Supplemental Methods

Mouse models. Ephexin5$^{+/-}$ mice (1) were crossed to hAPP mice (Mucke et. al 2000, B6.Cg-Tg(PDGFB-APPSwInd)20Lms/2Mmjax) (source of mice is The J. David Gladstone Institute) to yield mice heterozygous for both hAPP and Ephexin5. These mice (hAPP^{+}/E5$^{+/-}$ and E5$^{+/-}$) were used as breeding pairs to produce WT, hAPP^{+}/E5$^{+/+}$, hAPP^{+}/E5$^{-/-}$, and E5$^{-/-}$ mice. E5$^{-/-}$ adult mice showed no overt health or behavioral issues (Supplemental Figure 3D).To label neurons fluorescently for spine analysis, hAPP^{+}/E5$^{+/-}$ mice were crossed into the Thy1-EGFP line (Jackson STOCK Tg(Thy1-EGFP)MJrs/J, 007788). WT, hAPP, hAPP/E5$^{-/-}$ and E5$^{-/-}$ mice positive for EGFP were used in analysis. Both sexes were used for all experiments and no sex-specific alterations were discovered (Supplemental Figure 3B and Supplemental Figure 3C). Litter sizes and genotype ratios in the presence or absence of hAPP expression were obtained as expected indicating that the loss of Ephexin5 expression in these mice did not cause any overt change to the health of these mice.

Human brain samples. Human hippocampal tissue samples were acquired from Harvard Brain Bank. Information about the tissue itself is included in Supplemental Table 1. In short, the tissue samples were lysed in RIPA containing protease inhibitors and phosphatase inhibitors. Samples were spun to remove the insoluble fraction and the concentration of protein was quantified using a Bradford assay. Samples were run for Western by loading equal protein and were also normalized to actin signal.

Amyloid preparation. For *in vivo* injection and additional to hippocampal neurons, $A\beta_{(1-42)}$ (Millipore) was dissolved in DMSO and diluted in PBS to desired concentration (approximately 1:3). This solution was kept at 37°C for one week to promote oligomerization. As a control, $A\beta_{(42-1)}$ (Invitrogen) was treated identically to the forward peptide.

Neuronal culture and immunocytochemistry. Dissociated neurons were cultured in Neurobasal Medium supplemented with B27 were transfected with GFP expressing construct using the Lipofectamine method (Invitrogen) according to the manufacturer's suggestions. For Aβ stimulations in dissociated cultured neurons, 1µg/mL $A\beta_{1-42}$ was added for six days. For controls, 1µg/mL $A\beta_{42-1}$ in Neurobasal/B27 was applied to neurons. Neurons were paraformaldehyde fixed in PBS. For Ephexin5 density measurement, fixed neurons were incubated with α-Ephexin5 antibody followed by Cy3-α-rabbit (Jackson ImmunoResearch) (1:200 each in 1× GDB (30 mM phosphate buffer pH 7.4 containing 0.2% gelatin, and 0.8 M NaCl) for 1 hr at 25 °C) to visualize the primary antibodies.

Microdissection. Hippocampi from adult transgenic animals were microdissected as described previously (2). Samples were dissociated in RIPA buffer containing protease and phosphatase inhibitors, immediately placed in SDS sample buffer, and analyzed by western blot. Westerns were run in technical duplicate.

Behavioral Assays. All behavioral tests were performed in the evening (18:00-23:00). Animals were numbered and genotyped, but the genotype was not paired with the animal until after experiments, allowing the experimenter to be blinded to genotype during both testing and data analysis.

Novel Place Preference. Mice were allowed to habituate to the testing room on each day of testing for 20 minutes. Mice were habituated to the testing arena for 30 minutes in the testing room. The testing arena was a 10" x 10" box with a small visual cue on one side to orient the box. Between each mouse, the arena was cleaned with 70% ethanol. Twenty-four hours later, mice were exposed to 3 different objects for 10 minutes. An additional twenty-four hours later, mice were placed in the testing arena with the same 3 objects for 10 minutes, one object having been moved across the arena.

Both days with the objects were video recorded. A blinded observer scored the amount of time each mouse spent investigating the objects using ODLog (Macropod Software). Investigation was defined as time spent with the mouse's nose within 3 mm of the object, with orientation toward the object.

Passive Avoidance*.* Mice were habituated to the chamber for 15 seconds before opening of the guillotine door separating the lit chamber from a dark chamber (Coulbourn Instruments). As mice entered the dark chamber, the door closed, mice were shocked (0.3mA, 2 seconds) and immediately removed. 24 hours later, mice were placed in the lit box and allowed up to 5 minutes to enter the shock chamber. Latency on both days was recorded. Both chambers cleaned with 70% ethanol between mice.

In vivo surface biotinylation and streptavidin pulldown*.* Mice aged 6-8 months were euthanized, and hippocampi were prepared for *ex vivo* surface biotinylation pulldown experiments as previously described (3). Briefly, hippocampi were dissected and kept on ice. Each hippocampi was sliced on a tissue chopper (*company)* into 350 micron sections. Slices were rested for 15 minutes before addition of 0.5mg/ml biotin (Thermo Scientific) and incubated with agitation at 4◦C for 15 minutes. Slices were quenched with glycine, and dissociated and lysed in RIPA containing protease (Roche*)* and β-glyercol phosphate. Protein concentrations were determined via Bradford (Bio-Rad) and equal amounts protein were loaded onto neutravidin beads (Thermo Scientific*).* Protein bound to the beads was analyzed via western blot.

Antibodies/western analysis*.* Western analysis was accomplished using antibodies to actin (Abcam, ab8226), APP (Millipore MABN10, clone W0-2), Transferrin (Life technologies, 136800), Ephexin5 and EphB2 (both gifted by the Dr. Michael Greenberg, Harvard University Department of Neurobiology, Boston, MA). Westerns were quantified using ImageJ.

Perfusion. For *in vivo* immunofluorescence analysis and plaque analysis, mice were perfused with 0.1M phosphate buffer, then 4% paraformaldehyde (PFA). Brains were post-fixed in 30% sucrose and 4% PFA for 3 days and frozen down in OCT (Thermo Scientific). Brains were sectioned into 30-micron coronal slices.

Dendritic spine analysis. For spine quantification, Thy1-EGFP positive sections were stained for GFP, as previously described (4) with a few alterations. Blocking buffer contained 5% goat serum instead of BSA. Concentration of primary (anti-GFP, Aves Labs 1020) was 1:500, with 1:300 Alexa-Fluor 488-conjugated secondary (Novex, A-11039), and mounted in Fluoromount-G (Southern Biotech). Hoescht staining was used to label nuclei. For dendritic spine assays, a z series projection of each neuron was made using approximately 25 sections (1 μm/section), each averaged two times at 63x. Experimenter was blinded to genotype during imaging. To measure spine density, an experimenter measured between five and seven randomly chosen segments for quantification per mouse per brain region, with three mice per genotype. For quantification of dendritic spines, images blinded to the experimenter were analyzed using Metamorph (Universal Imaging Corporation) by manually tracing the dimensions of all spines measured in spine density quantification. Spine length (distance of shaft from dendrite to tip to the spine), head width (width of spine 10% of distance from tip), and density (number of protrusions per micron) were recorded. Mushroom and thin spines were classified as spines of lengths between 0.7 μm and 2.0 μm, to remove stubby and filopodial spines.

Plaque analysis. Free-floating sections were incubated with 1% H_2O_2 to quench endogenous peroxidase activity, and treated with 70% formic acid for 10 minutes as an antigen retrieval step. Sections were stained according to manufacturer's instructions (M.O.M Kit, Vector Laboratories, Inc.) with the 6E10 antibody. Staining was visualized using the DAB staining kit (Thermo Fischer Scientific), before sections were dehydrated

and cleared for imaging. Three hippocampi per animal were imaged and quantified using ImageJ.

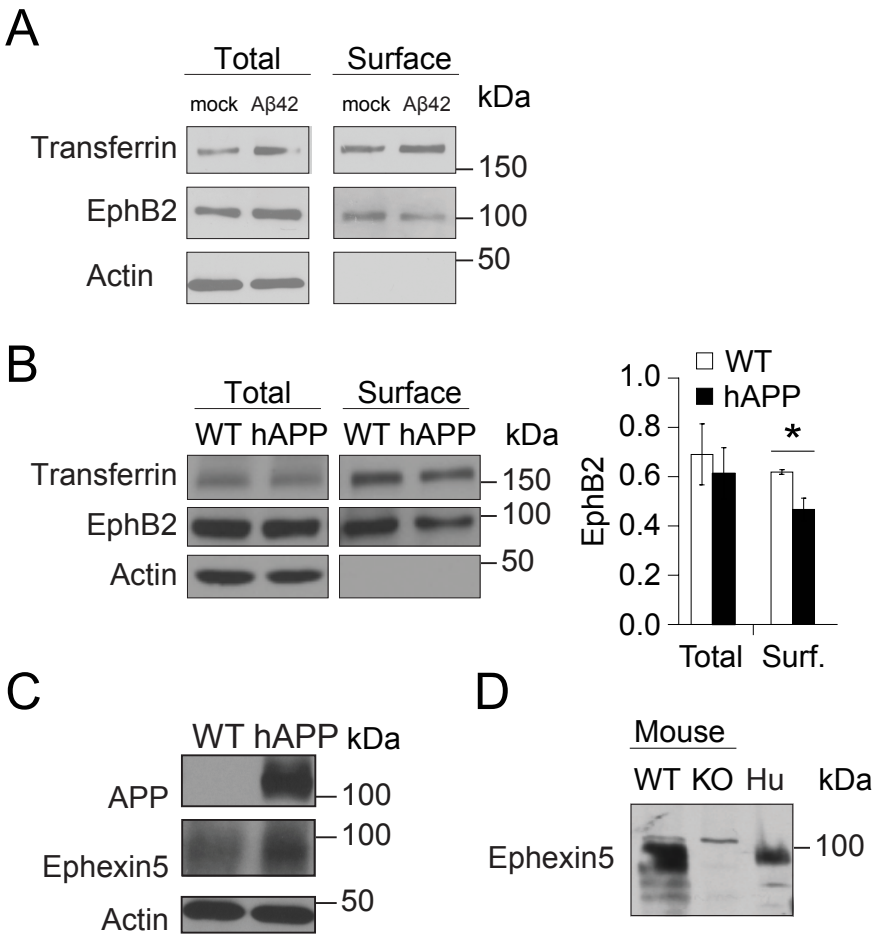
Amyloid measurements. Hippocampi from 12 month old WT, hAPP, and hAPP/E5$^{-/-}$ mice were lysed in Tris lysis buffer (Mesoscale Discovery) spiked with protease inhibitors (Roche) and β-glycerol phosphate. Samples were spun at 4 degrees, and the supernatant was used to measure soluble beta-amyloid with the 6E10 antibody according to manufacturer's instructions (Mesoscale Discovery). No amyloid was measured in WT controls.

Surgery and stereotactic injections. All stereotactic experiments were double blinded to injected virus and genotype of mice being injected from the surgery until after data analysis was completed. Conditions were revealed after behavioral analysis. Mice were injected bilaterally with lentivirus expressing either a shRNA hairpin to Ephexin5 (1) or a scrambled hairpin, and GFP directly into DG (Bregma 2.12mm M/L, 2.54mm P, 1.96mm V). Mice were allowed to recover and age for 2 months before behavioral testing and tissue collection. Behavioral testing was performed as described above.

Statistics. All analyses for biochemistry were Student's t-tests, two-tailed with equal variance, when not noted separately. Data represent mean ± SEM. Where applicable, statistical analysis appropriately corrects for multiple comparisons when comparing more than two groups and corrects for repeated measures when comparing multiple measurements within subjects.

References:

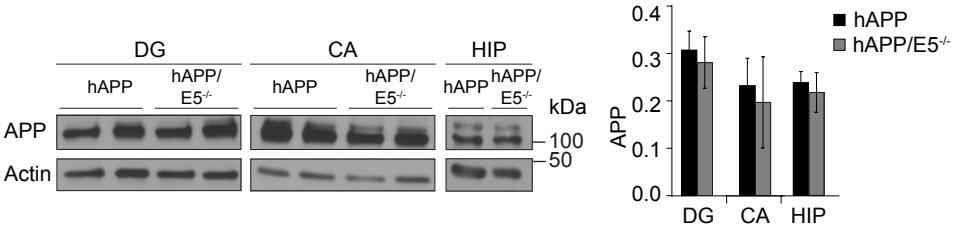
1. Margolis, S.S., Salogiannis, J., Lipton, D.M., Mandel-Brehm, C., Wills, Z.P., Mardinly, A.R., Hu, L., Greer, P.L., Bikoff, J.B., Ho, H.Y., et al. 2010. EphB-mediated degradation of the RhoA GEF Ephexin5 relieves a developmental brake on excitatory synapse formation. *Cell* 143:442-455.
2. Hagihara, H., Toyama, K., Yamasaki, N., and Miyakawa, T. 2009. Dissection of hippocampal dentate gyrus from adult mouse. *J. Vis. Exp.*
3. Gabriel, L.R., Wu, S., and Melikian, H.E. 2014. Brain slice biotinylation: an ex vivo approach to measure region-specific plasma membrane protein trafficking in adult neurons. *J Vis Exp*.
4. Vuksic, M., Del Turco, D., Bas Orth, C., Burbach, G.J., Feng, G., Muller, C.M., Schwarzacher, S.W., and Deller, T. 2008. 3D-reconstruction and functional properties of GFP-positive and GFP-negative granule cells in the fascia dentata of the Thy1-GFP mouse. *Hippocampus* 18:364-375.



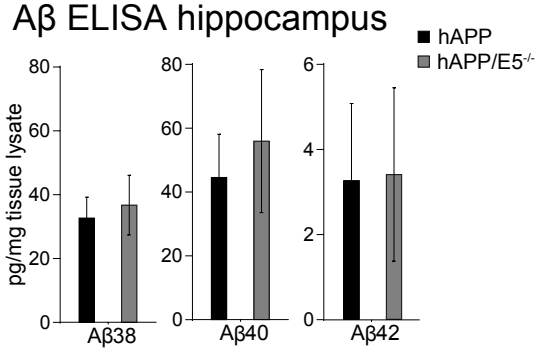
Supplemental Figure 1. (A) Surface biotinylation and streptavidin isolation of surface proteins in cultured hippocampal neurons treated with $A\beta_{1\text{-}42}$ (Aβ42) or mock conditions (mock), showing EphB2, transferrin, and actin both surface and total levels. **(B)** Surface biotinylation and streptavidin isolation of surface proteins hippocampi from WT and hAPP mice (WT n=3, hAPP n=4) showing EphB2 with actin as an internal control as well as transferrin as a surface control. **(C)** Example Western blot showing Ephexin5 levels in whole hippocampus of WT and hAPP mouse (experiment conducted 3 times). **(D)** A

western blot showing the specificity of the Ephexin5 antibody and its recognition of human protein.

A hAPP Western hippocampus



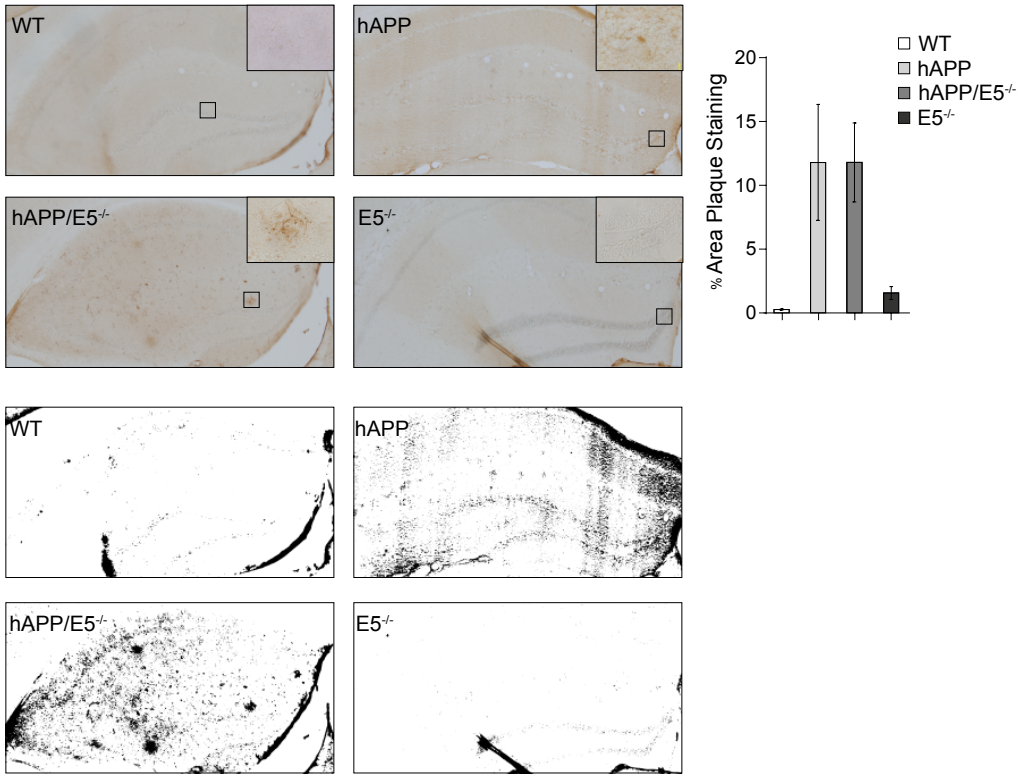
B Aβ ELISA hippocampus



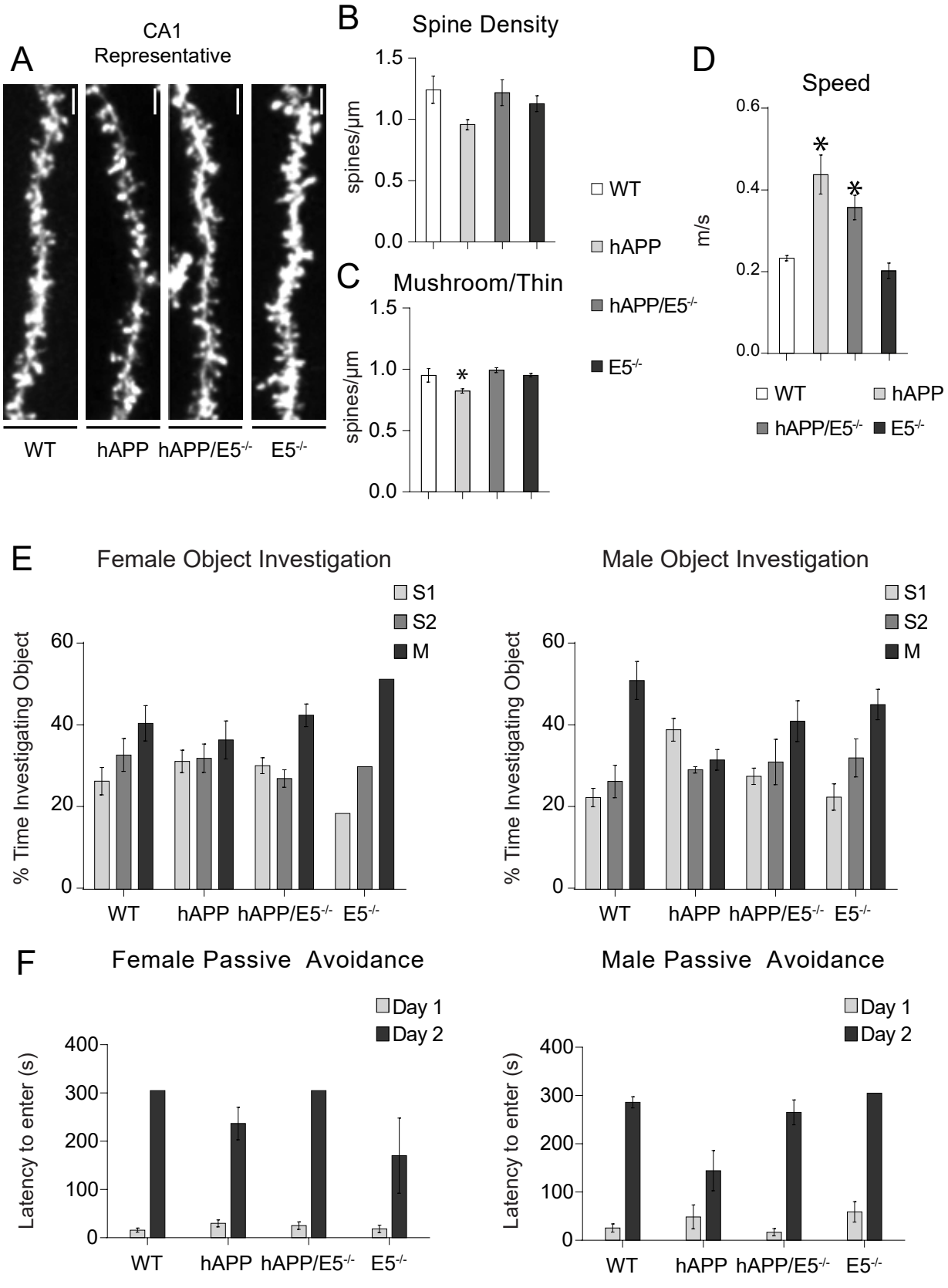
C IHC hippocampus (Plaque analysis)

Raw Images

Rendering



Supplemental Figure 2. Removal of Ephexin5 does not significantly alter amyloid production or aggregation in vivo. (A) Western blot of APP in hAPP (n=6) and hAPP/E5-/- (n=6) microdissected hippocampus. On the right, quantification of APP levels normalized to actin. Tissue from WT and $E5^{-/-}$ had no detectable APP signal (data not shown). **(B)** Soluble amyloid 38, 40, and 42 was quantified via ELISA and shown in pg/mg of tissue lysate. WT (not shown) had no measurable amyloid of any type. (WT n=4, hAPP n=4, and hAPP/$E5^{-/-}$ n=3). Samples from WT had no detectable Aβ (not plotted). **(C)** Example images showing amyloid IHC (top panel) as well as the regions quantified for staining (lower panel) for WT (n=2), hAPP (n=3), hAPP/$E5^{-/-}$ (n=3), and $E5^{-/-}$ (n=2) animals. The quantification on the right, showing the percent of measured region that is positive for staining.


A
CA1
Representative
WT
hAPP
hAPP/E5-/-
E5-/-
B
Spine Density
spines/µm
1.5
1.0
0.5
0.0
C
Mushroom/Thin
spines/µm
1.5
1.0
0.5
0.0
*
WT
hAPP
hAPP/E5-/-
E5-/-
D
Speed
m/s
0.6
0.4
0.2
0.0
*
*
WT
hAPP
hAPP/E5-/-
E5-/-
E
Female Object Investigation
% Time Investigating Object
60
40
20
0
S1
S2
M
WT
hAPP
hAPP/E5-/-
E5-/-
Male Object Investigation
% Time Investigating Object
60
40
20
0
S1
S2
M
WT
hAPP
hAPP/E5-/-
E5-/-
F
Female Passive Avoidance
Latency to enter (s)
400
300
200
100
0
Day 1
Day 2
WT
hAPP
hAPP/E5-/-
E5-/-
Male Passive Avoidance
Latency to enter (s)
400
300
200
100
0
Day 1
Day 2
WT
hAPP
hAPP/E5-/-
E5-/-

Supplemental Figure 3. Ephexin5 ameliorates Aβ induced spine morphology deficits in CA1, does not alter hyperactivity in hAPP mice and sex does not alter learning and memory phenotypes (**A**) CA1 representative dendrite segments **(B**) CA1 total spine density. (**C**) CA1 mushroom/thin spine density. Scale bar in A, 2μm. Data represent mean ± SEM. For all genotypes n=3. *P<0.05 using one-way ANOVA with a Tukey's correction. **(D)** Animals were placed into a Y maze arena and allowed to explore all 3 arms freely. The speed for each animal was quantified (WT n=8, hAPP n=10, hAPP/E5$^{-/-}$ n=11, E5-/- n=8). **(E)** Percent time investigating objects was separated by sex for each genotype (Female WT n=5, hAPP n=4, hAPP/E5$^{-/-}$ n=9, E5$^{-/-}$ n=1, Male WT n=3, hAPP n=6, hAPP/E5$^{-/-}$ n=5, E5$^{-/-}$ n=8). **(F)** Latency to enter the dark half of the passive avoidance arena was determined and separated by sex for each genotype (Female WT n=5, hAPP n=11, hAPP/E5$^{-/-}$ n=6, E5$^{-/-}$ n=4, Male WT n=9, hAPP n=7, hAPP/E5$^{-/-}$ n=6, E5$^{-/-}$ n=5).