

Supplemental Digital Content 1

Table S1 Primer Sequences Used for Real Time Polymerase Chain Reaction

<i>Gene</i>	<i>Direction</i>	<i>Primer (5' to 3')</i>	<i>Annealing temp(°C)</i>
<i>Tac1</i>	Fwd.	CTA AAT TAT TGG TCC GAC TG	60
	Rev.	TTC TGC ATT GCG CTT CTT TC	
<i>NR1</i>	Fwd.	CAG GAG CGG GTA AAC AAC GCAAC	58
	Rev.	GAC AGC CCC ACC AGC AGC CAC AGT	
<i>NR2A</i>	Fwd.	AGC CCC CTT CGT CAT CGT AGA	60
	Rev.	CAG AAG GGG AAA CAG TGC CAT TA	
<i>NR2B</i>	Fwd.	TCC GCC GTG AGT CTT CTG TCT ATG	58
	Rev.	CTG GGT GGT AAA GGG TGG GTT GTC	
<i>NR2C</i>	Fwd.	GAT GCC GCC GTC CTC AAC TAC A	60
	Rev.	GCT CCC AGG CAA AGA CCA GAA GG	
<i>Per1</i>	Fwd.	CGG ATT GTC TAT ATT TCG GAG CA	60
	Rev.	GGG CAC CCC GAA ACA CA	
<i>Per2</i>	Fwd.	CTT GCC TCC GAA ATA ACT CCT G	60
	Rev.	GCA CGG CTG TCT GAG CGT	
<i>Bmal1</i>	Fwd.	TCG TTG CAA TCG GGC G	60
	Rev.	CCG TAT TTC CCC GTT CGC	
<i>Clock</i>	Fwd.	CTT TCC TTC CTT AGA GAC GAG ACT G	60
	Rev.	GGT CTT GGT GCT CAT GTG CA	
<i>Npas2</i>	Fwd.	TGC TTA GAC AGC TCT GCG CTC	60
	Rev.	ACA CCT CCC ACT AAA ACA ATG TTG	
<i>Rev-erb α</i>	Fwd.	GATAGCTCCCCTTCTTCTGCATCATC	60
	Rev.	TTCCATGGCCACTTGTAGACTTC	
S18	Fwd.	CTT TGG TCG CTC GCT CCT C	60
	Rev.	CTG ACC GGG TTG GTT TTG AT	

Table S2 Primer Sequences Used for ChIP Assay

<i>Target Sequence</i>	<i>Direction</i>	<i>Primer (5' to 3')</i>	<i>Annealing temp</i> (°C)
F-p (- 1338 — - 900)	Fwd.	TGGCTTGCAAACACTTCTTTC	55
	Rev.	CACAGAAGGTAGAGGGAGTTGC	
F-p (- 885 — - 785)	Fwd.	AGCTTCAGAGGAACGAAAGAGG	55
	Rev.	AGAACTTACCCACCAGCCATTC	
F-p (- 822 — - 627)	Fwd.	ATGCTACAGCGTCCTTGAATG	55
	Rev.	TAGAGGAGGAAAGCAGACTTGC	
F-p (- 358 — - 203)	Fwd.	CCAAAGTCCGAGGCATGAG	55
	Rev.	TTAGGGAAGTCGAGGTCTTGC	
F-p (- 133 — - 25)	Fwd.	CTACATCACGCAAGCGAAAGG	55
	Rev.	ATTATCTCAGGCGTGACGAGC	

F-p: fragment of *Tac1* gene promoter.

Table S3 Rhythm Parameters for Messenger RNA Expression of Genes in Dorsal Root Ganglia and Spinal Cord in Wild Type Mice

Time-Effect analysis by ANOVA* and Cosine Regression Analysis#													
Gene	Tissue [^]	ANOVA			Cosine Regression Analysis							R Squared	
		F	p	T [^]	F	p [^]	M [^]	AMP [^]	f(h) [^]	2Amp% [^]	95% CI for peak [^]		
											L (h)	U (h)	
<i>Bmal1</i>	DRG	614	<0.001	24	11.74	0.019	0.31	0.46	0.61	298.22	-2.79	4.01	0.7
<i>Clock</i>	DRG	0.18	0.05	24	27.64	0.003	0.6	0.44	3.1	147.67	0.19	6.01	0.85
<i>Npas2</i>	DRG	13.55	<0.001	24	7.61	0.04	0.44	0.35	0.63	160.46	-3.05	4.31	0.6
<i>Tac1</i>	DRG	38.27	<0.001	24	12.41	0.017	0.55	0.38	2.77	139.37	-0.45	5.99	0.71
<i>Per1</i>	DRG	12.75	<0.001	24	5.3	0.05	0.47	0.25	10.86	104.47	6.93	14.8	0.52
<i>Per2</i>	DRG	110.41	<0.001	24	15.2	0.011	0.47	0.32	13.02	138.15	9.77	16.27	0.75
<i>Rev-erb α</i>	DRG	398.17	<0.001	24	19.5	0.007	0.33	0.41	7.7	250.46	4.83	10.58	0.8
<i>NR1</i>	DRG	18.3	<0.001	24	2.84	0.15	0.6	0.29	2.82	94.84	-1.67	7.31	0.36
<i>NR1</i>	DRG	18.3	<0.001	12	15	0.012	0.58	0.4	1.96	137.69	0.36	3.57	0.75
<i>NR2A</i>	DRG	337.14	<0.001	24	0.08	0.79	0.32	0.06	11.26	38.1	10.56	12	0.01
<i>NR2A</i>	DRG	337.14	<0.001	12	27.2	0.003	0.24	0.45	0.05	371.07	-1.37	1.47	0.83
<i>NR2B</i>	DRG	29.42	<0.001	24	0.08	0.792	0.35	0.06	12.36	32.95	9.36	15.36	0.02
<i>NR2B</i>	DRG	29.42	<0.001	12	27.62	0.003	0.28	0.43	23.99	301.06	22.53	25.45	0.85
<i>NR2C</i>	DRG	11.19	<0.001	24	0.65	0.457	0.59	0.17	0.14	58.11	-2.69	2.97	0.12
<i>NR2C</i>	DRG	11.19	<0.001	12	197.11	<0.001	0.55	0.5	0.28	183.85	-2.57	3.13	0.98
<i>NK1</i>	DRG	5.02	0.01	24	6.07	0.06	0.75	0.16	7.66	43.26	5.12	10.2	0.55
<i>NK1</i>	DRG	5.02	0.01	12	0.75	0.427	0.74	0.08	6.34	21.86	3.8	8.89	0.13

<i>Tac1</i>	SC	2	0.15	24	3.55	0.118	0.73	0.16	0.86	43.25	-2.02	3.74	0.42
<i>Tac1</i>	SC	2	0.15	12	3.96	0.103	0.73	0.17	21.45	46.09	20.08	22.83	0.44
<i>Bmal1</i>	SC	13.06	<0.001	24	29.77	0.03	0.68	0.31	2.81	90.27	0.09	5.53	0.86
<i>Clock</i>	SC	1.48	0.266	24	2.47	0.177	0.85	0.1	14.77	22.51	12.04	17.51	0.33
<i>Clock</i>	SC	1.48	0.266	12	7.56	0.04	0.85	0.14	17.12	32.82	15.89	18.36	0.6
<i>NR1</i>	SC	20.22	<0.001	24	2.46	0.178	0.57	0.17	6.96	58.54	4.4	9.53	0.33
<i>NR1</i>	SC	20.22	<0.001	12	3.8	0.11	0.57	0.19	18.17	68.43	16.94	19.4	0.43
<i>NR2A</i>	SC	6.22	0.005	24	38	0.002	0.85	0.18	5.65	42.84	3.17	8.14	0.88
<i>NR2A</i>	SC	6.22	0.005	12	1.64	0.257	0.85	0.05	14.61	12.66	13.28	15.95	0.09
<i>NR2B</i>	SC	24.41	<0.001	24	0.75	0.43	0.67	0.11	12.62	33.58	4.75	20.5	0.13
<i>NR2B</i>	SC	24.41	<0.001	12	94.06	<0.001	0.7	0.31	13.68	88.95	12.25	15.12	0.95
<i>NR2C</i>	SC	6.93	0.003	24	1.65	0.26	0.78	0.13	10.55	32.99	4.92	16.19	0.25
<i>NR2C</i>	SC	6.93	0.003	12	9.34	0.03	0.78	0.22	15.21	55.97	13.56	16.87	0.65
<i>NK1</i>	SC	35.63	<0.001	24	1.89	0.228	0.46	0.28	3.34	119.74	-1.73	8.41	0.27
<i>NK1</i>	SC	35.63	<0.001	12	4.2	0.096	0.45	0.35	2.73	155.9	0.73	4.73	0.46

* Analysis of Variance for gene expression difference among the experiment time points. # Analysis of Circadian (24 hrs) or Circasemidian (12 hrs) for gene expression using Cosine Regression Analysis (CRA) (refer to Materials and Methods for details). ▲ RNA was isolated and purified from dorsal root ganglion (DRG), spinal cord (SC) tissues. ▲ Period for CRA model. ◀ *p* value for CRA. ▼ Mesor of CRA model. ♠ Half range from trough to peak of CRA model. ♣ Time (in hour) of peak from CRA model. ♥ double amplitude = range from trough to peak of CRA model, as % of mesor. ♦ 95% Confidence Interval for peak (in hour).

Table S4 Rhythm Parameters for Substance P Expression in Spinal Cord Dorsal Horn in Wild Type Mice and *Per2^{Brdm1}* Mutant Mice

Time-Effect analysis by ANOVA* and Cosine Regression Analysis [#]													
G [▲]	S [▲]	ANOVA			Cosine Regression Analysis								
		F	<i>p</i>	T [◄]	F	<i>p</i> [▼]	M [♠]	AMP [♣]	<i>f</i> (<i>h</i>) [♥]	2Amp% [♦]	95%CI for peak [•]		R Squared
											L (h)	U (h)	
WT	N	3.33	0.007	24	28.4	0.003	52108.1	5017.37	17.82	19.26	15.11	20.53	0.85
WT	A-F-1h	0.71	0.618	24	13.03	0.015	63478.6	1893.7	17.12	5.97	14.16	20.08	0.72
WT	F-I	2.2	0.05	24	16.02	0.01	11370.5	3174.82	6.23	55.84	3.38	9.08	0.76
P2M	N	2.26	0.05	24	13.36	0.015	64695.3	5879.84	2.45	18.18	-0.76	5.66	0.73
P2M	A-F-1h	7.43	<0.001	24	3.84	0.11	73369.3	5639.82	23.43	15.37	19.09	27.76	0.43
P2M	F-I	5.3	<0.001	24	3.2	0.14	8673.96	4457.31	18.76	102.77	14.72	22.8	0.3

* Analysis of Variance for SP expression difference among the experiment time points. # Analysis of Circadian (24h) for SP expression using Cosine Regression Analysis (CRA) (refer to methods

for details). ▲ G: genotype of animals. WT, wild type mice; P2M, *Per2^{Brdm1}* mutant mice. ▲ S: SP expression. N, SP expression in naïve status; A-F-1h, SP expression after formalin injection 1 hr;

F-I, the increment SP expression induced by formalin injection. ◄ Period for CRA model. ▼ *p* value for CRA. ♠ Mesor of CRA model. ♣ Half range from trough to peak of CRA model. ♥ Time (in

hour) of peak from CRA model. ♦ double amplitude = range from trough to peak of CRA model, as % of mesor. • 95% Confidence Interval for peak (in hour).

Table S5 Rhythm Parameters for Formalin-induced Nociceptive Behavioral Response in Wild Type Mice and *Per2^{Brdm1}* Mutant Mice

Time-Effect analysis by ANOVA* and Cosine Regression Analysis#													
G [▲]	B [▲]	ANOVA			Cosine Regression Analysis								
		F	<i>p</i>	T [◄]	F	<i>p</i> [▼]	M [♠]	AMP [♣]	<i>f</i> (<i>h</i>) [♥]	2Amp% [♦]	95%CI for peak [•]		R Squared
											L (h)	U (h)	
WT	AP-F	2.45	0.05	24	40.82	0.001	171.02	34.16	10.47	39.95	7.49	13.45	0.89
WT	TP-F	3.16	0.017	24	23.941	0.005	452.86	53.36	5.82	23.57	3.06	8.57	0.83
WT	TP-F-S	4.53	0.004	24	56.54	0.001	408.86	114.23	5.46	55.88	2.85	8.08	0.92
WT	TP-F-A	0.14	0.982	24	7.52	0.064	198.3	8.55	16.7	8.62	15.06	18.34	0.6
P2M	AP-F	0.484	0.79	24	1.8	0.238	157.6	9.211	5.81	11.69	0.95	10.67	0.26
P2M	TP-F	11.1	<0.001	24	12.75	0.016	312.35	94.56	17.58	60.55	14.64	20.52	0.72

* Analysis of Variance for pain behavioral response difference among the experiment time points. #Analysis of Circadian (24 hrs) or Circasemidian (12 hrs) for nociceptive behavioral response

using Cosine Regression Analysis (CRA) (refer to methods for details). ▲ G: genotype of animals. WT, wild type mice; P2M, *Per2Brdm1* mutant mice. ▲ B: nociceptive behavioral response.

AP-F, acute phase of formalin-induced nociceptive response; TP-F, tonic phase of formalin-induced nociceptive response; TP-F-S, tonic phase control for formalin-induced nociceptive response with vehicle intrathecal administration; TP-F-A, tonic phase of formalin-induced nociceptive response after antagonist (L-732,138) intrathecal administration. ◄ Period for CRA model. ▼ *p* value for CRA. ♠ Mesor of CRA model. ♣ Half range from trough to peak of CRA model. ♥ Time (in hour) of peak from CRA model. ♦ double amplitude = range from trough to peak of CRA model, as % of mesor. • 95% Confidence Interval for peak (in hour).