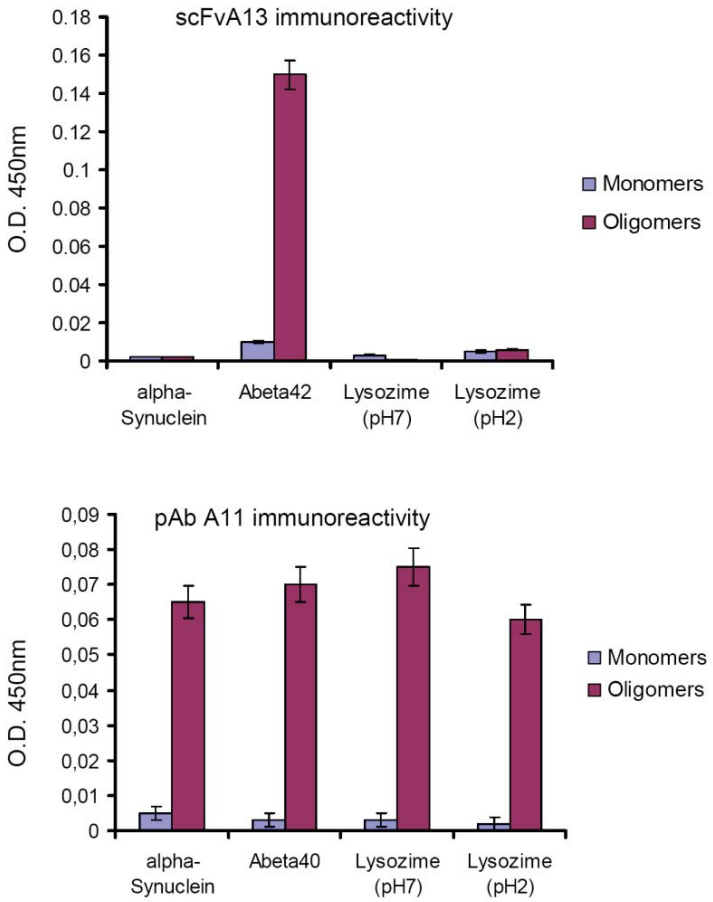


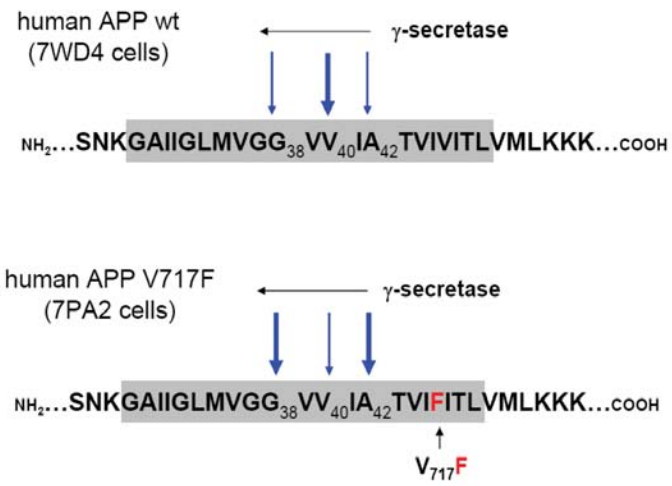
Supplementary Figure 1

The scFvA13 is specific for AβOs and does not react with other aggregating proteins.

ELISA against monomers and oligomers of human α-synuclein, human Aβ and Lysozime by using the anti-AβOs scFvA13[1] (upper panel), or the generic anti-oligomer polyclonal antibody (pAb) A11[2] (lower panel) as primary antibodies. The scFvA13 is revealed through its C-terminal V5 tag. The scFvA13 is both conformation-sensitive, due the selective recognition of Aβ Oligomers in comparison to Aβ monomers[1] and sequence-specific, due to the lack of cross-reactivity with oligomers and monomers of other proteins, according to results of DotBlot assay (see **Fig.1a**). As a control, the well-established generic anti-oligomer A11 cross-reacts with all the proteins, distinguishing only between oligomers or monomers, and attesting the oligomeric state of the assayed proteins.

The ELISA was performed as previously described[1] by coating in PBS (pH7.4) at 4°C overnight, 90ng/well of different proteins (1.3μg/ml). Oligomers of Aβ, α-Synuclein and Lysozime are prepared as described in the Methods section.

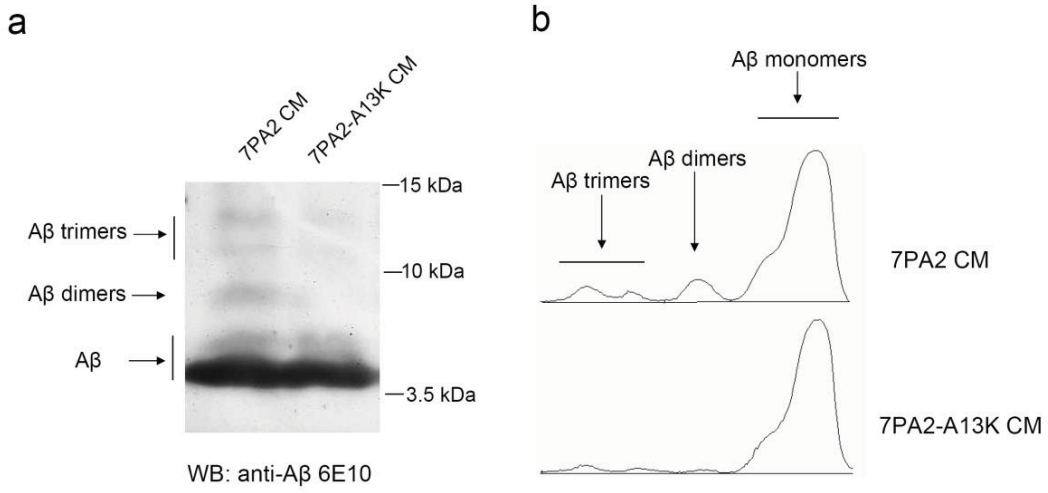
The histograms show the absorbance (O.D. 450nm) mean values ± s.e.m. (n=3).



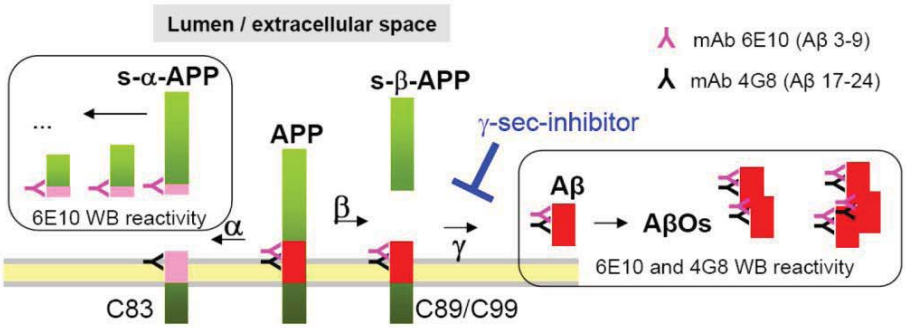
Supplementary Figure 2

Different γ-secretase cleavage in the APP-RIP cell models used.

The APP transmembrane domain (sequence in the grey box) undergoes γ-secretase cleavage at different sites. The blue vertical arrows indicate some biologically relevant clevages (with the numbers 38, 40, 42 referred to the C-terminal of Aβ products) and the horizontal arrows indicate the direction of cleavage (stepwise mechanism)[3]. In 7WD4 (CHO-hAPPwt) and 7PA2 (CHO-hAPPV717F) cells a different γ-secretase cleavage occurs: the thick arrow at 40 (generating Aβ40) is predominant in 7WD4 and thin vertical arrows indicate secondary cleavages; in the 7PA2 fAD cell line the proportions of cleavage change and the profile of Aβ peptides is unbalanced in favor of pathological forms (such as Aβ42).



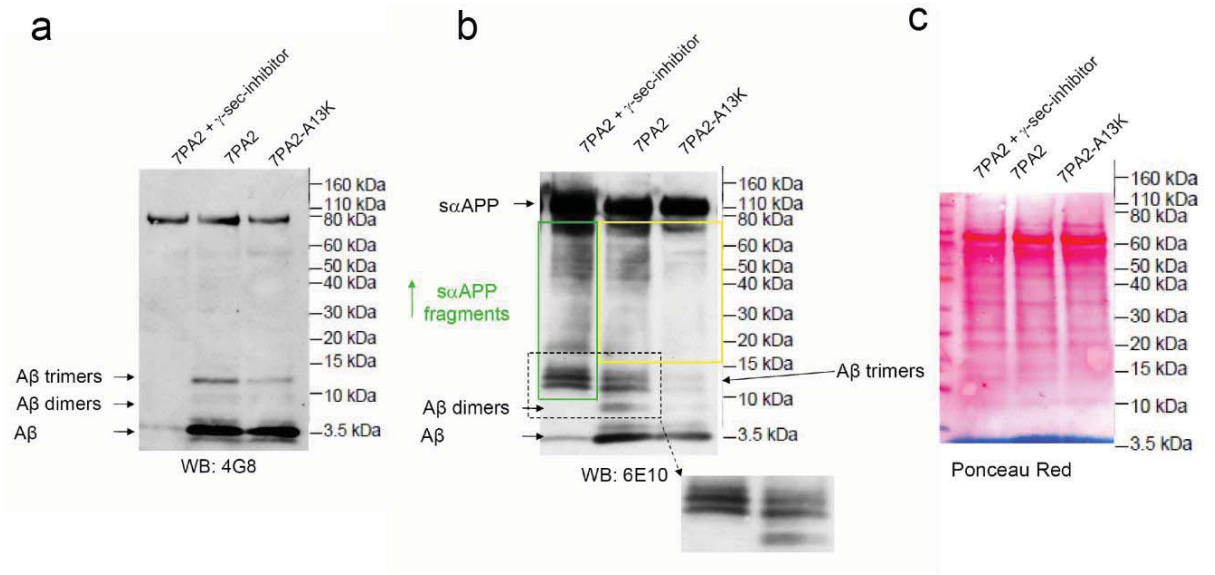
Supplementary Figure 3
Direct analysis of soluble Aβ species secreted by 7PA2 cells.
a. WB analysis of straight (non-concentrated) conditioned medium (CM) derived from 7PA2 cells (lane on the left) and from 7PA2 cells stably expressing the scFvA13-KDEL intrabody (referred to as 7PA2-A13K cells). In 7PA2 CM, the soluble Aβ species mainly run in the gels as monomeric bands while the Aβ oligomeric bands (LDS/SDS-stable Aβ dimers and trimers) are a small fraction, being <20% out of the total (calculated by densitometric analysis of band intensities).
b. Plot profile derived from the densitometric analysis of each WB lane.
In 7PA2-A13K CM the Aβ monomeric band has intensity similar to that of 7PA2 CM, while the LDS/SDS-stable Aβ dimers and trimers in 7PA2-A13K CM are decreased. However, Aβ dimers and trimers are difficult to detect in straight CM and variations are hard to quantify; to overcome this limit, the sensitivity of WB analysis was increased by loading CM subjected to preliminary steps of concentration (Supplementary **Fig.S4**).



Supplementary Figure 4

Scheme of generation of soluble APP and Aβ species.

Soluble APP (sAPP) fragments are generated from α– or β- secretase cleavages of APP, and Aβ peptides are generated from the sequential β- and γ- secretase cleavages; AβOs derive from the assembly of Aβ peptides. The recognition of anti-Aβ mAbs 6E10 and 4G8 is represented: they can recognize also the Aβ moiety contained in full length APP and in its fragments. As for soluble species, the mAb 6E10 (recognizing the 3-9 Aβ epitope) can react with Aβ and AβOs but also with the soluble α-APP and other soluble α-APP-fragments, while the mAb 4G8 (recognizing the 17-23 Aβ epitope) reacts with Aβ and AβOs but not with soluble α-APPs. A γ-secretase inhibitor abolish the Aβ synthesis, and as a consequence also AβOs are not formed.



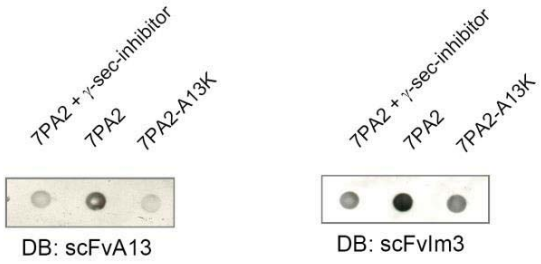
Supplementary Figure 5

Detection of Aβ and AβOs in Conditioned Media by WB analysis.

Representative full-length WB analysis of concentrated CM, blotted with mAbs 4G8 (**a**) and 6E10 (**b**), shows that the 4kDa Aβ monomeric band is dramatically reduced and the ~8kDa and ~12kDa Aβ oligomeric bands disappear in 7PA2 cells treated with the γ-secretase inhibitor L-685,458[4] (first lane). mAb 4G8 antibody (recognizing the 17-23 Aβ epitope) does not light up Aβ-specific bands higher than 12kDa: a ~80kDa band does not change after L-685,458 [4] treatment, suggesting that it is due to 4G8 cross-reactivity. mAb 6E10 antibody (recognizing the 3-9 Aβ epitope) reacts also with soluble N-terminal APP fragments which are increased after the L-685,458 treatments (see green box and the detail, showing the slightly different (molecular weights) MWs of sAPP bands in comparison to Aβ trimers). A co-presence of large AβOs and N-terminal APP fragments at MW>12kDa in CM from cells non-treated with γ-secretase inhibitor (yellow box) can not be excluded. The analysis of AβOs modulation in CM discussed in the main text (**Fig.3a**) was performed on undoubted Aβ dimers and Aβ trimers, detected both by mAbs 4G8 and 6E10. The 4KDa Aβ WB bands in CM derive both from real monomers and LDS/SDS unstable oligomers.

A major presence of LDS/SDS unstable AβOs in 7PA2 CM would explain why the differences of Aβ monomeric species measured by 6E10+4G8 SELDI-TOF MS between 7PA2 and 7PA2-A13K CM (showed in **Fig.3c**), are not reproduced by denaturing WB analysis of 4kDa Aβ bands.

c. Ponceau Red staining of the nitrocellulose membrane, before the incubation with mAbs4G8 and 6E10 antibodies (for WB analysis of CM described above). Similar loads of proteins can be observed, as normalization control.



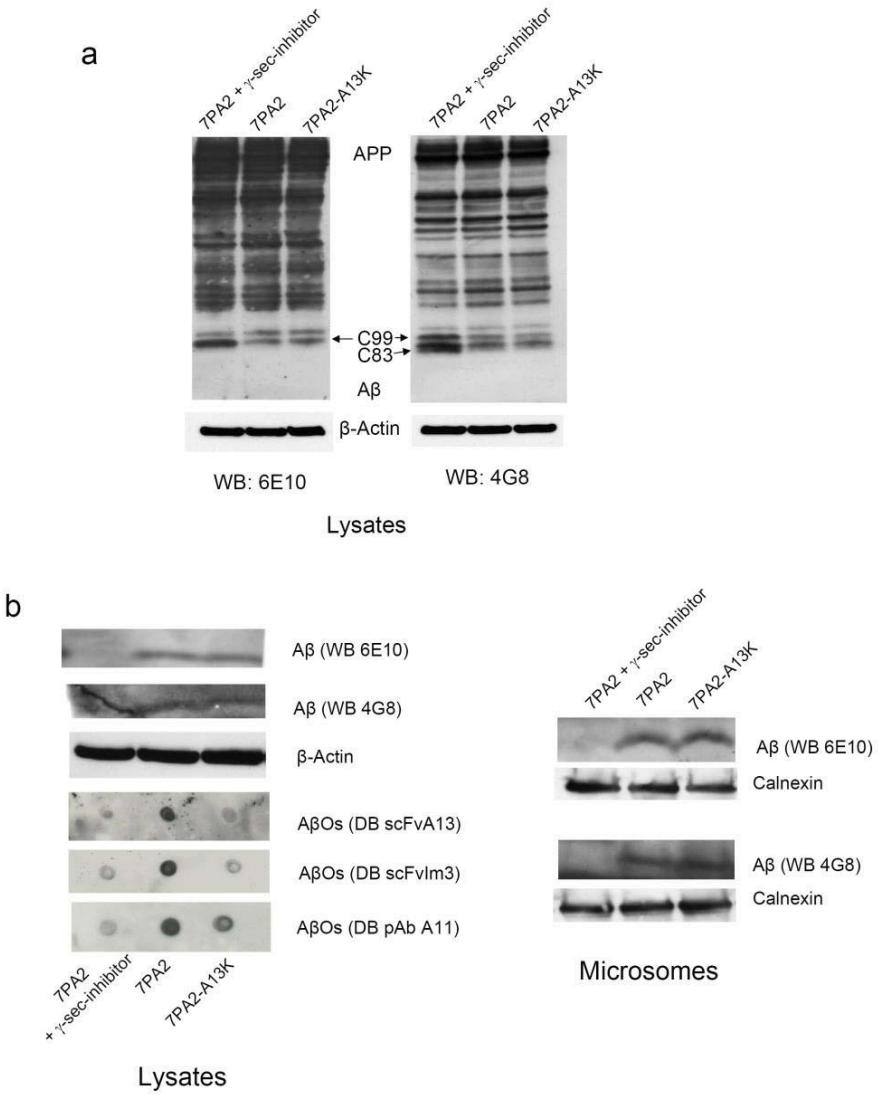
Supplementary Figure 6

Conformational Dot Blot (DB) analysis of conditioned media.

DB analysis is a widely used method to detect conformational assemblies, avoiding the denaturing steps of SDS-PAGE and improving the immunobinding of conformation-sensitive primary antibodies to the adsorbed antigens. Here, we exploited new DB immunoassays by using scFvA13 and scFvIm3 anti-AβOs as primary antibodies (and detected through anti-His tag).

In 7PA2 cells treated with L-685,458[4] the levels of AβO conformers detected by the scFv-DB analysis are dramatically decreased.

The variations of AβO conformers in 7PA2-A13K CM (vs 7PA2 CM) are described in the main text (**Fig.3b**) and reflect the selective variations of Aβ dimers and trimers in WB (at difference to the Aβ monomeric bands), attesting the oligomeric specificity of the new DB assay.



Supplementary Figure 7

WB and DB analysis of intracellular Aβ and AβOs

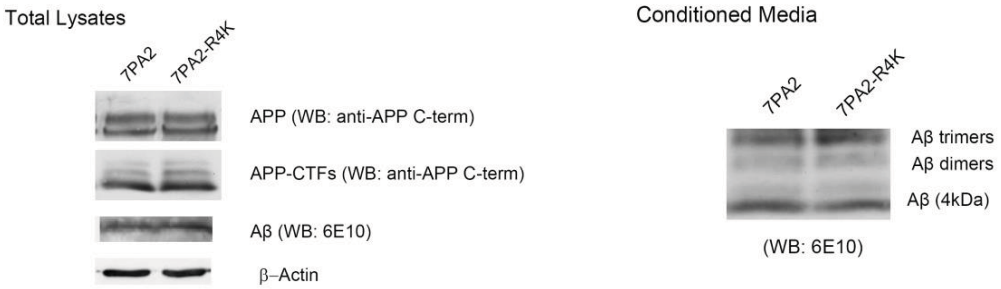
a. WB analysis (mAb 6E10 on the left and mAb 4G8 on the right) of total cell lysates of 7PA2 (treated or not with the γ-secretase inhibitor L-685,458[4]) and of 7PA2-A13K cells. The main immunoreactivity of 6E10 and 4G8 is with APP fragments. The lack of APP cleavage by γ-secretase (L-685,458 treated 7PA2 cells) determines a significant increase of APP-CTFs C89/99 and C83. No Aβ bands are detected under 100 μg of total proteins/lane loading.

b. Aβ and AβOs in cell lysates and microsomes. The WB analysis detects undoubted 4kDa Aβ monomeric bands, which are almost absent in L-685,458-treated cells (γ-secretase inhibitor). Instead, a clear detection of Aβ dimers and trimers by WB in lysates or microsomes is very difficult. The 4kDa Aβ band is likely representative of the largest pool of intracellular (in lysates) or subcellular (in microsomes, showed on the right) Aβ species.

In 7PA2 cells treated with L-685,458 the levels of both Aβ (4kDa) and AβO conformers detected by the scFv-DB and A11-DB analysis are dramatically decreased.

The analysis of Aβ and AβO conformers in 7PA2-A13K vs 7PA2 cells is described in the main text (**Fig.4**). Here, WB and DB analyses of lysates show the same samples reported in **Fig.4** with the addition of L-685,458-treated 7PA2 cells.

The DB assay was optimized by loading 500ng of total proteins/dot.

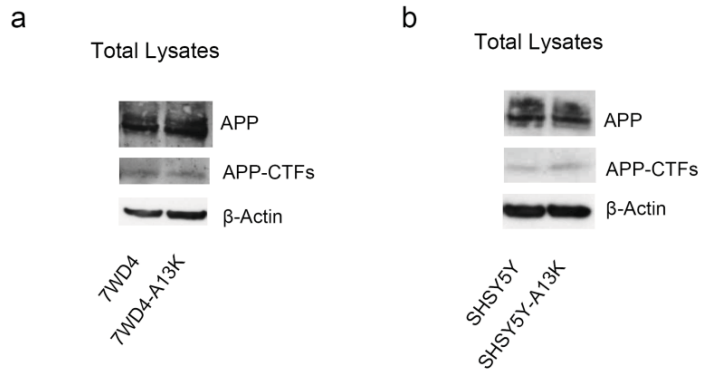


Supplementary Figure 8

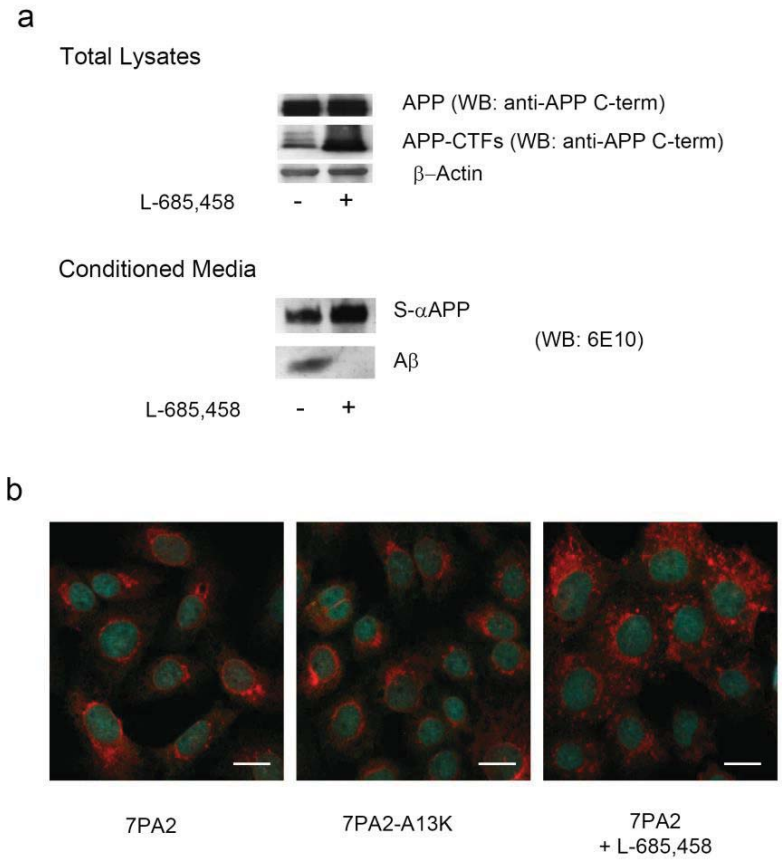
The Aβ-unrelated scFvR4-KDEL intrabody in 7PA2 cells.

The expression of scFvR4-KDEL Aβ-unrelated scFv control does not affect the Aβ and AβOs levels (WB by 6E10) and the APP-RIP (WB by anti-APP C-terminal).

The scFvR4 (see Methods) is an anti-βGalactosidase antibody fragment and it is used as Aβ-unrelated scFv control.



Supplementary Figure 9
Effects of the expression scFvA13-KDEL in 7WD4 and SHSY5Y cells.
WB analysis (pAb anti-APP C-terminal) of total cell lysates of 7WD4 and 7WD4-A13K cells (**a**), and of SHSY5Y and SHSY5Y-A13K cells (**b**).
The expression of scFvA13-KDEL in both cell lines does not affect the APP-RIP: no significant changes in the levels of full-length APP and of APP-CTFs are observed.

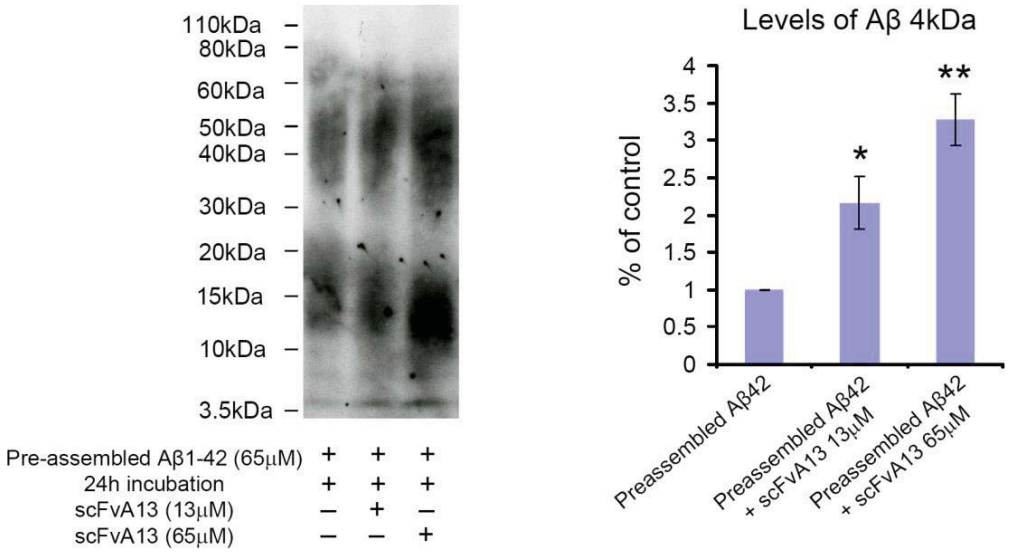


Supplementary Figure 10

The effect of the γ-secretase inhibitor L-685,458[4] on 7PA2 cells.

The γ-secretase inhibitor L-685,458 was administered (1μM, 18h) to the 7PA2 cells. As previously discussed in Supplementary **Figs.S3-S7**, the extracellular and intracellular Aβ levels, both of Aβ monomers and AβOs, are dramatically reduced by L-685,458. Moreover this compound, similarly to other γ-secretase inhibitors, has a dramatic side effect on the levels of intracellular APP-CTFs, which are massively increased (see panel **a**) and on the levels of soluble APP fragments (see conditioned media in panel **a** and the Supplementary **Fig.S5b** (6E10 WB)). Furthermore, APP and APP-CTFs are mis-distributed inside the cells treated with L-685,458: the panel **b** shows a comparative confocal microscopy analysis of 7PA2, 7PA2-A13K and 7PA2 cells treated with L-685,458 (indirect immunofluorescence of APP/APP-CTFs by using the anti-APP C-terminal as primary antibody; scale bar 15μm, magnification 63X).

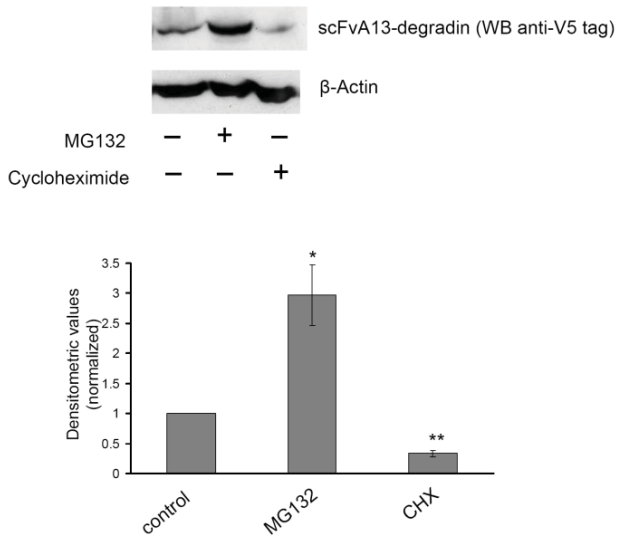
We conclude that, at variance with the scFvA13-KDEL intrabody mechanism, this interference approach (based on a chemical compound acting as γ-secretase inhibitor) is not selective for AβOs, modulating also the levels of Aβ monomers and of soluble APPs, and dramatically altering the processing and trafficking of APP and APP-CTFs.



Supplementary Figure 11

Actions of the scFvA13 on preformed AβOs *in vitro.*

The human Aβ1-42 peptide (100μM) was preaggregated at 22°C for 24h; then it was incubated (at the final concentration of 65μM) for additional 24h at 22°C without scFvA13, or in the presence of substoichimetric (13μM) or stoichiometric concentrations (65μM) of scFvA13. Both LMW and HMW AβOs undergo a reduction of their MWs (ranges of Aβ smearing in WB), accompanied by a significant increase of free Aβ monomers, when incubated with scFvA13. The scFvA13 antibody acts in a concentration-dependent manner. WB analysis (mAb anti-Aβ 6E10). The histogram shows the variations of Aβ monomers obtained from densitometric analysis. Mean values ± s.e.m., *$p<0.05$, **$p<0.01$. Student's t-Test, n=3

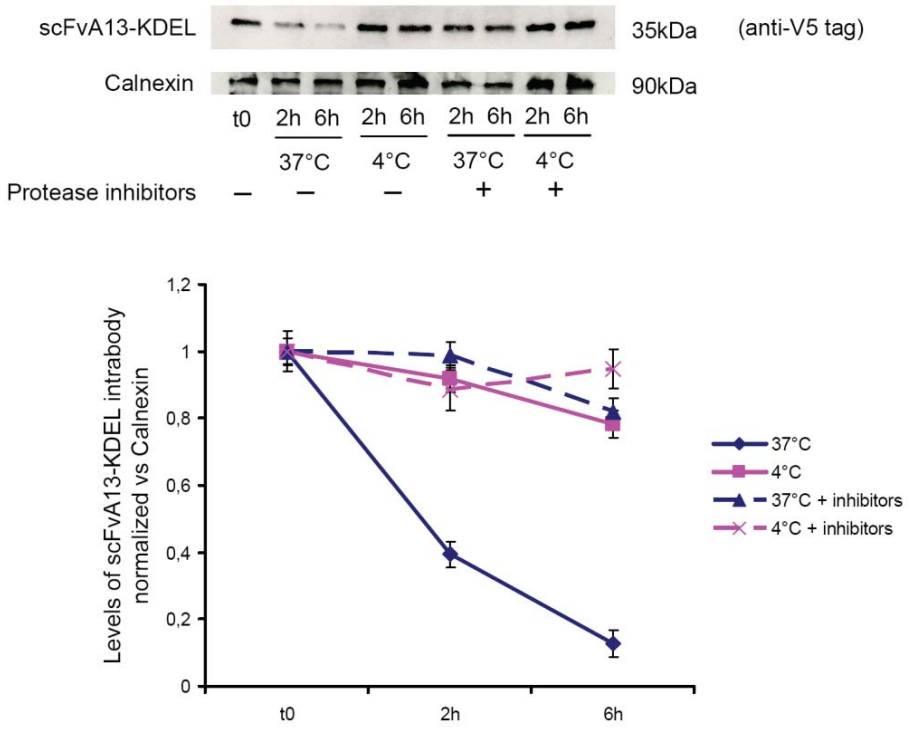


Supplementary Figure 12

ER associated degradation of the intrabody "scFvA13-degradin".

The so-called "scFvA13-degradin" is a C-terminal fusion of the scFvA13 with the moiety of SEL1L protein, which is involved in the retrotranslocation process from ER to the cytosol. We stably expressed in 7PA2 cells the intrabody scFvA13-degradin, and we observed a significant increase of the intrabody levels after 6 hours MG132 treatment. Thus, the scFvA13-degradin undergoes a significant ERAD and proteasome degradation, differently than scFvA13-KDEL.

The figure shows the WB analysis (anti-V5 tag) of scFvA13-degradin (stably expressed in 7PA2 cells) after 6h MG132 (20μM) treatment (to block the proteasome and study the ERAD pathway) or after 6h Cycloheximide (CHX, 40μM) to block the protein synthesis. The histogram shows the levels of scFvA13-degradin in the different treatments (values normalized from densitometric analysis). Mean values ± s.e.m., n=3; $p<0.01$ (Student's t-Test) * (MG132 treatment both vs control and vs CHX); ** (CHX treatment both vs control and vs MG132).



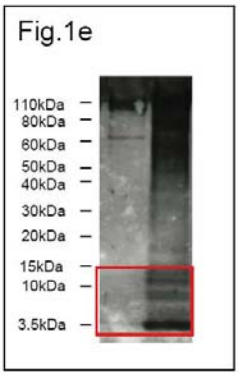
Supplementary Figure 13

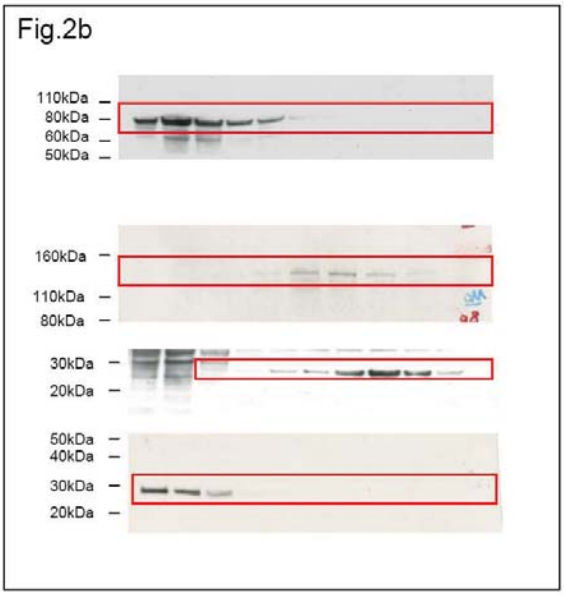
Time-dependent degradation of scFvA13-KDEL intrabody in ER-enriched fractions.

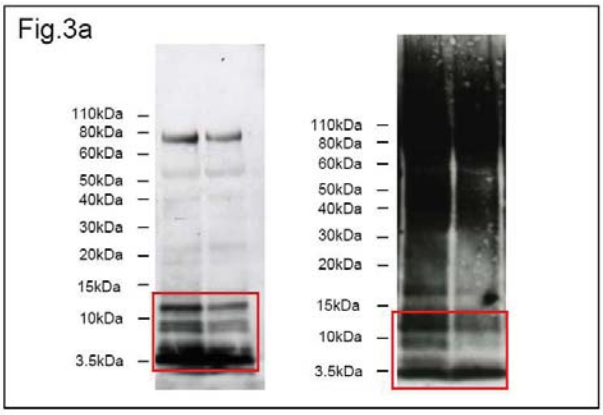
ER-enriched fractions prepared from subcellular fractionation gradients were subjected to incubations for 2 hours or 6 hours at 37°C or at 4°C, in presence or not of proteases inhibitors (cocktail by Roche). The levels of the scFvA13-KDEL intrabody were measured by WB (anti-V5 tag), and normalized against the levels of Calnexin. We observe a massive and time-dependent reduction of the levels of scFvA13-KDEL only in the ER-fractions incubated at 37°C. In all the other experimental points, the levels of scFvA13-KDEL do not undergo significant variations.

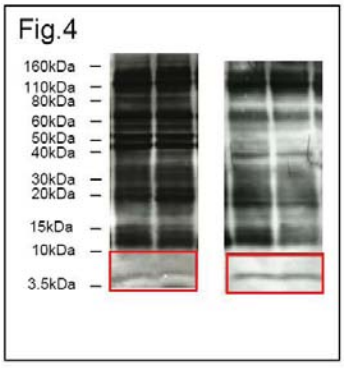
The results are in accordance with the dramatic reduction of the scFvA13-KDEL intrabody after 6 hours treatment with Cycloheximide (see **Fig.8b**). The block of degradation, at 4°C or through the addition of proteases inhibitors, suggests that the scFvA13-KDEL intrabody undergoes mechanisms of degradation mediated by endogenous proteases present in the ER-fractions, and likely resident in the ER. This explains the short half-life of the scFvA13-KDEL intrabody, and is in accordance with its exclusive presence in the ER and cis-Golgi, but not in endosomes, lysosomes or in ERAD pathways.

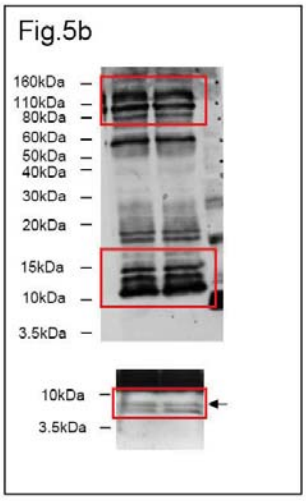
An intrabody-mediated degradation of AβOs through ER-resident proteases can not be excluded a priori. However, AβOs seems less prone to the proteolitic degradation in comparison to Aβ monomers and when Aβ undergoes degradation (i.e. by Neprilysin) an altered pattern of Aβ fragments can be measured [5] which is not the case of Aβ fragments secreted by 7PA2-A13K cells.

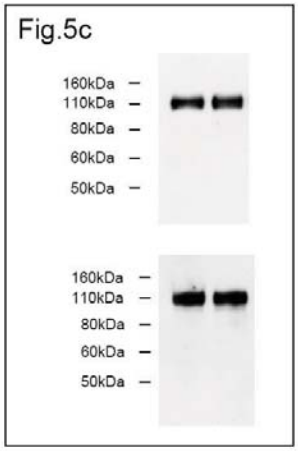




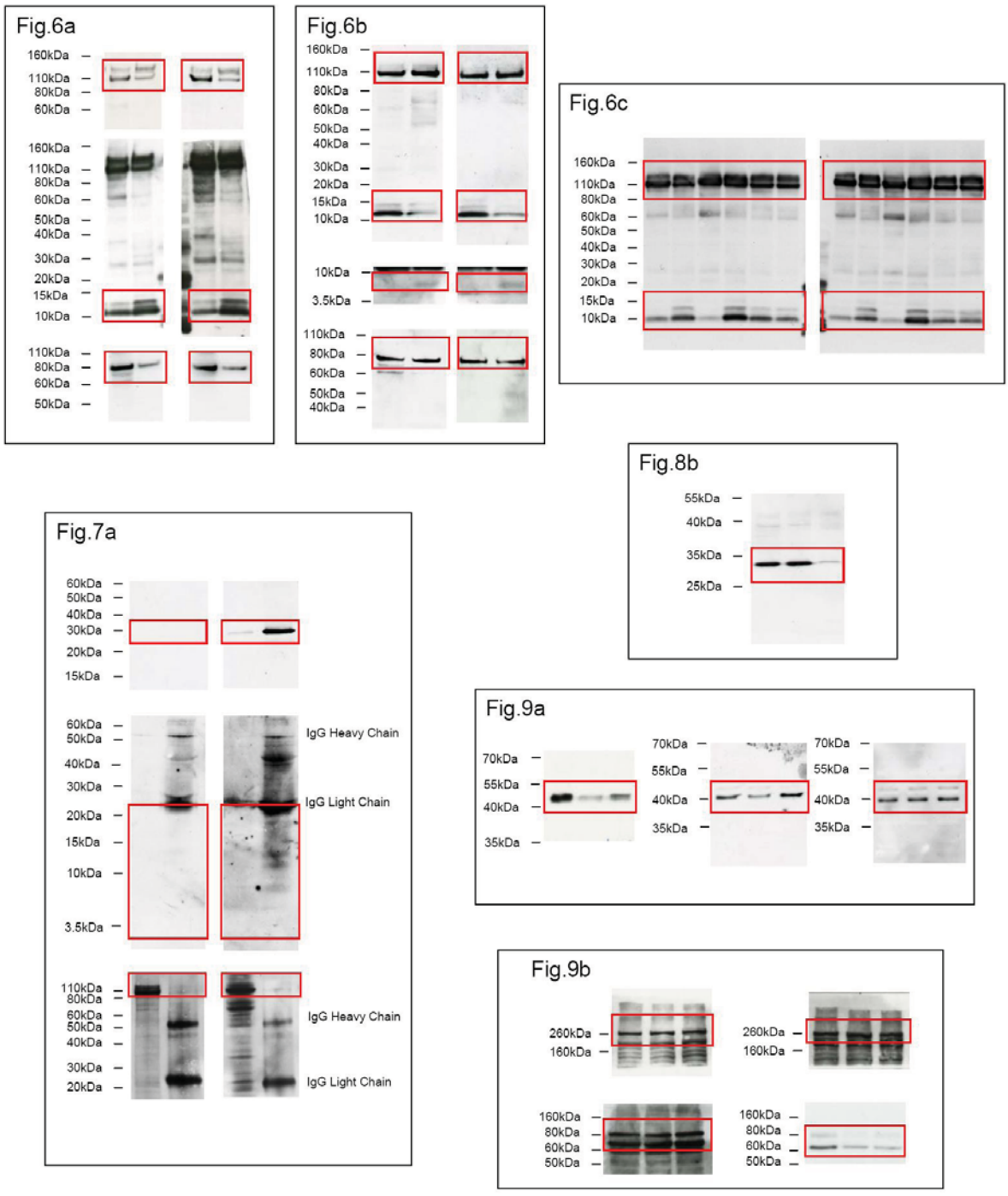








Supplementary Figure 14
Full length blots corresponding to the indicated main figures.



Supplementary Figure 15
Full length blots corresponding to the indicated main figures.

Supplementary Table 1.

Aβ monomers, N-terminally and C-terminally truncated Aβ measured by the anti-Aβ 6E10+4G8 SELDI-TOF MS in 7WD4 CM and 7PA2 CM.

	% of total Aβ [a] *7WD4 CM*	*% of total Aβ* [a] *7PA2 CM*	*Ratios* [b] *7PA2 CM/7WD4 CM*
Aβ1-17	1	1.2	4.8
Aβ1-18	4	1.8	1.8
Aβ1-19	2	1.3	2.6
Aβ11-40	2.9	2.1	2.9
Aβ11-42	0.5	1.1	8.9
Aβ1-33	7.1	7.8	4.5
Aβ1-34	8.4	11.2	5.5
Aβ1-37	8.9	7.5	3.4
Aβ1-38	9.7	20.7	8.8
Aβ1-39	8.2	12.5	6.3
Aβ1-40	44.2	28.2	2.6
Aβ1-42	3.1	4.7	6.3

Footnotes:

(a) Value calculated from the intensity of each immunoproteomic peak (corresponding to a single Aβ monomer or truncated form) as the percentage of the sum of all immunoproteomic peaks (total Aβ).

(b) Value is the ratio between the intensity of each immunoproteomic peak (corresponding to a single Aβ monomer or truncated form) measured in 7PA2 CM and in 7WD4 CM.

Supplementary Table 2.

Aβ monomers, N-terminally and C-terminally truncated Aβ measured by the anti-Aβ 6E10+4G8 SELDI-TOF MS in 7PA2 CM and 7PA2-A13K CM.

	% of total Aβ [a] *7PA2 CM*	*% of total Aβ* [a] *7PA2-A13K CM*	*Ratios* [b] *7PA2-A13K CM/7PA2 CM*
Aβ1-17	1.2	1.1	1.3
Aβ1-18	1.8	1.9	1.5
Aβ1-19	1.3	1.3	1.4
Aβ11-40	2.1	2.4	1.6
Aβ11-42	1.1	1.6	2
Aβ1-33	7.8	5.9	1.1
Aβ1-34	11.2	8.9	1.1
Aβ1-37	7.5	8	1.5
Aβ1-38	20.7	20.9	1.4
Aβ1-39	12.5	13	1.5
Aβ1-40	28.2	29.7	1.5
Aβ1-42	4.7	5.3	1.6

Footnotes:

(a) Value calculated from the intensity of each immunoproteomic peak (corresponding to a single Aβ monomer or truncated form) as the percentage of the sum of all immunoproteomic peaks (total Aβ).

(b) Value is the ratio between the intensity of each immunoproteomic peak (corresponding to a single Aβ monomer or truncated form) measured in 7PA2-A13K CM and in 7PA2 CM.

Supplementary Table 3.

Overall modulation of Aβ and AβO species measured by WB, DB and SELDI-TOF MS in 7PA2-A13K cells.

	Extracellular		*Intracellular*	
	Aβ species	*Variations in 7PA2-A13K vs 7PA2 cells*	*Aβ species*	*Variations in 7PA2-A13K vs 7PA2 cells*
WB	Aβ (4kDa) Aβ dimers and trimers	= ↓	Aβ (4kDa)	=
DB	AβOs	↓	AβOs	↓
SELDI-TOF MS	Aβ monomers and truncated forms	↑		

Supplementary References

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