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Supplemental information

Loss of circadian rhythmicity in *bdnf* knockout zebrafish larvae

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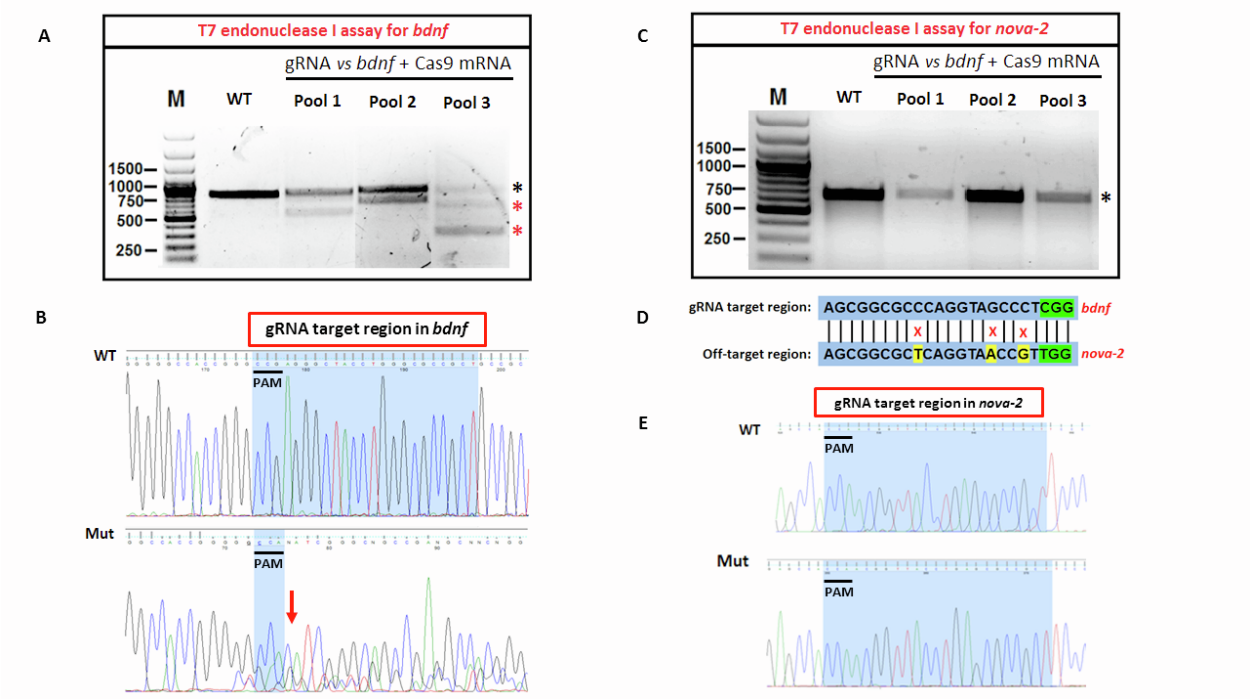

Figure S1. T7EI assay and Sanger sequencing, related to STAR Methods.
Evidence of InDels in *bdnf locus*. A) T7EI assay showed the presence of multiple bands (red asterisks) in three independent pools of injected embryos (Pool 1-2-3) compared to the single band (black asterisk) of the wt control pool of embryos. B) The presence of InDels introduced by the gRNA in the genomic *locus* of *bdnf* gene was confirmed by Sanger sequencing. wt and injected embryos (mut) sequence chromatograms are reported, the red arrow indicates the point where multiple picks appear few nucleotides downstream the PAM sequence. The target region is highlighted in light blue. M stay for 100 bp DNA ladder. Analysis of the off-target site in *nova2 locus*. C) T7EI assay showed the absence of multiple bands in *nova2* for three independent pools of injected embryos (Pool 1-2-3) compared to the wt control pool of embryos. D) Predicted off-target region in *nova2* by "CRISPR DESIGN" tool. The gRNA targeting *bdnf* is not able to recognize with perfect match the predicted off-target site; the PAM sequence and the mismatch point are highlighted in green and yellow, respectively. E) The comparison between sequence chromatograms of wt and injected embryos showed no differences in the nucleotide composition. The target region is highlighted in light blue. M stays for 100 bp DNA ladder.

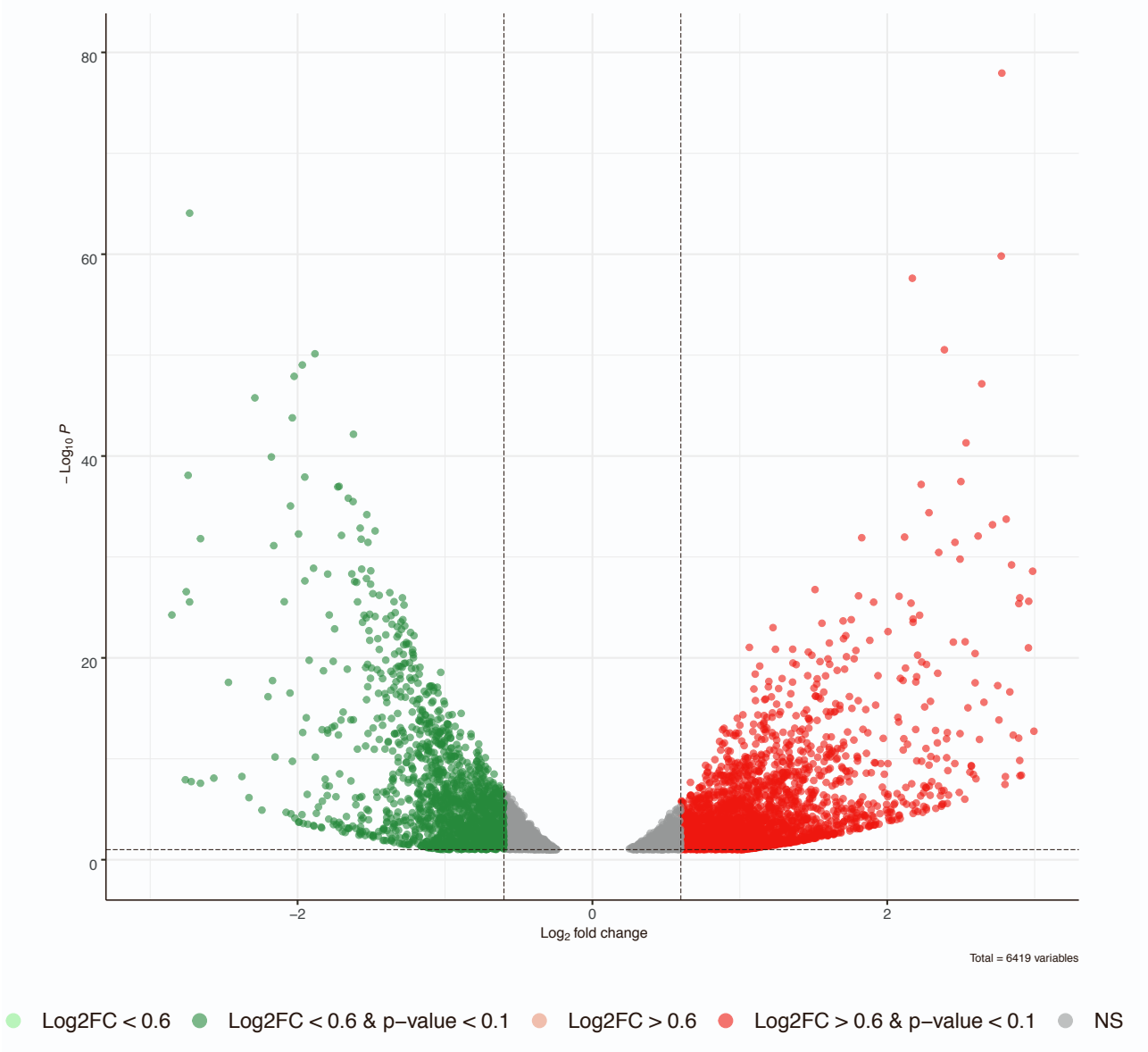

Figure S2. Volcano plot showing differential expressed genes at embryonic stage 48 hpf, related to STAR Methods.

Volcano Plot shows the relationship between log2 fold change and the statistical significance. The red and green dots in the plot represent the differentially expressed mRNAs with statistical significance.

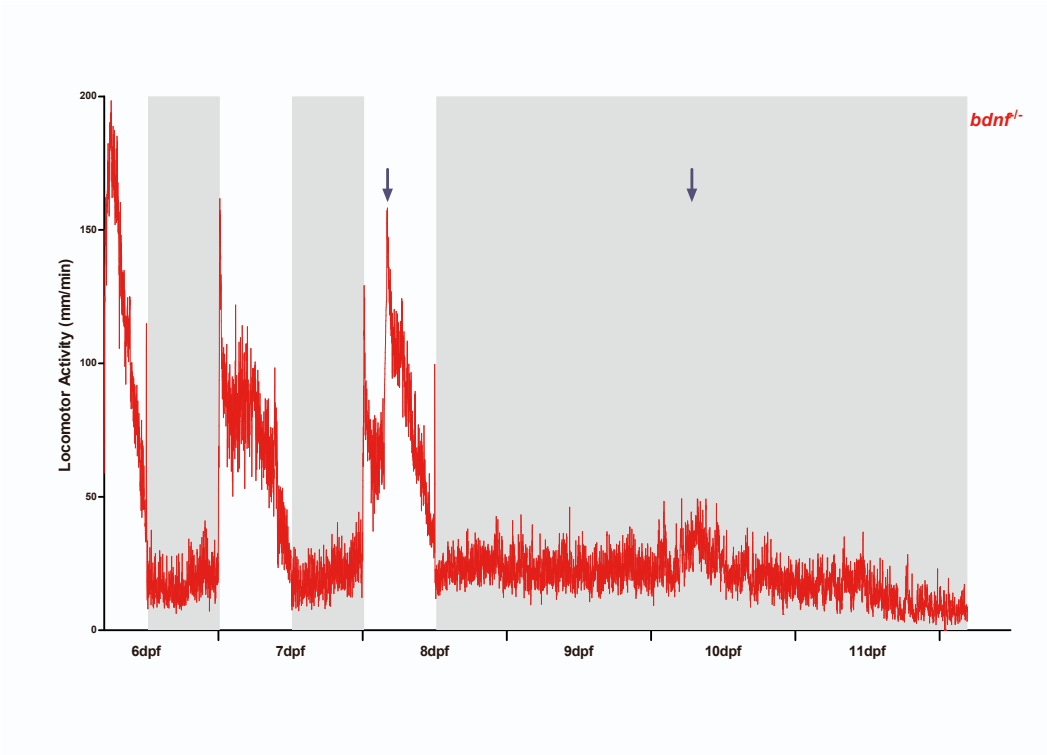

Figure S3. Locomotor activity in DD and starvation test in *bdnf*$^{-/-}$ zebrafish larvae, related to Figure 6.

Mean waveform of locomotor activity of *bdnf*$^{-/-}$ under 12:12 LD cycles from 6 to 8 dpf and DD from 9 to 11 dpf (n = 24). Vertical axis shows averaged distance moved (mm/1 min), while X-axis indicates time in recording. White and grey bars show light and dark phase, respectively. Black arrows indicate feeding time. Data are expressed as mean ± SEM.

Table S1. Sequence of insert-oligonucleotides used for the customized gRNA-expression vector synthesis, related to STAR Methods.

gRNA name	Oligo sequence (5' → 3')	PAM sequence
Dr_bdnf_gRNA	F: **TA**GGCGGCGCCCAGGTAGCCCT	CGG
	R: **AAAC**AGGGCTACCTGGGCGCCG	

Sticky-end are highlighted in bold.

Table S2. Primers sequences for PCR screening of mutations in *bdnf* gene, related to STAR Methods.

Target gene	Primer sequence (5' → 3')	wt amplicon size
Standard PCR Dr_bdnf	F: GAAGAGTGATGACCATCCTG	228 bp
	R: ATGACCTGCTCGAAAGTGTCCG	

Table S3. List of primers used T7 endonuclease I assay, related to STAR Methods.

Target gene	Primer sequence (5' → 3')	wt amplicon size
Dr_bdnf_T7I	F: GAAGAGTGATGACCATCCTG	828 bp
	R: GTGTACACTATCTGCCCC	
Dr_nova2_T7I	F: ACAGCCTCCAGTCTCCTGGG	678 bp
	R: TAGCCCACCTTCTGCGGATTGG	

Table S6. Statistical analysis of clock and clock-controlled gene expression, related to Figure 4 and Figure 5.
A: acrophase, ZT: zeitgeber time. The symbol (-) indicates the absence of peak.

LD	*bdnf*$^{+/+}$		*bdnf*$^{-/-}$	
	P	A (ZT)	P	A (ZT)
Arntl1	<0.00001	9	<0.003	9
Clock1a	<0.0003	15	<0.00001	15
Clock2	<0.00001	9	<0.00001	9
Per1b	<0.00001	21	<0.0002	21
Per2	<0.0001	3	<0.003	3
Cry1a	<0.00001	3	<0.00001	3
Nilf3-5	<0.0002	9	<0.0002	9
Nilf3-6	<0.0001	3	<0.001	3
Aanat2	<0.0001	21	<0.0001	21
Mtnr1aa	<0.05	3	<0.05	9

DD	*bdnf*$^{+/+}$		*bdnf*$^{-/-}$	
	P	A (CT)	P	A (CT)
Arntl1	<0.00001	9	<0.00001	9
Clock1a	<0.005	15	0.07	-
Clock2	<0.0002	15	0.09	-
Per1b	<0.00001	3	<0.00001	3
Per2	<0.0001	3	<0.002	3
Cry1a	<0.0001	3	<0.0001	3
Nilf3-5	<0.001	15	<0.0001	9
Nilf3-6	<0.002	3	<0.0001	3
Aanat2	<0.0001	15	>0.05	-
Mtnr1aa	<0.01	3	>0.8	-

Table S11. List of primers used for qPCR experiments, related to Figure 4 and Figure 5.

Genes	Forward	Reverse
Per1b	CCGTCAGTTTCGCTTTTCTC	ATGTGCAGGCTGTAGATCCC
Clock1a	CTGGAGGATCAGCTGGGTAG	CACACACAGGCACAGACACA
Arntl1a	TAGAGCGCTGTTTGCTGATG	GACCCGTGGACTTCAGTGAC
Per2	ATGTCGATGGCTTTAGGCAG	CGAGACATCCAGAAGGTGCT
Cry1a	TCCGCTGTGTGTACATCCTC	CAAACACTGCAGCAAAAACC
Clock2	AGAACTGCTGAGGCTGCTGT	TAACGTTGTGCTAGTCCCCT
Aanat2	CGTTTATCTCGGTGTCCGGTGAATGTC	CTGTTCTAGTTTCTCTTTATCCCAGCC
Mtnr1aa	CTGGTGATTTTCTCCGTCTACAGA	CCGCCACTGCCAAACTC
Nfil3-5	CTTCCAACCCAAAACAGCGG	GCAGCCTCGTTATTCTTGCG
Nfil3-6	TTTGCTTGCACGCTCACTTC	TTACACGGCGTTTCTCACGA
18S	ACCACCCACAGAATCGAGAAA	GCCTGCGGCTTAATTTGACT
Ef1α	GACAAGAGAACCATCGAG	CCTCAAACTCACCGACAC