SUPPLEMENTARY INFORMATION to the manuscript: Natural selection against a circadian clock gene mutation in mice, by Kamiel Spoelstra, Martin Wikelski, Serge Daan, Andrew S.I. Loudon, and Michaela Hau

Experimental set-up and methods

Enclosures. Enclosures (figure S1, S2) were shielded with 1.5 m high sheet metal walls (0.8 m deep into the ground) topped with a three-line electrical fence to keep out ground predators. Vegetation was mown frequently in order to prevent mice use vegetation to climb the walls. Water was supplied *ad libitum* with chicken water systems. Feeders were designed such that mice could not access any food without passing the antenna coils (figure S3). Mice were fed with LabDiet Mouse Diet 9F (www.labdiet.com), designed for maximizing production in breeding colonies.
At the start of the experiment, it took on average 9.5±0.5 days before a mouse was first recorded at the feeder; mice were able to forage on food provided directly in the hay filled sheds to ensure high survival rate directly after release. Trapping was done by catching mice inside the feeders such that mice were never longer than 15 min. inside before release. During the final trapping at the end of the experiment additional Longworth traps (Penlon ltd., Londen, UK) were used.

Transponder recording: transponders (Trovan ID100) were recorded with ø 40 mm antenna coils connected to LID665N decoders (DorsetID, Aalten, the Netherlands) in a RS485 network. Data were logged to two separate CF cards with two Acumen Databridge SDR2-CF data recorders (Acumen Instruments Corporation, Ames, Iowa, US). The setup was capable of recording multiple transponders simultaneously with different antenna coils. Transponder losses were low: just one of the 238 mice released lost its transponder injected a month before. It never happened that we caught a mouse which was not recorded during the two previous days.

Data analysis: transponder data were processed with custom software written by KS. Repetitive transponder recordings, which occur if a mouse stays right in the middle of the antenna coil, were filtered. A recording was only used if the same code was not recorded during the previous three seconds. Chronobiological analysis was done with the

ChronoShop 1.1 program (courtesy of Kamiel Spoelstra, freely available at https://nioo.knaw.nl/nl/employees/kamiel-spoelstra and final statistical calculations were done in R 3.1.1 (R Development Core Team, 2014; http://cran.r-project.org/).

Genotyping and phenotyping: Mice were genotyped by isolating DNA from ear tissue with Viagen DirectPCR Lysis Reagent (www.viagenbiotech.com) and running PCR with GoTaq green mix (www.promega.com), primer "a" (5'-CACCTGGGCATTGGTGAGT-3') detecting wild-type and "b" (5'-GGAGGTCAAGGGGCCAGT-3') detecting *Ck1ε*^{tau} alleles. Within the release cohort, the entrained phenotype and free-running circadian period in DD of 26 mice was measured. The phenotype related genotypes matched the PCR outcome impeccably.

Figure S1. Aerial view of enclosure with sheds and feeders.

Figure S2. Shed, feeders and runways in enclosure

Figure S3. Feeder entrance with antenna coil