

SUPPLEMENTARY INFORMATION

Figure S1

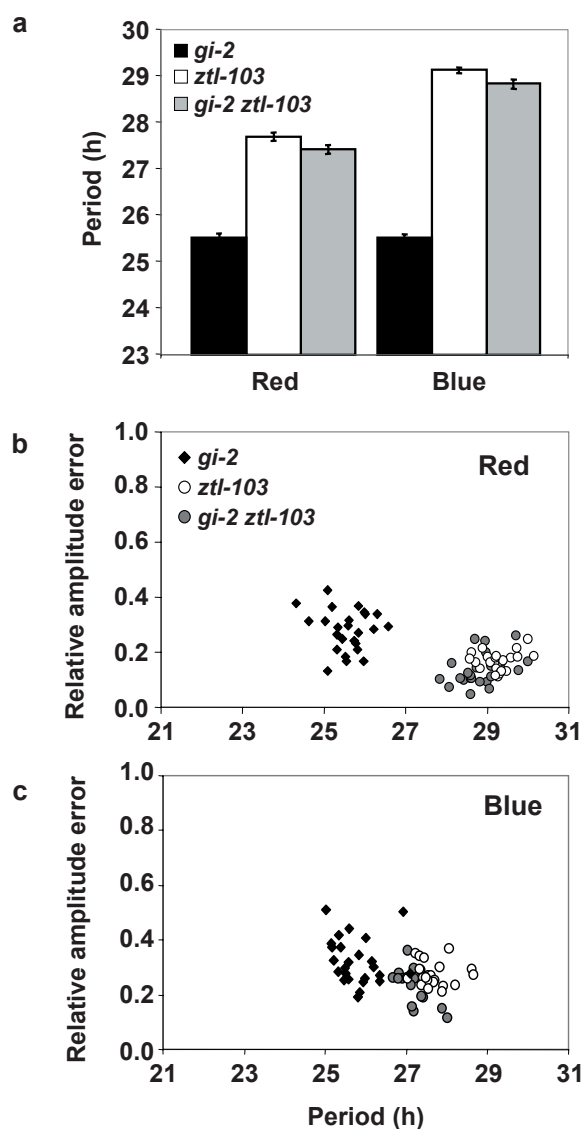


Figure S1: ZTL is epistatic to GI. (a) Free-running circadian period in the *gi-2*, *ztl-103* and *gi-2 ztl-103* backgrounds in constant red light (Red) blue light (Blue) (b). Relative amplitude error as a function of period in red and (c) blue light. Period and relative amplitude error estimates as described (Methods).

Figure S2

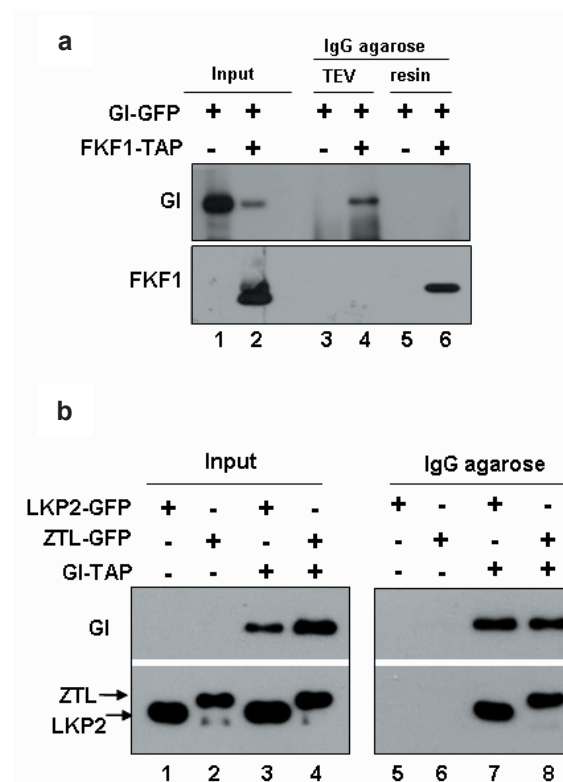


Figure S2: In planta interactions between GI, FKF1 and LKP2. (a) Total protein extracts from *N. benthamiana* infiltrated leaves constitutively expressing GI-GFP alone (lane 1) or co-expressed with FKF1-TAP (lane 2) show *in vivo* interaction (compare lanes 3, 4; upper panel) following incubation with IgG agarose and TEV protease. Cleavage with TEV removes TAP tag; FKF1 is not detectable in lane 4. Post-digestion resin (lanes 5,6) shows residual FKF1-TAP bound (lane 6, lower panel) demonstrating FKF1-TAP binding; GI not detectable under these conditions (lane 6, upper panel). (b) Total protein extracts from *N. benthamiana* leaves constitutively expressing LKP2-GFP (lanes 1,3) or ZTL-GFP (lanes 2,4) show *in vivo* interaction with GI-TAP (lanes 3,4) following incubation with IgG agarose and 3C protease (lanes 7,8). Extracts probed with α -His6 antisera (lanes 5-8; upper panel), α -GFP antisera (lanes 5-8; lower panel) or α -PAP antisera (lanes 1-4).

Figure S3

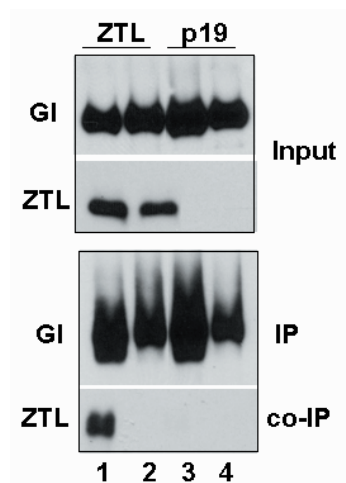


Figure S3: The GI/ZTL interaction occurs in vivo. Immunoprecipitation by α -GFP antibody was performed using four different *N. benthamiana* protein extracts. GI-GFP and ZTL were transiently co-expressed in the same leaves (lane 1), or individually expressed, ground separately and mixed (1:1) (lane 2). Tissue was harvested at ZT4-6 three days after infiltration and immune complexes probed for GI-GFP (IP) and ZTL (co-IP). Lanes 3 and 4 show GI-GFP co-infiltrated with *Agrobacteria* expressing p19 alone, or individually expressed, ground separately and mixed (1:1) and immunoprecipitated, respectively. Representative of three trials are shown.

Figure S4

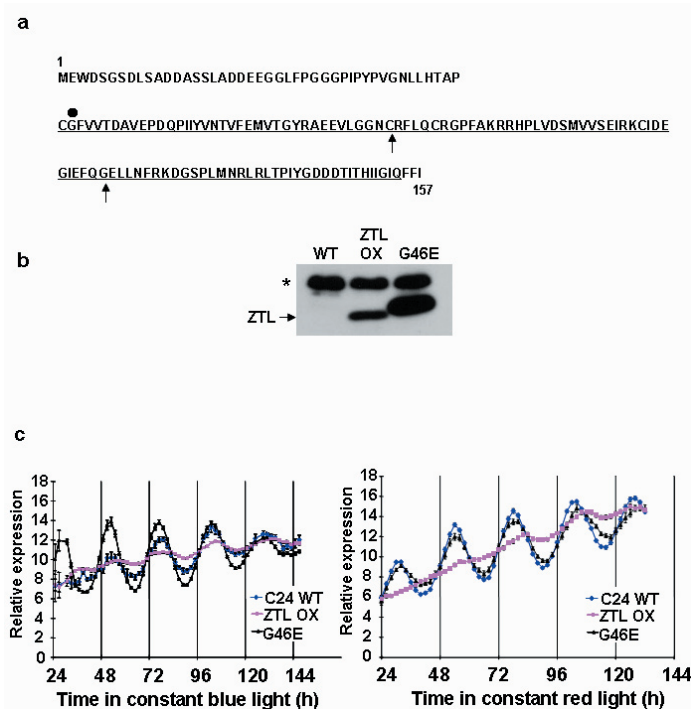


Figure S4: The G46E mutation rescues the ZTL OX phenotype. (a) First 157 residues of ZTL. LOV domain underlined. (*) indicates position of glycine 46; arrows indicate the highly conserved cysteine 82 and glycine 119 (site of *ztl-21* mutation). (b) Immunoblot showing ZTL abundance in WT, 35S::ZTL(ZTL OX) and 35S::*ztl*^{G46E}(G46E) plants. WT levels of ZTL not detectable under these

conditions. Note slower migration of *ztl*^{G46E} protein. Non-specific bands present at (*). (c) Free-running rhythms of *cab2::luciferase* expression in C24 WT, ZTL OX and G46E plants under constant red or blue light. Data normalized to mean (x10) within each genotype. n = 4-29.

Figure S5

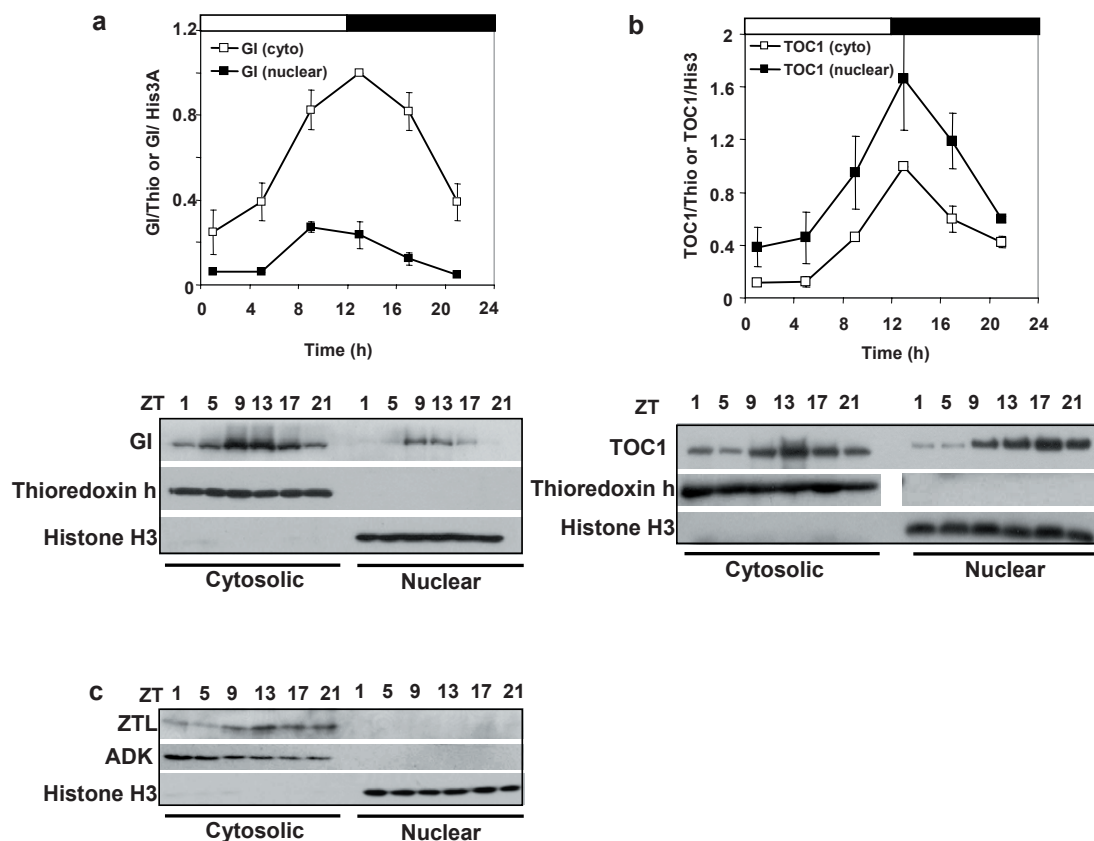


Figure S5: Cell fractionation localizes GI, TOC1 and ZTL. (a) *GI::GI-HA*, (b) *TOC1-YFP* and (c) WT plants grown under 12 h light / 12 h dark were harvested at times indicated. Cytosolic and nuclear protein extracts from them were probed with (a) α -HA, (b) α -GFP and (c) α -ZTL antiserum. α -Thioredoxin h (a and b) or α -ADK (c), and α -histone H3 (a-c) blots were used as controls for cytoplasmic and nuclear protein abundances, respectively. Abundances of (a)

GI-HA and (b) *TOC1-YFP* in nuclei relative to those in cytosol were normalized to cytosolic levels at ZT13 and shown as means of three trials $\pm$ s.e.m. Periods of light and dark entrainment indicated by white and black bars, respectively, above each figure. (c) ZTL was not detected in nuclear fraction. Representative blots are shown in which cytoplasmic and nuclear extracts were loaded with ratio of 1:6 (*GI-HA*) and 1:2 (*TOC1-YFP* and ZTL).

Figure S6

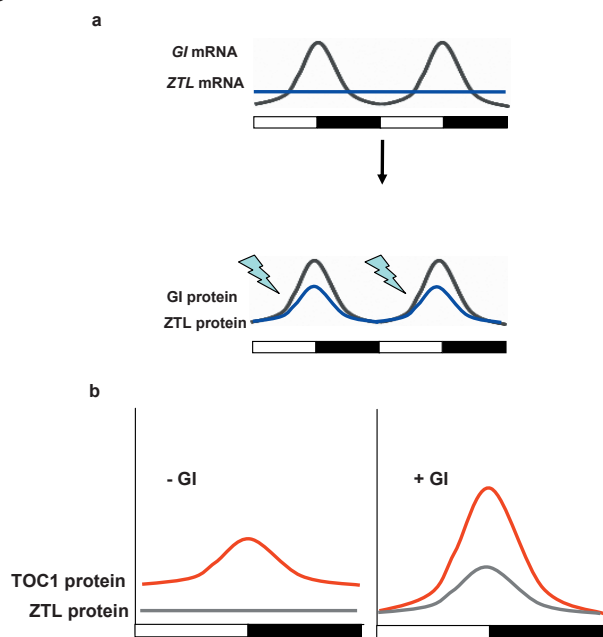


Figure S6: Post-translational control of circadian cycling through cooperative stabilization of ZTL and GI. (a) mRNA levels of GI (blue line) are rhythmic under LD in contrast to constitutive expression of ZTLmRNA (grey). Clock-controlled *GI* mRNA rhythms results in GI protein, which interacts with ZTL. Cooperative stabilization increases levels of ZTL and GI proteins, with enhancement of the interaction in the light by blue light photoactivation of ZTL (bolt), and a diminished interaction in the dark. This results in robust oscillations of both proteins. (b) One consequence of ZTL oscillations is to refine or “sculpt” the TOC1 protein profile (red line). In the absence of GI (-GI) ZTL levels are very low and non-cyclic. TOC1 protein oscillates with low amplitude, tracking its own message rhythms. In the WT (GI+), ZTL protein rhythms lead to high amplitude TOC1 protein cycles, resulting in a normal circadian period and phasing of clock-controlled outputs.