



A distinct astrocyte subtype in the aging mouse brain characterized by impaired protein homeostasis

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The aging brain exhibits a region-specific reduction in synapse number and plasticity. Although astrocytes play central roles in regulating synapses, it is unclear how changes in astrocytes contribute to age-dependent cognitive decline and vulnerability to neurodegenerative diseases. Here, we identified a unique astrocyte subtype that exhibits dysregulated autophagy and morphology in aging hippocampus. In these autophagy-dysregulated astrocytes (APDAs), autophagosomes abnormally accumulate in swollen processes, impairing protein trafficking and secretion. We found that reduced mammalian target of rapamycin (mTOR) and proteasome activities with lysosomal dysfunction generate APDAs in an age-dependent manner. Secretion of synaptogenic molecules and astrocytic synapse elimination were significantly impaired in APDAs, suggesting that APDAs have lost their ability to control synapse number and homeostasis. Indeed, excitatory synapses and dendritic spines associated with APDAs were significantly reduced. Finally, we found that mouse brains with Alzheimer's disease showed a significantly accelerated increase in APDAs, suggesting potential roles for APDAs in age- and Alzheimer's disease-related cognitive decline and synaptic pathology.

Astrocytes—the main glial cell population in the brain—are key players in maintaining synaptic homeostasis by regulating synapse function and number¹. Astrocytic fine processes closely contact individual synapses and respond to local synaptic transmission, regulating neurotransmitter and ion concentrations and directly modulating synaptic activity². Moreover, astrocytes mediate synapse turnover and plasticity by inducing synapse formation through secreting synaptogenic molecules as well as eliminating unnecessary synapses through phagocytic engulfment^{3–8}. Due to these important roles of astrocytes, it has been suggested that dysregulated astrocytic functions may be critical in the development of synaptic abnormalities associated with aging and neurodegenerative diseases, such as Alzheimer's disease, where astrocytes undergo reactive status with dramatic gene expression changes⁹.

Several studies have reported age-related gene expression changes and revealed highly heterogeneous astrocytic responses across different brain regions^{10,11}. Astrocytes in the aged hippocampus showed greater upregulation of reactive genes compared with astrocytes in other brain regions¹¹. In fact, the aged hippocampus shows impaired synapse turnover and plasticity with defective learning and memory formation¹². In Alzheimer's disease, it has also been shown that the hippocampus is one of the earliest brain regions to be affected during disease progression¹³. However, it is still unclear whether these age-related mRNA changes in astrocytes contribute directly to the selective vulnerability of the hippocampus during aging and Alzheimer's disease progression. Moreover, since most attention has been directed to reactive astrocytes and proinflammatory responses in the aging brain^{10,11,14}, whether additional astrocyte subtypes undergo progressive functional impairment during aging is unknown, and, for

any astrocyte subtypes identified, the cause of their appearance in specific brain regions is unclear.

In this study, by performing single-cell RNA sequencing (scRNA-seq) with 2-year-old mouse hippocampi, we identified a unique astrocyte subtype that significantly accumulates autophagosomes inside swollen processes. This APDA population was found specifically in the hippocampus, and increased gradually during aging, with defective protein trafficking and degradation. Remarkably, applying mTOR or proteasome inhibitors to 9-month-old wild-type (WT) mice was sufficient to induce the appearance of APDAs specifically in the hippocampus without affecting other cell types. In agreement with this finding, we discovered that critical components in protein homeostasis, mTOR, proteasome and lysosomal activities, were all significantly reduced in aged hippocampal astrocytes, providing the mechanisms of the dramatic autophagosome accumulation observed in APDAs. Importantly, astrocyte-derived molecules that can regulate synaptogenesis were not properly secreted from APDAs. Furthermore, we found that MEGF10, an astrocytic membrane receptor that plays essential roles in synapse elimination, was trapped inside these accumulated autophagosomes, decreasing their ability to eliminate synapses. The number of excitatory synapses and dendritic spines was reduced selectively when they were closely associated with APDAs, suggesting that APDAs fail to maintain synapse number and plasticity in the aged hippocampi. Finally, in APP/PS1 mice, the appearance of APDAs was significantly accelerated compared with that in normal aging, suggesting that amyloid- β functions as a disease-related inducer of APDAs in the hippocampus. Taken together, our results show that astrocytes with accumulated autophagosomes lose their normal ability to regulate synaptic homeostasis, contributing to aging- and Alzheimer's disease-related synaptic pathology.

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Results

scRNA-seq with aged hippocampi. To identify new characteristics of astrocytes in aged mouse brains, we carried out scRNA-seq analysis of hippocampi obtained from 2-year-old mice (Extended Data Fig. 1a). Hippocampal cells were partitioned into 16 clusters, and the identification of these cell clusters was validated by the expression of cell type-specific genes (Fig. 1a). Single-cell populations with astrocyte-specific gene expression were segregated into three different clusters: 2, 3 and 6. Then, we determined the differentially expressed genes (DEGs) among these astrocyte subtypes as shown in dot, violin and volcano plots (Fig. 1b–e).

In aged brain, it has been shown that a portion of astrocytes exhibits reactive phenotypes and upregulates proinflammatory genes^{10,11}. In agreement with these previous findings, we found that cluster 3 astrocytes in our analysis showed the most significant increase in reactive (Pan: general, A1: LPS-induced and A2: ischemia-induced reactive astrocyte status) and inflammatory genes compared with cluster 2 and 6 astrocytes (Fig. 1c,d)^{15,16}. Cluster 2 astrocytes showed the lowest level of those reactive genes, suggesting they represent nonreactive resting astrocyte population. However, we noticed that cluster 6 astrocytes showed a significantly upregulated mRNA expression for general astrocytic property and synapse related genes (Fig. 1b). These genes include astrocytic markers (*Aqp4*, *Aldh1l1*, *Aldoc* and *ApoE*), homeostasis genes (*Slc1a2*: EAAT2 and *Slc1a3*: EAAT1), and receptors and channels (*Gja1*: Connexin 43, *Gjb6*: Connexin 30, *Grm3*: Glutamate metabotropic receptor 3 and *Gabbr1*: GABA B receptor subunit 1) (Fig. 1b). Interestingly, genes involved in synaptogenesis such as *Thbs2* and *Thbs4* and *Hevin* (*Sparcl1*), as well as synapse elimination (*Tgfb2*, *Megf10* and *Mfge8*) were also upregulated in cluster 6 astrocytes (Fig. 1b). These specific upregulations of mRNAs related to basal astrocytic functions prompted us to hypothesize that cluster 6 astrocytes may play critical roles in regulating synapse homeostasis in aged brains. To determine whether the cluster 6 population is specific to the aged hippocampus, we compared our scRNA-seq data from 2-year-old hippocampus with the published scRNA-seq data obtained from 2-month-old hippocampus (Extended Data Fig. 1b,c)¹⁷. This comparison revealed that the characteristic gene expression changes in the cluster 6 astrocytes is unique to the aged hippocampus, since we could not identify a specific astrocyte cluster in 2-month-old hippocampus, which resembles cluster 6 astrocyte-like gene expression profiles.

Next, we decided to validate the presence and location of cluster 6 astrocytes in the aged hippocampus by performing immunohistochemistry with antibodies against the upregulated candidate molecules in cluster 6 astrocytes (Fig. 1e).

During these experiments, we found that subsets of hippocampal astrocytes in 18-month-old WT brains indeed showed significantly increased expression and protein accumulation of several cluster 6 astrocyte-related molecules, such as Cx43, APOE, HIF1 α , MEGF10, SCRG1, CLDN10 and GSTM1 (Extended Data Fig. 1f,g,i,j–m). These astrocytes were morphologically distinct from normal-looking astrocytes and contained numerous vacuoles with high

colocalization of those proteins (Fig. 1f–k). Among the accumulated proteins present in the vacuoles, we further utilized SCRG1, GSTM1 and CLDN10 as markers for the cluster 6 astrocytes, and found that their protein expression levels showed a gradual increase during aging (Extended Data Fig. 1d,e).

Due to their swollen processes with vacuoles, the vacuolated astrocytes showed a reduced density of fine astrocytic processes (Fig. 1l and Extended Data Fig. 2e,f). When we used SCRG1 as a marker to detect vacuolated astrocytes in various brain regions from 18-month-old mice, we found that only hippocampal astrocytes in the stratum radiatum (SR) and stratum lacunosum moleculare (SLM) regions showed this distinct phenotype (Extended Data Fig. 2a,c). Moreover, even in the hippocampal SR and SLM regions, other cell types, such as microglia, neurons and oligodendrocyte precursor cells, did not show SCRG1 upregulation or vacuole formation (Extended Data Fig. 2d). As they gradual increase in number during aging, SCRG1-positive vacuolated astrocytes account for around 20% of the total astrocytes in the SR regions of 18-month-old hippocampi (Extended Data Fig. 2b). Thus, our findings suggest that, in the aged hippocampus, a subset of astrocytes exhibited selectively upregulated cluster 6 genes, as well as distinct morphological changes and vacuole formation, although the expression of reactive and inflammatory genes was not increased significantly (Fig. 1c,d and Extended Data Fig. 1h).

Protein trafficking defects with autophagosome accumulation. Since proteome homeostasis is altered during the aging process^{18–20}, we hypothesized that our unique astrocytes with cluster 6 marker accumulation in their vacuoles may exhibit defects in protein trafficking and degradation. To test our hypothesis, we first examined endolysosomal proteins through immunostaining. Although the total levels of Rab5 (early endosome-related protein) and Lamp2 (lysosome-associated membrane protein) did not differ, we found that a significant portion of Rab5 and Lamp2 proteins were recruited to the vacuoles in the swollen processes of APDAs (Fig. 2a,b,d–g). In addition, ubiquitin-conjugated proteins, which are generated from polyubiquitination during proteolysis²¹, were highly concentrated in astrocytic vacuoles, similar to Rab5 and Lamp2 (Fig. 2c,h,i).

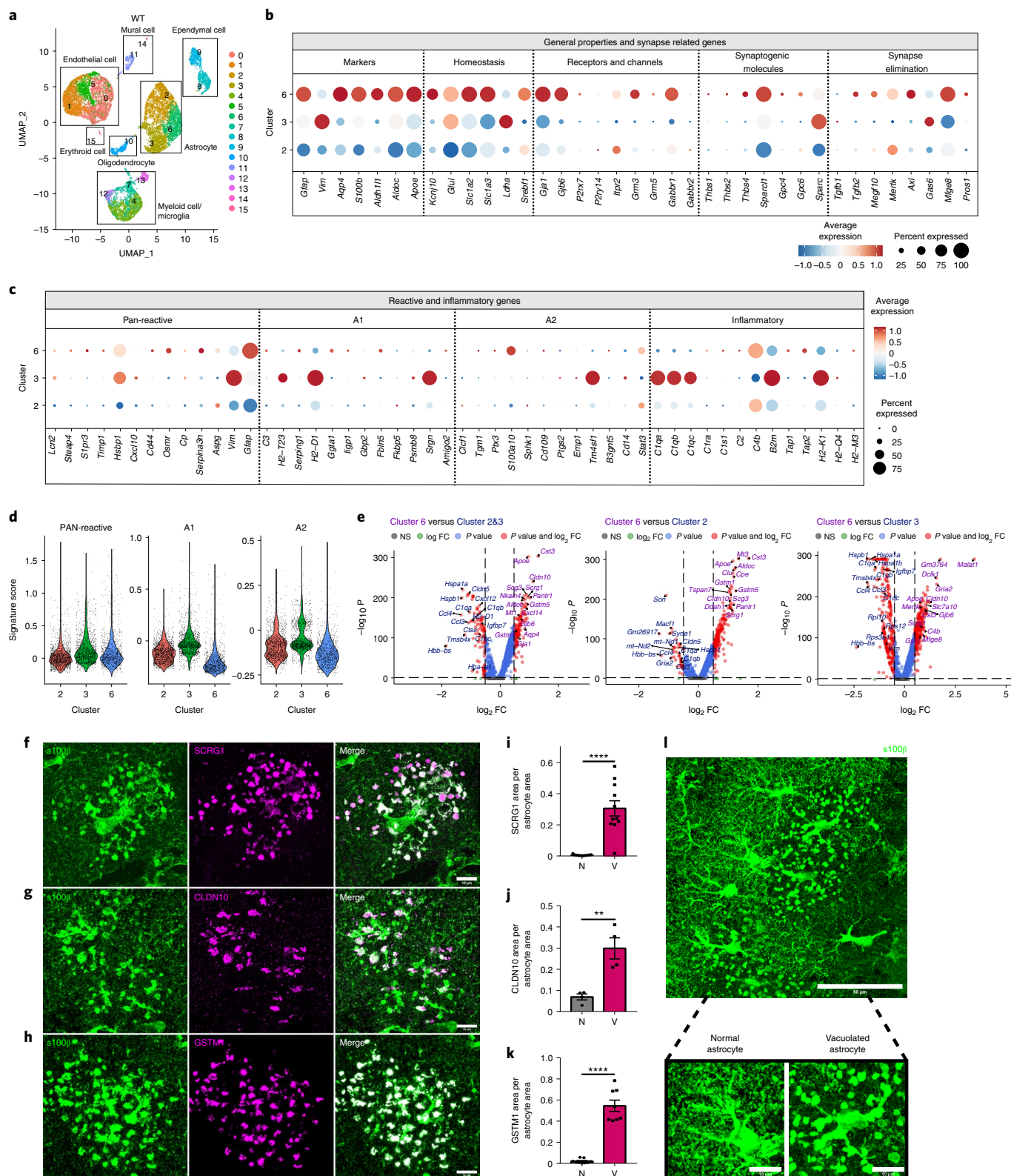
When we examined the localization of several membrane receptors in astrocytes, we found that some of the receptors were profoundly mislocalized. In vacuolated astrocytes, MEGF10, a phagocytic receptor that plays critical roles in eliminating excitatory synapses during developmental⁷ and adult stages²², accumulated notably in vacuoles of the swollen processes of APDAs and did not localize properly to the astrocytic membrane (Fig. 2j). Low-density lipoprotein receptor (LDLR), a membrane receptor of apolipoprotein E (ApoE)²³ in the brain, also accumulated notably inside astrocytic vacuoles (Fig. 2k). In contrast, EAAT2, a glutamate transporter expressed predominantly in glial cell membranes²⁴, appeared to maintain a membrane localization in vacuolated astrocytes (Fig. 2l).

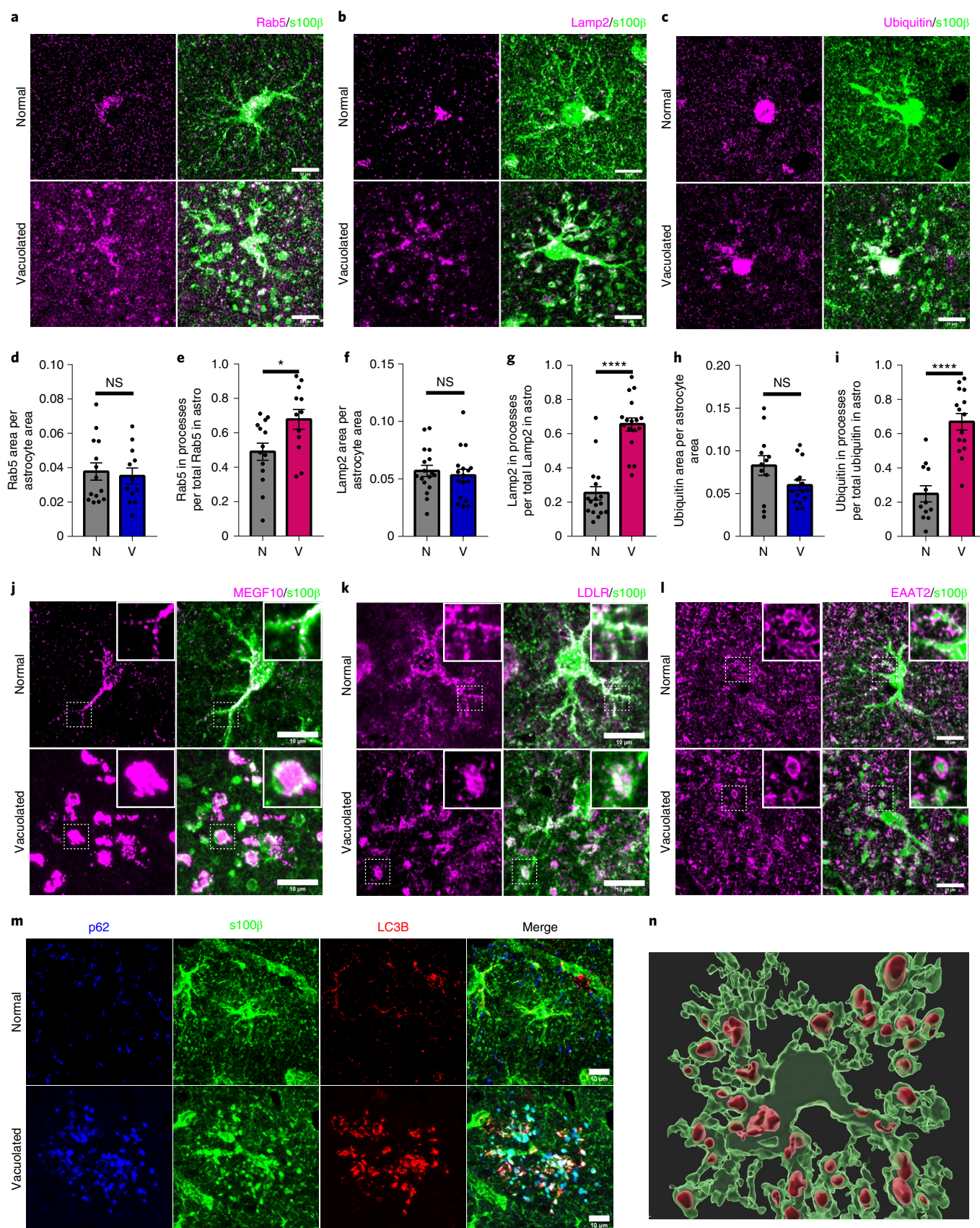
What then are those vacuoles that appear in the subset of aged hippocampal astrocytes? Interestingly, we found that the vacuoles contained autophagic proteins, such as LC3B and p62, showing that

Fig. 1 | scRNA-seq reveals a unique subtype of astrocytes in the aged hippocampus. **a**, A UMAP plot of 14,046 single cells from the hippocampi of 2-year-old mice. Cells were separated into the clusters. **b**, Gene expression related to general characteristics of astrocytes and synaptic regulation. **c**, Expression of reactive and inflammatory genes in astrocytes. **d**, Violin plots showing the scores for signature genes of reactive astrocytes. **e**, Volcano plots comparing DEGs among cluster 6 versus cluster 2 and/or 3. FC, fold change. **f–h**, Representative confocal z-stack images of astrocytes (s100 β , green) obtained from 18-month-old hippocampi exhibiting swollen processes with vacuoles colocalized with SCRG1 (**f**), CLDN10 (**g**) and GSTM1 (**h**) (red). Scale bar, 10 μ m. **i–k**, Quantification of SCRG1, CLDN10 and GSTM1 areas normalized by astrocyte area in the imaging fields. SCRG1 (**i**), CLDN10 (**j**) and GSTM1 (**k**) were highly upregulated in vacuolated astrocytes (V) compared with normal (N) astrocytes ($n=3$ mice, dots on the bar graphs represent data obtained from individual cells). **l**, Representative confocal z-stack images of the hippocampal SR region containing both normal and vacuolated astrocytes (s100 β , green). Vacuolated astrocytes showed a reduced density of astrocytic fine processes (right image in lower panel). Scale bars, 50 μ m (upper panel) and 10 μ m (lower panel). **i–k**, Two-tailed Student's *t*-test. **** $P < 0.0001$ (**i**), ** $P = 0.0044$ (**j**), **** $P < 0.0001$ (**k**); mean $\pm$ s.e.m.

they were formed by accumulated autophagosomes (Fig. 2m). These autophagosome-accumulating astrocytes were neither apoptotic nor necrotic, as indicated by a lack of caspase 3 activation, TUNEL or HMGB1 expression (Extended Data Fig. 3a–c). Moreover, through three-dimensional (3D) reconstruction of these astrocytes, we found that each autophagosome-filled vacuolar structure was

connected by thin astrocytic processes (Fig. 2n), showing that the astrocytic membranes were not fragmented or ruptured. Together, our data show that we have identified a unique astrocyte subtype in the aging hippocampus that dramatically accumulates autophagosomes with distinct morphological changes. Therefore, we decided to name these unique astrocytes APDAs.





APDA induction by inhibiting mTOR and proteasome activity. Next, to understand the mechanisms by which APDAs are generated in aging brains, we investigated cell signaling pathways that modulate autophagosome formation and clearance. First, we

attempted to suppress mTOR signaling by injecting 9-month-old *ALDH1L1-eGFP* mice with rapamycin—a well-known inhibitor of the mTOR pathway. In addition to a group receiving rapamycin injection, another group received both rapamycin and chloroquine

Fig. 2 | Vacuolated astrocytes show protein trafficking defects with autophagosome accumulation. **a–c**, Representative confocal z-stack images of Rab5 (**a**), Lamp2 (**b**) and ubiquitin (**c**) (red) in normal and vacuolated astrocytes (s100 β , green) obtained from 18-month-old hippocampi. **d–i**, Quantification of Rab5 (**d,e**), Lamp2 (**f,g**) and ubiquitin (**h,i**) areas normalized by astrocyte area in the imaging fields. The total amounts of Rab5, Lamp2 and ubiquitin were comparable between normal and vacuolated astrocytes (**d,f,h**), and these proteins were highly localized inside vacuole structures (**e,g,i**) ($n=3$ mice, dots on the bar graphs represent data obtained from individual cells). **j–l**, Representative confocal z-stack images of MEGF10 (**j**), LDLR (**k**) and EAAT2 (**l**) (red) with astrocytes (s100 β , green). MEGF10 (**j**) and LDLR (**k**) accumulate in the swollen processes of vacuolated astrocytes (lower panels) instead of localizing properly to the normal astrocytic membrane (upper panels). EAAT2 (**l**) appears to maintain its membrane localization in normal and vacuolated astrocytes. Insets, magnified part of normal (upper) and vacuolated (lower) astrocytes processes. **m**, Representative confocal z-stack images of autophagosome markers p62 (blue) and LC3B (red) with astrocytes (s100 β , green). Compared with normal astrocytes (upper panels), vacuolated astrocytes (lower panels) are filled with p62 (blue)- and LC3B (red)-positive autophagosomes in their swollen processes. Scale bar, 10 μ m. **n**, Representative 3D images of vacuolated astrocytes (green) containing p62 (red)-positive autophagosomes. **d–i**, Means $\pm$ s.e.m., two-tailed Student's t -test. $P=0.71$ (**d**), $*P=0.02$ (**e**), $P=0.59$ (**f**), $****P<0.0001$ (**g**), $P=0.08$ (**h**), $****P<0.0001$ (**i**). NS, not significant.

(CQ) injections to inhibit mTOR signaling and block the fusion process between autophagosomes and lysosomes, respectively. We found that systemic administration of rapamycin alone was sufficient to induce the appearance of vacuolated astrocytes (~18.93%) in the hippocampal SR regions of the 9-month-old mice (Fig. 3a,b). Coinjection of rapamycin with CQ did not further increase the number of these astrocytes (~14.54%). Similar to APDAs in the aged hippocampus, mTOR inhibition induced dramatic autophagosome formation only in hippocampal astrocytes (Fig. 3c) with SCRG1-, CLDN10- and GSTM1-positive vacuoles within their processes (Fig. 3d–f) along with the increased expression of MEGF10 and HIF1 α (Extended Data Fig. 4a,b).

Autophagy and the ubiquitin-proteasome system (UPS) are the two main pathways through which proteins and organelles are degraded, maintaining proteome homeostasis. Importantly, these two systems play mutually complementary roles, such that inhibition of proteasome activity results in the upregulation of autophagosome formation²⁵. Therefore, as the second cellular pathway, we manipulated the UPS. To our surprise, when we injected MG132, a potent proteasome inhibitor, into the ventricles of 9-month-old *ALDH1L1-eGFP* mice, we found that treatment with MG132 alone or MG132 with CQ recapitulated APDA phenotypes only in hippocampal astrocytes (~16.11% for MG132 alone and ~17.61% for MG132 + CQ in the hippocampal SR regions) (Fig. 3g,h). Similar to the aged or rapamycin-injected brains, APDAs treated with MG132 injection led to the accumulation of SCRG1, CLDN10 and GSTM1 in autophagosomes (Fig. 3i–l) as well as the increased expression of additional APDA markers (Extended Data Fig. 4c,d). MG132-induced APDAs were not found in other brain regions except the hippocampus, as in the WT aging brain (Extended Data Fig. 4j). Interestingly, when we injected CQ alone, we found that the number of APDAs were significantly increased as well (~11.68%, Extended Data Fig. 4e), but to a lesser extent compared with the

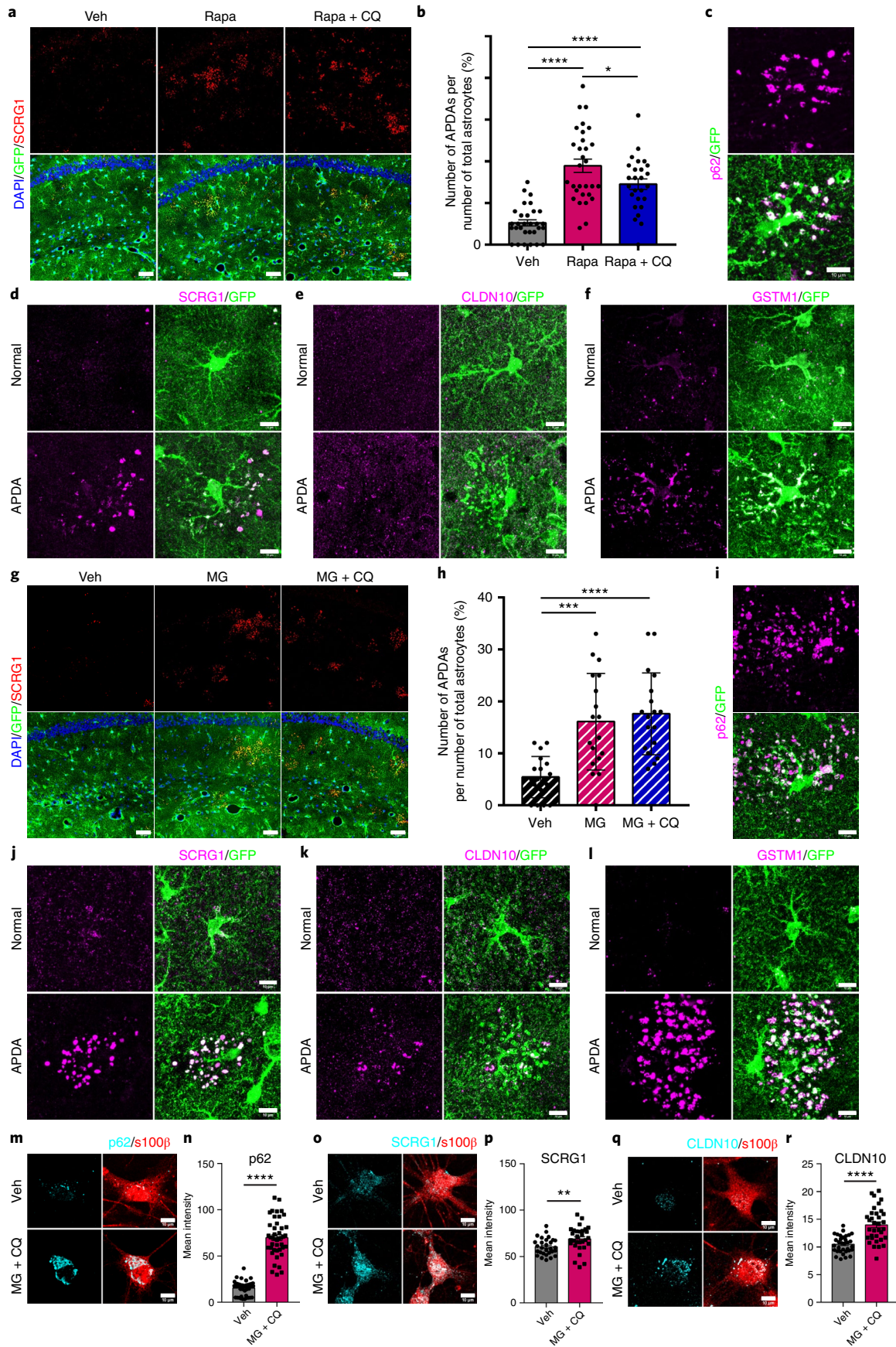
rapamycin+CQ or MG132+CQ injection cases (Fig. 3), suggesting that the decreased mTOR/proteasome and lysosomal activities are cooperatively involved in the generation of APDAs.

However, in sharp contrast, when we administered the same injections to 4-month-old *ALDH1L1-eGFP* mice, we found that neither rapamycin nor MG132 injection alone induced the acquisition of APDA phenotypes, suggesting that aging itself is a critical factor in inducing autophagosome accumulation in astrocytes (Extended Data Fig. 4f–i,k,l). Coinjection with MG132 and CQ resulted in a small but significant increase in APDA formation even in 4-month-old *ALDH1L1-eGFP* mice (Extended Data Fig. 4i). In agreement with this result, primary rat astrocytes purified from early postnatal stages (postnatal day7, P7) also showed accumulation of autophagosomes and upregulation of SCRG1 and CLDN10 expression when cultured in medium containing both MG132 and CQ (Fig. 3m–r). Taken together, our data show that APDAs can be induced artificially in the aging hippocampus by inhibiting either mTOR or proteasome activity, revealing that, among all brain cells, hippocampal astrocytes are selectively vulnerable to these challenges during aging.

Reduced levels of mTOR and proteasome activities in APDAs. Why are hippocampal astrocytes vulnerable to autophagosome accumulation during the aging process? Considering our results showing that mTOR or proteasome inhibition induces autophagosome accumulation only in aging hippocampal astrocytes, we hypothesized that endogenous mTOR signaling and proteasome activity are maintained differently in hippocampal astrocytes compared with other cell types in aging brains.

To evaluate whether mTOR signaling was affected differentially in hippocampal astrocytes during aging, the intensity of phospho-S6 (pS6), one of the downstream components in the mTOR signaling pathway, was measured in the hippocampi of 4-, 9- and

Fig. 3 | APDAs are induced by inhibiting either mTOR signaling or proteasome activity. **a**, Representative confocal z-stack images of 9-month-old *Aldh1l1-eGFP* hippocampi injected with vehicle (Veh, left), rapamycin (Rapa, middle) or rapamycin plus CQ (Rapa + CQ, right) and stained for nuclei (DAPI, blue), GFP (green) or SCRG1 (red). Scale bars, 50 μ m. **b**, Quantification showing the relative number of APDAs per total astrocytes in the SR hippocampal layers ($n=3$ mice). **c**, Representative confocal z-stack images of APDAs stained for p62 (red) together with GFP (green) in the Rapa + CQ group. Scale bar, 10 μ m. **d–f**, Representative confocal z-stack images of SCRG1 (**d**), CLDN10 (**e**) and GSTM1 (**f**) (red) with cells (GFP, green): normal astrocytes (upper panels) and APDAs induced by Rapa+CQ injections (lower panels). Scale bar, 10 μ m. **g**, Representative confocal z-stack images of 9-month-old *Aldh1l1-eGFP* hippocampi injected with vehicle (Veh, left), MG132 (MG, middle) or MG132 and CQ (MG + CQ, right) stained for nuclei (DAPI, blue), GFP (green) or SCRG1 (red). Scale bars, 50 μ m. **h**, Quantification showing the relative number of APDAs per total astrocytes in the SR hippocampal layers ($n=3$ mice). **i**, Representative confocal z-stack images of APDA stained for p62 (red) together with GFP (green) in the MG+CQ group. Scale bar, 10 μ m. **j–l**, Representative confocal z-stack images of SCRG1 (**j**), CLDN10 (**k**) and GSTM1 (**l**) (red) with cells (GFP, green): normal astrocytes (upper panels) and APDAs induced by MG+CQ injections (lower panels). Scale bars, 10 μ m. **m–r**, Representative confocal z-stack images (**m,o,q**) of primary rat astrocytes (s100 β , red) stained for p62 (**m**), SCRG1 (**o**) and CLDN10 (**q**) (scale bars, 10 μ m) with quantification analysis results (**n,p,r**) shown the mean intensity of p62 (**n**), SCRG1 (**p**) and CLDN10 (**r**) in astrocytes treated with vehicle (Veh) or MG132 plus CQ (MG132 + CQ) ($n=5$ independent experiments; dots on the bar graph indicate data obtained from individual ROIs). **b,h**, One-way ANOVA followed by Tukey's multiple comparison test. $****P<0.0001$ (Veh-Rapa, Veh-Rapa + CQ), $****P=0.04$ (Rapa-Rapa + CQ) (**b**); $***P=0.0002$ (Veh-MG), $****P<0.0001$ (Veh-MG + CQ) (**h**). **n,p,r**, Two-tailed Student's t -test. $****P<0.0001$ (**n**), $**P=0.006$ (**p**), $****P<0.0001$ (**r**); means $\pm$ s.e.m.



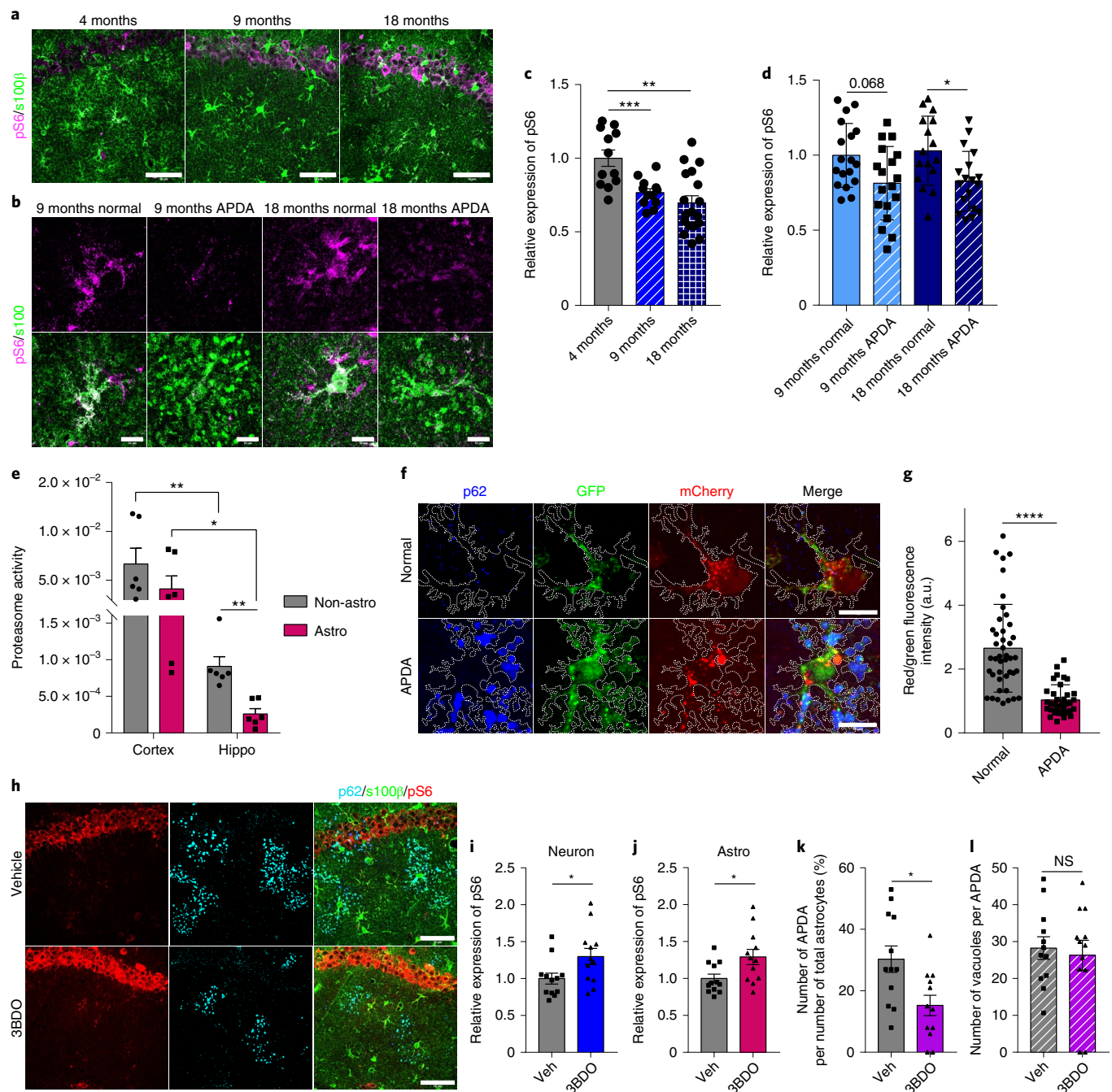


Fig. 4 | Multiple protein homeostasis components are selectively impaired in aged hippocampal astrocytes. **a**, Representative confocal z-stack images of 4-, 9- and 18-month-old hippocampi stained for pS6 (red) or s100 β (green). Scale bars, 50 μ m. **b**, Representative magnified confocal z-stack images showing pS6 (red) with s100 β (green) in individual normal astrocytes and APDAs. Scale bars, 10 μ m. **c,d**, Quantification of the mean intensity of pS6, comparing total hippocampal astrocytes (**c**) and comparing normal astrocytes versus APDAs (**d**) from different ages. Mean intensity of pS6 was normalized with 4-month-old astrocytes in (**c**) and 9-month-old normal astrocytes in (**d**) ($n = 4$ mice for each group). **e**, Quantification of the proteasome activity of astrocytes (Astro) and non-astrocyte cells (non-Astro) purified from 18-month-old cortices and hippocampi (Hippo) ($n = 6$ biologically independent samples). **f**, Representative confocal z-stack images of astrocytes expressing mCherry-GFP-LC3B in MG + CQ-injected 9-month-old hippocampi. Scale bars, 10 μ m. **g**, Quantification of the relative ratio of mCherry (red) intensity to GFP (green) fluorescence intensity ($n = 6$). **h**, Representative confocal z-stack images of 18-month-old hippocampi obtained from mice injected with vehicle or 3BDO stained for p62 (cyan), s100 β (green) or pS6 (red). Scale bars, 50 μ m. **i-j**, Quantification of the mean intensity of pS6. pS6 intensity was significantly increased in both CA1 neurons (**i**) and astrocytes (**j**) in the 3BDO-injected group. Mean intensity of pS6 was normalized with vehicle-injected group. **k**, Quantification showing the relative number of APDAs per total number of astrocytes in the SR layer of the hippocampus. **l**, Quantification of the number of vacuoles in the remaining APDAs (**i-l**, $n = 4$ mice). Dots on the graph indicate data obtained from individual ROIs. One-way ANOVA followed by Tukey's multiple comparisons test: *** $P = 0.0006$ (4 months–9 months), ** $P = 0.006$ (4 months–18 months) (**c**), $P = 0.068$ (9 months–normal versus 9 months–APDA), * $P = 0.049$ (18 months–normal versus 18 months–APDA) (**d**). Two-tailed multiple t-test: ** $P = 0.0016$ (non-Astro versus Astro in 18 month–Hippo), ** $P = 0.0051$ (Cortex versus Hippo in 18 month–non-Astro), * $P = 0.016$ (Cortex versus Hippo in 18 month–Astro) (**e**). Two-tailed Student's t-test: **** $P < 0.0001$ (**g**), * $P = 0.03$ (**i**), * $P = 0.02$ (**j**), * $P = 0.01$ (**k**), * $P = 0.71$ (**l**), NS, not significant; mean $\pm$ s.e.m.

18-month-old WT mice. As previously suggested, the pS6 intensity in the CA1 pyramidal neuronal layer was progressively enhanced with aging (Fig. 4a)^{26,27}. However, in contrast to neuronal pS6, the intensity of pS6 colocalized with s100 β in the hippocampi of 9- and 18-month-old mice was found to be reduced compared with that in the hippocampi of 4-month-old mice (Fig. 4c). Moreover, even among astrocytes in the hippocampi of 9- and 18-month-old mice, the intensity of pS6 was further reduced in APDAs compared with that in neighboring astrocytes with normal appearance (Fig. 4b,d).

Next, when we measured proteasome activity in cells purified from 18-month-old WT hippocampi and cortices, we found that hippocampal cells, in general, showed lower proteasome activity compared with cortical cells (Fig. 4e). Importantly, even among cells in the hippocampus, astrocytes exhibited the lowest level of proteasome activity (Fig. 4e). Thus, these data suggest that aging hippocampal astrocytes selectively lose their brake on autophagy induction, becoming prone to form autophagosomes.

During autophagic flux, autophagosomes fuse with lysosomes and are cleared by the autolysosomes that are formed²⁸. Since CQ cotreatment with MG132 significantly boosted APDA formation in 4-month-old mice and in *in vitro* astrocytes purified from P7 rats, we wondered whether APDAs have additional defects in lysosomal function. To test this idea, we expressed a pH-sensitive sensor consisting of mCherry and green fluorescent protein (GFP) dually linked to LC3B^{29,30} specifically in hippocampal astrocytes, and injected MG132 and CQ to induce APDA formation. During normal autolysosome formation, GFP signals were reduced compared with those of mCherry due to the weak stability of GFP under acidic lysosomal conditions. However, in APDAs, GFP signals tended to remain significantly higher in the accumulated autophagosomes, resulting in a decreased mCherry/GFP ratio compared with that in normal astrocytes (Fig. 4f,g). Thus, our results show that the age-dependent selective vulnerability of hippocampal astrocytes for autophagosome accumulation stems from multiple coinciding processes of impaired protein homeostasis, such as inhibited mTOR signaling and reduced proteasome and lysosomal activities.

Finally, we investigated whether mTOR activation can ameliorate the appearance of APDAs in the aged brain. We administered 3-benzyl-5-((2-nitrophenoxy) methyl)-dihydrofuran- 2(3 H)-one (3BDO), a mTOR signaling activator^{31,32}, to 18-month-old WT mice for 8 weeks. We confirmed that 3BDO injection successfully activated the mTOR signaling pathway as indicated by both neurons and astrocytes showing enhanced pS6 signal intensity (Fig. 4h–j). Surprisingly, we found that the number of APDAs in the 3BDO-injected group was significantly diminished compared with that in the vehicle-injected group (Fig. 4k,l), suggesting that APDAs can be

converted back to normal astrocytes in aged brains upon activation of mTOR signaling.

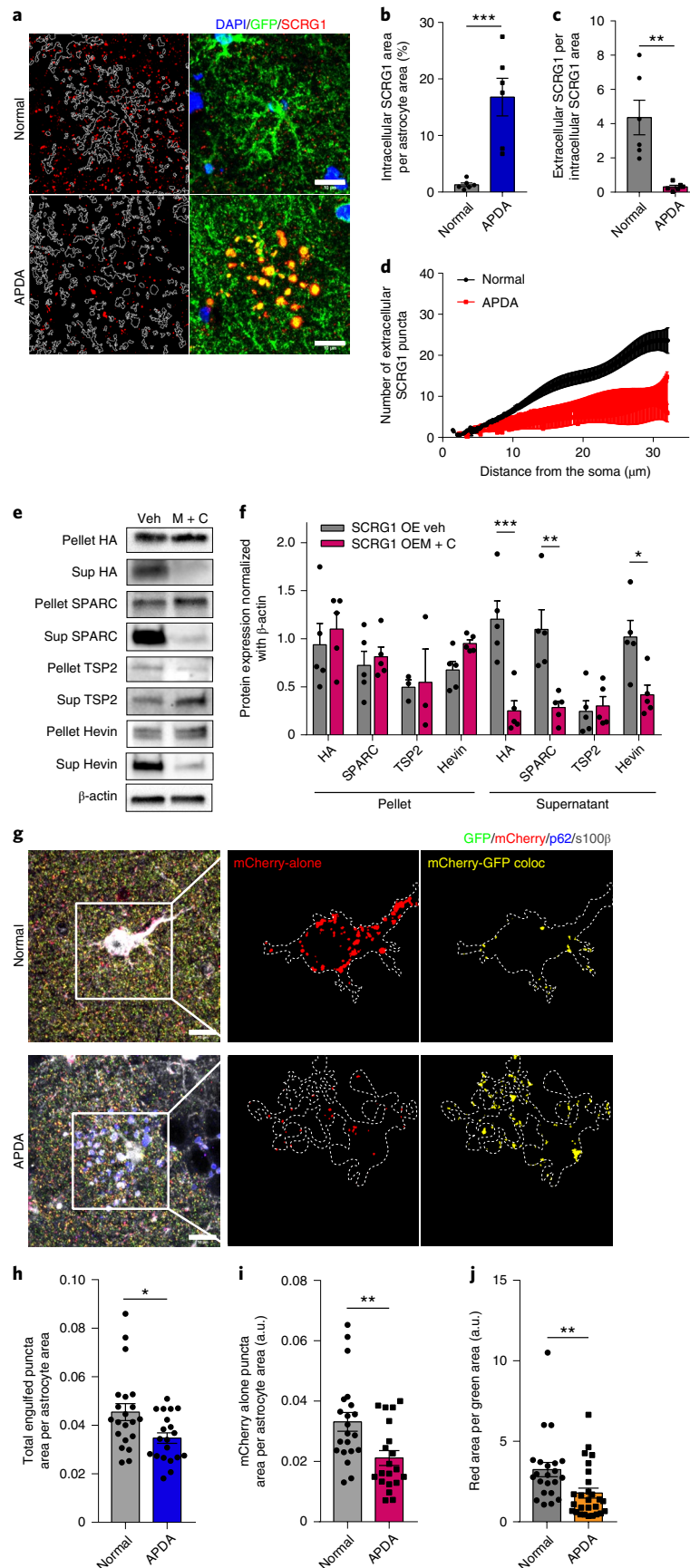
APDAs have impaired ability to regulate synapse number. Astrocytes can modulate synaptic plasticity and numbers by participating directly in the formation and elimination of synapses. According to our scRNA-seq data, cluster 6 astrocytes show significant upregulation in the mRNA levels of secreted factors controlling synaptogenesis, such as Thrombospondins, Sparcl1 (Hevin) or Sparc, or synapse phagocytosis machinery such as MEGF10. However, as shown for MEGF10 (Fig. 2), in APDAs, membrane receptors were profoundly mislocalized in autophagosomes, potentially preventing their normal actions. Interestingly, when we stained for SCRG1 in 9-month-old hippocampi, we noticed many extracellular SCRG1 puncta around astrocytes, suggesting that SCRG1 can be secreted normally, as previously suggested³³. In fact, when we expressed hemagglutinin (HA)-tagged SCRG1 sparsely in hippocampal astrocytes, HA was detected outside of SCRG1-HA-expressing astrocytes (Extended Data Fig. 5a–c). In contrast, when APDAs were induced by MG132 and CQ coinjection, we found that the level of extracellular SCRG1 was significantly reduced around APDAs, and most SCRG1 appeared to be trapped inside autophagosomes (Fig. 5a–d). To directly test whether APDAs have general defects in protein secretion, we cultured primary astrocytes and expressed HA-tagged SCRG1 with or without MG132 and CQ cotreatment. After washing out the MG132 and CQ, the astrocytes were further cultured to allow protein secretion for 3 days, and protein levels of SCRG1-HA and other secreted molecules were then determined in the cell lysate pellet and supernatant. Although the level of SCRG1-HA in the pellet was unchanged, the level of SCRG1-HA in the supernatant was significantly reduced in the astrocytes treated with MG132 and CQ. Importantly, we also found that the levels of SPARC and Hevin were significantly reduced only in the supernatant, not in the pellets, harvested from the MG132 and CQ cotreated astrocytes (Fig. 5e,f). Indeed, when we quantified the amount of extracellular Hevin and SPARC in the 18-month-old mouse hippocampus, we found that there was a significant reduction in the number of extracellular Hevin and SPARC puncta adjacent to APDAs compared with normal astrocytes (Extended Data Fig. 5d–g), suggesting that APDAs have defects in secreting these proteins.

Next, to evaluate the synapse engulfment ability of APDAs, we used an AAV-based synapse phagocytosis reporter that we had previously developed²². By labeling the hippocampal CA3–CA1 excitatory presynapses with mCherry-GFP, and isolating only mCherry puncta inside astrocytes, we found that APDAs showed reduced synapse phagocytosis capacity compared with that of neighboring

Fig. 5 | APDAs show defective regulation of synapse formation and elimination. **a**, Representative confocal z-stack images of normal astrocytes and APDAs (GFP, green) obtained from 9-month-old *Aldh1l1-eGFP* mice injected with MG + CQ and stained for nuclei (DAPI, blue), GFP (green) or SCRG1 (red). The lines indicate the boundaries of each astrocyte. Scale bars, 10 μ m. **b**, Quantification of the astrocytic intracellular area with SCRG1 staining normalized by the astrocyte area in the imaging field. **c**, Quantification of extracellular SCRG1 area normalized to the astrocytic intracellular SCRG1 area in the imaging field ($n = 3$ mice). **d**, The number of extracellular SCRG1 puncta plotted based on the distance from the soma. **e, f**, Western blots (**e**) and corresponding quantification (**f**) showing the level of proteins in the pellets and supernatants (Sup) in cultured astrocytes treated with MG132 and CQ (M + C) or vehicle (Veh); β -actin served as the loading control. SCRG1-HA, SPARC, TSP2 and Hevin levels were normalized to the level of β -actin (five independent experiments). The samples derive from the same experiment and gels/blots were processed in parallel. **g**, Representative confocal z-stack images of normal astrocytes (upper panels) and APDAs (lower panels) obtained from 18-month-old hippocampi injected with AAV9:hsyn promoter-driven synaptophysin-mCherry-GFP. Left panels show merged images stained for astrocytes (s100 β , gray), autophagosomes (p62, blue) and synapses (mCherry and GFP). The middle panel depicts astrocyte-engulfed synapses (only mCherry puncta, red). Right panels depict astrocyte-engulfed synapses that contain mCherry and GFP (yellow). Dotted lines indicate the boundaries of the astrocytes. Scale bars, 10 μ m. **h**, Quantification of the total engulfed synapse area (only mCherry- and mCherry-GFP double-positive puncta) in astrocytes normalized to the astrocyte area. **i**, Quantification of the mCherry-only punctate area normalized to the astrocyte area. **j**, Quantification of the relative area of only mCherry puncta per mCherry-GFP double-positive puncta engulfed by astrocytes ($n = 3$ mice, **h–j**). Dots on the bar graphs indicate data obtained from individual ROIs. Two-tailed Student's *t*-test: *** $P = 0.0009$ (**b**), ** $P = 0.0026$ (**c**). Two-way ANOVA followed by Sidak's multiple comparison test: *** $P = 0.0001$ (Sup HA), ** $P = 0.0014$ (Sup SPARC), * $P = 0.0349$ (Sup Hevin) (**f**). Two-tailed Student's *t*-test: * $P = 0.01$ (**h**), ** $P = 0.004$ (**i**), ** $P = 0.008$ (**j**); means $\pm$ s.e.m.

normal astrocytes (Fig. 5g–i). We also found that in the APDAs, the GFP signals tended to remain significantly higher in the engulfed synapse population, resulting in a decreased mCherry/GFP ratio

(Fig. 5j). These data indicate that APDAs neither engulf nor digest synapses efficiently, supporting our previous results indicating that APDAs have impaired lysosomal activity. Taken together, our data



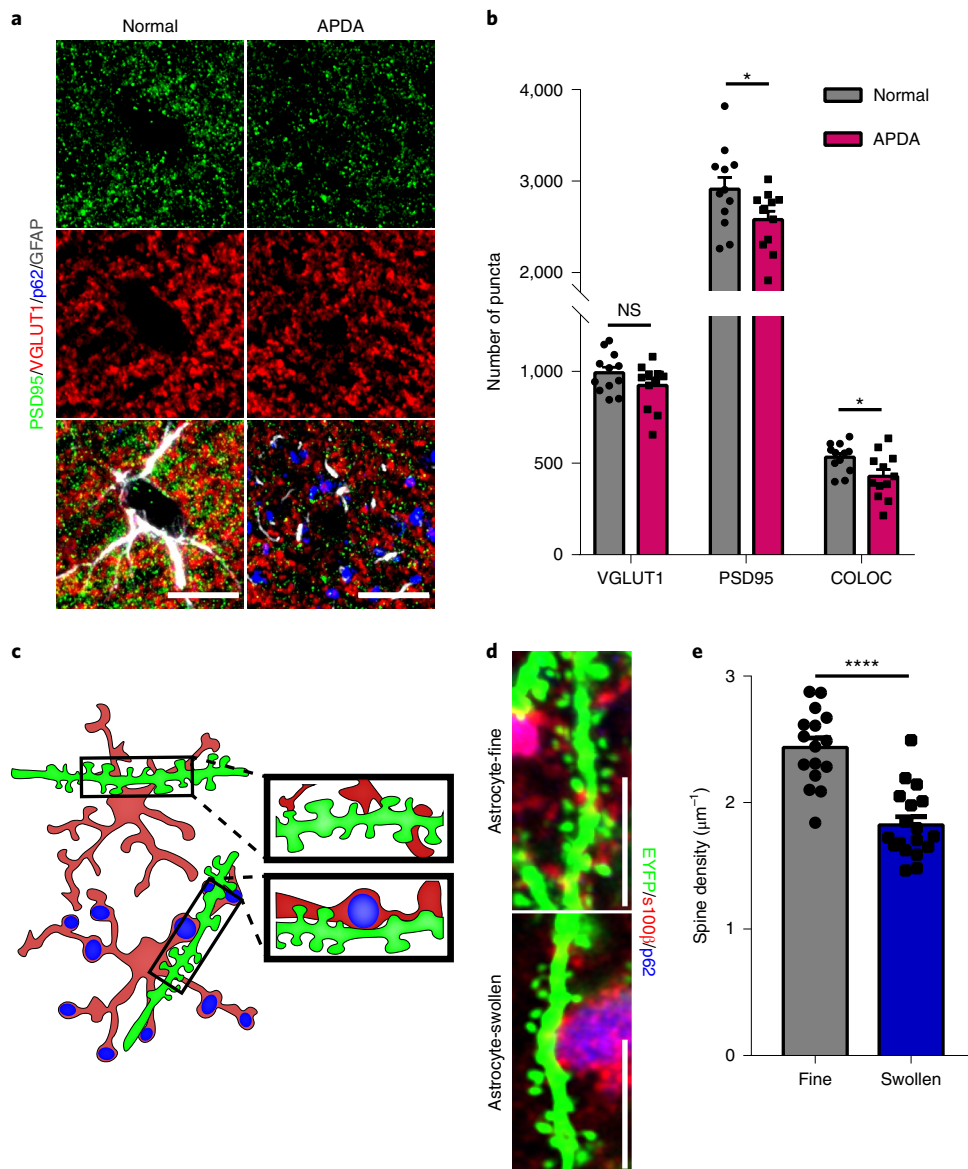


Fig. 6 | Reduced number of excitatory synapses in areas associated with APDAs. **a**, Representative confocal z-stack images of excitatory synapses stained with VGLUT1 (red) and PSD95 (green) around normal astrocytes and APDAs in the hippocampi of 18-month-old mice. Astrocytes were stained with GFAP (gray) and APDAs were identified by p62 (blue) accumulation. Scale bars, 10 μm . **b**, Quantification of the number of excitatory synapses around normal astrocytes and APDAs ($n=3$ mice; dots on the bar graph indicate data obtained from individual ROIs). The number of excitatory postsynapses (PSD95-positive puncta) and excitatory synaptic contacts (VGLUT1/PSD95-double-positive puncta) were reduced significantly around APDAs compared with normal astrocytes. **c**, Schematic diagram illustrating Thy1-EYFP-positive dendritic spines associated with astrocytes and APDAs. **d**, Representative confocal z-stack images of dendritic trees and spines (EYFP, green) in contact with fine astrocyte processes (upper panel) and with the swollen APDA processes (lower panel). The processes of astrocytes were labeled with s100 β (red), and swollen processes were labeled with p62 (blue). Scale bars, 10 μm . **e**, Quantification of the number of dendritic spines normalized to dendrite length ($n=7$ mice, dots on the bar graph indicate data obtained from individual ROIs). Multiple t-tests followed by Holm-Sidak method: $*P=0.048$ (PSD95), $*P=0.023$ (COLOC) (**b**). Two-tailed Student's t-test: $****P<0.0001$; mean $\pm$ s.e.m. (**e**).

suggest that, even though APDAs upregulate mRNAs for general astrocytic property and synapse related genes, most likely due to compensatory responses (Fig. 1c), APDAs have lost the normal astrocytic ability to regulate synapse formation and elimination by impaired protein trafficking and mislocalization.

Excitatory synapse loss near APDAs. Synapse dynamics—the rate of synapse formation and elimination—have been shown previously to be substantially altered by aging^{12,34}. To investigate how APDAs affect neighboring synapses, we measured the number of excitatory

pre- and postsynapses closely associated with the swollen processes of APDA. Surprisingly, we found that the number of excitatory postsynapses (PSD95-positive puncta) and excitatory synaptic contacts (puncta showing a certain overlap between VGLUT1 and PSD95) was significantly reduced in the region with APDAs (Fig. 6a,b). Furthermore, when we analyzed dendritic spines in CA1 hippocampal neurons obtained from 18-month-old *Thy1-EYFP-H* transgenic mouse lines (Fig. 6c), we found that EYFP-positive dendritic spines were disorganized and significantly reduced in their numbers when p62-positive swollen processes of APDAs made close contact with

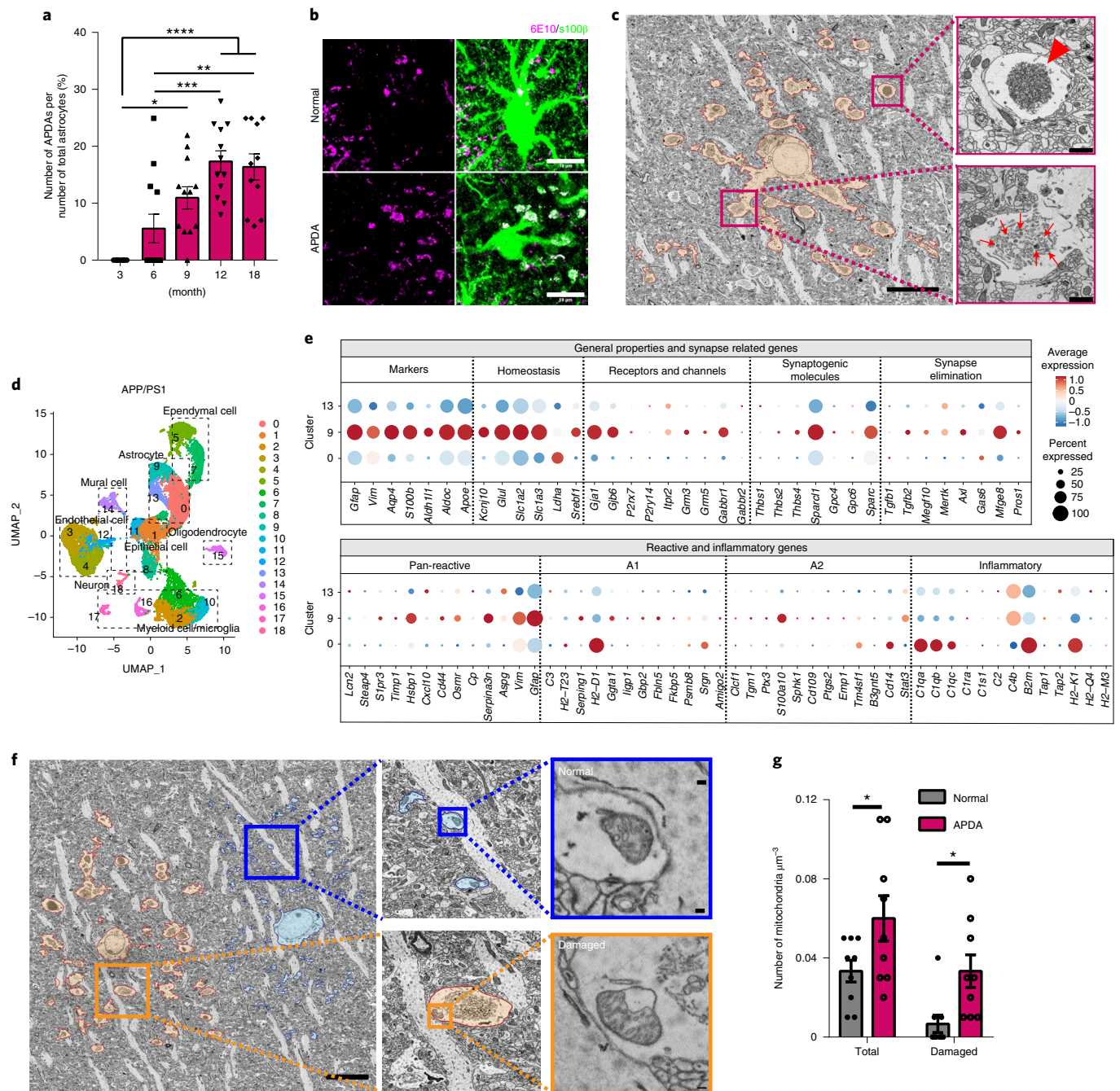


Fig. 7 | Accelerated appearance of APDAs in APP/PS1 hippocampi. **a**, Quantification of the relative number of APDAs per total astrocytes in 3-, 6-, 9-, 12- and 18-month-old APP/PS1 hippocampi ($n=3$, dots on the bar graph indicate data obtained from individual ROIs). **b**, Representative confocal z-stack images of normal astrocytes and APDAs obtained from 18-month-old APP/PS1 hippocampi stained for s100 β (green) and amyloid- β (6E10, red). Amyloid- β accumulated in the swollen processes of APDAs. Scale bars, 10 μm . **c**, Representative electron microscopy images showing an Alzheimer's disease APDA containing amyloid- β fibrils and autophagosomes inside of its vacuoles (orange). Scale bars, 10 μm (left panel) and 1 μm (right panel). Red arrowhead marks amyloid- β fibrils (right upper panel) and red arrows mark autophagic vesicles (right lower panel) in the swollen processes. **d**, A UMAP plot of 18,059 cells from the hippocampi of 2-year-old APP/PS1 mice. Cells were separated into 19 clusters. **e**, Gene expression related to general characteristics of astrocytes and synaptic regulation (upper panel), and reactive and inflammatory astrocytic responses (lower panel). **f**, Representative electron microscopy images showing mitochondria inside an Alzheimer's disease APDA (orange) and normal astrocytes (light blue). Normal and damaged mitochondria are indicated in the upper panel and the lower panel of the enlarged images, respectively. Scale bars, 10 μm (left panel) and 0.1 μm (right panel). **g**, Quantification of the number of mitochondria in astrocytes per unit volume. Both the number of total mitochondria and damaged mitochondria were significantly increased in APDAs compared with normal astrocytes ($n=9$, dots on the bar graph indicate data obtained from individual ROIs). One-way ANOVA followed by Tukey's multiple comparisons test (**a**). The P values are indicated in the source data file. Two-way ANOVA followed by Sidak's multiple comparisons tests: $*P=0.046$ (Normal versus APDA, total), $*P=0.046$ (Normal versus APDA, damaged) (**g**); means $\pm$ s.e.m.

dendritic trees (Fig. 6d,e). Interestingly, the number of inhibitory pre- and postsynapses labeled with VGAT and Gephyrin, respectively, was not changed in regions covered with both APDAs and normal astrocytes (Extended Data Fig. 6a,b).

As we discovered that APDAs can be induced artificially by inhibiting either mTOR or proteasome activity, we quantified the number of excitatory synapses associated with rapamycin-induced APDAs. Interestingly, although we found that there was a tendency to decrease, the changes in the number of excitatory postsynapses was not statistically significant (Extended Data Fig. 6c), suggesting that 10 days of mTOR inhibition alone was not sufficient to recapitulate the synaptic phenotype found in the aged hippocampus.

Accelerated appearance of APDAs in Alzheimer's disease hippocampi. Aging is a high risk factor for Alzheimer's disease. A recent scRNA-seq study revealed that the disease-associated astrocyte (DAA) cluster in the Alzheimer's disease hippocampus can also be found in normal aging brains, suggesting that similar changes in the status of astrocytes may be evident in aging and Alzheimer's disease³⁵. Therefore, we investigated whether APDAs can be found in Alzheimer's disease using the APP/PS1 mouse model of Alzheimer's disease³⁶. Surprisingly, we found that SCRG1-, CLDN10- and GSTM1-positive APDAs with autophagosome accumulation appeared even in the 6-month-old APP/PS1 hippocampi (Extended Data Fig. 7a–c), much earlier than in the WT hippocampi (Fig. 7a).

To better characterize Alzheimer's disease APDAs, we additionally performed scRNA-seq with 2-year-old APP/PS1 hippocampi. Similar to aged WT hippocampi, in Alzheimer's disease brains, we found that cluster9 astrocytes exhibit the upregulated APDA markers such as SCRG1, GSTM1 and CLDN10 (Fig. 7d,e and Extended Data Fig. 7e). Interestingly, cluster9 astrocytes also showed a tendency to upregulate the reactive astrocytic genes (Fig. 7e), suggesting that APDAs in Alzheimer's disease brains may have adopted characteristics of reactive astrocytes during disease progression.

Next, we performed electron microscopy (EM) imaging with 2-year-old APP/PS1 hippocampi and were able to identify vacuolated APDA populations in hippocampal SR regions. Interestingly, although the APDAs were not closely localized with amyloid- β plaques (Extended Data Fig. 7d), we found that 6E10-positive amyloid- β fibrils had accumulated significantly in the autophagosomes of Alzheimer's disease APDAs (Fig. 7b). Similarly, EM images of these APDAs also revealed amyloid- β fibril structures inside the vacuoles of Alzheimer's disease APDAs (Fig. 7c). These data suggest that, although amyloid- β oligomers and fibrils can be engulfed by APDAs, they accumulate inside APDA autophagic vesicles and are not properly degraded, most likely due to the reduced proteasomal and lysosomal activities of APDAs.

In addition, EM images of Alzheimer's disease APDAs confirmed that these APDAs do not show characteristic features of cell death, such as nuclear condensation or membrane fragmentation/rupture, supporting our claims that APDAs do not undergo apoptosis/necrosis (Extended Data Fig. 7f). Interestingly, when we examined the EM images of Alzheimer's disease APDAs in detail, we found that the APDAs had a significantly greater number of damaged mitochondria with broken and scarce cristae (Fig. 7f). The total number of mitochondria was also significantly higher in APDAs than in normal astrocytes (Fig. 7g). Since previous studies have demonstrated that damaged mitochondria are removed by mitophagy³⁷, our data indicate that APDAs also have impaired in mitophagy, resulting in the accumulation of damaged mitochondria. Finally, unlike normal astrocytic fine processes that show close physical association with synapses, we found that the vacuolated processes of APDAs fail to form tripartite synapses (Extended Data Fig. 8). This data indicate that normal astrocytic controls of synaptic functions, such as neurotransmitter uptakes and regulating synaptic potentiation, are likely disrupted in

APDAs, potentially contributing to the reduced synapse numbers and dysmorphic spines that we discovered near the swollen processes of APDAs (Extended Data Fig. 7g,h).

Discussion

The mechanisms that mediate cognitive decline in normal aging are not well characterized. Recent RNA-seq experiments revealed that astrocytes show region-specific transcriptional heterogeneity in aged brains with a tendency for the upregulation of genes involved in astrocyte reactivity¹⁰. However, it is still unknown whether aging astrocytes could be defined as just Pan/A1/A2 astrocytes and whether there is a distinct astrocyte subtype that shows progressive functional impairment during aging. In this study, although we also initiated our analysis by performing scRNA-seq with 2-year-old mouse hippocampi, we identified a unique astrocyte subtype with severe defects in protein localization and trafficking due to the dramatic accumulation of autophagosomes within their processes. These astrocytes, which we termed APDAs, show characteristic morphological changes, including the beading and disappearance of distal fine processes with swelling and increased vacuolization. It had been suggested that astrocytes in neuropathological conditions, such as severe trauma and ischemia, undergo clasmotodendrosis with morphological changes similar to the APDAs in this study^{38,39}. Interestingly, the morphological changes in clasmotodendrosis were thought to be due to fragmentation of astrocytic processes during the degeneration or necrosis of astrocytes^{40,41}. By examining various markers and cellular features with 3D confocal and EM images, we showed that the APDAs do not show any sign of apoptosis and necrosis. Rather, the vacuolization of astrocytic processes was due to the accumulation of various organelles including autophagosomes.

We also showed that APDAs were defective in protein trafficking and secretion, including synaptogenic molecules. In addition, we found that MEGF10, a membrane receptor critical for synapse elimination, mislocalized and accumulated in the autophagosomes, reducing the ability of APDAs to eliminate synapses. Since astrocytes have been shown to play critical roles in the constant formation and elimination of hippocampal synapses during learning and memory formation, APDAs may have lost the astrocytic ability to maintain synaptic homeostasis, resulting in reduced synaptic turnover and plasticity. Furthermore, the vacuolated processes of APDAs do not form tripartite synapses, suggesting that normal astrocytic controls of synaptic functions, such as neurotransmitter uptakes and aiding synaptic plasticity, are likely disrupted. Therefore, we propose that these impaired abilities of APDAs contribute to the reduced number of excitatory synapses that we identified in the aged/Alzheimer's disease hippocampal regions with APDAs.

In an attempt to identify the mechanisms of APDA formation, we found that the action of three critical components in protein homeostasis, the mTOR signaling pathway, the UPS and lysosomal activity, was profoundly diminished in aged hippocampal astrocytes. The combination of reduced mTOR signaling and UPS activity seems to be critical for inducing excessive autophagosome formation in astrocytes. Moreover, the content in overproduced autophagosomes failed to be cleared because of a reduction in lysosomal activity. Future work is necessary to determine exactly how these three components cooperate to induce APDA development and whether the same mechanisms play important roles in clasmotodendrosis in various brain injuries.

Recently, important progress has been made in characterizing critical interactions between astrocytes and other immune cells in healthy and diseased brains. For example, astrocytes interact with microglia, in controlling microglial phagocytic capacity⁴² as well as inflammatory responses¹⁶. Moreover, astrocytes induce immune cell infiltration by inflammatory cytokine production in experimental autoimmune encephalitis (EAE)⁴³, and in stroke⁴⁴, and potentially in neurodegenerative diseases. Since the intercellular crosstalk is based

on proper protein secretion and receptor localization in astrocytes, it is easily conceivable that APDAs would fail to communicate with these other immune cells. How the impaired function of APDAs contributes to the various disease-induced neuroinflammation in the aged brains would be fascinating subjects for future studies.

In summary, our study reveals that hippocampal astrocytes are selectively vulnerable during aging processes due to their reduced mTOR signaling and proteasome activity. APDAs can appear in the Alzheimer's disease hippocampus much earlier than in the normal brain, suggesting that APDAs may contribute to aging- and Alzheimer's disease-related cognitive decline. Our work also shows that general rapamycin treatment in the population of older people in an attempt to increase lifespan may produce APDAs in the hippocampus, thereby generating unwanted side effects, such as local defects in synaptic turnover. Therefore, to control mTOR-dependent cellular aging process, it is necessary to modulate the mTOR signaling pathway in a cell-specific manner, especially since we found that activating the mTOR signaling pathway in the aged brain can lead to the conversion of APDAs into normal astrocytes.

Methods

Mice and drug treatments. WT (C57BL/6J) mice were maintained by the Animal Breeding Center of Korea Advanced Institute of Science and Technology (KAIST). Additionally, 9-month-old mice were purchased from Daehan Biolink Co., and 16- to 18-month-old mice were purchased from Korea Basic Science Institute (KBSI). *APP^{swe}/PSEN1^{ΔE9}* (APP/PS1) double-transgenic Alzheimer's disease mice with a B6 × C3 background (MMRRC stock No. 034829-JAX) were provided by J.-H. Han's Laboratory at KAIST. *Aldh1l1-eGFP-L10a* transgenic mice were purchased from the Jackson Laboratory (catalog no. 030248). The 4- and 9-month-old *Aldh1l1-eGFP-L10a* mice were used to investigate the effects of autophagy activators and proteasome inhibitors. *Thy1-eYFP-H* transgenic mice were purchased from the Jackson Laboratory (catalog no. 003782). *Thy1-eYFP-H* mice were used to count the dendritic spine associated with astrocytes in the hippocampus. All lines were maintained by breeding with C57BL/6J mice. Both mouse sexes were used in this study.

To activate autophagic flux, 4- and 9-month-old *Aldh1l1-eGFP* mice were treated with rapamycin (20 mg kg⁻¹ per day by intraperitoneal injection for 10 days, LC laboratories) dissolved in 4% ethanol + 5% polyethylene glycol 300 + 5% Tween 80 + Dulbecco's PBS solution. CQ (60 mg kg⁻¹ per day; Sigma, catalog no. C6628) was injected intraperitoneally with rapamycin in the same solution. To inhibit the proteasome, a single dose of 0.5 μl of MG132 (2 μM, Sigma) was injected stereotactically into the hippocampus (anterior-posterior (AP) -2.0 mm and medial-lateral (ML) -1.2 mm from the bregma and dorsal-ventral (DV) -1.0 mm from the brain surface) 24 h before tissue collection. For CQ treatment, the daily injection was performed for 10 days before tissue collection. To induce apoptosis and necrosis for verification of apoptotic or necrotic marker, 0.5 μl of ATP (1 μM, Sigma) was injected stereotactically into 4-month-old WT brains.

As previously reported³², 3BD0 dissolved in DMSO at 200 mg ml⁻¹ (diluted with corn oil) was administered (100 mg kg⁻¹ per day by intraperitoneal injection) to 18-month-old WT mice for 8 weeks. All procedures and protocols were approved by the Animal Ethics Committee at KAIST. All experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC, KA2019-67).

scRNA-seq library preparation. Hippocampal tissue dissection. Hippocampal cells were isolated from 2-year-old WT mouse hippocampi (*n* = 3) and APP/PS1 mouse hippocampi (*n* = 2) as previously described⁴⁵. In brief, the hippocampus was dissected from each mouse brain, minced into small pieces, dissociated enzymatically (using papain ~20 U ml⁻¹) and dissociated mechanically to generate a single-cell suspension. Myelin was removed using myelin removal beads (Miltenyi Biotec), and the resulting single-cell suspension was sent to Macrogen for scRNA-seq.

10x scRNA v3.1 chemistry (single index). A LUNA-FL Automated Fluorescence Cell Counter (logos biosystems) was used in consultation with the 10x Genomics Single Cell Protocols: Cell Preparation Guide and the Guidelines for Optimal Sample Preparation flowchart (Documents CG00053 and CG000126, respectively) to prepare cells. A total of 14,759 cells were captured from the WT samples and 25,005 cells were captured from APP/PS1 samples. The number of cells sequenced from each individual mouse after passing through the QC filter are as follows: WT no. 1: 5,976 cells; WT no. 2: 1,045 cells; WT no. 3: 7,025 cells; APP/PS1 no. 1: 4,453 cells and APP/PS1 no. 2: 13,606 cells. Libraries were prepared using a Chromium controller according to the 10x Chromium Next GEM Single Cell 3' v3.1 protocol (CG000204). Briefly, cell suspensions were diluted in nuclease-free water to achieve a targeted cell count of 10,000. The cell suspension was mixed with Master Mix

and loaded with Single Cell 3' v3.1 Gel Beads and partitioning oil into Chromium Next GEM chip G. RNA transcripts from single cells were uniquely barcoded and reverse-transcribed within droplets. cDNA molecules were pooled, and the cDNA pool was subjected to an end-repair process, the addition of a single 'A' base, and ligation of adapters. The products were then purified and enriched by PCR to create the final cDNA library. The purified libraries were quantified using qPCR according to the qPCR Quantification Protocol Guide (KAPA) and qualified using an Agilent Technologies 4200 TapeStation (Agilent Technologies). Then, the libraries were sequenced using a HiSeq platform (Illumina) according to the read length, as specified in the Illumina user guide.

Single-cell gene expression analysis. We performed single-cell gene expression analysis using Cell Ranger: <https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/what-is-cell-ranger>. Briefly, raw BCL files obtained from Illumina HiSeq sequencing were demultiplexed to generate FASTQ files using the 'cellranger (v3.1.0) mkfastq' pipeline. Then, the raw FASTQ files were analyzed using 'cellranger count'.

Raw count matrices from wild-type data were imported into the Seurat v4.0.3 software package. Raw counts with all genes expressed in three or more cells and all cells with at least 200 detected genes were used in a downstream analysis. Since we found a rare subset of cells with an outlier level of high mitochondrial percentage and low unique molecular identifier content, we eliminated cells with percent. mito greater than 0.5. After deletion of this subset, the selected count value was normalized using the SCTransform method⁴⁶. Next, 3,000 integration features were selected for each sample separately and data were integrated using IntegrateData function with normalization.method = "SCT" option. With successfully integrated data, principal component analysis (PCA) was performed, and we decided to use first 15 principal components (PCs) for further analysis based on elbow plot. Then we visualized the data by the uniform manifold approximation and projection (UMAP) dimension reduction method. To define the cell clusters, we ran the FindNeighbors and FindClusters with Resolution=0.35 options. Then, significant markers for every cluster, compared with all remaining cells, were determined using the minimum fraction of min.pct cells and Wilcoxon rank sum test; only positive markers are reported. The significance of the differential expression data was determined using Wilcoxon rank sum test and the FindMarkers function in Seurat. The false discovery rate (FDR) was controlled by adjusting the *P* value via Bonferroni correction.

To compare the gene expression pattern of astrocyte subtype between young and old wild-type mice, we separately processed the published young wild-type mouse scRNA-seq data (GSE114000)⁴⁷ with the method described above to visualize. After visualization, we annotated the cells with published metadata. Then, we made dot plots from each dataset with upregulated genes in aged astrocyte clusters.

We analyzed the APP/PS1 scRNA-seq dataset using a method similar to that described above, but several different values were used on the parameters to optimize the data, as follows: (1) percent.mito greater than 0.5, (2) first 13 PCs used and (3) Resolution=0.4

Viral vector construction. To generate a pLenti-GfaABC1D-mCherry-GFP-LC3B construct, mCherry-GFP-LC3B sequences from pBABE-puro mCherry-EGFP-LC3B (Addgene, catalog no. 22418) were amplified by PCR and subcloned into pLenti-GfaABC1D-IRES-ZsGreen (the lentiviral backbone was a gift from W.D. Heo's laboratory at KAIST) at *NheI* and *MfeI* restriction sites. The primers for amplification were as follows: forward, 5'-GAGGGCTAGCCGGATCTGCCACATCG-3', reverse, 5'-GAGGCAATTGGGGTCGACTTACACTGAC-3'.

To generate pLenti-GfaABC1D-SCRG1-HA, an HA tag was inserted at the C-terminus of *Mus musculus Scrg1* with a short GSGSGS linker and subcloned into pLenti-GfaABC1D-IRES-ZsGreen at *NheI* and *EcoRI* restriction sites. For *Scrg1* cDNA, total RNA was extracted from APP/PS1 mouse hippocampi using a RNeasy mini kit (Qiagen), and cDNA synthesis was performed using reverse transcriptase (Enzynomics). For HA-tagged *Scrg1* cDNA with the GSGSGS linker, SCRG1-GSGSGS sequences and GSGSGS-HA sequences were amplified by PCR, and these two sequences were merged by overlapping PCR and subcloned into pLJM-EGFP (Addgene, catalog no. 19319) at *NheI* and *BsrGI* restriction sites. Then, the SCRG1-HA construct was subcloned into the pLenti-GfaABC1D-IRES-ZsGreen vector. The following primers were used for *Scrg1* cDNA: forward, 5'-GAGGGCTAGCATGATGAAATCTGTGGTACT-3', and reverse, 5'-GAGGACCGTGGGTGGTGTGTGCAGGGGATCA-3'. The following primers were used for the overlapping PCR: *NheI*-SCRG1-forward, 5'-GAGGGCTAGCGCCACCATGATGAAATCTGTGGTACT-3'; GSlinker-SCRG1-reverse, 5'-GCCGCTGCCGCTGCCGCTGCCGCTGCCGTGGTGTGTGCAGGG-3'; GSlinker-HA-forward, 5'-AGCGGCAGCGGCAGCGGCAGCGGCAGCTACCCATACGATGTTCCAG-3'; and *BsrGI*-HA-reverse: 5'-GAGGTGTACATCAAGCGTAATCTGGAACATCG-3'.

Lentivirus production. Lentivirus was produced as previously described⁴⁷. Briefly, we cotransfected four plasmids encoding lentiviral genes (VSVG, TAT, REV and Gag/Pol) and target plasmids into HEK293T cells using the PEI (1 mg ml⁻¹)-based

transfection method. The HEK293T cells were maintained in DMEM (Welgene) containing 10% fetal bovine serum (FBS, Gibco). The medium was replaced with serum-free medium during transfection (for 6–8 h). The transfected cells were incubated in an incubator (37 °C, 5% CO₂) for 48 h. The cell culture supernatant was harvested, and cell debris was removed by centrifugation and filtered. Then, we added as much as 12 ml of filtered supernatant to separate tubes (Beckman Coulter) and centrifuged the tubes for 2 h in an SW41 swinging bucket rotor at 82,700g and 4 °C. The supernatants were removed, and pellets were dissolved in Dulbecco's PBS. The viral titer was estimated by counting cells in a purified astrocyte culture that had been infected with the viral solution and incubated for 48–72 h after plating.

AAV production. AAV was produced as previously described⁴⁸. In brief, we cotransfected a capsid plasmid (pAAV9), a helper plasmid (pAd deltaF6) and target plasmids into HEK293T cells using the PEI (1 mg ml⁻¹)-based transfection method. pAAV::hSyn-Synaptophysin-mCherry-eGFP²² were packaged with AAV9 to label excitatory presynapses. The HEK293T cells were maintained in DMEM containing 10% FBS. The medium was replaced with serum-free medium during transfection (for 6–8 h). The transfected cells were incubated in an incubator (37 °C, 5% CO₂) for 72 h. Cells were collected and resuspended with hypotonic buffer (50% DMEM, 50% water, 0.04% DNase I (Worthington)). Then, cells were lysed with freeze-thaw cycles. To purify virus from the cell lysates and supernatants, a polyethylene glycol (PEG)-based purification method was used. Purified AAVs were concentrated using ultracentrifugation tubes (50 kDa filter, Millipore). AAV titer was measured using an AAV titration kit (Takara).

Virus injection. Mice were anesthetized with 0.5 ml isoflurane in a sealed plastic box and secured in a stereotaxic apparatus (KOPF Instruments). During surgical procedures, anesthesia was maintained with 1–2% isoflurane using a veterinary vaporizer (SurgiVet). All lentiviruses (pLenti-GfaABC1D-mCherry-GFP-LC3B and pLenti-GfaABC1D-SCRG1-HA) were injected unilaterally into the hippocampal SR region (AP –2.0 mm and ML –1.2 mm from the bregma and DV –1.4 mm from the brain surface) of 9-month-old WT mice. AAV9::hSyn-Synaptophysin-mCherry-eGFP was stereotactically injected unilaterally into the hippocampal CA3 region (AP –2.0 mm and ML 2.35 mm from the bregma and DV –2.25 mm from brain surface) of 18-month-old WT mice to label the CA3-CA1 excitatory presynapses. All infusions were performed with a motorized Hamilton syringe pump (Harvard Apparatus), and glass pipettes (WPI) that had been pulled by a motorized pipette puller (Sutter Instruments) were used as needles. After infusion, the surgical incision sites were closed with silk sutures, and the mice were allowed to rest in a heated cage for recovery.

Primary astrocyte culture. Astrocytes were purified by immunopanning and culturing as described previously^{49,50}. In brief, cortices were dissected from p7 rats and dissociated into single-cell suspensions both enzymatically (using papain) and mechanically. Single-cell suspensions were incubated on negative immunopanning plates to remove endothelial cells, microglia and oligodendrocyte lineage cells and then astrocytes were positively selected. The isolated astrocytes were cultured in serum-free medium (50% neurobasal medium and 50% DMEM with 100 U ml⁻¹ penicillin, 100 µg ml⁻¹ streptomycin, 1 mM sodium pyruvate, 292 µg ml⁻¹ L-glutamine, 1× SATO and 5 µg ml⁻¹ N-acetyl cysteine). Heparin-binding EGF-like growth factor (HBEGF, PeproTech (100–47) at 5 ng ml⁻¹) was added to this medium as an astrocytic survival factor. Purified astrocytes were transduced with virus on DIV7, and drugs were administered on DIV14.

Proteasome activity assay with cortical and hippocampal cells. Cortices and hippocampi were dissected from 4-, 9- and 18-month-old mice and dissociated into single-cell suspensions using papain and mechanical trituration with Pasteur pipettes. Myelin debris was removed from freshly dissociated cells using myelin removal beads II (Miltenyi Biotec) according to the manufacturer's instructions (with LS magnetic columns). Astrocytes were isolated from the flow-through cells using an ACSA-2 kit (Miltenyi Biotec) according to the manufacturer's protocol. Then, the astrocytes were frozen in liquid nitrogen and stored in a deep freezer (–80 °C). For the proteasome activity assay, all samples were homogenized by pipetting using 0.5% NP-40, and the supernatants were collected by centrifugation. Total protein concentration was measured using a Bradford protein assay (Bio-Rad). Proteasome activity was determined using a proteasome activity assay kit (Abcam) based on chymotrypsin-like activity. Briefly, 10 µl of each proteasome lysate was incubated with the proteasomal substrate with or without the proteasomal inhibitor MG132 (as a negative control). Fluorescence was measured at 350/440 nm, and proteolytic activity was evaluated after 2 h of incubation at 37 °C. The specific activity of the proteasomes was obtained by subtracting the fluorescence value of the MG132-treated sample from the total fluorescence value of the sample not treated with MG132. Values were further normalized to the protein concentration in each sample.

Immunohistochemistry. Animals were anesthetized with avertin (2,2,2-tribromoethanol, Sigma) by intraperitoneal injection and perfused with PBS followed by 4% paraformaldehyde (PFA, Wako). The brains were isolated,

postfixed overnight in 4% PFA at 4 °C and then transferred to 30% sucrose (Sigma) in PBS for a 48-h incubation at 4 °C. The brain samples were embedded in OCT compound (Leica) and then cryosectioned into 10-µm or 30-µm slices. Each brain section was treated with blocking buffer (4% bovine serum albumin (BSA) and 0.3% Triton X-100 in PBS) for 1 h at room temperature and then incubated with the appropriate primary antibody diluted in blocking buffer for 24–72 h at 4 °C. The primary antibodies used for immunohistochemistry were rabbit anti-SCRG1 (Proteintech, catalog no. 14038-1-AP, 1:500), rabbit anti-GSTM1 (Proteintech, catalog no. 12412-1-AP, 1:200), rabbit anti-CLDN10 (Thermo, catalog no. 38-8400, 1:100), rabbit anti-s100β (Abcam, catalog no. ab52642, 1:500), guinea pig anti-s100β (Synaptic Systems, catalog no. 287 004, 1:500), mouse anti-NeuN (clone A60) (Merck, catalog no. MAB377, 1:500), goat anti-Iba1 (Novus Biologicals, catalog no. NB100-1028, 1:500), goat anti-PDGFRα (R&D Systems, catalog no. AF1062-SP, 1:300), rabbit anti-Rab5 (Abcam, catalog no. ab18211, 1:300), rat anti-LAMP2 (Abcam, catalog no. ab13524, 1:300), rabbit anti-ubiquitin (Cell Signaling, catalog no. CST 3933, 1:500), rabbit anti-MEGF10 (Millipore, catalog no. ABC10, 1:500), mouse anti-LDLR (clone C7) (Novus, catalog no. NBP1-78159, 1:500), mouse anti-EAAT2 (clone 20/EAAT2) (BD, catalog no. 611654, 1:500), mouse anti-p62/SQSTM1 (clone 2C11) (Novus Biologicals, catalog no. H00008878-M01, 1:500), rabbit anti-LC3B (Novus Biologicals, catalog no. NB100-2220, 1:500), rabbit anti-pS6 (Cell Signaling, catalog no. 2211S, 1:500), chicken anti-GFP (Aves, catalog no. GFP-1020, 1:1,000), rat anti-mCherry (Invitrogen, catalog no. M11217, 1:500), rabbit anti-PSD95 (Invitrogen, catalog no. 51-6900, 1:500), guinea pig anti-VGLUT1 (Merck, catalog no. ab5095, 1:2000), rabbit anti-Gephyrin (Synaptic Systems, 147 008, 1:500), guinea pig anti-VGAT (Synaptic Systems, 131 003, 1:500), chicken anti-GFAP (Aves, GFAP, 1:1,000), rat anti-HMGB1 (R&D Systems, MAB16901-SP, 1:200) and mouse anti-beta-amyloid (clone 6E10) (BioLegend, 803001, 1:500), rabbit anti-Cx43 (Sigma, C6219, 1:500), rabbit anti-HIF1α (Novus, NB100-479s, 1:300), rabbit anti-HA (clone C29F4) (Cell Signaling, 3724, 1:500), goat anti-SPARC (R&D Systems, AF942-SP, 1:200), goat anti-APOE (Millipore, AB947, 1:100) and goat anti-Hevin (SPARCL1, R&D Systems, AF2836-SP, 1:200). The brain sections were washed five times with PBST (0.1% Tween 20 in PBS) and then incubated with the appropriate secondary antibodies: Donkey anti-Rabbit IgG (H + L) Alexa Fluor 594 (Invitrogen, catalog no. A21207, 1:500), Donkey Anti-Rabbit IgG (H + L) Alexa Fluor 405 (Abcam, catalog no. ab175649, 1:500), Donkey anti-Rabbit IgG (H + L) Alexa Fluor 647 (Invitrogen, catalog no. A31573, 1:500), Donkey anti-Rabbit IgG (H + L) Alexa Fluor 488 (Invitrogen, catalog no. A21206, 1:1,000), Goat anti-Chicken IgY (H + L) Alexa Fluor 488 (Invitrogen, catalog no. A11039, 1:1,000), Donkey Anti-Goat IgG (H + L) Alexa Fluor 405 (Abcam, catalog no. ab175665, 1:500), Donkey anti-Mouse IgG (H + L) Alexa Fluor 488 (Invitrogen, catalog no. A21202, 1:1,000), Donkey Anti-Mouse IgG H&L Alexa Fluor 405 (Abcam, catalog no. ab175658, 1:500) Donkey anti-Rat IgG (H + L) Alexa Fluor 594 (Invitrogen, catalog no. A21209, 1:500), Donkey anti-Guinea Pig IgG (H + L) Alexa Fluor 594 (Jackson Laboratory, catalog no. 706-585-148, 1:500) and Goat anti-guinea pig IgG (H + L) Alexa Fluor 405 (Abcam, catalog no. ab175678, 1:500) for 12 h at 4 °C. The sections were rewashed five times and treated with TrueBlack (Biotium) in 70% ethanol for 3 min each time at room temperature to remove autofluorescent lipofuscin. After a brief wash with PBS, the brain sections were mounted with an antifade mounting solution with or without 4,6-diamidino-2-phenylindole (DAPI) (Vectashield). TUNEL staining was performed using CF dye TUNEL Assay Apoptosis Detection Kits (Biotium).

All images were acquired with a Zeiss LSM880 Airyscan confocal microscope.

Image quantification. All images were processed using the Fiji distribution function in ImageJ software. Each imaging data was acquired from 12–16 astrocytes in the hippocampal SR region. Between two and six brain slices were imaged per mouse. Mice and images for analysis were assigned to groups randomly and blindly.

For the colocalization and comparative analysis of the upregulated proteins in astrocytes and APDAs, individual astrocyte confocal images were acquired, and each single-plane image from the confocal z-stack was evaluated with the DiAna plugin in ImageJ⁵¹.

To calculate the relative number of APDAs per the total number of astrocytes in a hippocampal SR layer of CA1 region, confocal single-plane images were merged into one z-stack image and the number of APDAs was counted and normalized by the total number of astrocytes in the region of interest (ROI). APDAs were selected as astrocytes containing more than three vacuoles with swollen processes.

To evaluate the intensity of pS6 in neurons or astrocytes, the ROIs in the CA1 neuronal layer and individual astrocytes in the hippocampal SR region were determined using the DiAna plugin and the intensity of the pS6 signals was measured in each ROI.

To measure extracellular SCRG1 protein, the ROIs containing SCRG1 puncta and astrocytes were established using the DiAna plugin and the intracellular SCRG1 puncta colocalized with astrocytes were then removed from the total SCRG1 puncta in the ROIs. The area of extracellular SCRG1 puncta was measured using the DiAna plugin, and the number of extracellular SCRG1 puncta over the distance from the astrocyte cell somas was determined using the Sholl analysis plugin⁵².

To evaluate lysosomal dysfunction, mCherry intensity was normalized to the GFP intensity in the ROI of mCherry-GFP-LC3B puncta expressed in hippocampal SR astrocytes. In normal astrocytes, ROIs were selected in the astrocytic area containing mCherry-GFP puncta. In APDAs, ROIs were selected in the astrocytic area containing mCherry-GFP puncta along with p62 aggregation. Then, mCherry (red) and GFP (green) intensities in each ROI were measured.

To quantify the astrocytic engulfment of synapses, mCherry sole puncta and mCherry-GFP colocalized puncta inside of hippocampal CA1 astrocytes were measured as previously described²². In brief, all combined images (p62, GFP, mCherry and s100 β) were divided into individual channels, then mCherry-GFP colocalized puncta images were acquired using the DiAna plugin. Then, mCherry-only puncta were determined by subtracting the mCherry-GFP colocalized puncta from the total mCherry-positive puncta. For measuring the area of the total engulfed puncta in astrocytes, mCherry-only puncta and mCherry-GFP colocalized puncta inside of astrocytes were acquired separately and combined through the DiAna plugin. Each puncta area was normalized with the area of astrocytes and the area of mCherry-GFP labeled synapses (to compensate potential difference in AAV injection). To compensate the potentially different expression levels of the AAV synaptic reporters, mCherry and GFP images were obtained with similar threshold values. The intensities of mCherry (red) and GFP (green) were measured in the ROI obtained from the mCherry-positive puncta images and the GFP-positive puncta images.

To compare the number of synapses near APDAs and normal astrocytes, five single-plane confocal images with uniform synapse images among confocal z-stack images were selected. After obtaining the thresholded images of presynapses (excitatory: VGLUT1, and inhibitory: VGAT) and postsynapses (excitatory: PSD95, and inhibitory: Gephyrin), pre- and postsynaptic puncta were counted using the DiAna plugin. The number of presynaptic only, postsynaptic only puncta as well as the number of colocalized puncta (pre- and postsynaptic together) were measured by the DiAna plugin. Pre- and postsynaptic markers should be separated by synaptic cleft (about 20 nm); however, with current confocal imaging resolution, synapses seem to show a certain degree of overlap between pre- and postsynaptic markers, which we detect and count using the DiAna plugin.

To quantify dendritic spines near astrocytes, dendrites of CA1 neurons near normal astrocytes and APDAs in *Thy1-EYFP-H* transgenic mice were imaged, and the dendritic spines were counted using RECONSTRUCT software³³. In particular, the number of dendritic spines in 10- μ m-long dendritic trees that were in close contact with the swollen processes of APDAs or the fine process of normal astrocytes was measured and compared.

Western blot analysis. Astrocytes isolated by immunopanning were transduced with lentiviruses expressing SCRG1-HA and treated with MG132 and CQ at DIV14 for 24 h. The cell culture medium was replaced with protein-free medium (50% neurobasal and 50% DMEM with 100 U ml⁻¹ penicillin, 100 μ g ml⁻¹ streptomycin, 1 mM sodium pyruvate and 292 μ g ml⁻¹ L-glutamine) and incubated for an additional 3 days. Cell pellets were collected and lysed in EzRIPA lysis buffer (Atto) containing protease inhibitors and phosphatase inhibitor cocktail (Atto). Cell supernatants were also collected, and cell debris was removed from the supernatants by centrifugation. To enrich the protein in the medium, the supernatants were concentrated approximately 30 times with a centrifugal concentrator (Vivaspin 5 kDa, GE healthcare). The protein concentration of the cell lysate pellets and supernatants was quantified by Bradford assay (Bio-Rad). The lysates and supernatants were mixed with 5 $\times$ SDS sample buffer (250 mM Tris-HCl pH 6.8, 5% 2-mercaptoethanol, 10% sodium dodecyl sulfate, 0.5% bromophenol blue and 50% glycerol) and loaded on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) gels (4–20% gels, Bio-Rad). The gels were transferred to polyvinylidene fluoride (PVDF, Atto) membranes, and the blots were incubated in blocking solution (10% skim milk in Tris-buffered saline (TBS), Dako) at room temperature for 1 h. The blots were incubated with the appropriate primary antibodies in blocking solution (5% skim milk in TBS) overnight at 4°C. The primary antibodies used for western blotting were as follows: rabbit anti-HA (Cell Signaling, catalog no. 3724, 1:1,000), goat anti-SPARC (R&D Systems, catalog no. AF942-SP, 1:500), mouse anti-Thrombospondin 2 (Clone 4) (BD, catalog no. 611150, 1:500), goat anti-Hevin (SPARCL1, R&D Systems, catalog no. AF2836-SP, 1:500) and mouse β -actin (clone C4) (SantaCruz, catalog no. sc-47778, 1:1,000). The blots were washed three times with TBST (0.1% Tween 20 in TBS) and incubated with m-IgGk BP-HRP (SantaCruz, catalog no. sc-516102, 1:1,000) mouse anti-rabbit IgG-HRP (SantaCruz, catalog no. sc-2357, 1:1,000) and Donkey anti-goat IgG-HRP (R&D Systems, catalog no. HAF109, 1:1,000) in blocking solution (5% skim milk in TBS) at RT for 2 h. The blots were re-washed three times and developed using ECL Prime western blot detection reagent (GE Healthcare). Visualization and imaging of the blots were performed with ChemiDoc XRS+ (Bio-Rad). Data quantification was performed with Fiji software.

EM imaging and data analysis. Tissue preparation for scanning electron microscopy. APP/PS1 Alzheimer's disease model mice were anesthetized and perfused with 0.15 M cacodylate buffer (pH 7.4) and then pre-fixed with 2% PFA/2.5% glutaraldehyde in 0.15 M cacodylate buffer. The brain was fixed with same solution overnight at 4°C and sectioned into 150- μ m-thick slices using a

vibratome (Leica). Fixed tissues were postfixed for scanning electron microscopy (SEM) in 2% osmium tetroxide (OsO₄; EMS) and 1.5% potassium ferrocyanide (Sigma) in 0.15 M cacodylate buffer for 1 h on ice. The tissues were stained with 1% thiocarbohydrazide (TCH; TCI) for 20 min and 2% OsO₄ in distilled water for 30 min at room temperature. Thereafter, the tissues were placed in 2% uranyl acetate (EMS) overnight at 4°C and incubated in the lead aspartate solution for 30 min. The tissues were dehydrated with a graded ethanol series (20%, 50%, 70%, 90% and 100%) on ice. After dehydrating with gradient ethanol, the samples were infiltrated with 100% acetone, mixture of acetone/epoxy resin (3:1.1:1.1:3), 100% resin and then embedded with an EMBED-812 embedding kit (EMS) following the manufacturer's instructions.

Imaging APDA in hippocampus of APP/PS1 Alzheimer's disease model mice using SEM. For imaging of APDA in hippocampus, serial sections (100 nm) were prepared from the embedded samples using an ultramicrotome (EM UC7; Leica) and mounted on indium-tin-oxide (ITO)-coated glass coverslips. The serial sections were imaged by the Zeiss Atlas5 software for Gemini 300 SEM (Zeiss) at an accelerating voltage of 3.5 kV. Astrocyte with amyloid- β plaque in their process was visualized the SR region about 100–150 μ m from the hippocampal CA1 pyramidal cell. Image resolution in the x-y plane was 5 nm per pixel. A total of 83 SEM images from the APP/PS1 mice were fine-tune aligned using the Fiji software TrakEM2 plug-in. To visualize 3D structure, APDAs in the hippocampus were tracked manually and 3D structure was reconstructed by Reconstruct software.

Analysis of damaged mitochondria in APDA using SEM. The number of normal and damaged mitochondria in astrocytes was counted in ten serial-EM images. For analysis of mitochondria, the efficient and unbiased stereological counting method was applied³⁴. The number of normal and damaged mitochondria per 100 μ m² from APDAs and normal astrocytes was measured by using unbiased counting frame with Reconstruct software.

Statistics and reproducibility. Statistical analysis was performed using GraphPad Prism v.7 software. For comparisons between two samples, data were analyzed by unpaired Student's *t*-test or multiple *t*-tests. Statistical significance determined using Holm-Sidak method in multiple *t*-test analysis was $\alpha = 0.05$. We analyzed each row individually, without assuming a consistent s.d.

For comparisons between more than two samples, data were analyzed by one-way or two-way analysis of variance (ANOVA) with Tukey's multiple comparisons test. The data are presented as the mean values $\pm$ s.e.m. A 95% confidence interval was used for statistical comparisons (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$). Statistical outliers were identified using the ROUT test with $Q = 1\%$. Data distribution was assumed to be normal but this was not formally tested. The imaging experiment and western analysis were repeated three or more times, and the proteasome activity assay was repeated twice. The results were reproducible.

No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to previous studies, including our own^{22,35}. A minimum of three mice in each group were used in vivo and a minimum of five independent batches in vitro.

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

Data availability

The raw bulk RNA-seq data are available at the Gene Expression Omnibus under the accession code [GSE183042](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE183042). Source data is provided with this document. All other data supporting the findings of this study are available from the corresponding author upon reasonable request.

Received: 9 September 2021; Accepted: 21 June 2022;

Published online: 01 August 2022

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Acknowledgements

We thank all members in Chung's laboratory for helpful discussion. This work was supported by grants from the Samsung Science and Technology Foundation (SSTF-BA1701-18, W.-S.C.). This work was also supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (Ministry of Science and ICT, MSIT) [2022R1A2C1009376 (J.Y.M.)], KBRI basic research program [22-BR-01-03 (J.Y.M.)] funded by MSIT, the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI) and Korea Dementia Research Center (KDRC), funded by the Ministry of Health and Welfare (MOHW) and MSIT, Republic of Korea (HU20C0290) and International Collaboration Initiative grant (KAIST-N11210255) supported by KAIST. scRNA-seq experiments were conducted with help from MacroGen and Amyloid Solution Inc. Imaris software was provided by KAIST Bio Core Center.

Author contributions

W.-S.C., E.L. and Y.-J.J. designed the project. E.L. performed all the experiments including primary cell isolation, plasmid cloning, virus production, cell culture, immunohistochemistry, confocal imaging and western blot. Y.-J.C. performed stereotaxic injection with virus and drugs. S.Y.L. provided the brain slices injected with virus and performed behavior analysis. Y.R.P. and J.Y.M. performed electron microscopy experiments. S.L. and C.H.K. performed scRNA-seq analysis. W.-S.C. and E.L. wrote the paper.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s43587-022-00257-1>.

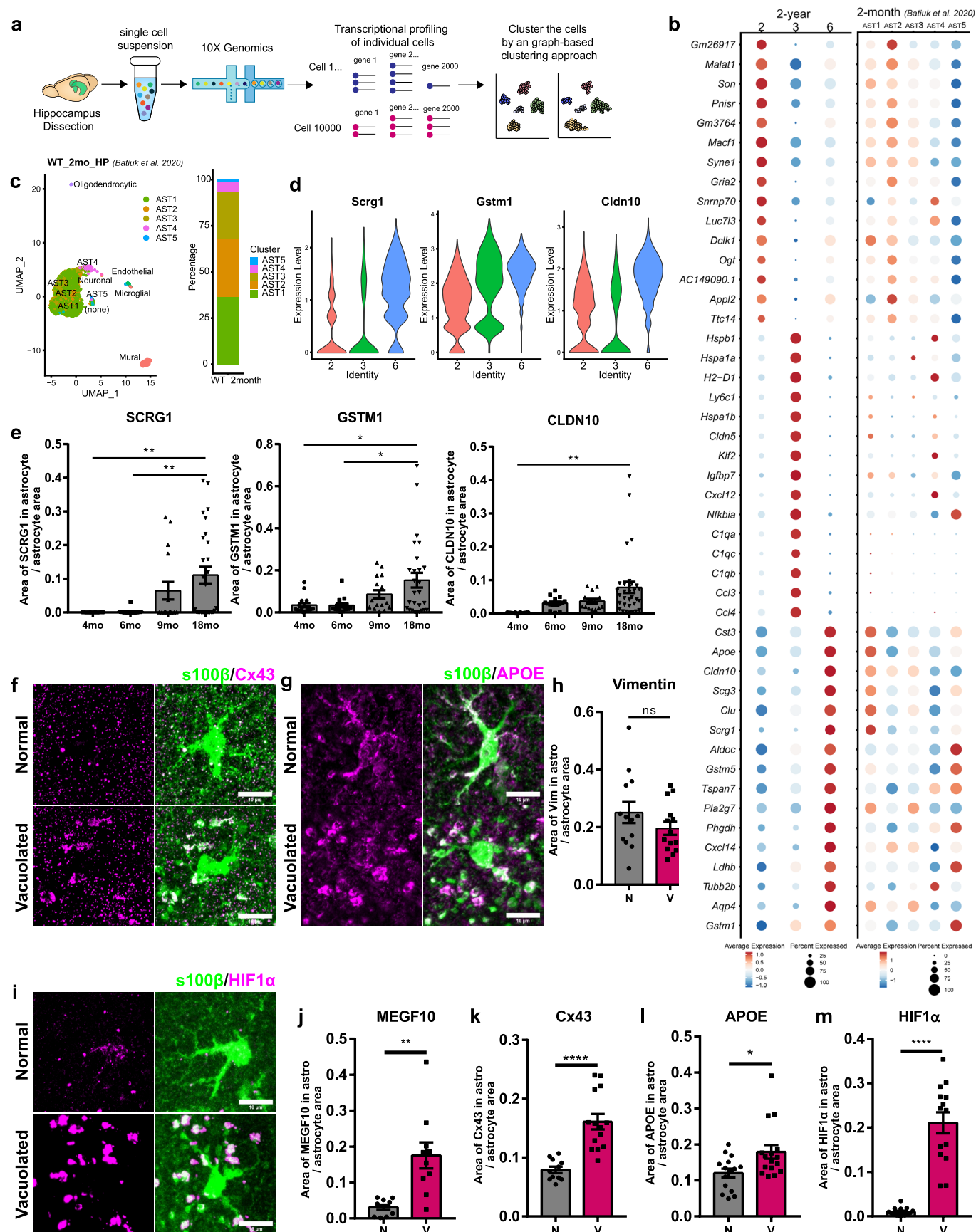
Correspondence and requests for materials should be addressed to Won-Suk Chung.

Peer review information *Nature Aging* thanks Andras Lakatos, Cagla Eroglu and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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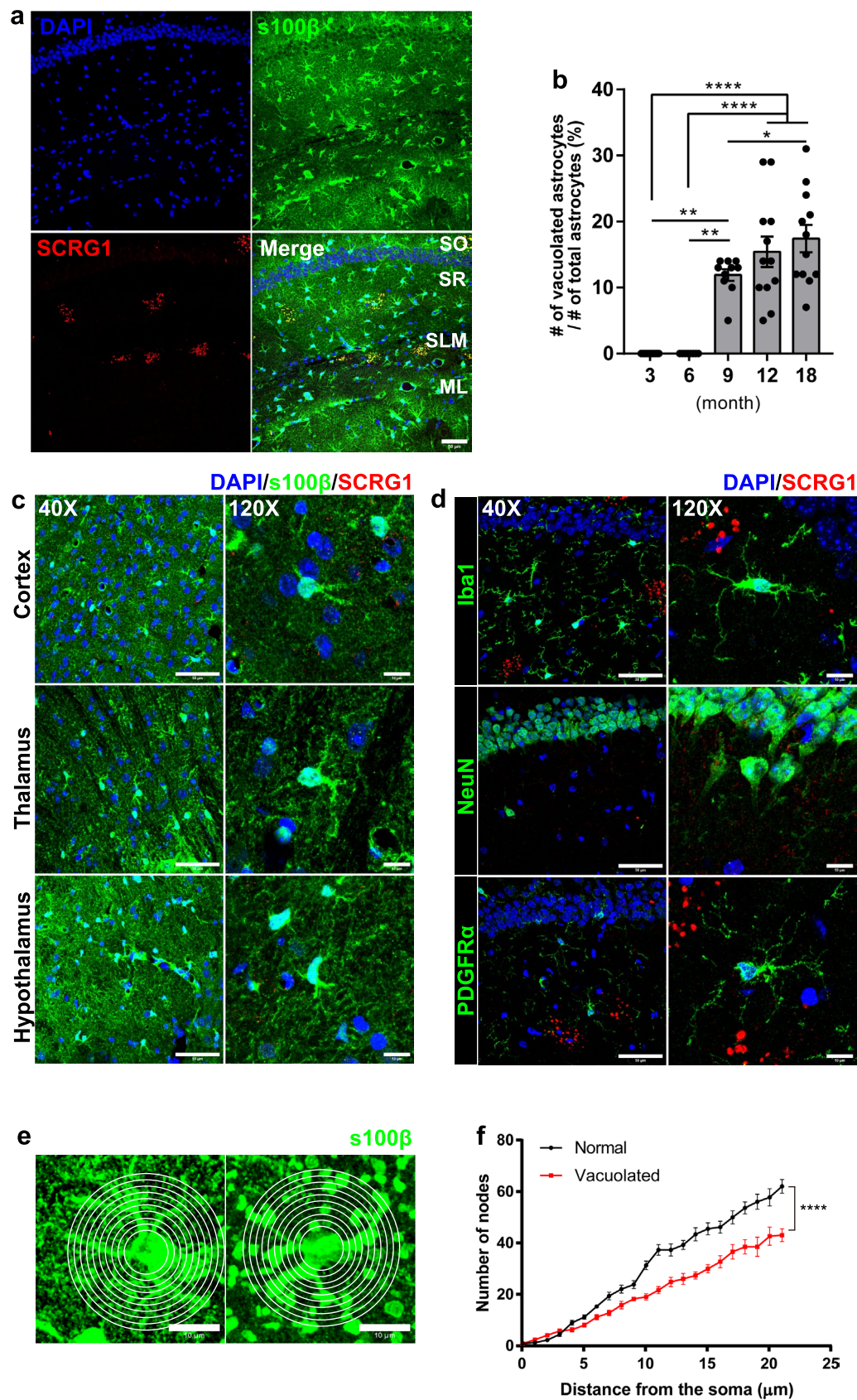
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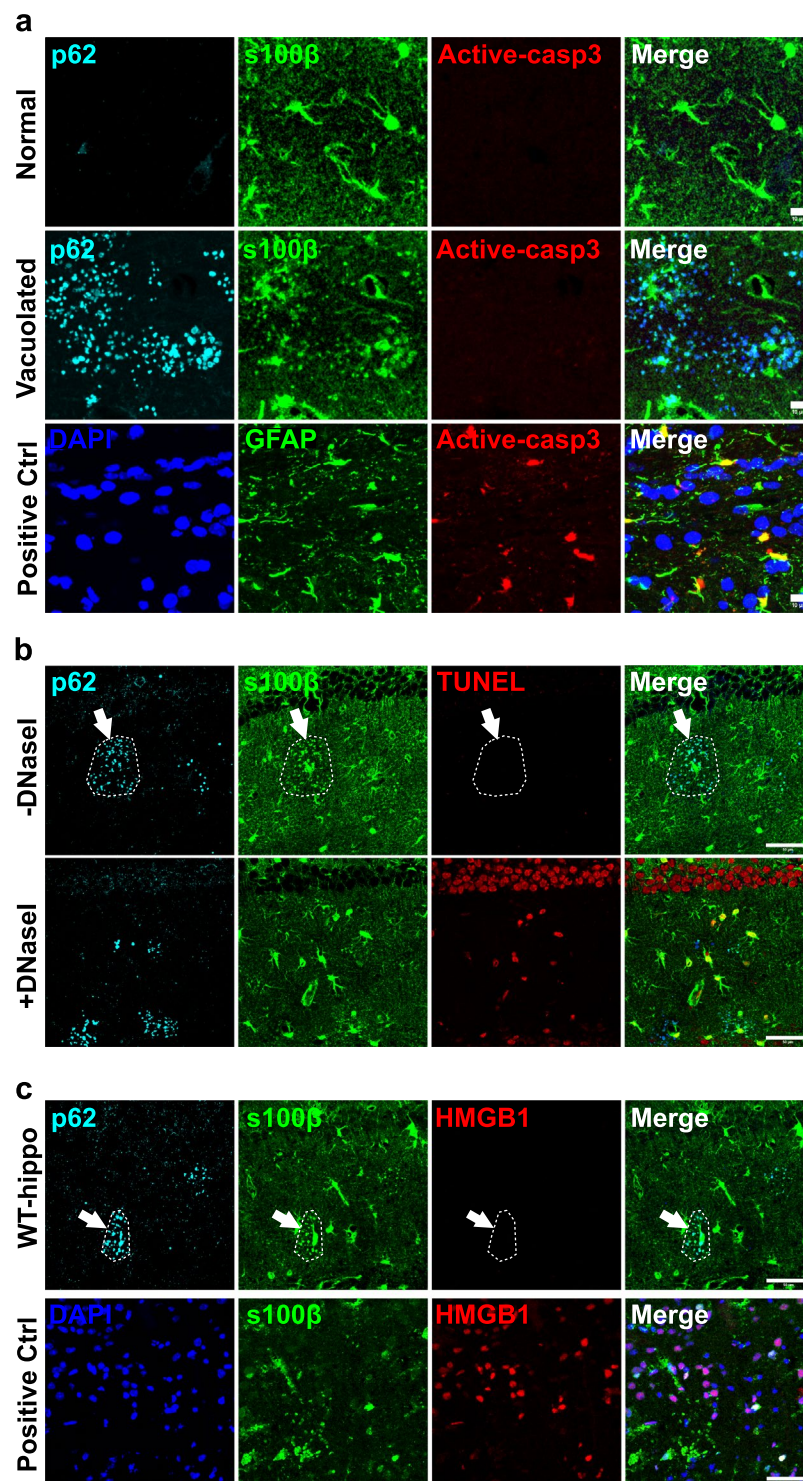
Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | Investigation of vacuolated astrocytes in the young and aged hippocampus. **a**, Schematic illustration of the scRNA-seq experiments. **b**, Comparison of upregulated genes in astrocyte clusters from 2-year-old hippocampi with scRNA-seq dataset obtained from 2-month-old hippocampus. Note that characteristic gene expression changes in cluster 3 and 6 are unique to the aged hippocampus. **c**, A UMAP plot of single cells from the 2-month-old hippocampi (left panel). Bar graph showing the relative percentages of five different astrocyte clusters (right panel). **d**, Violin plots showing the expression of *Scrg1*, *Gstm1* and *Cldn10* in cluster 2, 3 and 6 from 2-year-old hippocampi. **e**, Quantification of SCRG1, GSTM1 and CLDN10 areas normalized by astrocyte area in 4-, 6-, 9- and 18-month-old WT hippocampi ($n=3$, dots on the bar graph indicate data obtained from individual ROIs). **f,g,i** Representative confocal z-stack images of normal and vacuolated astrocytes obtained from 18-month-old WT hippocampi stained for s100 β (green), Cx43 (**f**, red), APOE (**g**, red) and HIF1 α (**i**, red). These proteins are accumulated in the swollen processes of vacuolated astrocytes. Scale bar, 10 μ m. **h,j-m**, Quantification of vimentin (**h**), MEGF10 (**j**), Cx43 (**k**), APOE (**l**) and HIF1 α (**m**) areas normalized by astrocyte area in 18-month-old WT hippocampi ($n=5$ mice, dots on the bar graphs represent data obtained from individual cells). **e**, One-way ANOVA followed by Tukey's multiple comparison test. The P -values are indicated in the source data file. **h,j-m**, Two-tailed Student's t -test. $P=0.21$ (**h**), $**P=0.001$ (**j**), $****P<0.0001$ (**k**), $*P=0.015$ (**l**), $****P<0.0001$ (**m**). ns, not significant. Mean $\pm$ s.e.m.

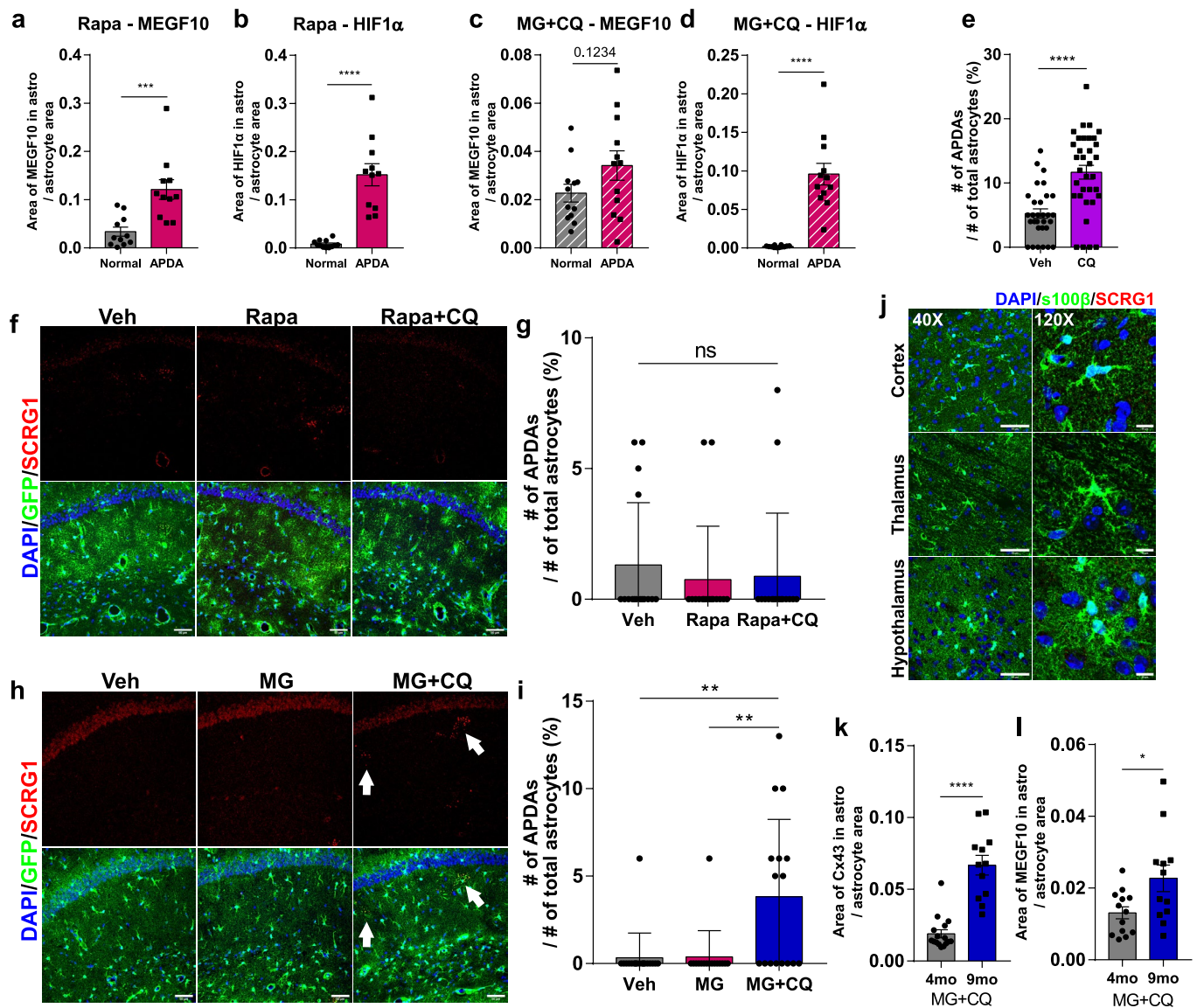


Extended Data Fig. 2 | See next page for caption.

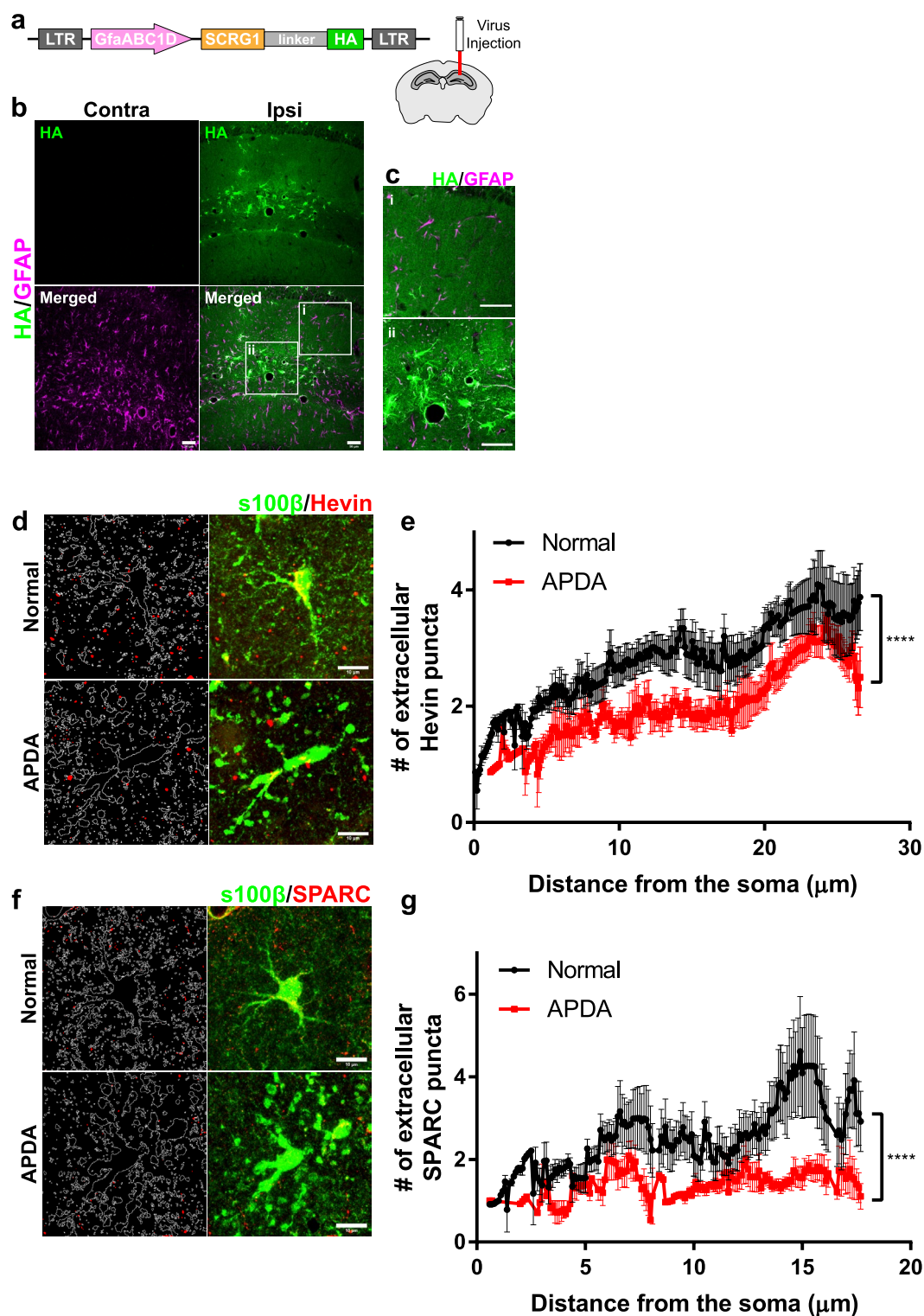
Extended Data Fig. 2 | Selective appearance of vacuolated astrocytes in the hippocampus. **a**, Representative confocal z-stack images of 18-month-old hippocampi stained for nuclei (DAPI, blue), s100 β (green) and SCRG1 (red). Astrocytes with upregulated SCRG1 expression were found only in the SR and SLM layers of the hippocampus. Scale bar, 50 μ m. **b**, Quantification of the number of vacuolated astrocytes normalized to the total number of astrocytes in the imaging fields ($n=3$, dots on the bar graph indicate data obtained from individual ROIs). **c**, Representative magnified confocal z-stack images showing the absence of SCRG1 (red)-expressing cells in other brain regions, such as the cortex, thalamus and hypothalamus. **d**, Representative magnified confocal z-stack images showing the absence of SCRG1 (red) expression in other cell types, such as microglia (Iba1, upper), neurons (NeuN, middle) and oligodendrocyte precursor cells (PDGFR α , lower). For both **c** and **d**, the left panels show representative images taken at $\times 40$ magnification (scale bar, 50 μ m), and the right panels show representative single astrocytes (s100 β , green) taken at $\times 120$ magnification (scale bar, 10 μ m). DAPI (blue) stains the nucleus. **e**, Sholl analysis of normal (left) and vacuolated (right) astrocytic processes stained for s100 β (green). The concentric circles were placed at 1 μ m apart, with the astrocytic soma in the middle (white lines). **f**, Quantification of the number of nodes where the processes intersect with the concentric circles ($n=3$, ROIs obtained from twelve images). **g**, One-way ANOVA followed by Tukey's multiple comparisons test. **h**, Two-way ANOVA followed by Sidak's multiple comparisons test. The P -values are indicated in the source data file (**b,h**). Mean $\pm$ s.e.m.



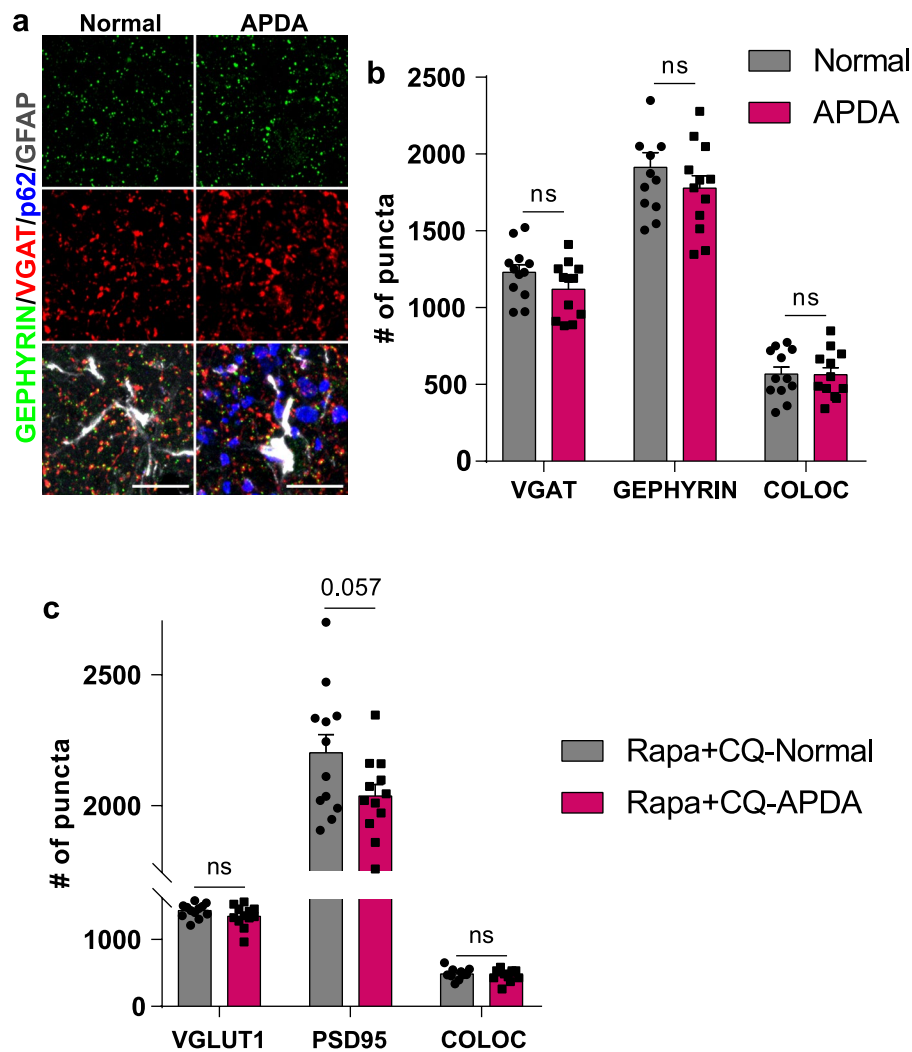
Extended Data Fig. 3 | Vacuolated astrocytes were neither apoptotic nor necrotic. **a**, Representative confocal z-stack images of 18-month-old hippocampi showing normal astrocytes (upper panels) and vacuolated astrocytes (middle panels). Autophagosomes (p62, cyan) accumulated in vacuolated astrocytes (s100β, green). However, these vacuolated astrocytes were not positive for activated caspase 3 signals (red). Lower panels were representative confocal images from 4-month-old cortex injected with ATP stained for nuclei (DAPI, blue), GFAP (green) and active caspase-3 (red). Scale bar, 10 μm. **b**, TUNEL staining of brain sections from 18-month-old WT mice along with a positive control of DNase I-treated brain sections (lower panels) stained for s100β (green) and p62 (cyan). APDA marked with a white arrow was not positive for TUNEL staining (red). Scale bar, 50 μm. **c**, Representative confocal z-stack images of 18-month-old hippocampi (upper panels) stained for p62 (cyan), s100β (green) and HMGB1 (red). APDA marked with a white arrow was not positive for HMGB1, a marker for necrosis. Lower panels were representative confocal images from 4-month-old cortex injected with ATP stained for nuclei (DAPI, blue), s100β (green) and HMGB1 (red). Scale bar, 50 μm. Dotted lines indicate APDA's territory (**b-c**).



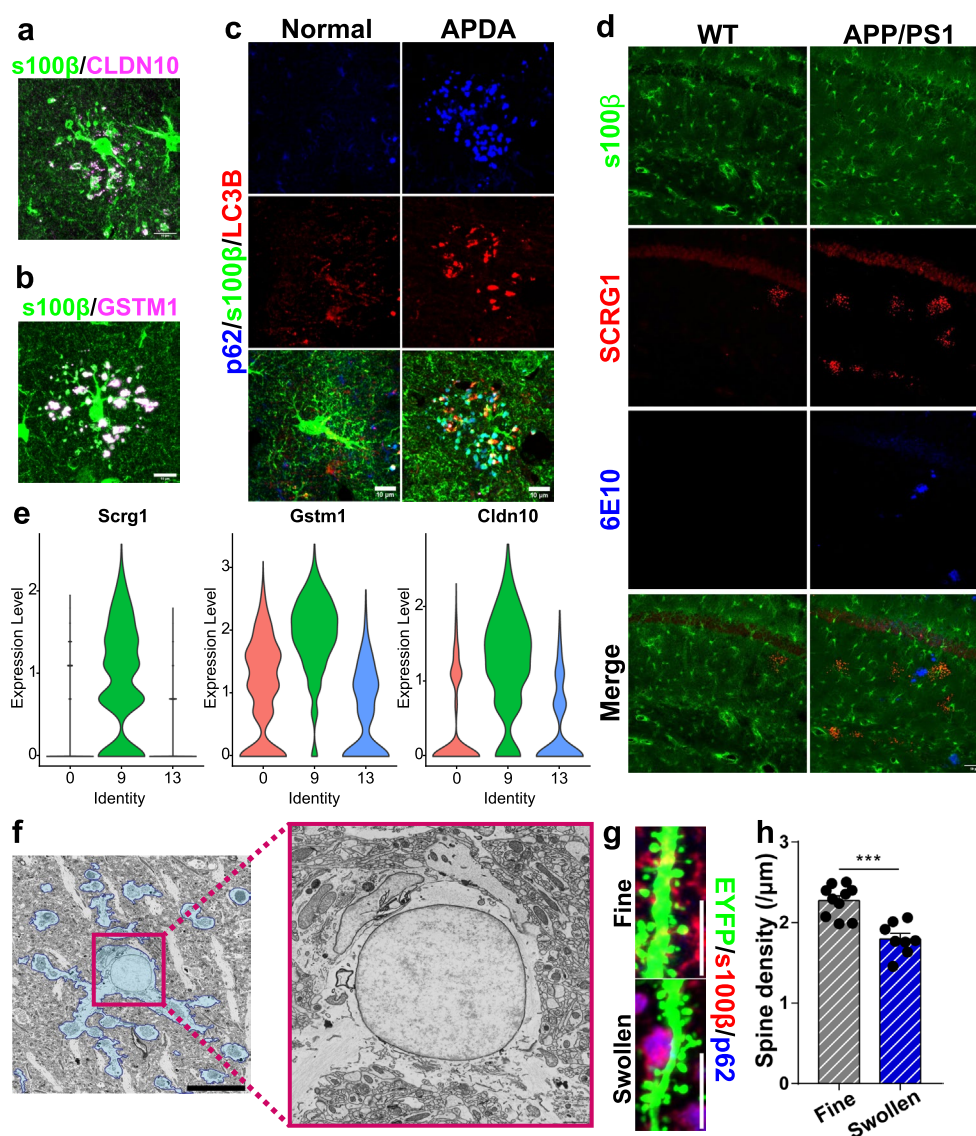
Extended Data Fig. 4 | Age dependent appearance of APDAs by mTOR and proteasome inhibition. **a–d**, Quantification of MEGF10 (**a,c**) and HIF1α (**b,d**) areas normalized by astrocyte area in the imaging fields. Normal astrocytes and APDAs were obtained from 9-month-old *Aldh1l1-eGFP* hippocampi injected with rapamycin (Rapa, **a,b**), and MG132 and CQ (MG+CQ, **c,d**). MEGF10 (**a,c**) and HIF1α (**b,d**) were highly upregulated in APDAs compared to normal astrocytes. **e**, Quantification showing the relative number of APDAs per total astrocytes in the SR hippocampal layers of 9-month-old *Aldh1l1-eGFP* hippocampi injected with CQ ($n=3$, dots on the bar graphs indicate data obtained from individual ROIs). ($n=3$ mice, dots on the bar graphs indicate data obtained from individual ROIs). **f**, Representative confocal z-stack images of 4-month-old *Aldh1l1-eGFP* hippocampi injected with vehicle (Veh, left), rapamycin (Rapa, middle), rapamycin and chloroquine (Rapa+CQ, right) stained for nuclei (DAPI, blue), GFP (green) and SCRG1 (red). Scale bar, 50 μ m. **g**, Quantification of the relative number of APDAs per the total number of astrocytes in the SR hippocampal layers ($n=4$ mice; dots on the bar graphs indicate data obtained from individual ROIs). **h**, Representative confocal z-stack images of 4-month-old *Aldh1l1-eGFP* hippocampi injected with vehicle (Veh, left), MG132 (MG, middle), MG132 plus chloroquine (MG+CQ, right) and stained for nuclei (DAPI, blue), GFP (green) and SCRG1 (red). Scale bar, 50 μ m. White arrows indicate APDAs in the hippocampus injected with MG+CQ. **i**, Quantification showing the relative number of APDAs per the total number of astrocytes in the SR hippocampal layers ($n=3$ mice; dots on the bar graphs indicate data obtained from individual ROIs). **j**, Representative magnified confocal z-stack images showing the absence of SCRG1 (red)-expressing cells in other brain regions, such as the cortex, thalamus and hypothalamus of 9-month-old *Aldh1l1-eGFP* hippocampi injected with MG132 and CQ. **k–l** Quantification of Cx43 (**k**) and MEGF10 (**l**) areas normalized by astrocyte area. Cx43 and MEGF10 were highly upregulated in 9-month-old *Aldh1l1-eGFP* mice injected with MG132 and CQ compared to 4-month-old mice ($n=3$ for 9-month-old and $n=4$ for 4-month-old mice, dots on the bar graphs indicate data obtained from individual ROIs). **a–e,k,l** two-tailed Student's *t*-test. *** $P=0.0009$ (**a**), **** $P<0.0001$ (**b**), $P=0.1234$ (**c**), **** $P<0.0001$ (**d**), **** $P<0.0001$ (**e**), **** $P<0.0001$ (**k**), * $P<0.023$ (**l**). **g,i** One-way ANOVA followed by Tukey's multiple comparisons test. The *P*-values are indicated in the source data file (**g,i**). Mean $\pm$ s.e.m.



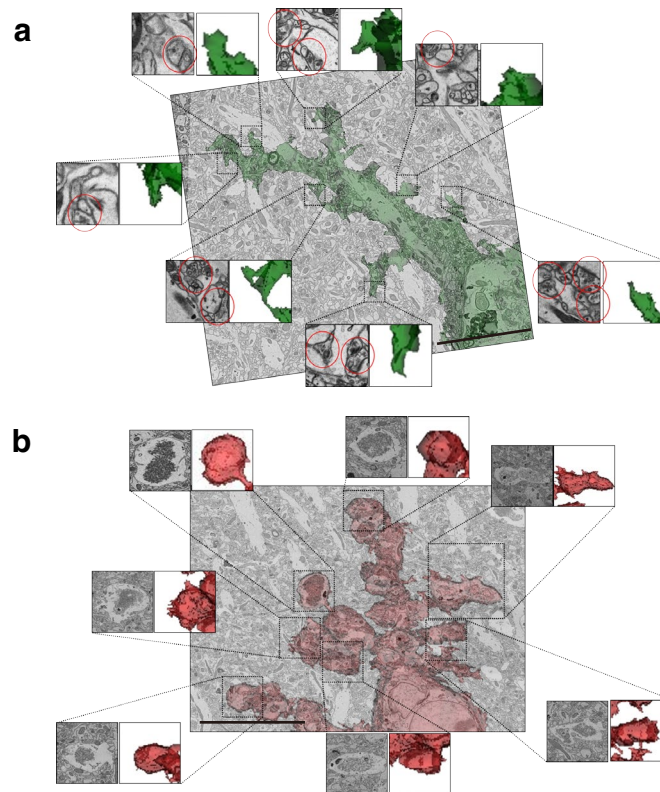
Extended Data Fig. 5 | In vivo secretion of SCRG1, Hevin and SPARC from normal astrocytes and APDAs. **a**, Schematic diagram of a lentiviral construct expressing SCRG1-HA under the *Gfap* promoter injected into the hippocampal CA1 region. **b**, Representative confocal z-stack images of contralateral and ipsilateral hippocampal regions injected with virus expressing SCRG1-HA. Extracellular SCRG1-HA (HA, green) signal was found outside of SCRG1-HA-expressing astrocytes (GFAP, red) on the ipsilateral side of the hippocampus. Scale bar, 50 μ m. **c**, Enlarged images of the white boxes shown in **b**. The signal of SCRG1-HA (HA, green) can be found in regions (i) where there were no SCRG1-HA-expressing astrocytes (ii, images of astrocytes expressing SCRG1-HA). Scale bar, 50 μ m. **d,f** Representative confocal z-stack images of normal astrocytes and APDAs (s100 β , green) stained for Hevin (**d**, red) and SPARC (**f**, red) in the 18-month-old hippocampi. **e,g**, The number of extracellular Hevin (**e**) and SPARC (**g**) puncta were plotted based on the distance from the soma. ($n=12$ cells examined over three mice) Unpaired Student's *t*-test. **** $P < 0.0001$ (**e,g**), Mean $\pm$ s.e.m.



Extended Data Fig. 6 | The number of inhibitory synapses in 18-month-old mice and the number of excitatory synapses in 9-month-old mice injected with rapamycin and CQ are not changed near APDAs. a, Representative confocal z-stack images of inhibitory synapses stained for VGAT (red) and Gephyrin (green) around normal astrocytes and APDAs. Astrocytes were stained with GFAP (gray), and APDAs were recognized by p62 (blue) accumulation. Scale bar, 10 μ m. **b,** Quantification of the number of inhibitory synapses around normal astrocytes and APDAs. (n = 3 mice, dots on the bar graph indicate data obtained from individual ROIs). **c,** Quantification of the number of excitatory synapses around normal astrocytes and APDAs in the hippocampi of 9-month-old mice injected with rapamycin and CQ (n = 3 mice, dots on the bar graph indicate data obtained from individual ROIs). **b,c** multiple *t*-tests followed by Two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli. The *P*-values are indicated in the source data file (**b,c**). Mean $\pm$ s.e.m.



Extended Data Fig. 7 | Effects of AD APDAs on neighboring synapses in APP/PS1 mice. **a,b** Representative confocal z-stack images of APDAs in 18-month-old APP/PS1 hippocampi stained for CLDN10 (**a**, red), GSTM1 (**b**, red) and *s100β* (green). Scale bar, 10 μm. **c**, Representative confocal z-stack images of normal astrocytes and APDAs (*s100β*, green) stained with p62 (blue) and LC3B (red) in the hippocampi of 18-month-old APP/PS1 mice. Scale bar, 10 μm. **d**, Representative confocal z-stack images of the 18-month-old WT (left panels) and APP/PS1 (right panels) hippocampi stained for *s100β* (green), SCRG1 (red) and 6E10 (blue). Scale bar, 50 μm. **e**, Violin plots showing the expression of *Scrg1*, *Gstm1* and *Cldn10* in astrocyte clusters from 2-year-old APP/PS1 mice. **f**, Representative electron microscopy images showing nucleus inside an AD APDA (light blue). The lower panel of the enlarged image of nucleus show neither apoptotic nor necrotic feature such as large chromatin clump or nuclear condensation/fragmentation. **g**, Representative confocal z-stack images of dendrites (EYFP, green) in contact with the fine processes of astrocytes (upper panel) and swollen processes of APDAs (lower panel) from 18-month-old *Thy1-EYFP-H*; APP/PS1 mice. The processes of astrocytes were labeled with *s100β* (red), and swollen processes were labeled with p62 (blue). Scale bar, 10 μm. **h**, Quantification of the number of dendritic spines normalized to dendrite length ($n=6$ mice, dots on the bar graph indicate data obtained from individual ROIs). **h**, two-tailed Student's *t*-test. *** $P=0.0001$ Mean $\pm$ s.e.m.



Extended Data Fig. 8 | Ultra-fine 3D structures of astrocytic processes from normal astrocytes and APDAs. a,b, Representative electron microscopy images showing processes of normal astrocyte (**a**, green) and APDA (**b**, red) from 18-month-old APP/PS1 hippocampi. Insets show magnified views of astrocytic processes. Normal astrocytic processes show close physical association with synapses (**a**, red circles), whereas APDA's vacuolated processes fail to form contacts with synapses (**b**). Scale bar, 10 μ m.

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| ☐ | ☒ 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 |

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Software and code

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Data collection	A LUNA-FL™ Automated Fluorescence Cell Counter (logos biosystems) was used in consultation with the 10x Genomics Single Cell Protocols: Cell Preparation Guide and the Guidelines for Optimal Sample Preparation flowchart (Documents CG00053 and CG000126, respectively) to prepare cells. Libraries were prepared using a Chromium controller according to the 10x Chromium Next GEM Single Cell 3' v3.1.1 protocol (CG000204). The cell suspension was mixed with Master Mix and loaded with Single Cell 3'V3.1 Gel Beads and partitioning oil into Chromium Next GEM chip G. The purified libraries were quantified using qPCR according to the qPCR Quantification Protocol Guide (KAPA) and qualified using an Agilent Technologies 4200 TapeStation (Agilent Technologies). Then, the libraries were sequenced using a HiSeq platform (Illumina) according to the read length, as specified in the Illumina user guide. ZEN software (ZEISS Image acquisition software, v2.3), ZEISS Atlas5 software for Gemini 300 SEM
Data analysis	Genomics; https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/what-is-cell-ranger). Briefly, raw BCL files obtained from Illumina HiSeq sequencing were demultiplexed to generate FASTQ files using the 'cellranger (v3.1.0) mkfastq' pipeline. Then, the raw FASTQ files were analyzed using 'cellranger count'. Raw count matrices from wild type data were imported into the Seurat 4.0.3 software package. Image J (NIH, v1.52p) and its plugin (e.g. DiAna, TrakEM2, Sholl analysis), IMARIS (Bitplane, v9.2), GraphPad Prism (v7.0), EM image reconstruct software (https://synapseweb.clm.utexas.edu/software-0 , Version 1.1.0.0, 2007), RECONSTRUCT software (v1.1.0.0)

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

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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
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Raw and processed scRNA-seq data are available on the Gene Expression Omnibus (GEO) under the accession GSE183042. And the published young wildtype mouse scRNA-seq data we used is available under the accession GSE114000.

Full list of antibodies used in this study are described in method sections.

All data that support the findings of this study are available from the corresponding author upon reasonable request.

Field-specific reporting

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Life sciences study design

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

Sample size	No statistical method was used to predetermine the sample sizes, but our sample size per group was determined from previous publications using similar methods. A minimum of 3 mice in each group were used in vivo and a minimum of 5 independent batches in vitro. The sample size was based on estimation from previous studies, including our own published studies (Lee et al, Nature 2020 & Park et al. Embo J 2021).
Data exclusions	For scRNA-seq analysis, no animal or sample was excluded from the analysis. As routinely done, specific single cell RNA-seq libraries were excluded based on low technical quality (low number of transcripts and genes captured) or after identification of these libraries as doublets. For imaging analysis, Data were excluded out based on outliers test (Grubbs' test, 95% confidence) supplied by GraphPad.
Replication	Biological replicates (three different mice) of the scRNA-seq libraries were made, and used as validations. For imaging analysis, each imaging data was acquired from 12-16 astrocytes in the hippocampus. Two to six brain slices were imaged per mouse. Experiments (imaging, western, and proteasome activity assay) were repeated at least twice and the results were reproducible.
Randomization	Mice and images for analysis were randomly allocated into groups.
Blinding	During experimentation and analysis, all experiments were performed blindly.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	rabbit anti-SCRG1 (Proteintech, 14038-1-AP, 1:500), rabbit anti-GSTM1 (Proteintech, 12412-1-AP, 1:200), rabbit anti-CLDN10 (Thermo, 38-8400, 1:100), rabbit anti-s100 β (Abcam, ab52642, 1:500), guinea pig anti-s100 β (Synaptic Systems, 287 004, 1:500), mouse anti-NeuN (clone A60) (Merck, MAB377, 1:500), goat anti-Ibal (Novus Biologicals, NB100-1028, 1:500), goat anti-PDGFR α
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(R&D Systems, AF1062-SP, 1:300), rabbit anti-Rab5 (Abcam, ab18211, 1:300), rat anti-LAMP2 (Abcam, ab13524, 1:300), rabbit anti-ubiquitin (Cell Signaling, CST 3933, 1:500), rabbit anti-MEGF10 (Millipore, ABC10, 1:500), mouse anti-LDLR (clone C7) (Novus, NBP1-78159, 1:500), mouse anti-EAAT2 (clone 20/EAAT2) (BD, 611654, 1:500), mouse anti-p62/SQSTM1 (clone 2C11) (Novus Biologicals, H00008878-M01, 1:500), rabbit anti-LC3B (Novus Biologicals, NB100-2220, 1:500), rabbit anti-pS6 (Cell Signaling, 2211S, 1:500), chicken anti-GFP (Aves, GFP-1020, 1:1000), rat anti-mCherry (Invitrogen, M11217, 1:500), rabbit anti-PSD95 (Invitrogen, 51-6900, 1:500), guinea pig anti-VGLUT1 (Merck, ab5095, 1:2000), rabbit anti-Gephyrin (Synaptic Systems, 147 008, 1:500), guinea pig anti-VGAT (Synaptic Systems, 131 003, 1:500), chicken anti-GFAP (Aves, aves-GFAP, 1:1000), rat anti-HMGB1 (R&D Systems, MAB16901-SP, 1:200) and mouse anti-beta-amyloid (clone 6E10) (BioLegend, 803001, 1:500), rabbit anti-Cx43 (Sigma, C6219, 1:500), rabbit anti-HIF1 α (Novus, NB100-479ss, 1:300), rabbit anti-HA (clone C29F4) (Cell Signaling, 3724, 1:500), goat anti-SPARC (R&D Systems, AF942-SP, 1:200), goat anti-APOE (Millipore, AB947, 1:100) and goat anti-Hevin (SPARCL1, R&D Systems, AF2836-SP, 1:200), mouse β -actin (clone C4) (Santacruz, sc-47778, 1:1000), mouse anti-Thrombospondin 2 (Clone 4) (BD, 611150, 1:500)

Donkey anti-Rabbit IgG (H+L) Alexa Fluor 594 (Invitrogen, A21207, 1:500), Donkey Anti-Rabbit IgG (H+L) Alexa Fluor 405 (Abcam, ab175649, 1:500), Donkey anti-Rabbit IgG (H+L) Alexa Fluor 647 (Invitrogen, A31573, 1:500), Donkey anti-Rabbit IgG (H+L) Alexa Fluor 488 (Invitrogen, A21206, 1:1000), Goat anti-Chicken IgY (H+L) Alexa Fluor 488 (Invitrogen, A11039, 1:1000), Donkey AntiGoat IgG (H+L) Alexa Fluor 405 (Abcam, ab175665, 1:500), Donkey anti-Mouse IgG (H+L) Alexa Fluor 488 (Invitrogen, A21202, 1:1000), Donkey Anti-Mouse IgG H&L Alexa Fluor 405 (Abcam, ab175658, 1:500) Donkey anti-Rat IgG (H+L) Alexa Fluor 594 (Invitrogen, A21209, 1:500), Donkey anti-Guinea Pig IgG (H+L) Alexa Fluor 594 (Jackson lab, 706-585-148, 1:500), Goat anti-guinea pig IgG (H+L) Alexa Fluor 405 (abcam, ab175678, 1:500), m-IgGk BP-HRP (Santacruz, sc-516102, 1:1000) mouse anti-rabbit IgG-HRP (Santacruz, sc-2357, 1:1000), Donkey anti-goat IgG HRP (R&D Systems, HAF109, 1:1000)

Validation

Staining with secondary antibody alone was used as a negative control to perform full validation and exclude non-specific staining.

anti-SCRG1 (Proteintech) and anti-CLDN10 (Thermo) are validated by manufacturer for immunostaining.

anti-GSTM1 (Proteintech) was validated for immunostaining by Kano et al. Science Signaling (2019).

anti-s100 β (Abcam) was validated for immunostaining by Williamson MR et al. Cell Rep (2021).

anti-s100 β (Synaptic Systems) was validated for immunostaining by Lee et al. Nature (2021).

anti-NeuN (Merck) was validated for immunostaining by Saunders, A et al. eLife (2015).

anti-Iba1 (Novus) was validated for immunostaining by Fang et al. Nat. Neurosci (2019).

anti-PDGFR α (R&D systems) was validated for immunostaining by Gibson et al. Science (2014).

anti-Rab5 (Abcam) was validated for immunostaining by Pensalfini A et al. Cell Rep (2020).

anti-LAMP2 (Abcam) was validated for immunostaining by Chen J et al. Nature (2018).

anti-ubiquitin (Cell Signaling) was validated for immunostaining by Jingyu Yao, et. al. Autophagy (2015).

anti-MEGF10 (Millipore) was validated for immunostaining by Morizawa, Y. M. et al. Nat. Comm (2017).

anti-LDLR (Novus) was validated for immunostaining by Lebeau et al. J. Biol. Chem (2018).

anti-EAAT2 (BD) was validated for immunostaining by Sosunov et al. J. Neurosci (2014).

anti-p62/SQSTM1 (Novus) was validated for immunostaining by Schweitzer GG et al. FASEB J (2018).

anti-LC3B (Novus) was validated for immunostaining by Wang IF et al. Proc Natl Acad Sci U S A (2012).

anti-pS6 (Cell Signaling) was validated for immunostaining by Anne Biever, et. al. J Neurosci (2015).

anti-GFP (Aves) was validated for immunostaining by Dhaliwal et al. Sci Rep (2018).

anti-mCherry (Invitrogen) was validated for western blot by manufacturer.

anti-PSD95 (Invitrogen) was validated for western blot by manufacturer.

anti-VGLUT1 (Merck) was validated for immunostaining by Turner et al. Nature (2015).

anti-Gephyrin (Synaptic Systems) was verified through KO experiments by manufacturer.

anti-VGAT (Synaptic Systems) was validated for western blot, ICC and IHC by manufacturer. Reacts with: rat (O35458), mouse (O35633), zebrafish, ape.

anti-GFAP (Aves) was verified through KO experiments by manufacturer.

anti-HMGB1 (R&D Systems) was validated for western blot by manufacturer. Detects human, mouse, and rat HMGB1/HMG-1 in Western blot.

anti-beta-amyloid (clone 6E10) (BioLegend) was validated for western blot and immunostaining by Forny-Germano L, et al. J Neurosci (2014).

anti-Hevin (R&D Systems) was validated for western blot and immunostaining by Jong-Heon Kim et al. Cell Death Differ (2021).

anti-SPARC (R&D Systems) was validated for western blot and immunostaining by manufacturer.

anti-HA (Cell Signaling) was validated for western blot and immunostaining by manufacturer.

anti-beta-actin (Santa Cruz) was validated for western blot by Yanxiang Meng et al. Nat Comm (2021).

anti-HIF1 α (Novus) was validated for immunostaining by Maggie A Khoo et al. Sci Rep (2021).

anti-Cx43 (Sigma) was validated for immunostaining by W C Sin et al. Oncogene (2015).

anti-APOE (Millipore) was validated for immunostaining by manufacturer.

anti-Thrombospondin2 (BD) was validated for western blot by Karen S. Christopherson et al. Cell (2005).

All secondary antibodies were validated for immunostaining by manufacturer.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)

HEK293T (ATCC, CRL-3216)

Authentication

None of the cell lines used were authenticated

Mycoplasma contamination

None of the cell lines used were not tested for mycoplasma contamination

Commonly misidentified lines
(See [ICLAC](#) register)

Cell lines that were generally misidentified were not used in this study.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

In this study, C57BL/6J WT mice (at age :3, 6, 9, 12, and 18 months old), Aldh1l1-eGFP-L10a transgenic mice (at age :4 and 9 months old), Thy1-eYFP-H transgenic mice (at age : 12 months old), and APPswe/PSEN1dE9 (APP/PS1) double-transgenic AD mice (at age :3, 6, 9, 12, and 18 months old) were used. Genders were not considered. Mice of all strains were housed in an environment with controlled temperature and humidity, on 12 hours light/dark cycles (lights on at 7:00), and, unless stated otherwise, fed with regular rodent's chow and sterilized tap water ad libitum. All experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee. (IACUC, KA2019-67)

Wild animals

No wild animals were used in this study

Field-collected samples

No field-collected samples were used in this study

Ethics oversight

All procedures and protocols were approved by the Animal Ethics Committee at KAIST. All experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC).

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