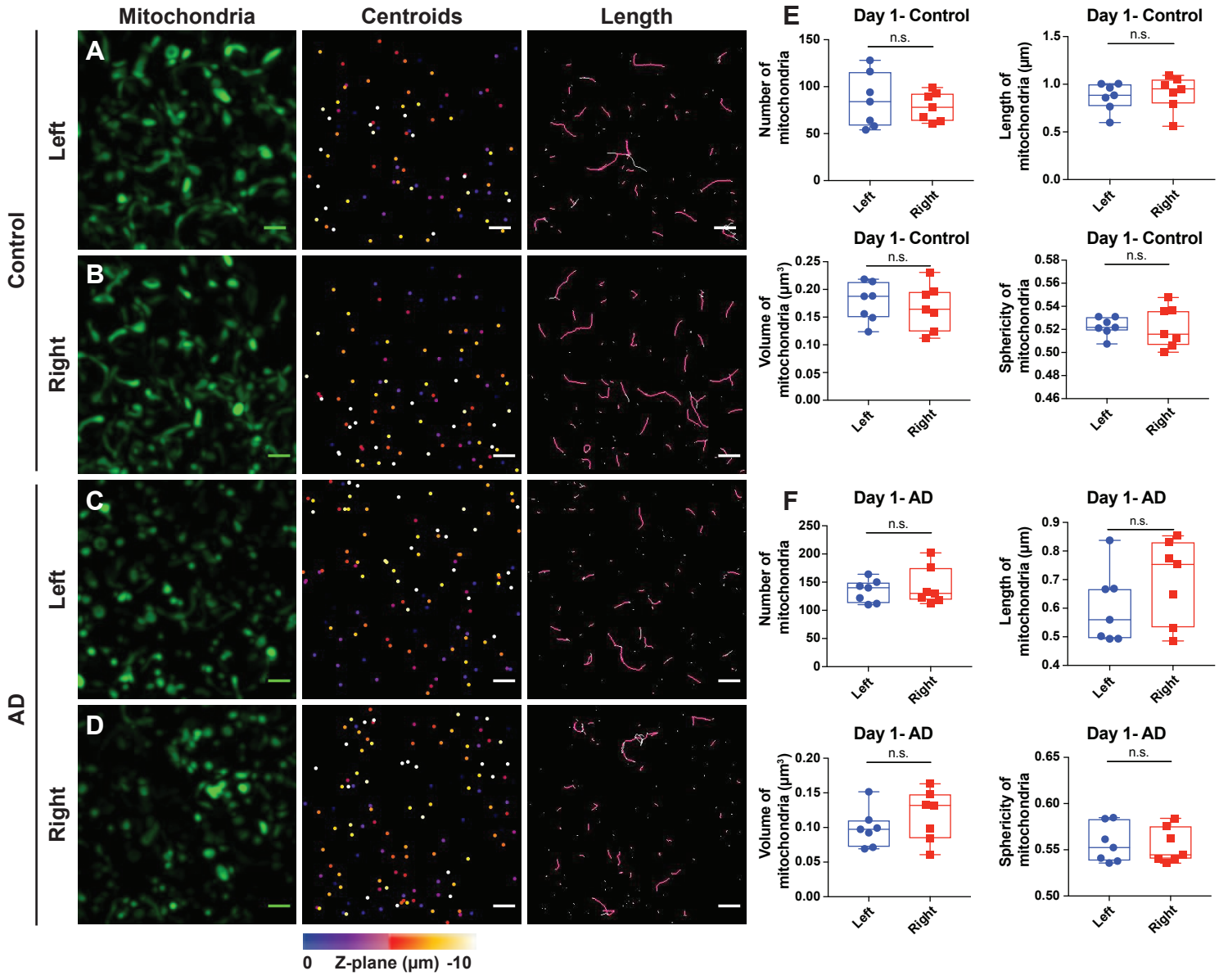


Wang et al., Figure S1

Figure S1. No hemispheric differences in mitochondrial morphology in control and Aβ42-expressing flies

(A) Maximum projection images of individual mitochondria, mitochondrial centroids and their lengths in a 10 μm^3 ROI from the somatic region in the left hemisphere of a control brain at 1 day-of-age. Genotype: *UAS*-mito-GFP/+; *R13F02*-Gal4/+. Scale bar 1 μm.

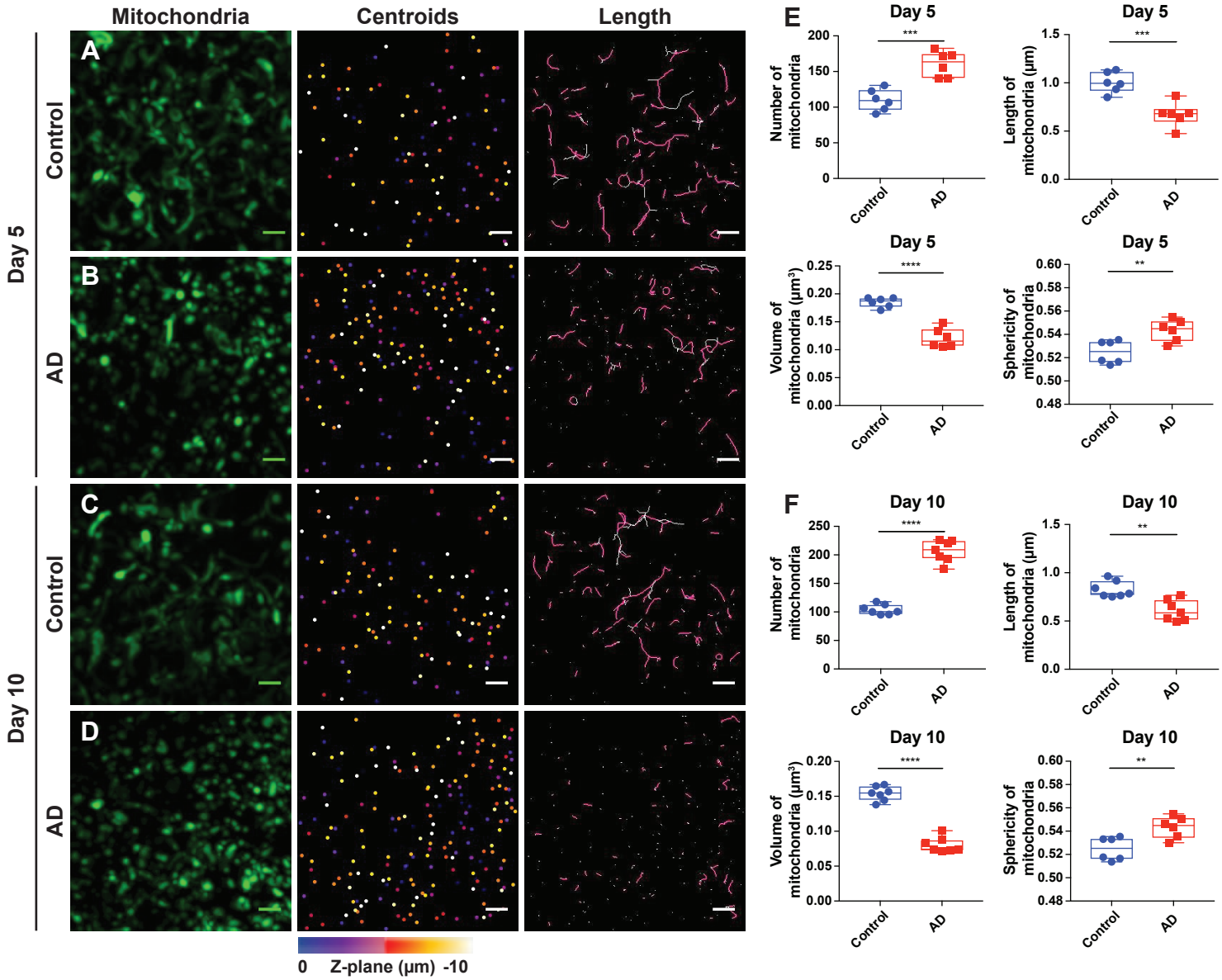
(B) Same as panel A for the somatic region in the right hemisphere of a control brain at 1 day-of-age.

(C) Same as panel A for the somatic region in the left hemisphere of an Aβ42-expressing brain at 1 day-of-age. Genotype: *UAS*-mito-GFP/ *UAS*-Aβ42; *R13F02*-Gal4/ *UAS*-Aβ42.

(D) Same as panel C for the somatic region in the right hemisphere of a Aβ42-expressing brain at 1 day-of-age.

(E) Box plots of somatic mitochondrial parameters in both hemispheres of control brains at 1 day-of-age. No differences were observed in any of the parameters. Mann-Whitney U, n=7. Each box plot shows the median, interquartile range and the range.

(F) Same as panel E for both hemispheres of Aβ42-expressing brains at 1 day-of-age. No differences were observed in any of the parameters. Mann-Whitney U, n=7.



Wang et al., Figure S2

Figure S2. Mitochondrial morphology in the soma of MBn expressing Aβ42 at 5- and 10-days of age.

(A) Maximum projection images of individual mitochondria, mitochondrial centroids and their lengths in a 10 μm^3 ROI from the somatic region of a control brain at 5 days-of-age. Genotype: *UAS*-mito-GFP/+; *R13F02*-Gal4/+. Scale bar 1 μm.

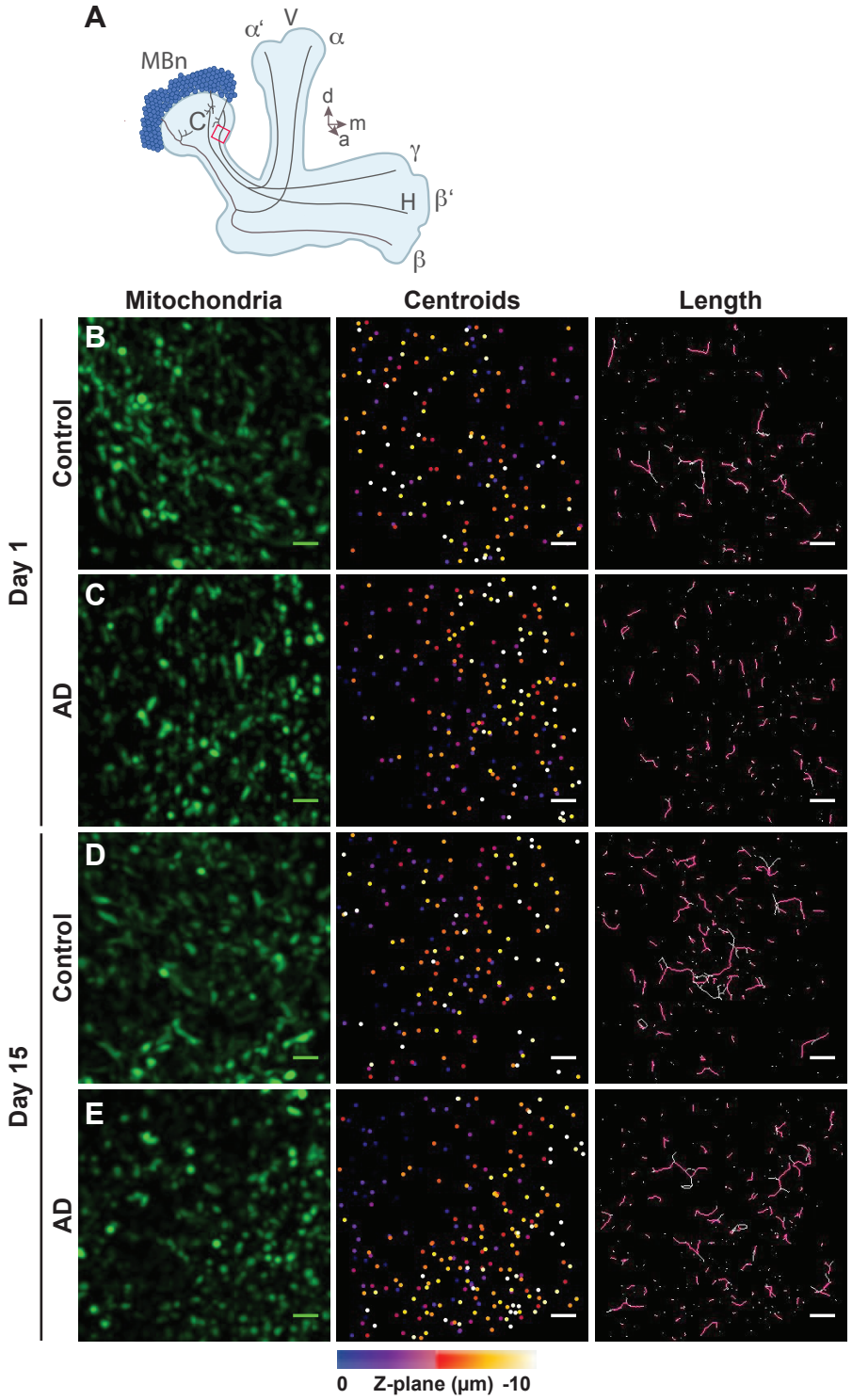
(B) Same as panel A for the somatic region of a Aβ42-expressing brain at 5 days-of-age. Genotype: *UAS*-mito-GFP/*UAS*-Aβ42; *R13F02*-Gal4/*UAS*-Aβ42.

(C) Same as panel A for a control brain at 10 days-of-age.

(D) Same as panel B for an Aβ42-expressing brain at 10 days-of-age.

(E) Box plots of somatic mitochondrial parameters in control and Aβ42-expressing flies at 5 days-of-age. Mitochondria are more numerous (***P< 0.001), shorter (***P< 0.001) and of reduced volume (****P< 0.0001) with increased sphericity (**P< 0.01) in the Aβ42-expressing flies. Mann-Whitney U, n=6. Each box plot shows the median, interquartile range and the range.

(F) Same as panel F for flies at 10 days-of-age. Mitochondria are more numerous (****P< 0.0001), shorter (**P< 0.01) and of reduced volume (****P< 0.0001) with increased sphericity (**P< 0.01) in the Aβ42-expressing flies. Mann-Whitney U, n=6.



Wang et al., Figure S3

Figure S3. Mitochondrial morphology in the dendrites of MBn expressing Aβ42.

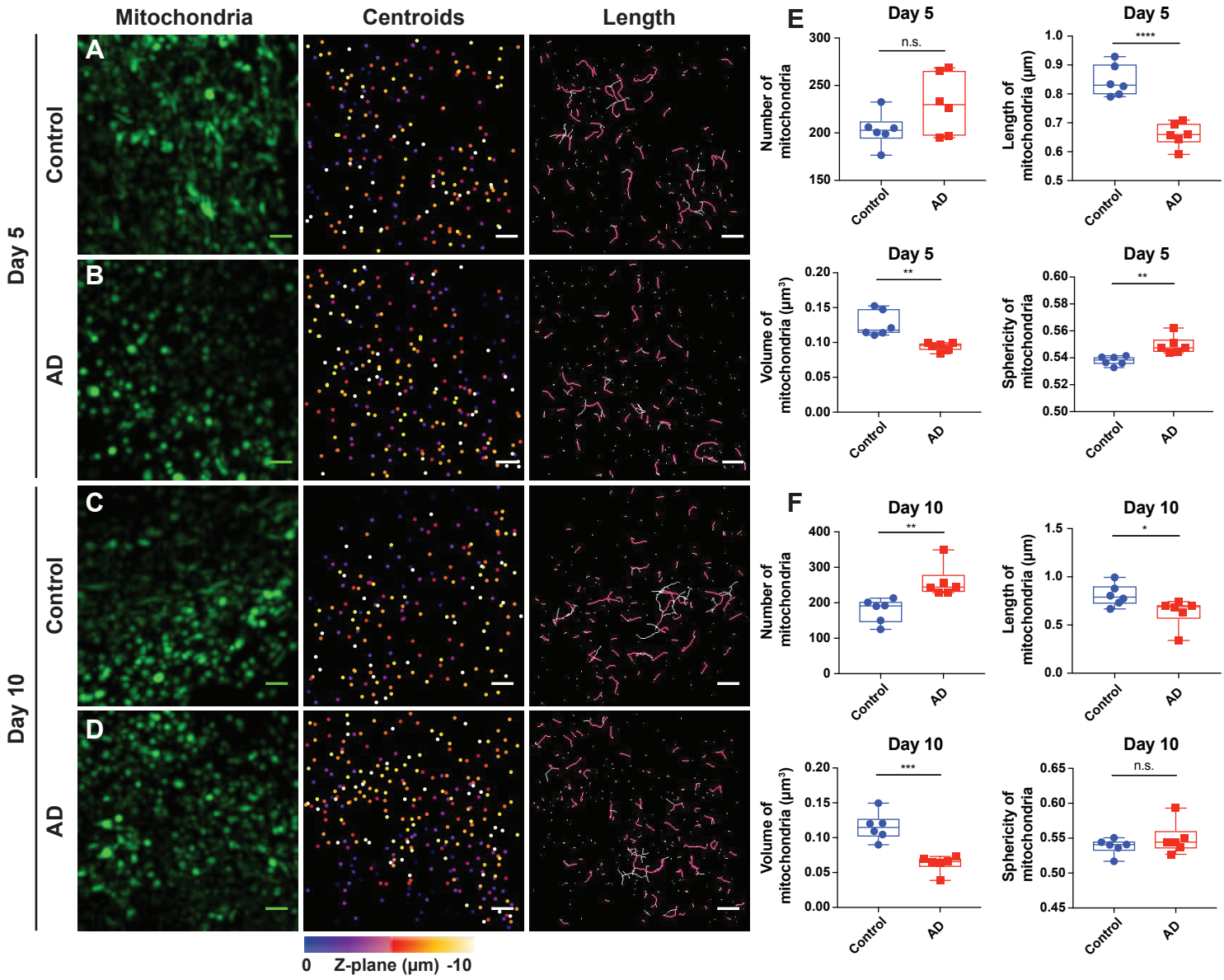
(A) ROI for characterizing dendritic mitochondria. See Figure 1.5A for more description.

(B) Maximum projection images of individual mitochondria, mitochondrial centroids and their lengths in a 10 μm^3 ROI from the dendritic region of a control brain at 1 day-of-age. Genotype: *UAS*-mito-GFP/+; *R13F02*-Gal4/+. Scale bar 1 μm. Quantification of image data is found in Figure 1.5B.

(C) Same as panel B for an Aβ42-expressing brain at 1 day-of-age. Genotype: *UAS*-mito-GFP/*UAS*-Aβ42; *R13F02*-Gal4/*UAS*-Aβ42. Quantification of image data is found in Figure 1.5E.

(D) Same as panel B for a control brain at 15 days-of-age. Quantification of image data is found in Figure 1.5C.

(E) Same as panel C for an Aβ42-expressing brain at 15 days-of-age. Quantification of image data is found in Figure 1.5F.



Wang et al., Figure S4

Figure S4. Mitochondrial morphology in the dendrites of MBn expressing Aβ42 at 5- and 10-days of age.

(A) Maximum projection images of individual mitochondria, mitochondrial centroids and their lengths in a 10 μm^3 ROI from the dendritic region of a control brain at 5 days-of-age. Genotype: *UAS*-mito-GFP/+; *R13F02*-Gal4/+. Scale bar 1 μm.

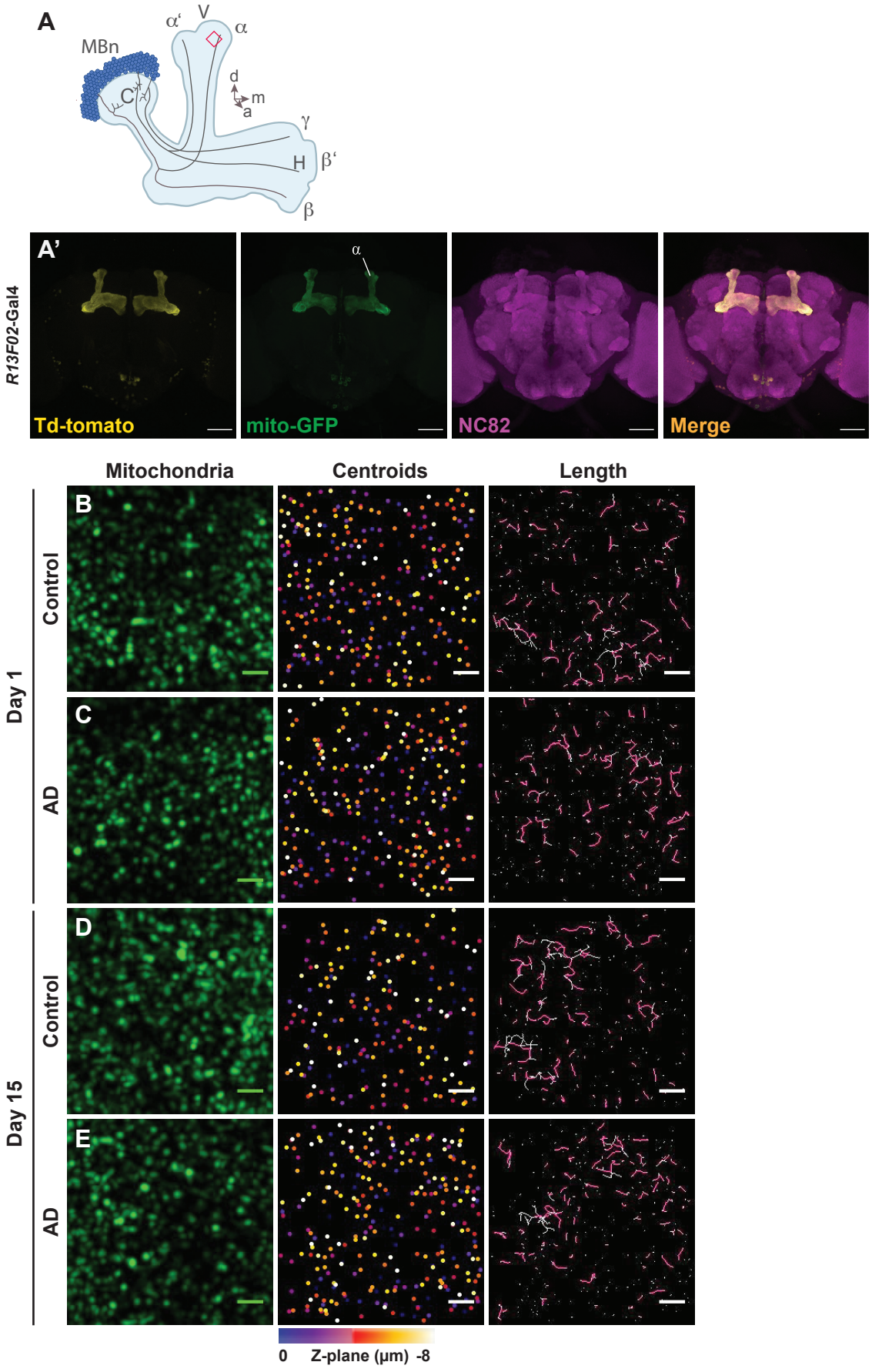
(B) Same as panel A for an Aβ42-expressing brain at 5 days-of-age. Genotype: *UAS*-mito-GFP/*UAS*-Aβ42; *R13F02*-Gal4/*UAS*-Aβ42.

(C) Same as panel A for a control brain at 10 days-of-age.

(D) Same as panel B for an Aβ42-expressing brain at 10 days-of-age.

(E) Box plots of dendritic mitochondrial parameters in control and Aβ42-expressing flies at 5 day-of-age. No difference was observed in number, probably due to experimental variability. The mitochondria were shorter (****P< 0.0001) and of reduced volume (**P< 0.01) with increased sphericity (**P< 0.01) in the Aβ42-expressing flies. Mann-Whitney U, n=6. Each box plot shows the median, interquartile range and the range.

(F) Same as panel F for control and Aβ42-expressing flies at 10 days-of-age. Mitochondria are more numerous (**P< 0.01), shorter (*P< 0.05) and of reduced volume (***P< 0.001) in the Aβ42-expressing flies at this age. Mann-Whitney U, n=6.



Wang et al., Figure S5

Figure S5. Mitochondrial morphology in the axons of MBn expressing Aβ42.

(A) Diagram of the MB in the fly's right hemisphere. See the legend of Figure 1A for a complete description. The eight μm^3 volume ROI (red box), located at a standardized and central location in the bulbous tip of the α lobe as viewed from a frontal perspective, was used to characterize the axonal mitochondria. d=dorsal, m=medial, a=anterior.

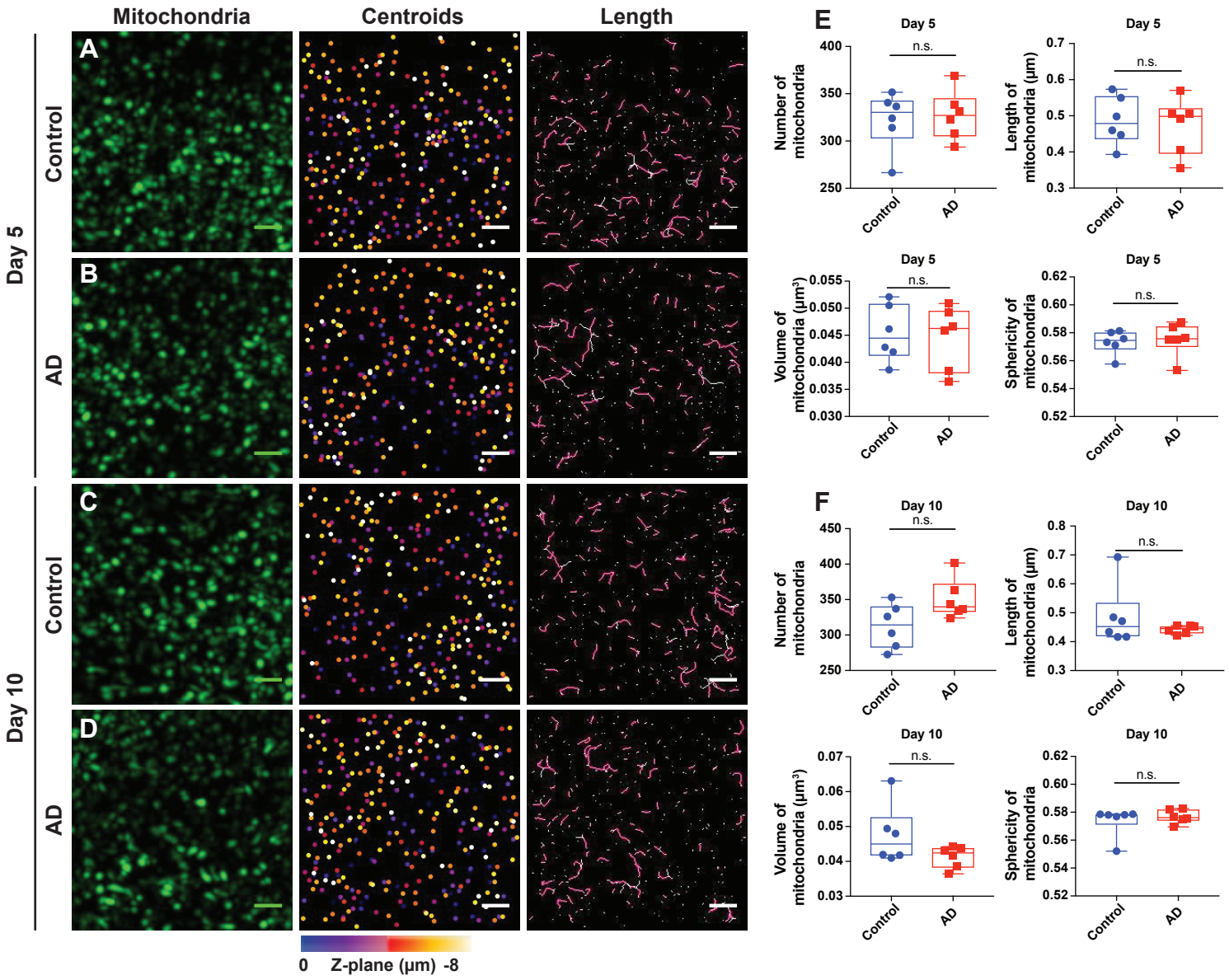
(A') Low resolution maximum projection images of the α/β, α'/β', and γ lobes of the adult MB in both hemispheres. The *R13F02*-Gal4 expression was visualized using *UAS-myr-Td-tomato* and *UAS*-mito-GFP. Brain neuropil was stained with anti-NC82. Scale bar 50 μm.

(B) Maximum projection images of individual mitochondria, mitochondrial centroids and their lengths in an 8 μm^3 ROI from the axonal region of a control brain at 1 day-of-age. Genotype: *UAS*-mito-GFP/+; *R13F02*-Gal4/+. Scale bar 1 μm.

(C) Same as panel B for an Aβ42-expressing brain at 1 day-of-age. Genotype: *UAS*-mito-GFP/*UAS*-Aβ42; *R13F02*-Gal4/*UAS*-Aβ42.

(D) Same as panel B for a control brain at 15 days-of-age.

(E) Same as panel C for an Aβ42-expressing brain at 15 days-of-age.



Wang et al., Figure S6

Figure S6. Mitochondrial morphology in the axons of MBn expressing Aβ42 at 5- and 10-days of age.

(A) Maximum projection images of individual mitochondria, mitochondrial centroids and their lengths in an 8 μm^3 ROI from the axonal region of a control brain at 5 days-of-age. Genotype: *UAS*-mito-GFP/+; *R13F02*-Gal4/+. Scale bar 1 μm.

(B) Same as panel A for an Aβ42-expressing brain at 5 days-of-age. Genotype: *UAS*-mito-GFP/*UAS*-Aβ42; *R13F02*-Gal4/*UAS*-Aβ42.

(C) Same as panel A for a control brain at 10 days-of-age.

(D) Same as panel B for an Aβ42-expressing brain at 10 days-of-age.

(E) Box plots of axonal mitochondrial parameters in control and Aβ42-expressing flies at 5 days-of-age. No difference was observed in number, length, volume or sphericity in the Aβ42-expressing flies. Mann-Whitney U, n=6. Each box plot shows the median, interquartile range and the range.

(F) Same as panel E for control and Aβ42-expressing flies at 10 days-of-age. No difference was observed in number, length, volume or sphericity in the Aβ42-expressing flies. Mann-Whitney U, n=6.

STAR★METHODS

KEY RESOURSE TABLE

REAGENT OR RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse monoclonal anti-GFP	Invitrogen	Cat. #A11120; RRID: AB_221568
Rabbit polyclonal anti-GFP	Invitrogen	Cat. #A11122; RRID: AB_221569
Chicken polyclonal anti-GFP	Molecular Probes	Cat. #A10262; RRID: AB_2534023
Mouse monoclonal anti-NC82	Developmental Studies Hybridoma Bank	RRID: AB_2314866
DsRed polyclonal antibody	Clontech (Takara)	Cat. #632496
Rabbit polyclonal anti-cleaved *Drosophila* Dcp-1 (Asp216)	Cell signaling Technology	Cat. #9578S
Alexa 488 goat polyclonal anti-rabbit lgG	Invitrogen	Cat. #A11008; RRID: AB_143165
Alexa 488 goat polyclonal anti-mouse lgG	Invitrogen	Cat. #A11029; RRID: AB_2534088
Alaxa 488 goat anti-chicken lgG	Invitrogen	Cat. #A11039; RRID: AB_2534096
Alexa 594 goat anti-rabbit lgG	Molecular Probes	Cat. #A11012; RRID: AB_2534079
Alexa 633 goat polyclonal anti-mouse lgG	Invitrogen	Cat. #A21052; RRID: AB_2535719
Other Reagents		
Thioflavin S	Sigma	Cat. #T1892-25G CAS: 1326-12-1
Poly-D-lysine hydrobromide	Sigma	Cat. #P7280-5MG CAS: 27964-99-4
Methyl salicylate	Sigma	Cat. #M6752-250ML CAS: 119-36-8
Fly lines (Backcrossed to *WCS10*)		
WCS10; R13F02-Gal4	Bloomington *Drosophila* Stock Center	48571
WCS10; *UAS*-mito-GFP	Bloomington *Drosophila* Stock Center	8442

WCS10; *UAS*-myr-Td-tomato	Bloomington *Drosophila* Stock Center	32221
WCS10; *UAS*-mitoGCaMP3, *UAS*-RFP/Cyo	[30]	N/A
WCS10; *UAS*-Aβ42;	Bloomington *Drosophila* Stock Center	33769
WCS10; *UAS*-Aβ42	Bloomington *Drosophila* Stock Center	32037
Other supplies		
High precision 18×18 mm coverslips	Marienfeld-Superior	Cat. # 0107032
Mounting medium	Vector Laboratories	Cat. #: H-1000
Schneider's medium (S2)	Life Technologies	Cat. #21720024

Immunohistochemistry and Confocal Imaging

Brains were dissected and processed as previously described [49]. Brains of indicated genotypes were dissected in cold Schneider's medium (S2) and placed in S2 medium with 1% paraformaldehyde (PFA) and rotated at 4 °C overnight. The fixed brains were rinsed quickly in cold PAT3 (0.5% TritonX-100, 0.5% BSA, in phosphate buffered saline) before being transferred into 1 ml PAT3 for 1 h at 23 °C. This step was repeated before transferring the brains to 1 ml blocking buffer (3% normal goat serum in PAT3) for 90 m at 23 °C. Then the brains were then incubated with primary antibodies and 3% normal goat serum in PAT3. They were rotated at 23 °C for 3 h before continuing the incubation at 4 °C overnight. The brains were rinsed in PAT3 and transferred to 1 ml PAT3 for a 1 h incubation at 23 °C. This step was repeated once before adding secondary antibodies and 3% normal goat serum in PAT3 for 3 h at 23 °C. Brains were then incubated in PAT3 at 4 °C for 4 d. After incubation with secondary antibodies, the brains were rinsed in 1 ml PAT3 and incubated in PAT3 for 1 h at 23 °C. This step was repeated once before adding 1 ml 1×PBS as a wash. One ml dH_2O was then used to rinse the brains quickly. They were then mounted between two coverslips with 102 μm spacers. Brains were imaged on Leica TCS SP8 confocal microscope with a 25X water immersion objective using 488 nm and 633 nm excitation lines. Fiji Image J was used for image analysis. A region of interest

(ROI) of identical size was used for both control and Aβ42-expressing brains. Data were imaged from both hemispheres of each brain and averaged to obtain one value for each metric per brain.

Structured illumination microscopy (SIM) and image analysis

Brains were dissected and immunostained as described above. Before dehydration, high precision 18×18 mm coverslips were coated with 1% poly-D-lysine for 24-48 h at 37 °C. The immunostained brains were then placed on the 18×18 mm coverslips, fixed to the coverslips, and dehydrated in an ethanol series (5 m each: 20%, 30%, 50%, 70%, 95%, 100%, 100%). The brains were then cleared in methyl salicylate overnight at 23 °C. They were then mounted in methyl salicylate for SIM. The brains were imaged with a ZEISS ELYRA PS1 Super Resolution Imaging System equipped with the Zeiss Efficient navigation (ZEN) software using a 63X oil immersion objective and 488 nm laser excitation. Z-stacks were imaged at 0.10 μm intervals. The acquired images were then processed using ZEN software followed by Fiji ImageJ analysis. For this step, all of the images were first converted to an 8-bit format. The image background was subtracted followed by a 0.3% enhancement of contrast. The intensity was then adjusted by setting the gamma level to 1.65. The region of interest (ROI) in the cell bodies, dendrites or axons was the selected. The threshold of all the images was adjusted to 21. Two plugins were used for ImageJ, 3D Objects Counter and Skeleton, to obtain the number, surface area, volume and length of individual mitochondria. The following formula was used to calculate the sphericity (Ψ) of mitochondria: $\Psi = \frac{\pi^{\frac{1}{3}}(6V_p)^{\frac{2}{3}}}{S_p}$, where Ψ is sphericity, Vp is volume of the particle, and Sp is surface area of the particle. The values for surface area, volume, length and sphericity were obtained by averaging data across both hemispheres for each fly.

Mitochondrial calcium import

Brains were isolated with saline solution (124 mM NaCl, 3 mM KCl, 20 mM MOPS, 1.5

mM $CaCl_2$, 4 mM $MgCl_2$, 5 mM $NaHCO_3$, 1 mM NaH_2PO_4, 10 mM trehalose, 7 mM sucrose, 10 mM glucose; adjusted to pH 7.2 at 25 °C). Then the brain was placed onto a round coverslip in a small chamber filled with a saline solution. The coverslips were precoated with 1% poly-D-lysine for 24-48 h at 37 °C to facilitate attaching the brains to the coverslips to reduce movement. KCl stimulation was performed by perfusing brains with the 15% KCl prepared in saline solution. Each brain was imaged with a 25X water immersion objective 488 and 633 nm laser excitation on Leica TCS SP8 confocal microscope. The mito-GCaMP3 emission was acquired at 510-550 nm and RFP at 610-700 nm. For the analysis of mitochondrial calcium import, the $\%\Delta F/F_o$ for both mito-GCaMP3 and RFP emission responses were calculated. The initial 5 frames of each recording were averaged as the F_o with the ΔF calculated from the response detected in each frame relative to this baseline. The $\%\Delta F/F_o$ of RFP was subtracted from the mito-GCaMP3 for each frame. The maximum response was calculated as the normalized peak response (averaged across 5 frames at the peak) minus the normalized baseline response.

Cell death assay

Brains were dissected and processed as previously described [49]. After immunostaining as described in the above Section “Immunohistochemistry and Confocal Imaging”, the brains were placed to 1 ml 50% ethanol/dH_2O with 0.25% Thioflavin S and rotated at 4 °C overnight. The brains were then destained in 1 ml 50% ethanol/dH_2O for 10 m and washed in 1 ml 1 X PBS for 5 m. This washing step was repeated twice before mounting the brains. Brains were mounted with mounting medium for fluorescence before imaging. Brains were imaged with a 25X water immersion objective using 488 and 633 nm laser excitation on Leica TCS SP8 confocal microscope. The amyloid burden was calculated as the value of fluorescence in the cell body region of MBn (10×10 μm^2) divided by the value of fluorescence in a standard region outside of MBn (10×10 μm^2). Both hemispheres from each brain were analyzed to obtain one value per brain.

Learning and memory assay

Flies were collected after eclosion. They were maintained in bottles to the indicated ages for aversive olfactory conditioning experiments. The flies were separated into vials (~60 flies per vial) 16 h prior to conditioning. The experiments were performed in a behavioral room under dim red light with 70-75% humidity and held at 25 °C. Before training, flies were transferred to fresh food and placed in the behavioral room for 30 m. Groups of flies were placed into tubes where they received 30 s of air, 1 m of the first odor paired with electric shocks (12 shocks, 90 V, 1.25 s for each shock and 5 s intervals; defined as CS+), 30 s of air, 1 m of the second odor without electric shock (defined as CS-), and 30 s of air. After 3 m, flies of indicated genotypes were placed into a T-maze for a 1 m rest and then allowed to choose between two arms that contained the first and the second odor, respectively. Odors used in the experiment were benzaldehyde (BEN, concentrations: 0.055%-0.085%) and 3-octanol (OCT, concentration: 0.15%). For data analysis, the number of flies in both arms was counted and the performance index (PI) calculated as ((CS-)-(CS+))/ ((CS-)+(CS+)).

Statistical analysis

Statistics were calculated using Graphpad Prism software. The Mann-Whitney U test or a one-way ANOVA followed by Tukey's multiple comparisons were used as indicated in the figure legends.