Short Title: AtMED17 role during the DNA damage response

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Arabidopsis Mediator subunit 17 connects transcription with DNA repair after UV-B exposure

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35 **One sentence summary:** In Arabidopsis, MED17 regulates the DNA damage response
36 after UV-B exposure transcriptionally modulating the expression of genes and possibly
37 also physically interacting with DNA repair proteins.

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39 **Author contributions**

40

41 M.G., P.Ce. and P.Ca. designed the research; M.G., S.N.F., A.J., M.L., J.M., M.L.F.F.,
42 and G.R. performed the research; M.S., G.R., P.Ce. and P.Ca. analyzed and interpreted
43 the data; and P.C. and M.G. wrote the manuscript.

44

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54

55 Abstract

56 Mediator 17 (MED17) is a subunit of the Mediator complex that regulates
 57 transcription initiation in eukaryotic organisms. In yeast and humans, MED17 also
 58 participates in DNA repair, physically interacting with proteins of the Nucleotide
 59 Excision DNA Repair system. We here analyzed the role of MED17 in Arabidopsis
 60 plants exposed to UV-B radiation, which role has not been previously described.
 61 Comparison of *med17* mutant transcriptome to that of WT plants showed that almost
 62 one third of transcripts with altered expression in *med17* plants are also changed by UV-
 63 B exposure in WT plants. To validate the role of MED17 in UV-B irradiated plants,
 64 plant responses to UV-B were analyzed, including flowering time, DNA damage
 65 accumulation and programmed cell death in the meristematic cells of the root tips. Our
 66 results show that *med17* and *OE MED17* plants have altered responses to UV-B; and
 67 that MED17 participates in various aspects of the DNA damage response (DDR).
 68 Increased sensitivity to DDR after UV-B in *med17* plants can be due to altered
 69 regulation of UV-B responsive transcripts; but additionally MED17 physically interacts
 70 with DNA repair proteins, suggesting a direct role of this Mediator subunit during
 71 repair. Finally, we here also show that MED17 is necessary to regulate the DDR
 72 activated by ATR, and that PDCD5 overexpression reverts the deficiencies in DDR
 73 shown in *med17* mutants. Together, the data presented demonstrates that MED17 is an
 74 important regulator of the DDR after UV-B radiation in Arabidopsis plants.

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77 Introduction

78 Mediator is a multi-subunit protein complex that regulates transcription initiation.
 79 Mediator acts as a molecular bridge between transcription factors bound at enhancers
 80 and RNA polymerase II (RNA pol II), but also regulates chromatin architecture, recruits
 81 epigenetic marks and participates in RNA processing. Structurally, Mediator is
 82 composed by four different modules, known as the head, middle, tail, and cyclin-
 83 dependent kinase 8 (CDK8) modules (reviewed in Buendía-Monreal and Gillmor, 2016;
 84 Malik et al., 2017; Mao et al., 2019). While the head module is thought to initially
 85 interact with RNA pol II to start transcription, the middle module has an important
 86 structural function and also binds to RNA pol II after its initial interaction with the
 87 head. The tail function is to associate with gene-specific transcription factors, whereas
 88 the CDK8 module is dissociable in response to different stimuli.

89 Even though the modular structure of the Mediator complex is conserved in
 90 eukaryotes, the composition of its subunits varies among species, but also changes in
 91 response to environmental and tissue-specific inputs, suggesting that different Mediator
 92 structures may have diverse functions (Mao et al., 2019). For instance, in plants, studies
 93 have shown that some Mediator proteins regulate cell division, cell fate and
 94 development, while others have a role in hormone signaling or are involved in biotic
 95 and abiotic stress responses. In plants, the Mediator complex comprises about 34
 96 subunits (Malik et al., 2017). Despite mutations in different Mediator subunits are not
 97 lethal, altered expression of particular MED subunits can lead to important changes in
 98 gene expression. Therefore, med mutants show different phenotypes, for example they
 99 have altered growth, development and stress responses (Yang et al., 2016; Dolan and
 100 Chapple, 2017). For example, Arabidopsis mutants in *MED5*, *MED16* and *MED23* have
 101 decreased accumulation of phenylpropanoid compounds (Stout et al., 2008; Dolan et al.,
 102 2017); *med16* mutants are deficient in cellulose biosynthesis and iron homeostasis
 103 (Sorek et al., 2015; Yang et al., 2014; Zhang et al., 2014), while in *med15* and *cdk8*
 104 plants, lipid biosynthesis is altered (Kim et al., 2006; Zhu et al., 2014; Kong and Chang,
 105 2018). Interestingly, several *med* mutants have shown contrasting metabolome profiles,
 106 which may relate to their different molecular function (Davoine et al., 2017).

107 MED17 is a subunit of the head Mediator module, and in the yeast and human
 108 complexes, it is an important protein which interacts with several other Mediator
 109 subunits (Guglielmi et al., 2004; Cevher et al., 2014). In Arabidopsis, MED17 seems to
 110 be a key scaffold component of the whole complex (Maji et al., 2019). AtMED17 was

111 demonstrated to participate in the production of small and long noncoding RNAs (Kim
112 et al., 2011). MED17 is required for small RNA biogenesis recruiting Pol II to
113 promoters of miRNA genes, and also for the repression of heterochromatic loci,
114 activating Pol II-mediated production of long noncoding RNAs (Maji et al., 2019).
115 These results suggest that MED17 may have a role not only in transcription but also in
116 genome stability. In yeast, MED17 participates in DNA repair through a physical
117 interaction with Xeroderma pigmentosum group G protein (XPG), an endonuclease that
118 participates in Nucleotide Excision DNA Repair (NER; Eyboullet et al., 2013).
119 Moreover, *med17* mutants show increased sensitivity to UV radiation. Thus, MED17
120 participates in DNA repair recruiting XPG to transcribed genes (Eyboullet et al., 2013).
121 In human cells, MED17 was shown to interact with the DNA helicase Xeroderma
122 pigmentosum group B protein (XPB), which is a subunit of the transcription factor II H
123 (TFIIH) and it is essential for both transcription and NER (Kikuchi et al., 2015).
124 MED17 colocalizes with the NER factors XPB and XPG after UV-C exposure *in vivo*,
125 and they also physically interact in *in vitro* assays, suggesting that, similarly as it was
126 described in yeasts, MED17 plays essential roles in the switch between transcription
127 and DNA repair (Kikuchi et al., 2015). The major DNA lesions that occur after UV
128 exposure are the formation of cyclobutane pyrimidine dimers (CPDs) and pyrimidine
129 (6-4) pyrimidones (6-4 PPs), which occur on two adjacent pyrimidine bases. In plants,
130 these photoproducts are mainly repaired through photoreactivation, which is the direct
131 reversal of major lesions by different types of photolyases that absorb light and reverse
132 the formation of CPDs or 6-4 PPs (Spampinato, 2017). In Arabidopsis, UVR2 is a CPD
133 photolyase, while UVR3 catalyzes the photoreactivation of 6-4 PPs. However, other
134 DNA repair systems that are independent of light absorption, such as the NER system,
135 which removes damaged nucleotides together with surrounding nucleotides; the Base
136 Excision Repair (BER) system, which removes damaged bases, and other repair systems
137 like the Mismatch Repair (MMR) system that recognizes and corrects of DNA
138 mismatches have been also shown to repair DNA damage after UV exposure
139 (Spampinato, 2017; Lario et al., 2011).

140 In this manuscript, we investigated the role of MED17 in Arabidopsis plants
141 exposed to UV-B radiation. Using *med17* mutants, we analyzed their transcriptome and
142 compared it to that of WT plants. Our results demonstrate that transcripts that encode
143 proteins that participate in UV-B responses and DNA repair genes showed altered
144 expression in *med17* mutants. *med17* mutants also showed altered phenotypes after

145 UV-B exposure, in particular during the DNA damage response. We here show that
 146 MED17 is necessary for the correct expression of genes after UV-B exposure, probably
 147 by interacting with other Mediator proteins and transcription factors, but it also binds
 148 proteins that participate in DNA repair in Arabidopsis, suggesting that MED17 may
 149 have a direct role during DNA repair. In addition, MED17 is required for DDR
 150 activation through Ataxia telangiectasia and Rad3 related (ATR); and overexpression of
 151 Programmed cell death 5 (PDCD5), a regulator of the DDR in humans and in
 152 Arabidopsis, overcomes the deficiency of responses to UV-B in *med17* mutants.
 153 Together, the data presented here demonstrates that MED17 is a key regulator of the
 154 DNA damage response after UV-B radiation in Arabidopsis plants.

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156

157 Results

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159 Transcriptome analysis of *med17* mutants

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161 As MED17 is a subunit of the transcriptional co-regulator Mediator complex, we were
 162 first interested in studying the global role of MED17 on gene expression. Thus, we
 163 compared the transcriptome of *med17* mutants and WT seedlings grown under white
 164 light for 10 days. Three biological replicates were used for RNA extraction and RNA-
 165 seq analysis. We identified 6822 differentially expressed genes in *med17* mutants in
 166 comparison to WT plants (Figure 1, A; Supplemental Table S1), 3534 (51.8%) were
 167 downregulated, whereas 3288 (48.2%) were upregulated. MED17 responsive genes
 168 were enriched in GO terms related to red, far-red, blue and UV-B light responses,
 169 among others (Supplemental Table S2). These results suggested a possible role for
 170 MED17 in UV responses. As described in the Introduction, MED17 was reported to
 171 participate in DNA repair after UV exposure in yeast and humans. Therefore, we
 172 compared the transcriptome changes of *med17* mutants to those of Col-0 plants grown
 173 under white light in our experiments (Supplemental Figure S1) to those previously
 174 reported for Col-0 plants after UV-B exposure (Tavridou et al., 2020). As shown in
 175 Figure 1, A, out of the 5079 UV-B regulated transcripts in WT plants (Tavridou *et*
 176 *al.*, 2020), 2184 showed altered expression in *med17* mutants, which was significant
 177 by a Fisher Exact Test ($p=4.6e-53$). Interestingly, 56% of this overlapping set of
 178 transcripts were up-regulated by UV-B in WT plants and down-regulated in *med17*
 179 mutants (Supplemental Figure S1, A), which was highly significant (Fisher Exact Test
 180 $p<1e-242$; Figure 1, D). The overlap between up-regulated genes in *med17* mutants and
 181 down-regulated in Col-0 plants by UV-B was also significant, although not to the same
 182 extent ($p < 0.005$; Figure 1, D). On the other hand, the overlap between the other two
 183 possible comparisons (up by UV-B vs up by *med17*; down by UV-B vs down by *med17*
 184 was 26 % altogether, and not statistically significant (Figure 1, D). Furthermore, the
 185 Odds ratio, which represents the strength of the association or correlation of the overlap,
 186 was higher for genes that are increased by UV-B and decreased in *med17* than for all the
 187 other comparisons (Figure 1, D). These results show that the transcripts that increase in
 188 response to UV-B require MED17 for maximal expression in the WT, suggesting a role
 189 for MED17 in UV-B responses.

Figure 1 Analysis of global gene expression differences between *med17* compared to WT plants grown without UV-B (Control), and WT plants exposed to UV-B radiation (UV-B) compared to non treated plants (Control). A, Venn diagram of comparisons between transcripts with altered expression in *med17* mutants and UV-B-responsive genes in Arabidopsis plants. Sets of genes were selected using the criteria described in Materials and methods. B, Heatmap comparing transcripts changed in *med17* compared to WT plants; and WT plants after UV-B exposure. The color saturation reflects the magnitude of the log2 expression ratio for each transcript. C, Clusters of expression profiles. Each graph displays the mean pattern of expression of transcripts in the cluster in blue and the standard deviation of average expression (orange and grey lines). The number of transcripts in each cluster is at the top left corner of each graph. The y-axis represents log2 of gene-expression levels relative to those in WT Col-0 plants under control conditions without UV-B. D, Fisher Exact Test and Odds Ratio of the overlap between differentially expressed transcripts in *med17* compared to WT plants and UV-B regulated transcripts in WT plants.

190 We then performed a cluster analysis of the 2184 overlapping genes shown in
 191 the Heatmap (Figure 1, B. Figure 1, C, and Supplemental Table S2). Genes were
 192 clustered in 5 groups, with clusters 1 and 4 including transcripts with decreased
 193 expression in *med17* compared to WT plants grown under white light conditions, and
 194 up-regulated by UV-B in Col-0 plants (Figure 1C). These two clusters mainly differ in
 195 the magnitude of the change observed; cluster 1 includes a lower number of transcripts,

but showing stronger changes compared to WT plants, while cluster 4 includes more transcripts with smaller differences. Interestingly, when the clusters were analyzed by GO terms, cluster 1 included the categories UV-B responsive genes, flavonoid and secondary metabolites biosynthesis, and oxidoreduction reactions. This cluster contains highly upregulated genes in WT plants exposed to UV-B compared to plants under white light, but downregulated in *med17* mutants compared to Col-0, both grown in the absence of UV-B. Among genes included in cluster 1, we found *EARLY LIGHT-INDUCIBLE PROTEIN 2 (ELIP2)*, which was shown to be activated by UV-B through UVR8 (Brown, 2005), with a log₂ fold change of 6.39 in WT under UV-B, and -3.29 in *med17* plants (Supplemental Table S2). *REPRESSOR OF UV-B PHOTOMORPHOGENESIS 2 (RUP2)* was also found in this cluster. RUP2, along with RUP1, provides an UVR8 negative feedback regulation, balancing UV-B responses (Gruber, 2010). RUP2 showed a 3.20 log₂ fold change in WT exposed to UV-B, while *med17* plants showed a log₂ fold change of -1.45 (Supplemental Table S2). On the other hand, cluster 4 included GO terms related to light responses (light stimulus, high light, red, far-red, blue, photosynthesis), oxidative stress and response to reactive oxygen species, amongst others (Supplemental Table S2). Remarkably, in this cluster we found genes induced by UV-B like *DREB2A* (Ulm et al., 2004), and *RUP1*, mentioned above. This analysis further validates the requirement of MED17 for increased expression of genes that participate in UV-B responses.

Photomorphogenic responses are altered in *med17* mutants.

Because *med17* mutants had altered expression of some genes regulated by UV-B radiation, we studied UV-B responses in *med17* plants. First, we analyzed *med17* seedling lethality after UV-B exposure. Seedlings were grown on MS-agar plates under light conditions; a group of plants was irradiated with UV-B for 1h while a different group was kept in the dark for the same period. A third group of plants was grown under normal light conditions. Although *med17* seedlings germinated and grew similar to WT seedlings under white light illumination (100 $\mu\text{E m}^{-2}\text{s}^{-1}$); darkness affected the growth of some *med17* mutants. However, a UV-B treatment provoked lethality of most *med17* plants, while WT seedlings were very low affected by the same treatment (Figure 2, A).

Figure 2 *med17* plants show higher UV-B sensitivity and DNA damage after UV-B than WT plants. A, Representative images of *med17* and WT Col-0 seedlings grown under light conditions and after 15 days were UV-B irradiated (UV-B) or kept under dark conditions (darkness) as described in Materials and methods. Alternatively, plants were grown under normal photoperiod (light conditions, and then kept under normal growth conditions after UV-B or kept under dark conditions. B and C, Relative CPD levels in the DNA of WT Col-0, *med17* and OE MED17 plants grown under control conditions or immediately after a 4-h UV-B treatment under light conditions (B), or immediately after a 4-h UV-B treatment under dark conditions and 2 h after recovery in the dark or in the light to allow photorepair (C). Results represent averages $\pm$ S.E.M. of six independent biological replicates. Different letters indicate statistically significant differences applying ANOVA test ($P < 0.05$), asterisks indicate statistically significant differences applying T-Student test ($P < 0.05$).

229 Flowering time is delayed by UV-B radiation in Arabidopsis (Dotto et al., 2018;
 230 Arongaus et al., 2018). As previously reported in Kim et al. (2011) and similarly as it
 231 was also demonstrated for other Mediator subunit mutants such as *med25* and *med18*
 232 (Iñigo et al., 2012; Zheng et al., 2013), *med17* mutants flowered later than WT plants
 233 (Supplemental Figure S2, A-C). After growth under UV-B conditions, while WT plants
 234 showed a delay in flowering time, *med17* mutants showed a similar flowering time as
 235 that observed under control conditions. Thus, MED17 is a regulator of this
 236 developmental pathway and it may have a role in the regulation of flowering time under

237 UV-B conditions. Together, the phenotypes analyzed show that *med17* mutants are
238 sensitive to UV-B exposure.

239

240 Plants with altered expression of *MED17* are deficient in the DNA damage response.

241

242 We then focused on the role of MED17 in the DNA damage response after UV-B
243 exposure. We first investigated if MED17 participates in DNA damage and repair using
244 both *med17* mutants and transgenic plants that overexpress *MED17* under the control of
245 the 35S promoter (*OE MED17*; Supplemental Figure S3). While all plants showed very
246 low and similar levels of CPDs under control conditions in the absence of UV-B, *med17*
247 mutants accumulated higher DNA damage after a UV-B treatment than WT plants when
248 the treatments were done under conditions that allowed photoreactivation (Figure 2, B).
249 On the contrary, *OE MED17* plants had less CPDs after UV-B exposure under the same
250 conditions. *med17* mutants also accumulated more CPDs than WT plants when the UV-
251 B treatments were done in the dark to prevent DNA repair by photolyases, and 2h after
252 the end of the UV-B treatment, either when recovery was done under light or dark
253 conditions (Figure 2, C). Thus, *med17* mutants accumulate more CPDs after UVB
254 exposure, and MED17 role during DNA damage and repair seems to be important not
255 only during dark repair but also during photoreactivation.

256 We next analyzed whether plants with altered *MED17* expression showed
257 differences in programmed cell death (PCD) after UV-B exposure. When DNA damage
258 occurs, DNA damage responses (DDR) are triggered that converge to a PCD pathway,
259 so as to avoid propagation of mutations in case the damage is not properly repaired
260 (Furukawa et al., 2010). As shown in Figure 3, A, C, one day after a UV-B treatment,
261 WT primary roots accumulated a higher number of dead cells after UV-B exposure than
262 *med17* roots, and lower than *OE MED17* lines; while none of the analyzed lines showed
263 any dead cells in untreated roots. However, 4 days after the treatment, although UV-B
264 irradiated WT and *OE MED17* roots recovered and dead cells were almost undetectable;
265 the number of dead cells in UV-B irradiated *med17* mutants was higher than in WT
266 roots (Figure 3, B, C). Thus, MED17 is required for a proper activation of the PCD
267 pathway after UV-B exposure.

268 Another consequence of the DDR in plants is the inhibition of cell proliferation
269 (Culligan et al., 2006). Thus, we analyzed the effect of UV-B on cell proliferation in the
270 primary root meristems of plants with altered *MED17* expression. *med17* mutants had a

Figure 3 Programmed cell death in meristematic root cells in WT Col-0, *med17* and *OE MED17* plants after UV-B exposure. A and B, Number of stem cells that are dead after 1 day (A) or 4 days (B) of UV-B exposure in WT Col-0, *med17* and *OE MED17* roots. Results represent the average of at least 50 biological replicates $\pm$ S.E.M. Different letters indicate statistically significant differences applying analysis of variance test ($p < 0.05$). C, Representative images of stem cells and adjacent daughter cells from WT Col-0, *med17* and *OE MED17* seedlings that were scored for intense PI staining to count dead stem cells per root 1 day and 4 days after a UV-B treatment or under control conditions. Scale bar represents 100 μ m.

271 smaller meristematic zone in the primary root than WT plants, which was significantly
 272 decreased after a UV-B treatment (Supplemental Figure S4, A and 5). The decrease in
 273 the meristematic zone size was higher in *med17* than in WT plants, in contrast to *OE*
 274 *MED17* plants, which were less inhibited by UV-B (Supplemental Figure 4, A and B).
 275 The higher decrease in the meristem size of *med17* roots by UV-B was a consequence
 276 of a higher inhibition of cortex cell proliferation than that measured in WT roots; and
 277 the opposite was observed in *OE MED17* roots (Supplemental Figure 4, C and D), while

the increase in cortex cell length determined in the meristems of all plants by UV-B was similar (Supplemental Figure 4, E and F). In this way, MED17 also regulates cell proliferation after UV-B exposure, altering root meristem size.

med17 and *OE MED17* plants show altered UV-B regulation of gene expression

Next, we investigated how the expression of genes that respond to this radiation and regulate its responses was affected in *med17* seedlings after UV-B exposure. First, we analyzed the expression of three transcripts encoding enzymes that participate in DNA repair by UV-B, *UVR2* and *UVR3*, which encode CPD and 6-4 photoproduct photolyases, respectively; and *UVR7* (or *ERCC1*), which encodes a DNA repair endonuclease of the NER system. *UVR3* belongs to cluster 4 in Figure 1, C, showing decreased expression in *med17* plants (Supplemental Table S2). While the expression of *UVR2* was similar in *med17* and WT seedlings after UV-B exposure; *UVR3* and *UVR7* showed significantly lower levels than WT plants under control and UV-B conditions (Figure 4, A-C). Thus, the higher accumulation of CPDs by UV-B in *med17* plants could be due to decreased expression of DNA repair enzymes. As flowering time was also affected in *med17* plants, we analyzed the expression of *FLC*, which encodes a master repressor of flowering time (Michaels and Amasino, 1999). *FLC* levels were significantly higher in *med17* both under control conditions and after UV-B, with similar levels under both conditions (Figure 4, D); hence, the delay in flowering time in *med17* under both conditions could be explained by increased levels of this protein. Transcript levels of the DNA damage response kinases ATR and ATM, and the transcription factor SOG1, a master regulator of the DDR (Furukawa et al., 2010), were also analyzed. Fig 4, E-G, shows that both *ATR* and *ATM* levels were higher in *med17* than in WT plants, both under control conditions and after UV-B exposure. However, *SOG1* expression was significantly decreased in *med17* under both conditions analyzed, suggesting that decreased levels of this transcription factor may affect the DNA responses after UV-B exposure. On the contrary, *MAPK6* levels in *med17* mutants were not different to those in WT plants (Figure 4, H), this kinase is also a regulator of some UV-B responses, so the phenotypes observed in the mutants are not due to changes in the expression of this enzyme.

UVR8, which encodes the UV-B photoreceptor that regulates mostly photomorphogenic responses and which levels were not changed by UV-B in WT plants

Figure 4 UV-B effect on expression of genes that participate in UV-B responses in WT *Col-0* and *med17* seedlings. Relative expression levels of *UVR2* (A), *UVR3* (B), *UVR7* (C), *FLC* (D), *ATM* (E), *ATR* (F), *SOG1* (G), *MAPK6* (H), *UVR8* (I), *HY5* (J), *CHS* (K) and *PDCD5* (L) analyzed by RT-qPCR in WT *Col-0* and *med17* seedlings under control conditions or immediately after a 4 h-UV-B treatment (UV-B). Results represent the average $\pm$ SEM. Different letters indicate statistically significant differences applying an ANOVA test ($P < 0.05$). Data represent at least three biological replicate experiments. Each RT-qPCR was repeated at least three times on each biological replicate.

312 (Figure 4, I), was similarly expressed in *med17* and WT seedlings under control
313 conditions, but showed a small but significant repression after UV-B exposure in the
314 mutant. *HY5*, which encodes a transcription factor that regulates UV-B responses in the
315 UVR8 pathway and showed decreased expression in *med17* plants in the RNA seq data
316 (Supplemental Table S1), also showed decreased levels after UV-B exposure (Figure 5,
317 J). Thus, under UV-B conditions, *med17* mutants have decreased expression of
318 important regulators of the UV-B photomorphogenic pathway. Finally, *CHALCONE*
319 *SYNTHASE* (*CHS*) transcript levels in *med17* were analyzed. *CHS* is a target of *HY5*

Figure 5 UV-B effect on expression of genes that participate in UV-B responses in WT Col-0 and OE MED17 seedlings. Relative expression levels of *UVR3* (A), *UVR7* (B), *HY5* (C), *PDCD5* (D), *SOG1* (E), *ATR* (F) and *ATM* (G) analyzed by RT-qPCR in WT Col-0 and OE MED17-4 seedlings under control conditions or immediately after a 4 h-UV-B treatment (UV-B). Results represent the average $\pm$ SEM. Different letters indicate statistically significant differences applying an ANOVA test ($P < 0.05$). Data represent at least three biological replicate experiments. Each RT-qPCR was repeated at least three times on each biological replicate.

320 and encodes the first enzyme in the flavonoid pathway; these specialized metabolites
321 provide UV-B protection in plants (Falcone Ferreyra et al., 2012). *CHS* expression was
322 not altered in *med17* mutants, neither under control conditions nor after exposure
323 (Figure 4, K). Interestingly, other transcripts that encode enzymes in the flavonoid
324 pathway, such as *FLAVONOL SYNTHASE 1*, *CHALCONE ISOMERASE 1 and 3*,
325 *FLAVONOID 3'-MONOOXYGENASE (CYP75B1)*; and the transcription factor *MYB*
326 *111*, which regulates the expression of enzymes in the flavonoid pathway, belong to
327 cluster 1 in the RNAseq data (Figure 1, B, Supplemental Table S1). These transcripts
328 show decreased expression in *med17* mutants under control conditions. Despite this,
329 flavonoid levels in *med17* and OE MED17 plants were similar to those in WT plants
330 (Supplemental Figure S2, D), correlating with the expression patterns of *CHS*. In this
331 way, increased sensitivity to UV-B in *med17* plants is not due to changes in flavonoid
332 accumulation.

On the other hand, when transcript levels of DNA repair enzymes with altered expression in *med17* mutants were analyzed in *OE MED17* plants, results showed that *UVR3* and *UVR7* were highly expressed in the transgenic plants, both under control conditions and after UV-B exposure (Figure 5, A, B), suggesting that lower CPD levels in *OE MED17* plants after UV-B exposure are due to high expression of these DNA repair enzymes. Interestingly, UV-B up-regulation of both genes is lost in the *OE MED17* plants; and this is also true for other UV-B responsive genes such as *HY5* and *SOG1* (Figure 5, C-E). Therefore, MED17 could mediate UV-B regulation of at least some UV-B marker genes, this may be through its regulation of *HY5* expression. On the contrary, both *ATR* and *ATM*, which were expressed at high levels in *med17* mutants, showed very low levels in *OE MED17* plants, in particular after UV-B exposure (Figure 5, F, G); thus, MED17 is a negative regulator of both DDR kinases.

Together, qRT-PCR results demonstrate that, for at least some genes, in particular *HY5*, *UVR3*, and *UVR7*, the upregulation after UV-B is lost in *med17* and constitutively increased in *OE MED17* seedlings; therefore, at least some of the phenotypes observed in UV-B irradiated plants could be due to altered expression these genes.

MED17 interacts with nuclear proteins with roles during DNA repair

As described in the Introduction, in humans and yeasts, MED17, besides being a transcriptional regulator, also physically interacts with proteins that participate in DNA repair through the NER system. Therefore, to analyze if in Arabidopsis there is also a physical interaction of MED17 with DNA repair enzymes after UV-B exposure, Arabidopsis nuclei were obtained from UV-B irradiated *med17* mutants expressing *MED17* fused to *GFP* under the *35S* promoter. Expression of this fusion protein complemented the mutant phenotypes suggesting it is active *in vivo* (Supplemental Figure S3).

MED17 was co-immunoprecipitated from purified nuclei using anti-GFP antibodies, and the output was analyzed by LC-MS/MS (Smaczniak et al., 2012). Sixty-five nuclear proteins coimmunoprecipitated with MED17-GFP in at least 2 of 3 biological replicates from UV-B treated plants (Supplemental Table S3). In this group, there were other Mediator proteins, such as MED8, MED37 A/B, C and F; transcription initiation and splicing factors, chromatin associated proteins and other transcription

factors (Supplemental Table S3). Interestingly, several proteins which were previously described to have a role during DNA repair were identified, such as the cohesin factor PDS5C (Pradillo et al., 2015), the histone chaperones NAP 1; 3 and NRP 1 and 2 (Casati and Gomez, 2021), two DEK domain-containing chromatin associated proteins (Waidmann et al., 2014), a sister chromatid cohesion 1 protein 4 (SYN4; da Costa-Nunes et al., 2004); a DNA repair ATPase-related protein and a replication factor C subunit 4 (Chen et al., 2018; Table 1, Supplemental Table S3). LC-MS data showed that MED17 is in a same complex with the transcription initiation factor TFIID subunit 9 and the transcription initiation factor IIE subunit alpha; therefore, MED17, together with these proteins, may be required for correct TCR repair as previously described in other species. Moreover, a cell division cycle 5-like protein (CDC5) was also found to immunoprecipitate with MED17 in plants exposed to UV-B, this protein has a role in cell cycle control and also in the response to DNA damage (Table 1; Lin et al., 2007). Thus, MED17 may not only directly participate in DNA repair, but it may also regulate the DNA damage response by interacting with other proteins acting downstream in the DDR pathway. This data suggests that, as demonstrated in yeasts and humans, AtMED17, besides having a role in transcription regulation by UV-B as shown in Figures 4 and 5, it could have a direct role interacting with nuclear proteins during DNA repair.

atr mutant phenotypes are suppressed in the absence of MED17

To further analyze the role of MED17 in the DDR, we crossed *med17* mutants with either *atm* or *atr* plants to generate the corresponding double mutants. Ataxia telangiectasia mutated (ATM) is usually activated by double-strand breaks in the DNA; while ATR is mainly triggered by single-strand breaks or stalled replication forks such as CPDs and 6-4PPs that occur after UV-B exposure; and both independently regulate the DNA damage response in plants (Culligan et al., 2006). For the *med17 atm* crosses, only heterozygous mutants in either or both genes were obtained after screening 60 plants, but no double homozygous mutant plants were recovered, suggesting that *med17* deficiency generates lesions that require ATM to allow cell viability. On the contrary, *med17 atr* double homozygous mutants were obtained (Supplemental Figure S6). *med17 atr* plants were smaller than WT and *atr* plants when grown under standard growth conditions in the growth chamber, but they looked like *med17* mutants

(Supplemental Figure S6, A). As *med17* single mutants, the double mutants had smaller leaves than WT and *atr* plants (Supplemental Figure S6, A). In addition, both the single *med17* and double *med17 atr* mutants displayed a significant reduction in the siliques length compared to WT and *atr* plants (Supplemental Figure S6, B and C). The number of seeds per silique in *med17* and *med17 atr* mutants was also decreased compared to WT and *atr* (Supplemental Figure S6, D). When the seeds in each silique were observed, *med17* and *med17 atr* mutants showed both fertilized seeds and aborted embryos, which correlates with the failure observed in seed production (Supplemental Figure S6, B, E and F). In contrast, both WT and *atr* mutants showed low and similar number of aborted seeds (Supplemental Figure S6, E and F). In order to analyze if embryos had dead cells, seeds were stained with propidium iodide and analyzed by confocal microscopy (Supplemental Figure S7). While WT and *atr* seeds showed a very low number of stained cells, both *med17* and *med17 atr* seeds showed higher staining. These results could explain the decreased fertility observed in *med17* and *med17 atr* mutants.

When plants were exposed to UV-B radiation, all mutants accumulated similar levels of CPDs and higher than those in WT plants (Figure 6, A). On the other hand, PCD was analyzed in the meristematic zone of the primary roots one day after a UV-B treatment and *med17 atr* mutants showed significantly fewer dead cells than *atr* and WT plants (Supplemental Figure S8). Moreover, 4 days after the treatment, *atr* mutants still had higher number of dead cells than the other lines under study, while *med17* and *med17 atr* mutants had a similar number of dead cells and higher than those in WT plants, which were almost recovered (Figure 6, B and C).

The effect of UV-B was investigated on the meristematic zone of the primary roots. The size of the meristematic zone of the primary roots from *atr* seedlings was similar to that of WT primary roots under control conditions in the absence of UV-B, while the size of that in the double mutant was significantly smaller (Figure 7a). In UV-B treated roots, there was a significant decrease in the meristematic zone size in all lines; however, the decrease observed was significantly higher in *atr* mutants, while *med17 atr* plants showed a similar decrease as that in *med17* plants (Figure 7, A and B). This higher decrease in the meristem size in *atr* plants was a consequence of a higher decrease in the cortex cell number in the root meristem than that in *med17*, *med17 atr* and WT plants by UV-B, while all lines showed a similar increase in the cell area after the treatment (Figure 7, C-F). Similarly as for all other parameters analyzed, MED17 is

Figure 6 Characterization of DNA damage responses in double *med17 atr* mutant plants. A, Relative CPD levels in the DNA of WT Col-0, *med17*, *atr* and *med17 atr* plants immediately after a 4-h UV-B treatment under light conditions. Results represent averages $\pm$ S.E.M. of six independent biological replicates. B, Programmed cell death in meristematic root cells in Col-0, *med17*, *atr* and *med17 atr* plants 4 days after UV-B exposure. Results represent the average of at least 50 biological replicates $\pm$ S.E.M. Different letters indicate statistically significant differences applying analysis of variance test ($p < 0.05$). C, Representative images of stem cells and adjacent daughter cells from WT Col-0, *med17*, *atr* and *med17 atr* seedlings that were scored for intense PI staining

435 required for the higher inhibition of cell proliferation in the meristematic zone of the
 436 primary roots observed in *atr* mutants under UV-B conditions. Therefore, *atr*
 437 phenotypes are suppressed in the absence of MED17. Interestingly, *med17* seedlings
 438 show increased *ATR* expression (Fig 6f); thus, the similar phenotypes observed in
 439 *med17* and *med17 atr* double mutants are independent of *ATR* levels.

440

Figure 7 UV-B root meristematic zone of *med17 atr* is similarly affected by UV-B as *med17* seedlings but differently than *atr* mutants. A, Average of meristematic root zone length; C, cortex cell number; E, cortex cell length in the root meristem from WT Col-0, *med17*, *atr* and *med17 atr* seedlings after 4 days of a UV-B treatment or under control condition. B, D and F, Ratio between meristematic root zone length (B), cortex cell number (D), and cortex cell area values (F) measured after UV-B exposure vs those under control conditions are shown. Results represent the average $\pm$ S.E.M. Different letters indicate statistically significant differences applying analysis of variance test ($P < 0.05$).

441 MED17 deficiency is overcome by PDCD5 overexpression during DNA damage
442 conditions after UV-B exposure

443

444 In addition, we investigated whether the role of MED17 in the DDR after UV-B
445 exposure was affected in plants with altered expression of *PDCD5*. Previously, we
446 showed that AtPDCD5 participates in the DNA damage response after UV-B exposure

Figure 8 Characterization of DNA damage responses in *OE PDCD5 med17* plants. A, Relative CPD levels in the DNA of WT Col-0, *med17*, *OE PDCD5* and *OE PDCD5 med17* plants immediately after a 4-h UV-B treatment under light conditions. Results represent averages $\pm$ S.E.M. of six independent biological replicates. B, Programmed cell death in meristematic root cells of WT Col-0, *med17*, *OE PDCD5* and *OE PDCD5 med17* plants 1 day after UV-B exposure. Results represent the average of at least 50 biological replicates $\pm$ S.E.M. Different letters indicate statistically significant differences applying analysis of variance test ($P < 0.05$).

447 in Arabidopsis (Falcone Ferreyra et al., 2016). Plants that overexpressed AtPDCD5
 448 accumulated lower levels of DNA damage after UV-B exposure and showed more PCD
 449 in root tips upon UV-B exposure (Falcone Ferreyra et al., 2016). Thus, we obtained
 450 transgenic plants that overexpressed PDCD5 in a *med17* mutant background by genetic
 451 crosses. When we analyzed DNA damage accumulation after UV-B in these plants, they
 452 showed lower amounts of CPDs than *med17* mutants, and even lower than WT plants,
 453 with CPD levels similar to those measured in *OE PDCD5* lines in a WT background
 454 (Figure 8, A). Moreover, when PCD was analyzed in the *OE PDCD5 med17* line one
 455 day after a UV-B treatment, these transgenic plants had a higher number of dead cells in
 456 the meristematic root zone than WT and *med17* roots, and similar to *OE PDCD5* plants

457 in a WT background (Figure 8, B). Thus, after UV-B exposure, *PDCD5* overexpression
458 counteracts the deficiency of *MED17* during the DDR.

459 When the expression of *PDCD5* was analyzed in *med17* and *OE MED17*
460 seedlings, transcripts were significantly lower in *med17* than in WT after UV-B
461 exposure, despite the opposite was observed under control conditions (Figure 4, L);
462 while in *OE MED17* plants *PDCD5* expression was significantly higher than in WT
463 plants, both under control conditions and after UV-B exposure (Figure 5, D). In this
464 way, some of the phenotypes of *med17* and *OE MED17* plants after UV-B could be the
465 result of altered *PDCD5* expression.

466

467

468 Discussion

469

470 MED17 is a subunit of the head module of the Mediator complex, it has an important
 471 role interacting with several other Mediator subunits, being a key scaffold component of
 472 the whole complex (Guglielmi et al., 2004; Cevher et al., 2014; Maji et al., 2019). In
 473 Arabidopsis, MED17 is required for smRNA biogenesis and for the repression of
 474 heterochromatic loci, suggesting that this Mediator subunit, besides having a role in
 475 transcription, it would also participate in genome stability (Kim et al., 2011). In this
 476 work, we aimed to investigate the role of AtMED17 in UV-B responses. The analysis of
 477 the transcriptome profile of *med17* mutants compared to that of WT plants grown under
 478 white light conditions showed that of the 6822 genes that showed altered expression in
 479 *med17* mutants, 32% were also UV-B regulated in WT Col-0 plants reported in
 480 previous experiments (Tavridou et al., 2020). Of these 2184 genes, about 56% showed
 481 low expression in *med17* mutants and were up-regulated by UV-B in WT plants,
 482 suggesting that MED17 has a positive role in the regulation of UV-B responsive genes
 483 in Arabidopsis plants. In this group and as shown in Supplemental Figure S1, C, we
 484 found *RUP1* and *RUP2*, which encode two highly related WD40-repeat proteins that are
 485 negative regulators of the UVR8 photoreceptor in UV-B photomorphogenic responses
 486 (Gruber et al., 2010). *HY5* and *HYH*, which encode transcription factors that mediate
 487 UV-B responses of the UVR8-dependent pathway and activate the expression of *RUP1*
 488 and *RUP2*, also showed decreased expression in *med17* mutants. CRY1 and CRY3,
 489 which are blue light photoreceptors in the nuclei (CRY1) and in the chloroplasts and
 490 mitochondria (CRY3; Liu et al., 2011), were also down-regulated in *med17* plants. In
 491 particular, CRY3 belongs to the CRY-DASH clade of the photolyase/cryptochrome
 492 superfamily, and besides acting as a photoreceptor, it may also have single-strand DNA
 493 repair activity (Selby and Sancar, 2006; Pokorny et al., 2008). Interestingly, *UVR3*,
 494 which encodes an enzyme with 6–4 photolyase activity in Arabidopsis (Nakajima *et al.*,
 495 1998), increased in response to UV-B in WT, but not in *med17* plants, and was
 496 constitutively highly expressed in *OE MED17* plants. On the other hand, other genes
 497 that encode DNA recombination and repair, such as *RADA* and *RAD23 B-D* (Ishibashi
 498 et al., 2006; Lahari et al., 2018) showed up-regulation in *MED17* deficient plants,
 499 demonstrating that MED17 is required for proper expression of DNA repair enzymes.

500 In yeast, MED17 physically interacts with Rad2/XPG and participates in DNA
 501 repair after UV exposure, recruiting Rad2 to transcribed genes (Eyboulet et al., 2013).

Moreover, in human cells, MED17 interacts with a DNA helicase XPB subunit of TFIIF, which is essential for both transcription and NER, and similarly as it was described in yeast, MED17 plays an important role switching between transcription and DNA repair (Kikuchi et al., 2015). After immunoprecipitation studies using transgenic plants expressing *MED17-GFP*, we demonstrated that in Arabidopsis, MED17 also directly or indirectly interacts with proteins that participate in DNA repair, such as the histone chaperones NAP 1; 3 and NRP 1 and 2 (Casati and Gomez, 2021) and a DNA repair ATPase-related protein and a replication factor C subunit 4 (Chen et al., 2018). Moreover, the results presented here show that *med17* mutants accumulate higher DNA damage than WT plants, while plants that overexpress *MED17* accumulate lower amounts of CDPs after UV-B exposure. In this way, and similarly as MED17 from yeast and humans, AtMED17 has a role in DNA repair. In yeast and humans, MED17 participates in transcription-coupled DNA repair (TCR) by NER DNA repair pathway, which removes DNA lesions that interfere with the progression of the RNA polymerase through transcribed genes (Eyboulet et al., 2013; Kikuchi et al., 2015; Hanawalt and Spivak, 2008). In Arabidopsis, *med17* plants are deficient not only in dark repair, which in plants is mostly achieved by NER, but also during light conditions, mostly accomplished by photolyases (Spampinato, 2017). Our results show that AtMED17 associates in a same complex with the transcription initiation factors TFIID subunit 9 and TFIIE subunit alpha, which may participate in TCR repair. Additionally, MED17, directly or indirectly interacting with the Replication Factor C subunit 4, could have a role in DNA repair by the NER system in Arabidopsis. AtMED17 also co-immunoprecipitated with histone chaperones and other chromatin associated factors, which may be required for proper dark DNA repair but also during photoreactivation. Thus, *med17* plants may be deficient in DNA repair due to decreased TCR repair and because MED17 may be necessary to interact with chromatin proteins during DNA repair. In addition, *med17* plants may accumulate more DNA damage after UV-B exposure because they express lower levels of some photolyases and other DNA repair proteins.

In addition, *med17* mutants showed a higher inhibition of cell proliferation in the root meristems after a UV-B treatment, and the meristems had less dead cells 1 day after, while they still presented dead cells after 4 days, in contrast to WT primary roots which showed more dead cells 1 day after the treatment but completely recovered after 4 days. These results suggest that MED17 may also participate in other aspects of the

DDR besides DNA repair. A low accumulation of dead cells persisting after exposure under genotoxic conditions was also previously observed in *sog1* mutants (Johnson et al., 2018). SOG1 is a transcription factor that, in *Arabidopsis thaliana*, is a master regulator of genes that participate in the DNA damage response, including in the activation of programmed cell death in the meristematic cells in the roots. Johnson et al. (2018) demonstrated that, in contrast to WT plants, *sog1* mutants are defective in damage-induced programmed cell death and fail to undergo cell division in the primary roots meristems. A similar response was also observed in *med17* mutants in our experiments. Interestingly, *SOG1* transcript levels were significantly decreased in *med17* plants, suggesting that the PCD phenotype and the inhibition of cell proliferation in the primary root meristems after UV-B exposure in the *MED17* deficient plants can be due to decreased expression of this transcription factor.

Interestingly, other Mediator subunits have been shown to participate in PCD and root development, for example MED18, which is also a subunit of the Mediator head. *med18* mutants show a reduction in primary root growth, with an increase in lateral root formation and root hair development (Raya-Gonzalez et al., 2018). *med18* roots had altered cell division and elongation with an increased auxin response and transport at the root tip. Moreover, *med18* seedlings showed PCD in the root meristem in the absence of any genotoxic stress, which increased with age and/or exposition to DNA-damaging agents (Raya-Gonzalez et al., 2018). Despite MED17 and MED18 are both components of the Mediator head, they have different roles at least during DNA damage conditions. For example, while *med18* roots show high PDC in the meristematic primary root zone even in the absence of any genotoxic agent; *med17* roots show an opposite phenotype, with very low number of dead cells after UV-B exposure, and undetectable dead cells under control conditions in the absence of UV-B. Moreover, while meristematic cells in the primary roots of *med18* seedlings are bigger than those in WT roots (Raya-Gonzalez et al., 2018); cells in the meristematic zone of *med17* plants are similar to those in WT plants. *med17* root meristems are shorter than WT root meristems because they have less cells, and they show a higher decrease in the number of cells after UV-B exposure, suggesting that while MED18 may have an important role controlling cell size, MED17 may mostly control cell proliferation.

In *Arabidopsis*, both ATR and/or ATM regulate DNA damage responses after UV-B exposure (Furukawa et al., 2010). The double *med17 atr* mutant showed similar phenotypes as those of *med17* mutants, both under control conditions and after UV-B

570 exposure; thus, MED17 is required for a proper activation of the DNA response
571 mediated by ATR. It is possible that MED17 could act upstream ATR, for example
572 interacting with DNA repair proteins during DNA damage recognition. The absence of
573 proper damage recognition due to MED17 deficiency may therefore affect the activation
574 of the DDR through ATR. However, we cannot rule out that MED17 may modulate
575 ATR activity in other ways, for instance regulating the expression of proteins required
576 for ATR activation of the DDR.

577 We previously characterized a UV-B inducible protein, AtPDCD5, which is
578 similar to human PDCD5, a PCD-associated protein (Falcone Ferreyra et al., 2016; Xu
579 et al., 2009). In humans, PDCD5 interacts with a histone acetyltransferase of the MYST
580 family, TIP60, which are together recruited to chromatin in response to DNA damage,
581 where they participate in different stages of repair (Murr et al., 2006; Xu et al., 2009).
582 On the other hand, PDCD5 is also involved in the activation of PCD in the cytosol
583 (Zhuge et al., 2011). *pcd5* mutants accumulate higher levels of CPDs than WT plants
584 after UV-B exposure but lower PCD in the primary root tip; these phenotypes are
585 similar to those of *med17* mutants. On the contrary, plants overexpressing *AtPDCD5*
586 were less sensitive to DNA damage and showed more dead cells in the root meristems
587 after UV-B exposure (Falcone Ferreyra et al., 2016). Our results show that
588 overexpression of *PDCD5* counteracts the deficiency in MED17 levels. As *PDCD5*
589 levels are affected in plants with altered *MED17* expression, some of the phenotypes
590 after UV-B exposure in *med17* and *OE MED17* plants could be, at least in part, the
591 result of altered *PDCD5* expression.

592 In summary, our results demonstrate that MED17 regulates different plant
593 responses to UV-B in Arabidopsis plants, in particular the DDR. According to the
594 presented data, MED17 not only transcriptionally modulates the expression of genes of
595 the DDR and the UV-B pathway, but it also physically interacts with transcription
596 initiation factors and/or chromatin proteins that could facilitate DNA repair. Finally, we
597 here show that MED17 is required for the *atr* mutant phenotypes, and that its deficiency
598 is overcome by *PDCD5* overexpression. The interaction of MED17 with ATR and
599 PDCD5 during the DDR may be regulating gene expression of proteins in the pathway,
600 but it may also be during the early recognition of DNA damage through the binding
601 with DNA repair proteins, which may be necessary for the activation of the pathway.

602

603 **Materials and methods**

604

605 Plant material, growth conditions and UV-B treatments

606

607 *A. thaliana* ecotype Col-0 was used for all experiments. *med17-1* (SALK_102813)
608 mutants were provided by Dr. Xuemei Chen (University of California, Riverside, USA).
609 *atr-2* (SALK_03284) and *atm-2* (SALK_006953) seeds were provided by Dr. Roman
610 Ulm (University of Geneva, Switzerland). *OE PDCD5* transgenic plants (Falcone
611 Ferreyra et al., 2016), *atr-2* or *atm-2* single mutants were crossed with *med17* mutants
612 and the F2 population was screened by PCR using specific primers for *MED17*, *ATR*,
613 *ATM* and *PDCD5* genes (Supplemental Table S4). For all experiments, F3 plants were
614 used.

615 Arabidopsis plants were sown on soil, stratified for 3 days at 4 °C and they were
616 then moved to a growth chamber. Plants were grown at 22 °C under a 16-h-light/8-h-
617 dark photoperiod (100 $\mu\text{E m}^{-2}\text{s}^{-1}$). For root and hypocotyl studies, plants were
618 germinated and grown on petri plates containing Murashige and Skoog salt (MS)-agar
619 (0.7 % w/v) medium for 5 days.

620 For all UV-B treatments except for flowering time assays, plants were irradiated
621 with UV-B lamps using fixtures mounted 30 cm above the plants (2 W m^{-2} UV-B and
622 0.6 W m^{-2} UV-A, Bio-Rad ChemiDoc™XRS UV-B lamps, catalogue 1708097). The
623 lamps have emission spectra from 290 to 310 nm, with a maximum emission peak at
624 302 nm. The bulbs were covered using cellulose acetate filters (100 mm extra-clear
625 cellulose acetate plastic, Tap Plastics, Mountain View, CA); the cellulose acetate filters
626 absorb wavelengths lower than 290 nm without removing UV-B from longer
627 wavelengths. As a control treatment without UV-B, plants were exposed for the same
628 period of time under lamps covered with a polyester plastic that absorbs UV-B at
629 wavelengths lower than 320 nm. For root and hypocotyl elongation assays, seedlings
630 were irradiated for 1 h. For DNA damage analysis, 4-week-old plants were irradiated
631 with UV-B for 4 h, and leaves were collected immediately and after 2 h of recovery in
632 the absence of UV-B, either under light or dark conditions. UV radiation was measured
633 using a UV-B/UV-A radiometer (UV203 AB radiometer; Macam Photometrics).

634 For flowering time analysis, white light was supplemented with 2 W m^{-2} of UV-
635 B (311 nm; Phillips narrowband TL/01 lamps) during 1 h every day starting from day 9
636 after transferring to the growth chamber until flowering, the zeitgeber time of UV-B
637 treatments was 4 h in long day conditions.

For seedling lethality analysis, seeds were sown in agar plates and stratified for 3 days. Then, they were irradiated with white light ($100 \mu\text{E m}^{-2} \text{s}^{-1}$) for 1 h and, after that, they were kept 24 h in the dark. Next, plates were treated with UV-B for 1 h, transferred to darkness for 48 h and then they were finally allowed to grow in the growth chamber for 15 days under normal light conditions (UV-B irradiated plants). Alternatively, a different group of seedlings were grown under the same conditions but they were not UV-B irradiated (darkness treated plants). Additionally, seedlings were stratified for 3 days and they there were grown under normal growth conditions for 18 days (light grown plants).

UV radiation was measured using a UV-B/UV-A radiometer (UV203 AB radiometer; Macam Photometrics).

DNA damage analysis

12 DAS leaf samples from plants treated with UV-B or kept under control conditions were collected immediately or 2 hours after the end of the treatment and immersed in liquid nitrogen. CPD accumulation in the DNA purified from the collected samples was analyzed as described previously (Lario et al., 2013). UV-B treatments were performed both under light and dark conditions; plants irradiated under dark conditions were allowed to recover for 2 h under light or dark conditions. 0.1 g were collected and extracted DNA was dot blotted onto a nylon membrane (Perkin-Elmer Life Sciences). The blot was incubated with monoclonal antibodies specific to CPDs (TDM-2) from Cosmo BioCo (1:2,000 in TBS). Quantification was achieved by densitometry of the dot blot using Image-Quant software version 5.2.

Root meristem analysis and programmed cell death after UV-B exposure

Seedlings were grown for 5 days in vertically oriented Murashige and Skoog plates, and were then irradiated with UV-B light or kept without UV-B. UV-B-irradiated and control seedlings were then incubated for 24 hr or 96 hr in the growth chamber, and then PCD was analyzed as described by Furukawa et al. (2010). Root tips were stained using a modified pseudo-Schiff propidium iodide staining protocol and visualized by confocal laser scanning microscopy (Nikon C1) under water with a 40× objective. The

excitation wavelength for propidium iodide-stained samples was 488 nm, and emission was collected at 520 to 720 nm. Dead (intensely Propidium iodide (PI)-staining) cells in the vicinity of the quiescent centre were counted and scored as dead cells per root.

675

676 Generation of Arabidopsis transgenic plants

677

cDNA was obtained from leaf tissues of WT plants grown under continuous light growth. *MED17* cDNA without its stop codon was amplified using specific primers including *KpnI* and *XhoI* restriction sites (Table S1). PCR was done using Pfu (Invitrogen) polymerase under the following conditions: 94°C for 5 min; 40 cycles of 94°C for 30 sec, 55°C for 20 sec and 72°C for 30 sec; and finally, one cycle at 70°C for 2 min. PCR product was purified from the gel, cloned in a pBluescript vector and sequenced. The construct was then transformed into *E. coli* DH5α and then the plasmid was purified and digested with *KpnI* and *XhoI*. The digestion fragment corresponding to *MED17* was subcloned into the pCardo plasmid, and the construct expressing *MED17* under the 35S promoter was transformed into *Agrobacterium tumefaciens* strain GV3101. Col 0 was transformed using the floral dip method (Clough and Bent, 1998). In addition, the pBluescript vector with *MED17* cDNA was digested using *Kpn* and *Sall* and the fragment was cloned into pCS052_GFP_pCHF3 (a modified version of pCHF3; with the GFP coding sequence without the start codon inserted into *Sall-PstI* sites). The resulting construct, pCHF3:*MED17-GFP*, was transformed into *E. coli* DH5α and purified. The construct was transformed into *Agrobacterium tumefaciens* GV3101 and Col 0 and *med17* plants were transformed using the floral dip method. Transformed seed (T1) were identified by selection on solid MS medium containing kanamycin (30 mg L⁻¹, pCHF3) or BASTA (3 mg mL⁻¹, pCardo, and finally plants were transferred to soil. The presence of *Pro35S:MED17* (*OE MED17*) and *Pro35S:MED17-GFP* (*OE MED17-GFP*) transgenes in T2 plants was screened by PCR using genomic DNA (Table S1).

700

701 Flowering time analysis

702

Flowering time was determined by counting the number of rosette leaves or the number of days until the first flower opens, similar to previous reports (Dotto et al., 2018).

704

705 Flowering time was counted as the number of rosette leaves at the moment of flowering
706 or the number of days until the first flower opens.

707

708 Seed analysis

709

710 Seeds were phenotypically analyzed using a Nikon SMZ-10 microscope. For silique
711 analyzes, 56 siliques from each genotype were analyzed, and the number of aborted
712 seeds per silique were counted. After that, seeds were stained using PI stain and they
713 were then observed using confocal laser scanning microscopy, using a Confocal Nikon
714 C1 microscope.

715

716 RNA-seq experiments

717 For the RNA-seq experiments, WT and *med17* seeds were sown on MS medium with
718 0.8 % (w/v) agar, kept at 4°C for three days and then grown for 10 days at 23°C under
719 long day (16 h light, 8 h dark, 100 $\mu\text{E m}^{-2} \text{s}^{-1}$) of white fluorescent light. Three
720 independent biological replicates for each genotype were harvested 2 h before the start
721 of the night period and the seedlings were immediately frozen in liquid nitrogen. Total
722 RNA was prepared using a Plant Total RNA Mini Kit (YRP50).

723 FASTQC v0.11.5 was used for quality control of the FASTQ sequence files
724 (Andrews, 2010). Illumina 150-bp paired-end reads were mapped to the *A. thaliana*
725 reference genome assembly (assembly version TAIR10) with HISAT2 (Kim et al.,
726 2015) and raw read counts per gene were then estimated with htseq-count (Anders et al.,
727 2015) and normalized according to trimmed mean of M-values (TMM) (Robinson and
728 Oshlack, 2010). Over 22 million reads were obtained for each sample, with an overall
729 alignment rate of 92%. Genes with more than five reads per million in only two or
730 fewer samples were eliminated from the analysis. Differential expression analysis of the
731 remaining genes was carried out with the R Package EdgeR (Robinson et al., 2010)
732 using a quasi-likelihood negative binomial generalized log-linear model (EdgeR
733 function glmQLFit) (Lun et al., 2016). Genes with FDR<0.05 were selected. A
734 complete list of identified transcripts is in Supplemental Table S1. Venn diagrams were
735 generated with VennDiagram R package (Chen and Boutros, 2011), and the Heatmap
736 was generated with Complex Heatmap R package (Gu et al., 2016). GO analysis was
737 performed using DAVID bioinformatics resources (Huang et al., 2009).

RNA seq data from Arabidopsis Col-0 plants UV-B irradiated was obtained from Tavridou et al. (2020). FASTQ files were obtained from Gene Expression Omnibus (GEO) repository, and processed as med17 files. Statistical analysis of the overlapping differentially expressed genes was done using the R Package GeneOverlap.

qRT-PCR analysis

Analysis was done as described in Maulion et al. (2019). Briefly, Total RNA was isolated using the TRIzol reagent (Invitrogen). 0.5 to 1.0 mg of total RNA was reverse transcribed using SuperScript II reverse transcriptase (Invitrogen) and oligo (dT) as a primer. The resultant cDNA was used as for quantitative PCR amplification in a StepOne™ System apparatus (ThermoFisher Scientific), using SYBRGreen I (Invitrogen) as a fluorescent reporter and Platinum taq polymerase (Invitrogen). Transcript levels were normalized to those of the *A. thaliana* calcium-dependent protein kinase3 (Supplemental Table S4) and to values in Col-0 plants grown under control conditions in the absence of UV-B.

Coimmunoprecipitation studies and MS analysis

For coimmunoprecipitation analyses, 3 g of Arabidopsis leaves were homogenized in a buffer containing 0.4M saccharose, 10mM Tris-HCl, pH 8.0, 10mM MgCl₂ and 1mM phenylmethylsulfonyl fluoride (PMSF). The extract was filtered through Miracloth and next was centrifugated for 20 min at 4500xg. The pellet was resuspended in buffer 2 (0.25M saccharose, 10mM Tris-HCl, pH 8.0, 10mM MgCl₂, 0.15% (v/v) Triton X-100, 5mM 2 mercaptoethanol and 0.1 mM PMSF). After 5 min of incubation in ice, the extract was centrifuged at 5000xg for 10 min. The pellet was resuspended in buffer 3 containing 0.44 M saccharose, 25mM Tris-HCl, pH 7.6, 10mM MgCl₂, 0.5% (v/v) Triton X-100 and 10mM 2-mercaptoethanol. The supernatant was discarded, and the pellet was resuspended in buffer 4 (0.44 M saccharose, 50mM Tris-HCl, pH 7, 5mM MgCl₂, 20% (v/v) glycerol and 10mM 2-mercaptoethanol) and centrifuged for 10 min at 12500xg. Finally, the pellet was resuspended in 200 µl of lysis buffer (10mM Tris-HCl, pH 7.5, 50mM NaCl, 0.1% (v/v) Triton X-100, 10% (v/v) glycerol and 1mM PMSF) and sonicated. Then, the extract was centrifugated at 17500xg for 15 min. After centrifugation, 1 mL of crude extract (0.75 mg of total protein) was incubated with 6 µL

772 (3 mg) of affinity-purified rabbit polyclonal antibody raised against GFP for 3 h at 4°C
 773 with gentle agitation. After this, 20 µL of protein A agarose was added, and the samples
 774 were incubated at 4°C with gentle agitation for 1 h. The agarose beads were pelleted by
 775 centrifugation and washed four times with 200 µL of lysis buffer (100 mM Tris-HCl,
 776 pH 7.5, 1 mM EDTA, 150 mM NaCl, and 1% (v/v) Triton X-100) for 5 min at 4°C and
 777 once with LNDET buffer (250 mM LiCl, 1% Nonidet P-40, 1% [w/v] deoxycholic acid,
 778 1 mM EDTA, and 10mM Tris-HCl, pH 8.0) for 5 min at 4°C. Proteins were eluted by
 779 incubation at 95°C for 5 min in 50 µL of SDS sample buffer. Samples were loaded on
 780 10% SDS-PAGE gels and run at 100 V for 15 min to allow proteins to migrate less than
 781 1 cm into the resolving gel. Gels were stained with colloidal Coomassie Brilliant Blue
 782 stain, and the immunoprecipitated protein-loaded lane was cut into one rectangular slice
 783 of less than 1 cm of height.

784 The gel slices were subjected to in-gel digestion (Link and LaBaer, 2009;
 785 <http://cshprotocols.cshlp.org/content/2009/2/pdb.prot5110.abstract>) with trypsin
 786 (porcine, side chain protected; Promega). Briefly, specific excised samples were washed
 787 once with 50% (v/v) acetonitrile in 50 mM NH₄HCO₃ and then dehydrated with pure
 788 acetonitrile. The gel samples were next reduced with dithiothreitol (DTT; 10 mM in 25
 789 mM NH₄HCO₃, 65°C for 30 min) and alkylated with iodoacetamide (55 mM in 25 mM
 790 NH₄HCO₃, room temperature for 30 min). Then, the gel pieces were incubated with
 791 acetonitrile, and rehydrated in 50 µL of digestion buffer (12 ng mL trypsin in 25 mM
 792 NH₄HCO₃) After overnight digestion at 37°C, peptides were extracted once with a
 793 solution containing 66% (v/v) acetonitrile and 5% (v/v) formic acid. The supernatants
 794 were concentrated to 5 µL by centrifugation under vacuum. The digests were analyzed
 795 by capillary HPLC-MS/MS.

796

797 HPLC-MS/MS

798

799 The peptide mixtures were analyzed in data-dependent mode on a Q-Exactive HF mass
 800 spectrometer coupled to an Ultimate 3000 nanoHPLC. A volume of 4 µL of peptide
 801 samples was loaded by the LC system. Peptides were desalted online on a reverse-phase
 802 C18 cartridge using buffer A (0.1% (v/v) formic acid) as running buffer, and then
 803 resolved on a 15-cm long PepMap nanocolumn (EASY-Spray ES801, Thermo) at a
 804 flow rate of 0.3 µL/min. Peptide elution was achieved with a gradient of buffer B (100%
 805 acetonitrile containing 0.1% formic acid). Total run time was 150 min and programmed

806 as follows: 15 min column equilibration in 96% buffer A, 4% buffer B, followed by a
807 100 min gradient from 4% buffer B to 35%. Then, a steeper gradient from 35% buffer B
808 to 90% was carried out in 25 min. 90% buffer B was maintained for 5 min and finally,
809 the system was allowed to reach initial conditions in 5 min.

810 For mass spectrometric analysis in the Q Exactive HF mass spectrometer, the
811 following tune method was used: full scan spectral range from m/z 375 to 1600,
812 automatic gain control (AGC) target value set at 3×10^6 , and a mass resolving power of
813 120,000 for full spectra. MS/MS were analyzed in data-dependent mode with a
814 resolution of 30,000 and an AGC target of 5×10^5 . Up to 20 precursors were selected
815 for dissociation in the high-energy collisional dissociation chamber using a normalized
816 collision energy of 27. Ion selection was performed applying a dynamic exclusion
817 window of 15 sec.

818 For protein identification, all raw LC-MS/MS data were analyzed by MaxQuant
819 v1.6.17.0 using the Andromeda Search engine and searched against the *A. thaliana*
820 database downloaded from Uniprot (August 2020 release with 39,346 protein
821 sequences). Parameters for MS/MS spectra assignment were as follows: full-trypsin
822 specificity, maximum of two missed cleavages, instrument default parameters set for
823 Orbitrap, carbamidomethylated cysteine as a fixed modification, and oxidized
824 methionine and N-acetylation of protein termini as variable modifications. False
825 discovery rate at both peptide and protein levels was set to 1%. Data filtering,
826 processing and interpretation were performed in Perseus v1.6.14.0.

827

828 Quantification of UV absorbing compounds

829

830 One-half gram of fresh leaf tissue was frozen in liquid nitrogen and ground to a powder
831 with a mortar and pestle. The powder was extracted for 8 hr with 3 mL of acidic
832 methanol (1% HCl in methanol), and then by a second extraction with 6 mL of
833 chloroform and 3 mL of distilled water. The extracts were vortexed and then centrifuged
834 2 min at $3,000 \times g$. UV-B absorbing compounds were quantified by absorbance at 330
835 nm.

836

837 Statistical analysis

838

839 Statistical analysis was done using analysis of variance models (Tukey test) or
840 alternatively Student's *t* test (Welch's *t* tests), using untransformed data.

841

842 Accession numbers

843

844 Sequence data from this article can be found in the The Arabidopsis Information
845 Resource under accession number At5G20170.

846

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848

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853 Arabidopsis plants and Mariana Giro for help in confocal microscope imaging.

854

855

856 Supplemental data

857

858 **Supplemental Figure S1** Venn diagrams of comparisons between transcripts with
859 altered expression in *med17* mutants and UV-B-responsive genes in Arabidopsis plants.

860

861 **Supplemental Figure S2** Analysis of *med17* and *OE med17* plants after UV-B
862 exposure.

863

864 **Supplemental Figure S3** Representative pictures of individual WT Col-0, *med17* and
865 *OE MED17* plants.

866

867 **Supplemental Figure S4** UV-B effect on cell proliferation in the root meristematic
868 zone of WT Col-0, *med17* and *OE MED17* seedlings 4 days after UV-B exposure.

869

870 **Supplemental Figure S5** UV-B effect on the root meristematic zone of WT Col-0 and
871 *med17* 1 day after UV-B exposure.

872

873 **Supplemental Figure S6** Characterization of double *med17 atr* mutant plants.

874

875 **Supplemental Figure S7** Confocal microscopy of PI stained seeds from WT Col-0,
876 *med17, atr* and *med17 atr* plants.

877

878 **Supplemental Figure S8** Programmed cell death in meristematic root cells in WT Col-
879 0, *med17, atr* and *med17 atr* plants 1 day after UV-B exposure.

880

881 **Supplemental Table S1** List of transcripts expressed in *med17* mutants and comparison
882 with expression levels in WT Col-0 plants.

883

884 **Supplemental Table S2** Cluster and GO analysis of transcripts with differential
885 expression both in *med17* mutants compared to WT plants and after UV-B exposure in
886 WT Col-0 plants.

887

888 **Supplemental Table S3** List of potential MED17 interaction proteins enriched in the
889 MED17-GFP IP experiments.

890

891 **Supplemental Table S4** Primers used in for the experiments described in this work.

892

893

Table 1 Proteins with a putative DNA repair role enriched in the MED17-GFP IP experiments.

Protein ID	Protein name	Accession number
Q93ZX1	Replication factor C subunit 4 (RFC4)	At1g21690
Q94K07	Nucleosome assembly protein 1;3 (NAP1;3)	At5g56950
Q9CA59	NAP1-related protein 1 (NRP1)	At1g74560
Q8GUP3	Precocious Dissociation of Sisters 5 (ATPDS5C)	At4g31880
P92948	Cell division cycle 5-like protein (CDC5)	At1g09770
F4K4Y5	DEK domain-containing chromatin associated protein	At5g55660
Q9SUA1	DEK domain-containing chromatin associated protein (DEK 3)	At4g26630
Q8LC68	NAP1-related protein 2 (NRP 2)	At1g18800
Q8W1Y0	Sister chromatid cohesion 1 protein 4 (SYN 4)	At5g16270
Q9ZQ26	DNA repair ATPase-related	At2g24420
Q9SYH2	Transcription initiation factor TFIID subunit 9 (TAF9)	At1g54140
Q93ZW3	Transcription initiation factor TFIIE subunit alpha	At1g03280

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897

898

FIGURE LEGENDS:

900

Figure 1 Analysis of global gene expression differences between *med17* compared to WT plants, and WT plants exposed to UV-B radiation. A, Venn diagram of comparisons between transcripts with altered expression in *med17* mutants and UV-B-responsive genes in Arabidopsis plants. Sets of genes were selected using the criteria described in Materials and Methods. B, Heatmap comparing transcripts changed in *med17* compared to WT plants; and WT plants after UV-B exposure. The color saturation reflects the magnitude of the log2 expression ratio for each transcript. C, Clusters of expression profiles. Each graph displays the mean pattern of expression of transcripts in the cluster in blue and the standard deviation of average expression (orange and grey lines). The number of transcripts in each cluster is at the top left corner of each graph. The y-axis represents log2 of gene-expression levels relative to those in WT Col-0 plants under control conditions without UV-B.

913

Figure 2 *med17* plants show higher UV-B sensitivity and DNA damage after UV-B than WT plants. A, Representative images of *med17* and WT Col-0 seedlings grown under light conditions and after 15 days were UV-B irradiated (UV-B) or kept under dark conditions (darkness) as described in Materials and methods. Alternatively, plants were grown under normal photoperiod (light conditions, and then kept under normal growth conditions after UV-B or kept under dark conditions. B and C, Relative CPD levels in the DNA of WT Col-0, *med17* and *OE MED17* plants grown under control conditions or immediately after a 4-h UV-B treatment under light conditions (B), or immediately after a 4-h UV-B treatment under dark conditions and 2 h after recovery in the dark or in the light to allow photorepair (C). Results represent averages $\pm$ S.E.M. of six independent biological replicates. Different letters indicate statistically significant differences applying ANOVA test ($P < 0.05$).

Figure 3 Programmed cell death in meristematic root cells in WT Col-0, *med17* and *OE MED17* plants after UV-B exposure. A and B, Number of stem cells that are dead after 1 day (A) or 4 days (B) of UV-B exposure in WT Col-0, *med17* and *OE MED17* roots. Results represent the average of at least 50 biological replicates $\pm$ S.E.M. Different letters indicate statistically significant differences applying analysis of variance test ($p < 0.05$). C, Representative images of stem cells and adjacent daughter cells from WT Col-0, *med17* and *OE MED17* seedlings that were scored for intense PI staining to count dead stem cells per root 1 day and 4 days after a UV-B treatment or under control conditions. Scale bar represents 100 μ m.

Figure 4 UV-B effect on expression of genes that participate in UV-B responses in WT Col-0 and *med17* seedlings. Relative expression levels of *UVR2* (A), *UVR3* (B), *UVR7* (C), *FLC* (D), *ATM* (E), *ATR* (F), *SOG1* (G), *MAPK6* (H), *UVR8* (I), *HY5* (J), *CHS* (K) and *PDCD5* (L) analyzed by RT-qPCR in WT Col-0 and *med17* seedlings under control conditions or immediately after a 4 h-UV-B treatment (UV-B). Results represent the average $\pm$ SEM. Different letters indicate statistically significant differences applying an ANOVA test ($P < 0.05$). Data represent at least three biological replicate experiments. Each RT-qPCR was repeated at least three times on each biological replicate.

Figure 5 UV-B effect on expression of genes that participate in UV-B responses in WT Col-0 and *OE MED17* seedlings. Relative expression levels of *UVR3* (A), *UVR7* (B),

948 *HY5* (C), *PDCD5* (D), *SOG1* (E), *ATR* (F) and *ATM* (G) analyzed by RT-qPCR in WT
949 Col-0 and *OE MED17-4* seedlings under control conditions or immediately after a 4 h-
950 UV-B treatment (UV-B). Results represent the average $\pm$ SEM. Different letters indicate
951 statistically significant differences applying an ANOVA test ($P < 0.05$). Data represent
952 at least three biological replicate experiments. Each RT-qPCR was repeated at least
953 three times on each biological replicate.

954
955 **Figure 6** Characterization of DNA damage responses in double *med17 atr* mutant
956 plants. A, Relative CPD levels in the DNA of WT Col-0, *med17*, *atr* and *med17 atr*
957 plants immediately after a 4-h UV-B treatment under light conditions. Results represent
958 averages $\pm$ S.E.M. of six independent biological replicates. B, Programmed cell death in
959 meristematic root cells in Col-0, *med17*, *atr* and *med17 atr* plants 4 days after UV-B
960 exposure. Results represent the average of at least 50 biological replicates $\pm$ S.E.M.
961 Different letters indicate statistically significant differences applying analysis of
962 variance test ($p < 0.05$). C, Representative images of stem cells and adjacent daughter
963 cells from WT Col-0, *med17*, *atr* and *med17 atr* seedlings that were scored for intense
964 PI staining to count dead stem cells per root 4 days after a UV-B treatment or under
965 control conditions.

966
967 **Figure 7** UV-B root meristematic zone of *med17 atr* is similarly affected by UV-B as
968 *med17* seedlings but differently than *atr* mutants. A, Average of meristematic root zone
969 length; C, cortex cell number; E, cortex cell length in the root meristem from WT Col-0,
970 *med17*, *atr* and *med17 atr* seedlings after 4 days of a UV-B treatment or under control
971 condition. B, D and F, Ratio between meristematic root zone length (B), cortex cell
972 number (D), and cortex cell area values (F) measured after UV-B exposure vs those
973 under control conditions are shown. Results represent the average $\pm$ S.E.M. Different
974 letters indicate statistically significant differences applying analysis of variance test (P
975 < 0.05).

976
977 **Figure 8** Characterization of DNA damage responses in *OE PDCD5 med17* plants. A,
978 Relative CPD levels in the DNA of WT Col-0, *med17*, *OE PDCD5* and *OE PDCD5*
979 *med17* plants immediately after a 4-h UV-B treatment under light conditions. Results
980 represent averages $\pm$ S.E.M. of six independent biological replicates. B, Programmed
981 cell death in meristematic root cells of WT Col-0, *med17*, *OE PDCD5* and *OE PDCD5*

982 *med17* plants 1 day after UV-B exposure. Results represent the average of at least 50
983 biological replicates $\pm$ S.E.M. Different letters indicate statistically significant
984 differences applying analysis of variance test ($P < 0.05$).

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