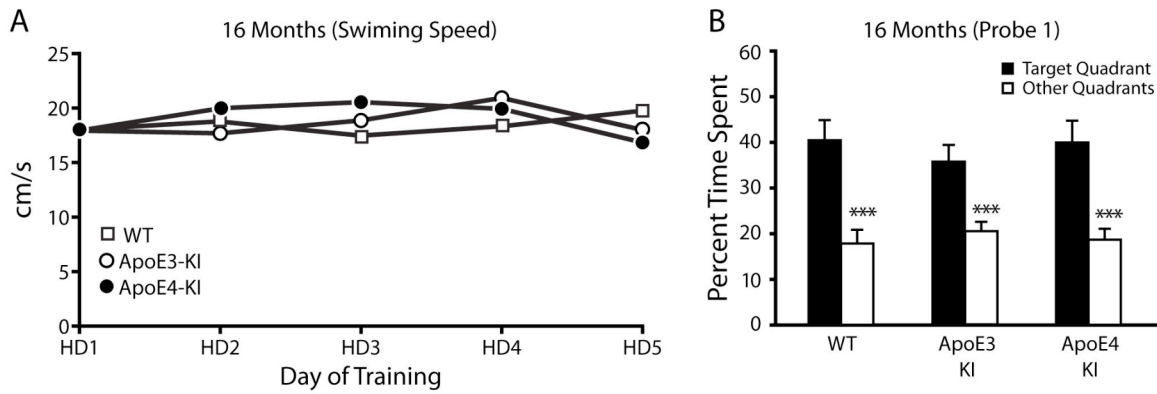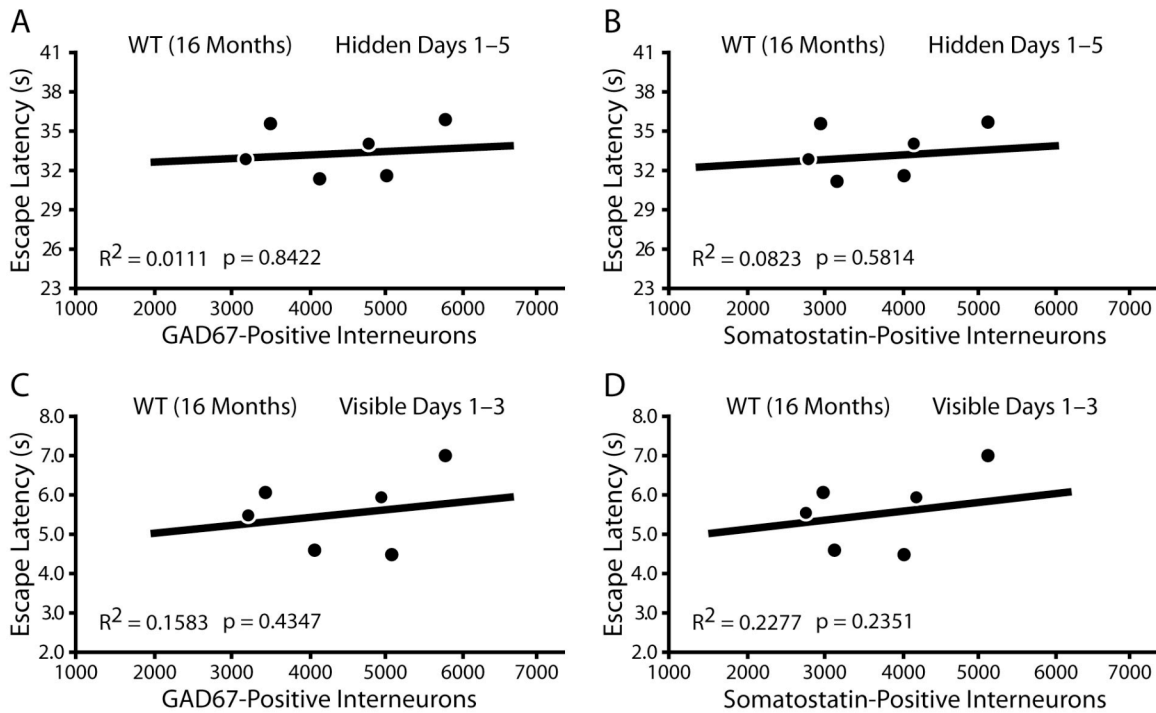**SUPPLEMENTAL DATA**

Apolipoprotein E4 Causes Age- and Tau-Dependent Impairment of GABAergic Interneurons, Leading to Learning and Memory Deficits in Mice

Yaisa Andrews-Zwilling, Nga Bien-Ly, Qin Xu, Gang Li, Aubrey Bernardo, Seo Yeon Yoon, Daniel Zwilling, Tonya Xue Yan, Ligong Chen, and Yadong Huang
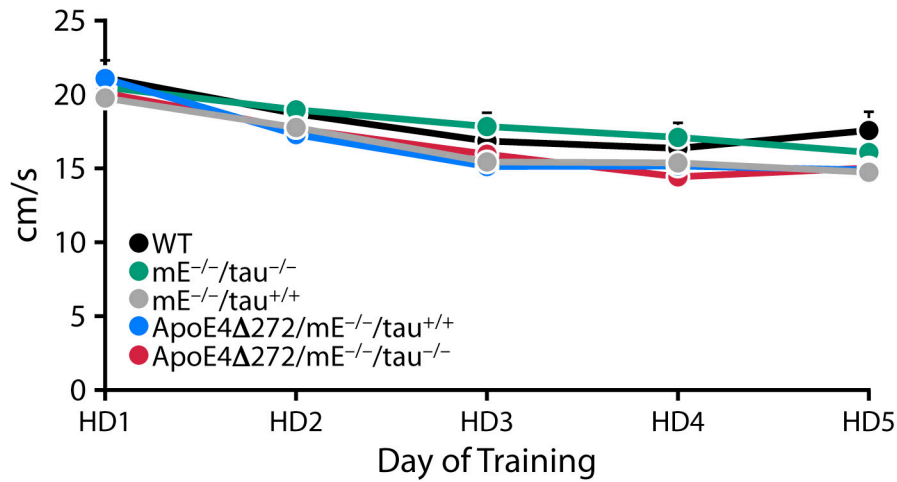
Supplemental Figure 1. *A,* There is no difference in swim speeds during the hidden platform trials among wildtype, apoE3-KI, and apoE4-KI mice. Nine wildtype, ten apoE3-KI, and 12 apoE4-KI mice at 16 months of age (all females) were tested in the Morris water maze. The swim speeds were calculated as cm/s during the hidden platform trials. *B*, The probe trials of female wildtype, apoE3-KI, and apoE4-KI mice at 16 months of age were performed 24 h (probe 1) after the last hidden platform training. Percent time spent in the target quadrant versus the average time spent in other quadrants did not differ by genotype in probe 1. Values are mean ± SEM. ***p < 0.005 (*t* test).

Supplemental Figure 2. Performance in the hidden and cued platform trials does not correlate with the number of hilar GABAergic interneurons in wildtype mice at 16 months of age. Female mice at 16 months of age were tested in the Morris water maze. *A, B*, Escape latency in hidden platform days 1–5 did not correlate with the number of GAD67-positive (A) and somatostatin-positive (B) hilar GABAergic interneurons in wildtype mice at 16 months of age. *C, D*, Performance in the cued platform trial did not correlate with the number of GAD67-positive (C) and somatostatin-positive (D) hilar GABAergic interneurons in wildtype mice at 16 months of age.
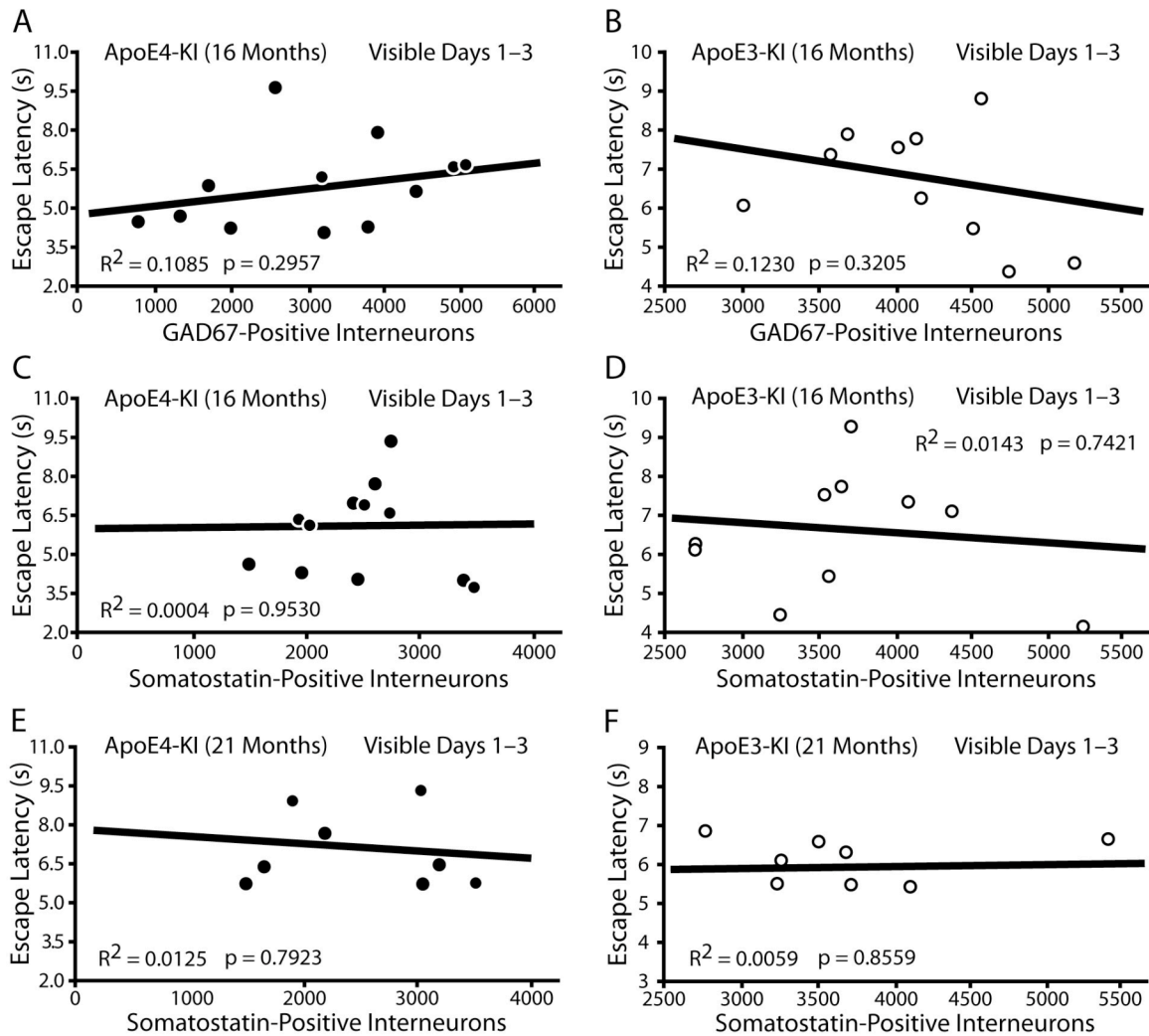
Supplemental Figure 3. Performance in the cued platform trial does not correlate with the number of hilar GABAergic interneurons in apoE3-KI and apoE4-KI mice at 16 and 21 months of age. Ten apoE3-KI and 12 apoE4-KI mice at 16 months of age and eight apoE3-KI and eight apoE4-KI mice at 21 months of age (all females) were tested in the Morris water maze. *A, B*, Performance in the cued platform trial did not correlate with the number of GAD67-positive GABAergic interneurons in apoE4-KI mice (A, n = 12) or apoE3-KI mice (B, n = 10) at 16 months of age. *C, D*, Performance in the cued platform trial did not correlate with the number of somatostatin-positive GABAergic interneurons in apoE4-KI mice (C, n = 12) or apoE3-KI mice (D, n = 10) at 16 months of age. *E, F*, Performance in the cued platform trial did not correlate with the number of somatostatin-positive GABAergic interneurons in apoE4-KI mice (E, n = 8) or apoE3-KI mice (F, n = 8) at 21 months of age.

Supplemental Figure 4. There is no difference in swim speeds during the hidden platform trials among different groups of mice. Nine $mE^{-/-}Tau^{+/+}$, 10 apoE4(Δ272–299)$mE^{-/-}Tau^{+/+}$, 12 apoE4(Δ272–299)$mE^{-/-}Tau^{-/-}$, six $mE^{-/-}Tau^{-/-}$, and eight wildtype mice (all females) were tested in the Morris water maze at 12 months of age. The swim speeds were calculated as cm/s during the hidden platform trials.
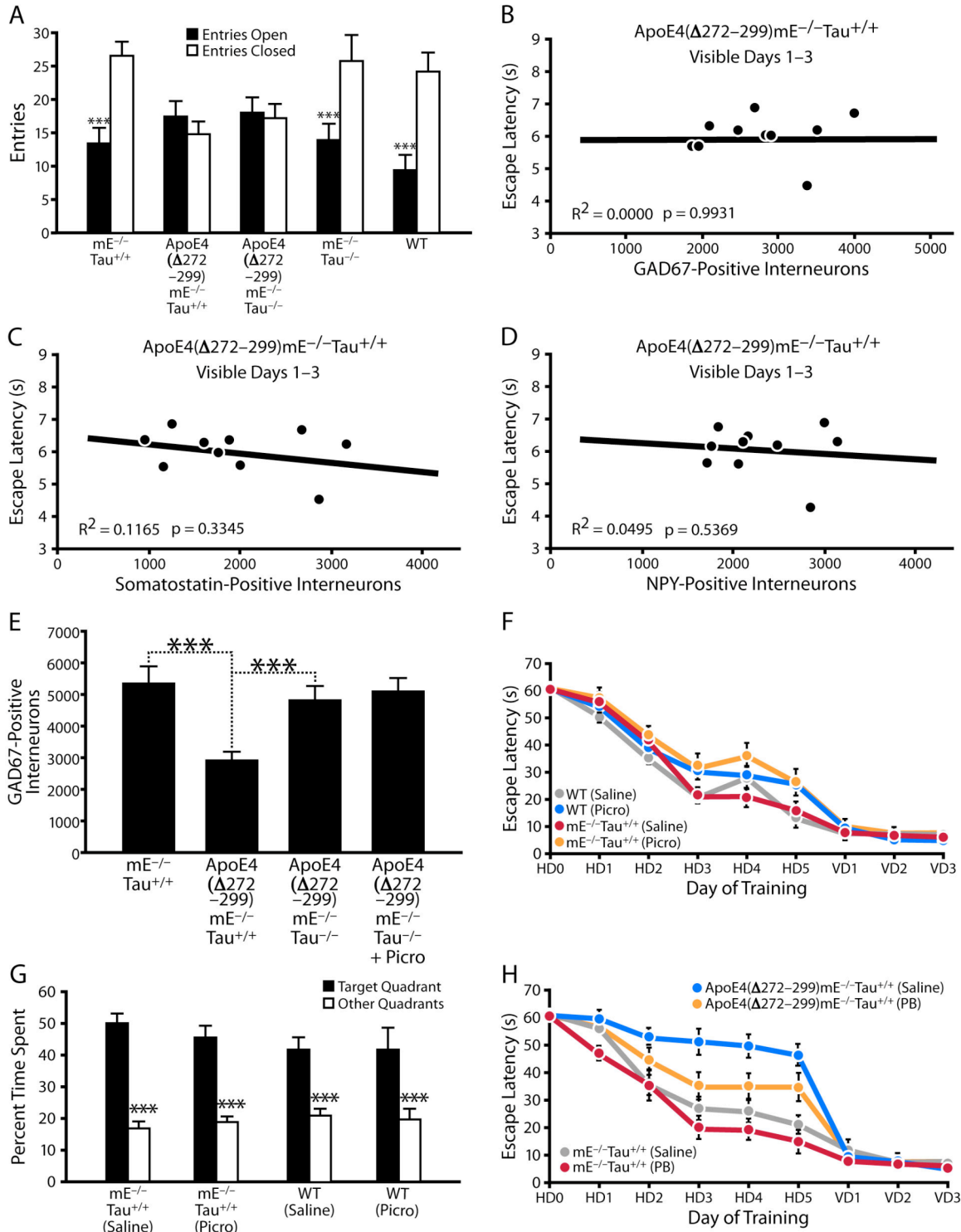
Supplemental Figure 5. *A*, Eliminating tau does not rescue apoE4 fragment-caused abnormal anxiety in apoE4(Δ272–299)mE$^{-/-}$Tau$^{+/+}$ mice. Nine mE$^{-/-}$Tau$^{+/+}$, 10 apoE4(Δ272–299)mE$^{-/-}$Tau$^{+/+}$, 12 apoE4(Δ272–299)mE$^{-/-}$Tau$^{-/-}$, six mE$^{-/-}$Tau$^{-/-}$, and eight wildtype mice (all females) were tested in an elevated plus maze at 12 months of

age. Values are mean ± SEM. ***p < 0.001 (*t* test). ***B–D***, Ten female apoE4(Δ272–299)mE$^{-/-}$Tau$^{+/+}$ were tested at 12 months of age in the Morris water maze. Performance in the cued platform trial did not correlate with the number of GAD67-positive (B), somatostatin-positive (C), or NPY-positive (D) hilar GABAergic interneurons in apoE4(Δ272–299)mE$^{-/-}$Tau$^{+/+}$ mice. ***E***, Treatment with the GABA$_A$ receptor antagonist picrotoxin (Picro) does not alter the number of hilar GABAergic interneurons in ApoE4(Δ272–299)mE$^{-/-}$Tau$^{-/-}$ mice. Female apoE4(Δ272–299)mE$^{-/-}$Tau$^{-/-}$ mice at 12 months of age were treated with picrotoxin (Picro, 1 mg/kg i.p.) or saline (n = 6–8 per group) for 3 days before the Morris water maze test and every day during the test. Age-matched, saline-treated apoE4(Δ272–299)mE$^{-/-}$Tau$^{+/+}$ and mE$^{-/-}$Tau$^{+/+}$ mice (n = 6–8 per group) served as controls. Total number of GAD67-positive interneurons in the hilus was quantified after the behavioral test. Values are mean ± SEM. ***p < 0.005 (*t* test). ***F, G***, Treatment with a low dose of picrotoxin does not alter the learning and memory performance in wildtype and mE$^{-/-}$Tau$^{+/+}$ mice. Female wildtype and mE$^{-/-}$Tau$^{+/+}$ mice at 12 months of age were treated with intraperitoneal injections of picrotoxin (Picro, 1 mg/kg) or saline (n = 8 per group) for 3 days before the Morris water maze test and every day during the test. Age-matched, saline-treated wildtype and mE$^{-/-}$Tau$^{+/+}$ mice (n = 8 per group) served as controls. There was no significant difference among the learning curves (F). In the probe trial performed 24 h after the last hidden platform training, the time spent in the target quadrant versus the other quadrants does not differ by genotypes or treatment (G). Values are mean ± SEM. ***p < 0.005 (*t* test). ***H***, Treatment with GABA$_A$ receptor potentiator pentobarbital rescues the learning deficit in apoE4(Δ272–299)mE$^{-/-}$Tau$^{+/+}$ mice. Female mE$^{-/-}$Tau$^{+/+}$ and apoE4(Δ272–299)mE$^{-/-}$Tau$^{+/+}$ mice at 12 months of age were treated with intraperitoneal injections of pentobarbital (PB, 20 mg/kg) or saline (n = 7–9 per group) for 21 days before the Morris water maze test and every day during the test. In the hidden platform sessions, learning curves differed significantly by genotype and treatment (p < 0.01, repeated-measures ANOVA). In post-hoc comparisons, apoE4(Δ272–299)mE$^{-/-}$Tau$^{+/+}$ mice learned poorly versus mE$^{-/-}$Tau$^{+/+}$ mice (p < 0.005). ApoE4(Δ272–299)mE$^{-/-}$Tau$^{+/+}$ mice treated with pentobarbital learned better than saline-treated apoE4(Δ272–299)mE$^{-/-}$Tau$^{+/+}$ mice (p < 0.01). Values are mean ± SEM.