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1 **Supplementary Information**

2 **Materials and methods**

3 **Southern blot analysis**

4 Southern blot analysis of organelle-specific DNA repair was based on the quantitative comparison of
5 the intensity, as determined by densitometry analysis, of T4 endonuclease V-treated and untreated
6 DNA.[1] T4 endonuclease V cleaves single DNA strands at CPD sites. Genomic DNA was isolated from
7 shoots of irradiated or non-irradiated seedlings as described previously.[2] Genomic DNA was digested
8 with *Bam*HI. The digested DNA was divided into two equal aliquots; one aliquot was treated with T4
9 endonuclease V, and the other was left untreated. The samples were resolved by alkaline agarose gel
10 electrophoresis, the DNA was blotted onto a nylon membrane as described previously.[3]

11 To generate probes for Southern blot analysis, a mitochondrial-specific gene (*COX1*: ATMG01360)
12 and a nuclear-specific gene (*PHT2;1*: AT3G26570) were amplified using gene-specific primers
13 (Supplementary Table 2). The amplified fragments were subcloned into the pGEM-T easy vector
14 (Promega), and the sequences were confirmed. The digested inserts were labelled with [α-^{32}P] dCTP
15 and hybridized individually to a nylon membrane as described previously.[3] Data are presented as the
16 ratio of [(intensity of untreated DNA) – (intensity of T4 endonuclease V-treated DNA)] to intensity of
17 untreated DNA [% fragments with enzyme-sensitive sites (ESS)].

18

19 **Carbonyl cyanide m-chlorophenyl hydrazone treatment**

20 Whole plants of transgenic *Arabidopsis* lines with MT-GFP, including roots, were pulled out from the
21 soil. A 10 mM MES-NaOH (pH 5.5) solution with or without 10 μM CCCP was infiltrated into leaves
22 using a 1-ml syringe. The seedlings were immersed in the same solutions with filter paper laid on top
23 to keep them submerged, and were then returned to the growth chamber for 6 h. The same amount of
24 DMSO as CCCP was added to the control. The mesophyll cells in the area infiltrated with CCCP were
25 observed by LSCM.

26

27 **Monitoring of autophagic flux**

The mitochondria-targeted yellow fluorescent protein (MT-YFP) driven by Pro35S was introduced into *Arabidopsis thaliana* ecotype Columbia (WT). The transgenic *Arabidopsis* line harboring MT-YFP (MtY-WT) was grown vertically on ½-MS medium agar plates in chambers at 23°C under a 16-h light (6 a.m. to 10 p.m.)/8-h dark (10 p.m. to 6 a.m.) photoperiod under white fluorescent lamps (140 μmol m^{-2} s^{-1}). Fourteen-day-old seedlings including the roots were treated with CCCP or UV-B irradiation as described below. The fourteen-day-old seedlings were immersed in 10 mM MES-NaOH (pH 5.5) solution with or without 10 μM CCCP, and then returned to the growth chamber for 6 h. The non-treated or treated seedlings were immersed in 10 mM MES-NaOH (pH 5.5) containing 0.2 μM DALGreen (Dojindo) and incubated in light-tight box for 4 h at 23°C. Seedlings were then washed three times with 10 mM MES-NaOH (pH 5.5) and observed by LSCM.[4].

Fourteen-day-old seedlings including the roots were irradiated for 1 h with narrowband light with UV-B (1.5 W m^{-2}) supplied by a xenon light source [MAX-303 (UV-VIS); Asahi Spectra Co., Ltd.] filtered through a 280 nm band pass filter (LX0280; Asahi Spectra Co., Ltd.). After UV-B irradiation, the irradiated seedlings were incubated in the growth chamber for 5 h, and then immersed in 10 mM MES-NaOH (pH 5.5) containing 0.2 μM DALGreen and incubated in light-tight box for 4 h at 23°C. Seedlings were then washed three times with 10 mM MES-NaOH (pH 5.5) and observed by LSCM.[4]. Images of the late elongation zone and neighboring cells in the differentiation zone of the roots were acquired. Fluorescence images of DALGreen (excitation 405 nm, emission 500–550 nm), and of YFP (excitation 488 nm, emission 500–550 nm) were obtained.

References

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62 **Supplementary figure legends**

63 **Supplementary Figure 1. UV-B-induced cell death in leaf mesophyll of *Arabidopsis* lines.** UV-B-
64 induced cell death in chloroplast stroma–targeted (CT)-GFP-expressing transgenic lines of wild-type
65 Colombia (WT), *atg5* and *phr1* mutants, *atg5 phr1* double mutant, and AtPHR1-overexpressing
66 transgenic (AtPHR1ox) seedlings as observed by LSCM at 2 d after UV-B exposure (280 nm, 0.5 or
67 1.5 W m^{-2} for 1 h). Green, GFP; magenta, chlorophyll. Arrows indicate dead cells (entirely green due
68 to distribution of GFP throughout cells after vacuolar degradation). Scale bars, 20 µm. Proportion of
69 dead cells out of total cells per fixed area (see Materials and Methods) is shown in Fig. 1c.
70

71 **Supplementary Figure 2. Repair of CPDs in mitochondrial DNA induced by UV-B exposure by**
72 **CPD photolyase, PHR1.** Fourteen-day-seedlings of WT, *atg5*, and *phr1* were non-irradiated (No UV-
73 B) or exposed to UV-B (1.5 W m^{-2} for 1 h) (UV-B). UV-B-irradiated seedlings were returned to the
74 growth chamber with white light for repair of CPDs (UV-B + 10 h and UV-B + 24 h). To measure the
75 level of CPD photolyase-independent repair, UV-B-irradiated seedlings were placed in a light-tight
76 box and returned to the growth chamber (UV-B + 24 h Dark). Genomic DNA was isolated and digested
77 with restriction enzymes, then digested DNA was incubated with (+) or without (−) T4 endonuclease
78 V. The membrane was incubated with ^{32}P-labeled probes for mitochondrial-encoded *COX1* and
79 nuclear-encoded *PHT2;1*. Percentage (%) fragments with enzyme-sensitive sites were calculated as
80 the ratio of intensity of T4 endonuclease V-treated to the intensity of untreated DNA. Data are means ±
81 SE. Asterisks indicate significant difference (*$p < 0.05$). ns., not significant.

82

83 **Supplementary Figure 3. Induction of mitochondrial fragmentation by carbonyl cyanide m-**
84 **chlorophenyl hydrazone.** (a) Mt-WT and Mt-*atg5* were non-treated (control) or treated with 10 μM
85 carbonyl cyanide m-chlorophenyl hydrazone (CCCP), a mitochondrial uncoupler that results in rapid
86 and dramatic fragmentation of mitochondria. Laser scanning confocal microscopy images were taken
87 at 6 h after treatment. Magenta, chlorophyll; green, mitochondria. Images are representative of three
88 independent plants. Scale bars, 20 μm. (b) At 6 h after treatment, the number of mitochondria per cell
89 was determined by Imaris software (number of MT-GFP dots within each cell in a fixed area; see
90 Materials and Methods) (n = 6 leaves). (c) The volume of each mitochondrion was estimated by 3D
91 conversion of MT-GFP dots by Imaris software (n = 6 leaves). Data are means ± SE. Asterisks indicate
92 a significant difference based on one-way ANOVA (**$p < 0.01$, ***$p < 0.001$). ns., not significant.
93

94 **Supplementary Figure 4. More effective removal of CPDs from mitochondrial DNA in AtPHR1-**
95 **overexpressing line (AtPHR1ox).** Transgenic plant stably over-expressing *AtPHR1*was non-
96 irradiated (No UV-B) or exposed to UV-B (1.5 W m^{-2} for 1 h) (UV-B). UV-B-irradiated seedlings
97 were returned to the growth chamber with white light for repairing CPD (UV-B + 10 h and UV-B +
98 24 h). To measure the level of CPD photolyase-independent repair, UV-B-irradiated seedlings were
99 placed in a light-tight box and returned to the growth chamber (UV-B + 24 h Dark). Genomic DNA
100 was isolated and digested with restriction enzymes, then digested DNA was incubated with (+) or
101 without (−) T4 endonuclease V. The membrane was incubated with ^{32}P-labeled probes for
102 mitochondrial-encoded *COX1* and nuclear-encoded *PHT2;1*. Percentage (%) fragments with enzyme-
103 sensitive sites was calculated as the ratio of intensity of T4 endonuclease V-treated to the intensity of
104 untreated DNA. Data are means ± SE. Asterisks indicate significant difference (*$p < 0.05$). ns., not
105 significant. Data for WT are same as in Supplementary Figure 2.
106

107 **Supplementary Figure 5. Comparison of UV-B-induced mitochondrial deactivation between**
108 **WT and CPD photolyase-overexpressing AtPHRo*x* plants.** (a) Transgenic plants stably over-

109 expressing mitochondrial matrix MT-GFP (Mt-WT and Mt-AtPHR1ox) were non-irradiated or
110 exposed to UV-B (1.5 W m^{-2} for 1 h), then co-stained 24 h later with mitochondrial membrane
111 potential indicator TMRE. Laser-scanning confocal microscope images of leaf mesophyll cells are
112 shown. Images are representative of three independent experiments. Green, mitochondria; magenta,
113 chlorophyll; orange, active mitochondria. Scale bars, 10 μm. (b) Numbers of mitochondria (GFP dots)
114 or active mitochondria (TMRE-signal dots) per cell as counted with Imaris software (number of
115 inactive mitochondria = total number of mitochondria – number of active mitochondria). Images from
116 three replicates were used for calculations. Data represent means ± SE ($n \geq 20$ cells). Asterisks denote
117 significant differences based on two-way ANOVA (*$p < 0.05$, ***$p < 0.001$). ns, not significant.

118

119 **Supplementary Figure 6. Autophagosome activity in *Arabidopsis* plants treated with CCCP or**
120 **exposed to UV-B radiation.** Transgenic plants stably over-expressing mitochondria-targeted YFP
121 (MtY-WT) were non-treated (No Treatment) or treated with CCCP (10 μM for 6 h) (CCCP Treatment)
122 in the growth chamber, then stained with DALGreen 4 h later. MtY-WT were exposed to UV-B (1.5
123 W m^{-2} for 1 h). After UV-B irradiation, the irradiated seedlings were incubated in the growth chamber
124 for 5 h, and then stained with DALGreen 4 h later (UV-B). Laser - scanning confocal microscope
125 images of root cells. Fluorescence images of DALGreen (excitation 405 nm, emission 500–550 nm),
126 and of YFP (excitation 488 nm, emission 500–550 nm) were obtained. Images are representative of
127 three independent experiments. Yellow, mitochondria; Green, active autophagosome. Scale bars,
128 20 μm.

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