

Supplementary Material

Supplementary Table S1

Primary antibodies used in this work and their commercial source and reference. The listed antibodies were used in immunofluorescence and/or western blot techniques to check for the differential expression of the corresponding proteins between *Epm2b*^{-/-} and wt samples of 16 months-old animals. The antibodies are arranged according to the corresponding inflammatory pathway where they are related. The antibodies that are in the column “No differences” were also used in immunofluorescence experiments but we found no differential signal in *Epm2b*^{-/-} samples in comparison to wt (see Supplementary Fig. S8). The secondary antibodies used in this work are also listed.

Supplementary Table S2

Plasma samples from two *Epm2b*^{-/-} and two wt mice of 16 months of age were analyzed by using a high throughput mouse cytokine array, which contains 111 captured antibodies against inflammatory mediators (Mouse XL Cytokine Array Kit, R&D). The crude average intensity of all the spots, which was measured by using the Quick Spot software provided by the manufacturer is presented. Values were ordered according to fold change. The SEM and p-value are also presented. Only CCL20 and IL15 (in grey) had a p-value ≤ 0.05 .

Supplementary Figure S1

(A) Representative microscopy bright field vertical images of polyglucosans (PG) using *Periodic acid–Schiff* (PAS) stain in samples from 16 months-old *Epm2b*^{-/-} and wt mice hippocampus. Neural nuclei were stained with hematoxylin (in blue). Black arrows highlight the presence of Lafora bodies (pink) only in the *Epm2b*^{-/-} mouse model. A squared dashed line indicates the area that is magnified in the right panels. The scale bar corresponds to 50 μ m. (B) Schematic representation of the different areas within the hippocampus where the intensity signal of the corresponding antibodies has been quantified after immunofluorescence, as indicated in Methods: A, containing the radiatum, the lacunosum-moleculare and the molecular dentate gyrus layers (RLM+MoDG area); B, the granular layer of the dentate gyrus (GrDG area); C, Pyramidal layer of the Cornus ammonis 1-3 and the polymorph layer of the dentate gyrus (Py+PoDG area).

Supplementary Figure S2

Representative confocal images of the whole hippocampus derived from 16 months-old *Epm2b*^{-/-} or wt mice, stained by immunofluorescence with the following antibodies: (A)

anti-TNFalpha, (B) anti-p65, (C) anti-phospho (Thr180/Tyr182)-P38 (pP38), or (D) anti-phospho (Thr202/Tyr204)-ERK1/2 (pERK1/2) (all in green). Five independent samples from each genotype were analyzed in the same way. The scale bar corresponds to 100µm. A squared dashed line indicates the area that is magnified. Signal quantifications are included in Figure 1 and Supplementary Fig. S3.

Supplementary Figure S3

Quantification of the intensity signal in the GrDG and Py+PoDG areas (see Supplementary Figure S1B) for TNFalpha (A and B), p65 (C and D) represented as arbitrary units (a.u.), or the percentage of positive cells for pP38 (E and F) and pERK1/2 (G and H). Results are expressed as median with a range of five independent samples including male and female mice from both genotypes. The differences between the two groups (wt and *Epm2b*^{-/-} mice) were analyzed by Mann-Whitney non-parametric t-test. Not significant (ns) differences were observed for any of the quantifications. Signal quantification of the RLM+MoDG area is included in Figure 1.

Supplementary Figure S4:

Representative confocal images of the whole hippocampus derived from 16-month-old *Epm2b*^{-/-} or wt mice, stained by immunofluorescence using the anti-Caspase-8 (green) antibody. Five independent samples from each genotype were analyzed in the same way. The scale bar corresponds to 100µm. A squared dashed line indicates the area that is magnified. (B and C) Signal quantification in the GrDG and Py+PoDG areas for caspase-8 represented as arbitrary units (a.u.). Results are expressed as median with a range of five independent samples including male and female mice from both genotypes. The differences between the two groups (wt and *Epm2b*^{-/-} mice) were analyzed by Mann-Whitney non-parametric t-test. Not significant (ns) differences were observed for any of the quantifications. Signal quantification of the RLM+MoDG area is included in Figure 2.

Supplementary Figure S5:

Representative confocal images of the whole hippocampus derived from 16-month-old *Epm2b*^{-/-} or wt mice, stained by immunofluorescence using the following antibodies: (A) anti-caspase-11 (green), or (B) anti-GSDM-D (green). Five independent samples from each genotype were analyzed in the same way. The scale bar corresponds to 100µm. A squared dashed line indicates the area that is magnified. (C and D) Signal quantification in the GrDG and Py+PoDG areas for GSDM-D represented as arbitrary units (a.u.). Results are expressed as median with a range of five independent samples

including male and female mice from both genotypes. The differences between the two groups (wt and *Epm2b*^{-/-} mice) were analyzed by Mann-Whitney non-parametric t-test. Not significant (ns) differences were observed for any of the quantifications. Signal quantification of the RLM+MoDG area is included in Figure 3.

Supplementary Figure S6:

Representative confocal images of the whole hippocampus derived from 16 old month *Epm2b*^{-/-} or wt mice stained by immunofluorescence with the following antibodies: (A) anti-Interleukin-6 (IL6), (B) anti-phospho (Tyr1007/Tyr1008)-JAK2 (pJAK2), (C) anti-phospho (Tyr-701)-STAT1 (pSTAT1), (D) anti-phospho (Tyr-705)-STAT3 (pSTAT3), or (E) anti-SOCS3 (green). Five independent samples from each genotype were analyzed in the same way. The scale bar corresponds to 100µm. A squared dashed line indicates the area that is magnified. Signal quantifications are included in Figure 4 and Supplementary Fig. S7.

Supplementary Figure S7

Quantification of the intensity signal in the GrDG and Py+PoDG areas (see Supplementary Figure S1B) for Il6 (A and B), pJAK (C and D), pSTAT1 (E and F) and SOCS3 (I and J) represented as arbitrary units (a.u.), or the percentage of positive cells for pSTAT3 (G and H), referred to the total nuclei in each corresponding area. Results are expressed as median with a range of five independent samples including male and female mice from both genotypes. The differences between the two groups (wt and *Epm2b*^{-/-} mice) were analyzed by Mann-Whitney non-parametric t-test. **P<0.01; ns, not statistically significant differences. Signal quantification of the RLM+MoDG area is included in Figure 4.

Supplementary Figure S8

Immunofluorescence images of hippocampal section of the brain of wt and *Epm2b*^{-/-} mice using the indicated antibodies. In the case that the antibodies did not show differences between two initial wt and *Epm2b*^{-/-} mice samples, we collected representative images from the immunofluorescence analyses. For some of these antibodies, we extended the analyses to three additional samples and quantified the signal in the RLM+MoDG area. Bars indicate 100 µm unless indicated. In the case of anti-IFNalpha and anti-IFNgamma, as they did not give any signal in IF, we performed a western blot analysis.

Supplementary Table S1
Primary antibodies

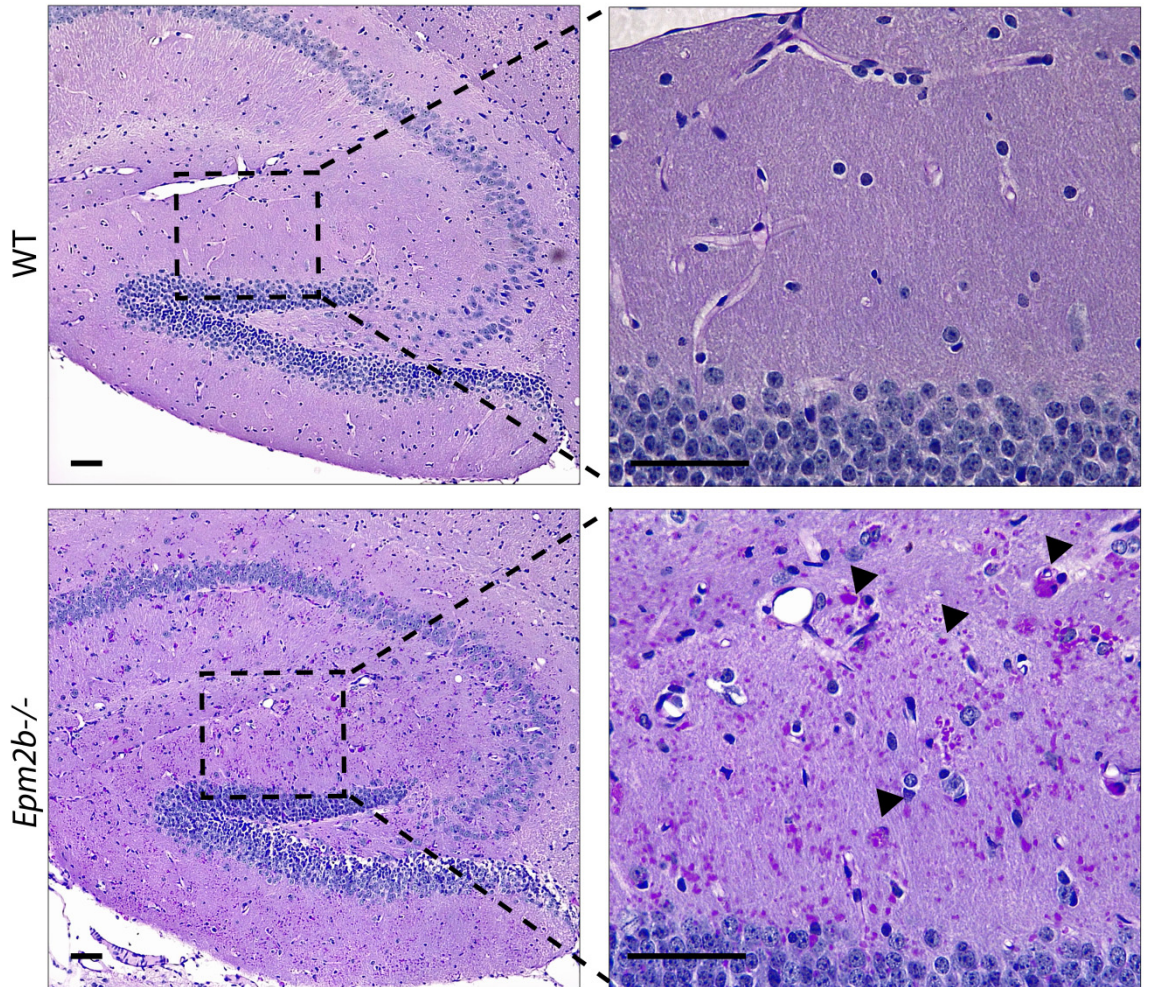
Pathway	Increased in <i>Epm2b</i> ^{-/-} mice	No differences <i>Epm2b</i> ^{-/-} vs WT mice
TNF	TNF (ab1793) p65 (F6) (SC-8008) Caspasa-8 (Cell signaling 9746) Phospho (Ser345)-MLKL (Millipore MABC1158)	TNFR1 (Invitrogen PA5-95585) Caspase-3 (Cell signaling 9662)
TLR/IL1RL1		HMGB1 (ab18256) IL1RL1 (Tyr496) (bs-5394R) IL1beta [ERP16805-15] (ab234437) MyD88 (ab133739) TLR4 (ab13556) TLR7 (Invitrogen PA5-95258) IRF3 (ab68481) IRF7 (Invitrogen PA5-20280) INF γ (bioss bs-0480R) INF α (bioss bs-7023R)
MAP kinases	pERK1/2 (Thr180/Tyr182) (Cell signaling 4376) pP38 (Thr180/Tyr182) (Cell signaling 9211)	ERK2 (SC-1647) P38 (SC-535) p-JNK-SAPK (Thr183/Tyr185) (Cell signaling 4668) JNK-SAPK (Cell signaling 9252)
IL6/JAK/STAT	IL6 (ab6672) pJAK2 (Tyr1007-Tyr1008) (SC-16566R) p-STAT1 (Tyr 701) (ab30645) p-STAT-3 (Tyr705) (Cell signaling 9145) SOCS3 (ab16030)	
Inflammasome	Caspase-11(NB120-10454) GSDMD (ab219800)	AIM2 (ab93015) NAIP (RD systems AF7608) Caspase-1 (NBP1-45433) NLRP3/NALP3 (NBP2-12446)
Lymphocyte infiltration	CD3 [CD3-12] (ab11089) CD4 [EPR19514] (ab183685) CD8 alpha (ab217344)	
Granulocytes		Ly-6G (BD pharmingen 551459)
Macrophages		F4/80 (ab6640)
BBB		Albumin (ab192603)
Reactive glia	Iba1 (wako 019-19741) S100 β (ab52642) GFAP (synaptic system 173 004)	
Chemokine	CXCL10 (Lahuerta et. al; 2020) CCL5 (Lahuerta et. al; 2020) LCN2 (Lahuerta et. al; 2020)	

Secondary antibodies

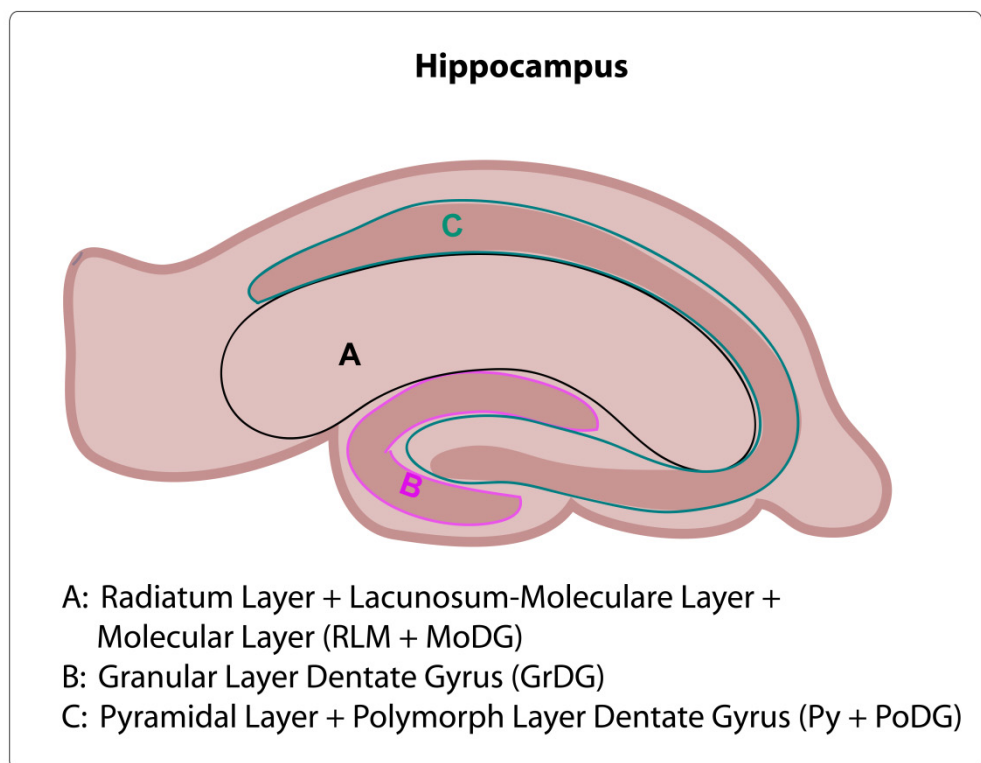
Secondary antibody	Reference
Alexa-Fluor 488 Goat anti Mouse IgG Fc	115-S45-164 Jackson
Alexa-Fluor 488 Goat anti Rabbit IgG Fc	111-S45-046 Jackson
Alexa-Fluor 568 Donkey anti Mouse IgG (H+L)	A10037 Thermo Fisher Scientific
Alexa-Fluor 568 Goat anti Guinea Pig IgG (H+L)	A-11075 Invitrogen
Alexa-Fluor 633 Goat anti Guinea Pig IgG (H+L)	A-21105 Invitrogen
Alexa-Fluor 633 Goat anti Rabbit IgG (H+L)	A-21071 Thermo Fisher
Alexa-Fluor 594 Donkey anti Rat IgG (H+L)	A-21209 Thermo Fisher
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anti Rabbit-HRP	NA934 Amersham
anti Rat-HRP	NA935 Amersham

Supplementary Figure S1

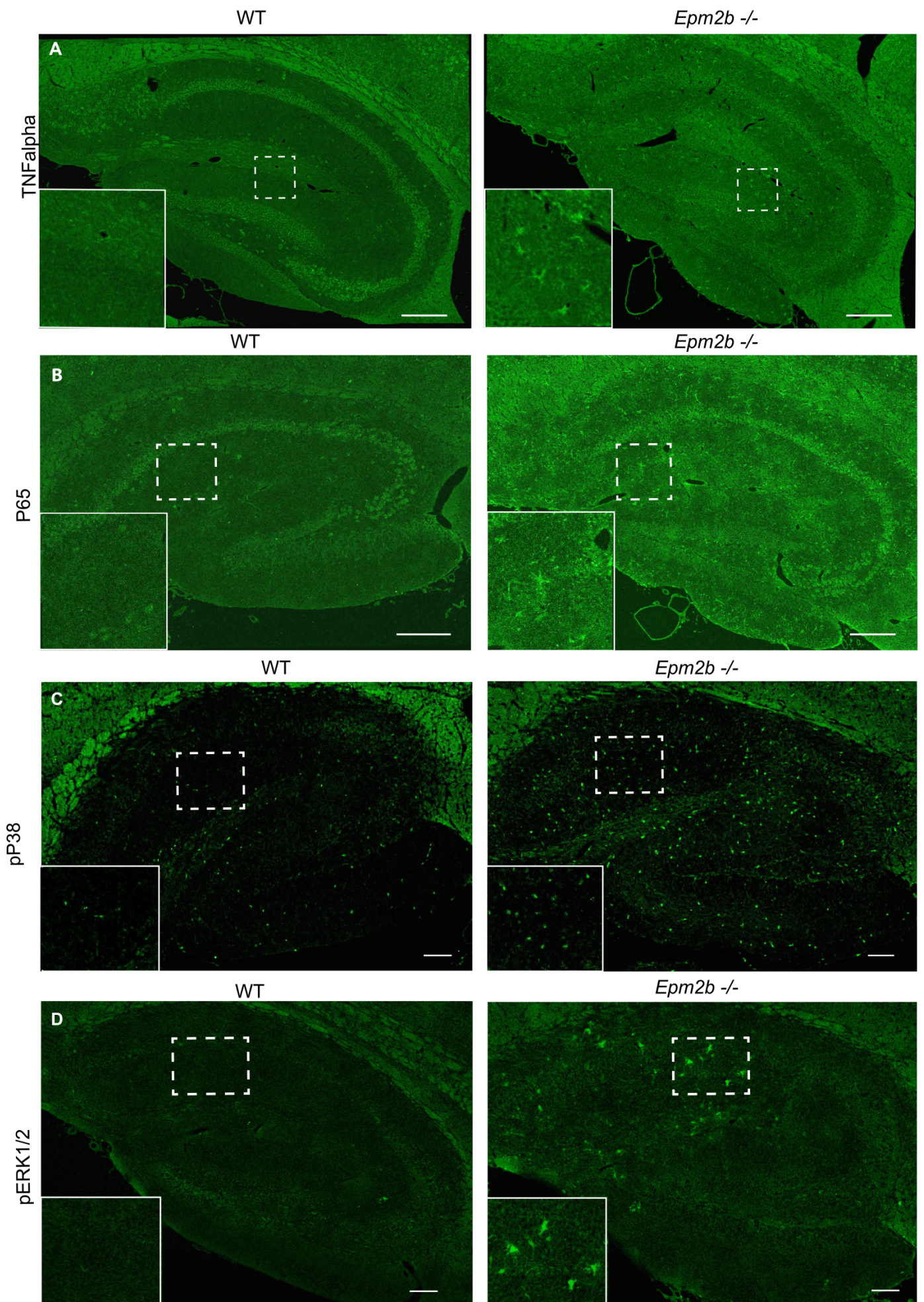
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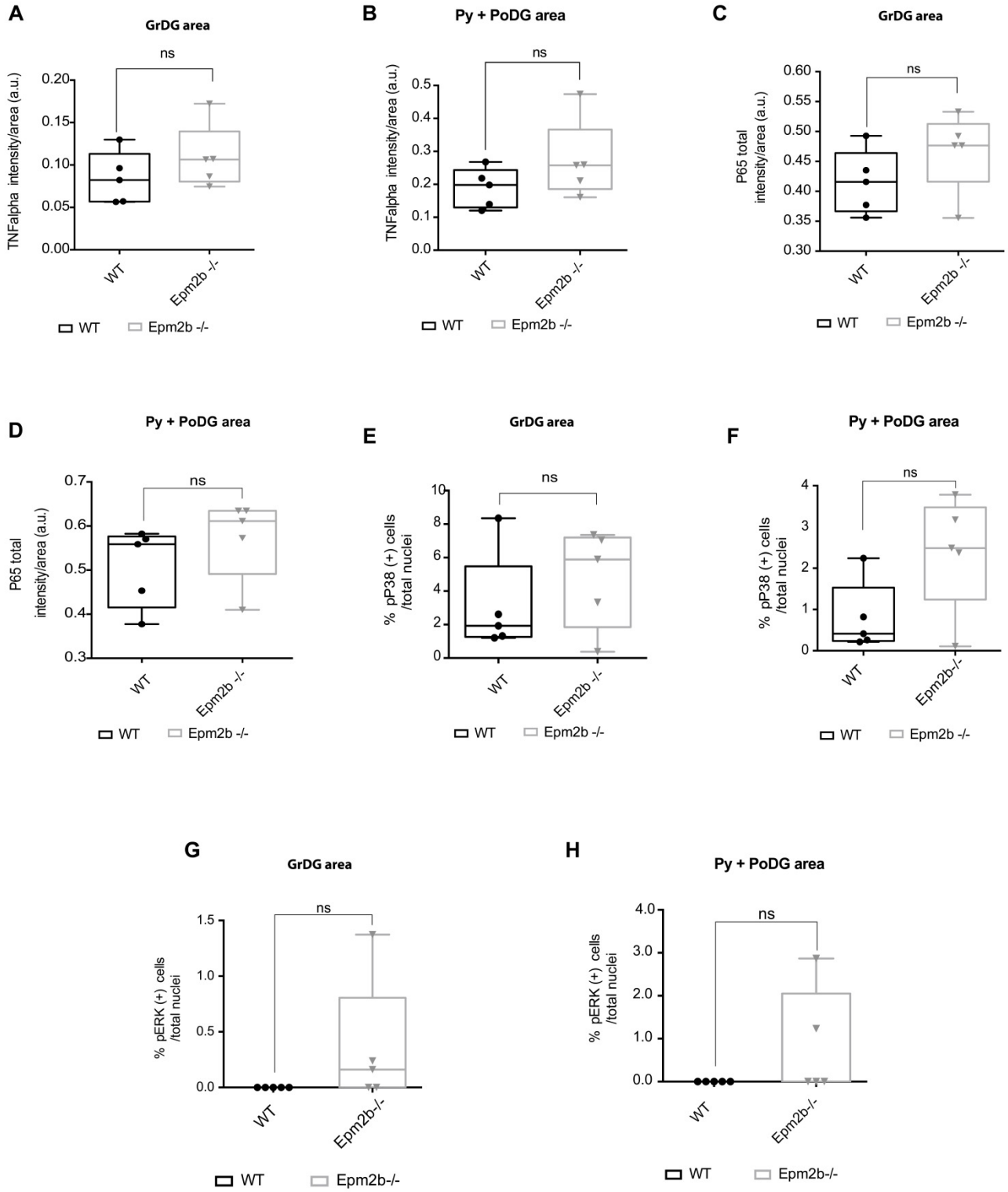
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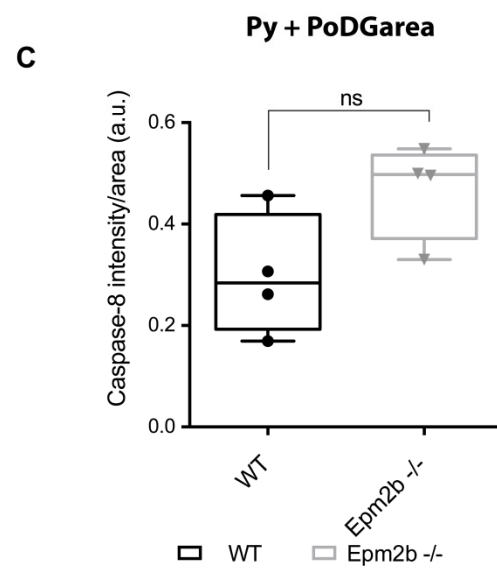
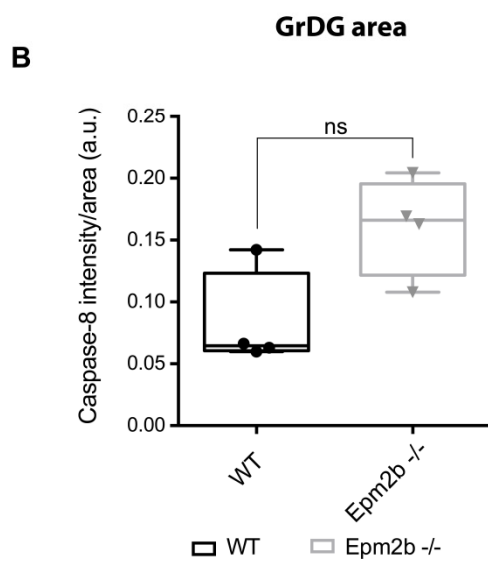
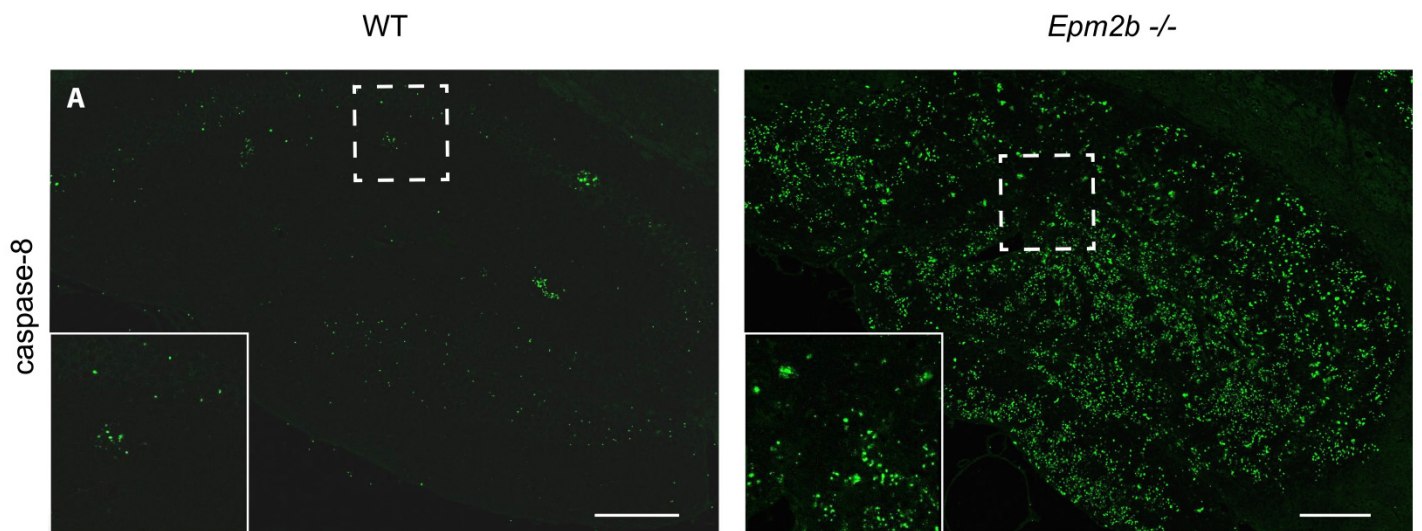
Supplementary Figure S2



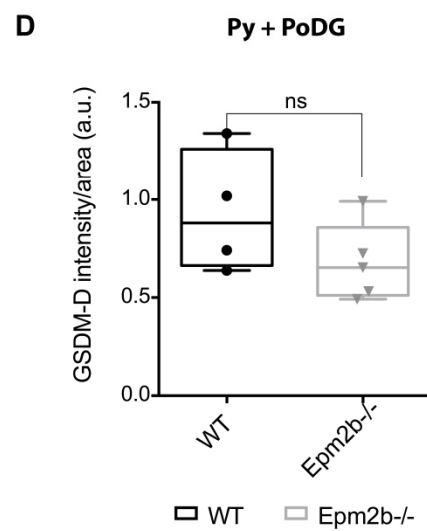
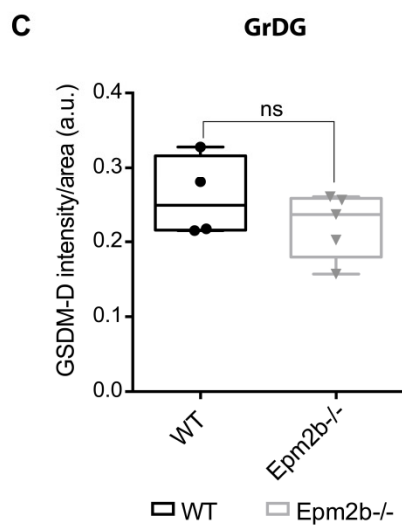
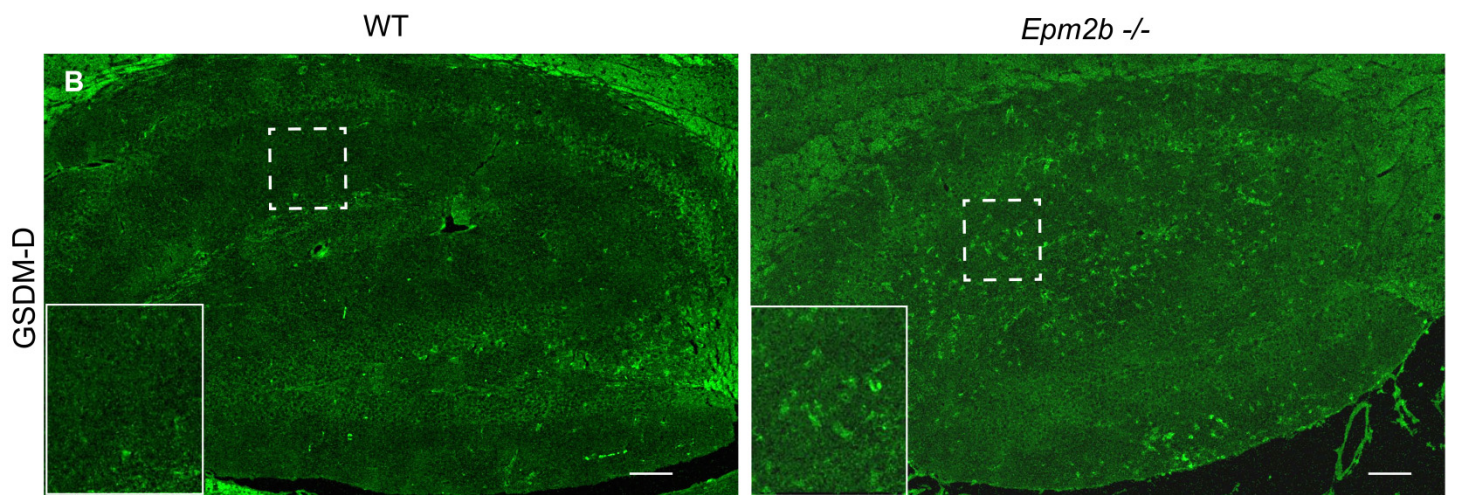
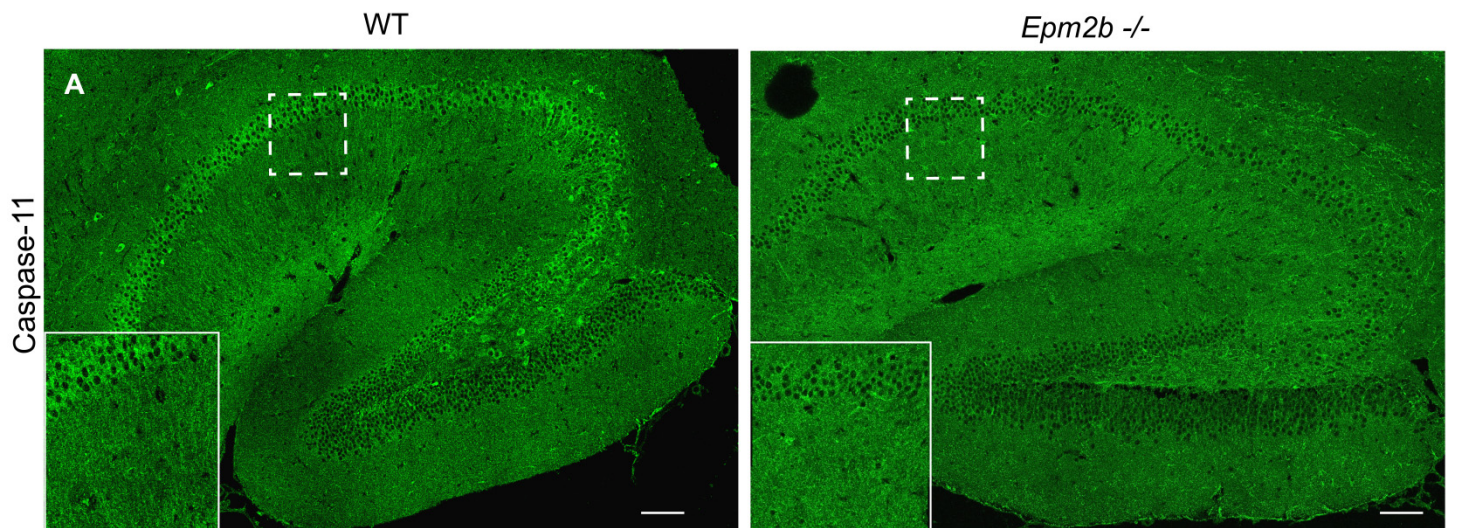
Supplementary Figure S3



Supplementary Figure S4



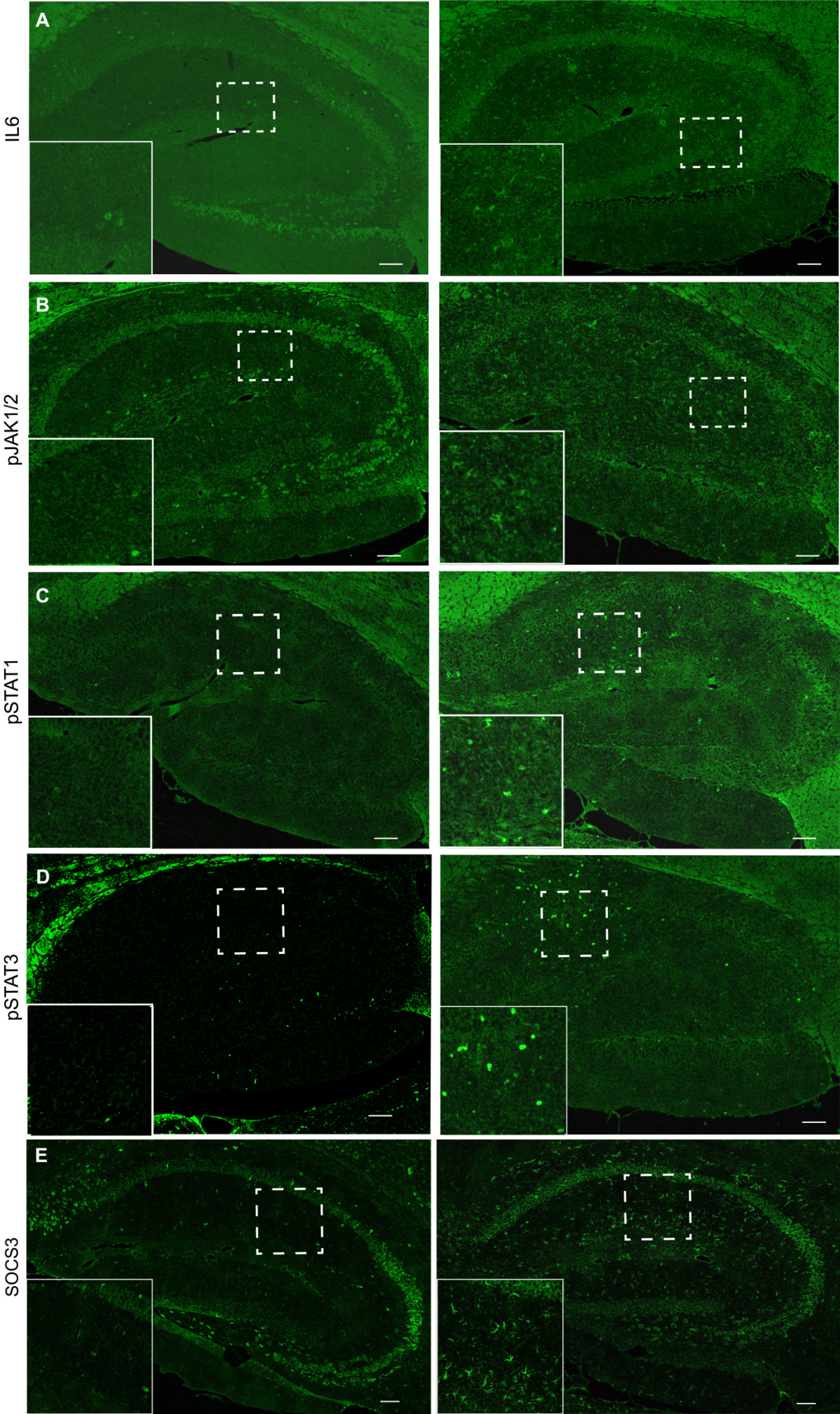
Supplementary Figure S5



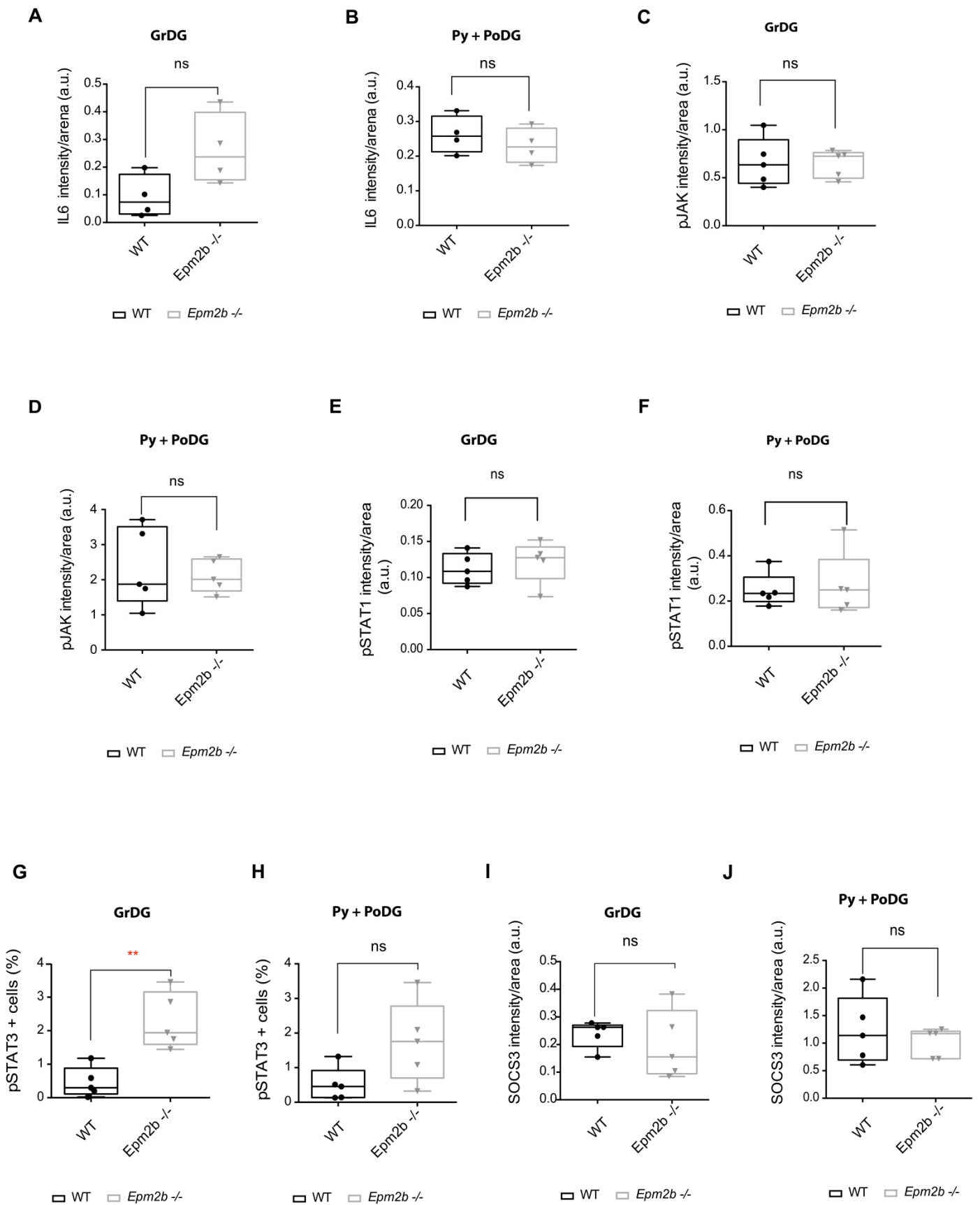
Supplementary Figure S6

WT

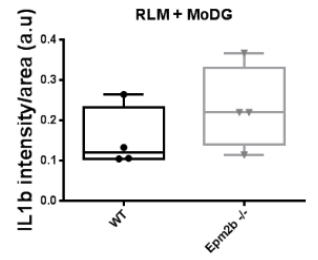
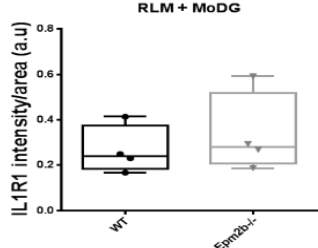
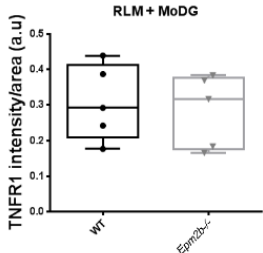
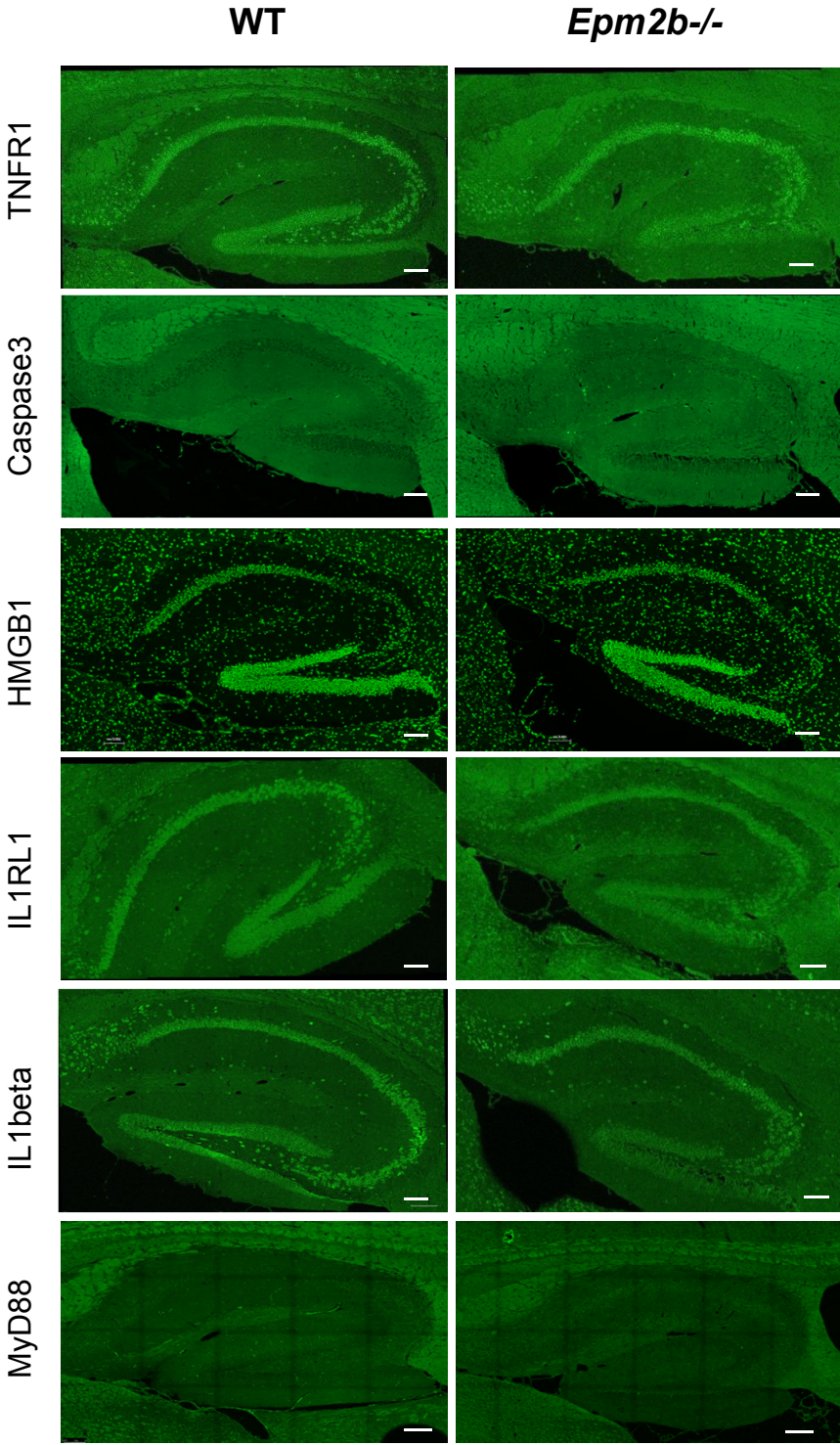
Epm2b^{-/-}



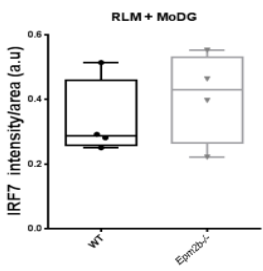
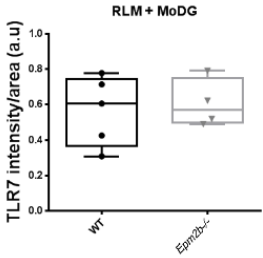
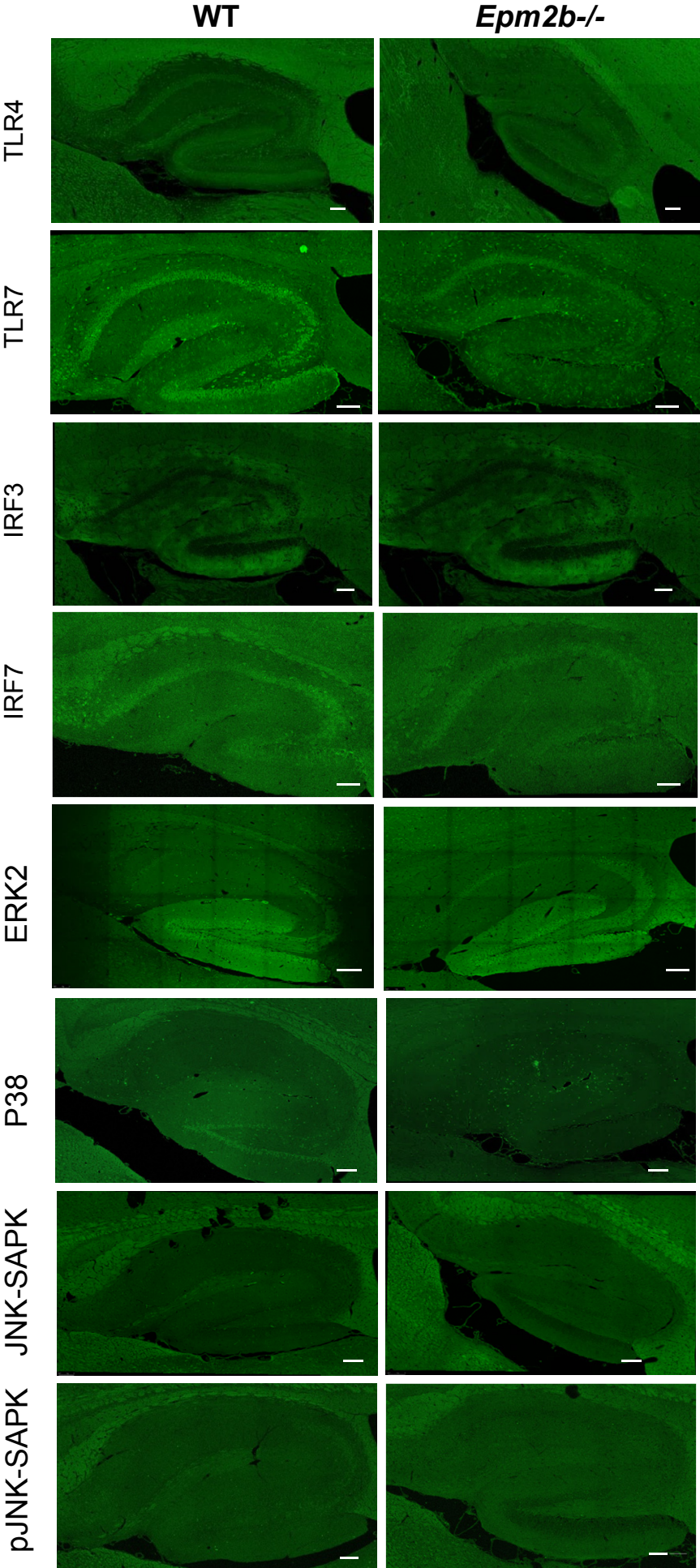
Supplementary Figure S7



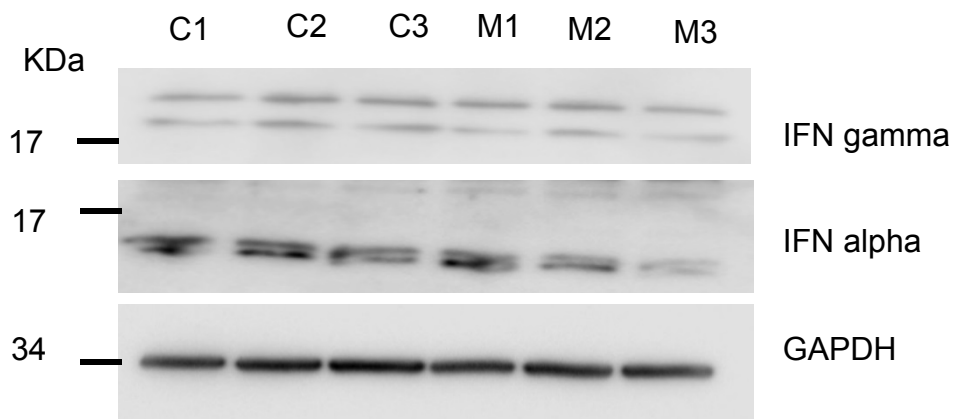
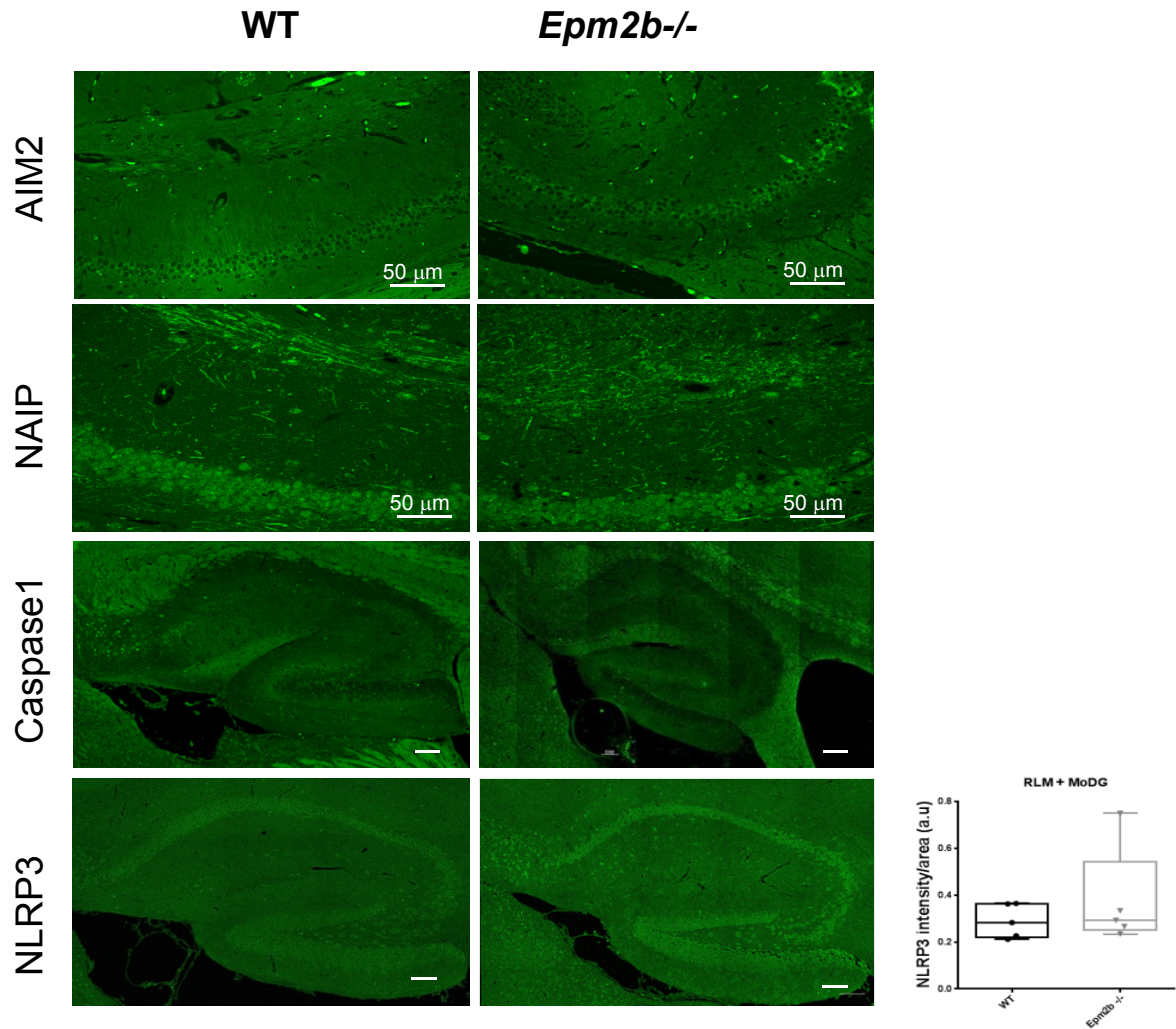
Supplementary Fig. S8 (1)



Supplementary Fig. S8 (2)



Supplementary Fig. S8 (3)



Supplementary Fig. S8 (4)

