

Mitophagy Regulates the Circadian Rhythms by Degrading NR1D1 in Microgravity and Isolation Environments

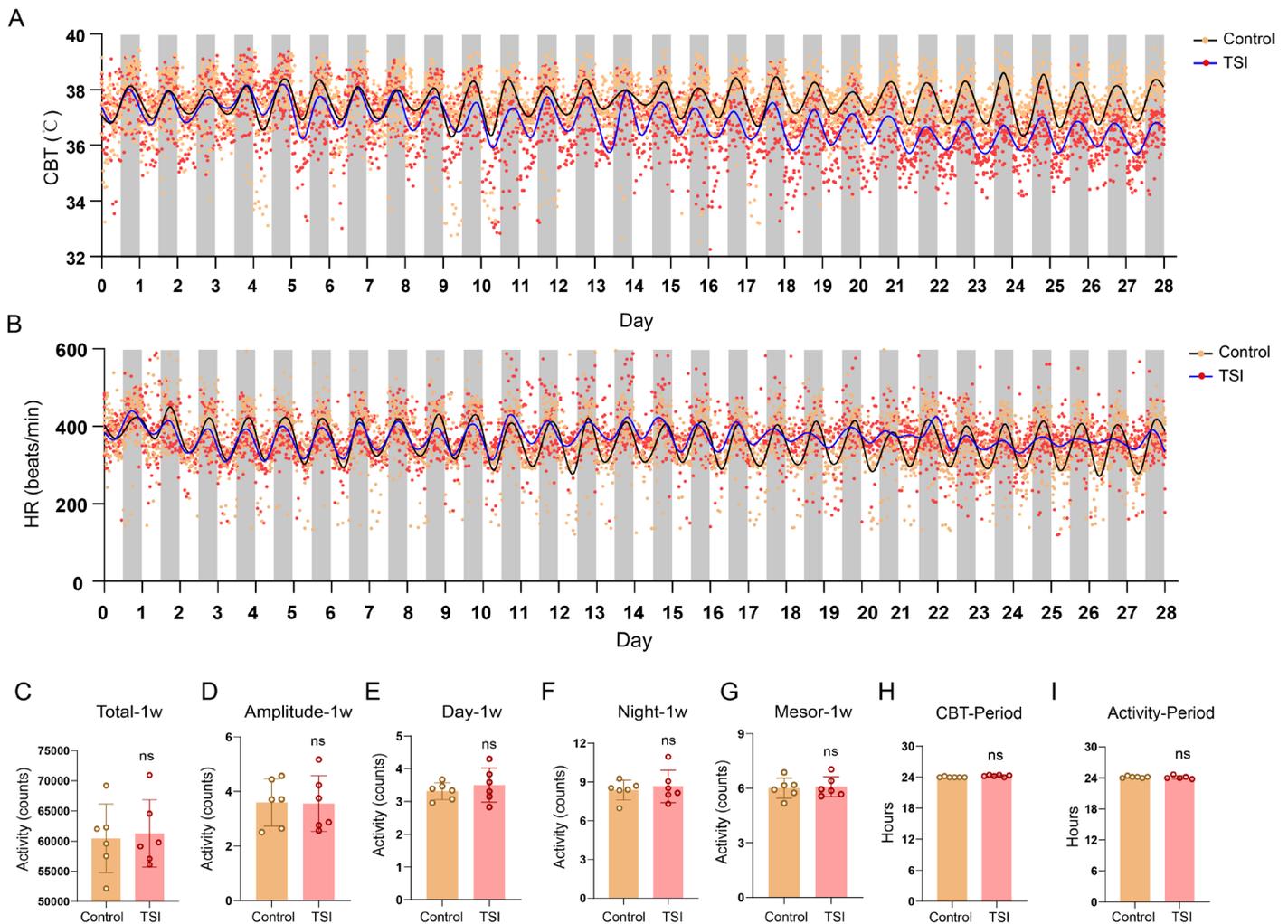
Sihai Zhou ^{1,2,†}, Xiaopeng Li ^{2,†}, Fengji Liang ², Guohua Ji ², Ke Lv ², Yanhong Yuan ², Yujie Zhao ², Na Yan ², Chuanjie Zhang ², Shiou Cai ², Shuhui Zhang ², Xu Liu ², Bo Song ^{1,*} and Lina Qu ^{2,*}

¹ Department of Pathology and Forensics, Dalian Medical University, Dalian 116044, China; zshdoct@163.com (S.Z.)

² State Key Laboratory of Space Medicine, China Astronaut Research and Training Center, Beijing 100000, China. lixiaopeng_acc@163.com (X.L.); freejohnnyliang@126.com (F.L.); jgh1682004@126.com (G.J.); lvke_med@aliyun.com (K.L.); yyh_yuan@163.com (Y.Y.); zhaoyujie9797@163.com (Y.Z.); yanna202204@163.com (N.Y.); zhangchuanjie96@163.com (C.Z.); caishiou19910228@163.com (S.C.); sherylhuhui@sina.com (S.Z.); xuxu_lh@163.com (X.L.)

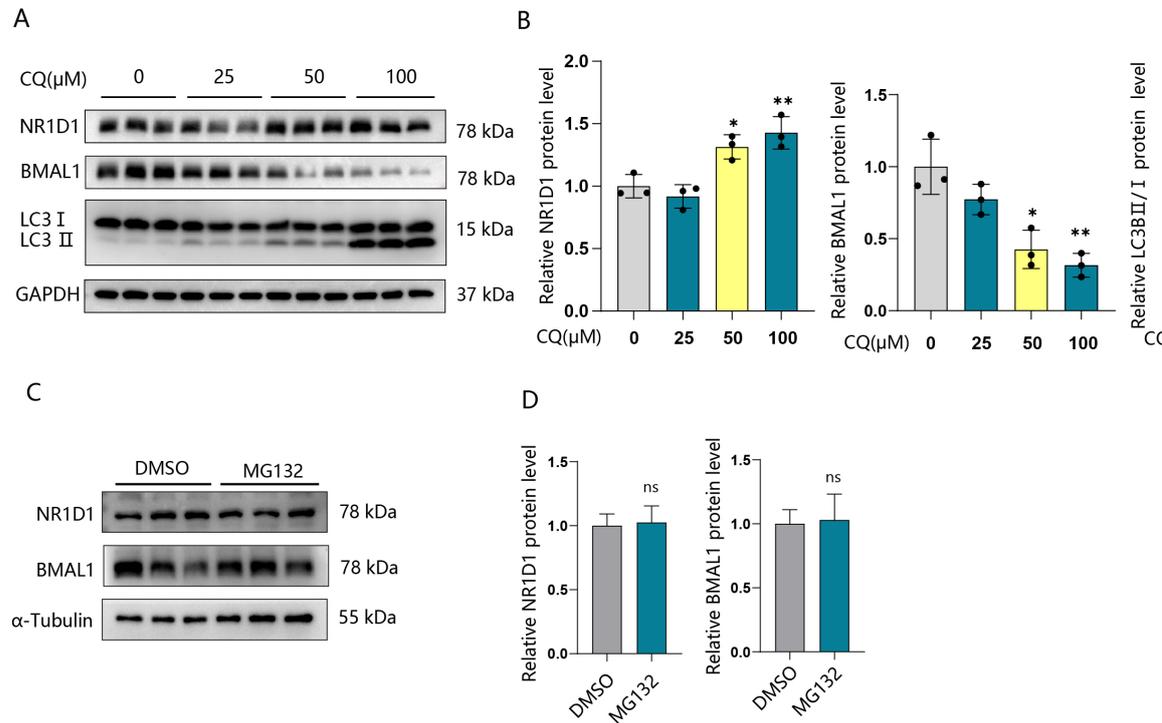
* Correspondence: bosong@dmu.edu.cn (B.S.); linaqu@263.net (L.Q.)

† These authors contributed equally to this work.

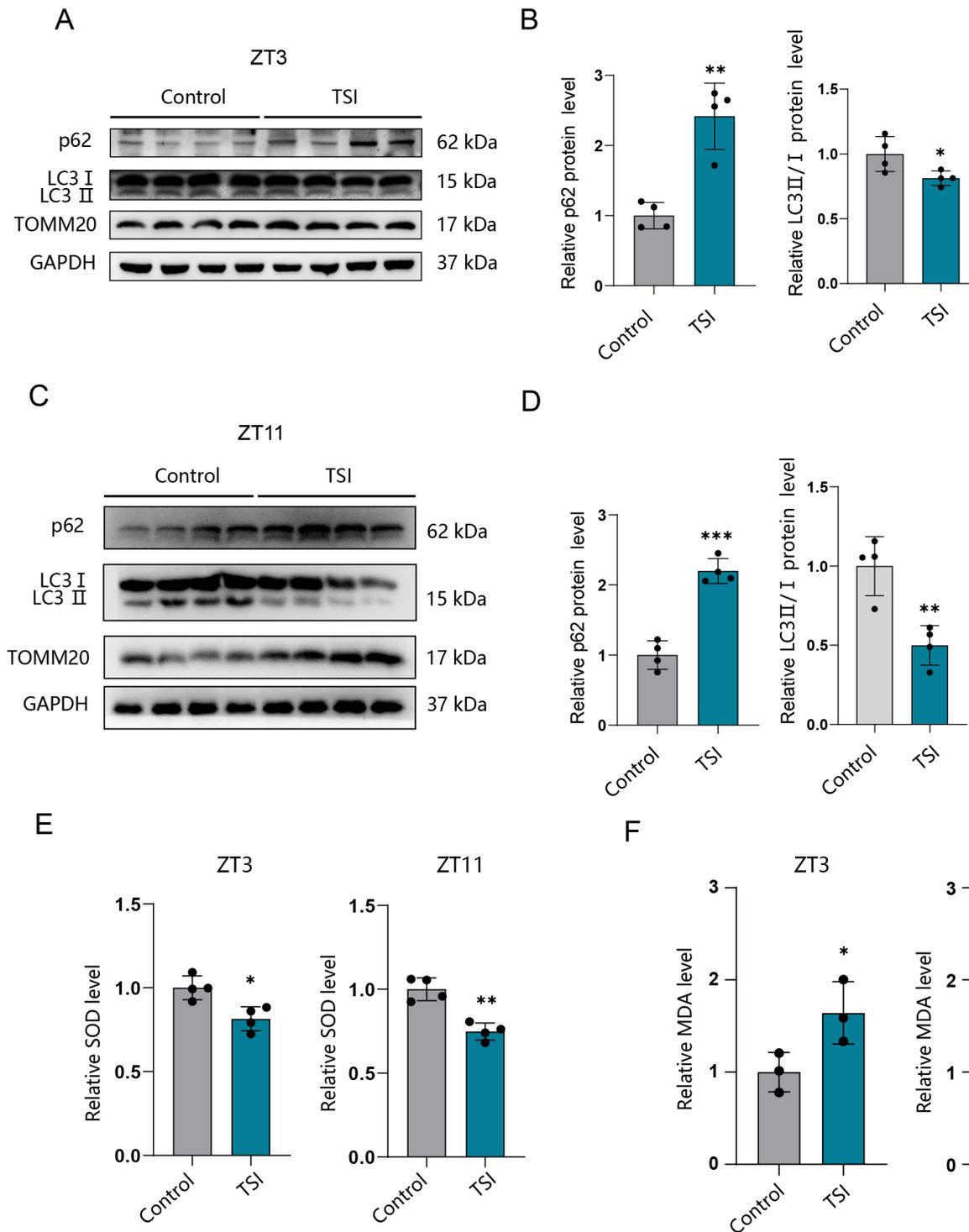


Supplementary Figure S1. TSI induces the disturbance of circadian rhythms in rats. (A, B) The curve fitting of the CBT (A) or HR (B) data of rats during the 28-day period from control and TSI groups (Each point represents the average CBT or HR values corresponding to each hourly time point from control and TSI rats). (C-G) Analysis of total (C), amplitude (D), daytime (E), nighttime (F) and mesor (G) of the locomotor activity data during the initial 7-day period. (H) Analysis of period (tau) of the CBT data during the final 7-

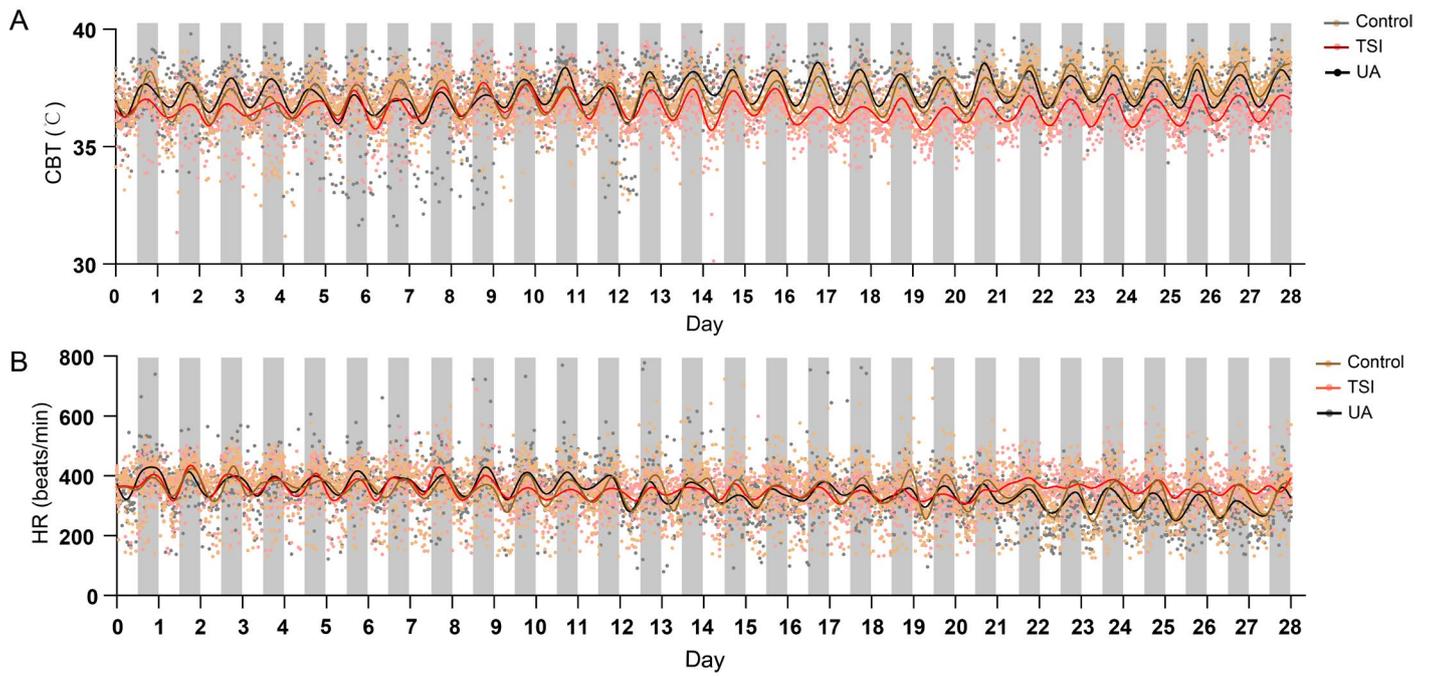
day period. **(I)** Analysis of period (τ) of the HR data during the final 7-day period. $n = 6$. ns: none significance. Two-sided Student's t tests were used to measure the significance.



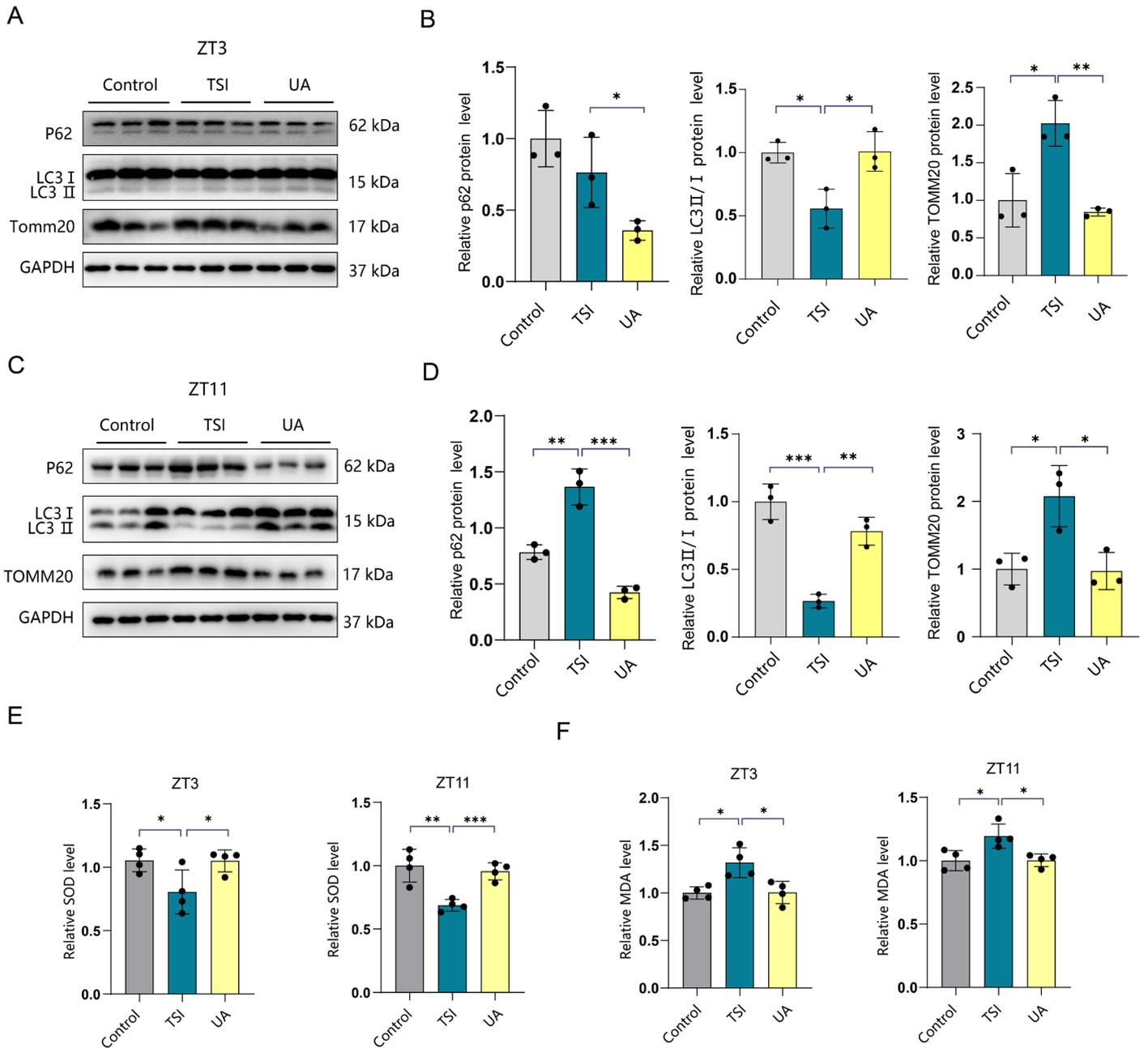
Supplementary Figure S2. NR1D1 is degraded by autophagy. **(A)** Western blotting analysis of the protein NR1D1, BMAL1 and LC3 after using CQ (25 μ M, 50 μ M, 100 μ M or vehicle) in NIH3T3 cells for 24 h. **(B)** Quantification of NR1D1, BMAL1 and LC3 II/I protein expression ($n = 3$). **(C)** Western blotting analysis of the protein NR1D1, BMAL1 after treating with MG132 (5 μ M) in NIH3T3 cells for 24 h. **(D)** Quantification of NR1D1, BMAL1 protein expression ($n = 3$). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. ns: none significance. Data are mean \pm S.E.M.. Two-sided Student's t tests were used to measure the significance.



Supplementary Figure S3. (A) The TSI environment causes mitophagy deficiency and mitochondrial dysfunction in neurons of the SCN. Western blotting analysis of the protein p62, LC3 II/I and TOMM20 of the SCN from control and model groups at ZT3 (n = 4). (B) Quantification of p62, LC3 II/I and TOMM20 protein expression at ZT3 (n = 4). (C) Western blotting analysis of the protein p62, LC3 II/I and TOMM20 of the SCN from control and model groups at ZT11 (n = 4). (D) Quantification of p62, LC3 II/I and TOMM20 protein expression at ZT11 (n = 4). (E-F) Quantification of the activity of SOD (E) and MDA (F) in the SCN tissues from control and model groups at ZT3 and ZT11. *p < 0.05; **p < 0.01; ***p < 0.001. Data are mean \pm S.E.M., n = 4. Two-sided Student's t tests were used to measure the significance.



Supplementary Figure S4. UA Ameliorates the Disturbance of SCN Rhythms. (A, B) The curve fitting of the CBT (A) or HR (B) data of rats during the 28-day period from control, TSI and UA-treated groups (Each point represents the average CBT or HR values corresponding to each hourly time point from control, TSI and UA-treated rats). $n = 6$. Data are mean \pm S.E.M.. Two-sided Student's t tests were used to measure the significance).



Supplementary Figure S5. UA ameliorates the disturbance of circadian rhythms through promote the degradation of NR1D1 in rats. (A, C) Western blotting analysis of the protein p62, LC3 II/I and TOMM20 of the SCN from TSI and UA-treated group (n = 3) at ZT3 and ZT11. (B, D) Quantification of the normalized p62, LC3 II/I and TOMM20 protein expression at ZT3 (n = 3) at ZT3 and ZT11. (E, F). Quantification of the activity of SOD (E) and MDA (F) in the SCN tissues from control and model groups at ZT3 and ZT11 (n = 4). ns: none significance. *p < 0.05; **p < 0.01; ***p < 0.001. Data are mean \pm S.E.M.. Two-sided Student's t tests were used to measure the significance.