**LEGENDS FOR SUPPLEMENTAL FIGURES**

**Figure S1**. Sleep profiles of lkb1 knockout mutants. (A) A diagram representation of Lkb1 knock-out mutants lkb1T1. (B) Relative Lkb1 mRNA levels in lkb1T1/+ (blue) and wt (black) flies. lkb1T1/T1 was lethal. (C) Sleep profiles of lkb1T1/+ (blue, n=71) and wt (black, n=84) flies. (D-G) Statistical analysis of sleep duration, sleep bout number, sleep bout duration and latency to sleep in lkb1T1/+ (blue, n=71) and wt (black, n=84) flies. Open bars denote daytime, filled bars nighttime. (D) Sleep duration. Nighttime sleep duration of lkb1T1/+ was lower than that of wt flies. (E) Sleep bout number. Daytime and nighttime sleep bout numbers of lkb1T1/+ were not significantly from those of wt flies. (F) Sleep bout duration. Nighttime sleep bout duration of lkb1T1/+ was lower than that of wt flies. (G) Latency to sleep. Latency to sleep after light-off of lkb1T1/+ was longer than that of wt flies. Unpaired t test was used. n.s. denotes p>0.05, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001. Error bars represent SEM

**Figure S2.** Sleep profiles of lkb1T1/T2 mutants. (A) Relative Lkb1 mRNA levels in lkb1T2/T2 (red), lkb1T1/T2 (dark red), lkb1T2/+ (blue), lkb1T1/+ (yellow) and wt (black) flies. (B) Sleep profiles of lkb1T2/T2 (red, n=48), lkb1T1/T2 (dark red, n=34), lkb1T2/+ (blue, n=46), lkb1T1/+ (yellow, n=47) and wt (black, n=48) flies. (C-F) Statistical analysis of sleep duration, sleep bout number, sleep bout duration and latency to sleep in lkb1T2/T2 (red, n=48), lkb1T1/T2 (dark red, n=34), lkb1T2/+ (blue, n=46), lkb1T1/+ (yellow, n=47) and wt (black, n=48) flies. Open bars denote daytime, filled bars nighttime. (C) Sleep duration. Daytime and nighttime sleep duration of lkb1T1/T2 was lower than that of wt, lkb1T1/+ and lkb1T2/+ flies. (D) Sleep bout number. Daytime sleep bout numbers of lkb1T1/T2 was lower than that of wt, lkb1T1/+ and lkb1T2/+ flies. (E) Sleep bout duration. Nighttime sleep bout duration of lkb1T1/T2 was lower than that of wt, lkb1T1/+ and lkb1T2/+ flies. (F) Latency to sleep. Latency to sleep after light-off of lkb1T1/T2 was longer than that of wt, lkb1T1/+ and lkb1T2/+ flies. One-way ANOVA was used. n.s. denotes p>0.05, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001. Error bars represent SEM

**Figure S3.** Genotype confirmation for lkb1T2. (A) A diagram of lkb1T2 and the PCR primers used to detect inserted sequences. (B) Polymerase chain reaction (PCR) confirmation of the inserted sequences.

**Figure S4.** Lkb1-sgRNA. A diagram of Lkb1 sgRNA. Three sgRNA were designed.

**Figure S5.** Sleep phenotypes of mutants with neuronal overexpression of lkb1. (A) Relative Lkb1 mRNA levels in heads of 57C10/UAS-Lkb1(red), 57C10/+(blue), UAS-Lkb1/+ (yellow) and wt (black) flies. (B) Sleep profiles of 57C10/UAS-Lkb1(red, n=43), 57C10/+(blue, n=47), UAS-Lkb1/+ (yellow, n=44) and wt (black, n=42) flies. (C-F) Statistical analysis of sleep duration, sleep bout number, sleep bout duration and latency to sleep in 57C10/UAS-Lkb1(red, n=43), 57C10/+(blue, n=47), UAS-Lkb1/+ (yellow, n=44) and wt (black, n=42) flies. Open bars denote daytime, filled bars nighttime. (C) Sleep duration. Daytime and nighttime sleep duration of 57C10/UAS-Lkb1 was no difference than those of 57C10/+ flies. (D) Sleep bout number. Daytime and nighttime sleep bout numbers of 57C10/UAS-Lkb1 was no difference than those of 57C10/+ flies. (E) Sleep bout duration. Daytime and nighttime sleep bout duration of 57C10/UAS-Lkb1 was no difference than those of 57C10/+ flies. (F) Latency to sleep. Latency to sleep of 57C10/UAS-Lkb1 was no difference than those of 57C10/+ and UAS-Lkb1/+ flies. One-way ANOVA was used. n.s. denotes p>0.05, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001. Error bars represent SEM.

**Figure S6.** Sleep profiles of lkb1-CKO in mice. (A) Time spent in wakeness of Cre+ Lkb1fl/fl (red, n=10), GFP+ Lkb1fl/fl (yellow, n=5), Cre+ Lkb1fl/+ (blue, n=7) and Cre+ Lkb1+/+ (black, n=4) mice in a 12 h light/12 h dark (LD) cycle. (B) Time spent in NREM of Cre+ Lkb1fl/fl (red, n=10), GFP+ Lkb1fl/fl (yellow, n=5), Cre+ Lkb1fl/+ (blue, n=7) and Cre+ Lkb1+/+ (black, n=4) mice in an LD cycle. (C) Time spent in REM of Cre+ Lkb1fl/fl (red, n=10), GFP+ Lkb1fl/fl (yellow, n=5), Cre+ Lkb1fl/+ (blue, n=7) and Cre+ Lkb1+/+ (black, n=4) mice in an LD cycle.

**Figure S7.** Circadian rhythm in lkb1 mutant flies. (A) Relative rhythmic power in wt (black, n=42, 4/42 arrhythmic), lkb1T2/+ (blue, n=44, 0/44 arrhythmic) and lkb1T2/T2 (red, n=37, 0/37 arrhythmic) flies. The Power of lkb1T2/+ and lkb1T2/T2 were significantly higher than wt flies. (B) Period length in wt (black, n=42), lkb1T2/+ (blue, n=44) and lkb1T2/T2 (red, n=37) flies. The period of lkb1T2/T2 was not significantly different from those of wt and lkb1T2/+ flies. (C-E) Double-plotted actograms showing locomotor activity in wt (n=42), lkb1T2/+ (n=44) and lkb1T2/T2 (n=37) flies switching from 12hr LD to constant darkness. Open bars denote daytime, filled bars denote nighttime and grey background denotes DD. One-way ANOVA was used. n.s. denotes p>0.05, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001. Error bars represent SEM.