www.sciencetranslationalmedicine.org/cgi/content/full/5/200/200ra115/DC1

Supplementary Materials for

Molecular Mechanism for Age-Related Memory Loss: The Histone-Binding Protein RbAp48

Elias Pavlopoulos, Sidonie Jones, Stylianos Kosmidis, Maggie Close, Carla Kim, Olga Kovalerchik, Scott A. Small,* Eric R. Kandel*

*Corresponding author. E-mail: erk5@columbia.edu (E.R.K.); sas68@columbia.edu (S.A.S.)

Published 28 August 2013, *Sci. Transl. Med.* **5**, 200ra115 (2013)
DOI: 10.1126/scitranslmed.3006373

The PDF file includes:

SUPPLEMENTARY MATERIALS

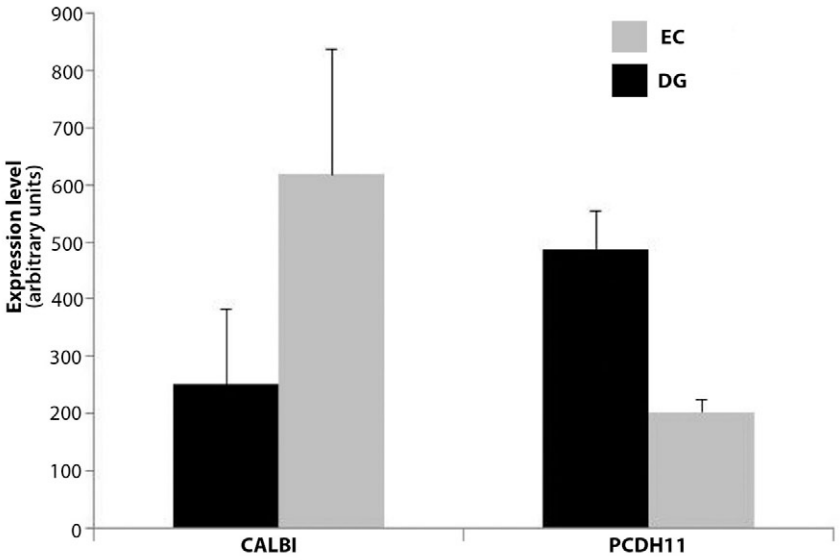

Fig. S1 Specificity of the microdissections from human postmortem tissue. Comparison of the expression levels of calbindin1 (CALB1), a gene differentially expressed in the dentate gyrus (DG) *(50)*, and PCDH11, which is differentially expressed in the EC (Allen Brain Atlas). Data are expressed as mean±SEM (N=8 subjects; 1 microarray experiment for each subject and subregion). Both genes were found in our microarray dataset. PCDH11 levels were significantly higher in the EC compared to DG (paired t-test: t=4.6, p=0.002), while the expression of CALB1 was significantly higher in DG compared to EC (paired t-test; t=2.73, p=0.03)

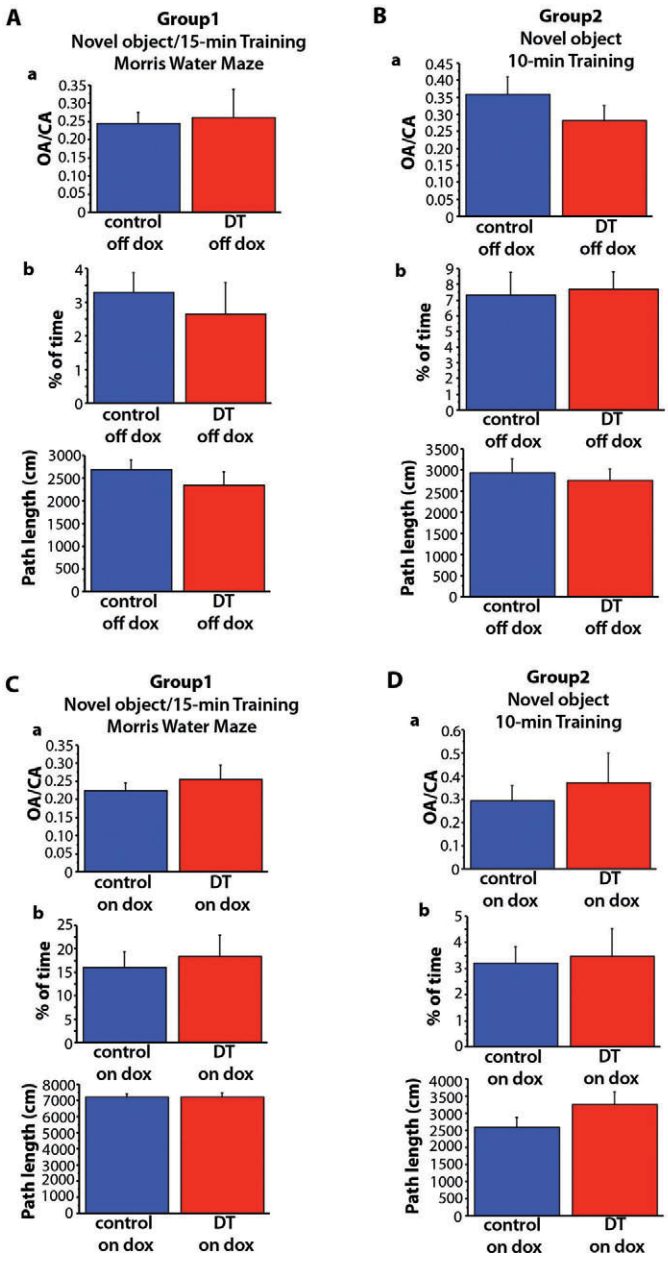

Fig. S2 Anxiety in mice expressing the dominant-negative inhibitor of RbAp48 in their forebrain. Data from the elevated plus maze (a) and an open field test (b) of RbAp48-DN DT mice and their control littermates tested off dox (same mice as in figures 3 and 4). (a) Averaged ratio (± SEM) of the time spent in open arms versus closed arms. (b) Percentage of time spent in the center of the open field and total path length (± SEM). The anxiety of the mice was examined once and prior to the cognitive tasks.

(A) Group of mice tested off dox in the 15-minute training novel object paradigm and the Morris water maze [same mice as in figures 3A(a) and 4A; DT: N=11 and Controls: N=22

(tetO=6, tTA=8, wt=8)]. DT mice off dox (DT; RbAp48-DN expression) and control animals off dox (control) spent comparable time in the closed and open arms of the maze (a) (ANOVA; no genotype effect; p=0.815). (b) Both groups exhibited similar performance in the open field (ANOVA; no genotype effect; p>0.37).

(B) Group of mice tested off dox in the 10-minute training novel object paradigm [same mice as in figure 3A(b); DT: N=12 and Controls: N=12 (tetO=5, tTA=4, wt=3)]. DT off dox and control off dox showed similar open arms/closed arms ratio (a) (ANOVA; no genotype effect; p=0.2660). Similar performance was also observed in the open field (b) (ANOVA; no genotype effect; p>0.68).

(C) Mice tested on dox in the 15-minute training novel object paradigm and the Morris water maze [same mice as in figures 3B(a) and 4B; DT on dox: N=10 and Controls: N=17 (tetO=5, tTA=5, wt=7)]. No differences were observed for DT mice on dox and controls on dox in the elevated plus maze (a) and the open field (b) (ANOVA; no genotype effect; p>0.46).

(D) Mice tested on dox in the 10-minute training novel object paradigm [same mice as in figure 3B(b); DT on dox: N=12 and Controls: N=21(tetO=7, tTA=7, wt=7)]. DT and control on dox showed similar performance in the elevated plus maze (a) and the open field (b) (ANOVA; no genotype effect; p>0.17). For detailed statistics see Table S3.

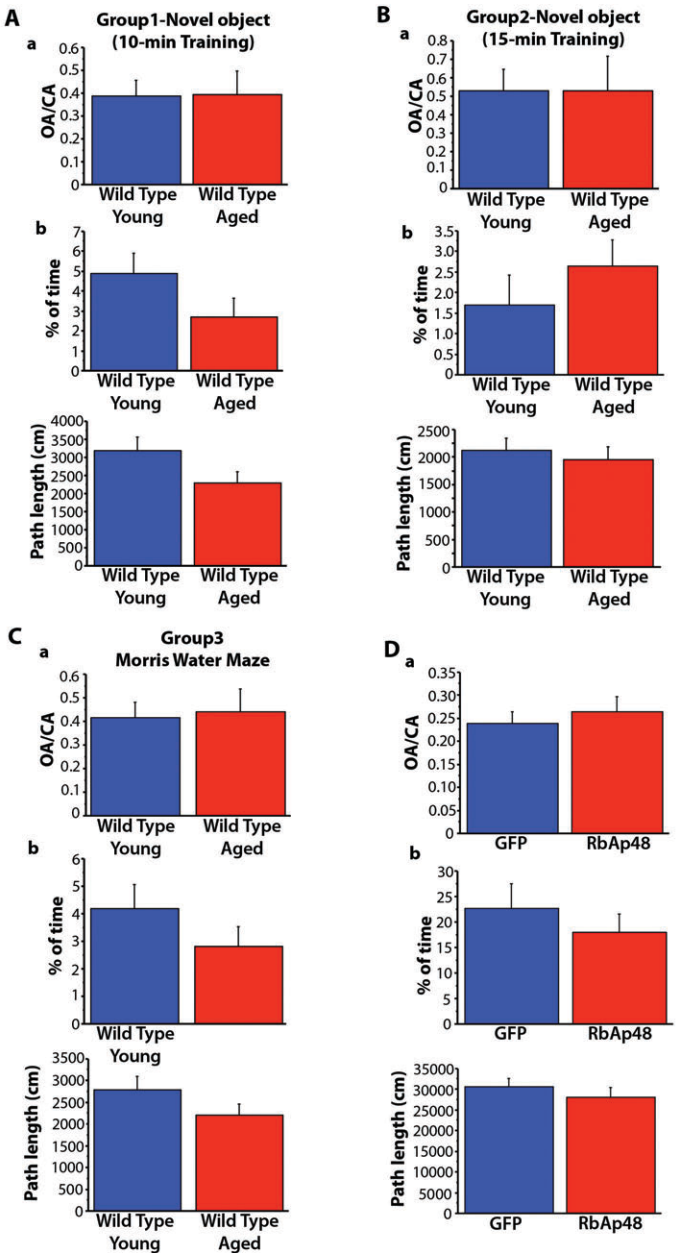

Fig. S3 Anxiety of wild-type mice tested in hippocampal-dependent memory tasks. Data from the elevated plus maze (a) and the open field (b) of wild-type mice (same groups as in Fig. 3,4 and 6). Averaged ratio (± SEM) of the time spent in open arms (OA) versus closed arms (CA) (a), and percentage of time spent in the center of the open field and total path length (± SEM) (b). The anxiety of the mice was examined once and prior to the cognitive tasks.

(A) Group of mice tested in the 10-minute training novel object paradigm (same mice as in figure 3D(b); N=10 mice/age) (ANOVA; no genotype effect; $p>0.075$).

(B) Similar performance between young and aged mice in the 15-minute training protocol (same mice as in figure 3D(a); N=8 mice/age). Aged and young mice exhibited similar performance (ANOVA; no genotype effect; $p>0.54$).

(C) Group of mice tested in the Morris water maze (same mice as in figure 4C; N=14/age). Aged and young mice performed similarly in the elevated plus maze (a) and the open field (b) (ANOVA; no genotype effect; $p>0.16$).

(D) Aged wild-type mice that were injected in their dentate gyrus with lentiviruses expressing either GFP (control) or RbAp48 (same mice as in figure 6; WT aged/RbAp48-HA injected in DG: N=12 and WT aged/GFP injected in DG: N=10). No significant differences were observed between groups (ANOVA; no genotype effect; $p>0.42$). For detailed analysis see Table S3.

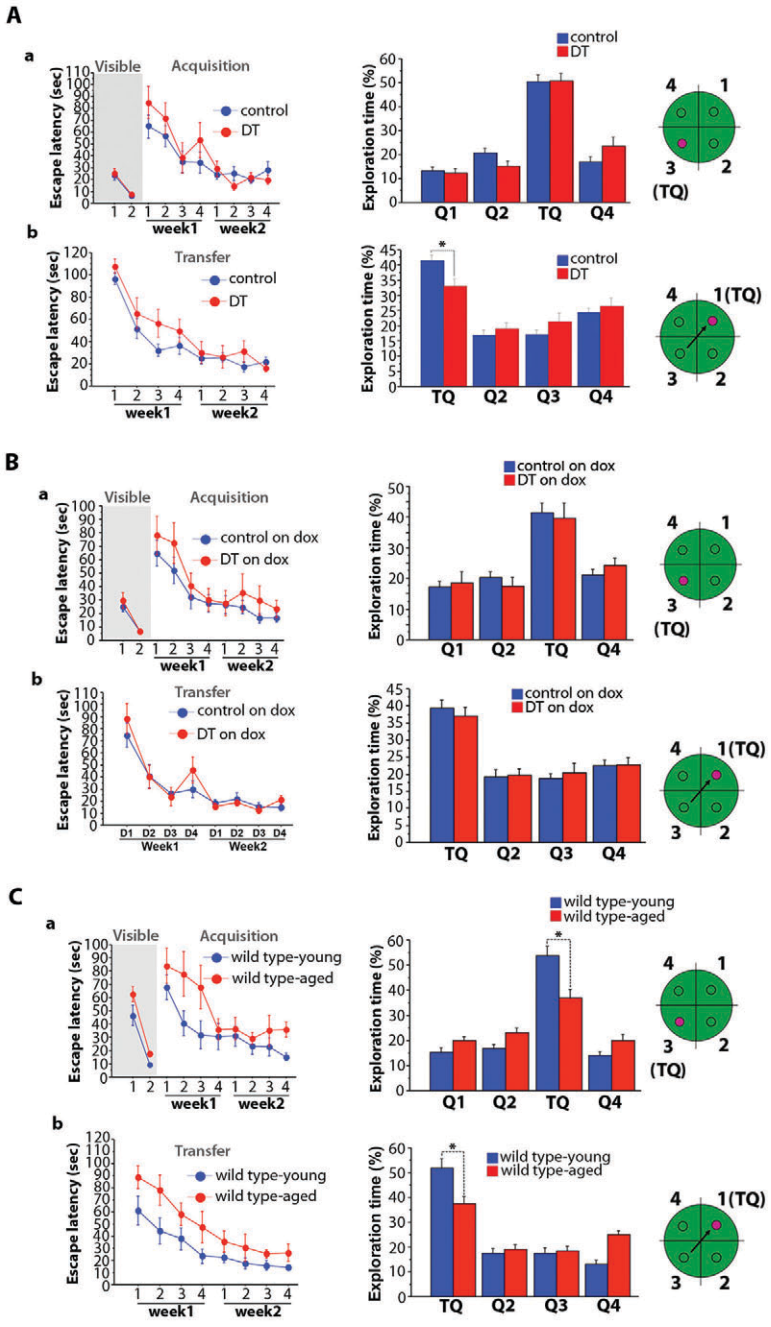

Fig. S4 Data from the Morris water maze that complement Fig.4. (left) Mean escape latencies (± SEM) across days for mice to reach the platform in the visible (a), the hidden (a) and the transfer phases (b) of the task. (right) Percentage of time (mean ± SEM) spent in quadrants during probe trials one day after the end of training (week2/day5).

(A) Group of DT mice and control littermates kept off dox during the task [same mice as in figure 4A; DT: N=11 and controls: N=22 (tetO=6, tTA=8, wt=8); one experiment]. DT and controls performed equally well in the visible platform (a) as well as in the acquisition (a) and

the transfer (b) phases of the hidden platform version of the task (repeated-measures ANOVA; no genotype effect: $p>0.1$). (a) During the probe trial after the end of acquisition, DT and controls spent similar time in the training quadrant (TQ) (repeated-measures ANOVA; no significant genotype or genotype*quadrant effects: $p=0.85$ and $p=0.2434$, respectively). DT, however, formed a less accurate knowledge of the platform location (see figure 4A(a)). (b) DT explored the training quadrant less than controls (repeated-measures ANOVA; genotype*quadrant interaction effect: $p=0.0269$; t-test for TQ: *$p= 0.012$). See also figure 4A(b) for significant effect for platform crossings.

(B) Group of DT and control mice kept on dox during the task [RbAp48-DN OFF in adulthood; same mice as in figure 4B; DT on dox: N=10 and controls: N=17 (tetO=5, tTA=5, wt=7); one experiment]. DT and control on dox displayed similar performance during the acquisition (a) and the transfer (b) phases of the hidden platform version of the task [repeated-measures ANOVA; no genotype ($p>0.14$)]. Both groups explored equally the training quadrant during the probe trials (repeated-measures ANOVA for hidden/acquisition and hidden/transfer; no significant genotype*quadrant effect: $p>0.76$).

(C) Young adult (3.5 months) and aged (15 months) wild-type mice (same mice as in figure 4C; N=14 mice/age; one experiment). Aged mice showed higher escape latencies than young mice (repeated-measures ANOVA for visible, hidden/acquisition and hidden/transfer; $p<0.03$). This effect is likely explained by the significantly lower swim speed of the aged mice (see Fig. S7). Both groups learned the visible platform (repeated-measures ANOVA; significant day effect; $p\leq0.0002$). The path lengths were similar between the two groups (see Fig. 4C). In the acquisition and the transfer phases of the water maze, the latencies of aged mice were reduced and reached plateau by the end of training, indicating that the mice learned the platform location equally well to young animals (repeated-measures ANOVA; significant day effect; $p<0.0016$). Consistent with equal learning skill between young and aged mice the path lengths

were similar between the two groups (see figure 4C). However, the aged mice did not form a good memory of the platform locations as evidenced by their significantly lower exploration time in the training quadrant in the probe trials [repeated-measures ANOVA; hidden-probe trial: significant age*quadrant effect ($p=0.0001$), t-test for TQ, $p=0.003$; transfer-probe trial: significant age*quadrant effect ($p<0.0001$), t-test for TQ, $p=0.0037$]. For platform crossings, see Fig.4C. *$p<0.0037$. See table S3 for detailed analysis.

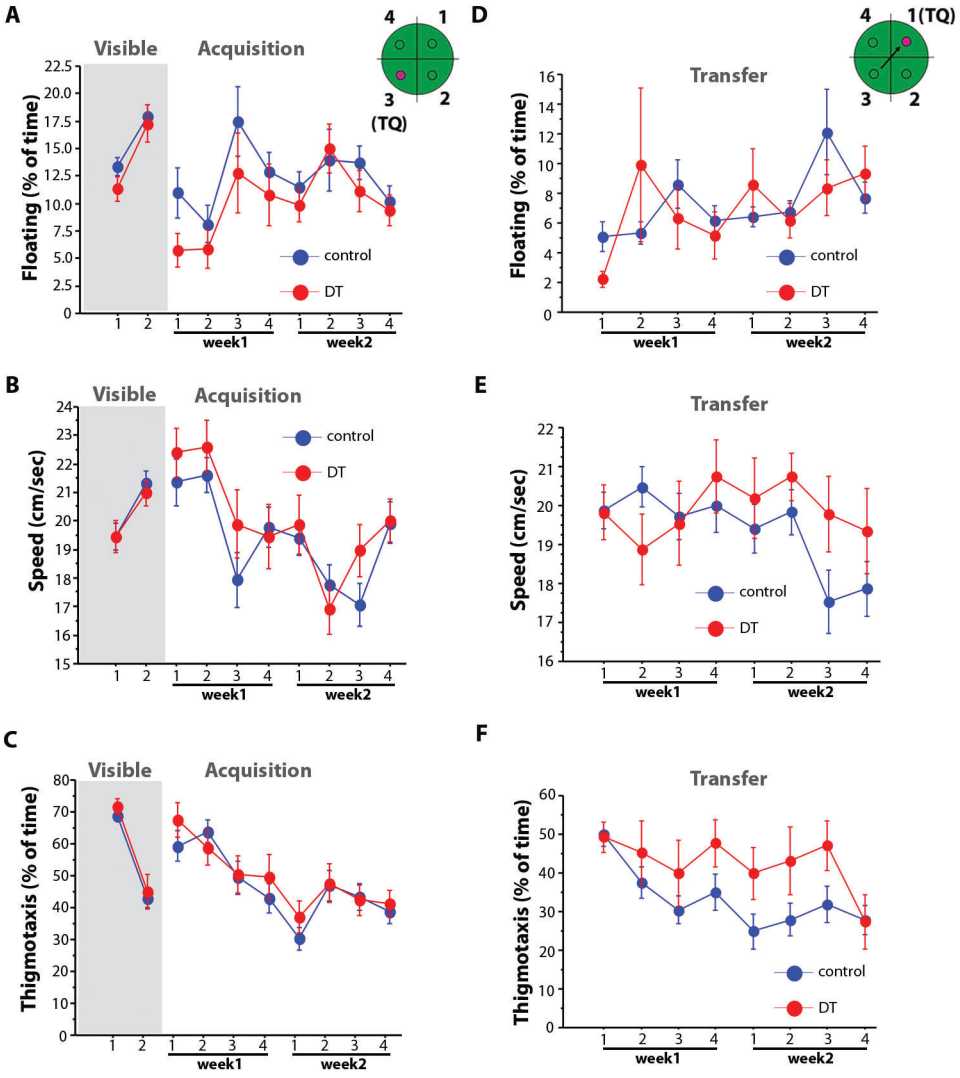

Fig. S5 Noncognitive parameters from the Morris water maze of DT mice and control littermates tested off doxycycline. (A-C) Visible platform and hidden platform/acquisition. **(D-F)** Transfer phase. Same mice as in figure 4A and figure S4A (DT: N=11 and controls: N=22 (tetO=6, tTA=8, wt=8); one experiment). Mean±SEM is shown. Repeated-measures ANOVA did not reveal significant genotype effect [Visible: $p>0.24$ (floating), $p=0.7991$ (speed) and $p=0.4366$ (thigmotaxis); Hidden/Acquisition: $p=0.0745$ (floating), $p=0.2567$ (speed) and $p=0.3855$ (thigmotaxis); Hidden/Transfer: $p=0.8263$ (floating), $p=0.4777$ (speed) and $p=0.0787$ (thigmotaxis)]. See table S3 for detailed analysis.

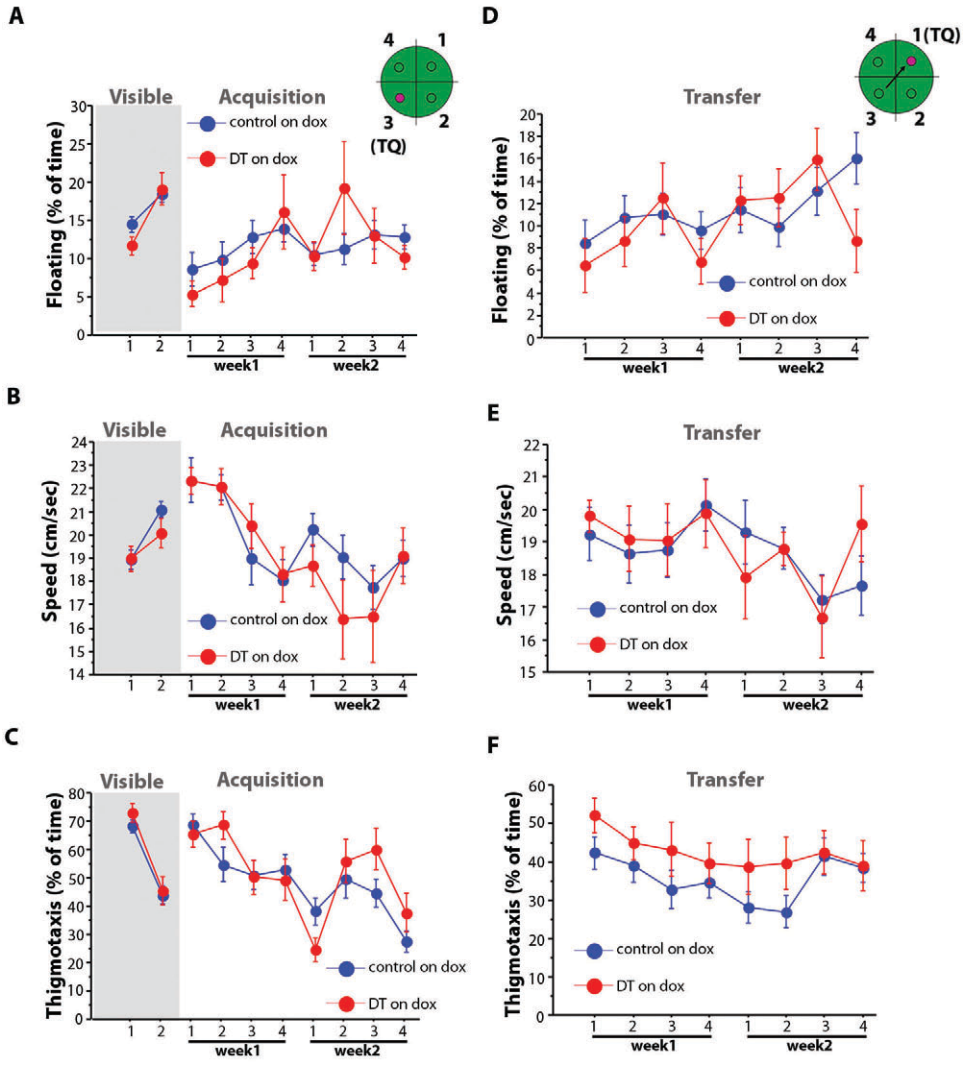

Fig. S6 Noncognitive parameters from the Morris water maze of DT mice mice and control littermates tested on doxycycline. (A-C) Visible platform and Hidden platform/acquisition. **(D-F)** Transfer phase. Same mice as in figure 4B and figure S4B [DT on dox: N=10 and controls on dox: N=17 (tetO=5, tTA=5, wt=7); one experiment]. Mean±SEM is shown. Repeated-measures ANOVA did not reveal significant genotype effect [Visible: p=0.4697 (floating), p=0.2394 (speed) and p=0.4621 (thigmotaxis); Hidden/Acquisition: p=0.8825 (floating), p=0.5031 (speed) and p=0.3981 (thigmotaxis); Hidden/Transfer: p=0.5289 (floating), p=0.8811 (speed) and p=0.0593 (thigmotaxis)]. See table S3 for detailed statistics.

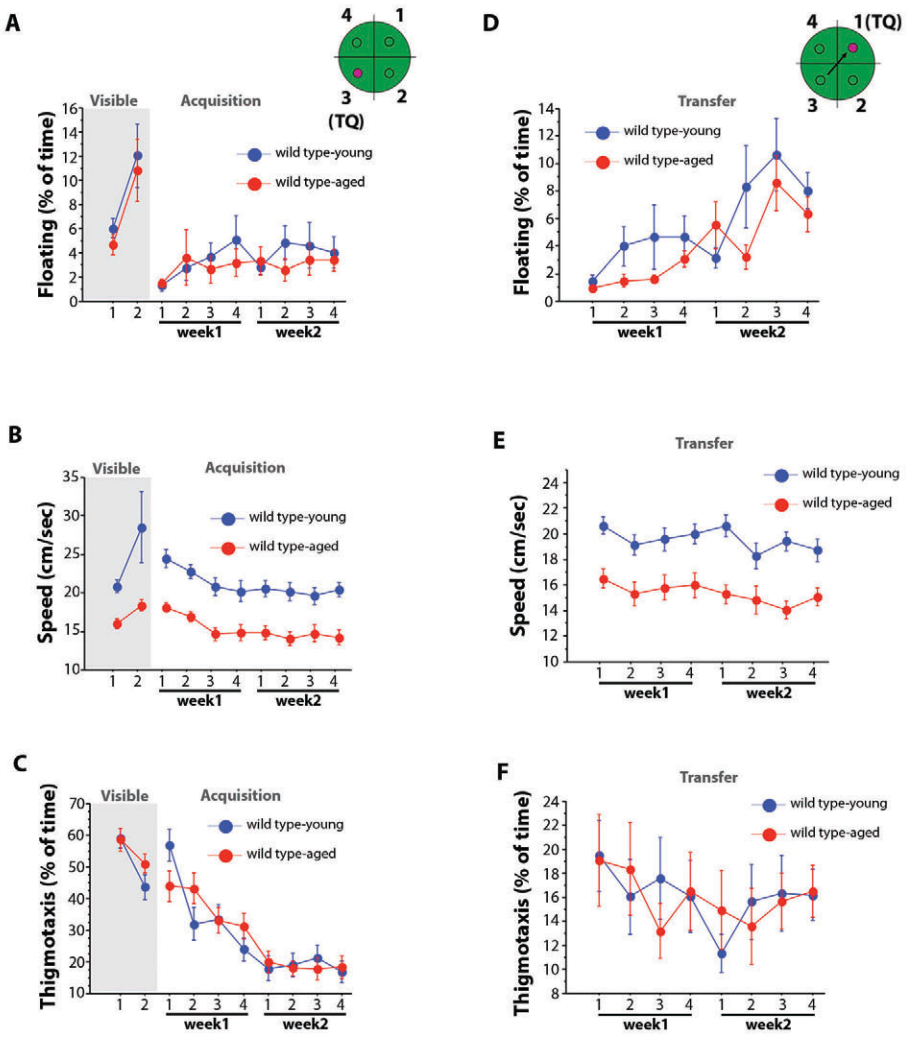

Fig. S7 Noncognitive parameters from the Morris water maze of young and aged wild-type mice. (A-C) Visible platform and Hidden platform/acquisition. **(D-F)** Transfer phase. Same mice as in figure 4C and figure S4C (WT young: N=14 and WT aged: N=14; one experiment). Mean±SEM is shown. Repeated-measures ANOVA did not reveal significant age effect for floating and thigmotaxis (Visible: $p>0.34$; Hidden/Acquisition: $p>0.46$; Hidden/Tranfer: $p>0.095$). The speed of aged mice was significantly lower than that of young animals (repeated-measures ANOVA; significant age affect; Visible: $p=0.0061$; Hidden-Acquisition: $p<0.0001$; Hidden-Transfer: $p<0.0001$). See table S3 for detailed analysis.

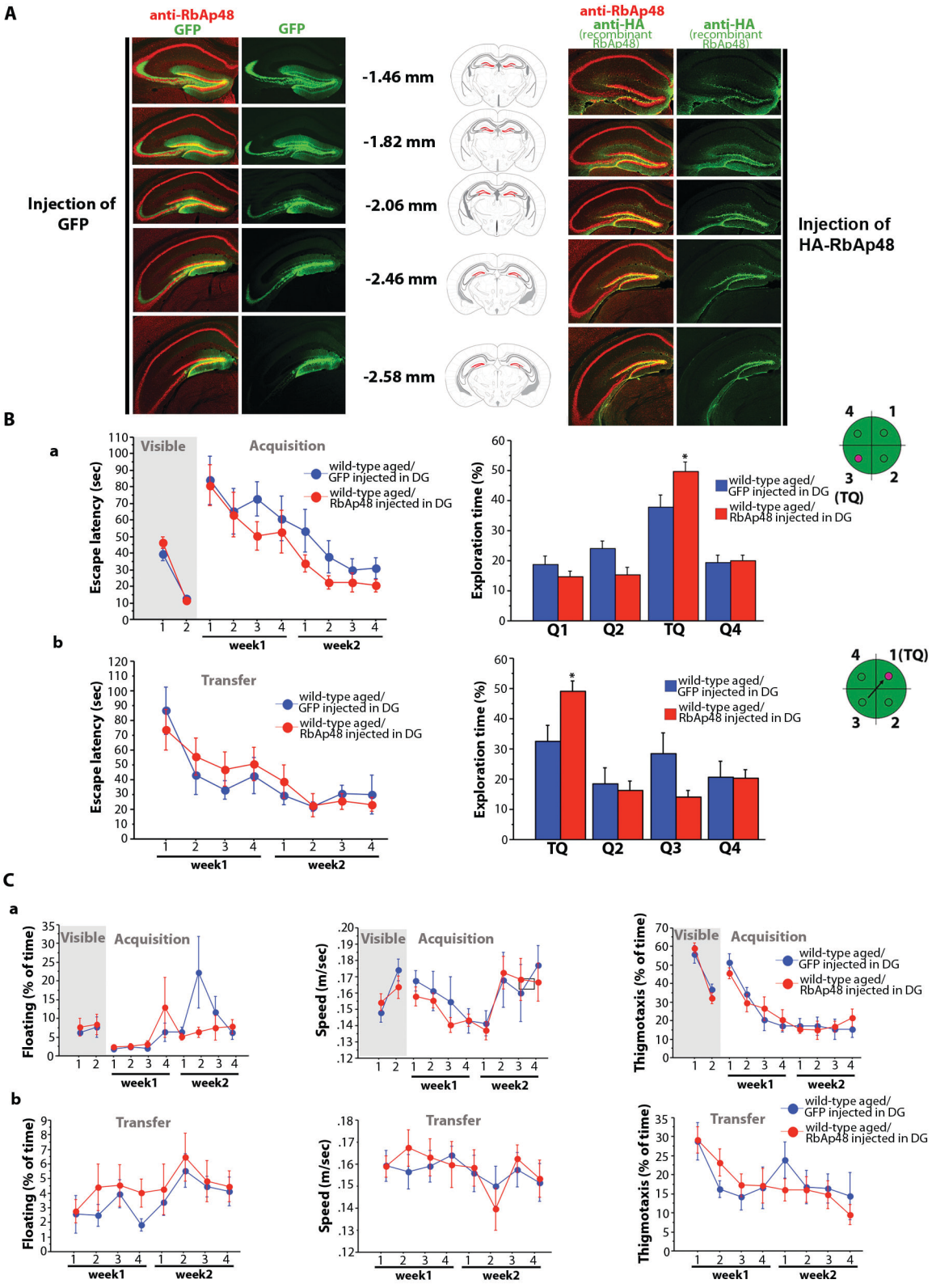

Fig. S8 Effect of lentivirus-mediated up-regulation of RbAp48 in the DG of aged wild-type mice on age-related loss of hippocampus-dependent memory. Data complement those in figure 6. Aged wild-type mice injected in their DG with either RbAp48-HA or GFP.

(A) Representative confocal images showing the distribution of the lentiviral expression of RbAp48-HA and GFP in the DG along the anterior-posterior axis.

(B and C) Data from the Morris water maze (same mice as in Fig.6B; WT aged/RbAp48-HA injected in DG: N=12 and WT aged/GFP injected in DG: N=10; one experiment). **(B)** Mean escape latencies (± SEM) in the visible (a), the hidden (a) and the transfer (b) phases of the task. The percentage of time spent in quadrants during probe trials one day after the end of training (week2/day5) is also shown (mean ± SEM). The latencies were similar between RbAp48-HA (RbAp48) and GFP-injected mice in all versions of the task (repeated-measures ANOVA; no genotype effect; Visible: p=0.3521; Acquisition/hidden: p=0.0577; Transfer: p=0.7587). During the probe trials, the RbAp48 mice spent significantly more time in the training quadrant (TQ) compared to the GFP age-matched control littermates (repeated-measures ANOVA; Hidden/aquisition: injection*quadrant interaction effect: p=0.0115 and t-test for TQ: p=0.0285; Hidden/transfer: injection*quadrant interaction effects: p=0.0271 and t-test for TQ: p=0.0119).

(C) Comparison of non-cognitive parameters of the Morris water maze task across days (mean±SEM). Repeated-measures ANOVA did not reveal differences (no injection effect; Visible: p>0.69; Hidden/ Acquisition: p>0.42; Hidden/Transfer: p=0.40). (B and C) See Table S3 for detailed statistics.

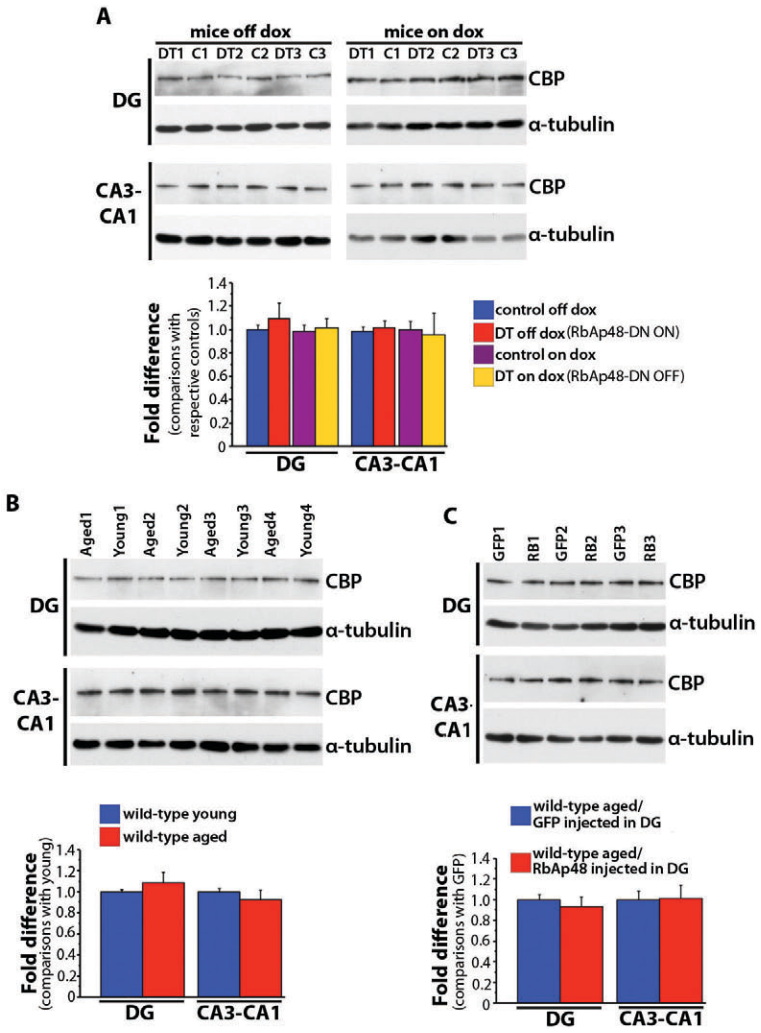

Fig. S9 RbAp48 effect on the protein levels of CBP. Western blot analysis and averaged data (± SEM) of the total levels of CBP from DG and CA3-CA1 lysates used for the CBP-specific IPs and HAT assays described in figure 7. The 1/40 of the CA3-CA1 lysates and the 1/25 of the DG lysates were analyzed. Anti-α-tubulin: control for loading and normalization. Each lane represents one mouse.

(A) DT1-3 and C1-3: three DT and three control littermates, respectively. Repeated-measures ANOVA did not reveal significant genotype and genotype*treatment effects ($p>0.44$; DT off dox: N=3, DT on dox: N=3, control off dox: N=3, control on dox: N=3; one experiment).

(B) Aged1-4 and Young1-4: four 15-month-old and four 3½-month-old wild-type mice, respectively. No differences were observed in the DG and CA3-CA1 (ANOVA; $p>0.4$; WT Aged: N=4 and WT Young: N=4; one experiment).

(C) RB1-3: three 15-month old wild-type mice virally expressing RbAp48-HA in the DG (DG-specific RbAp48 upregulation). GFP1-3: three 15-month old wild-type mice expressing GFP in their DG (control). ANOVA did not reveal any difference ($p>0.53$; WT aged/RbAp48-HA injected in DG: N=3 and WT aged/GFP injected in DG: N=3; one experiment). See Table S3 for detailed statistics.

Table S1. Comparative studies of histone acetylation.

acetylated Histone H2BK20			
Genotype & treatment (on or off dox)	***# of animals***	***# of sections (total)***	***# of sections/mouse***
DT off dox	5	27	≥ 4 (Every second slice/thickness of slice: 30µm)
DT on dox	3	15	≥ 4 (Every second slice/thickness of slice: 30µm)
controls off dox (pooled)	7 *single tetO:3* *single tTA: 4*	45 *single tetO:18* *single tTA: 24*	≥ 4 (Every second slice/thickness of slice: 30µm)
controls on dox (pooled)	4 *single tetO: 2* *single tTA: 2*	15 *single tetO:7* *single tTA: 8*	≥ 3 (Every second slice/thickness of slice: 30µm)
RbAp48-HA-injected aged wild-type mice	3	12	4 (Every second slice/thickness of slice: 30µm)
GFP-injected aged wild-type mice	3	12	4 (Every second slice/thickness of slice: 30µm)
acetylated Histone H4K12			
Genotype & treatment (on or off dox)	***# of animals***	***# of sections (total)***	***# of sections/mouse***
DT off dox	5	44	≥ 6 (Every second slice/thickness of slice: 30µm)
DT on dox	3	15	≥ 3 (Every second slice/thickness of slice: 30µm)
controls off dox (pooled)	7 *single tetO:3* *single tTA: 4*	52 *single tetO:24* *single tTA: 28*	≥ 6 (Every second slice/thickness of slice: 30µm)
controls on dox (pooled)	4 *single tetO: 2* *single tTA: 2*	11 *single tetO:6* *single tTA: 5*	≥ 2 (Every second slice/thickness of slice: 30µm)
RbAp48-HA-injected aged wild-type mice	3	12	4 (Every second slice/thickness of slice: 30µm)
GFP-injected aged wild-type mice	3	12	4 (Every second slice/thickness of slice: 30µm)
acetylated Histone H3K9			
Genotype & treatment (on or off dox)	***# of animals***	***# of sections (total)***	***# of sections/mouse***
DT off dox	5	16	≥ 3 (Every second slice/thickness of slice: 30µm)
controls off dox (pooled)	7 *single tetO:3* *single tTA: 4*	28 *single tetO:14* *single tTA: 14*	≥ 3 (Every second slice/thickness of slice: 30µm)
RbAp48-HA-injected aged wild-type mice	3	12	4 (Every second slice/thickness of slice: 30µm)
GFP-injected aged wild-type mice	3	12	4 (Every second slice/thickness of slice: 30µm)

Table S2. Oligonucleotides used for RNA in situ hybridization and PCR cloning.

Oligonucleotides used for RNA in situ hybridizations	
Flag-RbAp48-DN	5'-GCCGATGAATGATCTTATCGTCGTCATC CTTGTAATCCAT-3
RbAp48	oligo1: 5'- CCCGTTCTTCCACTGCGTCGTCAAAGGCCGCTTCC TTGTCAGCCA -3' oligo 2: 5'-CCCACTGAGATTTGGATTCCAAGAAAGCC CATAACCTTCCTTCTG-3' oligo 3: 5'- GGAGCAGATGCCAGGACACGTCCTCCACTACTGCTGTATGCCCCG-3'
Oligonucleotides used for cloning of the mouse Flag-RbAp48DN into pMM400 plasmid	
Forward primer	5'- GAAGATCTTCCACCATGGATTACAAGGATGACGACGATAAGATCATTCATCGGCTTGTCCTGGG-3' *(Includes the Flag epitope coding sequence)*
Reverse primer	5'- GAAGATCTGAGTCTAGGATCACAGGTGC-3'

Table S3. Detailed statistical analysis of behavioral, biochemical, and immunohistochemical studies.

ANXIETY TESTS **DT and Controls OFF DOX** N: DT=11 and Controls=22 (single tetO=6, single tTA=8, wild type=8) *(Same group as in the 15MIN-training novel object recognition task and the Morris water maze task)*	
Elevated plus maze **ANOVA** **Time spent in open arms versus closed arms:** No genotype effect: $F_{(1,31)}$=0.056, p=0.815	**Open field** **ANOVA** **Time spent in the center:** No genotype effect: $F_{(1,31)}$=0.377, p=0.543 **Path length:** No genotype effect: $F_{(1,31)}$=0.803, p=0.377
ANXIETY TESTS **DT and Controls OFF DOX** N: DT=12 and Controls =12 (single tetO=5, single tTA=4, wild type=3) *(Same group as in the 10MIN-training novel object recognition task)*	
Elevated plus maze **ANOVA** **Time spent in open arms versus closed arms:** No genotype effect: $F_{(1,22)}$=1.302, p=0.2660	**Open field** **ANOVA** **Time spent in the center:** No genotype effect: $F_{(1,22)}$=0.047, p=0.8311 **Path length:** No genotype effect: $F_{(1,22)}$=0.169, p=0.6852
ANXIETY TESTS **DT and Controls ON DOX** N: DT on dox=10 and Controls=17 (single tetO= 5, single tTA= 5, WT=7) *(Same group as in the 15MIN-training novel object recognition task and the Morris water maze task)*	
Elevated plus maze **ANOVA** **Time spent in open arms versus closed arms:** No genotype effect: $F_{(1,25)}$=0.550, p=0.4651	**Open field** **ANOVA** **Time spent in the center:** No genotype effect: $F_{(1,25)}$=0.162, p=0.691 **Path length:** No genotype effect: $F_{(1,25)}$=0.003, p=0.9594
ANXIETY TESTS **DT and Controls ON DOX** N: DT on dox =12 and Controls=21 (single tetO=7, single tTA=7, WT=7) *(Same group as in the 10MIN-training novel object recognition task)*	
Elevated plus maze **ANOVA** **Time spent in open arms versus closed arms:** No genotype effect: $F_{(1,31)}$=0.370, p=0.5470	**Open field** **ANOVA** **Time spent in the center:** No genotype effect: $F_{(1,31)}$=0.05, p=0.8254 **Path length:** No genotype effect: $F_{(1,31)}$=1.952, p=0.1723
ANXIETY TESTS **WT young (N=8) and WT aged (N=8)** *(Same group as in the 15MIN-training novel object recognition task)*	
Elevated plus maze **ANOVA**	**Open field**

Time spent in open arms versus closed arms: No genotype effect: $F_{(1,14)}$=0.0005, p=0.9944	**ANOVA** **Time spent in the center:** No genotype effect: $F_{(1,14)}$=0.968, p=0.543 **Path length:** No genotype effect: $F_{(1,14)}$=0.274, p=0.6087

ANXIETY TESTS

WT young (N=10) and WT aged (N=10)

(Same group as in the 10MIN-training novel object recognition task)

Elevated plus maze **ANOVA** **Time spent in open arms versus closed arms:** No genotype effect: $F_{(1,18)}$=0.004, p=0.9526	**Open field** **ANOVA** **Time spent in the center:** No genotype effect: $F_{(1,18)}$=2.411, p=0.1379 **Path length:** No genotype effect: $F_{(1,18)}$=3.54, p=0.0762

ANXIETY TESTS

WT young (N=14) and WT aged (N=14)

(Same group as in the Morris water maze task)

Elevated plus maze **ANOVA** **Time spent in open arms versus closed arms:** No genotype effect: $F_{(1,26)}$=0.04, p=0.8437	**Open field** **ANOVA** **Time spent in the center:** No genotype effect: $F_{(1,26)}$=1.530, p=0.2272 **Path length:** No genotype effect: $F_{(1,26)}$=2.021, p=0.1670

ANXIETY TESTS

WT aged/RbAp48-HA injected in DG (N=12) and WT aged/GFP injected in DG (N=10)

(Same group as in the 10MIN-training novel object recognition task and the Morris water maze task)

Elevated plus maze **ANOVA** **Time spent in open arms versus closed arms:** No injection effect: $F_{(1,20)}$=0.351, p=0.5601	**Open field** **ANOVA** **Time spent in the center:** No injection effect: $F_{(1,20)}$=0.623, p=0.4393 **Path length:** No injection effect: $F_{(1,20)}$=0.656, p=0.4275

NOVEL OBJECT

15-MIN Training

DT and Controls OFF DOX

N: DT=11 and Controls=22 (single tetO=6, single tTA=8, wild type=8)

The memory of DT mice for novel object recognition was impaired in the 48-hour test

Repeated-measures ANOVA **Discrimination index:** Significant genotype effect: $F_{(1,31)}$=6.95, p=0.013 ***Significant genotype*test effect:*** $F_{(2,62)}$=5.267, p=0.0077; t test for 48hr test, p=0.0001	**Repeated-measures ANOVA** **Exploration time:** no genotype effect: $F_{(1,31)}$=0.503, p=0.4834 no genotype*session effect: $F_{(2,62)}$=0.543, p=0.5837

Control genotypes showed similar performance and were pooled

Repeated measures ANOVA for control genotypes:
Discrimination index: no genotype effect ($F_{(2,19)}$=0.143, p=0.8678); no genotype*test effect ($F_{(4,38)}$=0.481, p=0.7498)
Exploration time: no genotype effect ($F_{(2,19)}$=0.721, p=0.4991); no genotype*session effect ($F_{(4,38)}$=0.234, p=0.731)

NOVEL OBJECT

10-MIN Training

DT and Controls OFF DOX

N: DT=12 and Controls =12 (single tetO=5, single tTA=4, wild type=3)

The memory of DT mice for novel object recognition was impaired in the 24-hour test	
Repeated-measures ANOVA **Discrimination index:** ***Significant genotype effect:*** $F_{(1,22)}$=7.791, p=0.0106 ***Significant genotype*test effect:*** $F_{(1,22)}$=6.835, p=0.0158; t test for 24hr test, p=0.0023	**Repeated-measures ANOVA** **Exploration time:** no genotype effect: $F_{(1,22)}$=0.020, p=0.8900 no genotype*session effect: $F_{(1,22)}$=0.524, p=0.4768
Control genotypes showed similar performance and were pooled Repeated measures ANOVA for control genotypes: Discrimination index: no genotype effect ($F_{(2,9)}$=0.235, p=0.7954); no genotype*test effect ($F_{(2,9)}$=0.516, p=0.6133) Exploration time: no genotype effect ($F_{(2,9)}$=2.04, p=0.1729); no genotype*session effect ($F_{(2,9)}$=0.321, p=0.7333)	

NOVEL OBJECT
15-MIN Training
DT ON DOX and controls ON DOX

N: DT on dox=10 and Controls=17 (single tetO= 5, single tTA= 5, WT=7)

DT on dox and control on dox animals had similar performance	
Repeated-measures ANOVA **Discrimination index:** no genotype effect: $F_{(1,25)}$=0.001, p=0.9913 no genotype*session effect: $F_{(2,50)}$=0.106, p=0.8994	**Repeated-measures ANOVA** **Exploration time:** no genotype effect: $F_{(1,25)}$=0.05, p=0.8244 no genotype*session effect: $F_{(2,50)}$=0.093, p=0.9111
Control genotypes showed similar performance and were pooled Repeated measures ANOVA for control genotypes: Discrimination index: no genotype effect ($F_{(2,12)}$=0.192, p=0.7404); no genotype*test effect ($F_{(4,24)}$=0.467, p=0.7596) Exploration time: no genotype effect ($F_{(2,12)}$=0.192, p=0.8280); no genotype*session effect ($F_{(4,24)}$=0.283, p=0.8858)	

NOVEL OBJECT
10-MIN Training
DT ON DOX and controls ON DOX

N: DT on dox =12 and Controls=21 (single tetO=7, single tTA=7, WT=7)

DT on dox and control on dox animals had similar performance	
Repeated-measures ANOVA **Discrimination index:** no genotype effect: $F_{(1,31)}$=2.142, p=0.1634 no genotype*session effect: $F_{(1,31)}$=0.271, p=0.6066	**Repeated-measures ANOVA** **Exploration time:** no genotype effect: $F_{(1,31)}$=0.074, p=0.7868 no genotype*session effect: $F_{(1,31)}$=0.08, p=0.7793
Control genotypes showed similar performance and were pooled Repeated measures ANOVA for control genotypes: Discrimination index: no genotype effect ($F_{(2,18)}$=0.099, p=0.9060); no genotype*test effect ($F_{(2,18)}$=1.638, p=0.1943) Exploration time: no genotype effect ($F_{(2,18)}$=0.616, p=0.5509); no genotype*session effect ($F_{(2,18)}$=0.451, p=0.6443)	

NOVEL OBJECT
15-MIN Training
WT young (N=8) and WT aged (N=8)

The aged mice showed significantly lower memory performance during the 48-hour memory test	
Repeated-measures ANOVA **Discrimination index:** ***Significant age effect:*** $F_{(1,14)}$=14.068, p=0.0022 ***Significant age*session effect:*** $F_{(2,28)}$=6.425, p=0.0052; t test for 48hr test, p=0.0002	**Repeated-measures ANOVA** **Exploration time:** no genotype effect: $F_{(1,14)}$=1.433, p=0.2512 no genotype*session effect: $F_{(2,28)}$=0.249, p=0.7816

NOVEL OBJECT
10-MIN Training
WT young (N=10) and WT aged (N=10)

The aged mice showed significantly lower memory performance during the 24-hour memory test	
Repeated-measures ANOVA **Discrimination index:** ***Significant age*session effect:*** $F_{(1,18)}$=12.916, p=0.0021; t test for 24hr test, p=0.01	**Repeated-measures ANOVA** **Exploration time:** no genotype effect: $F_{(1,18)}$=0.015, p=0.9042 no genotype*session effect: $F_{(1,18)}$=0.110, p=0.7442

NOVEL OBJECT
10-MIN Training
WT aged/RbAp48-HA injected in DG (N=12) and WT aged/GFP injected in DG (N=10)

The RbAp48-injected aged mice performed significantly better than their GFP-injected littermates during the 24-hour memory test

Repeated-measures ANOVA **Discrimination index:** ***Significant injection*session effect:*** $F_{(1,20)}$=6.486, p=0.0192; t test for 24hr memory test, p=0.0275	**Repeated-measures ANOVA** **Exploration time:** no injection effect: $F_{(1,20)}$=0.884, p=0.3584 no injection*session effect: $F_{(1,20)}$=0.031, p=0.8617

MORRIS WATER MAZE
DT and Controls OFF DOX

N: DT=11 and Controls=22 (single tetO=6, single tTA=8, wild type=8)

(Same group as in the 15MIN-training novel object recognition task)

VISIBLE PLATFORM
The path lengths and escape latencies were similar among controls and DT

Repeated-measures ANOVA **Path length:** significant training day effect: $F_{(1,31)}$=29.05, p<0.0001 DT: $F_{(1,10)}$=22.740, p=0.0008; Controls: $F_{(1,21)}$=16.241, p=0.0006 no genotype effect: $F_{(1,31)}$= 0.5, p=0.48	**Repeated-measures ANOVA** **Escape latency:** significant day effect: $F_{(1,31)}$=32.94, p<0.0001 DT: $F_{(1,10)}$=23.637, p=0.0007; Controls: $F_{(1,21)}$=19.474, p=0.0002 no genotype effect: $F_{(1,31)}$= 0.325, p=0.5727

Control genotypes showed similar performance and were pooled

Repeated measures ANOVA for control genotypes:
Path length: no genotype effect ($F_{(2,19)}$=1.16, p=0.3348); no genotype*day effect ($F_{(2,19)}$=1.446, p=0.2604)
Latency: no genotype effect ($F_{(2,19)}$=1.176, p=0.3299); no genotype*day effect ($F_{(2,19)}$=1.579, p=0.2322)

Non-cognitive parameters
No significant genotype effect
Repeated measures ANOVA
Floating: $F_{(1,31)}$=1.402, p=0.2453; Speed: $F_{(1,31)}$=0.066, p=0.7991; Thigmotaxis: $F_{(1,31)}$=0.621, p=0.9268

HIDDEN PLATFORM-ACQUISITION
Similar path lengths and escape latencies between DT and controls

Repeated-measures ANOVA **Path length:** significant day effect: $F_{(7,217)}$=11.31, p<0.0001 DT: $F_{(7,70)}$=6.701, p<0.0001; Controls: $F_{(7,147)}$=4.998, p<0.0001 no genotype effect: $F_{(1,31)}$=1.695, p=0.20	**Repeated-measures ANOVA** **Escape latency:** significant day effect: $F_{(7,217)}$=10.36, p<0.0001 DT: $F_{(7,70)}$=6.228, p<0.0001; Controls: $F_{(7,147)}$=4.52, p=0.0001 no significant genotype effect: $F_{(1,31)}$=1.77, p=0.19

Control genotypes showed similar performance and were pooled

Repeated measures ANOVA for control genotypes:
Path length: no genotype effect ($F_{(2,19)}$=0.364, p=0.456); no genotype*day effect ($F_{(14,133)}$=1.009, p=0.2670)
Latency: no genotype effect ($F_{(2,19)}$=0.383, p=0.444); no genotype*day effect ($F_{(14,133)}$=1.049, p=0.3482)

Non-cognitive parameters
No significant genotype effect
Repeated-measures ANOVA
Floating: $F_{(1,31)}$=1.402, p=0.0745; Speed: $F_{(1,31)}$=0.066, p=0.2567; Thigmotaxis: $F_{(1,31)}$=0.621, p=0.3855

Probe Trial at week2/day 5 (one day after the end of training)

DT mice had less accurate knowledge of the platform position compared to controls

Repeated-measures ANOVA **Searching time:** Both groups showed significant preference for TQ compared with each of the other quadrants (Scheffe's test: DT:p<0.0001; Controls:p<0.0001) no significant genotype*quadrant effect: $F_{(3,93)}$=1.415, p=0.2434	**Repeated-measures ANOVA** **Platform crossings:** ***significant genotype*quadrant effect*:*** $F_{(3,93)}$=2.748, p=0.04; t test in TQ, p=0.017

Control genotypes showed similar performance and were pooled

Repeated measures ANOVA for control genotypes:
Searching time: no genotype effect ($F_{(2,19)}$=0.128, p=0.8805); no genotype*quadrant effect ($F_{(6,57)}$=1.025, p=0.2867)
Platform crossings: no genotype effect ($F_{(2,19)}$=0.567, p=0.5765); no genotype*quadrant effect ($F_{(6,57)}$=0.967, p=0. 3434)

HIDDEN PLATFORM-TRANSFER

Similar path lengths and escape latencies between DT and controls.

Repeated-measures ANOVA **Path length:** significant day effect: $F_{(7,217)}$=23.82, p<0.0001 DT: $F_{(7,70)}$=10.263, p<0.0001; Controls: $F_{(7,147)}$=16.51, p<0.0001 no genotype effect: $F_{(1,31)}$=2.32, p=0.138	**Repeated-measures ANOVA** **Escape latency:** significant day effect: $F_{(7,217)}$=30.28, p<0.0001 DT: $F_{(7,70)}$=12.598, p<0.0001; Controls: $F_{(7,147)}$=20.83, p<0.0001 no significant genotype effect: $F_{(1,31)}$=2.75, p=0.1

Control genotypes showed similar performance and were pooled

Repeated measures ANOVA for control genotypes:
Path length: no genotype effect ($F_{(2,19)}$=1.439, p=0.2618); no genotype*day effect ($F_{(7,133)}$=0.445, p=0.9566)
Latency: no genotype effect ($F_{(2,19)}$=1.564, p=0.2351); no genotype*day effect ($F_{(7,133)}$=0.477, p=0. 9419)

Non-cognitive parameters
No significant genotype effect
Repeated measures ANOVA
Floating: $F_{(1,31)}$=0.049, p=0.8263; Speed: $F_{(1,31)}$=0.517, p=0.4777; Thigmotaxis: $F_{(1,31)}$=0.621, p=0.0787

Probe Trial at week2/day 5 (one day after the end of training)

DT mice showed a bad memory of the new platform location

Repeated-measures ANOVA **Searching time:** Controls showed significant preference for the TQ compared with each of the other quadrants (Scheffe's test, p<0.0001), while the DT did not (Scheffe's test: TQ-Q2:p=0.013, TQ-Q3:p=0.476, TQ-Q4:p=0.4258). ***significant genotype*quadrant effect:*** $F_{(3,93)}$=3.201, p= 0.0269; t test for TQ,, p= 0.012	**Repeated-measures ANOVA** **Platform crossings:** ***significant genotype*quadrant effect*:** $F_{(3,93)}$=3.023, p=0.03; t test for TQ, p=0.035

Control genotypes showed similar performance and were pooled

Repeated measures ANOVA for control genotypes:
Searching time: no genotype effect ($F_{(2,19)}$=0.747, p=0.4872); no genotype*quadrant effect ($F_{(6,57)}$=0.508, p=0.801)
Platform crossings: no genotype effect ($F_{(2,19)}$=0.312, p=0.7354); no genotype*quadrant effect ($F_{(6,57)}$=1.012, p=0. 4266)

MORRIS WATER MAZE

DT ON DOX and controls ON DOX

N: DT on dox=10 and Controls=17 (single tetO= 5, single tTA= 5, WT=7)

(Same group as in the 15MIN-training novel object recognition task)

VISIBLE PLATFORM

The path lengths and escape latencies were similar among controls and DT mice

Repeated-measures ANOVA **Path length:** significant training day effect: $F_{(1,25)}$=41.991, p<0.0001 DT on dox: $F_{(1,9)}$=14.748, p=0.0004; Controls on dox: $F_{(1,16)}$=28.428, p<0.0001 no genotype effect: $F_{(1,25)}$=0.389, p = 0.5384	**Repeated-measures ANOVA** **Escape latency:** significant day effect: $F_{(1,25)}$=50.134, p<0.0001 DT on dox: $F_{(1,9)}$=17.969, p=0.0002; Controls on dox: $F_{(1,16)}$=33.375, p<0.0001 no genotype effect: $F_{(1,25)}$=0.568, p=0.4583

Control genotypes showed similar performance and were pooled

Repeated measures ANOVA for control genotypes:
Path length: no genotype effect ($F_{(2,14)}$=1.225, p=0.3234); no genotype*day effect ($F_{(2,14)}$=0.582, p=0.5716)
Latency: no genotype effect ($F_{(2,14)}$=0.865, p=0.4423); no genotype*day effect ($F_{(2,14)}$=0.413, p=0.6697)

Non-cognitive parameters
No significant genotype effect
Repeated measures ANOVA
Floating: $F_{(1,25)}$=0.539, p=0.4697; Speed: $F_{(1,25)}$=1.453, p=0.2394; Thigmotaxis: $F_{(1,25)}$=0.558, p=0.4621

HIDDEN PLATFORM-ACQUISITION

The path lengths and escape latencies were similar among controls on dox and DT on dox mice

Repeated-measures ANOVA **Path length:** significant training day effect: $F_{(7,175)}$=11.335, p<0.0001 DT on dox: $F_{(7,63)}$=4.524, p=0.0004; Controls on dox: $F_{(7,112)}$=7.304, p<0.0001	**Repeated-measures ANOVA** **Escape latency:** significant day effect: $F_{(7,175)}$=10.293, p<0.0001 DT on dox: $F_{(7,63)}$=4.221, p=0.0007; Controls on dox: $F_{(7,112)}$=6.388, p<0.0001

no genotype effect: $F_{(1,25)}$=0.562, p = 0.1645	no genotype effect: $F_{(1,25)}$=0.290, p=0.1427
Control genotypes showed similar performance and were pooled Repeated measures ANOVA for control genotypes: Path length: no genotype effect ($F_{(2,14)}$=1.181, p=0.3358); no genotype*day effect ($F_{(14,98)}$=0.701, p=0.7682) Latency: no genotype effect ($F_{(2,14)}$=1.07, p=0.3694); no genotype*day effect ($F_{(14,98)}$=0.595, p=0.8634)	
Non-cognitive parameters *No significant genotype effect* **Repeated measures ANOVA** Floating: $F_{(1,25)}$=0.022, p=0.8825; Speed: $F_{(1,25)}$=0.462, p=0.5031 Thigmotaxis: $F_{(1,25)}$=0.739, p=0.3981	
Probe Trial at week2/day 5 (one day after the end of training) *DT on dox and control on dox mice showed similar performance*	
Repeated-measures ANOVA **Searching time:** Both groups showed significant preference for TQ compared with each of the other quadrants (Scheffe's test: DT on dox:p<0.0009; Controls on dox:p<0.0001) no significant genotype*quadrant effect: $F_{(3,75)}$= 0.385, p=0.7639	**Repeated-measures ANOVA** **Platform crossings:** no significant genotype*quadrant effect: $F_{(3,75)}$=1.119, p = 0.3470
Control genotypes showed similar performance and were pooled Repeated measures ANOVA for control genotypes: Searching time: no genotype effect ($F_{(2,14)}$=1.074, p=0.3626); no genotype*quadrant effect ($F_{(6,42)}$=1.025, p=0.3389) Platform crossings: no genotype effect ($F_{(2,14)}$=0.870, p=0.3906); no genotype*quadrant effect ($F_{(6,42)}$=0.725, p=0. 4389)	
HIDDEN PLATFORM-TRANSFER *The path lengths and escape latencies were similar among controls on dox and DT on dox*	
Repeated-measures ANOVA **Path length:** significant training day effect: $F_{(7,175)}$=16.065, p<0.0001 DT on dox: $F_{(7,63)}$=9.941, p<0.0001; Controls on dox: $F_{(7,112)}$=7.935, p<0.0001 no genotype effect: $F_{(1,25)}$=0.245, p=0.6253	**Repeated-measures ANOVA** **Escape latency:** significant training day effect: $F_{(7,175)}$=20.039, p<0.0001 DT on dox: $F_{(7,63)}$=11.065, p<0.0001; Controls on dox: $F_{(7,112)}$=10.291, p<0.0001 no genotype effect: $F_{(1,25)}$=0.507, p=0.4829
Control genotypes showed similar performance and were pooled Repeated measures ANOVA for control genotypes: Path length: no genotype effect ($F_{(2,14)}$=0.997, p=0.3936); no genotype*day effect ($F_{(14,98)}$=0.766, p=0.7029) Latency: no genotype effect ($F_{(2,14)}$=0.694, p=0.5159); no genotype*day effect ($F_{(14,98)}$=0.737, p=0.7324)	
Non-cognitive parameters *No significant genotype effect* **Repeated measures ANOVA** Floating: $F_{(1,25)}$=0.388, p=0.5389; Speed: $F_{(1,25)}$=0.023, p=0.8811; Thigmotaxis: $F_{(1,25)}$=1.75, p=0.1	
Probe Trial at week2/day 5 (one day after the end of training) *DT on dox and control on dox mice showed similar performance*	
Repeated-measures ANOVA **Searching time:** Both groups showed significant preference for TQ compared with each of the other quadrants (Scheffe's test: DT on dox:p<0.001; Controls on dox:p<0.0001) no significant genotype*quadrant effect: $F_{(3,75)}$=0.221, p=0.8813	**Repeated-measures ANOVA** **Platform crossings:** no significant genotype*quadrant effect: $F_{(3,75)}$=0.375, p=0.7716
Control genotypes showed similar performance and were pooled Repeated measures ANOVA for control genotypes: Searching time: no genotype effect ($F_{(2,14)}$=1.262, p=0.3135); no genotype*quadrant effect ($F_{(6,42)}$=1.141, p=0.3560) Platform crossings: no genotype effect ($F_{(2,14)}$=1.139, p=0.3101); no genotype*quadrant effect ($F_{(6,42)}$=0.503, p=0. 8027)	
MORRIS WATER MAZE **WT young (N=14) and WT aged (N=14)**	
VISIBLE PLATFORM *The path lengths, but not the escape latencies, were similar between aged and young wild type mice.* *The groups learned the task equally well*	
Repeated-measures ANOVA	**Repeated-measures ANOVA**

Path length: significant training day effect: $F_{(1,26)}$=81.357, p<0.0001 Aged: $F_{(1,13)}$=41.671, p<0.0001; Young: $F_{(1,13)}$=39.694, p<0.0001 no age effect: $F_{(1,26)}$=1.172, p=0.2890	**Escape latency:** significant training day effect: $F_{(1,26)}$=79.703, p<0.0001 Aged: $F_{(1,13)}$=60.917, p<0.0001; Young: $F_{(1,13)}$=26.715, p=0.0002 significant age effect: $F_{(1,26)}$=5.143, p=0.0319
Non-cognitive parameters *The swimming speed of aged mice was significantly lower compared to young mice* **Repeated measures ANOVA** no significant age effect for Floating: $F_{(1,26)}$=0.447, p=0.5098 ***significant age effect for Speed:*** $F_{(1,26)}$=8.922, p=0.0061 no significant age effect for Thigmotaxis: $F_{(1,26)}$=0.933, p=0.3429	
HIDDEN PLATFORM-ACQUISITION *The path lengths, but not the escape latencies, were similar among aged and young wild type mice.* *The groups learned the task equally well*	
Repeated-measures ANOVA **Path length:** significant training day effect: $F_{(7,182)}$=9.481, p<0.0001 Aged: $F_{(7,91)}$=5.101, p<0.0001; Young: $F_{(7,91)}$=4.834, p=0.0001 no age effect: $F_{(1,26)}$=0.714, p=0.4058	**Repeated-measures ANOVA** **Escape latency:** significant day effect: $F_{(7,182)}$=6.526, p<0.0001 Aged: $F_{(7,91)}$=3.680, p=0.0015; Young: $F_{(7,91)}$=5.790, p=0.0011 significant age effect: $F_{(1,26)}$=14.984, p=0.0007
Non-cognitive parameters *The swimming speed of aged mice was significantly lower than that of young mice* **Repeated measures ANOVA** no significant age effect for Floating: $F_{(1,26)}$=0.539, p=0.4693 ***significant age effect for Speed:*** $F_{(1,26)}$=29.995, p<0.0001 no significant age effect for Thigmotaxis: $F_{(1,26)}$=0.051, p=0.8239	
Probe Trial at week2/day 5 (one day after the end of training) ***The aged mice displayed significantly lower performance compared to young animals***	
Repeated-measures ANOVA **Searching time:** Both groups showed preference for TQ compared with each of the other quadrants (Scheffe's test: Aged:p<0.02; Young:p<0.0001) ***significant age*quadrant effect:*** $F_{(3,78)}$=7.666, p=0.0001; t-test for TQ, p=0.003	**Repeated-measures ANOVA** **Platform crossings:** ***significant age effect*:** $F_{(1,26)}$=7.946, p<0.0091 ***significant age*quadrant effect*:** $F_{(3,78)}$=6.939, p=0.0003; t test for TQ, p=0.0015
HIDDEN PLATFORM-TRANSFER *The path lengths, but not the escape latencies, were similar between aged and young wild type mice.* *Both groups learned the task equally well*	
Repeated-measures ANOVA **Path length:** significant training day effect: $F_{(7,182)}$=14.342, p<0.0001 Aged: $F_{(7,91)}$=9.766, p<0.0001; Young: $F_{(7,91)}$=5.251, p=0.0001 no age effect: $F_{(1,26)}$=2. 808, p=0.1058	**Repeated-measures ANOVA** **Escape latency:** significant day effect: $F_{(7,182)}$=14.342, p<0.0001 Aged: $F_{(7,91)}$=6.353, p<0.0001; Young: $F_{(7,91)}$=5.472, p=0.0001 ***significant age effect:*** $F_{(1,26)}$=15.682; p<0.0005
Non-cognitive parameters *The swimming speed of aged mice was significantly lower compared to young mice* **Repeated measures ANOVA** no significant age effect for Floating: $F_{(1,26)}$=2.964, p=0.1070 ***significant age effect for Speed:*** $F_{(1,26)}$=27.602, p<0.0001 no significant age effect for Thigmotaxis: $F_{(1,26)}$=0.003, p=0.9569	
Probe Trial at week2/day 5 (end of training) ***The aged mice displayed significantly lower performance compared to young animals***	
Repeated-measures ANOVA **Searching time:** Both groups showed preference for TQ compared with each of the other quadrants (Scheffe's test: Aged:p<0.014; Young:p<0.0001) ***significant age*quadrant effect:*** $F_{(3,78)}$=8.333, p<0.0001; t-test for TQ, p=0.0037	**Repeated-measures ANOVA** **Platform crossings:** ***significant age effect*:** $F_{(1,26)}$=5.206, p=0.0309 ***significant age*quadrant effect*:** $F_{(3,78)}$=7.560, p=0.0002; t test for TQ, p=0.002

MORRIS WATER MAZE

WT aged/RbAp48-HA injected in DG (N=12) and WT aged/GFP injected in DG (N=10)

(Same group as in novel object task)

VISIBLE PLATFORM

Both groups showed similar escape latencies and path lengths

Repeated-measures ANOVA **Path length:** significant training day effect: $F_{(1,20)}$=109.191, p<0.0001 RbAp48: $F_{(1,11)}$=63.891, p<0.0001; GFP: $F_{(1,9)}$=55.119, p<0.0001 no injection effect: $F_{(1,20)}$=0.838, p=0.36	**Repeated-measures ANOVA** **Escape latency:** significant training day effect: $F_{(1,20)}$=205.467, p<0.0001 RbAp48: $F_{(1,11)}$=129.115, p<0.0001; GFP: $F_{(1,9)}$=83.728, p<0.0001 no injection effect: $F_{(1,20)}$=0.908, p=0.3521

Non-cognitive parameters

No significant injection effect

Repeated measures ANOVA

Floating: $F_{(1,20)}$=0.161, p=0.6927; Speed: $F_{(1,20)}$=0.089, p=0.7688; Thigmotaxis: $F_{(1,20)}$=0.005, p=0.9433

HIDDEN PLATFORM-ACQUISITION

The path lengths and escape latencies were similar between the two groups of injected mice

Repeated-measures ANOVA **Path length:** significant training day effect: $F_{(7,140)}$=6.94, p<0.0001 RbAp48: $F_{(7,77)}$=5.384, p<0.0001; GFP: $F_{(7,63)}$=2.422, p=0.0292 no significant injection effect: $F_{(1,20)}$= 3.657, p=0.0703	**Repeated-measures ANOVA** **Escape latency:** significant day effect: $F_{(7,140)}$=8.565, p<0.0001 RbAp48: $F_{(7,77)}$=5.804, p<0.0001; GFP: $F_{(7,63)}$=3.332, p=0.0044 no injection effect: $F_{(1,20)}$=4.055, p=0.0577

Non-cognitive parameters

No significant injection effect

Repeated measures ANOVA

Floating: $F_{(1,20)}$=0.654, p=0.4282; Speed: $F_{(1,20)}$=0.163, p=0.6910; Thigmotaxis: $F_{(1,20)}$=0.064, p=0.8026

Probe Trial at week2/day 5 (end of training)

The aged mice injected with RbAp48 in their DG formed a better memory of the platform location than did GFP-injected aged mice (controls)

Repeated-measures ANOVA **Searching time:** Both groups showed preference for the TQ compared with each of the other quadrants (Scheffe's test, RbAp48:p<0.0001; GFP: p<0.008) ***significant injection*quadrant effect:*** $F_{(3,60)}$=4.004, p=0.0115; t test for TQ: p=0.0285	**Repeated-measures ANOVA** **Platform crossings:** ***significant injection*quadrant effect*:** $F_{(3,60)}$=4.179, p=0.0094; t test for TQ: p=0.0133

HIDDEN PLATFORM-TRANSFER

The path lengths and escape latencies were similar between the two groups of injected mice

Repeated-measures ANOVA **Path length:** significant training day effect: $F_{(7,140)}$=6.56, p<0.0001 RbAp48: $F_{(7,77)}$=3.476, p=0.0027; GFP: $F_{(7,63)}$=3.732, p=0.0019 no significant injection effect: $F_{(1,20)}$= 0.153, p=0.6996	**Repeated-measures ANOVA** **Escape latency:** significant training day effect: $F_{(7,140)}$=7.438, p<0.0001 RbAp48: $F_{(7,77)}$=3.701, p=0.0017; GFP: $F_{(7,63)}$=4.342, p=0.0006 no injection effect: $F_{(1,20)}$=70.097, p=0.7587

Non-cognitive parameters

No significant injection effect

Repeated-measures ANOVA

Floating: $F_{(1,20)}$=0.736, p=0.4011; Speed: $F_{(1,20)}$=0.018, p=0.8954; Thigmotaxis: $F_{(1,20)}$=0.029, p=0.8672

Probe Trial at week2/day 5 (one day after the end of training)

The aged mice injected with RbAp48 in their DG formed a good memory of the new platform location

Repeated-measures ANOVA **Searching time:** The RbAp48-injected mice showed significant preference for the new TQ compared with each of the other quadrants (Scheffe's test, p<0.0001). The GFP-injected littermates did not (Scheffe's test: p>0.5).	**Repeated-measures ANOVA** **Platform crossings:** ***significant injection*quadrant effect*:** $F_{(3,60)}$=2.86, p=0.0443; t test for TQ: p=0.0160

significant injection*quadrant effect: $F_{(3,60)}$=3.275, p=0.0271; t-test for TQ: p=0.0119	

HISTONE ACETYLATION

Transgenic mice off and on dox

For number of mice and number of slices see Table S1

AcH2B (lys20) and AcH4(lys12)

Reduced levels in the DG of DT off dox compared to all of the controls. No difference in CA1

ANOVA (DG) AcH2B: $F_{(3,95)}$=5.069, p=0.0027 AcH4: $F_{(3,83)}$=7.458, p=0.0002)	**ANOVA (CA1)** AcH2B: $F_{(3,95)}$=0.469, p=0.7043 AcH4: $F_{(3,83)}$=2.192, p=0.0951

No difference was observed between DT on dox and controls on and off dox

ANOVA AcH2B: CA1: $F_{(2,69)}$=1.014, p=0.3683 DG: $F_{(2,69)}$=0.261, p=0.7707	AcH4: CA1: $F_{(2,62)}$=0.419, p=0.6598 DG: $F_{(2,62)}$=0.452, p=0.6382

AcH3 (lys9)

No differences were observed between DT off dox and controls off dox

ANOVA

CA1: $F_{(1,42)}$=0.052, p=0.8214 DG: $F_{(1,42)}$=0.637, p=0.4292

Control genotypes were similar and pooled

ANOVA for control genotypes off dox

AcH2B: CA1: $F_{(1,40)}$=0.923, p=0.504 and DG: $F_{(1,40)}$=1.064, p=0.3550; AcH4: CA1: $F_{(1,37)}$=0.893, p=0.3509 and DG: $F_{(1,37)}$=1.122, p=0.2740
AcH3: CA1: $F_{(1,26)}$=0.485, p=0.4924 and DG: $F_{(1,26)}$=0.821, p=0.3732

ANOVA for control genotypes on dox

AcH2B: CA1: $F_{(1,13)}$=0.087, p=0.7723 and DG: $F_{(1,13)}$=1.195, p=0.2613; AcH4: CA1: $F_{(1,9)}$=1.09, p=0.3238 and DG: $F_{(1,9)}$=0.145, p=0.7126

HISTONE ACETYLATION

WT aged/RbAp48-HA injected in DG and WT aged/GFP injected in DG

For number of mice and number of slices see Table S1

DG-specific increase of the levels of AcH2B (lys20) and AcH4(lys12), but not AcH3(Lys9), in the RbAp48-injected mice

ANOVA AcH2B: CA1: $F_{(1,46)}$=1.998, p=0.1643 **DG: $F_{(1,46)}$=11.482, p=0.0014**	**ANOVA** AcH4: CA1: $F_{(1,46)}$=1.276, p=0.2648 **DG: $F_{(1,46)}$==16.696, p=0.0002**	**ANOVA** AcH3: CA1: $F_{(1,46)}$=2.9, p=0.0953 DG: $F_{(1,46)}$=1.294, p=0.2611

CBP HAT ASSAYS

DT and controls off and on dox

N: DT off dox=3, DT on dox=3, Control off dox =3 (single tTA=1, single tetO=1, WT=1) and Control on dox =3 (single tTA on dox=1, single tetO on dox =1, WT on dox=1)

Three CPM measurements for each IP/HAT assay

The CBP HAT activity was significantly reduced in the DG of DT off dox compared either to control off dox and on dox

ANOVA for fold difference of HAT activity

DT off dox vs control off dox:
DG: $F_{(1,16)}$=21.294, p=0.0003; CA3-CA1: $F_{(1,16)}$=2.274, p=0.1511 DT off dox vs control on dox:
DG: $F_{(1,16)}$=20.080, p=0.0004; CA3-CA1: $F_{(1,16)}$=1.414, p=0.2518The

CBP HAT activity in both DG and CA3-CA1 was similar between DT on dox and controls on and off dox

ANOVA for fold difference of HAT activity

DT on dox vs control on dox:
DG: $F_{(1,16)}$=0.015, p=0.9035; CA3-CA1: $F_{(1,16)}$=0.058, p=0.8135
DT on dox vs control off dox:
DG: $F_{(1,16)}$=0.694, p=0.4169; CA3-CA1: $F_{(1,16)}$=0.062, p=0.8064

The CBP HAT activity was significantly increased in the DG of DT on dox compared to DT off dox. Similar CBP HAT activity in CA3-CA1

ANOVA for fold difference of HAT activity

DG: $F_{(1,16)}$=18.5, p=0.0005; CA3-CA1: $F_{(1,16)}$=2.8, p=0.1137

Controls off and on dox were similar (no dox treatment effect)

ANOVA for fold difference of HAT activity
DG: $F_{(1,16)}$=0.981, p=0.3367; CA3-CA1: $F_{(1,16)}$=0.004, p=0.9489

The control genotypes off dox as well as the control genotypes on dox did not show significance difference and were pooled
ANOVA for fold difference of HAT activity
Controls off dox: DG: $F_{(2,6)}$=0.351, p=0.7176; CA3-CA1: $F_{(2,6)}$=2.131, p=0.1998
Controls on dox: DG: $F_{(2,6)}$=2.003, p=0.2156; CA3-CA1: $F_{(2,6)}$=2.153, p=0.1593

CBP HAT ASSAYS
WT Aged (N=4) and WT Young (N=4)

Three CPM measurements for each IP/HAT assay

The CBP HAT activity was significantly reduced in the DG of aged mice

ANOVA for fold difference of HAT activity
DG: $F_{(1,22)}$=15.847, p=0.0006; CA3-CA1: $F_{(1,22)}$=1.357, p=0.0925

CBP HAT ASSAYS
WT aged/RbAp48-HA injected in DG (N=3) and WT aged/GFP injected in DG (N=3)

Three CPM measurements for each IP/HAT assay

The CBP HAT activity was significantly increased in the DG of RbAp48-HA-injected mice

ANOVA for fold difference of HAT activity
DG: $F_{(1,16)}$=26.059, p=0.0001; CA3-CA1: $F_{(1,16)}$=2.147, p=0.1623

CBP PROTEIN LEVELS
DT and controls off and on dox

N: DT off dox=3, DT on dox=3, control off dox=3 (single tTA=1, single tetO=1, WT=1) and control on dox=3 (single tTA on dox=1, single tetO on dox =1, WT on dox=1)

(Same group of mice as in HAT assays)

The CBP protein levels were similar among all groups of mice

ANOVA for fold difference of protein levels
DG: $F_{(3,8)}$=0.176, p=0.9096; CA3-CA1: $F_{(3,8)}$=0.855, p=0.5024

Repeated measures ANOVA for fold difference of protein levels
No genotype effect:
DG: $F_{(1,4)}$=0.394, p=0.5645; CA3-CA1: $F_{(1,4)}$=0.210, p=0.6704
*No genotype*treatment interaction effect (no dox-diet effect):*
DG: $F_{(1,4)}$=0.027 p=0.8767; CA3-CA1: $F_{(1,4)}$=1.07, p=0.4402

CBP PROTEIN LEVELS
WT Aged (N=4) and WT Young (N=4)

(Same group of mice as in HAT assays)

The CBP protein levels were similar between the two groups

ANOVA for fold difference of protein levels
No age effect:
DG: $F_{(1,6)}$=0.250, p=0.6306; CA3-CA1: $F_{(1,6)}$=0.776, p=0.4041

CBP PROTEIN LEVELS
WT aged/RbAp48-HA injected in DG (N=3) and WT aged/GFP injected in DG (N=3)

(Same group of mice as in HAT assays)

The CBP protein levels were similar between the two groups

ANOVA for fold difference of protein levels
No age effect:
$F_{(1,4)}$=0.460, p=0.5347; CA3-CA1: $F_{(1,4)}$=0.003, p=0.9621