Supplemental Materials

A novel diabetic mouse model for real-time monitoring of clock gene oscillation and blood pressure circadian rhythm

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Running Title: Desynchrony of tissue oscillators in diabetes

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Table S1. Real-time PCR primer information.

Gene	Primer	Sequence
Bmal1	Forward	5'-ATCAGCGACTTCATGTCTCC-3'
	Reverse	5'-CTCCCTTGCATTCTTGATCC-3'
ROCK1	Forward	5'-GACTGGGGACAGTTTTGAGAC-3
	Reverse	5'-ATCCAAATCATAAACCAGGGCAT-3'
ROCK2	Forward	5’-TTTCTAAACATGCGAAGAATCTCATATG-3’
	Reverse	5’-CTTCTACCCCATTTCTTCCAAGTC-3’
Calponin-1	Forward	5'-GCACATTTTAACCGAGGTCCT-3'
	Reverse	5'-CTGATGGTCGTATTTCTGGGC-3'
Calponin-2	Forward	5'-GCGGGAACATGACACAGGT-3'
	Reverse	5'-CATGGTGGCGTCGTCAAAGT-3'
Calponin-3	Forward	5’-AGGCAGAATACCCCGATGAA-3’
	Reverse	5’-GGTCGTCGCCATACTGGTACTC-3’
Tropomyosin 1 (a)	Forward	5'-CTGGTTGAGGAGGAGTTGGA-3'
	Reverse	5'-ATGTGCTTGGCCTCTTTCAG-3'
Tropomyosin 2 (b)	Forward	5’-AGGCCACCGACGCTGAA-3’
	Reverse	5’-CCTGTGCCCGATCCAACT-3’
SM22a	Forward	5’-ACCGTGGAGATCCCAACTGGTTTA-3’
	Reverse	5’-CATTTGAAGGCCAATGACGTGCT-3’

Bmal1: Brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein 1; ROCK1/2: Rho kinase 1/2; SM22α: Smooth muscle protein 22-α.

Table S2. The daily oscillations in systolic blood pressure (SBP) and diastolic blood pressure (DBP) were diminished in *db/db-mPer2^{Luc}* mice.

Blood Pressure	Circadian Rhythm	*db/+-mPer2^{Luc}*	*db/db-mPer2^{Luc}*	P value
SBP	Amplitude (mmHg)	7.421±1.546	4.353±0.5485	0.001 *
	Acrophase (ZT time)	18.34±0.5734	19.17±0.731	0.0555
	Robustness (%)	67.93±7.997	44.17±9.646	0.0009 ***
DBP	Amplitude (mmHg)	6.596±1.425	3.728±0.4713	0.0004 ***
	Acrophase (ZT time)	17.96±0.5435	18.54±0.8214	0.1741
	Robustness (%)	66.58±10.39	46.22±9.543	0.0054 **

The SBP and DBP were recorded by radiotelemetry. The amplitude, acrophase, and robustness were calculated by Cosinor analysis. *, $P<0.05$; **, $p<0.01$, ***, $p<0.001$.

Table S3. No significant changes were detected in the period and amplitude of mPer2 oscillations in most explanted peripheral tissues from the *db/db-mPer2^{Luc}* mice.

Tissues	Circadian Rhythm	*db/+-mPer2^{Luc}*	*db/db-mPer2^{Luc}*	P value
Aorta	Period (h)	23.76±0.2235	24.05±0.186	0.3409
	Amplitude (counts)	139.3±29.33	208.3±21.45	0.0706
MA	Period (h)	24.41±0.2349	24.4±0.1887	0.9672
	Amplitude (counts)	26.6±2.005	28.11±3.485	0.7458
Kidney	Period (h)	24.5±0.5553	23.78±0.2905	0.2608
	Amplitude (counts)	8.072±2.38	25.06±3.207	0.0049 **
Liver	Period (h)	21.08±0.3492	21.01±0.2061	0.8555
	Amplitude (counts)	44.42±10.53	55.54±11.6	0.5471
WAT	Period (h)	24.41±0.2349	24.4±0.1887	0.9672
	Amplitude (counts)	26.6±2.005	28.11±3.485	0.7458
Thymus	Period (h)	25.23±0.9905	23.96±0.6772	0.3133
	Amplitude (counts)	23.83±10.15	20.5±5.679	0.7641
Lung	Period (h)	23.9±0.7106	23.85±0.3052	0.9431
	Amplitude (counts)	22.37±2.039	33.32±2.855	0.0233 *
AG	Period (h)	22.00±0.4155	20.67±1.014	0.1821
	Amplitude (counts)	5.882±1.914	16.16±7.113	0.1004
SCN	Period (h)	23.47±0.1764	23.88±0.273	0.311
	Amplitude (counts)	11.4±2.262	11.82±2.781	0.9212

The aorta, mesentery artery (MA), kidney, liver, white adipose tissues (WAT), thymus, lung, adrenal gland (AG), and suprachiasmatic nucleus (SCN) were isolated from the *db/db-mPer2^{Luc}* and *db/+-mPer2^{Luc}* control mice and cultured in organ culture. The mPer2 bioluminescence was monitored and recorded by the LumiCycle system. The period and amplitude of these tissues were analyzed by using the LumiCycle analysis software. *: $p<0.05$; **: $p<0.01$

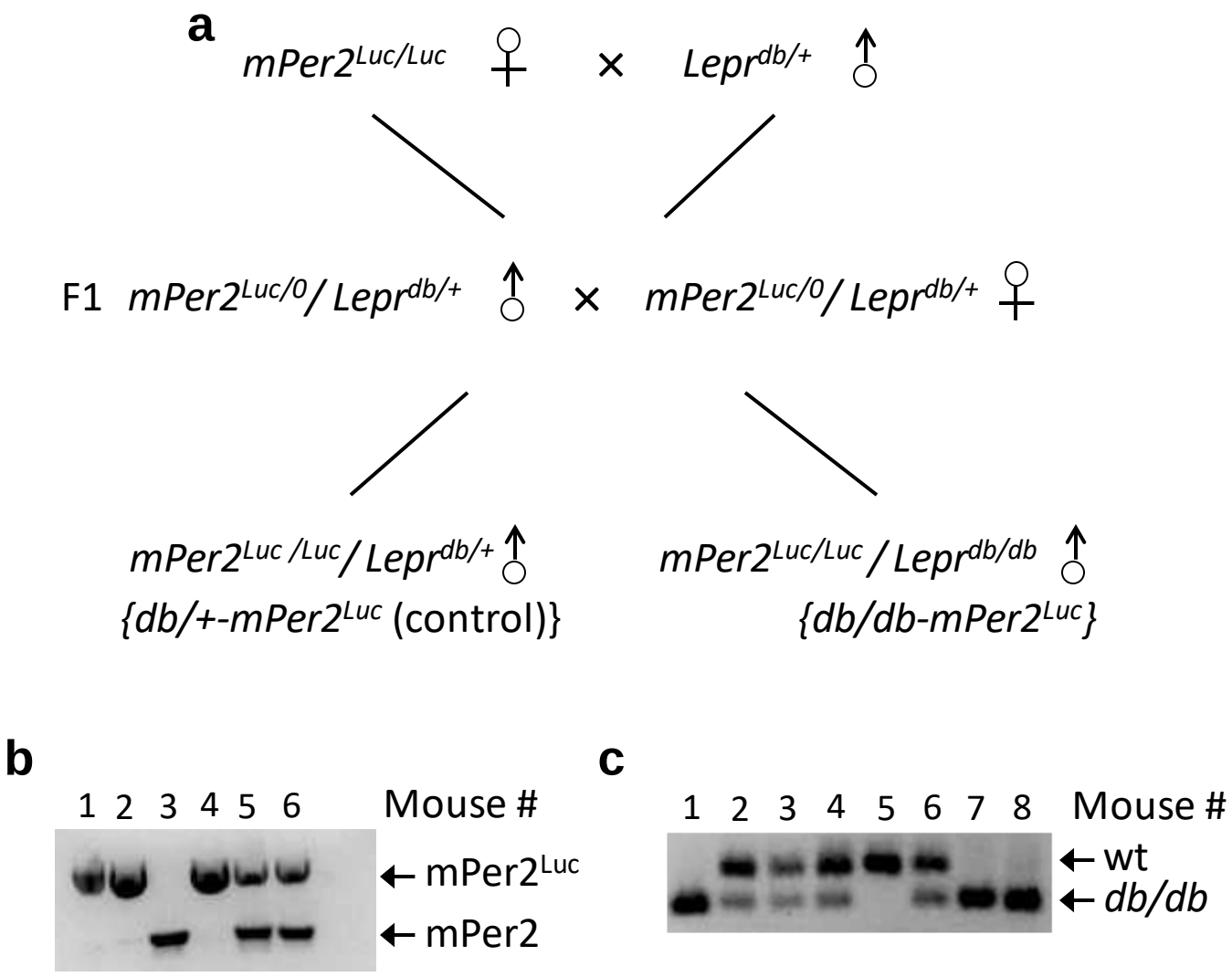

Figure S1. Generation of *db/db*-mPer2^{Luc} mice. (**a**) Breeding strategy to generate *db/db-mPer2^{Luc}* and control male mice. (**b**) Representative image for genotyping *mPer2^{Luc}* mice. Of note, mouse # 1, 2, and 4 are homozygous *mPer2^{Luc}* mice. (**c**) Representative image for genotyping *db/db* (*lepr$^{-/-}$*) mice. Of note, mouse #1, 7, and 8 are *db/db* mice whereas mouse # 2, 3, 4, and 6 are *db/+* control mice.

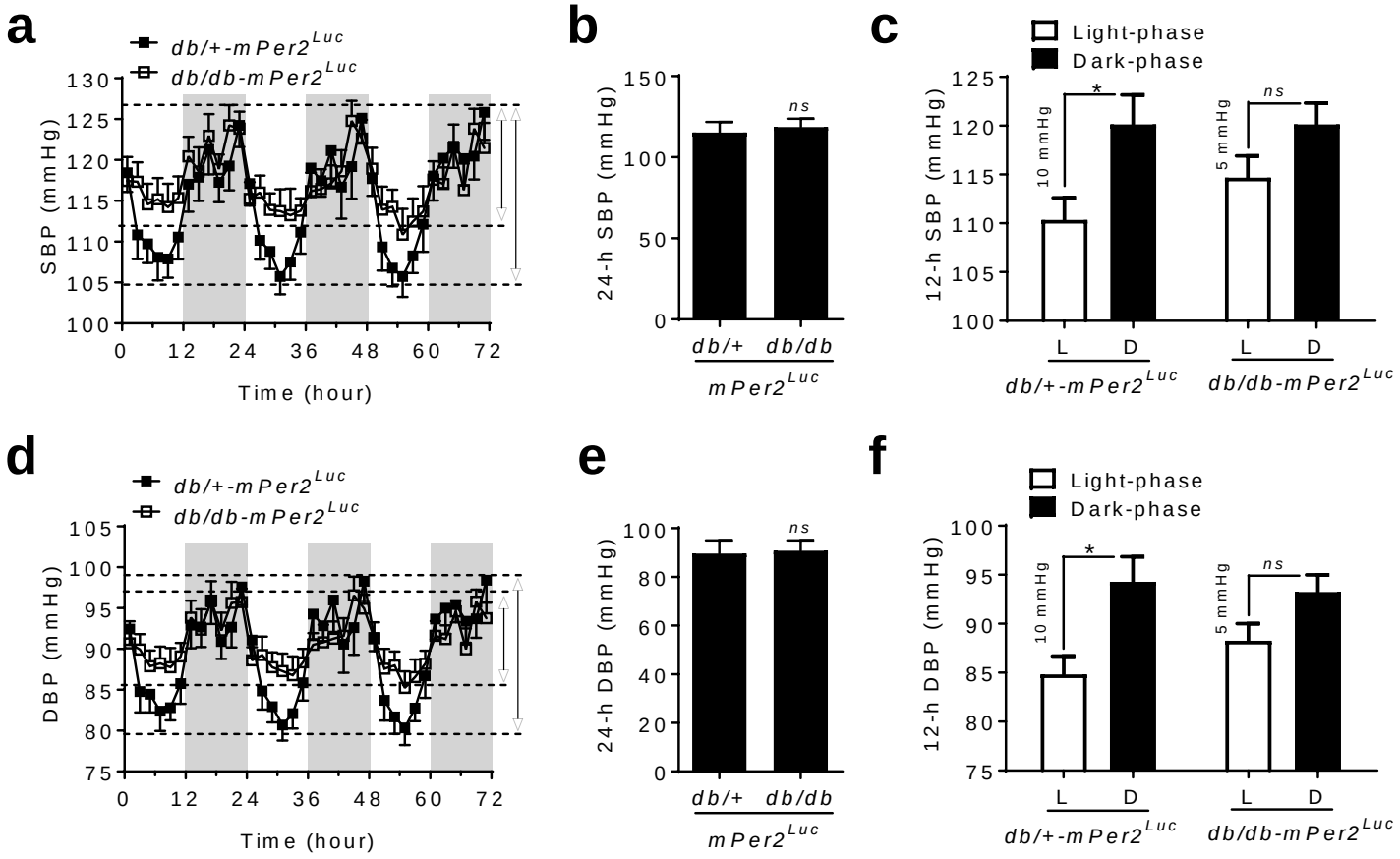

Figure S2. The daily oscillations of systolic blood pressure (SBP) and diastolic blood pressure (DBP) are diminished in *db/db-mPer2^{Luc}* mice. SBP (**a**, **b**, and **c**) and DBP (**d**, **e**, and **f**) were recorded by radiotelemetry. The 72-hour recording of SBP (**a**) and DBP (**d**) where the grey box indicates the dark phase and the length of the arrowhead lines indicate the difference of BP between the peak and trough in the two strains of mice. The 24-hour average of SBP (**b**) and DBP (**e**). The 12-hour SBP (**c**) and DBP (**f**) during the light phase (L) and dark phase (D). The difference in the day and night BP was indicated in the figures. All data were expressed as mean ± SEM (N = 6). Unpaired t test was used for (b and e). Two-way ANOVA was used for (c and f). The difference between two mouse strains was indicated in the figures. *, $p<0.05$; ns, $p>0.05$.

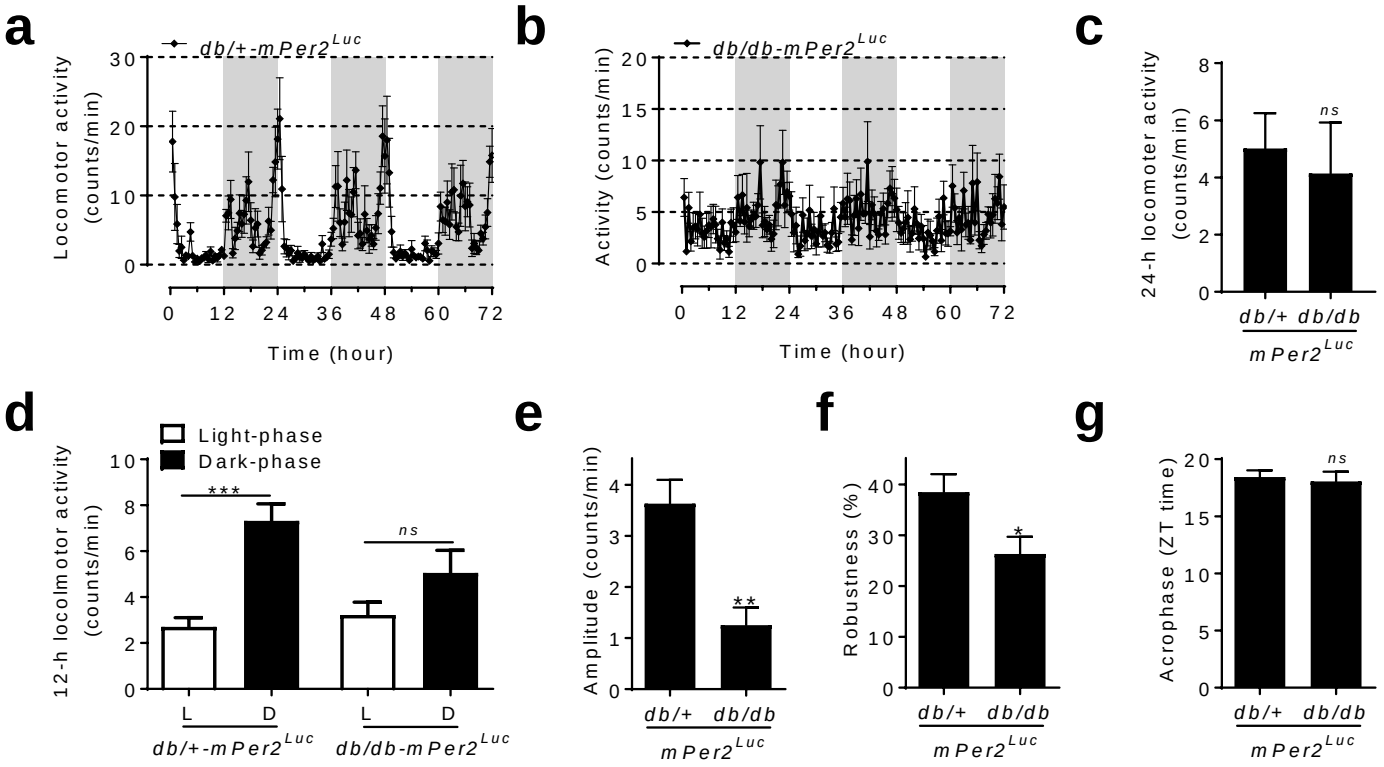

Figure S3. The daily oscillation of locomotor activity is diminished in the *db/db-mPer2^{Luc}* mice. Locomotor activity was recorded by radiotelemetry under 12:12 light/dark cycle in the *db/db-mPer2^{Luc}* and control *db/+-mPer2^{Luc}* mice for 72 consecutive hours. The 72-hour recording of locomotor activity in the control mice (**a**) and *db/db-mPer2^{Luc}* mice (**b**) where the grey box indicates the dark-phase. **c.** The 24-hour locomotor activity. **d.** The 12-hour locomotor activity during the light-phase (L) and dark-phase (D). The Cosinor analysis of locomotor activity daily rhythm for amplitude (**e**), robustness (**f**), and acrophase (**g**). All data were expressed as mean ± SEM (n=6). Two-way ANOVA was used for (d). Unpaired t test was used for (c, e, f, and g). *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ns, $p>0.05$.

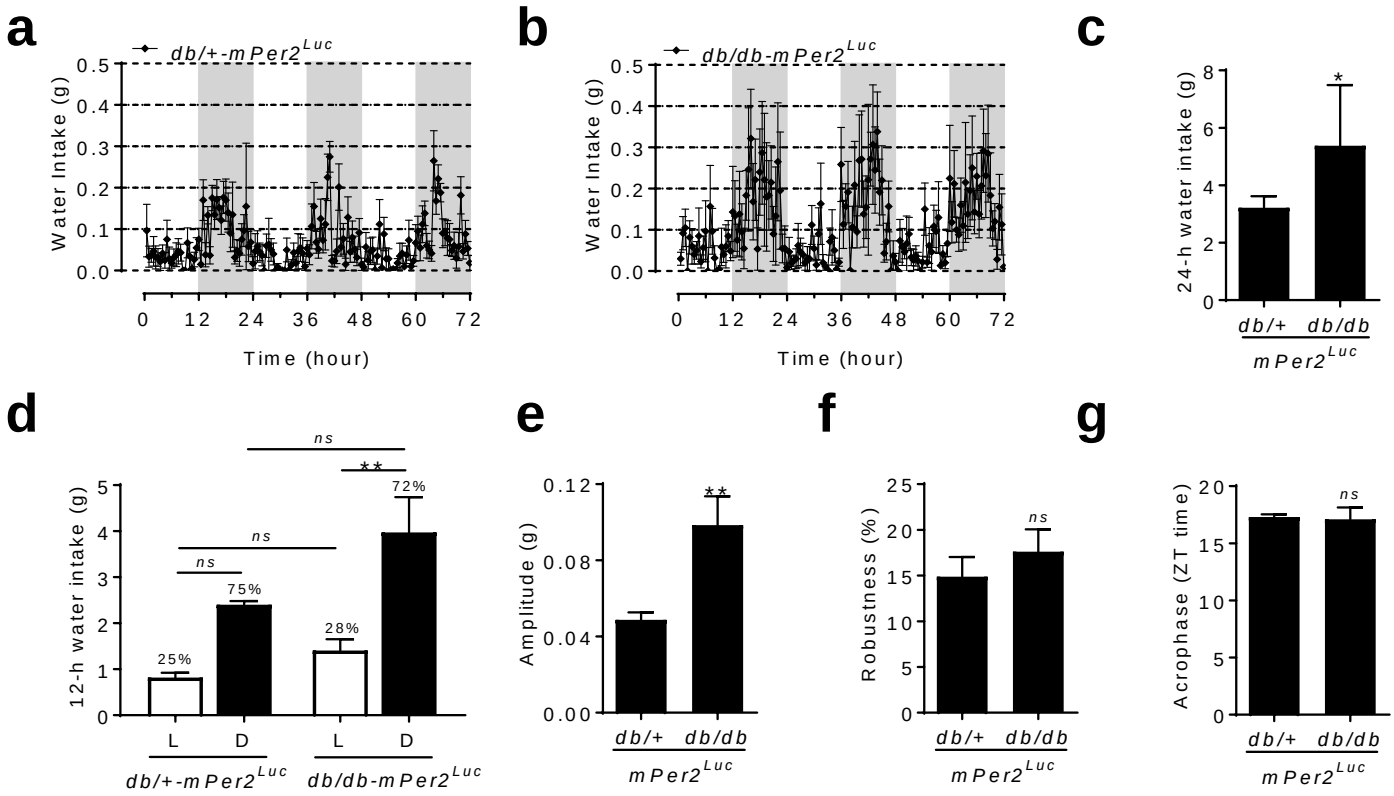

Figure S4. The daily oscillation of water intake is not diminished in the *db/db-mPer2*^{Luc} mice. Water intake was measured by indirect calorimetry every 30 min under 12:12 light/dark cycle in the *db/db-mPer2*^{Luc} and control *db/+-mPer2*^{Luc} mice for 72 consecutive hours. The 72-hour recording of water intake in the control mice (**a**) and *db/db-mPer2*^{Luc} mice (**b**) where the grey box indicates the dark-phase. **c.** The 24-hour water intake. **d.** The 12-hour water intake during the light-phase (L) and dark-phase (D), where the percentage numbers above each bar are the percentage of daily water intake. The Cosinor analysis of water intake daily rhythm for amplitude (**e**), robustness (**f**), and acrophase (**g**). All data were expressed as mean ± SEM (n=6). Two-way ANOVA was used for (d). Unpaired t test was used for (c, e, f, and g). *, $P < 0.05$; **, $P < 0.01$; ns, not significant.

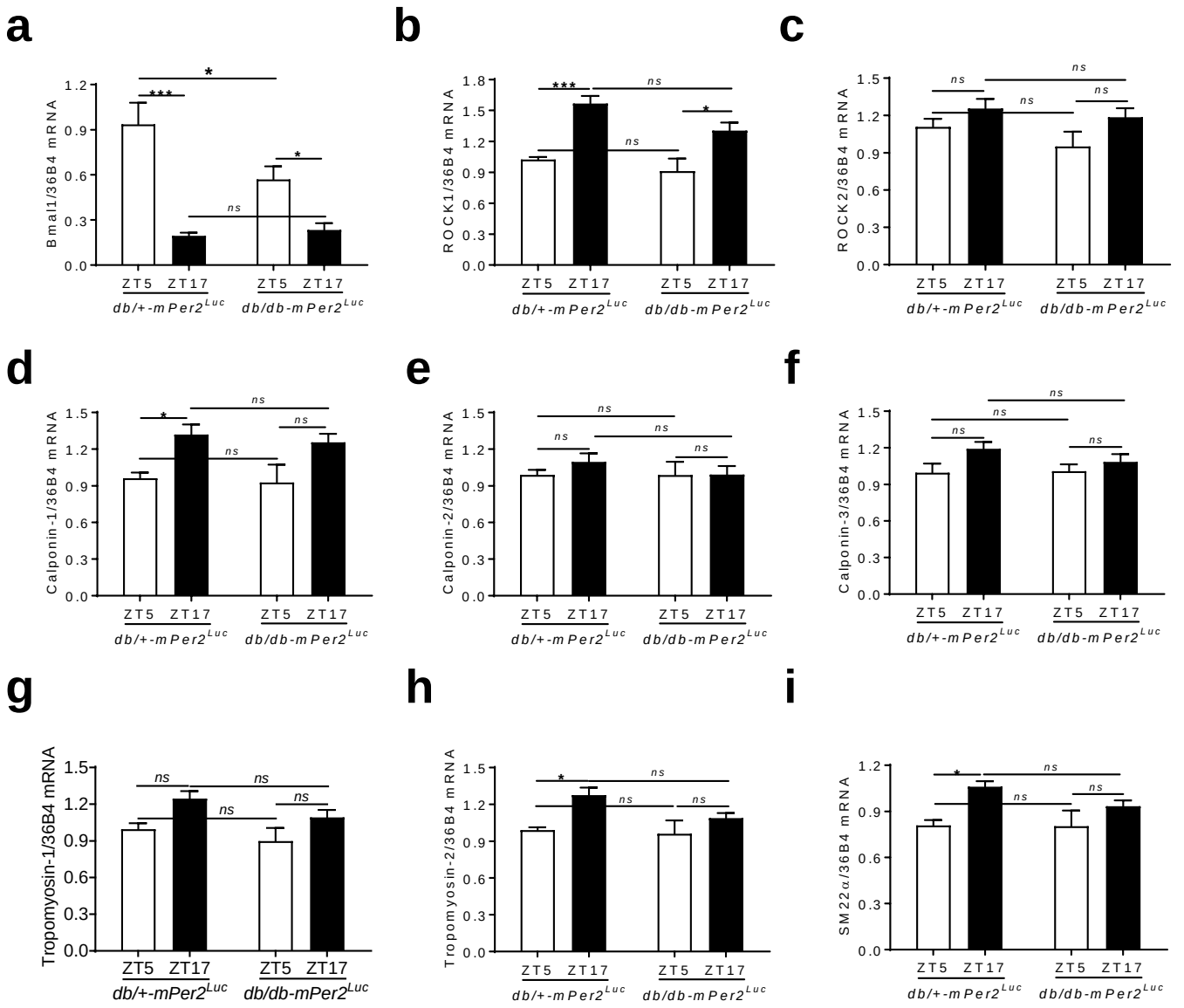

Figure S5. Altered time-of-day variations of gene expressions in the mesenteric arteries from the *db/db-mPer2^{Luc}* mice. Control and *db/db-mPer2^{Luc}* mice were euthanized at ZT5 and ZT17 and mesenteric arteries were harvested. Mesenteric artery mRNAs were quantified using real-time PCR. (**a**) Bmal1. (**b**) ROCK1. (**c**) ROCK2. (**d**) calponin-1. (**e**) calponin-2. (**f**) calponin-3. (**g**) tropomyosin-1. (**h**) tropomyosin-2. (**i**) smooth muscle protein 22-α (SM22α). N = 4-5 for each mouse strain at each time point. Data were analyzed by 2-way ANOVA. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.