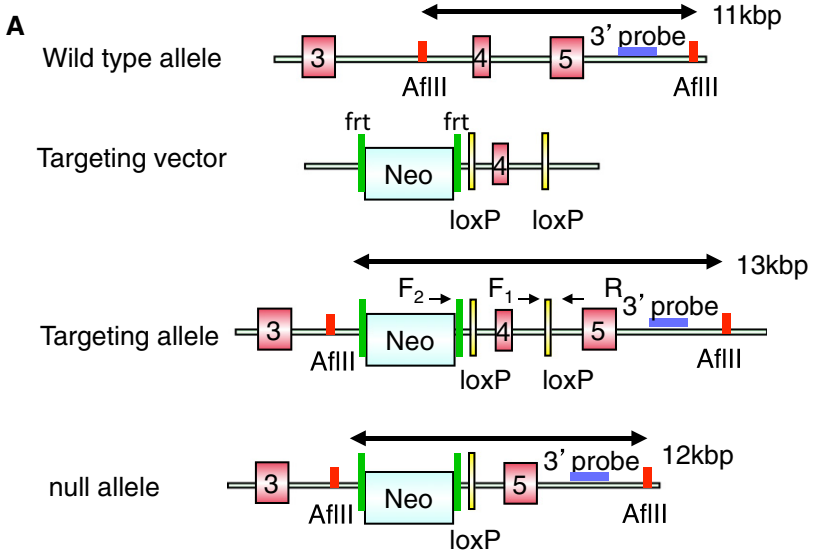
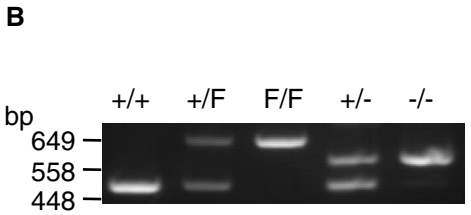
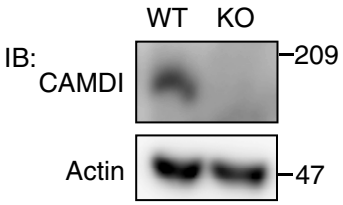
Expanded View Figures







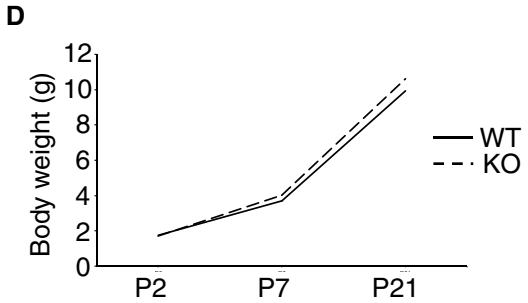


Figure EV1. Generation of CAMDI-KO mice.
Related to Fig 1.

A Targeting strategy for CAMDI-KO mice. The 3′ probes are used for Southern blot analysis. F1, F2, and R mark the primers used for genomic PCR.

B Genotypes of CAMDI mutant mice were determined by PCR on tail DNAs.

C Western blot analysis of E16 mouse brain lysate probed with anti-CAMDI-specific antibody to demonstrate an absence of CAMDI protein by CAMDI KO.

D Body weight analysis revealed normal body weight at birth and during juvenile age. n = 8 mice for each genotype.

Figure EV2. No significant difference in progenitor cell proliferation, cell fate, and callosal projection by CAMDI deletion.
Related to Fig 1.

A Abnormal distribution of Cux1-positive neurons in CAMDI-KO mice at P21. Expressions of Cux1 and CTIP2 in the somatosensory cortex were compared between P21 WT and CAMDI-KO mice. Scale bar, 20 μm.

B Quantification of the number of Cux1- and CTIP2-positive neurons of P21. n = 3 for WT mice (Cux1: 2,309 cells, CTIP2: 4,421 cells) and n = 4 for KO mice (Cux1: 2,324 cells, CTIP2: 4,393 cells). One-way ANOVA with Bonferroni's *post hoc* test. Data are presented as mean ± SEM.

C Quantification of the number of phospho-histone H3-positive cells at E16. n = 3 mice/genotype (WT = 96 cells, KO = 150 cells). One-way ANOVA with Bonferroni's *post hoc* test. Data are presented as mean ± SEM. Scale bar, 50 μm.

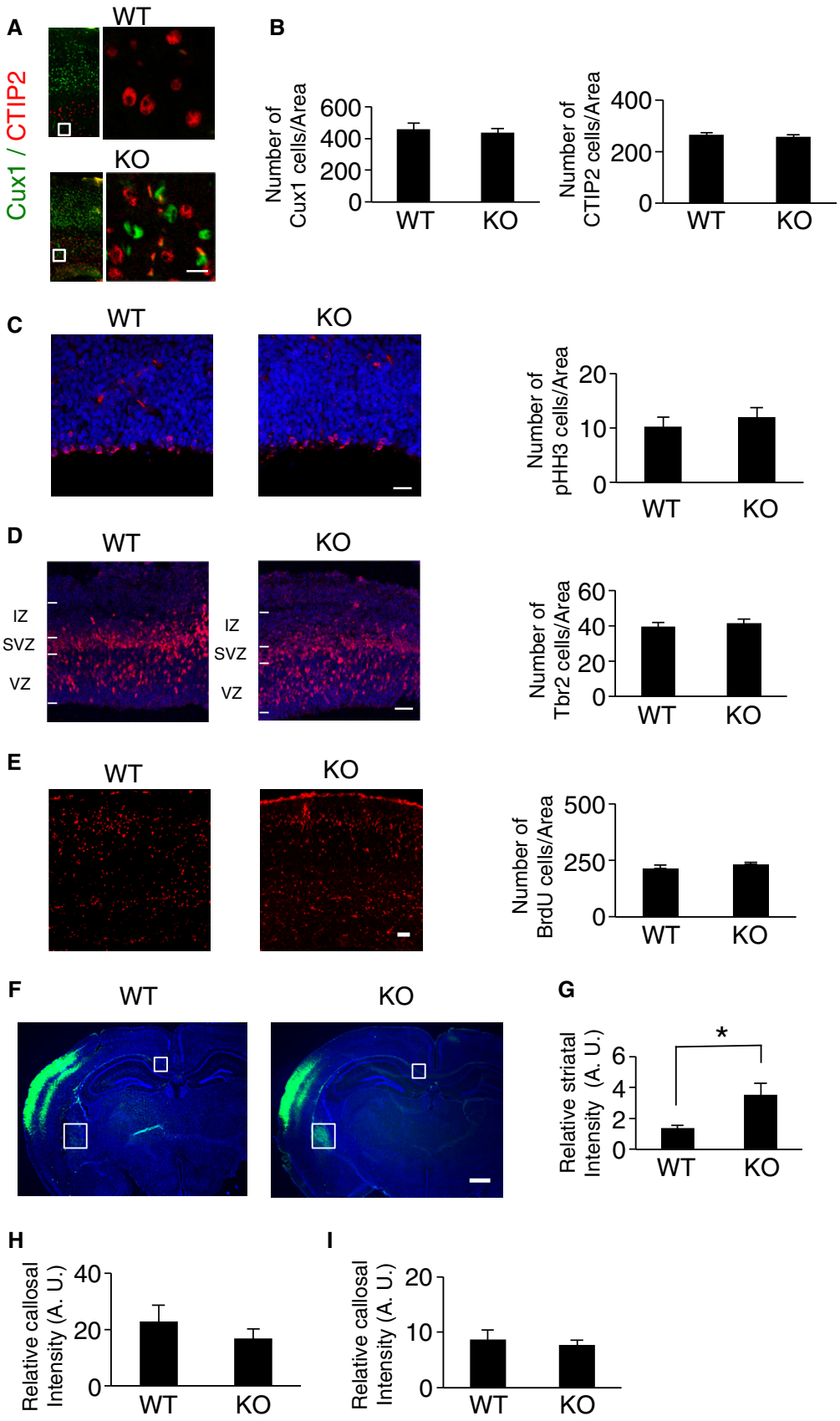
D Quantification of the number of TBR2-positive cells at E16. n = 3 mice/genotype (WT = 316 cells, KO = 327 cells). One-way ANOVA with Bonferroni's *post hoc* test. Data are presented as mean ± SEM. Scale bar, 100 μm.

E Quantification of the number of BrdU-positive cells at E18. BrdU injection was performed at E14. n = 3 mice/genotype (WT = 1,953 cells, KO = 2,103 cells). One-way ANOVA with Bonferroni's *post hoc* test. Data are presented as mean ± SEM. Scale bar, 100 μm.

F Coronal sections through the somatosensory cortex of P56 WT and CAMDI-KO mice were analyzed following *in utero* electroporation of EGFP plasmid at E14.5. Scale bar, 1 mm.

G Quantification of the striatal intensity at P56. n = 3 mice/genotype. *$P < 0.05$; one-way ANOVA with Bonferroni's *post hoc* test. Data are presented as mean ± SEM.

H, I Quantification of the callosal intensity at P56 (H) and P21 (I). n = 3 mice/genotype. One-way ANOVA with Bonferroni's *post hoc* test. Data are presented as mean ± SEM.


A
WT
KO
Cux1 / CTIP2
B
Number of Cux1 cells/Area
600
400
200
0
WT
KO
Number of CTIP2 cells/Area
400
200
0
WT
KO
C
WT
KO
Number of pHH3 cells/Area
20
10
0
WT
KO
D
WT
KO
IZ
SVZ
VZ
IZ
SVZ
VZ
Number of Tbr2 cells/Area
60
40
20
0
WT
KO
E
WT
KO
Number of BrdU cells/Area
500
250
0
WT
KO
F
WT
KO
G
Relative striatal Intensity (A. U.)
*
6
4
2
0
WT
KO
H
Relative callosal Intensity (A. U.)
40
20
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I
Relative callosal Intensity (A. U.)
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WT
KO

Figure EV2.

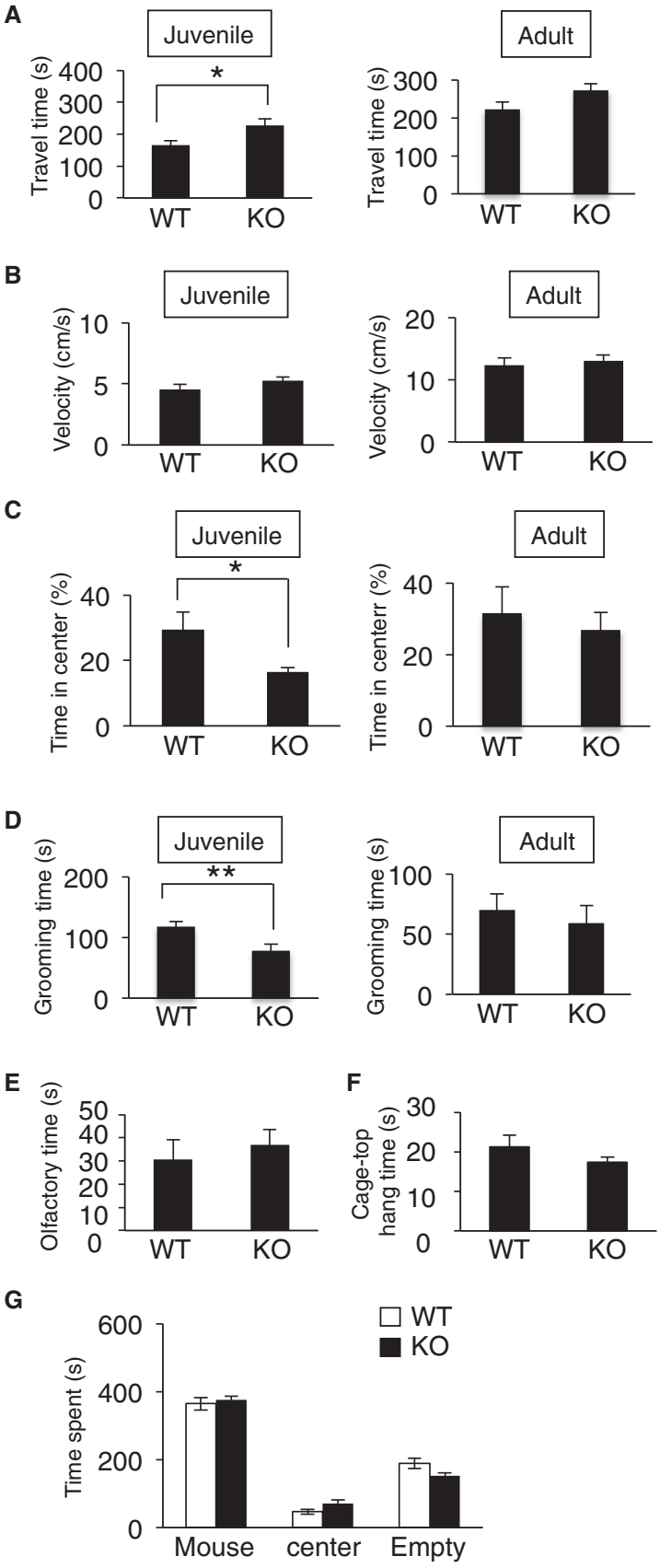

Figure EV3. The behavioral differences between WT and CAMDI-KO mice at juvenile and adult stages.

Related to Fig 2.

A Travel time on open-field test at juvenile and adult stages. For juvenile, n = 13 for WT mice and n = 13 for KO mice. For adult, n = 8 for WT mice and n = 7 for KO mice. *$P < 0.05$; one-way ANOVA with Bonferroni's *post hoc* test (juvenile: $F(1, 24) = 5.87$, adult: $F(1, 13) = 2.55$). Data are presented as mean ± SEM.

B The velocity in the open-field test at juvenile and adult stages. For juvenile, n = 13 for WT mice and n = 13 for KO mice. For adult, n = 8 for WT mice and n = 7 for KO mice. One-way ANOVA with Bonferroni's *post hoc* test (juvenile: $F(1, 24) = 1.74$, adult: $F(1, 13) = 0.17$). Data are presented as mean ± SEM.

C Time in center (%) on open-field test at juvenile and adult stages. For juvenile, n = 13 for WT mice and n = 13 for KO mice. For adult, n = 8 for WT mice and n = 7 for KO mice. *$P < 0.05$; one-way ANOVA with Bonferroni's *post hoc* test (juvenile: $F(1, 24) = 5.75$, adult: $F(1, 13) = 0.63$). Data are presented as mean ± SEM.

D Grooming time in open-field test at juvenile and adult stages. For juvenile, n = 13 for WT mice and n = 13 for KO mice. For adult, n = 8 for WT mice and n = 7 for KO mice. **$P < 0.01$; one-way ANOVA with Bonferroni's *post hoc* test (juvenile: $F(1, 24) = 7.84$, adult: $F(1, 13) = 0.31$). Data are presented as mean ± SEM.

E Olfactory function test at adult. n = 13 for WT mice and n = 21 for KO mice. One-way ANOVA with Bonferroni's *post hoc* test ($F(1, 32) = 0.21$). Data are presented as mean ± SEM.

F Cage-top hang test at adult. n = 10 for WT mice and n = 16 for KO mice. One-way ANOVA with Bonferroni's *post hoc* test ($F(1, 24) = 0.32$). Data are presented as mean ± SEM.

G Three-chamber social interaction test at adult. Total time spent in each chamber. n = 19 for WT mice and n = 20 for KO mice. Two-way ANOVA followed by Scheffe's *post hoc* test (main effect of genotype $F(1, 96) = 0.E+0$, main effect of chamber $F(2, 96) = 282$, interaction $F(2, 96) = 3.26$). Data are presented as mean ± SEM.

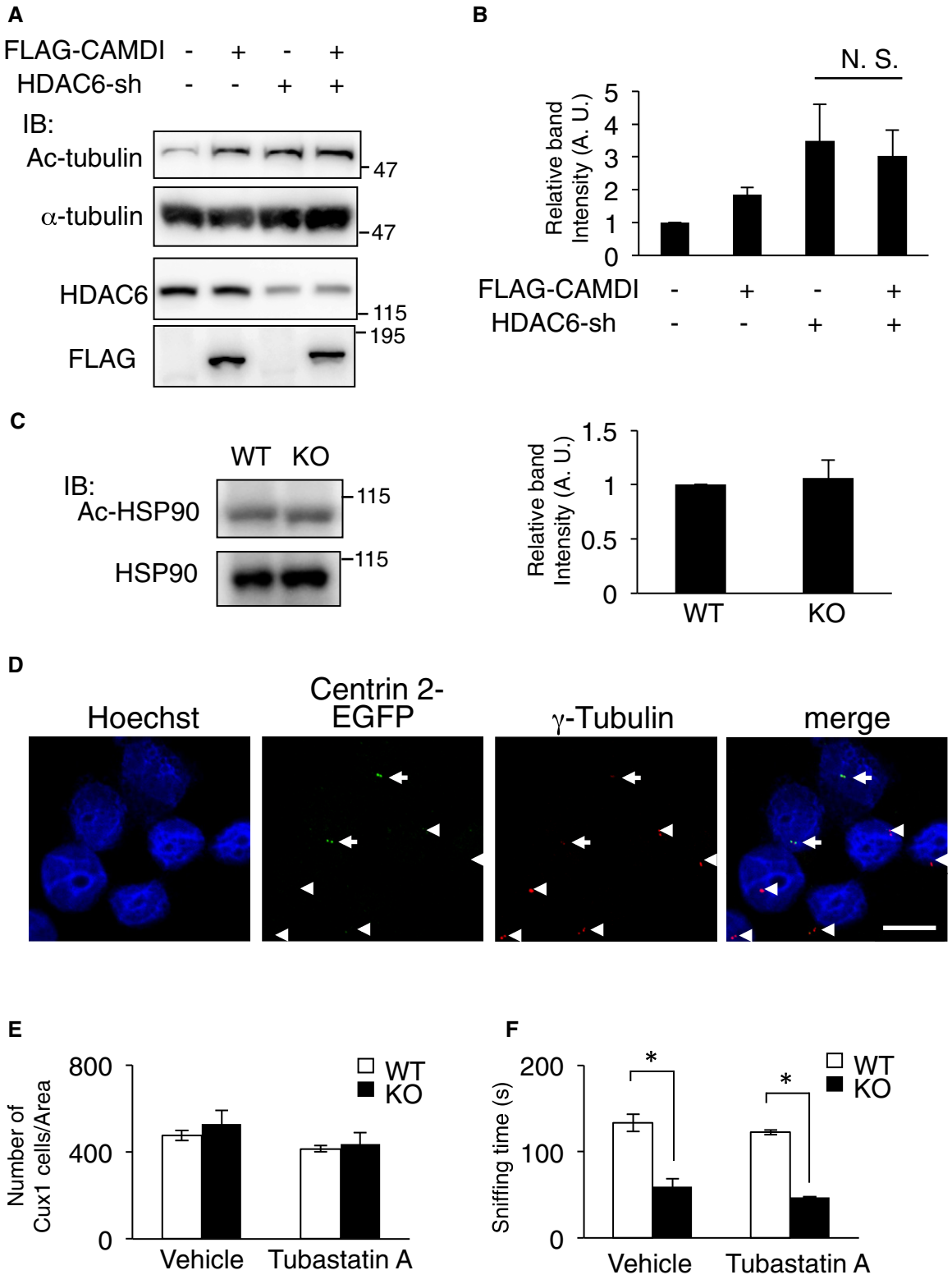

Figure EV4.

Figure EV4. Effects of HDAC6 overexpression on the γ-tubulin at centrosome.
Related to Figs 5–7.

A, B CAMDI overexpression has no effect on tubulin acetylation in the condition of HDAC6 knockdown by shRNA. SH-SY5Y cells were transfected with indicated vectors, and IP and IB assays were performed using the indicated antibodies. $n = 3$ independent experiments. N. S., not significant. Two-way ANOVA followed by Scheffe's *post hoc* test. Data are presented as mean ± SEM.

C No significant change in Ac-HSP90 level in CAMDI-KO cerebral cortex. Ac-HSP90 level was assessed by immunoblot analysis. $n = 3$ independent experiments. One-way ANOVA followed with Bonferroni's *post hoc* test. Data are presented as mean ± SEM.

D HeLa cells were transfected with centrin 2-EGFP and HDAC6-HA and immunostained with anti-EGFP (green) and anti-γ-tubulin antibodies (red). DNA was stained with Hoechst 33258. Arrow: HDAC6-HA and centrin 2-EGFP transfected cells. Arrowhead: non-transfected cells. Scale bar, 10 μm.

E Quantification of the number of Cux1-positive neurons at P2. $n = 3$ mice/genotype (WT (vehicle) = 1,426 cells, KO (vehicle) = 2,116 cells, WT (Tubastatin A) = 1,245 cells, KO (Tubastatin A) = 1,311 cells). Two-way ANOVA followed by Scheffe's *post hoc* test. Data are presented as mean ± SEM.

F Three-chamber social interaction test. The time spent sniffing with stranger mouse. $n = 8$ for WT (vehicle) mice, $n = 6$ for KO (vehicle) mice, $n = 5$ for WT (Tubastatin A) mice, and $n = 3$ for KO (Tubastatin A) mice. $^*P < 0.05$; two-way ANOVA followed by Scheffe's *post hoc* test (main effect of genotype $F(1, 18) = 11.11$, main effect of drug $F(1, 18) = 0.24$, interaction $F(1, 18) = 0.064$). Data are presented as mean ± SEM.

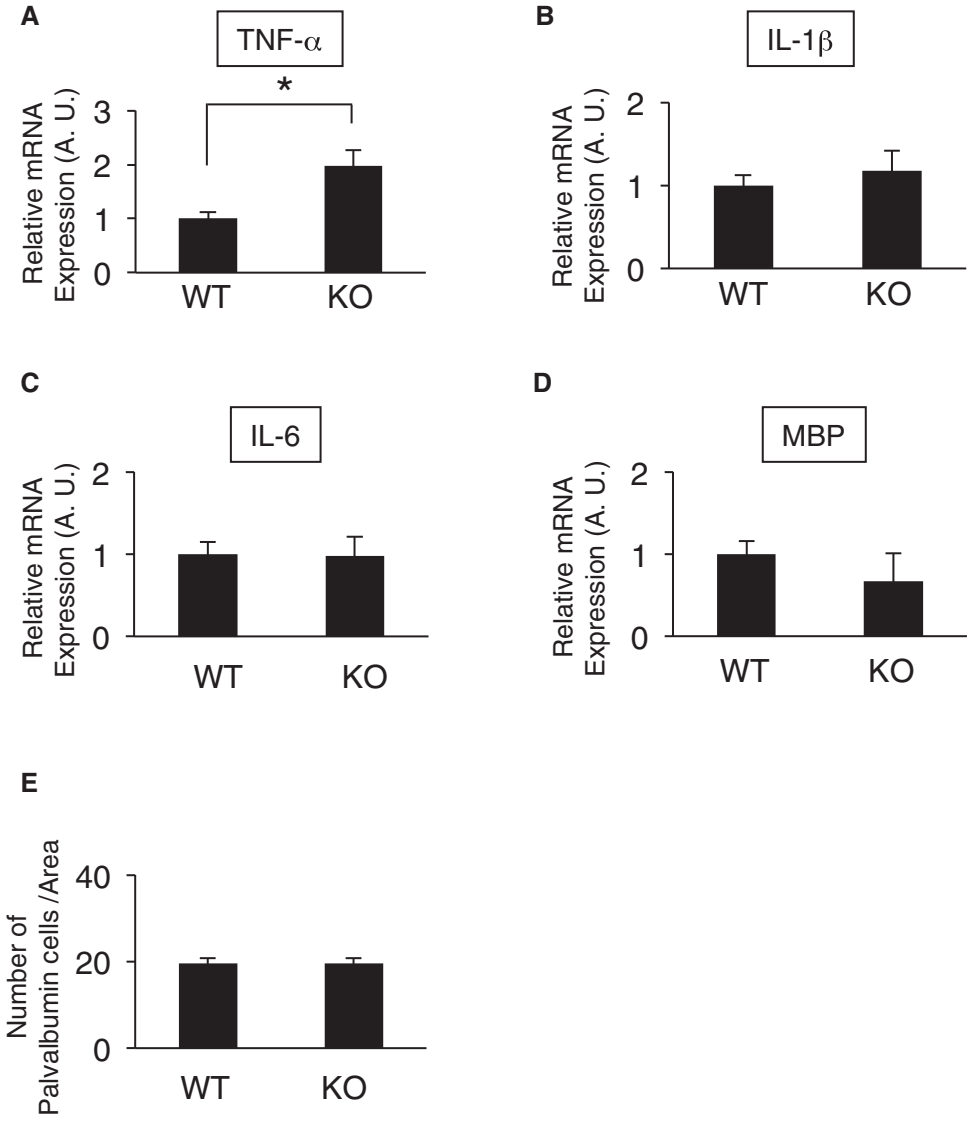

Figure EV5. Analysis of biological alterations in CAMDI-KO brain relevant to psychiatric disorders.

A Real-time PCR quantification of the transcript for TNF-α. n = 3 mice/genotype. *$P < 0.05$; one-way ANOVA with Bonferroni's *post hoc* test. Data are presented as mean ± SEM.

B Real-time PCR quantification of the transcript for IL-1β. n = 3 mice/genotype. One-way ANOVA with Bonferroni's *post hoc* test. Data are presented as mean ± SEM.

C Real-time PCR quantification of the transcript for IL-6. n = 3 mice/genotype. One-way ANOVA with Bonferroni's *post hoc* test. Data are presented as mean ± SEM.

D Real-time PCR quantification of the transcript for MBP (myelin basic protein). n = 3 mice/genotype. One-way ANOVA with Bonferroni's *post hoc* test. Data are presented as mean ± SEM.

E Quantification of the number of Parvalbumin-positive neurons. Note the abnormal distribution of neurons in deep cortical layers of CAMDI-KO mice. n = 3 mice/genotype (WT = 816 cells, KO = 438 cells). One-way ANOVA with Bonferroni's *post hoc* test. Data are presented as mean ± SEM.