Elevated levels of Secreted Frizzled Related Protein 1 contribute to Alzheimer's disease pathogenesis

Pilar Esteve^{1,2*}, Javier Rueda-Carrasco^{1,2}, María Inés Mateo^{1,2}, María Jesús Martín-Bermejo^{1,2}, Jonathan Draffin¹, Guadalupe Pereyra^{1,2}, África Sandoz^{1,2}, Inmaculada Crespo¹, Inmaculada Moreno³, Ester Aso⁴, Paula García-Esparcia⁴, Estrella Gómez-Tortosa⁵, Alberto Rabano⁶, Juan Fortea⁷, Daniel Alcolea⁷, Alberto Lleó⁷, Michael T. Heneka^{8,9}, José M. Valpuesta¹⁰, José A. Esteban¹, Isidro Ferrer⁴, Mercedes Domínguez³ and Paola Bovolenta^{1,2*}

¹ Centro de Biología Molecular “Severo Ochoa”, CSIC-UAM, c/ Nicolás Cabrera, 1, Campus de la Universidad Autónoma de Madrid, Madrid 28049, Spain

² CIBER de Enfermedades Raras (CIBERER)

³ Unidad de Inmunología Microbiana, Área de Inmunología, CNM, Instituto de Salud Carlos III, Madrid, Spain

⁴ Neuropathology Institute, Bellvitge Biomedical Research Institute (IDIBELL)-Hospital Universitari de Bellvitge, Universitat de Barcelona, Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), L'Hospitalet de Llobregat, Barcelona, Spain

⁵ Department of Neurology, Fundación Jiménez Díaz, Madrid, Spain

⁶ Neuropathology Laboratory, Fundación CIEN, Alzheimer Center Reina Sofía Foundation, Madrid, Spain

⁷ Department of Neurology, Institut Investigacions Biomèdiques Sant Pau - Hospital de Sant Pau, Barcelona, Spain; Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED)

⁸ Universitätsklinikum Bonn, Neurology

⁹ German Center for Neurodegenerative Diseases (DZNE) 53127 Bonn, Germany.

¹⁰ Centro Nacional de Biotecnología, CNB-CSIC, c/ Darwin 3, Campus de la Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain.

* corresponding authors at pesteve@cbm.csic.es and pbovolenta@cbm.csic.es

33 **Abstract**

34

35 The deposition of aggregated A β peptides -derived from the pro-amyloidogenic processing of
36 the Amyloid Precursor Protein (APP)- into characteristic amyloid plaques (APs) is distinctive
37 to Alzheimer's disease (AD). Alternative APP processing via the metalloprotease ADAM10
38 prevents A β formation. We tested whether down-regulation of ADAM10 activity by its
39 secreted endogenous inhibitor SFRP1 is a common trait of sporadic AD. We demonstrate that
40 SFRP1 is significantly increased in the brain and cerebrospinal fluid of AD patients,
41 accumulates in APs and binds to A β , hindering A β protofibril formation. Sfrp1
42 overexpression in an AD-like mouse model anticipates the appearance of APs and dystrophic
43 neurites, whereas its genetic inactivation or the infusion of α -Sfrp1 neutralizing antibodies
44 favours non-amyloidogenic APP processing. Decreased Sfrp1 function lowers AP
45 accumulation, improves AD-related histopathological traits and prevents LTP loss and
46 cognitive deficits. Our study unveils SFRP1 as a crucial player in AD pathogenesis and a
47 promising AD therapeutic target.

48

Introduction

Alzheimer's Disease (AD), a devastating condition and a worldwide leading cause of dementia¹, exists in two forms: a rare genetic form of early onset caused by mutations in few genes (APP, PS1, PS2); and a sporadic, late onset, slowly progressing and common form, for which age is the most accepted risk factor². By 2050, sporadic AD is expected to affect 150 million individuals worldwide, becoming an unsustainable socio-economical problem. The urgent need for an effective treatment has spurred a large number of clinical trials³, many of which have targeted one of the most salient disease hallmarks: the accumulation of A β peptides in the form of extracellular amyloid plaques (APs)^{1, 4}. A β peptides are produced mainly in neurons via sequential processing of APP by β - and γ -secretase although it is unclear whether this is the primary cause of the disease or its consequence⁵. Notwithstanding, reducing/blocking the different A β forms seems to delay the disease in animal models and to slow clinical decline in prodromal or mild AD patients. Unfortunately, the disease is not arrested^{6, 7}, perhaps because the factors that favour A β production and trigger their aggregation in toxic forms are still not fully understood¹.

ADAM10, the constitutive α -secretase of the brain⁸, cleaves APP within the A β sequence blocking the generation of toxic peptides and releasing a soluble extracellular fragment, known as sAPP α ⁹. Mutations in the ADAM10 pro-domain, which lower its α -secretase activity and shift APP processing towards the pro-amyloidogenic pathway¹⁰, co-segregate with some AD cases^{10, 11}. This raises the possibility that impaired ADAM10 activity might be among the common triggers of amyloid deposition, contributing to AD pathogenesis.

Here, we tested this possibility focusing on SFRP1, a small (35 kDa) secreted and highly dispersible protein¹² composed of two domains, one of which has homology with tissue inhibitors of metalloproteases¹³. Sfrp1 has a dual function: modulation of Wnt signalling¹³ and regulation of the enzymatic activity of ADAM10¹⁴. SFRP1 is expressed in the CNS and down-modulates the proteolytic processing of all the ADAM10 substrates so far tested, including APP¹⁴⁻¹⁶, thus being the only endogenous ADAM10 regulator hitherto described¹⁴.

We hypothesized that upregulation of SFRP1 levels in the brains of AD patients could enhance pro-amyloidogenic APP processing and the cascade of stereotyped brain alterations, that, in addition to AP deposition, include synaptic dysfunction, brain inflammation and the

82 formation of dystrophic neurites, among others traits¹. By combining studies in human AD
83 cases and in mouse models, we demonstrated that SFRP1 is indeed a novel player in AD
84 pathogenesis. Mechanistically, SFRP1 interferes with neuronal ADAM10-mediated anti-
85 amyloidogenic APP processing and further interacts with A β peptides hampering the
86 formation of A β protofibrils. Antibody mediated neutralization of SFRP1 activity suffices to
87 prevent synaptic dysfunction, pointing to a novel therapeutic avenue worthy of being
88 explored.

89

Results

SFRP1 levels in AD patients positively correlate with amyloidogenic APP processing

With the aim of determining whether SFRP1 levels are elevated in the brain AD patients, we first generated α -SFRP1 mAbs and developed a specific and sensitive ELISA (Fig. S1). Application of this ELISA showed a correlation between AD pathological state and SFRP1 upregulation. Indeed, SFRP1 protein was elevated in both the TBS and RIPA-soluble fractions of entorhinal and frontal cortex extracts from AD patients at pre-symptomatic, mild and advanced neuropathological stages of the disease (Braak and Braak (BB)I-II/0-A; III-IV/0-C; V-VI/B-C; Table S1), when compared to age-matched control samples (Fig. 1A,C). The increase was particularly evident in both fractions of the entorhinal cortex at early and intermediate disease stages, but less so in the soluble fraction at late stages, when SFRP1 levels were instead the highest in the RIPA fraction (Fig. 1A). The SFRP1 increase observed in the soluble fraction positively correlated with the amount of A β 42 found in the same extracts (Fig. 1E), whereas there was only a tendency towards a negative correlation when plotted against the levels of sAPP α (Fig. 1F) measured in the same samples. Nevertheless, sAPP α levels were significantly lower in extracts from AD patients (37.75 ± 5.299 , n=13) than those of controls (97.74 ± 19.83 ; n=5; $p \leq 0.001$; Fig. 1G). A tendency toward amyloidogenic APP processing was further confirmed by Western blot analysis of extracts from entorhinal cortical samples of AD patients as compared to those from age-matched controls (Fig. S2A-E).

SFRP1 protein increase was paralleled by its transcriptional up-regulation especially at BBV-VI in a similar characterized cohort of AD patients and age-matched controls (Fig. 1B,D), with no parallel significant changes in the expression of *BACE1*, *APP* and *ADAM10* at least at BBI-II and III-IV (Fig. S2F). Furthermore, an *in silico* search found that *SFRP1* was listed among the genes with a progressive enhanced hippocampal expression in patients with incipient or advanced AD (Supplementary information in¹⁷).

Immunohistochemical analysis of the human frontal cortex from AD patients revealed a consistent SFRP1 immunoreactivity in auto-fluorescent elastin+¹⁸ blood vessels (Fig. S3A,B) colocalizing with A β deposits (Fig. 1K). SFRP1 was also present in some GFAP+ reactive astrocytes surrounding the APs (Fig. 1L; Video S1) and in activated Iba1+ microglia infiltrated in the APs (Fig. 1M; Video S2). By contrast, immuno-positive signal was undetectable in the cortex of control individuals (Fig. S3D) although SFRP1 was found in

extracts of the choroid plexus at levels similar to those found in samples from AD individuals (Fig. S3E). The choroid plexus is the main source of CSF proteins and its function is unbalanced in AD patients¹⁹. We therefore measured SFRP1 content in CSF samples of another characterized cohort AD patients and control individuals (Table S2), finding a significantly higher protein concentration in patients than in controls (Fig. 1H).

Together these data demonstrate a correlation between elevated SFRP1 content and the presence of AD.

SFRP1 accumulates in APs and binds to A β hindering the formation of A β protofibrils

Of note, immunohistochemical analysis revealed a strong and specific accumulation of SFRP1 also in the core of A β + (Fig. 1I,J; S3F; staining with the 6E10 mAb) and ThioS+ plaques (APs; Fig. S3G). Positive signal was also observed in the AP halo (Fig. 1J, arrow; Fig. S3F,G arrows), where small A β oligomers seem to localize²⁰, and in ThioS-negative aggregates (Fig. S3H). This distribution appeared to be AD related, as it was never observed in control brains (Fig. S3D).

Together these observations suggested a possible interaction between SFRP1 and A β peptides as well as potential formation of SFRP1 aggregates. To explore these possibilities, we performed *in vitro* aggregation assays followed by SDS-PAGE and Western blot analysis with antibodies against SFRP1 and A β . Indeed, the two molecules formed SDS-resistant complexes with molecular weights compatible with combinations of both SFRP1 and A β 1–42 multimers (Fig. 2A-B). Binding between the two molecules was apparently stronger than that previously reported between A β 1–42 and albumin²¹ (Fig. S4A,B). Because SDS-PAGE can only resolve relatively low molecular weight A β aggregates^{22, 23}, we used transmission electron microscopy (TEM) analysis to determine the possible time-dependent formation of larger A β 1–42 and SFRP1 aggregates (Fig. 2C-E; Fig. S4C). A β alone formed aggregates following the path described for amyloid proteins²⁴⁻²⁶, from small oligomers to large protofibrils (Fig. 2C; Fig. S4C). SFRP1 alone also appeared to oligomerize over time but in a disordered manner, giving rise to spherical structures of different sizes (Fig. 2E; Fig. S4D). In notable contrast, when the two proteins were incubated together, the latter structures and the protofibrils were no longer visible (Fig. 2D; Fig. S4C). To further corroborate these findings, we used dot blot analysis of different concentrations of A β 1-42 and SFRP1 mixtures probed with antibodies to detect the presence and relative abundance of A β 1-42 fibril/protofibril forms (OC antibody) and low molecular weight amyloid oligomers (A11,

conformation antibody). This analysis confirmed that A β 1-42 fibril/protofibril forms increased with time but their relative abundance was lower in the presence of SFRP1 in a concentration dependent manner (Fig. S4D). Conversely, oligomeric A β 1-42 forms appeared to prevail in the presence of SFRP1 (Fig. S4D), supporting TEM observations. Unexpectedly, the A11 antibody recognized SFRP1 alone (Fig. S4D), raising the possibility that the protein might acquire amyloid characteristics, at least *in vitro*.

Together these data suggest that the two proteins influence each other's aggregation: A β prevents the formation of SFRP1 spherical structures and SFRP1 limits the formation of A β 1-42 protofibrils, favouring the presence of oligomeric forms.

Glial cell derived Sfrp1 localizes to the synaptic compartment in APP;PS1 mouse brains

To analyse whether SFRP1 is causally linked to AD progression, we turned to APP;PS1 transgenic mice²⁷ that are commonly used as a model of amyloidosis. Similar to what is observed in human tissue, a mouse-adapted and specific ELISA using RIPA homogenates from APP;PS1 brains showed an age-dependent significant increase of Sfrp1 levels, already evident in pre-symptomatic 2 months-old animals (Fig. 3A,B), in agreement with what is observed in the entorhinal cortex of BI-II stage patients (Fig. 1A). This increase was further confirmed by WB analysis of brain extracts from APP;PS1 brains (Fig. S5A). Co-immunostaining with α -Sfrp1 and glial specific markers in wt (Fig. S5B,C) and APP;PS1 (Fig. 3C,D) mice showed that Sfrp1 localized to the wall of the lateral ventricle and GFAP+ astrocytes and Iba1+ microglial cells as well as to laminin+ blood vessels (Fig. S5D,E). As observed in human samples, a strong and very specific Sfrp1+ signal was also detected in A β + and ThioS+ APs (Fig. 3E,F-H'; Fig. S5F-I) and in the choroid plexus of APP;PS1 mice with an intensity that increased with age (Fig. 3I-K). Co-IP studies using cortical extracts of 6 months-old APP;PS1 mice confirmed that Sfrp1 and A β interact *in vivo* (Fig. 3L; Fig. S6A).

In APP;PS1 brains- but also in AD patients- APP expression and processing occurs mostly in neurons²⁸, including in the synaptic compartment in which APP and ADAM10 localize^{29, 30} (Fig. 3M). Consistent with this idea and in contrast to what observed in wt animals, Sfrp1 was found in the synaptosomal preparations from 8 months-old APP;PS1 mice (Fig. 3M; Fig. S6B), where it specifically co-immunoprecipitated with ADAM10 (Fig. 3N; Fig. S6C).

Double RNAScope ISH with probes for *Iba1* and *Sfrp1* mRNA and *Sfrp1* ISH coupled to anti-GFAP further showed that in APP;PS1 brains *Sfrp1* is produced in astrocytes,

microglial and choroid plexus cells, likely at higher levels than in wt (Fig. 4A-C). Notably, cells surrounding ThioS+ plaques appeared to express twice as much *Sfrp1* mRNA than those located far from the plaques (Fig. 4D-F).

This observation supports the idea that *Sfrp1* released by glial cells influences ADAM10 activity in neurons, thereby modulating APP processing.

Forced expression of Sfrp1 in APP;PS1 mouse brains accelerates the appearance of APs

Given that *Sfrp1* expression and distribution in APP;PS1 mice mimicked that observed in human AD samples, we next tested if the forced increase of *Sfrp1* levels in the brains of young APP;PS1 mice would accelerate AP deposition. We first used heterozygous APP;PS1 mice, because of their late and slow AP build-up²⁷. Mice were transduced with lentiviral preparations expressing either *GFP* (LV-*GFP*) or *Sfrp1* (LV-*Sfrp1*-IRES-*GFP*) at 2.5 months of age and analysed 3 months later. At this final age, A β + APs were barely detectable in the cortex of het-APP;PS1 mice (not shown) or of mice transduced with LV-*GFP* (Fig. 4G,I,K). In contrast, mice exposed to LV-*Sfrp1*-IRES-*GFP* presented a significant build-up of A β + APs, surrounded by CD45+ activated microglial cells (Fig. 5H,J,L,M) as commonly observed around APs^{31,32}. There was also an increase in the number and size of accumulations positive for LAMP1 (Fig. 5I,J,N), a lysosomal glycoprotein that accumulates in dystrophic neurites³¹. A similar effect was observed when 2 months-old homozygous APP;PS1 mice were transduced with either one of the two LV preparations and analysed 1 month after (Fig. S7A-K). In LV-*Sfrp1*-IRES-*GFP* transduced animals, A β + APs, reactive gliosis and pTau+ dystrophic neurites outnumbered the few found in LV-*GFP* transduced (Fig. S7A-K) or non-transduced (not shown) mice.

Together these results support the contention that elevated *Sfrp1* levels contribute to AP formation.

Sfrp1 inactivation prevents the appearance of pathological traits in APP;PS1 mice

To further test the link between *Sfrp1* and amyloid deposition, we performed the converse experiment using APP;PS1;*Sfrp1*^{-/-} mice, generated by crossing the APP;PS1 line with *Sfrp1*^{-/-} mice³³, which are viable, fertile and show no sign of neurodegeneration. Taking advantage of the *lacZ* reporter present in the *Sfrp1* null allele³³, we first confirmed that both GFAP+ astrocytes and Iba1+ microglial cells, but not NeuN+ neurons, are indeed a source of *Sfrp1* (Fig. S7L-N), particularly abundant in the proximity of ThioS+ APs (Fig S7O,P). Next, we

immunostained the cortex of 4.5, 9 and 20 months-old APP;PS1 and APP;PS1;*Sfrp1*^{-/-} mice with an anti-Aβ42 antibody (H31L21) that recognizes only the longer and more pathogenic Aβ forms present in APs and the surrounding protofibrillary accumulations³⁴. APP;PS1;*Sfrp1*^{-/-} cortex developed significantly fewer, smaller and more compact Aβ42+ (Fig. 5A-F,G; Fig. S8A) or ThioS+ (Fig. S8B-H) APs than their APP;PS1 counterparts. Similar results were found in brains of a different group of APP;PS1 and APP;PS1;*Sfrp1*^{-/-} mice probed with antibodies that recognize Aβ1-40/42 peptides (Fig. S8I-M). In all cases, AP reduction correlated with a comparably significant decrease in LAMP1, Iba1, CD45 and GFAP immunoreactivity (Fig. 5H-S; Fig. S9).

Consistent with the observation that in the absence of *Sfrp1* APs are reduced, the amount of Aβ42 present in the RIPA fractions (Fig. 6A) and the levels of other β-secretase-derived APP fragments, i.e. CTFβ, present in 6 months-old APP;PS1;*Sfrp1*^{-/-} brains were significantly reduced when compared to those of age matched APP;PS1 brains (Fig. 6C,D; Fig. S10A-C), whereas those of sAPPα and CTFα were significantly increased (Fig. 6B,E,F; S10B,C). Similarly, the proteolysis of Cdh2, another ADAM10 substrate, implicated in synapse stabilization²⁹, was also significantly enhanced in APP;PS1;*Sfrp1*^{-/-} when compared to APP;PS1 (Fig. S10D). These changes were not related to differences in brain *App* and *Adam10* expression, as the respective mRNA levels were similar in the two genotypes (Fig. S11A). Similarly, no significant changes in APP, BACE1 and ADAM10 protein expression were detected (Fig. S10A,C; S11B,C).

Together these data indicate that SFRP1 interacts with ADAM10 in neurons and interferes with the proteolytical processing of APP and Cdh2, as previously shown in other contexts^{14, 16}. Thus, in the absence of *Sfrp1*, APP processing is shifted towards the non-amyloidogenic pathway, decreasing the accumulation of APP toxic forms, in agreement with the idea that α- and β- secretases compete for APP processing^{10, 35}. In further support of this interpretation, SFRP1 activity appeared independent from its well-established additional role as a Wnt signalling modulator^{13, 36}, given that mRNA and protein levels of *Axin2*, a read-out of Wnt/βcatenin signalling activation, were comparable in the brains of both genotypes (Fig. S11A,D).

To determine if *Sfrp1* inactivation protects APP;PS1 mice also from the appearance of behavioural deficits, we compared recognition and spatial learning and memory in 8 month-old wt, *Sfrp1*^{-/-}, APP;PS1 and APP;PS1;*Sfrp1*^{-/-} mice, using the novel object recognition (ORT) and Morris water maze tests. The behaviour of *Sfrp1*^{-/-} mice was indistinguishable

from that of wt mice, with proficient ORT discrimination indexes similar to those reported in other studies³⁷ and with comparable spatial and locomotor skills (Fig. S12A-C). In notable contrast with the already reported³⁸ and evident cognitive decline observed in APP;PS1, APP;PS1;*Sfrp1*^{-/-} mice showed a behaviour similar to that of age-matched wt mice in both tests (Fig. 6G,H; S12D), despite a slightly lower locomotor performance (Fig. S12E).

Together these data indicate that knocking *Sfrp1* out largely prevents the appearance of pathogenic characteristics in APP;PS1 mice, strongly delaying their progressive dysfunctions.

Antibody-mediated neutralization of SFRP1 activity counteracts the appearance of AD pathogenic traits in mice

To rule-out a possible relation of these results with an *Sfrp1* developmental function, we sought to interfere with Sfrp1 activity using a neutralizing mAb, given that systemically administered antibodies have been shown to successfully enter and bind to the brain parenchyma⁷. Among the generated mAbs, clone 10.5.6 secreted an IgG1 with Sfrp1 neutralizing activity (Fig. S13). Biotinylation of the 10.5.6 mAb showed the mAb successfully reached the brain parenchyma of APP;PS1 mice 24 hrs after systemic injection through the retro-orbital sinus, accumulating around A β + plaques (Fig. S14A-F). Treating 2 month-old APP;PS1 mice with a weekly administration of 10.5.6 mAb (100 μ g/injection) for 2 months, via the same route, significantly reduced their cortical A β 42 and ThioS + AP burden when compared to animals treated with a control unspecific mouse IgG1 (Fig. 7A-D,G,H), without promoting microglial activation or macrophages infiltration (Fig. S14G-V), which are reported secondary effects of some α -A β immunotherapies³⁹. The AP decrease was associated with a comparable significant reduction in the number and size of LAMP1+ accumulations around each APs (Fig. 7E,F,I,J), indicating that α -Sfrp1 treatment limits the area of dystrophic neurites that contribute to brain degeneration.

In a second set of experiments we treated animals with the same protocol but for three additional months and then tested whether SFRP1 neutralization would rescue synaptic plasticity deficits already documented for the APP;PS1 mice used in this study⁴⁰. Synaptic function was assessed by electrophysiological recordings of field excitatory postsynaptic potential (fEPSPs) between hippocampal CA3 and CA1 cells in acute slices prepared from wt or APP;PS1 treated mice (see representative traces in Fig. 7K). APP;PS1 animals injected with the control IgG showed a strong impairment in long-term potentiation (LTP), as

288 compared to wt (Fig. 7K-M). APP;PS1 mice treated with α -Sfrp1 antibodies instead
289 presented a LTP response comparable to that of wt (Fig. 7K-M).

290 Taken together these data indicate that antibody-mediated neutralization of SFRP1
291 activity is sufficient to counteract morphological and functional traits of AD.

Discussion

There are many hypotheses that attempt to explain AD pathogenesis. Among them, the amyloid cascade hypothesis has found strong support from genetic, pathological and biochemical studies¹. According to this hypothesis A β production and aggregation has a pivotal role in triggering the cascades of brain alterations that eventually lead to dementia¹. Nevertheless, why A β production and aggregation occurs in AD is still not fully understood⁴. Our data unveil the contribution of SFRP1 in these two processes and provide a proof of concept that neutralization of SFRP1 activity counteracts AP formation and synaptic dysfunction observed in AD mice, representing a therapeutic avenue worth exploring.

Our complementary observations in AD patients and in a mouse model of amyloidosis support a model in which SFRP1, produced and secreted by activated microglia, reactive astrocytes and cells of the choroid plexus, diffuses into the brain parenchyma to interfere with ADAM10-mediated APP processing in neurons, thereby enhancing pro-amyloidogenic APP processing and, thus, the production of toxic A β products. Notably, SFRP1 accumulates in APs, forms molecular aggregates and interacts with A β peptides, with the net result of hampering the formation of proto-fibrillary forms of A β , which are considered to be less harmful⁴¹. A β formation and aggregation further contribute to activate glial cells⁴² and, thus, to further SFRP1 production, suggesting that SFRP1 is part of the feed-forward mechanism that sustains AD pathogenesis (Fig. S15).

Based on our previous findings that *Sfrp1* interact with ADAM10 and thereby modulates the amount of sAPP α derived from ADAM10-mediated non-amyloidogenic APP processing, we have now shown that in AD patients SFRP1 levels positively correlate with the amount of soluble A β . Conversely, lack of *Sfrp1* activity in the brain of APP;PS1 mice strongly reduces soluble A β peptides and the presence of other products of amyloidogenic APP processing, while increasing the levels of sAPP α . These observations support our model that SFRP1 hinders APP processing by ADAM10, allowing for the production of toxic APP products. This mechanism by itself could explain the observed reduction of APs and neuroinflammation in APP;PS1;*Sfrp1*^{-/-} mice as well as their almost normal cognitive functions, due to the feed-forward mechanism of AD progression¹. However, the consequences of a diminished ADAM10 activity may not be directly limited to amyloid deposition, as the list of ADAM10 substrates is long⁴³. For example, the proteins TREM2 and Fractalkine -critical for microglial activation⁴²- and N-cadherin and Neuroligin -involved in

synaptic plasticity- are among its well-recognized targets⁴⁴. Besides, ADAM10 genetic inactivation impairs learning and memory⁴⁴. In support of a possible direct implication of SFRP1 in synaptic function, we have shown that SFRP1 immunoprecipitates with ADAM10 in synaptosomal preparations and that the absence or neutralization of Sfrp1 activity has remarkable effects in preventing cognitive loss and synaptic dysfunction.

The interaction of SFRP1 with A β peptides, its accumulation in APs and its significant upregulation in the brain parenchyma and CSF of AD patients also advocate for SFRP1 implication in disease pathogenesis. Our studies point to two concomitant mechanisms. Indeed, besides favouring amyloidogenic APP processing, SFRP1 seems to influence A β aggregation, limiting the formation of A β 1–42 protofibrils and favouring the presence of oligomeric forms. On the other hand, A β also seems to prevent the formation of SFRP1 spherical structures and SFRP1 may unexpectedly have amyloid characteristics. The significance of these observations for the time being is a matter of speculation. However, oligomeric forms of A β have been shown to be toxic for neurons, whereas proto-fibrillary forms are thought to be less harmful^{24, 25}. This raises the possibility that SFRP1 might indirectly increase A β promoting the existence of its oligomeric forms, a hypothesis that could be tested in the future.

Several anti-A β immunotherapies have given discouraging results^{45, 46}. Nevertheless, recent results obtained either with the administration of a different α -A β mAb (aducanumab)⁷ that targets toxic A β oligomeric forms, or the replacement of plasma albumin, which clears A β peptides⁴⁷, points to the benefit of decreasing soluble oligomeric A β and AP burden. In mice, Sfrp1 neutralization results in an effective maintenance of synaptic function without causing harmful macrophage infiltration³⁹, providing the proof of concept that lowering Sfrp1 levels has a positive effect, at least when applied at early stages of the disease. Thus, in a speculative view, targeting SFRP1 alone or in combination with other promising anti-A β strategies⁷ may represent a therapeutic avenue for pre-symptomatic AD patients, though unwanted side effects remain a possibility. Indeed, SFRP1 acts on different pathways and many of the ADAM10 substrates are implicated in tumour formation and general inflammation⁴⁴. Nonetheless, recent studies proposed that moderate ADAM10 activation might help preventing AD onset⁴⁸. SFRP1 is not a full inhibitor but rather a negative modulator of ADAM10 activity¹⁴. Its targeting might thus provide the right balance between beneficial and harmful effects of increasing ADAM10 activity.

Acknowledgements: We are in debt with I. Torres-Aleman, Instituto Cajal-CSIC, for donating a breeding pair of APP;PS1 mice and with J. Avila, C. Dotti, ML Toribio, S. Knafo and E. Palomer (CBMSO) for their advices during the course of this study. We also acknowledge the generosity of M.L de Ceballos, Instituto Cajal-CSIC, and M. Llorens, CBMSO, for sharing some tissue samples and C. Bovolenta (MolMed) for advice on lentiviral production. We thank O. Lanchó and ML. Toribio for the Sfrp1 lentiviral construct and M. Guerra of the CBMSO EM facilities for help with TEM. We wish to thank C. Dotti, J. Garcia de Yébenes, S.R. de Cordoba (CIB-CSIC), C. Bovolenta and M. Nieto (CNB, CSIC) for critical reading the manuscript. This work was supported by grants from the Spanish MINECO (BFU2013-43213-P; BFU2016-75412-R with FEDER support), Fundación Tatiana Pérez de Guzmán el Bueno and CIBERER to PB and PE, and FIS PI11/3035 to AL. JRC (BES-2011-047189), MIM (BES-2014-068797) and GP (BES-2017-080318) were supported by FPI fellowships from the MINECO. We also acknowledge a CBMSO Institutional grant from the Fundación Ramon Areces.

Authors' contributions: PE and PB set up the research concept, designed the experiments and wrote the manuscript. PE, JRC, MIM, IC, AS, MJMB and GP carried out the experiments except those in Fig. 1B,D; 2M-R and S2, which were performed by PGE and EA under the supervision of IF. IM and MD produced monoclonal antibodies and contributed to set-up ELISA assays. JD performed LTP analysis under the supervision of JAE. PE, JRC, MIM, IC, GP and PB interpreted the data. JRC, PE and PB assembled the figures. IF and MTH provided invaluable conceptual advice whereas JMV contributed to TEM studies and interpretation. IF and AR provided human tissue samples and MTH, AL, JF, DA and EGT provided CSF and other samples.

Competing Interests Statement: The authors declare no competing interests

References

1. Selkoe, D.J. & Hardy, J. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med* 8, 595-608 (2016).
2. Masters, C.L., et al. Alzheimer's disease. *Nat Rev Dis Primers* 1, 15056 (2015).
3. Cummings, J., Lee, G., Ritter, A. & Zhong, K. Alzheimer's disease drug development pipeline: 2018. *Alzheimers Dement (N Y)* 4, 195-214 (2018).
4. Benilova, I., Karran, E. & De Strooper, B. The toxic Abeta oligomer and Alzheimer's disease: an emperor in need of clothes. *Nat Neurosci* 15, 349-357 (2012).
5. Haass, C. & Selkoe, D.J. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol* 8, 101-112 (2007).
6. Fowler, S.W., et al. Genetic Modulation of Soluble Abeta Rescues Cognitive and Synaptic Impairment in a Mouse Model of Alzheimer's Disease. *J Neurosci* 34, 7871-7885 (2014).
7. Sevigny, J., et al. The antibody aducanumab reduces Abeta plaques in Alzheimer's disease. *Nature* 537, 50-56 (2016).
8. Jorissen, E., et al. The disintegrin/metalloproteinase ADAM10 is essential for the establishment of the brain cortex. *J Neurosci* 30, 4833-4844 (2010).
9. Lichtenthaler, S.F. Alpha-secretase in Alzheimer's disease: molecular identity, regulation and therapeutic potential. *J Neurochem* 116, 10-21 (2011).
10. Suh, J., et al. ADAM10 missense mutations potentiate beta-amyloid accumulation by impairing prodomain chaperone function. *Neuron* 80, 385-401 (2013).
11. Kim, M., et al. Potential late-onset Alzheimer's disease-associated mutations in the ADAM10 gene attenuate alfa-secretase activity. *Hum Mol Genet* 18, 3987-3996 (2009).
12. Esteve, P., et al. Secreted frizzled-related proteins are required for Wnt/beta-catenin signalling activation in the vertebrate optic cup. *Development* 138, 4179-4184 (2011).
13. Bovolenta, P., Esteve, P., Ruiz, J.M., Cisneros, E. & Lopez-Rios, J. Beyond Wnt inhibition: new functions of secreted Frizzled-related proteins in development and disease. *Journal of cell science* 121, 737-746 (2008).
14. Esteve, P., et al. SFRPs act as negative modulators of ADAM10 to regulate retinal neurogenesis. *Nat Neurosci* 14, 562-569 (2011).
15. Esteve, P., Crespo, I., Kaimakis, P., Sandonis, A. & Bovolenta, P. Sfrp1 Modulates Cell-signaling Events Underlying Telencephalic Patterning, Growth and Differentiation. *Cereb Cortex* 29, 1059-1074 (2019).
16. Marcos, S., et al. Secreted frizzled related proteins modulate pathfinding and fasciculation of mouse retina ganglion cell axons by direct and indirect mechanisms. *J Neurosci* 35, 4729-4740 (2015).
17. Blalock, E.M., et al. Incipient Alzheimer's disease: microarray correlation analyses reveal major transcriptional and tumor suppressor responses. *Proc Natl Acad Sci U S A* 101, 2173-2178 (2004).
18. Zipfel, W.R., et al. Live tissue intrinsic emission microscopy using multiphoton-excited native fluorescence and second harmonic generation. *Proc Natl Acad Sci U S A* 100, 7075-7080 (2003).
19. Simon, M.J. & Iliff, J.J. Regulation of cerebrospinal fluid (CSF) flow in neurodegenerative, neurovascular and neuroinflammatory disease. *Biochim Biophys Acta* 1862, 442-451 (2016).

20. Tammineni, P., Ye, X., Feng, T., Aikal, D. & Cai, Q. Impaired retrograde transport of axonal autophagosomes contributes to autophagic stress in Alzheimer's disease neurons. *Elife* 6 (2017).
21. Biere, A.L., et al. Amyloid beta-peptide is transported on lipoproteins and albumin in human plasma. *J Biol Chem* 271, 32916-32922 (1996).
22. Jan, A., Hartley, D.M. & Lashuel, H.A. Preparation and characterization of toxic Abeta aggregates for structural and functional studies in Alzheimer's disease research. *Nat Protoc* 5, 1186-1209 (2010).
23. Upadhaya, A.R., Lungrin, I., Yamaguchi, H., Fandrich, M. & Thal, D.R. High-molecular weight Abeta oligomers and protofibrils are the predominant Abeta species in the native soluble protein fraction of the AD brain. *J Cell Mol Med* 16, 287-295 (2012).
24. Ahmed, M., et al. Structural conversion of neurotoxic amyloid-beta(1-42) oligomers to fibrils. *Nat Struct Mol Biol* 17, 561-567 (2010).
25. Bieschke, J., et al. Small-molecule conversion of toxic oligomers to nontoxic beta-sheet-rich amyloid fibrils. *Nat Chem Biol* 8, 93-101 (2011).
26. Sot, B., et al. The chaperonin CCT inhibits assembly of alpha-synuclein amyloid fibrils by a specific, conformation-dependent interaction. *Sci Rep* 7, 40859 (2017).
27. Jankowsky, J.L., et al. Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide in vivo: evidence for augmentation of a 42-specific gamma secretase. *Hum Mol Genet* 13, 159-170 (2004).
28. Guo, Q., et al. Amyloid precursor protein revisited: neuron-specific expression and highly stable nature of soluble derivatives. *J Biol Chem* 287, 2437-2445 (2012).
29. Malinverno, M., et al. Synaptic localization and activity of ADAM10 regulate excitatory synapses through N-cadherin cleavage. *J Neurosci* 30, 16343-16355 (2010).
30. Guenette, S., Strecker, P. & Kins, S. APP Protein Family Signaling at the Synapse: Insights from Intracellular APP-Binding Proteins. *Front Mol Neurosci* 10, 87 (2017).
31. Gowrishankar, S., et al. Massive accumulation of luminal protease-deficient axonal lysosomes at Alzheimer's disease amyloid plaques. *Proc Natl Acad Sci U S A* 112, E3699-3708 (2015).
32. Ferrer, I. Defining Alzheimer as a common age-related neurodegenerative process not inevitably leading to dementia. *Prog Neurobiol* 97, 38-51 (2012).
33. Satoh, W., Gotoh, T., Tsunematsu, Y., Aizawa, S. & Shimono, A. Sfrp1 and Sfrp2 regulate anteroposterior axis elongation and somite segmentation during mouse embryogenesis. *Development* 133, 989-999 (2006).
34. Condello, C., Yuan, P., Schain, A. & Grutzendler, J. Microglia constitute a barrier that prevents neurotoxic protofibrillar Abeta42 hotspots around plaques. *Nat Commun* 6, 6176 (2015).
35. Colombo, A., et al. Constitutive alpha- and beta-secretase cleavages of the amyloid precursor protein are partially coupled in neurons, but not in frequently used cell lines. *Neurobiol Dis* 49, 137-147 (2013).
36. Esteve, P. & Bovolenta, P. The advantages and disadvantages of sfrp1 and sfrp2 expression in pathological events. *Tohoku J Exp Med* 221, 11-17 (2010).
37. Epis, R., et al. Blocking ADAM10 synaptic trafficking generates a model of sporadic Alzheimer's disease. *Brain* 133, 3323-3335 (2010).
38. Serneels, L., et al. gamma-Secretase heterogeneity in the Aph1 subunit: relevance for Alzheimer's disease. *Science* 324, 639-642 (2009).

39. Wang, Y., et al. Lessons from Anti-Amyloid-beta Immunotherapies in Alzheimer Disease: Aiming at a Moving Target. *Neurodegener Dis* 17, 242-250 (2017).
40. Li, Q., et al. Metaplasticity mechanisms restore plasticity and associativity in an animal model of Alzheimer's disease. *Proc Natl Acad Sci U S A* 114, 5527-5532 (2017).
41. Selkoe, D.J. Soluble oligomers of the amyloid beta-protein impair synaptic plasticity and behavior. *Behav Brain Res* 192, 106-113 (2008).
42. Song, W.M. & Colonna, M. The identity and function of microglia in neurodegeneration. *Nat Immunol* 19, 1048-1058 (2018).
43. Kuhn, P.H., et al. Systematic substrate identification indicates a central role for the metalloprotease ADAM10 in axon targeting and synapse function. *Elife* 5 (2016).
44. Saftig, P. & Lichtenthaler, S.F. The alpha secretase ADAM10: A metalloprotease with multiple functions in the brain. *Prog Neurobiol* 135, 1-20 (2015).
45. Godyn, J., Jonczyk, J., Panek, D. & Malawska, B. Therapeutic strategies for Alzheimer's disease in clinical trials. *Pharmacol Rep* 68, 127-138 (2016).
46. St-Amour, I., Cicchetti, F. & Calon, F. Immunotherapies in Alzheimer's disease: Too much, too little, too late or off-target? *Acta Neuropathol* 131, 481-504 (2016).
47. Boada, M., et al. Efficacy and Safety of Plasma Exchange with 5% Albumin to Modify Cerebrospinal Fluid and Plasma Amyloid-beta Concentrations and Cognition Outcomes in Alzheimer's Disease Patients: A Multicenter, Randomized, Controlled Clinical Trial. *J Alzheimers Dis* 56, 129-143 (2017).
48. Endres, K. & Fahrenholz, F. Upregulation of the alpha-secretase ADAM10--risk or reason for hope? *FEBS J* 277, 1585-1596 (2010).
49. Vojdani, A. & Vojdani, E. Amyloid-Beta 1-42 Cross-Reactive Antibody Prevalent in Human Sera May Contribute to Intraneuronal Deposition of A-Beta-P-42. *Int J Alzheimers Dis* 2018, 1672568 (2018).

Figure legends

Figure 1. SFRP1 is upregulated in human samples from AD patients correlating with pro-amyloidogenic APP processing. (A, C) The graphs show ELISA determination of SFRP1 protein levels in TBS and RIPA-soluble fractions of entorhinal and frontal cortex from controls (C) and AD patients at different BB stages as indicated in the graphs. Data were analysed with two-way ANOVA followed by Bonferroni test. * $p < 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$ (entorhinal $n=8$, frontal $n=7$ cortex; entorhinal cortex; soluble fraction: I-II versus C $P=0.0011$; III-IV versus C $P=0.0001$; RIPA fraction: III-IV versus C $P=0.0217$, V-VI versus C $P=0.01$; frontal cortex RIPA fraction: V-VI versus C; $P=0.00319$). (B, D) The graphs show fold enrichment of *SFRP1* mRNA levels in the entorhinal and frontal cortex of AD at different BB stages when compared with controls. Values were determined by Taqman PCR assays, normalized against the levels of HPRT and AARS housekeeping genes. Results were analysed with Kruskal-Wallis followed by post hoc Mann-Whitney test. Differences between groups were considered statistically significant at p-value: * $p \leq 0.05$; ** $p \leq 0.01$ (Entorhinal cortex: Control $n=6$, I-II $n=5$, III-IV $n=4$, V-VI $n=5$; Frontal cortex: Control $n=11$, I-II $n=5$, III-IV $n=8$, V-VI $n=20$). (E, F) Scatter plot of A β 42 (E) and sAPP α (F) versus SFRP1 levels in samples of human entorhinal cortex. SFRP1, A β 42 and sAPP α levels were determined by specific ELISA on the same samples. Note the significant positive correlation between SFRP1 and A β 42 levels (E) and the tendency towards a negative correlation between SFRP1 and sAPP α (F). The data were analysed with liner regression method. (G) The graph shows ELISA based determination of sAPP α levels in human control ($n=5$) and AD entorhinal ($n=13$) cortex extracts. Data are means and error bars the SEM analysed with two-sided T-test *** $p \leq 0.001$. (H) The graph shows SFRP1 levels in CSF samples from cohorts of AD patients ($n=40$) and age-matched controls ($n=43$; Table S2). Data are means and error bars the SEM analysed with two-sided T-test, significance is indicated in the graph. (I-M) Confocal images of frozen sections from the frontal cortex of AD patients (BBV-VI) co-immunostained with antibodies against SFRP1 (red) and A β (clone 6E10) (I-K), GFAP (L) or Iba1 (M) (all in green). In each row, single and merged channels are shown. Note that SFRP1 accumulates in the core of the A β + plaque (arrowheads in I, J), forms aggregates in the AP halo (arrow in J), is associated to blood vessels (K) and localizes to GFAP+ (arrowhead in L) and Iba1+ cells (arrowhead in M; see also Video S1 and S2). These experiments were repeated three times obtaining similar results. Scale bar: 50 μ m.

Figure 2. SFRP1 binds to A β hindering the formation of A β proto-fibrils. **A, B)** Western blot analysis showing hSFRP1 and hA β 1-42 interaction after *in vitro* incubation in TBS solution for 24 hrs at 37°C. Note the formation of SDS-resistant complexes between the two proteins. Note also that the anti-A β 42 mAb recognizes SFRP1. This cross-reactivity has been reported for other proteins⁴⁹. **C-E)** Transmission electron microscopic analysis of A β 1-42 (**C**) and SFRP1 (**E**) alone or in combination (**D**) after incubation at 37°C for the indicated times. Note that SFRP1 favours the oligomeric (red arrows) vs proto-fibrillary (black arrows) form of A β 1-42. SFRP1 alone also tends to aggregate (**E**). All experiments were repeated three times, obtaining similar results. Scale bar 80 nm.

Figure 3. Progressive upregulation of brain Sfrp1 correlates with the age of APP;PS1 mice brains and its forced expression accelerates the appearance of APs. **(A)** ELISA determination of Sfrp1 levels in brain extracts from 10-12 months old mice of the indicated genotypes. WT (n=11); APP;PS1 (n=7); *Sfrp1*^{-/-} and APP;PS1;*Sfrp1*^{-/-} (n=3); *****p*≤0.0001. Data are means and error bars the SEM analysed with two-way ANOVA followed by Bonferroni test. **(B)** ELISA determination of Sfrp1 protein levels in brain extracts from wt, APP;PS1 and *Sfrp1*^{-/-} and APP;PS1;*Sfrp1*^{-/-} (used as negative controls) mice at different ages. Data are means and error bars represent the SEM analysed with two-way ANOVA followed by Bonferroni test. APP;PS1 versus APP;PS1;*Sfrp1*^{-/-} at 2 months *P*=0.0007; *****p*≤0.0001. **(C-E)** Frontal cortical sections from 4.5 months-old APP;PS1 brains co-immunostained as indicated in each panel. Note that Sfrp1 co-localizes with some Iba1+ microglial cells (**C**), GFAP+ astrocytes (**D**), A β + AP (**E**). **(F-K)** Frontal cryostat sections from mouse cortex (age and genotype indicated in the panels) immunostained for Sfrp1 and stained for ThioS (**F-H'**). Positive signal localizes to the ThioS+ APs (**G, G',H, H'**) and choroid plexus (**I-K**) of APP;PS1 mice but is poorly detected in wt. **L)** Cropped images of Western blot analysis of A β and Sfrp1 co-immunoprecipitations from cortical extracts of APP;PS1 and APP;PS1;*Sfrp1*^{-/-} mice as indicated in the panels. **M)** Cropped images of Western blot analysis showing the presence of APP, Sfrp1, ADAM10 in the Snap25+ synaptic fractions. **N)** Cropped images of Western blot analysis of co-IP studies showing that Sfrp1 specifically interacts with ADAM10 at the synapses of APP;PS1 animals. The experiments illustrated in this figure were repeated at least three times obtaining similar results. Scale bars 100 μ m; F-H'; 50 μ m C-E; I-K

Figure 4. *Sfrp1* is expressed by glial cells in APP;PS1 mice and its forced brain expression accelerates the appearance of APs. **A-E)** Frontal cryostat sections of the cortex from 6 months-old APP;PS1 and wt mice subjected to RNAscope ISH for *Sfrp1* and *Iba1* or hybridized for *Sfrp1* and stained with ThioS or antibodies against GFAP, as indicated in the panels. *Sfrp1* mRNA (red signal) is present at low level in the cortex (A) and choroid plexus (B) of wt but it is abundantly present in the choroid plexus and *Iba1*+ (blue signal) microglial cells of APP;PS1 brains (black arrows in A). *Sfrp1*+ red signal is also found in *Iba1* negative cells (green arrows in A). *Sfrp1*+ red signal also colocalizes with GFAP+ immunostaining (C). Note that cells close to the plaque present a significantly higher number of dots (E) compared to cells far from the plaque (D), reflecting an increased presence of *Sfrp1* mRNA. The experiments were repeated 3 times with similar results. Scale bar: 100 μ m. **F)** The graph shows the quantification of the number of *Sfrp1*+ dots in cells close (n=23) or far (n=27) from a ThioS+ plaque. Data are means $\pm$ SEM, analyzed with two-sided Student-t test; p=0.00042. **G-L)** Frontal cryostat sections from the cortex of 5.5 months-old heterozygous APP;PS1 transduced at 2.5 months of age with lentiviruses (LV) carrying *GFP* (n=3) (**G,I,K**) or *Sfrp1*-IRES-GFP (n=3) (**H,J,L**). Sections were co-immunostained for A β 42, LAMP1 or the activated microglial marker CD45, as indicated in the panels. **M, N)** Quantification of LAMP1+ area (P=0.009) and A β 42+ hotspots (P=0.0029) in APP;PS1 transduced animals. Data are means $\pm$ SEM, analyzed with two-sided Student-t test; *p $\leq$ 0.05; **p $\leq$ 0.01. Scale bars: 100 μ m

Figure 5. *Sfrp1* inactivation prevents the appearance of pathological traits in APP;PS1 mice. **(A-F)** Frontal cryostat sections of the cortex from (n=6), 9 (n=4) and 20 (n=3) months-old APP;PS1 and APP;PS1;*Sfrp1*^{-/-} mice immunostained for A β 42, as indicated in the panels. **G)** Quantification of A β 42 hotspots as illustrated in (A-F). Data are means $\pm$ SEM analyzed with two-way ANOVA followed by Bonferroni test; ****p $\leq$ 0.0001; APP;PS1;*Sfrp1*^{-/-} versus APP;PS1 at 20 months-old p=0.0006. **H-S)** Frontal cryostat sections of the cortex from 4.5 months-old APP;PS1 and APP;PS1;*Sfrp1*^{-/-} mice immunostained for LAMP1 (n=3), *Iba1* (n=3), CD45 (n=4) and GFAP (n=5) and relative quantification of immune-positive signals (J, M, P, S). Data are means $\pm$ SEM analyzed with two-sided Student-t test. LAMP1, P=0.004; *Iba1*, P=0.0096; CD45, P=0.004; GFAP, P=0.004. Scale bars: 100 μ m

Figure 6. *Sfrp1* inactivation in APP;PS1 mice shifts APP processing towards the non-amyloidogenic pathway and prevents cognitive decline. **A-B)** The graphs show ELISA based determination of A β 42 (n=4) and sAPP α (n=3) levels present in cortical extracts from 6 months-old APP;PS1 and APP;PS1;*Sfrp1*^{-/-} mice. Data are means $\pm$ SEM analyzed with two-sided Student t-test. **** $p \leq 0.0001$. sAPP α , P=0.0062. **C)** Cropped images of Western blot analysis of TBS-TX100 extracts from 6 months-old APP;PS1 (n=4) and APP;PS1;*Sfrp1*^{-/-} (n=4) brains immunoprecipitated (IP) with anti-A β (6E10) antibodies and visualized with anti-APP C-terminal (anti-CTF) and anti-A β (4G8) antibodies (extended information, Fig S13). **D)** Quantification of immunoreactive bands as those shown in C normalized to full-length APP levels. Data are means $\pm$ SEM analyzed with two-way ANOVA followed by Bonferroni test **** $p \leq 0.0001$. **E)** Cropped images of Western blot analysis of CTF α , CTF β and APP content in cortical extracts from APP;PS1 (n=3) and APP;PS1;*Sfrp1*^{-/-} (n=3) mice. Membranes probed with the anti-APP C-ter antibody. **F)** Quantification of immunoreactive bands of WB studies as shown in E. Data are means $\pm$ SEM analyzed with two-sided Student-t test, P= 0.0011. **G-H)** The graphs depict temporal learning skills (**G**), and recognition index (**H**) of wt (n=6), APP;PS1 (n=8), and APP;PS1;*Sfrp1*^{-/-} (n=6) 8 months-old mice subjected to Morris water maze (**G**) and ORT (**H**) (Extended information in Fig S12). Data are means $\pm$ SEM analyzed with one- or two-way ANOVA followed by Tukey method. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

Figure 7. Antibody-mediated neutralization of Sfrp1 activity counteracts the appearance of AD pathogenic traits in mice. **(A-F)** Frontal cryostat sections of the cortex from 4-months-old APP;PS1 mice treated for 2 months with an unspecific mouse IgG1 (n=6) or the α -Sfrp1 mAb 10.5.6 (n=6), immunostained as indicated in the panels. Scale bar: 100 μ m. **G,H)** Quantification of the number of ThioS+ (P= 0.0063) and A β 42+ (P= 0.0021) APs present in the cortex of APP;PS1 mice after treatment with IgG1 (n=7) or α -Sfrp1(n=9). **I, J)** The graphs show the percent of LAMP1+ area (P=0.0028) and the size (P= 0.0256) distribution of LAMP1+ spots in the cortex of APP;PS1 after treatment with IgG1 (n=502, from 6 animals) or α -Sfrp1 (n=132, from 6 animals). Note that Sfrp1 neutralization prevents the appearance of larger LAMP1 hotspots. Data are means $\pm$ SEM analysed with two-sided Student's t-test, * $p \leq 0.05$; ** $p \leq 0.01$. **K-M)** Blockade of SFRP1 function rescues synaptic plasticity in APP;PS1 mice. **K).** Representative traces for electrophysiological recordings with acute hippocampal slices from wt (left; n=4) or APP;PS1 mice treated with mouse IgG1

(middle, n=3) or the α -Sfrp1 mAb (right; n=3). Baseline responses (light traces) are taken between 10 and 0 minutes before LTP induction, and potentiated responses (dark traces) are taken between 50 and 60 minutes after LTP induction. **L)** Time course of CA3-to-CA1 synaptic transmission (fEPSP slope) during an LTP experiment with acute hippocampal slices from wt (white symbols) or APP;PS1 mice treated with mouse IgG1 (black symbols) or the α -Sfrp1 mAb (red symbols). LTP was induced by θ -burst stimulation (TBS) and recorded for 60 min post-induction, following at least 20 min of stable baseline. Data are means $\pm$ SEM analysed by two-sided Student's t-test. **M)** Quantification of average synaptic potentiation from the last 10 minutes of the time course shown in **L**. Data are means $\pm$ SEM analysed with Mann-Whitney test. WT vs IgG treated, P=0.0164; IgG vs α -Sfrp1, P=0.0023.

Methods

Selection of cases and general human tissue samples processing. Brain tissue was obtained from the Institute of Neuropathology HUB-ICO-IDIBELL and Clinic Hospital-IDIBAPS Biobanks and the CIEN Tissue Bank, CIEN Foundation, Instituto de Salud Carlos III, following the guidelines of Spanish legislation on this matter and the approval of the local ethics committees. CSFs were collected at the Dept. of Neurology outpatient unit for neurodegenerative disease (KBFZ) of the University of Bonn by MTH. Details of data collections and criteria used to classify the samples are provided in Supplementary methods.

RNA purification: RNA was purified with RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, DE) following the manufacturer's protocol. Samples' concentration was determined at A260 using a Nanodrop 2000 spectrophotometer (Thermo Fisher, Waltham, Massachusetts, USA). RNA integrity was determined using the Agilent 2100 BioAnalyzer (Agilent, Santa Clara, CA, USA).

Retrotranscription reaction: Samples with RIN values from 6.4 to 9.1 were retro-transcribed in the presence or the absence (to assess genomic DNA contamination) of MultiScribe Reverse Transcriptase using the High capacity cDNA Archive kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's guidelines and using Gene Amp® 9700 PCR System thermocycler (Applied Biosystems, Foster City, CA, USA).

TaqMan Real Time PCR: TaqMan PCR assays were performed in duplicate in 384-well optical plates using an ABI Prism 7900 Sequence Detection system (Applied Biosystems, Foster City, CA, USA) and the probes listed in Table S3. The TaqMan reaction mixture (10 µl) contained 4.5 µl cDNA was mixed with 0.5µl 20x TaqMan Gene Expression Assays and 5 µl of 2x TaqMan Universal polymerase chain reaction (PCR) Master Mix (Applied Biosystems, Foster City, CA, USA). HPRT (hypoxanthine phosphoribosyltransferase) and AARS (alanyl-tRNA synthetase) probes were used in parallel assays performed in each sample and used as housekeeping controls. The reactions were performed using the following parameters: 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s and 60°C for 1 min. All TaqMan PCR data were captured using the Sequence Detector Software (SDS version 1.9, Applied Biosystems, Foster City, CA, USA). Taqman probes and context sequences are listed in Supplementary Table S3. Samples were analyzed with the double delta CT ($\Delta\Delta CT$) method. Delta CT (ΔCT) values represent normalized target gene levels with respect to internal housekeeping controls (HPRT1 and AARS), selected for the replicating efficiency in

human post-mortem brain tissue⁵⁰. $\Delta\Delta\text{CT}$ values were calculated as the ΔCT of each test sample minus the mean ΔCT of the calibrator samples for each target gene. The fold change was calculated using the equation $2^{(-\Delta\Delta\text{CT})}$. Results were analyzed with Kruskal-Wallis followed by post hoc Mann-Whitney test. Differences between groups were considered statistically significant at p-value: * $p < 0.05$.

Mice. Double chimeric transgenic APP;PS1 mice, expressing the human mutated APP (APP695swe) and presenilin1 (PS1-dE9) genes under the prion promoter that directs expression to the CNS²⁷, were crossed with *Sfrp1*^{+/-} mice, generated as described⁵ and maintained in B6/129 mixed background, to obtain APP;PS1;*Sfrp1*^{-/-} and APP;PS1;*Sfrp1*^{+/+} mice. Initial breeding pairs of the APP;PS1 mice were kindly provided by Dr. Torres-Aleman, Instituto Cajal, CSIC, Madrid. Homozygous male mice of age comprised between one and twenty months of age were used throughout the study, unless otherwise stated, with no inclusion/exclusion criteria other than genotype, sex and age. Within these criteria animals were randomly chosen among the available males. Animals were housed and treated according to European Communities Council Directive of 24 November 1986 (86/609/EEC) regulating animal research. All procedures were approved by the Bioethics Subcommittee of Consejo Superior de Investigaciones Científicas (CSIC, Madrid, Spain) and the Comunidad de Madrid under the following protocol approval number (PROEX 100/15; RD 53/2013). The number of animals used for each experiment is indicated in the figures or their legends. The minimum number of animals required in each experiment was predicted using the G3*Power 3.1 program, as detailed in the statistical section of this methods.

Antibodies. The following monoclonal antibodies were used: mouse anti-APP (clone 22C11, N-terminal, 1:1000, Chemicon, cat #MAB348, batch LV1634989), mouse anti-A β (clone 4G8, that recognizes amino acids 17-24 of the human and mouse A β (1:500, Covance, cat #SIG-39220, batch D11DF00836), mouse monoclonal anti-A β (clone 6E10, that recognizes amino acids 1-16 of the human A β and sAPP α (1:500, Covance, cat #SIG-39320), rabbit anti-A β (H31L21, that recognizes amino acids 36-42 of the human and mouse A β (1:1000, Thermo Fisher, cat #700254, batch RH240023), mouse anti-sAPP α (clone 2B3, which recognizes sAPP α , 1:500, IBL, cat #11088, batch 11-227), rabbit anti-SFRP1 (1:2000, Abcam, cat #ab126613), rat anti-CD45 (clone IBL-3/16, 1: 500, AbDSerotec, cat #MCA1388), rat anti-LAMP1 (clone ID4B, 1:200, DSHB), mouse anti- α tubulin (clone B-5-1-2, 1:5000, Sigma-Aldrich), mouse anti-NeuN (clone A60, 1:500, Millipore, cat #MAB377, batch LV1457494); rabbit anti-Axin2 (1:1000, Abcam #ab109307; lot GR1783799020); mouse

anti-Cdh2 (Clone 3B9 1:500 Invitrogen #180224; lot 1553634A). Rabbit polyclonal antibodies were the following: anti-sAPP β (1:500, BioLegend, cat #813401) anti-A β 1-40/42 (1:500, Millipore, cat #AB5076, batch 2584867), anti-APP C-terminal (1:500, Sigma-Aldrich, cat #A8717, batch 045M4785V), anti-Sfrp1 (1:500, Abcam, cat #ab4193), anti-GFAP (1:3000, DAKO, cat #Z0334, batch 20028619), anti-Iba1 (1:2000; Wako, cat #019-19741, batch LKG5732); anti-BACE1 (1:1000 Calbiochem #195111 batch D0903996), anti-Amyloid fibril OC (1:1000; Millipore, cat #AB2886), anti-Amyloid oligomer $\alpha\beta$ (A11) (1:1000; Millipore, cat #AB9234, batch 3108666). Chick anti- β Gal (1:500, Abcam, cat #ab9361, batch 365032). Secondary antibodies were the followings: Alexa-488, Alexa-594- conjugated affinity-purified secondary anti-rabbit or mouse antibodies (1:2000 Molecular Probes) or biotin-conjugated anti-rabbit and anti-mouse IgG (1:500; Jackson ImmunoResearch, Cambridgeshire, UK) and visualized with peroxidase conjugated streptavidin (1:500, Jackson ImmunoResearch). All antibodies used in the study were validated by the commercial sources, except the mAb against SFRP1 we generated that are validated in Fig. S1.

Generation of anti-SFRP1 monoclonal antibodies (mAb). Detailed in supplementary methods.

Semi-quantitative PCRs. Detailed in supplementary methods.

In situ hybridization (ISH) and immunohistochemistry (IHC). Mice were anesthetized with pentobarbital or ketamine/xilacine and transcardiacally perfused with 4% paraformaldehyde (PFA) in phosphate buffer (PB). Brains were extracted and post-fixed for 24h at 4°C. Tissue was then washed in PB saline (PBS), incubated in a 30% sucrose/PBS solution, embedded and frozen in a 7.5% gelatin, 15% sucrose solution. ISH was performed with RNAscope 2.5 Duplex detection technology (ACDbio) in 10-15 μ m cryostat sections, using a probe for mouse *Sfrp1* mRNA (channel C1, detected in red) and a probe for mouse *Iba1* mRNA (channel C2, detected in blue). ISH for *Sfrp1* was combined with IHC for GFAP as described below. IHC was performed on free-floating 50 μ m vibratome or 6 μ m paraffin sections from frontal human cortices and on 10-15 μ m mouse brain cryostat sections. In the case of A β and Sfrp1 co-immunostaining, sections were treated in 90% formic acid for 10 minutes or boiled in citrate buffer (10mM, pH:6) and then with 0.3% H₂O₂ in methanol for 20 min. After several washes in PBS, sections were treated with M.O.M. blocking reagent (Vector Labs), incubated in PBS containing 0.5% Triton X100, 5% FCS and 1% BSA for 2h at RT, and thereafter with anti-Sfrp1 10.5.6 mAb (1:2000, Supplementary Methods) or anti-Sfrp1 rabbit

polyclonal (Abcam) and anti-A β _{1-40/42} (4G8 or rabbit polyclonal, 1:500) antibodies diluted in the same buffer overnight at 4°C. After washing, sections were incubated with secondary antibodies for 2h. AP in human and mouse tissues were visualized with 0.01% ThioflavinS (Sigma-Aldrich) in 50% EtOH and differentiated in 50% EtOH. In the case of Sfrp1 and GFAP or Iba1 and A β 42 and CD45 or LAMP1 co-immunostaining, sections were pre-treated as above and incubated with appropriate antibodies overnight at 4°C. Tissue was analyzed with fluorescent conventional or confocal microscopy.

Synaptosome isolation. Synaptosomes were isolated from wt and APP;PS1 hemispheres as previously described⁵¹ with minor modifications. The synaptosome enriched fractions were solubilized in 147 mM NaCl, 3 mM KCl, 10 mM Glucose, 2 mM MgSO₄, 2mM CaCl₂, 20 mM Hepes, 0.1% SDS, 0.1% Na-Deoxicolate, 1% Triton X-100 plus protease inhibitors (Roche).

Immunoprecipitation and western blot analysis. To detect APP and its derived proteolytic peptides, cortical hemispheres from wt, APP;PS1;Sfrp1^{-/-} and APP;PS1 mice were homogenized in Tris-NaCl soluble buffer with protease inhibitors and centrifuged. The resulting pellets were homogenized in RIPA lysis buffer with protease inhibitors and processed for immunoprecipitation as described⁵² with the following modifications. After immunoprecipitation with anti-A β 6E10 mAb, beads were re-suspended in NuPAGE LDS sample buffer (4X) (Thermo Fisher, Waltham, MA, USA) without 2-mercaptoethanol, heated at 97°C on a shaker for 10 min, centrifuged and the supernatants were resolved in 10-20% Tris-Tricine gels (BIO-RAD). Gels were transferred onto nitrocellulose membranes by dry iBlot (Invitrogen). The membranes were then boiled in PBS for 7 min, stained with Ponceau red solution, washed in TBST, and incubated sequentially in PBS containing 0.05% Tween, 10% nonfat milk and 0.1% BSA and then with a6E10 mAb solution, followed by peroxidase-conjugated secondary antibody. Signal was visualized with the ECL Advanced Western Blotting Detection Kit (Amersham). Membranes were stripped with Re-Blot Plus Strong Solution (Millipore) and re-blotted with the 4G8 mAb or anti-APP-C-terminal polyclonal antibody. To detect Sfrp1-A β interaction, co-IP studies were performed using RIPA brain extracts from APP;PS1 and APP;PS1; Sfrp1^{-/-} using either anti-A β (6E10), anti-Sfrp1 or IgG isotype control antibodies. Co-IPs were analyzed by Western Blot with either anti-Sfrp1 or anti-A β antibodies, respectively. Co-IP studies to determine the possible interaction of Sfrp1 to ADAM10 in the synaptosome-enriched fraction from wt and APP;PS1 were performed with the same procedure. To detect APP and the derived proteolytic peptides in human tissue

by Western blot, entorhinal cortex samples were homogenized in RIPA lysis buffer supplemented with a proteinase inhibitor mixture (Roche) and PMSF (Sigma). Protein content was quantified with the Kit BCA (Thermo Scientific) and tissue extracts (20 µg) were separated with SDS-PAGE electrophoresis. Proteins were transferred onto nitrocellulose membranes. After blocking with TBS containing 0.1% Tween and 5% non-fat milk, the membranes were incubated overnight at 4°C with primary antibodies to detect sAPP α , sAPP β , APP and APP C-terminal fragments. Membranes were washed and then incubated with peroxidase-conjugated secondary antibodies followed by ECL Advanced Western Blotting Detection kit (GE Healthcare). The immune-reactive bands were quantified by densitometry.

Protein-protein binding assays. Nanomolar concentrations of recombinant human SFRP1 (RD cat: 5396-SF-025) and human A β 1-42 peptides (Life Technologies, cat: 03111, prepared as described in the data sheet to obtain minimal peptide aggregation) were mixed in protein LoBind tubes (Eppendorf) for 24 hrs at 37°C. NuPAGE LDS sample buffer (4x) was added to the tubes, boiled for 5 min and subjected to Western blot analysis. Microplate-based solid-phase protein-protein binding assays⁵³ was performed by immobilizing in wells 200 ng/well of either human A β 1-42 peptides, BSA, used as a positive control because previously described as an A β interactor²¹; or heparin, a described SFRP1 binding partner^{54, 55}. Immobilization was performed overnight at 4°C. The wells were then washed with TBS 3x and incubated with two different SFRP1 concentrations for 2 hrs at RT. In the converse experiment, BSA, heparin or SFRP1 were immobilized overnight at 4°C and two different concentrations of A β 1-42 were added for 2 hrs at RT. The wells were washed 6x in TBS and 2x sample buffer containing β -mercaptoethanol was added to the well. The content was recovered by scrapping, boiled for 5min and analyzed by SDS-PAGE followed by WB using anti-Sfrp1 or anti-A β antibodies, respectively.

Transmission electron microscopy analysis. Lyophilized human A β 1-42 peptides (Life Technologies, Cat:0311) were prepared in conditions that allow minimal peptide aggregation as indicated by the provider. Briefly, peptides were dissolved in 100% Hexafluoroisopropanol (HFIP), dried, and dissolved again with at 1mM in Dimethyl sulfoxide (DMSO) (1mM stock solution). For aggregation studies, A β 1-42 from the stock solution and human SFRP1 (RD, Cat:5396-SF) were diluted in TBS in protein LoBind tubes (Eppendorf) at 100µg/ml and 7µg/ml (200nM), respectively, and mixed when needed. Samples were incubated at 37 °C for the indicated times. For electron microscopy, aliquots of the different samples (A β 1-42,

SFRP1 and A β 1-42 + SFRP1) were placed onto 400-mesh carbon-coated grids (EMS; G400H-Cu), incubated for 3 min, negatively stained with 2% uranyl acetate for 2 min, and dried. Grids were analysed and photographed were taken with a JEM 1010 transmission electron microscope operated at 120 kV and recorded at 60,000x magnification.

Dot blot assays. A β 1-42 peptides and SFRP1 protein samples were prepared as described for TEM. Protein and peptides, alone or in combination, in TBS buffer were incubated for 24 hr at 37°C. Aliquots of each sample (2 μ l) were spotted on nitrocellulose membranes and allowed to dry for 15 min. The membranes were blocked for 1 hr with 5% non-fat dry milk in TBS-T (TBS Tween 0.1%) or with 1% gelatine from porcine skin (SIGMA) in TBS-T when the A11 antibody was used. Membranes were incubated overnight at 4°C with the following primary antibodies: anti-Amyloid fibril OC, anti-Amyloid oligomer $\alpha\beta$ (A11), anti-N-terminal A β (6E10), anti-SFRP1. Membranes were then washed with TBS-T 5X for 10 min, incubated for 1 hr with HRP-linked secondary antibodies diluted in 5% non-fat dry milk in TBS-T or in 1% gelatine in TBS-T (as above), washed 5X for 10 min in TBS-T, incubated with ECL Select reagent (Amersham) for 5 min and finally exposed to X-ray films.

Lentiviral production and injection. Lentiviral preparations expressing GFP or SFRP1-IRES-GFP were obtained by transient transfection of mycoplasma-free HEK-293T cells, originally obtained from the ATCC. Cells were transfected employing a three plasmid HIV-derived and VSV pseudotyped lentiviral system kindly provided by M.K. Collins, University College London, UK; A. Thraser, Institute of Child Health, UK; and D. Trono, Ecole Polytechnique Fédérale de Lausanne, Switzerland. Culture supernatants were collected two and three days after transfection and ultra-centrifuged. The pellets containing the lentiviral particles were re-suspended in PBS (1×10^8 TU/ml). Mice were anesthetized and placed in a stereotaxic frame. Small volumes (2.5 μ l) of Sfrp1-GFP or GFP lentiviral particles were injected unilaterally in the frontal cortex using appropriate stereotaxic coordinates. Sham-operated animals served as additional controls. One- or three-months post-injection, mice were sacrificed and their brains were analyzed by IHC as described above. Six male mice were used for each experimental condition.

ELISA assays. Human CSF samples: to assess the levels of SFRP1 in human CSF, we developed an ELISA assay using mAb 10.5.6 (IgG1) and 17.8.13 (IgG2b) for capture and detection, respectively. 96-well microtiter plates (Nunc Roskilde Denmark) were coated overnight at 4°C with 50 μ l/well of goat anti-mouse IgG1 (SouthernBiotech AL USA) at 3 μ g/ml in PBS. Plates were washed 3 times with PBS containing 0.05% Tween 20 (PBST)

and blocked (37°C, 30 min) with 100µl/well of 2% BSA in PBST (Sigma-Aldrich-Aldrich MO, USA). Plates were washed again with PBST and incubated (2 hrs, 37°C) with 50 µl/well of anti-Sfrp1 mAb 10.5.6 (1:10 dilution of hybridoma supernatant in blocking buffer). Wells were washed with PBST and incubated (2 hr, 37°C) with 50µl/well of samples diluted 1:10 and 1:20. After extensive washing with PBST, wells were incubated (1 hr, 37°C) with 50µl/well of anti-Sfrp1 17.8.13 (undiluted hybridoma supernatant). Plates were further washed with PBST and finally incubated (30 min, RT) with 50µl/well of goat anti-mouse IgG2b HRP (SouthernBiotech AL USA; 1:2000). After 3 washes with PBST, the enzymatic reaction was developed in the dark for about 5 min using 100 µl/well of O-phenylenediamine (OPD; 0.4 mg/ml diluted in Citrate-phosphate buffer pH5 0,1M; Sigma-Aldrich, Missouri, USA) and 1/1000 v/v of H₂O₂ (33%). The reaction was terminated by the addition of 50µl/well of 3N H₂SO₄ and the resulting product measured at 492 nm in a microtiter plate ELISA reader.

Mouse and human cortical samples: Human SFRP1 levels from cortex samples and mouse Sfrp1 levels of brain lysates were determined in soluble (TBS) and RIPA fractions prepared as described⁵⁶. The resulting fractions were analyzed in a modified capture ELISA performed as follow. 96-well microtiter plates (Nunc Roskilde Denmark) were coated overnight at 4°C with 50µl/well of 1.5µg/ml purified anti-SFRP1 mAb 10.5.6 in PBS. Plates were washed 3X with PBST and incubated for 3hrs with 2% BSA in PBST at RT, washed with PBST, and incubated 2hrs at 37°C with 50µl/well of brain tissue samples, at protein concentration of 0.1µg/µl, previously quantified by BCA protein assay kit (Thermo Fisher, Waltham, MA, USA). Wells were washed with PBST and incubated for 1hr at 37°C with 50µl/well of biotin-labelled anti-SFRP1 mAb 17.8.13 (using the EZ-Link NHS-PEO4-Biotinylation Kit, Thermo Fisher Waltham, MA, USA, following the manufacturer's procedure) at 1µg/ml. Plates were further washed with PBST and incubated for 1h at RT with 50µl/well of Streptavidin-POD 1:25000 (Jackson Laboratory). After extensive washing with PBST, the enzymatic reaction was developed at RT in the dark for 20 min using 100µl/well of Tetra-methyl-benzidine liquid substrate (TMB slow kinetic form, Sigma-Aldrich Aldrich). The reaction was terminated by the addition of 100µl/well of HCl 2N and the resulting product was measured at 450nm in a microtiter plate ELISA reader FLUOstar OPTIMA (BMG LABTECH).

ELISA assays for Aβ1-42 and sAPPα. The levels of Aβ1-42 and sAPPα in human cortical samples and in the cortex from APP;PS1;*Sfrp1*^{-/-}, APP;PS1 and APP;PS1 mice treated with antibodies were determined in the soluble (TBS) fraction using the human Aβ42 ELISA Kit

(Invitrogen, cat: KHB3441), the human-rat A β 42 ELISA kit (WAKO, cat: 292-64501) and the human sAPP α ELISA kit (MyBioSource, San Diego, CA, USA, cat: MBS915453). Samples were analyzed blindly and in duplicates.

Determination of mAb 10.5.6 Sfrp1 neutralizing activity. To determine the effect of possible neutralizing function among the mAb, generated as above described, we tested the ability of the mAb to favor the generation of sAPP α in the culture medium of primary cultures of telencephalic neuroepithelium from E13.5 wt embryos. Parallel cultures from *Sfrp1*^{-/-} embryos were used as control. See the legend of Fig. S13 for further explanations. Cultures were obtained as follow. Brains were removed in ice-cold Hank's solution (HBSS) and cortices were dissected, after removing the meninges. The samples were trypsinized, plated at a density of 5 $\times$ 10⁵ cells/cm² in dishes coated with poly-D-lysine (20 μ g/ml; Sigma-Aldrich) and cultured in Neurobasal supplemented with B27 nutrients (Gibco BRL). After 24h, the medium was replaced with Neurobasal/B27 alone or containing hybridoma supernatant (1:1 volume) or unspecific mouse IgG1 and cultured for additional 48 hr. The media were collected and the cells lysed, and all samples further processed for western blot analysis as described above.

Intravenous administration of anti-Sfrp1. In a first set of experiments, anti-Sfrp1 mAb 10.5.6 or an unspecific mouse IgG1 used as control were labelled with biotin using the EZ-Link NHS-PEO4-Biotinylation Kit (Thermo Fisher, cat: 21455) according to manufacturer's protocol. The antibodies were injected through the retro-orbital sinus and the animals were perfused after 24 hours and prepared for histological analysis as described above. The presence of the biotinylated mAb was determined by incubating the corresponding cryostat sections with streptavidin-POD (1:500) followed by incubation with tyramide (Cell Signaling). In a second set of experiments two months-old APP;PS1 mice were injected through the retro-orbital sinus once a week for two months, with 100 μ l of purified anti-Sfrp1 mAb 10.5.6 or a unspecific IgG1 as a control (1 μ g/ μ l). After treatment, animals were perfused and prepared for histological analysis as described above. In a third set of experiments, 2 months-old animals were injected once a week with 100 μ g of purified α -Sfrp1 mAb 10.5.6 or an unspecific IgG1 as a control for 5 months. After treatment, animals were prepared as described below for electrophysiology recordings and biochemical studies.

Electrophysiology recordings: Coronal acute hippocampal slices (300 μ m thick) were prepared from mice after antibodies treatments described above. Mice were briefly anaesthetized using dry ice sublimated with water, and the brain removed and placed in partially frozen Ca²⁺-free dissection medium (10 mM D-Glucose, 4 mM KCl, 26 mM

NaHCO₃, 234 mM sucrose, 5 mM MgCl₂, 1:1000 Phenol Red) saturated with 5% CO₂/ 95% O₂. Coronal slices were cut using a Leica VT1200S vibratome and let recover for 1 hr at 32°C in a submersion-type incubation chamber containing artificial cerebrospinal fluid (ACSF: 119 mM NaCl, 2.5 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgCl₂, 26 mM NaHCO₃, 1 mM NaH₂PO₄, 11 mM glucose; pH 7.4). Slices were then transferred to the recording chamber, and maintained at 25 °C under perfusion with ACSF saturated with 5% CO₂/95% O₂. Electrically-evoked field excitatory postsynaptic potentials (fEPSPs) were recorded in the CA1stratum radiatum. Bipolar stimulation electrodes were placed among Schaffer collaterals and glass recording electrodes (1–2 MΩ; filled with ACSF) placed ~200 μm away in the direction of fiber projection. Stimulation frequency during baseline was 0.066 Hz. After acquiring a stable baseline transmission of at least 20 min, LTP was induced with a theta-burst protocol (4 pulses at 100 Hz, with the bursts repeated at 5 Hz and each tetanus including three 10-burst trains separated by 15 s).

Behavioral studies. Detailed in the supplementary methods.

Quantification procedures and Statistical Analysis. For each type of experiment, we performed a pilot study. We then used the freely available statistical power analysis program G3*Power 3.1⁵⁷ to determine the minimal sample size needed in each set of experiment. We used the criteria of a p value of a maximum of 0.05 and a power of at least 0.8. If not otherwise stated, data distribution was assumed to be normal but this was not formally tested. ISH or IHC preparations were visualized and captured, at the same exposure settings for each antibody, with a DM5000 microscope equipped with a DFC350Fx monochrome camera or a DFC500 color camera (Leica Microsystems). Captured areas from at least six sections separated by 200 μm were analyzed per each animal. The number of quantified animals is indicated in each plot and exemplifies a representative group among the total of analyzed animals (5-10 per group). Intensity of immune-positive signals, number of AP burden and area of LAMP1+ dystrophic neurites were quantified using ImageJ software (U.S. National Institutes of Health, Bethesda, Maryland, USA, <https://imagej.nih.gov/ij>). Results were generally analyzed by Student's t-test but statistical tests used in each case are indicated in the corresponding figure legends. Statistically significant differences were considered and represented by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$. Three blinded independent researchers analyzed the experiments. Behavioral tests were statistically analyzed as follow. The normality of the distribution was tested by Kolmogorov-Smirnov test and homoscedasticity was tested by Bartlett's test. Differences between groups were analyzed by

ANOVA and two-sided test. Analysis was performed with the SPSS statistical package version 20.0, using a significance level of $p \leq 0.05$.

Data Availability Statement

The data that support the findings of this study are available from the corresponding authors upon request

Methods only References

50. Barrachina, M., Castano, E. & Ferrer, I. TaqMan PCR assay in the control of RNA normalization in human post-mortem brain tissue. *Neurochem Int* 49, 276-284 (2006).
51. Agostini, F., et al. Prion protein accumulation in lipid rafts of mouse aging brain. *PLoS One* 8, e74244 (2013).
52. Sherman, M.A. & Lesne, S.E. Detecting abeta*56 oligomers in brain tissues. *Methods Mol Biol* 670, 45-56 (2011).
53. Biesiadecki, B.J. & Jin, J.P. A high-throughput solid-phase microplate protein-binding assay to investigate interactions between myofilament proteins. *J Biomed Biotechnol* 2011, 421701 (2011).
54. Uren, A., et al. Secreted frizzled-related protein-1 binds directly to Wingless and is a biphasic modulator of Wnt signaling. *J Biol Chem* 275, 4374-4382 (2000).
55. Zhong, X., et al. Regulation of secreted Frizzled-related protein-1 by heparin. *J Biol Chem* 282, 20523-20533 (2007).
56. Kleinberger, G., et al. TREM2 mutations implicated in neurodegeneration impair cell surface transport and phagocytosis. *Sci Transl Med* 6, 243ra286 (2014).
57. Faul, F., Erdfelder, E., Buchner, A. & Lang, A.G. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav Res Methods* 41, 1149-1160 (2009).