

1 Modulating glial genes involved in synaptic function mitigates pathogenesis and behavioral deficits in a
2 *Drosophila* model of Huntington's Disease

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23 **Abstract**

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25 Most research on neurodegenerative diseases has focused on neurons, yet glia help form and maintain the

26 synapses whose loss is so prominent in these conditions. To investigate the contributions of glia to

27 Huntington's disease (HD), we studied transcriptomic changes in HD human, HD mice, and

28 *Drosophila* expressing human mutant *Huntingtin* (*mHTT*) in either glia, neurons or both. A large portion

29 of conserved genes are concordantly dysregulated across the three species; we tested these genes in a

30 high-throughput behavioral assay and found that downregulation of genes involved in synapse assembly

31 mitigated pathogenesis and behavioral deficits. To our surprise, mitigating glial pathogenesis by *dNRXN3*

32 knockdown was sufficient to improve the phenotype of flies expressing *mHTT* in neurons, suggesting that

33 *mHTT*'s toxic effects in glia ramify throughout the brain. This supports a model in which dampening

34 synaptic function is protective because it attenuates the excitotoxicity that characterizes HD.

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41 **Introduction**

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43 Neurodegenerative conditions involve a complex cascade of events that takes many years to unfold.
44 Even in the case of inherited disorders due to mutation in a single gene, such as Huntington's Disease
45 (HD), the downstream ramifications at the molecular level are astonishingly broad. Caused by a CAG
46 repeat expansion in *Huntingtin* (*HTT*) (The Huntington's Disease Collaborative Research Group, 1993),
47 HD pathology is prominent in the striatum and cortex, yet transcriptomic studies consistently reveal
48 thousands of changes in gene expression across the brain and different neuronal cell types, involving
49 pathways ranging from autophagy to vesicular trafficking (Saudou and Humbert, 2016). To disentangle
50 changes that are pathogenic from those that represent the brain's effort to compensate for the disease, we
51 recently integrated transcriptomics with *in silico* analysis and high-throughput *in vivo* screening using a
52 *Drosophila* model of HD (Al-Ramahi et al., 2018). This study demonstrated that HD pathogenesis is
53 driven by upregulation of genes involved in the actin cytoskeleton and inflammation, but that neurons
54 compensate by downregulating the expression of genes involved in synaptic biology and calcium
55 signaling.

56 The finding that synaptic changes were protective caught our attention, because HTT itself is
57 necessary for normal synaptogenesis and maintenance within the cortico-striatal circuit (McKinstry et al.,
58 2014), largely through its role in retrograde axonal trafficking of neurotrophic factors (Saudou and
59 Humbert, 2016). But synapses involve more than just neurons: glial cells also contribute to synapse
60 formation, function, and elimination (Filipello et al., 2018; McKinstry et al., 2014; Oceau et al., 2018;
61 Stogsdill et al., 2017). There is, in fact, emerging evidence that various glial subtypes affect outcomes in
62 HD. The accumulation of mHTT in astrocytes and oligodendrocytes hinders their development and
63 function and contributes to disease pathophysiology (Benraiss et al., 2016; Ferrari Bardile et al., 2019;
64 Osipovitch et al., 2019; Wood et al., 2018). Conversely, healthy glia can improve the disease phenotype
65 in HD mice (Benraiss et al., 2016). Recent studies using single-cell sequencing in astrocytes isolated from
66 post-mortem tissue from HD patients and mouse models of HD (Al-Dalahmah et al., 2020; Diaz-Castro et
67 al., 2019) developed molecular profiles that distinguish HD-affected astrocytes from astrocytes found in
68 healthy brain tissue, but the physiological consequences of the gene expression changes were unclear.
69 Whether mHTT affects glial participation in synapse formation or maintenance remains unknown, but
70 then, we are only just now beginning to understand the range of glial types and their functions (Bayraktar
71 et al., 2020; Darmanis et al., 2015).

72 The combination of synaptic degeneration in HD and the fact that both HTT and glia contribute to
73 synaptic formation and maintenance led us to further investigate the influence of *mHTT* in glia. Because
74 *Drosophila* have been used to elucidate glial biology (Freeman and Doherty, 2006; Olsen and Feany,
75 2019; Pearce et al., 2015; Ziegenfuss et al., 2012) and are a tractable model system for studying HD and

76 other neurodegenerative diseases (Al-Ramahi et al., 2018; Bondar et al., 2018; Donnelly et al., 2020;
77 Fernandez-Funez et al., 2000; Filimonenko et al., 2010; Goodman et al., 2019; Ochaba et al., 2014; Olsen
78 and Feany, 2019; O'Rourke et al., 2013; Rousseaux et al., 2018; Yuva-Aydemir et al., 2018), we decided
79 to generate flies that express *mHTT* solely in glia so that we could compare their transcriptomic signature
80 with that of flies expressing *mHTT* in neurons. We took an unbiased approach, first establishing the
81 repertoire of evolutionarily conserved genes that show concordant expression changes across HD human
82 and mouse striata and HD fly brains. We then integrated this comparative transcriptomic data with high-
83 throughput *in vivo* behavioral screening to acquire insight into glial contributions to HD pathogenesis and
84 identify disease-modifying targets that mitigate the HD phenotype.

85

86 **Results**

87 *The HD transcriptome is conserved among evolutionarily distant model systems*

88 To study the contributions of neurons and glia to HD pathogenesis, we first needed to define a
89 transcriptomic signature that would enable us to move across species (human, mouse, and fly) (Figure
90 1A). We began with human tissue. Since the striatum is the brain region most prominently affected in
91 HD, we compared the gene expression profiles of human post-mortem striatal samples from healthy
92 individuals and patients with HD, from different stages of the disease (i.e., Vonsattel Grade 0-4) (Hodges
93 et al., 2006; Vonsattel et al., 1985). We identified 1,852 downregulated and 1,941 upregulated
94 differentially expressed genes (DEGs) in patients with HD compared to healthy individuals (Figure 1B).

95 We then reanalyzed published RNA-seq data from mouse striata, using an allelic series of knock-in
96 mouse models with varying CAG-repeat lengths at six months of age (Langfelder et al., 2016). Because it
97 is unclear which CAG tract length in mice most faithfully recapitulates HD pathogenesis, the triplet repeat
98 length was treated as a continuous trait, and we narrowed our analysis to DEGs that correlate with
99 increasing CAG repeat length. Comparing the striata of wild-type mice to the knock-in HD mouse
100 models, there were 3,575 downregulated and 3,634 upregulated DEGs (Figure 1B). (The greater genome
101 coverage provided by RNA-seq (Miller et al., 2014) yielded larger datasets for mouse and, below, for
102 *Drosophila* than for humans.)

103 We performed RNA-seq leveraging *Drosophila* HD models (Kaltenbach et al., 2007; Romero et al.,
104 2008) (see Methods) to compare the effect of expressing *mHTT* in either neurons or glia. The binary
105 GAL4-*UAS* system was used to drive the expression of human *mHTT* either in neurons (*elav*>*GAL4*) or
106 glia (*repo*>*GAL4*). Both full-length (*HTT*^{FLQ200}) and N-terminal (*HTT*^{NT231Q128}) models were used in this
107 set of experiments, since both the full protein and N-terminal HD fragments accumulate in the human
108 brain as a result of proteolysis and mis-splicing (Kim et al., 2001; Neueder et al., 2017; Sathasivam et al.,
109 2013; Wellington et al., 2002). Principle component analysis (PCA) showed that the greatest differences

110 between samples are attributable to the cell-specific drivers, and not to the use of N-terminal vs. full-
111 length protein (Figure S1). Expressing *mHTT* in neurons resulted in 3,058 downregulated and 2,979
112 upregulated DEGs, while expressing *mHTT* in glia resulted in 3,127 downregulated and 3,159
113 upregulated DEGs. There were also DEGs common to both neurons and glia expressing *mHTT*: 1,293
114 downregulated and 1,181 upregulated (Figure 1B).

115 With these transcriptomic signatures in hand, we were able to compare gene expression profiles
116 across the three species. We focused on genes with significantly altered expression (using a FDR<0.05;
117 see Methods) in the same direction (i.e., upregulated or downregulated) in response to *mHTT* expression
118 across these three species, including both *Drosophila* HD models. We call genes that meet this criterion
119 concordantly altered DEGs (Table S1).

120 We compared DEGs using a graph-based approach (see Methods) that allows for evolutionary
121 divergence and convergence, instead of imposing one-to-one relationships. 815 upregulated DEGs
122 observed in HD patient-derived striatal tissue had an orthologous gene in the HD mouse model and at
123 least one *Drosophila* model of HD that was concordantly upregulated. Similarly, 791 DEGs identified in
124 HD patients had an orthologous gene in mouse and *Drosophila* models that was concordantly
125 downregulated (Figure 1C). About 40% of the alterations in gene expression in patient striatal samples are
126 concordant with orthologous genes in both *Drosophila* and mice models of HD. To determine whether
127 this result could be an artifact of overlapping a large number DEGs in each model, we randomly selected
128 and overlapped 815 and 791 orthologous genes across the three species 20,000 times. Based on the
129 resulting distribution, we concluded that the overlap of concordant, orthologous DEGs across the various
130 HD models was not random ($p=6.37 \times 10^{-158}$ and $p=1.66 \times 10^{-165}$, Probability Distribution Test).

131 To compare the consequence of expressing *mHTT* in glia versus neurons, we recalculated the
132 overlaps between the three species, distinguishing DEGs from the neuron-only and glia-only *HTT*-
133 expressing *Drosophila*. There were 425 concordantly upregulated and 545 concordantly downregulated
134 DEGs in glia. We also found 522 upregulated DEGs and 453 downregulated specific to neurons. Out of
135 these groups of DEGs, 310 were upregulated and 320 were downregulated in both neurons and glia. To
136 acknowledge the proportion of transcriptional alterations we excluded by specifying concordant
137 expression with the HD *Drosophila* models, we also calculated the overlap between concordant DEGs
138 observed only in striata from HD patients and mice. We found that 83.7% of upregulated DEGs and
139 77.7% of downregulated DEGs that were altered concordantly in human and mouse HD striata were also
140 concordantly altered in the brains of the neuronal and/or glial HD *Drosophila* models (Figure 1D). Of the
141 genes that showed concordantly altered expression only in human and mouse striata, 64 (40%) of the
142 upregulated and 68 (30%) of the downregulated DEGs did not have an ortholog in *Drosophila*.

143

144 *Network analysis identifies biological processes disrupted by mHTT toxicity in glia*

145 To investigate the cellular pathophysiology represented by DEGs in neurons and glia, we
146 constructed protein-protein interaction (PPI) networks using the STRING-db database (Szklarczyk et al.,
147 2015). The upregulated and downregulated networks of DEGs responding to *mHTT* expression in neurons
148 or glia had a significant PPI enrichment compared to networks constructed from an equivalent number of
149 random genes selected from a whole-proteome background (Table S2A). To control for potential artifacts
150 that could arise from using the whole proteome background, we performed a more stringent analysis
151 using only proteins that are found in the striatum (Al-Ramahi et al., 2018). Using average node degree
152 and betweenness as proxies for connectivity, we found that the glial and neuronal networks show higher
153 network connectivity than expected by random chance among proteins present in the striatum (Table
154 S2B).

155 This high connectivity suggested that the networks are enriched in specific biological processes
156 and/or pathways. We therefore clustered the glial *mHTT* response and neuronal *mHTT* response networks
157 using the InfoMap random walks algorithm (iGraph Package for R and Python) (Rosvall and Bergstrom,
158 2007). Clusters that had fewer than four nodes were filtered out of subsequent analysis. The glial
159 networks formed 23 and 24 clusters for upregulated and downregulated DEGs, respectively. Both the
160 upregulated and downregulated neuronal networks formed 29 clusters. We applied this clustering method
161 to the networks of randomly selected striatal proteins in order to determine the expected number of
162 clusters for networks of a similar size. Both the glial and neuronal networks formed significantly more
163 clusters than would be expected from random selection (Table S2B).

164 To gain insight into biological processes represented by each cluster, we queried the five most
165 significantly enriched terms (FDR<0.05) using the GO Biological Process and Kyoto Encyclopedia of
166 Genes and Genomes (KEGG) terms within each cluster (Table S3). A synthesis of these terms was used
167 to identify clusters in both the glial and neuronal networks (Table S3, Figures S2B and S2C). We
168 compared the membership within clusters across the glial and neuronal networks using a pair-wise
169 hypergeometric test and identified fourteen clusters of upregulated DEGs common to both glial and
170 neuronal networks. Similarly, there were fifteen clusters of downregulated DEGs common to the both
171 networks (Figure S2A).

172 Given the aims of our study, the clusters of DEGs specific to glia (represented by nodes in Figure 2)
173 were of particular interest to us. Six clusters were specifically upregulated in response to *mHTT*
174 expression, enriched in genes involved in transcription and chromatin remodeling, amino acid
175 metabolism, cell proliferation, cytokine signaling/innate immunity, arachidonic acid metabolism, and
176 steroid synthesis (Figure 2A). Six clusters were downregulated in response to glial *mHTT* expression,
177 containing genes involved in synapse assembly, calcium ion transport, immune system regulation,

178 phagocytosis, mRNA processing, and fatty acid degradation (Figure 2B).

179 We applied the same network analysis to genes that had concordantly altered expression in HD
180 patient striata and HD mouse model striata but not in HD *Drosophila* models (Figure S3A). We observed
181 that clusters comprising DEGs specific to the HD patients and the mouse models were functionally
182 related to DEGs in both the glial and neuronal networks (Figure S3B).

183

184 *Distinguishing glia-specific gene expression alterations from bulk tissue profiles*

185 Gene expression data from bulk tissue does not provide the resolution required to define cell-
186 autonomous gene expression alterations resulting from mHTT toxicity. Therefore, we compared DEGs
187 (FDR<0.1) in human embryonic stem cells from individuals with HD (carrying 40-48 CAG repeats) with
188 healthy embryonic stem cells that have been differentiated into either CD140+ oligodendrocyte
189 progenitor cells (OPCs) or CD44+ astrocyte progenitor cells (APCs) (Osipovitch et al., 2019). We
190 compared the resulting list of DEGs identified in the HD OPCs (1,439 genes) and HD APCs (193 genes)
191 to the list of conserved HD DEGs from flies expressing *mHTT* in glia.

192 We identified 46 upregulated and 91 downregulated DEGs in common (Figure 3A). Astrocyte
193 progenitor cells had 4 upregulated and 12 downregulated genes in common. We next asked whether any
194 clusters in the fly glial networks were enriched in genes dysregulated in HD OPCs or APCs. The Synapse
195 Assembly cluster (Figure 2B) was significantly enriched in genes with reduced expression in HD OPCs
196 (Fisher's Exact Test, $p < 0.001$), including *SYT13*, *LRRTM1*, *GRM1*, *EPB41L2*, *DLGAP3*, and *AGAP2*; the
197 only gene of this cluster that was upregulated in HD OPCs was *NRXN3* (Figure 3B and 3C).

198 In sum, by using a comparative, network-based analysis of the HD transcriptome we associated
199 dysregulation of several biological processes with the expression of *mHTT* in glia. Layering the gene
200 expression profile of homogenous glial populations affected by mHTT onto these networks, we were able
201 to extract from the bulk-tissue analysis a cluster of genes related to synaptic assembly that are altered in
202 response to glial mHTT toxicity.

203

204 *Downregulation of synapse assembly genes is compensatory in HD*

205 The next question we sought to answer is whether changes in expression of synaptic assembly
206 genes are compensatory or pathogenic. We reasoned that if lowering the expression of a downregulated
207 HD DEG aggravated mHTT-induced toxicity, then the downregulation of that gene is pathogenic.
208 Conversely, if reducing the expression of a DEG led to an improvement in HD-related phenotypes, we
209 considered that reduction to be compensatory. We previously used this approach, which takes advantage
210 of the genetic tractability of *Drosophila* and the availability of high-throughput behavioral screening as a
211 proxy for neurological function, to discover modifier genes that reduce HTT protein levels in HD patient

212 cells (Al-Ramahi et al., 2018). Here we assessed the effect of various genetic changes in the same group
213 of animals over time, following the expression of mHTT in either glia, neurons, or both cell types. We
214 used a custom, robotic assay system which video-records flies climbing upwards to the top of a vial after
215 being knocked to the bottom (negative geotaxis) to track the behavior of individual *Drosophila* in real
216 time and measure several motor metrics including speed (see Methods). Healthy flies reliably climb to the
217 top at a steady rate until the effects of aging gradually reduce their speed. In contrast, animals expressing
218 mHTT specifically in glia or neurons show much more rapid, if still age-dependent, loss of climbing
219 speed compared to animals expressing a non-targeting hairpin RNA (hpRNA). While we only focus on
220 the effect of these genetic perturbations on speed, we also observe impairments in coordination, balance,
221 and direction (output as number of turns and stumbles) in *Drosophila* expressing mHTT (data not shown).

222 The expression of *SYT13*, *LRRTM1*, *GRM1*, *EPB41L2*, *DLGAP3*, and *AGAP2* is reduced in HD
223 OPCs derived from human embryonic stem cells (Osipovitch et al., 2019), which is consistent with the
224 expression patterns we observed in patient-derived striatal tissue, knock-in mouse model striatal tissue,
225 and in neuronal tissue from *Drosophila* expressing mHTT in glia (Figure 3C). We performed genetic
226 perturbation analysis on the *Drosophila* orthologs of these genes to assess whether their downregulation
227 was pathogenic or compensatory in glia. Diminishing expression of the *Drosophila* orthologs of these six
228 genes mitigated the behavioral deficits induced by mHTT expression in glia (Figure 3D, additional
229 controls in Figure S4B). We concluded that reduced expression of these genes is a compensatory response
230 to mHTT expression in glia.

231 There were additional protein interactors in Synapse Assembly whose expression was not altered in
232 the HD-affected OPCs or APCs compared with controls but which were nonetheless downregulated
233 across all three HD models. In our behavioral assay, reducing expression of these interactors, including
234 *NLGN3*, *NLGN4X*, *HOMER1*, and *SLITRK5*, was also protective against glial mHTT toxicity (Figure
235 S4A, Table S4; additional controls in Figure S4B).

236 In sum, comparative transcriptomic analysis indicated that genes within the Synapse Assembly
237 cluster are associated with the glial response to HD, and the high-throughput behavioral assay further
238 defined this response as compensatory.

239

240 *Decreasing Neurexin expression in glia mitigates mHTT-induced pathogenesis in both neurons and glia*

241 *NRXN3* was identified as a DEG in both our cross-species comparative transcriptomic analysis and
242 in the gene expression profile of the HD glial progenitor population. *NRXN3* expression was lower in the
243 bulk HD transcriptome across species compared to their respective controls, but it was more highly
244 expressed in the HD OPCs than in controls. This discordance between the bulk and single-cell type gene
245 expression profiles might be a result of time-dependent changes in gene expression as neurons age, but it

246 prevented us from classifying the *NRXN3* expression changes as being compensatory or pathogenic. We
247 were particularly interested in neurexins, including *NRXN3*, because they mediate contact between pre-
248 and post-synaptic neurons (Ushkaryov et al., 1992; Zeng et al., 2007).

249 We therefore asked whether downregulation of *Drosophila NRXN3* (*dNRXN3*, also known as *nrx-1*)
250 is damaging or protective when both neurons and glia express *mHTT*. In the *Drosophila* behavioral assay,
251 heterozygous loss of *dNRXN3* function in animals expressing *mHTT* in both neurons and glia mitigated
252 *mHTT* toxicity and improved behavior (Figure 4A, left panel). Reproducing this experiment with flies
253 expressing *mHTT* only in glia yielded the same benefit (Figure 4A, middle panel). The obvious next
254 question, given its canonical role in neuron-neuron contact, was whether *dNRXN3* heterozygosity would
255 protect against *mHTT* pathogenesis in neurons. Interestingly, the answer was no (Figure 4A, right panel).
256 Consistent with this, glia-specific knockdown of *dNRXN3* (using the *repo-GAL4* driver) mitigated *mHTT*
257 toxicity in glia (Figure 4B, left panel), but neuron-specific knockdown (using the *elav-GAL4* driver) of
258 *dNRXN3* did not mitigate *mHTT* toxicity in neurons (Figure 4B, right panel). In sum, reducing *dNRXN3*
259 in both neurons and glia protects against glial pathogenesis—and the combination of neuronal and glial
260 pathogenesis—but not neuronal pathogenesis. This implies that *mHTT* disrupts some aspect of glial-
261 neuronal interaction that is driven by the glia, since lowering expression of *dNRXN3* in glia is necessary
262 and sufficient to mitigate behavioral impairments caused by *mHTT*.

263 To investigate whether *Nrxn3* is expressed in astrocytes in the striatum of HD mice, we performed
264 *in situ* hybridization in coronal sections of striatal tissue taken from a mouse model of HD (*Hdh^{zQ175/+}*) to
265 probe *Nrxn3* mRNA. *Nrxn3* was expressed in striatal astrocytes (Figure 4C and 4D). In conclusion,
266 modulating the expression genes other than *mHTT* in glia could be an effective strategy for ameliorating
267 HD-induced CNS dysfunction.

268
269 *Reducing SERPINA1 function mitigates behavioral impairments in neurons and glia, and lowers mHTT*
270 *protein levels*

271 We were curious to identify modifiers that concordantly affect *mHTT*-induced pathogenesis in
272 both neurons and glia, as these might be particularly attractive therapeutic targets for HD. We were
273 particularly interested to discover whether any such shared modifiers exert their effect by reducing *mHTT*
274 levels, which is considered a promising approach to therapy (Al-Ramahi et al., 2018; Barker et al., 2020;
275 Caron et al., 2020; Li et al., 2019; Tabrizi et al., 2019; Wang et al., 2014; Wood et al., 2018; Yamamoto
276 et al., 2000; Yao et al., 2015). We therefore again integrated network analysis with high-throughput
277 experimentation.

278 Genes were sampled from both the neuronal and glial *mHTT* response networks by prioritizing
279 those candidates with high centrality (calculated as a cumulative rank-score of node betweenness and

280 node degree) within each cluster. When available, we used alleles that perturb the expression or activity
281 of the *Drosophila* orthologs in the same direction as the gene expression change in the HD patient
282 population (Figure 5A). We screened 411 alleles, representing 248 *Drosophila* genes homologous to 211
283 human genes, for perturbations that improve the age-dependent behavior of *Drosophila* expressing mHTT
284 in neurons or glia (Table S5). Alleles that ameliorated neuronal or glial function were verified in a
285 subsequent trial in animals expressing mHTT across the CNS (in both neurons and glia). In all, we
286 identified 25 genes with altered expression in HD that suppressed mHTT-induced behavioral deficits in
287 neurons, glia, or both (Figure 5B and 5C, Figure S5A, Table S6).

288 In a secondary screen, we tested whether these disease modifiers common to both neurons and glia
289 exerted their beneficial effects by lowering levels of the mutant HTT protein. We collected protein lysates
290 from *Drosophila* expressing mHTT across the CNS that also bore alleles that suppressed mHTT-induced
291 behavioral deficits in both neurons and glia. We assessed the quantity of mHTT protein in these lysates by
292 western blot, comparing experimental (candidate modifiers) and control animals (carrying a non-targeting
293 hpRNA). This secondary screen identified *Spn42De* as a modifier whose knockdown lowered mHTT
294 levels. *Spn42De* is one of the four *Drosophila* homologues of human *SERPINA1* (which encodes alpha-1-
295 antitrypsin, a member of a large group of protease inhibitors). *Spn42De*, human *SERPINA1* and mouse
296 *Serpina1* are all upregulated in HD and they are part of the Wound Healing and Inflammation cluster in
297 both the neuronal and glial mHTT response networks (Figure S2C). Knockdown of *Spn42De* (henceforth
298 *dSERPINA1*) in *Drosophila* expressing mHTT in both neurons and glia mitigated behavioral impairments
299 (Figure 6A). In independent immunoblots, *dSERPINA1* knockdown consistently reduced mHTT protein
300 levels in lysates extracted from the heads *Drosophila* expressing mHTT in both neurons and glia (Figure
301 6B and 6C). As a control, we performed immunoblot analysis of lysates from a green fluorescent protein
302 (GFP) reporter line to ensure that this allele of *dSERPINA1* did not reduce the function of the *GAL4-UAS*
303 system (Figure S6).

304 To validate this observation across model systems we performed homogenous time-resolved
305 fluorescence (HTRF) on *Hdh^{Q111/Q7}* mouse striatal cell lysates that were treated with either a pool of non-
306 targeting scramble siRNAs, a pool of siRNAs against *Htt*, or a pool of siRNAs against *Serpina1a* (the
307 murine ortholog of *SERPINA1*). *Serpina1a* knockdown significantly reduced mHTT signal (Figure 6D).
308 Knockdown of *SERPINA1* thus protected against mHTT toxicity in neurons and glia by reducing levels of
309 mutant HTT. Verifying this effect in multiple model organisms increases confidence in this observation
310 and suggests that *SERPINA1* could potentially prove useful as a target for treating HD. Interestingly,
311 *SERPINA1* expression is low in the healthy brain but it is upregulated in several disease conditions,
312 consistent with a potential role in neuroinflammation (Abu-Rumeileh et al., 2020; Cabezas-Llobet et al.,
313 2018; Gollin et al., 1992; Peng et al., 2015), and we previously showed that other genes in the subnetwork

314 implicated in neuroinflammation can be manipulated to lower mHTT protein levels (Al-Ramahi et al.,
315 2018). *SERPINA1* may thus warrant investigation as a target for other neurological disorders as well.

316

317 **Discussion**

318 We found a high degree of overlap of differentially expressed genes (DEGs) across tissues from
319 human HD brains, brains of HD mice, and flies that express *mHTT* in glia. This observation is consistent
320 with previous evidence that *Drosophila* glia perform many of the same functions as mammalian
321 astrocytes, oligodendrocytes, and microglia (Chung et al., 2020; Freeman and Doherty, 2006; Ziegenfuss
322 et al., 2012). Several studies have also shown that wild-type glial cells ameliorate disease when
323 transplanted into HD mice, and mHTT exerts a deleterious effect on glial development and function,
324 which in turn influences HD pathogenesis (Benraiss et al., 2016; Bradford et al., 2009; Garcia et al., 2019;
325 Huang et al., 2015; Osipovitch et al., 2019). More recently, it was discovered that transcription factors
326 involved in glial differentiation and myelin synthesis are downregulated in glial progenitor cells
327 (Osipovitch et al., 2019). Yet despite this progress, the overall contributions of glial genes to synaptic
328 impairments and other key neurodegenerative pathologies remain poorly understood. The genetic
329 malleability of *Drosophila* enabled us to thoroughly examine the neuron-glia interface from both the glial
330 and the neuronal directions.

331 Synaptic dysfunction is a common theme among many neurodegenerative disorders (McInnes et
332 al., 2018; Phan et al., 2017; Prots et al., 2018). While it is clear that the dysfunction of the glia-synapse
333 interface is central to the pathophysiology of neurodegeneration (Filipello et al., 2018; Garcia et al., 2019;
334 Lian et al., 2015; Litvinchuk et al., 2018), the underlying mechanisms remain underexplored relative to
335 the interactions between pre- and post-synaptic neurons. Our results support the observation that the
336 expression of *mHTT* in glia is sufficient to drive synaptic dysfunction (Wood et al., 2018). In HD,
337 presynaptic motor neurons release elevated levels of glutamate into the synapse, driving medium spiny
338 neurons (MSNs) into excitotoxicity (Estrada Sánchez et al., 2008; Hong et al., 2016). Hyperactivity of
339 receptors at the post-synaptic densities sensitizes MSNs to excitotoxicity, further contributing to
340 neurodegeneration (Estrada Sánchez et al., 2008). Astrocytic *mHTT* expression may contribute to
341 neuronal excitotoxicity by elevating levels of glutamate, potassium, and calcium at the synapse (Garcia et
342 al., 2019; Jiang et al., 2016; Tong et al., 2014).

343 Modifiers of mHTT-induced pathogenesis identified in our study, such as metabotropic glutamate
344 receptors and the scaffold protein HOMER1, regulate calcium and glutamate signaling in astrocytes
345 (Buscemi et al., 2017; Spampinato et al., 2018). Reducing the expression of these genes could prevent
346 excess calcium and glutamate from accumulating at the synapse. Indeed, we previously found that HD
347 neurons downregulate the expression of genes involved in calcium signaling in an effort to compensate

348 for HD pathogenesis (Al-Ramahi et al., 2018). Glial calcium signaling can also influence neuronal
349 activity, however, at the neuronal soma (Weiss et al., 2019). In *Drosophila*, cortical glia modulate
350 neuronal activity through potassium buffering, a process that is regulated by calcium-mediated
351 endocytosis of potassium channels (Weiss et al., 2019). Glia can also physically disrupt synapses in
352 disease states: Förster resonance energy transmission (FRET) *in vivo* revealed that, in HD, the distances
353 between astrocytes and pre-synaptic neurons are increased at the cortico-striatal circuit (Octeau et al.,
354 2018). Thus, knocking down the genes in the Synapse Assembly cluster could reduce physical interaction
355 between glia and synapses, promoting normal synaptic function.

356 If in HD synapses grow more fragile and fewer in number as the disease progresses, why would
357 down-regulating the expression of glial genes required for synapse formation and function be protective?
358 We postulate it is for the same reason that downregulating calcium-signaling genes is compensatory (Al-
359 Ramahi et al., 2018): the brain is attempting to protect against the excitotoxicity described above. Mutant
360 HTT disrupts neuronal development (Ring et al., 2015) and skews embryonic neurogenesis toward
361 producing more neurons (Barnat et al., 2020); by the time HD mutations carriers reach the age of six
362 years, they have greatly enlarged striata and functional hyperconnectivity to the cerebellum
363 (Tereshchenko et al., 2020). The more hyperconnected, the more abrupt the loss of these connections, and
364 the more rapid the striatal atrophy that follows (Tereshchenko et al., 2020). The hyperfunction of a given
365 brain region puts considerable strain on the circuit, and it seems that over the course of a lifetime, the
366 brain keeps trying to compensate for the abnormalities that arise at different stages of HD. The recent
367 observation that deletion of astrocytic neurexin-1 α attenuates synaptic transmission but not synapse
368 number supports this hypothesis (Trotter et al., 2020).

369 We do not think that the protection provided by modifiers in this cluster is limited to modulating
370 neurotransmission. In astrocytes, calcium signaling also controls the activity of reactive astrocytes
371 (Buscemi et al., 2017). Astrogliosis, or the proliferation of immune active astrocytes, is typically
372 observed at later stages of HD (Al-Dalahmah et al., 2020; Buscemi et al., 2017). These immune-activated
373 glia not only eliminate synapses (Liddelow et al., 2017; Sofroniew, 2009) but can also transmit mHTT
374 aggregates through the synapse (Donnelly et al., 2020). In *Drosophila*, knockdown of *draper* prevents
375 astrocytic phagocytosis and stops the spread of mHTT protein aggregates from pre-synaptic neurons to
376 the post-synaptic compartment (Donnelly et al., 2020; Pearce et al., 2015). mHTT protein can also enter
377 the synaptic space by endosomal/lysosomal secretion mediated by Syt7 (Trajkovic et al., 2017). In our
378 present study, we observed that knockdown of synaptotagmins in *Drosophila* ameliorates glial mHTT-
379 induced dysfunction. Thus, knocking down genes in the Synapse Assembly cluster could also benefit the
380 circuit by reducing the transmission of aggregated mHTT protein from pre- to post-synaptic neurons.

381 Interestingly, loss-of-function variants in *NRXN1-3*, *NLGN1*, *NLGN3*, *DLGAP3* and *LRRTM1*
382 have been associated with various disorders of synaptic dysfunction, including Autism Spectrum Disorder
383 (ASD), schizophrenia, and obsessive compulsive disorder (OCD) (Nakanishi et al., 2017; Paris Autism
384 Research International Sibpair Study et al., 2003; Südhof, 2008; Vaags et al., 2012; Wang et al., 2018;
385 Windrem et al., 2017). We speculate that the consequences of loss of function of these genes depend on
386 both dosage and context: modest reductions of gene expression can be protective in the context of HD
387 pathogenesis, whereas a more severe loss of function results in ASD and OCD. Context could also relate
388 to the affected cell-type. Future studies should investigate whether these loss of function variants
389 associated with neurodevelopmental and psychiatric disorders alter the age of disease onset in patients
390 with HD. It could be of particular interest to assess if these neurodevelopmental and psychiatric-
391 associated variants prevent neurodevelopmental changes observed early in HD or blunt synaptic
392 hyperactivity later in disease.

393

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411 Health.

412

413 **Author Contributions**

414 J.B. conceived, supervised and provided resources for the study; Z.L. supervised bioinformatic studies;
415 T.O., A.L., G.A., B.L., I.A.-R., Z.L. and J.B. contributed to experimental design; T.O., A.L., H.Z., R.K.,
416 H.K., J.W., M.M., A.P., M.H., H.W. Y.-W.W. performed experiments; T.O. and J.B. wrote the paper.

417

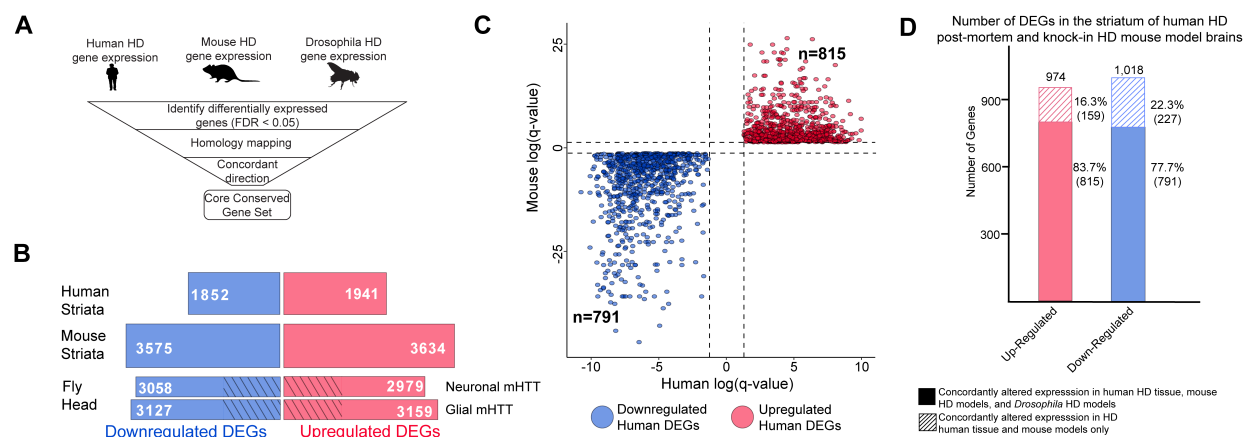
418 **Declarations of Interest**

419 The authors declare no competing financial interests.

420

421
422
423

Figures



424
425

426 **Figure 1. Differentially expressed genes (DEGs) in HD human striatal tissue are concordantly**
427 **altered in mouse and *Drosophila* HD models.**

428 A) Our approach to identifying orthologous genes in tissues from humans, mice, and *Drosophila* with
429 concordant expression changes (i.e., upregulated or downregulated in all three systems) following *mHTT*
430 expression.

431 B) The number of differentially expressed genes (DEGs) in each species-specific dataset that are
432 downregulated (blue) or upregulated (red) (see Methods). *Drosophila* DEGs were from flies expressing
433 either the N-terminal (*HTT^{NT231Q128}*) or full-length *mHTT* (*HTT^{FLQ200}*) in neurons (*elav-GAL4*) or glia
434 (*repo-GAL4*). The DEGs in flies are grouped according to the cell type expressing *mHTT*, rather than
435 *mHTT* model. The cross-hatched regions of the *Drosophila* bars represent DEGs shared between the
436 neuronal and glial sets: 1,293 downregulated genes and 1,181 upregulated genes.

437 C) Points in the scatterplot represent human DEGs identified by the strategy outlined in (A) that are
438 concordantly dysregulated across all three species. Red nodes represent upregulated DEGs (n=815),
439 whereas blue nodes represent downregulated genes (n=791). The overlap of these concordant DEGs
440 represents approximately forty percent of genes with altered expression in the human HD transcriptome
441 that are upregulated (p=6.37x10⁻¹⁵⁸) or downregulated (p=1.66x10⁻¹⁶⁵). The p-value was calculated using a
442 random background probability distribution over 2x10⁵ random samplings.

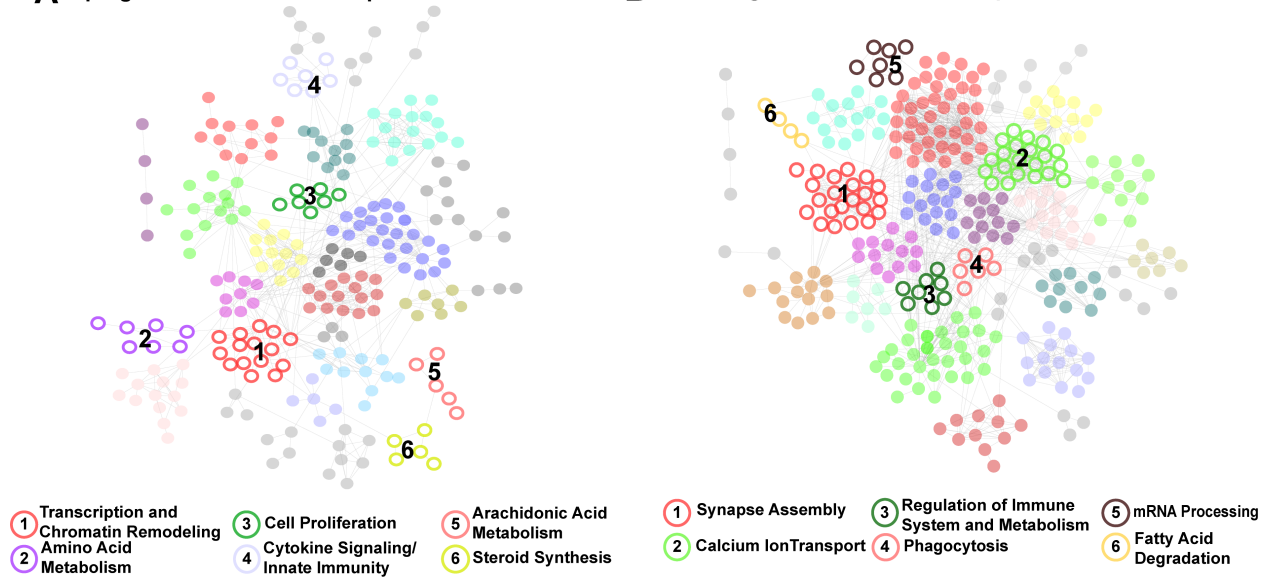
443 D) The stacked bar graph highlights that a large majority of concordant DEGs in human HD striata and
444 knock-in HD mouse models are also concordantly altered in *Drosophila* models of HD.

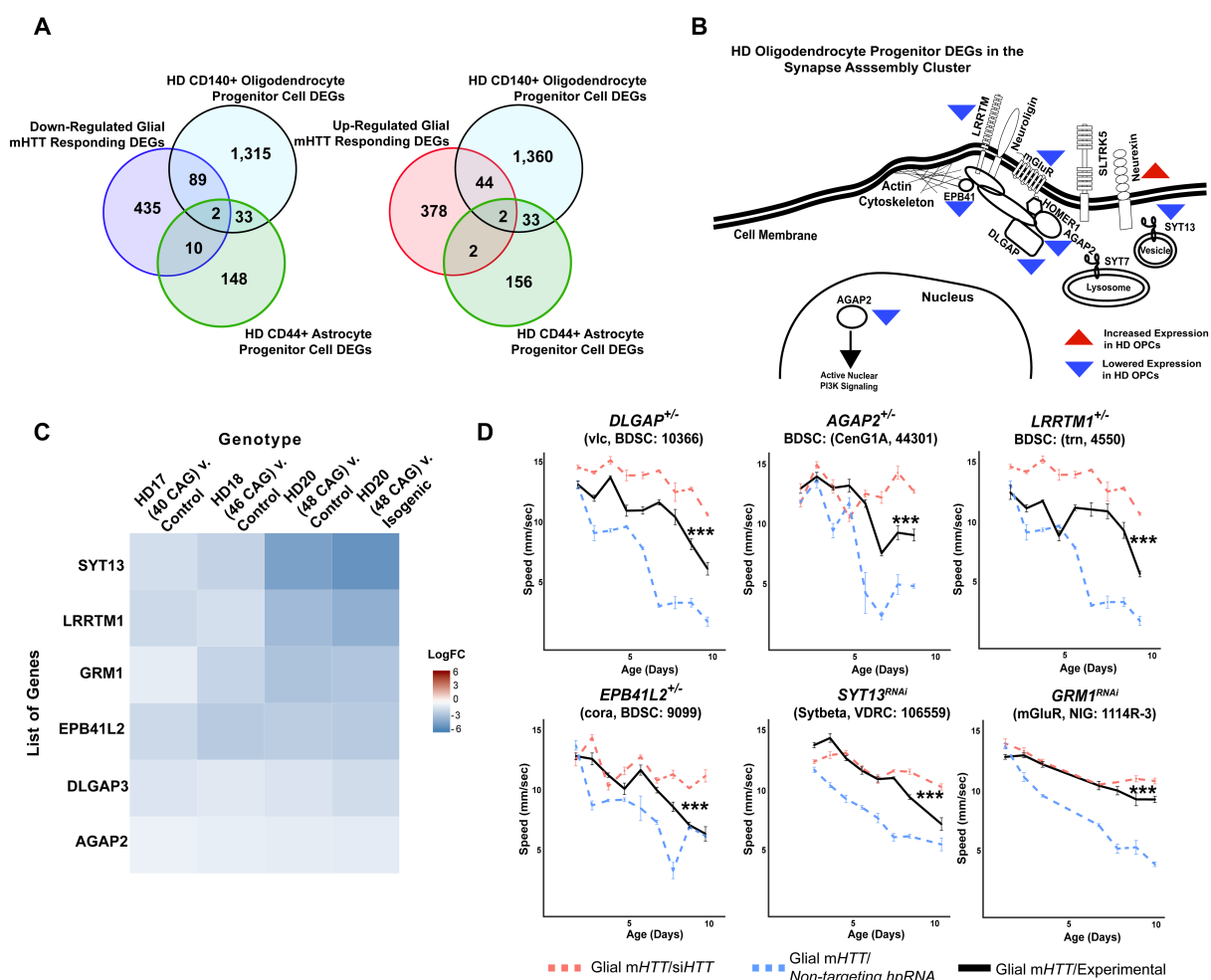
445 **Related to Figure 1, Figure S1:** Expressing *mHTT* in *Drosophila* glia or neurons leads to distinct gene
446 expression profiles. In contrast, the full-length and N-terminal models show relatively similar gene
447 expression profiles.

448

A Upregulated Glial mHTT Response Network

B Downregulated Glial mHTT Response Network





464

465 **Figure 3. Reducing the expression of Synapse Assembly cluster genes in glia mitigates mHTT-**
 466 **induced behavioral impairments.**

467 A) Overlaps between concordant DEGs from the cross-species analysis defined as responding to mHTT
 468 expression in glia and DEGs identified in HD human embryonic stem cells (hESCs) that have been
 469 differentiated into either CD140+ oligodendrocyte progenitor cells (OPCs) or CD44+ astrocyte progenitor
 470 cells (APCs) (Osipovitch et al. 2018).

471 B) Model placing Synapse Assembly cluster proteins into cellular context. The Synapse Assembly cluster
 472 was significantly enriched for DEGs in HD OPCs (Fisher's Exact Test, $p < 0.001$). Only one gene, *NRXN3*,
 473 was upregulated in HD OPCs compared to controls (upward red triangle); the rest (*AGAP2*, *GRM1*,
 474 *LRRTM1*, *EPB41L2*, *DLGAP3*, and *SYT13*) were downregulated (downward blue triangles).

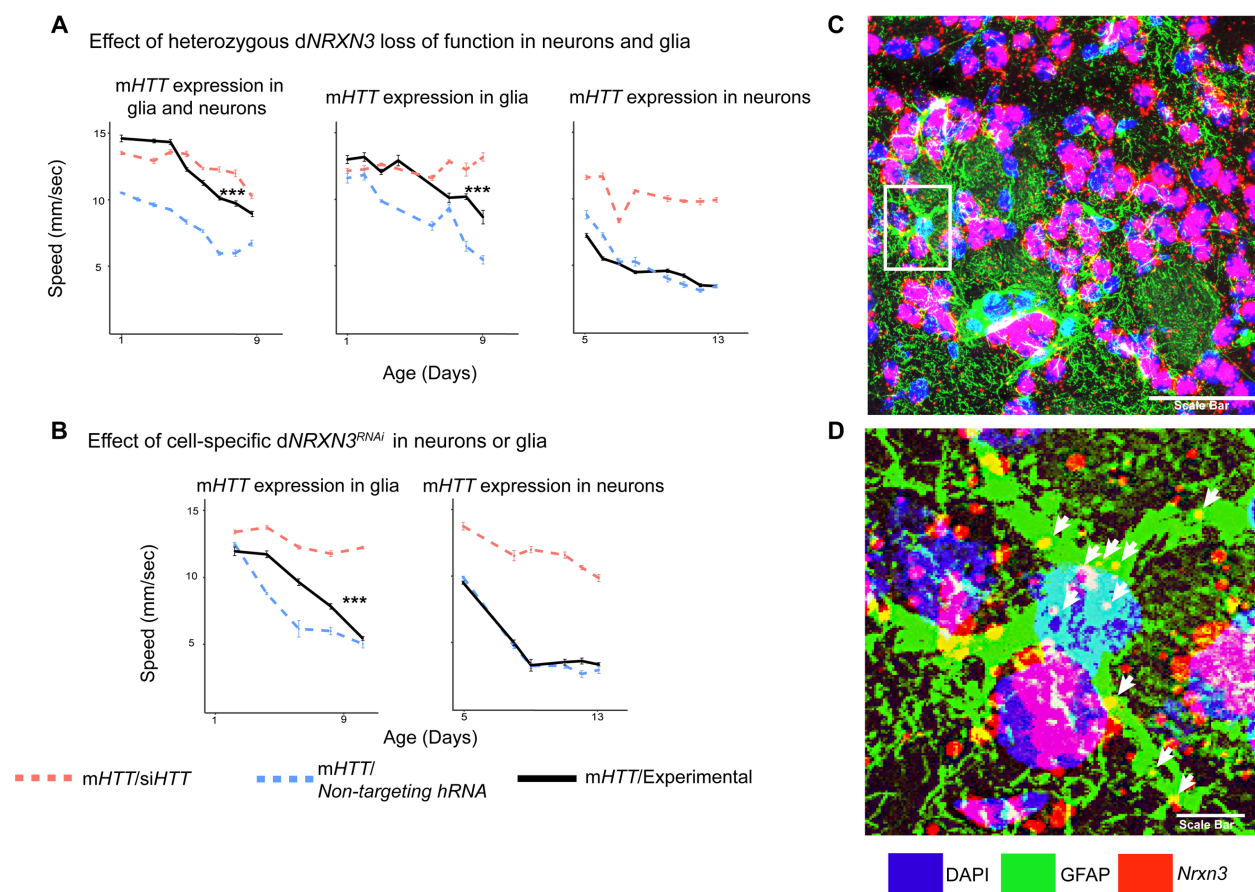
475 C) Heatmap representing genes with lower expression in HD OPCs compared to controls (presented as
 476 LogFC) that belong to the Synapse Assembly cluster. Each row is one downregulated gene; each column
 477 is a different HD human embryonic stem cell line, with CAG repeat length ranging from 40-48, compared
 478 to respective controls (Osipovitch et al 2018).

479 **Figure 3 continued.**

480 D) Behavioral assessment of fruit flies that express *mHTT* only in glia, after reducing the expression of
481 the overlapping DEGs in HD OPCs and the Synapse Assembly cluster. Plots show climbing speed as a
482 function of age. *** $p < 0.001$ between positive control and experimental (by linear mixed effects model
483 and post-hoc pairwise comparison (see Methods). Points and error bars on the plot represent the
484 mean \pm SEM of the speed for three technical replicates. Each genotype was tested with 4-6 replicates of 10
485 animals. Modifying alleles in (D) are listed in the **Key Resources Table**. Additional climbing data for
486 these genes can be found in **Figure S4A** and a summary of statistical analysis for this data can be found in
487 **Table S4**. Control climbing data for these alleles can be found in **Figure S4B**.

488 *Drosophila* Genotypes: positive control ($w^{1118}; UAS\text{- non-targeting } hpRNA/+; repo\text{-}GAL4, UAS\text{-}$
489 $HTT^{NT231Q128}/+$), treatment control ($w^{1118}; repo\text{-}GAL4, UAS\text{- } HTT^{NT231Q128}/UAS\text{-}siHTT$), and experimental
490 ($w^{1118}; repo\text{-}GAL4, UAS\text{- } HTT^{NT231Q128}/modifier$).

491



492

493 **Figure 4. Glia-specific *dNRXN3* knockdown mitigates impairments caused by *mHTT* expression.**

494 A) Behavioral assays (climbing speed as a function of age) showing that *dNRXN3* heterozygous loss-of-
 495 function (LOF) ameliorates behavioral impairments caused by expression of *mHTT* in both neurons and
 496 glia and in glia alone, but not in neurons alone.

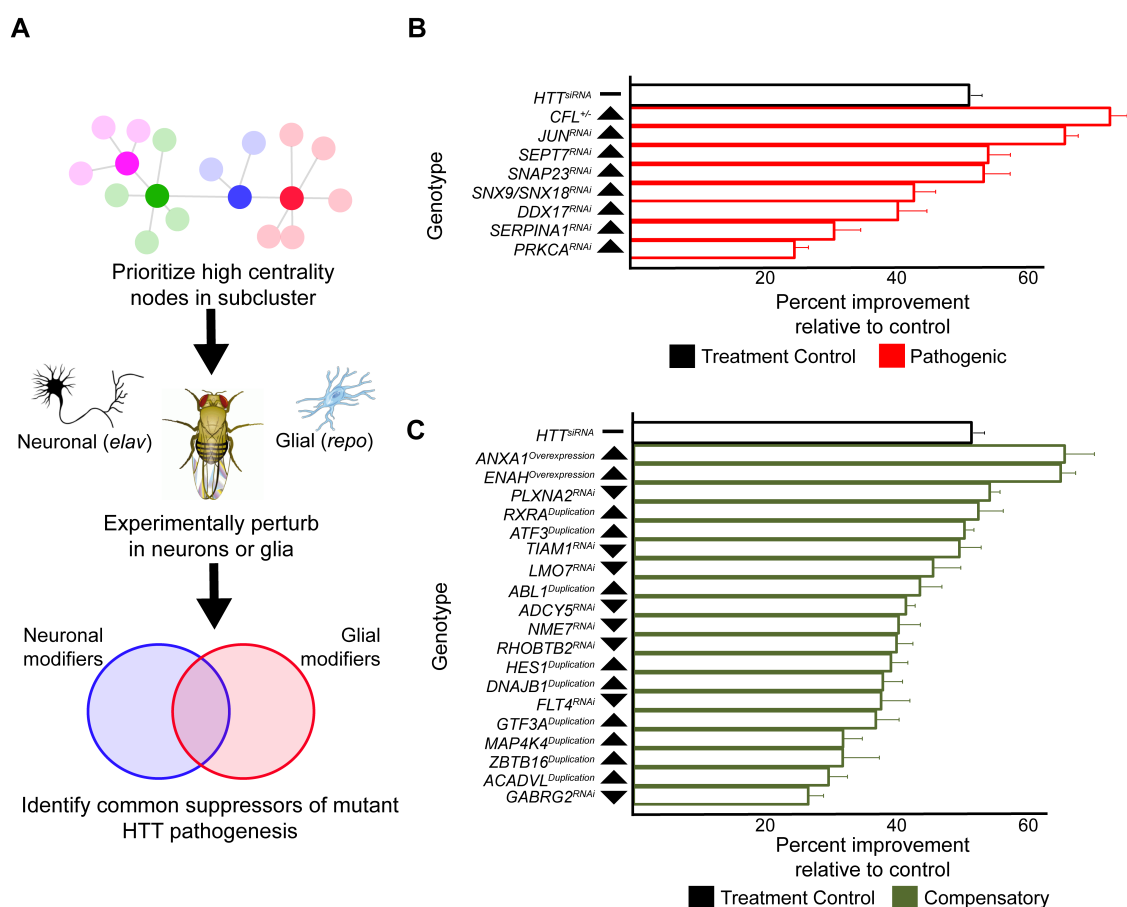
497 B) Glia-specific *dNRXN3* knockdown mitigates behavioral impairments caused by *mHTT* expressed
 498 solely in glia; however, neuron-specific knockdown of *dNRXN3* does not affect impairments induced by
 499 *mHTT* expressed solely in neurons. *** <0.001 between positive control and experimental by linear
 500 mixed effects model and post-hoc pairwise comparison (see **Methods**). Points and error bars on the plot
 501 represent the mean±SEM of the speed for three technical replicates. Each genotype was tested with 4-6
 502 replicates of 10 animals. A full summary of the statistical analysis for this data can be found in **Table S4**.
 503 Control climbing data for these alleles can be found in **Figure S4B**.

504 C) Astrocytes in the striatum of 6-month old knock-in HD mice (*Hdh*^{Q175/+}) expressing *Nrxn3*. *In situ*
 505 probe for *Nrxn3* mRNA is in red (appears magenta when overlapping with the DAPI channel) astrocytes
 506 are immunostained using an antibody specific for glial fibrillary acidic protein (GFAP) in green, and
 507 DAPI in blue. Image was taken at 63x magnification using a Leica SP8 confocal microscope. Scale bar
 508 (in white on the bottom right) represents 50 μm.

509 **Figure 4 continued.**

510 D) Magnified image of the astrocyte highlighted in the white box in (C). White arrows indicate yellow
511 puncta where *Nrxn3* mRNA localizes to astrocytes. Scale bar (in white on the bottom right) represents 5
512 μm .

513 *Drosophila* Genotypes: *dNRXN3* loss-of-function allele ($y^1 w^*$; $Mi\{y^{+mDint2}=MIC\}nrx-I^{M102579}$ or $nrx-I^{LOF}$,
514 BDSC: 61696), *dNRXN3* RNAi allele ($UAS-nrx-I^{hpRNA}$, VDRC: 36326), neuronal and glial HD model
515 with *dNRXN3* mutant ($elav^{c155}-GAL4/y^1 w^*$; $repo-GAL4,UAS-HTT^{NT231Q128}/Experimental\ allele$), glial HD
516 model with *dNRXN3* mutant ($w^{1118}/y^1 w^*$; $repo-GAL4,UAS-HTT^{NT231Q128}/Experimental\ allele$), and
517 neuronal model with *dNRXN3* mutant ($elav^{c155}-GAL4/y^1 w^*$; $UAS-HTT^{NT231Q128}/Experimental\ allele$).
518



519

520 **Figure 5. Compensatory and pathogenic gene expression changes shared by neurons and glia in**

521 **response to mHTT expression.**

522 A) Our approach for identifying modifiers of mHTT-induced behavioral impairments common to both

523 neurons and glia. Genes that were central to their respective clusters were prioritized and manipulated in

524 *Drosophila* expressing mHTT ($HTT^{NT231Q128}$) in either neurons (*elav-GAL4*) or glia (*repo-GAL4*).

525 B) Red bars represent the percent improvement in behavior over a 9-day trial, compared to positive

526 control (non-targeting hpRNA) in *Drosophila* expressing mHTT in neurons and glia, after we antagonized

527 pathogenic gene expression changes.

528 C) Green bars represent the percent improvement in behavior over a 9-day trial, compared to control (see

529 B), after we mimicked compensatory gene expression alterations.

530 In (B) and (C), the top black bars represent the effect of directly targeting the mHTT transgene using an

531 siRNA. Arrowheads indicate the direction of the conserved, concordant altered expression for each gene

532 as a result of mHTT expression in humans, mice and *Drosophila*. Behavioral assay graphs corresponding

533 to the data presented in (B) and (C) can be found in **Figure S5A**. Corresponding statistical analysis for

534

535

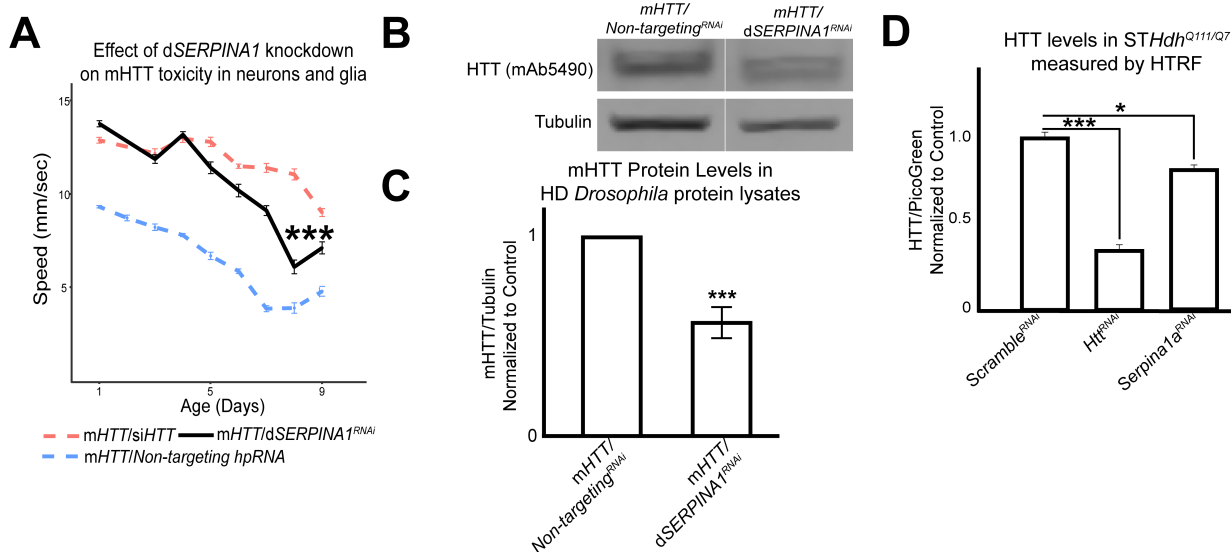
536 **Figure 5 continued.**

537 (B) and (C) can be found in **Table S6**. Corresponding controls for behavioral data can be found in **Figure**
538 **S5B and C**.

539 *Drosophila* Genotypes: Positive control (*elav^{c155}-GAL4/w¹¹¹⁸; UAS- non-targeting hpRNA/+; repo-*
540 *GAL4, UAS-HTT^{NT231Q128}/+*), treatment control (*elav^{c155}-GAL4/w¹¹¹⁸; repo-GAL4, UAS- HTT^{NT231Q128}/UAS-*
541 *siHTT*) and experimental (*elav^{c155}-GAL4/w¹¹¹⁸; repo-GAL4, UAS- HTT^{NT231Q128}/modifier*).

542

543



544
 545 **Figure 6. Antagonizing the pathogenic overexpression of *SERPINA1* in neurons and glia mitigates**
 546 **mHTT-induced behavioral impairments and lowers mHTT protein levels in *Drosophila* and HD**
 547 **mouse striatal cells.**

548 A) Behavioral assays following knockdown of *dSERPINA1* in *Drosophila* expressing mHTT in neurons
 549 and glia. *** indicates $p < 0.001$ by linear mixed effects model and post-hoc pairwise comparison between
 550 positive control and experimental animals. Points and error bars on the plot represent the mean \pm SEM of
 551 three technical replicates. Each genotype was tested with 4-6 replicates of 10 animals.

552 B) Representative western blot showing lower levels of mHTT following knockdown of *dSERPINA1* in
 553 *Drosophila* expressing mHTT in neurons and glia.

554 C) Quantification of five independent immunoblots showing the effect of *dSERPINA1* knockdown on
 555 mHTT levels in *Drosophila* head protein lysates. *** $p < 0.001$ between positive control and *dSERPINA1*
 556 knockdown by one-way T-test.

557 D) Quantification of HTT protein levels in HD mouse striatal-derived cells (*STHdh*^{Q111/Q7}) measured by
 558 HTRF following treatment with a pool of scramble siRNAs (negative control), a pool of siRNAs against
 559 *Htt*, and a pool of siRNAs against *Serpina1a*. Quantification is presented as a ratio of the emission signal
 560 from the fluorescent D2 dye (HTT)/PicoGreen (number of cells per well). $n = 9$ for each treatment group.
 561 * $p < 0.05$ and *** $p < 0.001$ between genotypes by Fisher's LSD test.

562 *Drosophila* Genotypes: *dSERPINA1* RNAi allele (*UAS-Spn42De*^{hpRNA}, VDRC: 102622), positive control
 563 (*elav*^{c155}-*GAL4/w*¹¹¹⁸; *UAS-non-targeting hpRNA/+*; *repo-GAL4, UAS-HTT*^{NT231Q128/+}), treatment control
 564 (*elav*^{c155}-*GAL4/w*¹¹¹⁸; *repo-GAL4, UAS-HTT*^{NT231Q128/UAS-siHTT}), and *dSERPINA1* experimental
 565 (*elav*^{c155}-*GAL4/w*¹¹¹⁸; *UAS-Spn42De*^{hpRNA/+}; *repo-GAL4, UAS-HTT*^{NT231Q128/+}).

566
 567

568 **Materials and Methods**

569 *Lead Contact and Material Availability*

570 Further information and requests for resources and reagents should be directed to and will be fulfilled by
571 the Lead Contact, Juan Botas (jbotas@bcm.edu).

572

573 *Drosophila models*

574 We began with *Drosophila* models expressing either N-terminal human HTT ($HTT^{NT231Q128}$) or
575 full-length (FL) HTT (HTT^{FLQ200}) (Kaltenbach et al., 2007; Romero et al., 2008). The mHTT was
576 expressed using either a pan-neuronal (*elav*) or a pan-glial driver (*repo*). Mutant strains for screening
577 were obtained from Bloomington *Drosophila* Stock Center, GenetiVision, and the Vienna *Drosophila*
578 Resource Center. All strains were maintained at 18°C in standard molasses, yeast extract, agar media until
579 their experimental use. For RNA-sequencing, the full-length models were raised at 29°C and the N-
580 terminal models were raised at 28°C. All behavioral experiments were performed on females raised at
581 28°C.

582 In figure 3D, we used the following mutants to assess the effect of reduced expression of synaptic
583 genes in mHTT animals on behavior: *UAS-non-targeting^{hpRNA}* (Vienna *Drosophila* Resource Center,
584 ID:13974), *CenGIA^{LOF}* or *y¹w^{*};Mi{MIC}CenGIA^{M106024}* (Bloomington *Drosophila* Stock Center, ID:
585 44301), *vlc^{LOF}* or *y¹w^{67e23};P{w^{+mc}=lacW}vlc^{k01109}/CyO* (Bloomington *Drosophila* Stock Center, ID:
586 10366), *trn^{LOF}* or *y¹w^{67e23};P{w^{+mc}=lacW}trn^{S064117}/TM3, Sb¹ Ser¹* (Bloomington *Drosophila* Stock Center,
587 ID: 4550), *cora^{LOF}* or *P{ry^{t7.2}=neoFRT}43D cora¹⁴/CyO* (Bloomington *Drosophila* Stock Center, ID:
588 9099), *UAS-Sybeta^{hpRNA}* (Vienna *Drosophila* Resource Center, ID:106559), and *UAS-mGluR^{RNAi}* (National
589 Institute of Genetics, Japan, ID: 11144-R3).

590 To generate *Drosophila* that expressed a small interfering RNA that knocked down human *HTT*
591 (*UAS-siHTT*), we cloned a 378bp inverted EcoRI, XbaI fragment of N-terminal Htt into the pMF3 vector
592 (*Drosophila* Genome Resource Center). This fragment maps to base pairs 406 to 783 of the human
593 mRNA *Huntingtin*, which we cloned using the following primers:

594 Forward 5'-gaattcGCACCGACCAAAGAAAGAAC-3'

595 Reverse 5'-tctagaGCAGAAGGTTCCACCAGGTA-3'

596 We first digested the PCR product with EcoRI and ligated it with itself to obtain inverted repeats.
597 We then digested the inverted repeat with XbaI and pasted the fragment into the pMF3 vector (also cut
598 with XbaI); the resulting plasmid was injected into *Drosophila* embryos using standard methods (Dietzl et
599 al., 2007). We validated that this line lowers mHTT levels.

600

601

602 *STHdh*^{Q111/Q7} mouse striatal cells

603 Immortalized mouse striatal cells heterozygous for mHTT (*STHdh*^{Q111/Q7}) were obtained from
604 Coriell Cell Repositories (Camden, NJ) and cultured in DMEM (Life Technologies, cat. no. 11965)
605 supplemented with 10% fetal bovine serum (Life Technologies, cat. no. 10082–147).

606
607 *DEG identification in Drosophila HD models*

608 We performed RNA-seq on head tissue collected from *Drosophila* expressing N-terminal (*UAS-*
609 *HTT*^{NT231Q128}) or full-length (*UAS-HTT*^{FLQ200}) human mHTT in neurons (*elav-GAL4*) or glia (*repo-GAL4*).
610 For each combination of HD model and driver, RNA-seq was performed at three time points to capture
611 the early, middle, and late phases of disease pathogenesis, corresponding to behavioral deficits caused by
612 mHTT-induced neuronal or glial dysfunction. At each timepoint, samples for HD and age-matched
613 controls were collected in triplicate. *Drosophila* expressing the N-terminal construct and corresponding
614 controls were obtained at 7,9, and 11 days post-eclosion for the neuronal driver, and at 5, 7, and 8 days
615 post-eclosion for the glial driver. *Drosophila* expressing the full-length construct, samples were obtained
616 at 18, 20, and 22 days post-eclosion for both the neuronal and glial driver. For RNA-seq the neuronal N-
617 terminal, glial N-terminal, and glial full-length model *Drosophila* were raised at 28°C. The neuronal full-
618 length model *Drosophila* were raised at 29°C. For each genotype at each timepoint, we collected an
619 equivalent number of control animals (*elav-GAL4* or *repo-GAL4*) that were raised in the same conditions.

620 Three replicates of 50 virgin females were collected for each genotype and timepoint. Animals were
621 aged in the appropriate temperature and were transferred to fresh food daily until tissue was harvested. At
622 the selected ages, animals were transferred to 1.5ml tubes, flash frozen in liquid nitrogen, vigorously
623 shaken and then sieved to collect 50 heads/genotype/replica (~5mg tissue/replica). Total RNA was
624 extracted using the miRNeasy Mini Kit (Qiagen Cat. # 210074).

625 RNA-seq profiling and preprocessing was performed by Q2 Solutions (Morrisville, North
626 Carolina). Samples were converted into cDNA libraries using the Illumina TruSeq Stranded mRNA
627 sample preparation kit (Illumina Cat. #20020595) and were sequenced using HiSeq-Sequencing-2x50bp-
628 PE. Initial analysis was performed using Q2 Solution in-house mRNA_{v7} pipeline with a median of 49
629 million actual reads. After adapter sequences were removed, the reads were aligned to the *Drosophila*
630 *melanogaster* transcriptome using Bowtie version 0.12.9 (Langmead and Salzberg, 2012). Expression was
631 quantified using RSEM version 1.1.19, resulting in a median of 11,214 genes and 18,604 isoforms
632 detected (Li and Dewey, 2011).

633
634 *Homology mapping of HD DEGs by network-based intersection*

635 Three homology maps were constructed to define conserved genes that were concordantly

636 dysregulated in response to mHTT toxicity: a *Drosophila*-human map, a *Drosophila*-mouse map, and a
637 mouse-human map. The *Drosophila*-human map and *Drosophila*-mouse map were both obtained from
638 DIOPT version 6.0.2 (Hu et al., 2011). To capture homology that results from evolutionary convergence
639 and divergence we included lower DIOPT scores between *Drosophila* and mammals, instead of fitting
640 one-to-one mappings between these species. The mouse-human homology mapping was obtained from
641 the Mouse Genome Informatics (MGI) database hosted by Jackson Laboratories (Blake et al., 2017).

642 We integrated these three homology maps by representing each map as an undirected bipartite
643 graph, where nodes are genes of one species and edges represent homology between two genes across
644 species. All components were then merged to form an undirected graph where each node represents a
645 gene name and corresponding species. We applied this integrated homology map consisting of nodes
646 representing the *Drosophila*, mouse, and human dysregulated genes, and all edges induced by the
647 corresponding nodes, to obtain a subgraph consisting of multiple connected components. If any individual
648 connected component contained nodes that belong to all three species, we characterized all genes within
649 the connected component as concordant.

650

651 *Protein-protein interaction network and clustering*

652 To examine how the upregulated and downregulated core genes interact functionally, we used
653 STRING v10.5 (Szklarczyk et al., 2015). Only high-confidence interactions (edge weight > 0.7) were
654 considered. Each node is converted from an ENSEMBL ID to human Entrez ID via the provided mapping
655 file (v10, 04-28-2015). Four subgraphs of STRING were then induced on each core gene set separately.
656 Nodes were further clustered with the InfoMap community detection algorithm (Rosvall and Bergstrom,
657 2008), implemented in the Python iGraph package, with the default settings (trials = 10) (Csardi and
658 Nepusz).

659

660 *Drosophila behavioral assay*

661 We crossed female virgins that carried the mutant HTT (mHTT) transgene under the control of
662 either the neuronal or glial driver, or the cell-specific driver alone, to males carrying the experimental
663 allele. We introduced a heat-shock-induced lethality mutation on the Y chromosome ($Y^{P\{hs-hid\}}$) to the
664 disease and cell-specific driver stocks to increase the efficiency of virgin collection (Starz-Gaiano et al.,
665 2001). For crosses involving alleles that were lethal or sterile mutations on the X chromosome, this
666 mating strategy was reversed. For behavioral assays, $elav>HTT^{NT231Q128}$ and $repo>HTT^{NT231Q128}$ animals
667 were raised and maintained at 28.5°C. $Elav,repo>HTT^{NT231Q128}$ animals were raised and maintained at
668 25°C.

669 The negative geotaxis climbing assay was performed using a custom robotic system (SRI

670 International, available in the Automated Behavioral Core at the Dan and Jan Duncan Neurological
671 Research Institute). The robotic instrumentation elicited negative geotaxis by “tapping” *Drosophila*
672 housed in 96-vial arrays. After three taps, video cameras recorded and tracked the movement of animals
673 at a rate of 30 frames per second for 7.5 seconds. For each genotype, we collected 4 to 6 replicates of 10
674 animals to be tested in parallel. Each trial was repeated three times. The automated, high-throughput
675 system is capable of assaying 16 arrays (1,536 total vials) in ~3.5 hours. To transform video recordings
676 into quantifiable data, individual *Drosophila* were treated as an ellipse, and the software deconvoluted the
677 movement of individuals by calculating the angle and distance that each ellipse moves between frames.
678 The results of this analysis were used to compute more than two dozen individual and population metrics,
679 including distance, speed, and stumbles.

680 Software required to run and configure the automation and image/track the videos include: Adept
681 desktop, Video Savant, MatLab with Image Processing Toolkit and Statistics Toolkit, RSLogix (Rockwell
682 Automation), Ultraware (Rockwell Automation). Additional custom designed software includes: Assay
683 Control – SRI graphical user interface for controlling the assay machine; Analysis software bundles:
684 FastPhenoTrack (Vision Processing Software), TrackingServer (Data Management Software),
685 ScoringServer (Behavior Scoring Software), Trackviewer (Visual Tracking Viewing Software).

686

687 *In situ and immunofluorescence in HD mouse brain sections*

688 mRNA in situ hybridization (ISH) and immunofluorescence were performed on 25- μ m thick
689 coronal brain sections cut from fresh-frozen brain harvested from a 6-month old *Hdh^{Q175/+}* mouse.
690 We generated digoxigenin (DIG)-labeled mRNA antisense probes against *Nrxn3* using reverse-
691 transcribed mouse cDNA as a template and an RNA DIG-labeling kit from Roche (Sigma). Primer
692 and probe sequences for the *Nrxn3* probe is available in Allen Brain Atlas ([http://www.brain-](http://www.brain-map.org)
693 [map.org](http://www.brain-map.org)). ISH was performed by the RNA In Situ Hybridization Core at Baylor College of Medicine
694 using an automated robotic platform as previously described (Yaylaoglu et al., 2005) with modifications
695 of the protocol for fluorescent ISH. In brief: after the described washes and blocking steps, the DIG-
696 labeled probe was visualized using a tyramide-Cy3 Plus kit (1:50 dilution, 15-minute incubation, Perkin
697 Elmer). Following washes in phosphate buffered saline (PBS) the slides were stained with 1:500 anti-
698 GFAP rabbit polyclonal antibody (DAKO, Z0334) diluted in 1% blocking reagent in Tris buffered saline
699 (Roche Applied Science, 11096176001) overnight at 4°C. After washing, slides were treated with 1:500
700 anti-rabbit IgG Alexa 488 secondary antibody for 30 min at room temperature (Invitrogen, A-11008). The
701 slides were stained with DAPI and cover slipped using ProLong Diamond (Invitrogen, P36970). Images
702 were taken at 63x magnification using a Leica SP8 confocal microscope.

703

704 *Immunoblot of Drosophila lysates*

705 For all immunoblot experiments, *Drosophila* were raised and maintained at 25°C. Female F1
706 progeny were collected and flash-frozen 24 hours after eclosion. Heads were separated by genotype and
707 divided into 8 individuals per replicate. *Drosophila* heads were lysed and homogenized in 30uL of lysis
708 buffer (1x NuPage LDS Sample Buffer, 10% beta-mercapethanol) and boiled at 100 °C for 10 minutes.
709 Lysates were loaded on a 4-12% gradient Bis-Tri NuPage (Invitrogen) gels and run at a constant voltage
710 of 80V for an hour and then 120V for 30 minutes. For mHTT levels, a 20% methanol transfer buffer was
711 used to transfer proteins at 4°C overnight using a 200mA current. For mCD8::GFP, proteins were
712 transferred using a 10% methanol buffer for 2 hours at 4°C a 200mA current.

713 Prior to antibody treatment, all membranes were treated with blocking solution (5% non-fat milk in
714 1x TBST). For primary antibody treatment all antibodies were diluted in blocking solution. To assess
715 mHTT levels, membranes were the treated with a 1:500 mouse anti-HTT solution (mAb5490, EMD
716 Millipore) overnight. For a loading control, membranes were subsequently treated with a 1:1000 alpha-
717 tubulin antibody (Abcam EP1332Y). 1:1000 Rabbit anti-GFP (ThermoFisher A-11122) was used to
718 assess levels of mCD8::GFP, and 1:1000 anti-lamin C (Hybridoma Bank LC28.26) was used as a loading
719 control. All blots were treated with 1:5000 Goat anti-Mouse (IRDye® 800CW Goat anti-Mouse IgG) and
720 Goat anti-Rabbit (RDye® 680RD Goat anti-Rabbit IgG) secondary antibodies diluted in blocking solution
721 for 1 hour and imaged using the Odyssey CLx imager (LI-COR Biosciences).

722

723 *Knockdown of Serpina1a and homogenous time-resolved fluorescence in STHdh^{Q111/Q7} cells*

724 *STHdh^{Q111/Q7}* cells were reverse transfected with pooled small interfering RNAs (siRNAs) using
725 Lipofectamine 2000 (Life Technologies, cat. no. 11668). Cells were treated with a pool of four small
726 siRNAs per gene with the following sequences (Qiagen 1027280):

- 727 • *Htt*
- 728 1. 5'-GAAAUUAAGGUUCUGUUGA-3'
- 729 2. 5'-CCACUCACGCCAACUAUAA-3'
- 730 3. 5'-GAUGAAGGCUUUCGAGUCG-3'
- 731 4. 5'-UAACAUGGCUCAUUGUGAA-3'
- 732 • *Serpina1a*
- 733 1. 5'-GAAUAUAACUUGAAGACAC-3'
- 734 2. 5'-GGGCUGACCUCUCCGGAU-3'
- 735 3. 5'-UGGUAGAUGCCACACAUAA-3'
- 736 4. 5'-GAAAGAUAGCUGAGGCGGU-3'
- 737 • *Scramble*

738

739 Following siRNA treatment, cell lysis buffer (1X phosphate buffered saline (PBS) with 1%
740 TrintonX-100 and 1% EDTA-free protease inhibitor (Calbiochem, #539134)) was added to each well and
741 the plate was put on ice for 30 minutes. After incubation, cells were homogenized and lysates were
742 extracted. Separately, HTRF assay buffer was prepared using 50 mM NaH₂PO₄(pH7.4), 400 mM KF,
743 0.1% bovine serum albumin (BSA), 0.05% Tween-20 and Quant-ITTM Picogreen(1:1500). The donor
744 antibody, 2B7 conjugated to terbium, was diluted in HTRF assay buffer to a concentration 0.023 ug/mL
745 of and the acceptor antibody, mAb2166 (SigmaAldrich) conjugated to fluorescent dye D2, was diluted for
746 a final concentration of 1.4 µg/mL. 5 µl of the HTRF buffer was added to 5uL of cell lysates (5 µl) in
747 each well of a 384-well plate. Lysates were then incubated at 4°C overnight.

748 HTRF was performed in a PerkinElmer EnVision multilabel plate reader (model #2104), measuring
749 the 615nm and 665nm, as well as the Picogreen signal at 485nm. Each sample was measured following
750 30 cycles of the excitation at an interval of 16.6 ms.

751

752 *DEG identification in Drosophila HD models*

753 Differential expression analysis used the DESeq2 R package on a total of twelve comparisons (two
754 HD models, two cell-specific drivers, and three timepoints) (Love et al., 2014). Outlier detection was
755 performed using principle component analysis on normalized gene expression data, resulting in one
756 sample being removed. To establish a list of upregulated and downregulated DEGs in *Drosophila*, we
757 examined the false discovery rate (FDR) at every time point in both genetic models. If the FDR was less
758 than 0.05 at any data point in the HD models compared to control, we established that that gene was
759 dysregulated due to the presence of mHTT in either neurons or glia. We did not take the magnitude of
760 fold-change into account, only the direction (upregulated or downregulated) (Langfelder et al., 2016).

761

762 *Reanalysis of HD patient-derived and knock-in mouse model transcriptomes*

763 The identification of DEGs from humans was based on microarray data from brain tissue collected
764 post-mortem in patients with HD and age-matched, healthy individuals. For consistency with the reported
765 results, we examined the summary statistics of the caudate probe on the Affymetrix U133 A and B
766 microarrays. We computed the FDR by applying the Benjamini-Hochberg procedure to the p-values
767 reported in Hodges et al. 2006. A probe was said to be dysregulated if the absolute value of its Fold
768 Change was greater than 1.2 (or log₂FC >0.263) and the FDR was less than 0.05. Since multiple
769 Affymetrix probes can match to the same Entrez ID, we specified if that an Entrez-identified human gene
770 was dysregulated, if there exists a matching probe that is also dysregulated.

771 We established lists of upregulated and downregulated DEGs in mice from RNA-seq data presented

772 in Langfelder et al. (Langfelder et al., 2016), where the authors profiled mRNA of an allelic series in a
773 HD knock-in mouse model. We reanalyzed data from the striatum at 6 months, identifying gene
774 expression alterations with that were significant ($FDR < 0.05$) in the continuous-Q case, a summary
775 regression variable derived from DESeq that tests the association of the expression profile with Q-length
776 as a numeric variable (Love et al., 2014).

777

778 *Connectivity of the mHTT responding networks compared to a striatal proteome background*

779 We randomly sampled 471 proteins (equivalent to the average number of input proteins in the
780 mHTT Responding networks) 1,000 times, from 15,884 proteins that are expressed in the striatum.
781 Implementing the same parameters that were used for the mHTT responding networks, we constructed
782 clustered PPI networks with the random striatal protein lists as inputs. We calculated the average node
783 degree and average node betweenness within each network of random genes and compiled a distribution
784 using these results. A Z-score was calculated using the distribution compiled from the random striatal
785 networks. These Z-scores were then used to calculate the p-values that were reported in Supplemental
786 Table 2. All simulations and statistical calculations were performed in R.

787

788 *Analysis of behavioral screen in Drosophila*

789 We assessed behavior in *Drosophila* as the speed at which individual animals within one vial
790 moved as a function of age and genotype using a non-linear random mixed effects model regression.
791 Specifically, we looked at differences in regression between genotypes with time (additive effect,
792 represented by a shift in the curve) or the interaction of genotype and time (interactive effect, represented
793 by a change in the slope of the curve). We reported p-values representative of the pairwise post-hoc tests
794 for testing whether all possible pairs of genotype curves are different in both models. We considered
795 differences between positive controls and experimental perturbations of $p < 0.001$ to be significant. P-
796 values were adjusted for multiplicity using Holm's procedure. Code for this analysis is available upon
797 request from the Botas Laboratory. All graphing and statistical analyses were performed in R.

798

799 *Statistical analysis for western blot and homogenous time-resolved fluorescence*

800 Images of western blots were analyzed using the Image Studio Lite software. We used an
801 equivalent area to measure signal intensity across all replicates. We present proteins of interests as a ratio
802 of the target protein to loading control. Experimental replicates were compared to controls using a one-
803 sided Student's T-Test. For HTRF, levels of mHTT were calculated by taking the ratio of the fluorescence
804 signals (665nM/615nM) and normalizing to the Picogreen signal in experimental groups after subtracting
805 the signal from wells containing only sample buffer and HTRF buffer, without protein lysates. Results are

806 presented as the average and standard error of the mean of the ΔF (%) (ΔF (%) = (Sample ratio – blank
807 ratio) / blank ratio \times 100). P-values were calculated using Fisher's LSD test.

808

809 **Data and Software Availability**

810

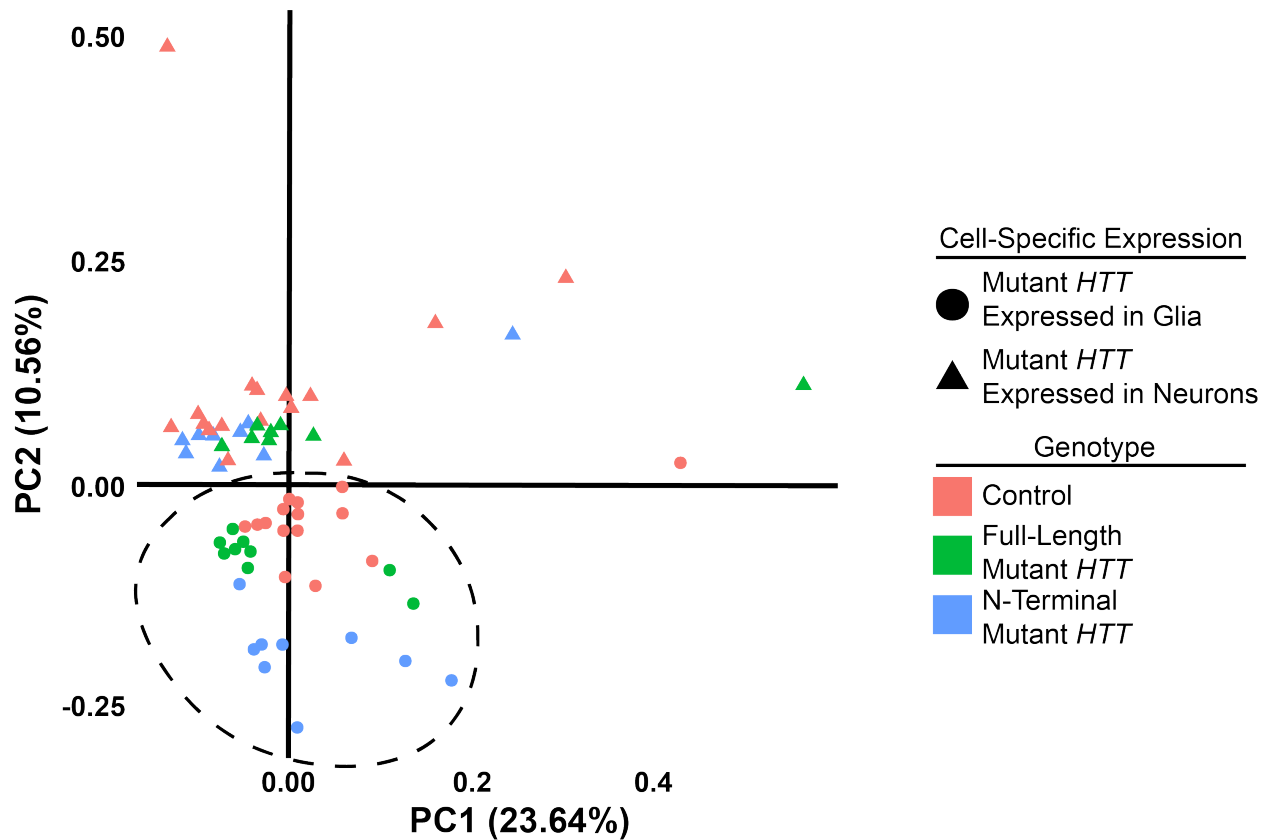
811 Raw data from *Drosophila* and mouse *STHdh*^{Q111/Q7} analysis available upon request. RNA-

812 sequencing from the HD *Drosophila* models is available in NCBI GEO under accession GSE157287.

813 Code for analysis of *Drosophila* behavioral assay in R available upon request.

814 **Supplemental Figures**

815



816

817 **Supplemental Figure 1. Expressing mHTT in *Drosophila* glia or neurons leads to distinct gene**
818 **expression profiles. In contrast, the full-length and N-terminal models show relatively similar gene**
819 **expression profiles, Related to Figure 1.**

820 Principal component analysis (PCA) plot of RNA-sequencing samples. Circles represent samples with
821 transgenic expression in glia (*repo-GAL4*), while triangles represent samples transgenic expression in
822 neurons (*elav-GAL4*). Red points represent control w^{1118} controls, green points represent animals
823 expressing the full-length protein mHTT transgene (HTT^{FLQ200}), and blue points represent animals
824 expressing the N-terminal mHTT fragment transgene ($HTT^{NT231Q128}$). Outliers skew the first component on
825 the x-axis (23.64% of the variability). Samples expressing mHTT in glia separate from those expressing it
826 in neurons along the second component on the y-axis (10.56% of the variability; dashed circle).

827

A

Whole proteome background

	mHTT expressed in neurons		mHTT expressed in glia	
	Up	Down	Up	Down
Input proteins	521	452	424	536
Edges	889	993	589	1410
PPI enrichment	<2E-16	<2e-16	1.63E-12	<2E-16

B

Striatal background

	mHTT expressed in neurons		mHTT expressed in glia	
	Up	Down	Up	Down
Average degree enrichment in striatum	1.01E-06	9.68E-08	1.17E-03	3.37E-15
Average betweenness enrichment in striatum	3.44E-06	3.63E-03	3.23E-03	5.11E-04
Number of clusters enrichment in striatum	3.09E-06	3.09E-06	1.23E-02	4.31E-03

828

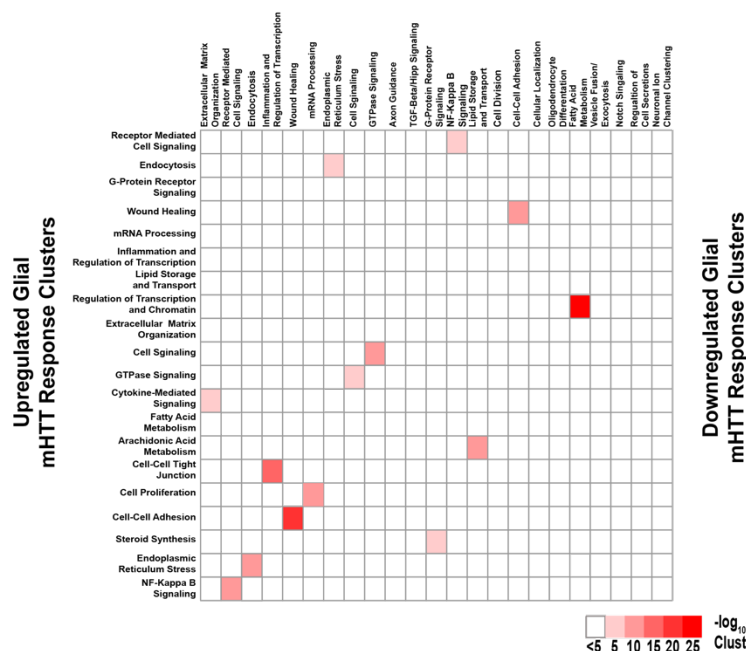
829 **Supplemental Table 2. Network connectivity of DEGs responding to glial or neuronal mHTT**

830 **expression, Related to Figure 2.**

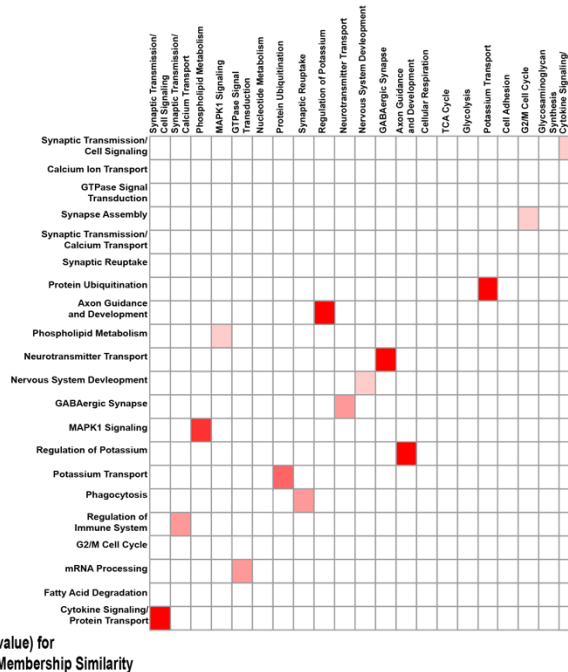
831 Protein-protein interaction (PPI) networks (STRING-db) built from HD DEGs are significantly more
 832 connected (Probability Distribution Test) than networks of equivalent size built from proteins selected
 833 randomly out of a whole proteome (A) or striatal (B) background. HD DEGs are concordantly
 834 upregulated or downregulated in HD human striatal tissue, knock-in HD mouse striatal tissue, and tissue
 835 taken from *Drosophila* expressing mHTT in neurons or glia.

836

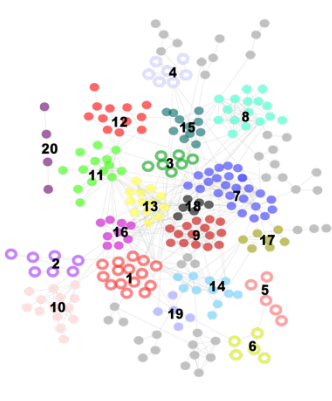
A 837 **Upregulated Neuronal mHTT Response Clusters**



Downregulated Neuronal mHTT Response Clusters



B



Clusters Upregulated in Response to mHTT in Glia Only

- | | |
|--|--------------------------------------|
| 1 Transcription and Chromatin Remodeling | 4 Cytokine Signaling/Innate Immunity |
| 2 Amino Acid Metabolism | 5 Arachidonic Acid Metabolism |
| 3 Cell Proliferation | 6 Steroid Synthesis |

Clusters Upregulated in Response to mHTT in Neurons or Glia

- | | |
|--|--|
| 7 Receptor Mediated Cell Signaling | 14 Lipid Transport and Storage |
| 8 Endocytosis | 15 GTPase Signaling/Actin Cytoskeleton |
| 9 G-Protein Coupled Receptor Signaling | 16 Cell Signaling |
| 10 mRNA Processing | 17 Fatty Acid Metabolism |
| 11 Wound Healing and Inflammation | 18 Adhesion |
| 12 Extracellular Matrix Organization and Signaling | 19 Endoplasmic Reticulum Stress |
| 13 Inflammation/Regulation of Transcription | 20 NF-Kappa B Signaling |

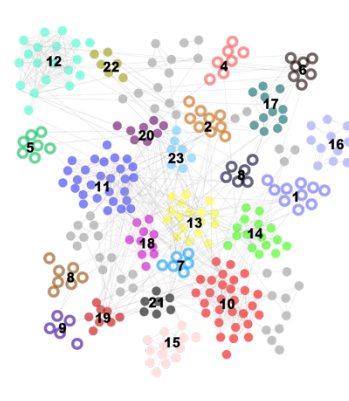
Clusters Downregulated in Response to mHTT in Glia Only

- | | |
|--|--------------------------|
| 1 Synapse Assembly | 4 Phagocytosis |
| 2 Calcium Ion Transport | 5 mRNA Processing |
| 3 Regulation of Immune System and Metabolism | 6 Fatty Acid Degradation |

Clusters Downregulated in Response to mHTT in Neurons or Glia

- | | |
|---|---|
| 7 Synaptic Transmission/Cell Signaling | 15 Synaptic Reuptake |
| 8 GTPase Signal Transduction | 16 Axon Guidance and Development |
| 9 Synaptic Transmission/Calcium Transport | 17 GABAergic Synapse |
| 10 Phospholipid Metabolism | 18 Potassium Transport |
| 11 Protein Ubiquitination | 19 Regulation of Potassium |
| 12 MAPK1 Signaling | 20 Cytokine Singaling and Protein Transport |
| 13 Nervous System Development | 21 G2/M Cell Cycle Transition |
| 14 Neurotransmitter Transport | |

C



Clusters Upregulated in Response to mHTT in Neurons Only

- | | |
|---|-----------------------------------|
| 1 TGF-Beta and Hippo Signaling Pathways | 6 Vesicle Fusion and Exocytosis |
| 2 Cell Signaling/Axon Guidance | 7 Notch Signaling |
| 3 Cellular Localization | 8 Regulation of Secretions |
| 4 Cell Division | 9 Neuronal Ion Channel Clustering |
| 5 Oligodendrocyte Differentiation | |

Clusters Upregulated in Response to mHTT in Neurons or Glia

- | | |
|--|---|
| 10 Extracellular Matrix Organization and Signaling | 17 GTPase Signaling/Actin Cytoskeleton |
| 11 Receptor Mediated Cell Signaling | 18 Cell Signaling |
| 12 Endocytosis | 19 G-Protein Coupled Receptor Signaling |
| 13 Inflammation/Regulation of Transcription | 20 NF-Kappa B Signaling |
| 14 Wound Healing and Inflammation | 21 Cell-Cell Adhesion |
| 15 mRNA Processing | 22 Fatty Acid Metabolism |
| 16 Endoplasmic Reticulum Stress | 23 Lipid Transport and Storage |

Clusters Downregulated in Response to mHTT in Neurons Only

- | | |
|-------------------------|-------------------------------|
| 1 Nucleotide Metabolism | 4 Tricarboxylic Acid Cycle |
| 2 Glycolysis | 5 Cell Adhesion |
| 3 Cellular Respiration | 6 Glycosaminoglycan Synthesis |

Clusters Downregulated in Response to mHTT in Neurons or Glia

- | | |
|---|---|
| 7 Synaptic Transmission/Cell Signaling | 15 GABAergic Synapse |
| 8 Synaptic Transmission/Calcium Transport | 16 Synaptic Reuptake |
| 9 MAPK1 Signaling | 17 Potassium Transport |
| 10 Phospholipid Metabolism | 18 Axon Guidance and Development |
| 11 GTPase Signal Transduction | 19 Neurotransmitter Transport |
| 12 Protein Ubiquitination | 20 G2/M Cell Cycle Transition |
| 13 Regulation of Potassium | 21 Cytokine Singaling and Protein Transport |
| 14 Nervous System Development | |

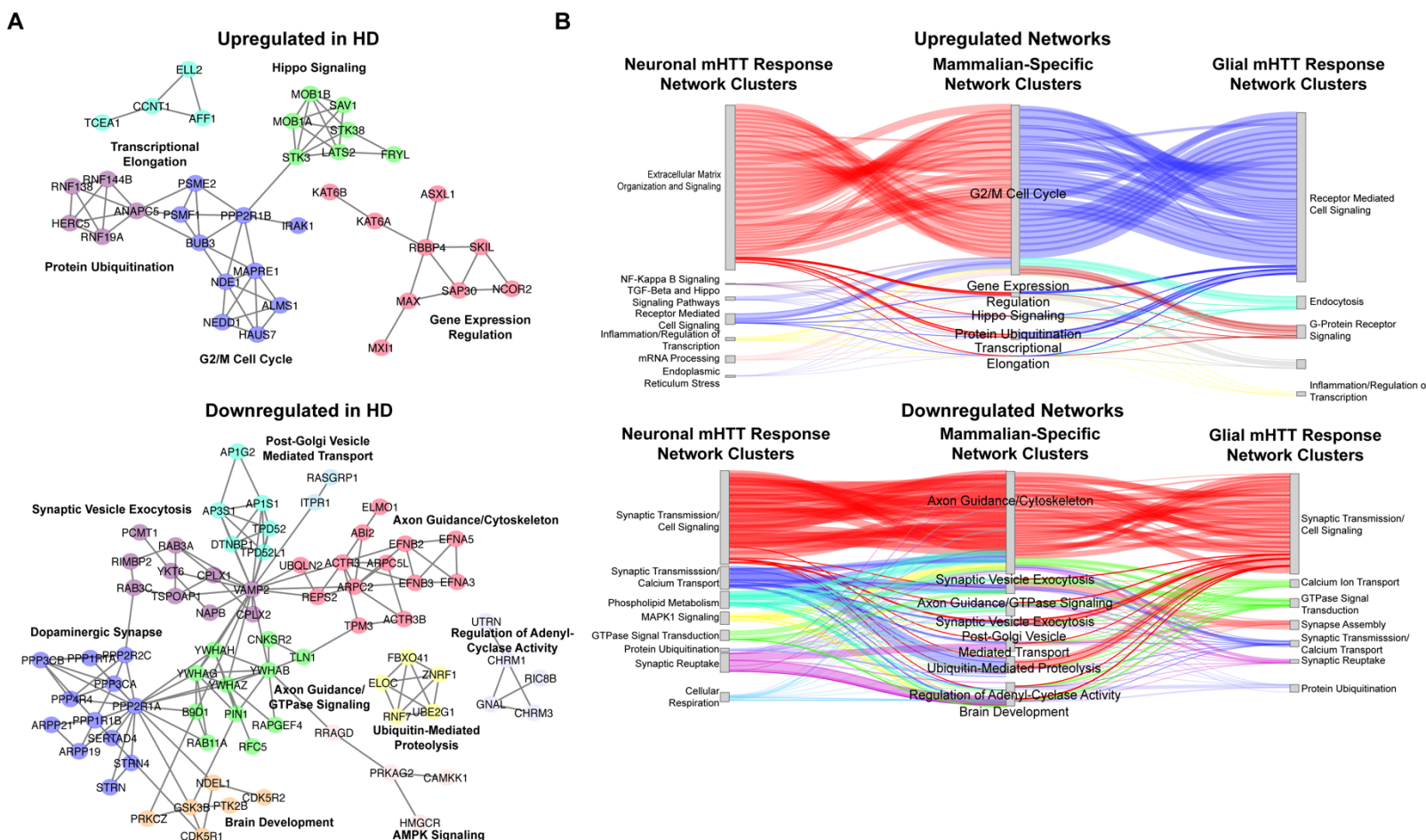
838 **Supplemental Figure 2. Network of DEGs responding concordantly to *mHTT* expression in glia or**
839 **neurons, Related to Figure 2.**

840 A) Heatmap representing all pairwise comparisons between clusters of upregulated (left) and
841 downregulated (right) DEGs in response to glial (x-axis) or neuronal (y-axis) *mHTT* expression. Clusters
842 are labeled using annotations for biological processes represented by DEGs within each cluster (Refer to
843 **Tables S3**). Color is scaled to represent the significance of membership similarity calculated using a
844 hypergeometric distribution. Colors represent the $-\log_{10}(\text{p-value})$ and range from white ($5 <$) to dark red
845 (>25).

846 B) PPI Network (STRING-db) of upregulated (top) and upregulated DEGs in human HD tissue, HD
847 mouse models, and *Drosophila* expressing *mHTT* in glia.

848 C) PPI Network (STRING-db) of upregulated (top) and upregulated DEGs in human HD tissue, HD
849 mouse models, and *Drosophila* expressing *mHTT* in neurons.

850 Refer to **Supplemental Table 3** for gene membership, as well as GO Panther and KEGG terms enriched
851 genes within each cluster. Numbers in (B) and (C) correspond to annotations. Hollow circles correspond
852 to clusters of DEGs that are dysregulated in response to *mHTT* (*HTT^{FLQ200}* or *HTT^{NT231Q128}*) expression in
853 only glia (*repo-GAL4*) or neurons (*elav-GAL4*). Solid circles correspond to clusters of DEGs that are
854 dysregulated in response to *mHTT* expression in neurons or glia.

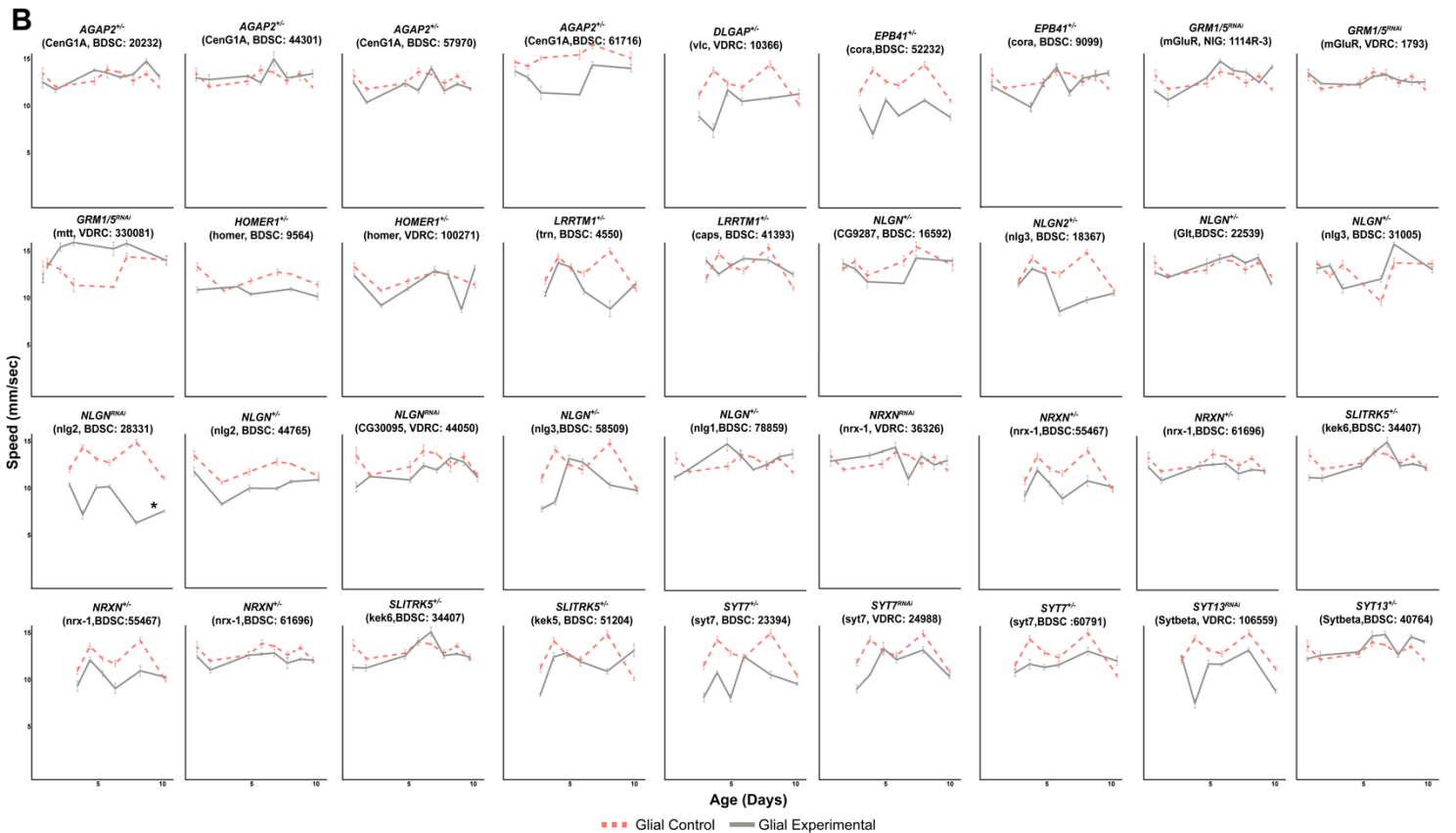
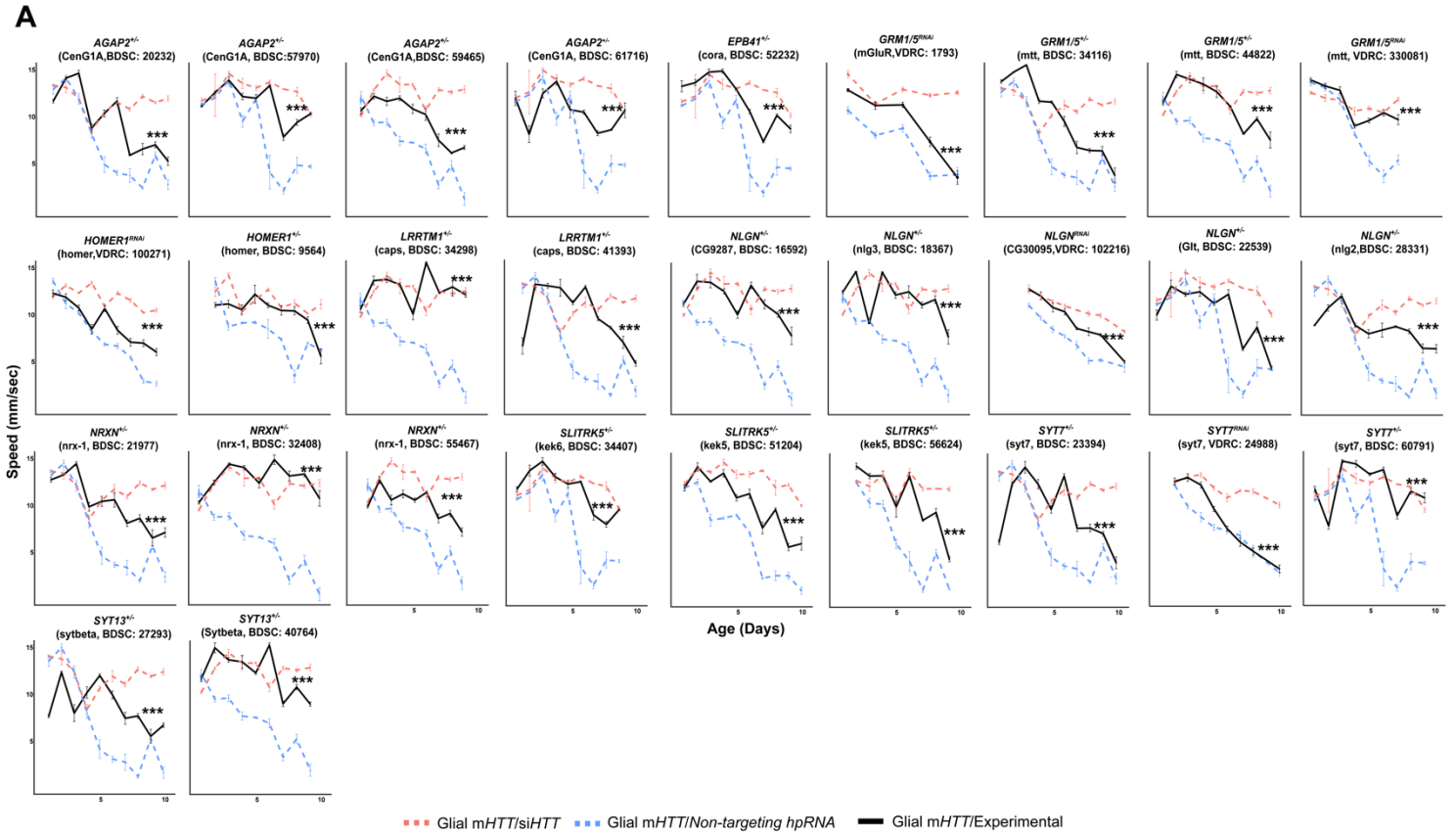


855
 856 **Supplemental Figure 3. Network of DEGs concordantly altered in human HD tissue and HD mouse**
 857 **models, but not in *Drosophila* HD models, Related to Figure 2.**

858 A) Clustered, annotated PPI network (STRING-db) of DEGs concordantly upregulated (top) and
 859 downregulated (down) in human HD striatal tissue collected post-mortem (Hodges et al. 2006) and the
 860 allelic series of knock-in HD mouse models (Langfelder et al. 2016), but not in the *Drosophila* models
 861 (this study). Clusters are annotated for the synthesis of the top five most significantly enriched GO
 862 Panther Biological Process and KEGG terms (FDR<0.05).

863 B) Sankey plot for genes concordantly upregulated (top) and upregulated (bottom) in HD patients and
 864 mouse model only (middle) connecting to cluster of DEGs that are concordantly upregulated in patients,
 865 mice, and *Drosophila* as a consequence of expressing *mHTT* (HTT^{FLQ200} or $HTT^{NT231Q128}$) in neurons (*elav*)
 866 (left) or in glia (*repo*) (right). Edges represent protein-protein interactions between connected DEGs
 867 (STRING-db).

868
 869



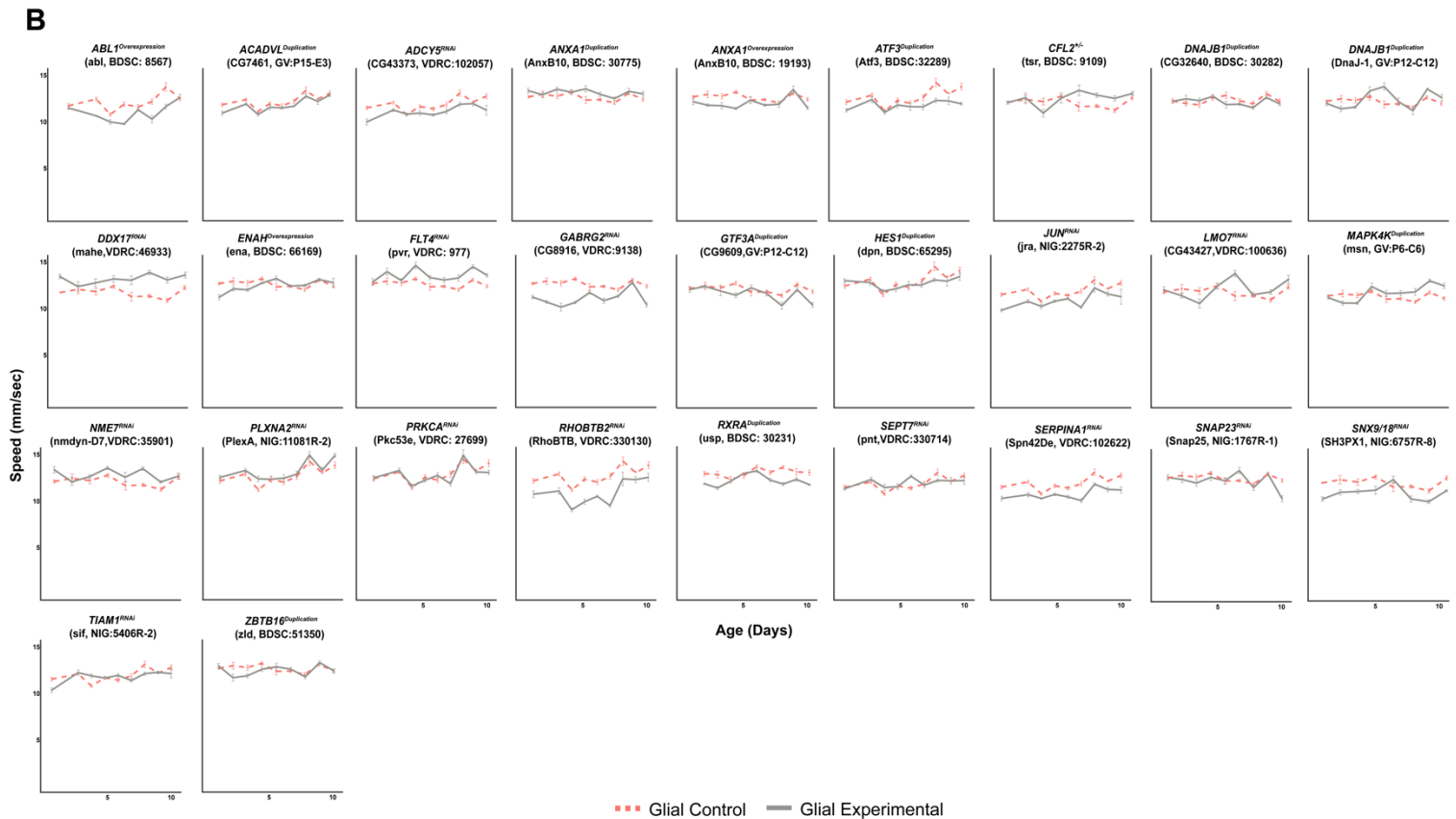
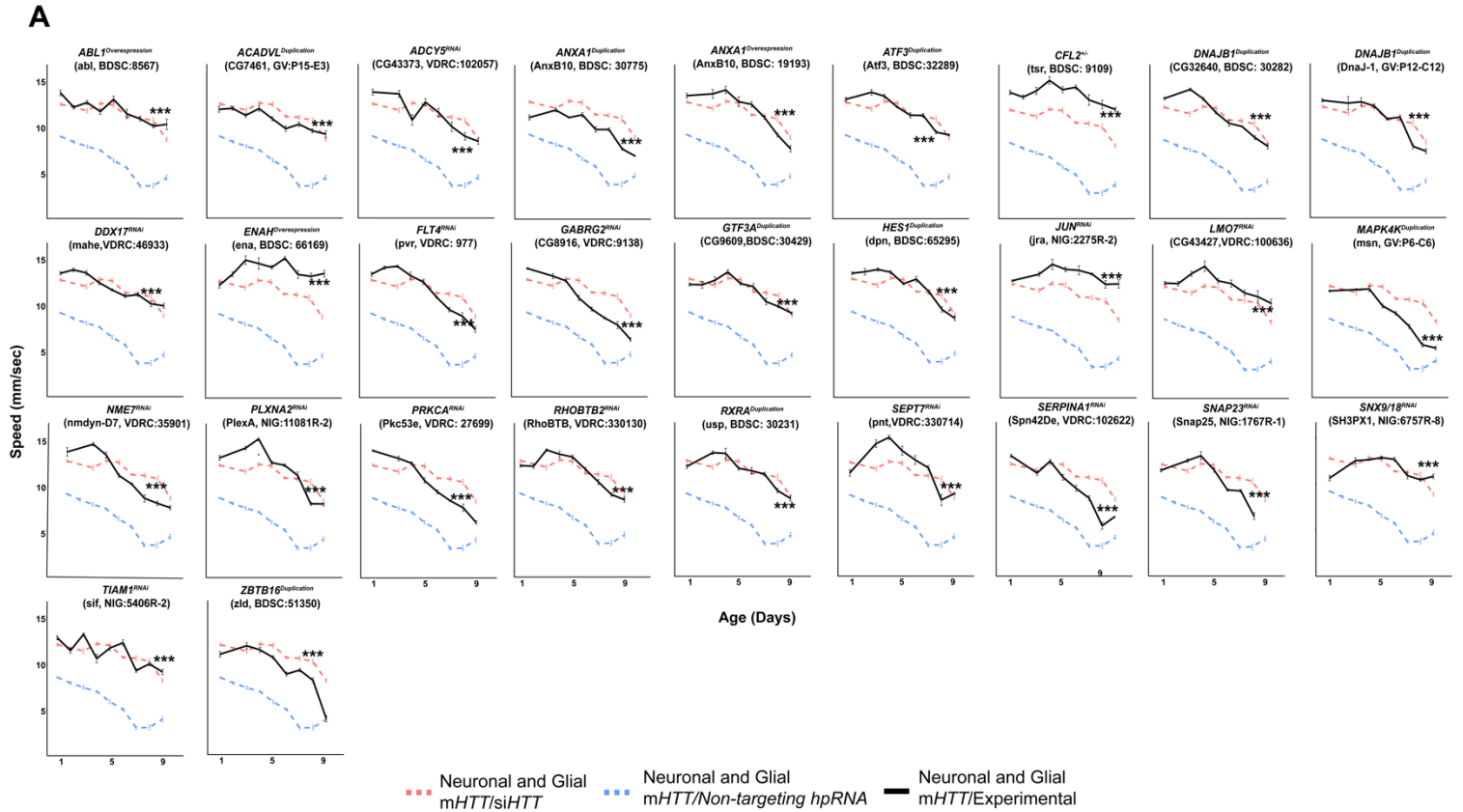
871 **Supplemental Figure 4. Suppressors of glial mHTT-induced behavioral impairments among DEGs**
872 **in the Synapse Assembly cluster, Related to Figure 3.**

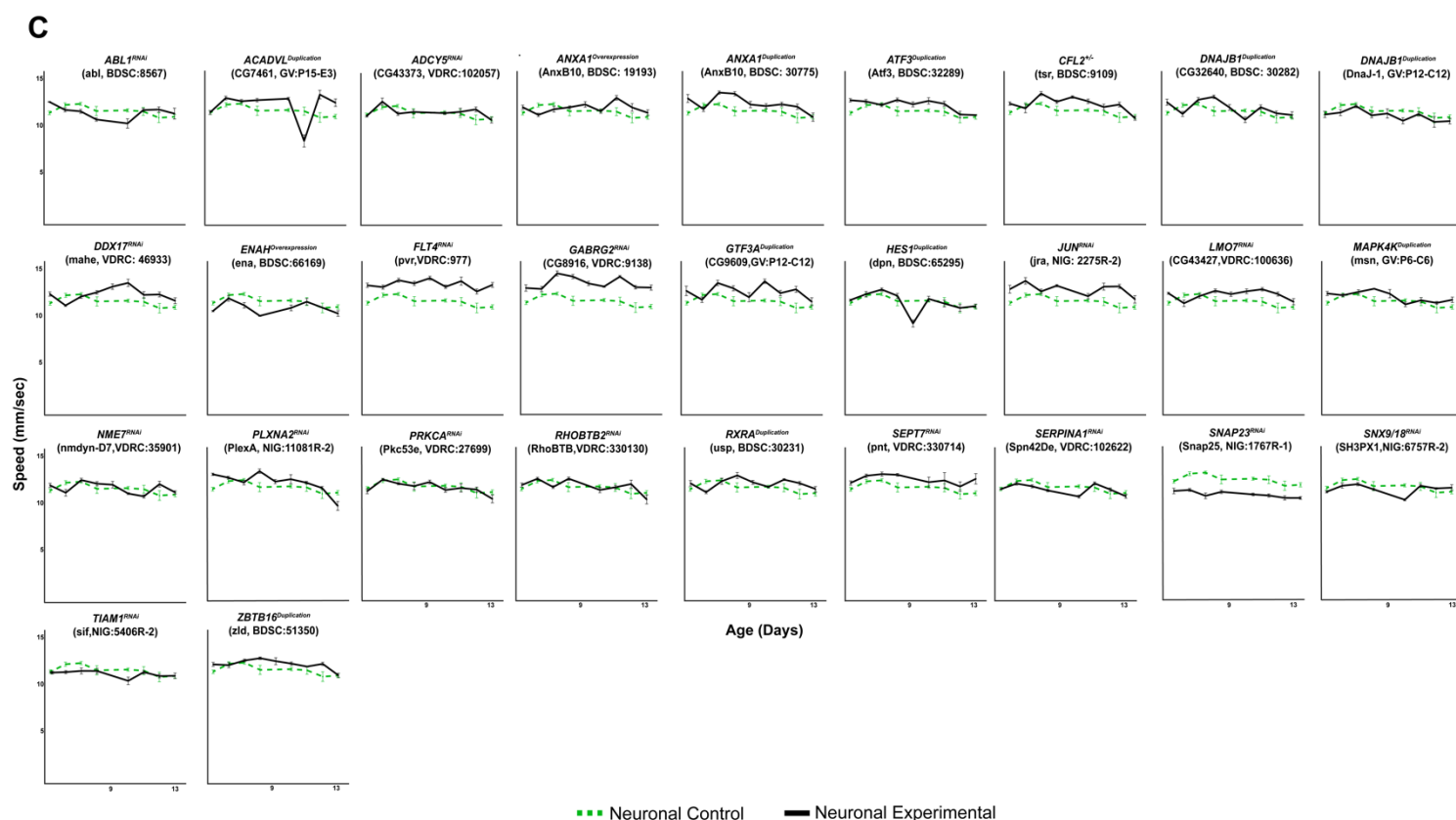
873 A) Representative graphs of behavioral assays (climbing speed as a function of time) of *Drosophila*
874 expressing mHTT in glia (*repo>HTT^{NT231Q128}*) and alleles knocking down genes in the synapse assembly
875 cluster. *** $p < 0.001$ between the positive control (dashed blue line) and experimental allele (solid black
876 line) by linear mixed effects model and post-hoc, pairwise analysis (see Methods). Refer to
877 **Supplemental Table 4** for a summary of the full statistical analysis.

878 B) Representative graphs of the climbing speed of wildtype *Drosophila* as a function of time with the
879 glial driver (*repo-GAL4*) expressing alleles that suppress glial mHTT-induced behavioral impairments.
880 Points and error bars on the plot represent the mean speed \pm SEM of three technical replicates. Each
881 genotype was tested with 4-6 replicates of 10 animals. The gray line represents climbing speed of animals
882 expressing the modifier allele in the *repo-GAL4* background.

883 *Drosophila* Genotypes: Positive control (*w¹¹¹⁸/+; UAS- non-targeting hpRNA/+; repo-GAL4, UAS-*
884 *HTT^{NT231Q128}/+*), treatment control (*w¹¹¹⁸; repo-GAL4, UAS- HTT^{NT231Q128}/UAS-siHTT*), experimental
885 (*w¹¹¹⁸; repo-GAL4, UAS- HTT^{NT231Q128}/modifier*), negative control (*w¹¹¹⁸; UAS- non-targeting hpRNA/+;*
886 *repo-GAL4/+*), and experimental control (*w¹¹¹⁸; repo-GAL4/modifier*).

887





889 **Supplemental Figure 5. Genetic modifiers suppress behavioral impairments caused by *mHTT***

890 **expression in neurons and glia, Related to Figure 5.**

891 A) Representative graphs of the climbing speed of animals expressing mutant *HTT* in neurons and glia in
 892 combination with common modifiers as a function of time. *** $p < 0.001$ between the positive control
 893 (dashed blue line) and experimental allele (solid black line) by linear mixed effects model and post-hoc,
 894 pairwise analysis. Refer to **Supplemental Table 6** for full statistical analysis of each genotype in neurons,
 895 glia, and both.

896 B) Representative graphs of the speed of wildtype *Drosophila* with the glial driver (*repo-GAL4*) and
 897 alleles that suppressed mutant *HTT*-induced behavioral impairments in both neurons and glia. The dashed
 898 red line represents the longitudinal climbing speed of animals expressing a non-targeting hpRNA in glia,
 899 while the gray line represents climbing speed of animals expressing the common modifier allele in the
 900 *repo-GAL4* background.

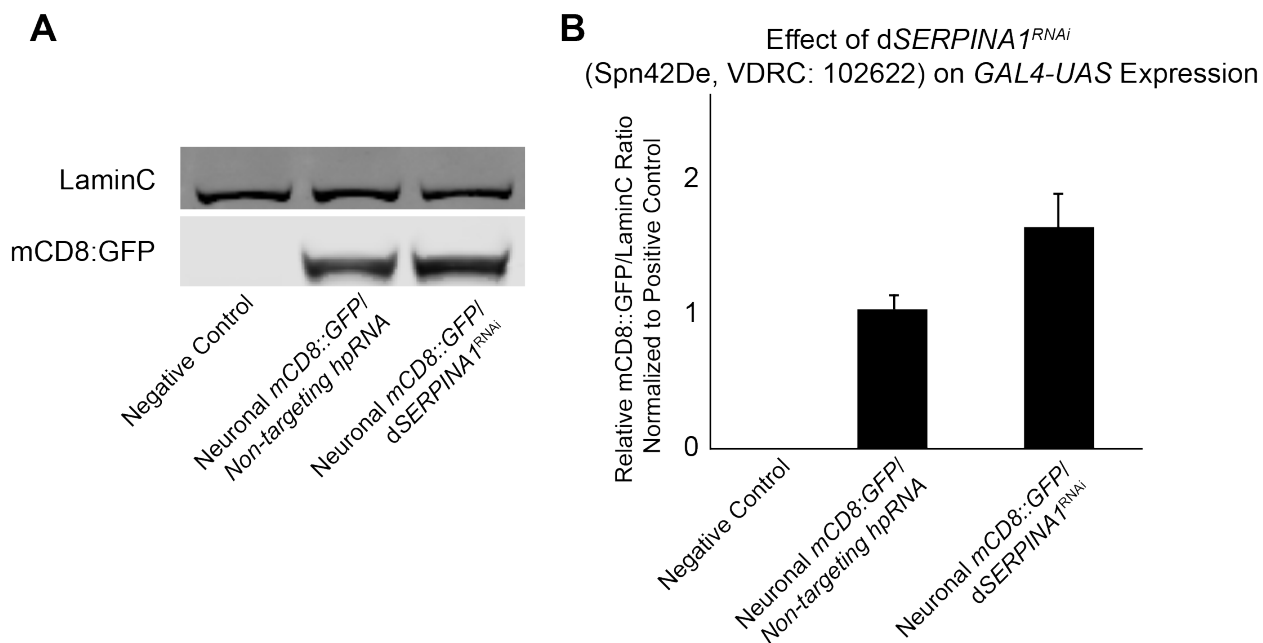
901 C) Representative graphs of the speed of wildtype *Drosophila* with the neuronal driver (*elav-GAL4*)
 902 expressing the alleles that suppressed mutant *HTT*-induced behavioral impairments in both neurons and
 903 glia as a function of time. The dashed green line represents the climbing speed of animals expressing a
 904 non-targeting hpRNA in neurons, while the black line represents climbing speed of animals expressing
 905 the common modifier allele in the *elav-GAL4* background.

906 **Supplemental Figure 5 continued.**

907 Points and error bars on the plot represent the mean speed \pm SEM of three replicates. Each genotype was
908 tested with 4-6 replicates of 10 animals.

909 *Drosophila* Genotypes: Neuronal and glial mHTT positive control (*elav^{c155}-GAL4/w¹¹¹⁸;UAS-non-*
910 *targeting hpRNA/+; repo-GAL4, UAS- HTT^{NT231Q128}/+*), neuronal and glial mHTT treatment control
911 (*elav^{c155}-GAL4/w¹¹¹⁸; repo-GAL4, UAS- HTT^{NT231Q128}/UAS-siHTT*), neuronal and glial experimental
912 (*elav^{c155}-GAL4/w¹¹¹⁸; repo-GAL4, UAS- HTT^{NT231Q128}/modifier*), glial control (*elav^{c155}-GAL4/w¹¹¹⁸; UAS-*
913 *non-targeting hpRNA/+*), glial experimental control (*elav^{c155}-GAL4/w¹¹¹⁸; modifier/+*), neuronal control
914 (*w¹¹¹⁸; UAS-non-targeting hpRNA/+; repo-GAL4/+*), and neuronal experimental control (*w¹¹¹⁸; repo-*
915 *GAL4/modifier*).

916



917

918 **Supplemental Figure 6. *dSERPINA1* knockdown does not reduce the expression of the *GAL4-UAS***
919 **system, Related to Figure 6.**

920 A) Representative immunoblot performed on *Drosophila* protein lysates assessing mCD8::GFP protein
921 levels as a proxy for *GAL4-UAS* expression. *mCD8::GFP* was expressed in neurons using the *elav-GAL4*
922 driver. The negative control in this experiment was *elav>GAL4* driving the expression of a non-targeting
923 hpRNA (left lane). The positive control was *elav>GAL4* expressing the *mCD8::GFP* construct and a non-
924 targeting hpRNA (middle lane). The experimental was *elav>GAL4* driving the expression of the
925 *mCD8::GFP* construct and the *dSERPINA1^{RNAi}* (Spn42De, VDRC: 102622) allele that reduced mutant
926 protein levels in **Figure 6** (right lane). LaminC was used as a loading control in this immunoblot.

927 B) Quantification of the three replicates from the immunoblot in (A) represented as the ratio of
928 mCD8::GFP to LaminC, normalized to the average of the positive control.

929

930 **List of Supplemental Tables**

931

932 The following data sets (with the exception of Table S2 which can be found on page 33) are available at
933 http://bit.ly/Onur_et_al_supplement.

934

935 Table S1. Mappings of orthologous HD human, mouse, and *Drosophila* DEGs, Related to Figure 1.

936 Table S2. Network connectivity of DEGs responding to glial or neuronal *mHTT* expression, Related to
937 Figure 2.

938 Table S3. Cluster membership and summary of cluster annotations for DEGs responding to glial or
939 neuronal *mHTT* expression, Related to Figure 2.

940 Table S4. Summary of statistical analysis for alleles in the Synapse Assembly cluster that modify glial
941 *mHTT*-induced behavioral impairments, Related to Figure 3.

942 Table S5. Summary of alleles screened and results for common modifiers of *mHTT*-induced behavioral
943 impairments in neurons and glia, Related to Figure 5.

944 Table S6. Summary of statistics for alleles are common suppressors of neuronal and glial *mHTT*-induced
945 behavioral impairments, Related to Figure 5.

946 **References**

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