Science Bulletin xxx (xxxx) xxx

Contents lists available at ScienceDirect

Science Bulletin

journal homepage: www.elsevier.com/locate/scib

Short Communication

Endothelial FAM171A2 is required for maintaining autophagy flux and blood–brain barrier integrity

Si-Da Han [a,d,1], Ning Ding [b,1], Jia-hui Huang [b], Yi-Wei Feng [a], Kai-Min Wu [a], Shi-Dong Chen [a], Qiang Dong [a], Mei Cui [a], Ting-ting Wang [c,*], Peng Yuan [b,*], Jin-Tai Yu [a,*]

[a] Department of Neurology, Huashan Hospital, State Key Laboratory of Medical Neurobiology and MOE Frontiers Center for Brain Science, Fudan University, Shanghai 200040, China
[b] Department of Rehabilitation Medicine, Huashan Hospital, State Key Laboratory of Medical Neurobiology, Institute for Translational Brain Research, MOE Frontiers Center for Brain Science, MOE Innovative Center for New Drug Development of Immune Inflammatory Diseases, Fudan University, Shanghai 200032, China
[c] Department of Pharmacy, Huashan Hospital, Fudan University, Shanghai 200040, China
[d] Department of Neurology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200080, China

ARTICLE INFO

Article history:
Received 22 October 2025
Received in revised form 25 February 2026
Accepted 28 May 2026
Available online xxxx

© 2026 Science China Press. Published by Elsevier B.V. and Science China Press. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

Our previous work indicated that a single-nucleotide polymorphism of the *FAM171A2* gene is associated with increased risk for Parkinson's disease, Alzheimer's disease, and frontotemporal dementia [1]. Mechanistically, FAM171A2 in neurons promotes the uptake and propagation of pathological α-synuclein [2], suggesting that reducing FAM171A2 may offer therapeutic benefits for Parkinson's disease. However, homozygous whole-body knockout of *Fam171a2* in mice is lethal, indicating essential physiological functions of this gene that remain poorly understood. Besides neurons, FAM171A2 is also highly expressed in vascular endothelial cells [1], but its role in this cell type has not been investigated.

We first examined the localization of FAM171A2 in cultured endothelial cells and found that FAM171A2 proteins were localized to vesicular structures (Fig. S1a–e online). Immunostaining with markers of various types of vesicles demonstrated that FAM171A2 was present on endosomes (RAB5, RAB7), autophagosomes (LC3, p62), and lysosomes (LAMP1). Among these compartments, FAM171A2 exhibited the highest degree of colocalization with early endosomes (Fig. S1f online). To investigate its functional role, we generated a *FAM171A2* overexpression virus (Fig. S2 online) and a strain of endothelial-specific *Fam171a2* conditional knockout (CKO) mice by crossing *Fam171a2*-floxed mice with Tie2-CreERT mice (Fig. S3a online). All animal experiments were approved by the Animal Care and Use Committee at Fudan University (Approval number: 202109004S, 2023-HSYY-451JZS). Tamoxifen induction led to efficient and specific loss of FAM171A2 in the vasculature, confirmed by immunofluorescence (Fig. S3b online) and by mRNA and protein analysis from isolated cerebral endothelial cells (Fig. S3c–e online).

Having established these tools for FAM171A2 modulation, we then examined the effect of FAM171A2 overexpression on lysosomes in cultured primary endothelial cells using the fluorescent dye LysoTracker-568. We found that elevated FAM171A2 caused lysosomal enlargement without changing their numbers (Fig. 1a). Consistently, flow cytometry showed increased lysosomal fluorescence intensity, suggesting enhanced acidity (Fig. 1b). To assess autophagosome-lysosome fusion, we infected endothelial cells with a lentiviral LC3-GFP-RFP reporter to label autophagosomes, in which GFP is quenched upon fusion with lysosomes while RFP remains stable. *Fam171a2* knockout markedly reduced the red-to-green fluorescence ratio and decreased autolysosome (RFP^+ only) puncta, indicating impaired fusion (Fig. 1c, d). Conversely, FAM171A2 overexpression increased both the red-to-green fluorescence ratio and the number of autolysosome puncta (Fig. 1c, d). These data indicate that FAM171A2 is likely involved in the process of autophagosome-lysosome fusion.

To further test the role of FAM171A2 in regulating autophagosome-lysosome fusion, we examined levels of p62 and LC3, two canonical autophagosome markers. p62 binds to ubiquitinated proteins destined for degradation and is incorporated into autophagosomes and lysosomes. Immunoblotting analysis showed that *Fam171a2* knockout led to p62 accumulation and a mild reduction in LC3-II/LC3-I ratio (Fig. 1e, f), whereas FAM171A2 overexpression reduced p62 levels (Fig. 1g, h). In the presence of chloroquine (CQ, which blocks autophagic degradation),

* Corresponding authors.
E-mail addresses: wangtths@fudan.edu.cn (T.-t. Wang), pyuan@fudan.edu.cn (P. Yuan), jintai_yu@fudan.edu.cn (J.-T. Yu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.scib.2026.06.002
2095-9273/© 2026 Science China Press. Published by Elsevier B.V. and Science China Press. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

Please cite this article as: S.-D. Han, N. Ding, Jia-hui Huang et al., Endothelial FAM171A2 is required for maintaining autophagy flux and blood–brain barrier integrity, Science Bulletin, https://doi.org/10.1016/j.scib.2026.06.002

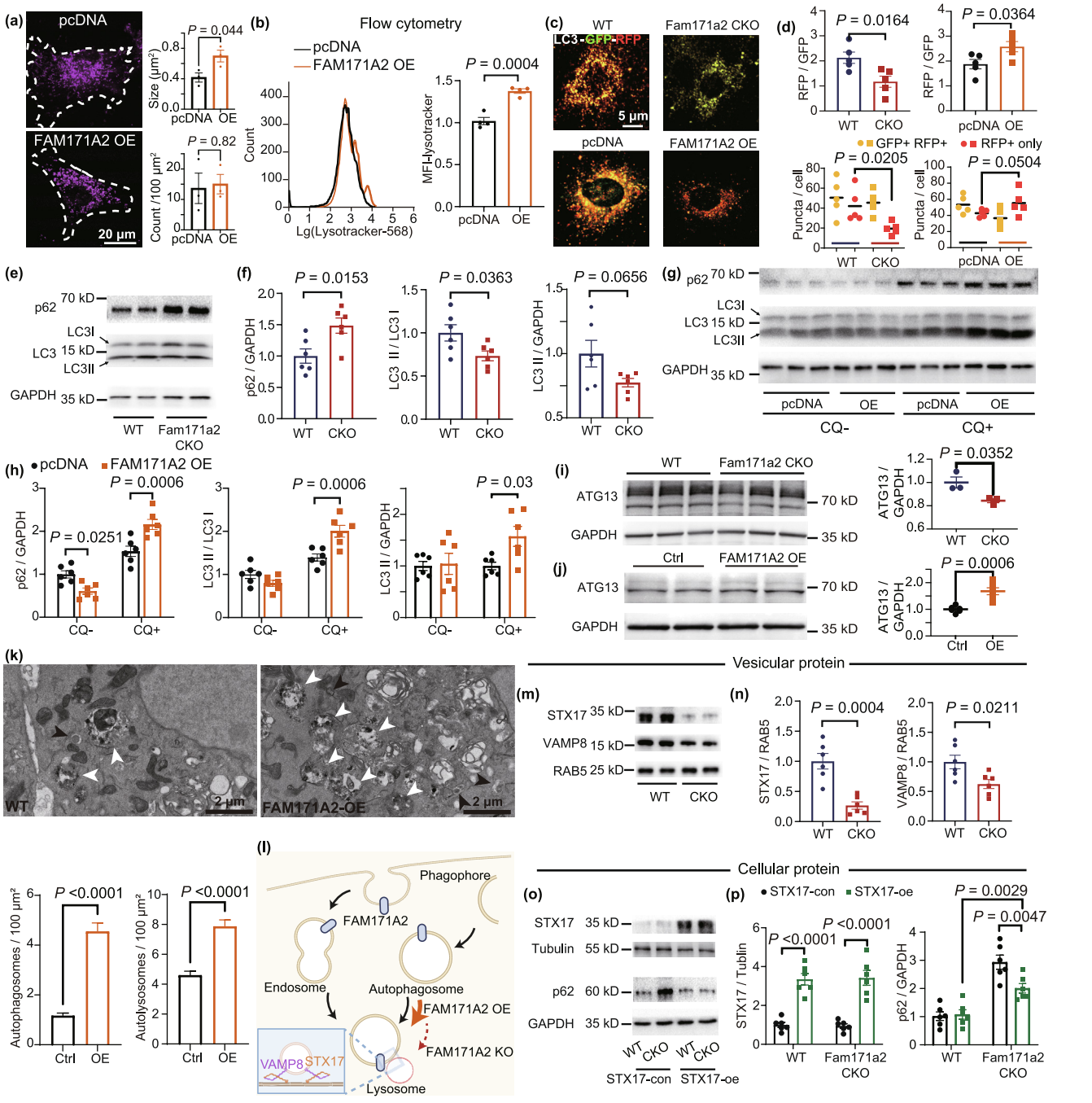

Fig. 1. FAM171A2 promotes autophagy flux in endothelial cells. (a) Confocal images of LysoTracker-568 in cultured endothelial cells with or without FAM171A2 OE. Right, quantifications of lysosome size and density. *n* = 3 experiments. (b) Flow cytometry of LysoTracker-568 intensity. *n* = 4 experiments. (c) Confocal images of LC3-GFP/RFP in endothelial cells from WT and *Fam171a2* CKO mice, and from cells with control or FAM171A2 OE transfection. (d) Quantification of GFP/RFP ratio and autophagosomes (GFP$^+$/RFP$^+$) and autolysosomes (RFP$^+$ only) numbers per cell from (c). *n* = 5 experiments. (e) Western blots of p62, LC3, and GAPDH in cerebral vascular endothelial cells from WT and *Fam171a2* CKO mice. (f) Quantification of p62 expression (left), LC3-II/LC3-I ratio (middle), and LC3-II expression (right) from (e). *n* = 6 experiments per group. (g) Western blots of p62, LC3, and GAPDH in endothelial cells with or without FAM171A2 OE and CQ treatment. (h) Quantification of p62 expression (left), LC3-II/LC3-I ratio (middle), and LC3-II expression (right) from (g). *n* = 6 experiments per group. WT and pcDNA&CQ– groups normalized to 1. (i) Western blots and quantification of ATG13 and GAPDH in brain tissue from WT and *Fam171a2* CKO mice. *n* = 3 mice. (j) Western blots and quantification of ATG13 and GAPDH in bEnd.3 cells with or without FAM171A2 OE. (k) Representative transmission electron microscopy images of WT, FAM171A2-OE bEnd.3 cells after 4 h EBSS starvation. Black arrows, autophagosomes; white arrows, autolysosomes. Quantification for the numbers of autophagosomes and autolysosomes (WT: *n* = 100 cell sections; OE: *n* = 96 cell sections) is shown as mean ± SEM. Scale bars: 2 μm. (l) Schematic of FAM171A2 promoting autophagosome–lysosome fusion in endothelial cells (Created with BioRender.com). (m) Western blots of STX17, VAMP8 and RAB5 on vesicles from cerebral vascular endothelial cells of WT and *Fam171a2* CKO mice. (n) Quantification of STX17 and VAMP8 on vesicles; RAB5 as loading control. *n* = 6 experiments per group. WT normalized to 1. (o) Western blots of STX17, p62, Tubulin, and GAPDH in primary endothelial cells from WT and *Fam171a2* CKO mice with or without STX17 OE. (p) Quantification of STX17 and p62. *n* = 6 experiments per group. WT normalized to 1. Data are mean ± SEM. *P* values (shown in the figure) were calculated by unpaired *t*-tests or two-way ANOVA followed by Bonferroni's multiple comparison test. WT, wild type; CKO, conditional knockout; pcDNA, plasmid control DNA; OE, overexpression; GFP, green fluorescence protein; RFP, red fluorescence protein; CQ, chloroquine; Ctrl, control.

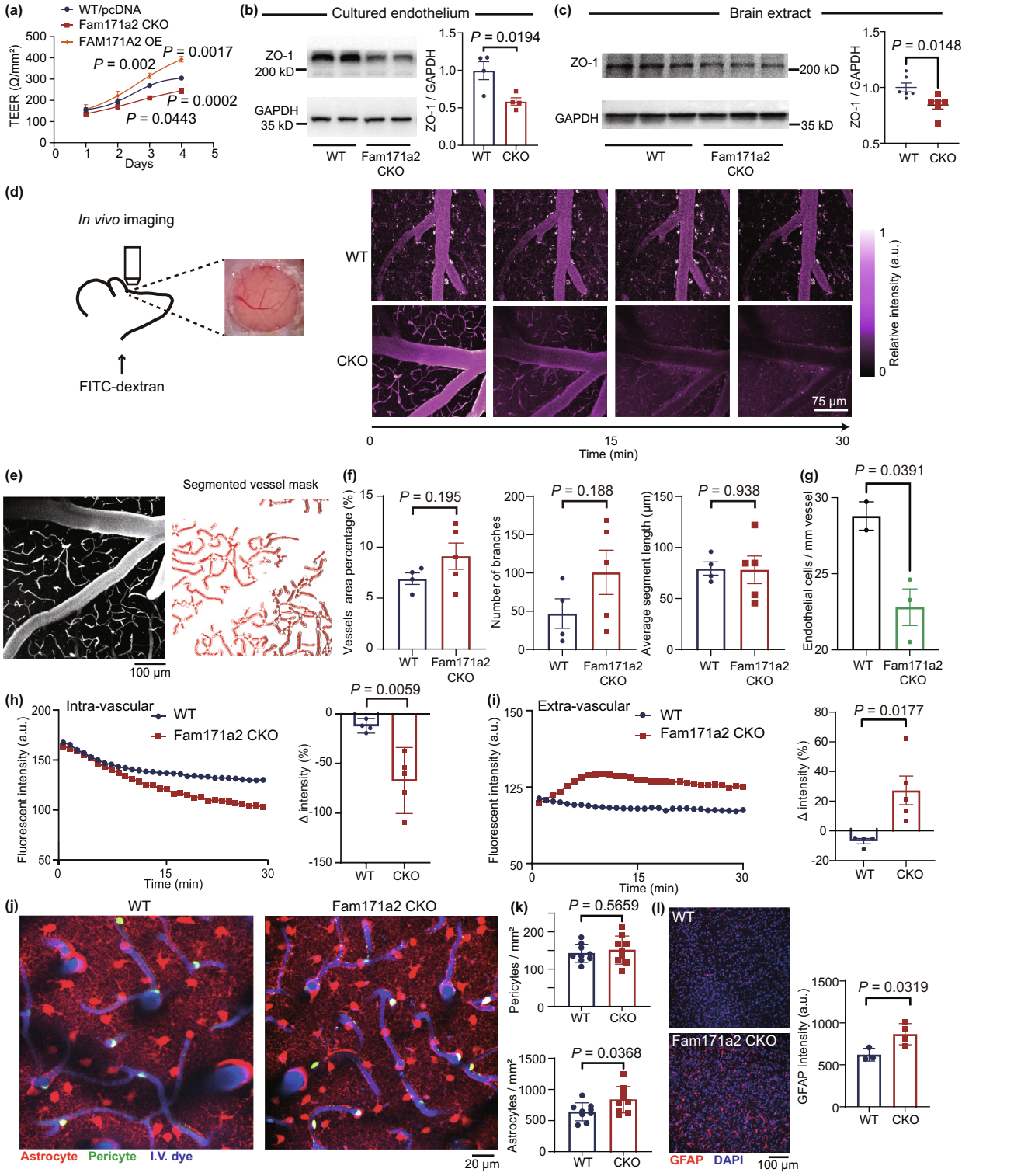

Fig. 2. FAM171A2 deficiency in endothelial cells causes blood–brain barrier leakage. (a) Trans-endothelial electrical resistance (TEER) in cultured endothelial cells from WT, *Fam171a2* CKO mice, and cells with FAM171A2 OE. $n = 5$ experiments. (b) Western blots of tight junction protein ZO-1 and GAPDH in primary endothelial cells from WT and *Fam171a2* CKO mice. Right, quantification of ZO-1 expression. $n = 4$ experiments per group. WT normalized to 1. (c) Western blots of ZO-1 and GAPDH in brain tissue from WT and *Fam171a2* CKO mice. Right, quantification of ZO-1 expression. $n = 6$ mice per group. WT normalized to 1. (d) Schematic of *in vivo* two-photon imaging setup and representative time-lapse images of intravascular dye fluorescence in cerebral vasculature over 30 min. (e) Schematic of quantification strategy for vascular morphology. (f) Quantification of vascular parameters: vessels area percentage, total number of branches, and average vessel length. (g) Quantification of endothelial cells per vessel length (mm) in cortices of WT and CKO mice. (h) Quantification of intravascular changes of fluorescent intensity (Δintensity) over 30 min and at endpoint. (i) Quantification of extravascular changes of fluorescent intensity (Δintensity) over 30 min and at endpoint. (j) *In vivo* two-photon images of cortical vasculature, astrocytes (red) and pericytes (green) in WT and CKO mice. (k) Quantification of pericyte and astrocyte densities from *in vivo* imaging. $n = 9$ fields of view from 3 mice for each group. (l) GFAP expression (left) and quantification (right) in cortex in WT and CKO mice. $n = 3$ or 4 mice per group. Data are mean ± SEM. *P* values (shown in the figure) were calculated by unpaired *t*-tests or two-way ANOVA followed by Bonferroni's multiple comparison test. WT, wild type; CKO, conditional knockout; OE, overexpression.

FAM171A2 overexpression increased both the LC3-II/LC3-I ratio and p62 level (Fig. 1g, h), indicating enhanced autophagosome formation. Consistently, the expression of ATG13, an early autophagosome protein, was also reduced in the brain tissue of *Fam171a2* CKO mice and increased in bEnd.3 cells upon FAM171A2 overexpression (Fig. 1i, j). To directly visualize autophagosome formation, we performed transmission electron microscopy (TEM) on bEnd.3 cells treated with CQ. TEM confirmed that FAM171A2 overexpression increased the number of double-membrane autophagosomes (Fig. 1k). Taken together, these data indicate that FAM171A2 promotes autophagic flux, including autophagosome formation, autophagosome-lysosome fusion, and lysosomal function (Fig. 1l).

We further investigated the mechanism underlying FAM171A2-mediated autophagosome-lysosome fusion. We purified intracellular vesicles from primary endothelial cells using density gradient centrifugation. *Fam171a2* knockout did not significantly affect vacuolar protein sorting components (VPS34) or autophagy-related proteins (ATG5, ATG16, ATG12) on vesicles (Fig. S4a–c online), which are critical for the initiation and formation of endosomes and primary autophagosomes [3]. On the other hand, levels of Syntaxin 17 (STX17) and vesicle-associated membrane protein 8 (VAMP8), two SNARE proteins essential for autophagosome-lysosome fusion [4], were markedly reduced in the vesicular fractions from *Fam171a2* knockout endothelial cells (Fig. 1m, n). Overexpression of STX17 in *Fam171a2* knockout cells partially reduced p62 accumulation (Fig. 1o, p), indicating that impaired fusion can be rescued by restoring STX17. These results suggest that the impaired autophagosome-lysosome fusion caused by FAM171A2 insufficiency is at least partly attributable to reduced vesicular recruitment of STX17.

Previous studies have shown that autophagy is essential for the survival of endothelial cells and that disruption of autophagy can induce endothelial apoptosis [5]. We therefore assessed apoptosis in endothelial cells of *Fam171a2* CKO mice. TUNEL staining revealed a significant increase in apoptotic endothelial cells in the brains of CKO mice compared to wild-type littermates (Fig. S5a, b online). Primary endothelial cells from *Fam171a2* CKO mice also exhibited a higher proportion of TUNEL-positive cells and increased levels of cleaved caspase-3 (Fig. S5c–f online). These results suggest that loss of FAM171A2 promotes endothelial cell apoptosis, potentially compromising vascular integrity. Given that STX17 overexpression partially restored autophagic flux in Fam171a2-deficient cells, we next examined whether it could also reduce the apoptosis described above. Indeed, overexpression of STX17 in Fam171a2-deficient primary endothelial cells partially reduced caspase-3 cleavage (Fig. S5g, h online). Thus, FAM171A2 deficiency triggers endothelial apoptosis via blocking autophagic flux.

We next investigated whether this endothelial cell loss affects vascular function *in vivo*, focusing on the blood–brain barrier (BBB). In cultured endothelial cells, Fam171a2 knockdown reduced trans-endothelial electrical resistance while overexpression had opposite effects (Fig. 2a). Fam171a2 insufficiency decreased expression of ZO-1, a crucial protein for tight junctions, in cultured endothelial cells and the mouse brain (Fig. 2b, c). To visualize BBB integrity, we performed two-photon imaging through a cranial window after retro-orbital injection of fluorescent dextran (Fig. 2d). As tamoxifen was given in adulthood, developmental angiogenesis was unlikely affected. Indeed, vascular length and branching patterns were unchanged in CKO mice, though endothelial cell density was slightly reduced (Fig. 2e–g). These data indicate that Fam171a2 insufficiency does not induce overwhelming structural degeneration in the brain vasculature. We then performed time-lapse imaging in these mice. We found that within 30 min after dye injection, control mice exhibited a slow (approximately 20%) decline in intravascular fluorescence due to peripheral clearance. In contrast, littermate CKO mice showed rapid loss of intravascular signal and a gradual increase of fluorescence in the perivascular cerebral parenchyma, indicating dye leakage (Fig. 2h, i). This phenotype was independently confirmed using thioflavin S as the intravascular label (Fig. S6a online). Furthermore, we examined the changes in vasculature-associated pericytes and astrocytes by specific labeling via NeuroTrace [6] and sulforhodamine 101, respectively. There was no overt pericyte loss or morphological change in astrocyte, but mild astrogliosis was observed (Fig. 2j, k). Immunostaining of GFAP confirmed widespread astrocyte activation (Fig. 2l). Fibrinogen extravasation and microglial activation were also elevated in *Fam171a2* CKO brains (Fig. S6b, c online).

Our findings reveal a physiological role of FAM171A2 in brain endothelial cells: it promotes autophagic flux, including autophagosome formation, autophagosome-lysosome fusion, and lysosomal function. Loss of FAM171A2 in endothelial cells disrupts autophagy, causes apoptosis, and compromises BBB integrity. While our previous studies suggested that FAM171A2 suppression might be a therapeutic approach for neurodegenerative diseases [1], the present study argues against non-selective suppression, consistent with the embryonic lethality of whole-body *Fam171a2* knockout [1]. Future studies should explore cell-type-specific or site-specific modulation of FAM171A2 as viable strategies for treatment.

As shown in our data, autophagy could be necessary for cell survival, as accumulation of misfolded proteins and dysfunctional organelles could generate toxicity [7]. Autophagy may also recycle small-molecule nutrients, thereby helping maintain metabolic homeostasis and energy supply [8]. In addition, degradation of pathological protein aggregates, such as β-amyloid, α-synuclein, and TDP-43, may require autophagosome and lysosome functions [9]. Therefore, nonspecific suppression of FAM171A2 in many cases could disrupt essential cell functions.

BBB breakdown can be observed at the preclinical stage of Alzheimer's disease, even before the deposition of β-amyloid. Postmortem studies show perivascular leakage of fibrin, albumin, immunoglobulin, and thrombin, all of which are blood-derived proteins, in the cortex and hippocampus of Alzheimer's disease patients [10]. All these phenomena indicate the close relationship between BBB dysfunction and neurodegenerative diseases. It is worth noting that various receptors exist on endothelial cells that act as transporters for crossing the BBB, including LDL receptor-related protein-1 (LRP1) and the receptor for advanced glycation end products (RAGE) for β-amyloid [11]. Considering our previous report that FAM171A2 can directly bind to α-synuclein and initiate its endocytosis [2], it could be possible that FAM171A2 on endothelial cells facilitates the entry of α-synuclein into, or its exit from, the brain. The complex features of FAM171A2's involvement in physiological and pathological processes warrant further studies to clarify.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (82271471, 82530047, 82588301, 82501500, 32371036, 82271471, 82401676, 82201468, and 82401677).

Author contributions

Jintai Yu, Peng Yuan, and Sida Han conceptualized the study. Sida Han, Yiwei Feng, Kaimin Wu, and Tingting Wang performed

experiments. Ning Ding and Jiahui Huang performed the *in vivo* imaging study and analysis. Sida Han and Tingting Wang analyzed the results. Qiang Dong, Mei Cui, Peng Yuan, and Jintai Yu managed the project. Peng Yuan and Jintai Yu acquired the resources and supervised the study. Sida Han and Ning Ding prepared the illustrations. Sida Han wrote the first draft of the manuscript. Tingting Wang, Peng Yuan, and Jintai Yu edited the manuscript.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scib.2026.06.002.

References

[1] Xu W, Han SD, Zhang C, et al. The FAM171A2 gene is a key regulator of progranulin expression and modifies the risk of multiple neurodegenerative diseases. Sci Adv 2020;6:eabb3063.
[2] Wu KM, Xu QH, Liu YQ, et al. Neuronal FAM171A2 mediates α-synuclein fibril uptake and drives Parkinson's disease. Science 2025;387:892–900.
[3] Nishimura T, Tooze SA. Emerging roles of ATG proteins and membrane lipids in autophagosome formation. Cell Discov 2020;6:32.
[4] Itakura E, Kishi-Itakura C, Mizushima N. The hairpin-type tail-anchored SNARE syntaxin 17 targets to autophagosomes for fusion with endosomes/lysosomes. Cell 2012;151:1256–69.
[5] Mameli E, Martello A, Caporali A. Autophagy at the interface of endothelial cell homeostasis and vascular disease. FEBS J 2022;289:2976–91.
[6] Damisah EC, Hill RA, Tong L, et al. A fluoronissl dye identifies pericytes as distinct vascular mural cells during *in vivo* brain imaging. Nat Neurosci 2017;20:1023–32.
[7] Dikic I, Elazar Z. Mechanism and medical implications of mammalian autophagy. Nat Rev Mol Cell Biol 2018;19:349–64.
[8] Mizushima N, Levine B, Cuervo AM, et al. Autophagy fights disease through cellular self-digestion. Nature 2008;451:1069–75.
[9] Maiuri MC, Zalckvar E, Kimchi A, et al. Self-eating and self-killing: crosstalk between autophagy and apoptosis. Nat Rev Mol Cell Biol 2007;8:741–52.
[10] Erickson MA, Banks WA. Blood-brain barrier dysfunction as a cause and consequence of Alzheimer's disease. J Cereb Blood Flow Metab 2013;33:1500–13.
[11] Storck SE, Meister S, Nahrath J, et al. Endothelial LRP1 transports amyloid-β(1-42) across the blood-brain barrier. J Clin Invest 2016;126:123–36.