

Naturally Occurring Antibodies Against Bim Are Decreased in Alzheimer's Disease and Attenuate Ad-type Pathologies in a Mouse Model

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Research

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Abstract

Background Alzheimer's disease (AD) is the most popular neurodegenerative disease affecting cognitive functions of the elderly population. Neuronal apoptosis is an important pathological process during the development of AD. The Bcl-2-interacting mediator of cell death (Bim) mediates Amyloid-beta ($A\beta$)-induced neuronal apoptosis. Naturally occurring antibodies against Bim (NAbs-Bim) exist in human blood, with their levels and functions unknown in AD.

Methods This study investigated the clinical relevance of plasma NAbs-Bim to AD in 55 AD patients, 28 patients with non-AD dementia, and 70 cognitively normal subjects. Furthermore, the pathophysiological functions of NAbs-Bim were explored in APP/PS1 mice and SY5Y cell lines overexpressing human amyloid precursor protein (APP).

Results We found that plasma levels of NAbs-Bim were lower in AD patients in comparison with patients with non-AD dementia and cognitively normal controls. Plasma levels of NAbs-Bim were negatively associated with brain amyloid burdens and positively associated with cognitive functions. NAbs-Bim purified from intravenous immunoglobulin rescued behavioral deficits of APP/PS1 mice. NAbs-Bim ameliorated $A\beta$ deposition, Tau hyperphosphorylation, microgliosis and neuronal apoptosis in APP/PS1 mice. *In vitro* investigations demonstrated that NAbs-Bim exerted neuroprotective effects against AD through neutralizing Bim-directed neuronal apoptosis and amyloidogenic processing of amyloid precursor protein.

Conclusions The decrease of NAbs-Bim might contribute to the pathogenesis of AD and immunotherapies targeting Bim may hold promise for the treatment of AD.

Background

Alzheimer's disease (AD) is a devastating and incurable neurodegenerative disease, which affects the cognitive function of the older population [1]. Senile plaque comprising Amyloid-beta ($A\beta$) peptide and neurofilament tangles consisted of hyperphosphorylated Tau protein are the major pathological hallmarks of AD. Most of the current therapeutic strategies attempt to target the two lesions, however, no clinical trial has been successful [2], partly because dramatic neuronal loss has been happening when the interventions are given [3, 4]. These had led to an increased attention to searching for alternative therapeutic targets of AD. Neuronal apoptosis is one of the terminal pathological processes that contribute to cognitive impairment, thus rescuing neuronal apoptosis may be a potential therapeutic strategy for AD. Currently, several drugs in clinical trials are targeting this process [2].

Apoptosis is regulated by various proteins, among those are primarily the B cell lymphoma-2 (Bcl-2) regulatory protein family [5]. The Bcl-2-interacting mediator of cell death (Bim) is a pro-apoptotic protein in the Bcl-2 family and is ubiquitously expressed in the central nervous system [6]. Bim is an essential mediator of $A\beta$ -induced neuronal apoptosis [7]. It is suggested that the expression of Bim, but not other pro-apoptotic proteins of the Bcl-2 family, was selectively increased in the brain of an AD mouse model

[8]. Postmortem studies also found that Bim is elevated in the AD brain [9]. These findings indicate that Bim may play a pivotal role in the pathogenesis of AD.

The permeability of the blood-brain barrier (BBB) increases with aging [10], thus promoting the exposure of brain antigens to the immune system, which may generate autoantibodies targeting brain antigens. These autoantibodies may in return enter the brain through the disrupted BBB and direct pathophysiological effects in the brain [11]. A recent study screened out a panel of naturally occurring antibodies that were altered in AD patients [12], suggesting that humoral autoimmunity may participate in the pathogenesis of AD. Naturally occurring antibodies against Bim (NAbs-Bim) exist in human blood and may be involved in the pathogenesis of diseases such as malignant pleura effusion [13]. We found in this study that circulating NAbs-Bim were decreased in AD patients, with the pathophysiological functions of these antibodies to be demonstrated. Therefore, we further investigated the clinical relevance of NAbs-Bim to AD and the functions of these antibodies in regulating AD-type pathologies.

Materials And Methods

See **Supplementary Information** for detailed descriptions.

The research protocols were approved by the institutional review boards of Daping Hospital, Third Military Medical University. Briefly, 55 AD patients and 28 patients with non-AD dementia were recruited from Department of Neurology, Daping hospital. 70 cognitively normal (CN) subjects were recruited from the Health Examination Center, Daping Hospital. Written consents for participation, blood sampling, and ApoE genotyping were obtained from the subjects or their legal relatives. The diagnosis of AD was made in accordance with the 2011 version of the NIA-AA standard [14] with confirming by PiB-PET positivity. Enzyme-linked immunosorbent assay (Elisa) was used to determine plasma levels of NAbs-Bim. NAbs-Bim were isolated from intravenous immunoglobulin for functional experiments. The effects of NAbs-Bim on APP processing and neuronal apoptosis were examined in SH-SY5Y-APP695 cell lines. 8 mon-aged APP/PS1 transgenic mice were used to test the effects of NAbs-Bim on AD-type pathologies. At 9 mon of age, mice were subjected to behavioral tests, histological and biochemical analyses. The behavioral performance of mice was tested using Y-maze and open field protocols, as described in our previous work [15]. Brain A β burden, APP processing, Tau hyperphosphorylation, neuroinflammation, and neuronal apoptosis et al. were assessed with histological or biochemical methods. Unless otherwise stated, the results were presented as mean \pm SEM. Comparisons between two groups were conducted using Student t-test, or Mann-Whitney u test, where appropriate. The comparisons among 3 groups were tested using one-way ANOVA or Kruskal-Wallis test. $P < 0.05$ was considered statistically significant. Data analyses were performed using SPSS software (version 25.0).

Results

Plasma NAbs-Bim levels are reduced in AD patients

To investigate whether plasma NAbs-Bim levels are altered in AD patients, 55 AD patients, 28 patients with non-AD dementia, and 70 CN subjects were recruited. There was no significant difference in age, sex, education, and the frequencies of diabetes mellitus, hypertension and stroke among the three groups. The AD group had a higher proportion of ApoE ϵ 4 carriers and lower Mini-mental State Examination (MMSE) scores than the other two groups.

The existence of NAbs-Bim in human plasma was verified by Western Blot (**Fig. S1a**). Through ELISA analyses, we found that plasma levels of NAbs-Bim in AD patients were significantly lower than in the CN group. AD patients also had slightly lower plasma levels of NAbs-Bim than subjects with non-AD dementia, with no significance being achieved (**Fig. 1a**). After adjusting for ApoE ϵ 4 carrier status and co-existing disorders, the difference in plasma levels of NAbs-Bim between the AD and CN group was still significant.

We next investigated the associations of plasma NAbs-Bim levels with cognitive functions as determined by MMSE scores, brain amyloid burden as reflected by Standard Uptake Value Ratio (SUVR) of PiB-PET, and plasma AD biomarkers. Plasma NAbs-Bim levels had a positive correlation with MMSE scores (**Fig. 1b**) and a negative correlation with PiB-PET SUVR (**Fig. 1c**). Besides, plasma NAbs-Bim levels were significantly correlated with plasma A β 42 levels, but not with A β 40 or t-tau levels (**Fig. 1d-f**). The above findings indicated that NAbs-Bim were negatively associated with the severity of AD, suggesting a possible protective role of these antibodies in AD.

NAbs-Bim rescue behavioral deficits in APP/PS1 mice

NAbs-Bim intervention experiments were performed to investigate the effects of NAbs-Bim on behavioral performances of APP/PS1 mice. 5 μ g (at a concentration of 1 μ g/ μ L) NAbs-Bim purified from IVIg or 5 μ L PBS were injected into the right lateral ventricle of mice from 8 mon of age. The mice were subjected to behavioral analyses at 9-mon old when extensive A β pathologies and significant behavioral deficits could be observed. APP/PS1 mice treated with NAbs-Bim had more entries into the novel arm in the Y-maze test, which reflected a better spatial recognition memory. But the mice did not show significantly different performances in the spontaneous exploration test in Y-maze (**Fig. 2a**). In open-field tests, NAbs-Bim treated mice showed a longer traveling distance, a higher number of rearing and grooming, and an increased ratio of time spent in the central zone to that in the peripheral zone than control APP/PS1 mice, indicating an enhanced locomotor activity and a reduced anxiety-like behavior (**Fig. 2b and c**). The above findings indicated that NAbs-Bim treatment could protect against behavioral deficits in APP/PS1 mice.

NAbs-Bim attenuate A β pathologies in APP/PS1 mice

We first confirmed that NAbs-Bim can exactly bind to Bim by immunofluorescence (IF) co-staining of Bim, NeuN, and DAPI (**Fig. S1b**). To explore whether NAbs-Bim treatment could reduce A β deposition in brains of APP/PS1 mice, we performed A β immunohistochemical (IHC) staining (6E10) for total A β plaques and Congo red staining for compact A β plaques. There was no significant difference in area fractions and the total plaque density of total A β plaques either in the neocortex or in the hippocampus between NAbs-Bim

and the control group. Compared with APP/PS1 controls, mice treated with NAbs-Bim displayed a significant reduction in area fractions of compact plaque in the neocortex, but not in the hippocampus. No significant difference was observed in the compact plaque density either in the neocortex or in the hippocampus (**Fig. 3a and b**). Taking together, our findings indicated that NAbs-Bim could attenuate A β pathologies in APP/PS1 mice.

We next measured amyloid precursor protein (APP) and its metabolites in brain homogenates of APP/PS1 mice. We found that A β , soluble APP (sAPP α + β), and C-terminal fragments (CTF)- β levels were significantly decreased in the brain of mice treated with NAbs-Bim when compared with controls, while full-length APP (APP_{fl}), sAPP α , and CTF- α had no differences (**Fig. 3c**). These results indicated that NAbs-Bim inhibited A β production via decreasing amyloidogenic processing of APP.

We determined levels of secretases responsible for APP processing. Beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) is the major β -secretase that catalyzes β -cleavage and promotes amyloidogenic processing of APP [16]. NAbs-Bim treatment group had significantly lower levels of BACE1 in brain homogenates in comparison with APP/PS1 controls. However, no significant differences were observed in a Disintegrin and Metalloproteinase 10 (ADAM10) or presenilin 1 (PS1, which is the catalytic subunit of γ secretase) levels (**Fig. 3d**). These results implied that the decrease in amyloidogenic processing of APP might be due to the reduction of BACE1. We also tested A β -degrading enzymes, including insulin-degrading enzyme (IDE) and neprilysin (NEP), and A β transporters, including low-density lipoprotein receptor-related protein-1 (LRP1) and receptor for advanced glycation end products (RAGE). RAGE was found to be decreased in NAbs-Bim treated mice in comparison with controls, while no differences were found in IDE, NEP, and LRP1 levels (**Fig. 3e**), suggesting that NAbs-Bim might reduce the receptor-mediated influx of A β through the blood-brain barrier (BBB).

Additionally, to confirm that NAbs-Bim could indeed inhibit the Bim-mediated apoptosis pathway in mice, we evaluated the abundance of Bim and its downstream protein in brain homogenates. NAbs-Bim treated group had significantly lower levels of extracellular Bim, total Bim, BAX, and BAK levels than APP/PS1 controls (**Fig S1c**).

NAbs-Bim attenuate neuroinflammation, Tau hyperphosphorylation, and synaptic degeneration in APP/PS1 mice

We then investigated whether NAbs-Bim could affect other AD-type pathologies. Activated microglia (CD68⁺) was significantly decreased after NAbs-Bim treatment both in the neocortex and in the hippocampus (**Fig. 4a**). NAbs-Bim treatment mildly reduced the activation of astrocytes (GFAP⁺) but no significance was achieved either in the neocortex or in the hippocampus (**Fig. 4b**). NAbs-Bim significantly reduced area fractions of pT231-positive neurons in the hippocampus of NAbs-Bim treated mice when compared to controls (**Fig. 4c**). Meanwhile, the levels of total tau (Tau5) and phosphorylated tau at multiple epitopes, including pS396 and pT231, were reduced in the NAbs-Bim treated group (**Fig. 4d**).

Furthermore, the NAbs-Bim treated mice displayed increased NeuN and Map-2 positive area fractions and a decreased activated caspase-3 positive area fraction in the hippocampus (**Fig. 4e-h**). Synapse-related proteins, including PSD93, PSD95, Snap, SYN1, and VAMP1, were also measured. There was a significant difference in PSD95 between NAbs-Bim treated mice and controls (**Fig. 4i**). Taking together, these findings suggested that NAbs-Bim protected against neuroinflammation, tau hyperphosphorylation, and synaptic degeneration in APP/PS1 mice.

NAbs-Bim antagonize Bim-induced neuronal apoptosis and amyloidogenic processing of APP *in vitro*

Bim is suggested to be a proapoptotic protein that actions mainly in intracellular compartments, which might not be accessible to NAbs-Bim. Furthermore, no evidence is now available regarding the role of Bim in APP metabolism. Therefore, to reveal the mechanisms of the protective effects of NAbs-Bim, we first investigated the pathological effects of extracellular Bim. SH-SY5Y-APP695 cells were treated with Bim protein, Bim protein plus NAbs-Bim, or PBS. Mitochondrial staining found that Bim treated group displayed a significantly lower positive area fraction than the other two groups (**Fig. 5a**), indicating that extracellular Bim protein could have pro-apoptotic effects, which can be antagonized by NAbs-Bim. Furthermore, Bim treated group showed increased levels of A β , CTF- β , sAPP α + β , and BACE-1 when compared to the other groups, which could be attenuated by NAbs-Bim (**Fig. 5b and c**). These results indicated that extracellular Bim protein could enhance A β generation through promoting amyloidogenic processing of APP and NAbs-Bim may exert protective effects through neutralizing extracellular Bim.

In SH-SY5Y-APP695 cell lines without extraneous Bim protein treatment, NAbs-Bim groups showed increased mitochondrial positive area fractions in a dose-dependent manner (**Fig. S1d**). To investigate whether NAbs-Bim would influence the activity of endogenous extracellular Bim, we performed IF co-staining for Bim and DAPI. The siRNA-Bim, which could inhibit the Bim expression, treated group was selected as a positive control. We found that the Bim-positive area fraction of the NAbs-Bim treated group was significantly lower than the control group and higher than the siRNA-Bim treated group (**Fig. 6a**). Furthermore, the NAbs-Bim group had a significantly higher mitochondrial positive area fraction than the control group, but had no difference with the siRNA group (**Fig. 6b**). These findings indicated that NAbs-Bim could inhibit the activity of Bim and increase neuronal survival.

We next measured APP metabolites and APP cleavage enzymes in cell protein extracts. NAbs-Bim group displayed lower levels of A β , sAPP α + β , CTF- β , and BACE-1 than control. Compared with the siRNA group, the NAbs-Bim group had higher levels of sAPP α + β . No significant differences were found in other APP metabolites and APP cleavage enzymes among groups (**Fig. 6c and d**). Levels of A β -degrading enzymes (IDE and NEP) and A β transport receptors across BBB (LRP-1 and RAGE) were also measured. NAbs-Bim group and siRNA group had significantly lower levels of RAGE than the control group, while there were no differences in other proteins among groups (**Fig. 6e**). These results were consistent with the *in vivo* experiments and supported that NAbs-Bim promoted the survival of neurons by reducing A β production via decreasing Bim-induced amyloidogenic processing of APP.

Collectively, the above findings suggested that extracellular Bim could simultaneously promote neuronal apoptosis and the amyloidogenic processing of APP. NAbs-Bim might actions through antagonizing these pathological effects of extracellular Bim, thus exerting neuroprotective effects in AD.

Discussion

Over the past decades, the Amyloid cascade hypothesis has gained traction as one of the major contributors to AD pathogenesis [17]. Cerebral A β accumulation is regarded as the initiator of AD and has been selected as one of the major therapeutic targets in clinical trials, represented by anti-A β immunotherapies [18]. However, these clinical trials have not succeeded yet [19]. Therefore, therapeutic strategies targeting other pathological processes of AD are emerging, including those targeting Tau hyperphosphorylation, neuroinflammation, and oxidative stress et al [2]. Neuronal apoptosis is a key pathogenic pathway in AD [20], and it remains to be seen whether interventions targeting this pathway will be disease-modifying. Bim plays a pivotal role in the transduction of pro-apoptotic signals upon the stimulation of pro-apoptotic factors such as A β [21]. Bim is upregulated in the AD brain [9], and inhibition of the Bim signaling pathway ameliorates AD-type pathologies and rescues cognitive deficits [22], suggesting that Bim is an important pathogenic agent and a potential therapeutic target of AD.

Previous studies have identified a panel of autoantibodies that are associated with AD, such as autoantibodies against A β [23, 24]. Postmortem studies found that brain-reactive autoantibodies are prevalent in human blood [25]. These findings suggest a potential role of humoral immunity in the pathogenesis of AD. NAbs-Bim is suggested to exist in human blood, with its pathophysiological role unknown [9]. We found that plasma levels of NAbs-Bim were decreased in AD patients and were negatively associated with the severity of AD, raising a possibility that NAbs-Bim may exert protective effects against AD. Therefore, we further investigated how NAbs-Bim might work in the AD brain. We believe that the most direct effect of NAbs-Bim is to antagonize the pathological effects of Bim via antibody-antigen interactions. Indeed, Bim levels were significantly reduced after treatment with NAbs-Bim both *in vivo* and *in vitro*. Bim directly binds and activates the pro-apoptotic effectors BAX and BAK, further activating the caspase cascade and inducing neuronal apoptosis [26]. NAbs-Bim significantly reduced the amount of BAX and BAK, and finally rescued neurons from apoptosis. However, it is suggested that Bim actions mainly in the cellular compartments, which might not be accessible to antibodies. We found in this study that Bim also exists in the extracellular space and extracellular Bim could be neutralized by NAbs-Bim. Furthermore, extracellular Bim was also toxic to neurons, and its neurotoxicity could be antagonized by NAbs-Bim. NAbs-Bim significantly reduced both total and extracellular Bim, thus ameliorating its pro-apoptotic effects.

We found in this study that extraneous Bim protein significantly promotes the expression of BACE1 and the production of A β *in vitro*. This finding is consistent with a previous study which found that BACE1 expression is increased during apoptosis [27]. Furthermore, a previous study suggests that Bim interacts with A β and promotes the formation of A β protofibrils [28]. Therefore, A β overproduction and neuronal apoptosis may form a vicious circle during the development of AD. We found that NAbs-Bim could

significantly reduce Bim-induced BACE1 upregulation and A β overproduction. These findings suggest another protective role of NAbs-Bim against AD through antagonizing the pro-amyloidogenic effects of Bim.

This study was conducted in APP/PS1 mice aged 8 mon, at which stage the amyloidosis has initiated and A β has deposited to a considerable amount. Upon 4 doses of intraventricular treatment, NAbs-Bim improved behavioral deficits and reduced the amyloid burden of APP/PS1 mice. As described above, NAbs-Bim may help control brain amyloidosis by inhibiting the amyloidogenic process. Intraneuronal neurofilament tangles (NFT) caused by Tau hyperphosphorylation is another major pathological hallmark of AD [29, 30]. In this study, NAbs-Bim was found to reduce NFT formation and suppress Tau hyperphosphorylation at several sites. Furthermore, other pathologies subsequent to A β or Tau, including overactivation of glia cells, down-regulation of synaptic proteins, and dendritic damage et al. were also improved after NAbs-Bim treatment. NAbs-Bim may act on A β and suppresses its toxicity of triggering Tau hyperphosphorylation and subsequent pathological changes. These findings imply that immunotherapies targeting the apoptotic process may hold promise for the treatment of AD.

Conclusions

In this study, we reported that plasma levels of NAbs-Bim were decreased in AD patients. Plasma levels of NAbs-Bim were negatively correlated with the severity of AD. NAbs-Bim rescued behavioral deficits and alleviated AD-type pathologies in an AD mouse model. Based on the neuroprotective effects of NAbs-Bim against AD, we supposed that the decreased levels of NAbs-Bim in AD patients might contribute to the disease progression. Furthermore, this study found out a potential therapeutic strategy by targeting neuronal apoptosis. There are several limitations of this study. The first is that the clinical relevance of NAbs-Bim was investigated in a cross-sectional observation, thus we could not address whether the decrease of NAbs-Bim would contribute to the disease progression from a longitudinal perspective. The second is that we investigated the therapeutic effects of NAbs-Bim through ventricular administration at a relatively high dose. This is invasive and cannot be clinically used, thus further studies through peripherally administrations of NAbs-Bim are needed to address a more solid conclusion about the clinical use potential of this antibody.

Abbreviations

AD: Alzheimer's disease; A β : Amyloid-beta; Bcl-2: B cell lymphoma-2; Bim: Bcl-2-interacting mediator of cell death; BBB: Blood-brain barrier; NAbs-Bim: Naturally occurring antibodies against Bim; CN: Cognitively normal; Elisa: Enzyme-linked immunosorbent assay; MMSE: Mini-mental State Examination; SUVR: Standard Uptake Value Ratio; IF: Immunofluorescence; IHC: Immunohistochemical; APP: Amyloid precursor protein; sAPP α + β : Soluble APP; CTF: C-terminal fragments; APPfl: Full-length APP; BACE1: Beta-site amyloid precursor protein cleaving enzyme 1; ADAM10: Disintegrin and Metalloproteinase 10; PS1: Presenilin 1; IDE: Insulin-degrading enzyme; NEP: Neprilysin; LRP1: Low-density lipoprotein receptor-

related protein-1; RAGE: Receptor for advanced glycation end products; NFT: Neurofilament tangles; Neoco.: Neocortex; Hippo.: Hippocampus; Snap: Synaptophysin; SYN1: Synapsin1.

Declarations

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Author Contributions

WYJ and JJM designed this study and drafted the manuscript. JJM, FDY and TDY conducted the experiments. CY, SPY, ZGH, and HCY conducted statistical analyses. WYR, ZJ and YXQ had critical reading of the manuscript.

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Availability of data and materials

All primary data are available upon reasonable request.

Ethics approval and consent to participate

The research protocols were approved by the institutional review boards of Daping Hospital, Third Military Medical University. Written consents for participation, blood sampling, and ApoE genotyping were obtained from the subjects or their legal relatives.

Consent for publication

All authors qualified the authorship and approved the publication of this study.

Competing interests

The authors declared no conflict of interest.

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Figures

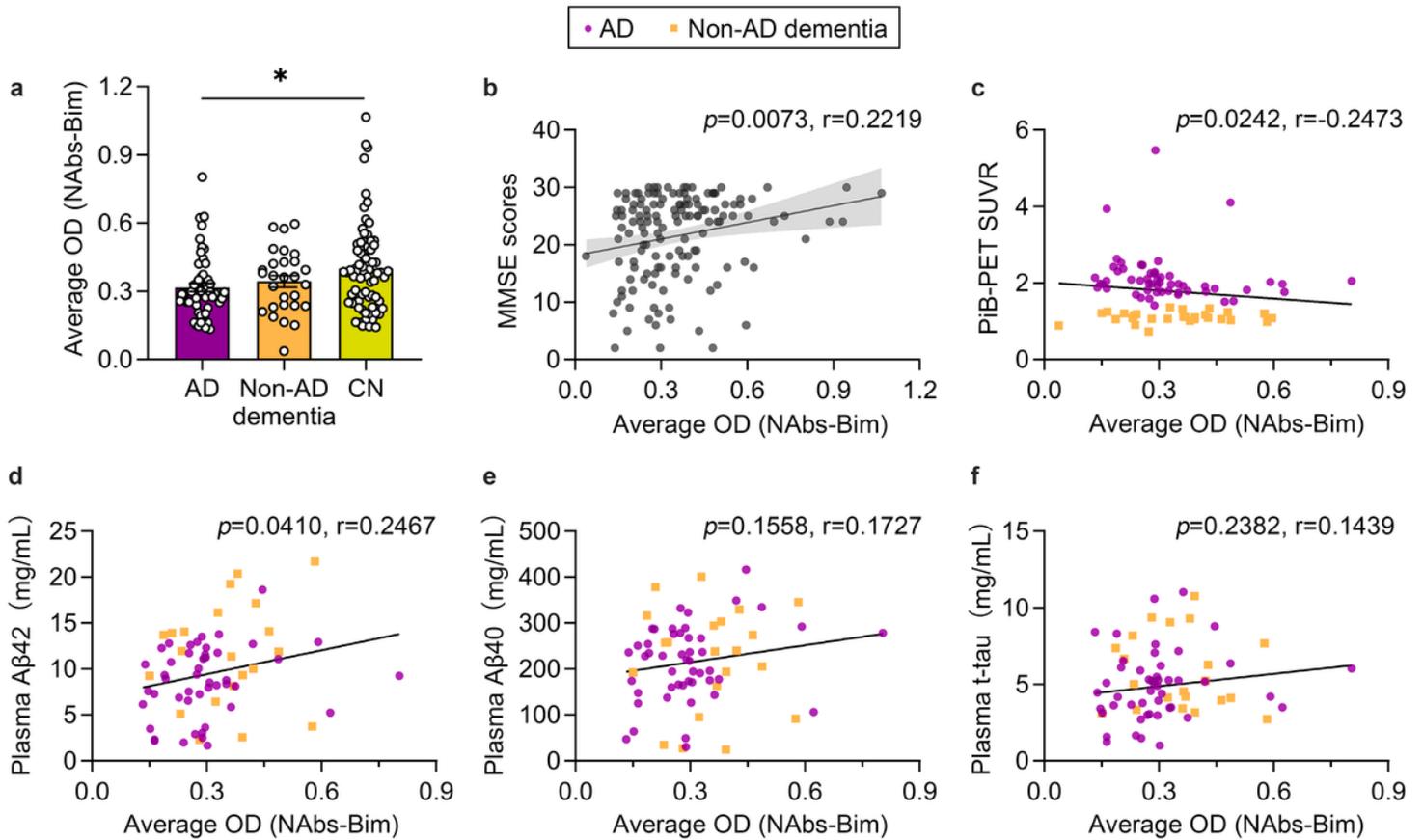


Figure 1

Plasma NAbs-Bim levels were reduced in AD patients and associated with cerebral amyloidosis. a Comparisons of the plasma NAbs-Bim levels among the AD, non-AD dementia, and CN group. Kruskal-Wallis test, $*P<0.05$. Bars express mean \pm SEM. b Correlation of plasma NAbs-Bim levels with MMSE scores in the total cohort. c Correlation of plasma NAbs-Bim levels with PiB-PET SUVR in AD and non-AD dementia group. d-f Correlation of plasma NAbs-Bim levels with plasma levels of A β 42, A β 40, and t-tau in AD and non-AD dementia group. Spearman correlation analysis, Shadows express 95% confidence interval.

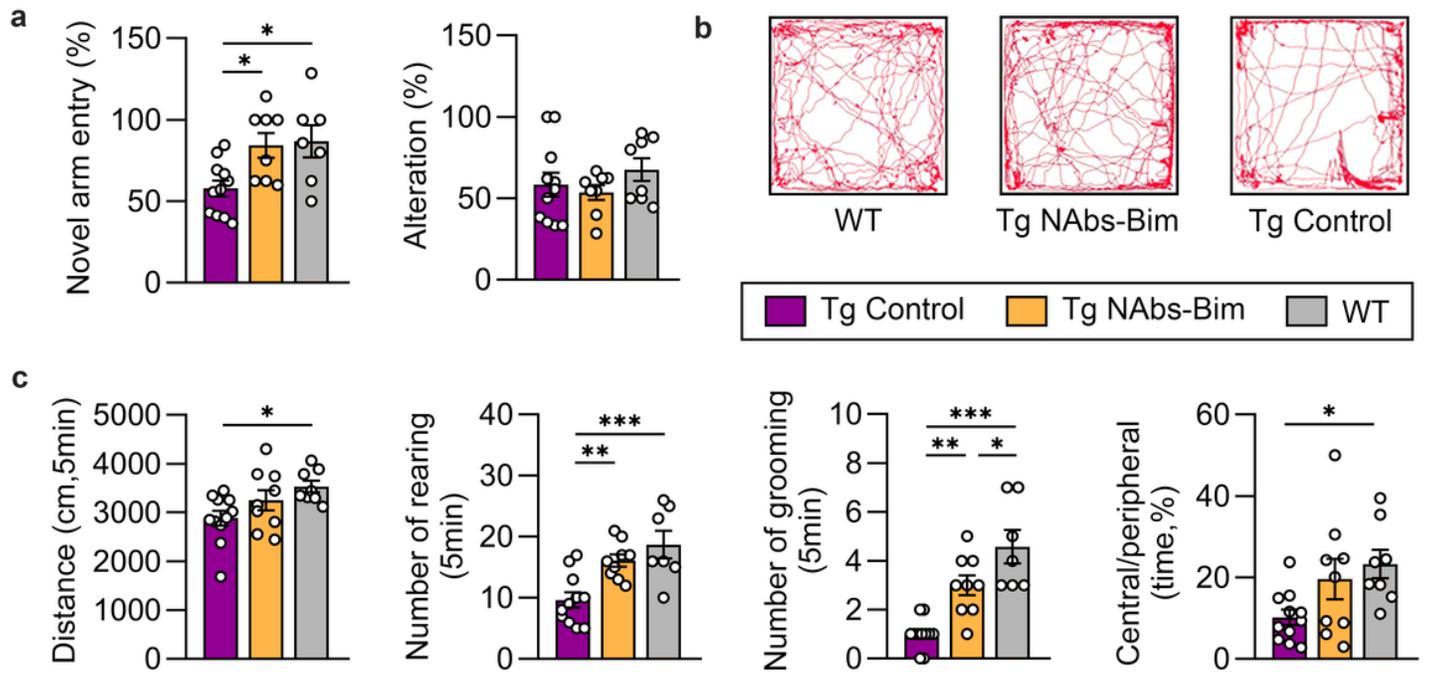


Figure 2

NAbs-Bim rescue behavioral deficits in APP/PS1 mice. a Percentage of novel arm entries and alteration in novel arm in Y-maze test. b Representative tracing graphs in the open-field test. c Distance traveled, number of rearing and grooming, the ratio of time spent in central and peripheral areas in the open-field test. One-way ANOVA, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Bars express mean \pm SEM.

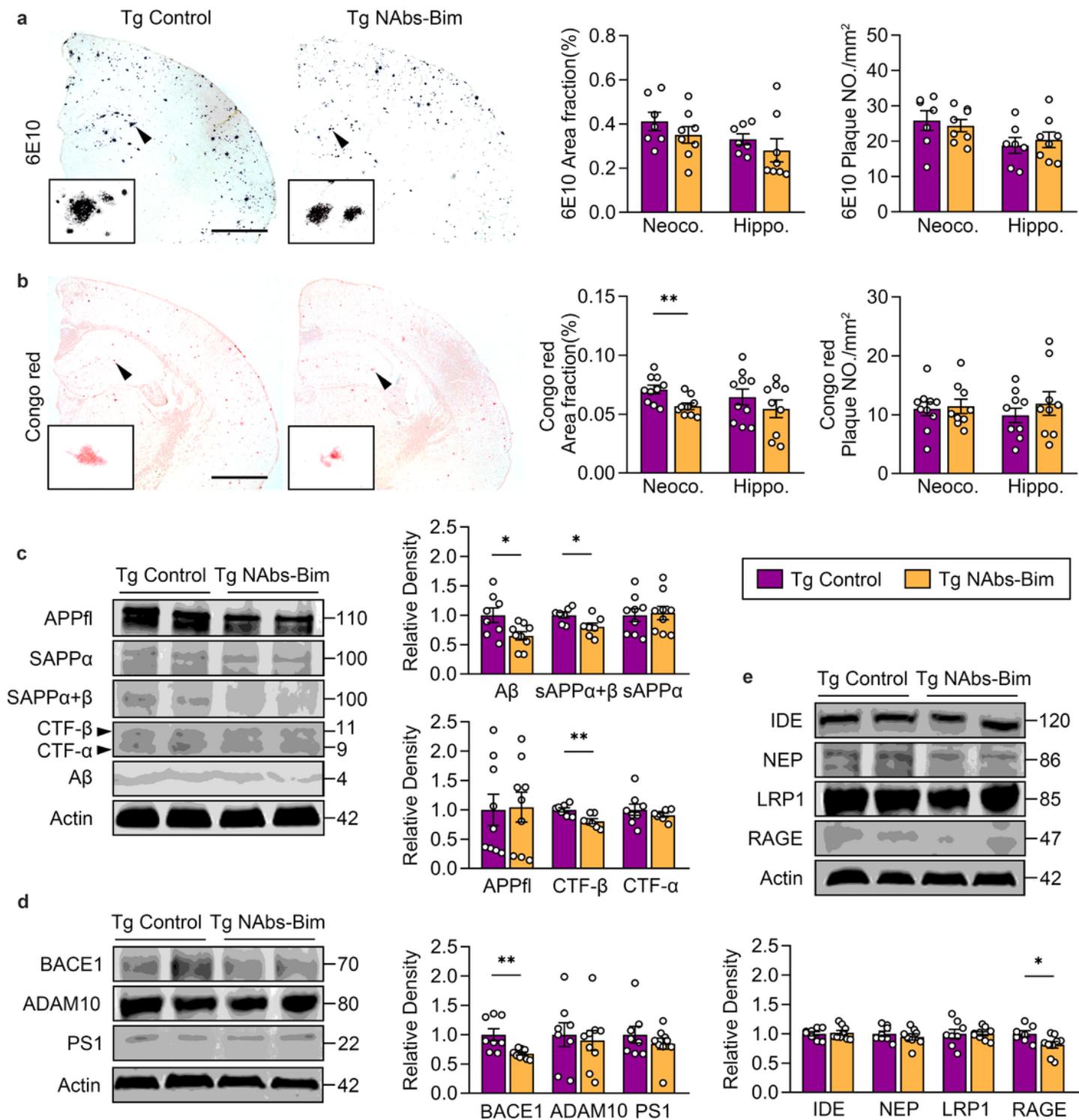


Figure 3

NAbs-Bim attenuate A β pathologies in APP/PS1 mice. a and b Immunostaining and quantification of 6E10 and Congo red in the neocortex (Neoco.) and hippocampus (Hippo.) of 9 mon controls and NAb-Bim treated mice. Insets show the representative morphology at a higher magnification. Scale bars 500 μ m. c Western blots and quantification for APP and its metabolites in brain homogenates. d Western blots and quantitative analysis for APP cleavage enzymes. e Western blots and quantitative analysis for

A β -degrading enzymes and A β transporters. Two-tailed t-test, *P<0.05, **P<0.01. Bars express mean \pm SEM.

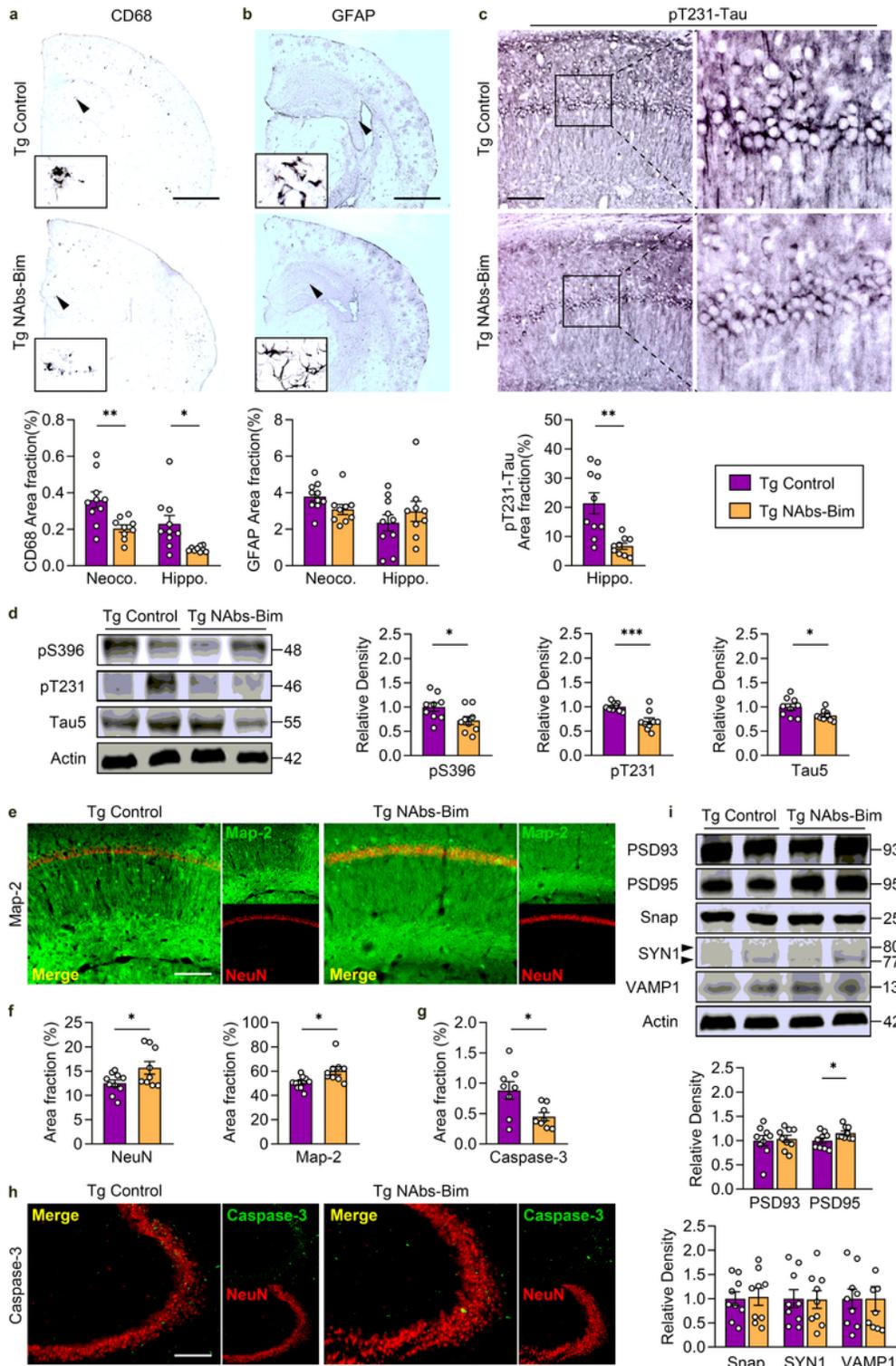


Figure 4

NAb-Bim attenuate neuroinflammation, Tau hyperphosphorylation, and synaptic degeneration in APP/PS1 mice. a and b Immunostaining and quantification of activated microgliosis (CD68) and astrocytes (GFAP) in the neocortex (Neoco.) and hippocampus (Hippo.) of 9 mon controls and NAb-Bim

treated mice. Insets show the representative morphology at higher magnification. Scale bars 500 μm . c Immunostaining and quantification of pT231 in the hippocampus of 9 mon controls and NAbs-Bim treated mice. Insets show the representative morphology at higher magnification. Scale bars 50 μm . d Western blots and quantification of phosphorylated tau at multiple sites (pS396 and pT231) and total tau (Tau5) in brain homogenates. e and f Representative images and quantification of the neurons and dendrites in the CA1 region of the hippocampus stained with anti-NeuN and anti-MAP-2 immunofluorescence. Scale bars 50 μm . g and h Representative images and quantification of neuronal apoptosis in the CA3 region of the hippocampus as illustrated with activated caspase-3 immunofluorescence. Scale bars 50 μm . i Western blots and quantification of synapse-associated proteins, including PSD93, PSD95, synaptophysin (Snap), synapsin1 (SYN1), and VAMP1, in the brain homogenates of NAbs-Bim treated and control mice. Two-tailed t-test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Bars express mean \pm SEM.

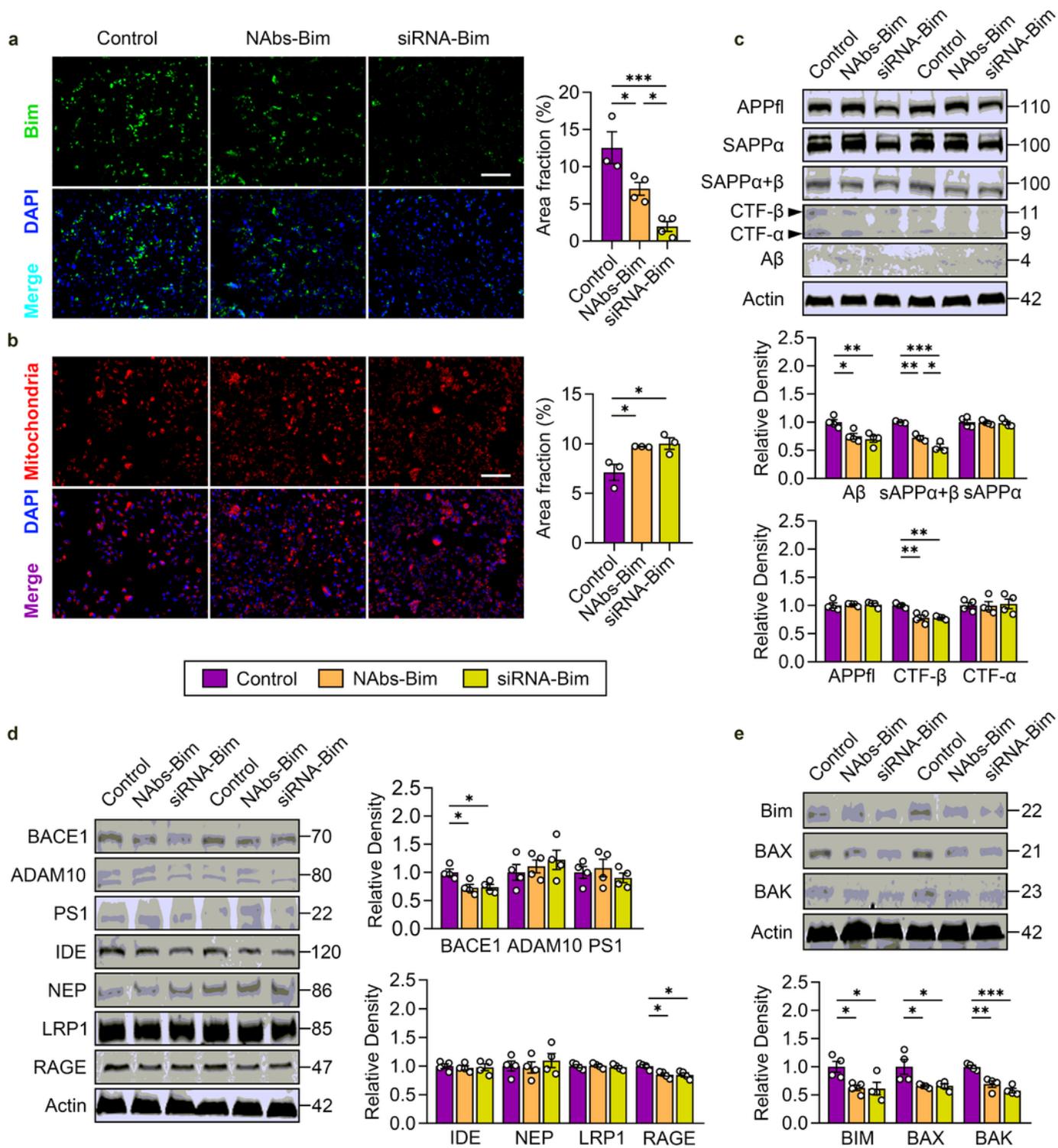


Figure 5

Bim upregulates A β production by promoting BACE-1 expression. a Representative images and quantification of mitochondria and neuron stained with mitochondrial staining solution and DAPI immunofluorescence in control, Bim plus with NAbs-Bim treated and Bim treated SH-SY5Y-APP695 cells. Scale bars 100 μ m. b Western blots and quantification for APP and its metabolites in SH-SY5Y-APP695

cells. c Western blots and quantification for APP cleavage enzymes. One-way ANOVA, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Bars express mean \pm SEM.

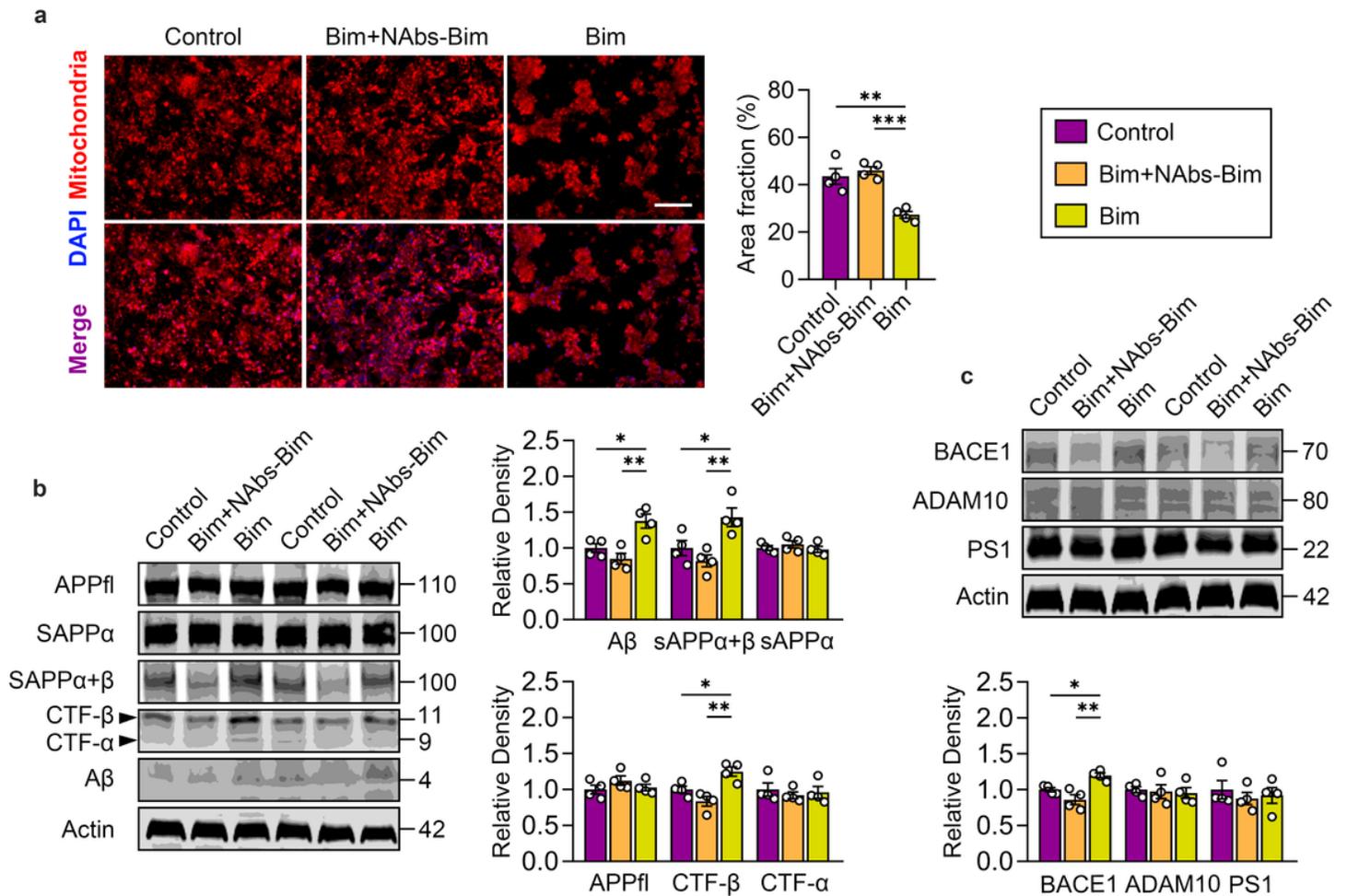


Figure 6

NAbs-Bim attenuates neuronal apoptosis and inhibits A β generation via decreasing amyloidogenic processing of APP. a Immunofluorescence co-staining and quantification of Bim and DAPI in control, NAbs-Bim treated and siRNA treated SH-SY5Y-APP695 cells. Scale bars 100 μ m. b Representative images and quantification of mitochondria and neurons stained with mitochondrial staining solution and DAPI immunofluorescence in control, NAbs-Bim treated and siRNA treated SH-SY5Y-APP695 cells. Scale bars 100 μ m. c Western blots and quantification for APP and its metabolites in SH-SY5Y-APP695 cells. d Western blots and quantification for APP cleavage enzymes, A β -degrading enzymes, and A β -transporting receptors in the blood-brain barrier (BBB). e Western blots and quantification for Bim and its downstream protein, including BAX, BAK. One-way ANOVA, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Bars express mean \pm SEM.

Supplementary Files

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- [Supplementaryinformation.pdf](#)