1 Aβ toxicity rescued by protein retention in the ER

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21 **Running Title:** Protein retention in the ER rescues Aβ toxicity in *Drosophila*.

22

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26 Abstract

27 Accumulation of A β in the brain is one of the hallmarks of Alzheimer's disease (AD). In the
 28 adult *Drosophila* brain, human A β over-expression is toxic and leads to deterioration of
 29 climbing ability and shortened lifespan. However, it remains unknown if A β is inherently
 30 toxic or if it triggers toxic downstream pathways that lead to neurodegeneration. Here, we
 31 describe a novel, and previously unidentified, protective role of intracellular laminin chain
 32 accumulation. Despite high A β levels, over-expression of the extracellular matrix protein
 33 subunit Laminin B1 (LanB1) resulted in a robust rescue of toxicity, highlighting a potential
 34 protective mechanism of resistance to A β . Over-expression of other Laminin subunits and
 35 a Collagen IV subunit also significantly rescued A β toxicity, while combining LanB1 with
 36 these subunits led to an even larger rescue. Imaging revealed that LanB1 was retained in
 37 the ER but had no effect on the secretion of A β into the extracellular milieu. LanB1
 38 rescued toxicity independently of the IRE1 α /XBP1-mediated branch of the ER stress
 39 response. Interestingly, over-expression of ER-targeted GFP also rescued A β toxicity,
 40 indicating a potentially broader benefit of ER protein retention. Finally, in proof-of-principle
 41 lentiviral transduction experiments using murine organotypic hippocampal slice cultures,
 42 over-expression of mouse Lamb1 resulted in ER-retention in transduced cells, highlighting
 43 a conserved mechanism. Typically, retention of proteins in the ER is detrimental to cellular
 44 health, but in the context of neuronal A β toxicity it may prove to be beneficial and a new
 45 therapeutic avenue for AD.

46 Introduction

47 Despite decades of intensive research and a large number of clinical trials, Alzheimer's
 48 disease (AD) has no cure. Most cases are sporadic, with age being the biggest risk factor.
 49 The neuropathological hallmarks include accumulation of Amyloid β ($A\beta$) plaques and
 50 neurofibrillary tau tangles¹. AD is thought to be triggered by the accumulation of $A\beta$
 51 peptides, derived from the misprocessing of amyloid precursor protein (APP), resulting in
 52 increased cellular stress, accumulation of toxic hyperphosphorylated tau, and eventual
 53 neuronal cell death². Characteristic features of AD include aggregates of unfolded
 54 proteins, increased reactive oxygen species and metabolic dysregulation in the affected
 55 neurons^{3,4}. The endoplasmic reticulum (ER) recognizes these alterations in neuronal
 56 homeostasis, and consequently AD brains display many signs of ER stress^{5,6}, which
 57 appears early in AD progression^{7,8}. ER stress generally occurs upon the accumulation of
 58 unfolded or misfolded proteins in the ER lumen. The ER chaperone BiP (Grp78)
 59 dissociates from membrane sensors (Inositol-requiring enzyme 1 α (IRE1 α), Protein
 60 kinase-like endoplasmic reticulum kinase (PERK), and Activating Transcription Factor 6
 61 (ATF6)), and binds to these misfolded proteins⁹. With the removal of BiP, IRE1 α
 62 oligomerises then catalyses the “unconventional” splicing of X-box binding protein 1
 63 (Xbp1) mRNA, generating XBP1s, a transcription factor that regulates genes involved in
 64 the ER stress response, including BiP¹⁰.

65 $A\beta$ pathology is largely extracellular but there is also a lively debate^{11,12} in the AD field
 66 about intracellular $A\beta$, which may play a major role in neurodegeneration^{13,14}. Although the
 67 existence of intracellular $A\beta$ has become less controversial, its role in the induction of the
 68 ER stress response is unresolved, and the mechanisms by which protein misfolding
 69 contributes to AD pathogenesis are still unclear¹⁵.

70 The detailed molecular mechanisms underlying the aetiology of AD remain to be
 71 confirmed, and many *in vitro* and *in vivo* models have been developed to study them^{16,17}.
 72 *Drosophila melanogaster* has been widely used as a model system to study
 73 neurodegenerative disorders, including AD¹⁶. In the adult fly brain, neuronally expressed
 74 $A\beta$ is predominantly found in the cell body of neurons, but can also be observed in the
 75 neuropil when glial function is affected¹⁸, indicating that glia, which account for ~10% of
 76 the cells¹⁹ yet cover a large area of the adult fly brain²⁰, play an important role clearing $A\beta$.

77 In flies, the ER stress response is activated in response to neuronal A β , with increased
78 Xbp1 splicing and BiP levels^{21–23}. Indeed, Xbp1 acts to buffer the toxic effects of A β since
79 over-expression of spliced Xbp1 reduces A β levels and partially rescues toxicity, while
80 Xbp1 knockdown increases A β levels and exacerbates toxicity^{21,23}.

81 The ER is involved in the secretion of many proteins, including those targeted to the
82 extracellular milieu, such as collagens and laminins²⁴. The assembly of laminins and
83 collagens from their individual subunits occurs in the ER before secretion into the trans-
84 Golgi network and then toward their final destination outside the cell, where they form the
85 extracellular matrix (ECM)²⁵. Laminins are obligate heterotrimers consisting of large α , β ,
86 and γ subunits that combine via the triple-helical coiled-coil domain in the centre of each
87 chain to form cruciform-shaped structures. The expression of the β 1 subunit of the ECM
88 protein Laminin (LamB1) appears restricted to regions of the brain that are susceptible to
89 neurodegeneration, especially the hippocampal tri-synaptic circuit^{26,27}. Indeed, modulating
90 LamB1 expression has been associated with changes in memory formation²⁸, while A β -
91 induced memory deficits are rescued by LamB1 modulation²⁹. Under normal conditions
92 laminin interacts with APP³⁰. Additionally, anti-laminin immunoreactivity levels in human
93 cerebrospinal fluid have been shown to correlate with the pathogenesis of AD and
94 vascular dementia³¹. *In vitro*, the laminin heterotrimer can inhibit A β fibrillation and
95 depolymerise A β , although without reduction of toxicity^{32–34}. Notably, A β can induce
96 memory deficits that can be rescued by ECM manipulation^{29,35–37}. The complexity of
97 neuron-matrix interactions makes it difficult to recapitulate ECM organisation and function
98 in cell culture. It is, therefore, important to design experiments to evaluate these
99 interactions *in vivo*.

100 Laminin in the brain is typically found extracellularly at basement membranes in the
101 vasculature, and at the blood brain barrier³⁸. However, synaptic laminin has also been
102 characterised at the neuromuscular junction (NMJ)^{39,40}. In the hippocampus, synaptic α 5-
103 laminin was found to be necessary and sufficient to stabilize dendritic spine dynamics⁴¹.
104 Surprisingly, intraneuronal laminin expression has also been identified in the hippocampus
105 and may have functions other than extracellular structural integrity^{28,42}. Indeed, laminin has
106 been observed in hippocampal neuronal perikarya and other susceptible regions of the
107 brain important in the development of AD^{43,44}. Laminin expression was also observed to be
108 increased in the AD prefrontal cortex compared to non-disease controls⁴⁵. Similarly,
109 Collagen VI α 1 chain was upregulated in dentate gyrus soma in mice expressing hAPP,

110 and could rescue A β toxicity *in vitro* by sequestering A β into large aggregates in the
111 extracellular milieu³⁵.

112 Vertebrates have multiple genes for all three laminin subunits; five α -chains (α 1– α 5), four
113 β -chains (β 1– β 4) and three γ -chains (γ 1– γ 3) are known, and they combine to form at least
114 16 different laminin heterotrimers⁴⁶, not including novel spliced forms⁴⁷. In *Drosophila*,
115 there are two α (LanA and wb), one β (LanB1), and one γ (LanB2) subunits, resulting in
116 only two laminin heterotrimers⁴⁸ (diagram in **Fig. 1h**). Laminin trimerization occurs in the
117 ER where the β and γ subunits assemble and require α subunit incorporation to form a
118 functional laminin heterotrimer before secretion^{49–51}. If either the β or γ subunit is missing,
119 the other accumulates intracellularly and the α subunit can be secreted as a monomer.
120 Knockdown of α laminin also results in β and γ subunit retention in the ER⁵². Similarly,
121 over-expression of laminin monomers in glial cells leads to ER expansion and triggers ER
122 stress, impairing correct development and locomotion in *Drosophila* larvae⁵³. Collagen IV
123 is another obligate heterotrimer that can accumulate intracellularly when subunit
124 stoichiometry is altered⁵⁴.

125 If ER stress is part of the AD pathogenic cascade, and it is also activated in response to
126 modulation of laminin/collagen subunit stoichiometry, then experimentally increasing
127 ectopic ECM protein subunit expression in adult neurons might be expected to overwhelm
128 the ER and exacerbate AD pathogenesis. On the contrary, we found that neuronal over-
129 expression of *Drosophila* laminin and collagen subunits led to pronounced intra-ER
130 accumulation of these subunits, yet could robustly ameliorate the toxic effects of A β , such
131 as reduced lifespan and climbing ability, without reducing A β levels. We also found that
132 over-expression of mouse Lamb1 in murine organotypic hippocampal slice cultures
133 resulted in ER-retention of these monomers, indicating a conserved mechanism.
134 Interestingly, over-expression of ER-targeted GFP also rescued A β toxicity, indicating a
135 potentially broader benefit of ER protein retention. Protein accumulation in the ER is
136 typically seen as detrimental to cellular health, but in the context of neuronal A β toxicity it
137 may prove to be beneficial.

138 Results

139 Over-expression of the laminin β -chain LanB1 rescues the toxic effect of 140 neuronal A β ₄₂ expression

141 To test if modulation of ECM components could alter the toxicity of A β ₄₂ *in vivo*, we took
142 advantage of the A β ₄₂-shortened lifespan of *Drosophila* as a read-out. We used a drug-
143 inducible *Drosophila* AD model that expressed the highly aggregative Arctic A β ₄₂ (A β ^{Arc})⁵⁵,
144 which when expressed in the neurons of adult flies shortens lifespan and induces
145 behavioural defects and neurodegeneration^{21,56}. The A β sequence contains a signal
146 peptide sequence from the *necrotic* gene⁵⁵ to target it to the secretory pathway, resulting in
147 A β that is secreted into the extracellular milieu. The pan-neuronal Elav-GeneSwitch
148 (ElavGS) driver can be induced by RU486 ('RU', a steroid drug inducer) to switch on gene
149 expression. We generated ElavGS; A β ^{Arc} recombinant flies and used these to screen
150 ECM-related genes by driving co-expression in neurons during adulthood. We observed a
151 pronounced rescue of lifespan with co-expression of A β and the β subunit of Laminin
152 (LanB1), using the EP-600 line with a P-element inserted 548 bp upstream of the *LanB1*
153 gene⁴⁸, compared to the A β ^{Arc}-alone controls (**Supplementary Fig. 1a**). We examined
154 further Laminin transgenic lines and found that two independent LanB2 RNAi lines also
155 significantly ameliorated A β ^{Arc} toxicity, but not to the same extent as LanB1 co-expression
156 (**Supplementary Fig. 1b**). As LanB1 co-expression induced the most consistent and
157 pronounced rescue of A β ^{Arc} toxicity, we took this line forward for further study.

158 Previous studies have shown that during *Drosophila* development laminin is predominantly
159 produced by the fat body, haemocytes, and glia^{53,57,58}. Information about laminin
160 expression in the adult fly brain is less abundant. However, by examining gene expression
161 in the adult fly brain using [Sscope](#)⁵⁹, a single-cell transcriptome atlas of the adult
162 *Drosophila* brain, we found that expression of laminin subunits appeared to be specific to
163 haemocytes and a subset of glial cells, while endogenous expression in neurons was low
164 (**Supplementary Fig. 2a and b**), indicating that neuronal expression of these subunits is
165 mainly ectopic.

166 To establish the robustness of the rescue of A β toxicity and to eliminate potential
167 confounding effects of genetic background, we tested further fly lines in the standardised
168 Dahomey (*w^{Dah}*) genetic background. The UAS-LanB1^{EP-600} mutant was backcrossed, and
169 the lifespan rescue experiment repeated, further including the genetically identical,

uninduced controls. Co-expression of LanB1 and A β^{Arc} in females resulted in a significant rescue of the short-lived phenotype (**Fig. 1a**, repeat experiments, **Fig. 1b** and **Supplementary Fig. 1c**). The rescue was also significant in male flies (**Fig. 1c**). We next assessed the ability of LanB1 co-expression to rescue the shortened lifespan in a different fly AD model, in which two copies of wild-type, tandem A β_{42} (A β^{X2})²¹ with an export sequence from the *argos* gene, are expressed in adult neurons. Co-expression of an RFP-tagged LanB1 significantly rescued the short lifespan with A β^{X2} induction (**Fig. 1d** and **Supplementary Fig. 1d**). To assess if these neuronal effects were specific to the ElavGS driver, we used a second, independent, pan-neuronal GeneSwitch driver, NsybGS. LanB1 significantly rescued the shortened lifespan with A β^{Arc} induction in NsybGS flies (**Fig. 1e**).

To assess the effects of LanB1 on neuromuscular performance in AD flies, we measured their climbing behaviour. Expression of A β^{Arc} in adult neurons led to a significant decline in climbing ability compared to uninduced controls (**Fig. 1f**), and LanB1 co-expression significantly rescued the deficit. We also examined the feeding behaviour of the flies, using the Capillary Feeder (CAFE) assay⁶⁰. Flies were induced/uninduced for 3 weeks and then feeding behaviour was measured with all flies eating food without RU. A β^{Arc} flies ingested a significantly smaller amount of food over 7 days compared to uninduced controls, while LanB1 co-expression completely rescued feeding behaviour to uninduced levels (**Fig. 2e**).

Expression of A β can also have toxic effects during development. For instance, expression of A β^{X2} using the GMR-GAL4 driver causes degeneration of the developing eye, resulting in a smaller, glassier appearance compared to the large eye and highly ordered ommatidial lattice in control flies. LanB1 co-expression led to a rescue of both the size and organisation of the eye (**Fig. 1g**). LanB1 could therefore rescue multiple toxic effects of A β expression.

A β is not toxic in glia in adult flies

We also induced adult-onset expression of A β in the other major cell-type in the fly brain, glia, and examined whether LanB1 co-expression could rescue any deleterious phenotypes. Pan-glial expression of A β^{Arc} did not reduce lifespan compared to uninduced controls, nor did A β and LanB1 co-expression (**Supplementary Fig. 1e**). This result is in line with the recent finding that A β produced by glia was less toxic despite having a much higher brain load than with neuronal expression^{23,61}.

201 *LanB1*, and not *cdc14*, an adjacent gene in the opposite genomic orientation,
202 is responsible for the rescue of A β toxicity

203 The *LanB1*^{EP-600} P-element (P{GSV1}) contains UAS sequences at both ends oriented
204 outwards (**Supplementary Fig. 3a**)⁶², and could potentially drive expression of *cdc14*, an
205 adjacent gene in the opposite genomic orientation, previously reported to be involved in
206 stress-resistance and lipid metabolism⁶³. To determine if the expression of *cdc14* was
207 affected we performed qPCR on the heads of *ElavGS>A β ^{Arc}* flies with and without RU-
208 induction and examined expression of *LanB1* and *cdc14*. We confirmed ~10-fold increase
209 in *LanB1* gene expression when induced in both UAS-*LanB1*^{EP-600} and UAS-*LanB1*:RFP
210 flies (**Supplementary Fig. 3b**), and no change in UAS-*cdc14*^{EY10303} flies. As predicted,
211 *cdc14* expression was significantly up-regulated in both UAS-*cdc14*^{EY10303} and UAS-
212 *LanB1*^{EP-600} flies, but not in UAS-*LanB1*:RFP transgenic flies (**Supplementary Fig. 3c**).

213 To determine if *LanB1*, *cdc14* or both were protective against A β toxicity, we expressed
214 them singly and examined the effect on eye phenotypes. Up-regulation of *cdc14* alone
215 during development, using UAS-*cdc14*^{EY10303}, did not rescue the toxic effects of A β ^{X2}, while
216 UAS-*LanB1*:RFP rescued both the size and organisation of the eye to the same extent as
217 in UAS-*LanB1*^{EP-600} flies (**Supplementary Fig. 3d** and quantified in **3e**). We also tested
218 whether *cdc14* alone could rescue the short lifespan of *ElavGS>A β ^{Arc}* flies. Up-regulation
219 of *cdc14* caused a small rescue of A β toxicity compared to induced *ElavGS>A β ^{Arc}* controls,
220 although a similar small rescue was observed with cytoplasmic GFP-overexpression
221 (**Supplementary Fig. 3f**). *LanB1* up-regulation, via either UAS-*LanB1*^{EP-600} or UAS-
222 *LanB1*:RFP, caused equivalently pronounced rescue of A β toxicity. Thus, *LanB1* and not
223 *cdc14* was responsible for the rescue of A β toxicity in UAS-*LanB1*^{EP-600} flies.

224 Co-expression of *LanB1* does not lower levels of A β

225 A potential explanation for the rescue of A β toxicity by *LanB1* is that less A β transcript was
226 generated due to GAL4 titration by the second UAS- transgene. We therefore measured
227 A β mRNA and protein levels. A β was increased >5-fold when induced in either the
228 presence or absence of the *LanB1* transgene (**Fig. 2a**), ruling out GAL4 titration as a factor
229 in the rescue of A β toxicity. We then examined the dynamics of A β peptide accumulation
230 over a 3-week period by ELISA and found no difference between flies expressing A β alone
231 and those co-expressing *LanB1* (**Fig. 2b**). We then measured the levels of soluble and
232 insoluble A β by western blot (**Fig. 2c**) and ELISA (**Fig. 2d**). There was no significant

233 difference in the levels of soluble or insoluble A β between flies expressing A β alone and
234 those co-expressing LanB1. Thus, there was no difference in A β expression at either the
235 RNA or protein level between flies expressing A β alone and those co-expressing LanB1,
236 and nor was the solubility state of A β affected, indicating that LanB1 rescued A β toxicity
237 rather than A β expression.

238 Rescue of A β toxicity by acute LanB1 over-expression implicates soluble A β
239 as the main driver of toxicity

240 Experiments where expression of A β is temporarily induced have shown that high levels of
241 insoluble A β persist in the brain long after induction has ceased, while soluble A β appears
242 to be rapidly cleared⁶⁴. Induction of A β in the first weeks after eclosion results in reduced
243 climbing ability and survival⁶⁴. It remains unclear if the detrimental effects of A β on
244 climbing and lifespan in later-life are caused by long-lasting toxic effects of soluble A β
245 during the early-life induction period, or from the insoluble A β that persists in the ageing
246 brain, or both. We found that 21 days of A β induction resulted in a significantly reduced
247 lifespan compared to uninduced controls (**Fig. 2f**), although the reduction was not as great
248 as with chronic A β induction. Co-expression of both A β and LanB1 for 21 days, in contrast,
249 resulted in a lifespan extension compared to uninduced controls (**Fig. 2f**). These results
250 indicate that the rescue occurred during the early induction period, and that therefore
251 soluble A β during the induction phase, rather than effects of accumulated insoluble A β ,
252 may have been responsible for the later effect on lifespan.

253 Laminin accumulates in the ER

254 The laminin heterotrimer is a canonical ECM protein and is secreted into the extracellular
255 space⁶⁵. Over-expression of monomeric RFP-tagged LanB1, however, leads to
256 intracellular retention²⁵. To determine the cellular localization of LanB1, adult brains
257 expressing A β and LanB1:RFP were dissected, stained and imaged. In agreement with
258 previous studies^{18,55,61}, we found that over-expression of the A β^{Arc} peptide resulted in
259 aggregation of A β in neurons (**Fig. 3a**). A β did not overlap with the nuclear protein histone
260 H3. LanB1 accumulated intracellularly, and partially overlapped with A β (**Fig. 3b**).

261 Upon closer inspection, LanB1 appeared to accumulate in discrete intracellular
262 puncta/compartments. To verify the intracellular expression of LanB1 in neurons, we used
263 the inducible Dilp2-GS driver, which drives expression in the Dilp2 neurons. *Drosophila*
264 insulin-like peptide 2 (Dilp2) neurons consist of 10-14 insulin-like peptide producing cells

located in the dorsal brain *pars intercerebralis* that project their axons ventrally⁶⁶. Driving LanB1 expression in these neurons allowed us to observe whether or not LanB1 was secreted into the extracellular space and/or accumulated intracellularly. We verified that A β and LanB1 were induced in these neurons only when the flies were fed RU486 (**Supplementary Fig. 4**) and that A β in Dilp2 neurons accumulated predominantly in the soma (**Fig. 3c,d**). Similar to A β , LanB1 accumulated intraneuronally but did not completely overlap with A β (**Fig. 3e-g**). LanB1 was not uniformly distributed through the cytoplasm of these cell bodies and appeared to accumulate in discrete intracellular compartments.

As laminin β and γ subunits cannot be secreted from the ER without first forming a heterotrimer^{67,68}, over-expressing LanB1 could have led to its accumulation in the ER⁵³. Indeed, intracellular accumulation of LanB1 protein has been previously reported for expression of the LanB1:RFP transgene in fat body²⁵. Similarly, the expression of the LanB1^{EP-600} element also led to intracellular laminin accumulation^{53,69}. To test if the intracellular accumulation of LanB1 was in the ER, we co-expressed LanB1:RFP and a GFP-tagged ER marker, KDEL:GFP, in adult neurons. We found a strong overlap of KDEL:GFP and LanB1:RFP expression, indicating that LanB1 accumulated in the ER (**Fig. 4**). We also found intracellular accumulation, probably in the ER, of LanB2:GFP in Dilp2 neurons when we examined these flies by live 2-photon imaging (**Supplementary Fig. 6**).

To determine whether intracellular A β aggregates were localized to the ER, we co-expressed A β and KDEL:GFP in adult Dilp2 neurons. A β did not colocalize with KDEL:GFP, indicating that A β did not accumulate in the ER (**Supplementary Fig. 7**). These results are in line with previous studies in adult fly brains that demonstrated that A β mainly accumulates in lysosomes^{70,71}, and it also does so in mice expressing intraneuronal A β ⁷².

LanB1 does not reduce secretion of A β from neurons

Since A β protein levels, both soluble and insoluble, were not reduced in LanB1 co-expressing flies, we hypothesised that the intracellular accumulation of LanB1 might have prevented the normal secretion of the toxic A β peptide into the extracellular space, despite the presence of a signal peptide. We used the Dilp2-GS line to drive expression during the first 3 weeks of adulthood in Dilp2 neurons, and then measured A β fluorescence in the area outside of Dilp2-expressing cells. We found that, when induced, A β was highly expressed in the cell bodies of Dilp2 neurons and also displayed a diffuse punctate pattern

of staining, compared to very little staining in uninduced flies (**Fig. 5a**). When quantified, we found a significant increase in A β fluorescence in induced flies compared to uninduced controls, indicating that A β is secreted into the extracellular environment (**Fig. 5b**). LanB1 co-expression had no effect on secretion of A β . In summary, A β aggregated strongly in the cell bodies of Dilp2 neurons and was also secreted into the extracellular milieu (**Fig. 5c**). Therefore, the beneficial effects of LanB1 co-expression were not due to the prevention of A β secretion.

Increased expression of a Collagen IV subunit also rescues A β toxicity in adult neurons

The trimerisation of laminins takes place in the ER and all three subunits are required for secretion to occur⁴⁹. We hypothesized that over-expressing protein subunits of similar, large, obligate heterotrimers that assemble in the ER may lead to amelioration of A β toxicity. Collagen IV, the main component of basement membranes, is another obligate heterotrimeric protein formed by three α chains (two α chains of Collagen at 25C (Cg25C) and one α chain Viking (Vkg)), and is present in all metazoans^{73,74}. Similar to laminins, expression of collagen IV subunits in the adult fly brain was predominantly in haemocytes and a subset of glial cells, while endogenous expression in neurons was low (**Supplementary Fig. 2c**), indicating that pan-neuronal expression of these subunits by ElavGS or NsybGS is ectopic. The RFP-tagged Cg25C transgenic over-expression line (*UAS-Cg25C:RFP*) rescued the short lifespan from induction of A β^{Arc} (**Fig. 6a**) and A β^{X2} (**Fig. 6b**), and to the same degree as LanB1, potentially indicating a similar mechanism of action.

We next examined if the rescue of degeneration in the fly eye due to A β toxicity (**Fig. 1f**) was specific to LanB1. Co-over-expression of mCD8:GFP or mCD8:RFP alone, as controls for GFP/RFP transgene overexpression, had no significant effect on A β toxicity (**Supplementary Fig. 5a** and quantified in **5b**). First, we assessed the effect of modulation of other laminin/collagen subunits on the rough eye phenotype. We confirmed that LanB1 co-expression (either using *UAS-LanB1^{EP-600}* or *UAS-LanB1:RFP*) significantly rescued A β toxicity. Additionally, we found that co-expression of laminin subunits, LanA (*UAS-LanA^{EY02207}*) and LanB2 (*UAS-LanB2:GFP*), and the collagen IV subunit Cg25C (*UAS-Cg25C:RFP*) separately all rescued A β toxicity (**Supplementary Fig. 5**). Knockdown of laminin α -chain subunits, LanA and wb, using TRiP RNAi transgenics had no effect on toxicity, while knockdown of γ -chain LanB2 led to a small but significant rescue

330 **(Supplementary Fig. 5).** Next we generated recombinant transgenic lines for co-
 331 expression of LanB1, i.e. 'LanB1^{EP-600} + Cg25C:RFP', 'LanB1^{EP-600} + LanA^{OE}', 'LanB1^{EP-600}
 332 + LanB2^{TRiP}', and 'LanB1^{EP-600} + wb^{TRiP}'. Although Cg25C and LanA could individually
 333 rescue eye size, modulation of these other subunits had no effect on the ability of LanB1 to
 334 protect against A β toxicity, indicating that LanB1 was the primary cause of the toxicity
 335 rescue in the context of the developing eye **(Supplementary Fig. 5).**

336 Enhanced rescue of A β toxicity with combination of laminin and collagen 337 subunits

338 As both LanB1 and Cg25C individually rescued A β toxicity to a similar extent **(Fig. 6a,b)**,
 339 and the combination of both did not further rescue A β toxicity in the developing eye
 340 **(Supplementary Fig. 5)**, we hypothesized that these proteins may have been epistatic,
 341 i.e. acting via the same mechanism. We therefore examined the effect of the combination
 342 of LanB1 and Cg25C in adult neurons on lifespan. We found that LanB1 and Cg25C
 343 together resulted in a greater rescue of A β toxicity compared to individual co-expression
 344 **(Fig. 6c)**. Cox Proportional Hazard analysis showed a significant interaction between
 345 LanB1 and Cg25C, indicating that, although the enhanced rescue was partially additive, it
 346 was also acting via a shared pathway.

347 Laminin A (LanA) is one of two laminin α -chains in *Drosophila*, the other being *wing blister*
 348 (*wb*), which form heterotrimers with LanB1 (β -chain) and LanB2 (γ -chain)⁴⁸. We found that
 349 over-expression of LanA alone could significantly rescue A β toxicity, but not to the same
 350 degree as LanB1 or Cg25C alone **(Fig. 6d and Supplementary Fig. 8a)**. We generated
 351 double transgenic flies with both LanA and LanB1, which resulted in an even greater
 352 rescue of toxicity, indicating an additive effect. However, Cox Proportional Hazard analysis
 353 showed a significant interaction between LanB1 and LanA, indicating the enhanced rescue
 354 is partly acting via a shared pathway. LanA also rescued A β toxicity in the developing fly
 355 eye when compared to A β ^{X2} alone, though not to the same extent as LanB1
 356 **(Supplementary Fig. 5)**. These results indicate that LanA can rescue A β toxicity, but to a
 357 lesser degree than LanB1. Overall, the collagen IV subunit Cg25C and the laminin α chain
 358 LanA led to enhanced rescue of A β toxicity in combination with the laminin β chain LanB1.
 359 These effects were epistatic, suggesting an overlapping molecular pathway.

360 LanB1 rescue of A β toxicity is independent of the BiP/Xbp1 ER stress 361 response pathway

362 A β induces ER stress markers, including the ER chaperone BiP, and leads to increased
363 alternative splicing of Xbp1^{21–23}. Increased BiP can exacerbate A β toxicity while reduction
364 of BiP has been shown to have beneficial effects^{22,75}. To assess if BiP reduction was
365 behind the LanB1 rescue of A β toxicity, we examined BiP mRNA and protein levels. After
366 3-weeks of A β induction, BiP was significantly up-regulated at both the mRNA (**Fig. 7a**)
367 and protein level (**Fig. 7b,c**) in fly heads. Co-expression of LanB1 with A β had no effect on
368 BiP mRNA or protein levels. Therefore, LanB1 rescued A β toxicity independently of the ER
369 stress regulator, BiP.

370 An increase in active spliced Xbp1, a marker of IRE1 α activation, can ameliorate A β
371 toxicity, while a decrease exacerbates A β toxicity^{21,23}. To assess whether the LanB1
372 rescue was acting via Xbp1, we examined the effect of Xbp1-knockdown on A β toxicity.
373 We used the A β -induced rough eye phenotype, and A β -induced short-lifespan as read-
374 outs. Knockdown of Xbp1 significantly exacerbated A β toxicity in the developing eye (**Fig.**
375 **7d**), and also led to a significantly shortened lifespan compared to A β alone (**Fig. 7e,f**).
376 Over-expression of LanB1 significantly rescued the short lifespan of A β flies with Xbp1-
377 knockdown (**Fig. 7f**), and there was no significant difference in lifespan between these
378 rescue flies and A β -alone controls ($p = 0.067$, log-rank). Thus, the ability of LanB1 to
379 rescue A β toxicity was independent of Xbp1. Since LanB1 did not affect BiP levels in the
380 context of neuronal A β , and since LanB1 could rescue A β toxicity independently of Xbp1,
381 LanB1 must have acted independently of the IRE1 α /XBP1s arm of the ER stress response
382 pathway.

383 Proteins that are retained in the ER can rescue A β toxicity

384 Since LanB1 over-expression resulted in intra-ER accumulation, we investigated whether
385 over-expression of other proteins restricted to the ER could result in the same phenotype.
386 To test this, we compared the effects of GFP localised to different cellular compartments,
387 namely membrane-targeted mCD8:GFP, LanB2:GFP (laminin γ chain) which accumulates
388 in the ER, KDEL:GFP which is retained in the ER, and secr:GFP which is secreted
389 extracellularly, and measured their effect on A β ^{X2} toxicity in the developing eye. As with
390 LanB1 over-expression, expression of LanB2:GFP rescued A β toxicity (**Fig. 8a** and
391 quantified in **8b**) compared to mCD8:GFP controls. Expressing KDEL:GFP led to a rescue
392 of eye size while secr:GFP exacerbated toxicity. In adult neurons LanB2 significantly
393 rescued the short lifespan induced by A β ^{Arc} toxicity (**Fig. 8c**). Again, KDEL:GFP rescued

394 A β^{Arc} toxicity and secr:GFP exacerbated A β^{Arc} toxicity (**Fig. 8c**). LanB2 also significantly
 395 rescued the short lifespan with A β^{X2} induction (**Supplementary Fig. 8b**). Thus, the
 396 retention of over-expressed proteins in the ER ameliorated A β toxicity. Overall, these
 397 results suggest that ER-retention is a promising avenue of AD research.

398 Lamb1 also accumulates in the ER when overexpressed in mouse neural
 399 tissue

400 Disruption of laminin subunit production results in intracellular accumulation of the
 401 remaining subunits⁵³. To our knowledge, however, there are no published data examining
 402 the over-expression of Laminin subunits and their expression pattern in mammalian
 403 models. We used *ex-vivo* mouse organotypic hippocampal slice cultures to transduce
 404 mouse Lamb1 using engineered lentivirus. Organotypics were generated from P10
 405 wildtype (C57/BL6J) mouse pups and incubated with mouse Lamb1 (mLamb1 + GFP)
 406 recombinant lentivirus or control recombinant lentivirus (GFP only). Cultures were
 407 transfected with virus at 14 days *in vitro*, and fixed after a further 14 days. Over-expression
 408 of mLamb1 resulted in marked intracellular accumulation compared to controls and did not
 409 result in a uniform cytoplasmic distribution, indicating compartmentalisation (**Fig. 9a**).
 410 Lamb1 colocalised with the ER marker Calnexin in some areas, indicating retention of
 411 Lamb1 in the ER, though there were also areas in these cells with no overlap. Therefore,
 412 the ER-retention of over-expressed laminin β chain is a conserved process.

413 Discussion

414 Here, we showed that ectopic, neuronal over-expression of laminin (and collagen IV)
 415 monomers provided robust protection in an *in vivo Drosophila* model of AD, and that
 416 ectopic over-expression of LanB1 resulted in ER-retention in these neurons. Over-
 417 expression of mouse Lamb1 in *ex vivo* mouse organotypic hippocampal slice cultures also
 418 resulted in ER-retention of these monomers, highlighting a conserved process. In the fly,
 419 LanB1 rescued A β toxicity without reducing A β levels (soluble or insoluble) and without
 420 affecting A β secretion into the extracellular milieu. LanB1 also rescued A β toxicity in
 421 combination with other laminin subunits and a collagen IV subunit and acted independently
 422 of the IRE1 α /XBP1s ER stress response branch. Finally, expression of other proteins
 423 targeted for retention in the ER could significantly rescue A β toxicity. Overall, we have
 424 described a novel mechanism whereby ER-retention of proteins, typically detrimental to
 425 cellular health, is a potential therapeutic target for AD.

426 For nearly 3 decades, A β has been one of the major targets for AD therapy with most
 427 major clinical trials aiming to reduce or detoxify A β ⁷⁶. Similarly, most studies in *Drosophila*
 428 showing rescue of A β toxicity appear to work, at least partially, via reduction of A β
 429 levels^{23,56,75,77}. Previous studies in both flies and humans, however, have demonstrated
 430 that A β load can be uncoupled from toxicity^{21,22,78}. Intriguingly, LanB1 was able to
 431 substantially rescue A β toxicity without altering levels of A β , either soluble or insoluble,
 432 and also without reducing secretion of A β into the extracellular milieu indicating that LanB1
 433 expression increased neuronal resistance to A β toxicity. This result also suggests that,
 434 since LanB1 accumulated in the ER, the beneficial effect of LanB1 over-expression may
 435 also occur in the ER. Further work examining the intracellular/extracellular availability of
 436 LanB1 may inform some of the debate surrounding intracellular vs extracellular A β
 437 toxicity¹¹.

438 Collagen VI rescues A β toxicity by sequestering A β into large aggregates in the
 439 extracellular milieu³⁵. Hence, LanB1 could have promoted the formation of A β fibrils from
 440 the more toxic oligomeric form. However, we observed no difference in the levels of
 441 soluble or insoluble A β levels with LanB1 co-expression, indicating that LanB1 was not
 442 altering the ratio of soluble/insoluble A β . It remains to be seen whether A β is binding to
 443 LanB1, or if LanB1 is sequestering toxic oligomeric A β into less toxic structures. Crucially,
 444 a rescue of A β toxicity was also observed with Cg25C, LanA, LanB2, and KDEL:GFP, the

445 last of which likely does not bind A β , indicating mechanisms other than A β sequestration
446 by LanB1.

447 Since glia are the other major cell type in the adult fly brains and can produce laminins, we
448 determined if LanB1 over-expression in these cells could also rescue A β toxicity. We could
449 not verify this, however, as we did not observe A β toxicity using GliaGS, and co-
450 expression of LanB1 also had no effect. This result was consistent with recent studies that
451 found glial A β was less toxic than neuronal A β despite having a much higher brain load
452 than those produced by neurons^{23,61}.

453 ER stress has been implicated in the progression of AD⁴, although the precise
454 mechanisms that underlie protein misfolding contributions to AD pathogenesis are still
455 unclear¹⁵. Here, we found that BiP was not affected at the mRNA/protein level by LanB1
456 despite the rescue of A β toxicity. It is possible that BiP expression was at the upper limit
457 and LanB1 could not increase BiP expression further. Regardless, it is likely that BiP was
458 chronically high throughout adult lifespan, which is detrimental to neuronal health when co-
459 expressed with A β ⁷⁵. Intriguingly, BiP may be a molecular chaperone of laminin assembly
460 in the ER⁷⁹, which may indicate that LanB1 over-expression sequesters BiP from having
461 toxic effects when upregulated chronically.

462 The importance of Xbp1 in the response to A β is evident with knockdown of Xbp1, which
463 has been shown to enhance A β toxicity^{21,23}. However, Lanb1 could rescue the enhanced
464 toxicity of A β +Xbp1^{RNAi}, indicating that Lanb1 could act independently of Xbp1. In addition,
465 knocking down Xbp1 increased A β protein levels while over-expression reduced A β
466 protein levels²³. However we found no difference in A β levels with LanB1 co-expression,
467 further indicating that the beneficial effects are independent of Xbp1. Further work
468 analyzing epistatic interactions between LanB1 over-expression and the ER stress
469 response branches will be necessary to deduce the mechanism underlying the rescue of
470 A β toxicity.

471 LanA also rescued A β toxicity, but not to the same extent as LanB1. Previous studies have
472 shown that the laminin α -chain can be secreted as a monomer without the requirement for
473 heterotrimerisation^{49,50}. Thus, it is possible that LanA is secreted from the ER in
474 monomeric form and so the ER may not accumulate as many large proteins, and hence
475 has a reduced ability to rescue A β toxicity. If this were the case, it would be expected that
476 over-expression of the other laminin α -chain (wb) might exhibit the same degree of A β

477 rescue as LanA. Collagen IV also forms an obligate heterotrimer with 2 chains of Cg25C
478 and one Vkg chain. Similar to LanA, Vkg can be secreted as a monomer⁵⁸ and it is thought
479 that Cg25C requires Vkg for secretion (like LanB1/LanB2). We suspect that over-
480 expression of LanB1/LanB2/Cg25C led to ER retention of these subunits and resulted in
481 ER expansion (illustrated in **Fig. 9b**). We predict that if Vkg can be secreted as a
482 monomer, it may also show a lesser rescue than Cg25C.

483 Laminin or collagen upregulation is considered detrimental in most contexts^{40,80} and
484 especially in relation to cancer invasiveness^{81,82}. Indeed research examining disease-
485 causing mutations in laminin/collagen has focused on defective ECM-receptor signalling
486 as the underlying cause of pathology^{80,82}. Other studies have focused on receptor-
487 independent effects of laminin/collagen loss and have found that mutations triggered ER
488 stress, potentially due to defective secretion of remaining subunits^{53,67,83}. A case study of a
489 patient with porencephaly (cystic brain lesions) carrying a collagen IV $\alpha 2$ mutation, which
490 caused intracellular accumulation of the COL4A2 chain, found ER stress and UPR
491 activation which could be rescued by chemical chaperone treatment⁸³. Interestingly, the
492 patient's father carrying the same allele also displayed basement membrane defects but
493 no disease symptoms, indicating that intracellular accumulation of COL4A2 rather than
494 extracellular effects led to disease⁸³.

495 Our hypothesis that LanB1 retention in the ER in neurons is beneficial is augmented by the
496 finding that ER-retained GFP (KDEL:GFP) also reduced A β toxicity. Interestingly, GFP
497 targeted for extracellular secretion (secr:GFP) exacerbated A β toxicity. It is possible that
498 extracellular GFP is degraded by glia and thus reduces the capacity of glia to clear A β ,
499 leading to increased toxicity. In agreement with our finding that secr:GFP exacerbated A β
500 toxicity, Fernandez-Funez *et al.* showed that eyes of GMR>A β +secr:GFP flies were
501 smaller when compared to GMR>A β +LacZ⁸⁴. In addition, prion protein, a molecule located
502 extracellularly, exacerbated A β toxicity when co-expressed, and caused massive A β
503 deposition in the brain⁸⁵.

504 We have discovered that increased expression of ER-retained proteins, typically seen as
505 detrimental, rescued A β toxicity in neurons, and could be a new therapeutic avenue for AD
506 research.

507 Methods

508 *Drosophila* stocks and fly husbandry

509 The wild-type stock Dahomey was collected in 1970 in Dahomey (now Benin) and has
510 since been maintained in large population cages with overlapping generations on a
511 12L:12D cycle at 25°C. The white Dahomey (w^{Dah}) stock was derived by incorporation of
512 the w^{1118} deletion into the outbred Dahomey background by successive backcrossing.

513 The following stocks were obtained from the Bloomington *Drosophila* Stock Center: UAS-
514 LanB1^{EP-600} (#43428), UAS-LanA^{EY02207} (#20141), UAS-cdc14^{EY10303} (#16450), UAS-
515 Col13A1^{EY09983} (#17628), GMR-GAL4 (#9146), UAS-GFP (#1521), UAS-H3:GFP
516 (#68241), UAS-mCD8:GFP (#5137), UAS-mCD8:RFP (#27392), UAS-KDEL:GFP (#9898).

517 The following stocks were obtained from the Vienna *Drosophila* Resource Center: UAS-
518 Mys^{VDRC} (β 1-integrin) (#29619), UAS-Mew^{VDRC} (α PS1-integrin) (#44890), UAS-wb^{VDRC}
519 (wb^{RNAi 1}) (#3141), UAS-Xbp1^{VDRC} (#109312). The following stocks were obtained from the
520 Transgenic RNAi Project at Harvard Medical School: UAS-LanA^{TRiP} (#28071), UAS-
521 LanB2^{TRiP} (LanB2^{RNAi 1}) (#55388), UAS-LanB2^{TRiP} (LanB2^{RNAi 2}) (#62002), UAS-wb^{TRiP}
522 (wb^{RNAi 2}) (#29559). UAS-A β ₄₂^{Arctic} was a gift from Dr. Damian Crowther (University of
523 Cambridge, UK)⁵⁵. UAS-A β ₄₂^{X2} (tandem wildtype A β) was a gift from Dr. Pedro Fernandez-
524 Funez (University of Minnesota, USA)²¹. UAS-Mys (β 1-integrin) was a gift from Dr.
525 Rongwen Xi (NIBS, China)⁸⁶. UAS-LanB1:RFP, UAS-Cg25C:RFP, UAS-LanB2:GFP, and
526 UAS-secr:GFP (SP^{Wg}:GFP) lines were a gift from Dr. José Carlos Pastor-Pareja (Tsinghua
527 University, China)^{25,87}. ElavGS driver line was a gift from Dr. Hervé Tricoire (CNRS,
528 France)⁸⁸. NsybGS driver line was a gift from Dr. Amita Sehgal (UPenn, USA)⁸⁹. GliaGS
529 driver line (GSG3285-1) was a gift from Dr. Minoru Saitoe (TMiMS, Japan) originally
530 generated by Dr. Haig Keshishian (Yale, USA)⁹⁰. Dilp2GS driver line (stock #3 on the
531 second chromosome) was a gift from Dr. Heinrich Jasper (Buck Institute, USA)⁹¹.

532 Mutants and transgenic lines were backcrossed into the w^{Dah} *Wolbachia*-positive strain for
533 at least six generations. Fly stocks were maintained and all experiments were conducted
534 at 25°C on a 12h:12h light/dark circadian cycle at constant 65% humidity using standard
535 sugar/yeast/agar (SYA) medium (containing 10% (w/v) brewer's yeast, 5% (w/v) sucrose
536 and 1.5% (w/v) agar)⁹². For all experiments involving Mifepristone, the steroid drug inducer
537 'RU' (RU486 – Sigma, Poole, Dorset, UK), the compound was dissolved in EtOH to make
538 100 mM stock solution and added to molten fly food for a final concentration of 200 μ M.

539 RU food was then dispensed into plastic vials, allowed to cool, then sealed and stored in a
540 4°C cold room. RU food was brought to room temperature before use with flies.

541 Lifespan assay

542 Flies were reared at a standard density before being used for lifespan experiments as
543 previously described^{92,93}. All experiments were performed with flies (females and males)
544 that were allowed 48 h to mate after emerging as adults. Flies were subsequently lightly
545 anaesthetized with CO₂, sorted into single sexes and counted at 15 per vial into 10 vials
546 for a total of 150 flies per condition. In all cases, flies were transferred to fresh food at least
547 three times a week, at which point deaths/censors were scored. For some survival assays,
548 vials were kept in DrosoFlippers (drosoflipper.com) for ease of regular transfer to fresh
549 vials. Microsoft Excel (lifespan template available at piperlab.org/resources/) was used to
550 calculate survival proportions. Log-rank tests of survivorship curves were performed in
551 Excel (Microsoft), and Cox proportional hazards analysis for multiple comparisons was
552 performed in R Studio (R Core Team).

553 Climbing assay

554 For each condition analysed in climbing assays, five vials of control food and five of food
555 containing RU, each containing 15 flies, were housed side-by-side in a single
556 DrosoFlipper. Flies were maintained as in lifespan studies and then were filmed for
557 climbing assays once to twice per week. For climbing assays, the flies were kept in
558 DrosoFlippers and transferred to empty vials on each side of the flipper, creating a
559 standard vertical column 20cm in height for each set of flies. Flies were tapped to the
560 bottom of the vials and allowed to climb upwards for 15 s before a still camera image was
561 captured. The heights of individual flies were then assessed by manual multi-point
562 selection in Fiji software⁹⁴, with each height in pixels calibrated to a height in cm from a
563 ruler placed next to the vials during filming.

564 *Drosophila* rough eye phenotype analysis

565 Virgin females expressing Aβ^{X2} under the control of the GMR-Gal4 driver were crossed to
566 males of the relevant genotype. Before imaging, flies were briefly snap frozen in liquid
567 nitrogen to aid in correct positioning and avoid any movement artefacts. Eye images of 7-
568 day-old flies were taken using a Leica M165 FC stereomicroscope equipped with a
569 motorized stage and a multi-focus tool (Leica application suite software). For eye size

analysis, wide-field images at the same magnification were taken of flies for each genotype and eye size was measured using Fiji⁹⁴.

Capillary feeder (CAFE) assay

A 7 mL bijou vial filled with 1 ml of (1%) agar, to ensure humid conditions, was sealed with Parafilm (Alpha Laboratories Ltd, Hampshire, UK). Four holes in the Parafilm that were equally spaced apart, were made using a 26-gauge needle to ensure adequate air circulation. Through the Parafilm was inserted a truncated 200 µl pipette tip which held a graduated 5 µl disposable glass capillary tube (Camag, Muttenz, Switzerland) containing liquid food (2% (w/v) yeast and 5% (w/v) sugar) supplemented with blue food dye (Langdale, Market Harborough, UK) to aid measurement of feeding. For all experiments, a mineral oil overlay (0.1 µl) was used to minimize evaporation. Food ingestion was measured every 24-hr. Each experiment included an identical, CAFE chamber without flies to determine evaporative losses (typically 10% of ingested volumes), which were subtracted from experimental readings⁶⁰.

Brain dissection, immunohistochemistry, and imaging of the fly brain

We followed the dissection and staining protocol for Aβ detection in the adult *Drosophila* brain from Ray *et al.* 2017¹⁸. Using forceps, fly heads were removed from the bodies in cold PBS containing 4% paraformaldehyde (Pierce, Thermo Fisher) + 0.01% Triton X-100 (Sigma). The proboscis was then removed from the fly head to allow fixative and detergent to fix and permeabilise the fly brain. The fly heads were transferred to a 0.5 mL microcentrifuge tube, fixed in 400 µL 4% paraformaldehyde + 0.01% Triton X-100 and rocked for 16 mins at room temperature. The heads were washed with PBST-1 (PBS containing 0.01% Triton-X-100) for 3 x 2 mins at room temperature. The brains were dissected from the fly heads in ice cold PBST-1 in a dissection dish under a light stereomicroscope. The brains were transferred to a new 0.5 mL microcentrifuge tube and fixed in 400 µL 4% paraformaldehyde + 0.1% Triton X-100 and rocked for 20 mins at room temperature. The brains were then washed in PBST-2 (PBS containing 0.1% Triton X-100) for 3 x 2 mins and blocked with SeaBlock blocking buffer (Thermo Fisher) for 15 mins at room temperature. SeaBlock blocking buffer was removed and the brains were incubated in primary antibody in PBST-2 overnight, washed in PBST-2 for 3 x 20 mins and incubated in secondary antibody in PBST-2 for 1 hour in the dark. The brains were washed in PBST-2 for 3 x 20 mins at room temperature in the dark and mounted on a microscope slide using Vectashield mounting media containing DAPI (Vector Labs). The following

antibodies were used. Primary antibodies: mouse anti-A β 6E10 (1:500; BioLegend #803001). Secondary antibodies: Alexa Fluor 488 donkey anti-mouse (1:400; Thermo Fisher #A21202), Alexa Fluor 594 goat anti-mouse (1:400; Thermo Fisher #A11005). Images were captured with a Zeiss LSM 700 confocal laser scanning microscope (Zeiss, Germany) or with a Leica TCS8 confocal microscope (Leica, Wetzlar, Germany) with a 20x or 63x oil immersion objective. Images were taken as stacks and are shown as single sections or maximum intensity projections of the complete stack. All images for one experiment were taken at the same settings.

Live 2-photon imaging

Adult flies were fixed ventral side down to microscope slides using dental composite (3M Espe Sinfony Enamel Effect Material) and cured using a curing light (3TECH LED-1007 #104-0028). A small piece of cuticle was removed from the posterior side of the head (cuticular window) to reveal the fly brain. GFP fluorescence resulting from LanB2:GFP expression in Dilp2 neurons was imaged with a Leica TCS SP8 MP 2-photon microscope (Leica, Wetzlar, Germany).

Quantitative real-time PCR (qPCR)

Total RNA was isolated from adult fly heads using standard TRIzol (Invitrogen) protocols. RNA samples were treated with Turbo DNase (Invitrogen) and converted to cDNA using oligo-dT primers and Superscript II reverse transcriptase (Invitrogen). Quantitative RT-PCR was performed using Power SYBR Green PCR Master Mix (ABI) in the Quant Studio 6 Flex system (Applied Biosystems, Thermo Fisher Scientific). Each sample was analyzed in duplicate, and values are the mean of four or five independent biological repeats. Relative quantities of transcripts were determined using the relative standard curve method normalized to Tub84B or Rp49. The following primer sequences (Eurofins, UK) were used in the analysis: A β ₄₂: 5'-CGATCCTTCTCCTGCTAACC-3', 5'-CACCATCAAGCCAATAATCG-3'; LanB1 (PP29286): 5'-CTCGCCGGAGAGATTCTGC-3', 5'-TTGTACGGATCATGCTTGGTC-3'; cdc14 (PP30015): 5'-GACTTTGGTCCGCTCAACATA-3', 5'-CGGATTCATGGAGGTGTAGTGA-3'; BiP: 5'-TCTTGTACACACCAACGCAGG-3', 5'-CAAGGAGCTGGGCACAGTGA-3'; Tub84B: 5'-TGGGCCCCGTCTGGACCACAA-3', 5'-TCGCCGTACCGGAGTCCAT-3'; Rp49: 5'-GACAATCTCCTTGCGCTTCT-3', 5'-CCAGTCGGATCGATATGCTAA-3'. LanB1 (PP29286) and cdc14 (PP30015) primer sequences were obtained from the Fly Primer Bank⁹⁵ (flyrnai.org/flyprimerbank).

636 Quantification of total, soluble and aggregated A β ₄₂

637 To extract total A β ₄₂, five fly heads were homogenised in 50 μ l GnHCl extraction buffer (5
638 M Guanidinium HCl, 50 mM HEPES pH 7.3, protease inhibitor cocktail (Sigma, P8340)
639 and 5 mM EDTA), centrifuged at 21,000 $\times$ g for 5 min at 4°C, and cleared supernatant
640 retained as the total fly A β ₄₂ sample. Alternatively, soluble and insoluble pools of A β ₄₂
641 were extracted using a protocol adapted from previously published methods^{56,96}: 50 fly
642 heads were homogenised in 50 μ L tissue homogenisation buffer (250 mM sucrose, 20 mM
643 Tris base, 1 mM EDTA, 1 mM EGTA, protease inhibitor cocktail (Sigma)) then mixed
644 further with 200 μ l DEA buffer (0.4% DEA, 100 mM NaCl and protease inhibitor cocktail).
645 Samples were centrifuged at 135,000 $\times$ g for 1 hr at 4°C (Beckman Optima™ Max
646 centrifuge, TLA120.1 rotor), and supernatant retained as the cytosolic, soluble A β ₄₂
647 fraction. Pellets were further resuspended in 400 μ l ice-cold formic acid (70%) and
648 sonicated for 2 $\times$ 30 s on ice. Samples were re-centrifuged at 135,000 $\times$ g for 1 hr at 4°C,
649 then 210 μ l of supernatant diluted with 4 ml FA neutralisation buffer (1 M Tris base, 0.5 M
650 Na₂ HPO₄, 0.05% NaN₃) and retained as the aggregated, formic acid-extractable A β ₄₂
651 fraction. Total, soluble or aggregated A β ₄₂ content was measured using the Ultrasensitive
652 hAmyloid- β ₄₂ ELISA kit (Thermo Fisher, # KHB3544). Total and soluble A β ₄₂ samples were
653 diluted 1:10, and aggregated A β ₄₂ samples diluted 1:5 in sample/standard dilution buffer
654 and the ELISA performed according to the manufacturers' instructions. Protein extracts
655 were quantified using the BCA Protein Assay Kit (Pierce) and the amount of A β ₄₂ in each
656 sample expressed as a ratio of the total protein content (pmol/g total protein).

657 Western blots

658 For protein extracts, fly heads (10-20 per biological sample) were homogenised in 1x
659 NuPAGE LDS sample buffer (Thermo Fisher) with 200 mM DTT (Sigma) and boiled at
660 95°C for 5 min. With the assumption that fly heads of the same sex contain comparable
661 levels of total protein, we lysed 1 fly head per 10 μ L sample buffer. Equal quantities of
662 protein for each sample were then separated on 4%–12% NuPAGE Bis-Tris gels
663 (Invitrogen) and transferred to a nitrocellulose membrane (GE Healthcare). For A β
664 westerns antigen retrieval was necessary, so membranes were boiled in 1X PBS for 5 min
665 in a microwave. Membranes were blocked in 5% bovine serum albumin (BSA, Sigma) in
666 Tris-buffered saline (TBS) with 0.1% Tween-20 (TBST) for 1 hr at room temperature, after
667 which they were probed with primary antibodies overnight at 4°C. The following primary
668 antibodies were used: mouse anti-A β 6E10 (1:500; BioLegend #803001 previously

669 #39320), rabbit anti-BiP (1:1000; Novus Biologicals #NBP1-06274), rabbit anti-Actin
670 (1:5,000; Abcam #ab1801), mouse anti-Tubulin (1:2000, Sigma #T6199). Membranes
671 were then probed with secondary anti-mouse HRP (1:10,000; Abcam #ab6789) or anti-
672 rabbit HRP antibodies (1:10,000; Abcam #ab6721) for 1 hr at room temperature. Blots
673 were developed using Luminata Crescendo (Millipore) and the ImageQuant LAS 4000
674 system. Densitometric analysis of blot images was carried out using Fiji software⁹⁴.

675 Single-cell transcriptomic data for the adult fly brain

676 Single-cell atlas images were obtained by gene name searches in *S*Scope⁵⁹, the
677 vizualisation and analysis tool from the Aerts lab (<http://scope.aertslab.org/>).

678 Mice

679 P10 wildtype mice (C57/BL6J) were used for slice culture experiments. Animals were
680 culled by humane schedule one methods and brain tissue removed for the generation of
681 organotypic slice cultures. All animal experiments conformed to national and institutional
682 guidelines including the Animals [Scientific Procedures Act] 1986 (UK), and the Council
683 Directive 2010/63EU of the European Parliament and the Council of 22 September 2010
684 on the protection of animals used for scientific purposes, and had full Home Office ethical
685 approval. Mice were bred in-house and group-housed on a 12h/12h light/dark cycle with
686 *ad libitum* access to food and water.

687 Organotypic slice cultures

688 Organotypic cultures of the hippocampus and surrounding cortex were taken from
689 humanely sacrificed P10 mouse pups of either sex according to previously described
690 protocols^{97–99}. Briefly, brains were rapidly removed and kept in dissection buffer (Earle's
691 Balanced Salt Solution (EBSS) + 25 mM HEPES + 1X Penicillin/Streptomycin) on ice.
692 From this point, until plating, all equipment and tissue was kept ice cold. Brains were
693 bisected at the midline then the cut sides glued (Loctite super glue), face down onto a
694 vibratome stage and flooded with dissection media. 350 µm sagittal slices (6 per brain)
695 were taken using a Leica VT1200S Vibratome; the hippocampus with surrounding cortex
696 was dissected out using sterile syringe needles. The dissected slices were then
697 transferred (using a sterile 3 ml plastic pipette - modified to widen the opening) to Falcon
698 tubes full of ice-cold dissection medium and stored until plating. To plate, slices were
699 transferred (3 slices from the same pup per dish, 2 dishes per pup (split randomly between
700 control/ test conditions)) onto sterile 0.4 µm pore membranes (Millipore #PICM0RG50) in

35 mm culture dishes (Nunc). Inserts were kept in 1 ml of maintenance medium (50% MEM with Glutamax-1 (Life Tech:42360-024), 25% Heat-inactivated horse serum (Life Tech: 26050-070), 23% EBSS (Life Tech: 24010-043), 0.65% D-Glucose (Sigma: G8270), 2% Penicillin-Streptomycin (Life Tech: 15140-122) and 6 units/ml Nystatin (Sigma: N1638)) and cultures were maintained in incubators at 37°C, 5% CO₂ for 4 weeks. Two 100% medium exchanges occurred (5 hr after plating and 4 days *in vitro* and a 50% media exchange occurred each week thereafter. At 14 days *in vitro*, LamB1 or control lentivirus was applied at a viral titer of 8.33x10⁷ per dish (diluted in the culture medium with a few drops added on top of the slice to ensure complete perfusion of the tissue). Culture medium was changed 24 hours later, and cultures maintained for a further 14 days *in vitro* before fixation.

Immunofluorescence staining of organotypics

Slice cultures were fixed for 20 mins in 4% paraformaldehyde in PBS. Slices were washed twice in PBS, blocked for 1 h in blocking solution (PBS with 0.5% Triton X-100 and 3% Goat Serum) then incubated in 200 µl primary antibody diluted in blocking solution overnight at 4°C with shaking. Slices were washed 3 times in PBS before being incubated (2 hr, RT in the dark) with secondary antibodies in blocking solution. In the second of the final 3 PBS washes, slices were incubated with DAPI (Sigma, 1:10,000 in PBS). Images were captured using a Leica TCS8 confocal microscope (Leica, Wetzlar, Germany). Primary antibodies used: rat Lamb1 (1:250; Abcam #ab44941), rabbit Calnexin (1:250; Abcam #ab13504). Secondary antibodies used: goat anti-rabbit Alexa Fluor 594 (1:400; Invitrogen #A11037) and goat anti-rat Alexa Fluor 647 (1:400; Invitrogen #A21247).

Statistics

Data were grouped for each genotype and the mean (+/- SEM) calculated. Log-rank, Cox Proportional Hazard, Analysis of Variances (ANOVA) and Tukey's HSD (honestly significant difference) post-hoc analyses were performed. Statistical analyses were performed in Excel (Microsoft) or Prism (GraphPad, La Jolla, CA), except for Cox Proportional Hazards which were performed in R Studio (R Core Team). A statistical difference of p<0.05 was regarded as significant.

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740 Author contributions

741 Conceived and designed the experiments: JHC LP. Performed the experiments: JC LM SA
 742 SJM SM MCD AR NSW MLA MA CD. Analysed the data: JHC. Contributed
 743 reagents/materials/analysis tools: JHC TLSJ LP. Wrote the paper: JHC LP.

744 References

- 745 1. Huang, Y., and Mucke, L. (2012). Alzheimer Mechanisms and Therapeutic
746 Strategies. *Cell* 148, 1204–1222.
- 747 2. Hardy, J., and Higgins, G. (1992). Alzheimer's disease: the amyloid cascade
748 hypothesis. *Science* (80-.). 256, 184–185.
- 749 3. Hetz, C., and Mollereau, B. (2014). Disturbance of endoplasmic reticulum
750 proteostasis in neurodegenerative diseases. *Nat. Rev. Neurosci.* 15, 233–249.
- 751 4. Gerakis, Y., and Hetz, C. (2017). Emerging roles of ER stress in the etiology and
752 pathogenesis of Alzheimer's disease. *FEBS J.* 285, 995–1011.
- 753 5. Hamos, J.E., Oblas, B., Pulaski-Salo, D., Welch, W.J., Bole, D.G., and Drachman,
754 D.A. (1991). Expression of heat shock proteins in Alzheimer's disease. *Neurology*
755 41, 345.
- 756 6. Hoozemans, J.J.M., van Haastert, E.S., Nijholt, D.A.T., Rozemuller, A.J.M.,
757 Eikelenboom, P., and Scheper, W. (2009). The Unfolded Protein Response Is
758 Activated in Pretangle Neurons in Alzheimer's Disease Hippocampus. *Am. J. Pathol.*
759 174, 1241–1251.
- 760 7. Unterberger, U., Höftberger, R., Gelpi, E., Flicker, H., Budka, H., and Voigtländer, T.
761 (2006). Endoplasmic Reticulum Stress Features Are Prominent in Alzheimer
762 Disease but Not in Prion Diseases In Vivo. *J. Neuropathol. Exp. Neurol.* 65, 348–
763 357.
- 764 8. Cornejo, V.H., and Hetz, C. (2013). The unfolded protein response in Alzheimer's
765 disease. *Semin. Immunopathol.* 35, 277–292.
- 766 9. Bertolotti, A., Zhang, Y., Hendershot, L.M., Harding, H.P., and Ron, D. (2000).
767 Dynamic interaction of BiP and ER stress transducers in the unfolded-protein
768 response. *Nat. Cell Biol.* 2, 326–332.
- 769 10. Uddin, M.S., Tewari, D., Sharma, G., Kabir, M.T., Barreto, G.E., Bin-Jumah, M.N.,
770 Perveen, A., Abdel-Daim, M.M., and Ashraf, G.M. (2020). Molecular Mechanisms of
771 ER Stress and UPR in the Pathogenesis of Alzheimer's Disease. *Mol. Neurobiol.* 57,
772 2902–2919.
- 773 11. Strobel, G. (2011). Intraneuronal A β : Was It APP All Along? Alzforum,
774 <https://www.alzforum.org/webinars/intraneuronal-av>.
775 <https://www.alzforum.org/webinars/intraneuronal-av-was-it-app-all-along>.
- 776 12. Bowman Rogers, M. (2015). Mistakes Prompt Retraction of Controversial Paper,
777 and Publication Ban. Alzforum, [https://www.alzforum.org/news/research-](https://www.alzforum.org/news/research-news/mistak)
778 [news/mistak](https://www.alzforum.org/news/research-news/mistakes-prompt-retraction-controversial-paper-and-publication-ban). [https://www.alzforum.org/news/research-news/mistakes-prompt-](https://www.alzforum.org/news/research-news/mistakes-prompt-retraction-controversial-paper-and-publication-ban)
779 [retraction-controversial-paper-and-publication-ban](https://www.alzforum.org/news/research-news/mistakes-prompt-retraction-controversial-paper-and-publication-ban).
- 780 13. Ji, L., Zhao, X., Lu, W., Zhang, Q., and Hua, Z. (2016). Intracellular A β and its
781 Pathological Role in Alzheimer's Disease: Lessons from Cellular to Animal Models.
782 *Curr. Alzheimer Res.* 13, 621–630.
- 783 14. Takahashi, R.H., Nagao, T., and Gouras, G.K. (2017). Plaque formation and the
784 intraneuronal accumulation of β -amyloid in Alzheimer's disease. *Pathol. Int.* 67, 185–
785 193.
- 786 15. Scheper, W., and Hoozemans, J.J.M. (2015). The unfolded protein response in
787 neurodegenerative diseases: a neuropathological perspective. *Acta Neuropathol.*
788 130, 315–331.
- 789 16. Fernandez-Funez, P., de Mena, L., and Rincon-Limas, D.E. (2015). Modeling the
790 complex pathology of Alzheimer's disease in *Drosophila*. *Exp. Neurol.* 274, 58–71.
- 791 17. Gama Sosa, M.A., De Gasperi, R., and Elder, G.A. (2011). Modeling human
792 neurodegenerative diseases in transgenic systems. *Hum. Genet.* 131, 535–563.
- 793 18. Ray, A., Speese, S.D., and Logan, M.A. (2017). Glial Draper Rescues A β Toxicity in
794 a *Drosophila* Model of Alzheimer's Disease. *J. Neurosci.* 37, 11881–11893.

- 795 19. Kremer, M.C., Jung, C., Batelli, S., Rubin, G.M., and Gaul, U. (2017). The glia of the
796 adult *Drosophila* nervous system. *Glia* 65, 606–638.
- 797 20. Awasaki, T., Lai, S.-L., Ito, K., and Lee, T. (2008). Organization and Postembryonic
798 Development of Glial Cells in the Adult Central Brain of *Drosophila*. *J. Neurosci.* 28,
799 13742–13753.
- 800 21. Casas-Tinto, S., Zhang, Y., Sanchez-Garcia, J., Gomez-Velazquez, M., Rincon-
801 Limas, D.E., and Fernandez-Funez, P. (2011). The ER stress factor XBP1s prevents
802 amyloid- β neurotoxicity. *Hum. Mol. Genet.* 20, 2144–2160.
- 803 22. Niccoli, T., Cabecinha, M., Tillmann, A., Kerr, F., Wong, C.T., Cardenes, D., Vincent,
804 A.J., Bettendi, L., Li, L., Grönke, S., et al. (2016). Increased Glucose Transport into
805 Neurons Rescues A β Toxicity in *Drosophila*. *Curr. Biol.* 26, 2291–2300.
- 806 23. Marcora, M.S., Belfiori-Carrasco, L.F., Bocai, N.I., Morelli, L., and Castaño, E.M.
807 (2017). Amyloid- β 42 clearance and neuroprotection mediated by X-box binding
808 protein 1 signaling decline with aging in the *Drosophila* brain. *Neurobiol. Aging* 60,
809 57–70.
- 810 24. Rios-Barrera, L.D., Sigurbjörnsdóttir, S., Baer, M., and Leptin, M. (2017). Dual
811 function for Tango1 in secretion of bulky cargo and in ER-Golgi morphology.
- 812 25. Liu, M., Feng, Z., Ke, H., Liu, Y., Sun, T., Dai, J., Cui, W., and Pastor-Pareja, J.C.
813 (2017). Tango1 spatially organizes ER exit sites to control ER export. *J. Cell Biol.*
814 216, 1035–1049.
- 815 26. Indyk, J.A., Chen, Z.L., Tsirka, S.E., and Strickland, S. (2003). Laminin chain
816 expression suggests that laminin-10 is a major isoform in the mouse hippocampus
817 and is degraded by the tissue plasminogen activator/plasmin protease cascade
818 during excitotoxic injury. *Neuroscience* 116, 359–371.
- 819 27. Sharif, K.A., Baker, H., and Gudas, L.J. (2004). Differential regulation of laminin b1
820 transgene expression in the neonatal and adult mouse brain. *Neuroscience* 126,
821 967–978.
- 822 28. Yang, Y.C., Ma, Y.L., Liu, W.T., and Lee, E.H.Y. (2011). Laminin- β 1 Impairs Spatial
823 Learning through Inhibition of ERK/MAPK and SGK1 Signaling.
824 *Neuropsychopharmacology* 36, 2571–2586.
- 825 29. Hsu, W.-L., Ma, Y.-L., Hsieh, D.-Y., Liu, Y.-C., and Lee, E.H.Y. (2013). STAT1
826 Negatively Regulates Spatial Memory Formation and Mediates the Memory-
827 Impairing Effect of A β . *Neuropsychopharmacology* 39, 746–758.
- 828 30. Kibbey, M.C., Jucker, M., Weeks, B.S., Neve, R.L., Van Nostrand, W.E., and
829 Kleinman, H.K. (1993). beta-Amyloid precursor protein binds to the neurite-
830 promoting IKVAV site of laminin. *Proc. Natl. Acad. Sci.* 90, 10150–10153.
- 831 31. Matsuda, K., Tashiro, K., Hayashi, Y., Monji, A., Yoshida, I., and Mitsuyama, Y.
832 (2002). Measurement of Laminins in the Cerebrospinal Fluid Obtained from Patients
833 with Alzheimer's Disease and Vascular Dementia Using a Modified Enzyme-Linked
834 Immunosorbent Assay. *Dement. Geriatr. Cogn. Disord.* 14, 113–122.
- 835 32. Bronfman, F.C., Garrido, J., Alvarez, A., Morgan, C., and Inestrosa, N.C. (1996).
836 Laminin inhibits amyloid- β -peptide fibrillation. *Neurosci. Lett.* 218, 201–203.
- 837 33. Monji, A., Tashiro, K., Yoshida, I., Hayashi, Y., and Tashiro, N. (1998). Laminin
838 inhibits A β 42 fibril formation in vitro. *Brain Res.* 788, 187–190.
- 839 34. Monji, A., Tashiro, K., Yoshida, I., Kaname, H., Hayashi, Y., Matsuda, K., and
840 Tashiro, N. (1999). Laminin inhibits both A β 40 and A β 42 fibril formation but does not
841 affect A β 40 or A β 42-induced cytotoxicity in PC12 cells. *Neurosci. Lett.* 266, 85–88.
- 842 35. Cheng, J.S., Dubal, D.B., Kim, D.H., Legleiter, J., Cheng, I.H., Yu, G.-Q., Tessier, I.,
843 Wyss-Coray, T., Bonaldo, P., and Mucke, L. (2009). Collagen VI protects neurons
844 against A β toxicity. *Nat. Neurosci.* 12, 119–121.
- 845 36. Lane-Donovan, C., Philips, G.T., Wasser, C.R., Durakoglugil, M.S., Masiulis, I.,
846 Upadhyaya, A., Pohlkamp, T., Coskun, C., Kotti, T., Steller, L., et al. (2015). Reelin

- protects against amyloid β toxicity in vivo. *Sci. Signal.* 8, ra67–ra67.
37. Végh, M.J., Heldring, C.M., Kamphuis, W., Hijazi, S., Timmerman, A.J., Li, K., van Nierop, P., Mansvelder, H.D., Hol, E.M., Smit, A.B., et al. (2014). Reducing hippocampal extracellular matrix reverses early memory deficits in a mouse model of Alzheimer's disease. *Acta Neuropathol. Commun.* 2, 76.
38. Xu, L., Nirwane, A., and Yao, Y. (2018). Basement membrane and blood–brain barrier. *Stroke Vasc. Neurol.* 4, 78–82.
39. Tsai, P.-I., Wang, M., Kao, H.-H., Cheng, Y.-J., Lin, Y.-J., Chen, R.-H., and Chien, C.-T. (2012). Activity-dependent retrograde laminin A signaling regulates synapse growth at *Drosophila* neuromuscular junctions. *Proc. Natl. Acad. Sci.* 109, 17699–17704.
40. Patton, B.L. (2000). Laminins of the neuromuscular system. *Microsc. Res. Tech.* 51, 247–261.
41. Omar, M.H., Kerrisk Campbell, M., Xiao, X., Zhong, Q., Brunken, W.J., Miner, J.H., Greer, C.A., and Koleske, A.J. (2017). CNS Neurons Deposit Laminin $\alpha 5$ to Stabilize Synapses. *Cell Rep.* 21, 1281–1292.
42. Chen, Z.-L., Indyk, J.A., and Strickland, S. (2003). The Hippocampal Laminin Matrix Is Dynamic and Critical for Neuronal Survival. *Mol. Biol. Cell* 14, 2665–2676.
43. Yamamoto, T., Iwasaki, Y., Yamamoto, H., Konno, H., and Isemura, M. (1988). Intraneuronal laminin-like molecule in the central nervous system: demonstration of its unique differential distribution. *J. Neurol. Sci.* 84, 1–13.
44. Brandan, E., and Inestrosa, N.C. (1993). Extracellular matrix components and amyloid in neuritic plaques of Alzheimer's disease. *Gen. Pharmacol. Vasc. Syst.* 24, 1063–1068.
45. Palu, E., and Liesi, P. (2002). Differential distribution of laminins in Alzheimer disease and normal human brain tissue. *J. Neurosci. Res.* 69, 243–256.
46. Domogatskaya, A., Rodin, S., and Tryggvason, K. (2012). Functional Diversity of Laminins. *Annu. Rev. Cell Dev. Biol.* 28, 523–553.
47. Hamill, K.J., Langbein, L., Jones, J.C.R., and McLean, W.H.I. (2009). Identification of a Novel Family of Laminin N-terminal Alternate Splice Isoforms. *J. Biol. Chem.* 284, 35588–35596.
48. Urbano, J.M., Torgler, C.N., Molnar, C., Tepass, U., Lopez-Varea, A., Brown, N.H., de Celis, J.F., and Martin-Bermudo, M.D. (2009). *Drosophila* laminins act as key regulators of basement membrane assembly and morphogenesis. *Development* 136, 4165–4176.
49. Yurchenco, P.D., Quan, Y., Colognato, H., Mathus, T., Harrison, D., Yamada, Y., and O'Rear, J.J. (1997). The α -chain of laminin-1 is independently secreted and drives secretion of its β - and γ -chain partners. *Proc. Natl. Acad. Sci.* 94, 10189–10194.
50. Kumagai, C., Kadowaki, T., and Kitagawa, Y. (1997). Disulfide-bonding between *Drosophila* laminin β and γ chains is essential for α chain to form $\alpha\beta\gamma$ trimer. *FEBS Lett.* 412, 211–216.
51. Morita, A., Sugimoto, E., and Kitagawa, Y. (1985). Post-translational assembly and glycosylation of laminin subunits in parietal endoderm-like F9 cells. *Biochem. J.* 229, 259–264.
52. De Arcangelis, A., Neuville, P., Boukamel, R., Lefebvre, O., Keding, M., and Simon-Assmann, P. (1996). Inhibition of laminin $\alpha 1$ -chain expression leads to alteration of basement membrane assembly and cell differentiation. *J. Cell Biol.* 133, 417–430.
53. Petley-Ragan, L.M., Ardiel, E.L., Rankin, C.H., and Auld, V.J. (2016). Accumulation of Laminin Monomers in *Drosophila* Glia Leads to Glial Endoplasmic Reticulum Stress and Disrupted Larval Locomotion. *J. Neurosci.* 36, 1151–1164.

- 899 54. Boot-Handford, R.P., and Briggs, M.D. (2009). The unfolded protein response and
900 its relevance to connective tissue diseases. *Cell Tissue Res.* 339, 197–211.
- 901 55. Crowther, D.C., Kinghorn, K.J., Miranda, E., Page, R., Curry, J.A., Duthie, F.A.I.,
902 Gubb, D.C., and Lomas, D.A. (2005). Intraneuronal A β , non-amyloid aggregates and
903 neurodegeneration in a *Drosophila* model of Alzheimer's disease. *Neuroscience*
904 132.
- 905 56. Sofola, O., Kerr, F., Rogers, I., Killick, R., Augustin, H., Gandy, C., Allen, M.J.,
906 Hardy, J., Lovestone, S., and Partridge, L. (2010). Inhibition of GSK-3 Ameliorates
907 A β Pathology in an Adult-Onset *Drosophila* Model of Alzheimer's Disease. *PLoS*
908 *Genet.* 6.
- 909 57. Bunt, S., Hooley, C., Hu, N., Scahill, C., Weavers, H., and Skaer, H. (2010).
910 Hemocyte-Secreted Type IV Collagen Enhances BMP Signaling to Guide Renal
911 Tubule Morphogenesis in *Drosophila*. *Dev. Cell* 19.
- 912 58. Pastor-Pareja, J.C., and Xu, T. (2011). Shaping Cells and Organs in *Drosophila* by
913 Opposing Roles of Fat Body-Secreted Collagen IV and Perlecan. *Dev. Cell* 21.
- 914 59. Davie, K., Janssens, J., Koldere, D., De Waegeneer, M., Pech, U., Kreft, L., Aibar,
915 S., Makhzami, S., Christiaens, V., Bravo González-Blas, C., et al. (2018). A Single-
916 Cell Transcriptome Atlas of the Aging *Drosophila* Brain. *Cell* 174.
- 917 60. Ja, W.W., Carvalho, G.B., Mak, E.M., de la Rosa, N.N., Fang, A.Y., Liong, J.C.,
918 Brummel, T., and Benzer, S. (2007). Prandiology of *Drosophila* and the CAFE
919 assay. *Proc Natl Acad Sci U S A* 104, 8253–8256.
- 920 61. Jonson, M., Nyström, S., Sandberg, A., Carlback, M., Michno, W., Hanrieder, J.,
921 Starkenberg, A., Nilsson, K.P.R., Thor, S., and Hammarström, P. (2018).
922 Aggregated A β 1-42 Is Selectively Toxic for Neurons, Whereas Glial Cells Produce
923 Mature Fibrils with Low Toxicity in *Drosophila*. *Cell Chem. Biol.* 25.
- 924 62. Toba, G., Ohsako, T., Miyata, N., Ohtsuka, T., Seong, K.-H., and Aigaki, T. (1999).
925 The Gene Search System: A Method for Efficient Detection and Rapid Molecular
926 Identification of Genes in *Drosophila melanogaster*; *Genetics*
927 151, 725 LP – 737.
- 928 63. Neitzel, L.R., Broadus, M.R., Zhang, N., Sawyer, L., Wallace, H.A., Merkle, J.A.,
929 Jodoin, J.N., Sitaram, P., Crispi, E.E., Rork, W., et al. (2018). Characterization of a
930 *cdc14* null allele in *Drosophila melanogaster*. *Biol. Open* 7.
- 931 64. Rogers, I., Kerr, F., Martinez, P., Hardy, J., Lovestone, S., and Partridge, L. (2012).
932 Ageing Increases Vulnerability to A β 42 Toxicity in *Drosophila*. *PLoS One* 7.
- 933 65. Hellewell, A.L., and Adams, J.C. (2016). Insider trading: Extracellular matrix proteins
934 and their non-canonical intracellular roles. *BioEssays* 38.
- 935 66. Nässel, D.R., Kubrak, O.I., Liu, Y., Luo, J., and Lushchak, O. V. (2013). Factors that
936 regulate insulin producing cells and their output in *Drosophila*. *Front. Physiol.* 4.
- 937 67. Chen, Y.M., Zhou, Y., Go, G., Marmarstein, J.T., Kikkawa, Y., and Miner, J.H.
938 (2013). Laminin β 2 Gene Missense Mutation Produces Endoplasmic Reticulum
939 Stress in Podocytes. *J. Am. Soc. Nephrol.* 24, 1223–1233.
- 940 68. Miner, J.H., and Yurchenco, P.D. (2004). LAMININ FUNCTIONS IN TISSUE
941 MORPHOGENESIS. *Annu. Rev. Cell Dev. Biol.* 20, 255–284.
- 942 69. de Celis, J.F., and Molnar, C. (2010). A cautionary tale on genetic screens based on
943 a gain-of-expression approach: The case of LanB1. *Fly (Austin)*. 4, 24–29.
- 944 70. Ling, D., Song, H.-J., Garza, D., Neufeld, T.P., and Salvaterra, P.M. (2009).
945 Abeta42-Induced Neurodegeneration via an Age-Dependent Autophagic-Lysosomal
946 Injury in *Drosophila*. *PLoS One* 4, e4201.
- 947 71. Ling, D., and Salvaterra, P.M. (2010). Brain aging and A β 1–42 neurotoxicity
948 converge via deterioration in autophagy–lysosomal system: a conditional *Drosophila*
949 model linking Alzheimer's neurodegeneration with aging. *Acta Neuropathol.* 121,
950 183–191.

- 951 72. Abramowski, D., Rabe, S., Upadhaya, A.R., Reichwald, J., Danner, S., Staab, D.,
952 Capetillo-Zarate, E., Yamaguchi, H., Saido, T.C., Wiederhold, K.-H., et al. (2012).
953 Transgenic Expression of Intraneuronal A 42 But Not A 40 Leads to Cellular
954 A Lesions, Degeneration, and Functional Impairment without Typical Alzheimer's
955 Disease Pathology. *J. Neurosci.* 32, 1273–1283.
- 956 73. Fidler, A.L., Darris, C.E., Chetyrkin, S. V, Pedchenko, V.K., Boudko, S.P., Brown,
957 K.L., Gray Jerome, W., Hudson, J.K., Rokas, A., and Hudson, B.G. (2017). Collagen
958 IV and basement membrane at the evolutionary dawn of metazoan tissues. *Elife* 6.
- 959 74. Natzle, J.E., Monson, J.M., and McCarthy, B.J. (1982). Cytogenetic location and
960 expression of collagen-like genes in *Drosophila*. *Nature* 296, 368–371.
- 961 75. Sekiya, M., Maruko-Otake, A., Hearn, S., Sakakibara, Y., Fujisaki, N., Suzuki, E.,
962 Ando, K., and Iijima, K.M. (2017). EDEM Function in ERAD Protects against Chronic
963 ER Proteinopathy and Age-Related Physiological Decline in *Drosophila*. *Dev. Cell*
964 41, 652–664.e5.
- 965 76. Selkoe, D.J., and Hardy, J. (2016). The amyloid hypothesis of Alzheimer's disease
966 at 25 years. *EMBO Mol. Med.* 8, 595–608.
- 967 77. Finelli, A., Kelkar, A., Song, H.-J., Yang, H., and Konsolaki, M. (2004). A model for
968 studying Alzheimer's A β 42-induced toxicity in *Drosophila melanogaster*. *Mol. Cell.*
969 *Neurosci.* 26, 365–375.
- 970 78. Arboleda-Velasquez, J.F., Lopera, F., O'Hare, M., Delgado-Tirado, S., Marino, C.,
971 Chmielewska, N., Saez-Torres, K.L., Amarnani, D., Schultz, A.P., Sperling, R.A., et
972 al. (2019). Resistance to autosomal dominant Alzheimer's disease in an APOE3
973 Christchurch homozygote: a case report. *Nat. Med.* 25, 1680–1683.
- 974 79. Kumagai, C., and Kitagawa, Y. (1997). Potential molecular chaperones involved in
975 laminin chain assembly. *Cytotechnology* 25, 173–182.
- 976 80. Yu, W.-M., Yu, H., and Chen, Z.-L. (2007). Laminins in Peripheral Nerve
977 Development and Muscular Dystrophy. *Mol. Neurobiol.* 35, 288–297.
- 978 81. Rousselle, P., and Scoazec, J.Y. (2020). Laminin 332 in cancer: When the
979 extracellular matrix turns signals from cell anchorage to cell movement. *Semin.*
980 *Cancer Biol.* 62, 149–165.
- 981 82. Huang, H., Kovacs, M.E., Y. Aguilera, K., and Brekken, R.A. (2019). Collagen
982 Signaling in Cancer. *Protein Kinase Inhib. as Sensitizing Agents Chemother.*, 89–
983 108.
- 984 83. Murray, L.S., Lu, Y., Taggart, A., Van Regemorter, N., Vilain, C., Abramowicz, M.,
985 Kadler, K.E., and Van Agtmael, T. (2013). Chemical chaperone treatment reduces
986 intracellular accumulation of mutant collagen IV and ameliorates the cellular
987 phenotype of a COL4A2 mutation that causes haemorrhagic stroke. *Hum. Mol.*
988 *Genet.* 23, 283–292.
- 989 84. Fernandez-Funez, P., Sanchez-Garcia, J., de Mena, L., Zhang, Y., Levites, Y.,
990 Khare, S., Golde, T.E., and Rincon-Limas, D.E. (2016). Holdase activity of secreted
991 Hsp70 masks amyloid- β 42 neurotoxicity in *Drosophila*. *Proc. Natl. Acad. Sci.* 113,
992 E5212–E5221.
- 993 85. Younan, N.D., Chen, K.-F., Rose, R.-S., Crowther, D.C., and Viles, J.H. (2018).
994 Prion protein stabilizes amyloid- β (A β) oligomers and enhances A β neurotoxicity in a
995 *Drosophila* model of Alzheimer's disease. *J. Biol. Chem.* 293, 13090–13099.
- 996 86. Lin, G., Zhang, X., Ren, J., Pang, Z., Wang, C., Xu, N., and Xi, R. (2013). Integrin
997 signaling is required for maintenance and proliferation of intestinal stem cells in
998 *Drosophila*. *Dev. Biol.* 377, 177–187.
- 999 87. Ke, H., Feng, Z., Liu, M., Sun, T., Dai, J., Ma, M., Liu, L.-P., Ni, J.-Q., and Pastor-
1000 Pareja, J.C. (2018). Collagen secretion screening in *Drosophila* supports a common
1001 secretory machinery and multiple Rab requirements. *J. Genet. Genomics* 45, 299–
1002 313.

1003 88. Latouche, M., Lasbleiz, C., Martin, E., Monnier, V., Debeir, T., Mouatt-Prigent, A.,
1004 Muriel, M.-P., Morel, L., Ruberg, M., Brice, A., et al. (2007). A Conditional Pan-
1005 Neuronal Drosophila Model of Spinocerebellar Ataxia 7 with a Reversible Adult
1006 Phenotype Suitable for Identifying Modifier Genes. *J. Neurosci.* 27, 2483–2492.
1007 89. Bai, L., Lee, Y., Hsu, C.T., Williams, J.A., Cavanaugh, D., Zheng, X., Stein, C.,
1008 Haynes, P., Wang, H., Gutmann, D.H., et al. (2018). A Conserved Circadian
1009 Function for the Neurofibromatosis 1 Gene. *Cell Rep.* 22, 3416–3426.
1010 90. Nicholson, L., Singh, G.K., Osterwalder, T., Roman, G.W., Davis, R.L., and
1011 Keshishian, H. (2008). Spatial and Temporal Control of Gene Expression in
1012 Drosophila Using the Inducible GeneSwitch GAL4 System. I. Screen for Larval
1013 Nervous System Drivers. *Genetics* 178, 215–234.
1014 91. Karpac, J., Hull-Thompson, J., Falleur, M., and Jasper, H. (2009). JNK signaling in
1015 insulin-producing cells is required for adaptive responses to stress in Drosophila.
1016 *Aging Cell* 8, 288–295.
1017 92. Bass, T.M., Grandison, R.C., Wong, R., Martinez, P., Partridge, L., and Piper,
1018 M.D.W. (2007). Optimization of dietary restriction protocols in Drosophila. *J Gerontol*
1019 *A Biol Sci Med Sci* 62, 1071–1081.
1020 93. Clancy, D.J., and Kennington, W.J. (2001). A simple method to achieve consistent
1021 larval density in bottle cultures. *Dros. Inf Serv.*
1022 94. Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T.,
1023 Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., et al. (2012). Fiji: an open-
1024 source platform for biological-image analysis. *Nat. Methods* 9, 676–682.
1025 95. Hu, Y., Sopko, R., Foos, M., Kelley, C., Flockhart, I., Ammeux, N., Wang, X.,
1026 Perkins, L., Perrimon, N., and Mohr, S.E. (2013). FlyPrimerBank: An Online
1027 Database for Drosophila melanogaster Gene Expression Analysis and Knockdown
1028 Evaluation of RNAi Reagents. *G3: Genes|Genomes|Genetics* 3, 1607–
1029 1616.
1030 96. Burns, M., Gaynor, K., Olm, V., Mercken, M., LaFrancois, J., Wang, L., Mathews,
1031 P.M., Noble, W., Matsuoka, Y., and Duff, K. (2003). Presenilin Redistribution
1032 Associated with Aberrant Cholesterol Transport Enhances β -Amyloid ProductionIn
1033 Vivo. *J. Neurosci.* 23, 5645–5649.
1034 97. De Simoni, A., and MY Yu, L. (2006). Preparation of organotypic hippocampal slice
1035 cultures: interface method. *Nat. Protoc.* 1, 1439–1445.
1036 98. Harwell, C.S., and Coleman, M.P. (2016). Synaptophysin depletion and
1037 intraneuronal A β in organotypic hippocampal slice cultures from huAPP transgenic
1038 mice. *Mol. Neurodegener.* 11, 44.
1039 99. Durrant, C.S. (2020). Preparation of Organotypic Hippocampal Slice Cultures for the
1040 Study of CNS Disease and Damage BT - Axon Degeneration: Methods and
1041 Protocols. In, E. Babetto, ed. (Springer US), pp. 133–144.

1042 **Figures**

1046 significantly ($p = 1.31 \times 10^{-70}$; log rank test) shortened lifespan compared to uninduced
1047 controls. LanB1 and $A\beta^{Arc}$ co-expression resulted in a significant rescue ($p = 1.38 \times 10^{-50}$;
1048 log rank test) of the short-lived phenotype. **b** Repeat of experiment in **a**. LanB1
1049 significantly rescued $A\beta^{Arc}$ toxicity ($p = 8.31 \times 10^{-56}$; log rank test). **c** LanB1 significantly
1050 rescued $A\beta^{Arc}$ toxicity in male flies ($p = 8.31 \times 10^{-56}$; log rank test). **d** Induction of $A\beta^{X2}$
1051 significantly ($p = 5.99 \times 10^{-69}$; log rank test) shortened lifespan compared to uninduced
1052 controls. LanB1 and $A\beta^{X2}$ co-expression resulted in a significant rescue ($p = 8.04 \times 10^{-40}$;
1053 log rank test). **e** Induction of $A\beta^{Arc}$ with NsybGS significantly ($p = 3.38 \times 10^{-67}$; log rank test)
1054 shortened lifespan compared to uninduced controls. LanB1 significantly rescued $A\beta^{Arc}$
1055 toxicity ($p = 6.03 \times 10^{-40}$; log rank test). Dashed lines represent uninduced 'RU-' controls,
1056 solid lines represent induced 'RU+' conditions. For all lifespan experiments $n = 150$ flies
1057 per condition. **f** Climbing ability was measured until day 51. Climbing ability of $A\beta^{Arc}$ flies
1058 was lower than that of all other groups ($p < 0.0001$, two-way ANOVA with Tukey's post-hoc
1059 test). LanB1 significantly rescued the $A\beta^{Arc}$ -induced decline in climbing ability, though not
1060 to the same level as uninduced controls ($p < 0.0001$, two-way ANOVA with Tukey's post-
1061 hoc test). Data are shown as mean $\pm$ SEM ($n = 37-70$ flies measured per time point). **g**
1062 LanB1 suppressed $A\beta$ toxicity in the eyes of flies raised at 29°C. Control flies displayed a
1063 highly ordered ommatidia lattice (left). Expression of $A\beta^{X2}$ using the eye-specific GMR-
1064 GAL4 driver resulted in small, glassy eyes that accumulated necrotic spots (middle). Co-
1065 expression of $A\beta^{X2}$ with LanB1 rescued most of the size and organisation defects (right). **h**
1066 *Drosophila* laminins and their assembly in the ER. Created using BioRender.

Fig. 2 LanB1 did not affect A β RNA or protein levels. **a** A β mRNA was significantly up-regulated upon RU induction ($p < 0.0001$; one-way ANOVA with Tukey's post-hoc test)

1070 and was not affected by co-expression of LanB1. Data indicate the mean (n = 5 biological
1071 replicates per condition). **b** Total A β ₄₂ protein levels increased significantly over time with
1072 RU induction compared to uninduced controls (p < 0.0001, two-way ANOVA with Tukey's
1073 post-hoc test), and was unaffected by LanB1 co-expression. Data are shown as mean $\pm$
1074 SEM (n = 4 biological replicates per time point). **c** Western blot with quantification of
1075 soluble A β . Green arrow indicates the distinctive crescent shape of A β used for
1076 quantification. Soluble A β levels increased in induced conditions and was unaffected by
1077 LanB1 co-expression. Data indicate the mean (n = 4 biological replicates per condition). **d**
1078 After 3 weeks of induction, neither soluble nor insoluble A β levels were affected by LanB1
1079 co-expression. Data are shown as mean $\pm$ SEM (n = 7 biological replicates per condition).
1080 **e** After 3-weeks of RU, induced A β ^{Arc} flies consumed a significantly smaller amount of food
1081 over 7 days compared to uninduced controls (p < 0.0001, two-way ANOVA with Tukey's
1082 post-hoc test). The amount of food ingested by flies with a history of LanB1 and A β ^{Arc}
1083 induction was not significantly different to uninduced controls. Food consumption was
1084 measured for 10 flies per condition every 24-hr. Data are shown as mean $\pm$ SEM. **e**
1085 Expression of A β for the first 3 weeks of adulthood significantly reduced lifespan compared
1086 to uninduced controls (p = 4.07 x 10⁻¹²; log rank test). Three-week induction of A β and
1087 LanB1 together resulted in a small but significant lifespan extension compared to
1088 uninduced controls (p = 5.04 x 10⁻⁰⁶; log rank test).

Fig. 3 Intracellular accumulation of A β and LanB1. **a** A β accumulated intracellularly and did not co-localise with the nuclear marker histone H3:GFP. Arrow indicates non-nuclear expression of A β . **b** Similarly, LanB1 accumulated intracellularly, not in the nucleus, and partially overlapped with A β expression. Arrow indicates co-localisation of A β and LanB1. **a,b** Representative confocal fluorescence z projections taken at 20x magnification of whole brains from 21-day-old female flies stained with A β (6E10 – green) and DAPI (blue). Yellow box inset shows a single section of the same brain taken at 63x magnification. Endogenous fluorescence (i.e. without staining) of H3:GFP and LanB1:RFP is shown. H3:GFP has been false-coloured red to aid comparison. **c** Z-projection and **d** single section showing intracellular accumulation of A β in Dilp2 neurons. **e** Z-projection and **f,g** single sections showing intracellular accumulation of A β and LanB1 in Dilp2 neurons. LanB1 expression was not uniformly distributed through the cytoplasm of these cell bodies and appeared to accumulate in discrete intracellular compartments. Arrows highlight co-localisation of A β and LanB1, while arrowheads highlight areas with no overlap, often in the same cell. **c-g** Representative confocal images taken at 63x magnification of Dilp2 neurons from 21-day-old female flies stained with A β (6E10 – green) and DAPI (blue). Endogenous fluorescence (i.e. without staining) of LanB1:RFP is shown. Genotypes: **a**, *ElavGS>A β^{X2} + H3:GFP*; **b**, *ElavGS>A β^{X2} + LanB1:RFP*; **c,d**, *Dilp2-GS>A β^{Arc}* ; **e-g**, *Dilp2-GS>A β^{Arc} + LanB1:RFP*. Scale bar, 100 μ m for **a,b**, 10 μ m for **c-g**.

Fig. 4 LanB1 accumulates in the ER. **a** Z-projection and **b** single section showing neuronal expression of LanB1 with the ER marker, KDEL:GFP, using the ElavGS driver. **c** Magnified view of the dashed yellow box inset in **b**. LanB1 colocalizes with KDEL:GFP. **d** without RU486 induction, there was very little induction of LanB1 and/or KDEL:GFP

1114 expression. Representative confocal fluorescence images taken at 63x magnification from
 1115 7-day-old female flies. Images show the antennal lobe and surrounding brain regions.
 1116 Endogenous fluorescence (i.e. without staining) of KDEL:GFP and LanB1:RFP is shown.
 1117 Genotype: *ElavGS>KDEL:GFP + LanB1:RFP*. Scale bar, 50 μ m for **a,b,d**, 10 μ m for **c**.

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c

Fig. 5 Quantification of Aβ secretion from Dilp2 neurons. **a** Aβ expression in Dilp2 neurons was highest in the cell body area, but there was also diffuse, punctate staining of Aβ outside the cell bodies. LanB1 co-expression had no effect on Aβ fluorescence outside Dilp2 neurons. Without RU induction, there was no Aβ found in Dilp2 neurons or the

1123 surrounding area. Representative confocal fluorescence z projections taken at 63x
 1124 magnification of whole brains from 21-day-old female flies stained with A β (6E10 – green)
 1125 and DAPI (blue). Dashed yellow area indicates the area of fluorescence measurement.
 1126 White asterisk in the top row of images indicates strong staining of oesophageal muscle.
 1127 Endogenous fluorescence (i.e. without staining) of LanB1:RFP is also shown. **b**
 1128 Quantification of A β fluorescence outside Dilp2 neurons. There was a significant increase
 1129 in diffuse, punctate A β staining outside Dilp2 neurons when induced ($p = 0.048$; one-way
 1130 ANOVA). LanB1 co-expression had no effect on A β staining. Biological replicate numbers
 1131 for each condition are labelled within the bars. **c** Diagram of the proposed secretion of A β
 1132 from Dilp2 neurons into the extracellular space. Created with BioRender.com. Genotypes:
 1133 *Dilp2-GS>A β^{Arc}* ; and *Dilp2-GS>A β^{Arc} + LanB1:RFP*. Scale bar, 50 μ m.

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Fig. 6 Enhanced rescue of A β toxicity with combined laminin/collagen IV subunit over-expression. **a** LanB1 significantly rescued A β toxicity ($p = 2.85 \times 10^{-62}$; log rank). Cg25C significantly rescued A β toxicity ($p = 3.42 \times 10^{-63}$; log rank). There were small but significant extensions of lifespan in the uninduced controls (LanB1, $p = 0.036$; Cg25C, $p = 7.51 \times 10^{-6}$; log rank vs *ElavGS>A β ^{Arc}* alone). **b** LanB1 significantly rescued A β toxicity ($p = 8.04 \times 10^{-40}$; log rank). Cg25C significantly rescued A β toxicity ($p = 1.25 \times 10^{-35}$; log rank). **c** LanB1 significantly rescued A β toxicity ($p = 3.77 \times 10^{-33}$; log rank). Cg25C significantly rescued A β toxicity ($p = 2.24 \times 10^{-30}$; log rank). LanB1 + Cg25C had a partially additive effect on the rescue of A β ^{Arc} toxicity. Cox Proportional Hazard analysis showed a significant interaction between LanB1 and Cg25C ($p < 0.001$). **d** LanB1, Cg25C, and LanA rescued A β toxicity (LanB1, $p = 3.34 \times 10^{-65}$; Cg25C, $p = 2.11 \times 10^{-55}$; LanA, $p = 1.10 \times 10^{-32}$; log rank vs *ElavGS>A β ^{Arc}* alone). LanB1 + Cg25C, and LanB1 + LanA had a partially additive effect on the rescue of A β ^{Arc} toxicity. Cox Proportional Hazard analysis showed a significant interaction with LanB1 for Cg25C ($p < 0.001$) and LanA ($p = 0.022$). There was significant extension of lifespan in the uninduced controls (LanB1, $p = 6.45 \times 10^{-10}$; Cg25C, $p = 1.57 \times 10^{-11}$; LanA, $p = 1.40 \times 10^{-10}$; LanA+LanB1, $p = 1.66 \times 10^{-39}$; LanB1+Cg25C, $p = 0.026$; log rank vs *ElavGS>A β ^{Arc}* alone). Dashed lines represent uninduced 'RU-' controls,

1152 solid lines represent induced 'RU+' conditions. For all lifespan experiments n = 150 flies
1153 per condition.

Fig. 7 LanB1 rescue of A β toxicity is independent of the BiP/Xbp1 ER stress response pathway. **a** *BiP* mRNA was significantly upregulated upon A β induction ($p < 0.01$; one-way ANOVA with Tukey's post-hoc test) and this was not changed by the overexpression of

1158 LanB1:RFP. Data are shown indicating the mean (n = 4-5 biological replicates per
1159 condition). **b** Western blot of BiP protein levels, and quantification in **c** confirm that BiP
1160 protein is also significantly upregulated with A β induction and this is not changed by
1161 LanB1:RFP overexpression (p < 0.01; one-way ANOVA with Tukey's post-hoc test). Data
1162 are shown indicating the mean (n = 3-4 biological replicates per condition). **d** Co-
1163 expression of A β^{X2} with Xbp1^{VDRC} in the developing eye. Knockdown of Xbp1 significantly
1164 exacerbated A β toxicity and these flies exhibited very small and depigmented eyes. **e**
1165 Knockdown of Xbp1 exacerbated A β toxicity and significantly shortened lifespan compared
1166 to controls (p = 3.03 x 10⁻³⁰; log rank). **f** Knockdown of Xbp1 exacerbated A β toxicity and
1167 significantly shortened lifespan compared to controls (p = 1.68 x 10⁻⁴⁴; log rank). Over-
1168 expression of LanB1 rescued the shorter lifespan of Xbp1^{VDRC} (A β +LanB1:RFP+Xbp1^{VDRC}
1169 vs A β +Xbp1^{VDRC}, p = 9.13 x 10⁻²²; log rank). For display purposes, the control micrograph
1170 in **d** is the same as that in **Supplementary Fig. 5**. For all lifespan experiments n = 150
1171 flies per condition. 'VDRC' indicates an RNAi transgene. Genotypes: **a**, *ElavGS>A β^{Arc}* ;
1172 *ElavGS>A β^{Arc} + LanB1:RFP*; **b,c**, *NsybGS>A β^{Arc}* ; *NsybGS>A β^{Arc} + LanB1:RFP*.

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1174 **Fig. 8** Protein accumulation in the ER may be responsible for the rescue of Aβ toxicity. **a,b**
1175 Co-expression of Aβ^{X2} with LanB2:GFP or KDEL:GFP rescued Aβ toxicity in the
1176 developing eye compared to mCD8:GFP controls, while secr:GFP exacerbated Aβ toxicity.
1177 **b** Quantification of eye sizes in **a**. mCD8:GFP did not rescue the rough eye phenotype
1178 compared to Aβ^{X2} alone (see **Supplementary Fig. 5**). LanB2:GFP and KDEL:GFP
1179 significantly rescued eye size while secr:GFP significantly reduced eye size compared to
1180 mCD8:GFP controls ($p < 0.0001$; one-way ANOVA with Dunnett's post-hoc test). Data are
1181 shown as mean $\pm$ SEM ($n = 34-46$ eyes measured per condition). For display purposes,
1182 two micrographs in **a** are the same as those in **Supplementary Fig. 5**. **c** Survival curves of
1183 female flies induced to express Aβ^{Arc} via NsybGS. LanB2:GFP and KDEL:GFP rescued Aβ
1184 toxicity while secr:GFP exacerbated Aβ toxicity compared to mCD8:GFP controls
1185 (LanB2:GFP, $p = 2.37 \times 10^{-26}$; KDEL:GFP, $p = 3.49 \times 10^{-17}$; secr:GFP, $p = 1.79 \times 10^{-19}$; log
1186 rank). Genotypes: **a,b**, $GMR>A\beta^{X2} + mCD8:GFP$; $GMR>A\beta^{X2} + LanB2:GFP$; $GMR>A\beta^{X2} +$
1187 $KDEL:GFP$; $GMR>A\beta^{X2} + secr:GFP$. For all lifespan experiments $n = 150$ flies per
1188 condition.

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b

1190 **Fig. 9** Intra-ER retention of overexpressed Lamb1 is conserved in mouse brain tissue. **a**
1191 Organotypic hippocampal slice cultures from 10-day old mouse pups were incubated for
1192 24 hours with lentivirus containing *mLamb1*+GFP or control lentivirus (GFP only), and then
1193 fixed 2-weeks later. Slices were then stained for Lamb1 and the ER-marker, Calnexin.
1194 Endogenous GFP expression was used to identify successful transduction. DAPI labelled
1195 nuclei. Top 2 rows show examples of control lentivirus without *mLamb1* induction. Bottom
1196 2 rows show examples of lentiviral *mLamb1* induction. Yellow arrowheads indicate areas
1197 of colocalization of Lamb1 and Calnexin. **b** Summary model of intra-ER laminin retention.
1198 Created with BioRender.com. Scale bar, 10 μ m.

1199 **Supplementary Figures**

Supplementary Fig. 1 Uncovering a role for LanB1 in the amelioration of Aβ toxicity. **a** ECM-related transgenic lines were crossed with flies expressing pan-neuronal Aβ^{Arc} with the ElavGS driver. LanB1 co-expression significantly rescued the short-lifespan phenotype compared to the induced Aβ^{Arc}-alone control ($p = 2.86 \times 10^{-44}$; log rank test). **b** Additional Laminin transgenic lines were examined for their effect on Aβ toxicity. LanB1 co-expression significantly rescued toxicity compared to induced controls ($p = 1.08 \times 10^{-54}$; log rank test). Both independent LanB2 RNAi lines also exhibited significant rescue of Aβ toxicity (LanB2^{RNAi 1} $p = 7.94 \times 10^{-35}$; LanB2^{RNAi 2} $p = 4.13 \times 10^{-41}$ log rank test). **c** Induction of Aβ^{Arc} significantly ($p = 8.34 \times 10^{-67}$; log rank test) shortened lifespan compared to uninduced controls. LanB1 and Aβ^{Arc} co-expression resulted in a significant rescue ($p =$

1211 1.48 x 10⁻⁵⁶; log rank test) of the short-lived phenotype. **d** Expression of two copies of
 1212 wildtype Aβ₄₂ (Aβ^{X2}) in neurons. Aβ^{X2} significantly (p = 4.52 x 10⁻⁶⁸; log rank test)
 1213 shortened lifespan compared to uninduced controls. There was a significant difference
 1214 between uninduced control lines (p = 4.00 x 10⁻¹²; log rank test), but in the reverse
 1215 direction to the rescue. LanB1 and Aβ^{X2} co-expression resulted in a significant rescue (p =
 1216 6.47 x 10⁻¹⁵; log rank test). **e** Glial expression of Aβ was not toxic. Using a glial
 1217 GeneSwitch driver (GliaGS), flies with adult-onset glial Aβ expression had slightly
 1218 extended lifespan compared to uninduced controls (Aβ p = 0.0074; Aβ+LanB1 p = 0.0074;
 1219 log rank test). LanB1 co-expression did not affect this extension (comparison in induced
 1220 conditions p = 0.14; log rank test). For lifespan experiments n = 150 flies per condition.

Supplementary Fig. 3 Expression of *LanB1* and not *cdc14*, an adjacent gene in the opposite orientation, rescued A β toxicity. **a** Schematic of the *LanB1*^{EP-600} locus located 548

1234 bases upstream of the 5' UTR of the *LanB1* gene. The P{GSV1} element contains UAS
1235 sequences at both ends oriented outwards. **b** *LanB1* was significantly upregulated in the
1236 heads of UAS-*LanB1*^{EP-600} and UAS-*LanB1*:RFP flies compared to uninduced controls ($p <$
1237 0.0001 ; one-way ANOVA with Tukey's post-hoc test). **c** *Cdc14* was significantly up-
1238 regulated in the heads of UAS-*LanB1*^{EP-600} and in UAS-*cdc14* (*cdc14*^{EY10303}) heads
1239 compared to uninduced controls ($p < 0.0001$; one-way ANOVA with Tukey's post-hoc test).
1240 *Cdc14* up-regulation was not observed in UAS-*LanB1*:RFP heads. Mild up-regulation in
1241 uninduced UAS-*cdc14* heads may be due to compensatory up-regulation as the
1242 *cdc14*^{EY10303} P-element is inserted 395 bases into the 5' UTR of *cdc14*. Relative qPCR
1243 values were normalized to *rp49* expression. Data are shown as mean $\pm$ SEM ($n = 4$
1244 biological replicates per condition). **d** Co-expression of $A\beta^{X2}$ with *LanB1* (via UAS-
1245 *LanB1*^{EP-600} and UAS-*LanB1*:RFP) using the eye-specific GMR-GAL4 driver rescued most
1246 of the size and organisation of the eye. *Cdc14* did not rescue $A\beta^{X2}$ toxicity. For display
1247 purposes, three micrographs in **d** are the same as those in **Fig 1a**. **e** Quantification of eye
1248 sizes in **d**. There was no significant difference in eye size between $A\beta^{X2}$ alone or with
1249 *cdc14* co-expression. *LanB1* (via UAS-*LanB1*^{EP-600} and UAS-*LanB1*:RFP) significantly
1250 rescued eye size ($p < 0.0001$; one-way ANOVA with Tukey's post-hoc test). Data are
1251 shown as mean $\pm$ SEM ($n = 30$ eyes measured per condition). **f** The considerable rescue
1252 from $A\beta$ toxicity by *LanB1*^{EP-600} was due to *LanB1* and not *cdc14*. Although co-expression
1253 of cytoplasmic GFP (BDSC #1521) or *cdc14* with $A\beta^{Arc}$ led to small but significant rescues
1254 of lifespan compared to $A\beta^{Arc}$ alone controls (cytoplasmic GFP, $p = 2.44 \times 10^{-12}$; *cdc14*, $p =$
1255 2.64×10^{-6} log rank test), *LanB1* and $A\beta^{Arc}$ co-expression (via UAS-*LanB1*^{EP-600} and UAS-
1256 *LanB1*:RFP) exhibited equivalently large rescues of $A\beta$ toxicity (*LanB1*^{EP-600}, $p = 1.39 \times 10^{-$
1257 61 ; *LanB1*:RFP, $p = 2.85 \times 10^{-62}$ log rank test). $n = 150$ flies per condition.

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1259 **Supplementary Fig. 4** Inducible expression of A β and LanB1 in Dilp2 neurons using the
1260 Dilp2-GS driver. Without RU induction, there was no induction of A β and/or LanB1
1261 expression. When fed RU, A β and LanB1 were expressed. Representative confocal
1262 fluorescence z projections taken at 20x magnification of whole brains from 21-day-old
1263 female flies stained with A β (6E10 – green) and DAPI (blue). Yellow box inset shows a
1264 higher magnification of the Dilp2 neuron cluster area in the dorsal brain. Endogenous
1265 fluorescence (i.e. without staining) of LanB1:RFP is shown. Genotypes: *Dilp2-GS>A β^{ARC}* ;
1266 and *Dilp2-GS>A β^{ARC} + LanB1:RFP*. Scale bar, 50 μ m.

1268 **Supplementary Fig. 5** Laminin-induced rescue of A β toxicity in the developing eye is not
1269 affected by modulation of other laminin subunits. **a** All flies shown expressed A β^{X2} using
1270 GMR-GAL4. The degree of rescue from LanB1 over-expression (via UAS-LanB1^{EP-600} and
1271 UAS-LanB1:RFP) was not affected by expression of the indicated genes. **b** Quantification
1272 of eye sizes in **a**. Eye size for each condition was quantified and compared to the
1273 *ElavGS>A β^{X2} /w^{Dah}* control. Over-expression or knockdown of other Laminin subunits in
1274 combination with LanB1 had no significant effect on the rescue. Conditions marked 'ns'
1275 were not significantly different compared to controls, while the other lines were significantly
1276 different (one-way ANOVA with Tukey's post-hoc test). Plus (+) symbol indicates presence
1277 of labelled transgene. Bars containing red indicate presence of a LanB1 over-expression
1278 line. Dashed grey line indicates the A β control, while dashed red line indicates the LanB1-
1279 rescue level. 'TRiP' indicates an RNAi transgene. Data are shown as mean $\pm$ SEM (n =
1280 34-62 eyes measured per condition).

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Supplementary Fig. 6 Live 2-photon imaging of a fly producing LanB2:GFP in Dilp2 neurons. LanB2 accumulated intracellularly in discrete compartments. *Top*, dorsal view of fly with cuticle removed to visualize the brain. To prevent dehydration, saline gel was added with a small coverslip on top. *Middle*, closer dorsal view of live fly brain. *Bottom left*, z-projection of the cell bodies of the Dilp2 neurons expressing LanB2:GFP. *Bottom right*, single slice of these cell bodies showing LanB2 accumulated in discrete cellular compartments and not diffusely in the cytoplasm. Arrows show discrete intracellular accumulations of LanB2:GFP. Genotype: *Dilp2-GS>LanB2:GFP*. In all images anterior is left, posterior is right. Scale bar, top 500 μm , middle 100 μm , bottom, 20 μm .

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Supplementary Fig. 7 Aβ does not accumulate in the ER. Inducible expression of Aβ and the ER marker, KDEL:GFP, in Dilp2 neurons using the Dilp2-GS driver. *Top two rows*, when fed RU, Aβ and KDEL:GFP were present in the cell bodies of Dilp2 neurons. Arrows

1295 highlight areas with no overlap of A β and KDEL:GFP. Arrowheads highlight punctate areas
 1296 of ER with no corresponding A β expression. *Bottom two rows*, without RU486 induction,
 1297 there was no induction of A β and/or KDEL:GFP expression. Representative confocal
 1298 fluorescence z projections taken at 63x magnification from 21-day-old female flies stained
 1299 with A β (6E10 – green). Endogenous fluorescence (i.e. without staining) of KDEL:GFP is
 1300 shown. Genotype: *Dilp2-GS>A β^{Arc} + KDEL:GFP*. Scale bar, 10 μ m.

Supplementary Fig. 8 Laminin γ -chain (LanB2) also rescued A β toxicity. **a** LanB1, Cg25C, and LanA rescued A β toxicity (LanB1, $p = 2.85 \times 10^{-62}$; Cg25C, $p = 3.43 \times 10^{-63}$; LanA, $p = 1.58 \times 10^{-17}$; log rank vs *ElavGS>A β ^{Arc}* alone). There were significant extensions of lifespan in the uninduced controls (LanB1, $p = 0.036$; Cg25C, $p = 7.50 \times 10^{-66}$; LanA, $p = 7.33 \times 10^{-66}$; log rank vs *ElavGS>A β ^{Arc}* alone). **b** LanB2 and A β ^{X2} co-expression using *NsybGS* resulted in a significant rescue ($p = 1.00 \times 10^{-20}$; log rank test) compared to induced A β ^{X2} controls. For all lifespan experiments $n = 150$ flies per condition.