

Supplemental Materials for

A familial natural short sleep mutation promotes healthy aging and extends lifespan in *Drosophila*

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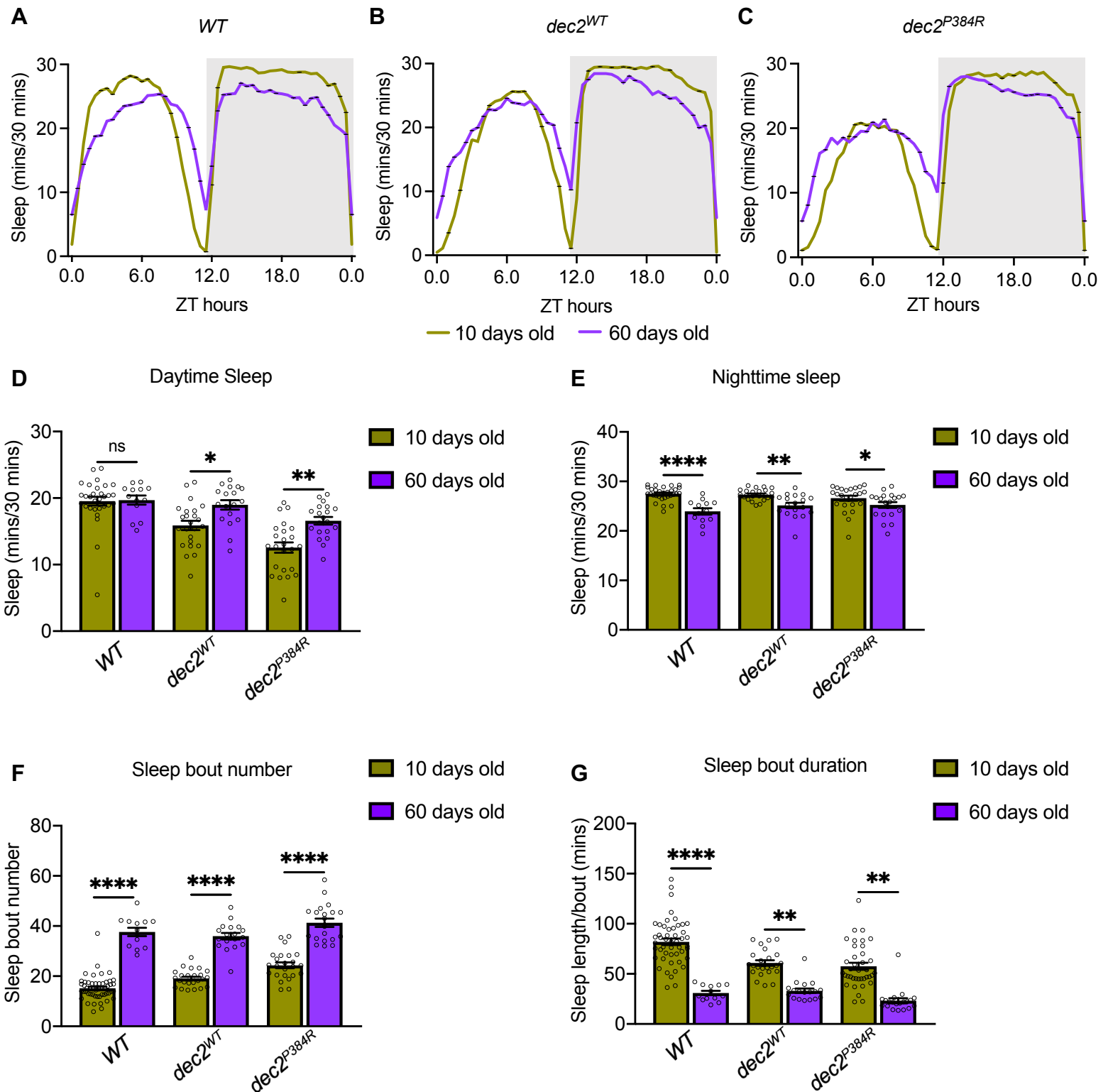
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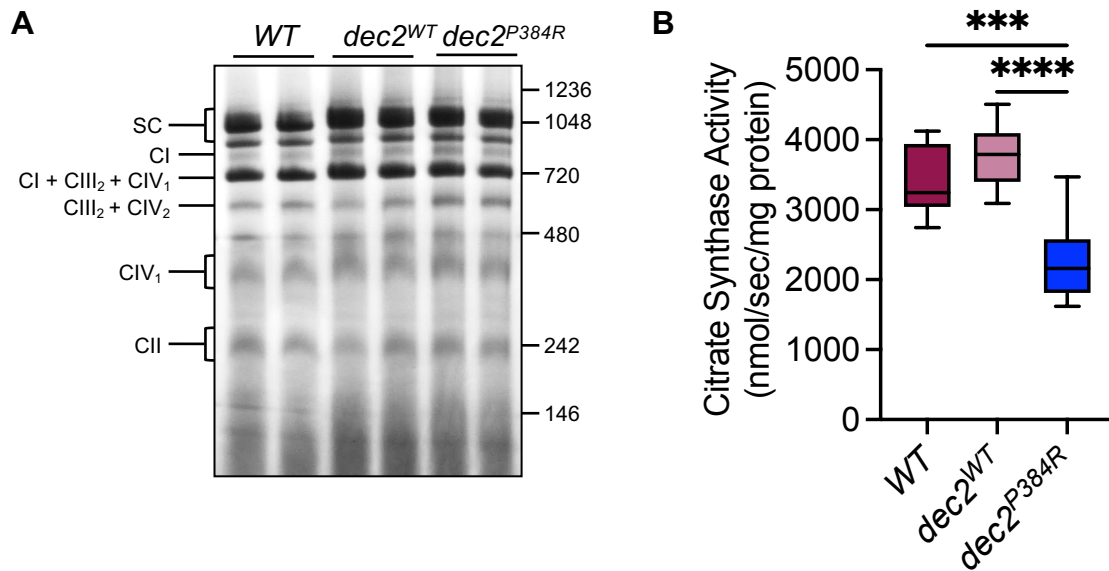
Figs. S1 to S6

Other Supplementary Materials for this manuscript include the following:

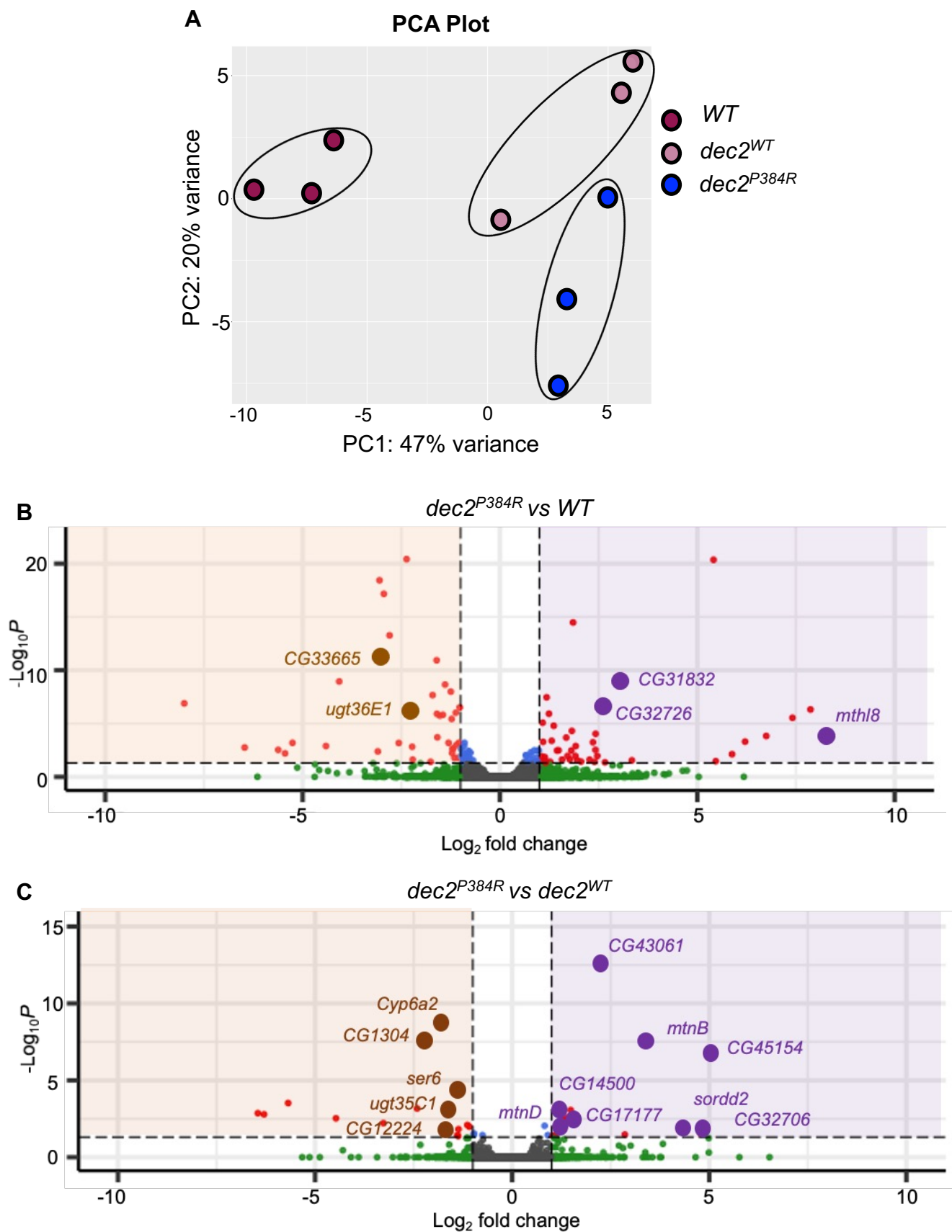
Tables S1 to S4



Supplemental Figure 1: *dec2^{P384R}* mutants exhibit age-dependent changes in sleep. **A-C.** Sleep analysis in 12:12h L:D condition at 10 and 60 days old for *WT* (A), *dec2^{WT}* (B) and *dec2^{P384R}* (C) genotypes. **D-E.** Average daytime (D) and nighttime (E) sleep for the genotypes indicated at 10 days and 60 days. ns=not significant, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$; one-way ANOVA with Tukey's comparisons. **F-G.** Average sleep bout number (F) and sleep length/bout (G) of the genotypes indicated at 10 days and 60 days. ns=not significant, ** $p < 0.01$, **** $p < 0.0001$; one-way ANOVA with Tukey's comparisons. *WT* 10 days ($n=30$) and 60 days ($n=13$); *dec2^{WT}* 10 days ($n=24$) and 60 days ($n=16$); *dec2^{P384R}* 10 days ($n=24$) and 60 days ($n=20$).



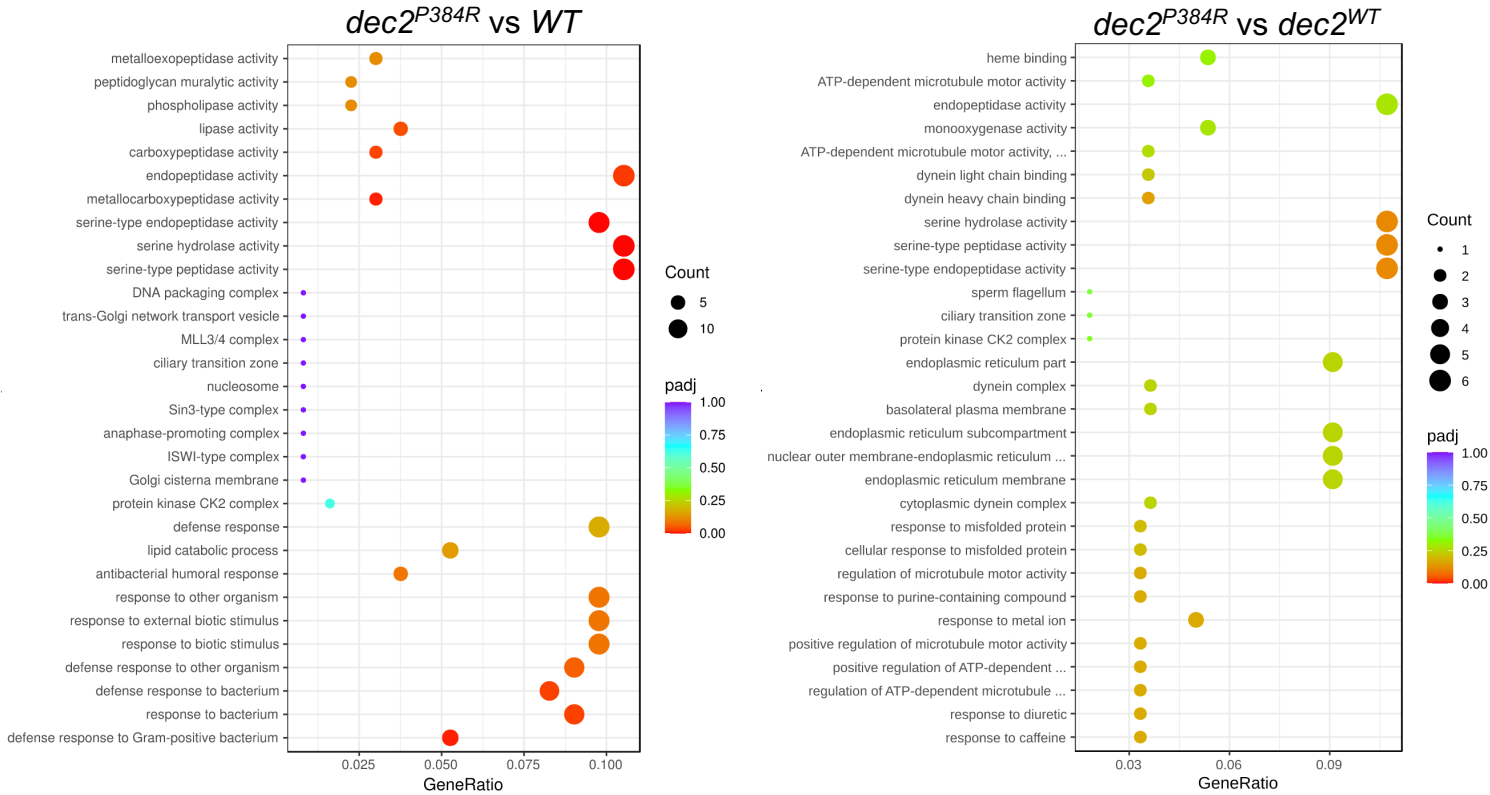
Supplemental Figure 2: Supercomplex formation and citrate synthase activity. A. Representative blue native gel of higher-order organization of the mitochondrial electron transport chain. **B.** Citrate synthase activity as a measure of relative mitochondrial abundance. ns=not significant, *** $p < 0.001$, **** $p < 0.0001$; one-way ANOVA with Tukey's multiple comparisons.



Supplemental Figure 3: Differential gene expression analyses. **A.** Principal Component Analysis (PCA) plot of Illumina-based gene expression among WT, *dec2*^{WT} and *dec2*^{P384R} genotypes (n=3). **B-C.** Volcano plots of DEGs in *dec2*^{P384R} vs. WT (B) and *dec2*^{P384R} vs. *dec2*^{WT} (C) identified in the Nanopore analysis. Significantly down-regulated genes are on negative side (left), significantly up-regulated genes are on positive side (right). Cutoff ranges: log fold changes of -1.5 and +1.5 padj-value of 0.05.

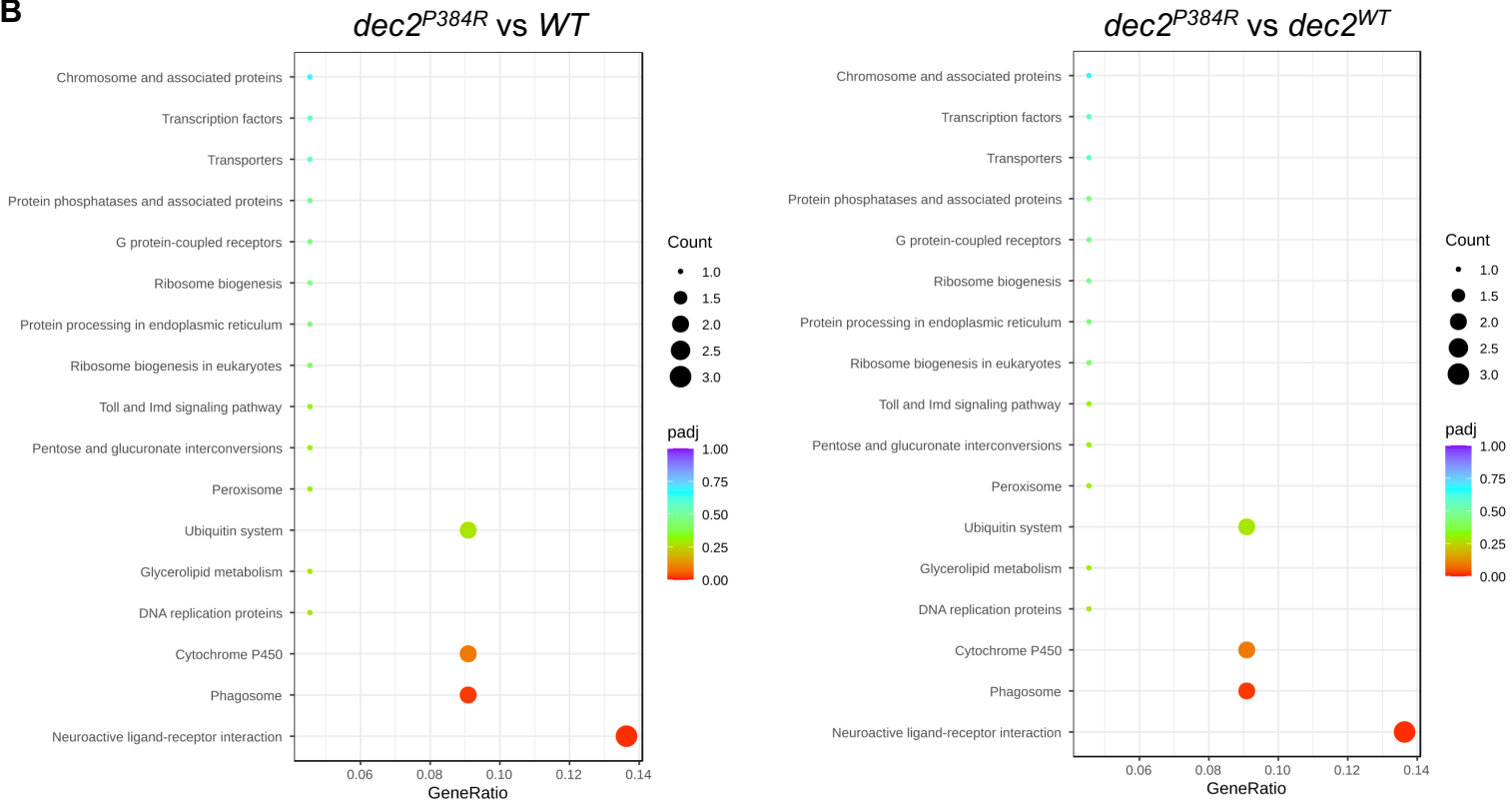
A

GO Enrichment Analysis

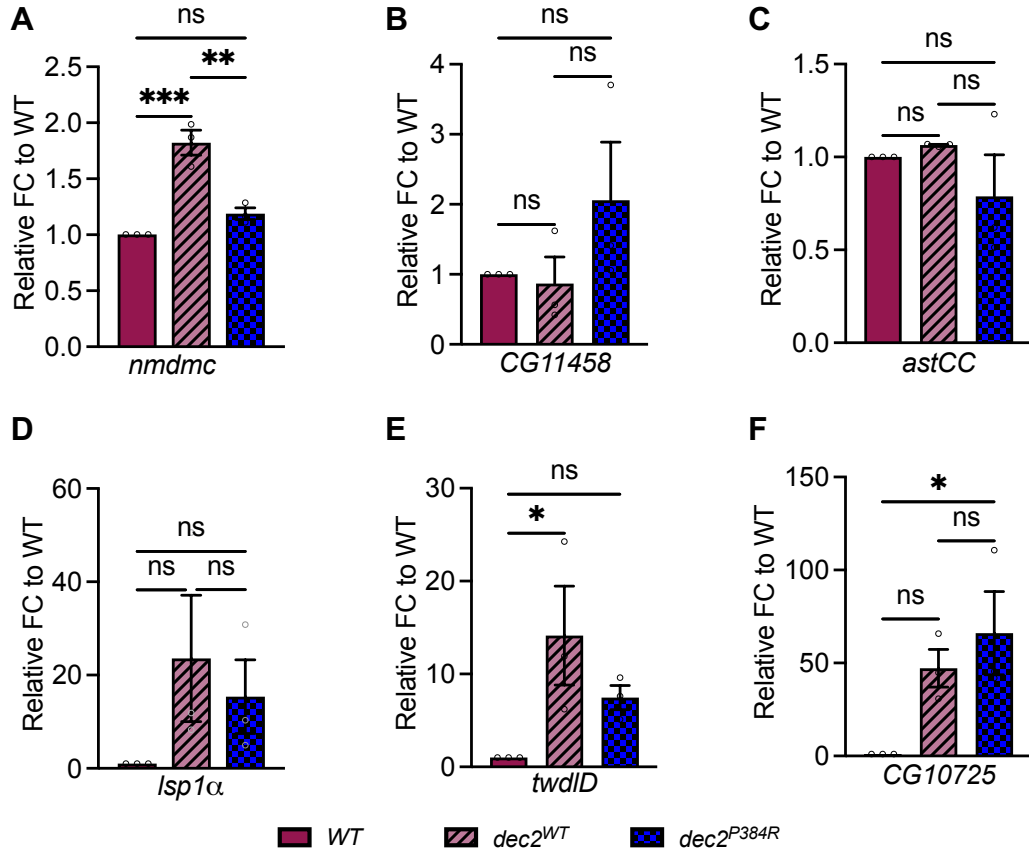


KEGG Pathway Enrichment Analysis

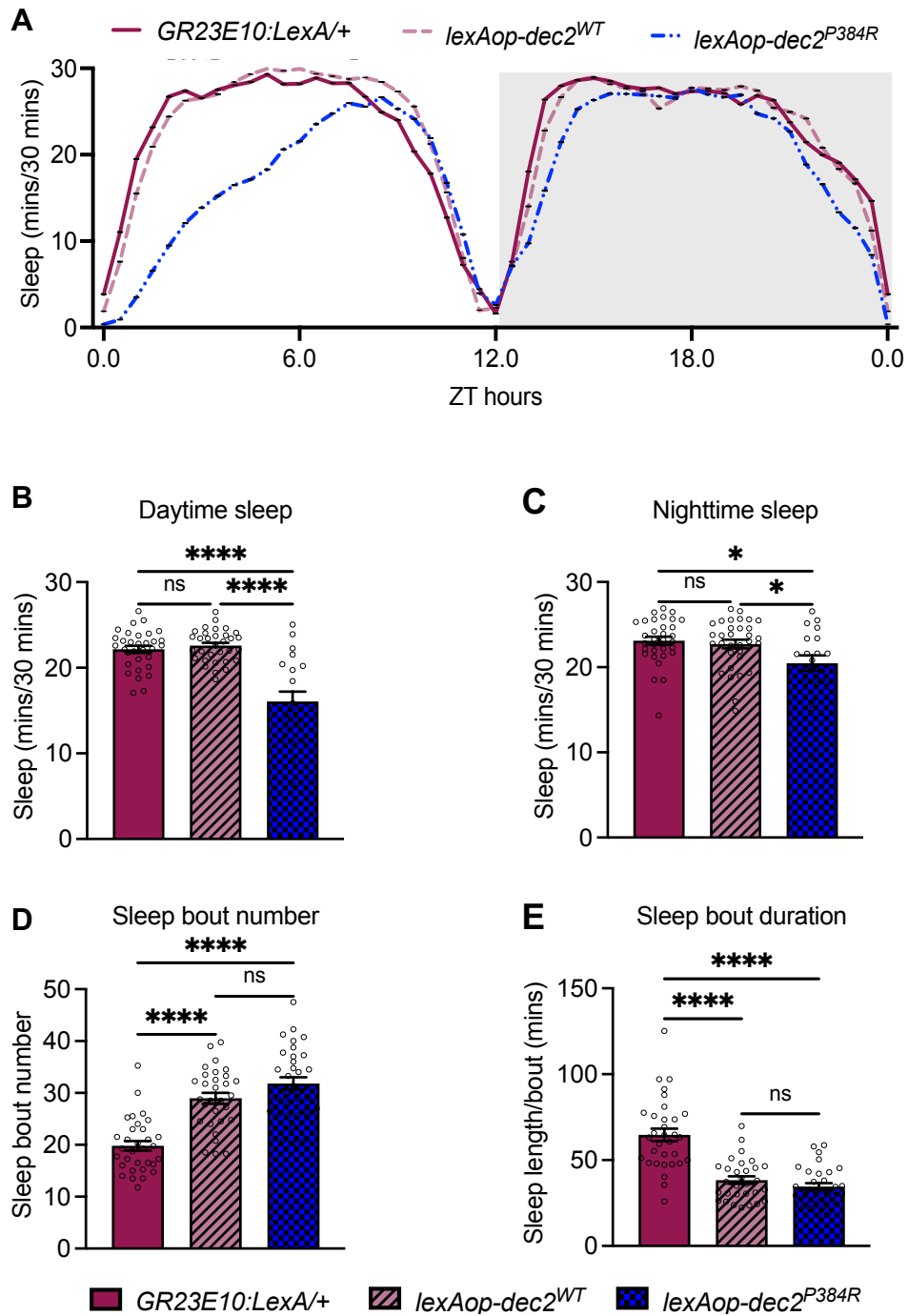
B



Supplemental Figure 4: Enrichment analyses for the Illumina sequencing data. A-B. Gene ontology (GO) enrichment scatter plot (A) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment scatter plot (B) for *dec2^{P384R}* vs *WT* groups and *dec2^{P384R}* vs *dec2^{WT}* groups. GO terms and KEGG pathway with *p*-adj < 0.05 are significantly enriched.



Supplemental Figure 5: qPCR analysis of candidate genes from the RNAseq data. A-F. Expression of *nmdmc* (A), *CG11458* (B), *astCC* (C), *lsp1α* (D), *twdID* (E) and *CG10725* (F) measured by qPCR (n=3). FC=fold change, ns=not significant, *p<0.05, **p<0.01, ***p<0.001; one-way ANOVA with Tukey's comparisons.



Supplemental Figure 6: Sleep analysis of *dec2* transgenes expressed in sleep neurons with the *lexA/lexAop* expression system. A. Sleep analysis in 12:12h L:D condition for the *GR23E10:LexA/+* (n=32), *lexAop-dec2^{WT}* (n=32) and *lexAop-dec2^{P384R}* (n=20). **B-C.** Average sleep during daytime (B) and nighttime (C) of the genotypes indicated. **D-E.** Average sleep bout number (D) and sleep length/bout (E) in the genotypes indicated. ns=not significant, *p<0.05, ****p<0.0001; one-way ANOVA with Tukey's comparisons.