Mosser et al., Supplementary Figure 1 (related to Figure 1)

Human RTN4

Uniprot ID: Q9NQC3
Sequence coverage: 5%

MEDLDQSPLV SSSDSPPRPQ PAFKYQFVRE PEDEEEEEEE EEEDEDEDLE ELEVLERKPA
AGLSAAPVPT APAAGAPLMD FGNDFVPPAP RGPLPAAPPV APERQPSWDP SPVSSTVPAP
SPLSAAAVSP SKLPEDDEPP ARPPPPPPAS VSPQAEPVWT PPAPAPAAPP STPAAPKRRG
SSGSVDETLF ALPAASEPVI RSSAENMDLK EQPGNTISAG QEDFPSVLLE TAASLPSLSP
LSAASFKEHE YLGNLSTVLP TEGTLQENVS EASKEVSEKA KTLLIDRDLT EFSELEYSEM
GSSFSVSPKA ESAVIVANPR EEIIVKNKDE EEKLVSNNIL HNQQELPTAL TKLVKEDEVV
SSEKAKDSFN EKRVAVEAPM REEYADFKPF ERVWEVKDSK EDSDMLAAGG KIESNLESKV
DKKCFADSLE QTNHEKDSES SNDDTSFPST PEGIKDRSGA YITCAPFNPA ATESIATNIF
PLLGDPTSEN KTDEKKIEEK KAQIVTEKNT STKTSNPFLV AAQDSETDYV TTDNLTKVTE
EVVANMPEGL TPDLVQEACE SELNEVTGTK IAYETKMDLV QTSEVMQESL YPAAQLCPSF
EESEATPSPV LPDIVMEAPL NSAVPSAGAS VIQPSSSPLE ASSVNYESIK HEPENPPPYE
EAMSVSLKKV SGIKEEIKEP ENINAALQET EAPYISIACD LIKETKLSAE PAPDFSDYSE
MAKVEQPVPD HSELVEDSSP DSEPVDLFSD DSIPDVPQKQ DETVMLVKES LTETSFESMI
EYENKEKLSA LPPEGGKPYL ESFKLSLDNT KDTLLPDEVS TLSKKEKIPL QMEELSTAVY
SNDDLFISKE AQIRETETFS DSSPIEIIDE FPTLISSKTD SFSKLAREYT DLEVSHKSEI
ANAPDGAGSL PCTELPHDLS LKNIQPKVEE KISFSDDFSK NGSATSKVLL LPPDVSALAT
QAEIESIVKP KVLVKEAEKK LPSDTEKEDR SPSAIFSAEL SKTSVVDLLY WRDIKKTGVV
FGASLFLLLS LTVFSIVSVT AYIALALLSV TISFRIYKGV IQAIQKSDEG HPFRAYLESE
VAISEELVQK YSNSALGHVN CTIKELRRLF LVDDLVDSLK FAVLMWVFTY VGALFNGLTL
LILALISLFS VPVIYERHQA QIDHYLGLAN KNVKDAMAKI QAKIPGLKRK AE

Human HSPA5

Uniprot ID: P11021
Sequence coverage: 58%

MKLSLVAAML LLLSAARAEE EDKKEDVGTV VGIDLGTTYS CVGVFKNGRV EIIANDQGNR
ITPSYVAFTP EGERLIGDAA KNQLTSNPEN TVFDAKRLIG RTWNDPSVQQ DIKFLPFKVV
EKKTKPYIQV DIGGGQTKTF APEEISAMVL TKMKETAEAY LGKKVTHAVV TVPAYFNDAQ
RQATKDAGTI AGLNVMRIIN EPTAAAIAYG LDKREGEKNI LVFDLGGGTF DVSLLTIDNG
VFEVVATNGD THLGGEDFDQ RVMEHFIKLY KKKTGKDVRK DNRAVQKLRR EVEKAKRALS
SQHQARIEIE SFYEGEDFSE TLTRAKFEEL NMDLFRSTMK PVQKVLEDSD LKKSDIDEIV
LVGGSTRIPK IQQLVKEFFN GKEPSRGINP DEAVAYGAAV QAGVLSGDQD TGDLVLLDVC
PLTLGIETVG GVMTKLIPRN TVVPTKKSQI FSTASDNQPT VTIKVYEGER PLTKDNHLLG
TFDLTGIPPA PRGVPQIEVT FEIDVNGILR VTAEDKGTGN KNKITITNDQ NRLTPEEIER
MVNDAEKFAE EDKKLKERID TRNELESYAY SLKNQIGDKE KLGGKLSSED KETMEKAVEE
KIEWLESHQD ADIEDFKAKK KELEEIVQPI ISKLYGSAGP PPTGEEDTAE KDEL

Human APMAP1

Uniprot ID: Q9HDC9
Sequence coverage: 16%

MSEADGLRQR RPLRPQVVTD DDGQAPEAKD GSSFSGRVFR VTFLMLAVSL TVPLLGAMML
LESPIDPQPL SFKEPPLLLG VLHPNTKLRQ AERLFENQLV GPESIAHIGD VMFTGTADGR
VVKLENGEIE TIARFGSGPC KTRDDEPVCG RPLGIRAGPN GTLFVADAYK GLFEVNPWKR
EVKLLLSSET PIEGKNMSFV NDLTVTQDGR KIYFTDSSSK WQRRDYLLLV MEGTDDGRLL
EYDTVTREVK VLLDQLRFPN GVQLSPAEDF VLVAETTMAR IRRVYVSGLM KGGADLFVEN
MPGFPDNIRP SSSGGYWVGM STIRPNPGFS MLDFLSERPW IKRMIFKLFS QETVMKFVPR
YSLVLELSDS GAFRRSLHDP DGLVATYISE VHEHDGHLYL GSFRSPFLCR LSLQAV

Human ATP1α1
Uniprot ID: P05023
Sequence coverage: 29%

MGKGVGRDKY EPAAVSEQGD KKGKKGKKDR DMDELKKEVS MDDHKLSLDE LHRKYGTDLS
RGLTSARAAE ILARDGPNAL TPPPTTPEWI KFCRQLFGGF SMLLWIGAIL CFLAYSIQAA
TEEEPQNDNL YLGVVLSAVV IITGCFSYYQ EAKSSKIMES FKNMVPQQAL VIRNGEKMSI
NAEEVVVGDL VEVKGGDRIP ADLRIISANG CKVDNSSLTG ESEPQTRSPD FTNENPLETR
NIAFFSTNCV EGTARGIVVY TGDRTVMGRI ATLASGLEGG QTPIAAEIEH FIHIITGVAV
FLGVSFFILS LILEYTWLEA VIFLIGIIVA NVPEGLLATV TVCLTLTAKR MARKNCLVKN
LEAVETLGST STICSDKTGT LTQNRMTVAH MWFDNQIHEA DTTENQSGVS FDKTSATWLA
LSRIAGLCNR AVFQANQENL PILKRAVAGD ASESALLKCI ELCCGSVKEM RERYAKIVEI
PFNSTNKYQL SIHKNPNTSE PQHLLVMKGA PERILDRCSS ILLHGKEQPL DEELKDAFQN
AYLELGGLGE RVLGFCHLFL PDEQFPEGFQ FDTDDVNFPI DNLCFVGLIS MIDPPRAAVP
DAVGKCRSAG IKVIMVTGDH PITAKAIAKG VGIISEGNET VEDIAARLNI PVSQVNPRDA
KACVVHGSDL KDMTSEQLDD ILKYHTEIVF ARTSPQQKLI IVEGCQRQGA IVAVTGDGVN
DSPALKKADI GVAMGIAGSD VSKQAADMIL LDDNFASIVT GVEEGRLIFD NLKKSIAYTL
TSNIPEITPF LIFIIANIPL PLGTVTILCI DLGTDMVPAI SLAYEQAESD IMKRQPRNPK
TDKLVNERLI SMAYGQIGMI QALGGFFTYF VILAENGFLP IHLLGLRVDW DDRWINDVED
SYGQQWTYEQ RKIVEFTCHT AFFVSIVVVQ WADLVICKTR RNSVFQQGMK NKILIFGLFE
ETALAAFLSY CPGMGVALRM YPLKPTWWFC AFPYSLLIFV YDEVRKLIIR RRPGGWVEKE
TYY

Human Tspan6
Uniprot ID: O43657
Sequence coverage: 22%

MASPSRRLQT KPVITCFKSV LLIYTFIFWI TGVILLAVGI WGKVSLENYF SLLNEKATNV
PFVLIATGTV IILLGTFGCF ATCRASAWML KLYAMFLTLV FLVELVAAIV GFVFRHEIKN
SFKNNYEKAL KQYNSTGDYR SHAVDKIQNT LHCCGVTDYR DWTDTNYYSE KGFPKSCCKL
EDCTPQRDAD KVNNEGCFIK VMTIIESEMG VVAGISFGVA CFQLIIFLAY CLSRAITNNQ
YEIV

Human Vamp2
Uniprot ID: P63027
Sequence coverage: 21%

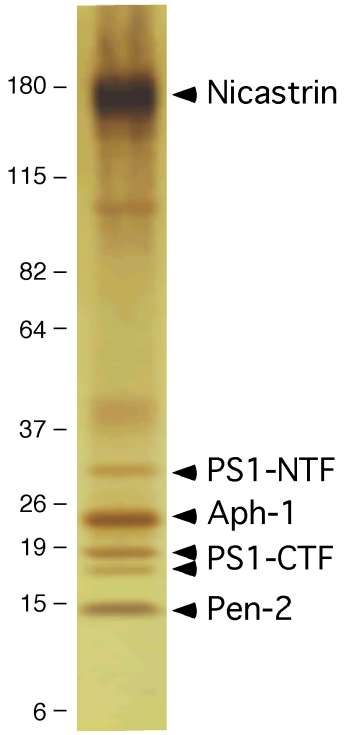
MSATAATAPP AAPAGEGGPP APPPNLTSNR RLQQTQAQVD EVVDIMRVNV DKVLERDQKL
SELDDRADAL QAGASQFETS AAKLKRKYWW KNLKMMIILG VICAIILIII IVYFST

Human Vamp3
Uniprot ID: Q15836
Sequence coverage: 24%

MSTGPTAATG SNRRLQQTQN QVDEVVDIMR VNVDKVLERD QKLSELDDRA DALQAGASQF
ETSAAKLKRK YWWKNCKMWA IGITVLVIFI IIIIVWVVSS

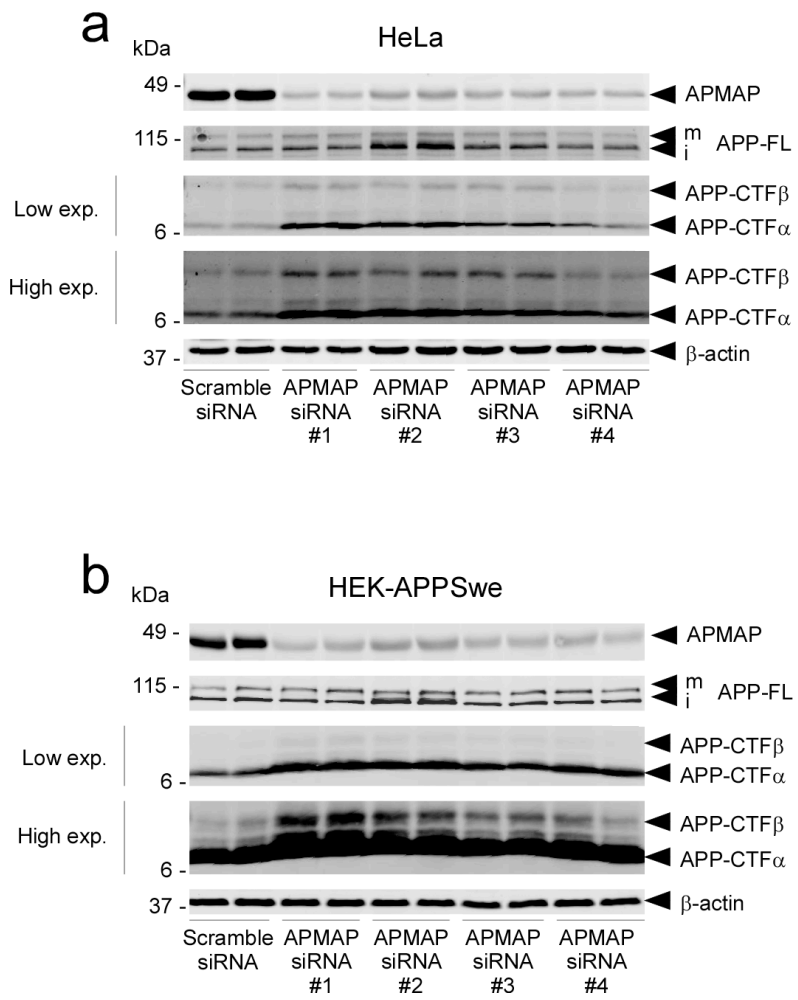
Sup. Fig. 1. Mass spectrometric identification of γ-secretase-interacting proteins. Peptides identified by LC-MS/MS after tryptic digestion of γ-secretase purified from CHO cells are highlighted in yellow in the primary human sequences. Trypsin cleavage sites are indicated in bold.

Mosser et al., Supplementary Figure 2 (related to Figure 1)



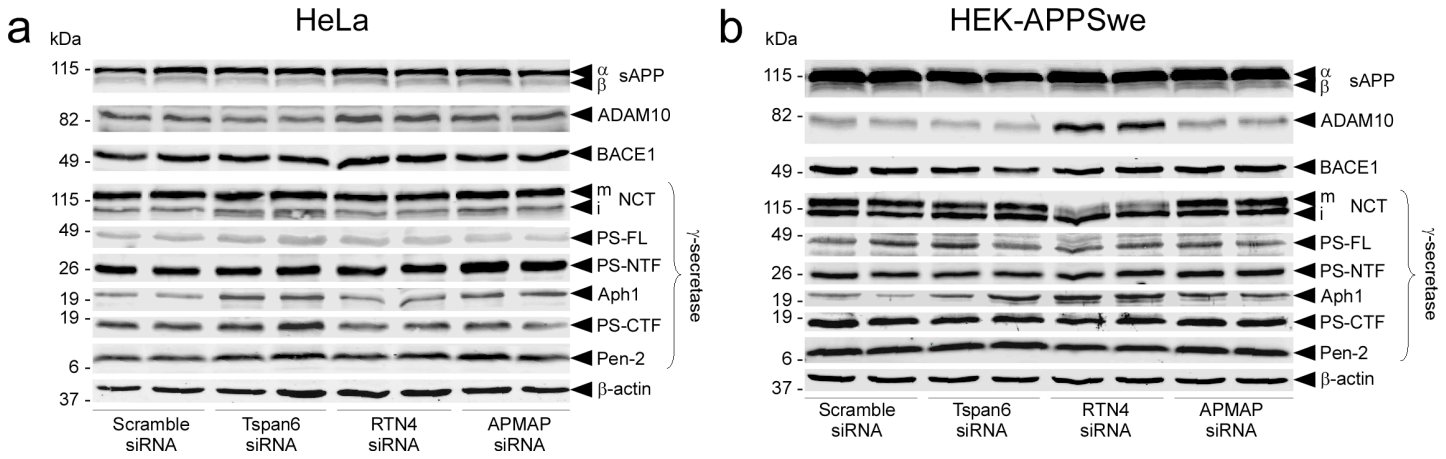
Sup. Fig. 2. Characterization by SDS-PAGE of purified γ-secretase used for the BN-PAGE analysis in Fig. 1a. γ-Secretase purified as described in the Methods section was analyzed by silver-stained SDS-PAGE on a NuPAGE Novex® 4-12% Bis-Tris gel (Life technologies). Western blot and mass spectrometric analyses confirmed that predominant proteins are γ-secretase subunits (labeled bands).

Mosser et al., Supplementary Figure 3 (related to Figure 1)



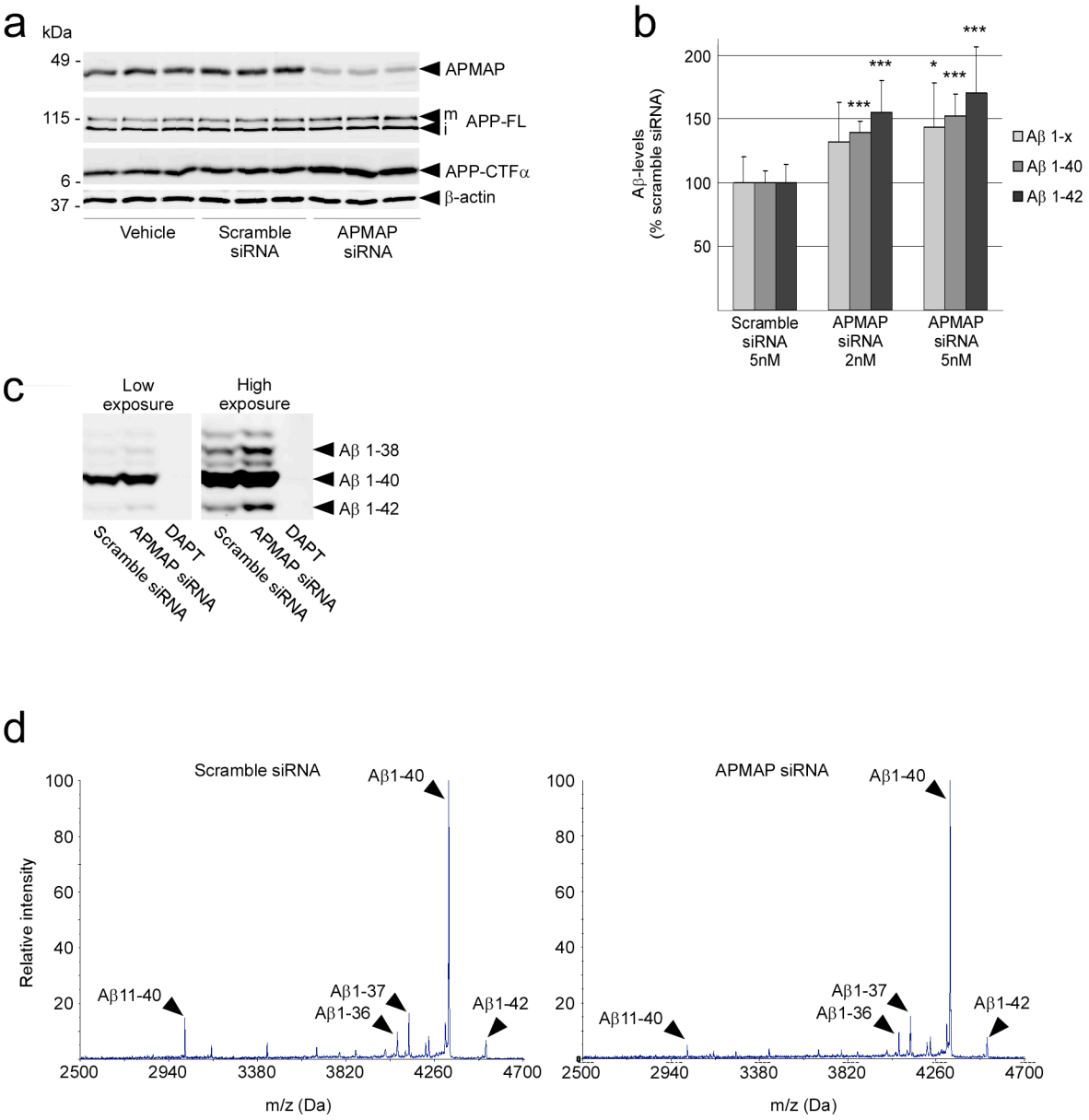
Sup. Fig. 3. Partial depletion of APMAP by different siRNAs raises the levels of APP-CTFs. HeLa cells (a) or HEK-APPSwe cells (b) were treated for 3 days with either negative control scramble siRNA or APMAP siRNA 1 (5'-TTCACCGATTCTAGCAGCAAA-3'), APMAP siRNA 2 (5'-UGAAGUAAAUCCCUGGAAA-3'), APMAP siRNA 3 (5'-GCAGAAAGGCUGUUUGAAA-3'), or APMAP siRNA 4 (5'-GGAAGAACAUGUCCUUUGU-3') duplexes. APMAP siRNA 1 was used for experiments described in Figs 1 & 3. β-Actin served as a protein loading control. mAPP-FL and iAPP-FL: mature and immature APP full-length.

Mosser et al., Supplementary Figure 4 (related to Figure 1)



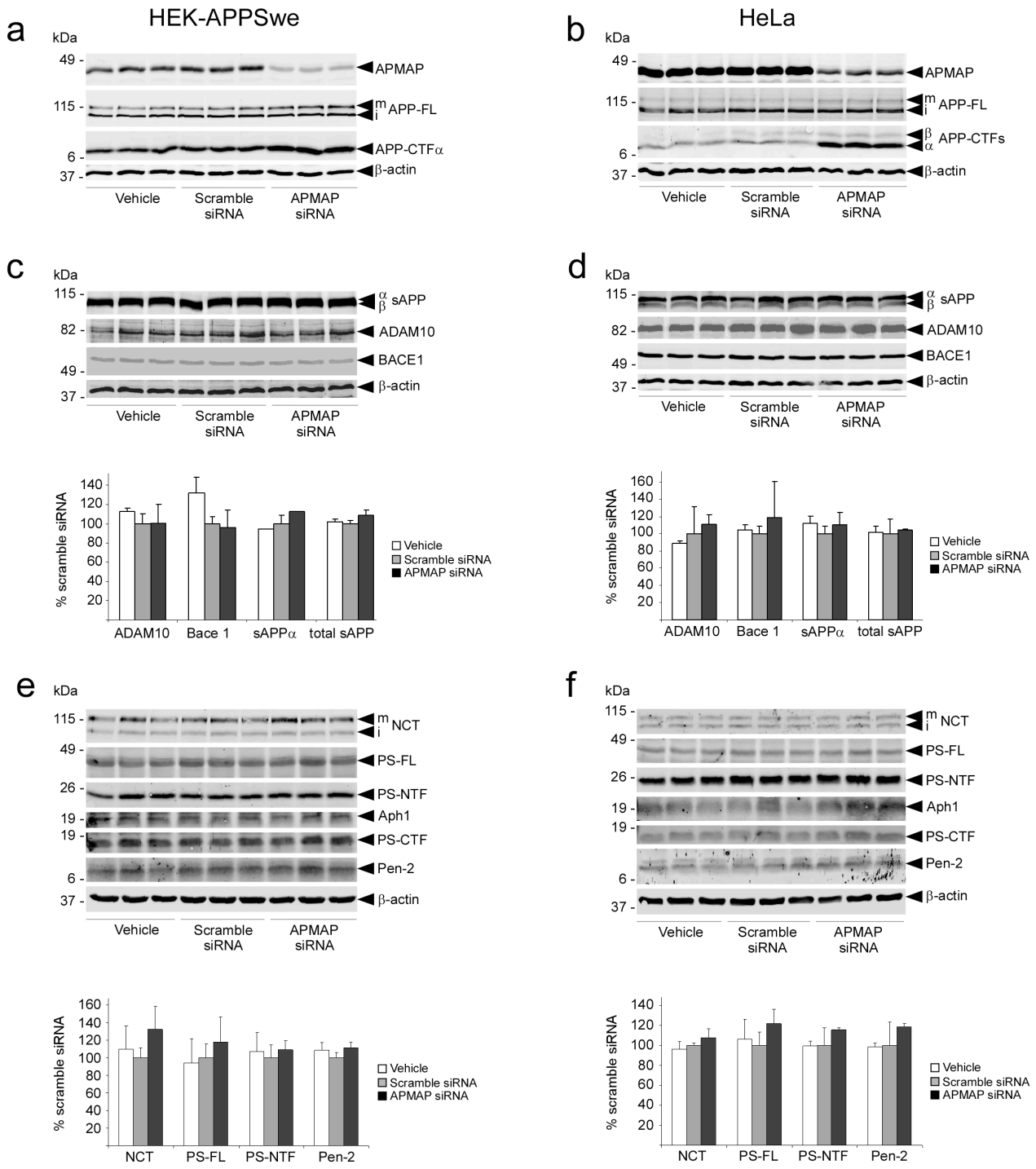
Sup. Fig. 4. Partial depletion of Tspan6, RTN4, and APMAP does not affect the maturation or protein levels of α-, β-, and γ-secretases. The exception to this result was RTN4, in which reduced levels are associated with increased ADAM10 levels. a) HeLa cells; b) HEK-APPSwe cells. β-Actin served as a protein loading control. mNCT and iNCT: mature and immature Nicastrin.

Mosser et al., Supplementary Figure 5 (related to Figure 1)



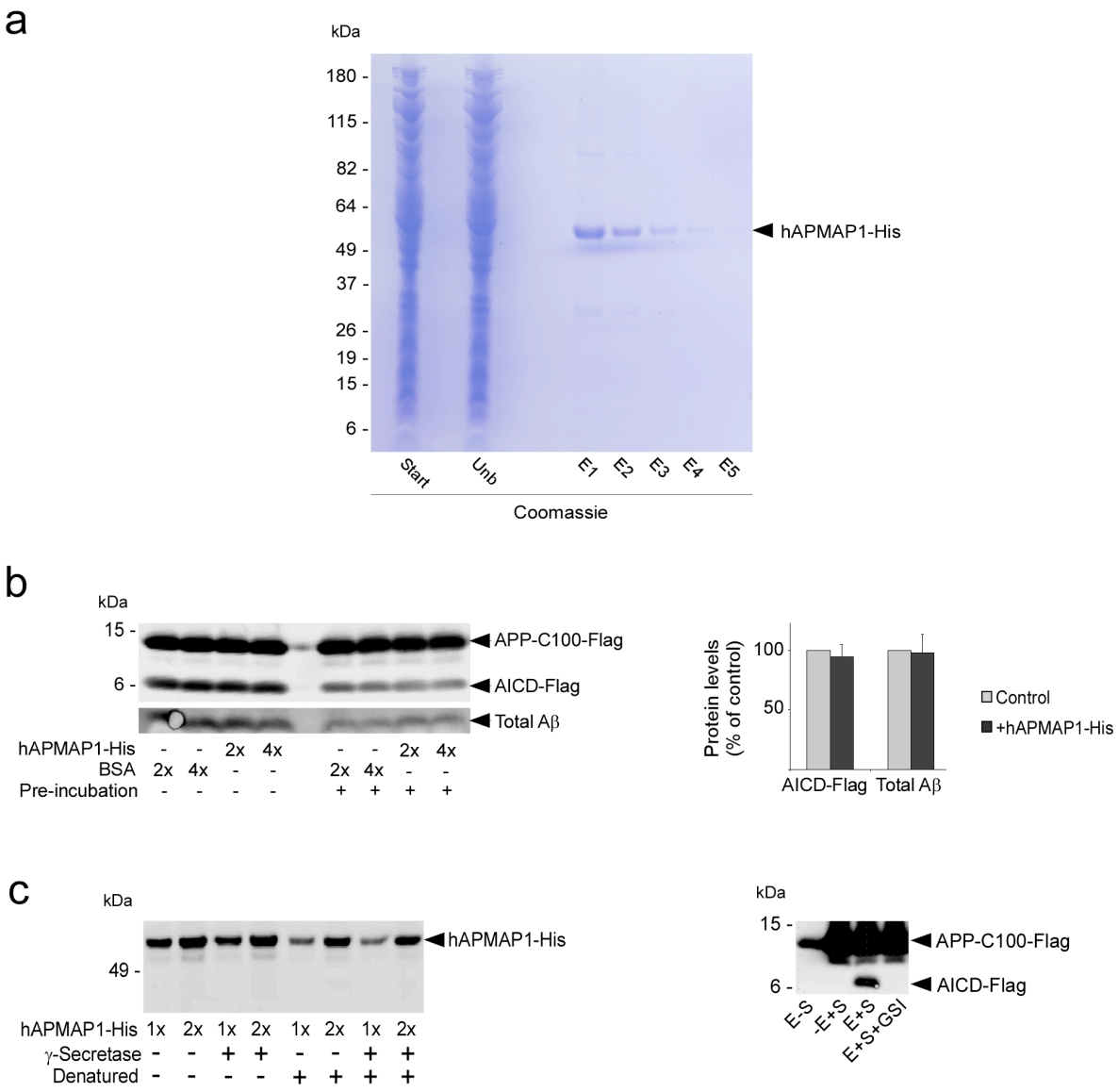
Sup. Fig. 5. APMAP is a negative regulator of Aβ production. In HEK-APPSwe cells treated with APMAP siRNA, increased levels of APP-CTFs (a) are associated with increased secretion of total Aβ (Aβ1-x), Aβ1-40, and Aβ1-42, as determined by either ELISA (b) or Western blot on a urea gel (c). The mass spectrometric analysis of secreted Aβ revealed no changes in the profiles of these peptides (d). (a) Biological triplicates are shown. (b): Student's *t*-test was applied for statistical analyses; mean +/- SD; *P<0.05; ***P<0.001; n=4/group. β-Actin served as a protein loading control.

Mosser et al., Supplementary Figure 6 (related to Figure 1)



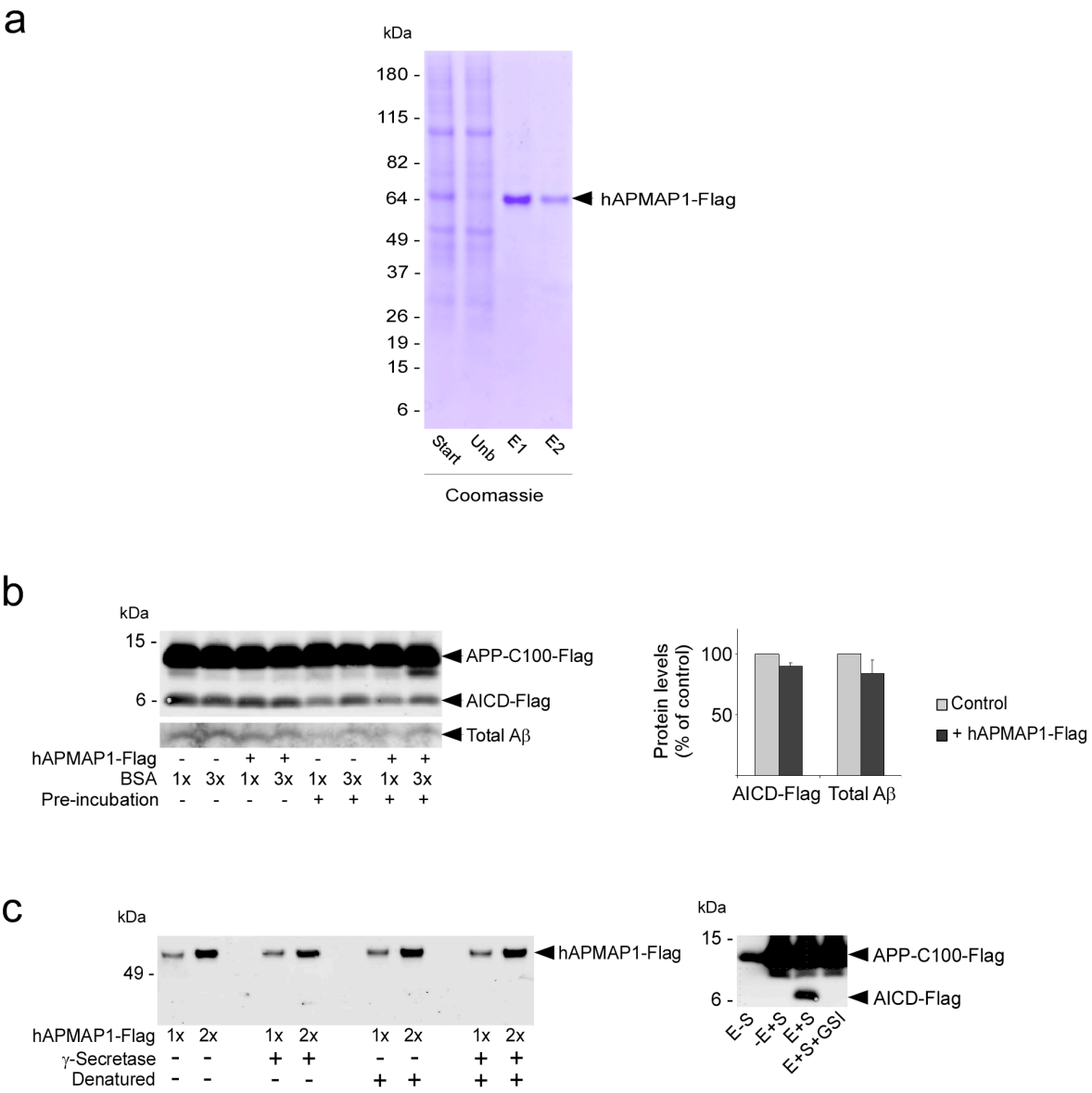
Sup. Fig. 6. The depletion of APMAP in HEK-APPSwe and HeLa cells does not affect the maturation or protein levels of α-, β-, and γ-secretases. Levels of ADAM10, BACE1, and γ-secretase components are shown in HEK-APPSwe cells (a, c, e) or HeLa cells (b, d, f) treated with either negative control scramble siRNA or APMAP siRNA. Biological triplicates are shown. (c-f) Bands revealed by Western blot analysis (upper panels) were quantified by densitometry (lower panels). Student's *t*-test was applied for statistical analyses (mean +/- SD), and revealed no significant differences in ADAM10, BACE1, γ-secretase, and sAPPα/β protein levels.

Mosser et al., Supplementary Figure 7 (related to Figure 1)



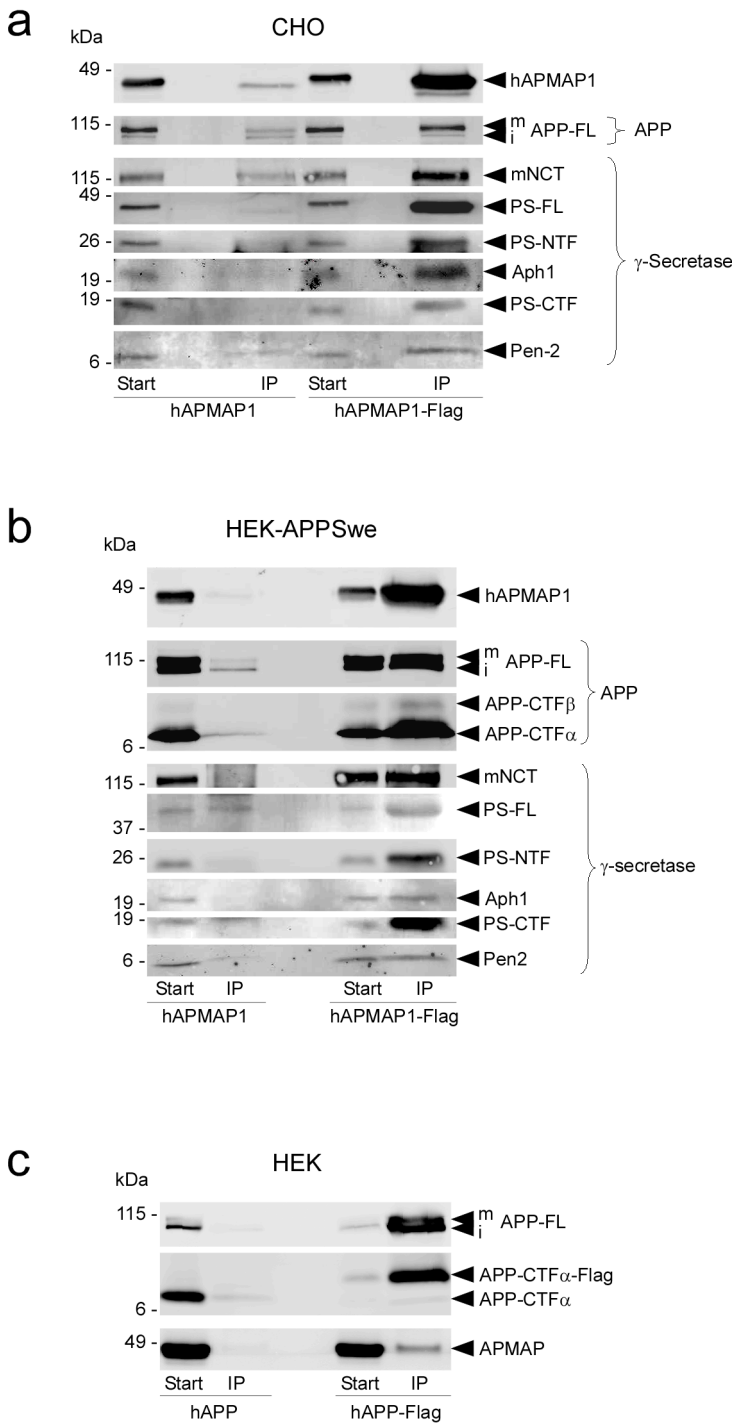
Sup. Fig. 7. APMAP1 purified from *E. coli* is not a substrate for γ-secretase and does not affect the processing of APP-CTFβ by purified γ-secretase. a) Affinity purification of human APMAP-His6 expressed in *E. coli*. The starting material (Start), unbound fraction (Unb), and five elution fractions (E1-5) were resolved by SDS-PAGE on a Tris-glycine gel stained with Coomassie blue. b) Cell-free γ-secretase activity assays with increasing concentrations of purified hAPMAP1-His6 (2x, 4x), pre-incubated (+) or not (-), at 37°C for 60 min before the addition of the substrate APP-C100-Flag. Bands detected by Western blot analysis (left panel) were quantified by densitometry (right panel). Student's *t*-test was applied for statistical analyses (mean +/- SD; n=4/group), and revealed no significant differences in AICD-Flag or total Aβ levels. c) Purified hAPMAP1-His6 is not a substrate for γ-secretase. Left panel: Increasing concentrations of purified hAPMAP1-His6 (1x, 2x), denatured at 65°C in 0.5% SDS (+) or not (-), were incubated for 4 h at 37°C in the presence (+) or absence (-) of purified γ-secretase and phospholipids (PC+PE). Right panel: APP-C100-Flag served as a positive control for γ-secretase activity. E-S: Enzyme in the absence of substrate; -E+S: Substrate in the absence of enzyme; E+S: Enzyme in the presence of substrate; E+S+GSI: Enzyme in the presence of substrate and 10μM γ-secretase inhibitor (GSI) DAPT. Cleavage products were detected with an anti-His6 antibody (hAPMAP1-His6) or anti-Flag antibody (APP-C100-Flag).

Mosser et al., Supplementary Figure 8 (related to Figure 1)



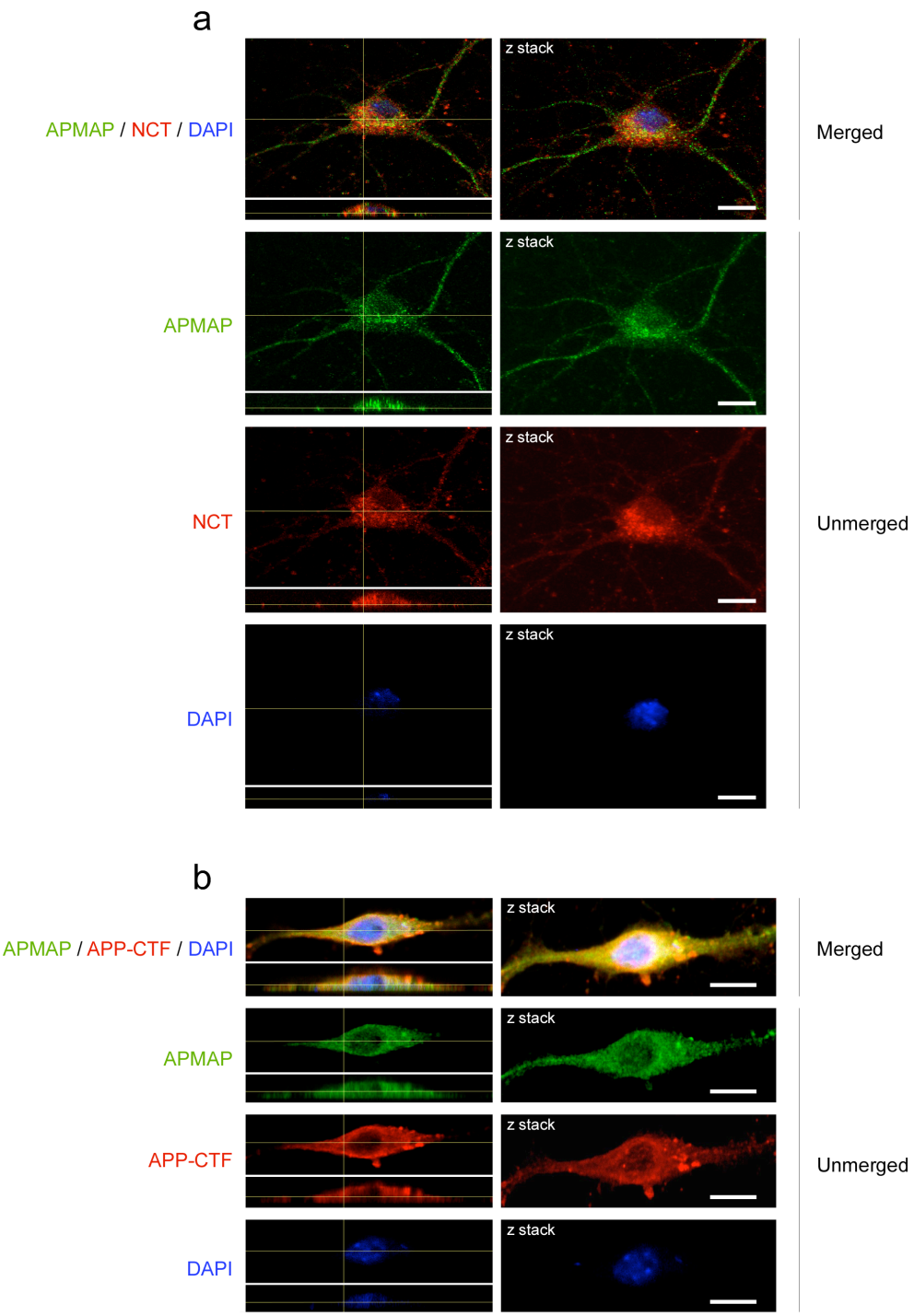
Sup. Fig. 8. APMAP1 purified from CHO cells is not a substrate for γ-secretase and does not affect the processing of APP-CTFβ by purified γ-secretase. a) Affinity purification of human APMAP1-Flag stably overexpressed in CHO cells. The starting material (Start), unbound fraction (Unb), and two elution fractions (E1-2) were resolved by SDS-PAGE on a Tris-glycine gel stained with Coomassie blue. b) Cell-free γ-secretase activity assays with increasing concentrations of purified hAPMAP1-Flag (1x, 3x), pre-incubated (+) or not (-) at 37°C for 60 min before addition of the substrate APP-C100-Flag. Bands detected by Western blot analysis (left panel) were quantified by densitometry (right panel). Student's *t*-test was applied for statistical analyses (mean +/- SD; n=4/group), and revealed no significant differences in AICD-Flag or total Aβ levels. c) Purified hAPMAP1-Flag is not a substrate for γ-secretase. Left panel: Increasing concentrations of purified hAPMAP1-Flag (1x, 2x), denatured at 65°C in 0.5% SDS (+) or not (-), were incubated for 4 h at 37°C in the presence (+) or absence (-) of purified γ-secretase and phospholipids (PC+PE). Right panel: APP-C100-Flag served as a positive control for γ-secretase activity. E-S: Enzyme in the absence of substrate; -E+S: Substrate in the absence of enzyme; E+S: Enzyme in the presence of substrate; E+S+GSI: Enzyme in the presence of substrate and 10μM γ-secretase inhibitor (GSI) DAPT. Cleavage products were detected with an anti-Flag antibody.

Mosser et al., Supplementary Figure 9 (related to Figure 2)



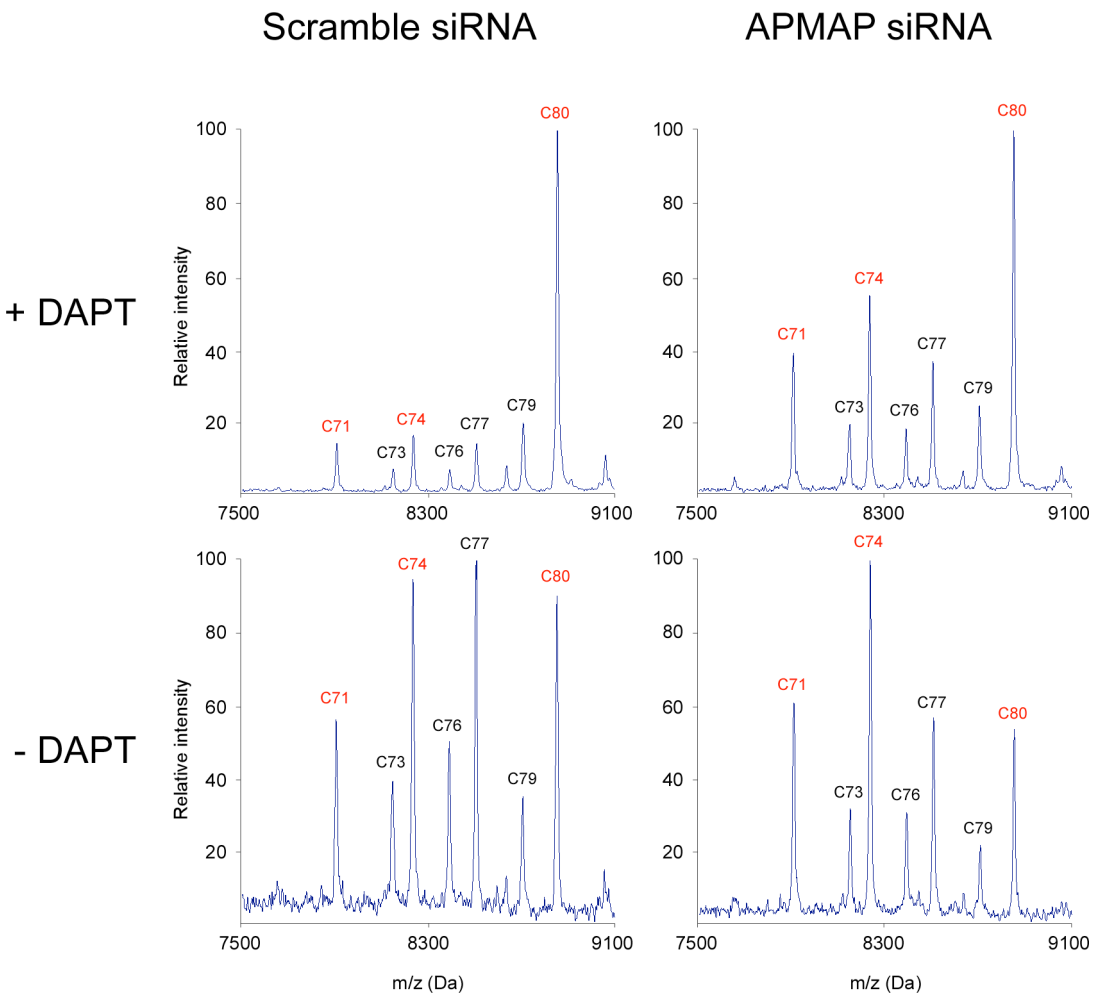
Sup. Fig. 9. APMAP interacts physically with γ-secretase, APP-FL, and APP-CTFs. Co-immunoprecipitation with anti-Flag beads of all γ-secretase components, APP-FL, and APP-CTFs with a Flag-tagged version of hAPMAP1 (hAPMAP1-Flag) overexpressed in CHO cells (a) or HEK-APPSwe cells (b). Untagged APMAP (hAPMAP1) served as a control for the specific co-precipitation. (c) Co-immunoprecipitation of endogenous APMAP with a Flag-tagged version of hAPP (hAPP-Flag) overexpressed in HEK cells. Untagged APP (hAPP) served as a control for the specific co-precipitation.

Mosser et al., Supplementary Figure 10 (related to Figure 2)



Sup. Fig. 10. Immunohistochemical co-localization of APMAP (green) with the γ-secretase subunit Nicastrin (red, panel a) or APP (red, panel b) in 14 days *in vitro* mouse primary cortical neurons. Scale bar: 10 μm. Both confocal images (left panels) and Z-stack projections (right panels) are shown with a microscopy objective magnification of 40X. Un-merged images for APMAP, NCT, APP-CTFs, and DAPI are also shown for comparison.

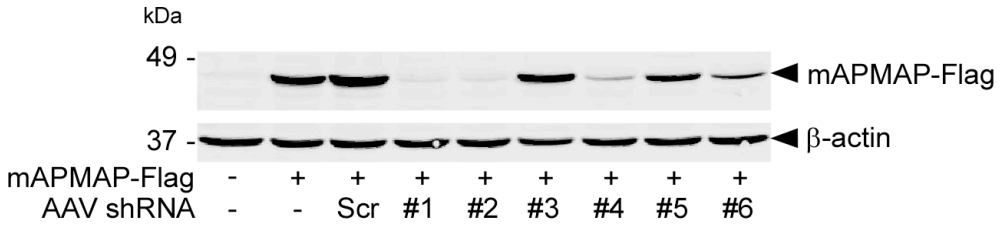
Mosser et al., Supplementary Figure 11 (related to Figure 3)



Sup. Fig. 11. APMAP controls the stability of APP-CTFs. MALDI-TOF mass spectrometric analysis of endogenous APP-CTFs immunoprecipitated from HeLa cells treated with scramble or APMAP siRNAs, in the presence (+ DAPT) or absence (- DAPT) of 1 μM of the γ-secretase inhibitor DAPT. Main changes (increased APP-C71:APP-C80 and APP-C74:APP-C80 ratios) are highlighted in red.

Mosser et al., Supplementary Figure 12 (related to Figure 4)

a

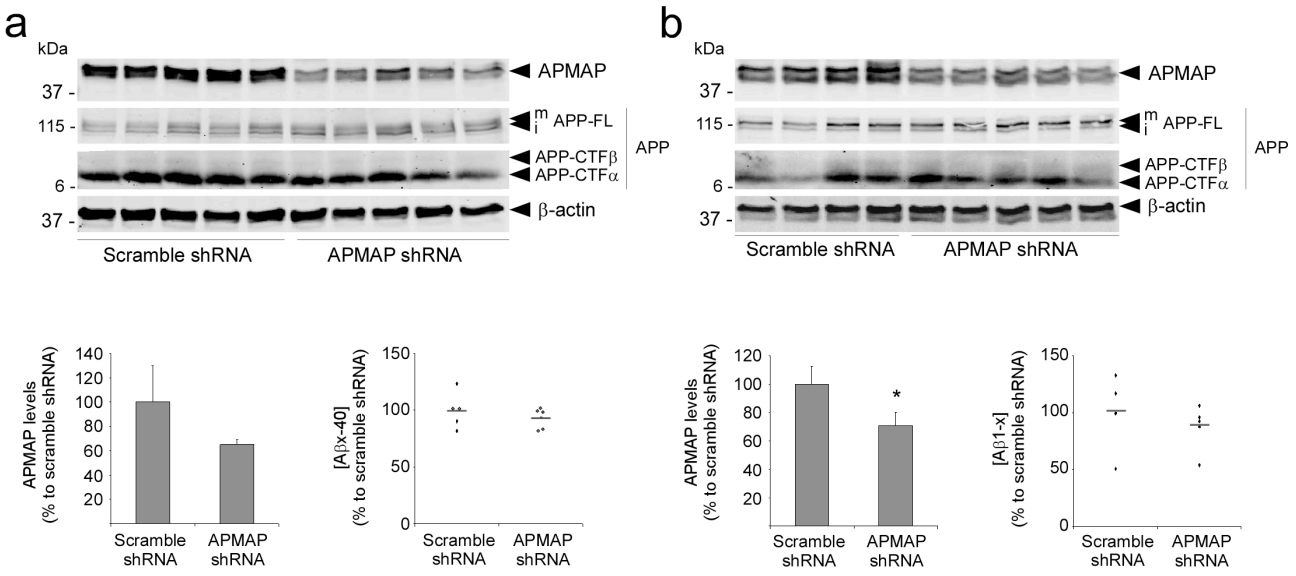


b

shRNA #	shRNA sequence (sense-loop-antisense)
Scramble	5'-CAACAAGATGAAGAGCACCAA-GTGTGCTGTCC-TTGGTGCTCTTCATCTTGTTG-3'
1*	5'-TGGACCAGAATCCATAGTAAA-GTGTGCTGTCC-TTTACTATGGATTCTGGTCCA-3'
2*	5'-TGAAGTTTGTGCCACGATATA-GTGTGCTGTCC-TATATCGTGGCACAAACTTCA-3'
3	5'-ACCAACTTAGTGGACCAGA-GTGTGCTGTCC-TCTGGTCCACTAAGTTGGT-3'
4	5'-ACGAAGTTGCGGCAAGCAG-GTGTGCTGTCC-CTGCTTGCCGCAACTTCGT-3'
5	5'-TCGAAGTAAATCCTCAGAA-GTGTGCTGTCC-TTCTGAGGATTTACTTCGA-3'
6	5'-GAAAGCCATTTCTCTTTAA-GTGTGCTGTCC-TTAAAGAGAAATGGCTTTC-3'

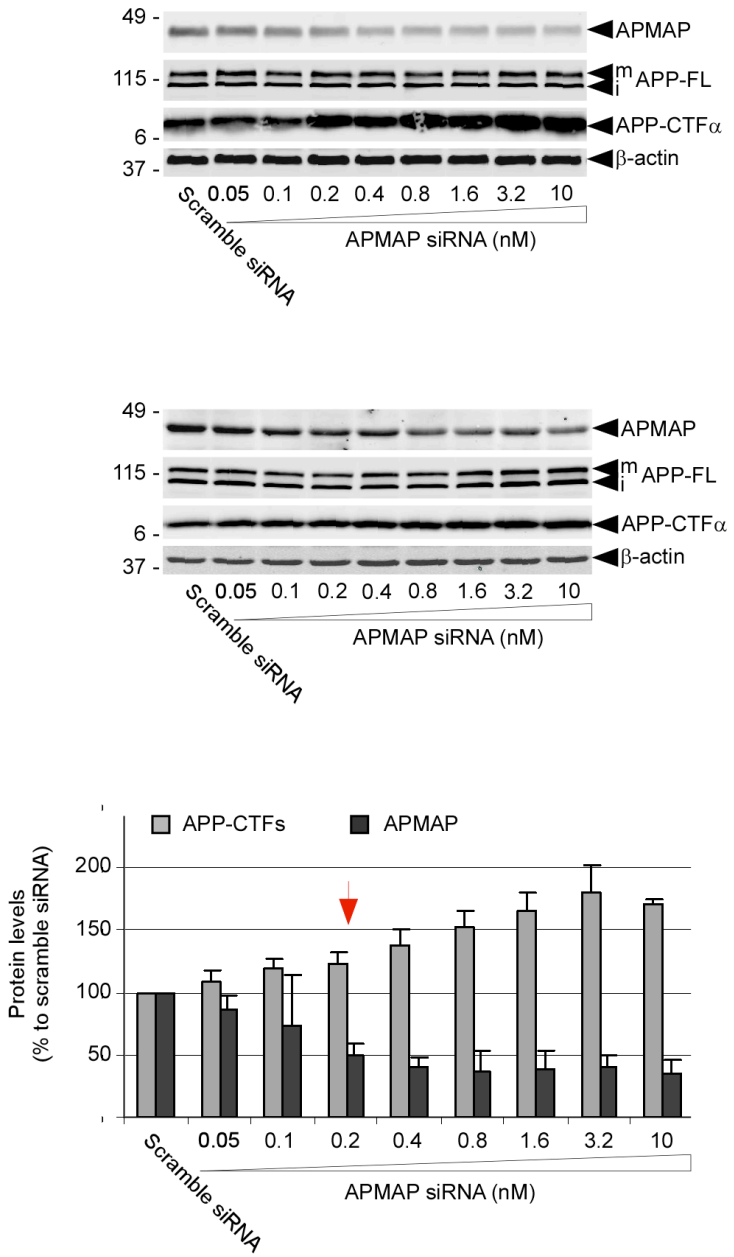
Sup. Fig. 12. Validation of shRNAs targeting mouse APMAP in HEK cells. (a) In this validation experiment, mouse APMAP-Flag was co-expressed in HEK cells (+) with a scramble shRNA (Scr) or with an AAV9-specific vector encoding six different shRNAs targeting mAPMAP (#1-6). After 48 h of culture, cells were collected, and the levels of mAPMAP-Flag were estimated by Western blot analysis with an anti-Flag antibody. (b) Sequences for all shRNAs targeting mAPMAP, tested in HEK cells. The shRNAs # 1 and 2 (labelled with an asterisk) were used for the *in vivo* experiments in WT and APP/PS1 mice, respectively (Fig.4).

Mosser et al., Supplementary Figure 13 (related to Figure 4)



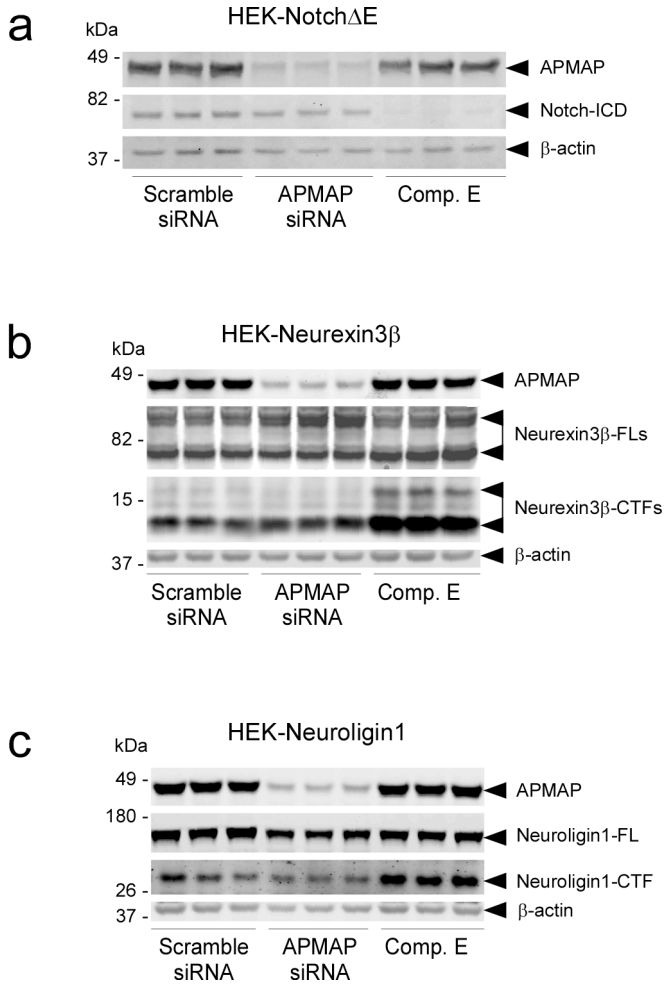
Sup. Fig. 13. APMAP does not affect Aβ production in wild-type (a) or APP/PS1 transgenic (b) female mice. Five-week-old animals were injected bilaterally in the dorsal hippocampus with AAV9 expressing APMAP shRNA or a scrambled shRNA, together with a GFP reporter. Four weeks post-injection, wild-type (a) or APP/PS1 (b) females displayed a reduction in APMAP expression (mean +/- SD; *P<0.05; n=4-5/group), associated with unchanged Aβ levels. Student's *t*-test was applied for statistical analyses. β-Actin served as a protein loading control.

Mosser et al., Supplementary Figure 14 (related to Figure 4)



Sup. Fig. 14. Dose-dependent depletion of APMAP1 and accumulation of APP-CTFs. HEK-APPSwe cells were treated for 3 days with either negative control scramble siRNA or increased concentrations of APMAP siRNA. The two upper panels show Western blots from two independent experiments. The densitometric analysis of APMAP and APP-CTF Western blot bands (lower panel) revealed a critical step for APMAP depletion at ~50% depletion (red arrow), above which small changes in APMAP expression are associated with big changes in APP-CTFs accumulation. β-Actin served as a protein loading control.

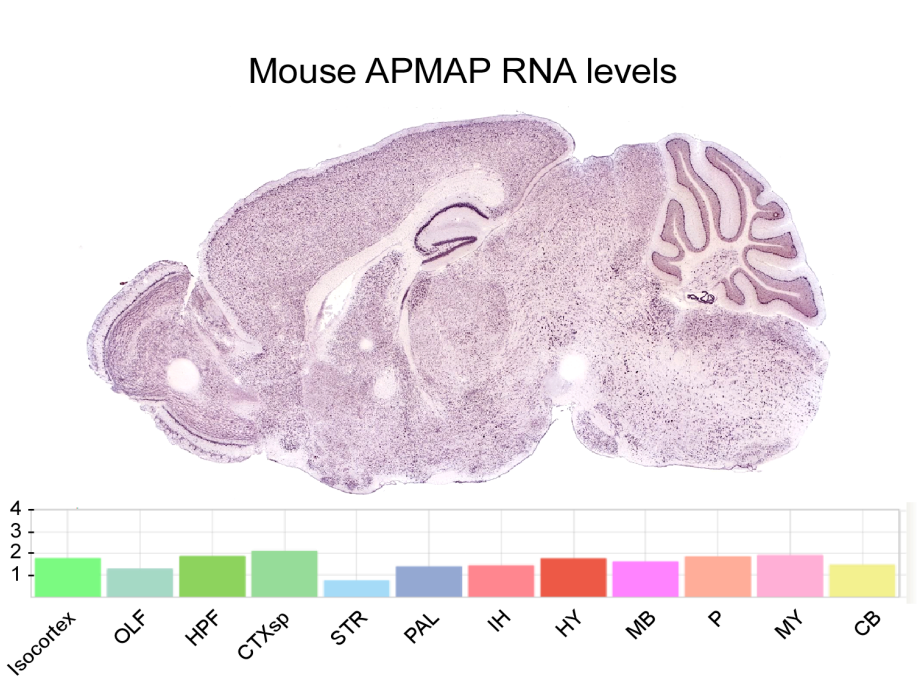
Mosser et al., Supplementary Figure 15 (related to the discussion)



Sup. Fig. 15. APMAP does not affect the processing of the Notch-1 receptor or the synaptic cell adhesion proteins Neurexin3β and Neuroligin1. In contrast to the γ-secretase inhibitor Compound E (Comp. E), APMAP knockdown did not interfere (a) with the processing of the γ-secretase substrate NotchΔE and Notch-intracellular domain (Notch-ICD) production in HEK-N7 stable cells, or (b, c) with the processing of the γ-secretase substrates Neurexin3β and Neuroligin1 transiently expressed in HEK cells. β-Actin served as a protein loading control.

Mosser et al., Supplementary Figure 16 (related to the discussion)

a



b

Human APMAP1/2 protein levels

Central nervous system (Brain)		
Cerebral cortex	Neuronal cells	
Cerebral cortex	Endothelial cells	
Cerebral cortex	Glial cells	
Cerebral cortex	Neuropil	
Hippocampus	Neuronal cells	
Hippocampus	Glial cells	
Lateral ventricle	Neuronal cells	
Lateral ventricle	Glial cells	
Cerebellum	Purkinje cells	
Cerebellum	Cells in granular layer	
Cerebellum	Cells in molecular layer	

Level of antibody staining/expression

High

Medium

Low

Not detected

Sup. Fig. 16. APMAP gene and protein expression patterns in mouse and human brains. (a) APMAP gene expression maps in the mouse brain, according to the Allen Brain Atlas (www.brain-map.org/). (b) APMAP protein expression maps in the human brain, according to the Human Protein Atlas (www.proteinatlas.org/).