

Supplementary Online Material

Aberrant Development of the Suprachiasmatic Nucleus and Circadian Rhythms in Mice Lacking the Homeodomain Protein Six6.

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Supplemental Table S1

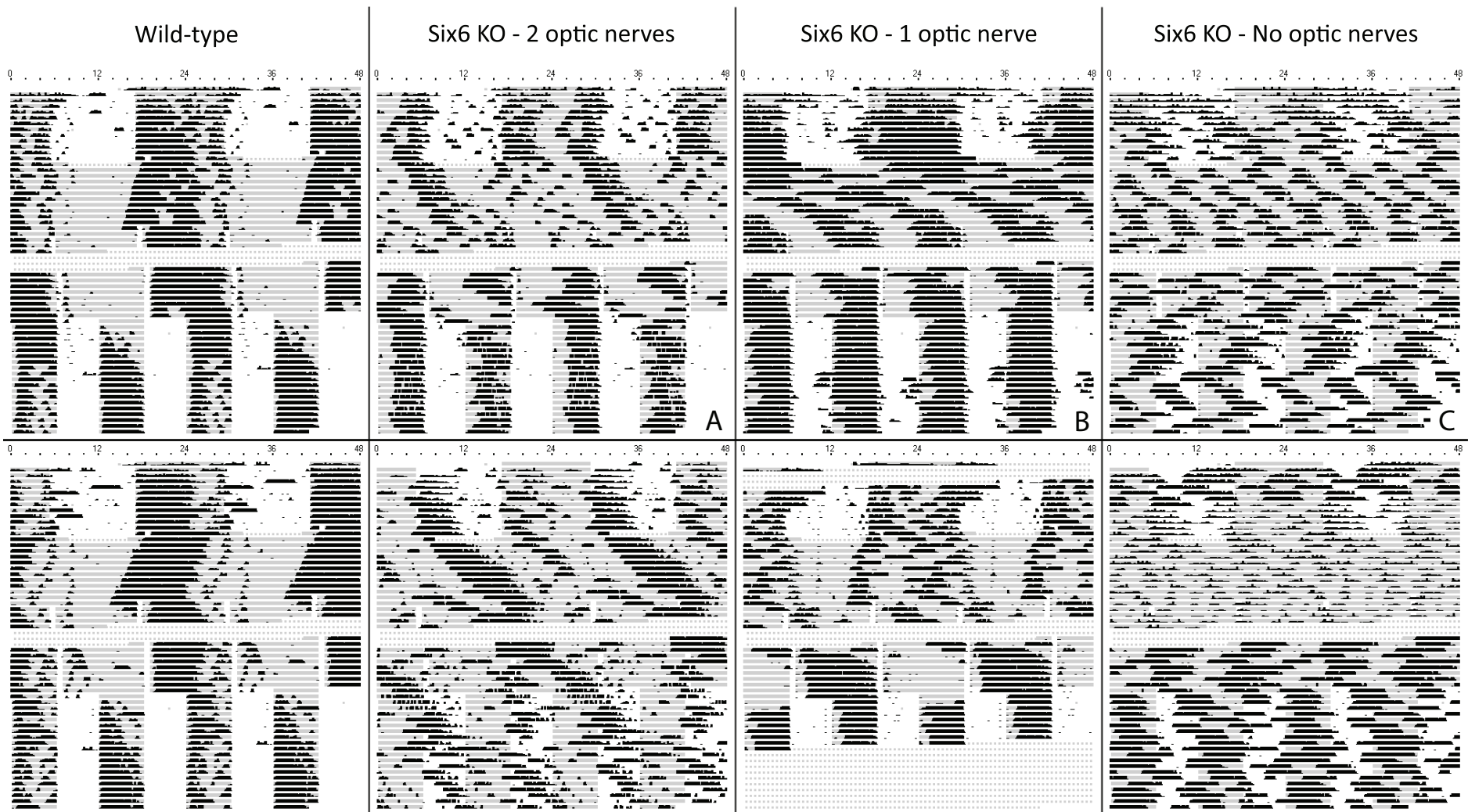
Supplemental Figures S1, S2 and S3

Supplemental Table S1

<u>Gene Symbol</u>	<u>Accession</u>	<u>Primer</u>	<u>Sequence</u>
Six6	NM_011384.4	Six6_F	TCGATGTTCCAGCTGCCCAT
Six6	NM_011384.4	Six6_R	TGGAAAGCCACGATGGCTCT
Six3	NM_011381.4	Six3_F	CCGGAAGAGTTGTCCATGTT
Six3	NM_011381.4	Six3_R	CGACTCGTGTTTGTGATGG
Clock	NM_007715.5	Clock_F	AAAGATGACACAGCGGAGGT
Clock	NM_007715.5	Clock_R	CTCTATCATCCGTGTCCGCT
Arntl	NM_007489.3	Bmal1_F	ATTCCAGGGGGAACCAGAG
Arntl	NM_007489.3	Bmal1_R	CCCTCCATTTAGAATCTTCTTGC
Per2	NM_011066.3	Per2_F	CAAAGGCACCTCCAACATG
Per2	NM_011066.3	Per2_R	AAAGTATTTGCTGGTGTGACTTG
Gapdh	NM_008084.2	GapDH_F	TGCACCACCAACTGCTTAG
Gapdh	NM_008084.2	GapDH_R	GGATGCAGGGATGATGTTC
H2afz	NM_016750.2	H2afz_F	TCACCGCAGAGGTACTIONGAG
H2afz	NM_016750.2	H2afz_R	GATGTGTGGGATGACACCA
Ppia	NM_008907.1	Ppia_F	AAGTTCCAAAGACAGCAGAAAAC
Ppia	NM_008907.1	Ppia_R	CTCAAATTTCTCTCCGTAGATGG

Sequences of DNA oligonucleotides.

Gene symbol, accession number, and sequence (5'-3') is provided for each primer used in the quantitative PCR experiments.

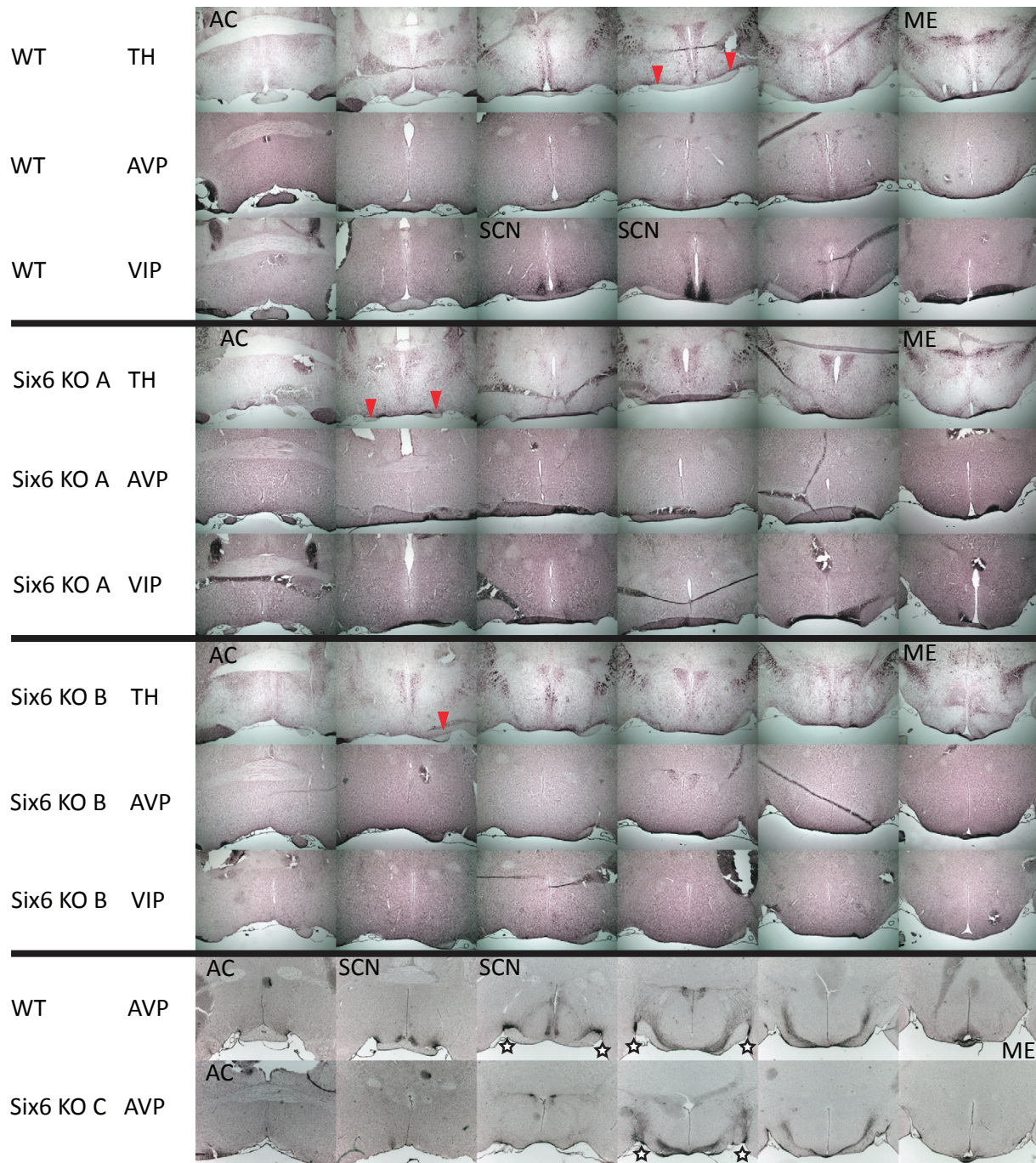


Full activity profile for two representative wild-type and all six *Six6*-null mice subjected to photoperiod manipulations.

Light gray background indicates lights off, white background indicates lights on, and dashed regions indicate no data for those times. The bottom panel in column “Six6 KO – 2 optic nerves” indicates data from a mouse that had thin, threadlike optic nerves. As this mouse displayed an apparent progressive loss of sensitivity toward the light schedule, its optic nerves may have been in the process of deteriorating over the course of the recording.

Six6 KO mice A, B, and C (see manuscript figure legends) are indicated in the bottom right corner of each appropriate record.

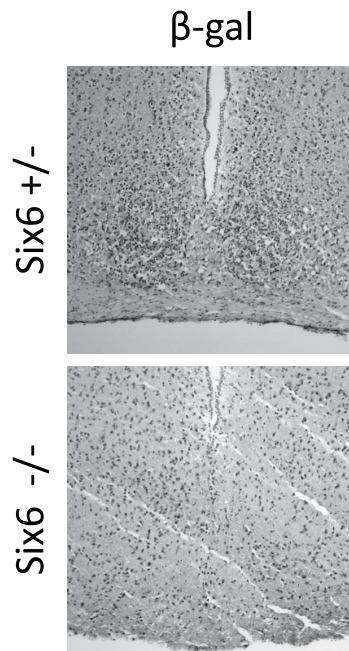
Supplemental Figure S2



Immunohistochemical staining of wild-type and *Six6*-null mouse hypothalamic.

The leftmost column of images shows coronal sections of the hypothalamus at the level of the anterior commissure (AC). The rightmost column of images shows coronal sections to the level of the median eminence. Optic nerves are indicated by red arrows. Wild-type mice display optic chiasmata, while KO mice do not. The bottom two rows of images were stained with a separate lot of the AVP antibody. Supraoptic nuclei in the bottom two rows are located above the black stars.

Supplemental Figure S3



SCN of heterozygous and Six6-null mice stained for β -galactosidase.

Heterozygous mice display neuronal β -galactosidase in the SCN.

β -galactosidase staining was observed in the Six6-null mice, though the SCN was not observed in any section. N = 3 for each genotype, and representative photographs are shown.