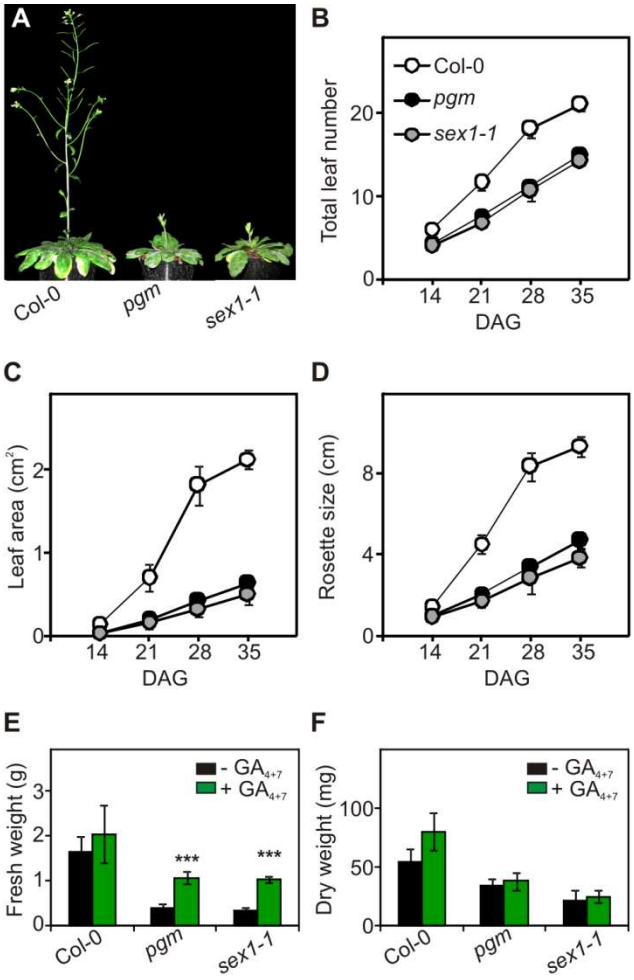
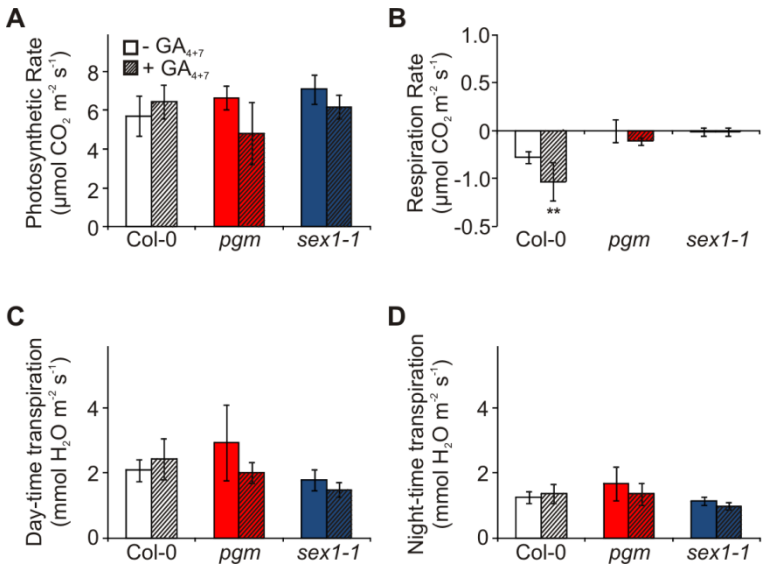
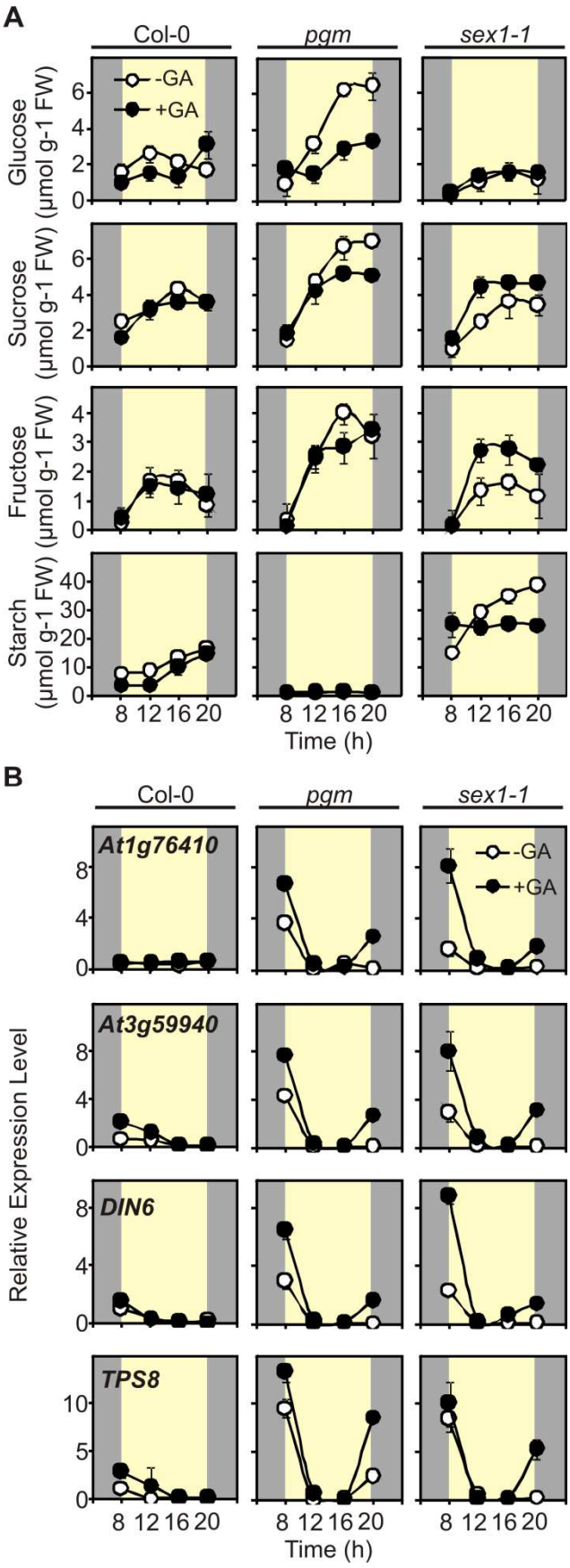


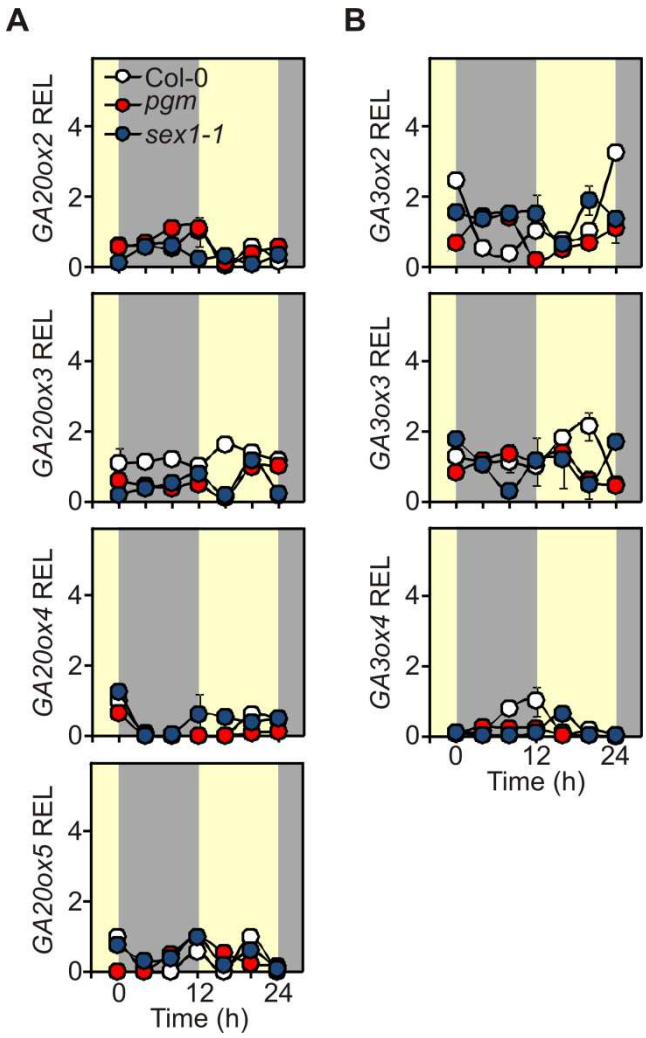
Supplemental Figure 1. Phenotypes of *pgm* and *sex1-1* mutants grown in a 12hL:12hD photoperiod.
(A) Photograph of 6-week-old Col-0, *pgm* and *sex1-1* plants. Note the delay in flowering of both mutants.
(B-D) Total rosette leaves number **(B)**, area of the first two leaves **(C)**, and diameter of the entire rosette **(D)** in each genotype from 14 to 35 days after germination. Data are the average (± SD) of independent plants (n = 10).
(E-F) Fresh weight **(E)** and dry weight **(F)** of 4-week-old plants treated without ($-GA_{4+7}$) or with 10 µM GA_{4+7} ($+GA_{4+7}$), as described in Methods section. Results are means (± SD) of three independent experiments (n = 20). Asterisks indicate significant differences between control and treated plants (2-way ANOVA; ***P < 0.001).



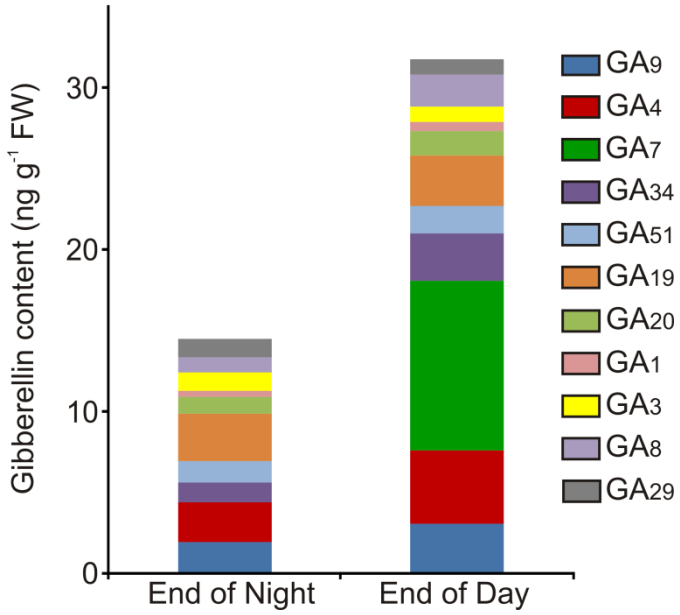
Supplemental Figure 2. Effect of GA treatment on photosynthesis, respiration, and transpiration rates in leaves of Col-0, *pgm* and *sex1-1* plants.
(A) Photosynthetic CO_2 fixation was quantified over the entire light phase.
(B) Respiratory CO_2 release was quantified over the entire dark phase.
(C-D) Day-time **(C)** and night-time **(D)** H_2O transpiration, and consequently stomatal conductance, in each genotype and condition.
Measures were taken after two weeks of treatment with GAs. Values represent means (± SD) of 15 measurements on 4 separate plants for each genotype. None of the differences between control and treated plants are significant, except for the Col-0 in B (2-way ANOVA, **P < 0.01).


A
Col-0
pgm
sex1-1
-GA
+GA
Glucose (µmol g-1 FW)
Sucrose (µmol g-1 FW)
Fructose (µmol g-1 FW)
Starch (µmol g-1 FW)
8 12 16 20
Time (h)
B
Col-0
pgm
sex1-1
At1g76410
At3g59940
DIN6
TPS8
Relative Expression Level
-GA
+GA
8 12 16 20
Time (h)

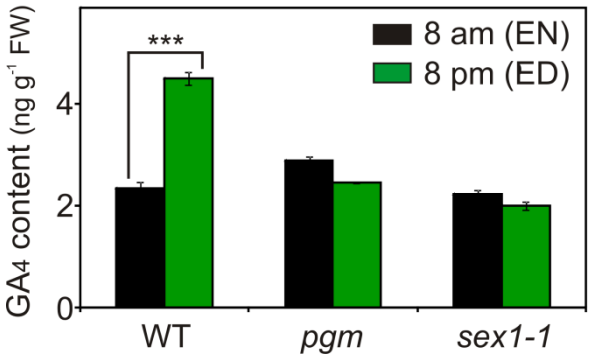
Supplemental Figure 3. Exogenous GA-treatment disrupts the normal expression pattern of starvation genes.
(A) Plants were grown in a 12hL:12hD photoperiod and treated with GA_{4+7} for 2 weeks. Plants were sampled every 4-h during the light phase and levels of glucose, sucrose, fructose and starch were measured in leaves of control plants (*open circles*) and GA-treated plants (*filled circles*). The same analysis was performed in Col-0, *pgm* and *sex1-1*. Each point represents the mean (± SD) of three biological replicates.
(B) Transcript levels of four starvation-related genes (*At1g76410*, *At3g59940*, *DIN6*, and *TPS8*) (Usadel et al., 2008) in GA-treated Col-0, *pgm* and *sex1-1* plants. Expression levels are expressed as relative units, assuming as unitary the value of -GA Col-0 at the beginning of the day. Each value is the mean (± SD) of three independent measurements. Background shading: yellow, day; grey, night.



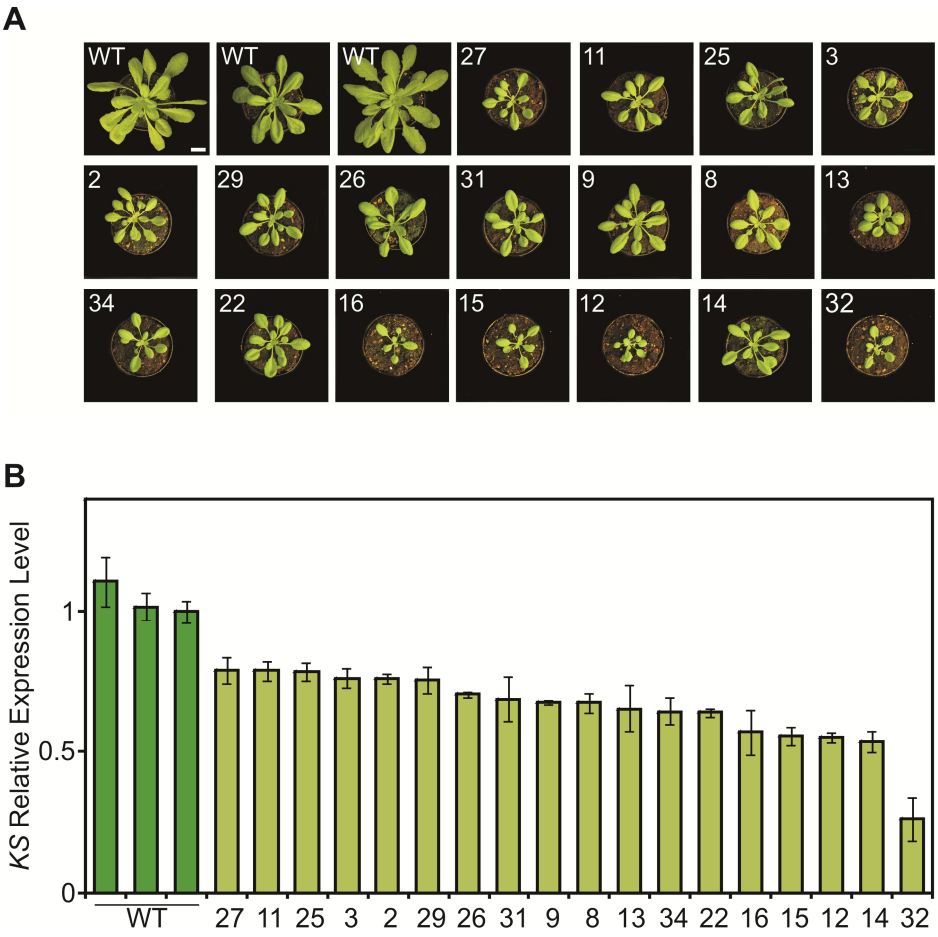
Supplemental Figure 4. Diurnal changes in the relative expression levels of genes encoding enzymes involved in the late steps of GA biosynthesis in Col-0 (*empty circles*), *pgm* (*red circles*) and *sex1-1* (*blue circles*) plants grown under 12hL:12hD photoperiod.
RT-qPCR analysis of relative transcript levels of **(A)** *GA20ox* genes (*GA20ox2*, *At5g51810*; *GA20ox3*, *At5g07200*; *GA20ox4*, *At1g60980*; *GA20ox5*, *At1g44090*), and **(B)** *GA3ox* genes (*GA3ox2*, *At1g80340*; *GA3ox3*, *At4g21690*; *GA3ox4*, *At1g80330*).
Expression levels are expressed as relative units assuming as unitary the value of wild-type (Col-0) at the beginning of the day (0h). Data are mean of three replicates (± SD). Yellow background, day; grey background, night. (see Supplemental Table 4 online for RT-qPCR primers list).



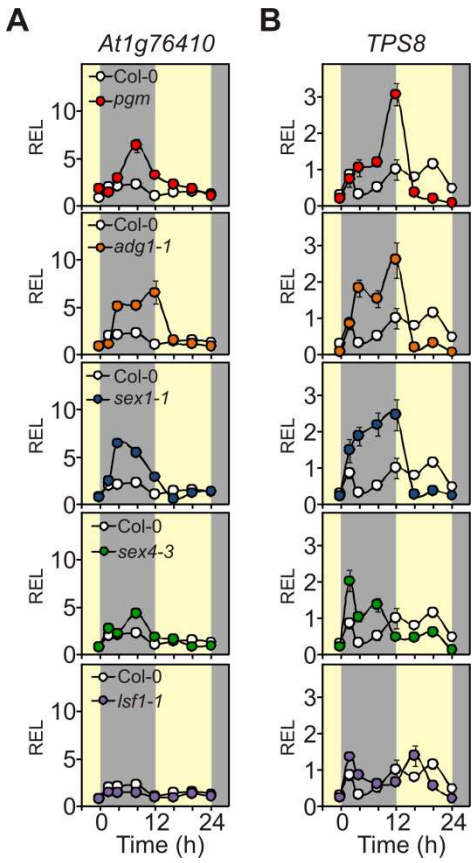
Supplemental Figure 5. Level of GAs (ng g^{-1} FW) in Col-0 at the end of night (8 am, EN) and end of day (8 pm, ED). Values are means of three independent replicates (see Supplemental Table 2 online).



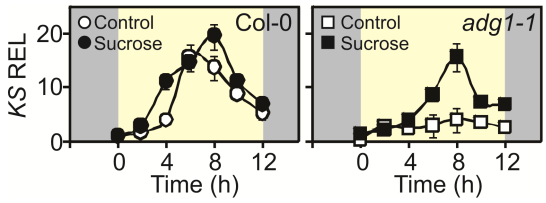
Supplemental Figure 6. Comparison of GA_4 content (ng g^{-1} FW) in Col-0, *pgm* and *sex1-1* at the end of night (8 am, EN) and end of day (8 pm, ED). Values are means of three replicates (± SD). Asterisks indicate significant differences from the wild-type at 8 am (2-way ANOVA; ***P < 0.001).



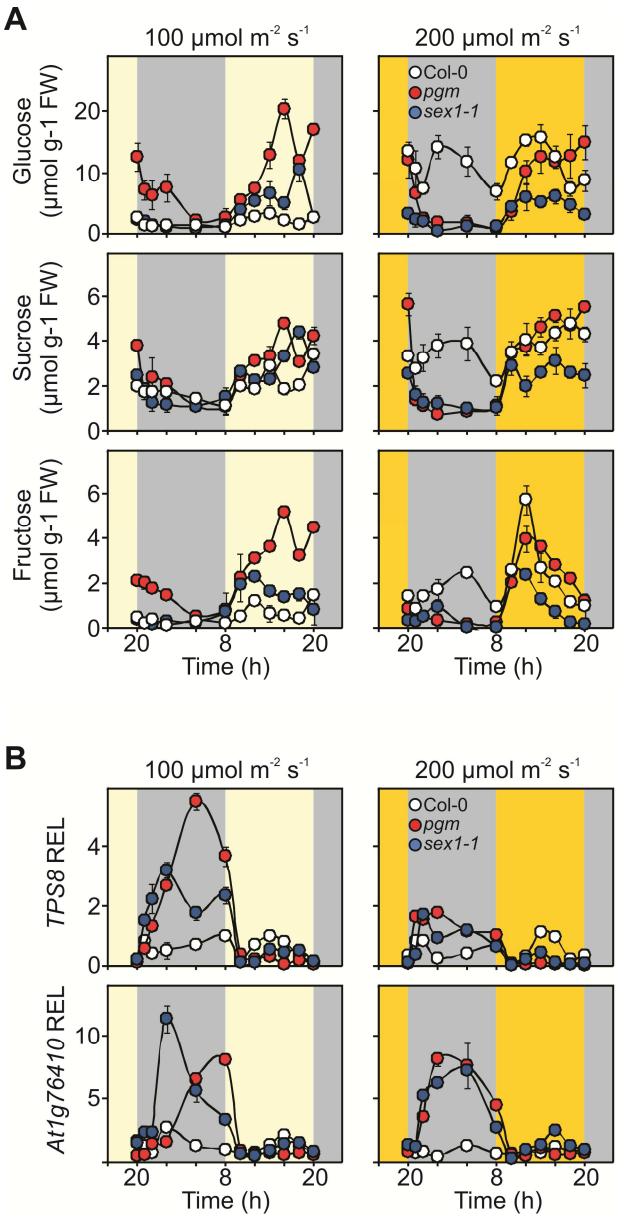
Supplemental Figure 7. Screening of *KS*i lines grown in a 12hL:12hD photoperiod. Comparison of phenotype and *KS* transcript levels.
(A) Photograph of 4-week-old Col-0 and transgenic *KS*i plants. White bar = 1.5 cm.
(B) RT-qPCR analysis of relative transcript levels of *KS* in wild-type plants and transgenic confirmed lines at 4 pm. X-axis values correspond to individual plants shown in **(A)**. Expression levels are expressed as relative units assuming as unitary the value of the second wild-type (WT) in panel A. (see Supplemental Tables 4 and 5 online for RT-qPCR and screening primers list). Data are mean of three replicates (± SD).



Supplemental Figure 8. Altered expression of sugar-starvation marker genes in starch mutants.
The transcript levels of two sugar-starvation marker genes (Usadel et al., 2008) in Col-0 and the starch mutants were analyzed. Plants were grown in 12hL:12hD photoperiod (grey/yellow background corresponds to night/day). See Figure 4 legend for a detailed description of the experiment.
(A) *At1g76410*
(B) *TPS8*
The relative expression level (REL) at the end of night of wild-type (Col-0) was set to 1. Values are means (± SD) of three independent replicates. Background shading: yellow, day; grey, night.



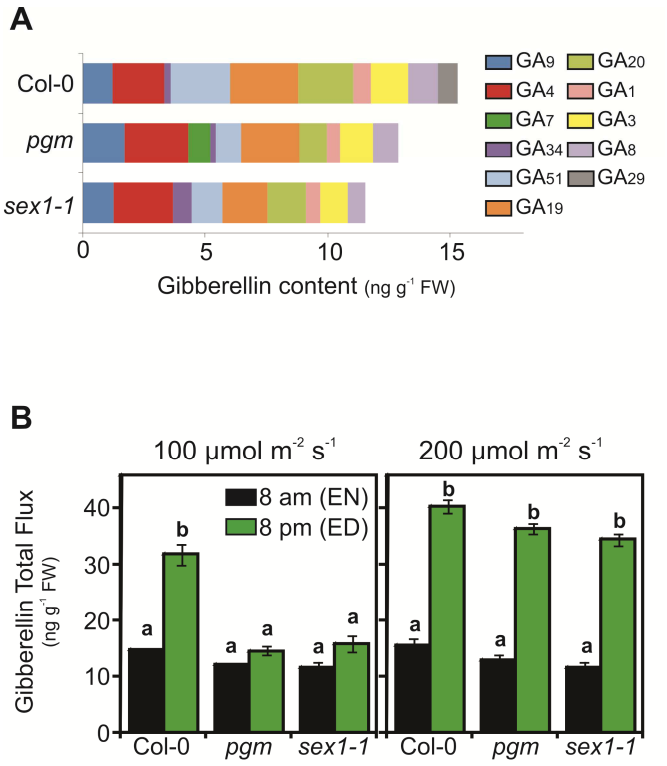
Supplemental Figure 9. Sucrose supplementation in *adg1-1* mutant the night before restores the afternoon *KS* expression peak the day after. The transcript levels of *KS* in Col-0 and the starchless *adg1-1* mutant treated with 1% sucrose starting 13h before dawn (*filled symbols*). Control plants (*empty symbols*) were sprayed with H_2O (See Figure 4 legend for a detailed description of the experiment). The relative expression level (REL) at the end of night of wild-type (Col-0) was set to 1. Values are means (± SD) of three independent replicates. Background shading: yellow, day; grey, night.



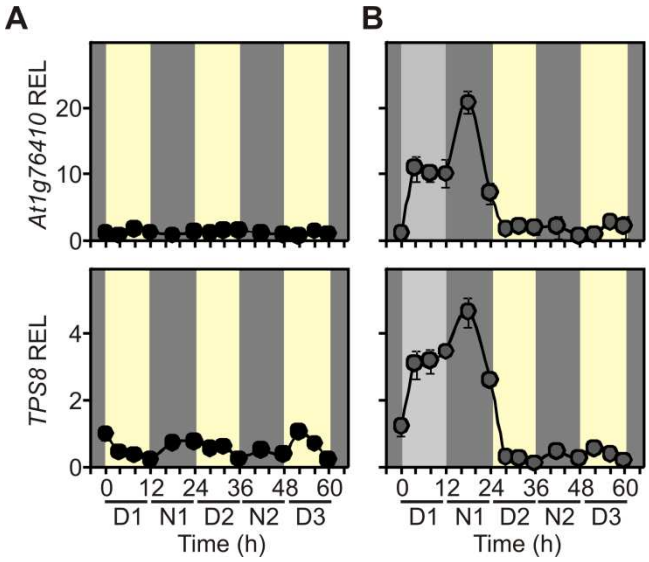
Supplemental Figure 10. Effect of light intensity on soluble sugar levels and on the expression of sugar-starvation marker genes in Col-0, *pgm* and *sex1-1* plants.

(A) Levels of glucose, sucrose, and fructose were measured in leaves of Col-0 (*empty circles*), *pgm* (*red circles*) and *sex1-1* (*blue circles*). Each point represents the mean (± SD) of three biological replicates.

(B) Transcript levels of *TPS8* and *At1g76410* in Col-0, *pgm*, and *sex1-1* plants grown at 100 or 200 µmol m^{-2} sec^{-1} light intensity. The relative expression level (REL) at the end of night of the wild-type (Col-0) was set to 1. Values are means (± SD) of three independent replicates. Background shading: light yellow, day at 100 µmol m^{-2} sec^{-1} irradiance; dark yellow, day at 200 µmol m^{-2} sec^{-1} irradiance; grey, night. (See Figure 5 legend for a detailed description of the experiment).

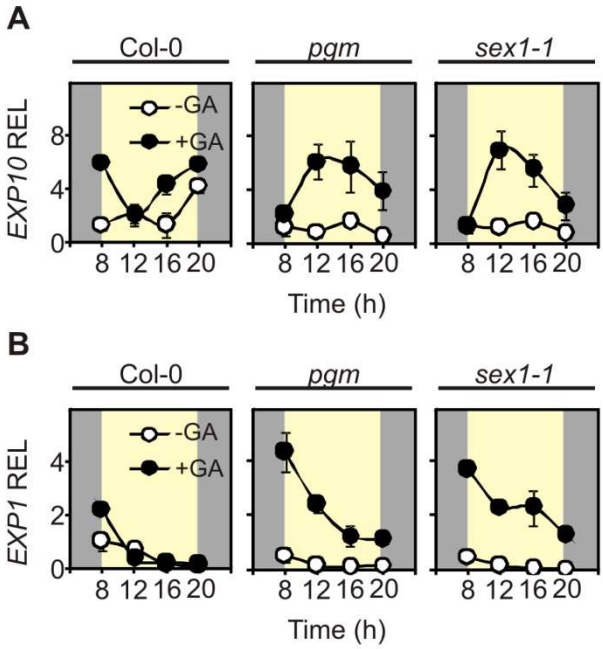


Supplemental Figure 11. Effect of light intensity on the GAs content in Col-0, *pgm* and *sex1-1* plants.
(A) Levels of GAs (ng g^{-1} FW) in Col-0, *pgm* and *sex1-1* grown at 200 µmol m^{-2} sec^{-1} irradiance at the end of night (8 am). Values are means (± SD) of three independent replicates.
(B) Comparison of gibberellins total flux (ng g^{-1} FW) at the end of night (8 am) and end of day (8 pm). Levels of gibberellins were measured in leaves of Col-0, *pgm* and *sex1-1* plants grown at 100 µmol m^{-2} sec^{-1} irradiance (*left panel*) and at 200 µmol m^{-2} sec^{-1} irradiance (*right panel*). Differences among genotypes at both light irradiance conditions and at the two time-point were evaluated by 2-way ANOVA (Bonferroni posttest, $P < 0.05$). Bars represent means (± SD) of three biological replicates.



Supplemental Figure 12. Expression of sugar-starvation marker genes in plants exposed under low light intensity for one day.

(A) Col-0 plants grown in 12hL:12hD photoperiod at 100 µmol m^{-2} sec^{-1} light intensity were used as controls. **(B)** Another group of plants was subjected to a 12-h treatment at low light intensity (10 µmol m^{-2} sec^{-1}; light grey background) and then transferred again to normal light intensity (yellow background). See Figure 6 for details about this experiment. D = day, N = night. Expression in the control set of plants **(A)** at time 0 was set to 1. Values are means (± SD) of three replicates from two independent experiments.



Supplemental Figure 13. Expression of *EXP10* and *EXP1* is modulated by gibberellins.
Plants were grown in a 12hL:12hD photoperiod and treated with GA_{4+7} for 2 weeks before sampling. Plants were harvested every 4-h during the light phase.
Transcript levels of *EXP10* **(A)** and *EXP1* **(B)** were measured in leaves of control (*open circles*) and GA-treated samples (*filled circles*). The same analysis was performed in Col-0, *pgm* and *sex1-1*. Expression levels (REL) are expressed as relative units, assuming as unitary the value of Col-0 control (–GA) plants at the beginning of the day. Each value is the mean (± SD) of three independent measurements. Background shading: yellow, day; grey, night.

Supplemental Table 1. List and description of the mutants used in the present work.

Mutant	Locus (AGI)	Gene	Description	References
pgm	*At5g51820*	*PGM*	Deficient in starch due to inactivation of a chloroplastic phosphoglucomutase isozyme, which converts the Glc 6-P into Glc 1-P.	(Caspar et al., 1985)
adg1-1	*At5g48300*	*ADG1*	Low starch mutant with a monogenic recessive mutation that has no measurable ADPGlc pyrophosphorylase (ADGase) activity	(Lin et al., 1988a;b; Wang et al., 1998)
sex1-1	*At1g10760*	*GWD1*	Mutant unable to metabolize the starch because of a point mutation decreasing the activity of α-glucan water dikinase, required for starch degradation	(Caspar et al., 1991; Yu et al., 2001; Ritte et al., 2002)
sex4-3	*At3g52180*	*SEX4*	T-DNA insertion in this phosphoglucan phosphatase results in a starch excess phenotype (SALK_102567 line)	(Kötting et al., 2009)
lsf1-1	*At3g01510*	*LSF1 (LIKE SEX4)*	T-DNA insertion in the promoter region causes a null mutation and a starch excess phenotype	(Comparot-Moss et al., 2011)

Supplemental Table 2. Levels of GAs (ng g^{-1} FW) at the end of night (EN) and at the end of day (ED) in wild-type plants grown at 100 µmol m^{-2} sec^{-1} irradiance.

	EN	ED
GA_9	0.00 (±0.01)	3.10 (±0.50)
GA_4	2.33 (±0.13)	4.51 (±0.13)
GA_7	0.00 (±0.01)	16.24 (±0.98)
GA_{34}	1.58 (±0.05)	2.93 (±0.01)
GA_{51}	2.96 (±0.03)	1.75 (±0.07)
GA_{19}	2.05 (±0.01)	3.06 (±0.30)
GA_{20}	0.93 (±0.01)	1.54 (±0.04)
GA_1	0.79 (±0.35)	0.51 (±0.03)
GA_3	1.22 (±0.06)	1.03 (±0.06)
GA_8	1.70 (±0.01)	1.89 (±0.03)
GA_{29}	0.96 (±0.07)	0.45 (±0.04)
Total flux	**14.55 (±0.72)**	**36.22 (±2.18)**

Endogenous GAs was quantified in wild-type Col-0 plants both at the end of the night (EN, 8 am) and the end of the day (ED, 8 pm) by GC-MS/MS. All GAs of the table are part of the early 13-hydroxylation and non-13-hydroxylation pathways. GA_1, GA_3, GA_4, and GA_7 are bioactive GAs, whereas the other GAs are precursors and deactivated GAs (GA_8 and GA_{34}). Values shown are average ± SD of three replicates.

Supplemental Table 3. Levels of GAs (ng g^{-1} FW) at the end of day in Col-0, *pgm* and *sex1-1* mutants.

	Col-0	*pgm*	Ratio (Col-0/*pgm*)	*sex1-1*	Ratio (Col-0/*sex1-1*)
GA_9	3.10 (±0.49)	1.91 (±0.01)	0.62	1.54 (±0.13)	0.49
GA_4	4.51 (±0.13)	2.45 (±0.01)	0.54	2.00 (±0.08)	0.44
GA_7	10.45 (±0.98)	n.d.	n.d.	n.d.	n.d.
GA_{34}	2.93 (±0.01)	1.20 (±0.01)	0.41	1.30 (±0.31)	0.44
GA_{51}	1.74 (±0.07)	1.36 (±0.04)	0.78	3.20 (±0.30)	1.84
GA_{19}	3.06 (±0.30)	2.94 (±0.09)	0.96	3.15 (±0.04)	1.03
GA_{20}	1.54 (±0.049)	1.03 (±0.11)	0.67	1.37 (±0.47)	0.89
GA_1	0.51 (±0.04)	0.42 (±0.01)	0.82	0.40 (±0.10)	0.78
GA_3	1.03 (±0.06)	1.06 (±0.13)	1.03	1.22 (±0.07)	1.18
GA_8	1.90 (±0.04)	0.98 (±0.23)	0.51	1.12 (±0.40)	0.59
GA_{29}	0.95 (±0.02)	1.15 (±0.04)	1.21	0.36 (±0.04)	0.38
Total flux	31.72 (±0.18)	14.5 (±0.06)	0.46	15.66 (±0.18)	0.49

Endogenous GAs was quantified in wild-type Col-0, *pgm* and *sex1* plants by GC-MS/MS. All GAs of the table are part of the early 13-hydroxylation and non-13-hydroxylation pathways. GA_1, GA_3, GA_4, and GA_7 are bioactive GAs, whereas the other GAs are precursors and deactivated GAs (GA_8 and GA_{34}). Values shown are average ± SD of three replicates. n.d., below the detection limit.

Supplemental Table 4. Primers used for gene expression analysis using real-time quantitative RT-PCR.

Gene (AGI)	Forward primer	Reverse primer
At1g13440 (GAPDH)	GAATCAACGGTTTCGGAAGA	CTCGGTGGTGATGAAAGGAT
At2g37270 (40SrRNA)	TCGACGCTGAGATTCAACAG	CGTAACCGAAACGTCATCAA
At4g02780 (CPS)	CCAGAAGCGGTTCCATACAT	CCGTCCGTTAGGTTTCTCAA
At1g79460 (KS)	TTCGGTTGCTTCTGGTTTCT	GAGTCCAATCTCCGTTCCAA
At5g25900 (KO)	CTAGTTCCCATTCGCTACGC	TGAAGAGCACCAGCACAAAC
At1g15550 (GA3ox1)	CCGAAGGTTTCACCATCACT	GAGGGTGGAGTCGGTATGAG
At1g80340 (GA3ox2)	CCAGCCACCACCTCAAATACTGTG	ATTAGGCCCGGCCCATTGTATG
At4g21690 (GA3ox3)	TCATGCCGAGTTCTGCAATGTG	CCTAACGAGCCCATCAACATGC
At1g80330 (GA3ox4)	CCTCATGATCACACCAAGTACTGC	TCTTCCACGGTGACACCAAGTG
At4g25420 (GA20ox1)	GGTTTCTTCCTCGTGGTCAA	TTTCGGAGAGAGGCATATCAA
At5g51810 (GA20ox2)	CAAGAGTTCGAGCAGTTTGGGAAG	TCGGAAATAGTCTCGGTTTACGC
At5g07200 (GA20ox3)	ACATAGGCGACACCTTCATGGC	TCCTTTCTCTCTCGCTGTTCACC
At1g60980 (GA20ox4)	GAACATTGGCGACACTTTAATGGC	TGGTGGCTTCACCACTTTGTCC
At1g44090 (GA20ox5)	AACGTTGGAGACACCTTCATGGC	ACTGCCCTGTGGTAACAACTCC
At1g76410	TCCGGTCGTTACCGAAACTCAC	AGCACACTCCACGAGTTTCTCC
At3g59940	ATACGATTCGGCTTCCGGTGAC	ACCGCTTGGCCACTCAAACTTC
At3g47340 (DIN6)	AAGGTGCGGACGAGATCTTTGG	ACTTGTGAAGAGCCTTGATCTTGC
At1g70290 (TPS8)	GTGGTTGTCAAGAGAGGTCAACAC	AGCTAGACCTTTGCTTACACCTTG
At1g26770 (EXP10)	AGCGAACAATAATGGCGGTTGG	CTTCTCCTGCAAGGAACCCTTC

At1g69530 (EXP1)	CAATGGGAGGTGCTTGTGGA	GTTGTTCGGTAAGGCGTTGT

Supplemental Table 5. Primers used to test the insertion in the AGRIKOLA RNAi lines.

Primer name	Position	Sequence 5'-3'
Agri51	35S promoter	CAACCACGTCTTCAAAGCAA
Agri56	Pdk intron	CTGGGGTACCGAATTCCTC
Agri64	Cat intron	CTTGCGCTGCAGTTATCATC
Agri69	OCS terminator	AGGCGTCTCGCATATCTCAT