Supplementary Fig. 2. Quantitative real–time PCR (qPCR) detection of Npas2 expression in the SCN.

a. PCR strategy. Schematic of wild–type (Npas2⁺) and mutant Npas2 (Npas2⁻) alleles (adapted from reference 9). Boxes, coding exons (E1 – 3); lines, introns. The exon encoding the bHLH domain of NPAS2 was replaced with a LacZ/Neomycin (lacZ/neo) cassette⁹. The green line denotes the qPCR amplicon that spans E2 and E3.

b. Specificity of qPCR detection of Npas2 mRNA. Real–time PCR amplification curves using Npas2 (blue) or Gapdh (black) detectors on mRNA obtained from the SCN of three wild–type mice (solid lines) or three Npas2⁻/+ (dotted lines) mice. Delta Rn is the change
in fluorescence from the previous cycle. The \textit{Npas2} signal produced from \textit{Npas2}^{-/-} SCN represent less than 0.1\% of those from wild–type SCN.

c. \textit{Npas2} expression in wild type (\textit{Clock}^{+/+}) and CLOCK–deficient (\textit{Clock}^{-/-}) SCN. SCN were dissected from mice, housed in a 12–hr light: 12–hr dark cycle, at Zeitgeber Time (ZT) 6 (white bars) or ZT 14 (black bars). qPCR data are plotted relative to \textit{Gapdh} and normalized to the average wild–type signal during peak expression time. Each value is the mean ± SEM of four SCN. * indicates within–genotype expression differences between time points (p < 0.05; two–way, unpaired t–test).

d. \textit{Npas2} expression in Cortex (CTX) samples dissected from the same sections as the SCN samples shown in C.

e. \textit{mPer1} expression in the same SCN samples as in C. * indicates within–genotype expression differences between time points (p < 0.05; two–way, unpaired t–test).

f. \textit{Bmal1} expression levels in the same SCN samples as in C. * indicates within–genotype expression differences between time points (p < 0.05; two–way, unpaired t–test).

Results:

\textit{Npas2} mRNA is expressed in the SCN of wild–type mice, similar to the results of others\textsuperscript{1,2}, and in the SCN of CLOCK–deficient mice, with mid–dark levels higher that mid–light levels for both genotypes (Supplementary Fig. 2c). In contrast, there is no light–dark difference of \textit{Npas2} expression in cortex from either genotype (Supplementary Fig. 2d). qPCR analysis of \textit{mPer1} expression (Supplementary Fig. 2e) and \textit{Bmal1} expression (Supplementary Fig. 2f) from the same SCN samples verified at the molecular level that each sample is mainly SCN: antiphase mRNA oscillations occur in the wild–type SCN, which are altered in CLOCK–deficient SCN, as found previously by \textit{in situ} hybridization\textsuperscript{3}. Taken together, these data indicate that \textit{Npas2} is rhythmically expressed in the SCN of wild–type and CLOCK–deficient mice. Similar data were obtained from mice studied during the first day in constant darkness (data not shown).

References:
