Materials and Methods

Plant materials and growth conditions

The following *Arabidopsis* mutants were used in this study: *gun1-1* (Susek et al., 1993), *gun5-1* (Mochizuki et al., 2001), *gun6-1D* (Woodson et al., 2011), the T-DNA insertion lines *ptm-1* (SALK_013123, Sun et al., 2011) and *ptm-2* (SALK_073799), together with wild-type (WT) *Arabidopsis* Col-0. For experiments in Kyoto, WT (Col-0) and *ptm-1* (*ptm-1* OL) lines were also obtained from Lixin Zhang (Chinese Academy of Sciences). A second allele of *gun1* (SAIL_742_A11) was obtained from the Arabidopsis Biological Resource Center. The presence of the T-DNA insertion was confirmed by PCR (see Supplemental Table S1 for primers), and by sequencing. This line has been described in previous studies as *gun1* (Sun et al., 2011), or *gun1-2* (Dietzel et al., 2015), and we re-named it here as *gun1-103* (with permission of the corresponding author of Dietzel et al., 2015) as *gun1-2* had been used previously (Koussevitzky et al., 2007).

For Norflurazon (NF) experiments in Southampton, four different growth conditions were used: (1) seeds were sown onto half-strength Linsmaier and Skoog (LS) medium (Melford Laboratories, Ipswich, UK) supplemented with 1% (w/v) sucrose and 0.8% (w/v) agar (pH5.7), with or without 5 µM NF, and grown in continuous low white light (25 µmol.m^{-2}.s^{-1}) for 7 d at 23 °C (Woodson et al., 2011; though note that Woodson et al used 0.6% (w/v) agar); (2) seeds were sown onto half-strength LS medium supplemented with 2% (w/v) sucrose and 0.8% (w/v) agar (pH 5.8), with or without 5 µM NF, and incubated in WL (120 µmol.m^{-2}.s^{-1}) for 2 h, then 4 d in the dark, followed by 3 d in WL, all at 23 °C (Sun et al., 2011); (3) seeds were sown onto half-strength Murashige and Skoog (MS) medium supplemented with 0.8% agar (pH 5.8), with or without 5 µM NF, and in the presence or absence of 1.5% (w/v) sucrose, and incubated in WL for 2 h, then 3 d in the dark, followed by 3 d in WL, all at 23 °C (McCormac and Terry, 2004); (4) seeds were sown onto half-strength Murashige and Skoog (MS) medium supplemented with 1% (w/v) agar (pH 5.8), with or without 1µM NF in the presence of 1 % (w/v) sucrose, and incubated

in WL for 2 h, then 2 d in the dark, followed by 3 d in WL (100 µmol.m^{-2}.s^{-1}), all at 22 °C.

For Lincomycin (Lin) experiments in Southampton, two different growth conditions were used: (1) seeds were sown onto half-strength Linsmaier and Skoog (LS) medium (Melford Laboratories, Ipswich, UK) supplemented with 2% (w/v) sucrose and 0.8% (w/v) agar (pH 5.8), with or without 0.5 mM Lin, incubated in WL for 2 h, and grown in continuous darkness for 5 d at 22 °C (Sun et al., 2011); (2) seeds were sown onto half-strength Murashige and Skoog (MS) medium supplemented with 1% (w/v) sucrose and 1% agar (pH 5.8), with or without 0.5 mM Lin and incubated 2 d in dark, followed by 3 d in WL, all at 22 °C. For experiments in Kyoto, seeds were sown onto MS medium supplemented with 2% (w/v) sucrose and 0.8% (w/v) agar (pH 5.8), with or without 2.5 µM NF or 560 µM Lin under continuous white light (100 µmol.m^{-2}·sec^{-1}) at 23°C.

DNA extraction, genotyping and sequencing

Genomic DNA was prepared from rosette leaves as described previously (Edwards et al., 1991). The *ptm-1* and *ptm-2* mutants were genotyped by PCR using primers listed in Supplemental Table S1. DNA fragments were analysed using QIAxcel system (Qiagen, Hilden, Germany). Amplicons were sequenced to confirm the precise T-DNA insertion sites, as shown in Supplemental Figure S1. *ptm-2* has at least two inverted T-DNA fragments, with left border (LB) sequences located at each end.

RNA extraction and qRT-PCR

Total RNA was extracted from whole seedlings using the Agencourt Chloropure System (Beckman Coulter, Miami, USA) following the manufacturer's instructions (Kyoto), or according to McCormac et al. (2001) (Southampton). cDNA was synthesised with oligo(dT)12-18 using Transcriptor first-strand synthesis kit (Roche, Basel, Switzerland) according to the manufacturer's instructions (Kyoto), or with oligo(dT) and random nonamer primers using the nanoScript2 reverse transcription kit (Primerdesign, Southampton, UK) according to the

manufacturer's instructions (Southampton). Quantitative real-time PCR (qRT-PCR) in Kyoto was performed using LightCycler 480 SYBR Green I Master (Roche) and a LightCycler 96 (Roche) with the following standard thermal profile: 95°C for 5 min, followed by 40 cycles of 95°C for 5 s, 55°C for 10 s, and 72°C for 20 s. qRT-PCR in Southampton was performed using PrecisionPLUS and PrecisionFAST Sybr Green mastermix (Primerdesign) and a StepOnePlus™ Real Time PCR System (Applied Biosystems, Foster City, USA), with the following thermal profile: 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min or 95 °C for 2 min, followed by 40 cycles of 95 °C for 3 s and 60 °C for 30 s when using the Fast Sybr Green mastermix. Primers used for qRT-PCR are listed in Supplemental Table S1 and further experimental details are provided in the accompanying MIQE checklist.

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