

<i>pdfGAL4 with UAS-DICER2</i>											<i>timGAL4 with UAS-DICER2</i>											
	N	Period (hrs)	SD	SE	OFF	OFF SD	RI	RI SD	RS	RS SD	%Rhy	N	Period (hrs)	SD	SE	OFF	OFF SD	RI	RI SD	RS	RS SD	%Rhy
Drivers	20	24.7	0.3	0.11	2.0	2.0	0.5	0.1	10.6	1.5	70%	24	24.0	0.3	0.1	2.8	0.8	0.5	0.1	11.4	2.5	92%
<i>pabp2</i>	26	25.5	0.4	0.11	2.3	0.8	0.4	0.1	9.3	2.3	92%	24	24.4	1.5	0.61	1.7	3.0	0.4	0.1	7.9	2.6	50%
<i>NAT1B</i>	28	27.1	0.8	0.29	3.2	0.8	0.3	0.1	7.3	2.2	57%	53	27.7	0.4	0.23	1.8	0.8	0.4	0.1	8.8	2.0	45%
<i>NAT1A</i>	32	24.8	0.6	0.24	2.9	0.6	0.3	0.1	7.3	2.6	63%	31	23.4	1.1	0.46	1.1	1.3	0.3	0.2	7.2	3.6	26%
<i>paip2a</i>	20	25.7	1.0	0.34	3.4	1.8	0.4	0.1	8.4	2.7	90%	20	24.5	0.4	0.14	2.7	1.6	0.5	0.1	10.6	2.6	90%
<i>eif4a</i>	21	24.7	1.1	0.48	2.5	0.5	0.3	0.1	5.7	1.2	52%	lethal in tim										
<i>eif4g</i>	20	24.7	1.4	0.54	2.9	0.9	0.3	0.1	7.3	2.1	70%	30	24.8	1.2	0.43	3.4	1.5	0.3	0.1	6.9	2.2	67%
<i>FMRI</i>	26	24.3	0.2	0.04	3.7	1.7	0.5	0.1	10.5	2.1	92%	24	24.2	0.3	0.08	3.3	1.4	0.5	0.1	10.7	2.4	92%
<i>eif5c</i>	26	26.0	0.8	0.30	3.3	1.8	0.5	0.1	11.8	2.9	67%	24	24.5	0.4	0.12	3.1	1.2	0.5	0.1	11.3	2.3	88%
<i>eif3.10</i>	23	25.2	0.9	0.35	4.2	1.5	0.4	0.1	9.1	2.7	64%	20	24.0	0.2	0.05	4.5	0.7	0.5	0.1	11.3	2.2	100%
<i>lk6</i>	28	25.6	1.2	0.34	3.1	1.5	0.4	0.1	9.7	2.8	86%	21	24.6	0.2	0.07	2.9	1.3	0.5	0.1	11.9	2.1	90%
<i>bel</i>	24	24.5	0.4	0.10	3.1	1.8	0.5	0.1	9.9	2.8	100%	30	24.2	0.2	0.08	4.9	1.1	0.5	0.1	11.8	1.5	100%
<i>thor</i>	25	24.4	0.2	0.05	2.6	1.5	0.5	0.1	11.0	2.2	100%	22	23.9	0.3	0.11	3.2	1.1	0.5	0.1	10.5	2.3	92%
<i>eEF2</i>	24	23.4	0.6	0.34	4.7	1.8	0.3	0.0	5.8	0.7	17%	20	24.5	0.3	0.09	4.5	0.8	0.5	0.1	11.3	1.6	91%
<i>akt</i>	23	25.7	0.6	0.21	3.8	0.9	0.4	0.1	9.4	2.9	64%	30	24.5	0.8	0.30	2.2	1.0	0.4	0.2	9.6	3.6	63%

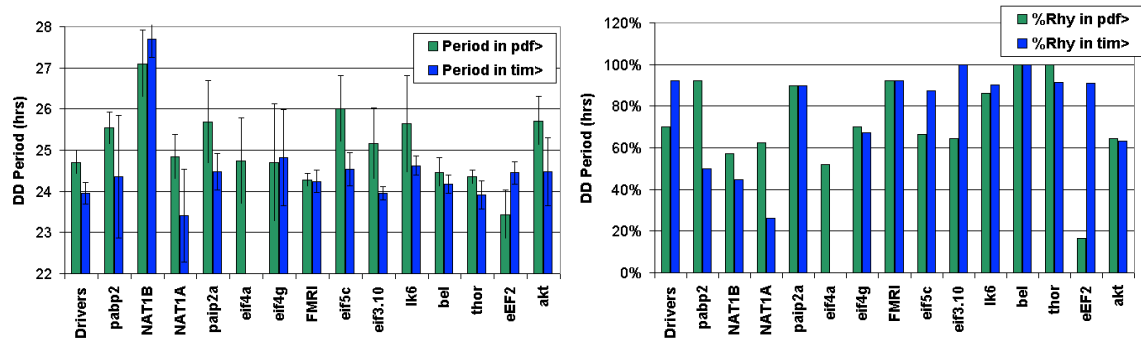


Figure S1 A subset of interesting RNAi lines were tested in combination with *PDF-GAL4* or *TIM-GAL4* with *UAS-DICER2*. In general the effects of the knockdown were more severe, with substantially fewer highly rhythmic flies. Significant period lengthening effects were revealed for *AKT* and *LK6*, which were not apparent without *UAS-DICER2*. Surprisingly, some effects on period were reduced in severity, perhaps due to non-specific effects of *DICER2* overexpression. This was the case for the second line targeting *NAT1*, which produced a less severe period-lengthening effect in combination with *tim>UAS-DICER2*, and *eEF2*, which was no longer lethal with the addition of *DICER2* overexpression.

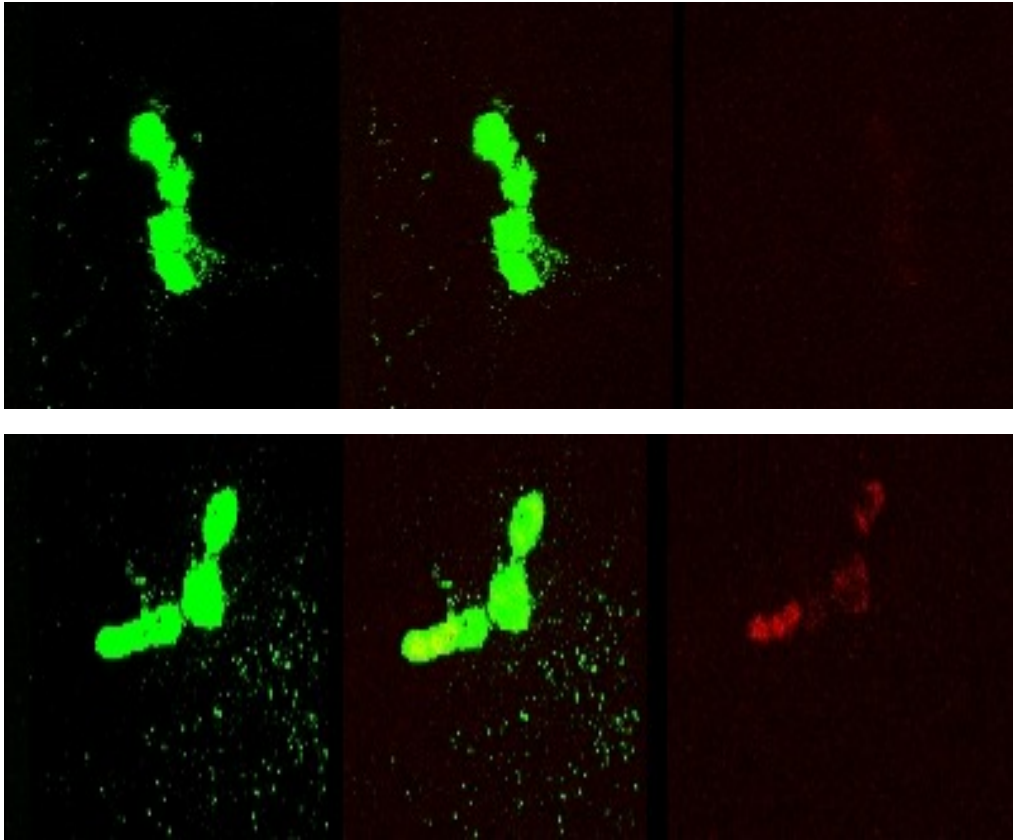


Figure S2 Staining for PDF (green) and PER (red) in PDF+ LNV cells at CT3 of DD2 reveals much lower expression in *tim>NAT1RNAi* fly brains (Top) compared to *tim-GAL4* controls (Bottom). This indicates that the decrement in PER expression presented in Figure 4 is not due to a phase delay resulting from the slower period length in these flies, but instead to lower expression of PER in *NAT1* knockdown.

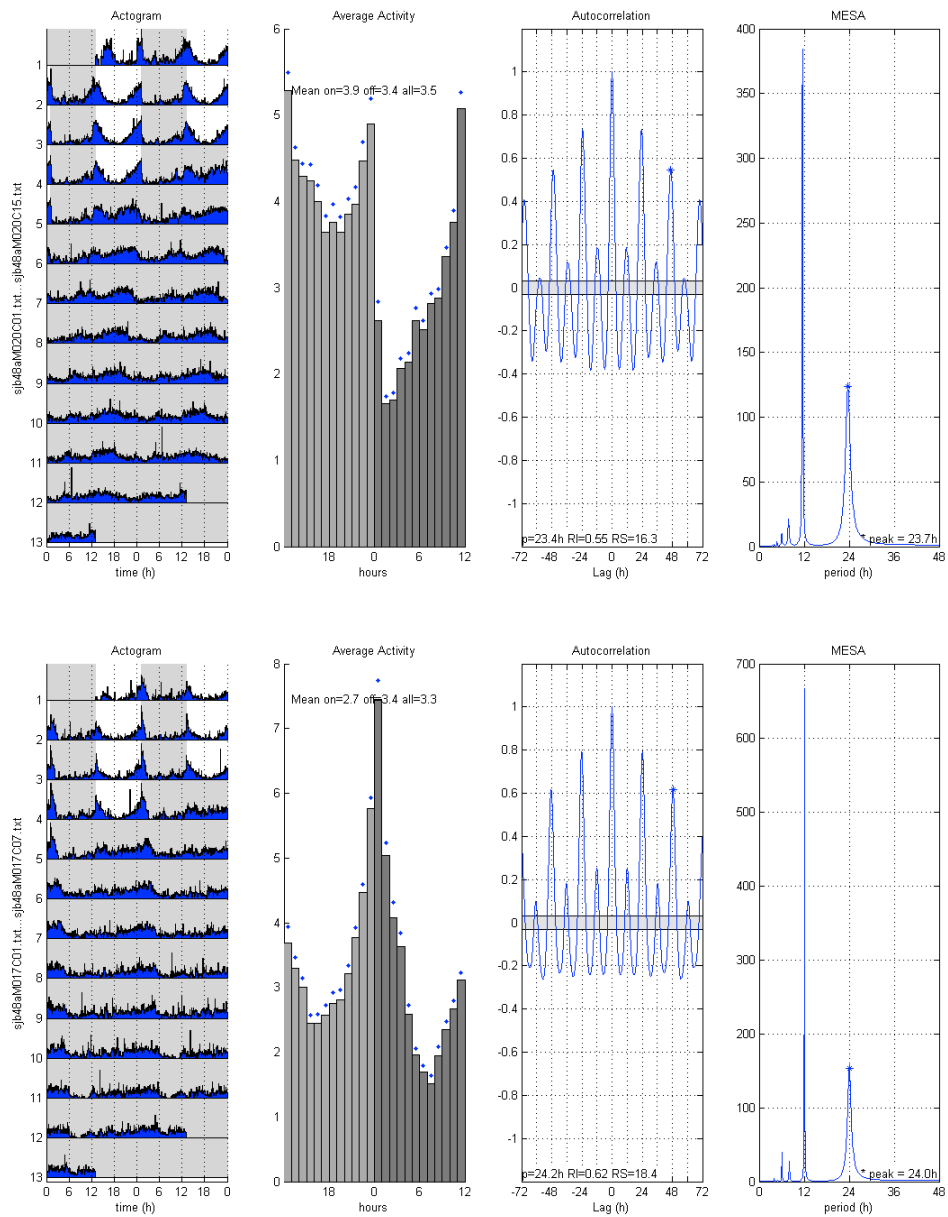
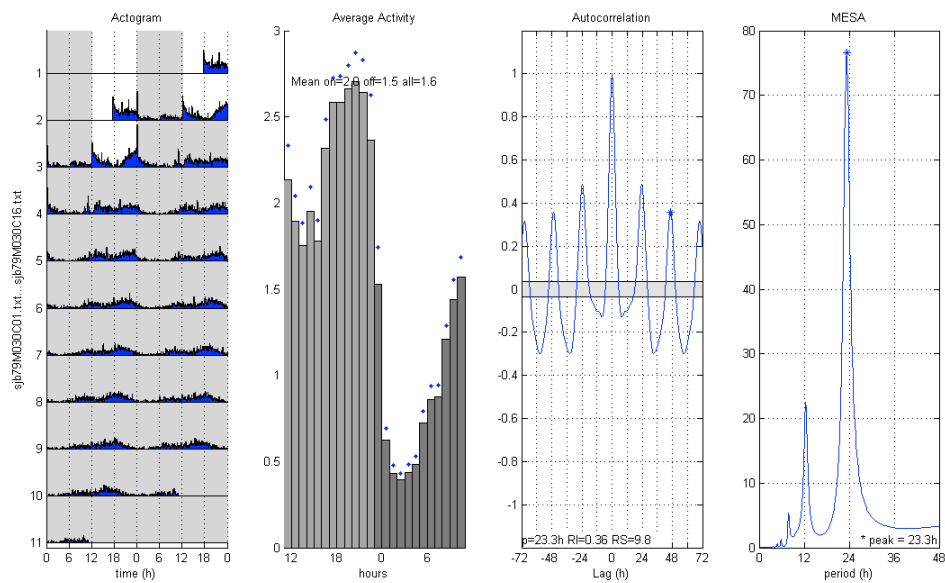


Figure S3 Flies homozygous for *uasNAT1* transgenes. (Top) Flies carrying two copies of a full-length *uasNAT1* construct have fast rhythms and persistent morning peaks. (Bottom) Elements in the 5'UTR of *NAT1* are required for the effect, as a 5' truncation of the transgene produces normal rhythms and a rapidly dampened morning peak.

Genotype	n	mean	sd	se	off	off sd	ri	ri sd	rs	rs sd	%Rh
dNAT1 P2: w[1118]; P[w[+mGT]=GT1]NAT1[BG02338]	24	23.3	0.2	0.1	2.4	1.1	0.4	0.1	8.5	2.0	75%
P2/P1	24	23.4	0.3	0.1	3.5	1.9	0.5	0.1	13.1	3.6	100%
P2/P1cyo	22	24.2	0.3	0.1	2.1	0.7	0.5	0.1	12.3	3.4	91%
P2/P3	16	24.2	1.4	0.6	3.8	3.0	0.5	0.1	12.6	3.3	75%
dNAT P1: cn[1] P[ry[+7.2]=PZ]NAT1[O1424]/CyO; ry[506]	12	23.7	0.5	0.2	2.2	1.1	0.5	0.1	11.6	2.6	100%
P1/P3	15	24.0	0.3	0.1	1.7	0.9	0.5	0.1	11.3	2.9	80%
P1cyo/P3	20	24.2	0.3	0.1	2.9	1.7	0.5	0.1	10.1	1.6	70%
dNAT1 P3: y[1] w[67c23]; P[w[+mC] y[+mDint2]=EPgy2]NAT1[EY08892]	13	24.0	0.2	0.1	3.0	0.8	0.5	0.1	11.4	1.6	77%
+;pdfGAL4/cyo;UAS-NAT1R/UAS-NAT1R	22	25.7	0.3	0.1	3.4	1.3	0.4	0.1	8.5	2.5	91%
+;pdfGAL4/P[w[+mC] y[+mDint2]=EPgy2]NAT1[EY08892];UAS-NAT1R/+	15	25.6	0.3	0.1	3.7	1.3	0.4	0.1	8.9	2.6	100%
+;pdfGAL4/P[ry[+7.2]=PZ]NAT1[O1424];UAS-NAT1R/+	43	25.6	0.4	0.2	3.9	0.5	0.3	0.1	7.1	1.3	56%
+;pdfGAL4/P[w[+mGT]=GT1]NAT1[BG02338];UAS-NAT1R/+	30	25.5	0.4	0.1	2.5	1.3	0.4	0.2	10.2	4.0	73%



genotype	n	mean	sd	se	off	off sd	ri	ri sd	rs	rs sd	%Rhy
+; pdfGAL4/UAS-FMRPRNAi; UAS-NAT1aRNAi/+	26	25.0	0.4	0.1	4.1	1.8	0.5	0.1	10.2	2.0	73%
+; pdfGAL4/+; UAS-NAT1aRNAi/UAS-NAT1bRNAi	24	25.6	0.8	0.3	3.3	1.8	0.4	0.2	8.4	3.1	58%
+; pdfGAL4/UAS-eIF4ARNAi; UAS-NAT1aRNAi/+	27	25.1	0.9	0.5	4.4	1.2	0.5	0.2	10.5	3.4	26%
+; pdfGAL4/UAS-eIF4GRNAi; UAS-NAT1aRNAi/+	23	25.4	0.8	0.3	2.7	1.9	0.3	0.1	6.5	1.5	61%
+; pdfGAL4/+; UAS-NAT1aRNAi/UAS-BELRNAi	20	25.4	0.4	0.1	2.2	1.6	0.4	0.1	9.0	2.2	90%
+; pdfGAL4/UAS-THORRNAi; UAS-NAT1aRNAi/+	21	24.7	0.4	0.2	4.4	3.3	0.4	0.1	7.6	2.6	62%
+; pdfGAL4/UAS-eIF5CRNAi; UAS-NAT1aRNAi/+	20	24.9	0.8	0.3	1.4	1.3	0.4	0.1	9.1	2.3	65%
+; pdfGAL4/UAS-PAIP2RNAi; UAS-NAT1aRNAi/+	26	25.1	0.2	0.1	1.9	0.7	0.5	0.1	11.6	2.9	81%
+; pdfGAL4/+; UAS-NAT1aRNAi/UAS-eIF3.10RNAi	21	25.3	1.0	0.3	3.7	1.8	0.4	0.1	8.9	1.9	71%
+; pdfGAL4/+; UAS-NAT1aRNAi/UAS-AKTRNAi	22	24.4	0.6	0.2	1.5	0.6	0.4	0.1	8.4	1.3	64%
+; pdfGAL4/UAS-LK6RNAi; UAS-NAT1aRNAi/+	24	25.2	0.4	0.1	2.1	1.2	0.5	0.1	10.7	2.4	83%

Figure S4 P-element insertions at the NAT1 locus combined with *pdf>NAT1RNAi* provided no additional increase in period lengthening. (Top) Various combinations of mutant alleles, where the most severe period phenotype is present with two copies of *UAS-NAT1RNAi*. Behavior metrics are calculated for flies with a DD rhythmic index of 0.2 or more. (Middle) only one mutant allele, dNAT P2, provided a period effect on its own, which was faster than WT controls. (Bottom) Combinations of interesting RNAi lines in PDF cells do not provide significant alterations from the *pdf>NAT1RNAi* long-period phenotype, although an increased fraction of arrhythmic flies was found. Behavior metrics are calculated for flies with a DD rhythmic index of 0.2 or more.

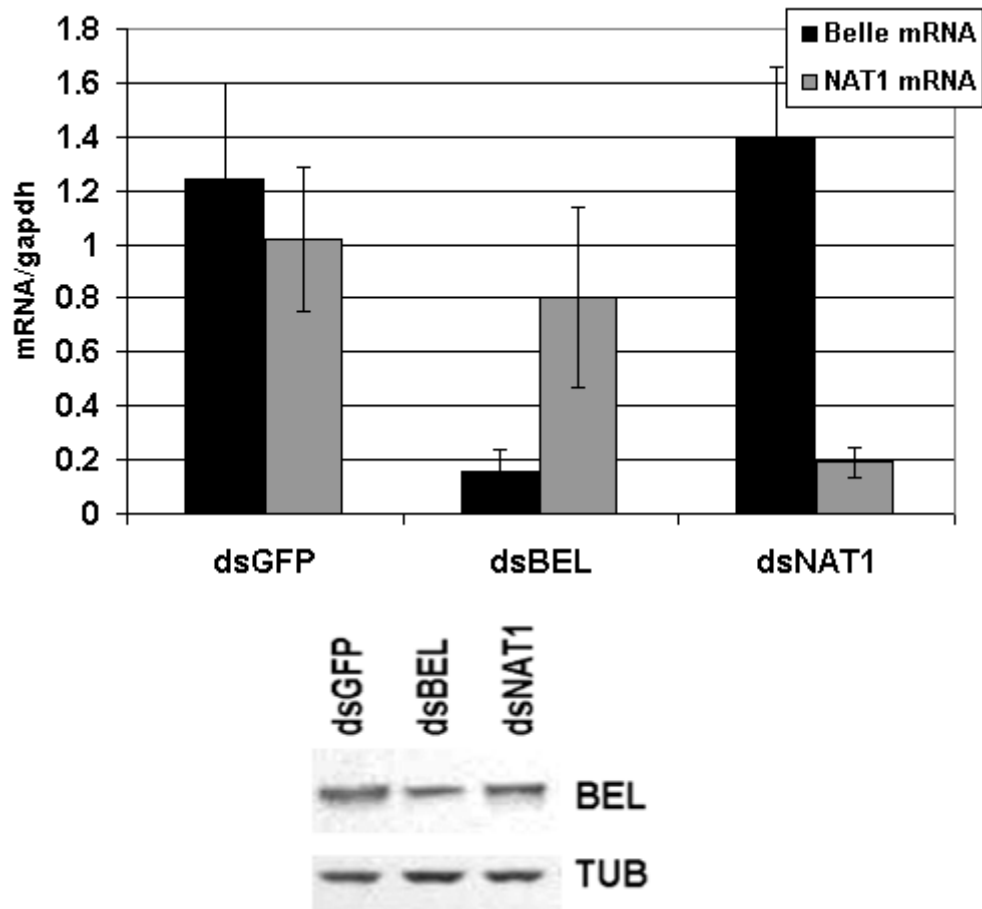


Figure S5 *drosophila* S2 cells were treated with double-stranded (ds)RNA against Belle, NAT1 or GFP as a control. (Top) RNAi of NAT1 or BEL causes a dramatic decrease in the mRNA for these genes. (Bottom) Western blotting confirms the knockdown of BEL, and shows that NAT1 RNAi causes some reduction of BEL levels, although less pronounced than the effect found in fly heads.

Table S1

Available for download at <http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.112.143248/-/DC1>.