Regulatory properties of ADP glucose pyrophosphorylase are required for adjustment of leaf starch synthesis and hence normal growth in different photoperiods[1][W]

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SUPPORTING INFORMATION

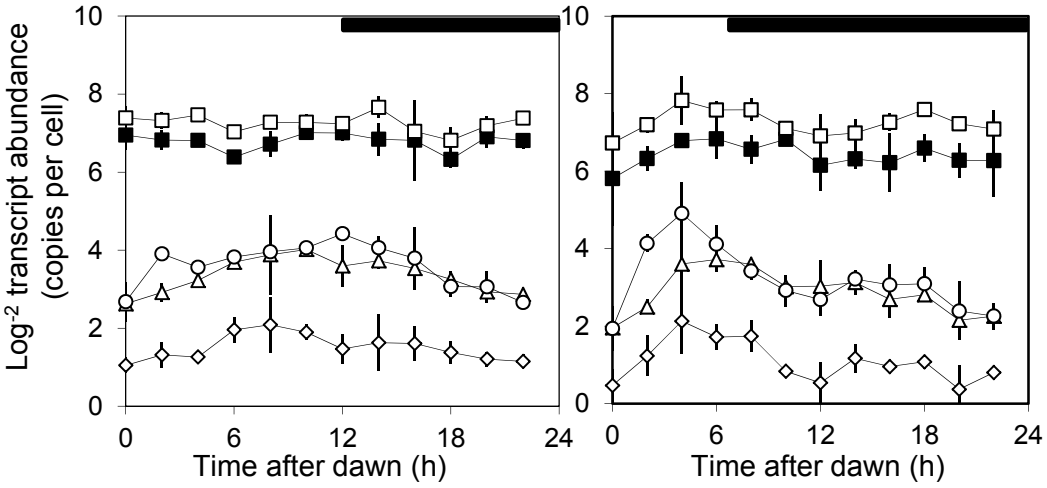

Figure S1. Diel changes in levels of transcripts encoding AGPase large subunits.

qRT-PCR measurements of transcript levels of AGPase subunits in rosettes of plants grown in 12:12 (left graph) or 6:18 (right graph) conditions and harvested at two-h intervals. Black bars indicate the hours of darkness. Values are means ± the difference of two biological replicates. Closed symbols: *APS1*. Open symbols: squares *APL1*, circles *APL3*, triangles *APL2*, diamonds *APL4.*

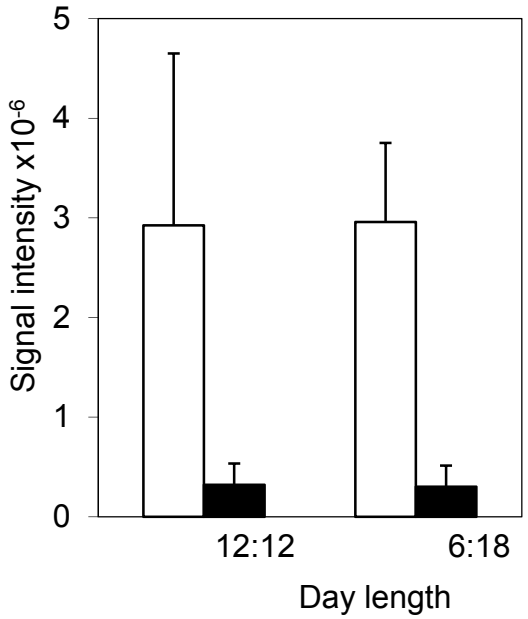

Figure S2. Immunoprecipitation with an antiserum to the AGPase small subunit.

Abundance of APS1 (white bars) and APL1 (black bars) peptides in proteins immunoprecipitated with an APS1 antiserum, from MALDI-ToF MS analysis of tryptic fragments. MALDI-ToF analysis. Proteins the range 50 to 60 kD were separated by SDS-PAGE from preparations from rosettes of plants grown in either 12:12 or 6:18 conditions, excised from the gel and subjected to tryptic digestion. Values are means ± SE of measurements on three separate immunoprecipitates from one extract. Similar results were obtained with a second, independent extract..

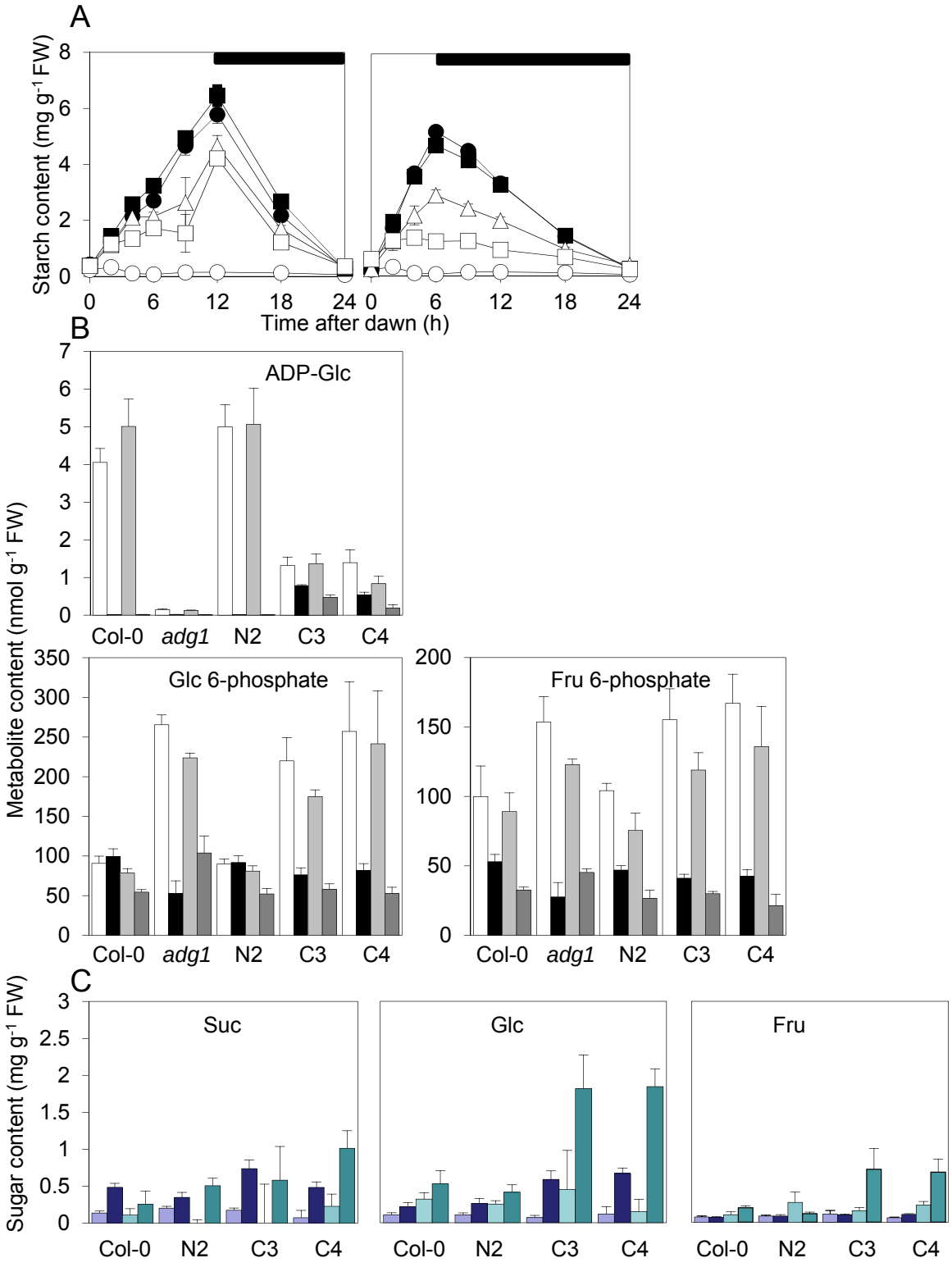

Figure S3. Metabolite levels in wild-type and GlgC-TM plants grown in different day lengths. A, Starch content over 24 h in plants grown in 12:12 (left: 21-day old plants) or 6:18 (right: 28-day old plants) conditions. ●, wild-type (Col-0); ■, *APS1*-expressing line N2; Δ, *GlgC-TM*-expressing line C3; □, *GlgC-TM*-expressing line C4; ○, *adg2*. This is a repetition of the experiment in Figure 4b, on a separately-grown batch of plants. Values are means ± SE of measurements made on six plants. B, Metabolite contents of wild-type (Col-0) plants, *APS1*-expressing line N2 and *GlgC-TM*-expressing lines C3 and C4. Plants were grown in 12:12 conditions and harvested at ZT3 (white) or ZT15 (black) or grown in 6:18 conditions and harvested at ZT3 (light grey) or ZT9 (dark grey). Values are means ± SD of measurements on five plants. Hexose phosphate data are also presented in Table S1. C, Sugar contents of plants as in (b), grown in 12:12 conditions and harvested at ZT0 (light indigo) or ZT6 (dark indigo) or grown in 6:18 conditions and harvested at ZT0 (light aqua) or ZT6 (dark aqua).

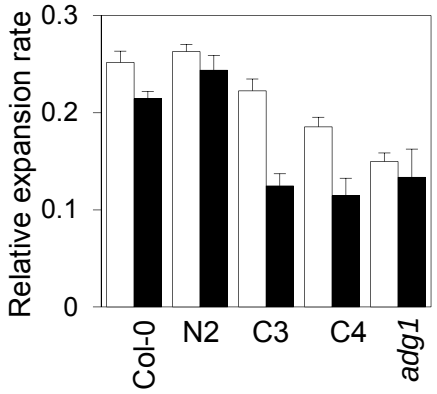

Figure S4. Relative rates of rosette expansion of wild-type and GlgC-TM plants grown in different day lengths.

Relative rosette expansion rates were measured in wild-type (Col-0) plants, *adg1* mutants, *APS1*-expressing line N2 and *GlgC-TM* expressing lines C3 and C4. Plants were photographed after 13 and 16 days of growth in 12:12 conditions (white bars) and after 20 and 23 days in 6:18 conditions (black bars). These time intervals were chosen because they give similar values in the two conditions for wild-type plants. Rosette areas were measured from photographs, and the relative expansion rate (cm^2 cm^{-2}) between the two time points was calculated as $((\ln(A_2)-(\ln(A_1))/t$; where A_1 and A_2 = area at first and second time point, t= time between them. Values are means ± SE calculated from measurements on 53 to 72 replicate plants per genotype.

Table S1

Rates of starch synthesis in different photoperiods. Linear regression was used to calculate mean rates of starch synthesis over the light period, standard errors of means (SE), and the significance of the difference in rate between photoperiods (p-value). Values were taken from the Figures indicated.

Rates of starch synthesis from Figure 1a				
Genotype	Photoperiod	Rate of synthesis (mg h^{-1} g^{-1} fresh weight)	SE	*p*-value vs. 12:12
WT Col-0	12:12	0.45	0.03	
	06:18	0.68	0.04	<0.001
WT Ws	12:12	0.60	0.03	
	06:18	0.96	0.05	<0.001
gi-201	12:12	0.58	0.04	
	06:18	0.85	0.08	0.098
fkf1	12:12	0.44	0.02	
	06:18	0.48	0.04	0.108
lhy cca1	12:12	0.29	0.02	
	06:18	0.57	0.06	<0.001
co-10	12:12	0.36	0.02	
	06:18	0.57	0.04	0.001
elf3	12:12	0.37	0.02	
	06:18	0.50	0.10	0.011
pif4	12:12	0.47	0.02	
	06:18	0.77	0.05	<0.001

Rates of starch synthesis from Figure 1b				
Genotype	Photoperiod	Rate of synthesis (mg h^{-1} g^{-1} fresh weight)	SE	*p*-value vs. 12:12
Wt Col0	12:12	0.42	0.06	
	06:18	0.85	0.08	<0.001
gi-201	12:12	0.31	0.10	
	06:18	0.37	0.06	0.971
gi-2	12:12	0.46	0.06	
	06:18	0.48	0.08	0.234
fkf1	12:12	0.37	0.03	
	06:18	0.39	0.10	0.758
co-10	12:12	0.35	0.06	
	06:18	0.74	0.08	<0.001

Rates of starch synthesis from Figure 3b				
Genotype	Photoperiod	Rate of synthesis (mg h^{-1} g^{-1} fresh weight)	SE	*p*-value vs. 12:12
WT	12:12	0.46	0.02	
	06:18	0.84	0.03	<0.001
N2	12:12	0.50	0.02	
	06:18	0.74	0.03	<0.001
C3	12:12	0.32	0.03	
	06:18	0.44	0.04	0.125
C4	12:12	0.26	0.04	
	06:18	0.10	0.05	0.934

adg1-1	12:12	n/a	
	06:18	n/a	0.245

Rates of starch synthesis from Figure 5a

Genotype	Photoperiod	Rate of synthesis (mg h^{-1} g^{-1} fresh weight)	SE	p-value vs. 12:12
WT	12:12	0.33	0.02	
	06:18	0.94	0.07	<0.001
Line A	12:12	0.37	0.03	
	06:18	0.99	0.05	<0.001
Line B	12:12	0.32	0.02	
	06:18	0.90	0.05	<0.001

Rates of starch synthesis from Figure 5b

Genotype	Photoperiod	Rate of synthesis (mg h^{-1} g^{-1} fresh weight)	SE	*p*-value vs. 12:12
WT col-0	12:12	0.61	0.02	
	06:18	1.08	0.10	<0.001
adg2	12:12	0.37	0.03	
	06:18	0.54	0.06	0.015
adg1-1	12:12	n/a		
	06:18	n/a		<0.001*

* a significant effect of photoperiod on starch content was detected in this experiment, but there was no net synthesis during the day so this does not equate to a difference in the rate of synthesis)

Table S2. Metabolite contents.

Metabolite contents of wild-type (Col-0) plants, *APS1*-expressing line N2 and *GlgC-TM*-expressing lines C3 and C4. Plants were grown in 12:12 conditions and harvested at ZT3 or ZT15 or grown in 6:18 conditions and harvested at ZT3 or ZT9. S6P is sedoheptulose 6-phospahte, Gly3P is glycerol 3-phosphate, 2-OG is 2-oxoglutarate. Values for hexose phosphates are also presented in Figure 4b and Figure S3b. Values are means ± SD of measurements on five plants. Data were analyzed using a general linear model and ANOVA to test for the effects of genotype, including time of day and photoperiod as covariants. Metabolites that show significant differences between genotypes with the endogenous AGPase (WT and N2) and those with the GlgC-TM AGPase (C3 and C4) are indicated by the *p*-value displayed in the table. ND = not detected.

Day length and time of day	Genotype	Metabolite								
		nmol g^{-1} fresh weight								
		Glc 1P	Glc 6P	Fru 6P	Tre 6P	S6P	Gal 6P	Gly3P	UDPGlc	Man6P
p-value		<0.001	<0.001	0.001	<0.001	0.018	0.221	0.018	0.457	0.534
12:12, ZT3	Wt	18.6 ± 3.3	91 ± 9	100 ± 22	0.46 ± 0.10	1.27 ± 0.37	2.55 ± 0.75	21.0 ± 5.4	108 ± 20	19.3 ± 1.1
	adg1	38.4 ± 4.1	266 ± 12	154 ± 18	0.37 ± 0.01	3.87 ± 0.89	2.87 ± 0.17	18.7 ± 1.9	78 ± 7	26.5 ± 2.4
	N2	21.0 ± 0.9	90 ± 6	104 ± 5	0.50 ± 0.13	1.46 ± 0.17	3.02 ± 0.52	24.6 ± 2.7	132 ± 4	22.6 ± 3.7
	C3	37.9 ± 6.5	220 ± 29	155 ± 22	0.99 ± 0.11	2.31 ± 0.31	2.65 ± 0.29	23.7 ± 5.8	98 ± 18	23.5 ± 3.5
	C4	39.4 ± 4.0	257 ± 63	167 ± 21	0.97 ± 0.22	2.34 ± 0.19	3.02 ± 0.78	24.4 ± 2.9	115 ± 18	26.7 ± 3.4
12:12, ZT15	Wt	22.2 ± 3.0	100 ± 10	53 ± 5	0.23 ± 0.05	1.25 ± 0.17	4.46 ± 0.48	33.4 ± 2.3	141 ± 13	25.7 ± 0.6
	adg1	12.4 ± 4.1	53 ± 16	28 ± 10	0.19 ± 0.07	0.49 ± 0.15	2.73 ± 0.88	23.2 ± 4.8	104 ± 33	13.3 ± 3.8
	N2	18.6 ± 1.3	92 ± 8	47 ± 3	0.23 ± 0.02	1.17 ± 0.13	3.87 ± 0.25	36.3 ± 8.4	114 ± 5	26.5 ± 2.8
	C3	18.1 ± 1.3	76 ± 9	41 ± 3	0.15 ± 0.01	0.69 ± 0.09	4.00 ± 0.26	26.1± 0.3	128 ± 8	22.2 ± 1.8
	C4	17.7 ± 1.9	82 ± 8	43 ± 5	0.11 ± 0.02	0.62 ± 0.02	3.74 ± 0.41	31.2 ± 2.5	136 ± 25	23.1 ± 2.9
6:18, ZT3	Wt	15.0 ± 2.1	79 ± 9	89 ± 13	0.28 ± 0.33	0.91 ± 0.17	2.10 ± 0.39	17.0 ± 2.3	92 ± 14	15.4 ± 1.5
	adg1	28.1 ± 1.1	224 ± 6	123 ± 4	0.41 ± 0.02	2.25 ± 0.15	2.33 ± 0.16	14.1 ± 0.6	66 ± 5	18.8 ± 1.0
	N2	14.5 ± 2.0	81 ± 7	76 ± 12	0.22 ± 0.04	0.81 ± 0.19	1.94 ± 0.29	15.8 ± 2.0	96 ± 18	15.2 ± 1.1
	C3	28.4 ± 3.7	175 ± 8	119 ± 12	0.69 ± 0.08	2.02 ± 0.36	2.19 ± 0.39	17.1 ± 2.3	87 ± 17	20.0 ± 1.4
	C4	31.0 ± 5.4	242 ± 67	136 ± 29	0.69 ± 0.16	1.82 ± 0.32	2.16 ± 0.41	18.1 ± 3.7	78 ± 16	25.1 ± 8.1
6:18, ZT9	Wt	11.8 ± 0.6	54 ± 3	33 ± 2	0.08 ± 0.01	0.35 ± 0.02	2.94 ± 0.23	29.2 ± 0.6	78 ± 9	19.8 ± 1.8
	adg1	12.8 ± 0.4	104 ± 21	45 ± 3	0.29 ± 0.01	0.44 ± 0.01	2.81 ± 0.27	12.4 ± 1.8	96 ± 17	16.6 ± 1.8
	N2	9.2 ± 1.6	52 ± 7	27 ± 6	0.06 ± 0.01	0.26 ± 0.04	2.38 ± 0.60	24.9 ± 2.4	67 ± 23	19.0 ± 2.7
	C3	11.5 ± 0.3	58 ± 7	30 ± 2	0.08 ± 0.01	0.23 ± 0.01	2.50 ± 0.23	21.6 ± 2.4	93 ± 12	17.1 ± 1.3
	C4	7.5 ± 3.6	53 ± 8	21 ± 8	0.11± 0.08	0.13 ± 0.05	1.40 ± 0.88	12.0 ± 6.1	57 ± 41	12.0 ± 4.1

Table S2 continued.

Day length and time of day	Genotype	Metabolite								
		nmol g^{-1} fresh weight								
		PEP	shikimate	aconitate	Iso-citrate	pyruvate	2-OG	3-PGA	Fru 1,6BP	succinate
p-value		0.155	0.270	0.275	0.944	0.020	>0.001	0.303	0.150	0.004
12:12, ZT3	Wt	23.6 ± 2.4	38.3 ± 1.6	109 ± 8	125 ± 7	115 ± 6	64 ± 4	290 ± 31	15.2 ± 0.4	240 ± 27
	adg1	29.9 ± 1.8	29.9 ± 2.6	145 ± 15	140 ± 9	102 ± 13	77 ± 4	274 ± 17	18.5 ± 1.7	343 ± 81
	N2	23.9 ± 7.3	42.5 ± 3.3	142 ± 25	154 ± 16	174 ± 35	78 ± 10	311 ± 43	14.9 ± 0.5	429 ± 229
	C3	21.1 ± 2.1	40.9 ± 4.1	106 ± 12	123 ± 18	121 ± 28	83 ± 7	345 ± 66	14.0 ± 2.2	275 ± 49
	C4	30.6 ± 9.6	40.0 ± 3.2	119 ± 11	132 ± 32	122 ± 28	102 ± 18	374 ± 58	17.4 ± 4.1	346 ± 45
12:12, ZT15	Wt	12.4 ± 3.1	49.0 ± 6.1	156 ± 27	136 ± 27	148 ± 12	81 ± 5	131 ± 19	3.6 ± 0.4	545 ± 16
	adg1	5.7 ± 1.7	54.4 ± 14.1	199 ± 35	167 ± 44	106 ± 24	159 ± 37	53 ± 9	1.4 ± 0.8	1527 ± 165
	N2	11.8 ± 0.6	50.8 ± 1.8	155 ± 18	137 ± 29	128 ± 17	80 ± 4	128 ± 5	3.0 ± 0	443 ± 11
	C3	6.4 ± 1.4	49.6 ± 3.8	152 ± 5	124 ± 3	116 ± 3	118 ± 42	80 ± 9	1.4 ± 0.9	697 ± 93
	C4	9.4 ± 0.4	49.3 ± 7.3	162 ± 32	147 ± 11	115 ± 9	142 ± 16	92 ± 4	1.6 ± 0.1	780 ± 147
6:18, ZT3	Wt	10.4 ± 1.6	38.8 ± 4.0	102 ± 15	140 ± 41	111 ± 13	48 ± 2	237 ± 35	9.5 ± 1.7	144 ± 26
	adg1	31.8 ± 3.8	29.1 ± 0.3	119 ± 3	155 ± 28	94 ± 8	66 ± 2	277 ± 14	18.0 ± 0.7	513 ± 41
	N2	17.8 ± 1.3	40.0 ± 6.4	95 ± 19	129 ± 43	106 ± 20	50 ± 5	271 ± 37	10.4 ± 0.5	148 ± 20
	C3	21.4 ± 6.2	35.0 ± 3.6	98 ± 11	132 ± 21	103 ± 15	74 ± 7	290 ± 5	11.6 ± 1.7	186 ± 38
	C4	36.1 ± 7.5	39.1 ± 7.9	95 ± 17	151 ± 48	93 ± 19	72 ± 22	346 ± 61	23.8 ± 5.0	211 ± 40
6:18, ZT9	Wt	18.9 ± 4.3	42.6 ± 4.3	131 ± 13	142 ± 6	107 ± 7	53 ± 5	128 ± 6	Nd	419 ± 42
	adg1	11.8 ± 5.4	42.7 ± 7.0	129 ± 0	201 ± 10	103 ± 13	134 ± 22	91 ± 23	1.7 ± 0.9	1750 ± 237
	N2	20.3 ± 3.0	36.7 ± 5.0	105 ± 20	151 ± 30	82 ± 18	46 ± 7	113 ± 18	Nd	328 ± 17
	C3	20.9 ± 4.9	38.9 ± 1.3	119 ± 11	166 ± 44	95 ± 19	83 ± 10	116 ± 9	Nd	642 ± 44
	C4	15.2 ± 3.3	37.9 ± 12.9	89± 48	144 ± 74	83 ± 34	94 ± 19	79 ± 28	0.8 ± 0	748 ± 477

Table S2 continued.

Day length and time of day	Genotype	Metabolite		
		µmol g^{-1} fresh weight		
		citrate	malate	fumarate
p-value		0.211	>0.001	>0.001
12:12, ZT3	Wt	13.0 ± 1.1	7.5 ± 1.4	3.5 ± 1.1
	adg1	14.2 ± 1.2	4.9 ± 0.6	3.8 ± 0.9
	N2	15.2 ± 2.4	9.1 ± 1.4	3.6 ± 0.5
	C3	12.9 ± 0.3	4.6 ± 0.4	3.1 ± 0.2
	C4	15.3 ± 2.6	5.1 ± 0.7	3.0 ± 0.6
12:12, ZT15	Wt	15.1 ± 0.8	14.8 ± 1.1	9.3 ± 1.0
	adg1	17.3 ± 3.7	11.0 ± 2.1	7.4 ± 1.1
	N2	15.1 ± 1.0	11.5 ± 0.3	5.6 ± 0.8
	C3	15.5 ± 1.0	10.9 ± 1.2	6.3 ± 0.8
	C4	16.4 ± 1.8	10.2 ± 1.2	5.0 ± 0.6
6:18, ZT3	Wt	12.4 ± 1.1	7.1 ± 0.7	3.9 ± 0.6
	adg1	12.9 ± 0.1	3.7 ± 0.1	3.0 ± 0.1
	N2	11.5 ± 1.8	6.2 ± 1.2	2.9 ± 0.7
	C3	12.3 ± 0.7	3.8 ± 0.4	2.3 ± 0.4
	C4	14.0 ± 3.0	3.5 ± 0.8	2.1 ± 0.3
6:18, ZT9	Wt	13.7 ± 1.0	7.4 ± 0.5	4.6 ± 0.3
	adg1	15.6 ± 0.9	5.6 ± 0.1	4.3 ± 0.3
	N2	11.8 ± 2.0	5.6 ± 0.8	3.3 ± 0.5
	C3	13.2 ± 1.2	4.6 ± 0.3	2.3 ± 0.2
	C4	13.3 ± 0.4	3.8 ± 2.0	2.3 ± 1.5

Enzyme		Wild-type		N2		C3		C4	
	p-value	ZT3	ZT15	ZT3	ZT15	ZT3	ZT15	ZT3	ZT15
Phosphoglucomutase	0.311	8447 ± 379	8180 ± 562	8376 ± 593	8959 ± 292	9106 ± 246	8651 ± 349	8097 ± 741	9784 ± 303
Phosphoglucoisomerase total	0.267	1110 ± 105	1142 ± 121	1146 ± 158	1232 ± 72	1252 ± 60	1272 ± 96	1096 ± 174	1406 ± 30
Phosphoglucoisomerase cytosolic	0.901	746 ± 53	792 ± 48	792 ± 90	806 ± 25	802 ± 27	795 ± 50	704 ± 60	862 ± 41
Phosphoglucoisomerase plastidial (derived)	0.122	364 ± 73	350 ± 81	354 ± 77	427 ± 73	450 ± 51	477 ± 54	392 ± 132	544 ± 25
Rubisco initial	0.242	10.3 ± 0.9	13.0 ± 0.6	10.6 ± 0.8	14.0 ± 0.9	11.6 ± 0.6	11.3 ± 0.9	10.1 ± 1.0	12.2 ± 0.6
Rubisco maximal	0.115	12.1 ± 0.7	13.8 ± 0.8	13.1 ± 0.8	17.0 ± 0.9	11.8 ± 0.2	13.7 ± 1.1	11.7 ± 0.5	15.3 ± 0.4
Rubisco activation (derived)	n/d	85	94	81	82	98	82	86	80
Glucokinase	0.672	90 ± 12	140 ± 7	91 ± 14	132 ± 8	107 ± 29	95 ± 17	123 ± 15	155 ± 18
Fructokinase	0.080	110 ± 6	152 ± 13	132 ± 9	140 ± 8	123 ± 14	145 ± 7	145 ± 10	184 ± 12
UDPglucose pyrophosphorylase	>0.001	8464 ± 405	7635 ± 555	7604 ± 589	8340 ± 394	9908 ± 308	9661 ± 728	10958 ± 673	11815 ± 735
Triose phosphate isomerase	0.712	15.7 ± 0.7	16.2 ± 0.9	15.6 ± 1.0	16.8 ± 0.6	16.1 ± 0.4	16.1 ± 0.4	14.2 ± 1.3	17.1 ± 0.6
Transketolase	0.015	5825 ± 318	5677 ± 335	6507 ± 301	6897 ± 153	5830 ± 246	5471 ± 263	5836 ± 263	5659 ± 156
Sucrose phosphate synthase	0.626	273 ± 17	267 ± 23	312 ± 29	343 ± 14	298 ± 14	295 ± 17	276 ± 21	305 ± 11
Nitrate reductase	0.811	1052 ± 77	564 ± 56	1023 ± 99	695 ± 56	1044 ± 24	642 ± 55	914 ± 56	709 ± 41

Table S3. Activities of enzymes of primary metabolism.

Measurements were made on wild-type plants and on *adg1* mutants expressing either APL1 (line N2) or GlgC-TM (lines C3 and C4). Values are means ± SE of measurements on four to six rosettes of plants grown in 12:12 conditions and harvested 3 h after dawn (ZT3) or 3 h after the start of the night (ZT15). All values are nmol min^{-1} g^{-1} fresh weight except those for Rubisco and triose phosphate isomerase which are µmol min^{-1} g^{-1} fresh weight and those for Rubisco activation which are percentages. Plastidial phosphoglucomutase activity was calculated by subtracting cytosolic from total activity (Kruckeberg et al., 1989; Gibon et al., 2009). Rubisco activation is initial activity expressed as a percentage of total activity (measured according to Sulpice et al., 2007). Data were analyzed using a general linear model and ANOVA to test for the effects of genotype, including time of day as a covariant. Enzyme activities that show significant differences between genotypes with the endogenous AGPase (WT and N2) and those with the GlgC-TM AGPase (C3 and C4) are indicated by the *p*-value displayed in the table. n/d= not determined.

Table S4. qPCR primers used in this study. *ACT2* was used as the control in the experiment shown in Figure 2a. GAPDH and spike-in controls were used in the experiment shown in Figure S1 (see Czechowski et al., 2004).

Gene/spike name	forward primer	Forward primer sequence 5' to 3':	reverse primer	reverse primer sequence 5' to 3':
APL1	At5g19220-f	GTTCCCATTGGAATAGGAGAGAACAC	At5g19220-r	GACCTATCTGCTTCTTGTATTCCCTC
APL2	At1g27680-f	TGGTCATAGCGAATGCAGATGGCGTG	At1g27680-r	AATGGTGGCGTTCTTCAGCACAACGG
APL4	At2g21590-f	GCTAATTTAGCTCTTGTTGAGGAGCG	At2g21590-r	GGAGGAACCGAGGAGAAGTGTAGAAC
APL3	At4g39210-f	CGGATTGTAAATTCGGTAATCTCAC	At4g39210-r	GCTCCTAACATAAGAGTATCCTGAAG
APS1	At5g48300-f	CTACACACAGCCGCGTTATTTACCAC	At5g48300-r	TATGCAGGAACGGAGTCCAACCACAG
GlgC-TM		TGGCGAGTATGGGTATCTACG		GTGGGATTGTACGCAAGAGAG
ACT2 155	H282-155-ACT2	AACTCTCCCGCTATGTATGTCGC	H283-155-ACT2	CAATACCGGTTGTACGACCACTG
ACT2 633	H284-633-ACT2	ACTTTCATCAGCCGTTTTGA	H285-633-ACT2	ACGATTGGTTGAATATCATCAG
Spike1	H251-A1780s1-f1	GATGCCCGACCATCATTTAG	H252-A1780s1-r1	GTGAGGGTAATGTCGCGTTC
Spike2	H253-A1780s2-f1	ATTGCGCTCGCCATATACAC	H254-A1780s2-r1	GCTGGGATCAGGAGGAGAAG
Spike3	H255-A1780s3-f1	GATCGTTTGCCTGCATTACC	H256-A1780s3-r1	GAGAGCGTCAGCCATACCAC
Spike4	H257-A1780s4-f1	TACGTCGCACAACCACAATC	H258-A1780s4-r1	CAGCGCCACTAACCCACTAC
Spike5	H302-S5f-2	ACAAAGGCAGCGTTGAAAAC	H301-S5r-1	TAGTGTCTGCACGCCATACC
Spike6	Spike-6f-2	CGCAAAGTCTCTCCTCTTGG	Spike-6r-2	CAGTAGCCATTGCGGAAGAT
Spike7	H313-S7f-3	CTGAACCAGACTGCACATGG	H314-S7r-3	ACGCTCATGGGCTTGTTTAT
Spike8	H315-S8f-3	TCATTAAAGCGGAAGGCAAT	H316-S8r-3	AAATCTTCCAGCACCGTCAG