantStudio 6 Flex Real-Time PCR sequence detection system
426 (Applied Biosystems) and FastStart Universal SYBR green Master mix (Roche). Data were normalized against
427 two reference genes (YLS8, UBC) using the Biogazelle qbase software. Gene-specific oligonucleotides used
428 for qPCR reactions are listed in Table S1.

429

430 **ChIP-qPCR**

431 Seedlings were harvested and cross-linked as described in (Bourbousse *et al.* 2012). Subsequent steps of
432 chromatin immune-precipitation were performed as described in (Fiorucci *et al.* 2020) using an anti-HA
433 antibody (Santa Cruz Biotechnology, Inc., Dallas, TX, USA; sc-7392 X). For the long-term LRFR treatment
434 (Supplementary Figure 1) 4-day-old long-day grown *p35S-PIF4-3XHA* (Lorrain *et al.* 2008) seedlings were
435 either kept in the same conditions for an additional 3 days or transferred for 3 days into LRFR before

harvesting. For the short-term LRFR treatment (Fig. 1) 10-day-old *pPIF4p::PIF4-3XHA* (in *pif4-101*) seedlings were grown in long days and either kept in high R/FR or shifted at ZT2 to LRFR for 2h before harvesting in liquid nitrogen. Oligonucleotides used for ChIP-qPCR reactions are listed in S1 Table.

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Accession numbers

The Arabidopsis Genome Initiative numbers for the genes mentioned in this article are as follows: AT2G18790 (PHYB), AT2G43010 (PIF4), AT3G59060 (PIF5), AT5G61270 (PIF7), AT3G14440 (NCED3), AT1G30100 (NCED5), AT1G52340 (ABA2), AT4G17870 (PYR1), AT5G46790 (PYL1), AT2G26040 (PYL2), AT2G38310 (PYL4), AT5G05440 (PYL5), AT5G53160 (PYL8), AT4G26080 (ABI1), AT5G57050 (ABI2), AT1G72770 (HAB1), AT3G11410 (PP2CA), AT3G50500 (SNRK2.2), AT5G66880 (SNRK2.3), AT4G33950 (SNRK2.6), AT2G20875 (EPF1), AT1G34245 (EPF2), AT4G00360 (CYP86A2), AT1G29910 (CAB3), AT3G54220 (SCR), AT5G07700 (MYB76).

Figure legends

Figure 1. LRFR induces PIF-mediated ABA biosynthesis in leaves

(A) ABA concentration in entire leaves 1 and 2 of wild-type (Col-0) in high R/FR (blue) versus low R/FR (red) conditions at 0 hours (ZT3) and 2 hours (ZT5) after start of treatment. Plants were grown for 14 days in standard long-day [LD, 16-h light, 8-h dark (16/8)] conditions. ZT0 corresponds to the beginning of the light period on day 15. Shade treatment started on day 15 at ZT3 by adding FR light to decrease the R/FR ratio. Each bar plot represents data from 4 biological replicates. Per replicate, 40 leaves (leaves 1 and 2) from 20 plant individuals were harvested and frozen in liquid nitrogen. (B) Relative fold expression of *NCED3* from leaf 3 of Col-0 plants in high R/FR (blue) versus low R/FR (red) conditions over time. Gene expression values were calculated as fold induction relative to a petiole sample at time=1h (ZT4) in high R/FR conditions. Plants

were grown for 15 d in standard long-day [LD, 16-h light, 8-h dark (16/8)] conditions. ZT0 corresponds to the beginning of the light period on day 16. Shade treatment started on day 16 at ZT3 by adding FR light to decrease the R/FR ratio. Petioles and lamina of leaf 3 were separately pooled into three biological replicates and frozen in liquid nitrogen. (C) ABA concentration in rosettes of Col-0 versus *nced3nced5* double mutant plants in high R/FR (blue) versus low R/FR (red) conditions at 2 hours (ZT5) after start of treatment. Plants were grown for 14 d in standard long-day [LD, 16-h light, 8-h dark (16/8)] conditions. ZT0 corresponds to the beginning of the light period on day 15. Shade treatment started on day 15 at ZT3 by adding FR light to decrease the R/FR ratio. Each bar plot represents data from 4 biological replicates. Per replicate, 15 and 20 entire rosettes of Col-0 and *nced3nced5* plants, respectively, were harvested and frozen in liquid nitrogen. (D) ABA concentration in rosettes of Col-0 and *pif4pif5pif7* triple plants in high R/FR (blue) versus low R/FR (red) conditions at 2 hours (ZT5) after start of treatment. Plants were grown for 14 d in standard long-day [LD, 16-h light, 8-h dark (16/8)] conditions. ZT0 corresponds to the beginning of the light period on day 15. Shade treatment started on day 15 at ZT3 by adding FR light to decrease the R/FR ratio. Each bar plot represents data from 4 biological replicates. Per replicate, 15 entire rosettes were harvested and frozen in liquid nitrogen. (E) Relative fold expression of *NCED3* in Col-0 and *pif4pif5pif7* from leaves 1 and 2 in high R/FR (blue) versus LRFR (red) conditions. Gene expression values were calculated as fold induction relative to a sample in high R/FR conditions. Plants were grown for 14 d in standard long-day [LD, 16-h light, 8-h dark (16/8)] conditions. ZT0 corresponds to the beginning of the light period on day 15. Shade treatment started on day 15 at ZT3 by adding FR light to decrease the R/FR ratio. At ZT5 (+/- 2h shade), entire leaves 1 and 2 were separately pooled into three biological replicates and frozen in liquid nitrogen. (F) Schematic representation of the *NCED3* and *HFR1* genes. Regions amplified by qPCR and relative positions of G- and PBE-boxes are depicted relative to the start codon. (G) PIF4-HA binding to the *NCED3* and *HFR1* promoter regions. Input and immunoprecipitated DNA was extracted from 10 day-old *PIF4::PIF4-HA(pif4-101)* seedlings exposed to +/- 2 hours of LRFR from ZT2 and quantified by qPCR. PIF4-HA enrichment is presented as IP/input. Bars represent the mean from 3 technical replicates. T-tests were performed and asterisks indicate significant difference ($P < 0.05$) between peak and control considering shade conditions. (A-E, G) Error bars represent the twofold SE of mean estimates. Two-way ANOVAs followed by Tukey's Honestly Significant Difference (HSD) test were performed and different letters were assigned to significantly different groups.

Figure 2. Diel and shade-induced hyponasties both require a functional ABA biosynthetic pathway

(A) Leaf elevation angle of leaves 1 and 2 in Col-0 (black) and *nced3nced5* mutant (blue) plants in high R/FR (solid) versus low R/FR (dashed) conditions. Leaf elevation angles are mean values ($n=44-58$). (B) Leaf elevation angle of leaves 1 and 2 in Col-0 (black) and *aba2* mutant (orange) plants in high R/FR (solid) versus low R/FR (dashed) conditions. Leaf elevation angles are mean values ($n=44-60$). (A-B) Plants were grown for 14 days in standard long-day (LD, 16/8) conditions. Imaging started on day 15 at ZT0 ($t=0$), plants were maintained in LD. Shade treatment started at ZT3 by adding FR light to decrease the R/FR ratio. Col-0

plants analyzed in (A) and (B) are same. Opaque bands around mean lines represent the 95% confidence interval of mean estimates. Vertical gray bars represent night periods. (C) Boxplots representing the amplitude of leaf movement between maximum and minimum leaf elevation angles over the time period from t=3 to t=16 and computed for each individual leaf analyzed in (A) and (B). Solid and dashed plots represent data from high R/FR and low R/FR conditions, respectively. Two-way ANOVA followed by Tukey's HSD test were performed and different letters were assigned to significantly different groups (p-value < 0.05).

Figure 3. Functional ABA signaling is required for diel and shade-induced hyponasties

(A) Boxplots representing the amplitude of leaf movement between maximum and minimum leaf elevation angles over the time period from t=3 to t=16 of Col-0 (black), *pyr1pyl1pyl2pyl4* quadruple mutant (1124, red) and *pyr1pyl1pyl2pyl4pyl5pyl8* sextuple mutant (112458, green). Data was computed for each individual leaf analyzed in Figure S4 A, B. Solid and dashed plots represent data from high R/FR and low R/FR conditions, respectively. (B, C) Boxplots representing the amplitude of leaf movement between maximum and minimum leaf elevation angles over the time period from t=3 to t=16 (solid plots, high R/FR) or from t=27 to t=40 (dashed plots, low R/FR) for Col-0 (black), *abi1abi2hab1pp2ca* mutant (B, brown, *Qabi2*) or *snrk2.2snrk2.3snrk2.6* mutant (C, blue) plants and computed for each individual leaf analyzed in Figure S3C (B) or in Figure S3F (C). (A-C) Two-way ANOVA followed by Tukey's HSD test were performed and different letters were assigned to significantly different groups (p-value < 0.05).

Figure 4. Hormone treatments reveal dysfunctional auxin-induced hyponasty in ABA mutants and reduced sensitivity to ABA in LRFR.

(A) Leaf elevation angle of leaves 1 and 2 in Col-0 (black) and *nced3nced5* mutant (blue) plants treated with mock solution (solid lines) or 10 μ M IAA (dashed lines). At ZT3 on day 15 (t=3) a 1- μ L drop of the corresponding solution was applied to the leaf tip (adaxial side). Leaf elevation angles are mean values (n=22-30). (B, C) Leaf elevation angle of leaves 1 and 2 in Col-0 plants treated with mock (black), 1 μ M ABA (B, red) and 10 μ M ABA (C, green) solutions in high R/FR (solid) versus low R/FR (dashed) conditions. Leaf elevation angles are mean values (n=26-30). At ZT3 on day 15 (t=3), the corresponding solution was sprayed on the entire leaves (adaxial side). Shade treatment started at t=3 by adding FR light to decrease the R/FR ratio. Col-0 plants analyzed in (B) and (C) are same. (A-C) Plants were grown for 14 days in standard long-day (LD, 16/8) conditions. Imaging started on day 15 at ZT0 (t=0), plants were maintained in LD. Opaque bands around mean lines represent the 95% confidence interval of mean estimates. Vertical gray bars represent night periods.

Figure 5. Transpiration and stomata opening during LRFR-induced hyponasty

(A) Transpiration of the aerial parts of 21-day-old Col-0 plants grown in hydroponics system in equinoctial (12:12) conditions, measured by infrared gas analysis (illumination at 150 μ mol m⁻² s⁻¹, 20 °C, 60% relative humidity, 380 ppm CO₂). Mean values from three and four biological replicates ($\pm$ SD) are given for high R/FR

and low R/FR treatments, respectively. Vertical gray bars represent night periods. Supplementation with FR light 3 h into the second day is indicated. (B) Stomatal pore width in high and low R/FR at ZT8 (low R/FR from ZT3), from dental paste imprints of 2-week-old plants grown in LD. P-value given by a T-test without assumption of equal variance. (C) Leaf elevation angle of leaves 1 and 2 in Col-0 (black), *epf1epf2* double mutant (orange), *EPF2-OX* mutant (blue) plants in high R/FR then low R/FR conditions. Shade treatment started on day 16 at t=27 (ZT3) by adding FR light to decrease the R/FR ratio. Leaf elevation angles are mean values (n=29-60). Plants were grown for 14 days in standard long-day (LD, 16/8) conditions. Imaging started on day 15 at ZT0 (t=0), plants were maintained in LD. Opaque bands around mean lines represent the 95% confidence interval of mean estimates. Vertical gray bars represent night periods.

Figure 6. ABA signaling in multiple tissues modulates the hyponastic response

(A-D) Leaf elevation angle of leaves 1 and 2 in Col-0 (A-D, black), *CAB3::abi1-1* mutant (A, red, line #MT36-11), *pCYP86A2::abi1-1* mutant (B, blue, line #MT39-03), *pMYB76::abi1-1* mutant (C, green, line #MT41-13), *pSCR::abi1-1* mutant (D, orange, line #MT37-24) plants in high R/FR then low R/FR conditions. Shade treatment started on day 16 at t=27 (ZT3) by adding FR light to decrease the R/FR ratio. Leaf elevation angles are mean values (A, n=18-24; B, n=6-30; C, n=16-28; D, n=16-26). (E-G) Leaf elevation angle of leaves 1 and 2 in Col-0 (E-G, black), *pCAB3::abi1-1* mutant (E, red, line #MT36-11), *pCYP86A2::abi1-1* mutant (F, blue, line #MT39-03), *pMYB76::abi1-1* mutant (G, green, line #MT41-13) plants sprayed with mock solution (solid lines) or 10 μ M ABA (dashed lines). At ZT3 on day 15 (t=3) mock or ABA solutions were sprayed on the entire rosette (adaxial side). Col-0 plants analyzed in (E) and (G) are same. Leaf elevation angles are mean values (E, n=26-28; F, n=25-28; G, n=21-27). (A-G) Plants were grown for 14 days in standard long-day (LD, 16/8) conditions. Imaging started on day 15 at ZT0 (t=0), plants were maintained in LD. Opaque bands around mean lines represent the 95% confidence interval of mean estimates. Vertical gray bars represent night periods.

562 **Supporting Information**

563

564 **Figure S1.** Detailed analysis of LRFR induced and PIF-mediated ABA biosynthesis in leaves

565 **Figure S2.** Diel and shade-induced hyponasties in *nced* single mutants

566 **Figure S3.** Functional ABA signaling is required for diel and shade-induced hyponasties

567 **Figure S4.** Detailed analysis of auxin-induced hyponasty in ABA biosynthetic mutants

568 **Figure S5.** Validation of stomatal measurement methodology

569 **Figure S6.** Dysfunctional ABA signaling in internal leaf tissues affects light-modulated hyponastic responses

570 **Table S1.** Primers used in this study

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References

- Adams S, Grundy J, Veflingstad SR, Dyer NP, Hannah MA, Ott S, Carre IA (2018) Circadian control of abscisic acid biosynthesis and signalling pathways revealed by genome-wide analysis of LHY binding targets. *New Phytol* 220 (3):893-907. doi:10.1111/nph.15415
- Antoni R, Gonzalez-Guzman M, Rodriguez L, Peirats-Llobet M, Pizzio GA, Fernandez MA, De Winne N, De Jaeger G, Dietrich D, Bennett MJ, Rodriguez PL (2013) PYRABACTIN RESISTANCE1-LIKE8 plays an important role for the regulation of abscisic acid signaling in root. *Plant Physiol* 161 (2):931-941. doi:10.1104/pp.112.208678
- Ballare CL, Pierik R (2017) The shade-avoidance syndrome: multiple signals and ecological consequences. *Plant Cell Environ* 40 (11):2530-2543. doi:10.1111/pce.12914
- Barberon M, Vermeer JE, De Bellis D, Wang P, Naseer S, Andersen TG, Humbel BM, Nawrath C, Takano J, Salt DE, Geldner N (2016) Adaptation of Root Function by Nutrient-Induced Plasticity of Endodermal Differentiation. *Cell* 164 (3):447-459. doi:10.1016/j.cell.2015.12.021
- Benschop JJ, Millenaar FF, Smeets ME, van Zanten M, Voeseek LA, Peeters AJ (2007) Absciscic acid antagonizes ethylene-induced hyponastic growth in Arabidopsis. *Plant Physiol* 143 (2):1013-1023. doi:10.1104/pp.106.092700
- Bourbousse C, Ahmed I, Roudier F, Zabulon G, Blondet E, Balergue S, Colot V, Bowler C, Barneche F (2012) Histone H2B monoubiquitination facilitates the rapid modulation of gene expression during Arabidopsis photomorphogenesis. *PLoS Genet* 8 (7):e1002825. doi:10.1371/journal.pgen.1002825
- Cagnola JJ, Ploschuk E, Benech-Arnold T, Finlayson SA, Casal JJ (2012) Stem transcriptome reveals mechanisms to reduce the energetic cost of shade-avoidance responses in tomato. *Plant Physiol* 160 (2):1110-1119. doi:10.1104/pp.112.201921
- Casal JJ (2013) Photoreceptor signaling networks in plant responses to shade. *Annu Rev Plant Biol* 64:403-427. doi:10.1146/annurev-arplant-050312-120221
- Casal JJ, Balasubramanian S (2019) Thermomorphogenesis. *Annu Rev Plant Biol* 70:321-346. doi:10.1146/annurev-arplant-050718-095919
- Casal JJ, Questa JJ (2018) Light and temperature cues: multitasking receptors and transcriptional integrators. *New Phytol* 217 (3):1029-1034. doi:10.1111/nph.14890
- Cheng MC, Kathare PK, Paik I, Huq E (2021) Phytochrome Signaling Networks. *Annu Rev Plant Biol* 72:217-244. doi:10.1146/annurev-arplant-080620-024221
- Crawford AJ, McLachlan DH, Hetherington AM, Franklin KA (2012) High temperature exposure increases plant cooling capacity. *Curr Biol* 22 (10):R396-397. doi:10.1016/j.cub.2012.03.044
- de Wit M, Keuskamp DH, Bongers FJ, Hornitschek P, Gommers CMM, Reinen E, Martinez-Ceron C, Fankhauser C, Pierik R (2016) Integration of Phytochrome and Cryptochrome Signals Determines Plant Growth during Competition for Light. *Curr Biol* 26 (24):3320-3326. doi:10.1016/j.cub.2016.10.031
- de Wit M, Ljung K, Fankhauser C (2015) Contrasting growth responses in lamina and petiole during neighbor detection depend on differential auxin responsiveness rather than different auxin levels. *New Phytol* 208 (1):198-209. doi:10.1111/nph.13449
- Dickinson PJ, Knerova J, Szczotka M, Stevenson SR, Burgess SJ, Mulvey H, Bagman AM, Gaudinier A, Brady SM, Hibberd JM (2020) A bipartite transcription factor module controlling expression in the bundle sheath of Arabidopsis thaliana. *Nat Plants* 6 (12):1468-1479. doi:10.1038/s41477-020-00805-w
- Dornbusch T, Michaud O, Xenarios I, Fankhauser C (2014) Differentially phased leaf growth and movements in Arabidopsis depend on coordinated circadian and light regulation. *Plant Cell* 26 (10):3911-3921. doi:10.1105/tpc.114.129031
- Duan L, Dietrich D, Ng CH, Chan PM, Bhalerao R, Bennett MJ, Dinneny JR (2013) Endodermal ABA signaling promotes lateral root quiescence during salt stress in Arabidopsis seedlings. *Plant Cell* 25 (1):324-341. doi:10.1105/tpc.112.107227
- Fiorucci AS, Galvao VC, Ince YC, Boccaccini A, Goyal A, Allenbach Petrolati L, Trevisan M, Fankhauser C (2020) PHYTOCHROME INTERACTING FACTOR 7 is important for early responses to elevated temperature in Arabidopsis seedlings. *New Phytol* 226 (1):50-58. doi:10.1111/nph.16316

- 625 Frey A, Effroy D, Lefebvre V, Seo M, Perreau F, Berger A, Sechet J, To A, North HM, Marion-Poll A (2012)
- 626 Epoxycarotenoid cleavage by NCED5 fine-tunes ABA accumulation and affects seed dormancy and
- 627 drought tolerance with other NCED family members. *Plant J* 70 (3):501-512. doi:10.1111/j.1365-
- 628 313X.2011.04887.x
- 629 Fujii H, Zhu JK (2009) Arabidopsis mutant deficient in 3 abscisic acid-activated protein kinases reveals critical
- 630 roles in growth, reproduction, and stress. *Proc Natl Acad Sci U S A* 106 (20):8380-8385.
- 631 doi:10.1073/pnas.0903144106
- 632 Gao C, Liu X, De Storme N, Jensen KH, Xu Q, Yang J, Liu X, Chen S, Martens HJ, Schulz A, Liesche J (2020)
- 633 Directionality of Plasmodesmata-Mediated Transport in Arabidopsis Leaves Supports Auxin
- 634 Channeling. *Curr Biol* 30 (10):1970-1977 e1974. doi:10.1016/j.cub.2020.03.014
- 635 George GM, Kolling K, Kuenzli R, Hirsch-Hoffmann M, Flutsch P, Zeeman SC (2018) Design and Use of a
- 636 Digitally Controlled Device for Accurate, Multiplexed Gas Exchange Measurements of the Complete
- 637 Foliar Parts of Plants. *Methods Mol Biol* 1770:45-68. doi:10.1007/978-1-4939-7786-4_3
- 638 Gonzalez CV, Ibarra SE, Piccoli PN, Botto JF, Boccalandro HE (2012) Phytochrome B increases drought
- 639 tolerance by enhancing ABA sensitivity in Arabidopsis thaliana. *Plant Cell Environ* 35 (11):1958-1968.
- 640 doi:10.1111/j.1365-3040.2012.02529.x
- 641 Gonzalez-Grandio E, Poza-Carrion C, Sorzano CO, Cubas P (2013) BRANCHED1 promotes axillary bud
- 642 dormancy in response to shade in Arabidopsis. *Plant Cell* 25 (3):834-850.
- 643 doi:10.1105/tpc.112.108480
- 644 Gonzalez-Guzman M, Apostolova N, Belles JM, Barrero JM, Piqueras P, Ponce MR, Micol JL, Serrano R,
- 645 Rodriguez PL (2002) The short-chain alcohol dehydrogenase ABA2 catalyzes the conversion of
- 646 xanthoxin to abscisic aldehyde. *Plant Cell* 14 (8):1833-1846. doi:10.1105/tpc.002477
- 647 Gonzalez-Guzman M, Pizzio GA, Antoni R, Vera-Sirera F, Merilo E, Bassel GW, Fernandez MA, Holdsworth MJ,
- 648 Perez-Amador MA, Kollist H, Rodriguez PL (2012) Arabidopsis PYR/PYL/RCAR receptors play a major
- 649 role in quantitative regulation of stomatal aperture and transcriptional response to abscisic acid.
- 650 *Plant Cell* 24 (6):2483-2496. doi:10.1105/tpc.112.098574
- 651 Grunwald Y, Wigoda N, Sade N, Yaaran A, Torne T, Gosa SC, Moran N, Moshelion M (2021) Arabidopsis leaf
- 652 hydraulic conductance is regulated by xylem sap pH, controlled, in turn, by a P-type H(+) -ATPase of
- 653 vascular bundle sheath cells. *Plant J* 106 (2):301-313. doi:10.1111/tpj.15235
- 654 Hayes S, Pantazopoulou CK, van Gelderen K, Reinen E, Tween AL, Sharma A, de Vries M, Prat S, Schuurink RC,
- 655 Testerink C, Pierik R (2019) Soil Salinity Limits Plant Shade Avoidance. *Curr Biol* 29 (10):1669-1676
- 656 e1664. doi:10.1016/j.cub.2019.03.042
- 657 Hepworth C, Doheny-Adams T, Hunt L, Cameron DD, Gray JE (2015) Manipulating stomatal density enhances
- 658 drought tolerance without deleterious effect on nutrient uptake. *New Phytol* 208 (2):336-341.
- 659 doi:10.1111/nph.13598
- 660 Holalu SV, Finlayson SA (2017) The ratio of red light to far red light alters Arabidopsis axillary bud growth and
- 661 abscisic acid signalling before stem auxin changes. *J Exp Bot* 68 (5):943-952. doi:10.1093/jxb/erw479
- 662 Holalu SV, Reddy SK, Blackman BK, Finlayson SA (2020) Phytochrome interacting factors 4 and 5 regulate
- 663 axillary branching via bud abscisic acid and stem auxin signalling. *Plant Cell Environ* 43 (9):2224-2238.
- 664 doi:10.1111/pce.13824
- 665 Hopkins R, Schmitt J, Stinchcombe JR (2008) A latitudinal cline and response to vernalization in leaf angle and
- 666 morphology in Arabidopsis thaliana (Brassicaceae). *New Phytol* 179 (1):155-164. doi:10.1111/j.1469-
- 667 8137.2008.02447.x
- 668 Huang J, Zhao X, Chory J (2019) The Arabidopsis Transcriptome Responds Specifically and Dynamically to High
- 669 Light Stress. *Cell Rep* 29 (12):4186-4199 e4183. doi:10.1016/j.celrep.2019.11.051
- 670 Keller MM, Jaillais Y, Pedmale UV, Moreno JE, Chory J, Ballare CL (2011) Cryptochrome 1 and phytochrome B
- 671 control shade-avoidance responses in Arabidopsis via partially independent hormonal cascades.
- 672 *Plant J* 67 (2):195-207. doi:10.1111/j.1365-313X.2011.04598.x
- 673 Kinoshita T, Toh S, Torii KU (2021) Chemical control of stomatal function and development. *Curr Opin Plant*
- 674 *Biol* 60:102010. doi:10.1016/j.pbi.2021.102010
- 675 Kohnen MV, Schmid-Siebert E, Trevisan M, Petrolati LA, Senechal F, Muller-Moule P, Maloof J, Xenarios I,
- 676 Fankhauser C (2016) Neighbor Detection Induces Organ-Specific Transcriptomes, Revealing Patterns
- 677 Underlying Hypocotyl-Specific Growth. *Plant Cell* 28 (12):2889-2904. doi:10.1105/tpc.16.00463

678 Kolling K, George GM, Kunzli R, Flutsch P, Zeeman SC (2015) A whole-plant chamber system for parallel gas
679 exchange measurements of Arabidopsis and other herbaceous species. *Plant Methods* 11:48.
680 doi:10.1186/s13007-015-0089-z

681 Lee KH, Piao HL, Kim HY, Choi SM, Jiang F, Hartung W, Hwang I, Kwak JM, Lee IJ, Hwang I (2006) Activation of
682 glucosidase via stress-induced polymerization rapidly increases active pools of abscisic acid. *Cell* 126
683 (6):1109-1120. doi:10.1016/j.cell.2006.07.034

684 Leon-Kloosterziel KM, Gil MA, Ruijs GJ, Jacobsen SE, Olszewski NE, Schwartz SH, Zeevaart JA, Koornneef M
685 (1996) Isolation and characterization of abscisic acid-deficient Arabidopsis mutants at two new loci.
686 *Plant J* 10 (4):655-661. doi:10.1046/j.1365-313x.1996.10040655.x

687 Lorrain S, Allen T, Duek PD, Whitelam GC, Fankhauser C (2008) Phytochrome-mediated inhibition of shade
688 avoidance involves degradation of growth-promoting bHLH transcription factors. *Plant J* 53 (2):312-
689 323. doi:10.1111/j.1365-313X.2007.03341.x

690 McClung CR (2013) Beyond Arabidopsis: the circadian clock in non-model plant species. *Semin Cell Dev Biol*
691 24 (5):430-436. doi:10.1016/j.semcdb.2013.02.007

692 Merlot S, Mustilli AC, Genty B, North H, Lefebvre V, Sotta B, Vavasseur A, Giraudat J (2002) Use of infrared
693 thermal imaging to isolate Arabidopsis mutants defective in stomatal regulation. *Plant J* 30 (5):601-
694 609. doi:10.1046/j.1365-313x.2002.01322.x

695 Miao R, Russinova E, Rodriguez PL (2022) Tripartite hormonal regulation of plasma membrane H(+)-ATPase
696 activity. *Trends Plant Sci.* doi:10.1016/j.tplants.2021.12.011

697 Michaud O, Fiorucci AS, Xenarios I, Fankhauser C (2017) Local auxin production underlies a spatially restricted
698 neighbor-detection response in Arabidopsis. *Proc Natl Acad Sci U S A* 114 (28):7444-7449.
699 doi:10.1073/pnas.1702276114

700 Millenaar FF, Van Zanten M, Cox MCH, Pierik R, Voeseek L, Peeters AJM (2009) Differential petiole growth
701 in Arabidopsis thaliana: photocontrol and hormonal regulation. *New Phytol* 184 (1):141-152.
702 doi:10.1111/j.1469-8137.2009.02921.x

703 Moreno JE, Tao Y, Chory J, Ballare CL (2009) Ecological modulation of plant defense via phytochrome control
704 of jasmonate sensitivity. *Proc Natl Acad Sci U S A* 106 (12):4935-4940. doi:10.1073/pnas.0900701106

705 Mullen JL, Weinig C, Hangarter RP (2006) Shade avoidance and the regulation of leaf inclination in
706 Arabidopsis. *Plant Cell Environ* 29 (6):1099-1106. doi:10.1111/j.1365-3040.2005.01484.x

707 Negin B, Yaaran A, Kelly G, Zait Y, Moshelion M (2019) Mesophyll Absciscic Acid Restrains Early Growth and
708 Flowering But Does Not Directly Suppress Photosynthesis. *Plant Physiol* 180 (2):910-925.
709 doi:10.1104/pp.18.01334

710 Nozue K, Tat AV, Kumar Devisetty U, Robinson M, Mumbach MR, Ichihashi Y, Lekkala S, Maloof JN (2015)
711 Shade avoidance components and pathways in adult plants revealed by phenotypic profiling. *PLoS*
712 *Genet* 11 (4):e1004953. doi:10.1371/journal.pgen.1004953

713 Orsel M, Eulenbuck K, Krapp A, Daniel-Vedele F (2004) Disruption of the nitrate transporter genes AtNRT2.1
714 and AtNRT2.2 restricts growth at low external nitrate concentration. *Planta* 219 (4):714-721.
715 doi:10.1007/s00425-004-1266-x

716 Ortiz-Alcaide M, Llamas E, Gomez-Cadenas A, Nagatani A, Martinez-Garcia JF, Rodriguez-Concepcion M
717 (2019) Chloroplasts Modulate Elongation Responses to Canopy Shade by Retrograde Pathways
718 Involving HY5 and Absciscic Acid. *Plant Cell* 31 (2):384-398. doi:10.1105/tpc.18.00617

719 Pantazopoulou CK, Bongers FJ, Kupers JJ, Reinen E, Das D, Evers JB, Anten NPR, Pierik R (2017) Neighbor
720 detection at the leaf tip adaptively regulates upward leaf movement through spatial auxin dynamics.
721 *Proc Natl Acad Sci U S A* 114 (28):7450-7455. doi:10.1073/pnas.1702275114

722 Park SY, Fung P, Nishimura N, Jensen DR, Fujii H, Zhao Y, Lumba S, Santiago J, Rodrigues A, Chow TF, Alfred
723 SE, Bonetta D, Finkelstein R, Provart NJ, Desveaux D, Rodriguez PL, McCourt P, Zhu JK, Schroeder JI,
724 Volkman BF, Cutler SR (2009) Absciscic acid inhibits type 2C protein phosphatases via the PYR/PYL
725 family of START proteins. *Science* 324 (5930):1068-1071. doi:10.1126/science.1173041

726 Park SY, Peterson FC, Mosquna A, Yao J, Volkman BF, Cutler SR (2015) Agrochemical control of plant water
727 use using engineered abscisic acid receptors. *Nature* 520 (7548):545-548. doi:10.1038/nature14123

728 Pedmale UV, Huang SC, Zander M, Cole BJ, Hetzel J, Ljung K, Reis PAB, Sridevi P, Nito K, Nery JR, Ecker JR,
729 Chory J (2016) Cryptochromes Interact Directly with PIFs to Control Plant Growth in Limiting Blue
730 Light. *Cell* 164 (1-2):233-245. doi:10.1016/j.cell.2015.12.018

731 Polko JK, van Zanten M, van Rooij JA, Maree AF, Voesenek LA, Peeters AJ, Pierik R (2012) Ethylene-induced
732 differential petiole growth in *Arabidopsis thaliana* involves local microtubule reorientation and cell
733 expansion. *New Phytol* 193 (2):339-348. doi:10.1111/j.1469-8137.2011.03920.x

734 Prado K, Cotellet V, Li G, Bellati J, Tang N, Tournaire-Roux C, Martinieri A, Santoni V, Maurel C (2019)
735 Oscillating Aquaporin Phosphorylation and 14-3-3 Proteins Mediate the Circadian Regulation of Leaf
736 Hydraulics. *Plant Cell* 31 (2):417-429. doi:10.1105/tpc.18.00804

737 Preuten T, Hohm T, Bergmann S, Fankhauser C (2013) Defining the site of light perception and initiation of
738 phototropism in *Arabidopsis*. *Curr Biol* 23 (19):1934-1938. doi:10.1016/j.cub.2013.07.079

739 Qin X, Zeevaart JA (2002) Overexpression of a 9-cis-epoxycarotenoid dioxygenase gene in *Nicotiana*
740 *plumbaginifolia* increases abscisic acid and phaseic acid levels and enhances drought tolerance. *Plant*
741 *Physiol* 128 (2):544-551. doi:10.1104/pp.010663

742 Quint M, Delker C, Franklin KA, Wigge PA, Halliday KJ, van Zanten M (2016) Molecular and genetic control of
743 plant thermomorphogenesis. *Nat Plants* 2:15190. doi:10.1038/nplants.2015.190

744 Raghavendra AS, Gonugunta VK, Christmann A, Grill E (2010) ABA perception and signalling. *Trends Plant Sci*
745 15 (7):395-401. doi:10.1016/j.tplants.2010.04.006

746 Rauf M, Arif M, Fisahn J, Xue GP, Balazadeh S, Mueller-Roeber B (2013) NAC transcription factor speedy
747 hyponastic growth regulates flooding-induced leaf movement in *Arabidopsis*. *Plant Cell* 25 (12):4941-
748 4955. doi:10.1105/tpc.113.117861

749 Rubio S, Rodrigues A, Saez A, Dizon MB, Galle A, Kim TH, Santiago J, Flexas J, Schroeder JI, Rodriguez PL (2009)
750 Triple loss of function of protein phosphatases type 2C leads to partial constitutive response to
751 endogenous abscisic acid. *Plant Physiol* 150 (3):1345-1355. doi:10.1104/pp.109.137174

752 Tardieu F, Simonneau T, Parent B (2015) Modelling the coordination of the controls of stomatal aperture,
753 transpiration, leaf growth, and abscisic acid: update and extension of the Tardieu-Davies model. *J*
754 *Exp Bot* 66 (8):2227-2237. doi:10.1093/jxb/erv039

755 van Zanten M, Voesenek LA, Peeters AJ, Millenaar FF (2009) Hormone- and light-mediated regulation of heat-
756 induced differential petiole growth in *Arabidopsis*. *Plant Physiol* 151 (3):1446-1458.
757 doi:10.1104/pp.109.144386

758 Voesenek LA, Colmer TD, Pierik R, Millenaar FF, Peeters AJ (2006) How plants cope with complete
759 submergence. *New Phytol* 170 (2):213-226. doi:10.1111/j.1469-8137.2006.01692.x

760 Weiner JJ, Peterson FC, Volkman BF, Cutler SR (2010) Structural and functional insights into core ABA
761 signaling. *Current Opinion in Plant Biology* 13 (5):495-502. doi:10.1016/j.pbi.2010.09.007

762 Yoshida T, Christmann A, Yamaguchi-Shinozaki K, Grill E, Fernie AR (2019) Revisiting the Basal Role of ABA -
763 Roles Outside of Stress. *Trends Plant Sci* 24 (7):625-635. doi:10.1016/j.tplants.2019.04.008

764 Zhang B, Holmlund M, Lorrain S, Norberg M, Bako L, Fankhauser C, Nilsson O (2017) BLADE-ON-PETIOLE
765 proteins act in an E3 ubiquitin ligase complex to regulate PHYTOCHROME INTERACTING FACTOR 4
766 abundance. *Elife* 6. doi:10.7554/eLife.26759

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Figure 1. LRFR induces PIF-mediated ABA biosynthesis in leaves

(A) ABA concentration in entire leaves 1 and 2 of wild-type (Col-0) in high R/FR (blue) versus low R/FR (red) conditions at 0 hours (ZT3) and 2 hours (ZT5) after start of treatment. Plants were grown for 14 days in standard long-day [LD, 16-h light, 8-h dark (16/8)] conditions. ZT0 corresponds to the beginning of the light period on day 15. Shade treatment started on day 15 at ZT3 by adding FR light to decrease the R/FR ratio. Each bar plot represents data from 4 biological replicates. Per replicate, 40 leaves (leaves 1 and 2) from 20 plant individuals were harvested and frozen in liquid nitrogen. (B) Relative fold expression of *NCED3* from leaf 3 of Col-0 plants in high R/FR (blue) versus low R/FR (red) conditions over time. Gene expression values were calculated as fold induction relative to a petiole sample at time=1h (ZT4) in high R/FR conditions. Plants were grown for 15 d in standard long-day [LD, 16-h light, 8-h dark (16/8)] conditions.

778 ZT0 corresponds to the beginning of the light period on day 16. Shade treatment started on day 16 at ZT3 by adding FR
779 light to decrease the R/FR ratio. Petioles and lamina of leaf 3 were separately pooled into three biological replicates and
780 frozen in liquid nitrogen. (C) ABA concentration in rosettes of Col-0 versus *nced3nced5* double mutant plants in high
781 R/FR (blue) versus low R/FR (red) conditions at 2 hours (ZT5) after start of treatment. Plants were grown for 14 d in
782 standard long-day [LD, 16-h light, 8-h dark (16/8)] conditions. ZT0 corresponds to the beginning of the light period on
783 day 15. Shade treatment started on day 15 at ZT3 by adding FR light to decrease the R/FR ratio. Each bar plot represents
784 data from 4 biological replicates. Per replicate, 15 and 20 entire rosettes of Col-0 and *nced3nced5* plants, respectively,
785 were harvested and frozen in liquid nitrogen. (D) ABA concentration in rosettes of Col-0 and *pif4pif5pif7* triple plants in
786 high R/FR (blue) versus low R/FR (red) conditions at 2 hours (ZT5) after start of treatment. Plants were grown for 14 d
787 in standard long-day [LD, 16-h light, 8-h dark (16/8)] conditions. ZT0 corresponds to the beginning of the light period on
788 day 15. Shade treatment started on day 15 at ZT3 by adding FR light to decrease the R/FR ratio. Each bar plot represents
789 data from 4 biological replicates. Per replicate, 15 entire rosettes were harvested and frozen in liquid nitrogen. (E)
790 Relative fold expression of *NCED3* in Col-0 and *pif4pif5pif7* from leaves 1 and 2 in high R/FR (blue) versus LRFR (red)
791 conditions. Gene expression values were calculated as fold induction relative to a sample in high R/FR conditions. Plants
792 were grown for 14 d in standard long-day [LD, 16-h light, 8-h dark (16/8)] conditions. ZT0 corresponds to the beginning
793 of the light period on day 15. Shade treatment started on day 15 at ZT3 by adding FR light to decrease the R/FR ratio.
794 At ZT5 (+/- 2h shade), entire leaves 1 and 2 were separately pooled into three biological replicates and frozen in liquid
795 nitrogen. (F) Schematic representation of the *NCED3* and *HFR1* genes. Regions amplified by qPCR and relative positions
796 of G- and PBE-boxes are depicted relative to the start codon. (G) PIF4-HA binding to the *NCED3* and *HFR1* promoter
797 regions. Input and immunoprecipitated DNA was extracted from 10 day-old *PIF4::PIF4-HA(pif4-101)* seedlings exposed
798 to +/- 2 hours of LRFR from ZT2 and quantified by qPCR. PIF4-HA enrichment is presented as IP/input. Bars represent
799 the mean from 3 technical replicates. T-tests were performed and asterisks indicate significant difference ($P < 0.05$)
800 between peak and control considering shade conditions. (A-E, G) Error bars represent the twofold SE of mean estimates.
801 Two-way ANOVAs followed by Tukey's Honestly Significant Difference (HSD) test were performed and different letters
802 were assigned to significantly different groups.

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804

Figure 2. Diel and shade-induced hyponasties both require a functional ABA biosynthetic pathway

(A) Leaf elevation angle of leaves 1 and 2 in Col-0 (black) and *nced3nced5* mutant (blue) plants in high R/FR (solid) versus low R/FR (dashed) conditions. Leaf elevation angles are mean values (n=44-58). (B) Leaf elevation angle of leaves 1 and 2 in Col-0 (black) and *aba2* mutant (orange) plants in high R/FR (solid) versus low R/FR (dashed) conditions. Leaf elevation angles are mean values (n=44-60). (A-B) Plants were grown for 14 days in standard long-day (LD, 16/8) conditions. Imaging started on day 15 at ZT0 (t=0), plants were maintained in LD. Shade treatment started at ZT3 by adding FR light to decrease the R/FR ratio. Col-0 plants analyzed in (A) and (B) are same. Opaque bands around mean lines represent the 95% confidence interval of mean estimates. Vertical gray bars represent night periods. (C) Boxplots representing the amplitude of leaf movement between maximum and minimum leaf elevation angles over the time period from t=3 to t=16 and computed for each individual leaf analyzed in (A) and (B). Solid and dashed plots represent data from high R/FR and low R/FR conditions, respectively. Two-way ANOVA followed by Tukey's HSD test were performed and different letters were assigned to significantly different groups (p-value < 0.05).

Figure 3. Functional ABA signaling is required for diel and shade-induced hyponasties

(A) Boxplots representing the amplitude of leaf movement between maximum and minimum leaf elevation angles over the time period from t=3 to t=16 of Col-0 (black), *pyr1pyl1pyl2pyl4* quadruple mutant (1124, red) and *pyr1pyl1pyl2pyl4pyl5pyl8* sextuple mutant (112458, green). Data was computed for each individual leaf analyzed in Figure S4 A, B. Solid and dashed plots represent data from high R/FR and low R/FR conditions, respectively. (B, C) Boxplots representing the amplitude of leaf movement between maximum and minimum leaf elevation angles over the time period from t=3 to t=16 (solid plots, high R/FR) or from t=27 to t=40 (dashed plots, low R/FR) for Col-0 (black), *abi1abi2hab1pp2ca* mutant (B, brown, *Qabi2*) or *snrk2.2snrk2.3snrk2.6* mutant (C, blue) plants and computed for each individual leaf analyzed in Figure S3C (B) or in Figure S3F (C). (A-C) Two-way ANOVA followed by Tukey's HSD test were performed and different letters were assigned to significantly different groups (p-value < 0.05).

Figure 4. Hormone treatments reveal dysfunctional auxin-induced hyponasty in ABA mutants and reduced sensitivity to ABA in LRFR.

(A) Leaf elevation angle of leaves 1 and 2 in Col-0 (black) and *nced3nced5* mutant (blue) plants treated with mock solution (solid lines) or 10μM IAA (dashed lines). At ZT3 on day 15 (t=3) a 1-μL drop of the corresponding solution was applied to the leaf tip (adaxial side). Leaf elevation angles are mean values (n=22-30). (B, C) Leaf elevation angle of leaves 1 and 2 in Col-0 plants treated with mock (black), 1μM ABA (B, red) and 10μM ABA (C, green) solutions in high R/FR (solid) versus low R/FR (dashed) conditions. Leaf elevation angles are mean values (n=26-30). At ZT3 on day 15 (t=3), the corresponding solution was sprayed on the entire leaves (adaxial side). Shade treatment started at t=3 by adding FR light to decrease the R/FR ratio. Col-0 plants analyzed in (B) and (C) are same. (A-C) Plants were grown for 14 days in standard long-day (LD, 16/8) conditions. Imaging started on day 15 at ZT0 (t=0), plants were maintained in LD. Opaque bands around mean lines represent the 95% confidence interval of mean estimates. Vertical gray bars represent night periods.

Figure 5. Transpiration and stomata opening during LRFR-induced hyponasty

(A) Transpiration of the aerial parts of 21-day-old Col-0 plants grown in hydroponics system in equinoctial (12:12) conditions, measured by infrared gas analysis (illumination at 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 20 °C, 60% relative humidity, 380 ppm CO₂). Mean values from three and four biological replicates ($\pm$ SD) are given for high R/FR and low R/FR treatments, respectively. Vertical gray bars represent night periods. Supplementation with FR light 3 h into the second day is indicated. (B) Stomatal pore width in high and low R/FR at ZT8 (low R/FR from ZT3), from dental paste imprints of 2-week-old plants grown in LD. P-value given by a T-test without assumption of equal variance. (C) Leaf elevation angle of leaves 1 and 2 in Col-0 (black), *epf1epf2* double mutant (orange), *EPF2-OX* mutant (blue) plants in high R/FR then low R/FR conditions. Shade treatment started on day 16 at t=27 (ZT3) by adding FR light to decrease the R/FR ratio. Leaf elevation angles are mean values (n=29-60). Plants were grown for 14 days in standard long-day (LD, 16/8) conditions. Imaging started on day 15 at ZT0 (t=0), plants were maintained in LD. Opaque bands around mean lines represent the 95% confidence interval of mean estimates. Vertical gray bars represent night periods.

Figure 6. ABA signaling in multiple tissues modulates the hyponastic response

(A-D) Leaf elevation angle of leaves 1 and 2 in Col-0 (A-D, black), *CAB3::abi1-1* mutant (A, red, line #MT36-11), *pCYP86A2::abi1-1* mutant (B, blue, line #MT39-03), *pMYB76::abi1-1* mutant (C, green, line #MT41-13), *pSCR::abi1-1* mutant (D, orange, line #MT37-24) plants in high R/FR then low R/FR conditions. Shade treatment started on day 16 at t=27 (ZT3) by adding FR light to decrease the R/FR ratio. Leaf elevation angles are mean values (A, n=18-24; B, n=6-30; C, n=16-28; D, n=16-26). (E-G) Leaf elevation angle of leaves 1 and 2 in Col-0 (E-G, black), *pCAB3::abi1-1* mutant (E, red,

865 line #MT36-11), pCYP86A2::*abi1-1* mutant (F, blue, line #MT39-03), pMYB76::*abi1-1* mutant (G, green, line #MT41-13)
866 plants sprayed with mock solution (solid lines) or 10 μ M ABA (dashed lines). At ZT3 on day 15 (t=3) mock or ABA solutions
867 were sprayed on the entire rosette (adaxial side). Col-0 plants analyzed in (E) and (G) are same. Leaf elevation angles
868 are mean values (E, n=26-28; F, n=25-28; G, n=21-27). (A-G) Plants were grown for 14 days in standard long-day (LD,
869 16/8) conditions. Imaging started on day 15 at ZT0 (t=0), plants were maintained in LD. Opaque bands around mean
870 lines represent the 95% confidence interval of mean estimates. Vertical gray bars represent night periods.
871