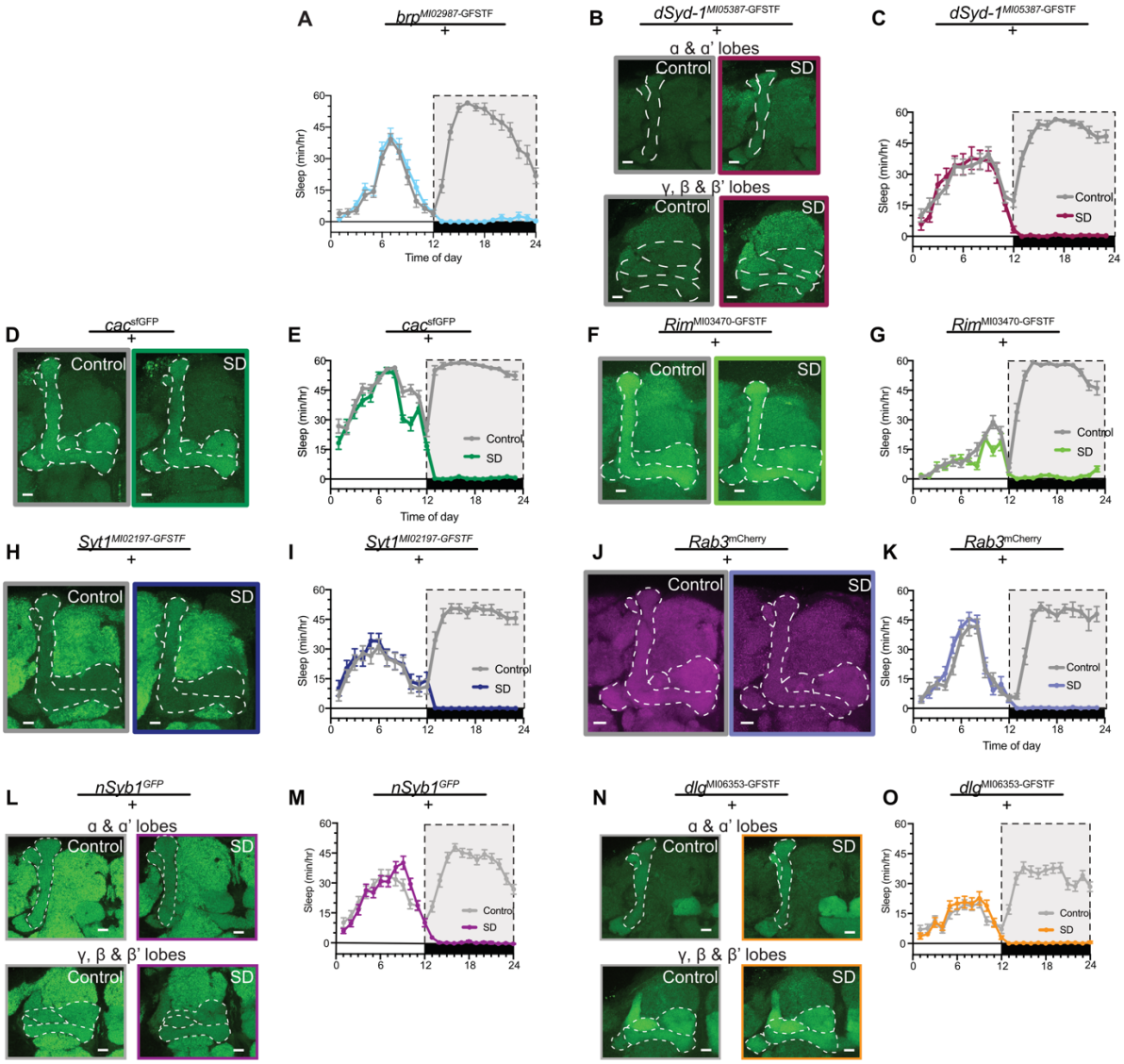

Figure S1 - Representative images and sleep patterns of synaptic protein reporters, related to Figures 1-2

(A) Hourly sleep traces of *brp*$^{MI02987\text{-}GFSTF}$/+ flies. Flies were either allowed ad libitum sleep (gray) or were sleep deprived overnight for 12 hours (blue) before dissection. Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(23,3013)}$=133.5, p<0.0001, n=59 control, 74 SD).

(B-C) Example images of dSyd-1::GFP (A) and sleep traces (B) from both sleep deprived and control *dSyd-1*$^{MI05387\text{-}GFSTF}$/+ flies. Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(23,2553)}$=100.2, p<0.0001, n=64 control, 49 SD).

(D-E) Confocal images of Cac::sfGFP (C) and hourly sleep measurements (D) from *cac*^{sfGFP}/+ flies that were allowed either *ad libitum* sleep or 12-h overnight sleep deprivation before dissection. Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(23,2093)}$=122.2, p<0.0001, n=48 control, 45 SD).

(F-G) Rim::GFP images (E) and hourly sleep traces (F) from sleep deprived and control *Rim*$^{MI03470\text{-}GFSTF}$/+ flies before dissection. Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(23,2392)}$=97.51, p<0.0001, n=58 control, 48 SD).

(H-I) Example images of Syt1::GFP (G) and pre-dissection sleep traces (H) from both sleep deprived and rested control *Syt1*$^{MI02197\text{-}GFSTF}$/+ flies. Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(23,1932)}$=84.32, p<0.0001, n=37 control, 49 SD).

(J-K) Representative Rab3::mCherry images (I) and hourly sleep patterns prior to dissection (J) from *Rab3*^{mCherry}/+ flies. Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(23,1587)}$=76.46, p<0.0001, n=24 control, 28 SD).

(L-M) Representative Nsyb::GFP images (I) and hourly sleep patterns before dissection (M) from *nSyb*^{GFP}/+ flies. Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(23,2070)}$=63.05, p<0.0001, n=44 control, 48 SD).

(N-O) Dlg::GFP images (N) and sleep timecourses before dissection (O) from *dlg*$^{MI06353\text{-}GFSTF}$/+ flies. Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(23,1748)}$=44.06, p<0.0001, n=40 control, 38 SD).

Scale bars depict 10 μm; error bars represent SEM for all panels.

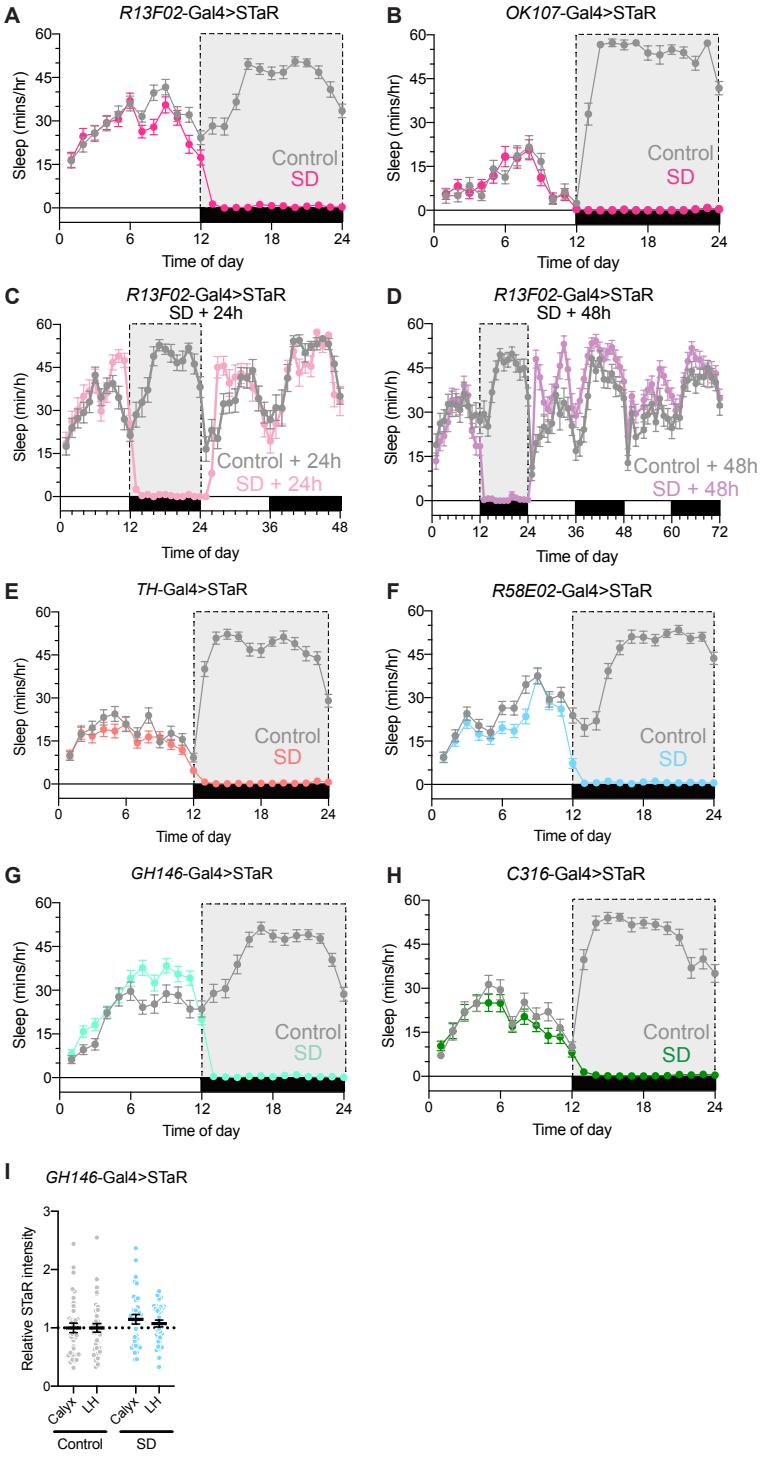

Figure S2 – Sleep patterns of flies expressing STaR reporter in different MB cell types prior to dissection, related to Figure 3

(A) Hourly sleep traces of *R13F02*-Gal4>STaR flies that were allowed ad libitum sleep (gray) or were sleep deprived overnight for 12 hours (magenta). Two-way repeated-measures ANOVA of

hourly sleep time course finds a significant time-by-SD interaction ($F_{(23,1564)}$=54.88, p<0.0001, n=36 control, 34 SD).
(B) Hourly sleep traces of *OK107*-Gal4>STaR flies that were allowed ad libitum sleep (gray) or were sleep deprived overnight for 12 hours (magenta). Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(23,1012)}$=95.18, p<0.0001, n=24 control, 22 SD).
(C) Hourly sleep traces of *R13F02*-Gal4>STaR flies that were allowed ad libitum sleep (gray) or were sleep deprived overnight for 12 hours (pink), and 24 hours of recovery. Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(47,1222)}$=27.94, p<0.0001, n=15 control, 13 SD).
(D) Hourly sleep traces of *R13F02*-Gal4>STaR flies that were allowed ad libitum sleep (gray) or were sleep deprived overnight for 12 hours (pink), and 48 hours of recovery. Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(71,2414)}$=21.46, p<0.0001, n=20 control, 16 SD).
(E) Hourly sleep traces of *TH*-Gal4>STaR flies that were allowed ad libitum sleep (gray) or were sleep deprived overnight for 12 hours (salmon). Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(23,2208)}$=90.49, p<0.0001, n=45 control, 53 SD).
(F) Hourly sleep traces of *R58E02*-Gal4>STaR flies that were allowed ad libitum sleep (gray) or were sleep deprived overnight for 12 hours (blue). Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(23,2070)}$=84.49, p<0.0001, n=44 control, 48 SD).
(G) Hourly sleep traces of *GH146*-Gal4>STaR flies that were allowed ad libitum sleep (gray) or were sleep deprived overnight for 12 hours (light green). Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(23,2093)}$=97.84, p<0.0001, n=42 control, 51 SD).
(H) Hourly sleep traces of *C316*-Gal4>STaR flies that were allowed ad libitum sleep (gray) or were sleep deprived overnight for 12 hours (green). Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(23,1886)}$=68.95, p<0.0001, n=35 control, 49 SD).

(I) Relative *GH146*-Gal4>STaR intensity in olfactory projection neuron axons in the MB calyces (Calyx) and lateral horn (LH). Data from rested controls shown in gray; flies dissected after overnight sleep deprivation depicted in light blue. Two-way repeated measures ANOVA finds no significant main effect for sleep deprivation ($F_{(1,70)}$=1.202, p=0.2767, n=34-38).

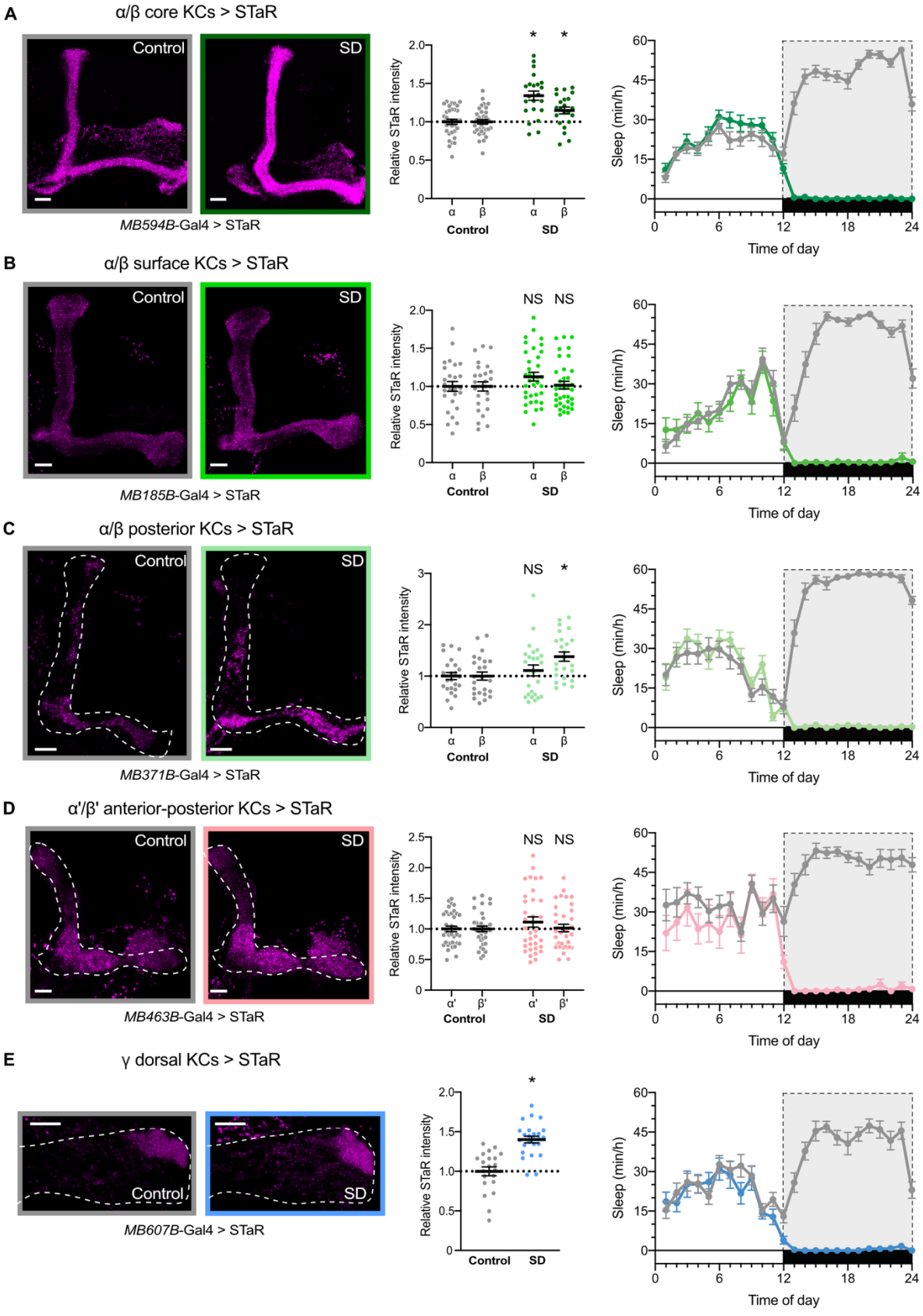
A
α/β core KCs > STaR
Control
SD
MB594B-Gal4 > STaR
Relative STaR intensity
α
β
Control
SD
Sleep (min/h)
Time of day
B
α/β surface KCs > STaR
NS
MB185B-Gal4 > STaR
C
α/β posterior KCs > STaR
MB371B-Gal4 > STaR
D
α'/β' anterior-posterior KCs > STaR
α'
β'
MB463B-Gal4 > STaR
E
γ dorsal KCs > STaR
MB607B-Gal4 > STaR

Figure S3 – Effect of SD on BRP-positive punctae in KC subtypes, related to Figure 3

(A) Representative images (left) and quantification of BRP::V5 intensity (center) from α/β core KCs in *MB594B*>STaR flies after *ad libitum* sleep (gray) or 12 hours of overnight SD (green). Panel on right shows sleep traces for rested and sleep-deprived *MB594*>STaR flies during the 24-h prior to dissection. Two-way ANOVA finds a significant effect of SD on BRP::V5 ($F_{(1,54)}$=21.52, p<0.0001, n=22-34 hemispheres/group).

(B) Representative images (left) and quantification of BRP::V5 intensity (center from α/β surface KCs in *MB185B*>STaR flies after 12 hours of rest (gray) or 12 hours of overnight SD (green). Right hand panel depicts sleep patterns for *MB185B*>STaR flies during the 24-h before dissection. Two-way ANOVA finds no significant effect of SD on BRP::V5 ($F_{(1,60)}$=0.9211, p=0.341, n=26-36 hemispheres/group)

(C) Representative images (left), and quantification of BRP::V5 intensity (center) from α/β posterior KCs *MB371B*>STaR flies after 12 hours of rest (gray) or 12 hours of overnight SD (green). Panel on right shows sleep traces for rested and sleep-deprived *MB371B*>STaR flies during the 24-h prior to dissection. Two-way ANOVA finds a significant effect of SD ($F_{(1,46)}$=4.497, p=0.0394, n=24 hemispheres/group).

(D) Representative images (left) and quantification of BRP::V5 intensity (centerfrom α'/β' anterior-posterior KCs of *MB463B*>STaR flies after 12 hours of rest (gray) or 12 hours of overnight SD (pink). Sleep traces for *MB463B*>STaR from both experimental groups is shown in the right panel. Two-way ANOVA finds no significant effect of SD on BRP::V5 ($F_{(1,68)}$=0.6134, p=0.4362, n=34-36 hemispheres/group).

(G) Representative images (left) and BRP::V5 quantification (center) from γ dorsal neurons of *MB607B*>STaR flies after 12 hours of rest (gray) or 12 hours of overnight SD (blue). Presynapses labelled by STaR (BRP::V5) in magenta. Sleep traces for both experimental groups are shown in right-hand panel. Two-tailed T-test finds a significant effect of SD on BRP::V5 in γ dorsal neurons (t=5.818, p<0.0001, n=21-25 hemispheres/group).

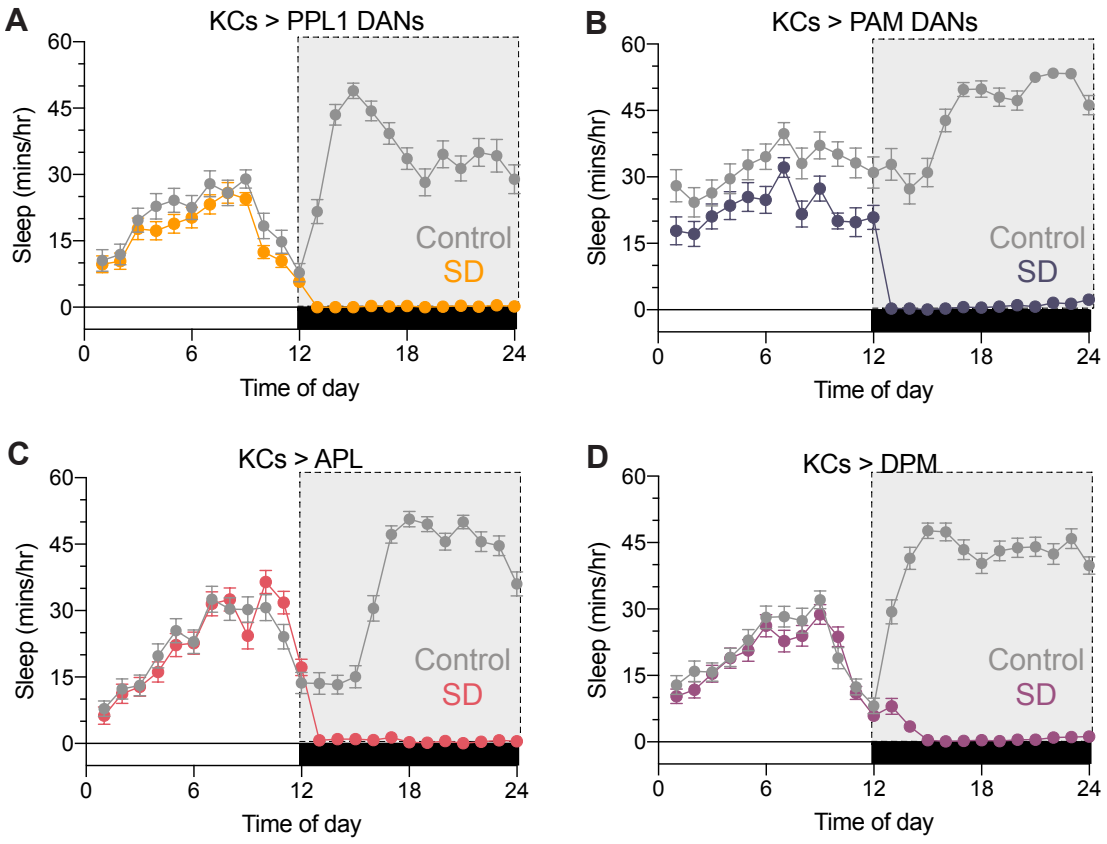

Figure S4 – Sleep patterns of flies expressing GRASP between KCs and non-MBON cell types prior to dissection, related to Figure 4

(A) Hourly sleep traces of GRASP/+; *TH*-Gal4/*MB*-LexA flies that were allowed ad libitum sleep (gray) or were sleep deprived overnight for 12 hours (orange). Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(23,1748)}$=48.89, p<0.0001, n=34 control, 44 SD).

(B) Hourly sleep traces of GRASP/+; *R58E02*-Gal4/*MB*-LexA flies that were allowed ad libitum sleep (light gray) or were sleep deprived overnight for 12 hours (dark gray). Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(23,1978)}$=49.86, p<0.0001, n=42 control, 46 SD).

(C) Hourly sleep traces of *GH146*-Gal4/GRASP; *MB*-LexA/+ flies that were allowed ad libitum sleep (gray) or were sleep deprived overnight for 12 hours (pink). Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(23,2208)}$=69.97, p<0.0001, n=42 control, 56 SD).

(D) Hourly sleep traces of GRASP/+; *C316*-Gal4/*MB*-LexA flies that were allowed ad libitum sleep (light gray) or were sleep deprived overnight for 12 hours (maroon). Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(23,2806)}=77.93$, $p<0.0001$, n=69 control, 55 SD).

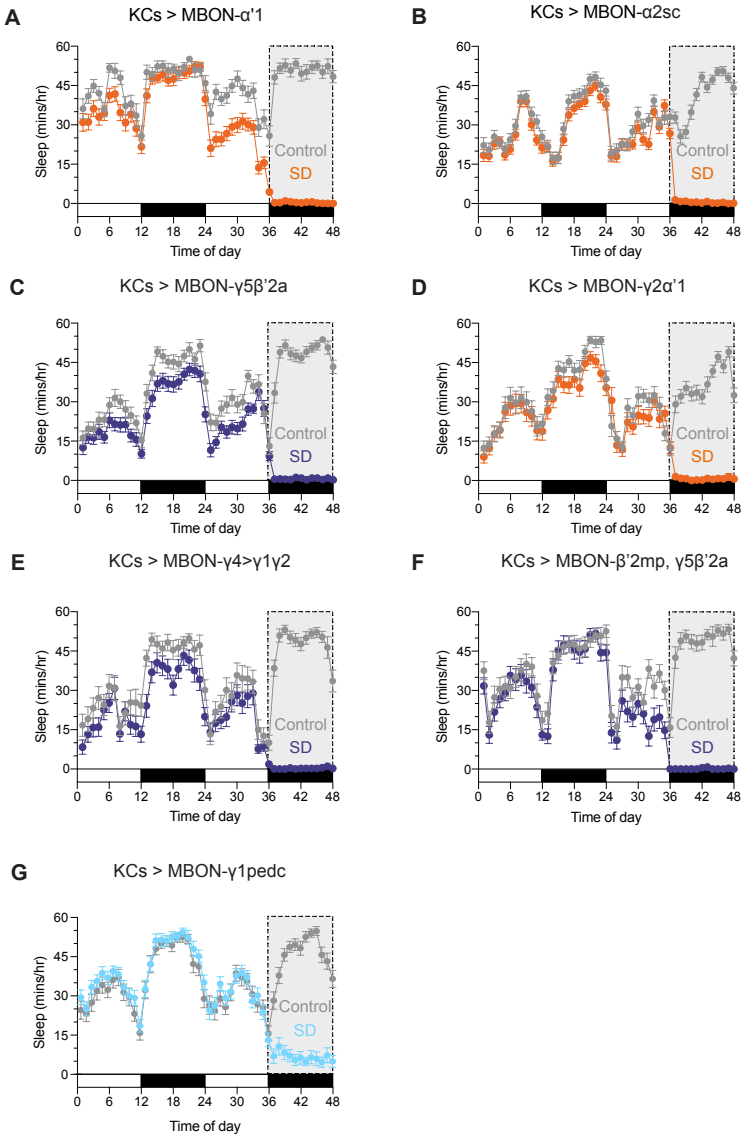

Figure S5 – Sleep patterns of flies expressing KC>MBON GRASP prior to dissection, related to Figure 5

(A) Hourly sleep traces of GRASP/+; *MB543B*-Gal4/*MB*-LexA flies that were allowed 24 hours of baseline sleep, followed by either ad libitum sleep (gray) or sleep deprivation overnight for 12 hours (orange). Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(47,2726)}=36.6$, $p<0.0001$, n=25 control, 35 SD).

(B) Hourly sleep traces of GRASP/+; *R71D08*-Gal4/*MB*-LexA flies that were allowed 24 hours of baseline sleep, followed by either ad libitum sleep (gray) or sleep deprivation overnight for 12

hours (orange). Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(47,4982)}$=42.01, p<0.0001, n=46 control, 64 SD).
(C) Hourly sleep traces of GRASP/+; *R66C08*-Gal4/*MB*-LexA flies that were allowed 24 hours of baseline sleep, followed by either ad libitum sleep (gray) or sleep deprivation overnight for 12 hours (dark blue). Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(47,3008)}$=32.57, p<0.0001, n=26 control, 40 SD).
(D) Hourly sleep traces of GRASP/+; *R25D01*-Gal4/*MB*-LexA flies that were allowed 24 hours of baseline sleep, followed by either ad libitum sleep (gray) or sleep deprivation overnight for 12 hours (orange). Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(47,3572)}$=26.24, p<0.0001, n=37 control, 41 SD).
(E) Hourly sleep traces of GRASP/+; *MB434B*-Gal4/*MB*-LexA flies that were allowed 24 hours of baseline sleep, followed by either ad libitum sleep (gray) or sleep deprivation overnight for 12 hours (dark blue). Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(47,1974)}$=20.94, p<0.0001, n=20 control, 24 SD).
F) Hourly sleep traces of GRASP/+; *MB011B*-Gal4/*MB*-LexA flies that were allowed 24 hours of baseline sleep, followed by either ad libitum sleep (gray) or sleep deprivation overnight for 12 hours (dark blue). Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(47,1598)}$=25.25, p<0.0001, n=17 control, 19 SD).
(G) Hourly sleep traces of GRASP/+; *R12G04*-Gal4/*MB*-LexA flies allowed 24h of baseline sleep followed by either ad libitum sleep (gray) or overnight sleep deprivation for 12h (light blue). Two-way repeated-measures ANOVA of hourly sleep time course finds a significant time-by-SD interaction $F_{(47,2538)}$=34.38, p<0.0001, n=27-29 flies/group).