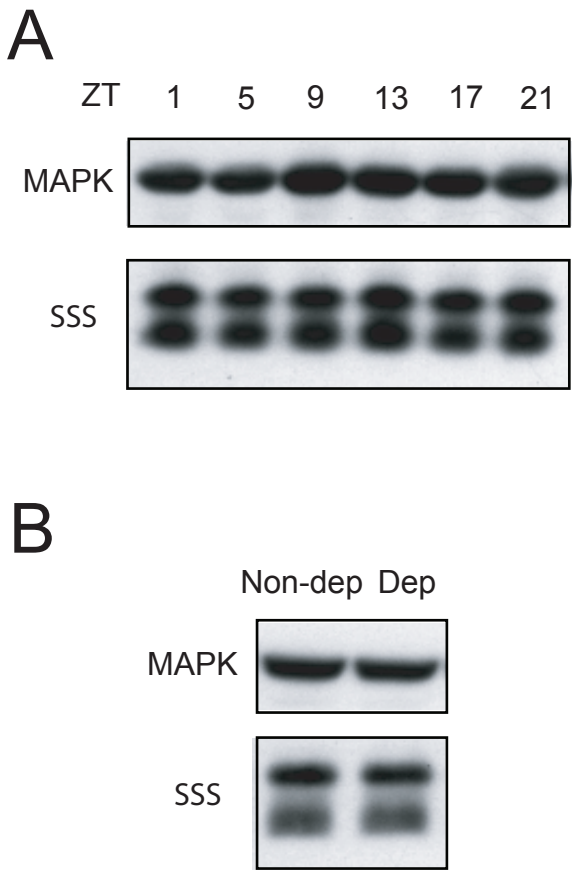

fig. S1. Regulation of SSS protein. (**A**) Circadian profile of SSS protein in head extracts. Wild-type fly heads were collected at indicated Zeitgeber times (ZT), and SSS levels were determined by Western blot analysis. (**B**) SSS protein levels in head extracts do not change in response to sleep deprivation. The SSS protein level of wild-type (*iso31*) flies that were deprived of sleep for 8 hours during ZT 12-20 (Dep) is comparable to that of wild-type flies that were not deprived (Non-dep). Each of these experiments was performed 3 times with similar results.

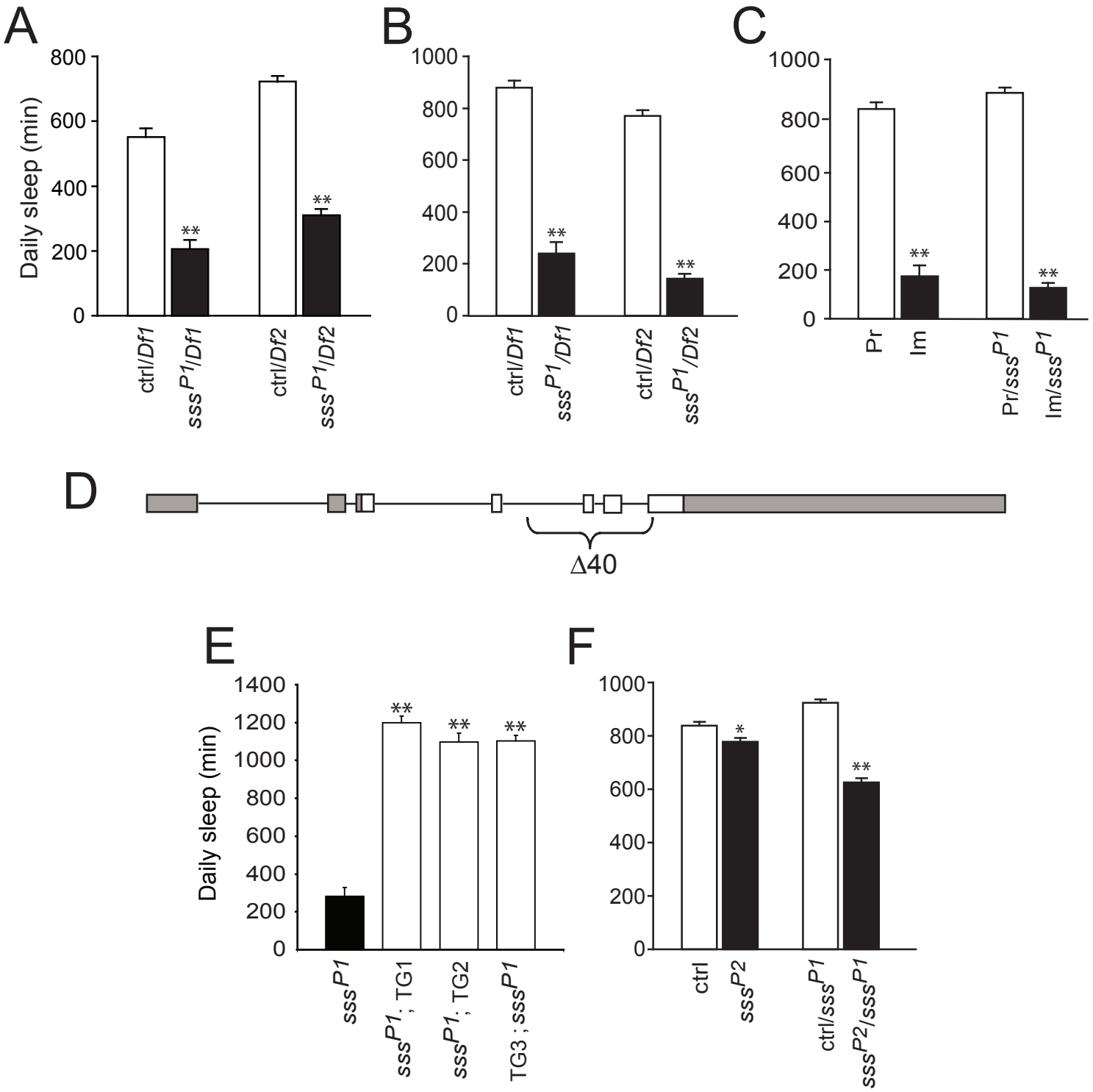

fig. S2. Genetic analysis of *sss* flies. **(A-B)** Deficiency mapping of the *sss* mutation. Daily sleep in minutes is shown for *Df(2R)ED2219* (*Df1*) and *Df(2R)en-B* (*Df2*) flies crossed to either background control (ctrl) or sss^{P1} flies. 21-65 female (A) and 18-42 male (B) flies were tested in each condition. (**C**) Daily sleep amount for precise excision (Pr; n=25), $sss^{\Delta 40}$ imprecise excision (Im, n=18) , precise/sss^{P1} (Pr/ sss^{P1}, n=29), and imprecise/sss^{P1} (Im/sss^{P1}, n=33) male flies. (**D**) Schematic of the *sss* genomic region and the imprecise excision allele, Δ*40*. The bracket indicates bases deleted in the Δ*40* allele. (**E**) Daily sleep amount for male sss^{P1} mutant flies with (sss^{P1};TG1-3, n=16, 8, 15, respectively) or without (sss^{P1}, n=13) a genomic *sss* transgene. TG1-3 refer to three independent transgenic insertions and either 1 or 2 copies of the transgene were present in the flies tested. **(F)** Daily sleep amount for sss^{P2} (n=106) versus background control (ctrl, n=80), as well as ctrl/sss^{P1} (n=79) versus sss^{P2}/ sss^{P1} (n=112) male flies. $^{*}P < 0.05$; $^{**}P < 0.0001$.

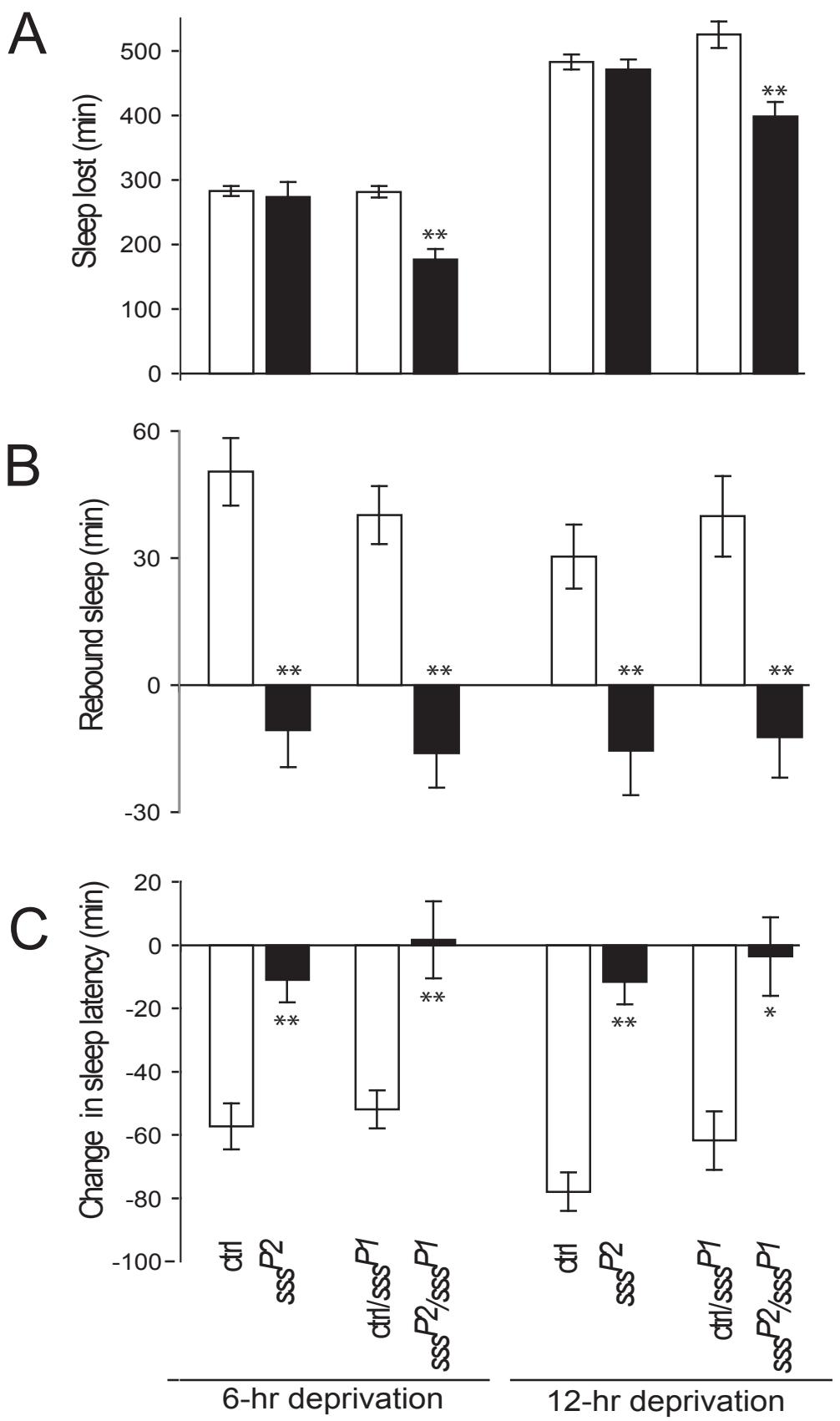

fig. S3. Reduced homeostatic response to sleep deprivation in male *sss* mutants. **(A)** Amount of sleep lost during 6 or 12 hours of deprivation at the end of the dark period for background control (ctrl), sss^{P2}, ctrl/sss^{P1}, and sss^{P2}/sss^{P1} flies. Data from 18-60 male flies are presented. **(B)** Amount of sleep gained during 6 hours of recovery following deprivation as in (A). **(C)** Change in sleep latency following deprivation, compared to undisturbed controls as in (A). $*P < 0.05$; $**P < 0.001$.

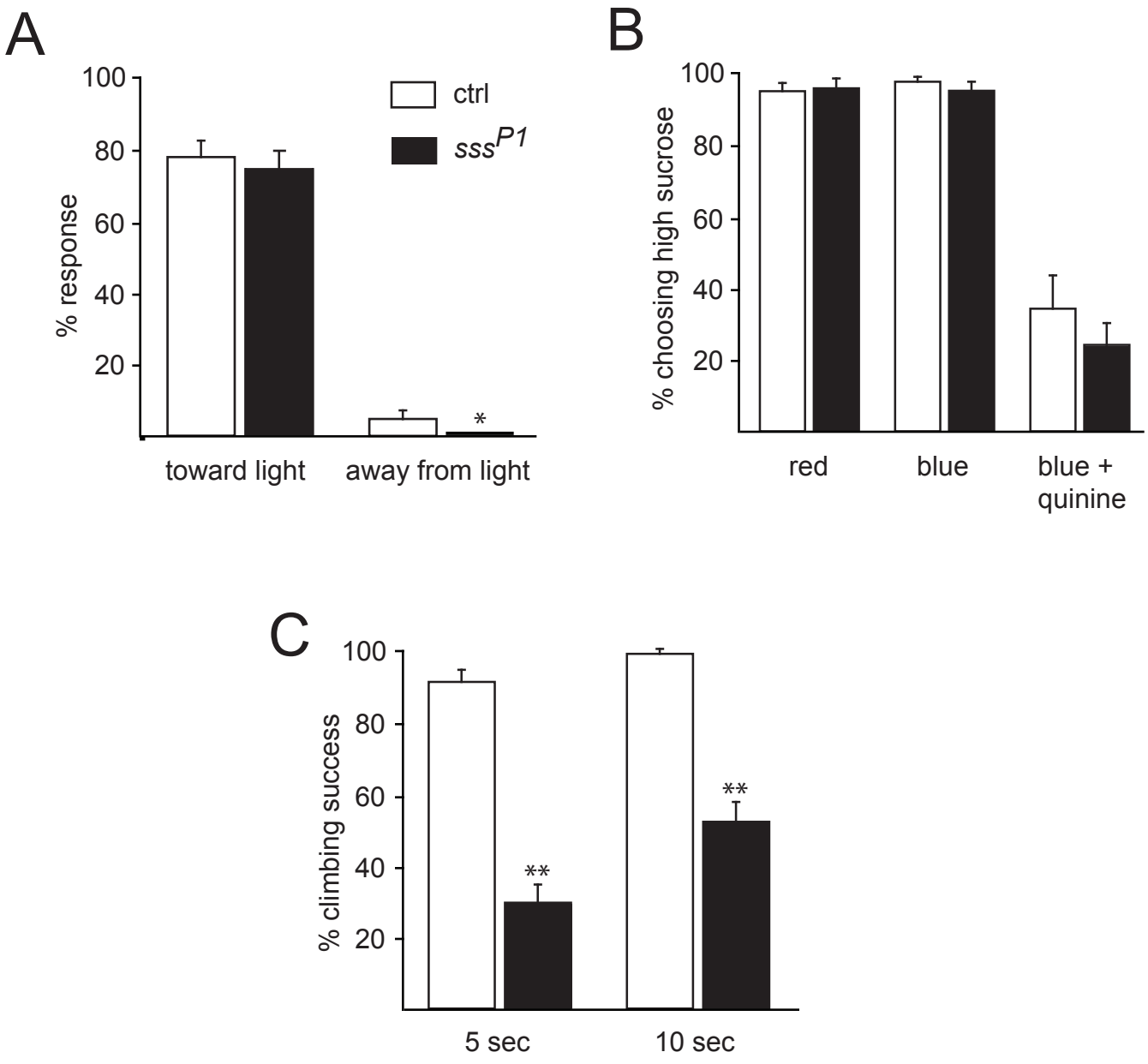

fig. S4. General behavioral assays for *sss* mutants. (**A**) Percent of control and sss^{P1} flies that run toward a distal light source or away from a proximal light source is shown. 79-82 flies were tested in each condition. (**B**) Percent of control and sss^{P1} flies that choose 5 mM over 1 mM sucrose is shown. Addition of food coloring (red or blue) or quinine to the 5 mM sucrose condition is denoted below the results of each of three experiments. No preference is observed for color of food. Control and mutant flies have an equivalent preference for 5 mM over 1 mM sucrose and an equivalent avoidance of 1 mM quinine in the presence of the higher concentration of sugar. 26-95 animals were used in each condition. (**C**) Percent of control and sss^{P1} flies that climb 9 cm in either 5 or 10 seconds is shown. 74-83 animals were tested in each condition. In all three panels, white depicts control and black depicts sss^{P1} animals. $*P < 0.05$; $**P < 0.0001$.

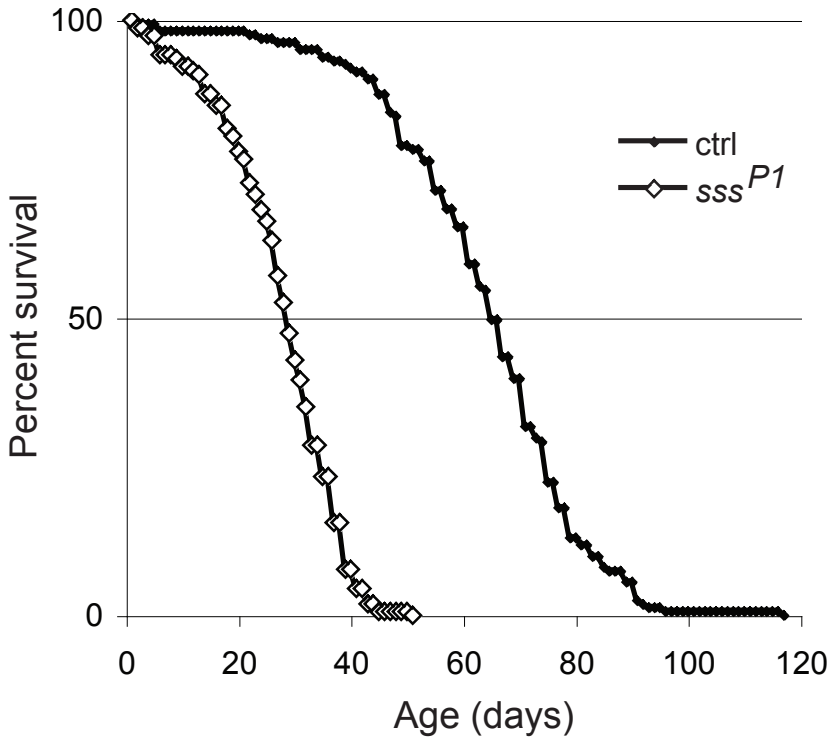

Fig. S5. Survivorship curves of background control (ctrl, closed diamonds) and sss^{P1} (open diamonds) flies. Male sss^{P1} flies (n=154) show a significantly shorter lifespan ($P < 0.0001$) than male control flies (n=161).

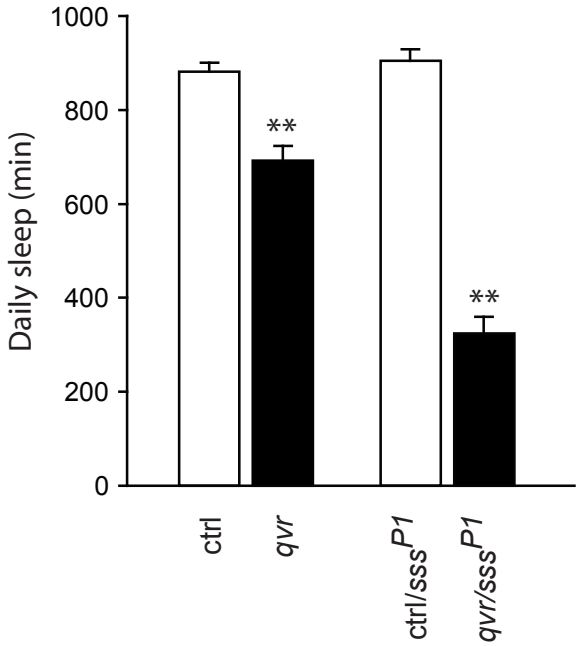

fig. S6. Daily sleep amount for *qvr* (n=32) versus background control (ctrl, n=29), as well as ctrl/sss^{P1} (n=30) versus *qvr*/sss^{P1} (n=31) male flies. $^{**}P < 0.0001$.