New Phytologist

***New Phytologist* Supporting Information Figs S1–S6 and Table S1**

Article title: Silencing *Nicotiana attenuata LHY* and *ZTL* alters circadian rhythms in flowers

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The following Supporting Information is available for this article:

Fig. S1 Silencing efficiency and hypocotyl length of irLHY and irZTL lines.

Fig. S2 Protein alignment of CAB2 orthologs in *Nicotiana attenuata* and *Arabidopsis thaliana*.

Fig. S3 Flower morphology of the clock-silenced lines.

Fig. S4 Flower opening in the clock-silenced lines under LL and LD conditions.

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Fig. S6 Vertical movement in the clock-silenced flowers under LL and LD conditions.

Table S1 Insertion fragments of irLHY and irZTL silenced lines

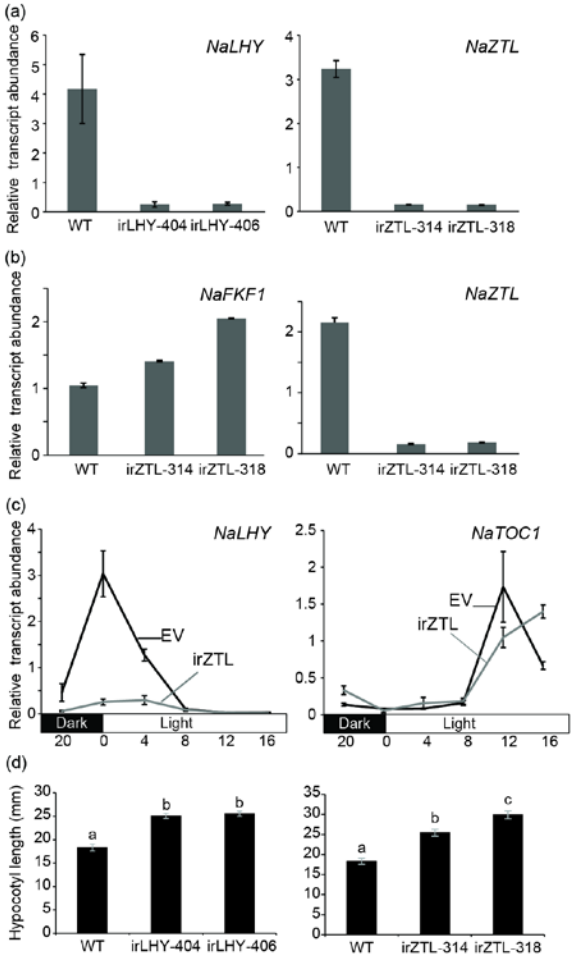

Fig. S1 Silencing efficiency and hypocotyl length of irLHY and irZTL lines. (a) Mean (± SE) levels of transcript accumulation of *NaLHY* and *NaZTL* in irLHY and irZTL lines, respectively. Plants were grown under 16 h : 8 h, light : dark conditions, and leaf samples were collected at ZT0 for irLHY, at ZT12 for irZTL lines. (b) Mean (± SE) levels of transcript accumulation of *NaFKF1* in irZTL lines collected at ZT12. (c) Mean (± SE) levels of transcript accumulation of *NaLHY* and *NaTOC1* in irZTL-314. Plants were grown under 16 h : 8 h, light : dark conditions, and leaf samples were harvested every 4 h for 1 d. (d) Mean (± SE) length of hypocotyl in EV, irLHY-406, and irZTL-314 seedlings grown under the dim light conditions. Different letters indicate significant differences in hypocotyl length among the lines (P <0.05, one-way ANOVA with Bonferroni *post hoc* test).

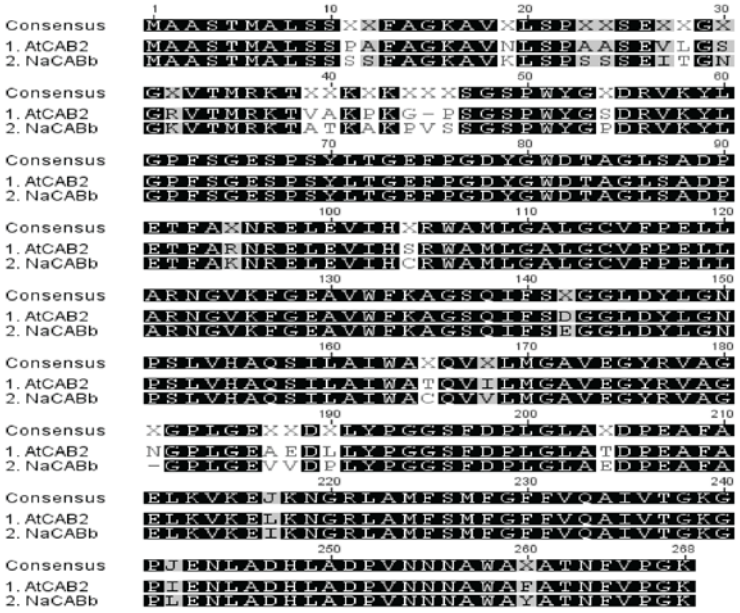

Fig. S2 Protein alignment of CAB2 orthologs in *Nicotiana attenuata* and *Arabidopsis thaliana*. Full-length amino acid sequences were aligned using the Geneious software V5.7.7 (www.geneious.com). TAIR accession number of *A. thaliana* CAB2 (AtCAB2) is At1g29920. CAB2, CHLOROPHYLL A/B BINDING PROTEINS 2.

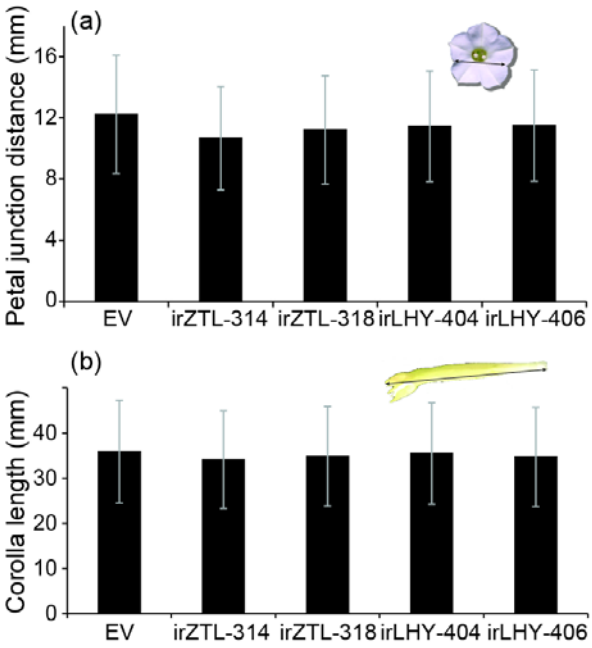

Fig. S3 Flower morphology of the clock-silenced lines. (a) Mean (± SE) distance between petal junctions on corolla limbs of EV, irLHY-406, irLHY-404, irZTL-314, and irZTL-318 flowers. (b) Mean (± SE) corolla length of EV, irLHY-406, irLHY-404, irZTL-314, and irZTL-318 flowers.

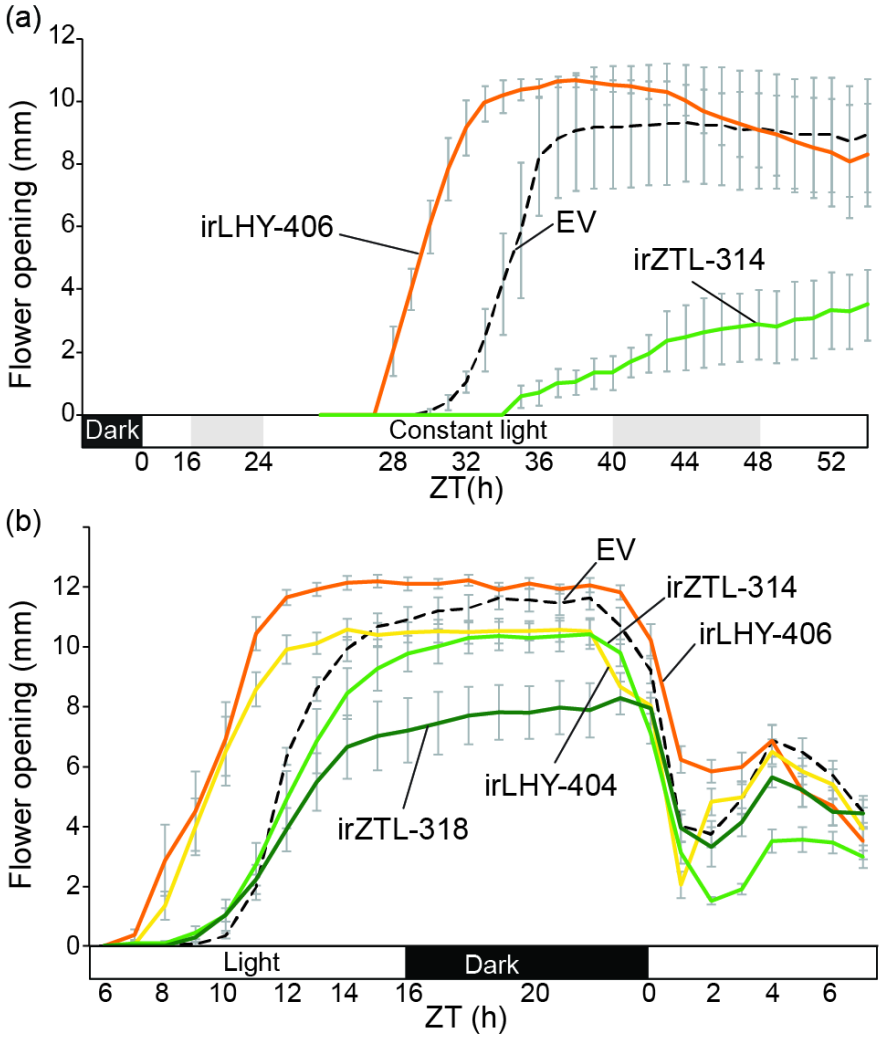

Fig. S4 Flower opening in the clock-silenced lines under LL and LD conditions. (a) Flower opening and closing of *N. attenuata* clock-silenced lines under LL conditions. Mean (± SE) distance between petal junctions on corolla limbs of EV, irLHY-406, and irZTL-314. We exposed LD-grown flowering plants to LL conditions for 24 h and then measured flower opening/closing. A gray box indicates the subjective dark period under LL conditions. (b) Mean (± SE) distance between petal junctions on corolla limbs of EV, irLHY-406, irLHY-404, irZTL-314, and irZTL-318 plants grown under LD conditions. LD, 16 h : 8 h, light : dark; LL, constant light. ZT, zeitgeber time.

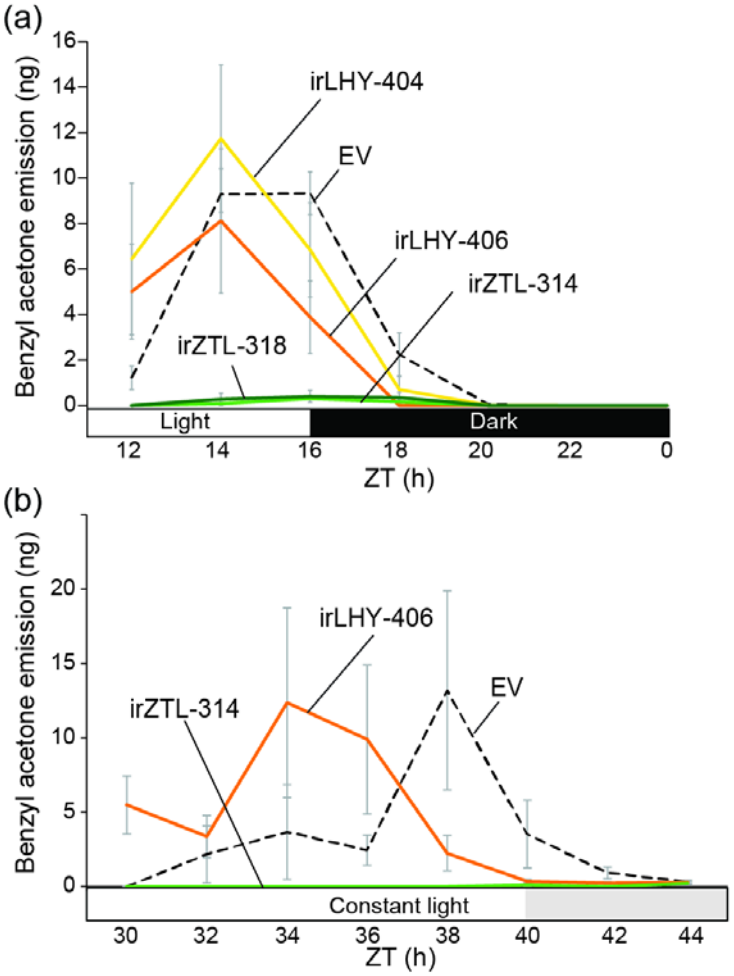

Fig. S5 Benzyl acetone emission in the clock-silenced flowers. (a) Mean (± SE) levels of BA emission of flowers in EV, irLHY-406, irLHY-404, irZTL-314 and irZTL-318 under LD conditions, BA emission measured using a z-Nose™ instrument for real time measurements. (b) Mean (± SE) levels of BA emission of flowers in EV, irLHY-406, and irZTL-314 under LL conditions. We exposed LD-grown flowering plants to LL condition for 24 h and measured BA emission using a PDMS trapping and TDU-GC-MS instrument. LD, 16 h : 8 h, light : dark; LL, constant light. ZT, zeitgeber time.

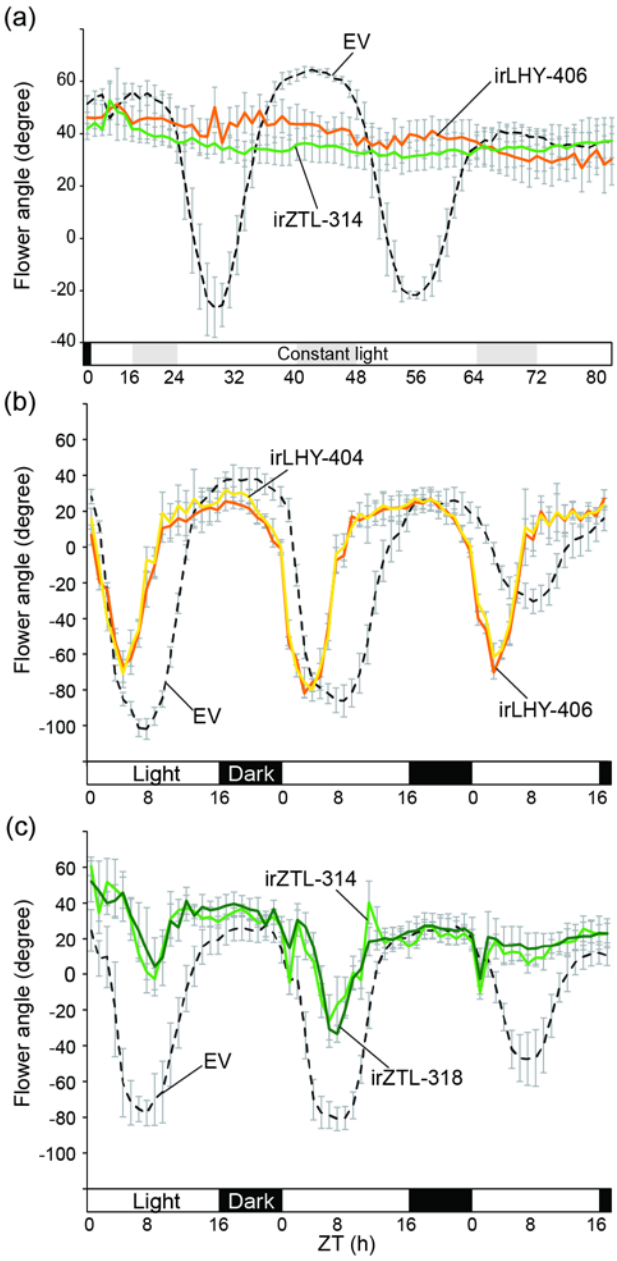

Fig. S6 Vertical movement in the clock-silenced flowers under LL and LD conditions. (a) Mean (± SE) angle of flowers in EV, irLHY-406, and irZTL-314 under LL conditions. We exposed LD-grown flowering plants to LL condition for 24 h and measured flower movement. A gray box indicates the subjective dark period of LL conditions. Mean (± SE) angle of flowers in (b) *NaLHY*-silenced lines and (c) *NaZTL*-silenced lines under LD conditions. LD, 16 h : 8 h, light : dark; LL, constant light. ZT, zeitgeber time.

Table S1 Insertion fragments of irLHY and irZTL silenced lines

Target gene	NCBI accession	Inserted fragment for silencing construct
LHY	JQ424913	GGTAAAGAAGAGCCTCAAGAACCTAATGTTAACCTTCTAGCTGGAGATGCTGG GAATCGGCGTGGTAGGAATTGCATCAGTCCAAATGATTCTTGGAAAGAAGTCT CCGAAGGGGGACGGATAGCGTTCCAGGCTCTTTTCACCAGAGAGAAGTTGCCT CAAAGCTTTTCTCCTTCAAATGATCCGAAAAATAAGGGAACAATCAATCTTGA AAACGTTAAGCAAAAGCCAGACGAGAAAGGTCTAAGTGGATCGCAGTTAGAC CTTAATGATCAGGCATCCGACATCTGTTCCAGTCATCAAGCAGTGGAAGATAA TGTGTTAGTAATTGGC
ZTL	JQ424912	GCGAGGAAGAACCATGCTGGAGATGTGTTACAGGAAGTGGAATGCCTGGTGCC GGAAATCCTGGAGGTGTTGCTCCTCCACCAAGGCTTGATCACGTGGCAGTAAG TCTCCCTGGTGGCAGAATTCTGGTCTTTGGTGGGTCCGTTGCTGGTCTCCACTC AGCATCTCAGCTCTACATTTTGGATCCAACAGAAGAGAAGCCTACATGGAGGA TATTGAATGTACCTGGTCGGCCTCCAAGATTTGCTTGGGGACATAGCACATGTA TTGTTGGAGGAACTAGAGCAATAGTCCTCGGAGGTCAAACTGGTG

Specific fragments used for the inverted repeated constructs to silence NaLHY and NaZTL. NCBI accession numbers of each gene sequence.