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Seasonal regulation of the lncRNA *LDAIR* modulates self-protective behaviours during the breeding season

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Supplementary Information for:

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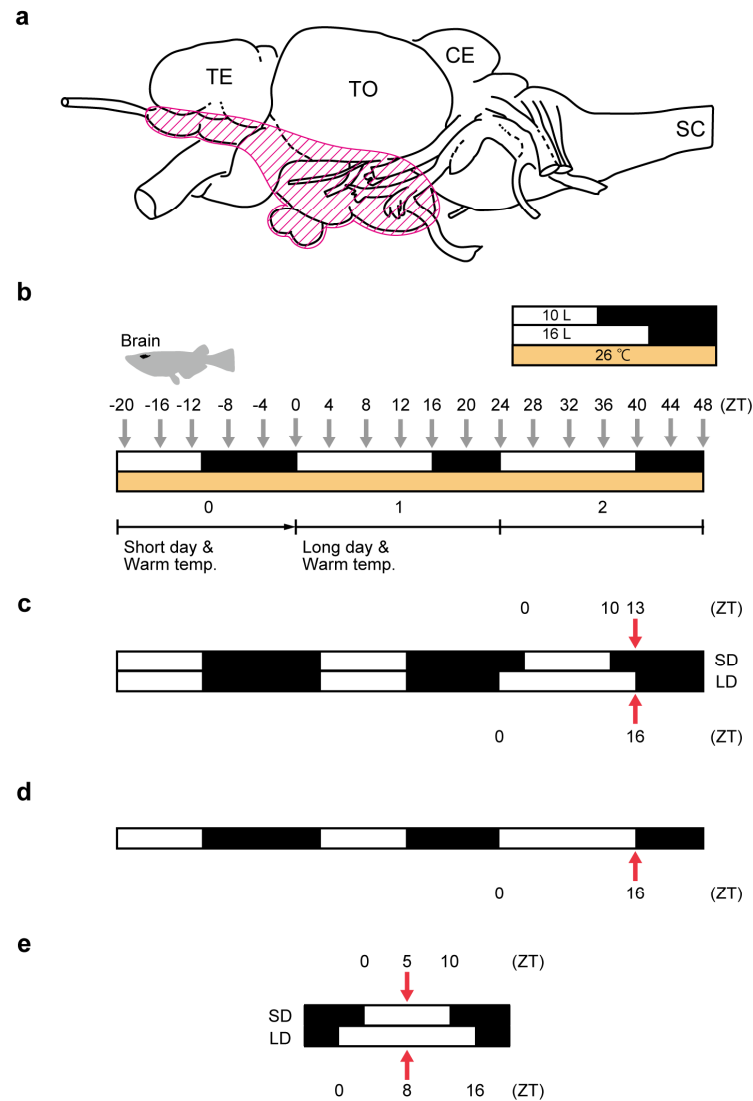
Supplementary Figure 3

Supplementary Figure 4

Supplementary Figure 5

Supplementary Figure 6

Supplementary Table 1



Supplementary Figure 1. Schematic drawing of the brain region dissected for expression analysis and experimental design.

a The hatched pink area represents the brain region dissected for DNA microarray, RNA-seq, Ribo-seq and qPCR analysis. This region includes the ventral part of the telencephalon and the hypothalamus and pituitary. The lateral view of medaka brain was modified from the Medaka Histological Atlas (Yuji Ishikawa, Masami Yoshimoto, and Hironobu Ito, Medaka Brain Atlas, edited by the Editorial Board of Medaka Histological Atlas of NBRP Medaka, https://shigen.nig.ac.jp/medaka/medaka_atlas/). TE, telencephalon; TO, tectum opticum; CE, corpus cerebelli; SC, spinal cord.

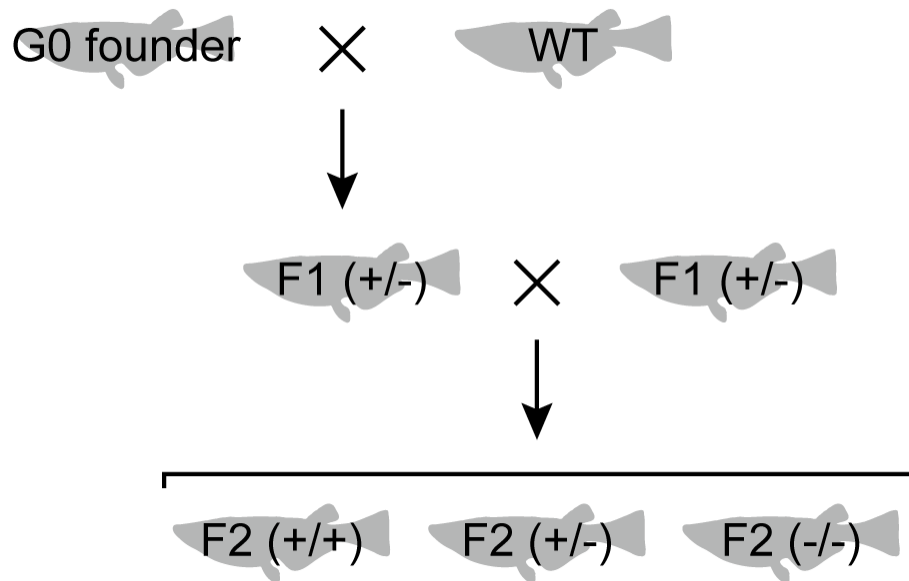
b Experimental design for Figure 1. Medaka kept under short day and warm temperature conditions (SD: 10 h light / 14 h dark; 26 °C) were transferred to long day and warm

temperature conditions (LD: 16 h light / 8 h dark; 26 °C). Brain samples were collected from six fish every 4 h for three days during this transition. White and black bars represent the day-night cycle, and arrows above the bars indicate sampling time points, where ZT0 is defined as ‘lights on’ on the first long day (SD time points include ZT-20 to ZT-4, and LD time points include ZT0 to ZT48).

c Experimental design for Figure 2a and Supplementary Figure 4. Medaka kept under SD conditions were either maintained in SD or transferred into LD conditions. Brains were collected 16 hours after the transition. This timing was selected because *LDAIR* expression is the highest at this time of day under LD conditions. White and black bars represent the day-night cycle, and arrows indicate sampling time points (ZT13 for SD and ZT16 for LD conditions).

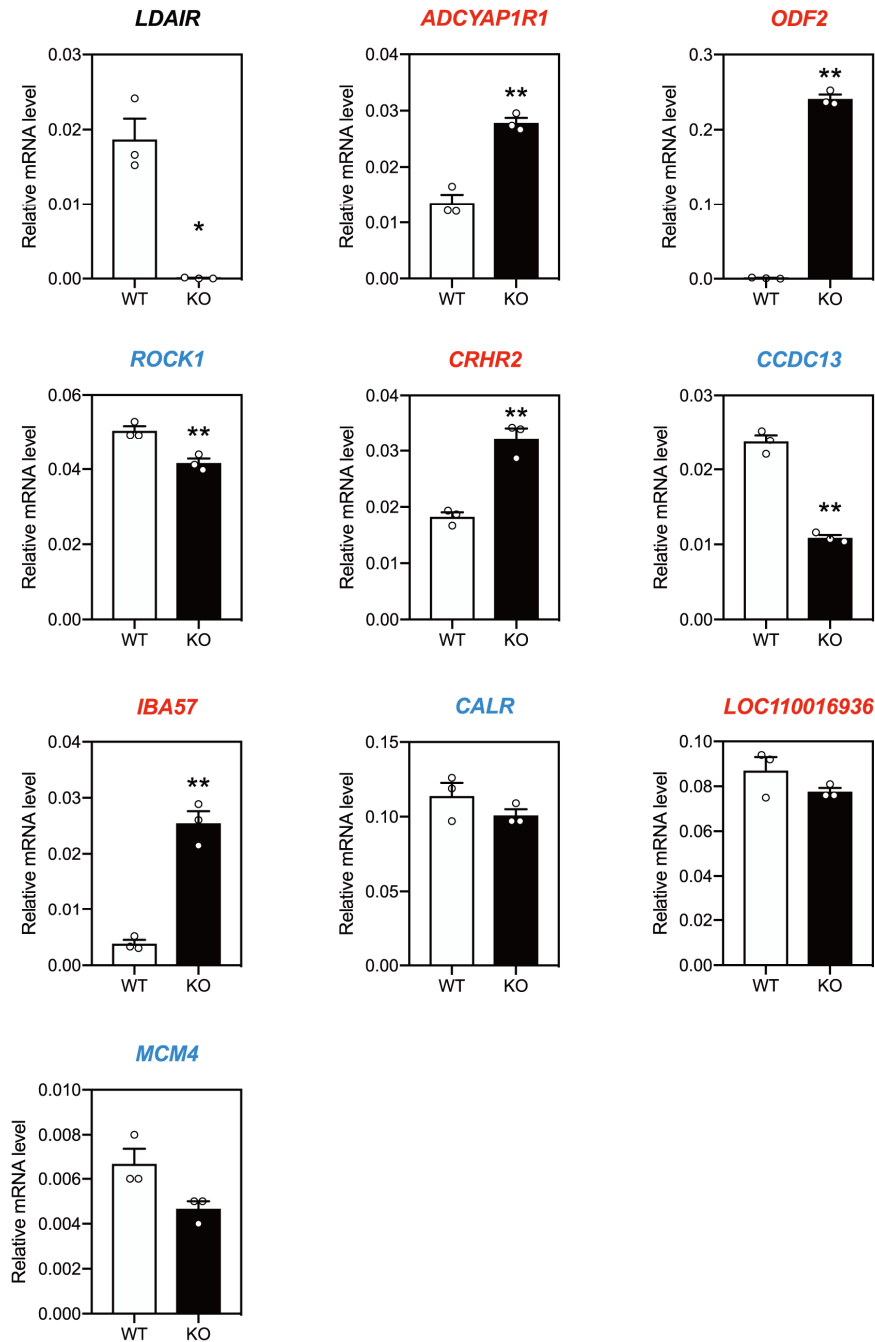
d Experimental design for Figures 2b, 3c and Supplementary Figure 3. Medaka kept under SD conditions were transferred into LD conditions and were sacrificed at 16 hours after lights on (ZT16). This timing was selected because *LDAIR* expression is the highest at this time of day under LD conditions. White and black bars represent the day-night cycle, and arrow indicates sampling time point.

e Experimental design for Figure 4a and Supplementary Figure 5. Medaka were either maintained in SD or LD conditions and were sacrificed at the middle of the day. Since cortisol levels and gonadal weight (due to morning oviposition) change dramatically at light onset and the phase of circadian clock is affected by photoperiod, samples were collected in the middle of the day (ZT5 for SD and ZT8 for LD conditions).



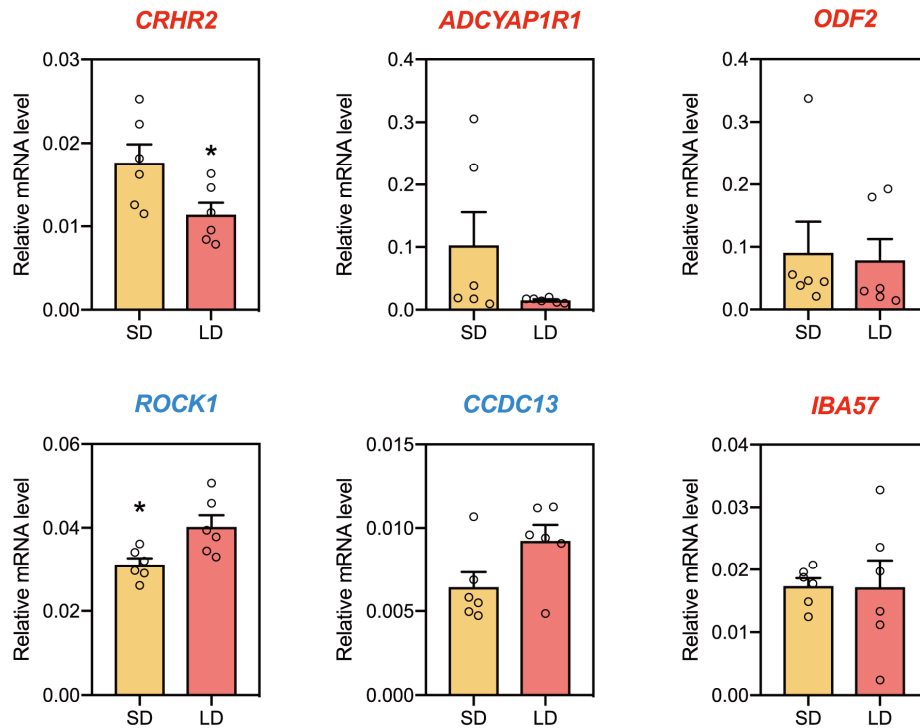
Supplementary Figure 2. Schematic drawing of the steps to establish *LDAIR* KO medaka.

Single guide RNAs (sgRNAs) and the Cas9 mRNA were microinjected into one-cell stage embryos. Mutant G0 founders were raised to adulthood and outcrossed to wild-type (WT) medaka to obtain F1 heterozygotes (+/-). F1 heterozygotes (+/-) were then incrossed to obtain F2 WT (+/+) and knockout (KO) (-/-) siblings, which were used in our experiments.



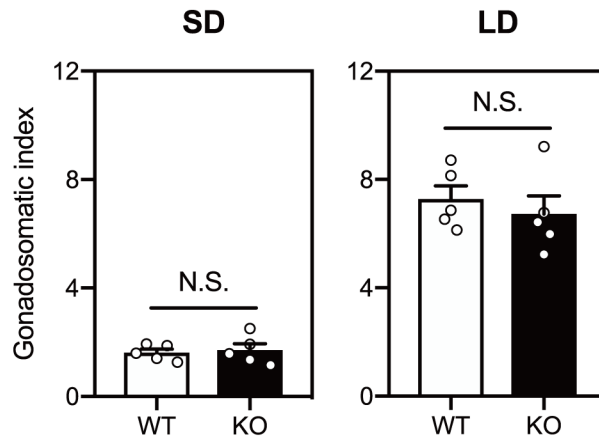
Supplementary Figure 3. qPCR verification of selected differentially-expressed genes within the *LDAIR* locus in WT and KO medaka.

Relative gene expression levels in the brain (ventral telencephalic area, hypothalamus and pituitary) were measured by qPCR using medaka kept under LD conditions (* $P < 0.05$, ** $P < 0.01$, t -test; mean + SEM, $n = 3$). Each dot indicates individual data points collected at ZT16.



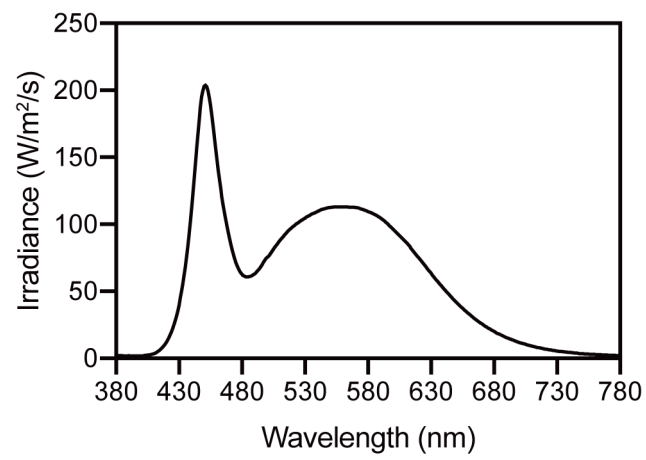
Supplementary Figure 4. Effect of changing daylength on expression of *LDAIR* neighboring genes.

Relative gene expression levels in the brain (ventral telencephalic area, hypothalamus and pituitary) were measured by qPCR using medaka kept under SD or LD conditions (* $P < 0.05$, t -test; mean + SEM, $n = 6$). Each dot indicates individual data points collected at ZT13 for SD and ZT16 for LD conditions.



Supplementary Figure 5. Effect of changing daylength on gonadosomatic index in WT and *LDAIR* KO medaka.

Gonadosomatic index (gonad weight / total body weight) of WT and *LDAIR* KO female medaka was similar under SD and LD conditions (ZT5 for SD and ZT8 for LD conditions) (*t*-test between KO and WT; mean + SEM, *n* = 5).



Supplementary Figure 6. Spectral analysis of white light used in this study.

Spectral quality of white light was measured by a Light Analyzer LA-105 (Nippon Medical & Chemical Instruments Co., Ltd).

Supplementary Table 1. qPCR primer sequences.

Gene symbol	Primer sequence (forward)	Primer sequence (reverse)
<i>RPL7</i>	aactggccttgtcatcagg	cggagaagctgcagaactt
<i>LDAIR</i>	gctctgtttgtcctcatggtca	tggaatgtgctggagggtact
<i>ADCYAP1R1</i>	acatcgccaaccaggatcag	ccgtccatgcaggagaaact
<i>ODF2</i>	gaaggctttccctgaactgc	tgaacgaactgggacttgtca
<i>ROCK1</i>	ggaagaggccaacaacatgc	atggtcttcccatctccga
<i>CRHR2</i>	gcctacatcaacggggtgaa	tctcaaagcccacgtcca
<i>CCDC13</i>	ggaggctcagtgacagcaat	gggctgctgttgcacttg
<i>IBA57</i>	gagggaaacagacccttg	actccctcaggaacgcctat
<i>CALR</i>	tacagcccagacgcaaaca	aagttgtcgaagacgggtccc
<i>LOC110016936</i>	ggacaccccagatgacttg	ctccagcgagtcggttcat
<i>MCM4</i>	tgcagctctgtcgtgcatt	gggatcctcgctattcggtg