SNARE-dependent mitochondria-lysosome contacts regulate glial mitochondrial dynamics and dopaminergic neuron survival

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24 **Summary**

25 Mitochondria are dynamic organelles that undergo fission and fusion, enabling swift
 26 structural modification to adapt cellular needs. Disturbances in mitochondrial
 27 dynamics, frequently defects ascribed to neurons, have been associated with
 28 pathological contexts such as neurodegeneration in Parkinson's disease. Nonetheless,
 29 the mechanism of glial mitochondrial dynamics contributing to neurodegeneration
 30 remains unclear. Here we present evidence that the *Drosophila* R-SNARE VAMP7
 31 regulates glial mitochondrial dynamics and dopaminergic neuron survival via
 32 modulating the dynamic of mitochondria-lysosome contact, which determines the
 33 mitochondrial fission site. Independent of its characterized role in
 34 autophagosome-lysosome fusion, glial VAMP7 depletion causes mitochondrial
 35 elongation and dysfunction, increased ROS levels, and production of lipid droplets.
 36 These conferred changes in glia in turn affects nearby dopaminergic neuron survival.
 37 Glial VAMP7 genetically interacts with the mitochondrial fission/fusion factors Drp1
 38 and Marf1 and controls glial mitochondrial dynamics via regulating the frequency and
 39 duration of mitochondria-lysosome contact. Our findings indicate that SNARE
 40 proteins, although not direct mediators on mitochondrial fusion, provide spatial cues
 41 to modulate glial mitochondrial fission via organelle contacts, impacting on neuron
 42 survival in a non-cell-autonomous manner.

43

44 **Keywords**

45 VAMP7; Glia; Mitochondrial Dynamics; Mitochondria-Lysosome Contact;

46 Neurodegeneration

47 **Introduction**

48 Mitochondria are dynamic organelles that undergo structural and functional changes
 49 in response to a variety of cellular signals such as energy production, metabolism, or
 50 survival. They constantly remodel and interact with other organelles so that their
 51 morphologies are swiftly modified to adapt the cellular needs. Different aspects of
 52 mitochondrial dynamics have been uncovered including fission (division), fusion, or
 53 shape transition, generating different forms of mitochondria that function in various
 54 cellular contexts (1-3). These dynamic changes in morphology and function are under
 55 tight regulation and have been extensively linked to catastrophic consequences such
 56 as neurodegeneration and death (4, 5). For instance, mitochondrial fission regulated
 57 by dynamin-related protein-1 (Drp1), a GTPase that dynamically associates with the
 58 endoplasmic reticulum (ER) and mitochondria (6-10), suppresses oxidative damage
 59 and safeguards neuron survival (11). It is generally considered that the fission process
 60 generates smaller mitochondria easier to be targeted by mitophagy, the
 61 autophagosomal degradation of damaged mitochondria mediated by Parkinson's
 62 disease (PD) risk genes *PINK1* and Parkin (12-14). On the other hand, elongated
 63 mitochondria due to the lack of fission accumulate oxidative damage and lose
 64 respiratory function, transforming into large spheres resistant to mitophagy.
 65 Interestingly, most of these mitochondrial dynamic defects underlying
 66 neurodegeneration are ascribed to neurons, whereas how mitochondrial dynamics in
 67 glia, the other major brain cell type important for neuron survival, contributes to

68 neurodegeneration, remains very limitedly explored.

69 In addition to ER, mitochondrial fission sites are also contacted by lysosomes,
70 which help to restrict mitochondria motility by recruiting the lysosome GTPase Rab7
71 (15, 16). It has been shown that Rab7 GTP hydrolysis is crucial for the untethering of
72 lysosome and mitochondria (16). Upon mitochondria-lysosome contact untethers,
73 mitochondrial fission occurs. These findings implicate a role for lysosome in
74 regulating mitochondrial dynamics. In regard to this aspect, whether lysosome and its
75 related factors play a role in glial mitochondrial fission is largely unknown; how
76 mitochondrial dynamic is controlled in glia has not been extensively studied.

77 The *Drosophila* vesicular R-SNARE (soluble N-ethylmaleimide-sensitive factor
78 attachment protein receptors), VAMP7, is homologous to mammalian VAMP7 and
79 VAMP8, and the latter exhibits a prominent expression in microglia (17, 18).
80 Mammalian VAMP7 or VAMP8 localizes on the late endosome and lysosome and
81 forms a complex with the cytosolic Synaptosomal-associated protein 29 (SNAP-29)
82 and the autophagosomal Syntaxin-17 (Syx17); together this complex regulates the
83 autophagosome-lysosome fusion in autophagy (19, 20). Despite a role in vesicle
84 fusion, whether VAMP7 plays additional roles with respect to its lysosomal origin
85 remains unclear. Here we present evidence that VAMP7 regulates glial mitochondrial
86 dynamics via modulating the dynamics of mitochondria-lysosome contacts. Lack of
87 glial VAMP7 causes mitochondrial elongation independent of its autophagy function,
88 increased ROS levels and the production of lipid droplets (LDs), and regulates nearby
89 dopaminergic (DA) neuron survival. Glial VAMP7-mediated mitochondrial

elongation is suppressed by expressing the fission factor Drp1 or downregulating the expression of the mitochondrial assembly regulatory factor Marf, indicating a role for glial VAMP7 in mitochondrial fission/fusion. Considering that SNARE proteins mediate membrane fusion, our findings uncover a new role for a canonical SNARE factor in glial mitochondrial fission, demonstrating its importance in glial contribution to neuron survival.

Results

Glia deficient of VAMP7 exhibit mitochondrial elongation

Previously characterized as a fusion factor regulating autophagosome-lysosome fusion, we first confirm if VAMP7 functions similarly in glia. Two independent RNAi lines: *vamp7*-RNAi^{#38300} and *vamp7*-RNAi^{#43543} were used to selectively silence *vamp7* expression. Expression of either RNAi in glia abolished *vamp7* transcription levels, validating the efficacy of RNAi (Figure S1A). Consistent to previous findings, glial autophagic flux monitored by a dual reporter *UAS-GFP-mCherry-Atg8a* in the absence of glial VAMP7 was strikingly impaired. The values of Pearson's coefficient indicating colocalization of GFP and mCherry fluorescent signals were significantly increased upon glial VAMP depletion, indicating non-quenched GFP signals due to impaired fusion (Figure S1B and S1C). These results confirm a role for VAMP7 in glial autophagosome-lysosome fusion.

Taking advantage of the RNAi lines, we next investigated thoroughly the consequences of lacking glial VAMP7 in the brains. Striking defects were detected

when analyzing Transmission Electron Microscopy (TEM) images of adult fly brains expressing *vamp7*-RNAi in glia. In addition to the presence of LDs and mitophagic vacuoles, mitochondria were elongated, indicating a possible defect in fission or fusion (yellow arrows, Figure 1A). The morphologies of mitochondria were categorized and quantified by comparing the values of mitochondrial length over diameter (L/D). Interestingly, the ratio of mitochondria with a L/D number lower than 2.5 decreased, whereas the ones with a L/D number higher than 2.5 increased upon glial VAMP7 depletion (Figures 1A and 1B). These results indicate that mitochondria exhibit elongated morphology in the absence of glial VAMP7. Furthermore, two independent mitochondrial markers: *UAS-mito-HA-GFP* (*mito.GFP*) and *UAS-mitoOMM-mCherry* (*mitoOMM.mCherry*), that expresses a mitochondrial import signal and labels the outer mitochondrial membrane, respectively, were used to assess glial mitochondrial dynamics in the absence of VAMP7. Consistent with the EM results, mitochondrial area and volume were significantly increased when expressing *vamp7*-RNAi in glia, indicating that mitochondria are elongated (Figures 1C-1E and S1D-S1F). Taken together, these results indicate that lack of glial VAMP7 disrupts mitochondrial dynamics, possibly due to a defect in fission or fusion.

Given that VAMP7 is a characterized fusion factor in autophagy, we next determine if VAMP7-mediated glial mitochondrial dynamics depends on autophagosome-lysosome fusion. Interestingly, mitochondrial area and volume in glia were not altered in the presence of Bafilomycin A1 (BafA1), a drug blocking autophagosome-lysosome fusion (21, 22) (Figures 1F-1H). Moreover, glial inhibition

134 of the other factor involved in autophagosome-lysosome fusion, Syx17, did not cause
135 mitochondrial elongation (Figures S1G-S1I). Taken together, these results indicate
136 that VAMP7 mediates glial mitochondrial dynamics independent of its function in
137 autophagosome-lysosome fusion.

138

139 **Lack of glial VAMP7 leads to mitochondrial dysfunction**

140 To further demonstrate that VAMP7-mediated mitochondrial elongation is
141 physiologically relevant, the calcium sensor *mitoGCamp5.0* was expressed in glia to
142 monitor the mitochondrial calcium activity (23). Whereas an increase in the
143 *mitoGCamp5.0* signal was detected in the control young animals, the increase was
144 blocked when silencing glial *vamp7* expression (Figure 2A). The increased
145 *mitoGCamp5.0* signal was similarly blocked by glial VAMP7 depletion when flies
146 aged (Figure 2B). These results indicate that glial VAMP7 regulates mitochondrial
147 calcium activity. As mitochondria are the major sites for ROS production, adult fly
148 brains of control and *repo>vamp7-RNAi* animals were examined for ROS levels with
149 the dihydroethidium (DHE) staining. Consistent with previous findings, DHE-positive
150 fluorescent intensities increased when expressing the RNAi targeting *superoxide*
151 *dismutase 1* (*sod1*), the enzyme that regulates ROS levels by catalyzing the
152 conversion of superoxide into oxygen and hydrogen peroxide (Figure 2C).
153 Interestingly, inhibiting *vamp7* expression in glia caused similar increase, indicating
154 that glial VAMP7 expression regulates cellular ROS levels (Figure 2C). In addition,
155 the transcriptional levels of genes related to the mitochondrial fatty acid β -oxidation

156 pathway, including those encoding acyl-coA dehydrogenases (*CG17544* and *CG9527*)
 157 and fatty acid synthase (*FASN*), were increased upon glial *vamp7*-RNAi expression,
 158 indicating altered mitochondrial function (24, 25) (Figure 2D). Notably, the levels of
 159 BODIPY-positive LDs increased in both larval and adult fly brains in the absence of
 160 glial VAMP7 (Figures 2E-2G), with a similar increase in the overall LD
 161 triacylglycerols (TAG) content (Figure 2H). The increased LD number was
 162 suppressed in the presence of α -Lipoic acid, a compound known for suppressing LD
 163 formation (Figures 2I and 2J), indicating that glial VAMP7 regulates LD formation.
 164 Taken together, these results indicate that glial VAMP7 regulates mitochondrial
 165 function, ROS levels, and LD production via modulating mitochondrial dynamics in
 166 glia.

167

168 Glial VAMP7 contributes to DA neuron survival

169 As glia interact with neurons and the glia-neuron crosstalk often contributes to
 170 neuronal function and survival, we next investigated if VAMP7-mediated glial
 171 mitochondrial dynamics contributes to DA neuron survival using the *Drosophila* PD
 172 model established in the lab. DA neurons are located in clusters and named upon their
 173 relative positions in adult fly brains (26, 27). These clusters include protocerebral
 174 posterior lateral (PPL)1, PPL2, protocerebral posterior medial (PPM)1, PPM2, and
 175 PPM3 (Figure 3A). Interestingly, lack of glial VAMP7 causes DA neuron loss at
 176 PPM1/2, PPM3, and PPL1 clusters in an age-dependent manner (Figures 3B-3D).
 177 These results suggest that glial VAMP7 contributes to DA neuron survival. Given that

the degeneration of DA neurons in the PPM1/2 cluster has been implicated in a *Drosophila* PD model overexpressing α -Synuclein (28), it is conceivable that glial VAMP7 plays important roles in regulating DA neuron survival under pathological conditions.

Glial VAMP7 regulates mitochondrial fission and fusion

Given that lack of glial VAMP7 induces mitochondrial elongation, possibly due to decreased fission or increased fusion, we tested genetically if glial VAMP7 interacts with the fission factor Drp1 or the fusion factor Marf. Interestingly, increased Drp1 expression suppressed the mitochondrial elongation in the absence of glial VAMP7 (Figures 3E-3G), suggesting that increasing fission helps to prevent VAMP7-mediated mitochondrial elongation. Moreover, decreased Marf expression in the presence of glial *vamp7*-RNAi also suppressed the mitochondrial elongation. These results indicate that Drp1 and Marf are epistatically downstream of glial VAMP7 in controlling mitochondrial fission and fusion. Interestingly, endogenous protein levels of Fission, mitochondrial 1 (Fis1), Drp1, Marf, and Optic atrophy 1 (Opa1) were not affected by the absence of glial VAMP7, suggesting that VAMP7 does not regulate glial mitochondrial dynamics via tuning fission/fusion proteins levels (29) (Figures S2A-S2H). These results suggest that glial VAMP7 acts upstream of these mitochondrial factors in regulating mitochondrial fission/fusion; mitochondrial elongation caused by the lack of glial VAMP7 is potentially due to a change in the dynamic or balance of fission and fusion.

200

201 **Glial VAMP7 regulates the dynamic of mitochondria-lysosome contact**

202 It has been reported that lysosomes contact mitochondria to restrict its motility and
 203 determine the fission site (16, 30). Given that VAMP7 is localized on the lysosome,
 204 we next investigated whether glial VAMP7 regulates mitochondrial dynamic via the
 205 mitochondria-lysosome contact. Two reporters: *UAS-mitoOMM-mCherry* and
 206 *UAS-Lamp1-GFP*, were co-expressed to label mitochondria and lysosomes
 207 simultaneously, such that mitochondria-lysosome contact can be visualized under
 208 various conditions. Interestingly, colocalization of mitoOMM and Lamp1 increased
 209 upon glial VAMP7 depletion, indicating an increase in mitochondria-lysosome contact
 210 (Figures 4A and 4B). Importantly, these dual positive contacts were not positive for
 211 the autophagosome marker Atg8a, indicating that these do not represent mitochondria
 212 undergoing mitophagy (Figures S2I-S2K). Whereas the value of Pearson's coefficient
 213 for mitoOMM and Lamp1 increased significantly (Figure S2J), the colocalization
 214 values for Atg8a and mitoOMM decreased upon glial VAMP7 depletion (Figure S2K).
 215 These results indicate that lack of glial VAMP7 increases mitochondria-lysosome
 216 contact and blocks mitophagy, consistent with the notion that elongated mitochondria
 217 are resistant to and slows down mitophagy.

218 In addition, the dynamics of colocalized mitoOMM and Lamp1 were analyzed by
 219 time lapse analysis *in-vivo*. Interestingly, mitochondria and lysosomes contacted each
 220 other and dissociated over a course of 100 seconds, whereas the duration of these
 221 contacts persisted and prolonged up to 400 seconds upon glial VAMP7 depletion

(Figures 4C, 4D, and Movie S1-S3). The frequency of mitochondria and lysosome contacting each other and the duration of these contacts increased significantly (Figures 4E and 4F) and a higher number of contacts persisting more than 5 min was also detected (Figure 4G). Consistently, mitochondria and lysosome were significantly less mobile in the absence of glial VAMP7 as indicated by their moving speed (Figures 4H and 4I). Taken together, these results indicate that glial VAMP7 regulates the dynamics of mitochondria-lysosome contacts.

Discussion

SNARE proteins are canonical regulators of membrane fusion, yet to date no evidence indicates that they mediate mitochondrial fusion. Mitochondrial fission, however, has been shown to rely on the activity of SNARE protein Syx17. Upon starvation-induced autophagy, Syx17-Drp1 interaction is disrupted, causing a block in mitochondrial fission, hence the elongated mitochondria resistant to mitophagy (31). Syx17 also mediates fusion between the mitochondrial-derived vesicles (MDVs) and the late endosome/lysosome under stress conditions (32). These results indicate that SNARE proteins are possible mediators in mitochondrial dynamics. *Drosophila* VAMP7 is highly homologous with mammalian VAMP8, which exhibits prominent expression in microglia. Our findings reveal that lack of glial VAMP7 causes elongated mitochondria, a phenotype genetically suppressed by increasing the expression of the fission factor Drp1. These elongated mitochondria are dysfunctional as mitochondrial calcium activity is significantly disrupted and the levels of ROS and

lipid droplets are increased, endangering the survival of nearby DA neurons. Despite a role in autophagosome-lysosome fusion, glial VAMP7-mediated mitochondrial dynamics does not depend on its fusion function as inhibiting autophagosome-lysosome fusion by BafA1 does not alter mitochondrial length.

Mitochondrial fission site is determined by contacts between mitochondria and ER or lysosomes. With its lysosomal localization, it is intriguing to speculate that VAMP7 regulates mitochondria-lysosome contacts, determines the mitochondrial fission site, in turn controls the fate of mitochondria undergoing fission, fusion, or inter-mitochondrial clustering. Multiple lines of evidence support this hypothesis: first, elongated mitochondria were aggregated and exhibit increasing contacts with lysosomes upon glial VAMP7 depletion, suggesting abnormal tethering among mitochondria themselves or between mitochondria and lysosomes. These abnormal clusterings do not represent mitophagy events as no Atg8a-positive autophagosomes are detected at these sites. Rather, the phenomenon suggests defective mitochondria-lysosome contacts, which prevent mitochondria from fission, hence the elongation. Similarly, the critical regulator Rab7 in mitochondria-lysosome contact also appears to be mislocalized and aggregated in the absence of glial VAMP7, further suggesting the disruptive formation of mitochondria-lysosome contact (Figure S3A). It is noteworthy to mention that lysosomes exhibit abnormal acidity in the absence of glial VAMP7 (Figures S3B and S3C). Moreover, as contact sites between mitochondria and ER are known as sites for calcium transfer, inhibiting the glial expression of TRPML, a mucolipin channel on the lysosomal membrane that releases

266 calcium, does not cause mitochondrial elongation (Figures S3D-S3F). These findings
267 suggest that lysosomal calcium transfer might not be responsible for the persisted
268 contacts and mitochondrial elongation induced by glial VAMP7 depletion.
269 Mitochondria-lysosome contacts might provide a more direct and physical cue to
270 modulate glial mitochondrial dynamics, via a route different from the calcium transfer
271 between mitochondria and ER.

272 Similar to neurons, glia, particularly astrocytes, possess SNARE machineries for
273 their vesicular trafficking function. These SNARE machineries, some in the same
274 composition as in neurons but some different, are responsible for slow secretory
275 exocytosis rather than fast and immediate release of signaling molecules (33). Very
276 importantly, they regulate synaptic transmission and plasticity, antigen presentation in
277 neuro-inflammation, and control different types of behavior such as sleep. Our
278 findings unexpectedly reveal a new role for the canonical SNARE protein VAMP7 in
279 glial mitochondrial dynamics. Considering that mitochondria are present in astrocytic
280 processes in close proximity to neurons and contribute to local calcium signaling, ATP
281 production, and metabolism of signaling molecules, it is conceivable that
282 VAMP7-mediated glial mitochondrial dynamics subsequently impacts mitochondrial
283 function and participates in the metabolic process supporting neuron survival. Given
284 that DA neurodegeneration is one of the hallmarks in PD, our findings illustrate a
285 SNARE-dependent mechanism controlling organelle contacts, glial mitochondrial
286 dynamics, and DA neuron survival, providing the mechanistic basis underlying DA
287 neurodegeneration. Canonical SNARE proteins not only regulate membrane fusion,

but also important mediators in mitochondrial dynamics; future work is warranted to explore additional roles of SNARE proteins in various cellular contexts in both cell-autonomous and non-cell-autonomous fashions.

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Author Contributions

H.W, S.Z, and M.S.H conceived and designed the study. H.W, L.W, and S.Y performed the experiments. H.W and M.S.H analyzed the data. M.S.H wrote the paper

310 with input from H.W. All authors read and approved the manuscript.

311

312 **Declaration of Interests**

313 The authors declare no competing interests.

314

315 **Figure Legends**

316 **Figure 1. Lack of glial VAMP7 causes mitochondrial elongation (A and B)**

317 Representative TEM images (A) and quantifications (B) of mitochondrial morphology

318 in control and *repo>vamp7-RNAi*⁴³⁵⁴³ adult fly brains of 10-day-old. Note the

319 presence of lipid droplets (red arrows), mitophagic vacuoles (pink arrows), and

320 elongated mitochondria (yellow arrows) in the absence of glial VAMP7. Note that the

321 ratio of mitochondria with a length to diameter (L/D) number lower than 2.5 is

322 significantly lower while the ratio for L/D number above 2.5 is significantly higher

323 upon glial VAMP7 depletion. (C-E) Representative confocal images (C) and

324 quantifications (D and E) of glial mitochondrial morphology monitored by

325 *UAS-mito.GFP* in control and *repo>vamp7-RNAi*⁴³⁵⁴³ adult fly brains. Note that the

326 average mitochondrial area and volume quantified by Imaris are significantly larger

327 than the control animals when expressing *vamp7-RNAi* in glia. (F-H) Representative

328 confocal images (F) and quantifications (G and H) of glial mitochondrial morphology

329 in the absence or presence of Bafilomycin A1 (BafA1). Note that no change in the

330 mitochondrial area and volume is observed when autophagosome-lysosome fusion is

331 blocked by BafA1. Insets represent enlarged view of single mitochondria. Scale bar:

332 500 nm in A, 10 μ m in C and F. Mitochondrial area and volume are quantified by
333 Imaris as detailed in the Materials and Methods. The numbers of brains analyzed and
334 quantified are indicated in the Figures. For TEM, 6 independent fly brains for each
335 genotype were analyzed. Data are shown as mean $\pm$ SEM. P-values of significance
336 (indicated with asterisks, ns no significance, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$)
337 are calculated by two-tailed unpaired t-test. Approximately 20 confocal Z-stack
338 sections were taken with 0.125 μ m each. Merged images are shown. All data in
339 subsequent figures are present and analyzed as described above unless stated
340 otherwise.

341

342 **Figure 2. Lack of glial VAMP7 disrupts mitochondrial function and increases**
343 **ROS levels and lipid droplet production (A and B)** The reporter
344 *UAS-mitoGCamp5.0* was expressed together with *UAS-mitoOMM.mCherry* in glia to
345 monitor mitochondrial calcium activity (*mitoOMM.mCherry*; *repo-Gal4*,
346 *mitoGCamp5.0*). Note that the increased mitochondrial calcium activity is blocked in
347 both young and old flies when inhibiting glial VAMP7 expression. (C) DHE
348 fluorescence intensities representing cellular ROS levels were significantly increased
349 upon glial VAMP7 depletion. (D) RT-qPCR analysis reveals increased transcription
350 levels of genes related to mitochondrial function including the ones encoding
351 acyl-coA dehydrogenases (*CG17544* and *CG9527*) and fatty acid synthase (*FASN*).
352 (E-H) The numbers of LDs labelled by the BODIPY dye in both larval and adult fly
353 brains (E-G) and the TAG content in the LDs (H) are significantly increased in the

354 absence of glial VAMP7. **(I and J)** Glial VAMP7 regulates LD formation as addition
 355 of α -lipoic acid suppresses LD formation in both control and *repo>vamp7-RNAi*⁴³⁵⁴³
 356 adult fly brains of 3-day-old. Scale bar: 100 μ m in 3rd instar larvae in E and I, 50 μ m
 357 in Day 3 in E. The numbers of brains analyzed and quantified are indicated in the
 358 Figures. For α -lipoic acid treatment, 8 independent fly brains for each genotype were
 359 analyzed. Data are shown as mean $\pm$ SEM. P-values of significance (indicated with
 360 asterisks, ns no significance, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$) are calculated by
 361 two-tailed unpaired t-test.

362

363 **Figure 3. VAMP7 regulates glial mitochondrial dynamics and DA neuron**
 364 **survival** **(A)** An schematic diagram illustrating the DA neuron clusters in adult fly
 365 brains. The black dashed box encloses the area visualized in B. **(B-D)** The numbers of
 366 DA neurons in PPM1/2, PPM3, and PPL1 are significantly decreased (white arrows in
 367 B) in the aged flies lacking glial VAMP7. **(E-G)** Epistatic analysis of VAMP7 and
 368 mitochondrial fission/fusion factors Drp1, Marf, and Opa1 in regulating mitochondrial
 369 dynamics. Note that increasing expression of the fission factor Drp1 or
 370 downregulating the expression of the fusion factor Marf or Opa1 causes increased
 371 fission, opposite to the mitochondrial elongation phenotype in the absence of glial
 372 VAMP7. Co-expression of *Drp1*, *Marf*-RNAi, or *Opa1*-RNAi and *vamp7*-RNAi in
 373 glia all leads to suppression on mitochondrial elongation. Insets represent enlarged
 374 view of single mitochondria. Scale bar: 50 μ m in B, 10 μ m in E. The numbers of
 375 brains analyzed and quantified are indicated in the Figures. For DA neurons statistics,

15 independent fly brains for each genotype were analyzed (C and D). Data are shown as mean $\pm$ SEM. P-values of significance (indicated with asterisks, ns no significance, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$) are calculated by ordinary one-way ANOVA followed by Tukey's multiple comparisons test.

Figure 4. VAMP7 regulate glial mitochondrial dynamics via mitochondria-lysosome contacts (A and B) Two reporters *UAS-mitoOMM.mCherry* (labels the mitochondrial outer membrane, red) and *UAS-Lamp1* (labels lysosome, green) are co-expressed to visualize mitochondria-lysosome contact. Note that significant increase in colocalization of mitoOMM and Lamp1 upon glial VAMP7 depletion analyzed by Imaris. Co-localization traces are illustrated in yellow in A. (C-I) Representative images (C and D) and quantifications (E-I) of the dynamic of mitochondria-lysosome contact monitored by *in-vivo* time lapse analysis. Note that mitochondria and lysosome contact each other and separate within the course of 100 seconds, while the contact persists up to 400 seconds upon glial VAMP7 depletion. Both the frequency and the duration of the contacts increase, while the mobility of both organelles decrease, when lacking glial VAMP7. Parameters in E-I are quantified using Imaris and ImageJ. Scale bar: 10 μ m in A, 5 μ m in A (colocalization panel), 1 μ m in C and D. The numbers of brains analyzed and quantified are indicated in the Figures. For Lamp1.GFP and mitoOMM.mCherry colocalization statistics, 9 independent fly brains for each genotype were analyzed (B). Data are shown as mean $\pm$ SEM. P-values of significance (indicated with asterisks, ns no significance, *

398 $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$) are calculated by two-tailed unpaired t-test.

399

400 **Figure S1. Glia deficient of VAMP7 exhibit mitochondrial elongation (A)**

401 RT-qPCR analysis indicates that expression of either *vamp7*-RNAi, #38300 or #43543,

402 in glia reduces the *vamp7* transcription levels significantly. (B and C) Glial

403 autophagic flux monitored by the dual reporter *UAS-GFP-mCherry-Atg8a* is impaired

404 upon glial VAMP7 depletion, suggesting impaired fusion. Note that the Pearson's

405 coefficient value indicating colocalization of GFP and mCherry increases when

406 lacking glial VAMP7 (B). (D-F) Glial mitochondrial dynamic is monitored by

407 expressing the reporter *UAS-mitoOMM-mCherry* in glia. Consistent with the other

408 reporter *UAS-mito.GFP*, Imaris analysis reveals an increase in the mitochondrial area

409 and volume in the absence of glial VAMP7. (G-I) Glial inhibition of *syx17* expression

410 does not cause mitochondrial elongation. Note that mitochondrial area and volume

411 quantified using the reporter *UAS-mito.GFP* remain unaffected in the presence of

412 *syx17*-RNAi. Scale bar: 10 μ m in C, D and G. Mitochondrial area and volume are

413 quantified by Imaris as detailed in the Materials and Methods. The numbers of brains

414 analyzed and quantified are indicated in the Figures. Data are shown as mean $\pm$ SEM.

415 P-values of significance (indicated with asterisks, ns no significance, * $p < 0.05$, **

416 $p < 0.01$, and *** $p < 0.001$) are calculated by two-tailed unpaired t-test or ordinary

417 one-way ANOVA followed by Tukey's multiple comparisons test. Approximately 20

418 confocal Z-stack sections were taken with 0.125 μ m each. Merged images are shown.

419 All data in subsequent figures are present and analyzed as described above unless

420 stated otherwise.

421

422 **Figure S2. VAMP7 regulates glial mitochondrial dynamics via**

423 **mitochondria-lysosome contact (A-H)** Western blot analysis of endogenous

424 fission/fusion protein levels in the control and *repo>vamp7*-RNAi animals. Knock-in

425 lines at the original locus of Drp1, Opa1, and Marf created with an insertion of the HA

426 tag for protein detection and the Fis1 antibodies are gifts from Chun-Hong Chen. **(I-K)**

427 Two reporters *UAS-mitoOMM.mCherry* (labels the mitochondrial outer membrane,

428 red) and *UAS-Lamp1* (labels lysosome, green) are co-expressed to visualize

429 mitochondria-lysosome contact. Note that significant increase in colocalization of

430 mitoOMM and Lamp1 upon glial VAMP7 depletion analyzed by Imaris (J).

431 Immunostaining with anti-Atg8a antibodies reveals a decrease in the colocalization of

432 Atg8a and mitoOMM, suggesting that mitophagy is not induced (K). Note that no

433 Atg8a signals are detected in contact puncta positive for mitoOMM and Lamp1

434 (insets in I). Scale bar: 10 μ m in I. The numbers of brains analyzed and quantified are

435 indicated in the Figures. Data are shown as mean $\pm$ SEM. P-values of significance

436 (indicated with asterisks, ns no significance, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$)

437 are calculated by two-tailed unpaired t-test or ordinary one-way ANOVA followed by

438 Tukey's multiple comparisons test.

439

440 **Figure S3. VAMP7 regulates the dynamic of mitochondria-lysosome contact (A)**

441 Two reporters *UAS-mitoOMM.mCherry* (labels the mitochondrial outer membrane,

red) and *UAS-Rab7.GFP* (labels late endosome and lysosome, green) are co-expressed to visualize the dynamic of Rab7 contacting mitochondria. Note that Rab7 exhibit aggregated and mislocalized pattern upon glial VAMP7 depletion. (**B** and **C**) Lysosomal acidity monitored by the LysoTracker Red dye is significantly impaired when expressing *vamp7*-RNAi in glia. Note that LysoTracker Red fluorescent intensities increase upon glial VAMP7 depletion. (**D-F**) Mitochondrial dynamic monitored by the reporter *UAS-mito.GFP* is significantly affected by *trpml*-RNAi expression in glia. Note that mitochondrial area and volume are reduced, indicating rounder and shorter morphology, opposite to mitochondrial elongation. Scale bar: 10 μ m in A, B and D. The numbers of brains analyzed and quantified are indicated in the Figures. Data are shown as mean $\pm$ SEM. P-values of significance (indicated with asterisks, ns no significance, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$) are calculated by two-tailed unpaired t-test or ordinary one-way ANOVA followed by Tukey's multiple comparisons test.

Movie S1. In-vivo time lapse analysis of mitochondria-lysosome contact in the control flies. Two reporters *UAS-mitoOMM.mCherry* (labels the mitochondrial outer membrane, red) and *UAS-Lamp1* (labels lysosome, green) are co-expressed to visualize mitochondria-lysosome contact. A representative contact is shown. A Z-stack of 10 images was taken at an interval of 19 seconds for 10 minutes. Note that mitochondria and lysosomes contact and dissociate over the imaging period.

464 **Movie S2. *In-vivo* time lapse analysis of mitochondria-lysosome contact in flies**

465 **lacking glial VAMP7.** Two reporters *UAS-mitoOMM.mCherry* (labels the
466 mitochondrial outer membrane, red) and *UAS-Lamp1* (labels lysosome, green) are
467 co-expressed to visualize mitochondria-lysosome contact. A representative contact is
468 shown. A Z-stack of 10 images was taken at an interval of 19 seconds for 10 minutes.
469 Note the significant increase in colocalization of mitoOMM and Lamp1 upon glial
470 VAMP7 depletion.

471

472 **Movie S3. *In-vivo* time lapse analysis of mitochondria-lysosome contact in flies**

473 **lacking glial VAMP7.** Two reporters *UAS-mitoOMM.mCherry* (labels the
474 mitochondrial outer membrane, red) and *UAS-Lamp1* (labels lysosome, green) are
475 co-expressed to visualize mitochondria-lysosome contact. A representative contact
476 different from Movie S2 is shown. A Z-stack of 10 images was taken at an interval of
477 19 seconds for 10 minutes. Note the significant increase in colocalization of
478 mitoOMM and Lamp1 upon glial VAMP7 depletion.

479

480 **Materials and Methods**

481 ***Drosophila* genetics**

482 Flies were raised under standard conditions at 25°C with 70% humidity. All fly crosses
483 were carried out at 25°C with standard laboratory conditions unless noted otherwise.
484 All strains were obtained from Bloomington Drosophila Stock Center (BDSC), the
485 Vienna Drosophila RNAi Center (VDRC), Tsinghua Fly Center, or as gifts from

colleagues. The following fly strains were used in this study: *repo-GAL4* (a pan-glial driver), *UAS-luciferase-IR* (BL31603), *UAS-lacZ* (BL1777), *UAS-mito-HA-GFP* (BL8442), *UAS-mito.OMM.mCherry* (BL66532), *UAS-Lamp1.GFP*, *UAS-vamp7 RNAi* (BL38300 and BL43543), *UAS-marf RNAi* (BL31157), *UAS-opa1 RNAi* (THU0811), *UAS-sod1 RNAi* (THU3207), *UAS-Rab7.GFP* (BL42705); *UASp-GFP-mCherry-Atg8a* (Henry Sun); *UAS-HRP* (Aike Guo); *UAS-drp1* (Chao Tong); *drp1-HA*, *opa1-HA*, and *marf-HA* (Chun-Hong Chen).

493

494 **Molecular biology**

495 qRT-PCR: Total RNAs were extracted from adult fly heads using TransZol Up (Cat. #ET111-01, TransGen, Beijing, China). cDNAs were reversely transcribed using HiScript III RT SuperMix (Cat. #R323-01, Vazyme, Nanjing, China). qRT-PCR reactions were performed using ChamQ Universal SYBR qPCR Master Mix (Cat. #Q711-02, Vazyme) and ABI 7500 RT-PCR system. The mRNA levels were normalized to *rp49*, respectively. The expression levels were analyzed by $\Delta\Delta CT$ method. Primers used for amplification:

501 *rp49*-F: CCACCAGTCGGATCGATATGC

502 *rp49*-R: CTCTTGAGAACGCAGGCGACC

503 *vamp7*-F: GGCTCGCAATTGGATAGTAGT

504 *vamp7*-R: TGCTTATCGGGGAATGAGCC

505 *CG17544*-F: GTACTATGCTCTGACGCGCT

506 *CG17544*-R: GGTGGTCATGTGCTTCTCCA

507 *CG9527*-F: CTGCTTCCCCTGCTGAAGAA

508 *CG9527-R: GACGTTTCCATCGCTCATGC*

509 *FASN-F: CGCTCCACTCCAAGAACTCG*

510 *FASN-R: CAGGTTTAGTTGTAGGGGCTAGA*

511

512 **Immunohistochemistry**

513 Adult fly brains were dissected and fixed in 4% formaldehyde for 45 minutes, then
514 washed with PBT (PBS + 0.3% TX-100) for 3 times and dissected further to remove
515 additional debris in PBS solution. Clean and fixed brains were blocked in PBT
516 solution with 5% Normal Donkey Serum (NDS) or 10% Bovine Serum Albumin
517 (BSA) and subsequently stained with primary antibodies at 4°C overnight and then
518 secondary antibodies at room temperature for 2 hours. Primary antibodies used:
519 mouse anti-Atg8a (1:1000, gift from Chao Tong) and rabbit anti-TH (1:1000, Cat.
520 #NB300-109, Novus). For all samples, secondary antibodies used were from Jackson
521 ImmunoResearch (West Grove, PA, USA): donkey anti-rabbit Cy3 (1:1000, Cat.
522 #711-166-152), donkey anti-mouse Cy5 (1:500, Cat. #715-175-150). All samples
523 were mounted in Vectashield mounting media (Cat. #H-1500-10, Vector Laboratories,
524 Burlingame, CA, USA).

525

526 ***In-vivo* time lapse analysis**

527 Live adult fly brains were dissected and kept in PBS solution. All samples were
528 mounted in PBS, and immediately scanned anteriorly to posteriorly (top to bottom)
529 using Nikon C2 confocal microscope (Tokyo, Japan) with a 60X oil objective. The

530 number of contacts were analyzed by Imaris using Autoregressive motion tracking
531 mode. Duration of contacts were analyzed by ImageJ using Time stamper.

532

533 **Calcium imaging**

534 Live adult fly brains expressing mitoGCamp5.0 were dissected and kept in PBS
535 solution. All samples were mounted in PBS, and immediately scanned in a single
536 focal plane using Nikon A1 confocal microscope (Tokyo, Japan) with a 60X oil
537 objective. The fluorescence intensity were analyzed by ImageJ using Time series
538 analyzer V3.

539

540 **Live fluorescent dye imaging**

541 Live adult fly brains were dissected and kept in PBS solution. For the analysis of
542 LDs, brains were incubated with 1 µg/mL BODIPY™ 493/503 (Cat. #D3922,
543 Invitrogen) for 5 minutes at 25°C, then immediately scanned by the Nikon C2
544 confocal microscope (Tokyo, Japan) with a 20X objective. To analyze LD formation,
545 brains were also treated with 10 µM α-Lipoic acid (Cat #D118666, Aladdin) for 3
546 days, followed by 1 µg/mL BODIPY™ 493/503 (Cat. #D3922, Invitrogen) for 5
547 minutes at 25°C, then immediately scanned by the Nikon C2 confocal microscope
548 (Tokyo, Japan) with a 20X objective. For the analysis of mitochondria-derived ROS
549 levels, brains were incubated with 5 µM DHE (Cat. #D11347, Invitrogen) for 5
550 minutes at 25°C, then immediately scanned using the Nikon A1 confocal microscope
551 (Tokyo, Japan) with a 20X objective. For the analysis of lysosome acidity, brains

were incubated with LysoTracker Red DND-99 (1:1000, Cat. #40739ES50, Yeasen)
solution in PBS for 5 minutes at 25°C, then immediately scanned using Nikon C2
confocal microscope (Tokyo, Japan) with a 60X oil objective.

Transmission electron microscopy

Adult fly brains were fixed, embedded, stained, and dehydrated according to previous
protocols (34). Ultrathin sections (70 nm) of each brain were cut with a
Diatomediamond knife on a Leica EM UC7 ultra-microtome (Leica Microsystems,
Germany) and collected on copper grids. Images were acquired with Talos L120C
transmission electron microscope (Thermo Fisher Scientific, Waltham, MA, UAS)
operating at an acceleration voltage of 120 kV.

Biochemistry

Adult fly heads were homogenised using a motorised pestle (Cat. #116005500, MP
Biomedicals, Irvine, CA, USA) in lysis buffer (0.4% NP-40, 0.2 mM EDTA, 150 mM
NaCl, 20% glycerol, 100 mM Tris-HCl pH7.5, 2% Tween 20, 0.5 mM
phosphodiesterase inhibitors, and 1 mM PMSF). Proteins were separated on
SDS-PAGE gels and transferred to PVDF membranes (Cat. #IPFL00010, Millipore,
Billerica, MA, USA). The membranes were blocked by 5% fat-free milk diluted in
PBS with 0.1% Tween-20 for 40 minutes. Samples were incubated with the primary
antibodies at 4°C overnight and HRP-conjugated secondary antibodies at room
temperature for 2 hours. Primary antibodies used: rat anti-HA-HRP (1:1000, Cat.

574 #3F10, Roche), mouse anti- α -Tubulin (1:5000, Cat. #T9026, Sigma). Bands were
575 visualized by Clarity Western ECL Substrate (Cat. #WBKLS0500, Millipore). Results
576 are representative of at least three biological replicates. For BafilomycinA1 treatment:
577 BafA1 (Cat. #BML-CM110-0100, ENZO) was diluted in DMSO (Cat. #D8371-50,
578 solarbio) in a concentration of 1 mM, then added to 0.5 ml of liquid yeast for a final
579 concentration of 4 μ M. Yeast containing BafA1 was added to the top of the fly food in
580 standard vials kept overnight to allow the evaporation of residual DMSO. Flies of
581 different genotypes (20 females and 20 males) were then put in each vial and kept at
582 25°C for 5 days. After treatment, flies were collected for western blot analysis. The
583 TAG content in the LD was quantified by an triglyceride assay kit (Cat #E1103,
584 Applygen Technologies).

585

586 Confocal microscopy and statistical analysis

587 Images of adult fly brains were acquired by scanning a serial Z-stack of average 10-20
588 sections, each of 0.8 or 0.125 μ m thickness, using Nikon A1, C2 confocal microscope
589 (Tokyo, Japan) with the 20X or 60X oil objective. The whole brain was positioned so
590 that they can be scanned anteriorly to posteriorly (top to bottom). For statistical
591 analyses of mitochondrial area and volume, original and unmodified images were
592 imported into Imaris 9.6 (Oxford Instruments) using Surface to create parameters to
593 batch data from different sample groups. For statistical analyses of LD number and
594 intensity, original and unmodified images were imported into ImageJ (National
595 Institutes of Health), and the intensity threshold for the relevant channel was set so

that maximum number of dots were selected without miscounting two adjacent dots into one. For colocalization measurements, primary images were imported into ImageJ (National Institutes of Health) or Imaris 9.6 (Oxford Instruments), and analyzed automatically using the colocalization plugin with the intensity correlation tool, showing as Pearson's correlation coefficient (R) or colocalized trace. All data were imported into GraphPad Prism 7 and shown in scatter plots or column bar graphs. For calculating the statistical significance, two-tailed unpaired t-test or ordinary one-way ANOVA followed by Tukey's multiple comparisons test was used. P value less than 0.05 is considered significant. ns: no significance, $p \geq 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$.

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