PNAS
www.pnas.org

Supplementary Information for

Circadian Regulation of c-MYC in Mice

Zhenxing Liu[a], Christopher P. Selby[a], Yanyan Yang[a], Laura A. Lindsey-Boltz[a], Xuemei Cao[a], Khagani Eynullazada[a] and Aziz Sancar[a,1]

[a]Department of Biochemistry and Biophysics, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599-7260

[1]To whom correspondence may be addressed. Email: Aziz_Sancar@med.unc.edu.

This PDF file includes:

Supplementary Information
Figures S1 to S4

www.pnas.org/cgi/doi/10.1073/pnas.2011225117

Supplementary Information

Mice

All mice were synchronized to a standard light:dark 12 h:12 h schedule at a constant temperature of 21 ℃ to 23 ℃, with food and water ad libitum, both males and females were used interchangeably in this study. Traditionally, ZT0 (zeitgeber time) is the time of lights-on and ZT12 is the time of lights-off. Mice were handled according to the guidelines of the NIH and the University of North Carolina School of Medicine (Institutional Animal Care and Use Committee).

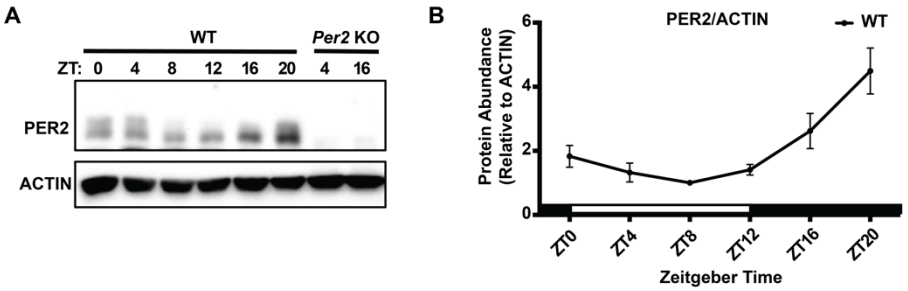

Fig. S1. PER2 is rhythmically expressed in WT spleen. (**A**)**.** Endogenous PER2 was detected by Western Blotting in spleen of wild-type (WT) mice using ACTIN as a loading control. Samples were collected at the indicated time points (ZT= Zeitgeber Time). (**B**)**.** Quantification of PER2 levels in wild-type (WT) mouse spleens at the indicated time points. For each genotype and time point, three mice were used for quantification. White and black bars indicate lights-on and lights-off, respectively. Error bars correspond to SEM (standard error of the mean). Data were normalized to a value of 1 for WT at ZT8.

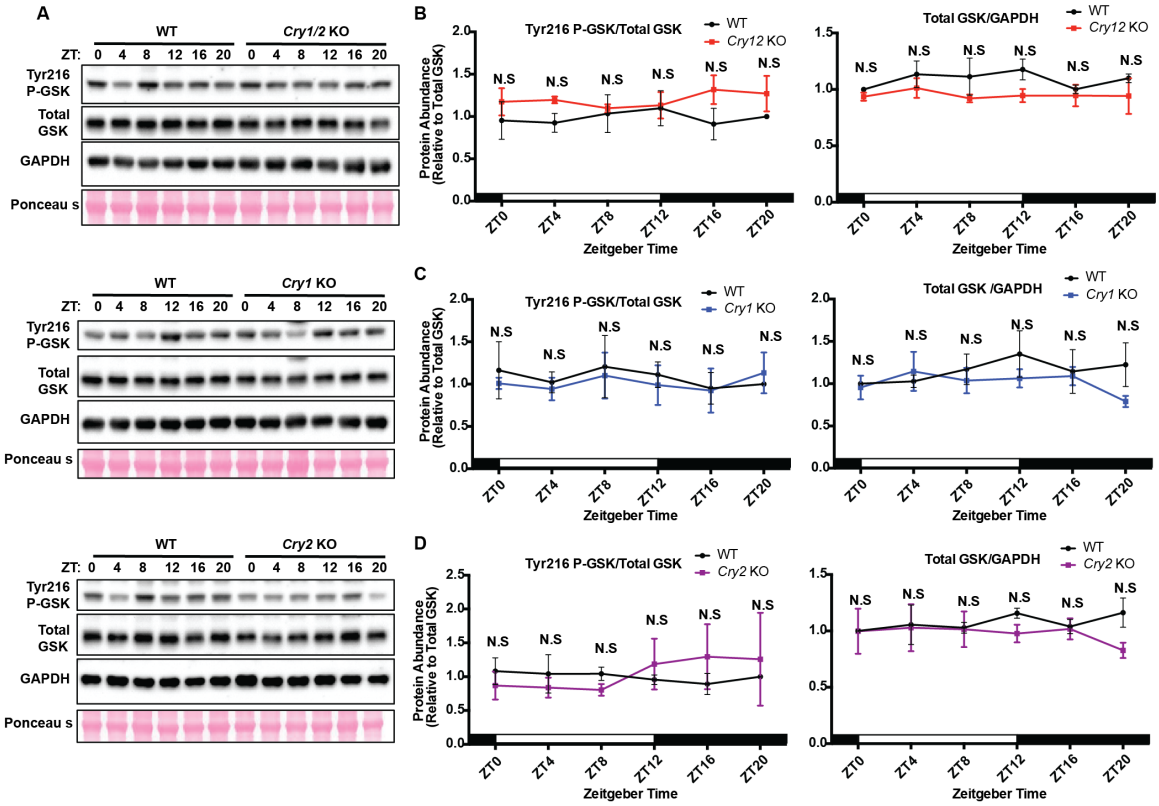

Fig. S2. Active form of GSK-3β and total GSK-3β levels are similar in wild-type (WT) and three *Cry* mutants. (A). Active form of GSK-3β (Tyr216 p-GSK) and total GSK-3β levels were detected by Western Blotting in spleens of wild-type (WT) and three *Cry* mutants using GAPDH and Ponceau S as loading controls. Samples were collected at the indicated time points (ZT= Zeitgeber Time). **(B-D).** Quantification of active form of GSK-3β and total GSK-3β levels in the indicated mouse genotypes**.** For each genotype and timepoint, four mice were used for quantification**.** White and black bars indicate lights-on and lights-off, respectively. Error bars correspond to SEM. For active form of GSK-3β: Data were normalized to a value of 1 for WT at ZT20, for total GSK-3β: Data were normalized to a value of 1 for WT at ZT0. N.S, not significant, as determined by *t* test.

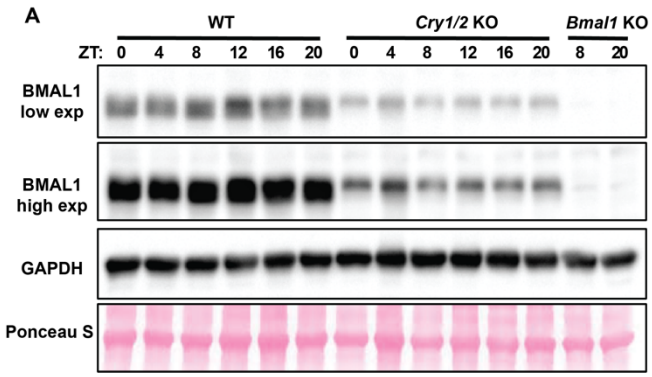

Fig. S3. Expression of phosphorylated and non-phosphorylated BMAL1 in wild-type (WT) and *Cry1/2* KO mice. (A). Endogenous BMAL1 was probed by Western Blotting of spleen from WT, *Cry1/2 KO* and *Bmal1 KO* mice using GAPDH as a loading control. Samples were collected at the indicated time points (ZT= Zeitgeber Time).

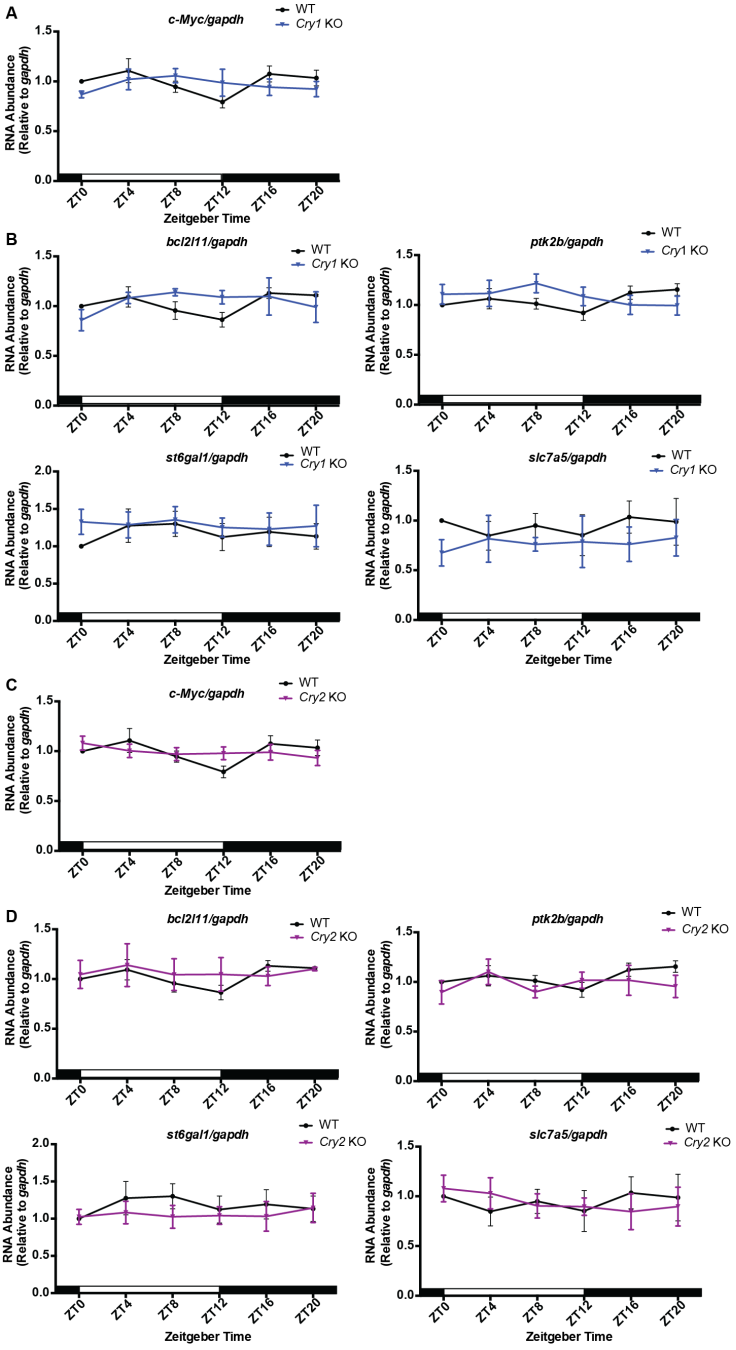

Fig. S4. *Cry1* and *Cry2* single deletions do not affect *c-Myc* transcription or c-MYC target gene mRNA levels. (A and C). *c-Myc* mRNA levels in the spleen samples of WT, *Cry1 KO* and *Cry2 KO* mice detected by reverse transcription-qPCR. Three biological repeats were used for quantification. White and black bars indicate lights-on and lights-off, respectively. Error bars correspond to SEM. Data were normalized to a value of 1 for WT at ZT0. (**B and D).** mRNA levels of c-MYC target genes in the spleens of WT, *Cry1* KO and *Cry2* KO. For each genotype and time point, at least three mice were used for quantification**.** Error bars correspond to SEM. Data were normalized to a value of 1 for WT at ZT0.