**B. Supplemental Figures and Legends, Tables, Experimental Procedures, and References**

**Supplemental Figures and Legends**

**Figure S1, related to Figure 2**

**Figure S1**. ES derived neurons are sensitive to Aβ42 oligomer induced cell death. (A) Cell viability detected using a resuflourin based assay in relative fluorescent units (RFU) after 24 hour treatment with indicated concentration of Aβ42 oligomer in reduced serum media.



**Figure S2**. sAPPβ antibodies are specific. (A) To selectively detect soluble APP derived from β-secretase-mediated cleavage, we synthesized two peptides comprising the β-secretase cleavage site of APPwt and APPsw. These were used to generate the sAPPβ antibodies sβwt and sβsw, which were selected against binding to sAPPβ. (B) Neuro2a-BACE cells were transiently transfected with empty vector, SEAP-APPwt, or SEAP-APPsw. Culture media was immunoprecipitated with pre-immune serum (P) or the indicated sAPPβ antibody (I) and visualized on Western blot with anti-HA (SEAP-APP constructs contain N-terminal HA tags). sβsw antibodies were generated by immunizing rabbits with keyhole limpet hemocyanin-conjugated peptides corresponding to the C-terminal region of secreted APPβsw (C)GGGISEVNL. This antibody detects APPβsw specifically and does not detect full-length APP. (C) To show that sβwt does not bind to sAPPα, a sandwich ELISA was performed using sβwt to capture purified recombinant sAPPα or sAPPβ (Sigma), and the pan-APP antibody LN27 was used for detection. A secondary antibody conjugated to HRP was used for colorimetric detection. (D) Lysate from wild type N2a cells or N2a expressing the APPsw transgene (Tg +) were immunoprecipitated using the sβsw antibodies R1 or R2 in tandem with lysate from mouse brain from wild type or Tg2576 (Tg +) mice. (\*) indicates non specific IgG bands from antibody cross reactivity. The antibodies were characterized in Neuro2a cells stably overexpressing human BACE1 (Figure S2B).

**Supplemental Experimental Procedures**

**Directed Differentiation of ES Cells into Pyramidal Neurons**. For ESN differentiation, methods were adapted and optimized from the published protocol(Bibel et al., 2004; Bibel et al., 2007). ES cells were cultured in the presence of 1,000 U/ml Leukemia Inhibitory Factor (LIF) (Millipore) in medium containing DMEM (Millipore Specialty Media); 15% ES grade FBS (Thermo Fisher Scientific Hyclone); non-essential amino acids (Millipore Specialty Media); nucleosides (Millipore Specialty Media); glutamine (Millipore Specialty Media) and β-mercaptoethanol (Gibco) on mitomycin C (Sigma) treated primary mouse embryonic fibroblasts (PMEF). Cell culture dishes were coated with 0.1% gelatin (Chemicon) for 30 min. ES medium was changed every day. Prior to differentiation, newly defrosted ES cells were cultured as above for two passages and subsequently deprived of PMEF for two additional passages. After 2 feeder-free passages, cells could be frozen and used to begin embyoid body (EB) formation immediately after thawing. *Embryoid body formation*: Feeder-free ES cells were trypsinized (0.025% trypsin/0.75mM EDTA without calcium and magnesium; Chemicon), harvested in EB medium containing DMEM (Millipore Specialty Media); 10% ES grade FBS (Thermo Fisher Scientific Hyclone); non-essential amino acids (Millipore Specialty Media); nucleosides (Millipore Specialty Media); glutamine (Millipore Specialty Media) and β-mercaptoethanol (Gibco) and plated in EB medium on non-adherent bacterial Petri dishes (Greiner Bio-One) at a concentration of 3 x 106 cells/100 mm. Medium was changed every 2 days for 8 days total and 5 µM retinoic acid (Sigma) was added to EB medium on days 4 and 6. *Plating of neurons*: Tissue culture plates and slides were coated with 10μg/ml poly-D-Lysine (Sigma) in borate buffer (150mM, pH 8.6) and 3μg/ml laminin (Sigma). Embryoid bodies were collected in EB medium, washed twice with PBS (Gibco) and trypsinized for 5 min at 37ºC. EBs were dissociated in EB medium, and centrifuged at 1,500 rpm at RT for 5 min. The pellet was resuspended in Advanced DMEM/F12 (Gibco) supplemented with N2 (Gibco) and filtered through a 40μM nylon strainer. Cells were plated at a density of 1.5x105 cells/cm2 in 6 well (surface area(SA)/well=10 cm2), 12 well (SA/well=4 cm2), 35mm (SA/well=4 cm2), 24 well (SA/well=2 cm2), and 96 well (SA/well=0.3 cm2) plates. N2 supplemented medium was changed after 2 and 24 hrs. Media was changed to Neurobasal medium (Gibco) supplemented with B27 (Gibco), glutamine, penicillin and streptomycin 48 hours after plating. Subsequently, neuronal media was changed every 2 days. After 7 days neuronal medium was supplemented with 25 uM β-mercaptoethanol (Gibco).

**Immunocytochemistry.** Neuronal β-Tubulin III was detected by TUJ1 antibody from Covance, MAP-2 antibody was from BD Biosciences, and the antibody recognizing EMX1, a pyramidal neuron marker (Chan et al., 2001) was from Santa Cruz Biotechnology. GFAP and PSD-95 antibodies were from Thermo Scientific and the synaptophysin antibody was from Epitomics. At indicated days in vitro (DIV), cells were fixed with 4% paraformaldehyde (Electron Microscopy Sciences), permiabilized with 0.1% Triton X-100 (Roche), blocked in normal goat serum (EMD) and incubated with primary antibodies in normal goat serum in PBS with 0.1% (or 0.2% for detection of MAP2B) Triton X-100. Detection was achieved using fluorescent Alexa Fluor secondary antibodies (Invitrogen/Molecular Probes). Confocal images were collected with a Nikon C1 digital scanning laser confocal system attached to an Olympus IX71 inverted scope using a 100x objective.

**Western Blot analysis.** CamKIIα antibody was from BD biosciences; APP (human) was detected using 6e10 from Covance and mouse APP, and CTFs were detected using CT-Max (Landman et al., 2006; Okada et al., 2010). The BACE1 antibody was a generous gift from R. Vassar (Northwestern University) and PS1-loop antibody was a generous gift from J. Lah and A. Levey (Emory University). Actin antibody was from Sigma. Cells were grown for the indicated DIV and harvested in lysis buffer containing 10mM Tris-HCl, pH 7.4, 150mM NaCl, 1% Triton X-100, 0.25% NP-40 and 2mM EDTA. Lysate was centrifuged at 14,000 xg for 15 minutes at 4°C and the protein concentration of the supernatant was determined using BCA protein assay (Pierce) and diluted with Laemmli sample buffer. Protein was separated by SDS-PAGE on 4-20% Tris/Glycine gels (Invitrogen) and transferred to PVDF membranes for Western blot analysis.

**Transmission Electron Microscopy**. Neurons were plated in a 35mm dish as above and fixed with 2.5% glutaraldehyde in 0.1M Sorenson’s buffer (PH 7.2) for at least one hour. Cells were then postfixed with 1% OsO4 also in Sorenson’s buffer for one hour. Enblock staining was performed using 1% tannic acid. After dehydration, cells were embedded in a mixture of Lx-112 (Ladd Research Industries, Inc.) and Embed-812 (EMS, Fortwashington, PA). Thin sections were cut on a MT-7000 ultramicrotome. The sections were stained with uranyl acetate and lead citrate and examined under a JEOL JEM-1200 EXII electron microscope. Pictures were taken on an ORCA-HR digital camera (Hamamatsu) and recorded with an AMT Image Capture Engine.

**Miniaturization of Aβ assay in Tg2576 ESNs**

Tg2576 ESNs were plated at 1.5x105 cells/cm2 in a 96 well plate and after 10 *days in vitro* (DIV) neurons were treated with BSI or GSI for 24 hours. Media was collected and subjected to Aβ40 and Aβ42 detection using human specific ELISA (Invitrogen). Data were analyzed using Graph Pad Prism software to fit log (inhibitor) vs. normalized response curves.

**Cell viability assay.** Neurons were grown in 96 well plates and incubated with Cell Quanti-Blue reagent from Bio Assay Systems. Fluorescence was detected using a Tecan Infinite 200 PRO multimode plate reader.

**Supplemental References**

see main text