SUPPLEMENTAL MATERIAL

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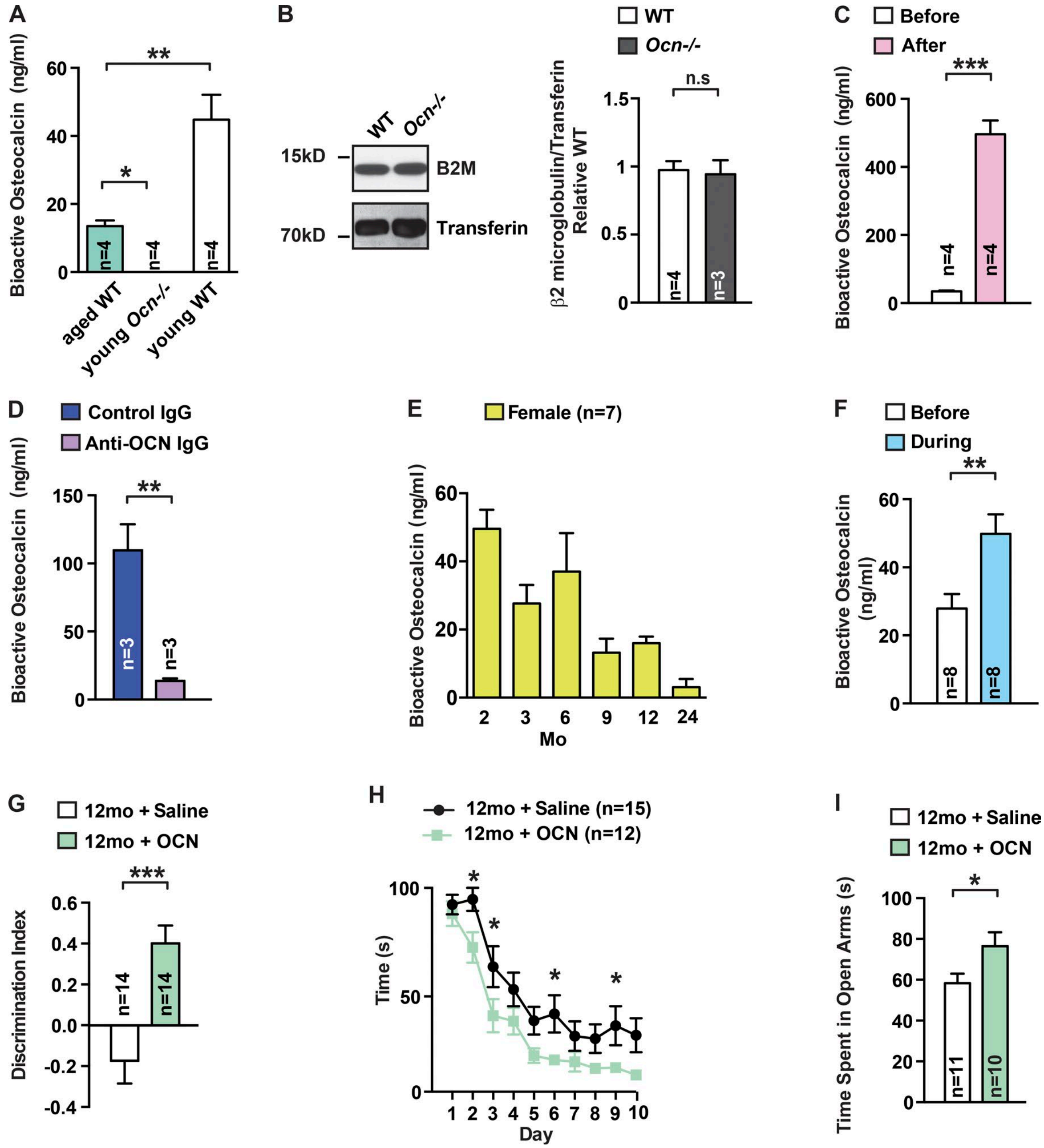

Figure S1. **OCN is sufficient to improve cognitive function and anxiety-like behaviors.** (A) Bioactive OCN content in plasma pooled from young WT, young *Ocn*−/−, or older WT used for the treatment of older mice (one-way ANOVA compared with older WT mice, followed by Bonferroni's post hoc test, *n* = 4 mice per group). (B) β_2 microglobulin accumulation (representative Western blot, left) and quantification of band intensities (right) in 3-mo-old *Ocn*−/− (*n* = 3) and WT (*n* = 4) plasma (Student's *t* test). Transferrin was used as a loading control. (C) Bioactive OCN content in the serum of older mice 24 h before or 10 min after injection with plasma from young *Ocn*−/− supplemented with 90 ng/g BW of OCN (Student's *t* test, *n* = 4 mice per group). (D) Bioactive OCN content in plasma from young mice immunodepleted with IgG or anti-OCN antibody (Student's *t* test, *n* = 3 per group). (E) Bioactive OCN content in serum of female 3–24-mo-old mice (*n* = 5–7 per group). (F) Bioactive OCN content in the serum of 16-mo-old mice before and during peripheral OCN treatment (90 ng/hr) via osmotic minipump (Student's *t* test, *n* = 8 per group). (G) NOR performed in 12-mo-old WT mice treated with saline or OCN (30 ng/hr) for 2 mo. Discrimination index was measured (Student's *t* test, *n* = 14 per group). (H) MWMT in 12-mo-old WT mice treated with saline (*n* = 15) or OCN (*n* = 12; 30 ng/hr) for 2 months. The graph shows the time to localize a submerged platform in the swimming area (two-way repeated-measures ANOVA followed by Fisher's least significantly different post-hoc test). (I) EPMT performed in 12-mo-old WT mice treated with saline (*n* = 11) or OCN (*n* = 10; 30 ng/hr) for 2 mo. Time spent in the open arms was measured (Student's *t* test). Results are given as mean ± SEM. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

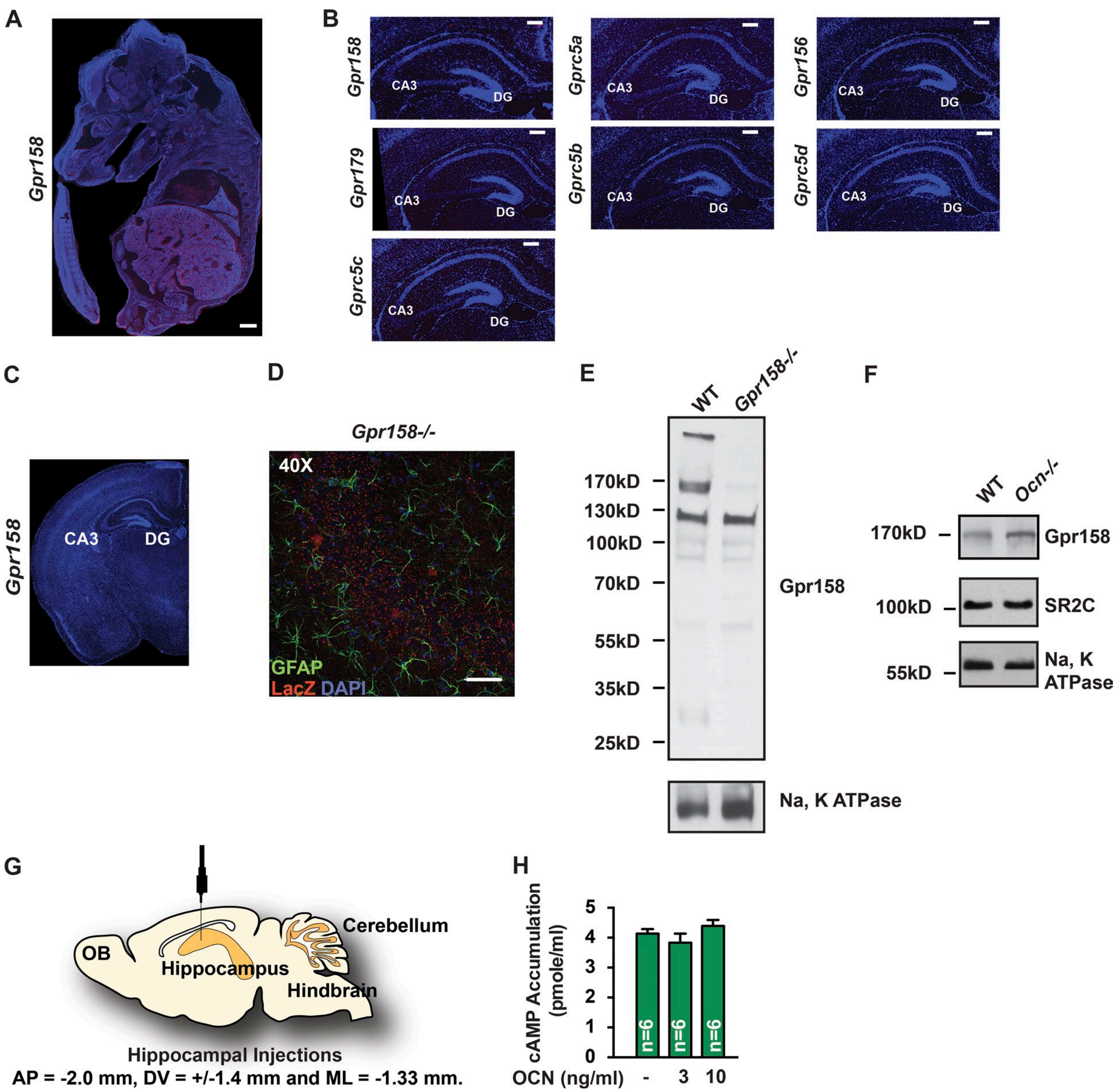

Figure S2. **Identification of a receptor for OCN in the brain.** (A) Expression (in situ hybridization) of *Gpr158* in E14.5 mouse embryos (sense probe). Bar, 0.5 mm. (B) Expression (in situ hybridization) of *Gpr156*, *Gpr179*, *Gprc5a*, *Gprc5b*, *Gprc5c*, and *Gprc5d* in the brain of 10-d-old mice (sense probe). Bar, 250 mm. (C) Expression (in situ hybridization) of *Gpr158* in the brain of 3-mo-old mice (sense probe). Bar, 250 mm. (D) Immunofluorescence of LACZ and GFAP in brain slices of 3-mo-old *Gpr158*−/− mice. Bar, 50 mm. (E) Western blot using an anti-Gpr158 antibody in membrane preparations of WT or *Gpr158*−/− hippocampi. Na,K ATPase channel was used as a loading control. (F) Gpr158 and serotonin receptor 2C (SR2C) accumulation (representative Western blot) in *Ocn*−/− and WT hippocampi. Na,K ATPase channel was used as a loading control. (G) Representation of stereotaxic coordinates for injection in the anterior hippocampus. AP, anteroposterior; DV, dorsal ventral; ML, medial lateral; OB, olfactory bulb. (H) cAMP accumulation in primary hippocampal neurons treated with either saline or OCN for 30 min (Student's *t* test, n = 6 per group). Results are given as mean ± SEM.

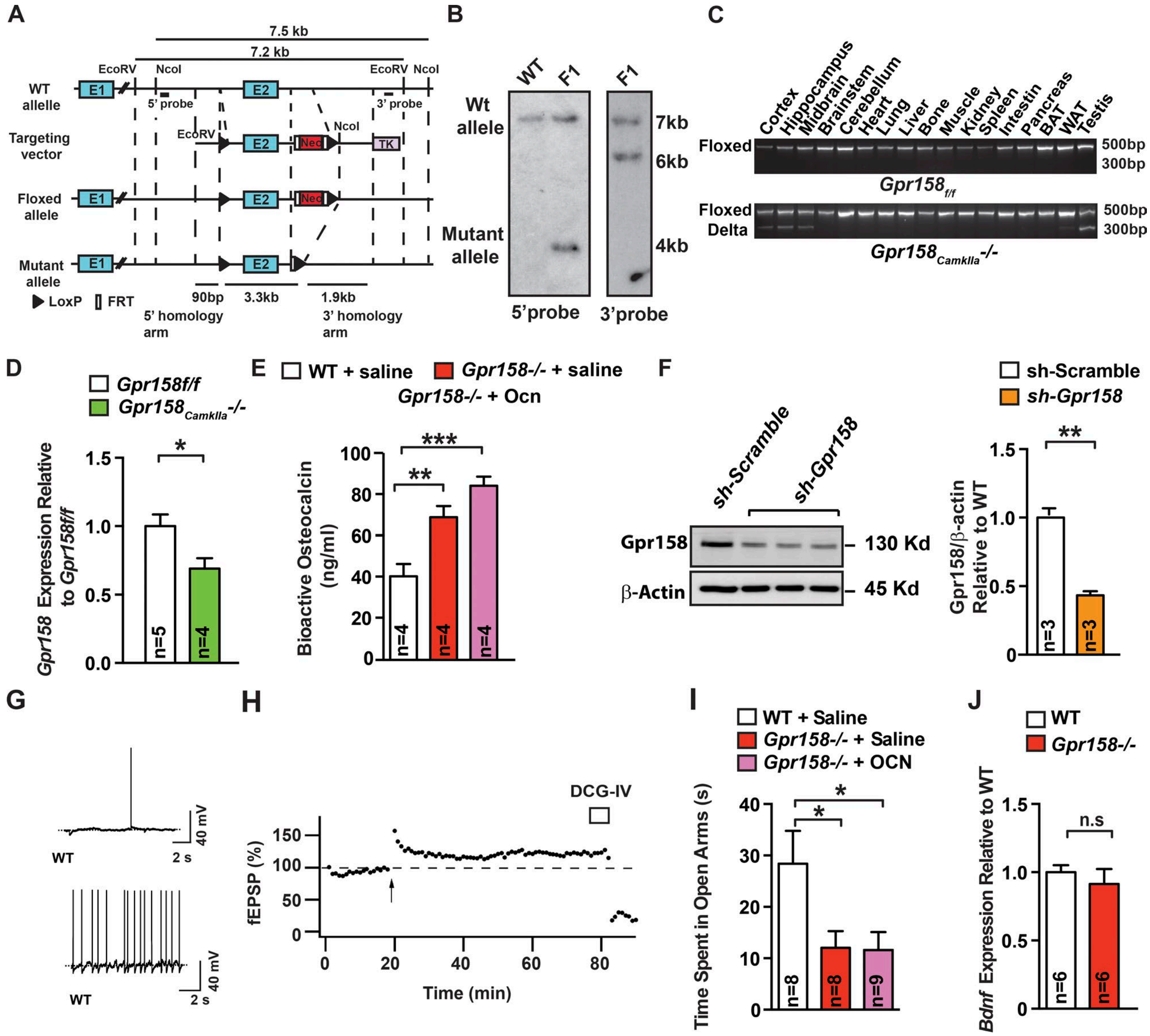

Figure S3. **Functional evidence that OCN signals through Gpr158.** (A) Targeting strategy used to generate a floxed allele of *Gpr158*. Bold lines represent locations of probes used for Southern blotting (5′ and 3′) to detect the mutated allele. (B) Southern blots showing germline transmission of the *Gpr158* floxed allele. (C) Detection of *Gpr158* mutated allele in genomic DNA isolated from tissues of *Gpr158f/f* and *Gpr158*$_{CamkIIa}$*−/−* mice. (D) *Gpr158* expression in hippocampi of 3-mo-old *Gpr158f/f* (n = 5) and *Gpr158*$_{CamkIIa}$*−/−* (n = 4) mice (Student's t test). (E) Bioactive osteocalcin content in the serum of 3-mo-old WT, *Gpr158−/−* mice during peripheral osteocalcin treatment (90 ng/hr) via osmotic minipump (one-way ANOVA compared with WT saline, followed by Bonferroni's post hoc test, n = 4 mice per group). (F) Representative Western blot (left) and quantification of band intensities (right) of Western blot performed in the hippocampus of WT mice injected with shRNA scramble or shRNA Gpr158 (Student's t test, n = 3 per group). β-Actin is used as a loading control. (G) Electrophysiogram of a single AP before osteocalcin treatment (top) and of a train of APs during osteocalcin treatment (bottom) in WT CA3 pyramidal neurons. (H) Time-course experiment showing the blockade of fEPSPs by an mGluR2/mGluR3-specific agonist, DCG-IV, after the induction of LTP at MF-CA3 synapses. Arrow, application of two trains of high-frequency stimulation. Bar, application of DCG-IV (5 μM) to the recording chamber. (I) EPMT performed in 4-mo-old WT mice treated with saline (n = 8) or *Gpr158−/−* mice treated with saline (n = 8) or OCN (n = 9; 90 ng/hr) for 1 mo. Time spent in the open arms was scored (one-way ANOVA followed by a Bonferroni test compared with WT saline treated). (J) Quantitative PCR analysis of Bdnf expression in the brainstem of *Gpr158−/−* and WT (Student's t test, n = 6 mice per group). Results are given as mean ± SEM. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.