Supplemental Data

Alterations in the endogenous ascorbic acid content affect flowering time in *Arabidopsis thaliana*

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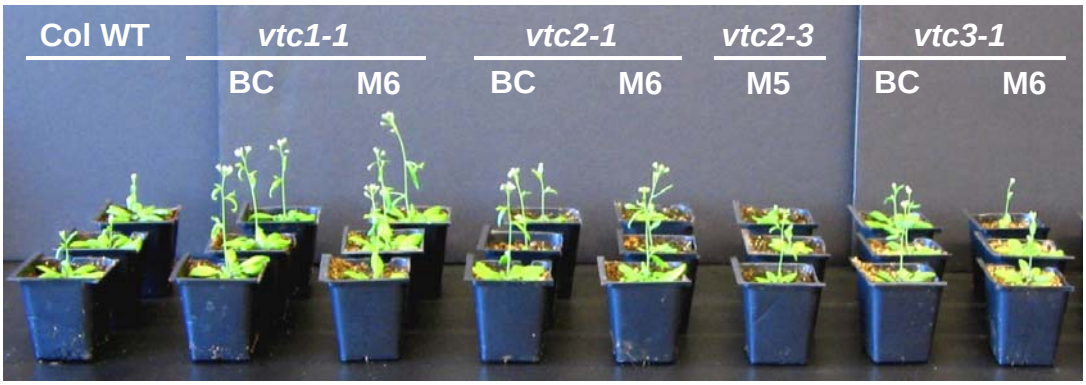
Supplemental Table I. Sequences of oligonucleotide primers used for mutant identification.

Name	Primer sequence	ATG number
CO-2-F	5'-CCACGTGTAGGCACTCAGGA-3'	AT5G15840
CO-2-R	5'-GAACAGCCACGAAGCAACCT-3'	
FCA1-F	5'-CCCGTTAGGTGGTTATGGTGTTCC-3'	AT4G16280
FCA1-R	5'-TTGGTTTGGTTGCTGCATAGACTG-3'	
FT sequ-F	5'-CCTGCTACAACTGGAACAACC-3'	AT1G65480
FT sequ-R	5'-ATAGGCATCATCACCGTTCG-3'	
GI sequ-F*	5'-CTGAACCCTTGGAAGCCTACCT-3'	AT1G22770
GI sequ-R*	5'-ATGCACTTGCGAGAATCACCAG-3'	
GI sequ-F2*	5'-GCGCTATGCAATGTCGTATCTG-3'	
VTC1-F2	5'-ACATTTTTAGCAGCTGGTATTGAG-3'	AT2G39770
VTC1-R2	5'-AGGTAAGAACTGGCAGACTAAAG-3'	

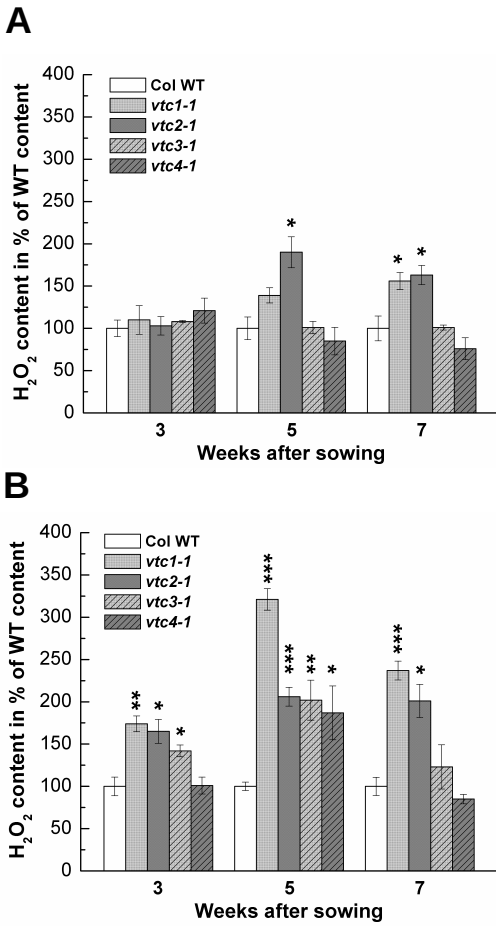
*PCR product was generated using GI sequ-F and GI sequ-R primers, but GI sequ-F2 was used for sequencing.

Supplemental Table II. Sequences of oligonucleotide primers used for gene expression analysis.

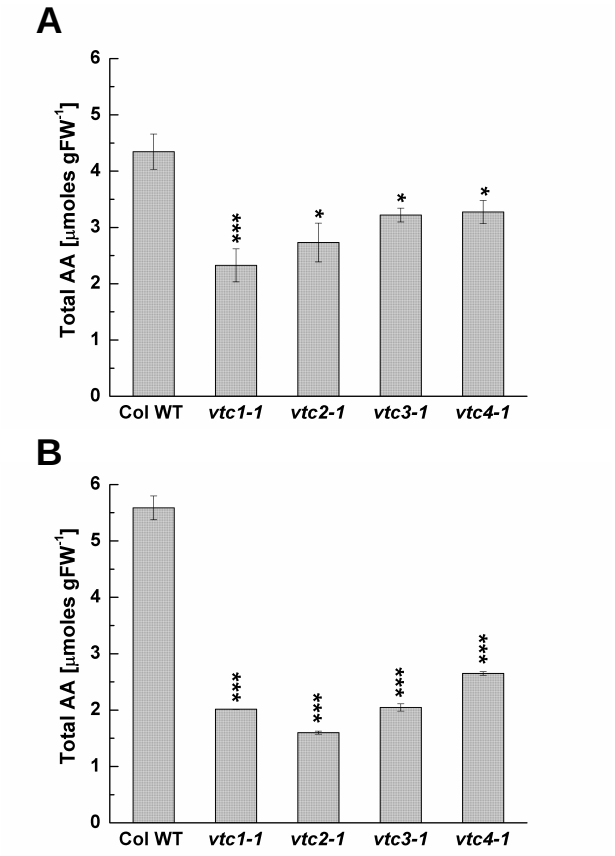
Name	Primer sequence	ATG number
ACTIN2-F	5'-ATGGCTGAGGCTGATGATATTCAAC-3'	AT3G18780
ACTIN2-R	5'-GAAACATTTTCTGTGAACGATTCCT-3'	
CO-F	5'-TCCCCCGTAGCTCGTCTGTGGTA-3'	AT5G15840
CO-R	5'-GCGTGCTCCGGCTGCTTTTT-3'	
CRY1-F	5'-ATCTGGTTGTGGTTCTGGTGGTTG-3'	AT4G08920
CRY1-R	5'-TCAGGGTCATAAGGCATACTAAGA-3'	
CRY2-F	5'-GCTATCTGCTACAATCTCATCA-3'	AT1G04400
CRY2-R	5'-TCTTAGGGGAATCGGTTTA-3'	
FCA1-F	5'-CCCGTTAGGTGGTTATGGTGTTCC-3'	AT4G16280
FCA1-R	5'-TTGGTTTGGTTGCTGCATAGACTG-3'	
FLC-F	5'-TACAAACGCTCGCCCTTATCAG-3'	AT5G10140
FLC-R	5'-CGCATCCGTCGCTCTTCTC-3'	
FT-F	5'-ATGTCTATAAATATAAGAGAGC-3'	AT1G65480
FT-R	5'-CTAAAGTCTTCTTCCTCCGCAG-3'	
GA20OX1-F	5'-GAGCGCCATTGATTTTCCACA-3'	AT4G25420
GA20OX1-R	5'-ACCCGCTTCTTTGATATGCCTCTC-3'	
GA3OX2-F	5'-TGAAGCACGCTCGGGAAGATT-3'	AT1G80340
GA3OX2-R	5'-TCAAGGCGGCTCGGTCAGA-3'	
GA3OX4-F	5'-TGGCCTTGTCGAAAACCTCATACT-3'	AT1G80330
GA3OX4-R	5'-AGGCGCCATACGACTAAACCACTA-3'	
GI-F	5'-GAAGCCACGGCAAGAGCAATAC-3'	AT1G22770
GI-R	5'-CAACGCCGGTGGGAGTGAT-3'	
LFY-F	5'-TCATTTGCTACTCTCCGCCGCT-3'	AT5G61850
LFY-R	5'-CATTTTTCGCCACGGTCTTTAG-3	
LHY-F	5'-AATGCAACTACTGATTCGTG-3'	AT1G01060
LHY-R	5'-GAGACAAGACATGGGGTAAT-3'	
PHYA-F	5'-CTGCGTCCCAAATACTTCTCCTAA-3'	AT1G09570
PHYA-R	5'-GCATGACGCCGGGTTTCCTA-3'	
PHYB-F	5'-GGGCTCCTCATGGTTGTCACTCTC-3'	AT2G18790
PHYB-R	5'-ACCCCGCATCGCCTAAACTATCAG-3'	
TOC1-F	5'-CTTGGTCACCGGCAGGAAATC-3'	AT5G61380
TOC1-R	5'-TGAGCCGCAAGAGCCAACAT-3'	



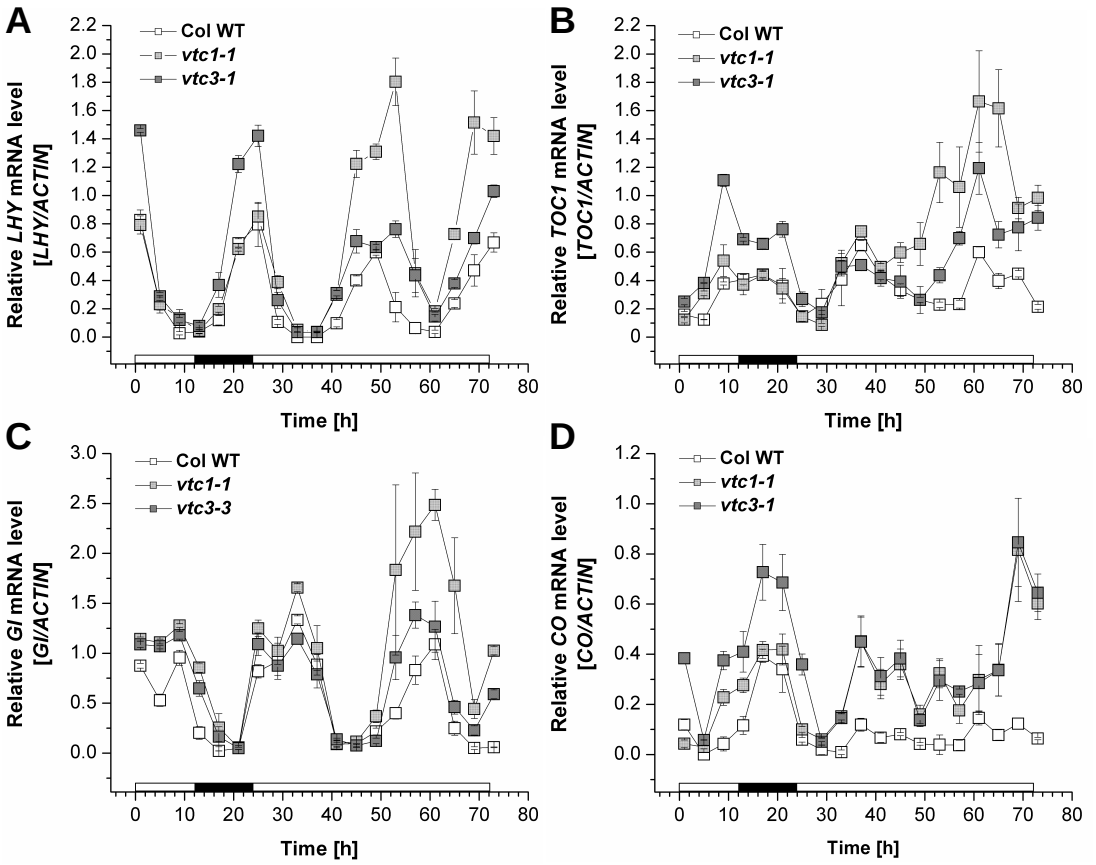
Supplemental Figure 1. Flowering phenotype of Columbia-0 wild type (Col WT) and *vtc* mutants under long days. BC denotes back cross and M indicates ethyl methanesulfonate-mutagenized seeds with numbers indicating the number of back crosses to Col WT and the progeny of M seeds, respectively.



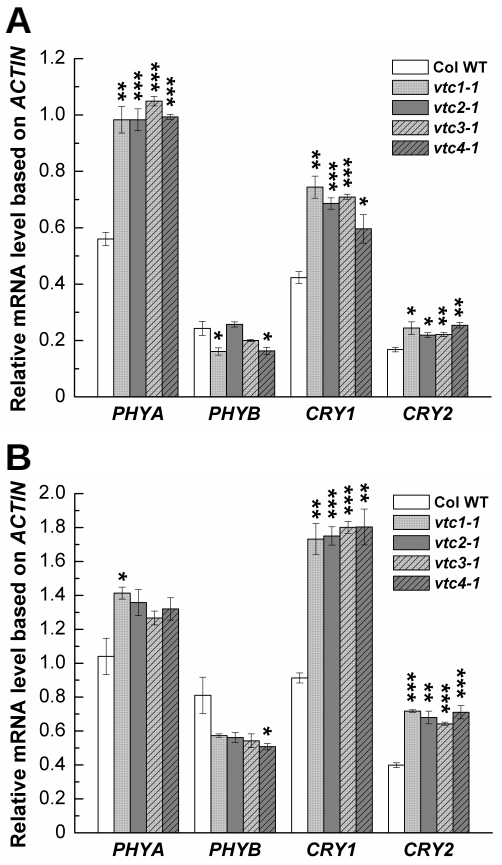
Supplemental Figure 2. Hydrogen peroxide (H_2O_2) content in Columbia-0 wild type (Col WT) and *vtc* mutants grown under short and long days. H_2O_2 content in three-, five- and seven-week-old leaves of the wild type and *vtc* mutants grown under short days (A) and long days (B), respectively. Whole rosette leaves were harvested 4 h after growth chamber lights turned on. Means ± SE of three to seven independent replicates are depicted. Significant differences in comparison to the wild type are indicated with asterisks: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Student's *t*-test.



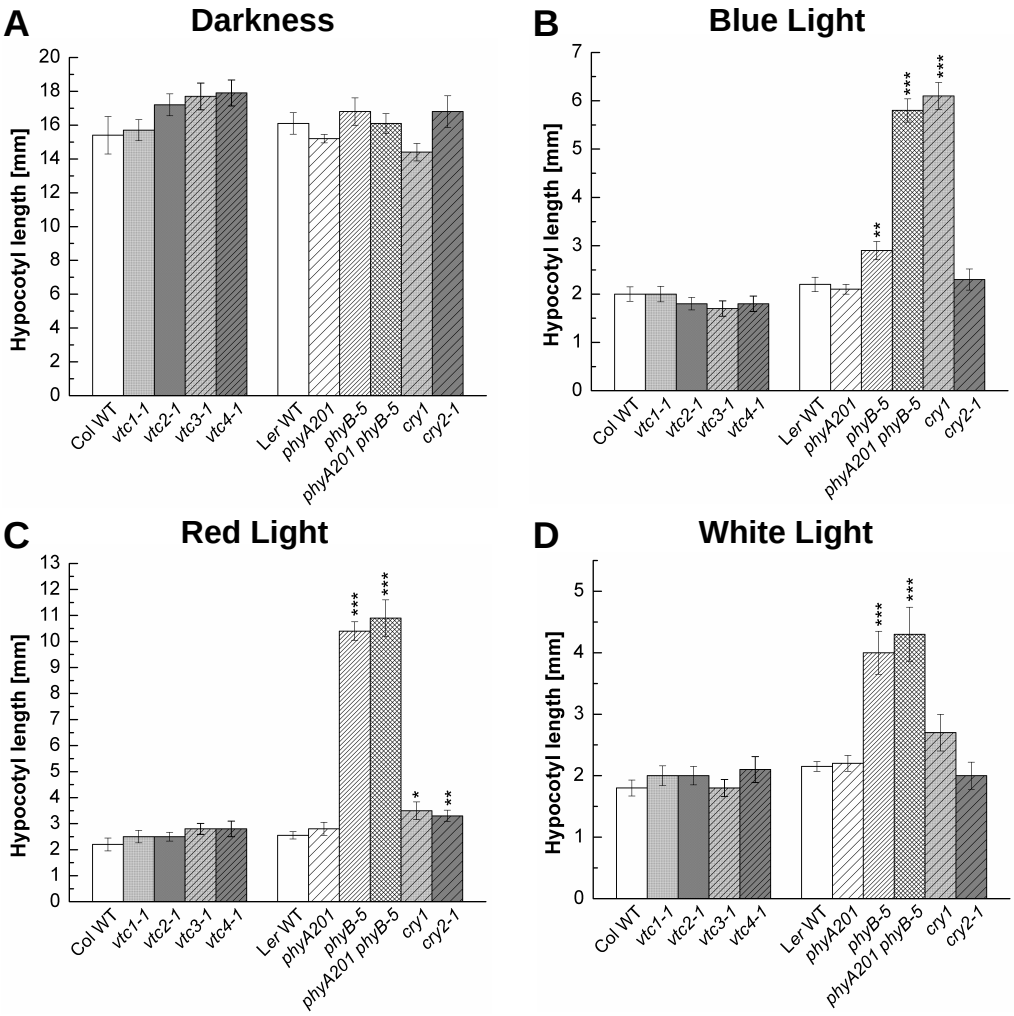
Supplemental Figure 3. Total ascorbic acid (AA) content in three-week-old Columbia-0 wild-type (Col WT) plants and *vtc* mutants grown under short (A) or long (B) days. Whole rosettes were harvested 4 h after growth chamber lights turned on. Means ± SE of three independent replicates are shown. Significant differences in comparison to the wild type are indicated with asterisks: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Student's *t*-test.



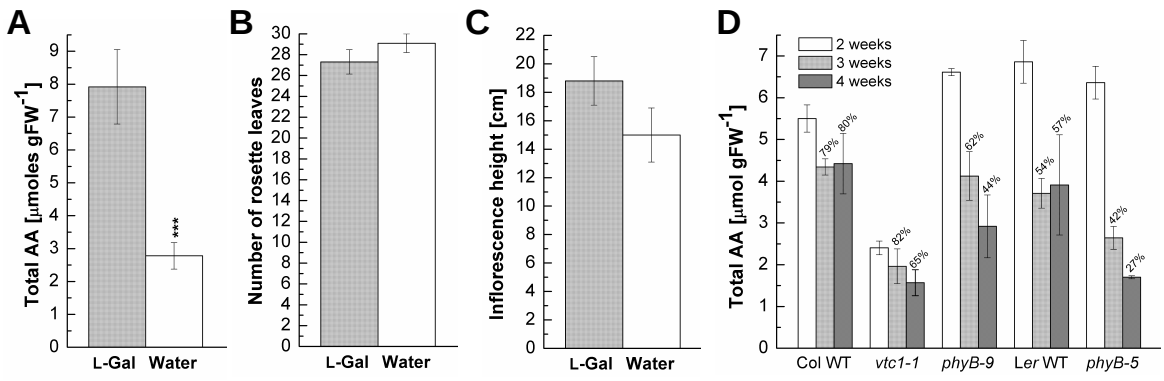
Supplemental Figure 4. Circadian rhythms of clock and photoperiod pathway genes in Columbia-0 wild type (Col WT) and *vtc* mutants. Wild type, *vtc1-1* and *vtc3-1* were grown in 12 h dark/12 h light cycles for three weeks and then transferred to constant light for an additional two days. Collection of leaf tissue started in the last 12 h dark/12 h light cycle, indicated by the white 0 h to 12 h bar and the black 12 h to 24 h bar on the x-axis, and continued throughout the 48 h constant light period, which is indicated by the white 24 h to 72 h bar. Relative transcript levels based on *ACTIN* of the circadian clock genes *LHY* (A) and *TOC1* (B) as well as photoperiodic pathway genes *GI* (C) and *CO* (D) are shown. Data represent means ± SE of three independent replicates.



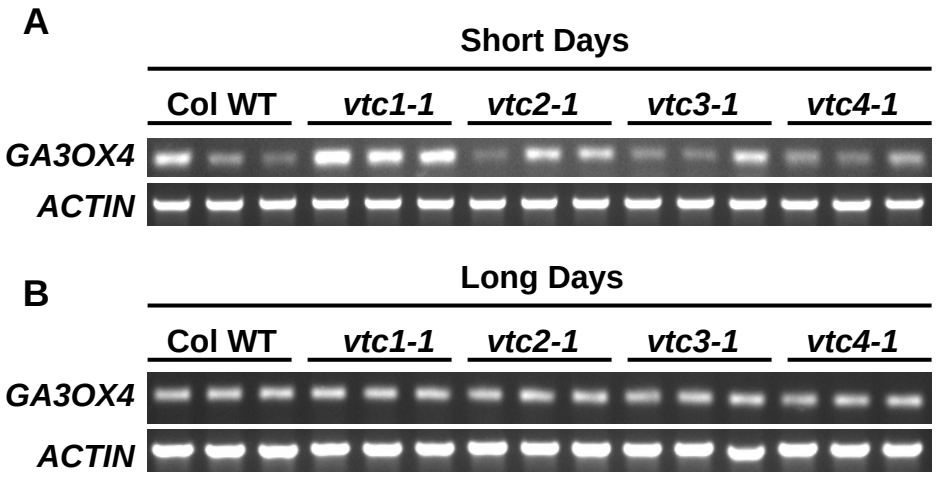
Supplemental Figure 5. Expression analysis of phytochrome and cryptochrome genes in Columbia-0 wild type (Col WT) and *vtc* mutants. Plants were grown under short days (A) and long days (B), respectively. Relative mRNA levels of *PHYA*, *PHYB*, *CRY1* and *CRY2* based on *ACTIN* were determined in leaves of five-week-old plants harvested 4 h after lights turned on. Means ± SE of three independent replicates are depicted. Significant differences in comparison to the wild type are indicated with asterisks: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Student's *t*-test.



Supplemental Figure 6. Hypocotyl length of eight-day-old seedlings of Columbia-0 wild type (Col WT), Landsberg *erecta*-0 wild type (L*er* WT), *vtc* as well as phytochrome (*phyA201*, *phyB-5*, *phyA201 phyB-5*) and cryptochrome (*cry1*, *cry2-1*) mutants. Hypocotyl length was measured in seedlings grown in darkness (A), blue light (B), red light (C), and white light (D). Seedlings were grown under long days. Results illustrate means ± SE of ten independent replicates. Mutants are presented next to their respective wild-type controls. Significant differences in comparison to the wild type are indicated with asterisks: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Student's *t*-test.

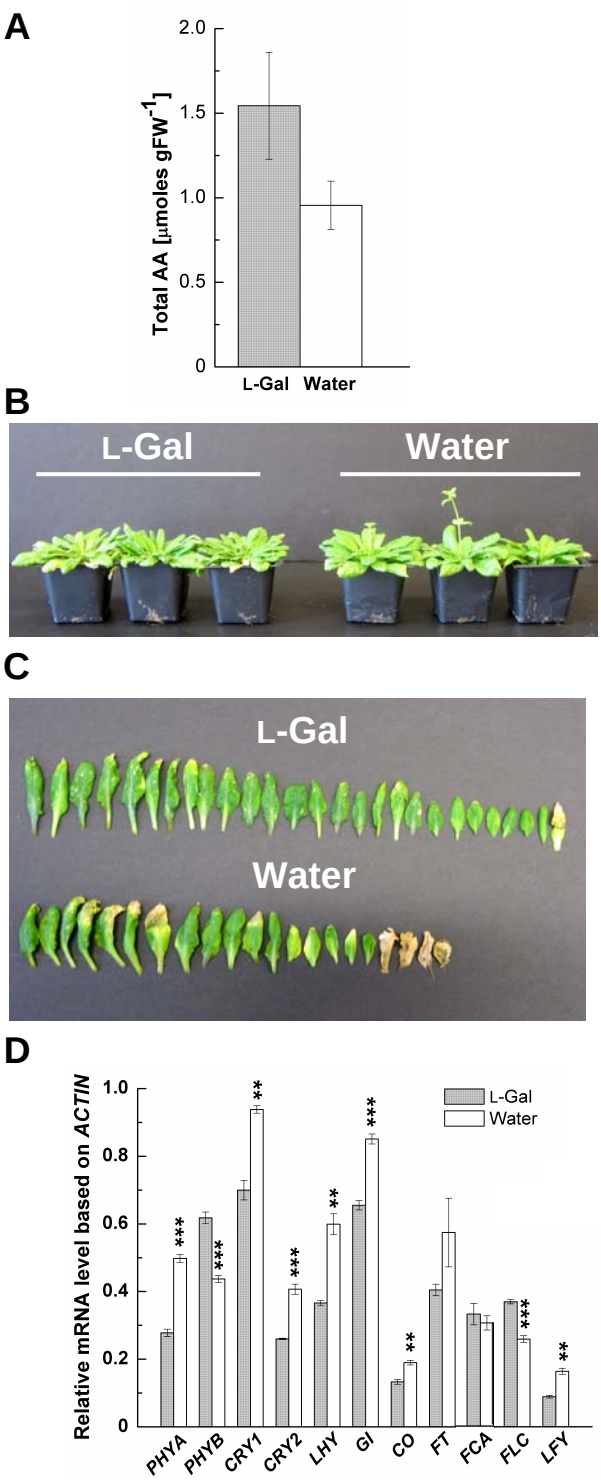


Supplemental Figure 7. Effect of L-galactose (L-gal) treatment on flowering time in *phyB-9* mutants (A-C) and developmental changes in the ascorbic acid (AA) content in wild type, *vtc1-1* and *phyB* mutants grown under long days. (A) Total ascorbic acid content in four-week-old *phyB-9* plants sprayed with L-Gal or water. Means ± SE of three independent replicates are shown. Significant differences between L-gal and water treatments are indicated with asterisks: *** $P < 0.001$, Student's *t*-test. (B) Number of rosette leaves of four-week-old L-Gal- and water-treated *phyB-9* mutants. Data represent means ± SE of 16 independent replicates. The difference is not significant. (C) Inflorescence height in four-week-old L-Gal- and water-sprayed *phyB-9* mutants. Results illustrate means ± SE of 16 independent replicates. Results are not significantly different. (D) Total ascorbic acid content in Columbia-0 wild type (Col WT), *vtc1-1* (Col background), *phyB-9* (Col background), Landsberg *erecta*-0 wild type (L*er* WT), and *phyB-5* (L*er* background) during development. Two, three, and four weeks after sowing, whole rosettes were harvested 4 h after growth chamber lights turned on. Percentages on top of bars indicate differences compared to the respective two-week-ascorbic acid content. Means ± SE of three independent replicates are shown.



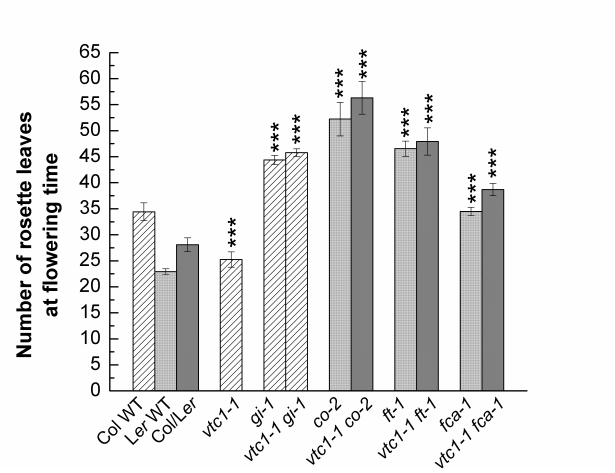
Supplemental Figure 8. Expression analysis of gibberellin 3-oxidase 4 *(GA3OX4)*, an ascorbic acid-dependent gibberellin oxidase gene involved in gibberellin biosynthesis, in Columbia-0 wild type (Col-0 WT) and *vtc* mutants. (A) Transcript levels of *GA3OX* in eleven-week-old wild type and *vtc* mutants grown under short days. (B) Messenger RNA levels of *GA3OX4* in five-week-old plants grown under long days. Inflorescence tissue was harvested 4 h after lights turned on. Transcript levels were assessed in three individual plants. *ACTIN* served as an internal control.

Kotchoni_Supplemental Fig. 9



Supplemental Figure 9. Effect of L-galactose (L-Gal) treatment on flowering time in short day-grown plants. (A) Total content of ascorbic acid (AA) in 13-week-old Columbia-0 wild-type plants sprayed with L-Gal or water. Data represent means ± SE of three independent replicates. The difference is not significant. (B) Flowering phenotype of L-Gal- and water-treated plants. (C) Number of leaves and senescence phenotype of L-Gal- and water-sprayed plants. (D) Expression analysis of photoreceptor (*PHYA*, *PHYB*, *CRY1*, *CRY2*), circadian clock (*LHY*), photoperiodic pathway (*GI*, *CO*, *FT*), autonomous (*FCA*, *FLC*) and floral meristem identity (*LFY*) genes in plants treated with L-Gal or water. Leaf and inflorescence tissues of five-week-old plants were harvested 4 h after lights turned on. To assess expression of *FT*, 33 PCR amplification cycles were run. Transcript levels were based on *ACTIN*. Results represent means ± SE of three independent replicates. Significant differences between L-gal and water treatments are indicated with asterisks: ** $P < 0.01$, *** $P < 0.001$, Student's *t*-test.

Kotchoni_Supplemental Fig. 10



Supplemental Figure 10. Effect of the *vtc1-1* mutation on flowering time in the background of photoperiodic and autonomous pathway mutants grown under short days. Total rosette leaf number of all genotypes at flowering time is shown. Results depict means ± SE of 12 to 17 independent plants per genotype. Shading patterns indicate plants of the same genetic background, allowing for easier statistical comparison of single and double mutants to their respective wild-type controls. Asterisks denote significant differences: *** $P < 0.001$, Student's *t*-test.