Transmembrane protein 184B (TMEM184B) promotes expression of synaptic gene networks in the mouse hippocampus

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Running head: TMEM184B in hippocampal synaptic gene expression

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Abstract

In Alzheimer's Disease (AD) and other dementias, hippocampal synaptic dysfunction and loss contribute to the progression of memory impairment. Recent analysis of human AD transcriptomes has provided a list of gene candidates that may serve as drivers of disease. One such candidate is the membrane protein TMEM184B. To evaluate whether TMEM184B contributes to neurological impairment, we asked whether loss of TMEM184B in mice causes gene expression or behavior alterations, focusing on the hippocampus. Because one major risk factor for AD is age, we compared young adult (5-month-old) and aged (15-month-old) wild type and *Tmem184b*-mutant mice to assess the dual contributions of age and genotype. TMEM184B loss altered expression of pre- and post-synaptic transcripts by 5 months and continued through 15 months, specifically affecting genes involved in synapse assembly and neural development. Wnt-activated enhancer elements were enriched among differentially expressed genes, suggesting an intersection with this pathway. Few differences existed between young adult and aged mutants, suggesting that transcriptional effects of TMEM184B loss are relatively constant. To understand how TMEM184B disruption may impact behaviors, we evaluated memory using the novel object recognition test and anxiety using the elevated plus maze. Young adult *Tmem184b*-mutant mice show normal object discrimination, suggesting a lack of memory impairment at this age. However, mutant mice showed decreased anxiety, a phenotype seen in neurodevelopmental disorders. Taken together, our data suggest that TMEM184B is required for proper synaptic gene expression and function but may not be causal for AD and related dementias.

Introduction

Alzheimer's Disease (AD) is a devastating neurodegenerative disease for which we have a paucity of treatment options. In 2022, it is estimated that 10.7% of adults over 65 have Alzheimer's-induced dementia [1]. The burden on patients, their families and health systems is expected to grow significantly in the next 20 years; this trend is already apparent [2].

Alzheimer's Disease causes the accumulation of amyloid beta plaques and neurofibrillary tangles in diverse regions of the brain [3]. Many clinical trials have focused on the depletion of amyloid as a key therapeutic strategy. However, trials in which amyloid plaques are successfully diminished have not produced a reduction or delay in cognitive impairment in trial participants [4]. This highlights the need for the identification of new targets that may contribute to early cognitive impairment and that may offer hope at alternative strategies for stopping the progression of Alzheimer's Disease.

Large-scale human genome-wide association and RNAseq analysis to identify network drivers of disease has yielded many plausible candidates that could contribute to AD [5–7]. With these candidates in hand, the task for the AD field now shifts to identifying which candidates cause bona fide alterations in relevant gene expression pathways or in behaviors associated with AD. Through a recent RNAseq analyses, the transmembrane protein TMEM184B was predicted as a candidate protein that could promote AD progression [8]. TMEM184B is a 7-pass transmembrane protein expressed broadly in the nervous system and is thought to play a role in neuronal excitability, synaptic structure, and expression of key developmental and adult pathways involved in neuronal differentiation [9, 10]. Using analysis of the ROSMAP and MAYO clinic human cohorts, *TMEM184B* was predicted to drive AD-associated gene expression patterns in the dorsolateral prefrontal cortex and temporal cortex, respectively [8]. However, knockdown of *TMEM184B* in human induced pluripotent stem cell-derived neurons did not directly affect Aβ levels [5], suggesting that this gene may play a more indirect role.

In this study we sought to directly test the hypothesis that TMEM184B regulates gene networks that could contribute to AD, using a mutant mouse model in which the *Tmem184b* gene is disrupted [11]. We compared how TMEM184B-regulated gene networks are influenced by aging, focusing on the hippocampus as a key location involved in memory. We identified impairments in pathways controlling synapse assembly and function in TMEM184B-deficient hippocampus. We also evaluated memory and anxiety behaviors in these mice. Our results identify a clear effect of TMEM184B on the expression of synaptic gene networks at both young adult and older ages. We also uncovered an unexpected effect of TMEM184B loss on anxiety behavior. However, at young adulthood TMEM184B mutant mice do not have impaired memory. Our work places TMEM184B into a gene regulatory network that could impact synaptic connectivity and influence cognitive function but whose loss likely does not precipitate memory impairment.

Materials and Methods

Animal Assurances

All animal experiments are approved by and were performed in accordance with the University of Arizona Institutional Animal Care and Use Committee (IACUC) Protocol 17-216.

Hippocampal RNA Isolation

Male and female mice were humanely euthanized with carbon dioxide, and hippocampi were removed within 10 minutes of euthanasia and immediately frozen for subsequent RNA isolation. Total RNA was extracted using Trizol (Invitrogen) using the manufacturer's protocol. Following initial quality check via Nanodrop analysis, total RNA samples were frozen and shipped to Novogene (Sacramento, CA) for sequencing. Quality control checks were done by Novogene to ensure that sequenced samples had high RNA quality. Sequencing was performed using paired end 150bp reads, 30 million reads/sample, on the Illumina platform. Raw reads data were provided by Novogene.

Data Processing and Statistical Analysis

Alignment to the mouse genome (mm10.0) was done using Salmon and run on the High Performance Computing cluster at the University of Arizona. Differential gene expression analysis was done using DESeq2 on Galaxy servers (http://usegalaxy.org). PCA plots were generated by Galaxy's DESeq2 default settings, or done in R (https://cran.r-project.org/) using the stats R package.

For GO analysis, Panther pathway analysis, and KEGG pathway analysis, ribosomal genes were manually removed from the lists prior to submission. GO analysis (http://geneontology.org/) was performed with a background list of genes consisting of the full list of mapped genes identified by pseudoalignment via the Salmon algorithm from the datasets used for each comparison. KEGG analysis was performed using Enrichr online tools (https://maayanlab.cloud/Enrichr/). Transcription factor binding analysis involved two Enrichr tools: ChEA (2016) and Transcription Factor Perturbations. For both analyses, adjusted P-values were exported and plotted in Graphpad Prism.

Data Visualization

Fold change values were imported into Cytoscape and mapped onto networks (Biogrid Protein-Protein interaction Network as derived from https://downloads.thebiogrid.org/ or GOCAM Gene Ontology network, as derived from https://github.com/geneontology/gocam-sif-pyexport). To create subnetworks for visualization, we first identified hub proteins that were themselves differentially expressed and also contained multiple differentially expressed genes in their nearest neighbors. Colorization of levels of fold change used a continuous scale from red to blue, with grey representing genes not differentially expressed in the comparison groups. For biological processes, the default color palette was changed such that darker blues are more significant adjusted P-values, while lighter greens to white are less significant.

Graphs were made in either R (ggplot2 package) or in Graphpad Prism. Venn diagrams were created using online tools available at the University of Gent, Belgium (https://bioinformatics.psb.ugent.be/webtools/Venn/).

Novel Object Recognition

Our protocol for novel object recognition was adapted from Leger et al. [12]. Eight to ten mice of each genotype were used for this study; these mice were 5-7 months old and were approximately sex balanced (50/50 or 60/40). Mice were acclimated to the testing room and to the experimenter for two sessions (two days). On day 3, mice were individually placed in an empty square chamber and allowed to explore for 5 minutes. Videos were recorded from above the box in ambient light and with the experimenter outside the room. On day 4 (training/association), mice were returned to the chamber to explore two identical objects for ten minutes. On day 5 (24 hours after the day 4 training trial), mice were returned to the chamber which contained one familiar object from the day before, along with one novel object. Ten minutes of object exposure was captured via video. Videos were analyzed offline in Ethovision. To quantify mouse behavior, we used a discrimination index, which is calculated as (total time at novel object – total time at familiar object) / (total object exploration time). Positive values indicate recognition of a novel object.

Elevated Plus Maze

Mice were placed at the distal end of a closed arm and allowed to walk on an elevated X-shaped platform (50cm from the ground, 35 cm arm length, made of gray non-reflective non-odorous poly-methyl methacrylate (PMMA)) for five minutes. Two of the arms of the platform are enclosed by walls, while two are open. The number of times a mouse enters the open or closed arms is recorded. The ratio of open vs. closed entries, and time spent in open versus closed arms, are calculated as an index of anxiety. Benchpads are used under the open arms to cushion any unexpected falls.

Results

TMEM184B promotes expression of synapse assembly genes in the mouse hippocampus.

TMEM184B expression is required for maintenance of synaptic structure at neuromuscular junctions in both mouse and fly. We wondered if this role would extend to the hippocampus, a center for learning and memory processing. TMEM184B is expressed in neuronal layers of the hippocampus, suggesting it may contribute here as well. We compared gene expression in the hippocampus in wild type and *Tmem184b* gene-trap mutant mice (which have less than 5% mRNA of *Tmem184b* remaining [10, 11]) at 5 months of age (Fig 1 and Supplementary File 1). Wild type mice showed high clustering in both principal components (Fig 1a), while mutant mice separated well from wild type mice in principal component 1 (PC1). In total, 1153 genes were differentially expressed in *Tmem184b*-mutant hippocampi (Fig 1b-c). When considering those genes with significant hippocampus expression, a group of developmentally important transcripts emerged including Shank1 (involved in post-synaptic density scaffolding), Neurexin 1 (involved in synaptic assembly and adhesion), and Somatostatin (a marker of subtypes of interneurons).

To evaluate the biological processes likely affected by TMEM184B in the hippocampus, we used gene ontology analysis to pinpoint processes with significant enrichment of differentially expressed genes (DEGs) in our dataset (Fig 1d). This showed a large predicted effect on synapse assembly and neural development. Other processes that may be affected in *Tmem184b*-mutant mice include memory/cognition, synapse function, behavior, and neuronal cell death. We performed pathway analysis on these data by querying the KEGG database (Fig 1e). This analysis identified many key pathways affecting both glutamatergic (excitatory) and GABAergic (inhibitory) synapses. Furthermore, it suggested that the pathways dysregulated in *Tmem184b*-mutants were enriched for those contributing to neurodegenerative diseases including Parkinson's disease and Alzheimer's Disease. Taken together, our analysis of

transcriptomic changes in *Tmem184b*-mutant hippocampus predicts significant dysregulation of synapses that may be linked to neurological disease.

Synaptic Protein-Protein Interaction Networks and Biological Processes rely on TMEM184B for proper hippocampal expression.

We sought to take a closer look at the relatedness of genes that were differentially expressed in the pathways described above, specifically synapse assembly and neural development. Using Cytoscape, we visualized our gene expression changes on the predicted mouse protein-protein interaction network annotated by Biogrid (Fig 2-B). We noticed a significant enrichment of altered transcripts of proteins that are predicted to interact with two key neurodevelopmental proteins: Neurogenin3 and with Shank1. However, contrary to our expectations, *Tmem184b*-mutant mice show upregulation of proteins in these networks (shown in circles with pink and red shading). Neurogenin 3 is a transcription factor that promotes dendritogenesis in cultured hippocampal neurons [13]. Shank1, along with its family members, are critical scaffolds for postsynaptic density proteins and glutamate receptors, and they are implicated in autism spectrum disorders [14, 15]. The upregulation of these proteins could be a direct effect of TMEM184B or a compensatory effect following other synaptic disruptions; our data does not distinguish between these possibilities. Nevertheless, the correct levels of these factors are crucial for maintenance of proper circuit assembly and function; both up- and down-regulation of both proteins is deleterious [13, 16].

In addition to mapping our data onto known protein-protein interactions, we also mapped our data onto gene ontology networks and assessed which neuronal or synapse-related processes were predicted to be altered by TMEM184B expression. The most differentially expressed GO Biological Processes associated with synapses were in synaptic transmission and plasticity. This prediction is consistent with data showing that TMEM184B mutation causes hyperexcitability at glutamatergic synapses in *Drosophila* [9]. Within the search term “neuron”

194 these processes were enriched for neurogenesis and projection (axon/dendrite) development.
195 Overall, our analysis suggests that TMEM184B maintains appropriate expression of
196 interconnected networks of synaptic and neurodevelopmental genes in the hippocampus.
197
198 **Analysis of Transcription Factors Upstream of Hippocampal Differentially Expressed**
199 **Genes implicates Wnt signaling alterations.**
200 To understand why loss of TMEM184B causes gene expression changes in the
201 hippocampus at 5 months, we performed an *in silico* analysis to identify enriched transcription
202 factor target genes in our DEGs using two platforms. In the first (ChEA 2016), we identified four
203 transcription factors with enriched targets (DMRT1, RARB, ZFP281, and TCF7) (Fig 3A-B).
204 DMRT1 is not expressed in the brain and so is unlikely to have an effect. ZFP281 is a
205 transcriptional repressor that maintains the pluripotency of stem cell populations and is highly
206 expressed. RARB, or retinoic acid receptor beta, is a steroid hormone receptor responsive to
207 retinoic acid (RA) and plays roles in embryonic development. Disruptions to RARB lead to
208 neurodevelopmental delay and are associated with autism spectrum disorder [17]. TCF7 is a
209 canonical Wnt signaling pathway mediator [18]. In a separate analysis, we used the EnrichR
210 platform to examine the overlap between DEGs from our data set with mouse transcription
211 factor manipulations performed by others. We identified many transcription factors whose gene
212 expression alterations significantly resemble that of *Tmem184b*-mutant hippocampus.
213 Interestingly, multiple associations were identified with transcription factors known to promote
214 Wnt signaling (NEUROD1, TCF3, PLAGL2) [19][20, 21]. This is consistent with prior work
215 suggesting that Wnt pathway dysfunction could participate in TMEM184B-dependent
216 phenotypes [10]. Wnt/β-catenin signaling is critical for hippocampal neurogenesis [22, 23], so
217 this suggests that one effect of *Tmem184b* disruption may be loss of neurons. Other factors,
218 such as NFIA, are known contributors to stem cell maintenance and proliferation. Adult
219 neurogenesis in the hippocampus is required for long-term spatial memory in mice [24]. Taken

220 together, this analysis suggests a possible role for TMEM184B in the generation of hippocampal
221 neurons via promotion of Wnt pathway signaling.
222
223 **Identification of Common and Unique Aging Signatures in *Tmem184b*-mutant**
224 **hippocampus**
225 The biggest risk factor for the development of Alzheimer's Disease is advanced age. To
226 identify unique signatures of TMEM184B that may affect the aging brain, we compared genes
227 that were altered upon aging (15 months of age) in wild type mice with those in *Tmem184b*-
228 mutant mice. The *Tmem184b* transcript itself was not significantly altered across wild type
229 aging, although it was slightly reduced in older mice (log_2FC = -0.13; Adjusted P value = 0.074).
230 After filtering out ribosomal transcripts, a total of 2171 genes were significantly altered in
231 15-month-old *Tmem184b*-mutant mice compared to age-matched wild types (Supplementary
232 File 2). Of these, 222 transcripts were altered at both ages (Fig 4A-B and Supplementary File
233 3). We identified the most enriched processes in this group using gene ontology (Fig 4C-D).
234 This analysis confirmed the strong effects of TMEM184B loss on synaptic processes, including
235 postsynaptic density organization, glutamate receptor and neuroligin binding activity, and
236 synaptic plasticity. Interestingly, both young and aged TMEM184B mutant mice have a small but
237 significant upregulation of ApoE transcripts (log_2FC of 0.23 and 0.30, respectively). This
238 comparison confirms that, throughout aging, TMEM184B continues to influence synaptic gene
239 expression.
240 If we compare 5-month-old and 15-month-old mutant mice, very few genes are
241 significantly different (104 total) (Fig 4E and Supplementary File 4). In this group, no biological
242 pathways, molecular functions, or cellular components reached statistical significance (all had
243 adjusted p values > 0.05). This indicates that the changes that have occurred in TMEM184B
244 mutant mice primarily occur by 5 months, and gene expression is relatively constant beyond
245 that point. We used the Molecular Signatures database (MSigDB) to query whether alterations

246 in this small group of genes were similar to other published transcriptomic datasets. This
247 analysis revealed that the most similar datasets are mouse models of neurological disease,
248 including two Huntington's Disease models (R6/2 and Q175) and spinal cerebellar ataxia
249 models caused by disruption of Ataxin-1 (Fig 4F). This suggests that the few genes that are
250 altered by aging in TMEM184B mutants may affect similar pathways to those affected in HD and
251 Ataxia.
252
253 **Somatostatin, and other neuronal genes, are similarly regulated in both central and**
254 **peripheral neurons**
255 To evaluate common signatures of TMEM184B-dependent gene expression across
256 disparate types of neurons, we compared the differentially expressed genes (adjusted $p < 0.05$)
257 between our 5 month hippocampus data set and one we have previously reported from 6 month
258 adult dorsal root ganglia [10]. Among differentially expressed genes, we identified 12 genes
259 (including *Tmem184b* itself) that were similarly regulated (Fig 5 and Supplementary File 5). In
260 many cases, the fold change in the hippocampus was somewhat less than that in the DRG,
261 which could reflect a greater percentage of non-neuronal cells in the hippocampus that may
262 dilute the overall fold change. Nevertheless, we noticed that Somatostatin, a neuropeptide used
263 as a marker of GABAergic inhibitory interneurons in the hippocampus (but which marks
264 excitatory, pruriceptive neurons in the DRG), was downregulated in both data sets, suggesting a
265 common means of regulation in these two tissues. One common upregulated gene, Tsukushi
266 (*Tsku*), is a negative regulator of the Wnt pathway [25]. Taken together with our earlier
267 analyses, this again implicates a loss of Wnt signaling in TMEM184B-associated phenotypes.
268 Finally, Bc1, a long non-coding RNA that is upregulated in TMEM184B-deficient neurons, has
269 been shown to promote translation of amyloid precursor protein (APP) and cause spatial
270 learning and memory defects [26].
271

TMEM184B loss does not affect spatial memory but alters anxiety behaviors in middle aged mice

Finally, we sought to evaluate how these gene expression changes in the hippocampus affect behaviors driven by this brain region, including anxiety and object-oriented memory. In prior work, we established that six-month-old *Tmem184b*-mutant mice show no deficiencies on the rotarod assay, but show difficulty in an inverted screen test, indicating that some sensorimotor impairment is present [10, 11]. Prior to performing memory assays, we first evaluated mobility of mice in an open field, using the same arena we planned to use for spatial memory testing. We did not observe any significant differences in mobility (total distance traveled) (Fig 6A), indicating that we could use this paradigm and arena for novel object recognition, a classical object-oriented memory paradigm. However, we did not see any difference between wild type and *Tmem184b*-mutant mice in their ability to recognize novel objects (both showed a positive discrimination index, indicating more time at the novel object) (Fig 6B). This indicates that at six months of age, TMEM184B loss does not alter memory.

Next, we evaluated anxiety using the elevated plus maze. Anxious mice spend more time in closed arms (with walls) than in open arms (without walls). Surprisingly, we identified a decreased anxiety overall in *Tmem184b*-mutant mice when compared to wild types. *Tmem184b*-mutant mice spent considerably more time in the open arms of the maze (Fig 6C). This indicates that TMEM184B promotes appropriate anxiety levels in the elevated plus maze assay.

Discussion

TMEM184B was predicted in human transcriptome analyses to be associated with deleterious gene expression that could drive the progression of Alzheimer's dementia [8]. Our experiments sought to evaluate this hypothesis using RNAseq analysis across aging in young

and aged *Tmem184b*-mutant and wild type mice. Our results show that TMEM184B has significant effects on synaptic gene expression in the hippocampus in both young and old mice.

Alteration of post-synaptic density gene transcripts occurred in all of our pathway analyses. Proteins in this group, such as SHANK1, SHANK3, and PSD95/DLG4 contribute to the scaffolding of neurotransmitter receptors. Interestingly, transcripts of all three of these genes are over-expressed in *Tmem184b*-mutant mice. This upregulation of mRNA could reflect a compensation for presynaptic dysfunction, an inappropriate dendritic overgrowth, or a failure to prune exuberant synapses. In fly models of mutation of the TMEM184B ortholog, *Tmep*, extra active zones and synaptic release sites (boutons) are observed at neuromuscular junctions [9], suggesting that additional synapses may be a common effect of TMEM184B disruption. Further detailed analysis of hippocampal synaptic morphology and physiology will be necessary to parse apart these possibilities.

Somatostatin (*Sst*), a common downregulated gene across both central and peripheral TMEM184B-deficient data sets, plays many distinct roles in the nervous system. In dorsal root ganglia, it is a neurotransmitter for a subset of itch-activated sensory neurons [27]. TMEM184B loss in DRG neurons causes disruption of sensation of interleukin-31, a key cytokine involved in atopic dermatitis (eczema) that is detected exclusively by SST+ neurons [27, 28]. In contrast, in the dentate gyrus, somatostatin reduction occurs in genetic models of temporal lobe epilepsy [29]. Reintroduction of SST in this model reverses the epileptic phenotypes, arguing for a causal role in suppression of seizures. *Drosophila* models of TMEM184B deficiencies show ectopic firing and elevated synaptic calcium, indicating a role in restraining excitability [9]. Thus, phenotypes of SST and TMEM184B overlap substantially; our determination that TMEM184B regulates *Sst* expression in multiple cell types could explain this concordance. It would be of future interest to determine whether restoration of SST in TMEM184B mutant mice could restore normal gene expression and/or behaviors.

322 Another common theme among our data is the connection between TMEM184B and
323 Wnt pathway regulation. In our prior work, we showed that TMEM184B expression positively
324 influences the expression of many Wnt signaling components, as well as their downstream
325 targets, in developing somatosensory neurons [10]. In this work, we also find dysregulation of
326 Wnt components in the hippocampus, including the loss of Wnt pathway targets as well as
327 upregulation of Wnt pathway inhibitors such as Tsukushi. Wnt signaling deficiency contributes to
328 AD phenotypes in mouse models [30], and it has also been identified as a key pathway altered
329 in human AD samples [31]. While our data does not directly link TMEM184B to the development
330 of AD pathology, it suggests that TMEM184B dysfunction disrupts synaptic gene regulatory
331 networks and also influences expression of genes with more direct links to AD.
332 The mechanism of action of the TMEM184B protein is yet unknown. Clues to its role
333 include localization to endosomes and synaptic vesicles, accumulation of multilamellar
334 structures in mutant presynaptic terminals, and additional autophagosomes and lysosomes in
335 skeletal muscle in its absence. Because Wnt signaling occurs within endosomes and
336 contributes to dendritic growth, we hypothesize that a failure of Wnt signaling (perhaps due to a
337 blockade in the endolysosomal pathway) may account for some of the phenotypes we observe.
338 While we did not complete an exhaustive behavioral analysis, we found that TMEM184B
339 disruption causes reduced anxiety in the elevated plus maze paradigm. Reduced anxiety is
340 seen in some models of neuroatypical development including autism spectrum disorders and in
341 mice predisposed to depression [32, 33]. We speculate that TMEM184B loss may disrupt
342 anxiety circuits to cause this phenotype.
343 We did not observe memory impairment when TMEM184B was disrupted in 6-month-old
344 mice. It is still possible that older mice may show impairments. However, perhaps more likely,
345 we suspect that TMEM184B disruption itself is not causative for memory loss. Instead, we
346 hypothesize that compromising TMEM184B function, in the presence of other susceptibility loci,
347 may increase the likelihood of developing dementia. Given its strong effects on synaptic gene

348 expression networks as well as the disruptions to synaptic morphology and function seen in
349 other studies, it will be of future interest to directly evaluate synaptic function via electrical
350 recording in the hippocampus and to evaluate its role in the context of mouse models of AD.
351

352 **Data Availability**

353 The data sets generated and analyzed in the current study have been deposited to NCBI
354 GEO at the following accession: GSE204831. All custom-written code in R for RNAseq analysis
355 is available at GitHub (https://github.com/eriklarsen4/ggplot-scripts.git and
356 https://github.com/martharcb/). Dorsal root ganglion data to which we compared our
357 hippocampal RNAseq data can be found on NCBI GEO (GSE154316).
358

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370 **Conflict of Interest**

371 The authors do not report any conflicts of interest pertaining to the content of this manuscript.
372

373 **References:**

374 1. (2022) 2022 Alzheimer's disease facts and figures. Alzheimer's Dement 18:700–789.
375 https://doi.org/10.1002/ALZ.12638
376 2. Hebert LE, Beckett LA, Scherr PA, Evans DA (2001) Annual incidence of Alzheimer
377 disease in the United States projected to the years 2000 through 2050. Alzheimer Dis
378 Assoc Disord 15:169–173. https://doi.org/10.1097/00002093-200110000-00002
379 3. Price JL, Davis PB, Morris JC, White DL (1991) The distribution of tangles, plaques and
380 related immunohistochemical markers in healthy aging and Alzheimer's disease.
381 Neurobiol Aging 12:295–312. https://doi.org/10.1016/0197-4580(91)90006-6
382 4. Knopman DS, Jones DT, Greicius MD (2021) Failure to demonstrate efficacy of
383 aducanumab: An analysis of the EMERGE and ENGAGE trials as reported by Biogen,
384 December 2019. Alzheimers Dement 17:696–701. https://doi.org/10.1002/ALZ.12213
385 5. Mostafavi S, Gaiteri C, Sullivan SE, et al (2018) A molecular network of the aging human
386 brain provides insights into the pathology and cognitive decline of Alzheimer's disease.
387 Nat Neurosci 21:811–819. https://doi.org/10.1038/S41593-018-0154-9
388 6. Wang M, Li A, Sekiya M, et al (2021) Transformative Network Modeling of Multi-omics
389 Data Reveals Detailed Circuits, Key Regulators, and Potential Therapeutics for
390 Alzheimer's Disease. Neuron 109:257-272.e14.
391 https://doi.org/10.1016/J.NEURON.2020.11.002
392 7. Harold D, Abraham R, Hollingworth P, et al (2009) Genome-wide association study
393 identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet
394 41:1088–1093. https://doi.org/10.1038/NG.440
395 8. AMP-AD AMP in AD Agora AD Knowledge Portal. https://agora.adknowledgeportal.org/.
396 Accessed 17 Apr 2022
397 9. Cho TS, Beigaitė E, Klein NE, et al (2022) The Putative Drosophila TMEM184B Ortholog
398 Tmep Ensures Proper Locomotion by Restraining Ectopic Firing at the Neuromuscular
399 Junction. Mol Neurobiol. https://doi.org/10.1007/S12035-022-02760-3

400 10. Larsen EG, Cho TS, McBride ML, et al TMEM184B is necessary for IL-31-induced itch.
401 PAIN (in Press. https://doi.org/10.1101/2020.01.25.919902
402 11. Bhattacharya MRC, Geisler S, Pittman SK, et al (2016) TMEM184b Promotes Axon
403 Degeneration and Neuromuscular Junction Maintenance. J Neurosci 36:4681–9.
404 https://doi.org/10.1523/JNEUROSCI.2893-15.2016
405 12. Leger M, Quiedeville A, Bouet V, et al (2013) Object recognition test in mice. Nat Protoc
406 8:2531–2537. https://doi.org/10.1038/NPROT.2013.155
407 13. Salama-Cohen P, Arévalo MÁ, Grantyn R, Rodríguez-Tébar A (2006) Notch and
408 NGF/p75NTR control dendrite morphology and the balance of excitatory/inhibitory
409 synaptic input to hippocampal neurones through Neurogenin 3. J Neurochem 97:1269–
410 1278. https://doi.org/10.1111/J.1471-4159.2006.03783.X
411 14. Sheng M, Kim E (2000) The Shank family of scaffold proteins. J Cell Sci 113 (Pt
412 11):1851–1856. https://doi.org/10.1242/JCS.113.11.1851
413 15. Vyas Y, Cheyne JE, Lee K, et al (2021) Shankopathies in the Developing Brain in Autism
414 Spectrum Disorders. Front Neurosci 15:775431.
415 https://doi.org/10.3389/fnins.2021.775431
416 16. Harris KP, Akbergenova Y, Cho RW, et al (2016) Shank Modulates Postsynaptic Wnt
417 Signaling to Regulate Synaptic Development. J Neurosci 36:5820–5832.
418 https://doi.org/10.1523/JNEUROSCI.4279-15.2016
419 17. Doan RN, Lim ET, De Rubeis S, et al (2019) Recessive gene disruptions in autism
420 spectrum disorder. Nat Genet 2019 517 51:1092–1098. https://doi.org/10.1038/s41588-
421 019-0433-8
422 18. Hoppler SP, Kavanagh CL (2007) Wnt signalling: variety at the core. J Cell Sci 120:385–
423 393. https://doi.org/10.1242/JCS.03363
424 19. Arredondo SB, Valenzuela-Bezanilla D, Mardones MD, Varela-Nallar L (2020) Role of
425 Wnt Signaling in Adult Hippocampal Neurogenesis in Health and Disease. Front Cell Dev

426 Biol 8:860. https://doi.org/10.3389/FCELL.2020.00860/BIBTEX
427 20. Wang YP, Guo PT, Zhu Z, et al (2017) Pleomorphic adenoma gene like-2 induces
428 epithelial-mesenchymal transition via Wnt/β-catenin signaling pathway in human
429 colorectal adenocarcinoma. Oncol Rep 37:1961–1970.
430 https://doi.org/10.3892/OR.2017.5485
431 21. Li N, Li D, Du Y, et al (2019) Overexpressed PLAGL2 transcriptionally activates Wnt6 and
432 promotes cancer development in colorectal cancer. Oncol Rep 41:875–884.
433 https://doi.org/10.3892/OR.2018.6914
434 22. Lie DC, Colamarino SA, Song HJ, et al (2005) Wnt signalling regulates adult hippocampal
435 neurogenesis. Nature 437:1370–1375. https://doi.org/10.1038/NATURE04108
436 23. Kalani MYS, Cheshier SH, Cord BJ, et al (2008) Wnt-mediated self-renewal of neural
437 stem/progenitor cells. Proc Natl Acad Sci U S A 105:16970–16975.
438 https://doi.org/10.1073/PNAS.0808616105
439 24. Deng W, Saxe MD, Gallina IS, Gage FH (2009) Adult-Born Hippocampal Dentate
440 Granule Cells Undergoing Maturation Modulate Learning and Memory in the Brain. J
441 Neurosci 29:13532–13542. https://doi.org/10.1523/JNEUROSCI.3362-09.2009
442 25. Ohta K, Ito A, Kuriyama S, et al (2011) Tsukushi functions as a Wnt signaling inhibitor by
443 competing with Wnt2b for binding to transmembrane protein Frizzled4. Proc Natl Acad
444 Sci U S A 108:14962–14967. https://doi.org/10.1073/PNAS.1100513108
445 26. Zhang T, Pang P, Fang Z, et al (2018) Expression of BC1 Impairs Spatial Learning and
446 Memory in Alzheimer's Disease Via APP Translation. Mol Neurobiol 55:6007–6020.
447 https://doi.org/10.1007/S12035-017-0820-Z
448 27. Huang J, Polgár E, Solinski HJ, et al (2018) Circuit dissection of the role of somatostatin
449 in itch and pain. Nat Neurosci 21:707–716. https://doi.org/10.1038/s41593-018-0119-z
450 28. Grimstad Ø, Sawanobori Y, Vestergaard C, et al (2009) Anti-interleukin-31-antibodies
451 ameliorate scratching behaviour in NC/Nga mice: a model of atopic dermatitis. Exp

452 Dermatol 18:35–43. https://doi.org/10.1111/j.1600-0625.2008.00766.x
453 29. Kim S, Kim H, Park D, et al (2020) Loss of IQSEC3 Disrupts GABAergic Synapse
454 Maintenance and Decreases Somatostatin Expression in the Hippocampus. Cell Rep
455 30:1995-2005.e5.
456 https://doi.org/10.1016/J.CELREP.2020.01.053/ATTACHMENT/D265A14F-832D-4B2B-
457 AD52-23B0CEC64DD4/MMC1.PDF
458 30. Marzo A, Galli S, Lopes D, et al (2016) Reversal of Synapse Degeneration by Restoring
459 Wnt Signaling in the Adult Hippocampus. Curr Biol 26:2551–2561.
460 https://doi.org/10.1016/J.CUB.2016.07.024
461 31. Bai B, Wang X, Li Y, et al (2020) Deep Multilayer Brain Proteomics Identifies Molecular
462 Networks in Alzheimer's Disease Progression. Neuron 105:975-991.e7.
463 https://doi.org/10.1016/J.NEURON.2019.12.015
464 32. McFarlane HG, Kusek GK, Yang M, et al (2008) Autism-like behavioral phenotypes in
465 BTBR T+tf/J mice. Genes Brain Behav 7:152–163. https://doi.org/10.1111/J.1601-
466 183X.2007.00330.X
467 33. Fernandez SP, Gaspar P (2012) Investigating anxiety and depressive-like phenotypes in
468 genetic mouse models of serotonin depletion. Neuropharmacology 62:144–154.
469 https://doi.org/10.1016/J.NEUROPHARM.2011.08.049
470
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Figure Legends:

Figure 1. TMEM184B-dependent gene expression alterations in the mouse hippocampus. **A**, Principal component analysis (PCA) of wild type and mutant samples taken at 5 months of age shows strong clustering of wild types (black circles), and loose clustering of mutant samples (green circles). **B**, volcano plot showing both up- and down-regulation of genes based on differential expression analysis. Purple indicates adjusted P-values of $p < 0.05$. **C**, MA-plot of base mean expression value versus Log_2(Fold Change) from DeSeq2. Red, Adjusted $p < 0.05$; blue, adujsted $p < 0.01$. Selected genes of interest are labeled. **D**, GO Biological Process Fold Enrichment (FE) scores of categories above 2.0 FE. Numbers indicate average FE; parentheses denote number of processes in that category. **E**, KEGG Pathways analysis (done using Enrichr). Top 10 pathways are shown, ranked by p-value.

Figure 2. TMEM184B loss disrupts key synaptic gene networks. A-B, mouse protein interaction networks as defined by BioGRID. Node color represents log_2 of fold change (darker red indicates higher over-expression in *Tmem184b*-mutant; blue shades indicate downregulation in *Tmem184b*-mutant). Circles indicate adjusted P-values where $p < 0.05$; rectangles indicate $p > 0.05$. Edge lengths have no significance. **C-D**, GO Biological Process network maps (from GoCAM) showing terms (gene sets) having significant alteration in *Tmem184b*-mutant mice versus controls. Darker blue indicates stronger fold change, while larger nodes indicate stronger significance (all nodes shown have Adjusted $p < 0.05$). Edge lengths have no significance. GO Biological Process terms disrupted in *Tmem184b*-mutant hippocampi identified with the search term “synapse” (in C) or “neuron” (in D).

Figure 3. Analysis of Transcription Factors Upstream of Hippocampal DEGs identifies Wnt pathway alterations. A, Chromatin Enrichment Analysis (ChEA) for mouse transcription

factors (TFs) with binding in promoters of differentially expressed genes from *Tmem184b*-mutant hippocampi at 5 months of age. Shown are TFs for which enrichment scores were statistically significant (Adjusted P < 0.05). **B**, Base mean expression in 5-month-old hippocampus of each transcription factor in A, graphed against its adjusted P-value. **C**, top 10 mouse data sets from transcription-factor-manipulated genetic backgrounds that show significant overlap with TMEM184B differentially expressed genes. Adjusted P values calculated by Enrichr.

Figure 4. Gene Expression Changes in 15-month *Tmem184b*-mutant vs wild type mice. A, MA-plot of gene expression changes between 15-month-old *Tmem184b*-mutant and wild type hippocampus. Red dots show genes with adjusted p < 0.05, while blue dots show adjusted p < 0.01. **B**, Venn diagram showing overlap between 5-month and 15-month genotype-induced changes. **C-D**, evaluation of enrichment among the 222 overlapping genes using gene ontology for Biological Processes (C) and Cellular Components (D). Shown is Combined Score reported by Enrichr. This score takes into account both P-value and Odds Ratio. Asterisks indicate level of statistical significance (Adjusted P-value, **** <0.0001, *** < 0.001, ** < 0.01). **E**, Four-way comparison of differentially expressed genes (no ribosomal gene filtering). **F**, gene set categories identified by Molecular Signatures Database (MSigDB) among genes different between 5- and 15-month *Tmem184b*-mutant mice. Abbreviations: HD, Huntingon's Disease model mice; Oligo, oligodendrocytes; Ataxin, Ataxin-1 mutant mice. The full list of molecular signatures is shown in Supplementary Table 5.

Figure 5. TMEM184B similarly regulates somatostatin and other transcripts in both central and peripheral neurons. Data for DRG is available at NCBI GEO (NCBI GEO GSM4668859) Log_2FC of genes identified in each data set (hippocampus, black bars; adult

DRG, grey bars). A full list of genes differentially regulated in both data sets can be found in Supplementary Table 6.

Figure 6. Memory and Anxiety Behaviors in *Tmem184b*-mutant mice. All graphs show mice of both sexes and are approximately sex balanced. **A**, Open field habituation over five minutes for mice 5-7 months of age. Total distance traveled in centimeters. WT, wild type, Mut, *Tmem184b*-gene trap mutant mice. **B**, Memory test at 24 hours using the novel object recognition task. Discrimination index is calculated as described in materials and methods. Each dot shows an individual mouse. N = 10 wild type and 8 mutant mice. **C**, measurement of anxiety using the elevated plus maze. For all graphs, statistical evaluation used unpaired t-test with Welch's correction. Numbers in bars (in A and C) indicate sample size. Error bars show standard error of the mean (SEM) for all panels.

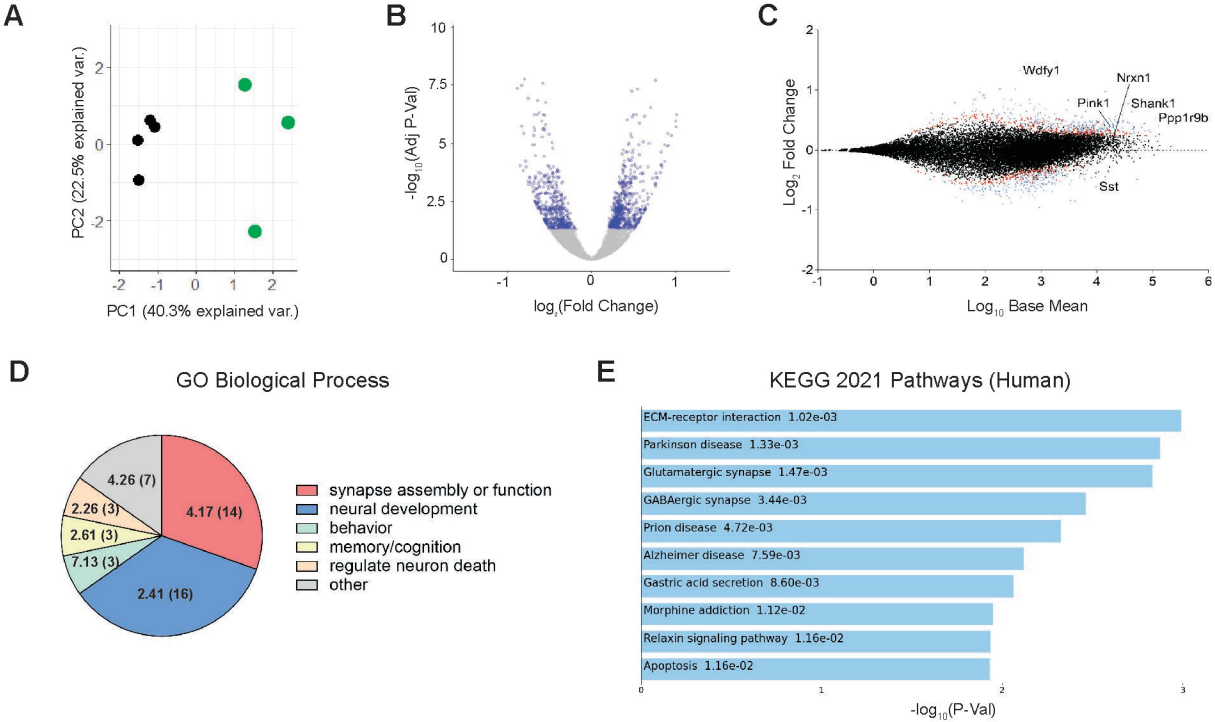
Supplementary Material

Supplementary File F1. DESeq2 output of differential gene expression analysis between the hippocampi of 5-month-old *Tmem184b*-mutant mice and age-matched wild types.

Supplementary File F2. DESeq2 output of differential gene expression analysis between the hippocampi of 15-month-old *Tmem184b*-mutant mice and age-matched wild types.

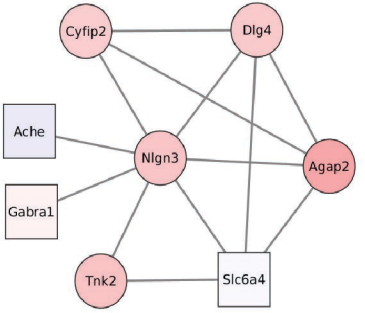
Supplementary File F3. Differentially expressed genes in *Tmem184b*-mutant mice common to both the 5-month-old and 15-month-old comparison groups. Ribosomal transcripts have been omitted.

549 **Supplementary File F4.** DESeq2 output of differential gene expression analysis between the
550 hippocampi of 15-month-old *Tmem184b*-mutant mice and 5-month-old *Tmem184b*-mutant mice.
551
552 **Supplementary File F5.** Analysis of 104 differentially expressed genes across aging in
553 *Tmem184b*-mutant mice using the Molecular Signatures Database (MsigDB).
554
555 **Supplementary File F6.** All genes identified as differentially expressed in both adult dorsal root
556 ganglia and hippocampus of *Tmem184b*-mutant mice compared to wild type mice.
557
558


A
PC2 (22.5% explained var.)
PC1 (40.3% explained var.)
B
-log10(Adj P-Val)
log2(Fold Change)
C
Wdfy1
Nrxn1
Pink1
Shank1
Ppp1r9b
Sst
Log2 Fold Change
Log10 Base Mean
D
GO Biological Process
4.26 (7)
4.17 (14)
2.26 (3)
2.61 (3)
7.13 (3)
2.41 (16)
synapse assembly or function
neural development
behavior
memory/cognition
regulate neuron death
other
E
KEGG 2021 Pathways (Human)
ECM-receptor interaction 1.02e-03
Parkinson disease 1.33e-03
Glutamatergic synapse 1.47e-03
GABAergic synapse 3.44e-03
Prion disease 4.72e-03
Alzheimer disease 7.59e-03
Gastric acid secretion 8.60e-03
Morphine addiction 1.12e-02
Relaxin signaling pathway 1.16e-02
Apoptosis 1.16e-02
-log10(P-Val)

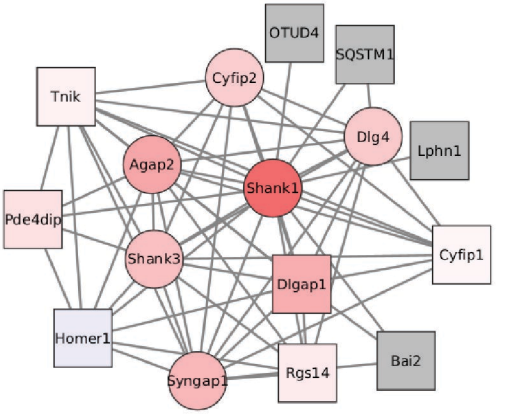
A

Neurogenin 3 Subnetwork



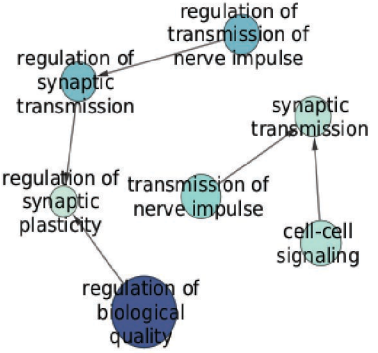
B

Shank1 Subnetwork



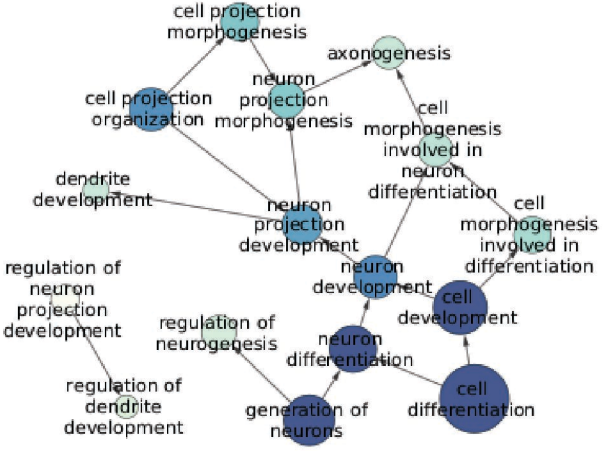
C

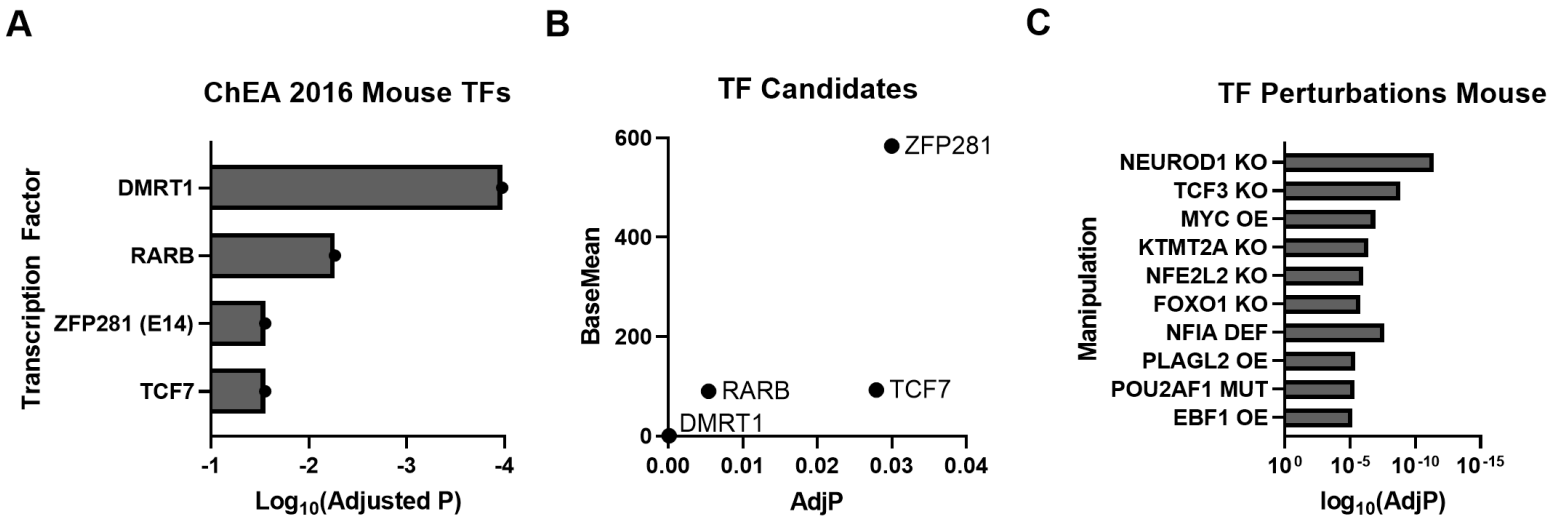
GO Biological Process "Synapse" Subnetwork

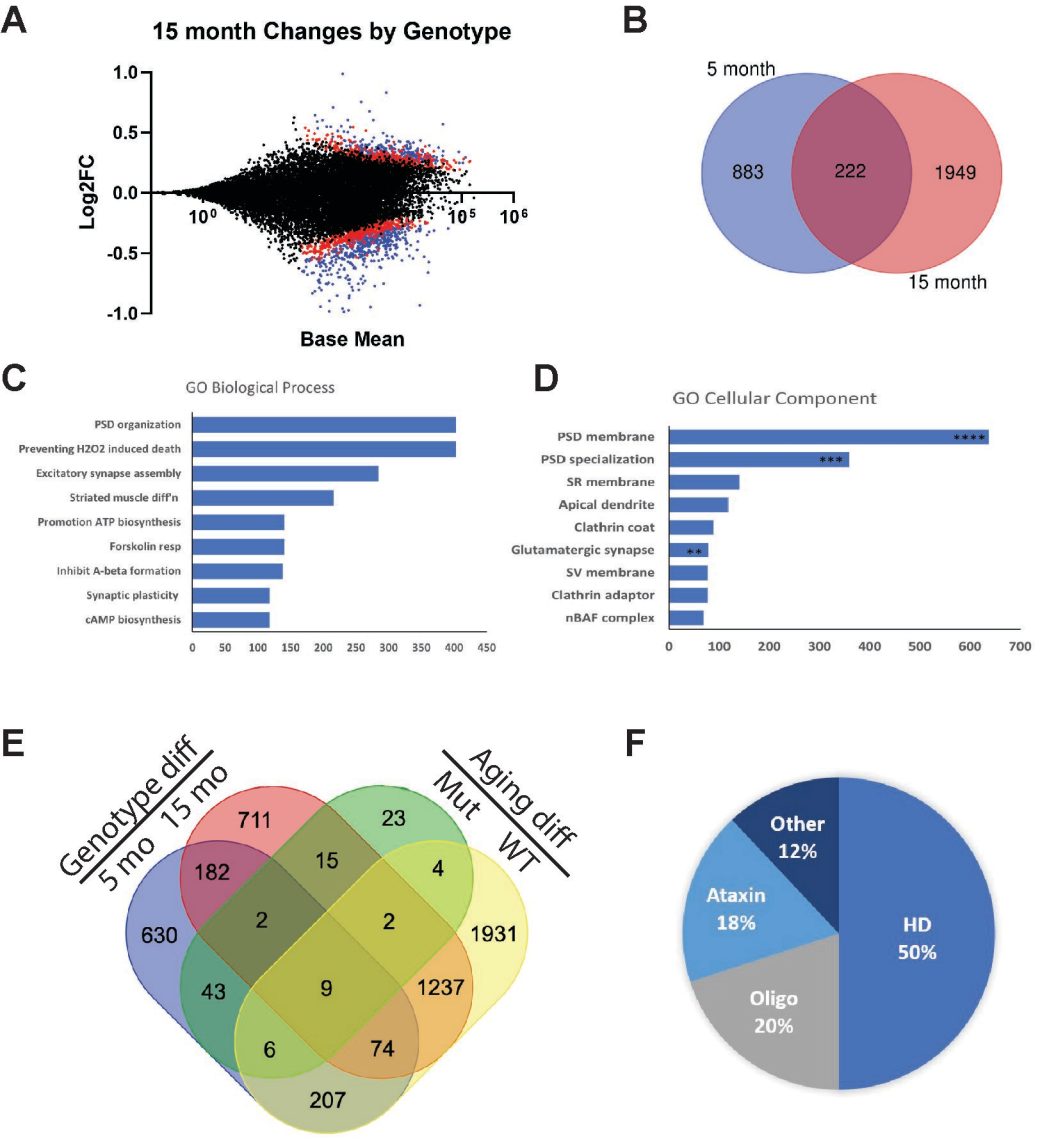


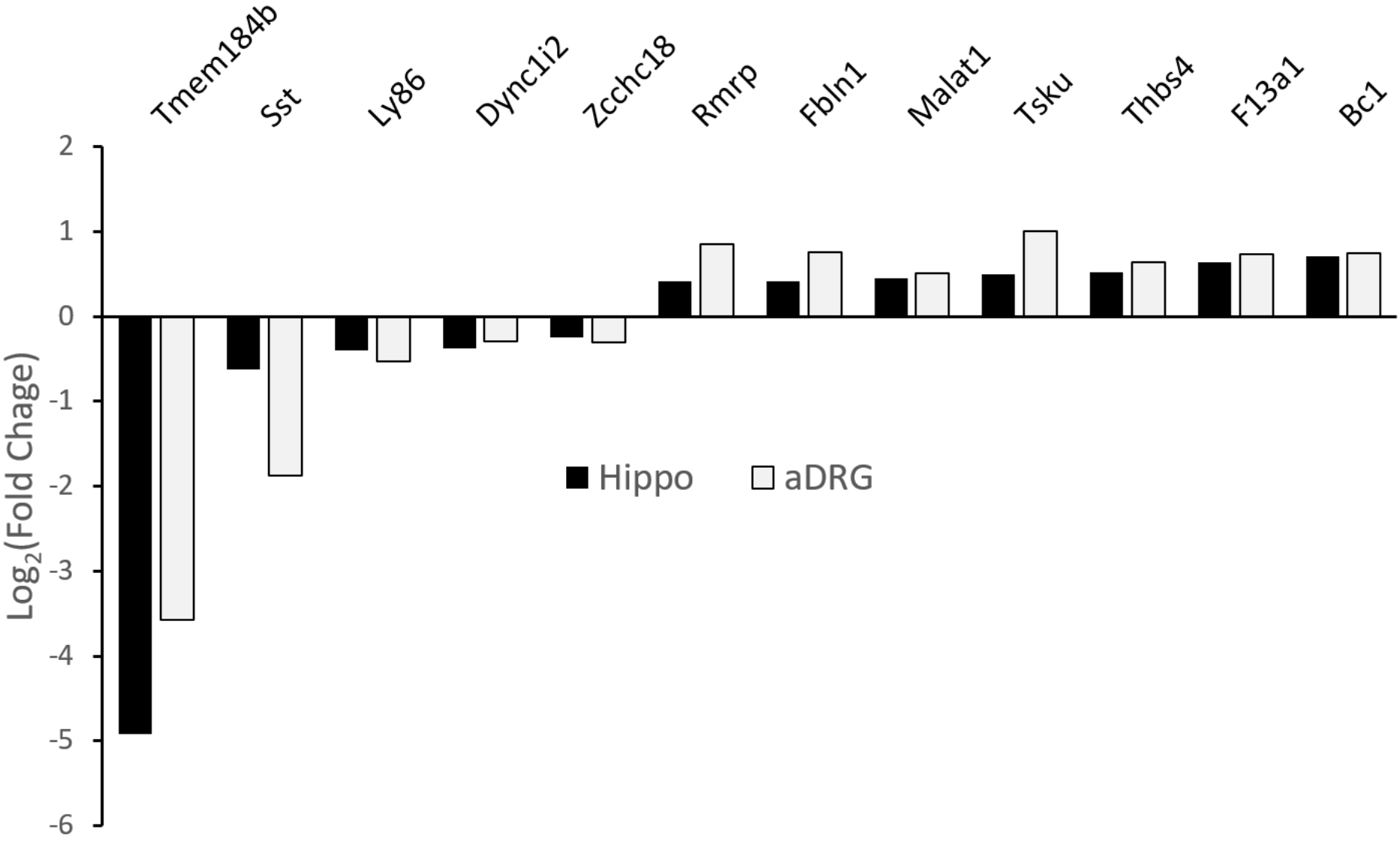
D

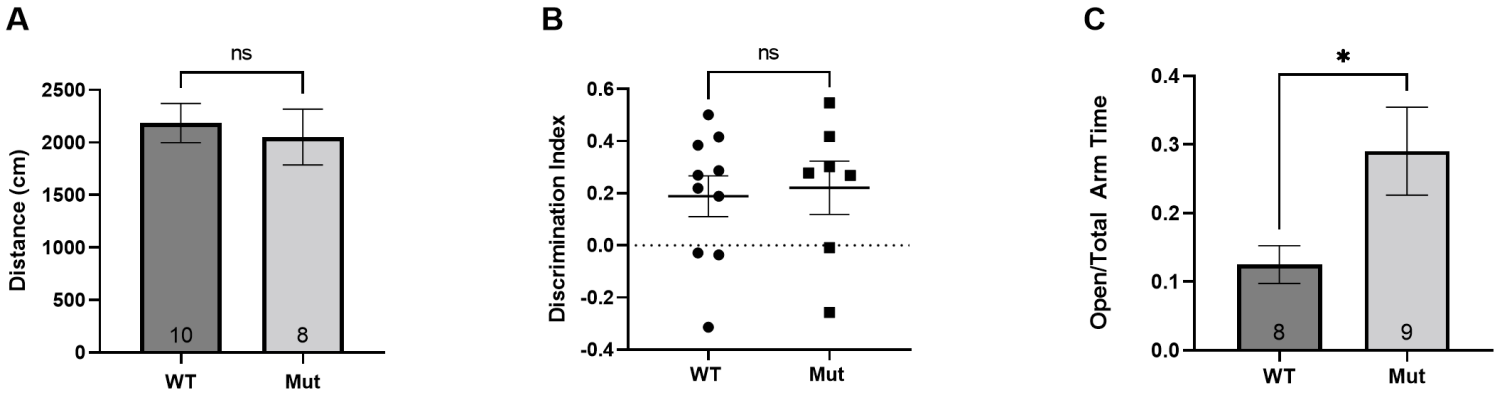
GO Biological Process "Neuron" Subnetwork




A
ChEA 2016 Mouse TFs
Transcription Factor
DMRT1
RARB
ZFP281 (E14)
TCF7
-1
-2
-3
-4
Log10(Adjusted P)
B
TF Candidates
BaseMean
600
400
200
0
ZFP281
RARB
TCF7
DMRT1
0.00
0.01
0.02
0.03
0.04
AdjP
C
TF Perturbations Mouse
Manipulation
NEUROD1 KO
TCF3 KO
MYC OE
KTMT2A KO
NFE2L2 KO
FOXO1 KO
NFIA DEF
PLAGL2 OE
POU2AF1 MUT
EBF1 OE
10^0
10^-5
10^-10
10^-15
log10(AdjP)


A
15 month Changes by Genotype
Log2FC
1.0
0.5
0.0
-0.5
-1.0
10^0
10^5
10^6
Base Mean
B
5 month
883
222
1949
15 month
C
GO Biological Process
PSD organization
Preventing H2O2 induced death
Excitatory synapse assembly
Striated muscle diff'n
Promotion ATP biosynthesis
Forskolin resp
Inhibit A-beta formation
Synaptic plasticity
cAMP biosynthesis
0 50 100 150 200 250 300 350 400 450
D
GO Cellular Component
PSD membrane ****
PSD specialization ***
SR membrane
Apical dendrite
Clathrin coat
Glutamatergic synapse **
SV membrane
Clathrin adaptor
nBAF complex
0 100 200 300 400 500 600 700
E
Genotype diff
5 mo 15 mo
Aging diff
Mut WT
711
23
182
15
4
630
2
2
1931
43
9
1237
6
74
207
F
Other 12%
Ataxin 18%
HD 50%
Oligo 20%


Tmem184b
Sst
Ly86
Dync1i2
Zcchc18
Rmrp
Fbln1
Malat1
Tsku
Thbs4
F13a1
Bc1
2
1
0
-1
-2
-3
-4
-5
-6
Log2(Fold Chage)
Hippo
aDRG


A
ns
Distance (cm)
2500
2000
1500
1000
500
0
10
8
WT
Mut
B
ns
Discrimination Index
0.6
0.4
0.2
0.0
-0.2
-0.4
WT
Mut
C
*
Open/Total Arm Time
0.4
0.3
0.2
0.1
0.0
8
9
WT
Mut