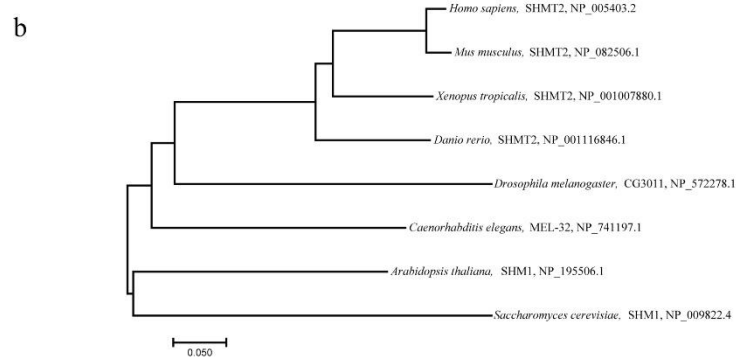
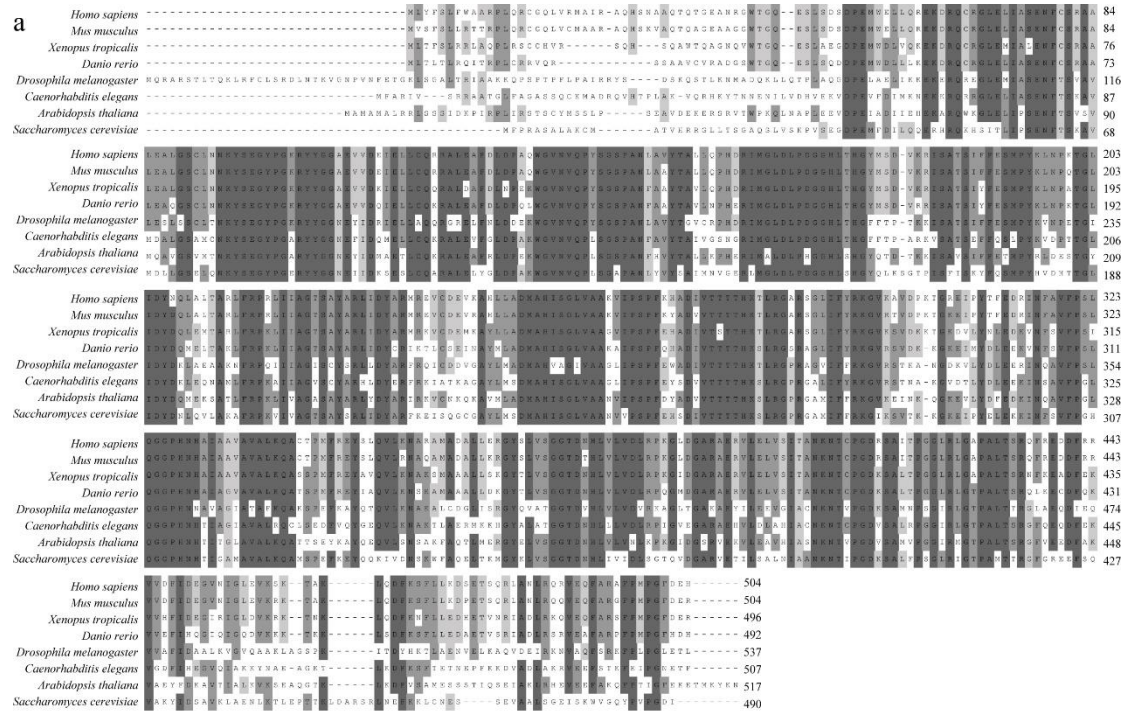


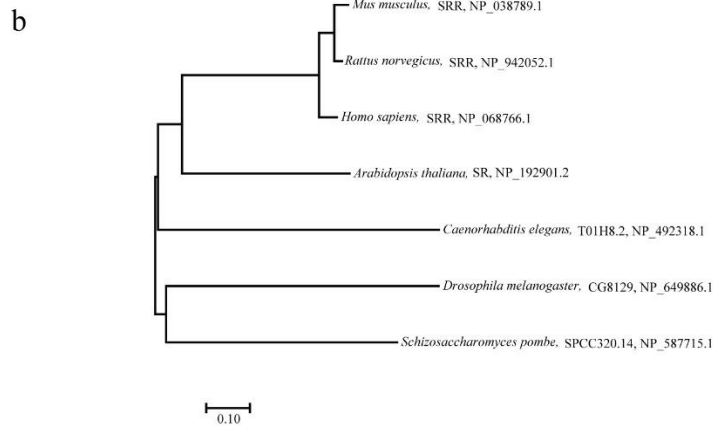
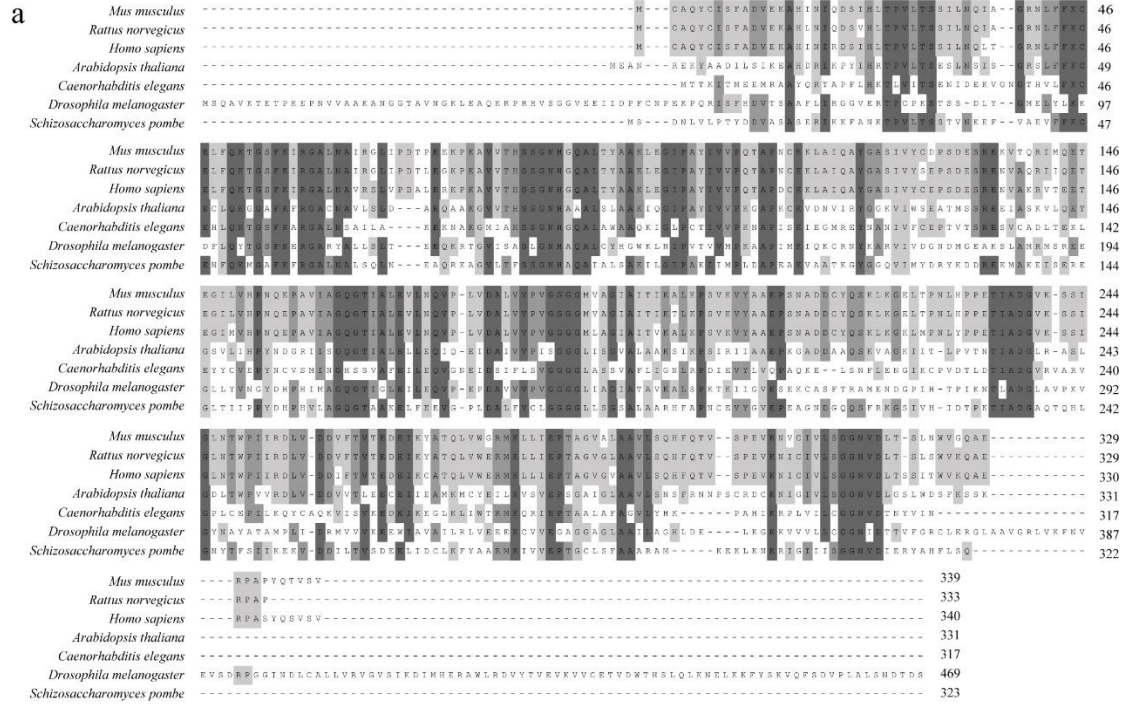
Supplementary Information

A Novel Physiological Role in Sleep for D-Serine Made by Serine Racemase in *Drosophila* Intestine

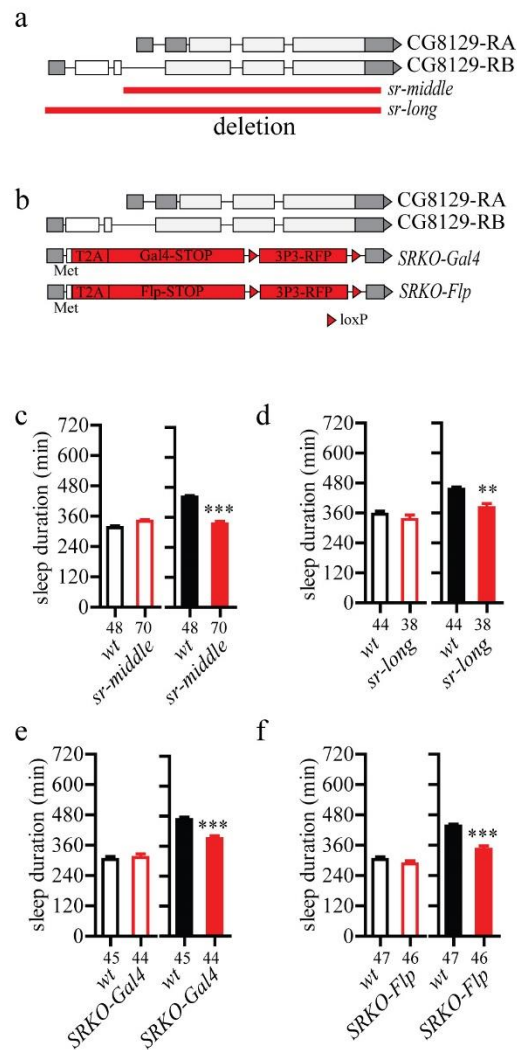
Xihuimin Dai, Enxing Zhou *et al.*



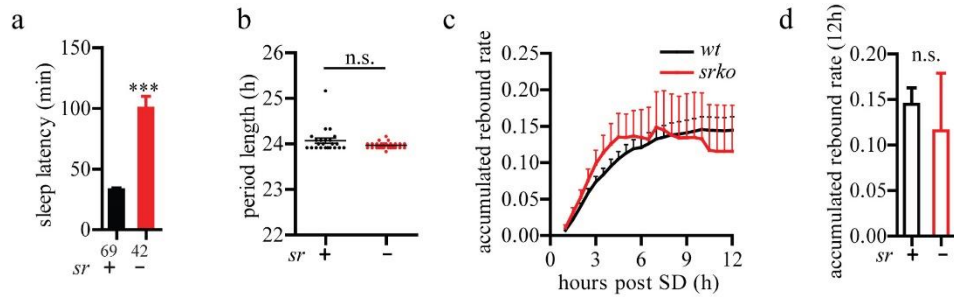
Supplementary Figure 1. Alignment of SHMT proteins. **a,** Alignment of the predicted amino acid (aa) sequence of *Drosophila CG3011* isoform PA (GenBank number NP_572278) with SHMT from other species using Clustal Omega. Identical aa residues are shaded in black, and similar residues in gray. **b,** A phylogenetic tree of SHMT proteins. The scale bar represents 5% aa substitutions per residue. *Drosophila* SHMT protein is 57% identical to *Homo sapiens* SHMT2 (NP_005403), 56% identical to *Mus musculus* SHMT2 (NP_082506), 58% identical to *Xenopus tropicalis* SHMT2 (NP_001007880), 56% identical to *Danio rerio* SHMT2 (NP_001116846), 56% identical to *Caenorhabditis elegans* MEL-32 (NP_741197), 51% identical to *Arabidopsis thaliana* SHM1 (NP_195506), 49% identical to *Saccharomyces cerevisiae* SHM1 (NP_009822).



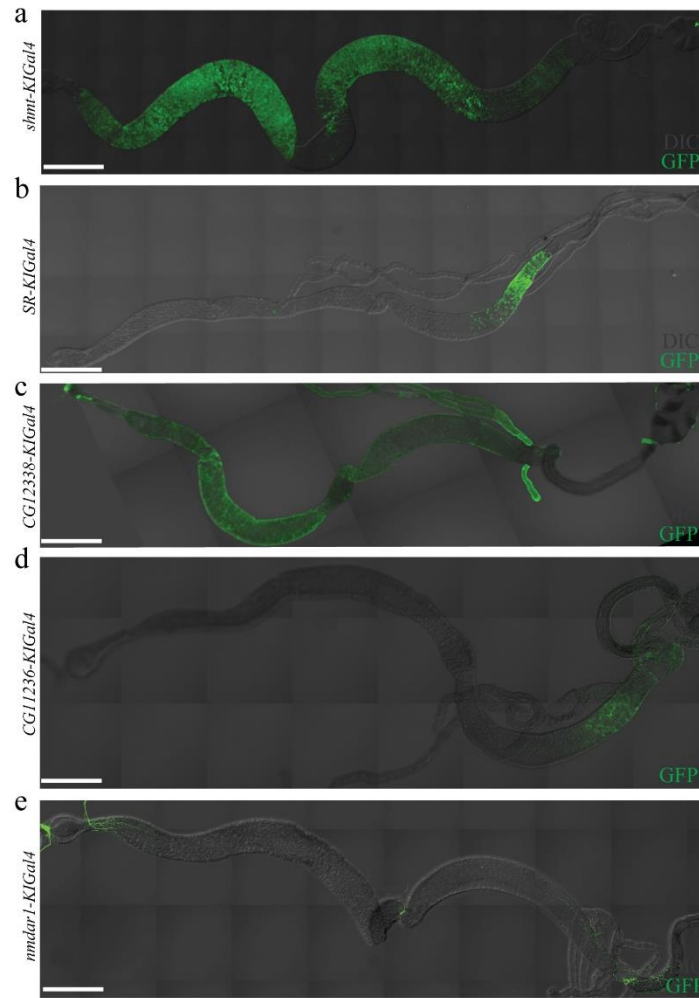
Supplementary Figure 2. Alignment of SR Proteins. **a**, Alignment of the predicted amino acid sequence of *Drosophila* CG8129 isoform PB (GenBank number NP_649886) with SRs from other species using Clustal Omega. Identical amino acid residues are in black, and similar residues in gray. **b**, A phylogenetic tree of SRs. The scale bar represents 10% amino acid substitutions per residue. *Drosophila* SR is 35% identical to *Homo sapiens* SRR (NP_068766), 33% identical to *Mus musculus* SRR (NP_038789), 34% identical to *Rattus norvegicus* SRR (NP_942052), 28% identical to *Caenorhabditis elegans* T01H8.2 (NP_492318), 34% identical to *Arabidopsis thaliana* SR (NP_192901), 32% identical to *Schizosaccharomyces pombe* SPCC320.14 (NP_587715).



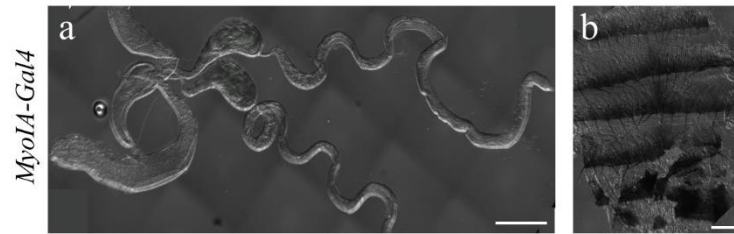
Supplementary Figure 3. Sleep Phenotypes of *sr* Mutants. **a-b**, Schematic representations of four different *sr* mutants, including two deletion mutants (**a**) and two insertion mutants (**b**). **c-f**, Nighttime sleep duration was significantly decreased in four different *sr* mutants: *sr-middle* (**c**), *sr-long* (**d**), *SRKO-Gal4* (**e**), and *SRKO-Flp* (**f**). Open bars denote daytime sleep, filled bars denote nighttime sleep. Numbers below each bar represent the number of flies tested. Mann Whitney test, *** $P < 0.001$, ** $P < 0.01$. Error bars represent s.e.m. Male flies were used.



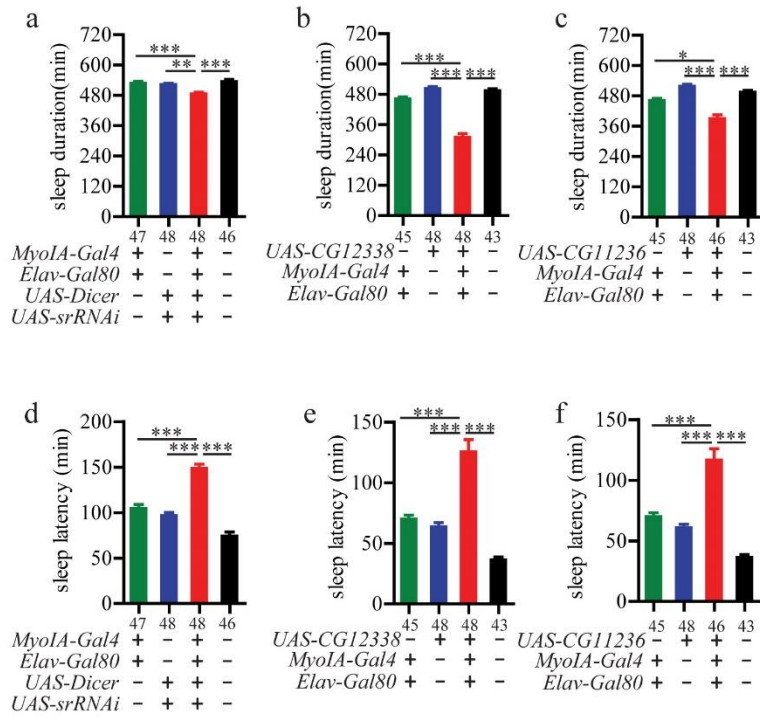
Supplementary Figure 4. Sleep and Circadian Phenotypes of *srko* Flies. **a**, Sleep latency of *srko* (red) (n=42) flies was significantly longer compared to *wt* (black) (n=69) flies. **b**, Circadian period length of *srko* (red) (n=26) had no significant difference with *wt* (black) (n=23) flies. Circadian rhythms were calculated in ActogramJ¹. **c**, Accumulated rebound curves of *srko* (red) (n=52) and *wt* (black) (n=77) flies after one night of sleep deprivation. **d**, Statistical analysis of rebound rate at 12 h post-sleep deprivation. No significant difference was detected between *srko* and *wt* flies. Mann Whitney test, n.s. $P > 0.05$. Error bars represent s.e.m. Male flies were used in **(a-b)**. Female flies were used in **(c-d)**.



Supplementary Figure 5. Expression Patterns of *shmt*, *sr*, *daao* and *nmdar1* in the Gut. **a-e**, Expression patterns of *shmt-KIGal4* (**a**), *SR-KIGal4* (**b**), *CG12338-KIGal4* (**c**), *CG11236-KIGal4* (**d**), and *nmdar1-KIGal4* (**e**) labeled by mCD8::GFP in the gut. The guts were immunostained with an anti-GFP antibody. Scale bars are 500µm.



Supplementary Figure 6. No Expression of *MyoIA-Gal4* was Detected in the Genital (a) and the Internal Abdominal Cuticle Surface (b). Expression patterns of *MyoIA-Gal4* were labeled by mCD8::GFP. The tissues were immunostained with anti-GFP. Scale bars are 200 μ m.



Supplementary Figure 7. Sleep Phenotypes of Gut-specific D-Ser Downregulation.

a-c, Sleep duration was significantly decreased in gut-specific *sr* knockdown (**a**) and gut-specific *daao* overexpression (**b-c**). **d-f**, Sleep latencies were significantly increased in gut-specific *sr* knockdown (**d**) and gut-specific *daao* overexpression (**e-f**).

Numbers below each bar represent the number of flies tested. Mann Whitney test, ***

$P < 0.001$, ** $P < 0.01$, * $P < 0.05$. Error bars represent s.e.m. Male flies were used.

Supplementary Table 1. List of genotypes of generated fly lines. Targeted region is based on FB2018_02, released Apr 3, 2018 by FlyBase.

Mutated sequences are in red characters.

Constructed line	targeted region	manipulation
<i>srko</i>	3R: 9366409..9367626	deleted
<i>CG12338ko</i>	2R:10729640..10730764	deleted
<i>CG11236ko</i>	2L:6803390..6804121	deleted
<i>nmdar1ko</i>	3R: 5514628..5516383	replaced with 2A-Gal4-STOP
<i>shmt-KIGal4</i>	X:5913046..5913199	replaced with 2A-Gal4-STOP
<i>SR-KIGal4</i>	3R: 9367596..9367646	replaced with 2A-Gal4-STOP
<i>SR-KILexA</i>	3R: 9367596..9367646	replaced with 2A-LexA-STOP
<i>CG12338-KIGal4</i>	2R:10729128..10729641	replaced with 2A-Gal4-STOP
<i>CG11236-KIGal4</i>	2L:6804256..6804630	replaced with 2A-Gal4-STOP
<i>nmdar1-KIGal4</i>	3R: 5508989..5509789	replaced with 2A-Gal4-STOP
<i>SRKO-Gal4</i>	3R: 9365491..9367646	replaced with 2A-Gal4-STOP
<i>sr-middle</i>	3R: 9365859..9367626	deleted
<i>sr-long</i>	3R: 9365158..9367626	deleted
<i>SRKO-Flp</i>	3R: 9365491..9367646	replaced with 2A-Flp-STOP
	Genotype	Protein Sequence
<i>wildtype</i>	ATGCAGCGGGCGCGCTCTACACTGACACAAAAGC TTCGGTTTTGCCTTAGTCGGGACCTGAACACCAA AGTTGGCAACCCGGTTAAC...	MQRARSTLTQKLRFCLSRDLNTKVGNPV
<i>shmt-es</i>	ATGCAGCGGGCGCGCTCTACACTGACACAAAAGC TTCGGTTTTGCCTTAGTCGGGACCTGAACACCA G TTGGCAACCCGGTTAA...	MQRARSTLTQKLRFCLSRDLNT SWQPG*

Supplementary Table 2. List of the Sequences of Primers Used for Identification of Generated Lines.

Constructed line	Forward primer	Reverse primer	Length
<i>srko</i>	SR-F: 5'-aatgggcgtgtcttcgaact-3'	SR-R:5'-acgtccgaggacttgacaac-3'	2140bp
<i>sr-middle</i>			1590bp
<i>sr-long</i>			889bp
<i>CG12338ko</i>	12338-F: 5'-ggagagcggatttgataagagc-3'	12338-R: 5'-atgcaacgcaccaactgttc-3'	867bp
<i>CG11236ko</i>	11236-F: 5'-aacgaaagggtccgagagttt-3'	11236-R: 5'-atgcttgcatgacccaatc-3'	950bp
<i>nmdar1ko</i>	NMDAR1KO-F: 5'-ccctcatctgcctctt-3'	GAL4-R: 5'-tctgtgacggcatcttt-3'	2961bp
<i>shmt-KIGal4</i>	SHMTKI-F: 5'-ttccgaagtgcgtcag-3'		2537bp
<i>SR-KIGal4</i>	SRKI-F: 5'-aacgcataccttccatgac-3'		2422bp
<i>CG12338-KIGal4</i>	12338KI-F: 5'-ttgattatccctgtcacgtc-3'		2387bp
<i>CG11236-KIGal4</i>	11236KI-F: 5'-gtcaaagggcatgaaggat-3'		2980bp
<i>nmdar1-KIGal4</i>	NMDAR1KI-F: 5'- aaagtgtagcgaagcagagg-3'		2598bp
<i>SRKO-Gal4</i>	SRKO-F: 5'-tggcagcattgtggagaacagt-3'		2170bp
<i>SRKO-Flp</i>	SRKO-F: 5'-tggcagcattgtggagaacagt-3'	Flp-R: 5'-tgccgcagttgatgaat-3'	2448bp
<i>SR-KILexA</i>	SRKI-F: 5'-aacgcataccttccatgac-3'	LexAR: 5'-tcctggcaacgactacctg-3'	2511bp

Supplementary References:

1. Schmid, B., Helfrich-Förster, C. & Yoshii, T. A new ImageJ plug-in ‘ActogramJ’ for chronobiological analyses. *J. Biol. Rhythms* **26**, 464–467 (2011).