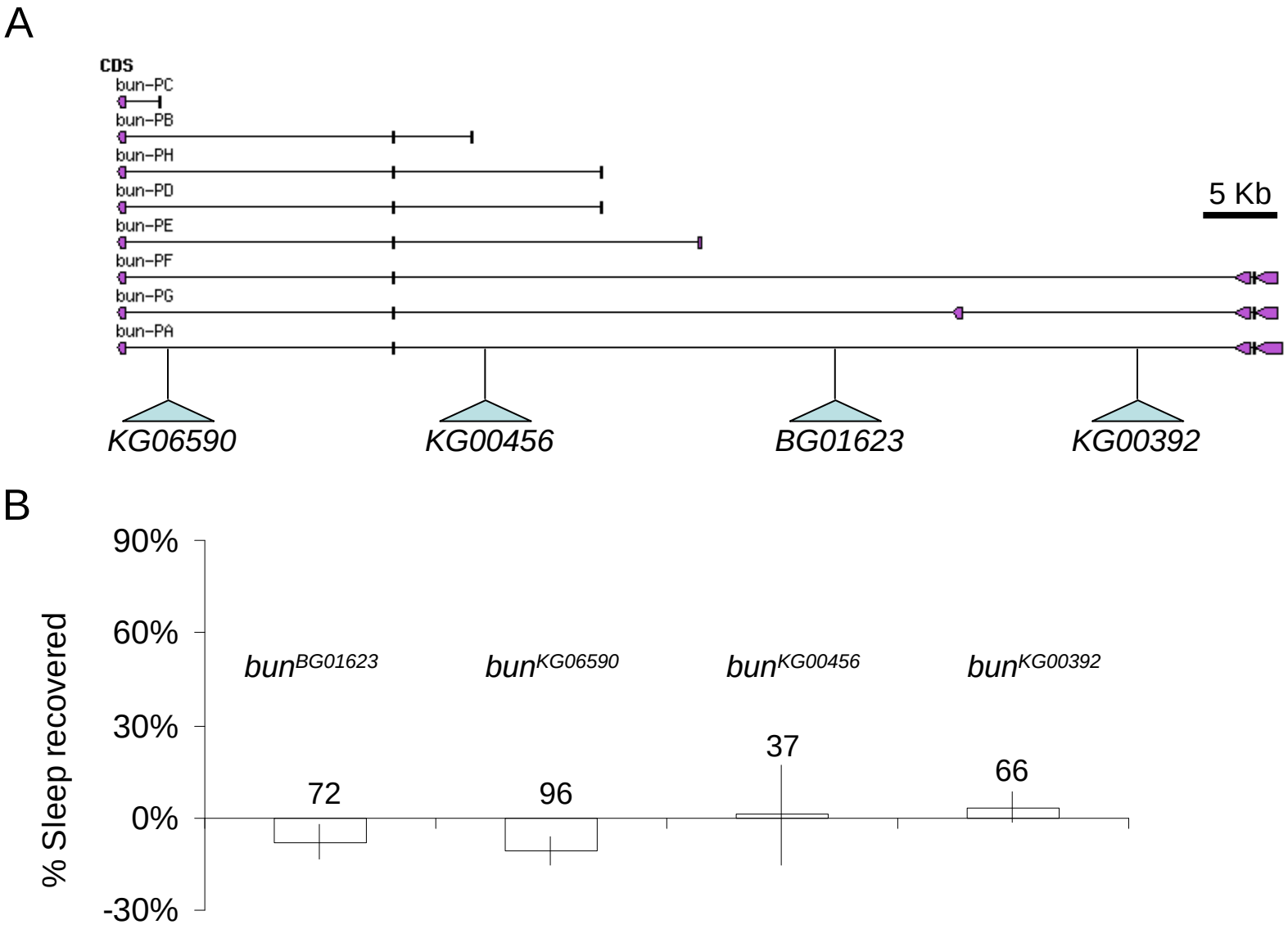
Supplemental Table S1 related to Figures 1, 2 and 4: Sleep data for *bunched, Notch* and *Delta* mutant conditions

Genotype	Latency	total sleep	Day bout	Night bout	day bout #	night bout #	48h recovery
bun^{R2}	29,3	783,2	16,7	43,3	17,1	16,7	45%
	5,7	53,5	2,3	8,8	1,7	2,3	10%
bun^{BG01623}	18,1	758,1	19,4	80,4	13,3	9,6	-8%
	4,1	34,6	2,6	13,7	0,8	1,5	6%
bun^{KG06590}	7,9	904,4	11,9	58,9	23,5	16,7	1%
	1,2	41,9	0,9	12,0	1,7	2,0	3%
bun^{KG06590}*/Df*	12,6	900,0	13,6	95,3	17,8	9,6	-26%
	0,9	42,9	2,0	18,6	2,4	1,4	6%
bun^{KG06590}*/+;Dl*^{X}*/+*	14,9	1070,4	28,0	83,0	20,2	13,0	38%
	3,7	31,6	4,5	21,7	2,5	2,0	12%
UAS-bunX/+	21,8	689,3	11,9	83,5	8,0	16,9	24%
	3,4	28,5	2,5	45,2	1,6	1,9	6%
c309/+	13,4	924,0	10,9	79,9	9,1	10,3	70%
	1,3	43,1	1,2	11,3	1,5	1,6	15%
UAS-bunX/+; c309/+	10,8	701,5	7,2	68,8	8,1	10,1	8%
	1,4	22,2	0,4	25,6	1,5	1,6	4%
UAS-bunX/+; 247/+	16,6	892,1	12,6	85,4	17,5	12,6	3%
	1,8	58,1	1,7	17,3	2,4	1,9	6%
247/+	27,8	756,0	15,9	49,6	10,9	16,6	65%
	3,4	23,2	1,7	11,4	1,3	1,3	8%
UAS-Delta/+	18,0	679,5	6,9	85,3	5,6	12,0	40%
	1,1	17,8	0,5	14,7	1,3	1,7	12%
UAS-Delta/+;247/+	19,7	616,3	7,7	31,4	8,6	21,3	0%
	3,2	31,3	0,4	4,3	2,0	1,3	17%
MBSW/UAS-Dl RU-	15,1	561,1	12,7	23,4	10,0	22,2	44%
	2,5	46,5	3,0	3,1	1,6	1,6	22%
MBSW/UAS-Dl RU+	12,9	730,7	30,2	26,7	15,6	24,2	-6%
	3,3	48,0	18,2	3,9	2,0	2,1	11%
Cs	23,7	718,2	8,1	67,7	11,3	13,7	60%
	3,0	24,8	0,5	9,4	1,7	1,3	10%
N^{spl1}	14,7	831,8	11,1	26,1	25,9	23,8	-11%
	2,7	44,8	0,8	2,5	1,5	1,4	20%
N^{Ax59b}*/+*	53,9	646,4	8,7	53,3	12,3	12,3	74%
	9,1	34,5	0,8	6,1	1,8	1,4	14%
N^{nd-2}*/+*	26,1	673,6	10,8	89,1	15,6	11,9	65%
	2,7	33,5	1,7	36,4	2,2	2,0	11%
N^{nd-1}	40,5	892,9	12,7	65,1	21,2	12,8	67%
	14,8	63,1	1,4	13,8	2,6	1,6	23%
N^{5419}*/+*	21,4	763,8	9,3	98,2	14,7	11,8	80%
	4,1	41,0	1,1	23,2	2,2	1,9	12%
N^{CO}*/+*	32,1	875,4	13,2	41,3	27,1	15,4	55%
	6,7	48,1	1,3	5,9	1,5	1,6	14%
Eaat1Gal4/+	25,0	762,2	8,9	42,8	18,3	17,1	52%
	2,7	23,5	0,7	4,0	1,6	1,1	13%
UAS-NICD/+	23,6	729,9	9,8	48,8	15,1	18,0	42%
	2,7	41,6	1,0	8,5	1,6	1,5	15%
UAS-NICD/+; Eaat1Gal4/+	41,2	545,5	8,3	25,3	10,8	19,4	-13%
	10,0	37,3	0,5	2,6	1,7	1,3	12%

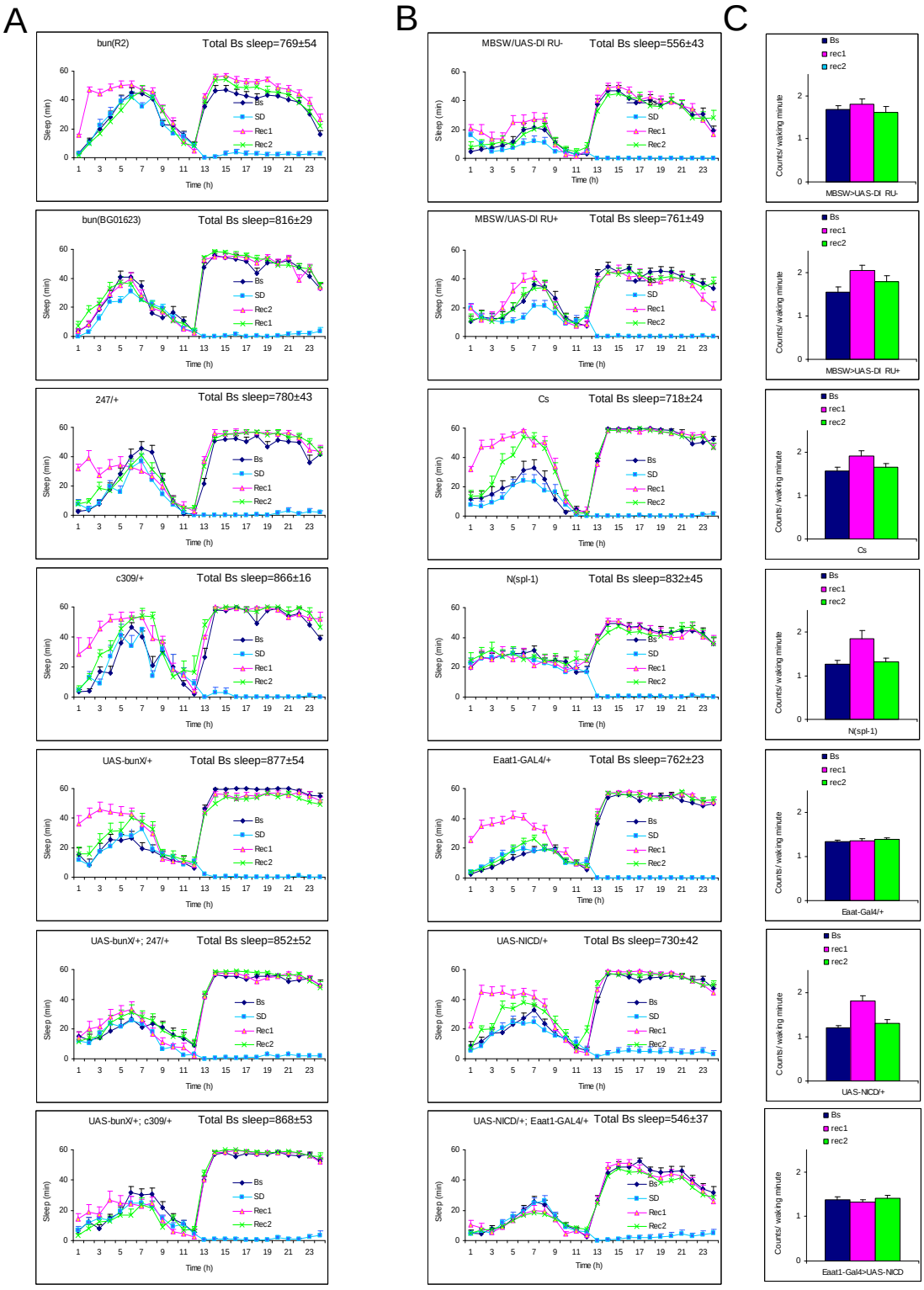
Latency: interval in minutes between lights off and the first sleep bout of the night
Total sleep: total sleep/24h
Day bout: average sleep bout duration during the light phase
Night bout: average sleep bout duration during the dark phase
Day bout #: average number of bout during the light phase
Night bout #: average number of bout during the dark phase
48h recovery: % sleep recovered in 48h after 12h of sleep deprivation

Supplemental Table S2, related to Figure 2 and Figure 4: Control metrics for *Notch* and *Delta* mutant conditions

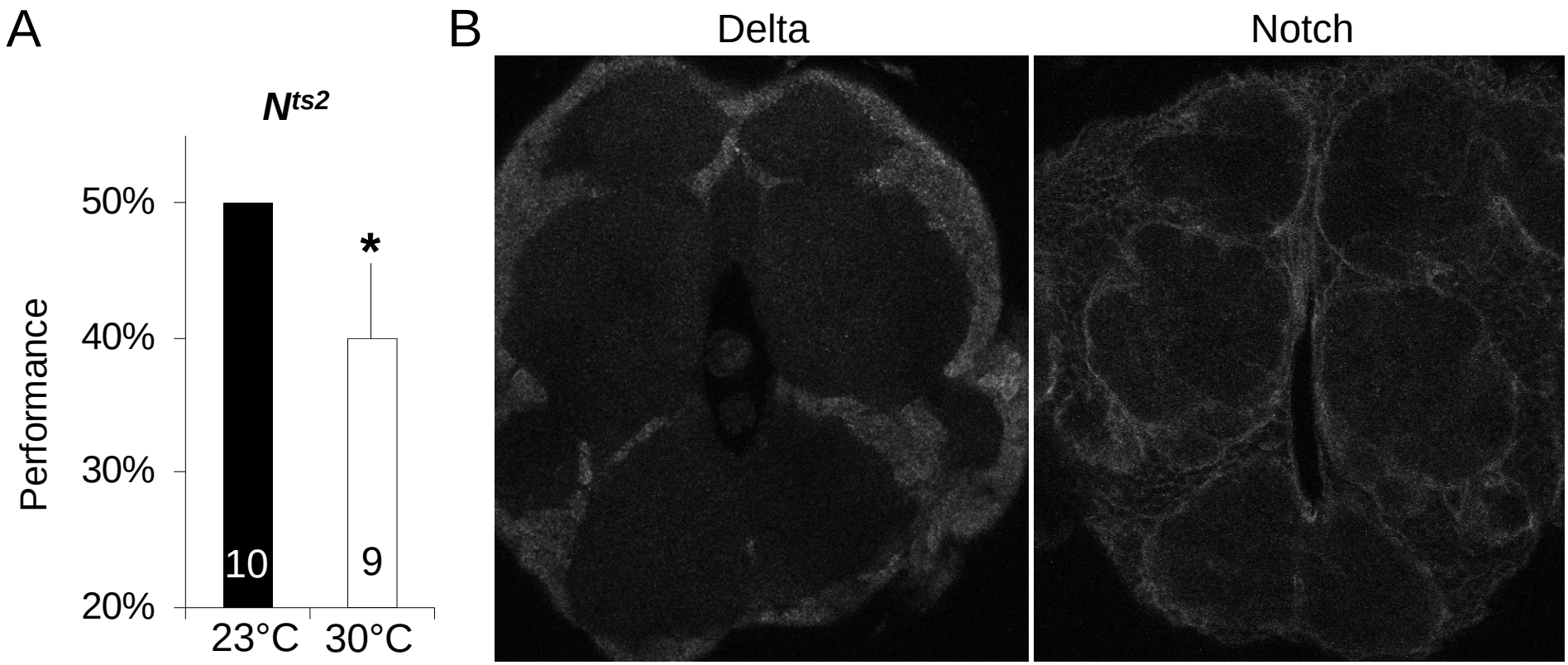
	PI (Phototaxis index)				QSI (Quinine sensitivity index)			
	mean	±	s.e.m.	n	mean	±	s.e.m	n
247/+	83%	±	2%	6	238	±	8	5
UAS-Dl/+	92%	±	2%	5	283	±	9	6
247/UAS-Dl	90%	±	3%	5	285	±	6	6
Cs	94%	±	4%	5	244	±	14	5
N^{spl-1}	83%	±	2%	6	277	±	13	6
N^{ts1} *23˚C*	85%	±	2%	6	287	±	6	6
N^{ts1} *31˚C*	85%	±	2%	6	280	±	10	6
N^{ts2} *23˚C*	82%	±	3%	6	281	±	17	6
N^{ts2} *31˚C*	78%	±	3%	6	256	±	21	6
Eaat1-GAL4/+	80%	±	3%	6	298	±	1	6
UAS-NICD/+	78%	±	2%	6	275	±	16	6
UAS-NICD/+; Eaat1-GAL4/+	77%	±	2%	6	278	±	16	6



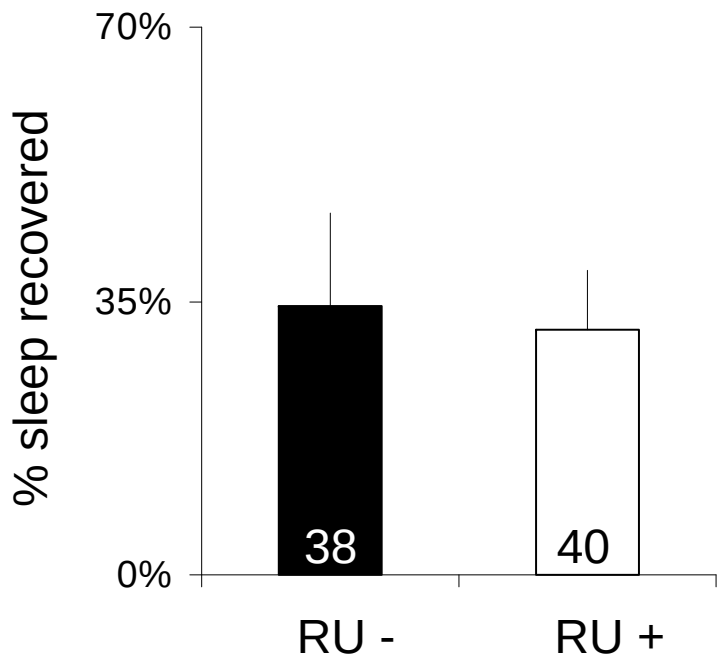
Supplemental Figure S1: Sleep homeostasis in *bunched* mutants. **A**, localization of the P-element insertions in the *bunched* locus. **B**, % sleep recovered in 48h following 12h of sleep deprivation for viable P element insertions in the *bunched* locus. All lines failed to show a sleep rebound. Number indicates N. Mean±s.e.m. is shown



Supplemental Figure S2, related to Figure 1,2 and 4: **Recovery after sleep deprivation in *bunched*, *Notch* and *Delta* mutant conditions**. **A-B**; sleep in minute/hr on baseline day (Bs, blue diamonds), on sleep deprivation day (SD, light blue squares), on recovery day1 (Rec1, pink triangles), on recovery day 2 (Rec2, green crosses) for *bunched* (A) and *Notch* (B) mutant conditions. **C,** average daily counts (infrared beam crossing in the Trikinetics system) per waking minute on baseline day (Bs, blue), on recovery day1 (rec1, pink), on recovery day 2 (rec2, green) in the corresponding *Notch* mutant conditions shown in A. mean ± s.e.m is shown.



Supplemental Figure S3 related to Figure 2, 3: A, Flies mutant for the thermo-sensitive allele N^{ts2} are learning impaired at non-permissive temperature. Learning is significantly impaired in N^{ts2} flies tested at non permissive temperature (30°C, white) compared to flies of the same genotype tested at permissive temperature (23°C). N^{ts2} displayed normal PSI and QSI at both permissive and non permissive temperature (supplemental table1). N is indicated in the bars, Mean±s.e.m. is shown * p=0.05 (Student's t-test). **B, Whole brain view of Delta and Notch immuno-localization.** Confocal images showing representative sagital sections of the brain. Delta is most abundantly present in the cortical regions of the brain (left) while Notch intracellular domain (right) is enriched in the membranes surrounding the brain neuropiles and the cell bodies of the cortex.



Supplemental Figure S4:Expressing *UAS-NICD* in neurons does not reduce the homeostatic response to sleep loss. *UAS-NICD/+;Elav-Switch/+* flies were fed control food (RU-) or RU 486 containing food (RU+) for 48h before being sleep deprived for 12h. % sleep recovered in 48h is shown

N is indicated in the bars, Mean±s.e.m. is shown

Experimental procedures:

Fly stocks, sleep monitoring and sleep deprivation: *Canton-S* (*Cs*), N^{spl-1}, N^{ts2} , *Eaat1-GAL4, c309-GAL4,* $bun^{BG01623}$, $bun^{KG06590}$, $bun^{KG00456}$ and $bun^{KG00392}$ *bun Deficiency, ,Df(2L)prd1.7* flies were obtained from the Bloomington *Drosophila* stock center (Indiana). Flybase provided P element insertion locations (Supplemental Figure S1). $bun^{BG01623}$ hopout (bun^{R2}) were generated by standard procedures. We obtained *247* and *elav-Switch* from Aaron DiAntonio (Washington University School of Medicine, Missouri), N^{ts1}*, UAS-Notch intra-cellular domain (NICD)* from Ross Cagan (Mount Sinai School of Medicine, New york), N^{5419} and *UAS-Delta* flies from James Skeath (Washington University School of Medicine, Missouri), N^{ts1}, *y v* , and $Dp(1;2)^{w+51b}$ were obtained from Pascal Heitzler (Strasbourg University, France), *UAS-bun2* and *UAS-bunX* were obtained from Leonard Dobens (University of Missouri, Kansas City), *MB-Switch* was obtain from Ron Davis (Scripps Research institute, Florida). N^{ts1} flies were kept recombined with *y* and *v* and compared with *yv* flies. For GAL4>UAS experiments, parental lines were outcrossed to *Cs* flies. Flies were cultured at 25°C, 50 to 60% humidity, in 12h:12h Light:Dark cycle, on a standard food containing yeast, dark corn syrup, molasses, dextrose and agar. Three day old female flies were individually placed into 65mm glass tubes and monitored using the Trikinetics activity monitoring system as previously described [1] (www.Trikinetics.com). Flies were sleep deprived using the Sleep Deprivation Nullifying Apparatus (SNAP) from Zeitgeber Time (ZT) 12 (lights-out) to ZT 0 (Lights-on). For *MB-Switch* and *elav-Switch* experiments, RU486 (mifepristone, Sigma) was diluted in Ethanol (50mg/ml) and then diluted in food (100µg/ml). Flies were fed RU486 for 48h prior to testing.

Learning test: Aversive Phototaxic Suppression (APS) is an operant paradigm requiring short term memory and the ability to inhibit a potent attraction towards light [2, 3]. Flies are tested individually in a T-maze where they are allowed to enter either a dark or a lighted vial. In the lighted vial, an aversive stimulus is provided by a filter paper wetted with a 10-1M Quinine hydrochloride solution (Sigma, St. Louis, Missouri). After entering the dark or lighted vial, the choice is recorded and the fly is quickly removed from the vial and placed back at the entrance of the maze. The performance index is the percentage of visits to the dark vial during the last block of 4 trials of a 16 trial test. For an experiment, learning was evaluated by the same experimenter who was blind to genotype and condition. All flies were tested in the morning between ZT0 and ZT4. For sleep deprivation experiments, flies were sleep deprived from ZT12 to ZT0 and until each fly was tested for learning. Learning scores are normally distributed [3]. Thus, Statistical analyses were performed using Systat (Systat, Chicago, Illinois). Differences were assessed using either a Student's t-test or Analyses of variance (ANOVA) which were followed by a modified Bonferroni correction. Time to complete test was similar for all the genotypes and within the normal range observed for wild type flies. Photosensitivity was evaluated in the T-maze over 10 trials in the absence of filter paper. For Quinine/humidity sensitivity, flies were individually placed at the bottom of a 14 cm cylindrical tube with each half of the apparatus containing separate pieces of filter paper either wetted with quinine or kept dry. The QSI was determined by calculating the time that the fly spent on the dry side of the tube when the other side had been wetted with quinine, during a 5 min period.

Immunohistochemistry and confocal mircoscopy: Brains were dissected in cold PBS, fixed for 20 minutes in a 4% paraformaldehyde Phosphate Buffered Saline, washed in 3% triton X100 PBS (PBS-T) and blocked for at least 45 min in 3% Goat Serum PBS-T. Monoclonal antibodies against the intracellular domain of Notch (C17.9C6, dilution 1:10), against the extracellular domain of Delta (C594.9B, dilution 1:50), and against repo (8D12, dilution 1:30) were obtained from Hybridoma Bank, university of Iowa. GFP was detected using a Rabbit anti-GFP antibody (1:2000; Molecular Probes, Carlsbad, California) and *lacZ* using an anti-βgalactosidase (1:2000, Cappel). After primary antibody incubation, brains were washed in PBS-T and incubated with Alexa 488 and Alexa 568 conjugated secondary antibodies (Molecular probes). Brains were mounted in hard set vectashield (Vector laboratories, Burlingame, California) and imaged using a Fluoview confocal microscope (Olympus, Center Valley, Pennsylvania). Confocal stacks were processed using Metamorph 6.2 software.

QPCR

Total RNA was isolated from 20 fly heads with Trizol (Invitrogen, Carlsbad, California) and DNAse I digested. cDNA synthesis was performed in quadruplicate using superscript III (Invitrogen), according to manufacturer protocol. To evaluate the efficiency of each reverse transcription, equal amounts of cDNA were used as a starting material to amplify *RP49* as previously described [4]. cDNA from comparable reverse transcription reactions were pooled and used as a starting material to run four QPCR replicates. Expression values for *RP49* were used to normalize results between groups. For each experimental condition and unless otherwise mentioned, two independent groups of flies were collected and processed independently for QPCR. Both experimental and untreated circadian matched controls were collected at the exact same circadian time ZT0-1. The following primers were used:
RP49, exon 1-2: Forward: aagaagcgcaccaagcacttcatc ; Reverse: tctgttgtcgataccttgggctt
bun, exon 1: Forward: agcagcagctagtgagcagtaaca ; Reverse : aacgtgctccattattgttgggcg

Human samples

Nine healthy human adult volunteers (seven men and two women) were enrolled for the expermiment. The study was carried out at the Sleep Medicine Center, Department of Neurology, Washington University School of Medicine and was approved by the Institutional Review Board at Washington University School of Medicine. The subjects were randomly separated in two groups, which where scheduled to alternate 2 weekends of either normal sleep or 28 h of continuous waking. On the normal-sleep weekend, the volunteers were allowed to fall asleep at 10:00 p.m. Normal sleep architecture was confirmed by standard polysomnography. The SD group remained awake and was allowed free access to water during the night. Saliva was collected from plain (noncitric acid) cotton Salivettes (Sarstedt, Newton, NC), rapidly frozen over dry ice and kept at −80°C until assayed. RNA was isolated from cell-free supernatant and processed for QPCR as described [5]. Control experiments removing either mRNA or reverse transcriptase from the reaction failed to result in amplification (data not shown) indicating that the signal was mRNA and not due to contamination. Results were evaluated using the Wilcoxon signed rank

test. Expression values for *ACTB* were used to normalize results. The following primers were used:
TSC22D, exon 3: Forward: aggatggtgtcctactgtggatga; Reverse: caggctccattggatcaaagccat
ACTB, exon 5-6: Forward: cccagcacaatgaagatcaa; Reverse: cgatccacacggagtacttg

References:

1. Shaw, P.J., Cirelli, C., Greenspan, R.J., and Tononi, G. (2000). Correlates of sleep and waking in Drosophila melanogaster. Science *287*, 1834-1837.
2. Le Bourg, E., and Buecher, C. (2002). Learned suppression of photopositive tendencies in Drosophila melanogaster. Anim Learn Behav *30*, 330-341.
3. Seugnet, L., Suzuki, Y., Stidd, R., and Shaw, P.J. (2009). Aversive Phototaxic Suppression: evaluation of a short-term memory assay in Drosophila melanogaster. Genes Brain Behav.
4. Shaw, P.J., Tononi, G., Greenspan, R.J., and Robinson, D.F. (2002). Stress response genes protect against lethal effects of sleep deprivation in Drosophila. Nature *417*, 287-291.
5. Seugnet, L., Boero, J., Gottschalk, L., Duntley, S.P., and Shaw, P.J. (2006). Identification of a biomarker for sleep drive in flies and humans. Proc Natl Acad Sci U S A *103*, 19913-19918.