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## Supplemental information

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# Supplementary Information

**Triosephosphate export from chloroplasts and cellular sugar content regulate anthocyanin biosynthesis during high light acclimation**

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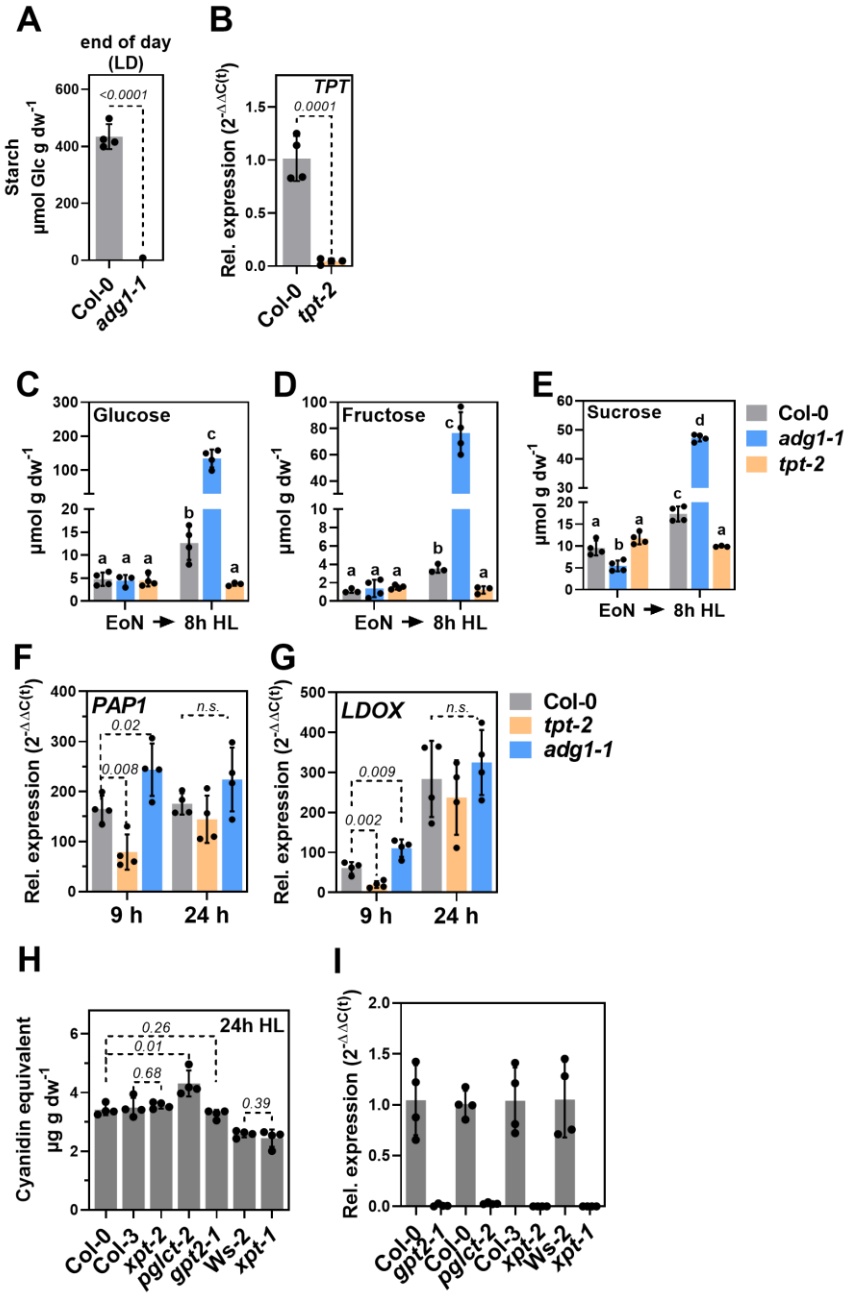


**Figure S1**: (**A**) Analysis of starch content in the *adg1-1* mutant at the end of day (long-day), (**B**) *TPT* expression in Col-0 and *tpt-2* mutant, (**C**) glucose, (**D**) fructose, (**E**) sucrose content in Col-0, *adg1-1,* and *tpt-2*. Plants grown in SD were analyzed at the end of the night (EoN) and after 8h HL shift. Statistical significance between genotypes was analyzed by two-way ANOVA (Tukey's multiple comparisons test) analysis (n≥3) and significance groups are indicated by letters ($p$<0.01). (**F-G**) Relative expression of *PAP1* (**F**) and *LDOX* (**G**) in WT, *adg1-1* and *tpt-2* after HL treatment. Plants were shifted at EoN. Changes in gene expression were calculated using the $2^{-\Delta\Delta(C(t))}$ method and *SAND* as reference gene and are expressed relative to Col-0 before the HL shift. Statistical significance between Col-0 and mutant was analyzed using Student's t-test. Values are mean ±SD (n=4) and the $p$-values are shown. n.s., not significant. (**H**) Anthocyanin content (represented as cyanidin equivalents) in mutants for plastid-localized transporters after 24h HL treatment. The *xpt-1* mutant was in Wassilewskija (Ws-2) and *xpt-2* in Col-3 background. (**I**) Confirmation of gene knockout in mutants shown in (H). For (A) Relative gene expression was calculated using the $2^{-\Delta\Delta(C(t))}$ method and *SAND* as reference gene relative to Col-0. For (H) the control was the respective WT background. For (A), (B) and (H), statistical significance between wild-type and mutant(s) was analyzed using student's t-test. Values are mean ±SD (n=4) and the $p$-values are shown.

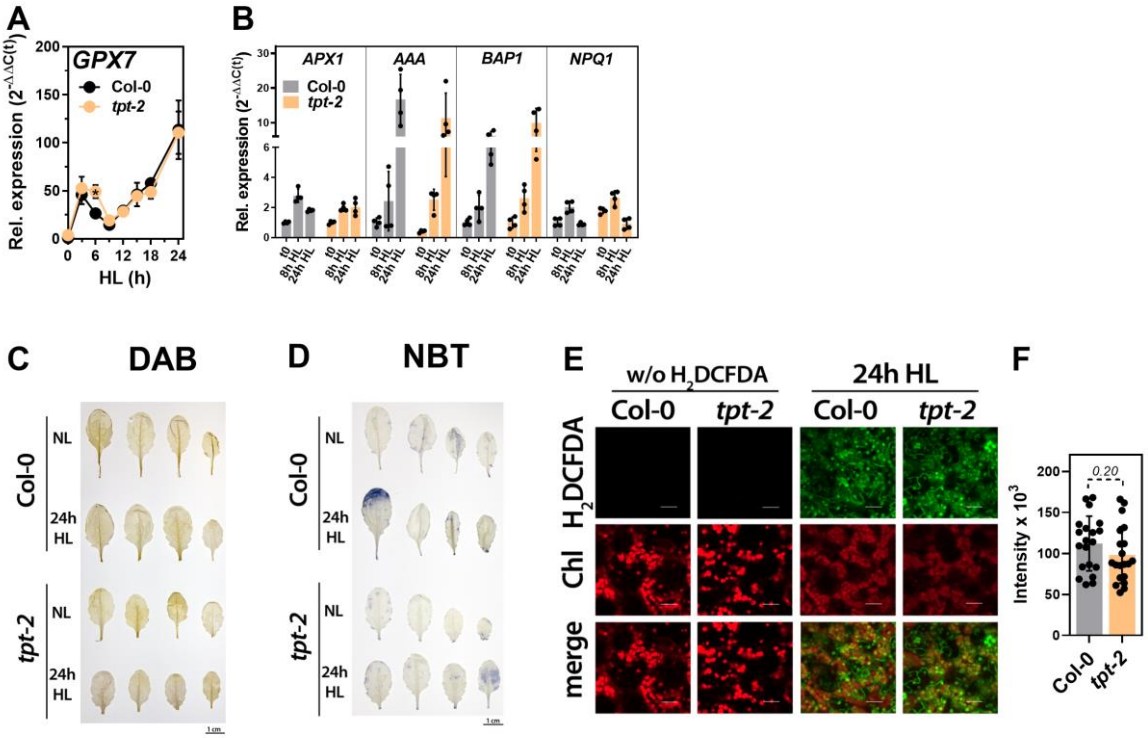


**Figure S2**: The *tpt-2* mutant showed a WT-like ROS response during HL shifts. (**A**) Expression of *GLUTATHIONE PEROXIDASE 7 (GPX7,* AT4G31870*)* during a 24h HL shift experiment (compare Fig. 2), (**B**) *ASCORBATE PEROXIDASE1 (APX1,* AT1G07890*), AAA-ATPase (AAA,* AT3G28580*), BON ASSOCIATION PROTEIN 1 (BAP1,* AT3G61190*) and VIOLAXANTHIN DEEPOXIDASE (NPQ1,* AT1G08550*)* in Col-0 and *tpt-2* at t0 and the indicated time-points after the HL shift. Changes in gene expression were calculated using the $2^{-\Delta\Delta(C(t))}$ method relative to Col-0 before the HL shift (t0) and *SAND* as reference gene. Values are mean ±SD (n=4). (**C**) 3,3'-Diaminobenzidine (DAB), (**D**) Nitro blue tetrazolium chloride (NBT) and (**E**) 2′,7′-dichlorofluorescein diacetate ($H_2$DCF-DA) staining of leaves from normal light (NL) conditions and after 24h of continuous HL. Signals for chlorophyll autofluorescence (Chl, red, emission 650-680 nm) and DCF (green, emission 500-575 nm) were recorded using a confocal laser scanning microscope and 488 nm excitation wavelength. Further details are given in the materials and methods section. Leaf samples were analyzed with the same settings. Scale bar 1 cm (C and D) and 20 µM (E). (**F**) Densitometric analysis of the mean fluorescence signals for DCF in the chloroplasts. The means are shown ±SD (n=20 from two independent leaves per genotype). Statistical significance between Col-0 and *tpt-2* was analyzed using student's t-test and the p-value is shown.

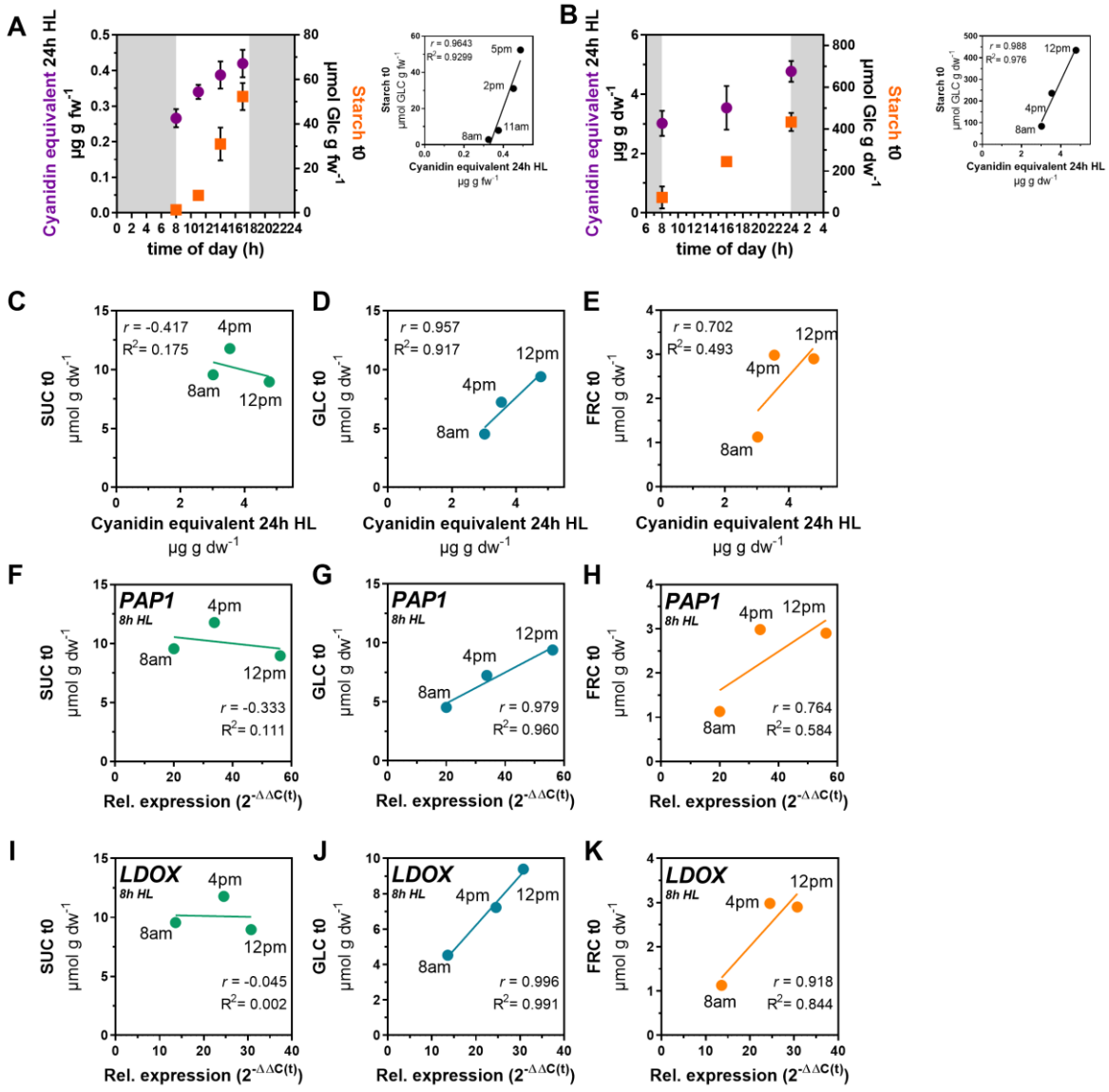


**Figure S3:** Induction of flavonoid biosynthesis in HL correlated with starch and sugar contents before the HL shift. (**A**) Short-day and (**B**) long-day grown *Arabidopsis* wild-type were subjected to 24h HL treatment at different time points during the day. Samples for starch analysis (orange) were harvested before the HL shift. After 24 h of continuous HL treatment, anthocyanin contents (purple) were quantified. Correlation analysis of starch contents before (t0) and anthocyanin contents after 24 h HL treatment are shown to the right of each graph. Values in (A) represent the mean ±SEM for n=8 samples from three independent experiments. In (B), the mean ±SD for n≥3 are shown. (**C-E**) Correlation analysis of anthocyanin content after 24 h HL treatment and (C) sucrose (SUC), (D) glucose (GLC) and (E) fructose (FRC) contents before the HL shift. To reduce the complexity of the graphs, only the mean values of n≥3 samples are shown. (**F-K**) Relative expression of *PAP1* (**F-H**) and *LDOX* (**I-K**) in Col-0 after 8h of HL treatment was correlated with SUC (F and I), GLC (G and J) and FRC (H and K) contents before the HL shift (t0). Anthocyanin contents are expressed as cyanidin equivalents. Changes in gene expression were calculated relative to the expression values at 8 am prior to the HL shift. Gene expression was calculated using the $2^{-\Delta\Delta(C(t))}$ method and *SAND* as reference gene. To reduce the complexity of the graphs, only the mean values for n≥3 samples are shown. Pearson correlation coefficient (*r*) and the linear regression correlation coefficient ($R^2$) are shown.

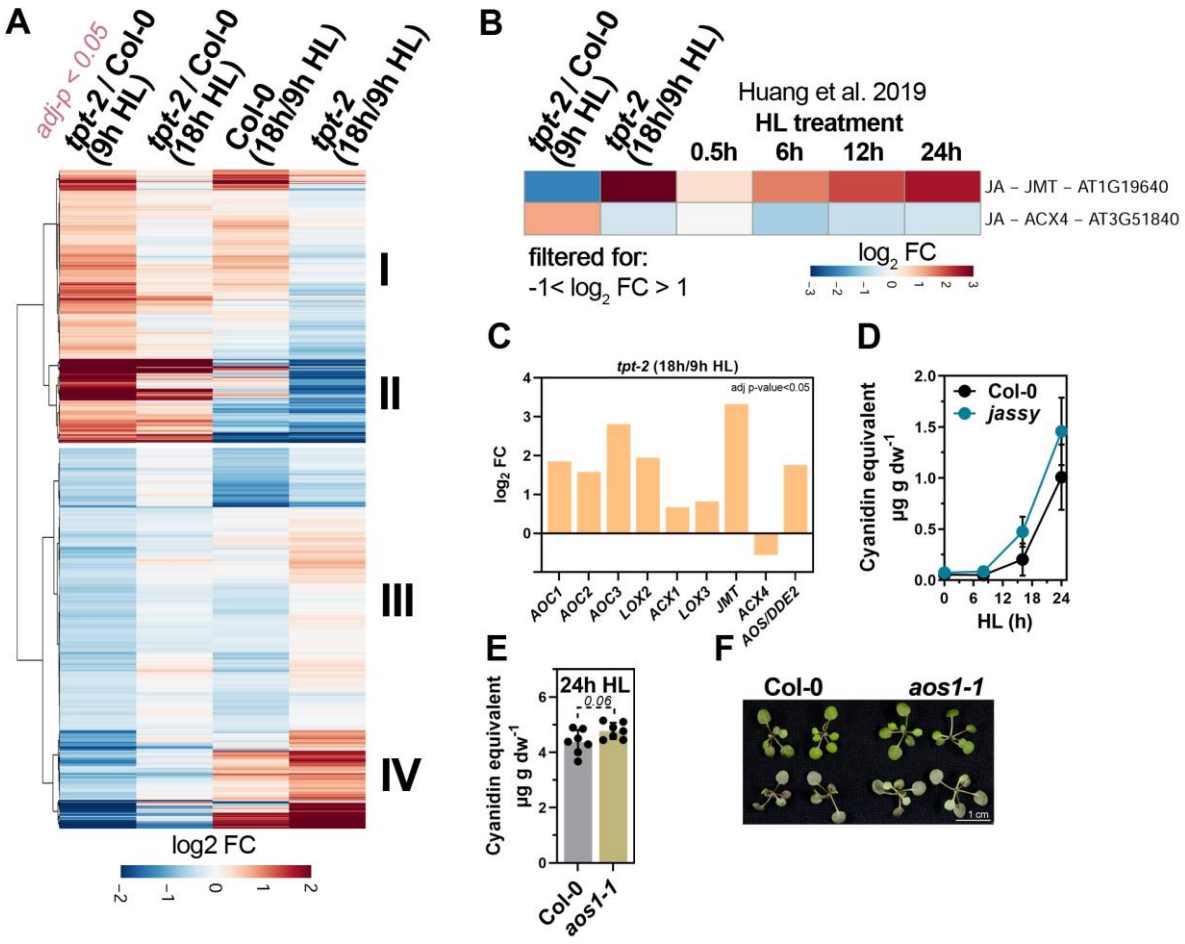


**Figure S4: (A)** RNA-seq analysis of significantly differentially expressed genes (DEGs) in *tpt-2* and Col-0 at different time-points of the HL kinetic. Transcripts were filtered for DEGs (adjusted p-value<0.05) in *tpt-2* relative to Col-0 at 9 h of the HL treatment, and changes of expression in the indicated comparisons are shown. Cluster I and II contain moderately to highly induced transcripts in *tpt-2*/Col-0 at 9 h HL whose expression is largely repressed after 18h of HL treatment in *tpt-2* relative to 9 h HL treatment (last column). Cluster III and IV encompass transcripts that were moderate to strongly downregulated in *tpt-2*/Col-0 at 9 h HL but were induced in *tpt-2* after 18h of HL treatment relative to 9 h HL. (**B**) Relative expression changes for *JASMONIC ACID CARBOXYL METHYLTRANSFERASE (JMT)* and *ACYL-COA OXIDASE 4 (ACX4).* The list of DEGs in *tpt-2*/Col-0 (9 h HL) was filtered (-1<$\log_2$ FC>1, adj-p<0.05) for hormone biosynthesis genes and downstream targets of hormone signalling and only *JMT* and *ACX4* were found to be deregulated in *tpt-2* at 9h HL (Huang et al. 2019, see also Supplementary Dataset S4). Changes in gene expression are given as $\log_2$ fold change (FC) relative to the control and hierarchical row clustering (Euclidean distance) using ward.D method was applied. (**C**) Expression of DEG involved in jasmonate biosynthesis in *tpt-2* 18 h/9 h HL (adjusted p-value<0.05). *JASMONIC ACID CARBOXYL METHYLTRANSFERASE (JMT)* and *ACYL-COA OXIDASE (ACX1* and *4), LIPOXYGENASE* (*LOX2* and *3*), *ALLENE OXIDE SYNTHASE* (*AOS*) and *ALLENE OXIDE CYCLASE* (*AOC1-3*). Accumulation of anthocyanins (expressed as cyanidin equivalent) in Col-0 (black) and (**D**) jasmonate-deficient *jassy* (blue) mutant and (**E**) *aos1-1* (beige) and (**F**) phenotype of Col-0 and *aos1-1* after a 24h HL shift. Top row adaxial; bottom row: abaxial surface. Values are given as mean ±SD for n≥3 samples and p-value for student's T-test are shown.

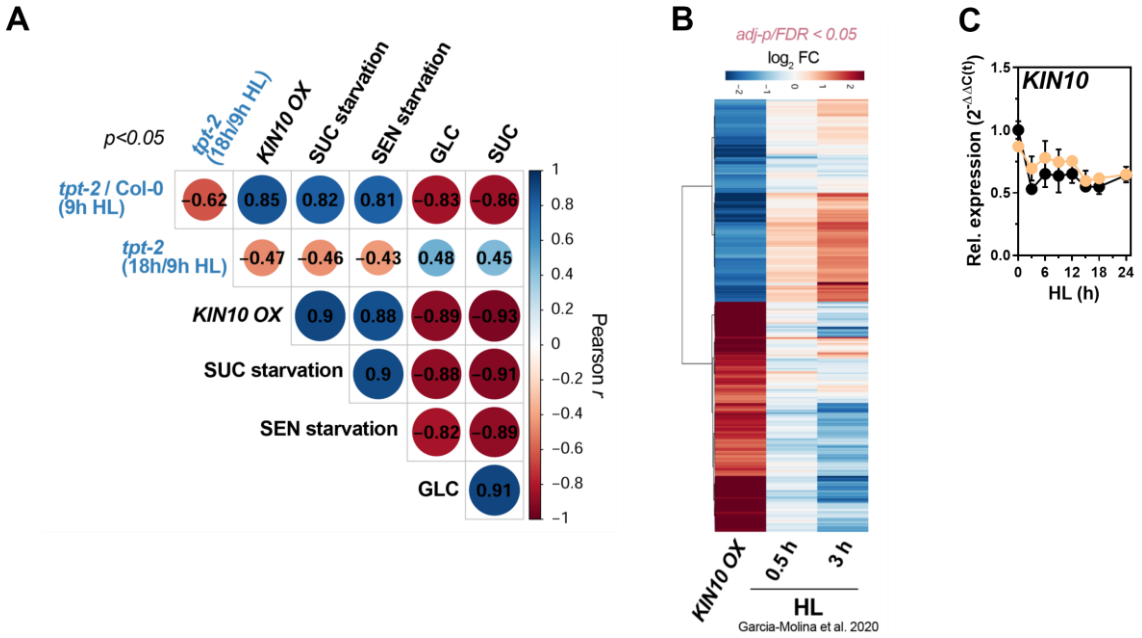


**Figure S5**: (**A**) Correlation plot of transcriptomes shown in Figure 5B. The size and colors represent the Pearson correlation coefficient (*r*, depicted inside the circles, p<0.05). Note that the changes in the *tpt-2* relative to Col-0 at 9 h HL were positively correlated with transcriptome changes induced by *KIN10* overexpression in protoplasts and starvation conditions but negatively correlated with gene expression changes stimulated by glucose (GLC) and sucrose (SUC) feeding. In contrast, compared to 9 h HL prolonged HL treatment induced changes in gene expression in *tpt-2,* leading to a negative correlation with *KIN10 OX* and starvation but a positive correlation with sugar feeding. (**B**) Heatmap comparing the relative expression of DEGs in protoplasts expressing *KIN10* (*KIN10 OX*, Baena-Gonzalez et al., 2007) and in Arabidopsis Col-0 exposed to 0.5 and 3 h of HL (Garcia-Molina et al., 2020) (false discovery rate (FDR) <0.05). 504 of 1024 DEGs in *KIN10ox* were found in the WT data set and used for comparison (Supplementary Dataset S7). (**C**) Expression of the catalytic SnRK1 subunit *KIN10* in Col-0 (black) and *tpt-2* (orange) through a HL shift kinetic (compare Figure 2).

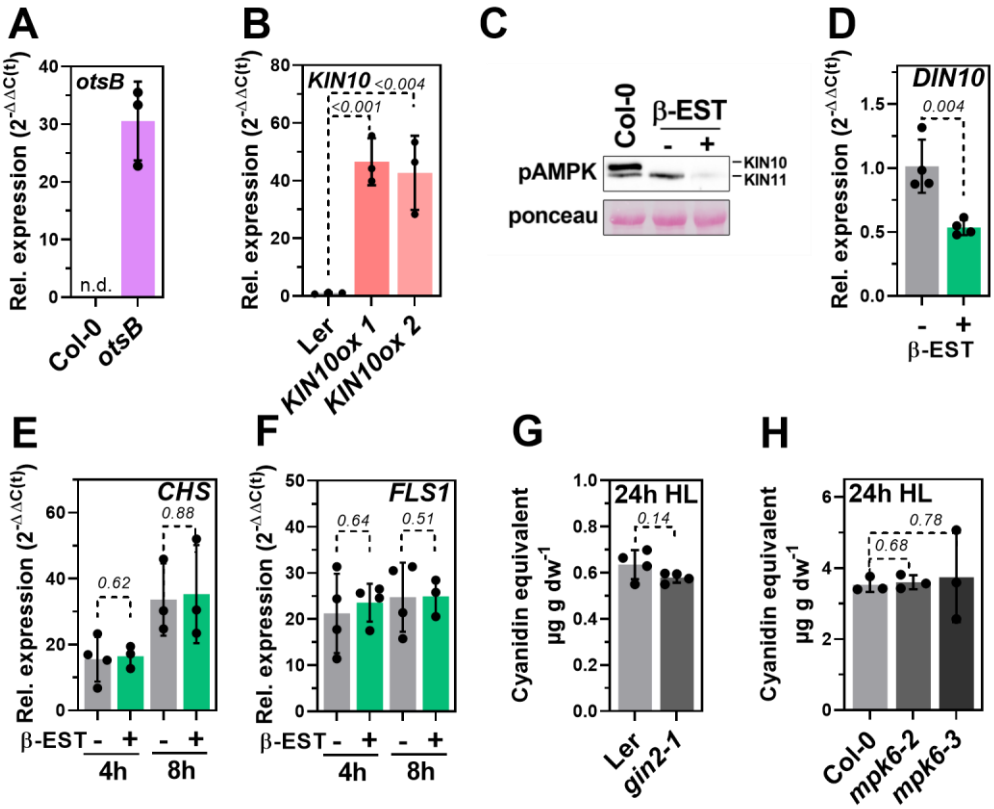


**Figure S6:** Confirmation of (**A**) *otsB* expression in *otsB* and (**B**) *KIN10* overexpression in two independent *KIN10ox* lines. (**C**) Western-blot confirming the knockout of KIN10/*SnRK$\alpha$1* and knockdown of KIN11/*SnRK$\alpha$2* in *snrk1$\alpha$1-3* amiRNAi *KIN11* induced by ß-EST application. The content of both catalytic SnRK1 subunits was analyzed using an antibody raised against the phosphorylated T-loop (T172) of human AMP-activated protein kinase (pAMPK) recognizing also phosphorylated SnRK1α1$^{T175}$/SnRK1α2$^{T176}$. (**D**) Expression of *DIN10* in *snrk1a1-3* amiRNAi *KIN11* at the end of a night (14h dark). Expression of (**E**) *CHS* and (**F**) *FLS1* in *snrk1a1-3* amiRNAi *KIN11* during a HL shift. Gene expression was calculated using the $2^{-\Delta\Delta(C(t))}$ (for A, D, E, F) and $2^{-\Delta(C(t))}$ (for B) method relative to the expression in the WT/untreated control, and *SAND* as reference gene. (**G**) Anthocyanin content in Ler and *gin2-1* after 24h HL (n=4). (**H**) Anthocyanin content in Col-0, *mpk6-2* and *mpk6-3* mutants after 24 h HL (n=3). For (B and D-H) statistical significance between WT and mutant(s) was analyzed using student's t-test and the p-values are shown. Values are mean ±SD (n≥3). n.d., not detectable.