Supplementary Table 1: Sources and sequences of siRNAs.

	Provider	Cat #	Target sequence
Non-targeting siRNA	GE Healthcare Dharmacon, Inc.	#D-001810-02	UGGUUUACAUGUUGUGUGA
GSK3β siRNA B1	GE Healthcare Dharmacon, Inc.	#D-041080-02	GAGGAGAGCCCAAUGUUUC
GSK3β siRNA B2	GE Healthcare Dharmacon, Inc.	#D-041080-03	GCACCAGAGUUGAUCUUUG
BDNF siRNA	GE Healthcare Dharmacon, Inc.	#D-042566-01	UAUGUACACUGACCAUUAA
		#D-042566-02	GAGCGUGUGUGACAGUAUU
		#D-042566-03	GAACUACCCAAUCGUAUGU
		#D-042566-04	UCAUAAGGAUAGACACUUC
HDAC1 siRNA	GE Healthcare Dharmacon, Inc.	#J-040287-22	GGGAGAAGGUGGUCGCAAG
		#J-040287-23	ACUAUGGUCUCUACCGAAA
		#J-040287-24	UGAACUACCCACUGCGAGA
		#J-040287-25	CCAGAACACUAACGAGUAC
HDAC2 siRNA	GE Healthcare Dharmacon, Inc.	#J-046158-05	CCAAUGAGUUGCCAUAUAA
		#J-046158-06	CAAUUGGGCUGGAGGACUA
		#J-046158-07	ACAGGAGACUUGAGGGAUA
		#J-046158-08	CAAAAGUGAUGGAGAUGUA
HDAC3 siRNA	GE Healthcare Dharmacon, Inc.	#J-043553-05	GGGAAUGUGUUGAAUAUGU
		#J-043553-06	CGGCAGACCUCCUGACGUA
		#J-043553-08	GCACCCGCAUCGAGAAUCA
		#J-043553-17	UAUAAGAAGAUGAUCGUCU
HDAC4 siRNA	GE Healthcare Dharmacon, Inc.	#J-043626-05	GGUUAUGCCUAUCGCAAAU
		#J-043626-06	GUGGAUAGCGACACCAUAU
		#J-043626-07	GAAAUUACGCUCAAGGCUU
		#J-043626-08	CAACAUGGCUUUCACGGGU
IGF2 siRNA	GE Healthcare Dharmacon, Inc.	#J-043709-09	GGCCAGAUAAGGAGAUCGA

Supplementary Table 2: Primers used for qRT-PCR analyses

	Forward	Reverse
Igf2	TGTGCTGCATCGCTGCTTAC	CGGTCCGAACAGACAAACTGA
Kcne2	CATCCTGTACCTCATGGTGATG	TGGCCTTGGAGTCTTCCAGAT
Sostdc1	TACACCCGTCAGCACAACGA	CTCAGACTGTGCTTGCTGGATT

Supplemental Figure 1. Glycogen synthase kinase-3β (GSK3β) immunohistochemistry

We tested if intranasal administration of GSK3β siRNA lowered GSK3β levels in the hippocampus or perirhinal cortex of wild-type (WT) mice or *Fmr1*$^{-/-}$ mice. **A.** Representative staining of GSK3β-labeled neurons in WT mice treated with scrambled siRNA (n=7) (A) dentate gyrus (DG), (B) CA3, and (C) CA1, with GSK3β siRNA-B1 (n=5) (D) DG, (E) CA3, and (F) CA1, or with GSK3β siRNA-B2 (n=6) (G) DG, (H) CA3, and (I) CA1. GSK3β-labeled neurons in *Fmr1*$^{-/-}$ mice treated with scrambled siRNA (n=5) (J) DG, (K) CA3, and (L) CA1, treated with GSK3β siRNA-B1 (n=6) (M) DG, (N) CA3, and (O) CA1, or treated with GSK3β siRNA-B2 (n=5) (P) DG, (Q) CA3, and (R) CA1. (S) Quantitation of GSK3β-labeled neurons in the hippocampus DG (WT: $F_{(2,17)}$=3.19, p<0.05; *Fmr1*$^{-/-}$: $F_{(2,15)}$=7.59, p<0.05), CA3 (WT: $F_{(2,17)}$=9.17, p<0.01; *Fmr1*$^{-/-}$: $F_{(2,15)}$=6.20, p<0.05), and CA1 (WT: $F_{(2,17)}$=8.11, p<0.01; *Fmr1*$^{-/-}$: $F_{(2,15)}$=22.73, p<0.01) in WT and *Fmr1*$^{-/-}$ mice. Values are means±SEM. (*p<0.05 compared to scrambled siRNA-treated values in the same genotype) (cc:corpus callosum; siB1: GSK3β siRNA sequence 1; siB2:GSK3β siRNA sequence 2). Scale bars: 400 μm in all images; dashed lines delineate area of interest.

Representative staining of GSK3β-labeled neurons in perirhinal cortex in wild-type (WT) mice treated with (T) scrambled siRNA (n=7), (U) GSK3β siRNA-B1(n=5), or (V) GSK3β siRNA-B2 (n=6). GSK3β-labeled neurons in perirhinal cortex in *Fmr1*$^{-/-}$ mice treated with (W) scrambled siRNA (n=5), (X) GSK3β siRNA-B1 (n=5), or (Y) GSK3β siRNA-B2 (n=5). (Z) Quantitation of GSK3β-labeled neurons in the perirhinal cortex (WT: F(2,17)=7.08, p<0.01; *Fmr1*$^{-/-}$: F(2,15)=10.23, p<0.01) in WT and *Fmr1*$^{-/-}$ mice. Values are means±SEM. (*p<0.05 compared to scrambled siRNA-treated values in the same genotype) (ec:external capsule; siB1: GSK3β siRNA sequence 1; siB2:GSK3β siRNA sequence 2). Scale bars: 400 μm in all images. Each symbol represents the value from an individual mouse.

Supplemental Figure 2. Histone deacetylase-2 (HDAC2) immunohistochemistry

We tested if intranasal administration of HDAC2 siRNA lowered HDAC2 levels in the hippocampus of wild-type (WT) mice or glycogen synthase kinase-3 knockin (KI) mice. Representative staining of HDAC2-labeled neurons in the hippocampus. Labeled neurons in wild-type (WT) mice treated with scrambled siRNA (n=5) (A) dentate gyrus (DG), (B) CA3, and (C) CA1, or treated with HDAC2 siRNA (n=5) (D) DG, (E) CA3, and (F) CA1. Labeled neurons in GSK3 knockin (KI) mice treated with scrambled siRNA (n=5) (G) DG, (H) CA3, and (I) CA1, or

treated with HDAC2 siRNA (n=5) (J) DG, (K) CA3, and (L) CA1. (M) Quantitation of HDAC2-labeled neurons in the hippocampus DG (WT: $t_{(8)}$=4.34, p<0.01; KI: $t_{(8)}$=3.54, p<0.01), CA3 (WT: $t_{(8)}$=5.28, p<0.01; KI: $t_{(8)}$=2.51, p<0.05), and CA1 (WT: $t_{(8)}$=5.58, p<0.01; KI: $t_{(8)}$=3.15, p<0.05) in WT and GSK3 KI mice. Values are means±SEM. *p<0.05 compared to scrambled siRNA-treated values in the same genotype. (cc:corpus callosum) Scale bars: 400 μm in all images; dashed lines delineate area of interest. Each symbol represents the value from an individual mouse.

Supplemental Figure 3. Histone deacetylase-1 (HDAC1) immunohistochemistry

We tested if intranasal administration of HDAC1 siRNA lowered HDAC1 levels in the hippocampus of wild-type (WT) mice or glycogen synthase kinase-3 knockin (KI) mice. Representative staining of HDAC1-labeled neurons in the hippocampus. Labeled neurons in wild-type (WT) mice treated with scrambled siRNA (n=5) (A) dentate gyrus (DG), (B) CA3, and (C) CA1, or treated with HDAC1 siRNA (n=6) (D) DG, (E) CA3, and (F) CA1. Labeled neurons in GSK3 knockin (KI) mice treated with scrambled siRNA (n=4) (G) DG, (H) CA3, and (I) CA1, or treated with HDAC1 siRNA (n=4) (J) DG, (K) CA3, and (L) CA1. (M) Quantitation of HDAC1-labeled neurons in the hippocampus DG (WT: $t_{(9)}$=3.10, p<0.05; KI: $t_{(6)}$=4.14, p<0.01), CA3 (WT: $t_{(9)}$=5.30, p<0.01; KI: $t_{(6)}$=3.38, p<0.05), and CA1 (WT: $t_{(9)}$=2.94, p<0.05; KI: $t_{(6)}$=2.40, p<0.05) in WT and GSK3 KI mice. Values are means±SEM. *p<0.05 compared to scrambled siRNA-treated values in the same genotype (cc:corpus callosum) Scale bars: 400 μm in all images; dashed lines delineate area of interest. Each symbol represents the value from an individual mouse.

Supplemental Figure 4. Histone deacetylase-3 (HDAC3) immunohistochemistry

We tested if intranasal administration of HDAC3 siRNA lowered HDAC3 levels in the hippocampus of wild-type (WT) mice or glycogen synthase kinase-3 knockin (KI) mice. Representative staining of HDAC3-labeled neurons in the hippocampus. Labeled neurons in wild-type (WT) mice treated with scrambled siRNA (n=4) (A) dentate gyrus (DG), (B) CA3, and (C) CA1, or treated with HDAC3 siRNA (n=4) (D) DG, (E) CA3, and (F) CA1. Labeled neurons in GSK3 knockin (KI) mice treated with scrambled siRNA (n=4) (G) DG, (H) CA3, and (I) CA1, or treated with HDAC3 siRNA (n=5) (J) DG, (K) CA3, and (L) CA1. (M) Quantitation of HDAC3-labeled neurons in the hippocampus DG (WT: $t_{(6)}$=3.75, p<0.01; KI: $t_{(7)}$=3.07, p<0.05), CA3 (WT: $t_{(6)}$=2.75, p<0.05; KI: $t_{(7)}$=2.82, p<0.05), and CA1 (WT: $t_{(6)}$=3.63, p<0.05; KI: $t_{(7)}$=2.81, p<0.05)

in WT and GSK3 KI mice. Values are means±SEM. *p<0.05 compared to scrambled siRNA-treated values in the same genotype (cc:corpus callosum) Scale bars: 400 μm in all images; dashed lines delineate area of interest. Each symbol represents the value from an individual mouse.

Supplemental Figure 5. Histone deacetylase-4 (HDAC4) immunohistochemistry

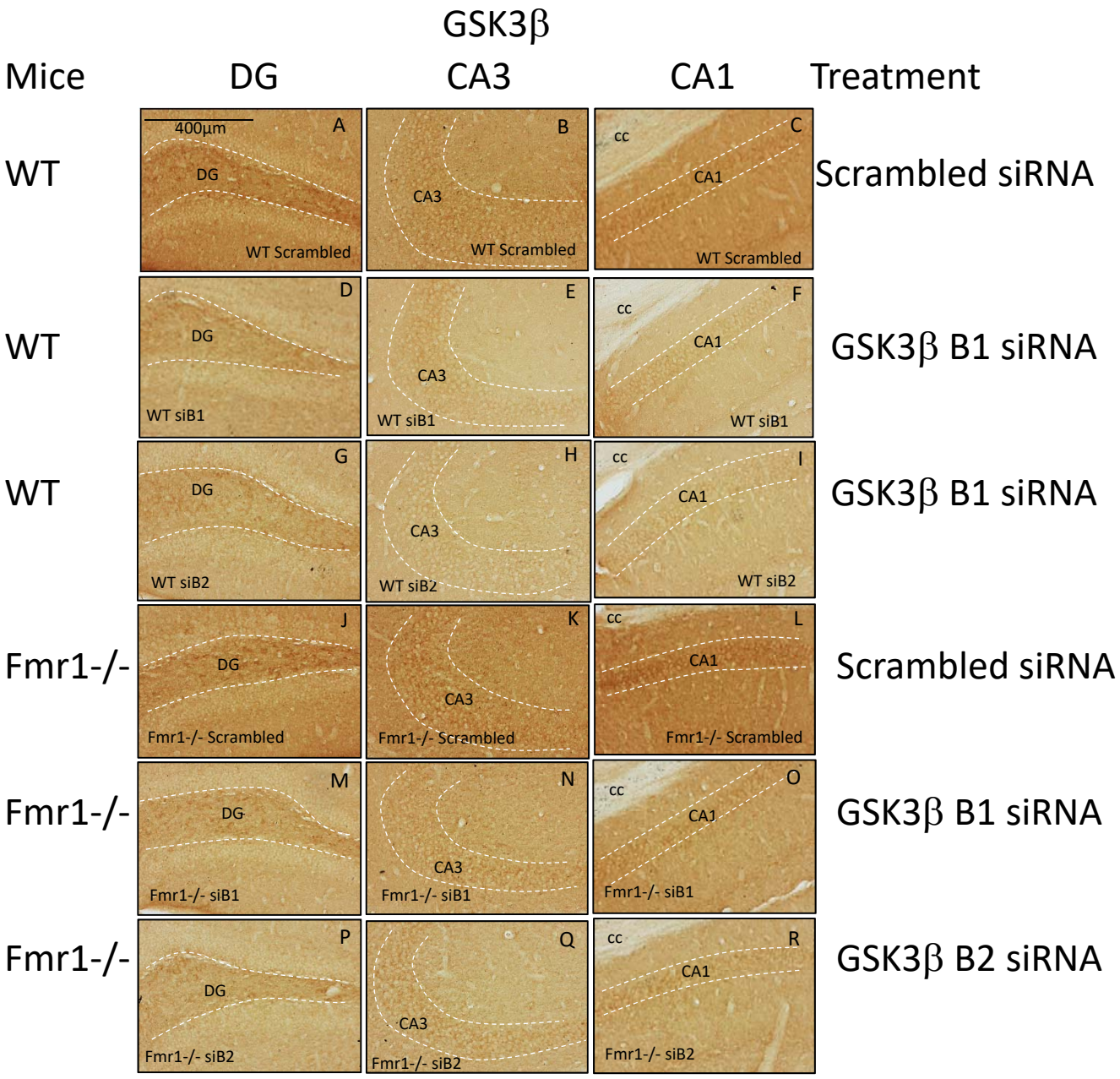
We tested if intranasal administration of HDAC4 siRNA lowered HDAC4 levels in the hippocampus of wild-type (WT) mice. Representative staining of HDAC4-labeled neurons in the hippocampus. Labeled neurons in wild-type (WT) mice treated with scrambled siRNA (n=4) (A) dentate gyrus (DG), (B) CA3, and (C) CA1, or treated with HDAC4 siRNA (n=5) (D) DG, (E) CA3, and (F) CA1. (G) Quantitation of HDAC4-labeled neurons in the hippocampus DG ($t_{(7)}$=4.74, p<0.01), CA3($t_{(7)}$=3.50, p<0.05), and CA1 ($t_{(7)}$=6.62, p<0.01), in WT mice. Values are means±SEM. *p<0.05 compared to scrambled siRNA-treated values. (cc:corpus callosum) Scale bars: 400 μm in all images; dashed lines delineate areas of interest. Each symbol represents the value from an individual mouse.

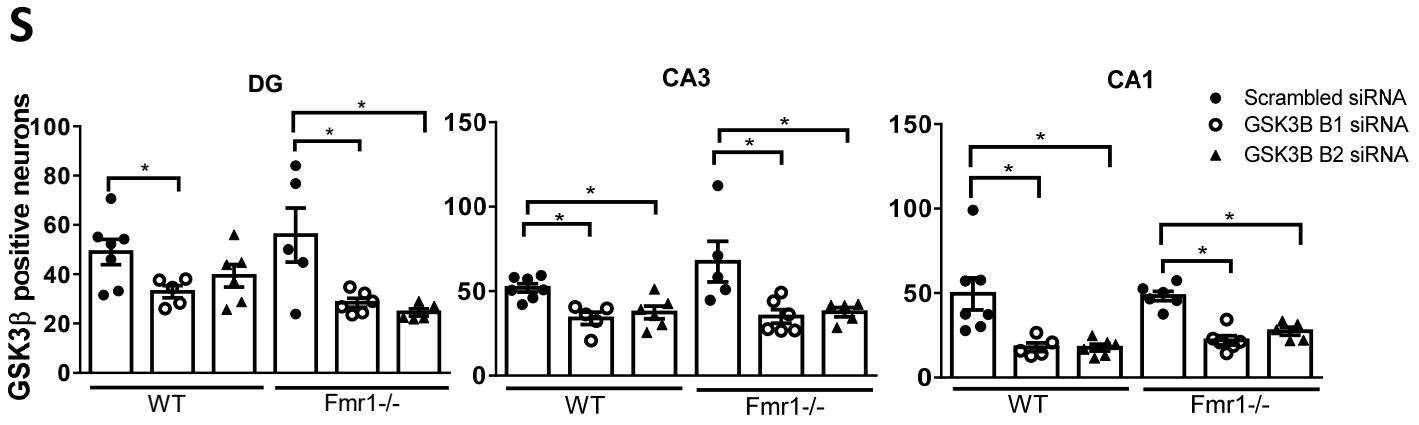
Supplemental Figure 6. Effects of intranasal treatment with histone deacetylase-4 (HDAC4) or brain-derived neurotrophic factor (BDNF) siRNA on gene expression.

qRT-PCR was used to measure the hippocampal mRNA levels of Sostdc1, Kcne2 and insulin-like growth factor-2 (IGF2) in wild-type mice treated intranasally with scrambled siRNA (n=5), HDAC4 siRNA (n=4), or BDNF siRNA (n=4). Wild-type mice were treated intranasally with scrambled siRNA or siRNA targeting HDAC4 or BDNF and hippocampal gene expression was analyzed. (A) Heat map presentation of gene-expression profiles of the genes with significant differences in expression measured by qRT-PCR (red, high; green, low). HDAC4 and BDNF siRNA treatments reduced mRNA levels of (B) IGF2 (one-way ANOVA; $F_{(2,12)}$=5.12, p<0.05) (*p<0.05 compared to scrambled siRNA-treated WT mice), (C) Kcne2 (one-way ANOVA; $F_{(2,12)}$=8.09, p<0.01) (*p<0.01 compared to scrambled siRNA-treated WT mice), and (D) Sostdc1 (one-way ANOVA; $F_{(2,12)}$=11.44, *p<0.01) (*p<0.01 compared to scrambled siRNA-treated WT mice). Values are means±SEM. The number of mice (n) for each value is shown within each bar.

Supplemental Figure 7. Cognitive performance of wild-type mice after intranasal insulin-like growth factor-2 (IGF2) treatment.

We tested if intranasal IGF2 administration altered the performance of wild-type (WT) mice in novel object recognition, temporal ordering, or coordinate and categorical spatial processing. WT mice received intranasal vehicle or IGF2 (0.1 μg/mouse/day) 24 hr and 1 hr prior to behavioral testing (Scheme). (A) WT mice spent significantly more time exploring the novel (N) object than the familiar (F) object regardless of treatment (*$p<0.01$) (vehicle: n=8, $t(14)=7.49$, *$p<0.01$; IGF2: n=8, $t(14)=4.75$, *$p<0.01$). (B) Discrimination index is shown for novel object recognition ($t(14)=1.15$, $p=0.27$. (C) WT mice spent significantly more time exploring the first object presented (1) than the most recent object (3) regardless of treatment (*$p<0.01$) (vehicle: n=8, $t(14)=5.27$, *$p<0.01$; IGF2: n=8, $t(14):3.68$, *$p<0.01$). (D) Discrimination index is shown for temporal ordering ($t(14)=0.30$, $p=0.77$). IGF2 treatment of WT mice did not alter (E) coordinate spatial processing (n=8, $t(14)=0.90$, $p=0.39$), or (G) categorical spatial processing (n=8, $t(14)=0.88$, $p=0.39$). Values are means±SEM. Each symbol represents the value from an individual mouse.





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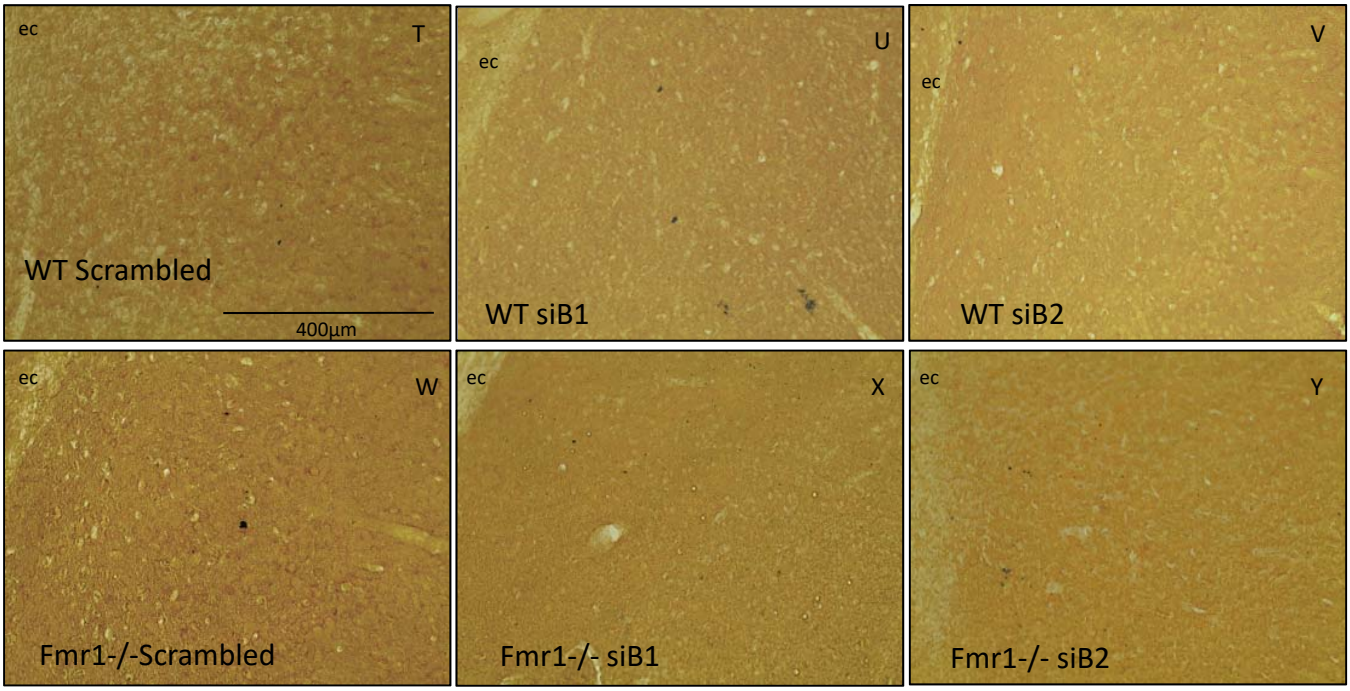

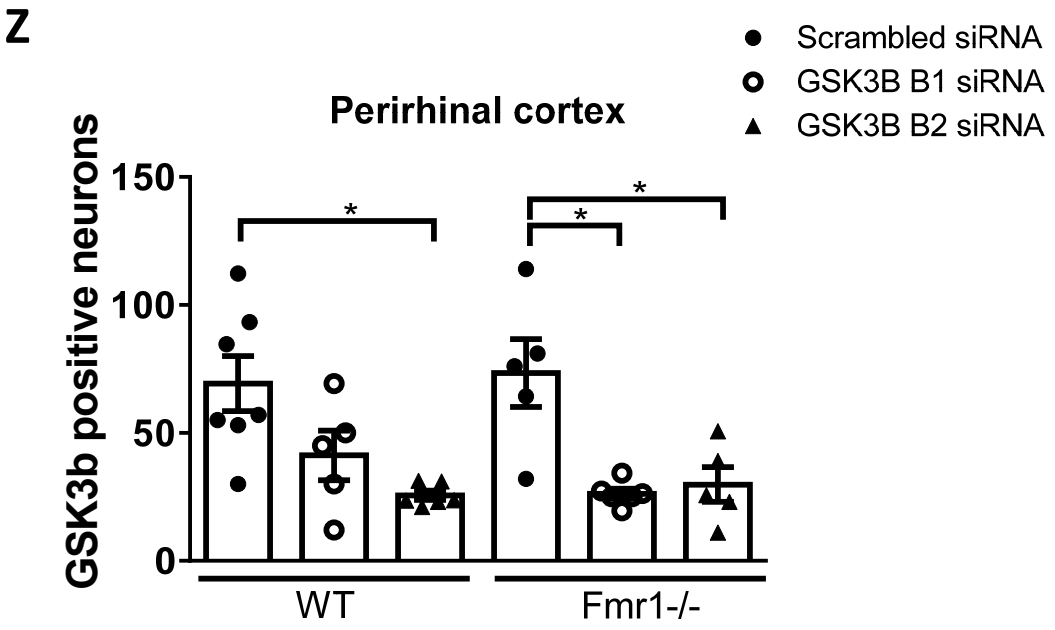

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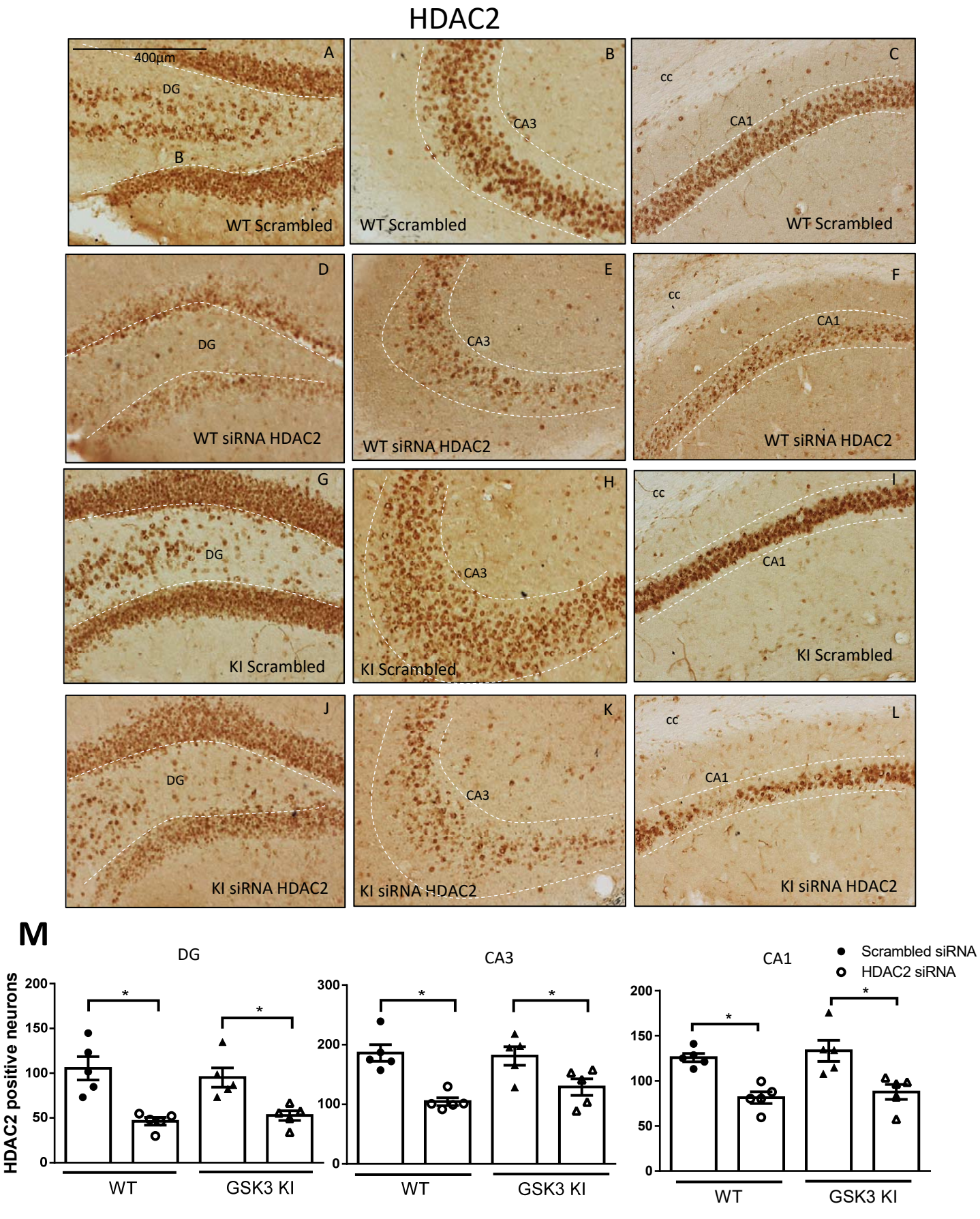

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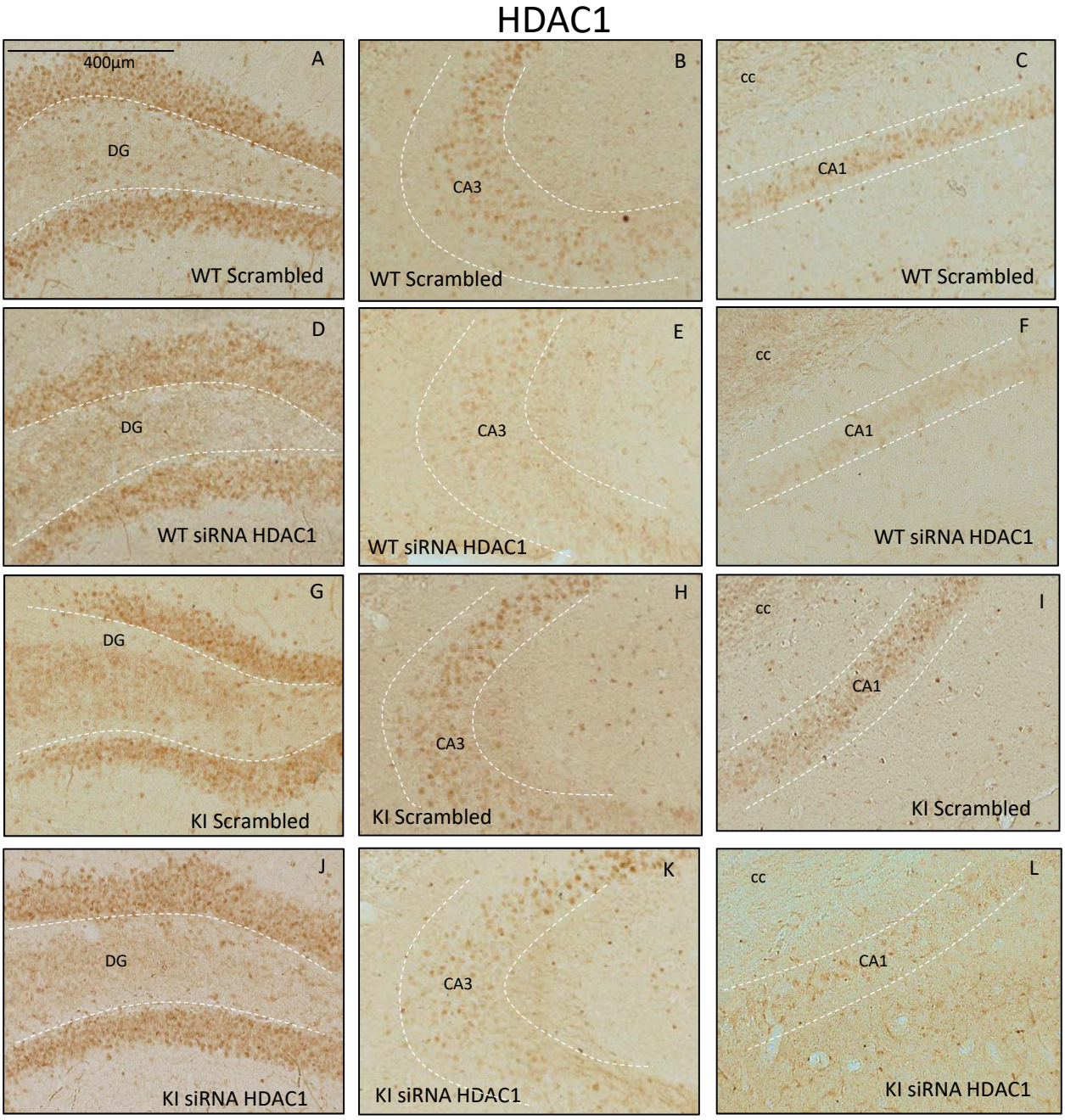

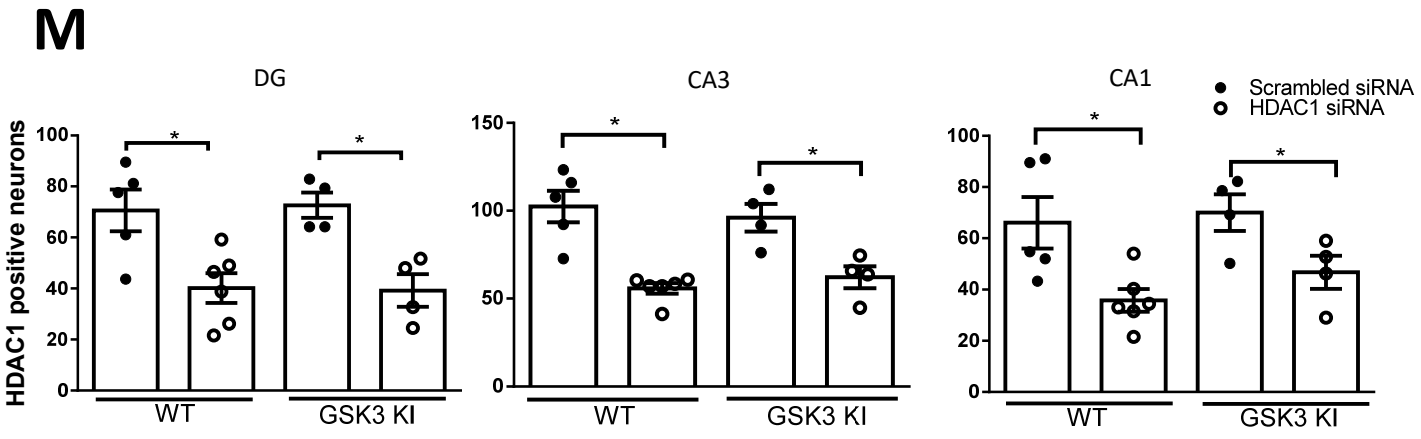

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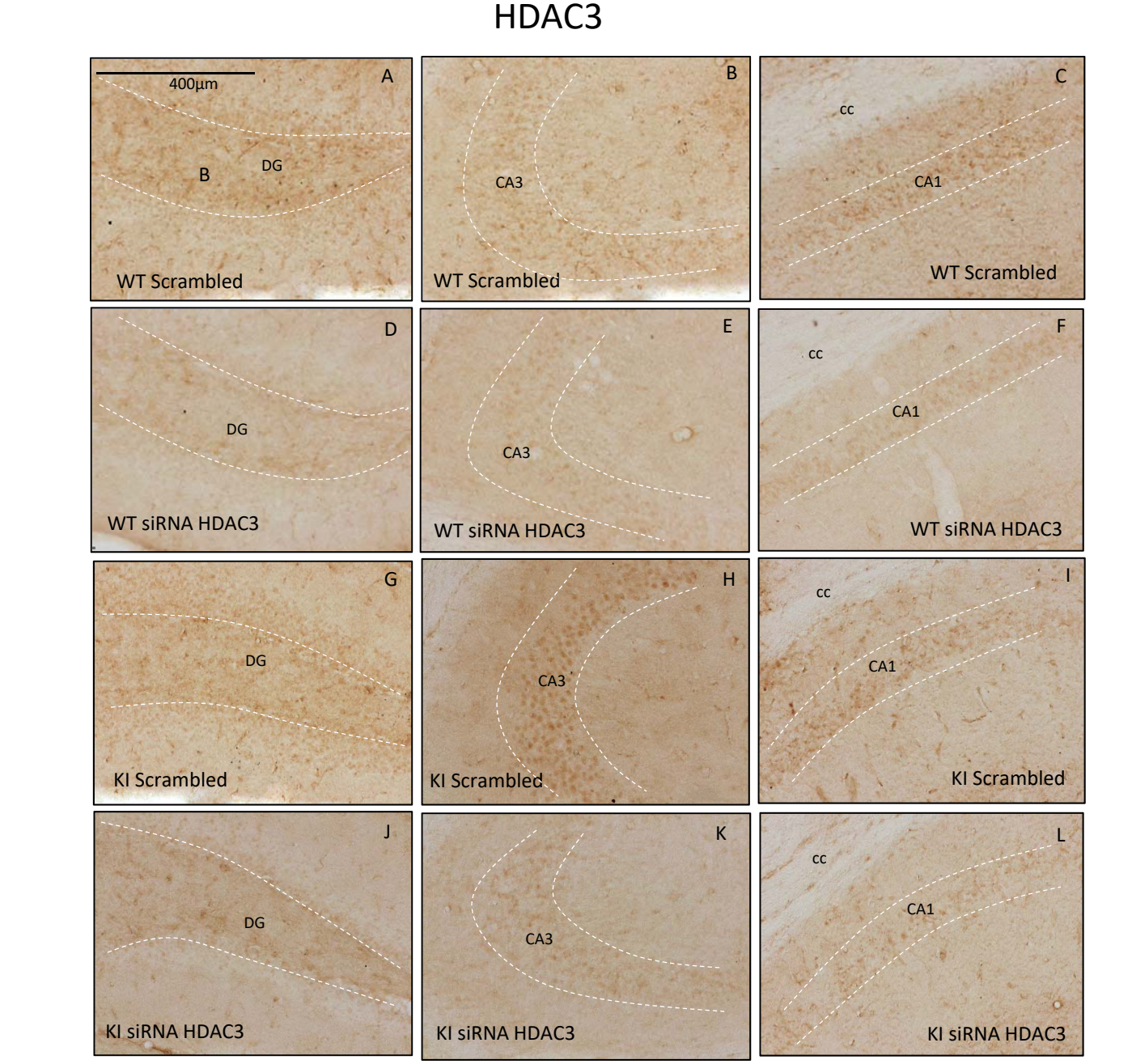
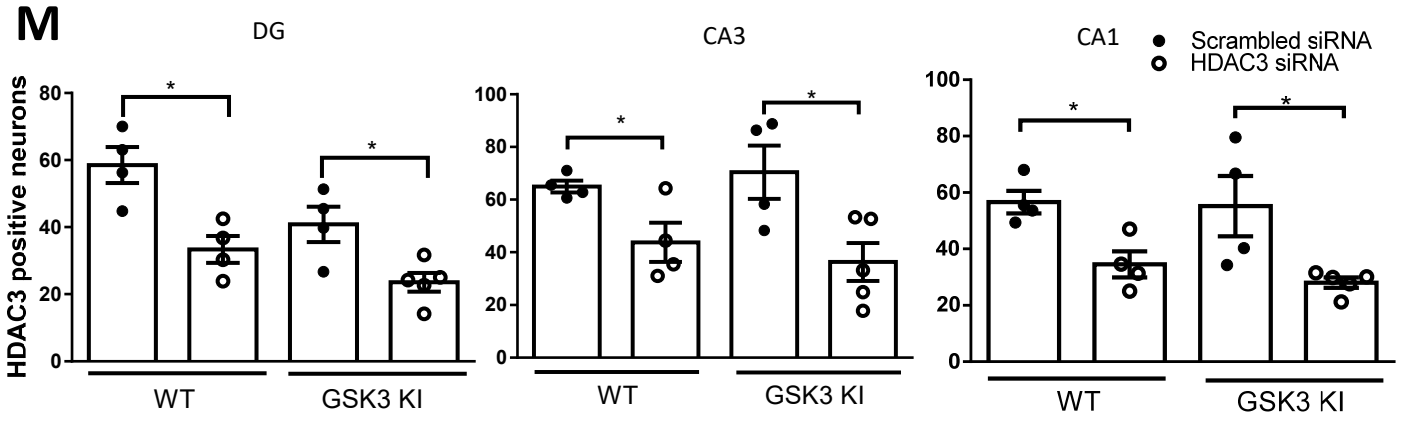
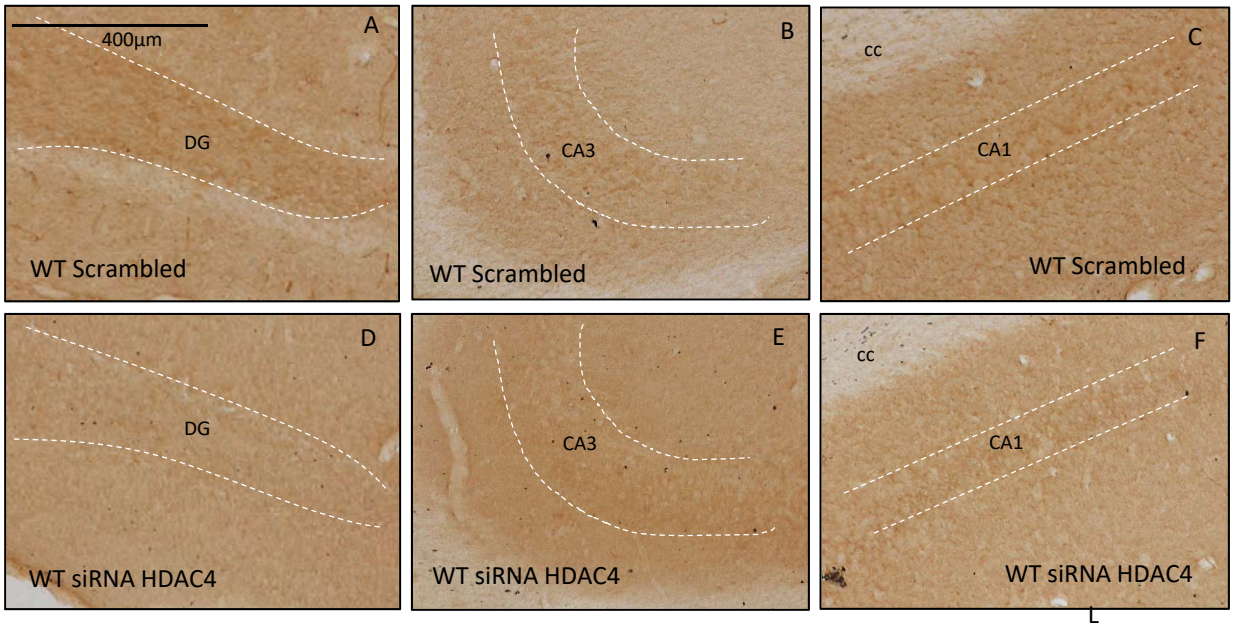
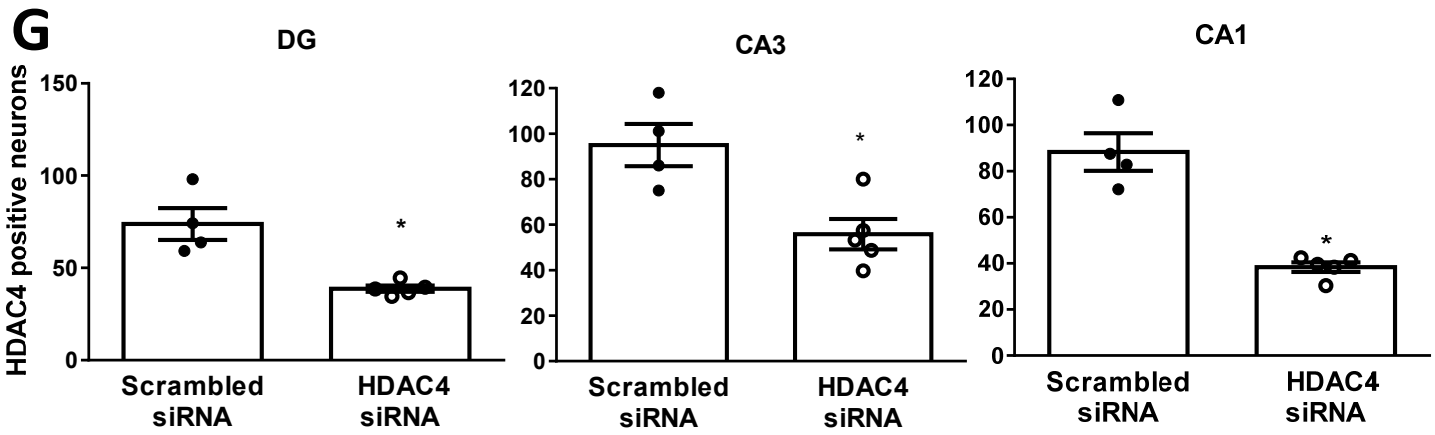




Supplemental Figure 4

HDAC4





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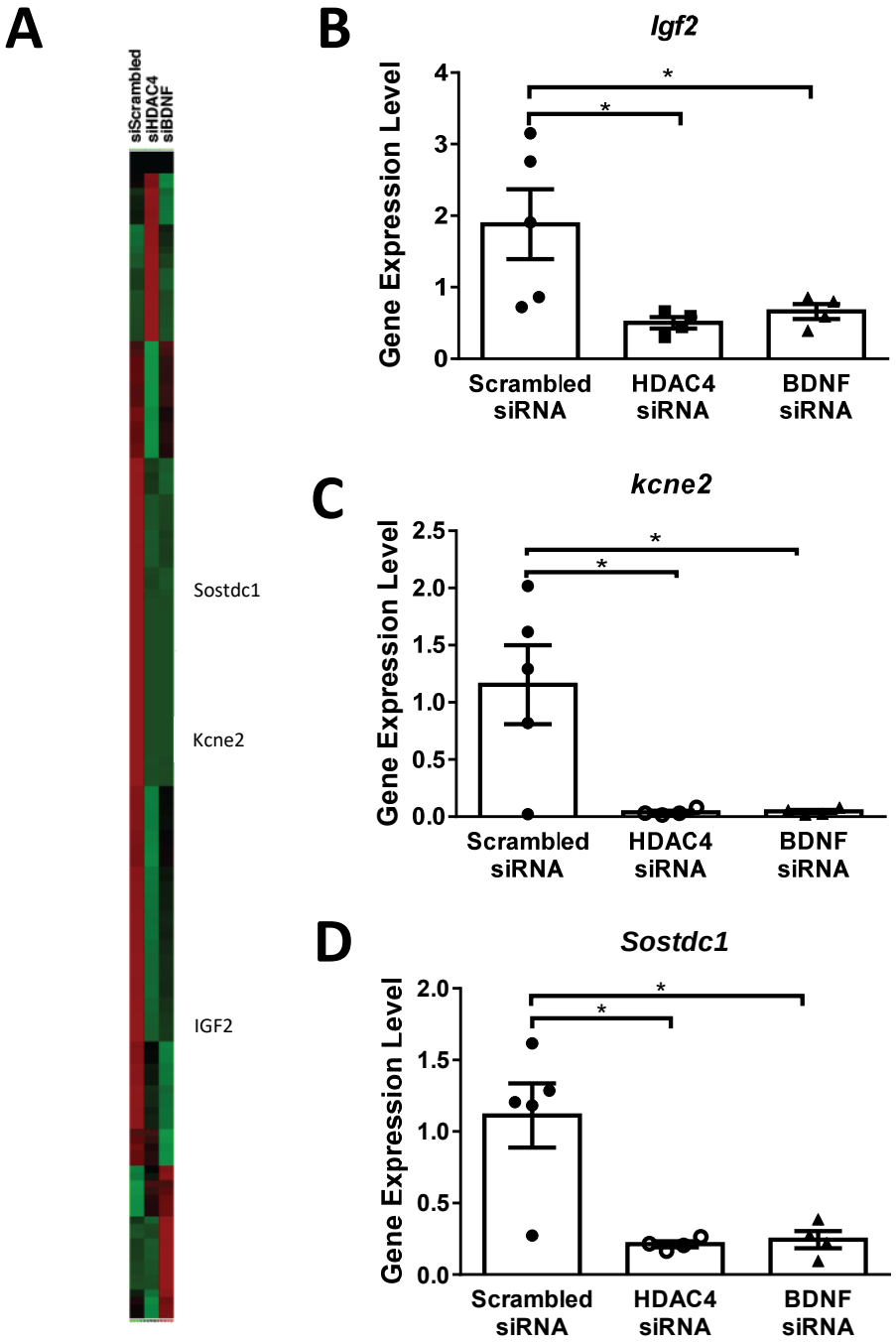

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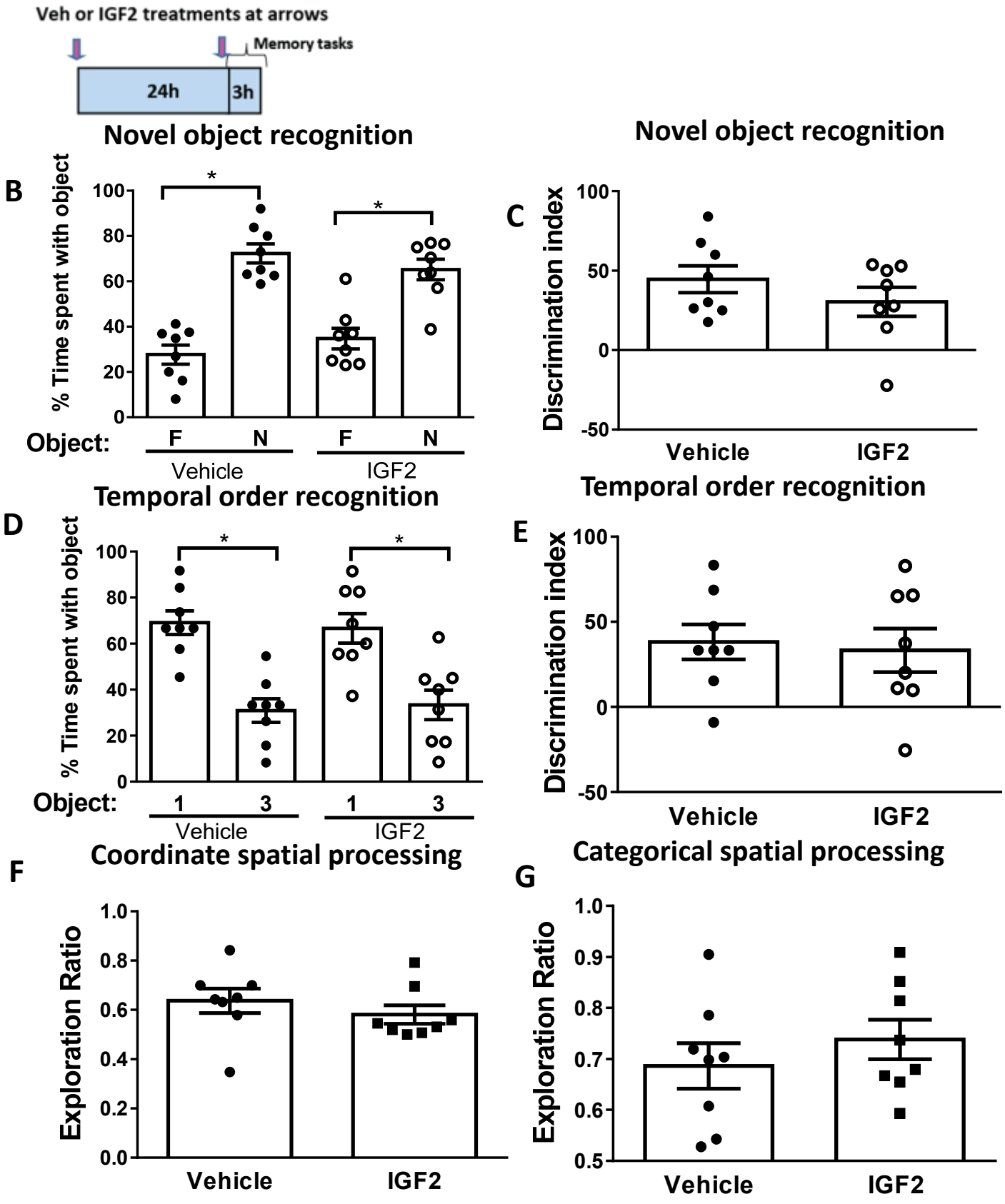

Supplemental Figure 7