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Angiotensin Converting Enzyme Inhibitors and Angiotensin Receptor Blockers rescue memory defects in *Drosophila* expressing Alzheimer's disease-related transgenes independently of the canonical Renin Angiotensin System

Shin-Hann Lee[1,2*], Sarah M. Gomes[1,3*], Judy Ghalayini[1,2], Konstantin G. Iliadi[1] and Gabrielle L. Boulianne[1,2,3]

1. Program in Developmental & Stem Cell Biology, The Hospital for Sick Children, M5G 0A4
2. Department of Molecular Genetics, University of Toronto, M5S 1A1
3. Institute of Medical Science, University of Toronto, M5S 1A1

Corresponding author: Gabrielle L. Boulianne, Program in Developmental & Stem Cell Biology, Peter Gilgan Centre for Research and Learning, The Hospital for Sick Children, 686 Bay Street, Toronto, ON, M5G 0A4, gboul@sickkids.ca

*These authors contributed equally to this work.

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Conflict of Interest

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Abstract

Alzheimer's disease (AD) is a degenerative disorder that causes progressive memory and cognitive decline. Recently, studies have reported that inhibitors of the mammalian renin angiotensin system (RAS) result in a significant reduction in the incidence and progression of AD by unknown mechanisms. Here, we used a genetic and pharmacological approach to evaluate the beneficial effects of Angiotensin Converting Enzyme Inhibitors (ACE-Is) and Angiotensin Receptor Blockers (ARBs) in *Drosophila* expressing AD-related transgenes. Importantly, while ACE orthologs have been identified in *Drosophila*, other RAS components are not conserved. We show that captopril, an ACE-I, and losartan, an ARB, can suppress a rough eye phenotype and brain cell death in flies expressing a mutant human C99 transgene. Captopril also significantly rescues memory defects in these flies. Similarly, both drugs reduce cell death in *Drosophila* expressing human Aβ42 and losartan significantly rescues memory deficits. However, neither drug affects production, accumulation or clearance of Aβ42. Importantly, neither drug rescued brain cell death in *Drosophila* expressing human Tau suggesting that RAS inhibitors specifically target the amyloid pathway. Of note, we also observed reduced cell death and a complete rescue of memory deficits when we crossed a null mutation in *Drosophila Acer* into each transgenic line demonstrating that the target of captopril in *Drosophila* is Acer. Altogether, these studies demonstrate that captopril and losartan are able to modulate AD related phenotypes in the absence of the canonical RAS pathway and suggest that both drugs have additional targets that can be identified in *Drosophila*.

Significance Statement

AD is a devastating neurodegenerative disorder for which there is no cure. Recently, studies have reported a significant reduction in the incidence of AD and dementia among patients taking ACE-Is and ARBs. Given the enormous and immediate potential of ACE-Is and ARBs for AD therapeutics, it is imperative that we understand how they function and why they are beneficial in some patients but not others. Here we show that captopril, an ACE-I, and losartan, an ARB, can restore memory defects in flies expressing human AD transgenes in the absence of the canonical RAS pathway. These studies provide us with a unique opportunity to identify novel targets of ACE-Is and ARBs and evaluate their therapeutic effectiveness in robust models of AD.

Introduction

Alzheimer's disease (AD) is a degenerative disorder of the central nervous system that causes progressive memory and cognitive decline during mid to late adult life. Mutations in three genes, *APP*, *presenilin 1* and *presenilin 2* (*PS1* and *PS2*), cause early-onset autosomal dominant AD, which accounts for less than 5% of familial AD cases (Goate *et al*., 1991). APP encodes a single-pass transmembrane protein that is cleaved by two proteases, β-secretase and γ-secretase, to generate amyloid peptides. PSs encode the catalytic component of γ-secretase (Wolfe *et al*., 1999), which cleaves the C-terminal fragment of APP (APP-CTF, C99) to produce Aβ peptides. Generally, longer Aβ peptides (Aβ42) are prone to self-aggregation and are concentrated in amyloid plaques, which are associated with brain atrophy, regional hypometabolism, network dysfunction, inflammation, and oxidative stress (Holtzman, 2011). Therefore, Aβ42 and plaques are often used as a diagnostic tool for AD prognosis and progression (Hansson *et al*., 2007; Lewczuk *et al*., 2015).

Recently, biochemical studies have shown that additional proteins can associate with PS and γ-secretase to modulate its assembly and/or interaction with specific targets (Bursavich *et al*., 2016; Tan *et al*., 2016). Proteins that modulate γ-secretase assembly would provide valuable insight into the function of this important complex during development and disease. Similarly, proteins that modulate the interaction of γ-secretase with specific targets such as APP, or affect the production of Aβ peptides or their clearance, might allow for the development of new therapeutic targets for AD. Although extremely promising, only a few PS and γ-secretase modulators have been identified and their mechanism of action remains largely unknown.

Using a genetic approach in *Drosophila*, we previously identified *Acer* and *Ance-5,* two orthologs of human angiotensin converting enzyme (ACE), as modifiers of PS and C99 (van de Hoef *et al*., 2009). ACE is a metalloprotease that cleaves angiotensin 1, a major component of the renin-angiotensin system (RAS) that regulates blood pressure in humans. Importantly, while ACE orthologs

have been identified in *Drosophila*, other components of the RAS are not conserved. Interestingly, several studies have established a link between RAS-targeting anti-hypertensive drugs, such as ACE-Is and ARBs, and AD (Ohrui *et al*., 2004; Davies *et al*., 2011; Abdalla *et al*., 2013; Qiu *et al*., 2013; Yasar *et al*., 2013; de Oliveira *et al*., 2014; Wharton *et al*., 2015). For example, both ACE-Is and ARBs have been shown to delay the onset of cognitive impairment and neurodegeneration in mouse models of AD and in some patients, although the mechanism of action remains unclear (Alvarez *et al*., 1999; Ohrui *et al*., 2004; Hajjar *et al*., 2005; Edwards *et al*., 2009; Miners *et al*., 2009; Belbin *et al*., 2011; Qiu *et al*., 2013; Soto *et al*., 2013; Yasar *et al*., 2013; de Oliveira *et al*., 2014; Kauwe *et al*., 2014; O'Caoimh *et al*., 2014; Wharton *et al*., 2015; Ho *et al*., 2017).

Here, we have examined the effects of ACE-Is and ARBs in *Drosophila* that express human AD-related transgenes. We show that captopril, an ACE-I and losartan, an ARB, suppress a rough eye phenotype and cell death in the brains of flies expressing a human C99 transgene carrying a London mutation. Moreover, captopril significantly rescues memory deficits in these flies. Similarly, both drugs reduce cell death and losartan significantly rescues memory deficits in *Drosophila* expressing human Aβ42. Importantly, neither drug affects the levels or clearance of Aβ42. We also observed no effects of either drug on degenerative phenotypes observed in *Drosophila* expressing human Tau suggesting that the beneficial effects are specific to APP-CTF and Aβ42 expressing flies. Importantly, we found that an *Acer* null mutant was able to rescue cell death and memory deficits in *Drosophila* expressing Aβ42 consistent with Acer being the target of captopril in *Drosophila*. However, since the downstream targets of Acer including angiotensin and the angiotensin receptor are not conserved, we could not use a similar approach to identify the target/s of losartan. Altogether, these studies demonstrate that captopril and losartan are able to modulate AD related phenotypes in *Drosophila*. Moreover, since

these beneficial effects are observed in the absence of the canonical RAS, these studies suggest that captopril and losartan may have additional targets that can be identified in *Drosophila*.

Materials and Methods

Drosophila stocks. Stocks and crosses were maintained on standard media with or without drug treatment at 29°C for eye models and at 25°C for CNS models with 65% relative humidity and a 12/12-h light/dark cycle. *gmr-GAL4;UAS-mCD8GFP/SM5CyO* recombinant line was generated as described (Burr *et al*., 2014) (referred to as *gmr-GAL4-UAS-GFP*). *UAS-APP*^{C99J4}, *UAS-APP*^{C99J6} (referred to as UAS-*C99*^{wt}) and *UAS-APP*^{C99V717I} London mutation (referred to as UAS-*C99*^{V717I}) have been previously described (Finelli *et al*., 2004). *elav-GAL4/CyO* (8765), *elav-GAL4*^{C155} (458), *UAS-APP*$^{Abeta42.B}$ (33769) (referred to as *UAS-Aβ42*), *UAS-Tau*$^{wt1.13}$ (51362) (expresses the 2N4R isoform of human Tau referred to as *UAS-Tau*), *w*^{1118} and *Canton-S* (referred to as wt) were obtained from the Bloomington Stock Center. The *Acer* null allele (*Acer*^{A168}) was obtained from (Carhan *et al*., 2011) and crossed to *elav-GAL4*^{C155}, *UAS-APP*^{C99V717I} and *UAS-Aβ42* flies to generate fly lines expressing AD-related transgenes with an *Acer* null mutation. *elav-GAL4*^{C155} driver was used instead of *elav-GAL4/CyO* for *Acer* null-related experiments for the purpose of generating a homozygous *Acer* null mutation.

Drug Treatments. All adult flies were maintained on standard media with or without addition of either captopril (5mM) (Sigma Aldrich, Oakville, ON) or losartan (1mM) (U.S. Pharmacopeial Convention, Rockville, MD) from the first day after eclosion (DAE=0).

GFP and REP Imaging. Heads from 7-day old adults were removed using spring scissors and slide mounted using double-sided tape. Heads were imaged at room temperature using a confocal Leica TCS

159 SP5 microscope (Leica Microsystems Inc., Concord, ON), with 20X objective and standard GFP filters
160 with Leica Application Suite (LAS X) software (Leica Microsystems Inc., Concord, ON). Images were
161 processed using ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda,
162 Maryland, USA, http://imagej.nih.gov/ij/, 1997-2016.). GFP expression was analyzed using Corrected
163 Total Cell Fluorescence (CTCF) calculations (based on Burgess *et al*., 2010). Rough eye phenotype
164 images were captured with a 4X objective using a Nixon SMZ-2T light microscope (Japan) and an
165 OptixCam Summit K2 microscope (Roanoke, VA) camera with ToupView software (by ToupTek
166 Photonics, China).
167
168 ***TUNEL labelling.*** Brains from 28-day old adults were dissected in cold PBS with 0.5% TritonX-100
169 and fixed in 4% PFA at room temperature for 30 minutes. Brains were then rinsed twice in PBS with
170 0.5% TritonX-100 for 10 minutes each and washed once in H_2O plus 0.5% TritonX-100 and 0.1%
171 Sodium Citrate solution for 15 minutes at 4°C followed by two washes in PBS with 0.5% TritonX-100
172 for 10 minutes each. TUNEL staining was performed according to the manufacturer instructions
173 (Roche, *in situ* cell death detection kit, Cat# 11684795910). Images were captured as a Z-stack and
174 compressed into a single image using a Nikon A1R confocal microscope. Cell death was manually
175 counted for statistical analysis.
176
177 ***Courtship Conditioning Assay.*** All experiments and analyses were performed double-blind as
178 previously described (Kamyshev *et al*., 1999). Experimental flies were collected within 6h after
179 eclosion and kept individually in culture vials on standard media with or without drugs (captopril or
180 losartan) for 28 days until the experiment was performed. One day before the experiment, 3-5 day old
181 *Canton-S* virgin females were mated with same age males. Mated females were then used for training
182 and testing. All behavioral experiments were performed within a 3-hour time window (between 16:00 –

19:00 h) in an environmental control room. Male courtship behavior was observed in a custom-made Perspex chamber (15mm diameters, 5mm high) with a sliding opaque partition that divided the chamber into two halves, with two lateral entries (3 mm diameter) with stoppers. Before training or testing, each chamber was cleaned with 50% ethanol and dried. For training, a naïve male (with no sexual experience) was placed into an experimental chamber together with a 5-day-old mated *Canton S* female. After several minutes to recover from the transfer the divider was withdrawn and the flies were left together for 1 hour. After training, an experimental male was isolated for 30 minutes and then tested for short-term memory performance with a mated female during 10 minutes. Courtship behavior during the test session was video-recorded using a color camera (EverFocus EQ.610, Polistar II) that was fitted with a CCTV lens (Computar, VariFocal TG4Z2813 FCS-IR) and fixed on a mounting bracket about 50 cm above the chamber. The distance of the camera to the object as well as the zoom, focus and iris aperture were optimized for video-recording. Subsequent video analysis of time spent performing courtship behavior and all statistical comparisons were done using computer software (*Drosophila* Courtship Lite 1.4, developed by N.G. Kamyshev, Russian Academy of Science). Courtship Index (CI) was defined as the percentage of time spent performing courtship behavior during the observation period. Memory Index (MI) was calculated as: [100(1-(CI with training/Mean of CI without training)] (Kamyshev *et al*., 1999; Lim *et al* ., 2018).

***Western Blots*.** Ten heads (5 male, 5 female) from 7 and 28-day old adults were lysed in 2X Tricine Sample Buffer (Bio-Rad Cat# 1610739), boiled for 5 minutes and run on 16.5% Tris-Tricine gels (Bio-Rad Cat# 4563066) with 1X SDS/Tris/Tricine Running Buffer (Bio-Rad Cat#1610744). Protein was transferred onto 0.2 µm nitrocellulose membranes (Bio-Rad Cat# 1620168) using standard transfer buffer. Membranes were boiled 3 minutes in 1X PBS then blocked for 1 hour using 1X TBST with 5% skim milk. Primary antibody detection was done overnight at 4°C using Aβ-6E10 (1:500) (Biolegend

Cat# 803001) and anti-α-Tubulin (1:1000) or anti-β-actin (1:1000) in 1X TBST 5% skim milk. Membranes were washed 3X in 1X TBST for 10 minutes each. Secondary antibody detection was done using anti-mouse-HRP for 2 hours at 4°C (1:10,000). Membranes were then washed 3X in 1X TBST for 10 minutes each. Signal was detected using chemiluminesence substrates (BioRad Cat# 1705060) and membranes were imaged using LI-COR Odyssey Fc imager.

ELISA Assays. Aβ42 peptide levels were determined using human Aβ specific ELISA kits (Invitrogen, Cat# 3441) as per manufacturer's instructions. Forty heads from 28-day old CNS models maintained at 25°C were lysed in 1x RIPA buffer with a complete protease inhibitor (Roche) containing 50mM Tris, 150mM NaCl, 1% SDS, 1% NP-40 and 0.5% sodium deoxycholate, pH 8.0. The homogenates were diluted 2-fold before loading onto the plate. The signals were measured at 450 nm using a microplate reader. The whole experiment was performed as described previously (Van de Hoef *et al*., 2009).

Plaque staining

Flies expressing *Aβ42* in the CNS were maintained on standard media with or without drugs (captopril or losartan) for 28 days post eclosion and subjected to plaque staining using the amyloid specific luminescent conjugated oligiohiophene (LCO), p-FTAA, as previously described (Jonson *et al*., 2018). Fly brains were dissected in cold PBS and fixed in 96% ethanol for 10 minutes. Samples were then rehydrated following a step wash with 70%, 50%, 0% ethanol, then washed with PBS and stained with p-FTAA diluted 1:1000 in PBS for 30 minutes. After incubation with p-FTAA, samples were washed in PBS and mounted using DAKO mounting medium. Z-stack images of whole brains were acquired using a Sp8 confocal microscope and images were analyzed using volocity software. Levels of amyloid deposits were determined by measuring total pixel count over set threshold across z-stacks.

Statistics. Statistical analyses were done using GraphPad Prism or SPSS. Two-tailed Student's *t* test was used to analyze differences between two groups. One-way ANOVA with Bonferroni *post hoc* analysis was used for multiple comparisons. Kruskal-Wallis ANOVA followed by Dunn's multiple comparisons *post hoc* test were used for non-parametric analyses. Data are graphically reported as mean ± SEM. Kruskal-Wallis ANOVA test followed by Dunn's multiple comparisons test and Mann-Whitney U test were used for statistical comparisons for the courtship conditioning assay. Data are graphically reported as mean/median and the Box-and-whisker plots for CIs show 10^{th}, 25^{th}, 75^{th} and 90^{th} percentiles. MIs are shown as mean ± SEM.

Results

Characterization of $C99^{wt}$, $C99^{V717I}$ and $A\beta42$ phenotypes

To determine whether pharmacological inhibition of the RAS pathway using ACE-Is and ARBs can exert any beneficial effects in fly models of AD, we used the *GAL4-UAS* system to target expression of human AD related transgenes in the compound eye and CNS of *Drosophila* (Brand & Perrimon, 1993). Previous studies have shown that expression of these transgenes in the compound eye results in a rough eye phenotype, characterized by changes in the size of the eye that can be due to changes in photoreceptor neurons, loss of interomatidial bristles and pigmentation, and necrotic tissue (Prübing *et al*., 2013; Iyer *et al*., 2016). Expression of AD related transgenes in the CNS has also been shown to lead to Aβ aggregation, plaque formation, neurodegeneration, shortened lifespan and deficits in learning and memory (Ye *et al*., 1999; Finelli *et al*., 2004; Greeve *et al*., 2004; Iijima *et al*., 2004; Iijima *et al*., 2008; Chakraborty *et al*., 2011; Prüßing *et al*., 2013).

253 To quantitate the rough eye phenotype generated by expression of human AD related
254 transgenes, we crossed each UAS-transgenic line with flies expressing membrane bound UAS-GFP to a
255 *gmr-GAL4* driver that targets expression in the developing eye. In previous studies, GFP intensity has
256 been shown to be negatively correlated with retinal cell death (Burr *et al.,* 2014). We found that
257 expression of both *gmr>C99*^{V717I} and *gmr>Aβ42* resulted in a significant decrease in mean GFP
258 intensity (46.67 ± 2.96% and 40.32 ± 3.39%, respectively) compared to a driver-control (97.82 ±
259 4.22%) (Figure 1) while expression of *gmr>C99*^{wt} showed intermediate levels of GFP intensity (73.01
260 ± 4.15 %) compared to controls (Figure 1).
261
262 We also examined the pathological effects associated with expression of human AD transgenes
263 in the CNS using the pan-neuronal *elav-GAL4* driver (Figure 2). We first examined brain cell death
264 using TUNEL analysis and found that expression of *elav>C99*^{V717I} or *elav>Aβ42* resulted in a
265 significant increase in cell death within the adult brain (11.5 ± 1.6 and 11.8 ± 0.7, respectively)
266 compared to that observed in flies expressing *elav>C99*^{wt} or *wt* (2.3 ± 0.7 and 0.6 ± 0.4, respectively)
267 (Figure 2A, B). These results are consistent with previously reported data (Finelli *et al*., 2004; Iijima *et*
268 *al*., 2004; Iijima *et al*., 2008; Chakraborty *et al*., 2011; PruBing *et al*., 2013). We also examined
269 memory performance using a conditioned courtship suppression paradigm (Siegel and Hall, 1979;
270 Kamyshev *et al*., 1999). This associative learning paradigm is based on the observation that previous
271 unsuccessful attempts at courtship in males reduces subsequent courtship activity towards new females.
272 Courtship index (CI) is the fraction of time a male spends in courtship behavior during the observation
273 period. Kruskal-Wallis ANOVA test did not show any significant difference among naïve males from
274 all experimental groups (H: (3, N= 104) =2.39 p=0.5014), demonstrating that the sexual activity of
275 these males was equal. Both *elav>C99*^{wt} and *elav>C99*^{V717I} as well as *elav>Aβ42* males showed no

significant decrease in courtship activity compared to their naive counterparts (*elav>C99*^{wt} CI_{naive} =33.133 vs $CI_{trained}$ = 17.194 U=196.5, p=0.0891; *elav*>C99^{V717I} CI_{naive} =32.650 vs $CI_{trained}$ = 14.189, U=175, p=0.0504; *elav>AB42* CI_{naive} =38.889 vs $CI_{trained}$ = 29.487 U=333.5, p=0.1252) while *elav>W*^{1118} driver-control males showed a significant decrease in courtship activity (*elav>W*^{1118} CI_{naive} =33.340 vs $CI_{trained}$ = 3.704, U=130, p<0.0001) (Figure 2C).

Since all tests for trained males were done in the span of 30 minutes after a 1-hour training session it can be defined as a test for short-term memory (STM) performance (Kamyshev *et al*., 1999; McBride *et al*., 2005). The difference between CIs of trained and naïve males can be represented as a memory index (MI) (Kamyshev *et al*., 1999; Lim *et al*., 2018). Kruskal-Wallis ANOVA test revealed significant differences in memory performance between driver control line and transgenic lines (H: (3, N= 107) =19.09 p< 0.001). We found that males expressing *elav>C99*^{V717I} and *elav>Aβ42* transgenes showed a significant loss in STM compared to *elav>W*^{1118} driver control line (p<0.05 and p<0.001, respectively). However, it has to be noted that males expressing wild type C99 also exhibited a reduction in STM performance, although this difference was not statistically significant (Figure 2D).

Altogether these data suggest that expression of *Aβ42* either in fly eyes (*gmr-GAL4*) or pan-neuronally (*elav-GAL4*) produced the most pathological phenotypes while expression of the London mutation C99^{V717I} generally produced more severe phenotypes compared to wild type C99. Thus, our results support previous findings (Finelli *et al*., 2004; Iijima *et al*., 2004; Iijima *et al*., 2008; Chakraborty *et al*., 2011; PruBing *et al*., 2013) and provide us with models to evaluate the effect of RAS inhibitors on the development of AD-related phenotypes.

Captopril and Losartan suppress degenerative phenotypes observed in mutant *C99^{V717I}* and *Aβ42* flies

To determine whether captopril or losartan could suppress the rough eye phenotype observed in *Drosophila* expressing AD-related transgenes, we raised flies on medium with and without drugs and examined GFP intensity as described (Figure 1). We did not observe any effect of either drug on GFP intensity in flies expressing *C99^{wt}* or *Aβ42* (Figure 3). In contrast, *gmr>C99^{V717I}* flies exhibited significant increases in retinal GFP expression (26% and 41%, respectively) after administration of either captopril or losartan. Similarly, both drugs significantly reduced the number of TUNEL-labeled brain cells in 4-week old *elav>C99^{V717I}* flies (Figure 4). Moreover, a similar effect was observed in *elav>Aβ42* flies that were fed with losartan for 28 days whereas *elav>C99^{wt}* flies showed no differences in TUNEL-labelled brain cells regardless of drug condition (Figure 4).

Altogether, these data demonstrate that known inhibitors of the RAS signaling pathway in humans (captopril and losartan) can suppress toxic phenotypes observed in the eye and CNS of flies expressing AD-related transgenes.

Captopril and Losartan selectively rescue STM in mutant *C99^{V717I}* and *Aβ42* flies

To determine whether captopril or losartan could restore cognitive function in our AD models we examined short-term memory (STM) using the courtship conditioning paradigm described in Figure 2 (Siegel and Hall, 1979; Kamyshev *et al*., 1999). Since lack of courtship activity in naive males may significantly skew the results of courtship conditioning, we first analysed the potential differences in male sexual activity among naïve males of different genotypes and drug conditions. A two-way ANOVA did not reveal any significant effects for genotype ($F_{(3, 272)} = 0.624$, $p = 0.599$), drug condition ($F_{(2, 272)} = 0.577$, $p = 0.563$) or their interaction ($F_{(6, 272)} = 0.668$, $p = 0.596$). Courtship and memory

indices for all comparable groups are shown (Figure 5; note that we have also included the data from Figure 2 for "no drug" condition for comparative purposes). We found that administration of either drug (captopril or losartan) did not significantly change 30-minute short-term memory in the *elav>W*^{1118} control flies (Figure 5), whereas for the transgenic lines these drugs exert a selectiv effect. Administration of both drugs in these flies resulted in an increased MI, similar to that observed in *elav>W*^{1118} controls. However due to large variance within the *elav>C99*^{wt} expressing flies the multiple comparison test revealed statistical significance only for losartan. Opposite effect was observed in flies expressing *elav>C99*^{V717I}, captopril shows a significant memory improvement while losartan does not. *elav>Aβ42* flies showed obvious increase of MI in response to both drugs, althouth only for losartan the effect was statistically significant (Figure 5). Overall, these data demonstrate that known inhibitors of the RAS pathway in humans, can significantly improve memory performance in *Drosophila* expressing AD-related transgenes.

Captopril and Losartan do not suppress degenerative phenotypes observed in *Tau* flies

To determine whether captopril and losartan exert beneficial effects in other forms of AD, we examined their ability to suppress brain cell death in flies expressing human Tau protein. Previous studies have shown expression of human Tau in animal models leads to several neurodegenerative phenotypes similar to human AD cases including an increase in cell death, shrinkage in brain size and defects in cognitive ability (Wittmann *et al*., 2001; Gistelinck *et al*., 2012). We found that neither drug affected the number of TUNEL-labelled brain cells when maintained on either captopril or losartan for 28 days in *elav>Tau* flies (Figure 6) suggesting that the beneficial effects of RAS inhibitors are specific to APP-CTF and Aβ42 expressing flies.

Captopril and Losartan do not affect APP-CTF or *Aβ42*

Previous studies have suggested that ACE-Is may be beneficial in AD by regulating the production, degradation, conversion and/or clearance of Aβ peptides. Whether ARBs have similar effects is unknown. To determine whether the beneficial effects of RAS inhibitors on brain cell neurodegeneration and STM in our AD-related transgenic flies occur through similar mechanisms we first used Western Blot to quantitate the levels of C99 in the presence or absence of drugs. We found that administration of either captopril or losartan throughout the adult lifespan of both $C99^{wt}$ and mutant $C99^{V717I}$ flies had no effects on the levels of C99 in either fly eyes (*gmr-GAL4* driver) or in the central nervous system (*elav-GAL4* driver) (Figure 7A, B respectively).

We then asked whether captopril or losartan affect the levels of Aβ peptides by measuring the soluble Aβ42 levels from lysates of adult fly heads using Western Blot and enzyme-linked immunosorbent assay (ELISA). We found that administration of either captopril or losartan throughout the adult lifespan of *gmr>*$C99^{V717I}$ and *gmr>Aβ42* flies had no effect on the levels of Aβ42 at 7 days post eclosion (Figure 8A). Similarly, neither drug had significant effect on the levels of Aβ42 in *elav>Aβ42* flies at 28 days post eclosion (Figure 8B); Aβ42 was undetected in both *elav>*$C99^{wt}$ and mutant *elav>*$C99^{V717I}$ regardless of drug treatment. To examine the effects of both drugs on insoluble Aβ42, we measured and compared Aβ aggregates in the brains of *elav>Aβ42* flies with or without drug treatment using the amyloid-specific LCO, p-FTAA stain, to detect Aβ plaques at 28 days post eclosion. Comparison across different conditions revealed no significant changes (Figure 9). Altogether, these results suggest that the beneficial effects of captopril and losartan are independent of APP-CTF processing or accumulation/clearance of Aβ42.

A null mutation in *Drosophila Acer* recapitulates the beneficial effects of captopril in $C99^{V717I}$ and *Aβ42* flies

To determine whether components of RAS underlie the beneficial effects of captopril (ACE-I) in our *Drosophila* AD models, we obtained an *Acer* null mutant (Carhan *et al*., 2011) and recombined it with our AD transgenic lines $elav\text{-}GAL4^{C155}>UAS\text{-}C99^{V717I}$ or $elav\text{-}GAL4^{C155}>A\beta42$. $elav\text{-}GAL4^{C155}$ driver was used instead of *elav-GAL4/CyO* for genetic recombination purposes and generated flies expressing $C99^{V717I}$ or *Aβ42* in a homozygous *Acer* null background. Since $elav\text{-}GAL4^{C155}$ endogenously drives expression of GAL4 at higher levels, the phenotypes observed in our transgenic lines were more severe than those previously observed using *elav-GAL4/CyO*, which expresses GAL4 at lower levels. Of note, although there are several ACE homologs in *Drosophila*, we focused on *Acer* since previous studies have shown that it contains the N-terminal catalytic site observed in human ACE and can be inhibited by captopril *in vitro* (Houard *et al*., 1998). We found that a null mutation in *Acer* significantly reduced brain cell death in both 4-week old $elav^{C155}>C99^{V717I}$ and $elav^{C155}>A\beta42$ flies similar to what we observed after captopril treatment (Figure 10A, B). Similarly, an *Acer* null mutation also rescued STM in both 4-week old $elav^{C155}>C99^{V717I}$ and $elav^{C155}>A\beta42$ flies ($p<0.0001$; $p=0.0001$, respectively compared to no drug treatment) (Figure 11). Importantly, we did not observe any additive effects when the same flies were fed captopril for 28 days post eclosion (Figure 10A, B, 11). Interestingly, we also observed that flies heterozygous for the *Acer* null mutation also suppressed brain cell death in 4-week old $elav^{C155}>A\beta42$ flies similar to captopril treatment and no additive effects were found when fed with either captopril or losartan (Figure 10C). Altogether, these data are consistent with Acer being the target of captopril that mediates the beneficial effects observed in our transgenic lines expressing AD-related transgenes. Whether losartan acts in the same downstream pathway remains to be determined and requires further targets to be discovered.

Discussion

Recent studies have shown that administration of antihypertensive medications such as ACE-Is and ARBs are associated with reduced onset and progression of Alzheimer's disease. However, the mechanisms by which these drugs lead to beneficial effects in AD are unclear. Here, we examined the effects of captopril (ACE-I) and losartan (ARB) in *Drosophila* that express human AD-related transgenes in the eye and CNS. We found that administration of either drug significantly reduced cell death within the brain and improved short-term memory. We also found that the beneficial effects were most pronounced in flies expressing Aβ42 peptides although neither drug affected the production, accumulation or clearance of Aβ42. We also observed no effects of either drug on degenerative phenotypes in *Drosophila* expressing human Tau suggesting that the beneficial effects are specific to APP-CTF and Aβ42 expressing flies. Finally, we found that the beneficial effects observed upon captopril treatment could be completely recapitulated by introducing an *Acer* null mutation into our AD fly models consistent with Acer being the target of captopril in *Drosophila*. Interestingly, while ACE orthologs have been identified in *Drosophila* the renin angiotensin system (RAS), which includes downstream effectors of ACE including angiotensin I/II and the angiotensin receptor, are not conserved. This suggests that the beneficial effects of ACE-Is and ARBs in *Drosophila* may involve mechanisms that are distinct from those mediated by the canonical RAS.

Several studies have shown that use of ACE-Is and ARBs correlates with decreased incidence and improved cognitive outcomes in AD patients (Ohrui *et al*., 2004; Davies *et al*., 2011; Qiu *et al*., 2013;Yasar *et al*., 2013; de Oliveira *et al*., 2014; Wharton *et al*., 2015; Ho *et al*., 2017). Importantly, only brain-penetrating ACE-Is and ARBs have been shown to delay the onset of cognitive impairment and neurodegeneration in mice models and humans, demonstrating that their beneficial effects are

independent of their role in regulating blood-pressure (Alvarez *et al*., 1999; Ohrui *et al*., 2004; Hajjar *et al*., 2005; Edwards *et al*., 2009; Miners *et al*., 2009; Belbin *et al*., 2011; Davies *et al*., 2011; Qiu *et al*., 2013; Soto *et al*., 2013; Yasar *et al*., 2013; de Oliveira *et al*., 2014; Kauwe *et al*., 2014; O'Caoimh *et al*., 2014; Wharton *et al*., 2015; Ho *et al*., 2017). Several *in vitro* studies have suggested that ACE may be involved in Aβ degradation, conversion and clearance (Kehoe *et al*., 1999; Hemming & Selkoe, 2005; Liu *et al*., 2014). *In vivo* studies however, are controversial with some studies demonstrating that ACE-Is promote Aβ42 deposition (Zou *et al*., 2007; Bernstein *et al*., 2014), have little to no effect on Aβ42 peptide levels or plaque deposition (Hemming *et al*., 2007; Dong *et al*., 2011), and reduce Aβ deposits in the hippocampus (Abdalla *et al*., 2013). Despite this conflicting evidence, ACE-Is have consistently demonstrated improved cognitive outcomes in mice models of AD and in patients (Ohrui *et al*., 2004; Hajjar *et al*., 2005; El Sayed *et al*., 2009; Yamada *et al*., 2010; Dong *et al*., 2011; AbdAlla *et al*., 2013; Soto *et al*., 2013; de Oliveira *et al*., 2014; O'Caoimh *et al*., 2014). Similarly, ARBs have also been reported to improve cognitive function in rodent models (Takeda *et al*., 2009; Tsukuda *et al*., 2009; Shindo *et al*., 2012; Bild *et al*., 2013; Singh *et al*., 2013; Royea *et al*., 2017) but do not appear to alter Aβ levels (Ongali *et al*., 2014) or aggregation (Ferrington *et al*., 2011).

Given the known role of ACE-Is and ARBs in modulating RAS, several *in vivo* studies have examined the effect of regulating specific components of RAS on AD related phenotypes. These studies demonstrated toxic effects associated with Ang II/AT1R signaling in the brain resulting in an increase in the levels and deposition of Aβ42 (Faraco *et al*., 2016), increased oxidative stress and enhanced cognitive defects (Bild *et al*., 2013; Royea *et al*., 2017). On the other hand, protective effects including a decrease in neuronal degeneration and improved cognitive function, were observed with enhanced Ang II/AT2R and Ang IV/AT4R signaling (Bild *et al*., 2013; Royea *et al*., 2017). In line with these findings, studies have also shown beneficial roles of ACE-Is and ARBs in animal models of AD

436 whereby the drugs prevented Ang II production and inhibited Ang II/AT1R signaling (Tsukuda *et al*.,
437 2009; AbdAlla *et al*., 2013; Royea *et al*., 2017). Altogether, these studies suggest that the protective
438 effects of ACE-Is and ARBs in AD may be associated with inhibition of Ang II/AT1R signaling
439 however, the role of RAS in AD pathology is still unclear.

440 We first identified two ACE-like factors in *Drosophila*, *Acer* and *Ance-5,* in a genetic screen for
441 PS and C99 modifiers (van de Hoef *et al*., 2009). Interestingly, although *Drosophila* have ACE
442 orthologs, the canonical renin angiotensin system that includes Angiotensin I/II and the Angiotensin
443 Receptor is not conserved. Importantly, only *Acer* and *Ance-5* were identified in our screen and, of
444 these, Acer shares greater amino acid similarity and identity to human ACE and also retains the ACE
445 active site and enzyme activity (Coates *et al*., 2000). In addition, ACE inhibitors are significantly more
446 potent towards Acer (Cornell *et al*.,1995; Hourad *et al*., 1999). Indeed, we found that ACE-Is can
447 significantly reduce cell death within the brain and improve short-term memory in *Drosophila*
448 expressing AD-related transgenes except *Tau*. Moreover, we observed similar beneficial effects in
449 *Drosophila* treated with an ARB, even though the Angiotensin Receptor is not conserved. At present,
450 the mechanism by which ACE-Is and ARBs function in *Drosophila* is unclear. Both captopril and
451 losartan consistently suppress AD-related phenotypes in flies expressing either human C99 carrying a
452 London mutation or Aβ42 however, these beneficial effects are not associated with any changes in the
453 production, accumulation or clearance of Aβ42. This finding is consistent with previous *in vivo* studies
454 in mice and humans demonstrating that ACE-Is and ARBs improved cognitive function without
455 affecting Aβ levels (Hemming *et al*., 2007; Wharton *et al*., 2012) but contrasts with *in vitro* studies,
456 demonstrating that ACE-Is lead to increased Aβ42 production and aggregation (Kehoe *et al*., 1999;
457 Hemming & Selkoe, 2005; Zou *et al*., 2007; Liu *et al*., 2014). Therefore, based on our findings, it is
458 unlikely that these drugs are modulating AD-related phenotypes through γ-secretase cleavage of C99.

It is also unlikely that the ability of ACE-Is and ARBs to rescue cell death and cognitive dysfunction in *Drosophila* is due to effects on Angiotensin receptors since, other than ACE, the canonical RAS is not conserved in *Drosophila.* At present, the function of *Acer* in *Drosophila* is not fully understood. Some ACE-like factors have been shown to be affected by ACE-Is including *Acer* and *Ance* (Williams *et al*., 1996; Houard *et al*., 1998) however, the targets of either protein have yet to be identified. *Acer* null mutants have also been shown to exhibit disruptions in night-time sleep and sleep fragmentation (Carhan *et al*., 2011) as well as altered behavioural and metabolic responses to diet (Glover *et al*, 2019). However, these flies develop normally to adulthood, suggesting that major developmental or signaling pathways have not been affected. Flies lacking *Ance* have also been shown to develop normally without any obvious physiological defects (Kim *et al*., 2017). Similarly, the target for ARBs in *Drosophila* is currently unknown as no homologue of ATR has been discovered. Altogether, our data demonstrate that ACE-Is and ARBs can alleviate toxic phenotypes in *Drosophila* expressing human AD transgenes. Since these beneficial effects are observed in the absence of the canonical RAS this also suggests that captopril and losartan may be acting on a more ancestral function of this pathway and have additional targets that can be identified in *Drosophila*.

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References

AbdAlla S, Langer A, Fu X, Quitterer U (2013). ACE Inhibition with Captopril Retards the Development of Signs of Neurodegeneration in an Animal Model of Alzheimer's Disease. Intl J Mol Sci 14: 16917-16942.

483 Alvarez R, Alvarez V, Lahoz CH, Martínez C, Peña J, Sánchez JM et al (1999). Angiotensin
484 converting enzyme and endothelial nitric oxide synthase DNA polymorphisms and late onset
485 Alzheimer's disease. J Neurol, Neurosurg, Psych 67: 733-736.
486 Bernstein KE, Koronyo Y, Salumbides BC, Sheyn J, Pelissier L, Lopes DHJ et al(2014). Angiotensin-
487 converting enzyme overexpression in myelomonocytes prevents Alzheimer's-like cognitive
488 decline. J Clin Invest 124: 1000-1012.
489 Belbin O, Brown K, Shi H, Medway C, Abraham R, Passmore P, Kehoe PG (2011). A multi-center
490 study of ACE and the risk of late-onset Alzheimer's disease. J Alz Dis 24: 587-597.
491 Bild W, Hritcu L, Stefanescu C, Ciobica A (2013). Inhibition of central angiotensin II enhances
492 memory function and reduces oxidative stress status in rat hippocampus. Prog
493 Neuropsychopharmacol Biol Psychiatry 43: 79-88.
494 Boulianne GL, Livne-Bar I, Humphreys JM, Liang Y, Lin C, Rogaev E, St. George-Hyslop P (1997).
495 Cloning and characterization of the Drosophila presenilin homologue. Neurorep 8: 1025-1029.
496 Braszko JJ, Karwowska-Polecka W, Halicka D, Gard PR (2003). Captopril and enalapril improve
497 cognition and depressed mood in hypertensive patients. J Basic Clin Physiol Pharmacol 14:
498 323-343.
499 Brand AH, Perrimon N (1993). Targeted gene expression as a means of altering cell fates and
500 generating dominant phenotypes. Development 118: 401-415.
501 Burr AA, Tsou W-L, Ristic G, Todi SV (2014). Using membrane-targeted green fluorescent protein to
502 monitor neuros protein-dependent degeneration of *Drosophila* eyes. J Neurosci Res 92: 1100-
503 1109.
504 Burgess A, Vigneron S, Brioudes E, Labbé J-C, Lorca T, Castro A (2010). Loss of human Greatwall
505 results in G2 arrest and multiple mitotic defects due to deregulation of the cyclin B-Cdc2/PP2A
506 balance. Proc Natl Acad Sci USA 107: 12564-12569.

507 Burdick D, Soreghan B, Kwon M, Kosmoski J, Knauer M, Henschen A et al (1992). Assembly and
508 aggregation properties of synthetic Alzheimer's A4/beta amyloid peptide analogs. J Biol Chem
509 267: 546-554.
510 Bursavich MG, Harrison BA, Blain J-F (2016). Gamma Secretase Modulators: New Alzheimer's Drugs
511 on the Horizon? J Med Chem 59(16), 7389–7409.
512 Carhan A, Tang K, Shirras CA, Shirras AD, Isaac RE (2011). Loss of Angiotensin-converting enzyme-
513 related (ACER) peptidase disrupts night-time sleep in adult *Drosophila* melanogaster. J Exp
514 Biol 214: 680-686.
515 Chakraborty R, Vepuri V, Mhatre SD, Paddock BE, Miller S, Michelson SJ et al (2011).
516 Characterization of a *Drosophila* Alzheimer's disease model: pharmacological rescue of
517 cognitive defects. PLoS One 6; e20799.
518 Choma MA, Suter MJ, Vakoc BJ, Bouma BE, Tearney GJ (2011). Physiological homology between
519 *Drosophila melanogaster* and vertebrate cardiovascular systems. Dis Model Mech 4: 411-420.
520 Coates D, Isaac RE, Cotton J, Siviter R, Williams TA, Shirras A et al (2000). Functional conservation
521 of the active sites of human and *Drosophila* angiotensin Iconverting enzyme. Biochem 39(30),
522 8963–8969.
523 Cornell MJ, Williams TA, Lamango NS, Coates D, Corvol P, Soubrier F et al (1995). Cloning and
524 expression of an evolutionary conserved single-domain angiotensin converting enzyme from
525 *Drosophila melanogaster*. J Biol Chem 270(23), 13613–13619.
526 Davies NM, Kehoe PG, Ben-Shlomo Y, Martin RM (2011). Associations of anti- hypertensive
527 treatments with Alzheimer's disease, vascular dementia, and other dementias. J Alz Dis 26:
528 699-708.
529 de Oliveira FF, Bertolucci PHF, Chen ES, Smith MC (2014). Brain-penetrating angiotensin-converting

530 enzyme inhibitors and cognitive change in patients with dementia due to Alzheimer's disease. J
531 Alz Dis 42 Suppl 3: S321-324.
532 Dong Y-F, Kataoka K, Tokutomi Y, Nako H, Nakamura T, Toyama K et al (2011). Perindopril, a
533 centrally active angiotensin-converting enzyme inhibitor, prevents cognitive impairment in
534 mouse models of Alzheimer's disease. FASEB J 25: 2911-2920.
535 Edwards TL, Pericak-Vance M, Gilbert JR, Haines JL, Martin ER, Ritchie MD (2009). An association
536 analysis of Alzheimer disease candidate genes detects an ancestral risk haplotype clade in ACE
537 and putative multilocus association between ACE, A2M, and LRRTM3. Am J Med Genet Part
538 B, Neuropsych Genet 150B: 721-735.
539 El Sayed NS, Kassem LA, Heikal OA (2009). Promising therapy for Alzheimer's disease targeting
540 angiotensinconverting enzyme and the cyclooxygense-2 isoform. Drug Discoveries &
541 Therapeutics 3: 307-315.
542 Faraco G, Park L, Zhou P, Luo W, Paul SM, Iadecola C (2016). Hypertension enhances Abeta-induced
543 neurovascular dysfunction, promotes beta-secretase activity, and leads to amyloidogenic
544 processing of APP. J Cereb Blood Flow Metab 36: 241-252.
545 Ferrington L, Miners JS, Palmer LE, Bond SM, Povey JE, Kelly PA, Kehoe PG (2011). Angiotensin II-
546 inhibiting drugs have no effect on intraneuronal Aβ or oligomeric Aβ levels in a triple
547 Transgene mouse model of Alzheimer's disease. Am J Transl Res 3: 197-208.
548 Finelli A, Kelkar A, Song H-J, Yang H, Konsolaki M (2004). A model for studying Alzheimer's
549 Abeta42-induced toxicity in *Drosophila melanogaster*. Mol Cell Neurosci 26: 365-375.
550 Gistelinck M, Lamber JC, Callaerts P, Dermaut B, Dourlen P (2012). *Drosophila* models of taupathies:
551 what have we learned? Int J Alzheimers Dis https://doi.org/10.1155/2012/970980.
552 Goel R, Bhat SA, Hanif K, Nath C, Shukla R (2016). Perindopril Attenuates Lipopolysaccharide-
553 Induced Amyloidogenesis and Memory Impairment by Suppression of Oxidative Stress and

554 RAGE Activation. ACS Chem Neurosci 7:206-217.
555 Goel R, Bhat SA, Hanif K, Nath C, Shukla R (2018). Angiotensin II Receptor Blockers Attenuate
556 Lipopolysaccharide-Induced Memory Impairment by Modulation of NF-kB-Mediated
557 BDNF/CREB Expression and Apoptosis in Spontaneously Hypertensive Rats. Mol Neurobiol
558 55: 1725-1739.
559 Gao Y, O'Caoimh R, Healy L, Kerins DM, Eustace J, Guyatt G, Sammon D, Molloy DW (2013).
560 Effects of centrally acting ACE inhibitors on the rate of cognitive decline in dementia. BMJ
561 Open 3: pii: e002881.
562 Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, Fidani L et al (1991). Segregation of a
563 missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease.
564 Nature 349: 704-706.
565 Glover Z, Hodges MD, Dravecz N, Cameron J, Askwith H, Shirras A, Broughton SJ (2019). Loss of
566 angiotensin-converting enzyme-related (ACER) peptidase disrupts behavioural and metabolic
567 responses to diet in *Drosophila melanogaster*. J Exp Biol https://doi.org/10.1242/jeb.194332.
568 Griffith LC, Ejima A (2009). Courtship learning in *Drosophila melanogaster*: diverse plasticity of a
569 reproductive behavior. Learn Mem 16: 743-750.
570 Greeve I, Kretzschmar D, Tschape JA, Beyn A, Brellinger C, Schweizer M, Nitsch RM, Reifegerste R
571 (2004). Age-dependnet neurodegeneration and Alzheimer-amyloid plaque formation in
572 transgenic *Drosophila.* J Neurosci 24: 3899-3906.
573 Guo Y, Livne-Bar I, Zhou L, Boulianne GL (1999). *Drosophila* presenilin is required for neuronal
574 differentiation and affects notch subcellular localization and signaling. J Neurosci 19: 8435-
575 8442.
576 Hansson O, Zetterberg H, Buchhave P, Andreasson U, Londos E, Minthon L, Blennow K (2007).
577 Prediction of Alzheimer's disease using the CSF Abeta42/Abeta40 ratio in patients with mild

578 cognitive impairment. Dementia and Geriatric Cognitive Dis 23: 316-320.
579 Hajjar I, Catoe H, Sixta S, Boland R, Johnson D, Hirth V et al (2005). Cross- sectional and longitudinal
580 association between antihypertensive medications and cognitive impairment in an elderly
581 population. J Geront Series A Biol Sci and Med Sci 60: 67-73.
582 Hemming ML, Selkoe DJ, Farris W (2007). Effects of prolonged angiotensin-converting enzyme
583 inhibitor treatment on amyloid beta-protein metabolism in mouse models of Alzheimer disease.
584 Neurobiol Dis 26: 273-281.
585 Hemming ML, Selkoe DJ (2005). Amyloid beta-protein is degraded by cellular angiotensin-converting
586 enzyme (ACE) and elevated by an ACE inhibitor. J Biol Chem 280: 37644-37650.
587 Ho JK, Nation DA, Alzheimer's Disease Neuroimaging Initiative. (2017). Memory is preserved in
588 older adults taking AT1 receptor blockers. Alz Res Ther 9: 33.
589 Holtzman DM, Morris JC, Goate AM (2011). Alzheimer's disease: the challenge of the second
590 century. Sci Transl Med 3: 77sr1. https://doi.org/10.1126/scitranslmed.3002369
591 Hong CS, Koo EH (1997). Isolation and characterization of *Drosophila* presenilin homolog.
592 Neuroreport 8: 665-668.
593 Houard X, Williams TA, Michaud A, Dani P, Isaac RE, Shirras AD, Coates D, Corvol P (1998). The
594 *Drosophila melanogaster*-related angiotensin-I-converting enzymes Acer and Ance. Eur J
595 Biochem 257: 599-606.
596 Iijima K, Chiang HC, Hearn SA, Hakker I, Gatt A, Shenton C, Granger L, Leung A, Iijima-Ando K,
597 Zhong Y (2008). Abeta42 mutants with different aggregation profiles induce distinct
598 pathologies in *Drosophila*. PLoS One 3:e1703.
599 Iijima K, Liu HP, Chiang, AS, Hearn S, Konsolaki M, Zhong, Y. (2004). Dissecting the pathological
600 effects of human Aβ40 and Aβ42 in *Drosophila*: A potential model for Alzheimer's disease.

PNAS 101: 6623-6628.

Iyer J, Wang Q, Le T, Pizzo L, Grönke S, Ambegaokar SS et al (2016). Quantitative Assessment of Eye Phenotypes for Functional Genetic Studies Using *Drosophila melanogaster*. G3 (Bethesda) 6: 1427-1437.

Jenkins TA, Chai SY (2007). Effect of chronic angiotensin converting enzyme inhibition on spatial memory and anxiety-like behaviours in rats. Neurobiol Learn Mem 87: 218-224.

Jonson M, Nyström S, Sandberg A, Carlback M, Michno W, Hanrieder J et al (2018) Aggregated Aβ1-42 Is Selectively Toxic for Neurons, Whereas Glial Cells Produce Mature Fibrils with Low Toxicity in *Drosophila*. Cell Chem Biol 25: 595-610.

Kauwe JSK, Bailey MH, Ridge PG, Perry R, Wadsworth ME, Hoyt KL et al (2014). Genome-Wide Association Study of CSF Levels of 59 Alzheimer's Disease Candidate Proteins: Significant Associations with Proteins Involved in Amyloid Processing and Inflammation. PLoS Genet 10: e1004758.

Kamyshev NG, Iliadi KG, Bragina JV (1999). *Drosophila* conditioned courtship: two ways of testing memory. Learn mem 6: 1-20.

Kehoe PG, Passmore PA (2012). The renin-angiotensin system and antihypertensive drugs in Alzheimer's disease: current standing of the angiotensin hypothesis? J Alz Dis 30: Suppl 2: S251-268.

Kehoe PG, Miners S, Love S (2009). Angiotensins in Alzheimer's disease- friend or foe? Trends Neurosci 32: 619-628.

Kehoe PG, Wilcock GK (2007). Is inhibition of the renin-angiotensin system a new treatment option for Alzheimer's disease? Lancet Neurol 6: 373-378.

Kehoe PG, Russ C, McIlory S, Williams H, Holmans P, Holmes C et al (1999). Variation in DCP1,

encoding ACE, is associated with susceptibility to Alzheimer disease. Nat Genet 21: 71-72

Keleman K, Vrontou E, Krüttner S, Yu JY, Kurtovic-Kozaric A, Dickson BJ (2012). Dopamine neurons modulate pheromone responses in *Drosophila* courtship learning. Nature 489: 145-149.

Kim AR, Choi EB, Kim MY, Choi KW (2017). Angiotensin-converting enzyme Ance is cooperatively regulated by Mad and Pannier in *Drosophila* imaginal discs. Sci Rep 7: 13174.

Lewczuk P, Lelental N, Spitzer P, Maler JM, Kornhuber J (2015). Amyloid-β 42/40 cerebrospinal fluid concentration ratio in the diagnostics of Alzheimer's disease: validation of two novel assays. J Alz Dis 43: 183-191.

Lim J, Fernandez AI, Hinojos SJ, Aranda GP, James J, Seong CS, Han KA (2018). The mushroom body D1 dopamine receptor controls innate courtship drive. Genes Brain Behav 17: 158-167.

Liu S, Liu J, Miura Y, Tanabe C, Maeda T, Terayama Y et al (2014). Conversion of Aβ43 to Aβ40 by the successive action of angiotensin-converting enzyme 2 and angiotensin-converting enzyme. J Neurosci Res 92: 1178-1186.

McBride SM, Choi CH, Wang Y, Liebelt D, Braunstein E, Ferreiro D et al. (2005). Pharmacological rescue of synaptic plasticity, courtship behavior, and mushroom body defects in a *Drosophila* model of fragile X syndrome. Neuron 45: 753-764.

Miners S, Ashby E, Baig S, Harrison R, Tayler, H, Speedy E et al. (2009). Angiotensin-converting enzyme levels and activity in Alzheimer's disease: differences in brain and CSF ACE and association with ACE1 genotypes. Am J Transl Res 1: 163-177.

Nikolova JG, Getova DP, Nikolov FP (2000). Effects of ACE-inhibitors on learning and memory processes in rats. Folia Medica 42(1), 47-51.

O'Caoimh R, Healy L, Gao Y, Svendrovski A, Kerins DM, Eustace J et al. (2014). Effects of centrally acting angiotensin converting enzyme inhibitors on functional decline in patients with Alzheimer's disease. J Alz Dis 40: 595-603.

Ohrui T, Tomita N, Sato-Nakagawa T, Matsui T, Maruyama M, Niwa K et al. (2004). Effects of brain-penetrating ACE inhibitors on Alzheimer disease progression. Neurol 63: 1324-1325.

Ongali B, Nicolakakis N, Tong X-K, Aboulkassim T, Papadopoulos P, Rosa-Neto P. et al (2014). Angiotensin II type 1 receptor blocker losartan prevents and rescues cerebrovascular, neuropathological and cognitive deficits in an Alzheimer's disease model. Neurobiol Dis 68: 126-136.

Parks AL, Curtis D (2007). Presenilin diversifies its portfolio. Trends Genet 23: 140-150.

Prüßing K, Voigt A, Schulz JB (2013). *Drosophila melanogaster* as a model organism for Alzheimer's disease. Mol Neurodegen 8: 35.

Qiu WQ, Mwamburi M, Besser LM, Zhu H, Li H, Wallack M et al. (2013). Angiotensin converting enzyme inhibitors and the reduced risk of Alzheimer's disease in the absence of apolipoprotein E4 allele. *J. Alzheimer's Dis: JAD*, 37: 421-428.

Royea J, Zhang L, Tong XK, Hamel E (2017). Angiotensin IV Receptors Mediate the Cognitive and Cerebrovascular Benefits of Losartan in a Mouse Model of Alzheimer's Disease. J Neurosci 37: 5562-5573.

Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT (2011). Neuropathological Alterations in Alzheimer Disease. Cold Spring Harb Perspect Med https://doi.org/10.1101/cshperspect.a006189.

Shindo T, Takasaki K, Uchida K, Onimura R, Kubota K, Uchida N et al. (2012). Ameliorative effects of telmisartan on the inflammatory response and impaired spatial memory in a rat model of Alzheimer's disease incorporating additional cerebrovascular disease factors. Biol. Pharmaceut. Bull. 35: 2141-2147.

Siegel RW, Hall JC (1979). Conditioned responses in courtship behavior of normal and mutant *Drosophila*. Proc Natl Acad Sci 76: 3430-3434.

672 Singh B, Sharma B, Jaggi AS, Singh N (2013). Attenuating effect of lisinopril and telmisartan in
673 intracerebroventricular streptozotocin induced experimental dementia of Alzheimer's disease
674 type: possible involvement of PPAR-γ agonistic property. J. Renin-Angiotensin-Aldosterone
675 System: JRAAS 14: 124-136.
676 Soto ME, Abellan van Kan G, Nourhashemi F, Gillette-Guyonnet S, Cesari M, Cantet C et al. Vellas,
677 B. (2013). Angiotensin-Converting Enzyme Inhibitors and Alzheimer's Disease Progression in
678 Older Adults: Results from the Réseau sur la Maladie d'Alzheimer Français Cohort. J. Amer.
679 Geriatrics Soc. 61: 1482-1488.
680 Takeda S, Sato N, Takeuchi D, Kurinami H, Shinohara M, Niisato K et al. (2009). Angiotensin
681 receptor blocker prevented beta-amyloid-induced cognitive impairment associated with
682 recovery of neurovascular coupling. Hypertension 54: 1345-1352.
683 Tan Y, Zhang Q, Wong SG, Hua Q (2016). Anti-Alzheimer Therapeutic Drugs Targeting γ-Secretase.
684 Curr. Topics Med. Chem. 16(5), 549–557.
685 Tota S, Kamat PK, Saxena G, Hanif K, Najmi AK, Nath C (2012). Central angiotensin converting
686 enzyme facilitates memory impairment in intracerebroventricular streptozotocin treated rats.
687 Behav Brain Res. 226: 317-330.
688 Tsukuda K, Mogi M, Iwanami J, Min L-J, Sakata A, Jing F et al. (2009). Cognitive deficit in amyloid-
689 beta-injected mice was improved by pretreatment with a low dose of telmisartan partly because
690 of peroxisome proliferator-activated receptor-gamma activation. Hypertension 54: 782-787.
691 van de Hoef DL, Hughes J, Livne-Bar I, Garza D, Konsolaki M, Boulianne GL (2009). Identifying
692 genes that interact with *Drosophila* presenilin and amyloid precursor protein. Genesis 47: 246-
693 260.
694 WhartonW, Goldstein FC, Zhao L, Steenland K, Levey AI, Hajjar I (2015). Modulation of Renin-
695 Angiotensin System May Slow Conversion from Mild Cognitive Impairment to Alzheimer's

696 Disease. J Am Geriatr Soc. 63: 1749-1756.
697 Wharton W, Stein JH, Korcarz C, Sachs J, Olson SR, Zetterberg H,et al. (2012). The effects of
698 ramipril in individuals at risk for Alzheimer's disease: results of a pilot clinical trial. J
699 Alzheimers Dis. 32: 147-156.
700 Williams TA, Michaud A, Houard X, Chauvet MT, Soubrier F, Corvol P (1996). *Drosophila*
701 *melanogaster* angiotensin I-converting enzyme expressed in *Pichia pastoris* resembles the C
702 domain of the mammalian homologue and does not require glycosylation for secretion and
703 enzymic activity. Biochem J, 318: 125-131.
704 Wittmann CW, Wszolek MF, Shulman JM, Salvaterra PM, Lewis J, Hutton M, Feany MB (2001).
705 Taupathy in *Drosophila*: neurodegeneration without neurofibrillary tangles. Science 293: 711-
706 714.
707 Wolfe MS, Xia W, Ostaszewski BL, Diehl TS, Kimberly WT, & Selkoe DJ (1999). Two
708 transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-
709 secretase activity. Nature 398: 513-517.
710 Yang WN, Han H, Hu XD, Feng GF, Qian YH (2013). The effects of perindopril on cognitive
711 impairment induced by d-galactose and aluminum trichloride via inhibition of
712 acetylcholinesterase activity and oxidative stress. Pharmacol Biochem Behav. 114-115: 31-36.
713 Yasar S, Xia J, Yao W, Furberg CD, Xue Q-L, Mercado CI, Ginkgo Evaluation of Memory (GEM)
714 Study Investigators (2013). Antihypertensive drugs decrease risk of Alzheimer disease: Ginkgo
715 Evaluation of Memory Study. Neurology 81: 896-903.
716 Yamada K, Uchida S, Takahashi S, Takayama M, Nagata Y, Suzuki N et al. (2010). Effect of a
717 centrally active angiotensin-converting enzyme inhibitor, perindopril, on cognitive performance
718 in a mouse model of Alzheimer's disease. Brain Res. 1352: 176-186.
719 Ye Y, Fortini, ME (1999). Apoptotic activities of wild-type and Alzheimer's disease-related mutant

720 presenilins in *Drosophila melanogaster*. J Cell Biol. 146: 1351-1364.

721 Zou K, Yamaguchi H, Akatsu H, Sakamoto T, Ko,M, Mizoguchi, K et al. (2007). Angiotensin-

722 converting enzyme converts amyloid beta-protein 1-42 Abeta(1- 42) to Abeta(1-40), and its

723 inhibition enhances brain Abeta deposition. J. Neurosci. 27: 8628-8635.

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Figure Legends

Figure 1. *gmr-GAL4 Drosophila* model of AD. Confocal GFP (top row) and light microscope (bottom row) images of 7-day old *gmr-GAL4-UAS-GFP>W^{1118}*, *gmr-GAL4-UAS-GFP>UAS-C99^{wt}*, *gmr-GAL4-UAS-GFP>UAS-C99^{V717I}* and *gmr-GAL4-UAS-GFP>UAS-Aβ42* fly heads as labelled. Kruskal-Wallis ANOVA analysis of GFP quantification showed significant differences between transgenes (p<0.0001). Multiple comparison analysis using Dunn's Corrected Multiple Comparison test showed flies expressing *C99^{wt}* (N=41), *C99^{V717I}* (N=56) and *Aβ42* (N=30) have a significant decrease in GFP expression compared to *wt* (N=88) (p=0.0388, p<0.0001, p<0.0001, respectively). Data are shown as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, n.s. not significant.

Figure 2. *elav-GAL4 Drosophila* model of AD. (A) TUNEL labeling in brains of 28-day old flies. **(B)** Kruskal-Wallis ANOVA analysis with Dunn's multiple comparisons test showed that flies expressing *C99^{V717I}* (N=6) and *AB42* (N=10) have a significant higher amount of TUNEL-labelled cell death compared to *wt* (N=5) (p=0.0091, p=0.0015, respectively). **(C)** Courtship indexes (CI) were calculated by dividing the time a male spent in courtship to a total given time. Trainer and tester females: -, none; m, mated female. Box-and-whisker plots for CI show 10th, 25th, 75th and 90th percentiles and mean (+). (D) Memory indexes (MI) were calculated as [100[1-(CI with training/Mean of CI without training)] . Kruskal-Wallis ANOVA test followed by Dunn's multiple comparisons test were used for statistical comparisons (N≥20 for each genotype). *elav-GAL4>UAS-C99^{V717I}* and *elav-GAL4>UAS-Aβ42* flies flies showed statistically significant lower MIs when compared to *elav-GAL4>W^{1118}* but not *elav-GAL4>UAS-C99^{wt}* (p=0.0423, p=0.0001, and p=0.1859, respectively) Data are shown as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001. n.s. not significant.

Figure 3. Captopril and losartan increase retinal GFP in flies expressing AD London Mutation, *C99^{V717I}*. Confocal GFP and light microscope images of 7-day *gmr-GAL4-UAS-GFP>UAS-C99^{wt}*, *gmr-GAL4-UAS-GFP>UAS-C99^{V717I}* and *gmr-GAL4-UAS-GFP>UAS-Aβ42* fly heads shown as labelled with or without drug treatments (Top panel). One-Way ANOVA of GFP quantification in *gmr>C99^{wt}* flies showed no significant differences when administered either drug (N=49 for captopril; N=34 for losartan, p=0.2374). Similar results were found for *gmr>Aβ42* flies (N=25 for captopril; N=28 for losartan, p=0.182). However**,** One-Way ANOVA of GFP quantification in *gmr>C99^{V717I}* flies showed a significant effect of drug condition (p=0.0006). *Post hoc* analysis using Bonferroni's Multiple Comparison test showed that both captopril (N=51) and losartan (N=61) significantly increased retinal GFP (p=0.0363, p=0.0003, respectively). Data are shown as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001. n.s. not significant.

Figure 4. Captopril and losartan reduce TUNEL-labelled brain cell death in flies expressing AD London Mutation, *C99^{V717I}* and *Aβ42*. Confocal microscope images of 28-day *elav-GAL4>UAS-C99^{wt}*, *elav-GAL4>UAS-C99^{V717I}* and *elav-GAL4>UAS-Aβ42* fly brains with or without drug treatments are shown as labelled. Kruskal-Wallis ANOVA analysis showed that flies expressing *C99^{wt}* (N≥7 per condition) had no significant difference in the number of cell death when compared between no drug versus drugs (p=0.768). However, Kruskal-Wallis analysis with Dunn's multiple comparisons test showed that flies expressing *C99^{V717I}* (N≥6 per condition) had significant lower number of cell death in drug treated flies when compared between captopril to no drug and losartan to no drug (p=0.0343 and p=0.0035, respectively). Similarly, for flies expressing *Aβ42* (N≥8 per condition), a significant lower number of cell death was observed in losartan treated flies when compared to no drug (p=0.0066). Data are shown as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001. n.s. not significant.

773 **Figure 5. Captopril and losartan selectively rescue STM in *elav>C99^{V717I}* and *elav>Aβ42 flies*. (A)**
774 Percentage of Courtship Indexes (CI). Courtship indexes were calculated by dividing the time a male
775 spent in courtship to a total given time. Trainer and tester females: -, none; m, mated female. Box-and-
776 whisker plots for CI show 10th, 25th, 75th and 90th percentiles and mean (+)**. (B)** Percentage of
777 Memory indexes (MI). Memory indexes were calculated as [100[1-(CI with training/Mean of CI
778 without training)]. . Kruskal-Wallis test followed by Dunn's multiple comparisons test were used for
779 statistical comparisons ($N \geq 20$ per genotype per condition). *elav-GAL4>W^{1118}* flies showed no
780 significant difference in MIs when compared no drug to captopril ($p=0.5171$) and losartan ($p>0.9999$)
781 conditions. *elav-GAL4>UAS-C99^{wt}* flies showed no significant difference in MIs when compared no
782 drug to captopril ($p=0.5171$) but losartan ($p=0.0436$). *elav-GAL4>UAS-C99^{V717I}* flies showed
783 statistically significant MIs when compared no drug to captopril ($p=0.0271$) but losartan conditions
784 ($p=0.333$). *elav-GAL4>UAS-Aβ42 flies* showed no significant difference in MIs when compared no
785 drug to captopril ($p=0.2459$) but losartan ($p=0.045$). Data are shown as mean ± SEM. *$p<0.05$,
786 **$p<0.01$, ***$p<0.001$. n.s. not significant.
787
788 **Figure 6. Captopril and losartan do not affect number of TUNEL-labelled brain cell death in**
789 **flies expressing *Tau*.** TUNEL labeling in brains of 28-day old flies are shown as labelled. Kruskal-
790 Wallis ANOVA analysis with Dunn's multiple comparisons test showed that flies expressing *Tau* +/-
791 captopril or losartan have a significant higher amount of TUNEL-labelled cell death compared to *wt*
792 ($N \geq 5$ per condition) ($p=0.0035$, $p=0.0064$ and $p=0.0404$, respectively). However, no significant change
793 was observed when compared captopril or losartan treated flies to no drug ($N \geq 5$ per condition)
794 ($p>0.9999$ and $p>0.9999$, respectively).
795

Figure 7. Captopril and losartan do not change C99 levels in either *gmr* or *elav* model of C99 expressing flies. (A) Western Blots using samples from *gmr-GAL4-UAS-GFP>UAS-C99^{wt}* and *gmr-GAL4-UAS-GFP>UAS-C99^{V717I}* heads with or without drug treatments are shown as labelled. Each condition was tested with 2 technical replicates each time with a total of 3 biological replicates (N=3, n=2). Kruskal-Wallis ANOVA analysis showed that both captopril and losartan had no significant effects on the levels of C99 in both *gmr>C99^{wt}* and *gmr>C99^{V717I}* flies at 7 days (p=0.9929 and p=0.5429, respectively). **(B)** Western Blots using samples from *elav-GAL4>UAS-C99^{wt}* and *elav-GAL4>UAS-C99^{V717I}* heads with or without drug treatments are shown as labelled. Each condition was tested with 2 technical replicates each time with a total of 3 biological replicates (N=3, n=2). Kruskal-Wallis ANOVA analysis showed that both captopril and losartan had no significant effects on the levels of C99 in both *elav>C99^{wt}* and *elav>C99^{V717I}* flies at 28 days (p=0.8786 and p=0.7214, respectively). Data are shown as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001. n.s. not significant.

Figure 8. Captopril and losartan do not change soluble Aβ42 levels in flies expressing Aβ42 using a *gmr* or *elav* driver. (A) Western Blots using samples from *gmr-GAL4-UAS-GFP>UAS-C99^{V717I}* and *gmr-GAL4-UAS-GFP>UAS-Aβ42* heads with or without drug treatments are shown as labelled. Each condition was tested with 3 biological replicates ($N=3$). Kruskal-Wallis ANOVA analysis showed that both captopril and losartan had no significant effects on the levels of soluble Aβ42 in both *gmr>C99^{V717I}* and *gmr>Aβ42* flies at 7 days (p=0.6286 and p=0.2964, respectively). **(B)** Levels of Aβ42 in *elav-GAL4>UAS-Aβ42* heads at 28 days post eclosion were measured using human Aβ42 ELISA. The two-tailed unpaired t test showed that captopril had no significant effect on Aβ42 levels when compared to no drug condition (p=0.31). A similar result was observed in *elav-GAL4>UAS-Aβ42* flies treated with losartan (p=0.5182). Each condition was tested with 3 technical replicates and 2

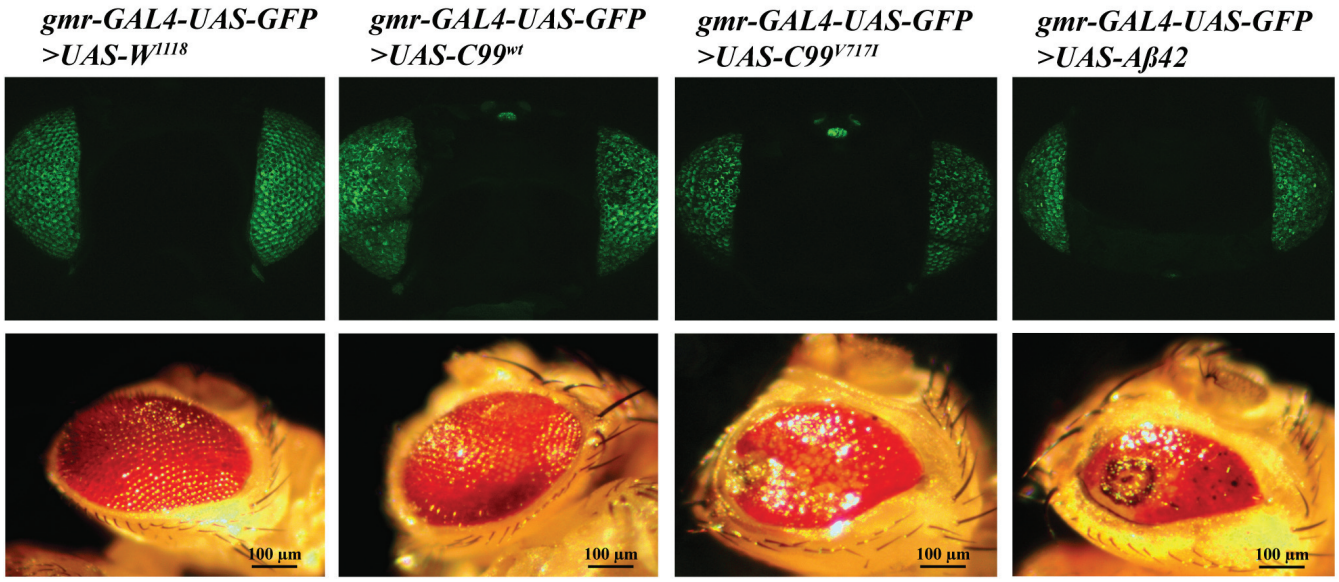
biological replicates in total (N=2, n=3). Data are shown as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001. n.s. not significant.

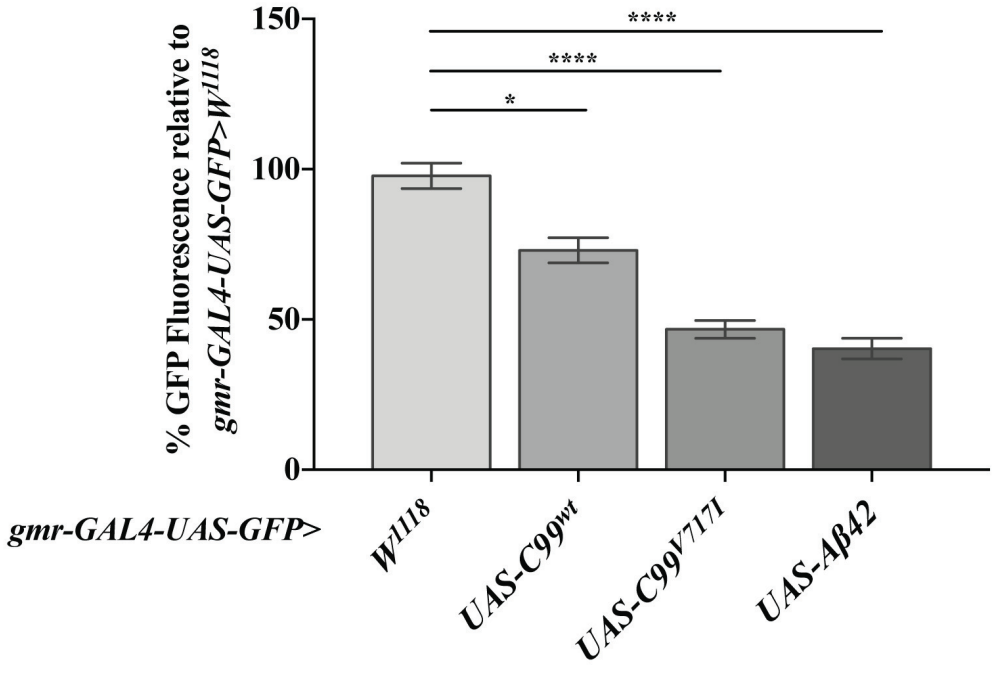
Figure 9. Captopril and losartan do not change Aβ aggregates in *elav>Aβ42* flies. Whole *Drosophila* brain staining with the amyloid-specific LCO, p-FTAA (green) in *elav-GAL4>W^{1118},* and *elav-GAL4>UAS-Aβ42* flies are shown as labelled. Staining reveal Aβ aggregates in *elav-GAL4>UAS-Aβ42* flies (white arrows). Quantification and comparison of Aβ aggregates (p-FTAA pixels) in *elav-GAL4>UAS-Aβ42* flies with or without drug treatment at 28 days post eclosion using Kruskal-Wallis ANOVA analysis revealed no significant changes (p=0.9516) (N≥5 per condition). Data are shown as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001. n.s. not significant.

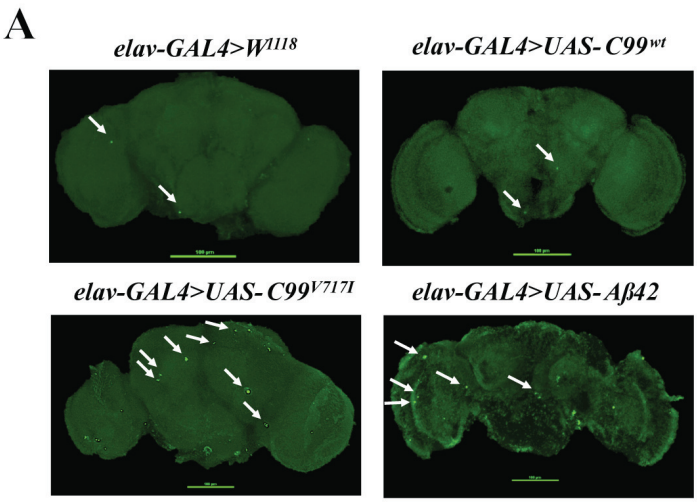
Figure 10. A homozygous *Acer* null mutant reduces brain cell death in flies expressing *C99^{V717I}* and *Aβ42*. Confocal microscope images of 28-day (**A**) *elav-GAL4^{C155}>UAS-C99^{V717I}* and (**B**) *elav-GAL4^{C155}>UAS-Aβ42* fly brains in the presence or absence of captopril and an *Acer* null mutation are shown as labelled. Mann-Whitney analysis showed that *C99^{V717I}* flies (N≥6 per condition) treated with captopril as well as those carrying an *Acer* null mutant +/-captopril had significantly lower numbers of cell death than compared to control flies on no drug (p<0.0001, p<0.0001 and p=0.0031, respectively). A similar effects was observed in *Aβ42* flies (N≥7 per condition) treated with captopril or in flies carrying an *Acer* null mutations +/- captopril (p=0.003, p=0.0001 and p=0.0004, respectively). (**C**) *elav-GAL4^{C155}>UAS-Aβ42* fly brains with an *Acer* heterozygous null mutation in the presence or absence of captopril and losartan are shown as labelled (N≥9 per condition). Kruskal-Wallis ANOVA analysis with Dunn's multiple comparisons test showed that an *Acer* heterozygous null mutant had significantly lower numbers of cell death compared to *elav^{C155}> Aβ42* flies on no drug (p=0.0156). No

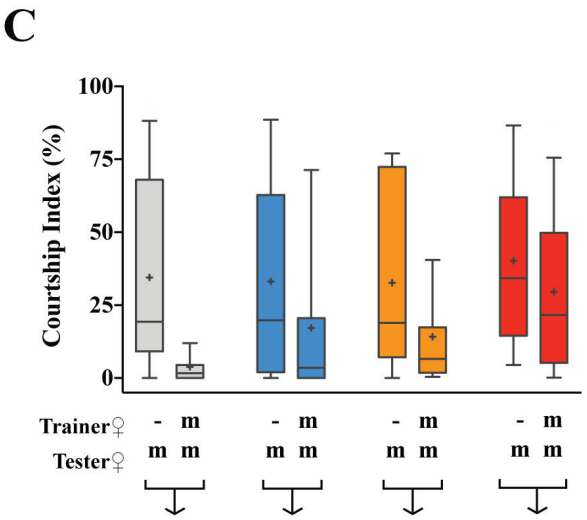
significant difference was found when compared to either plus captopril or losartan or an *Acer* homozygous null mutant (p>0.9999 for all comparisons). Data are shown as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. n.s. not significant.

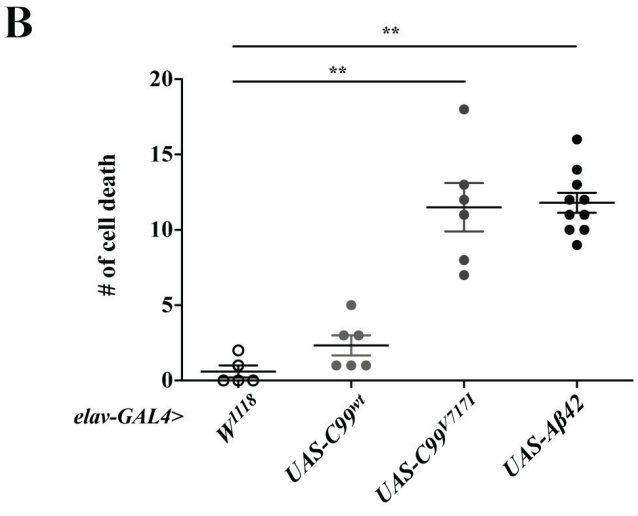
Figure 11. A homozygous *Acer* null mutant rescues STM in flies expressing $C99^{V717I}$ and *Aβ42*. Percentage of Courtship Indexex(CI) and Memory Indexes (MI) are shown as labelled for **(A)** *elav-GAL4^{C155}>UAS-C99^{V717I}* and **(B)** *elav-GAL4^{C155}>UAS-Aβ42* flies. Courtship indexes were calculated by dividing the time a male spent in courtship to a total given time. Trainer and tester females: -, none; m, mated female. Box-and-whisker plots for CI show 10th, 25th, 75th and 90th percentiles and mean (+). Memory indexes were calculated as [100[1-(CI with training/Mean of CI without training)]. Kruskal-Wallis test followed by Dunn's multiple comparisons test were used for statistical comparisons (N≥20 per genotype per condition). *elav-GAL4^{C155}>UAS-C99^{V717I}* flies treated with captopril as well as those carrying an *Acer* null mutant +/- captopril had significantly higher MIs when compared to no drug condition (p=0.0005, p<0.0001 and p<0.0001, respectively). A similar effects was observed in *Aβ42* flies treated with captopril or in flies carrying an *Acer* null mutant +/- captopril (p=0.0001, p<0.0001 and p=0.0001, respectively). Data are shown as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. n.s. not significant.

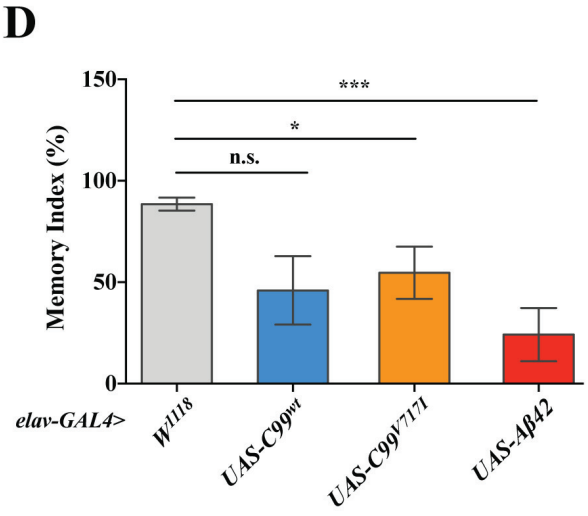


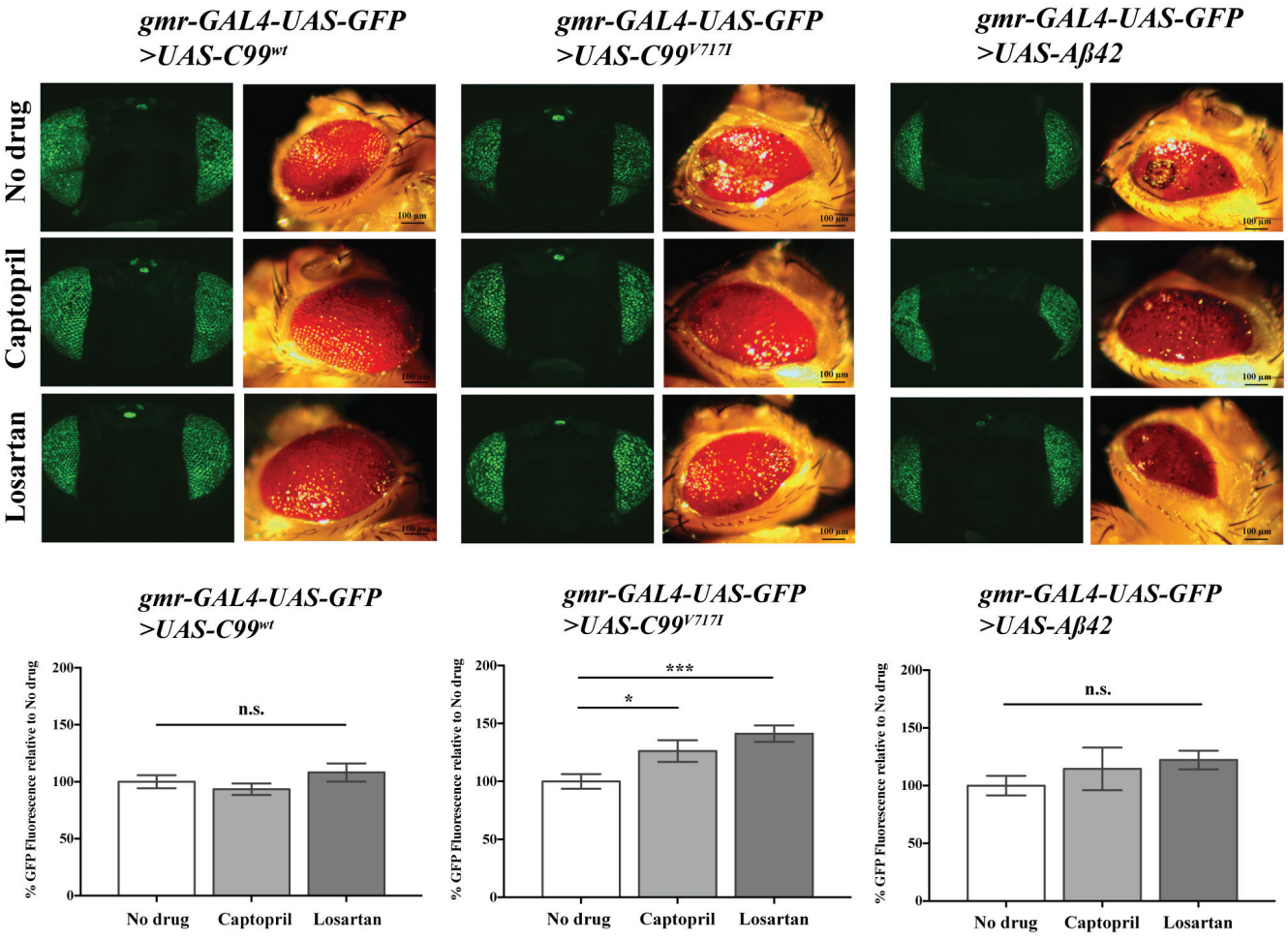


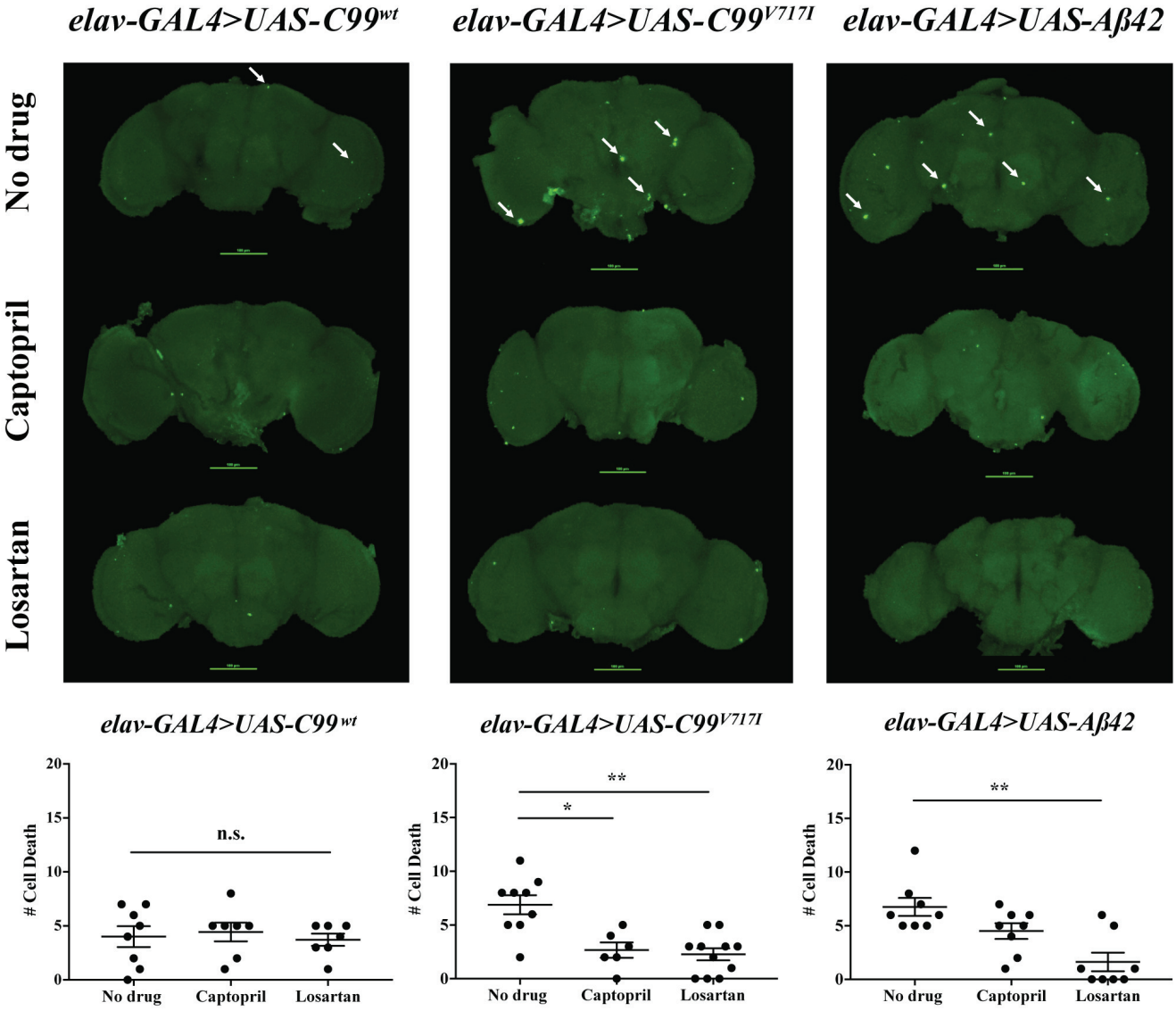


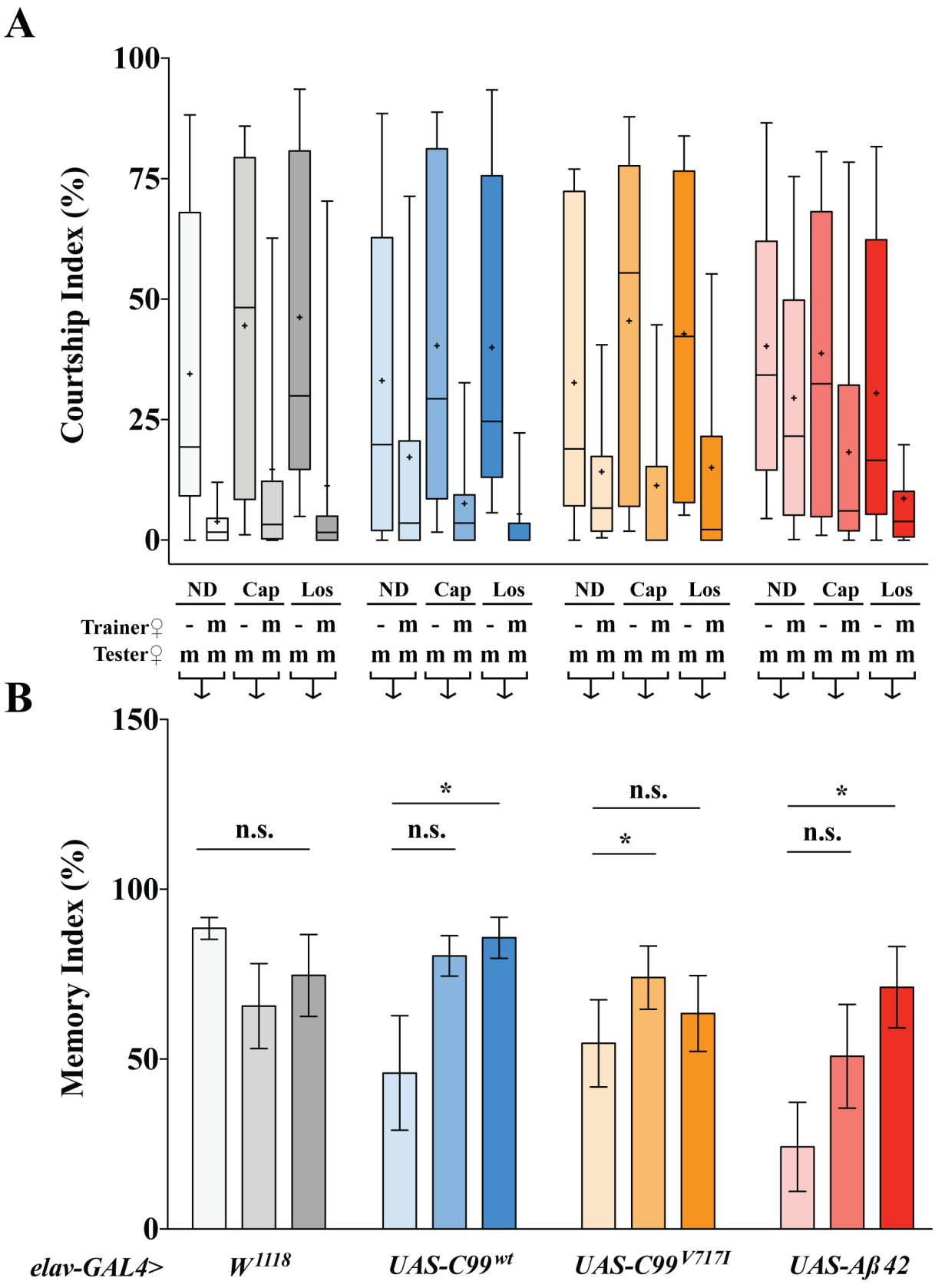


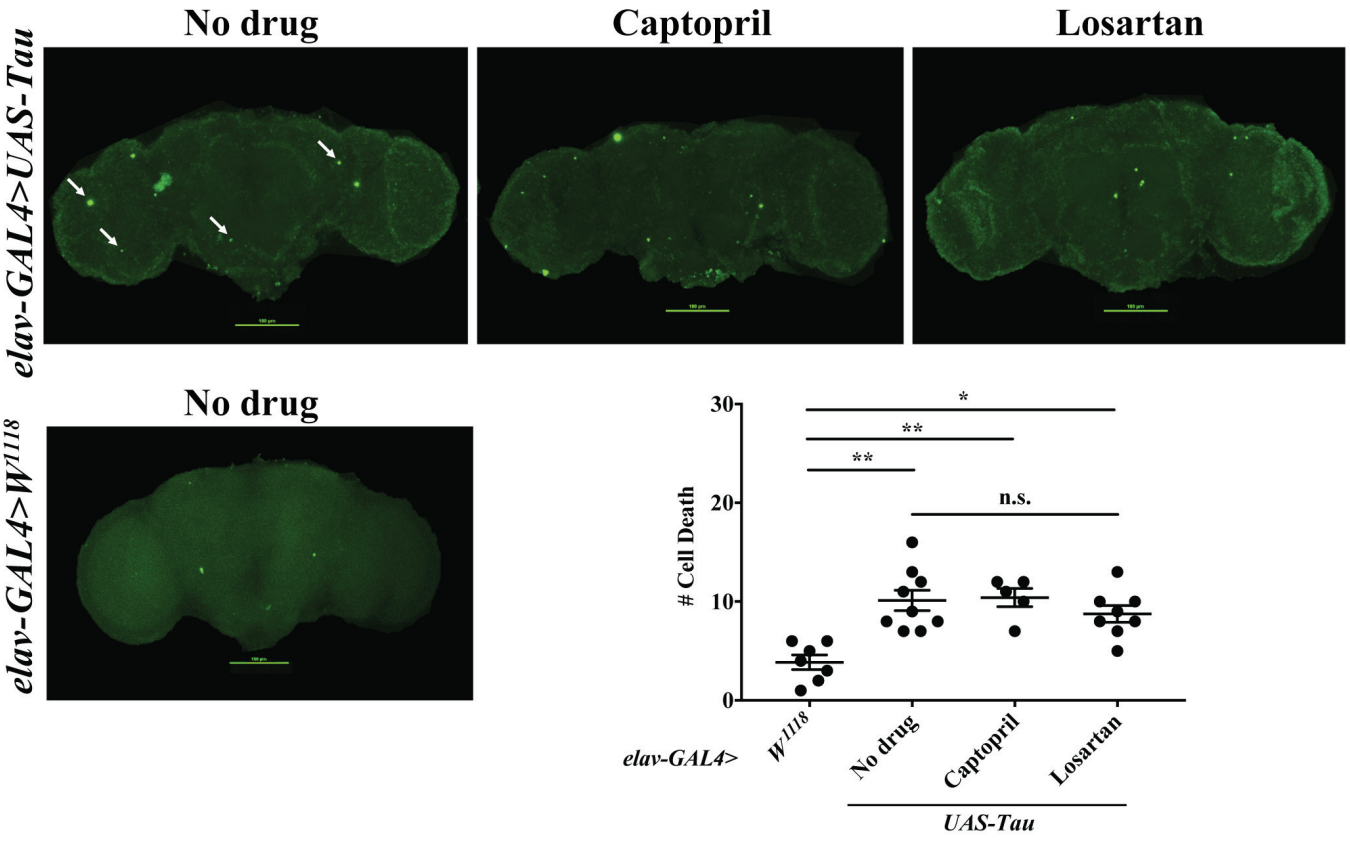


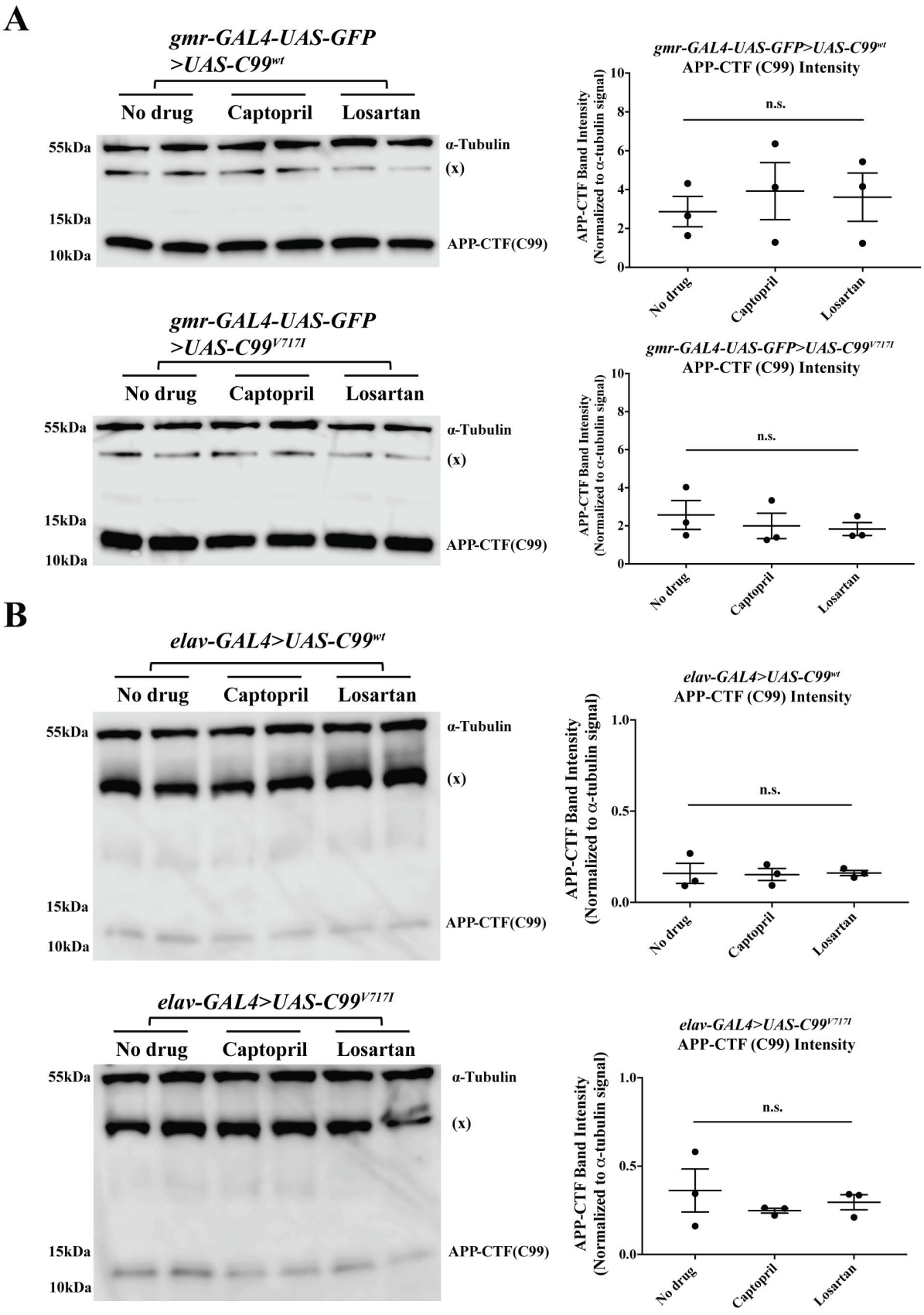












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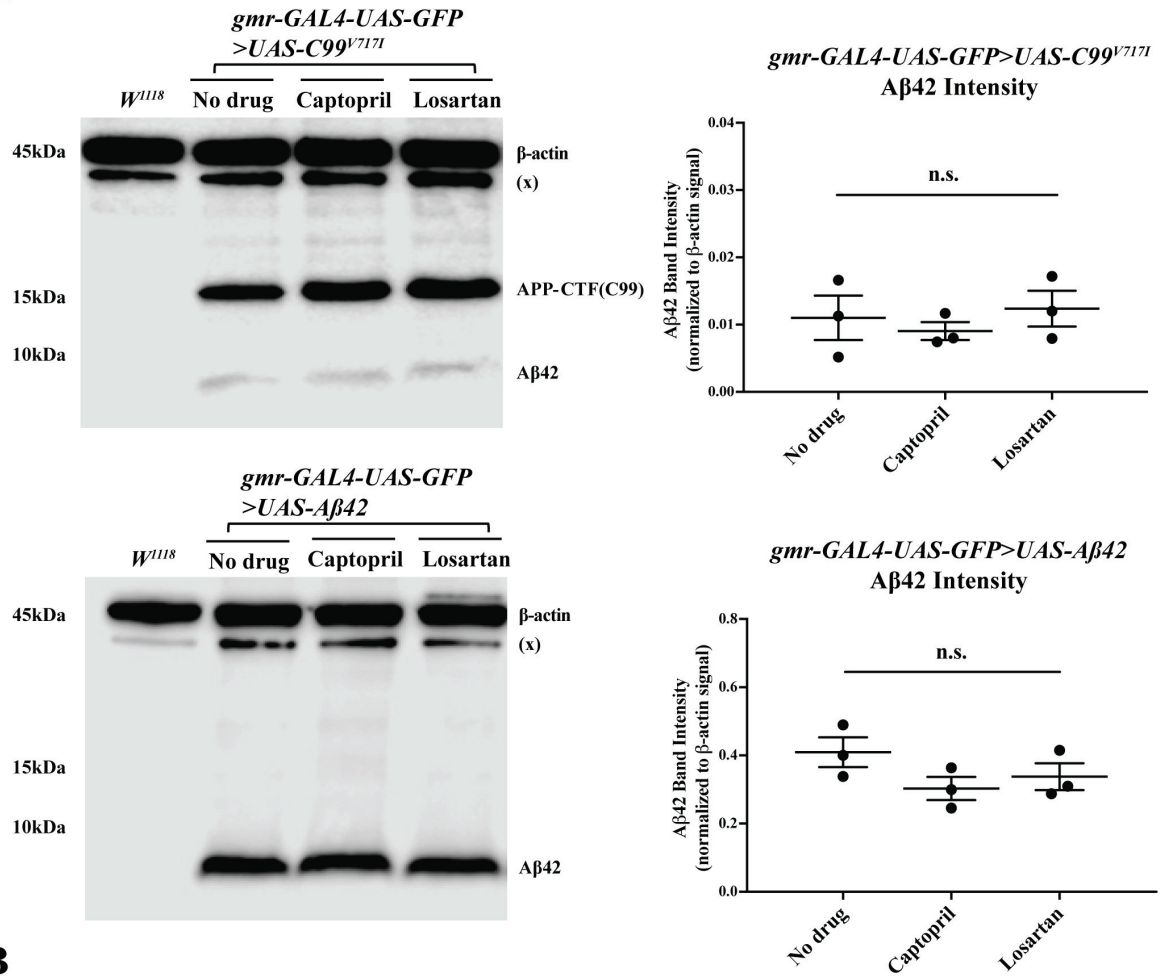

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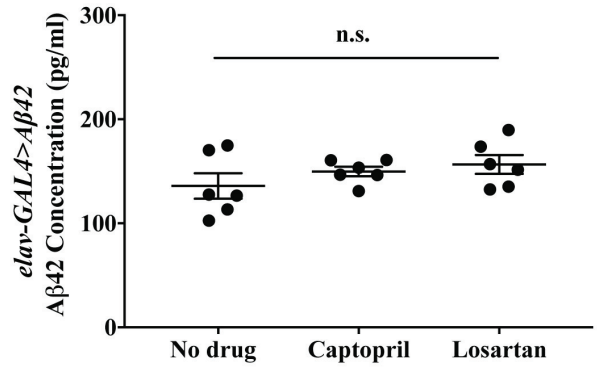

Genotype	Treatment	Aβ42(pg/ml)
elav-GAL4>Aβ42	No drug	135.9 ± 12.20
elav-GAL4>Aβ42	Captopril	149.8 ± 4.561
elav-GAL4>Aβ42	Losartan	156.6 ± 9.038