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1 Short title: *ptm* is not a *gun* mutant
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3 Corresponding author: Professor Matthew J. Terry
4 Biological Sciences
5 University of Southampton
6 Highfield Campus
7 Southampton SO17 1BJ, UK
8 Tel: +44 (0)23 80592030
9 e-mail: mjt@soton.ac.uk
10

11 Seedlings lacking the PTM protein do not show a *genomes uncoupled* 12 *(gun)* mutant phenotype[1]

13

14 Mike T. Page[2], Sylwia M. Kacprzak, Nobuyoshi Mochizuki, Haruko Okamoto, Alison G. Smith
15 and Matthew J. Terry*

16

17 Biological Sciences, University of Southampton, Southampton SO17 1BJ, UK (M.T.P., S.K.,
18 H.O., M.J.T.); Graduate School of Science, Kyoto University, Kyoto, Japan (N.M.); Institute for
19 Life Sciences, University of Southampton, Southampton SO17 1BJ, UK (H.O., M.J.T.) and
20 Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, UK (A.G.S.)

21

22 Summary sentence: The *ptm* mutant of Arabidopsis does not show a *genomes uncoupled*
23 mutant phenotype and PTM is therefore unlikely to function in chloroplast-to-nucleus
24 signalling as previously reported.

25

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30 [2]Present address: Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ,
31 UK.

32 *Address correspondence to mjt@soton.ac.uk

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34 Author contributions: M.J.T., A.G.S. and N.M. conceived the study; M.T.P, S.K. and N.M.
35 performed the analyses; M.T.P, S.K., H.O., N.M. and A.G.S. analysed and interpreted the data
36 and contributed to writing the article. M.J.T. analysed and interpreted the data and wrote
37 the article.
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39
40 Chloroplast development requires communication between the nucleus and the
41 developing chloroplast to ensure that this process is optimised (Jarvis and López-Juez, 2013;
42 Chan et al., 2016). This is especially true during de-etiolation as mis-regulation of chloroplast
43 development can lead to seedling death from photo-oxidative damage. Retrograde signalling
44 from the developing chloroplast (plastid) to the nucleus, which is termed biogenic signalling
45 (Pogson et al., 2008), can be revealed using either the bleaching herbicide Norflurazon (NF),
46 an inhibitor of carotenoid synthesis, or the plastid translation inhibitor, lincomycin (Lin) to
47 damage the plastid. Under these conditions there is a strong down regulation of hundreds of
48 nuclear genes (Koussevitzky et al., 2007; Aluru et al., 2009; Page et al., 2016). Despite
49 decades of research, the biogenic retrograde signalling pathway is still very poorly
50 understood. What we do know has mostly come from an innovative screen by the group of
51 Joanne Chory in which *genomes uncoupled* (*gun*) mutants were identified that retained
52 nuclear gene expression of chloroplast-related genes after NF treatment (Susek et al., 1993).
53 This screen now defines the *gun* phenotype: increased expression, compared to wild-type
54 (WT), of nuclear genes following chloroplast damage. In total six original *gun* mutants have
55 been described. GUN1 is a pentatricopeptide repeat protein with a still unknown function
56 (Koussevitzky et al, 2007). The other GUNs are all related to the tetrapyrrole pathway
57 (Mochizuki et al, 2001; Larkin et al, 2004; Woodson et al., 2011). Further analysis of these
58 mutants has supported the idea that tetrapyrroles are important for plastid signalling (Vinti
59 et al., 2000; Strand et al., 2003; Moulin et al., 2008; Mochizuki et al., 2008; Voigt et al., 2010)
60 and our current understanding is that the synthesis of heme by ferrochelatase 1 results in a
61 positive signal that promotes expression of nuclear-encoded chloroplast genes (Woodson et
62 al., 2011; Terry and Smith, 2013).

63 Additional mutants identified through screens for a *gun* phenotype are the blue-light
64 photoreceptor mutant *cry1* (Ruckle et al., 2007) and the *coe1* mutant lacking a functional
65 mitochondrial transcription termination factor 4 (Sun et al., 2015). A number of *happy on*
66 *norflurazon* (*hon*) mutants were also identified by screening seedlings grown on NF under
67 lower light intensities (Saini et al., 2011). This identified one *hon* mutation in the ClpR4
68 subunit of the chloroplast-localized Clp protease complex (Saini et al., 2011). Other mutants
69 with a *gun* phenotype have been identified *via* informed approaches to test potential
70 signalling components. These include the transcription factor mutants *abi4* (Koussevitzky et
71 al, 2007), *hy5* (Ruckle et al., 2007) and *glk1glk2* (Waters et al., 2009). Interestingly,
72 *GOLDEN2-LIKE* (*GLK*) overexpressing plants (Leister and Kleine, 2016) have also been
73 reported to show *gun* phenotypes, perhaps reflecting the complex relationship between the

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74 anterograde signals by which the nucleus controls chloroplast development and retrograde
75 signalling (Martin et al., 2016).

76 In 2011, Sun et al identified a PHD transcription factor associated with the
77 chloroplast envelope, called PTM, which they proposed mediates chloroplast signals to the
78 nucleus through cleavage in response to changes in plastid status. Accumulation of the N-
79 terminus of the protein in the nucleus would then inhibit nuclear gene expression.
80 Consistent with this, they reported that the *ptm* mutant has a *gun* phenotype with elevated
81 expression compared to WT of *Lhcb* on both NF and Lin. This was a significant result for the
82 field as it defined a mechanism for plastid signalling, and is unsurprisingly included in
83 numerous models for this pathway (*e.g*. Chan et al, 2016; Bobik and Burch-Smith, 2015;
84 Terry and Smith, 2013; Barajas-López et al, 2013). Subsequent studies from the same group
85 have suggested that PTM functions in retrograde signalling from the chloroplast to regulate
86 flowering under high light (Feng et al, 2016) and in the integration of light and chloroplast
87 retrograde signalling during de-etiolation (Xu et al, 2016). However, the demonstration that
88 PTM shows a *gun* phenotype and is involved in retrograde signalling has yet to be supported
89 by additional experimental data from other groups.

90 Given the potential importance of PTM for our understanding of plastid signalling
91 we have further examined the role of PTM in responses to NF and Lin in two different
92 laboratories. For the experiments at Southampton, it was necessary for us to isolate the
93 same insertional *ptm* mutant allele described in Sun et al (2011) from the SALK collection
94 because this was no longer available from the authors. Isolation of the *ptm* mutant for this
95 study, which we name here as *ptm-1*, is described in Figure S1. Analysis of gene expression
96 after NF treatment was then performed. As shown in Figure 1A, 5 µM NF treatment using
97 the experimental conditions (1% sucrose, 25 $\mu mol.m^{-2}.s^{-1}$ white light (WL) for 7 d) of
98 Woodson et al (2011) resulted in no change in gene expression for a suite of five
99 photosynthesis-related genes (including *LHCB2.1* used by Sun et al (2011) for their real-time
100 PCR experiments) in *ptm-1* compared to WT seedlings, whereas there was clear rescue of
101 gene expression in the control *gun5* and *gun6* mutants. Next we repeated the experiment
102 under identical conditions (2% sucrose, 4d dark followed by 3d 120 $\mu mol.m^{-2}.s^{-1}$ WL) to those
103 reported in Sun et al (2011). Under these conditions we also saw rescue of gene expression
104 in *gun5* and *gun6*, but not in *ptm-1* (Figure 1B). These studies were performed using *ADF2* as
105 a reference gene. To confirm that the lack of a *gun* phenotype in *ptm1* was not related to
106 the choice of reference gene, we also normalised the data using *YLS8*, which gave essentially
107 identical results (Figure S2). Finally, we examined expression under conditions we have

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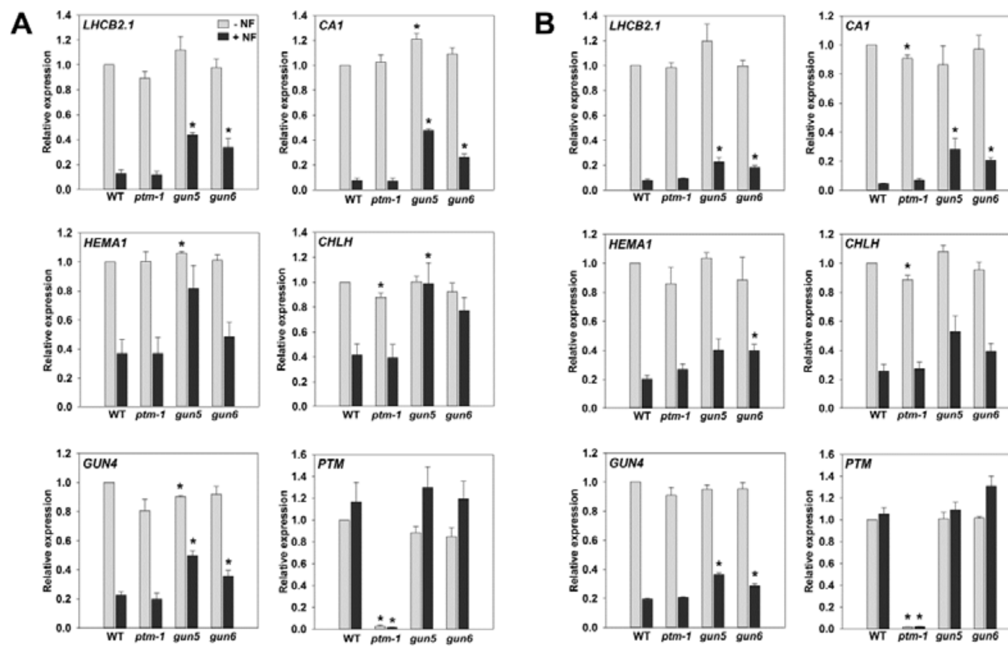

Figure 1. The *ptm-1* mutant does not show a *gun* phenotype on Norflurazon (NF). Seedlings were grown on half-strength Linsmaier and Skoog medium (A) supplemented with 1% sucrose and 0.8% agar (pH 5.7) with (dark grey bars) or without (light grey bars) 5 µM NF under continuous low white light (LWLc, 25 µmol m^{-2} s^{-1}) for 7 d, or (B) supplemented with 2% sucrose and 0.8% agar (pH 5.8) with (dark grey bars) or without (light grey bars) 5 µM NF under the following conditions: an initial 2 h WL treatment (120 µmol m^{-2} s^{-1}) to stimulate germination, 4 d dark, 3 d WLc (120 µmol m^{-2} s^{-1}). For (A) and (B), *genomes uncoupled 5* (*gun5*) and *gun6* mutants were included as positive controls (known to rescue nuclear gene expression on NF). Expression was determined with qRT-PCR and is relative to WT -NF and normalised to *ACTIN DEPOLYMERISING FACTOR 2* (*ADF2*, At3g46000). Data shown are the means +SEM of three independent biological replicates. Asterisks denote a significant difference *vs.* WT for the same treatment (-NF or +NF), Student's *t*-test ($p<0.05$).

108 previously described (McCormac and Terry, 2004). With 3d dark followed by 3d 120 µmol.m^{-2}.s^{-1} WL we also saw no *gun* phenotype for *ptm-1* either in the presence or absence of
110 sucrose (Figure S3). Only under one particular set of conditions did we see any indication of
111 a rescue of gene expression in *ptm-1* after NF treatment. Under these conditions (1%
112 sucrose, 2d dark followed by 3d 100 µmol.m^{-2}.s^{-1} WL with a lower NF concentration of 1 µM)
113 we saw a very small, but statistically significant increase for *LHCB2.1* and *HEMA1*, but not for
114 the other three genes tested (Figure S4). Given that under these conditions *gun1-1* rescue
115 was complete for both genes (>300% for *HEMA1*) we do not believe this one exception
116 supports a role for PTM in the plastid signalling pathway exposed by NF treatment.

117 The *ptm-1* mutant was also reported to result in elevated gene expression compared
118 to WT seedlings when grown on Lin (Sun et al., 2011). We therefore also tested *ptm-1* under
119 these conditions. As shown in Figure 2, *ptm-1* failed to result in elevated gene expression on
120 Lin while *gun1-1* (Koussevitzky et al., 2007) and *gun1-103* (see methods) control seedlings,
121 both showed strong rescue of gene expression (Figure 2). This was true whether seedlings
122 were grown in the dark (Figure 2A) or in the light (Figure 2B), and was independent of the
123 reference gene used (Figure S5).

124 To verify further whether we could detect a *gun* mutant phenotype for *ptm*
125 mutants, we also performed experiments in parallel in Kyoto. For this set of experiments

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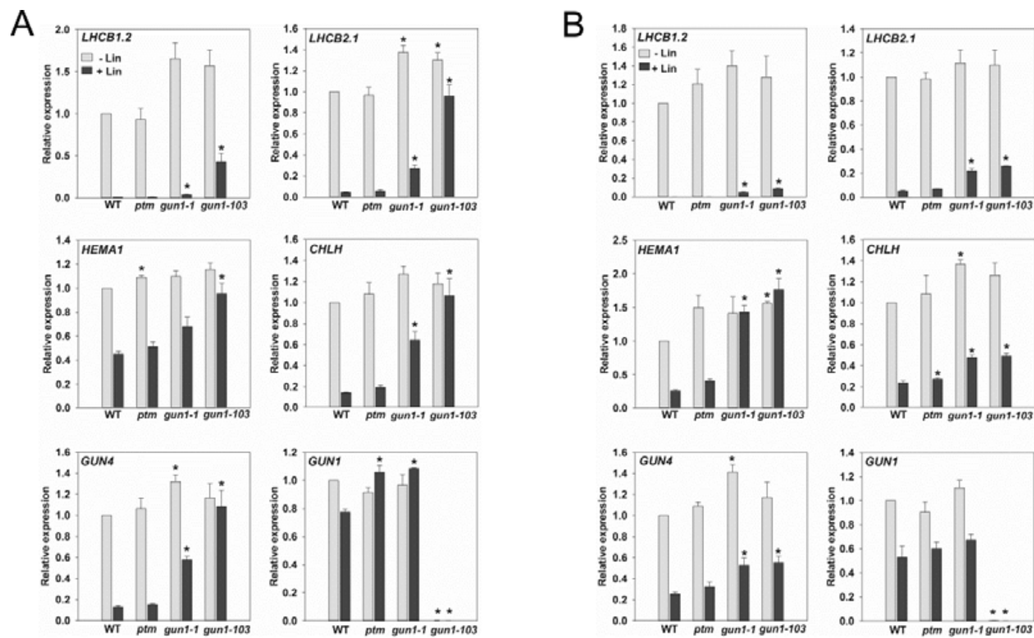

Figure 2. The *ptm* mutant does not show a *gun* phenotype on lincomycin (Lin). Seedlings were grown on half-strength Linsmaier and Skoog medium supplemented with 2% sucrose and 0.8% agar (pH 5.8) with (dark grey bars) or without (light grey bars) 0.5 mM Lin in dark for 5 d (A), or (B) on half-strength Murashige and Skoog medium supplemented with 1% sucrose and 1% agar (pH 5.8) with (dark grey bars) or without (light grey bars) 0.5 mM Lin under the following conditions: 2 d dark, 3 d Wlc (100 μmol $m^{-2}s^{-1}$). For (A) and (B), two alleles of *genomes uncoupled 1* (*gun1-1 and gun1-103*) mutants were included as positive control (known to rescue gene expression on Lin). Expression is relative to WT -Lin and normalised to *ACTIN2 (ACT2*, At3g18780*)* used in Sun et al (2011). Data shown are means +SEM of three independent biological replicates. Asterisks denote a significant difference *vs.* WT for the same treatment (-Lin or + Lin), Student's *t*-test ($p<0.05$).

126 two *ptm* alleles were used, the original *ptm* mutant (*ptm-1* OL) was obtained from Lixin
127 Zhang (CAS, Beijing; Sun et al., 2011) and independently from the SALK collection (*ptm-1*)
128 and, in addition, a second *ptm* allele, *ptm-2*, was also identified from the SALK collection (Fig
129 S1). As shown in Figure 3 none of the *ptm* mutants showed an elevation of *LHCB1.2*
130 (although the primer set used is also likely to detect *LHCB1.1* and *LHCB1.3)* or *LHCB2.1*
131 expression after NF or Lin treatment compared to WT, while a strong increase was observed
132 in the *gun1-1* control.

133 In conclusion, rigorous testing of the phenotype of *ptm* mutants on NF and Lin
134 shows that the *ptm* mutant does not show elevated expression of photosynthetic genes
135 compared to WT. This was true whether using the conditions described in the original
136 publication or other conditions used routinely to test plastid signalling responses. One
137 possible difference between our study and that of Sun et al (2011) is that they used RNA gel
138 blot analysis for most of their experiments. The probe used should preferentially detect
139 *LHCB1.1*, but might also be expected to detect *LHCB1.2* and *LHCB1.3,* and possibly other
140 *LHCB* genes. In our experiments we have tested both *LHCB1.1* and *LHCB1.2,* so it remains
141 possible that changes in another *LHCB* gene could account for the observed phenotype in
142 the original paper (Sun et al., 2011). However, Sun et al (2011) also reported the same gene
143 expression phenotype for *ptm* using real-time PCR and a primer pair that most closely
144 matches *LHCB2.1,* and we did not detect an increase in expression for this gene in our

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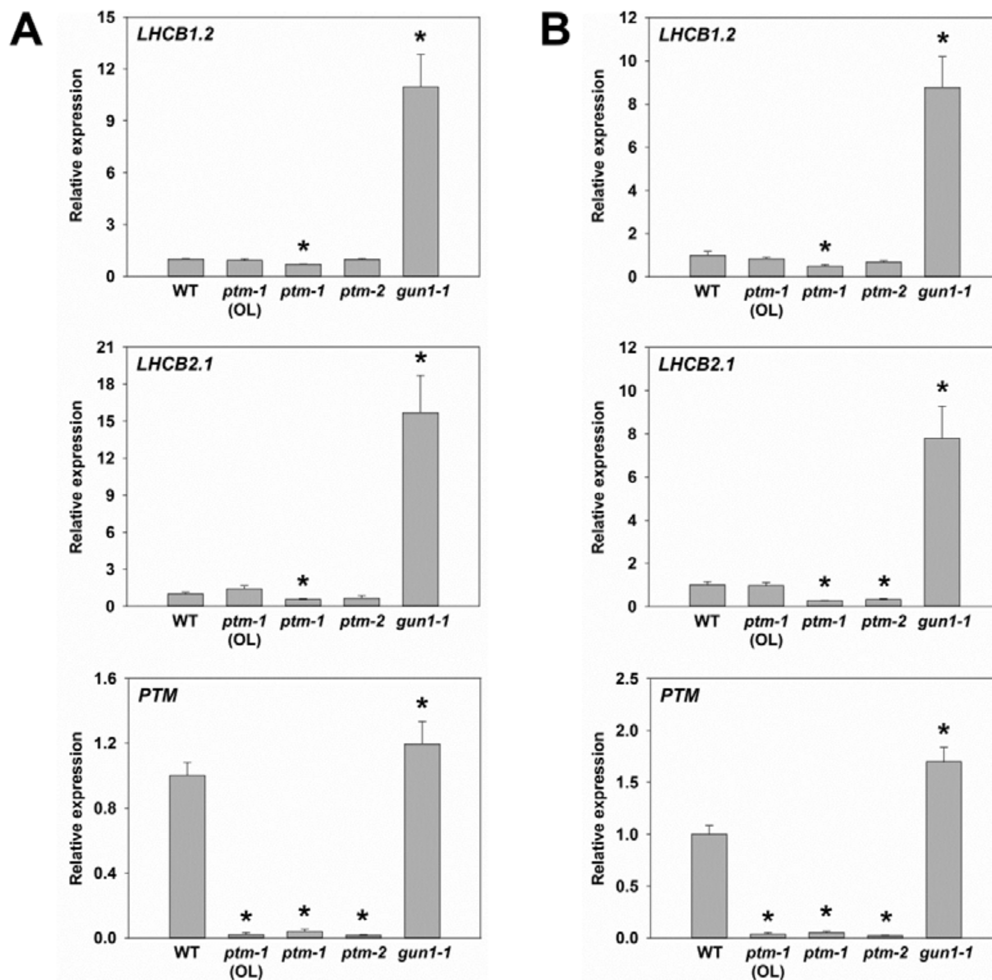

Figure 3. A second *ptm* mutant allele does not show a *gun* phenotype on Norflurazon (NF) or lincomycin (Lin). Seedlings were grown on Murashige and Skoog medium supplemented with 2% sucrose and 0.8% agar (pH 5.8), and either (A) 2.5 µM NF or (B) 560 µM Lin. All seedlings were grown under continuous white light (WLc, 100 µmol m^{-2} s^{-1}) for 4 d at 23 °C. Three *ptm* mutant lines were tested: *ptm-1* (OL) is the original line as used in Sun *et al*., 2011; *ptm-1* is the same insertion line as *ptm-1* (OL), Salk_013123, but obtained independently from the stock centre; *ptm-2* is a second insertion line, Salk_073799. The *genomes uncoupled 1-1* (*gun1-1*) mutant was included as a positive control (known to rescue nuclear gene expression on NF and Lin). Expression was determined with qRT-PCR and is relative to WT +NF and normalised to *TUBULIN BETA CHAIN 2* (*TUB2*, At5g62690). Data shown are the means +SEM of five independent biological replicates. Asterisks denote a significant difference *vs*. WT +NF, Student's *t*-test ($p<0.05$).

145 experiments (with one exception). We therefore believe it is unlikely that differences in
146 detection methods or genes tested can account for the observed differences in phenotype.
147 Moreover, if PTM is to be considered an important player in plastid signalling, the *gun*
148 phenotype of *ptm* should be robust enough to withstand this level of scrutiny. We have not
149 tested other results reported by Sun et al (2011). However, we note that the 3-fold elevation
150 of expression of *PTM* on NF measured using *PTM:GUS* was not apparent in our experiments
151 (Figures 1 and S3) and the reduction in *PTM* expression in *gun1* after NF and Lin treatment
152 was also not observed (Figure 3). In fact *PTM* expression was moderately (but significantly)

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153 elevated in *gun1-1* in our study (Figure 3). Whether our result has implications for other PTM
154 signalling roles (Feng et al., 2016; Xu et al., 2016) is currently unknown, but should be the
155 subject of further scrutiny.

156 The signalling pathway by which the status of the developing chloroplast is relayed
157 to the nucleus is one of the few remaining plant signalling pathways that we know of, but for
158 which we have little idea of the signalling components involved. We believe this study
159 resolves one of the major discrepancies in plastid signalling research by eliminating a major
160 role for PTM, and paves the way for more focussed studies that build on recent progress on
161 the role of tetrapyrroles and chloroplast protein homeostasis in plastid retrograde signalling
162 (Woodson et al., 2011; Murata et al., 2015; Ibata et al., 2016; Tadini et al., 2016).

163

164 **Supplemental data**

165 **Supplemental Table.** Primers used in this study.

166 **Supplemental Figure S1.** Characterisation of the *ptm* T-DNA insertion mutants

167 **Supplemental Figure S2.** The phenotype of *ptm-1* after NF treatment using the Sun et al
168 (2011) method normalised to *YLS8*

169 **Supplemental Figure S3.** The phenotype of *ptm-1* after NF treatment using the McCormac &
170 Terry (2004) method in the presence and absence of sucrose

171 **Supplemental Figure S4.** The phenotype of *ptm-1* after NF treatment using a modification of
172 the McCormac & Terry (2004) method in the presence of sucrose

173 **Supplemental Figure S5.** The phenotype of *ptm-1* after Lin treatment normalised to *YLS8*

174

175 **Acknowledgments**

176 We would like to thank Tania Garcia-Becerra for technical support. Thanks also to Joanne
177 Chory and Jesse Woodson (SALK Institute) for the *gun1-1*, *gun5* and *gun6* mutants used in
178 this study. N.M. thanks Lixin Zhang (Chinese Academy of Sciences) for the *ptm* (*ptm-1* OL)
179 mutant.

180

181 **Figure Legends**

182 **Figure 1**. **The *ptm-1* mutant does not show a *gun* phenotype on Norflurazon (NF).**
183 Seedlings were grown on half-strength Linsmaier and Skoog medium (A) supplemented with

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184 1% sucrose and 0.8% agar (pH 5.7) with (dark grey bars) or without (light grey bars) 5 µM NF
185 under continuous low white light (25 µmol.m^{-2}.s^{-1}) for 7 d, or (B) supplemented with 2%
186 sucrose and 0.8% agar (pH 5.8) with (dark grey bars) or without (light grey bars) 5 µM NF
187 under the following conditions: an initial 2 h WL treatment (120 µmol.m^{-2}.s^{-1}) to stimulate
188 germination, 4 d dark, 3 d WLc (120 µmol m^{-2} s^{-1}). For (A) and (B), *genomes uncoupled 5*
189 (*gun5*) and *gun6* mutants were included as positive controls (known to rescue nuclear gene
190 expression on NF). Expression was determined with qRT-PCR and is relative to WT -NF and
191 normalised to *ACTIN DEPOLYMERISING FACTOR 2* (*ADF2*, At3g46000). Data shown are the
192 means +SEM of three independent biological replicates. Asterisks denote a significant
193 difference *vs*. WT for the same treatment (-NF or +NF), Student's *t*-test ($p<0.05$).
194

195 **Figure 2. The *ptm* mutant does not show a *gun* phenotype on lincomycin (Lin).** Seedlings
196 were grown on half-strength Linsmaier and Skoog medium supplemented with 2% sucrose
197 and 0.8% agar (pH 5.8) with (dark grey bars) or without (light grey bars) 0.5 mM Lin in dark
198 for 5 d (A), or (B) on half-strength Murashige and Skoog medium supplemented with 1%
199 sucrose and 1% agar (pH 5.8) with (dark grey bars) or without (light grey bars) 0.5 mM Lin
200 under the following conditions: 2 d dark, 3 d WL (100 µmol.m^{-2}.s^{-1}). For (A) and (B), the
201 *genomes uncoupled, gun1-1* and *gun1-103* mutants were included as positive controls
202 (known to rescue gene expression on Lin). Expression is relative to WT -Lin and normalised
203 to *ACTIN2* (*ACT2*, At3g18780) used in Sun et al. (2011). Data shown are means +SEM of three
204 independent biological replicates. Asterisks denote a significant difference vs. WT for the
205 same treatment (-Lin or + Lin), Student's t-test ($p<0.05$).
206

207 **Figure 3. A second *ptm* mutant allele does not show a *gun* phenotype on Norflurazon (NF)**
208 **or lincomycin (Lin).** Seedlings were grown on Murashige and Skoog medium supplemented
209 with 2% sucrose and 0.8% agar (pH 5.8), and either (A) 2.5 µM NF or (B) 560 µM Lin. All
210 seedlings were grown under continuous white light (WLc, 100 µmol.m^{-2}.s^{-1}) for 4 d at 23 °C.
211 Three *ptm* mutant lines were tested: *ptm-1* (OL) is the original line as used in Sun *et al*., 2011;
212 *ptm-1* is the same insertion line as *ptm-1* (OL), Salk_013123, but obtained independently
213 from the stock centre; *ptm-2* is a second insertion line, Salk_073799. The *genomes*
214 *uncoupled 1-1* (*gun1-1*) mutant was included as a positive control (known to rescue nuclear
215 gene expression on NF and Lin). Expression was determined with qRT-PCR and is relative to
216 WT +NF and normalised to *TUBULIN BETA CHAIN 2* (*TUB2*, At5g62690). Data shown are the

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217 means +SEM of five independent biological replicates. Asterisks denote a significant
218 difference *vs*. WT +NF, Student's *t*-test (p<0.05).
219

220 **Supplemental Fig. S1**. **Characterisation of the *ptm* T-DNA insertion mutants.** (A) *PTM* gene
221 structure, with black boxes representing exons. The approximate location of the
222 Salk_013123 (*ptm-1*) and Salk_073799 (*ptm-2*) T-DNA inserts, genotyping primers (LB, LP, RP)
223 and qRT-PCR primers (qF1, qR1, qF2, qR2) are indicated. The *ptm-2* mutant has tandem T-
224 DNA insertions with a 24 bp deletion, in which the LB primer binding site is located at each
225 end of the tandem insertion. Precise T-DNA insertion sites in (B) *ptm-1* and (C) *ptm-2* as
226 revealed by sequencing. For (B) and (C) amino acid single letter codes are given above DNA
227 sequences, with the T-DNA sequences underlined in black. Sequence is given from the LP
228 and RP sides of the *ptm-2* T-DNA insertion in (C), to demonstrate the site of the 24 bp
229 deletion (underlined in red in the WT sequence). (D) PCR genotyping of *ptm-1* and *ptm-2*
230 mutants. Primers shown in (A) were used to amplify the following: *ptm-1* - WT band (LP1 +
231 RP1, predicted size 1,098 bp) and mutant band (LB + RP1, predicted size 687 bp); *ptm-2* - WT
232 band (LP2 + RP2, predicted size 1,142 bp) and two mutant bands (LB + RP2, predicted size
233 661 bp, and LB + LP2, predicted size 904 bp). MW = molecular weight marker. (E) Expression
234 of *PTM* in WT and *ptm-1* seedlings as determined by qRT-PCR. This analysis was repeated
235 under the conditions used in this study: the growth conditions in McCormac & Terry, 2004
236 (white bars), Sun *et al*., 2011 (grey bars) and Woodson *et al*., 2011 (black bars), all in the
237 absence of NF. Expression is relative to WT for each condition and normalised to *ACTIN*
238 *DEPOLYMERISING FACTOR 2* (*ADF2*, At3g46000). Data represent the mean + SEM of three
239 independent biological replicates, asterisks indicate a significant difference *vs*. WT (p < 0.05,
240 Student's *t*-test).
241

242 **Supplemental Fig. S2**. **Normalisation of expression data to a different reference gene does**
243 **not reveal a *gun* phenotype for *ptm-1*.** Seedlings were grown on half-strength Linsmaier and
244 Skoog medium supplemented with 2% sucrose and 0.8% agar (pH 5.8) with (dark grey bars)
245 or without (light grey bars) 5 µM NF under the following conditions: an initial 2 h WL
246 treatment (120 $\mu mol.m^{-2}.s^{-1}$) to stimulate germination, 4 d dark, 3 d WLc (120 $\mu mol\ m^{-2}\ s^{-1}$).
247 *genomes uncoupled 5* (*gun5*) and *gun6* mutants were included as positive controls (known
248 to rescue nuclear gene expression on NF). Expression was determined with qRT-PCR and is
249 relative to WT -NF and normalised to *YELLOW LEAF SPECIFIC GENE 8* (*YLS8*, At5g08290).
250 Data shown are the means +SEM of three independent biological replicates. Asterisks

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251 denote a significant difference *vs*. WT for the same treatment (-NF or +NF), Student's *t*-test
252 (p<0.05).
253

254 **Supplemental Fig. S3**. **Growth under a third set of conditions fails to find a *gun* phenotype**
255 **in *ptm-1*.** Seedlings were sown onto half-strength Murashige and Skoog medium
256 supplemented with 0.8% agar (pH 5.8) with (dark grey bars) or without (light grey bars) 5 µM
257 NF, and either in the presence (A) or absence (B) of 1% sucrose. For (A) and (B), seedlings
258 were grown under the following conditions: an initial 2 h WL treatment (120 $\mu mol.m^{-2}.s^{-1}$) to
259 stimulate germination, 3 d dark, 3 d WLc (120 $\mu mol.m^{-2}.s^{-1}$). *genomes uncoupled 5* (*gun5*)
260 and *gun6* mutants were included as positive controls (known to rescue nuclear gene
261 expression on NF). Expression was determined with qRT-PCR and is relative to WT -NF and
262 normalised to *ACTIN DEPOLYMERISING FACTOR 2* (*ADF2*, At3g46000). Data shown are the
263 means +SEM of three independent biological replicates. Asterisks denote a significant
264 difference *vs*. WT for the same treatment (-NF or +NF), Student's *t*-test (p<0.05).
265

266 **Supplemental Figure S4. The *ptm* mutant shows a very weak *gun* phenotype for some**
267 **genes under low (1 µM) Norflurazon (NF).** Seedlings were grown on half-strength
268 Murashige and Skoog medium supplemented with 1% sucrose and 1% agar (pH 5.8) with
269 (dark grey bars) or without (light grey bars) 1 µM NF under the following conditions: 2 d dark,
270 3 d WLc (100 $\mu mol.m^{-2}.s^{-1}$). The *genomes uncoupled 1* (*gun1-1*) mutant was included as
271 positive control (known to rescue gene expression on NF). Expression is relative to WT -NF
272 and normalised to *YELLOW LEAF SPECIFIC GENE 8 (YLS8,* At5g08290). Data shown are means
273 +SEM of three independent biological replicates. Asterisks denote a significant difference vs.
274 WT for the same treatment (-NF or + NF), Student's t-test (p<0.05).
275

276 **Supplemental Figure S5. The *ptm* mutant does not show a *gun* phenotype on lincomycin**
277 **(Lin).** Seedlings were grown on half-strength Linsmaier and Skoog medium supplemented
278 with 2% sucrose and 0.8% agar (pH 5.8) with (dark grey bars) or without (light grey bars) 0.5
279 mM Lin in dark for 5 d (A), or (B) on half-strength Murashige and Skoog medium
280 supplemented with 1% sucrose and 1% agar (pH 5.8) with (dark grey bars) or without (light
281 grey bars) 0.5 mM LIN under the following conditions: 2 d dark, 3 d Wlc (100 $\mu mol.m^{-2}.s^{-1}$).
282 For (A) and (B), two alleles of *genomes uncoupled 1* (*gun1-1* and *gun1-103*) mutants were
283 included as positive control (known to rescue gene expression on Lin). Expression is relative

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284 to WT -Lin and normalised to *YELLOW LEAF SPECIFIC GENE 8 (YLS8,* At5g08290). Data shown
285 are means +SEM of three independent biological replicates. Asterisks denote a significant
286 difference vs. WT for the same treatment (-Lin or + Lin), Student's t-test ($p<0.05$).
287
288

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