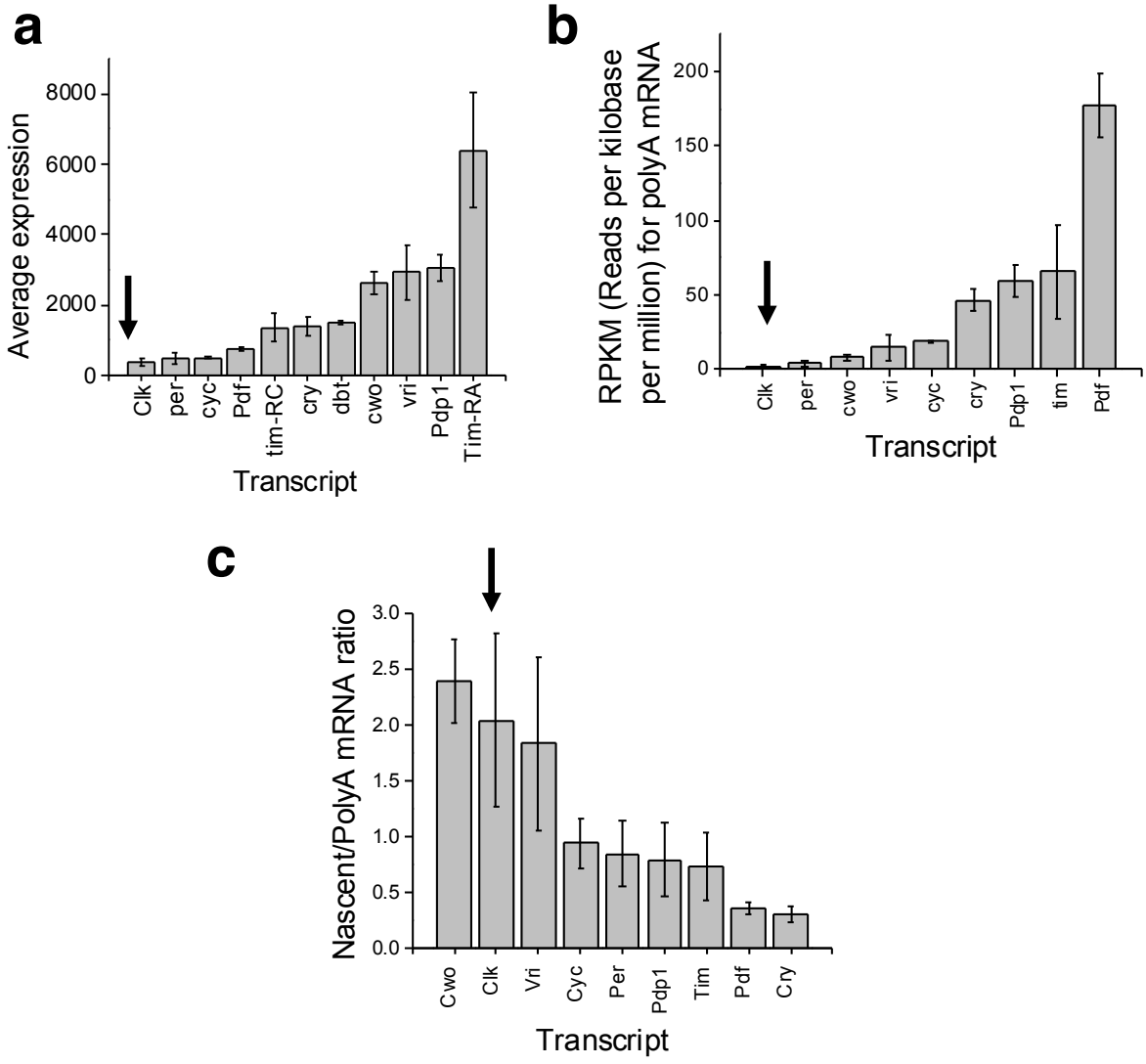
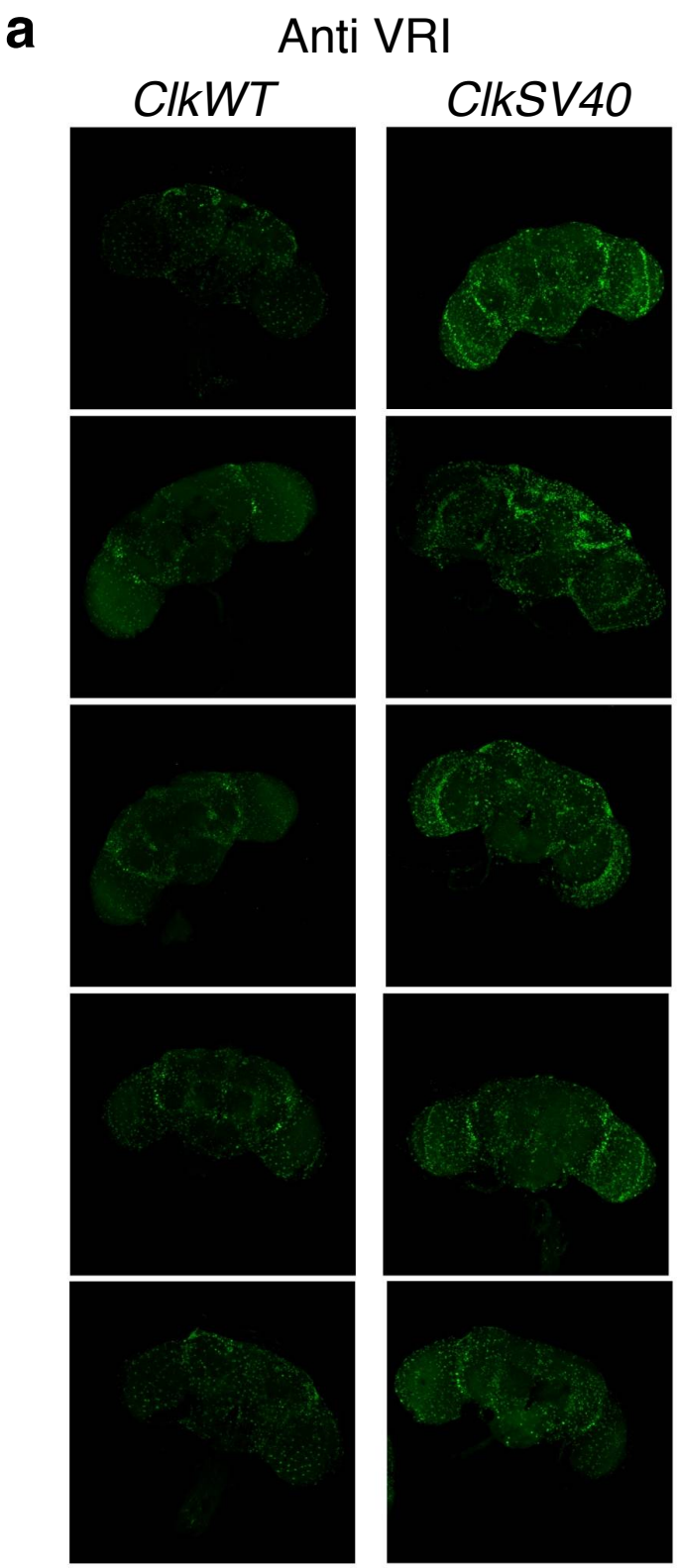
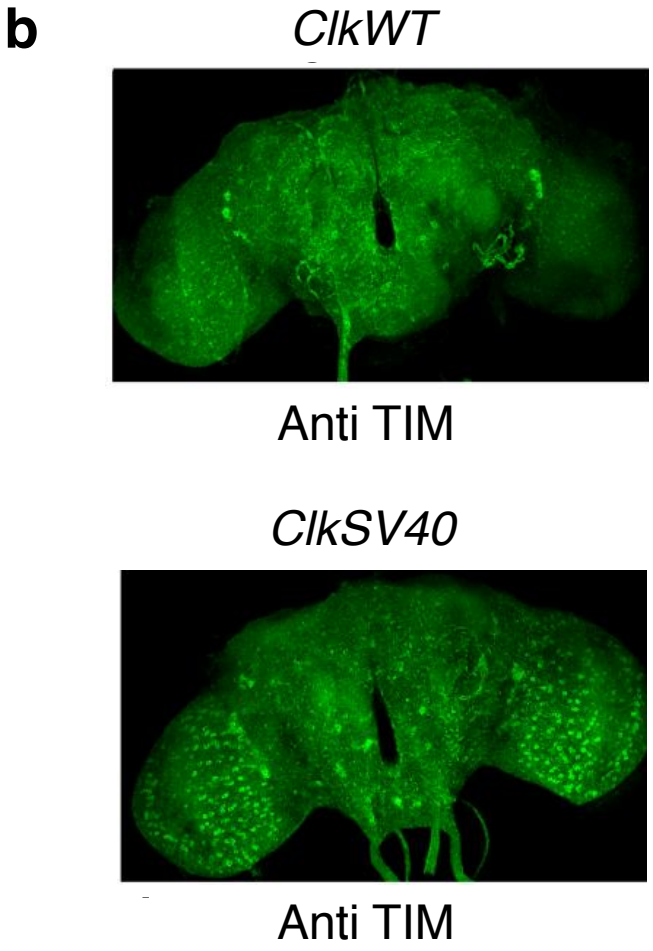
Supplementary Fig. 1



Supplementary Figure 1. ***Clk*** **is under strong strong post-transcriptional control. a.** Comparison of mRNA levels of the core *Drosophila* circadian components. Previously published microarray data was used to compare expression levels of core circadian components in fly heads[1]; an average of six time points ± SE in LD conditions (ZT3, 7, 11, 15, 19, 23) were analyzed. **b.** Analysis of sequencing data of brain polyA+ RNA at four time points (ZT0, 6, 12, 18)[2]. **c.** Ratio of nascent to polyA+ RNAs[3] at six time points with multiple replicas (ZT 2,6,10,14,18,22). In all panels, the arrow indicates the position of *Clk* in the bar graph.

Supplementary Fig. 2





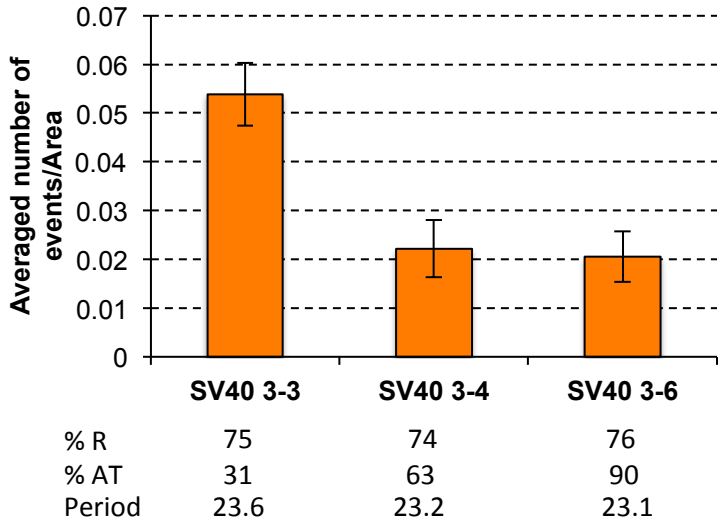
Supplementary Figure 2. Ectopic expression of clock genes in *ClkSV40* flies. a. Representative pictures of VRI immunostaining for five different insertions of the *ClkWT* and *ClkSV40* transgenes. The flies were entrained for three days in LD, and brains dissected at ZT15. Brains were visualized by confocal microscopy. **b.** TIM immunostaining of *ClkSV40* and *ClkWT* flies. The flies were entrained for three days in LD, and brains dissected at ZT18. Brains were visualized by confocal microscopy.

Supplementary Fig. 3

a

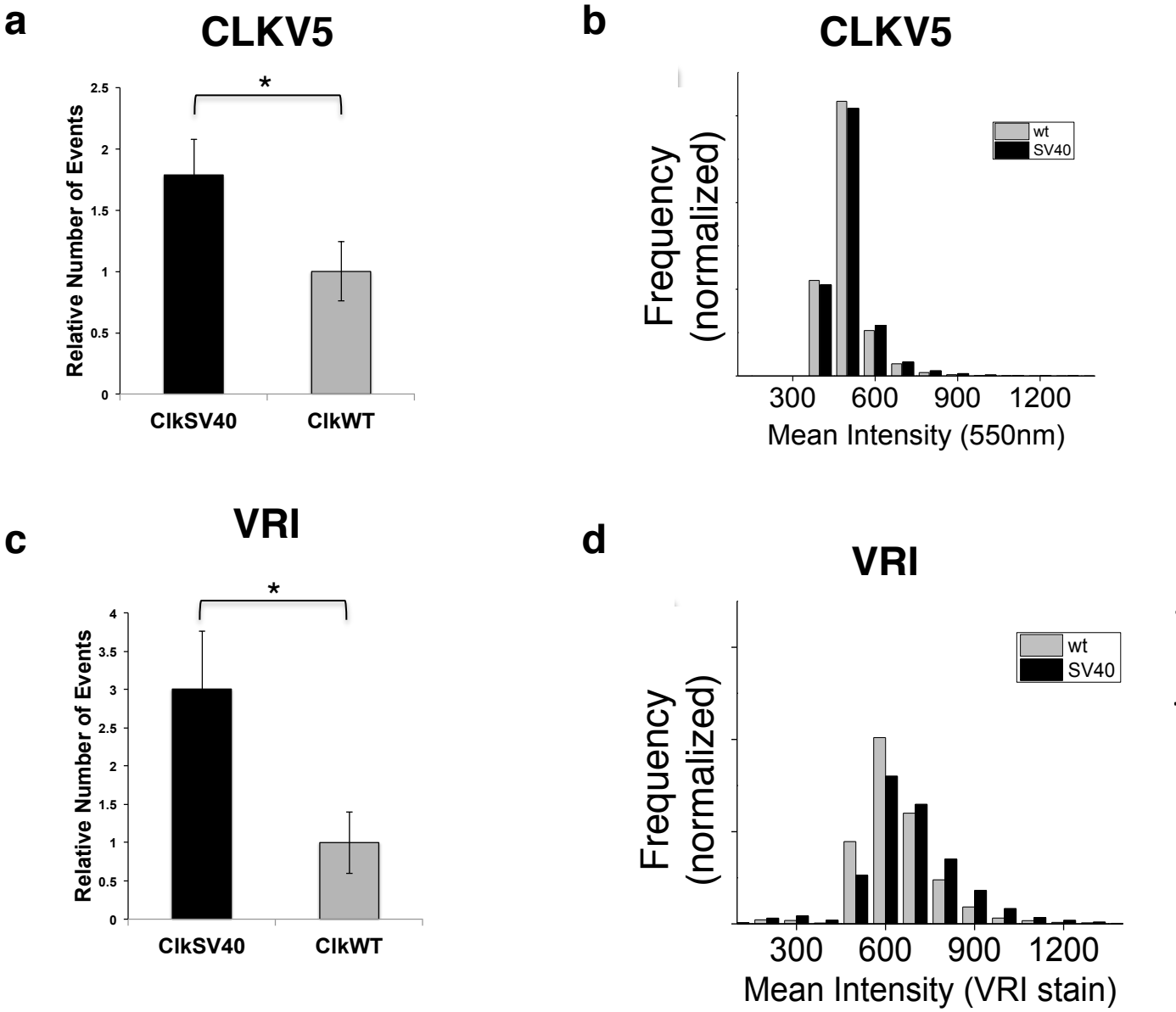
	DD 1-5		DD 1-10	
	%R (n)	Period	% Rhythmic normal (n)	% Rhythmic atypical (n)
WT 3-1	90.2 (92)	23.98±0.09	91.4 (53)	8.6 (5)
WT 1-1	92.8 (28)	23.47±0.13	91.7 (22)	8.3 (2)
SV40 2-8	93.1 (29)	23.28±0.09	48 (12)	52 (13)
SV40 3-1	90.2 (92)	23.14±0.07	55.9 (38)	44.1 (30)
SV40 3-2	84.4 (90)	23.24±0.43	51.5 (35)	48.5 (33)
SV40 3-3	75 (80)	23.57±0.11	69.2 (36)	30.8 (16)
SV40 3-4	74.5 (47)	23.20±0.11	37 (10)	63 (17)
SV40 3-5	51.7 (29)	22.70±0.14	16.7 (1)	83.3 (5)
SV40 3-6	76 (23)	23.10±0.13	10 (1)	90 (9)
SV40 3-7	93.1 (29)	23.18±0.16	71.4 (10)	28.6 (4)
SV40 3-8	66.7 (30)	23.31±0.17	38 (8)	62 (13)
SV40 3-9	86.7 (15)	23.16±0.10	60 (6)	40 (4)

b



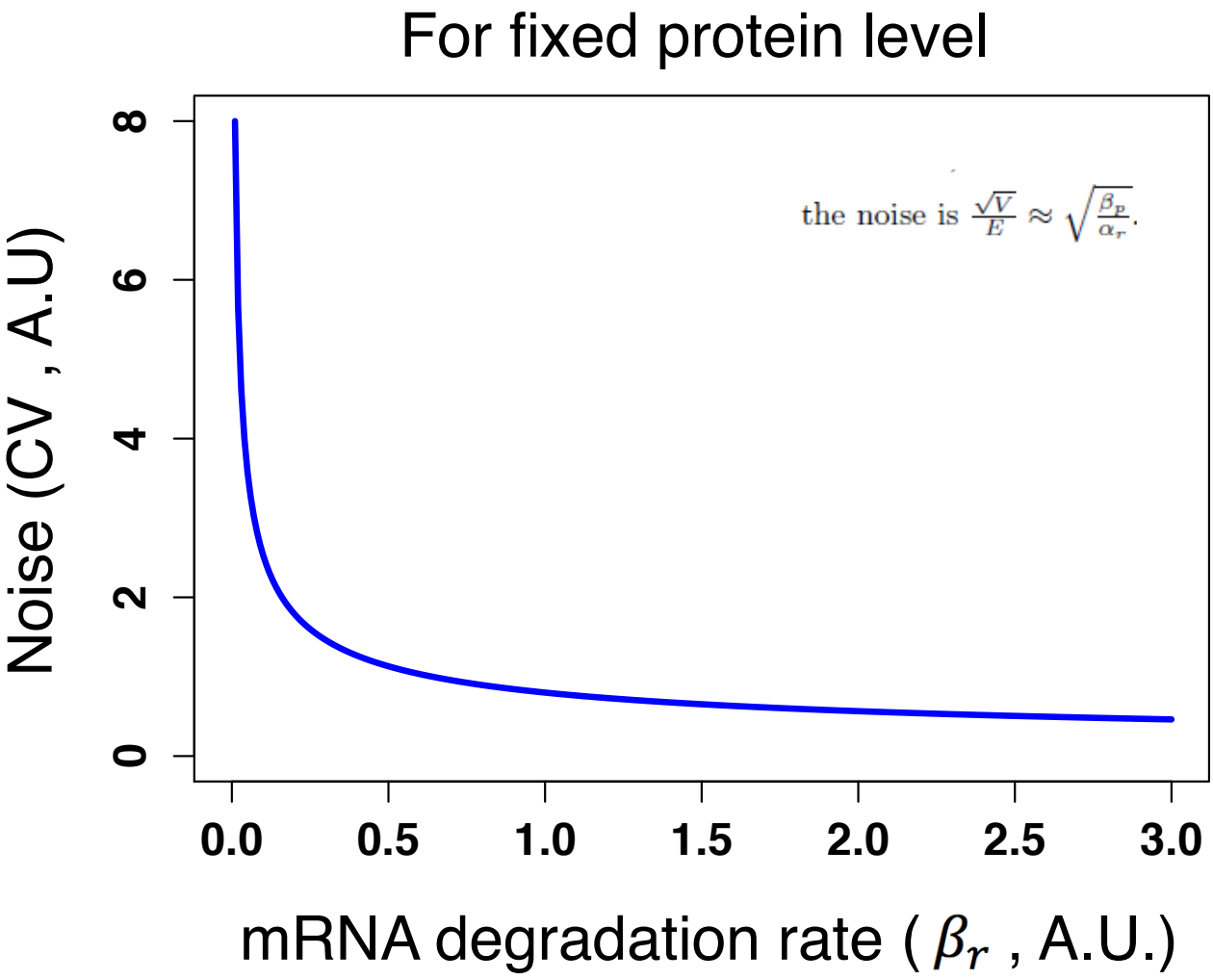
Supplementary Figure 3. ***ClkSV40*** **flies display variable circadian behavior. a.** Locomotor activity behavioral analysis of *ClkSV40* and *ClkWT* flies. **b.** Comparison between the extent of ectopic expression and the abnormalities in circadian behavior in a set of *ClkSV40* lines. In order to minimize effects due to differences in brain area, the number of events was normalized to the brain area. VRI stainings (performed at ZT15) were utilized for quantifying the number of circadian cells.

Supplementary Fig. 4



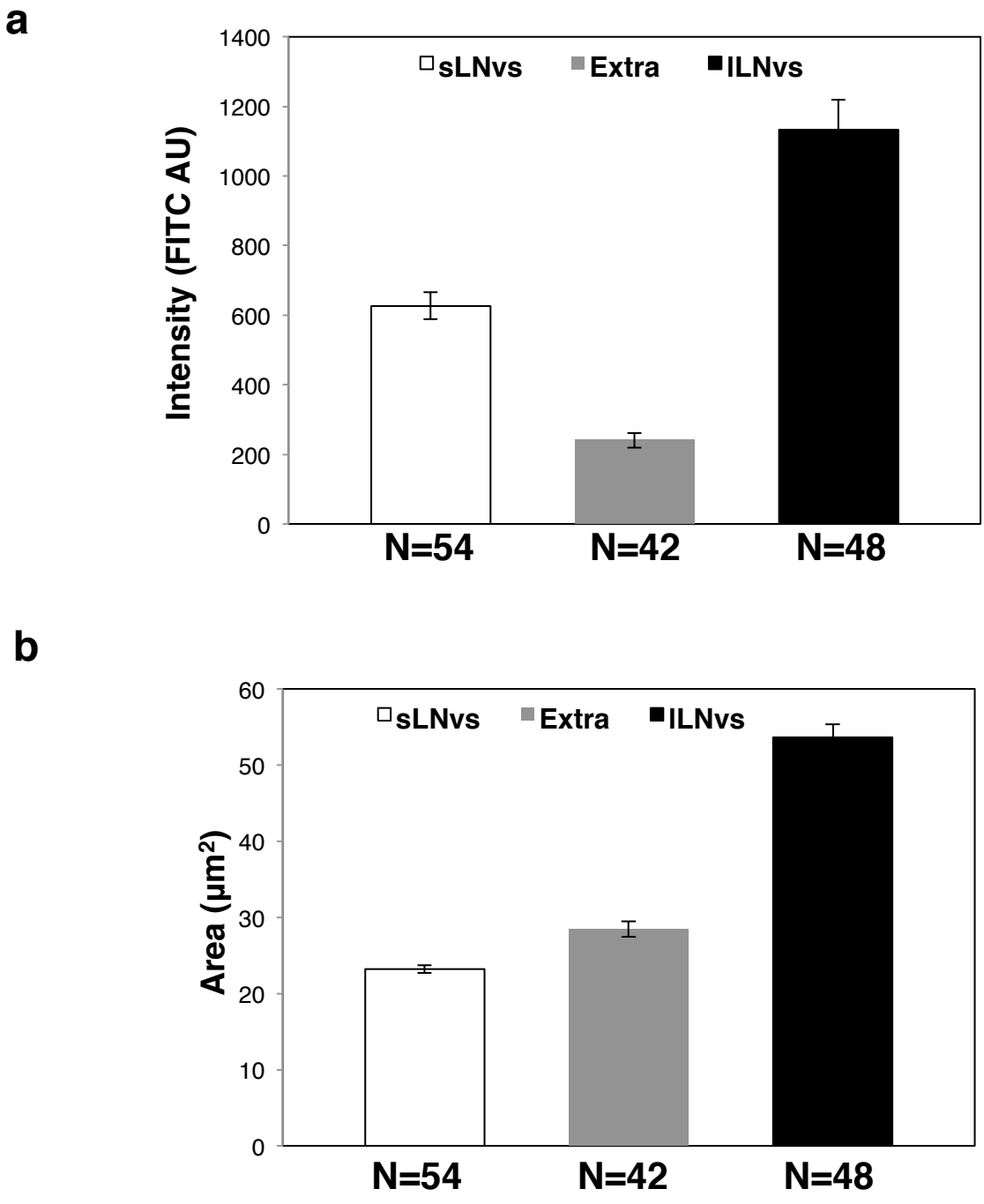
Supplementary Figure 4. Characterization of clock cells in *ClkSV40* flies. a,b. *ClkSV40* flies have more CLKV5-positive cells than *ClkWT* flies but similar levels of CLKV5 per cell. **a.** Relative number of CLKV5 positive "events" per brain (calculated from confocal pictures taken every 3 μm) from two *ClkWT* and three *ClkSV40* fly lines (6 brains from each line). A statistically significant difference was observed between *ClkWT* and *ClkSV40* flies (Student's *t*-test, p=0.047015). **b.** Histogram of CLKV5-positive event intensities for *ClkWT* (sum of the data obtained for four independent transgene insertion) and *ClkSV40* (sum of the data obtained for five independent transgene insertion) flies. **c,d** *ClkSV40* flies have more VRI-positive cells and higher levels of VRI per cell than *ClkWT* flies. **c.** Relative number of VRI-positive "events" per brain (calculated from confocal pictures taken every 3 μm) from two *ClkWT* and three *ClkSV40* fly lines (6 brains from each line). A statistically significant difference was observed between *ClkWT* and *ClkSV40* flies (Student's *t*-test, p=0.00519). **d.** Histogram of VRI-positive event intensities for *ClkWT* (sum of the data obtained for four independent transgene insertion) and *ClkSV40* (sum of the data obtained for five independent transgene insertion) flies. The same transgenic lines have been used in the measurements of VRI and CLK presented in **a** and **c**.

Supplementary Fig. 5



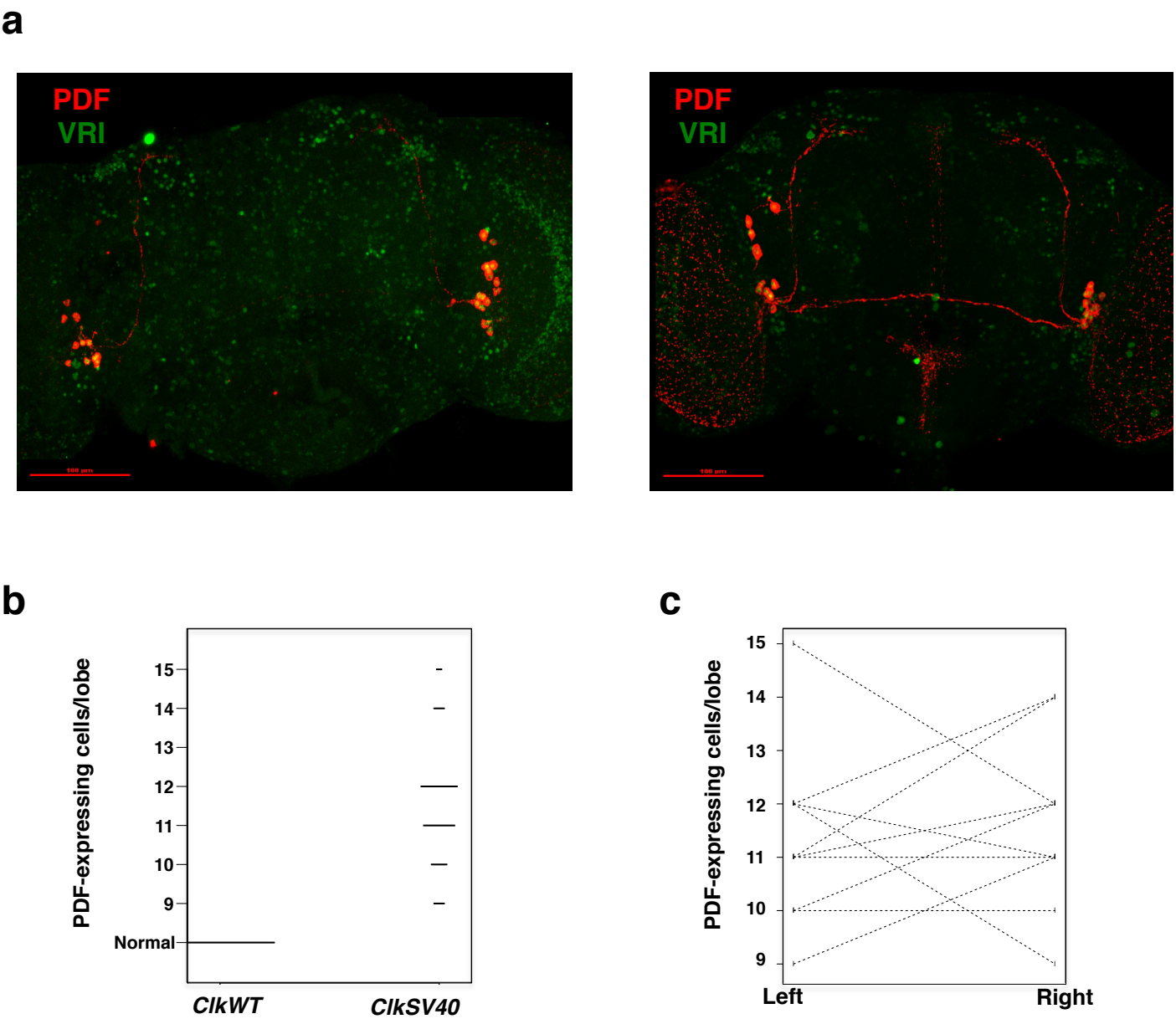
Supplementary Figure 5. The formulated mathematical model predicts a role for mRNA degradation rate in decreasing transcriptional noise. Graph representing the levels of noise in *Clk* expression relative to mRNA degradation rate for a fixed level of CLK.

Supplementary Fig. 6



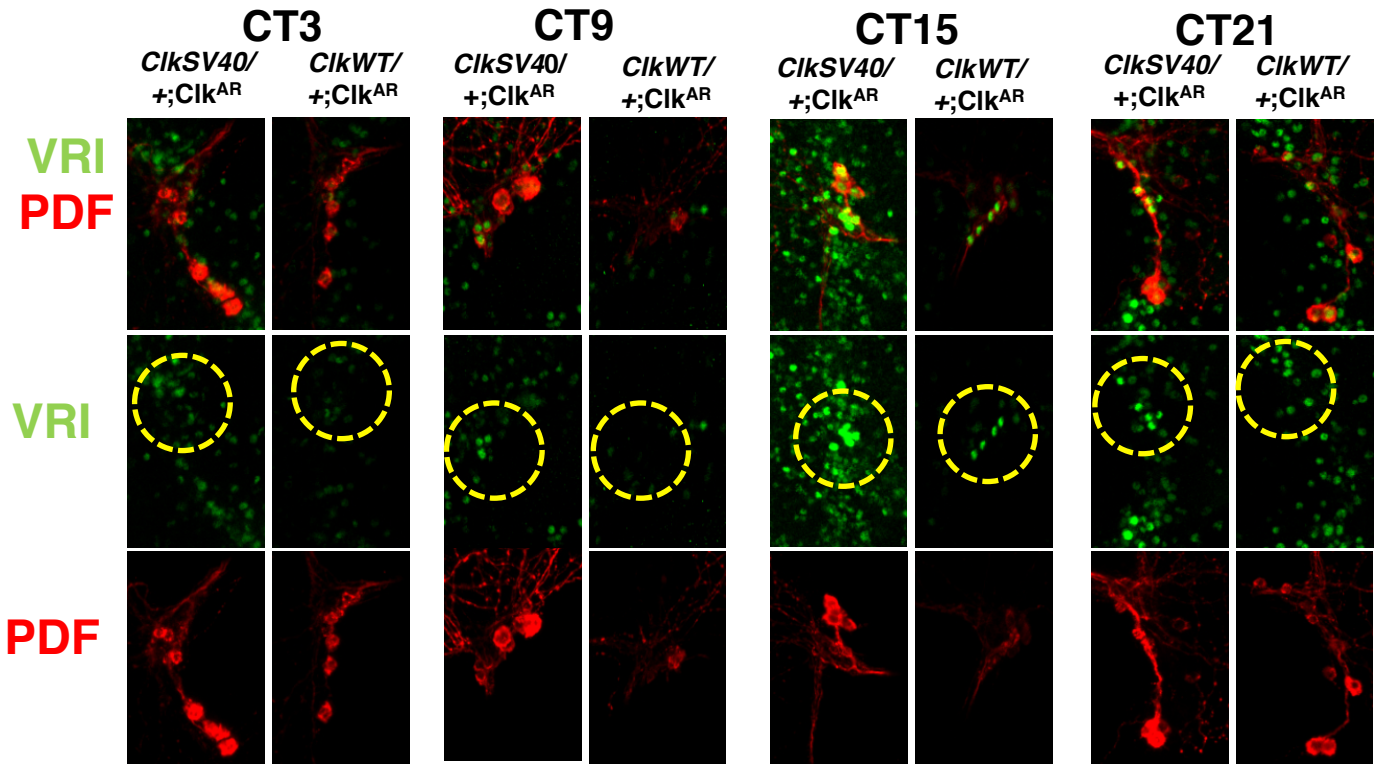
Supplementary Figure 6. Characterization of the *pdf*-expressing in *ClkSV40* flies. a. Quantification of the intensity of the PDF staining in the different cell groups in brains of *ClkSV40 2-8* flies. sLNvs= small Lateral ventral neurons; lLNvs= large Lateral ventral neurons; extra= ectopic cells. We plotted the average of the indicated number of measurements (N). Error = standard error of the mean. **b.** Area measurement of the different types of *pdf*-expressing cells in brains of *ClkSV40 2-8* flies.

Supplementary Fig. 7



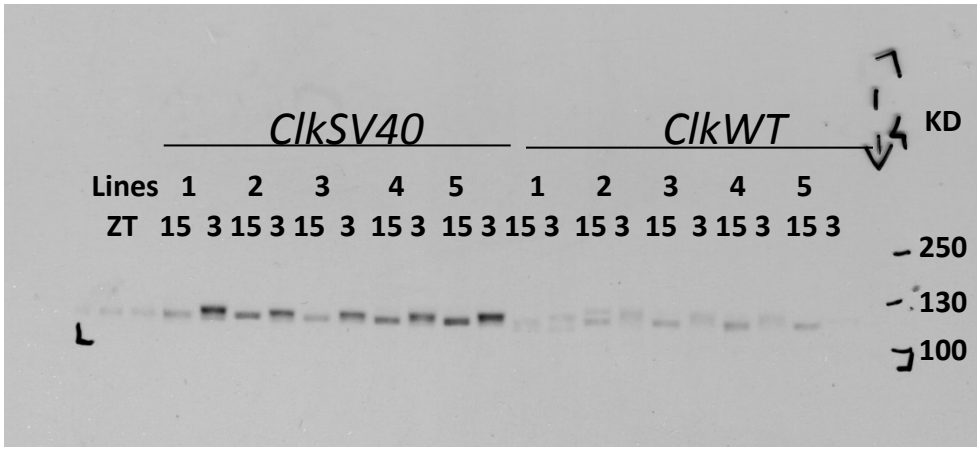
Supplementary Figure 7. Characterization of pdf cells in *ClkSV40* pupae. a. Representative example of two pupae brains of the *ClkSV40* strain in which additional *pdf*-expressing cells were observed. The red bar represents 100µm. **b.** Number of *pdf*-positive cell bodies in the brains of pupae from *ClkWT* and *ClkSV40* flies. Data is from the *ClkSV40* (2-8) and *ClkWT* (1-1) fly strains. **c.** Comparison of left and right hemispheres in *ClkSV40* pupae based on PDF immunofluorescent staining.

Supplementary Fig. 8

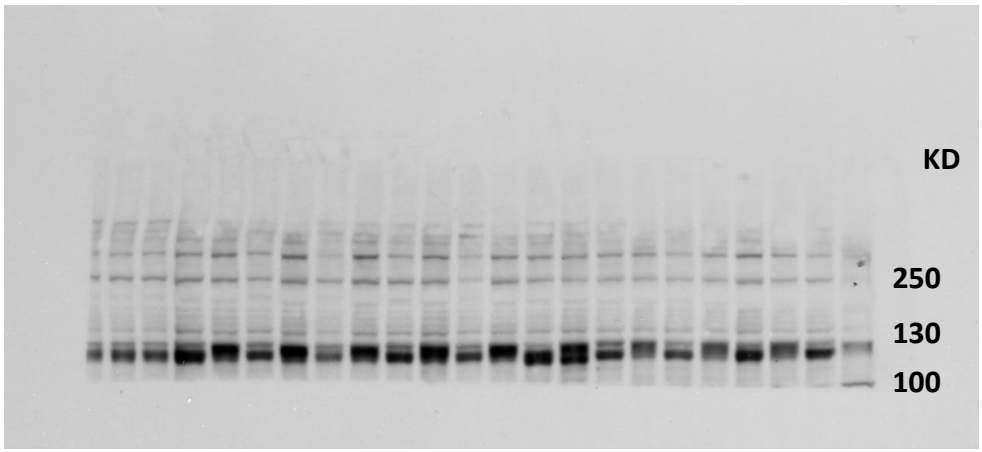


Supplementary Figure 8. ***ClkSV40*** **flies display variable VRI expression in the sLNvs.** VRI immunofluorescence (IF) in sLNvs at 4 time points (day 10 in DD), in *ClkSV40* (line 2-8) /+;Clk^{AR} and *ClkWT* (line 1-1) /+;Clk^{AR} flies. PDF IF was used for detection of sLNvs.

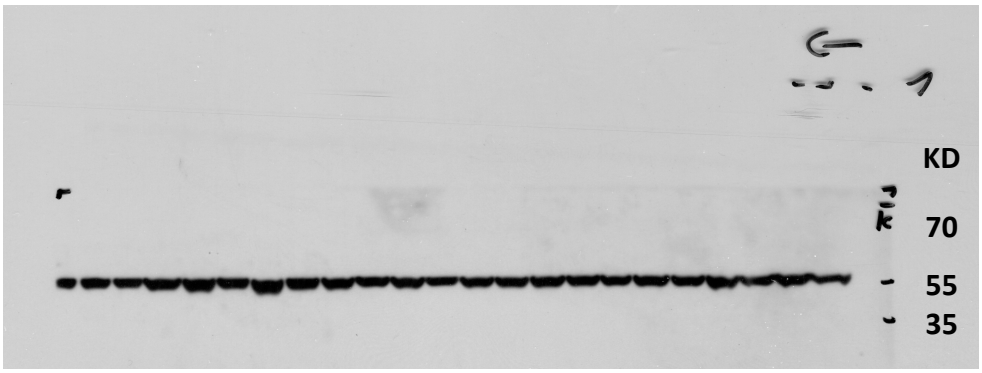
Supplementary Fig. 9



α-V5



α-Clk



α-tubulin

Supplementary Figure 9. Complete blots Figure 3e

SUPPLEMENTARY TABLE 1

Oligonucleotide sequences Utilized in this study

Primer pairs for assessment of post-transcriptional control

	Exonic Primer Pair	Intronic Primer Pair
Clock	5'-GGAAATCAATTGTTGCGTGA-3'	5'-ATACGGATACACAAGGGTTG-3'
	5'-GCAAACGAGAAGAACCGAAG-3'	5'-AGCAATAATCCCAAAACTCA-3'
timeless	5'-CAGGTGGAAAATCAGGAAT-3'	5'-GCTGGCCGATTACAGGATAAC-3'
	5'-CGTCTAAGCTCCTTTTTGTC-3'	5'-AGTAAAACAGCGGCACACTCA-3'
cycle	5'-ATGAACACGTACATCAACGA-3'	5'-AACGAGCTGTAAGTGTGTGC-3'
	5'-GAAGCTAGGCCGGTAATC-3'	5'-GTGTGGATTTCCCGTGTAAC-3'
cryptochrome	5'-CCAAGAATGTGGGTTACAAT-3'	5'-CTTGTGAAAATGGAAAGAGG-3'
	5'-AGACGCACTTGCTCATGTA-3'	5'-ATATCTCTCTCCCAGCGATT-3'
vrille	5'-GATCCCAGTAGCTCTCGTC-3'	5'-GTCTAATTCTCGCTCCCTCT-3'
	5'-GCTATGGAGATGGAATGATG-3'	5'-GAACTTTCTTTGTTCGTTGG-3'
α-tub84B	5'-ACAACGAGGCTATCTACGAC-3'	5'-CTTCGACGCATAACTGTAGA-3'
	5'-AACTCAGTCAGATCCACGTT-3'	5'-AAAATCGATAATTGCAGAGC-3'
Epsilon 14-3-3	5'-CGTACAAGAATGTGATTGGA-3'	5'-GCAATTAGGTGCAGAGTACC-3'
	5'-CGTTCAGTATATCCGAGCAG-3'	5'-AAACGAGATGGCTAGTGTGT-3'
Sdh	5'-GATATGGACGAGCATTCTTC-3'	5'-ACCAGCGAGGTTATGTTAT-3'
	5'-CTCGGTGATGAGACATCC-3'	5'-TCCGATTAAGCGGTACTTAT-3'

Primer pairs for gene expression

Clk 1	5'- TGGAGTCTCTCGATGGTTTTA -3'	5'- CGGTGTGGGATTCATAAAGAT -3'
Clk 2	5'- CGACAAGGATGATACAAAAAG -3'	5'- ATGATTTTTCAGGAAGGCTA -3'
ClkV5	5'-CGAATCCCTTTCTCAACAGTC-3'	5'-GGGTTAGGGATAGGCTTACC-3'
Clk only Endogenous	5'-CGAATCCCTTTCTCAACAGTC-3'	5'-GGTATACGCTATTGACTACTGC-3'
vri	5'-CACGCTGGAACAGAAAGTGA-3'	5'-TGTTGCTTTGAGCTTGGATG-3'
tim pre-mRNA	5'-AAAGGGTACATCTTGGAGA-3'	5'-GAGCCAATGCTGTAAAAGAA-3'
tim	5'-GCAGCAAATGCAATCATC-3'	5'-GTTCAGGCTCAAAGTGGTT-3'
cyc	5'-ACGTTGATGCTAGTCGATGT-3'	5'-CGTCGTAGTTTTCATCTTCG-3'
cry	5'-GCGAATGTGATTTGGTTTC-3'	5'-CGATTGTAACCCACATTCTT-3'
RP49 (Rpl32)	5'-TACAGGCCCAAGATCGTGAA-3'	5'-CCATTTGTGCGACAGCTTAG -3'
RpS18	5'-CCTTCTGCCTGTTGAGGA -3'	5'-TGCACCGAGGAGGAGGTC -3'
H1	5'-CCACCAGCGACAGTTGAG-3'	5'-TGGCGGATGTGACGGCGT-3'
18S rRNA	5'-TGGTCTTGTACCGACGACAG-3'	5'-GCTGCCTTCCTTAGATGTGG-3'

SUPPLEMENTARY NOTES 1

1. Steady state analysis for a stochastic transcription and translation model

1.1. **An analytical model of transcription and translation.** Assume that transcription events are independent and that given the mRNA state, translation events are conditionally independent. Assume also that mRNA and protein decay events are independent.

We characterize an mRNA molecule by a pair $(s,l) \in \mathbb{R} \times [0,\infty) = \Theta$, s being time of transcription and l life time. Consider a single, potential, mRNA molecule $\theta \in \Theta$. Denote by I_θ the indicator of whether it has existed.

Let P_θ^t be a random variable denoting the number of proteins translated from this molecule which were active at time t (i.e translated before time t and degraded after time t). First, we assume that this molecule did exist, i.e $I_\theta = 1$. So the conditioned distribution of $P_\theta^t | \, I_\theta = 1$ is Poisson. To see this, we may consider this variable to be the number of successes in an infinite number of Bernoulli experiments $\{ B_a | \, a \in [s,l] \}$. Each of these experiments corresponds to a potential protein translated by θ, and the experiment is successful if the protein was translated and also active at time t. According to Lemma 3 in the appendix, this variable is Poisson with expectation that can be calculated according to the particular translation and protein degradation model which we did not fix yet. Denote this parameter by λ_θ^t, so $\lambda_\theta^t = E\left[P_\theta^t | \, I_\theta = 1 \right]$.

We drop the assumption $I_\theta = 1$ in order to analyse $P_{total}^t = \sum_\theta P_\theta^t$, the total number of proteins translated from all mRNA molecules which are active at time t. Each term in the sum is zero with probability 1, but we may analyze P_{total}^t using Lemma 4. Let $\rho : \Theta \to \mathbb{R}$ be the rate of existence of mRNA molecules (i.e the rate of I_θ) as in Definition 1. The rate ρ is found by analysis of the particular transcription and mRNA degradation model. According to Lemma 4 we have $E\left[P_{total}^t\right] = \int \lambda_\theta^t \rho\left(\theta\right) d\theta$ and $Var\left[P_{total}^t\right] = \int \left(\lambda_\theta^t + \left(\lambda_\theta^t\right)^2 \right) \rho\left(\theta\right) d\theta$. To conclude, calculate $S_i^t = \int \left(\lambda_\theta^t\right)^i \rho\left(\theta\right) d\theta$ for $i = 1,2$ to find $E\left[P_{total}^t\right] = S_1^t$ and $Var\left[P_{total}^t\right] = S_1^t + S_2^t$.

1.2. **Analysis of steady state.** We now consider the steady state. Let α_r be the constant transcription rate, α_p the translation rate per mRNA molecule and β_r, β_p the rates of expontential degradation of mRNA and protein, respectively.

Let L be a random variable denoting the life time of an mRNA molecule. Then the probability density function of L is $PDF\left(l\right) = \beta_r e^{-\beta_r l}$. Considering an mRNA molecule $\theta = (s,l)$, we have

$$\rho\left(s,l\right) = \alpha_r \cdot PDF\left(l\right) = \alpha_r \beta_r e^{-\beta_r l}$$

We find $\lambda_{s,l}^t$ by analysis of constant rate translation and protein degradation. For $t \leq s$, we have $\lambda_{s,l}^t = 0$. For $s \leq t \leq s+l$, $\lambda_{s,l}^t = \frac{\alpha_p}{\beta_p}\left(1 - e^{-\beta_p (t-s)}\right)$. For $l+s \leq t$, we have exponential decay with no generation of new protein so $\lambda_{s,l}^t = \lambda_{s,l}^l \cdot e^{-\beta_p (t-l)}$. Together,

$$\lambda_{s,l}^t = \begin{cases} 0 & t \leq s \\ \frac{\alpha_p}{\beta_p}\left(1 - e^{-\beta_p (t-s)}\right) & s \leq t \leq s+l \\ \frac{\alpha_p}{\beta_p}\left(e^{-\beta_p (t-l)} - e^{-\beta_p (t-s)}\right) & s+l \leq t \end{cases}$$

Now, we wish to calculate

$$S_i^t = \int \left(\lambda_{s,l}^t\right)^i \rho\left(\theta\right) d\theta = \int_0^\infty \int_{-\infty}^\infty \left(\lambda_{s,l}^t\right)^i \cdot \alpha_r \cdot PDF_L\left(l\right) dsdl$$

for $i = 1, 2$. The result for $i = 1$ is $E\left[P_{total}^t\right] = S_i^t = \frac{\alpha_r \alpha_p}{\beta_r \beta_p}$ as expected, and the details are omitted.

Consider $i = 2$. $P_{s,l}^t$ has the same distribution as $P_{0,l}^{t-s}$ so $\lambda_{s,l}^t = \lambda_{0,l}^{t-s}$ and we may integrate over t instead by substitution of variables:

$$S_i^t = \int_0^\infty \int_{-\infty}^\infty \left(\lambda_{0,l}^{t-s}\right)^2 \cdot \alpha_r \cdot PDF_L\left(l\right) dsdl = \alpha_r \int_0^\infty \left(\int_{-\infty}^\infty \left(\lambda_{0,l}^s\right)^2 ds\right) PDF_L\left(l\right) dl =$$

$$= \alpha_r E_L\left[\int_{-\infty}^\infty \left(\lambda_{0,L}^s\right)^2 ds\right]$$

Note that this expression is independent of t as expected in steady state. Let $T\left(l\right)$ be the nested integral.

We evaluate $T\left(l\right)$ separately over the two intervals where $\lambda_{s,l}^t$ is non zero. First,

$$\int_0^l \left(\lambda_{0,l}^t\right)^2 dt = \int_0^l \frac{\alpha_p^2}{\beta_p^2}\left(1 - e^{-\beta_p t}\right)^2 dt = \frac{\alpha_p^2}{\beta_p^2}\int_0^l \left(1 - 2e^{-\beta_p t} + e^{-2\beta_p t}\right) dt =$$

$$= \frac{\alpha_p^2}{\beta_p^2}\left(t + \frac{2}{\beta_p}e^{-\beta_p t} - \frac{1}{2\beta_p}e^{-2\beta_p t}\right)\Bigg|_{t=0}^l =$$

$$= \frac{\alpha_p^2}{\beta_p^2}\left(l + \frac{2}{\beta_p}e^{-\beta_p l} - \frac{1}{2\beta_p}e^{-2\beta_p l} - \frac{2}{\beta_p} + \frac{1}{2\beta_p}\right)$$

and secondly

$$\int_l^\infty \left(\lambda_{0,l}^t\right)^2 dt = \int_l^\infty \frac{\alpha_p^2}{\beta_p^2}\left(1 - e^{-\beta_p l}\right)^2 e^{-2\beta_p (t-l)} dt = \frac{\alpha_p^2}{\beta_p^2}\left(1 - e^{-\beta_p l}\right)^2 \frac{1}{2\beta_p}$$

Together

$$T\left(l\right) = \frac{\alpha_p^2}{\beta_p^3}\left(\frac{1}{2} - e^{-\beta_p l} + \frac{1}{2}e^{-2\beta_p l} + \beta_p l + 2e^{-\beta_p l} - \frac{1}{2}e^{-2\beta_p l} - \frac{3}{2}\right) =$$

$$= \frac{\alpha_p^2}{\beta_p^3}\left(e^{-\beta_p l} + \beta_p l - 1\right)$$

Taking expectation with respect to L (details omitted) we find

$$S_2 = \alpha_r \frac{\alpha_p^2}{\beta_p^3}\left(\frac{\beta_r}{\beta_r + \beta_p} + \frac{\beta_p}{\beta_r} - 1\right) =$$

$$= \alpha_r \frac{\alpha_p^2}{\beta_p^3}\left(-\frac{\beta_p}{\beta_r + \beta_p} + \frac{\beta_p}{\beta_r}\right) = \alpha_r \frac{\alpha_p^2}{\beta_p^2} \cdot \frac{-\beta_r + \beta_r + \beta_p}{\beta_r\left(\beta_r + \beta_p\right)} = \frac{\alpha_r \alpha_p^2}{\beta_r \beta_p\left(\beta_r + \beta_p\right)}$$

To conclude,

$$E\left[P_{total}^t\right] = \frac{\alpha_r \alpha_p}{\beta_r \beta_p}$$

and

$$V\left[P_{total}^t\right] = E\left[P_{total}^t\right] + \frac{\alpha_r \alpha_p^2}{\beta_r \beta_p\left(\beta_r + \beta_p\right)}$$

If we may assume that $\beta_r \gg \beta_p$ (i.e mRNA half life is much shorter than protein half life) and that $\frac{\alpha_p}{\beta_r} \gg 1$ (i.e mean number of proteins generated from each mRNA molecule is $\gg 1$) then the variance further simplifies to $V\left[P^t_{total}\right] \approx \frac{\alpha_r \alpha_p^2}{\beta_r^2 \beta_p}$. In this case, the noise is $\frac{\sqrt{V}}{E} \approx \sqrt{\frac{\beta_p}{\alpha_r}}$.

1.3. **Application to our experimental results.**

1.3.1. *In WT.* The noise equation $\frac{\sqrt{V}}{E} = \sqrt{\frac{\beta_p}{\alpha_r}}$ shows that by increasing the transcription rate α_r and increasing the mRNA degradation rate β_r by the same factor, the noise is decreased while the protein expression remains unchanged. This suggests that the high rates α_r and β_r of *Clk* serve to decrease noise.

1.3.2. *In the ClkSV40 system.* The removal of post transcriptional control increases β_r. The vri feedback response achieves normal Clk protein levels by decreasing α_r. According to the noise equation, the noise is indeed increased.

2. Appendix

Let B_θ be independent non negative random variables for $\theta \in \Theta$ when $\Theta \subseteq \mathbb{R}^n$ is an arbitrary index set. For $A \subseteq \Theta$, let $C_A = \sum_{\theta \in A} B_\theta$. Assume that $Pr\left[B_\theta > 0\right] = 0$ for all $\theta \in \Theta$ and that $E\left[C_\Theta\right] < \infty$.

Definition 1. $\rho : \Theta \to \mathbb{R}$ is the rate function of $\{B_\theta\}$ if it is a continous (or measurable) function so that $E\left[B_A\right] = \int_A \rho$ for all measurable $A \subseteq \Theta$.

Definition 2. For a measurable $\rho : \Theta \to \mathbb{R}$, denote $\bar{\rho}\left(A\right) = \int_A \rho$. This is a measure.

Lemma 3. *Assume that B_θ are Bernoulli or Poisson variables and let ρ be the rate function of $\{B_\theta\}$. Then $C_\Theta \sim Poisson\left(\bar{\rho}\left(\Theta\right)\right)$.*

Lemma 4. *Assume that there exist measurable functions $\rho_I, \lambda : \Theta \to \mathbb{R}$ so that $B_\theta = I_\theta Q_\theta$ with $Q_\theta \sim Poisson\left(\lambda\left(\theta\right)\right)$ and I_θ a Bernoulli variable with zero probability of success such that ρ_I is the rate function of $\{I_\theta\}$. Then $E\left[C_\Theta\right] = \int_\Theta \rho_I \cdot \lambda$ and $Var\left[C_\Theta\right] = E\left[C_\Theta\right] + \int_\Theta \rho_I \cdot \lambda^2$.*

Both lemmas may be proven by a discretization of the problem (similarly to Poisson limit theorem or Le Cam's theorem). The result regarding the variance stems from the limit

$$\frac{Var\left[I \cdot Q\right]}{p} \underset{p \to 0}{\longrightarrow} \lambda + \lambda^2$$

for $I \sim \text{Bernoulli}\left(p\right)$ with $p > 0$ and $Q \sim Poisson\left(\lambda\right)$.

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