Alzheimer’s disease (AD) and diabetes are clinically positively correlated. However, the connection between them is not clarified. Here, using Drosophila as a model system, we show that reducing insulin signaling can effectively suppress the toxicity from Aβ (Amyloid beta 42) expression. On the other hand, Aβ accumulation led to the elevation of fly insulin-like peptides (ILPs) and activation of insulin signaling in the brain. Mechanistically, these observations are attributed to a reciprocal competition between Drosophila insulin-like peptides and Aβ for the activity of insulin-degrading enzyme (IDE). Intriguingly, peripheral insulin signaling is decreased despite its heightened activity in the brain. While many upstream factors may modify Aβ toxicity, our results suggest that insulin signaling is the main downstream executor of Aβ damage, and thus may serve as a promising target for Alzheimer’s treatment in non-diabetes patients. This study explains why more Alzheimer’s cases are found in diabetes patients.
Alzheimer’s disease (AD) is the most common type of neurodegenerative disease among the elderly, leading to decline of memory, behavioral performance, and cognition. The extracellular senile plaque, an aggregation of β-amyloid peptide (Aβ), and intracellular neurofibrillary tangles (NFTs), comprised of microtubule-associated protein Tau, are considered the two major hallmarks of this progressive neuropathology. Among factors that may affect late-onset AD, age is the major risk factor. AD incidence increases to 15% among people aged over 65 and further increases to 40–50% among people aged over 85[1–4]. At present, it is not clear how aging may increase the risk of AD and which factors of the aging process are involved in the progress and pathology of AD.

Diabetes is also a risk factor for AD and dementia[5–7]. In particular, type 2 diabetes increases the risk of developing cognitive impairment and dementia[8,9]. Type 2 diabetes and AD share several common symptoms such as hyperglycemia, hyperinsulinemia, and insulin resistance. Type 2 diabetes may facilitate the progress of AD, and AD increases the risk of developing type 2 diabetes[10,11]. Obviously, there is an intimate link between AD and type 2 diabetes, but it is not clear how they are mechanistically linked. Although AD patients tend to be physiologically overall insulin resistant, evidence also suggests that the level of insulin/insulin signaling is unchanged or increased in AD brains[12], and downstream activity like AKT (also known as PKB, protein kinase B) is upregulated[13,14]. Consistently, insulin level in the serum of AD patients is higher than in healthy subjects[15,16], and hyperinsulinemia is associated with increased risk of AD[17]. Generally speaking, the current literature is very confusing with regard to how AD and insulin are connected. It has been shown that insulin treatment can modestly improve cognition, but also worsen AD pathology[10,17–19], whereas increasing insulin signaling can aggravate or protect from AD pathology[20]. The relationship between insulin signaling and AD thus starts to get bewildering. If type 2 diabetes is associated with AD, then reduced insulin signaling might underlie AD, but why would hyperinsulinemia or insulin treatment of type 2 diabetes aggravate AD?

In the course of studying how ageing relates to AD in Drosophila, we found reduction of insulin signaling efficiently ameliorates Aβ toxicity. Downregulation of Drosophila insulin-like peptides (ILPs), which are secreted by neurons in the brain[21], and multiple other insulin signaling components, could drastically suppress Aβ toxicity. As insulin signaling has been established as an important player in ageing, our findings link ageing, AD, and type 2 diabetes, and indicate insulin signaling is the common underpinning of these biological phenomena. We speculate that in type 2 diabetes patients insulin insensitivity may not uniformly occur in all tissues. It is therefore possible that compensatory effects due to type 2 diabetes may hyperactivate insulin signaling in certain brain regions where insulin signaling was originally less affected, thus aggravating AD.

**Results**

*chico downregulation mitigates Aβ toxicity*. Since ageing is the single most important contributing factor to AD, and ageing and neurodegeneration share common features, we explored whether ageing factors underlie AD. A group of upstream activation sequence (UAS)-driven RNA interference (RNAi) transgenes targeting genes known to be involved in ageing (Supplementary Table 1) were crossed into the AD model fly Elav-Gal4: UAS-Aβ42, which expresses Aβ42 in the central nervous system (CNS) (Elav-Gal4 specifically directs UAS-fused gene expression in the CNS, Supplementary Fig. 1a)[22]. Expression of Aβ42 in the fly CNS causes neurodegeneration and brain vacuolization, resulting in severe climbing disability and lifespan reduction[22]. For technical reasons, we mainly examined locomotion loss and degree of vacuolization as indicators of Aβ42 toxicity.

We found downregulation of fly ageing genes chico, Indy (I’m not dead yet) and mth (methuselah) significantly improved the climbing ability of AD flies (Supplementary Fig. 1b), indicating downregulation of these genes could modify Aβ toxicity. As chico is the homolog of mammalian insulin receptor substrate (IRS), and the only one with a clearly characterized pathway, we decided to further analyze this gene and its likely pathway. Knocking-down chico improved the climbing ability of Aβ flies, from 16% to 60% without any adverse effect on the climbing ability of wild-type (WT) flies (Fig. 1a). RNAi efficacy was confirmed by reverse transcription-PCR (RT-PCR) (Supplementary Fig. 2). Expression of human Aβ42 in the fly CNS driven by Elav-Gal4 causes severe neurodegeneration including brain vacuolization[23,24]. In order to evaluate neurodegeneration rescue by chico knock-down, sections of fly heads were stained with hematoxylin–eosin (H&E staining) and examined under the microscope for degenerative vacuolization. We found that chico RNAi dramatically decreased the number of vacuoles in Aβ fly brains (Fig. 2a, b), indicating it could suppress neurodegeneration of the Aβ fly.

Insulin signaling pathway mediates Aβ toxicity. To ascertain whether other components in the insulin pathway besides chico are involved in AD, we first tested the effect of RNAi and overexpression of *InR* (insulin receptor) (Supplementary Fig. 2). Knocking-down *InR* in Aβ flies dramatically improved the climbing ability of Aβ flies (Fig. 1b); two independent lines improved the climbing ability of Aβ flies from 15% to 60% and 58%, respectively (Fig. 1b). As a control, the effects of *InR* RNAi on normal flies were tested. One *InR* RNAi line (line 2#) did not affect the climbing ability of normal flies, whereas the other RNAi line (line 1#) slightly improved the climbing ability of WT flies (Fig. 1b), probably as a result of *InR*’s effects on aging and related climbing disability. However, overexpression of *InR* in Aβ flies did not significantly worsen the climbing disability of Aβ flies (Fig. 1b), likely because the climbing disability was already very severe in the aged Aβ flies (~30 days old flies). Consistently, Drosophila ILPs knock-down also suppressed Aβ toxicity. It has been reported that ILP1, 2, 3, and 5 are all expressed in the fly brain[24]. Individual knock-down of Drosophila ILP1, ILP2, and ILP5 by using Elav-Gal4 (Elav-Gal4 directs expression in the brain including the insulin-producing cells (IPCs), Supplementary Fig. 1c), could effectively rescue the climbing disability of Aβ flies (Fig. 1c); the climbing ability was restored from 20% to ~40–50% of the normal, whereas knocking-down ILP1, ILP2, and ILP5 did not affect the climbing ability of the control flies (Fig. 1c). ILP3 RNAi, however, had some side effects on its own (causing the impairment of the climbing ability in the control flies), making the analysis difficult. We therefore did not pursue ILP3 further.

We then analyzed some downstream components of insulin signaling such as phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) and target of rapamycin (Tor)[25] for their Aβ-toxicity-rescuing effects. Knocking-down Pi3K components Pi3Kδ8D and Pi3Kδ21B could both improve the climbing ability of Aβ flies, whereas their effects on the WT flies were not significant (Fig. 1d). Tor downregulation had similar climbing ability-rescuing effects (Fig. 1e).

We additionally used Cha-Gal4 (expressed in fly cholinergic neurons) to confirm the results. Knocking-down of *InR* and *chico* by Cha-Gal4 could also mitigate the climbing disability of the Aβ expression fly (Supplementary Fig. 3a).
To exclude the possibility that the rescue effect was a secondary effect of early stage gene expression, we adopted the RU486-induced gene switch system to examine insulin signaling effects on Aβ toxicity. With this drug-induced expression system, we could analyze the effect of genes specifically on adult stages, bypassing the early development period. Knocking-down insulin signaling components in the adult stage could still rescue the climbing disability caused by Aβ expression, as InR, chico, Pi3K21B, and ILP1/2 RNAi all could suppress Aβ toxicity (Supplementary Fig. 3b and Supplementary Fig. 3c).

Fig. 1 Genetic screening identifies insulin signaling as a significant modifier of Aβ toxicity. a Effects of chico RNAi on the climbing ability of Aβ flies. Elav-Gal4 was used to drive the expression of Aβ and the knock-down of chico. Data represent mean ± SEM, **p < 0.01. Elav-Gal4 was used to express Aβ and knock-down ILPs in fly CNS. The corresponding genotypes: “Aβ+/+” is Elav-Gal4>UAS-Aβ+/+; “chico-R/Aβ” is Elav-Gal4>UAS-Aβ/UAS-chico RNAi; “WT” is Elav-Gal4+/+; “chico-R/+” is Elav-Gal4>UAS-chico RNAi/+. b Effects of InR manipulation on the climbing ability of Aβ flies. Elav-Gal4 was used to drive the overexpression of InR, Aβ and the knock-down of InR. Data represent mean ± SEM, **p < 0.01. The corresponding genotypes: “Aβ+/+” is Elav-Gal4>UAS-Aβ+/+; “InR-R/Aβ” is Elav-Gal4>UAS-Aβ/UAS-InR RNAi; “InR-RE/Aβ” is Elav-Gal4>UAS-InR/Aβ; “Ap+/+” is Elav-Gal4>UAS-Aβ+/+; “InR-RE/+/+” is Elav-Gal4>UAS-InR+/+; “InR-RE/+/+” is Elav-Gal4>UAS-InR+/+. c Effects of inhibitors on the climbing ability of Aβ flies. Elav-Gal4 was used to express Aβ and knock-down ILPs in fly CNS. Data represent mean ± SEM, **p < 0.01. d Effects of fly PI3K68D and PI3K92E RNAi on the climbing ability of Aβ flies. Elav-Gal4 was used to express Aβ and knock-down PI3K in fly CNS. Data represent mean ± SEM, **p < 0.01. e Effects of fly Tor RNAi on the climbing ability of Aβ flies. Elav-Gal4 was used to express Aβ and knock-down Tor in fly CNS. Data represent mean ± SEM, **p < 0.01. f Effects of inhibitors on the climbing ability of Aβ flies. InR inhibitor GSK19045297 and PI3K inhibitor Wortmannin could improve the mobility of Aβ flies. Elav-Gal4 was used to express Aβ in fly CNS. Data represent mean ± SEM, **p < 0.01. 0.01. Elav-Gal4 was used to drive Aβ expression in fly CNS.
**Fig. 2** Downregulating insulin signaling reduces neurodegeneration in Aβ flies. **a–d** Modulations of fly insulin signaling by RNAi or overexpression of the downstream components can effectively affect the number of vacuoles in the brains of Aβ flies. *Elav-Gal4* was used to overexpress and knock-down the corresponding genes. **a, c** Paraffin brain sections were stained with H&E. Green arrow heads indicate the degenerative vacuoles in flies’ brains. Scale bar: 50 μm. **b** Quantification of (**a**). Data represent mean ± SEM, *p* < 0.05, **p** < 0.01, ***p*** < 0.001. **d** The quantification of (**e**). Data represent mean ± SEM, ***p*** < 0.001. **e, f** H&E-stained paraffin brain sections of the Aβ, and Aβ, ILPs RNAi flies. *Elav-Gal4* was used to express Aβ and knock-down the corresponding genes. Green arrow heads indicate the degenerative vacuoles in flies’ brains. Scale bar: 50 μm. **f** Quantification of (**e**). Data represent mean ± SEM, **p** < 0.01, ***p*** < 0.001.
In addition to genetic interference, we further used chemical drugs to examine the effects of insulin signaling on Aβ toxicity. Inhibitors of both InR (GSK19045297) and PI3K (Wortmannin) significantly improved the climbing ability of Aβ flies (Fig. 1f), confirming that inhibition of insulin signaling can rescue Aβ toxicity.

The degenerative process in the brain of Aβ flies was further analyzed. RNAi of InR, PI3K21B, and Tor reduced the number of brain vacuoles in Aβ flies (Fig. 2a–d). H&E staining of brain sections revealed similar rescuing effects for ILPs RNAi: the number of brain vacuoles in Aβ flies was significantly reduced when ILP1, ILP2, and ILP5 were knocked-down (Fig. 2e, f), indicating insulin signaling, i.e., insulin receptor and its downstream components, mediates the toxicity of Aβ in the fly’s brain.

The above results obtained with both genetic techniques and chemical drugs proved that insulin signaling mediates Aβ toxicity; reduction of insulin signaling suppresses, instead of enhancing, Aβ fly phenotypes.
Insulin signaling is mis-regulated in Aβ flies. As Aβ toxicity is related to the activity of insulin signaling, we asked whether Aβ expression could alter the level of insulin and insulin signaling. In the literature, changes of insulin level and insulin signaling in AD brains are still debated and inconsistent. By using RT-PCR analyses, we found that the head level of thor (4EBP homolog in the fly), which is reduced by insulin, was decreased in Aβ flies (Fig. 3a); the level of head tobi (target of brain insulin) was increased in Aβ flies (Fig. 3a). These results suggest Aβ expression indeed positively affects InR signaling pathway in the head. To confirm this, we further analyzed the levels of phosphorylated AKT (p-AKT), whose phosphorylation state is regulated in InR signaling. Our results showed that the downstream component AKT was activated in the head of Aβ flies, as revealed by elevated p-AKT (Fig. 3b).

To test whether the effect of Aβ on insulin signaling relies on upstream activation by ligands, we examined the levels of ILP2 and ILP5, two of the major ILPs in the brain. The protein levels of ILP2 and ILP5 were indeed elevated in Aβ flies when compared with those in control flies (Fig. 3c), indicating that Aβ expression led to an increase of ILP2 and ILP5. Immunostaining of Repo and ILP2 indicated that the glial cells and IPCs were not significantly affected in AD flies (Supplementary Fig. 4a and Supplementary Fig. 4b), but ILP2 appeared to be more accumulated in IPCs (Supplementary Fig. 4c).

The level of insulin signaling in peripheral tissues of AD flies was then assayed. tobi mrna level was significantly reduced in the fat body of AD flies (Fig. 3d), but that of thor increased, indicating a reduction of insulin signaling in peripheral tissues. This reduction is in contrast to the increase in the brain. Western blot indicated that circulating ILP2 protein signals, albeit very weak, appeared reduced in the hemolymph of Aβ flies (Fig. 3e). We, however, cannot be certain that this is also the case for the other ILPs in the body, given undetectable level of ILP5 and lack of appropriate antibodies to the other ILPs.

We next tested whether ILPs suppression could reciprocally reduce Aβ levels. We separated the soluble and insoluble fractions of Aβ from fly heads and tested by dot blot their individual levels. Both fractions of Aβ were significantly reduced when ILP1 and ILP5 were knocked-down (Fig. 3f). Results from Aβ immunostaining also confirmed this observation (Fig. 3g, h). We conclude that ILPs reduction effectively downregulates Aβ toxicity and decreases Aβ level.

Aβ competes with Drosophila ILPs for IDE degradation. To explore the mechanism of reciprocal regulation between ILPs and Aβ, we focused our attention on a metalloprotease named insulin-degrading enzyme (IDE), which might regulate the level of both Aβ and mammalian insulin. Although it has been reported that IDE regulates the level of fly ILPs in vivo, we did not know whether Drosophila IDE could regulate the level of Aβ, and if so whether competition between insulin and Aβ for IDE contributed to Aβ toxicity in vivo. To ascertain this, we separated the soluble and insoluble Aβ fractions from the heads of Aβ flies, and tested the level of Aβ with an antibody (Fig. 4a, b). IDE is broadly expressed in the adult fly brain, including the neurons of the mushroom body (Supplementary Fig. 5a). We modulated IDE expression by using Elav-Gal4 (Supplementary Fig. 5b). Overexpression of Drosophila IDE (Supplementary Fig. 5b) significantly reduced both the soluble and insoluble Aβ levels, indicating that the total levels of Aβ were reduced in IDE-overexpressing flies (Fig. 4a). We also tested Aβ deposition in fly brains. Immunostaining showed that Aβ deposition was reduced when IDE was overexpressed (Fig. 4c, d). Correspondingly, RNAi of IDE (Supplementary Fig. 5b) led to an increase of Aβ deposition (Fig. 4b, e, f). These results indicate that the level of Aβ in fly brains is regulated by Drosophila IDE.

As insulin and Aβ are both substrates of IDE, a hypothesis relating insulin, Aβ and IDE has been proposed, but never tested. According to this model, IDE can degrade Aβ and insulin, and these two substrates may compete for the degrading enzyme. To address this possibility, direct competition between Aβ and Drosophila ILPs was tested by an in vitro degradation assay wherein Escherichia coli-expressed Drosophila IDE and full-length ILPs proteins were co-incubated. These purified ILPs were capable of functionally inducing insulin signaling in vitro to some extent (Supplementary Table 2 and Supplementary Fig. 6). Competition experiments did indeed show that Drosophila IDE could degrade both Drosophila ILPs (ILP1, ILP2, and ILP5) and Aβ in vitro (Fig. 5a, b). Moreover, Aβ inhibited the degradation of fly ILPs by IDE (Fig. 5a), and conversely fly ILPs (like ILP1, ILP2, and ILP5) inhibited Aβ degradation (Fig. 5b). A subsequent semi-in-vitro degradation assay, wherein protein extracts from IDE overexpression flies and control flies were co-incubated with Aβ and fly ILPs, further supported this conclusion. Although both Aβ and fly ILPs could be degraded by WT fly extracts, the extract from IDE-overexpressing flies showed enhanced degrading activity (Fig. 5c). Furthermore, Aβ and ILPs antagonized degradation of each other (Fig. 5d, e). The degradation of ILP1 and ILP2 by fly extract was partially blocked by the addition of Aβ (Fig. 5d). Similarly, Aβ degradation by fly protein extract could be largely inhibited by ILPs addition (Fig. 5e). Interestingly, ILPs and Aβ could not completely block the degradation of each other, indicating there are other degrading enzymes in the fly protein extract.

In order to prove that the degradation was due to the function of IDE, 6bk, a compound that inhibits IDE, was used. Degradation of ILP1 and Aβ by the protein extracts was indeed blocked by 6bk (Fig. 5f), especially in the case of the IDE-overexpressed extract. Altogether, these results show that Aβ and Drosophila ILPs compete for the activity of Drosophila IDE, resulting in reciprocal accumulation. Accumulation of ILPs caused by Aβ could also explain why insulin and insulin signaling were elevated in Aβ flies.

Considering that our recombinant ILPs are not mature in nature and fly in origin, we further tested competition between bovine mature insulin and human Aβ for the action of IDE. The result indicated that bovine insulin could inhibit Aβ degradation by fly IDE protein in vitro (Fig. 5g).
To ascertain whether ILPs/InR signaling could mitigate the effects of IDE on Aβ flies, we recombined IDE RNAi and InR RNAi together in the Aβ fly. IDE RNAi reduced the climbing ability of Aβ flies; however, the effect of IDE RNAi on Aβ flies was completely suppressed by InR RNAi (Fig. 6e). The increase of brain vacuoles caused by IDE RNAi was also suppressed by InR RNAi (Fig. 6f, g). The epistatic relationship between IDE and InR indicated that the effects of IDE on Aβ toxicity are mediated by the ILPs/InR signaling axis.

S6K is involved in the action of IDE-ILPs (insulin)/InR on Aβ toxicity. Downstream insulin/InR signaling branches into forkhead box, sub-group O (FOXO) and Ribosomal protein S6 kinase (RpS6K, S6K). It has been shown that activation of insulin signaling may result in FOXO phosphorylation and inactivation37. If FOXO inactivation mediates the effects of IDE-insulin/InR on Aβ toxicity, overexpressing FOXO may reduce Aβ toxicity, whereas repressing FOXO may aggravate Aβ toxicity. Therefore, we tested whether overexpression and RNAi of fly

Fig. 4 IDE regulates the level of Aβ in vivo. a-d Manipulating the level of IDE could affect the level of Aβ in fly. Elav-Gal4 was used to drive the overexpression or knock-down of IDE. a, b The levels of Aβ in IDE OE flies and IDE RNAi flies were analyzed by dot blotting. Actin was used as the loading control (uncropped images of the blots are presented in Supplementary Fig. 11e and Supplementary Fig. 11f). Elav-Gal4 was used to drive the overexpression or knock-down of IDE. Results are reproducible in three independent dot blotting experiments, and only one is shown here. c-f The levels of Aβ aggregates were analyzed with immunostaining. (c, d) The level of Aβ aggregates in IDE-overexpression flies, n = 6. Scale bar = 50 μm. f The quantification of (e). Data represent mean ± SEM, *p < 0.05.
**Fig. 5** Aβ and ILPs compete for the activity of IDE. **a** Aβ inhibits the ILPs degradation in vitro. Recombinant and purified ILPs-HA were co-incubated with IDE or IDE and Aβ peptide for 12 h before western blotting analyses. **b** ILPs inhibit the Aβ degradation in vitro. Aβ peptides were incubated with IDE in the presence or absence of ILPs-HA, and the levels of Aβ were analyzed by western blotting. **c** Aβ and ILPs can be degraded by protein extracts from the control and IDE-overexpression flies. ILP1-HA, ILP5-HA, ILP2-HA and Aβ were incubated with the protein extracts from control and IDE overexpression flies. After incubation, levels of ILPs and Aβ were analyzed by western blotting. Actin was used as the loading control. **Elav-Gal4** was used to drive the overexpression of fly IDE in the CNS. Protein extracts were prepared from fly heads. **d** Aβ inhibits the semi-in-vitro degradation of ILP1 and ILP2. ILP1-HA and Aβ were analyzed by western blotting. ILP5-HA and Aβ peptides were co-incubated with IDE and Aβ in the presence or absence of ILPs. Levels of Aβ were analyzed by western blotting. **f** 6bk inhibits the effects of IDE-overexpression on ILP1 and Aβ degradation in vitro. ILP1-HA and Aβ peptides were co-incubated with IDE and Aβ in the presence or absence of 6bk. Levels of Aβ and ILP1-HA were analyzed by western blotting. Uncropped images of the western blots (**a-f**) are presented in Supplementary Fig. 12a-Supplementary Fig. 12f. **g** Bovine insulin inhibited the Aβ degradation in vitro. Recombinant and purified IDE protein was used to perform the in vitro cleavage assay. Aβ was co-incubated with IDE or IDE and bovine insulin for 12 h before western analyses. b-insulin bovine insulin. All western blot results here are repeated three times, and only one is shown. *p < 0.05, **p < 0.01, ***p < 0.001
Fig. 6 IDE-insulin/InR signaling regulates Aβ toxicity in vivo. a Overexpression of Drosophila IDE improved the climbing ability of Aβ flies. Data represent mean ± SEM, **p < 0.01. Elav-Gal4 was used to drive Aβ and fly IDE expression in fly CNS. OE overexpression. b Knocking-down Drosophila IDE exacerbated the climbing disability of Aβ flies. IDE-R RNAi of Drosophila IDE. IDE RNAi was driven by Elav-Gal4. Data represent mean ± SEM, **p < 0.01. c, d Manipulation of the level of IDE affected the degenerative process in the brain of Aβ flies. c Hematoxylin and eosin (H&E)-stained paraffin brain sections of these Aβ, Aβ/IDE-OE, and Aβ/IDE-RNAi flies. Elav-Gal4 was used to drive gene overexpression or knocking-down in fly CNS. Green arrow heads indicate only some of the many degenerative vacuoles in the brain. Scale bar: 50 μm. d The quantification of (c). Data represent mean ± SEM, **p < 0.01. e The effect of IDE and InR double RNAi on the climbing ability of Aβ flies. Elav-Gal4 was used to overexpress and knock-down the corresponding genes. Data represent mean ± SEM, *p < 0.05, **p < 0.01. f, g H&E-stained paraffin brain sections of the IDE and InR double RNAi, Aβ-expression flies. Green arrow heads indicate the degenerative vacuoles in flies’ brains. Scale bar: 50 μm. g The quantification of (f). Data represent mean ± SEM, *p < 0.05, **p < 0.01.
FOXO could change the toxicity of Aβ. Unfortunately, overexpression of FOXO in the fly CNS led to larval death at the first instar stage. Similarly, overexpression of FOXO-GFP (green fluorescence protein) in the fly CNS also caused developmental arrest, with many flies dying on the pupal stage. Nevertheless, some of the FOXO-GFP-expressing flies did manage to eclose, although with abnormal wing posture. Importantly, expression of FOXO-GFP in the fly CNS did not alter the climbing abnormality of these eclosed Aβ flies despite their abnormal wing posture (Supplementary Fig. 7a). Knock-down of FOXO or expression of the phosphorylated form of FOXO did not aggravate the toxicity of Aβ (Supplementary Fig. 7b); instead, they both reduced the climbing abnormalities of Aβ flies. Altogether, these results suggest that FOXO inactivation may not boost Aβ toxicity. If anything, FOXO inactivation mitigates Aβ toxicity, likely in an indirect manner.

S6K is another downstream branched effector of insulin signaling, also involved in the aging process 38. S6K is activated when insulin signaling activity is elevated. We tested whether S6K was involved in IDE-ILPs (insulin)/InR’s effects on Aβ toxicity. Our results indicated S6K knock-down improved the climbing ability of Aβ flies (Fig. 7a). Brain sections also revealed S6K manipulation could reduce Aβ toxicity in the flies (Fig. 2c, d). Furthermore, western blot revealed that the level of phosphorylated S6K (p-S6K) was elevated in Aβ flies (Fig. 7b), indicating S6K was activated in Aβ flies, consistent with the elevation of fly insulin signaling.

To ascertain whether S6K could mitigate the effect of IDE on Aβ flies, we introduced IDE RNAi and S6K RNAi into the Aβ fly. IDE RNAi reduced the climbing ability of Aβ flies. However, the effect of IDE RNAi on Aβ was suppressed by S6K RNAi (Fig. 7c). S6K RNAi improved the climbing ability upon IDE knock-down, indicating that S6K mediates the effect of IDE on Aβ toxicity and this IDE-ILPs (insulin)/InR-S6K signaling axis is involved in Aβ toxicity. Consistently, chico RNAi decreased the level of p-S6K in Aβ flies (Supplementary Fig. 8). Interestingly, when mth or Indy were knocked-down, the level of p-S6K was also reduced in Aβ flies (Supplementary Fig. 8), indicating S6K may also be involved in the effects of mth and Indy on Aβ toxicity, and in the crosstalk between mth and insulin signaling.

Discussion
Mammalian insulin/insulin-like growth factor 1 (IGF-1) is in the brain is not fully understood. Changes in insulin signaling may not only affect InR signaling, but also IGF-1 receptor signaling 39. The model organism Drosophila is endowed with a comparably simpler insulin/insulin signaling pathway, which may facilitate understanding of the connection between AD and insulin/insulin signaling; in particular, the connection between toxicity of Aβ and insulin/insulin signaling.

In this work, we presented evidence that Aβ and ILPs compete for fly IDE activity and that Aβ causes accumulation of fly ILPs...
and elevation of fly InR-S6K signaling. This IDE-ILPs (insulin)/ InR-S6K signaling axis is involved in Aβ toxicity, and knockdown of ILPs, InR or the downstream components S6K is an effective means to suppress Aβ toxicity (Fig. 7d, model). As insulin signaling is an ageing factor, our results help to connect several important phenotypes, namely ageing, AD and diabetes, through insulin signaling.

The role of insulin and insulin signaling in human AD is debated. It is not fully clear whether the alteration of insulin signaling is the cause or the consequence of AD.26 Decreased insulin signaling occurs in patients with type II diabetes, where higher insulin levels are often elicited, likely as a compensatory action. These patients overall have a higher incidence of AD. On the other hand, it has been reported that insulin signaling is less affected in AD patients than in healthy persons.15 Interestingly, clinical insulin treatment is associated with increased AD rate10,16–18. How insulin and insulin signaling connect with AD mechanistically is confusing. Is decreased insulin signaling sensitivity such as in diabetes a cause of AD increase? Or is elevated insulin and insulin signaling can effectively reduce, instead of aggravate, Aβ pathology (Fig. 7d, model), favoring the latter possibility.

It has been reported that AD brains tend to be insulin resistant, accompanied by decline of insulin signaling. The decline of insulin signaling has been proposed as a consequence of AD and a cause further aggravating AD pathology.28 However, our observation that reducing insulin signaling can reduce, instead of aggravate, toxicity of Aβ suggests an alternative explanation: the association of insulin resistance or decrease of insulin signaling with AD may be considered a protective response to minimize the damage from Aβ. Considering that reduction of insulin signaling can improve cell survival and activation can trigger cell death, reduction of insulin signaling as a protective approach to avoid the more severe damage and prolong cell survival in AD brain seems reasonable.

It has been reported that IDE activity is reduced in some tissues during the aging process and it is connected with AD pathology. Reducing the level of IDE or genetic variations of IDE alter AD pathology. In mouse models, Aβ level was elevated when IDE was knocked out, and AD-related pathology was exacerbated under that condition. In our hands, we found that while manipulation of IDE could reduce Aβ toxicity, knockdown of the insulin receptor and some of its downstream components could to a much greater extent suppress the defects of the Aβ flies and the effects of IDE RNAi. In the absence of Aβ, knocking-down IDE by itself could cause some deleterious effects, such as the drop of climbing ability in aged flies (Supplementary Fig. 9), consistent with the notion that accumulation of ILPs/insulin and other substrates is a central theme not only to the pathophysiology of Aβ flies, but also to some aspects of ageing in normal flies. Besides IDE, other factors like Neprilysin (NEP) might also contribute to the degradation of insulin and Aβ. This explains why sole manipulation of IDE level does not affect Aβ toxicity as significantly as that of Drosophila ILPs (insulin), InR and some of other downstream signaling components.

It appears therefore that the involvement of insulin signaling in human AD pathology may consist of a complex repertoire of different states (Fig. 8, model), which may explain the conflicting results in this field. In some or probably most AD patients, insulin signaling is not affected. In some AD patients, insulin signaling is reduced overall accompanied by type 2 diabetes. However, we speculate that in some regions of the brain insulin signaling is not affected as much as in the rest of the body. In this scenario, compensatory insulin secretion in the rest of the body or insulin treatment will more than offset the loss of insulin signaling in the brain.
brain and exacerbate Aβ toxicity. In still some other AD patients, insulin signaling in the brain may be comparably more severely affected than in the rest of the body, affording a protective measure. In other words, compensation of the signaling loss in the body will not be enough to remedy the brain signaling loss in this case.

According to our RNAi-based genetic screening results, besides the insulin signaling component chico, the other two aging-related genes, mth and Indy, could also reduce the toxicity of Aβ. The mechanism of lifespan extension by mth and Indy remains to be elucidated. However, some evidence suggests that mth and Indy may crossstalk with insulin signaling43,44. Mth and its ligand Sun (stunted) can modulate physiological insulin levels in response to nutrient inputs in the larvae, helping to understand the crossstalk between mth and insulin signaling; decreasing Indy levels may be coupled with reduced insulin signaling. From this perspective, it is possible that Mth and Indy act through the insulin connection to modulate Aβ toxicity, and indeed we found p-S6K was decreased in mth and Indy RNAi flies. Nevertheless, how big an effect this is, i.e., whether Mth and Indy regulate Aβ toxicity, and whether it is only via insulin signaling, is unclear.

It has been suggested that downstream insulin signaling in AD may switch to mammalian target of rapamycin (mTOR) signaling45. Our results indicate that S6K is at least partially involved in the pathological process of Aβ pathology, and mediates the effects of IDE-insulin/InR signaling on Aβ toxicity. The downstream targets of insulin and insulin signaling remain incompletely elucidated46,47. At present, we still do not know how many downstream components of insulin signaling are involved in AD. Some results are still controversial and unclarified due to the complexity of insulin actions in mammals48,49. Our results indicate that FOXO inactivation may not be involved in insulin action on Aβ toxicity. Interestingly and paradoxically, it has been reported that, instead of inactivation, FOXO is activated by Aβ and mediates Aβ’s effect on cell death through Bim50, and this activation is likely caused by oxidative stress51,52, which is elevated by Aβ53. Our results indicate S6K, activated in AD flies, is involved and contributes to the pathological process of Aβ. Supporting this, it was also reported that S6K is activated in AD patients54. The mechanism of lifespan extension by S6K inhibition is thought to involve AMPK activation55,56, consistent with our results showing that AMPK inhibition can enhance Aβ toxicity (climbing disability, Supplementary Fig. 10).

In summary, we demonstrated that brain IDE-ILPs (insulin)/InR-S6K signaling is involved in AD pathology, which implies the mechanism contributing to age-related AD may be the same as the cause of ageing in the brain. These experimental findings offer better understanding of AD, ageing and diabetes, and may provide new AD-relevant strategies and therapeutic targets in the future.

Methods
Fly stocks and genetics. Fly stocks used in this study were mainly obtained from the Bloomington Drosophila Stock Center, the Vienna Drosophila Resource Center (VDRC), and the Drosophila Stock Center of Tsinghua University. The RNAi flies of chico (CG5688), Tor (CG3092), and PI3K86D (CG11621), PI3K21B (CG2699), IPI (CG14173), ILP2 (CG1167), IPI) (CG14167), and ILPS (CG3272) were obtained from Tsinghua Drosophila Stock Center. UAS-Inv by and RNAi flies of Inv (CG18402) (line 1# and line 2#), UAS-FOXO (CG3143), UAS-FOXO RNAi, UAS-pFOXO, UAS-FOXO-GFP. Flag, UAS-S6K (CG10539), and S6K RNAi flies were obtained from the Bloomington Drosophila Stock Center. IDE (CG5517) RNAi fly was obtained from the VDRC. IDE-GFP expression GFP under the control of the fly IDE promoter (and UAS-IDE fly and the corresponding background w- flies57) was a kind gift from Dr. Pablo Wappner (Universidad de Buenos Aires). Elav-geneswitch and UAS-Aβ2 fly was from Dr. Yi. Zhong (Tsinghua University). Control WT flies corresponded, respectively, to the background flies of each resource, and were labeled as WT or w-.

In order to introduce various transgenes to Aβ fly background, UAS-Aβ flies were first crossed with Elav-Gal4 flies to obtain Elav-Gal4; UAS-Aβ/Cyo, which were then used to crossed in different transgenes. As the controls, the corresponding background flies of these transgenes were crossed with Elav-Gal4 or crossed with Elav-Gal4, UAS-Aβ/Cyo, to obtain Elav-Gal4/+ in background flies, or Elav-Gal4+/+: UAS-Aβ/+ in background flies. For the transgenes from VDRC, the VDRC w- fly was used as the background fly; for the transgenes from Bloomington Drosophila Stock Center, Bloomington w- was used as the background; for the transgenes from Tsinghua Drosophila Stock Center, the same background w- fly was used, and for UAS-IDE, the corresponding background w- fly was used.

Flies were raised with standard corn meal under 25 ±1 °C unless noted. GSK19045297 and Wortmannin were added in standard corn meal food at 5 μg/mL. RU486 was administered and performed as described58. RU486 (mifepristone) was used at 20 μg/mL final concentration, and in the control, the same volume of solvent ETOH was added.

Climbing ability assay. Flies were maintained at 25°C on standard corn media. The climbing ability assay was as previously described. Flies were aged for ~4 weeks at 25 °C. The climbing ability represented the total number of flies that were able to climb 7 cm in 8 s, divided by the total number of flies in every assayed group (%). For each genotype, at least three individual repeats were assayed. For GSK19045297 and Wortmannin fed flies, flies were aged for ~4 weeks at 25 °C, and then assayed for climbing ability. At least three individual repeats were performed for each drug treated group. For RU486 fed flies, their climbing ability was defined as the total number of flies able to climb 7 cm in 8 s, divided by the total number of flies in every assayed group (%).

Fly protein extraction and western blot. For AKT and ILPS hybridization, at least 30 adult heads (aged for 4 weeks) were collected and homogenized in NP40 lysis buffer containing protease inhibitors and phenylmethane sulfonyl fluoride. After centrifugation at 12,000 g, 4 °C for 10 min, the supernatants were collected, mixed with sodium dodecyl sulfate (SDS) loading buffer and then heated at 75 °C. After centrifugation at 10,000 g for 5 min, the protein samples were loaded into 13% SDS–polyacrylamide gel electrophoresis (SDS-PAGE) gel for electrophoresis separation. Separated proteins were transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore) and hybridized with antibodies after blocking with 5% milk in phosphate buffered saline with Tween 20 (PBST) buffer. AKT and p-AKT antibodies were generously provided by Xun Huang’s Lab (Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China). Anti-actin antibody was purchased from ZSGB-BIO company (China), Tubulin and ILPS antibodies were purchased from Abcam company (UK), ILPS antibody was purchased from Ambart (Shanghai, China), and p-S6K antibody was purchased from Cell Signaling Technology Company (CST, USA). Secondary antibodies were peroxidase-labeled anti-mouse IgG, or anti-rabbit IgG (CST, USA). Immunoblot signals were developed by enhanced chemiluminescence ( Pierce, USA).

Soluble and insoluble Aβ fractions were separated as described57. Briefly, 50 fly heads were collected and homogenized in NP40 lysis buffer (containing 1% SDS), centrifuged at 10,000 g to remove the debris. The supernatants were collected and centrifuged again at 10,000 g for 30 min. The supernatants (soluble fractions) were then mixed with SDS loading buffer, and the remaining pellets (the insoluble fractions) were solubilized by 70% formic acid and later sonicated to disperse the β-amyloid aggregates. After evaporating the formic acid, the protein samples were divided in 2 x SDS loading buffer. Both the soluble and insoluble fractions were heated at 75 °C before loading onto the PVDF membrane and hybridizing with the anti-Aβ42 antibody (Cell Signaling Technology, USA), or running into the 15% SDS-PAGE gel for western blot. Similar procedures were followed with AKT and ILPS western blot. The intensity of gel bands was quantitated using Imagel (U.S. National Institutes of Health, Bethesda, MD, USA).

Drosophila head sectioning and H&E staining. Drosophila head sectioning and H&E staining were performed as previously described58. Briefly, before the experiment, fly were aged for 4 weeks, fly heads were collected and fixed with Carnoy fixation solution (ethanol: chloroform: acetic acid = 6:3:1) for 4 h, and the samples were then dehydrated twice by 100% ethanol for 30 min, once by dry alcohol (absolute), then centrifuged at 1 h, and ethanol was replaced by methanol for 1 h, before embedding into melted paraffin. The embedded fly heads were sectioned into 8 μm continuous sections using the Leica section apparatus (RM2235, Germany). H&E (ZSGB-BIO, China) staining was used to facilitate the observation of the vacuoles in the brains.

Constructs, protein expression, purification, and in vitro degradation assay. Drosophila ILP1, ILP2, ILP5, and IDE gene coding regions were amplified from fly cDNA by overlap extension into the pGBKT7 vector (pGBKT7 vector was purchased from Promega, Madison, WI, USA), and inserted into pGBKT7 vector. Protein expression and purification were performed as previously described59. Briefly, proteins were purified with chitin beads (NEB) as described by vendor brochure, and finally dissolved in 0.1 M PBS (pH = 7.4) buffer containing 1 mg/ml dithiothreitol (DTT) after desalt treatment using the Zeba™ desalt column (Zebadisk, Thermo Scientific). In vitro degradation assay was performed by adding the purified proteins and the Aβ peptide (from Yanmei Li’s Lab, Tsinghua University, 0.1 M PBS, pH = 7.4), mixing and overnight incubation. The protein
levels were analyzed by western blot. For bovine insulin competitive assay, bovine insulin was purchased from Macklin Company (China).

**Immunostaining.** Immunostaining assay was performed as described[8]. Briefly, fly heads were collected and dissected to obtain the brains in phosphate buffered saline (PBS). After fixing in 4% paraformaldehyde, the brains were incubated in PBS containing 0.3% Triton X100 for 30 min, washed 3 times in PBS, and then blocked with 10% normal goat serum for 1 h, before incubating with the primary antibodies overnight (for Aβ immunostaining, the Aβ antibody was diluted at 1:300). Samples were washed with PBST (0.1% Tween 20) for three times, and then incubated with fluorescein isothiocyanate or tetramethylrhodamine-labeled anti-mouse or anti-rabbit IgG for 1.5 h. After washing with PBST (0.1% Tween 20) for three times, fly brains were examined under the confocal microscope (Zeiss 710 META) for image capturing.

**Fly mRNA extraction and RT-PCR.** For total head RNA extraction, at least 20 fly heads were collected. To extract total RNA from fly fat body, at least 20 fly abdomens were collected and the fat bodies were separated. Fly mRNA extraction and RT-PCR were performed as previously described[60]. Briefly, total RNA was extracted with TRizol reagent (Invitrogen, Carlsbad, CA, USA). cDNA was reverse transcribed by the Transcript Reverse Transcriptase (TransGen Biotech Co., Beijing, China). Semi-quantitative RT-PCR was performed using gene-specific primers to amplify partial regions of the target genes. RNA isolation, reverse transcription[8], and semi-quantitative RT-PCR were performed independently for three times. The intensity of gel bands was quantitated using ImageJ (U.S. National Institutes of Health, Bethesda, MD, USA). Primer sequences used in RT-PCR are listed in Supplementary Table 3.

**Statistics.** Data are presented as mean ± S.E.M. Differences among groups were analyzed by the GraphPad Prism 5 software with Student’s t-test (comparison of two groups) or ANOVA test (three groups or more). *p < 0.05, **p < 0.01, ***p < 0.001.

**Data availability.** The data are generated and/or analyzed during the current study are available from the authors upon request. The source data underling the graphs and charts presented in the main figures are presented in Supplementary Data 1.

Received: 9 April 2018 Accepted: 3 December 2018 Published online: 08 January 2019

**References**

1. Association, A. a. Alzheimer’s disease facts and figures. Alzheimer’s Dement. J. Alzheimer’s Assoc. 7, 208 (2011).

2. Evans, D. A. et al. Prevalence of Alzheimer’s disease in a community population of older persons. Higher than previously reported. JAMA 262, 2551–2559 (1989).

3. Yaffe, K. et al. Mild cognitive impairment, dementia, and their subtypes in the population of older persons. Higher than previously reported. Arch. Intern. Med. 167, 25–31 (2007).

4. Cormons, M., Brookmeyer, R., Berlau, D., Paganini-Hill, A. & Kawas, C. Prevalence of dementia after age 90 results from the 90+ study. Neurology 71, 337–343 (2008).

5. Arvanitaki, Z., Wilson, R. S., Bienias, J. L., Evans, D. A. & Bennett, D. A. Diabetes mellitus and risk of Alzheimer disease and decline in cognitive function. Arch. Neurol. 61, 661–666 (2004).

6. Grodstein, F., Chen, J., Wilson, R. S. & Manson, J. E. Type 2 diabetes and cognitive function in community-dwelling elderly women. Diabetes Care 24, 1060–1065 (2001).

7. Lee, S. et al. Type 2 diabetes as a risk factor for dementia in women compared with men: a pooled analysis of 2.3 million people comprising more than 100,000 cases of dementia. Diabetes Care 39, 300–307 (2016).

8. Mcnay, E. C. & Recknagel, A. K. Brain insulin signaling: a key component of insulin resistance. Curr. Opin. Endocrinol. Diabetes Obes. 15, 371–376 (2008).

9. Conejo, R. & Lorenzo, M. Insulin signaling leading to proliferation, survival, and resistance develop in Alzheimer’s disease. Cell 158, 1688–1705 (2014).

10. Vekrellis, K. et al. Neurons regulate extracellular levels of amyloid β protein via proteolysis by insulin-degrading enzyme. J. Neurosci. 20, 1657–1665 (2000).

11. Farris, W. et al. Insulin-degrading enzyme regulates the levels of insulin, amyloid β protein, and the β-amylloid precursor protein intracellular domain in vivo. Proc. Natl Acad. Sci. USA 101, 896–901 (2004).

12. Galagovsky, D. et al. The Drosophila insulin-degrading enzyme restricts growth by modulating the PI3K pathway in a cell-autonomous manner. Mol. Biol. Cell 25, 916–924 (2014).

13. Mairani, A. et al. Anti-diabetic activity of insulin-degrading enzyme inhibitors mediated by multiple hormones. Nature 511, 94–98 (2014).

14. Lizzano, J. M. & Alessi, D. R. The insulin signalling pathway. Curr. Biol. 10, 325–328 (2000).

15. McQuary, P. R. et al. C. elegans S6K mutants require a creatine-kinase-like effector for lifespan extension. Cell Rep. 14, 2039–2067 (2016).

16. Comejo, R. & Lorenzo, M. Insulin signaling leading to proliferation, survival, and membrane ruffling in C2C12 myoblasts. J. Cell. Physiol. 187, 96–108 (2001).

17. Runyan, K., Duckworth, W., Kibach, A. & Huff, G. The effect of age on insulin-degrading activity in rat tissue. Diabetes 28, 324–325 (1979).
42. Ertekin-Taner, N. et al. Genetic variants in a haplotype block spanning IDE are significantly associated with plasma Aβ42 levels and risk for Alzheimer disease. *Hum. Mutat.* 23, 334–342 (2004).
43. Ling, Y., Morgan, K. & Kalshkeer, N. Amyloid precursor protein (APP) and the biology of proteolytic processing: relevance to Alzheimer’s disease. *Int. J. Biochem. Cell Biol.* 35, 1505–1535 (2003).
44. Delanoue, R. et al. Drosophila insulin release is triggered by adipose stunted ligand to brain Methuselah receptor. *Science* 353, 1553–1556 (2016).
45. Wang, P. Y. et al. Long-lived Indy and calorie restriction interact to extend life span. *Proc. Natl Acad. Sci. USA* 106, 9262–9267 (2009).
46. Oddo, S. The role of mTOR signaling in Alzheimer disease. *Curr. Opin. Genet. Dev.* 11, 287–292 (2001).
47. Gems, D. & Partridge, L. Insulin/IGF signalling and ageing: seeing the bigger picture. *Curr. Opin. Genet. Dev.* 14, 941–952 (2012).
48. Giesels, D. & Partridge, L. Insulin/IGF signaling and ageing: seeing the bigger picture. *Curr. Opin. Genet. Dev.* 11, 287–292 (2001).
49. Rincon, M., Rudin, E. & Barzilai, N. The insulin/IGF-1 signaling in mammals and its relevance to human longevity. *Exp. Gerontol.* 40, 873–877 (2005).
50. Takeda, S., Sato, N., Rakugi, H. & Morishita, R. Molecular mechanisms linking diabetes mellitus and Alzheimer disease: beta-amyloid peptide, insulin signaling, and neuronal function. *Mol. Biostem.* 7, 1822–1827 (2011).
51. Sanphui, P. & Biwas, S. C. FoxO3a is activated and executes neuron death via Bim in response to beta-amyloid. *Cell death & Dis.* 4, e625 (2013).
52. Gomez-Crisostomo, N. P., Rodriguez Martinez, E. & Rivas-Arancibia, S. Oxidative stress activates the transcription factors FoxO1a and FoxO3a in the hippocampus of rats exposed to low doses of ozone. *Oxid. Med. Cell Longev.* 2014, 805764 (2014).
53. Miranda, S. et al. The role of oxidative stress in the toxicity induced by amyloid beta-peptide in Alzheimer’s disease. *Prog. Neurobiol.* 62, 633–648 (2000).
54. An, W. L. et al. Up-regulation of phosphorylated/activated p70 S6 kinase and its relationship to neurofibrillary pathology in Alzheimer’s disease. *Am. J. Pathol.* 163, 591–607 (2003).
55. Selman, C. et al. Ribosomal protein S6 kinase 1 signaling regulates mammalian life span. *Science* 326, 140–144 (2009).
56. Copeland, J. M. et al. Extension of Drosophila life span by RNAi of the mitochondrial respiratory chain. *Curr. Biol.* 19, 1591–1598 (2009).
57. Sudo, S., Frosch, M. P. & Wolf, B. A. Differential effects of proteases involved in intracellular degradation of amyloid β-protein between detergent-soluble and-insoluble pools in CHO-695 cells. *Biochemistry* 41, 1091–1099 (2002).
58. Huang, Y. et al. Zinc binding directly regulates tau toxicity independent of tau hyperphosphorylation. *Cell Rep.* 8, 831–842 (2014).
59. Wang, X., Wu, Y. & Zhou, B. Dietary zinc absorption is mediated by ZnT1 in Drosophila melanogaster. *FASEB J.* 23, 2650–2661 (2009).
60. Tang, X. & Zhou, B. Ferritin is the key to dietary iron absorption and tissue iron detoxification in Drosophila melanogaster. *FASEB J.* 27, 288–298 (2013).
61. Cui, Y., Zhao, S., Wang, X. & Zhou, B. A novel Drosophila mitochondrial carrier protein acts as a Mg(2+) exporter in fine-tuning mitochondrial Mg(2+) homeostasis. *Biochim. Biophys. Acta* 1863, 30–39 (2016).

**Acknowledgements**

This study was supported by the National Natural Science Foundation of China (91649118 and 31700883), China Postdoctoral Science Foundation (2013M02052), and the Postdoctoral Fellowship from Tsinghua-PKU Centre for life sciences. We are grateful to Dr. Yi Zhong of Tsinghua University for the Aβ1-42 story, Dr. Pablo Wappnera of Universidad de Buenos Aires for the IDE overexpression story, and the Bloomington Drosophila Stock Center, VDRC, and Tsinghua University fly center for the other fly stocks. We thank the Biomedical Analysis Center of Tsinghua University and Tsinghua University Laboratory Animal Centre for their help and services, and Dr. Xun Huang of Institute of Genetics and Developmental Biology, Chinese Academy of science and Dr. Yangmei Li of Tsinghua University, for their help of reagents, and Wenming Wang for the preparation of ILP proteins. Our thanks also go to Dr. Jose Pastor for his help in manuscript writing.

**Author contributions**

Y.H. and B.Z. designed the experiments. Y.H. performed most of the experiments. Z. Wan and Z. Wang helped with some of the experiments. Y.H. and B.Z. analyzed the data. Y.H. and B.Z. wrote the manuscript.

**Additional information**

Supplementary information accompanies this paper at https://doi.org/10.1038/s42003-018-0253-x.

**Competing interests:** The authors declare no competing interests.

**Reprints and permission** information is available online at http://npg.nature.com/reprintsandpermissions/

**Publisher’s note:** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.