Supplemental Figure S1. Microarray analysis of *Clic4* expression and response to ethanol in PFC of C57BL6/J and DBA/2J mice. Robust multichip analysis (RMA) expression values for *Clic4* probeset ID 94254_at are presented from data published in Kerns et al. (Kerns *et al.*, 2005).

Supplemental Figure S2. Representative time-course of negative geotaxis in the continuous presence of vapor in flies. eRING assays were performed in Control w^{1118} (○), $Clic^{G0472}$/+ (●) and $Clic^{EY04209}$/+ (■) flies. Data from flies exposed to water vapor are shown at 0 (zero) minutes of ethanol exposure. Data from flies exposed to ethanol are shown at 1-11 minutes of ethanol exposure.

Supplemental Figure S3. Ethanol sensitivity in additional studies with *Drosophila Clic* transposon mutants. Ethanol sensitivity (T50 values) from eRING studies in hemizygous males (A, B) and homozygous females (C, D) were reared at 20°C to bypass the lethality associated with strong loss of function in *Clic* and then tested at 25°C using standard conditions. In all panels the *Clic* mutants have higher T50 values than Control w^{1118} flies.

Supplemental Figure S4. Rapid tolerance to ethanol is not affected in *Drosophila Clic* mutants. Rapid ethanol tolerance (calculated as $[(T50_{EE}/T50_{E}\text{-}1) \times 100\%]$ where $T50_E$ and $T50_{EE}$ are determined, respectively, during a first and second exposure to ethanol separated by 4 hours of recovery without ethanol) in $Clic^{G0472}$/+ (A) and $Clic^{EY04209}$/+ (B) was not significantly altered compared to Control w^{1118} flies.

Supplemental Figure S5. Locomotor (climbing) behavior in the absence of ethanol in *Drosophila Clic* mutants. (A, C) Climbing speed was not significantly altered in $Clic^{G04722}$/+ (A) or $Clic^{EY04209}$/+ (C) compared to Control w^{1118} flies. (B, D) Climbing latency (time to initiate

climbing) was significantly shorter in *Clic*^{G04722}/+ mutants compared to Control *w*^{1118} flies, but was not significantly altered in *Clic*^{EY04209}/+ flies.

Supplemental Figure S6. Initial sensitivity to ethanol and locomotor behavior in the absence of ethanol *C. elegans* N2 controls and *Clic* mutants. (A) Data from Figure 5 replotted to show increased relative locomotor speed in *exc-4(rh133)* compared to N2 controls during the first 5 minutes of ethanol exposure. Other mutant genotypes are not shown for clarity. (B) Locomotor speed in the absence of ethanol in N2 control, *exc-4(rh133)*, *exl-1(ok857)* and *exc-4(rh133);exl-1(ok857)*. Data are from 4 independent experiments with 10 animals each per genotype.

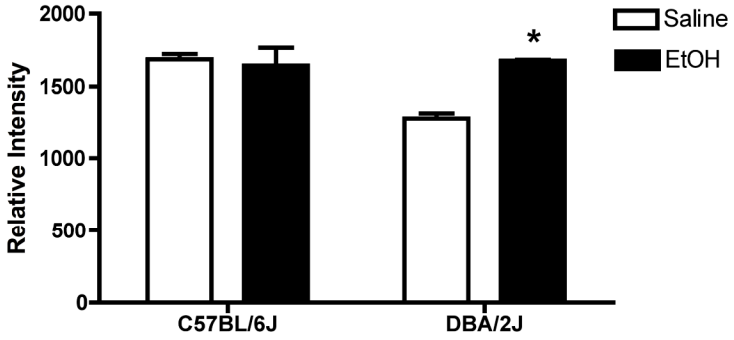
Supplemental Figure S7. Expression of *Clic4* correlates with expression of RNA processing genes in mouse PFC. Microarray expression analysis of PFC from saline-treated BXD mouse strains (Wolen and Miles, unpublished) was used to identify genes having highly significant (FDR < 0.1%) Pearson correlations with *Clic4* expression. Ingenuity Pathway Analysis identified a highly significant over-representation of genes functioning in RNA processing. The network diagram displays the gene-gene interactions for this group of genes as determined by literature association analysis within IPA. Red and green coloration of gene symbol hubs reflect, respectively, positive or negative correlations with *Clic4* expression.

Supplemental Tables

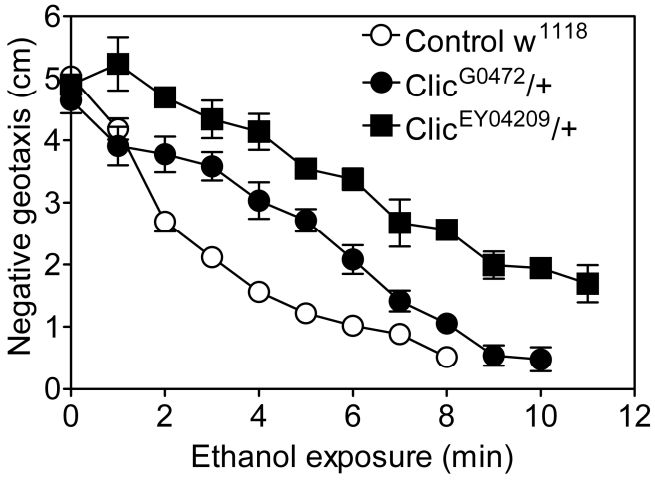
Supplemental Table 1. Gene probesets having significant correlations with *Clic4* expression in PFC of saline treated BXD strains. Data analysis is described in Methods. This gene list was used for functional over-representation within Ingenuity Pathway Analysis (Figure S7).

Supplemental Table 2. ToppGene over-representation analysis of genes with correlated basal expression with Clic4 across the BXD panel of mice in PFC. Genes were selected and analyzed for functional group over-representation as described in Methods.

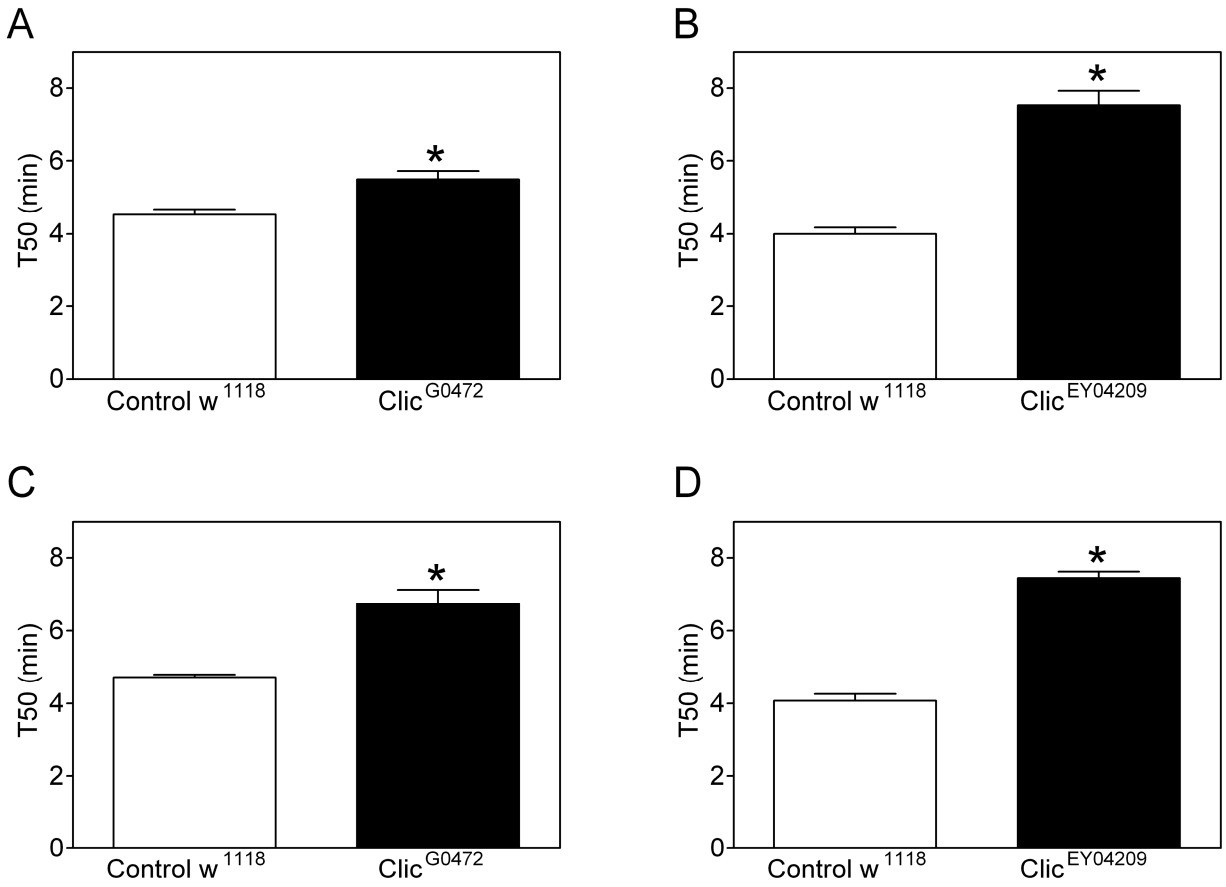
Bhandari Figure S1



Bhandari Figure S2

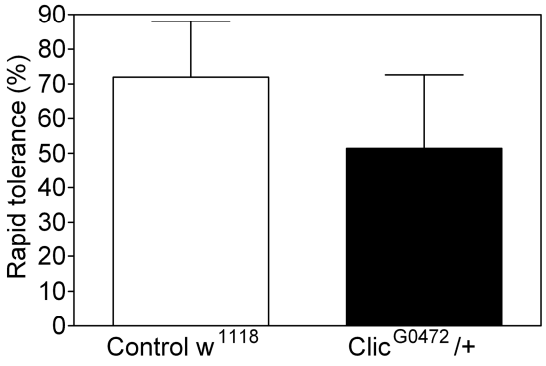


Bhandari Figure S3



Bhandari Figure S4

A



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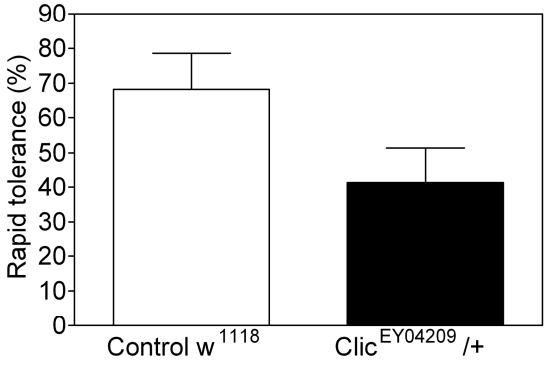
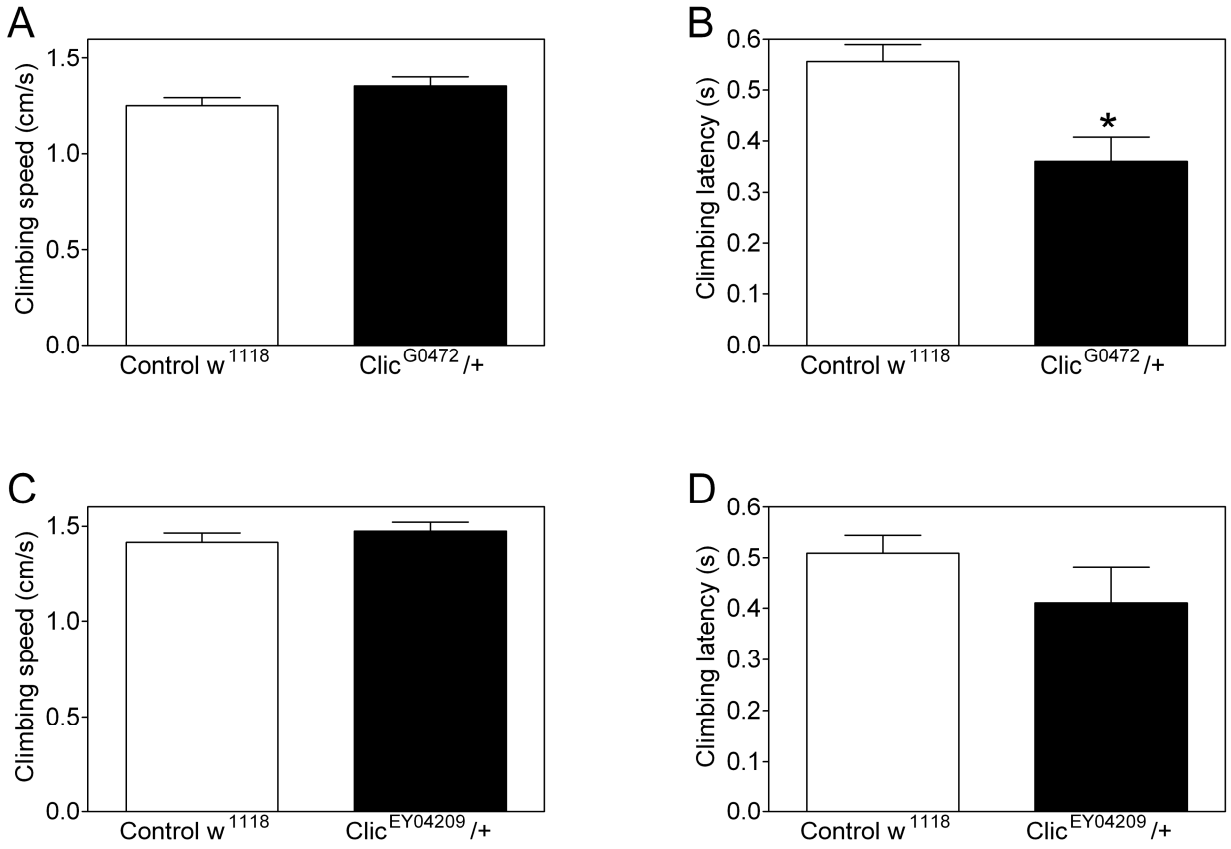
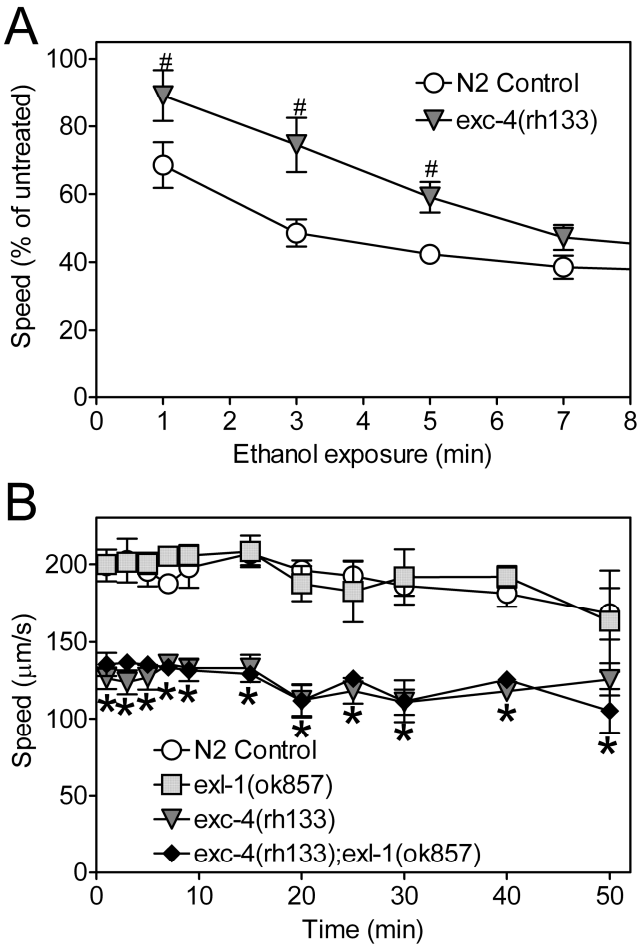


Bhandari Figure S5



Bhandari Figure S6



Bhandari Figure S7