

Aberrant excitatory neuronal ERBB4 promotes Alzheimer's disease pathology

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Neuroinflammation and synapse loss are associated with cognitive decline in Alzheimer's disease (AD). Although microglial hyperphagocytic activity has been implicated in synapse loss^{1–4}, the mechanisms underlying these pathologies remain obscure. Here we demonstrate that, during AD progression in mice, astrocytes and microglia increase phagocytic elimination of excitatory synapses while reducing elimination of inhibitory synapses, suggesting that neuroinflammation alone may be dispensable for early AD synapse loss. Instead, single-nucleus RNA-sequencing analysis identified the emergence of early-responsive excitatory neurons (EREN), characterized by expression of ectopic Erb-B2 receptor tyrosine kinase 4 (*ErbB4*), as one of the earliest major alterations in AD mouse models. Selective *ErbB4* deletion in AD excitatory neurons abrogated abnormal neuronal network activities and synapse loss, as well as reactive gliosis, amyloid plaque deposition and cognitive deficits. Conversely, *ErbB4* overexpression in wild-type excitatory neurons recapitulated these core AD-like phenotypes without amyloid plaques. Mechanistically, these effects required mammalian target of rapamycin (mTOR) signalling downstream of ERBB4. Subsequent transcriptomic analyses showed that excitatory neuronal *ErbB4* is both necessary and sufficient to induce EREN and reactive gliosis. Directed mediation analysis of human AD transcriptomic data further support a model in which excitatory neuronal ERBB4 contributes to a pathogenic cascade that links amyloid pathology to tau propagation and cognitive decline. These findings identify aberrant *ErbB4* expression in excitatory neurons as an early driver of AD pathophysiology and a potential therapeutic target across neurodegenerative diseases.

Synapse loss is evident across multiple brain regions from early stages of AD^{5,6}. A prevailing model proposes that amyloid- β (A β) oligomers induce reactive microglial states and activate complement-dependent phagocytic programs, leading to excessive synapse elimination^{1–4}. Previously, we revealed that astrocytes have a major role in the continuous elimination of both excitatory and inhibitory synapses in the normal adult hippocampus⁷. This astrocytic elimination of adult hippocampal synapses is highly dependent on hippocampal activity and has a critical role in maintaining circuit homeostasis and memory function⁷. As most research on the mechanisms of synapse loss in AD has focused on microglia and excitatory synapses, we initially set out to determine the contribution of astrocytes to the elimination of both excitatory and inhibitory synapses during AD progression.

Here we found that altered glial synapse elimination arises as a result of gene expression and functional changes in AD excitatory neurons. Using single-nucleus RNA-sequencing (snRNA-seq), gene set enrichment analysis (GSEA), weighted gene correlation network analysis

(WGCNA) and candidate gene approach, we identified a single responsible pathway, an ectopically expressed ERBB4 tyrosine kinase receptor in excitatory neurons, as a key disease-promoting factor orchestrating broad aspects of AD pathophysiology. CRISPR-based *ErbB4* knock-out in excitatory neurons of the 5 $\times$ FAD mouse model substantially normalized excitatory neuronal hyperactivity, inhibitory neuronal hypoactivity, abnormal synapse elimination, reactive gliosis and even A β accumulation, leading to significant recovery of cognitive functions. Conversely, restricted *ErbB4* overexpression in wild-type (WT) excitatory neurons reproduced major AD-like phenotypes in the absence of A β plaques through downstream mTOR signalling. Human AD tissue and transcriptomic analyses further linked excitatory neuronal ERBB4 to amyloid and tau pathology and cognitive decline. These findings support a model in which early excitatory neuronal dysfunction, mediated by aberrant *ErbB4* expression, precipitates maladaptive glial responses and synaptic imbalance, promoting cognitive decline and neurodegeneration.

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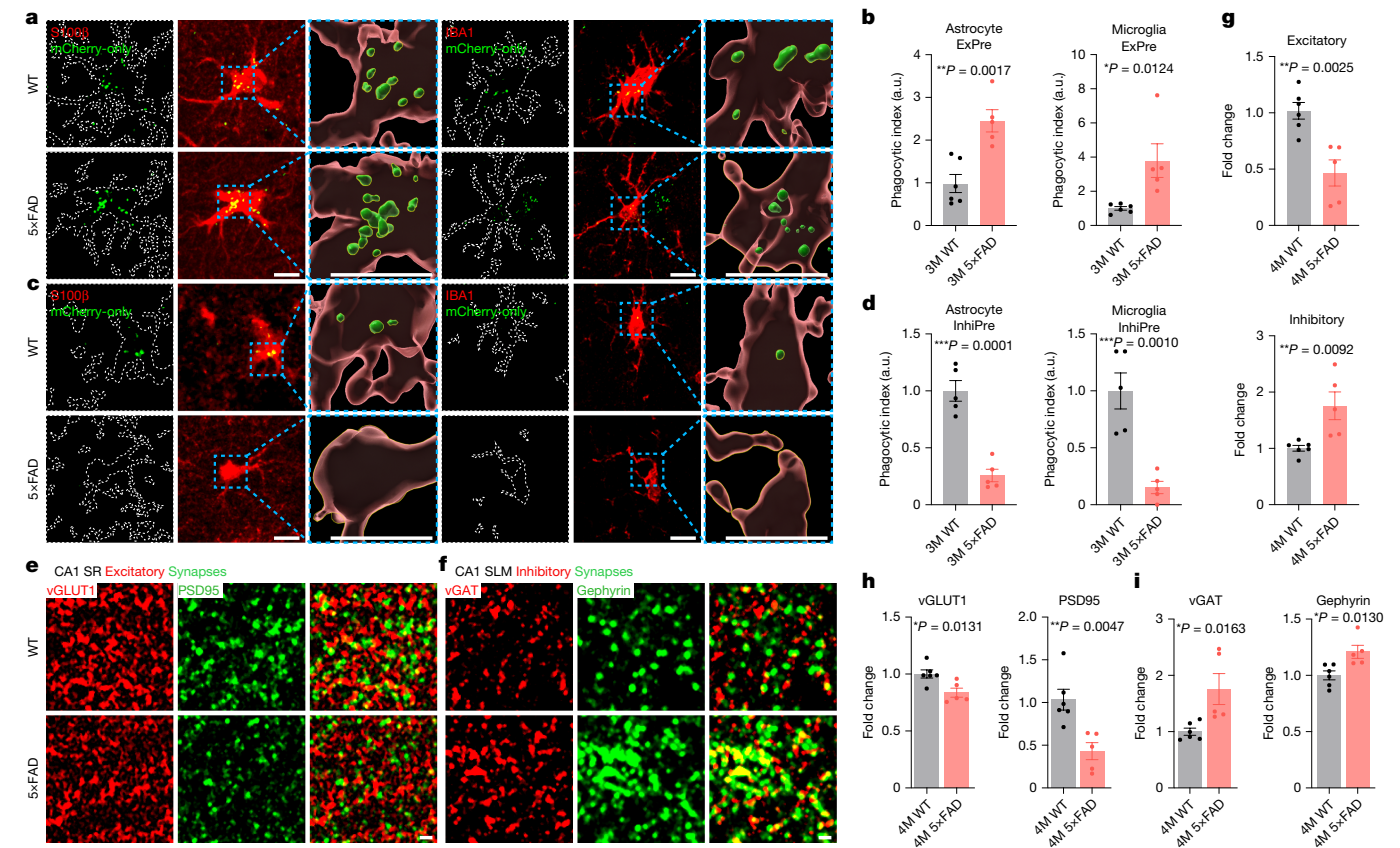


Fig. 1 | Differential elimination of excitatory and inhibitory synapses by AD glia. **a, c.** Representative confocal z-stack images of mCherry-only puncta from ExPre (**a**, green) and InhiPre (**c**, green), along with astrocytes (S100 β) and microglia (IBA1) (red) in the CA1 of 3-month-old (3M) WT and 5x-FAD mice. The white dotted lines indicate the outlines of glial cells. Right, enlarged three-dimensional reconstructions of the blue dotted boxes in the middle panels, highlighting engulfed mCherry-only puncta (green) inside glial cells (burgundy). Scale bars, 10 μ m. **b, d.** Quantification of engulfed excitatory (**b**) and inhibitory (**d**) pre-synapses by astrocytes (left) and microglia (right). $n = 6$ (WT) and

5 (5x-FAD) (**b**); $n = 5$ (WT) and 5 (5x-FAD) (**d**). a.u., arbitrary units. **e, f.** Representative confocal single-plane images of excitatory (**e**; pre-synapse: vGLUT1, red; post-synapse: PSD95, green) and inhibitory (**f**; pre-synapse: vGAT, red; post-synapse: gephyrin, green) synapses in the CA1 of 4-month-old WT and 5x-FAD mice. Scale bars, 1 μ m. **g–i.** Quantification of the excitatory (**h**) and inhibitory (**i**) synapses with their colocalization (excitatory (**g**, top) and inhibitory (**g**, bottom)). $n = 6$ and 5 (**g–i**). Statistical analysis was performed using two-sided unpaired Student's *t*-tests; * $P < 0.05$, ** $P < 0.01$. Data are mean $\pm$ s.e.m. n values represent the number of mice per group.

Opposing synapse elimination by AD glia

We monitored glial synapse engulfment using ExPre and InhiPre reporters, in which acid-labile eGFP undergoes denaturation and loses fluorescence in acidic phagolysosomes, whereas acid-resistant mCherry remains fluorescent, thereby distinguishing intact from engulfed pre-synapses^{7,8} (Extended Data Fig. 1a). ExPre was expressed in hippocampal CA3 excitatory projections and analysed in the stratum radiatum (SR) of the hippocampal CA1, where Schaffer collateral synapses predominate. InhiPre was expressed in CA1 inhibitory neurons and analysed in the stratum lacunosum moleculare (SLM), which is enriched in somatostatin (SST)-positive inhibitory synapses⁹. After reporter delivery to WT and APP/PS1 mice, we quantified astrocytic and microglial engulfment from 3 to 18 months to compare ageing- and AD-associated changes (Extended Data Fig. 1b–e).

Across APP/PS1 progression, excitatory pre-synapse elimination by both astrocytes and microglia progressively increased, whereas inhibitory pre-synapse elimination decreased (Extended Data Fig. 1b–e). Astrocytic changes were evident by 6 months and preceded comparable microglial changes. A similar opposing pattern emerged in the more rapidly progressing 5x-FAD model at 3 months, but not at 2 months, coinciding with the appearance of hippocampal A β plaques (Fig. 1a–d and Extended Data Fig. 1f–i). Astrocytes engulfed more synapses than microglia across synapse types, ages and mouse models (Extended Data Fig. 1j, k), suggesting that they are major early responders to synaptic

perturbation. No sex-dependent differences in phagocytosis were detected (Extended Data Fig. 2a–d).

Synapse numbers were unchanged in the 2- and 3-month-old cohorts but, by 4 months, 5x-FAD mice had fewer excitatory synapses in the SR and more inhibitory synapses in the SLM compared with the WT controls (Fig. 1e–i and Extended Data Fig. 2e–i). These findings, in which changes in synapse numbers are negatively correlated with the extent of synapse engulfment by glial cells, suggest that glial phagocytosis may regulate excitatory as well as inhibitory synapse numbers in AD brains. Furthermore, the selective elimination of excitatory synapses, coupled with reduced elimination of inhibitory synapses, raises the possibility that intrinsic neuronal states may guide glial phagocytic behaviour and that indiscriminate neuroinflammation is unlikely to be the sole driver of synapse elimination in AD.

Notably, excitatory neurons become hyperactive, whereas inhibitory neurons become hypoactive, early in AD^{10–12}. As glial synapse elimination is neuronal activity dependent^{7,13,14}, we hypothesized that early alterations in neuronal activity in AD brains may misguide glial phagocytic processes, leading to aberrant synapse elimination. To modulate intrinsic neuronal activity, human G_q- or G_i-coupled muscarinic designer receptors (hM3Dq or hM4Di) were expressed selectively in excitatory or inhibitory neurons, followed by four daily clozapine-*N*-oxide (CNO) injections and confirmation of activity changes by FOS immunoreactivity (Extended Data Fig. 3). Importantly, glial synapse phagocytosis was increased with heightened neuronal

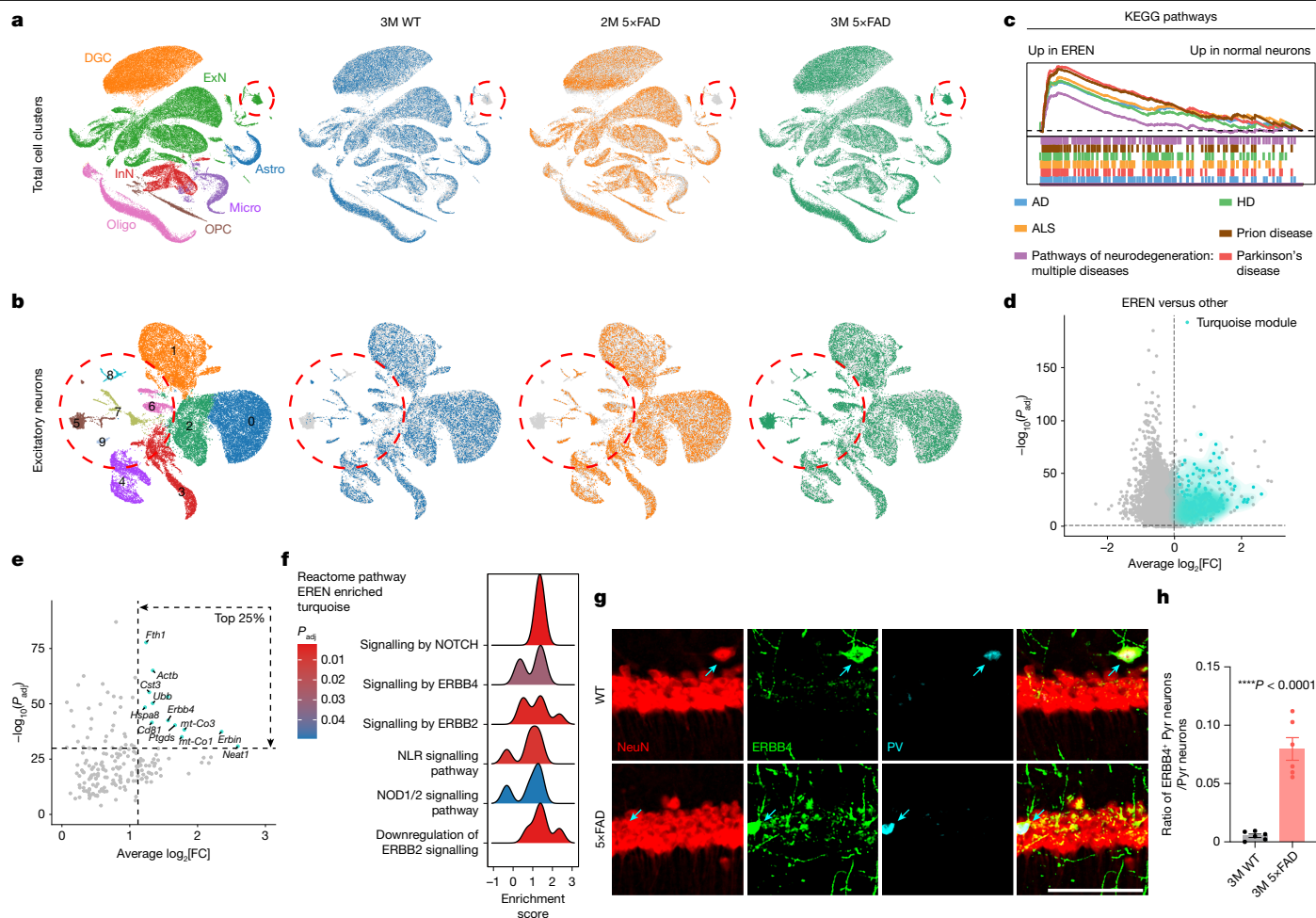


Fig. 2 | snRNA-seq reveals aberrant *Erbb4* expression in ERENs. a, UMAP plots showing total cell clusters and annotated brain cells from the hippocampi of 3-month-old WT, 2-month-old 5x FAD and 3-month-old 5x FAD mice. DGC, dentate granule cells; ExN, excitatory neurons; InN, inhibitory neurons; astro, astrocytes; micro, microglia; oligo, oligodendrocytes; OPC, oligodendrocyte progenitor cells. The red dotted circles indicate UMAP differences in the excitatory neuronal populations across groups. **b**, UMAP plots of the excitatory neuronal clusters with annotations. The red dotted circles indicate clusters enriched in the excitatory neurons of 3-month-old 5x FAD mice. **c**, GSEA results based on KEGG pathways enriched in ERENs. **d**, DEGs between EREN clusters and other excitatory neuronal clusters. Genes from the turquoise module are highlighted by turquoise-coloured clouds. **e**, DEGs of ERENs; selected genes are

annotated on the basis of adjusted P value and average $\log_2$ -transformed fold change (FC). **f**, GSEA results based on Reactome pathways enriched in turquoise module genes. All snRNA-seq comparisons used cells from two mice per group; cell numbers are reported in the Methods. **g**, Representative confocal z-stack images of NeuN (red), ERBB4 (green) and PV (cyan) in the CA1 of 3-month-old WT and 5x FAD mice. The cyan arrows indicate ERBB4⁺ PV neurons. Scale bar, 100 μ m. **h**, Quantification of the ratio of ERBB4⁺ pyramidal neurons to the number of pyramidal neurons. $n = 6$ per group. Statistical analysis was performed using one-sided permutation tests (**c**, **f**), one-sided Wilcoxon rank-sum tests (**d**, **e**) and two-sided unpaired Student's t -tests (**h**). **** $P < 0.0001$. P_{adj} , adjusted P . Data are mean $\pm$ s.e.m. n values represent the number of mice per group.

activity and decreased when neuronal activity was suppressed in both excitatory and inhibitory neurons (Extended Data Fig. 3). These data suggest that imbalanced neuronal activity, characterized by excitatory neuronal hyperactivity and inhibitory neuronal hypoactivity, may shape abnormal patterns of glial synapse elimination in AD brains.

To test whether astrocytic phagocytosis directly contributes to excitatory synapse loss, we knocked down the astrocyte-enriched phagocytic receptor MEGF10 in CA1 of 5x FAD mice using two AAV-encoded shRNAs⁷ (Extended Data Fig. 4a–c). *Megf10* knockdown increased the number of excitatory synapses without affecting inhibitory synapses (Extended Data Fig. 4d–g), consistent with a selective requirement for MEGF10 in excitatory synapse elimination⁶.

snRNA-seq reveals *Erbb4* in ERENs

To determine the molecular mechanisms underlying early alterations in neuronal activity and corresponding synapse elimination in AD

brains, we performed hippocampal snRNA-seq analysis in 3-month-old WT, 2-month-old 5x FAD and 3-month-old 5x FAD mice. After SysVI batch integration¹⁵, excitatory neurons showed the most prominent disease-associated shift on uniform manifold approximation and projection (UMAP) plots (Fig. 2a). Subclustering separated excitatory neurons into ten clusters (Fig. 2b and Supplementary Fig. 1a,b) and identified clusters 5–9 as selectively enriched in 3-month-old 5x FAD mice (Extended Data Fig. 5a). Their differentially expressed genes (DEGs) were enriched for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways associated with AD, Huntington's disease (HD), Parkinson's disease and amyotrophic lateral sclerosis (ALS) (Fig. 2c). As these transcriptional alterations appeared early and were more pronounced than changes in astrocytes or microglia (Extended Data Fig. 5b), we designated these clusters early-responsive excitatory neurons (ERENs).

Next, we performed WGCNA^{16,17} of excitatory neuronal genes and identified ten distinct gene modules (Extended Data Fig. 5c,d). Among these, ERENs exhibited a significantly elevated module score only for

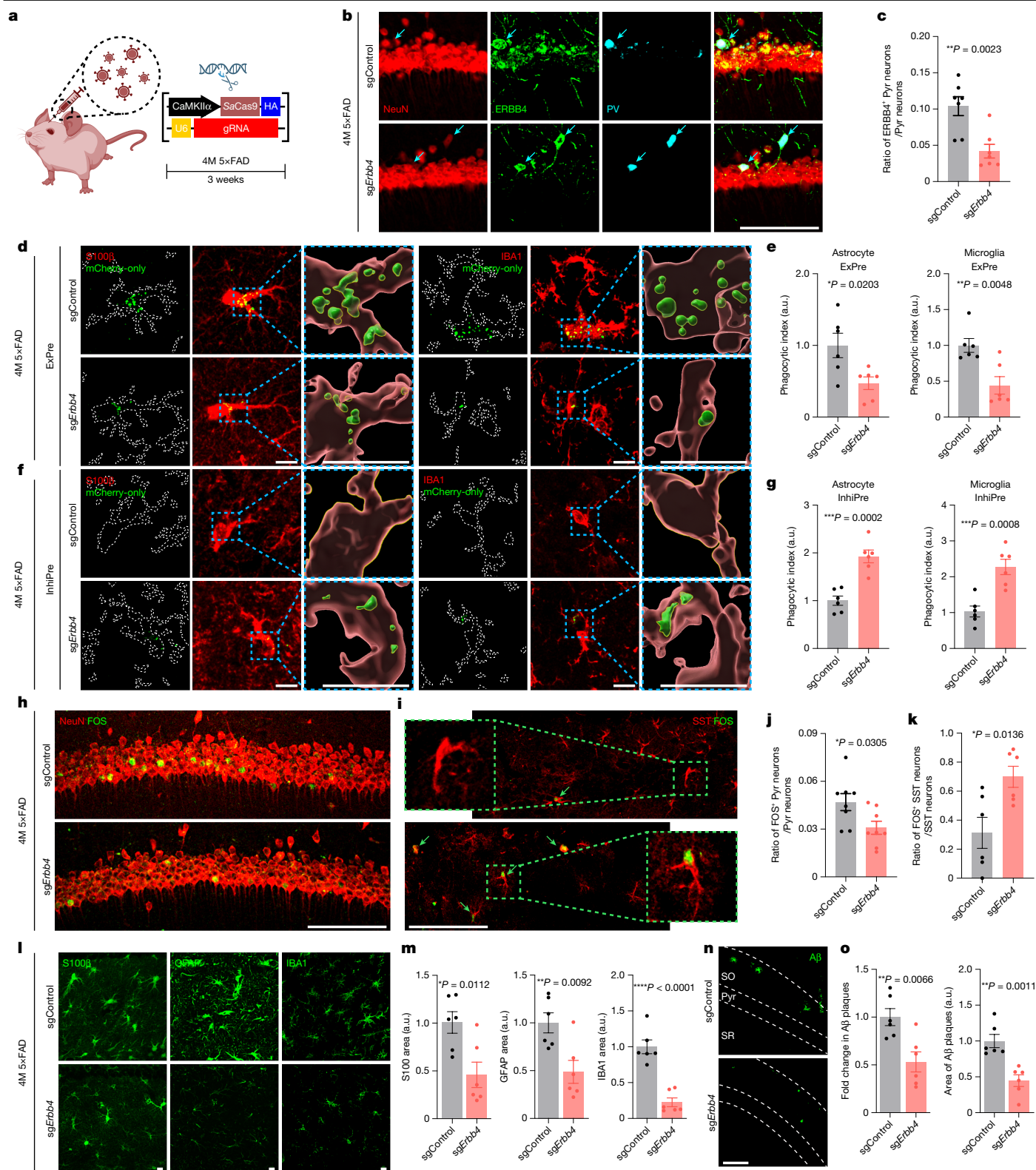


Fig. 3 | See next page for caption.

the turquoise module (Fig. 2d and Extended Data Fig. 5c). For further analysis, we selected the top 25% of turquoise module genes that were differentially expressed between ERENs and other excitatory neuronal clusters, ranked by adjusted *P* value and expression level (Fig. 2e and Supplementary Fig. 1c). Among these top-ranked genes, *Erbb4* was the only gene that overlapped with Reactome pathway terms enriched by

GSEA, prompting us to prioritize ERBB4 signalling for subsequent analyses (Fig. 2f). Independent analysis of cortical neurons from 7-month-old 5xFAD mice¹⁸ similarly identified an expanded *Erbb4*^{high} excitatory subset and firebrick module enriched in this subset (Extended Data Fig. 5e,f). Reactome GSEA again identified signalling by ERBB4 as the only significantly enriched pathway among firebrick-module genes

Fig. 3 | Reducing *Erbb4* in ERENs rescues AD pathophysiology. **a**, Schematic of CRISPR-AAV-mediated *Erbb4* deletion in the CA1 of 4-month-old 5×FAD mice. The diagram was created using BioRender; Chung, W. <https://biorender.com/jotrdxh> (2026). **b**, Representative confocal z-stack images of NeuN (red), ERBB4 (green) and PV (cyan). The cyan arrows indicate ERBB4⁺ PV neurons. Scale bar, 100 μm. **c**, Quantification of ERBB4⁺ pyramidal neurons as the proportion of total pyramidal neurons. *n* = 7 mice per group. **d, f**, Representative confocal z-stack images of mCherry-only puncta from ExPre (**d**, green) and InhiPre (**f**, green), along with astrocytes (S100β) or microglia (IBA1) (red). The white dotted lines indicate the outlines of glial cells. Right, magnified three-dimensional reconstructions of the blue dotted boxes in the middle panels, highlighting engulfed mCherry-only puncta (green) inside glial cells (burgundy). Scale bars, 10 μm. **e, g**, Quantification of the engulfed excitatory (**e**) and inhibitory

(**g**) pre-synapses by astrocytes (left) and microglia (right). *n* = 6 mice per group. **h, i**, Representative confocal z-stack images of FOS (green) with NeuN (**h**, red) or SST (**i**, red). The green dotted boxes indicate the magnified regions (**j**). Scale bars, 100 μm. **j, k**, Quantification of the ratio of FOS⁺ pyramidal neurons (**j**) and FOS⁺ SST neurons (**k**) to the number of pyramidal or SST neurons, respectively. *n* = 8 mice per group (**j**), and *n* = 6 mice per group (**k**). **l**, Representative confocal z-stack images of S100β (left), GFAP (middle) and IBA1 (right). Scale bars, 10 μm. **m**, Quantification of the areas of S100β (left), GFAP (middle) and IBA1 (right). *n* = 6 mice per group. **n**, Representative confocal z-stack images of Aβ plaques. SO, stratum oriens; Pyr, pyramidal layer. Scale bar, 100 μm. **o**, Quantification of the fold changes in the number (left) and area (right) of Aβ plaques. *n* = 6 mice per group. Statistical analysis was performed using two-sided unpaired Student's *t*-tests. Data are mean ± s.e.m.

(Extended Data Fig. 5g, h), supporting ERBB4 signalling as a conserved feature of EREN-like states.

While *Erbb4* has been known to be exclusively expressed in parvalbumin (PV)⁺ inhibitory neurons in the central nervous system, where it contributes to synaptogenesis through the mTOR pathway¹⁹, its expression and function in excitatory neurons remain unclear. To validate our findings, we performed immunohistochemistry and observed a significant upregulation of ERBB4 in the CA1, but not in the CA3, of 3-month- and 4-month-old 5×FAD mice compared with WT mice (Fig. 2g, h and Extended Data Fig. 5i). Consistently, fluorescence in situ hybridization (FISH) analysis revealed a significant increase in nuclear *Erbb4* mRNA in pyramidal neurons within the CA1 hippocampus of 5×FAD mice (Extended Data Fig. 5j, k).

EREN abundance was unchanged in *Trem2*-deficient 5×FAD mice¹⁸ (Extended Data Fig. 5l), arguing against an essential role for TREM2-dependent disease-associated microglia (DAM) in their generation. Conversely, CA1 injection of Aβ oligomers induced excitatory neuronal ERBB4 within 2 days (Extended Data Fig. 5m, n). These findings suggest that Aβ can promote ectopic *Erbb4* expression in excitatory neurons, at least partly independently of TREM2-mediated microglial activation.

Reducing *Erbb4* mitigates AD pathology

To determine the functional role of ERBB4 in AD pathology, we selectively deleted *Erbb4* expression in excitatory neurons by delivering an AAV encoding a CaMKIIα promoter-driven *Staphylococcus aureus* Cas9 (*SaCas9*) along with a U6 promoter-driven single guide RNA (sgRNA) targeting *Erbb4* into CA1 of 4-month-old 5×FAD mice (Fig. 3a and Extended Data Fig. 6a). This approach reduced ERBB4 in pyramidal neurons without altering its expression in PV interneurons (Fig. 3b, c and Extended Data Fig. 5j, k). Importantly, *Erbb4* deletion reduced excitatory pre-synapse elimination and increased inhibitory pre-synapse elimination by both astrocytes and microglia in the AAV-injected 5×FAD mice, restoring values towards those in WT mice (Fig. 3d–g). It also rescued the loss of excitatory pre-synapses and post-synapses and the increase in inhibitory pre-synapses (Extended Data Fig. 6l–o).

Consistent with previous reports²⁰, assessment of neuronal activity through FOS immunoreactivity in the CA1 revealed an increased number of FOS⁺ excitatory neurons in 5×FAD mice compared with in WT mice (Extended Data Fig. 6d, f). Notably, *Erbb4* deletion in excitatory neurons strongly normalized the hyperactivity of excitatory neurons in 5×FAD mice (Fig. 3h, j). Conversely, the number of FOS⁺ SST neurons, which was significantly lower in 5×FAD mice than in WT mice (Extended Data Fig. 6e, g), was also strongly restored to WT levels after excitatory neuronal *Erbb4* deletion (Fig. 3i, k). The number of FOS⁺ PV neurons remained unchanged in 4-month-old 5×FAD mice, regardless of *Erbb4* manipulation (Extended Data Fig. 6h–k), indicating subtype-specific FOS changes in AD interneurons. These findings place aberrant excitatory neuronal ERBB4 upstream of excitatory hyperactivity, SST hypoactivity and the corresponding changes in synapse-selective glial phagocytosis. The restoration of SST activity despite *Erbb4* deletion

being confined to excitatory neurons further indicated that ERBB4-dependent dysfunction propagates through local circuit interactions rather than remaining cell-autonomous.

Given the association between abnormal neuronal activity and neuroinflammation in AD²¹, we next evaluated reactive gliosis by quantifying S100β, GFAP and IBA1 levels in 5×FAD mice with or without CA1 excitatory neuronal *Erbb4* deletion. Notably, the 5×FAD mice with excitatory neuronal *Erbb4* deletion exhibited significantly reduced levels of all three reactive gliosis markers (Fig. 3l, m), along with a reduction in AXL⁺ microglia, indicative of a diminished DAM population (Extended Data Fig. 6p, q). Moreover, we found that amyloid plaque burden, measured by both plaque area and number, was strongly reduced in 4-month-old 5×FAD mice after excitatory neuronal *Erbb4* deletion (Fig. 3n, o). These results suggest that aberrant *Erbb4* expression in ERENs not only disrupts synaptic homeostasis but also drives gliosis and plaque deposition. Importantly, the same AAV-mediated deletion of *Erbb4* in WT CA1 excitatory neurons did not elicit detectable changes in synapse phagocytosis or reactive gliosis (Extended Data Fig. 6r–w), thereby highlighting excitatory neuronal ERBB4 as a promising therapeutic target for AD pathophysiology.

To determine whether normalization of excitatory activity was sufficient to reproduce the effects of *Erbb4* deletion, we expressed hM4Di in CA1 excitatory neurons of 5×FAD mice. After four consecutive days of CNO injections, we found that activating hM4Di in AD excitatory neurons not only reduced the number of FOS⁺ excitatory neurons, but also increased the number of FOS⁺ SST neurons in 5×FAD mice (Extended Data Fig. 7a–e), supporting abnormal excitatory control over SST neurons in AD brains. Excitatory neuronal *Erbb4* deletion also reduced the number of FOS⁺ vasoactive intestinal peptide (VIP) interneurons (Extended Data Fig. 6b, c). As VIP interneurons inhibit SST neurons²², these results are consistent with a potential excitatory neuron–VIP–SST disinhibitory circuit linking ERBB4-dependent excitatory hyperactivity to SST hypoactivity. Suppression of excitatory neuronal activity also decreased excitatory synapse phagocytosis by astrocytes and increased inhibitory synapse phagocytosis by both astrocytes and microglia (Extended Data Fig. 7f–i). However, despite normalization of neuronal activity and synapse phagocytosis, acute hM4Di activation did not reduce gliosis or amyloid plaques (Extended Data Fig. 7j–m), in contrast to *Erbb4* deletion. These data suggest that ERBB4-expressing excitatory neurons initiate multiple aspects of AD pathophysiology by disrupting neuronal network activity. However, reactive gliosis may arise through ERBB4-dependent but neuronal-activity-independent mechanisms that have yet to be identified.

Finally, we tested durability and therapeutic timing. *Erbb4* deletion at 3 months reduced reactive gliosis, AXL⁺ or TREM2⁺ DAM, and amyloid deposition when mice were analysed at 7 months (Extended Data Fig. 8a–h). Delivery at 8 months likewise reduced these pathological features at 10 months (Extended Data Fig. 8i–p). Thus, reducing excitatory neuronal *Erbb4* produced sustained benefits and remained effective when initiated after substantial disease progression.

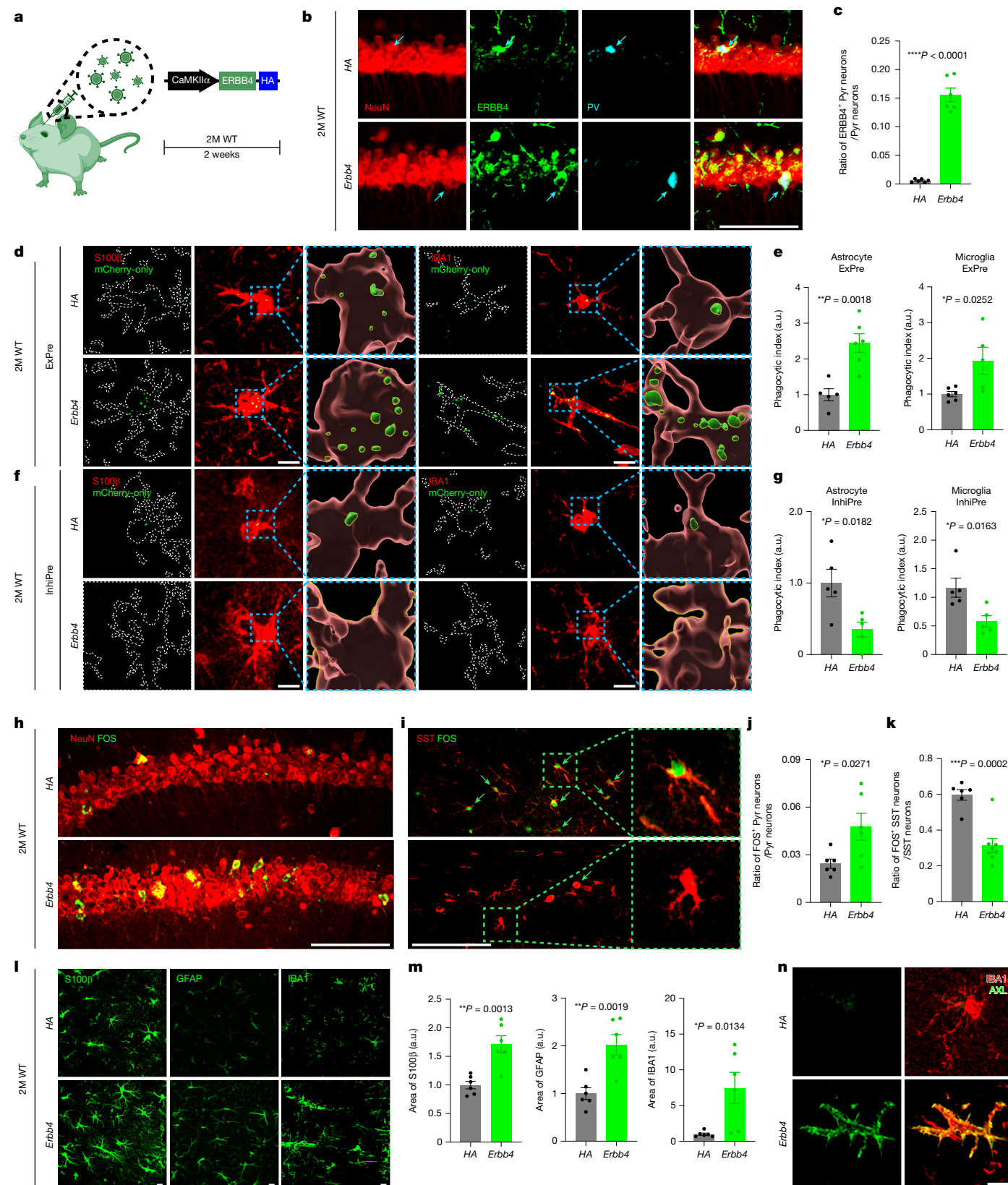


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Ectopic *ErbB4* induces AD-like pathology

We next examined whether ectopic *ErbB4* expression in WT excitatory neurons was sufficient to induce AD-like changes. A CaMKIIα-driven

ErbB4 AAV was delivered to the CA1 of 2-month-old WT mice, with the viral dosage adjusted so that 5–15% of pyramidal neurons expressed ERBB4, matching the proportion observed in 5×FAD mice (Fig. 4a–c). Importantly, ERBB4 overexpression increased excitatory and decreased

Fig. 4 | Ectopic *Erbb4* expression in excitatory neurons induces AD-like pathophysiology. **a**, Schematic of *Erbb4*-expressing AAV-injection into the CA1 of 2-month-old WT mice. The diagram was created using BioRender; Chung, W. <https://biorender.com/jotrdxh> (2026). **b**, Representative confocal z-stack images of NeuN (red), ERBB4 (green) and PV (cyan). The cyan arrows indicate ERBB4⁺ PV neurons. Scale bar, 100 μ m. **c**, Quantification of the ratio of ERBB4⁺ pyramidal neurons to the number of pyramidal neurons. $n = 6$ mice per group. **d,f**, Representative confocal z-stack images of mCherry-only puncta from ExPre (**d**, green) and InhiPre (**f**, green), and glial cells (red, astrocytes: S100 β ; microglia: IBA1). The white dotted lines indicate the outlines of glial cells. Right, magnified three-dimensional reconstructions of the blue dotted boxes in the middle panels, highlighting engulfed mCherry-only puncta (green) inside glial cells (burgundy). Scale bars, 10 μ m. **e,g**, Quantification of the engulfed excitatory (**e**)

and inhibitory (**g**) pre-synapses by astrocytes (left) and microglia (right). $n = 5$ (HA) and 6 (*Erbb4*) (**e**, left), $n = 6$ mice per group (**e**, right), and $n = 5$ mice per group (**g**). **h,i**, Representative confocal z-stack images of FOS (green) with NeuN (**h**, red) or SST (**i**, red). The green dotted boxes indicate the enlarged regions (**i**). Scale bars, 100 μ m. **j,k**, Quantification of the ratio of FOS⁺ pyramidal neurons (**j**) and FOS⁺ SST neurons (**k**) to the number of pyramidal neurons and SST neurons, respectively. $n = 6$ mice per group (**j**), and $n = 6$ (HA) and 8 (*Erbb4*) (**k**). **l**, Representative confocal z-stack images of S100 β (left), GFAP (middle) and IBA1 (right). Scale bars, 10 μ m. **m**, Quantification of the areas of S100 β (left), GFAP (middle) and IBA1 (right). $n = 6$ mice per group. **n**, Representative confocal z-stack images of AXL (green) and IBA1 (red). Scale bar, 10 μ m. Statistical analysis was performed using two-sided unpaired Student's *t*-tests. ****P* < 0.001. Data are mean $\pm$ s.e.m. *n* values represent the number of mice per group.

inhibitory synapse engulfment by both astrocytes and microglia (Fig. 4d–g), increased FOS⁺ excitatory neurons, reduced FOS⁺ SST neurons and left PV activity unchanged (Fig. 4h–k and Extended Data Fig. 9a,b). Along with these changes, the number of excitatory synapses was decreased while the number of inhibitory synapses was increased (Extended Data Fig. 9c–f), recapitulating the excitatory–inhibitory imbalance observed in 5 $\times$ FAD mice. ERBB4 overexpression also elevated GFAP, S100 β , IBA1 and AXL⁺ microglia without detectable cleaved caspase-3⁺ apoptosis (Fig. 4l–n and Extended Data Fig. 9g,h). By contrast, a kinase-dead ERBB4 mutant (K751M) did not alter synapse phagocytosis or gliosis (Extended Data Fig. 9i–q). Thus, ERBB4 kinase activity in excitatory neurons is sufficient to drive major AD-like neuronal, synaptic and glial phenotypes.

Erbb4 expression in cortical excitatory neurons similarly increased neuronal FOS activity and S100 β , GFAP and IBA1 levels (Extended Data Fig. 9r–u). Together with the cortical EREN-like population identified in 7-month-old 5 $\times$ FAD mice, these findings indicate that ectopic ERBB4 can promote AD-like neuronal and glial changes beyond the hippocampus.

ERBB4 alters excitatory neuron function

As aberrant ERBB4 signalling altered both excitatory and inhibitory synapse numbers, we next examined whether these structural changes altered synaptic input onto CA1 pyramidal neurons. Whole-cell recordings were performed at 4 months, when ERBB4-dependent synapse changes first became evident in 5 $\times$ FAD mice. Analysis of miniature excitatory postsynaptic currents (mEPSCs) revealed a reduced event frequency in 5 $\times$ FAD mice compared with in the WT controls, and this reduction was restored to WT levels by excitatory-neuron-specific *Erbb4* deletion (Extended Data Fig. 7n–p). Conversely, *Erbb4* overexpression in WT excitatory neurons reduced the mEPSC frequency to levels comparable to those observed in 5 $\times$ FAD mice (Extended Data Fig. 7n–p). By contrast, the mEPSC amplitude remained unchanged across groups (Extended Data Fig. 7o). Together, these data indicate that ERBB4 primarily decreases the number of functional excitatory inputs rather than changing postsynaptic strength at individual synapses.

Despite increased inhibitory presynaptic density in the SLM, both the frequency and amplitude of miniature inhibitory postsynaptic currents (mIPSCs) recorded from 5 $\times$ FAD CA1 pyramidal neurons were reduced compared with WT controls (Extended Data Fig. 7q–s). Moreover, *Erbb4* modulation in 5 $\times$ FAD mice did not produce statistically significant changes in either mIPSC frequency or amplitude (Extended Data Fig. 7q–s). However, *Erbb4* overexpression in WT excitatory neurons showed a trend toward increased mIPSC frequency, consistent with the increased inhibitory synapse number observed under this condition ($P = 0.0504$, unpaired Student's *t*-test). Thus, the lack of a corresponding increase in mIPSC frequency despite the increased inhibitory presynaptic density suggests that these additional inhibitory contacts in 5 $\times$ FAD mice may be immature, silent or otherwise

functionally ineffective. Moreover, broader AD-associated dysfunction of PV or other interneuron populations could also contribute to the reduced inhibitory transmission^{23,24}.

Although the ERBB4-dependent reduction in mEPSC frequency is consistent with excitatory synapse loss, this synaptic input phenotype alone cannot readily explain the increased FOS activity in excitatory neurons (Extended Data Fig. 6d,f). We therefore examined whether aberrant ERBB4 expression alters the intrinsic neuronal excitability of CA1 pyramidal neurons after *Erbb4* modulation. Input resistance was unchanged, but 5 $\times$ FAD and *Erbb4*-overexpressing WT pyramidal neurons fired many more action potentials during depolarizing current injection, whereas *Erbb4* deletion restored 5 $\times$ FAD firing to WT levels (Extended Data Fig. 7t–v). These findings indicate that aberrant ERBB4 directly increases intrinsic excitability, providing a mechanism for excitatory hyperactivity. Hyperactivity may then promote glial synapse elimination and subsequent synapse loss, consistent with chemogenetic experiments showing that G_q-driven activation enhances, whereas G_i-driven suppression reduces, synapse phagocytosis (Extended Data Figs. 3 and 7a–i).

mTOR mediates aberrant ERBB4 pathology

ERBB4 activates several downstream pathways, including the mTOR^{19,25} and mitogen-activated protein kinase (MAPK) signalling cascades^{26,27}. Notably, neuronal mTOR hyperactivation is associated with seizures, excitatory–inhibitory imbalance, Down syndrome and AD^{28,29}, prompting us to test whether mTOR signalling acts downstream of ERBB4 to mediate AD-related pathological effects. We deleted the gene encoding regulatory-associated protein of mTOR (*Rptor*), an essential component of the mTOR complex 1 (mTORC1), in CA1 excitatory neurons of 4-month-old 5 $\times$ FAD mice using CaMKII α -SaCas9 and *sgRptor* (Extended Data Fig. 10a). *Rptor* deletion reduced phosphorylated ribosomal protein S6 (p-S6), confirming suppression of mTORC1 activity (Extended Data Fig. 10b,c). Similar to *Erbb4* deletion, *Rptor* deletion normalized excitatory and inhibitory synapse elimination, restored corresponding synapse numbers, reduced excitatory activity and increased SST activity (Extended Data Fig. 10d–o). Moreover, *Rptor* deletion significantly attenuated gliosis, reduced both the AXL⁺ microglia population and amyloid plaque burden (Extended Data Fig. 10p–u). The close correspondence between *Rptor* and *Erbb4* deletion phenotypes placed mTORC1 at a major convergence point linking excitatory neuronal signalling to synaptic, glial and amyloid-related outcomes.

We next examined whether excitatory neuronal mTOR signalling is required for the pathological effects induced by ectopic *Erbb4* overexpression. To this end, we co-expressed *Erbb4* with *sgRptor* in CA1 excitatory neurons of 2-month-old WT mice (Extended Data Fig. 11a). Despite robust ERBB4 expression, *Rptor* deletion suppressed p-S6 and prevented excitatory hyperactivity, SST hypoactivity, gliosis, AXL⁺ microglia, and changes in excitatory and inhibitory synapse numbers (Extended Data Fig. 11b–o). Together, these findings demonstrate that mTOR signalling is a critical downstream mediator of aberrant ERBB4

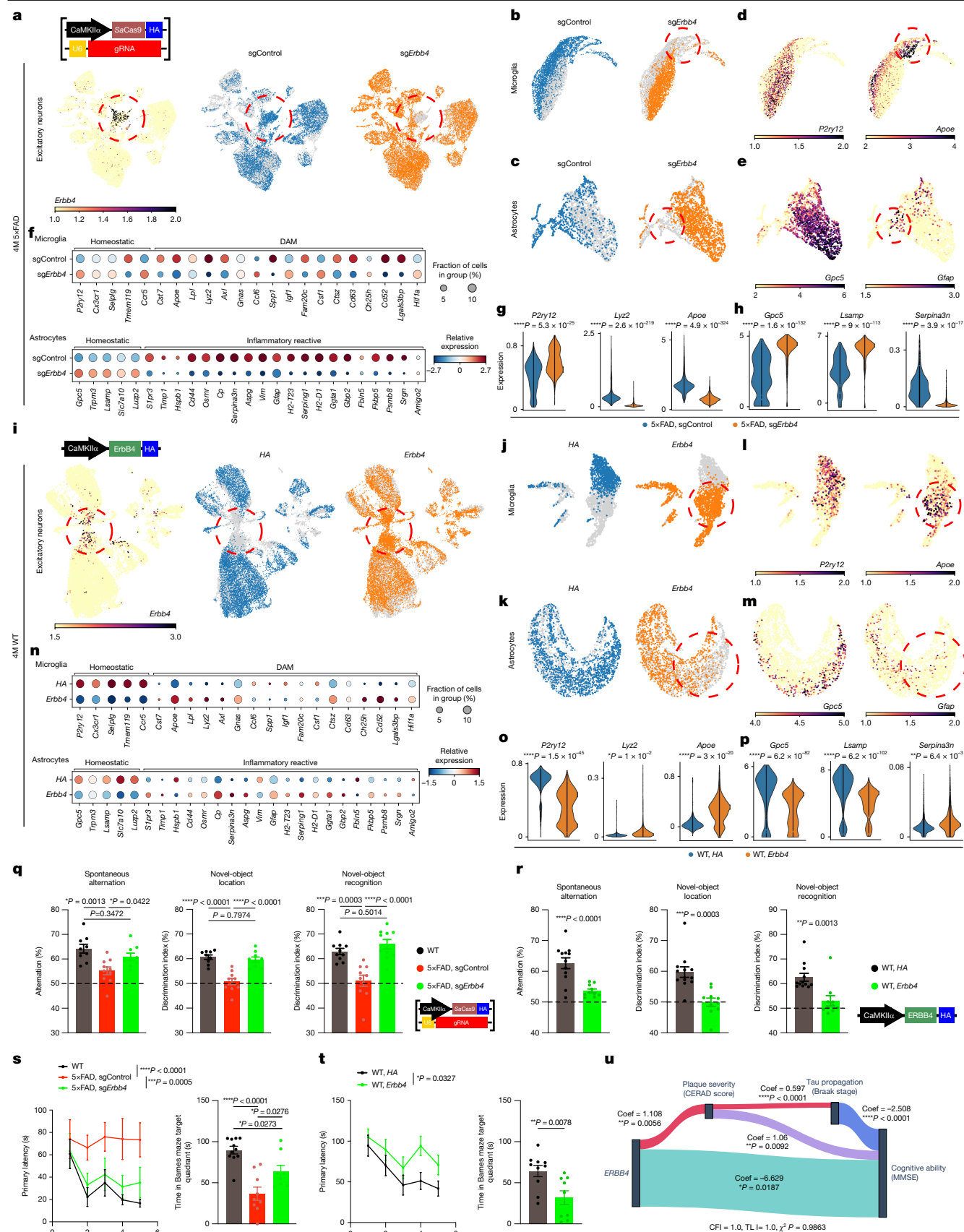


Fig. 5 | See next page for caption.

Fig. 5 | Aberrant *Erbb4* alters the cellular landscape and cognitive function. **a,i**, UMAPs of *Erbb4* expression (left) within excitatory neurons of sgControl- and sg*Erbb4*-injected 5×FAD (**a**) and HA- and *Erbb4*-injected WT (**i**) mice. The red dotted circles highlight *Erbb4*⁺ populations. **b,c,j,k**, UMAPs of microglial (**b,j**) and astrocytic (**c,k**) clusters in the 5×FAD (**b,c**) and WT (**j,k**) mice described in **a** and **i**. **d,e,l,m**, UMAP feature plots of *P2ry12* (**d,l**, left), *Apoe* (**d,l**, right), *Gpc5* (**e,m**, left) and *Gfap* (**e,m**, right) in microglia and astrocytes in the 5×FAD (**d,e**) and WT (**l,m**) mice described in **a** and **i**. The red dotted circles mark altered clusters. **f,n**, Homeostatic and DAM (top) and homeostatic and inflammatory reactive astrocyte (bottom) genes in the 5×FAD (**f**) and WT (**n**) mice described in **a** and **i**. **g,h,o,p**, Homeostatic (**g,o**, left) and DAM (**g,o**, middle and right) microglial and homeostatic (**h,p**, left and middle) and inflammatory reactive astrocyte (**h,p**, right) genes in the 5×FAD (**g,h**) and WT (**o,p**) mice described in **a** and **i**. All snRNA-seq comparisons used cells from three mice per group; cell numbers are reported in the Methods. Statistical analysis was performed using the one-sided Wilcoxon rank-sum test with Benjamini–Hochberg adjustment. **q**, Performance of 4-month-old WT, 5×FAD injected with sgControl or sg*Erbb4* mice in the spontaneous alternation (left), novel-object location (middle) and novel-object recognition (right) tests. $n = 9$ (WT), 10 (5×FAD; sgControl) and

8 (5×FAD; sg*Erbb4*) (left); $n = 9$ (WT), 10 (5×FAD; sgControl) and 9 (5×FAD; sg*Erbb4*) (middle); and $n = 10$ mice per group (right). **r**, Performance of 2-month-old WT mice injected with HA or *Erbb4* in the spontaneous alternation (left), novel-object location (middle) and novel-object recognition (right) tests. $n = 12$ (HA) and 10 (*Erbb4*). **s**, Performance of 4-month-old WT and 5×FAD injected with sgControl or sg*Erbb4* during Barnes maze learning (left) and the probe test (right). $n = 11$ (WT), 9 (5×FAD; sgControl) and 8 (5×FAD; sg*Erbb4*). **t**, Performance of 2-month-old WT mice injected with HA or *Erbb4* during Barnes maze learning (left) and the probe test (right). $n = 10$ (HA) and 9 (*Erbb4*). **u**, Path model constructed using directed mediation analysis and structural equation modelling in 446 human patients with AD from the ROSMAP cohort. Coef., regression coefficient. A one-sided χ^2 test was used for structural equation modelling. Two-sided bootstrapping was used for mediation analysis. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple-comparisons test (**q**), two-way ANOVA followed by Tukey's multiple-comparisons test (**s** and **t** (left)) and two-sided unpaired Student's *t*-tests (**r** and **t** (right)). Data are mean $\pm$ s.e.m. n values represent the number of mice per group.

signalling and is necessary for the induction of multiple AD-like pathophysiological features.

Consistent with an ERBB4–mTOR signalling axis, we found that ERENs and excitatory neurons from *Erbb4*-overexpressing WT mice showed transcriptional changes that resembled those seen in *Tsc2*-deficient neurons³⁰ (Extended Data Fig. 12a). Notably, these overlapping changes involved genes related to neuronal excitability, including those encoding voltage-gated K⁺ channels, inwardly rectifying K⁺ channels, γ -aminobutyric acid type A (GABAA) receptors and GABAB receptors. By contrast, excitatory neurons from *Erbb4*-deficient 5×FAD mice showed opposing changes in these gene sets (Extended Data Fig. 12a). Given that these ion channels and inhibitory receptors are critical for regulating neuronal excitability^{31–34}, our findings suggest that aberrant ERBB4–mTOR signalling may promote excitatory neuronal hyperactivity, at least in part, by disrupting transcriptional programs that normally constrain neuronal excitability.

Erbb4 alters glial states and behaviour

To better understand how *Erbb4* contributes to multiple aspects of AD pathophysiology, we performed CA1 snRNA-seq analysis in 4-month-old 5×FAD mice with or without *Erbb4* deletion and in WT mice with or without *Erbb4* overexpression. First, in 5×FAD mice, UMAP data readily showed *Erbb4* loss within the excitatory neuronal cluster after introduction of a CRISPR AAV targeting *Erbb4* (Fig. 5a and Supplementary Fig. 1a,b). Moreover, deleting *Erbb4* in 5×FAD excitatory neurons substantially altered the UMAP distribution of astrocytes and microglia (Fig. 5b,c). Importantly, DAM-associated genes, including *Cst7*, *Lpl*, *Lyz2* and *Apoe*, and reactive astrocyte-associated genes, including *Vim*, *Gfap*, *Serpina3n* and *Cp*, were reduced. Conversely, homeostatic microglial markers such as *P2ry12*, *Cx3cr1* and *Tmem119* and astrocytic genes including *Gpc5*, *Luzp2* and *Lsamp* were increased^{35–37} (Fig. 5d–h).

In parallel, excitatory neuronal *Erbb4* overexpression in WT mice generated a clearly separated *Erbb4*-expressing excitatory subcluster, analogous to that in 5×FAD mice (Figs. 2a and 5i and Supplementary Fig. 1a,b). Similarly, excitatory neuronal *Erbb4* overexpression in WT mice was sufficient to shift microglia and astrocytes towards a more-reactive state (Fig. 5j,k). Microglia in *Erbb4*-overexpressing WT mice upregulated DAM-associated genes and concomitantly downregulated homeostatic genes (Fig. 5l,n,o). Likewise, astrocytes in this group upregulated inflammatory reactive-astrocyte-associated genes and reduced homeostatic gene expression (Fig. 5m,n,p). Notably, the expression levels of genes involved in the APP-processing pathway³⁸, including *App*, *Psen1*, *Ncstn* and *Bace1*, were significantly reduced in *Erbb4*-deleted 5×FAD excitatory neurons (Extended Data Fig. 12b),

potentially providing a mechanistic explanation for the reduced A β plaque burden after excitatory neuronal *Erbb4* deletion in 5×FAD mice.

Finally, we assessed hippocampus-dependent cognition using spontaneous alternation, novel-object location, novel-object recognition and Barnes maze tests^{39–41}. Notably, excitatory-neuron-specific *Erbb4* deletion strongly rescued all behavioural deficits in 4-month-old 5×FAD mice, whereas *Erbb4* overexpression impaired WT performance across these assays (Fig. 5q–t).

To test generalizability, *Erbb4* was deleted in CA1 excitatory neurons of 7-month-old APP/PS1 mice, and behaviour was measured at 10 months. Control APP/PS1 mice were impaired relative to age-matched WT mice, whereas *Erbb4* deletion improved performance in all behaviour tasks (Extended Data Fig. 12c,d). Taken together, these results underscore the pivotal role of aberrant excitatory neuronal ERBB4 in orchestrating gene expression changes not only in neurons but also in glial cells, ultimately leading to cognitive impairments through disrupting neural network activity and synapse homeostasis.

ERBB4 mediates cognitive decline in AD

To assess the clinical relevance of our findings, we first assessed excitatory neuronal ERBB4 in human brain tissue. FISH analysis of human brain sections revealed increased ERBB4 signals in *SLC17A7*⁺ excitatory neurons from patients with AD compared with control individuals without dementia (Extended Data Fig. 12e,f). We next investigated whether ERBB4-expressing excitatory neurons in human patients with AD also show correlation with AD pathological metrics, including Consortium to Establish a Registry for Alzheimer's Disease (CERAD) score, Braak stage and Mini-Mental State Examination (MMSE). To address this, we analysed snRNA-seq data of *CUX2*⁺ and *CUX2*[−] excitatory neurons from the dorsolateral prefrontal cortex of 446 participants in the Religious Orders Study and Rush Memory and Aging Project (ROSMAP) cohort^{42–44}. After integration¹⁵ with adjustment for confounding covariates such as age at death, sex and race, ERBB4^{high} clusters were identified within both *CUX2*⁺ and *CUX2*[−] excitatory neuronal populations (Extended Data Fig. 12g,h). Notably, the abundance of these clusters was positively correlated with plaque severity as assessed by CERAD scores, and negatively correlated with cognitive performance measured by MMSE (Extended Data Fig. 12i,j). These correlations suggest that ectopic ERBB4 expression in excitatory neurons is linked to pathological burden and cognitive impairment in human AD.

We next used directed mediation analysis within the established amyloid-to-tau-to-cognitive-decline framework^{45–47}. Two non-mutually exclusive configurations were evaluated: one in which amyloid burden induces excitatory neuronal ERBB4, followed by tau pathology and

cognitive decline; and another in which *ERBB4* acts upstream of amyloid, tau and cognition. Both configurations showed a direct association between *ERBB4* and cognitive decline, but the data provided stronger support for the upstream-*ERBB4* model (Fig. 5u). In this configuration, *ERBB4* was also indirectly associated with cognitive decline through sequential mediation by plaque severity and tau burden, whereas no plaque-independent direct association between *ERBB4* and tau was detected.

On the basis of these results, we constructed a path model describing the relationships among excitatory neuronal *ERBB4* expression and amyloid pathology, subsequent tau propagation and cognitive decline, and evaluated it using structural equation modelling. The model showed an excellent fit to the data (comparative fit index = 1.0, Tucker–Lewis index = 1.0; χ^2 test, $P = 0.9863$), supporting the plausibility of the proposed pathway (Fig. 5u). Importantly, replacing *ERBB4* with *ERBB2*, another EGFR family member, did not yield an adequate model fit (comparative fit index = 0.9684, Tucker–Lewis index = 0.8895; χ^2 test, $P = 0.0046$), underscoring the specificity of *ERBB4* in this framework. Collectively, these findings support a model in which aberrant *ERBB4* expression in excitatory neurons forms a vicious cycle with amyloid pathology, acting as both a consequence and an amplifier of plaque accumulation, thereby promoting tau pathology and cognitive decline in human AD.

Discussion

AD involves interconnected neuronal, glial and pathological changes, and therapies targeting a single component may therefore have limited efficacy. Although A β immunotherapies reduce brain amyloid⁴⁸, their cognitive benefits remain modest⁴⁹. A β accumulation may initiate multiple self-sustaining cascades that are not readily reversed by later plaque reduction^{50–52}. These findings highlight the need to identify core disease drivers downstream of A β accumulation.

Here we propose that the emergence of *Erbb4*-expressing excitatory neurons from the early stages of AD represents such a mechanism. Functional studies using CRISPR-mediated *Erbb4* deletion revealed that excitatory neuronal *ERBB4* is integral not only to synaptic regulation but also to excitatory hyperactivity, inhibitory hypoactivity, reactive gliosis, DAM induction and amyloid plaque deposition. Moreover, overexpression of *Erbb4* in WT excitatory neurons recapitulated these pathological features, even in the absence of amyloid plaques. We further identified mTOR signalling as a key downstream effector of excitatory neuronal *ERBB4*.

Although we believe that our findings provide key mechanistic insight and a long-sought answer to the core drivers of early AD pathophysiology, several questions remain unresolved. First, while our data indicate that A β may act as a primary inducer of aberrant *Erbb4* expression in excitatory neurons, the identity of the relevant *ERBB4* ligands and the transcriptional regulation mechanisms governing *Erbb4* remain to be elucidated. Second, our results suggest that inhibitory synaptic remodelling in 5 $\times$ FAD mice may follow a more temporally or compartmentally complex trajectory than the excitatory phenotypes examined here. Analyses across interneuron subtypes, dendritic compartments and disease stages should clarify how it relates to excitatory dysfunction. Finally, given that our mouse study used amyloidogenic AD models, future studies should determine whether EREN-like populations arise in tauopathies and other neurodegenerative models and drive comparable pathology.

Notably, our transcriptomic analyses suggest that *Erbb4*-expressing excitatory neurons may not be unique to AD. Consistent with KEGG-GSEA results from mouse data (Fig. 2c), preliminary analyses of public human snRNA-seq datasets identified EREN-like excitatory neuronal clusters with elevated *ERBB4* expression in the cortex of patients with progressive supranuclear palsy⁵³ (Extended Data Fig. 12k–m). Similarly, snRNA-seq data from patients with HD⁵⁴ revealed a linear correlation

between excitatory neuronal *ERBB4* and disease severity (Extended Data Fig. 12n). These observations suggest that ERENs may represent a convergent, disease-promoting neuronal state across diverse neurodegenerative conditions.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-026-10964-z>.

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Article

Methods

Mice

All mouse experiments were performed according to protocols approved by the Institutional Animal Care and Use Committees (IACUC) at Korea Advanced Institute of Science and Technology (KAIST) and the Institute for Basic Science (IBS), adhering strictly to ethical guidelines. B6.Cg-Tg(APPswe, PSEN1dE9)85Dbo/Mmjax (APP/PS1) mice and B6.Cg-Tg(APPswF1Lon, PSEN1^{M146L}*L286V)6799Vas/Mmjax (5×FAD) mice were produced in our laboratory and maintained by breeding with C57BL/6J mice. Mice were housed under a 12 h–12 h light–dark cycle. Both male and female mice were used unless otherwise specified in the figure legends. All mice were randomly assigned to experiments, which were performed by investigators blinded to experimental conditions. Gender was not considered unless explicitly noted in figure legends.

Human tissue sections

Human formalin-fixed paraffin-embedded (FFPE) sections were provided by the SNUH Brain Bank and use of these sections for this study was reviewed and determined to be exempt from review by the Public Institutional Review Board designated by the Ministry of Health and Welfare, Republic of Korea. All procedures involving human-derived materials were conducted in accordance with relevant regulations.

Cell lines

HEK293T cells (ATCC) were used solely for AAV production. Cells were maintained in our laboratory and were not independently authenticated for this study. HEK293T cells were confirmed to be free of mycoplasma contamination.

Antibodies and reagents

The antibodies used and their dilution factor were as follows: anti-S100β (Abcam, ab52642, Synaptic System, 287-004; Aves Labs, S100B-0020; 1:500), anti-IBA1 (Wako, 019-19741; Novus, NB100-1028; 1:500), anti-mCherry (Invitrogen, M11217; Aves Labs, mCherry-0020; 1:1,000), anti-vGLUT1 (Millipore, AB5905; 1:1,000), anti-PSD95 (Invitrogen, 51-6900; 1:500), anti-vGAT (Synaptic System, 131-004; 1:1,000), anti-gephyrin (Synaptic System, 147-008; 1:500), anti-FOS (Cell Signaling, 2250S; Synaptic System, 226-308; Synaptic System, 226-017; 1:500), anti-MEGF10 (Merck, ABC10; 1:500), anti-ERBB4 (Abcam, ab32375; Cell Signaling, 4795T; 1:500), anti-parvalbumin (Swant, GP72; 1:1,000), anti-beta amyloid (BioLegend, 803001; Cell Signaling, 2454S; 1:1,000), anti-GFAP (Abcam, ab4674; 1:1,000), anti-AXL (R&D systems, AF854; 1:100), anti-somatostatin (BMA Biomedicals, T-4103; 1:500), anti-VIP (Synaptic System, 443-005; 1:500), anti-NeuN (Sigma-Aldrich, ABN91; 1:500), anti-cleaved caspase-3 (Cell Signaling, 9661S; 1:500), anti-TREM2 (R&D systems, AF1729; 1:500), anti-HA (Cell Signaling, 3724S; 1:500) and anti-pS6 (Cell Signaling, 2211S; 1:500).

Secondary antibodies: donkey anti-goat IgG (H&L) Alexa Fluor 405 (Abcam, ab175665), donkey anti-goat IgG (H&L) Alexa Fluor 488 (Jackson Laboratory, 705-545-003), donkey anti-chicken DyLight 405-conjugated AffiniPure, donkey anti-chicken IgY (IgG) (H&L) (Jackson Laboratory, 703-475-155), donkey anti-chicken IgG (H&L) Alexa Fluor 488 (Jackson Laboratory, 703-545-155), donkey anti-chicken IgG (H&L) Alexa Fluor 594 (Jackson Laboratory, 709-585-155), donkey anti-rat IgG (H&L) Alexa Fluor 594 (Invitrogen, A-21209), donkey anti-rat IgG (H&L) Alexa Fluor 647 (Abcam, ab150155), donkey anti-rabbit IgG (H&L) Alexa Fluor 405 (Abcam, ab175649), donkey anti-rabbit IgG (H&L) Alexa Fluor 488 (Invitrogen, A-21206), donkey anti-rabbit IgG (H&L) Alexa Fluor 594 (Invitrogen, A-21207), donkey anti-guinea pig IgG (H&L) Alexa Fluor 488 (Jackson Laboratory, 706-545-148), donkey anti-guinea pig IgG (H&L) Alexa Fluor 594 (Jackson Laboratory, 706-585-148), donkey anti-guinea pig IgG (H&L) Alexa Fluor 647 (Jackson Laboratory, 706-605-148), donkey anti-sheep IgG H&L (Alexa Fluor 488) (Abcam, ab150177), donkey

anti-mouse IgG (H&L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594 (Invitrogen, A-21203). All Alexa-Fluor-488-conjugated secondary antibodies were used at a dilution of 1:1,000, whereas all other secondary antibodies were used at 1:500. The following reagents were used: HistoVT One (Nacalai, 06380-05), CNO (Sigma-Aldrich, C0832-5mg).

Python packages for data analysis

The following packages were used: Python v.3.10.0, numpy v.2.0.2, pandas v.2.2.3, matplotlib v.3.9.2, collections (built-in module in Python), scanpy v.1.10.4, scvi v.1.3.0, gseapy v.1.1.8, semopy v.2.3.11, pyWGCNA v.2.2.1, statsmodels v.0.14.5, scipy v.1.15.2, scikit-learn v.1.6.1 and magic-impute v.3.0.0.

snRNA-seq analysis

snRNA-seq data were analysed using Cell Ranger v.8.0.1. In brief, raw BCL files from Illumina HiSeq were demultiplexed to generate FASTQ files using ‘cellranger mkfastq’. FASTQ files were further processed with ‘cellranger count’, involving mapping to the mouse reference genome (mm10-2020-A), quantifying gene expression through unique molecular identifiers and cell barcodes, cell clustering and differential gene expression analysis. Multiple sequencing runs were combined using cellranger aggr.

Raw count matrices from 10x Genomics were imported into Scanpy v.1.10.4. Genes expressed in at least 3 cells and cells with 200–8,000 detected genes were used for downstream analysis. Cells with mitochondrial gene content over 5% were excluded. Doublets were removed by scanpy.pp.scrublet by default. After filtering, the selected count value was normalized by Pearson normalization, followed by highly-variable-gene identification with scanpy.experimental.pp.normalize_pearson_residuals and scanpy.experimental.pp.highly_variable_genes, respectively, in Scanpy. With Pearson-normalized counts and about 3,000 highly variable genes, we performed SysVI according to the package tutorial (<https://docs.scvi-tools.org/en/stable/tutorials/notebooks/scrna/sysvi.html>). Integrations with total cell clusters were performed with 100–200 epochs, whereas integrations with a single cell type such as excitatory neuron, astrocyte or microglia were performed with 5–30 epochs to obtain the minimum reconstruction loss between training set and validation set. Clustering and UMAP analysis were performed using scanpy.tl.leiden and scanpy.tl.umap by default. DEGs were obtained by scanpy.tl.rank_genes_groups using the Wilcoxon rank-sum method and then filtered by adjusted *P* value and log-transformed fold changes of their expressions. WGCNA was performed in Python with pyWGCNA v.2.2.1, as shown in the vignettes (https://github.com/mortazavilab/PyWGCNA/blob/main/tutorials/Quick_Start.ipynb). GSEA was performed with gseapy v.1.1.8 according to the tutorial (<https://gseapy.readthedocs.io/en/latest/introduction.html>). We used squared-rooted-normalized counts (numpy.sqrt(scanpy.pp.normalize_total(anndata, inplace=False)['X'])) for dot plots, UMAP plots and DEG analysis. We used magic-imputed counts³⁵ for visualization of violin plots, as shown in the vignettes (<https://magic.readthedocs.io/en/stable/tutorial.html>).

Analysed cell numbers

Cell numbers were as follows: Fig. 2a, 23,217 (3-month-old WT mice), 22,718 (2-month-old 5×FAD mice) and 33,623 (3-month-old 5×FAD mice) cells were analysed; Fig. 2b, 9,069 (3-month-old WT mice), 10,231 (2-month-old 5×FAD mice) and 14,044 (3-month-old 5×FAD mice) excitatory neurons were analysed. For each group, hippocampal samples were derived from two mice.

Figure 5a, 6,763 (sgControl) and 12,826 (sg*Erbb4*) excitatory neurons; Fig. 5b, 3,199 (sgControl) and 2,840 (sg*Erbb4*) microglia; Fig. 5c, 729 (sgControl) and 1,436 (sg*Erbb4*) astrocytes; Fig. 5i, 8,570 (*HA*) and 10,442 (*Erbb4*) excitatory neurons; Fig. 5j, 1,037 (*HA*) and 1,450 (*Erbb4*) microglia; Fig. 5k, 1,857 (*HA*) and 1,267 (*Erbb4*) astrocytes. For each comparison, cells were derived from three mice per group.

Stereotaxic injection for adeno-associated virus transduction

All AAVs were produced in the laboratory as described previously⁷. In brief, we co-transfected pAAV9 capsid plasmid, helper plasmid for virus assembly and target plasmids into HEK293T (Korean Cell Line Bank) cells using a PEI-based (0.3 mg ml⁻¹) transfection method⁵⁶. HEK293T cells were maintained with FBS-containing (Gibco) Dulbecco's modified Eagle's medium (DMEM, Welgene), which was replaced with serum-free medium during transfection (6–12 h). Transfected cells were incubated in a 37 °C, 5% CO₂ conditioned cell incubator. Then, 72 h after medium replacement, culture medium was collected and fresh medium was added. Collected medium was stored at 4 °C. After 48 h, the culture medium and HEK293T cells were collected and purified using the polyethylene-glycol-mediated purification method⁵⁷. Purified AAVs were concentrated using a 100 kDa Amicon ultra centrifugal filter tube (Millipore) to 200 µl.

For sgRNA targeting *ErbB4*, the TTAGCGATATTCTTAACTA sequence was cloned into the SaCas9 vector. The sequence GGTCGGGGCGTATG CGTCTA was cloned into the SaCas9 vector. For sgRNA targeting *Rptor*, the sequence TGCAGGTCGTATATGGACAG⁵⁸ was cloned into the SaCas9 vector. For control sgRNA, we used a non-targeting sgRNA sequence with no predicted target sites on the mouse genome, based on previously reported screening⁵⁹. For overexpression of *ErbB4*, the *ErbB4* cDNA sequence was purchased from Sino Biological (MG51064) and cloned into an AAV target vector. For the kinase-dead mutant of *ErbB4*, Lys751 was mutated to methionine manually. For control shRNA, CATT GCTGGCAGCAAGATTGAC and GTAGCAGAGCACCGTTTACATG were used. For shRNA targeting *Megf10*, TGAATCTTAAAAATGTGAATCC and GTTATTACAGAACCTAAGTGA were used.

Stereotaxic injection for delivering AAVs into the mouse brains was performed as described previously^{7,37}. In brief, mice were anaesthetized with isoflurane (Piramal) using a veterinary vaporizer (Surgivet). Heads were cleaned using 70% ethanol followed by hair removal and incision of the skin. For ExPre and chemogenetic approaches with hSyn promoter, AAVs were injected at CA3 (medialateral (ML), -2.5 mm; anteroposterior (AP), -2.0 mm from bregma; dorsoventral (DV), -2.2 mm from the brain surface). For InhiPre, chemogenetic approaches with the *Gad67* promoter, SaCas9 with sgRNAs, shRNAs, *ErbB4* overexpression and oligomeric Aβ injections were performed at the CA1 (ML, -1.25 mm; AP, -2.0 mm from bregma; DV, -1.5 mm from the brain surface). We injected viruses bilaterally only for behavioural experiments. For oligomeric Aβ injection, mice were euthanized 2 days after injection. For *ErbB4* overexpression experiments, mice were euthanized at 2 weeks after injection. For all other AAV-delivered experiments, mice were euthanized at 3 weeks after injection, or as otherwise reported in the figure legend. For chemogenetic approaches, CNO was injected at 0.5 mg per kg concentration for 4 consecutive days before euthanasia. We used same titre for each cohort. The incision was closed with Reflex 7 mm Wound Clips (ROBOZ) after injection.

Immunohistochemistry, FISH, antigen retrieval and image analysis

Mice were anaesthetized with avertin (20 µl g⁻¹) by intraperitoneal injection and perfused with 1× PBS (Welgene) followed by 4% PFA. Brains were post-fixed in 4% PFA at 4 °C overnight and transferred to 30% sucrose in 1× PBS for 48 h. Brains were embedded in OCT compound (Leica), sectioned into 30 µm coronal slices on cryo-stat microtomes (Leica). The sections were permeabilized by blocking buffer (4% BSA, 0.3% Triton X-100 in 1× PBS) for 1 h at room temperature followed by incubation with appropriate primary antibodies for 24 h at 4 °C. The sections were washed with PBST (0.1% Tween-20 in 1× PBS) and stained with the appropriate secondary antibodies conjugated with Alexa Fluor (Invitrogen, Abcam, Jackson ImmunoResearch) in PBST for 2 h at room temperature. The sections were washed and mounted onto slide glasses. TrueBlack (Biotium) diluted to 1/20 in 70% ethanol

was applied to the sections for 2 min at room temperature to remove lipofuscin autofluorescence. The sections were washed with distilled water, and Vectashield with or without DAPI (Vector Lab) was used as the mounting medium. The samples were stored at 4 °C before imaging on a confocal laser-scanning microscope.

For immunohistochemistry analysis of ERBB4, we applied antigen retrieval before conventional immunohistochemistry. In brief, slices were placed in an Eppendorf tube filled with 500 µl 1× HistoVT One solution, and incubated at 70 °C for 20 min. After incubation, the slices were moved to a 24-well plate where conventional immunohistochemistry was performed.

For FISH, tissue sections were cut at a thickness of 10 µm and mounted onto SuperFrost Plus glass slides (Thermo Fisher Scientific). The sections were fixed in 4 °C PFA for 15 min, followed by dehydration through a graded ethanol series (50%, 70%, 100% and 100% ethanol; 5 min each) at room temperature. Human FFPE sections were deparaffinized before proceeding according to the same RNAscope workflow. After air-drying, FISH was performed using the RNAscope Multiplex Fluorescent Assay kits (Advanced Cell Diagnostics) according to the manufacturer's instructions, with minor modifications. The slides were subjected to protease treatment for 30 min, followed by hybridization with target-specific RNAscope probes. All hybridization, amplification and wash steps were carried out manually at room temperature or at the temperatures specified by the manufacturer. Signal detection was achieved using Opal TSA fluorophores (Akoya Biosciences) diluted 1:1,000, depending on the probe and channel requirements. Nuclear counterstaining was performed using DAPI.

All confocal images from the brain sections were acquired using Zeiss LSM880 (×10 lens, ×20 lens or ×40 oil-immersion optical lens) for quantification as described below. Data indicate mice otherwise reported in figure legend.

To quantify *ErbB4* mRNA levels in pyramidal neurons in mouse tissues, confocal single-plane images of *Gad1* and *ErbB4* within the pyramidal layer were isolated. Colocalization between *Gad1* and *ErbB4* was quantified using the DiAna plugin. To exclude *ErbB4* mRNA originating from *Gad1*⁺ inhibitory neurons, the colocalized areas were subtracted from the total *ErbB4*⁺ areas.

To quantify *ERBB4* mRNA levels in excitatory neurons in human tissue, confocal single-plane images of *ERBB4*, *SLC17A7* and DAPI signals were analysed. *SLC17A7*⁺ excitatory neurons were identified by the presence of *SLC17A7* puncta in DAPI⁺ nuclei. *ERBB4* puncta colocalized with *SLC17A7*⁺ DAPI⁺ signal were then measured using the DiAna⁶⁰ plugin.

To quantify synapse phagocytosis by glial cells, confocal single-plane images of GFP intensity subtracted from mCherry intensity (mCherry intensity – GFP intensity; mCherry alone) along with glial cells (S100β and IBA1) were separately isolated, and the areas of colocalization (mCherry alone⁺, glial cell⁺) were measured using the DiAna⁶⁰ plugin. To compensate for differences in the injected viruses, the colocalization areas were normalized to GFP areas.

To quantify synapse numbers, confocal single-plane images of excitatory synapses (vGLUT1 and PSD95) and inhibitory synapses (vGAT and gephyrin) were separately isolated, and the areas of themselves and their colocalization were measured using the Analyze Particle function in ImageJ and DiAna plugin, respectively.

To quantify the areas of GFAP, S100β, IBA1 and Ab, confocal single-plane images of these markers were isolated, and their areas were measured by Analyze Particle function.

To quantify the areas of pS6 in the pyramidal neurons and PV⁺ neurons, confocal single-plane images of pS6, PV and NeuN were isolated, and their colocalization (pS6⁺PV⁺NeuN⁺ or pS6⁺PV⁺NeuN⁺) was measured using the DiAna plugin.

To quantify the areas of DAM population (AXL⁺/TREM2⁺), confocal single-plane images of AXL, TREM2 and IBA1 were isolated, and their colocalization (AXL⁺IBA1⁺ or TREM2⁺IBA1⁺) was measured using the DiAna plugin.

To quantify MEGF10 in shRNA-applied samples, confocal single-plane images of MEGF10 and TagBFP were separately isolated, and the colocalization areas were measured using the DiAna plugin. To compensate for differences in the labelled astrocytes, the colocalization areas were normalized to TagBFP areas.

To quantify cleaved caspase-3⁺, FOS⁺ or ERBB4⁺ neurons, confocal z-stacked images of cleaved caspase-3, FOS or ERBB4 with neuronal markers (PV, SST or VIP with NeuN) were merged, and the numbers were manually counted.

Slice electrophysiology

Mice were anaesthetized with isoflurane and decapitated. The brains were quickly removed and transferred into ice-cold artificial cerebrospinal fluid (ACSF) continuously bubbled with 95% O₂ and 5% CO₂. The ACSF consisted of 125 mM NaCl, 2.5 mM KCl, 1.25 mM NaH₂PO₄, 25 mM NaHCO₃, 1 mM MgCl₂, 2 mM CaCl₂ and 15 mM glucose. Coronal slices were cut in ice-chilled ACSF using a vibratome (VT1200S, Leica). After slicing, the sections were allowed to recover in ACSF at 34 °C for 20 min, followed by incubation at room temperature for at least 40 min. For recordings, slices were placed into a submerged recording chamber and continuously perfused with oxygenated ACSF at a flow rate of 2–3 ml min⁻¹. The chamber temperature was maintained at 30–31 °C, and recordings were performed within 4–5 h after the recovery period. Neurons were visualized using infrared differential interference contrast microscopy with an upright microscope (BX51WI, Olympus). Whole-cell voltage-clamp recordings were obtained with borosilicate glass pipettes (2.5–3.5 MΩ) filled with a Cs⁺-based low-Cl⁻ internal solution containing 135 mM CsMeSO₃, 10 mM HEPES, 1 mM EGTA, 3.3 mM QX-314, 0.1 mM CaCl₂, 4 mM Mg-ATP, 0.3 mM Na₂-GTP, 8 mM Na₂-phosphocreatine (290–300 mOsm, pH 7.3 adjusted with CsOH). Recordings were included in the analysis only when the access resistance was between 10–20 MΩ and remained stable, with less than 20% change throughout the experiment. Whole-cell patch-clamp recordings were acquired using the Multiclamp 700B (Molecular Devices) system and signals were filtered at 2 kHz and digitized at 10 kHz (NI PCIe-6259, National Instruments). Data were monitored and acquired by WinWCP (Strathclyde Software), further analysed using Clampfit v.10.7 (Molecular Devices) and OriginPro 2017 (OriginLab). To measure the mEPSCs and mIPSCs, Nav channel blocker (TTX, 500 nM, Alomone Labs, T-550) and NMDA receptor antagonist (D-AP5, 25 μM, Tocris, 0106) were added to ACSF and applied throughout the recording session. Both mEPSCs and mIPSCs were recorded in the same cell. mEPSCs were obtained at -70 mV (reversal potential of chloride), and mIPSCs were subsequently obtained at 0 mV (reversal potential of ionotropic glutamate receptors). mEPSCs and mIPSCs were recorded for 3 min each. mEPSCs were analysed over the entire 3 min recording period, whereas mIPSCs were analysed during a 60 s segment from 60 s after the start of recording.

For intrinsic excitability recordings, coronal slices containing the dorsal hippocampal CA1 (300 μm) were cut in ice-cold oxygenated sucrose-based cutting solution containing 75 mM sucrose, 76 mM NaCl, 2.5 mM KCl, 25 mM NaHCO₃, 25 mM glucose, 1.25 mM NaH₂PO₄, 7 mM MgSO₄, 0.5 mM CaCl₂ with pH 7.3, equilibrated with 95% O₂ and 5% CO₂, using a vibratome (Leica, VT1200S), and then recovered in the same solution for 30 min at 32 °C. Slices were then transferred to an incubation chamber filled with oxygenated ACSF containing 124 mM NaCl, 2.5 mM KCl, 1.3 mM MgCl₂, 2.5 mM CaCl₂, 1.0 mM NaH₂PO₄, 26.2 mM NaHCO₃ and 20 mM glucose with pH 7.4 at room temperature and slices were kept for less than 6 h before recordings.

For patch-clamp recordings, slices were transferred to a recording chamber perfused with oxygenated ACSF at 30–32 °C controlled by a peristaltic pump. Patch microelectrodes were pulled from borosilicate glass (Harvard Apparatus, 30-0065) using a micropipette puller (Narishige, PC-10). Patch microelectrodes had a resistance of 5.0–7.0 MΩ. Signals were recorded using a patch-clamp amplifier (Multiclamp 700B,

Molecular Devices) and digitized with Digidata 1550 digitizer (Molecular Devices) using Clampex software (Molecular Devices). Signals were amplified, filtered at 2 kHz and sampled at 10 kHz.

In current-clamp recordings, the membrane potential was held at -70 mV with intracellular solution: 135 mM K-gluconate, 7 mM NaCl, 10 mM HEPES, 0.5 mM EGTA, 2 mM Mg-ATP, 0.3 mM Na₂-GTP and 10 mM Na-phosphocreatine with pH 7.3 and 295 mOsm. Current-clamp experiments were recorded 5 min after obtaining whole-cell configuration. To evaluate intrinsic excitability, 500 ms depolarizing currents were injected from -200 to 500 pA with increments of 50 pA, and the mean firing rate was calculated based on the number of evoked action potentials in response to a depolarizing current injection. The input resistance (*R*_{in}) was estimated as the slope of the *I*-*V* relationship derived by measuring the difference between the baseline and the steady-state.

Behavioural test

Both novel-object recognition test and novel-object location test were performed in a square box (acrylic box, 30 cm × 30 cm × 28 cm, *W* × *D* × *H*, custom-made). One side of the box has a stripe to give information about location. Both tasks are composed of three steps: habituation, training and testing.

For the novel-object location test, mice were habituated to the testing box by freely moving in the open-field arena for 10 min. Then, 24 h after habituation, the mice were placed into the box, which contained two identical objects, for 10 min for training. Then, 24 h after training, the mice were again placed in the box, in which one object was moved to a new location for 10 min.

For the novel-object recognition test, mice were habituated under the same conditions as for the novel-object location test. At the training step, mice were exposed to two identical objects. For the testing step, one of the two objects was replaced with a novel object.

For spontaneous alternation, mice were placed on the centre of a Y-maze (acrylic box, 5 cm × 30 cm × 15 cm, *W* × *D* × *H* for one arm, custom-made) for 8 min.

To quantify performance in the novel-object location test and novel-object recognition test, the time spent interacting with the objects for both the old and novel object and location were measured. Then, the discrimination index was calculated according to the following equation:

$$\frac{\text{Time interacting with novel object or object at novel location} \times 100}{\text{Time interacting with old object or object at old location} + \text{Time interacting with novel object or object at novel location}}$$

For quantification of spontaneous alternation, arm entry for all arms was quantified and alternation percentages were calculated by the following equation:

$$\frac{\text{Spontaneous alternation} \times 100}{\text{Total arm entries} - 2},$$

where spontaneous alternation was defined as sequential arm entry for all arms.

For the Barnes maze, we followed the shortened protocol as described previously⁴¹. In brief, the maze consisted of a circular white platform (1 m diameter) elevated approximately 1 m above the floor, with 20 evenly spaced holes along the perimeter, one of which led to a dark escape cage. The task was performed in three phases: habituation, training and probe. On the habituation day, the mice were placed at the centre of the maze for 30 s and then gently guided to the target hole to allow familiarization with the escape cage. During the training phase, the mice were placed inside a transparent cylinder at the centre of the maze for 10 s, after which the cylinder was removed. Mice were allowed

to explore the maze for up to 2 min per trial to locate the target hole. Training consisted of five trials over 2 days (three trials on day 1 and two trials on day 2), with an inter-trial interval of approximately 20 min. If a mouse failed to enter the escape cage within the allotted time, it was gently guided to the target hole. Primary latency, defined as the time from trial onset to the first nose poke at the target hole, was recorded as a measure of spatial learning. One day after final training session, a probe trial was conducted in which the escape cage was removed, and mice were allowed to explore the maze for 2 min. Time spent in each quadrant was recorded as a measure of spatial memory.

All behavioural tests were performed by researchers blinded to the groups assignments.

Illustrations

All illustrations were generated using a BioRender with a licence (<https://www.biorender.com>).

Software and statistical analysis

Zen (Zeiss) software acquisition system was used for acquisition of confocal images. For image analysis, ImageJ (NIH) and its plugin DiAna⁶⁰ were used.

No statistical methods were used to predetermine sample sizes. All statistical analyses were performed using GraphPad Prism 10 with 95% confidence. The Shapiro–Wilk test was first performed for all data to determine normality of the data distribution. If the data passed normality testing, comparisons between two groups were performed using two-tailed unpaired Student's *t*-tests. For data that did not follow a normal distribution, the Mann–Whitney *U*-test was used. For comparisons of markers measured from different mouse brains, unpaired tests were applied, whereas for measurements obtained from the same mouse brain following bilateral injection of different AAVs, paired tests were used. For comparison of more than three groups with normal distribution, one-way ANOVA followed by Tukey's multiple-comparison test was used. For comparison of more than two groups with two independent variables, two-way ANOVA followed by Tukey's multiple-comparison test was used. For comparison of cell numbers in the excitatory neuronal cluster (Extended Data Fig. 5a,f), the generalized estimating equation test was performed using statsmodels v.0.14.5 in Python v.3.10. For comparison of excitability (Extended Data Fig. 7v), a linear mixed model was used and corrected using the 'fdr_bh' method in statsmodels and scipy v.1.15.2 in Python. For directed mediation analysis, statsmodels and semopy v.2.3.11 were used in Python. Linear regression was used by scikit-learn v.1.6.1 in Python. The statistical test used for each experiment is reported in the results.

Ethics statement

All animal experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committees of the Korea Advanced Institute of Science and Technology and the Institute for Basic Science. Human FFPE hippocampal sections were provided by the SNUH Brain Bank. Their use in this study was reviewed and determined to be exempt from protocol review by the Public Institutional Review Board designated by the Ministry of Health and Welfare, Republic of Korea. All procedures involving human-derived materials were conducted in accordance with relevant regulations.

Reporting summary

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

Data availability

The sequencing data generated in this study have been deposited into the NCBI's Gene Expression Omnibus (GEO) under accession number GSE292137. snRNA-seq data from the patients with HD were retrieved from the GEO under accession number GSE242197. snRNA-seq data from the patients with progressive supranuclear palsy were obtained from CZ CELLxGENE (<https://cellxgene.cziscience.com/collections/c53573b2-eff4-4c5e-9ad0-b24d422dfd9b>). snRNA-seq data from the *Tsc2*-knockout mice were obtained from the GEO (GSE286912). snRNA-seq data from excitatory neurons of ROSMAP cohort were obtained from Synapse portal under accession numbers SYN53694054 and SYN53694068. Source data are provided with this paper.

Code availability

The analysis of RNA-seq data followed tutorials from Scanpy, pyWGCNA and SysVI, as described in the Methods in more detail. No new software or code was generated and used for data collection and analysis.

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Author contributions W.-S.C. and S.Y.L. designed projects and wrote the manuscript. H.-E.L. performed mEPSC and mIPSC recording. S.K. and Y.Y. performed intrinsic excitability recording. K.L., K.-J.Y., S.P., E.K. and J.-I.K. provided reagents, mice and discussion. Y.-J.C. designed shRNAs for *Megf10*. J.P. performed immunohistochemistry. E.P. performed behaviour experiments and FISH. S.Y.L. performed all other experiments including cloning, stereotaxic injection, scRNA-seq analysis and virus production.

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Competing interests S.Y.L. and W.-S.C. have filed a patent application based on this work. The other authors declare no competing interests.

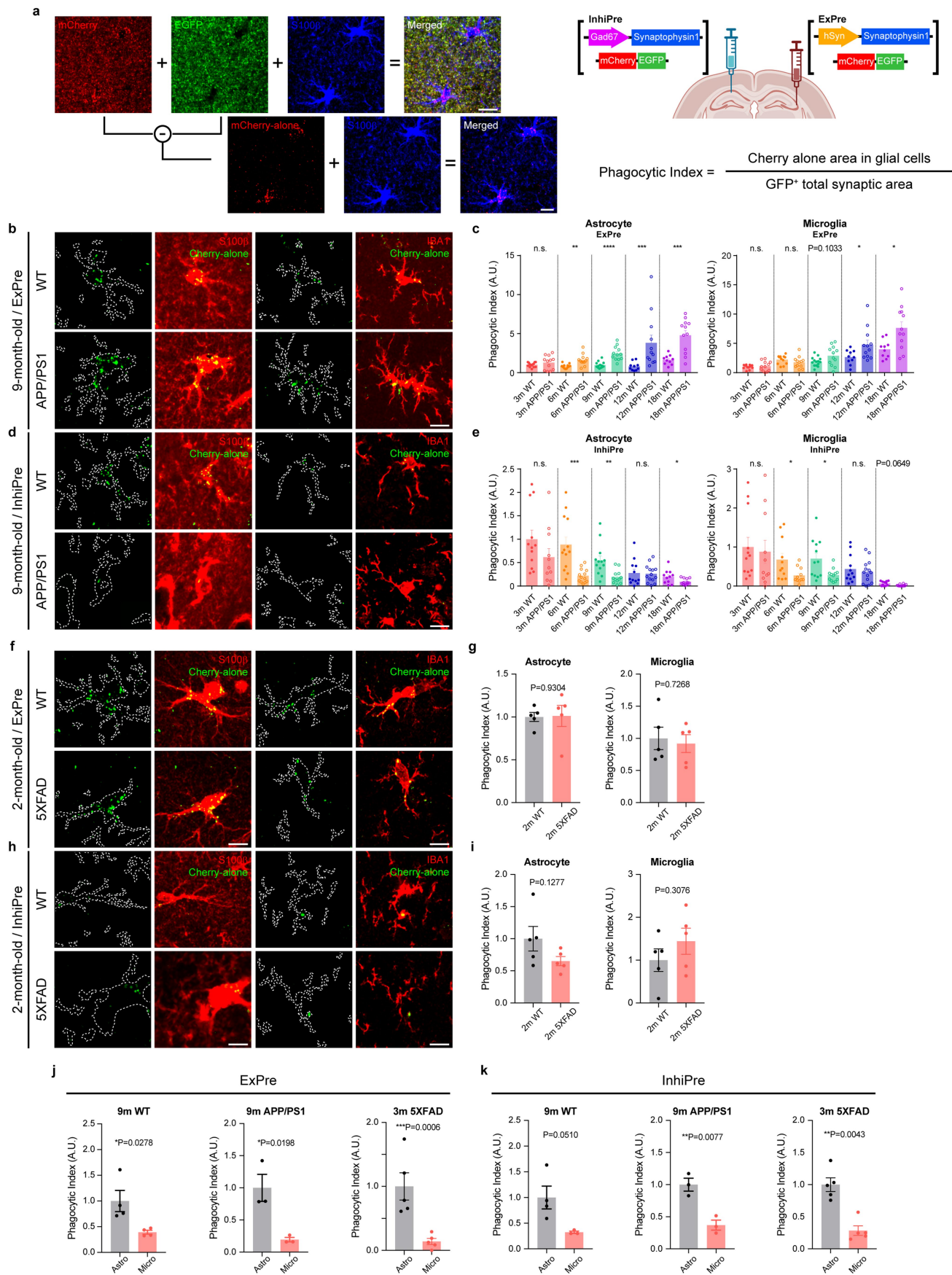
Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41586-026-10964-z>.

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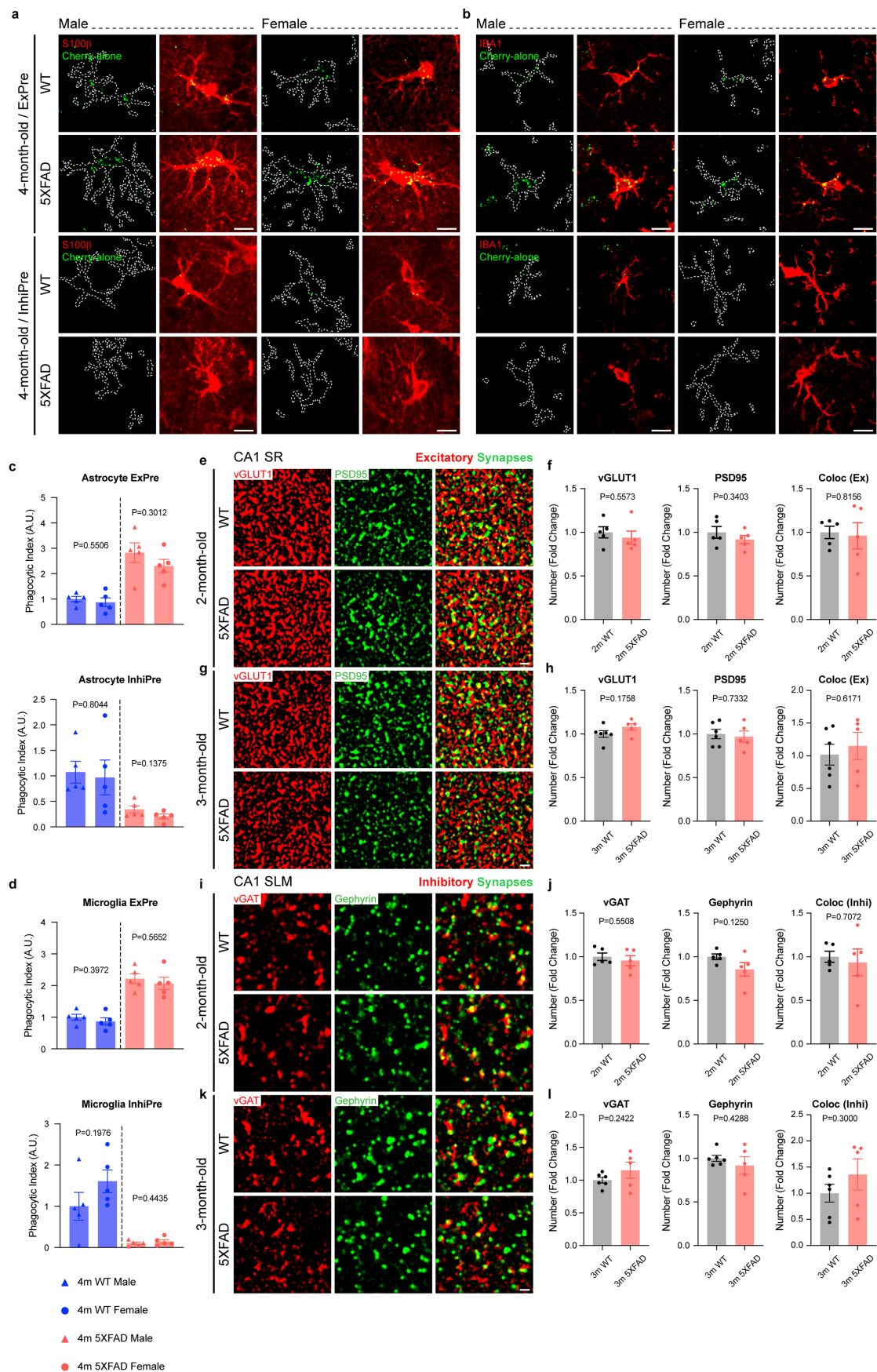
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Extended Data Fig. 1 | Excitatory and Inhibitory Synapse Elimination Across Multiple AD Models and Ages. **a**, Schematic illustration of the mCherry-EGFP signal processing workflow (left). Representative confocal z-stack images of mCherry (red), EGFP (green) and S100 β (blue). Schematic illustration of AAV-injection into the hippocampus (right, top). Equation used to calculate the phagocytic index (right, bottom). Scale bar, 100 μ m. This diagram was created in Biorender; Chung, W. (2026). <https://Biorender.com/jotrdrhx>. **b, d, f, h**, Representative confocal z-stack images of mCherry-alone puncta from ExPre (**b, f**, green) and InhiPre (**d, h**, green), and glial cells (red, astrocytes: S100 β ; microglia: IBA1) in the CA1 of 9-month-old WT and APP/PS1 (**b, d**) and

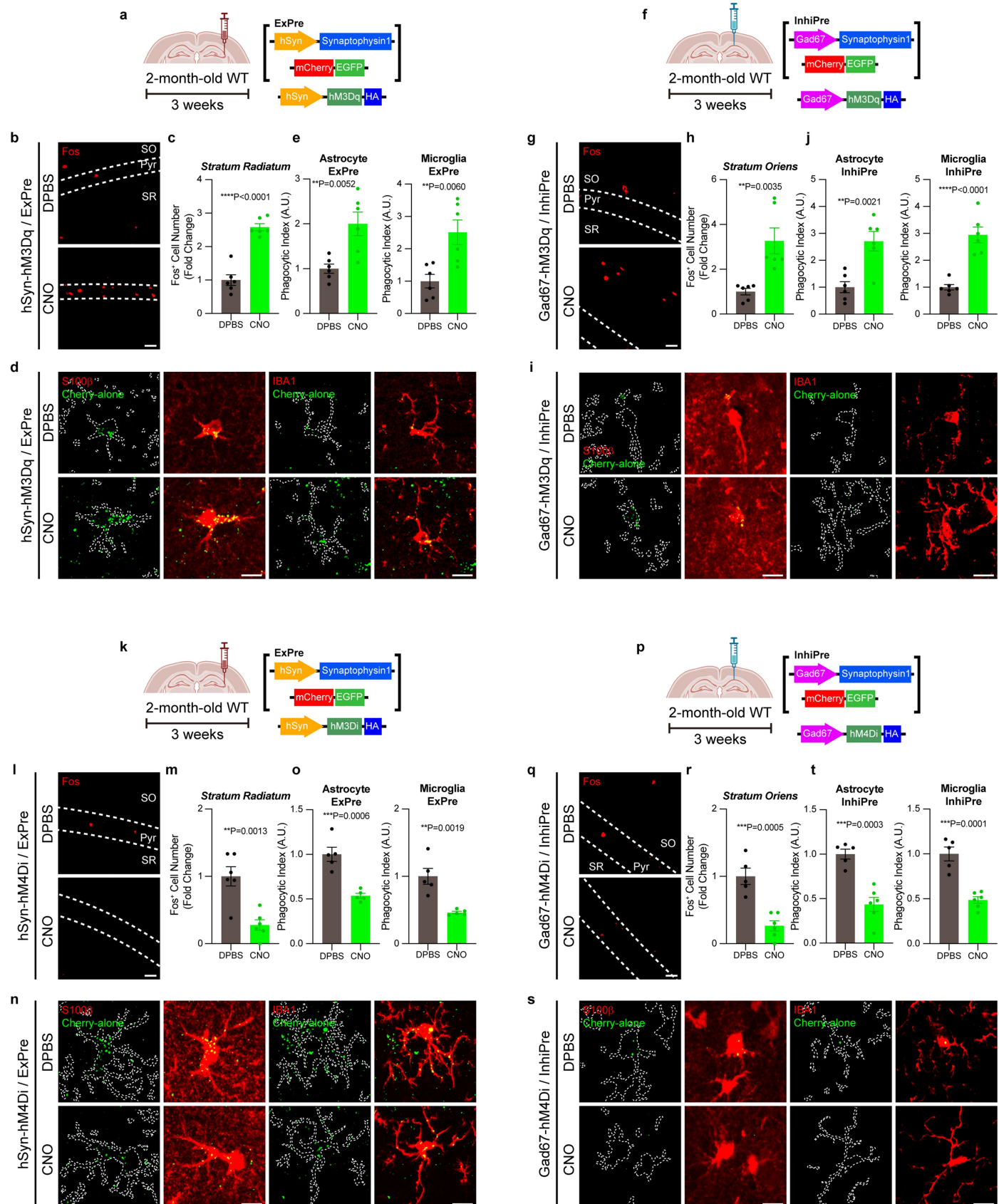
2-month-old WT and 5xFAD (**f, h**). White dotted lines indicate outlines of glial cells. Scale bar, 10 μ m. **c, e, g, i-k**, Quantification of the engulfed excitatory (**c, g**) and inhibitory (**e, i**) pre-synapses by astrocytes (left) and microglia (right). $n = 14, 12, 12, 12, 13, 12, 10, 12, 10, 12$ (**c**). $n = 12, 11, 13, 13, 13, 12, 14, 11, 12$ (**e**). $n = 5$ mice per group (**g, i**). $n = 4$ mice per group (**j**, left). $n = 3$ mice per group (**j**, middle). $n = 5$ mice per group (**j**, right). $n = 4$ (Astrocytes), 3 (Microglia) (**k**, left). $n = 3$ mice per group (**k**, middle). $n = 5$ mice per group (**k**, right). Two-sided unpaired Student's t-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. Mean $\pm$ s.e.m.; n value represents independent experiments from 3–5 mice per group (**b-e**). Otherwise, n value represents mice number per group.



Extended Data Fig. 2 | See next page for caption.

Extended Data Fig. 2 | Lack of Sex Differences in Synapse Elimination and Synapse Numbers in Young 5xFAD Mice. **a, b**, Representative confocal z-stack images of mCherry-alone puncta from ExPre (**a, b**, top, green) and InhiPre (**a, b**, bottom, green), and glial cells (red, astrocytes: S100 β ; microglia: IBA1) in the CA1 of 4-month-old WT and 5xFAD (**a**: male, **b**: female). White dotted lines indicate outlines of glial cells. Scale bar, 10 μ m. **c, d**, Quantification of the engulfed excitatory (top of **c, d**) and inhibitory (bottom of **c, d**) pre-synapses by astrocytes (**c**) and microglia (**d**). $n = 5$ mice per group (**c, d**). **e, g, i, k**, Representative confocal

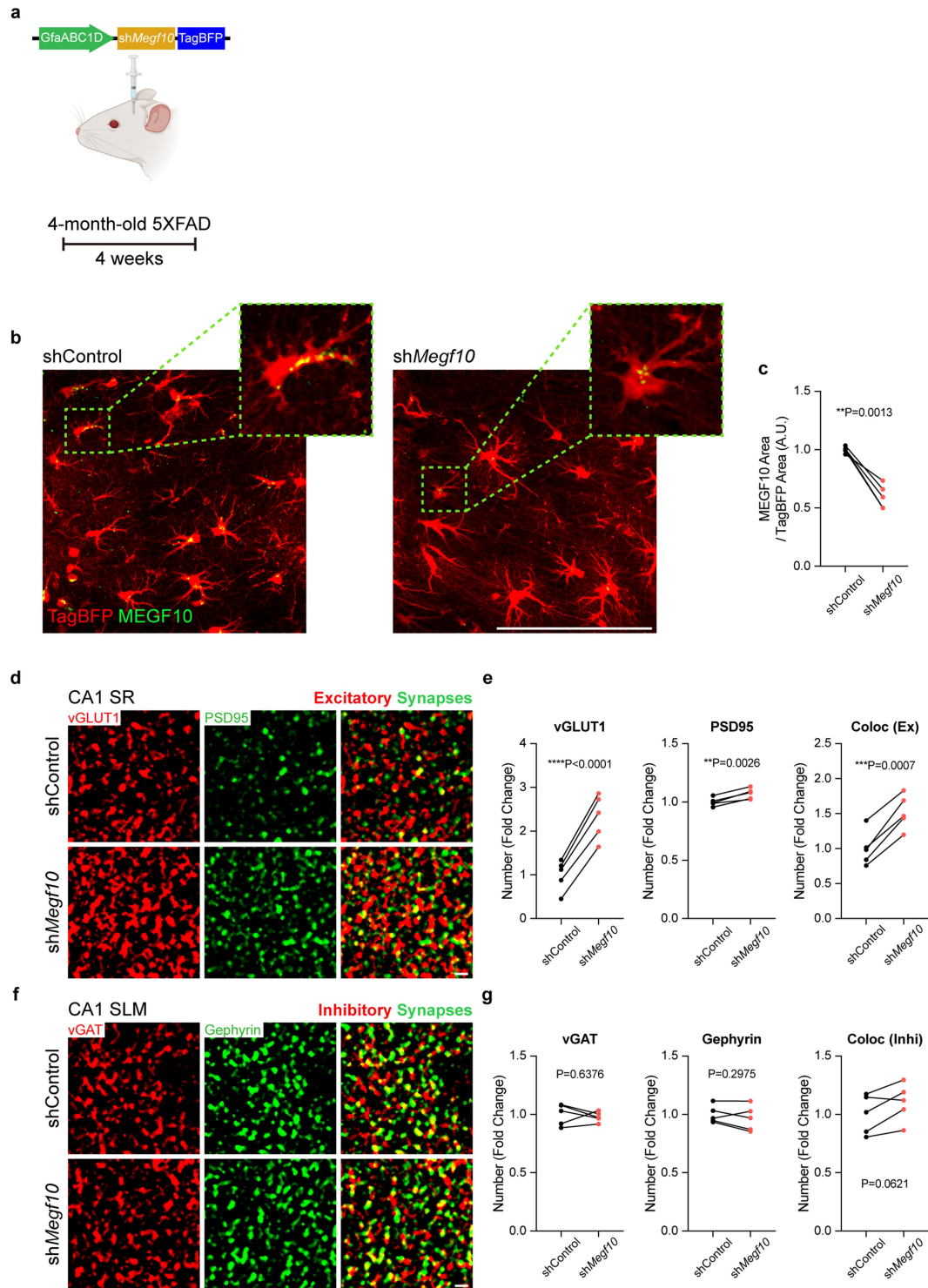
single plane images of excitatory (**e, g**, pre-synapse: vGLUT1, red, post-synapse: PSD95, green) and inhibitory (**i, k**, pre-synapse: vGAT, red, post-synapse: Gephyrin, green) synapses in the CA1 of 2-month-old (**e, i**) and 3-month-old (**g, k**) WT and 5xFAD. SR, *Stratum Radiatum*. SLM, *Stratum Lacunosum Moleculare*. Scale bar, 1 μ m. **f, h, j, l**, Quantification of the excitatory (**f, h**) and inhibitory (**j, l**) synapses. $n = 5$ mice per group (**f, j**). $n = 6$ (WT), 5 (5xFAD) (**h, l**). Two-sided unpaired Student's t-test. Mean $\pm$ s.e.m.; n value represents the number of mice per group.



Extended Data Fig. 3 | See next page for caption.

Extended Data Fig. 3 | Neuronal Activity Modulates Synapse Elimination by Glial Cells. a, f, k, p. Schematic illustration of AAV-injections into the CA1 of 2-month-old WT. This diagram was created in Biorender; Chung, W. (2026). <https://biorender.com/jotrdxh>. **b, g, l, q.** Representative confocal z-stack images of c-Fos (red) in the CA1 of DPBS- and CNO-injected WT. SO, *Stratum Oriens*. Pyr, pyramidal layer. SR, *Stratum Radiatum*. Scale bar, 10 μ m. **c, h, m, r.** Quantification of the fold changes in c-Fos⁺ cell numbers. $n = 6$ mice per group (**c, h, m**). $n = 5$ (DPBS), 6 (CNO) (**r**). **d, i, n, s.** Representative confocal z-stack images of

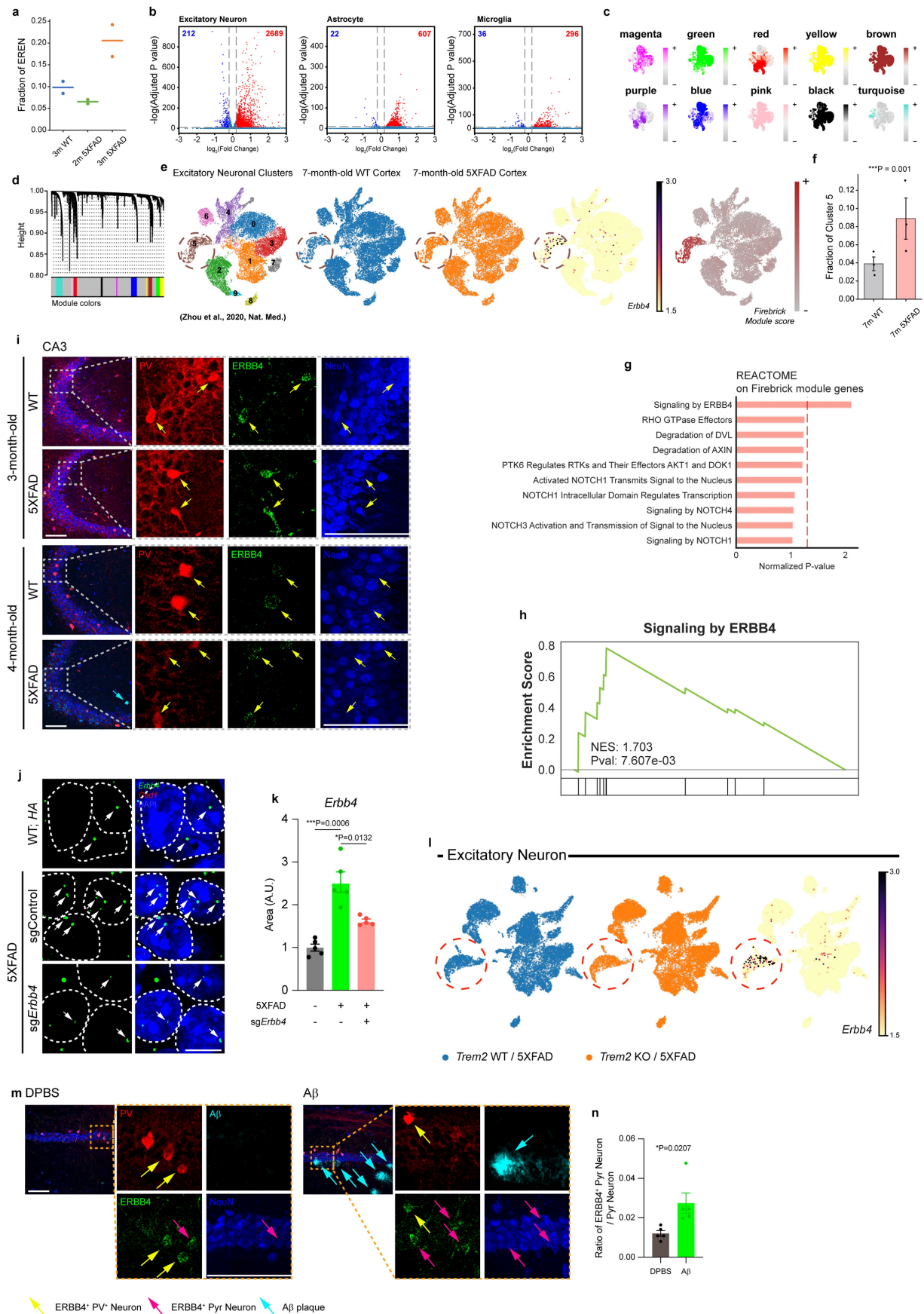
mCherry-alone puncta from ExPre (**d, n**, green) and InhiPre (**i, s**, green), and glial cells (red, astrocytes: S100 β ; microglia: IBA1) in the CA1 of DPBS- and CNO-injected WT. White dotted lines indicate outlines of glial cells. Scale bar, 10 μ m. **e, j, o, t.** Quantification of the engulfed excitatory (**e, o**) and inhibitory (**j, t**) pre-synapses by astrocytes (left) and microglia (right). $n = 6$ mice per group (**e, j**). $n = 5$ mice per group (**o**). $n = 5$ (DPBS), 6 (CNO) (**t**). Two-sided unpaired Student's t-test. * $P < 0.05$, ** $P < 0.01$. Mean $\pm$ s.e.m.; n value represents the number of mice per group.



Extended Data Fig. 4 | Synapse Change in AD Requires Glial Phagocytosis.

a, Schematic illustration showing shRNA-expressing AAV-injection into the CA1 of 4-month-old 5xFAD. This diagram was created in Biorender; Chung, W. (2026). <https://biorender.com/jotrdxh>. **b**, Representative confocal z-stack images of TagBFP (red) and MEGF10 (green) in the CA1 of shControl- and shMegf10-injected 5xFAD. Green dotted boxes indicate enlarged regions. Scale bar, 10 μ m. **c**, Quantification of MEGF10 area colocalized with TagBFP, normalized to TagBFP area. **d, f**, Representative confocal single plane images

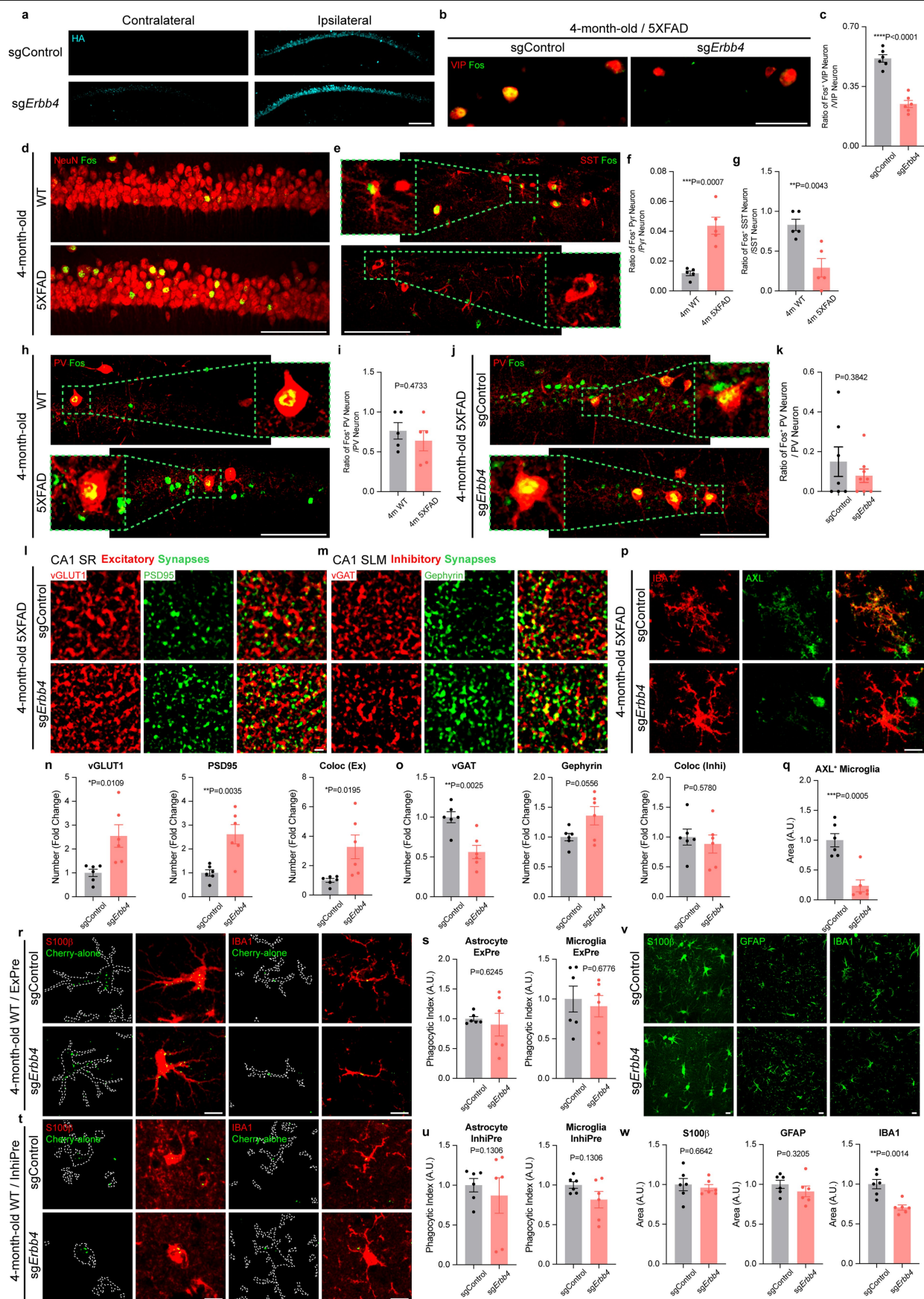
of excitatory (**d**, pre-synapse: vGLUT1, red, post-synapse: PSD95, green) and inhibitory (**f**, pre-synapse: vGAT, red, post-synapse: Gephyrin, green) synapses in the CA1 of shControl- and shMegf10-injected 5xFAD. SR, *Stratum Radiatum*. SLM, *Stratum Lacunosum Moleculare*. Scale bar, 1 μ m. **e, g**, Quantification of the excitatory (**e**) and inhibitory (**g**) synapses. $n = 5$ mice per group (**c, e, g**). Two-sided paired Student's t-test. * $P < 0.05$, ** $P < 0.01$. Mean $\pm$ s.e.m.; n value represents the number of mice per group.



Extended Data Fig. 5 | See next page for caption.

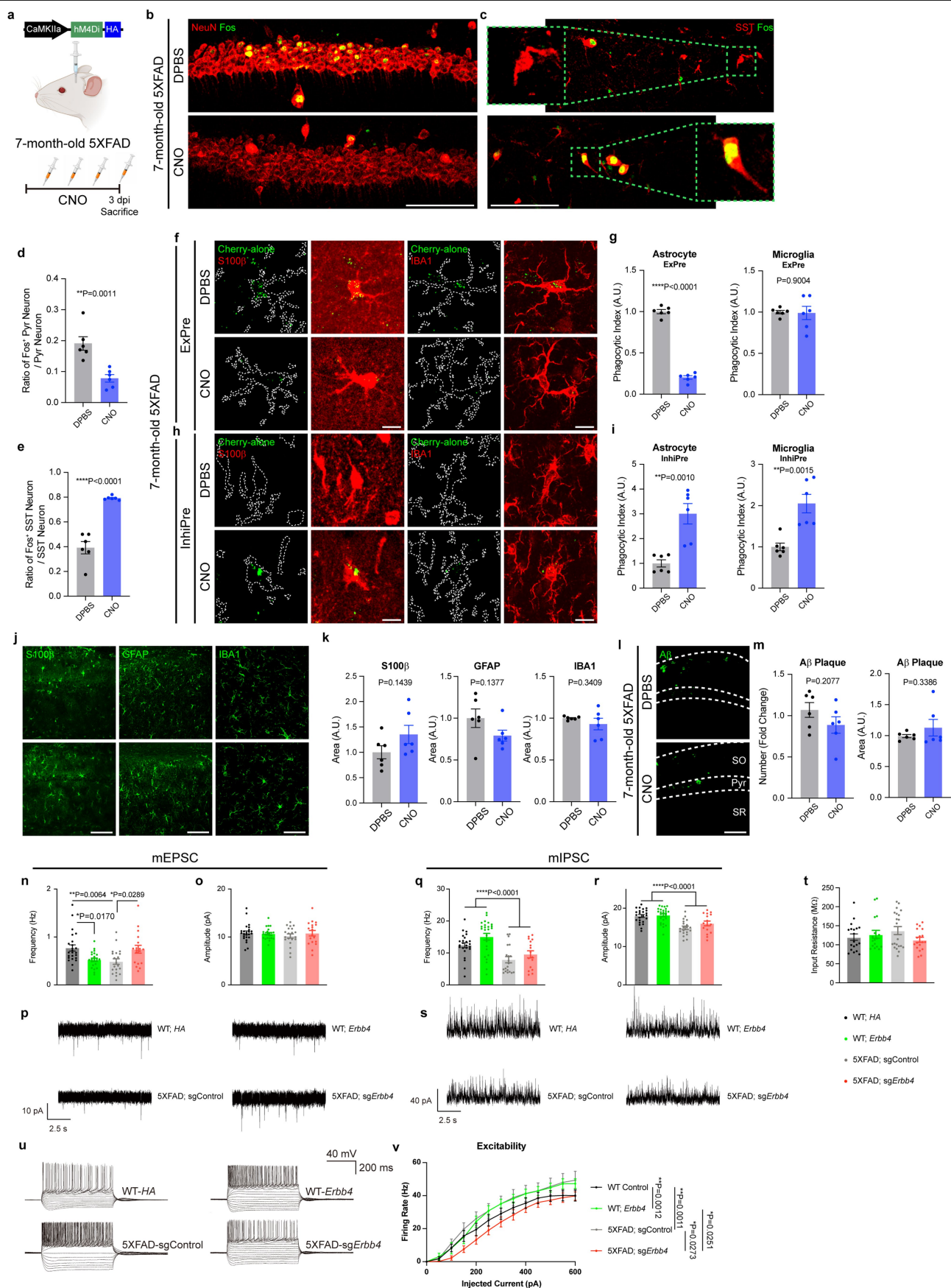
Extended Data Fig. 5 | snRNAseq Analysis Reveals that A β , but Not DAM, Induces *ErbB4* Expression in Excitatory Neurons. **a**, Bar graphs of the fraction of EREN population from 3-month-old WT, 2-month-old 5xFAD, and 3-month-old 5xFAD. $n = 2$ mice per group. **b**, Volcano plots showing DEGs of excitatory neurons (left), astrocytes (middle), and microglia (right) from 3-month-old 5xFAD compared to 2-month-old 5xFAD and 3-month-old WT. Red dots represent upregulated genes, while blue dots represent downregulated genes. One-sided Wilcoxon rank-sum. **c**, UMAP plots of the excitatory neuronal clusters showing the distribution of colour-coded modules. **d**, Colour-coded modules identified by WGCNA and separated by a dendrogram. **e**, UMAP plots of the excitatory neurons from 7-month-old WT, 5xFAD, their cluster annotations, *ErbB4* expression, and Firebrick module score. Brown dotted circles indicate EREN-like population. A total of 17996 cells were analysed, derived from two groups with $n = 3$ mice per group. **f**, Bar graphs of the fraction of excitatory neurons in cluster 5 from 7-month-old WT and 5xFAD. $n = 3$ mice per group. Two-sided generalized estimating equation. **g**, **h**, Bar plot and GSEA plot showing enrichment of ERBB4 signalling in firebrick module genes. One-sided permutation test. **i**, Representative confocal z-stack images of PV (red), ERBB4 (green), and NeuN (blue) in the CA3 of 3-month-old (top) and 4-month-old (bottom) WT and 5xFAD. Images are representative of three independent experiments, each including two mice per group, with similar results.

Grey dotted boxes indicate enlarged regions. Cyan arrow indicates amyloid plaque, and yellow arrows indicate ERBB4⁺ PV neurons. Scale bar, 100 μ m. **j**, Representative confocal single-plane images of *ErbB4* (green), *Gad1* (red), and DAPI (blue) in the CA1 of 4-month-old *HA*-injected WT (top), sgControl- and sg*ErbB4*-injected 5xFAD (middle and bottom). White dotted lines indicate boundaries of individual neuronal nuclei. Images are representative of two independent experiments; the first and second experiments included three and two mice per group, respectively, with similar results. White arrows indicate *ErbB4* mRNA puncta in the pyramidal neurons. Scale bar, 10 μ m. **k**, Quantification of the *ErbB4* mRNA area in pyramidal layer. $n = 5$ mice per group. Two-way ANOVA followed by Tukey's multiple comparisons test. **l**, UMAP plots of the excitatory neurons from 7-month-old *Trem2*-WT/5xFAD and *Trem2*-KO/5xFAD, and their *ErbB4* expression. Red dotted circles indicate EREN-like population. **m**, Representative confocal z-stack images of PV (red), ERBB4 (green), and A β (cyan) in the CA1 of DPBS- and A β -injected 2-month-old WT. Orange dotted boxes indicate enlarged regions. Yellow arrows indicate ERBB4⁺ pyramidal neurons. Magenta arrows indicate ERBB4⁺ PV neurons. Cyan arrows indicate A β plaques. **n**, Quantification of the ratio of ERBB4⁺ pyramidal neurons to the number of pyramidal neurons. $n = 5$ mice per group. Two-sided unpaired Student's t-test. * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$. Mean $\pm$ s.e.m.; n value represents the number of mice per group (**e-i**).



Extended Data Fig. 6 | Effects of *ErbB4* Deletion in 5xFAD and WT Excitatory Neurons on Synapse Elimination and Reactive Gliosis. **a**, Representative confocal z-stack images of the HA (cyan) in the contralateral and ipsilateral CA1 of sgControl- and sg*ErbB4*-injected 4-month-old WT. Scale bar, 1 mm. **b, d, e, h, j**, Representative confocal z-stack images of VIP (**b**, red), NeuN (**d**, red), SST (**e**, red), PV (**h, j**, red) and c-Fos (green) in the CA1 of 4-month-old WT and 5xFAD (**d, e, h**) and sgControl- and sg*ErbB4*-injected 4-month-old 5xFAD (**b, j**). Green dotted boxes indicate enlarged regions. Scale bar, 100 μ m. **c, f, g, i, k**, Quantification of the ratio of c-Fos⁺ VIP neuron (**c**), c-Fos⁺ pyramidal neurons (**f**), c-Fos⁺ SST neurons (**g**), c-Fos⁺ PV neurons (**i, k**) to the number of VIP neurons, pyramidal neurons, SST neurons, and PV neurons, respectively. *n* = 6 mice per group (**c**). *n* = 5 mice per group (**f, g, i**). *n* = 7 (sgControl), 8 (sg*ErbB4*) (**k**). **l, m**, Representative confocal single plane images of excitatory (**l**, pre-synapse: vGLUT1, red, post-synapse: PSD95, green) and inhibitory (**m**, pre-synapse: vGAT, red, post-synapse: Gephyrin, green) synapses in the CA1 of sgControl- and sg*ErbB4*-injected 5xFAD. SR, *Stratum Radiatum*.

SLM, *Stratum Lacunosum Moleculare*. Scale bar, 1 μ m. **n, o**, Quantification of the excitatory (**n**) and inhibitory (**o**) synapses. *n* = 6 mice per group. **p**, Representative confocal z-stack images of the AXL (green) and IBA1 (red) in the CA1 of sgControl- and sg*ErbB4*-injected 5xFAD. Scale bar, 10 μ m. **q**, Quantification of the area of AXL colocalized with IBA1. *n* = 6 mice per group. **r, t**, Representative confocal z-stack images of mCherry-alone puncta from ExPre (**r**, green) and InhiPre (**t**, green), and glial cells (red, astrocytes: S100 β ; microglia: IBA1) in the CA1 of sgControl- and sg*ErbB4*-injected 4-month-old WT. White dotted lines indicate outlines of glial cells. Scale bar, 10 μ m. **s, u**, Quantification of the engulfed excitatory (**s**) and inhibitory (**u**) pre-synapses by astrocytes (left) and microglia (right). *n* = 6 mice per group. **v**, Representative confocal z-stack images of S100 β (left), GFAP (middle), and IBA1 (right) in the CA1 of sgControl- and sg*ErbB4*-injected WT. Scale bar, 10 μ m. **w**, Quantification of the areas of the S100 β (left), GFAP (middle), and IBA1 (right). *n* = 6, 6. Two-sided unpaired Student's t-test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001. Mean $\pm$ s.e.m.; *n* value represents the number of mice per group.

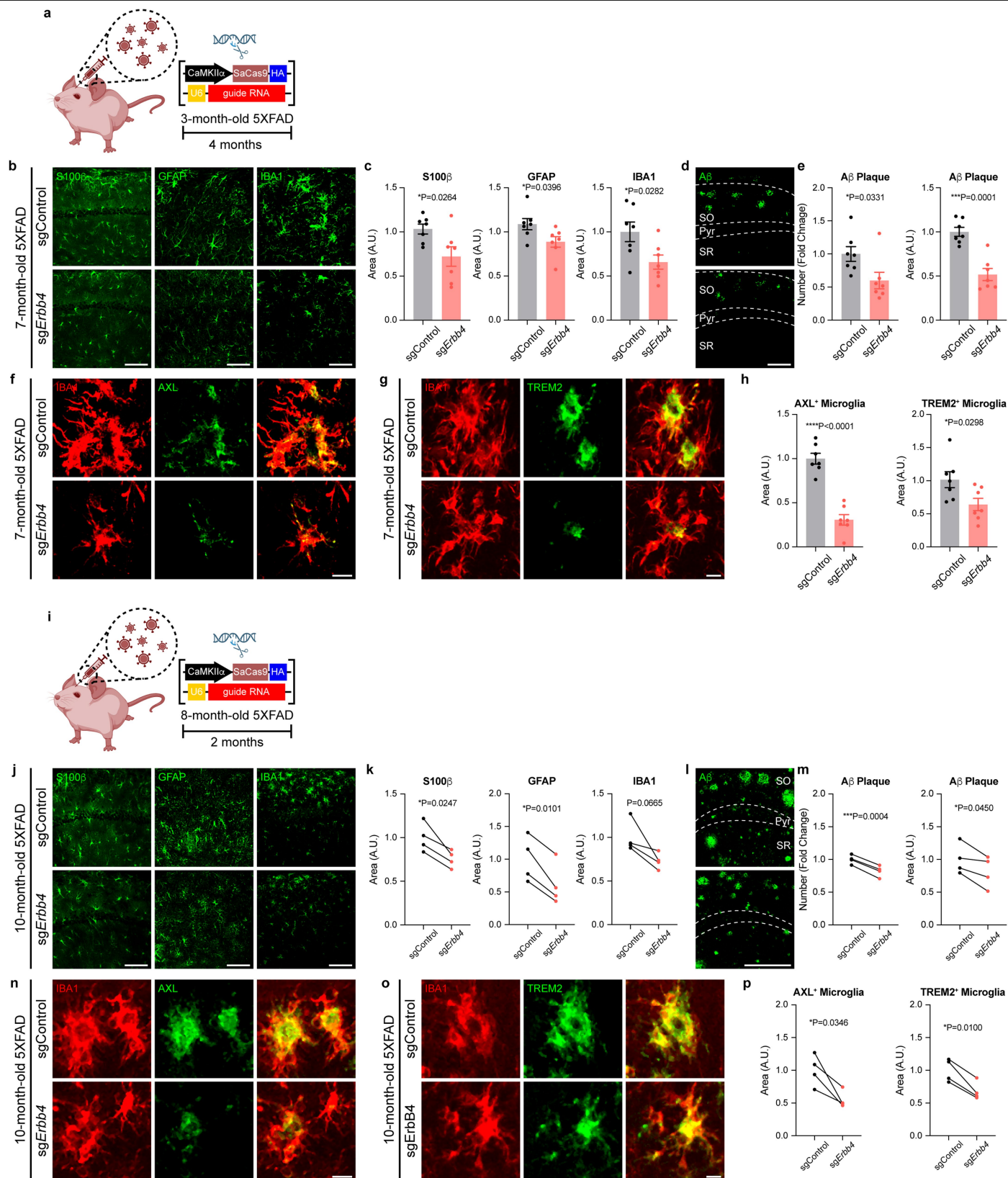


Extended Data Fig. 7 | See next page for caption.

Article

Extended Data Fig. 7 | Excitatory Neuronal Activity Is Required for Synapse Elimination but Not for Reactive Gliosis in 5xFAD Mice. **a**, Schematic illustration of the chemogenetic experiments in 7-month-old 5xFAD. This diagram was created in Biorender; Chung, W. (2026). <https://Biorender.com/jotrxdxh>. **b, c**, Representative confocal z-stack images of NeuN (**b**, red), SST (**c**, red), and c-Fos (green) in the CA1 of DPBS- and CNO-injected 5xFAD. Green dotted boxes indicate enlarged regions. **d, e**, Quantification of the ratio of c-Fos⁺ pyramidal neurons (**d**) and c-Fos⁺ SST neurons (**e**) to the number of pyramidal neurons and SST neurons, respectively. $n = 6$ mice per group. **f, h**, Representative confocal z-stack images of mCherry-alone puncta from ExPre (**f**, green) and InhiPre (**h**, green), and glial cells (red, astrocytes: S100 β ; microglia: IBA1) in the CA1 of DPBS- and CNO-injected 5xFAD. White dotted lines indicate outlines of glial cells. Scale bar, 10 μ m. **g, i**, Quantification of the engulfed excitatory (**g**) and inhibitory (**i**) pre-synapses by astrocytes (left) and microglia (right). $n = 6$ mice per group. **j**, Representative confocal z-stack images of S100 β (left), GFAP (middle), and IBA1 (right) in the CA1 of DPBS- and CNO-injected 5xFAD. Scale bar, 10 μ m. **k**, Quantification of the areas of S100 β (left), GFAP (middle), and IBA1 (right). $n = 6$ mice per group. **l**, Representative confocal z-stack images of A β

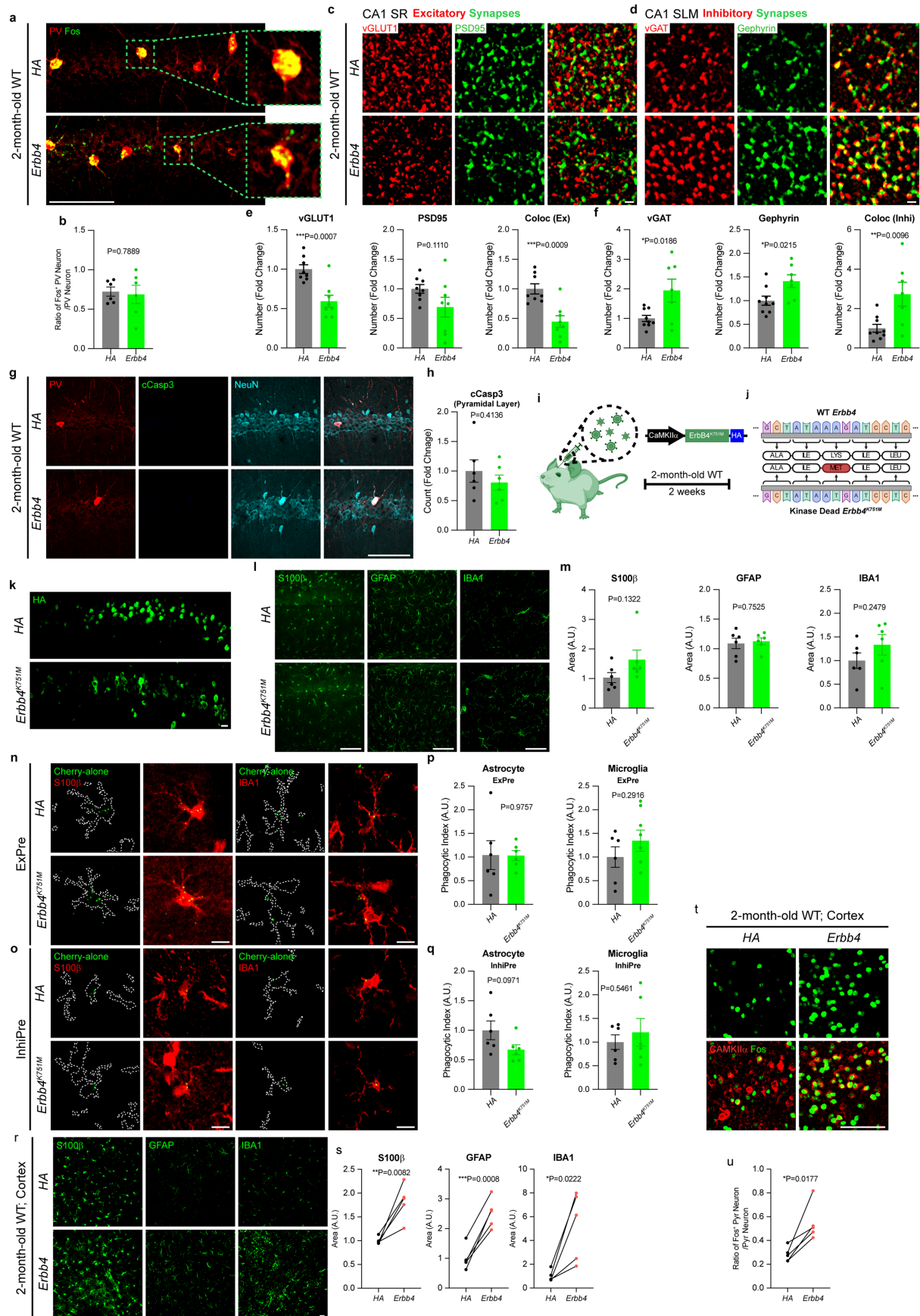
plaques in the CA1 of DPBS- and CNO-injected 5xFAD. SO, *Stratum Oriens*. Pyr, pyramidal layer. SR, *Stratum Radiatum*. Scale bar, 100 μ m. **m**, Quantification of the fold-changes in number (left) and area (right) of A β plaques in DPBS- and CNO-injected 5xFAD. $n = 6$ mice per group. **n, o, q, r**, mEPSC frequency (**n**), mEPSC amplitude (**o**), mIPSC frequency (**q**), and mIPSC amplitude (**r**) of CA1 pyramidal neurons in *HA*- and *ErbB4*-injected WT and sgControl- and sg*ErbB4*-injected 5xFAD. $n = 20, 18, 24, 23$ (**n, o**). $n = 20, 16, 24, 26$ (**q, r**). **p, s**, Representative traces of mEPSC (**p**) and mIPSC (**s**) from *HA*- and *ErbB4*-injected WT and sgControl- and sg*ErbB4*-injected 5xFAD. **t**, Input resistance of CA1 pyramidal neurons in *HA*- and *ErbB4*-injected WT and sgControl- and sg*ErbB4*-injected 5xFAD. **u, v**, Current-firing curves of CA1 pyramidal neurons in *HA*- and *ErbB4*-injected WT and sgControl- and sg*ErbB4*-injected 5xFAD. $n = 19, 17, 21, 18$ (**t, v**). Two-sided unpaired Student's t-test (**a-m**). Two-way ANOVA followed by Tukey's multiple comparisons test (**n, o, q, r, v**). * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$. Mean $\pm$ s.e.m.; n value represents the number of mice per group (**a-m**). For **d, e, g, i, k, m**, n value represents the number of mice per group. For **n, o, q, r**, n value represents the number of neurons from 4 mice per group. For **t, v**, n value represents the number of neurons from 3 mice per group.



Extended Data Fig. 8 | See next page for caption.

Extended Data Fig. 8 | Preventive and Therapeutic Effects of *ErbB4* Deletion in 5xFAD. **a, i**, Schematic illustration of AAV-injection into the CA1 of 3-month-old (**a**) and 8-month-old (**i**) 5xFAD. This diagram was created in Biorender; Chung, W. (2026). <https://biorender.com/jotr dxh>. **b, j**, Representative confocal z-stack images of S100 β (left), GFAP (middle), and IBA1 (right) in the CA1 of sgControl- and sg*ErbB4*-injected 5xFAD. Scale bar, 100 μ m. **c, k**, Quantification bar graphs (**c**) and paired dot plots (**k**) of the areas of S100 β (left), GFAP (middle), and IBA1 (right). **d, l**, Representative confocal z-stack images of A β plaques in the CA1 of sgControl- and sg*ErbB4*-injected 5xFAD. SO, *Stratum Oriens*. Pyr, pyramidal layer. SR, *Stratum Radiatum*. Scale bar, 100 μ m. **e, m**, Quantification

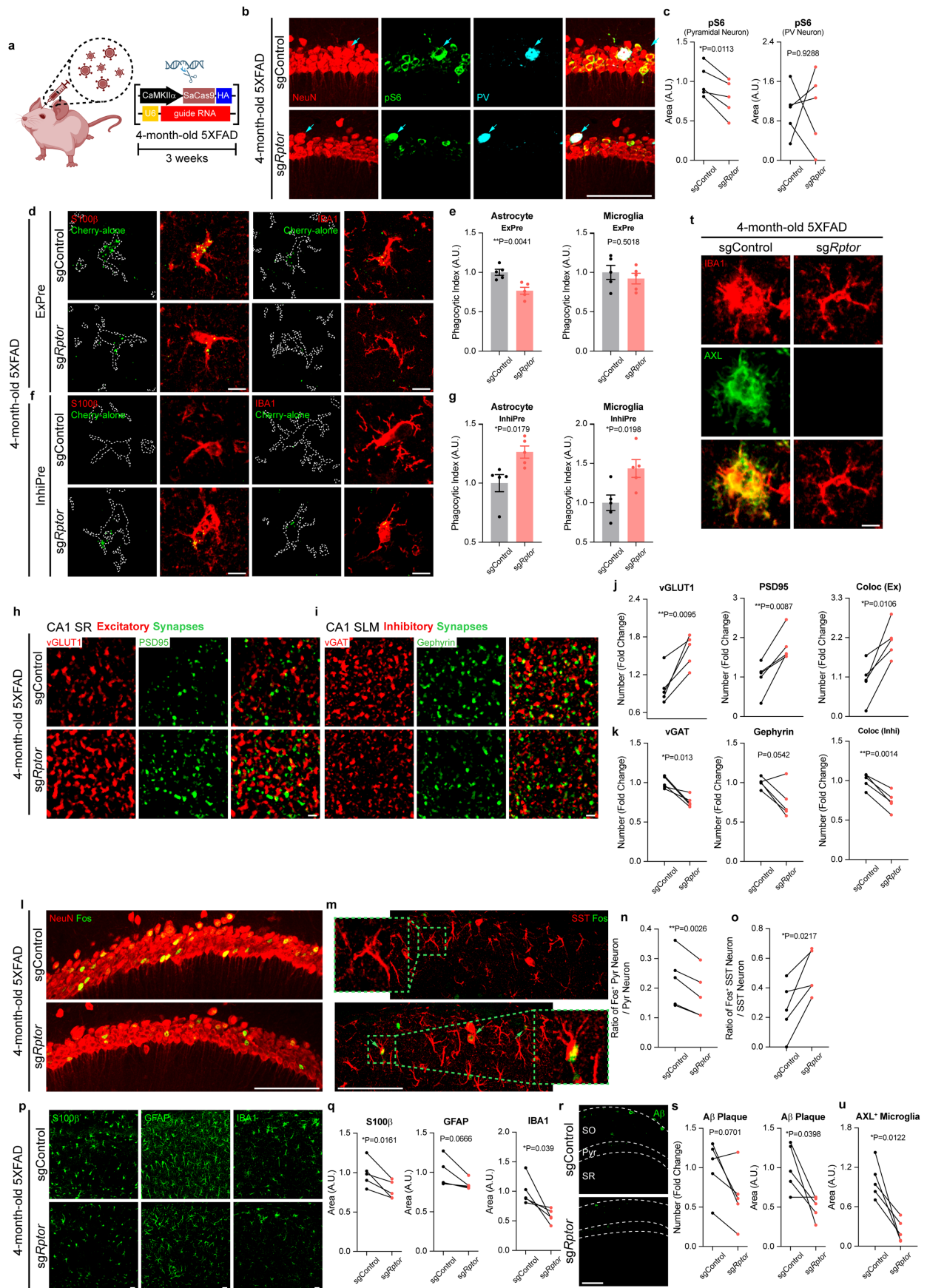
bar graphs (**e**) and paired dot plots (**m**) of the fold changes in number (left) and area (right) of A β plaques in sgControl- and sg*ErbB4*-injected 5xFAD. **f, g, n, o**, Representative confocal z-stack images of the AXL (**f, n**, green), TREM2 (**g, o**, green) and IBA1 (red) in the CA1 of sgControl- and sg*ErbB4*-injected 5xFAD. Scale bar, 10 μ m. **h, p**, Quantification bar graphs (**h**) and paired dot plots (**p**) of the area of the AXL (**h**) and TREM2 (**p**) colocalized with IBA1. $n = 7$ mice per group (**c, e, h**). $n = 4$ mice per group (**k, m, p**). Two-sided unpaired (**c, e, h**) and paired (**k, m, p**) Student's t-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. Mean $\pm$ s.e.m.; n value represents the number of mice per group.



Extended Data Fig. 9 | See next page for caption.

Extended Data Fig. 9 | Kinase Activity is Necessary for the Pathophysiological Effects of ERBB4. **a**, Representative confocal z-stack images of PV (red) and c-Fos (green) in the CA1 of *HA*- and *ErbB4*-injected 2-month-old WT. Green dotted boxes indicate enlarged regions. Scale bar, 100 μ m. **b**, Quantification of the ratio of c-Fos⁺ PV neurons to the number of PV neurons. $n = 6$ mice per group. **c, d**, Representative confocal single plane images of excitatory (**c**, pre-synapse: vGLUT1, red, post-synapse: PSD95, green) and inhibitory (**d**, pre-synapse: vGAT, red, post-synapse: Gephyrin, green) synapses in the CA1 of *HA*- and *ErbB4*-injected WT. SR, *Stratum Radiatum*. SLM, *Stratum Lacunosum Moleculare*. Scale bar, 1 μ m. **e, f**, Quantification of the excitatory (**e**) and inhibitory (**f**) synapses. $n = 8$ mice per group (**e**). $n = 9$ (*HA*), 7 (*ErbB4*) (**f**). **g**, Representative confocal z-stack images of PV (red), cleaved Caspase-3 (green), and NeuN (cyan) in the CA1 of *HA*- and *ErbB4*-injected WT. **h**, Quantification of the fold change in the number of cleaved Caspase-3⁺ pyramidal neurons. $n = 6$, 6. **i**, Schematic illustration of kinase dead *ErbB4*-injection into the CA1 of 2-month-old WT. **j**, Schematic illustration of mutagenesis for cloning of kinase dead mutant *ErbB4* from WT *ErbB4*. These diagrams were created in Biorender; Chung, W. (2026). <https://biorender.com/jotrdxh>. **k**, Representative confocal z-stack images of HA (green)

in the CA1 of *HA*- and *ErbB4*^{K751M}-injected WT. **l**, Representative confocal z-stack images of S100 β (left), GFAP (middle), and IBA1 (right) in the CA1 of *HA*- and *ErbB4*^{K751M}-injected WT. Scale bar, 100 μ m. **m**, Quantification of the areas of S100 β (left), GFAP (middle), and IBA1 (right). $n = 6$, 6. **n, o**, Representative confocal z-stack images of mCherry-alone puncta from ExPre (**n**, green) and InhiPre (**o**, green), and glial cells (red, astrocytes: S100 β ; microglia: IBA1) in the CA1 of *HA*- and *ErbB4*^{K751M}-injected WT. White dotted lines indicate outlines of glial cells. Scale bar, 10 μ m. **p, q**, Quantification of the engulfed excitatory (**p**) and inhibitory (**q**) pre-synapses by astrocytes (left) and microglia (right). $n = 6$ mice per group (**m, p, q**). **r**, Representative confocal z-stack images of S100 β (left), GFAP (middle), and IBA1 (right) in the cortex of *HA*- and *ErbB4*-injected WT. Scale bar, 100 μ m. **s**, Quantification of the areas of S100 β (left), GFAP (middle), and IBA1 (right). $n = 5$ mice per group. **t**, Representative confocal z-stack images of c-Fos (green) and CAMKII α (red) in the cortex of *HA*- and *ErbB4*-injected WT. Scale bar, 100 μ m. **u**, Quantification of the c-Fos⁺ pyramidal neurons to the number of pyramidal neurons in the cortex of *HA*- and *ErbB4*-injected WT. $n = 5$ mice per group (**s, u**). Two-sided unpaired (**a-q**) and paired Student's t-test (**s, u**). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Mean $\pm$ s.e.m.; n value represents the number of mice per group.

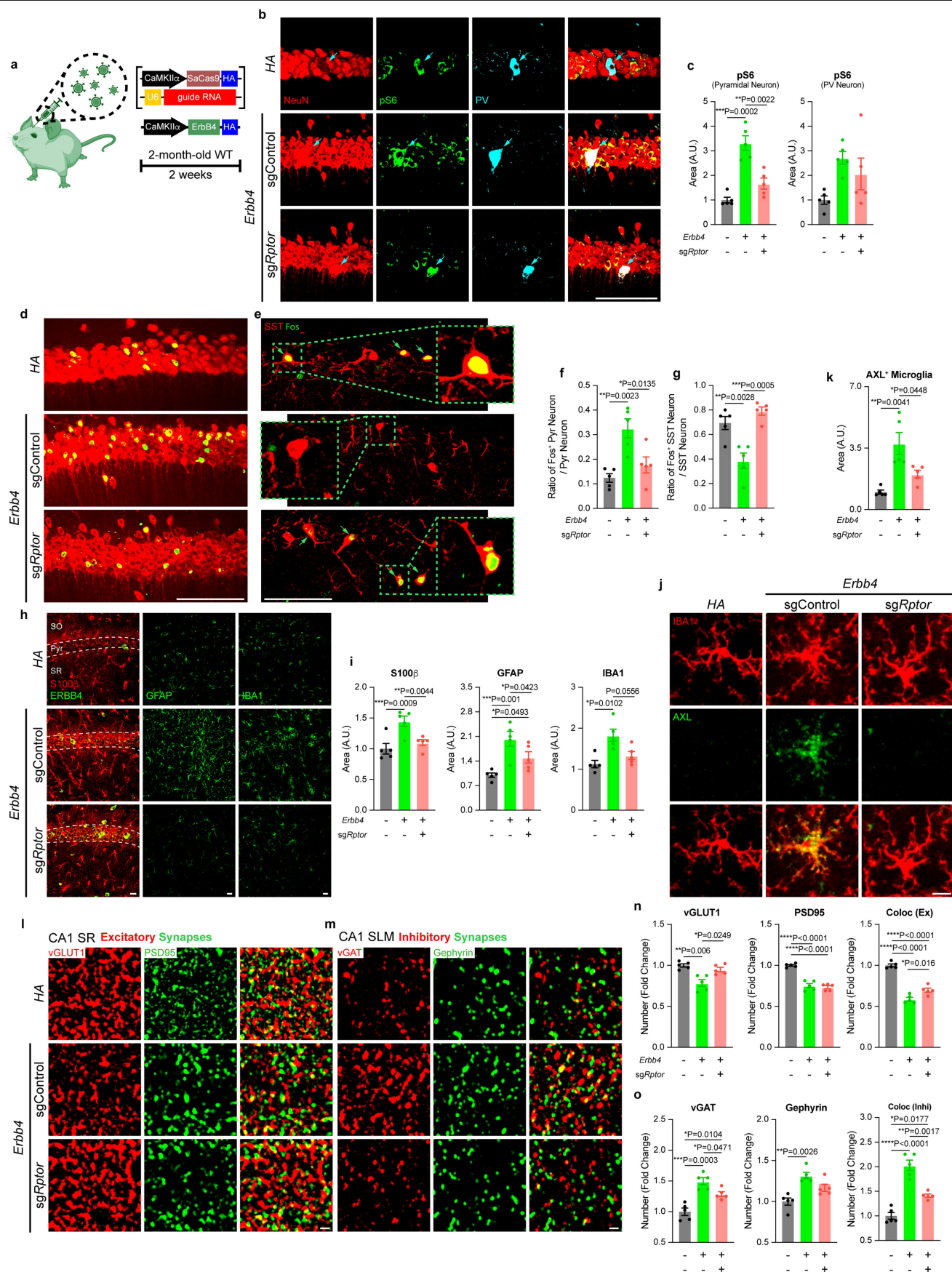


Extended Data Fig. 10 | See next page for caption.

Extended Data Fig. 10 | Reducing mTOR Signalling Rescues AD

Pathophysiology. **a**, Schematic illustration showing *Rptor*-deletion via CRISPR AAV-injection into the CA1 of 4-month-old 5xFAD. This diagram was created in Biorender; Chung, W. (2026). <https://Biorender.com/jotrxdxh>. **b**, Representative confocal z-stack images of NeuN (red), pS6 (green), and PV (cyan) in the CA1 of sgControl- and sg*Rptor*-injected 5xFAD. Cyan arrows indicate PV neurons. Scale bar, 100 μ m. **c**, Quantification of the areas of pS6 in the pyramidal (left) and PV (right) neurons. $n = 5$ mice per group. **d, f**, Representative confocal z-stack images of mCherry-alone puncta from ExPre (**d**, green) and InhiPre (**f**, green), along with glial cells (red, astrocytes: S100 β ; microglia: IBA1) in the CA1 of sgControl- and sg*Rptor*-injected 5xFAD. White dotted lines indicate outlines of glial cells. Scale bar, 10 μ m. **e, g**, Quantification of the engulfed excitatory (**e**) and inhibitory (**g**) pre-synapses by astrocytes (left) and microglia (right). $n = 5$ mice per group. **h, i**, Representative confocal single plane images of excitatory (**h**, pre-synapse: vGLUT1, red, post-synapse: PSD95, green) and inhibitory (**i**, pre-synapse: vGAT, red, post-synapse: Gephyrin, green) synapses in the CA1 of sgControl- and sg*Rptor*-injected 5xFAD. SR, *Stratum Radiatum*. SLM, *Stratum Lacunosum Moleculare*. Scale bar, 1 μ m. **j, k**, Quantification of the excitatory (**j**) and inhibitory (**k**) synapses. $n = 5$ mice per group.

l, m, Representative confocal z-stack images of c-Fos (green) with NeuN (**l**, red) or SST (**m**, red) in the CA1 of sgControl- and sg*Rptor*-injected 5xFAD. Green dotted boxes indicate enlarged regions (**m**). Scale bar, 100 μ m. **n, o**, Quantification of c-Fos⁺ pyramidal neurons (**n**) and c-Fos⁺ SST neurons (**o**) to the number of pyramidal neurons and SST neurons, respectively. $n = 5$ mice per group. **p**, Representative confocal z-stack images of S100 β (left), GFAP (middle), and IBA1 (right) in the CA1 of sgControl- and sg*Rptor*-injected 5xFAD. Scale bar, 10 μ m. **q**, Quantification of the areas of S100 β (left), GFAP (middle), and IBA1 (right). $n = 5$ mice per group. **r**, Representative confocal z-stack images of A β plaques in the CA1 of sgControl- and sg*Rptor*-injected 5xFAD. SO, *Stratum Oriens*. Pyr, pyramidal layer. SR, *Stratum Radiatum*. Scale bar, 100 μ m. **s**, Quantification of the fold changes in number (left) and area (right) of A β plaques in sgControl- and sg*Rptor*-injected 5xFAD. $n = 5$ mice per group. **t**, Representative confocal z-stack images of the AXL (green) and IBA1 (red) in the CA1 of sgControl- and sg*Rptor*-injected 5xFAD. Scale bar, 10 μ m. **u**, Quantification of the area of the AXL colocalized with IBA1. $n = 5$ mice per group. Two-sided unpaired (**e, g**) and paired (**c, j, k, n, o, q, s, u**) Student's t-test. * $P < 0.05$, ** $P < 0.01$. Mean $\pm$ s.e.m.; n value represents the number of mice per group.

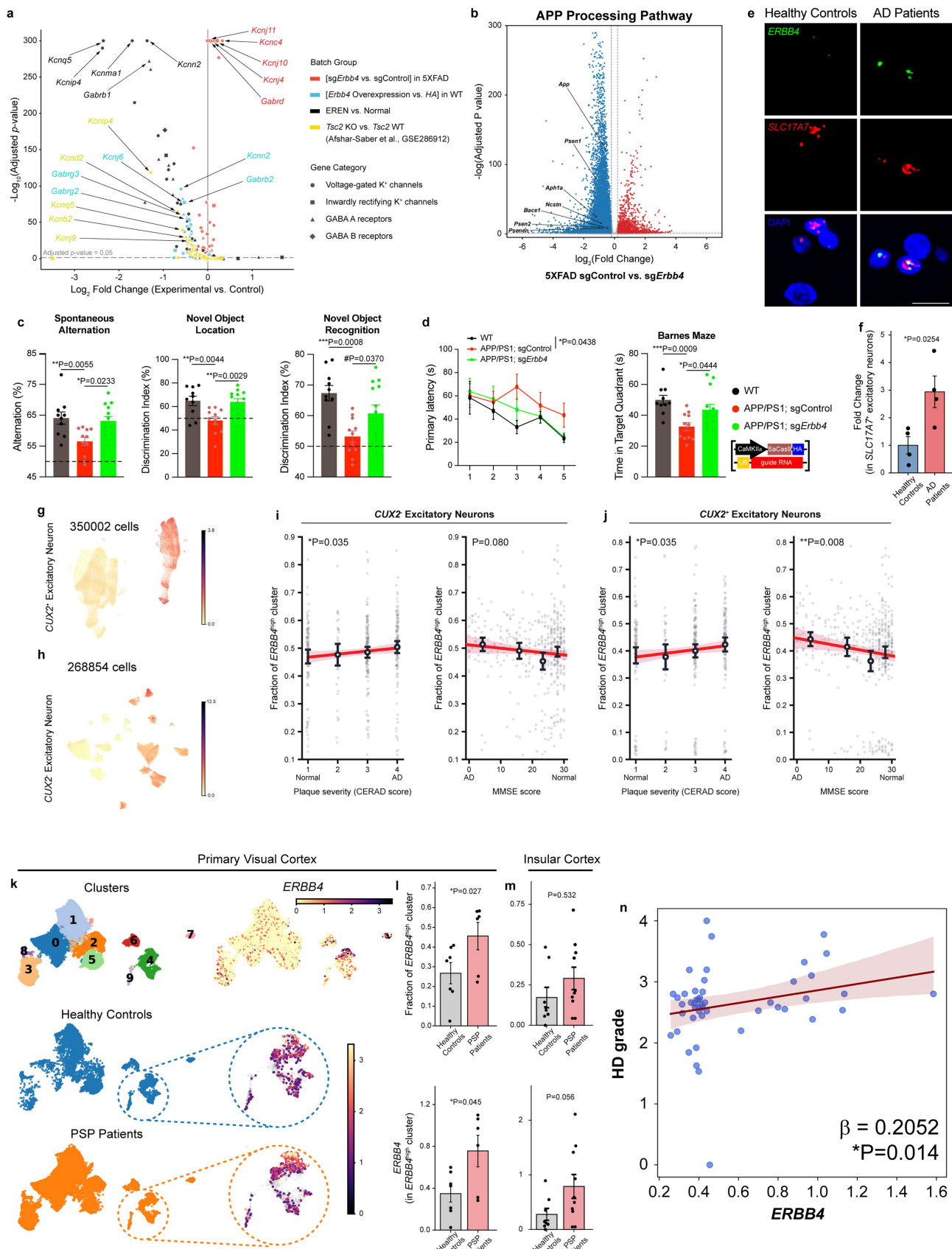


Extended Data Fig. 11 | See next page for caption.

Extended Data Fig. 11 | mTOR Signalling Mediates ERBB4-Induced AD-like

Pathophysiology. **a**, Schematic illustration showing co-injection of *sgRptor* and *ErbB4* AAV into the CA1 of 2-month-old WT. This diagram was created in Biorender; Chung, W. (2026). <https://Biorender.com/jotrdxh>. **b**, Representative confocal z-stack images of NeuN (red), pS6 (green), and PV (cyan) in the CA1 of WT mice injected with *HA* alone or co-injected with *ErbB4* and either *sgControl* or *sgRptor* AAVs. Cyan arrows indicate PV neurons. Scale bar, 100 μ m. **c**, Quantification of the areas of pS6 in the pyramidal (left) and PV (right) neurons. $n = 5$ mice per group. **d, e**, Representative confocal z-stack images of c-Fos (green) with NeuN (**d**, red) or SST (**e**, red) in the CA1 of WT mice injected with *HA* alone or co-injected with *ErbB4* and either *sgControl* or *sgRptor* AAVs. Green dotted boxes indicate enlarged regions (**e**). Scale bar, 100 μ m. **f, g**, Quantification of c-Fos⁺ pyramidal neurons (**f**) and c-Fos⁺ SST neurons (**g**) to the number of pyramidal neurons and SST neurons, respectively. $n = 5$ mice per group. **h**, Representative confocal z-stack images of S100 β (red) and ERBB4 (green) (left), GFAP (middle), and IBA1 (right) in the CA1 of WT mice injected with *HA* alone or co-injected with

ErbB4 and either *sgControl* or *sgRptor* AAVs. Scale bar, 10 μ m. **i**, Quantification of the areas of S100 β (left), GFAP (middle), and IBA1 (right). $n = 5$ mice per group. **j**, Representative confocal z-stack images of the AXL (green) and IBA1 (red) in the CA1 of WT mice injected with *HA* alone or co-injected with *ErbB4* and either *sgControl* or *sgRptor* AAVs. Scale bar, 10 μ m. **k**, Quantification of the area of the AXL colocalized with IBA1. $n = 5$ mice per group. **l, m**, Representative confocal single plane images of excitatory (**l**, pre-synapse: vGLUT1, red, post-synapse: PSD95, green) and inhibitory (**m**, pre-synapse: vGAT, red, post-synapse: Gephyrin, green) synapses in the CA1 of WT mice injected with *HA* alone or co-injected with *ErbB4* and either *sgControl* or *sgRptor* AAVs. SR, *Stratum Radiatum*. SLM, *Stratum Lacunosum Moleculare*. Scale bar, 1 μ m. **n, o**, Quantification of the excitatory (**n**) and inhibitory (**o**) synapses. $n = 5, 5, 5$. Two-way ANOVA followed by Tukey's multiple comparisons test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. Mean $\pm$ s.e.m.; n value represents the number of mice per group.



(Rexach et al., 2024, Cell)

Extended Data Fig. 12 | See next page for caption.

Article

Extended Data Fig. 12 | Relevance of Excitatory Neuronal ERBB4 in Human Patients with AD, PSP, and HD. **a**, Volcano plot showing DEGs encoding voltage-gated K⁺ channels, inwardly rectifying K⁺ channels, GABAA receptors, and GABAB receptors in excitatory neurons across *ErbB4*- and mTOR-related datasets. Colours indicate each comparison: red, *sgErbB4*- versus *sgControl*-injected 5xFAD excitatory neurons; cyan, *ErbB4*- versus *HA*-injected WT excitatory neurons; black, EREs versus normal excitatory neurons; yellow, *Tsc2* KO versus *Tsc2* WT excitatory neurons. The top five DEGs in each comparison are labelled. One-sided Wilcoxon rank-sum test. **b**, Volcano plot showing DEGs from excitatory neurons of *sgErbB4*-injected 5xFAD compared to *sgControl*-injected 5xFAD. Labelled genes are involved in the APP processing pathway. One-sided Wilcoxon rank-sum test. **c, d**, Behavioural performance measured by spontaneous alternation (**c**, left), NOL (**c**, middle), NOR (**c**, right), and Barnes maze (**d**; left: learning, right: probe) tests in *HA*-injected WT and *sgControl*- or *sgErbB4*-injected APP/PS1. *n* = 10 (WT), 11 (APP/PS1; *sgControl*), 13 (APP/PS1; *sgErbB4*). Two-sided two-way ANOVA followed by Tukey's multiple comparisons test. **e**, Representative confocal z-stack images of *ERBB4* (green), *SLC17A7* (red), and DAPI (blue) in hippocampus of healthy controls and AD patients. **f**, Bar graph of *ERBB4* expression level in *SLC17A7*⁺ excitatory neurons in the hippocampus of healthy controls and AD patients. *n* = 4 (healthy controls), 4 (AD patients). Scale bar, 10 μ m. Two-sided unpaired Student's t-test. **g, h**, UMAP plots showing *ERBB4* expression level of *CUX2*⁺ (**g**) and *CUX2*⁻ (**h**) excitatory neurons from 446 human AD patients in ROSMAP cohort (350002 and 268854 cells, respectively). **i, j**, Regression plots showing linear correlation between the

fraction of *ERBB4*^{high} clusters in *CUX2*⁺ (**i**) and *CUX2*⁻ (**j**) excitatory neurons with plaque severity (CERAD score; left) or cognitive performance (MMSE score; right). Grey dots indicate individual donors, black circles indicate the mean fraction within each CERAD score or MMSE bin. The red line indicates the fitted linear regression line, representing the predicted mean fraction, and the shaded band indicates the 95% confidence interval of the fitted mean. Pearson's correlation. *n* = 446. Red line indicates the linear regression fit, and shaded area represents standard deviation. Pearson's correlation. **k**, UMAP plots of the excitatory neurons from healthy controls and PSP patients, their cluster annotations, *ERBB4* expression. Blue and orange dotted circles indicate EREN-like population. A total of 25298 cells were analysed, derived from 7 healthy controls and 6 PSP patients. **l, m**, Bar graphs of the fraction of excitatory neurons in *ERBB4*^{high} clusters (**l, m**, top) and their *ERBB4* expression (**l, m**, bottom) in the primary visual cortex (**l**) and insular cortex (**m**) of healthy controls and PSP patients. *n* = 7 (healthy controls), 6 (PSP patients) (**l**), *n* = 8 (healthy controls), 10 (PSP patients) (**m**). Generalized estimating equation (**l, m**, top). Two-sided Student's t-test (**l, m**, bottom). **n**, Regression plot showing linear correlation between excitatory neuronal *ERBB4* expression level and HD grade. Both *ERBB4* expression level and HD grade were adjusted for confounding factors including age, the number of CAG repeat, and brain region. β indicates regression coefficient. Two-sided ordinary least squares regression. *n* = 49. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. #*P* < 0.05 by unpaired Student's t-test. Mean $\pm$ s.e.m.; *n* value represents the number of patients.

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	- ZEN software (ZEISS Image acquisition software, v2.3) - Cellranger (v8.0.1) - mkfastq (built-in function in Cellranger)
Data analysis	- Image J (NIH, v1.52g) and its plugin (Diana) - IMARIS (Bitplane, v9.2) - GraphPad Prism(v10) - Python 3.10.0 - numpy 2.0.2 - pandas 2.2.3 - matplotlib 3.9.2 - collections (built-in module in python) - scanpy 1.10.4 - scvi 1.3.0 - gseapy 1.1.8 - semopy 2.3.11 - pyWGCNA 2.2.1 - statsmodels 0.14.5 - scipy 1.15.2

- scikit-learn 1.6.1
- magic-impute 3.0.0

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All RNA sequencing raw data used in this study has been deposited into Gene Expression Omnibus (Accession number: GSE292137 / Reviewer Accession Token: adkxwkcixfwrhgl). snRNAseq data from the HD patients was retrieved from the GEO with accession GSE242197. snRNAseq data from the PSP patients was obtained from CZ CELLxGENE (<https://cellxgene.cziscience.com/collections/c53573b2-eff4-4c5e-9ad0-b24d422dfd9b>). snRNAseq data from the Tsc2 KO mice was obtained from GSE286912. snRNAseq data from excitatory neurons of ROSMAP cohort was obtained from Synapse portal with accession SYN53694054 and SYN53694068.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

For snRNA-seq analyses, publicly available human snRNAseq data were obtained from GEO and the Synapse portal. Sex was included as a covariate during SysVI batch-effect correction; gender identity information was not available. Human hippocampal FFPE sections were obtained based on tissue availability from the SNUH Brain Bank. The AD group comprised two male and two female donors, and the non-demented control group comprised three male and one female donor. Sex was not analysed as a biological variable for the FFPE cohort owing to the limited sample size.

Reporting on race, ethnicity, or other socially relevant groupings

For snRNAseq analyses, publicly available human snRNAseq data were obtained from GEO and the Synapse portal. Available race and ethnicity information was included as a covariate during SysVI batch-effect correction. For hippocampal FFPE sections, race and ethnicity information was not available for the brain-bank tissue donors and was not considered in the analyses.

Population characteristics

For snRNA-seq analyses, publicly available human snRNAseq data were obtained from GEO and the Synapse portal. Age was included as a covariate during SysVI batch-effect correction. Human hippocampal FFPE sections were obtained from four donors with AD (69-93 years; post-mortem interval, 5-13 h) and four non-demented donors (60-87 years; post-mortem interval, 5.2-9 h). AD cases showed AD neuropathological change (Thal phase 2-3, tau Braak stage 3-4 and CERAD score 2-3), whereas non-demented donors showed Thal phase 0, tau Braak stage 1-2 and CERAD score 0. These FFPE donor characteristics were not used as covariates or incorporated into the analyses.

Recruitment

Human hippocampal FFPE sections were obtained from the SNUH Brain Bank; we were not involved in donor recruitment. Publicly available human snRNA-seq data were obtained from GEO and the SYNAPSE portal.

Ethics oversight

N/A

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

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size

The anticipated effect size was based on findings from relevant previous experiments. This informed our power calculations and the selection of sample sizes that provided adequate statistical power while remaining practically feasible.

Data exclusions

For imaging analyses, potential outliers were identified using Grubbs' test in GraphPad Prism ($\alpha = 0.05$). This objective criterion was applied uniformly across groups to identify values statistically inconsistent with the remaining observations. The exclusion criterion was pre-established before statistical analysis and was not based on experimental outcome. For single nucleus RNA sequencing data analysis, to remove poor quality cells, we used filtering criteria described below.

- mitochondrial RNA fraction > 5%
 - minimal detected number of genes < 200
 - maximal detected number of genes > 8000
 Suspected doublets were also automatically removed by scrublet function in scanpy.

For ROSMAP dataset, we excluded samples from individuals with missing values in their pathological scores such as CERAD score, Braak stage, MMSE score, or age.

Replication

Non-behavioural animal experiments were independently performed in two cohorts, with cohort sizes of at least $n = 3$ and $n = 2$ mice per group, respectively. Behavioural experiments were independently performed in three cohorts, each comprising approximately 3-4 mice per group. The reported findings were reproducible across cohorts, and all planned replication attempts were successful.

Randomization

Age-matched male and female mice were randomly assigned to experimental and control groups for all in vivo experiments. As no sex-dependent differences in synapse phagocytosis were detected (Extended Data Fig. 2a-d), data from male and female mice were pooled for subsequent analyses. Brain sections for imaging were selected at random. Human FFPE tissues were obtained from the brain bank based on availability, and publicly available human snRNAseq datasets were analysed observationally; thus, no experimental allocation of human samples was applicable.

Blinding

Investigators were blinded to group allocation during in vivo data collection and analysis, including behavioural testing and imaging acquisition and quantification. Blinding was not applicable to analyses of publicly available snRNAseq datasets because the data and metadata were obtained from public repositories. Relevant metadata were included as covariates during SysVI batch-effect correction but did not determine any experimental allocation.

Reporting for specific materials, systems and methods

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

Materials & experimental systems

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

Methods

- | | |
|-------------------------------------|---|
| n/a | Involved in the study |
| ☒ | ☐ ChIP-seq |
| ☒ | ☐ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

Antibodies

Antibodies used

anti-S100b (Abcam ab52642, Synaptic System 287-004, Aves Labs S100B-0020) (1:500), anti-IBA1 (Wako 019-19741, Novus NB100-1028) (1:500), anti-mCherry (Invitrogen M11217, Aves Labs mCherry-0020) (1:1000), anti-vGLUT1 (Millipore AB5905) (1:1000), anti-PSD95 (Invitrogen 51-6900) (1:500), anti-vGAT (Synaptic System 131-004) (1:1000), anti-Gephyrin (Synaptic System 147-008) (1:500), anti-Fos (Cell Signaling 2250S, Synaptic System 226-308, Synaptic System 226-017) (1:500), anti-MEGF10 (Merck ABC10) (1:500), anti-ERBB4 (Abcam ab32375, Cell Signaling 4795T) (1:500), anti-Parvalbumin (Swant GP72) (1:1000), anti-beta amyloid (Biolegend 803001, Cell Signaling 2454S) (1:1000), anti-GFAP (Abcam ab4674) (1:1000), anti-AXL (R&D systems AF854) (1:100), anti-Somatostatin (BMA Biomedicals T-4103) (1:500), anti-VIP (Synaptic System 443-005) (1:500), anti-NeuN (Sigma Aldrich ABN91) (1:500), anti-cleaved Caspase3 (Cell Signaling 9661S) (1:500), anti-TREM2 (R&D systems AF1729) (1:500), anti-HA (Cell Signaling 3724S) (1:500), anti-pS6 (Cell Signaling 2211S) (1:500).
 Secondary antibodies: Donkey anti-Goat IgG (H&L) Alexa Fluor 405 (Abcam, ab175665). Donkey anti-Goat IgG (H&L) Alexa Fluor 488 (Jackson Laboratory, 705-545-003). Donkey anti-Chicken DyLight 405-conjugated AffiniPure. Donkey Anti-Chicken IgY (IgG) (H&L) (Jackson Laboratory, 703-475-155). Donkey anti-Chicken IgG (H&L) Alexa Fluor 488 (Jackson Laboratory, 703-545-155). Donkey anti-Chicken IgG (H&L) Alexa Fluor 594 (Jackson Laboratory, 709-585-155). Donkey anti-rat IgG (H&L) Alexa Fluor 594 (Invitrogen, A-21209). Donkey anti-rat IgG (H&L) Alexa Fluor 647 (Abcam, ab150155). Donkey anti-rabbit IgG (H&L) Alexa Fluor 405 (Abcam, ab175649). Donkey anti-rabbit IgG (H&L) Alexa Fluor 488 (Invitrogen, A-21206). Donkey anti-rabbit IgG (H&L) Alexa Fluor 594 (Invitrogen, A-21207). Donkey anti-guinea pig IgG (H&L) Alexa Fluor 488 (Jackson Laboratory, 706-545-148). Donkey anti-guinea pig IgG (H&L) Alexa Fluor 594 (Jackson Laboratory, 706-585-148). Donkey anti-guinea pig IgG (H&L) Alexa Fluor 647 (Jackson Laboratory, 706-605-148). Donkey Anti-Sheep IgG H&L (Alexa Fluor® 488) (Abcam, ab150177). Donkey anti-Mouse IgG (H&L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594 (Invitrogen, A-21203). All Alexa Fluor 488-conjugated secondary antibodies were used at a dilution of 1:1,000, whereas all other secondary antibodies were used at 1:500.

Validation

anti-S100b (Abcam ab52642): supported by several references including PubMed: 38082296
 anti-S100b (Synaptic System 287-004): verified through ICC/IHC experiment by manufacturer
 anti-S100b (Aves Labs S100B-0020): verified through ICC/IHC experiment by manufacturer
 anti-IBA1 (Wako 019-19741): supported by several references including PubMed: 33361813
 anti-IBA1 (Novus NB100-1028): supported by several references including PubMed: 33361813
 anti-mCherry (Invitrogen M11217): verified through ICC/IHC experiment by manufacturer

anti-mCherry (Aves Labs mCherry-0020): verified through ICC/IHC experiment by manufacturer
 anti-vGLUT1 (Millipore AB5905): supported by several references including PubMed: 33361813
 anti-PSD95 (Invitrogen 51-6900): supported by several references including PubMed: 33361813
 anti-vGAT (Synaptic System 131-004): supported by several references including PubMed: 33361813
 anti-Gephyrin (Synaptic System 147-008): supported by several references including PubMed: 33361813
 anti-Fos (Cell Signaling 2250S): verified through ICC/IHC experiment by manufacturer
 anti-Fos (Synaptic System 226-308): verified through ICC/IHC experiment by manufacturer
 anti-Fos (Synaptic System 226-017): verified through ICC/IHC experiment by manufacturer
 anti-Megf10 (Merck ABC10): verified through ICC/IHC experiment by manufacturer
 anti-ErbB4 (Abcam ab32375): supported by several references including PubMed: 28230865
 anti-ErbB4 (Cell Signaling 4795T): verified through ICC/IHC experiment by manufacturer
 anti-Parvalbumin (Swant GP72): supported by several references including PubMed: 36199089
 anti-beta amyloid (Biolegend 803001): verified through ICC/IHC experiment by manufacturer
 anti-beta amyloid (Cell Signaling 2454S): verified through ICC/IHC experiment by manufacturer
 anti-GFAP (Abcam ab4674): supported by several references including PubMed: 33361813
 anti-Axl (R&D systems AF854): verified through ICC/IHC experiment by manufacturer
 anti-Somatostatin (BMA Biomedicals T-4103): verified through ICC/IHC experiment by manufacturer
 anti-VIP (Synaptic System 443-005): verified through ICC/IHC experiment by manufacturer
 anti-NeuN (Sigma Aldrich ABN91): verified through ICC/IHC experiment by manufacturer
 anti-cleaved Caspase3 (Cell Signaling 9661S): verified through ICC/IHC experiment by manufacturer
 anti-TREM2 (R&D systems AF1729): verified through ICC/IHC experiment by manufacturer
 anti-HA (Cell Signaling 3724S): verified through ICC/IHC experiment by manufacturer
 anti-pS6 (Cell Signaling 2211S): verified through ICC/IHC experiment by manufacturer

Donkey anti-Goat IgG (H&L) Alexa Fluor 405 (Abcam, ab175665)
 Donkey anti-Goat IgG (H+L) Alexa Fluor 488 (Jackson Laboratory, 705-545-003)
 Donkey anti-Chicken DyLight 405-conjugated AffiniPure Donkey Anti-Chicken IgY (IgG) (H+L) (Jackson Laboratory, 703-475-155)
 Donkey anti-Chicken IgG (H+L) Alexa Fluor 488 (Jackson Laboratory, 703-545-155)
 Donkey anti-Chicken IgG (H+L) Alexa Fluor 594 (Jackson Laboratory, 709-585-155)
 Donkey anti-rat IgG (H+L) Alexa Fluor 594 (Invitrogen, A-21209)
 Donkey anti-rat IgG (H&L) Alexa Fluor 647 (Abcam, ab150155)
 Donkey anti-rabbit IgG (H&L) Alexa Fluor 405 (Abcam, ab175649)
 Donkey anti-rabbit IgG (H+L) Alexa Fluor 488 (Invitrogen, A-21206)
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 Donkey anti-guinea pig IgG (H+L) Alexa Fluor 647 (Jackson Laboratory, 706-605-148)
 Donkey Anti-Sheep IgG H&L (Alexa Fluor® 488) (Abcam, ab150177)
 Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594 (Invitrogen, A-21203)

All stated secondary antibodies were verified through IHC experiment by manufacturer.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	HEK293T cells were originally obtained from ATCC
Authentication	HEK293T cells have been maintained in our laboratory for an extended period and were not independently authenticated for this study.
Mycoplasma contamination	HEK293T cell line was confirmed for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell line was used in this study.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	All animal experiments used <i>Mus musculus</i> : C57BL/6J wild-type mice and C57BL/6J-background APP/PS1 and 5XFAD transgenic mice. APP/PS1 and 5XFAD mice were maintained by breeding with C57BL/6J mice. Both male and female mice were used; as no sex-dependent differences in synapse phagocytosis were detected (Extended Data Fig. 2a-d), data from both sexes were pooled for subsequent analyses. APP/PS1 mice aged 3-18 months, 5XFAD mice aged 2-10 months, and age-matched C57BL/6J wild-type controls were used as indicated for each experiment. For snRNAseq, 2-month-old 5XFAD and 3-month-old 5XFAD and wild-type mice were used. Mice were housed under standard conditions on a 12-h light-dark cycle at 22°C and 30% relative humidity, with food and water provided ad libitum.
Wild animals	No wild animals were used in this study.
Reporting on sex	In Extended Data Fig. 2a-d, we confirmed sex difference does not affect phenotype of interest. Therefore, we used both male and female randomly for other experiments.

Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	All mouse experiments were performed according to articles approved by Institutional Animal Care and Use Committees (IACUC) protocols from Korea Advanced Institute of Science and Technology (KAIST).

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A