# Modeling familial Alzheimer's disease with induced pluripotent stem cells

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# Introduction

- Alzheimer's disease (AD) is the most common form of age-related dementia
- The leading hypothesis for AD progression is accumulation of Aβ42 which causes an amyloid cascade leading to nerve cell death
- Mutations in the PS1, PS2 and the APP gene account for most of the familial early onset cases of AD

# Materials

- Two clones of iPSCs:  PS1 (A246E) and PS2 (N141I) by retroviral transduction of OCT4, SOX2, KLF4, LIN28 and NANOG.
- Immunofluorescence staining of iPS and derived neurons
- Microarray analysis and aCGH analysis

# Results: Generation of iPSC with presenilin mutations

- Used retroviral techniques and target genes identified in iPSC studies to create two clones with the PS1 mutations and PS2 mutations
  - Cells from human fibroblasts
  - PS1: A246E (PS1-2 iPSC and PS1-4 iPSC)
  - PS2: N141I (PS2-1 iPSC and PS2-2 iPSC)
  - Reprogrammed with OCT4, SOX2, KLF4, LIN28 and NANOG
- Created a line of control iPSC using cells from sporadic parkinson's and the 201B7 line for comparison using same retroviral technique
  - OCT4, SOX2, KLF4, and cMYC

# Results: Generation of iPSC with presenilin mutations

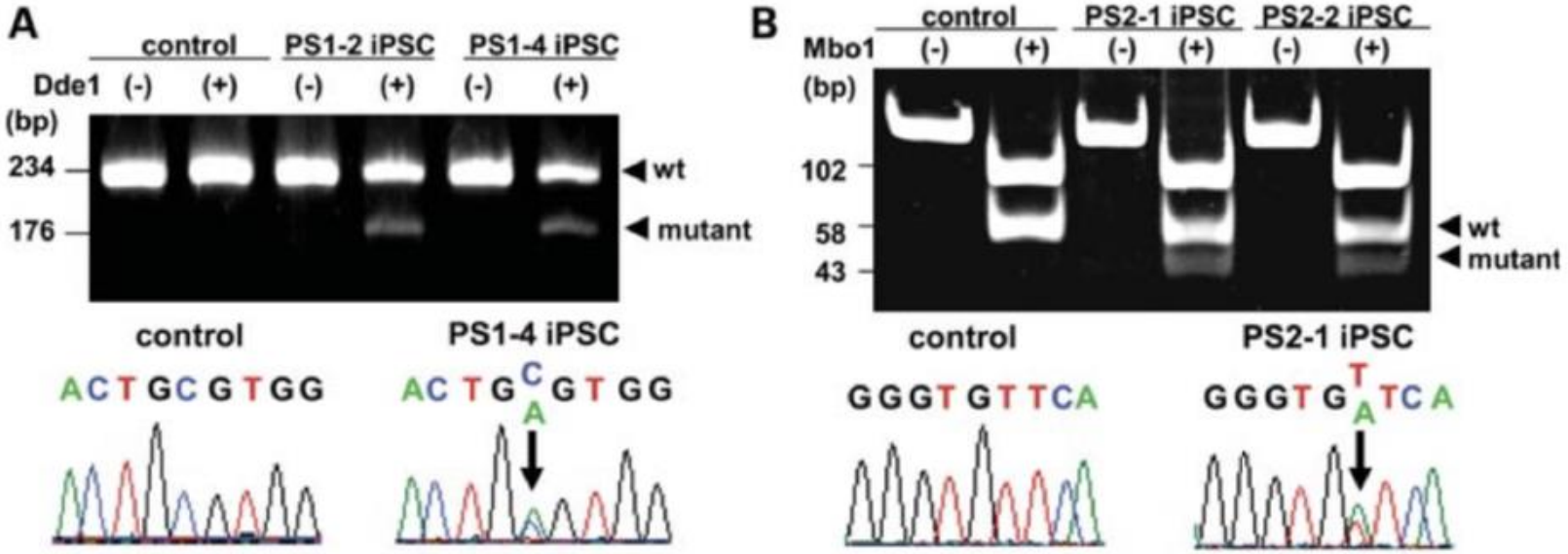



**FIG 1 A & B:** confirmation of genotyping using PCR-RFLP

# Results: Generation of iPSC with presenilin mutations

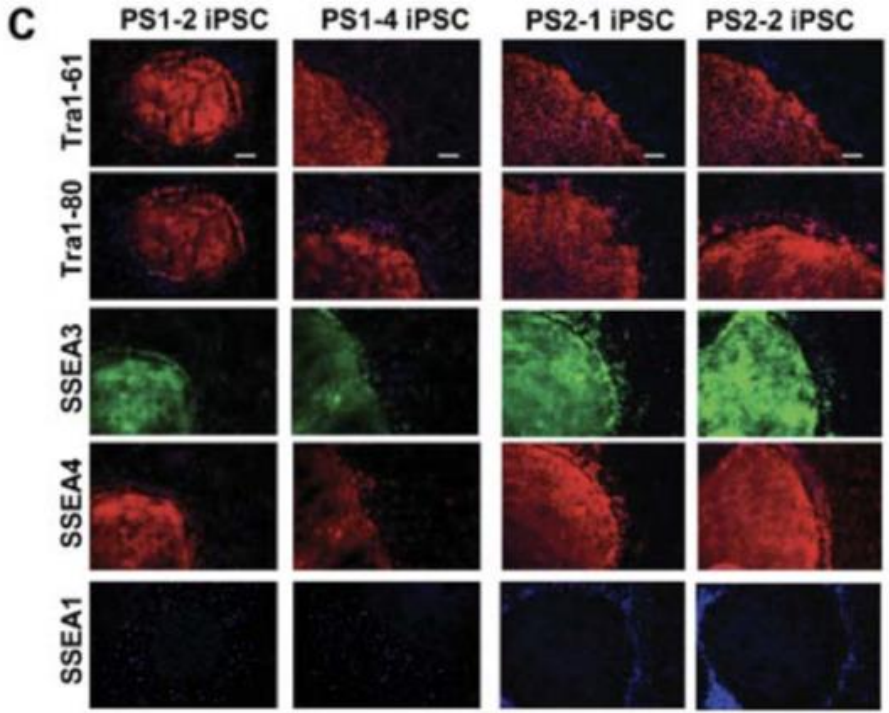



**FIG 1 C:** similar morphology expression in created iPSC lines

# Results: Generation of iPSC with presenilin mutations

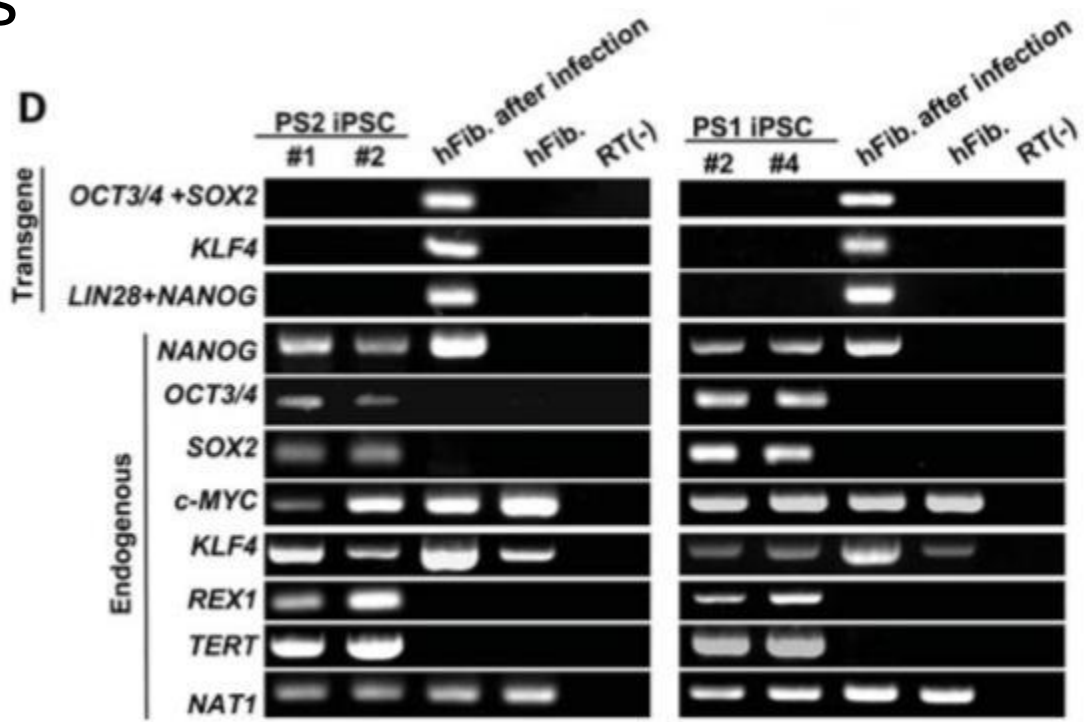



**FIG 1 D:** RT-PCR analysis of transgenes (silencing and pluripotency)

# Results: Generation of iPSC with presenilin mutations

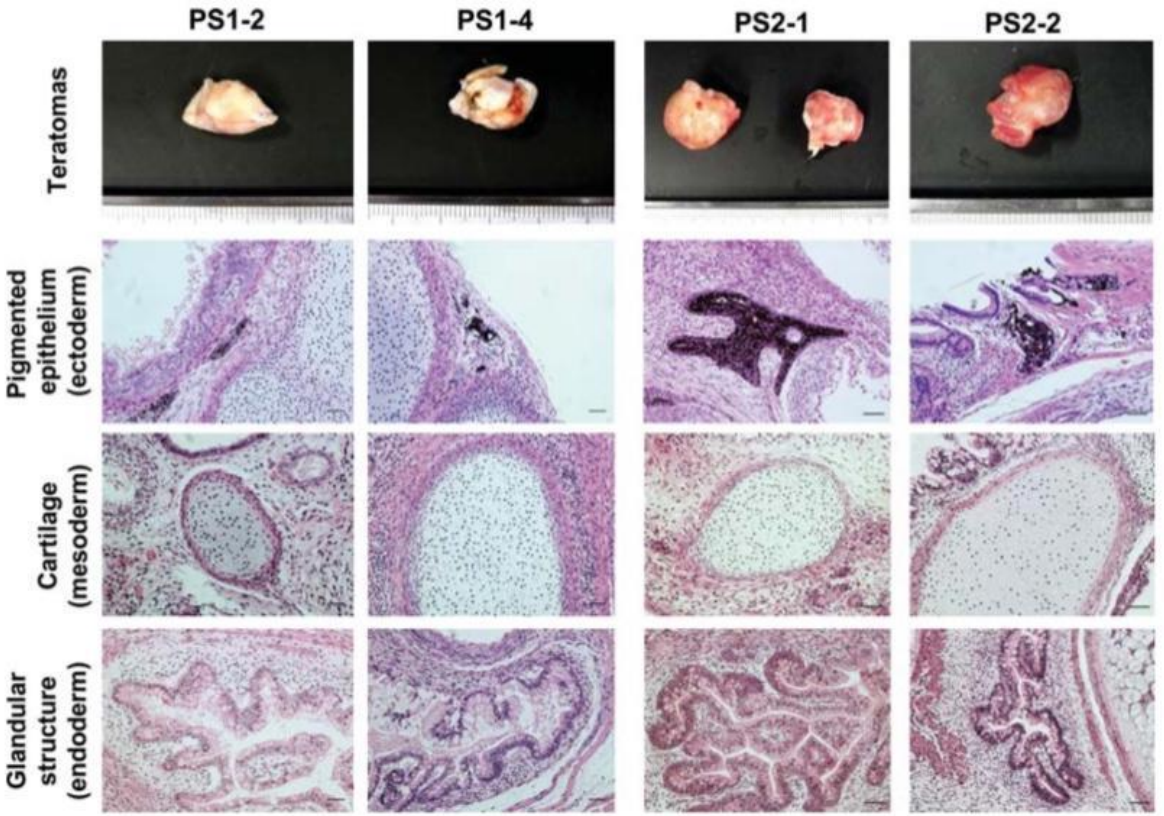



**FIG 2:** Confirmation of iPSC based on germ layer growth *in vivo*

# Results: Generation of iPSC with presenilin mutations

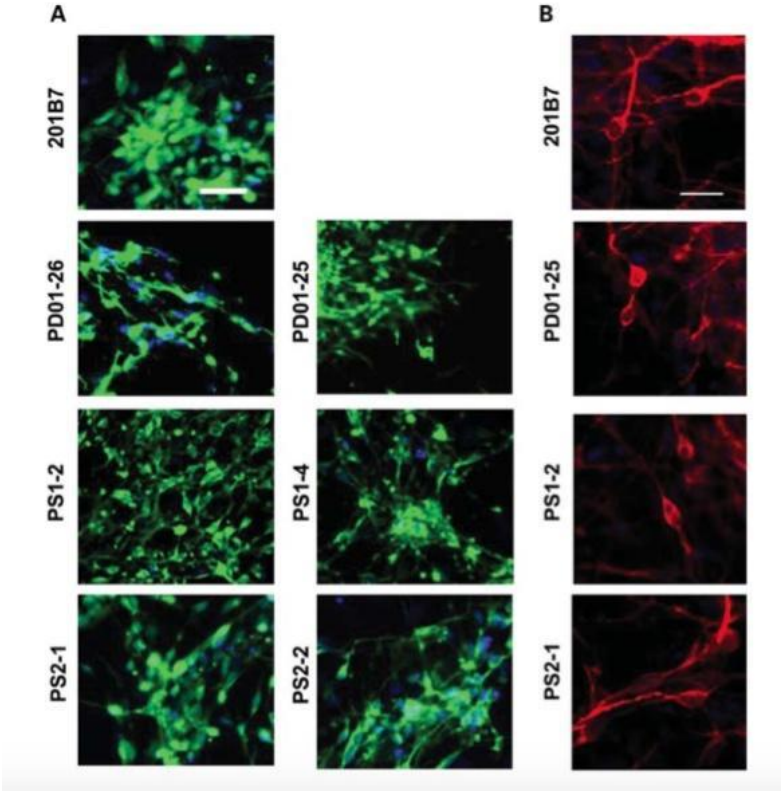
- Confirmed the viability and comparability of tissues in several other ways
  - Heat maps: global gene expression profiles
    - Similarities between iPSC of control and experimental lines
  - No significant difference in AD-related molecule expression
  - Array Comparative Genomic Hybridization (aCGH)
    - Compared PS2-1 and PS2-2 iPSCs and AG09908
    - 52, 61, and 102 aberrations respectively (out of ~17,000)
    - No aberrations in PS1, PS2, or APP genes

# Results: Differentiation of PS1 iPSC and PS2 iPSC into neurons

- Modeled the disease pathogenesis of AD *in vitro*
- All 4 PS1 and PS2 iPSC lines as well as controls cultured on Matrigel-coated dishes for 2 weeks
- Intended to induce neural cell differentiation and terminal differentiation

# Results: Differentiation of PS1 iPSC and PS2 iPSC into neurons

**FIG 3 A&B:** Confirming expression of βIII Tubulin (A) and MAP-2 (B)



# Results: Differentiation of PS1 iPSC and PS2 iPSC into neurons

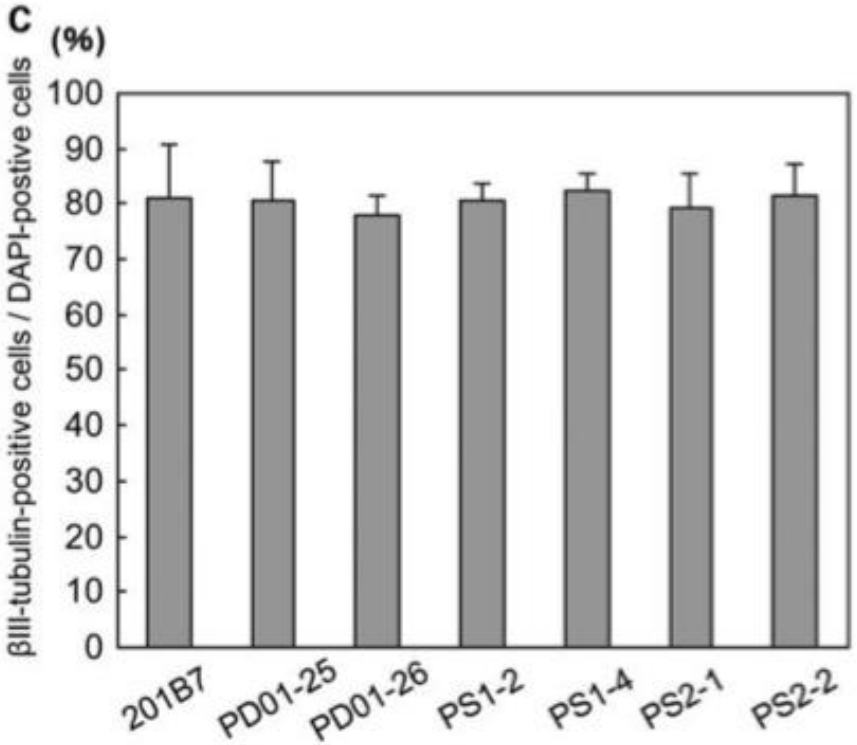



**FIG 3C:** Percent of βIII Tubulin positive cells

# Results: Production of Aβ secreted from iPSC-derived neurons

- Investigation of Aβ secretion of iPSC and iPSC-derived neurons
  - Unable to compare Aβ40 and Aβ42 among iPSC lines
  - Higher and measurable Aβ secretions in the differentiated neurons
    - Significant fluctuation during differentiation?

# Results: Production of Aβ secreted from iPSC-derived neurons

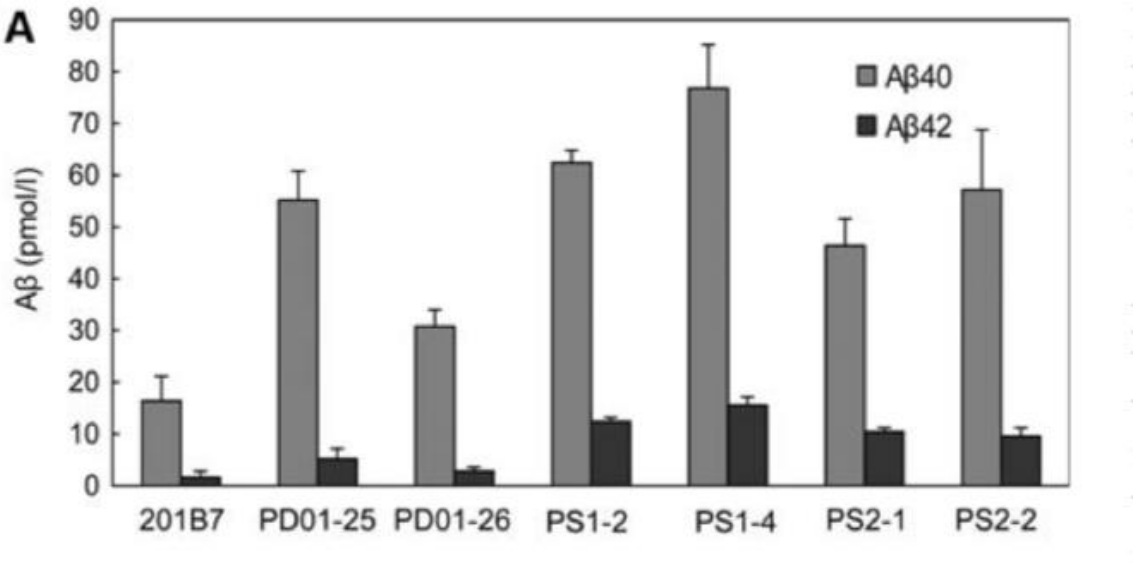



**FIG 4 A:** Some clonal variation of Aβ40 and Aβ42

# Results: Production of Aβ secreted from iPSC-derived neurons

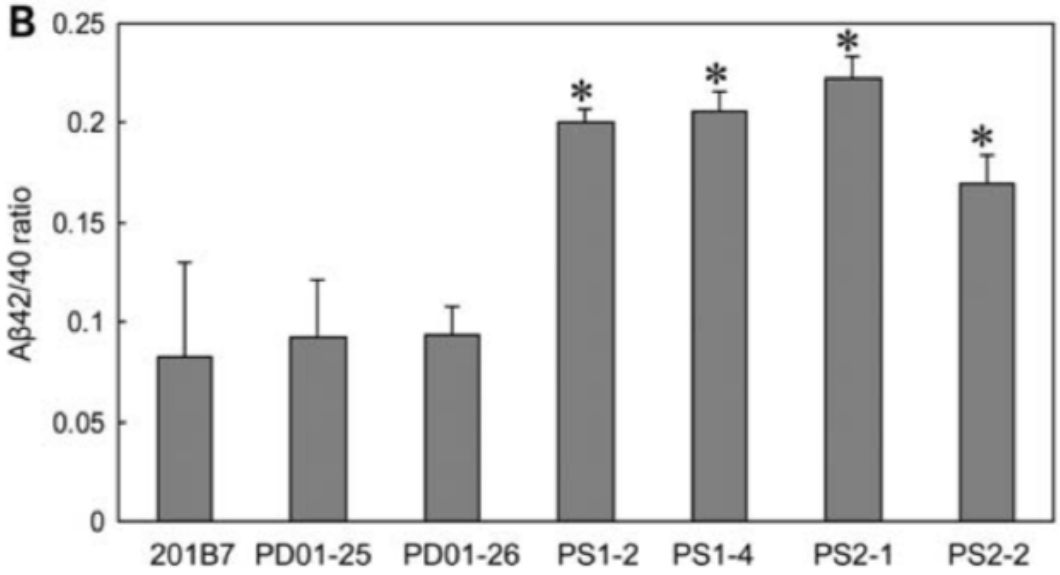


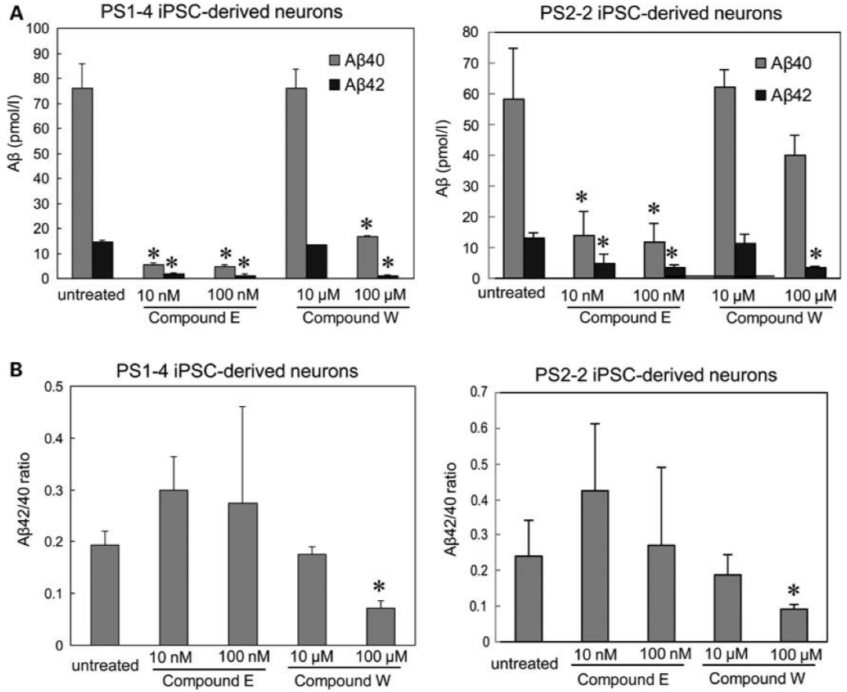

**FIG 4 A:** Ratios of Aβ40 and Aβ42 in the different cell lines

# Results: Pharmacological response to γ-secretase inhibitors in PS1 iPSC- and PS2 iPSC-derived neurons

- Tested how inhibitors impacted the secretion of Aβ
- Data indicated that both PS1 and PS2 iPSC-derived neurons respond to drug treatment in an expected manner and might be useful for drug screening

# Results: Pharmacological response to γ-secretase inhibitors in PS1 iPSC- and PS2 iPSC-derived neurons

**FIG 5 A&B:** Aβ40 and Aβ42 secretions with inhibitors and the ratios of secretion



# Results: Pharmacological response to γ-secretase inhibitors in PS1 iPSC- and PS2 iPSC-derived neurons

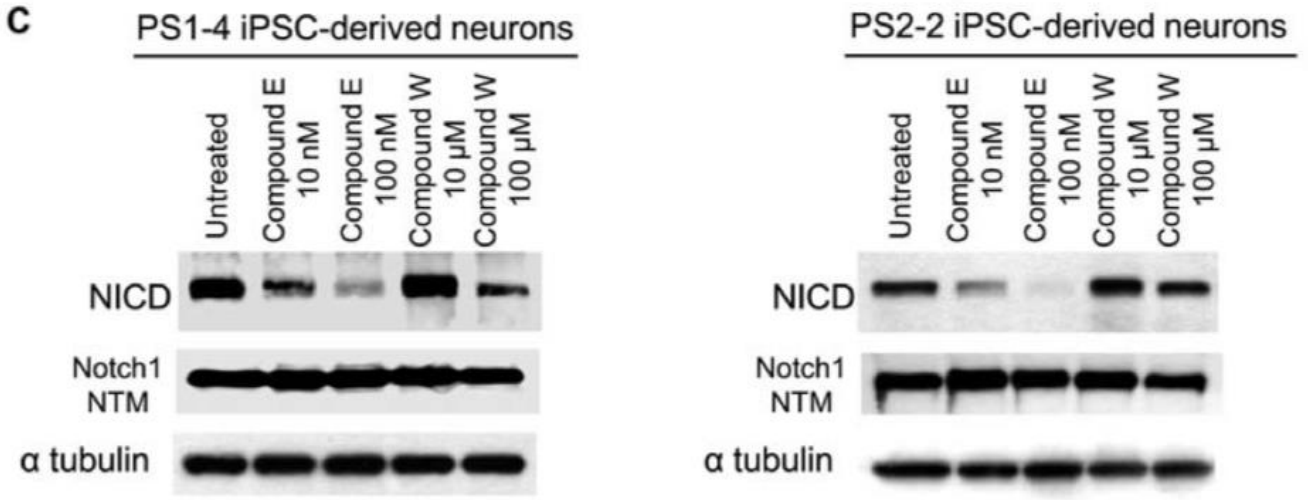



**FIG 5 A&B:** Western blotting of the E and W inhibitor secretions

# Conclusion

Patient-derived differentiated neurons increase Aβ42 secretion, recapitulating the pathological mechanism of FAD with PS1 and PS2 mutations

The findings demonstrate that the FAD–iPSC-derived neuron is a valid model for studying AD, and provides important clues for the identification and validation of candidate drugs