Supplementary online material

Synaptotagmin10-Cre, a driver to disrupt clock genes in the SCN

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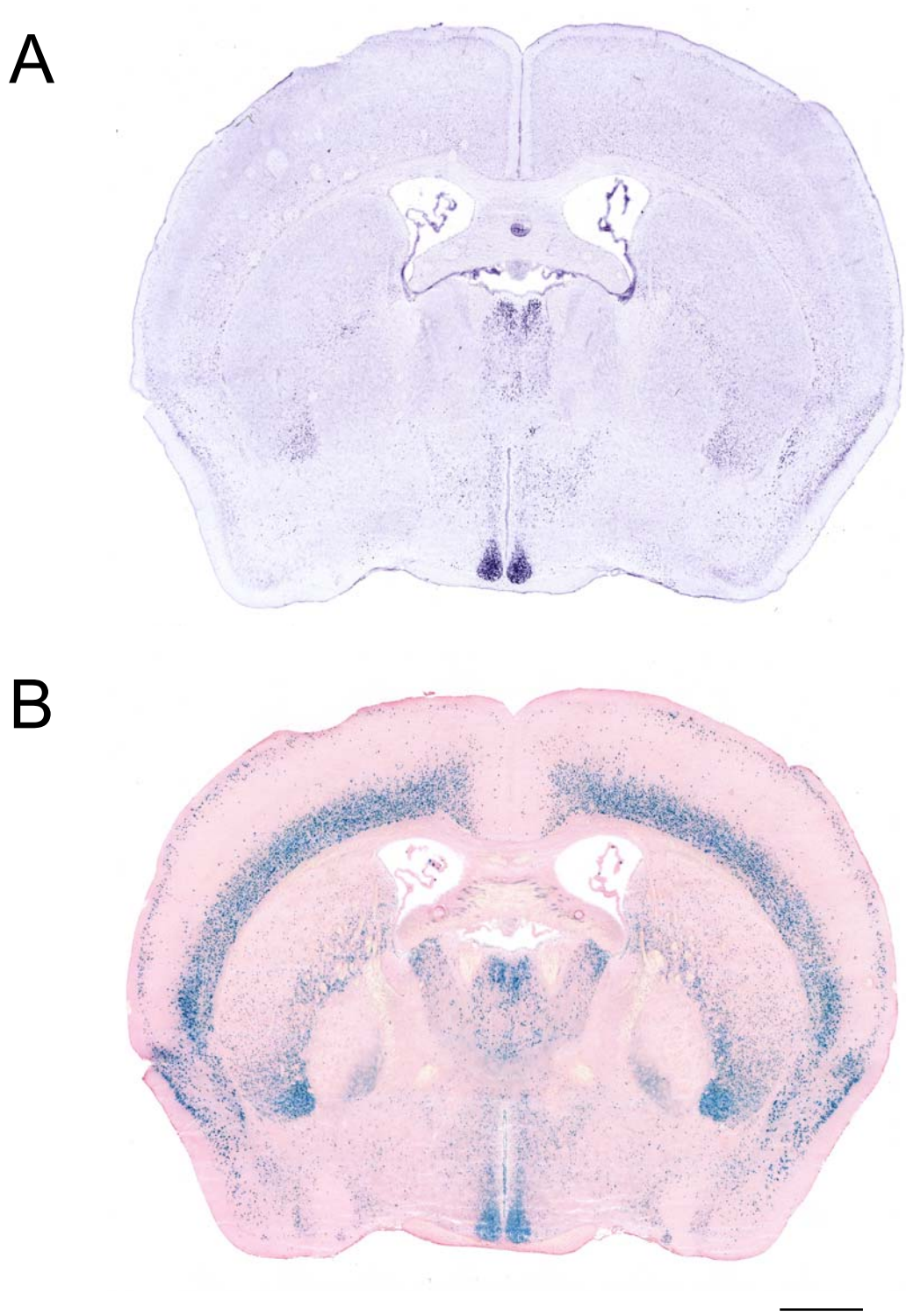
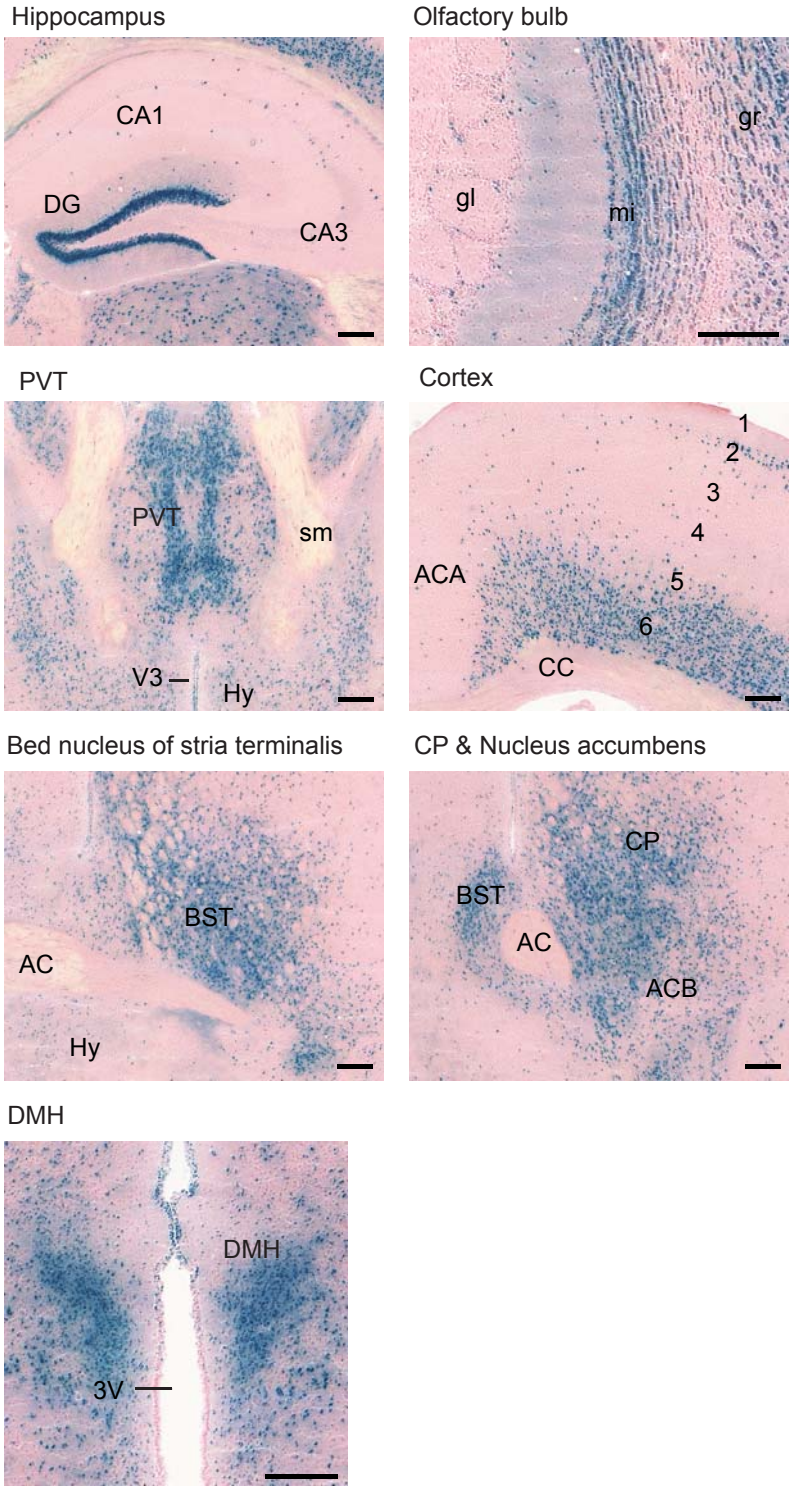


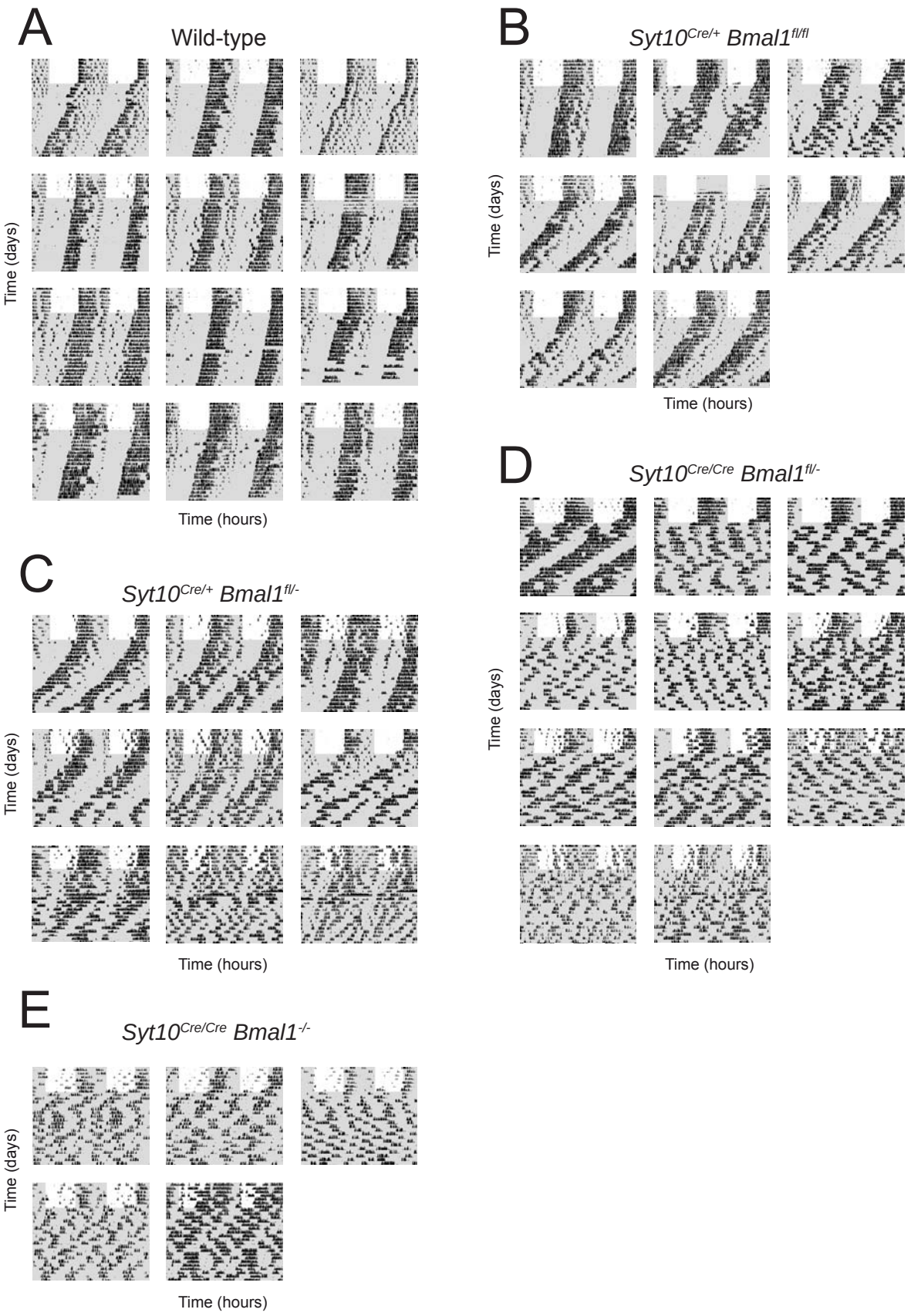
Supplementary Figure S1. Adjacent coronal sections comparing the pattern of *Syt10* mRNA and Cre activity.

Coronal brain sections at the level of the SCN showing *Syt10* expression (A) and Cre activity (B) as determined by *in situ* hybridization or ß-galactosidase staining. Sections shown here are 25 µm apart. Scale bar: 1 mm.



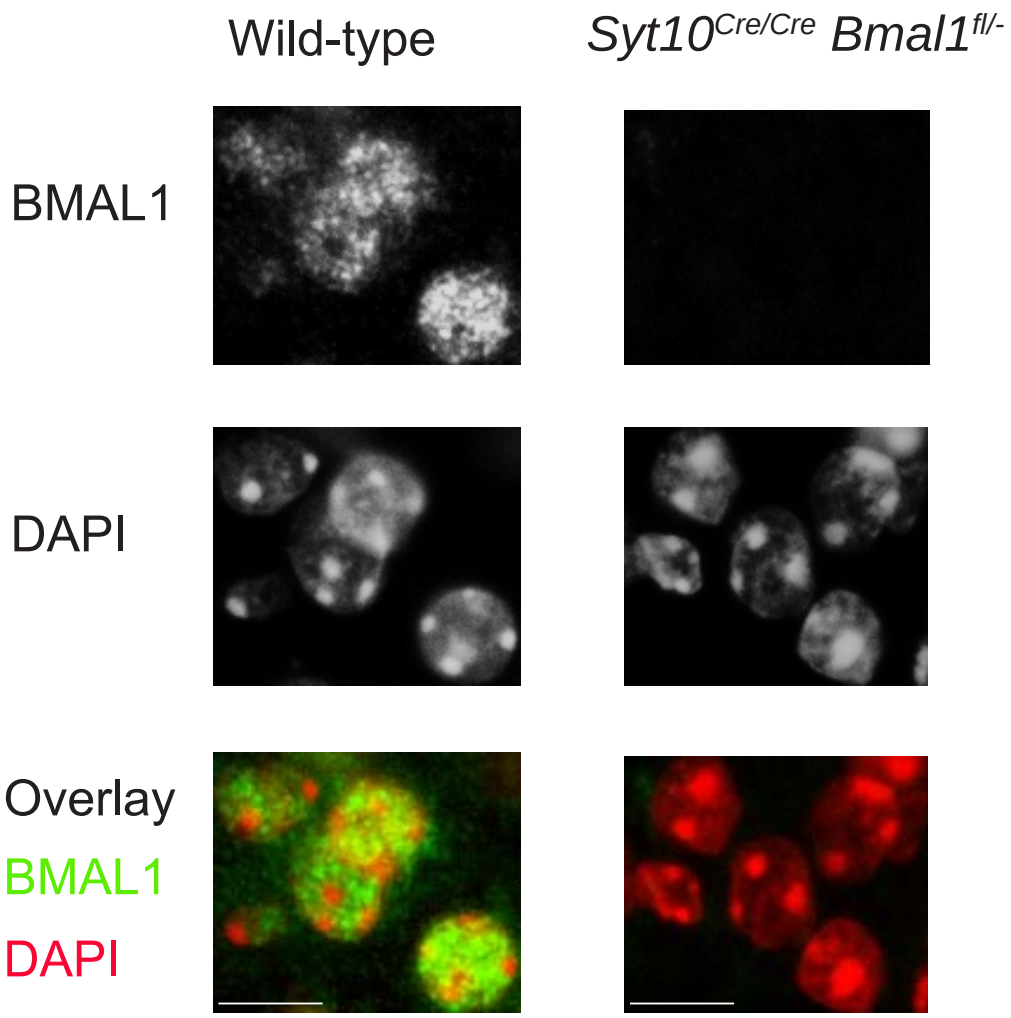
Supplementary Figure S2. Additional Cre activity sites in *Syt10*$^{Cre/+}$ *R26R*$^{LacZ/+}$ mice.

Expression of the reporter ß-galactosidase in various brain regions as determined by ß-galactosidase staining of coronal sections of *Syt10*$^{Cre/+}$ *R26R*$^{LacZ/+}$ mice . Scale bar: 0.2 mm. Abbreviations: 1-6, cortical layers 1-6; 3V, 3rd ventricle; AC, anterior commissure; ACA, anterior cingulate area; ACB, nucleus accumbens; BST, bed nucleus of the stria terminalis; CA1/CA3, Ammon's horn fields CA1/CA3; CC, corpus callosum; CP, caudoputamen; DG, Dentate gyrus; DMH, dorsomedial nucleus of the hypothalamus; Gl, glomerular layer of OB; Gr, granule layer of OB; Hy, Hypothalamus; Mi, mitral layer of OB; OB, olfactory bulb; PVT, paraventricular nucleus of the thalamus; Sm, stria medullaris.



Supplementary Figure S3. Actograms of all *Syt10^{Cre}* driven *Bmal1* knock-outs.

Double-plotted actograms of all individual mice used for the calculations in Fig. 5. Wild-type (A), *Syt10$^{Cre/+}$ Bmal1$^{fl/fl}$* (B), *Syt10$^{Cre/+}$ Bmal1$^{fl/-}$* (C), *Syt10$^{Cre/Cre}$ Bmal1$^{fl/-}$* (D) and *Syt10$^{Cre/Cre}$ Bmal1$^{-/-}$* (E). Light regimen: 7 days in LD 12:12 followed by 19 days in DD.



Supplementary Figure S4. Validation of the anti-BMAL1 antibody used for BMAL1 quantification.

Representative high-power images of SCN neurons in wild-type and *Syt10*$^{Cre/Cre}$ *Bmal1*$^{fl/-}$ mice. Top: anti-BMAL1 antibody; center: DNA counterstained with DAPI; bottom: overlay of BMAL1-immunoreactivity (green) and DNA (red). Scale bar: 10 µm. Note the absence of any signal in SCN cells from *Syt10*$^{Cre/Cre}$ *Bmal1*$^{fl/-}$ mice.

Supplementary Table S1: Breeding strategies

Genotype ♀	Genotype ♂	Genotype offspring of interest	Experimental question addressed
R26R$^{LacZ/LacZ}$	*Syt10*$^{Cre/Cre}$	*Syt10*$^{Cre/+}$ *R26R*$^{LacZ/+}$	LacZ reporter analysis
Syt10$^{Cre/+}$ *R26R*$^{LacZ/+}$	*Syt10*$^{Cre/+}$ *R26R*$^{LacZ/+}$	*Syt10*$^{Cre/+}$ *R26R*$^{LacZ/+}$ [1]	Ubiquitous LacZ staining
Syt10$^{Cre/+}$	*Syt10*$^{Cre/+}$	*Syt10*$^{Cre/+}$	Circadian phenotype of knock in mice
Syt10$^{Cre/+}$	*Syt10*$^{Cre/+}$	*Syt10*$^{Cre/Cre}$	Circadian phenotype of knock in mice
Syt10$^{Cre/+}$ *Bmal1*$^{fl/+}$	*Bmal1*$^{fl/fl}$	*Syt10*$^{Cre/+}$ *Bmal1*$^{fl/fl}$	Circadian phenotype of conditional *Bmal1* KO
Syt10$^{Cre/+}$ *Bmal1*$^{fl/f}$	*Syt10*$^{Cre/+}$ *Bmal1*$^{fl/fl}$	*Syt10*$^{Cre/+}$ *Bmal1*$^{fl/-}$ [2]	Circadian phenotype of conditional *Bmal1* KO
Syt10$^{Cre/+}$ *Bmal1*$^{fl/f}$	*Syt10*$^{Cre/+}$ *Bmal1*$^{fl/fl}$	*Syt10*$^{Cre/Cre}$ *Bmal1*$^{fl/}$ [2]	Circadian phenotype of conditional *Bmal1* KO
Syt10$^{Cre/Cre}$ *Bmal1*$^{fl/-}$	*Syt10*$^{Cre/Cre}$ *Bmal1*$^{fl/-}$	*Syt10*$^{Cre/Cre}$ *Bmal1*$^{-/-}$ [2]	Positive control for circadian phenotype

[1] As *Syt10*^{Cre} is active in the male germline (and thus in the father of our breeding pair) *Syt10*^{Cre} *R26R*^{LacZ} offspring from this breeding expresses the reporter in all cells of the body.

[2] The *Bmal1* knock-out allele in the offspring resulted from germline expression of *Syt10*^{Cre} in the father.