

Supplementary Information

Regulation of stomatal opening and histone modification by photoperiod in *Arabidopsis thaliana*

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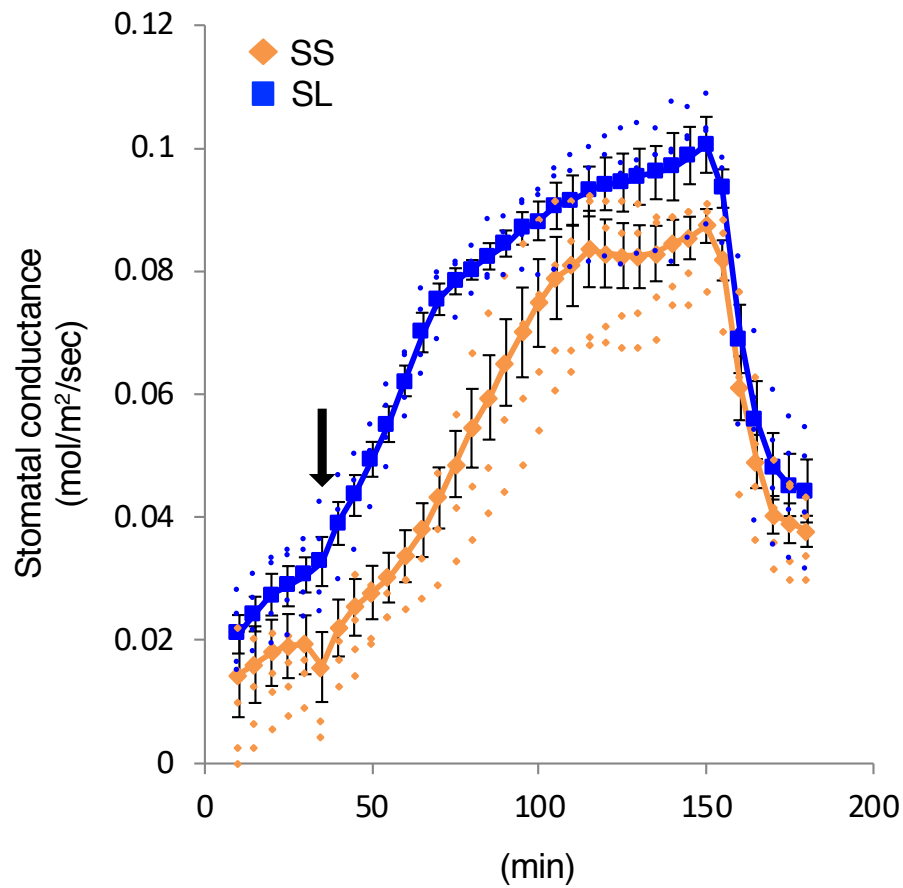
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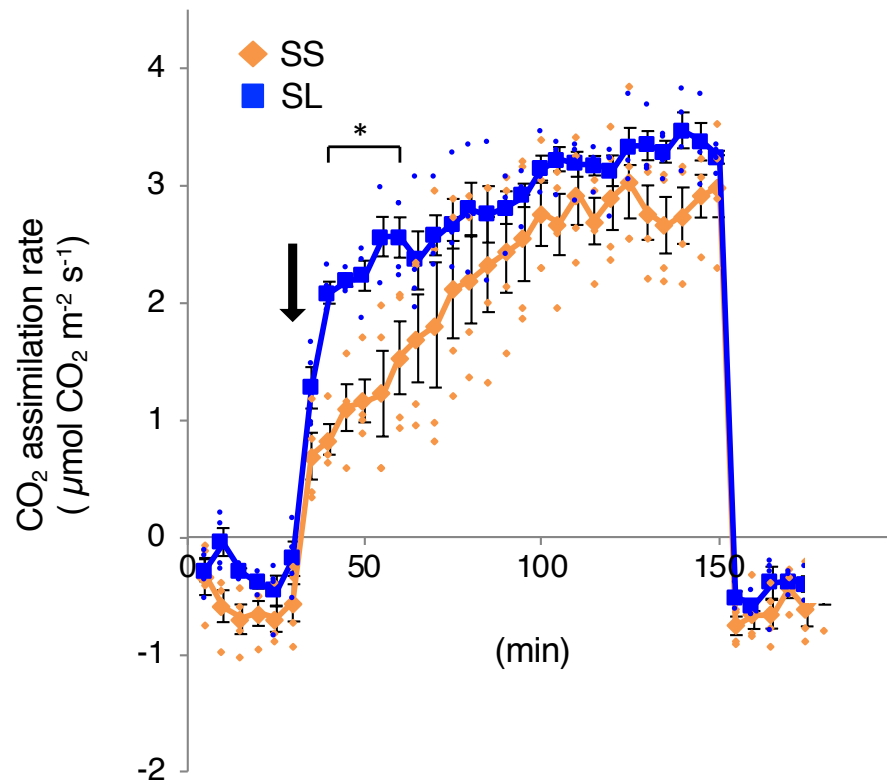
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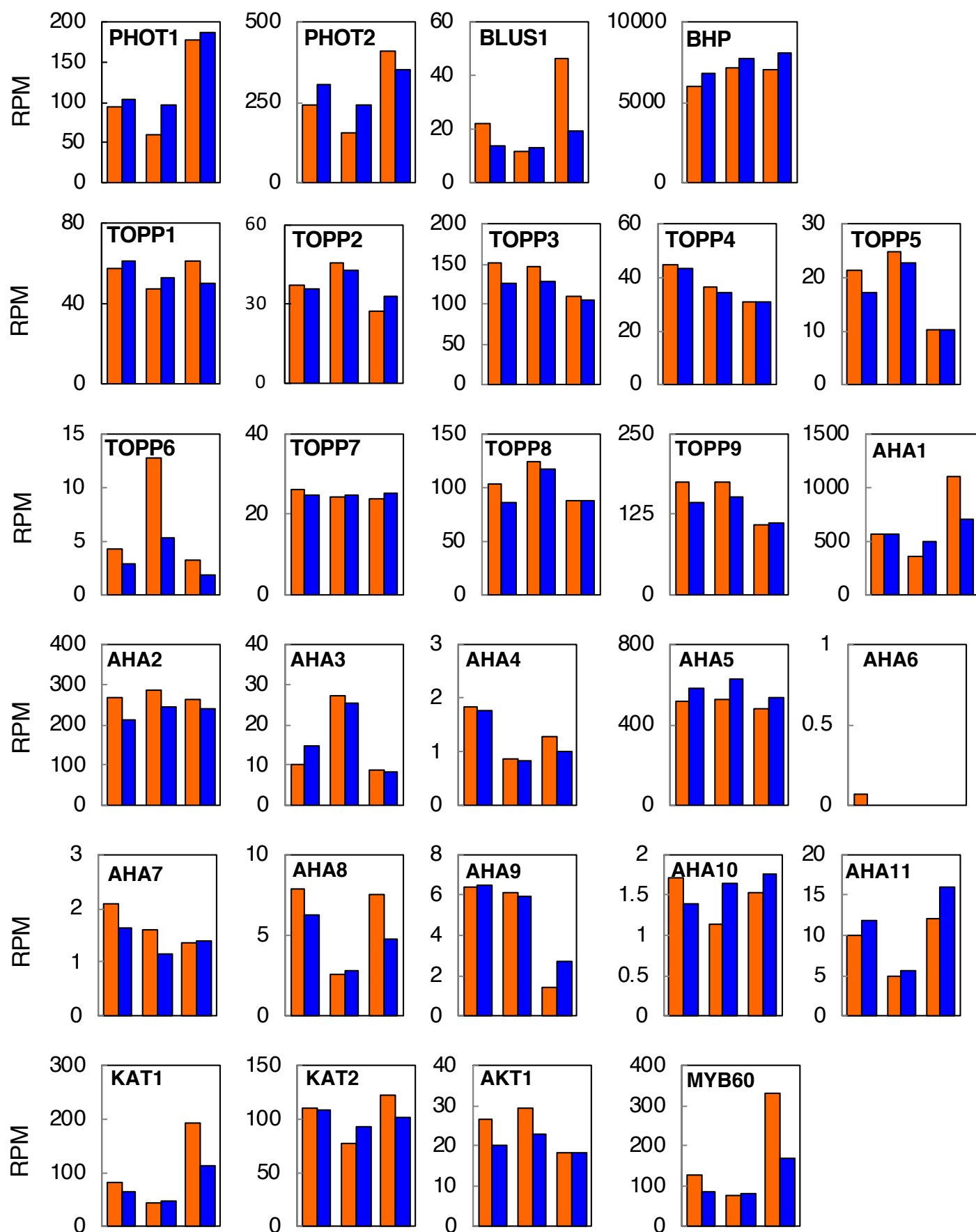
Supplementary Figure 1. Plant phenotypes grown under different conditions. a) Plants were grown under short-day (SD) conditions for 3 weeks, and then transferred to SD conditions for 2 weeks (SS). **b)** Plants were grown under SD conditions for 3 weeks, and then transferred to long-day (LD) conditions for 2 weeks (SL). Bars indicate 1 cm.



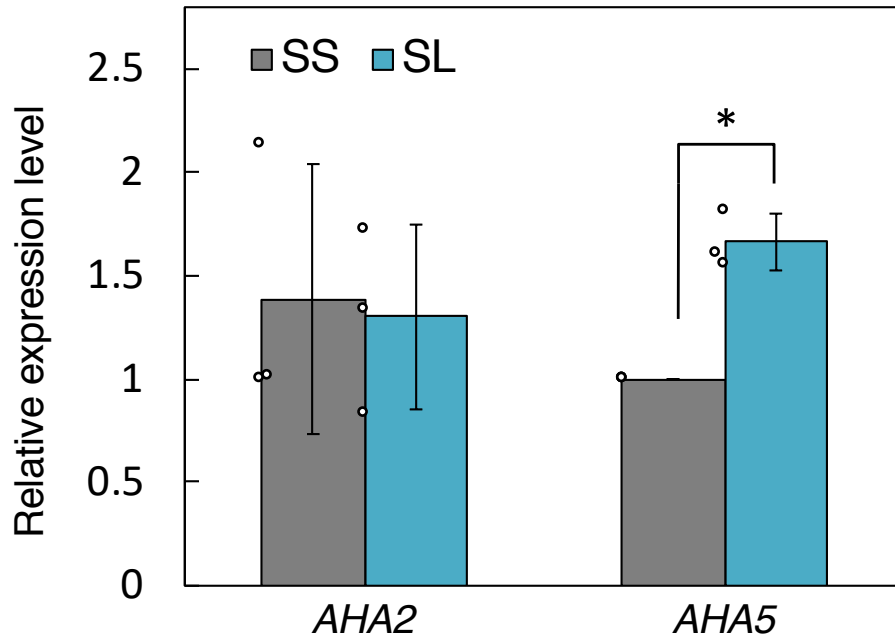
Supplementary Figure 2. Time course of light-induced increase of stomatal conductance in SS and SL plants. Data are means of four measurements in SS and SL plants $\pm$ SE. Fig. 1c was obtained from this figure. Arrow indicates start of light illumination. Circles indicate exact values for each sample.



Supplementary Figure 3. Photosynthetic activity in SS and SL plants. Time course of CO₂ assimilation in SS and SL plants. Data are means of four measurements in SS and SL plants $\pm$ SE (two-sided Student's *t*-test, **P* < 0.05). Arrow indicates start of light illumination. Circles indicate exact values for each sample.



Supplementary Figure 4. Individual RPM values of genes listed in Table 2. The bar graphs show the individual RPM values of three biological replicates that are used for means listed in Table 2.



Supplementary Figure 5. Expression levels of plasma membrane H⁺-ATPase isoforms (*AHA2* and *AHA5*). Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) analysis of *AHA2* and *AHA5* expression in guard cell protoplasts (GCPs) collected at ZT4 from SS and SL plants. Data are means of three independent experiments $\pm$ SD. Circles indicate exact values for each sample. Asterisks indicate statistical significance (two-sided Student's *t*-test, **P* = 0.001).

Supplementary Table 1. Stomatal density and leaf area in SS and SL plants

	Density (number of stomata per mm ²)	SD	Leaf area (cm ²)	SD
SS	91.9	± 9.4	1.5	± 0.35
SL	100.5	± 7.2	1.4	± 0.31

Plants were grown under short-day (SD) conditions for 3 weeks, and then transferred to separate SD conditions for 2 weeks (SS) or to long-day (LD) conditions for 2 weeks (SL). Stomatal density was counted from replicas of abaxial epidermis from mature leaves using varnish. Data represent means of densities in 5 different positions with SD (Student's t test, two-sided, $P = 0.13$). Leaf area was measured from scan image. Data represent means of leaf area in 18 individual plants (Student's t test, two-sided, $P = 0.38$).

Supplementary Table 2. Up-regulated genes in RNAseq analysis of GCPs (ZT4)

AGI No.	Name	RPM in SS	RPM in SL	Fold Change (SL/SS)	FDR	<i>P</i> value
AT1G01060	<i>LHY</i>	13.7 ± 11.3	63.5 ± 45.5	4.64	0.320	0.000
AT3G09600	<i>RVE8</i>	12.1 ± 7.97	43.9 ± 20.2	3.63	0.320	0.000
AT5G60910	<i>FUL</i>	4.62 ± 1.36	13.0 ± 2.95	2.82	0.320	0.000
AT1G32900	<i>GBSS1</i>	3.11 ± 1.27	7.38 ± 7.03	2.37	1.00	0.028
AT1G53560	<i>Ribosomal protein</i>	5.61 ± 0.31	11.4 ± 2.71	2.04	1.00	0.001
AT1G64500	<i>THRUMIN1</i>	6.73 ± 5.82	15.8 ± 8.16	2.34	1.00	0.019
AT1G73190	<i>TIP3;1</i>	4.24 ± 2.31	9.46 ± 5.72	2.23	1.00	0.014
AT2G45660	<i>SOC1</i>	8.33 ± 7.93	23.0 ± 14.3	2.76	1.00	0.010
AT2G46830	<i>CCA1</i>	80.3 ± 74.9	194 ± 145	2.42	1.00	0.027
AT3G15354	<i>SPA3</i>	26.3 ± 20.0	54.4 ± 23.9	2.07	1.00	0.022
AT3G22840	<i>ELIP1</i>	26.6 ± 28.3	95.1 ± 115	3.58	1.00	0.016
AT3G26790	<i>FUS3</i>	8.93 ± 1.69	21.8 ± 10.1	2.44	1.00	0.000
AT3G54500	<i>LNK2</i>	160 ± 81.4	390 ± 121	2.43	1.00	0.001
AT4G08950	<i>EXORDIUM</i>	58.5 ± 16.7	121 ± 50.8	2.06	1.00	0.002
AT4G10250	<i>ATHSP22.0</i>	16.5 ± 7.87	33.8 ± 29.9	2.05	1.00	0.041
AT4G15430	<i>Unknown</i>	9.14 ± 7.86	24.4 ± 19.6	2.67	1.00	0.014
AT4G38960	<i>BBX19</i>	8.31 ± 5.31	18.7 ± 9.98	2.25	1.00	0.014
AT5G15950	<i>SAMDC2</i>	8.53 ± 1.50	19.9 ± 8.99	2.33	1.00	0.001
AT5G18670	<i>BAM9</i>	10.8 ± 3.18	23.0 ± 12.7	2.14	1.00	0.007
AT5G37260	<i>RVE2</i>	26.0 ± 6.58	54.2 ± 11.6	2.09	1.00	0.001
AT5G42760	<i>Unknown</i>	3.12 ± 2.80	10.9 ± 7.50	3.49	1.00	0.002

GCPs from SS and SL plants were used for analysis. The actual read counts were normalized by TMM normalization and converted to reads per million (RPM). Data represent means of three independent experiments with SD. False discovery rate (FDR) and raw *p*-value (*P* value) are calculated with edgeR.

Supplementary Table 3. List of primers

qRT-PCR		
<i>SOC1</i>	Fw	CGAGAAGCTCTCTGAAAAGTGGGG
<i>SOC1</i>	Rv	GGGCTACTCTCTTCATCACCTCTTCC
<i>FT</i>	Fw	GAACTTCTATACTTTGGTTATGGTGGATC
<i>FT</i>	Rv	CACAATCTCATTGCCAAAGGTTG
<i>AHA2</i>	Fw	GCTTTGACTTACATTGACGGCAG
<i>AHA2</i>	Rv	CAACAAATTCCCATGGCG
<i>AHA5</i>	Fw	CATAGCTCAGCTGGTGGCG
<i>AHA5</i>	Rv	TCCACTCAAAACATAGCGGATTC
<i>TUB2</i>	Fw	GCGAGTTGCGGTAGATTCGT
<i>TUB2</i>	Rv	TCCCAGGCTCCAAATCCA
ChIP-qPCR		
<i>SOC1</i>	Fw	GTGAGGGGGCAAACTCAGATG
<i>SOC1</i>	Rv	GCTGGCGAATTCATAAAGTTTGCC