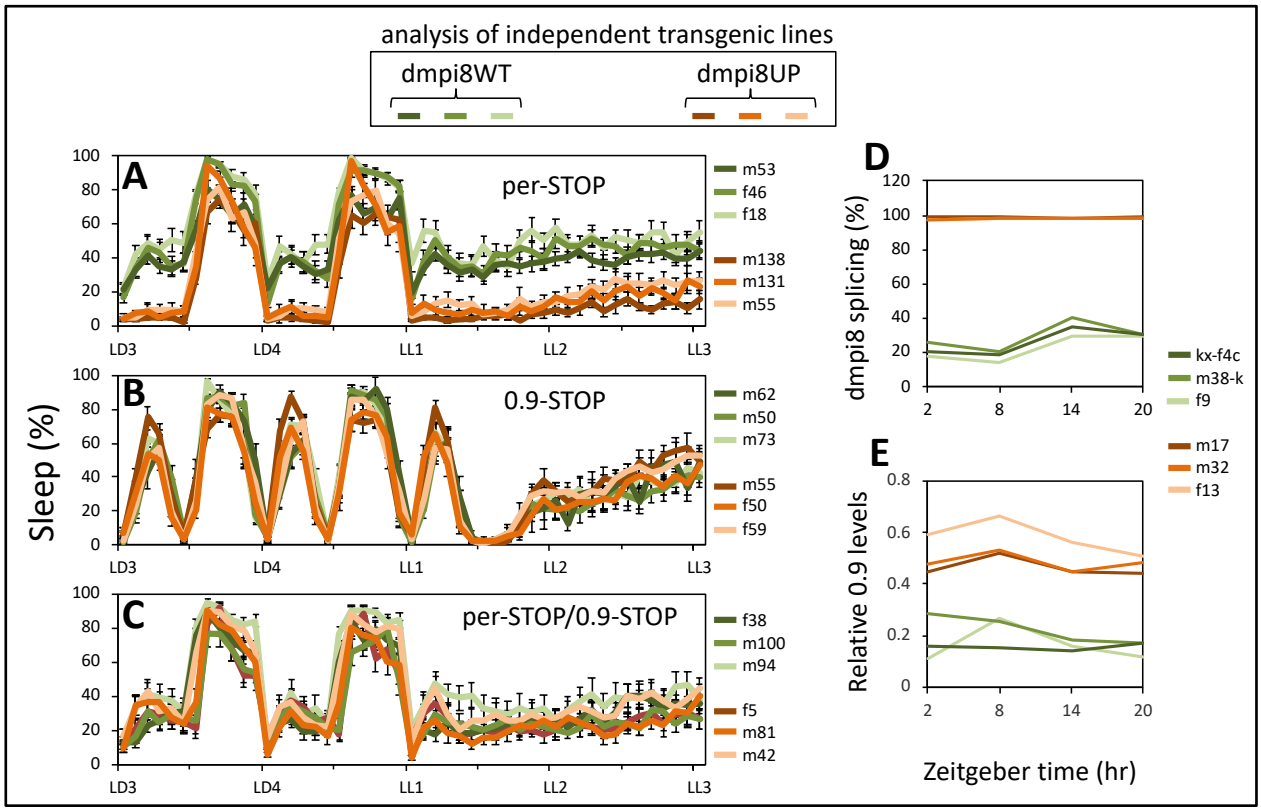

Figure S1. Behavioral and molecular analysis of individual transgenic lines, Related to Figure 1. Independent transgenic lines containing the dmpi8WT version are represented by different shades of green, whereas those with the dmpi8UP version are shown in different shades of orange (top). For each genotype, 3 independent insertion lines are shown. The identities of the individual lines used are indicated to the right of the panels. (A-C) Shown are results for the individual transgenic lines used to generate the average profiles depicted in Figure 1 C, D, and E. (D, E) Shown are results for the individual lines used to generate the average profiles depicted in Figure 2A, B. The results further support the findings that the differences in daytime sleep levels between dmpi8WT and dmpi8UP are due to 0.9 protein and not PER protein (A-C). In addition, although there is some variation between individual lines of the same genotype with regards to 0.9 levels, it is clear that 0.9 levels are significantly higher in flies with the dmpi8UP version compared to the dmpi8WT version (E).

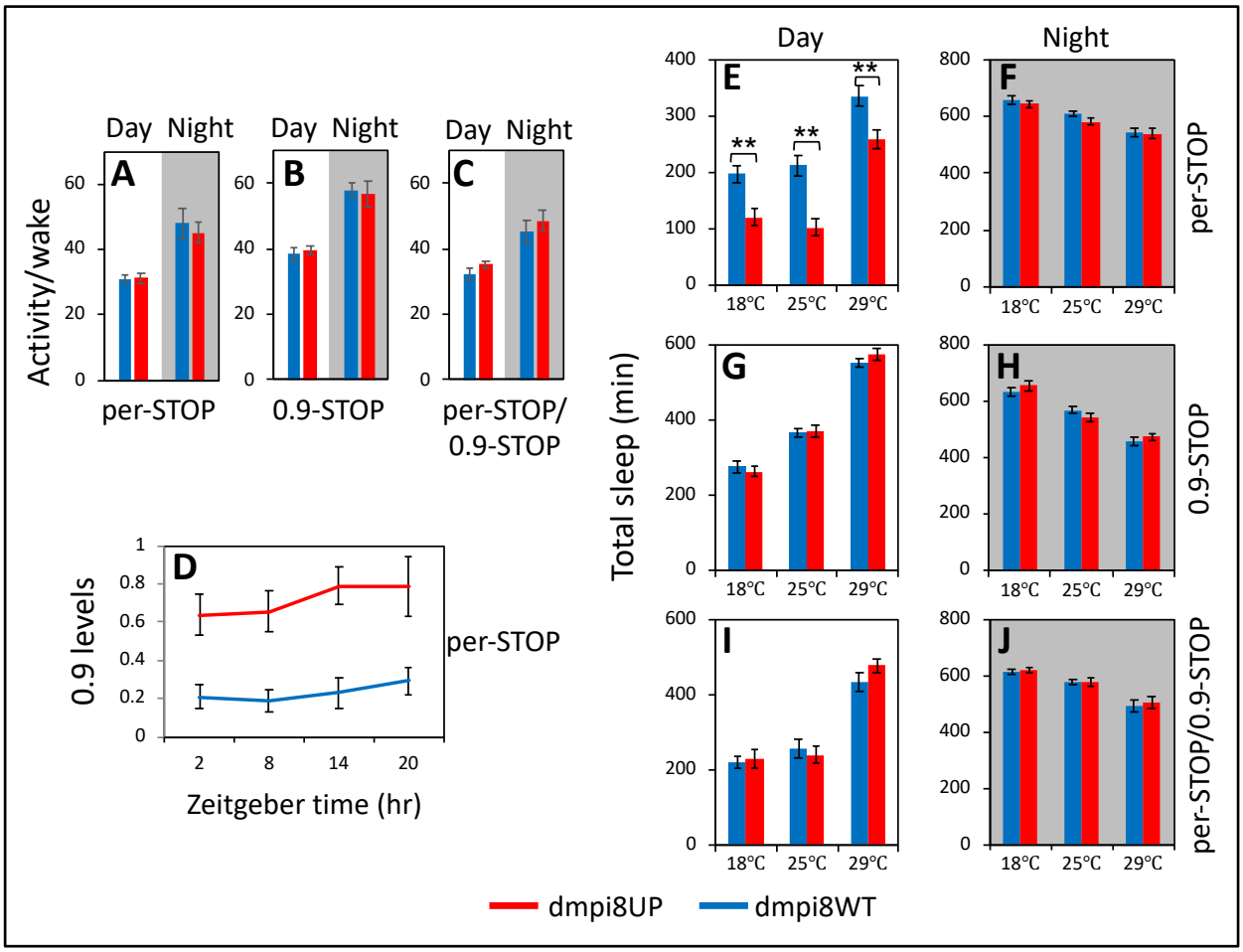

Figure S2. The effects of dmpi8 splicing efficiency on daytime sleep levels are mediated via 0.9 function over a broad range of temperatures and are not due to hyper-activity, Related to Figures 1 and 2. (A-C) Activity (beam counts) per wake 30 min period during 12 hr light or 12 hr dark periods in LD for the indicated genotypes (bottom of panels). Values were derived from the same activity data as that shown in figure 1C, D, E, and are the average of the last three days of LD. No significant differences in activity during wake periods was observed for any of the STOP-genotypes when comparing the dmpi8WT and dmpi8UP variants, as follows (Student's *t*-test, p values; A) per-STOP; day, 0.488; night, 0.30; B) 0.9-STOP; day, 0.269; night, 0.428; C) per-STOP/0.9-STOP; day, 0.20; night, 0.355. (D) Relative levels of 0.9 mRNA for per-STOP[dmpi8WT] and per-STOP[dmpi8UP] flies throughout a light-dark cycle at 25°C (ZT0 = start of 12 hr light period, ZT12 = start of 12 hr dark period). For each timepoint, 50-100 adult flies were collected and head extracts prepared, followed by measuring 0.9 levels. The graphs shown are an average of three independent experiments. The following independent transgenic lines were used; per-STOP[dmpi8WT], m53, f18, f46; per-STOP[dmpi8UP], m55, m131, m138. The levels of 0.9 transcripts are significantly higher in the dmpi8UP version compared to dmpi8WT (Student's *t*-test, p values); ZT2, 0.0075; ZT8, 0.0044; ZT14, 0.0023; ZT, 20, 0.013. (E-J) Male flies of the indicated genotype (right) were exposed to five days of LD at three temperatures (18°, 25°, 29°C). Data from the last three days of LD was averaged to determine total time sleeping during the 12 hr light period (left panels) and 12 hr dark period (right panels). For each independent fly line and temperature, activity data from 16 flies was used. Each genotype represents an average of five independent transgenic lines. For each genotype and temperature, the same transgenic fly lines were used as described in the legend to figure 1 (panels C, D, E). Thus, each bar graph represents data from 80 individual flies. Only per-STOP flies show a significant difference in daytime sleep (but not nighttime sleep) levels between the dmpi8WT and dmpi8UP versions, which is observed over a wide temperature range. **, $p < 0.01$; the following p-values were determined (Student's *t*-test); panel E; 18°C, 0.0013; 25°C, < 1.0 x 10-5; 29°C, 0.0038; panel F; 18°C, 0.23; 25°C, 0.15; 29°C, 0.46; panel G; 18°C, 0.31; 25°C, 0.45; 29°C, 0.16; panel H; 18°C, 0.23; 25°C, 0.11; 29°C, 0.20; panel I; 18°C, 0.42; 25°C, 0.38; 29°C, 0.20; panel J; 18°C, 0.33; 25°C, 0.49; 29°C, 0.41.

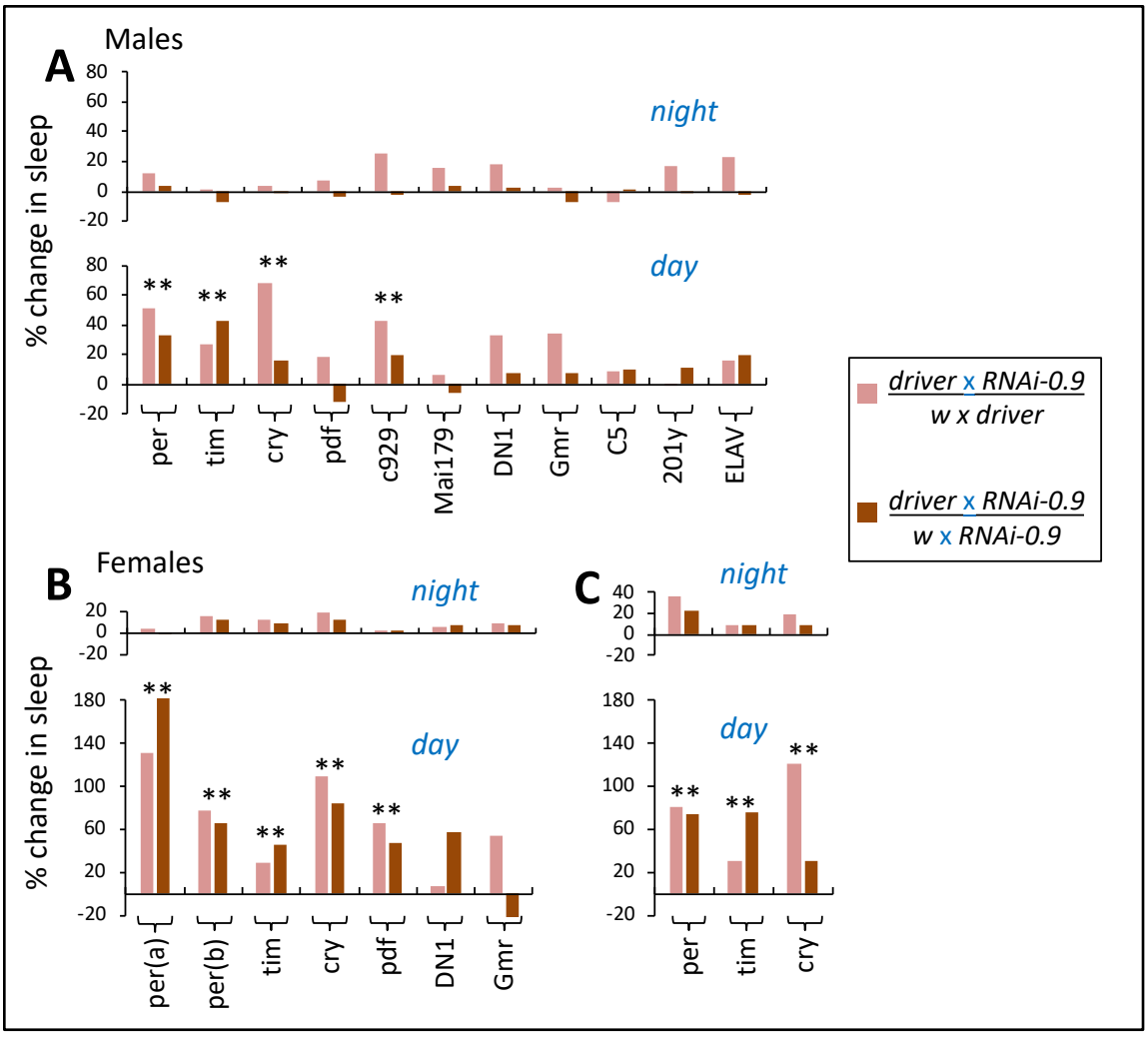

Figure S3. RNAi-0.9 in combination with widespread clock cell drivers increases daytime but not nighttime sleep levels in males and females, Related to Figure 3. (A-C) Shown are the changes in total daytime and nighttime sleep for the indicated experimental group (driver > RNAi-0.9) relative to its corresponding parental control crosses (w^{1118} x driver, light brown; w^{1118} x RNAi-0.9, dark brown). Per(a) and per(b) (panel B) refers to a cross between per-Gal4 and two independent RNAi-0.9 lines; a = VDRC (#105930); b = BDSC (#56988). The data is an average of the last three days of LD for male (A) or female (B, C) flies kept at either 18ºC (A, B) or 25ºC (C). **, $p < 0.01$ for experimental group compared to each parental control crosses for daytime values (Student's *t*-test); see Table S1 for p values. For each cross, activity data from 32 individual flies was used to obtain the group averages shown. Adult expression patterns of the different Gal4 drivers used in this study have been described, and include; *per-Gal4* [S1] and *tim(UAS)-Gal4* [S2], widespread expression in the approximately 150 brain 'clock' neurons, in addition to other cells; *cry16-Gal4* [S3, S4], *cry*-expressing clock neurons; *pdf-Gal4* [S5], *pdf*-expressing clock neurons; *c929* [S6], l-LNv clock neurons and small abdominal neurons; *Mai179* [S6, S7], s-LNv and some l-LNv clock neurons; *DN1-Gal4* (*R18H11*) [S8, S9], DN1 clock neurons; *Gmr-Gal4* [S10], eye; *C5-Gal4* [S11], dorsal fan-shaped body sleep-output neurons; *201y-Gal4* [S12], mushroom bodies sleep center; *ELAV-Gal4* [S13], pan-neural expression.

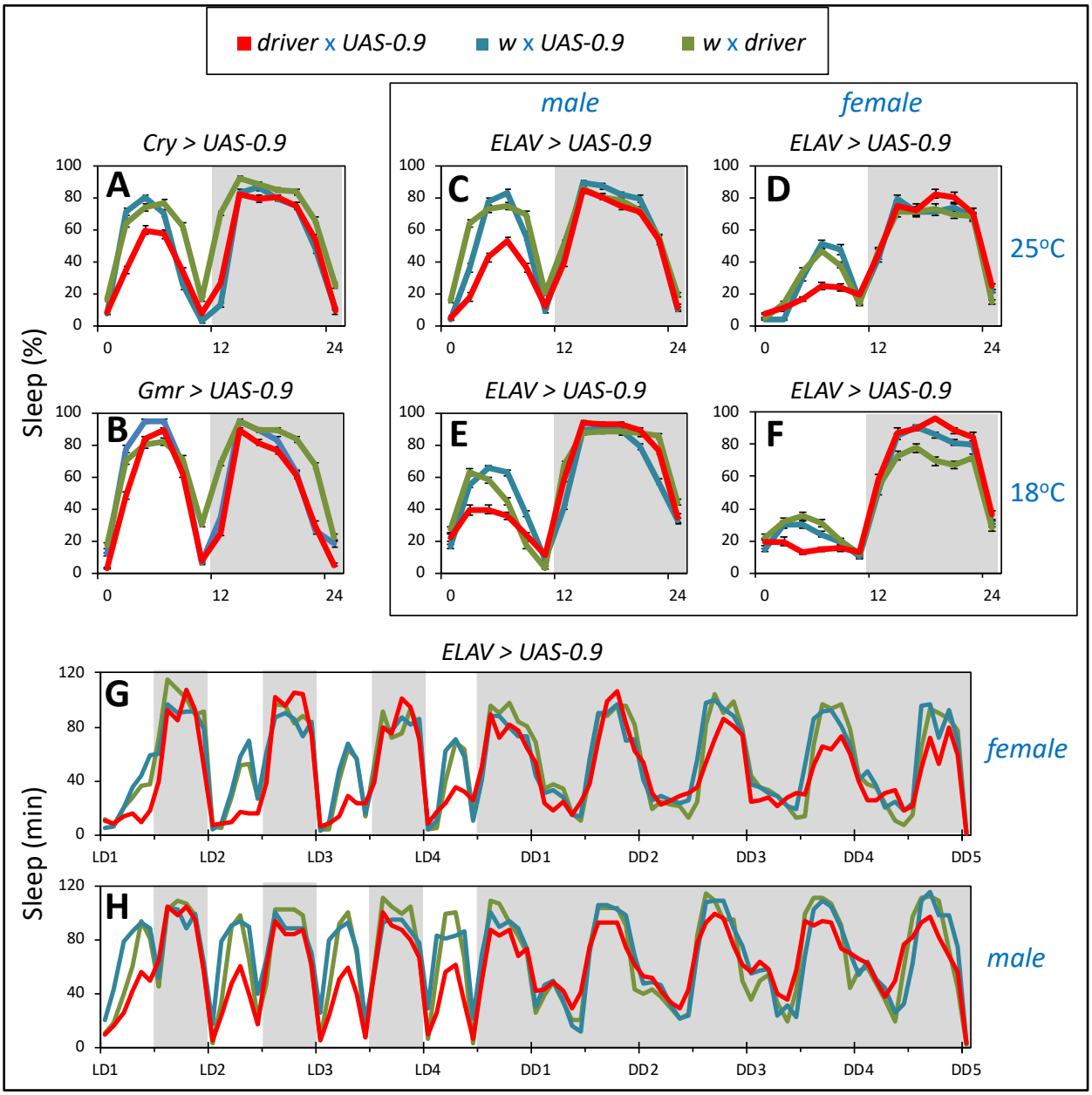

Figure S4. Overexpression of 0.9 reduces midday siesta in males and females, with no effect during constant darkness, Related to Figure 3. (A-H) Flies were kept for 5 days in LD at 25°C (A-D, G, H) or 18°C (E, F), followed by 5 days in complete darkness (DD; G, H). Shown are the daily sleep levels for male (A-C, E, H) of female (D, F, G) adult progeny for the indicated driver and UAS-0.9 (red), and the two parental control crosses between w^{1118} and the driver (green) or UAS-0.9 (blue). (A-F) The sleep profiles are an average of the last three days of LD based on pooling results from three separate experimental crosses with a different UAS-0.9 line (f79, f58, f57), using data collected from 32 individual flies for each cross. (G, H) The sleep profiles show each individual day during LD followed by DD for UAS-0.9 line f58 based on pooling data from 32 individual flies; a representative example is shown. Note that during DD, there is no significant effect of overexpressing 0.9. The different GAL4 drivers used were *cry16-Gal4* (BDSC; no. 24514), *Gmr-Gal4* (BDSC; no. 1104) and *ELAV-Gal4* (BDSC; no. 458).

	Day		Night	
Gal-4 driver	**driver**	**RNAi**	**driver**	**RNAi**
Figure S3A				
per	$<1x10^{-5}$	$<1x10^{-5}$	0.0073	0.23
tim	$<1x10^{-5}$	$<1x10^{-5}$	0.46	0.97
cry	$<1x10^{-5}$	0.013	0.17	0.691
Pdf	0.0356	0.94	0.069	0.79
C929	0.0005	0.010	0.0007	0.75
Mai179	0.18	0.87	0.0069	0.14
DN1	$5x10^{-4}$	0.091	0.010	0.34
Gmr	$1x10^{-4}$	0.093	0.36	0.89
C5	0.13	0.074	0.98	0.39
201Y	0.55	0.042	0.0008	0.54
Elav	0.0036	0.0030	< 0.001	0.29
Figure S3B				
per(1)	< 0.001	< 0.001	0.45	0.78
per(2)	< 0.001	0.0022	< 0.001	< 0.001
tim	0.0316	0.0096	< 0.001	< 0.001
cry	< 0.001	< 0.001	< 0.001	< 0.001
pdf	< 0.001	0.0057	0.45	0.28
DN1	0.28	0.0027	0.08	0.014
Gmr	0.0277	0.93	0.0053	0.018
Figure S3C				
per	< 0.001	< 0.001	< 0.001	< 0.001
tim	0.0048	< 0.001	0.1	0.088
cry	< 0.001	0.0015	0.011	0.066

Table S1. P values for Figure S3. Related to Figures 3 and S3. Student's *t*-test comparing total 12 hr day or night sleep (min) for experimental group (*Gal4-driver* > *RNAi-0.9*) compared to either driver control cross (w^{1118} x *Gal4-driver;* designated 'driver') or RNAi control cross (w^{1118} x *RNAi-0.9;* designated 'RNAi'). Sleep values for each cross were determined using activity data collected from 42 flies per cross. All experimental and control crosses were done contemporaneously. Only when the experimental cross was significantly different from both control crosses ($p < 0.05$) was it considered a significant effect of RNAi treatment on total sleep levels.

Primer	Source
P3177F (forward primer to generate per-STOP mutation) 5'-GCGTCGACGAGCCTAGGGGCA-3'	This paper
per0 R (reverse primer to generate per-STOP mutation) 5'-AGAAGGACGTAGCAACCGTTCTAGATGAGGAAGCGGTATGGCTTG-3'	This paper
P7312R (reverse primer to generate per-STOP mutation) 5'-AACCTTAGGGCTGAGAAGGGTGGT-3'	This paper
per0 F (forward primer to generate per-STOP mutation) 5-CAAGCCATACCGCTTCCTCATCTAGAACGGTTGCTACGTCCTTCT-3'	This paper
STOP-CG2650F (forward primer to generate 0.9-STOP mutation) 5'-CAGCTGGGTTTCCTGATGAGTGGACGCCTCCG-3'	This paper
STOP-CG2650R (reverse primer to generate 0.9-STOP mutation) 5'-CGGAGGCGTCCACTCATCAGGAAACCCAGCTG-3'	This paper
Bsu36I.R1 (reverse primer to construct 0.9-STOP containing transgenes) 5'-GCCCTAAGGTTTATATATCCG-3'	This paper
EcoRI.9300.F (forward primer to construct 0.9-STOP containing transgenes) 5'-TTGAATTCAATGTAAAATGGTT-3	This paper
CG2650.EcoRI.F (forward primer to construct UAS-0.9 plasmid) 5'-TAAGAATTCATGCAGCTAACCGGTGCC-3'	This paper
CG2650.XbaI.R (forward primer to construct UAS-0.9 plasmid) 5'-TATATCTAGATCATTCCTTTTCGAAGAACTCG-3'	This paper
P6851-StulF (forward primer to measure plasmid derived *per* transcript levels and dmpi8 splicing efficiency) 5'-ACACAGCACGGGGATGGGAGGC-3'	This paper
P6851f (forward primer to measure endogenous *per* transcript levels and dmpi8 splicing efficiency) 5'-ACACAGCACGGGGATGGGTAGT-3'	[S14, S15]
P7184r (reverse primer to measure either plasmid-derived or endogenous *per* transcript levels and dmpi8 splicing efficiency) 5'-GGCTTGAGATCTACATTATCCTC-3'	[S14, S15]
CG2650-F1 (forward primer to measure 0.9 transcript levels) 5'-CCAACTCGATGATGGTCAAGAG-3')	This paper
CG2650-R1 (reverse primer to measure 0.9 transcript levels) 5'- GTCGTTGAACAGATTCGACAGG-3')	This paper
CBP294F (forward primer to measure CBP20 transcript levels) 5'-TGATTGTGATGGGCCTGGACAAGT-3'	[S14, S15]
CBP536R (reverse primer to measure CBP20 transcript levels) 5'-GTCCAAGCGAGTGCCATTCACAAA-3')	[S14, S15]

Table S2. Primers used in this study, Related to STAR Methods

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