

Supplemental Table 1. Circadian rhythm behavior of glycolytic enzyme knockdown flies, related to Figure 1.

Genotype	Temp(°C)	N ^a	Tau ± SEM (h)	R (%) ^b	Power ± SEM ^c
<i>d2,tim-gal4>Hex-A Ri²¹⁵⁰⁴</i>	25	32	24.1 ± 0.08	81.3	92.7 ± 6.35
<i>d2,tim-gal4>Hex-A Ri⁰⁴⁶⁸⁰</i>	25	25	24.1 ± 0.10	60	80.6 ± 6.2
<i>d2,tim-gal4>Hex-C Ri⁵⁷⁴⁰⁴</i>	25	32	24.0 ± 0.05	100	149.3 ± 9.31
<i>d2,tim-gal4>Hex-C Ri³⁵³³⁸</i>	25	31	24.9 ± 0.07	67.7	70.9 ± 6.59
<i>d2,tim-gal4>Hex-C Ri³⁵³³⁷</i>	25	32	25.1 ± 0.08	71.9	73.1 ± 4.4
<i>d2,tim-gal4>Pfk Ri³⁴³³⁶</i>	25	31	24.7 ± 0.11	64.5	58.8 ± 4.31
<i>d2,tim-gal4>Pfk Ri¹⁰⁵⁶⁶⁶</i>	25	26	26.5 ± 0.19	26.9	63.8 ± 7.08
<i>d2,tim-gal4>Pyk Ri³⁵²¹⁸</i>	25	32	22.7 ± 0.09	50	107.4 ± 15.92
<i>d2,tim-gal4>Pyk Ri⁴⁹⁵³³</i>	25	32	23.0 ± 0.09	65.6	85.0 ± 5.34
<i>d2,tim-gal4>Pyk Ri³⁵¹⁶⁵</i>	25	27	23.9 ± 0.08	92.6	159.3 ± 13.99
<i>d2,tim-gal4/+</i>	25	32	23.9 ± 0.07	96.9	188.9 ± 9.73
<i>Hex-A Ri²¹⁵⁰⁴/+</i>	25	30	23.5 ± 0.04	96.7	226.5 ± 13.01
<i>Hex-A Ri¹⁰⁴⁶⁸⁰/+</i>	25	26	23.4 ± 0.03	100	281.0 ± 14.23
<i>Hex-C Ri⁵⁷⁴⁰⁴/+</i>	25	25	23.6 ± 0.05	96.0	233.0 ± 13.96
<i>Hex-C Ri³⁵³³⁸/+</i>	25	28	23.5 ± 0.03	96.4	292.0 ± 13.71
<i>Hex-C Ri³⁵³³⁷/+</i>	25	28	23.5 ± 0.04	100	227.9 ± 12.85
<i>Pfk Ri³⁴³³⁶/+</i>	25	26	23.4 ± 0.03	92.3	226.7 ± 13.69
<i>Pfk Ri¹⁰⁵⁶⁶⁶/+</i>	25	27	23.4 ± 0.03	100	219.9 ± 14.64
<i>Pyk Ri³⁵²¹⁸/+</i>	25	29	23.4 ± 0.08	100	185.6 ± 13.37
<i>Pyk Ri⁴⁹⁵³³/+</i>	25	26	23.4 ± 0.04	96.2	212.7 ± 14.31
<i>Pyk Ri³⁵¹⁶⁵/+</i>	25	28	23.6 ± 0.05	100	280.3 ± 10.9

^aTotal number of flies that survived until the end of the testing period.

^bPercentage of flies with activity rhythms with a power value ≥ 10 and a width ≥ 2.

^cMeasure of the strength or amplitude of the rhythm.

Supplemental Table 2. Circadian rhythm behavior of adult specific HexC and Pyk knockdown flies, related to Figure 1E and Figure 1F.

Genotype	Temp (°C)	N ^a	Tau ± SEM (h)	R (%) ^b	Power ± SEM ^c
<i>tub-gal80^{ts};tim-gal4</i>	20	29	23.6 ± 0.08	69	86.2 ± 9.69
<i>tub-gal80^{ts};tim-gal4>Hex-C Ri³⁵³³⁷</i>	20	32	23.5 ± 0.07	90.6	102.4 ± 7.27
<i>Hex-C Ri³⁵³³⁷/+</i>	20	30	23.7 ± 0.06	100	140.3 ± 9.93
<i>tub-gal80^{ts};tim-gal4</i>	30	15	23.9 ± 0.21	66.7	79.3 ± 14.55
<i>tub-gal80^{ts};tim-gal4>Hex-C Ri³⁵³³⁷</i>	30	32	23.4 ± 0.06	96.9	210.1 ± 13.12
<i>Hex-C Ri³⁵³³⁷/+</i>	30	32	23.4 ± 0.03	75.0	210.7 ± 13.89
<i>tub-gal80^{ts};tim-gal4</i>	20	31	23.5 ± 0.10	96.8	149.3 ± 9.31
<i>tub-gal80^{ts};tim-gal4>Pyk Ri³⁵²¹⁸</i>	20	30	23.2 ± 0.07	86.7	70.9 ± 6.59
<i>Pyk Ri³⁵²¹⁸/+</i>	20	31	23.4 ± 0.05	100	106.4 ± 7.31
<i>tub-gal80^{ts};tim-gal4</i>	30	28	23.6 ± 0.08	96.4	73.1 ± 4.4
<i>tub-gal80^{ts};tim-gal4>Pyk Ri³⁵²¹⁸</i>	30	32	22.5 ± 0.14	37.5	61.2 ± 5.34
<i>Pyk Ri³⁵²¹⁸/+</i>	30	32	23.1 ± 0.03	71.9	182.9 ± 14.79

^aTotal number of flies that survived until the end of the testing period.

^bPercentage of flies with activity rhythms with a power value ≥ 10 and a width ≥ 2.

^cMeasure of the strength or amplitude of the rhythm.

Supplemental Table 3. Circadian rhythm behavior of *Pyk* AKD;*Pyk* AOE flies, related to Figure 1G.

Genotype	Temp (°C)	N ^a	Tau ± SEM (h)	R (%) ^b	Power ± SEM ^c
<i>tub-gal80^{ts};tim-gal4</i>	30	23	23.5 ± 0.08	100	166.6 ± 13.88
<i>Pyk Ri³⁵²¹⁸/+</i>	30	28	23.4 ± 0.05	96.4	164.6 ± 11.22
<i>Pyk Ri::Pyk⁵/+</i>	30	32	23.2 ± 0.03	93.7	236.6 ± 15.42
<i>tub-gal80^{ts};tim-gal4>Pyk Ri³⁵²¹⁸</i>	30	17	22.7 ± 0.52	29.4	103.7 ± 35.97
<i>tub-gal80^{ts};tim-gal4>Pyk Ri³⁵²¹⁸::Pyk⁵</i>	30	24	23.2 ± 0.11	83.3	131.5 ± 16.43

^aTotal number of flies that survived until the end of the testing period.

^bPercentage of flies with activity rhythms with a power value ≥ 10 and a width ≥ 2.

^cMeasure of the strength or amplitude of the rhythm.

Supplemental Table 4. Circadian rhythm behavior of adult specific Pyk overexpression flies

Genotype	Temp (°C)	N ^a	Tau ± SEM (h)	R (%) ^b	Power ± SEM ^c
<i>tub-gal80^{ts};tim-gal4</i>	30	23	23.4 ± 0.1	82.6	123.6 ± 9.3
<i>tub-gal80^{ts};tim-gal4 > Pyk</i>	30	20	23.2 ± 0.08	85	122.1 ± 16.8

^aTotal number of flies that survived until the end of the testing period.

^bPercentage of flies with activity rhythms with a power value ≥ 10 and a width ≥ 2.

^cMeasure of the strength or amplitude of the rhythm.

Supplemental Table 5. Circadian rhythm behavior of adult specific *Pdf* and *Pyk* knockdown flies

Genotype	Temp (°C)	N ^a	Tau ± SEM (h)	R (%) ^b	Power ± SEM ^c
<i>tub-gal80^{ts};tim-gal4</i>	30	32	23.4 ± 0.06	90.6	266.5 ± 13.7
<i>Pdf Ri²⁵⁸⁰²/+</i>	30	29	23.4 ± 0.06	82.7	198.2 ± 16.18
<i>tub-gal80^{ts};tim-gal4 > Pdf Ri²⁵⁸⁰²</i>	30	29	23.0 ± 0.09	62.1	146.9 ± 15.22
<i>tub-gal80^{ts};tim-gal4</i>	30	29	23.5 ± 0.06	93.5	230 ± 10.17
<i>Pyk Ri³⁵²¹⁸/+</i>	30	28	23.4 ± 0.05	96.4	164.6 ± 11.22
<i>tub-gal80^{ts};tim-gal4 > Pyk Ri³⁵²¹⁸</i>	30	12	22.9 ± 0.12	42.3	163.6 ± 24.49

^aTotal number of flies that survived until the end of the testing period.

^bPercentage of flies with activity rhythms with a power value ≥ 10 and a width ≥ 2.

^cMeasure of the strength or amplitude of the rhythm.

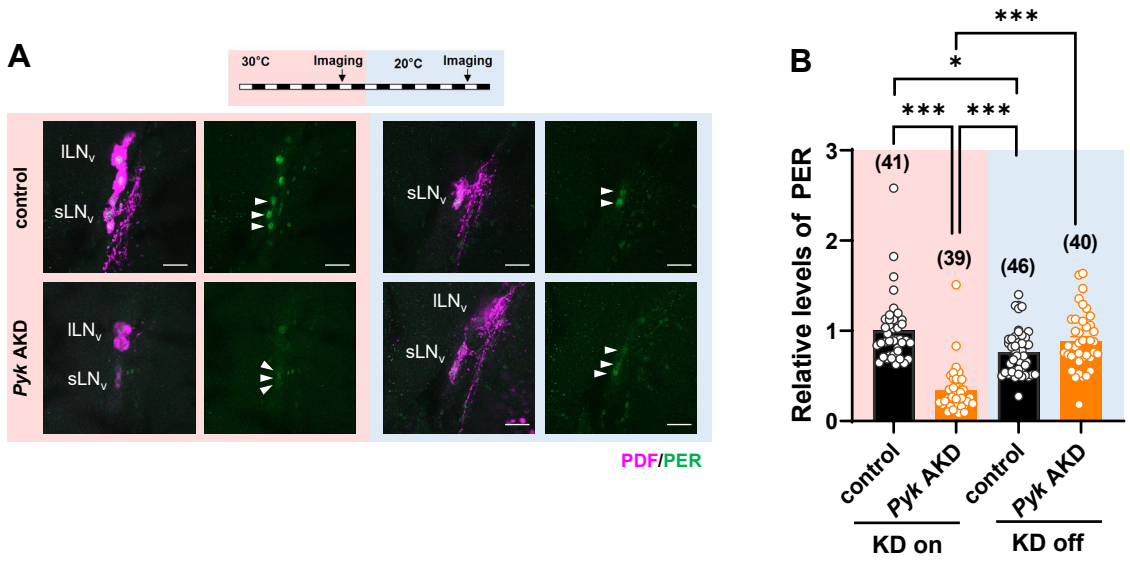
Supplemental Table 6. Circadian rhythm behavior of *Pyk* AKD, *Pyk* AKD;*tara* AOE, *tara* AOE flies

Genotype	Temp (°C)	N ^a	Tau ± SEM (h)	R (%) ^b	Power ± SEM ^c
<i>tub-gal80^{ts};tim-gal4</i>	30	30	23.4 ± 0.06	96.7	212.9 ± 14.42
<i>Pyk Ri³⁵²¹⁸/+</i>	30	28	23.4 ± 0.05	96.4	164.6 ± 11.22
<i>tara/+</i>	30	29	23.5 ± 0.04	86.2	176.1 ± 15.52
<i>Pyk Ri³⁵²¹⁸:tara/+</i>	30	32	23.4 ± 0.03	96.9	240 ± 12.41
<i>tub-gal80^{ts};tim-gal4>Pyk Ri³⁵²¹⁸</i>	30	31	23.1 ± 0.06	61.3	140.3 ± 20.69
<i>tub-gal80^{ts};tim-gal4>tara</i>	30	27	23.6 ± 0.12	74.1	135.6 ± 15.17
<i>tub-gal80^{ts};tim-gal4>Pyk Ri³⁵²¹⁸:tara</i>	30	32	23.4 ± 0.09	87.5	159.2 ± 12.94

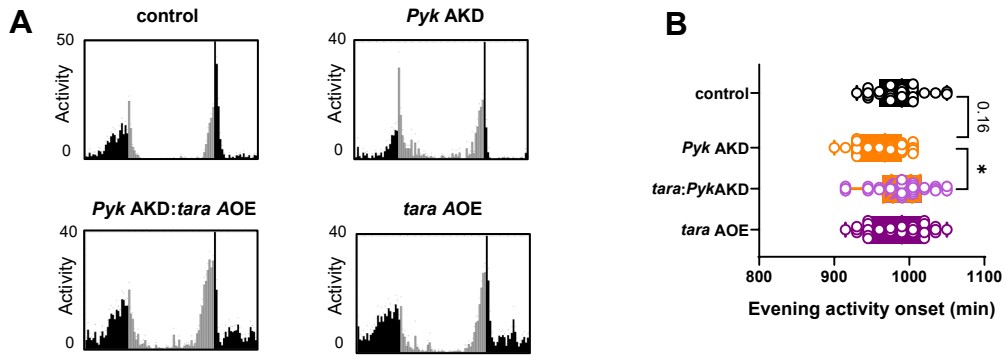
^aTotal number of flies that survived until the end of the testing period.

^bPercentage of flies with activity rhythms with a power value ≥ 10 and a width ≥ 2.

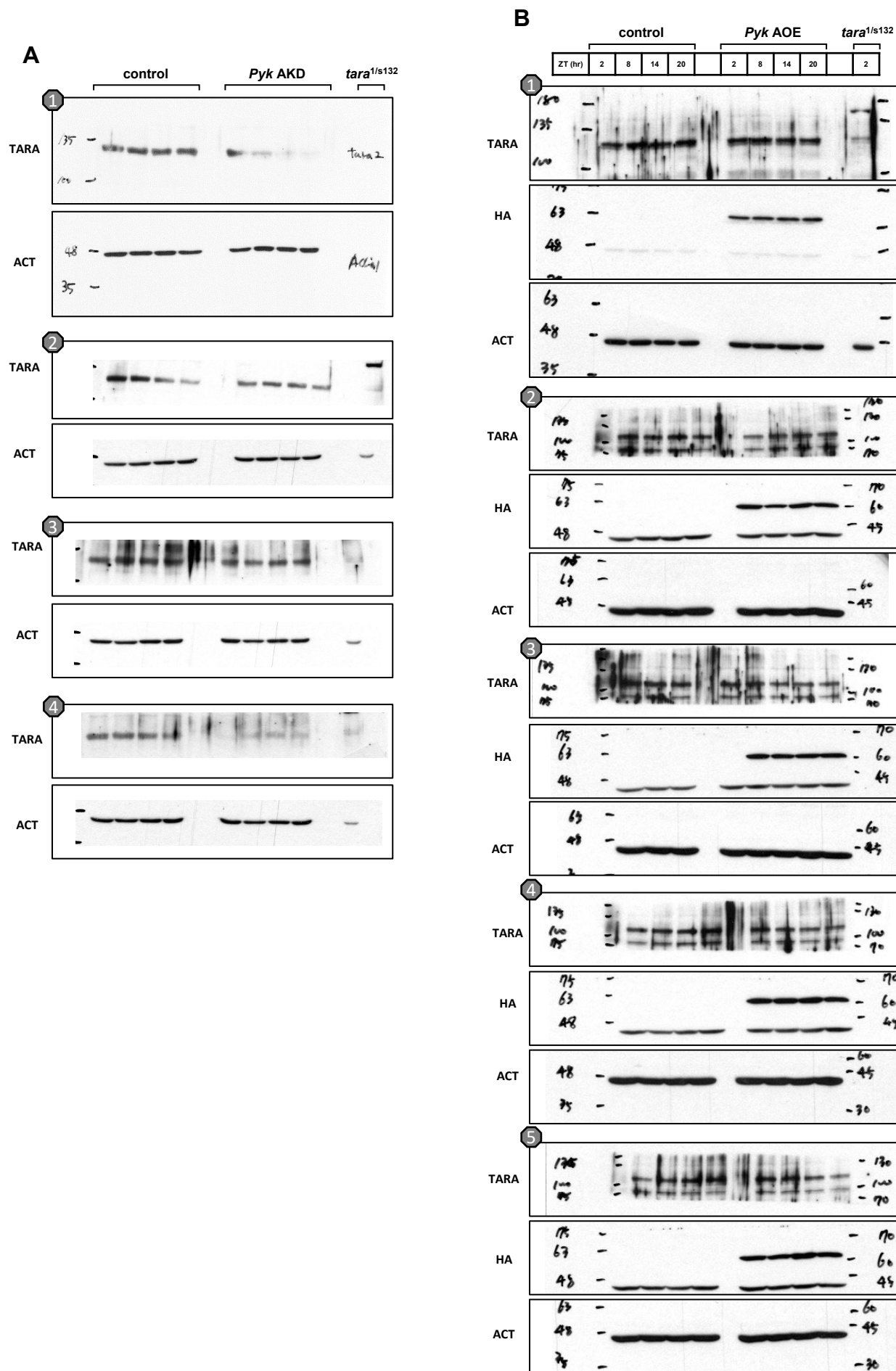
^cMeasure of the strength or amplitude of the rhythm.



Supplemental Figure 1. PER levels were highly responsive to *Pyk* expression. (A and B) *dcr2;tub-g80^{ts}; tim-GAL4* flies were crossed with *w¹¹¹⁸* (control) or UAS-*Pyk* Ri (*Pyk* AKD) flies and reared at 20°C. (A) F1 offsprings were maintained on a 12L:12D cycle at 30°C (KD on, red background) for five days and then shifted to 20°C (KD off, blue background) for five days. Brains were dissected at ZT2 on day 5 (arrow, “Imaging”) and stained with anti-PER (green) antibodies. PER intensities were significantly reduced in *Pyk* AKD flies compared to control at 30°C, but similar between control and *Pyk* AKD flies after temperature shift to 20°C. Arrowheads indicate sLN_vs. All scale bars represent 20 μm. (B) PER intensities were quantified, and bars indicate the mean ± SEM. n represents the number of cells analyzed for each genotype and is indicated above each bar. Statistical differences were determined using one-way ANOVA followed by a two-sided Tukey’s multiple comparisons test: **p* < 0.05, ***p* < 0.01, ****p* < 0.001. The source data for supplemental figure 1 is in Supplemental Data S1.



Supplemental Figure 2. Circadian rhythm behavior of *Pyk* overexpression flies. *dcr2;tub-G80^{ts}; tim-G4* flies were crossed with *w¹¹¹⁸* (control), UAS-*Pyk* Ri (*Pyk* AKD), or UAS-*tara* (*tara* AOE) either individually or in combination as indicated. Circadian rhythmic behavior of F1 offsprings were analyzed at 30°C. (A) Daily activity profiles of flies with the indicated genotypes on the 4th day of the LD cycle are shown. Light gray bars indicate locomotion during the light phase, and black bars indicate locomotion during the dark phase. (B) Evening activity onsets of flies with the indicated genotype are shown. Statistical differences were determined using the Kruskal-Wallis test followed by a two-sided Dunns multiple-comparisons test: ***p* < 0.01, ****p* < 0.001. The source data for supplemental figure 2 is in Supplemental Data S2.



Supplemental Figure 3. Uncropped and unedited blot images corresponding to Fig. 4. (A) Blots related to Fig. 4D. **(B)** Blots related to Fig. 4G.