

Supplementary Fig. S1. Correlation analyses of *Lhcb1* expression levels, ETR, as well as contents of MgProtoIX, soluble sugars and total carbohydrates. (**A**) *Lhcb1* expression *vs* MgProtoIX contents. MgProtoIX *vs* total carbohydrates (**B**), soluble sugars (**C**), or ETR (**F**). *Lhcb1* expression *vs s* total carbohydrates (**D**), soluble sugars (**E**), or ETR (**G**). The data were obtained from Figs. 1 to 3. Where necessary log2-ratios instead of absolute values were used. The colours represent Col-0 (black), *tpt-2* (blue), *adg1-1* (red) and *adg1-1/tpt-2* (dark purple).

Supplementary Fig. S2. Graphviz presentation of co-expression networks obtained with ATTEDII for data shown in Supplementary Table S7A and Supplementarty Document S3. The query genes were found amongst the commonly regulated genes 4h after LL/HLtransfer. `Query genes´ related to major CHO metabolism (up-regulated) (**A**), lipid metabolism up- (**B**) or down-regulated (**C**), are marked in light blue or purple colour, respectively. Co-expressed genes indentified as differentially regulated in the array experiment are marked in dark-blue or purple colour. All other genes within the coexpression network are marked in grey. The dark blue-coloured genes marked in (**B**) are associated with major CHO metabolism and were co-expressed with `query genes´ for lipid metabolism.

Supplementary Figure S3. Relative distribution of differentially regulated genes in publicly available microarray experiments compared to in-house expression data for Col-0 (**A**), *tpt-2* (B) , $adg1-1$ (C) and $adg1-1/tpt-2$ at t_{4h} and t_{48h} after LL/HL-transfer.

Supplementary Table S11. Contents of metabolites determined by GC/MS in leaves of **(A)** Col-0, **(B)** *adg1-1*, **(C)** *tpt-2* and **(D)** *adg1-1/tpt-2* grown either continuously in LL or HL or after a LL/HL-transfer at \mathfrak{t}_{4h} or \mathfrak{t}_{48h} . The data represent the mean of five independent samples ± SE. **A**

A (continued)

B

B (continued)

β-Alanine 0.187 [±] 0.008 0.862 [±] 0.083 1.800 [±] 0.092 1.360 [±] 0.134

C

C (continued)

D

D (continued)

Supplementary Document S1

Document S1 (Table 1). Statistical analysis (ANOVA/Tukey-Kramer) of photosynthetic electron transport (ETR) and F_v/F_m ratios of wild-type and mutant plants in a time series after transfer from LL conditions (*i.e*. a PFD of 30 µmol·m-2·s-1) to HL (*i.e.* a PFD of 300 µmol·m-2·s-1) within 172 h. The plant lines are denoted, *^a* = Col-0, *b* = *tpt-2*, *^c* ⁼*adg1-1*, *d* ⁼*adg1-1/tpt-2.* Significance levels of P < 0.05 or P < 0.01 are indicated by light or dark blue colours.

Document S1 (Table 2). Statistical analysis (ANOVA/Tukey-Kramer) of starch, soluble sugar, and anthocyanin levels of wild-type and mutant plants in a time series after tansfer from LL conditions (*i.e*. a PFD of 30 µmol·m-2·s-1) to HL (*i.e*. a PFD of 300 µmol·m-2·s-1) within 148 min. The plant lines are denoted, *^a* = Col-0, *b* = *tpt-2, ^c* ⁼*adg1-1*, *d* ⁼*adg1-1/tpt-2.* Significance levels of P < 0.05 or P < 0.01 are indicated by light or dark blue colours.

Document S1 (Table 3). Statistical analysis (ANOVA/Tukey-Kramer) of MgProtoIX contents and Lhcb1 transcript abundance of wild-type and mutant plants in a time series after transfer from LL conditions (*i.e.* a PFD of 30 µmol·m⁻²·s⁻¹) to HL (*i.e.* a PFD of 300 µmol·m⁻²·s⁻¹) within 480 min. The plant lines are denoted, *^a* = Col-0; *b* = *tpt-2, ^c* ⁼*adg1-1*, *d* ⁼*adg1-1/tpt-2.* Significance levels of P < 0.05 or P < 0.01 are indicated by light or dark blue colours.

Document S1 (Table 4). Statistical analysis (ANOVA/Tukey-Kramer) of contents of redox components of wild-type and mutant plants in a time series after transfer from LL conditions (*i.e.* a PFD of 30 µmol·m⁻²·s⁻¹) to HL (*i.e.* a PFD of 300 µmol·m⁻²·s⁻¹) within 4h compared to HL grown plants. The biotypes are denoted, *^a* = Col-0; *b* = *tpt-2*, *^c* ⁼*adg1-1*, *d* ⁼*adg1-1/tpt-2.* Significance levels of P < 0.05 or P < 0.01 are indicated by light or dark blue colours.

Document S1 (Table 5). Statistical analysis (ANOVA/Tukey-Kramer) of metabolite contents of wild-type and mutant plants in a time series after transfer from LL conditions (*i.e*. a PFD of 30 µmol·m-2·s-1) to HL (*i.e.* a PFD of 300 µmol·m-2·s-1) within 4h and 48h compared to HL- or LLgrown plants. The conditions are denoted, a = t₀ (LL); b = t_{4h} (HL), c = t_{48h} (HL), d = HL. Significance levels of P < 0.05 or P < 0.01 are indicated by light or dark blue colours.

Document S1 (Table 5), continued

Document S1 (Table 5), continued

Document S1 (Table 5), continued.

Document S1 (Table 6). Statistical analysis (ANOVA/Tukey-Kramer) of metabolite contents of wild-type and mutant plants in a time series after transfer from LL conditions (*i.e*. a PFD of 30 µmol·m-2·s-1) to HL (*i.e.* a PFD of 300 µmol·m-2·s-1) within 4h and 48h compared to HL- or LLgrown plants. The plant lines are denoted, *^a* = Col-0; *b* = *tpt-2*, *^c* ⁼*adg1-1*, *d* ⁼*adg1-1/tpt-2.* Significance levels of P < 0.05 or P < 0.01 are indicated by light or dark blue colours.

Document S1 (Table 6), continued

Document S1 (Table 6), continued

Organic acids Pyruvic acid (1MEOX) (1TMS) Malic acid, 2-methyl-, DL- (3TMS) Glutaric acid (2TMS) Succinic acid (2TMS) Fumaric acid (2TMS) Malonic acid (2TMS) Glyceric acid, DL- (3TMS) Butyric acid, 3-hydroxy- (2TMS) Gluconic/Galactonic acid (6TMS) Benzoic acid (1TMS)

Document S1 (Table 6), continued

Supplementary Document S2

Static assessment of global gene expression after LL/HL-transfer

(A) *adg1-1 vs* **Col-0**

The starch-free *adg1-1* single mutant contained the highest number of significantly altered genes in the static comparison, particularly under LL-conditions (Fig. 6A) or 48h after LL/HLtransfer. Under LL-conditions 16 gens belonging to the category `protein metabolism´ were differentially regulated as a part of the 144 highly altered genes. Interestingly, among these 16 genes, 12 genes related to `protein degradation´ were down-regulated and four genes related to `protein synthesis´ were up-regulated (Tables 1, Supplementary Table S2A), suggesting that in the absence of starch the maintenance of protein abundance is promoted.

The transfer from LL to HL had a profound impact on the functional categories of altered genes in the *adg1-1* single mutant. Although the number of differentially regulated genes dropped 4h after transfer to HL, 30 and 17 genes were up- and down-regulated, respectively, compared to the wild type (Table 1, Supplementary Table S3A). Among the 30 up-regulated genes, there were five genes related to `lipid metabolism´. Four of the five proteins reside in chloroplasts. Furthermore, four genes involved in `secondary metabolism´ (flavonoid biosynthesis) were up-regulated and five genes related to `stress´ were downregulated (Table 1, Supplementary Table S3A). These data suggest an elevated input into lipid formation and/or secondary metabolism as a consequence of a deficiency in the nightpath of photoassimilate export from the chloroplast.

The functional pattern of genes changed appreciably after 48h in HL (Fig. 6, Table 1, Supplementary Table S4A). There were a total of 209 genes specifically altered in *adg1-1* compared to the wild type. Interestingly, although the category `lipid metabolism´ was missing among the 58 highly up-regulated genes, there were seven genes belonging to this category among the 151 highly down-regulated genes, with four of these genes related to `lipid degradation´. Hence, the transient enhancement of lipid synthesis (i.e. 4h after LL/HLtransfer) was replaced by an inhibition of lipid degradation in the long term. Furthermore five genes involved in `major CHO metabolism´, 12 genes related to `proteins´ (including seven genes connected to `protein synthesis´), as well as six genes related to `regulation of transcription (RT)´ were highly up-regulated (Table 1). Moreover, the up-regulation of genes involved in `protein synthesis´ was accompanied by a down-regulation of 13 genes related to `protein degradation´, again suggesting an enhanced production and/or maintenance of proteins. Furthermore 21, 11, and seven genes related to `RT´, `stress´ and `signalling´,

respectively, were highly down-regulated in *adg1-1* 48h after LL/HL-transfer (Supplementary Table S4A). Moreover, the gene coding for the glucose 6-phosphate/phosphate translocator 2 (*GPT2*; At1g61800) was highly up-regulated in *adg1-1*.

(B) *tpt-2 vs* **Col-0**

Under LL-conditions, a limitation in the day-path of photoassimilate export from the chloroplast in the *tpt-2* mutant resulted only in the down-regulation of a single gene encoding a disulfide isomerase-like protein (AtPDIL 5-4, At4g27080; Supplementary Table S2B).

The number of highly altered genes in *tpt-2* was increased to 36, 4h after the plants were transferred to HL. There were only two genes down-regulated, amongst them again AtPDIL 5-4 and a protein of unknown function (At4g27080; Supplementary Table S3B). The 34 highly up-regulated genes comprised three genes related to `major carbohydrate metabolism´ and seven, significantly over-represented genes involved in `protein degradation´ (Table 1). After 48h in HL, again, there were more genes highly up-regulated (104) than down-regulated (3) (Supplementary Table S4B). Interestingly, AtPDIL 5-4 still belonged to the down-regulated genes. Within the group of up-regulated genes there were three significantly over-represented functional clusters, *i.e.* `cell wall´, `hormone metabolism´ and `stress´ (Table 1). Furthermore, genes related to `RT´ and `development´ were represented with at least five members. Ten genes, including a MAPkinase (At1g01560), were connected to `signalling´, in particular, `calcium signalling´ (nine genes).

(c) *adg1-1/tpt-2 vs* **Col-0**

Despite the relative high number of specifically altered genes in the *adg1-1* mutant, surprisingly, the combined deficiency in the day- and night-path of photoassimilate export resulted in an appreciable lower number of differentially regulated genes in *adg1-1/tpt-2*. Under LL-conditions there were only 21 genes highly up- or down-regulated in the double mutant (Supplementary Table S2C). Among the nine up-regulated genes in *adg1-1/tpt-2,* remarkably, there were three genes related to `abiotic stress´, sub-category heat. All three genes belong to the putative HSP20-type protein of unknown function (At1g53540, At3g46230, At5g12020). Of the 12 down-regulated genes one half is related to `histone proteins´ and `chromatin structure´, suggesting that parts of the DNA was not associated with proteins and/or the plants contained less DNA. The only gene dramatically downregulated in the overlapping area of *adg1-1/tpt-2* and *tpt-2* was, as expected, the *TPT* gene (Supplementary Table S2D). In the overlapping region between *adg1-1/tpt-2* and *adg1-1* there were 13 up- and 33 down-regulated genes found. Among the down-regulated genes there were six genes related to `chromatin structure´ as well as five genes involved in `protein degradation´, again suggesting a function of starch and/or soluble sugars in protein maintenance and chromatin structure.

After 4h in HL only 10 genes were highly altered in *adg1-1/tpt-2* (Fig. 6B, Supplementary Table S3C). The three up-regulated genes comprised a stress induced protein (At2g40170) involved in ABA metabolism and a chloroplast localised superoxide dismutase (At2g28190). Both genes were more pronounced up-regulated in *adg1-1/tpt-2* compared to *adg1-1* or *tpt-2*. Amongst the down-regulated genes there was a bHLH-type transcription factor (At4g17880; MYC4), which was highly and specifically down-regulated with a log2-ratio of - 5.17 in *adg1-1/tpt-2.* Strikingly the same gene was also highly down-regulated after 48h in HL (log2-ratio = -4.21) and even in LL (log2-ratio = -3.68). A closer inspection of the expression profiles (eFP browser, Winter *et al.*, 2007) revealed that this gene is highly regulated by various stress conditions, like for instance oxidative stress, and it responds to jasmonate (Fernández-Calvo *et al*., 2011). Moreover, the presence of externally fed Suc induces the expression of At4g17880. Furthermore, MYC4 has been identified to be one of the key players in the regulation of glucosinolate biosynthesis (Schweizer *et al.,* 2013). At t_{48h} there were eight more transcriptional regulators within the group of 112 highly downregulated genes in *adg1-1/tpt-2*. Moreover, the functional categories `amino acids´, `cell wall', and `major carbohydrate metabolism' were significantly over-represented (Table 1). Interestingly, among the 36 highly up-regulated genes, 22 were plastome-encoded and belonged to the categories `PS light reaction´ (16 genes), `protein biosynthesis´ (five genes) and `lipid metabolism´ (one gene). Moreover, there were four nuclear-encoded genes involved in `RT´ up-regulated in *adg1-1/tpt-2*.

Supplementary Document S3

Genes associated with `major carbohydrate´- and `lipid metabolism´ as well as `transport´ were commonly up-regulated 4h after LL/HL-transfer

The group of commonly regulated genes as a response to LL/HL-transfer comprised also metabolic genes. Strikingly, seven and 11 genes associated with `lipid-´ and `major CHO metabolism', respectively, were up-regulated only transiently at t_{4h} vs t₀ (Supplementary Table S7A).

Among the up-regulated genes associated with `lipid metabolism´, there were two genes involved in triacylglycerol (TAG) biosynthesis (At1g54570 and At2g19450), a sterol oxidase (At1g07420), probably anchored at the outer envelope (gene ontology, cellular component), a 16:0 delta9 desaturase (At2g31360) and a phospholipase A2 family protein (At2g06925). As an analysis with ATTED-II (version 6.1) revealed, all genes apart from a plastidial thioesterase (At1g54570) belong to a regulatory network (Supplementary Fig. S3B; Supplementary Table S7A).

Moreover, in the category `lipid metabolism´ there were ten and seven genes downregulated at t_{4h} *vs* t_0 and t_{48h} *vs* t_0 , respectively, with an overlap of five genes. The ten downregulated genes at t_{4h} *vs* t_0 form, with the exception of At5g08030, a large network (Supplementary Fig. S3C), whereas there is no evidence for any exceptional network formation with the seven down-regulated genes at t_{48h} *vs* t_0 (not shown).

In the category `major CHO metabolism´, the 11 up-regulated genes at t_{4h} *vs* t_0 were - in a broader sense - all involved in either starch synthesis or degradation, despite the fact that the *adg1-1* single mutant and the *adg1-1/tpt-2* double mutant lack starch (Supplementary Table S7A). Furthermore, all 11 genes belong to a single regulatory network (Supplementary Fig. S2A). The list of highly up-regulated genes comprised not only chloroplast-localised metabolic enzymes such as α-amylase (At1g69830), disproportionating enzyme 1 (DPE1, At5g64860; Stettler *et al.,* 2009), isoamylase/debranching enzyme (At4g09020; Streb *et al.,* 2008; Wattebled *et al.,* 2008), branching enzyme 1 and 2 (At3g20440; At2g36390; Walters *et al.,* 2004; Dumez *et al.,* 2006) and glucan phosphorylase (At3g29320), but also regulatory proteins such as glucan water dikinase (GWD; SEX1; At1g10760; Yu *et al.,* 2001) phosphoglucan, water dikinases (PWD; AtGWD3; At5g26570; Kötting *et al.,* 2005), involved in the phosphorylation of glucose residues in amylopectin at C_6 and C_3 (Ritte *et al.,* 2006), respectively, and a protein phosphatase (AtSEX4; At3g52180), involved in the dephosphorylation of the aforementioned glucose residues (Hejazi *et al.,* 2010). Moreover,

two genes associated with the cytosolic conversion of maltose to sucrose (*i.e.* disproportionating enzyme 2 [At2g40840; Lu & Sharkey, 2004] and glucan posphorylase 2 [At3g46970]) were highly up-regulated 4h after LL/HL-transfer in both wild-type and mutant plants. Again this regulation of genes involved in carbohydrate metabolism also occurred in the starch-free background (*i.e. adg1-1* and *adg1-1/tpt-2*). Interestingly, three of the starchrelated genes belong to the co-expression network of phospholipase A (At2g06925; Supplementary Table S7A). Moreover, within the co-regulation network of genes belonging to the category `major CHO metabolism´, there was a chloroplast localised AMP activated protein kinase induced 4h and 48h after transfer to HL (Supplementary Table S7, A and B). The respective gene (At5g39790) appears to contain a starch binding domain (SUBA3 database; Heazlewood *et al.,* 2007; Tanz *et al*., 2013) and might hence be involved in carbohydrate metabolism or signalling. Most strikingly, the expression of `starch related´ genes occurred independently from the presence of starch (*i.e.* in the starch-free background *adg1-1*), suggesting that the resulting proteins might have additional unknown functions.

In the category `secondary metabolism´ there were five and 11 genes up-regulated, related to `flavonoids' in a broader sense at t_{4h} *vs* t_0 and t_{48h} *vs* t_0 , respectively (Supplementary Table S7, A and B). Only three of these genes were commonly differently regulated at both time points. In addition, three more up-regulated genes belonged to the sub-category `isoprenoids´ and `miscellaneous´.

Genes associated with specific `transport processes´ were differentially expressed most pronounced 4h after LL/HL-transfer

Genes associated with `transport processes´ were de-regulated both at 4h and 48h after LL/HL-transfer. Of the 14 up-regulated genes at t_{4h} *vs* t_0 , only two genes were also found at t_{48h} *vs* t_0 . Likewise, of the 18 down-regulated transport associated genes at t_{4h} *vs* t_0 , only six were also found at t_{48h} vs t₀ (Supplementary Table S7, A and B). Interestingly two genes belonging to the phosphate translocator family were de-regulated after 4h in HL. The glucose 6-phosphate/phosphate translocator2 (GPT2; At1g61800) and the phosphoenolpyruvate/phosphate translocator2 (PPT2; At3g01550) were up- and downregulated, respectively. It has been demonstrated that *GPT2*, which is usually only expressed in generative tissue (eFP browser; Winter *et al.,* 2007), strongly responds to elevated soluble sugar levels (Kunz *et al.,* 2010, Schmitz *et al.,* 2012), *e.g.* in starch-free mutants or after feeding of exogenous sugars to the plants (Heinrichs *et al.,* 2012). *GPT2* was highly up-regulated with log2-ratios between 3.7 and 6.7 at t_{4h} vs t_0 in all plant lines (Supplementary Table S7). At 48h after LL-to-HL-transfer, the log2-ratios of *GPT2* expression in the wild-type and the *tpt-2* single mutant dropped to lower levels compared to t_{4h} *vs* t₀. However, in the *adg1-1* mutant the expression of *GPT2* was further increased from 6.7 at t_{4h} *vs* t_0 to 7.2 at t_{48h} *vs* t_0 , whereas in the double mutant *GPT2* was not significantly altered. The changes in the *GPT2* expression ratios in the time series correspond well with levels of soluble sugars in wild-type and mutant plants (Compare Fig. 1 and Table 4).

Of the 14 up-regulated transport-related genes, six genes encode proteins with a high probability of a mitochondrial localisation and only two are likely to be localised in the chloroplasts. The portion of organelle-localised gene products was further diminished in the group of down-regulated transport related genes (*i.e.* two mitochondrial and one plastidial).

Additional References (Supplementary Document S3)

- **Dumez S, Wattebled F, Dauvillee D, Delvalle D, Planchot V, Ball SG, D'Hulst C.** 2006. Mutants of Arabidopsis lacking starch branching enzyme II substitute plastidial starch synthesis by cytoplasmic maltose accumulation. *The Plant Cell* **18,** 2694-2709. http://www.ncbi.nlm.nih.gov/pubmed/17028209
- **Heazlewood JL, Verboom RE, Tonti-Filippini J, Small I and Millar AH.** 2007. SUBA: the Arabidopsis Subcellular Database. *Nucleic Acids Research.* **35(D),** 213-218. http://www.ncbi.nlm.nih.gov/pubmed/17071959
- **Hejazi M, Fettke J, Kötting O, Zeeman SC, Steup M.** 2010. The Laforin-like dual-specificity phosphatase SEX4 from Arabidopsis hydrolyzes both C6- and C3-phosphate esters introduced by starch-related dikinases and thereby affects phase transition of alphaglucans. *Plant Physiology* **152,** 711-722.

http://www.ncbi.nlm.nih.gov/pubmed/20018599

- **Kötting O, Pusch K, Tiessen A, Geigenberger P, Steup M, Ritte G.** 2005. Identification of a novel enzyme required for starch metabolism in Arabidopsis leaves. The phosphoglucan, water dikinase. Plant Physiology **137,** 242-252. http://www.ncbi.nlm.nih.gov/pubmed/15618411
- **Lu Y, Sharkey TD.** 2004. The role of amylomaltase in maltose metabolism in the cytosol of photosynthetic cells. *Planta* **218,** 466–473 http://www.ncbi.nlm.nih.gov/pubmed/14593480
- **Ritte G, Heydenreich M, Mahlow S, Haebel S, Kötting O, Steup M.** 2006. Phosphorylation of C6- and C3-positions of glucosyl residues in starch is catalysed by distinct dikinases. FEBS Letters **580,** 4872-4876.

http://www.ncbi.nlm.nih.gov/pubmed/16914145

- **Stettler M, Eicke S, Mettler T, Messerli G, Hortensteiner S, Zeeman SC.** 2009. Blocking the metabolism of starch breakdown products in Arabidopsis leaves triggers chloroplast degradation. *Molecular Plant* **2,** 1233-1246. http://www.ncbi.nlm.nih.gov/pubmed/19946617
- **Streb S, Delatte T, Umhang M, Eicke S, Schorderet M, Reinhardt D, Zeeman SC.** 2008. Starch granule biosynthesis in Arabidopsis is abolished by removal of all debranching enzymes but restored by the subsequent removal of an endoamylase. *The Plant Cell.* **20,** 3448-3466.

http://www.ncbi.nlm.nih.gov/pubmed/19074683

- **Tanz SK, Castleden I, Hooper CM, Vacher M, Small I; Millar, AH.** 2013. SUBA3: a database for integrating experimentation and prediction to define the SUBcellular location of proteins in Arabidopsis. *Nucleic Acids Research.* **41(D),** 1185-1191. http://www.ncbi.nlm.nih.gov/pubmed/23180787
- **Wattebled F, Planchot V, Dong Y, Szydlowski N, Pontoire B, Devin A, Ball S, D'Hulst C.** 2008. Further evidence for the mandatory nature of polysaccharide debranching for the aggregation of semicrystalline starch and for overlapping functions of debranching enzymes in Arabidopsis leaves. *Plant Physiology* **148,** 1309-1323. http://www.ncbi.nlm.nih.gov/pubmed/18815382
- **Yu T-S, Kofler H, Häusler RE, Hille D, Flügge UI, Zeeman SC, Smith AM, Kossmann J, Lloyd J, Ritte G, Steup M, Lue W-L, Weber A.** 2001. The Arabidopsis *sex1* mutant is defective in the R1 protein, a general regulator of starch degradation in plants, and not the chloroplast hexose transporter. *The Plant Cell* **13,** 1907-1918. http://www.ncbi.nlm.nih.gov/pubmed/11487701