The innate immunity protein IFITM3 modulates γ-secretase in Alzheimer’s disease

Innate immunity is associated with Alzheimer’s disease, but the influence of immune activation on the production of amyloid-β is unknown. Here we identify interferon-induced transmembrane protein 3 (IFITM3) as a γ-secretase modulatory protein, and establish a mechanism by which inflammation affects the generation of amyloid-β. Inflammatory cytokines induce the expression of IFITM3 in neurons and astrocytes, which binds to γ-secretase and upregulates its activity, thereby increasing the production of amyloid-β. The expression of IFITM3 is increased with ageing and in mouse models that express familial Alzheimer’s disease genes. Furthermore, knockout of IFITM3 reduces γ-secretase activity and the formation of amyloid plaques in a transgenic mouse model (5xFAD) of early amyloid deposition. IFITM3 protein is upregulated in tissue samples from a subset of patients with late-onset Alzheimer’s disease that exhibit higher γ-secretase activity. The amount of IFITM3 in the γ-secretase complex has a strong and positive correlation with γ-secretase activity in samples from patients with late-onset Alzheimer’s disease. These findings reveal a mechanism in which γ-secretase is modulated by neuroinflammation via IFITM3 and the risk of Alzheimer’s disease is thereby increased.

The γ-secretase enzyme complex consists of four obligatory subunits—presenilin (PS), nicastrin (NCT), anterior pharynx-defective 1 (APH-1) and presenilin enhancer 2 (PEN-2). Increasing evidence suggests that γ-secretase activity can be regulated by γ-secretase modulatory proteins. The identification of γ-secretase modulatory proteins is an actively evolving field. It has been suggested that IFITM3 is part of the γ-secretase complex and could be a potential risk factor for disease progression. IFITM3 may function as an immune switch to increase γ-secretase activity and amyloid-β production for its anti-microbial activity and consequently, contribute to Alzheimer’s disease pathogenesis.

**IFITM3 is part of the γ-secretase complex**

To identify GSM-binding proteins, E2012-BPyne (Fig. 1a) was incubated with cell membranes and photocrosslinked with its binding proteins. The photolabelled species was reacted with TAMRA-azide and analysed by gel electrophoresis (Fig. 1b). Two bands migrating at approximately 30 kDa and 15 kDa (Fig. 1b) were blocked by E2012. The 30-kDa protein is the PS1N-terminal fragment (PS1-NTF), as previously reported, and the 15-kDa protein was identified as IFITM3 by liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Extended Data Fig. 1a) and confirmed by western blot analysis (Fig. 1c). In addition, photolabelling was partially suppressed by the γ-secretase inhibitor (GSI) L-685,458.
IFITM3 interacts with IFITM3 in primary neurons as indicated by discrete red active γ-secretase captures not only PS1-NTF and NCT, but also IFITM3 body (Fig. 1d), indicating that IFITM3 interacts with the γ-secretase (L458) (Fig. 1c). The labelling of PS1-NTF is enhanced by L458 (Fig. 1c, Fig. 1d). Knock-out of IFITM3 had an opposite effect on two types (acid versus imidazole) of GSI and no effect on L458 (Extended Data Fig. 2d–f), indicating that IFITM3 not only affects γ-secretase activity, but also alters the selectivity to GSs. Notably, knockdown (Extended Data Fig. 2g, h) and knockout of IFITM3 (Extended Data Fig. 2j) increased γ-secretase activity and resultant NOTCH cleavage in cell-based assays (Extended Data Fig. 2h, i) and γ-secretase in vitro activity assay (Extended Data Fig. 2j), demonstrating that IFITM3 exhibits distinct modulatory effects on γ-secretase processing of APP and NOTCH.

Ageing and F AD upregulates IFITM3

The expression of IFITM3 significantly increased with age (2.9- and 2.3-fold at 28 months compared to 4 months in females and males, respectively) in wild-type mice, whereas levels of NCT and PS1-NTF were relatively consistent (Fig. 3a, Extended Data Fig. 3a). Furthermore, the subcellular distribution of IFITM3 and γ-secretase components does not change with age (Extended Data Fig. 3a). IFITM3 modulates γ-secretase activity

IFITM3 was knocked down by short interfering RNA (siRNA) in human embryonic kidney (HEK) cells stably expressed with amyloid precursor protein (HEK-APP) (Fig. 2a). Knock down of IFITM3 did not affect the levels of NCT, PS1-NTF and APP proteins (Fig. 2a, Extended Data Fig. 2a) but decreased the production of Aβ1-40 and Aβ1-42 by 17.1 ± 2.4% and 24.6 ± 1.3% (mean ± s.d.), respectively, compared to transfection with scrambled control siRNA (Fig. 2b). Next, IFITM3 was knocked out in U138 astrocytoma cells (Fig. 2c, lanes 3 and 4) and then reintroduced by transient overexpression (Fig. 2c, lanes 2 and 4). γ-secretase activity was determined using a recombinant APP substrate (Extended Data Fig. 2b). IFITM3 knockdown reduced γ-secretase activity for the production of Aβ1-40 and Aβ1-42 as compared to the empty vector guide RNA cell line by 36% and 27%, respectively (Fig. 2d). Notably, overexpression of IFITM3 in the knockout cell line rescued the loss of γ-secretase activity (Fig. 2d). Transient overexpression of IFITM3 in the empty vector cell line (Fig. 2c) significantly increased γ-secretase activity as measured by cleavage of recombinant APP substrate to Aβ1-40 and Aβ1-42 (Fig. 2d). Together, the level of IFITM3 is associated with increased γ-secretase activity leading to the cleavage of amyloid-β.

Next, we determined how IFITM3 affects the potency and selectivity of GSs and the GSI L458, which bind to the different sites of γ-secretase' (Extended Data Fig. 2c). E2012-BPyn selectivity (Aβ1-40/Aβ1-42) is 21-fold in γ-secretase from empty vector membranes compared to 57-fold in knockout membranes (Fig. 2e). Knockout of IFITM3 had an opposite effect on two types (acid versus imidazole) of GSI and no effect on L458 (Extended Data Fig. 2d–f), indicating that IFITM3 not only affects γ-secretase activity, but also alters the selectivity to GSs. Notably, knockdown (Extended Data Fig. 2g, h) and knock-out of IFITM3 (Extended Data Fig. 2j) increased γ-secretase activity and resultant NOTCH cleavage in cell-based assays (Extended Data Fig. 2h, i) and γ-secretase in vitro activity assay (Extended Data Fig. 2j), demonstrating that IFITM3 exhibits distinct modulatory effects on γ-secretase processing of APP and NOTCH.

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IFITM3 and γ-secretase activity in patients with Alzheimer’s

Analysis of gene expression patterns in the Mayo Clinic large cohort dataset revealed that IFITM3 expression is significantly higher in patients with Alzheimer’s disease than in controls in the temporal cortex (Fig. 4a). Moreover, the expression of IFITM3 is positively correlated with age in both the cortex and hippocampus of human brains in the Genotype-Tissue Expression (GTEx) cohort (Extended Data Fig. 4a). Next, the expression of IFITM3 in the frontal cortex of post-mortem tissues from human patients with LOAD (n = 18) and non-demented control participants (n = 10) (Supplementary Table 1) was examined. The mRNA level of IFITM3 was higher in LOAD samples than in controls (Fig. 4b). Notably, the astrocyte marker GFAP was also increased in LOAD samples, whereas levels of the neuronal marker MAP2 and microglia marker AIF1 were similar to control (Extended Data Fig. 4b), which is consistent with data from the Mayo Clinic large cohort dataset (Extended Data Fig. 4c). Protein levels of NCT and PS1-NTF were higher in the control than in LOAD samples (Extended Data Fig. 4d). IFITM3 protein is more highly expressed in LOAD samples, and although expression is similar throughout the control brains, there is large variation between LOAD samples (Fig. 4c). Because levels of IFITM3 are associated with γ-secretase activity, and IFITM3 protein is significantly increased in some patients with LOAD (Fig. 4c), LOAD brain samples were divided into two groups. Samples with IFITM3 levels greater than two standard deviations from the mean of control were defined as LOAD-high (LOAD-H, 8 patients), whereas the remaining samples were defined as LOAD-low (LOAD-L, 10 patients) (Fig. 4d, left, Supplementary Table 1). Notably, LOAD-L samples have similar levels of γ-secretase activity as the control brains, whereas LOAD-H samples exhibit 127.6% and 133.6% activity compared with control samples for Aβ40 and Aβ42, respectively (Fig. 4d, right). In addition, the expression of IFITM3 was correlated with amyloid-β load in the brain regions Brodmann area 22 (BA-22) (rho = 0.149, P = 0.0497) and BA-36 (rho = 0.170, P = 0.0204) in the Mount Sinai Brain Bank dataset (Supplementary Table 2). These findings demonstrate that there is an association between IFITM3 protein and γ-secretase activity for amyloid-β production and suggest that IFITM3 contributes to amyloid-β production in a subset of patients with LOAD.

Single nucleotide polymorphisms (SNPs) in IFITM3 have been associated with the severity of viral infection and gene expression level. We performed genotype analysis for IFITM3SNP variants rs12252 and rs34481144 in the human samples (Extended Data Fig. 4e, f, Supplementary Table 3). R analysis indicates that T allele dosage for rs34481144 does not affect the relationship between Alzheimer’s disease status (control, LOAD-L, LOAD-H) and IFITM3 protein expression in this sample set.

Induction of IFITM3 in neurons and astrocytes

The expression of IFITM3 is detected in mouse hippocampal neurons, human induced pluripotent stem (iPS) cell-derived neurons and primary human astrocytes (Extended Data Fig. 4g–j). The effect of interferon-γ (IFNγ) on IFITM3 and γ-secretase in mouse primary neuronal culture was investigated. IFNγ (at 10 and 100 ng ml⁻¹) significantly induced expression of IFITM3 but had little effect on NCT and PS1-NTF (Fig. 4e). Furthermore, treatment with IFNγ led to an increase in secreted levels of Aβ40 and Aβ42 (Fig. 4f), increasing the γ-secretase cleavage activity for Aβ40 and Aβ42 by 1.69- and 1.7-fold, respectively (Fig. 4g), and increased photolabelling of active γ-secretase (Fig. 4h). IFNα exhibited similar effects to IFNγ (Extended Data Fig. 4i–k). Because astrocytes are another source of amyloid-β, dissociated human astrocytes (hASTs) were treated with IL-6 and IL-1β, which also upregulated IFITM3 expression (Fig. 4i), and increased γ-secretase activity for Aβ40 and Aβ42 cleavages (Fig. 4j).
The association of IFITM3 with cytokines is supported by gene expression data from the Harvard Brain Tissue Resource Center, in which there is a correlation between transcription of ITIM3 and IL6, IL1B, IL10 and IL8 and type I IFN responsive genes (ITIM3 and ITIM2) (Supplementary Tables 4, 5). Furthermore, the expression level of ITIM3 is positively correlated with the expression level of the human herpes virus 6B (HHV-6B, rho = 0.248, P = 0.0014, male: **P = 0.0002, human herpes virus 6B (HHV-6B, rho = 0.248, **P = 0.0002, male: **P = 0.0002, female: ***P = 0.0001) in the Mount Sinai Brain Bank (MSBB) dataset 18 (Extended Data Fig. 4n), which suggests that cytokine induction of ITIM3 could be part of the immune response to such viruses.

**IFITM3 is near the active site of γ-secretase**

To examine whether ITIM3 is near the catalytic site of γ-secretase (Fig. 5a), four active site-directed photoaffinity inhibitors (Extended Data Fig. 5a) were used. Because all four probes specifically labelled PS1-NTF and ITIM3 (Fig. 5b), we conclude that ITIM3 interacts with γ-secretase near the active site. In addition, 11b that was used to label the docking site of γ-secretase was blocked by pep11, but not by E2012, which suggests that the E2012 binding site does not overlap with the docking site (Extended Data Fig. 5b).

Next, membranes isolated from human samples were labelled with L505, LS505 labelled both PS1-NTF and ITIM3 (Fig. 5c), indicating that ITIM3 is engaged in the γ-secretase complex in human brains as well. There was a positive correlation between the LS505-labelled PS1-NTF and γ-secretase activity for Aβ cleavage (R = 0.67) and Aβ cleavage (R = 0.66) (Extended Data Fig. 5c). Furthermore, the correlation coefficients between the LS505-labelled ITIM3 and γ-secretase activity for Aβ cleavage and Aβ cleavage were 0.71 and 0.73, respectively (Fig. 5d). Together, the strong correlations between the LS505-labelled ITIM3 and γ-secretase activity in LOAD brains highlights ITIM3 as a marker of γ-secretase activity.

To investigate whether ITIM3 is cross-linked with PS1, we used L631, which contains two photoreactive groups allowing for crosslinking of two labelled species (Fig. 5e). L631 labels three species: PS1-NTF, PS1 C-terminal fragment (PS1-CTF) and cross-linked PS1-NTF–PS1-CTF heterodimer (Fig. 5e). When cross-linked species were probed by an anti-ITIM3 antibody, three species were detected that co-migrate at molecular masses of approximately 15, 30 and 45 kDa (Fig. 5e), corresponding to ITIM3 monomer, dimer and crosslinked ITIM3–PS1-NTF (recognized by both ITIM3 and PS1-NTF antibodies), respectively. Although ITIM3 dimer has been observed, whether ITIM3 exists as monomer or dimer needs to be determined by structural studies.
The crosslinking of IFITM3 and PSI-NTF by L631 further confirms that IFITM3 binds near the active site of γ-secretase (Fig. 5f).

**Discussion**

Our work identifies IFITM3 as a γ-secretase modulatory protein associated with ageing and Alzheimer’s disease, and directly connects the production of amyloid-β with an emerging field in Alzheimer’s research: innate immunity and neuroinflammation. IFITM3 has a broad spectrum of antiviral activities and serves as the first line of defence against infection. IFITM3 is basally expressed in many cells and highly upregulated by both type I and II IFNs. IFITM3 has also previously been suggested as one of hundreds of potential PS1- and PS2-interacting proteins. However, whether IFITM3 regulates γ-secretase and is involved in Alzheimer’s disease was previously unknown.

Neuroinflammation has emerged as a crucial component of Alzheimer’s disease pathogenesis. Several studies have shown that TREM2 and CD33 regulate microglia-mediated clearance and uptake of amyloid-β. By contrast, our work reveals that neuroinflammation can also contribute to the production of amyloid-β through an IFITM3–γ-secretase complex. It has been reported that ageing—the biggest risk factor for Alzheimer’s disease—induces type I IFNs that modulate brain function. Therefore, neuroinflammation caused by ageing could lead to an increase in the level of IFITM3, which increases γ-secretase activity and resultant production of amyloid-β production in humans.

Increasing evidence indicates that amyloid-β peptides have antimicrobial and antiviral activities as part of the innate immune response in the brain. This raises the possibility that pathogenic challenge stimulates amyloid-β production as part of an innate immune response, and a secondary consequence is Alzheimer’s disease risk. Discovery that IFITM3 upregulates γ-secretase activity for amyloid-β production offers a mechanism as to how amyloid-β production is regulated in an innate immune response. Pathogenic challenge results in the production of cytokines by microglia, astrocytes and possibly other cell types. Pro-inflammatory cytokines induce the expression of IFITM3, which in turn augments γ-secretase in neurons and astrocytes, resulting in increased secretion of amyloid-β. This has two consequences: anti-infection, and an increased risk for Alzheimer’s disease.
IFITM3 and γ-secretase; IFITM3 is near the active site and can be crosslinked with PS1-NTF. g. IFITM3 connects infections and innate immunity with production of Aβ and Alzheimer’s disease risk. Left, pathogenic challenge, or other inflammatory conditions, induce the release of proinflammatory cytokines from astrocytes and microglia. Middle, cytokines upregulate γ-secretase expression in neurons and astrocytes that potentiates γ-secretase, increasing Aβ production. Right, part of an innate immune response, Aβ acts as an antimicrobial or antiviral peptide. In turn, Aβ accumulation also triggers Alzheimer’s disease pathology. All western blotting images and graphs are representative of three independent experiments (except e, which denotes two replicates).

Online content
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3. De Strooper, B. Aph-1, Pen-2, and Nicastrin with Presenilin generate an active γ-secretase activity (Fig. 4d) and L505-labelled IFITM3 (Fig. 5). Double cross-linking of γ-secretase and IFITM3 by the dual probe, L631. Western blotting analysis reveals several protein complex species containing IFITM3, PS1-NTF, PS1-CTF, IFITM3 homodimer (IFITM3), IFITM3 and PS1-NTF or PS1-NTF/PS1-CTF heterodimers. f. Schematic representation of the interaction between suggests that IFITM3 could be an indicator of γ-secretase activity in patients with LOAD. Importantly, the differential level of IFITM3 protein within patients with LOAD suggests that IFITM3 could potentially serve as a biomarker and therapeutic target for a specific subpopulation of patients LOAD. LOAD is a multifactorial disease; therefore, identification of subpopulations of individuals with Alzheimer’s disease will aid in studying the underlying mechanisms and developing precision medicine treatments.
were assayed on day 14.

in each well was replaced every other day. hNGN2-induced neurons

of any non-neuronal cells. Beginning at day 4, half of the medium

Sigma-Aldrich) was added for 48 h to the medium to inhibit prolifera-

DMEM/F12 medium supplemented with N2/B27/Glutamax (Invit-

On day 2, the culture medium was replaced by neuron medium:

3–4 h, the medium was replaced with fresh NPC medium. Doxycycline

genus at 1 × 10^6 pfu/ml per plasmid (multiplicity of infection (MOI) of 2)

P2A-PuroR (79049, Addgene) with CAGGs-rtTA lentivirus

(3803, ScienCell). hASTs were confirmed by immunofluorescence stain-

supplement (1852, ScienCell) and 1% penicillin/streptomycin solution

containing 2% fetal bovine serum (0010, ScienCell), 1% astrocyte growth

cells. hASTs were cultured in basal astrocyte medium (1800), cells were isolated from cerebral cortex and cryopreserved

from E. Chen) and HEK293-Notch∆E cells (a gift from D. S. Johnson);

The following cells were cultured in DMEM medium with high glucose,

penicillin, streptomycin and 10% FBS: MEF (wild type (WT) and PS1/PS2
double knockout (DKO), a gift from H. Xu); HEK293-APP695 cells (a gift

E. Chen) and HEK293-Notch∆E cells (a gift from D. S. Johnson);

U138 cells (U-138 MG human grade IV glioblastoma cells, HTB-16, ATCC). All
cells were cultured at 37 °C with 5% CO₂/95% air. All human cell lines
were authenticated by STR profile report and all cell lines were tested
negative for mycoplasma contamination by PCR mycoplasma detec-
tion kit (G238, Applied Biological Materials).

Neuronal culture. Mouse embryos at embryonic day 16 (E16) were
isolated from WT C57BL/6j or C57BL/6n mice as described previously.
In brief, cortices were collected from dissected brains and meninges
were removed. Cortices were trypsinized and cell were dissociated
and plated onto PDL/PLL (Sigma) coated chamber slides (Corning)
or cell culture plates. Primary mouse neurons were seeded in seeding
medium (neurobasal medium/L-glutamax/10% FBS). At day 1 in vitro
(DIV1), cell culture medium was changed for 100% to NBM complete
medium (neurobasal medium/L-glutamax/B-27/N2/FUD) and medium
was changed for 50% thereafter.

Human primary astrocytes. hASTs were obtained from ScienCell
(1800), cells were isolated from cerebral cortex and cryopreserved
hASTs. hASTs were cultured in basal astrocyte medium (1801, ScienCell)
containing 2% fetal bovine serum (0010, ScienCell), 1% astrocyte growth
supplement (1852, ScienCell) and 1% penicillin/streptomycin solution
(0503, ScienCell). hASTs were confirmed by immunofluorescence stain-
ing with GFAP and S100β (data not shown).

Generation of iNeurons from human IPS cells. Homogeneous
population of excitatory neurons were generated from neural pro-
genitor cells (NPC) derived from human IPS cells. NPCs were dis-
associated with accutase (Innovative Cell Technologies) and plated at
250,000 cells per well of a 24-well plate on day 2. Cells were plated on
Matrigel (BD Biosciences)-coated coverslips or plates in NPC medium
(DMEM/F12/N2/B27/FGF2, Invitrogen). On day 1, hNGN2 lentivirus
infection was started (1 μg ml⁻¹) and the infection was continued
with GFAP and S100β (data not shown).

Mice
All procedures were carried out in accordance with the National Insti-
tutes of Health ‘Guide for Care and Use of Laboratory Animals’ and
were approved by Research Animal Resource Center of Memorial Sloan Kett-
ting Cancer Center and the Animal Care and Use Committees of the
Rockefeller University. B6.CgTg(APPSwFlb/LwJ, PSEN1*M146L*L286V)679
9Vas/Mmjjax (5xFAD) mice were obtained from the Jackson Laboratory.
5xFAD mice were maintained as hemizygous by breeding with C57BL/6j.
Frozen Ifitm3−/− embryos were obtained from the European Mutant
Mouse Archive and rederived in pseudopregnant host mice. Ifitm3−/−
mice were bred with 5xFAD mice to generate Ifitm3−/−;5xFAD mice. All
mice were genotyped by PCR amplification of DNA extracted from tail.

For primary neuronal culture, wild type C57BL/6j or C57BL/6n mice
with timed pregnancy were obtained from the Jackson Laboratory and
Charles River Laboratories, respectively. Translating ribosome
affinity purification (TRAP) knock-in mice were generated by crossing
mice bearing a loxP-stop-loxP-EGFPRL10a sequence in the Ef1α1 pro-
moter (EF1α1–1SL.EGFPPL10a) with CCK-Cre (CCKtm1.1( cre)Zjh/J), glutamate
decarboxylase 2 (65 kDa)-Cre (B6N.Cg-Gad2tm1.1( cre)Zjh/J) and cortistatin-Cre
(Tg(Cort−tm1.1)M42Gsat) or parvalbumin-Cre (Pvalb−tm1.1( cre)Zjh/J) mice. RNA
isoform and qPCR analysis were done as previously described.

Mouse brain samples
Brains of C57BL/6 female and male mice at different age groups (mature
adult (4-month-old) and old (28-month-old)) were collected from NIA
Aged Rodent Tissue Bank and stored at −80 °C before use. Brains of
female wild-type and 5xFAD transgenic mice at 3 months (mature adult)
and 12 months (middle aged) of age were provided by R. J. Vassar. Brains
were extracted from wild-type and Ifitm3−/− at 18 months of age, and the
cortex was then dissected and flash frozen, and stored at −80 °C before
use. Cortical tissue from 4-month-old 5xFAD and Ifitm3−/−; 5xFAD mice was
extracted in the same way.

Human brain samples
Frontal cortices of human brains from control participants and patients
with LOAD were provided by the UCSD ADRC Neuropathology Core
after review and approval by the ADRC Biospecimen Review Committee.
All human samples were obtained from participants who consented to
autopsy for the UCSD ADRC. All tissue samples were de-identified and
contained a number code only.

LOAD samples included 6 females and 11 males with an average age
of 81 years, and controls included 7 females and 3 males, with an average
age of 83.2 years (Supplementary Table 1). On the basis of the quantifi-
cation of IFITM3 protein levels by western blotting, LOAD brain samples
were separated into two groups: LOAD-L (low, 10 patients) and LOAD-H
(high, 8 patients). Samples with IFITM3 levels greater than two standard
deviations from the mean of control samples were defined as LOAD-H,
and the remaining samples were defined as LOAD-L.

Human IFITM3 SNP genotyping
We determined whether IFITM3 SNP affect IFITM3 protein expression
levels in human brain samples (control, LOAD-L, and LOAD-H groups).
In brief, genomic DNA was extracted from frozen post mortem human
brain tissue using the KingFisher Flex System (ThermoFisher Scientific)
according to manufacturer’s instructions. TaqMan SNP genotyping
assays (ThermoFisher Scientific) were used to interrogate the human
IFITM3 SNPs rs12252 (C_175677529_10) and rs34481144 (C_26288451_10)
on the QuantStudio 7 Flex Real-Time PCR System (ThermoFisher Sci-
fientific) according to manufacturer’s instructions. Molecular grade
water and a standard in-house DNA sample were used as a non-template
control and positive control, respectively. Genotype calls and allelic
discrimination plots were generated using QuantStudio Software v.1.1
(ThermoFisher Scientific). Genotyping data were reported in REF/ ALT
format, which is consistent with the human reference genome.
The effect of SNP allele dosage on the relationship between Alzheimer’s disease status and IFITM3 protein expression levels was analysed using the lm() function in R (v.3.6.2)44. All samples have the same A/A genotype by IFITM3 SNP rs12252 genotyping, indicating that this SNP does not influence levels of IFITM3 protein expression in our cohort (Supplementary Table 3). The allelic discrimination plot for rs34481144 showed control contains homozygous T/T (equivalent to A/A on the negative strand of DNA) and heterozygous C/T (G/A) while LOAD-L and LOAD-H are homozygous C/C (G/G), heterozygous C/T (G/A), and homozygous T/T (A/A) (Extended Data Fig. 4e). Allele frequency of C and T in control and LOAD was calculated (Extended Data Fig. 4f). R analysis indicates T allele dosage for rs34481144 does not affect the relationship between Alzheimer’s disease status (control, LOAD-L, LOAD-H) and IFITM3 protein expression in our sample set.

**Photolabelling with clickable probe, LC–MS/MS and protein identification**

Photolabelling with E2012-Bpyne was prepared as previously described11,38. In brief, cell membranes were incubated with clickable probe, E2012 Bpyne in the absence or presence of the indicated compounds (GSMs or GSIs). Samples were cross-linked with 5 μg of purified PSI-NTF antibody, anti-IFITM3 antibody, monoclonal IFITM3 antibody (9D11, generated in our lab), ImmunoPure rabbit IgG (Pierce) or mouse IgG isotype control (Invitrogen) for overnight at 4 °C with rotation. Beads were added to samples for 1 h at room temperature with rotation. Beads were washed three times with 1 ml of immunoprecipitation buffer containing 1% CHAPS and the beads were eluted in 30 μl of SDS–PAGE sample buffer with DTT for 10 min at room temperature. The eluted samples were subjected to SDS–PAGE and western blotting (for source gels see Supplementary Fig. 1).

**Preparation of membranes and solubilization**

HeLa cell membranes (membrane fraction) were prepared from the HeLa cell pellet (BioVest International). All procedures were carried out on ice. MEF (wild-type and PSI/PS2 double knockout) cell pellets were resuspended in 5 mM Tris, pH 7.4, protease inhibitor cocktail, and PMSF, and incubated on ice for 1 h. After homogenization with a mechanical pestle homogenizer by 20 strokes, cells were centrifuged at 1,000 g for 30 min and the post-nuclear supernatants were centrifuged at 100,000 g for 1 h to yield the pellet. The pellet was washed with 1× MES (pH 6.0), protease inhibitor, and PMSF and the final pellet was collected after 10,000 g for 1 h, resuspended in buffer, and stored at ~80 °C. Brain tissue membranes were prepared as described previously with some modifications48. Whole brains (or dissected cortices) were homogenized by using TissueLyser II (Qiagen). In brief, brains were placed with 5 mm stainless steel bead in lysis buffer and homogenized by high-speed shaking at 30 per s for 2 min. Cell and tissue membranes were solubilized with 1% SDS and protein concentrations were determined by the Lowry protein assay. HeLa cell membranes were solubilized as described previously41,47,48.

**Capture of γ-secretase complex**

CHAPSO solubilized γ-secretase was pre-incubated with parent compounds (L458 or BMS708163) at 37 °C for 20 min in the buffer containing 0.25% CHAPSO, followed by the capture with GY6 or 163-BP-L-biotin at 37 °C for 1 h. Washed streptavidin beads were added and incubated with 4 °C for overnight. Beads were washed with 0.25% CHAPSO and eluted in SDS sample buffer and analysed by western blotting.

**Co-immunoprecipitation**

CHAPSO solubilized HeLa cell membranes (400 μg of protein per sample) were immunoprecipitated by anti-PS1-NTF or anti-IFITM3 antibody in immunoprecipitation buffer (20 mM HEPES (pH 7.5), 50 mM KCl, 2 mM EGTA, 0.25% CHAPSO and protease inhibitor mixture)44,49. In brief, protein A/G magnetic beads (Pierce) were washed then added to samples for 30 min at 4 °C with rotation as a pre-clear step. The supernatant from samples was incubated with 5 μg of purified PSI-NTF antibody, IFITM3 antibody, monoclonal IFITM3 antibody (9D11, generated in our lab), ImmunoPure rabbit IgG (Pierce) or mouse IgG isotype control (Invitrogen) for overnight at 4 °C with rotation. Beads were added to samples for 1 h at room temperature with rotation. Beads were washed three times with 1 ml of immunoprecipitation buffer containing 1% CHAPS and the beads were eluted in 30 μl of SDS–PAGE sample buffer with DTT for 10 min at room temperature. The eluted samples were subjected to SDS–PAGE and western blotting (for source gels see Supplementary Fig. 1).

**Subcellular fractionation**

Male wild-type C57BL/6 mouse hemibrains at 4 and 28 months were homogenized and layered on iodixanol gradient for subcellular fractionations as previously described40 with some modifications. In brief, brains were homogenized by 20 strokes on ice using a mechanical pestle homogenizer (Eberbach Corporation), followed by 10 passages through a 21-gauge needle (BD). Homogenates were centrifuged at 1,000 g for 10 min then the post-nuclear supernatant was centrifuged at 10,000 g for 15 min. The supernatant was layered on the top of 2.5–30% (w/v) iodixanol gradients (OptiPrep density gradient medium, Sigma-Aldrich) and were centrifuged at 126,000 g for 3 h (TH-641 rotor, Thermo Scientific). All centrifugation procedures were carried out at 4 °C. One-millilitre fractions were collected from the top and γ-secretase, IFITM3 and different subcellular markers were analysed by SDS–PAGE and western blotting (for source gels see Supplementary Fig. 1).

**Cytokine induction in neurons and astrocytes**

Primary mouse neuronal culture was treated with IFNγ (R&D Systems, 485-MI-100/CF) or IFNαx (R&D Systems, 12100-1) at the final concentrations of 10 ng ml⁻¹ and/or 100 ng ml⁻¹ was added to neuronal culture at DIV12 for 24 h. hAST cultures were treated with PBS, 1ng ml⁻¹ ‘IL-6 (R&D Systems, 206-IL-010/CF) or 10 ng ml⁻¹ ‘IL-1β (R&D Systems, 201-LB-005) for 48 h. After cytokine treatment, mouse neurons and hAST were either solubilized with RIPA buffer for western blotting or membranes were extracted for γ-secretase activity assay.

**Western blot analysis**

Protein samples were subjected to SDS–PAGE gels (Bio-Rad), electrophoresed, transferred to Immobilon-P PVDF (Millipore) or Immobilon-FL PVDF membranes (Millipore) and western blotting. The following primary antibodies were used: PSI-NTF (a gift from Merck Research Laboratories); PS1-CTF (MAB5232, Millipore); PS2-CTF (19871-1, Epitomics); nicastrin was generated in our laboratory; APH-1αL (38-3600, Invitrogen); PEN-2 (ab18189, Abcam); human IFITM3 (anti-Fragilis, ab109429, Abcam); mouse IFITM3 (anti-Fragilis, ab15592, Abcam); APP (22C11, MAB348, Millipore); SPP (317) was generated in our laboratory; cleaved Notch1 (Val1744), Cell Signaling Technology and SM320, generated in our laboratory; c-Myc (9E10, Roche Life Science); N-cadherin (D4R1H, I3116, Cell Signaling Technology); RAB7 (B-3, sc-376362, Santa Cruz Biotechnology); EEA1 (Ab2900, Abcam); β-actin (C4, sc-7778, Santa Cruz Biotechnology); β-tubulin III (T8578, Sigma-Aldrich); tubulin (ab56676, Abcam), Horseradish...
peroxidase (HRP)-conjugated anti-rabbit and mouse secondary antibodies (NA9340V, NA9314V, GE Healthcare) for ECL substrate (Pierce) and IRDye 800CW goat anti-rabbit IgG (H+L) or anti-mouse IgG (H+L) secondary antibodies (925-3221I, 925-32210, LI-COR) for Odyssey CLx Imaging (LI-COR) were used. For quantification, ImageJ and Image Studio Lite (LI-COR) were used, respectively (for source gels see Supplementary Fig. 1).

RNA interference
Silencer Select pre-designed siRNA oligonucleotides targeting IFITM3 were purchased from Ambion and tested: siRNA id s195033, s195034 and s195035 (sense sequence 5′-CCCAAGGUCCACACUCCU-3′ and antisense sequence 5′-GGGAAGUGAGAACUGUGGat-3′). IFITM3 siRNA (s195035) and scrambled siRNA (negative control) were used. siRNAs were transfected to HEK293-APP695 cells in triplicates as previously described. The conditioned medium was measured for secreted levels of Aβ40 and Aβ42, measured by Aβ peptide multiplex kits (6E10, MSD) according to manufacturer's instructions. γ-Secretase activity was shown in pg/mg lysate.

CRISPR–Cas9n and rescue
CRISPR constructs were purchased from the RNAi core at Memorial Sloan Kettering Cancer Center and purified with the HiSpeed Maxiprep kit (Qiagen). U138 cells were transfected with Lipofectamine LTX according to the manufacturer's instructions with px459 vector containing Cas9 and guide RNAs (either an empty vector or one of three IFITM3 guides). Transfected cells were incubated for 48 h before puromycin selection. Single cells were extracted and expanded. IFITM3 knockout was confirmed by sequencing and western blotting.

Plasmid DNAs for human IFITM3 (OriGene) were cloned into the pcDNA3.1 vector. U138 EV and U138/Ifitm3−/− cells were transfected with either pcDNA3.1 empty vector or pcDNA3.1 IFITM3 construct using Lipofectamine LTX according to the manufacturer's instructions and allowed to incubate for 48 h before membrane preparation.

Measurement of secreted Aβ levels
To measure γ-secretase activity in cells, cell culture medium was collected and levels of Aβ40 and Aβ42 were measured using Aβ peptide multiplex kits (4G8, MSD) according to the manufacturer's instructions. γ-secretase activity (secreted Aβ) was shown as the percentage of control or pg per mg lysate.

In vitro γ-secretase activity assay (Aβ40 and Aβ42 cleavage rate)
In vitro γ-secretase activity assay (Aβ40 and Aβ42 cleavage rate) was carried as previously described. In brief, to detect production of Aβ by the cleavage of γ-secretase, cells were lysed with 0.25% CHAPSO and incubated with the recombinant APP substrate Sb4 at 37 °C for 3 h. Lysates (20 μl) were incubated with 20 μl detection buffer (for Aβ40: anti-mouse IgG AlphaLISA acceptor beads (PerkinElmer), G2-10 antibody and streptavidin-conjugated Alpha donor beads (PerkinElmer), for Aβ42: Protein A AlphaLISA acceptor beads (PerkinElmer), 10G-3 antibody and streptavidin-conjugated Alpha donor beads (PerkinElmer)) overnight at room temperature. For solubilized γ-secretase, 0.25% CHAPSO solubilized membranes from cells (U138 empty vector and knockout cells, and hAST) and mouse brain tissues (wild-type, Ifitm3−/−, SxFAD and Ifitm3−/−;SxFAD) were used to incubate with a recombinant APP substrate. Aβ40 and Aβ42 levels were measured as AlphaLISA signals by EnVision Plate Reader (PerkinElmer). γ-secretase activity (the Aβ cleavage rate) was shown in AlphaLISA units per min per mg of protein (AU/min, mg) for U138 empty vector and knockout cells or normalized units (normalized to the average of wild-type for 18-month-old wild-type and Ifitm3−/−, to the average of SxFAD for 4-month-old SxFAD and Ifitm3−/−;SxFAD, and to the average of PBS treatment for PBS, IL-6 and IL-1β treated hAST cells). To measure IC50 values for Aβ cleavage, in vitro γ-secretase activity assay was carried out in the absence or presence of GSIs or GSIs with different dilutions.

To measure γ-secretase activity in 4- and 28-month-old C57BL/6 mouse brains, human brains, and primary neuronal culture, membranes were incubated in the same conditions as above except that C100-ΔID-Flag was used as a substrate. Aβ40 and Aβ42 levels were measured by Aβ peptide multiplex kits (6E10, MSD) according to the manufacturer's instructions. γ-Secretase activity was shown in pg/mg of protein.

In vitro γ-secretase activity assay
In vitro γ-secretase activity assay (NICTD production level) was carried as previously described. In brief, to detect NICTD production by the cleavage of γ-secretase, HEK293-NotchΔE cells were lysed with 0.25% CHAPSO after siRNA transfection. Five microlitres of lysates were incubated with 20 μl detection buffer (protein A-conjugated acceptor beads (PerkinElmer), NICTD antibody (SM320), 9E10 anti-human c-myc conjugated to biotin antibody and streptavidin-conjugated donor beads (PerkinElmer)) overnight at room temperature. NICTD levels were measured as AlphalISA signals by EnVision Plate Reader (PerkinElmer).

To detect NICTD production by the cleavage of γ-secretase in IFITM3-knockout cells, cell membranes were lysed with 0.25% CHAPSO and incubated with a recombinant Notch substrate, N1Sβ1, at 37 °C for 2 h. Lysates (20 μl) were incubated with 20 μl detection buffer (protein A-conjugated acceptor beads (PerkinElmer), NICTD antibody (SM320, generated in our laboratory) and streptavidin-conjugated donor beads (PerkinElmer)) overnight at room temperature. NICTD levels were measured as described above.

Gene expression analysis
To measure mRNA expression levels of genes (IFITM3, MAP2, GFAP and AIF1) in LOAD and control samples, RNA was extracted from human brain tissues using QIAzol Lysis Reagent (Qiagen) and RNAeasy Mini kit (Qiagen). RNA concentrations were measured by using NanoDrop 8000 spectrophotometer (Thermo Scientific) and cDNAs were synthesized by reverse transcription (High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor, Life Technologies). cDNA was mixed with TaqMan assays for human IFITM3 (Hs04194512_g1, Life Technologies), MAP2 (Hs00258900_m1, Life Technologies), GFAP (Hs00909233_m1, Life Technologies) and AIF1 (Hs00610419 g1, Life Technologies) in TaqMan Universal master mix II (Applied Biosystems). GAPDH (Hs04420632 _g1, Life Technologies) was used as a reference gene.

To measure IFITM3 gene expression in human IPS cell-derived neurons, human primary astrocyte cultures, and MEFs (WT and PS1/PS2 double knockout), RNA was extracted from cells with the NEasy mini kit (Qiagen) with on-column DNase digestion (Qiagen) and cDNA was synthesized by reverse transcription according to the manufacturer’s instructions (iScript cDNA Synthesis kit, Bio-Rad). Total gene expression levels were measured by real-time PCR (ABI7900HT Fast Real-Time PCR System or 7500 Fast Real-Time PCR System, Applied Biosystems). The comparative C method was used to calculate total mRNA expression levels of genes. Gene expression levels were normalized to GAPDH. For ME cells, mouse ifitm3 (Mm00847057_m1, Life Technologies) and Gapdh (Mm99999915_g1, Life Technologies) probes were used.

Alzheimer’s disease datasets
To understand the role of IFITM3 in the pathogenesis, we examined the expression of IFITM3 mRNA in Alzheimer’s disease and correlations between IFITM3 and genes known to be involved in disease progression.
in five transcriptomic datasets from three large cohorts studying Alzheimer’s disease. The three cohorts include: (1) the MSBB, which contains two brain regions: superior temporal gyrus (BA-22) and entorial area (BA-36); (2) the Harvard Brain Tissue Resource Center, which contains two brain regions: prefrontal cortex and visual cortex; and (3) the Mayo clinic cohort which contains one brain region: temporal cortex. The disease status of each sample was defined by the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD).

Differential gene expression and correlation analyses

We performed differential gene expression analysis between Alzheimer’s disease and control samples using R package limma (v.3.34.0) with default settings. Multiple tests were adjusted using the Benjamini–Hochberg’s false discovery rate method. Genes with a false discovery rate of less than 0.05 and fold change greater than 1.2 were considered significant.

Correlation analysis of IFITM3 and age

Correlation analyses of IFITM3 and age in cortex and hippocampus were performed based on the GTEx brain data. GTEx brain RNA-seq data were downloaded from the GTEx portal. Lowly expressed genes with expression levels at least 1 count per million in less than 20% of samples were removed. A total of 16,494 genes were included in the correlation analysis. Expression data were then normalized, log10-transformed and corrected for race and BMI. Spearman correlation coefficients and P-values were calculated between expression levels and age. Adjusted P-values were calculated based on a null distribution from 1,000 permutations.

Correlation analysis of IFITM3 and viruses

To study the effect of viruses on the expression level of IFITM3 in Alzheimer’s disease, we used the virus data from a previous study. Raw reads were normalized by library size of each sample, and further corrected for covariants (that is, age of death, race, RNA integrity number, sex, batch and post mortem interval), based on a linear regression model. Spearman correlation coefficients and P-values were calculated between expression levels of IFITM3 and HHV-6B or hepatitis C virus genotype 4, and nominal P-values were reported.

Immunofluorescence staining and microscopy

Proximity ligation assay. Interaction of PSI and IFITM3 was determined by proximity ligation assay in mouse primary neurons. Primary neurons at DIV13 were permeabilized in 0.4% CHAPS and proximity ligation assay (Sigma-Aldrich) was performed according to manufacturer’s instructions. After final washes, neurons were stained with Alexa Fluor 488 phalloidin (Invitrogen) for F-actin, mounted with a mounting medium containing DAPI (Invitrogen) as described and PLA signals were imaged by Axio Imager Z2 (ZEISS).

Human iPS cell-derived neurons and human primary astrocytes staining. Human iPS cell-derived neurons and human primary astrocytes at passage 4 (ScienCell, 1800) were fixed using 4% PFA (Electron Microscopy Sciences) or 10% formalin (Sigma-Aldrich), permeabilized with 0.01% Triton X-100, blocked with 10% donkey serum (Sigma-Aldrich) for 1 h, and incubated with primary antibodies (MAP2 (Sigma-Aldrich), S100β (Sigma-Aldrich)) for overnight at 4 °C and Alexa Fluor 488 and 594 conjugated secondary antibodies (Life Technologies) for 1–2 h at room temperature. Cells were counterstained using DAPI (Invitrogen), mounted with AquaPolymount mounting solution (Polysciences), and imaged with EVOS microscope.

Mouse perfusion. 12-month-old wild-type and 5xFAD mice and 4-month-old 5xFAD and Ifitm3−/−;5xFAD mice were anesthetized with a single dose of ketamine or xylazine (100 mg kg−1 or 5 mg kg−1, respectively and transcardially perfused with 50 ml of PBS followed by 50 ml of 4% PFA. Brains were removed and post fixed in 4% PFA overnight at 4 °C.

IFITM3 staining. 12-month-old wild-type and 5xFAD samples were then processed for paraffin embedding with tissue processor (Leica Biosystems, ASP6025) and 8-μm paraffin sections were obtained and mounted on slides for immunohistochemistry. The tissue sections were deparaffinized with EZPrep buffer (Ventana Medical Systems), antigen retrieval was performed with CC1 buffer (Ventana Medical Systems). Sections were blocked for 30 min with Background Buster solution (Innovex), followed by avidin-biotin blocking for 8 min (Ventana Medical Systems). For IFITM3 staining, sections were incubated with anti-Fragilis antibody (Abcam, ab15992, 1 μg ml−1) for 5 h, followed by 60 min incubation with biotinylated goat anti-rabbit IgG (Vector labs, PK6101) at 1:200 dilution. The detection was performed with Streptavidin-HRP D (part of DABMap kit, Ventana Medical Systems), followed by incubation with Tyramide Alexa 488 (Invitrogen, B40953).

For IFITM3 and GFAP or IBA1 colocalization staining, sections were first incubated with anti-GFAP antibody (DAKO, Z0334, 1 μg ml−1) or anti-IBA1 antibody (Abcam, ab178847, 0.1 μg ml−1) for 5 h, followed by 60 min incubation with biotinylated goat anti-rabbit IgG (Vector labs, PK6101) at 1:200 dilution. The detection was performed with Streptavidin-HRP D (DABMap kit, Ventana Medical Systems), followed by incubation with Tyramide Alexa Fluor 568 (Invitrogen, T20948). After staining, slides were counterstained with DAPI (Sigma Aldrich, D9542, 5 μg ml−1) for 10 min and a coverslip was added with Mowiol. Slides were imaged with MIRAX SCAN (Zeiss) and immunofluorescence was quantified using CaseViewer and ImageJ.

Thioflavin-S staining. Four-month-old 5xFAD and Ifitm3−/−;5xFAD samples were washed three times in PBS and incubated in 30% sucrose solution overnight at 4 °C. Brains were then flash frozen in OCT compound and stored at −80 °C. Samples were serially sectioned coronally at 30 μm and stored at −20 °C in tissue storage solution (30% sucrose, 30% ethylene glycol in 0.1 M phosphate buffer). Every sixth section was stained with thioflavin-S (0.1% in ddH2O for 8 min) and washed three times in PBS with a fourth wash overnight at 4 °C (samples were protected from light at all times). Stained sections were mounted on charged slides and imaged with MIRAX SCAN (Zeiss) and immunofluorescence (FITC) was visualized and quantified using CaseViewer and ImageJ. The number of plaques per section was counted and divided by total tissue area (in mm²), the average number of plaques per area across all sections was reported for each sample.

Statistical analysis

Statistical analysis was performed by using GraphPad Prism 7 and GraphPad Prism 8. Results were presented as mean ± s.d. Violin plots represent median (middle line) and interquartile range outer lines. P-values were calculated with two tailed unpaired Student’s t-test, one-way ANOVA followed by Fisher’s LSD or two-way ANOVA followed by Tukey. Significance was set as P < 0.05 and expressed as **P < 0.05, ***P < 0.01, ****P < 0.001 and "****P < 0.0001. The experiments were not randomized due to the nature and sample sizes. All human and mouse sample analysis was performed blinded: one investigator designed and prepared samples and a second blinded investigator performed the experiment.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Source data are provided with this paper. All other data are available from the corresponding authors upon reasonable request. Source data are provided with this paper.
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Author contributions J.-Y.H. and Y.-M.L. conceived the study. J.-Y.H., G.R.F. and Y.-M.L. planned, and J.-Y.H., G.R.F. and X.W. performed most of the experiments, and J.-Y.H., G.R.F. and Y.-M.L. analysed the data. X.W. and C.C. performed proteomics analysis. S.J.P. and E.W. cloned the constructs and S.J.P. expressed a recombinant substrate protein. E.W. performed a photolabelling study in primary neurons and Notch AlphaLISA assay in knockout cells. M.B. performed immunofluorescence. T.L. perfused mouse brains and T.L., Y.Z. and Y.K. helped with animal care. N.L. Nadon for mouse brains. We thank E. Sikora for Ape genotyping, D. Yanlin for immunostaining, S. Fujisawa and Y. Romin for help with microscopy, and S. Shulberg for human brain samples. We thank S. Wagner for providing NSP-75555. This work is supported by the JBRI Foundation (Y.-M.L., P.G. and A.G.), the Fisher Center for Alzheimer’s Research Foundation (P.G.), Cure Alzheimer’s Fund (Y.-M.L.), the National Institutes of Health R01NS069275 (Y.-M.L.), RFA1G057593 (Y.-M.L.), RFA1G061350 (Y.-M.L.), R01AG046170 (B.Z.), RFA1G057440 (B.Z.) and R01AG057907 (B.Z.). We also acknowledge the MSK Cancer Center Support Grant/Core Grant (grant P30 CA008748) the ADRC parent grant (P30 AG062429), Mr. William H. Goodwin and Mrs. Alice Goodwin and the Commonwealth Foundation for Cancer Research, the Experimental Therapeutics Center of MSKCC, and the William Randall Hearst Fund in Experimental Therapeutics.

Competing interests Y.-M.L. is co-inventor of intellectual property (assay for gamma secretase activity and screening method for gamma secretase inhibitors) owned by MSKCC and licensed to Jiangsu Continental Medical Development.

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Extended Data Fig. 1  | Identification of IFITM3 as γ-secretase binding protein. a, LC–MS/MS analysis of the 15-kDa band identified four peptides that match with human IFITM3. b, Western blotting analysis of PS1-NTF protein labelled with E2012-BPyne (500 nM). c, Structures of imidazole GSMs, acid GSM and GSIs. d, Western blotting analysis of E2012-BPyne-labelled proteins in the absence or presence of imidazole GSMs, acid GSM and GSIs. Labelled proteins were captured and analysed by western blotting for IFITM3. e, Structures of GY6 and 163-BP-l-biotin. f, IFITM3 co-immunoprecipitates with γ-secretase subunits. CHAPSO-solubilized cell membranes were immunoprecipitated with anti-IFITM3 antibody and probed with antibodies against PS2-CTF and PEN-2. Rabbit IgG was used as a negative control. g, IFITM3 does not co-immunoprecipitate with SPP. CHAPSO-solubilized cell membranes were immunoprecipitated with a monoclonal anti-IFITM3 antibody (9D11) and probed with antibodies against SPP and IFITM3. Mouse IgG was used as a negative control. h, Analysis of the total protein level in wild-type MEF or PS1/PS2 double knockout MEF cells. The same amount of membrane proteins was loaded and analysed by western blotting. i, Ifitm3 mRNA expression levels were measured by RT–PCR in wild-type MEFs or PS1/PS2 double knockout MEF cells (n = 6). All western blotting images and graphs are representative of three independent experiments (except in a and d, which represent two replicates). Data are mean ± s.d. not significant. P values were determined by two-sided Student’s t-test.
Extended Data Fig. 2 | Effect of IFITM3 knockdown and knockout on γ-secretase.

a, Quantification of western blotting (Fig. 2a) showed that IFITM3 knockdown did not change protein expression levels of APP, NCT and PS1-NTF in HEK-APP wild-type cells ($n = 3$).
b, Schematic representation of cell-free γ-secretase assay. γ-secretase is incubated with a recombinant APP substrate in the presence of 0.25% CHAPSO. Cleaved $\alpha$- and $\beta$-species are measured with cleavage specific antibodies and AlphaLISA technology.
c, Schematic model showing different GSM and GSI binding sites in γ-secretase: E2012 (imidazole GSM), GSM-1 (acid GSM), and L458 (transition state analogue inhibitor, GSI).
d–f, Comparison of IC$_{50}$ values of GSM-25 (EV: $n = 9$, KO: $n = 8$) for $\alpha$- and $\beta$-species (ns) (d), GSM-1 ($n = 6$) for $\alpha$- (ns (d)) and $\beta$-species (ns (d)) (e), and L458 ($n = 3$) for $\alpha$- (ns) and $\beta$-species (ns (f)) cleavages in the U138 EV or KO cell lines ($n \geq 3$).
g, IFITM3 knockdown (KD) does not affect expression of γ-secretase subunits. IFITM3 was knocked down by siRNA (6 pmol, $n = 3$) in HEK-NotchΔE cells and scramble siRNA (SC, $n = 3$) was used as a negative control. Cell lysates were probed by antibodies against NCT, PS1-NTF and IFITM3. β-Actin was used as a loading control.
h, Effect of IFITM3 knockdown on γ-secretase activity. IFITM3 knockdown increased γ-secretase cleaved product NICD, analysed by western blotting. Cell lysates were probed by antibodies against c-Myc (NotchΔE) and NICD and a representative quantification of NICD ($n = 8$) (**$P = 0.0039$) is shown (bottom).
i, Cell-based NICD AlphaLISA assay (left) revealed an increase in NICD production with IFITM3 knockdown. Quantification of NICD ($n = 8$) (**$P = 0.0039$) is shown in the right panel.
j, Effect of IFITM3 knockout on γ-secretase activity. Knockout cells lines have increased γ-secretase activity as compared to the EV cell line. The NICD cleavage in vitro was measured by AlphaLISA assay ($n = 3$) (**$P = 0.0096$). All western blotting images and graphs are representative of three independent experiments. Data are mean ± s.d. $P$ values were determined by two-sided Student’s $t$-test.
Extended Data Fig. 3 | Effect of ageing and the expression of APP/PS1 on the level of γ-secretase and IFITM3. 

(a) Western blotting for NCT and PS1-NTF (Fig. 3a) were quantified by Odyssey imaging (n = 5 mice pooled per group, except n = 4 for 28F, ns). 

(b) Effect of ageing on subcellular localizations of IFITM3. A hemibrain from male wild-type C57BL/6 mouse at 4 and 28 months (n = 1 per group) were homogenized and layered on iodixanol gradient (2.5–30%). Fractions were collected from the top and resolved by western blotting for γ-secretase, IFITM3 and different subcellular markers. 

(c) Western blotting for APP, NCT and PS1-NTF (Fig. 3e) were quantified by Odyssey imaging (n = 5 mice per group except n = 4 for WT at 12 months). APP: 3moWT-3mo5x: ****P<0.0001, 3mo5x-12mo5x: ****P<0.0001, 12moWT-12mo5x: ****P<0.0001. NCT: 3moWT-12mo5x: ****P<0.0001, 3mo5x-12mo5x: ****P<0.0001, 12moWT-12mo5x: ****P<0.0001. PS1-NTF: 3moWT-12mo5x: ****P<0.0001, 12moWT-12mo5x: ****P<0.0001. 

(d) Immunostaining of IFITM3 in mouse brains. Fluorescence microscopy of IFITM3 expression in 12-month-old PFA-perfused mice (WT, top; 5xFAD, bottom). Representative images of cortex, hippocampus and subiculum (left to right) show IFITM3 (green) and DAPI (blue). Scale bars, 1 mm, 200 μm, 100 μm (left to right). Total IFITM3 fluorescence area within the hippocampus and cortex of WT and 5xFAD was quantified using FIJI. Total IFITM3 was divided by tissue area and IFITM3 expression was normalized to average of WT (WT: n = 7, 5xFAD: n = 9) (cortex: **P<0.0035, hippocampus: ****P<0.0001). 

(e) IFITM3 expression in astrocytes and microglia is upregulated in 5xFAD mice compared to wild-type mice. Fluorescence microscopy of IFITM3, GFAP (top) and IBA1 (bottom) expression in 12-month-old PFA-perfused mice (WT, top; 5xFAD, bottom). Representative images of the hippocampus and cortex show IFITM3 (red), GFAP (green, top), IBA1 (green, bottom), and DAPI (blue). Scale bar, 500 μm. Inset panels (left to right) show GFAP or IBA1 (green), IFITM3 (red) and merged staining. Scale bar, 50 μm. All western blotting images and graphs are representative of three independent experiments (except in b, which denotes two replicates). Data mean ± s.d. P values were determined by two-sided Student’s t-test (except in c, which was by one-way ANOVA followed by Tukey).
Extended Data Fig. 4 | Expression profile of IFITM3 and other markers in LOAD and age-matched controls. 

a, Spearman’s correlation of mRNA expression of human IFITM3 gene with age was analysed in the cortex (n = 158) and hippocampus (n = 123) of normal human brains using the GTEx cohort.

b, mRNA expression in samples from non-demented control participants (n = 10) and patients with LOAD (n = 18) of MAP2 (ns), GFAP (**P = 0.0046), and AIF1 (ns) were measured, which were used in Fig. 4c, d, e. Expression profiles of MAP2 (ns), GFAP (**P = 0.0046), and AIF1 (ns) in the temporal cortex of samples from human control participants (n = 76) and patients with LOAD (n = 80) using the Mayo Clinic cohort data. Correlation analyses were carried out and P values were calculated. d, The protein levels of γ-secretase and IFITM3 were analysed by western blotting (n = 4 per group). β-tubulin III was used as a loading control. l, Effect of IFITM3 induction on γ-secretase activity for Aβ 40 (*P = 0.0116) and Aβ42 (*P = 0.0319) activity. Membranes from primary neurons were incubated with the recombinant APP substrate C100-∆ID-FLAG and γ-secretase activity (Aβ cleavage rate) was assayed by Odyssey imaging (n = 12, 10). m, JC8 whole-cell photolabelling. Neuronal membranes were photolabelled with JC8 in the absence or presence of L458 and analysed by anti-PS1-NTF antibody. Photolabelled PS1-NTF protein level was quantified by Odyssey imaging (n = 3, ***P = 0.0210). n, Spearman’s correlation between the expression level of IFITM3 and viruses. In the BA-22 region in the MSBB cohort, the expression level of IFITM3 is positively correlated with the expression level of HHV-6B (rho = 0.248, P = 0.044, n = 66). In the BA-36 region, the expression level of IFITM3 is positively correlated with the expression of hepatitis C virus genotype 4 (rho = 0.255, P = 0.033, n = 70). All western blotting images and graphs are representative of two independent experiments (except in b and e, which denotes one replicate; in h, data are pooled from two experiments; in k, which denotes three replicates; and in l, data are pooled from four experiments). Data are mean ± s.d. Violin plots represent median (middle line) and interquartile range (outer lines). Two-sided Student’s t-test (except in g, which was by one-way ANOVA followed by Tukey).
Extended Data Fig. 5 | Correlation between L505 labelled protein and γ-secretase activity. 

**a**, Structures of JC8, L505, L646, GY4 and L631. **b**, Western blotting analysis of 11Bt-labelled proteins in the absence or presence of its parent compound, a substrate binding site inhibitor pep11 and imidazole GSM, E2012. Labelled proteins were captured and analysed by western blotting for PS1-NTF (n = 1). **c**, Pearson’s correlation between γ-secretase activity (Aβ40, Aβ42 cleavage rates in Fig. 4d) and L505-labelled PS1-NTF (in Fig. 5c) in LOAD samples (n = 10). Linear regression analysis was used to calculate R and P values.
Reporting Summary

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- Study design: e.g., sample size, RCT
- Data collection: e.g., methods, number, follow-up period
- Data exclusions: e.g., how many and why
- Analysis methods: e.g., parameters, how they were tested
- Statistics: e.g., calculations, software, degrees of freedom, method of multiple comparisons, P values, effect sizes

Software and code

Policy information about availability of computer code

Data collection

No Software was used

Data analysis

GraphPad Prism 7 and 8; Microsoft Office 360 - Excel; ImageJ; Fiji; Chemoffice 16; R package V3.34.0; Image Studio Lite; Caseviewer, Adobe Illustrator, Adobe Photoshop, GIMP 2.10

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Source data for Figs. 1–5 and Extended Data Figs. 1–5 and table S1 – S5 are available with the paper. All other data are available from the corresponding authors upon reasonable request.
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

- **Sample size**: Sample sizes were not predetermined. Group sizes for biochemical, cellular, mouse samples and human samples were chosen based on numbers used in previous publications to generate statistical significant results.

- **Data exclusions**: Human brain samples: one sample was excluded because MAP2 mRNA levels were extremely low. Mouse brain tissue: one WT sample was excluded because nicastrin protein levels (determined by Western blot) were greater than 2 standard deviations below the mean value. The criteria of 2 standard deviations were pre-established.

- **Replication**: All data was reliably reproduced and the exact number of repeats and sample sizes are provided in each figure legend and methods.

- **Randomization**: The nature and sample sizes are not suitable for randomization.

- **Blinding**: All human and mouse sample analysis was performed blinded: one investigator designed and prepared samples and a second blinded investigator performed experiment and data analysis.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☑️ | Antibodies            |
| ☑️ | Eukaryotic cell lines |
| ☑️ | Palaeontology and archaeology |
| ☑️ | Animals and other organisms |
| ☑️ | Human research participants |
| ☑️ | Clinical data |
| ☑️ | Dual use research of concern |

### Antibodies

**Antibodies used**

PS1-NTF, PS1-CTF, nicastrin, Aph-1aL, Pen-2, human IFITM3, mouse IFITM3, APP (22C11, MAB348), β-actin (C4), β-tubulin III (T8578), tubulin (ab56676). SPP, human IFITM3 monoclonal antibody 9D11, G2-10, 10G3, SM320, cleaved Notch1 (Val1744), V-PLEX Aβ Peptide Panel 1 (4G8) Kit, V-PLEX Aβ Peptide Panel 1 (4G8) Kit, N-cadherin (D4R1H), Rab7 (B-3), EEa1 (Ab2900), NA9340V, NXA931V, IRDye 800CW goat anti-rabbit IgG (H+L), anti-mouse IgG (H+L) secondary antibodies

**Validation**

PS1-NTF (a kind gift from Dr. Min-Tain Lai, Merck Research Laboratories); J Biol Chem 278, 22475-22481 (2003).

PS1-CTF (MAB5232, Millipore); https://www.emdmillipore.com/US/en/product/Anti-Presenilin-1-Antibody-loop-a.a.-263-378-CT-clone-PS1-loop.MM_NF-MAB5232

PS2-CTF (1987-1, Epitomics); Cell reports 15, 2226-2238, doi:10.1016/j.celrep.2016.05.013 (2016).

nicastrin was generated in our laboratory; J Biol Chem 284, 2967-2977 (2009)

Aph-1aL (38-3600, Invitrogen); J Biol Chem 284, 2967-2977 (2009)
Pen-2 (ab18189, Abcam); https://www.abcam.com/pen2-antibody-ab18189.html

human IFITM3 (anti-Fragilis, ab109429, Abcam); https://www.abcam.com/fragilis-antibody-epr5242-ab109429.html

mouse IFITM3 (anti-Fragilis, ab15592, Abcam); https://www.abcam.com/fragilis-antibody-ab15592.html

APP (22C11, MA8348, Millipore); https://www.emdmillipore.com/US/en/product/Anti-APP-A4-Antibody-a.a.-66-81-of-APP-NT-clone-22C11,MM_NF-MAB348

β-actin (C4, sc-47778, Santa Cruz Biotechnology); https://www.scbt.com/p/beta-actin-antibody-c4

β-tubulin III (T8578, Sigma-Aldrich); https://www.sigmaaldrich.com/catalog/product/sigma/t8578

tubulin (ab56676, Abcam), https://www.abcam.com/tubulin-antibody-loading-control-ab56676.html

SPP (317, generated in our laboratory), ACS Chem Biol 10, 1925-1931, doi:10.1021/acschembio.5b00321 (2015).

human IFITM3 monoclonal antibody (9D11, generated in our laboratory). This antibody only detects IFITM3 in U138 cells, not in IFITM3 deficient U138 cells generated in this work, validating its specificity.

G2-10: Anti A 40-Specific
https://www.emdmillipore.com/US/en/product/Anti-Amyloid-40-Antibody-clone-G2-10,MM_NF-MABN11

10G3: Anti A 42-Specific
Brain 139, 563-577, (2016)

SM320, Notch cleavage antibody
J Biol Chem 287, 17288-17296, doi:10.1074/jbc.M111.300483 (2012)

cleaved Notch1 (Val1744)
https://www.cellsignal.com/products/primary-antibodies/cleaved-notch1-val1744-antibody/2421

V-PLEX Aβ Peptide Panel 1 (6E10) Kit:
https://www.mesoscale.com/en/products/v-plex-abeta-peptide-panel-1-6e10-kit-k15200e

V-PLEX Aβ Peptide Panel 1 (4G8) Kit:
https://www.mesoscale.com/en/products/v-plex-plus-abeta-peptide-panel-1-4g8-kit-k15199g/

N-cadherin (D4R1H, #13116, Cell Signaling Technology);
https://www.cellsignal.com/products/primary-antibodies/n-cadherin-d4r1h-xp-rabbit-mab/13116

Rab7 (B-3, sc-376362, Santa Cruz Biotechnology);
https://www.scbt.com/p/rab-7-antibody-b-3

EEA1 (Ab2900, Abcam)
https://www.abcam.com/eea1-antibody-early-endosome-marker-ab2900.html

HRP-conjugated anti-rabbit and mouse secondary antibodies (NA9340V, NXA931V, GE Healthcare)
Previously sold by GE Healthcare, now by Sigma Aldrich
NA9340V
https://www.sigmaaldrich.com/catalog/product/sigma/gena93401ml?lang=en&region=US
NXA931V
https://www.sigmaaldrich.com/catalog/product/sigma/genxa9311ml?lang=en&region=US

IRDye 800CW goat anti-rabbit IgG (H+L)
(925-32211, Li-COR)
https://www.licor.com/bio/reagents/irdye-800cw-goat-anti-rabbit-igg-secondary-antibody

anti-mouse IgG (H+L) secondary antibodies
(925-32210, Li-COR)
https://www.licor.com/bio/reagents/irdye-800cw-goat-anti-mouse-igg-secondary-antibody
Eukaryotic cell lines

Policy information about cell lines

| Cell line source(s) | MEF (WT and PS1/2 double KO) cells , HEK293-APP695 cells and HEK293-NotchΔE were obtained from collaborators. U-138 MG and HeLa cells were obtained from ATCC. |
| Authentication | All cell lines were authenticated by STR profile report. |
| Mycoplasma contamination | All cell lines tested negative for mycoplasma contamination. Applied biological materials (ABM) PCR mycoplasma detection kit (#G238) was used. |
| Commonly misidentified lines | Hek293, HeLa, U-138 MG |

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | 1. WT mice: C57BL/6J; purchased from The Jackson Laboratory; stock# 000664 For primary neuronal culture, wild type C57BL/6J or C57BL/6NJ female mice of 1.5-2 month old with timed pregnancy were obtained from the Jackson Laboratory and Charles River Laboratories. |
| 2. 5xFAD mice: B6.Cg-Tg(APPScs765PASf/J)J L6855Btm1Her/J; purchased from The Jackson Laboratories; stock# 34848-JAX |
| 3. Aged WT mouse brain samples were obtained from NIA. Brains of C57BL/6 female and male mice at different age groups (mature adult (4-month-old) and old (28-month-old)) were collected from NIA Aged Rodent Tissue Bank. |
| 4. Aged 5xFAD mouse brain samples were from two coauthors. Brains of female WT and 5xFAD Tg mice at 3- (mature adult) and 12-month-old (middle aged) were kindly provided by Dr. Robert J Vassar (Northwestern University). |
| ExFtg4G mice: Translating ribosome affinity purification (TRAP) knock-in mice were generated by crossing mice bearing a loxP-stop-loxP-EGFPPL10a sequence in the Eef1α1 promoter (Eef1α1-LSL-EGFPPL10a) with CCK-Cre (CCKtm1.1(cre)Zjh/J), Glutamate decarboxylase 2 (65 kDa)-Cre (B6.N.Cg-Gad2tm2(cre)J/Zjh/J), Cortistatin-Cre (Tg(Cortcre)M42Gsat) or parvalbumin-Cre (Pvalbtm1(cre)Arbr/J) mice. |
| 5. IFITM3−/− embryos were obtained from the European Mutant Mouse Archive and rederived in pseudopregnant host mice. |

Wild animals | This study did not involve wild animals. |

Field-collected samples | This study did not involve samples collected from the field. |

Ethics oversight | IACUC, Memorial Sloan Kettering Cancer Center and Rockefeller University |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

| Population characteristics | All human brain samples were provided by the Alzheimer's Disease Research Center (ADRC) Neuropathology Core at University of California, San Diego, after review and approval by the ADRC Biospecimen Review Committee. All human samples were obtained from subject who consented to autopsy for the UCSD ADRC. All tissues provided were deidentified and contained a number code only. |
| Recruitment | N/A |
| Ethics oversight | N/A |

Note that full information on the approval of the study protocol must also be provided in the manuscript.