## Methods

### Compounds, peptides and antibodies

GSM-1, GSM-1-BpB, NS-1017, GSM-1-amide-BpB, Fen-B and DAPT were synthesized as described [10,12,36]. L-685,458 and fenofibrate were purchased from Bachem and SIGMA, respectively. L-852,646 [37] was kindly provided from Dr. Y. Li (Sloan-Kettering Cancer Center). Synthetic longer Aβ peptides (i.e., β-amyloid (1-43, #23573), (1-45, #61956-01), (1-46, #62076-01), (1-48, #61965-01), (1-49, #61963-01) were purchased from Anaspec. Aβ (1-40) (#4307-v) and Aβ (1-42) (#4349-v) peptides were purchased from Peptide institute. The rabbit polyclonal antibodies anti-PS1 NTF (G1Nr5), anti-PS1 CTF (G1L3) and anti-Pen-2 (PNT3) were raised as described [38-40]. Anti-PS1 NTF (PS1NT) [41] and anti-SPP (SPPc) [42] were kindly gifted from Drs. G. Thinakaran (The University of Chicago) and T. Golde (University of Florida). Anti-nicastrin N1660 (SIGMA), anti-APP CTF (Immuno-Biological Laboratories), anti-Aph-1aL O2C2 (Covance), anti-human Aβ 82E1 (Immuno-Biological Laboratories) and anti-biotin (Bethyl) were purchased from indicated vendors. The monoclonal antibody anti-α-tubulin AA4.3 developed by Dr. C. Walsh was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD, National Institutes of Health, and maintained by The University of Iowa, Department of Biology, Iowa City, IA.

### Plasmid construction, cell culture manipulation and cell based assay

cDNAs encoding PS1 and APP carrying Swedish mutation (APPNL) were inserted into pMXs-puro [43]. cDNAs encoding mutant PS1 were generated by long PCR-based QuikChange™ strategy (Stratagene). To produce recombinant proteins, cDNAs encoding PS1 were cloned into pGEX-6P-1 vector (GE healthcare) [10]. Maintenance of cultured cells, transfection, retroviral infection, two-site enzyme-linked immunosorbent assay (ELISA), or immunoblotting using Urea/SDS-PAGE gel system as described [10,39,44,45].

### Photoaffinity labeling and SCAM experiments

Preparation of samples for photoaffinity labeling experiments [46] was performed as follows. Brains of C57J/B6 mouse (3-5 month age) or cultured cells were homogenized with homogenize buffer (20 mM HEPES (pH 7.0), 140 mM KCl, 250 mM sucrose, 0.5 mM diisopropyl fluorophosphate, 0.5 mM phenylmethylsulfonyl fluoride, 1 μg/ml tosyllysine chloromethyl ketone, 1 μg/ml antipain, 1 μg/ml leupeptin, 10 μg/ml phosphoramidon, 5 mM EDTA, 1 mM EGTA) using Potter-Elvehjem Tissue Grinder (Wheaton), and membrane fractions were collected by ultracentrifugation at 100,000 × g (Beckman) [10]. PAL experiments utilizing avidin-biotin catch principle [47] and thrombin digestion experiments after PAL were performed as previously described [10]. Briefly, after resuspension of the microsome in the homogenize buffer by 25G needle with syringe, protein content was measured by BCA assay (Thermo Fisher Scientific). 1 mL of microsome-containing solution (1 mg/ml protein) was preincubated with compounds for 30 min on ice. Then photoprobes were added and incubated for 10 min on ice under the dark condition. UV irradiation (352 nm) was performed on ice for 1 hr with a UV lamp (Model XX-15BLB, UVP). The approximate distance from UV lamp to the samples was 10 cm. The biotinylated proteins were precipitated by streptavidin sepharose (GE healthcare) in 1% SDS containing homogenization buffer. For SCAM, all methanethiosulfonate reagents (Toronto Research Chemicals) were dissolved in dimethyl sulfoxide at 200 mM prior to use or stored at 80 degree until use. The methods for SCAM and competition experiments using biotinylaminoethyl methanethiosulfonate have been described in detail before [10,11]. Briefly, stable DKO cells expressing cysteine mutant PS1 were grown on two 15-cm dishes per single analysis. Cells were scraped in PBS and resuspended in 2 ml of SCAM homogenization buffer (10 mM HEPES (pH 7.4), 150 mM NaCl, 10% glycerol, Complete protease inhibitor cocktail (Roche Biochemicals)). Cells were disrupted by a Polytron homogenizer (Hitachi), and nuclei and large cell debris were pelleted by centrifugation at 1,500 × g for 10 min. The postnuclear supernatants were centrifuged, and the resultant microsomal pellets were resuspended in 0.2 ml of PBS in a syringe, and 0.1 mM biotinylaminoethyl methanethiosulfonate was added to this fraction. After 30 min incubation at 4 degree, microsomes were centrifuged twice to wash out. The resultant pellets resuspended in 1% SDS/PBS were incubated with the streptavidin sepharose overnight and analyzed as in the intact cell biotinylation experiment. In PAL or SCAM experiments, we loaded 1.5 and 20% of samples as "input" and "bound", respectively, in all immunoblot analyses.

### Protein purification and binding assay

GST-fusion recombinant proteins were expressed in *E. Coli* (BL21 DE3) (Novagen) and purified by two step procedures using glutathione sepharose and mono Q columns (GE Healthcare) as manufacturer's instruction. All recombinant proteins were finally diluted with recombinant protein preparation buffer (10 mM HEPES (pH 7.4), 150 mM NaCl, 0.25% CHAPSO). C99-FLAG was purified from Sf9 cells infected with recombinant baculovirus encoding C99-FLAG and diluted at 1 μg/ml in recombinant protein preparation buffer. To perform binding assay, 0.5 μg of GST-fusion recombinant

proteins were mixed at 1 μg of C99-FLAG, 1 (for Aβ43, Aβ45, Aβ46, Aβ48 and Aβ49) or 10 (for Aβ40 and Aβ42) nM of synthetic Aβ in 1 ml of recombinant protein preparation buffer, and incubated at 4 degree overnight. After addition of glutathione sepharose, samples were then washed with the buffer and precipitates were eluted by boiling in sample buffer. For binding assay using native PS1 protein, PS1 or P88L mutant PS1 was expressed in DKO cells and solubilized in 10 mM HEPES buffer containing 1% CHAPSO. After addition of 2 ng of Aβ48 peptide, the solubilized fraction was incubated with anti-PS1 antibody G1Nr5 at 4 degree overnight. PS1-Aβ48 complex was then immunoprecipitated using Protein G sepharose 4 Fast Flow (GE Healthcare). Subsequently eluates (i.e., proteins bound to GST-fusion recombinant proteins or native PS1 proteins) were analyzed by immunoblotting. We loaded 0.75 and 20% of samples as "input" and "bound", respectively, in immunoblot of all pull down assay unless the amount of loaded proteins was otherwise indicated.

#### Abbreviations

AD: Alzheimer disease; CTF: Carboxyl-terminal fragment; DKO: *Psen1/Psen2* double knockout mouse immortalized fibroblasts; GSM: γ-secretase modulator; NTF: Amino-terminal fragment; mt: Mutant; PS: Presenilin; SCAM: Substituted cysteine accessibility method; TMD: Transmembrane domain.

#### Competing interests

The authors declare that they have no competing interests.

#### Authors' contributions

YO and TT designed the research. YO, AT, SO and TT performed biochemical experiments. NS, TH, SY and TF synthesized the compounds. YO, TT and TI wrote the paper. All authors read and approved the final manuscript.

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