Supplementary Materials

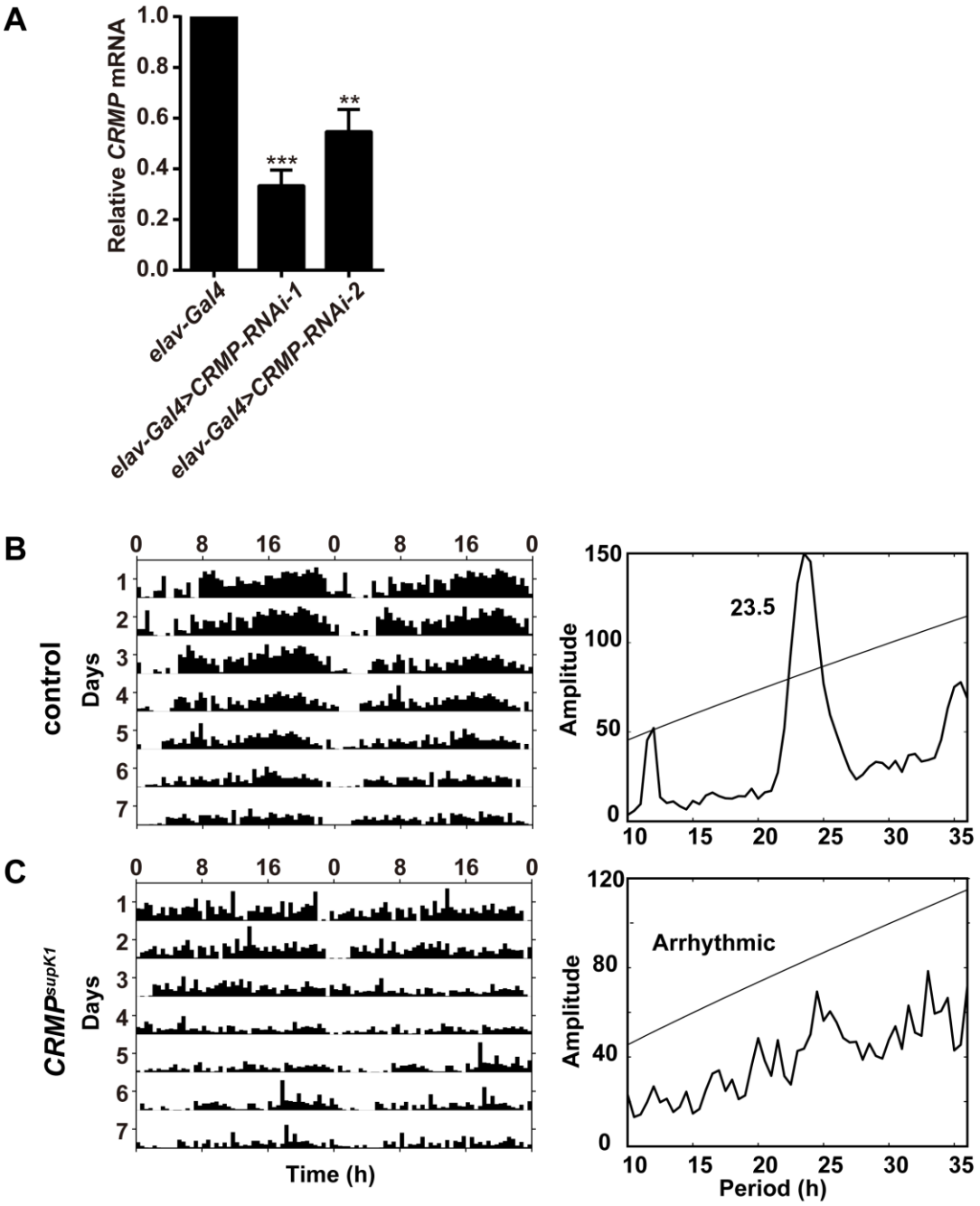

Fig. S1 A The relative *CRMP* mRNA levels in fly heads of *CRMP* RNAi lines driven by *elav-Gal4.* ***P* <0.01, ****P* <0.001, one-way ANOVA. *n* >30 flies per genotype. Data are shown as the mean ± SEM. **B, C** Representative actograms and χ^2 periodograms from control flies (**B**) and *CRMP* mutant flies (**C**) in constant darkness. Control flies display a normal locomotor activity rhythm and *CRMP* mutant flies lose rhythmicity. **D** Relative rhythmic power of genotypes displayed in **B** and **C**. *CRMP* mutant flies show impaired circadian locomotor activity relative to control flies. ***P* <0.01, unpaired *t*-test, *n* >30 flies per genotype. Data are shown as the mean ± SEM.

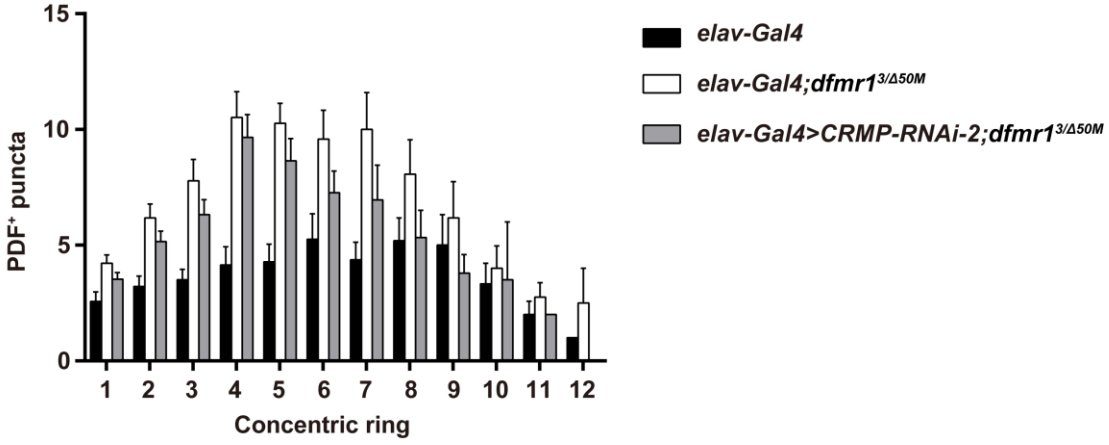

Fig. S2 *CRMP-RNAi-2* rescues structural deficits of sLN_v neurons in *dfmr1* mutants. Sholl analysis of the distribution of PDF-reactive puncta throughout the sLNv axonal arbors. The *CRMP-RNAi-2* line rescues the distribution defect in *dfmr1* mutant flies. *n* >20 hemispheres per genotype.

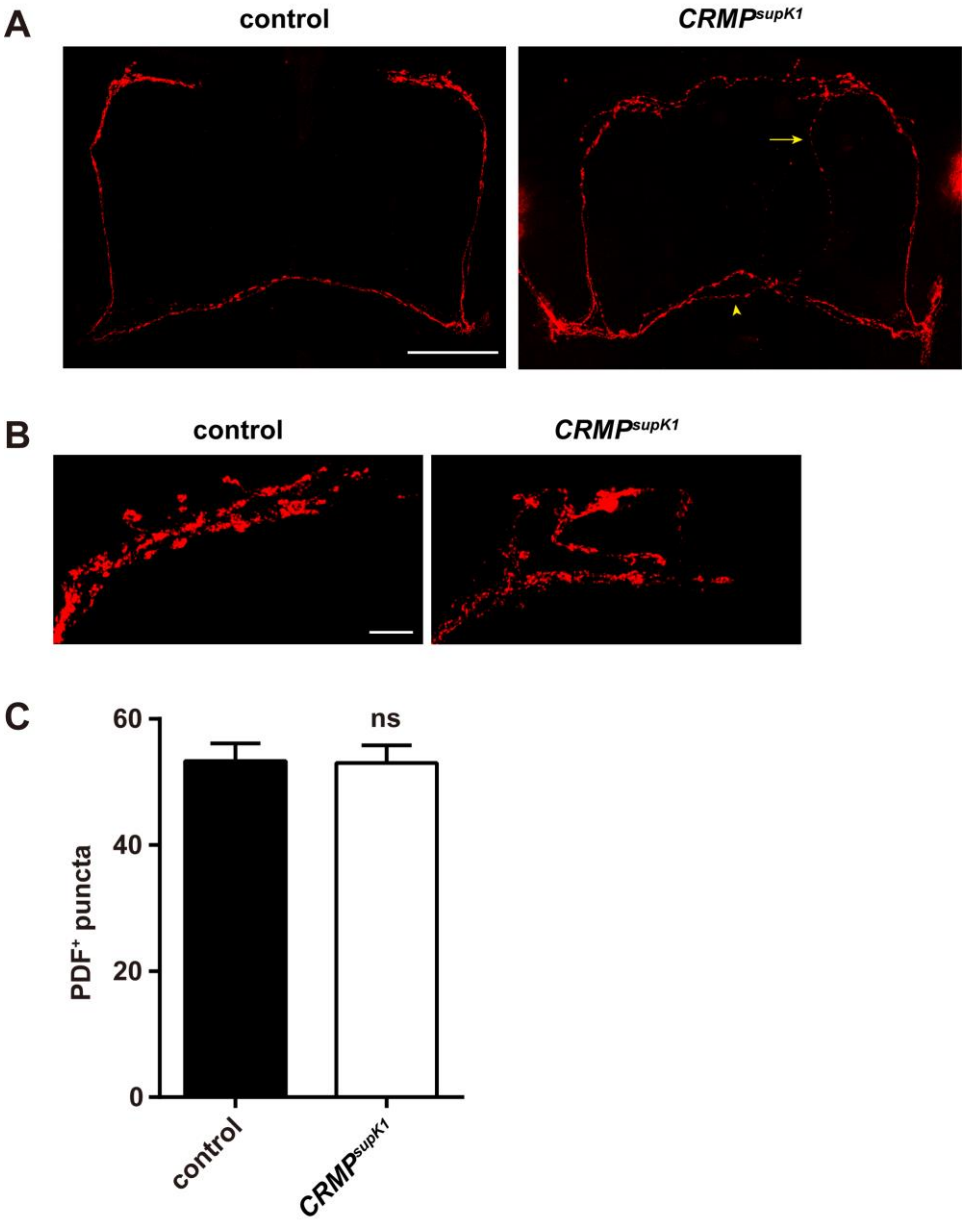

Fig. S3 *CRMP* mutant flies display structural deficits in LN_v neurons. **A** Representative split POT (arrow) and ectopic collateral branches (arrowhead) phenotype in *CRMP* mutant flies (scale bar, 75 μm). **B** *CRMP* mutant flies display normal terminal branch complexity (scale bar, 10 μm). **C** Total number of PDF puncta throughout the sLN_v axonal arbors shows no difference between *CRMP* mutants and control flies. ns, not significant.

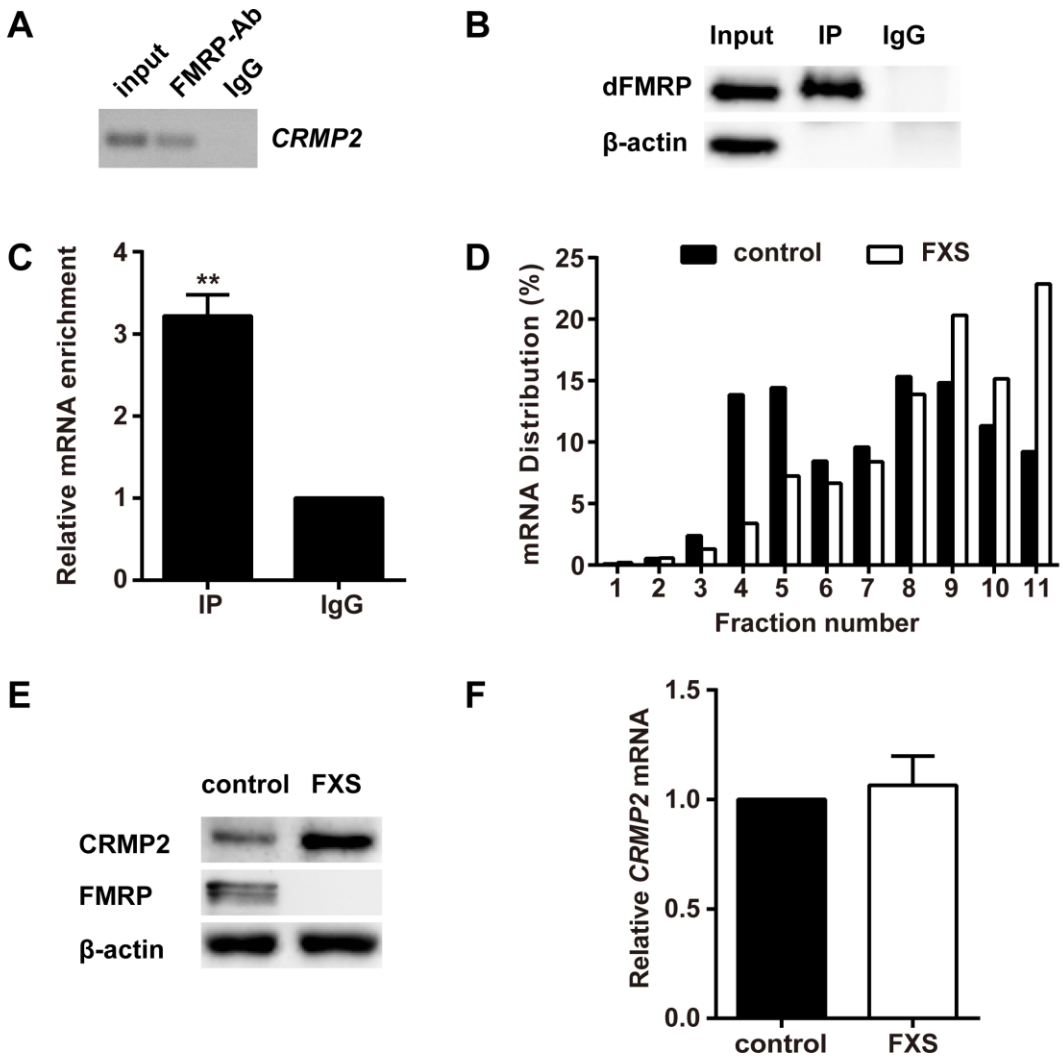

Fig. S4 FMRP interacts with *CRMP* mRNA in *Drosophila* brains and CRMP2 protein levels are elevated in FXS lymphoblastoid cells. **A** Gel electrophoresis showing that *CRMP2* mRNAs are specifically bound to the FMRP antibody but not the negative control IgG in HEK293 cell lysates. **B** Western blots of dFMRP in S2 cells indicate dFMRP immunoprecipitation by anti-dFMRP antibody with specificity, using IgG as a negative control. **C** qPCR analysis of *CRMP* mRNA levels co-precipitated with dFMRP in *Drosophila* brains. *CRMP* mRNAs display an enrichment in dFMRP antibody precipitates compared to the negative control IgG. **D** qRT-PCR quantification of *CRMP2* mRNA levels in each fraction of sucrose gradients of normal and FXS patient-

derived lymphoblastoid cells. In the FXS lymphoblastoid cells there is a clearly enhanced association in the polysomal fractions of *CRMP2* mRNAs in comparison to normal cells. E Western blots from normal and FXS patient lymphoblastoid cells show that CRMP2 protein levels are increased in FXS lymphoblastoid cells. β-actin was used as a loading control. **F** qRT-PCR quantification of *CRMP2* mRNA expression in FXS cells. No significant difference in *CRMP2* mRNA expression between FXS and control cells. *GAPDH* was used as a reference gene. $^{**}P < 0.01$, unpaired *t*-test, $n = 3$ independent experiments. Data are represented as the mean ± SEM.

Table S1 Circadian phenotypes of *CRMP* mutants in constant darkness

Genotype	Number	Average period (h)	% Arrhythmic flies
control	65	24.08 ± 0.07	7.69%
$CRMP^{supK1}$	78	23.79 ± 0.09	39.74%

Table S2 Penetrance of structural defects of LN_vs

Genotype	Number of brains	Collateral branching (percent of total half brains)	POT splitting (percent of total brains)
elav-Gla4	17	0	5.88%
elav-Gal4;dfmr1^{-}	29	12.07%	68.97%
elav-Gal4>CRMP-RNAi-	22	0	27.27%

1;dfmr1^{3}/dfmr1$^{\Delta 50M}$			
elav-Gal4>CRMP-RNAi-2;dfmr1^{3}/dfmr1$^{\Delta 50M}$	24	0	29.17%
elav-Gal4>CRMP-RNAi-1	23	0	17.39%
elav-Gal4>CRMP-RNAi-2	21	0	14.28%
control	15	3.33%	40.00%
CRMP^{supK1}	20	40.00%	80.00%