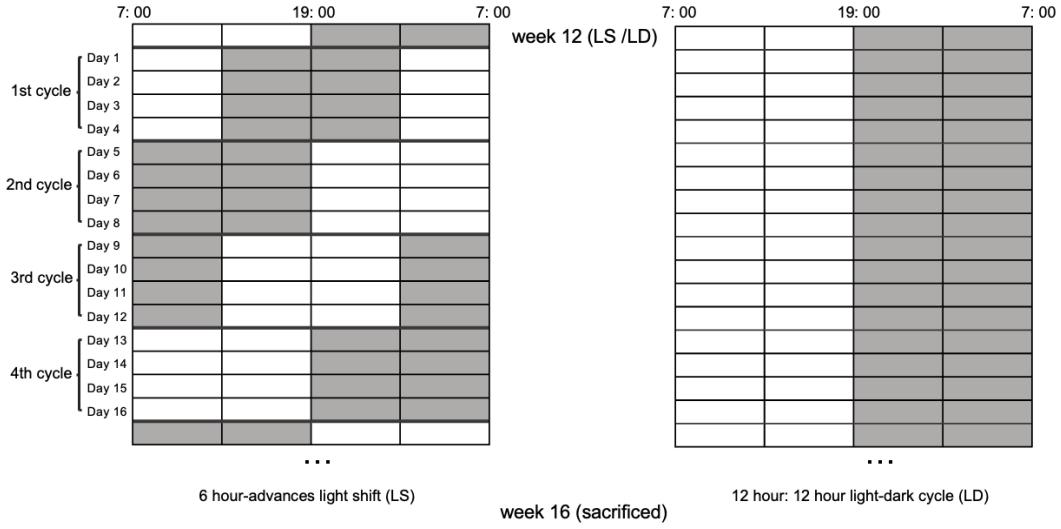
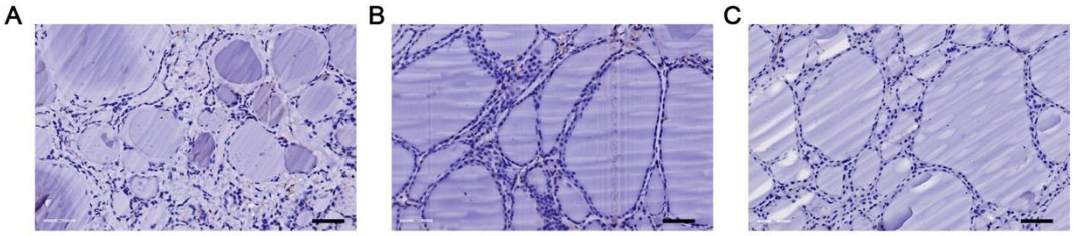
Supplementary Data



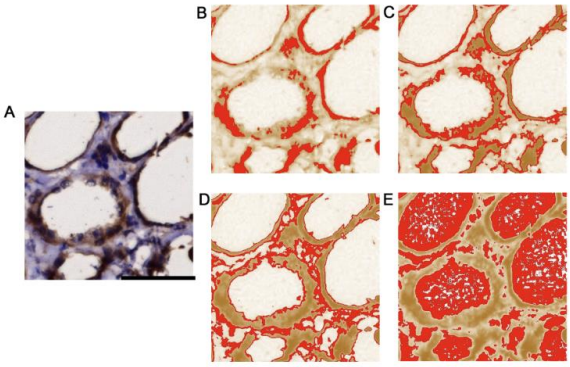
Supplementary Figure 1. Schemes of light shift. After a 4-day acclimation period, the light cycles of mice in the light-shift (LS) treatment groups were altered by 6-hour phase advance every 4 days, while those in the control group remained a normal light-dark cycle (12h light: 12h dark with lights on at 7:00 am; light intensity: 150-170 lux). After changing light phase for 24 days, the mice were maintained on a 12h light: 12h dark cycle with lights on at 7:00 am for 4 days and sacrificed at 16-week-old (4th day following the last shift).



Supplementary Figure 2. Representative IHC sections of negative controls. (A) Negative DAB staining by BMAL1 antibody without HRP-conjugated secondary antibody. (B) Negative DAB staining by PER2 antibody without HRP-conjugated secondary antibody. (C) Negative DAB staining by HRP-conjugated secondary antibody without primary antibodies. (magnification=×400; scale bar=50μm).

Supplementary Data 1. Quantifications of IHC staining

For each slide, 3 representative images at a 400× magnification (450 × 450 pixels) were selected for quantification of each marker. The quantification of staining intensities was conducted utilizing IHC Profiler, a plugin available in the ImageJ software (MD, USA) (21). The plugin automatically ascertains the percentage of positive area which is directly correlated with the concentration of DAB. Ranges of pixel values for high positive, positive, low positive and negative zones were 0-60, 61-120, 121-180 and 181-235, respectively (**Supplementary Figure 3**). A scale that ranges from 0 to 3 was applied to describe the intensity of staining in each field, where 0 means negative and 3 means high positive. The percentage of tissue with different score of intensity were recorded for each field, and an IHC score for each sample was calculated as follows: [3×%high positive area+2×% positive area+1 ×%low positive area]. A mean value was then derived for each sample.



Supplementary Figure 3. Different zones assigned for the scoring of IHC sections by IHC profiler profiles. (A) A representative DAB-stained thyroid section. (B) The red area highlights the strong positive zone (3+). (C) The red area highlights the positive zone (2+). (D) The red area highlights the low positive zone (1+). (E) The red area highlights the negative zone (0+).

Supplementary Table 1. Primers used for human and mouse studies.

mGapdh-F	AGGTCGGTGTGAACGGATTTG
mGapdh-F	TGTAGACCATGTAGTTGAGGTCA
mBmal1-F	CGTCGGGACAAAATGAACAG
mBmal1-R	GAACAGCCATCCTTAGCAC
mPer2-F	GCCTTCAGAACTCATGATGACAGA
mPer2-R	TTTGTGTGCGTCAGCTTTGG
mClock-F	CACTCTCACAGCCCCACTGTAC
mClock-R	CCCCACAAGCTACAGGAGCAGT
mCry1-F	CTGGCGTGGAAGTCATCGT
mCry1-R	CTGTCCGCCATTGAGTTCTATG
mRev-erba-F	TGGCATGGTGCTACTGTGTAAGG
mRev-erba-R	ATATTCTGTTGGATGCTCCGGCG
mRor-F	ATGGGGGACTCTCACGAAGAC
mRor-R	TCTTGCTGAACTCCGGTATCTC
h-BMAL1-F	AGGATGGCTGTTCAGCACATGA
h-BMAL1-R	CAAAAATCCATCTGCTGCCCTG
h-CLOCK-F	AAGTTAGGGCTGAAAGACGACGA
h-CLOCK-R	GAACTCCGAGAAGAGGCAGAAG
h-PER2-F	AAGCAGGTGAAAGCCAATGAAGA
h-PER2-R	CCACCGCAAACATATCGGCATT
h-CRY1-F	CTGCGTCTACATCCTGGACC
h-CRY1-R	GAAGCAAAAATCGCCACCTGT
h-REVERB-F	ACAGCTGACACCACCCAGATC
h-REVERB-R	CATGGGCATAGGTGAAGATTTCT
h-ROR-F	TCGCAGCGATGAAAGCTCAAAT
h-ROR-R	GTGGCATTGCTTTGCTGACT
h-GAPDH-F	TCGGAGTCAACGGATTTGGT
h-GAPDH-R	TTCCCGTTCTCAGCCTTGAC
h-IL-17-F	CAAGACTGAACACCGACTAAG
h-IL-17-R	TCTCCAAAGGAAGCCTGA
h-TNF-α-F	GAGCACTGAAAGCATGATCC
h-TNF-α-R	CGAGAAGATGATCTGACTGCC
h-IFN-γ-F	GACCAGAGCATCCAAAAGAGT
h-IFN-γ-R	ATTGCTTTGCGTTGGACATTC
h-CXCR4-F	AATCTTCCTGCCCACCATCTACT
h-CXCR4-R	CCGTCATGCTTCTCAGTTTCTTC
h-CXCL12-F	CATGAACGCCAAGGTCGTG
h-CXCL12-R	ACATGGCTTTCGAAGAATCGG

Supplementary Data 2. Quality control of q-PCR experiments

All reactions were performed in triplicate, negative control reactions with no template were generated to detect contamination. The efficiency of primers was validated by a standard curve of complementary DNA plotted against cycle threshold, and primers with efficiency of 90%-110% were selected for PCR experiments. The specificity of PCR products was evaluated by the presence of a single peak in the melting curve.

To identify stable reference genes for thyroid tissue, we utilized the RefFinder software to conduct an internal reference gene selection, and determined mouse glyceraldehyde-3-phosphate dehydrogenase (mGapdh) as a reliable reference gene for our experimental conditions (Supplementary Table 2).

Supplementary Table 2. Selection of stable references by RefFinder

Method	Ranking order (Better-Good-Average)			
	1	2	3	4
Delta CT	Gapdh	mRN18S	β-actin	mTBP
BestKeeper	Gapdh	β -actin	mRN18S	mTBP
Normfiner	Gapdh	mRN18S	β-actin	mTBP
Genorm	Gapdh|β -actin	-	mRN18S	mTBP
Comprehensive	Gapdh	β -actin	mRN18S	mTBP

Supplementary Table 3. Time of patient thyroid tissue sample collection.

Time (hours)	Control	AIT
Early morning (6-9)	11 (36.7%)	6 (20%)
Late morning (10-11)	7 (23.3%)	4 (13.3%)
Noon (12-14)	7 (23.3%)	13 (43.3%)
Afternoon (15-17)	3 (10%)	6 (20%)
Evening (18-21)	2 (6.7%)	1 (3.3%)

Supplementary Table 4. Cosinor analysis of clock genes in patients.

Gene		Amplitude	Acrophase	P cosinor
BMAL1	Control	0.2	8	0.6
	AIT	0.1	5	0.6
CLOCK	Control	0.3	17	0.8
	AIT	0.6	13	0.2
PER2	Control	0.7	5	0.1
	AIT	0.7	23	0.2
CRY1	Control	0.1	18	0.7
	AIT	0.3	11	0.6
REV-ERB	Control	1.1	10	0.5
	AIT	0.6	1	0.4
ROR	Control	2.1	0	0.3
	AIT	1.3	9	0.4

Supplementary Table 5. Levels of inflammatory cytokines in AIT and control group.

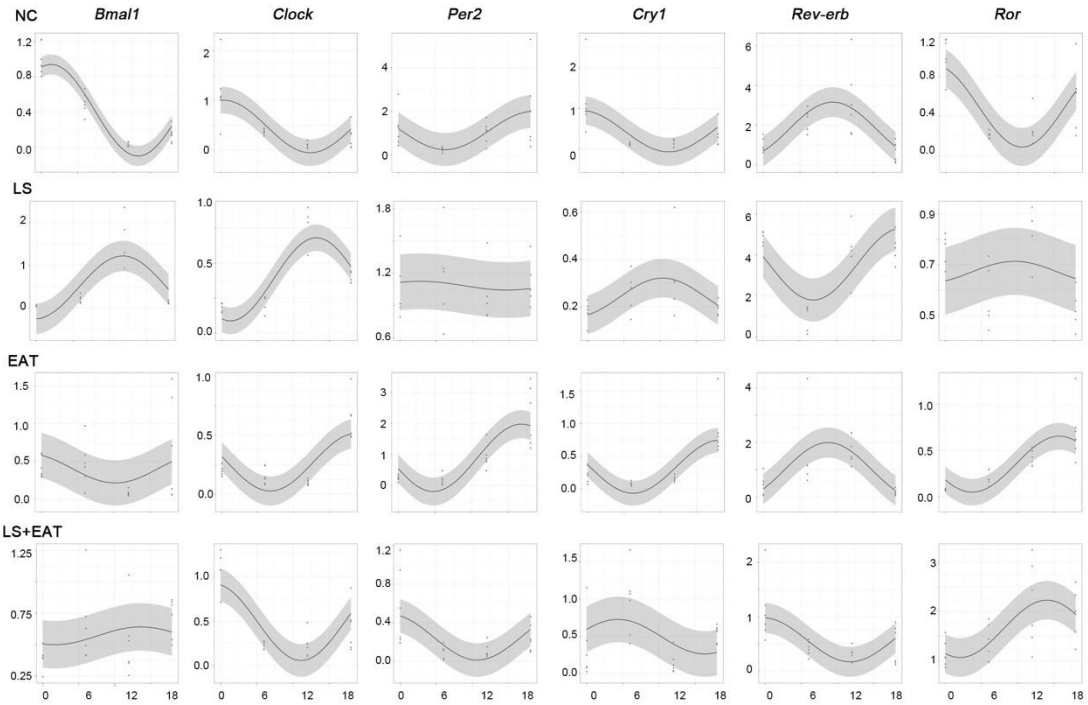
	Control	AIT	P
IL-1β (pg/mL)	4.93 (2.31-7.80)	9.72 (7.05-24.34)	<0.001
CXCL10 (pg/mL)	16.67 (13.14-25.48)	31.80 (22.18-39.84)	<0.001
IL-7 (pg/mL)	7.76 (2.83-13.01)	22.15 (15.19-29.18)	<0.001
IL-2 (pg/mL)	0.76 (0.47-1.09)	2.34 (1.75-29.18)	<0.001
IL-4 (pg/mL)	0.23 (0-0.45)	0.23 (0.68-0.42)	0.634
IL-6 (pg/mL)	4.39 (1.55-9.91)	5.99 (3.87-9.96)	0.139
IL-10 (pg/mL)	1.13 (0.33, 1.46)	4.60 (3.72-5.21)	<0.001
IL-17 (pg/mL)	0.34 (0.10-0.73)	1.89 (1.33-2.38)	<0.001
TNF-α (pg/mL)	4.07 (1.41-6.53)	10.72 (6.23-18.51)	<0.001
IFN-γ (pg/mL)	0.10 (0-0.13)	0.10 (0-0.33)	0.248

Supplementary Table 6. Circadian analysis of clock genes in mouse.

	Group	Acrophase (ZT)	Amplitude	Cosinor P	One way ANOVA P
Bmal1	NC	1.34	0.52	<0.001	<0.001
	LS	11.76	0.74	<0.001	<0.001
	EAT	22.18	0.19	0.278	0.106
	LS+EAT	13.65	0.07	0.639	0.107
Clock	NC	0.39	0.53	<0.001	<0.001
	LS	13.18	0.3	<0.001	<0.001
	EAT	18.74	0.24	<0.001	<0.001
	LS+EAT	23.12	0.43	<0.001	<0.001
Per2	NC	18.39	0.88	0.026	0.067
	LS	2.77	0.04	0.921	0.872
	EAT	16.77	1.09	<0.001	<0.001
	LS+EAT	22.65	0.24	0.009	0.006
Cry1	NC	23.30	0.51	0.005	0.001
	LS	10.36	0.08	0.061	0.055
	EAT	18.26	0.41	<0.001	<0.001
	LS+EAT	4.33	0.23	0.181	0.002
Rev-erb	NC	9.46	1.38	<0.001	0.001
	LS	18.81	1.76	0.002	<0.001
	EAT	8.85	1.01	<0.001	0.002
	LS+EAT	23.74	0.41	0.005	0.004
Ror	NC	22.51	0.41	<0.001	<0.001
	LS	9.58	0.04	0.710	0.028
	EAT	15.72	0.30	<0.001	<0.001
	LS+EAT	14.04	0.58	0.004	0.014

Supplementary Table 7. Effects of EAT and light shift on clock gene expression in mice.

Clock genes	Treatment group	Two-way ANOVA analysis p value			
		Time	EAT	LS	interaction between treatment and time
Bmal1	NC vs EAT	<0.001	0.894	-	<0.001
	NC vs LS	<0.001	-	0.384	<0.001
	EAT vs EAT+LS	0.027	-	0.089	0.302
Clock	NC vs EAT	<0.001	0.007	-	<0.001
	NC vs LS	0.003	-	0.188	<0.001
	EAT vs EAT+LS	<0.001	-	<0.001	<0.001
Per2	NC vs EAT	<0.001	0.344	-	0.323
	NC vs LS	0.128	-	0.890	0.098
	EAT vs EAT+LS	<0.001	-	<0.001	<0.001
Cry1	NC vs EAT	<0.001	0.307	-	<0.001
	NC vs LS	0.002	-	0.027	<0.001
	EAT vs EAT+LS	<0.001	-	0.057	<0.001
Rev-erb	NC vs EAT	<0.001	0.004	-	0.582
	NC vs LS	<0.001	-	<0.001	<0.001
	EAT vs EAT+LS	0.016	-	0.022	<0.001
Ror	NC vs EAT	<0.001	0.013	-	<0.001
	NC vs LS	<0.001	-	0.009	<0.001
	EAT vs EAT+LS	<0.001	-	<0.001	0.202



Supplementary Figure 4. Cosinor fitting curve of clock genes expression in thyroid.

The horizontal axis represents ZT time, and the vertical axis represents the relative expression of clock genes. n=5-6/group. NC, normal control; EAT, experimental autoimmune thyroiditis; LS, exposure to 4 weeks of light shift.