SUPPLEMENTARY INFORMATION
for

The intrinsic microglial molecular clock controls diurnal variations of synaptic strength and locomotor activity via the circadian expression of cathepsin S

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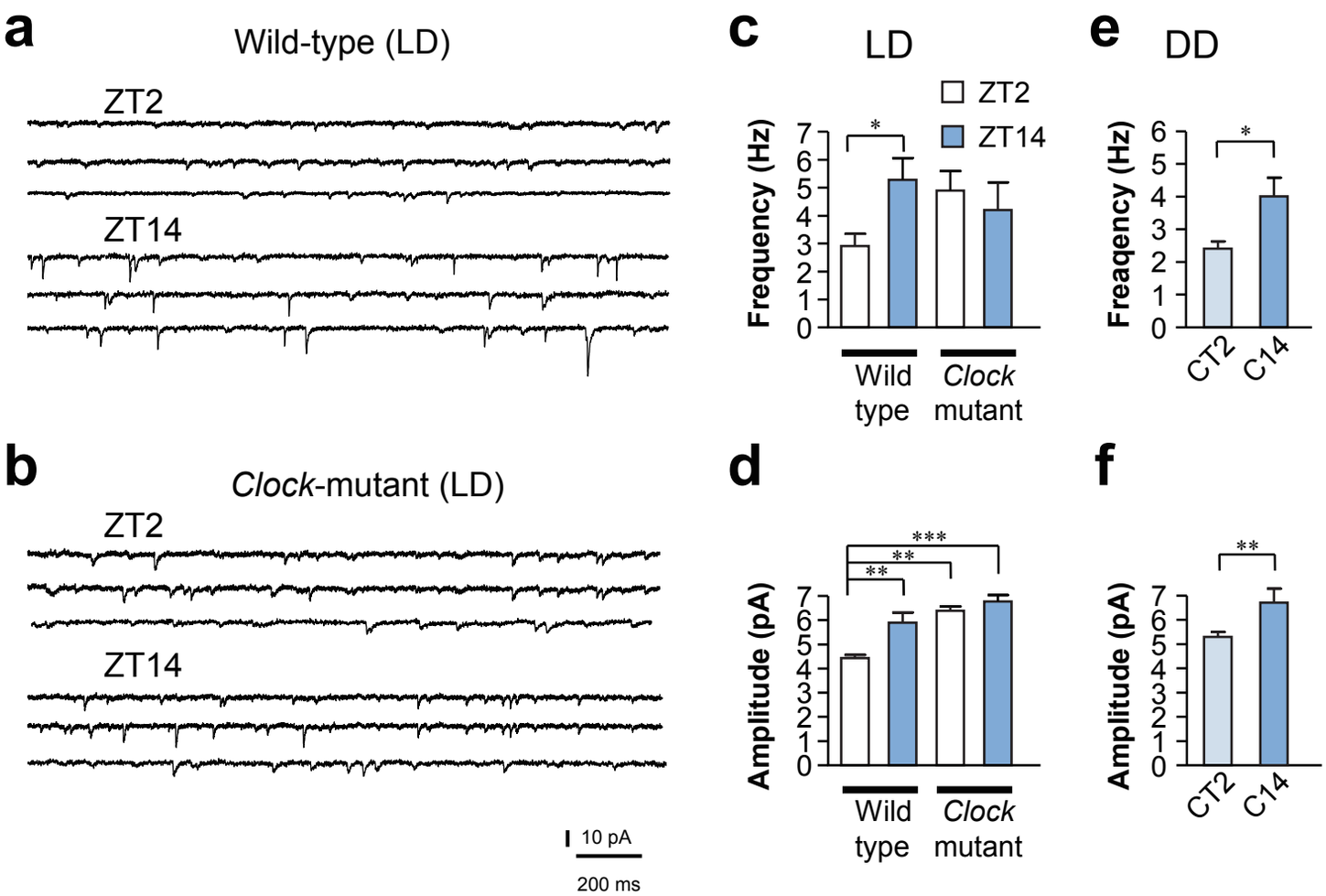
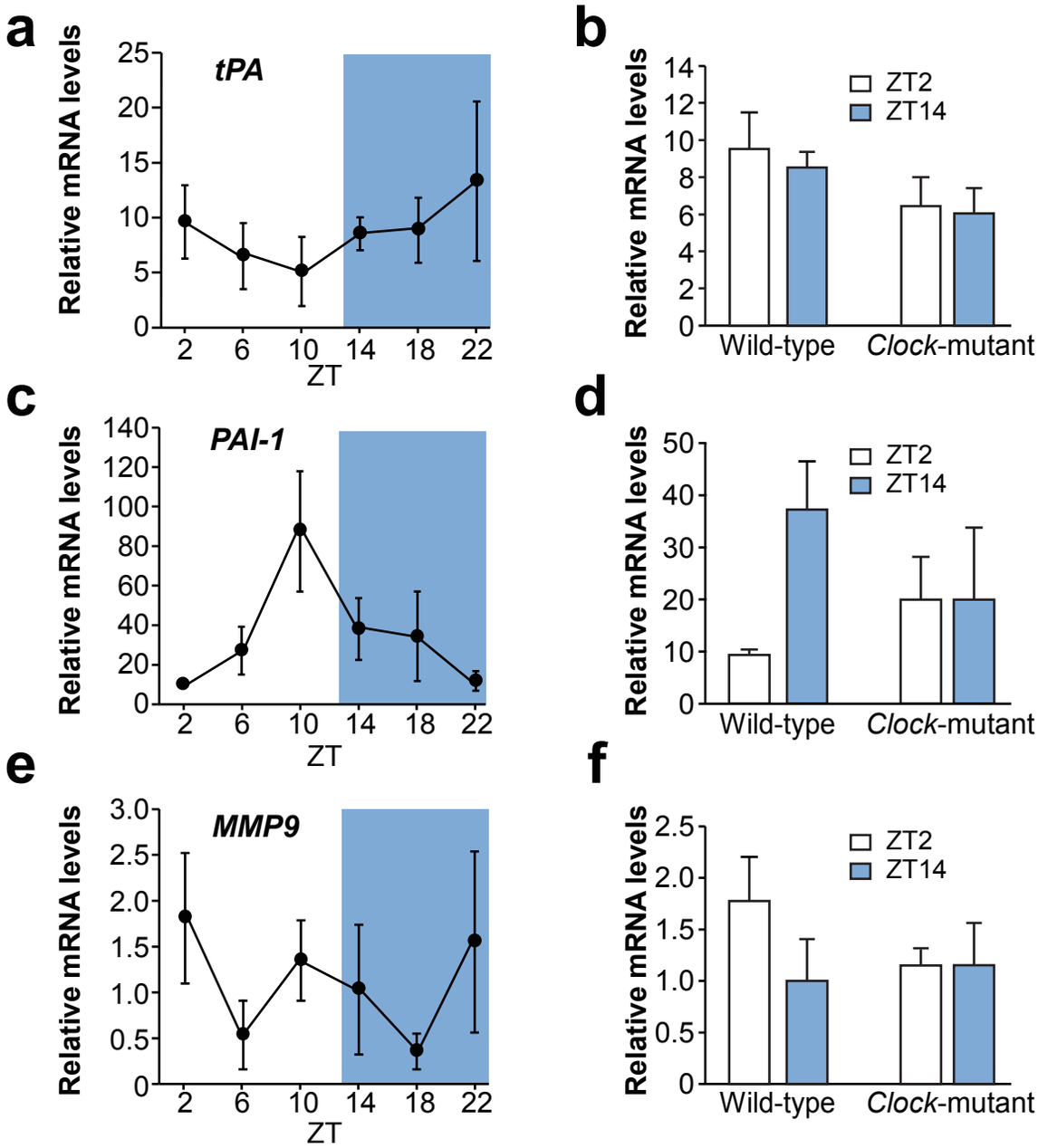
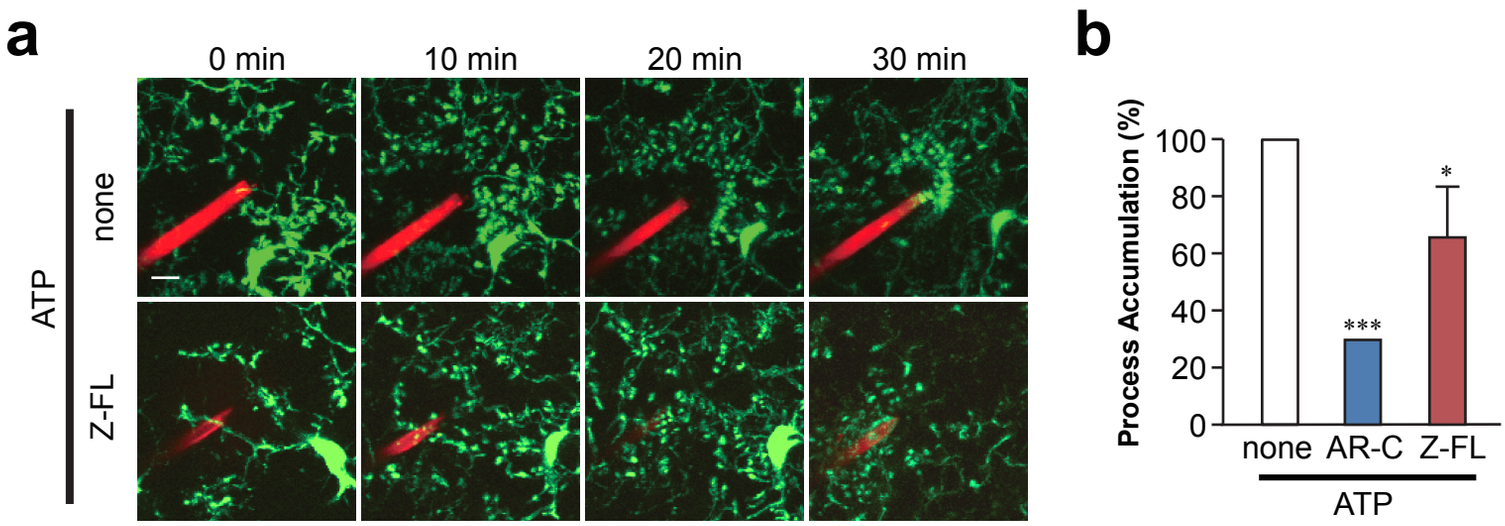
Supplementary information includes:
Supplementary Figures S1-S4
Supplementary Tables S1



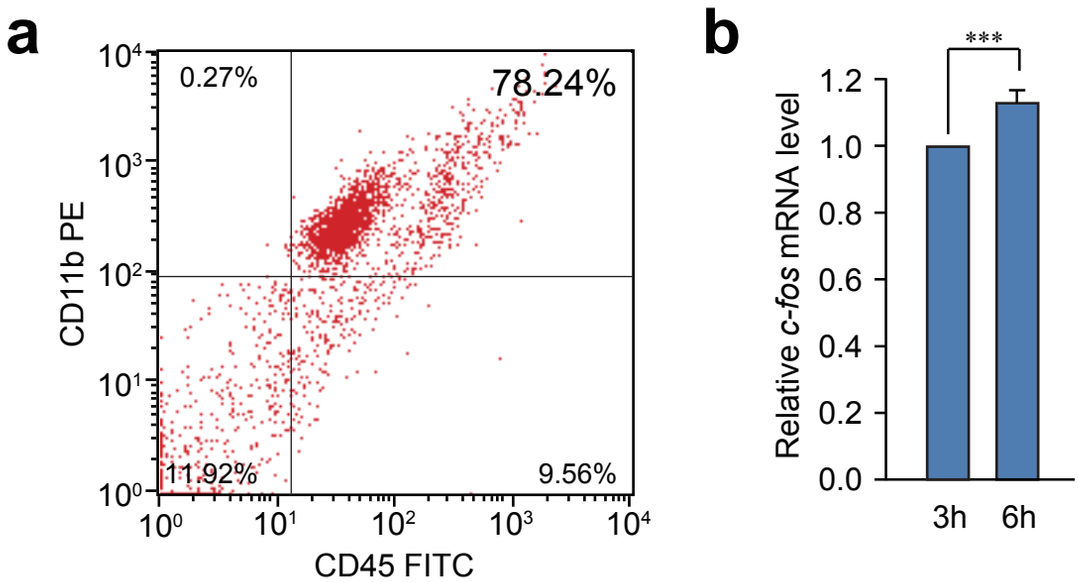
Supplementary Figure S1. Deficits in the circadian changes in the neuronal synaptic activities in *clock*-mutant mice. (**a and b**) Typical traces of mEPSCs recorded from cortical neurons from wilt-type (**a**) and *clock*-mutant mice (**b**) at ZT2 and 14. (**c and d**) The mean frequency (**c**) and amplitude (**d**) of mEPSCs recorded from wild-type and *clock*-mutant mice under the LD cycle. (**e and f**) The mean frequency (**e**) and amplitude (**f**) of mEPSCs recorded from wild-type mice under the DD cycle. The asterisks indicate statistically significant differences between values. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; two-way ANOVA (**c and d**). *, $P<0.05$; **, $P<0.01$; unpaired *t*-test (**e and f**) .The data are the means ± S.E.M. (animals = 3, n = 10-20 neurons, each).



Supplementary Figure S2. Circadian changes in the expression of *tPA*, *PAI-1* and *MMP-9* in cortical microglia. (a, c, and e) Neither *tPA* (**a**) nor *MMP-9* (**e**) showed circadian oscillation. On the other hand, *PAI-1*, an endogenous inhibitor of tPA, exhibited circadian oscillation (**c**), but this oscillation was not correlated with *Per1* or *Per2* oscillation. The data are the means ± S.E.M. (n=3, each, *P*=0.245 (**a**), 0.0015 (**c**), 0.087 (**e**), respectively. one-way ANOVA). (**b, d, and f**) The relative mean mRNA levels were normalized to the β-actin level. The data are the means ± S.E.M. (n=3 independent experiments, each).



Supplementary Figure S3. Examination of the possible involvement of CatS in the ATP-induced movenent of microglial processes in Iba1-EGFP mice using the two-photon imaging system. (**a**) GFP-expressing microglia in the somatosensory cortex were visualized in vivo using two-photon time-lapse microscopy. ATP (1 mM)-induced robust process extension toward the tip of the electrode (containing rhodamin-dextran, red), which was significantly inhibited by Z-FL-COCHO (Z-FL; a CatS inhibitor, 1 μM). Scale Bar: 10 μm. (**b**) The mean relative process accumulation of microglial processes at 30 min after the focal application of ATP (1 mM) in the presence and absence of AR-C 66096 (AR-C; a $P2Y_{12}R$ inhibitor, 10 μM) or Z-FL-COCHO (Z-FL; a CatS inhibitor, 1 μM). The asterisks indicate statistically significant differences from none-treated control (*, $P<0.05$; ***, $P<0.001$; one-way ANOVA).



Quad	Events	% Gated
UL	25	0.27
UR	885	9.56
LL	7240	78.24
LR	1103	11.92

Supplementary Figure S4. Isolation of cortical microglia from adult mice. (**a**) The purity of microglia preparation (78.24%) was assessed by a FACS analysis using PE-labeled anti-CD11b and FITC-labeled CD45. (**b**) Microglia were activated by the passage of preparation time during preparation. The preparations were completed by 3h and 6h. The expression levels of *c-fos* mRNA were measured as an index of microglial activation. The asterisks indicate statistically significant differences between values (n=3 independent experiments. ***, $P<0.001$; unpaired *t*-test).

Supplementary Table 1.

Gene	Direction	Primer sequence	Amplicons
Per1	Forward Reverse	5’-CCAGATTGGTGGAGGTTACTGAGT-3’ 5’-GCGAGAGTCTTCTTGGAGCAGTAG-3’	92 bp
Per2	Forward Reverse	5’-TTCCACTATGTGACAGCGGAGG-3’ 5’-CGTATCCATTCATGTCGGGCTC-3’	187 bp
Rev-erbα	Forward Reverse	5’-CCCTGGACTCCAATAACAACACA-3’ 5’-GCCATTGGAGCTGTCACTGTAG-3’	110 bp
Bmal1	Forward Reverse	5’-CTATCTTCCTCGGACACTGC-3 5’-CTTCTTGCCTCCTGGAGAAG-3’	216 bp
CatS	Forward Reverse	5’-ATGGCTGTTTTGGATGCCCC-3’ 5’-TTCCCAGATGAGACGCCGTA-3’	154 bp
tPA	Forward Reverse	5’-CAGCTCCCTGACTGGACAGA-3’ 5’-GCATGCATCGTGGAGGTCTT-3’	216 bp
PAI-1	Forward Reverse	5’-GGACACCCTCAGCATGTTCA-3’ 5’-TCTGATGAGTTCAGCATCCAAGA-3’	92 bp
MMP-9	Forward Reverse	5’-CAAGTGGGACCATCATAACA-3’ 5’-GCTTCGGGTCCGTACA-3’	150 bp
c-fos	Forward Reverse	5'- AGAGCGGGAATGGTGAAG -3' 5'- GGATTCTCCGTTTCTCTTCC -3'	106 bp
β-Actin	Forward Reverse	5’-CACACCTTCTACAATGAGCTGC-3’ 5’-CATGATCTGGGTCATCTTTTCA-3’	109 bp