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1 **DEK influences the trade-off between growth and**
2 **arrest via H2A.Z-nucleosomes in Arabidopsis**

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23

24

25 **Abstract**

26

27 The decision of whether to grow and proliferate or to restrict growth and
28 develop resilience to stress is a key biological trade-off. In plants, constitutive
29 growth results in increased sensitivity to environmental stress^{1,2}. The
30 underlying mechanisms controlling this decision are however not well
31 understood. We used temperature as a cue to discover regulators of this
32 process in plants, as it both enhances growth and development rates within a
33 specific range and is also a stress at extremes. We found that the conserved
34 chromatin-associated protein DEK plays a central role in balancing the
35 response between growth and arrest in Arabidopsis, and it does this via
36 H2A.Z-nucleosomes. DEK target genes show two distinct categories of
37 chromatin architecture based on the distribution of H2A.Z in +1 nucleosome
38 and gene body, and these predict induction or repression by DEK. We show
39 that these chromatin signatures of DEK target genes are conserved in human
40 cells, suggesting that DEK may act through an evolutionarily conserved
41 mechanism to control the balance between growth and arrest in plants and
42 animals.

43

44 **Main**

45 Plants are exposed to daily fluctuations in ambient temperature, which
46 influence growth, development and fitness³. Being sessile organisms, plants
47 frequently need to opt between growth and stress resilience, making them
48 good systems to analyse how these trade-offs are made.

49

50 Recently, it has become clear that the chromatin landscape—i.e. the
51 positioning of the nucleosome containing histone variants such as H2A.Z and
52 H3.3 across the gene body—is correlated to the responsiveness of a gene to
53 environmental variation^{3–7}. For instance, hypersensitive environmental genes
54 are enriched in H2A.Z-nucleosomes in their gene bodies, suggesting its
55 involvement in the regulation of environmental response^{4,7}. Warm
56 temperature results in H2A.Z-nucleosome eviction and large-scale
57 transcriptional activation in plants^{8,9}. Furthermore, H2A.Z is removed from +1
58 nucleosomes of temperature-induced genes upon temperature increase
59 allowing activation of expression by transcription factors⁹. The molecular
60 mechanism underlying this response likely involves a re-organisation of the
61 chromatin landscape by chromatin remodelling enzymes. In this paper, we
62 demonstrate that DEK-DOMAIN CONTAINING PROTEIN3 (DEK3), the most
63 abundant member of a family of four DEK-domain containing chromatin re-
64 modellers in Arabidopsis¹⁰, is a link between the chromatin landscape and
65 environmental responsiveness.

66

67 The mammalian orthologue DEK is an oncoprotein involved in the
68 development of cancer, inflammation and stem cell biology^{11,12}. Because of its
69 key role in cancer, DEK has been intensely studied in animals, and it has
70 been described to have roles as an H3.3 chaperone, co-transcriptional
71 regulator and in splicing^{13–16}. Despite these studies, the genome-wide targets
72 directly regulated by DEK are not known¹⁷, and it is also unclear whether DEK
73 serves as an activator or inhibitor of transcription. In Drosophila and humans
74 DEK can serve as a H3.3 chaperone, and it remodels nucleosomes into more
75 transcriptionally active chromatin through its histone chaperone activity¹⁵. By
76 contrast, in mammalian cell lines DEK acts as a positive regulator of

77 heterochromatin formation, maintaining the balance between heterochromatin
78 and euchromatin *in vivo*¹⁴. DEK therefore appears to act as either an activator
79 or a repressor depending on the context.

80 In Arabidopsis, DEK was identified as a component of the nucleolus by mass
81 spectrometry¹⁶. DEK3 can change the topology of protein free DNA *in vitro*
82 and cause transcriptional repression of specific loci by increasing nucleosome
83 density¹⁰. Correct *DEK3* expression is critical for the degree of response of
84 the plants to some stress conditions, including high salinity and heat shock.
85 Plants with constitutively elevated levels of DEK3 are more sensitive to high
86 salinity, whereas plants deficient in *DEK3* are more salt tolerant¹⁰.

87

88 To further understand the global effects of DEK3 on gene expression in
89 plants, we used chromatin immunoprecipitation DNA-sequencing (ChIP-seq)
90 and RNA-seq to investigate how DEK3 occupancy influences the
91 responsiveness of gene expression to temperature. We show that DEK3
92 affects temperature-dependent biological trade-offs in Arabidopsis, through
93 the up-regulation of stress response genes and by the suppression of thermo-
94 responsive induction of growth and development genes. Furthermore,
95 inherent chromatin landscape features are sufficient to predict whether a gene
96 will be up- or down-regulated by overexpression of DEK3. Additionally, we
97 show for the first time that DEK3 genetically and physically interacts with
98 H2A.Z and modifies H2A.Z-nucleosome distribution within the gene bodies of
99 DEK3 target genes. We suggest a model whereby feedback between the
100 chromatin landscape and chromatin re-modellers can affect trade-off between
101 growth and arrest, and therefore influence developmental plasticity.

102

103

104

105 **Results**

106 **H2A.Z and DEK interact**

107 Since increasing temperature does not cause H2A.Z-nucleosome loss *in*
108 *vitro*⁹, we sought to determine if other factors may contribute to this process in
109 plants. To find H2A.Z interacting proteins, we performed H2A.Z affinity
110 purification (from *HTA11::HTA11-FLAG* expressing lines) coupled with mass
111 spectrometry. This approach identified three homologous DEK-domain
112 containing chromatin re-modellers and 6 previously reported H2A.Z
113 interactors¹⁸ among 263 proteins found in complex with H2A.Z *in vivo* (Supp.
114 Fig. 1a, Supp. Table.1). Two of them, DEK3 and DEK2, have been found
115 among 7 proteins enriched significantly in samples collected at 27°C
116 compared to 17°C (Fig. 1a, Supp. Table.1) suggesting a possible role in
117 temperature pathway regulated through H2A.Z nucleosomes. There are four
118 DEK-domain containing proteins in *Arabidopsis thaliana*, among them, DEK3
119 shows a strong and abundant expression in all plant organs¹⁰. Interestingly,
120 DEK3 shows one of the highest fold change when comparing the binding
121 affinities to H2A.Z complexes between 27°C and 17°C (Fig. 1a, Supp. Table
122 1). We confirmed the interaction of DEK3 with H2A.Z by co-
123 immunoprecipitating H2A.Z- containing nucleosomes and H2A.Z protein
124 (purified from plants), with purified DEK3 (Fig. 1b). This validation set up
125 mimics *in vitro* binding experiments while preserving post-translational
126 modifications of H2A.Z and DEK3. We find that H2A.Z purified from histone
127 extract coimmunoprecipitated with DEK3 in the comparable levels to H2A.Z
128 nucleosomes obtained from nuclear extract, despite reduced levels of other
129 histones as determined by H3 levels (Fig. 1b). This suggests that other
130 protein factors might not be necessary for the interaction between these two
131 proteins.

132 Previously, immunopurification of DEK3 has identified its interaction with
133 histones H3 and H4, but not with H2A and H2B¹⁰. The use of an antibody
134 directed against all H2A variants in this previous study made the detection of
135 H2A.Z very difficult as H2A.Z levels are only approximately 10 % of those of
136 H2A¹⁹.

137

138 **Perturbing *DEK* expression levels affects growth and stress response**

139 Temperature has a strong effect on plant growth and development. Growth in
140 high ambient temperatures (below the threshold of inducing widespread heat
141 stress), results in faster growth and accelerated development
142 (thermomorphogenesis), illustrated by increased hypocotyl length and early
143 flowering²⁰. In contrast, heat stress induces metabolic imbalance,
144 accumulation of toxic by-products and, adversely influencing reproductive
145 growth and yield quality²¹. Plant resilience to stress conditions such as high
146 salinity and heat shock requires the correct level of *DEK3*¹⁰. Additionally, a
147 *DEK3* null allele (*dek3-2*) shows an exaggerated response to warmer (non-
148 stressful) temperature, while overexpression of *DEK3* reduces the thermal
149 responsiveness of seedlings, both as measured by elongation of hypocotyl
150 and flowering time (Fig. 1c-d, Supp. Fig. 1b-c). The altered responsiveness of
151 *DEK3* mis-expressing plants to temperature changes appears to be a general
152 property, since *DEK3* overexpressors are also unable to acclimate to cold
153 temperature and show increased sensitivity to cold stress, applied with and
154 without acclimation (Fig. 1e). *dek3-2* plants are less sensitive to freezing
155 without acclimation (Fig. 1e, middle panel), suggesting a key role of *DEK3* in
156 cold resistance pathways.

157 We observe similarly altered hypocotyl growth in *dek2* and *dek4* mutants,
158 suggesting a general role for this family of genes in controlling response to
159 temperature (Supp. Fig. 1d).

160

161 To further understand why plants with abnormal *DEK3* protein levels exhibit
162 altered temperature responses, we investigated gene expression in *dek3-2*
163 and *35S::DEK3* plants at 17 °C and 27 °C. Since the ambient temperature
164 transcriptome is dynamic²², we sampled the transcriptome over the 24 h day-
165 night cycle (Supp. Fig. 1e-g). Consistent with plant phenotypes, the largest
166 perturbation in gene expression occurs in *35S::DEK3* at 27 °C (Supp. Fig. 1e).
167 The overexpression of *DEK3* caused ~4,860 genes to be miss-expressed in
168 at least one time-point compared to WT plants when grown at warm
169 temperature (Supp. table 2).

170 We used Principal Component Analysis (PCA) to further understand
171 differences in gene expression. Principle Components (PC) 1 and 2 together
172 could explain 54% of the gene expression variance and primarily separate the
173 samples based on time of day into day and night time samples (Supp. Fig. 1f).
174 Among the night samples, the expression of differentially expressed genes in
175 *35S::DEK3* plants at 27 °C resembles the 17 °C transcriptomes of all
176 genotypes rather than that of warm temperature transcriptomes (Supp. Fig.
177 1f). Indeed, this is consistent with the phenotype of *35S::DEK3* plants, which
178 at 27 °C show perturbed hypocotyl elongation and flowering similar to the
179 plants grown at 17 °C (Fig. 1c-d, Supp. Fig. 1b-c).

180 Hierarchical clustering of differentially expressed genes reveals three major
181 categories: the first group includes warm temperature induced genes in Col-0,
182 whose expression was induced during the day (first group: clusters 2 and 3)
183 or during the night (Second group: clusters 6 and 7), and genes whose
184 expression was specifically induced only by *DEK3* overexpression at 27 °C
185 (third group: clusters 4 and 5) (Supp. Fig. 1g).

186 The first group is highly enriched for different GO terms related to metabolism,
187 translation and ribosome biogenesis, RNA methylation, nucleosome assembly
188 and histone modifications (Supp. Table 2); the second group of genes is
189 highly enriched for GO terms connected to shoot and meristem development,
190 regulation of growth, metabolism, transcription (RNA elongation and gene
191 silencing) and photosynthesis (Supp. Table 2). This cluster contains genes
192 encoding heat shock proteins such as *HSP70*, as well as genes related to
193 hypocotyl growth, like *LHY*. Genes in this group are more expressed in *dek3-2*
194 toward the end of the night; Genes in the third group respond only in
195 *35S::DEK3*, and are specifically enriched in GO terms related to response to
196 biotic stimuli and immune response (Supp. Fig. 1g, Supp. Table 2).

197

198 **DEK3 direct targets can be activated or repressed by elevated *DEK3*** 199 **expression**

200 Since the effects of *DEK3* on transcription may be indirect, we performed
201 CHIP-seq of *DEK3-CFP* expressed under its native promoter in *dek3-2*
202 mutant plants grown at 17 °C and 27 °C at the end of the night and day.

203 These time points are the most distinct according to a PCA analysis (Supp.
204 Fig. 1f), allowing us to capture the maximal diversity in DEK3 behaviour.
205 We observed DEK3 binding primarily in active chromatin states (Supp. Fig.
206 2a, the chromatin states 1, 3 and 7) characterized by open chromatin and
207 highly correlated with mRNA-encoding genes²³.
208 Since DEK3 primarily binds to DNA in shallow, broad peaks (not sharp peaks
209 like a transcription factor), a peak calling approach was not appropriate for
210 analyzing DEK3 binding profiles. Instead, we identified targeted genes by
211 clustering the DEK3 profiles over the gene bodies. DEK3 can be detected in
212 the majority of gene bodies with defined boundaries at the beginning and the
213 end of the genes (Fig. 2a, Supp. Fig. 2b). For many genes, there is an
214 enrichment for DEK3 in the 3' end of the gene (Fig. 2a, clusters 5 - 7), which
215 is consistent with its role as an H3.3 chaperone in *Drosophila* and
216 humans^{14,15}. Since overexpression of DEK3 protein caused such a dramatic
217 effect on plants physiology and transcriptome at warm temperatures (Fig. 1c-
218 e, Supp. Fig. 1b-c, 1e-g), we checked its chromatin binding profiles and
219 compared them to the profiles of the native DEK3 (Supp. Fig. 2b). Since
220 native and overexpressed DEK3 bind widely throughout the genome (Fig. 2a,
221 Supp. Fig. 2b) it is not possible to make a quantitative comparison in their
222 binding to chromatin, however, it is clear that they have different binding
223 patterns. Overexpression of DEK3 results in extension of binding beyond the
224 ends of the gene body (Supp. Fig. 2b) which might contribute to the altered
225 gene expression seen in *35S::DEK3* (Fig. 1b-d, Supp. Fig. 1b-c). We do not
226 observe a significant difference in the binding of DEK3 to chromatin that can
227 be associated with temperature or the time of day (Fig. 2a, Supp. Fig. 2b),
228 suggesting that other factors may be involved in temperature dependent gene
229 expression pattern of DEK3 targets during the day. Hence, we used all four
230 DEK3 ChIP-seq datasets from *DEK3::DEK3-CFP dek3-2* plants as biological
231 replicates for the following analysis in order to determine DEK3 direct targets
232 controlled by temperature.
233
234 We identified 2079 genes as potential direct DEK3 targets (Fig. 2b), as
235 defined by having a high DEK3 occupancy in both temperatures (clusters 3-5

236 in Fig. 2a) and being differentially expressed at least at one time point at 27
237 °C when *DEK3* is perturbed (Supp. Table 2).

238

239 Since we observe the highest influence of *DEK3* levels on gene expression
240 based on PCA and hierarchical cluster analysis at ZT0 (Supp. Fig. 1f-g), we
241 further analysed this timepoint. More than half of the potential direct *DEK3*
242 targets (1048 out of the 2079 genes) were differentially expressed at this time
243 point (Supp. Table 3). To analyse the mechanism of *DEK3* transcription
244 regulation at this time point, we focused on these 1048 genes for further
245 analysis.

246

247 These *DEK3* targets can be separated into three groups with distinct
248 transcriptional profiles (Fig. 2c, Supp. Table 3): Genes in Cluster 1 show
249 greatly enhanced expression at 27 °C in *35S::DEK3* compared to Col-0 and
250 *dek3-2* (Fig. 2c). Since these genes are already expressed at low levels in
251 Col-0, it would be challenging to detect a significant reduction in expression in
252 *dek3-2* compared to WT plants (Fig. 2c).

253 This group is dominated by seven members of the AP2-type transcription
254 factors, mainly of the *ETHYLENE RESPONSE FACTOR (ERF)* class whose
255 expression is induced by *DEK3* overexpression in combination with
256 temperature (Fig. 2d). Overall, the genes in this cluster are highly enriched for
257 stress genes (Supp. Fig. 2c). The miss-expression of the stress transcriptome
258 likely contributes to the observed enhanced sensitivity of *35S::DEK3* to
259 freezing (Fig. 1e).

260 Cluster 2 contains genes induced during the night at 27 °C in Col-0. These
261 genes are up-regulated in *dek3-2* earlier in the night compared to Col-0, while
262 not induced at all when *DEK3* is overexpressed. For example, the
263 temperature responsive auxin biosynthesis gene *YUCCA8* that is necessary
264 for hypocotyl elongation at 27 °C is directly repressed by *DEK3*. Prominent
265 transcription factors in cluster 2 include the AUXIN RESPONSE FACTORS
266 (ARF) 1 and 19, and the GATA transcription factors 2, 5 and 9. Many genes
267 also implicated in auxin signalling, for example *NAKED PINS IN YUCCA*
268 *MUTANT (NPY) 1, 3 and 5* are also repressed by *DEK3*. At the same time
269 these genes are induced even more during the night in *dek3-2* plants in

270 temperature dependent manner (Fig. 2d). Overall there is strong enrichment
271 for the GO-terms associated with growth, development and auxin (Supp. Fig.
272 2c), indicating that influence on growth caused by *DEK3* perturbations at 27
273 °C is direct. The final cluster is heterogeneous, but it is the only cluster that
274 contains genes that decrease their expression levels at elevated
275 temperatures.

276
277 *DEK3* affects growth and survival in salinity stress¹⁰, heat stress¹⁰, elevated
278 ambient temperature and cold stress (Fig. 1c-e, Supp. Fig. 1b-c), indicating
279 that *DEK3* controls genes playing a role in promoting stress responses and
280 inhibiting growth in a broad range of conditions. Consistent with this, using
281 publicly available transcriptomic data from the AtGenExpress consortium, we
282 found that the Cluster 1 genes were up-regulated and the Cluster 2 were
283 down-regulated in a wide range of abiotic stress conditions including UV-B,
284 salt, osmotic, cold, wounding, heat, drought, genotoxic and oxidative
285 stresses²⁴ (Supp. Fig. 4a).

286

287 **The activation or repression of *DEK3* targets is related to gene body** 288 **H2A.Z**

289 *DEK3* mediates the correct expression of a set of genes that are widely
290 responsive to abiotic stresses, while simultaneously repressing the induction
291 of genes promoting growth (Fig. 2c, Supp. Fig. 2c). This raises the question of
292 how a single factor can both activate and repress gene expression in a locus
293 specific manner. This behaviour is similar to that reported in mammals, where
294 DEK behaves as repressor in some cases and as an activator in others^{17,25}.
295 Since chromatin state is correlated with the degree of gene
296 responsiveness^{4,6,7}, we investigated whether the binding pattern of H2A.Z,
297 H3.3 and DNA accessibility were predictive of whether a direct *DEK3* target
298 would be activated (“stress” genes) or repressed (“growth” genes). To do this,
299 we built a conditional decision tree model that would take the distribution of
300 *DEK3*, H2A.Z and H3.3 (ChIP-seq) and the DNA accessibility (MNase-seq) in
301 WT as an input, and trained a model to predict whether a *DEK3* bound gene
302 would be up- or down- regulated in the *35S::DEK3* plants at 27 °C at the end

303 of the night (Fig. 3a, Supp. Fig. 3a-d), where the biggest difference in
304 expression could be observed (Supp. Fig. 1f).
305 Strikingly, the most important feature that distinguished between repressed
306 genes (referred to as the “growth” genes) and induced genes (referred to as
307 the “stress” genes) was the gene-body distribution of H2A.Z ($p < 0.001$) with
308 most DEK3 up-regulated “stress” genes having H2A.Z in the gene body,
309 whereas the repressed “growth” genes did not have H2A.Z in the gene body.
310 These “growth” genes predominantly have +1 H2A.Z nucleosomes (Fig. 3a),
311 some “stress” genes were found to have both gene body H2A.Z and strong +1
312 H2A.Z. H3.3 and chromatin occupancy (via MNase-seq) also contribute to
313 differences in “growth”, and “stress” genes. Specifically, genes with lower
314 H2A.Z levels, but open promoter regions are more likely to be “stress” genes
315 (Fig. 3a, node 2 and genes with lower levels of H3.3 are more likely to be
316 “growth” genes (Fig. 3a, node 6).

317

318 While the level of DEK3 binding does not determine whether a gene will have
319 H2A.Z in the gene body (Fig. 3a-b, Supp. Fig. 3e), perturbing *DEK3*
320 expression consistently changes the distribution of H2A.Z incorporation
321 around the +1 nucleosome and gene body (Fig. 3c-d, Supp. Fig. 3f). In *dek3-*
322 *2*, there is a larger ratio of gene body H2A.Z in “growth” genes compared to
323 WT plants (Fig. 3c-d (lower panel), Supp. Fig. 3f (lower panel)), which is
324 consistent with these genes being more easily induced in *dek3-2* compared to
325 Col-0 at 27 °C (Supp. Fig. 4b (upper panel)). This might explain the
326 phenotypic differences between these plants (Fig. 1c-d, Supp. Fig. 1b-c).
327 Conversely, there is lower enrichment for H2A.Z in both the +1 position and
328 gene bodies of “stress” related genes in plants over-expressing *DEK3*
329 compared to WT background (Fig. 3c-d (upper panel), Supp. Fig. 3f (upper
330 panel)).

331 It has been shown that transcription of genes with H2A.Z in gene bodies is
332 more easily induced by environmental cues and stress^{4,7}—these are the
333 genes that are induced by overexpression of *DEK3* and temperature (Fig. 3a,
334 node 1, Supp. Fig. 4b (lower panel)). In contrast, genes that have only +1
335 H2A.Z nucleosomes and H3.3 around TTS, are expected not to be easily
336 induced by environment but correlated with growth⁴. These genes are

337 suppressed by overexpression of DEK3 and induced in *dek3* in a temperature
338 specific way manner compared to Col-0 (Supp. Fig. 4b (upper panel)).

339

340 These observations are consistent with the interaction of DEK3 with H2A.Z
341 (Fig. 1a-b, Supp. Fig. 1a), and with decreased expression of the “growth”
342 genes and increased expression of the “stress” related genes in the absence
343 of H2A.Z (Fig. 4d-e) or when its incorporation into chromatin is reduced (Fig.
344 4f-g). Given that H2A.Z is known to accumulate in the gene body of
345 environmentally responsive genes (such as in drought stress responsive
346 genes)^{4,7,6}, a relationship between DEK3 and H2A.Z may suggest a
347 mechanism by which DEK3 perturbations could affect H2A.Z distribution in +1
348 nucleosomes and gene bodies, and in this way control inducibility of gene
349 expression by environment. This pattern doesn’t hold among strongly DEK3-
350 bound genes in other nodes (Fig. 3c, Supp. Fig. 3f), suggesting that the
351 chromatin landscape is likely to be changing as a result of the DEK3-H2A.Z
352 interaction, rather than as a consequence of changes in transcription.

353

354 ***DEK3* and *H2A.Z* interact genetically**

355 Our results suggest that DEK3 plays a role in driving H2A.Z-nucleosome
356 removal from the gene body of stress responsive genes. Supporting this
357 model (Supp. Fig. 4i), when H2A.Z levels or its deposition is perturbed in
358 mutants lacking H2A.Z or *ACTIN RELATED PROTEIN6 (ARP6)*, the
359 transcriptome shows similar mis-expression as in *35S::DEK3* (Fig. 4d-e), but
360 opposite to that of *dek3-2* (Fig. 4f-g). Furthermore, the *arp6-1* mutation is able
361 to partially suppress the effect of *dek3-2* on the transcriptome (Supp. Fig. 4g).
362 Since ARP6 might be present in several protein complexes including SWR1
363 and may have other roles^{26,27}, we also investigated the effect of removing
364 H2A.Z in plants on the *DEK3* dependent transcriptome. In the triple mutant for
365 *hta8,9,11*, we observe a consistent pattern over the time course, with up-
366 regulation of *35S::DEK3* activated genes and down-regulation of *35S::DEK3*
367 suppressed genes (Fig. 4c-e), similarly to what is observed in *arp6-1* (Fig. 4f-
368 g).

369

370 In general, “growth” related genes have less H2A.Z signal, and this is most
371 likely to be found near the TSS (Fig. 3a). These temperature dependent
372 genes are further induced by warm ambient temperature in *dek3-2* possibly by
373 increasing the levels of their H2A.Z gene body binding (Fig. 4d, Supp. Fig. 4b
374 (upper panel)). Reducing H2A.Z-nucleosome occupancy in the *h2a.z* and
375 *arp6-1* backgrounds slightly inhibits the temperature induction of “growth”
376 genes (Fig. 4d, 4f). However, when the levels of both proteins, H2A.Z and
377 DEK3, on chromatin are reduced in the *dek3-2 arp6-1* double mutant, the
378 temperature dependent activation of these genes is been impaired (Supp. Fig.
379 4g (upper panel)). In contrast, the “stress” related genes are enriched in
380 H2A.Z in their gene bodies (Fig. 3a) and up-regulated in the *35S::DEK3* plants
381 grown at 27°C, (Fig. 4e, Supp. Fig. 4b (lower panel)). These genes are also
382 up-regulated in *h2a.z* and *arp6-1* mutants in a temperature-dependent
383 manner (Fig. 4e, 4g), indicating that H2A.Z-nucleosome occupancy may
384 regulate their transcription. This increase is abrogated in *dek3-2 arp6-1*
385 plants, demonstrating the antagonistic interaction between DEK3 and ARP6
386 (Supp. Fig. 4g (lower panel)).

387

388 In light of the physical interaction between DEK3 and H2A.Z (Fig. 1a-b, Sup.
389 Fig. 1a) and in line with the transcriptional patterns of *dek3-2 arp6-1* double
390 mutants and *arp6-1* (Supp. Fig. 4g), we sought to determine if they also
391 interact genetically. The partial rescue of the *arp6-1* phenotype by the *dek3-2*
392 mutation at both an elevated temperature and cold stress (Fig. 4a, Supp. Fig.
393 4c-f) indicates that *ARP6* and *DEK3* might have opposite functions within the
394 same pathway. Over-expression of *DEK3* in *arp6-1* led to temperature-
395 dependent lethality (Supp. Fig. 4h), suggesting that normal levels of *DEK3*
396 expression are necessary for plants to survive with reduced H2A.Z-
397 nucleosomes when exposed to warm or cold temperature.

398

399 **H2A.Z deposition patterns in human DEK targets**

400 DEK proteins are conserved through evolution found in almost all higher
401 eukaryotes^{29,30}, and show similarity in domain structure between plants and
402 animals¹². Three of the four plant DEK proteins, DEK2, DEK3 and DEK4,
403 have been found in complex with H2A.Z by mass spec analysis (Fig. 1a,

404 Supp. Fig. 1a, Supp. Table 1) and showed similar growth phenotypes in
405 response to warm temperature (Fig. 1d, Sup. Fig. 1d), suggesting functional
406 redundancy between them. We therefore asked if our finding that the pattern
407 of H2A.Z-nucleosome occupancy on the gene body may be predictive of how
408 a gene is regulated by DEK3 in Arabidopsis also applies in humans.
409 Previously, human DEK (hDEK) has been shown to both activate and repress
410 expression of the genes involved in cancer and hematopoiesis^{30–38}
411 (summarised in Supp. Fig. 4j). We observe that hDEK up-regulated genes
412 possess mainly gene body H2A.Z, while hDEK down-regulated genes have
413 more +1 nucleosome H2A.Z binding (Supp. Fig. 4j, 4k). This suggests that the
414 interaction between DEK and H2A.Z may be functionally conserved, and that
415 the role of DEK in controlling the balance between growth and stress
416 resilience is conserved between plants and vertebrates. DEK in vertebrates
417 plays key roles in carcinogenesis^{7,9,10,13,25,31,36–38,39}, haematopoiesis^{25,30,33} and
418 inflammation^{11,46}, processes involving a fine-control of proliferation and stress
419 resilience. It will be interesting to determine if these functions of DEK are also
420 mediated via H2A.Z-nucleosomes.

421

422 Discussion

423 We find that altered levels of DEK3, an Arabidopsis ortholog of the onco-
424 protein DEK, perturbs developmental programming in *Arabidopsis thaliana*
425 grown in warm temperature by influencing expression of environmental
426 (stress related) and growth related (developmental) genes (Sup. Fig. 2c, Sup.
427 Fig. 4b). The effect of DEK3 on the transcriptome and development appears
428 to be mediated at least in part by changes in the relative distribution of H2A.Z-
429 nucleosomes on the gene bodies of target genes (Fig. 3c-d, Sup. Fig. 3f).
430 These results may explain the dual effect of DEK3 on the transcription of its
431 target genes, shedding light on the longstanding question regarding the
432 influence of DEK on gene expression^{12,17,25}. We also provide evidence for
433 physical and genetic interaction between DEK3 and H2A.Z in Arabidopsis
434 (Fig. 1a-b, Sup. Fig. 1a, Sup. Fig. 4c-g). Since DEK proteins are found in most
435 multicellular eukaryotes, as is the H2A variant H2A.Z, these findings may be

436 of broad relevance, and contribute towards understanding of hDEK as a
437 therapeutic target.

438

439 The chromatin landscape regulates accessibility of DNA to the transcriptional
440 machinery and transcription factors, controlling the transcriptome and how it
441 responds to a changing environment⁴⁷. Incorporation of the histone variants,
442 H2A.Z and H3.3, into chromatin is frequently associated with responses to
443 environmental perturbations^{4,7,8}. While it was reported previously that DEK is
444 a H3.3 chaperone and controls H3.3 deposition into chromatin¹⁴, the effect of
445 altered levels of DEK3 on more than half of the warm temperature
446 transcriptome can be explained by initial pattern of H2A.Z distribution (Fig. 3a,
447 Sup. Fig. 3f). Previous work suggests that DEK may interact with H3.3¹⁴ —
448 which is not inconsistent with this study as it is possible for a protein to have
449 multiple binding partners. It will be interesting to see if DEK3 is able to bind
450 H2A.Z and H3.3 simultaneously.

451

452 Our results suggest that DEK3 influences the chromatin landscape by
453 modulating the distribution of H2A.Z at specific genes thus controlling their
454 responsiveness to environmental signals (Fig. 3c-d, Sup. Fig. 3f). Our data
455 confirm and extend previous reports suggesting that the initial pattern of
456 H2A.Z on gene bodies is important for the proper induction of transcription in
457 response to environmental stress^{4,7}. We show that the levels of H2A.Z on
458 gene bodies is altered by DEK3, influencing gene expression responsiveness
459 in response to environmental signals (Fig. 3). *DEK3* overexpression for
460 example depletes H2A.Z-nucleosome occupancy, leading to enhanced
461 transcriptional responses to higher temperature (Fig. 3c-d, Supp. Fig. 3f,
462 Supp. Fig. 4b).

463

464 DEK3 may influence H2A.Z distribution through its role in H3.3 incorporation
465 into chromatin. In animal systems, lack of DEK causes enhanced H3.3
466 incorporation into the chromatin by HIRA and DAXX/ATRAX chaperones¹⁴.
467 H3.3 incorporation into chromatin might in turn lead to elevated DNA
468 methylation and subsequent prevention of H2A.Z incorporation into gene
469 bodies⁶. This model is not consistent with our finding that increased levels of

470 H2A.Z occur in the gene bodies of *dek3* plants, and conversely,
471 overexpressing *DEK3* results in reduced gene body H2A.Z, at least in the
472 subset of the DEK3 target genes (Fig. 3a, 3c-d, Sup. Fig. 3f). These
473 observations suggest another mechanism of regulation. Interestingly, H3.3
474 distribution was able to explain the effect of *DEK3* expression on the warm
475 temperature transcriptome for only a subset of the genes (Fig. 3a, node 6),
476 while the H2A.Z pattern alone predicts the behaviour of more than half of the
477 DEK3 target genes (Fig. 3a). This effect could partially be explained by
478 possible physical interaction between DEK3 and H2A.Z (Fig. 1a-b). Even
479 though we could not exclude the possibility of indirect physical interaction
480 between these two proteins, our genetic studies confirm the biological
481 relevance of this interaction (Sup. Fig. 4c-f, Sup. Fig. 4h). Many cancers are
482 associated with impaired DEK levels^{12,13,40,41,43,45,46,48-50,14,25,32-35,38,39}, and it
483 will be interesting to determine if these show a similar relationship for
484 transcriptional response and the distribution of hDEK and H2A.Z-
485 nucleosomes.

486

487 Our work suggests a model for growth promoting and growth inhibiting
488 responses to environmental changes (Sup. Fig. 4i), where genes with high
489 levels of gene body H2A.Z are responsive to environmental induction, and this
490 sensitivity is enhanced by DEK which can facilitate a decrease in H2A.Z
491 occupancy and higher gene expression. Genes with low gene body H2A.Z
492 occupancy are more likely to be resistant to activation by environmental
493 signals in presence of normal DEK3 levels. For plants this is important for
494 example in tuning the response of the transcriptome to ambient temperature.
495 For plants it is of particular importance to keep a balance between
496 developmental and metabolic transcriptomes under different conditions.
497 Enhanced growth as a result of elevated temperature may cause a reduction
498 in size of adult plant and seed yield⁵¹. Additionally, plants need to attenuate
499 their development rate in order to provide appropriate response to biotic
500 stress to obtain optimised fitness². However, it may also play a role in other
501 contexts, for example in the case of inflammation and cancer where changes
502 in the relative levels of nutrients and oxygen occur frequently, and cells must
503 respond appropriately.

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- 654

Fig. 1.

Chromatin protein DEK3 interacts with H2A.Z and plays a role in balancing the temperature response between growth and arrest in Arabidopsis

(a) Volcano plot showing the distribution of proteins identified by Mass Spec in complex with H2A.Z according to p -value and fold change binding. Horizontal line indicates significance level at p -value ≤ 0.05 , and vertical lines shows fold change of 2.

(b) Validation of H2A.Z and DEK3 binding. Nuclear extracts from 35S::DEK3-CFP expressing lines or from Col-0 were subjected to IP with anti-GFP antibodies, and mixed with H2A.Z-Flag purified from nuclear or histone plant extract. The western blot shows the presence of H2A.Z-Flag, the 35S::DEK3-CFP and H3 in the immune complexes and in input lysates. The amount of protein in the input represents 1% of the amount of proteins used for IP.

(c) Boxplots summarising days to bolting of Col-0, *dek3-2*, 35S::DEK3_1 and 35S::DEK3_2 (independent over-expression lines). The plants have been grown at 17°C, 22°C and 27°C. 35S::DEK3_1 and 35S::DEK3_2 plants haven't bolt within 100 days when grown at 27°C. The following numbers of plants have been used (17/22/27°C): Col-0 9/18/23; *dek3-2* 12/12/24; 35S::DEK3_1 18/12/24; 35S::DEK3_2 18/12/24. Box and whisker plots show median, inter-quartile ranges and 95% confidence intervals (t-test p -values are summarized in Supp. Table 5).

(d) Boxplots summarising hypocotyl length (mm) of Col-0, *dek3-2*, 35S::DEK3_1 and 35S::DEK3_2 (independent over-expression lines). The seedlings have been grown for 7 days under short day photoperiod at 17°C, 22°C and 27°C. The following numbers of seedlings have been used (17/22/27°C): Col-0 55/54/46; *dek3-2* 55/57/58; 35S::DEK3_1 16/18/16; 35S::DEK3_2 13/7/17. Box and whisker plots show median, inter-quartile ranges and 95% confidence intervals (t-test statistic p -values are summarized in Supp. Table 5).

(e) Summary of the cold sensitivity assays. Col-0, *dek3-2*, 35S::DEK3_1 and 35S::DEK3_2 (independent over-expression lines) seedlings have been grown for 21 days in SD at 22°C and either subjected to -6°C for 24hr, or subjected to -12°C for 4 days after 24 h acclimation at 4°C. The plants have been allowed to grow for a further 14 days under recovery conditions, to distinguish between plants that do and do not survive the treatment. All the pictures have been done at the same time and survival rates have been calculated. Percentages indicate survival rate. The survival rate of all control plants has been 100%. The following numbers of plants used in the assay: non-acclimated (survived/total) Col-0 19/27; *dek3-2* 23/27; 35S::DEK3_1 16/27; 35S::DEK3_2 14/27; acclimated (survived/total) Col-0 20/27; *dek3-2* 19/27; 35S::DEK3_1 6/27; 35S::DEK3_2 7/27.

Figure 1

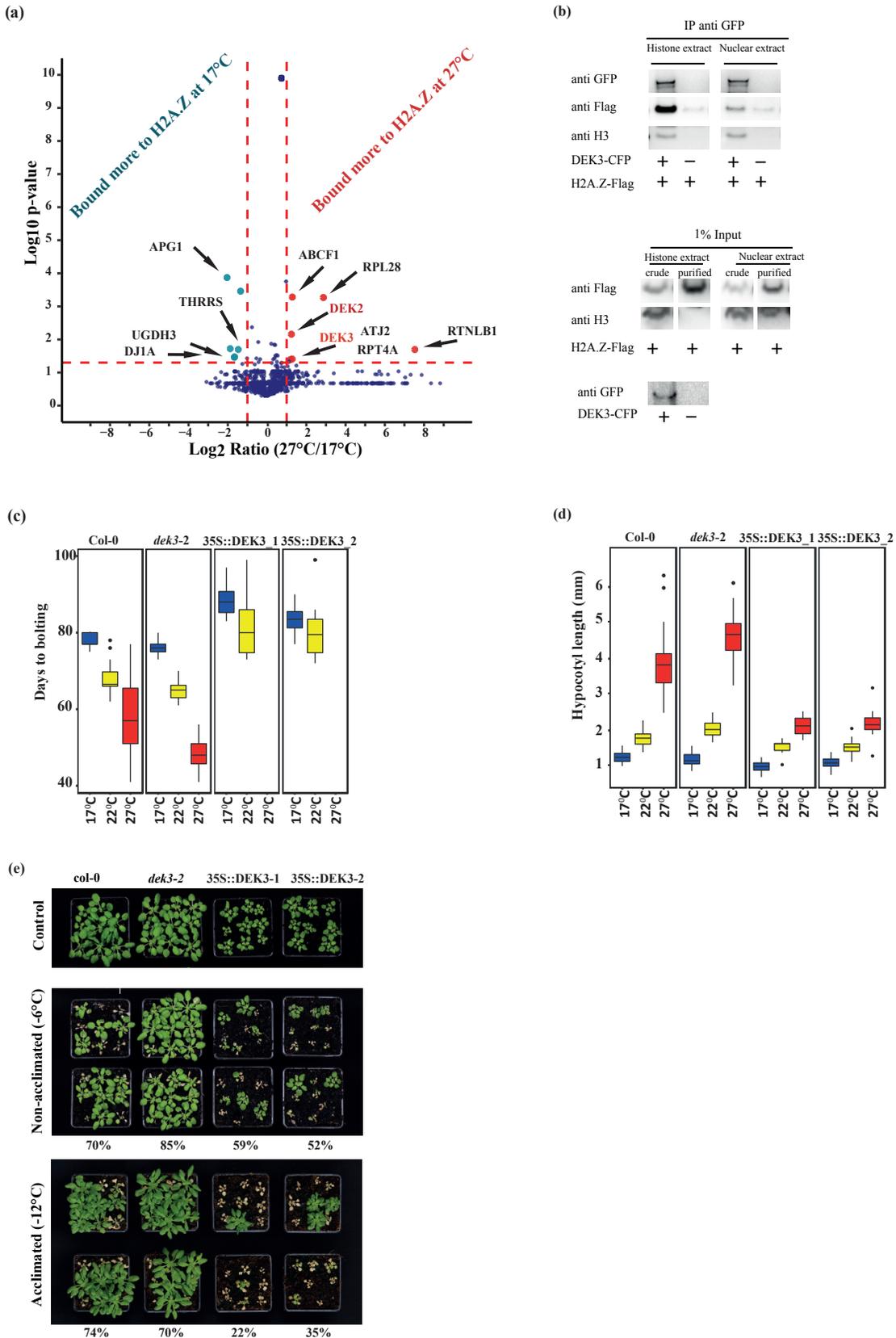


Fig.2.

DEK3 directly regulates the expression of “Growth” and “Stress” genes.

(a) ChIP-seq binding profiles of DEK3-CFP in seedlings grown at 17°C and 27°C and collected at the end of the night. The DEK3 binding patterns across all gene bodies (including 200 bp upstream of the transcription start site (TSS) and 200 bp downstream of the transcription termination site (TES) have been clustered. A large proportion of genes have an enrichment of DEK3 over their gene body, with clusters 3-5 showing the highest enrichment. The label colour bar on the right shows normalised read count, with the highest level in blue and lower in red.

(b) The overlap between the genes mis-expressed by perturbation in DEK3 levels (4,859 genes) and those that are bound directly by DEK3 (12,639 genes, cluster 3-5, from (a)). The overlap highlights DEK3’s direct targets mis-regulated in warm temperature when DEK3 is overexpressed (2,079 genes).

(c) Transcriptional patterns of DEK3 direct targets at the end of the night (1,048 genes) which were hierarchically clustered into 3 groups based on their z-scores calculated from transcript per million (TPM) values in all time points. Up-regulated genes are in red and down-regulated genes are in blue. The sidebar to the left of the heatmap indicates the 3 clusters of differentially expressed genes. Black bars on the top indicate night, white bars day.

(d) Expression of direct DEK3 targets belong to AP2 and auxin related families as summarised in Supp. Table 2, in Col-0, *dek3-2*, *35S::DEK3-CFP* plants grown at 17°C and 27°C under short photoperiod and collected at 8 different time points during 24 hr time course.

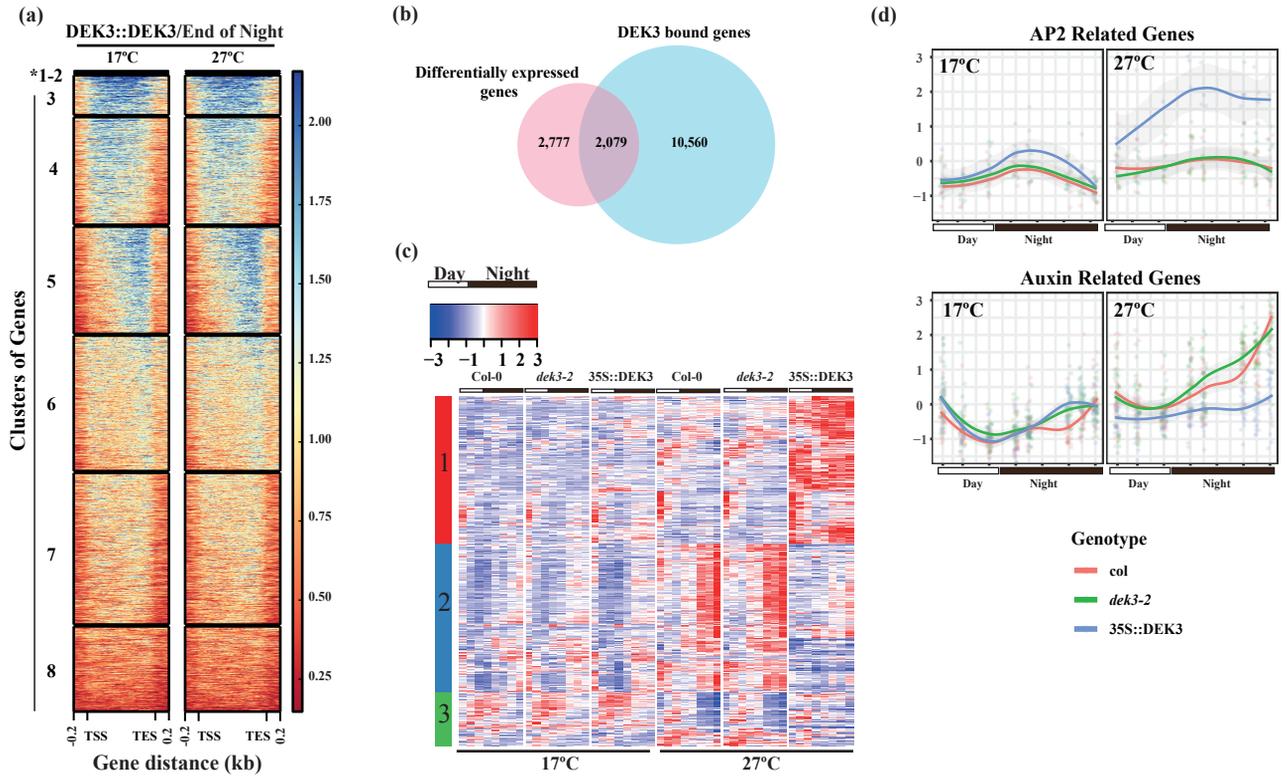
Figure 2

Fig. 3.

DEK3 affects H2A.Z distribution on target gene bodies

(a) The summary of decision tree predicting whether a DEK3 target gene is up- or down-regulated by overexpression of DEK3 in plants grown at 17°C. Each row in each of the clusters represents a gene, and each column represents a position along the gene body. At each branching point (node), the genes are split into two groups based on their ChIP-seq or MNase-seq profile. (b) The example ChIP-seq binding profiles of H2A.Z-Flag in Col-0, *dek3-2* and *35S::DEK3* plants grown at 27°C and collected at the end of the night. The binding profiles are ordered by the nodes identified by the decision tree. (c) Profiles of H2A.Z-Flag ChIP-seq coverage in *35S::DEK3* and *dek3-2* relative to Col-0 plants, depicted over the gene body, 200bp upstream (TSS) and downstream (TES). Plants were grown at 17°C and collected at the end of the night. (d) The violin plot summarizing the distribution of natively expressed H2A.Z-Flag on the gene bodies of the “stress” and “growth” genes in Col-0, *dek3-2* and *35S::DEK3* plants grown at 17°C and 27°C (Collected at the end of the night).

Fig.4.

DEK3 controls the expression of “growth” and “stress” genes antagonistically to H2A.Z

(a) Col-0, *dek3-2*, *arp6-1* and *dek3-2 arp6-1* seedlings were grown for 21 days in SD at 22°C and subjected to -12°C for 4 days after acclimation for 24 hr at 4°C. Percentages indicate survival rate. All control plants have survived. The numbers of plants used: acclimated (survived/total) Col-0 20/27; *dek3-2* 19/27; *arp6-1* 4/28; *dek3-2 arp6-1* 10/27.

(b) Venn diagram of overlap between the genes mis-expressed by perturbation in DEK3 levels (4,859 genes) and genes mis-expressed in *h2a.z* plants (3,609). The overlap highlights (1,079 genes) what are mis-regulated in both genotypes in warm temperature. (hypergeometric test p-value of the overlaps < 2.63e-65 for both groups).

(c) Expression profiles of DEK3 target genes in *35S::DEK3* and *h2a.z* genetic backgrounds were hierarchically clustered into two groups, based on the log₂ ratio compared to the values of Col-0 in transcript per million (TPM). Plants were grown at 27°C and collected at 8 time points over 24 h. Up-regulated genes are shown in red and down-regulated genes are shown in blue. The sidebar on the left of the heatmap indicates the major clusters. Black bars on the top indicate night, white bars day.

(d) (e) Expression of “Growth”(d) and “Stress”(e) genes in *35S::DEK3* and *h2a.z* genetic backgrounds were hierarchically clustered, based on the log₂ ratio compared to the values of Col-0 in transcript per million (TPM). Plants has been grown at 27°C and collected at 8 time points during 24 h. Up-regulated genes are shown in red and down-regulated genes are shown in blue. The sidebar on the left of the heatmap indicates major clusters. Black bars on the top indicate night, white bars day.

(f) (g) Expression, based on the log₂ ratio compared to the values of Col-0 in TPM, of “Growth” (f) and “Stress” (g) genes in *dek3-2* and *arp6-1* genetic backgrounds were hierarchically clustered as in (f) and (g) respectively. Plants were grown at 17°C and 27°C, and collected at the end of the day (white bar) or at the end of the night (black bar). Up-regulated genes are shown in red and down-regulated genes are shown in blue. The sidebar on the left of the heatmap indicates the major clusters.

Figure 4