SUPPLEMENTARY ONLINE MATERIAL
for

Testing Time: Can Ethanol-Induced Pulses of Proposed Oscillator Components Phase Shift Rhythms In *Arabidopsis*?

by

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Figures S1-S6

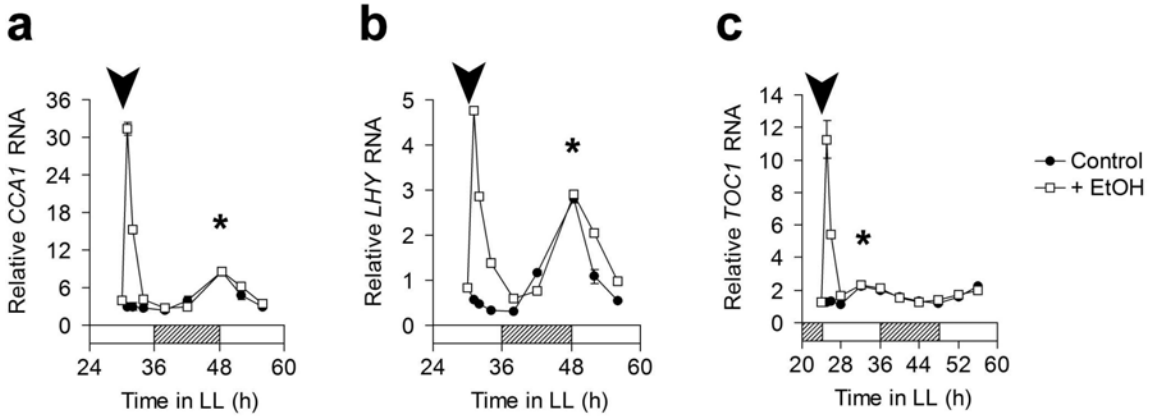

Figure S1. EtOH-induced RNA pulses of *CCA1*, *LHY* and *TOC1* in plants under LL. *Alc::CCA1* (a), *Alc::LHY* (b) and *Alc::TOC1* seedlings (c) were treated with EtOH at a point in the cycle when their expression is normally low (indicated by the arrowheads). Treatment times were at 30 h (CT 6) (a, b) and 24 h in LL (CT 0) (c). RNA abundance of the induced gene was measured by quantitative RT-PCR and normalized to *RH8*. Circadian peaks are marked by an asterisk for comparison to EtOH-induced peak levels. Closed circles, untreated plants; open squares, EtOH-treated. Error bars denote ± s.d. (n=3). White and hatched bars denote subjective day and night, respectively.

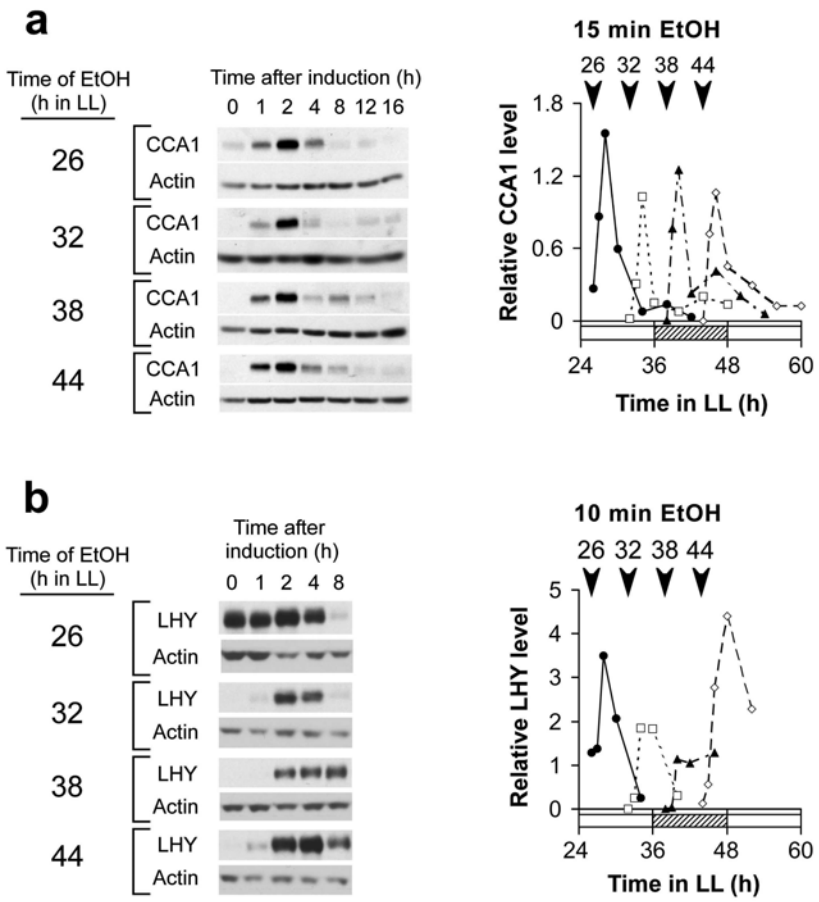

Figure S2. CCA1 and LHY protein expression can be induced by EtOH at any time in the circadian cycle. Seedlings were given an EtOH pulse at various times (26 h, 32 h, 38 h, and 44 h in LL). (a) Pulsed CCA1 expression in *Alc::CCA1* seedlings. Seedlings were exposed to 10% (v/v) EtOH for 15 min. (b) Pulsed LHY expression in *Alc::LHY* seedlings. Seedlings were exposed to 3% (v/v) EtOH for 10 min. Left, Western blots showing the transient accumulation of protein after induction. Actin was used as a loading control. Right, quantitation of relative protein levels from the blots shown. Arrowheads denote times of EtOH treatment for their respective plot. Induction times in LL: circles, 26 h; squares, 32 h; triangles, 38 h; diamonds, 44 h.

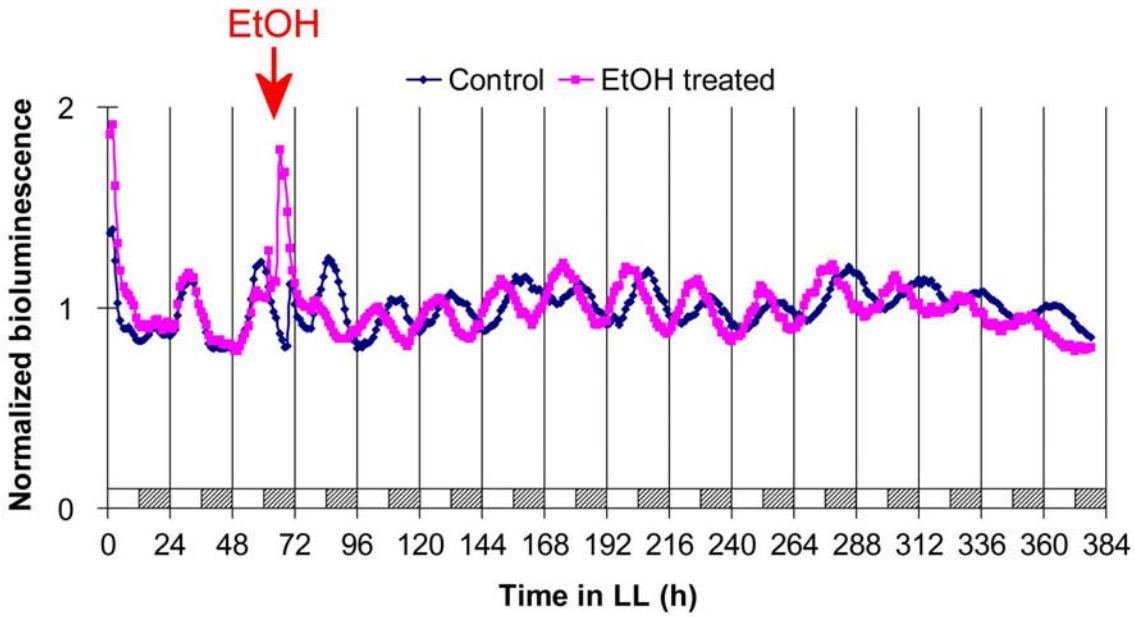

Figure S3. An LHY pulse during the subjective night stably shifts the phase of rhythms. *Alc::LHY* seedlings in the *CAB2::LUC* background were pulsed with EtOH at 65 h in LL (CT 13; red arrow). Plots represent the mean bioluminescence from 20 seedlings. cpm, counts per minute.

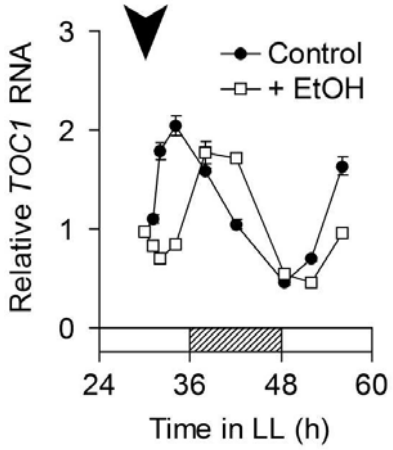

Figure S4. A pulse of *LHY* at CT 6 delays the peak of *TOC1* RNA in LL. Eight day-old *Alc::LHY* seedlings were given an EtOH pulse at 30 h in LL (CT 6, black arrowhead) and tissue was harvested over time for RNA extraction. Control plants were untreated. *TOC1* RNA abundance was measured by quantitative RT-PCR and normalized to *RNA HELICASE* 8 (*RH8*). Error bars denote ± s.d. (n=3). Subjective day and subjective night are denoted by white and hatched bars, respectively.

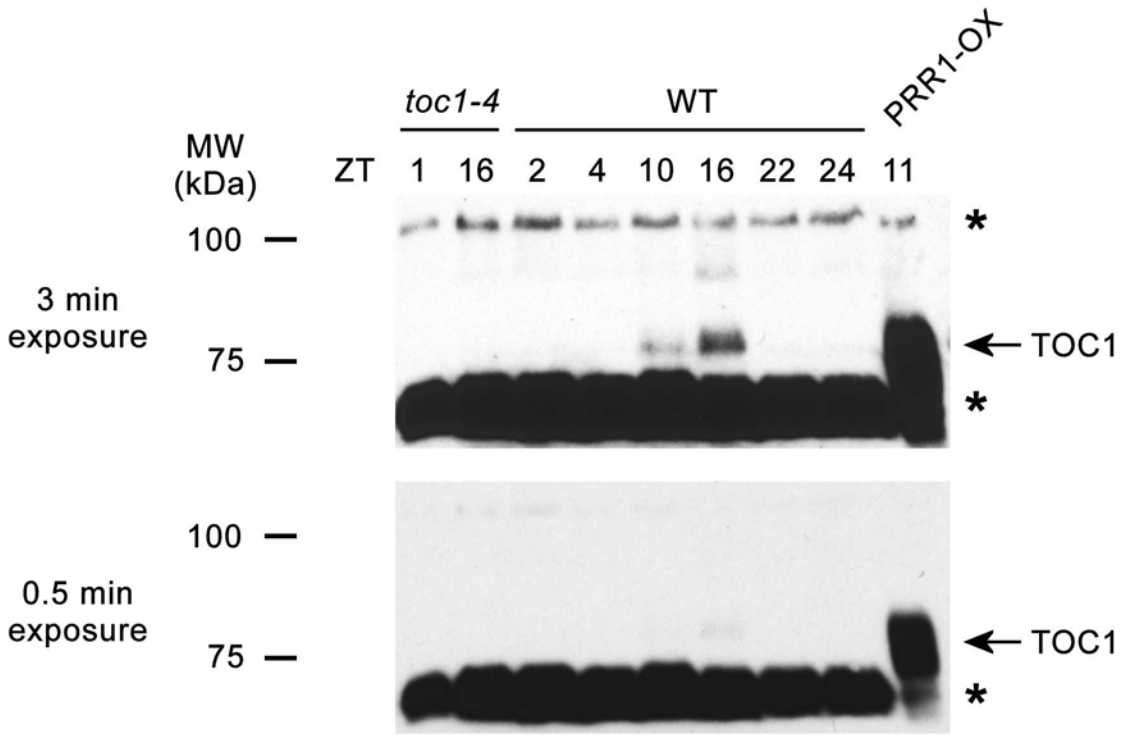

Figure S5. Specificity of anti-TOC1 antibodies. Protein extracts were prepared from two week-old WT, *toc1-4* and PRR1-OX (a TOC1-overexpressing line) seedlings that were harvested at different times in 12:12 LD. TOC1 protein was detected by Western blot using anti-TOC1 antibodies. Equal amounts of protein were loaded, with the exception of PRR1-OX, which was diluted 10-fold. Two exposures of a single Western blot are shown. The arrow indicates the TOC1 band and the asterisks indicate cross-reacting bands. Times of harvest are expressed as zeitgeber time (ZT, time (h) after lights-on).

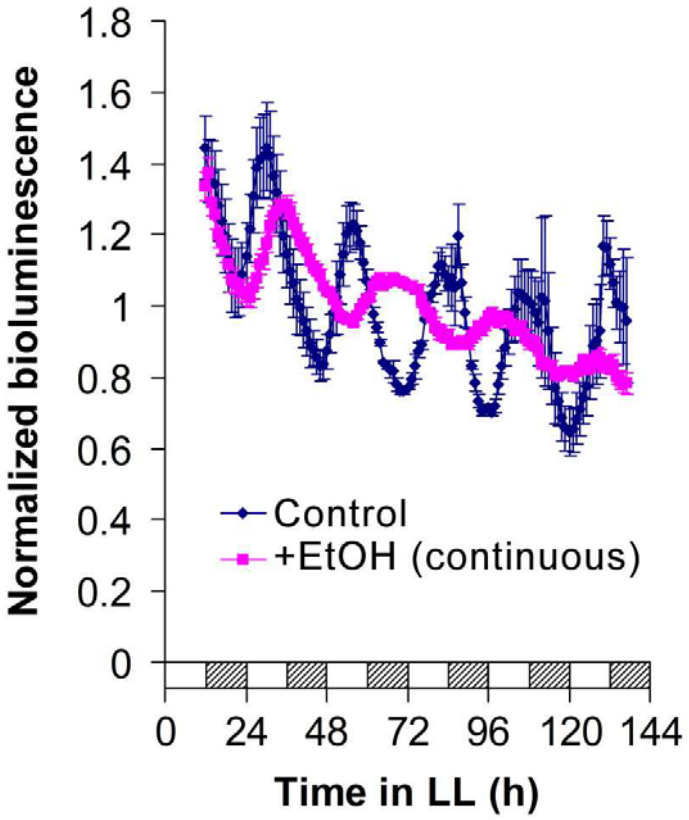

Figure S6. Continuous induction of *TOC1* in *Alc::TOC1* seedlings lengthens the period of circadian rhythms. Six day-old *Alc::TOC1* seedlings in the *CAB2::LUC* background were transferred to tissue culture plates containing either 0 (control) or 0.01% (v/v) EtOH for the experiment. Plots represent the normalized bioluminescence from groups of 20 seedlings. For each experiment the average luminescence was arbitrarily set to 1. Period length estimates (Lomb-Scargle method) were 25.10 ± 0.11 h for control seedlings and 31.18 ± 0.47 h for induced seedlings. Period length estimates and error bars are ± s.e.m. (control, *n*=3; 0.01% EtOH, *n*=12).