Supporting Information

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SI Materials and Methods

Fly Stocks. The wild-type strains *Canton-S* (*CS*) and *Oregon-R* (*OreR*) were lab strains, *Crimea* and *Pi2* flies were obtained from the Bloomington Stock Center. Recently caught wild-type strains were as described in (1). Northern blots using the flies collected from the wild were performed in 2000, shortly after their collection.

Hot-Stop PCR. This method allows for linear quantitation of alleles and was performed as described in (2). A high- and a low-expressing *takeout* strain were crossed and RNA extracted from progeny males. cDNA was synthesized as described below. Twenty rounds of PCR were performed with *takeout* specific, nonlabeled primers (3). One more round of PCR was performed with ^{32}P-labeled primers. PCR products were purified over a column (Qiagen) and digested with the restriction enzyme Fnu4H1. Only fragments originating from RNA produced by the low-expressing OreR strain contain the enzyme site and will show a smaller fragment size after digestion.

Immunohistochemistry was carried out as described (4) using the Vector ABC Elite staining kit. Unspecific fat body staining was blocked using the Vector Avidin/Biotin Blocking kit following the supplier's "fast protocol". Antibodies: Guinea pig anti-Pdp1 (GP40) (5) was used at 1:300 dilution, anti-guinea-pig (Vector) at 1:200. Rabbit anti-PER antibody (a gift of Michael Rosbash, Brandeis University, Waltham, MA) was preabsorbed against dissected heads of per^{01} flies and used at 1:4,000. Anti-Rabbit was from Vector and used as suggested by the ABC Elite staining protocol.

X-Gal staining was carried out as described (3).

1. Sureau G, Ferveur JF (1999) Co-adaptation of pheromone production and behavioural responses in Drosophila melanogaster males. *Genet Res* 74:129–137.
2. Uejima H, Lee MP, Cui H, Feinberg AP (2000) Hot-stop PCR: A simple and general assay for linear quantitation of allele ratios. *Nat Genet* 25:375–376.
3. Dauwalder B, Tsujimoto S, Moss J, Mattox W (2002) The Drosophila takeout gene is regulated by the somatic sex-determination pathway and affects male courtship behavior. *Genes Dev* 16:2879–2892.
4. Skoulakis EM, Kalderon D, Davis RL (1993) Preferential expression in mushroom bodies of the catalytic subunit of protein kinase A and its role in learning and memory. *Neuron* 11:197–208.
5. Benito J, Zheng H, Hardin PE (2007) PDP1epsilon functions downstream of the circadian oscillator to mediate behavioral rhythms. *J Neurosci* 27:2539–2547.

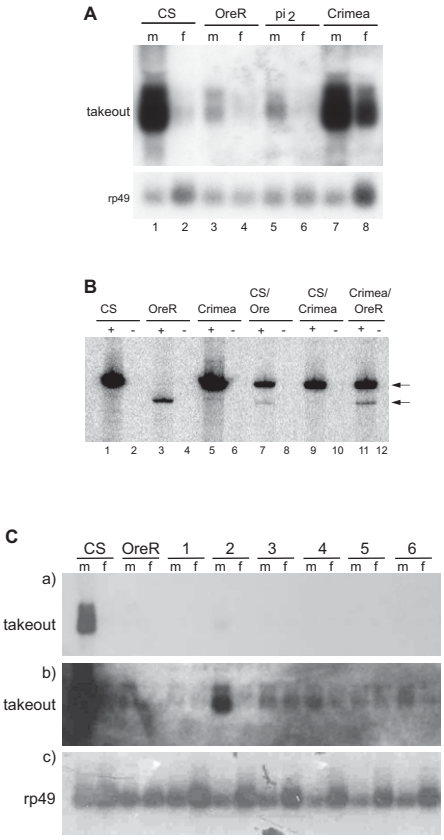

Fig. S1. Varying takeout RNA levels in wild-type strains are due to a *cis*-effect. Strains freshly collected from the wild show low levels of takeout expression. Some laboratory strains expressed takeout at the high levels reported earlier, whereas others showed much lower levels of expression. Two wild-type strains, *Canton-S* (*CS*) and *Crimea* showed high levels of expression, whereas the other lines showed much lower levels of *takeout* RNA. To examine whether this effect was due to the differential levels of a transcriptional regulator (a *trans* effect) or due to inherent properties of the *takeout* promoter (a *cis* effect) in these lines, we crossed high-expressers with low-expressers and examined *takeout* RNA expression from the corresponding alleles in the progeny. To distinguish between the two alleles we made use of single nucleotide polymorphisms that we had identified in the coding regions of the two *takeout* alleles. If the difference is due to a *trans* effect, we expect both alleles to be equally expressed in the heteroallelic flies. In contrast, in the case of *cis* regulation we expect each allele to be expressed like it is in the parental line. This is indeed what we observed (*B*). The *takeout* copy from the *CS* and *Crimea* strain were still expressed at the high levels observed in the respective strains, whereas copies from the other lines maintained their low expression levels. We conclude that the difference in expression levels is due to a *cis* regulatory effect. (*A*) takeout expression in males (m) and females (f) of different wild-type strains was examined by Northern blot. Ribosomal protein 49 (rp49) hybridization to the same blot is shown as a control for the amount of RNA loaded. (*B*) RT-PCR analysis of *takeout* transcripts of RNA from parents and progeny of crosses between different wild-type strains. A fragment of the *takeout* coding region was amplified using the "HotStop" method and digested with Fnu4H1. This site is only present in the *takeout* variant from the low-expressing OreR strain. (*C*) *takeout* expression in male and female whole flies collected from across Africa (1) was examined by Northern blot. RNA from the CS and OreR laboratory strains was included on the blot for comparison. Origin of strains: 1, Ivory coast (Tai strain); 2, Malawi; 3, Seychelles; 4, Madagascar; 5, Cotonou (Benin); 6, Guinea-Bisseau; (*a*) In a short exposure RNA is visible only in CS males. (*b*) long exposure shows low levels of *takeout* RNA in OreR and all of the other strains. (*c*) Ribosomal protein 49 (rp49) hybridization to the same blot is shown as a control for the amount of RNA loaded.

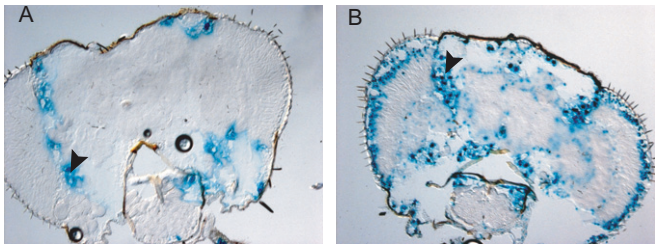

Fig. S2. Comparison of the t*imGal4* and the *Lsp2-Gal4* drivers. Cryosections of (*A*) Lsp2-Gal4/UAS-lacZ and (*B*) tim-Gal4/UAS-lacZ flies were stained side by side to compare the strength of the respective drivers. Lsp2-Gal4 shows slightly lower staining in fat body cells (marked by arrow).

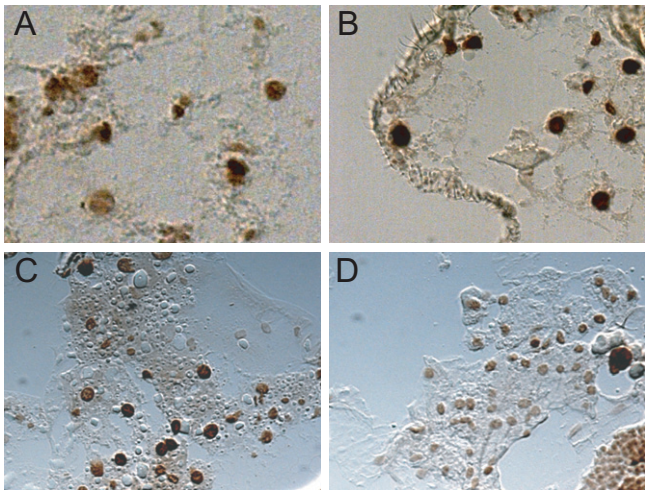

Fig. S3. Pdp1 protein expression in the fat body of *Lsp2-Gal4/UAS-Pdp1* flies and *Lsp2-Gal4-Pdp1i* flies. Cryosections of mutant/transgenic flies and CS control flies collected at ZT21 and mounted and processed side by side were stained with a Pdp1 antibody (5). Slightly elevated and decreased protein levels can be observed in the respective mutants. (*A* and *B*) Pdp1 immunoreactivity in fat body cells of *CS* (*A*) and *Lsp2-Gal4/UAS-Pdp1* (*B*) flies. (*C* and *D*) Pdp1 immunoreactivity in fat body cells of *CS* (*C*) and *Lsp2-Gal4-Pdp1i* (*D*) flies.

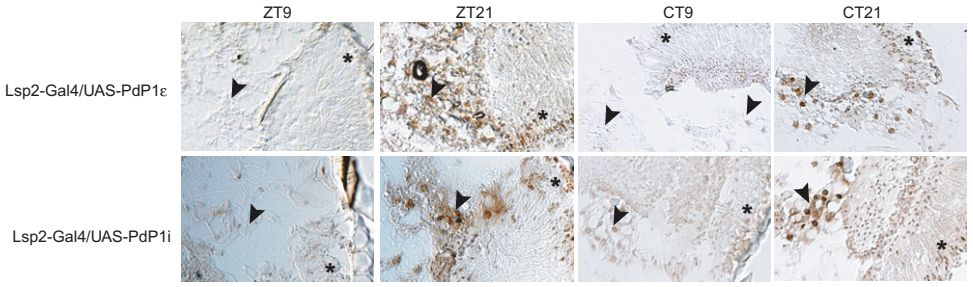

Fig. S4. PER protein amounts in fat body cycle in *Lsp2-Gal4/UAS-Pdp1i* and *Lsp2-Gal4/UAS-Pdp1* flies. Flies were entrained for 3 days and either collected at ZT9 and ZT21, or placed at DD for one day and collected at CT9 and CT21. Head cryosections were incubated with anti-PER antibody. Heads of flies collected at different time points were mounted and processed next to each other on the same slide. Fat body cells are indicated by arrowhead, photoreceptor cells are marked by asterix.