SUPPLEMENTARY INFORMATION

ADAM30 downregulates APP-linked defects through cathepsin D activation in Alzheimer's disease

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Supplementary experiment procedures

Human brain samples

Brains were obtained at autopsy from one hundred forteen patients with early- and late-onset sporadic AD accessioned from the Greater Manchester region of United Kingdom during years 1986-2001 (mean age at death = 73.1 ± 9.1 years old; mean age at onset = 65.9 ± 10.3 years old; 51% male). All patients were of Caucasian ethnic origin. Pathological diagnoses were made in accordance with CERAD Neuropathological Criteria for AD *(Mirra et al. 1991)*. All patients were at Braak stages 5 or 6 at time of death *(Braak & Braak 1991)*. The proportion of tissue area occupied by Aβx-40 and Aβx-42 was quantified in immunohistochemically stained section from Brodmann area 8/9 of the frontal cortex, as previously reported *(Lambert et al. 2001)*. Control brains were obtained from an initial set of one hundred sixty seven brains recruited from routine autopsies carried out at the Hospices Civils de Strasbourg (France, age at death: 80.1 ± 6.2, 42% male)*(Berr et al. 2001)*. Recruitment was designed to exclude cases of dementia (Individuals were not recruited from medical institutions where the majority of patients presented with dementia, but from a general hospital). Most cases were admitted less than 48 hours before death via emergency services and were living at home prior to their admission. Cases referred to autopsy for neurological pathologies were excluded. The neuropathological diagnosis for Alzheimer's disease followed CERAD Neuropathological criteria. In addition, Braak stages for neurofibrillary tangles *(Braak & Braak 1991)* were assessed in the whole series and only samples with a braak stage ≤2 was used for randomly selection. Again, all control subjects were Caucasian.

Transcriptomic Study design

The main goal of this study was to research for new actors of the APP metabolism on a biological convergence approach. One of the main difficulties in differential expression analyses is to determine whether a variation is due to the pathological process or is a consequence of an inter-individual variability or technical biases. From these considerations, we first followed a dye-swap strategy to minimize the variation of hybridization between the pathological and control samples and to systematically perform duplicate experiments. We postulated that the measure of an inter-individual variability in the controls was not relevant for our main objective and may even add some noise background. By opposition, the knowledge of this inter-individual variability in the brain of AD cases seemed to be more important to address our questioning. Furthermore, we determined that a statistical analysis (moderated t-statistic with empirical Bayes shrinkage of the standard errors) was well adapted to take into account the experiments altogether but also to evaluate the homogeneity of the difference in expression between the different AD cases. That is why accordingly to all these considerations, we decided to analyse each AD samples versus a pool of all the control samples *(Chapuis et al. 2009; Bensemain et al. 2009).*

Transcriptomic analyses

The main criteria of selection for the oligonucleotides were: (i) a length of 60 oligonucleotides; (ii) the hybridization temperature (between 65 and 75 °C); (iii) the specificity of the oligonucleotide sequence; (iv) inability to form a secondary structure at the hybridization temperature; (v) an oligonucleotide sequence close to the 3'-UTR end of the selected gene (Supplementary Table 3).

For the transcriptomic experiments, we followed a dye-swap strategy, with each AD sample being analyzed on two independent microarrays on which the same sample was labeled either by Cy3 or Cy5 fluorophores. For hybridization, 4 µl of cRNA from each AD case was mixed

with 4 µl of cRNA from the control pool. This mix was then dissolved in 22 µl of hybridization buffer (Supplier) to obtain a final concentration of 40 % formamide, 2.5xDenhardt's, 0.5% SDS and 4xSSC. After incubating at 95 °C for 5 min, the mix was applied to the slides under a cover slip. The slides were then placed in a hybridisation chamber (Corning®, USA), and 30 µl of hybridisation buffer was added to the chamber before sealing. The sealed chambers were incubated for 14-16 h in a water bath at 42 °C. The slides were then washed twice in SSC 2X and SDS 0.1% for 5 min at 42°C, once for one min in SSC 0.2X at room temperature and then once for 1 min in SSC 0.1 X at room temperature. Finally, the slides were dried by centrifugation at 1000 rpm for 5 min at room temperature. After hybridisation, arrays were scanned using an Affymetrix® 418 scanner and images were processed using ImaGene 6.0 (Biodiscovery) software. Raw data were then analysed using the LIMMA library (Linear Models for Microarray Data) *(Smyth et al. 2003)* running under the statistical language R v2.0.1 *(Ihaka & Gentleman 1996)*. A normalisation protocol, consisting of a within-array print-tip less normalisation to correct for dye and special effects *(Yang et al. 2002)*, was applied on the background subtracted median intensities of the unflagged spots. After normalization, identification of statistically significant regulation was performed using moderated t-statistic with empirical Bayes shrinkage of the standard errors *(Lonnstedt & Speed 2002)*.

Quantigene protocol

Briefly, capture and label extender probe sets specific for ADAM17, ADAM30, ADAM33, ADAMTS16, β-actin and β-glucuronidase mRNA (as furnished by the supplier) were combined and diluted to respectively 500 fmol/µl in a lysis buffer supplied in the QuantiGene bDNA Signal Amplification Kit (Bayer Diagnostics, USA). Total RNA (respectively, 0.8 µg for ADAM17, ADAM33 and ADAMTS16 and 2.5 µg ADAM30 and respectively 0.2 µg for

β-actin and β-glucuronidase in a final volume of 10 µl) was added to each well of a 96-well plate with 40 µl of capture buffer, 40 µl of lysis buffer and 10 µl of each diluted probe set. RNA was allowed to hybridize for at least 16h at 53°C. Plates were then washed at room temperature (600 µl of a wash buffer). Samples were then hybridized for 60 min at 46°C with the bDNA amplifier molecules (100 µl/well) diluted in an amplifier/label probe buffer (1:100). At room temperature, plates were then rinsed with the wash Buffer. Label probe (1:100 in a amplifier/label probe buffer) was added to each well (100 µl/well) and hybridized to the bDNA-RNA complex for 60 min at 46°C. Plates were again rinsed with wash buffer at room temperature. Alkaline phosphatase-mediated luminescence was triggered by the addition of a dioxetane substrate solution (100 µl /well). The enzymatic reaction was allowed to proceed for 30 min at 46°C, and luminescence was measured with the 1420 Victor light luminometer (Perkin Elmer®, France).

Plasmid constructions and site-directed mutagenesis

The pcDNA-APP^{695wt} vector was already described elsewhere . The pcDNA- ADAM30^{wt} vector was obtained as follow: the ADAM30^{wt} cDNA was supplied by the TrueClone® Society. Following validation of the sequence by direct sequencing, the ADAM30^{wt} cDNA was sub-cloned into a mammalian expression pcDNA3.1(+) vector (Invitrogen®, USA) using *Not*I restriction sites. Using the pcDNA3-ADAM30^{wt} vector as a template, the ADAM30 catalytic site was inactivated by mutations of the Histidines at positions 338, 342 and 348 into Leucines (ADAM30^{mut}) using PCR site-directed mutagenesis (see supplementary Table 5 for the mutagenesis oligonuclotide sequences). The pcDNA- ADAM30$^{wt/mut}$-GFP vector was obtained after sub-cloning of the GFP cDNA into the pcDNA3- ADAM30$^{wt/mut}$ vector using *Hind*III and *Eco*RV sites. This vector allowed the expression of a fusion protein of ADAM30^{wt} with a GFP in C-ter. Using the pcDNA3-APP^{695wt} vector as a template, the

Lysine at position 688 was mutated into a stop codon to express an APP protein truncated at its 8 last C-ter amino-acids and named $APP^{\Delta C8}$. From the same template, the Phenylalanine acid at position 690 was also mutated into a Serine (APP^{F690S}) and the Glutamic acid at position 691 mutated into a Valine (APP^{E691V}) (see supplementary Table 5 for the mutagenesis oligonuclotide sequences). All the mutagenesis products were performed using QuickChange II Site-Directed Mutagenesis kit (Stratagene®) according to the supplier's instructions and were validated by direct sequencing. ShRNA pU6/Entry vector was supplied by Invitrogen® society and oligonucleotidic sequence of shADAM30 5'-CACCGGATCCTATAAACACCCAAAGCCAACTTTGGGTGTTTATAGG-ATCC-3' was sub-cloned according to the supplier's instructions (Invitrogen®, USA).

Secretase activity

α-secretase activity was measured as previously described *(Cissé et al. 2006)*. HEK cells overexpressing Swedish mutated APP or mock-transfected controls were transiently transfected with cDNA encoding either wild-type or mutated ADAM30 (2µg in jetPrime reactive). 24 or 48 hours after transfection, cells were recovered, centrifuged (3000 rpm, 5 min) then homogenized (10 passages in a syringe) in 400µl of buffer (Hepes pH7.4 containing sucrose and EDTA). Lysates were left 15min at 0°C then spun (850 rpm, 5min) and the supernatant was recovered, centrigfuged for 1 hour at 20000g and the pellet ultimately resuspended in Tris 10mM, pH7.5. α-secretase (in 50µg of homogenate) was then fluorimetrically assayed with JMV2770 substrate.

BACE1 fluorometric assay was defined as previously described *(Andrau et al. 2003)*. Briefly, samples (30µg of proteins in acetate buffer, 25mM, pH4.5) were incubated in a final volume of 100µL of the above acetate buffer containing BACE1 substrate (7-methoxycoumarin-4-yl)acetyl-SEVNLDAEFRK(2,4-dinitrophenyl) -RRNH2; R&D Systems,

10µM) in the absence or presence of β-secretase inhibitor I (50µM, PromoCell). BACE1 activity corresponds to the β-secretase inhibitor-sensitive fluorescence recorded at 320 and 420 nm as excitation and emission wavelengths, respectively. The slopes of the initial linear phase were calculated and expressed as fluorometric units/mg/h*(Andrau et al. 2003)*.

γ-secretase activity was measured as previously described *(Sevalle et al. 2009)*. Briefly, cell pellets were suspended in Tris 10mM at pH 7.5 with inhibitors (Sigma), passed through a 25G needle and finally centrifugated for 10 min at 800g at 4°C. Resulting supernatant were centrifugated for 1 h at 20 000g at 4°C and then, pellets containing the membranes were resuspended in a solubilization buffer to yield a 1mg/mL protein concentration. The samples (5 µL) were diluted with sodium citrate buffer (5 µL, 150 mM, pP 6.4) and a reaction buffer (10 µL) containing dithiothreitol (20 mM), egg phosphatidyl choline (1 mg/mL), bovine serum albumin (0.2 mg/mL) and recombinant hAPP (50 µg/mL). After an incubation in constant agitation for 16 h t 37°C, 2X Tris-tricine loading buffer was added to samples (20 µL). Finally, after a 5 min boiling, samples were subjected to western blot for Aβ analysis with 6E10 monoclonal antibody.

Generation of HEK293 stably over-expressing ADAM30^{wt} or ADAM30^{mut}

ADAM30^{WT} and ADAM30^{mut} cDNA were excised from pcDNA3.1-ADAM30$^{wt/mut}$ (*Hind*III/ *Not*I) and inserted into the pLPCX (*Hind*III/ *Not*I)(Clontech) retroviral vector to generate pLPCX- ADAM30$^{wt/mut}$. Retroviruses were produced by transfection of HEK293-APP^{695wt} packaging cells as previously described (Augert *et al.*, 2009). Briefly, one day prior to transfection, 2.6 million HEK293-APP^{695wt} cells were plated in poly-lysine pre coated 10cm dishes. The next day, cells were transfected using Exgen 500 (Euromedex). For each condition, 24 µl of Exgen 500 was added to 600 µl of NaCl 0.9 % water and mixed. Ten minutes later, the solution was added to 600 µl NaCl 0.9 % water containing 1 µg of VSV and

5 µg of retroviral vector and mixed. Thirty minutes later, the solution was added onto HEK293-APP^{695wt} cells in Opti-Mem media (Invitrogen) and 6 hrs later the medium was removed and replaced by the routinely used medium. Two days later, the viral supernatant was collected, filtered (0,45µM; Millipore), mixed with fresh media (1/2) and supplemented with polybrene (8 µg/ml). The viral supernatant was finally added to the target cells for 7 hrs, removed and replaced by the routinely used medium. The eukaryotic selection started 48 hrs post infection with the appropriate antibiotics (Puromycin 1.5µg/ml).

Cell surface biotinylation

HEK293- APP695^{wt} cells were transfected using empty vector or ADAM30^{wt} cDNA in 100 mm dishes. After 48 hours of transfection, cell surface proteins were biotinylated using Sulfo-NHS-SS-Biotin, as per supplier's recommendations (Cell Surface Protein Isolation Kit, Pierce). Briefly, cells were incubed with clod PBS containing Sulfo-NHS-SS-Biotin for 30 minutes with gentle rocking at 4°C. Cells were then lysed and immunoprecipitated with streptavidin beads. Precipitated proteins were eluted from the avidin beads with loading buffer containing 50 mM DTT, and heated for 5 minutes at 95°C then applied to Western blotting analysis.

N-terminal COFRADIC experiments

The HEK293-APP695^{wt}-ADAM30^{wt} and HEK293-APP695^{wt}-ADAM30^{mut} cell lines were cultivated in DMEM:F12 (1:1) with 10% FCS, P/S and 100 ng/ml puromycin. After a few passages, cells were switched to SILAC medium (DMEM:F12 1:1, with dialyzed FCS, P/S and 100 ng/ml puromycin) supplemented with either $^{12}C_6$ arginine or $^{13}C_6$ arginine to label the cells. HEK293-APP695^{wt}-ADAM30^{mut} cells were labeled with $^{12}C_6$ arginine and HEK293-APP695^{wt}-ADAM30^{wt} cells were labeled with $^{13}C_6$ arginine. Cells were labeled for one week

in SILAC medium, detached from the cell culture flask, washed with PBS and lysed in a CHAPS-containing lysis buffer. To ensure complete lysis, we further subjected the samples to three freeze-thaw cycles. After lysis, the protein concentrations of each sample were measured and equal amounts of both samples were mixed. We then performed N-terminal COFRADIC analysis on this mixed sample.

Briefly, primary amino groups on lysines and protein N-termini are first acetylated using an N-hydroxysuccinimide ester of trideutero-acetate (AcD3). Next, the proteins are digested with trypsin, which will only cleave C-terminal to arginine as acetylated lysines are not recognized by trypsin. N-terminal peptides (and co-isolated internal peptides) in the digest thus end on a $^{12}C_6$ or $^{13}C_6$ labeled arginine, depending on whether it originated from the "mutant sample" or the "WT sample". Trypsin digestion generates two classes of peptides: those with a blocked (acetylated) N-terminus (peptides from protein N-termini) and those with a primary alpha-amino group (formed during digestion with trypsin). The former peptides are then enriched by strong cation exchange at pH 3 as they are not retained by the SCX resin, and further separated by a first RP-HPLC in step. The different peptides fractions hence obtained are then treated with TNBS, which modifies primary amino groups and increases the peptides' retention on the RP-HPLC column. These TNBS-modified fractions are then separated individually in a series of RP-HPLC runs identical to the first run. In this way, N-terminal peptides are isolated and further analyzed by LC-MS/MS using a LTQ Orbitrap mass spectrometer. The resulting tandem mass spectra were then identified using the Mascot algorithm and identified peptides were quantified with Mascot Distiller.

Here, since N-terminal peptides will have only one arginine (located at their C-terminus and either $^{12}C_6$- or $^{13}C_6$-labeled) and since equal amounts of both samples were mixed, most peptides should have a ^{12}C:^{13}C ratio of 1:1. However, cleavage of proteins in the "WT sample" by ADAM30 will generate peptides with a new N-terminus that are absent in the

"mutant sample". Thus, upon quantification these "new" N termini should appear only in the $^{13}C_6$-labelled WT fraction or have a a ratio ^{12}C:^{13}C ratio significantly lower than 1. In the present study, we identified 2,238 proteins. Of note is that the Mascot Distiller software does not always quantify peptides correctly, and so manual validation is required. Peptides with a primary NH_2 group are discarded because they are formed upon trypsin digestion and are not protein termini. True N-termini of proteins (or protein fragments) should bear an acetyl or trideutero-acetyl group, and peptides that start at position 1 or 2 of the protein typically correspond to the normal N-terminus and thus do not result from cleavage by ADAM30, whereas the opposite can be true for peptides starting downstream of these position. We sorted all N-terminal peptides according to their ratios and determined those peptides that have ratios that differ significantly from those of other peptides. According to the Huber distribution *(Staes et al. 2008)*, peptides with a ratio below 0.707 were considered to be significantly different (Fig, S15).

Recombinant ADAM30^{wt} and ADAM30^{mut} production

ADAM30$^{wt/mut}$ expressions were improved by gene codon optimization before sub-cloning into bacteria expression vectors (pGS21a). *E.coli* ArcticExpress (DE3) host strain was transformed with recombinant plasmids. Single colony of E. coli host was inoculated into LB medium containing ampicillin; culture tubes were shaked in 37°C at 200 rpm. Once cell density reached OD=0.6-0.8 at 600 nm, IPTG was introduced for induction. SDS-PAGE was employed to monitor the expression and His-Tag was used to facilitate purification. Purity was estimated about 80% by Coomassie blue-stained SDS-page gels.

RNA extraction and qRT-PCR in mice brains

Fifty mg of Cortex samples were homogenized in 1ml of TRIzol® Reagent using a power homogenizer according to the manufacturer's specifications (Invitrogen®). After total RNA precipitation, total RNA aqueous phase were purified using NucleoSpin® RNA II extraction kit (Macherey-Nagel®) with rDNAse. RNA were dissolved in 40 µl RNase-free water and stored at -80°C. After quantification, total RNA was added to reverse transcription reactions from High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems®). First-strand cDNA were used for quantitative PCR and run in 13 µl reactions using Brilliant II SYBR® Green QPCR Master Mix (Agilent Technologies®). qPCR primers were generated by Eurogentec (Supplementary Table 5). Genes were expressed as mRNA level normalized to two different standard housekeeping genes (NeuN or GAPDH).

Primary neuronal cultures

Young mice (2-3 month old) genotyped Cre or ADAM30/Cre were decapitated and their brain were rapidly removed and dissected under microscope to remove the cerebellum. Cell preparation was obtained from cortex and hippocampus following the protocol described elsewhere *(Brewer & Torricelli 2007; Liu et al. 2005)*. After tissue digestion by papain (20U/ml), neurons were purified following centrifugation at 1900 rpm for 15 min through a discontinuous density gradient of Optiprep[15]. Neuron-enriched fractions were collected and diluted into 5 ml of Neurobasal A/B27 medium[16] with 0.5mM Glutamax and 10µg/ml gentamycin. The cell were again centrifuged at 1000 rpm for 5 min to remove Optiprep. Finally, the cells were plate on glass coverslips treated with Poly-D Lys 0.1 mg/ml (135 KD Sigma). Seven days later the cell were fixed 30 min with PFA 4% at 4°C. For the ADAM30 MAP2 labeling, protocol for ADAM30 was first used followed by the Map2 one. Cells were permeabilized by overnight incubation in 5% donkey serum, 1% BSA and 0.05% saponin in PBS for ADAM30 labeling. The cell were incubated with the primary antibody against

ADAM30 (1/400 Abcam ab90504) overnight at 4°C. Slides were then washed with 5% donkey serum 1% BSA and 0.05% saponin in PBS, incubated 1 hr with an FITC-conjugated goat anti-rabbit antibody (1/800 Jackson Immunoresearch). After three washes PBS, slides were analysed by fluorescence microscope. For Map2 labeling, the cells were permeabilized with 5% goat serum 1% BSA and 0.01% triton in PBS overnight. After 3 washes, the cells were incubated with Map2 antibody (at 1/400 HM-2 Sigma) overnight and incubated 1 hr with an 488-conjugated goat anti-mouse antibody (at 1/800, Lifetechnology).

Met metabolism analysis

Mouse monoclonal antibody directed against C-terminal domain of Met (L41G3) was purchased from Cell Signaling Technology and mouse monoclonal anti GAPDH (6C5) antibody was purchased from Santa Cruz. Peroxidase conjugated antibody directed against mouse IgG was purchased from Jackson Immunoresearch Labs. Mouse monoclonal antibody directed against the N-terminal domain of Met (DL-21) was kindly provided by Dr. Sylvia Giordano (University of Torino Medical School, Italy).

HEK 293 cells were cultured for 1 day in DMEM-10% FBS-ZellShield (Minerva Biolabs) 1X in 6-well plate (500.000 cells/well). The next day, cells were transfected with 2 µg of DNA (empty vector; vector expressing human Met : hu Met pCAGGS ; vector expressing ADAM30) using FuGENE® HD Transfection Reagent (Promega) in 0% FBS medium. After 1 day, medium was collected and submitted to cold acetone precipitation (1:4 ratio). Cells were lysed with PY lysis buffer containing Tris-HCl (20mM ph7.5), NaCl (50mM), EDTA (5mM), Triton X-100 (0,1%), aprotinin (1%), PMSF (1mM), leupeptin (1µM), Na3VO4 (1mM), β-glycérophosphate (20mM). Precipitates and lysates were separated onto 10% SDS-polyacrylamide gels and transferred onto PVDF membrane. Membrane was blocked in casein 0.2% and then incubated with specific primary antibody. Membrane was then incubated with

secondary antibody conjugated with peroxidase and revealed by an enhanced chemiluminescence detection system. Soluble Met level in conditioned medium was measured by ELISA with “c-Met soluble ELISA kit, human” as described by the supplier (Novex KHO2031).

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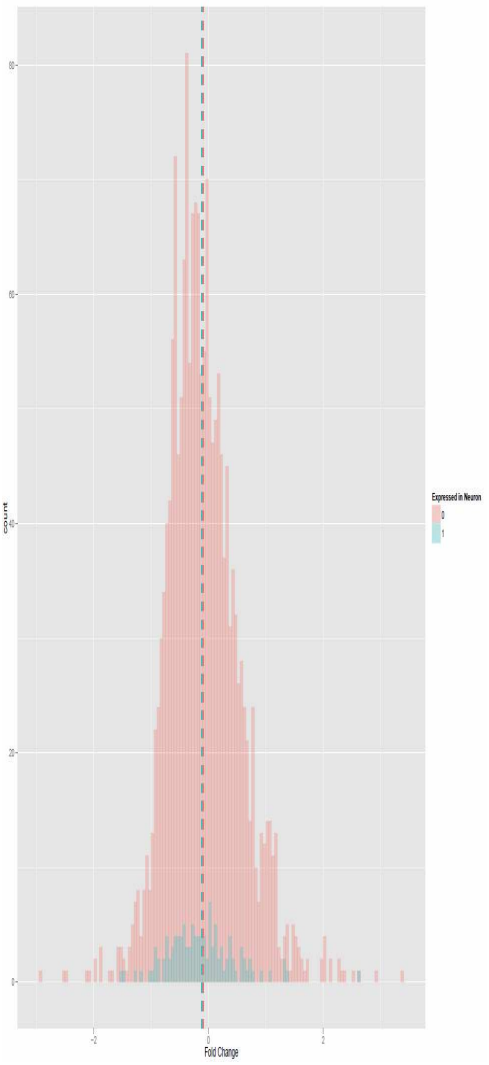
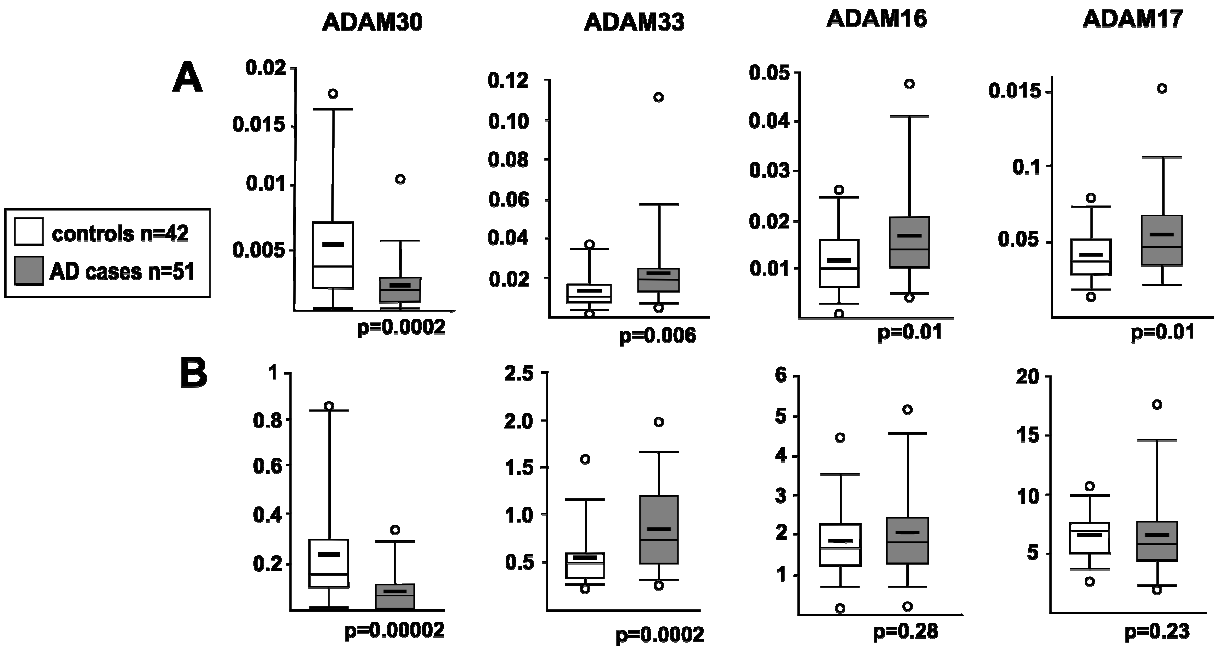
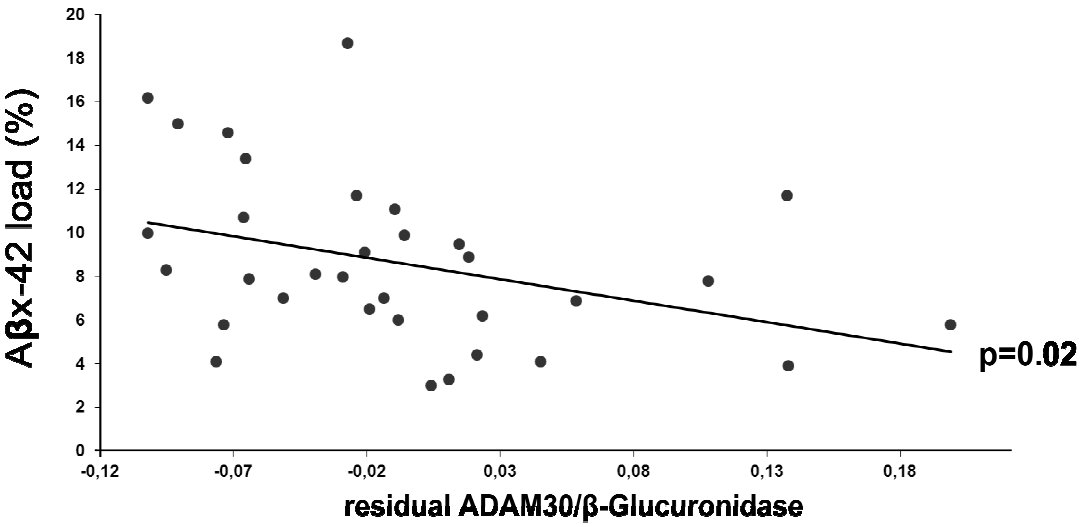
Supplementary Figure 1. Comparisons of fold-change expression between genes exclusively expressed in neurons (n=113, category 1 in the graph) or in other cerebral cell types (n=1789, category 2 in the graph) as defined in the Genesat database (*www.gensat.org)*. Data were generated from the list of genes analysed in the transciptomic experiments described in this paper and in *(Chapuis et al. 2009; Bensemain et al. 2009)* (see the material and method section). No difference was observed between the two groups analysed (-0.12 fold in average for genes only expressed in neurons and -0.09 fold in average in genes expressed in other cell types).

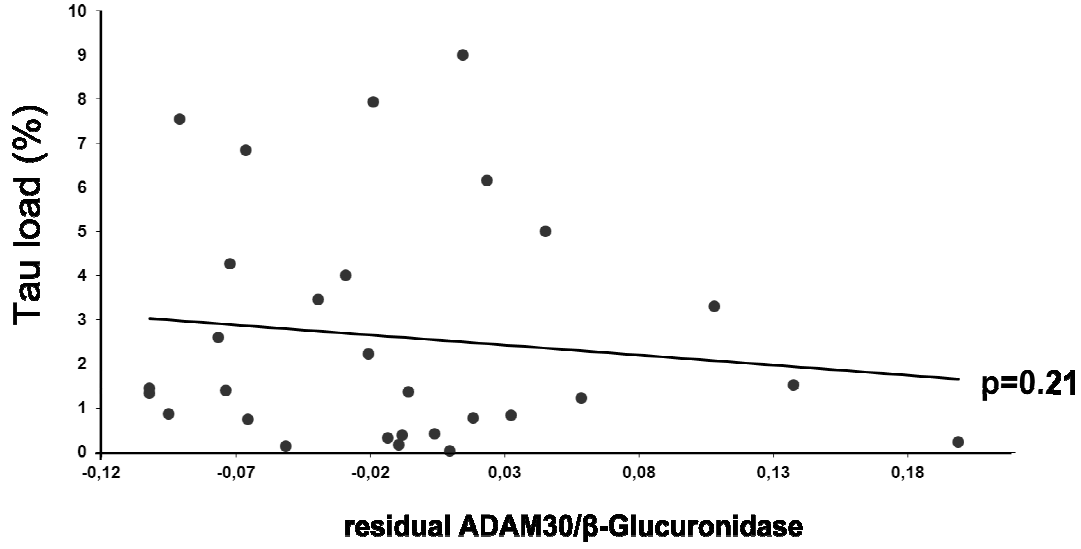


Supplementary Fig. 2: Expression level of ADAM30, ADAM33, ADAMTS16 and ADAM17 in the brain of AD cases and controls. All values were reported as arbitrary units following normalisation by **(A)** β-actin or **(B)** β-glucuconidase mRNA quantification. All mRNA quantifications were carried out in triplicate in all individuals (n=42 controls and n=51 cases). Thick line: median of mRNA quantification expression in cases and controls; Middle line: Mean; Upper horizontal line: inclusion of 75% of the individuals; lower horizontal line: inclusion of 25% of the individuals. circle: individuals exhibiting extreme values (out of the global distribution). P-values refer to a Mann-Whitney non-parametric test.

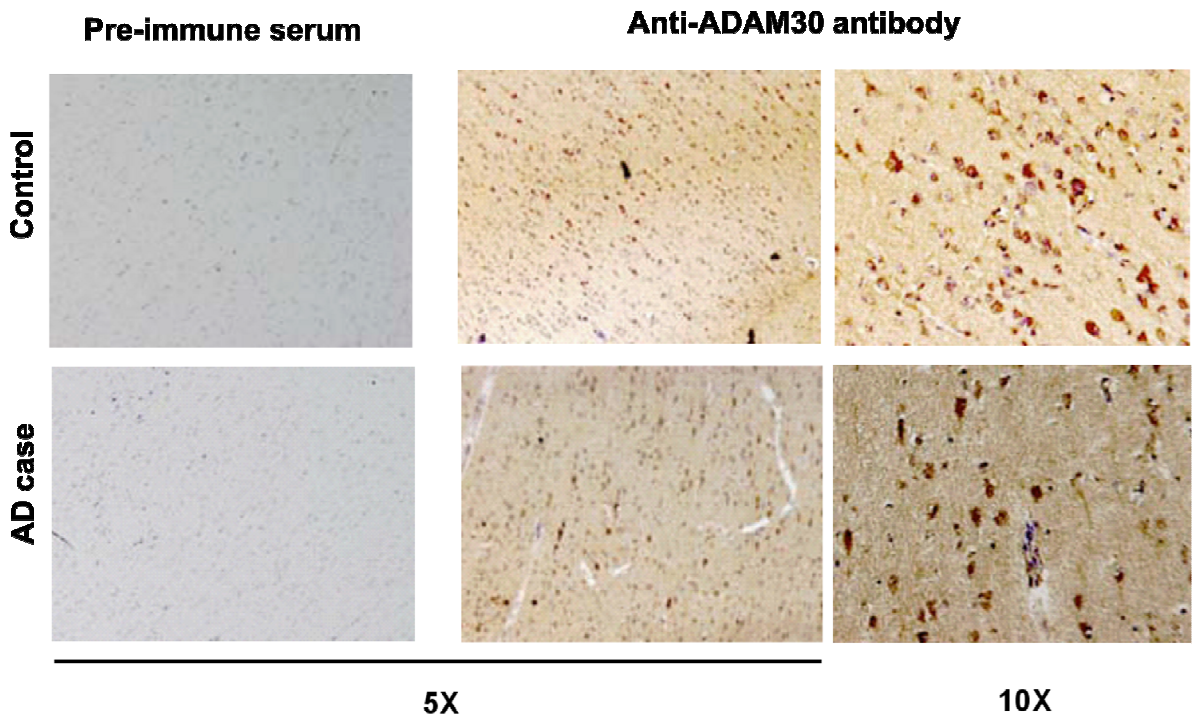


Supplementary Fig. 3: Association of $A\beta_{x-42}$ and Tau loads in the brain of AD cases with the expression of ADAM30 (residual correction), normalized against the expression of β-glucuronidase housekeeping gene. P-values refer to Spearman's non-parametric test.

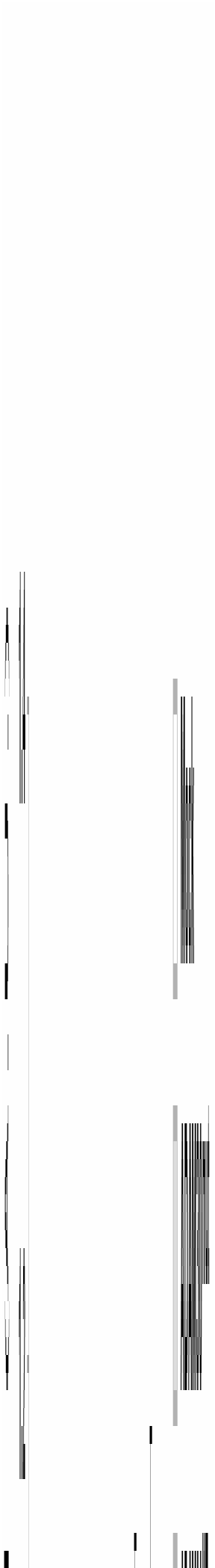




Supplementary Fig. 4: Representative ADAM30 immunolabelling in AD brains (n=3) or in control brains (n=3). This labelling is mostly restricted to neurons.



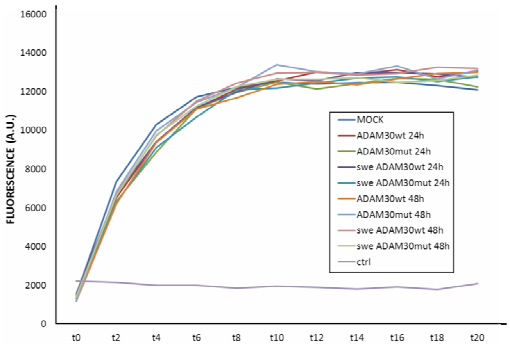
Supplementary Fig. 5: mRNA APP quantification after ADAM30 over-expression in SKNSH-SY5Y-APP^{695} and HEK293-APP^{695} cell lines. Cells were transfected with empty vector (Mock), ADAM30^{Mut} or ADAM30^{WT} cDNA. 48 hours after transfection RNA were extracted for mRNA APP quantification by Quantigene assay. NS, (Mann-Whitney non-parametric test).



Supplementary Fig. 6:

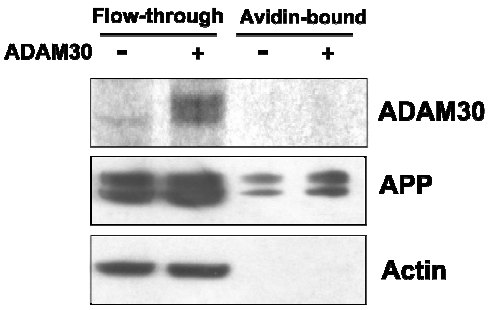
(A) α-secretase measured by fluorimetric assay with JMV2770 substrate (see supplementary materials). HEK cells overexpressing Swedish mutated APP or mock-transfected controls were transiently transfected with cDNA encoding either wild-type or mutated ADAM30 and fluorometry was measured 24 or 48 hours after transfection. **(B)** Search for localisation of $ADAM30^{WT}$ at the cell surface. SKNSH-SY5Y-APP^{695wt} cells were transfected with $ADAM30^{WT}$-GFP for 48 hour. Cells were incubated 1 hours at 4°C to accumulate protein at the cell surface. Then cells were fixed for immunofluorescne staining. APP staining (red) was used as control. To note, no accumulation of $ADAM30^{WT}$ (green) was observed at the cell surface. (**C)** Cell-surface-biotinylated proteins from HEK293-APP^{695wt} cells transiently transfected with $ADAM30^{WT}$ (+) or control empty plasmid (-) were precipitated with immobilized avidin and analyzed by WB using antibodies directed against APP, ADAM30 and Actin.

A

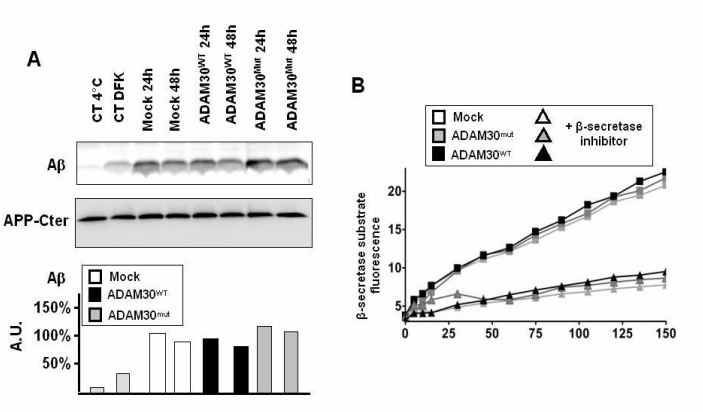


B

C

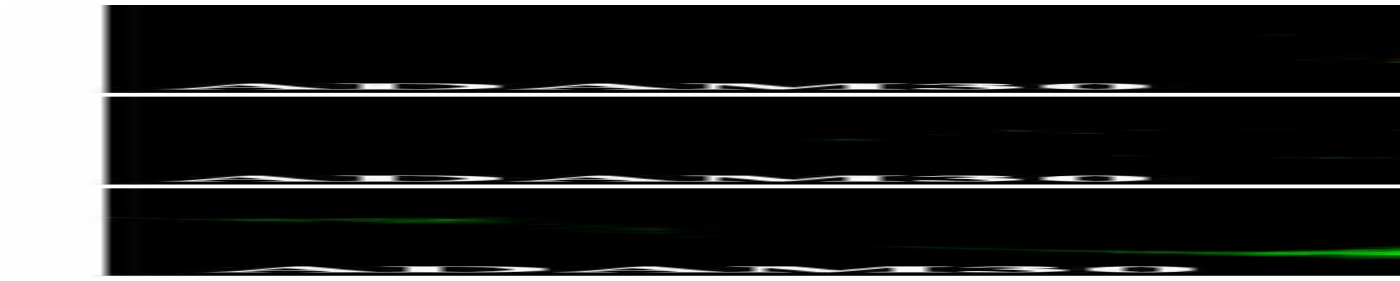


Supplementary Fig 7: (A) In vitro γ-secretase activity measurements as a function of ADAM30^{wt} or ADAM30^{mut} overexpression (24h or 48h after transfection). Solubilized membranes from HEK293 cells overexpressing ADAM30$^{wt/mut}$ (or not) were incubated with recombinant C100-flag[12]. The amounts of APP-cter and Aβ were quantified by densitometry. CT 4°C: solubilized membranes purified from non-transfected cells and incubated with C100-flag at 4°C; CT DFK: solubilized membranes purified from non-transfected cells and incubated with both C100-flag and DFK167 γ-secretase inhibitor; DNA3: solubilized membrane purified from mock-transfected cells; A30$^{wt/mut}$: solubilized membrane purified from ADAM30$^{wt/mut}$-transfected cells (after 24h or 48h) and incubated with C100-flag; **(B)** *In vitro* β-secretase activity as a function of ADAM30^{wt} or ADAM30^{mut} overexpression.

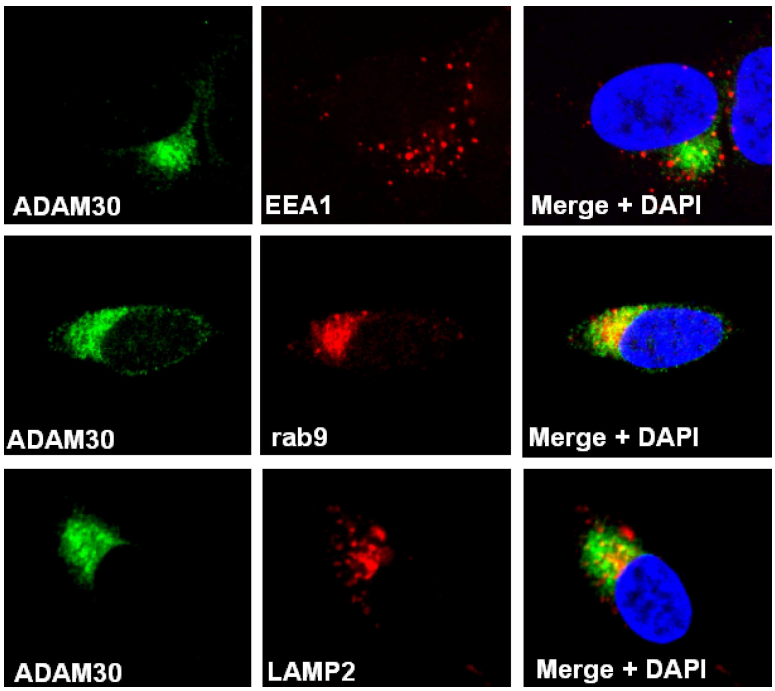


Supplementary Fig. 8: Immunofluorescence staining of (a) ADAM30^{wt} and (b) ADAM30^{mut} with early endosome (EEA1), late endosome (Rab7) or lysosome (Lamp2) marker in HEK293-APP695^{wt}. ADAM30^{wt} and ADAM30^{Mut} staining mainly co-localized with Rab7 showing the preferential localization of ADAM30^{Mut} in late endosome compartment. (n=3).

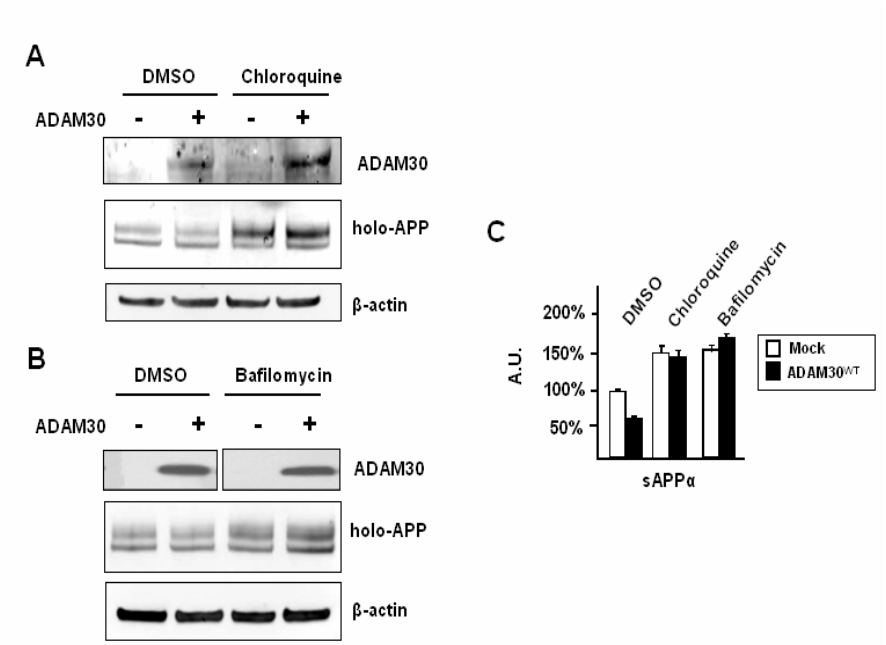
(a)



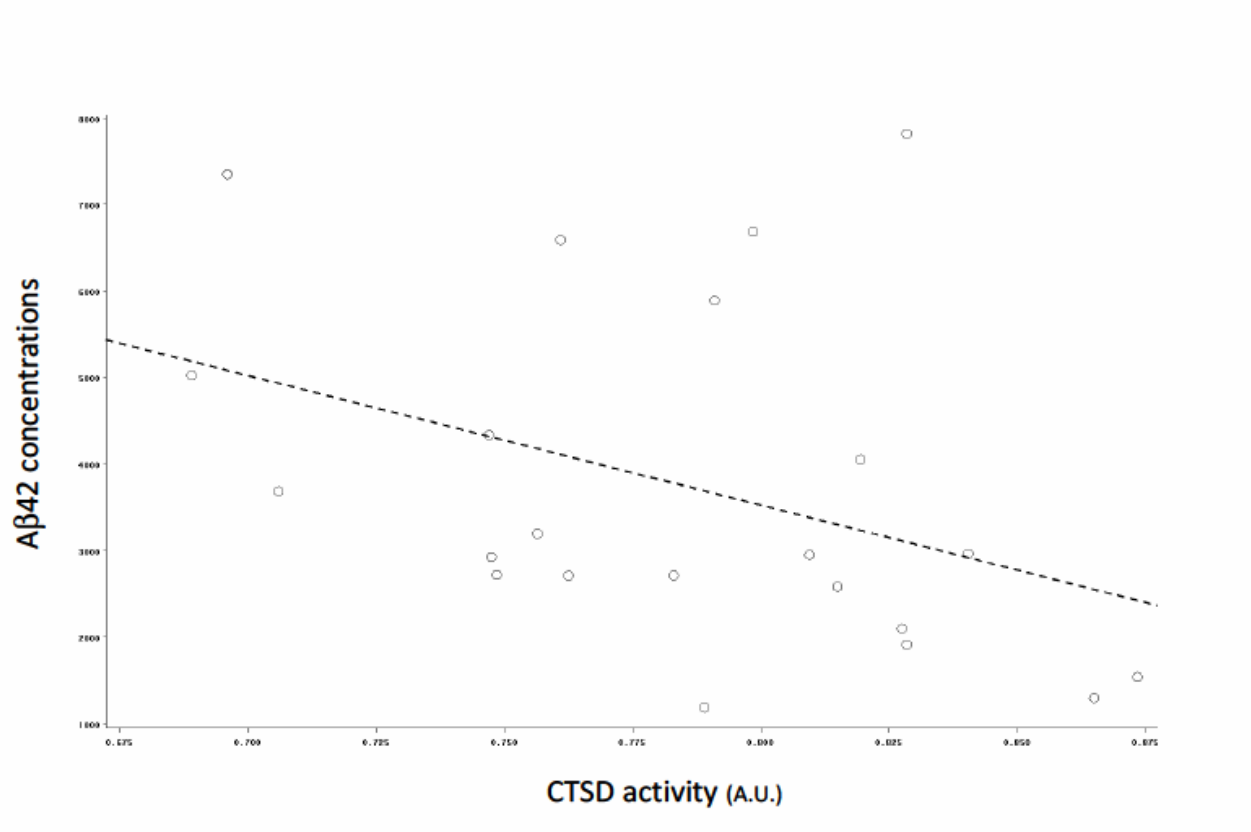
(b)



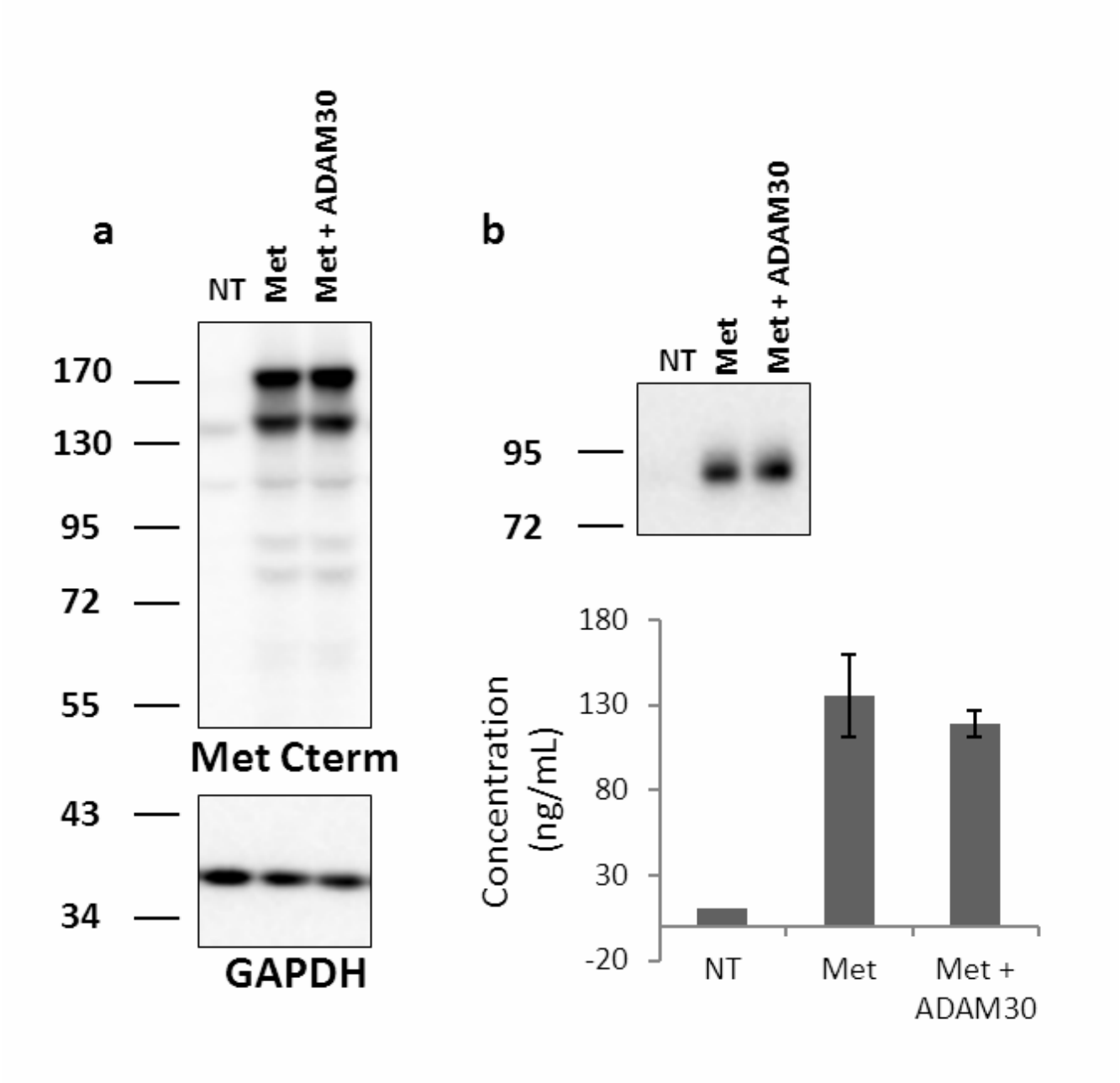
Supplementary Fig 9: Exposure to generic lysosome inhibitors e.g Bafilomicyn A1 or Chloroquine following the ADAM30^{WT} over-expression in HEK293-APP695^{WT} cell line.
Cells were transfected with ADAM30^{WT} (+) or the empty vector (Mock (-)). 24 hours after transfection cells were treated with Chloroquine (1 μM) or Bafilomycin (100nM) during 16 h. Cell extracts were analysed by western blot (**A** and **B**) and secreted sAPPα were measured by ELISA (**C**). Three independent experiments were performed in duplicate after 24 hours of transfection. NS (Mann-Whitney non-parametric test).



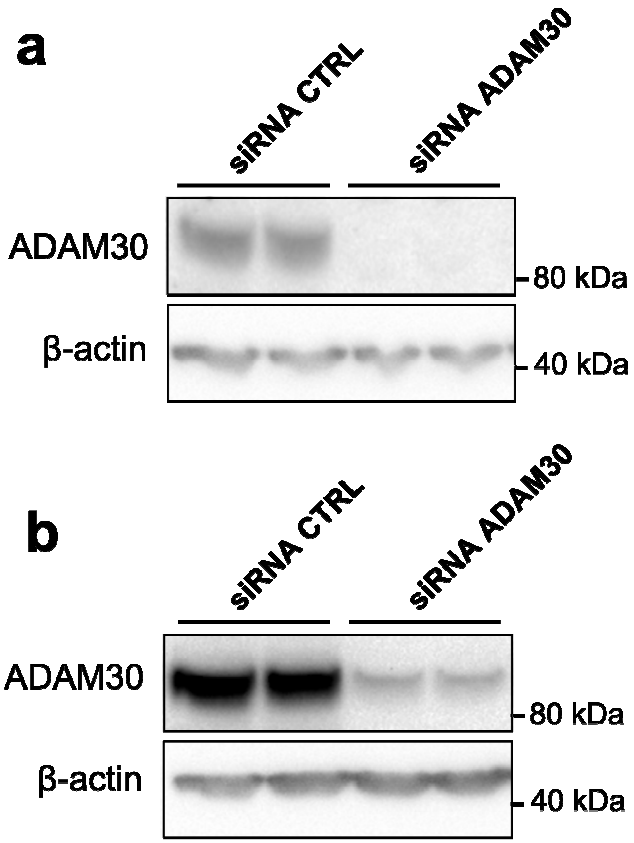
Supplementary Fig. 10: Correlation between CTSD activity measured in cortex and soluble Aβ42 concentrations in hADAM30^{wt}-hAPP^{Sw,Ind}-Cre mice. Spearman correlation test $p<0.05$.



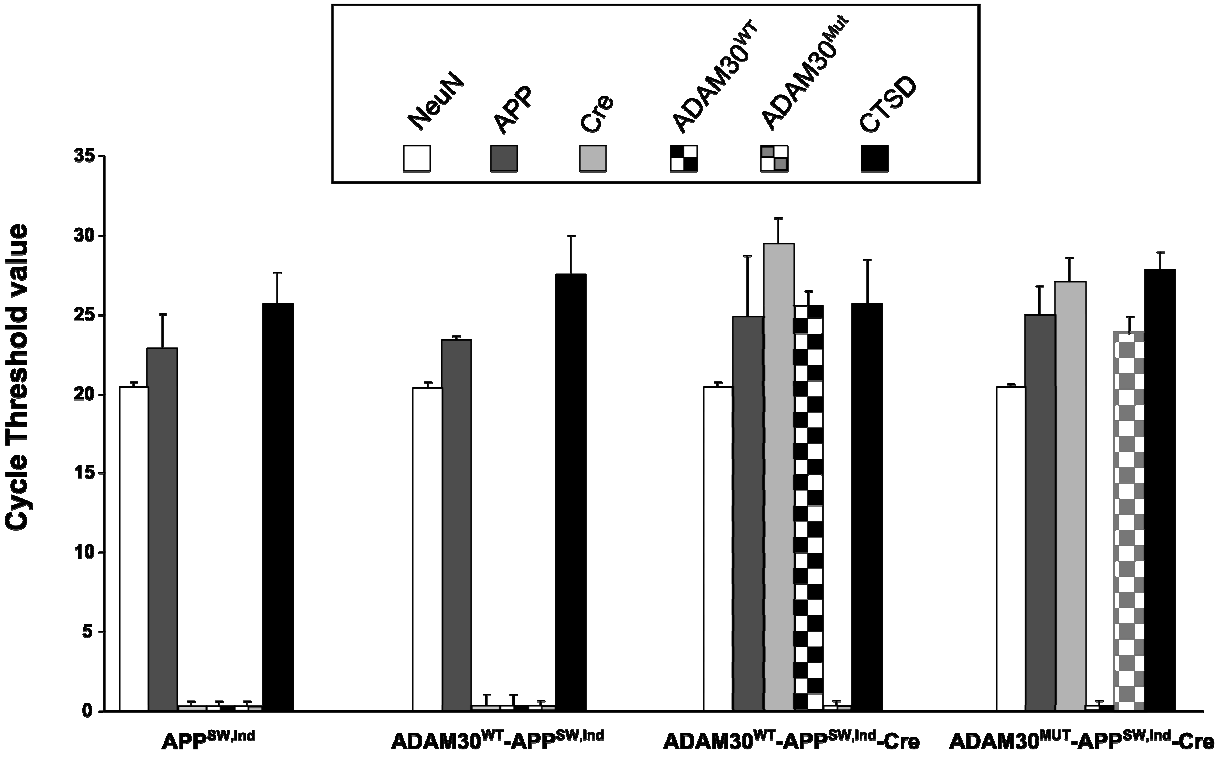
Supplementary figure 11: **(a)** A representative experiment measuring transfection of MET and ADAM30^{wt} mammalian expression vectors (MET secreted byproducts and β-actin). **(b)** Mean differences (±SEM) in the amounts of Met secreted byproducts. Three independent experiments were performed in duplicate after 24 hours of transfection. NS (Mann-Whitney non-parametric test).



Supplementary Fig 12: Representative Western Blot showing the silencing of ADAM30 expression level after transfection of siRNA in HEK293 (a) and SKNSH-SY5Y (b) over-expressing ADAM30 (n=3).



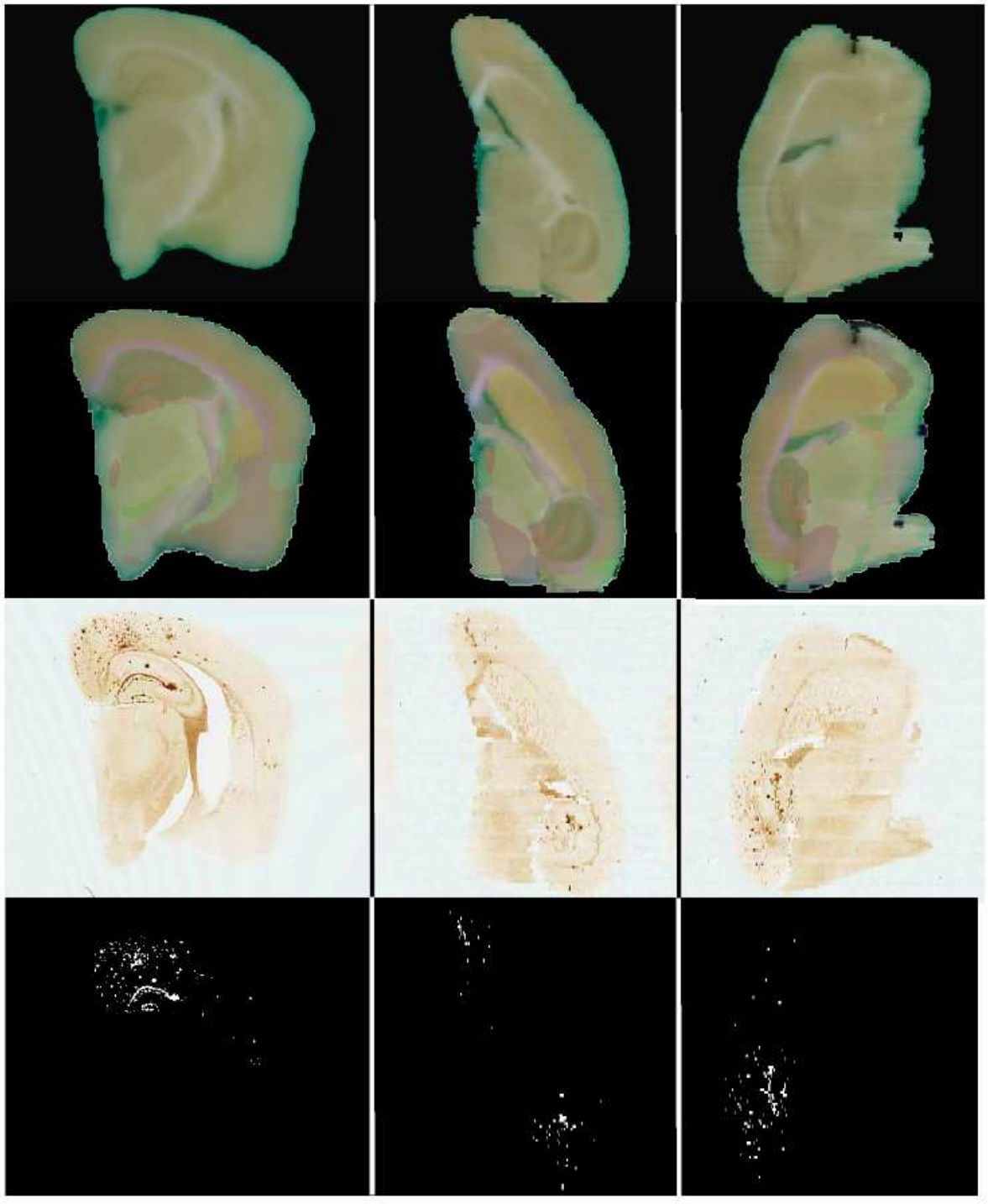
Supplementary Fig 13: Quantification by Q-PCR of NeuN, APP, Cre, ADAM30^{wt} , ADAM30^{mut} or CTSD expression levels in transgenic mice brain.



(b)

Supplementary Fig 14:

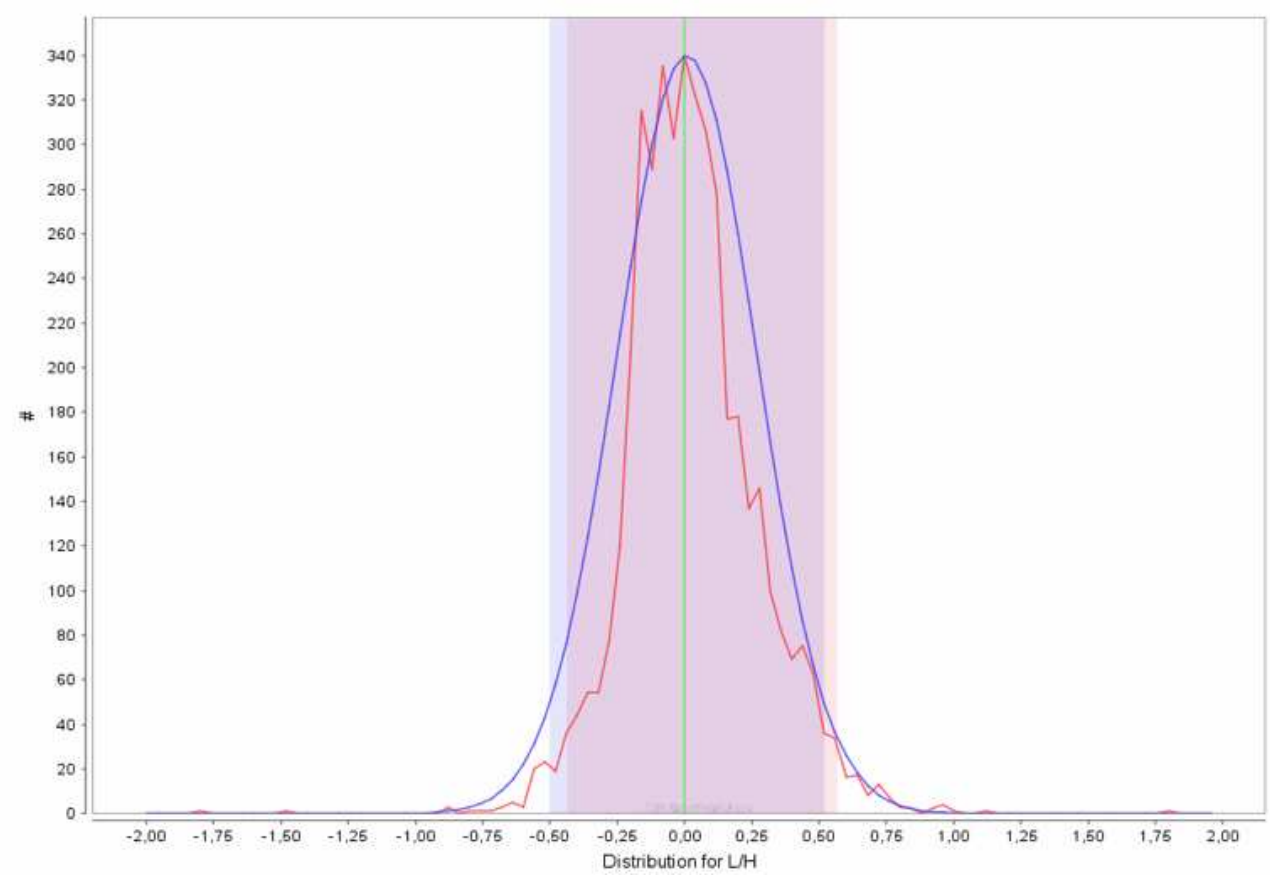
3D histopathology image processing steps. From left to right: coronal, axial and sagittal views. From top to bottom: block-face photography reconstructed volume; digital mouse brain atlas registered on the block-face photography volume; 6E10 immunohistochemistry volume after registration with the block-face photography volume; segmented Aβ deposits in the 6E10 immunohistochemistry volume.



Supplementary Fig 15:

Huber distribution. In red is the real distribution of the peptides. In blue is the statistically normalized distribution.

The darker background indicates the 95% confidence interval and the lighter (broader) background indicates the 99% confidence interval. The 95% confidence interval has been used to determine if a peptide had a deviating ratio from the other peptides.



Supplementary Table 1: Main characteristics of the brain samples used for transcriptomic analyses[5].

AD Case	Gender	Age at death (y)	28S/18S Ratio
AD1	F	68	1.4
AD2	M	86	1.0
AD3	M	67	1.9
AD4	F	66	1.6
AD5	M	66	1.4
AD6	F	84	1.7
AD7	M	77	1.1
AD8	M	71	1.2
AD9	M	65	1.3
AD10	F	64	1.0
AD11	F	85	1.0
AD12	F	77	1.3
	50%	73.0 ±	1.3 ± 0.3

Control	Gender	Age at death (y)	28S/18S Ratio
T1	F	74	1.0
T2	F	72	1.2
T3	M	75	1.0
T4	F	74	1.7
T5	F	70	1.1
T6	M	67	1.4
T7	M	69	1.8
T8	F	73	1.4
T9	F	80	1.1
T10	M	72	1.2
T11	M	78	1.2
T12	M	70	1.5
	50%	72.8 ±	1.3 ± 0.3

Supplementary Table 3: List of primary antibodies used.

Protein	Antibody used
ADAM30wt / mut	ADAM30 antibody [N1N2], N-term *GTX117694, GeneTex, Ivrine, USA*
EEA1	Ms mAb to EEA1 [1G11] *ab70521, Abcam, Cambridge, England*
Rab7	Ms mAb to RAB7 [Rab7 - 117] *ab50533, Abcam, Cambridge, England*
LAMP2	Ms mAb to LAMP2 [H4B4] *ab2563, Abcam, Cambridge, England*
APP	Ms β-Amyloid Antibody (LN27), Santa Cruz Biotechnology, USA Rb APPCter-C17, Ms Beta Amyloid, 1-16 (6E10) , CovanceE
CTSD	MS [CTD-19] to cathepsin D (ab6313) Abcam, *Cambridge, England*

Supplementary table 4: oligonucleotide sequence used for mutagenesis, mice genotyping and qPCRs.

Gene	Forward primer	Reverse primer
Mutagenesis primers		
Adam30-H338L	CTA CCT GGT CTG CTC TTG AGC TGG GTC ATG	CAT GAC CCA GCT CAA GAG CAG ACC AGG TAG
Adam30-H338-H342L	GCT CTT GAG CTG GGT CTT GCT GTA GGA ATG TCA C	GTG ACA TTC CTA CAG CAA GAC CCA GCT CAA GAG C
Adam30-H338-H342L-H348L	GTC TTG CTG TAG GAA TGT CAC TTG ATG AAC AAT ACT GCC AAT G	CAT TGG CAG TAT TGT TCA TCA AGT GAC ATT CCT ACA GCA AGA C
AppΔC8	CGG CTAC GAA AAT CCA ACC TAC TAG TTC TTT GAG CAG ATG CAG	CTG CAT CTG CTC AAA GAA CTA GTA GGT TGG ATT TTC GTA GCC G
App-F690S	CCA ACC TAC AAG TTC TCT GAG CAG ATG CAG AAC TAG	CTA GTT CTG CAT CTG CTC AGA GAA CTT GTA GGT TGG
App-E691V	CCT ACA AGT TCT TTG TGC AGA TGC AGA ACT AGA AGG GCG	CGC CCT TCT AGT TCT GCA TCT GCA CAA AGA ACT TGT AGG
Genotype primers		
Adam30^{wt}	CTG CTC ATG AGC TGG GTC A	GTG GAA CCA CAG TCA CAT TCC
Adam30^{mut}	GCG TTG GAT ATC CAG AGT TAG C	CTA CAG CAA GAC CCA GCT CAA
hApp$^{Swe/Ind}$ (hApp)	TCT TCT TCT TCC ACC TCA GC	GGT GAG TTT GTA AGT GAT GCC
Cre	TGG GCG GCA TGG TGC AAG TT	CCT GCG GTG CTA ACC AGC GTT
Rosa26	CAT GTC TTT AAT CTA CCT CGA TGG	CTC TTC CCT CGT GAT CTG CAA CTC C
QPCR primers		
Gapdh	GGC AAG CCC ATC ACC ATC TT	GCC TTC TCC ATG GTG GTG AA
NeuN	GGC AAT GGT GGG ACT CAA AA	GGG ACC CGC TCC TTC AAC
Adam30^{wt}	TGA TGC TCT TGC ATG GTC GTT TGG	CCT GAG CCC ATG ATG CAA TTA AGC C
Adam30^{mut}	GCG TTG GAT ATC CAG AGT TAG C	CTA CAG CAA GAC CCA GCT CAA
App695^{wt}	TCT TCT TCT TCC ACC TCA GC	GGT GAG TTT GTA AGT GAT GCC
Cre	AAC GCT GGT TAG CAC CGC AGG	CCC TTC CAG GGC GCG AGT TG
Cathepsin D	GCC AAG TTT GAT GGC ATC TTG	AAA GAC CGG AAG CAC GTT GT