Supplementary Table 1:

mutated *gypsy* "TRAP"	2-4-day	~14-day	~21-day	28-35-day
transformant line #1	0/15[(1)]	0/4	0/9	0/11
transformant line #2	0/8	0/2	-	0/3
gypsy "TRAP"	2-4-day	~14-day	~21-day	28-35-day
transformant line #1	0/15	0/1	-	4/16 [(2)]
transformant line #2	0/11	2/3 [(1)]	1/6	10/23

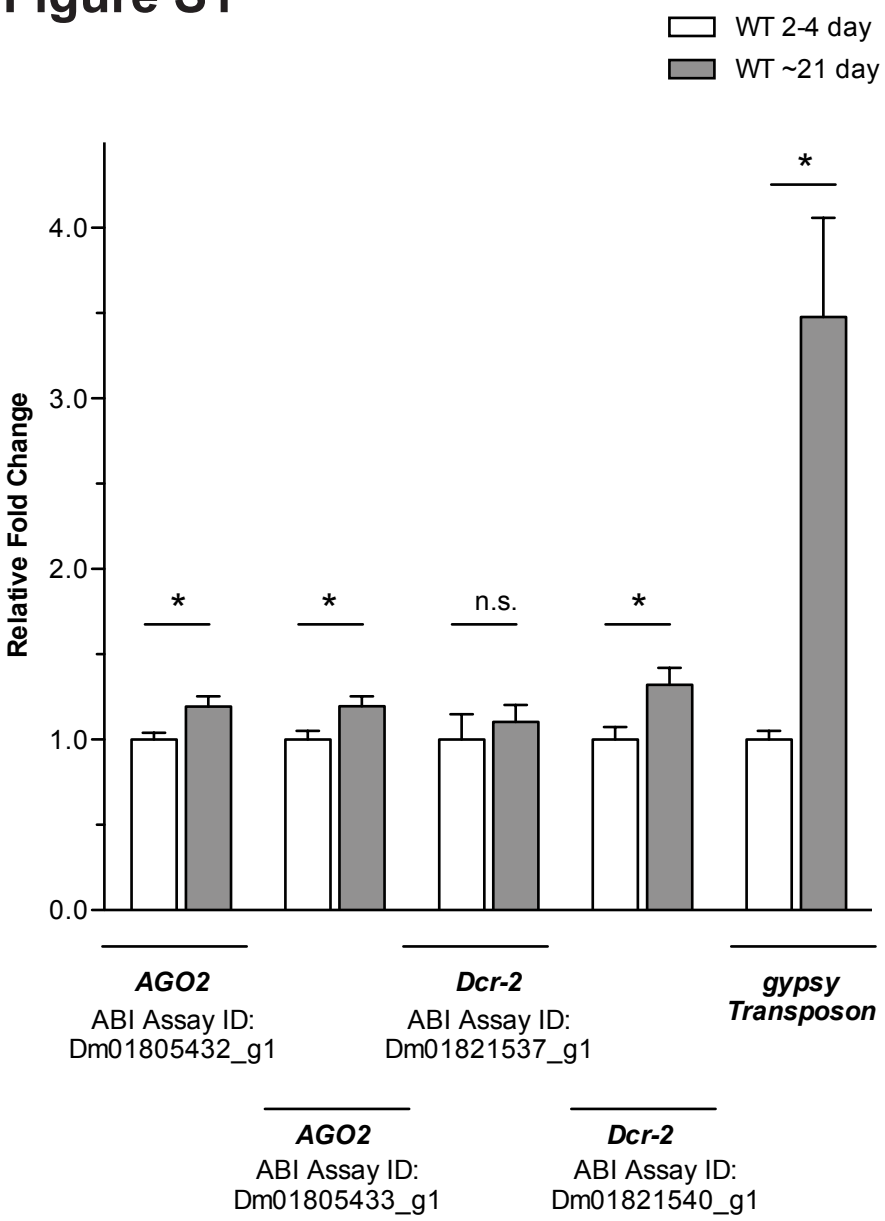
Supplementary Table 1: Mutated *"gypsy-TRAP"* transgenic lines with mutated Ovo binding sites or "*gypsy-TRAP"* transgensic lines with intact Ovo binding sites were crossed with *UAS::mCD8::GFP; MB247* animals. Brains were dissected and imaged from resulting progeny that inherited the "*gypsy-TRAP*" (or mutated "*TRAP*"), *MB247-GAL4* and *UAS::mCD8::GFP* transgenes. Number of brains with GFP-labeled Kenyon cell neurons/total number of brains imaged is shown for 2-4day, ~14 day, ~21 day and ~28 day time points (Chi-square analysis was used to compare the incidents in which GFP labeled brains are identified in 28-35-day old animals in "*TRAP*" vs. mutated transformant lines, $p<0.01$ and to compare incidents in which GFP labeled brains were identified in 2-4 day versus 28-35 day "*TRAP"* lines, $p<0.001$). [(1), (1), (2)] The indicated number (in the parentheses) of brains showed GFP labeling in a large number of MB neurons with dendrites restricted to one quadrant of the calyx, consistent with the loss of Gal80 expression during neurogenesis in one of the 4 neuroblasts giving rise to the MB structure. Such brains were not included in the analysis.

Supplementary Table 2:

Genotype	Olfactory Acuity			Shock Reactivity
	4-Methylcyclohexanol ($1.0X10^{-3}$ v/v)	Benzaldehyde ($0.5X10^{-3}$ v/v)	3-Octanol ($1.5X10^{-3}$ v/v)	60-volts
WT	70.48 ± 19.81	51.33 ± 24.31	66.88 ± 16.65	65.48 ± 5.16
dAgo2^{414}	65.60 ± 10.38	58.07 ± 14.27	64.98 ± 18.44	69.04 ± 5.73
dAgo2^{51B}	70.09 ± 15.45	62.98 ± 7.31	71.67 ± 10.63	66.80 ± 5.52

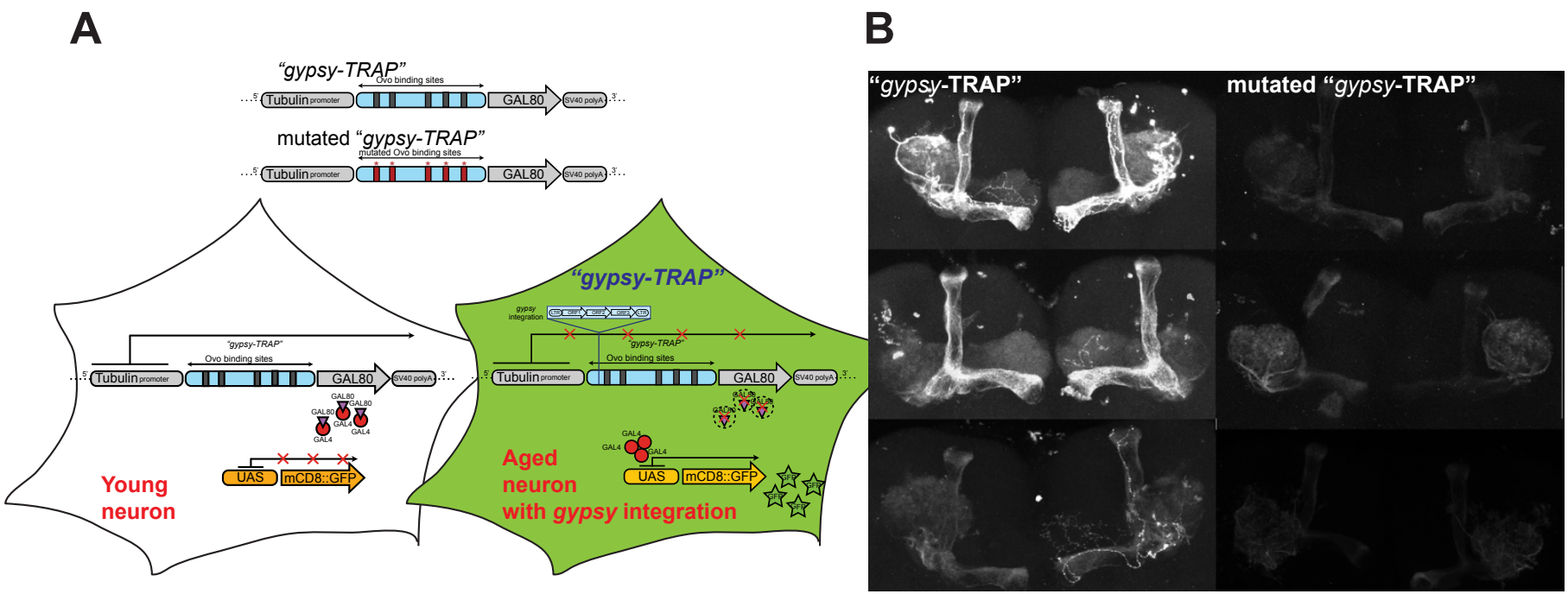
Supplementary Table 2: Olfactory acuity was quantified by exposing naïve flies to each odor versus air in the T-maze (*i.e.* odor from the left and air from the right). After 2 minutes, the number of flies in each arm of the T-maze was counted. Subsequently the directions of odor and air were alternated (*i.e.* odor from the right and air from the left) and another group of naïve flies were tested. A half PI was calculated by dividing the number of flies that chose air, minus the flies that chose the odor by the total number of flies in the experiment. The final avoidance index was calculated by averaging both reciprocal half PIs. Odor concentrations test for olfactory acuity were: 3-Octanol ($1.5X10^{-3}$ v/v), 4-Methylcyclohexanol ($1X10^{-3}$ v/v), Benzaldehyde ($0.5X10^{-3}$ v/v). Pure odors were purchased from Sigma and delivered as the stated concentrations with air flow at 750ml/min. (Group N=6 for each genotype, no differences was found between genotypes.) Shock reactivity was quantified by exposing naïve flies to two electrifiable grids with air flow at 750ml/min in the T-maze, while delivering a 60-volts electric shock to one of the grids. Flies were also allowed for choosing for 2 minutes. An avoidance index was calculated by dividing the number of flies that chose to avoid the shock, minus the flies that chose the shock by the total number of flies in the experiment. The orientation of delivery of electric shock was also alternated between different trials. (N=8 for each genotype, no differences was found between genotypes.)

Figure S1



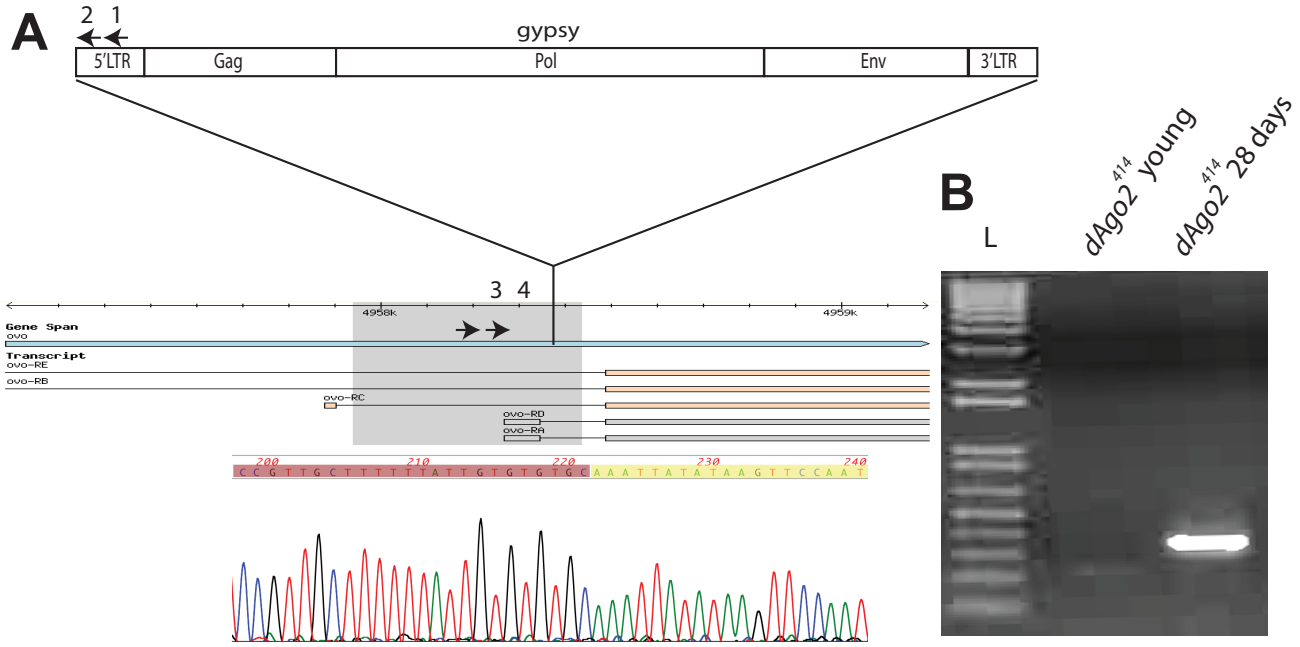
Supplementary Figure S1: Expression levels of *dAGO2* and *Dcr-2* in fly heads from both 2-4-day old young animals and ~21-day old aged animals were tested by QPCR. A moderate increase (*, $p<0.05$) of *dAGO2* expression was observed with two independent *dAGO2* Taqman assays tested. A moderate increase (*, $p<0.05$) of *Dcr-2* expression was observed with one of two independent *Dcr-2* Taqman assays tested.

Figure S2



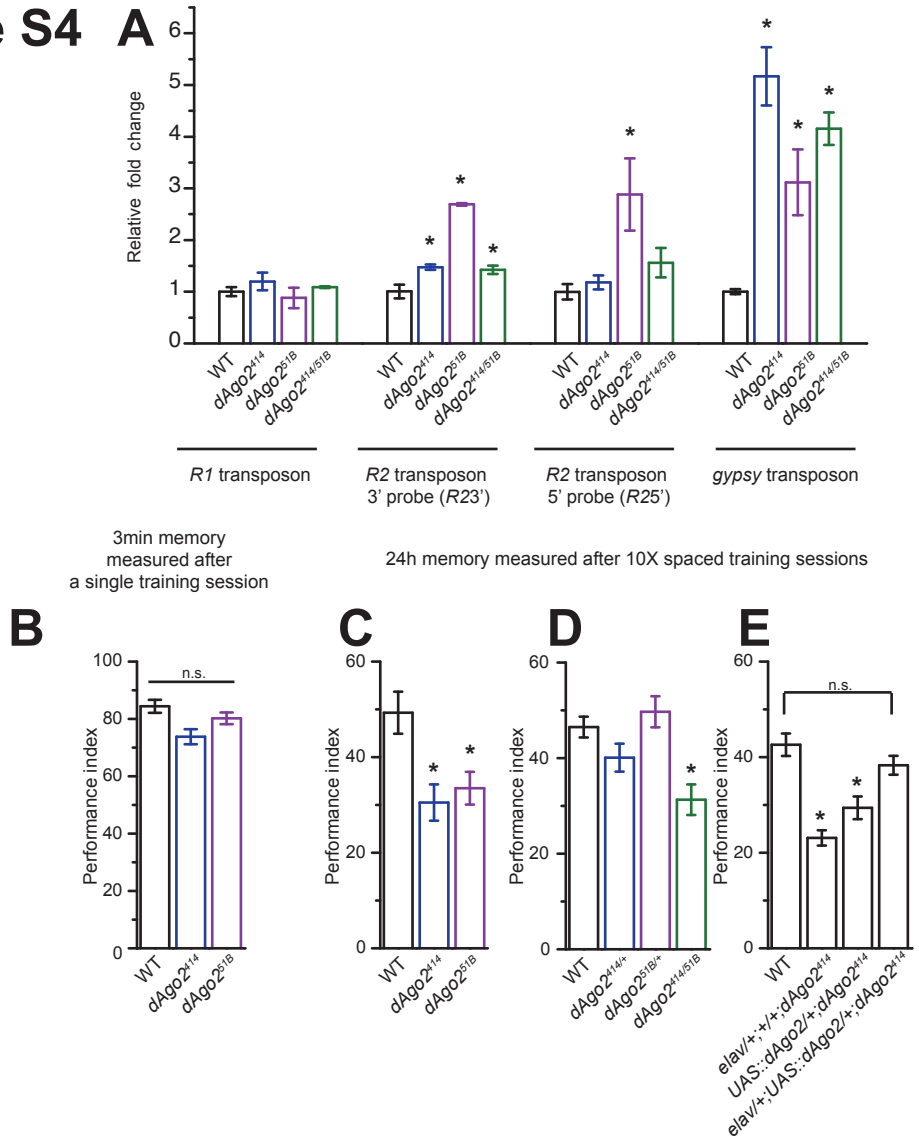
Supplementary Figure S2: (**A**) Illustration of the design of *"gypsy-TRAP".* A ~500bp fragment from the *ovo* regulatory region containing 5 Ovo binding sites is inserted between *Tub promoter* and *GAL80* gene. A mutated *"gypsy-TRAP"* construct contains mutations that disrupt each of the 5 Ovo binding sites. In the absence of *gypsy* insertions, GAL80 expression suppresses GAL4, and *UAS::mCD8::GFP* is not expressed. In the presence of *gypsy* integration into the *"gypsy-TRAP"*, GAL80 expression is blocked, and *UAS::mCD8::GFP* is turned on. (**B**) "*gypsy-TRAP*" reporter detects *de novo* integration in neurons in aged animals. As in Figure 2, the "*gypsy-TRAP*" reporter was combined with *MB247-GAL4* line, and *UAS::mCD8::GFP*. In brains from aged animals (28-35 day post eclosion) we consistently observe sparsely GFP labeled mushroom body neurons (three examples shown in left panels, see also Figure 2 and Supplementary Table S1). As is true in germline, insertion of *gypsy* into this cassette requires the presence of Ovo binding sites. No labeled neurons are seen with the mutated "*gypsy-TRAP*" construct in which these sites are mutated (three examples shown in right panels, see also Figure 2 and Supplementary Table S1).

Figure S3



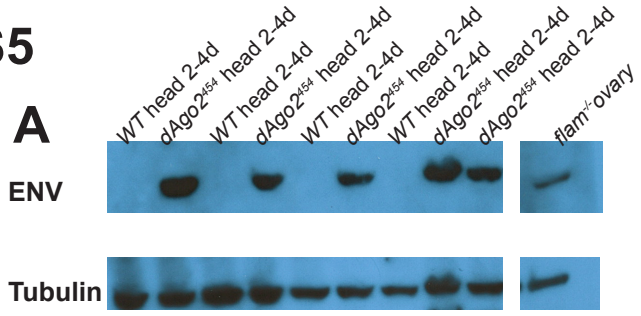
Supplemental Figure S3: *de novo* insertion of *gypsy* into the *ovo* locus is detected in *dAgo2*^{414} mutants with genomic PCR. (**A**) Cartoon of a flybase GBrowse screenshot showing a 2 Kb window of the *ovo* locus with the location and orientation of the *gypsy* retrotransposon insertion that was detected by nested genomic PCR. The region of the GBrowse highlighted in gray depicts the sequence present in the "*gypsy-TRAP*" construct. Sequence results, from clones of the nested PCR fragment from 28-day old *dAgo2*^{414} fly heads, at bottom show the *ovo/gypsy* junction with the *ovo* sequence highlighted in red followed by *gypsy* sequence in yellow. Arrows represent the primers used for nested PCR. (**B**) Ethidium bromide stained gel with a 1 Kb plus DNA ladder showing the presence of a 350bp *de novo* band from heads of 28-day old *dAgo2*^{414} mutant animals. This product is not present in young animals. Because the genomic DNA was extracted from whole heads, we cannot rule out the possibility that there is a low rate of *de novo* insertions in young animals that we are unable to detect it by genomic PCR.

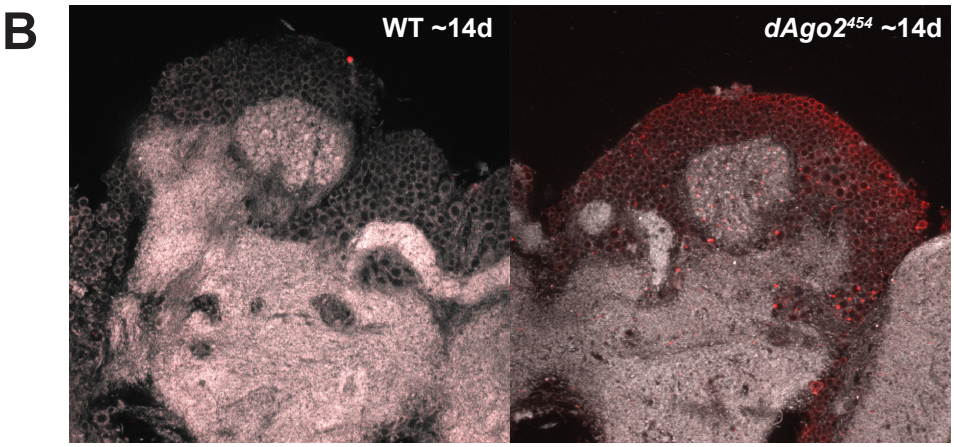
Figure S4

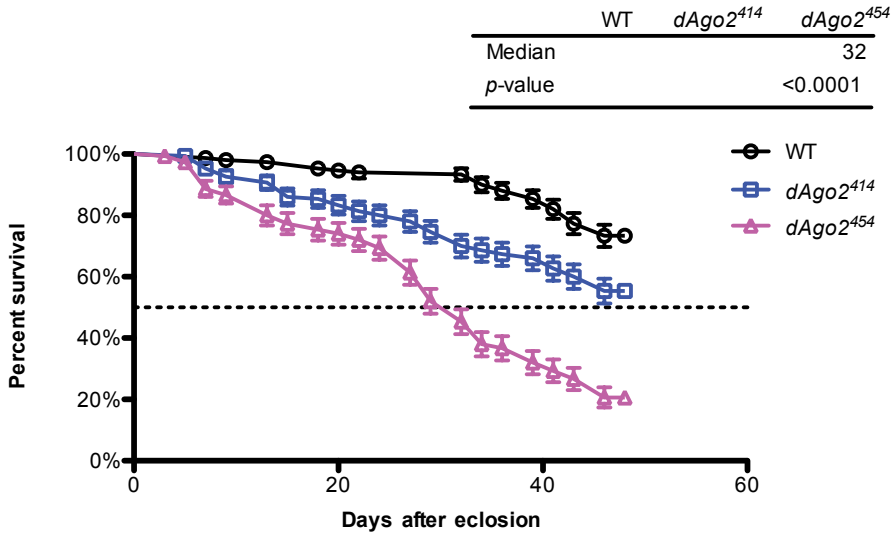


Supplementary Figure S4: *dAgo2* mutants have increased *R2* and *gypsy* expression and defective olfactory memory. (**A**) Levels of transcripts from *R1*, *R2* and *gypsy* were quantified in *dAgo2* mutant animals relative to wild type from 2-4-day old animals. For *R2*, two independent probes were designed to target the 5' *(R25')* and 3' *(R23')* end of the *R2* transcript. Significant elevated expression of *R23'* and *gypsy* are seen in $dAgo2^{414}$, $dAgo2^{51B}$ and $dAgo2^{414/51B}$ (*, p<0.05 and N=4). With *R25'*, a significant increase is seen in $dAgo2^{51B}$ (*, p<0.05 and N=8). (**B**-**E**) Behavioral performance indices (means ± SEM) are shown for aversive Pavlovian olfactory memory. (**B**) *dAgo2* mutant animals exhibit normal STM (3 minutes memory measured after one training session, Supplementary Materials) (n.s., not significant and N=8). (C-E) *dAgo2* mutant alleles exhibit defective LTM (24hr memory measured after 10X spaced training (Online Methods). (**C**) Both homozygous $dAgo2^{414}$ and $dAgo2^{51B}$ animals exhibit significantly lower LTM memory performance indices (*, p<0.05, N=8) relative to that of WT flies. (**D**) $dAgo2^{414}$ and $dAgo2^{51B}$ fail to complement each-other for LTM performance as $dAgo2^{414/51B}$ animals exhibit reduced performance (*; p<0.05 and N=16) relative to WT controls or to animals that are heterozygous either for $dAgo2^{414}$ or $dAgo2^{51B}$. (**E**) The LTM defect can be rescued by expressing a *UAS::dAgo2* transgene under control of the pan-neuronal *elav*-Gal4 line *(elav/+;UAS::dAgo2/+;*$dAgo2^{414}$*).* Animals that are homozygous for $dAgo2^{414}$ and heterozygous for either the *elav*-Gal4 line or *UAS::dAgo2 (elav/+;+/+;*$dAgo2^{414}$ *or UAS::dAgo2/+;*$dAgo2^{414}$*)* exhibit defective LTM (*, p<0.05 and N=16).

Figure S5

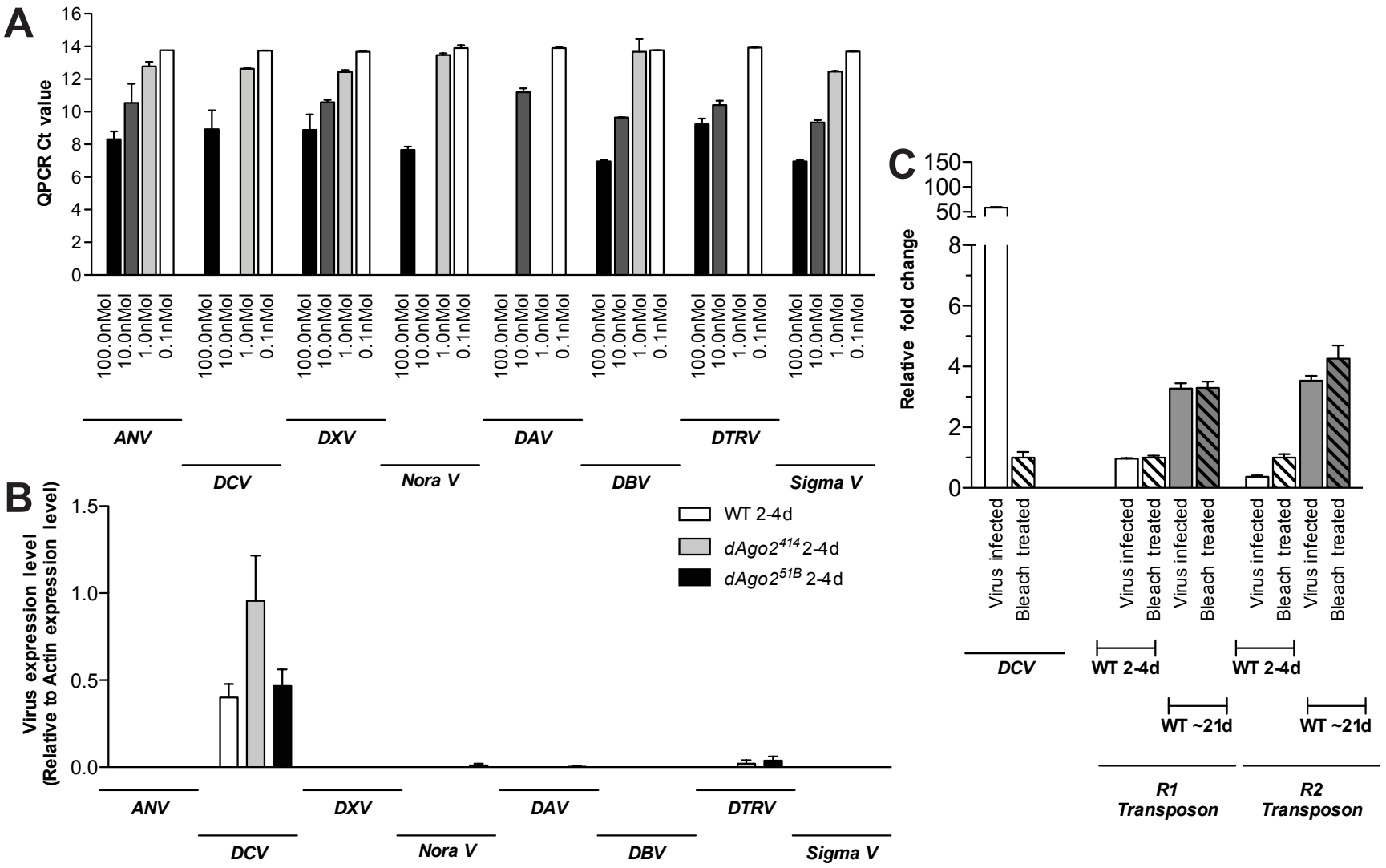






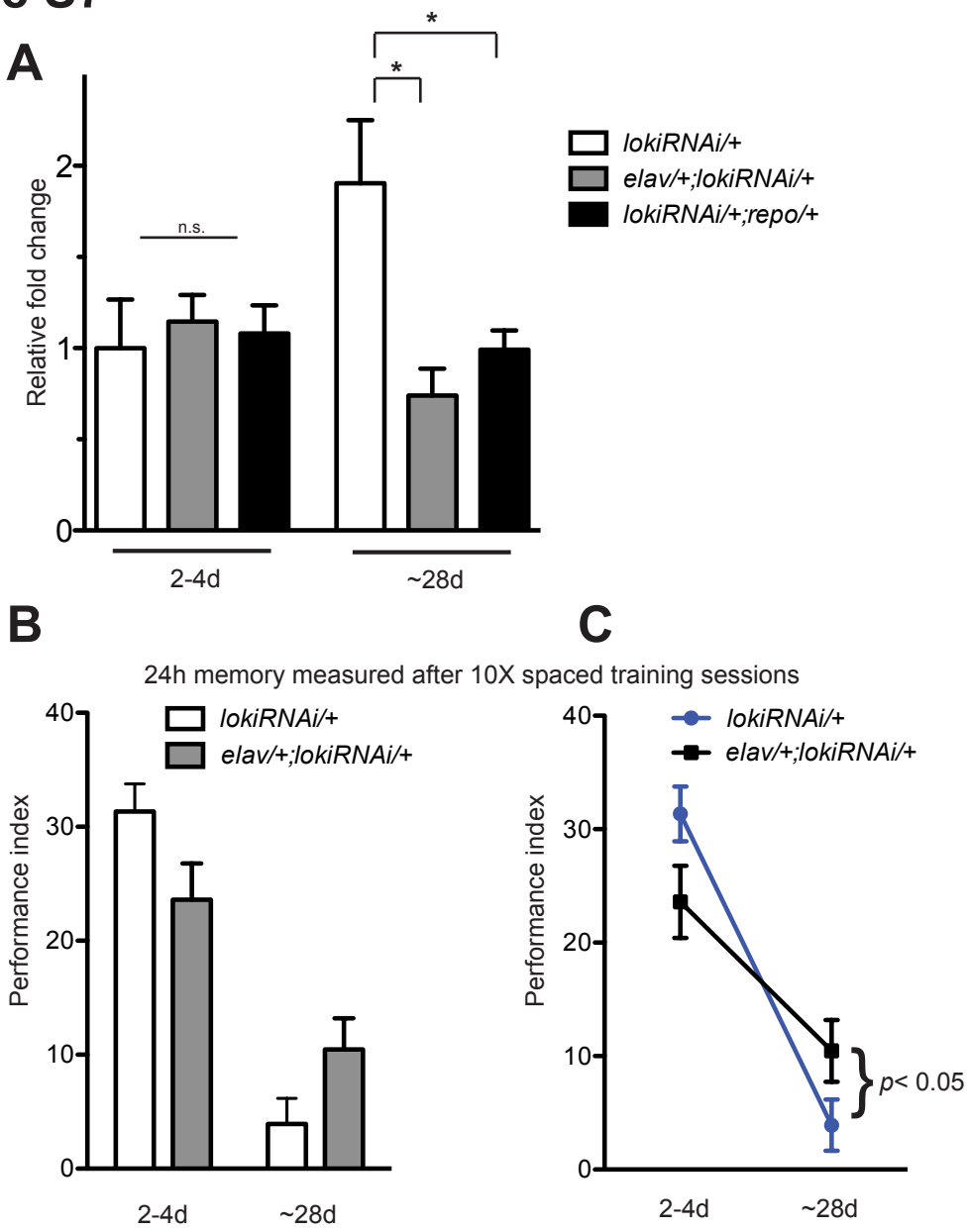
Supplementary Figure S5: Increased *gypsy* ENV protein level is detected in heads from 2-4-day old *dAgo2*^{454} animals by western blot. The detected *gypsy* ENV band from fly heads is identical in size to the *gypsy* ENV band from *flam*$^{-/-}$ ovaries (**A**). *dAgo2*^{454} allele is a recently identified *dAgo2* null allele (Hain 2010). Increased gypsy ENV expression is also detected in whole mount brains from 0-4-day (not shown), ~14-day and ~28-day (not shown) old *dAgo2*^{454} animals by ENV immunolabelling. Optical sections are shown for ~14-day WT (left panel) and *dAgo2*^{454} (right panel). Env (RED) and DiD counterstain (grey) are shown (**B**). This *dAgo2*^{454} allele exhibits severely shortened lifespan (**C** and log-rank test).

Figure S6



Spplementary Figure S6: We examined the expression of the 8 natural viruses that have been detected in *Drosophila melanogaster* strains and cells (Wu 2010). Only one of these, *Drosophila C virus (DCV)* was detected in our strains (**A,B**), but its expression levels do not correlate with age, genotype or TE expression. By bleach treating embryos (see online Methods) we are able to eliminate detectable *DCV* (**C**). Even after this treatment, we observe the age-dependent activation of TEs occurs in the absence of virus (**C**) and the effects of *dAgo2* on lifespan remain (**Figure 3E**). Thus the accelerated decline that we observe in *dAgo2* mutants neither correlates with nor depends upon presence of exogenous viruses. In contrast, the expression of *R2* and *gypsy* retrotransposons correlates with age dependent decline both in wild type and *dAgo2* mutants.

Figure S7



Supplementary Figure S7: *lokiRNAi* is effective in suppressing *loki* expression in heads from ~28-day old animals. When *lokiRNAi* is expressed in neurons with *elav-GAL4* or glia cells with *repo-GAL4*, reduced *loki* expression levels are observed in heads from ~28-day old animals (**A**). However, the effect on lifespan is only observed when *lokiRNAi* is expressed in neurons with *elav-GAL4* (Figure 3F), but not in glia cells with *repo-GAL4* (data not shown). 24hr memory measured after 10X spaced training sessions is measured for *lokiRNAi/+* and *elav/+;lokiRNAi/+* animals at both 2-4-day and ~28-day time point (**B,C**). The age-dependent memory decline in *elav/+;lokiRNAi/+* is significantly smaller than *lokiRNAi/+* animals (**C** and Two-Way ANOVA for age and genotype interaction).