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Supplemental Information

Neuronal Selection Based on Relative Fitness Comparison Detects and Eliminates Amyloid-β-Induced Hyperactive Neurons in *Drosophila*

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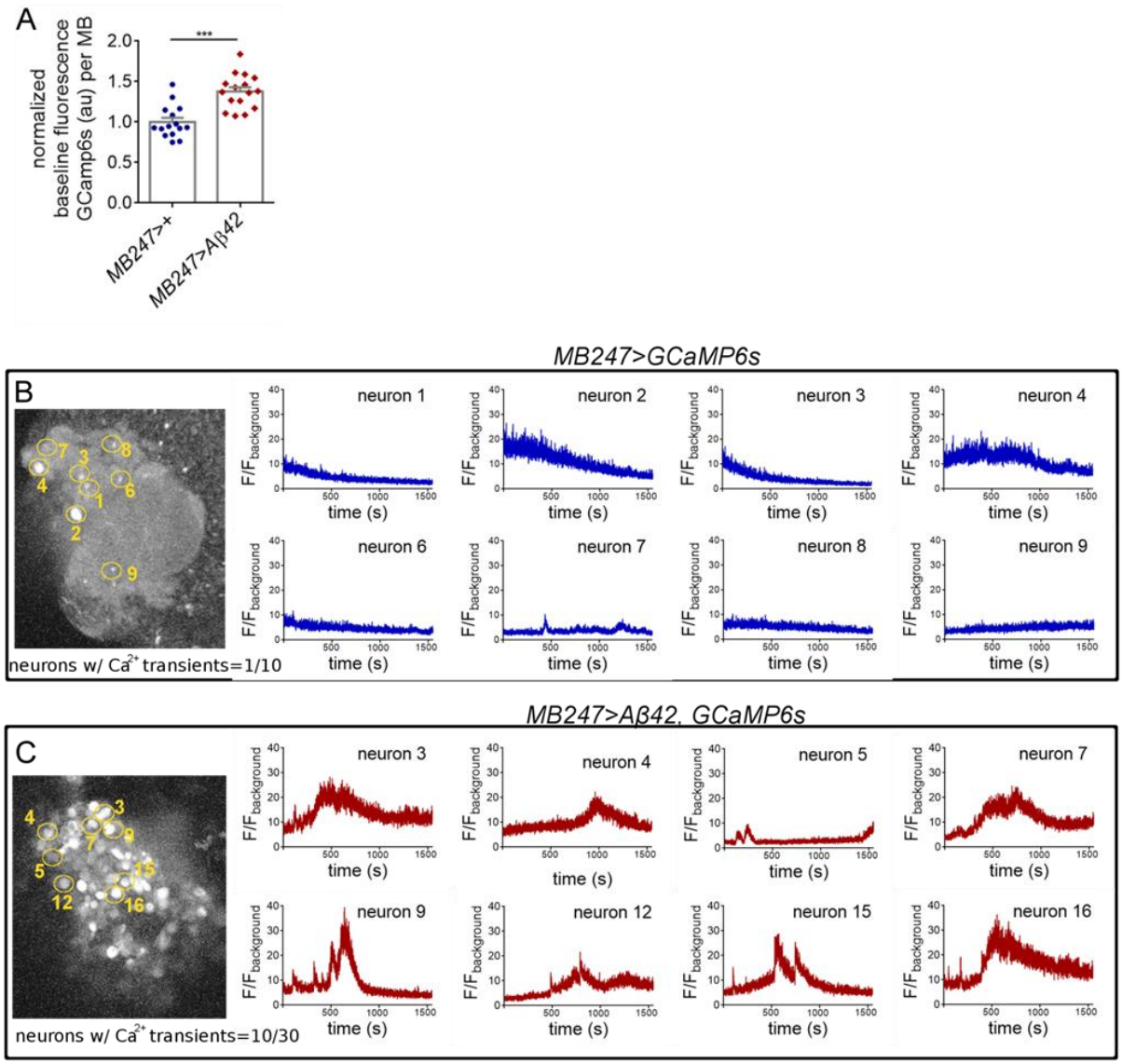

Figure S1. Two-photon Ca^{2+} imaging of *ex-vivo MB247>Aβ42* brains using GCaMP6s. Related to Figure 1.

(A) Mean intensity value of *GCaMP6s* fluorescence measured per mushroom body calyx in non-stimulated *ex-vivo* brains at baseline. Intensity values for *MB247>Aβ42* brains were normalized to the intensity values of control *MB247>+* brains. ***P<0.001, unpaired Student's t-test with Welch's correction.

(B) Plots depicting oscillations of GCaMP6s fluorescence recorded overtime by two-photon imaging for representative individual neurons in a plan of the mushroom body in *MB247>+ ex-vivo* brains. GCaMP6s fluorescence was acquired every 0.5s and normalized to the background fluorescence at each time point.

(C) Plots depicting oscillations of GCaMP6s fluorescence recorded overtime by two-photon imaging for representative individual neurons in a plan of the mushroom body in *MB247>Aβ42 ex-vivo* brains. GCaMP6s fluorescence was acquired every 0.5s and normalized to the background fluorescence at each time point.

Experiments represented in B and C were replicated more than 2 independent times and similar results were obtained.

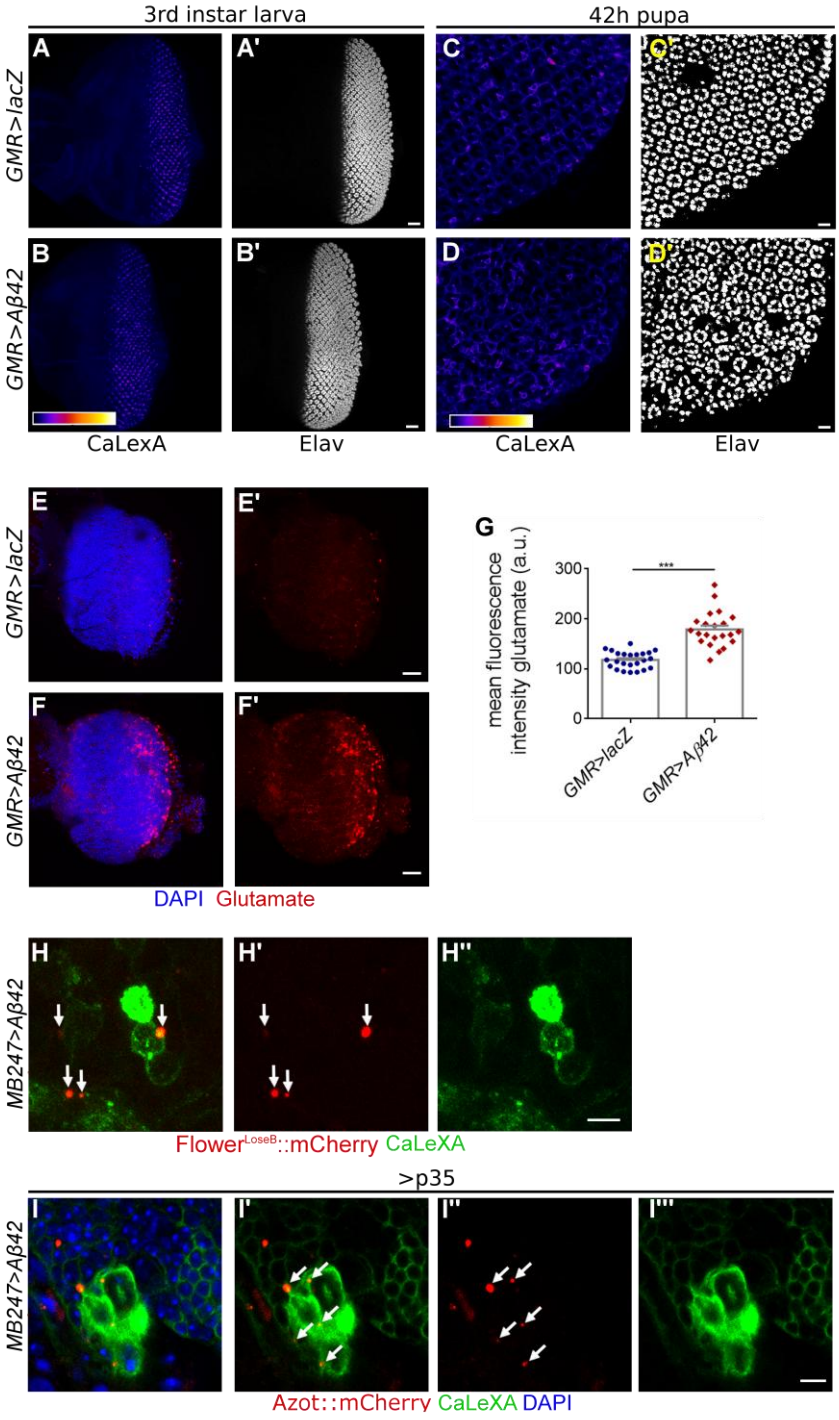

Figure S2. CaLexA pattern in neuronal tissue of the larva, pupa or adult in the presence of heterologous Aβ42. Related to Figure 1.

(A, B) Expression of the CaLexA system (pseudocolored fire) in the eye imaginal disc of *GMR>+* (A) or *GMR>Aβ42* (B) third instar larvae. Neuronal nuclei marked by the anti-Elav antibody are in white. Scale bar, 20µm.

(C,D) Expression of the CaLexA system (pseudocolored fire) in the pupal retina of *GMR>+* (C) *or GMR>Aβ42* individuals (D), at 42h pupation. Nuclei marked by Elav antibody are in white. Scale bar, 10µm.

(E,F) Immunohistochemistry with anti-glutamate antibody (red) in the optic lobe of *GMR>Aβ42 (F) or GMR>lacZ (E)* flies at 15 days of age. Nuclei are in blue. Scale bar: 20µm.

(G) Graph represents the mean fluorescence intensity for anti-glutamate staining per optic lobe for the genotypes *GMR>Aβ42 or GMR>lacZ.* ***P<0.001, unpaired Student's t-test with Welch's correction.

(H) Kenyon cells activating CaLexA (green) exhibit positive signal for the Flower^{LoseB}::mCherry (red) fusion protein at membranes of the soma and neuropile projections (arrows).

(I) Presence of Azot::mCherry positive-foci (red) at the membrane of Kenyon cells which are inducing the expression of the CaLexA system (green) - arrows. Apoptosis of less fit neurons is prevented in these flies by driving a *UAS-p35* construct.

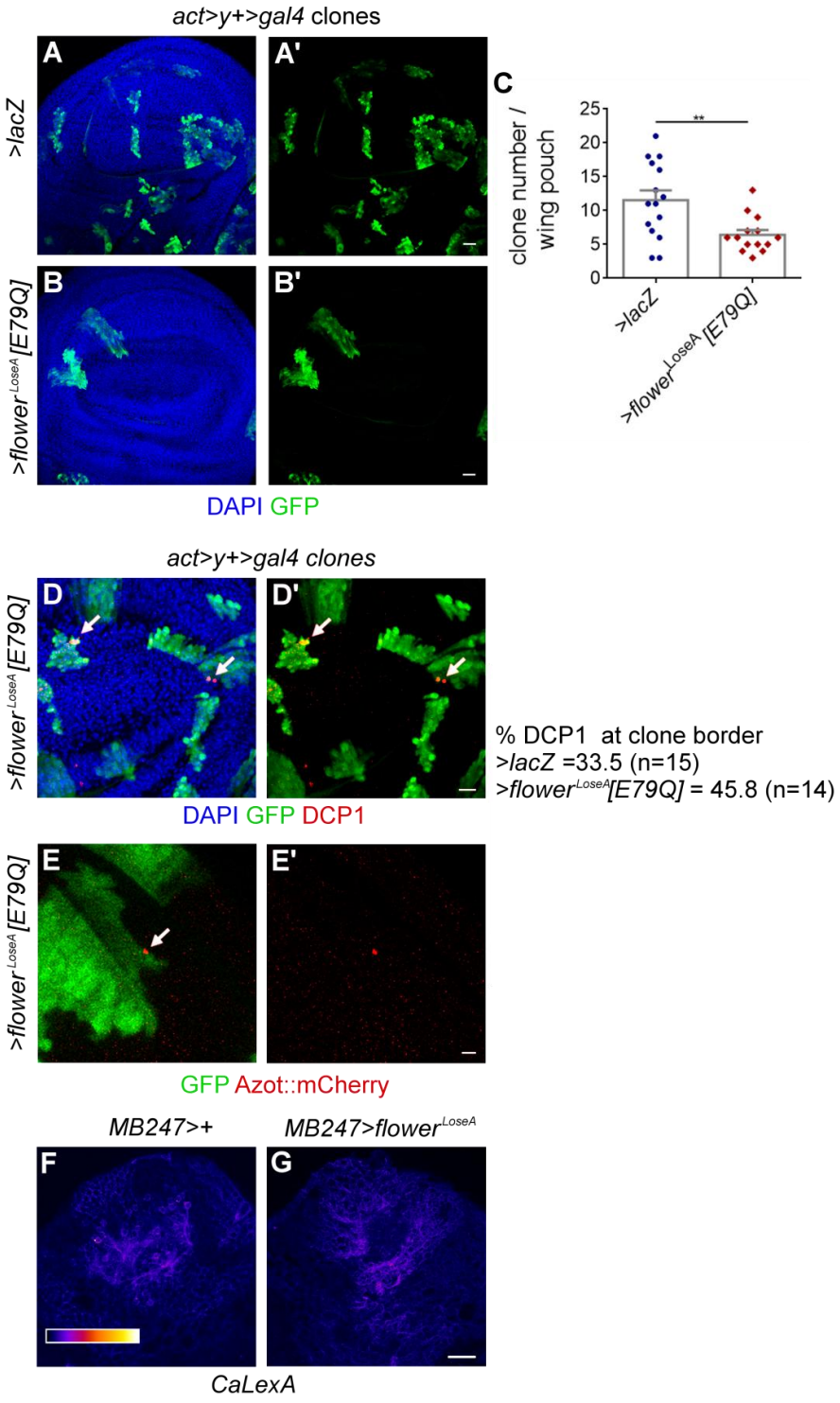

Figure S3. Clones overexpressing the mutant form *flower*$^{LoseA[E79Q]}$ are eliminated from wing discs. Related to Figure 2.

(A,B) Clones induced by heat-shock of the flip-out cassette *act>y+>gal4* and marked by *UAS-GFP* (green) are driving the expression of *UAS-lacZ (A)* or *UAS-flower*$^{LoseA[E79Q]}$ (B). Images show clones in the wing imaginal discs of third instar larvae at 72h ACI (after clone induction). DAPI is in blue. Scale bar: 20µm.

(C) The number of GFP clones per wing pouch was counted for each genotype at 72h ACI and plotted as shown. **P value<0.01, Mann-Whitney U test.

(D) Expression of the apoptotic marker DCP1 (red) at the borders of clones (arrows) overexpressing *UAS-flower*$^{LoseA[E79Q]}$ (green), 72h ACI. DAPI is in blue. Scale bar: 10µm.

(E) Azot-mCherry reporter (red) is detected at the borders of clones (arrow) expressing *UAS-flower*$^{LoseA[E79Q]}$ (green) at 72h ACI. Scale bar: 5µm.

(F,G) CaLexA activation (pseudocolored fire) in the mushroom body of *MB247>* + (F) or *MB247>UAS-flower*^{LoseA} (G) flies. Scale bar 10µm.

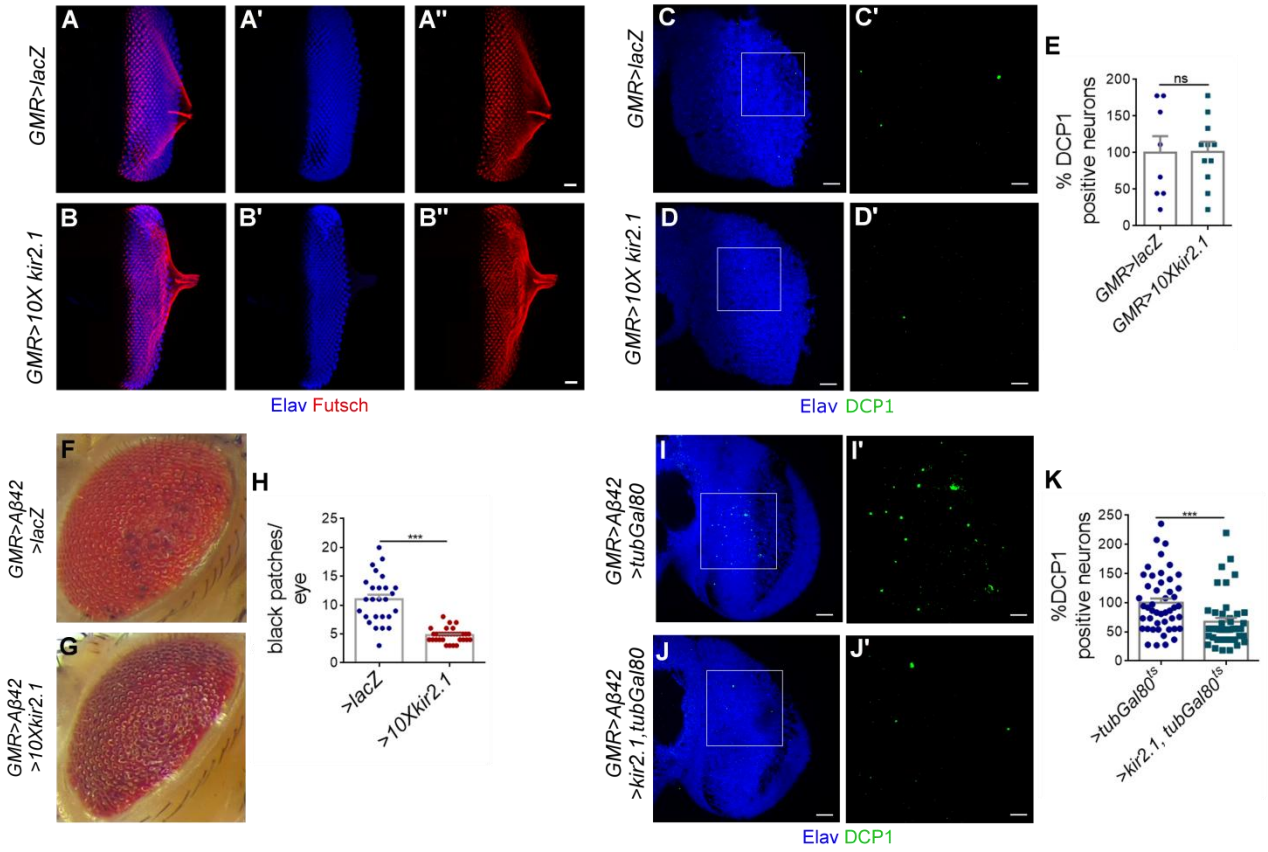

Figure S4. Ectopic expression of Kir2.1 reduces amyloid-β-induced cell death. Related to Figure 4.

(A-B) Staining for the neuronal markers Elav (blue) and Futsch (red) in eye imaginal discs of *GMR>UASlacZ* (A) or *GMR>10XUAS-kir2.1* (B) third instar larva. Scale bar: 20µm

(C,D) Expression of *10XUAS-kir2.1* under the control of the *GMR-Gal4* driver does not perturb differentiation of neurons and does not cause increased apoptosis in the optic lobe. DCP1 marks dying cells (green) and Elav labels differentiated neurons (blue). Scale bar: 20µm or 8 µm in the inset.

(E) Quantification of apoptotic cells marked by DCP1 (green) in the optic lobe of *GMR>UASlacZ* (C) or *GMR>10XUAS-kir2.1* (D) flies. Ns: no significant, Mann-Whitney U non-parametric test.

(F,G) Degenerative eye phenotype of 4-5 days old adult flies raised at 25ºC for the following genotypes: *GMR>Aβ42 / UAS-lacZ* (F) and *GMR>Aβ42 / 10XUAS-kir2.1* (G).

(H) Quantification of the number of black necrotic patches counted per eye for the genotypes indicated before in (F,G). ***P value<0.001, Krustal-Wallis test with Dunn's post-hoc test.

(I-K) Representative images of apoptotic cells marked by DCP1 (green) in the optic lobe of *GMR>Aβ42 / >UAStubGal80ts* (I) or *GMR>Aβ42 / UASkir2.1, tubGal80ts*(J) flies and respective quantification (K). To restrict Gal4 expression to adulthood, flies were raised at 18ºC and transferred to 30ºC 1-2 days after eclosion. Neurons are shown in blue. Scale bar: 20µm or 6µm in the inset. ***P value<0.001, Mann-Whitney U non-parametric test.

Error bars show standard error mean.

Transparent Methods

Genetics

All experimentation was conducted on *Drosophila melanogaster*. The number of flies used was the minimum necessary to achieve the objectives and the best practices were employed. Flies were treated in a humane manner, using CO_2 as anesthetic and were kept according to applicable European and Institutional guidelines.

All stocks were maintained on a standard cornmeal/molasses medium at 25°C in a 12 hr light/dark cycle, unless otherwise indicated. Male flies were used as subjects in all experiments. The following stocks were obtained from the Bloomington stock center: *GMR-Gal4* (second chr, RRID: BDSC_1104); *20XUAS-GCaMP6s* (second chr., BDSC #42746), *UAS-diap1* (second chr., RRID: BDSC_63820); *UAS-lacZ* (second chr., RRID: BDSC_3955); *MB247-Gal4* (third, RRID: BDSC_50742), *UAS-p35* (second chr., RRID: BDSC_5072). The *GMRGal4>(2x)UAS-Aβ42* model was generated for Casas-Tinto et al., 2011. The following transgenic lines were described in previous publication of our laboratory: *flower*^{LoseB}*::mCherry KI* (third Chr.) and *azot::mCherry* reporter line (third chr.) in Coelho et al., 2018; *UAS-flower*$^{LoseA/B}$ *long hairpin RNAi* in Merino et al., 2013; *UAS-flower*^{LoseA} in Rhiner et al., 2010. The CaLexA system whose complete genotype is *w;lexAop-CD8-GFP-2A-CD8-GFP/CyO; UAS-mLexA-VP16-NFATC(H-2), LexAop-CD2-GFP/TM6b* was designed for Masuyama et al., 2012. Flies carrying the translational reporter Flower$^{LoseB::}$Myc or the point mutation *UAS-flower*$^{LoseA[E79Q]}$ were gifts from Hugo Bellen (Yao et al., 2009). The *UAS-fwe dsRNA* transgenic line was obtained from the Vienna Drosophila Resource Center (second chr, GD collection VDRC #39596, RRID: FlyBase_FBst0463114). The stock *10XUAS-kir2.1::EGFP* was generated by the Gwyneth M.Card lab (von Reyn et al., 2014). Additional stocks were obtained from other research laboratories at Champalimaud: *UAS-trpA1* (second chr.) was obtained from the Chiappe lab; *UAS-kir2.1* inserted on the second chromosome and the recombinant lines *tubGal80,UAS-kir2.1* and *chat-Gal4,UAS-GFP* were obtained from the Vasconcelos lab.

Neuronal activation and neuronal silencing

To activate TRPA1, flies were collected 1 day after eclosion and transferred to 30ºC for 4days until dissection. Control non-activated flies were kept at 22ºC for the same time period.

When using the *tubGAL80*^{ts} transgene, flies were raised at 18°C and placed at 30ºC shortly after eclosion for Gal4 expression and *kir2.1* induction.

When monitoring neuronal activity with GCaMP6s or CaLexA, vials from different experimental groups were always kept side-by-side to ensure all flies were subjected to same environmental stimuli prior to experiments.

Immunohistochemistry and image acquisition

For clone induction, larvae were given a heat shock at 37ºC, 48h or 72h before dissection. For pupal dissections, white prepupae (0hr) were collected and maintained at 25°C for 42h. For adult brain dissections, males were collected 0-1 days after eclosion and maintained at 25ºC until the required age. Dissections were performed in chilled PBS, samples were fixed for 30min in formaldehyde (4% v/v in PBS) at room temperature and washed for 60min with PBT 0,4%Triton. For blocking, samples were incubated for 1h at room temperature in 10% normal goat serum (Sigma Aldrich, Cat# G9023) in PBT and incubated overnight with the primary antibody diluted in the same solution at 4ºC. The following antibodies were used: rat anti-Elav (1:50; Developmental Studies Hybridoma Bank, DSHB, Cat#7E8A10, RIDD AB_528218); mouse anti-Futsch (1:200, DSHB Cat#22C10, RRID:AB_528403); polyclonal anti-Myc-tag (1:50; Cell Signaling Cat#2272; RRID:AB_10692100), cleaved DCP1 (1:100, Cell Signaling Cat#9578,

RRID:AB_2721060), chicken anti-GFP (1:500 Abcam Cat#ab13970, RRID:AB_30079813970). TUNEL staining (Roche Cat#3333574001 and Roche Cat#11093070910) was performed according to the supplier's protocol and modified as previously (Lolo et al., 2012). Samples were mounted in Vectashield (Vectorlab Cat#H-1200) and imaged on a Zeiss LSM 880 using a 20X dry objective or a 40X oil objective.

Calcium imaging experiments with GCaMP6

For two-photon Ca^{2+} imaging experiments, flies expressed the Ca^{2+} indicator GCaMP6s in the mushroom body. To obtain *ex-vivo* preparations of the adult brain, flies were dissected in a saline solution composed of 108mM NaCl, 5mM KCl, 4mM $NaHCO_3$, 1mM NaH2PO4, 5mM Trehalose*$2H_20$, 10mM sucrose and 5mM HEPES. Isolated brains were immediately transferred to a coated Petri dish and covered with the same saline solution supplemented with 2mM $CaCl_2$*$2H_20$ and 8.2mM $MgCl_2$*$6H_2O$.

An Ultima two-photon laser-scanning microscope from Bruker (Billerica) and a Coherent Chameleon XR laser were used for imaging. Images were acquired with an Olympus BX61 microscope equipped with a 40X 0.8 NA objective. The image was zoomed to allow the selection of a region of interest (ROI) including the mushroom body calyx. No stimulation was performed. A z-stack of the mushroom body calyx was acquired for each sample at time=0s and without stimulation to obtain a measure of GCaMP6s fluorescence at baseline for each genotype. In addition, we recorded videos of the spontaneous oscillations of GCaMP6s fluorescence by acquiring images of the same ROI every 0.5s in a single plan for 30min.

Image analysis and Statistics

Image quantification and fluorescent intensity measurements were done with Fiji.

To determine GCaMP6s fluorescence, we delineated by hand and around the observable soma of each neuron a second ROI. We measured the baseline fluorescence per neuronal soma at time =0s and then calculated the mean intensity value at each time frame. To calculate the normalized oscillation in the relative fluorescence of *GCaMP6s* overtime, we divided the intensity fluorescence per each neuronal soma in the n^{th} frame by the background intensity of the same frame.

We quantified CaLexA signal on 30µm-wide maximum projections that included almost the total width of the mushroom body calyx in males at 10days (except for data in Fig.2A where males with 5, 10 or 15days were used). When acquiring images, we adjusted the gain so that the genotype with the strongest signal did not saturate the dynamic range of the PMT detector, and we imaged all the brains with the same settings and as close as possible time-wise. To quantify the number of saturated pixels for CaLexA as depicted in Fig.1, a histogram displaying the distribution of number of pixels per fluorescence intensity value was obtained for each z-projection. Pixels with intensities values above 75% of the maximum value were counted as saturated. We scored as CaLexA-activating neurons as shown in Fig2, Kenyon cells exhibiting intense signal of GFP in the soma comparing to other neurons in the vicinity. The number of CaLexA-activating neurons was scored blindly across different genotypes. The number of hyperactive neurons scored for each sample was divided by the mean number of CaLexA-positive neurons present in the control genotype of the same experimental replicate and represented as a percentage of it. In the end, results from all experimental replicates were pooled for each genotype and mean and SEM were calculated.

The number of positive cells for FlowerLoseB::mCherry or Azot::mCherry signal represented in Figures 3 or 4 was determined with the FIJI tool 'Find Maxima' in maximum-intensity projections of the optic lobe. Z-stacks spanned approximately 40µm of each optic lobe starting from the anterior side of the brain. Noise signal was removed

beforehand with a Gaussian blur filter (sigma =1 or sigma=1.5). Comparisons were made between data collected either from parallel experiments, with exactly the same solutions, timeline, and imaging conditions, or from different batches of experiments normalized to a common control. For example, data for Fig. 3 were collected separately, but within each batch there was always one group of UAS-lacZ at 30ºC, whose signal served as the common denominator.

The mean intensity fluorescence for glutamate in the optic lobe (or for GCaMP6s in the mushroom body) was determined by measuring the mean intensity per pixel in ROIs with the same size.

In images illustrating co-localization experiments, we show single slices (**Fig. 1K, Fig. 1L, Fig. 2B, Fig.S2H, Fig.S2I, Fig.S3D, Fig.S3E)** while for the rest of the panels, we show maximum-projections (with the same thickness when comparing between different genotypes).

All statistical analyses were performed using GraphPad Prism software version 6.0 (GraphPad Software). The number of Flower^{LoseB}::mCherry and Azot::mCherry positive cells in the adult optic lobes (**Fig. 3F,J**; **Fig. 4E,J**) and the number of black patches in the adult eye **(Fig.S4H**) were analyzed with a Krustal-Wallis test with Dunn's post-hoc paired comparisons. Mann-Whitney U non-parametric tests were performed to determine significant differences between two groups for the following variables: baseline fluorescence of GCaMP6s per neuron (**Fig.1C**), number of saturated CaLexA pixels (**Fig.1G**), number of TUNEL-positive cells in the adult optic lobe (**Fig. 4M**), clone number per wing pouch (**Fig. S3C**) and percentage of DCP-1 positive cells **(Fig.2F, Fig. S4C and Fig.S4K**). We compared the number of CaLexA-labelled neurons across time points with one-way ANOVA and Holm-Sidak's multiple comparisons tests (**Fig.2A**). An unpaired Student's t-test with Welch's correction was used to analyze the number of CaLexA-activating neurons (**Fig.2E, J**), the normalized baseline fluorescence of GCaMP6s per mushroom body (**Fig. S1A**), the fluorescence intensity of glutamate (**Fig. S2G**) number of TUNEL-positive cells in the adult optic lobe (**Fig.3M,P)**.

All the p-values are two tailed and all graphs are displayed as mean ± standard error.

Supplemental References

Casas-Tinto, S., Zhang, Y., Sanchez-Garcia, J., Gomez-Velazquez, M., Rincon-Limas, D.E., and Fernandez-Funez, P. (2011). The ER stress factor XBP1s prevents amyloid- neurotoxicity. Human Molecular Genetics *20*, 2144–2160.

von Reyn, C.R., Breads, P., Peek, M.Y., Zheng, G.Z., Williamson, W.R., Yee, A.L., Leonardo, A., and Card, G.M. (2014). A spike-timing mechanism for action selection. Nat Neurosci *17*, 962–970.