A *Drosophila* screen of schizophrenia-related genes highlights the requirement of neural and glial matrix metalloproteinases for neuronal remodeling

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Abstract

Schizophrenia (SCZ) is a multifactorial neuropsychiatric disorder of complex and mostly unknown etiology, affected by genetic, developmental and environmental factors. Neuroanatomical abnormalities, such as loss of grey matter, are apparent prior to the onset of symptoms, suggesting neurodevelopmental origin. Indeed, it has been hypothesized, and recently experimentally supported, that SCZ is associated with dysregulation of developmental synaptic pruning. Here, we explore the molecular link between SCZ-associated genes and developmental neuronal remodeling, focusing on the *Drosophila* mushroom body (MB), which undergoes stereotypic remodeling during metamorphosis. We conducted a loss-of-function screen in which we knocked down *Drosophila* homologs of human genes that genomic studies have associated with SCZ. Out of our 'positive hits', we focused on matrix metalloproteinases (MMPs), mostly known for their role in remodeling of the extracellular matrix. We found that both Mmp1 and Mmp2, which are closely-related to mammalian MMPs, are required in neurons and in glia for the pruning of MB axons. Our combinatorial loss-of-function experiments suggest that Mmp2 secretion from glia is the most important promoter of axon pruning. Our results shed new light on potential molecular players underlying neurodevelopmental defects in SCZ and highlight the advantage of genetically tractable model organisms in the study of human neurodevelopmental disorders.

Introduction

Following its initial establishment, the developing nervous system of both vertebrates and invertebrates undergoes remodeling to shape its mature connectivity. In humans, developmental neuronal remodeling occurs postnatally and includes degenerative events such as retraction of synapses and large-scale axon elimination. While these processes occur most prominently during the first two years of life, they continue until after adolescence (Luo & O'Leary, 2005; Schuldiner & Yaron, 2015). Defects in neuronal remodeling have long been hypothesized to be one of the underlying causes of neuropsychiatric disorders, including schizophrenia (SCZ; Feinberg, 1982; Penzes et al., 2011).

SCZ is a severe, multifactorial mental health condition affecting ~1% of the population. It is characterized by delusions, hallucinations, social withdrawal and cognitive deficits, typically onsetting during the late teens to early adulthood (McCutcheon et al., 2020). It is known from MRI studies that structural abnormalities in the brain, such as reduction in grey matter and ventricular enlargement, may precede the onset of psychotic symptoms, implying neurodevelopmental roots (Brent et al., 2013; Suzuki et al., 2002; Zikidi et al., 2020). It has been suggested, for many years, that the characteristic neuroanatomical alterations in individuals with SCZ are due to dysregulation of neuronal remodeling, specifically over-pruning of synapses (Feinberg, 1982; Hoffman & Dobscha, 1989; Keshavan et al., 1994). The first experimental evidence to support this hypothesis came several years ago, and associated SCZ with excessive expression of complement components leading to increased synapse elimination by microglia (Sekar et al., 2016). This work as well as others highlighted glia, including microglia and astrocytes, as key mediators of synapse pruning in normal development as well as in SCZ and other neuropathologies (Reviewed in Laricchiuta et al., 2024; Notter, 2021; Scott-Hewitt et al., 2023). Still, the molecular basis of SCZ risk and its link to abnormalities in neuronal remodeling remains mostly unknown.

Drosophila melanogaster is an ideal model to study neuronal remodeling, due to its unparalleled genetic toolkit combined with its massive stereotypic circuit remodeling during metamorphosis (Truman, 1990; Yaniv & Schuldiner, 2016). Our focus is on the *Drosophila* mushroom body (MB), a brain structure that functions as a center of olfactory

learning and memory (Heisenberg, 1998). The MB is comprised of three types of intrinsic neurons, known as Kenyon cells (KCs), which are sequentially born from identical neuroblasts (Fig. 1A). Out of the three KC types (γ, α'/β' and α/β), only the first-born γ-KCs undergo stereotypic remodeling. When they initially grow, in the embryonic and early larval stages, γ-KCs project axons that bifurcate to form vertical and medial lobes. At the early pupal stage (~6 hours after puparium formation; h APF) both axonal lobes begin to undergo pruning up to their branchpoint (which is over at ~18h APF). Subsequently, γ-KCs initiate axon regrowth to create the adult-specific medially projecting γ-lobe (Lee et al., 1999; Yaniv & Schuldiner, 2016; Fig. 1A). Importantly, glia were shown, by our lab and others, to actively participate in the remodeling of γ-KCs (Awasaki et al., 2011; Awasaki & Ito, 2004; Boulanger et al., 2021; Hakim et al., 2014; Marmor-Kollet et al., 2023; Watts et al., 2004). Three glial subtypes are present around the MB: cortex glia, ensheathing glia and astrocyte-like glia (hereafter referred to as astrocytes; Freeman, 2015). Cortex glia were shown to have an instructive role in pruning via secretion of the TGF-β ligand myoglianin, which subsequently activates a pruning program in the γ-KCs (Awasaki et al., 2011). Astrocytes, however, are the main cleaners of axonal debris following pruning (Hakim et al., 2014; Tasdemir-Yilmaz & Freeman, 2014). Moreover, we recently demonstrated that astrocytes actively infiltrate the axon bundle prior to pruning to facilitate axon defasciculation and pruning (Marmor-Kollet et al., 2023).

Importantly, *Drosophila* is routinely used to study human neurodegenerative disorders (Lessing & Bonini, 2009), and the Disrupted-in-Scizophrenia-1 (DISC1) gene has been shown to affect MB structure and its regulation of sleep (Furukubo-Tokunaga, 2009; Sawamura et al., 2008). Here, we harness the fly genetic toolkit and the well-characterized remodeling of the MB to delve into the molecular underpinnings of the long-standing hypothesis, and recent evidence supporting it, that defects in neuronal remodeling contribute to SCZ etiology. We systematically examine how MB remodeling is affected by perturbations in *Drosophila* genes whose human homologs have been associated with SCZ. Our findings highlight novel players in axon pruning, and provide potential candidates for future research on the neurodevelopmental molecular origin of SCZ.

Results

Drosophila homologs of human SCZ-related SNPs are dynamically expressed during the development of the mushroom body γ-KCs

We searched genome-wide association studies (GWAS) for candidate genes that have SNPs in SCZ patients (Wu et al., 2017; http://www.szdb.org/index.html). SNP-containing genes were converted to their homolog *Drosophila* genes using HumanMine (Lyne et al., 2022; Fig. 1B). To narrow our candidate list to genes that are potentially required for pruning, we used our previously generated transcriptional atlas of developing γ-KCs (Alyagor et al., 2018) to identify genes that are specifically upregulated before the onset of (0-6h APF), or during (6-18h APF), γ-axon pruning (Fig. 1B, Fig. 1C clusters III-VI highlighted). This process resulted in 32 candidate genes, out of which 29 had available RNAi lines for screening (Fig.1B).

Drosophila homologs of human SCZ-associated genes are required for γ-KC axon pruning

If gene upregulation prior to γ axon pruning is due to requirement in the pruning process, then knockdown (KD) is expected to inhibit pruning and result in persistence of larval γ axons in the adult brain. Therefore, we screened candidate genes using a loss-of-function (LOF) strategy by tissue-specific expression of RNAi-species via the Gal4-UAS system (Brand & Perrimon, 1993). For KD in γ-KCs, we used the pan-KC driver OK107-Gal4, which is strongly expressed in all KC types (α/β, α'/β' and γ), combined with a second, independent binary system (Riabinina & Potter, 2016) to visualize γ-KCs (using the more specific R71G10-QF2 driver, which is expressed in all γ-KCs and stochastically in α/β-KCs, to drive mtdT-HA expression). Screening the 29 RNAi lines using OK107-Gal4 resulted in 11 genes displaying varying degrees of pruning defects upon KD, and one gene whose KD led to abnormal MB morphology (Fig. 2). For 4 genes, KD resulted in lethality (Fig. 2K).

Drosophila Mmp2 is required in both KCs and glia for γ axon pruning

Many of the 'positive hits' in the screen are promising directions for further exploration. However, before deciding which genes to focus on in this study, we also reviewed the expression of the SCZ-related genes in glia - due to their known roles in neuronal

125 remodeling and synaptic mis-regulation in SCZ (Scott-Hewitt et al., 2023; Sekar et al.,
126 2016). Thus, we turned to our previously generated transcriptional profiling of astrocytes
127 in the *Drosophila* brain (Marmor-Kollet et al., 2023), and analyzed the larval vs. adult
128 expression of all of the SCZ homolog *Drosophila* genes (Fig. 3A). Genes that are more
129 highly expressed in late larval compared to adult astrocytes are good candidates to be
130 involved in γ axon pruning, which is initiated at early pupal stages. Interestingly, two genes
131 that came up in our KC screen as required for axon pruning, are also significantly
132 upregulated in larval astrocytes: Syntaxin13 and Matrix Metalloproteinase 2 (Mmp2; Fig.
133 3A and Fig. 2B,D). We decided to delve deeper into Mmp2, as a previous, CRISPR-based
134 screen done by our lab also found it to be required in γ-KCs for axon pruning (Meltzer et
135 al., 2019). To test whether Mmp2 is required in glia for γ axon pruning, we used the pan-
136 glial driver Repo-Gal4 to KD Mmp2 (in combination with R71G10-QF2-driven QUAS-
137 mtdT-HA to visualize the γ-KCs). Indeed, this resulted in a γ axon pruning defect,
138 indicating that Mmp2 originating from glia promotes MB γ axon pruning (Fig. 3C-D). We
139 further knocked down Mmp2 using the astrocyte-specific driver Alrm-Gal4, which also
140 resulted in a pruning defect, although milder (Fig. S1 A,C), validating its requirement in
141 astrocytes. The milder defect could be due to weaker RNAi expression in astrocytes by
142 the Alrm-Gal4 driver compared to Repo-Gal4, or because of Mmp2 secretion by additional
143 glial subtypes (e.g., cortex or ensheathing glia). Notably, the initial growth of γ-KCs, as
144 evident in the late larval stage, is unaffected by Mmp2 KD in either KCs or glia (Fig. S1
145 D,F,G,I).

146 ***Mmp1 is required in glia, and to a lesser extent in KCs, for γ axon pruning***

147 MMPs are extracellular proteases that can cleave extracellular matrix (ECM) components
148 (Page-McCaw et al., 2007). In mouse, there are 23 MMPs, while the zebrafish genome
149 encodes 25 MMPs[26]. The *Drosophila* genome, in contrast, encodes only two MMPs –
150 Mmp1 and Mmp2 – which together have 13 predicted isoforms, most of which are likely
151 secreted, while each Mmp also has a glycosylphosphatidylinositol (GPI)-anchored
152 isoform (LaFever et al., 2017). The significantly reduced complexity makes *Drosophila*
153 MMPs an ideal model system to uncover novel insights into their roles in neuronal
154 remodeling. Since both *Drosophila* MMPs are functionally similar, we tested the potential
155 requirement of Mmp1 in KCs as well as glia. Indeed, KD of Mmp1 in glia results in

156 significant γ axon pruning defect (Fig. 4A-C), while its KD in KCs results in a mild γ axon
157 pruning defect (Fig. 4D-F). As with Mmp2, astrocyte-specific Mmp1 knockdown with Alrm-
158 Gal4 also results in a pruning defect, but milder than with Repo-Gal4 (Fig. S1 A-B). Initial
159 growth of the γ-KCs is unaffected by Mmp1 KD in either KCs nor glia (Fig. S1 D-E, G-H).

160 ***While axon pruning of γ-KCs relies on both glial and neuronal MMPs, the main***
161 ***requirement is glial Mmp2***

162 Since most Mmp1 and Mmp2 isoforms are secreted, we speculated that both are secreted
163 in parallel from KCs and glia to promote axon pruning. Therefore, we preformed different
164 KD combinations of Mmp1 and Mmp2 in KCs and/or glia (Fig. 5). Our results draw two
165 main conclusions: first, while both Mmp1 and Mmp2 are required for pruning, the
166 contribution of Mmp2 is more significant as, in all conditions, the double KD of Mmp1 plus
167 Mmp2 did not further exacerbate the pruning defect of Mmp2 KD alone (Fig. 5 A-L).
168 Second, while Mmp1/2 expression from both neurons (KCs) and glia is required for
169 pruning of KC-axons, the main source is likely glia, as KD of a single or double Mmp in
170 both glia and neurons was not significantly more severe than the respective KD in glia
171 only (Fig. 5, quantified in M-O).
172 Taken together, our results suggest that while both MMPs affect pruning via secretion
173 from both neurons and glia, the strongest requirement for MB γ axon pruning comes from
174 glial-secreted Mmp2.

175 **Discussion**

176 Four decades ago, Feinberg hypothesized that SCZ is caused by aberrant synaptic
177 pruning during adolescence: “too many, too few or the wrong synapses are eliminated”
178 (Feinberg, 1982). In recent years, accumulating findings are providing experimental
179 support of this hypothesis (Keshavan et al., 2020; Sekar et al., 2016; Sellgren et al.,
180 2019). In this work, we aimed to further explore the association of SCZ with defective
181 neuronal remodeling, by utilizing the stereotypic remodeling of the *Drosophila* MB.
182 In a genetic LOF screen for *Drosophila* homologs of human genes that contain SNPs
183 associated with SCZ, over 40% of the genes we screened (12 out of 29) were found to
184 have a role in γ-KC development, most notably (11 out of 12) in axon pruning. This high
185 rate of ‘positive hits’ strengthens the link between SCZ and defects in developmental

neuronal remodeling. Notably, we focused on genes with upregulated expression prior to pruning, but future efforts to overexpress genes that are downregulated prior to pruning might also reveal interesting insights.

While many of the positive hits are fascinating candidates for further research, in this study we decided to focus on the role of MMPs in neuronal remodeling, for several reasons. First, in our screen, Mmp2 was found to be required for γ axon pruning (Fig. 2B), but is also highly expressed in larval astrocytes compared to adult ones. This is of particular interest due to the speculated role of glia in abnormal synaptic pruning in SCZ patients. Second, numerous reports have highlighted the link between MMPs and the pathophysiology of SCZ. Specifically, elevated levels of MMPs, most prominently Mmp9, were shown to correlate with SCZ risk and cognitive impairment (Dickerson et al., 2023; Kudo et al., 2020; Seitz-Holland et al., 2022), emphasizing their potential as biomarkers and therapeutic targets. While it has been suggested that this correlation is via MMP-dependent changes in dendritic spine morphology (Lepeta et al., 2017), the precise mechanism remains poorly understood. Third, the *Drosophila* MMPs appear to be quite evolutionary conserved (LaFever et al., 2017; Llano et al., 2002), highlighting the potential relevance to more translational contexts. As there are only 2 fly MMPs compared to 23 in mammals, *Drosophila* provides a simplified and powerful model system for mechanistic exploration.

Here, we found that the two *Drosophila* MMPs, expressed from both KCs and glia, are required for γ axon pruning in the MB. Mmp2, specifically from glia, seems to be the most significant requirement in this process.

MMPs are known for their roles in tissue remodeling by cleaving substrates in the extracellular matrix (ECM; Page-McCaw et al., 2007), and are well studied in the cancer field (Overall & López-Otín, 2002), but their mechanistic function in the developing nervous system is less characterized (e.g.,Broadie et al., 2011; Dear et al., 2016; Gore et al., 2021). MMPs are known to be secreted by neurons and glia, and are thought to facilitate synaptic remodeling of dendritic spines (Huntley, 2012). In the adult brains of rodent models, pharmacologically- (Szklarczyk et al., 2002) or injury- (Pijet et al., 2019) induced dendritic remodeling is suggested to occur via Mmp9. In the developmental remodeling of *Drosophila* dendritic arborization (da) neurons, both Mmp1 and Mmp2 were

217 shown to be required for the elimination of severed dendritic branches in a non-cell
218 autonomous manner (Kuo et al., 2005). Their source, as well as the mechanism by which
219 they promote dendrite removal, remain unknown (the authors speculate they are secreted
220 from phagocytic cells – at the time they suggested blood cells, but given more recent
221 studies from the same lab, epidermal cells are more likely; Han et al., 2014). Interestingly,
222 MMPs were also shown to mediate dendrite reshaping of da neurons in the mature fly via
223 local degeneration of the basement membrane, further reinforcing their significance in
224 refining the mature nervous system (Yasunaga et al., 2010).
225 The mechanism by which MMPs promote remodeling of MB γ-KCs, and specifically their
226 substrates in this context, remain to be uncovered. Since the main known role of MMPs
227 is cleaving ECM substrates, they may act as 'path cleaners' for the migration of cells and
228 molecules (Page-McCaw et al., 2007). This function can be relevant to axon pruning in
229 several, non-mutually exclusive ways. One option is that MMPs allow the migration of
230 Myoglianin (Myo) in the ECM. Myo is part of the pruning initiation cascade and was shown
231 to be secreted by cortex glia and astrocytes (Awasaki et al., 2011), and bind the Baboon
232 receptor on γ-KCs to eventually promote expression of EcR-B1, a key regulator of MB
233 remodeling (Yu et al., 2013; Zheng et al., 2003). Moreover, it was shown that the human
234 Mmp9 can proteolytically activate latent TGF-β, which is the human homolog of the
235 *Drosophila* Myo (Yu & Stamenkovic, 2000). However, we found that the expression of
236 EcR-B1 in γ-KC cell bodies in unaffected by Mmp1/Mmp2 knockdown, in neither glia nor
237 KCs (Fig. S2). Another option is that MMPs facilitate astrocyte infiltration. Astrocytes are
238 known to infiltrate the γ-KC axon bundle at the onset of pruning, likely assisting in its
239 defasciculation (Marmor-Kollet et al., 2023), and later engulf the axonal debris (Hakim et
240 al., 2014; Tasdemir-Yilmaz & Freeman, 2014). Thus, the pruning defects caused by MMP
241 KD in γ-KCs, as well as non-cell autonomously by KD in glia (including the astrocytes
242 themselves), may be due to failure of the astrocytes to infiltrate and defasciculate the
243 axonal bundle. MMPs were also shown to degrade adhesion molecules (Page-McCaw et
244 al., 2007), and our lab previously showed that adhesion molecules – specifically Fasciclin
245 II (FasII) – must be downregulated for pruning to occur properly (Bornstein et al., 2015).
246 It is thus possible that MMPs cleave FasII, or other adhesion molecules, to promote axon
247 pruning. Since most *Drosophila* MMP isoforms are secreted (LaFever et al., 2017),

together with the finding that they are required from both KC and glia origin, it is possible that MMPs are also secreted from additional cells within or around the MB circuit. All the above-mentioned options are promising directions for future research, in hopes of uncovering the mechanisms by which MMPs promote neuronal remodeling.

Notably, the main hypothesis in the literature is that SCZ is associated with increased synapse pruning, while in our study knockdown of SCZ-associated genes actually resulted in inhibition of pruning. However, SNPs can result in loss- but also gain-of-function, which could account for this apparent conflict. In fact, it was previously shown that the SCZ-related SNP in Mmp9 is in the 3' UTR, and affects Mmp9 mRNA folding which eventually results in higher Mmp9 expression compared to WT (Lepeta et al., 2017).

Understating the neurodevelopmental function of MMPs may provide insights into the changes that the ECM in the brain undergoes throughout development, and specifically during neuronal remodeling. The ECM consists of different components that allow efficient cell-cell communication as well as cell-ECM communication (Miller et al., 2020). It is therefore likely that MMPs, secreted from both glia and neurons, have a profound effect on the ECM during development, and thus have a pivotal role in the coordination of developmental processes and the etiology of neuropsychiatric disorders.

Materials and methods

Gene selection for the screen

Human genes that contain SNPs that are associated with SCZ were collected from two studies: PFC2 & CLOZUK (Wu et al., 2017; Supplemental excel 'screen data' sheet 'Human SCZ SNPs'). These genes were to their *Drosophila melanogaster* homologs converted using HumanMine (Supplemental excel 'screen data' sheet 'Fly homologs to human genes'). In cases of multiple fly homologs for a single human gene, we decided to include all.

Drosophila melanogaster rearing and strains

All fly strains were reared under standard laboratory conditions at 25°C on molasses-containing food. Males and females were chosen at random. Unless specifically stated

277 otherwise, the relevant developmental stage is adult, which refers to 3-5 days post
278 eclosion. All RNAi lines for the screen were from the TRiP collection (Zirin et al., 2020)
279 and obtained for the Bloomington *Drosophila* Stock Center (BDSC) – the full list is detailed
280 in table 1 below. The following constructs were also obtained from BDSC: Luciferase
281 RNAi (used as control for RNAi experiments; #35788), Mmp1 RNAi (#31489), Repo-Gal4
282 (#7415), OK107-Gal4 (#854) and QUAS-mtdTomato-3xHA (#30004). R71G10-QF2 was
283 previously generated by our lab (Bornstein et al., 2021).
284

Gene	**TRiP #**	**BDSC #**
tartan	HM05011	28525
Fish-lips	HM05054	28568
retinal degeneration A	JF03371	29435
Ppr-Y	HMS00819	33882
FER tyrosine kinase	HMS00249	33375
Matrix metalloproteinase 2	HMJ23143	61309
Phospholipase C at 21C	HMS00436	32438
Proteasome alpha3 subunit	HMS05889	77145
Regulatory particle non-ATPase 7	HMS00096	34787
Syntaxin 13	HMS01723	38525
anterior pharynx defective 1	HMS01693	38249
BNIP3	HMJ02058	42494
liquid facets	HMJ22079	58130
eukaryotic translation release factor 1	HMS05812	67900
DIP-kappa	HM04050	31740
MYPT-75D	HMS01166	34688
Protein phosphatase 19C	HMS01841	38372
GTPase activating protein and centrosome-associated	HMS01132	34976
Ran-binding protein M	HMC05142	61172
Na[+]-driven anion exchanger 1	HMC05184	62177
minidiscs	HMC05214	62207
Serine-arginine protein kinase at 79D	HMC04154	55881
Ypel	HM05209	29530
Akt1	HMS00007	33615
absent, small, or homeotic discs 2	HMC05816	64942
Fmr1	HMS00248	34944
Arginine methyltransferase 1	GL01072	36891
Ge-1	HMS00340	32349
crooked legs	HMS02202	41669

285 **Table 1.** RNAi lines used in the screen

286 *Immunohistochemistry and imaging*

287 *Drosophila* brains were dissected in cold ringer solution and placed on ice. Followed by
288 fixation in 4% paraformaldehyde (PFA) solution for 20 min in room temperature (RT).
289 Fixed brains were then washed with PB supplemented with 0.3% Triton X-(PBT) – 3 times,

290 20 min each. Next the brains were blocked for non-specific staining using 5% heat
291 inactivated normal goat serum (NGS). Antibody staining was performed as follows:
292 primary antibodies (4°C, overnight), PBT washes (X3 in RT, 20 min each), secondary
293 antibodies (RT, 2h), PBT washes (X3 in RT, 20 min each), mounting in SlowFade
294 (Invitrogen). Primary antibodies included mouse anti-FasII 1:25 (1D4; DSHB), mouse
295 anti-EcRB1 1:25 (AD4.4; DSHB) and rat anti-HA 1:250 (Sigma Aldrich, 11867423001).
296 Secondary antibodies included Alexa fluor 647 goat anti-mouse 1:300 (A-21236;
297 Invitrogen) and Alexa fluor 568 goat anti-rat 1:300 (A-21247; Invitrogen). All brains were
298 imaged on a Zeiss LSM980 confocal microscope. The images were processed using
299 ImageJ 2.14.0 (NIH).

300 *Quantification and statistical analysis*

301 Blind ranking of the pruning phenotypes for Fig. 3-5 was performed by an independent
302 scorer who reviewed the Z-projection. Ranking scores were as follows: 1 = WT-like
303 phenotype, 2 = very mild pruning defect (PD), 3 = mild PD, 4 = moderate PD, 5 = severe
304 PD.

305 A non-parametric statistical analysis was preformed: Kruskal Wallis H test followed by a
306 post hoc Mann Whitney U test with FDR correction.

307 *** = p value<0.001, ** = p value<0.01, * = p value<0.05, N.S = not significant. Specific p
308 values are indicated in the figure legends.

309

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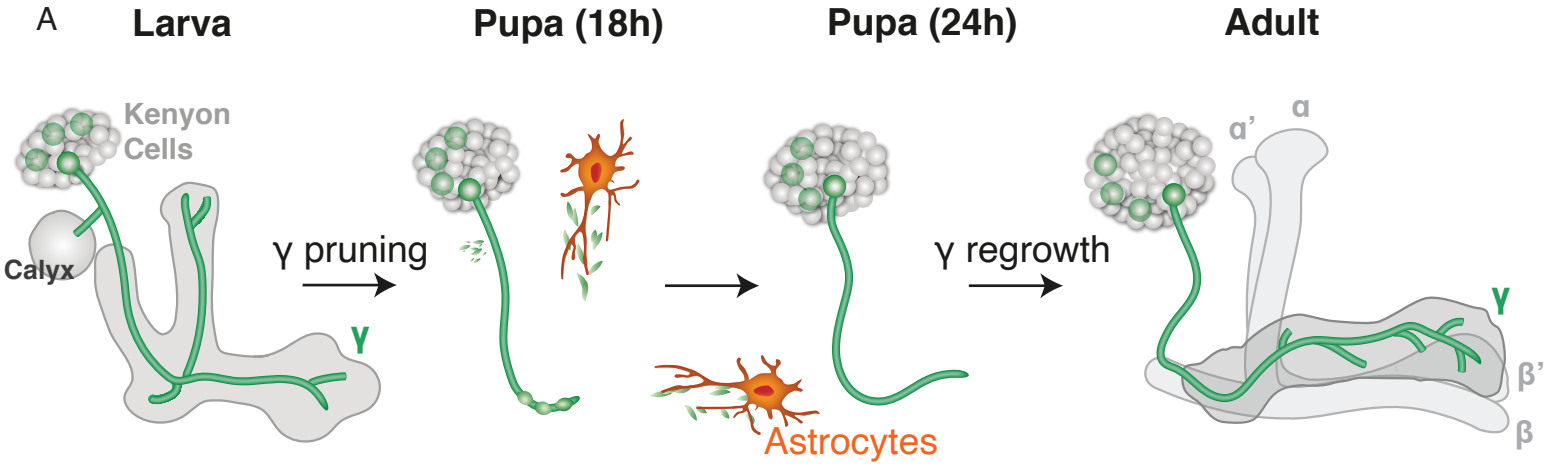
319 **Author contributions**

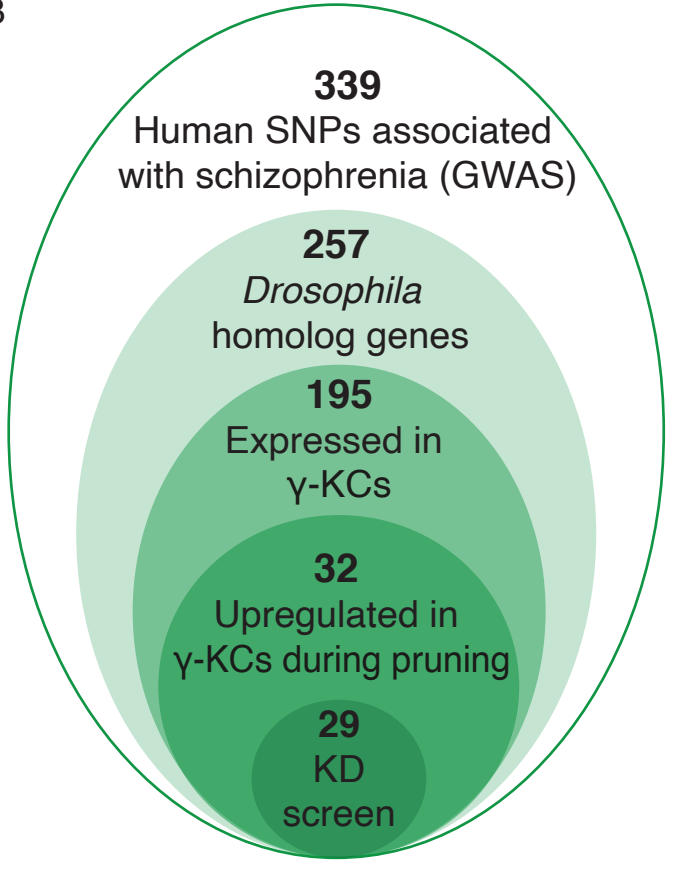
320 S.K. conceptualized the project, designed, performed and analyzed experiments,
321 performed bioinformatic and statistical analyses, and wrote the manuscript. H.M.
322 designed and performed experiments, interpreted results, and wrote the manuscript. N.M-
323 K. designed experiments and assisted with bioinformatic analyses. O.S. led the project,
324 designed experiments, interpreted results, wrote the manuscript, and procured funding.

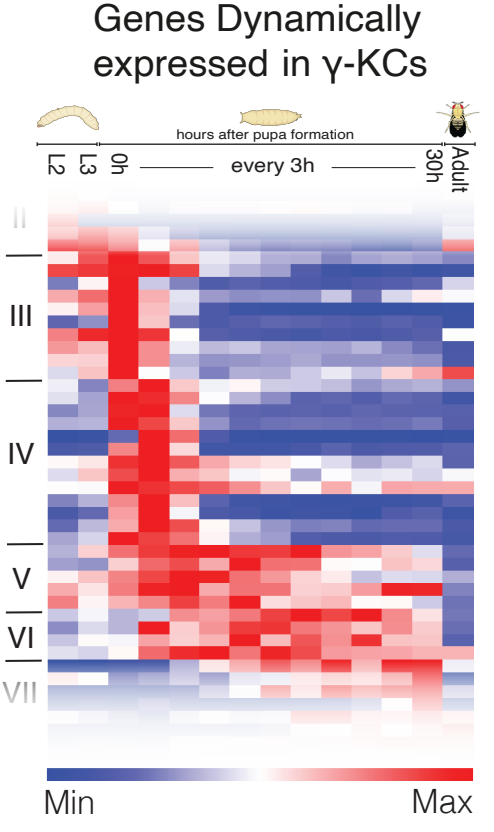
325 **Declaration of interests**

326 The authors declare that they have no conflict of interest.

327


A
Larva
Pupa (18h)
Pupa (24h)
Adult
Kenyon Cells
Calyx
γ
γ pruning
Astrocytes
γ regrowth
α'
α
γ
β'
β

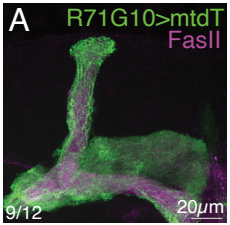

B
339
Human SNPs associated with schizophrenia (GWAS)
257
Drosophila homolog genes
195
Expressed in γ-KCs
32
Upregulated in γ-KCs during pruning
29
KD screen


C
Genes Dynamically expressed in γ-KCs
hours after pupa formation
L2
L3
0h
every 3h
30h
Adult
II
III
IV
V
VI
VII
Min
Max

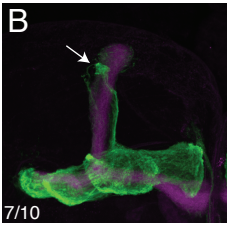
328 **Figure 1. *Drosophila* homologs of SCZ-related genes are dynamically expressed in**
329 **developing γ-KCs.**
330 (A) Schematic representation of *Drosophila* MB development. During early pupal stages,
331 the γ-KCs (green) prune their dendrites completely and their vertical and medial axonal
332 branches up to their branch-point. Subsequently, they reform dendrites in the calyx and
333 regrow axons to form the adult-specific medial lobe.
334 (B) A schematic representation describing the logic for selection of candidates for the
335 loss-of-function screen.
336 (C) A heatmap representing the expression of dynamically expressed genes along γ-KC
337 development (based on Alyagor et al., 2018). Each row represents a gene, with red and
338 blue indicating high and low relative expression, respectively. The genes in clusters III-VI
339 were selected as candidates for screening.
340

KC-Gal4> RNAi

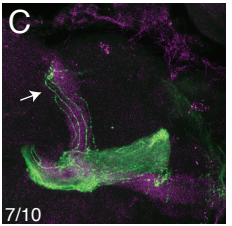
Control



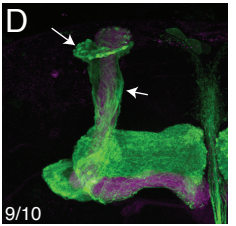
Mmp2



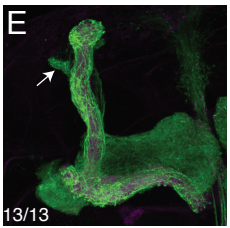
Plc21C



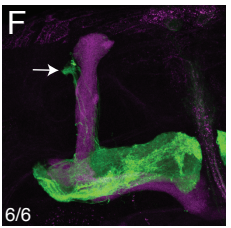
Syx13



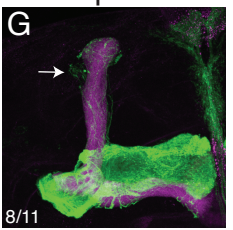
Pp4-19C



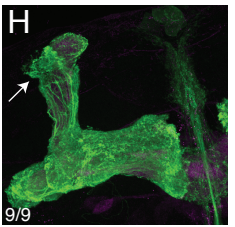
RanBPM



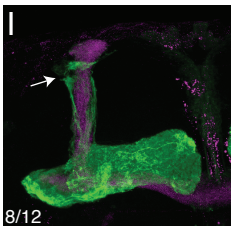
Ypel



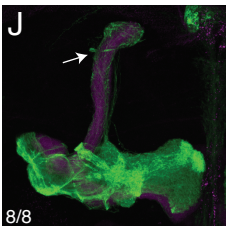
Ge-1



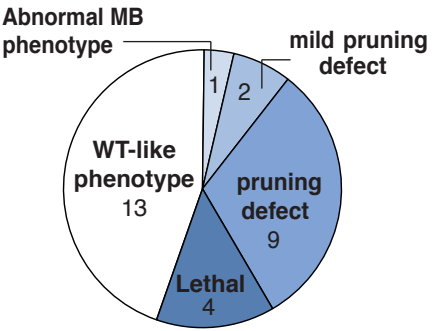
crol



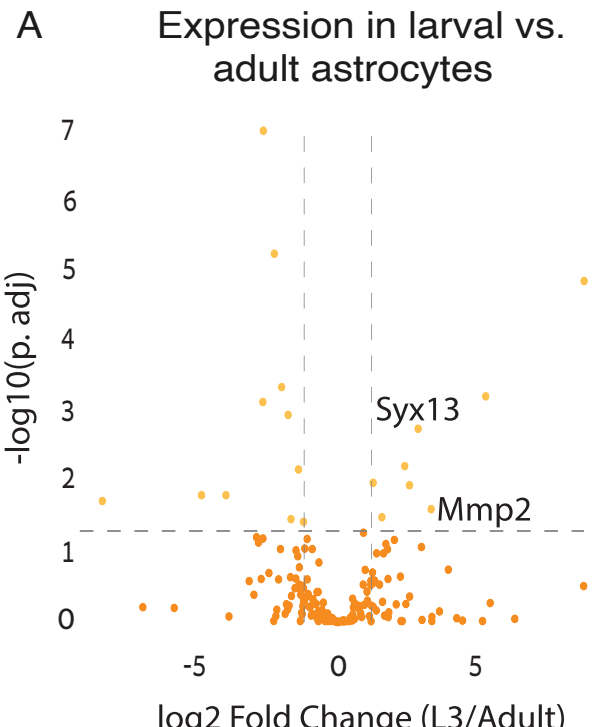
BNIP3

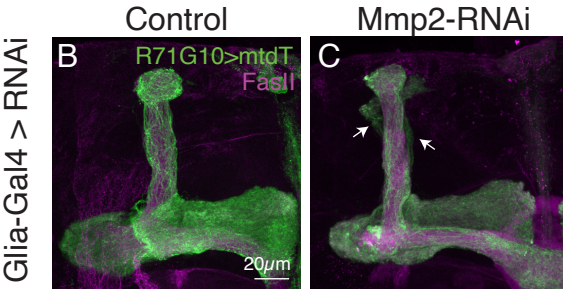


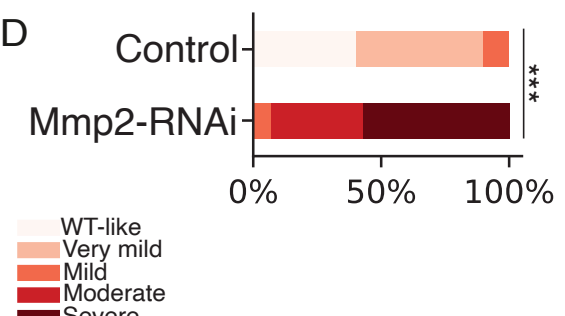
K



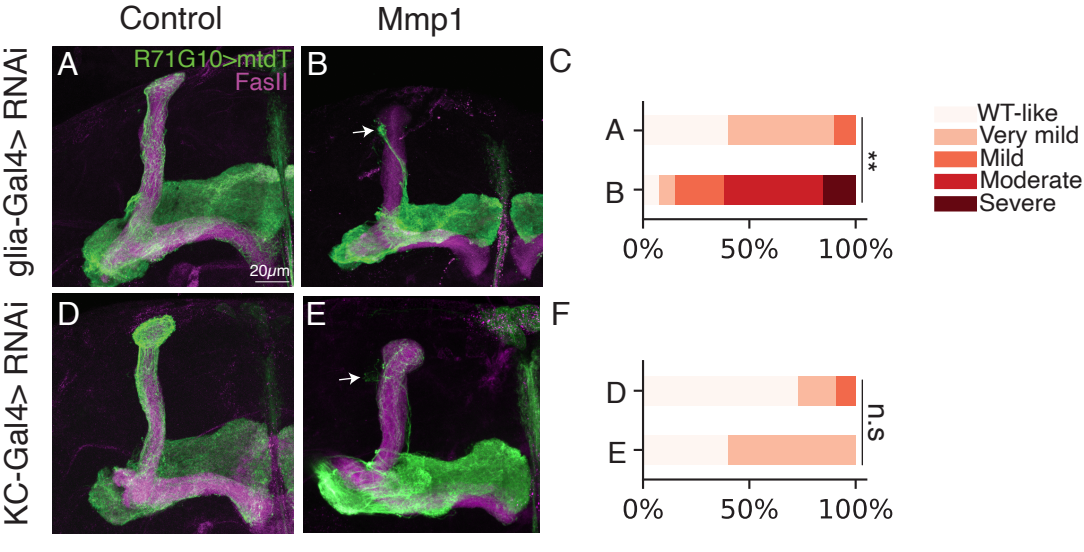
341 **Fig 2. *Drosophila* homologs of SCZ-associated genes are required for γ-axon**
342 **pruning.**
343 (A-J) Confocal z-projections of brains in which the indicated UAS-RNAi transgenes were
344 expressed in all KCs using the strong driver OK107-Gal4, while R71G10-QF2-drives the
345 expression of membranal tandem tomato (mtdT; green) in γ-KCs and stochastically in α/β-
346 KCs. The RNAi transgenes target the following genes: Matrix Metalloproteinase 2 (Mmp2;
347 B), Phospholipase C at 21C (Plc21C; B), Syntaxin 13 (Syx13; D), Protein phosphatase
348 19C (Pp4-19C; E), Ran-binding protein M (RanBPM; F), Yippee-like (Ypel; G), Ge-1 (Ge-
349 1; H), crooked legs (crol; I), BCL2 Interacting Protein 3 (BNIP3; J). Control is RNAi
350 targeting Luciferase (A). FasII antibody (magenta) strongly labels α/β-KCs and weakly
351 labels γ-KCs.
352 Arrows indicate unpruned axons. Scale bar corresponds to 20μm. The number of MBs
353 (each from an individual brain) showing the presented phenotype out of the total n for
354 each genotype is indicated.
355 (K) Pie chart representing the overall screen results.
356


A
Expression in larval vs. adult astrocytes
-log10(p. adj)
7
6
5
4
3
2
1
0
Syx13
Mmp2
-5
0
5
log2 Fold Change (L3/Adult)

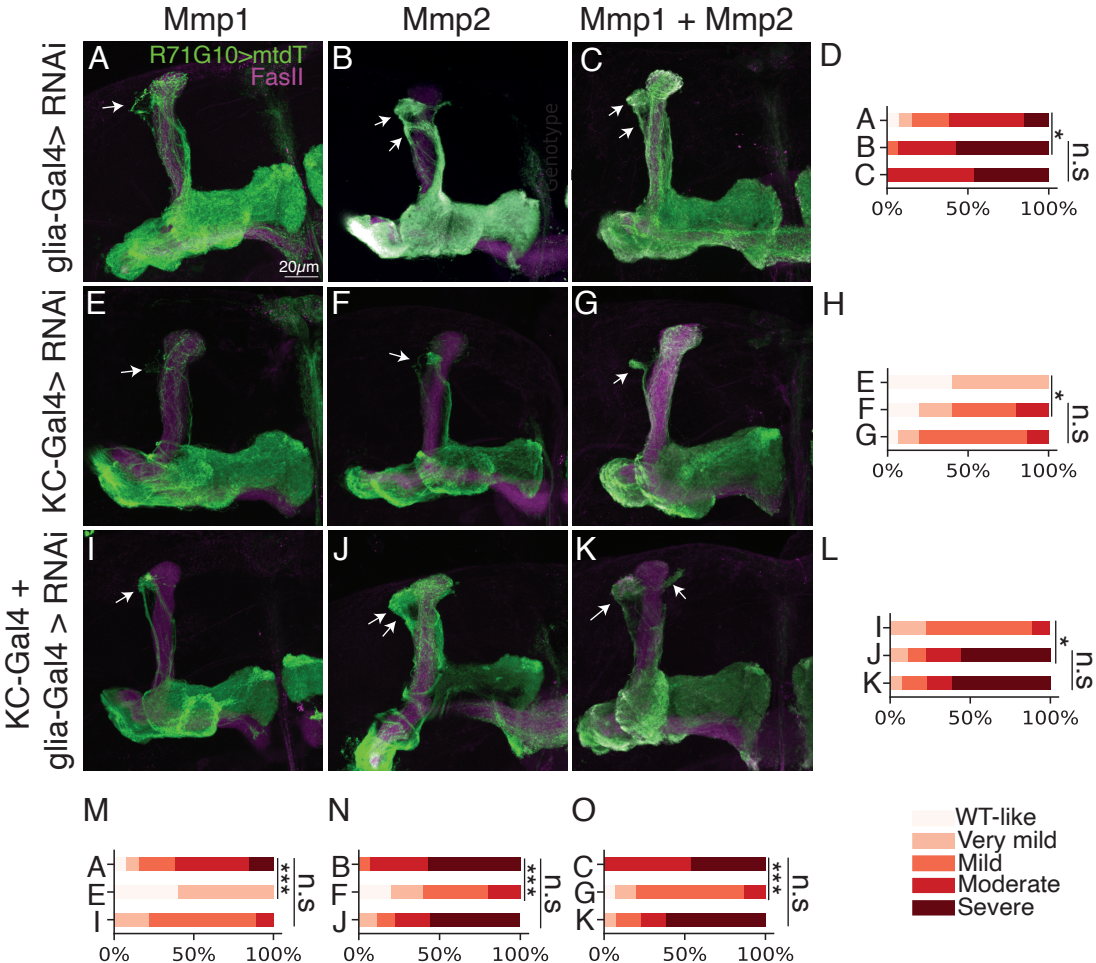

Control
Mmp2-RNAi
Glia-Gal4 > RNAi
B
R71G10>mtdT
FasII
20μm
C


D
Control
Mmp2-RNAi
***
0%
50%
100%
WT-like
Very mild
Mild
Moderate
Severe

357 **Fig. 3. Mmp2 is upregulated in larval astrocytes and required in glia for γ-axon**
358 **pruning.**
359 (A) Volcano plot representing the expression of candidates in astrocytes (based on
360 Marmor-Kollet et al., 2023). The x-axis represents log2 fold change of 3rd instar larvae
361 (L3) vs. adult astrocytes. The y-axis represents the significance of the change (-log10 of
362 p. adjust). The annotated genes are upregulated in larval compared to adult astrocytes,
363 as well as required in KCs for γ-axon pruning (See Fig. 2 and Supplemental excel 'screen
364 data' sheet 'Astrocytes - genes exp. data').
365 (B-C) Confocal z-projections of control (B) and Mmp2-RNAi (C) driven by the pan-glial
366 Repo-Gal4 while R71G10-QF2 drives expression of mtdT (green) to label the γ-KCs plus
367 some of the α/β-KCs.
368 FasII antibody (magenta) strongly labels α/β-KCs and weakly labels γ-KCs.
369 Arrows indicate unpruned axons. Scale bar corresponds to 20μm.
370 (D) Quantification of the pruning defect phenotypes, ranging from 1 (WT-like phenotype)
371 to 5 (severe pruning defect). *** p=0.0002
372


Control
Mmp1
glia-Gal4> RNAi
KC-Gal4> RNAi
A
R71G10>mtdT
FasII
20μm
B
C
D
E
F
A
B
**
0%
50%
100%
D
E
n.s
0%
50%
100%
WT-like
Very mild
Mild
Moderate
Severe

373 **Fig. 4**. **Mmp1 is required in glia, and to a lesser extent in KCs, for γ-axon pruning.**
374 (A-B, D-E) Confocal z-projections of adult MBs in which γ-KCs are labeled by R71G10-
375 QF2-driven QUAS-mtdT (green), and control or Mmp1-RNAi is expressed in all glia using
376 Repo-Gal4 (A, B), or in all KCs using OK107-Gal4 (D, E).
377 FasII antibody (magenta) strongly labels α/β-KCs and weakly labels γ-KCs.
378 Arrows indicate unpruned axons. Scale bar corresponds to 20μm.
379 (C, F) Quantification of the pruning defect phenotypes, ranging from 1 (WT-like
380 phenotype) to 5 (severe pruning defect). ** p=0.002, n.s = not significant.
381


Mmp1
Mmp2
Mmp1 + Mmp2
glia-Gal4> RNAi
KC-Gal4> RNAi
KC-Gal4 +
glia-Gal4 > RNAi
R71G10>mtdT
FasII
20μm
genotype
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
0%
50%
100%
*
n.s
***
WT-like
Very mild
Mild
Moderate
Severe

382 **Fig. 5. While axon pruning of γ-KCs relies on both glial and neuronal MMPs, the**
383 **main requirement is glial Mmp2.**
384 (A-C, E-G, I-K) Confocal z-projections of adult MBs in which γ-KCs are labeled by
385 R71G10-QF2-driven QUAS-mtdT (green), and UAS-RNAi species targeting MMP1 (A, E,
386 I), MMP2 (B, F J), or both MMPs (C, G, K) driven by Repo-Gal4 (A-C), OK107-Gal4 (E-
387 G), or both Gal4s simultaneously (I-K). FasII antibody (magenta) strongly labels α/β-KCs
388 and weakly labels γ-KCs. Arrows indicate unpruned axons.
389 (D, H, L-O) Quantification of the pruning defect phenotypes, ranging from 1 (WT-like
390 phenotype) to 5 (severe pruning defect). Genotypes are indicated with the letter of the
391 corresponding image within this figure. *** = $p<0.001$, * = $p<0.05$, n.s = not significant.
392 A vs. B $p=0.019$, B vs. C $p=0.814$, E vs. F $p=0.041$, F vs. G $p=0.659$, I vs. J $p=0.019$, J
393 vs. K $p=0.905$, A vs. E $p=0.0006$, A vs. I $p=0.088$, B vs. F $p=0.0007$, B vs. J $p=0.809$, C
394 vs. G $p=0.0002$, C vs. K $p=0.937$
395

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