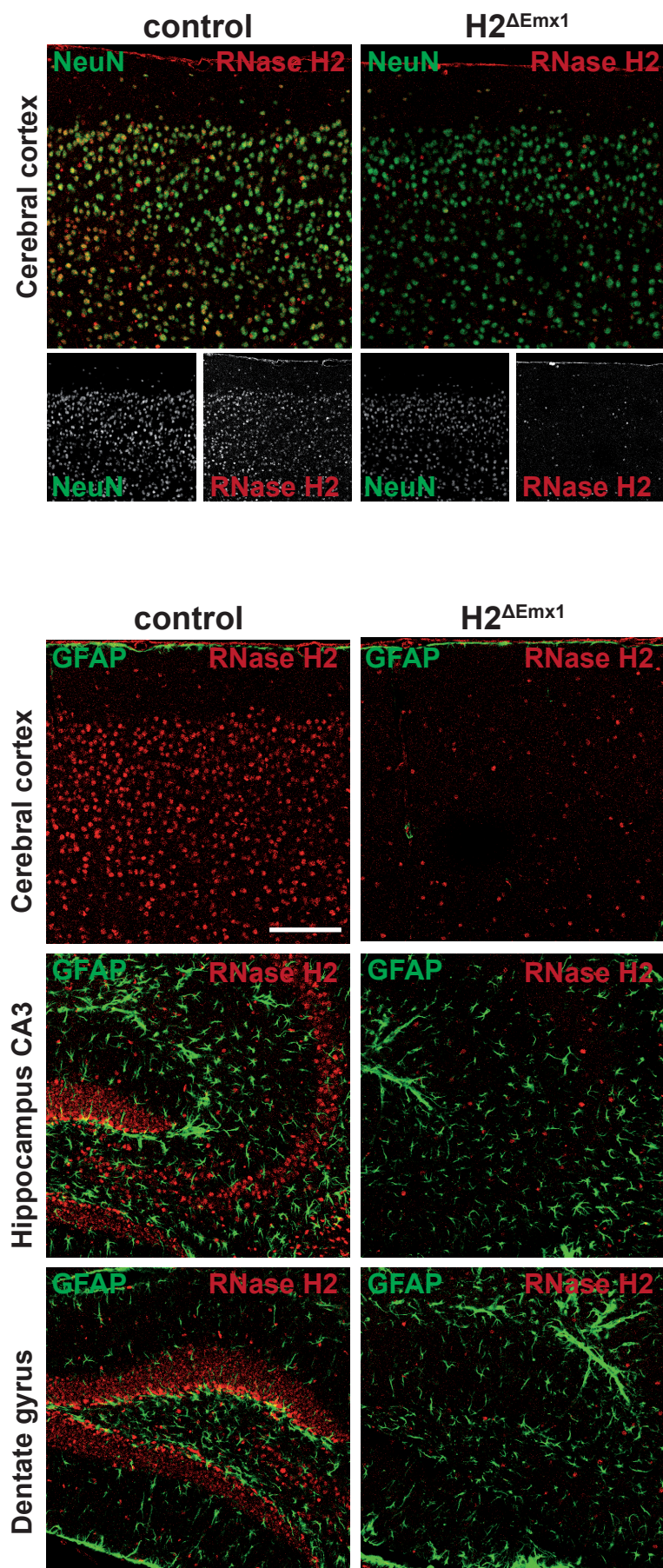
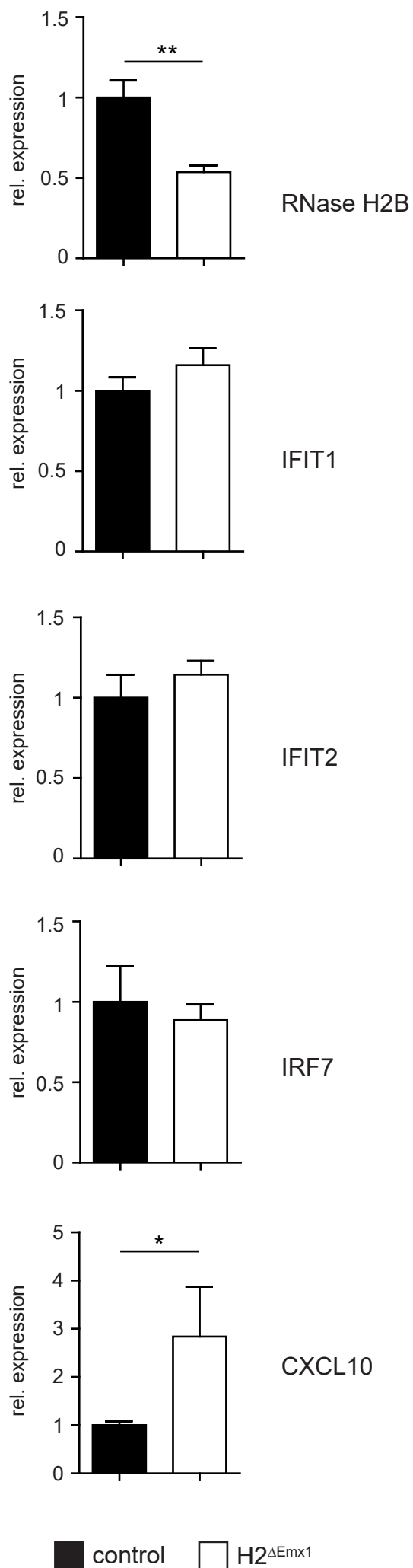


Suppl. Fig. 1:

A

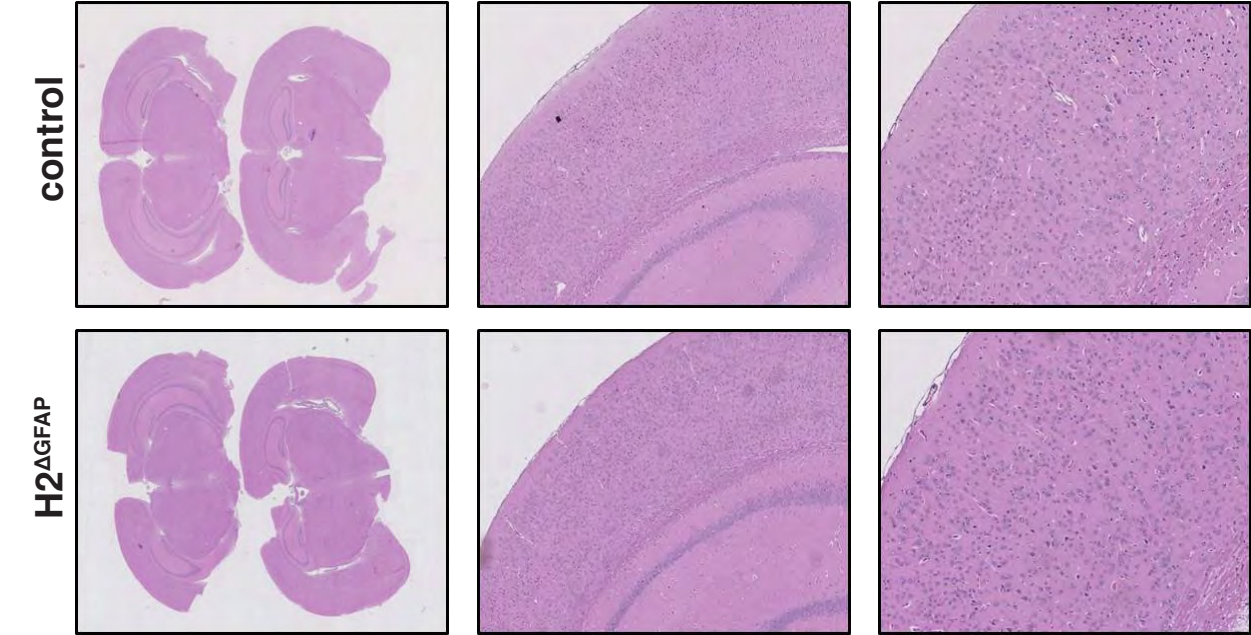


B



Suppl. Fig. 2:

A

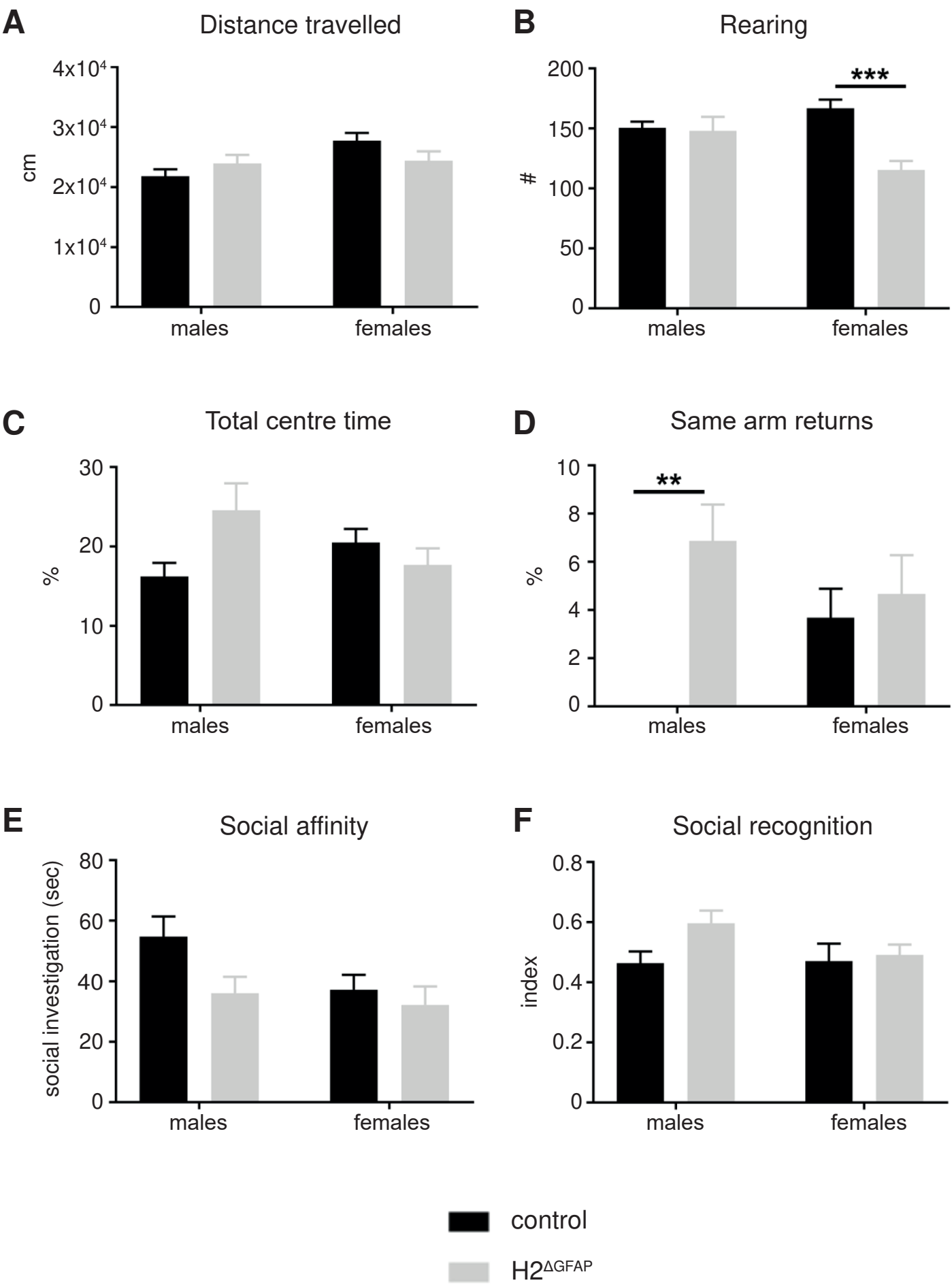


B

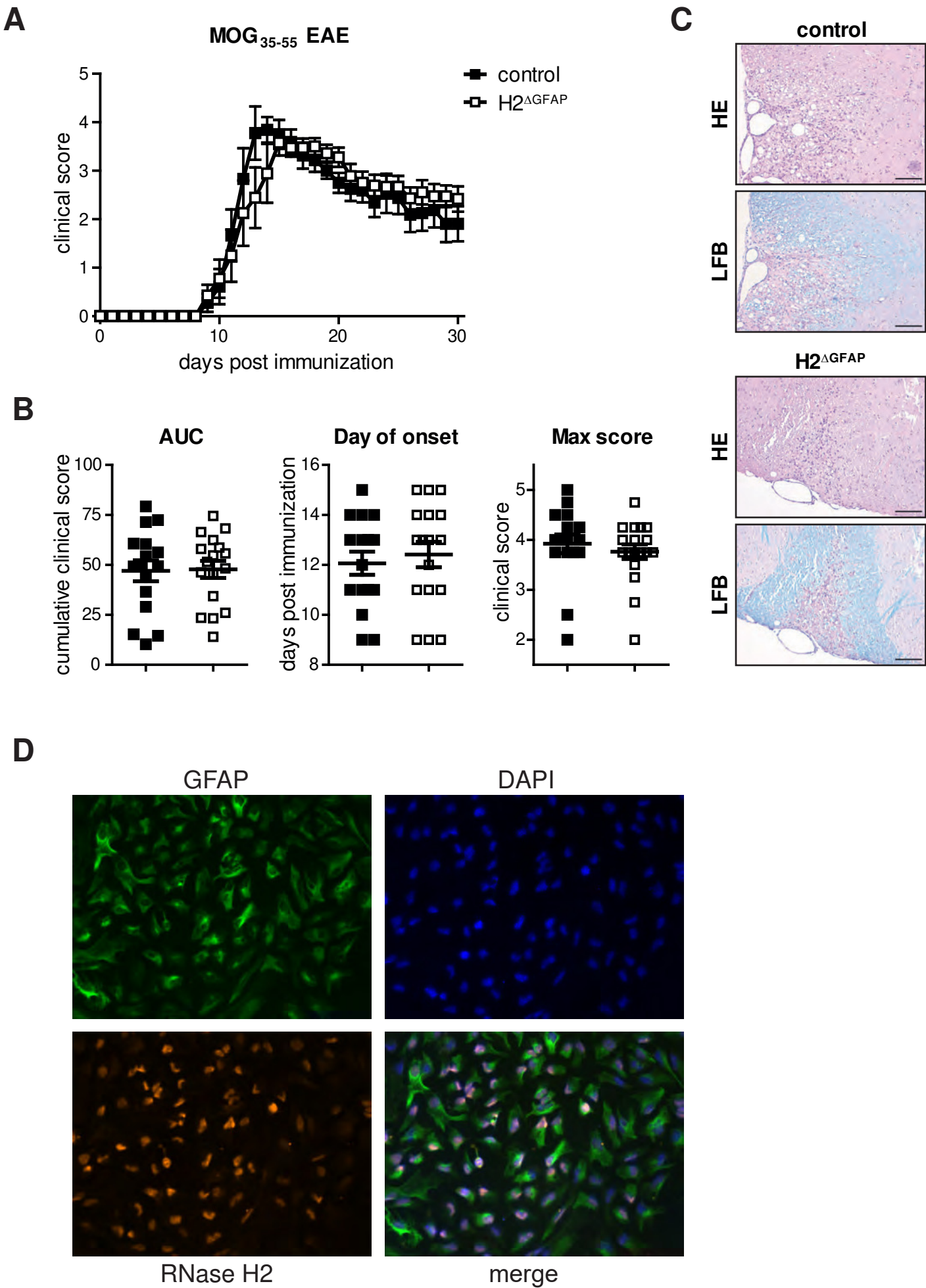
Test	Age (weeks)	Result
Open Field	8	Reduced rearing in females
SHIRPA	9	No differences
Grip strength	9	No differences
ASR/PPI	10	No differences
Rotarod	13	No differences
Hotplate	13	Subtle effect (slightly faster second reaction)
ABR	14	No differences
Balance Beam	52	Subtle findings for single beams (more traversing time beam 1, more stops of females on beams 1 and 2)
Y-Maze	53	Increased same arm entries in males
Social Discrimination	54	Subtle finding (trend for reduced social affinity in males)
Vertical Pole	57	No significant differences
Beam ladder	60	No significant differences

ASR/PPI: Acoustic startle response/Pre-pulse inhibition
ABR: Auditory brain stem response

Suppl. Fig. 3:



Suppl. Fig. 4 :



Supplementary Figure legends

Suppl. Figure 1: Basal analysis of RNase H2^{ΔEmx1} mice.

(A) Top: Absence of RNase H2 in the majority of neurons in the cerebral cortex of RNase H2^{ΔEmx1} mice (age 6 weeks). Neurons were co-stained with the neuronal marker NeuN. Bottom: Lack of RNase H2 in different brain regions of RNase H2^{ΔEmx1} mice. GFAP immunohistochemistry revealed no apparent astrogliosis. Astrogliosis would present as increased expression of GFAP, cellular hypertrophy with changes of astrocyte morphology or proliferation of astrocytes. (B) Comparable ISG expression in cortices of RNase H2^{ΔEmx1} mice, as quantified by qPCR. mRNA levels were normalized to GAPDH and indicated relative to control cortices ($x=1$). CXCL10 was slightly, but significantly upregulated. In line with neuronal RNase H2 loss, RNase H2B transcript levels were reduced in RNase H2^{ΔEmx1} cortices. Error bars are SEM, * $p < 0.05$, ** $p < 0.01$, t-test ($n=3$).

Suppl. Figure 2: No overt structural, neurological or behavioral changes in RNase H2^{ΔGFAP} mice.

A) HE staining of RNase H2^{ΔGFAP} brains showed no morphological differences between genotypes ($n=3$) at 8 months of age. B) Tabular summary of the neurological and behavioral analyses. Only subtle, sporadic and mostly sex-specific changes with no apparent relation to AGS were observed in RNase H2^{ΔGFAP} mice. For detailed reports see Suppl. Fig. 3 (behaviour screen) and Suppl. Data. (neurology screen).

Suppl. Figure 3: Detailed behavioral characterization of RNase H2^{ΔGFAP} mice.

Open Field test (A-C). Spontaneous locomotor activity in a novel environment as measured by total distance travelled (A) at the age of 8 weeks yielded no difference in forward locomotor activity, but a significantly reduced rearing activity (B) in female RNase H2^{ΔGFAP} mice only (Two-way ANOVA with Sidak's post-hoc test corrected for multiple comparisons; genotype \times sex interaction: $F(1,35) = 6.494$, $p < 0.05$; adjusted p-value post-hoc test females: $p < 0.001$). RNase H2-deficiency in the brain affected anxiety-related behavior as measured by the time spent in the centre of the Open Field (C) only subtly and in opposite directions in the two sexes (genotype \times sex interaction: $F(1,35) = 4.425$, $p < 0.05$; post-hoc tests n.s.). Spontaneous alternation in the Y-Maze did not reveal any difference in working memory, but an increase of same arm returns that was only significant in male RNase H2^{ΔGFAP} mice (D), suggesting an increase in repetitive behavior mice (genotype \times sex interaction: $F(1,29) = 4.498$, $p < 0.05$; adjusted p-value post-hoc test males: $p < 0.01$). Social discrimination test (E,F). Analysis of social recognition memory demonstrated a trend towards reduction in social affinity (E) that just missed significance (genotype effect: $F(1,29) = 4.165$, $p = 0.0505$), and no difference in the social recognition index (F). There were no genotype effects in sensorimotor gating behavior as measured by prepulse inhibition of the acoustic startle reflex (data not shown).

Several tests for basic neurological function did not reveal clear differences either. There were subtle significant changes in single parameters but no clear indication for neurological dysfunctions in the mice tested at the age of 9-14 weeks (see supplemental Neurology data). To exclude a later onset of symptoms, mice were analyzed at the age of 12-14 months again with additional motor tests but again showing only single alterations (see supplemental Neurology data).

Suppl. Fig. 4: No exacerbation of CNS autoimmunity in RNase H2^{ΔGFAP} mice.

A) Following immunization with myelin oligodendrocyte glycoprotein peptide (MOG35-55), RNase H2^{ΔGFAP} and control mice developed similar clinical signs of ascending paralysis. Disease course of control ($n=9$) versus RNase H2^{ΔGFAP} ($n=10$) mice after immunization with MOG/CFA/Ptx. Graph is representative of 2 independent experiments. B) Analysis of clinical

parameters, including disease onset, maximum and cumulative clinical score, revealed no differences between RNase H2^{ΔGFAP} and control mice. EAE disease parameters depicting the area under the curve (AUC), day of onset and maximum clinical score of EAE-immunized control (n=16) and RNase H2^{ΔGFAP} (n=18) mice. Data is summarized from both independent experiments. C) Similar clinical manifestation was further confirmed by the degree of demyelination in spinal cords of RNase H2^{ΔGFAP} versus control mice. Hematoxylin/Eosin (HE) and Luxol fast blue (LFB) stainings of spinal cord sections from EAE-afflicted control and RNase H2^{ΔGFAP} mice. Note the lesion areas as manifested by dense leukocyte infiltration (HE) co-localizing with apparent demyelination (LFB). Pictures are representative for 4 mice with similar disease scores examined in each group. Scale bar = 100μm. D) Primary cultures generated from P3/4 brains consistently contained > 90% of GFAP-positive murine astrocytes. A representative astrocyte preparation from an RNase H2-proficient control brain is shown. Cells were counter-stained with DAPI and rabbit anti-RNase H2 antiserum.

Supplementary tables

A Nucleotide sequences of qPCR primers used in the study

Gene	forward primer	reverse primer
Ifit1	GAACCCATTGGGGATGCACAACCT	CTTGTCCAGGTAGATCTGGGCTTCT
Ifit2	ATGAGTTTCAGAACAGTGAGTTTAA	AACTGGCCCATGTGATAGTAGACCC
Irf7	ATGCACAGATCTTCAAGGCCTGGGC	GTGCTGTGGAGTGCACAGCGGAAGT
Cxcl10	GCCGTCATTTTCTGCCTCA	CGTCCTTGCGAGAGGGATC
Cdkn1a (p21)	CCTGGTGATGTCCGACCTGTT	GGGGAATCTTCAGGCCGCTC
Trp53	CTAGCATTCAGGCCCTCATC	TCCGACTGTGACTCCTCCAT
Igfbp5	CGCGGGGTTTGCCCTAACGA	CTGCGGCAGGGGCCCTTGTC
Lamp1	TAATGGCCAGCTTCTCTGCCTCCTT	AGGCTGGGGTTCAGAAACATTTTCTT
Ctsa	GACTCCAAGCACTTCCACTACTGGT	CTGGCTGGATCAGAAAGGGGCCGTG
Gapdh	TTCACCACCATGGAGAAGGC	GGCATCGACTGTGGTCATGA
Rnaseh2b	AGGTTTCCAGGGACAAGGAAGAGGA	GTCAATGAAGCTGGAGGTTCTGGAAG

B Dilutions of antibodies used in the study

Antibody	Application	Dilution 1st AB	2nd AB	Dilution 2nd AB
α -mouse RNase H2 holoenzyme	IHC, IF	both 1:250	α -rabbit	1:5000
α -NeuN	IHC	1:1500	α -mouse	1:500
α -GFAP	IHC	1:500	α -mouse	1:500
α -Iba-1	IHC	1:500	α -rabbit	1:500
α -Ki-67	IHC	1:500	α -rat	1:500
α -RNase H2A	WB	1:1000	α -rabbit	1:5000
α -PARP1	WB	1:1000	α -rabbit	1:5000
α - γ H2AX	IF, FACS	both 1:75	α -rabbit	1:1000
α -LAMP1	WB	1:1000	α -mouse	1:5000
α -H-Ras	WB	1:500	α -mouse	1:5000

IHC: Immunohistochemistry (frozen sections)

IF: Immunofluorescence

WB: Western Blotting

FACS: Flow Cytometry

AB: Antibody