UVR8 interacts with de novo DNA methyltransferase and suppresses DNA methylation in Arabidopsis

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DNA methylation is an important epigenetic gene regulatory mechanism conserved in eukaryotes. Emerging evidence shows DNA methylation alterations in response to environmental cues. However, the mechanism of how cells sense these signals and reprogramme the methylation landscape is poorly understood. Here, we uncovered a connection between ultraviolet B (UVB) signalling and DNA methylation involving UVB photoreceptor (UV RESISTANCE LOCUS 8 (UVR8)) and a de novo DNA methyltransferase (DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2)) in Arabidopsis. We demonstrated that UVB acts through UVR8 to inhibit DRM2-mediated DNA methylation and transcriptional de-repression. Interestingly, DNA transposons with high DNA methylation are more sensitive to UVB irradiation. Mechanistically, UVR8 interacts with and negatively regulates DRM2 by preventing its chromatin association and inhibiting the methyltransferase activity. Collectively, this study identifies UVB as a potent inhibitor of DNA methylation and provides mechanistic insights into how signalling transduction cascades intertwine with chromatin to guide genome functions.

DNA methylation is a conserved mechanism for gene regulation and plays quintessential roles in transposon silencing, imprinting, development and environmental responses. The predominant form of DNA methylation in eukaryotes occurs on 5-methylcytosine (5mC), although adenine methylation has also been reported. In plants, methylation is present in transposons, repetitive sequences and gene bodies in three sequence contexts: CG, CHG and CHH (H = A, T or C). In Arabidopsis, the maintenance of DNA methylation is mediated by distinct pathways. CG and CHG methylation are maintained by METHYLTRANSFERASE 1 (MET1) and CHROMOMETHYLASE 3 (CMT3), respectively, while CHH methylation is maintained by DRM2 through the RNA-directed DNA methylation (RdDM) pathway and CHROMOMETHYLASE 2 (CMT2). While DRM2 is responsible for CHH methylation of euchromatic regions, short transposable elements (TEs) and the edges of long TEs, CMT2 preferentially methylates pericentromeric heterochromatin and the bodies of long TEs. DNA methylation in all sequence contexts is established by the de novo activity of DRM2 through the RdDM pathway.

Accumulating evidence suggests a functional link between DNA methylation and various developmental and environmental cues in both plants and mammals. Distinct DNA methylation patterns are established in different cell types and tissues and are tightly modulated during growth and development. For example, maternal nutritional status during early pregnancy causes persistent and systemic epigenetic changes as metastable epialleles in humans. In plants, distinct DNA methylation patterns have been reported in different cell types and tissues. For example, the genome of columella root cap cells is the most highly methylated in Arabidopsis, whereas soybean root hairs are more hypermethylated than stripped roots.

DNA methylation is also dynamically regulated by versatile environmental stimuli including herbicide, bacterial pathogen infection, salicylic acid treatment, ultraviolet radiation, heat stress and microgravity during spaceflight. In Arabidopsis, bacterial (Pseudomonas syringae) infection induces both hyper- and hypomethylation at numerous regions, among which many are correlated with gene expression changes. The Arabidopsis plants grown in the international space station have higher methylation levels in the context of CHG and CHH within protein-coding genes compared with those of ground grown plants. Despite the large amount of descriptive information regarding the dynamic DNA methylation patterns in response to environmental cues, the underlying mechanism is poorly understood.

UVB is an inherent part of sunlight that can penetrate the atmosphere of Earth and affect many biological processes. In plants, natural low-level and non-damaging UVB acts as a signal to regulate development and assimilation, such as inhibition of hypocotyl elongation and biosynthesis of flavonoids and anthocyanins for UVB protection. In Arabidopsis, UVR8 is the photoreceptor of UVB. UVR8 predominately exists in the cytosol as an inactive homodimer, and, upon UVB exposure, it undergoes monomerization due to the disruption of π–cation interactions after absorption of UVB by tryptophan chromophores and is imported into the nucleus.

In the absence of UVB, UVR8 monomers re-dimerize with the assistance of REPRESSOR OF UVB PHOTOMORPHOGENESIS (RUP), a class of WD40-repeat proteins. Downstream of UVB perception, UVR8 interacts with E3 ligase CONSTITUTIVELY PHOTOMORPHIC 1 (COP1) and transcription factors WRKY DNA BINDING PROTEIN 36/13 (WRKY36/13), MYB DOMAIN PROTEIN 73/77 (MYB73/77), BRI1-EMS-SUPPRESSOR...
1 (BES1) and BES1-INTERACTING MYC-LIKE 1 (BIM1) to transduce UVB signals34–36. Previous studies suggest a potential role of DNA methylation in adaptation to high UVB irradiation for high-altitude maize landraces32. High-altitude-related human disorders (for example, pulmonary oedema) are also associated with aberrant DNA methylation37. Despite the correlative information, how distinct DNA methylation patterns are established and maintained in response to UVB exposure remains unknown.

In this study, we found that UVB acts through DRM2 to suppress DNA methylation and de-repress several reporter genes in a UVR8-dependent manner. Genome-wide DNA methylation analysis further showed that UVB induced DNA hypomethylation preferentially around pericentromeric regions and TEs. The UVB photoreceptor UVR8 directly interacts with DNA methyltransferase DRM2 in the nucleus and this interaction is mediated by the ubiquitin-associated (UEA) domains of DRM2. Mechanistic dissection of UVR8 action on DRM2 revealed that UVR8 does not impact DRM2 protein stability and nuclear accumulation. Instead, UVR8 inhibits DRM2 chromatin association and catalytic activity. Collectively, this study identifies UVR8 as a negative regulator of DRM2 and establishes a mechanistic connection between light signalling and DNA methylation in plants.

Results

UVB induces DNA hypomethylation and de-represses reporter genes. Our previous immunoprecipitation coupled with mass spectrometry (IP-MS) experiments of DNA methyltransferase DRM2 identified ARGONAUTE 4 (AGO4)34 and the UVB photoreceptor, UVR8, which perceives UVB and induces physiological responses to secure plant acclimation and thus promotes survival in sunlight35 (Supplementary Data 1). This led us to hypothesize that UVB may be connected to DNA methylation. To determine whether UVB has an impact on DNA methylation, we first utilized a dual Cauliflower Mosaic Virus 35S promoter-driven luciferase (35S:LUC) reporter system (Fig. 1a), where 35S promoter regions are methylated and the LUC gene is transcriptionally inhibited36. A medium 35S:LUC line (LUCM) was used as it is methylated at a medium level and thus could reveal the change of DNA methylation in both directions (Extended Data Fig. 1a–e). When crossing LUCM into the drm1 drm2 (dd) background, LUC intensity was increased and DNA methylation at 35S promoter was reduced (Extended Data Fig. 1f,g). Upon UVB irradiation, we noted an increased LUC intensity in LUCM compared with the plants grown under white light (Fig. 1b,c). Further examination of the DNA methylation at the 35S promoter by McrBC (a nuclease that cleaves DNA containing 5mC) digestion and bisulphite sequencing revealed decreased DNA methylation levels in response to UVB treatment (Fig. 1d,e). Next, we asked whether UVB-induced DNA hypomethylation is mediated by DRM2. We utilized another reporter line where GFP expression is driven by the promoter of the SDC (SUPPRESSOR OF DRM2 CMT3) gene. The SDC gene has seven tandem repeats in its promoter region, is silent in wild-type plants and only becomes demethylated and transcriptionally reactivated when both DRM2 and CMT3 pathways are inactivated40. When introducing pSDC:GFP into the cmt3 null mutant41, we noted an increased GFP protein abundance accompanied by a significant increase in the endogenous SDC transcript levels with UVB treatment (Fig. 1f,g), suggesting that UVB acts through DRM2 to alter DNA methylation.

UVR8 mediates UVB-induced DNA hypomethylation. As UVR8 is the photoreceptor of UVB, we asked whether UVB-induced DNA methylation depends on UVR8. We crossed the LUCM with uvr8-6 and also cop1-6, in which the UVR8 signalling downstream component COP1 is mutated34. Under white light, we found that luciferase luminescence and LUC RNA transcript levels in uvr8-6 LUCM were both reduced compared with LUCM, while cop1-6 LUCM was similar to LUCM (Fig. 1h,i). We next assessed DNA methylation levels in these lines by McrBC digestion and found that the DNA methylation of 35S promoter regions was significantly increased in uvr8-6 LUCM compared with LUCM (Fig. 1j), indicating that UVR8 induces DNA hypomethylation. We next investigated the impact of UVB on LUC intensity and found that unlike LUCM and cop1-6 LUCM plants, the LUC intensity in uvr8-6 is similar in white light and UVB treatment (Fig. 1k), suggesting that LUC is insensitive to UVB when UVR8 is absent. It further suggests that UVR8 mediates UVB-induced DNA methylation reduction.

We next investigated whether UVR8 overexpression can lead to DNA methylation alteration. We transformed FLOWERING WAGENINGEN (FWA) transgene, which is not methylated and can be de novo methylated by the DRM2 pathway38, into 35S:UVR8-FLAG overexpressing lines (UVR8-OX, Supplementary Fig. 1). When grown under white light generated by fluorescent tubes producing a low level of UVB39, FWA/UVR8-OX plants showed a significant late-flowering phenotype compared with FWA/Col-0 in both T2 homozygous and T3 transgenic populations (Fig. 1l,m and Extended Data Fig. 2). Furthermore, the FWA transgene in high-altitude maize landraces32. High-altitude-related human disorders (for example, pulmonary oedema) are also associated with aberrant DNA methylation37. Despite the correlative information, how distinct DNA methylation patterns are established and maintained in response to UVB exposure remains unknown.

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lines expressing a constitutively monomeric UVR8W285A showed even later flowering compared with FWA/UVR8-OX (Extended Data Fig. 2b). These data indicate that UVR8 overexpression inhibited the de novo methylation of the FWA transgene. To determine whether UVR8 acts through DRM2 to regulate FWA methylation, we generated the uvr8-6 drm1 drm2 (udd) triple mutant. FWA/udd
transgenic plants demonstrated late flowering to a similar extent to FWA/dd (Extended Data Fig. 2a). Further examination of another locus (Chr1:23068006) methylated by DRM2 revealed increased DNA methylation in all CG, CHG and CHH contexts in uvr8-6, which was abolished in the triple udl mutant (Fig. 1n). Together, these data suggest that UVR8 mediates UVB-induced DNA hypomethylation.

UVB induces genome-wide DNA hypomethylation. To further investigate the impact of UVB on DNA methylation, we examined two endogenous loci: Chr1:23068006 and AtSN1, a well-characterized retrotransposon whose methylation is dependent on DRM2 (ref. 40). Upon UVB treatment, we noted a slight reduction of DNA methylation in Col-0 and the reduction was further enhanced in UVR8-OX plants (Fig. 2a), indicating that UVB reduces DNA methylation in a UVB-dependent manner. To determine whether UVB alters global DNA methylation, we performed whole-genome bisulfite sequencing (Supplementary Table 1) and found no notable global methylation difference between Col-0 plants grown under white light or UVB. In contrast, the UVB-treated UVR8-OX showed a strong reduction of CHH methylation, particularly at the centromeric and pericentromeric regions (Fig. 2b). Consistently, we found that UVB-induced differentially methylated regions (DMRs) in both Col-0 and UVR8-OX are mostly in the context of CHH with a majority of hyper DMRs (Fig. 2c). We next compared UVB-induced CHH hypo DMRs with that of the drm1 drm2 (dd) DMRs with that of the drm1 drm2 (dd), drm2-2 and cmt2-3. We found that ~55% and ~61% of UVB-induced DMRs in Col-0 overlap with dd-DMRs and drm2-2 DMRs, respectively (Fig. 2d; Extended Data Fig. 3a–c and Supplementary Data 2). Besides DRM2, we also found that 635 of 5,189 (~12%) UVB-induced CHH hypo DMRs are uniquely overlapped with cmt2 DMRs (Extended Data Fig. 3a). Significant CHH methylation reduction was observed in UVB-treated Col-0 and more drastically in UVR8-OX at both UVB-specific and overlapping DMRs with dd-DMRs (Fig. 2e; Extended Data Fig. 3a–c). Interestingly, dd-specific DMRs also showed a significant decrease of CHH methylation by UVB treatment compared with non-treated control in both Col-0 and UVR8-OX (Fig. 2d,e), suggesting that these regions are probably weak UVB targets despite being called as not significant DMRs. We further noticed that UVR8 overexpression alone without UVB treatment also showed a relatively mild CHH methylation reduction, both globally and at specific loci (Fig. 2d–f). In addition, we performed bisulfite sequencing of the uvr8-6 mutant with or without UVB treatment (Supplementary Data 2) and found very little DNA methylation changes at the genome-wide level comparing uvr8-6 with Col-0 under white light (Extended Data Fig. 3d). We also noticed that only a very small fraction of UVB-induced CHH hypo DMRs in uvr8-6 overlap with those in Col-0, suggesting that the UVB-induced DMRs mostly depend on UVR8 (Extended Data Fig. 3e).

We next profiled the genome distribution pattern of UVB-induced CHH hypo DMRs (UVB-DMRs) and found that they are enriched in promoters and TEs, similar to dd-DMRs (Fig. 2g). These UVB-DMRs were colocalized with TEs, especially in pericentromeric regions (Fig. 2h). TE regions containing UVB-induced DMRs tend to have a much higher CHH methylation level than those without UVB-DMRs (Fig. 2i), indicating that the TEs with high CHH methylation were more sensitive to UVB. Moreover, long TEs (>500 base pairs (bp)) and certain types of Class II DNA transposons were enriched with UVB-induced DMRs, similar to the pattern of TEs containing dd-DMRs (Extended Data Fig. 3f,g). Further comparison of UVB-induced DMRs with published ultraviolet C-induced DMRs (ref. 41) revealed a very small portion of overlapping DMRs (Extended Data Fig. 4a–d), suggesting that the UVB-induced DMRs identified in this study were not induced by DNA damage. In summary, these data demonstrate that UVB induces genome-wide DNA methylation reduction with preference over TEs with high CHH methylation and long Class II DNA transposons.

UVB and DRM2 de-repress a set of TEs. To determine the transactivation changes induced by UVB, we first checked the transcription of Romania T5 and AtCopia28, two TEs repressed by DNA methylation, and found that they were significantly upregulated in UVB-treated pSDC:GFP/cmt3 plants (Fig. 3a). We next performed RNA-sequencing (RNA-seq) on Col-0 and dd with or without UVB treatment (Fig. 3b; Extended Data Fig. 5a and Supplementary Table 2). The transcriptional responses to UVB are similar in Col-0 and dd with a large portion of overlapped differentially expressed genes (DEGs) and correlated fold-change of expression levels (Extended Data Fig. 5a,b). UVB-induced marker genes, including CHS, ELIP1, ELIP2 and RUP2, were upregulated in the UVB-treated plants, while high-dosage DNA-damaging UVB-induced genes were unchanged (Extended Data Fig. 5c–e). Comparison of our RNA-seq data of 10-d UVB treatment with that of short-term (6-h) treatment revealed that short-term UVB treatment has a stronger effect on gene expression in terms of both number of DEGs and fold-change (Extended Data Fig. 5f–h). Overlapping of the DEGs of UVB-treated Col-0 and dd revealed that only a few genes are commonly regulated by DRM2 and UVB, suggesting that genes are not major targets of UVB-induced DNA hypomethylation (Fig. 3b and Supplementary Data 3). This is consistent with the fact that DNA methylation primarily suppresses TEs in Arabidopsis42. Hence, we analysed TE expression in our RNA-seq data and noticed more upregulated TEs (269) than downregulated TEs (65) (Fig. 3c and Supplementary Data 3). Ninety-six upregulated TEs were significantly overlapped between the dd mutant and UVB-treated Col-0 (Fig. 3d–f). This was further confirmed by quantitative PCR with reverse transcription (RT–qPCR) of five selected TEs showing upregulation

Fig. 2 | UVB induces genome-wide CHH hypomethylation. a, McrBC–qPCR-based DNA methylation assay of Chr1:23068006 and AtSN1 in Col-0, UVR8-OX and uvr8-6 plants with WL or UVB treatment (1.5 µE for 10 d). Data are mean ± s.e.m. from two biological replicates with three technical replicates. The P values by Student’s t-test are shown. b, Metaplots showing average CHH methylation levels in Col-0 and UVR8-OX plants with or without UVB treatment. Chr1 to Chr5 represent five chromosomes. c, Numbers of DMRs in the context of CG, CHG and CHH. Col-0” is the control for drm1 drm2 (dd) from ref. 23. d, Boxplots of CHH methylation levels of UVB-specific, overlapping and dd-specific DMRs in different samples. The numbers of DMRs are indicated as ‘n’. The DMRs are the overlapping of Col-0, UVB-DMRs (upper panel) or UVR8-OX,UVB-DMRs (lower panel) (against Col-0, WL) and dd-DMRs. The lower and upper box edges correspond to the first and third quartiles, the horizontal lines indicate the median, and the lower and upper whiskers denote the smallest and largest values at most 1.5 × IQR, respectively. ***P < 0.001, by non-parametric Mann–Whitney–Wilcoxon test. e, Heat maps showing the CHH methylation levels of different samples in the regions corresponding to the indicated DMRs. f, Representative snapshots of UVB-induced CHH hypomethylation regions. The data range is [0,1]. g, The distribution of CHH hypo DMRs in the genome. h, The density of Col-0,UVB-CHH hypo DMRs and TEs on chromosomes. The density is the proportion of DMRs or TEs in a 100-kb window. i, CHH methylation levels of TE regions within or without UVB-induced CHH hypo DMRs. The lower and upper box edges correspond to the first and third quartiles, the horizontal lines indicate the median, and the lower and upper whiskers denote the smallest and largest values at most 1.5 × IQR, respectively. ***P < 0.001, by non-parametric Mann–Whitney–Wilcoxon test. IQR, interquartile range; UTR, untranslated region.
in UVB-treated Col-0 and UVR8-OX, but no response to UVB in uvr8-6 mutant (Fig. 3g). We further found a reduced CHH methylation level in UVB-treated UVR8-OX over the upregulated TEs (Extended Data Fig. 5i).

UVB photoreceptor UVR8 interacts with DRM2 in vitro and in vivo. Our previous DRM2 IP-MS experiment identified 6–7 unique UVR8 peptides (Supplementary Data 1 and Fig. 4a). UVR8 was also found to be specifically pulled down by DRM2, but not by

![Graphs and diagrams illustrating the interaction between UVR8 and DRM2, as well as the distribution and methylation levels of CHH markers in different conditions.](image-url)
other chromatin factors (Extended Data Fig. 6a). To verify our IP-MS data, we first extracted total proteins from transgenic *Arabidopsis* expressing N-terminal tagged 3xFLAG-9xMyc-DRM2 in the *dd* mutant background (3F9M-DRM2) and incubated them with recombinant full-length glutathione S-transferase (GST)-UVR8, which contains both monomeric and dimeric UVR8 (Extended Data Fig. 6b). DRM2 was coprecipitated with GST-UVR8, but not GST alone (Fig. 4b). Next, we co-infiltrated *Agrobacterium* carrying both 35S promoter-driven UVR8-HA (UVR8-HA) and DRM2 genomic sequence fused with 3xFLAG at the C terminus (DRM2-FLAG) in *Nicotiana benthamiana* leaves and found that UVR8 co-immunoprecipitated with DRM2 (Extended Data Fig. 6c). Similar copurification was detected when using transgenic *Arabidopsis* plants co-expressing UVR8-HA and 3F9M-DRM2.
UVR8–DRM2 interaction was further confirmed to be in the nucleus by co-immunoprecipitation (co-IP) using isolated nuclei from these transgenic Arabidopsis plants (Extended Data Fig. 6d). We also performed a split luciferase complementation assay by fusing N- and C-terminal domains of luciferase (nLuc and cLuc, respectively) to the full-length UVR8 and DRM2 and co-expressed

### Table 1

| Protein | Spectra | UniPepts | Percentage coverage | NSAFe5 |
|---------|---------|----------|----------------------|--------|
| DRM2    | 310     | 37       | 43.30                | 1,025.469 |
| AGO4α   | 35      | 20       | 22.10                | 78.439 |
| UVR8    | 9       | 6        | 16.40                | 42.357 |
| MSI4α   | 25      | 8        | 21.30                | 102.11 |
| HTB9α   | 10      | 5        | 35.30                | 138.052 |
| TPR2    | 11      | 7        | 5.40                 | 20.14  |

* Listed in Zhong et al. 34.
them in *N. benthamiana* leaves. The co-infiltration of UVR8-nLuc and cLuc-DRM2, as well as DRM2-nLuc and cLuc-UVR8 showed strong luminescence signals compared with the negative controls (Fig. 4d,e). To determine whether UVB has an impact on UVR8–DRM2 interaction, we performed a co-IP experiment using the nuclei isolated from *UVR8-HA*/*3F9M-DRM2* transgenic plants treated with or without UVB and found that UVB enhanced the UVR8–DRM2 interaction in the nucleus (Fig. 4f). We also irradiated only one half of an *N. benthamiana* leaf with UVB, with the other half of the same leaf covered with aluminium foil (Fig. 4g). Compared with the untreated side (-UVB), the half leaf treated with UVB showed stronger luciferase luminous intensity (Fig. 4h,i). This increased signal is due to enhanced interaction between DRM2 and UVR8 but not their protein level change (Extended Data Fig. 6e). Together, these data suggest that UVR8 interacts with DRM2 in vitro and in vivo.

**DRM2 interacts with UVR8 in the nucleus via its UBA domains.** We further investigated the subcellular localization of the DRM2–UVR8 interaction by performing bimolecular fluorescence complementation (BIFC) assays. Full-length UVR8 was fused with an N terminus of YFP (nYFP-UVR8), and DRM2 was fused with a C-terminal fragment of YFP (DRM2-cYFP). We found that UVR8 interacts with DRM2 in the nucleus in a pattern similar to the DRM2–DRM2 interaction (Fig. 5a), consistent with the dimerization of DRM2 (ref. 34). UVR8 is known to exist as a homodimer predominantly in the cytosol in normal conditions and undergoes monomerization upon UVB exposure23. We next investigated which form of UVR8 could interact with DRM2 by utilizing the UVR8W285A and UVR8W285F mutants, constitutive monomeric and dimeric forms, respectively24. The results of both split luciferase and BIFC assays showed that both UVR8W285A and UVR8W285F can interact with DRM2 in the nucleus (Fig. 5b and Extended Data Fig. 6f,g).

Arabidopsis DRM2 contains three tandem UBA domains at the N terminus and a rearranged catalytic domain at the C terminus43. To examine which domain mediates the interaction with UVR8, we generated two truncated DRM2 mutants containing only the UBA domains (DRM2UBA) or catalytic domain (DRM2CAT). The BIFC assay showed that the DRM2UBA, but not DRM2CAT, was able to interact with UVR8, suggesting that UBA domains are necessary and sufficient to mediate the DRM2–UVR8 interaction (Fig. 5a). We also truncated UVR8 into the core domain (1–396) and the C terminus, both of which are important for interaction with COP1 (ref. 44), and noted that both truncations can interact with DRM2 (Extended Data Fig. 7a). Interestingly, we found that UVR8 and its mutant forms all interact with DRM2 in certain nuclear bodies (Fig. 5c and Extended Data Fig. 7b). Notably, the UVR8–DRM2 nuclear body is distinct from the AB body (AGO4/NRPD1B-body)45 that is adjacent to the nucleolus and is also different from COP1 nuclear bodies (Extended Data Fig. 6h). Taken together, these results suggest that the UBA domains in DRM2 are necessary and sufficient for UVR8 interaction.
UVR8 inhibits the catalytic activity and chromatin association of DRM2. The direct UVR8–DRM2 interaction (Figs. 4 and 5) and inhibition of DRM2-mediated DNA methylation by UVR8 (Figs. 1 and 2) suggest that UVR8 might negatively regulate DRM2. To dissect the molecular mechanism, we first determined whether UVR8 regulates DRM2 protein stability. We found no noticeable difference in DRM2 protein level with and without UVB treatment (Fig. 6a). Similarly, plants without UVR8 exhibit similar DRM2 abundance as those with UVR8 (Fig. 6b). Next, we assessed whether UVR8 affects DRM2 nuclear localization. Co-expression of UVR8-GFP or DRM2-GFP respectively with and without UVB treatment (~1.5 μmol m⁻² s⁻¹ narrowband UVB for 4 h) in Arabidopsis. The concentration of DRM2 (residues 59–626) and His-tagged proteins is 0.2 μM and 3.0 μM, respectively. GFP-His and H₂O serve as controls. Data are mean ± s.e.m. from four biological replicates. The P values by Student’s t-test against H₂O control are shown. e, ChIP-qPCR showing DRM2 enrichment at selected loci with and without UVB treatment using DRM2-FLAG/dd and DRM2-FLAG/udd transgenic plants. ACT7 serves as a control. Data are mean ± s.e.m. from three biological replicates. *P < 0.05; **P < 0.01 by Student’s t-test. f, A working model of UVR8-mediated UVB perception and DNA methylation suppression. Under WL, UVR8 predominantly localizes in the cytosol as a homodimer with a small portion in the nucleus. The nuclear UVR8 interacts with DRM2 and induces DNA hypomethylation by inhibiting the methyltransferase activity and chromatin association of DRM2.
UVR8W285A-GFP with DRM2-mCherry did not change the nuclear localization of DRM2 (Supplementary Fig. 2a–c). We also generated DRM2-GFP transgenic Arabidopsis plants in both the dd and udd mutants, and showed that neither UVR8 nor UVB affects DRM2 nuclear localization (Fig. 6c and Supplementary Fig. 2d). There was also no significant difference in the transcription levels of DRM2, other RdDM components and proteins in the DNA demethylation pathway in response to UVB (Supplementary Fig. 3a–c). These results suggested that UVB and UVR8 do not regulate DRM2 transcription, protein stability and subcellular localization.

To test the role of UVR8 in DRM2 catalytic activity, we performed in vitro methyltransferase assays using recombinant DRM2 protein containing both UBA and CAT domains (residues 59–626). We found that DRM2 activity was similarly inhibited by UVR8, UVR8W285A and UVR8W285F (Fig. 6d). Interestingly, the DRM2 catalytic domain (DRM2CAT, residues 269–626) was insensitive to UVR8 (Supplementary Fig. 4a, b), consistent with the observation that UVR8 interacts with DRM2 via the UBA domains (Fig. 5). To test whether UVB regulates DRM2 chromatin association, we performed chromatin immunoprecipitation coupled with quantitative PCR (ChIP–qPCR) using the flowers of DRM2-FLAG in dd and udd with or without UVB treatment. DRM2 showed enrichment at several loci, including Chr1:23068006 and two TEs (AT4TE29620 and ATITE55145), with decreased DNA methylation in dd and UVB-irradiated samples (Fig. 2a, f). Interestingly, we noted significantly reduced DRM2 chromatin enrichment of these loci upon UVB irradiation (Fig. 6e). The DRM2 enrichment at these loci was not affected by UVB in udd when UVR8 was absent (Fig. 6e), suggesting that UVB-inhibited DRM2 chromatin association is dependent on UVR8 at these loci.

Discussion
In this study, we have identified a mechanistic connection between ultraviolet light signalling and DNA methylation involving UVB photoreceptor UVR8 and a key de novo DNA methyltransferase, DRM2. We showed that a direct physical interaction between UVR8 and DRM2 is critical for UVB-induced DNA methylation alternation and transcriptional de-repression. These findings suggest that UVR8 acts as a molecular sensor and transmits the UVB signalling to regulate DRM2-mediated DNA methylation. Here, we propose a working model wherein UVR8 predominantly localizes in the cytosol as a homodimer with a small portion in the nucleus to interact with DRM2 and exhibit a basal inhibitory activity on DRM2 under white light. Upon UVB exposure, UVR8 converts into an active monomer, which traffics into the nucleus and interacts with DRM2 to inhibit DRM2 activity, leading to DNA hypomethylation (Fig. 6f). The lower DNA methylation induced by UVB could be due to an inhibition of the maintenance process (passive demethylation) or an active DNA demethylation accompanied by an insufficient re-establishment of the methylation landscape. Whether UVB regulates active DNA demethylation needs to be further investigated, although UVB does not change the transcription of active DNA demethylation components (Supplementary Fig. 3b).

UVB has been reported to induce the dynamic DNA methylation change in different species. Consistent with our results in Arabidopsis, UVB exposure induces hypomethylation at several loci in maize, Norway spruce (Picea abies) and Artemisia annua (24–48). Some studies showed no DNA methylation reduction by UVB and even observed hypermethylation in grapes (49). Due to the limited number of tested loci in these species, it remains unclear whether UVB has distinct impacts on global DNA methylation in different plant species. Similar UVB-induced dynamic DNA methylation patterns have been reported in mammals, in which a UVB photoreceptor has yet to be discovered. In humans, UVB irradiation leads to decreased DNA methylation and DNMT1 expression in T cells of patients with systemic lupus erythematosus (51). It should be noted that current knowledge of specific methylation patterns in these species is largely based on the genetic analyses of a few loci. The molecular mechanism(s) of establishing such dynamic DNA methylation patterns in response to UVB exposure in diverse plant and animal species is unknown. The study presented here is a mechanistic investigation of the impact of UVB on DNA methylation and provides a molecular mechanism linking the UVB signalling pathway to DNA methylation.

Besides UVB, many other internal and external cues also alter the plant DNA methylome. Bacterial pathogen P. syringae pv. tomato DC3000 induces global hypomethylation at centromeric regions in Arabidopsis (49, 52). Herbicide treatment of Arabidopsis leads to more than 9,000 DMRs of which ~6,000 are dosage-dependent (53). Both soybean root hairs and stripped roots show hypomethylation after heat stress (40°C), especially in the CHH context (54). In rice, Pi starvation induces widespread changes in DNA methylation, especially in the TEs in proximity to Pi starvation-induced genes (55). Heavy metals such as cadmium induce DNA hypomethylation in industrial hemp (Cannabis sativa), clover (Trifolium repens) and seagrass (Posidonia oceanica) (56). The genome of Arabidopsis flown onboard the scientific satellite SJ-10 exhibited lower methylation levels, while the leaves of Arabidopsis grown in the International Space Station showed higher methylation levels in protein-coding genes (57). Together, these studies suggest that the plant epigenome is plastic and can be ‘edited’ by versatile environmental cues. Future mechanistic study is important to understand how cells modulate their epigenomes to generate adaptive responses.

In Arabidopsis, several DRM2-interacting proteins have been identified, including AGO4, RNA-directed DNA methylation 1 (RDM1) and U2AF56 associated protein 56 (UAP56) (refs. 34, 56–58). In the RdDM pathway, short interfering RNAs are loaded onto AGO4 and pair with complementary long Pol V transcripts (1). The interaction between AGO4 and DRM2 is thought to guide DRM2 to specific genomic regions to establish de novo DNA methylation (14). RDM1, also a component of the DDR complex, physically associates with both AGO4 and DRM2 and is proposed to promote the AGO4–DRM2 interaction (16). UAP56 is a DEAD box RNA helicase and partially colocalizes with DRM2 (ref. 17). Despite some chromatin-associated properties, its function in epigenome regulation remains unclear. In rice, OsDRM2 has been reported to interact with OsElf4A (an ATP-dependent RNA helicase) and SDG711 (an H3K27me3 methyltransferase in PRC2 complex) (9, 56). Interestingly, all currently known DRM2-interacting proteins play positive roles in facilitating DRM2-mediated DNA methylation. In contrast, multiple lines of genetic, genomic and biochemical evidence in this study demonstrated that UVR8 inhibits DRM2 function, highlighting a negative regulator of this plant DNA methyltransferase. Consistently, the de novo methyltransferase DNMT3A has been reported to interact with Mcf2p, which negatively regulates the activity of DNMT3A in humans (41).

UBA domains are found in many proteins involved in degradation pathways and ubiquitin-dependent signalling pathways by recognizing various ubiquitin forms, including poly- and mono-ubiquitin (52). The UBA domains of DRM2 have been reported to mediate its interactions with other proteins. For OsElf4A and SDG711, their interactions with OsDRM2 are mediated by the UBA domains (52). Given that UVR8 interacts with the UBA domains, we wonder whether UBA domains serve as a platform mediating interaction with multiple proteins to regulate the complex function in vivo. Similar to UVR8, we indeed found that the UBA domains, but not methyltransferase domain, interact with AGO4 (Supplementary Fig. 5a). Within DRM2, UBA domains also tend to self-associate and associate with the methyltransferase domain (Supplementary Fig. 5b). Consistent with this notion, the UBA domains are indispensable for DNA methylation catalysed by DRM2 in vivo (41). UBA domains are known to associate with ubiquitin; however, to our knowledge,
none of the currently known DRM2-interacting proteins have been reported to be ubiquitylated. Previously, DRM2 UBA domains were shown to bind to poly-ubiquitin chains with a preference for Lys63-linked chains in vitro45. While poly-ubiquitylation is often associated with protein degradation, mono-ubiquitylation has been implicated in a variety of pathways such as endocytosis, DNA repair and cell signalling46. Thus, it will be important to identify additional proteins associated with DRM2 UBA domains to further connect the DNA methylation and ubiquitylation fields.

It appears that UVR8 has a dual role in DRM2 function. In one capacity, UVR8 can inhibit the catalytic activity of DRM2 (Fig. 6d). In another capacity, UVR8 can inhibit DRM2 chromatin association (Fig. 6e). While the precise mechanism is unclear, several possibilities could account for this dual action. First, the UVR8 binding may induce a conformational change of DRM2, resulting in its dysfunction in both chromatin association and catalytic activity. Second, UVR8 binding of UBA domains may outcompete other chromatin and transcription factors and disable their association with DRM2. As discussed earlier, UBA domains are thought to be mediators of protein–protein interactions. UVR8 may either directly or indirectly via other factors inhibit DRM2 chromatin association. For example, UVR8 binding to transcription factors BES1, BIM1, MYB73/77 and WRKY36/13 repressed their association with chromatin28–31. While this makes sense with the chromatin binding, it raises an interesting question as to how UVR8 can inhibit the catalytic activity by binding to UBA domains. A possible mechanism is the allosteric inhibition of UVR8 on DRM2. The UBA domains may interact with the methyltransferase domain in an intra-molecular or inter-molecular manner to inhibit catalytic activity. The binding of UVR8 with UBA domains further enhances this negative regulation. This is consistent with the case of DNMT3A-MeCP2, where the interaction of MeCP2 with the ADD domain of DNMT3A stabilizes DNMT3A’s autoinhibitory conformation and, thus, inhibits its catalytic activity32–34.

In both UVB-treated Col-0 and dd mutant, we found that UVB could de-repress many TEs (Fig. 3). This is consistent with the idea that DNA methylation primarily represses the TEs to maintain genome stability in plants44–45. UVB activates the expression of a DNA transposon Mutator in maize46, consistent with our results that UVB-induced hypo DMRs are enriched in DNA transposons (Extended Data Fig. 3g). In nature, an Arabidopsis relative Crucichimalaya himalaica from the Qinghai–Tibet Plateau with high levels of UVB irradiation shows long terminal repeat retrotransposons expansion shortly after the dramatic uplift and climatic change of the Himalayas from the Late Pliocene to Pleistocene47. Transposon reactivation in response to stress could increase phenotypic diversity and adaptability to changing environments by transcriptional regulation of neighbouring genes and by novel transposon insertions48–49. For example, Capsella rubella exhibits a wider distribution and higher phenotypic diversity accompanied with highly enriched TEs compared with its congenic species, such as Capsella grandiflora45, suggesting that TE insertions can drive rapid phenotypic variation and potentially promote adaptation to changing environments. When these results are collectively reviewed along with our present findings, it is therefore likely that UVB-induced DNA methylation reduction and TE reactivation is a mechanism of plant adaptation to changing local environment by promoting phenotypic and genetic diversity.

Methods

Plant materials. For Arabidopsis thaliana, the Columbia-0 (Col-0) ecotype was used as the background for all mutant and transgenic plants. The mutant lines used were uvr8-6 (SALK_033468), drm1-2 drm2-2 (dd, SALK_037105, SALK_150863), cop1-6 (C099041) and fwa-4 (CS69041). The DNA methylation reporter lines, LUCH and LUCM reporter lines50 and pSDC-GFP/cmt3 (ref. 51) have been described previously. LUCM is from the same batch as LUCH and LUCM but characterized in this study. Multiple mutants and reporters under mutant backgrounds (uvr8-6 dd, uvr8-6 LUCM, cop1-6 LUCM) were generated by crossing and further genotyping. The transgenic lines used in this study include UVR8-FLAG (3S); UVR8-HA (3S) in Col-0 and 35S-MRM2/dd backgrounds; and FWa in Col-0, dd, uvr8-6, udd and UVR8-FLAG backgrounds.

UVB treatment. The UVB treatments were performed using Philips UVB lamps TL20W/01RS for narrowband UVB (305–315 nm, with peak at 312 nm) and TL40W/12RS for broadband UVB (290–315 nm). The UVB intensity on plants was manipulated by adding plastic tape and adjusting the distance between the plants and the lamp, and was measured by a UV/AB Light Meter (SPER Scientific Inc., model 850009). For narrowband UVB, the intensity unit of μmol m\(^{-2}\) s\(^{-1}\) was calculated approximately using the peak wavelength of 312 nm from μW cm\(^{-2}\). For plants used for whole-genomic bisulfite sequencing, RNA-seq and DNA methylation reporters (LUCM and pSDC-GFP/cmt3), seeds were planted on ½ Murashige and Skoog (MS) medium with 1% sucrose and 0.7% agar. The seeds were put directly under UVB lamps for 4 h and then imaged with a confocal microscope (Nikon A1R). For co-IP after UVB treatment, 10-d-old seedlings were put under a narrowband UVB lamp for 4 h and then directly subjected to nuclei isolating and co-IP. For ChIP–qPCR, the flowering gdm2-FLAG/dd and gdm2-FLAG/dd transgenic plants used with broad band UVB were grown in a growth chamber with 16-h light/8-h dark cycles and temperature of 22 °C. The flower buds were collected after 1.5 to 2 g of flower tissues were collected after 1 h. Then, 1.5–2 g of flower tissues were collected immediately. For UVB stress treatment, 10-d-old seedlings grown on ½ MS medium were treated with broadband UVB (430 μW cm\(^{-2}\) for 3.5 h in addition to white light (60 μW cm\(^{-2}\)) and then recovered under white light for 1 h. The plants with newly grown leaves were defined as survived plants.

Co-IP. Co-IP in Arabidopsis was performed with homozygous UVR8-HA transgenic plants in 35S-MRM2/dd or Col-0 backgrounds. For co-IP after isolating nuclei, fresh 10-d-old seedlings were chopped with a blade and then ground with a mortar and pestle in ice-cold nuclei-isolating buffer (10 mM MES-KOH, pH 5.5, 2.5 mM EDTA, 10 mM NaCl, 10 mM KCl, 0.2 M sucrose, 0.1 mM spermidine, 2.5 mM dithiothreitol). Then the samples were filtered through two layers of miracloth and centrifuged at 1,000g for 10 min at 4 °C. The pellet containing nuclei was then resuspended in co-IP binding buffer (20 mM HEPES, pH 7.5, 40 mM KCl, 1 mM EDTA, 1% Triton X-100 and protease inhibitor cocktail), lysed using a Dounce tissue grinder (Sigma) and rotated at 4 °C for 30 min. After centrifuging, the supernatant was incubated with 6 μl of FLAG magnetic beads for 1 h. The beads were then washed with co-IP wash buffer (20 mM HEPES, pH 7.5, 40 mM KCl, 1 mM EDTA, 1% Triton X-100) three times, and boiled at 95 °C in 1x SDS loading buffer. For co-IP using total proteins, 4-week-old rossette leaves were ground to a fine powder using a mortar and pestle in liquid nitrogen. Total proteins were then extracted by grinding the powder with liquid nitrogen using a Dounce tissue grinder (Sigma) and incubating with rotation at 4 °C for 30 min. After centrifuging, the supernatant was filtered through a 0.45-μm membrane and incubated with 10 μl of FLAG magnetic beads for 1 h. After washing with co-IP wash buffer five times, beads were boiled at 95 °C in 1x SDS loading buffer. For co-IP in N. benthamiana, the leaves were co-infiltrated with Agrobacterium carrying LUCM-HA and DRM2-FLAG. Total proteins were extracted with 2× extraction buffer and then immunoprecipitated with 5 μl of FLAG beads for 1 h at 4 °C. After washing with the same buffer five times, the beads were boiled in 1x SDS loading buffer.

Immunoblotting. Protein samples were run on SDS-PAGE gels and transferred to nitrocellulose membranes. The membranes were blocked with 5% non-fat milk, rinsed with TBST and then incubated with primary and/or secondary antibodies. The primary antibodies used were anti-FRAP-HRP (Sigma, 1:5,000), anti-HA-HRP (Roche, 1:5,000), anti-GFP (Roche, 1:1,000), anti-actin (Proteinitech, 1:5,000), anti-tubulin (Servicio, 1:5,000) and anti-H3 (Abcam, 1:1,000). All antibodies were used in 3% BSA in 1x TBST buffer. Chemiluminescence images were taken after adding Clarity Western enhanced chemiluminescence (ECL) substrate (Bio-Rad) with ImageQuant LAS4000 (GE).

Split luciferase and luciferase imaging. For split luciferase assays, Agrobacterium carrying nLuc and cLuc plasmids was cultured in liquid LB medium, resuspended to optical density of 0.2 (OD\(_{600}\)) and then cultured for 4 h at 35°C in liquid medium (20 mM HEPES, 200 μM acetoxyctyrretine). Then, equal volumes of nLuc- and cLuc-containing Agrobacterium suspensions were infiltrated into 3- to 5-week-old N. benthamiana. After 36–48 h, the leaves were sprayed with 2 μl muracitin (Promega) in 0.01% Triton X-100 solution and kept in darkness for 5 min. Chemiluminescence images were then taken with a 5× exposure. For luciferase imaging in Arabidopsis, 6- or 7-d-old seedlings were cultured on ½ Murashige and Skoog (MS) medium with 1% sucrose and 0.7% agar. The seeds were put directly under a broad band UVB lamp (430 μW cm\(^{-2}\)) for 3.5 h in addition to white light (60 μW cm\(^{-2}\)) and then recovered under white light for 1 h. For UVR8 and DRM2-GFP localization by UVB treatment, 7-d-old homoygous transgenic seedlings on ½ MS medium were put under a narrowband UVB lamp for 4 h and then imaged with a confocal microscope (Nikon A1R).
NATURE PLANTS

and a UVR8-OX transgenic line were planted on ½ MS medium, and treated with Bisulfite sequencing.

trimethylammonium bromide (CTAB) method70. An equal amount of genomic (DRM2CAT, 269–626) proteins, 1 DNA (12 repeats of TAC, annealed to form double-stranded DNA (dsDNA), 1450–522) and washed two times with 200 mM ammonium bicarbonate, two times were treated with broadband UVB for 1 h. Then, 1–2 g of flowers were ground into fine powder in liquid nitrogen with a mortar and pestle. The powder was then crosslinked in nuclei isolation buffer (10 mM HEPES, pH 8.0, 1 M sucrose, 200 mM -end adenylation and methylated adaptor ligation using Illumina TruSeq method as for the split luciferase assay. After 36–48 h, the infiltrated leaves were used. The excitation wavelengths for YFP/GFP and mCherry were 488 nm and 561 nm, respectively, and the emission wavelengths for YFP/GFP and mCherry were 500–550 nm and 570–620 nm, respectively. All confocal images were exported as TIFF images with single channel and merged multiple channels.

Quantitative PCR analysis. For RT-qPCR, plant total RNA was extracted using Ambion PureLink RNA Mini Kit (Invitrogen). The first-strand complementary DNA was then synthesized from 2 μg of the extracted total RNA using oligo(dT)15VN and random hexamer primers, and SuperScript III (Invitrogen) or ProtoScript II (NEB) reverse transcriptions. For qPCR following DNA digestion (Chop–qPCR), plant genomic DNA was extracted with the cetyl trimethylammonium bromide (CTAB) method. An equal amount of genomic DNA was then digested with MspI or HaeIII (NEB) for 6 h at 37 °C. For Chop-qPCR, 0.5 μl of immunoprecipitated DNA was used as template. The quantitative PCR was performed in triplicate using SYBR Green qPCR Master Mix (Vazyme) and a Bio-Rad CFX384 C1000 Real-Time system. The gene expression levels in RT-qPCR were normalized against wild-type and internal control ACT7 or a U-box gene (At5g14400). The relative methylation levels of Chop–qPCR were normalized to uncut control. The relative enrichment of each locus in ChIP-qPCR was normalized to Col-0.

DNA methyltransferase activity assay. The methyltransferase assay was carried out at 30 °C for 1 h in a total volume of 25 μl containing 5 μl of Sadenosyl-L-[methyl-3H] methionine (SAM) (14.4 Ci mmol−1; PerkinElmer), 1.5 μl of substrate DNA (12 repeats of TAC, annealed to form double-stranded DNA (dsDNA), 15 μM) and 0.2 μl of DRM2 full-length (59–626) or DRM2 methyltransferase (DRM2−ΔC177, −ΔC177) and a Bio-Rad CFX384 C1000 Real-Time system. The gene expression levels in RT-qPCR were normalized against wild-type and internal control ACT7 or a U-box gene (At5g14400). The relative methylation levels of Chop–qPCR were normalized to uncut control. The relative enrichment of each locus in ChIP-qPCR was normalized to Col-0.

ChIP. For DRM2-FLAG ChIP, flowers from gDRM2-FLAG/dd transgenic plants were used. For UVB-treated samples, plants were treated with broad-band UVB for 1 h. Then, 1–2 g of flowers were ground into fine powder in liquid nitrogen with a mortar and pestle. The powder was then crosslinked in nuclei isolation buffer (10 mM HEPES, pH 8.0, 1 M sucrose, 5 mM KCl, 5 mM MgCl2, 5 mM EDTA, 0.6% Triton X-100, 0.4 mM PMSF and protease inhibitor cocktail) with 1% formaldehyde for 15 min at room temperature. Crosslinking was stopped by adding 1 μl of cold SAM (NEB). A total of 11 μl from each reaction was applied onto DEAE Filtermat (PerkinElmer, 1450–522) and washed two times with 200 mM ammonium bicarbonate, two times with water and two times with ethanol. The paper was dried and placed into 4 μl of liquid scintillation cocktail (Fisher Scientific) and the activity was measured by a Liquid Scintillation Analyzer (PerkinElmer, Tri-Carb 2910 TR). High-throughput sequencing data analysis. Bisulfite sequencing reads were aligned to the TAIR10 genome using Bsam v2.9 (ref. 1). Reads were filtered for <5% characters and were further filtered using Trimomatic v0.39 (ref. 1) and then aligned to the TAIR10 genome using Bsam v2.9. For metaplots, we calculated average methylation level in CG, CHG and CHH contexts with bedtools (plotted with R package) (https://www.r-project.org/). For DMR calling, we used both MethyKit package66 and Fisher’s exact test to call DMRs, and the overlapped DMRs were then used for subsequent analysis. For RNA-seq analysis, we firstly filtered reads with Trimomatic v0.39 (ref. 1) and then aligned them to the TAIR10 genome using HISAT2 (v2.0.0-beta)1. The alignments were then filtered with a bash code to keep the uniquely mapped reads. The quantification of gene expression and the identification of DEGs were performed with Cufflinks v2.2.1 (ref. 1). The heat map was made using Heatmapper (http://www.heatmapper.ca/expression/). The snapshots of track data were made using the IGV browser.

Quantification, statistical analysis and reproducibility. Quantification of immunoblots and luciferase was carried out using ImageStudio and ImageJ. Statistical analyses were carried out using Excel, GraphPad Prism and R. Data are presented as mean ± s.e.m. or mean ± g.s.e.m., as indicated. All statistical tests used were two-sided. For the immunoblots and micrographs, at least two independent experiments were repeated with similar results.

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7. For bisulfite-Sanger sequencing, the genomic DNA was bisulfite converted using an EZ DNA Methylation-Gold kit (Zymo Research). PCR was then performed using a MyTaq Mix (Bioline). The PCR products were purified from agarose gels and ligated to pCR2.1 using a TOPO TA Cloning kit (Thermo Fisher Scientific). Then, 10–19 clones were sequenced (Genewiz) and analyzed with Kismeth (http://kathadin.nsmm.sis.kth.se/kismeth/revpajl.php).

RNA-seq. For RNA-seq, total RNA was extracted using Ambion PureLink RNA Mini Kit (Invitrogen) and treated with DNase I. RNA-seq libraries were constructed using a TruSeq RNA Library Preparation Kit (Illumina, RS-122-2002). In brief, messenger RNA was purified with RNA purification beads, and fragmented with Enul, Primer, Fragment Mix. Later, double-stranded cDNA was synthesized with SuperScript II (Invitrogen) followed by second-strand synthesis. End-repair, 3’-end adenylation, ligation of adaptors and PCR amplification for 12 cycles were then performed. Libraries were sequenced on a HiSeq 2500 sequencing system (Illumina) in the University of Wisconsin–Madison Biotechnology Center. Two biological replicates were performed for RNA-seq.

Data availability. All whole-genome bisulfite sequencing and RNA-seq data produced during this study were deposited into the Gene Expression Omnibus under accession number GSE132944. Source data are provided with this paper.

DNA availability. All whole-genome bisulfite sequencing and RNA-seq data produced during this study were deposited into the Gene Expression Omnibus under accession number GSE132944. Source data are provided with this paper.
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Author contributions
J.J. designed and performed most experiments, analysed data, prepared figures and wrote the manuscript draft. J.L. and D.S. performed the genomic data analysis. S.Q. provided reagents and edited the manuscript. W.R. and J.S. provided recombinant DRM2 proteins. F.L. conceived the project and edited the manuscript. X.Z. conceived the project, designed experiments, analysed data and wrote the manuscript.

Competing interests
The authors declare no competing interests.

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Characterization of LUCM reporter line. **a**, Luciferase images of Col-0 and three d35S:LUC reporter lines treated with DNA methylation inhibitor 5-Azacytidine (5-AzaC, 100 μM) for 7 days. LUCL and LUCH are previously reported (ref. 35) low and high LUC expressing lines, respectively. **b**, Copy number of 35S-LUC transgene in LUCL, LUCH, and LUCM lines revealed by qPCR of LUC using genomic DNA. Data is mean ± SD. **c**, McrBC-qPCR based DNA methylation assay of 35S promoter regions in LUCL, LUCH, and LUCM lines. Low amplification represents high DNA methylation level. Data is mean ± SD. **p < 0.01; ***p < 0.001 by Student’s t-test. **d**, Relative transcript level of LUC gene in LUCL, LUCH, and LUCM lines. Data is mean ± SD. **p < 0.01; ***p < 0.001 by Student’s t-test. **e**, Bisulfite sequencing of indicated regions (1 and 2) in LUCL, LUCH, and LUCM lines. **f**, Luciferase images of 5-d old LUCM and ddlUCM (drm1 drm2 LUCM) seedlings. **g**, McrBC-qPCR based DNA methylation assay of 35S promoter regions. SDC serves as a control for dd. Data is mean ± SD. ***p < 0.001 by Student’s t-test.
Extended Data Fig. 2 | Flowering phenotypes of *FWA* transgenic plants. a and b, The flowering time of *FWA* transgenic T1 plants from two replicates with replicate 1 in (a) and replicate 2 in (b). Indicated genotypes were transformed with *FWA* transgene and the flowering time of positive Basta-resistant T1 transformants were counted. The non-transformed plants serve as controls. The number of plants is indicated by n. *dd*, *drm1 drm2*; *udd*, *uvr8-6 drm1 drm2*; *UVR8-OX*, *35S:UVR8-FLAG* overexpression lines (#3 and #2); *UVR8W285A-OX*, *35S:UVR8W285A-FLAG* overexpression lines (#5 and #8). The blue dash line indicates median. Each dot represents a single plant. Significantly different (p < 0.05 by Student's t-test) groups are labelled with different letters.
Extended Data Fig. 3 | Analysis of UVB-induced differentially methylated regions. a, b, Venn diagrams showing the overlap of CHH hypo differential methylation regions (DMRs) among drm1 drm2 (dd), cmt2-3, and UVB-induced DMRs in Col-0 (a) and UVR8-OX (b). Data of dd and cmt2-3 are from ref. 77. c, Venn diagrams showing the overlap of CHH hypo DMRs among UVB-treated Col-0 (left) or UVR8-OX (right), dd, and drm2-2 mutant. Data of drm2-2 is from ref. 78. d, Venn diagrams showing the overlap of UVB-induced CHH hypo DMRs in Col-0 with hyper DMRs in uvr8-6 mutant. e, Venn diagrams showing the overlap of UVB-induced CHH hypo DMRs in Col-0 and uvr8-6. f, The enrichment of TEs containing CHH hypo DMRs based on length. ***, p < 0.001 by Fisher’s exact test. g, The enrichment of Class II (DNA) TEs containing CHH hypo DMRs. *** p < 0.001; ** p < 0.01; * p < 0.05; ns, not significant by Fisher’s exact test.
Extended Data Fig. 4 | Comparison of UVB- and UVC-induced DMRs. a, Comparison of CHH hypo DMRs by UVC treated Col-0 (compared to Col-0 without treatment) and drm1 drm2 (dd). The UVC-induced DMRs are from ref. 41 (GSE132750). b, Comparison of UVB- and UVC-induced CHH hypo DMRs in Col-0. c, Comparison of CHH hypo DMRs of UVB treated UVR8-OX (compared with Col-0,WL) and UVC. d, Overlapping of CHH hypo DMRs of UVB treated UVR8-OX (compared with UVR8-OX,WL) and UVC.
Extended Data Fig. 5 | Differential expressed genes (DEG) induced by UVB. a, Venn diagram showing the overlapping of DEGs induced by UVB in Col-0 and drm1 drm2 (dd). b, Correlation plot showing the expression level change (log2 FC) of common UVB-responsive DEGs in Col-0 and dd (n = 327). c, Expression levels of marker genes, which are up-regulated by UVB, in Col-0 and dd. Data is mean with 95% confidence interval. d, Expression levels of DNA-damage induced genes in Col-0 and dd. Data is mean with 95% confidence interval. e, Expression levels of genes in UVB-signaling pathway. Data is mean with 95% confidence interval. Different letters denote significant differences (p < 0.05 by Student’s t-test) among samples. f, Venn diagram showing the overlapping of DEGs induced by long-term (10d, this study) and short-term (6h, ref. 79) UVB treatment in Col-0. g and h, Heat map showing the expression of several UVB up-regulated (g) and down-regulated (h) DEGs in both long-term and short-term UVB treatment. i, Metaplots showing the CHH methylation level of UVB up-regulated Tes (n = 269). Data of 1 kb upstream and downstream of the TE body are shown.
Extended Data Fig. 6 | DRM2 interacts with UVR8 and its active form UVR8W285A. a, Heatmap showing the NSAF score (an indicator of normalized spectral abundance factor) of UVR8 and DRM2 in various immunoprecipitation-mass spectrometry (IP-MS) experiments. The IP-MS data of DRM2 is from ref. 34, DRM3 and NRPE1 are from ref. 80, CMT3 is from ref. 81, DRD1 and DMS3 are from ref. 82, MORC6 is from ref. 69, IDN2 is from ref. 83, HDA9 and PWR are from ref. 84, HOS15 is from ref. 85, HD2C is from ref. 86, EBS is from ref. 87. b, Coomassie bright blue staining of non-boiled GST-UVR8 proteins on SDS-PAGE. The GST-UVR8W285A proteins serve as control for monomer. c, Co-immunoprecipitation of UVR8 and DRM2 with FLAG beads from N. benthamiana leaves co-expressing UVR8-HA and DRM2-FLAG. d, Co-immunoprecipitation of UVR8 and DRM2 with FLAG beads from transgenic Arabidopsis plants co-expressing UVR8-HA and 3F9M-DRM2. UVR8-HA in Col-0 serves as a control. e, Immunoblots showing protein levels with or without UVB treatment. The ±UVB set-up is the same as that in Fig. 4g. Actin serves as an internal control. f, Split luciferase assay showing the interaction between DRM2 and UVR8W285A. The indicated constructs were co-expressed in N. benthamiana leaves and imaged after spraying with the luciferin. nLuc- and cLuc-only vectors serve as negative controls. Two biological replicates are shown. g, Bimolecular fluorescence complementation (BIFC) assays in N. benthamiana leaves showing the interaction between DRM2 and different forms of UVR8. Scale bar, 10 μm. h, BIFC assays co-expressing indicated proteins in N. benthamiana leaves. Scale bar, 10 μm.
Extended Data Fig. 7 | DRM2 interacts with both the core domain and the C-terminus of UVR8. a, Bimolecular fluorescence complementation (BIFC) assays in N. benthamiana leaves. nYFP-fused full length UVR8, UVR8 core domain (UVR8N396), and C-terminus (UVR8C44) were co-expressed with DRM2-cYFP. The arrow indicates the nuclei showing nuclear bodies. Scale bar, 10 μm. b, Magnified images showing the interaction of UVR8-DRM2, UVR8N396-DRM2 in nuclear bodies. Scale bar, 10 μm.
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Policy information about availability of computer code

Data collection | Illumina sequencing data was collected using the standard Illumina pipeline for the HiSeq4000 (BS-seq) and HiSeq 2500 (RNA-seq).

Data analysis | For high-throughput sequencing data analysis, the softwares used include: BSmap version 2.9; Trimmomatic version 0.39; R software (https://www.r-project.org/); Trimmomatic (version 0.39); MethylKit package; HISAT2 (version 2.0.0-beta); Cufflinks v2.2.1; Heatmapper (http://www.heatmapper.ca/expression/); IGV genome browser (v2.4.14). Other softwares include ImageJ (NIH); Image Studio (LICOR, v5.2.5); GraphPad Prism 8; Excel (Microsoft 365).

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All WGBS and RNA-seq data produced during this study were deposited into Gene Expression Omnibus under accession number GSE132944.
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### Antibodies

**Antibodies used**

- Anti-FLAG-HRP antibody (Sigma, A8592); Anti-HA-HRP antibody (Roche, 12013819001); Anti-GFP antibody (Roche, 11814460001); Anti-actin antibody (Proteintech, 60008-1); Anti-tubulin antibody (Servicebio, GB11200); Anti-H3 antibody (Abcam, ab1791)

**Validation**

All antibodies used are commercially available and validated by corresponding companies.