

1 Running Title:

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3 Functional redundancy of Trx *f* and NTRC systems

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40 **Thioredoxin *f1* and NADPH-dependent thioredoxin**
41 **reductase C have overlapping functions in regulating**
42 **photosynthetic metabolism and plant growth in response**
43 **to varying light conditions**

44
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56 One Sentence Summary:

57 There is a previously unrecognized communication between light and NADPH
58 dependent thiol-redox systems in regulating photosynthetic metabolism and growth in
59 response to varying light conditions.

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61 Footnotes:

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70 The author responsible for distribution of materials integral to the findings presented
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74 **ABSTRACT**

75

76 Two different thiol-redox-systems exist in plant chloroplasts, the ferredoxin-
77 thioredoxin system, which depends of ferredoxin reduced by the photosynthetic
78 electron-transport chain and, thus, of light, and the NADPH-dependent thioredoxin
79 reductase C (NTRC) system, which relies on NADPH and thus may be linked to
80 sugar metabolism in the dark. Previous studies suggested therefore that the two
81 different systems may have different functions in plants. We now report that there is a
82 previously unrecognized functional redundancy of thioredoxin-*f1* and NTRC in
83 regulating photosynthetic metabolism and growth. In *Arabidopsis* mutants, combined
84 - but not single - deficiencies of thioredoxin-*f1* and NTRC led to severe growth
85 inhibition and perturbed light acclimation, accompanied by strong impairments of
86 Calvin-Benson-cycle activity and starch accumulation. Light-activation of key-
87 enzymes of these pathways, fructose-1,6-bisphosphatase and ADP-glucose
88 pyrophosphorylase, was almost completely abolished. The subsequent increase in
89 NADPH/NADP⁺ and ATP/ADP ratios led to increased nitrogen assimilation, NADP-
90 malate dehydrogenase activation and light-vulnerability of photosystem I core-
91 proteins. In an additional approach, reporter studies show that Trx *f1* and NTRC
92 proteins are both co-localized in the same chloroplast substructure. Results provide
93 genetic evidence that light and NADPH dependent thiol-redox systems interact at the
94 level of thioredoxin-*f1* and NTRC to coordinately participate in the regulation of
95 Calvin-Benson-cycle, starch metabolism and growth in response to varying light
96 conditions.

97

98 **INTRODUCTION**

99

100 Reversible disulfide-bond formation between two cysteine residues regulates
101 structure and function of many proteins in diverse organisms (Cook and Hogg, 2013).
102 Thiol-disulfide exchange is controlled by thioredoxins (Trx), which are small proteins
103 containing a redox-active disulfide group in their active site (Holmgren, 1985;
104 Baumann and Juttner, 2002). The latter can be reduced to a dithiol by Trx reductases
105 using NADPH or ferredoxin (Fdx) as electron donors. Due to their low redox midpoint
106 potential, reduced Trxs are able to reductively cleave disulfide-bonds in many target
107 proteins and, thus, modulate their functions.

108

109 Plants contain the most versatile Trx system found in all organisms with respect to
110 the multiplicity of different isoforms and reduction systems (Buchanan and Balmer,
111 2005; Nikkanen and Rintamäki, 2014; Geigenberger and Fernie, 2014). The
112 Arabidopsis genome contains a complex family of Trxs, including up to 20 different
113 isoforms grouped into seven subfamilies (Schuermann and Buchanan, 2008; Dietz
114 and Pfannschmidt, 2011). Trxs *f*1-2, *m*1-4, *x*, *y*1-2 and *z* are located exclusively in the
115 chloroplast, Trx *o* exclusively in the mitochondria, while the eight Trx *h*
116 representatives are distributed between the cytosol, nucleus, endoplasmic reticulum
117 (ER), and mitochondria (Meyer et al., 2012). The different Trxs can be reduced by
118 two different redox systems, dependent on Fdx and Fdx-Trx reductase (FTR) in the
119 chloroplast or NADPH and NADPH-Trx reductase (NTRA and NTRB) in other cell
120 compartments (Buchanan and Balmer, 2005). More recently, a third type of NADPH-
121 Trx reductase (NTRC) has been identified which forms a separate Trx system in the
122 chloroplast (Serrato et al., 2004; Perez-Ruiz et al., 2006). NTRC is a bimodular
123 enzyme containing both an NTR and Trx domain on a single polypeptide (Serrato et
124 al., 2004). Its catalytic unit is a homodimer, transferring electrons from NTR to Trx
125 domains via inter-subunit pathways (Pérez-Ruiz and Cejudo, 2009). In vitro studies
126 suggest that NTRC is a Trx with its own Trx reductase, since it has not been shown
127 to interact with other free Trxs (Perez-Ruiz et al., 2006; Bohrer et al., 2012).

128

129 In chloroplasts, Trxs are reduced via FTR in a light-dependent manner, using
130 photosynthetic electrons provided by Fdx. The Fdx-Trx system with Trxs *f* and *m* was
131 originally discovered as a mechanism for the regulation of the Calvin-Benson cycle,

132 ATP synthesis and NADPH export in response to light-dark changes (Buchanan et
133 al., 1979; Buchanan, 1980). In numerous biochemical studies performed in vitro, the
134 roles of Trxs *f* and *m* were extended to the regulation of many other chloroplast
135 enzymes involved in various pathways of primary metabolism (Buchanan and
136 Balmer, 2005; Meyer et al., 2012). In-vitro experiments with purified proteins revealed
137 differences in biochemical specificities to different types of Trxs. Enzymes of the
138 Calvin-Benson cycle were found to be exclusively regulated by *f*-type Trxs (Collin et
139 al., 2003; Michelet et al., 2013; Yoshida et al. 2015), while key-enzymes involved in
140 related pathways such as starch synthesis (Fu et al., 1998; Ballicora et al., 2000;
141 Geigenberger et al., 2005; Thormählen et al., 2013), starch degradation (Mikkelsen et
142 al., 2005; Valerio et al., 2011; Seung et al., 2013; Silver et al., 2013), fatty acid
143 synthesis (Sasaki et al., 1997), amino acid synthesis (Lichter and Häberlein, 1998;
144 Choi et al., 1999; Balmer et al., 2003), chlorophyll synthesis (Ikegamie et al. 2007;
145 Luo et al. 2012), NADPH export (Collin et al., 2003; Yoshida et al. 2015) and
146 oxidative pentose-phosphate pathway (Nee et al. 2009) were found to be regulated
147 by both Trxs *f* and *m*, with *f*- being in most cases more effective than *m*-type. Other
148 plastidial isoforms belonging to the *x*- and *y*-types were found to be essentially
149 involved in antioxidant functions, being efficient electron donors to 2-Cys
150 peroxiredoxins (Prxs) and Prx Q, respectively, but unable to activate carbon
151 metabolism enzymes (Collin et al., 2003; Collin et al., 2004). The biochemical
152 properties of Trx *z* are not fully resolved yet. While this new type of Trx has been
153 identified to be part of the plastid-encoded RNA polymerase, implicating a role in the
154 transcription of the plastome (Arsova et al., 2010), it has also been found to act as an
155 electron donor for several antioxidant enzymes, indicating a role in plastid stress
156 responses (Chibani et al., 2011).

157

158 While most of the results mentioned above are based on biochemical studies, little is
159 known about the in-vivo relevance and specificity of the different chloroplast Trxs
160 isoforms in planta. Recent progress in this area was made by using reverse genetic
161 studies, including Arabidopsis mutants and transgenic plants. Intriguingly, these
162 genetic studies revealed specific roles of *m*-type Trxs in regulating photosynthetic
163 electron transport and developmental processes, rather than its expected roles in
164 primary metabolism. Arabidopsis lines with combined under-expression of Trxs *m1*,
165 *m2* and *m4* were defective in the biogenesis of photosystem II (Wang et al., 2013),

166 single mutants with deletions in Trx *m4* were affected in alternative photosynthetic
167 electron transport pathways (Courteille et al., 2013), while deletions in Trx *m3*
168 affected meristem development (Benitez-Alfonso et al., 2009). In addition to this,
169 Arabidopsis mutants with deletions in Trx *f1* leading to a more than 97% decrease in
170 Trx *f* protein level showed alterations in diurnal starch accumulation, rather than any
171 changes in photosynthetic parameters and growth (Thormählen et al., 2013). This is
172 surprising, given the exclusive regulation of individual steps of the carbon fixation
173 cycle by Trx *f1* in vitro (Collin et al., 2003; Michelet et al., 2013).

174

175 Compared to the Fdx-Trx system, relatively little is known on the more recently
176 identified chloroplast NADPH dependent NTRC system, which uses NADPH as a
177 source of electrons. So far only a few targets have been identified to be regulated by
178 NTRC, with 2-Cys Prxs involved in H₂O₂-detoxification (Perez-Ruiz et al., 2006),
179 ADPGlc pyrophosphorylase (AGPase) the key enzyme of starch biosynthesis
180 (Michalska et al., 2009; Lepisto et al., 2013) and enzymes of chlorophyll biosynthesis
181 (Richter et al., 2013; Perez-Ruiz et al., 2014) being the most elaborated ones.
182 Regulation of these processes by NTRC was confirmed in planta by analyzing an
183 insertional knockout mutant of NTRC, revealing (i) a decreased 2-Cys-Prx redox-
184 status and impaired H₂O₂-detoxification (Perez-Ruiz et al., 2006), (ii) an attenuation
185 of redox-activation of AGPase and starch accumulation (Michalska et al., 2009;
186 Lepisto et al., 2013), and (iii) impaired GluTR1, CHLM and Mg-chelatase activities
187 together with decreased chlorophyll levels (Richter et al., 2013; Perez-Ruiz et al.,
188 2014). Since most of these effects were operational in the dark, this suggests a role
189 of NTRC to regulate these pathways independently of light. In addition to the
190 NADPH-dependent NTRC system, 2-Cys Prx and AGPase have also been found to
191 be regulated by the light-dependent Fdx-Trx system with Trx *x* (Collin et al. 2003;
192 Bohrer et al. 2012) and Trx *f1* (Thormählen et al., 2013), respectively. However, little
193 is known on the interrelation of light and NADPH dependent chloroplast redox
194 systems in regulating these targets.

195

196 In this report, the interrelation between Trx *f1* and NTRC in regulating plant
197 metabolism and growth was investigated by using a genetic approach. Analysis of an
198 Arabidopsis *trxf1 ntrc* double mutant shows that combined inactivation of Trx *f1* and
199 NTRC leads to a strong inhibition in light-activation of the Calvin-Benson cycle and

200 related metabolic activities resulting in a severe limitation of growth, while these
201 responses were not or only weakly expressed in the single mutants. Reporter studies
202 show that both Trx *f1* and NTRC are expressed in the same tissues during
203 development and are co-localized in the same chloroplast substructure. This provides
204 evidence for a previously unknown redundant function of Trx *f1* and NTRC in
205 regulating photosynthetic metabolism and growth in response to varying light
206 conditions.

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209 RESULTS

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211 Combined inactivation of Trx *f1* and NTRC leads to a severe growth phenotype

212

213 To analyze the interrelation between Trx *f1* and NTRC in regulating growth and
214 metabolism of *Arabidopsis* plants, the well-characterized *trxf1* (SALK_128365;
215 Thormählen et al., 2013) and *ntrc* (SALK_012208; Serrato et al., 2004; Pérez-Ruiz et
216 al., 2006) T-DNA insertion lines were crossed to generate a *trxf1 ntrc* double mutant.
217 A homozygous *trxf1 ntrc* line was identified, where T-DNA insertions were present in
218 both genomic alleles (Fig. 1A), while protein content of both Trx *f1* and NTRC were
219 strongly decreased to detection limit (Fig. 1B). In comparison to this, expression of
220 NTRC and Trx *f1* was still detectable in the *trxf1* and *ntrc* single mutants,
221 respectively, although Trx *f1* protein levels were found to be slightly lower in the *ntrc*
222 background than in the wild-type (Fig. 1B). In the Western blots of Fig. 1B, a Trx *f*
223 antibody was used that gives similar signals with Trx *f1* and Trx *f2* (Thormählen et al.,
224 2013), indicating that Trx *f1* is the predominant Trx *f* isoform in *Arabidopsis*.

225

226 As previously reported, *trxf1* (Thormählen et al., 2013) and *ntrc* single mutants
227 (Perez-Ruiz et al., 2006; Lepisto et al., 2013) showed no or moderate growth
228 phenotypes, respectively, when grown in a 8 h photoperiod at 160 $\mu\text{mol photons m}^{-2}$
229 s^{-1} light intensity (Fig. 2B). In contrast to this, growth of the *trxf1 ntrc* double mutant
230 was very severely perturbed, when compared to the wild-type or the single mutants
231 (Fig. 2B). The rosette fresh-weights of the *trxf1 ntrc* double mutant decreased to
232 below 2% of wild-type level, while those of the *ntrc* mutant decreased to 25% and
233 those of the *trxf1* mutant remained unaltered (Fig. 2H). Despite this very strong
234 growth defect, *trxf1 ntrc* mutant plants were viable and produced seeds under these
235 conditions (Fig. 2G). Interestingly, the extent of the growth phenotypes differed
236 depending on the length of the photoperiod and the light intensity (Figs. 2 A-F). When
237 the length of the photoperiod was decreased from 8 to 4 h light, rosette fresh-weights
238 decreased significantly to 80% and 15% of wild-type level in the *trxf* and *ntrc* single
239 mutants, respectively, and to levels below the detection limit in the *trxf1 ntrc* double
240 mutant (Fig. 2H). Conversely, an increase in the length of the photoperiod from 8 to
241 24 h led to a partial relieve in the growth retardation of both the *ntrc* and the *trxf1 ntrc*
242 mutant. In the *ntrc* mutant, rosette fresh-weights increased from 25 to 50% of wild-

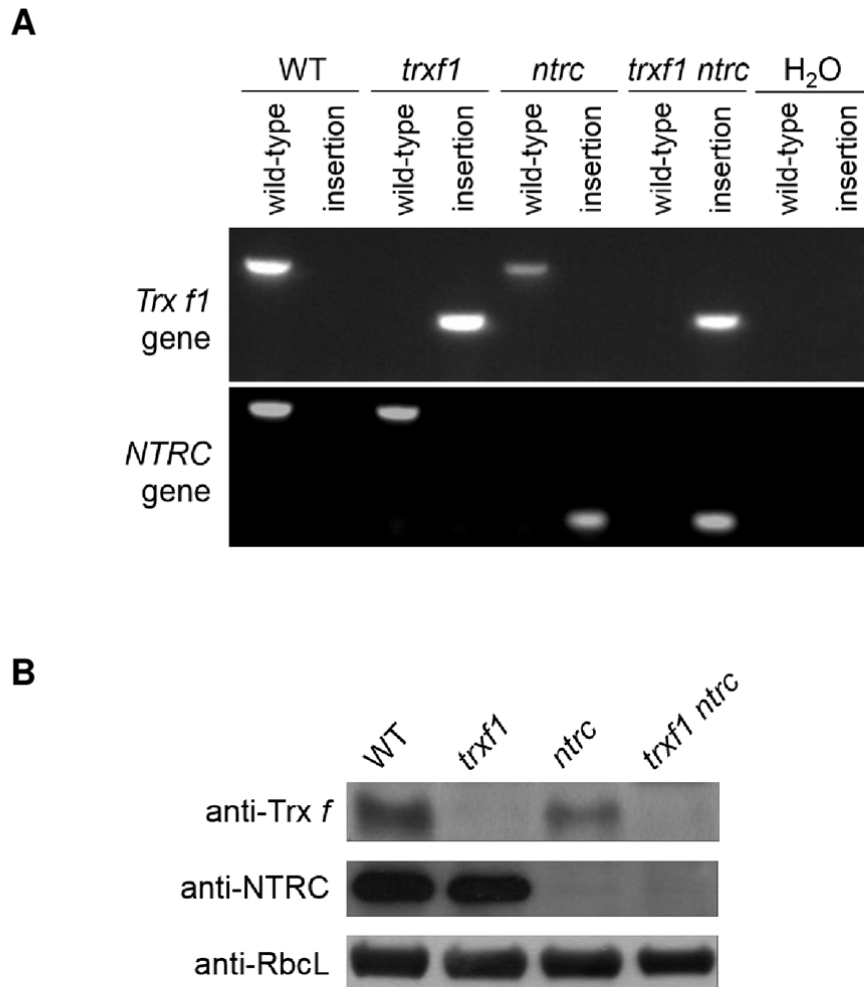


Figure 1: Molecular characterization of *trxf1*, *ntrc* and *trxf1 ntrc* Arabidopsis mutants compared to wild-type. **(A)** Genotyping by PCR analysis with different primer combinations (wild-type or insertion) for the identification of T-DNA insertions in *Trx f1* and *NTRC* genes. **(B)** Detection of *Trx f* and NTRC proteins using Western blot analysis. Representative Western blots are shown of measurements, which were performed in leaves of 5-week old plants grown in an 8h-day with 160 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ light regime harvested 4 h into the light period. Rubisco protein level is shown as control.

243 type level in 16 h and 24 h, compared to 8 h photoperiods (Fig. 2H), in agreement
 244 with previous studies (Lepisto et al., 2009). The *trxf1 ntrc* double mutant showed no
 245 significant change in fresh-weight when the photoperiod was increased from 8 h to
 246 16 h light, but there was an increase from 1 to 3% of wild-type level when the
 247 photoperiod was increased from 16 h to 24 h light (Fig. 2H). Also a change in the light

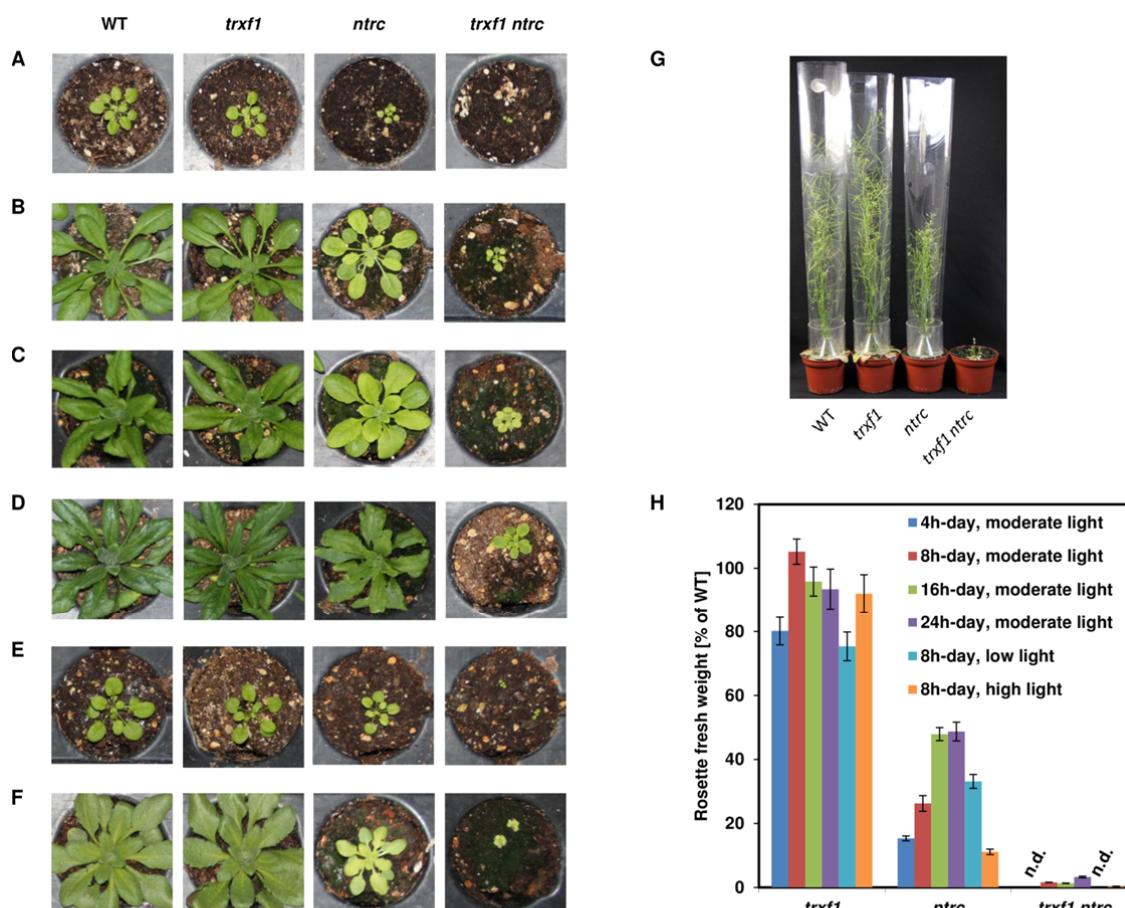


Figure 2: Growth analysis of wild-type, *trx1*, *ntrc* and *trx1 ntrc* Arabidopsis mutants across different light conditions. (A), (B), (E) and (F) correspond to 5 week-old plants, while (C) and (D) correspond to 4 week-old plants, and (G) to 7 week-old plants. In the first week, plants - except (G) - were grown in an 8 h-day and moderate light intensity regime before they were transferred for additional 3-4 weeks to the conditions indicated below: (A) 4 h-day and moderate light intensity, (B) 8 h-day and moderate light intensity, (C) 16 h-day and moderate light intensity, (D) 24 h-day and moderate light intensity, (E) 8 h-day and low light intensity, (F) 8 h-day and high light intensity, and (G) 16 h-day in greenhouse. In (H), rosette fresh-weights of plants corresponding to the conditions shown in (A) to (F) are given as percent of wild-type levels in the respective conditions. Results are the mean \pm SE, $n = 30$ -86 (wild-type), 15-44 (*trx1*), 5-44 (*ntrc*) or 9-111 (*trx1 ntrc*) different plants. All values are significantly different from wild-type according to the Student's *t*-test ($P < 0.05$), except the *trx1* mutant in 8 h-, 16 h- and 24 h-day regimes at moderate and high light intensities (see Suppl. Table S1). Low light intensity = $30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$; moderate light intensity = $160 \mu\text{mol photons m}^{-2} \text{s}^{-1}$; high light intensity = $950 \mu\text{mol photons m}^{-2} \text{s}^{-1}$; n.d. = not detectable (fresh-weight values were below the detection limit)

248 intensity at 8 h-photoperiod affected the growth phenotype of the mutants. When the
 249 light intensity was decreased from $160 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ to $30 \mu\text{mol photons m}^{-2}$
 250 s^{-1} , the rosette fresh weights dropped significantly to 75 and 35% of wild-type level in
 251 the *trx1* and *ntrc* mutants, respectively, and to levels below the detection limit in the
 252 *trx1 ntrc* double mutant, (Fig. 2H). When the light intensity was increased from 160 to
 253 $950 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, rosette fresh-weights dropped severely in the *trx1 ntrc*
 254 mutant, moderately in the *ntrc* mutant, while no effect was observed in the *trx1*
 255 mutant, compared to wild-type (Fig. 2H). Overall, the results show that knockout of
 256 Trx *f1* leads to a severe growth inhibition in the *ntrc*, but not in the wild-type
 257 background, suggesting a functional redundancy of both redox systems.
 258

259 **Combined deficiency of Trx f1 and NTRC leads to a strong impairment of**
260 **photosynthesis**

261

262 To investigate whether the severe growth phenotype of the *trxf1 ntrc* mutant is due to
263 an effect on photosynthesis, CO₂ assimilation rates were measured in leaves of the
264 different genotypes grown in an 8 h photoperiod at 160 μmol photons m⁻² s⁻¹ light
265 intensity using an open gas-exchange system. The light response-curves at ambient
266 CO₂ are shown in Fig. 3A. At light intensities between 150 and 1000 μmol photons m⁻²
267 s⁻¹, CO₂ fixation rates were strongly decreased in the *trxf1 ntrc* mutant relative to the
268 wild-type, the decrease being light intensity-dependent: 80% at 150, 60% at 200-300,
269 50% at 400-600, and 33% at 800-1000 μmol photons m⁻² s⁻¹. At light intensities
270 between 50-100 μmol photons m⁻² s⁻¹, CO₂ assimilation rates were below the
271 respiration rate in the double mutant, but not in the wild-type, the light compensation
272 point switching from 20 μmol photons m⁻² s⁻¹ in the wild-type to 120 μmol photons m⁻²
273 s⁻¹ in the double mutant. In the dark, CO₂ release rates were 4-fold higher in the
274 double mutant compared to wild-type. In contrast to the double mutant, the single
275 mutants showed no (*trxf1*) or only slight changes (*ntrc*) in CO₂ assimilation rates,
276 compared to wild-type. Deletion of NTRC led to a slight decrease in CO₂-assimilation
277 rates at all light intensities, which was statistically significant at 50 and 200 μmol
278 photons m⁻² s⁻¹ using the Student's *t*-test (Suppl. Table S2) and for all light intensities
279 using the two-way Anova test (Fig. 3A), confirming previous studies (Lepisto et al.,
280 2009). Overall, the results show that inactivation of Trx f1 led to a strong decrease in
281 CO₂ fixation rates in the *ntrc*, but not in the wild-type background, lending further
282 support to the proposal of a functional redundancy of both systems to regulate the
283 CO₂ fixation rate.

284

285 In Fig. 3B, leaf transpiration rates are shown across different light intensities and
286 genotypes. Compared to wild-type, there was a strong (up to 8-fold) increase in
287 transpiration rates in the *trxf1 ntrc* mutant, while the single mutants behaved like the
288 wild-type at all light conditions tested. Similar results were observed for stomal
289 conductance (data not shown) and intercellular CO₂ concentration (Fig. 3C), both
290 parameters being strongly increased in the double mutant relative to the wild-type.
291 These results show that the lower rate of CO₂ fixation caused by the combined
292 deficiency of Trx f1 and NTRC is not due to a restriction in CO₂ uptake rates or a

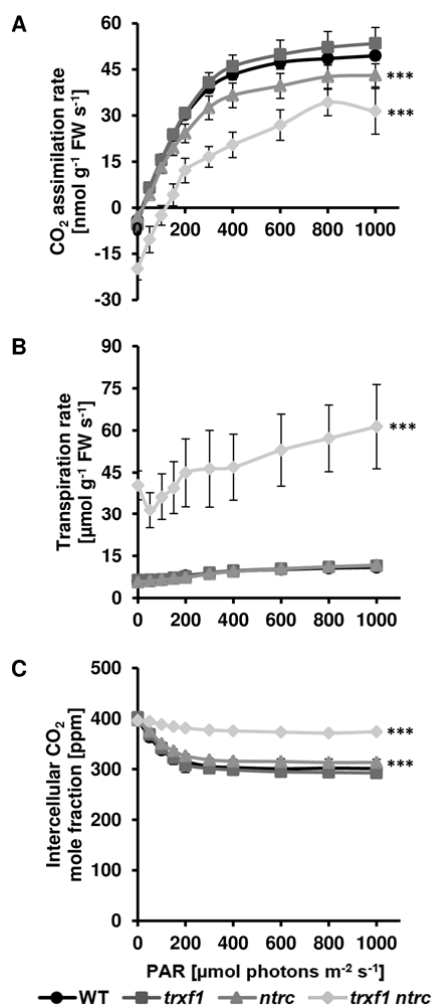


Figure 3: Changes in gas-exchange parameters in leaves of *trxf1*, *ntrc* and *trxf1 ntrc* Arabidopsis mutants compared to wild-type. **(A)** CO₂ assimilation rate, **(B)** transpiration rate, and **(C)** intercellular CO₂ concentration were measured at different light intensities in leaves from plants growing in an 8 h photoperiod with 160 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Results are the mean \pm SE, $n = 10$ (wild-type) or 5 (mutants) different plant replicates. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ (according to two-way analysis of variance [Anova]), Tukey test); for further statistical analysis see Suppl. Table S2;

PAR = photosynthetic active radiation

293 decrease in internal CO₂ concentrations, but most likely to a direct inhibition of the
 294 CO₂-fixation cycle.

295

296 To investigate whether the inhibition of CO₂-assimilation is accompanied by changes
 297 in photosynthetic light reactions, chlorophyll fluorescence parameters were measured
 298 by pulse-amplitude modulation (PAM) fluorimetry. A significant decrease of maximal
 299 (F_v/F_m) and effective quantum yield of PS II (Φ_{PSII}) was observed in the double
 300 mutant relative to the single mutants or the wild-type (Figs. 4A and 4B), indicating
 301 that the combined deficiency of Trx *f1* and NTRC led to a strong impairment of PSII
 302 functionality and photosynthetic electron transport rates. Correspondingly, quantum
 303 yields of regulated (Φ_{NPQ}) and non-regulated (Φ_{NO}) energy dissipation (Fig. 4B)
 304 were strongly increased in the double mutant. Similar to previous studies (Lepisto et
 305 al., 2009; Thormählen et al., 2013), no changes in chlorophyll fluorescence
 306 parameters were found in the *trxf1* mutant, while the *ntrc* mutant revealed moderate
 307 but significant changes in Φ_{PSII} and Φ_{NPQ} , relative to the wild-type (Fig. 4B).

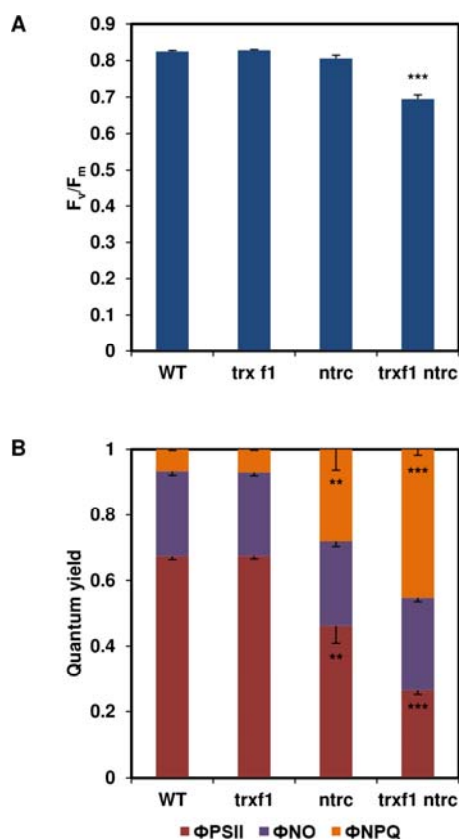


Figure 4: Changes in chlorophyll fluorescence parameters in leaves of *trx1*, *ntrc* and *trx1 ntrc* Arabidopsis mutants compared to wild-type. Plants growing in an 8 h photoperiod with 160 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ were dark adapted for 10 min, before exposure of a far red light saturation pulse (5,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 0.8 s) to single leaves. Afterwards the maximal chlorophyll a fluorescence was quenched by electron transport with an actinic red light of 166 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Within 10 min the steady state was reached and another saturation pulse was given. In the end, **(A)** the maximal PSII (F_v/F_m), and **(B)** the effective PSII (Φ_{PSII}), the non-regulated energy dissipation (Φ_{NO}) and the regulated energy dissipation (Φ_{NPQ}) quantum yields were calculated. Results are means \pm SE, $n = 11$ different plants.

*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ (according to Student's *t*-test)

308

309 We further investigated whether impaired photosynthetic light reactions were
 310 accompanied by decreased abundance of proteins involved in photosynthetic
 311 electron transport (Fig. 5). Western blot analyses showed that combined deficiency of
 312 Trx *f1* and NTRC led to a strong decrease in proteins of the PSI complex (PsaA and
 313 PsaB) down to approx. 25% of wild-type level and to more moderate decreases in
 314 proteins of PSII (PsbD), cytochrome b6-f (PetC), light-harvesting (Lhca1 and Lhcb1)
 315 and ATPase complexes (Atp β). In comparison to this, the *trx1* and *ntrc* single
 316 mutants were only weakly affected. Chlorophyll content was slightly decreased in the
 317 *trx1* mutant, and down to 55 and 45% of wild-type level in the *ntrc* single and *trx1*
 318 *ntrc* double mutant, respectively (Suppl. Fig. S1). This confirms previous studies
 319 showing chlorophyll levels to be decreased by 50% in the *ntrc* mutant relative to the
 320 wild-type (Perez-Ruiz et al., 2006; Lepisto et al., 2009), while a combined deficiency
 321 of Trx *f1* and NTRC only led to minor additional effects (Suppl. Fig. S1).

322

323 **Combined deficiency of Trx *f1* and NTRC affects NADP reduction and adenylate**
 324 **energy states**

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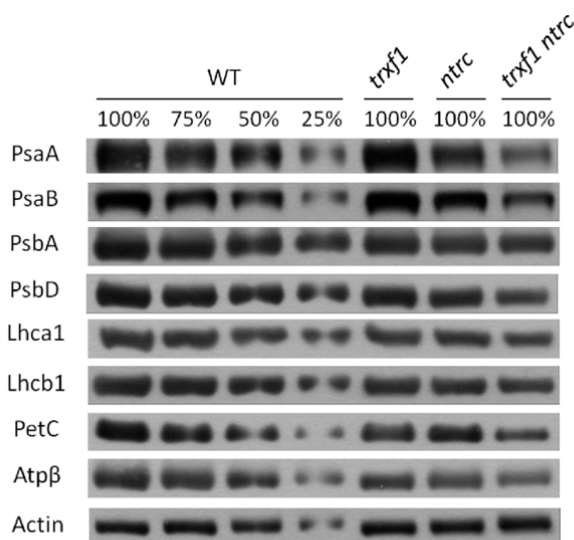


Figure 5: Changes in the levels of proteins involved in photosynthetic electron transport and ATP synthesis in leaves of *trxf1*, *ntrc* and *trxf1 ntrc* Arabidopsis mutants compared to wild-type. PsaA, PsaB, PsbA, PsbD, PetC, Lhca1, Lhcb1 and Atp β proteins were detected using specific antibodies. Representative Western blots are shown from 5-week old plants growing in an 8h-day with 160 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ light regime harvested 4 h into the light period. In the wild-type, different amounts of samples were loaded (25-100%) for comparison. Actin protein level is shown as control.

326 The Calvin–Benson cycle uses most of the ATP and NADPH delivered by the
 327 photosynthetic light reactions (Michelet et al., 2013). To investigate the relationship
 328 between photosynthetic activity and the function of the Calvin-Benson cycle, the
 329 levels of NAD(P)H, NAD(P)⁺, ATP and ADP were analyzed in leaves of wild-type and
 330 the different redox mutants (Fig. 6). In wild-type plants, the sum of NADPH and
 331 NADP⁺ increased at the end of the day relative to the end of the night (Fig. 6A), while
 332 the NADPH/NADP⁺ ratio decreased (Fig 6B), confirming previous studies (Liu et al.,
 333 2008; Beeler et al., 2014; Lintala et al., 2014). The light-induced decrease in the
 334 NADPH/NADP⁺ ratio is probably attributable to the Calvin-Benson cycle being
 335 activated under these conditions. In the *trxf1 ntrc* mutant, the diurnal changes in the
 336 sum of NADPH and NADP⁺ levels were strongly attenuated (Fig. 6A), while there was
 337 a clear increase in the NADPH/NADP⁺ ratio at the end of the day (3-fold) and at the
 338 end of the night (2-fold), compared to wild-type (Fig. 6B). No changes were observed
 339 in the *trxf1* mutant while in the *ntrc* mutant the NADPH/NADP⁺ ratio was slightly but
 340 significantly increased (Figs. 6A and 6B). The wild-type also showed diurnal changes
 341 in the sum of NADH and NAD⁺ (Fig. 6C) and in the NADH/NAD⁺ ratio (Fig. 6D), with
 342 the former decreasing and the latter increasing towards the end of the day. In the
 343 *trxf1 ntrc* mutant, the sum of NADH and NAD⁺ was further decreased, while the
 344 NADH/NAD⁺ ratio further increased, compared to wild-type.

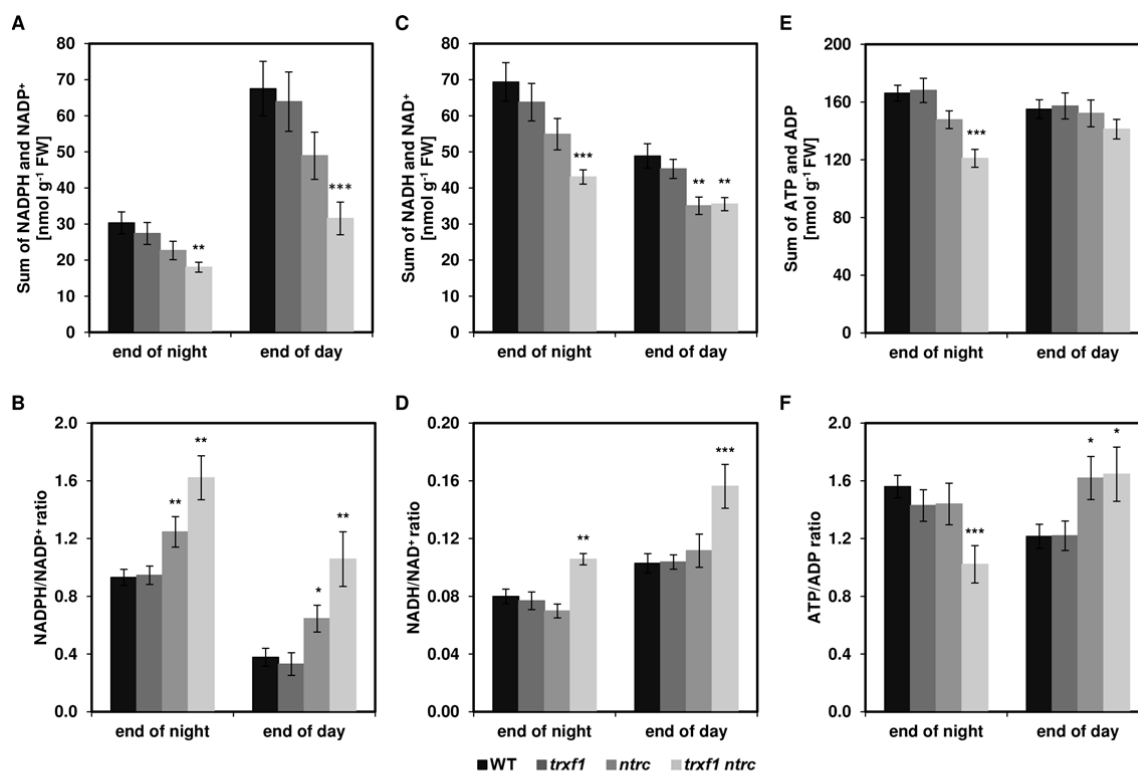


Figure 6: Changes in nucleotide levels in leaves of *trxf1*, *ntrc* and *trxf1 ntrc* Arabidopsis mutants compared to wild-type. (A) Sum and NADPH and NADP⁺, (B) NADPH/NADP⁺ ratio, (C) sum of NADH and NAD⁺, (D) NADH/NAD⁺ ratio, (E) sum of ATP and ADP, and (F) ATP/ADP ratio were measured in leaves harvested at the end of day and end of night. Results are means \pm SE, $n = 20-30$ (wild-type) or 10-15 (mutants) independent plant replicates. Plants were grown in an 8 h photoperiod with $160 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ (according to Student's *t*-test).

345

346 In the wild-type, the diurnal changes in NADP redox state were accompanied by
 347 corresponding changes in adenylate energy state, with ATP/ADP ratios being
 348 decreased at the end of the day as compared to the end of the night (Fig. 6F). A
 349 similar decrease in the ATP/ADP ratio from 1.5 to 1.2 in response to light was found
 350 in previous studies with leaves of wild-type Arabidopsis plants (Carrari et al. 2005).
 351 This is most probably attributable to light-activation of the Calvin-Benson cycle and
 352 other ATP-consuming biosynthetic processes. In the *trxf1 ntrc* mutant, diurnal
 353 changes in both adenylate levels (Fig. 6E) and ATP/ADP ratios (Fig. 6F) were
 354 opposite to the wild-type, being increased during the day relative to the night, while
 355 the *trxf1* and *ntrc* single mutants were largely similar to wild-type (Figs. 6E and 6F).

356

357 Overall these results show that a combined deficiency of Trx *f1* and NTRC causes
 358 major alterations in both NADPH/NADP⁺ and ATP/ADP ratios during the day,
 359 indicating that the primary cause for the strong impairment of photosynthesis is an
 360 inhibition of the Calvin-Benson cycle rather than the light reactions.

361

362 **Combined deficiency of Trx *f1* and NTRC strongly impairs redox-activation of**

363 **fructose-1,6-bisphosphatase while having no inhibitory effect on redox-**
364 **activation of NADP-malate dehydrogenase**

365

366 The above described results show that the combined deficiency of Trx *f1* and NTRC
367 causes impairment of photosynthetic parameters, the diurnal oscillation of energy
368 availability and carbon fixation rate. We then analyzed whether this could be due to
369 direct effects on enzymes of the Calvin-Benson cycle or NADPH export from the
370 chloroplast. To this end we focused on FBPase and NADP-MDH, representing key-
371 regulatory steps of these processes and classical targets of Trx *f* and Trx *m*,
372 respectively (Buchanan et al. 1979). Chloroplast FBPase (cpFBPase) is known to be
373 subject to exquisite light-activation via the Fdx-Trx *f* system, leading to reduction of
374 an intra-molecular disulfide that promotes activation of the enzyme (Zimmermann et
375 al., 1976; Buchanan et al. 1979). To analyze the effect of a combined deletion of
376 NTRC and Trx *f1* on redox regulation of FBPase, the redox status of the chloroplast
377 enzyme was analyzed in vivo by labeling of thiol groups with the alkylating agent MM-
378 PEG₂₄, which adds 1.5 kDa per thiol, thus causing a switch of the electrophoretic
379 mobility of the reduced form of the enzyme as compared to the oxidized form. In the
380 wild-type, cpFBPase protein was completely oxidized at the end of the night, while
381 more than 50% of the protein was in the reduced state at the end of the day (Fig.
382 7A), confirming light-induced reduction of its intra-molecular disulfide in vivo. This
383 response was strongly modified in the redox mutants. At the end of the day, the ratio
384 of reduced to oxidized cpFBPase protein was substantially decreased in *trxf1* and
385 *ntrc* single mutants relative to the wild-type, while there was an even stronger
386 decrease in the double mutant (Fig. 7A). No changes were observed between the
387 genotypes at the end of the night. Results from four independent experiments were
388 quantified and are summarized in Fig. 7B, showing that the ratio of reduced to
389 oxidized cpFBPase protein decreased significantly by 40% in *trxf1*, 20% in *ntrc* and
390 70% in *trxf1 ntrc* mutants relative to the wild-type, indicating an additive effect in the
391 double mutant. Finally, it was noticed that the content of cpFBPase protein in the
392 double mutant was slightly decreased as compared with the wild-type or single
393 mutants (Fig. 7A) suggesting minor effects on cpFBPase protein turnover in addition
394 to post-translational thiol-disulfide modulation.

395

396 We then investigated whether a deficiency in Trx *f1* and NTRC also affects FBPase

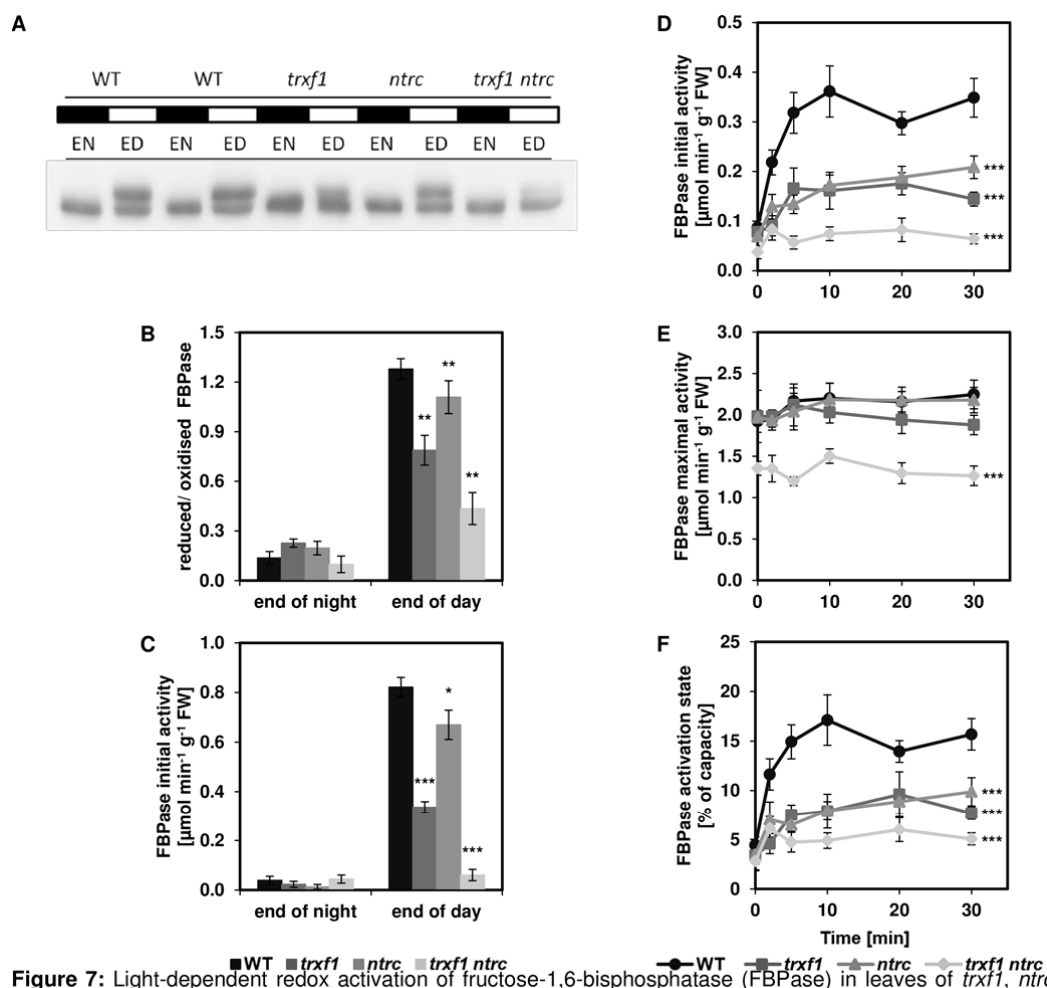


Figure 7: Light-dependent redox activation of fructose-1,6-bisphosphatase (FBPase) in leaves of *trxf1* and *trxf1 ntrc* Arabidopsis mutants compared to wild-type. **(A)** and **(B)** show the thiol-disulfide reduction state of chloroplast FBPase in leaves harvested at the end of night (EN) and end of day (ED) analyzed by using gel-shift assays: **(A)** Representative gel-shift blot using an antibody specific for chloroplast FBPase, and **(B)** calculated ratio of reduced to oxidized FBPase. **(C)** Corresponding initial enzyme activity of FBPase (assay without DTT) in leaves harvested at the end of night (EN) and end of day (ED). **(D)** to **(F)** Transient light activation of FBPase during a detailed time course. At the end of the night (0 min), plants were illuminated for different time-periods (2, 5, 10, 20 and 30 min) to measure FBPase activity using different assay conditions: **(D)** Initial activity without DTT additions in the assay, **(E)** maximal activity with 10 mM DTT included in the assay, and **(F)** estimated redox-activation state (initial/maximal activity*100). Results are means \pm SE, $n = 8$ (wild-type) or 4 (mutants) independent plant replicates **(B)**, $n = 20$ (wild-type) or 10 (mutants) independent plant replicates **(C)**, and $n = 10$ (wild-type) or 4-6 (mutants) independent plant replicates **(D)** to **(F)**. All plants were grown in an 8 h photoperiod with $160 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, according to Student's t -test **(B)** and **(C)** or two-way analysis of variance [Anova] Tukey test **(D)** to **(F)**, see Suppl Table S3. Estimation of the redox-activation state from enzyme assays **(F)** and the directly measured thiol-disulfide state of the protein **(B)** yielded different absolute values, which is most likely due to additional factors affecting FBPase activity during the enzyme assays.

397 enzyme activity. In leaves of the wild-type, FBPase activity was very low at the end of
 398 the night and increased 20-fold towards the end of the day (Fig. 7C), confirming
 399 previous studies on the light activation of cpFBPase (Zimmermann et al., 1976;
 400 Chiadmi et al., 1999). This response was strongly attenuated in the redox mutants. At

401 the end of the day, FBPase activity was progressively decreased down to 40, 80 and
402 7% of wild-type level in *trxf1*, *ntrc* and *trxf1 ntrc* mutants, respectively, while there
403 were no significant changes between these genotypes at the end of the night. In the
404 double mutant, no significant changes between nocturnal and day-time FBPase
405 activity were observed, indicating that light activation of FBPase has been abolished.
406 The data also show a correlation between FBPase activity and FBPase redox-state
407 across different genotypes and day-night conditions (compare Figs. 7B and 7C),
408 confirming the major role of thiol-disulfide modulation in regulating FBPase enzyme
409 activity in-vivo. It should be noted that the FBPase activity was measured in crude
410 extracts which will likely overestimate the residual chloroplastic activity in the dark, as
411 the redox-insensitive cytosolic FBPase will contribute to the measured activity.
412 However, analysis of Arabidopsis mutants lacking cytosolic FBPase show that under
413 these conditions 80% of the FBPase activity in crude leaf extracts is due to
414 chloroplastic cpFBPase (Rojas-Gonzalez et al. 2015).

415

416 To investigate transient light-activation of FBPase in a detailed time course, FBPase
417 activity was analyzed in leaves 0, 2, 5, 10, 20 and 30 min after illumination. As shown
418 in Fig. 7D, light led to a rapid increase in FBPase activity, reaching half-maximal
419 activity within 1 min after the start of illumination in the wild-type. Compared to wild-
420 type, the increase in FBPase activity was significantly delayed by approx. 50% in *trx*
421 *f1* and *ntrc* single mutants, reaching half-maximal activities 5 and 10 min after the
422 start of illumination, respectively. Between 10 and 30 min, FBPase activity showed no
423 further increase or increased only slightly, with *trxf1* and *ntrc* mutants both saturating
424 at approx. 50% of wild-type level. Intriguingly, combined deficiency of Trx *f1* and
425 NTRC led to a complete loss in light-activation of FBPase, with the double mutant
426 showing no significant increase in FBPase activity upon illumination. When FBPase
427 activity was measured in the presence of 10 mM DTT in the assay medium to fully
428 reduce the regulatory disulfide of the enzyme, no significant changes were detected
429 in the different genotypes and light conditions, except a slight decrease of the
430 maximal FBPase activity in the *trxf1 ntrc* mutant compared to wild-type and single
431 mutants (Fig. 7E). The ratio between the activities in the two assay conditions (minus
432 DTT versus plus DTT) is shown as a calculated redox-activation state in Fig. 7F. The
433 changes in the estimated redox-activation state followed similar curves as the initial
434 activities measured without DTT (compare Figs. 7D and 7F). The results show that

435 knockout of Trx *f1* led to a decreased efficiency in light activation of FBPase which is
436 in-line with earlier studies showing that chloroplast FBPase is redox-activated by *f*-
437 type Trx in vitro (Collin et al., 2003). However, deletion of Trx *f1* only led to a 50%
438 inhibition in FBPase activation, which is similar to the degree of inhibition in the *ntrc*
439 mutant, indicating that neither of these redox systems has an exclusive role in the
440 redox-regulation of FBPase in-vivo. Moreover, the almost complete loss of light-
441 dependent activation of FBPase in the *trxf1 ntrc* mutant strongly suggests the
442 cooperative effect of Trx *f* and NTRC in FBPase redox regulation. A comparison
443 between Figs. 7D and 7E also documents that the redox-sensitivity of the extracted
444 FBPase protein itself was not compromised in the mutants relative to the wild-type.

445

446 For comparative purposes, we also measured the activity of NADP-MDH, a
447 chloroplast enzyme involved in the export of NADPH to the cytosol via the malate
448 valve (Scheibe, 2004) and being subject to activation by Trxs *f* and *m* in-vitro (Collin
449 et al. 2003; Yoshida et al. 2015). In the wild-type, NADP-MDH initial activity was
450 higher at the end of the day compared to the end of the night (Fig. 8A), confirming
451 previous studies on the light-activation of this enzyme in the chloroplast stroma
452 (Scheibe, 2004). Interestingly, this response was promoted rather than inhibited in
453 the redox mutants. Compared to wild-type, *trxf1*, *ntrc* and *trxf1 ntrc* mutants showed
454 increased activation of NADP-MDH during the day, while there were no substantial
455 changes observed in the night. When NADP-MDH activity was measured in the
456 presence of 10 mM DTT in the assay medium to fully reduce the regulatory disulfides
457 of the enzyme, no substantial changes were detected across the different genotypes
458 and light conditions (Fig. 8B). The ratio between the activities in the two assay
459 conditions (minus DTT versus plus DTT) is shown as a calculated redox-activation
460 state (Fig. 8C). It followed a similar curve as the initial activities measured without
461 DTT (compare Figs. 8A and 8C). In the mutants, increased activation of NADP-MDH
462 is probably due to increased chloroplast NADPH/NADP⁺ ratios (Fig. 6), which
463 promote NADP-MDH redox-activation indirectly by acting on the redox-potential of its
464 regulatory disulfides (Faske et al., 1995).

465

466 **Combined deficiency of Trx *f1* and NTRC leads to decreased starch**
467 **accumulation and decreased redox-activation of ADPGlc pyrophosphorylase**

468

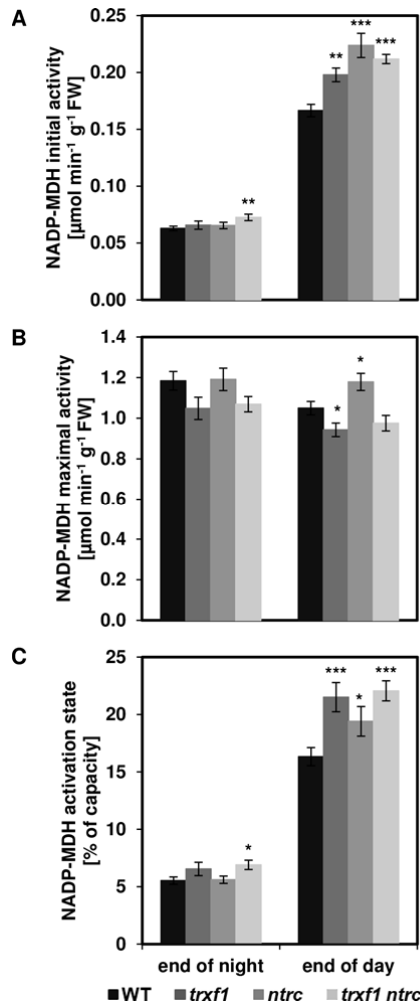


Figure 8: Light-dependent redox activation of NADP-dependent malate dehydrogenase (MDH) in leaves of *trxf1*, *ntrc* and *trxf1 ntrc* Arabidopsis mutants compared to wild-type. **(A)** Initial activity without DTT additions in the assay, **(B)** maximal activity with 10 mM DTT included in the assay, **(C)** redox-activation state (initial/maximal activity*100). Leaves were sampled at the end of night and end of day. Results are means \pm SE, $n = 24$ (wild-type) or 12 (mutants) independent plant replicates. Plants were grown in an 8 h photoperiod with $160 \mu\text{mol photons m}^{-2} \text{s}^{-1}$.

*: P<0.05, **: P<0.01, ***: P<0.001 (according to Student's t-test)

469 Following with our purpose of determining the function of Trx *f1* and NTRC in redox
 470 regulation of different carbon metabolic pathways, we investigated the effect of the
 471 combined deficiency of Trx *f1* and NTRC on the synthesis of photosynthetic end
 472 products, starch and sucrose. Wild-type leaves showed characteristic diurnal
 473 changes of starch (Fig. 9A) and sucrose levels (Fig. 9B), which increased by 3- and
 474 2-fold, respectively, towards the end of the day. These diurnal changes were
 475 attenuated in the redox-mutants. At the end of the day, *trxf1*, *ntrc* and *trxf1 ntrc*
 476 mutants showed a progressive decrease in starch accumulation down to 80, 65 and
 477 25% of wild-type levels, respectively (Fig. 9A), confirming previous studies showing
 478 attenuation of starch accumulation in *trxf1* (Thormählen et al., 2013) and *ntrc* single
 479 mutants (Michalska et al., 2009; Lepisto et al., 2013). The decrease in day-time
 480 starch content was additive in the double mutant (Fig. 9A), dropping to levels below
 481 those of the wild-type at the end of the night. At any time, the starch content in the
 482 double mutant did not exceed nocturnal wild-type levels. At the end of the night, *trxf1*,
 483 *ntrc* and *trxf1 ntrc* mutants showed a further progressive decrease in the remaining

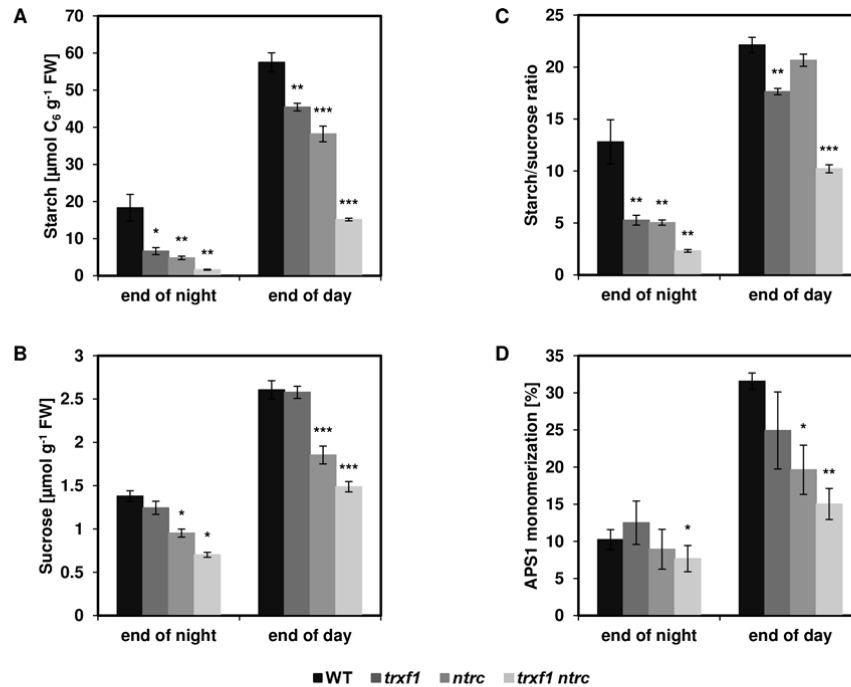


Figure 9: Changes in the accumulation of starch and sucrose and in the thiol-disulfide reduction state of the small subunits of ADP-glucose pyrophosphorylase (APS1) in leaves of *trxf1*, *ntrc* and *trxf1 ntrc* Arabidopsis mutants compared to wild-type. (A) Starch level, (B) sucrose level, (C) starch/sucrose ratio, and (D) APS1 monomerisation (APS1 monomer as percent of total APS1 [monomer + dimer]) were measured in leaves sampled at the end of night (EN) and end of day (ED). APS1 monomerisation was analyzed in non-reducing SDS gels, where reduced and oxidized APS1 can be separated as monomer and dimer, respectively. Results are means \pm SE, $n = 8$ (wild-type) or 4 (mutants) independent plant replicates growing in an 8 h photoperiod with $160 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ (according to Student's *t*-test)

484 starch content, reaching 35, 25 and less than 10% of the nocturnal wild-type level,
 485 respectively (Fig. 9A), showing that starch reserves were exhausted in the double
 486 mutant. The *trxf1 ntrc* mutant showed decreased accumulation of sucrose, which at a
 487 lower level was also affected in the *ntrc* mutant but not in the *trxf1* mutant (Fig. 9B).
 488 All mutants under analysis showed a decrease of the starch/sucrose ratio relative to
 489 the wild-type, the decrease being more pronounced in the *trxf1 ntrc* double mutant
 490 with 55% at the end of the day and 80% at the end of the night (Fig. 9C).

491

492 AGPase is a key-enzyme of starch synthesis, which is rapidly activated upon
 493 illumination by reduction of an intermolecular disulfide bond between the Cys-81
 494 residues joining the two small subunits (APS1) of this heterotetrameric enzyme
 495 (Hendriks et al., 2003; Kolbe et al., 2005; Hädrich et al. 2012). To investigate whether
 496 the inhibition of starch synthesis in the different genotypes is due to decreased
 497 redox-activation of AGPase, monomerisation of APS1 was analyzed in leaves
 498 harvested at the end of the night and at the end of the day. As seen in previous
 499 studies (Hendriks et al., 2003), wild-type leaves revealed a strong increase in the
 500 monomerization of APS1 during the day, while APS1 was almost completely
 501 dimerized in the night (Fig. 9D). Compared to wild-type, light-dependent

502 monomerisation of APS1 was attenuated in the *trxf1* and *ntrc* single mutants (Fig.
503 9D), confirming results from earlier studies (Michalska et al., 2009; Thormählen et al.,
504 2013). Compared to the single mutants, there was an additional attenuation of APS1
505 monomerisation in the *trxf1 ntrc* double mutant (Fig. 9D), indicating Trx *f*1 and NTRC
506 to act additively on the reduction of APS1 in vivo.

507

508 **Combined deficiency of Trx *f*1 and NTRC causes deep effects on metabolite**
509 **levels including an increase in amino acids at the expense of organic acids**

510

511 The above data clearly indicate the combined action of Trx *f* and NTRC on redox
512 regulation of different aspects of carbon metabolism. The impairment of the
513 regulation of the Calvin-Benson cycle and attendant starch synthesis in the mutants
514 under investigation is expected to provoke changes of in-vivo metabolite levels
515 indicative of regulatory steps in these pathways. In wild-type plants, the levels of 3-
516 phosphoglycerate (3PGA) (Fig. 10A), fructose 1,6-bisphosphate (FBP) (Fig. 10B) and
517 fructose 6-phosphate (F6P) (Fig. 10C) showed strong diurnal alterations, higher
518 levels being observed at the end of the day, which is in-line with the changes in
519 Calvin-Benson cycle activity. These diurnal changes in metabolite levels were
520 differentially modified in the *trxf1 ntrc* mutant. Compared to wild-type, the day-time
521 increase in the level of 3PGA, the first fixation product of Rubisco, was attenuated by
522 75% in the *trxf1 ntrc* mutant, while there were no changes in the *trxf1*, and only a
523 smaller decrease (by 35%) in the *ntrc* mutant (Fig. 10A). In contrast to this, the day-
524 time levels of FBP, the substrate of FBPase, were significantly increased by 50, 125
525 and 75% in *trxf1*, *ntrc* and *trxf1 ntrc* mutants, respectively (Fig. 10B), while those of
526 F6P, the product of FBPase, were only slightly increased in *trxf1* and *ntrc* single
527 mutants, or even decreased in the *trxf1 ntrc* double mutant, compared to wild-type
528 (Fig. 10C). Concerning the ratio between product and substrate of FBPase
529 (F6P/FBP), there was a significant and progressive decrease down to 80, 55 and
530 45% of wild-type level in *trxf1*, *ntrc* and *trxf1 ntrc* mutants, respectively (Fig. 10D),
531 indicating a progressive inhibition of plastidial FBPase in vivo and confirming the
532 decrease in FBPase activity and cpFBPase reduction state (Fig. 7). It should be
533 noted that whole-leaf levels of F6P and FBP were measured, which reflect the sum of
534 the chloroplastic and cytosolic pools, not just the chloroplastic pool. However, studies
535 with *Arabidopsis* mutants show that lack of chloroplastic FBPase leads to a decrease

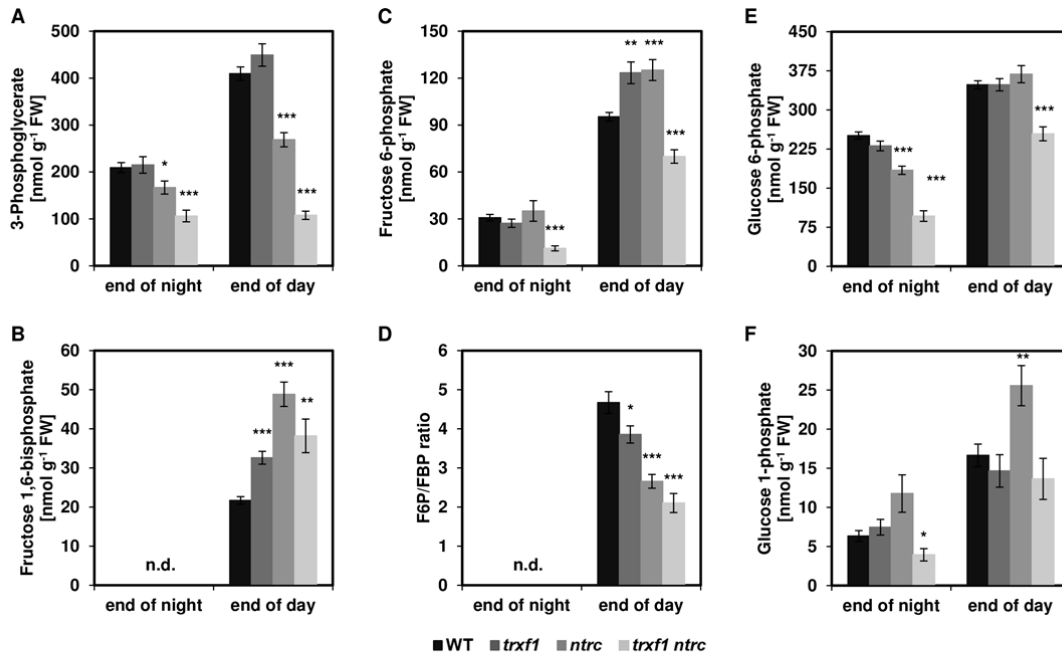


Figure 10: Changes in the in-vivo levels of phosphorylated intermediates in leaves of *trx1*, *ntrc* and *trx1 ntrc* Arabidopsis mutants compared to wild-type. (A) 3-phosphoglycerate (3PGA) level, (B) fructose 1,6-bisphosphate (FBP) level, (C) fructose 6-phosphate (F6P) level, (D) F6P/FBP ratio, (E) glucose 6-phosphate (G6P) level, and (F) glucose 1-phosphate (G1P) level were measured in leaves sampled at the end of the night and end of the day. Results are means \pm SE, $n = 20-30$ (wild-type) or 10-15 (mutants) independent plant replicates growing in an 8 h photoperiod with 160 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ (according to Student's *t*-test); n.d. = not detectable (values were below the detection limit)

536 in the overall F6P/FBP metabolite ratio, while there was no change in the F6P/FBP
 537 ratio in response to a lack of cytosolic FBPase (Rojas-Gonzalez et al. 2015). This
 538 demonstrates that a decrease in the overall F6P/FBP ratio is indicative for an
 539 inhibition of chloroplastic rather than cytosolic FBPase.

540

541 Compared to wild-type, glucose 1-phosphate (G1P), the substrate of AGPase,
 542 remained unchanged or increased slightly in the different genotypes, indicating that
 543 the inhibition of starch synthesis was unlikely to be due to a shortage of this substrate
 544 (Fig. 10F). At the end of the night, the levels of 3PGA (Fig. 10A) and hexose-
 545 phosphates (Figs. 10C, E, F) all showed a progressive decrease in *trx1*, *ntrc* and
 546 *trx1 ntrc* mutants relative to the wild-type, with the decrease being specifically
 547 pronounced in the *trx1 ntrc* double mutant. This is consistent with a progressive
 548 shortage of carbon in these mutants. Nocturnal levels of FBP were below the
 549 detection limit in all genotypes (Fig. 10B).

550

551 To gain a more in-depth insight into the global effects of Trx *f1* and NTRC on redox
 552 regulation of metabolism, GC-MS based metabolite profiling was performed. Suppl.
 553 Table S4 and Fig. 11A show significant changes in leaf metabolite levels at the end

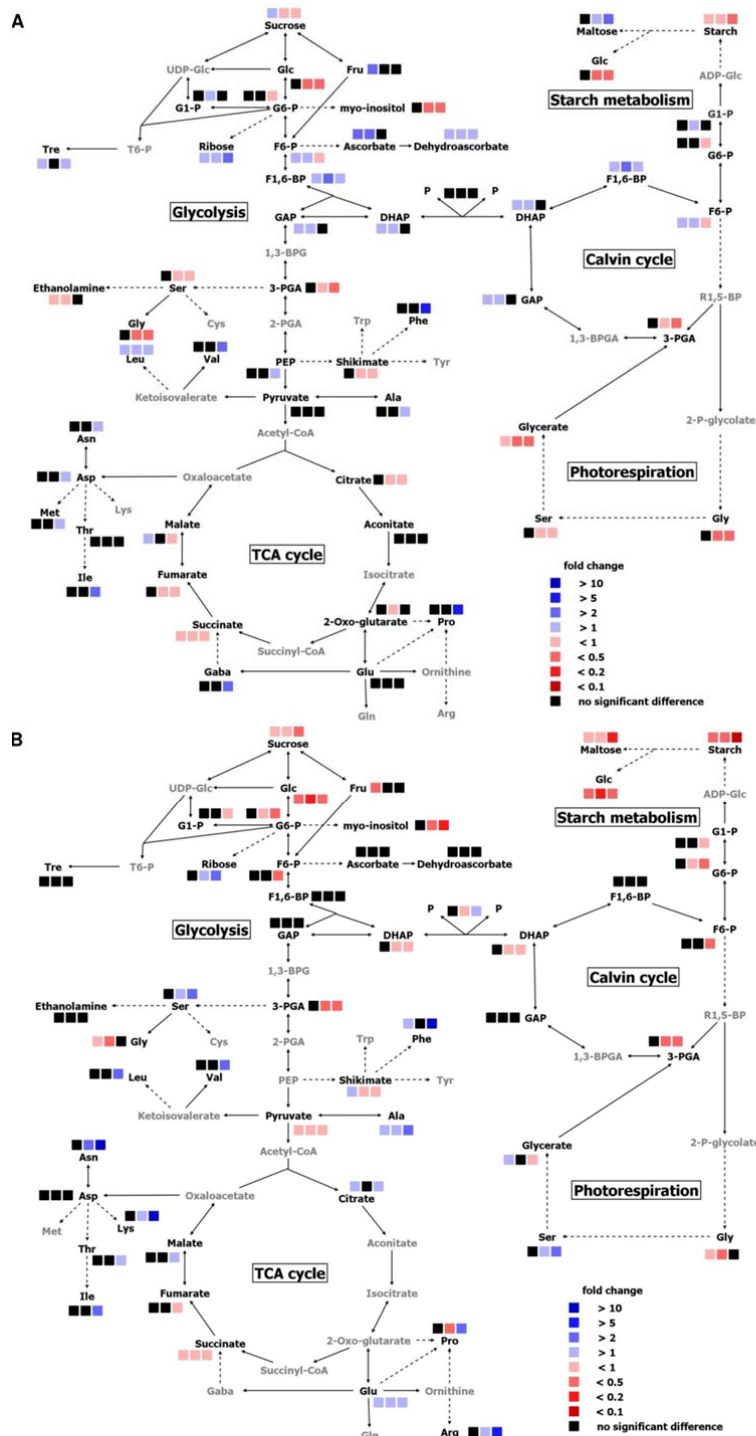


Figure 11: Overview of changes in metabolite profiles from leaves of *trx1*, *ntrc* and *trx1 ntrc* Arabidopsis mutants compared to wild-type. Results from leaves sampled at the end of day (**A**) and end of night (**B**) are visualized using Vanted diagrams. Metabolite levels which are significantly different from wild-type according to the student *t*-test ($P < 0.05$) are indicated in blue (increase) or red (decrease) color, while black color indicates no significant difference from wild-type. The order of the squares from left to right is *trx1*, *ntrc* and *trx1 ntrc* mutants being in first, second and third position, respectively. Data are taken from Supplemental Tables S4 – S7.

554 of the day in *trx1*, *ntrc* and *trx1 ntrc* mutants, relative to the wild-type. In the *trx1*
 555 *ntrc* double mutant, sugars like glucose and raffinose decreased by a factor of 2,
 556 while the levels of maltose and ribose were 3-times and the level of trehalose 2-times
 557 increased. Similar or less strongly expressed changes in sugar levels were observed

558 in the *ntrc* mutant, while sugar levels remained rather unchanged or increased
559 slightly in the *trxf1* mutant. Several organic acids showed a significant decrease in
560 both *ntrc* and *trxf1 ntrc* mutants, such as citrate, fumarate, glycerate, 2-oxoglutarate,
561 shikimate, succinate and threonate, while malate decreased only in the double
562 mutant. The decrease in glycerate (3-fold), suggests possible effects on
563 photorespiration. In contrast to this, organic acid levels were largely unchanged or
564 showed only slight alterations in the *trxf1* mutant. Also amino acids showed large and
565 significant alterations in the *trxf1 ntrc* mutant. With the exception of glycine and serine
566 which were both 2-fold decreased, most other amino acids were increased in the
567 double mutant, this is the case of alanine (1.4-fold), aspartate (1.8-fold), asparagine
568 (2-fold), isoleucine (2.3-fold), leucine (2-fold), methionine (1.4-fold), phenylalanine
569 (6.7-fold), proline (7.2-fold) and valine (2.2-fold). Amino acids remained unchanged or
570 showed only slight changes in the *trxf1* and *ntrc* single mutants, with the exception of
571 glycine and serine, which both decreased by a similar degree in the *ntrc* single and
572 *ntrc trxf1* double mutants. The strong increase in most of the amino acids at the
573 expense of organic acids indicates that combined deficiency of Trx *f1* and NTRC has
574 led to a stimulation of nitrogen assimilation, probably due to the increase in the NADP
575 redox and adenylate energy states. The decrease in glycine and serine is consistent
576 with combined effects on photorespiration. The strong increase in ascorbate which
577 was observed in the *trxf1* and *ntrc* single mutants was strongly attenuated in the
578 double mutant.

579

580 Metabolite levels were also determined at the end of the night where sugars derive
581 from the degradation of starch reserves (Suppl. Table S5 and Fig. 11B). Compared to
582 wild-type, *trxf1*, *ntrc* and *trxf1 ntrc* mutants showed a further progressive decrease in
583 the levels of various sugars, with the double mutant revealing a specifically strong
584 decrease in maltose (down to 19% of wild-type level) and sucrose (46% of wild-type
585 level) consistent with an increased shortage of carbon under these conditions. There
586 were significant decreases in the levels of various organic acids, which in most cases
587 were more severe in the double mutant, compared to the single mutants, specifically
588 fumarate, glycerate, pyruvate, shikimate and succinate. Large and significant
589 alterations were observed in the levels of various amino acids, which increased in the
590 double mutant, compared to wild-type or the single mutants. While glycine
591 decreased, there were increases in the levels of alanine (3-fold), arginine (7.5-fold),

592 asparagine (43.7-fold), glutamate (1.9-fold), isoleucine (4-fold), leucine (3.3-fold),
593 lysine (10.2-fold), phenylalanine (10.3-fold), proline (2.4-fold), serine (2.3-fold),
594 threonine (2-fold) and valine (2.6-fold). The more than 10-fold increase in
595 phenylalanine, while shikimate levels were 1.5 fold decreased, indicates aromatic
596 amino acid synthesis to be strongly stimulated by the combined deficiency of Trx *f1*
597 and NTRC.

598

599 **Reporter studies provide evidence for co-localization of Trx *f1* and NTRC in the**
600 **same chloroplast sub-structure and in the same tissues**

601

602 The above data clearly show combined functions of Trx *f1* and NTRC in regulating
603 photosynthetic metabolism. To investigate whether this is accompanied by a possible
604 co-localisation of Trx *f1* and NTRC, we analyzed the subcellular localization pattern
605 of the two proteins. Constructs were generated, containing the full length cDNA of Trx
606 *f1* fused to YFP and NTRC fused to cyan fluorescent protein (CFP), respectively.
607 Pairwise expression of these fusion proteins in *Nicotiana benthamiana* leaves
608 resulted in YFP and CFP fluorescence signals which were induced in discrete
609 regions inside the chloroplast (Fig. 12). As revealed by the merged picture, Trx*f1*:YFP
610 and NTRC:CFP fluorescence pattern were congruent to each other, indicating that
611 both Trx *f1* and NTRC co-localized in the same sub-chloroplast structure (Fig. 12).
612 We also analyzed the expression pattern of Trx *f1* at the tissue level. GUS analysis of
613 Trx *f1* expression (Suppl. Figure S2) reveals a pattern similar to NTRC expression in
614 Arabidopsis plants (Kirchsteiger et al., 2012). This indicates that Trx *f1* and NTRC are
615 both active in the same tissues, highlighting the biological relevance of their
616 cooperative function.

617

618

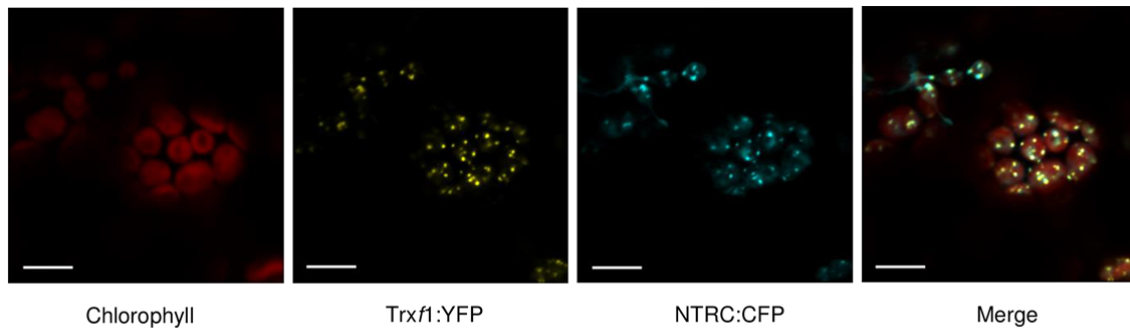


Figure 12: Suborganellar localization of Trx *f1* and NTRC proteins. Co-localization of Trx*f1*:YFP and NTRC:CFP fusions in transiently transformed leaf mesophyll cells of *Nicotiana benthamiana*. The red auto-fluorescence of chlorophyll, yellow fluorescence of YFP, blue fluorescence of CFP and the merge of the three fluorescent images are shown from left to right. Pictures were monitored in the channel mode with identical microscope settings. Bars = 10 μ m

619

DISCUSSION

620

621 Two different thiol redox-systems exist in plant chloroplasts, the Fdx-Trx, which is
 622 dependent of Fdx reduced by the photosynthetic electron transport chain and, thus,
 623 of light, and the NADPH-NTRC system, which relies on NADPH and thus may be
 624 operative also during the night. Previous studies led to the view that the two different
 625 systems may have different functions in plants. However, the possibility remains that
 626 both systems might act cooperatively. In the present work we have tested this
 627 possibility by using a genetic approach. Results provide evidence that light and
 628 NADPH dependent redox systems interact at the level of Trx *f1* and NTRC to
 629 coordinately participate in the regulation of photosynthetic carbon metabolism and
 630 growth in response to changes in light conditions.

631

632 **Trx *f1* and NTRC cooperatively participate in light regulation of the Calvin-**
 633 **Benson cycle and growth while having no effect on the redox regulation of the**
 634 **malate valve**

635

636 Our results show that single knockouts of Trx *f1* or NTRC cause no or only slight
 637 impairment of photosynthesis (Fig. 3 and 4), respectively, confirming previous studies
 638 (Lepisto et al., 2009; Thormählen et al., 2013). Interestingly, the combined deficiency
 639 of both thiol redox-regulators led to a more severe inhibition of photosynthetic CO₂-
 640 assimilation (Fig. 3), electron transport rates (Fig. 4) and growth (Fig. 2) than in both
 641 single knockouts. This was accompanied by an increase in both the NADPH/NADP⁺

642 and ATP/ADP ratios (Fig. 6), indicating that the primary cause for the strong
643 impairment of photosynthesis is an inhibition of the Calvin-Benson cycle rather than
644 the light reactions. Direct measurements of FBPase, a key enzyme of the Calvin-
645 Benson cycle, confirm this interpretation (Fig. 7). Light activation of FBPase was
646 attenuated by up to 50% in the *Trx f1* and NTRC single mutants, while it was almost
647 completely abolished in the double mutant. A similar picture emerged when the
648 redox-state of the regulatory disulfide of chloroplast FBPase was directly analyzed
649 using gel-shift assays in vivo. Inhibition of FBPase is also indicated at the metabolite
650 level, since it was accompanied by a decrease in F6P/FBP metabolite ratios in-vivo
651 (Fig. 10D). This shows that combined knockout of *Trx f1* and NTRC strongly impedes
652 light-dependent changes in FBPase redox transition (Fig. 7) and hence Calvin-
653 Benson cycle activity (Fig. 3) and plant growth (Fig. 2). Results are in-line with
654 previous studies on transgenic plants (Kossmann et al., 1994) or Arabidopsis mutants
655 (Livingston et al., 2010; Rojas-Gonzalez et al. 2015) with knock-down of chloroplast
656 FBPase showing that a decrease in chloroplast FBPase activity below 36% of wild-
657 type level impairs F6P/FBP metabolite ratios, photosynthetic CO₂ assimilation, starch
658 accumulation and growth.

659

660 In textbooks, *Trx f* is proposed to act as the exclusive thiol redox-regulator of FBPase
661 and the Calvin-Benson cycle, a scenario which is based on pioneering in-vitro
662 experiments of Buchanan and coworkers back in the seventies (Buchanan et al.,
663 1979; Buchanan, 1980) and subsequent comparative studies using a large set of
664 different recombinant purified *Trx* isoforms in vitro (Collin et al. 2003). Our in-vivo
665 studies show that this textbook view cannot be transferred to the more complex
666 situation in planta, where an almost complete deficiency of *Trx f* has been found to be
667 not sufficient enough to decrease light dependent redox activation of FBPase by
668 more than 50% (compare Fig. 1 and Fig. 7). The latter requires a combined
669 deficiency of *Trx f* and NTRC, indicating that both proteins have redundant functions
670 in light-regulation of FBPase and the Calvin-Benson cycle activity in planta. The
671 involvement of NTRC in redox-regulation of the Calvin-Benson cycle allows carbon
672 assimilation to be linked to the NADP redox state as an additional input, which is
673 influenced by light via FNR and by metabolic parameters.

674

675 In contrast to the strong effects on FBPase, single and combined knockouts of Trx *f1*
676 and NTRC did not lead to any inhibition in the redox-activation of NADP-MDH (Fig.
677 8), a key enzyme in the export of excess reducing equivalents from the chloroplast to
678 the cytosol via the malate valve (Scheibe, 2004). This indicates that neither Trx *f1* nor
679 NTRC affect NADP-MDH redox activation in planta, suggesting that the latter is
680 regulated by other Trx isoforms, such as *m*-type Trxs. This regulatory feature could
681 be important to prevent an imbalance of the chloroplast NADP redox state. If Trx *f1*
682 and NTRC would activate Calvin-Benson cycle and the malate valve simultaneously,
683 this could lead to a strong depletion in chloroplast NADPH levels with adverse effects
684 on the operation of the Calvin-Benson cycle. Our results therefore show that the
685 predominant role of Trx *f* in redox regulation of NADP-MDH which was proposed on
686 the basis of in-vitro studies (Collin et al. 2003; Yoshida et al. 2015) cannot be
687 translated to the situation in planta.

688

689 **Trx *f1* and NTRC cooperatively participate in light regulation of starch**
690 **metabolism and balancing of carbon and nitrogen metabolism**

691

692 Combined deficiency of Trx *f1* and NTRC led to a nearly complete inhibition of starch
693 accumulation. This was accompanied by an additive decrease in light activation of
694 the key enzyme of starch synthesis, AGPase (Fig. 9). The effect is most likely
695 attributable to both Trx *f1* and NTRC being able to reduce the small subunit of
696 AGPase with similar efficiencies, as observed by previous in-vitro studies
697 (Thormählen et al., 2013). In the *trxf1 ntrc* double mutant, the additive decrease in
698 redox-activation of AGPase will partly contribute to the strong decrease in starch
699 accumulation (compare Figs. 9A and 9D), while there will be an additional
700 contribution from the inhibition of the Calvin-Benson cycle (Fig. 3A) and the
701 subsequent decrease in the level of its first fixation product 3PGA (Fig. 10A), which is
702 a strong allosteric activator of AGPase (Preiss 1988). Changes in the redox (Hädrich
703 et al. 2012) and allosteric properties of AGPase (Obana et al. 2006; Mugford et al.
704 2014) have been found to substantially alter diurnal starch turnover in leaves of
705 *Arabidopsis* plants.

706

707 Interestingly, combined - but not single - deficiencies of Trx *f1* and NTRC led to a
708 strong and unexpected increase in the level of the starch degradation product

709 maltose during the light period (Fig. 11A and Table 1). This could indicate that Trx *f1*
710 and NTRC may participate in the diurnal regulation of starch degradation in addition
711 to starch synthesis. This is in-line to previous in-vitro studies reporting that several
712 enzymes in the pathway of starch degradation are subject to thiol redox-regulation
713 (Valerio et al., 2011; Glaring et al., 2012), including a plastid-targeted beta-amylase
714 which has been shown to be regulated by both Trx *f1* and NTRC (Valerio et al.,
715 2011). It remains to be determined whether a similar mechanism is operational in
716 vivo.

717

718 Unexpectedly, combined deletion of Trx *f1* and NTRC resulted in a strong increase in
719 the levels of most amino acids, while sugars and organic acids were mainly
720 decreased (Fig. 11), indicating an induction of nitrogen assimilation in the face of a
721 decreased carbon assimilation. It is unlikely that this is due to direct effects of Trx *f1*
722 and NTRC deficiencies on redox-activation of enzymes involved in nitrogen
723 assimilation, such as nitrate and nitrite reductase, glutamate:oxoglutarate
724 aminotransferase or glutamine synthetase, since this would have led to a decrease
725 rather than an increase in the activities of these enzymes (Lichter und Häberlein
726 1998; Choi et al. 1999). However, there will be indirect effects due to the elevated
727 NAD and NADP reduction and adenylate energy states as a consequence of the
728 inhibition of carbon assimilation (Fig. 6), which will promote nitrogen assimilation by
729 increasing the levels of its cofactors. This is accompanied by a decrease in the levels
730 of organic acids, which are used as substrates to provide carbon skeletons for amino
731 acid synthesis. These results suggest a combined role of Trx *f1* and NTRC in
732 balancing carbon and nitrogen assimilation as well as the levels of sugars and amino
733 acids to avoid an over-reduction of PS I and osmotic imbalances, respectively (Ferne
734 et al., 2002; Faix et al., 2012). Alternatively, the increase in amino acids may also be
735 attributable to (i) the overall attenuation of protein synthesis in the cell because of the
736 strongly reduced growth rate of the double mutant, or (ii) a specific stimulation of
737 protein degradation to mobilize additional carbon reserves when sugars are limiting.
738 However, in both of these scenarios, organic acid levels would increase rather than
739 decrease.

740

741 **Trx *f1* and NTRC cooperatively participate in light acclimation of photosystem I**

742

743 Impaired photosynthetic light reactions in the *trxf1 ntrc* mutant were most likely a
744 consequence of the inhibition of the Calvin-Benson cycle and the subsequent
745 increase in NADPH/NADP⁺ ratios (Figs. 4, 3 and 6B). Combined knockout of Trx *f1*
746 and NTRC led to a strong decrease in the abundance of PSI core proteins (Fig. 5),
747 which was most likely due to an inhibition of electron transfer at the acceptor side of
748 PSI, leading to its over-reduction. As shown by previous studies, PSI is very sensitive
749 to excess electrons delivered from PSII due to its limited capacity of regeneration by
750 protein turnover (Suorsa et al., 2012; Tikkanen and Aro, 2014). The moderate
751 decreases in the abundance of PSII core proteins (Fig. 5) and in chlorophyll levels
752 (Suppl. Fig. S1) are most likely part of an adaptive response to relieve the electron
753 pressure on PSI and to protect PSI from photo-damage (Grieco et al., 2012; Suorsa
754 et al., 2012; Tikkanen et al., 2014). The decreased ability of the *trxf1 ntrc* mutant to
755 adapt to high-light conditions (Fig. 2F) provides further evidence for a role of Trx *f1*
756 and NTRC in light-acclimation of PSI. Proteins involved in cyclic electron transport
757 around PSI may also be part of an adaptive response to preserve PSI, although their
758 specific functions remain to be clarified (Livingston et al., 2010; Suorsa et al., 2012;
759 Hertle et al., 2013). Interestingly, an Arabidopsis mutant deficient in chloroplast
760 FBPase showed impaired linear electron transport and increased cyclic electron flow
761 (Livingston et al., 2010). More studies are needed to resolve the roles of the stromal
762 redox-regulators Trx *f1* and NTRC in the regulatory network of plant thylakoid energy
763 transduction.

764

765 Alternatively, our data could indicate more specific effects of Trx *f1* and NTRC on
766 chloroplast protein synthesis. It has been shown in previous studies that light plays a
767 crucial role in regulating chloroplast protein translation, which most likely involves the
768 Fdx-Trx system as one of the underlying signaling pathways (Pfannschmidt and
769 Liere, 2005). More recently, a role of NTRC was proposed to regulate translation of
770 the D2 protein of PSII by thiol-disulfide modulation of chloroplast translation factors in
771 *Chlamydomonas reinhardtii* (Schwarz et al., 2012). However, in-vivo evidence to
772 support this conclusion is lacking at the moment.

773

774 **Trx *f1* and NTRC cooperatively participate in growth acclimation to varying**
775 **light conditions**

776

777 Combined - but not single - deficiencies of Trx *f1* and NTRC severely affected growth
778 acclimation to varying light conditions, leading to strongly impaired acclimation of
779 plant growth to a decrease in the length of the photoperiod or changes in light
780 intensities over a wide range of conditions (Fig. 2H). The ability to acclimate to a 4 h
781 photoperiod or to low light intensity ($30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) was almost completely
782 lost, as well as the ability to acclimate to high-light conditions ($950 \mu\text{mol m}^{-2} \text{s}^{-1}$). The
783 vulnerability of the *trxf1 ntrc* double mutant to low light conditions is most likely due to
784 its strongly impaired ability to activate the Calvin-Benson cycle in response to light.
785 Decreased photoperiods but also low light intensities require efficient mechanisms to
786 fully activate the Calvin-Benson cycle when light becomes available. The decreased
787 ability of the *trxf1 ntrc* double mutant to acclimate to high light is most likely
788 attributable to the increased sensitivity of PSI to photo-damage (see Fig. 5 and
789 discussion above). The strong depletion in soluble sugars prevailing in the double
790 mutant (Fig. 11) may also have contributed to the decrease in its ability for high-light
791 acclimation. As shown in previous studies, soluble sugars act as signals in the high-
792 light response of Arabidopsis plants, while high-light acclimation is impaired when
793 soluble sugar levels are decreased (Schmitz et al., 2014). Overall, these results
794 suggest a combined role of Trx *f1* and NTRC in growth acclimation to varying light
795 conditions.

796

797 **Trx *f1* and NTRC co-localize in the same chloroplast substructure**

798

799 The Trx*f1*:YFP and NTRC:CFP fusion proteins were found to be co-localized in
800 discrete regions within the chloroplast stroma (Fig. 12). The pattern is similar to that
801 previously reported for the interaction of NTRC with 2-Cys Prx (Bernald-Bayard et al.
802 2014) or the CHL-1 subunit of the Mg-chelatase complex (Perez-Ruiz et al. 2014)
803 and for immunogold-labelling of NTRC (Perez-Ruiz et al. 2009). The co-localization
804 of these clusters with specific chloroplast structures is not clear from these studies.
805 Our preliminary results indicate that the substructure where Trx *f1* and NTRC are co-
806 localized does not correlate with chloroplast nucleoids, but was found to be
807 associated to starch granules (data not shown). The vicinity of these clusters to
808 starch granules may be related to the combined function of Trx *f1* and NTRC in
809 regulating carbon assimilation and storage.

810

811 MATERIALS AND METHODS

812

813 Plant material and growth conditions

814

815 *Arabidopsis thaliana* T-DNA insertion lines *trxf1* (SALK_128365; Thormählen et al.,
816 2013), *ntrc* (SALK_012208; Perez-Ruiz et al., 2006), the double mutant *trxf1 ntrc*,
817 generated by cross breeding, and the respective Col-0 wild-types were grown for five
818 weeks on potting soil (Stender, Germany) in a growth chamber with 8 h photoperiod,
819 160 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, 20°C/16°C, and 60%/75% humidity (day/night), if not
820 indicated otherwise in the figure legends. For rosette fresh-weight determination,
821 plants were grown for the first week under the conditions indicated above, before
822 they were transferred to 16 h or 24 h photoperiods for additional 3 weeks or to 4 h
823 photoperiod, 30 or 950 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for additional 4 weeks using a growth
824 chamber at 21°C.

825

826 Homogenisation of plant material

827

828 For all the metabolite, DNA or protein extractions described below, leaves were
829 shock-frozen directly into liquid nitrogen, and subsequently homogenized to a fine
830 powder using a liquid nitrogen-cooled ball mill (MM 400, Retsch GmbH, Haan,
831 Germany).

832

833 Selection and molecular characterization of the knockout lines

834

835 The *trxf1 ntrc* mutant was selected after crossing the well-characterized homozygous
836 parental lines carrying T-DNA insertions in *Trx f1* (Thormählen et al., 2013) and
837 *NTRC* genes (Perez-Ruiz et al., 2006). The selection of a homozygous line with
838 insertion in both alleles was performed by PCR analyses of genomic DNA using
839 gene-specific primers for the *Trx f1* (At3g02730; 5'-TGTCAGTGTTGGTCAGGTGAC-
840 3' and 5'-AGAACCCATCCAACACACTTG-3') and *NTRC* (At2g41680; 5'-
841 TATTGAGCAACACCAAGGGAC-3' and 5'-CATAATTCCAGCTGCTTCAGC-3')
842 genes or oligonucleotides of the T-DNA (5'-ATTTTGCCGATTTCCGGAAC-3'). PCR
843 products were fractionated on 1 % agarose gels, and visualized by ethidium bromide
844 staining. Detection of *Trx f* and *NTRC* proteins was done by Western blot analysis

845 (Laemmli, 1970) using antibodies raised against pea Trx *f* (Hodges et al., 1994) and
846 rice NTRC (Serrato et al., 2004). To do this, frozen leaf powder was extracted with 2-
847 fold Laemmli buffer (Laemmli, 1970) including 20 mM DTT instead of β -
848 mercaptoethanol. After shaking the extract for 3 min at 90°C, each lane of the
849 polyacrylamide gels was loaded with sample corresponding to 1 mg fresh weight for
850 each genotype. The pea Trx-*f* antibody used in these experiments has been found in
851 previous studies to recognize Arabidopsis Trx *f*1 and Trx *f*2 recombinant proteins with
852 comparable efficiencies (Thormählen et al., 2013).

853

854 **Gas exchange measurements**

855

856 Photosynthesis-related gas exchange parameters were determined on 4 to 5 week
857 old plants with the portable GFS-3000 system (Heinz Walz GmbH, Effeltrich,
858 Germany). The control unit 3000-C with the measuring head 3010-S was used by
859 adapting the cuvette to Arabidopsis Chamber 3010-A. The conditions within the
860 cuvette were 22°C, 60% relative humidity and ambient CO₂ concentrations, while the
861 impeller speed was set to 7, and the flow rate to 750 $\mu\text{mol s}^{-1}$. The monitoring of the
862 light curve was started with darkened rosettes. When the CO₂ and H₂O system
863 parameters were stabilized, the light was switched on and changed in the following
864 order: 50, 100, 150, 200, 300, 400, 600, 800, 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The parameters of
865 CO₂ assimilation rate, transpiration rate and intercellular CO₂ mole fraction were
866 calculated by the software GFS-Win V3.50b (Heinz Walz GmbH, Effeltrich,
867 Germany).

868

869 **Chlorophyll fluorescence analysis**

870

871 For the in vivo chlorophyll a fluorescence measurement and the calculation of
872 standard photosynthesis parameters of PSII, a Dual PAM fluorometer (Dual-PAM
873 100, Walz GmbH, Effeltrich, Germany) was used as described previously
874 (Thormählen et al., 2013).

875

876 **Analysis of chlorophyll content**

877

878 The chlorophyll level was determined and calculated as described in Porra et al.
879 (1989). 25 mg frozen leaf material was extracted twice with 1 ml 80% acetone. For
880 each extraction step the samples were vortexed for two minutes, incubated for 10
881 min in darkness on ice, and 10 min centrifuged at 4°C. The collected supernatants
882 were pooled, and the light absorption measured at 663 nm, 645 nm, and 750 nm
883 (absorption at 750 nm was subtracted from the values at 665 nm and 652 nm) with
884 an UV/VIS spectrophotometer (Ultrospec 3100 pro, GE Healthcare Europe GmbH,
885 Freiburg, Germany).

886

887 **Enzyme-coupled analysis of metabolite levels by spectrophotometry**

888

889 For metabolite analysis, leaf samples were directly frozen in liquid nitrogen without
890 any shading allowing rapid quenching of metabolism. Extraction and analysis of the
891 pyridine nucleotides NAD, NADP, NADH and NADPH were performed as described
892 previously (Lintala et al., 2014). In brief, 25 mg frozen leaf powder was resuspended
893 in 250 µl 0.1 M HClO₄ (NAD⁺ and NADP⁺) or 250 µl 0.1 M KOH (for NADH and
894 NADPH, respectively) and incubated for 10 min on ice. Samples were centrifuged at
895 20,000 g for 10 min at 4°C and the supernatant was heated to 95°C for 2 min. The
896 pH was adjusted to 8.0 – 8.5 by addition of an equal volume 0.2 M Tris (pH 8.4), 0.1
897 M KOH or 0.2 M Tris (pH 8.4), 0.1 M HClO₄, respectively. The final detection mix for
898 NAD(H) contained 100 mM Tricine/KOH (pH 9), 4 mM EDTA, 500 mM EtOH, 0.1 mM
899 phenazine ethosulfate (PES), 0.6 mM methylthiazolyldiphenyl-tetrazolium bromide
900 (MTT), 6 U ml⁻¹ alcohol dehydrogenase (ADH). For NADP(H) the final mix consisted
901 of 100 mM Tricine/KOH (pH 9), 4 mM EDTA, 3 mM glucose 6-phosphate, 0.1 mM
902 PES, 0.6 mM MTT and 6 U ml⁻¹ G6PDH. Absorption was monitored at 570 nm at
903 30°C in a microplate reader (HT3, Anthos Mikrosysteme GmbH,
904 <http://www.anthos.de/>). To validate the method, small representative amounts (two to
905 threefold the endogenous content) of NAD⁺, NADP⁺, NADH and NADPH were added
906 to the plant material in the killing mixture of HClO₄ or KOH. The recoveries of these
907 metabolites from Arabidopsis leaves during extraction and assay were (as
908 percentage of the amount added): NAD⁺, 92 %; NADP⁺, 98 %; NADH, 79 % and
909 NADPH, 111 %. The extraction of ATP and ADP was performed according to
910 previous studies, where also the validity of this method has been documented (Jelitto
911 et al., 1992). In brief, 50 mg frozen leaf powder was extracted with ice-cold 16% TCA

912 (w/v), 5 mM EGTA by vortexing 1 h at 4°C. After centrifugation for 10 min at 4°C with
913 20,000 g, the supernatant was shortly mixed with 4 ml ice-cold, water-saturated
914 diethyl ether (DEE) and centrifuged again at 3,200 g at 4°C for 5 min. The upper
915 ether phase was discarded to repeat the washing step. The pH adjustment of the
916 remaining water phase was done with 5 M KOH, 1 M triethanolamine or 1 M HCl until
917 a pH of 6-7 was reached. The remaining DEE in the extract evaporated under the
918 hood for 1 h on ice. Directly after the extraction, ATP and ADP levels were measured
919 enzymatically as described previously (Stitt et al., 1989), with the exception that the
920 change in NAD(P)H levels was measured by fluorescence spectroscopy in 96-well
921 micro plates at 360 nm with a FilterMax F5 Multi-Mode Microplate reader (Molecular
922 Devices, Sunnyvale, USA). Starch and sucrose were measured photometrically by
923 NADPH absorption, hexose phosphates, FBP, triose phosphates and 3PGA by
924 NAD(P)H fluorescence as described previously (Thormählen et al., 2013). Each
925 individual plant sample was measured with at least 2 analytical replicates.

926

927 **GC-TOFMS analysis of polar primary metabolites**

928

929 GC-TOFMS-based analysis of primary metabolites was performed exactly as
930 described previously (Thormählen et al., 2013). For each biological replicate three
931 analytical replicates were measured. To visualize the metabolite changes within an
932 overview, we used the open source software VANTED version 2.1.0
933 (<http://vanted.ipk-gatersleben.de/>).

934

935 **Immunodetection of photosynthesis and starch related proteins**

936

937 Proteins involved in photosynthetic electron transport (PsaA, PsaB, PsbA, PsbD,
938 Lhca1, Lhcb1, PetC, Atp β), Rubisco (RbcL) and actin were detected by Western
939 blotting using specific antibodies (Agrisera, Vännäs, Sweden) according to company
940 instructions. Frozen leaf powder was extracted with 2-fold Laemmli buffer (Laemmli,
941 1970) including 20 mM DTT instead of β -mercaptoethanol. After shaking the extract
942 for 3 min at 90°C, each lane of the polyacrylamide gels was loaded with sample
943 corresponding to 1 mg fresh weight (100%) for each genotype. Immunoblotting of
944 APS1, detection and quantification of ECL signals were performed as described
945 previously (Thormählen et al., 2013).

946

947 **FBPase gel-shift assays**

948

949 For FBPase gel-shift assays proteins from leaves of wild-type and mutant plants,
950 harvested at the end of the night (EN) or the day (ED), were extracted in the
951 presence of 10% (v/v) trichloroacetic acid (TCA) and protein thiols were alkylated
952 with 10 mM MM(PEG)₂₄. For the Western blot analysis, samples were subjected to
953 SDS-PAGE (9.5% polyacrylamide) under non-reducing conditions, transferred onto
954 nitrocellulose membranes and probed with an anti-FBPase antibody kindly provided
955 by Mariam Sahrawy (Estación Experimental del Zaidín, CSIC, Granada, Spain).
956 Quantification of the protein band intensities was done by using the open source
957 software ImageJ version 1.49g (<http://imagej.nih.gov/ij/>).

958

959 **Enzyme activity measurements**

960

961 The activity of plastidial FBPase was determined as described in a previous study
962 (Holaday et al., 1992). In short, 20 mg frozen leaf powder was rapidly extracted with
963 1 ml ice-cold extraction buffer (10 mM MgCl₂, 1 mM EDTA, 0.05 % [v/v] Triton X-100,
964 100 mM Tris [pH 8, HCl], 1 mM fructose 1,6-bisphosphate) and centrifuged with
965 18.000 g at 4°C. 10 µl of the supernatant was added immediately to 190 µl assay
966 mixture in the well of 96-well microplate. The final assay contained 10 mM MgCl₂, 1
967 mM EDTA, 0.05 % Triton X-100, 100 mM Tris (pH8, HCl), 0.5 mM NADP⁺, 2 units ml⁻¹
968 ¹ G6PDH and 4 units ml⁻¹ PGI. For the maximal activity measurement, 10 mM DTT
969 was included in the assay. To start the reaction, FBP (0.1 mM for initial activity and 4
970 mM for the maximal activity) was added, while NADPH formation was measured
971 spectro-photometrically at 340 nm using a micro-plate reader (HT-3, Anthos
972 Mikrosysteme GmbH, Krefeld, Germany). The activity of NADP-malate
973 dehydrogenase was measured as described previously (Lintala et al., 2014). Every
974 individual plant sample was measured with at least 2 analytical replicates.

975

976 **Subcellular localization studies**

977

978 For co-localisation studies of NTRC and Trx *f1* the entire coding region of each gene
979 was amplified from Arabidopsis (Col-0) cDNA by PCR using the following primer

980 pairs (NTRC-fwd 5'-CACCATGGCTGCGTCTCCCAAGATAGGCATCGGTAT-
 981 3'/NTRC-rev 5'-TTTATTGGCCTCAATGAATTCTCGGTACTCTTT-3' and Trxf1-fwd
 982 5'-CACCATGCCTCTTTCTCTCCGTCTTTCTCCTTCGCC-3'/TRXf1-rev 5'-
 983 TCCGGAAGCAGCAGACTTCGCTGTTTCAATCGC-3'). The PCR products were
 984 inserted into the pENTR-D/TOPO (Invitrogen) entry vector and were checked by
 985 sequencing. Trx *f1* and NTRC entry vectors were used to generate C-terminal fusion
 986 gene constructs with full length YFP and CFP, respectively. As destination vectors,
 987 the GATEWAY-compatible 35S::Venus-pBar and 35S::sCFP3A-pBar were used
 988 (Zakharov et al., 2004). Each binary vector construct was separately transformed into
 989 *Agrobacterium tumefaciens* strain EHA105 for subsequent transient transformation of
 990 leaves of 4 week-old *Nicotiana benthamiana* grown in soil under greenhouse
 991 conditions. Specific fluorescence signals were monitored by Zeiss LSM 780 confocal
 992 laser scanning microscope 48 h after combined infiltration of the different gene fusion
 993 constructs into tobacco leaves. Fluorophore signals, chlorophyll autofluorescence
 994 and bright field images were scanned sequentially in channel mode to prevent any
 995 crosstalk between fluorescence channels. The lambda mode was used to confirm the
 996 spectral signature of the fluorophores.

997

998 Promoter-GUS analysis

999

1000 An 875 bp fragment of the *Trx f1* gene promoter was amplified from Arabidopsis (Col-
 1001 0) DNA by standard PCR method using a Phusion DNA polymerase (Thermo
 1002 Scientific) and the following primer pair (Tf1prom-fwd 5'-
 1003 TACTGCAGGCGGTGGAGTACGATTTAGGACAAAGAA-3'/Tf1prom-rev 5'-
 1004 TAGTCGACTGTTTGAGGAATTCAACAGAGAGACGAT-3'). The PCR product was
 1005 restricted with *Pst*I and *Sal*I (Thermo Scientific) and cloned into the pBAR binary
 1006 vector (Zakharov et al., 2004) containing the GUS-reporter expression cassette. The
 1007 construct was transformed into cells of the *Escherichia coli* DH5 α strain for
 1008 amplification and subsequent sequencing of the plasmids. Purified plasmids were
 1009 used for *Agrobacterium tumefaciens* mediated transformation of *Arabidopsis thaliana*
 1010 (Col-0) via the floral dip method (Clough and Bent, 1998). Transformed plants were
 1011 selected for homozygosity and assayed for GUS activity as described previously
 1012 (Jefferson et al., 1987). GUS stained specimens were bleached in 70% (v/v) ethanol
 1013 and either directly analyzed by stereomicroscopy (Zeiss Stereo Lumar.V12) or

1014 samples were cleared by mounting in Hoyer's solution (100 g chloral hydrate, 5 ml
1015 glycerol in 30 ml water). Cleared specimens were imaged by differential interference
1016 contrast microscopy (Zeiss Axio Imager.M2).

1017

1018 **Statistical Analysis**

1019

1020 The statistical data analyses were done with Microsoft Office Excel 2007 (Student's *t*-
1021 test) and SYSTAT Sigma Plot 11 (two way analysis of variance, Tukey's multiple
1022 comparison test). The Student's *t*-test was done as a two-tailed test assuming
1023 unequal or equal variance depending on the data (checked by performing an F-test in
1024 the beginning). When Western blot signals were quantified, the paired *t*-test was
1025 used.

1026

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1028

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1032 analyze photosynthetic proteins, Jürgen Soll (LMU Munich) for providing facilities for
1033 gas exchange measurement, and to Emmanuelle Issakidis-Bourguet (University
1034 Paris-Orsay, France) and Mariam Sahrawy (CSIC, Spain) for providing anti-Trx *f* and
1035 anti-FBPase antibodies, respectively.

1036

1037

1038 **LIST OF AUTHOR CONTRIBUTIONS**

1039

1040 I.T., T.M. and P.G. designed research. I.T., T.M, J.G. A.B.Ö., E.v.R.-L., B.N. and
1041 F.J.C. performed research. I.T., T.M, J.G. A.B.Ö., E.v.R.-L., B.N., F.J.C. and P.G.
1042 analyzed data. I.T., T.M., F.J.C. and P.G. wrote the article.

1043 **FIGURE LEGENDS**

1044

1045 **Figure 1:** Molecular characterization of *trxf1*, *ntrc* and *trxf1 ntrc* Arabidopsis mutants
 1046 compared to wild-type. **(A)** Genotyping by PCR analysis with different primer
 1047 combinations (wild-type or insertion) for the identification of T-DNA insertions in *Trx*
 1048 *f1* and *NTRC* genes. **(B)** Detection of *Trx f* and *NTRC* proteins using Western blot
 1049 analysis. Representative Western blots are shown of measurements, which were
 1050 performed in leaves of 5-week old plants grown in an 8h-day with 160 $\mu\text{mol photons}$
 1051 $\text{m}^{-2} \text{s}^{-1}$ light regime harvested 4 h into the light period. Rubisco protein level is shown
 1052 as control.

1053

1054 **Figure 2:** Growth analysis of wild-type, *trxf1*, *ntrc* and *trxf1 ntrc* Arabidopsis mutants
 1055 across different light conditions. **(A)**, **(B)**, **(E)** and **(F)** correspond to 5 week-old plants,
 1056 while **(C)** and **(D)** correspond to 4 week-old plants, and **(G)** to 7 week-old plants. In
 1057 the first week, plants - except **(G)** - were grown in an 8 h-day and moderate light
 1058 intensity regime before they were transferred for additional 3-4 weeks to the
 1059 conditions indicated below: **(A)** 4 h-day and moderate light intensity, **(B)** 8 h-day and
 1060 moderate light intensity, **(C)** 16 h-day and moderate light intensity, **(D)** 24 h-day and
 1061 moderate light intensity, **(E)** 8 h-day and low light intensity, **(F)** 8 h-day and high light
 1062 intensity, and **(G)** 16 h-day in greenhouse. In **(H)**, rosette fresh-weights of plants
 1063 corresponding to the conditions shown in **(A)** to **(F)** are given as percent of wild-type
 1064 levels in the respective conditions. Results are the mean \pm SE, $n = 30-86$ (wild-type),
 1065 $15-44$ (*trxf1*), $5-44$ (*ntrc*) or $9-111$ (*trxf1 ntrc*) different plants. All values are
 1066 significantly different from wild-type according to the Student's *t*-test ($P < 0.05$), except
 1067 the *trxf1* mutant in 8 h-, 16 h- and 24 h-day regimes at moderate and high light
 1068 intensities (see Suppl. Table S1). Low light intensity = $30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$;
 1069 moderate light intensity = $160 \mu\text{mol photons m}^{-2} \text{s}^{-1}$; high light intensity = $950 \mu\text{mol}$
 1070 $\text{photons m}^{-2} \text{s}^{-1}$; n.d. = not detectable (fresh-weight values were below the detection
 1071 limit)

1072

1073 **Figure 3:** Changes in gas-exchange parameters in leaves of *trxf1*, *ntrc* and *trxf1 ntrc*
 1074 Arabidopsis mutants compared to wild-type. **(A)** CO_2 assimilation rate, **(B)**
 1075 transpiration rate, and **(C)** intercellular CO_2 concentration were measured at different
 1076 light intensities in leaves from plants growing in an 8 h photoperiod with 160 μmol

1077 photons $\text{m}^{-2} \text{s}^{-1}$. Results are the mean \pm SE, $n = 10$ (wild-type) or 5 (mutants) different
 1078 plant replicates. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ (according to two-way analysis of
 1079 variance [Anova]), Tukey test); for further statistical analysis see Suppl. Table S2;
 1080 PAR = photosynthetic active radiation

1081

1082 **Figure 4:** Changes in chlorophyll fluorescence parameters in leaves of *trxf1*, *ntrc* and
 1083 *trxf1 ntrc* Arabidopsis mutants compared to wild-type. Plants growing in an 8 h
 1084 photoperiod with $160 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ were dark adapted for 10 min, before
 1085 exposure of a far red light saturation pulse ($5,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ for 0.8 s) to single
 1086 leaves. Afterwards the maximal chlorophyll a fluorescence was quenched by electron
 1087 transport with an actinic red light of $166 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Within 10 min the
 1088 steady state was reached and another saturation pulse was given. In the end, **(A)** the
 1089 maximal PSII (F_v/F_m), and **(B)** the effective PSII (Φ_{PSII}), the non-regulated energy
 1090 dissipation (Φ_{NO}) and the regulated energy dissipation (Φ_{NPQ}) quantum yields were
 1091 calculated. Results are means \pm SE, $n = 11$ different plants.

1092 *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ (according to Student's *t*-test)

1093

1094 **Figure 5:** Changes in the levels of proteins involved in photosynthetic electron
 1095 transport and ATP synthesis in leaves of *trxf1*, *ntrc* and *trxf1 ntrc* Arabidopsis mutants
 1096 compared to wild-type. PsaA, PsaB, PsbA, PsbD, PetC, Lhca1, Lhcb1 and Atp β
 1097 proteins were detected using specific antibodies. Representative Western blots are
 1098 shown from 5-week old plants growing in an 8h-day with $160 \mu\text{mol photons m}^{-2} \text{s}^{-1}$
 1099 light regime harvested 4 h into the light period. In the wild-type, different amounts of
 1100 samples were loaded (25-100%) for comparison. Actin protein level is shown as
 1101 control.

1102

1103 **Figure 6:** Changes in nucleotide levels in leaves of *trxf1*, *ntrc* and *trxf1 ntrc*
 1104 Arabidopsis mutants compared to wild-type. **(A)** Sum of NADPH and NADP, **(B)**
 1105 NADPH/NADP ratio, **(C)** sum of NADH and NAD, **(D)** NADH/NAD ratio, **(E)** sum of
 1106 ATP and ADP, and **(F)** ATP/ADP ratio were measured in leaves harvested at the end
 1107 of day and end of night. Results are means \pm SE, $n = 20-30$ (wild-type) or 10-15
 1108 (mutants) independent plant replicates. Plants were grown in an 8 h photoperiod with
 1109 $160 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ (according to Student's
 1110 *t*-test).

1111

1112 **Figure 7:** Light-dependent redox activation of fructose-1,6-bisphosphatase (FBPase)
 1113 in leaves of *trxf1*, *ntrc* and *trxf1 ntrc* Arabidopsis mutants compared to wild-type. **(A)**
 1114 and **(B)** show the thiol-disulfide reduction state of chloroplast FBPase in leaves
 1115 harvested at the end of night (EN) and end of day (ED) analyzed by using gel-shift
 1116 assays: **(A)** Representative gel-shift blot using an antibody specific for chloroplast
 1117 FBPase, and **(B)** calculated ratio of reduced to oxidized FBPase. **(C)** Corresponding
 1118 initial enzyme activity of FBPase (assay without DTT) in leaves harvested at the end
 1119 of night (EN) and end of day (ED). **(D)** to **(F)** Transient light activation of FBPase
 1120 during a detailed time course. At the end of the night (0 min), plants were illuminated
 1121 for different time-periods (2, 5, 10, 20 and 30 min) to measure FBPase activity using
 1122 different assay conditions: **(D)** Initial activity without DTT additions in the assay, **(E)**
 1123 maximal activity with 10 mM DTT included in the assay, and **(F)** estimated redox-
 1124 activation state (initial/maximal activity*100). Results are means \pm SE, $n = 8$ (wild-
 1125 type) or 4 (mutants) independent plant replicates **(B)**, $n = 20$ (wild-type) or 10
 1126 (mutants) independent plant replicates **(C)**, and $n = 10$ (wild-type) or 4-6 (mutants)
 1127 independent plant replicates **(D)** to **(F)**. All plants were grown in an 8 h photoperiod
 1128 with $160 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, according to
 1129 Student's *t*-test **(B)** and **(C)** or two-way analysis of variance [Anova] Tukey test **(D)** to
 1130 **(F)**, see Suppl Table S3. Estimation of the redox-activation state from enzyme assays
 1131 **(F)** and the directly measured thiol-disulfide state of the protein **(B)** yielded different
 1132 absolute values, which is most likely due to additional factors affecting FBPase
 1133 activity during the enzyme assays.

1134

1135 **Figure 8:** Light-dependent redox activation of NADP-dependent malate
 1136 dehydrogenase (MDH) in leaves of *trxf1*, *ntrc* and *trxf1 ntrc* Arabidopsis mutants
 1137 compared to wild-type. **(A)** Initial activity without DTT additions in the assay, **(B)**
 1138 maximal activity with 10 mM DTT included in the assay, **(C)** redox-activation state
 1139 (initial/maximal activity*100). Leaves were sampled at the end of night and end of
 1140 day. Results are means \pm SE, $n = 24$ (wild-type) or 12 (mutants) independent plant
 1141 replicates. Plants were grown in an 8 h photoperiod with $160 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$.
 1142 *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ (according to Student's *t*-test)

1143

1144 **Figure 9:** Changes in the accumulation of starch and sucrose and in the thiol-
 1145 disulfide reduction state of the small subunits of ADP-glucose pyrophosphorylase
 1146 (APS1) in leaves of *trxf1*, *ntrc* and *trxf1 ntrc* Arabidopsis mutants compared to wild-
 1147 type. **(A)** Starch level, **(B)** sucrose level, **(C)** starch/sucrose ratio, and **(D)** APS1
 1148 monomerisation (APS1 monomer as percent of total APS1 [monomer + dimer]) were
 1149 measured in leaves sampled at the end of night (EN) and end of day (ED). APS1
 1150 monomerisation was analyzed in non-reducing SDS gels, where reduced and
 1151 oxidized APS1 can be separated as monomer and dimer, respectively. Results are
 1152 means \pm SE, $n = 8$ (wild-type) or 4 (mutants) independent plant replicates growing in
 1153 an 8 h photoperiod with $160 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$
 1154 (according to Student's *t*-test)

1155

1156 **Figure 10:** Changes in the in-vivo levels of phosphorylated intermediates in leaves of
 1157 *trxf1*, *ntrc* and *trxf1 ntrc* Arabidopsis mutants compared to wild-type. **(A)** 3-
 1158 phosphoglycerate (3PGA) level, **(B)** fructose 1,6-bisphosphate (FBP) level, **(C)**
 1159 fructose 6-phosphate (F6P) level, **(D)** F6P/FBP ratio, **(E)** glucose 6-phosphate (G6P)
 1160 level, and **(F)** glucose 1-phosphate (G1P) level were measured in leaves sampled at
 1161 the end of the night and end of the day. Results are means \pm SE, $n = 20-30$ (wild-
 1162 type) or 10-15 (mutants) independent plant replicates growing in an 8 h photoperiod
 1163 with $160 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ (according to
 1164 Student's *t*-test); n.d. = not detectable (values were below the detection limit)

1165

1166 **Figure 11:** Overview of changes in metabolite profiles from leaves of *trxf1*, *ntrc* and
 1167 *trxf1 ntrc* Arabidopsis mutants compared to wild-type. Results from leaves sampled at
 1168 the end of day **(A)** and end of night **(B)** are visualized using Vanted diagrams.
 1169 Metabolite levels which are significantly different from wild-type according to the
 1170 Student's *t*-test ($P < 0.05$) are indicated in blue (increase) or red (decrease) color,
 1171 while black color indicates no significant difference from wild-type. The order of the
 1172 squares from left to right is *trxf1*, *ntrc* and *trxf1 ntrc* mutants being in first, second and
 1173 third position, respectively. Data are taken from Supplemental Tables S4 – S7.

1174

1175 **Figure 12:** Suborganellar co-localization of Trx *f1* and NTRC proteins. Co-
 1176 localization of Trx*f1*:YFP and NTRC:CFP fusions in transiently transformed leaf
 1177 mesophyll cells of *Nicotiana benthamiana*. The red auto-fluorescence of chlorophyll,

1178 yellow fluorescence of YFP, blue fluorescence of CFP and the merge of the three
 1179 fluorescent images are shown from left to right. Pictures were monitored in the
 1180 channel mode with identical microscope settings. Bars = 10 μm

1181

1182 SUPPLEMENTAL FIGURES

1183

1184 **Suppl. Figure S1:** Changes in chlorophyll content in leaves of *trxf1*, *ntrc* and *trxf1*
 1185 *ntrc* Arabidopsis mutants compared to wild-type. Results are the mean \pm SE, $n = 10$
 1186 (wild-type) or 5 (mutants) different plant replicates growing in an 8 h photoperiod with
 1187 $160 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ (according to Student's
 1188 *t*-test)

1189

1190 **Suppl. Figure S2:** Histochemical localization of GUS expression in Arabidopsis
 1191 plants transformed with a *Trxf1_{pro}*-GUS reporter gene. GUS staining of 10-day-old
 1192 seedlings grown in a 16 h photoperiod **(A)** to **(D)**, and of 6-week-old plants having
 1193 flowered and begun to set seed **(E)** to **(I)**. GUS staining is shown in emerging leaves
 1194 **(A)** and **(B)**, roots **(C)**, hypocotyl **(D)**, silique petiol **(E)**, silique **(F)**, flower **(G)**, sepal
 1195 **(H)** and stigma **(I)**. No GUS staining was observed in trichomes **(B)**. The following
 1196 microscopic techniques were used: **(A)**, **(E)** and **(F)** Stereomicroscopy, **(B)**, **(C)**, **(D)**,
 1197 **(G)**, **(H)** and **(I)** differential interference contrast microscopy, and **(G)** and **(H)** single
 1198 image merge. Bars = 1000 μm in **(A)**, **(E)**, **(F)**, **(G)** and **(H)**, and 100 μm in **(B)**, **(C)**,
 1199 **(D)** and **(I)**.

1200

1201 SUPPLEMENTAL TABLES

1202

1203 **Suppl. Table S1:** Statistical analysis for rosette fresh weights of *trxf1*, *ntrc* and *trxf1*
 1204 *ntrc* Arabidopsis mutants growing in different light conditions, compared to wild-type.
 1205 Values are based on the data presented in Figure 2H. Significantly different values
 1206 from wild-type according to the Student's *t*-test are indicated in bold ($P < 0.05$). n.d. =
 1207 not detectable.

1208

1209 **Suppl. Table S2:** Statistical analysis for gas exchange parameters of *trxf1*, *ntrc* and
 1210 *trxf1 ntrc* Arabidopsis mutants dependent on different light intensities, compared to
 1211 wild-type. Values are based on the data presented in Figure 3. Significantly different

1212 values from wild-type according to the Student's *t*-test ($P < 0.05$) are indicated in bold.

1213 PAR = photosynthetic active radiation.

1214

1215 **Suppl. Table S3:** Statistical analysis for the time course of fructose-1-6-
1216 bisphosphatase light activation in leaves of *trxf1*, *ntrc* and *trxf1 ntrc* Arabidopsis
1217 mutants, compared to wild-type. Values are based on the data presented in Figure
1218 7D-F. Significantly different values from wild-type according to the Student's *t*-test
1219 ($P < 0.05$) are indicated in bold.

1220

1221 **Suppl. Table S4:** Changes in GC-MS based metabolite profiles in leaves of *trxf1*,
1222 *ntrc* and *trxf1 ntrc* Arabidopsis mutants compared to wild-type. Leaves were sampled
1223 at the end of the day. Results are means \pm SD, $n = 12$. Values which are significantly
1224 different from wild-type according to the Student's *t*-test ($P < 0.05$) are indicated in
1225 bold (see also Figure 11A).

1226

1227 **Suppl. Table S5:** Changes in GC-MS based metabolite profiles in leaves of *trxf1*,
1228 *ntrc* and *trxf1 ntrc* Arabidopsis mutants compared to wild-type. Leaves were sampled
1229 at the end of the night. Results are means \pm SD, $n = 12$. Values which are
1230 significantly different from wild-type according to the Student's *t*-test ($P < 0.05$) are
1231 indicated in bold (see also Figure 11B).

1232

1233 **Suppl. Table S6:** Changes in the levels of phosphorylated intermediates and starch
1234 in leaves of *trxf1*, *ntrc* and *trxf1 ntrc* Arabidopsis mutants compared to wild-type,
1235 based on spectrophotometric measurements. Leaves were sampled at the end of the
1236 day. Results are normalized to wild-type level and represent means \pm SE, $n = 8-30$
1237 (wild-type) or 4-15 (mutants). Values which are significantly different from wild-type
1238 according to the Student's *t*-test ($P < 0.05$) are indicated in bold (see Figure 11A).

1239

1240 **Suppl. Table S7:** Changes in the levels of phosphorylated intermediates and starch
1241 in leaves of *trxf1*, *ntrc* and *trxf1 ntrc* Arabidopsis mutants compared to wild-type,
1242 based on spectrophotometric measurements. Leaves were sampled at the end of the
1243 night. Results are normalized to wild-type level and represent means \pm SE, $n = 8-30$
1244 (wild-type) or 4-15 (mutants). Values which are significantly different from wild-type

1245 according to the Student's t-test ($P < 0.05$) are indicated in bold (see also Figure 11B).

1246 n.d. = not detectable.

1247

1248

1249

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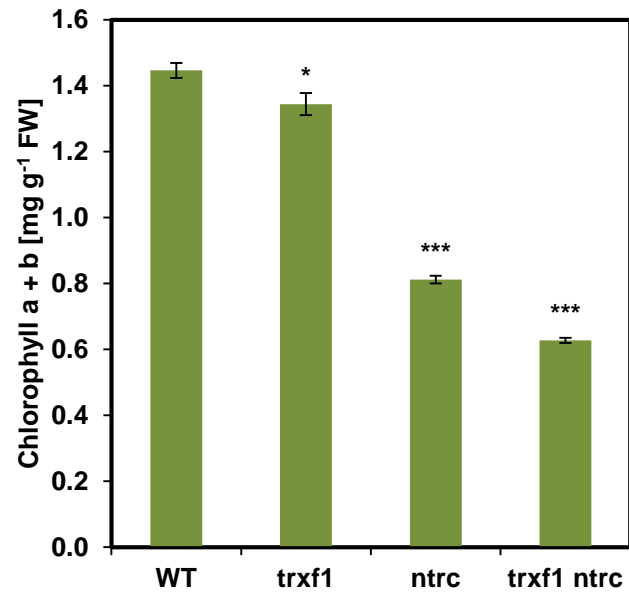
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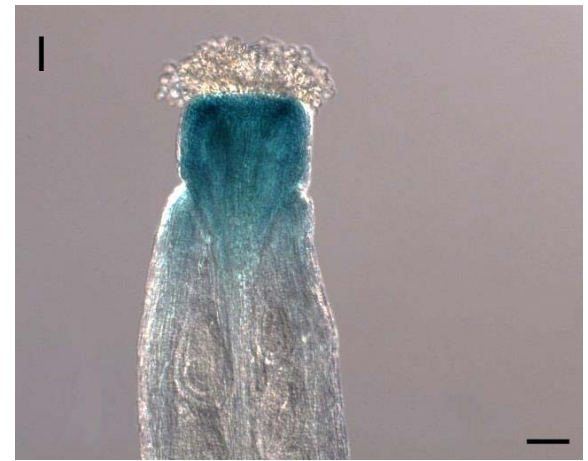
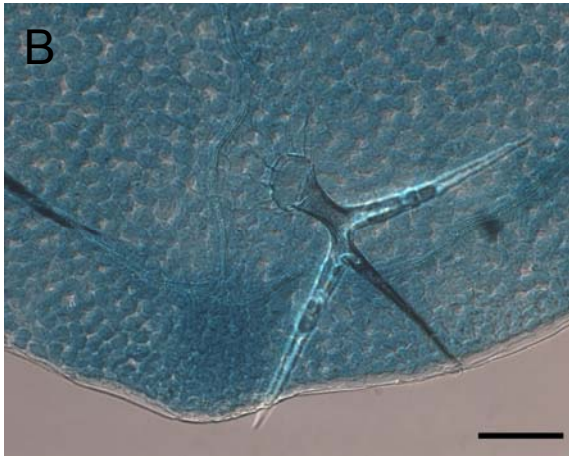
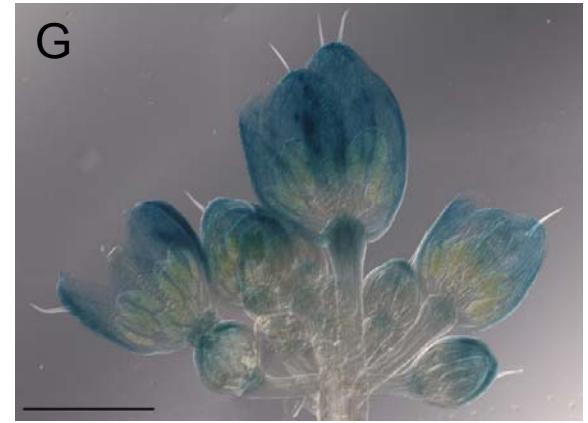
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Suppl. Figure S1: Changes in chlorophyll content in leaves of *trxf1*, *ntrc* and *trxf1 ntrc* Arabidopsis mutants compared to wild-type. Results are the mean \pm SE, $n = 10$ (wild-type) or 5 (mutants) different plant replicates growing in an 8 h photoperiod with $160 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ (according to Student's *t*-test)



Suppl. Figure S2: Histochemical localization of GUS expression in Arabidopsis plants transformed with a *Trxf1_{pro}*-GUS reporter gene. GUS staining of 10-day-old seedlings grown in a 16 h photoperiod **(A)** to **(D)**, and of 6-week-old plants having flowered and begun to set seed **(E)** to **(I)**. GUS staining is shown in emerging leaves **(A)** and **(B)**, roots **(C)**, hypocotyl **(D)**, silique petiol **(E)**, silique **(F)**, flower **(G)**, sepal **(H)** and stigma **(I)**. No GUS staining was observed in trichomes **(B)**. The following microscopic techniques were used: **(A)**, **(E)** and **(F)** Stereomicroscopy, **(B)**, **(C)**, **(D)**, **(G)**, **(H)** and **(I)** differential interference contrast microscopy, and **(G)** and **(H)** single image merge. Bars = 1000 μm in **(A)**, **(E)**, **(F)**, **(G)** and **(H)**, and 100 μm in **(B)**, **(C)**, **(D)** and **(I)**.

Suppl. Table S1:

Statistical analysis for rosette fresh weights of *trxf1*, *ntrc* and *trxf1 ntrc* *Arabidopsis* mutants growing in different light conditions, compared to wild-type. Values are based on the data presented in Figure 2H. Significantly different values from wild-type according to the Student's *t*-test ($P < 0.05$) are indicated in bold. n.d. = not detectable. Growth conditions see Figure 2H.

| Growth condition | <i>trxf1</i> | <i>ntrc</i> | <i>trxf1 ntrc</i> |
|-------------------------|------------------|------------------|-------------------|
| 4h-day, moderate light | 0.002 | <0.001 | n.d. |
| 8h-day, moderate light | 0.354 | <0.001 | <0.001 |
| 16h-day, moderate light | 0.484 | <0.001 | <0.001 |
| 24h-day, moderate light | 0.316 | <0.001 | <0.001 |
| 8h-day, low light | <0.001 | <0.001 | n.d. |
| 8h-day, high light | 0.781 | <0.001 | <0.001 |

Suppl. Table S2:

Statistical analysis for gas exchange parameters of *trxf1*, *ntrc* and *trxf1 ntrc* Arabidopsis mutants dependent on different light intensities, compared to wild-type. Values are based on the data presented in Figure 3. Significantly different values from wild-type according to the Student's *t*-test ($P < 0.05$) are indicated in bold. PAR = photosynthetically active radiation.

| PAR [$\mu\text{mol m}^{-2} \text{s}^{-1}$] | Assimilation rate | | | Transpiration rate | | | Intercellular CO ₂ mole fraction | | |
|---|-------------------|--------------|-------------------|--------------------|-------------|-------------------|---|--------------|-------------------|
| | <i>trxf1</i> | <i>ntrc</i> | <i>trxf1 ntrc</i> | <i>trxf1</i> | <i>ntrc</i> | <i>trxf1 ntrc</i> | <i>trxf1</i> | <i>ntrc</i> | <i>trxf1 ntrc</i> |
| 0 | 0.402 | 0.365 | 0.011 | 0.767 | 0.773 | 0.002 | 0.328 | 0.912 | 0.693 |
| 50 | 0.958 | 0.009 | 0.017 | 0.789 | 0.971 | 0.016 | 0.446 | 0.063 | <0.001 |
| 100 | 0.863 | 0.052 | 0.004 | 0.864 | 0.944 | 0.022 | 0.682 | 0.046 | <0.001 |
| 150 | 0.762 | 0.081 | 0.005 | 0.882 | 0.906 | 0.026 | 0.996 | 0.040 | <0.001 |
| 200 | 0.797 | 0.048 | 0.008 | 0.851 | 0.525 | 0.037 | 0.670 | 0.078 | <0.001 |
| 300 | 0.576 | 0.091 | <0.001 | 0.935 | 0.773 | 0.052 | 0.660 | 0.040 | <0.001 |
| 400 | 0.455 | 0.090 | <0.001 | 0.916 | 0.909 | 0.033 | 0.585 | 0.047 | <0.001 |
| 600 | 0.532 | 0.058 | <0.001 | 0.852 | 0.884 | 0.029 | 0.404 | 0.026 | <0.001 |
| 800 | 0.529 | 0.104 | 0.002 | 0.794 | 0.638 | 0.017 | 0.208 | 0.087 | <0.001 |
| 1000 | 0.493 | 0.077 | 0.071 | 0.718 | 0.456 | 0.028 | 0.170 | 0.056 | <0.001 |

Supplemental Table S3:

Statistical analysis for the time course of fructose-1,6-bisphosphatase light activation in leaves of *trxf1*, *ntrc* and *trxf1 ntrc* *Arabidopsis* mutants, compared to wild-type. Values are based on the data presented in Figure 7D-F. Significantly different values from wild-type according to the Student's *t*-test ($P < 0.05$) are indicated in bold. Time = time after illumination

| Time [min] | Initial activity | | | Maximal activity | | | Activation state | | |
|---------------|------------------|--------------|-------------------|------------------|-------------|-------------------|------------------|------------------|-------------------|
| | <i>trxf1</i> | <i>ntrc</i> | <i>trxf1 ntrc</i> | <i>trxf1</i> | <i>ntrc</i> | <i>trxf1 ntrc</i> | <i>trxf1</i> | <i>ntrc</i> | <i>trxf1 ntrc</i> |
| 0 | 0.802 | 0.503 | 0.070 | 0.837 | 0.811 | 0.014 | 0.521 | 0.480 | 0.255 |
| 2 | 0.011 | 0.044 | 0.008 | 0.813 | 0.939 | 0.006 | 0.019 | 0.097 | 0.060 |
| 5 | 0.053 | 0.001 | <0.001 | 0.878 | 0.642 | <0.001 | 0.023 | <0.001 | 0.002 |
| 10 | 0.039 | 0.006 | 0.002 | 0.589 | 0.965 | 0.023 | 0.051 | 0.006 | <0.001 |
| 20 | 0.010 | 0.007 | <0.001 | 0.343 | 0.936 | <0.001 | 0.075 | 0.010 | <0.001 |
| 30 | <0.001 | 0.021 | <0.001 | 0.227 | 0.793 | 0.002 | <0.001 | 0.026 | <0.001 |

Suppl. Table S4:

Changes in GC-MS based metabolite profiles in leaves of *trxf1*, *ntrc* and *trxf1 ntrc* Arabidopsis mutants compared to wild-type. Leaves were sampled at the end of the day. Results are means \pm SD, $n = 12$. Values which are significantly different from wild type according to the student t-test ($P < 0.05$) are indicated in bold (see also Figure 11A).

| Metabolites | WT | <i>trxf1</i> | <i>P</i> -value | <i>ntrc</i> | <i>P</i> -value | <i>trxf1 ntrc</i> | <i>P</i> -value |
|------------------------------|-----------------|-----------------------------------|-----------------|-----------------------------------|------------------|-----------------------------------|------------------|
| <u>Sugars</u> | | | | | | | |
| Fructose | 1.00 \pm 0.57 | 2.03 \pm 0.69 | 0.001 | 1.15 \pm 0.37 | 0.445 | 0.83 \pm 0.22 | 0.360 |
| Glucose | 1.00 \pm 0.63 | 1.43 \pm 0.63 | 0.109 | 0.39 \pm 0.08 | 0.006 | 0.45 \pm 0.24 | 0.014 |
| Maltose | 1.00 \pm 0.26 | 1.03 \pm 0.20 | 0.725 | 1.65 \pm 0.69 | 0.008 | 3.13 \pm 1.34 | <0.001 |
| Raffinose | 1.00 \pm 0.35 | 1.93 \pm 1.13 | 0.017 | 0.25 \pm 0.07 | <0.001 | 0.55 \pm 0.64 | 0.045 |
| Ribose | 1.00 \pm 0.08 | 1.15 \pm 0.18 | 0.016 | 1.55 \pm 0.51 | 0.003 | 2.64 \pm 1.14 | <0.001 |
| Sucrose | 1.00 \pm 0.07 | 1.11 \pm 0.10 | 0.004 | 0.86 \pm 0.14 | 0.007 | 0.89 \pm 0.07 | 0.001 |
| Trehalose | 1.00 \pm 0.49 | 1.38 \pm 0.23 | 0.028 | 1.20 \pm 0.44 | 0.304 | 1.88 \pm 0.75 | 0.003 |
| <u>Phosphate ester</u> | | | | | | | |
| Glycerol-3-phosphate | 1.00 \pm 0.27 | 1.14 \pm 0.17 | 0.143 | 1.22 \pm 0.47 | 0.174 | 1.85 \pm 1.02 | 0.016 |
| Phosphoenolpyruvate | 1.00 \pm 0.18 | 1.02 \pm 0.20 | 0.805 | 1.35 \pm 0.68 | 0.112 | 1.85 \pm 0.53 | <0.001 |
| <u>Organic acids</u> | | | | | | | |
| Aconitate (cis) | 1.00 \pm 0.29 | 0.99 \pm 0.32 | 0.956 | 0.84 \pm 0.12 | 0.086 | 1.05 \pm 0.36 | 0.711 |
| Benzoate | 1.00 \pm 0.24 | 1.14 \pm 0.32 | 0.257 | 1.33 \pm 0.58 | 0.096 | 1.61 \pm 0.29 | <0.001 |
| caffeate (cis) | 1.00 \pm 0.10 | 0.98 \pm 0.10 | 0.593 | 1.09 \pm 0.40 | 0.486 | 1.33 \pm 0.93 | 0.247 |
| caffeate (trans) | 1.00 \pm 0.36 | 1.03 \pm 0.26 | 0.871 | 0.92 \pm 0.20 | 0.594 | 1.10 \pm 0.26 | 0.530 |
| 3-caffeoyl-quininate (cis) | 1.00 \pm 0.10 | 1.06 \pm 0.16 | 0.306 | 0.88 \pm 0.15 | 0.033 | 0.91 \pm 0.18 | 0.138 |
| 3-caffeoyl-quininate (trans) | 1.00 \pm 0.22 | 1.23 \pm 0.41 | 0.112 | 0.59 \pm 0.12 | <0.001 | 0.74 \pm 0.19 | 0.005 |
| Citrate | 1.00 \pm 0.19 | 1.09 \pm 0.14 | 0.202 | 0.69 \pm 0.24 | 0.002 | 0.51 \pm 0.38 | 0.001 |

| | | | | | | | |
|-------------------------|--------------|--------------------|------------------|--------------------|------------------|--------------------|------------------|
| Fumarate | 1.00 ± 0.17 | 1.05 ± 0.10 | 0.404 | 0.80 ± 0.19 | 0.011 | 0.70 ± 0.22 | 0.001 |
| Galactonate | 1.00 ± 0.12 | 0.95 ± 0.06 | 0.268 | 0.88 ± 0.11 | 0.015 | 0.91 ± 0.06 | 0.029 |
| Glycerate | 1.00 ± 0.04 | 0.61 ± 0.07 | <0.001 | 0.36 ± 0.18 | <0.001 | 0.33 ± 0.44 | <0.001 |
| Gulonate | 1.00 ± 0.19 | 1.28 ± 0.19 | 0.002 | 1.28 ± 0.38 | 0.033 | 1.42 ± 0.36 | 0.002 |
| Malate | 1.00 ± 0.12 | 1.22 ± 0.19 | 0.003 | 1.00 ± 0.24 | 0.955 | 0.69 ± 0.28 | 0.003 |
| Maleate | 1.00 ± 0.51 | 0.85 ± 0.35 | 0.395 | 0.94 ± 0.36 | 0.726 | 1.51 ± 0.74 | 0.065 |
| 2-methyl-malate | 1.00 ± 0.09 | 0.93 ± 0.17 | 0.206 | 0.63 ± 0.18 | <0.001 | 0.61 ± 0.21 | <0.001 |
| 2-oxo-glutarate | 1.00 ± 0.18 | 0.92 ± 0.21 | 0.311 | 0.66 ± 0.12 | <0.001 | 0.83 ± 0.36 | 0.281 |
| Pyruvate | 1.00 ± 0.35 | 0.79 ± 0.18 | 0.079 | 0.85 ± 0.23 | 0.225 | 1.02 ± 0.23 | 0.853 |
| Shikimate | 1.00 ± 0.08 | 1.11 ± 0.23 | 0.160 | 0.52 ± 0.12 | <0.001 | 0.51 ± 0.32 | <0.001 |
| sinapate (cis) | 1.00 ± 0.16 | 0.95 ± 0.16 | 0.485 | 0.97 ± 0.13 | 0.573 | 1.07 ± 0.19 | 0.370 |
| sinapate (trans) | 1.00 ± 0.22 | 0.91 ± 0.17 | 0.290 | 1.00 ± 0.22 | 0.987 | 1.29 ± 0.40 | 0.040 |
| Succinate | 1.00 ± 0.16 | 0.80 ± 0.12 | 0.002 | 0.57 ± 0.15 | <0.001 | 0.58 ± 0.35 | 0.002 |
| Threonate | 1.00 ± 0.12 | 0.95 ± 0.18 | 0.416 | 0.62 ± 0.05 | <0.001 | 0.64 ± 0.14 | <0.001 |
| <u>Amino acids</u> | | | | | | | |
| Alanine | 1.00 ± 0.22 | 1.12 ± 0.37 | 0.357 | 1.07 ± 0.36 | 0.567 | 1.36 ± 0.48 | 0.032 |
| Aspartate | 1.00 ± 0.21 | 1.08 ± 0.33 | 0.496 | 1.24 ± 0.62 | 0.226 | 1.75 ± 0.60 | 0.001 |
| Asparagine | 1.00 ± 0.23 | 1.10 ± 0.17 | 0.368 | 1.21 ± 0.67 | 0.359 | 1.95 ± 0.48 | <0.001 |
| Glutamate | 1.00 ± 0.27 | 1.03 ± 0.27 | 0.797 | 0.85 ± 0.26 | 0.179 | 1.05 ± 0.33 | 0.676 |
| Glycine | 1.00 ± 0.13 | 1.24 ± 0.38 | 0.055 | 0.45 ± 0.17 | <0.001 | 0.43 ± 0.31 | <0.001 |
| 4-hydroxy-proline (cis) | 1.00 ± 0.28 | 0.77 ± 0.37 | 0.100 | 0.38 ± 0.05 | 0.000 | 0.85 ± 0.26 | 0.300 |
| Isoleucine | 1.00 ± 0.20 | 1.16 ± 0.30 | 0.136 | 1.48 ± 0.90 | 0.093 | 2.30 ± 1.16 | 0.003 |
| Leucine | 1.00 ± 0.172 | 1.23 ± 0.19 | 0.007 | 1.40 ± 0.46 | 0.018 | 1.96 ± 1.00 | 0.007 |
| Methionine | 1.00 ± 0.20 | 1.08 ± 0.31 | 0.470 | 1.12 ± 0.25 | 0.219 | 1.37 ± 0.44 | 0.018 |

| | | | | | | | |
|-----------------------|-------------|--------------------|------------------|--------------------|------------------|--------------------|------------------|
| Phenylalanine | 1.00 ± 0.28 | 1.21 ± 0.25 | 0.073 | 3.16 ± 4.21 | 0.104 | 6.74 ± 4.27 | 0.001 |
| Proline | 1.00 ± 0.42 | 1.58 ± 0.90 | 0.059 | 2.30 ± 2.91 | 0.153 | 7.16 ± 5.63 | 0.003 |
| Pyroglutamate | 1.00 ± 0.33 | 1.22 ± 0.20 | 0.066 | 1.14 ± 0.38 | 0.356 | 1.59 ± 0.51 | 0.003 |
| Serine | 1.00 ± 0.36 | 0.75 ± 0.32 | 0.086 | 0.50 ± 0.19 | <0.001 | 0.57 ± 0.19 | 0.001 |
| Threonine | 1.00 ± 0.21 | 1.06 ± 0.40 | 0.672 | 1.03 ± 0.39 | 0.845 | 1.19 ± 0.25 | 0.059 |
| Valine | 1.00 ± 0.15 | 1.12 ± 0.36 | 0.313 | 1.21 ± 0.71 | 0.339 | 2.16 ± 1.16 | 0.005 |
| <u>Sugar alcohols</u> | | | | | | | |
| Erythritol | 1.00 ± 0.14 | 1.34 ± 0.20 | 0.003 | 2.63 ± 2.82 | 0.072 | 7.40 ± 3.30 | <0.001 |
| Glycerol | 1.00 ± 0.14 | 0.99 ± 0.19 | 0.918 | 1.09 ± 0.29 | 0.328 | 1.41 ± 0.33 | 0.001 |
| Mannitol | 1.00 ± 0.20 | 1.04 ± 0.30 | 0.738 | 2.91 ± 3.02 | 0.051 | 8.07 ± 5.35 | 0.001 |
| myo-inositol | 1.00 ± 0.16 | 1.19 ± 0.41 | 0.164 | 0.44 ± 0.16 | <0.001 | 0.42 ± 0.36 | <0.001 |
| Sorbitol | 1.00 ± 0.09 | 0.87 ± 0.11 | 0.007 | 1.04 ± 0.15 | 0.449 | 1.24 ± 0.34 | 0.034 |
| <u>Others</u> | | | | | | | |
| 4-amino-butanoate | 1.00 ± 0.16 | 1.67 ± 1.31 | 0.105 | 1.17 ± 0.84 | 0.542 | 4.92 ± 2.95 | 0.001 |
| Ascorbate | 1.00 ± 0.52 | 3.40 ± 2.32 | 0.004 | 4.22 ± 2.19 | <0.001 | 1.02 ± 0.60 | 0.947 |
| Dehydroascorbate | 1.00 ± 0.20 | 1.44 ± 0.45 | 0.011 | 1.70 ± 0.64 | 0.003 | 1.76 ± 0.28 | <0.001 |
| Ethanolamine | 1.00 ± 0.26 | 0.59 ± 0.14 | <0.001 | 0.53 ± 0.17 | <0.001 | 1.00 ± 0.13 | 0.968 |
| Phosphate | 1.00 ± 0.13 | 1.02 ± 0.16 | 0.793 | 0.99 ± 0.18 | 0.847 | 1.07 ± 0.21 | 0.337 |
| Putrescine | 1.00 ± 0.29 | 1.06 ± 0.40 | 0.658 | 1.28 ± 0.52 | 0.124 | 2.02 ± 1.10 | 0.009 |
| Spermidine | 1.00 ± 0.67 | 1.13 ± 0.56 | 0.636 | 0.77 ± 0.47 | 0.355 | 0.74 ± 0.35 | 0.267 |
| Uracil | 1.00 ± 0.62 | 0.92 ± 0.48 | 0.763 | 1.24 ± 0.67 | 0.407 | 3.42 ± 3.29 | 0.028 |

Suppl. Table S5:

Changes in GC-MS based metabolite profiles in leaves of *trxf1*, *ntrc* and *trxf1 ntrc* Arabidopsis mutants compared to wild-type. Leaves were sampled at the end of the night. Results are means \pm SD, $n = 12$. Values which are significantly different from wild type according to the student t-test ($P < 0.05$) are indicated in bold (see also Figure 11B).

| Metabolites | WT | <i>trxf1</i> | <i>P</i> -value | <i>ntrc</i> | <i>P</i> -value | <i>trxf1 ntrc</i> | <i>P</i> -value |
|----------------------------|-----------------|-----------------------------------|------------------|-----------------------------------|------------------|-----------------------------------|------------------|
| <u>Sugars</u> | | | | | | | |
| Arabinose | 1.00 \pm 0.09 | 0.98 \pm 0.08 | 0.549 | 0.95 \pm 0.03 | 0.100 | 0.70 \pm 0.04 | <0.001 |
| Fructose | 1.00 \pm 0.34 | 0.38 \pm 0.04 | <0.001 | 1.14 \pm 0.46 | 0.397 | 1.14 \pm 0.68 | 0.535 |
| Galactose | 1.00 \pm 0.33 | 0.56 \pm 0.15 | <0.001 | 0.31 \pm 0.10 | <0.001 | 0.39 \pm 0.13 | <0.001 |
| Glucose | 1.00 \pm 0.38 | 0.35 \pm 0.04 | <0.001 | 0.20 \pm 0.04 | <0.001 | 0.34 \pm 0.12 | <0.001 |
| Glucose (beta-1,6-anhydro) | 1.00 \pm 0.19 | 0.83 \pm 0.16 | 0.025 | 0.80 \pm 0.16 | 0.011 | 1.15 \pm 0.21 | 0.085 |
| Maltose | 1.00 \pm 0.09 | 0.73 \pm 0.07 | <0.001 | 0.51 \pm 0.04 | <0.001 | 0.19 \pm 0.03 | <0.001 |
| Mannose | 1.00 \pm 0.32 | 0.94 \pm 0.19 | 0.615 | 0.81 \pm 0.14 | 0.079 | 0.99 \pm 0.17 | 0.905 |
| Psicose | 1.00 \pm 0.05 | 1.09 \pm 0.06 | <0.001 | 0.99 \pm 0.08 | 0.733 | 1.02 \pm 0.04 | 0.230 |
| Ribose | 1.00 \pm 0.17 | 1.04 \pm 0.11 | 0.464 | 1.17 \pm 0.11 | 0.011 | 2.71 \pm 0.45 | <0.001 |
| Sucrose | 1.00 \pm 0.03 | 0.82 \pm 0.09 | <0.001 | 0.53 \pm 0.04 | <0.001 | 0.46 \pm 0.04 | <0.001 |
| Trehalose | 1.00 \pm 0.28 | 1.11 \pm 0.19 | 0.283 | 0.86 \pm 0.21 | 0.168 | 1.08 \pm 0.14 | 0.386 |
| Xylose | 1.00 \pm 0.18 | 0.88 \pm 0.10 | 0.055 | 0.84 \pm 0.11 | 0.014 | 1.11 \pm 0.17 | 0.124 |
| <u>Organic acids</u> | | | | | | | |
| Citrate | 1.00 \pm 0.26 | 1.53 \pm 0.23 | <0.001 | 0.99 \pm 0.25 | 0.924 | 1.23 \pm 0.26 | 0.036 |
| Fumarate | 1.00 \pm 0.20 | 0.92 \pm 0.11 | 0.256 | 0.89 \pm 0.09 | 0.107 | 0.59 \pm 0.06 | <0.001 |
| Glycerate | 1.00 \pm 0.13 | 1.13 \pm 0.09 | 0.011 | 0.90 \pm 0.18 | 0.120 | 0.70 \pm 0.09 | <0.001 |
| Hexadecanoate | 1.00 \pm 0.55 | 0.58 \pm 0.22 | 0.026 | 0.54 \pm 0.17 | 0.016 | 0.57 \pm 0.18 | 0.022 |

| | | | | | | | |
|--------------------|-------------|--------------------|------------------|--------------------|------------------|---------------------|------------------|
| Malate | 1.00 ± 0.22 | 1.06 ± 0.12 | 0.437 | 1.12 ± 0.25 | 0.216 | 1.54 ± 0.15 | <0.001 |
| Maleate | 1.00 ± 0.14 | 1.00 ± 0.16 | 0.985 | 1.10 ± 0.20 | 0.167 | 1.87 ± 0.22 | <0.001 |
| Octadecanoate | 1.00 ± 0.62 | 0.69 ± 0.29 | 0.134 | 0.65 ± 0.23 | 0.088 | 0.70 ± 0.25 | 0.134 |
| Pyroglutamate | 1.00 ± 0.19 | 1.05 ± 0.13 | 0.435 | 1.66 ± 0.14 | <0.001 | 2.04 ± 0.14 | <0.001 |
| Pyruvate | 1.00 ± 0.29 | 0.75 ± 0.18 | 0.018 | 0.68 ± 0.18 | 0.003 | 0.71 ± 0.19 | 0.007 |
| Shikimate | 1.00 ± 0.07 | 1.17 ± 0.11 | <0.001 | 0.87 ± 0.09 | <0.001 | 0.69 ± 0.07 | <0.001 |
| Sinapate (cis) | 1.00 ± 0.11 | 1.00 ± 0.16 | 0.991 | 1.00 ± 0.06 | 0.993 | 1.45 ± 0.10 | <0.001 |
| Sinapate (trans) | 1.00 ± 0.14 | 1.11 ± 0.09 | 0.028 | 1.14 ± 0.11 | 0.015 | 1.68 ± 0.14 | <0.001 |
| Succinate | 1.00 ± 0.14 | 0.82 ± 0.10 | 0.002 | 0.86 ± 0.12 | 0.018 | 0.59 ± 0.06 | <0.001 |
| Threonate | 1.00 ± 0.11 | 1.24 ± 0.12 | <0.001 | 1.42 ± 0.45 | 0.009 | 0.94 ± 0.15 | 0.310 |
| <u>Amino acids</u> | | | | | | | |
| Alanine | 1.00 ± 0.13 | 1.25 ± 0.11 | <0.001 | 1.64 ± 0.15 | <0.001 | 3.03 ± 0.19 | <0.001 |
| Arginine | 1.00 ± 0.25 | 1.22 ± 0.29 | 0.057 | 1.27 ± 0.32 | 0.031 | 7.53 ± 3.62 | <0.001 |
| Asparagine | 1.00 ± 0.43 | 1.02 ± 0.28 | 0.881 | 2.41 ± 1.65 | 0.014 | 43.70 ± 9.51 | <0.001 |
| Aspartate | 1.00 ± 0.48 | 0.93 ± 0.36 | 0.686 | 0.77 ± 0.51 | 0.272 | 1.14 ± 0.62 | 0.543 |
| Glutamate | 1.00 ± 0.30 | 1.40 ± 0.18 | <0.001 | 1.48 ± 0.24 | <0.001 | 1.88 ± 0.12 | <0.001 |
| Glycine | 1.00 ± 0.43 | 0.67 ± 0.13 | 0.027 | 0.42 ± 0.06 | <0.001 | 0.77 ± 0.26 | 0.134 |
| Isoleucine | 1.00 ± 0.15 | 1.04 ± 0.29 | 0.665 | 1.15 ± 0.30 | 0.141 | 3.98 ± 0.54 | <0.001 |
| Leucine | 1.00 ± 0.16 | 0.99 ± 0.40 | 0.952 | 1.05 ± 0.51 | 0.731 | 3.28 ± 0.52 | <0.001 |
| Lysine | 1.00 ± 0.25 | 1.14 ± 0.28 | 0.215 | 1.52 ± 0.76 | 0.034 | 10.21 ± 3.01 | <0.001 |
| Phenylalanine | 1.00 ± 0.33 | 1.36 ± 0.37 | 0.019 | 0.82 ± 0.22 | 0.135 | 10.28 ± 1.84 | <0.001 |
| Proline | 1.00 ± 0.44 | 0.80 ± 0.33 | 0.225 | 0.49 ± 0.18 | 0.002 | 2.36 ± 0.38 | <0.001 |
| Serine | 1.00 ± 0.27 | 1.05 ± 0.16 | 0.547 | 1.25 ± 0.14 | 0.010 | 2.33 ± 0.28 | <0.001 |
| Threonine | 1.00 ± 0.20 | 1.07 ± 0.08 | 0.314 | 1.13 ± 0.14 | 0.087 | 1.99 ± 0.16 | <0.001 |

| | | | | | | | |
|-----------------------|-------------|--------------------|--------------|--------------------|------------------|---------------------|------------------|
| Valine | 1.00 ± 0.19 | 1.04 ± 0.17 | 0.578 | 1.13 ± 0.22 | 0.124 | 2.59 ± 0.24 | <0.001 |
| <u>Sugar alcohols</u> | | | | | | | |
| Erythritol | 1.00 ± 0.19 | 1.12 ± 0.07 | 0.051 | 1.63 ± 0.23 | <0.001 | 5.96 ± 0.77 | <0.001 |
| Glycerol | 1.00 ± 0.13 | 1.12 ± 0.18 | 0.081 | 0.99 ± 0.14 | 0.870 | 1.33 ± 0.16 | <0.001 |
| Inositol (myo) | 1.00 ± 0.11 | 1.00 ± 0.07 | 0.985 | 0.47 ± 0.06 | <0.001 | 0.19 ± 0.03 | <0.001 |
| Mannitol | 1.00 ± 0.64 | 0.74 ± 0.19 | 0.200 | 2.90 ± 0.90 | <0.001 | 18.26 ± 6.04 | <0.001 |
| Sorbitol | 1.00 ± 0.07 | 0.90 ± 0.06 | 0.001 | 0.96 ± 0.09 | 0.192 | 1.37 ± 0.07 | <0.001 |
| <u>Others</u> | | | | | | | |
| Ascorbate | 1.00 ± 0.68 | 0.66 ± 0.25 | 0.126 | 0.87 ± 0.81 | 0.680 | 1.03 ± 0.32 | 0.895 |
| Dehydroascorbate | 1.00 ± 0.25 | 0.86 ± 0.11 | 0.087 | 0.82 ± 0.23 | 0.083 | 1.14 ± 0.29 | 0.228 |
| Ethanolamine | 1.00 ± 0.34 | 0.85 ± 0.12 | 0.163 | 1.02 ± 0.17 | 0.888 | 1.34 ± 0.48 | 0.058 |
| Phosphate | 1.00 ± 0.15 | 1.01 ± 0.14 | 0.865 | 0.67 ± 0.14 | <0.001 | 1.88 ± 0.14 | <0.001 |
| Putrescine | 1.00 ± 0.36 | 1.06 ± 0.27 | 0.664 | 1.36 ± 0.48 | 0.048 | 3.65 ± 0.42 | <0.001 |
| Spermidine | 1.00 ± 0.54 | 1.34 ± 0.52 | 0.135 | 2.62 ± 0.99 | <0.001 | 2.44 ± 1.81 | 0.021 |
| Uracil | 1.00 ± 0.19 | 0.74 ± 0.18 | 0.002 | 0.80 ± 0.16 | 0.011 | 4.40 ± 0.56 | <0.001 |

Suppl. Table S6:

Changes in the levels of phosphorylated intermediates and starch in leaves of *trxf1*, *ntrc* and *trxf1 ntrc* *Arabidopsis* mutants compared to wild-type, based on spectrophotometric measurements. Leaves were sampled at the end of the day. Results are normalized to wild-type level and represent means \pm SE, $n = 8-30$ (wild-type) or 4-15 (mutants). Values which are significantly different from wild-type according to the student t-test ($P < 0.05$) are indicated in bold (see also Figure 11A).

| Metabolites | WT | <i>trxf1</i> | <i>P-value</i> | <i>ntrc</i> | <i>P-value</i> | <i>trxf1 ntrc</i> | <i>P-value</i> |
|----------------------------|-----------------|-----------------------------------|------------------|-----------------------------------|------------------|-----------------------------------|------------------|
| Dihydroxyacetone phosphate | 1.00 \pm 0.03 | 1.22 \pm 0.06 | 0.001 | 1.69 \pm 0.10 | <0.001 | 0.99 \pm 0.14 | 0.926 |
| Fructose 1,6-bisphosphate | 1.00 \pm 0.04 | 1.50 \pm 0.08 | <0.001 | 2.25 \pm 0.14 | <0.001 | 1.76 \pm 0.20 | 0.002 |
| Fructose 6-phosphate | 1.00 \pm 0.03 | 1.29 \pm 0.07 | 0.001 | 1.31 \pm 0.07 | <0.001 | 0.73 \pm 0.05 | <0.001 |
| Glucose 1-phosphate | 1.00 \pm 0.09 | 0.88 \pm 0.12 | 0.432 | 1.54 \pm 0.15 | 0.003 | 0.82 \pm 0.16 | 0.282 |
| Glucose 6-phosphate | 1.00 \pm 0.02 | 1.00 \pm 0.03 | 0.997 | 1.06 \pm 0.05 | 0.207 | 0.73 \pm 0.04 | <0.001 |
| Glyceraldehyde 3-phosphate | 1.00 \pm 0.04 | 1.25 \pm 0.09 | 0.006 | 1.68 \pm 0.11 | <0.001 | 0.92 \pm 0.09 | 0.389 |
| 3-Phosphoglycerate | 1.00 \pm 0.04 | 1.10 \pm 0.06 | 0.139 | 0.66 \pm 0.04 | <0.001 | 0.26 \pm 0.02 | <0.001 |
| Starch | 1.00 \pm 0.04 | 0.79 \pm 0.02 | 0.009 | 0.66 \pm 0.04 | <0.001 | 0.26 \pm 0.01 | <0.001 |

Suppl. Table S7:

Changes in the levels of phosphorylated intermediates and starch in leaves of *trxf1*, *ntrc* and *trxf1 ntrc* *Arabidopsis* mutants compared to wild-type, based on spectrophotometric measurements. Leaves were sampled at the end of the night. Results are normalized to wild-type level and represent means \pm SE, $n = 8-30$ (wild-type) or 4-15 (mutants). Values which are significantly different from wild-type according to the student t-test ($P < 0.05$) are indicated in bold (see also Figure 11B). n.d. = not detectable.

| Metabolites | WT | <i>trxf1</i> | <i>P</i> -value | <i>ntrc</i> | <i>P</i> -value | <i>trxf1 ntrc</i> | <i>P</i> -value |
|----------------------------|-----------------|-----------------------------------|-----------------|-----------------------------------|------------------|-----------------------------------|------------------|
| Dihydroxyacetone phosphate | 1.00 \pm 0.03 | 0.96 \pm 0.08 | 0.608 | 0.73 \pm 0.03 | <0.001 | 0.65 \pm 0.07 | <0.001 |
| Fructose 1,6-bisphosphate | n.d. | n.d. | | n.d. | | n.d. | |
| Fructose 6-phosphate | 1.00 \pm 0.07 | 0.88 \pm 0.09 | 0.322 | 1.14 \pm 0.21 | 0.549 | 0.36 \pm 0.05 | <0.001 |
| Glucose 1-phosphate | 1.00 \pm 0.11 | 1.18 \pm 0.16 | 0.364 | 1.86 \pm 0.38 | 0.053 | 0.62 \pm 0.12 | 0.042 |
| Glucose 6-phosphate | 1.00 \pm 0.03 | 0.92 \pm 0.04 | 0.106 | 0.73 \pm 0.03 | <0.001 | 0.38 \pm 0.04 | <0.001 |
| Glyceraldehyde 3-phosphate | n.d. | n.d. | | n.d. | | n.d. | |
| 3-Phosphoglycerate | 1.00 \pm 0.05 | 1.03 \pm 0.08 | 0.771 | 0.80 \pm 0.07 | 0.026 | 0.51 \pm 0.06 | <0.001 |
| Starch | 1.00 \pm 0.20 | 0.36 \pm 0.05 | 0.014 | 0.26 \pm 0.02 | 0.007 | 0.09 \pm 0.01 | 0.002 |