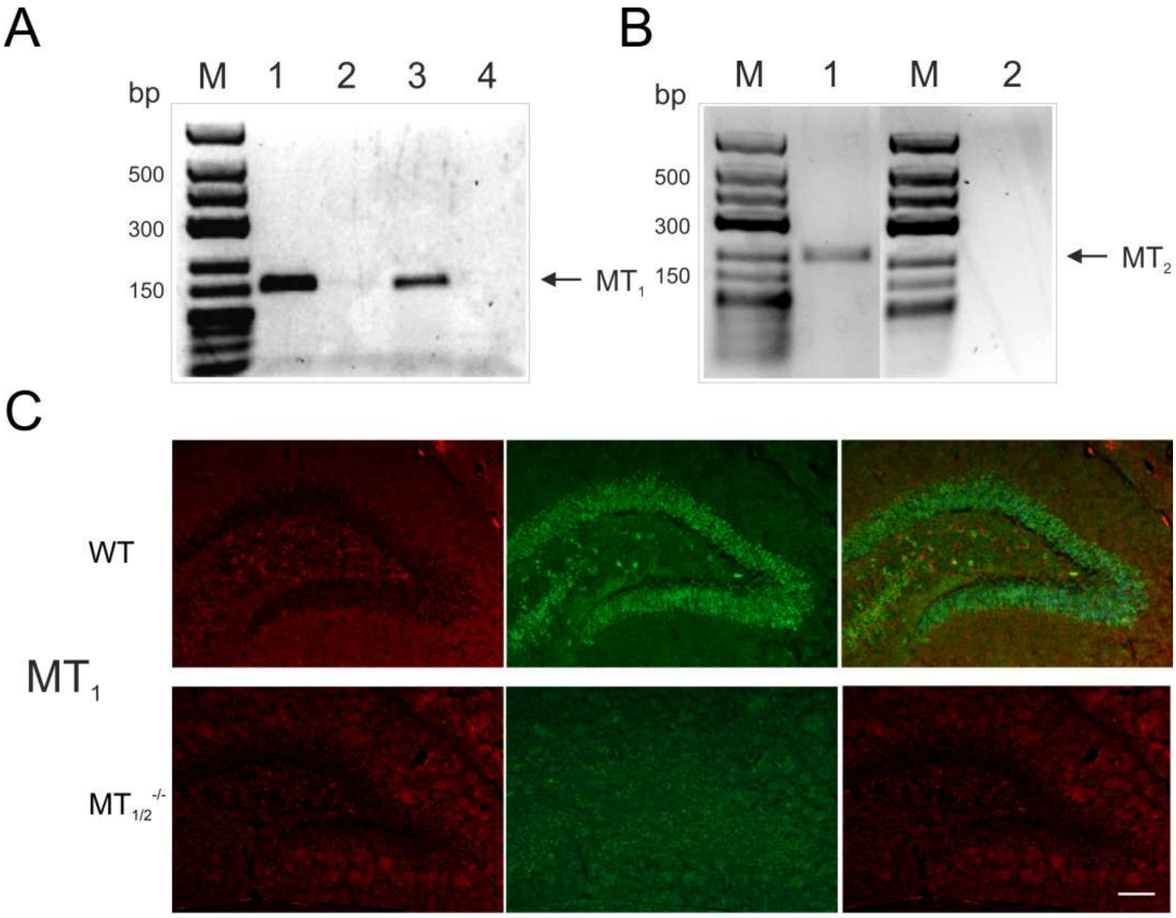
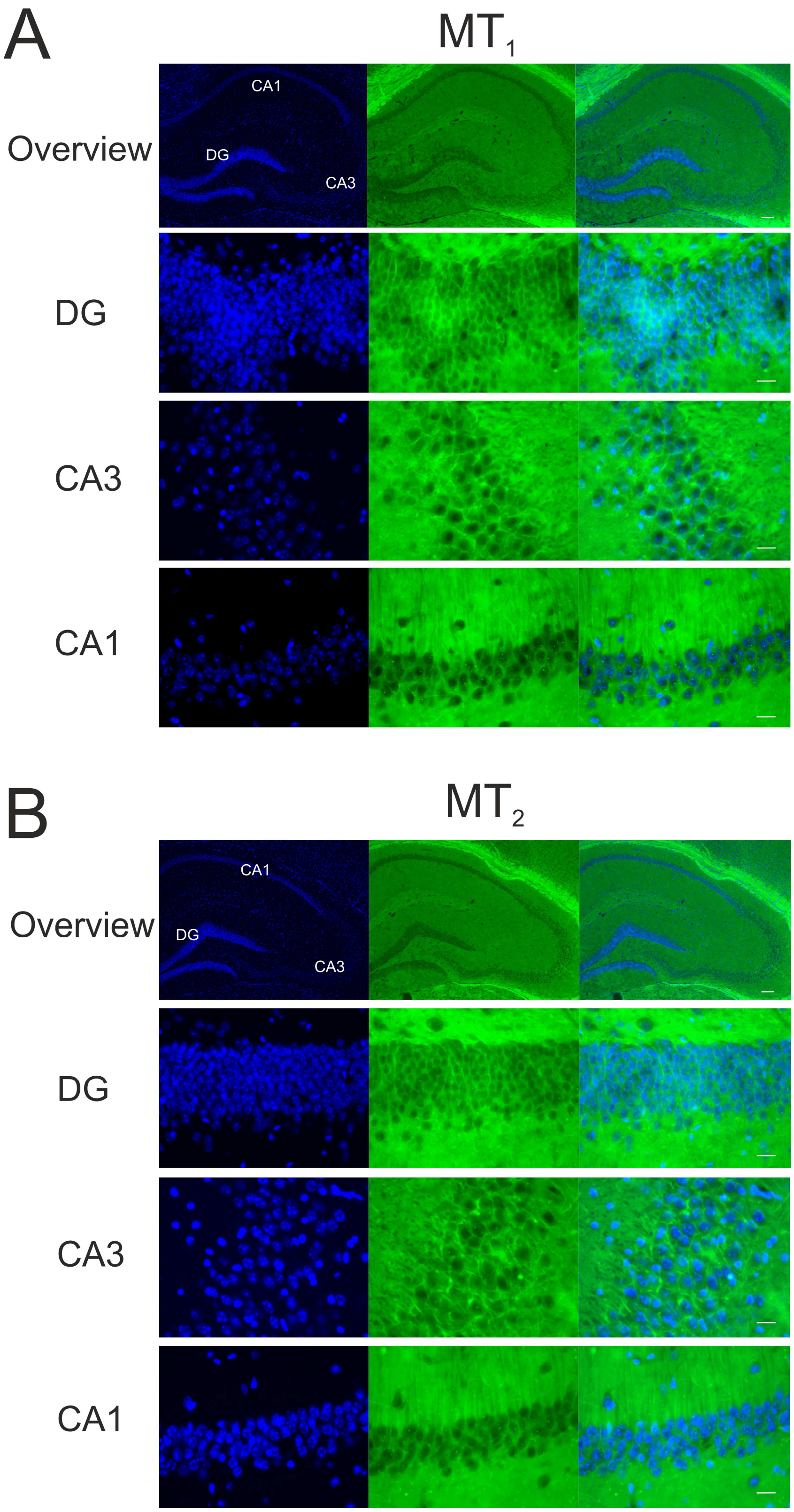
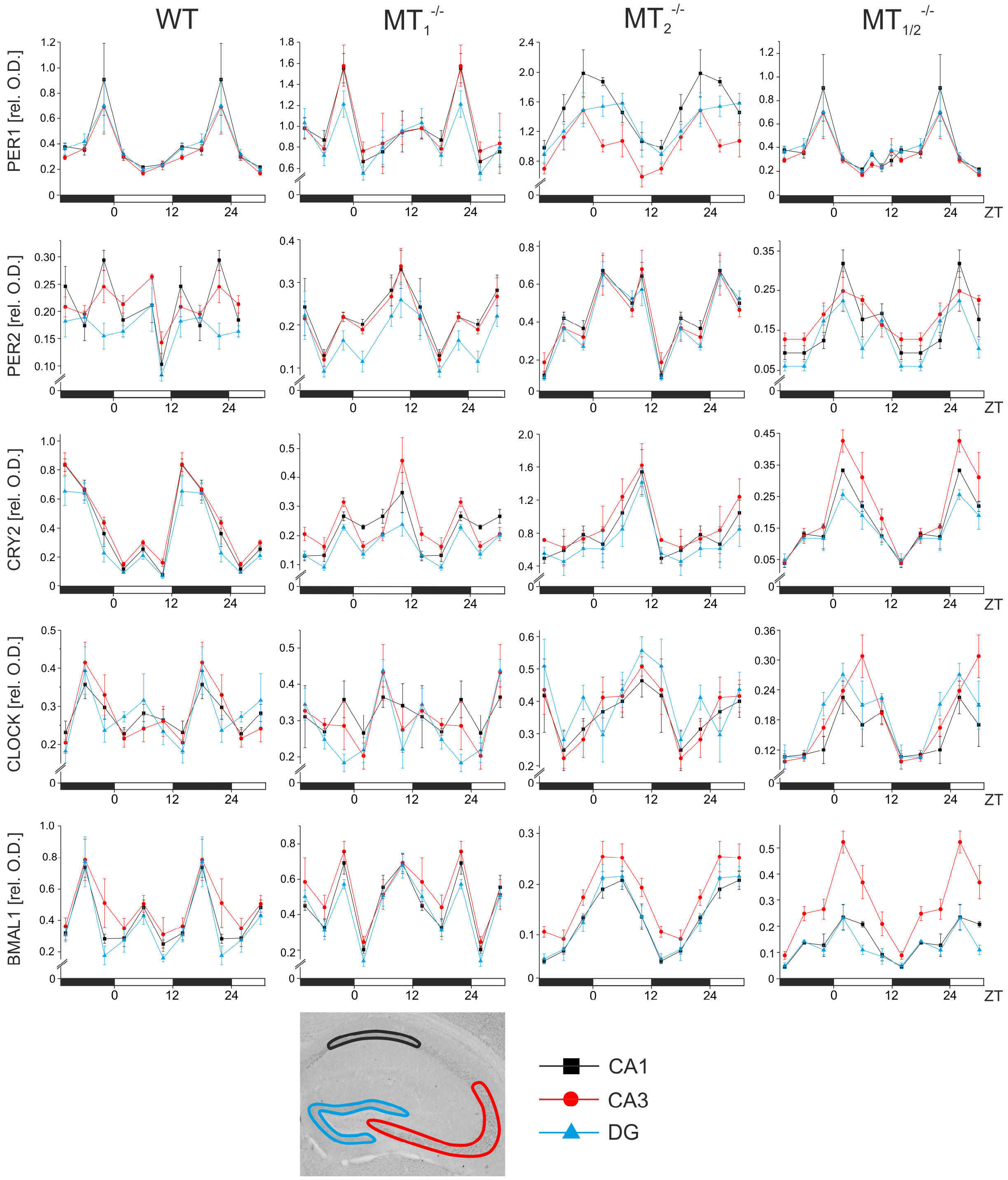
Supplemental Figures



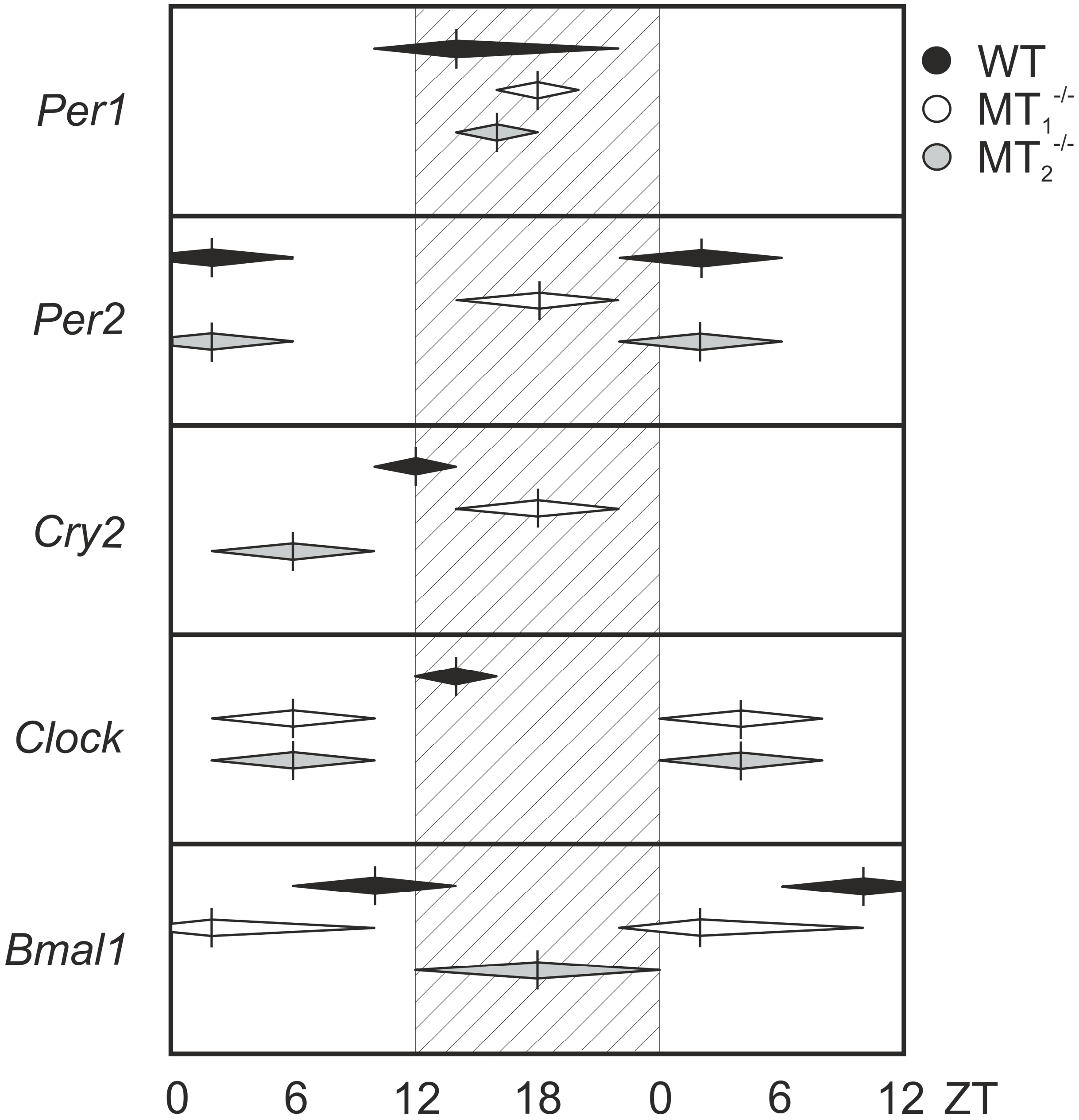
Suppl. Fig. 1: MT receptor expression in mouse hippocampus. (A) PCR products, obtained using MT_1 receptor-specific primers with mRNA extracted from SCN tissue (lanes 1, 2), or from hippocampus (lanes 3, 4) of WT mice (lanes 1, 3), or in $MT_1^{-/-}$ mice (lanes 2, 4). (B) PCR products, obtained using MT_2 receptor-specific primers with mRNA extracted from hippocampus of WT mice (lane 1), or $MT_2^{-/-}$ mice (lane 2). M, size marker; bp, base pairs as indicated on the right. N = 3. (C) Representative immunofluorescence staining for MT-receptors (green; middle panel; antibody specificity see: [49] and Tab.1) and MAP2 (red; left panel) in the hippocampus of WT (upper panel) and $MT_{1/2}^{-/-}$ (lower panel) mice. Right panel shows overlay of MT receptor and MAP2 staining. Scale bar: 100µm.



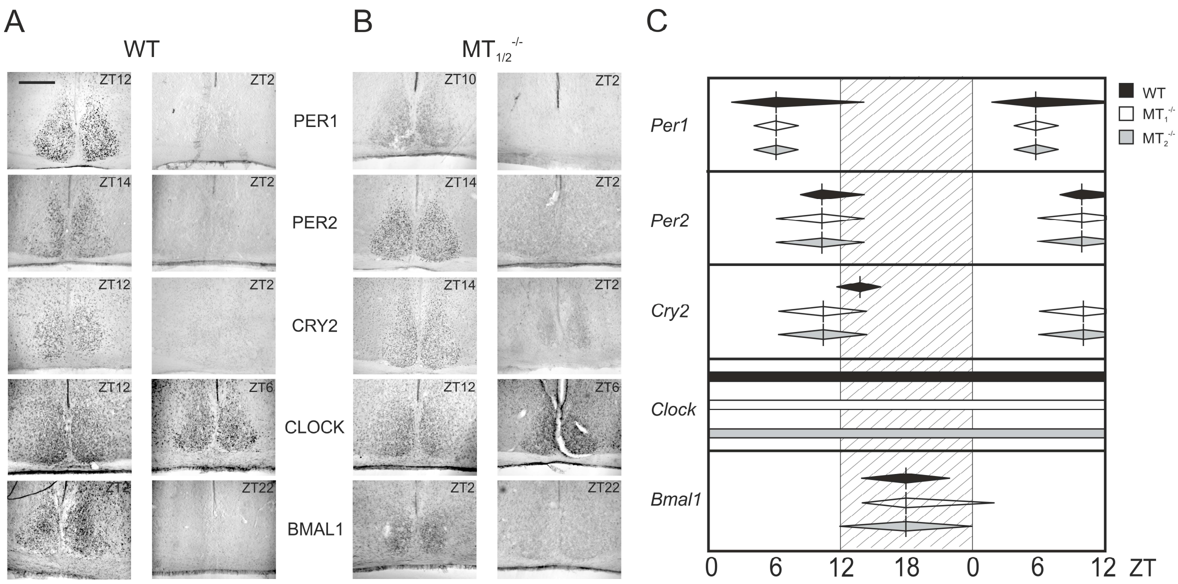
Suppl. Fig. 2: Representative immunohistochemical images of (A) MT_1 and (B) MT_2 receptors, respectively, within subregions of WT mice hippocampus, using antibody AMR-031 (see Tab. 1). Left column, DAPI, middle column, MT receptor staining, right column, overlay. Scale bars: overview, 100µm; DG, CA3, CA1, 20µm.



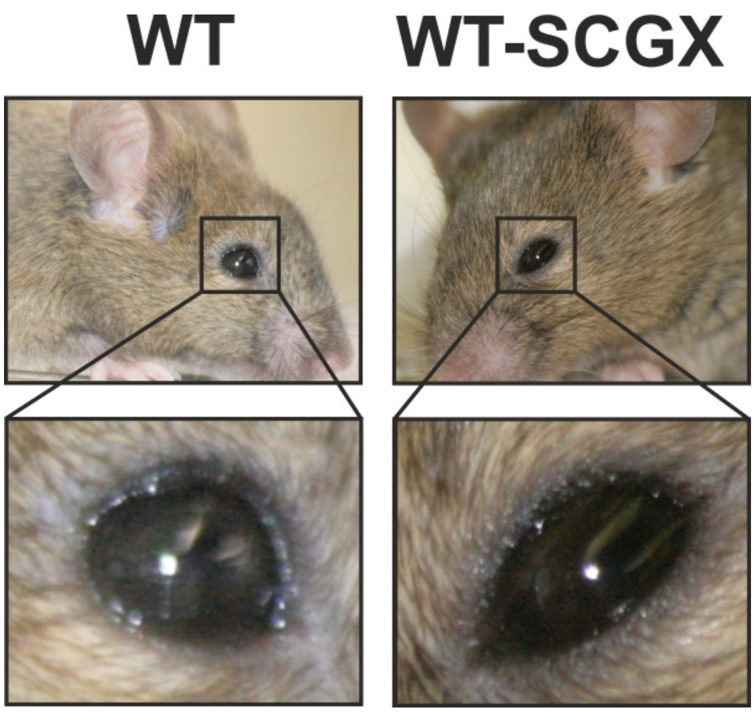
Suppl. Fig. 3: Time-of-day-dependent profiles of PER1, PER2, CRY2, CLOCK, and BMAL1 in clock gene protein levels in hippocampal sections, analyzed per subfield (CA1 [-■-], CA3 [-●-], and DG [-▲-]; see inset image) in WT, $MT_1^{-/-}$, $MT_2^{-/-}$, and $MT_{1/2}^{-/-}$ mice. Values were assessed by semiquantitative densitometric analyses of immunohistochemical signals and are expressed as relative optical densities (rel. O.D.; means ± SEM; n = 3). Data are double-blotted against Zeitgeber time (ZT) for clarity reasons. For significances see Suppl. Tab. 10.



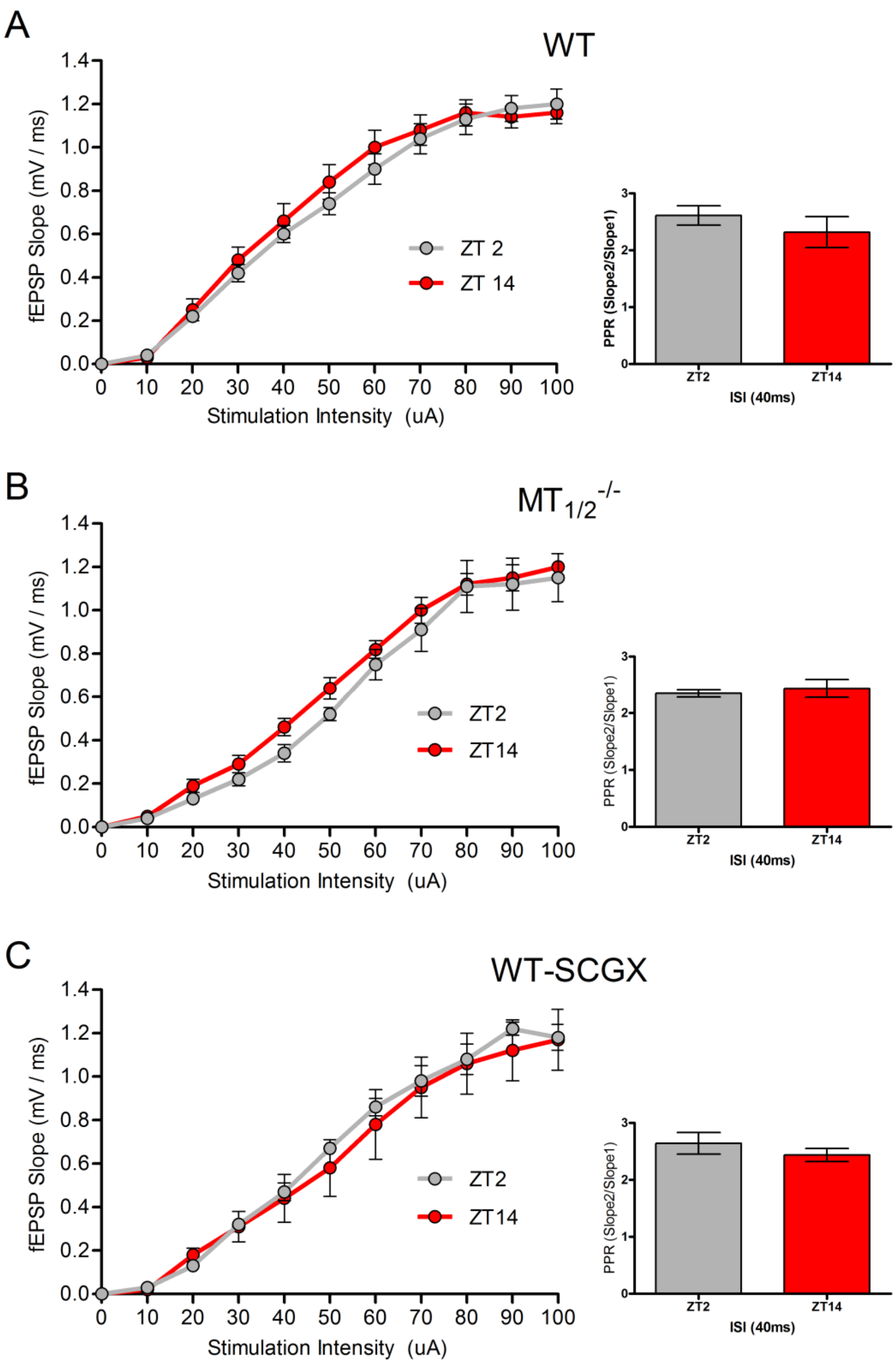
Suppl. Fig. 4: Phase-relationship of mouse hippocampal peak clock gene RNA expression values in WT, $MT_1^{-/-}$ and $MT_2^{-/-}$ mice. Peak values of clock gene RNA, analyzed by the second derivate maximum method (SDMM; for details see [34]), are shown. Vertical bars represent times of peak expression, horizontal extents of deltoids indicate the approximate times of the rise to, and the decline from maximal clock gene mRNA expression values, with levels estimated by eye fitting on the basis of the original data sets.



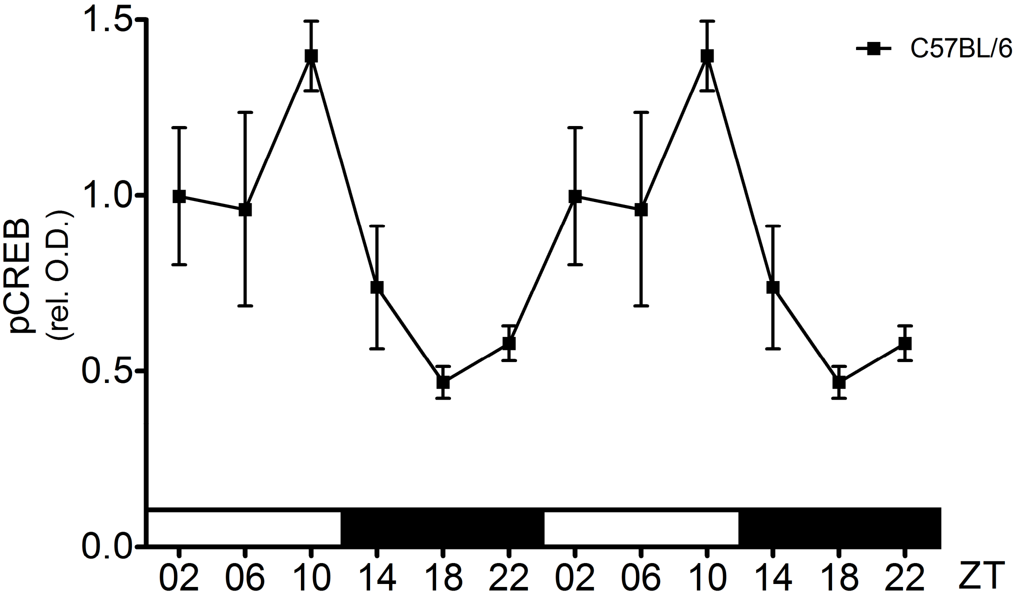
Suppl. Fig. 5: Clock gene expression in the SCN of WT and $MT^{-/-}$ mice. Comparison of peak (left panels) and trough (right panels) levels in clock gene protein levels for PER1, PER2, CRY2, CLOCK and BMAL1 in the SCN of WT (A) and $MT_{1/2}^{-/-}$ (B) mice. N = 3 with 3 sections/animal. (C) Comparison of phase-relationship of peak values in clock gene expression values in the SCN. Peak values of clock gene mRNA expression were assessed by second derivative maximum method (SDMM; for details see reference [18]). Vertical bars represent times of peak mRNA expression, horizontal extents of deltoids indicate the approximate times of the rise to, and the decline from maximal values, with levels estimated by eye fitting on the basis of the original data sets.



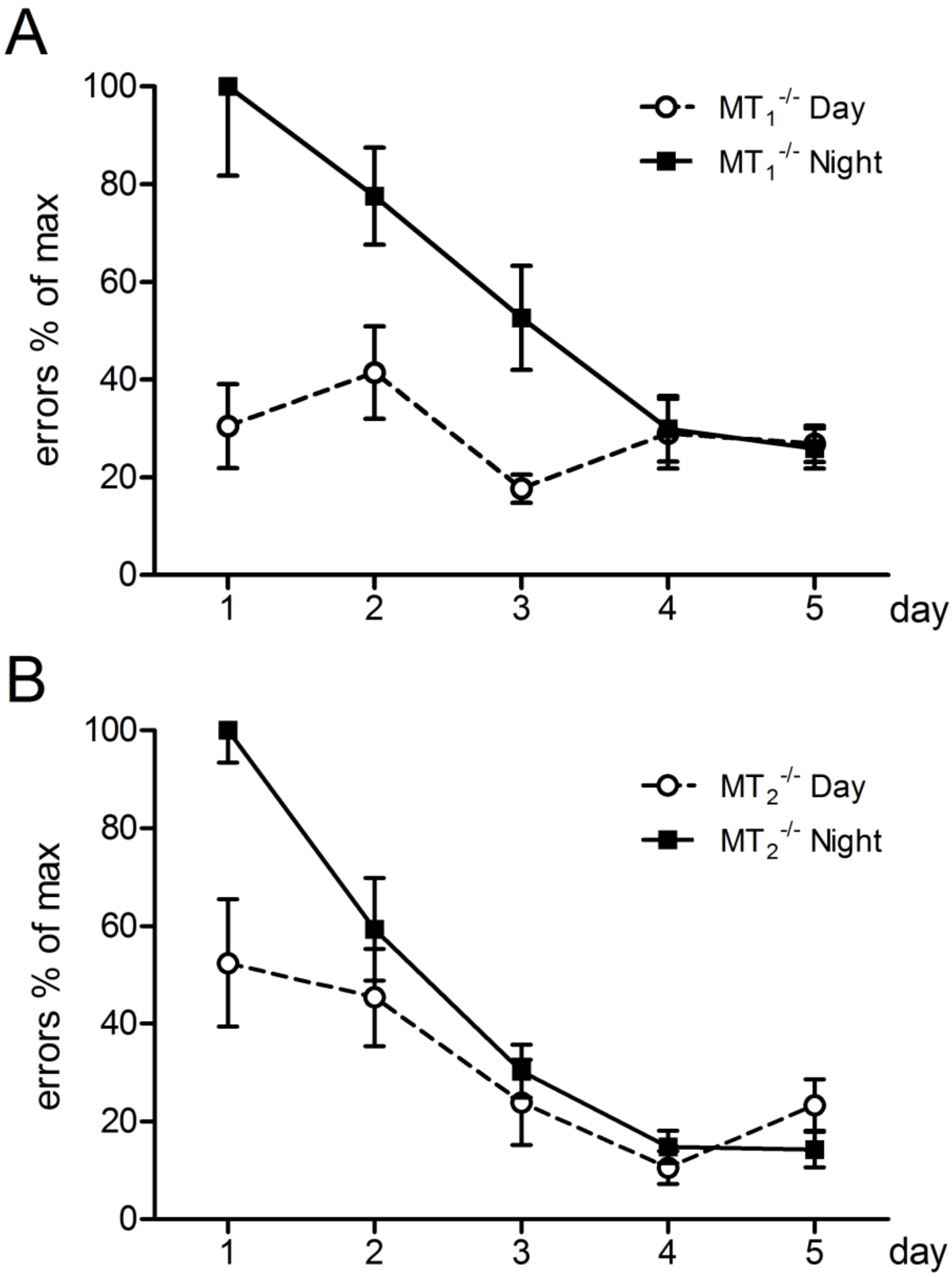
Suppl. Fig.6: Ptosis, miosis and enophthalmus (Horner's syndrome) in WT-SCGX mice as compared to WT controls.



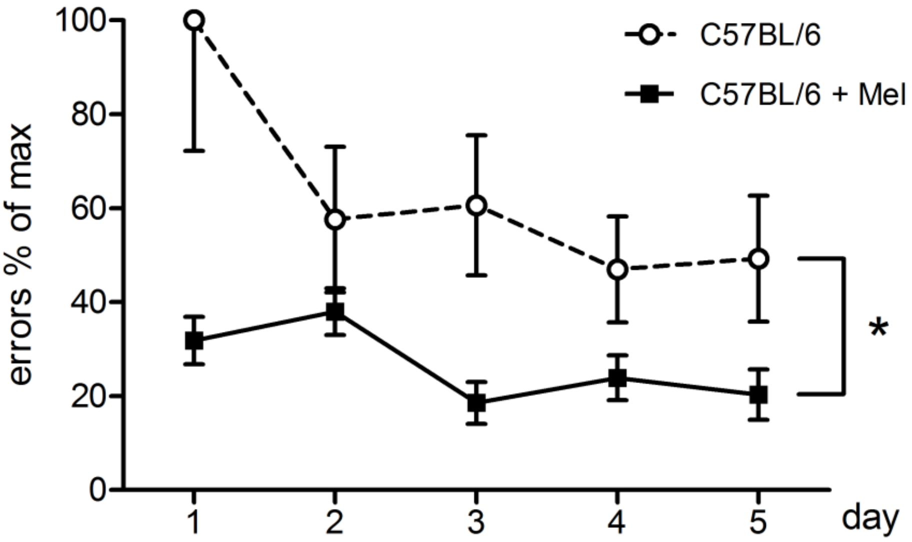
Suppl. Fig. 7. Day and night input-output strength and paired pulse facilitation at inter-stimulus interval in WT, $MT_{1,2}^{-/-}$ and WT-SCGX mice. Input-output strength and paired pulse facilitation at inter-stimulus interval of 40ms showed no significant differences between ZT2 and ZT14 in WT (A), $MT_{1,2}^{-/-}$ (B) or WT-SCGX mice (C). Values are described as mean ±SEM.



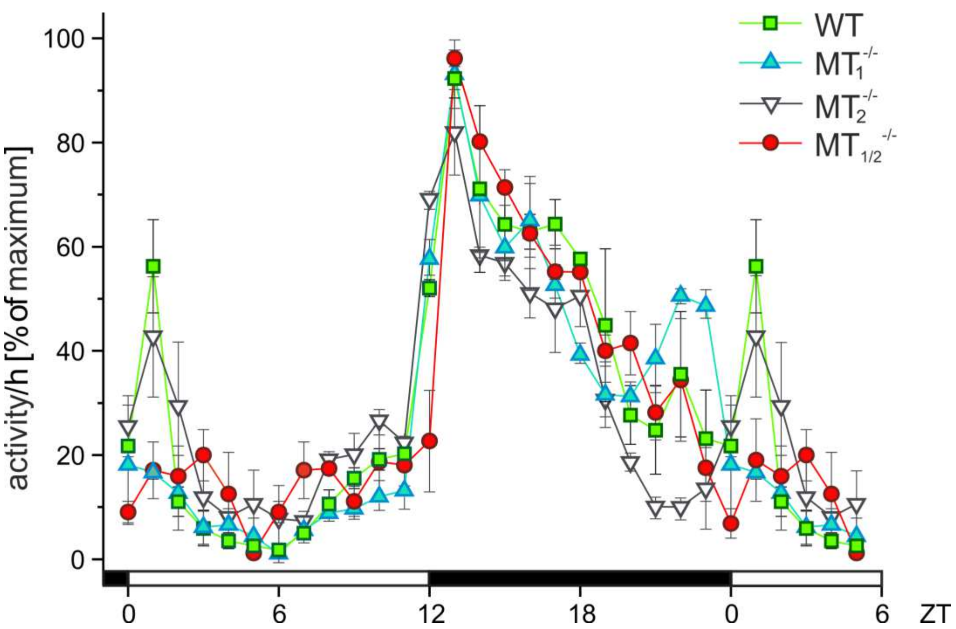
Suppl. Fig. 8: Time-of-day-dependent CREB phosphorylation in the hippocampus of C57BL/6 mice. Semiquantitative densitometric analysis of immunohistochemical signals in the hippocampus of C57BL/6 mice (n = 3) expressed as relative optical density (rel. O.D.). Data show a significant time-of-day-dependent rhythm in CREB phosphorylation ($P < 0.05$). Values are illustrated as mean ± SEM.



Suppl. Fig. 9: Spatial learning in $MT_1^{-/-}$ (A) and $MT_2^{-/-}$ mice (B) mice. Indicated are errors as percent of maximal values (errors % of max) in the 8-arm radial-arm maze test. Significant differences in the One-way ANOVA: A, Night: $P \leq 0.0001$; B, Day: $P \leq 0.05$, Night: $P \leq 0.0001$; significant differences in the Two-way ANOVA: A,B, Day vs. Night: $P \leq 0.0001$. Values are expressed as mean ± SEM.



Suppl. Fig 10: Spatial learning abilities of C57BL/6 mice with or without melatonin treatment. The effect of melatonin treatment (0.1 mg/ml) at nighttime (■), in the 8-arm radial maze test over 5 days of consecutive daytime training sessions (ZT02), compared to untreated melatonin-deficient C57BL/6 mice (○) training sessions (n = 7). (*: P ≤ 0.05 column factor two-way ANOVA). Values are expressed as mean ± SEM.



Suppl. Fig. 11: Diurnal activity patterns of here used mouse strains. Locomotor activity was assessed over 14 consecutive days using an infrared detection device. The accumulated averaged activity of a given animal, analyzed in 1 hour time bins, is shown. No differences are observed at any time points between strains tested. For clarity reasons, part of the data is double-plotted.

Supplemental Tables

Suppl. Tab. 1: Statistical analyses of dynamics in diurnal clock gene protein levels in mouse hippocampus. One-way ANOVA with Bonferroni test for each protein level (df = 5).

WT

PER1

ZT	**2**	**6**	**10**	**14**	**18**	**22**
2						0.05
6				0.05	0.05	0.05
10					0.05	0.05
14		0.05				
18		0.05	0.05			0.05
22	0.05	0.05	0.05		0.05	

PER2

ZT	**2**	**6**	**10**	**14**	**18**	**22**
2			0.05			
6						
10	0.05			0.05	0.05	0.05
14			0.05			
18			0.05			
22			0.05			

CRY2

ZT	**2**	**6**	**10**	**14**	**18**	**22**
2		0.05		0.05	0.05	0.05
6	0.05		0.05	0.05	0.05	
10		0.05		0.05	0.05	0.05
14	0.05	0.05	0.05			0.05
18	0.05	0.05	0.05			0.05
22	0.05		0.05	0.05	0.05	

CLOCK

ZT	**2**	**6**	**10**	**14**	**18**	**22**
2					0.05	
6				0.05	0.05	
10					0.05	
14		0.05			0.05	0.05
18	0.05	0.05	0.05	0.05		0.05
22				0.05	0.05	

BMAL1

ZT	**2**	**6**	**10**	**14**	**18**	**22**
2		0.05			0.05	
6	0.05		0.05		0.05	0.05
10		0.05			0.05	
14					0.05	
18	0.05	0.05	0.05	0.05		0.05
22		0.05			0.05	

$MT_1^{-/-}$

PER1

ZT	**2**	**6**	**10**	**14**	**18**	**22**
2			0.05	0.05		0.05
6						0.05
10	0.05					0.05
14	0.05					0.05
18						0.05
22	0.05	0.05	0.05	0.05	0.05	

PER2

ZT	**2**	**6**	**10**	**14**	**18**	**22**
2		0.05	0.05		0.05	
6	0.05				0.05	
10	0.05			0.05	0.05	0.05
14			0.05		0.05	
18	0.05	0.05	0.05	0.05		0.05
22			0.05		0.05	

CRY2

ZT	**2**	**6**	**10**	**14**	**18**	**22**
2			0.05			0.05
6			0.05		0.05	
10	0.05	0.05		0.05	0.05	
14			0.05			0.05
18		0.05	0.05			0.05
22	0.05			0.05	0.05	

CLOCK

ZT	**2**	**6**	**10**	**14**	**18**	**22**
2		0.05				
6	0.05		0.05		0.05	0.05
10		0.05				
14						
18		0.05				
22		0.05				

BMAL1

ZT	**2**	**6**	**10**	**14**	**18**	**22**
2		0.05	0.05	0.05	0.05	0.05
6	0.05		0.05		0.05	0.05
10	0.05	0.05		0.05	0.05	
14	0.05		0.05		0.05	0.05
18	0.05	0.05	0.05	0.05		0.05
22	0.05	0.05		0.05	0.05	

$MT_2^{-/-}$

PER1

ZT	2	6	10	14	18	22
2			0.05	0.05		
6				0.05		
10	0.05					0.05
14	0.05	0.05				0.05
18						
22			0.05	0.05		

PER2

ZT	2	6	10	14	18	22
2		0.05		0.05	0.05	0.05
6	0.05		0.05	0.05	0.05	0.05
10		0.05		0.05	0.05	0.05
14	0.05	0.05	0.05		0.05	0.05
18	0.05	0.05	0.05	0.05		
22	0.05	0.05	0.05	0.05		

CRY2

ZT	2	6	10	14	18	22
2		0.05	0.05			
6	0.05		0.05	0.05	0.05	0.05
10	0.05	0.05		0.05	0.05	0.05
14		0.05	0.05			
18		0.05	0.05			
22		0.05	0.05			

CLOCK

ZT	2	6	10	14	18	22
2			0.05			
6					0.05	
10	0.05				0.05	0.05
14					0.05	
18		0.05	0.05	0.05		
22			0.05			

BMAL1

ZT	2	6	10	14	18	22
2			0.05	0.05	0.05	0.05
6			0.05	0.05	0.05	0.05
10	0.05	0.05		0.05	0.05	
14	0.05	0.05	0.05			0.05
18	0.05	0.05	0.05			0.05
22	0.05	0.05		0.05	0.05	

$MT_{1/2}^{-/-}$

PER1

ZT	2	6	10	14	18	22
2						0.05
6				0.05	0.05	0.05
10					0.05	0.05
14		0.05				0.05
18		0.05	0.05			0.05
22	0.05	0.05	0.05	0.05	0.05	

PER2

ZT	2	6	10	14	18	22
2		0.05	0.05	0.05	0.05	0.05
6	0.05					
10	0.05			0.05	0.05	
14	0.05		0.05			
18	0.05		0.05			
22	0.05					

CRY2

ZT	2	6	10	14	18	22
2		0.05	0.05	0.05	0.05	0.05
6	0.05		0.05	0.05	0.05	0.05
10	0.05	0.05				
14	0.05	0.05				0.05
18	0.05	0.05				
22	0.05	0.05		0.05		

CLOCK

ZT	2	6	10	14	18	22
2				0.05	0.05	0.05
6				0.05	0.05	
10						
14	0.05	0.05				
18	0.05	0.05				
22	0.05					

BMAL1

ZT	2	6	10	14	18	22
2		0.05	0.05	0.05	0.05	0.05
6	0.05			0.05		
10	0.05					
14	0.05	0.05			0.05	
18	0.05			0.05		
22	0.05					

Suppl. Tab. 2: Comparison of clock gene protein expression in the hippocampus between WT and $MT^{-/-}$ mice. Two-way ANOVA with Bonferroni post test (genotype: df = 3; clock-gene proteins: df = 4).

WT	**$MT_1^{-/-}$**	**$MT^{2-/-}$**	**$MT_{1/2}^{-/-}$**
PER1	0.05	0.05	n.s.
PER2	n.s.	0.05	0.05
CRY2	0.05	0.05	0.05
CLOCK	0.05	0.05	0.05
BMAL1	n.s.	0.05	0.05

Suppl. Tab. 3: Time-of-day-dependent pCREB profiles in the hippocampus of WT, $MT_{1/2}^{-/-}$ and C57BL/6 mice. Shown are P values < 0.05 (One-way and Two-way ANOVA).

One-way analysis of variance: WT pCREB

P value = 0.0004 df = 5

Bonferroni's Multiple Comparison Test

ZT	2	6	10	14	18	22
2			0.05	0.05	0.05	
6			0.05	0.05	0.05	
10	0.05	0.05				
14	0.05	0.05				
18	0.05	0.05				0.05
22					0.05	

One-way analysis of variance: $MT_{1/2}^{-/-}$pCREB

P value = 0.002 df = 5

Bonferroni's Multiple Comparison Test

ZT	2	6	10	14	18	22
2						
6			0.05	0.05	0.05	
10		0.05				
14		0.05				
18		0.05				
22						

One-way analysis of variance: C57BL/6 pCREB

P value = 0.0183 df = 5

Bonferroni's Multiple Comparison Test

ZT	2	6	10	14	18	22
2						
6						
10					0.05	
14						
18			0.05			
22						

Two-way ANOVA: WT vs $MT_{1/2}^{-/-}$

Interaction	P = 0.2679	ns	df = 5
Genotype	P = 0.0338	*	df = 1
Time	P < 0.0001	****	df = 5

Bonferroni's Multiple Comparison Test:

ZT	
2	ns
6	ns
10	ns
14	ns
18	ns
22	ns

Suppl. Tab. 4: Statistical analysis of in the radial arm maze performance in WT mice.

One-way analysis of variance: WT day

P value	< 0.00001	df = 4

Bonferroni's Multiple Comparison Test

Day	1	2	3	4	5
1		0.05	0.05	0.05	0.05
2	0.05				
3	0.05				
4	0.05				
5	0.05				

One-way analysis of variance: WT night

P value	= 0.0110	df = 4

Bonferroni's Multiple Comparison Test

Day	1	2	3	4	5
1					0.05
2					
3					
4					
5	0.05				

Two-way ANOVA: WT day vs night

Interaction	P = 0.6429	ns	df = 4
Treatment	P = 0.0250	*	df = 1
Time	P < 0.0001	****	df = 4

Bonferroni's Multiple Comparison Test

day	
1	ns
2	ns
3	ns
4	ns
5	ns

Suppl. Tab. 5 Statistical analysis of in the radial arm maze performance in $MT_1^{-/-}$ mice.

One-way analysis of variance: $MT_1^{-/-}$ day

P value = 0.2069	df = 4

Bonferroni's Multiple Comparison Test

Day	1	2	3	4	5
1					
2					
3					
4					
5					

One-way analysis of variance: $MT_1^{-/-}$ night

P value < 0.0001	df = 4

Bonferroni's Multiple Comparison Test

Day	1	2	3	4	5
1			0.05	0.05	0.05
2				0.05	0.05
3	0.05				
4	0.05	0.05			
5	0.05	0.05			

Two-way ANOVA: $MT_1^{-/-}$ day vs night

Interaction	P = 0.0014	**	df = 4
Treatment	P < 0.0001	****	df = 1
Time	P < 0.0001	****	df = 4

Bonferroni's Multiple Comparison Test

day	
1	0.05
2	0.05
3	ns
4	ns
5	ns

Suppl. Tab. 6: Statistical analysis of in the radial arm maze performance in $MT_2^{-/-}$ mice.

One-way analysis of variance: $MT_2^{-/-}$ day

P value = 0.0102	df = 4

Bonferroni's Multiple Comparison Test

Day	1	2	3	4	5
1				0.05	
2					
3					
4	0.05				
5					

One-way analysis of variance: $MT_2^{-/-}$ night

P value < 0.0001	df = 4

Bonferroni's Multiple Comparison Test

Day	1	2	3	4	5
1		0.05	0.05	0.05	0.05
2	0.05		0.05	0.05	0.05
3	0.05	0.05			
4	0.05	0.05			
5	0.05	0.05			

Two-way ANOVA: $MT_2^{-/-}$ day vs night

Interaction	P = 0.0078	**	df = 4
Treatment	P = 0.0123	*	df = 1
Time	P < 0.0001	****	df = 4

Bonferroni's Multiple Comparison Test

day	
1	0.05
2	ns
3	ns
4	ns
5	ns

Suppl. Tab. 7: Statistical analysis of in the radial arm maze performance in $MT_{1/2}^{-/-}$ mice.

One-way analysis of variance: $MT_{1/2}^{-/-}$ day

P value = 0.0006	df = 4

Bonferroni's Multiple Comparison Test

Day	1	2	3	4	5
1		0.05	0.05	0.05	0.05
2	0.05				
3	0.05				
4	0.05				
5	0.05				

One-way analysis of variance: $MT_{1/2}^{-/-}$ night

P value = 0.8157	df = 4

Bonferroni's Multiple Comparison Test

Day	1	2	3	4	5
1					
2					
3					
4					
5					

Two-way ANOVA: $MT_{1/2}^{-/-}$ day vs night

Interaction	P = 0.0430	**	df = 4
Treatment	P = 0.0379	*	df = 1
Time	P = 0.0007	***	df = 4

Bonferroni's Multiple Comparison Test

day	
1	0.05
2	ns
3	ns
4	ns
5	ns

Suppl. Tab. 8: Statistical analysis of in the radial arm maze performance in C57BL/6 and in melatonin-treated C57BL/6 mice.

One-way analysis of variance: C57BL/6 day

P value = 0.2264	df = 4

Bonferroni's Multiple Comparison Test

Day	1	2	3	4	5
1					
2					
3					
4					
5					

One-way analysis of variance: C57BL/6 +Melatonin

P value = 0.0453	df = 4

Bonferroni's Multiple Comparison Test

Day	1	2	3	4	5
1					
2					
3					
4					
5					

Two-way ANOVA: C57BL/6 day vs C57BL/6 + Melatonin

Interaction	P = 0.3319	ns	df = 4
Treatment	P < 0.0001	****	df = 1
Time	P = 0.1034	ns	df = 4

Bonferroni's Multiple Comparison Test

day	
1	0.05
2	ns
3	ns
4	ns
5	ns

Suppl. Tab. 9: Time (seconds) that mice spent in open arms of the elevated plus maze (mean values ±SD).

	MW	**SD**
WT	18.35	11.13
$MT_1^{-/-}$	27.63	20.24
$MT_2^{-/-}$	21.32	9.50
$MT_{1/2}^{-/-}$	4.44	8.82
C57BL/6	9.19	2.22

Suppl. Tab. 10: Comparison of clock gene protein expression in the subregions of the hippocampus between WT and $MT^{-/-}$ mice. Two-way ANOVA comparing dynamics for each clock gene protein between three hippocampal subregions (CA1, CA3, DG) for each genotype (genotype: df = 3; clock-gene proteins: df = 4; hippocampal subregions: df = 2).

	WT	**$MT_1^{-/-}$**	**$MT_2^{-/-}$**	**$MT_{1/2}^{-/-}$**
PER1			0.05	
PER2	0.05	0.05		0.05
CRY2	0.05	0.05		0.05
CLOCK				
BMAL1			0.05	0.05