Supplementary Information:

Orexin signaling regulates both the hippocampal clock and the expression of Alzheimer's disease-risk genes

Zhixiong Ma[1, 2], Weiliang Jiang[3], & Eric Erquan Zhang[2*]

[1]*College of Life Sciences, Beijing Normal University, Beijing 100875, China.*

[2]*National Institute of Biological Sciences, Beijing 102206, China.*

[3]*Department of Gastroenterology, Shanghai First People's Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, 200080, China.*

*Corresponding Email: zhangerquan@nibs.ac.cn

Supplementary Figure Legends:

Supplementary Figure1: Orexin A shortens the period of hippocampal circadian clock.

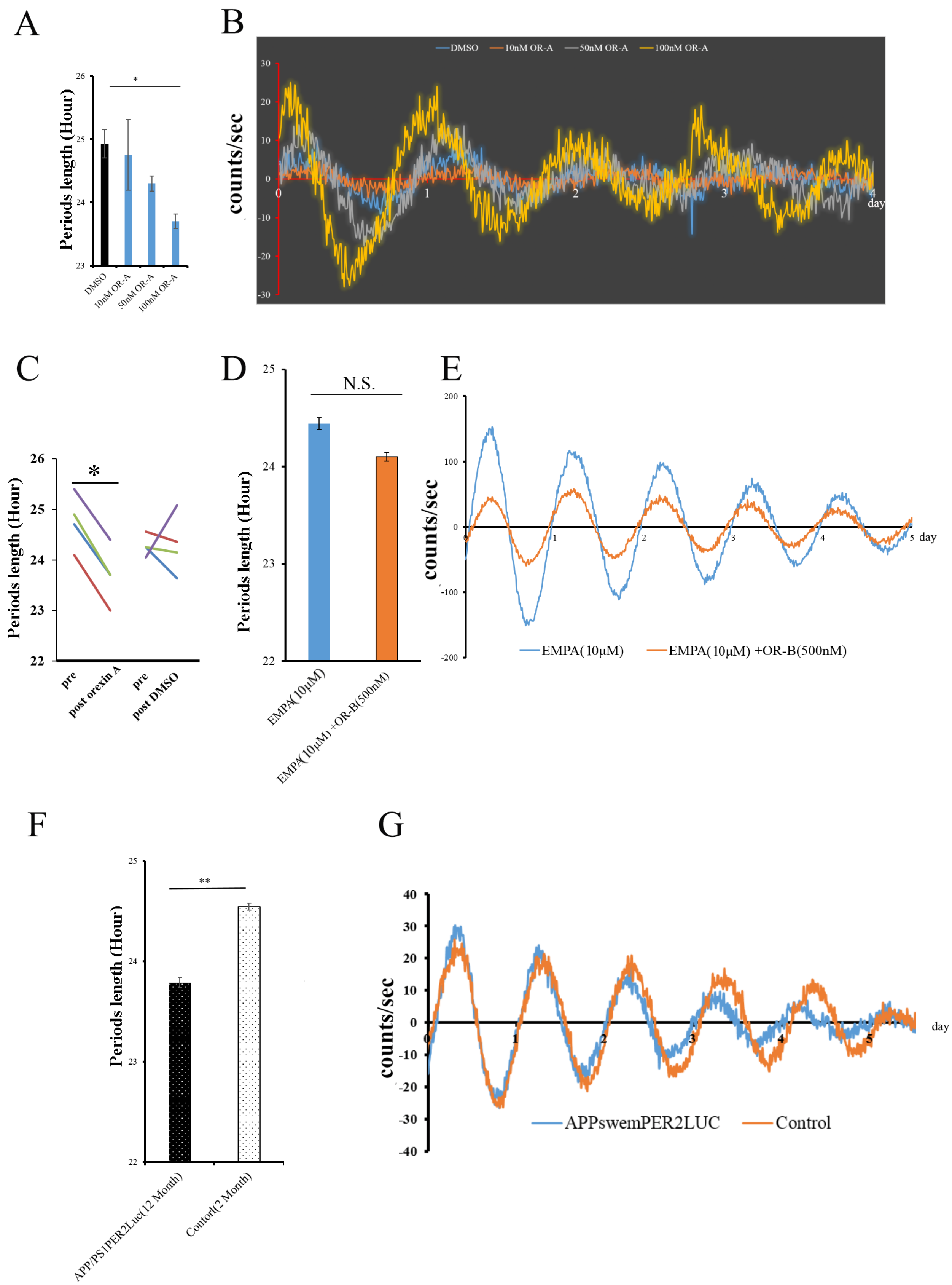
A and B：Orexin A shortens the period of hippocampal circadian clock in a dose dependent manner. (C) pre-orexin A treatment and post-orexin A shows the orexin A shortens the period of hippocampal circadian clock (n=4, * $p< 0.05$, paired T-test). D and E: The EMPA block the effect of orexin B induced shorted period in hippocampus (n=6-10, * $p<0.05$, student's T-test). F and G: The period of hippocampal circadian clock in aging APP/PS1 mice is shorter than the one in the normal WT mice (n=8-18, ** $p< 0.01$, student's T-test)

Supplementary Figure2: Diurnal expression pattern of *orexins*

(A)Diurnal expression pattern of *orexin precursor* gene in hypothalamus. . Expression orexin precursor mRNA was detected by qPCR in the hypothalamus of WT. the expression of the orexin precursor mRNA is significant different at ZT5 and ZT17 (n=4-5, * $p< 0.05$, student's T-test). (B) Immunostaining of orexin A peptide shows that the diurnal expression pattern of orexin A is consistent with the one of *orexin precursor* gene in later hypothalamus area.

Supplementary Figure3: The genes are insusceptible to the AD pathology. (A) Rhythmicity of BACE1 and GSK3α was unchanged in hippocampus of APP/PS1 mice (n=3-5, N.S. $p>0.05$, student's T-test). (B) Rhythmicity of Bmal1, Clock, NR1D1, and NR1D2 was unchanged in hippocampus of APP/PS1 mice (n=4, * $p< 0.05$; N.S. $p>0.05$, student's T-test).

Figure S1



A
B
C
D
E
F
G
Periods length (Hour)
DMSO
10nM OR-A
50nM OR-A
100nM OR-A
counts/sec
day
*
pre
post orexin A
pre
post DMSO
N.S.
EMPA(10μM)
EMPA(10μM) +OR-B(500nM)
**
APP/PS1PER2Luc(12 Month)
Contorl(2 Month)
APPswemPER2LUC
Control

Figure S2

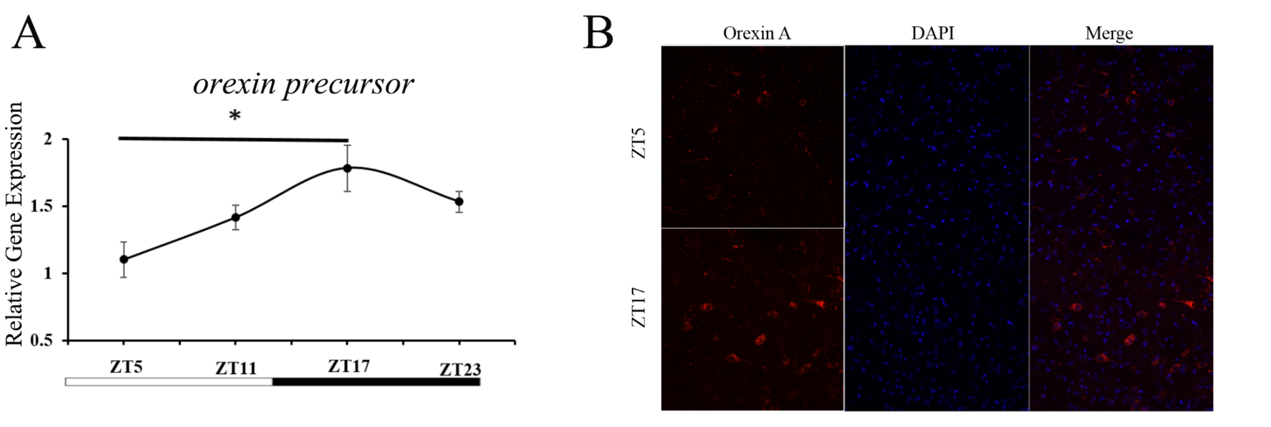
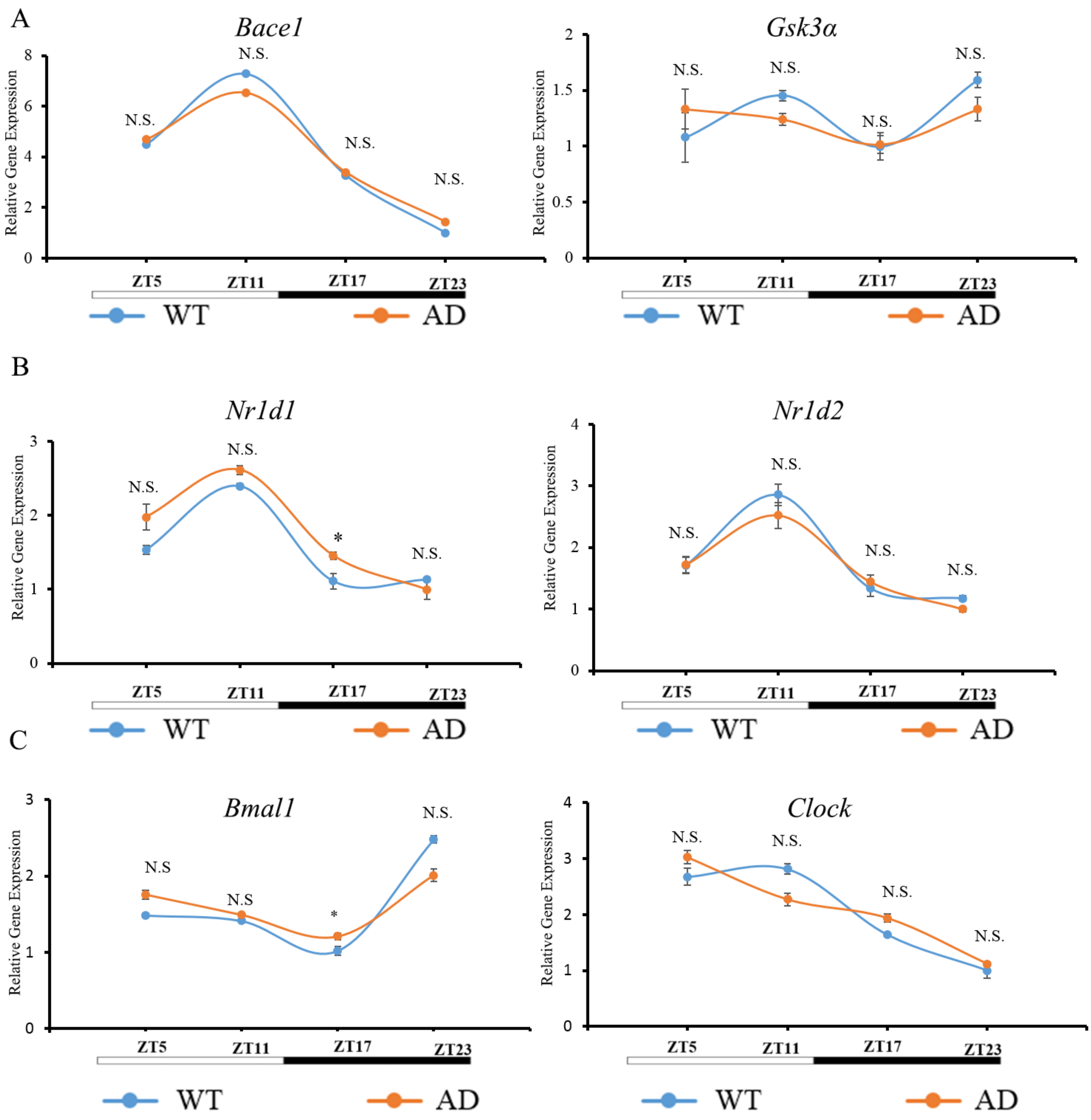

A
orexin precursor
*
Relative Gene Expression
2
1.5
1
0.5
ZT5
ZT11
ZT17
ZT23
B
Orexin A
DAPI
Merge
ZT5
ZT17

Figure S3



A
Bace1
Gsk3α
B
Nr1d1
Nr1d2
C
Bmal1
Clock
Relative Gene Expression
N.S.
N.S
*
ZT5
ZT11
ZT17
ZT23
WT
AD
0
0.5
1
1.5
2
3
4
6
8

Supplementary Tables:

Supplementary Table 1: qPCR primers of AD-risk genes

A2m-1F	TCAAAGTTGCAGTACCAGAG
A2m-1R	TCCCATAGGTGTATATGCCA
Abca1-1F	GTGTTTCTGGATGAACCAACC
Abca1-1R	CTTGACAATGCTTAGGGCAC
Ache-1F	CCTGAAGCCCTTAGAGGTG
Ache-1R	CTCGTCCAGAGTATCGGTG
Adam10-1F	CTGGACATATTTATGGTGAAGAAGG
Adam10-1R	CCACGAGTCTTGATGAAACC
Adam9-1F	AGATTGCCAGTTCCTTCCA
Adam9-1R	GAACCGTTGCAGTACTCAG
Apbb1-1F	ATTGTATCCGCCAGCTCTC
Apbb1-1R	GCAGATCCTTTCCCTCTCC
Apbb2-1F	GGGCAAAGACATGTACCTG
Apbb2-1R	GTAAGCAAAGTCCGTCTCAC
APH1A-1F	AGCTCCTTAAGAAGGCAGATG
APH1A-1R	TGATGATACCGAAGGACAGAC
Aplp1-1F	GCGAATGAATCAGAGCCTG
Aplp1-1R	AGAAGCTCCTGGATCTGTG
Aplp2-1F	GGAAATTGATGAGCTCCTTCAG
Aplp2-1R	GGGATCTCCTCACTCTCCT
Apoa1-1F	TGTGGATGCGGTCAAAGACA
Apoa1-1R	TCCCAGAAGTCCCGAGTCAA
Apoe-1F	CACATTGCTGACAGGATGC
Apoe-1R	TAATCCCAGAAGCGGTTCAG
Apba1-1F	GAATGGAATTATCTGCAGCCT
Apba1-1R	ATTTCGATGATCCGATGTCC
Apba3-1F	CTCACAGAGACAAGAGAAATCC
Apba3-1R	TACTGGTTGTTCCTGTCCC
App-1F	ATGTCCCAGGTCATGAGAG
App-1R	GATAACGGCCTTCTTGTCAG
Bace1-1F	GTTTCCAAGCTCAACATCCTG
Bace1-1R	AGTAGCGATGCAGGAAAGG
Bace2-1F	CACGAACATCTCTGATTCCA
Bace2-1R	GCGTTTCAGAATTTGTCCAG
Bche-1F	AAATATGGACATCCCAATGGG
Bche-1R	GGGAGCACGAAGTTTAGAG
BDNF-1F	TCATACTTCGGTTGCATGAAGG

BDNF-1R	ACACCTGGGTAGGCCAAGTT
Capn1-1F	CTATGAGTGGAACAAAGTGGAC
Capn1-1R	AACGACATCCAGAACTCCC
Casp3-1F	AATGGATTATCCTGAAATGGGC
Casp3-1R	GAGCGAGATGACATTCCAG
Casp4-1F	CAACAATTGCCACTGTCCA
Casp4-1R	TTCTCCAGAGTTCCCACCT
CDC2-1F	AAATTGGAGAAGGTACTTACGG
CDC2-1R	CTCCTTCTTCCTCGCTTTC
Cdk5-1F	GATTGTGAAGTCATTCCTCTTCC
Cdk5-1R	TTCAGGTCCCTATGTAGCAC
Cdkl1-1F	GCACCAGCAAGTATTTAGC
Cdkl1-1R	CAACTCAAGTGTTTCCATGTC
Chat-1F	TGGGATCTGGCAACTTCGTC
Chat-1R	AGCACCTCCTCACAGCTAGA
Clu-1F	ATACCTGCATGAAGTTCTATGC
Clu-1R	GGTTTAGAAACTCCTCTAGCTG
Ctsb-1F	TATCCCTCTGGAGCATGGA
Ctsb-1R	TGGTAAGCAGCCTACATGAG
Ctsc-1F	CACCTACCCTGATCTGCTG
Ctsc-1R	CTTCTGTTGCTTCCATCACC
Ctsd-1F	GATTATCAGAATCCCTCTGCG
Ctsd-1R	ATCAGGTCTTCCACAGAGC
Ctsg-1F	CTCCAGGAGGTGCAGCTAAG
Ctsg-1R	TCCATAGGAGACGATGCCCT
CTSL1-1F	GACTGTATGGCACGAATGAG
CTSL1-1R	TCTTCTCCCATATCGCTCTC
Ep300-1F	TGCGTCTGTAGAGCTGTGAG
Ep300-1R	TCTCTTCTCCGAAACGGGGT
Ern1-1F	AAGAAGATCCAGTCCTGCA
Ern1-1R	AAAGGGAAGTTTCGTCAGG
Gap43-1F	GGAGAAAGACGCTGTAGAC
Gap43-1R	CATGTTCTTGGTCAGCCTC
Gnao1-1F	CACTTCAGGCTGTTTGACG
Gnao1-1R	TGGAGTCGAAGAGCATGAG
GNAZ-1F	GGCAGAGGTCAGAACGCAAA
GNAZ-1R	GCTCGTCTGGTTGTCCTCATA
Gnb1-1F	CCACAAACAAGGTTCATGC
Gnb1-1R	CCACATAATTCCCAGAAGGAG

Gnb2-1F	ACACTGACCCAGATCACAG
Gnb2-1R	ACCTAGACATACTAAAGCAGGG
Gnb4-1F	GGGCTCCAGCTCTTCACTTG
Gnb4-1R	GAGGGATGGCGTGCATCTTA
Gnb5-1F	GTCTCCATCCTGTTTGGAC
Gnb5-1R	ATGCCCAAACTCTTAGGGT
Gng11-1F	CTTCACATCGAGGATCTGC
Gng11-1R	TTTAGATACCTGTTGTCTCTGC
Gng3-1F	TATGAGTATTGGTCAAGCACG
Gng3-1R	GCCTTGGACACCTTTATCC
Gng4-1F	GACCCTGAGTCAGCTTCTC
Gng4-1R	TTCATTCCTGCACTCCCTG
Gngt1-1F	AAAAACAGCTTCCCTGACAGAA
Gngt1-1R	AGCCTCCTTTGAGTTCCTTGA
Gngt2-1F	TGAGAAGGAGCTGTTGAGG
Gngt2-1R	CCTGTCTTGGAAATCAGATCAC
GSK3A-1F	AAGCTCTGCGATTTTGGCAGT
GSK3A-1R	GAGTTCTGGAGCACGGTAGTA
Gsk3b-1F	CTTGGACAAAGGTCTTCCG
Gsk3b-1R	AATGTGCACAAGCTTCCAG
Hsd17b10-1F	GTGGCCATTAAGACATACCAC
Hsd17b10-1R	AAGATTCACATTGATAACCCGC
Ide-1F	TGCATCAGGGATGAATGCA
Ide-1R	AAGAGATCGCATATAGGCCTC
Il1a-1F	ATGATCTGGAAGAGACCATCC
Il1a-1R	CGAGCTTCATCAGTTTGTATCTC
Insr-1F	TCAGAAAGTTTGCCCAACC
Insr-1R	GTCATCAGGTTCCGAACAG
Lpl-1F	AGAATTACTGGTTTGGATCCAG
Lpl-1R	AAATCAGCGTCATCAGGAG
Lrp1-1F	TGTATGAAGGTGGAGAGCC
Lrp1-1R	AGTTGGTAGGCTTGTCAGG
Lrp6-1F	GAGGAAGATCTTGATTTCAGAGG
Lrp6-1R	GTCCAATACATGTACCCAACC
Ntrk1-1F	CATACACAGACGCTTCATGAC
Ntrk1-1R	TGTGGGTACACCACAACTG
Ntrk2-1F	AGAACGAGTATGGGAAGGA
Ntrk2-1R	TTGGGTTTGTCTCGTAGTC
Pkp4-1F	TCCATGAAAGTGAGGGATCG

Pkp4-1R	CTTCCTGGTACCCACTGTC
Plat-1F	TTATTGTCGGAATCCAGATGGT
Plat-1R	TCACAGTATTCCCACGTCAG
Plau-1F	ACAATTACTGCAGGAACCC
Plau-1R	ATTCTTGGACAAACTGCCT
Plg-1F	CCACGTACCCAACTACTCTC
Plg-1R	TCATCATTGTCTGGGTTCCT
Prkca-1F	AGTCGGGAAATTTAAGGAGC
Prkca-1R	TTCAGATCCCTGTAAATGATCC
Prkcb-1F	AGAACCACAAATTCACCGC
Prkcb-1R	AAAGCAGCAGACTTGACAC
Prkcd-1F	TGAGTTCTGGCTGGATCTG
Prkcd-1R	GTTTAATGGCTCCACGACG
Prkce-1F	TTTGGCAAGGTCATGTTGG
Prkce-1R	CGTCTTGTAGGATAACGTCCT
PRKCG-1F	CCTGCAATGTCAAGTCTGTAG
PRKCG-1R	ACACTCGAAGGTCACAAATTC
Prkci-1F	ACGATGAGGATATCGATTGGG
Prkci-1R	CTGGAAGCAAGAATGCAGC
Prkcq-1F	AACCGTGGAACTCTACTCC
Prkcq-1R	TGAGGTTTCAGCTCTAACCA
Prkcz-1F	GTGCAGCGAAAGGATATGG
Prkcz-1R	GACAGAATCCATATGCCTCCT
Psen1-1F	GCTCTTATCTATTCCTCAACAATGG
Psen1-1R	CTTGGGTACCCTCCTTTGG
Psen2-1F	CAGAGATGGAAGAAGACTCCT
Psen2-1R	AAGATGAAGTCTCCCAGGC
Snca-1F	GAGTCCTCTATGTAGGTTCCA
Snca-1R	CTTGCTCTTTGGTCTTCTCAG
Sncb-1F	CTATGTCGGAAGCAAGACCA
Sncb-1R	CAGAGAACACAGCTCCTCC
Ubqln1-1F	AATCCTCAGCTGCAGAGTC
Ubqln1-1R	TTCACGGTTCAAGAATCCCA
Uqcrc1-1F	CTTTCAAGGGAACAAAGAATCG
Uqcrc1-1R	TGTAGGCATTAAGATGAGCC
Uqcrc2-1F	AGCAGGCAGTAGATATGAGG
Uqcrc2-1R	CCTTTGGTAGTCAAACTAGATGC
mINS-1 F1	CACCCCACCTGGAGACCTTA
mINS-1 R1	CCACACACCAGGTAGAGAGC

Supplementary Table 2: qPCR primers of core clock genes

mDBP_mrna-f	CCTGAGGAACAGAAGGATGA
mDBP_mrna-r	ATCTGGTTCTCCTTGAGTCTTCTTG
mCRY2-F1	GCCTCTGCCTCCCTTGATTT
mCRY2-R1	TGACATAAGCAGCATGGGCA
mCLOCK-F1	CACGCGCTCCCGTGAAAG
mCLOCK-R1	GTCCTTGTCATCTTCTTCCACCA
mCRY1-F2	AGCTCGTGTCCGTTCGTG
mCRY1-R2	CCTGCGCTTCCAAGAAAACC
mBMAL1_mrna-f	CTCGACACGCAATAGATGGGA
mBMAL1_mrna-r	CTTCCTTGGTCCACGGGTT
mPER1_mrna-f	TGAAGCAAGACCGGGAGAG
mPER1_mrna-r	CACACACGCCATCACATCAA
mPER2_mrna-f	GAAAGCTGTCACCACCATAGAA
mPER2_mrna-r	AACTCGCACTTCCTTTTCAGG
mNR1D1 qPCR F	TGGCATGGTGCTACTGTGTAAGG
mNR1D1 qPCR R	ATATTCTGTTGGATGCTCCGGCG
mNR1D2 qPCR F	GGAGTTCATGCTTGTGAAGGCTGT
mNR1D2 qPCR R	CAGACACTTCTTAAAGCGGCACTG

Supplementary Table 3: qPCR primers of housekeeping genes, *orexin precursor* gene and orexin receptors

mActin_mrna-nf	GGCTGTATTCCCCTCCATCG
mActin_mrna-nr	CAGTTGGTAACAATGCCATGT
mGapdh_mrna-nf	GACCTCAACTACATGGTCTACA
mGapdh_mrna-nr	ACTCCACGACATACTCAGCAC
mOrexin-PreF1	CCTGCCGTCTCTACGAACTG
mOrexinPreR1	GGTGCTAAAGCGGTGGTAGT
Hcrtr1 F3	CTGTGGCGCGATTATCTCTAC
Hcrtr1 R3	GCCAGGGACAGGTTGACAA
Hcrtr2 F3	GAGGATTCCCTCTCTCGTCG
Hcrtr2 R3	GGTGTAGGTATTCCCTCCACA

Supplementary Table 4: Primers for amplified the coding sequence of E4BP4 and the promotor regions of *BACE1* and *BACE2*

Human E4BP4 F	ACCGGCGGCCGCATGCAGCTGAGAAAAATGCA
Human E4BP4 R	ACCGGGCGCGCCTTACCCAGAGTCTGAAGCAG
BACE1 pro gen-REV R	AGCTAAGCTTTTGGGCCAAGGTGGGCCCCG
BACE1 pro 1k	ATCCGGTACCTTGCAGCCTGGAAAAACTCT
BACE2 pro gen-REV R	AGCTAAGCTTGTGCGCCCAAGCCCACGGCG
BACE2 pro 1.4k	ATCCGGTACCTTGTTTTTTAAAACAGCCAACATAA