Supporting data for

eEF2/eEF2K pathway in the mature dentate gyrus determines neurogenesis level and cognition

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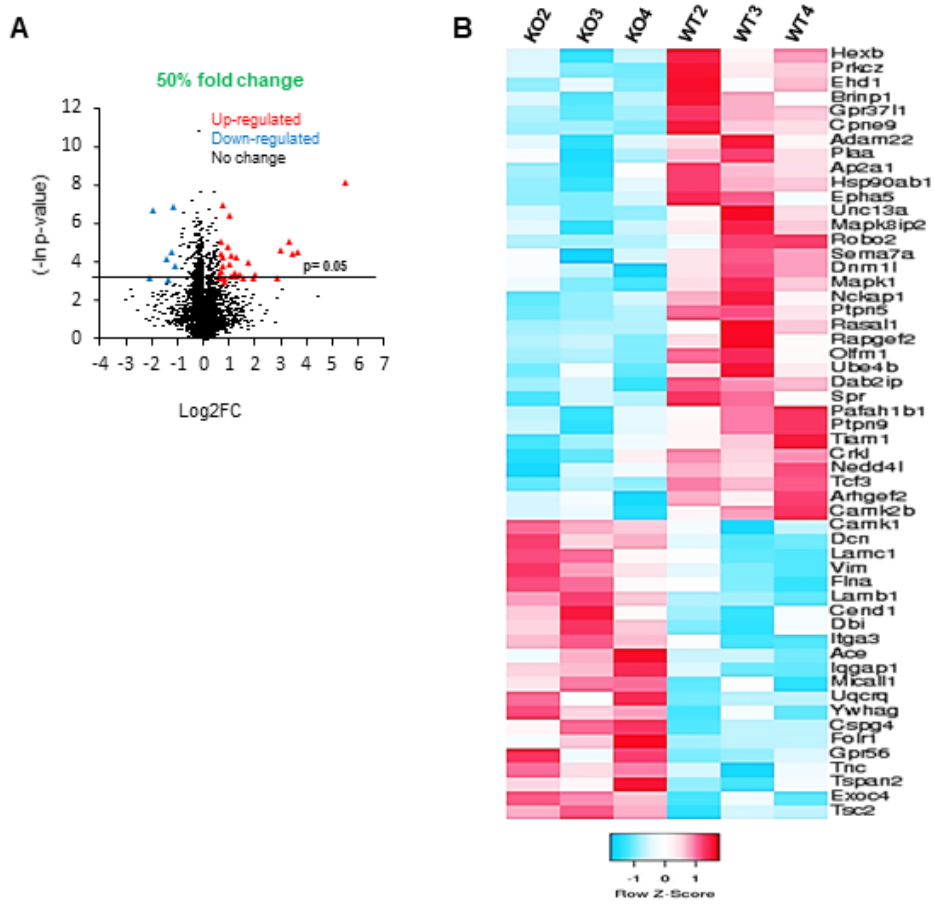
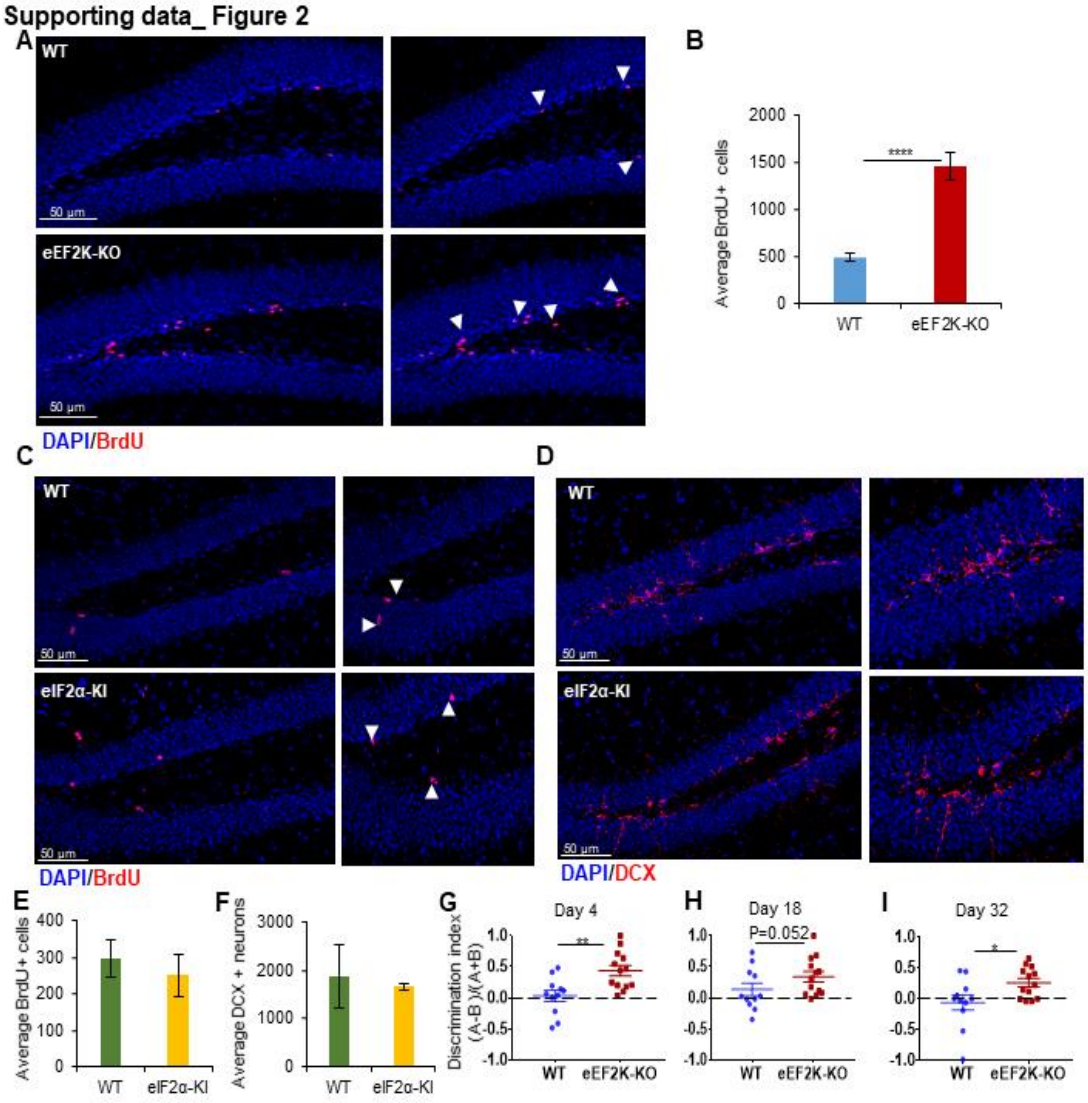
Supporting data_ Figure 1



Supporting data Figure 1, related to main Figure 1

(A) Volcano plot with a 50% cutoff showing the differentially expressed proteins between eEF2K-KO and WT mice. There are more up-regulated proteins in eEF2K-KO mice compared to WT mice.

(B) Heat-map showing the normalized intensity of 54 proteins identified as neurogenesis-related proteins in eEF2K-KO compared to WT mice.



Supporting data Figure 2, related to main Figure 1

(A) Representative coronal hippocampal sections immunostained for BrdU from eEF2K-KO and WT littermate mice. Scale bar, 50µm, 20x.

(B) Quantification of BrdU positive cells. eEF2K-KO mice show higher levels of BrdU positive cells. Mean±SEM are shown (t-test, n=8, $p<0.0001$)

(C-D) Representative coronal hippocampal sections immunostained for BrdU and DCX from eIF2α-KI and WT littermate mice. Scale bar, 50µm, 20x.

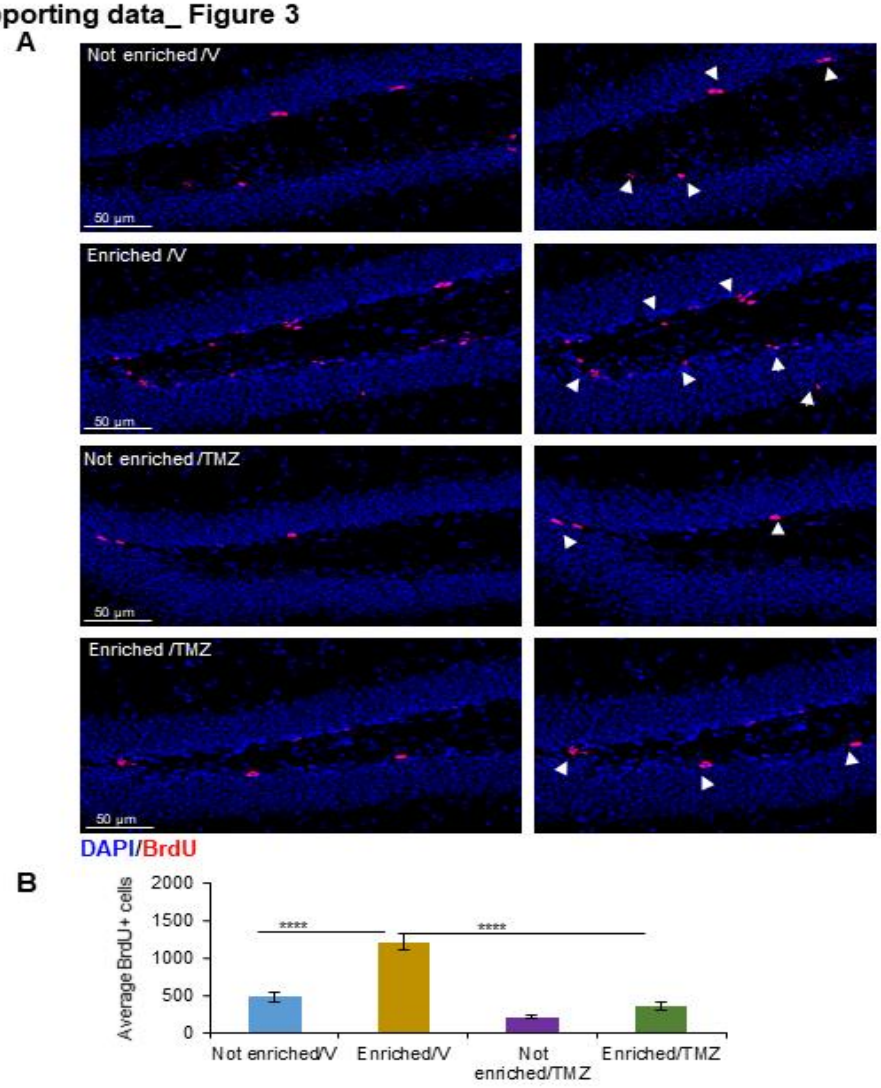
(E-F) There is no change in BrdU and DCX levels in eIF2α-KI compared to WT mice. Mean±SEM are shown (BrdU: Mann-Whitney U test, n=3, $p>0.05$; DCX: Mann-Whitney U test, n=3-4, $p>0.05$)

(G) Discrimination index analysis in eEF2K-KO and WT mice. Discrimination index was calculated as: (%freezing in context A -%freezing in context B)/(Total % of freezing in contexts A and B). eEF2K-KO mice have significantly better discrimination index between context A and B on day 4. Mean±SEM are shown (t-test, n=11-13 $p<0.01$).

(H) eEF2K-KO mice and WT mice have a similar discrimination index between context A and B on day 18. Mean±SEM are shown (Mann-Whitney U test, n=11-13 $p=0.052$).

(I) eEF2K-KO mice have a significantly better discrimination index between context A and B on day 32. Mean±SEM are shown (t-test, n=11-13 $p<0.05$).

Supporting data_ Figure 3

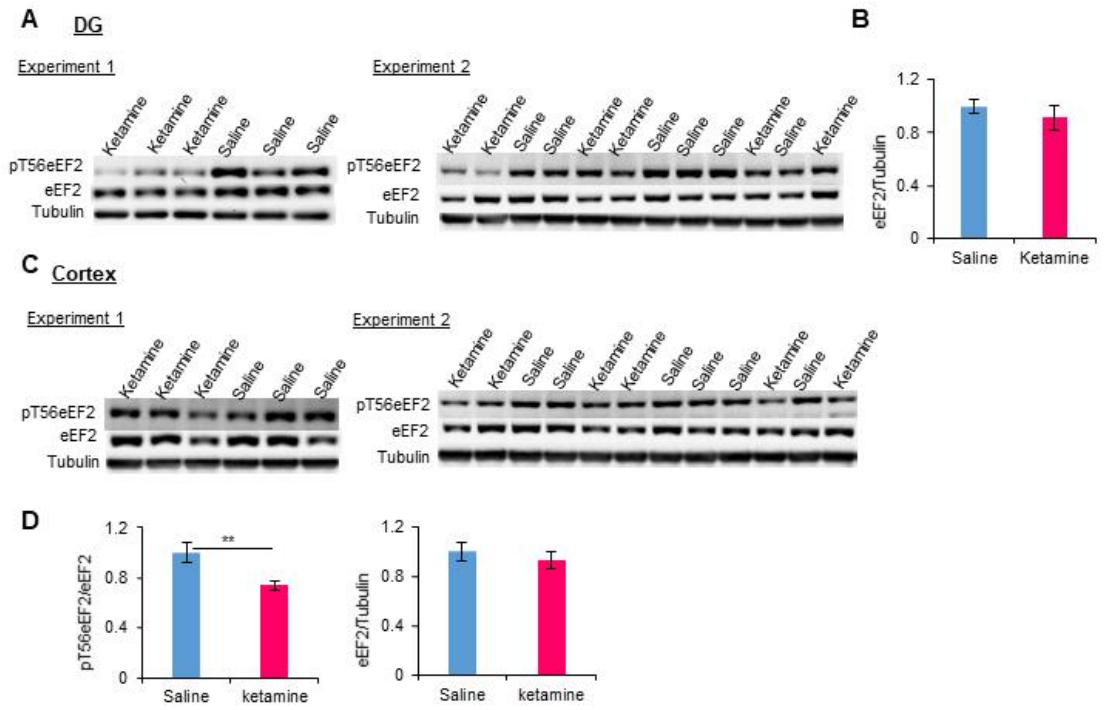


Supporting data Figure 3, related to main Figure 2

(A) Representative coronal hippocampal sections immunostained for BrdU from WT mice, four groups: 1. Not Enriched/vehicle; 2. Enriched/vehicle; 3. Not enriched/TMZ 4. Enriched/TMZ. Scale bar, 50µm, 20x.

(B) Quantification of BrdU positive cells in the different groups. Enriched environment induced an increase in BrdU positive cells, which was blocked by TMZ. Mean±SEM are shown (Two-way ANOVA, n=8, $p<0.0001$).

Supporting data_ Figure 4



Supporting data Figure 4, related to main Figure 2

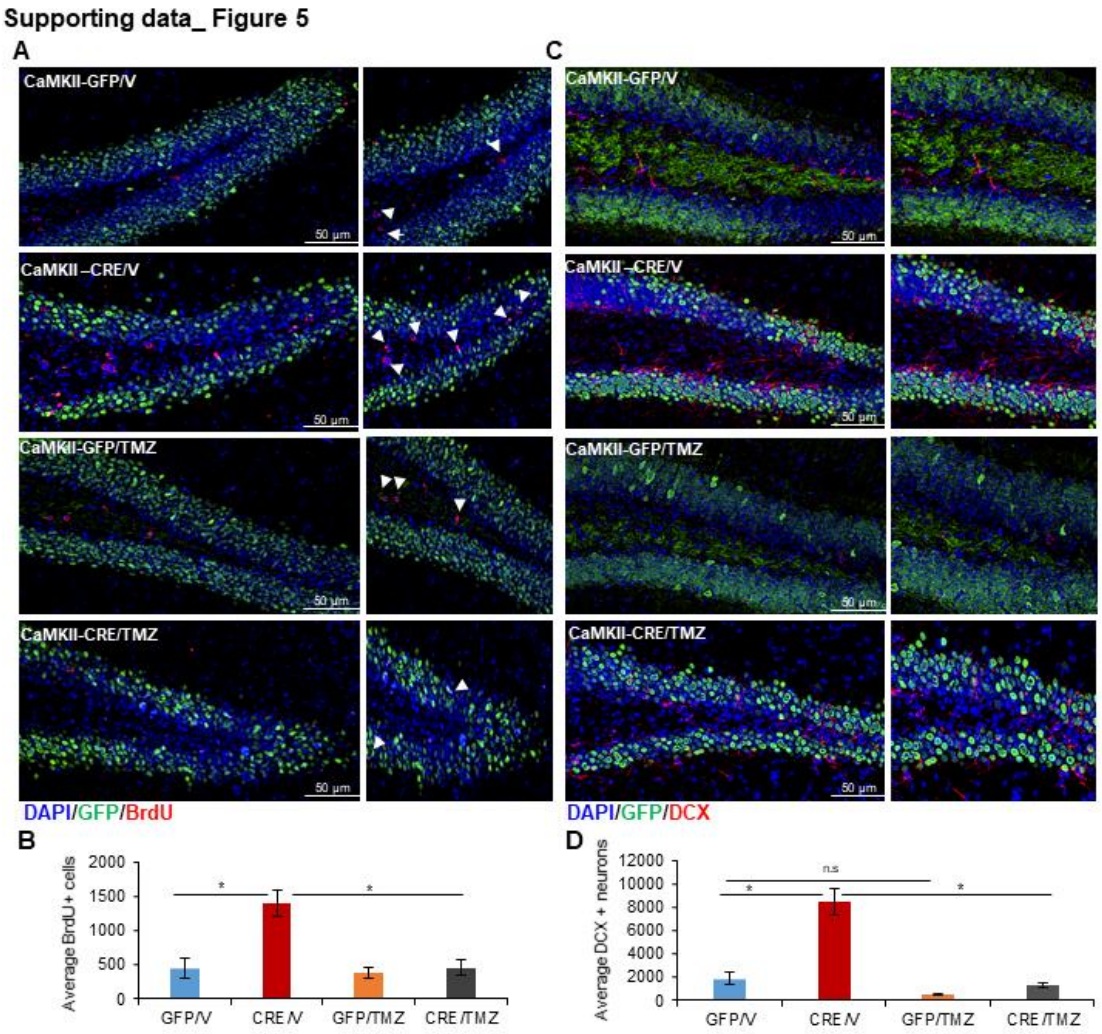
(A) Full length uncropped original immunoblots of pThr56eEF2, eEF2, and tubulin from DG samples collected 30min after saline/ketamine (5mg/kg) i.p. injection from two experiments.

(B) There is no change in eEF2 protein levels normalized to tubulin in ketamine-injected mice compared to saline-injected mice in DG samples. Mean±SEM are shown (Mann-Whitney U test, n= 9, $p>0.05$).

(C) Full length uncropped original immunoblots of pThr56eEF2, eEF2, and tubulin from cortical samples collected 30min after saline/ketamine (5mg/kg) i.p. injection from two experiments.

(D) (Left) pT56eEF2 normalized to eEF2 protein in cortex tissue following 30 min of ketamine injection. Ketamine, 5mg/kg, reduces pT56eEF2 levels in ketamine-injected mice compared to control. Mean±SEM are shown (t-test, n= 9, $p<0.01$). (Right) there

is no change in eEF2 protein level normalized to tubulin in ketamine-injected mice compared to control mice in cortex tissue. Mean±SEM are shown (t-test, n= 9, p>0.05).



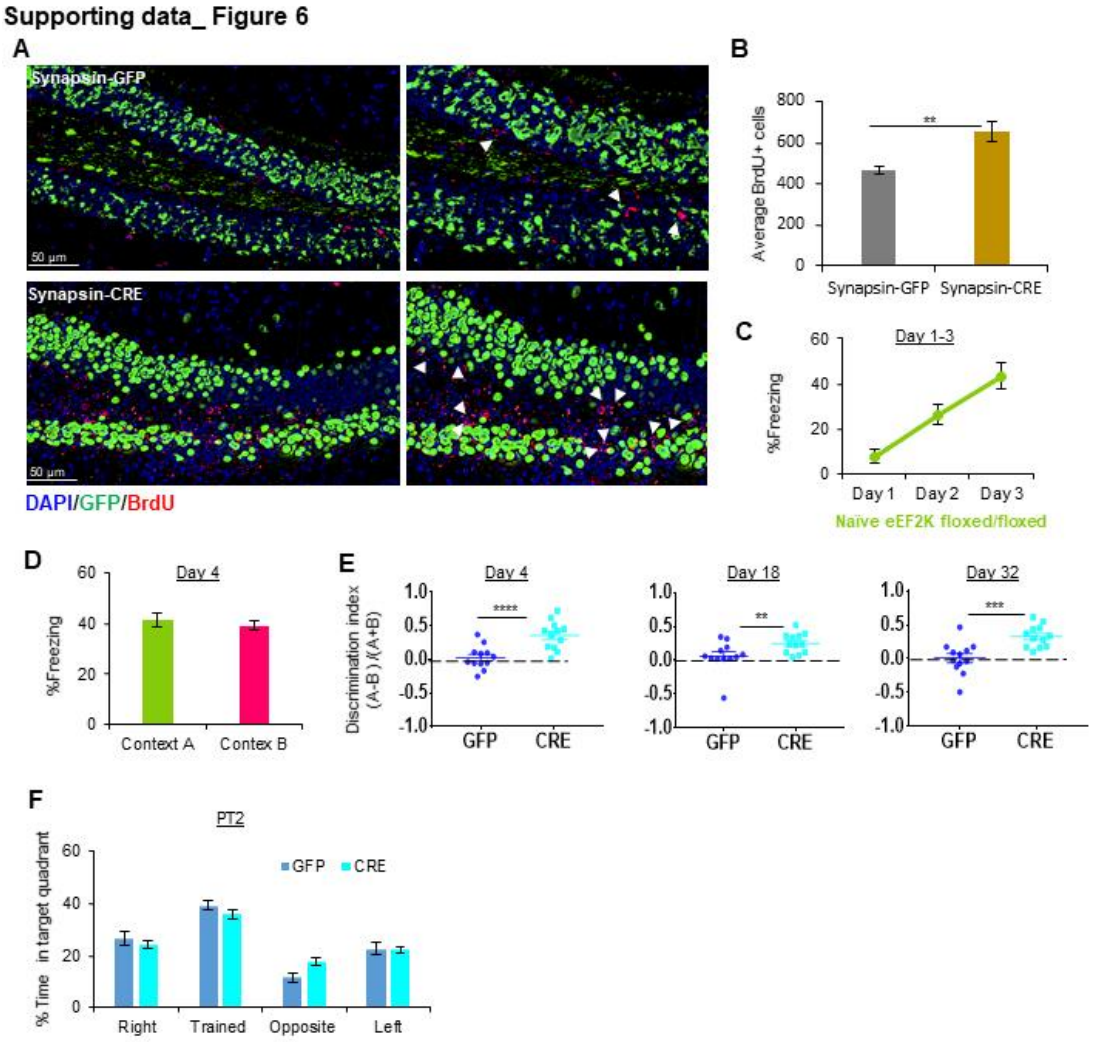
Supporting data Figure 5, related to main Figure 3

(A) Representative coronal hippocampal sections immunostained for BrdU from CaMKII-GFP- and CaMKII-Cre-injected mice treated with vehicle or TMZ for 6 weeks (See Methods), (n=3). Scale bar, 50µm, 20x.

(B) Quantification of BrdU positive cells. Reduced expression of eEF2K in excitatory DG neurons in eEF2K floxed mice increases BrdU positive cells which was occluded using TMZ. Mean±SEM are shown (Two-way ANOVA, n=3, p<0.05)

(C) Representative coronal hippocampal sections immunostained for DCX from CaMKII-GFP- and CaMKII-Cre-injected mice treated with Vehicle and TMZ for 6 weeks (See Methods), (n=4). Scale bar, 50µm, 20x.

(D) Quantification of DCX positive cells. Reduced expression of eEF2K in excitatory DG neurons in eEF2K floxed mice increases DCX positive cells which was occluded by TMZ. CaMKII-GFP treated with TMZ showed non-significant reduced levels of DCX positive cells compared to CaMKII-GFP treated with vehicle. Mean±SEM are shown (Two-way ANOVA, n=4, $p<0.05$).



Supporting data Figure 6, related to main Figure 3

(A) Representative coronal hippocampal sections immunostained for BrdU from Synapsin-GFP- and Synapsin-Cre-injected mice (n=5-6). Scale bar, 50μm, 20x.

(B) Quantification of BrdU positive cells. Reduced expression of eEF2K in DG neurons in eEF2K floxed mice increases BrdU positive cells. Mean±SEM are shown (t-test, n=5-6, $p<0.01$)

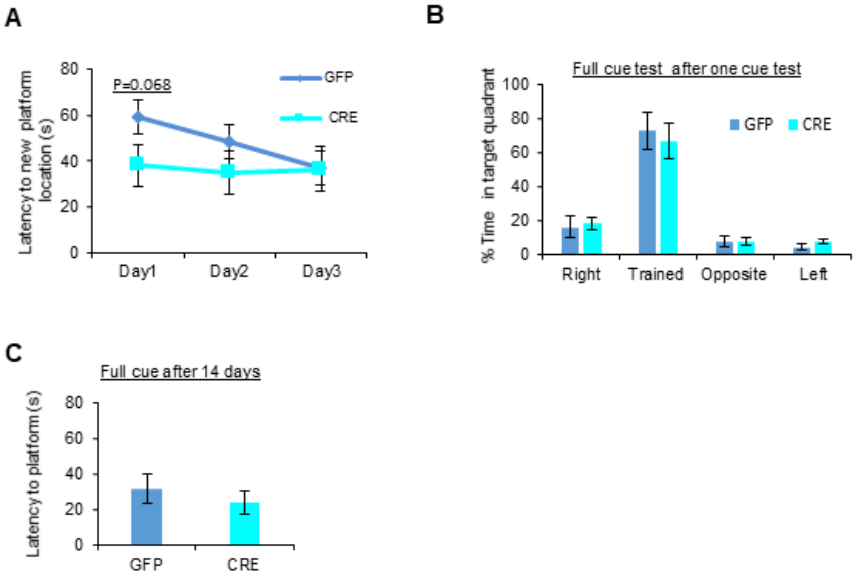
(C) Fear conditioning acquisition analysis of naïve eEF2K floxed/floxed mice (not injected) in context A. There is no difference between naïve eEF2K floxed/floxed mice and CaMKII-GFP-injected mice in context A acquisition for three days. Mean±SEM are shown (Repeated-measures ANOVA, n=7, within group $p<0.0001$)

(D) Context discrimination on day 4 between context A and B in Naïve eEF2K floxed/floxed mice. There is no difference in context discrimination of A and B between naïve eEF2K floxed/floxed mice and CaMKII-GFP-injected mice. Mean±SEM are shown (t-test, n=7, $p>0.05$).

(E) Discrimination index analysis on day 4, day 18, and 32 in CaMKII-Cre-injected mice and CaMKII-GFP-injected mice. CaMKII-Cre-injected mice show significantly higher discrimination index for one month. Mean±SEM are shown (Day4: t-test, n=12, $p<0.0001$; Day18: Mann-Whitney U test, n=12, $p<0.01$; Day32: t-test, n=12, $p<0.01$).

(F) Probe test (PT2) analysis on day 6 of the Morris water maze. CaMKII-Cre-injected mice and CaMKII-GFP-injected mice spent significantly more time in the target quadrant compared to other quadrants. Mean±SEM are shown (Kruskal-Wallis test, n=6, $p<0.05$).

Supporting data_ Figure 7



Supporting data Figure 7, related to main Figure 3

(A) Reversal Morris water maze analysis. CaMKII-Cre-injected mice and CaMKII-GFP-injected mice show similar learning of the new platform location, suggesting normal cognitive flexibility. Mean±SEM are shown (Friedman test, n=6, $p>0.05$).

(B) Probe test analysis of the Morris water maze with full cues after one cue test. This test was done 14 days following PT2. CaMKII-Cre-injected mice and CaMKII-GFP-injected mice spent significantly more time in the target quadrant compared to other quadrants. Mean±SEM are shown (Kruskal-Wallis test, n=6, $p<0.05$).

(C) Latency to platform location/zone in full cue conditions after 14 days of PT2. CaMKII-Cre-injected mice and CaMKII-GFP-injected mice exhibited similar latencies to platform zone after 14 days. Mean±SEM are shown (Mann-Whitney U test, n=6 $p>0.05$).

Extended statistical analysis

Figure S4	Number of mice	Statistics	P value
S4B	$BrdU^+$cells analysis GFP: n=9 Cre: n=9	GFP versus Cre: Mann-Whitney U test, U=3	P=0.001
S4C	Discrimination index, day 4 analysis, old mice GFP: n=12 Cre: n=12	two tailed *t* test, T_{22}=-0.577	P=0.570
S4D	Discrimination index, day 18 analysis, old mice GFP: n=12 Cre: n=12	Mann-Whitney U test, U=40	P=0.065
S4E	Discrimination index, day 32 analysis, old mice GFP: n=12 Cre: n=12	two tailed *t* test, T_{22}=-1.104	P=0.282

Table S11. Detailed statistical analysis, related to Figure S4.

Supporting data Figure 1	Number of mice	Statistics	P value
SuppD_Fig1A	WT: n=3, eEF2K-KO: n=3	Cut-off =±50%	P<0.05

Table S12. Detailed statistical analysis, related to Supporting data Figure 1.

Supporting data Figure 2	Number of mice	Statistics	P value
SuppD_Fig2B	$BrdU^+$ cells: WT: n=8, eEF2K-KO: n=8	WT versus eEF2K-KO, two tailed *t* test, T_{14}=-6.108	P=0.000027
SuppD_Fig2E	$BrdU^+$ cells: WT: n=3, eIF2α-KI: n=3	Mann-Whitney U test U=2	P=0.275

SuppD_Fig2F	DCX^+ cells: WT: n=4, eIF2α-KI: n=3	Mann-Whitney U test U=4	P=0.480
SuppD_Fig2G	Discrimination index, day 4 analysis WT: n=11, eEF2K-KO: n=13	two tailed *t* test, T_{22}=-3.236	P=0.004
SuppD_Fig2H	Discrimination index, day 18 analysis WT: n=11, eEF2K-KO: n=13	Mann-Whitney U test U=38	P=0.052
SuppD_Fig2I	Discrimination index, day 32 analysis WT: n=11, eEF2K-KO: n=13	two tailed *t* test, T_{22}=-2.365	P=0.027

Table S13. Detailed statistical analysis, related to Supporting data Figure 2.

Supporting data Figure 3	Number of mice	Statistics	P value
SuppD_Fig3B	$BrdU^+$ cells analysis: n=8 mice in each group.	Two-way ANOVA: model: $F_{3,28}$= 39.856, P<0.0001.	enriched/not enriched: $F_{1,28}$=40.090, P<0.0001. vehicle/TMZ: $F_{1,28}$ =62.661, P<0.0001, interaction: $F_{1,28}$ =16.816, P<0.0001. not enriched/V versus enriched/V: T_{14}=-5.759, P<0.0001, enriched/V versus enriched/TMZ: T_{14}=6.842, P<0.0001.

Table S14. Detailed statistical analysis, related to Supporting data Figure 3.

Supporting data Figure 4	Number of mice	Statistics	P value
SuppD_Fig4B	eEF2/tubulin analysis in DG: Saline: n=9 Ketamine: n=9	Mann-Whitney U test U=26	P=0.200
SuppD_Fig4D	(Left) pT56eEF2/eEF2 analysis in cortex.	(Left) two tailed *t* test, T_{16}=2.983	P=0.009

	(Right) eEF2/tubulin analysis in cortex Saline: n=9 Ketamine: n=9	(Right) two tailed *t* test, T_{16}=0.693	P=0.499

Table S15. Detailed statistical analysis, related to Supporting data Figure 4.

Supporting data Figure 5	Number of mice	Statistics	P value
SuppD_Fig5B	$BrdU^+$ cells analysis: n=3 mice in each group.	Two-way ANOVA: model: $F_{3,8}$=12.611, P=0.002.	CaMKII-Cre/CaMKII-GFP: $F_{1,8}$=13.931, P=0.006. vehicle/TMZ: $F_{1,8}$ =13.607, P=0.006, interaction: $F_{1,8}$ =10.295, P=0.012. GFP/V/ versus CRE/V:U=0.00, p=0.05, CRE/V versus CRE/TMZ: U=0.00, p=0.05.
SuppD_Fig5D	DCX^+ cells analysis: n=4 mice in each group.	Two-way ANOVA: model: $F_{3,12}$=34.510, P<0.0001.	CaMKII-Cre/CaMKII-GFP: $F_{1,12}$=35.488, P<0.0001. vehicle/TMZ: $F_{1,12}$=46.621, P<0.0001, interaction: $F_{1,12}$=21.421, P=0.001. GFP/V/ versus CRE/V: U=0.00, P=0.021, CRE/V versus CRE/TMZ: U=0.00, P=0.021, GFP/V/ versus GFP/TMZ: U=3, P=0.149

Table S16. Detailed statistical analysis, related to Supporting data Figure 5.

Supporting data Figure 6	Number of mice	Statistics	P value
SuppD_Fig6B	Synapsin-GFP: n=6, Synapsin-Cre: n=5	two tailed *t* test , T_9=-4.034	P=0.003

SuppD_Fig6C	Fear acquisition analysis in naïve floxed mice, n=7	within-subjects effects: Repeated-measures ANOVA, $F_{2,12}$=28.829	P<0.0001
SuppD_Fig6D	Day 4 analysis in naïve floxed, n=7	two tailed *t* test , T_{12}=0.758	Context A versus context B: p=0.463
SuppD_Fig6E Day 4	Discrimination index, day 4 analysis GFP: n=12 Cre: n=12	two tailed *t* test, T_{22}=-4.206	P<0.0001
SuppD_Fig6E Day18	Discrimination index, day 18 analysis GFP: n=12 Cre: n=12	Mann-Whitney U test, U=27	P=0.009
SuppD_Fig6E Day 32	Discrimination index, day 32 analysis GFP: n=12 Cre: n=12	two tailed *t* test, T_{22}=-3.838	P=0.001
SuppD_Fig6F	PT2 analysis GFP: n=6 Cre: n=6	NP-Kruskal-Wallis Test (all groups together in four quadrants). Right: χ^2_1=0.026, p=0.873 Trained; χ^2_1=0.778, p=0.378 Opposite: χ^2_1=2.564, p=0.109 Left: χ^2_1=0.026, p=0.873.	GFP: NP- Kruskal-Wallis Test: χ^2_3=11.530, p=0.009. Cre: one-way ANOVA, $F_{3,20}$=0.004, Post-hoc analysis; Tukey HSD test: Trained versus right, p=0.073; trained versus opposite, p=0.003; trained versus left, p=0.028

Table S17. Detailed statistical analysis, related to Supporting data Figure 6.

Supporting data Figure 7	Number of mice	Statistics	P value
SuppD_Fig7A	Reversal MWM GFP: n=6 Cre: n=6	NP-Friedman test, χ^2_2=1.721, p=0.423	Day1 analysis: Mann-Whitney U test, U=7, p=0.068
SuppD_Fig7B	Full cue analysis after one cue GFP: n=6	NP-Kruskal-Wallis Test (all groups together in four quadrants).	GFP: NP- Kruskal-Wallis Test: χ^2_3=10.987, p=0.012

	Cre: n=6	Right: χ^2_1=0.117, p=0.732 Trained; χ^2_1=0.467, p=0.494 Opposite: χ^2_1=0.263, p=0.608 Left: χ^2_1=0.467, p=0.494	Cre: NP- Kruskal-Wallis Test: χ^2_3=8.987, p=0.029
SuppD_Fig7C	Full cue after 14 days, latency GFP: n=6 Cre: n=6	Mann-Whitney U test, U=13	P=0.422

Table S18. Detailed statistical analysis, related to Supporting data Figure 7.