1	Appendix Table of Contents
2	Appendix Figure S1.
3	Appendix Figure S2.
4	Appendix Figure S3.
5	Appendix Figure S4.
6	Appendix Figure S5.
7	Appendix Figure S6.
8	Appendix Figure S7.
9	Appendix Figure S8.
10	Appendix Table S1. Primer list.
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	
21	
22	

# **Appendix Figures**



## 26

# 27 Appendix Figure S1. UV-B inhibits the growth of lateral root.

A-C. Phenotypic analysis. Seedlings of indicated genotype were grown in 1/2 MS with or
without UV-B (1 W/m<sup>2</sup>) for 2 weeks. Images are shown in (A); scale bar = 2 mm, and
average length of lateral roots of the indicated genotypes were measured and shown in (B).
SDs (n > 8) are indicated. (C) Average lateral root length ratios (White+UV-B/White) of the
quantified lateral root length in (B).

33 D. UV-B treatment breaks the function of IAA but not NAA. WT Seedlings were grown in 34 LD for 5 days, then transferred to new plates containing 1  $\mu$ M IAA (left) or 0.4  $\mu$ M NAA 35 (right) that were pre-irradiated by white light with or without UV-B for 7 days and kept in 36 continuous white light for 7 days. Scale bars = 2 mm.

E-G. High concentration of auxin inhibits the lateral root growth. Seedlings of WT were grown in LD condition for 5 days, then transplanted to new medium with the addition of a series of concentrations of NAA and kept in continuous white condition for 7 days. Images are shown in (E); scale bar = 2 mm. The lateral root density (number of lateral roots/length of primary root) (F) and average length of lateral roots (G) of the indicated genotypes were

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#### Appendix Figure S2. UV-B represses the auxin responses to inhibit the hypocotyl 46 47 elongation in a UVR8-dependent manner.

48 A. Images showing details of the covering root experiment.

49 B. Phenotypic analysis. Seedlings of WT and uvr8 were grown in LD condition for 5 days,

then transplanted to new medium containing 0.4 µM NAA and roots were covered, then kept 50

in white or white plus UV-B light condition  $(1 \text{ W/m}^2)$  for 10 days. Scale bar = 2 mm. 51

52 C-E. Phenotypic analysis. Seedlings of the indicated genotypes were grown in 1/2 MS with or without L-kyn (the inhibitor of auxin synthesis) in continuous white light condition and white 53 54 plus UV-B (2 W/m<sup>2</sup>) light condition. Images of the representative seedlings are shown in (C); 55 scale bar = 1 mm. And the hypocotyl lengths of the indicated genotypes were measured and

are shown in (D) and (E). Standard deviations (n > 15) are indicated. 56

- 57 F-H. uvr8 mutant is hypersensitive but rup1 rup2 is insensitive to PIC (auxin analogous)
- 58 under UV-B condition. WT, uvr8 and rup1 rup2 were grown in 1/2 MS with 200 µM L-kyn,

and with the addition of a series of concentrations of PIC in the continuous white light condition or white plus UV-B  $(2 \text{ W/m}^2)$  light condition for 6 d. Images of the representative seedlings are shown in (F); scale bar = 1 mm. The hypocotyl lengths of the indicated genotypes were measured and are shown in (G) and (H). Standard deviations (n > 15) are indicated.

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A. UVR8 inhibited auxin responses under UV-B in a root-autonomous way. WT and *uvr8*seedlings grown in LD for 5 days were used for reciprocal grafting. Seedlings were kept in
LD for 7 days after grafting, then transplanted to new medium containing 0.4 μM NAA and
covered the roots and kept in white plus UV-B light for 10 days. Scale bar=2 mm.

B. Transcriptome analysis of genes expression regulated by auxin, UV-B and UVR8. WT/
UV-B 0.5 h and WT/ UV-B 2 h showed the transcriptome of roots and only one biological
replicate was analyzed. The *uvr8*/ UV-B 1 h and WT auxin/ none showed the transcriptome of
seedlings and three biological replicates were analyzed. The parameter measured by color key
shows the Log-fold change.

C. qPCR assay showing that UV-B inhibits the expression of auxin-signaling genes. WT
protoplast were kept in white light condition or white light plus UV-B condition for 16h. The *ACT7* gene was analyzed as an internal control. Error bars are SD of three biological
replicates.

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Appendix Figure S4. UVR8 physically interacts with the R2R3 DNA-binding domain of 85 **MYB73/MYB77.** 86

87 A. Neighbor-joining phylogenetic analysis and alignment of amino acid sequence showing possible relationship between MYB73 and other MYB proteins. The bootstrap values are 88 indicated. The scale bar indicates substitution per site. 89

B and C. Co-IP assays showing that the interaction between UVR8<sup>R338A</sup> and MYB73 90 (B)/MYB77(C) is stronger than that between GR-UVR8<sup>W285F</sup> and MYB73/MYB77. 14-91 35S::MYB73-TAP/GR-UVR8<sup>W285F</sup> day-old transgenic seedlings expressing 92 35S::MYB77-TAP/GR-UVR8<sup>W285F</sup> 35S::MYB73-TAP/GR-UVR8<sup>R338A</sup> 93 and 35S::MYB73-TAP/GR-UVR8<sup>R338A</sup> grown in 1/2 MS with 20 µM DEX in long day condition
were moved to UV-B (1 W/m<sup>2</sup>) for 20 min. Total proteins were extracted and used in the
co-IP assay. Input: immunoblots showing the levels of UVR8, MYB73-TAP/MYB77-TAP in
the total protein extract. UVR8-IP: the IP products precipitated by the anti-UVR8 antibody.
Total proteins (Input) or IP products were probed, in immunoblots with antibodies to UVR8
or Myc.

- 100 D. Schematic representation of MYB73/MYB77 and UVR8 used in this work.
- MYB73/MYB77 contains a R2R3 domain and a C terminal. UVR8 contains seven repeats of
   a β-propeller fragment and a C terminal (including C27 domain).
- E. BiFC assays showing that both of UVR8N and UVR8C interact with the R2R3 DNA-binding domain of MYB73/MYB77.
- F and G. pull-down assays showing that R2R3 domain of MYB73 could bind the N terminal(F) and C terminal (G) of UVR8.
- H and I. Pull-down assays showing that both the R2R3 domain and the C terminal of MYB77
  could interact with the N terminal of UVR8 (H), but only the R2R3 domain of MYB77 could
  bind the C terminal of UVR8 (I).
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Appendix Figure S5. UV-B slightly induces the transcription of MYB73/MYB77 but does 115 not affect their protein stability. 116

A and B. qPCR assays showing that UV-B slightly induces the expression of MYB73 (A) and 117 MYB77 (B) in a UVR8-independent manner. 6-d-old seedlings grown in constant white light 118 were transferred to UV-B for the indicated time. The ACT7 gene was analyzed as an internal 119 control. Error bars are SD of three biological replicates. 120

C and D. Immunoblots showing that the lack of UV-B effects on the abundance of the 121 122 MYB73(C) /MYB77(D) protein. Transgenic plants expressing 35S:MYB73-TAP or 35S::MYB77-TAP plants were grown in 22°C continuous white light condition for 6 days, and 123 kept in white light or transferred to white plus UV-B light for a 5-h time course. Samples 124 were fractionated by 10% SDS-PAGE, blotted, and probed with the anti-MYC antibody. NS 125 (non-specific band). 126

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Appendix Figure S6. Genotyping of mutants 131

A-E. PCR results show the genotyping of the indicated mutants. Primers used in the PCR 132

reaction are labeled in the left. CK (control: without DNA template). 133

F. Immunoblots show UVR8 in indicated genotypes. Samples were fractionated by 10% 134 135 SDS-PAGE, blotted, and probed with the anti-UVR8 antibody. NS (non-specific band).

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auxin responses.



Appendix Figure S7. MYB73/MYB77 work redundantly to mediate UVR8-controlled 139

141 A. myb73 myb77 uvr8 can partially rescue the phenotype of uvr8. Seedlings of indicated genotypes were grown in LD condition for 5 days, then transplanted to new medium 142 containing 0.4 µM NAA and roots were covered, then kept in continuous white or white light 143 144 plus UV-B condition for 10 days; scale bar = 2 mm.

B and C. MYB73/MYB77 acts downstream of UVR8 to mediate UV-B signaling. Seedlings 145 of the indicated genotypes were grown in continuous white light or white plus UV-B light for 146 6d, Images of representative seedlings are shown in (B); scale bars = 2 mm. The hypocotyl 147 148 lengths of the indicated genotypes were measured and shown in (C). Standard deviations (n >15) are indicated. 149



*myb73*, *myb77* and *myb73myb77*. Seedlings were grown in continuous white light for 5 days, 151 152 then transferred to UV-B for 1 day with addition of 1 µM IAA. Error bars are SD of three 153 biological replicates.

E. qPCR assay analyses the expression of indicated genes in WT and myb73myb77. WT and 154 myb73 myb77 protoplasts were kept in white plus UV-B light condition for 16 h. ACT7 gene 155 was analyzed as an internal control. Error bars are SD of three biological replicates. 156

F. Quantitative RT-PCR analyses of genes expression of MYB73/MYB77 target genes. 5-d-old 157 158 seedlings grown in continuous white light were transferred to UV-B and treated with 1 µM IAA for 1d. Error bars are SD of three biological replicates. 159

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### 165 UVR8-dependent manner.

- A. DNA sequences of the probe including the MBSI motif. The *IAA19p* is the wild-type *IAA19* promoter contained MBSI (*IAA19p*) and mutated MBSI (*IAA19mp*) was used as
  competitor. Red represents the MBSI and the blue represents the mutated nucleotides.
- B. EMSA showing that UVR8 has a strong effect on inhibiting MYB73 to bind DNA under
  UV-B. MYB73 and UVR8 protein were expressed and purified from *E.coli*. The reaction
- 171 mixture was treated with UV-B light for 30 min.
- 172 C and D. EMSA results showing that UVR8<sup>W285A</sup> inhibits the MYB73 (C) and MYB77 (D) to
  173 bind the promoter of *IAA19* in vitro.
- 174 E and F. ChIP-qPCR showing that UV-B inhibits the DNA-binding activity of MYB77 (E) in
- 175 roots in a UVR8-dependent manner (F). ChIP-qPCR assays were performed using transgenic
- 176 seedlings expressing 35S::MYB77-TAP/WT or 35S::MYB77-TAP/uvr8, which were treated
- 177 with or without UV-B for 5 hours before harvesting samples. Chromatin fragments (~500bp)
- 178 were immunoprecipitated by anti-Myc antibody, and the precipitated DNA was analyzed by
- 179 qPCR using the primer pairs indicated. The IP/Input ratios are shown with SDs (n=3).
- 180 G. ChIP-qPCR showing that UVR8 cannot bind the promoters of MYB73/MYB77 target
  181 genes. The IP/Input ratios are shown with SDs (n=3).
- H and I. ChIP-qPCR results show monomeric UVR8 (UVR8<sup>R338A</sup>) can strongly inhibit the DNA-binding activity of MYB77(H)/MYB73(I). The IP/Input ratios are shown with SDs (n=3).
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# **Table S1. Primer list**

<i>myb73-</i> F	AGAGAGTGGAAGAAACGCTCC
myb73-R	CCTCCGGAGATAGCTGGTTAC
myb77-F	GATGAGCAGCTACGAAGGATG
myb77-R	TGGTTATGAATCACCAAAACAAG
MYB73q-F	GAGGAGTTACATGGCGGATT
MYB73q-R	CTTTGTGGCATACACGAACC
MYB77q-F	TAAGGCGGAAGTGAGGAGTT
MYB77q-R	TCCAATTTGACTCATCGGAA

	GGGGACAAGTTTGTACAAAAAGCAGGCTTC
MYB/3nYFP/nLUC-F	ATGTCAAACCCGACCCGTAAG
MVD72nVED/nI LIC D	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTCCATCTTC
MID/JIII//IILOC-K	CCAATTCCGAT
MVB77nVFP/nLUC-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCGGA
WID////////////////////////////////////	TCGTGTTAAAGGT
MYB77nVFP/nI LIC P	GGGGACCACTTTGTACAAGAAAGCTGG
	GTCCTCAACCTTAGGTGTTATTACTCCA
MYB70-nLUC-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTGG
	TTCGACCCGGA
MYB70-pLUC-R	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTCGATCCTA
MTD/0-IILOC-K	ССТААТССААТААА
MYB44-nLUC-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCTGA
	TAGGATCAAAGGTCC
MYB44-nLUC-R	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTCGATTCTC
	ССААСТССААТ
MYB73pCold-F	CCGCTCGAGATGTCAAACCCGACCCGTAAG
MYB73pCold-R	GGAATTCCTACTCCATCTTCCCAATTCCG
MYB77pCold-F	CCGCTCGAGATGGCGGATCGTGTTAAAGGT
MYB77pCold-R	GGAATTCCTACTCAACCTTAGGTGTTATTACTCCA
MVD72N »VED E	GGGGACAAGTTTGTACAAAAAGCAGGCTTC
MIID/JIN-IIIFF-F	ATGTCAAACCCGACCCGTAAG
MVB73N_nVFP_R	GGGGACCACTTTGTACAAGAAAGCTGGGTCACTTTGCCCT
	TCGACGCTG
MYB73C-nYFP-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTGTGA
	TTTTGGTGGTAATGGAGG
MYB73C-nYFP-R	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTCCATCTTC
	CCAATTCCGAT
MYB77N-nYFP-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCGGA
	TCGTGTTAAAGGT
MYB77N-nYFP-R	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTCCGTCTCC
MYB77C-nYFP-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTC
MYB77C-nYFP-R	GGGGAUCAUTTIGTAUAAGAAAGUTGG
MVD72N nCald E	
MYD72N - C-14D	
$\frac{1}{10} \frac{1}{10} \frac$	
MYD72C C LLD	
MYB/3C-pCold-R	GGAATICCTACTCCATCTTCCCAATTCCG
MYB77N-pCold-F	CCGCTCGAGATGGCGGATCGTGTTAAAGGT

MYB77N-pCold-R	GGAATTCCTACTCCGTCTCCGTCACCGTC
MYB77C-pCold-F	CCGCTCGAGATGGAAGATCAGGATCGGCCGA
MYB77C-pCold-R	GGAATTCCTACTCAACCTTAGGTGTTATTACTCCA
MYB73-TAP-F	TAGTTAATTAAATGTCAAACCCGACCCGTAA
MYB73-TAP-R	CGAGGCGCGCCACTCCATCTTCCCAATTCCGAT
MYB77-TAP-F	TAGTTAATTAAATGGCGGATCGTGTTAAAGG
MYB77-TAP-R	CGAGGCGCGCCACTCAACCTTAGGTGTTATTACTCCACA
IAA5q-F	AGAAAGAACAGTCTCGAACGGAC
IAA5q-R	AGAATTTGCAGAGCGGAAGC
SAUR20q-F	CTTGAATCTTTTCATACATCTTCAGAAG
SAUR20q-R	TAACTAGGAAGAAAAATGTTGGCTCA
SAUR23q-F	ATTCAAACTTTCAGACAAAAGAAATGG
SAUR23q-R	ACAAGGAAACAACTCTATCTCTAACT
SAUR63q-F	GTGTCTTTACTAGCAGCTCTTCTACTGT
SAUR63q-R	GTGATTGGTCCTTCCGTTGG
HAT2q-F	AACCATCACCACAATCACAGGC
HAT2q-R	CATCCAAAATTACAATGACCCCA
IAA19q-F	GATAAGCTCTTCGGTTTCCG
IAA19q-R	TCGCAGTTGTCACCATCTTT
GH3.2q-F	CCTCTTCAATCTCGGATGGT
GH3.2q-R	GTCAGGGTTCCGAGTCATTT
GH3.3q-F	TTGTCCAGACTCATCCCAAA
GH3.3q-R	CTTCGTGACGCATAAGGAGA
MBSI-F	TCGACATGGCGGTTATGTTAACTGCCATGG
MBSI-R	CCATGGCAGTTAACATAACCGCCATGTCGA
IAA19n-FMSA-F	CAATGATCCAACGGTCAGAGAAAAATCAGAAGAAAATTT
	CACAACCGTAGGATCTGTTCC
IAA19p-EMSA-R	GGAACAGATCCTACGGTTGTGAAATTTTCTTCTGATTTTTC
	TCTGACCGTTGGATCATTG
IAA19pm-EMSA-F	CAATGATCTGACGATCAGAGAAAAATCAGAAGAAAATTT
IAA19pm-EMSA-R	GGAACAGATCCTATGGTCATGAAATTTTCTTCTGATTTTTC TCTCATCCTCACATCATTC
IAA1CHIDE	
IAAICHIP-F	
IAATCHIP-R	
IAA/CIIIP-F	
IAA/UIIP-K	
IAAI/UIII'-I'	
IAAI9ChIP-F	LATUUUATUTTAUUAUAAUUAUAT

IAA19ChIP-R	TGTTATTCAGTTTGTGTGGGGAG
GH3.2ChIP-F	CGTTGATTAACTTAAAGTCACTCCG
GH3.2ChIP-R	TGTCACAGGGTTGTAGCCGAG