Supplemental Figures

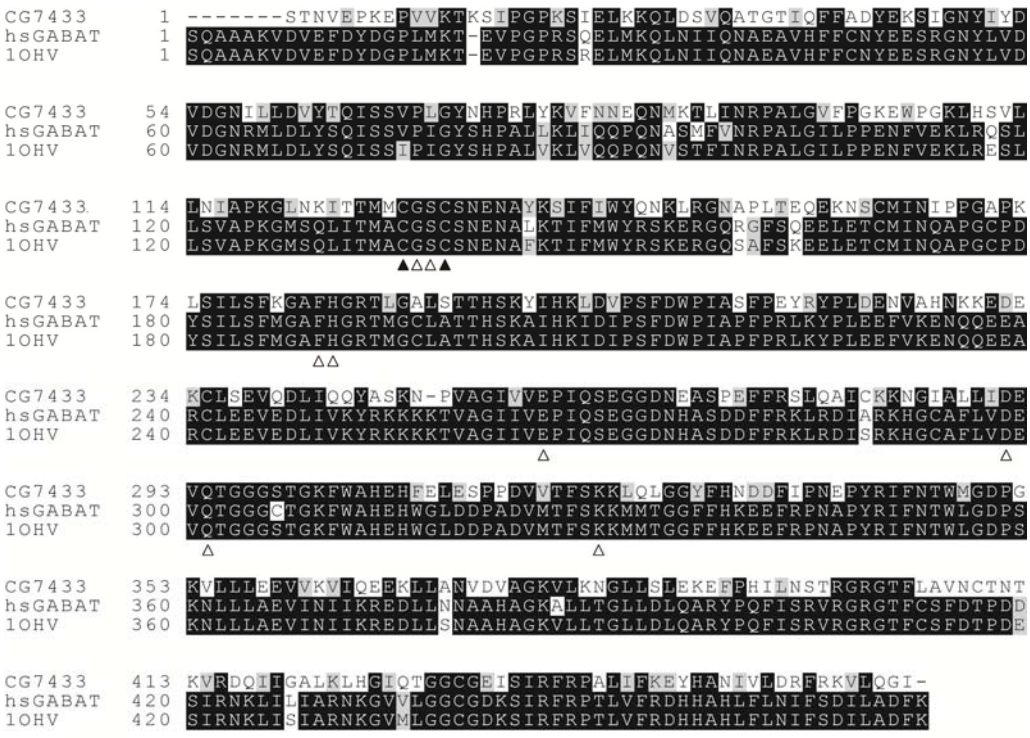

Figure S1. CG7433 is a mitochondrial protein highly similar to mammalian GABATs. Multiple sequence alignment of CG7433 with the GABATs from human and pig. Predicted mitochondrial signal peptides were removed from the proteins. The protein sequences were then multiple-aligned with ClustalW (1) and highlighted with BOXSHADE. A mitochondrial signal peptide of 22 amino acids (aa) was predicted in CG7433 by MitoProt (2). 1OHV denotes the primary structure of the crystallized pig GABAT (3). hsGABAT is the human GABAT (NCBI reference sequence NP_065737.2). Although MitoProt predicts a 35-aa mitochondrial signal peptide for hsGABAT

(MASMLLAQRL ACSFQHSYRL LVPGSRHISQ AAAKV), alignment with the primary structure of crystallized 1OHV suggests that the signal peptide of hsGABAT is more likely the first 28-aa, which have been removed in the figure. CG7433 shows high similarity with hsGABAT (56% identities, 75% positives) and 1OHV (56% identities, 76% positives). Open arrowheads (△) denote the pyridoxal 5'-phosphate binding sites predicted by NCBI protein BLAST. Close arrowheads (▲) denote the two conserved cysteine residues involved in homo-dimerization of GABAT via chelation with a [2Fe-2S] cluster as revealed by the 1OHV crystal structure.

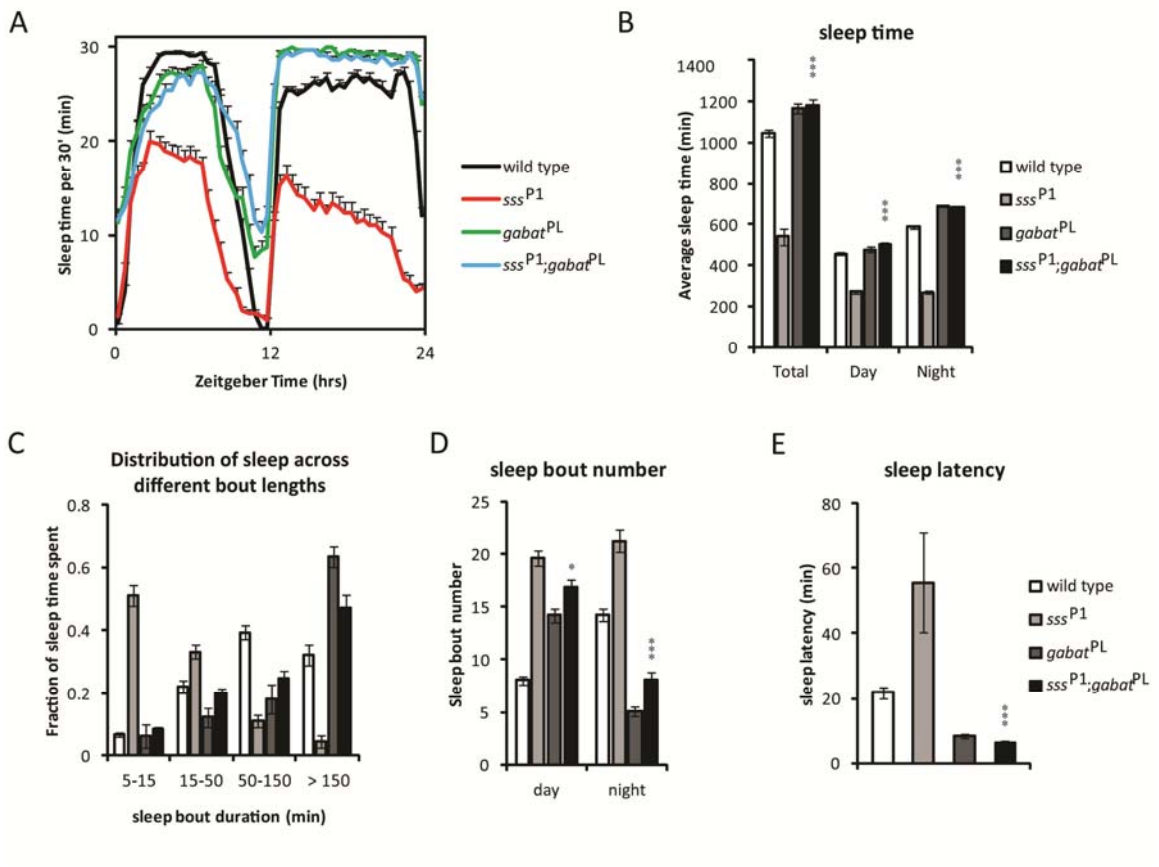

Figure S2. The *gabat* mutation rescues sleep behavior of *sss*^{P1}. (**A**) and (**B**) The *gabat*^{PL} mutation increases sleep in *sss*^{P1} mutants during both the day and the night.. With the incorporation of *gabat*^{PL} in *sss*^{P1}, sleep is also better consolidated during the night-time, as measured by the increased distribution of sleep in longer sleep bouts (**C**) and decreased sleep bout number (**D**). (**E**) *gabat*^{PL} rescues the long sleep latency phenotype of *sss*^{P1}. Asterisks show results of one-way ANOVA with Tukey's test on the sleep parameters between *sss*^{P1} and *sss*^{P1};*gabat*^{PL} (***:P<0.001; *, P<0.05). The MWU test was used to assay for differences in the distribution of sleep bouts among genotypes (MWU test with Bonferroni adjustment, *sss*^{P1} vs *sss*^{P1};*gabat*^{PL} P<0.0001; *gaba*^{PL} vs *sss*^{P1};*gabat*^{PL}, P=0.18).

Table S1. Circadian rhythm parameters of *gabat* mutants and controls.

	Con F01602	***gabat*^{F}**	**Con PL00338**	***gabat*^{PL}**
Tau (hr)	23.9	23.9	23.6	23.4
s.e.m.	0.1	0.1	0.1	0.1
Number of flies	31	17	29	23
% Rhythmic	96.9	53.1	90.6	71.9

χ^2 periodogram analysis was performed for each fly to determine the free-running period, tau. The control wild type flies were derived from the last outcross of the mutant into iso31 (see Methods).

Table S2. Circadian rhythm parameters affected by *gabat*^{F} in *sss*^{P1} flies.

	wild type	***sss*^{P1}**	***gabat*^{F}**	***sss*^{P1}*;gabat*^{F}**
Tau (hr)	23.6	23.7	23.8	23.8
s.e.m.	0.1	0.1	0.1	0.1
Number of flies	16	15	24	31
% Rhythmic	100.0	40.0	62.5	74.2

χ^2 periodogram analysis was performed for each fly to determine the free-running period, tau. The control wild type flies was iso31 (see Methods). The pooled activity records of these flies are shown in Figure S3F.

References:

1. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H *et al.* Clustal W and Clustal X version 2.0. *Bioinformatics* 2007 Nov 1; **23**(21)**:** 2947-2948.

2. Claros MG, Vincens P. Computational method to predict mitochondrially imported proteins and their targeting sequences. *Eur J Biochem* 1996 Nov 1; **241**(3)**:** 779-786.

3. Storici P, De Biase D, Bossa F, Bruno S, Mozzarelli A, Peneff C *et al.* Structures of gamma-aminobutyric acid (GABA) aminotransferase, a pyridoxal 5'-phosphate, and [2Fe-2S] cluster-containing enzyme, complexed with gamma-ethynyl-GABA and with the antiepilepsy drug vigabatrin. *J Biol Chem* 2004 Jan 2; **279**(1)**:** 363-373.