Supplementary Text

Detailed information for Materials and Methods

mRNA Expression analysis

For synthesis of cDNA, 2 µg of total RNA was primed using dT_{15} primer and cDNA synthesis was performed using the Superscript II reverse transcriptase enzyme from Invitrogen. cDNA was diluted to 150 µl with water, and 3 µl of diluted cDNA was used for PCR-amplification of *CO*, *FT*, *COP1* and *UBQ10* fragments using gene-specific primers. The *UBQ10* fragment was used as a control to normalize for the amount of cDNA used. *CO*, *FT* and *UBQ10* primers were described previously (An et al., 2004; Blazquez and Weigel, 1999; Nakagawa and Komeda, 2004; Suarez-Lopez et al., 2001). A *COP1* fragment was amplified using 5'-CAACCACTTCATGTCTTCAGTG-3' and 5'-GACTATTCGCAGTCAACATCGTG-3'. For all cDNAs, the exponential range of amplification was determined experimentally, then, appropriate numbers of cycles for *CO*, *FT*, *COP1*, and *UBQ10* amplification were used in the experiments. These were typically 25 cycles for *CO*, 27 cycles for *FT* and 18 cycles for *UBQ10*. PCR products were separated on agarose gels, transferred to nylon filters and hybridized with radioactively labelled probes. Images were visualized using a Phosporimager (Molecular Dynamics), band intensities were quantified using ImageQuant software and values were normalized to *UBQ10*.

In vitro binding assay

All proteins were synthesized using the TnT reticulocyte coupled transcription and translation system (Promega) in the presence of ^{35}S-labelled methionine. Protein synthesis and co-immunoprecipitations were conducted as previously described (Hoecker and Quail, 2001). Briefly, 10 µl each of bait and prey TnT reactions were added to 200 µl of binding buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM dithiotreitol (DTT), 0.1% Tween 20) and incubated on a rotary platform for 2 hours at 4°C. Co-immunoprecipitation was conducted by subsequently adding 0.4 µg of α-GAD antibody (Santa Cruz Biotechnology) and 8 µl of protein A-coated magnetic beads (Dynal, Oslo, Norway). Precipitates were washed five times with 1 ml of binding buffer (without DTT). Pellet and supernatant fractions were resolved by SDS-

PAGE and visualized using a phosphorimager (Molecular Dynamics).

FRET analysis

Laser-scanning confocal microscopy was performed using a Leica TCS SP2 system (Leica Microsystems, Heidelberg, Germany). YFP was excited with the 514 nm line and CFP was excited with the 405 nm line of a diode laser of an argon laser 20%. Images were taken with an objective HC PL APO CS 20.0x0.70 UV. Fluorescence was detected in case of YFP between 525-590 nm and in case of CFP between 454-503 nm.

For COP1-CO co-localization studies and FRET acceptor photobleaching, Arabidopsis leaf epidermal cells of 3-week-old, LD-grown plants were co-transfected ballistically with two plasmid constructs, respectively, encoding CFP-COP1, YFP-CO, CFP or YFP, and analyzed 24 hours after bombardment. Cells exhibiting co-expression of both fluorescent proteins were bleached in the acceptor YFP channel by scanning an ROI (region of interest) with 100% laser intensity. FRET efficiency was calculated directly by the TCS software using the following formula: $FRET_{Eff}=(D_{post}-D_{pre})/D_{post}$ for all $D_{post>}D_{pre.}$

Supplementary Figure 1. Legend.

Analysis of *CO* and *FT* mRNA levels under LDs.

A. *CO* mRNA levels in Columbia wild-type and *cop1-4* mutants.
B. FT mRNA levels in Columbia wild-type and *cop1-4* mutants.
In panels A and B the graphs show levels of *CO* or *FT* mRNA relative to levels of *UBQ10* mRNA**.** The gels in which each mRNA was detected are shown below each graph. Each cDNA was amplified by RT-PCR, separated on an agarose gel and detected with an appropriate radioactively labeled probe (Material and Methods). The photoperiod used is shown above each graph; white represents the photoperiod, black represents the night.
C. *CO* and *FT* mRNA levels in Columbia wild-type and transgenic Columbia plants carrying *pSUC2:COP1*. Both graphs show *CO* (dark line) and *FT* (light line) mRNA levels. The gels are shown below each graph (as for A and B) and the photoperiod is represented above each graph (as for A and B).

Supplementary Figure 2. Legend.

Comparison of *CO* mRNA and protein in the same SD-grown *cop1-4* plants. The graph illustrates *CO* mRNA level with a characteristic peak during the night in dark-grown plants. The vertical axis denotes *CO* mRNA levels relative to those of *UBQ10*, while the horizontal axis denotes hours after dawn. The plants were exposed to light for the first 8 hours and to dark for the following 16 hours as denoted by the shading. The gels below illustrate mRNA levels (top two gels) and protein levels (bottom two gels). mRNA and protein samples were extracted from the same samples at each of the times shown on the horizontal axis of the graph. The protein accumulates to highest levels after the peak in mRNA and persists at high levels in the dark for at least 4 hours after the mRNA declines. This demonstrates that the instability of CO protein that was previously described during the dark is severely reduced in *cop1-4* mutants.

Supplementary Figure 3. Legend.

Analysis of *phyB-9 cop1-6* plants.

A. Flowering times of *phyB-9 cop1-6* plants grown under LD or SD compared to control genotypes. Left hand panel, representative plants grown under LD. Right hand panel, representative plants grown under SD. The genotype of each plant is indicated in yellow. The flowering time of each genotype is indicated below each plant. The flowering times are represented as mean total leaf number plus or minus the standard error. In each case a population of at least 8 plants were grown.
B. Analysis of *CO* and *FT* mRNA in *cop1-6* and *cop1-6 phyB-9* plants grown under SDs and harvested 6 hours after dawn. No difference in *CO* mRNA is detected between the genotypes, although CO protein is much less abundant in *cop1-6* (see panel C). UBQ10 acts as a loading control.
C. CO protein abundance in *cop1-6* and *cop1-6 phyB-9* plants grown under SDs and harvested 6 or 16 hours after dawn. This is the same panel shown in Figure 5D, but is illustrated here for comparison with the RNA samples harvested at 6 hours after dawn. The comparison between panels B and C demonstrates that the reduction in CO

protein level in *cop1-6 phyB-9* 6 hours after dawn is not due to a reduction in *CO* mRNA.

References

An, H., Roussot, C., Suarez-Lopez, P., Corbesier, L., Vincent, C., Pineiro, M., Hepworth, S., Mouradov, A., Justin, S., Turnbull, C. and Coupland, G. (2004) CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of Arabidopsis. *Development*, **131**, 3615-3626.

Blazquez, M.A. and Weigel, D. (1999) Independent regulation of flowering by phytochrome B and gibberellins in Arabidopsis. *Plant Physiology*, **120**, 1025-1032.

Hoecker, U. and Quail, P.H. (2001) The phytochrome A-specific signaling intermediate SPA1 interacts directly with COP1, a constitutive repressor of light signaling in Arabidopsis. *Journal of Biological Chemistry*, **276**, 38173-38178.

Nakagawa, M. and Komeda, Y. (2004) Flowering of Arabidopsis cop1 mutants in darkness. *Plant and Cell Physiology*, **45**, 398-406.

Suarez-Lopez, P., Wheatley, K., Robson, F., Onouchi, H., Valverde, F. and Coupland, G. (2001) CONSTANS mediates between the circadian clock and the control of flowering in Arabidopsis. *Nature*, **410**, 1116-1120.

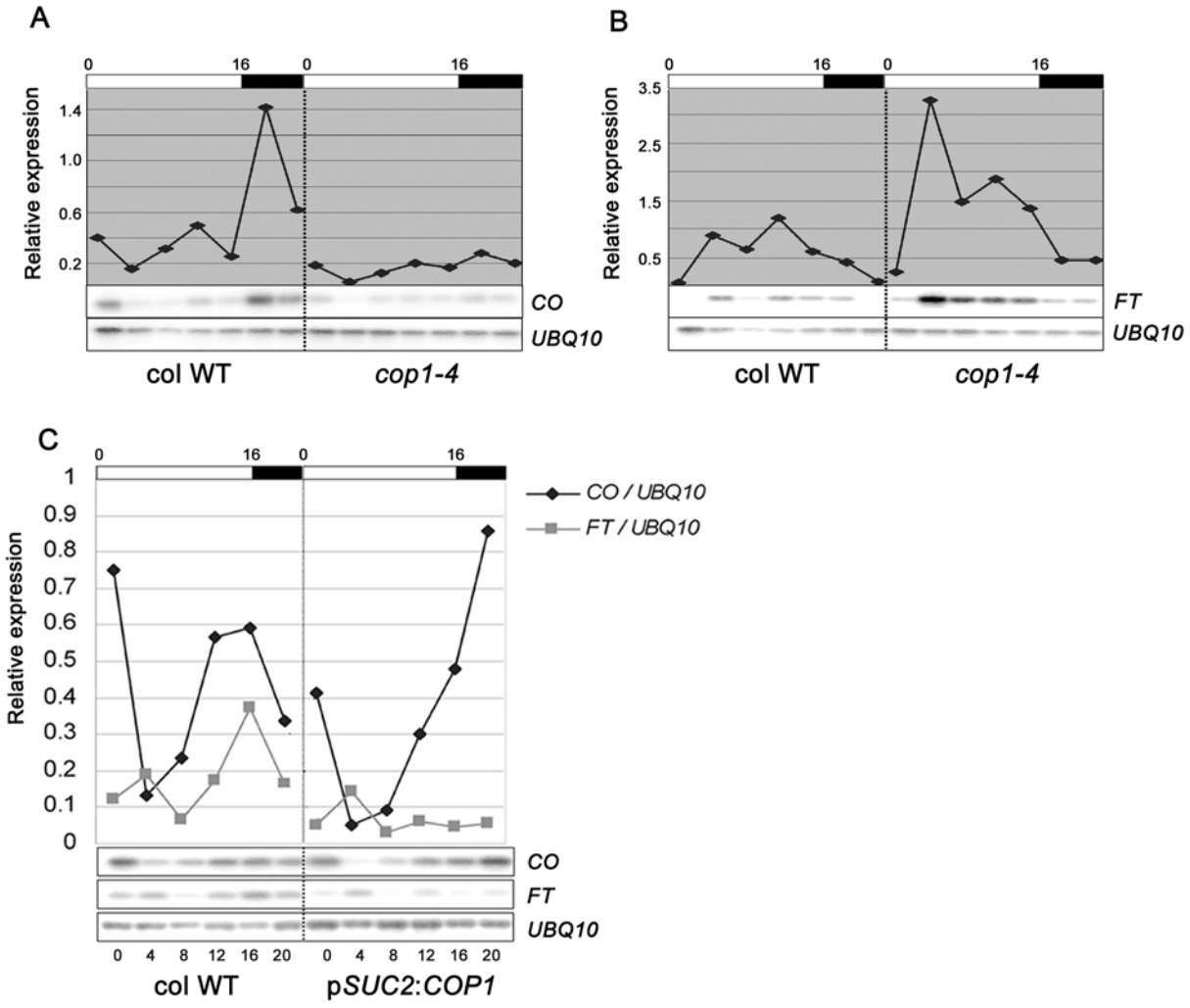
Supplementary Information

Table 1. Flowering times of transgenic plants and mutants under long or short days.

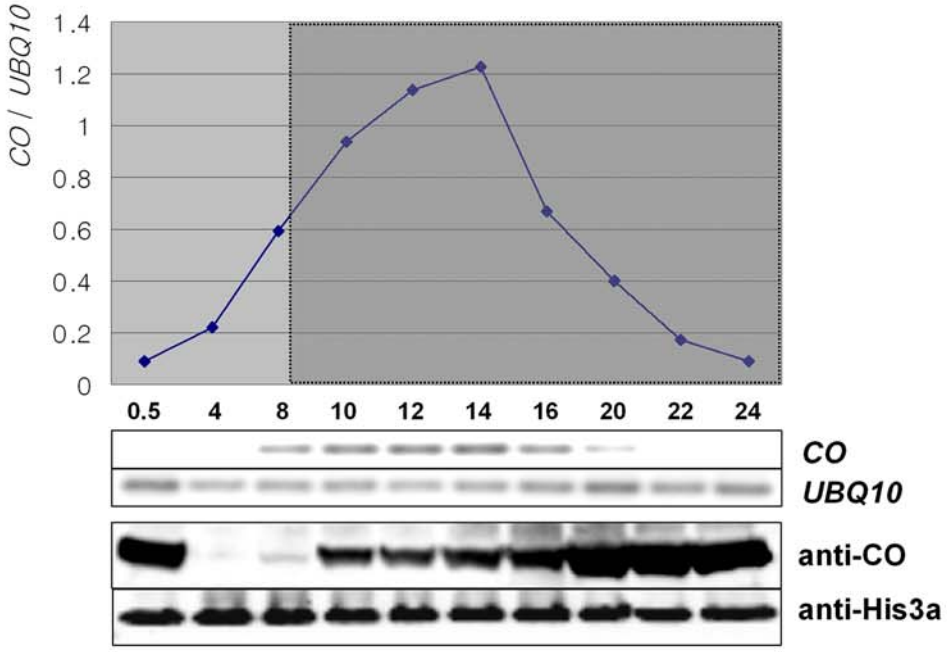
Genotype	LD (16h L)	SD (8h L)
Col WT (n= 10)	14.5 ± 0.6	60.3 ± 3.2
p*SUC2*:*CO* (n=10)	6.8 ± 0.1	7.4 ± 0.2
cop1-4 (n= 10)	9.7 ± 0.2	12.6 ± 0.3
p*SUC2*:*COP1* in *cop1-4*		
#1 (T3, n=12)	10.8 ± 0.5	25.3 ± 3.3
#2 (T3, n=15)	12.8 ± 0.5	32.2 ± 3.9
p*SUC2*:*CO* p*SUC2*:HA:*COP1*		
#1 (T3, n=8)	7.8 ± 0.2	14.8 ± 1.8
#2 (T3, n=9)	9.5 ± 0.3	22.1 ± 3.3
#3 (T3, n=9)	9.2 ± 0.3	19.8 ± 3.1
p*SUC2*:*COP1* in col WT		
#1 (T3, n=15)	20.3 ± 0.7	59.1 ± 5.5
#2 (T3, n=18)	17.1 ± 0.5	58.8 ± 5.2

Total leaf numbers are presented as the average for at least 8 homozygous plants ± SD. The specific number of plants used in each case is indicated (n). T3 denotes the generation used in the experiment (T1 is the original transformed plant).

Supplementary Figure 1

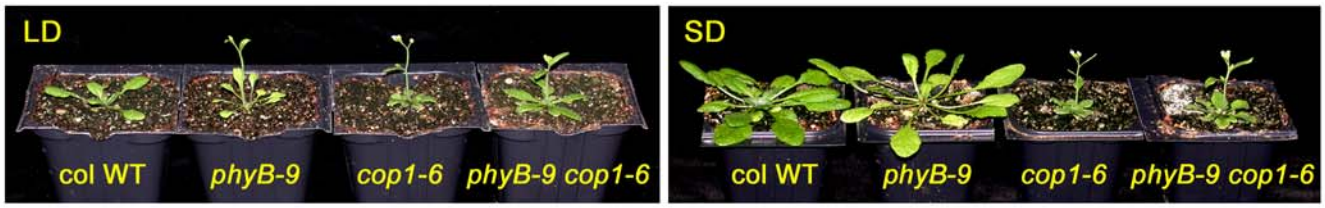


Supplementary Figure 2



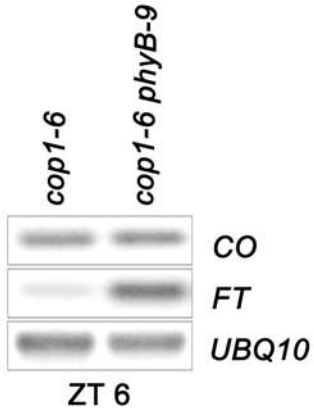
Supplementary figure 3

A



11.0 ± 0.8 8.6 ± 0.4 7.5 ± 0.2 10.6 ± 0.6 58.5 ± 4.5 47.2 ± 2.8 12.1 ± 0.3 13.0 ± 0.8

B



C