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Supplemental Information

RbAp48 Protein Is a Critical Component of GPR158/OCN Signaling and Ameliorates Age-Related Memory Loss

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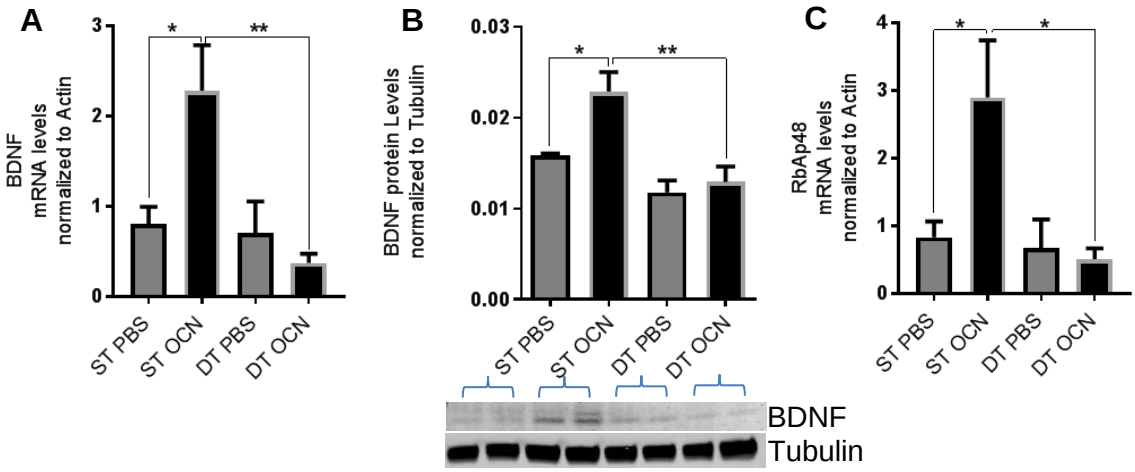
Figure S1 related to Figure 1



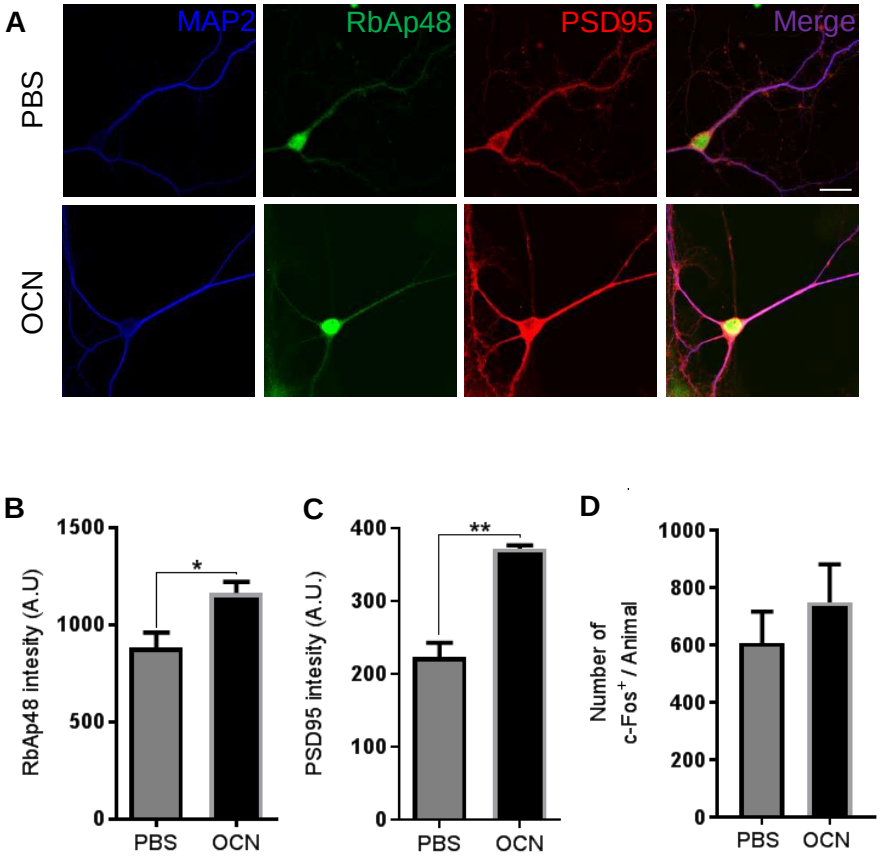
S1 related to Figure 1

(A) Quantitative real-time PCR (RT-PCR) of BDNF mRNA levels from dissected DG 24 h after injection of PBS or OCN in ST or DT mice. BDNF mRNA is upregulated in the DG of ST mice injected with OCN (One-way ANOVA, treatment between columns, post-hoc Bonferroni n=4-5 mice per treatment respectively, ST PBS vs ST OCN $p=0.02$ and ST OCN vs DT OCN $p=0.0027$).

(B) Quantification of BDNF protein levels from dissected DG 24 h after injection of PBS or OCN in ST or DT mice. BDNF protein is upregulated in the DG of ST mice injected with OCN (One-way ANOVA treatment between columns post-hoc Bonferroni n=4 mice, ST PBS vs ST OCN $p=0.039$ and ST OCN vs DT OCN $p=0.0035$).

(C) Quantification of RbAp48 mRNA levels from dissected DG 24 h after injection of PBS or OCN in ST or DT mice. RbAp48 mRNA is upregulated in the DG of ST mice injected with OCN (One-way ANOVA treatment between columns post-hoc Bonferroni n=4-5 mice, ST PBS vs ST OCN $p=0.0326$ and ST OCN vs DT OCN $p=0.0117$).

Figure S2 related to Figure 2



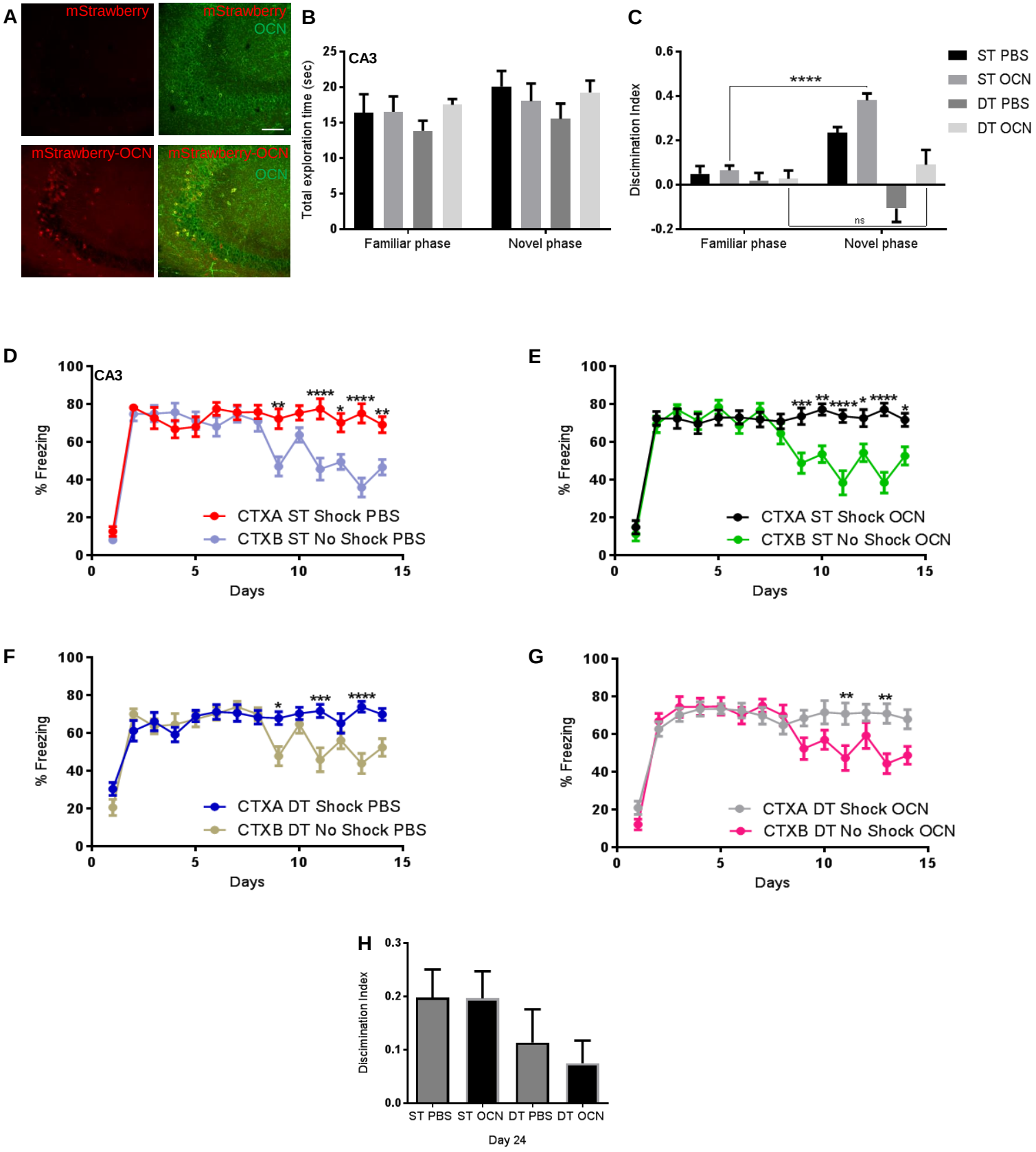
S2 related to Figure 2

(A) Representative images of MAP2 (blue), RbAp48 (green) and PSD95 (red) staining in hippocampal neuronal cultures (DIV 17) treated with PBS (*top*) or OCN (*bottom*). Right panels correspond to merged confocal channels (Scale bar 10μm).

(B-C) Quantification of RbAp48 and PSD95/μm intensities in 17DIV neuronal cultures (24h after and in triplicates) treated with OCN (5ng) or PBS. Treatment with OCN increases RbAp48 and PSD95 protein levels <120 neurons per treatment (Unpaired *t*-tests p=0.045 for RbAp48 and p=0.0017 for PSD95).

(D) Quantification of c-Fos positive neurons per animal in the DG of OCN- and PBS- injected animals. There was no significant difference in the total number of c-Fos positive neurons.

Figure S3 related to figure 3



S3 related to Figure 3

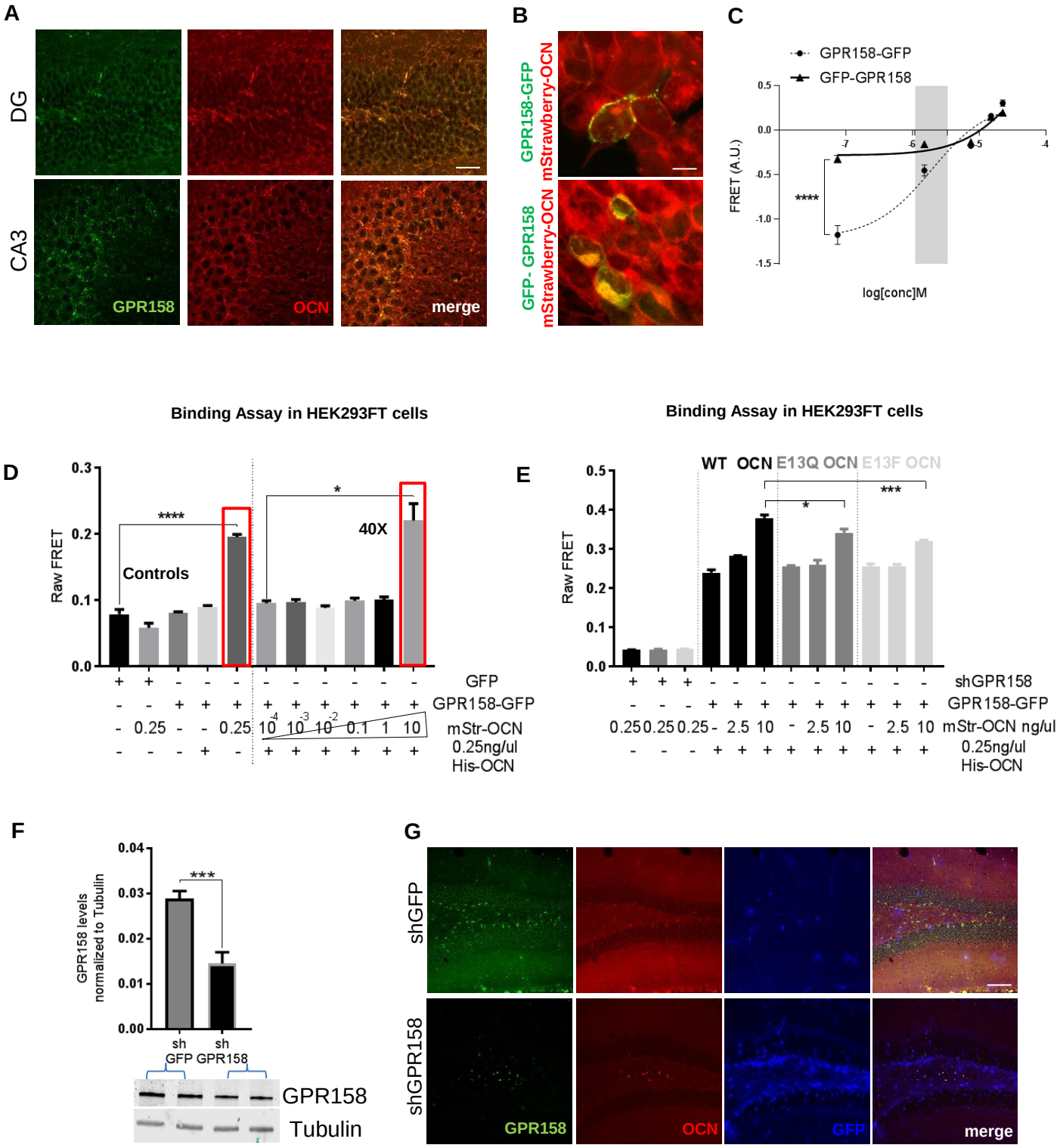
(**A**) Images of the CA3c showing accurate targeting of OCN (100 ng) 15 min after injection of mStrawberry alone (*top*) or fusion mStrawberry-OCN (100ng) (*bottom*). Total OCN (green) staining was performed as control (Scale bar 100μm).

(**B-C**) NOR test after injection of PBS or OCN in the CA3 of ST and DT mice (n=16 mice per group). No differences were detected in total exploration time between the familiarization and the testing phase (**B**), and (**C**) discrimination index for novel object. OCN had an effect only on ST mice (2-way RMANOVA comparisons between OCN or PBS and ST vs DT, post-hoc Bonferroni $p<0.0001$).

(**D-G**) Contextual Fear Discrimination in ST or DT mice injected with OCN in the CA3a. Both genotypes were able to discriminate equally between the two contexts. OCN treatment had no effect genotype. (2-way RMANOVA, post-hoc Bonferroni. 3-way RMANOVA, post-hoc Bonferroni for treatment *x* Contexts in ST mice and treatment *x* Contexts in DT mice; n=16 mice per group).

(**H**) Discrimination index of ST and DT mice injected in the CA3a with OCN or PBS, ten days after the end of Contextual Fear Discrimination. No differences were observed between genotype and treatment.

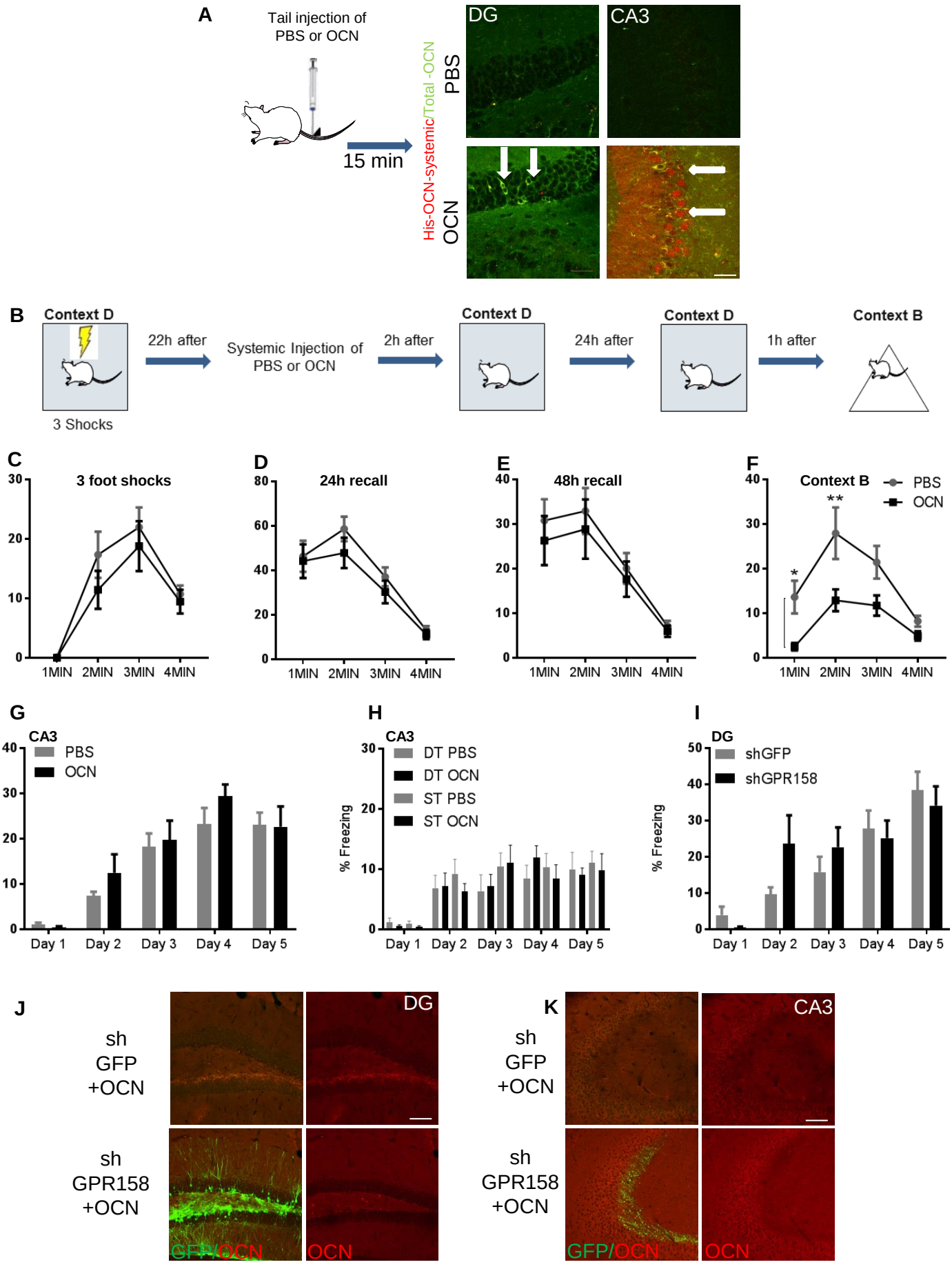
Figure S4 related to Figure 4



S4 related to Figure 4

(A) Images of DG (*top*) and CA3 (*bottom*) showing co-localization of OCN with GPR158 (Scale bar 20μm). (**B**) Co-localization of GPR158-GFP (*top*) and GFP-GPR158 (*bottom*) with WT mStrawberry-OCN in HEK293FT cells (Scale bar 5μm). (**C**) Raw FRET signal comparison between N- and C-terminus GFP-tagged GPR158 incubated with various concentrations of mStrawberry OCN (2-way ANOVA comparison between N- and C-terminal tagged GPR158 for concentrations 10^{-8} to 10^{-6}, post-hoc Bonferroni $p<0.0001$). (**D**) Quantification of FRET signal produced upon interaction of OCN with GPR158 under different conditions. mStrawberry-OCN produces significant FRET signal only in the presence of GPR158-GFP (2-way ANOVA comparison between bars 1-5, post-hoc Bonferroni $p<0.0001$). mStrawberry-OCN was added to HEK293FT cells transfected with GPR158-GFP, to displace His-tag-OCN (0.25 ng). FRET signal was produced only upon incubation with 10 ng/μl mStrawberry OCN (2-way ANOVA comparison between bars 6-11, post-hoc Bonferroni $p=0.02$). (**E**) FRET signal after GPR158 knock down and incubation with WT or mutant forms of OCN (bars 1-3) compared with HEK293FT cells transfected with GPR158-GFP and incubated with WT or mutant OCN (2-way ANOVA comparison between bars 1-3 and 4, 7, 10, post-hoc Bonferroni). Mutant forms of OCN cannot displace with the same efficiency unlabeled WT OCN (0.25ng/μl) from GPR158-GFP transfected HEK293FT (2-way ANOVA comparison between bars 6-9-12, post-hoc Bonferroni, $p=0.0116$ and $p=0.0005$). (**F**) Quantification of GPR158 protein from dissected DG after injection of shGFP (control) or shGPR158 (knock down) (Unpaired *t*-test $p=0.0004$). (**G**) Representative images of shGFP- (control, *top*) or shGPR158- (knock down, *bottom*) injected DG stained for GPR158 (green), OCN (red) and GFP (blue) (Scale bar 100μm).

Figure S5 related to Figure 5



S5 related to Figure 5

(A) Schematic of tail injection of PBS (top) or OCN (bottom) followed by immunostaining for total (green) and systemically delivered His-OCN (red) in the DG (*left*) and CA3 (*right*) (Scale bar 20μm).

(**B**) Schematic of 3-shock CFC paradigm.

(**C-F**) Quantification of freezing (n=16 mice per group). OCN treatment had an effect on behavior only after exposure to different context B (**F**) (2-way RMANOVA, post-hoc Bonferroni n=16 mice per group, Treatment p=0.0109 and p=0.0445 for 1^{st} min and p=0.0028 for 2^{nd} min).

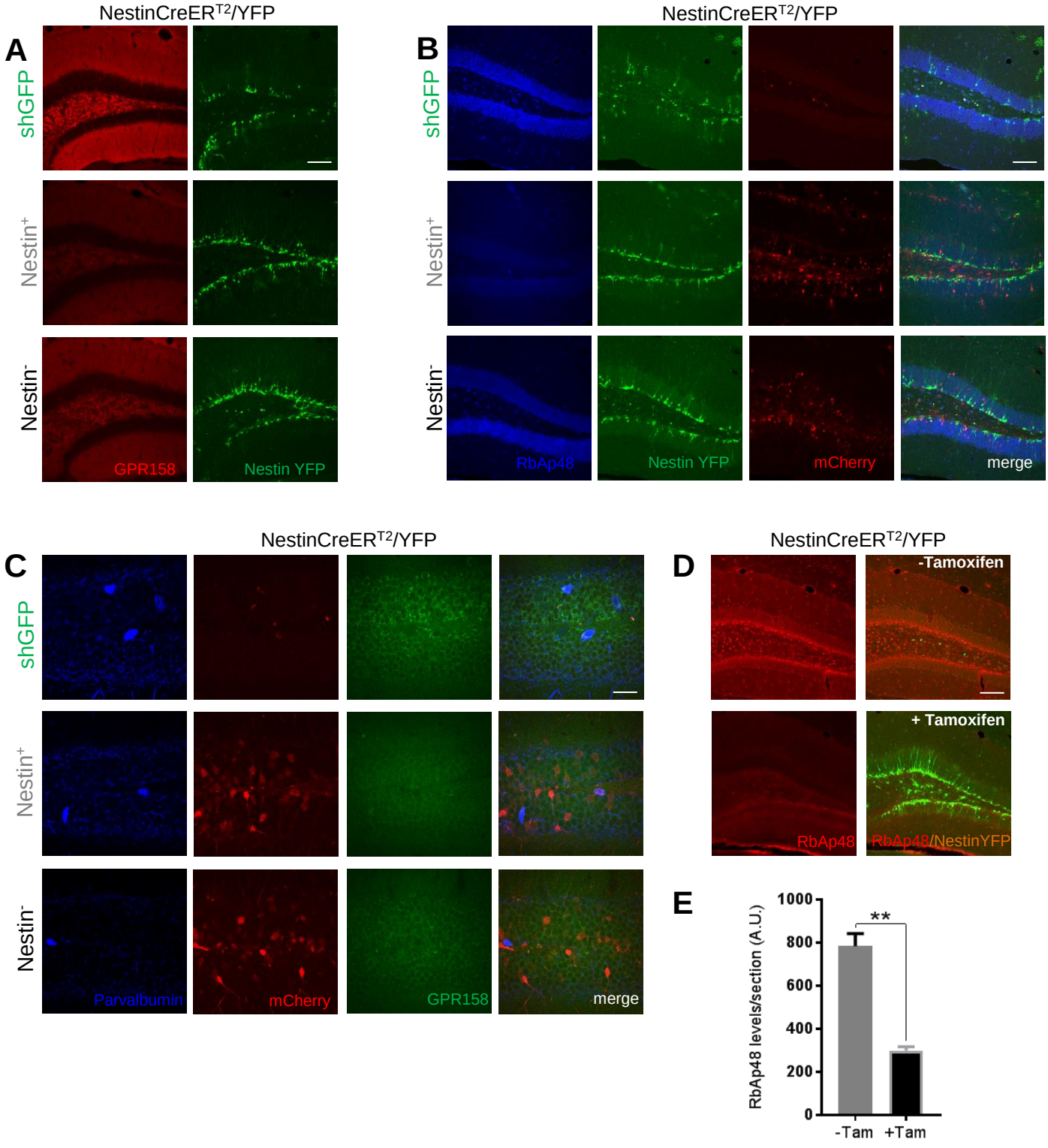
(**G**) Quantification of 5-day freezing levels of mice injected with PBS or OCN in CA3a and exposed to context D without foot shock for 10 min.

(**H**) Quantification of 5-day freezing levels of ST and DT mice injected with OCN or PBS in CA3 and exposed to context D without shock for 10 min.

(**I**) Quantification of 5-day freezing levels of shGFP and shGPR158 injected mice in the DG/CA3c and exposed to context D without foot shock for 10 min.

(J-K) Representative images from DG region **(J)** and from CA3a region **(K)** of shGFP- (control, *top*) or shGPR158- (knock down, *bottom*) animals injected in the DG after peripheral administration of OCN and pre-exposure mediated contextual fear conditioning, stained for GFP (green) and OCN (red) (Scale bar 100 μm).

Figure S6 related to Figure 6



S6 related to Figure 6

(A) Representative images of the DG from control and GPR158 knock down, in Nestin^{+} or Nestin^{-} cells. Staining for Nestin YFP (green) and GPR158 (red) proteins in the DG region (Scale bar 100μm).

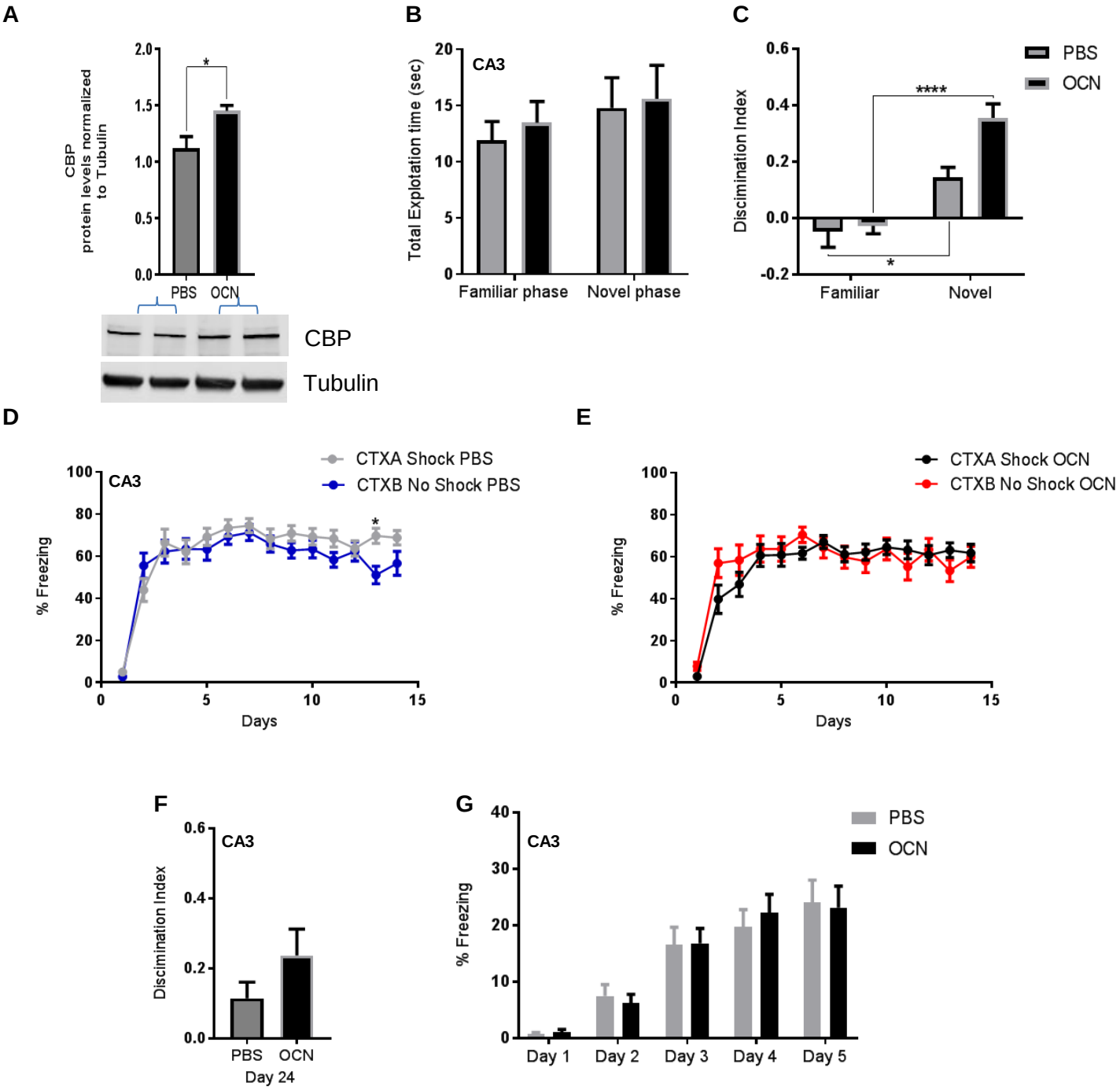
(**B**) Representative images of the DG from control and GPR158 knock down, in Nestin^{+} or Nestin^{-} cells. Staining for RbAp48 (blue), Nestin YFP (green) and mCherry (virus) (red) proteins in the DG region (Scale bar 100μm).

(**C**) Higher magnification representative images of DG from control and GPR158 knock down, in Nestin^{+} or Nestin^{-} cells. Staining for Parvalbumin (blue), mCherry (red), and GPR158 (green) proteins in the DG demonstrating GPR158 expression in Nestin^{-} cells (Scale bar 20μm).

(**D**) Staining for RbAp48 (red) and Nestin-YFP (GFP) in mice injected with GPR158 knockdown virus activated when Cre protein is present. Tamoxifen activates Cre in Nestin cells (Scale bar 100μm).

(**E**) Quantification of RbAp48 protein in mice with GPR158 knockdown in Nestin^{+} cells (+Tamoxifen) and control mice (-Tamoxifen) (Unpaired *t*-test p=0.001).

Figure S7 related to Figure 7



S7 related to Figure 7

(A) Quantification of CBP protein levels with Western blot after injection of OCN or PBS in the DG of 16-month-old WT mice. OCN increases CBP protein levels (n=5 mice per group Unpaired *t*-tests $p<0.0001$ and $p=0.0205$ respectively).

(**B-C**) No differences were observed between the genotypes in total exploration time in the familiarization or the testing phase (**B**). Discrimination index for novel object (**C**) after injections of PBS or OCN in the CA3a of 16-month-old WT mice (2-way RMANOVA comparisons, post-hoc Bonferroni, n=16 mice per group $p=0.0087$ Fam vs Novel OCN $p<0.0001$ and PBS 0.0141).

(**D-E**) Contextual Fear Discrimination in 16-month-old mice injected with OCN (**E**) or PBS (**D**) in CA3a (3-way RMANOVA for Context *x* treatment, $p=0.01$ for PBS n=16 mice per group).

(**F**) Contextual Fear Discrimination at Day 24 of the same mice (**D-E**) (Unpaired *t*-test; n=16 mice).

(**G**) Quantification of 5-day freezing levels of mice injected with PBS or OCN in CA3a and exposed to context D without foot shock for 10 min.