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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 BamHI. 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 $\left[\alpha^{-32}P\right] dCTP$ 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.

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27 Monitoring of autophagic flux

28 The mitochondria-targeted vellow fluorescent protein (MT-YFP) driven by Pro35S was introduced 29 into Arabidopsis thaliana ecotype Columbia (WT). The transgenic Arabidopsis line harboring MT-30 YFP (MtY-WT) was grown vertically on ¹/₂-MS medium agar plates in chambers at 23°C under a 16-31 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 32 µmol m⁻² s⁻¹). Fourteen-day-old seedlings including the roots were treated with CCCP or UV-B 33 irradiation as described below. The fourteen-day-old seedlings were immersed in 10 mM MES-NaOH 34 (pH 5.5) solution with or without 10 μ M CCCP, and then returned to the growth chamber for 6 h. The 35 non-treated or treated seedlings were immersed in 10 mM MES-NaOH (pH 5.5) containing 0.2 µM 36 DALGreen (Dojindo) and incubated in light-tight box for 4 h at 23°C. Seedlings were then washed 37 three times with 10 mM MES-NaOH (pH 5.5) and observed by LSCM.⁴.

38 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.] 39 40 filtered through a 280 nm band pass filter (LX0280; Asahi Spectra Co., Ltd.). After UV-B irradiation, 41 the irradiated seedlings were incubated in the growth chamber for 5 h, and then immersed in 10 mM 42 MES-NaOH (pH 5.5) containing 0.2 µM DALGreen and incubated in light-tight box for 4 h at 23°C. 43 Seedlings were then washed three times with 10 mM MES-NaOH (pH 5.5) and observed by LSCM.⁴. 44 Images of the late elongation zone and neighboring cells in the differentiation zone of the roots were 45 acquired. Fluorescence images of DALGreen (excitation 405 nm, emission 500-550 nm), and of YFP 46 (excitation 488 nm, emission 500–550 nm) were obtained.

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48 **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 μ m. Proportion of
69	dead cells out of total cells per fixed area (see Materials and Methods) is shown in Fig. 1c.
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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 \pm
81	SE. Asterisks indicate significant difference (* $p < 0.05$). ns., not significant.
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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 \pm 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 AtPHR1was 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 ³²P-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 \pm SE. Asterisks indicate significant difference (*p < 0.05). ns., not 105 significant. Data for WT are same as in Supplementary Figure 2.

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

109 expressing mitochondrial matrix MT-GFP (Mt-WT and Mt-AtPHR10x) 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 \pm SE ($n \ge 20$ cells). Asterisks denote 117 significant differences based on two-way ANOVA (*p < 0.05, ***p < 0.001). ns, not significant.

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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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