1   **Running head**: Photoperiod signaling via GRXS17

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3   Corresponding author: Pascal Rey

4   CEA, DSV, IBEB, Laboratoire d'Ecophysiologie Moléculaire des Plantes, Saint-Paul-lez-

5   Durance, F-13108, France

6   Phone: ++33 442254776

7   E-mail: pascal.rey@cea.fr

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9   **Research area:** Signaling and Response

10   **Secondary research area:** Genes, Development and Evolution

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Arabidopsis glutaredoxin S17 and its partner NF-YC11/NC2α contribute to maintenance of the shoot apical meristem under long-day photoperiod

Johannes Knuesting[1,†], Christophe Riondet[2,3†], Carlos Maria[4], Inga Kruse[5], Noëlle Bécuwe[6,7,8], Nicolas König[1], Carsten Berndt[9,10], Sébastien Tourrette[6,7,8], Jocelyne Guilleminot-Montoya[2,3], Enrique Herrero[4], Frédéric Gaymard[11], Janneke Balk[5], Gemma Belli[4], Renate Scheibe[1], Jean-Philippe Reichheld[2,3], Nicolas Rouhier[12,13] and Pascal Rey[6,7,8*]

[1]Department of Plant Physiology, FB5, University of Osnabrück, D-49069 Osnabrück, Germany

[2] Laboratoire Génome et Développement des Plantes, Université Perpignan Via Domitia, F-66860 Perpignan, France

[3]Laboratoire Génome et Développement des Plantes, CNRS, F-66860 Perpignan, France

[4] Departament de Ciències Mèdiques Bàsiques, IRBLleida, Universitat de Lleida, Montserrat Roig 2, 25008 Lleida, Spain

[5] Department of Biological Chemistry, John Innes Centre, Norwich NR4 7UH, United Kingdom

[6] CEA, DSV, IBEB, Laboratoire d'Ecophysiologie Moléculaire des Plantes, Saint-Paul-lez-Durance, F-13108, France

[7]CNRS, UMR 7265 Biologie Végétale & Microbiologie Environnementale, Saint-Paul-lez-Durance, F-13108, France

[8] Aix-Marseille Université, Marseille, F-13284, France

[9] Division for Biochemistry, Department for Medical Biochemistry and Biophysics, Karolinska Institutet, 17177 Stockholm, Sweden

[10] Heinrich-Heine-University, Department of Neurology, Medical Faculty, Merowinger Platz 1a, 40225 Düsseldorf, Germany

[11] Biochimie et Physiologie Moléculaire des Plantes, Centre National de la Recherche Scientifique, Institut National de la Recherche Agronomique, Université Montpellier, Montpellier Cedex 1, France

[12] Université de Lorraine, Interactions Arbres - Microorganismes, UMR1136, F-54500 Vandoeuvre-lès-Nancy, France

[13] INRA, Interactions Arbres - Microorganisms, UMR1136, F-54280 Champenoux, France

[†] The authors have equally contributed to the work

[*] Corresponding author: Pascal Rey

58 **Summary**:

59 The unusual multidomain glutaredoxin S17 and its partner, the nuclear factor NF-
60 YC11/NC2α, control plant development and flowering time in relation to photoperiod in
61 *Arabidopsis thaliana*.

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96 **Corresponding author** : Pascal Rey

97 CEA, DSV, IBEB, Laboratoire d'Ecophysiologie Moléculaire des Plantes, Saint-Paul-lez-

98 Durance, F-13108, France

99 Phone: ++33 442254776

100 E-mail: pascal.rey@cea.fr

101   **Abstract**

102   Glutaredoxins (GRXs) catalyse the reduction of protein disulfide bonds using

103   glutathione as a reductant. Certain GRXs are able to transfer iron-sulfur (Fe-S) clusters to

104   other proteins. To investigate the function of *Arabidopsis thaliana* GRXS17, we applied a

105   strategy combining biochemical, genetic and physiological approaches. GRXS17 was

106   localized in the nucleus and cytosol, and its expression was elevated in the shoot meristems

107   and reproductive tissues. Recombinant GRXS17 bound Fe_2S_2 clusters, a property likely

108   contributing to its ability to complement the defects of a yeast strain lacking the mitochondrial

109   GRX5. However, a *grxs17* knock-out Arabidopsis mutant exhibited only a minor decrease in

110   the activities of Fe-S enzymes, suggesting that its primary function is as a disulfide

111   oxidoreductase. The *grxS17* plants were sensitive to high temperature and long-day

112   photoperiod, resulting in elongated leaves, compromised shoot apical meristem, and delayed

113   bolting. Both environmental conditions applied simultaneously led to a growth arrest. Using

114   affinity chromatography and split-YFP methods, a nuclear transcriptional regulator termed

115   NF-YC11/NC2α was identified as a GRXS17 interacting partner. A mutant deficient in NF-

116   YC11/NC2α exhibited similar phenotypes to *grxs17* in response to photoperiod. Therefore,

117   we propose that GRXS17 interacts with NF-YC11/NC2α to relay a redox signal generated by

118   photoperiod to maintain meristem function.

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Introduction

Glutaredoxins (GRXs) are small oxidoreductases structurally related to thioredoxins (TRXs) and present in most organisms (Rouhier et al., 2008; Meyer et al., 2009). Their capacity to reduce disulfide bonds is usually dependent on glutathione (GSH) and relies on a 4-residue active-site motif comprising at least one redox-active cysteine (Rouhier et al., 2006). In higher plants, GRXs are encoded by multigene families and subdivided into four classes (Couturier et al., 2009). GRXs from classes I and II are present in all photosynthetic organisms and possess in most cases the motifs CPXC and CGFS as active sites, respectively. GRXs from class III are restricted to terrestrial plants and display a CCXX motif. Class-IV GRXs are present both in algae and in terrestrial plants and are composed of three domains, one N-terminal GRX module carrying a CXXC/S motif followed by two domains of unknown function.

Through their biochemical function as disulfide reductases, GRXs are thought to alter the activity of metabolic enzymes and transcriptional factors (Michelet et al., 2005; Murmu et al., 2010; Couturier et al., 2014). They also participate in the regeneration of thiol-dependent antioxidant enzymes (Rouhier et al., 2001; Gama et al., 2007; Tarrago et al., 2009; Couturier et al., 2011). Besides, other functions have been proposed for class I and II GRXs owing to their capacity to bind iron-sulphur (Fe-S) clusters (Rouhier et al., 2007; Bandyopadhyay et al., 2008; Rouhier et al., 2010). For instance, oxidized glutathione promotes Fe-S cluster disassembly from human GRX2 and restores disulfide reductase activity, therefore class-I GRXs may constitute redox sensors (Lillig et al., 2005). GRXs belonging to class II, also named monothiol GRXs, seem intimately linked to iron metabolism. Those present in mitochondria participate in Fe-S cluster assembly, most likely as Fe-S carriers from scaffold proteins to acceptor proteins (Rodriguez-Manzaneque et al., 2002; Mühlenhoff et al., 2003; Bandyopadhyay et al., 2008). In addition, nucleo-cytosolic monothiol GRXs participate in iron sensing and trafficking in yeast and animals (Ojeda et al., 2006; Pujol-Carrion et al., 2006; Kumanovics et al., 2008; Mühlenhoff et al., 2010; Haunhorst et al., 2013). However, it is not known if GRXs play a role in Fe-S cluster assembly or iron sensing in plants.

Recently, essential roles of plant GRXs have been unveiled in developmental processes and stress responses. Several *Arabidopsis thaliana* GRXs from class III participate in the tolerance to photooxidative stress (Laporte et al., 2012) and in defence against pathogens (Ndamukong et al., 2007; La Camera et al., 2011). Others are required for proper reproductive development through interaction with bZIP-type TGA transcription factors

160 (Xing and Zachgo, 2008; Murmu et al., 2010). Concerning class-I GRXs, an Arabidopsis
161 mutant deficient in both GRXC1 and C2 has a lethal phenotype due to impaired embryo
162 development (Riondet et al., 2012). Among the four class-II GRXs (S14, S15, S16 and S17),
163 the plastidial S14 isoform participates in arsenic tolerance in a hyper-accumulating fern
164 (Sundaram et al., 2008) and is induced in response to high temperature in *A. thaliana*
165 (Sundaram and Rathinasabapathi, 2010). Tomato plants silenced for the expression of
166 *GRXS16*, encoding another plastid-localized GRX, display increased sensitivity to osmotic
167 stress (Guo et al., 2010). GRXS14 and GRXS15 are presumed to participate in responses to
168 oxidative stress (Cheng et al., 2006; Cheng, 2008). Concerning GRXS17, Arabidopsis
169 knockout plants growing at 28°C exhibited impaired primary root growth, impaired flowering
170 and altered sensitivity to auxin (Cheng et al., 2011). Consistently, ectopic expression of
171 Arabidopsis GRXS17 in tomato plants resulted in enhanced thermo-tolerance (Wu et al.,
172 2012).

173   In this work, we examined the physiological role of Arabidopsis GRXS17, which
174 belongs to class II and has three CGFS active sites, in relation to its biochemical functions.
175 Recombinant GRXS17 incorporated Fe_2S_2 clusters and complemented the yeast *grx5* mutant.
176 However, in plants, GRXS17 had a minor role in Fe-S cluster metabolism, as the activities of
177 cytosolic Fe-S-dependent enzymes were not substantially altered in *grxs17* mutant plants. On
178 the other hand, *grxS17* plants exhibited severe developmental defects as a consequence of a
179 perturbed shoot meristem, specifically at elevated temperatures and in long-day conditions.
180 We show that GRXS17 interacts with a nuclear transcriptional factor, NF-YC11/NC2α, which
181 is also involved in the control of plant development as a function of photoperiod duration. Our
182 data indicate that GRXS17 plays an important role in meristem maintenance and suggest that
183 this role is fulfilled via the relay of a redox-dependent signal to NF-YC11/NC2α.

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Results

Expression of *GRXS17* in Arabidopsis plant organs and subcellular localization

To investigate the function of *GRXS17 in planta,* we first analyzed its expression pattern. Previously published quantitative RT-PCR and promoter-GUS fusion data showed high *GRXS17* expression in growing leaves and anthers of *A. thaliana* (Cheng et al., 2011). To gain more detail, the GRXS17 protein abundance in various organs was determined using a serum raised against the whole recombinant protein. The serum specifically recognized a protein at *ca.* 50 kDa as shown by Western blot analysis of flower protein extracts from WT and the signal was absent in homozygous *grxS17* plants (Fig. 1A; Fig. S1). GRXS17 was substantially more abundant in stems, young leaves and flowers. Note that the electrophoretic mobility of GRXS17 varies in leaf samples due to the proximity of RubisCO, which appears as a light grey background band in *grxS17* extracts (Fig. 1A-B). *In situ* hybridization was performed on shoot apical meristem (SAM) and flowers of Arabidopsis WT plants. A strong digoxigenin staining was found in all meristematic cells, particularly in stem cells (Fig. 1C-D), and in pollen and ovules (Fig. 1E-F). These data reveal that *GRXS17* is expressed in very different cell types localized in meristematic areas or in reproductive and vascular organs.

In order to determine the subcellular localization of GRXS17, transient expression in protoplasts and stable expression of *P35S:GRXS17:GFP* fusion were undertaken. The results indicated that the protein is targeted to both cytosol and nucleus (Fig. 2A-B). The nuclear localization is surprising considering the absence of a recognizable nuclear localization signal in GRXS17 sequence and the size of the fusion protein, which should be too big to freely diffuse through nuclear pores. Therefore, we prepared nuclear and cytosolic fractions from Arabidopsis inflorescences. Their relative purity was verified using sera against cytosolic and nuclear markers, TRXh5 (Marchal et al., 2014) and NUC1 (Pontvianne et al., 2010), respectively. GRXS17 was detected in both fractions in agreement with GRXS17-GFP localization (Fig. 2C). No signal for GRXS17 was detected in mitochondrial or chloroplastic extracts (Fig. S2).
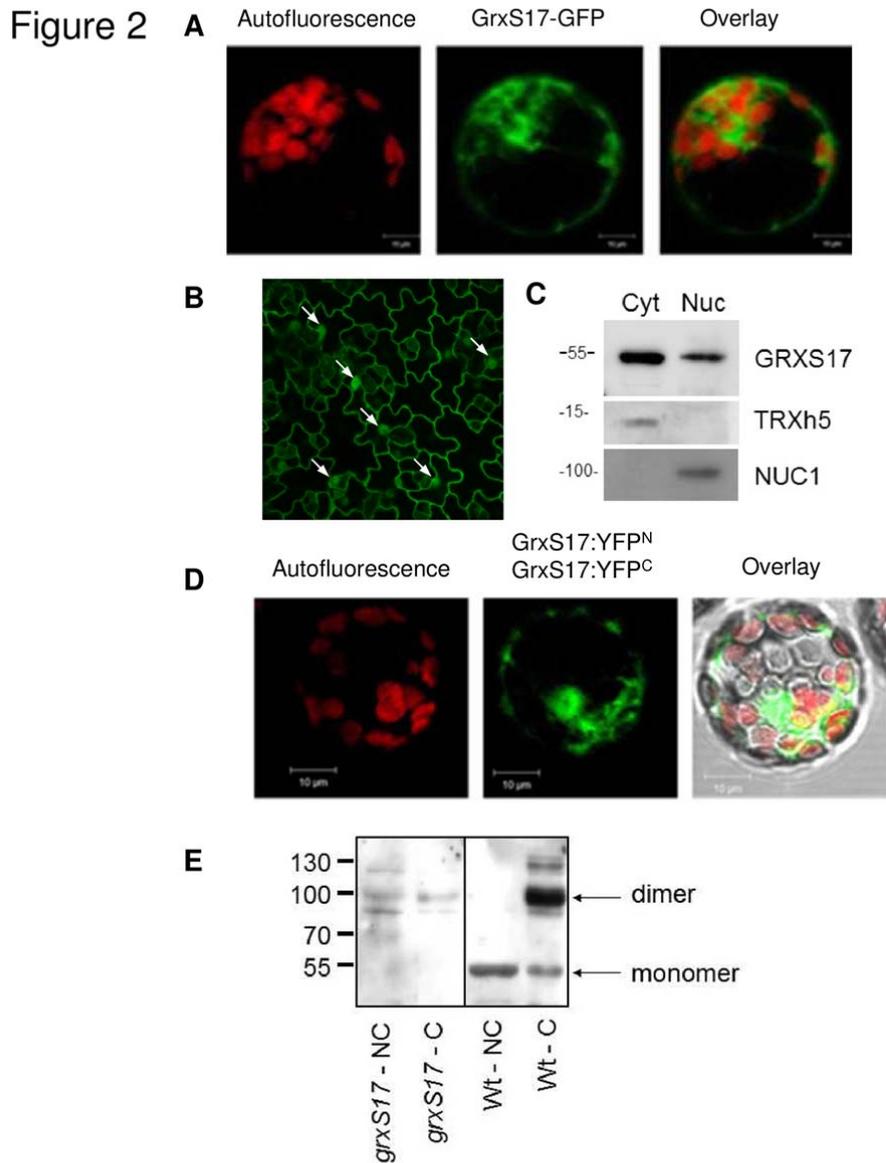
Figure 2. Subcellular localization and dimerisation of GRXS17.
(A) Transient expression of a GRXS17:GFP fusion in *A. thaliana* mesophyll protoplasts. Autofluorescence of chlorophyll indicates chloroplasts. (B) Stable expression of a GRXS17:GFP fusion in *Arabidopsis thaliana* leaves. White arrows indicate nuclei. (C) Immunodetection of GRXS17, thioredoxin TRXh5 and nucleolin NUC1 in cytosolic (Cyt) and nuclear (Nuc) extracts of Arabidopsis flower buds. (D) Bimolecular fluorescence complementation assay of GRXS17. Vectors encoding C- and N-terminal split-YFP fusions with GRXS17 were co-transformed into *Arabidopsis thaliana* mesophyll protoplasts. (E) Immunodetection of GRXS17 in *grxS17* and WT extracts cross-linked (C) or not (NC) with DMP.

214   Using bimolecular fluorescence complementation (BiFC) *i.e.*, transient expression of

215   two GRXS17 constructs fused to half YFPs in *Arabidopsis* protoplasts, we observed YFP

216   signal confirming the nucleo-cytosolic localization and indicating that GRXS17 forms dimers

217   in both compartments (Fig. 2D, Fig. S3). The ability of GRXS17 to dimerise *in vivo* was

Figure 1

Figure 1. Expression of *GRXS17* in *A. thaliana* plants.
(A-B) Western analysis of GRXS17 abundance. (A) Western blot analysis in flowers from WT and *grxS17* plants, and in organs of WT plants grown under standard conditions (20 μg per lane). The RubisCO large subunit (LSU) appears as a light grey background band in the *grxS17* lane. R, root from adult plant; S, stem from bolting plant; YL, young leaf; ML, mature leaf; OL, old leaf; B, floral bud; F, flower. (B) Loading controls: Coomassie blue stained gels (50-kDa region). (C-F) RNA *in situ* hybridization. Longitudinal sections of WT meristem inflorescences (C, D) and flower buds (E, F). Hybridizations were performed with antisense (C, E) or sense (D, F) GRXS17 cDNA probes.

218 further investigated in crude leaf extracts incubated with the cross-linker dimethyl
219 pimelimidate•2 HCl (DMP). Upon non-reducing SDS-PAGE and Western blot analysis, a
220 single band at 50 kDa was apparent in untreated WT samples and an additional 100-kDa

221 band, corresponding to a GRXS17 dimer, was specifically observed in the cross-linked extract

222 (Fig. 2E). These data indicate that the GRX dimer is not formed via disulfide bridging.

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224 **Development of the *grxS17* mutant in response to photoperiod**

225   To analyse the physiological function of AtGRXS17, we isolated homozygous *grxS17*

226 knockout plants from the Arabidopsis SALK_021301 line (Fig. S1) and transformed this line

227 with the *GRXS17* cDNA under the control of the CaMV-35S promoter. Two independent

228 *grxS17 GRXS17* complemented lines, termed 3.3 and 17.8, were generated and transgene

229 expression was confirmed at the protein level (Fig. S1). The phenotype characteristics of

230 *grxS17* mutant and complemented lines were investigated under various light and temperature

231 conditions. When cultivated under standard conditions (22°C/18°C regime, 8-h photoperiod

232 and 200 µmoles photons m^{-2} s^{-1}), all lines showed a similar development (Fig. 3A). Transfer

233 of 2.5-week old seedlings to 28°C and standard light for 2.5 weeks strongly impaired

234 development of all genotypes, which displayed thin and elongated leaves. In addition, the

235 *grxS17* mutant failed to form new leaves, which was partially or entirely complemented in

236 lines 3.3 and 17.8, respectively (Fig. 3B). These data indicate that GRXS17 is required for

237 maintenance of the shoot apical meristem at high temperature, consistent with the previously

238 reported thermo-sensitivity of the *grxS17* line (Cheng et al., 2011).

239   When plants were grown at 22°C and moderate light, but under long-day photoperiod

240 (16/8h), we observed that 4-week old *grxS17* plants displayed elongated and thickened lamina

241 (Fig. 3C). The development of the main floral spike (raceme) was delayed, which entirely

242 failed to form when plants were shifted to continuous light (Fig. 3D). To analyse whether the

243 phenotype appearance is due to day length or related to the total light inception, plants grown
244 for 2 weeks under standard conditions were transferred to high light (500 µmol photons m^{-2}
245 s^{-1}), 22°C and short-day conditions. After 3 weeks, there was no change in *grxS17*
246 development in this light regime (Fig. S4A), indicating that photoperiod duration is the

247   primary determinant for the observed phenotype. When plants were grown under the same

248   high light intensity under long days, *grxS17* exhibited strongly impaired development (Figs.

249   S4B, S5G). It is worth mentioning that the temperature measured at the plant level is elevated

250   by 2°C (24°C) in high-light conditions, thus possibly explaining the more severe phenotype in

Figure 3
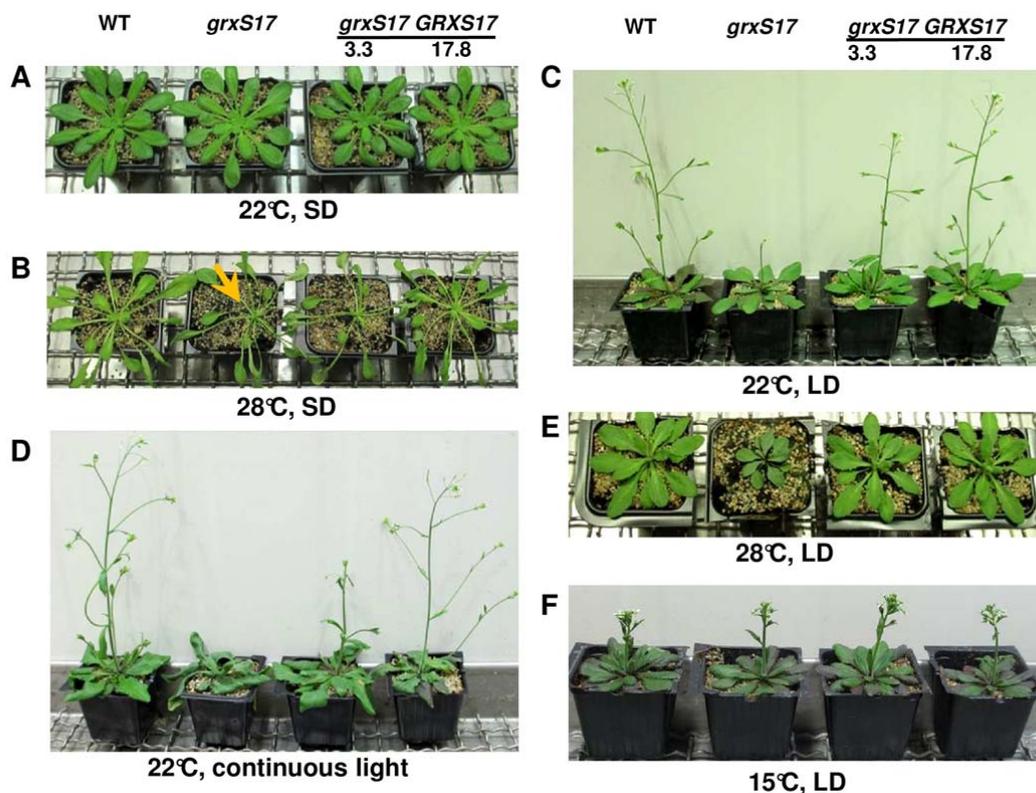
Figure 3. Growth and development of plants modified in *GRXS17* expression as a function of photoperiod and temperature.
(A) Five-week old plants grown in standard conditions (8-h photoperiod, 200 µmol photons.m^{-2}.s^{-1}) at 22°C. (B) Plants grown for 2.5 weeks in standard conditions and transferred to 28°C (8-h photoperiod, 200 µmol photons.m^{-2}.s^{-1}) for 2.5 weeks. The arrow indicates the absence of young leaves. (C) Plants grown in long-day photoperiod conditions (16 h, 200 µmol photons.m^{-2}.s^{-1}) at 22°C. (D) Plants grown for 2 weeks in standard conditions and transferred to continuous light (200 µmol photons.m^{-2}.s^{-1}, 22°C) for 3 weeks. (E) Plants grown for 2 weeks in standard conditions and transferred to 28°C and long-day photoperiod (16 h, 200 µmol photons.m^{-2}.s^{-1}) for 2.5 weeks. (F) Plants grown for 3 weeks in standard conditions and transferred to 15°C and long-day photoperiod (16 h, 200 µmol photons.m^{-2}.s^{-1}) for 4 weeks. WT, Wild-type plants; *grxS17*, homozygous SALK_021301 plants; *grxS17 GRXS17* 3.3 and 17.8, two independent *grxS17* lines expressing *GRXS17*. SD and LD, short (8 h) and long (16 h) day.

251 long day at high light compared to moderate light. Under this light regime, there was no
252 visual evidence of photooxidative damage in *grxS17* leaves (Fig. S4B). This was confirmed
253 by autoluminescence imaging (Fig. S6), which allows recording of the photon emission
254 associated with lipid peroxidation (Havaux et al., 2006). When combining high temperature

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255   and long day, we observed that *grxS17* growth stopped after a few days (Fig. 3E).
256   Noteworthy, the growth and reproductive development of *grxS17* plants cultivated for 4
257   weeks in long-day conditions at 15°C was not modified (Fig. 3F). Similarly, when young
258   plants were transferred to long-day conditions at 15°C and high light (500 µmol photons m^{-2}
259   s^{-1}), no alteration was noticed in *grxS17* (Fig. S4C). Altogether, these data reveal that plants
260   deficient in GRXS17 display sensitivity to a long-day regime in a temperature-dependent
261   manner.

262   When *grxS17* plants were grown under long-day and high-light conditions, a
263   significant delay of bolting was observed. These plants only formed secondary floral spikes
264   after some time (Fig. S5B, D, F and G). In short-day conditions, floral development was even
265   accelerated Fig. S5A, C and E), while *grxS17* vegetative growth was not affected.
266   Complemented lines exhibited contrasting phenotypes under short day, one line (3.3)
267   flowering like the mutant and the other (17.8) like WT. The difference could originate from
268   the much higher GRXS17 amount in the latter (Fig. S1). Collectively, these data point to the
269   central role of GRXS17 in conveying environmental variations, such as temperature and day
270   length, to coordinate the flowering response in plants.

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272   **The shoot apical meristem is compromised in the *grxS17* mutant**

273   As floral induction depends on the transition of the shoot apical meristem (SAM) from

274   vegetative to reproductive fate (Levy and Dean, 1998), we performed histological analysis of

275   SAM in *grxS17* mutants. The SAM overall structure was not altered in the mutant grown in

276   short-day conditions (Fig. S7). On the contrary, when grown under long days, the meristem

277   area was smaller in *grxS17* compared to WT (Fig. 4A). The cell numbers in L1, L2 and L3

278   layers were 45% lower in *grxS17* than in WT, revealing impairment in the division of stem

279   cells (Fig. 4C). Moreover, the size of meristematic cells was noticeably increased in the

280   mutant (Fig. 4A), suggesting that the lower cell division rates are associated with increased

281   cell expansion. These changes in meristematic cell size and numbers are consistent with the

282   high *GRXS17* expression level observed in the meristem (Fig. 1C) and likely lead to the

283   impaired development of *grxS17* plants observed under conditions of long-day photoperiod

284   and/or high temperature (Fig. 3; Fig. S5). Histological analysis of mesophyll cells in plants

Figure 4
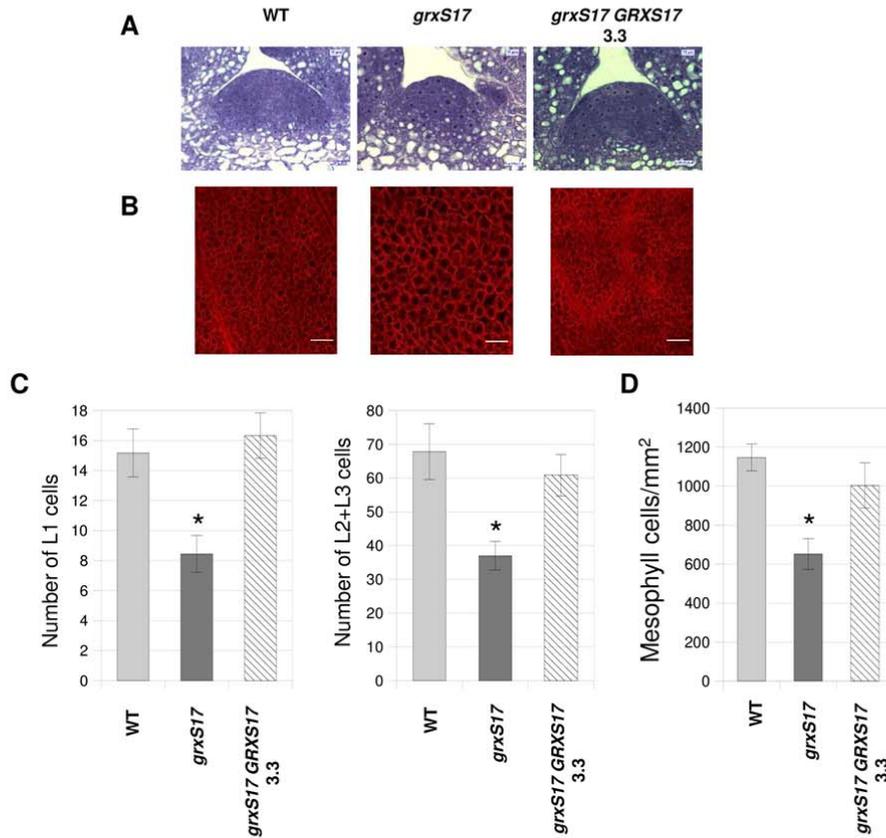
Figure 4. Structure of shoot apical meristem (SAM) and leaves in grxS17 plants.
(A) Histological structure of the SAM stained by toluidine blue in 7-day old wild-type, *grxS17* and *grxS17 GRXS17* (line 3.3) plants grown in long-day conditions (16 h) and high light (500 µmol photons.m^{-2}.s^{-1}). (B) Observation of mesophyll cells in leaves of 3-week old plants grown under long-day/high-light conditions. (C) Number of L1, L2 and L3 layer stem cells in SAM cross-sections shown in (A). Ten sections per genotype were analyzed. (D) Density of mesophyll cells in the sections shown in (B) (n: 12). *, value significantly different from Wt value with $p < 0.05$ (t-test).

285   grown under long-day and high-light conditions revealed a reduced cell density in mutant

286   plants (652 ± 79 cells.mm^{-2}) compared to WT (1148 ± 68 cells.mm^{-2}) and a much larger cell

287   size (Fig. 4B, D). These data indicate that GRXS17 is required for cell division under long-

288   day conditions.

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AtGRXS17 architecture and capacity to bind Fe-S clusters

To investigate the biochemical function of AtGRXS17, and how this could affect meristem development, we first analyzed the capacity of the protein to bind Fe-S clusters. *A. thaliana* GRXS17 possesses an N-terminal TRX-like domain with a WCDAS motif in place of the canonical WCGPC active site, followed by three GRX domains containing CGFS motifs (Fig. 5A). This architecture is unique to land plants, since mammalian, fungal and algal homologs consist of one TRX and maximally two GRX domains (Couturier et al., 2009). The GRX modules of GRXS17 share 62 to 65% identity and are subsequently referred to as M2, M3 and M4. From secondary structure prediction and 3D-structure modeling, the four AtGRXS17 domains all adopt a classical TRX fold and are connected by long linker sequences (Fig. S8). The capacity of recombinant AtGRXS17 to incorporate Fe-S clusters like other Arabidopsis CGFS GRXs (Bandyopadhyay et al., 2008) was analyzed after anaerobic *in vitro* reconstitution mediated by the cysteine desulfurase IscS in the presence of glutathione. Indeed, upon purification of GRXS17, the oxygen-sensitive Fe-S clusters are lost; therefore reconstitution of the clusters guarantees a sufficient amount of holo-GRXS17 for spectroscopy analysis. The UV-visible spectrum of the reconstituted GRXS17 showed absorbance peaks at 320 nm and around 420 nm similar to other Fe-S cluster coordinating GRXs and typical for Fe_2S_2 clusters (Fig. 5B). Estimation of the iron content in a freshly reconstituted WT protein indicated the presence of 2.48 ± 0.58 Fe atoms/monomer. To investigate the contribution of each domain to cluster binding, the active-site cysteines were replaced by serines, either individually or in all three GRX domains. Whereas the triple-Cys mutant (C179/309/416S) did not incorporate any Fe-S cluster upon *in vitro* reconstitution, variants carrying one single substitution all incorporated between 40 to 60% of clusters as assessed by relative absorbance measurements at 420 nm (Fig. 5C). Since each of the active site cysteines of the GRX-subunits contributed to Fe_2S_2 incorporation, these data - together with the quantification of Fe - indicate that AtGRXS17 dimers incorporate three Fe_2S_2 clusters *in vitro*, involving each GRX domain. Furthermore, the stoichiometry indicates that the GSH included in the reconstitution assay acts as a Fe-S cluster ligand as described for all other CGFS GRXs.

A. thaliana GRXS17 rescues most yeast _grx5_ mutant phenotypes

Figure 5
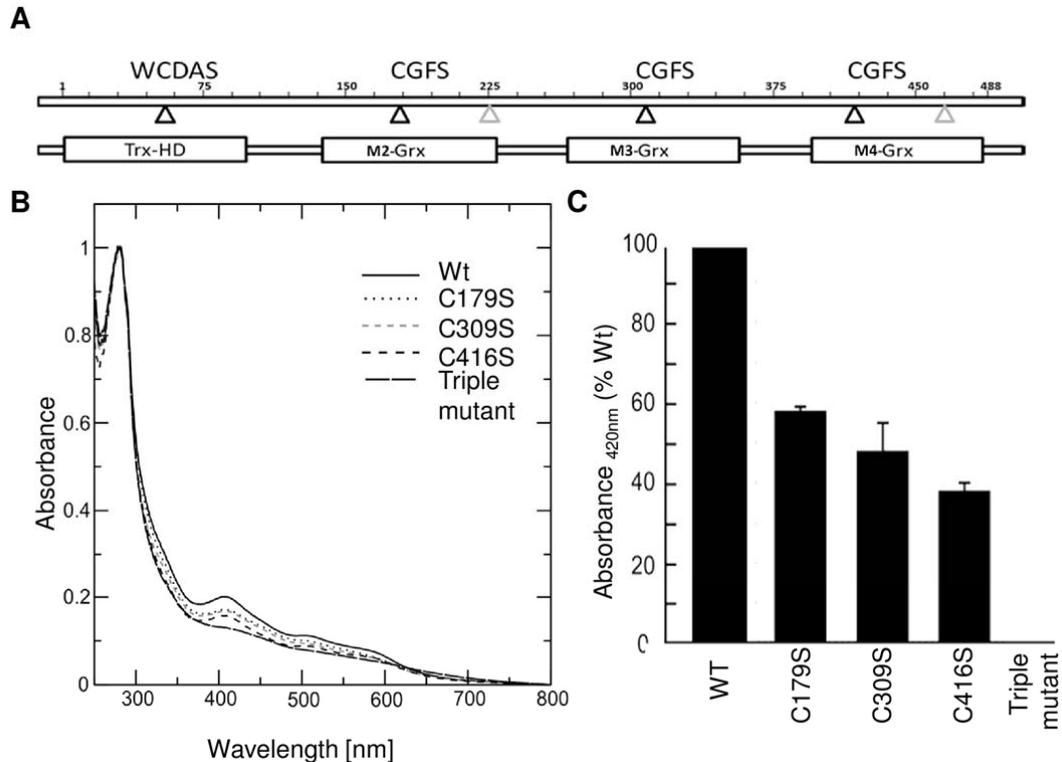
Figure 5. Incorporation of Fe-S clusters into recombinant WT and mutated AtGRXS17.
(A) Domain structure of GRXS17. TRX-HD: TRX-like homology domain; M2-GRX, M3-GRX and M4-GRX: three monothiol-GRX domains. Positions of active-site cysteines are indicated by black triangles and that of other cysteine by a grey triangle. (B) Absorption spectra of GRXS17 and cysteine mutants. UV-visible absorption spectra were recorded immediately after *in vitro* reconstitution in anaerobic conditions. The active-site cysteines of each GRX domain were individually or together substituted by serines (M2:C179S; M3: C309; M4: C416S; C179/309/416S). (C) Relative absorption at 420 nm of GRXS17 mutants.

321   To further investigate a possible role of GRXS17 as a Fe-S cluster carrier, its capacity

322   to rescue the defects of a yeast *grx5* mutant was examined. The entire protein or the three

323   individual GRX domains were fused to the Grx5 mitochondrial targeting sequence and a C-

324   terminal haemaglutinin (HA) tag. The constructs were expressed in Baker's yeast,

Figure 6
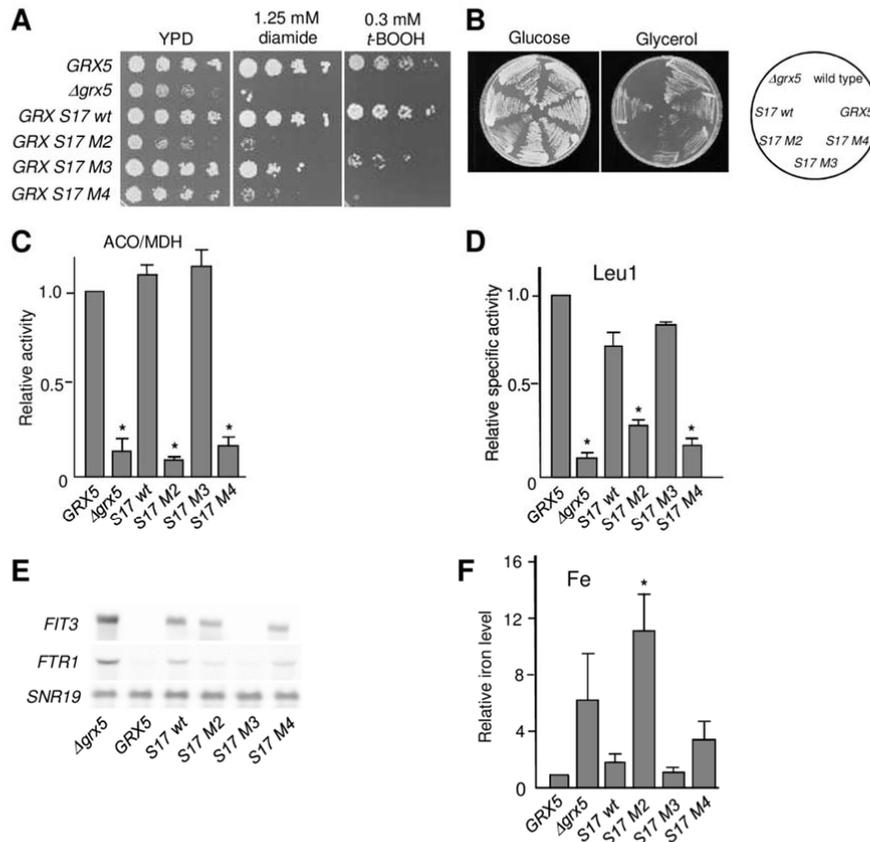
Figure 6. Rescue of the *S. cerevisiae grx5* mutant defects by AtGRXS17.
(A) Sensitivity to *t*-BOOH or diamide after 3 days at 30˚C on YPD plates. (B) Growth on glucose (YPD plates) or glycerol (YPGly plates) after 3 days at 30˚C. (C) Relative ratio between aconitase and malate dehydrogenase activities, normalized with respect to the ratio in the WT strain, inexponential cultures at 30˚C in YPGalactose medium. (D) Relative specific Leu1 activity. (E) Northern blot analysis of *FIT3* and *FTR1* mRNA levels from exponential cultures at 30˚C. Loading control: *SNR19* mRNA. (F) Relative iron content from exponential cultures at 30˚C in YPD medium. Mean ± SD (n: 3). Asterisks indicate statistically significant differences (Student's *t*-test, p<0.05) compared to WT (*GRX5*) cultures.

Saccharomyces cerevisiae and the localization of the Arabidopsis proteins in the mitochondrial matrix was confirmed by Western blot analysis (Fig. S9). Phenotype studies indicated that only the entire protein (WT), and the M3 module to a lesser extent, rescued the sensitivity to two externally added oxidants, for which yeast *grx5* mutant cells are

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329   hypersensitive (Rodriguez-Manzaneque et al., 2002), *tert*-butyl hydroperoxide (*t*-BOOH,

330   causing general oxidative damage on cellular macromolecules) and diamide (specific oxidant

331   of thiol groups) (Fig. 6A). When grown under obligate respiratory conditions (glycerol as

332   carbon source), both the M3 and M4 modules, in addition to the entire GRXS17 molecule,

333   totally or partially rescued the *S. cerevisiae grx5* defective phenotype (Fig. 6B). The M3

334   module also fully rescued, like GRXS17, the ability to express active aconitase holoenzyme

335   (Fig. 6C) and mostly restored isopropylmalate isomerase (Leu1) activity (Fig. 6D). These

336   two Fe-S containing enzymes are located in mitochondria and cytosol, respectively, and both

337   are dependent on the mitochondrial Fe-S cluster assembly pathway. We then checked the

338   expression of iron uptake genes by determining the transcript levels of two reporter genes of

339   the Aft1 regulon, *FTR1* and *FIT3*. Of the three modules, only M3 restored repression of both

340   genes in *grx5* cells, while the entire GRXS17 molecule was unable to repress *FTR1* and *FIT3*

341   expression (Fig. 6E), indicating that one component participating in iron status signalling is

342   still deficient and that this could be due to some steric incompatibility linked to the modular

343   architecture of GRXS17. Accordingly, the M3 domain suppressed iron accumulation in *grx5*.

344   Of note, expression of the entire GRXS17 protein also prevented iron accumulation (Fig.

345   6F). To summarize, the entire AtGRXS17 rescued most *grx5* phenotypes, notably the

346   activities of Fe-S containing enzymes.

347

348   **GRXS17 plays a minor role in maintaining the activity of cytosolic Fe-S enzymes**

349   Next, we investigated whether GRXS17 fulfils a role in Fe-S cluster assembly in *A.*
350   *thaliana*. Therefore, activities and/or abundance of Fe-S enzymes were analyzed, including
351   aconitases (one Fe_4S_4), aldehyde oxidases (two Fe_2S_2, FAD and molybdenum cofactors) and
352   photosystem I (PSI, three Fe_4S_4). All measurements were performed using 2-week old

353　　seedlings grown under conditions where mild phenotypic changes are visible (22°C and 16-h

354　　light) or under conditions leading to severely impaired growth in *grxS17* (plants shifted to

355　　28°C, 16-h light) (Fig. S10A). Two mutant alleles of *ATM3* (*ABC TRANSPORTER OF THE*

356　　*MITOCHONDRIA 3/ABCB25*), encoding a transporter that provides persulfide for cytosolic

357   Fe-S cluster assembly (Schaedler et al., 2014) were used for comparison. The *atm3-1* and

358   *atm3-4* mutants are strong and weak mutant alleles, respectively, that have significantly

359   decreased activities of aldehyde oxidases and cytosolic aconitase (Bernard et al., 2009).

360   Protein extracts from leaf samples were separated by native gel electrophoresis, followed by

Figure 7

Figure 7. Activities and levels of Fe-S enzymes in plants modified in *GRXS17* expression.
Wild type (WT), a *grxS17* knock-out mutant and two complemented lines (3.3 and 17.8) were grown for 14 days under standard conditions (22℃) or t transferred to 28℃ after 7 days (Fig. S10). Mutant alleles in the *ABC Transporter of the Mitochondria 3* (*atm3-1* and *atm3-4*)were used for comparison. (A) Activities of aconitase (ACO) and aldehyde oxidase (AO) isozymes visualized as a formazan precipitate in a native gel assay. Cytosolic (cyto) and mitochondrial (mito) isoforms of ACO are indicated. Equal protein amounts (160 μg for ACO and 60 μg for AO) were loaded per lane. Immunoblotting of ACO and PsaA (a subunit of PSI) in Arabidopsis seedlings (10 μg protein per lane) and loading control (Ponceau). (B) Relative activities of cytosolic and mitochondrial aconitase isoforms quantified using ImageJ software. The values are given as percentages of the sum of the band intensities per lane (mean ± SD). The data are representative of 3 independent experiments.

361   in-gel activity staining. Part of the same protein extract was subjected to denaturing SDS-
362   PAGE and Western blotting to estimate protein levels of aconitase and PSI. As expected for a
363   cytosolic protein, GRXS17 is not required for the maturation of mitochondrial (aconitases)

and plastidial (photosystem I) Fe-S proteins (Fig. 7A). In *grxS17* plants grown at 22°C, the amount of total aconitase protein was approximately 40% of WT, corresponding to a similar decrease in activity of the cytosolic isoform (Fig. 7A-B). Aldehyde oxidase activity was decreased to ca. 50% in *grxS17* at this temperature, compared to less than 5% in *atm3-4* (Fig. 7A, left panel; Fig. S10B). In the complemented 3.3 line, the activities at 22°C of cytosolic aconitase and aldehyde oxidase were partially restored, and fully restored in the 17.8 line. In *grxS17* plants grown at 28°C, the activities of the mitochondrial aconitase isoforms was increased and total aconitase protein levels were close to WT levels (Fig. 7A-B, right panels). Further, there was no difference in aldehyde oxidase activity at this temperature (Fig. S10B, right panel). In comparison with the *atm3* alleles, neither of which is a knock-out, the *grxS17* knock-out mutant displayed a relatively moderate decrease in cytosolic Fe-S enzymes in environmental conditions that lead to strongly impaired development. Taken together, these data suggest that GRXS17, despite its capacity to bind Fe-S clusters *in vitro* and to rescue the yeast *grx5* mutant, does not play a critical function in *de novo* synthesis of Fe-S clusters *in planta*.

Identification of a nuclear factor interacting with GRXS17

To explore other possibilities for how GRXS17 functions in meristem development, we performed affinity chromatography using a Ni-matrix loaded with His-tagged GRXS17 to identify interacting proteins. After applying a crude leaf extract, the bound proteins were eluted with DTT, and identified by mass-spectrometry. A number of proteins were repeatedly isolated in 15 independent affinity experiments (Table I). Interestingly, the predicted or experimentally determined localization of most proteins, *e.g.* cytosol or nucleus, is comparable to that of GRXS17. In accordance with a role of GRXS17 in redox signaling pathways and with the known interaction of GRX3/PICOT with protein kinase C in animal cells, one transcription factor and one kinase were identified. The At3g12480 gene product, NF-YC11/NC2α, isolated in 7 out of 15 experiments, displayed high peptide sequence coverage. This partner was selected for deeper investigations because proteins of the NF-Y family are known nuclear factors regulating developmental processes (Kumimoto et al., 2010). Transient expression of NF-YC11/NC2α-GFP in *A. thaliana* protoplasts indicated that the protein is localized both in cytosol and nucleus (Fig. 8A), like GRXS17. The interaction between both proteins was further confirmed using BiFC exclusively in the nucleus (Fig. 8B; Fig. S3).

Figure 8

A Autofluorescence   GFP-NF-YC11/NC2α   Overlay

B Autofluorescence   GrxS17:YFP^C NF-YC11/NC2α:YFP^N Overlay
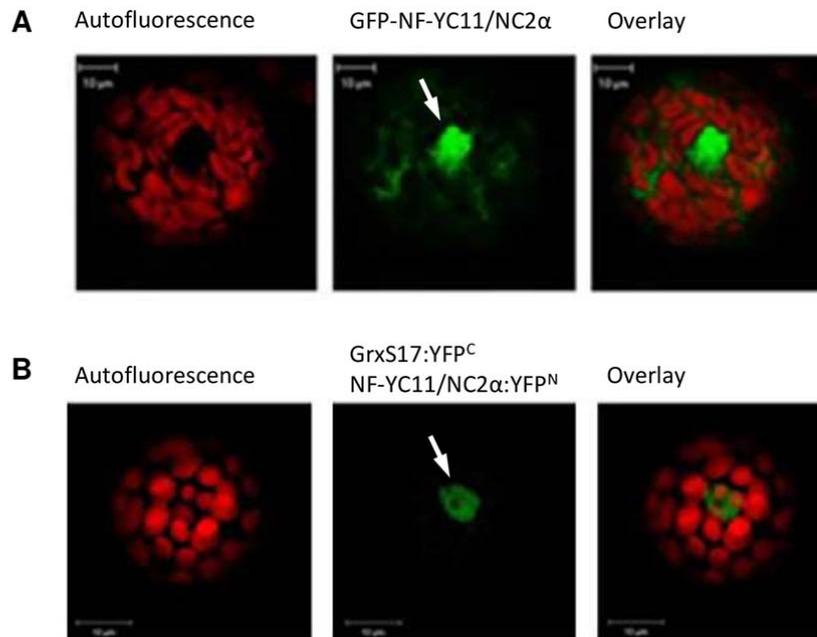
Figure 8. Localisation of NF-YC11/NC2α and interaction with GRXS17.
Isolated *A. thaliana* mesophyll protoplasts were transiently transformed with a NF-YC11/NC2α:GFP fusion (A) or with split-YFP constructs containing GRXS17 or NF-YC11/NC2α (B). White arrows indicate nuclei.

397

Development of *nf-yc11/nc2α* plants as a function of photoperiod

399   We isolated knockout plants for *NF-YC11/NC2α* expression from the GABI-Kat 042E02

400 line (Kleinboelting et al., 2012), and only 4% as opposed to the expected 25% homozygous

Figure 9
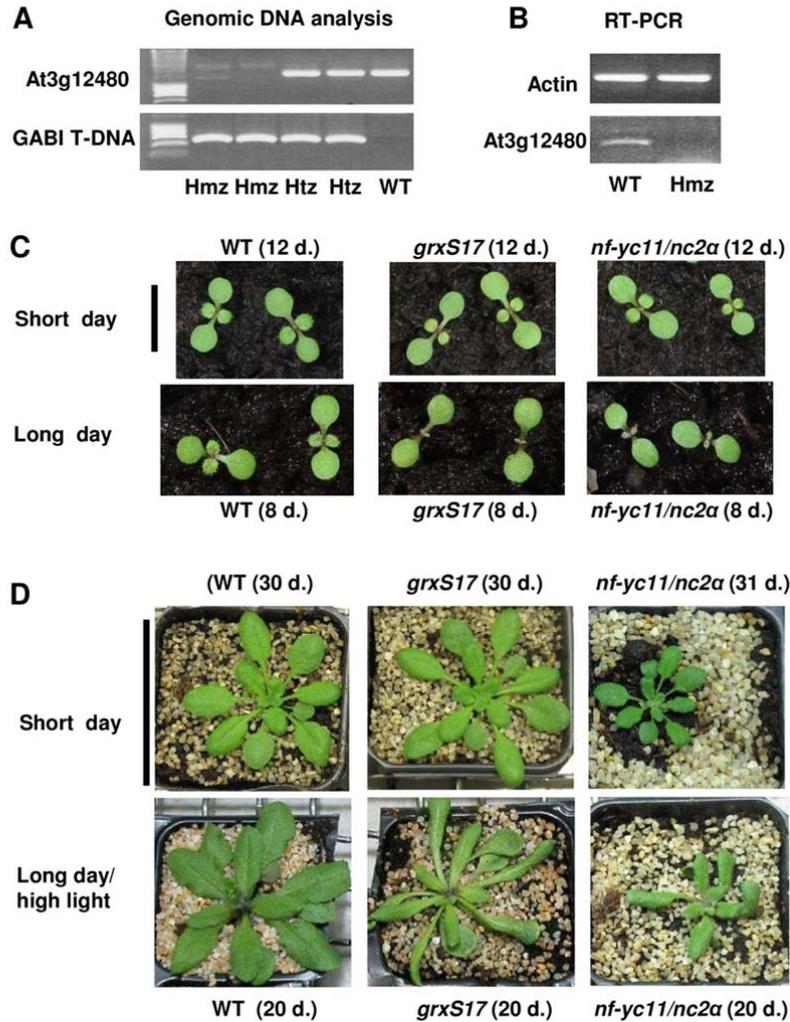
Figure 9. Characterization of a *nf-yc11/nc2α* mutant and comparison of the growth of *grxS17* and *nf-yc11/nc2α* plants as a function of photoperiod duration.
(A) PCR analysis of genomic DNA of At3g12480 and T-DNA in WT, heterozygous (Htz) and homozygous (Hmz) plants of the GABI-042E02 mutant line. (B) RT-PCR analysis of WT and homozygous plants. (C) Seedling development in short- and long-day conditions (8- and 16-h, respectively) at moderate light (200 µmol photons m^{-2} s^{-1}) and 22℃. The seedlings were grown simultaneously in the same conditions and the original photographs are shown in Fig. S 11. (D) Vegetative growth of plants in standard light conditions (short-day: 8-h photoperiod and moderate light, 200 µmol photons m^{-2} s^{-1}) and and in long-day and high-light conditions (16-h photoperiod, 500 µmol photons m^{-2} s^{-1}) at 22℃. The plant age is indicated for each genotyp e and culture condition. Scale bars: 1 and 6 cm in C and D, respectively.

401   *nf-yc11/nc2α* plants were recovered in the T2 progeny (Fig. 9A-B).When grown under short-
402   day conditions and moderate light, *nf-yc11/nc2α* plants showed slow and altered development
403   with a compact rosette, small leaves and a disorganized floral spike (Fig. 9D; Fig. S11C). We
404   transferred 33-day old *nf-yc11/nc2α* plants grown in short-day to long-day conditions and

31

405   observed a strongly reduced development of the main spike and many secondary

406   inflorescences compared to WT (Fig. S11C; Fig. 3C). We then compared the development of

407   WT, *nf-yc11/nc2α* and *grxS17* seedlings under short- and long-day conditions (Fig. 9C). We

408   did not observe any difference in germination and cotyledon development for the three

409   genotypes in both photoperiod conditions. While in short-day photoperiod, the first two leaves

410   appeared at the same time in the three lines, the development of these two leaves was

411   substantially delayed in *nf-yc11/nc2α* and *grxS17* plants in long-day conditions (Fig. 9C). We

412   analyzed the phenotype of *nf-yc11/nc2α* plants following sowing in long-day photoperiod and

413   high-light conditions. We observed that growth and development of these plants were more

414   perturbed than in control conditions (Fig. 9D). Particularly, *nf-yc11/nc2α* plants remained

415   much smaller than WT and *grxS17* plants. They exhibited elongated and distorted leaves, a

416   trait characteristic shared by the *grxS17* mutant. In these long-day conditions, no main floral

417   spike was observed and only secondary small spikes developed (Fig. S11C). Taken together,

418   these observations indicate that NF-YC11/NC2α participates in plant developmental processes

419   in relation to the photoperiod duration and reveal that *nf-yc11/nc2α* and *grxS17* plants share

420   similar developmental characteristics in long-day conditions.

421

422

423

424

425

Discussion

Physiological function of GRXS17 in relation to its biochemical properties

In this work, we showed that GRXS17 is a central element for plant development in relation to environmental factors such as photoperiod and temperature and we investigated whether a major function in Fe-S protein biogenesis may underpin its physiological role. Previous studies indicated that multi-domain GRX orthologs from yeast and vertebrates bind Fe-S clusters and modify the activity of iron-responsive transcriptional regulators (Ojeda et al., 2006; Pujol-Carron et al., 2006; Kumanovics et al., 2008; Mercier and Labbé 2009; Jbel et al., 2011), affecting the intracellular iron distribution through the maturation of most iron-containing proteins (Mühlenhoff et al., 2010; Haunhorst et al., 2013). Accordingly, we showed that AtGRXS17 is able to bind Fe_2S_2 clusters by *in vitro* reconstitution experiments and to complement the defects in Fe-S cluster maturation of the *S. cerevisiae grx5* strain (Figs 5-6). However, we found that GRXS17 in *A. thaliana* does not play a major role in Fe-S protein biogenesis. Although *grxS17* seedlings do have decreased activities of cytosolic aconitase and aldehyde oxidases in control conditions (Fig. 7), the effect is far less severe than that observed in the *atm3* mutant lines (Bernard et al., 2009). Moreover, the aldehyde oxidase activity, which depends on two Fe_2S_2 clusters, is not decreased in *grxS17* plants at 28°C, a temperature condition leading to a severe phenotype. Therefore, our data indicate that GRXS17 is not involved in *de novo* biosynthesis of cytosolic Fe_2S_2 clusters, in agreement with the viability of the knock-out mutant. Indeed, because many cytosolic and nuclear Fe-S proteins are essential, mutants in Fe-S cluster assembly are generally embryo lethal (Balk and Schaedler, 2014). An alternative explanation is that the function of GRXS17 is redundant or is compensated by another component of the Fe-S cluster assembly pathway. However, as GRXS17 is the only class-II GRX present in cytosol and nucleus, it is unlikely that such a function is fulfilled by another GRX. Taking into consideration the *in vitro* capacity of GRXS17 to bind Fe-S clusters and the variations observed in aconitase activity in *grxS17* plants, we can speculate that it protects Fe-S proteins from oxidative stress and destruction of clusters. Such a hypothesis will need further investigation.

We assumed a function of GRXS17 in connection with its redox properties and hypothesized that it participates in signalling pathways related to the changes in the cellular redox status occurring in response to environmental variations. Of note, AtGRXS17 has been recently reported as prone to H_2O_2-induced Cys sulfenation (Wasczak et al., 2014). Redox changes might affect the subcellular localization of AtGRXS17 or the set of interacting

459   partners through post-translational redox modifications. Concerning the first point, our
460   experiments (GFP-fusion and cellular fractionation) show that GRXS17 is localized in both
461   nucleus and cytosol (Fig. 2), and a former study indicated that high temperature induces
462   GRXS17 translocation from cytosol to nucleus (Wu et al., 2012). Thus, in response to
463   environmental signals or to a specific physiological state, the GRXS17 function might be
464   associated with nucleo-cytosolic shuttling. With regard to the 14 putative GRXS17 interaction
465   partners identified by affinity chromatography, their localization is consistent with an
466   interaction *in vivo*. Note that BOLA2, a possible transcriptional regulator interacting with
467   GRXS17 in binary yeast two-hybrid and BiFC experiments (Couturier et al., 2014), was not
468   isolated. This could originate from a low abundance or from the type of plant material used in
469   this work. Nevertheless, the data gained from affinity experiments clearly show the ability of
470   GRXS17 to interact with different types of partners, and thus to possibly modify their
471   conformation or regulate their activity through post-translational redox modification.
472

473   **GRXS17: a hub integrating hormonal and redox signals?**
474   Consistent with the pleiotropic phenotype of *grxS17* plants, Western data revealed the
475   presence of the GRXS17 protein in all organs, particularly those containing actively dividing
476   or elongating cells. In addition, *in situ* hybridization showed a high transcript level in apical
477   meristem and histological analyses of plants grown in long day specifically revealed a larger
478   cell size in both meristems and leaves of *grxS17* plants. All these data suggest a crucial role of
479   GRXS17 in meristem activity and cell division. Several studies provided evidence for a tight
480   relationship between intracellular redox status, disulfide reductases, and development
481   (Considine and Foyer, 2014). For instance, the development of root meristems is influenced
482   by changes in the overall redox status and auxin content/distribution (Vernoux et al., 2000;
483   Jiang et al., 2003; Yu et al., 2013). Another example is the *A. thaliana ntra ntrb cad2* triple
484   mutant, which is defective in TRX reduction and GSH synthesis, and exhibits strongly
485   impaired reproductive development in relation to altered auxin metabolism (Bashandy et al.,
486   2010). The plastidial TRXm3 is essential for meristem maintenance in Arabidopsis through a
487   role in symplastic permeability (Benitez-Alfonso et al., 2009) and the nucleoredoxin NRX1
488   participates in the establishment of pollen fertility (Qin et al., 2009; Marchal et al., 2014).
489   With regard to GRXs, the fate of germ cells in maize anthers is determined by the redox status
490   via a GRX, termed MSCA1, presumed to play a role in gene transcription (Kelliher and
491   Walbot, 2012). The nuclear class-III GRXs, ROXY1 and 2, are required for proper

492   development of floral organs in Arabidopsis likely through interaction with TGA transcription
493   factors (Xing and Zachgo, 2008; Hong et al., 2012). Altogether, these reports support the view
494   that disulfide reductases finely control plant development.
495   Arabidopsis *grxS17* plants display hypersensitivity to high temperature and altered auxin-
496   mediated signalling pathways in roots (Cheng et al., 2011). The present work unveils another
497   function for GRXS17 in integrating photoperiod signals for proper development. Temperature
498   is however an important determinant, since the phenotype is evident in long-day conditions at
499   22°C and 28°C, but not at 15°C. In this study, we mainly investigated the phenotype of aerial
500   parts of plants grown on soil and observed an altered shape of the leaves, which turned thick
501   and elongated and displayed a reduced number of large cells. Looking for mutants with a
502   similar phenotype (Bensmihen et al., 2008), we noticed that *drl1* and *elo* mutants exhibit
503   elongated leaves and a strongly reduced number of larger palisade cells compared to WT
504   (Nelissen et al., 2003; 2005). DRL1 regulates RNA polymerase II-mediated transcription
505   through the elongator complex, which is composed of several ELO proteins and displays
506   histone acetyltransferase activity preferentially in regions of auxin-related genes (Nelissen et
507   al., 2010). Importantly, the floral development of *drl1* plants is delayed and the root
508   development of both *elo* and *drl1* mutants is substantially reduced (Nelissen et al., 2003).
509   Interestingly, by investigating the genes co-expressed with *GRXS17* using Genevestigator
510   (Hruz et al., 2008), a high correlation was found with *ELO2* (At5g13680). It is thus tempting
511   to hypothesize that the *grxS17* phenotype is linked to defects in ELO- and/or DRL1-
512   dependent transcription mechanisms.
513
514   **Possible roles of GRXS17 in interaction with the NF-YC11/NC2α nuclear factor**

515   Among the GRXS17 partners identified using affinity chromatography, we focused on

516   the nuclear factor NF-YC11/NC2α. Because the interaction between this factor and the GRX

517   was confirmed by BiFC experiments in Arabidopsis protoplasts, a physiologically relevant

518   interaction can be assumed. The *A. thaliana* NF-Y family comprises three main types (A, B

519   and C). Initially named CCAAT-binding factor or Heme Activator Protein (HAP), they

520   usually form trimeric complexes of A, B and C subunits, binding to CCAAT-promoter

521   sequences and transcriptionally regulating genes participating in plant development and stress

522   responses (Dolfini et al., 2012; Laloum et al., 2013). In animal cells, they are central to cell

Figure 10
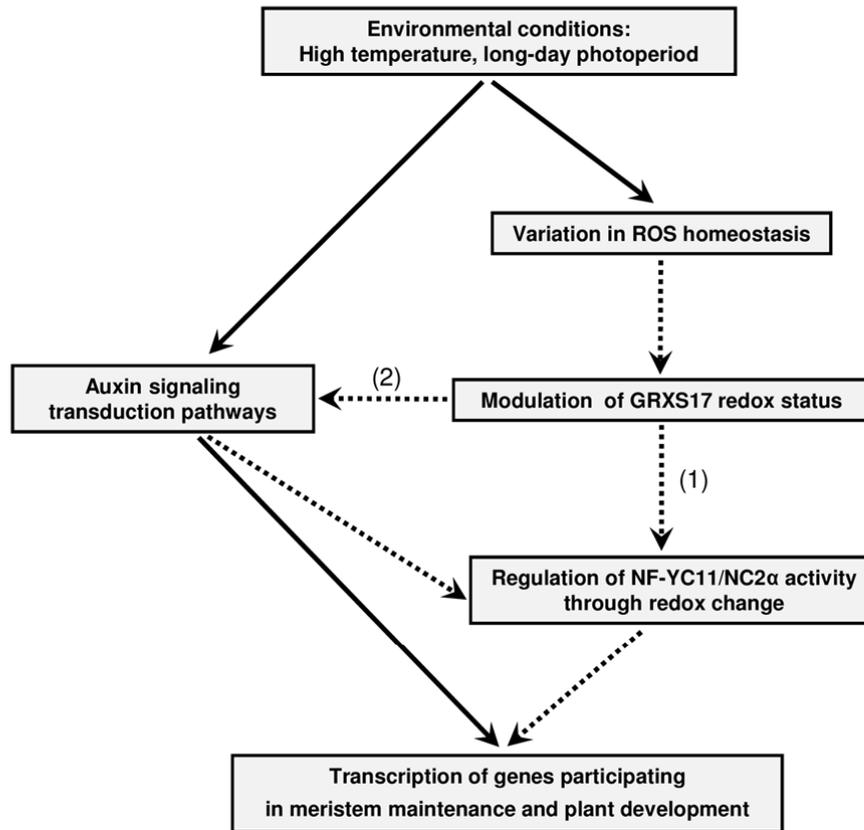
Figure 10. Suggested role of GRXS17 during plant development in connection with environmental conditions and auxin-related mechanisms.
Dotted lines indicate the proposed steps in signal transduction involving GRXS17. The model is based on the data presented in this work (1) and those reported in Cheng et al. (2011) (2).

523   cycle progression (Benatti et al., 2011) and in fungi, the corresponding trimeric complex
524   formed by HAP2, 3 and 5 includes a fourth HAP4 subunit. Interestingly, in *S. pombe*, the
525   function and subcellular localization of HAP4, which participates in iron homeostasis, is
526   under the control of the multidomain GRX4 (Mercier and Labbé, 2009). In relation to the

527 *grxS17* phenotype, it is worth mentioning that AtNF-YC11 specifically interacts with AtNF-

528 YB3, an isoform controlling flowering time (Kumimoto et al., 2008). To date, the only

529 evidence for redox control was obtained for a mammalian NF-YB whose association to NF-

530 YC is dependent on the reduction of an intermolecular disulfide bond (Nakshatri et al., 1996).

531 Interestingly, all plant NF-YC11 orthologs exhibit a unique N-terminal sequence clearly

532 distinguishing them from other NF-YCs. On this basis, they have been reclassified as

533 homologs to NC2α factors (Petroni et al., 2012). NC2α, together with another factor called

534 NC2β, forms a tight heterodimer able to associate with the TATA-binding complex and acts

535 as a transcription repressor as shown in *S. cerevisiae* and rice (Kim et al., 1997; Song et al.,

536 2002). Two cysteine residues are present in the NF-YC11/NC2α N-terminal extension (Fig.

537 S12). Of note, one is strictly conserved in plant orthologues and also in human NC2α protein,

538 while the position of the second varies in Dicotyledons and in Monocotyledons.

539 Unfortunately, all attempts to produce recombinant NF-YC11/NC2α failed which precluded

540 investigations on a possible redox-mediated interaction with GRXS17. The data gained from

541 the characterization of the Arabidopsis *nf-yc11*/*nc2α* mutant line (Fig. 9; Fig. S11) revealed

542 that the nuclear factor is a central element for proper plant development. In short-day

543 conditions, *nf-yc11*/*nc2α* plants display developmental defects (slow growth and altered floral

544 spikes). Most interestingly, when comparing the phenotypes of *grxS17* and *nf-yc11*/*nc2α*

545 plants, we noticed that both mutants share common photoperiod-dependent characteristics

546 such as delayed appearance of the first two leaves, abnormal leaf shapes and impaired

547 flowering (Fig. 9; Fig. S11). This phenotype resemblance, which is revealed in long day but

548 not in short-day conditions, gives further credence to a concerted action of GRXS17 and NF-

549 YC11/NC2α in the control of plant development in relation to environmental conditions. We

550 might thus speculate that GRXS17 modulates the NF-YC11/NC2α function by controlling its

551 redox state, and we propose a working model illustrating such a role for GRXS17 (Fig. 10).

552 Based on the resemblance of *grxS17* and *nf-yc11*/*nc2α* mutants, it is conceivable that

553 GRXS17-mediated redox changes modify the capacity of AtNF-YC11/NC2α to bind to a

554 NC2β subunit (AtNF-YB11-13), ultimately resulting in the modification of the transcription

555 level of genes involved in meristem maintenance and plant developmental programmes, such

556 as those related to auxin action (Fig. 10). Taken collectively, these data lead us to propose that

557 *A. thaliana* GRXS17 relays environmental signals, possibly via subtle changes in the

558 cellular/nuclear redox state, and then enters this information into the control of gene

559 transcription to initiate essential developmental steps. The use of mutant lines expressing

560 mutated GRXS17 and NF-YC11/NC2α forms will help to determine the precise mechanisms

561 underlying the functions of these two key actors in plant development in relation with the

562 presence and the redox status of their cysteines.

563

Material and Methods

Plant material and growth conditions

566 *Arabidopsis thaliana* Col-0 plants were grown in standard conditions under an 8-h

567 photoperiod and a photon flux density of 200 µmol photons $m^{-2} s^{-1}$ at 22°C. Other conditions

568 of light (500 µmol photons $m^{-2} s^{-1}$), temperature (15°C or 28°C) and photoperiod (16 h or

569 continuous light) were applied in controlled growth chambers either from sowing or on 2- to

570 3-week old plants grown under standard conditions.

571

Transformation of *A. thaliana* plants

573 The full-length GRXS17 cDNA (At4g04950) was cloned into the pB2GW7 vector

574 (GATEWAY, Invitrogen). Following transformation using *Agrobacterium tumefaciens* C58

575 strain (Clough and Bent, 1998), homozygous lines (T2) were obtained from resistance

576 segregation assays. Leaf genomic DNA was extracted using the DNeasy Plant Mini Kit

577 (Qiagen) to perform PCR using appropriate primers (Table SIII), Taq DNA polymerase (Life

578 Technologies) and the GeneAmp PCRSystem 2700 (Applied Biosystems). RT-PCR was

579 performed using Sensiscript III (Life Technologies) following leaf RNA extraction

580 (NucleoSpin, Macherey-Nagel).

581

Expression of recombinant WT and Cys-mutant GRXS17

583 AtGRXS17 cDNA was cloned into the pET-16b vector (Novagen, Merck Biosciences) for

584 expression in *E. coli* BL21(DE3)-pLysS. The protein was purified by nickel-chelate

585 chromatography (GE Healthcare). Site-directed mutagenesis was performed using the

586 QuikChange II protocol (Stratagene) and appropriate primers (Table SIII).

587

In vitro reconstitution assay of Fe-S clusters and analytical measurements

589 GRXS17 (50 µM) was reconstituted *in vitro* by incubation with $Fe(NH_4)_2(SO_4)_2$, cysteine,

590 GSH, pyridoxal phosphate, and *E. coli* IscS in the molar ratio of 1 GRX:6:9:10:0.04:0.02 in

591 50 mM sodium phosphate buffer pH 8.0, 300 mM NaCl, 5 mM DTT under argon atmosphere

592 for 2 h. After centrifugation (4 min, 4°C, 13,000 rpm), UV/visible spectra were recorded with

40

593 a Shimadzu UV-2100 spectrophotometer. Iron content was determined after reconstitution of
594 100 μM GRXS17 according to Fish (1988).

595

596 **Cytology and *in situ* hybridization**
597 Meristem cross sections were prepared using a microtome (Leica RM2255) from tissues fixed
598 with formaldehyde/glutaraldehyde and embedded in hydroxyethyl methacrylate (Technovit
599 7100; Heraeus Kulzer) and counterstained with Toluidine blue. Measurements of mesophyll
600 cells were performed after propidium iodide (PI) staining (5 ng.mL^{-1}). Confocal microscopic
601 observations were carried out using the Axio observer Z1 microscope with the LSM 700
602 scanning module, the ZEN 2010 software (Zeiss), and the PI (BP 566–1000) filters. *In situ*
603 hybridization was performed as in Bashandy et al. (2010). After fixation, dehydration and
604 embedding in paraffin wax, sample sections (7-mm thick) were attached to precoated glass
605 slides (DAKO). Probes were synthesized using digoxigenin (DIG)-UTP (Boehringer
606 Mannheim). Immunodetection was performed using an anti-DIG antibody coupled to alkaline
607 phosphatase.

608

609 **Transient transformation of protoplasts with GFP and split-YFP constructs**
610 Protoplasts were isolated from leaves of 6-week-old *A. thaliana* plants for transient expression
611 of fusion proteins (Wojtera-Kwiczor et al., 2013). cDNAs encoding GRXS17 and NF-
612 YC11/NC2α were cloned in sense direction into the pGFP2-vector for C-terminal GFP fusion.
613 The GRXS17 and NF-YC11/NC2α cDNAs were inserted in frame with YFP N- and C-
614 terminal parts in pSPYNE and pSPYCE vectors obtained from Jörg Kudla (University of
615 Muenster). Combinations of empty vectors and fusion constructs were used as controls. GFP
616 and chlorophyll autofluorescence were visualized with excitation at 488 nm and emission at
617 500-530 nm and 650-710 nm, respectively, using the cLSM 510 META (Carl Zeiss). YFP
618 was visualized with excitation at 514 nm and emission at 535-590 nm.

619

620 **Affinity chromatography and electrospray ionization (ESI) mass spectrometry**
621 His-tagged GRXS17 was bound to a Ni-NTA-column and used as affinity matrix. Leaves of
622 5-week old plants grown under short-day conditions were homogenized in 20 ml 50 mM
623 Bicine, pH 7.8, 100 mM sucrose, 50 mM NaCl. After filtration through Miracloth and
624 centrifugation (10 min, 6,000 *xg*, 4°C and 50 min, 4°C, 100,000 *xg*), the clarified supernatant
625 (30 mg protein) was applied to the matrix and incubated for 2 h at 4°C. Non-bound material

626 was removed by washing the column 4 times with 10 ml 20 mM Bicine, pH 7.8. Elution was

627 achieved with 4 ml of the same buffer containing 150 mM DTT. After tryptic digestion (50

628 µg proteins per analysis), the fragments were separated by reverse-phase HPLC and analyzed

629 by ESI-MS (Holtgrefe et al., 2008). Results were analyzed using the Brucker Daltronics

630 software.

631

632 **Biochemical methods**

633 Plant soluble proteins were prepared, separated by SDS-PAGE and electro-transferred onto a

634 nitrocellulose membrane (Pall Corporation) (Rey et al., 2005). Protein crosslinking was

635 achieved using dimethyl pimelimidate/2 HCl (DMP) (Thermo Fisher Scientific) (Riondet et

636 al., 2012). Polyclonal antibodies were raised in rabbit against His-tagged AtGRXS17

637 (Genecust). Immunodetection of AtGRXS17 was carried out using primary antibodies diluted

638 1:1000 and the goat anti-rabbit "Alexa Fluor® 680" IgG (1:10,000) (Invitrogen). Bound

639 antibodies were revealed at 680 nm using the Odyssey Infrared Imager (LiCor). For

640 immunodetection of aconitase (Bernard et al., 2009) and PsaA (Agrisera), horse-radish

641 peroxidase-conjugated   secondary   antibodies   and   chemiluminescence   were   used.

642 Autoluminescence imaging of lipid peroxidation was performed as in Collin et al. (2008).

643

644 **Yeast plasmids, strains and growth conditions**

645 The GRXS17 entire sequence and each GRX module, M2 ($S17_{167\text{-}252}$), M3 ($S17_{297\text{–}383}$) and

646 M4 ($S17_{404\text{-}488}$) were cloned in-frame in the yeast plasmid pMM221. pMM221 contains the

647 *S. cerevisiae* GRX5 mitochondrial targeting sequence plus a C-terminal 3HA/His6 tag, under

648 the control of the doxycycline-regulatory tetO2 promoter (Tables SI and SIII) (Molina et al.,

649 2004). pMM54 contains a yeast *GRX5*-3HA construction under its endogenous promoter

650 (Rodriguez-Manzaneque et al., 2002). Strains are described in Table SII. Plasmids were

651 linearized by *Cla*I previous to chromosomal integration. Samples were taken from cultures

652 grown exponentially (Molina et al., 2004) for at least 10 generations at 30˚C. Sensitivity to

653 oxidants was determined on YPD plates, by spotting 1:5 serial dilutions of exponential

654 cultures and recording growth after 2 days at 30˚C. Subfractionation of mitochondria was

655 performed as in Bandyopadhyay et al. (2008). Northern blot analyses using yeast RNA were

656 performed with digoxigenin (Belli et al., 1998). Gene probes were generated by PCR from

657 genomic DNA using appropriate oligonucleotides (Table SIII).

658

Enzyme activity determinations

Aconitase and malate dehydrogenase were assayed in extracts from yeast growing exponentially in YPGalactose medium (Robinson et al., 1987). Isopropylmalate isomerase activity was determined in extracts prepared from cells growing exponentially in SC medium supplemented with the specific auxotrophy requirement (Pierik et al., 2009). In the case of leucine, only 1/3 of the standard concentration was added into the medium to allow growth. In-gel activity assays for aldehyde oxidase and aconitase were as previously described (Bernard et al., 2009).

Figure legends

Figure 1. Expression of *GRXS17* in *A. thaliana* plants.

(A-B) Western analysis of GRXS17 abundance. (A) Western blot analysis in flowers from WT and *grxS17* plants, and in organs of WT plants grown under standard conditions (20 µg per lane). The RubisCO large subunit (LSU) appears as a light grey background band in the *grxS17* lane. R, root from adult plant; S, stem from bolting plant; YL, young leaf; ML, mature leaf; OL, old leaf; B, floral bud; F, flower. (B) Loading controls: Coomassie blue stained gels (50-kDa region). (C-F) RNA *in situ* hybridization. Longitudinal sections of WT meristem inflorescences (C, D) and flower buds (E, F). Hybridizations were performed with antisense (C, E) or sense (D, F) GRXS17 cDNA probes.

Figure 2. Subcellular localization and dimerisation of GRXS17.

(A) Transient expression of a GRXS17:GFP fusion in *A. thaliana* mesophyll protoplasts. Autofluorescence of chlorophyll indicates chloroplasts. (B) Stable expression of a GRXS17:GFP fusion in *Arabidopsis thaliana* leaves. White arrows indicate nuclei. (C)

692 Immunodetection of GRXS17, thioredoxin TRXh5 and nucleolin NUC1 in cytosolic (Cyt)

693 and nuclear (Nuc) extracts of Arabidopsis flower buds. (D) Bimolecular fluorescence

694 complementation assay of GRXS17. Vectors encoding C- and N-terminal split-YFP fusions

695 with GRXS17 were co-transformed into *Arabidopsis thaliana* mesophyll protoplasts. (E)

696 Immunodetection of GRXS17 in *grxS17* and WT extracts cross-linked (C) or not (NC) with

697 DMP.

698

699 **Figure 3. Growth and development of plants modified in *GRXS17* expression as a**

700 **function of photoperiod and temperature**.

701 (A) Five-week old plants grown in standard conditions (8-h photoperiod, 200 µmol photons

702 $m^{-2} s^{-1}$) at 22°C. (B) Plants grown for 2.5 weeks in standard conditions and transferred to 28°C

703 (8-h photoperiod, 200 µmol photons $m^{-2} s^{-1}$) for 2.5 weeks. The arrow indicates the absence of

704 young leaves. (C) Plants grown in long-day photoperiod conditions (16 h, 200 µmol photons

705 $m^{-2} s^{-1}$) at 22°C. (D) Plants grown for 2 weeks in standard conditions and transferred to

706 continuous light (200 µmol photons $m^{-2} s^{-1}$, 22°C) for 3 weeks. (E) Plants grown for 2 weeks

707 in standard conditions and transferred to 28°C and long-day photoperiod (16 h, 200 µmol

708 photons $m^{-2} s^{-1}$) for 2.5 weeks. (F) Plants grown for 3 weeks in standard conditions and

709 transferred to 15°C and long-day photoperiod (16 h, 200 µmol photons $m^{-2} s^{-1}$) for 4 weeks.

710 WT, Wild-type plants; *grxS17,* homozygous SALK_021301 plants; *grxS17 GRXS17* 3.3 and

711 17.8, two independent *grxS17* lines expressing *GRXS17*. SD and LD, short (8 h) and long (16

712 h) day.

713

714 **Figure 4. Structure of shoot apical meristem (SAM) and leaves in *grxS17* plants.**

715 (A) Histological structure of the SAM stained by toluidine blue in 7-day old wild-type,

716 *grxS17* and g*rxS17 GRXS17* (line 3.3) plants grown in long-day conditions (16 h) and high

717 light (500 µmol photons $m^{-2} s^{-1}$). (B) Observation of mesophyll cells in leaves of 3-week old

718 plants grown under long-day/high-light conditions. (C) Number of L1, L2 and L3 layer stem

719 cells in SAM cross-sections shown in (A). Ten sections per genotype were analyzed. (D)

720 Density of mesophyll cells in the sections shown in (B) (n: 12). *, value significantly different

721 from Wt value with $p < 0.05$ (t-test).

722

723 **Figure 5. Incorporation of Fe-S clusters into recombinant WT and mutated AtGRXS17.**

44

(A) Domain structure of GRXS17. TRX-HD: TRX-like homology domain; M2-GRX, M3-GRX and M4-GRX: three monothiol-GRX domains. Positions of active-site cysteines are indicated by black triangles and that of other cysteine by a grey triangle. (B) Absorption spectra of GRXS17 and cysteine mutants. UV-visible absorption spectra were recorded immediately after *in vitro* reconstitution in anaerobic conditions. The active-site cysteines of each GRX domain were individually or together substituted by serines (M2:C179S; M3: C309S; M4: C416S; C179/309/416S). (C) Relative absorption at 420 nm of GRXS17 mutants.

Figure 6. Rescue of the *S. cerevisiae grx5* mutant defects by AtGRXS17.
(A) Sensitivity to *t*-BOOH or diamide. The indicated yeast strains were spotted on YPD plates at 10x serial dilutions and grown for 3 days at 30˚C on YPD plates. (B) Growth on glucose (YPD plates) or glycerol (YPGly plates) after 3 days at 30˚C. (C) Relative ratio between aconitase and malate dehydrogenase activities, normalized with respect to the ratio in the WT strain, in exponential cultures at 30˚C in YPGalactose medium. (D) Relative specific Leu1 activity. (E) Northern blot analysis of *FIT3* and *FTR1* mRNA levels from exponential cultures at 30˚C. Loading control: *SNR19* mRNA. (F) Relative iron content from exponential cultures at 30˚C in YPD medium. Mean ± SD (n = 3). Asterisks indicate statistically significant differences (Student's *t*-test, p<0.05) compared to WT (*GRX5*) cultures.

Figure 7. Activities and levels of Fe-S enzymes in plants modified in *GRXS17* expression.
Wild type (WT), a *grxS17* knock-out mutant and two complemented lines (3.3 and 17.8) were grown for 14 days under standard conditions (22°C) or transferred to 28°C after 7 days (Fig. S10). Mutant alleles in the *ABC Transporter of the Mitochondria 3* (*atm3-1* and *atm3-4*)were used for comparison. (A) Activities of aconitase (ACO) and aldehyde oxidase (AO) isozymes visualized as a formazan precipitate in a native gel assay. Cytosolic (cyto) and mitochondrial (mito) isoforms of ACO are indicated. Equal protein amounts (160 μg for ACO and 60 μg for AO) were loaded per lane. Immunoblotting of ACO and PsaA (a subunit of PSI) in Arabidopsis seedlings (10 μg protein per lane) and loading control (Ponceau). (B) Relative activities of cytosolic and mitochondrial aconitase isoforms quantified using ImageJ software. The values are given as percentages of the sum of the band intensities per lane (mean ± SD). The data are representative of 3 independent experiments.

757 **Figure 8. Localisation of NF-YC11/NC2α and interaction with GRXS17.**

758 Isolated *A. thaliana* mesophyll protoplasts were transiently transformed with a NF-

759 YC11/NC2α:GFP fusion (A) or with split-YFP constructs containing GRXS17 or NF-

760 YC11/NC2α (B). White arrows indicate nuclei.

761

762 **Figure 9. Characterization of a *nf-yc11/nc2α* mutant and comparison of the growth of**

763 ***grxS17* and *nf-yc11/nc2α* plants as a function of photoperiod duration.**

764 (A) PCR analysis of genomic DNA of At3g12480 and T-DNA in WT, heterozygous (Htz) and

765 homozygous (Hmz) plants of the GABI-042E02 mutant line. (B) RT-PCR analysis of WT and

766 homozygous plants. (C) Seedling development in short- and long-day conditions (8- and 16-h,

767 respectively) at moderate light (200 µmol photons m^{-2} s^{-1}) and 22°C. The seedlings were

768 grown simultaneously in the same conditions and the original photographs are shown in Fig.

769 S 10. (D) Vegetative growth of plants in standard light conditions (short-day: 8-h photoperiod

770 and moderate light, 200 µmol photons m^{-2} s^{-1}) and in long-day and high-light conditions (16-h

771 photoperiod, 500 µmol photons m^{-2} s^{-1}) at 22°C. The plant age is indicated for each genotype

772 and culture condition. Scale bars: 1 and 6 cm in C and D, respectively.

773

774 **Figure 10. Suggested role of GRXS17 during plant development in connection with**

775 **environmental conditions and auxin-related mechanisms.**

776 Dotted lines indicate the proposed steps in signal transduction involving GRXS17. The model

777 is based on the data presented in this work (1) and those reported in Cheng et al. (2011) (2).

778

779

780 **Table I. Putative interaction partners of AtGRXS17.** The list includes the proteins
781 identified at least 4 times in 15 independent affinity chromatography experiments with His-
782 tagged AtGRXS17.

Accession number	Protein name	Function	Subcellular localization[a]	Sequence length (aa)	Protein identification (times)[b]	Sequence coverage (%)[c]
At4g04950	GRXS17	Cell redox homeostasis	CY,NU,PL	488	15	90%
At1g50570	Calcium-dependent lipid-binding family protein	Unknown	NU	388	13	68%
At3g12480	NF-YC11/NC2α	Transcription factor	CY,MI,NU,PL	293	7	59%
At4g25860	Oxysterol-binding protein-related protein 4A	Sterol transport	CY	386	11	56%
At3g13460	CIPK1 interacting protein ECT2	Unknown	CY,NU,PL	667	6	52%
At2g39960	Microsomal signal peptidase	Peptidase activity	CY,ER,MI,PM	192	5	45%
At1g13440	GapC2	Glycolysis	CY,MI,NU,PL,PX	338	9	41%
At5g46570	BR-signaling kinase 2	Protein phosphorylation	CY,PM	492	5	41%
At4g31180	Aspartate-tRNA ligase-like protein	Aspartyl-tRNA synthetase activity	CY,PL	270	4	31%
At5g11870	Alkaline phytoceramidase	Hydrolase activity	ES,MI,PL,PM	270	8	28%
At5g54050	Cysteine/histidine-rich C1 domain-containing protein	Protein-disulfide reductase activity	ES, NU	580	8	21%
At5g20830	Sucrose synthase 1	UDP-glycosyltransferase activity	CY,MI	808	4	19%
At1g24510	T-complex protein 1 subunit epsilon	Chaperone activity	CY,MI,PM	535	6	18%
At4g22030	Probable F-box protein	Unknown	CY,MI,PL	626	5	12%

[a] Sub-cellular localization predicted by BAR Cell eFP Browser. CY: Cytosol; ER: Endoplasmic reticulum; ES: Extracellular space; MI: Mitochondria; NU: Nucleus; PX: Peroxisome; PM: Plasma membrane; PL: Plastid

[b] Proteins were identified due to the presence of their peptides in the indicated number of experiments.

[c] Identified peptides with a MOWSE score greater 15 were used to calculate the total sequence coverage.

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788 **Supplemental data**

789 **Table SI. Plasmids employed for experiments on yeast.**

790

791 **Table SII. Yeast strains.**

792

793 **Table SIII. List of primers employed in the study.**

794

795 **Figure S1. Molecular characterization of *A. thaliana* plants modified in *GRXS17***
796 **expression and GRXS17 protein abundance.**

797 (A) PCR analysis of *GRXS17* gene and GRXS17 cDNA in the genomic DNA of WT plants,

798 of SALK_021301 T-DNA homozygous plants (*grxS17*) and of two independent lines of T-

799 DNA plants expressing the *GRXS17* cDNA under the control of the cauliflower mosaic virus

800 35S promoter (*grxS17 GRXS17* 3.3 and 17.8). (B) Western blot analysis of GRXS17

801 abundance in leaf soluble proteins (20 µg per lane). The grey band in *grxS17* lane is attributed

802 to a non-specific signal from the large RubisCO subunit which has almost the same size as

803 GRXS17.

804

805 **Figure S2. Subcellular localization of GRXS17.**

806 Immunodetection of GRXS17, nuclear histone H3, cytosolic thioredoxin h3 (TRXh3),

807 mitochondrial thioredoxin o1 (TRXo1) and plastidial 2-Cys peroxiredoxin (2-Cys PRX) in

808 nuclear (Nuc), cytosolic (Cyt), mitochondrial (Mit) and chloroplastic (Chl) fractions prepared

809 from Arabidopsis plants. The faint histone H3 band detected in mitochondrial fraction

810 probably indicates a slight contamination from nuclei.

811

812 **Figure S3. Controls of the bimolecular fluorescence complementation assay of GRXS17**
813 **and NF-YC11/NC2α.**

814 Vectors encoding C- and N-terminal split-YFP fusions with GRXS17 or NF-YC11/NC2α and

815 empty vectors were co-transformed into *A. thaliana* mesophyll protoplasts. Pictures were

816 taken 14 h after transformation.

817

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819

820 **Figure S4. Growth and development of _A. thaliana_ plants modified in _GRXS17_**
821 **expression as a function of light and temperature regimes**.
822 (A) Five-week old plants grown for 2 weeks in standard conditions and then transferred for 3
823 weeks to high-light (500 µmol photons m^{-2} s^{-1}) and short-day (8-h photoperiod) conditions at
824 22°C. (B) Three-week old plants grown in high-light (500 µmol photons m^{-2} s^{-1}) and long-day
825 (16-h photoperiod) conditions at 22°C. (C) Seven-week old plants grown for 3 weeks in
826 standard conditions and then transferred to 15°C, long-day photoperiod (16 h) and high-light
827 conditions (500 µmol photons m^{-2} s^{-1}) for 4 weeks. WT, wild-type plants; _grxS17_,
828 homozygous SALK_021301 plants; _grxS17 GRXS17_ 3.3 and 17.8, two independent KO lines
829 expressing _GRXS17_.
830

831 **Figure S5. Floral development of _A. thaliana_ plants modified in _GRXS17_ expression as a**
832 **function of photoperiod and light intensity.**
833 (A) 54-day old plants grown in standard conditions (8-h photoperiod, 200 µmol photons m^{-2}
834 s^{-1} at 22°C). (B) Four-week old plants grown in high-light (500 µmol photons m^{-2} s^{-1}) and
835 long-day (16-h photoperiod) conditions at 22°C. (C) and (D) Height of the main floral spike
836 in plants grown in standard conditions for 54 days and in high-light and long-photoperiod
837 conditions for 4 weeks, respectively. (E) and (F) Number of leaves at flowering in plants
838 grown in standard conditions and in high-light and long-day conditions, respectively. (G)
839 Five-week old _grxS17_ plant grown in high-light (500 µmol photons.m^{-2}.s^{-1}) and long-day (16-
840 h photoperiod) conditions at 22°C. WT, Wild-type plants; _grxS17_, homozygous
841 SALK_021301 plants; _grxS17 GRXS17_ 3.3 and 17.8, two independent KO lines expressing
842 _GRXS17_. ***, significantly different from the WT value with p < 0.01 (t test).
843

844 **Figure S6. Autoluminescence in _A. thaliana_ plants modified in _GRXS17_ expression.**
845 (A) Three-week old plants grown in high-light (500 µmol photons m^{-2} s^{-1}) and long-day (16-h
846 photoperiod) conditions at 22°C. (B) Autoluminescence imaging of lipid peroxidation in these
847 plants. The homogeneous blue/purple color of plants indicates the absence of photodamage
848 (compare panel D for positive control). WT, wild-type plants; _grxS17_, homozygous
849 SALK_021301 plants; _grxS17 GRXS17_ 3.3 and 17.8, two independent KO lines expressing
850 _GRXS17_. (C) Five-week old WT plant grown in standard conditions (8-h photoperiod, 200
851 µmol photons m^{-2} s^{-1}at 22°C) and then exposed to a photooxidative treatment for 1 day (8-h
852 photoperiod, 1250 µmol photons m^{-2} s^{-1} at 6°C). The red arrows indicate leaves displaying

49

853   bleaching resulting from photodamage. (D) Autoluminescence imaging of lipid peroxidation

854   in the plant shown in panel C. The damaged leaves appear in white/yellow color due to the

855   photon emission associated with lipid peroxidation.

856

857   **Figure S7. Structure of the shoot apical meristem (SAM) in the *grxS17* mutant.**

858   (A) Histological structure of the SAM stained by toluidine blue in WT, *grxS17* and *grxS17*

859   *GRXS17* (line 3.3) seedlings grown for 11 days in short-day (8 h) conditions and standard

860   light (200 µmol photons m^{-2} s^{-1}). (B) Number of L1, L2 and L3 layer stem cells in cross-

861   sections of SAM shown in (A). Ten sections per genotype were analyzed.

862

863   **Figure S8. Hypothetical structure of GRXS17 and amino acid alignment of the three**

864   **GRX domains M2, M3 and M4 from *A. thaliana*.** The structures of the single domains were

865   generated from 1XFL and 3L4N pdb entries for TRX and GRX proteins, respectively, and are

866   not experimentally confirmed. The linker regions and the relative positions of the TRX

867   domain are speculative.

868

869   **Figure S9. Analysis of the GRXS17 forms expressed in *S. cerevisiae*.**

870   (A) Western blot analysis with anti-HA antibodies (10 µg total proteins from cell extracts per

871   lane). Extracts from yeast cells expressing the homologous GRX5 protein under the native

872   promoter (Grx5 Sc) are included as control. Samples from cells grown in fermentative

873   conditions (Glu) and in respiratory conditions (Lac) were run in parallel. Molecular size of the

874   expressed S17 forms is indicated. (B) Subfractionation of extracts from *S. cerevisiae* cells

875   expressing the respective S17 forms and grown in respiratory conditions (lactose medium).

876   TE (total extracts, 25 µg), MT (total mitochondria, 5 µg), IMS (intermembrane mitochondrial

877   space, 5 µg), MX (mitochondrial matrix, 5 µg). Alpha-ketoglutarate dehydrogenase (α-KDH)

878   is shown as a control of a mitochondrial matrix protein.

879

880   **Figure S10. Growth phenotypes and quantification of aldehyde oxidase enzyme activity**

881   **in plants modified in *GRXS17* expression.**

882   *GrxS17* knockout seedlings were grown under normal and heat stress conditions and

883   compared to complemented lines (3.3 and 17.8) and wild type (WT). For comparison, a weak

884   (*atm3-4*) and a strong mutant allele (*atm3-1*) of *ATM3* were analysed, which are known to

885   have decreased activities of cytosolic Fe-S enzymes. Plants were grown in long photoperiod

886   (16 h) at 22°C for 14 days (white bars), or shifted to 28°C after 7 days (grey bars). (A) Images

887   of *grxS17* mutants and controls grown under standard conditions and under heat stress. Scale

888   bar = 1 cm. (B) Quantification of the band intensities in native gels (Fig. 7) reflecting the

889   major aldehyde oxidase activity using ImageJ. Activities are shown as percentage of WT

890   levels (mean ± SD; n = 3).

891

892   **Figure S11. Development of *nf-yc11/nc2α* plants as a function of light environment.**

893   (A) Development of seedlings in short- and long-day conditions (8-h and 16-h, respectively)

894   at moderate light (200 µmol photons m^{-2} s^{-1}) and 22°C. (B) Phenotype of plants grown in

895   long-day (16 h photoperiod) and moderate light (200 µmol photons m^{-2} s^{-1}) conditions at

896   22°C. (C) Reproductive development of *nf-yc11/nc2α* plants in short- or long-day conditions

897   at moderate or high light (200 or 500 µmol photons m^{-2} s^{-1}, respectively) and 22°C. Scale

898   bars: 6 cm. The plant age and culture condition are indicated for each genotype.

899

900   **Figure S12. Sequence alignment of plant, human and yeast proteins containing a NC2α**

901   **domain.**

902   Alignment was performed using the ClustalW software At, *Arabidopsis thaliana* (At3g12480,

903   NP_187854.2); Mt, *Medicago truncatula* (XP_003628014.1); St, *Solanum tuberosum*

904   (XP_006347683.1); Pt, *Populus trichocarpa* (XP_002304814.1); Vv, *Vitis vinifera*

905   (CAN71308.1); Os, *Oryza sativa* (AF464904); Bd, *Brachypodium distachyon*

906   (XP_003577492.1); Zm, *Zea mays* (NP_001105089.1); Hs, *Homo sapiens* (AAB02192.1); Sc,

907   *Saccharomyces cerevisiae* (NP_011086.3). Amino acids are given using standard single-letter

908   designation, and dashes indicate gaps. Stars and colons indicate identity and similarity of

909   amino acids, respectively. The cysteine conserved in plant and human proteins is highlighted

910   in yellow, the cysteines conserved in dicotyledonous and monocotyledonous plants are

911   highlighted in blue and green, respectively. The black line indicates the NC2α domain.

912

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Figure S1

Figure S1. Molecular characterization of *A. thaliana* plants modified in *GRXS17* expression and GRXS17 protein abundance.
(A) PCR analysis of *GRXS17* gene and GRXS17 cDNA in the genomic DNA of WT plants, of SALK_021301 T-DNA homozygous plants (*grxS17*) and of two independent lines of T-DNA plants expressing the *GRXS17* cDNA under the control of the cauliflower mosaic virus 35S promoter (*grxS17 GRXS17* 3.3 and 17.8). (B) Western blot analysis of GRXS17 abundance in leaf soluble proteins (20 µg per lane). The grey band in *grxS17* lane is attributed to a non-specific signal from the large RubisCO subunit which has almost the same size as GRXS17.

Figure S2

Figure S2. Subcellular localization of GRXS17.
Immunodetection of GRXS17, nuclear histone H3, cytosolic thioredoxin h3 (TRXh3), mitochondrial thioredoxin o1 (TRXo1) and plastidial 2-Cys peroxiredoxin (2-Cys PRX) in nuclear (Nuc), cytosolic (Cyt), mitochondrial (Mit) and chloroplastic (Chl) fractions prepared from Arabidopsis plants. The faint histone H3 band detected in mitochondrial fraction probably indicates a slight contamination from nuclei.

Figure S3

	Brightfield	Auto fluorescence	YFP-Signal	Overlay	
					GrxS17:YFP^N + GrxS17:YFP^C
					GrxS17:YFP^N + NF-YC11/NC2α:YFP^C
					YFP^N + NF-YC11/NC2α:YFP^C
					NF-YC11/NC2α:YFP^N + YFP^C
					YFP^N + GrxS17:YFP^C
					GrxS17:YFP^N + YFP^C
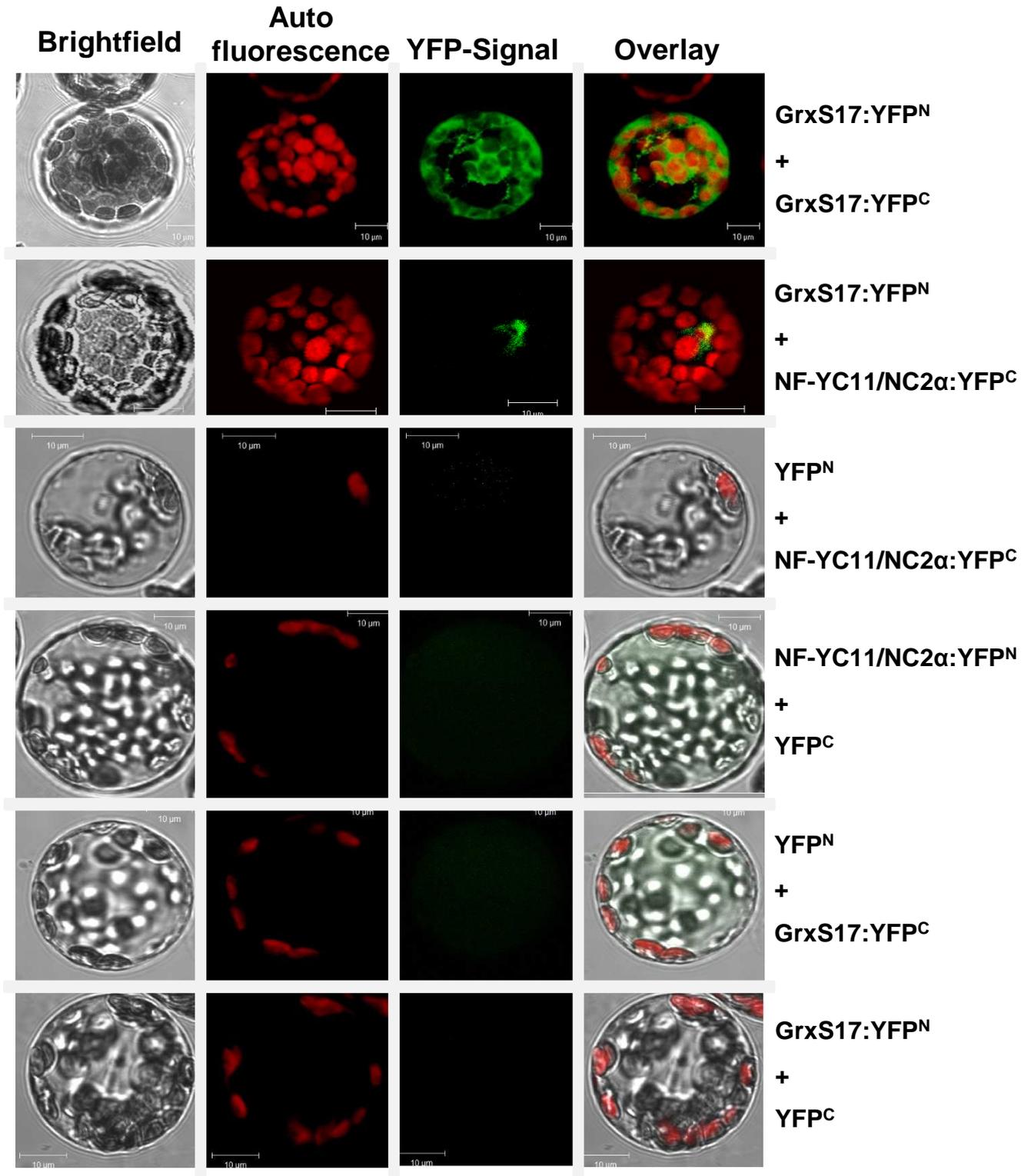
Figure S3. Controls of the bimolecular fluorescence complementation assay of GRXS17 and NF-YC11/NC2α. Vectors encoding C- and N-terminal split-YFP fusions with GRXS17 or NF-YC11/NC2α and empty vectors were co-transformed into *A. thaliana* mesophyll protoplasts. Pictures were taken 14 h after transformation.

Figure S4
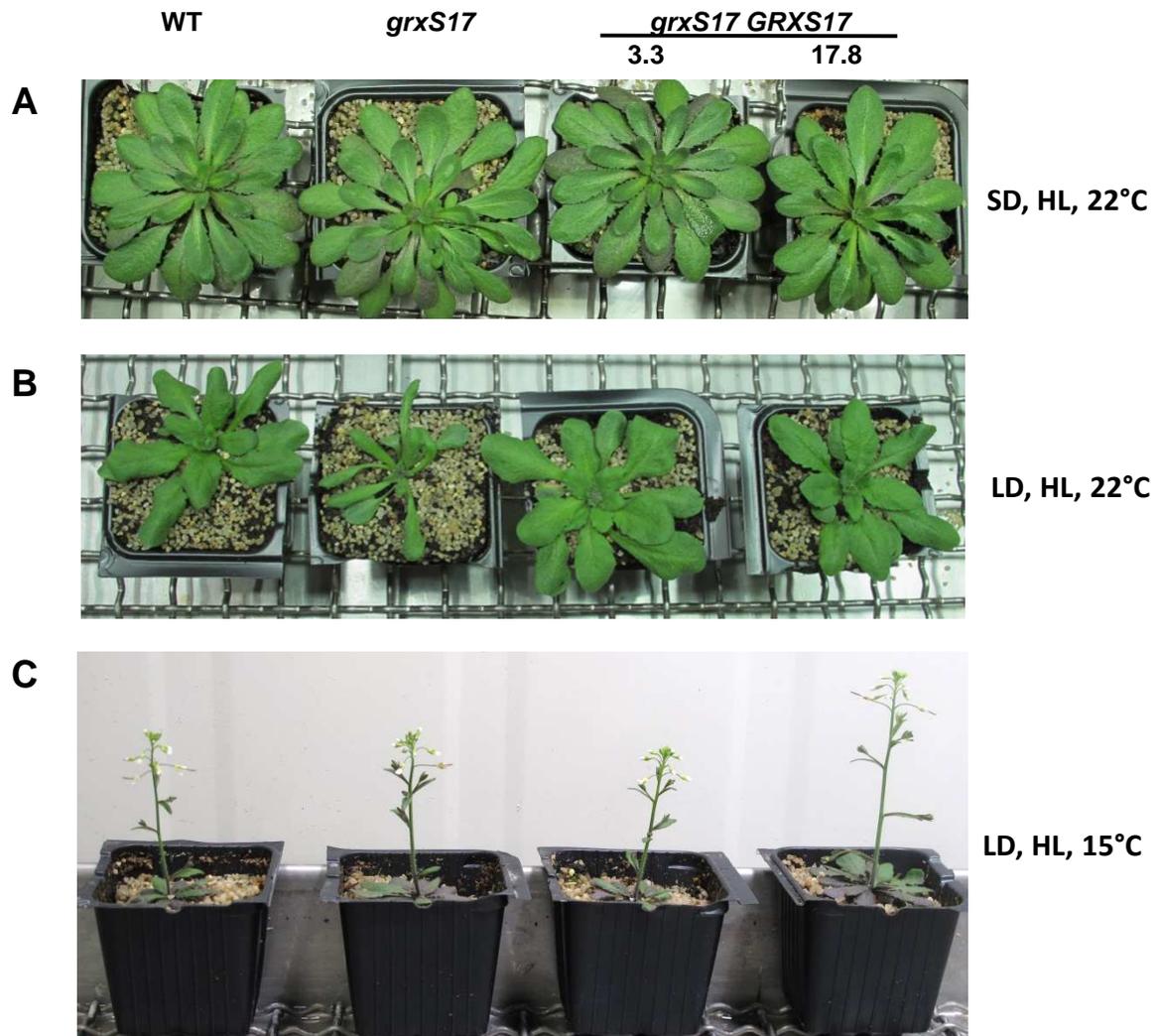
Figure S4. Growth and development of *A. thaliana* plants modified in *GRXS17* expression as a function of light and temperature regimes.
(A) Five-week old plants grown for 2 weeks in standard conditions and then transferred for 3 weeks to high-light (500 µmol photons.m^{-2}.s^{-1}) and short-day (8-h photoperiod) conditions at 22°C. (B) Three-week old plants grown in high-light (500 µmol photons.m^{-2}.s^{-1}) and long-day (16-h photoperiod) conditions at 22°C. (C) Seven-week old plants grown for 3 weeks in standard conditions and then transferred to 15°C, long-day photoperiod (16 h) and high-light conditions (500 µmol photons.m^{-2}.s^{-1}) for 4 weeks. WT, wild-type plants; *grxS17*, homozygous SALK_021301 plants; *grxS17 GRXS17* 3.3 and 17.8, two independent KO lines expressing *GRXS17*.

Figure S5
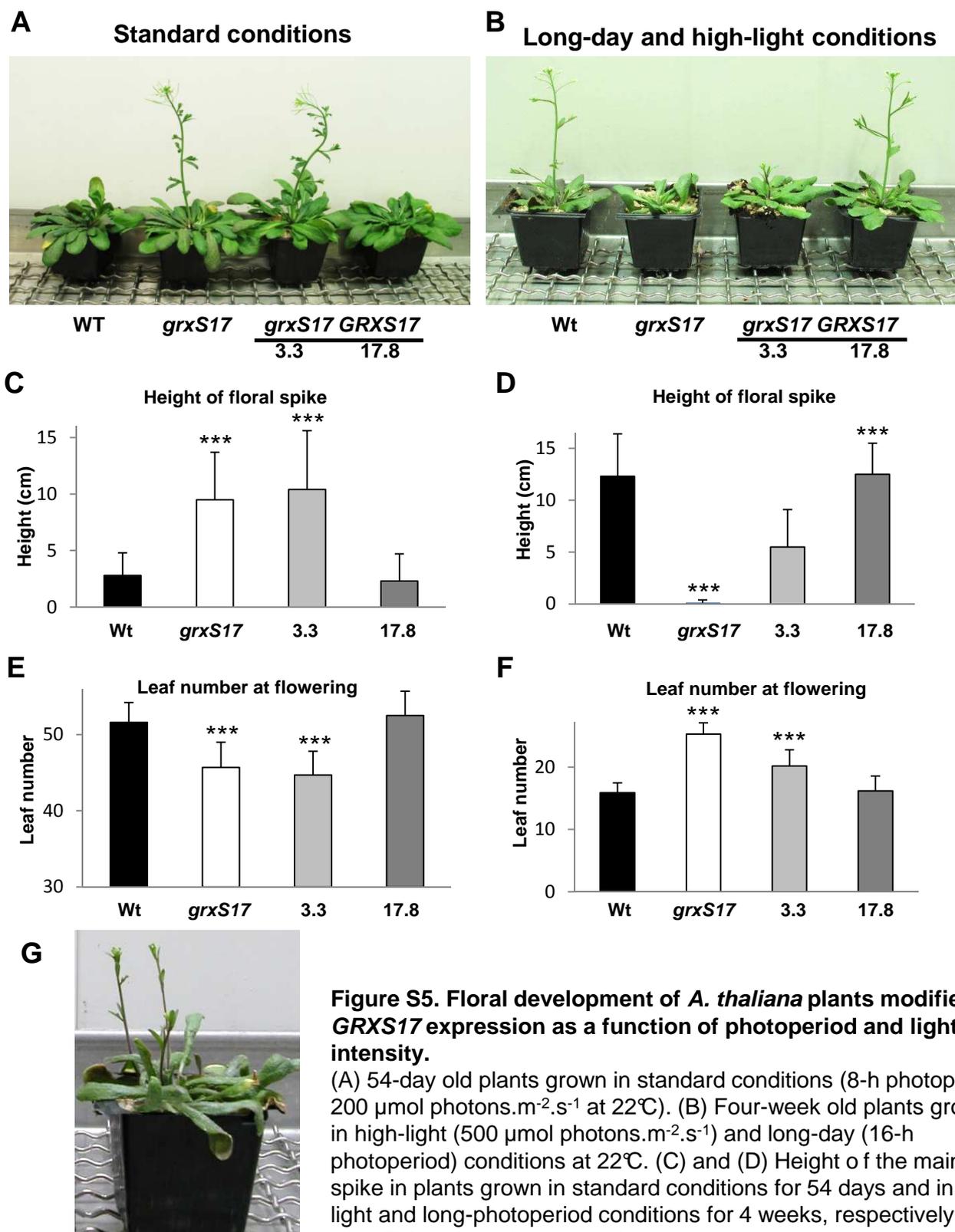
A **Standard conditions**

B **Long-day and high-light conditions**

WT   *grxS17*   <u>*grxS17 GRXS17*</u>
                     3.3   17.8

Wt   *grxS17*   <u>*grxS17 GRXS17*</u>
                     3.3   17.8

C Height of floral spike

D Height of floral spike

E Leaf number at flowering

F Leaf number at flowering

Figure S5. Floral development of *A. thaliana* plants modified in *GRXS17* expression as a function of photoperiod and light intensity.
(A) 54-day old plants grown in standard conditions (8-h photoperiod, 200 µmol photons.m^{-2}.s^{-1} at 22°C). (B) Four-week old plants grown in high-light (500 µmol photons.m^{-2}.s^{-1}) and long-day (16-h photoperiod) conditions at 22°C. (C) and (D) Height o f the main floral spike in plants grown in standard conditions for 54 days and in high-light and long-photoperiod conditions for 4 weeks, respectively. (E) and (F) Number of leaves at flowering in plants grown in standard conditions and in high-light and long-day conditions, respectively. (G) Five-week old *grxS17* plant grown in high-light (500 µmol photons.m^{-2}.s^{-1}) and long-day (16-h photoperiod) conditions at 22°C. WT, Wild-type plants; *grxS17*, homozygous SALK_021301 plants; *grxS17 GRXS17* 3.3 and 17.8, two independent KO lines expressing *GRXS17*. ***, significantly different from the WT value with $p < 0.01$ (t test).

Figure S6

A WT *grxS17*

grxS17 GRXS17
3.3   17.8

B WT *grxS17*

grxS17 GRXS17
3.3   17.8

C

Wt
Control

D

Wt
Photooxidative stress
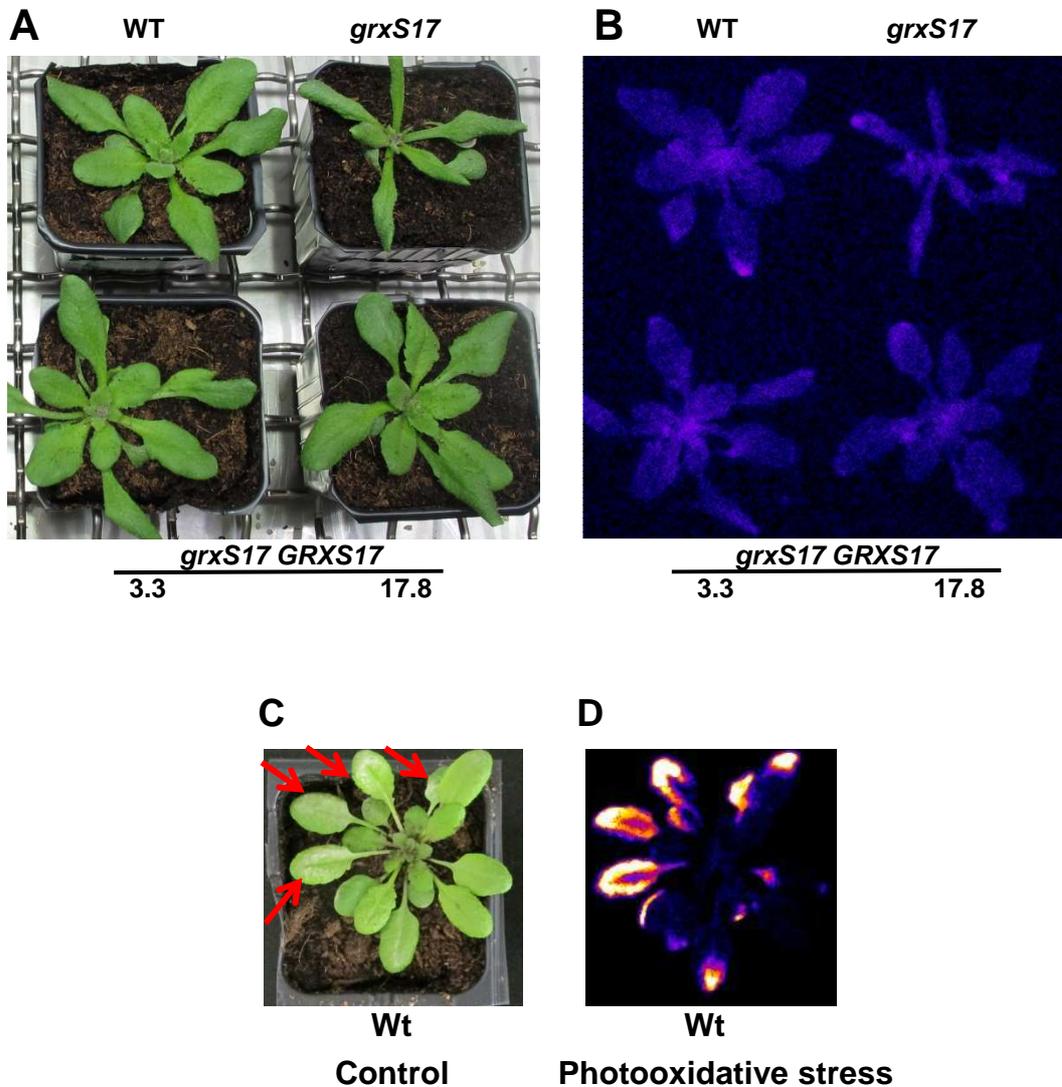
Figure S6. Autoluminescence in *A. thaliana* plants modified in *GRXS17* expression.
(A) Three-week old plants grown in high-light (500 µmol photons.m^{-2}.s^{-1}) and long-day (16-h photoperiod) conditions at 22℃. (B) Autoluminescence ima ging of lipid peroxidation in these plants. The homogeneous blue/purple color of plants indicates the absence of photodamage (compare panel D for positive control). WT, wild-type plants; *grxS17*, homozygous SALK_021301 plants; *grxS17 GRXS17* 3.3 and 17.8, two independent KO lines expressing *GRXS17*. (C) Five-week old WT plant grown in standard conditions (8-h photoperiod, 200 µmol photons.m^{-2}.s^{-1} at 22℃) and then exposed to a photooxidative treatment for 1 day (8-h photoperiod, 1250 µmol photons.m^{-2}.s^{-1} at 6℃). The red arrows indicate leaves displaying blea ching resulting from photodamage. (D) Autoluminescence imaging of lipid peroxidation in the plant shown in panel C. The damaged leaves appear in white/yellow color due to the photon emission associated with lipid peroxidation.

Figure S7

A

| WT | grxS17 | grxS17 GRXS17 3.3 |

B
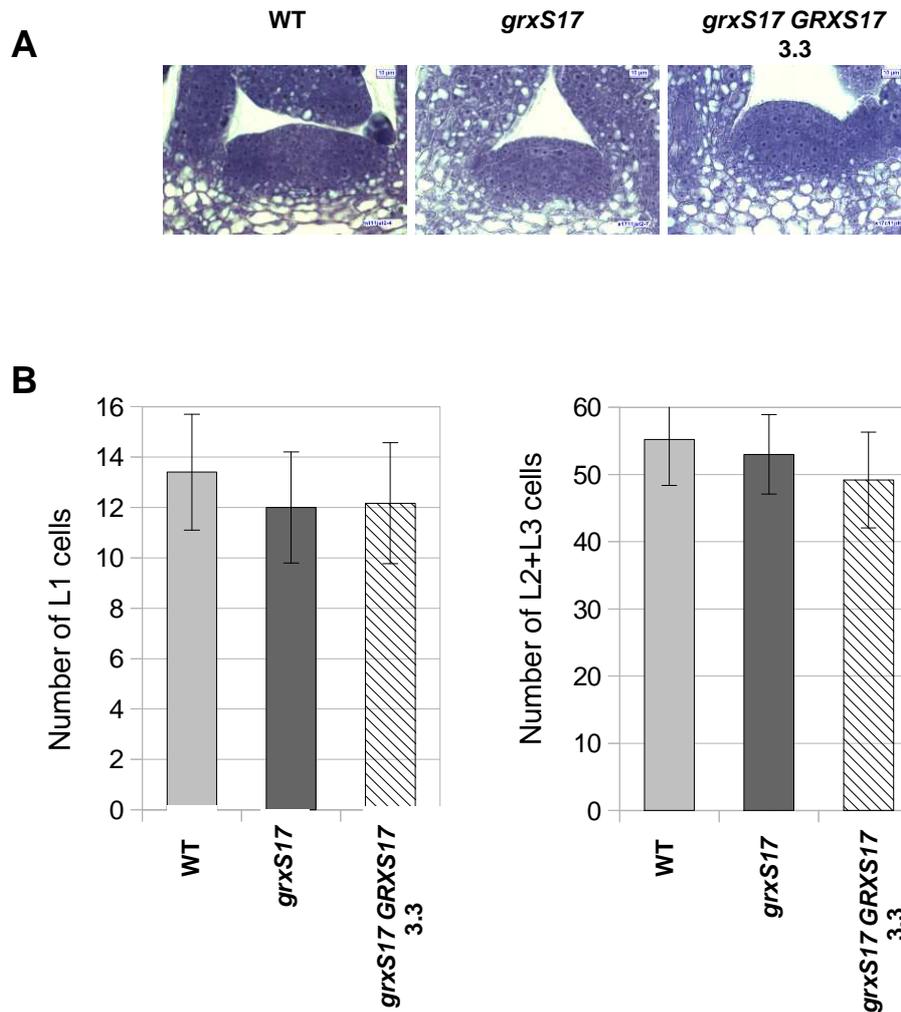
Figure S7. Structure of the shoot apical meristem (SAM) in the *grxS17* mutant.
(A) Histological structure of the SAM stained by toluidine blue in WT, *grxS17* and
grxS17 GRXS17 (line 3.3) seedlings grown for 11 days in short-day (8 h) conditions and
standard light (200 µmol photons.m^{-2}.s^{-1}). (B) Number of L1, L2 and L3 layer stem cells
in cross-sections of SAM shown in (A). Ten sections per genotype were analyzed.

Figure S8
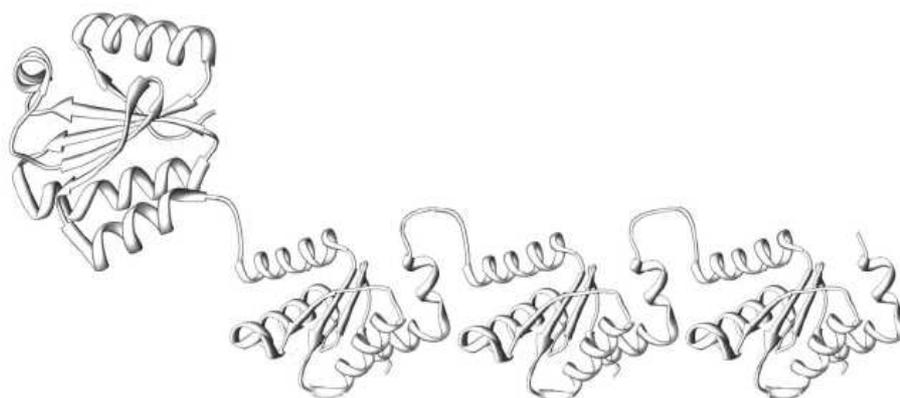

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M2    MLFMKGIPEEPRCGFSRKVVDILKEVNVDFGSFDILSDNEVREGLKKFSNWPTFPQLYCN 226
M3    MLFMKGRPEEPKCGFSGKVVEILNQEKIEFGSFDILLDDEVRQGLKVYSNWSSYPQLYVK 356
M4    MLFMKGSPDEPKCGFSSKVVKALRGENVSFGSFDILTDEEVRQGIKNFSNWPTFPQLYYK 463
      ****** *:**:**** ***. *.  ::.******* *:***:*:* :***.::**** :
M2    GELLGGADIAIAMHESGELKDAFKD 251
M3    GELMGGSDIVLEMQKSGELKKVLTE 381
M4    GELIGGCDIIMELSESGDLKATLSE 488
      ***:**.** : : :**:** .:.:
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Figure S8. Hypothetical structure of GRXS17 and amino acid alignment of the three GRX domains M2, M3 and M4 from *A. thaliana*. The structures of the single domains were generated from 1XFL and 3L4N pdb entries for TRX and GRX proteins, respectively, and are not experimentally confirmed. The linker regions and the relative positions of the TRX domain are speculative.

Figure S9

A

B
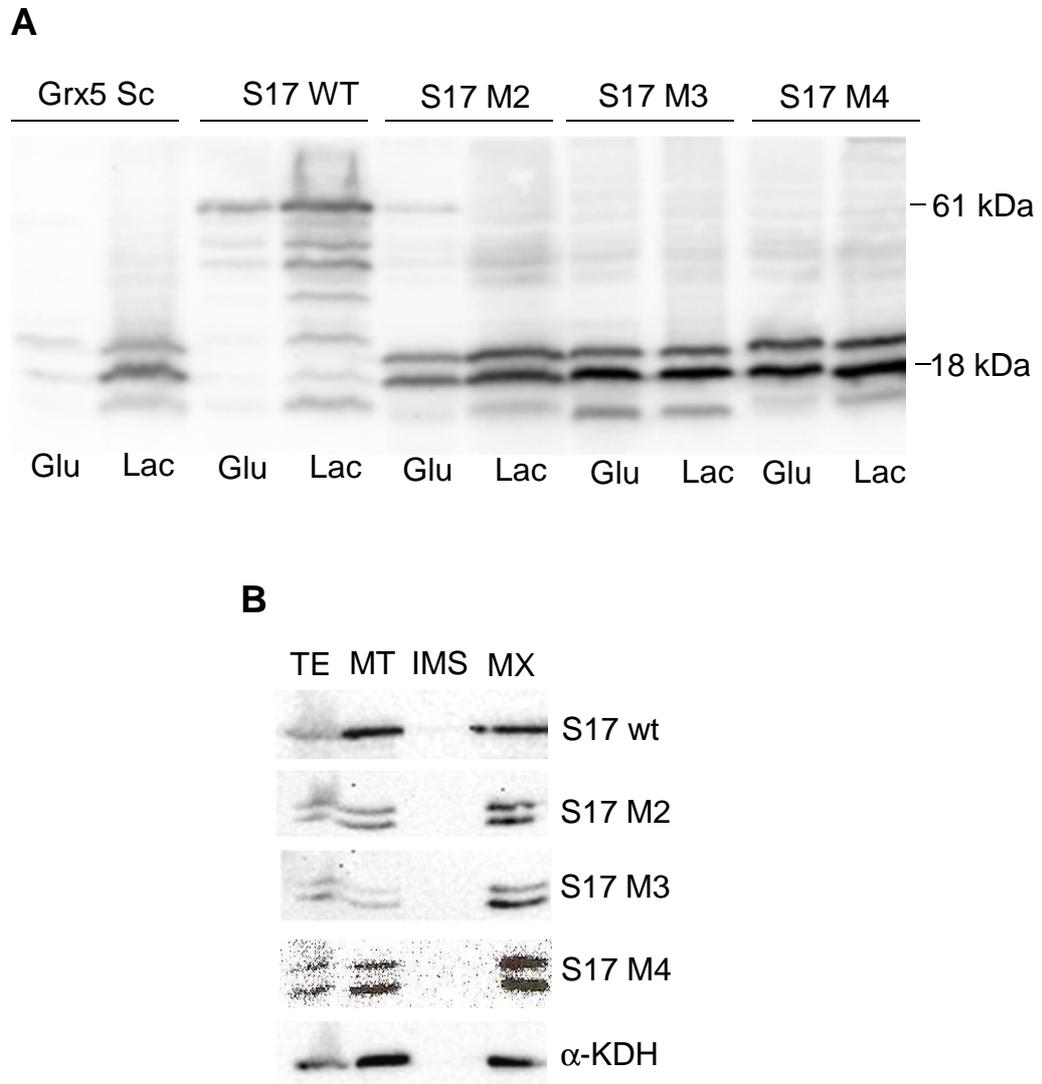
Figure S9. Analysis of the GRXS17 forms expressed in *S. cerevisiae*.
(A) Western blot analysis with anti-HA antibodies (10 μg total proteins from cell extracts per lane). Extracts from yeast cells expressing the homologous GRX5 protein under the native promoter (Grx5 Sc) are included as control. Samples from cells grown in fermentative conditions (Glu) and in respiratory conditions (Lac) were run in parallel. Molecular size of the expressed S17 forms is indicated. (B) Subfractionation of extracts from *S. cerevisiae* cells expressing the respective S17 forms and grown in respiratory conditions (lactose medium). TE (total extracts, 25 μg), MT (total mitochondria, 5 μg), IMS (intermembrane mitochondrial space, 5 μg), MX (mitochondrial matrix, 5 μg). Alpha-ketoglutarate dehydrogenase (α-KDH) is shown as a control of a mitochondrial matrix protein.

Figure S10

A

B
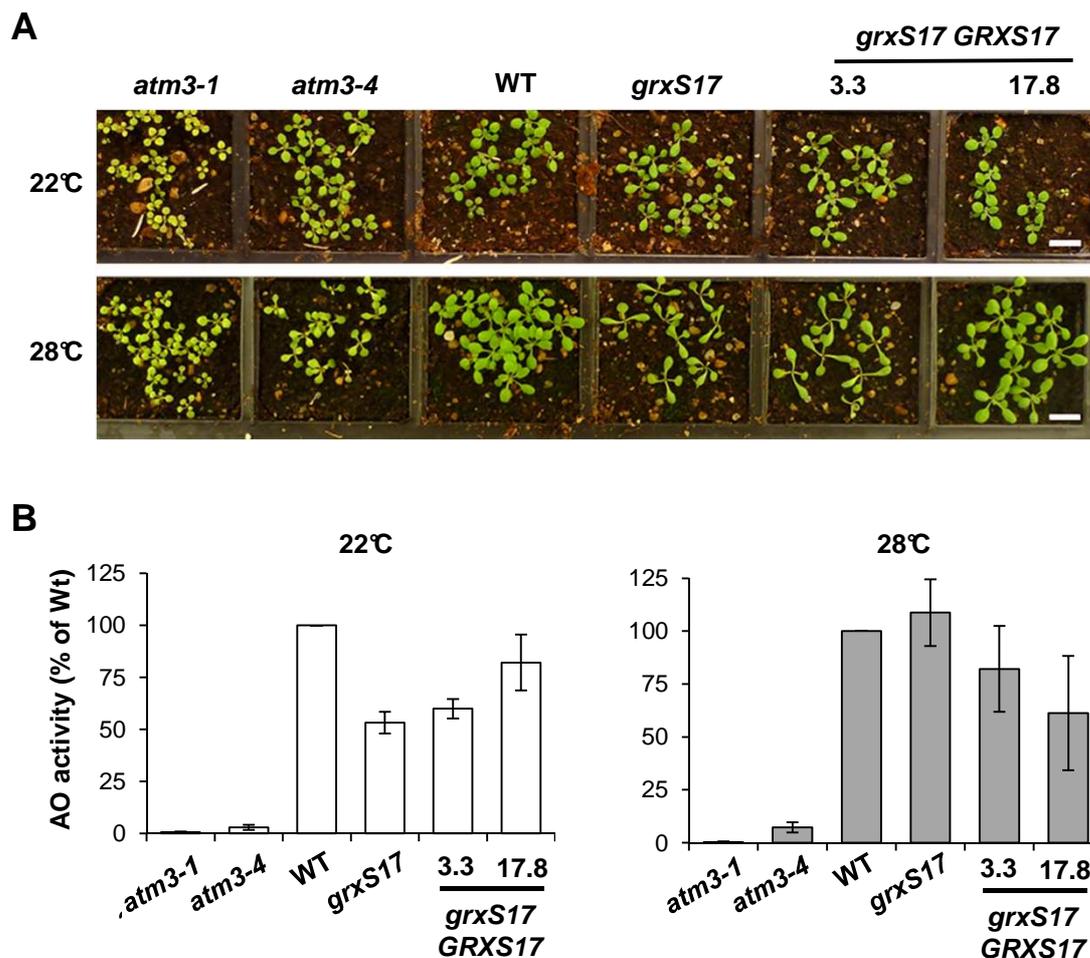
Figure S10. Growth phenotypes and quantification of aldehyde oxidase enzyme activity in plants modified in *GRXS17* expression.

grxS17 knockout seedlings were grown under normal and heat stress conditions and compared to complemented lines (3.3 and 17.8) and wild type (WT). For comparison, a weak (*atm3-4*) and a strong mutant allele (*atm3-1*) of *ATM3* were analysed, which are known to have decreased activities of cytosolic Fe-S enzymes. Plants were grown in long photoperiod (16 h) at 22°C for 14 days (white bars), or shifted to 28°C after 7 days (grey bars). (A) Images of *grxS17* mutants and controls grown under standard conditions and under heat stress. Scale bar = 1 cm. (B) Quantification of the band intensities in native gels (Fig. 7) reflecting the major aldehyde oxidase activity using ImageJ. Activities are shown as percentage of WT levels (mean ± SD; n = 3).

Figure S11

A

B

C
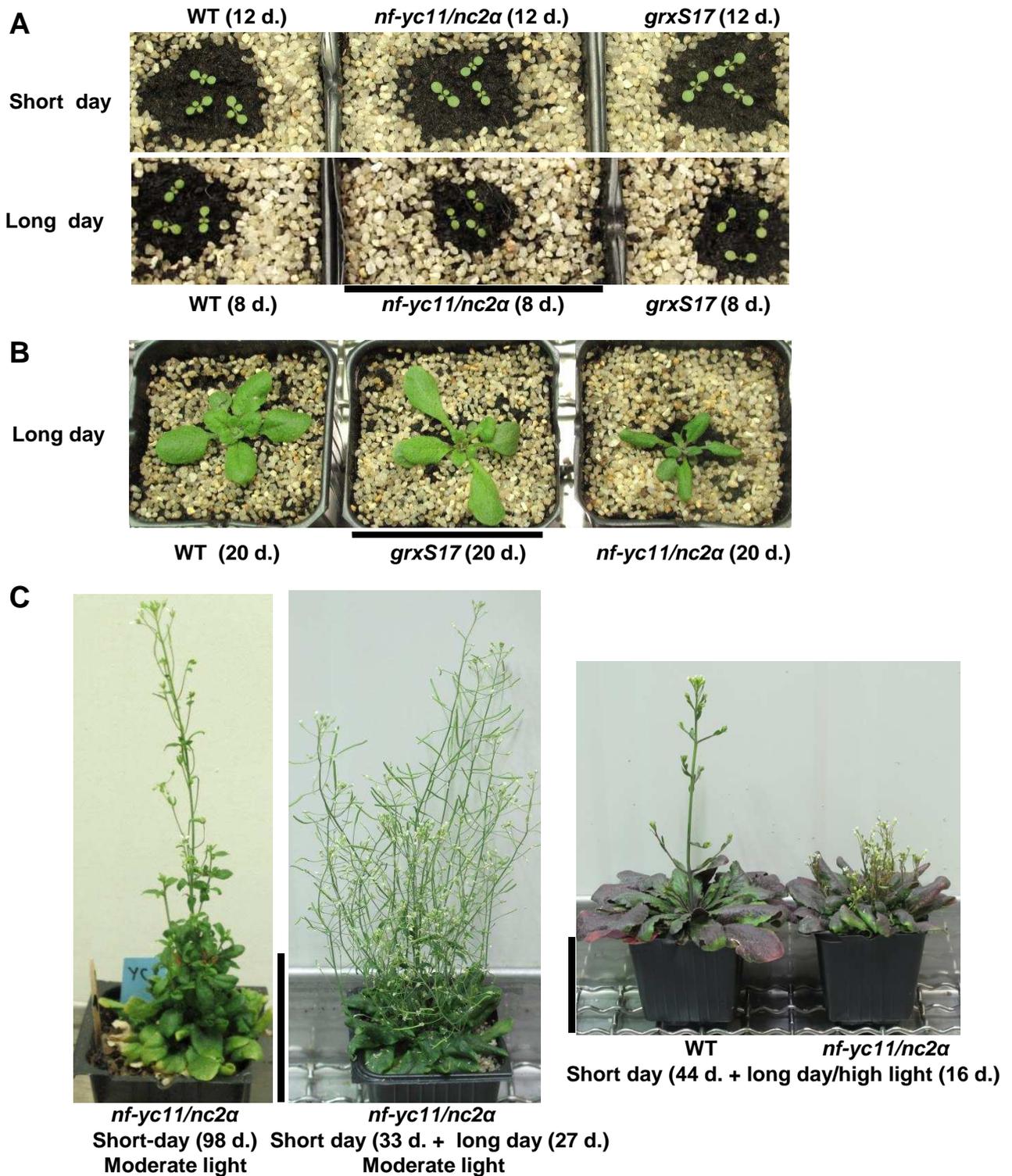
Figure S11. Development of *nf-yc11/nc2α* plants as a function of light environment
(A) Development of seedlings in short- and long-day conditions (8-h and 16-h, respectively) at moderate light (200 µmol photons.m m^{-2}.s^{-1}) and 22℃. (B) Phenotype of plants grown in long-da y (16 h photoperiod) and moderate light (200 µmol photons. m^{-2}.s^{-1}) conditions at 22℃. (C) Reproductive development of *nf-yc11/nc2α* plants in short- or long-day conditions at moderate or high light (200 or 500 µmol photons.m m^{-2}.s^{-1}, respectively) and 22℃. Scale bars: 6 cm. The plant ag e and culture condition are indicated for each genotype.

```
At   1  --------------------------------------------MRKKLDTRFPA-----ARIKKIMQADEDVGKIALAVPVLVSKSLELFLQDLCDRTYEITLERGAKTVSSLHLKHCVERYNVFDFLREVVSKVPDY  90
Mt   1  --------------------------------------------MKKKLDTRFPA-----ARIKKIMQADEDVGKIALAVPVLVSKALELFLQDLCDRTYEITLQRGAKTMNSLHLKHCVQSYNVFDFLKDVVSKVPDY  90
St   1  --------------------------------------------MKKKLDTRFPA-----ARIKKIMQADEDVGKIAMAVPVLVSKALELFLQDLCDRTYDITLRRGAKTVNSLHLKHCVQSYNVFDFLREVVSKVPDY  90
Pt   1  --------------------------------------------MKKKLDTRFPA-----ARIKKIMQADEDVGKIALAVPVLVSKALELFLQDLCDRTHEITLQRGAKTMSALHLKHCVQSYNVFDFLREIVSRVPDY  90
Vv   1  ------------------------------MHLGMQVMKKNFGGGGPCGLPWVSRIKKIMQADEDVGKIALAVPLLVSKALELFLQDLCDRTYQITLERGAKTMSSLHLKQCVQRFNVFDFLREIVSKVPDL 102
Os   1  --------------------------------------------MRKKLGTRFPA-----ARIKKIMQADEDVGKIALAVPVLVSRALELFLQDLIDRTYEITLQSGAKTLNSFHLKQCVRRYSSFDFLTEVVNKVPDL  90
Bd   1  --------------------------------------------MRKKLGTRFPA-----ARIKKIMQADEDVGKIALAVPVLVSRALELFLQDLIDRSYNITVQSGAKTLNSFHLKQCVKRYNSFDFLTEIVNKVPDL  90
Zm   1  --------------------------------------------MRKKLGTRFPA-----ARIKKIMQADEDVGKIALAVPVLVSRSLELFLQDLIDRTYEITLQSGAKTLNSFHLKQCVKRYSSFDFLTEVVSKVPDL  90
Hs   1  -----------------------------------------------MEFRIKKIMQTDEEIGKVAAAVPVIISRALELFLESLLKKACQVTQSRNAKTMTTSHLKQCIELEQQFDFLKDLVASVPDM  81
Sc   1  MADQVPVTTQLPPIKPEHEVPLDAGGSPVGNMGTNSNNNNELGDVFDRIKTHFPP-----AKVKKIMQTDEDIGKVSQATPVIAGRSLEFFIALLVKKSGEMARGQGTKRITAEILKKTILNDEKFDFLREGLCVEEGQ 134
                                                                      ::*****:**::**:: *.*:: .:::**:*: * .:: ::: .:* :.: **: : . **** :: .
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At   91  GHSQGQGHGDVTMDDRSISKRRKPISDEVNDSDEEYKKSKTQEIGSAKT---SGRGGRGRGRGRGGRAAKAAEREGLNREMEVEAANSGQPPPEDN--------------VKMHASESSPQEDEKKGIDGTAASNE 211
Mt   91  GHGHGHTDAGGAD-DQTIPKRRKAAGDDGNDSDEEAKRGKMLELGHTS----PTGRGRGRGRGRGRGRGRAIQREGHHQETESEPCPSVQQVSQNTTDTNVAILDGTESNELPKNNVVVLPADNSDSLLNIDLNANM 224
St   91  GHSDAAGEM------P---KRRKVAIEEHHDSEDEYKKSRTEMS------PVSSSGRGRGRGRGRGRGRVSRADKELARPDMQLESCTSAQQSGQQNPN----------PGTQTENCSEPKESPKQ-----DSTVGD 197
Pt   91  SHGHSDSTG-----DHRPLQKRKPTGDECNDSDEELKRSRMHEMSHAG----SSGRGRGRGRGRGRG-ARNIERESSSRDLEPESCTTVQQSIKNNTNPGVVMDNGSESKESVKENNRVSDATNQP-ERNFDLNAEV 218
Vv  103  GCSDAGGED------RSASKRRKVVDDEGNDSDEESKRSRMHETGHTVGSGSGSGSGRGRGRGRGRGRGRGSRTIERDTIAESEKPEDDTDMHTNNDNQSQNPETRDNGAEPEESKENISVVKNESKE-----NISVGK 230
Os   91  GGADSCGDD------RALPRRRKALPNGSDPENEESRSSKMAVRSANIS------PRGRGRGRGRGRPPTKRKEVGYVQFEDESSMFADQGE------------------------------------------AL 174
Bd   91  GGADSCGDE------RGLPRRRKLS-NESDPENEEPRSSKMPIRSLNTS-------PRGRGRGRGRGRPPTKRKEVGYVQFEDESSMFTEQSE-------------------------------------------SL 173
Zm   91  GGADSCGDE------RGLPRRRKSN--GSDPENDESRSSKMAIRNANIS------PRGRGRGRGRGRPPTKRKEVGYVQFEDESSMFAEQGE-------------------------------------------PL 172
Hs   82  QGDGE--------------------DNHMDGDKGARRGRKPGSG--------------GRKNGGMGTKSKDKKLSGTDSEQEDESEDTDTDG---------------------------------------------- 139
Sc  135  TQPEEESA----------------------------------------------------------------------------------------------------------------------------- 142
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At  212  DTKQHLQSPKEGIDFDLNAESLDLNETKLAPATGTTTTTTAATDSEEYSGWPMMDIS--KMDPAQLASLGKRIDEDEEDYDEEG 293
Mt  225  NENDDKKASTVANLTVTIPEAAN---PEAANPPVSEPPPPDSSHHEEIPGWSLSEVDKMAIDSMQLAQLGTRMDEDDEDYDEEG 305
St  198  KENSVVTTPNLKVDVDDSTD----KPAAPEIAPCNPSPRPVNEKAEEGPQWSL-EMDRMVIDPAHMSQLNTSVAEEEEDYDEEE 276
Pt  219  NDSEDAKAAAAPAAAAPAAAATTTTTTTTTTSAPSSSVEPAAETNHEEYPGWSLSEMDKMVIDPLQLAQLSKRLDEEEEDYDEEG 302
Vv  231  NADSCVRNFDLNVDLDENGDSTSILPAAPAPAPVTPSPKLTEMKHEEYPGWSLSDMEKMEIDPIQLANLNRRIDEDEDYDEE- 313
Os  175  PGEETVPETIHGTESVPPSTHPPAEAPSAAEIPAPNPKVEEAKNDDHQPDWPMPDAIGNIGVGPSGFGHLTVQVDEDEDYDNED 258
Bd  174  PGDDAIPETKCGSESIPQSANPPADAPSTGVPAAIS--KVEEASTNHQPDWPMPDAIGGIGVGPSSFGHLTVQVDEDEDYDNED 255
Zm  173  PGEETVQEING-NETMPQSTQPPVEAPPTALAQATTSSKAEEANSDHQSDWPMPDAIGSIGVVPSGFGHLTVQVE-DEDYDNED 254
Hs  140  -------------------------EEETSQPPPQASHPSAHFQSPPTPFLPFASTLPLPPAPPGPSAPDEEDEEDYDS-- 193
Sc       ---------------------------------------------------------------------------------
```

Figure S12: Sequence alignment of plant, human and yeast proteins containing a NC2α domain. Alignment was performed using the ClustalW software. At, *Arabidopsis thaliana* (At3g12480, NP_187854.2); Mt, *Medicago truncatula* (XP_003628014.1); St, *Solanum tuberosum* (XP_006347683.1); Pt, *Populus trichocarpa* (XP_002304814.1); Vv, *Vitis vinifera* (CAN71308.1); Os, *Oryza sativa* (AF464904); Bd, *Brachypodium distachyon* (XP_003577492.1); Zm, *Zea mays* (NP_001105089.1); Hs, *Homo sapiens* (AAB02192.1); Sc, *Saccharomyces cerevisiae* (NP_011086.3). Amino acids are given using standard single-letter designation, and dashes indicate gaps. Stars and colons indicate identity and similarity of amino acids, respectively. The cysteine conserved in plant and human proteins is highlighted in yellow, the cysteines conserved in dicotyledonous and monocotyledonous plants are highlighted in blue and green, respectively. The black line indicates the NC2α domain.

Table SI. Plasmids employed for experiments on yeast.

Plasmids	Characteristics	
pMM993	Sequence coding from amino acid 1-488 of GrxS17 cloned between *NotI-PstI* sites of pMM221	
pMM994	Sequence coding from amino acid 167-252 (M2) of GrxS17 cloned between *NotI-PstI* sites of pMM221	
pMM997	Sequence coding from amino acid 297-383 (M3) of GrxS17 cloned between *NotI-PstI* sites of pMM221	
pMM998	Sequence coding from amino acid 404-488 (M4) of GrxS17 cloned between *NotI-PstI* sites of pMM221	

Table SII. Yeast strains.

Strains	Relevant phenotype	Comments
W303-1A	*MAT***a** *ura3-1 ade2-1 leu2-3,112 trp1-1 his3-11,15*	Wild type
W303-1B	As W303-1A but *MATα*	Wild type
MML100	*MAT***a** *grx5::kanMX4*	Rodríguez-Manzaneque *et al.* (2002)
MML240	*MAT***a** *grx5::kanMX4* [pMM54(*GRX5-3HA*)]::*LEU2*	Rodríguez-Manzaneque *et al.* (2002)
MML289	*MATα grx5::kanMX4*	As MML100, but derived from W303-1B
MML1268	*MAT***a** [pMM997(*GrxS17-3HA*)]::*LEU2*	Integration of linear pMM997 in W303-1A
MML1272	*MAT***a** [pMM993(*GrxS17-3HA*)]::*LEU2*	Integration of linear pMM993 in W303-1A
MML1274	*MAT***a** [pMM994(*GrxS17-3HA*)]::*LEU2*	Integration of linear pMM994 in W303-1A
MML1276	*MAT***a** [pMM998(*GrxS17-3HA*)]::*LEU2*	Integration of linear pMM998 in W303-1A
MML1280	*MAT***a** *grx5*::*kanMX4* [pMM993(*GrxS17-3HA*)]::*LEU2*	Spore from a cross MML 289 x MML1272
MML1282	*MAT***a** *grx5*::*kanMX4* [pMM994(*GrxS17-3HA*)]::*LEU2*	Spore from a cross MML 289 x MML1274
MML1288	*MAT***a** *grx5*::*kanMX4* [pMM997(*GrxS17-3HA*)]::*LEU2*	Spore from a cross MML 289 x MML1268
MML1290	*MAT***a** *grx5*::*kanMX4* [pMM998(*GrxS17-3HA*)]::*LEU2*	Spore from a cross MML 289 x MML1276

Table SIII. List of primers employed in this study.

Name	Primers	Characteristics	Organism
MMO 1285	AAACAGCGGCCGCATTATTCATGAAAGGTATTCCT	Upstream oligo for cloning M2 module of *AtGRXS17* in pMM221. Contains NotI site	Yeast
MMO 1286	AAACACTGCAGAAGATCTTTGAAAGCATCTTT	Downstream oligo for cloning M2 module of *AtGRXS17* in pMM221. Contains PstI site	Yeast
MMO 1287	AAACAGCGGCCGCACTGTTCATGAAAGGAAGAC	Upstream oligo for cloning M3 module of *AtGRXS17* in pMM221. Contains NotI site	Yeast
MMO 1288	AAACACTGCAGCCCTTTCTCGGTCAAGAC	Downstream oligo for cloning M3 module of *AtGRXS17* in pMM221. Contains PstI site	Yeast
MMO 1289	AAACAGCGGCCGCACTATTCATGAAAGGTTCACC	Upstream oligo for cloning M4 module of *AtGRXS17* in pMM221. Contains NotI site	Yeast
MMO 1290	AAACACTGCAGCTCGGATAGAGTTGCTTTG	Downstream oligo for cloning M4/entire module of *AtGRXS17* in pMM221. Contains PstI site	Yeast
MMO 1291	AAACAGCGGCCGCAAGCGGTACGGTGAAGGAT	Upstream oligo for cloning the entire *AtGRXS17* in pMM221. Contains NotI site	Yeast
At4g04950 F	CCCCCATGAGCGGTACGGTGAAGGAT	Forward primer for cloning *AtGRXS17* and PCR analysis	Arabidopsis
At4g04950 R	CCCCGGATCTTACTCGGATAGAGTTGC	Reverse primer for cloning AtGRXS17 and PCR analysis	Arabidopsis
At4g04950 Rec-F	GGGAATTCCATATGAGCGGTACGGTGAAGG	Forward primer for cloning AtGRXS17 in pET-16b expression vector	Arabidopsis
At4g04950 Rec-R	CGGGATCCTTACTCGGATAGAGTTGCTTTG	Reverse primer for cloning AtGRXS17 in pET-16b expression vector	Arabidopsis
At4g04950 C33S F	CTTCTGGGCTTCTTGGAGTGATGCTTCGAAGCA	Forward primer for site-directed mutagenesis (Cys-33 to Ser)	Arabidopsis
At4g04950 C33S R	TGCTTCGAAGCATCACTCCAAGAAGCCCAGAAG	Reverse primer for site-directed mutagenesis (Cys-33 to Ser)	Arabidopsis
At4g04950 C179S F	CCTGAAGAGCCTAGGAGTGGGTTTAGCAGGA	Forward primer for site-directed mutagenesis (Cys-179 to Ser)	Arabidopsis
At4g04950 C179S R	TCCTGCTAAACCCACTCCTAGGCTCTTCAGG	Reverse primer for site-directed mutagenesis (Cys-179 to Ser)	Arabidopsis
At4g04950 C309S F	GACCAGAAGAACCAAAGAGTGGGTTCAGTGGGAAA	Forward primer for site-directed mutagenesis (Cys-309 to Ser)	Arabidopsis
At4g04950 C309S R	TTTCCCACTGAACCCACTCTTTGGTTCTTCTGGTC	Reverse primer for site-directed mutagenesis (Cys-309 to Ser)	Arabidopsis
At4g04950 C416S F	GGTTCACCAGATGAACCGAAAAGCGGATTTAGCT	Forward primer for site-directed mutagenesis (Cys-416 to Ser)	Arabidopsis
At4g04950 C416S R	AGCTAAATCCGCTTTTCGGTTCATCTGGTGAACC	Reverse primer for site-directed mutagenesis (Cys-416 to Ser)	Arabidopsis
At5g09810 F	AAAATGGCCGATGGTGAGGATAT	Forward primer for PCR analysis (Actin control)	Arabidopsis
At5g09810 R	CAATACCGGTTGTACGACCACT	Reverse primer for PCR analysis (Actin control)	Arabidopsis
At3g12480 F2	TCGACGATTTCTGGATTTGG	Forward primer for PCR and RT-PCR analyses (AtNF-YC11/NC2α)	Arabidopsis
At3g12480 R2	CCTTCTCTTGAGATGCTGC	Reverse primer for PCR and RT-PCR analyses (AtNF-YC11/NC2α)	Arabidopsis