

## Supplemental Information (SI) File

Six supplemental figures (S1-S6) and one supplemental table (S1)

Figure S1. Additional *dNab2-dfmr1* genetic interactions in negative geotaxis. Related to Figure 1.

Figure S2. Additional *dNab2-dfmr1* genetic interactions in mushroom body development. Related to Figure 1.

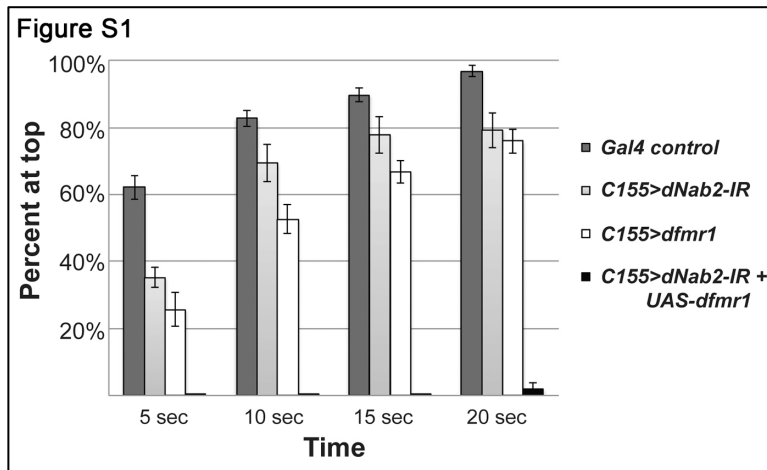
Figure S3. Effect of RNase on the coIP of dNab2 and dFMRP. Related to Figure 3. Figure S4.

Figure S4. Overexpression of dNab2 cannot compensate for the effect of dFMRP loss on the *CaMKII* 3'UTR. Related to Figure 4.

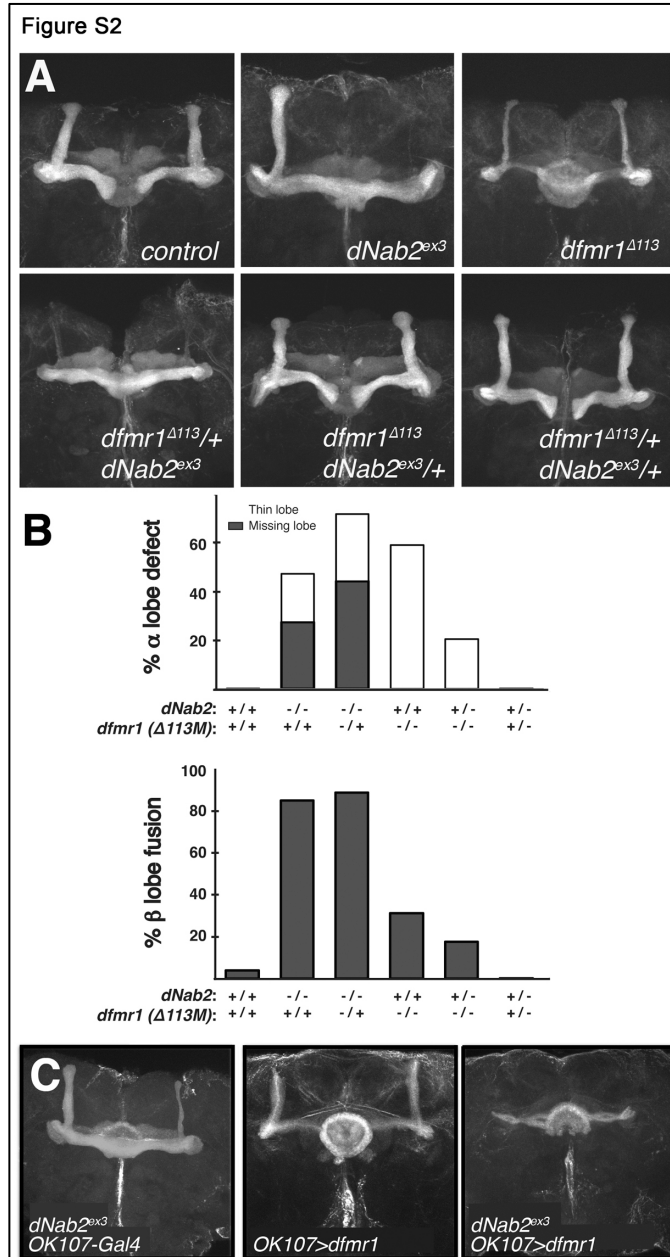
Figure S5. RNA-IP analysis of interaction between Flag-dNab2 and *futsch* mRNA. Related to Figure 5.

Figure S6. Association of cytoplasmic ZC3H14 with EDTA/puromycin-sensitive complexes. Related to Figure 7

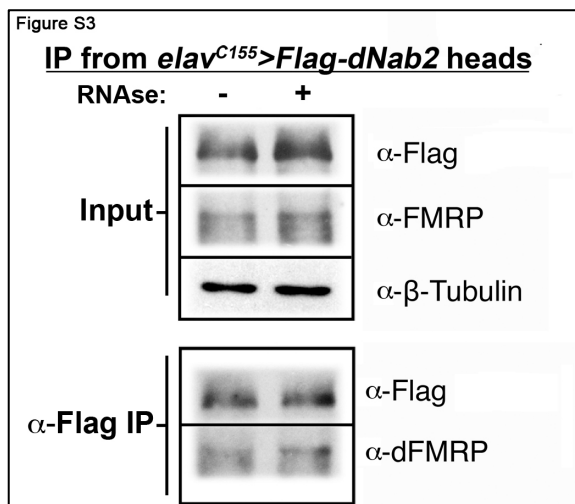
Table S1. Results of the GMR>dNab2 modifier screen. Related to Figure 1.



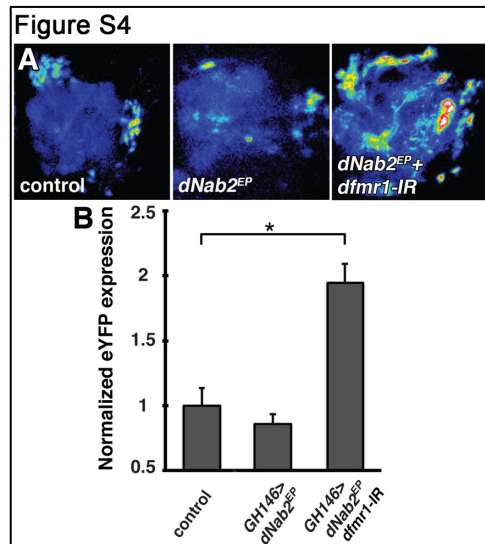
**Figure S1. Additional *dNab2-dfmr1* genetic interactions in negative geotaxis. Related to Figure 1.** Quantitation of negative geotaxis behavior among groups of 5-day old adult “Gal4 control” (*C155-Gal4*), pan-neuronal *dNab2* RNAi (*elavC155-Gal4,UAS-dNab2RNAi*), pan-neuronal overexpression of *dfmr1* (*elavC155-Gal4,UAS-dfmr1*), or *dNab2* and *dfmr1* (*elavC155-Gal4,UAS-dNab2RNAi,UAS-dfmr1*) flies. Data are presented as the percentage flies that reach the top of a cylinder at each time interval. Flies were assayed in groups of 10 and measured in at least 10 independent trials. Error bars = SD.



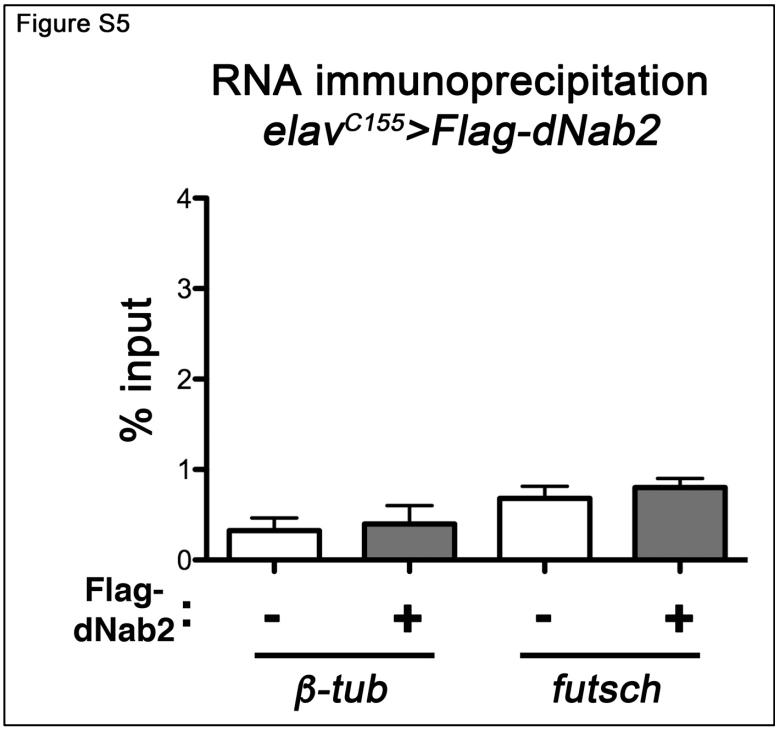
**Figure S2. Additional *dNab2-dfmr1* genetic interactions in mushroom body development. Related to Figure 1.** (A) Anti-Fas2 maximum intensity confocal projections of a *dNab2* wildtype brain (*pex<sup>41</sup>* isogenic control), a *dNab2* null brain (*dNab2<sup>ex3</sup>* homozygotes), a *dfmr1* null brain (*dfmr1<sup>Δ113</sup>* homozygotes), a *dNab2* null brain lacking one copy of *dfmr1* (*ex3,Δ113/ex3,+*), an *dfmr1* null lacking one copy of *dNab2* (*ex3,Δ113/+ ,Δ113*), or a *trans*-heterozygote brain (*ex3, Δ113/+ ,+*). (B) Penetrance of  $\alpha$ -lobe (missing or thinned) or  $\beta$ -lobe fusion defects in the same genotypes as in (A) with individual lobes counted as discrete events. At least 23 brains of each genotype were examined. (C) Anti-Fas2 maximum intensity confocal projections of MBs in *dNab2* nulls brains in the absence (*ex3/ex3,OK107-Gal4*) or presence of MB-specific *dfmr1* overexpression (*ex3/ex3,OK107>dfmr1*). The effect of MB-specific overexpression of *dfmr1* on Fas2+ MB structure is also shown (*OK107>dfmr1*).



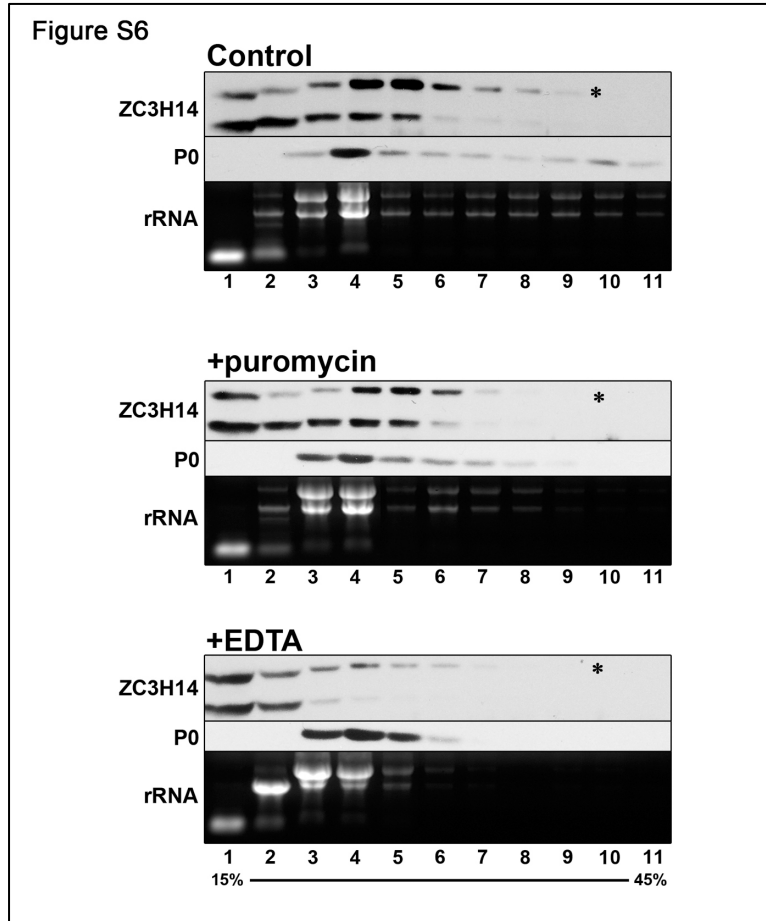
**Figure S3. Effect of RNase on the coIP of dNab2 and dFMRP. Related to Figure 3.** Western blot analysis of input lysates (top panel) and anti-Flag immunoprecipitates (bottom panel) with the indicated antibodies. Lysates were generated from adult heads expressing Flag-tagged dNab2 in neurons (*elav<sup>C155</sup>>UAS-Flag-dNab2*). Prior to IP, lysates were treated for 30 minutes at 37°C in the presence (+) or absence (-) of RNase at a final concentration of 50µg/ml.



**Figure S4. Overexpression of dNab2 cannot compensate for the effect of dFMRP loss on the *CaMKII* 3'UTR. Related to Figure 4. (A)** Confocal images showing expression of the *eYFP:CaMKII-3'UTR* reporter in control ALPNs (left panel; *GH146>eYFP:CaMKII-3'UTR*), in ALPNs that overexpress dNab2 (*GH146>dNab2<sup>EP</sup>*), or in ALPNs that overexpress dNab2 and lack dFMRP (*GH146>dNab2<sup>EP</sup>,dfmr1-IR*). **(B)** Mean eYFP fluorescence values for the indicated genotypes. Data are normalized to the mean fluorescence of control *GH146-Gal4,UAS-eYFP:CaMKII-3'UTR*. Error bars represent SEM (\* $p < 0.05$ ).



**Figure S5. RNA-IP analysis of interaction between Flag-dNab2 and *futsch* mRNA. Related to Figure 5.** Anti-Flag immunoprecipitation from lysates of control *elav<sup>C155</sup>>+* heads (-) or *elav<sup>C155</sup>>Flag-dNab2* heads (+) followed by qPCR to detect co-precipitated *β-tubulin* (*β-tub*) and *futsch* transcripts. The amount of RNA recovered (as a % of input) is indicated. Error bars represent SEM.



**Figure S6. Association of cytoplasmic ZC3H14 with EDTA/puromycin-sensitive complexes. Related to Figure 7.** Linear 15-45% sucrose gradient of cytoplasmic extracts of N2a cells prepared in the absence or presence of EDTA or puromycin, and immunoblotted with the indicated antibodies. Accompanying agarose gels shows the distribution of ribosomal RNA (rRNA) in each gradient. Note the complete loss of rRNA in fractions 7-11 in the presence of EDTA, and the more mild loss of rRNA from fractions 9-11 in the presence of puromycin.