Cell Reports

Supplemental Information

APP Metabolism Regulates Tau Proteostasis in Human Cerebral Cortex Neurons

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SUPPLEMENTARY MATERIALS:

1. **Supplementary Methods**
2. **Supplementary Figures**
3. **Supplementary Movies**

SUPPLEMENTARY METHODS

Pluripotent stem cell culture

Pluripotent stem cells were maintained on mouse embryonic fibroblasts (MEFs) (GlobalStem) in DMEM/F12 containing 20% KSR (vol/vol), 100 μm non-essential amino acids, 100 μM 2-mercaptoethanol, 50 U ml^{-1} penicillin and 50 mg ml^{-1} streptomycin (all Life Technologies) and 10 ng ml^{-1} FGF2.

Immunostaining and imaging

Cultures were fixed in 4% paraformaldehyde in phosphate buffered saline or methanol at -20°C before being processed for immunofluorescence staining and confocal microscopy. Antibodies used for immunofluorescence in this study were Tbr1 (Abcam, ab31940), CTIP2 (Abcam, ab18465), β3-tubulin (Covance, MMS-435P), and total tau (Dako Cytomation, A0024).

Calcium imaging

Calcium imaging was performed on familial AD (PSEN1 Y115C, Intron 4 and APPV717) and control (NDC) cultures aged between 8 and 9 weeks post-induction. Cells were loaded with the calcium indicator Oregon Green 488 BAPTA (OGB) by diluting a 0.7mM OGB stock solution to 3.2μM in N2B27 media containing 0.01% v/v Cremaphor EL and 0.4% Pluronic F-127 to make a loading solution. Cells were then bathed in the OGB loading solution for 1 hour at 37ºC and 7% CO_2 in the dark. The loading solution was then removed and the cells were washed over twice with N2B27 and incubated for a further 30 minutes at 37ºC and 7% CO_2 in the dark in N2B27. The cells were then imaged in artificial CSF using a Deltavision (Applied Precision), with an EMCCD camera and using softWoRx 5.0.0 (Applied Precision) software. The imaging chamber was heated to 37ºC and supplied with 5% CO_2. The recordings were 2 minutes in length and were captured at 10 Hz. The 2-minute movies were converted to AVI format playing at 150 frames per second using ImageJ software.

Western blot analysis

Whole cell protein extraction was performed by lysis of cell pellets in cell extraction buffer (Invitrogen) supplemented with PMSF (Sigma), protease inhibitors (Thermo Scientific) and phosphatase inhibitors (Thermo Scientific) before removal of the soluble fraction. Western blot analysis was carried out using the following antibodies: β-actin (Sigma, A2228), β3-tubulin (Covance, MMS-435P), APP C-terminal fragment (Covance, SIG-39152), total tau (Dako Cytomation, A0024), phospho-PHF-tau pS202/T205 - AT8 (Thermo, MN1020), anti-tau Phospho S396 (Abcam, ab109390) and anti-tau Phospho S404 (Abcam, ab92676). Detection of immunoblots was carried out using LI-COR Odyssey CLx Infrared Imaging System and Image Studio Software.

RT-PCR analysis of MAPT transcription

Total RNA was extracted using TRIzol before cDNA was prepared using from 500 ng of RNA superscript II (both Life Technologies). MAPT mRNA expression was assessed using the primer set, forward 5′-AAGTCGCCGTCTTCCGCCAAG-3′; reverse 5′-GTCCAGGGACCCAATCTTCGA-3′ (Iovino et al., 2010). PCR was performed in a final volume of 25 µl using the following protocol. 95°C for 15 min, 30 cycles at 94°C for 30 seconds, 60°C for 30 s and 74 °C for 90 s with a final 10 min extension at 74°C. GAPDH transcription was assessed as a control using the primer set, forward 5' – CTGGTAAAGTGGATATTGTTGCCAT- 3': reverse 5'-TGGAATCATATTGGAACATGTAAACC- 3'. Products were analysed on a 2% agarose gel and imaged with a Molecular Imager Gel Doc XR+ imaging system (BIO RAD)

IP-MALDI analysis of Aβ peptides

For analysis of cell culture media/supernatants, 4 µg of the Aβ-specific antibodies 6E10 and 4G8 (epitope in the N-terminal region and epitope 18-22, respectively, Signet Laboratories, Inc., Dedham, MA, USA) were separately added to 25 µL Dynabeads M-280 (Dynal) sheep anti-mouse according to the manufacturer's product description. Briefly, the IPs were conducted on 940 µL cell media, to which 10 µL 2.5% Tween-20 (Bio-Rad Laboratories Inc.) had been added. The beads/cell media solution (total volume 1 mL) was transferred to a KingFisher magnetic particle processor (polypropylene tubes,

Thermo Scientific) for washing and elution in a 5-step procedure. The collected supernatant was dried in a vacuum centrifuge and redissolved in 5 μL 0.1% formic acid in 20% acetonitrile. Samples were analyzed by MALDI TOF/TOF (Autoflex, Bruker Daltonics, Bremen, Germany) operating in reflector mode. FlexAnalysis (version 3.3, Bruker Daltonics) was used for automated integration of the peaks for each spectrum. Prior to the statistical analysis, the peak areas were normalized to the sum of the integrated peaks, duplicated samples were averaged, and the relative changes compared to baseline values were calculated. All solvents used were of HPLC quality.

REFERENCE:

Iovino, M., Patani, R., Watts, C., Chandran, S., and Spillantini, M.G. (2010). Human stem cell-derived neurons: a system to study human tau function and dysfunction. PLoS ONE *5*, e13947.

SUPPLEMENTARY FIGURES

Figure S1: Derivation and characterisation of *APP* and *PSEN1* mutant iPSC lines

A, B. Reprogrammed fibroblasts from patients with *APP* V717I or *PSEN1* (Y115C, M146I, Intron 4) endogenously express the pluripotency markers Oct3/4, Sox2, Nanog and c-Myc. mRNA expression data normalized to H9 ESC expression levels, error bars = SD. Embryoid body differentiation of each line confirmed pluripotency, assessed by a panel of markers as indicated. Related to **Figure 1A.**

Figure S2: *PSEN1* mutants are hypomorphic loss of function alleles

A-D. Representative IP-MALDI traces demonstrating the increase of Aβ14/15/16 in *PSEN1* mutant neurons compared to control neurons. Quantification of these data was used to generate the statistics presented in Figure 1L and M. **Related to Figure 1K-M.**

Figure S3: Tau proteostasis is altered in fAD neurons

A. Additional technical replicates of the immunoblots for soluble total, pS202/205, pS396 and pS404 tau in fAD and control neurons at day 90 post neural induction, related to **Figure 2A and C.**

B. RT-PCR for *MAPT* demonstrating that the increased tau protein levels observed in *APP* V717I, Ts21 and *APP* (dup) neurons is not a result of increased transcription. GAPDH is included as a control.

Figure S4: Responses of fAD neurons to γ-secretase manipulation

A-C. Quantification of the absolute amounts of Aβ40, Aβ42 and Aβ38 released from fAD neurons, assessed at day 90 post-induction following 20 days of treatment with either DMSO (Ctrl), DAPT (GSI) and E2012 (GSM). **, $p<0.01$, Student's *t*-test. Error bars = SD.

D-F. IP-MALDI analysis of media taken from Ctrl-, GSI- or GSM-treated neurons, demonstrating the efficacy of GSI and GSMs in human neurons.

G-H. Technical replicates of the immunoblots presented in **Figure 4 E-F** for **(G)** total tau and selected phosphorylation sites **(H)** APP-C83/99 and β3-tublin with the respective β-

actin loading control performed on soluble protein extracts of neurons following treatment with the indicated compounds.

Movie S1: fAD cortical progenitors form 3-dimensional neuronal networks

A. 3D rendering of a control neuronal culture at day 100 post induction following tissue clearing and immunostaining for tau (red) and DNA (DAPI, blue). Removal of tau positive neuronal processes reveals the cellular density achieved by this system after months in culture. Z= 145μm. **Related to Figure 1D**.

B. Serial Z sections of control neurons at day 100 post induction following tissue clearing and immunostaining for tau (red) and DNA (DAPI, blue). Z= 118 μm captured in 2 μm steps, scale bar = 62 μm. **Related to Figure 1D**.

Movie S2: fAD neurons form spontaneously active neuronal networks

A-D. Live imaging of neuronal cultures loaded with Oregon Green BAPTA to visualise calcium transients. Control neurons (day 61 post induction, **A**) demonstrate comparable network activity to *PSEN1* intron 4 (day 64 post induction, **B**), *PSEN1* Y115C (day 55 post induction, **C**) and *APP* V717I neurons (day 61 post induction, **D**). **Related to Figure 1A-D.**

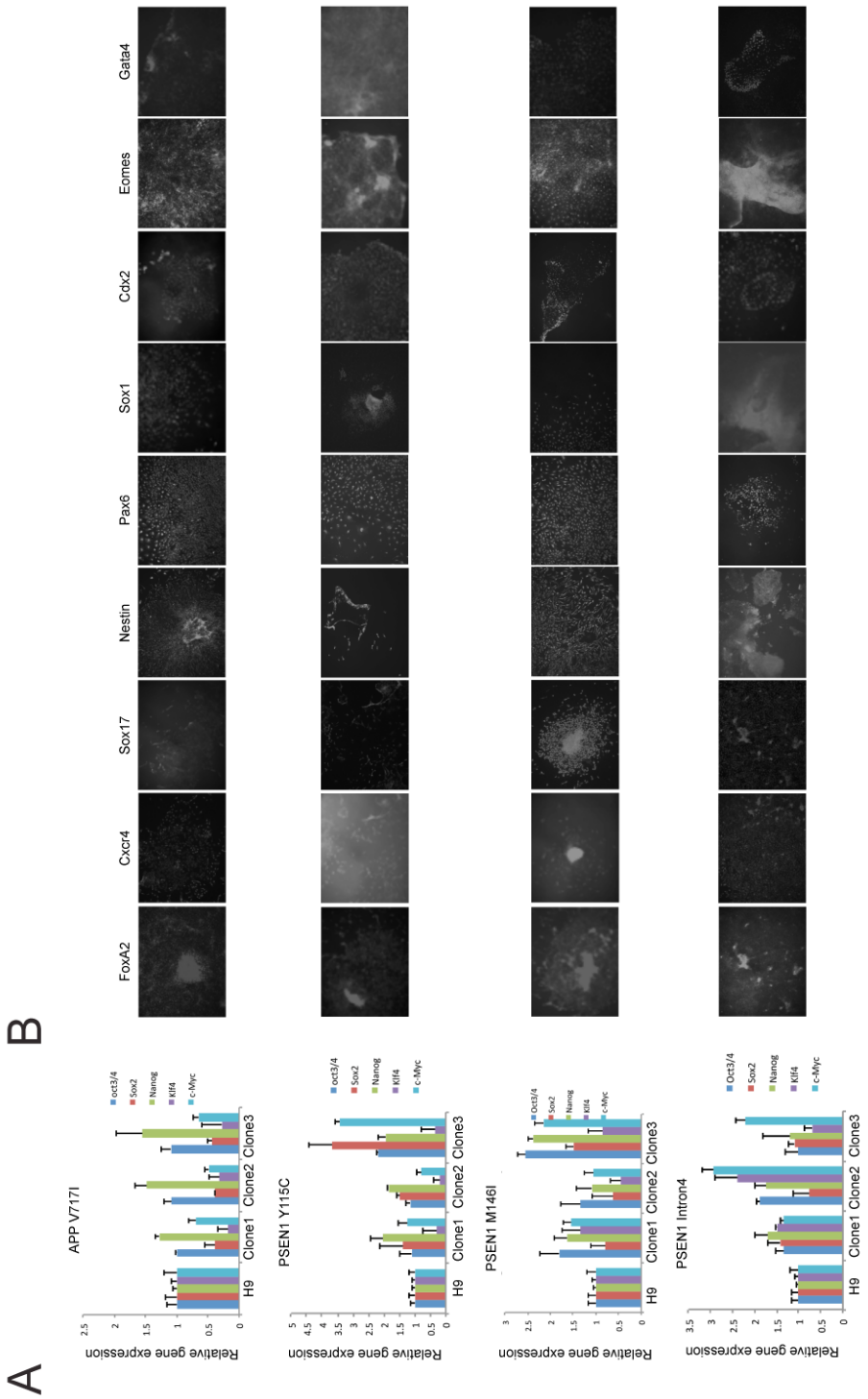
A
B
FoxA2
Cxcr4
Sox17
Nestin
Pax6
Sox1
Cdx2
Eomes
Gata4
APP V717I
PSEN1 Y115C
PSEN1 M146I
PSEN1 Intron4
Oct3/4
Sox2
Nanog
Klf4
c-Myc
Relative gene expression
H9
Clone1
Clone2
Clone3

Figure S1

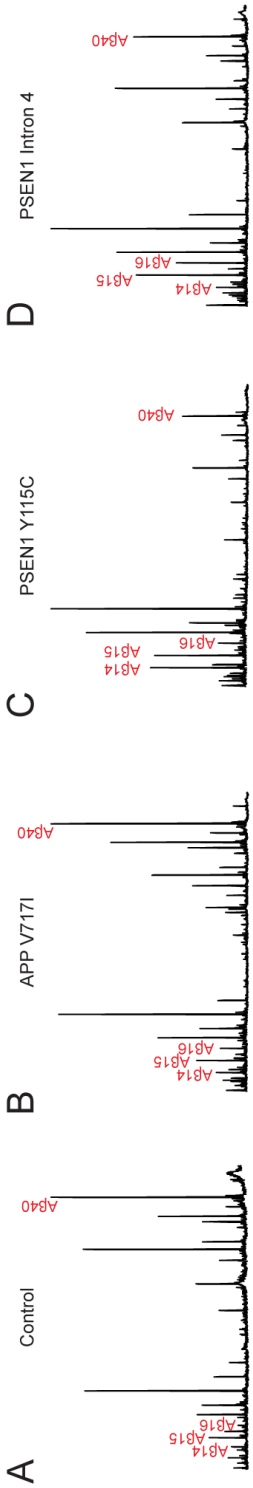
A
Control
Aβ40
Aβ14
Aβ15
Aβ16
B
APP V717I
Aβ40
Aβ14
Aβ15
Aβ16
C
PSEN1 Y115C
Aβ40
Aβ14
Aβ15
Aβ16
D
PSEN1 Intron 4
Aβ40
Aβ14
Aβ15
Aβ16

Figure S2

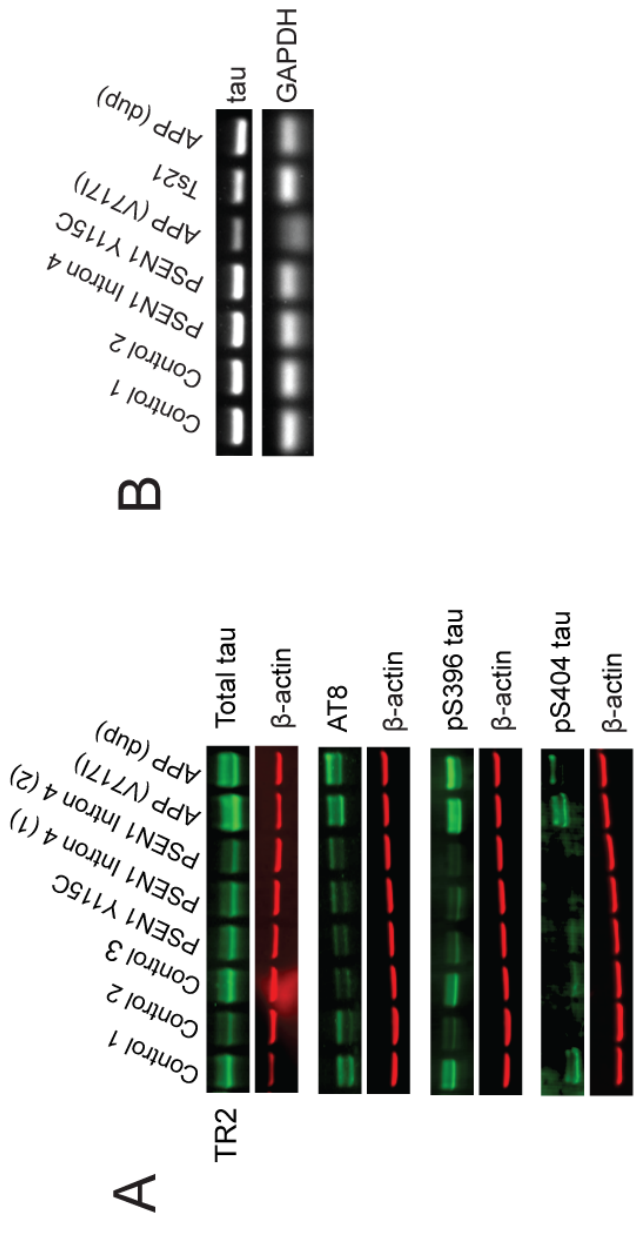
A
TR2
Total tau
β-actin
AT8
β-actin
pS396 tau
β-actin
pS404 tau
β-actin
Control 1
Control 2
Control 3
PSEN1 Y115C
PSEN1 Intron 4 (1)
PSEN1 Intron 4 (2)
APP (V717I)
APP (dup)
B
tau
GAPDH
Control 1
Control 2
PSEN1 Intron 4
PSEN1 Y115C
APP (V717I)
Ts21
APP (dup)

Figure S3

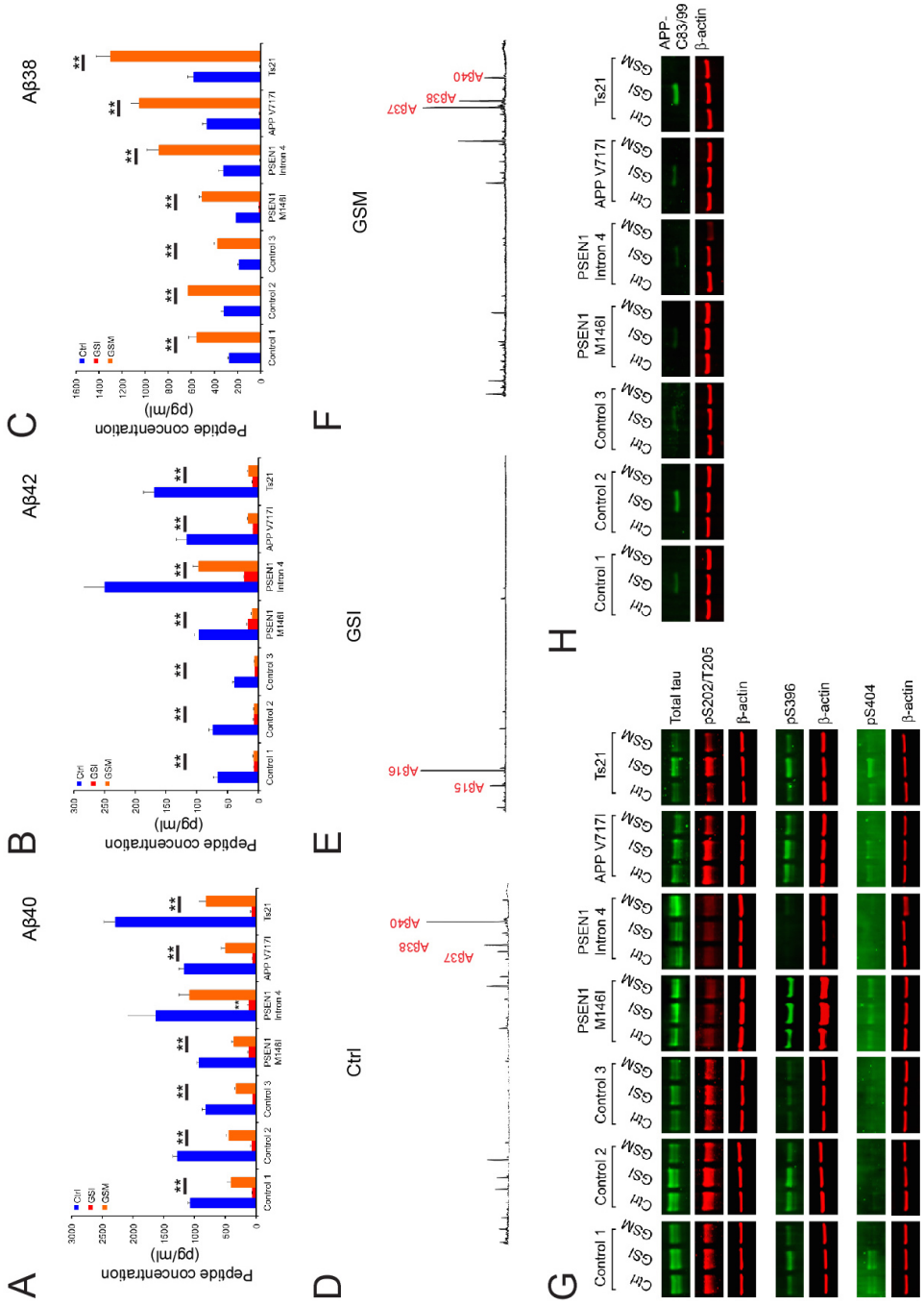
A
Aβ40
Peptide concentration (pg/ml)
Ctrl
GSI
GSM
Control 1
Control 2
Control 3
PSEN1 M146I
PSEN1 Intron 4
APP V717I
Ts21
B
Aβ42
C
Aβ38
D
Ctrl
Aβ37
Aβ38
Aβ40
E
GSI
Aβ15
Aβ16
F
GSM
G
Total tau
pS202/T205
β-actin
pS396
pS404
H
APP-C83/99

Figure S4