Downregulation of glial genes involved in synaptic function mitigates Huntington’s disease pathogenesis

Tarik Seref Onur\(^{1,2,3\dagger}\), Andrew Laitman\(^{2,4,5\dagger}\), He Zhao\(^2\), Ryan Keyho\(^2\), Hyemin Kim\(^2\), Jennifer Wang\(^2\), Megan Mair\(^{1,2,3}\), Huilan Wang\(^6\), Lifang Li\(^{1,2}\), Alma Perez\(^2\), Maria de Haro\(^{1,2}\), Ying-Wooi Wan\(^2\), Genevera Allen\(^{2,7}\), Boxun Lu\(^6\), Ismael Al-Ramahi\(^{1,2}\), Zhandong Liu\(^{2,4,5}\), Juan Botas\(^{1,2,3,4\ast}\)

\(^{1}\)Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, United States; \(^{2}\)Jan and Dan Duncan Neurological Research Institute at Texas Children’s Hospital, Houston, United States; \(^{3}\)Genetics & Genomics Graduate Program, Baylor College of Medicine, Houston, United States; \(^{4}\)Quantitative & Computational Biosciences, Baylor College of Medicine, Houston, United States; \(^{5}\)Department of Pediatrics, Baylor College of Medicine, Houston, United States; \(^{6}\)State Key Laboratory of Medical Neurobiology and MOE Frontiers Center for Brain Science, Fudan University, Shanghai, China; \(^{7}\)Departments of Electrical & Computer Engineering, Statistics and Computer Science, Rice University, Houston, United States

Abstract

Most research on neurodegenerative diseases has focused on neurons, yet glia help form and maintain the synapses whose loss is so prominent in these conditions. To investigate the contributions of glia to Huntington’s disease (HD), we profiled the gene expression alterations of *Drosophila* expressing human mutant *Huntingtin* (mHTT) in either glia or neurons and compared these changes to what is observed in HD human and HD mice striata. A large portion of conserved genes are concordantly dysregulated across the three species; we tested these genes in a high-throughput behavioral assay and found that downregulation of genes involved in synapse assembly mitigated pathogenesis and behavioral deficits. To our surprise, reducing dNRXN3 function in glia was sufficient to improve the phenotype of flies expressing mHTT in neurons, suggesting that mHTT’s toxic effects in glia ramify throughout the brain. This supports a model in which dampening synaptic function is protective because it attenuates the excitotoxicity that characterizes HD.

Introduction

Neurodegenerative conditions involve a complex cascade of events that takes many years to unfold. Even in the case of inherited disorders due to mutation in a single gene, such as Huntington’s disease (HD), the downstream ramifications at the molecular level are astonishingly broad. Caused by a CAG repeat expansion in *Huntingtin* (HTT) (The Huntington’s Disease Collaborative Research Group, 1993), HD pathology is prominent in the striatum and cortex, yet transcriptomic studies consistently reveal thousands of changes in gene expression across the brain and different neuronal cell types, involving pathways ranging from autophagy to vesicular trafficking (Saudou and Humbert, 2016). To disentangle changes that are pathogenic from those that represent the brain’s effort to compensate for the disease, we recently integrated transcriptomics with in silico analysis and high-throughput in vivo screening using a *Drosophila* model of HD (Al-Ramahi et al., 2018). This study demonstrated that HD pathogenesis is driven by upregulation of genes involved in the actin...
eLife digest When a neuron dies, through injury or disease, the body loses all communication that passes through it. The brain compensates by rerouting the flow of information through other neurons in the network. Eventually, if the loss of neurons becomes too great, compensation becomes impossible. This process happens in Alzheimer’s, Parkinson’s, and Huntington’s disease. In the case of Huntington’s disease, the cause is mutation to a single gene known as huntingtin. The mutation is present in every cell in the body but causes particular damage to parts of the brain involved in mood, thinking and movement.

Neurons and other cells respond to mutations in the huntingtin gene by turning the activities of other genes up or down, but it is not clear whether all of these changes contribute to the damage seen in Huntington’s disease. In fact, it is possible that some of the changes are a result of the brain trying to protect itself. So far, most research on this subject has focused on neurons because the huntingtin gene plays a role in maintaining healthy neuronal connections. But, given that all cells carry the mutated gene, it is likely that other cells are also involved. The glia are a diverse group of cells that support the brain, providing care and sustenance to neurons. These cells have a known role in maintaining the connections between neurons and may also have a role in either causing or correcting the damage seen in Huntington’s disease.

The aim of Onur et al. was to find out which genes are affected by having a mutant huntingtin gene in neurons or glia, and whether severity of Huntington’s disease improved or worsened when the activity of these genes changed. First, Onur et al. identified genes affected by mutant huntingtin by comparing healthy human brains to the brains of people with Huntington’s disease. Repeating the same comparison in mice and fruit flies identified genes affected in the same way across all three species, revealing that, in Huntington’s disease, the brain dials down glial cell genes involved in maintaining neuronal connections.

To find out how these changes in gene activity affect disease severity and progression, Onur et al. manipulated the activity of each of the genes they had identified in fruit flies that carried mutant versions of huntingtin either in neurons, in glial cells or in both cell types. They then filmed the flies to see the effects of the manipulation on movement behaviors, which are affected by Huntington’s disease. This revealed that purposely lowering the activity of the glial genes involved in maintaining connections between neurons improved the symptoms of the disease, but only in flies who had mutant huntingtin in their glial cells. This indicates that the drop in activity of these genes observed in Huntington’s disease is the brain trying to protect itself.

This work suggests that it is important to include glial cells in studies of neurological disorders. It also highlights the fact that changes in gene expression as a result of a disease are not always bad. Many alterations are compensatory, and try to either make up for or protect cells affected by the disease. Therefore, it may be important to consider whether drugs designed to treat a condition by changing levels of gene activity might undo some of the body’s natural protection. Working out which changes drive disease and which changes are protective will be essential for designing effective treatments.
from HD patients and mouse models of HD (Al-Dalahmah et al., 2020; Diaz-Castro et al., 2019) developed molecular profiles that distinguish HD-affected astrocytes from astrocytes found in healthy brain tissue, but the physiological consequences of the gene expression changes were unclear. Whether mHTT affects glial participation in synapse formation or maintenance remains unknown, but then, we are only just now beginning to understand the range of glial types and their functions (Bayraktar et al., 2020; Darmanis et al., 2015).

The combination of synaptic degeneration in HD and the fact that both HTT and glia contribute to synaptic formation and maintenance led us to further investigate the influence of mHTT in glia. Because Drosophila have been used to elucidate glial biology (Freeman and Doherty, 2006; Olsen and Feany, 2019; Pearce et al., 2015; Ziegenfuss et al., 2012) and are a tractable model system for studying HD and other neurodegenerative diseases (Al-Ramahi et al., 2018; Bondar et al., 2018; Donnelly et al., 2020; Fernandez-Funez et al., 2000; Filimonenko et al., 2010; Goodman et al., 2019; Ochaba et al., 2014; Olsen and Feany, 2019; O’Rourke et al., 2013; Rousseaux et al., 2018; Yuva-Aydemir et al., 2018), we decided to generate flies that express mHTT solely in glia so that we could compare their transcriptomic signature with that of flies expressing mHTT in neurons. We took an unbiased approach, first establishing the repertoire of evolutionarily conserved genes that show discordant expression changes across HD human and mouse striata and HD fly brains. We then integrated this comparative transcriptomic data with high-throughput in vivo behavioral screening to acquire insight into glial contributions to HD pathogenesis and identify disease-modifying targets that mitigate the HD phenotype.

**Results**

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

To study the contributions of neurons and glia to HD pathogenesis, we first needed to define a transcriptomic signature that would enable us to move across species (human, mouse, and fly) (Figure 1A). We began with human tissue. Since the striatum is the brain region most prominently affected in HD, we compared the gene expression profiles of human post-mortem striatal samples from healthy individuals and patients with HD, from different stages of the disease (i.e., Vonsattel Grade 0–4) (Hodges et al., 2006; Vonsattel et al., 1985). We identified 1852 downregulated and 1941 upregulated differentially expressed genes (DEGs) in patients with HD compared to healthy individuals (Figure 1B). (The greater genome coverage provided by RNA-seq [Miller et al., 2014] yielded larger datasets for mouse and, below, for Drosophila than for humans.)

We then reanalyzed published RNA-seq data from mouse striata using an allelic series of knock-in mouse models with varying CAG repeat lengths at 6 months of age (Langfelder et al., 2016). Because it is unclear which CAG tract length in mice most faithfully recapitulates HD pathogenesis, the triplet repeat length was treated as a continuous trait, and we narrowed our analysis to DEGs that correlate with increasing CAG repeat length. Comparing the striata of wildtype mice to the knock-in HD mouse models, there were 3575 downregulated and 3634 upregulated DEGs (Figure 1B). (The HD transcriptome is conserved among evolutionarily distant model systems)

We performed RNA-seq leveraging Drosophila HD models (Kaltenbach et al., 2007; Romero et al., 2008) (see Materials and methods) to compare the effect of expressing mHTT in either neurons or glia. The binary GAL4-UAS system was used to drive the expression of human mHTT either in neurons (elav >GAL4) or glia (repo >GAL4). Both full-length (HTT^FLO200) and N-terminal (HTT^NT231Q128) models were used in this set of experiments since both the full protein and N-terminal HD fragments accumulate in the human brain as a result of proteolysis and mis-splicing (Kim et al., 2001; Neude et al., 2017; Sathasivam et al., 2013; Wellington et al., 2002). Principal component analysis (PCA) showed that the greatest differences between samples are attributable to the cell-specific drivers, and not to the use of N-terminal versus full-length protein (Figure 1—figure supplement 1). Expressing mHTT in neurons resulted in 3058 downregulated and 2979 upregulated DEGs, while expressing mHTT in glia resulted in 3127 downregulated and 3159 upregulated DEGs. There were also DEGs common to both neurons and glia expressing mHTT: 1293 downregulated and 1181 upregulated (Figure 1B).
With these transcriptomic signatures in hand, we were able to compare gene expression profiles across the three species. We focused on genes with significantly altered expression (using a false discovery rate [FDR] < 0.05; see Materials and methods) in the same direction (i.e., upregulated or downregulated) in response to mHTT expression across these three species, including both Drosophila HD models. We call genes that meet this criterion concordantly altered DEGs (Supplementary file 1).

We compared DEGs using a graph-based approach (see Materials and methods) that allows for evolutionary divergence and convergence, instead of imposing one-to-one relationships. 815 upregulated DEGs observed in HD patient-derived striatal tissue had an orthologous gene in the HD mouse model and at least one Drosophila model of HD that was concordantly upregulated. Similarly, 791 DEGs identified in HD patients had an orthologous gene in mouse and Drosophila models that was concordantly downregulated (Figure 1C). About 40% of the alterations in gene expression in patient striatal samples are concordant with orthologous genes in both Drosophila and mice models of HD. To determine whether this result could be an artifact of overlapping a large number of DEGs in each model, we randomly selected and overlapped 815 and 791 orthologous genes across the three species 20,000 times. Based on the resulting distribution, we concluded that the overlap of concordant, orthologous DEGs across the various HD models was not random (p=6.37×10^{-158} and p=1.66×10^{-165}, probability distribution test).
To compare the consequence of expressing mHTT in glia versus neurons, we recalculated the overlaps between the three species, distinguishing DEGs from the neuron-only and glia-only HTT-expressing Drosophila. There were 425 concordantly upregulated and 545 concordantly downregulated DEGs in glia. We also found 522 upregulated DEGs and 453 downregulated specific to neurons. Out of these groups of DEGs, 310 were upregulated and 320 were downregulated in both neurons and glia. To acknowledge the proportion of transcriptional alterations we excluded by specifying concordant expression with the HD Drosophila models, we also calculated the overlap between concordant DEGs observed only in striata from HD patients and mice. We found that 83.7% of upregulated DEGs and 77.7% of downregulated DEGs that were altered concordantly in human and mouse HD striata were also concordantly altered in the brains of the neuronal and/or glial HD Drosophila models (Figure 1D). Of the genes that showed concordantly altered expression only in human and mouse striata, 64 (40%) of the upregulated and 68 (30%) of the downregulated DEGs did not have an ortholog in Drosophila.

Network analysis identifies biological processes disrupted by mHTT toxicity in glia

To investigate the cellular pathophysiology represented by DEGs in neurons and glia, we constructed protein-protein interaction (PPI) networks using the STRING-db database (Szklarczyk et al., 2015). The upregulated and downregulated networks of DEGs responding to mHTT expression in neurons or glia had a significant PPI enrichment compared to networks constructed from an equivalent number of random genes selected from a whole-proteome background (Supplementary file 2). To control for potential artifacts that could arise from using the whole proteome background, we performed a more stringent analysis using only proteins that are found in the striatum (Al-Ramahi et al., 2018). Using average node degree and betweenness as proxies for connectivity, we found that the glial and neuronal networks show higher network connectivity than expected by random chance among proteins present in the striatum (Supplementary file 2).

This high connectivity suggested that the networks are enriched in specific biological processes and/or pathways. We therefore clustered the glial mHTT response and neuronal mHTT response networks using the InfoMap random walks algorithm (Rosvall and Bergstrom, 2007). Clusters that had fewer than four nodes were filtered out of subsequent analysis. The glial networks formed 23 and 24 clusters for upregulated and downregulated DEGs, respectively. Both the upregulated and downregulated neuronal networks formed 29 clusters. We applied this clustering method to the networks of randomly selected striatal proteins in order to determine the expected number of clusters for networks of a similar size. Both the glial and neuronal networks formed significantly more clusters than would be expected from random selection (Supplementary file 2).

To gain insight into biological processes represented by each cluster, we queried the five most significantly enriched terms (FDR < 0.05) using the GO Biological Process and Kyoto Encyclopedia of Genes and Genomes (KEGG) terms within each cluster (Supplementary file 3). A synthesis of these terms was used to identify clusters in both the glial and neuronal networks (Supplementary file 3, Figure 2—figure supplement 1B, C). We compared the membership within clusters across the glial and neuronal networks using a pairwise hypergeometric test and identified 14 clusters of upregulated DEGs common to both glial and neuronal networks. Similarly, there were 15 clusters of downregulated DEGs common to the both networks (Figure 2—figure supplement 1A).

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

We applied the same network analysis to genes that had concordantly altered expression in HD patient striata and HD mouse model striata but not in HD Drosophila models (Figure 2—figure supplement 2A). We observed that clusters comprising DEGs specific to the HD patients and the mouse models were functionally related to DEGs in both the glial and neuronal networks (Figure 2—figure supplement 2B).
Distinguishing glia-specific gene expression alterations from bulk tissue profiles

Gene expression data from bulk tissue does not provide the resolution required to define cell-autonomous gene expression alterations resulting from mHTT toxicity. Therefore, we compared DEGs (false discovery rate [FDR] < 0.1) in human embryonic stem cells from individuals with HD (carrying 40–48 CAG repeats) with healthy embryonic stem cells that have been differentiated into either CD140+ oligodendrocyte progenitor cells (OPCs) or CD44+ astrocyte progenitor cells (APCs) (Osipovitch et al., 2019). We compared the resulting list of DEGs identified in the HD OPCs (1439 genes) and HD APCs (193 genes) to the list of conserved HD DEGs from flies expressing mHTT in glia.

We identified 46 upregulated and 91 downregulated DEGs in common (Figure 3A). APCs had 4 upregulated and 12 downregulated genes in common. We next asked whether any clusters in the fly glial networks were enriched in genes dysregulated in HD OPCs or APCs. The Synapse Assembly cluster (Figure 2B) was significantly enriched in genes with reduced expression in HD OPCs (Fisher’s
Figure 3. Reducing the expression of Synapse Assembly cluster genes in glia mitigates mutant Huntingtin (mHTT)-induced behavioral impairments. (A) Overlaps between concordant differentially expressed genes (DEGs) from the cross-species analysis defined as responding to mHTT expression in glia and DEGs identified in Huntington’s disease (HD) human embryonic stem cells (hESCs) that have been differentiated into either CD140+ oligodendrocyte progenitor cells (OPCs) or CD44+ astrocyte progenitor cells (APCs) (Osipovitch et al., 2019). (B) Model placing Synapse Assembly cluster proteins into cellular context. The Synapse Assembly cluster was significantly enriched for DEGs in HD OPCs (Fisher’s exact test, p<0.001). Only one gene, NRXN3, was upregulated in HD OPCs compared to controls (upward red triangle); the rest (AGAP2, GRM1, LRRTM1, EPB41L2, DLGAP3, and SYT13) were downregulated (downward blue triangles). (C) Heatmap representing genes with lower expression in HD OPCs compared to controls (presented as LogFC) that belong to the Synapse Assembly cluster. Each row is one downregulated gene; each column is a different HD human embryonic stem cell line, with CAG repeat length ranging from 40 to 48, compared to respective controls (Osipovitch et al., 2019). (D) Behavioral assessment of fruit flies that express mHTT only in glia, after reducing the expression of the overlapping DEGs in HD OPCs and the Synapse Assembly cluster. Plots show climbing speed as a function of age. **p<0.001 between positive control and experimental (by linear mixed effects model and post-hoc pairwise comparison; see Materials and methods). Points and error bars on the plot represent the mean ± SEM of the speed for three technical replicates. Each genotype was tested with 4–6 replicates of 10 animals. Modifying alleles in (D) are listed in the Key resources table. Additional climbing data for these genes can be found in Figure 3—figure supplement 1A, and a summary of statistical analysis for this data can be found in Figure 3 continued on next page.
Downregulation of synapse assembly genes is compensatory in HD

The next question we sought to answer is whether changes in expression of synaptic assembly genes are compensatory or pathogenic. We reasoned that if lowering the expression of a downregulated HD DEG aggravated mHTT-induced toxicity, then the downregulation of that gene is pathogenic. Conversely, if reducing the expression of a DEG led to an improvement in HD-related phenotypes, we considered that reduction to be compensatory. We previously used this approach, which takes advantage of the genetic tractability of Drosophila and the availability of high-throughput behavioral screening as a proxy for neurological function, to discover modifier genes that reduce HTT protein levels in HD patient cells (Al-Ramahi et al., 2018). Here we assessed the effect of various genetic changes in the same group of animals over time, following the expression of mHTT in either glia, neurons, or both cell types. We used a custom, robotic assay system that video-records flies climbing upwards to the top of a vial after being knocked to the bottom (negative geotaxis) to track the behavior of individual Drosophila in real time and measure several motor metrics including speed (see Materials and methods). Healthy flies reliably climb to the top at a steady rate until the effects of aging gradually reduce their speed. In contrast, animals expressing mHTT specifically in glia or neurons show much more rapid, if still age-dependent, loss of climbing speed compared to animals expressing a non-targeting hairpin RNA (hpRNA). While we only focus on the effect of these genetic perturbations on speed, we also observe impairments in coordination, balance, and direction (output as number of turns and stumbles) in Drosophila expressing mHTT (data not shown).

The expression of SYT13, LRRTM1, GRM1, EPB41L2, DLGAP3, and AGAP2 is reduced in HD OPCs derived from human embryonic stem cells (Osipovitch et al., 2019), which is consistent with the expression patterns we observed in patient-derived striatal tissue, knock-in mouse model striatal tissue, and in neuronal tissue from Drosophila expressing mHTT in glia (Figure 3C). We performed genetic perturbation analysis on the Drosophila orthologs of these genes to assess whether their downregulation was pathogenic or compensatory in glia. Diminishing expression of the Drosophila orthologs of these six genes mitigated the behavioral deficits induced by mHTT expression in glia (Figure 3D, additional controls in Figure 3—figure supplement 1B). We concluded that reduced expression of these genes is a compensatory response to mHTT expression in glia.

There were additional protein interactors in Synapse Assembly whose expression was not altered in the HD-affected OPCs or APCs compared with controls but that were nonetheless downregulated across all three HD models. In our behavioral assay, reducing expression of these interactors, including NLGN3, NLGN4X, HOMER1, and SLITRK5, was also protective against glial mHTT toxicity (Figure 3—figure supplement 1A, Supplementary file 4; additional controls in Figure 3—figure supplement 1B).
In sum, comparative transcriptomic analysis indicated that genes within the Synapse Assembly cluster are associated with the glial response to HD, and the high-throughput behavioral assay further defined this response as compensatory.

Decreasing neurexin expression in glia mitigates mHTT-induced pathogenesis in both neurons and glia

NRXN3 was identified as a DEG in both our cross-species comparative transcriptomic analysis and in the gene expression profile of the HD glial progenitor population. NRXN3 expression was lower in the bulk HD transcriptome across species compared to their respective controls, but it was more highly expressed in the HD OPCs than in controls. This discordance between the bulk and single-cell-type gene expression profiles might be a result of time-dependent changes in gene expression as neurons age, but it prevented us from classifying the NRXN3 expression changes as being compensatory or pathogenic. We were particularly interested in neurexins, including NRXN3, because they mediate contact between pre- and post-synaptic neurons (Ushkaryov et al., 1992; Zeng et al., 2007).

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

To investigate whether Nrxn3 is expressed in astrocytes in the striatum of HD mice, we performed in situ hybridization (ISH) in coronal sections of striatal tissue taken from a mouse model of HD (HdhQ175/+) to probe Nrxn3 mRNA. Nrxn3 was expressed in striatal astrocytes (Figure 4C, D, Figure 4—figure supplement 1). In conclusion, modulating the expression genes other than mHTT in glia could be an effective strategy for ameliorating HD-induced central nervous system (CNS) dysfunction.

Reducing SERPINA1 function mitigates behavioral impairments in neurons and glia, and lowers mHTT protein levels

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

Genes were sampled from both the neuronal and glial mHTT response networks by prioritizing those candidates with high centrality (calculated as a cumulative rank-score of node betweenness and node degree) within each cluster. When available, we used alleles that perturb the expression or activity of the Drosophila orthologs in the same direction as the gene expression change in the HD patient population (Figure 5A). We screened 411 alleles, representing 248 Drosophila genes homologous to 211 human genes, for perturbations that improve the age-dependent behavior of Drosophila expressing mHTT in neurons or glia (Supplementary file 5). Alleles that ameliorated neuronal or glial function were verified in a subsequent trial in animals expressing mHTT across the CNS (in both neurons and glia). In all, we identified 25 genes with altered expression in HD that suppressed...
Figure 4. Glia-specific dNRXN3 knockdown mitigates impairments caused by mutant Huntingtin (mHTT) expression. (A) Behavioral assays (climbing speed as a function of age) showing that dNRXN3 heterozygous loss of function (LOF) ameliorates behavioral impairments caused by expression of mHTT in both neurons and glia and in glia alone, but not in neurons alone. (B) Glia-specific dNRXN3 knockdown mitigates behavioral impairments caused by mHTT expressed solely in glia; however, neuron-specific knockdown of dNRXN3 does not affect impairments induced by mHTT expressed solely in neurons. ***p<0.001 between positive control and experimental by linear mixed effects model and post-hoc pairwise comparison (see Materials and methods). Points and error bars on the plot represent the mean ± SEM of the speed for three technical replicates. Each genotype was tested with 4–6 replicates of 10 animals. A full summary of the statistical analysis for this data can be found in Supplementary file 4. Control climbing data for these alleles can be found in Figure 3—figure supplement 1B. (C) Astrocytes in the striatum of 6-month-old knock-in HD mice (Hdh\textsuperscript{Q175/+}) expressing Nrxn3. In situ probe for Nrxn3 mRNA is in red (appears magenta when overlapping with the DAPI channel), astrocytes are immunostained using an antibody specific for glial fibrillary acidic protein (GFAP) in green, and DAPI in blue. Image was taken at x63 magnification using a Leica SP8 confocal microscope. Scale bar (in white on the bottom right) represents 50 μm. 3/5 (60%) of astrocytes in this field appear Nrxn3 positive. (D) Magnified image of the astrocyte highlighted in the white box in (C). White arrows indicate yellow puncta where Nrxn3 mRNA localizes to astrocytes. Scale bar (in white on the bottom right) represents 5 μm. See Figure 4—figure supplement 1 for additional images and quantification of Nrxn3 in situ signal in striatal astrocytes in Hdh\textsuperscript{Q175/+} mice. Drosophila genotypes: dNRXN3 LOF allele (y¹ w*; M{y¹ Dm02=MC}nrx-1\textsuperscript{1M02575} or nrx-1\textsuperscript{1-LOF}, BDSC: 61696), dNRXN3 RNAi allele (UAS-nrx-1\textsuperscript{hpRNA}, VDRC: 36326), neuronal and glial Huntington’s disease (HD) model with dNRXN3 mutant (elav\textsuperscript{c155}-GAL4/y¹ w*; repo-GAL4,UAS-HTT\textsuperscript{NT231Q128}/Experimental allele), glial HD model with dNRXN3 mutant (w\textsuperscript{1118}/y¹ w*; repo-GAL4,UAS-HTT\textsuperscript{NT231Q128}/Experimental allele), and neuronal model with dNRXN3 mutant (elav\textsuperscript{c155}-GAL4/y¹ w*; UAS-HTT\textsuperscript{NT231Q128}/Experimental allele).

The online version of this article includes the following source data and figure supplement(s) for figure 4:

Source data 1. Raw behavioral data for Drosophila expressing mutant Huntingtin (mHTT) following reduced expression of dNRXN.

Figure supplement 1. Additional images of astrocytes expressing Nrxn3 in the striatum of Hdh\textsuperscript{Q175/+} mice and quantification.
Figure 5. Compensatory and pathogenic gene expression changes shared by neurons and glia in response to mutant Huntingtin (mHTT) expression.

(A) Our approach for identifying modifiers of mHTT-induced behavioral impairments common to both neurons and glia. Genes that were central to their respective clusters were prioritized and manipulated in Drosophila expressing mHTT (HTTNT231Q128) in either neurons (elav-GAL4) or glia (repo-GAL4). (B) Red bars represent the percent improvement in behavior over a 9-day trial compared to positive control (non-targeting hpRNA) in Drosophila expressing mHTT in neurons and glia, after we antagonized pathogenic gene expression changes. (C) Green bars represent the percent improvement in behavior over a 9-day trial compared to control (see B), after we mimicked compensatory gene expression alterations. In (B) and (C), the top black bars represent the effect of directly targeting the mHTT transgene using a small interfering RNA (siRNA). Arrowheads indicate the direction of the conserved, concordant altered expression for each gene as a result of mHTT expression in humans, mice, and Drosophila. Behavioral assay graphs corresponding to the data presented in (B) and (C) can be found in Figure 5—figure supplement 1A. Corresponding statistical analysis for (B) and (C) can be found in Supplementary file 6. Corresponding controls for behavioral data can be found in Figure 5—figure supplement 1B, C. Drosophila genotypes: positive control (elav-GAL4/w1118; UAS-non-targeting hpRNA/+; repo-GAL4, UAS-HTTNT231Q128/+), treatment control (elav-GAL4/w1118; repo-GAL4, UAS-HTTNT231Q128/UAS-siHTT), and experimental (elav-GAL4/w1118; repo-GAL4, UAS-HTTNT231Q128/modifier).

The online version of this article includes the following source data and figure supplement(s) for figure 5:

Source data 1. Numerical data for bar charts summarizing the improvement in behavior in Drosophila expressing mutant Huntingtin (mHTT) in neurons and glia by manipulating common pathogenic and compensatory alterations.

Figure supplement 1. Genetic modifiers suppress behavioral impairments caused by mutant Huntingtin (mHTT) expression in neurons and glia.

Figure supplement 1—source data 1. Raw behavioral data for Drosophila expressing mutant Huntingtin (mHTT) in neurons and glia by manipulating common pathogenic and compensatory alterations.
mHTT-induced behavioral deficits in neurons, glia, or both (Figure 5B, C, Figure 5—figure supplement 1, Supplementary file 6).

Many of the modifiers common to neuronal and glial mHTT-induced dysfunction are involved in the regulation of the actin cytoskeleton (RHOC, TIAM1, ENAH, and CFL2), vesicular trafficking (SNAP23, SNX9, and SNX18), and inflammation (JUN, GTF3A, and ATF3). Multiple reports have implicated components of these pathways in the pathogenesis of not only HD, but in other neurodegenerative disorders as well (Al-Ramahi et al., 2018; Bardai et al., 2018; Bondar et al., 2018). We previously established an axis of genes with altered expression that regulate actin cytoskeleton and inflammation pathways driving forward HD pathogenesis (Al-Ramahi et al., 2018). Our current results would indicate that these pathways are not only critical to disease progression in neurons, but also in glia.

We previously observed that reducing the activity of RAC GTPase, a regulator of the actin cytoskeleton, and inflammation mediating nuclear factor kappa-light-chain-enhancer of activated B cells (NF Kappa-B) ameliorated pathogenesis by lowering mHTT protein levels through the activation of autophagy (Al-Ramahi et al., 2018). Thus, in a secondary screen we tested whether these disease modifiers common to both neurons and glia exerted their beneficial effects by lowering levels of the mutant HTT protein.

We collected protein lysates from Drosophila expressing mHTT across the CNS that also bore alleles that suppressed mHTT-induced behavioral deficits in both neurons and glia. We assessed the quantity of mHTT protein in these lysates by western blot, comparing experimental (candidate modifiers) and control animals (carrying a non-targeting hpRNA). This secondary screen identified Spn42De as a modifier whose knockdown lowered mHTT levels. Spn42De is one of the four Drosophila homologues of human SERPINA1 (which encodes alpha-1-antitrypsin, a member of a large group of protease inhibitors). Spn42De, human SERPINA1, and mouse Serpina1 are all upregulated in HD, and they are part of the Wound Healing and Inflammation cluster in both the neuronal and glial mHTT response networks (Figure 2—figure supplement 1C). Knockdown of Spn42De (henceforth dSERPINA1) in Drosophila expressing mHTT in both neurons and glia mitigated behavioral impairments (Figure 6A). In independent immunoblots, dSERPINA1 knockdown consistently reduced mHTT protein levels in lysates extracted from the heads of Drosophila expressing mHTT in both neurons and glia (Figure 6B, C). As a control, we performed immunoblot analysis of lysates from a green fluorescent protein (GFP) reporter line to ensure that this allele of dSERPINA1 did not reduce the function of the GAL4-UAS system (Figure 6—figure supplement 1).

To validate this observation across model systems, we performed homogenous time-resolved fluorescence (HTRF) on HdhQ111/Q7 mouse striatal cell lysates that were treated with either a pool of non-targeting scramble small interfering RNAs (siRNAs), a pool of siRNAs against Htt, or a pool of siRNAs against Serpina1a (the murine ortholog of SERPINA1). Serpina1a knockdown significantly reduced mHTT signal (Figure 6D). Knockdown of SERPINA1 thus protected against mHTT toxicity in neurons and glia by reducing levels of mutant HTT. Verifying this effect in multiple model organisms increases confidence in this observation and suggests that SERPINA1 could potentially prove useful as a target for treating HD. Interestingly, SERPINA1 expression is low in the healthy brain but it is upregulated in several disease conditions, consistent with a potential role in neuroinflammation (Abu-Rumeileh et al., 2020; Cabezas-Llobet et al., 2018; Gollin et al., 1992; Peng et al., 2015). We found increased Serpina1a protein staining in the striatum of HdhQ175/+ compared to wildtype mice at 8.5 months (Figure 6—figure supplement 2), confirming its upregulation from the transcriptomic data. Previously we had shown that other genes in the subnetwork implicated in neuroinflammation can be manipulated to lower mHTT protein levels (Al-Ramahi et al., 2018). SERPINA1 may thus warrant investigation as a target for other neurological disorders as well.

Discussion
We found a high degree of overlap of DEGs across tissues from human HD brains, brains of HD mice, and flies that express mHTT in glia. This may seem unexpected given obvious differences between vertebrate and Drosophila glia, such as a lack of documented microglia or distinct morphology of endothelial/glial cells forming the blood-brain barrier in Drosophila (Freeman and Doherty, 2006). Our observations are however consistent with previous evidence that Drosophila glia perform many of the same functions as mammalian astrocytes, oligodendrocytes, endothelial cells, and
microglia including phagocytosis (Chung et al., 2020; Freeman, 2015; Freeman and Doherty, 2006; Ziegenfuss et al., 2012). In fact, the overlap of concordant DEGs between mammalian and Drosophila glia may be underestimated in our analysis because it was limited to CD44+ and CD140+ cells from human embryonic stem cell-derived glial progenitors and therefore we may have missed DEG overlaps from other glial types, or from more mature state of oligodendrocytes or astrocytes.

Figure 6. Antagonizing the pathogenic overexpression of SERPINA1 in neurons and glia mitigates mutant Huntingtin (mHTT)-induced behavioral impairments and lowers mHTT protein levels in Drosophila and Huntington’s disease (HD) mouse striatal cells. (A) Behavioral assays following knockdown of dSERPINA1 in Drosophila expressing mHTT in neurons and glia. *** indicates p<0.001 by linear mixed effects model and post-hoc pairwise comparison between positive control and experimental animals. Points and error bars on the plot represent the mean ± SEM of three technical replicates. Each genotype was tested with 4–6 replicates of 10 animals. (B) Representative western blot showing lower levels of mHTT following knockdown of dSERPINA1 in Drosophila expressing mHTT in neurons and glia. (C) Quantification of five independent immunoblots showing the effect of dSERPINA1 knockdown on mHTT levels in Drosophila head protein lysates. ***p<0.001 between positive control and dSERPINA1 knockdown by one-way t-test. (D) Quantification of HTT protein levels in HD mouse striatal-derived cells (STHdhQ111/Q7) measured by homogenous time-resolved fluorescence (HTRF) following treatment with a pool of scramble small interfering RNAs (siRNAs) (negative control), a pool of siRNAs against Htt, and a pool of siRNAs against Serpina1a. Quantification is presented as a ratio of the emission signal from the fluorescent D2 dye (HTT)/PicoGreen (number of cells per well). n = 9 for each treatment group. *p<0.05 and ***p<0.001 between genotypes by Fisher’s Least Significant Difference (LSD) test. Drosophila genotypes: dSERPINA1 RNAi allele (UAS-Spn42De hpRNA, VDRC: 102622), positive control (elavc155-GAL4/w1118;UAS- non-targeting hpRNA/+; repo-GAL4,UAS-HTTNT231Q128/+), treatment control (elavc155-GAL4/w1118; repo-GAL4, UAS- HTTNT231Q128/UAS-siHtt), and dSERPINA1 experimental (elavc155-GAL4/w1118;UAS-Spn42De hpRNA/+; repo-GAL4, UAS-HTTNT231Q128/+).

The online version of this article includes the following source data and figure supplement(s) for figure 6:

Source data 1. Raw behavioral data for Drosophila expressing mutant Huntingtin (mHTT) in neurons and glia following knockdown of Spn42De.

Source data 2. Summary of numerical and raw data for western-blot protein lysates from Drosophila heads expressing mutant Huntingtin (mHTT) in neurons and glia with knockdown of Spn42De.

Source data 3. Summary of numerical and raw data for homogenous time-resolved fluorescence (HTRF) for protein lysates from STHdhQ111/Q7 treated with pooled small interfering RNAs (siRNAs) against Serpina1a.

Figure supplement 1. dSERPINA1 knockdown does not reduce the expression of the GAL4-UAS system.

Figure supplement 1—source data 1. Summary of numerical and raw data for western-blot protein lysates from Drosophila heads expressing mutant Huntingtin (mHTT) in neurons with knockdown of Spn42De.

Figure supplement 2. Serpina1a protein accumulates in the striata of HdhQ175/+ mice compared to wildtype littermates.

Figure supplement 2—source data 1. Summary of numerical and raw data for immunohistochemistry analysis of Serpina1 staining in 8.5-month-old mouse striata from wildtype and zQ175 mice.
influences HD pathogenesis (Benraiss et al., 2016; Bradford et al., 2009; Garcia et al., 2019; Huang et al., 2015; Osipovitch et al., 2019). More recently, it was discovered that transcription factors involved in glial differentiation and myelin synthesis are downregulated in glial progenitor cells (Osipovitch et al., 2019). Yet despite this progress, the overall contributions of glial genes to synaptic impairments and other key neurodegenerative pathologies remain poorly understood. The genetic malleability of Drosophila enabled us to thoroughly examine the neuron-glial interface from both the glial and the neuronal directions.

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

Modifiers of mHTT-induced pathogenesis identified in our study, such as metabotropic glutamate receptors and the scaffold protein HOMER1, regulate calcium and glutamate signaling in astrocytes (Buscemi et al., 2017; Spampinato et al., 2018). Reducing the expression of these genes could prevent excess calcium and glutamate from accumulating at the synapse. Indeed, we previously found that HD neurons downregulate the expression of genes involved in calcium signaling in an effort to compensate for HD pathogenesis (Al-Ramahi et al., 2018). Glial calcium signaling can also influence neuronal activity, however, at the neuronal soma (Weiss et al., 2019). In Drosophila, cortical glia modulate neuronal activity through potassium buffering, a process that is regulated by calcium-mediated endocytosis of potassium channels (Weiss et al., 2019). Glia can also physically disrupt synapses in disease states: Förster resonance energy transmission in vivo revealed that, in HD, the distances between astrocytes and pre-synaptic neurons are increased at the cortico-striatal circuit (Octeau et al., 2018). Thus, knocking down the genes in the Synapse Assembly cluster could reduce physical interaction between glia and synapses, promoting normal synaptic function.

If in HD synapses grow more fragile and fewer in number as the disease progresses, why would downregulating the expression of glial genes required for synapse formation and function be protective? We postulate it is for the same reason that downregulating calcium-signaling genes is compensatory (Al-Ramahi et al., 2018): the brain is attempting to protect against the excitotoxicity described above. Mutant HTT disrupts neuronal development (Ring et al., 2015) and skews embryonic neurogenesis toward producing more neurons (Barnat et al., 2020); by the time HD mutation carriers reach the age of 6 years, they have greatly enlarged striata and functional hyperconnectivity to the cerebellum (Tereshchenko et al., 2020). The more hyperconnected, the more abrupt the loss of these connections, and the more rapid the striatal atrophy that follows Tereshchenko et al., 2020. The hyperfunction of a given brain region puts considerable strain on the circuit, and it seems that over the course of a lifetime, the brain keeps trying to compensate for the abnormalities that arise at different stages of HD. The recent observation that deletion of astrocytic neurexin-1α attenuates synaptic transmission but not synapse number supports this hypothesis (Trotter et al., 2020).

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

Intriguingly, loss-of-function variants in NRXN1-3, NLGN1, NLGN3, DLGAP3, and LRRTM1 have been associated with various disorders of synaptic dysfunction, including autism spectrum disorder (ASD), schizophrenia, and obsessive compulsive disorder (OCD) (Nakanishi et al., 2017; Jamain et al., 2003; Südhof, 2008; Vaags et al., 2012; Wang et al., 2018; Windrem et al., 2017). We speculate that the consequences of loss of function of these genes depend on both dosage and context: modest reductions of gene expression can be protective in the context of HD pathogenesis, whereas a more severe loss of function results in ASD and OCD. It is interesting that many HD patients develop schizophrenia-like psychosis, suggesting that the compensatory mechanism at place in HD may eventually lead to schizophrenia-like symptoms (Connors et al., 2020; Tsuang et al., 2018). Future studies should investigate whether these loss-of-function variants associated with neurodevelopmental and psychiatric disorders alter the age of disease onset in patients with HD. It could be of particular interest to assess if these neurodevelopmental and psychiatric-associated variants ameliorate neurodevelopmental changes observed early in HD or blunt synaptic hyperactivity later in disease.

Materials and methods

### Key resources table

| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Antibody (mouse monoclonal)      | Anti-HTT    | EMD Millipore       | mAb5490, RRID: AB_2233522 | WB (1:500) |
| Antibody (rabbit polyclonal)     | Anti-GFP    | ThermoFisher        | A-11122, RRID: AB_221569  | WB (1:1000) |
| Antibody (mouse monoclonal)      | Anti-HTT    | Novartis            | 2B7         | HTRF (0.023 µg/mL) |
| Antibody (mouse monoclonal)      | Anti-laminC | Hybridoma Bank      | LC28.26, RRID: AB_528339  | WB (1:1000) |
| Antibody (rabbit polyclonal)     | Anti-GFAP   | DAKO                | Z0334, RRID: AB_10013382  | IF (1:500) |
| Antibody (rabbit polyclonal)     | Alpha-tubulin | Abcam            | EP1332Y, RRID: AB_922700  | WB (1:1000) |
| Antibody (mouse monoclonal)      | Anti-HTT    | SigmaAldrich        | mAb2166, RRID: AB_11213141 | HTRF (1.4 µg/mL) |
| Antibody (goat polyclonal)       | Anti-rabbit IgG Alexa 488 | Invitrogen | A-11008, RRID: AB_143165 | IF (1:500) |
| Antibody (rabbit polyclonal)     | Anti-Serpin1a | Invitrogen       | PA5-16661, RRID: AB_10985745 | IF (1:250) |
| Antibody (goat polyclonal)       | RDye 680RD anti-Rabbit IgG | LI-COR Biosciences | 925-68071, RRID: AB_2721181 | WB (1:5000) |
| Antibody (goat polyclonal)       | IRDye 800CW anti-Mouse IgG | LI-COR Biosciences | 925-32210, RRID: AB_2687825 | WB (1:5000) |
| Chemical compound, drug          | Lipofectamine 2000 | Life Technologies | 11668  |
| Chemical compound, drug          | EDTA-free protease inhibitor | Calbiochem | 539134  |
| Commercial assay, kit             | miRNeasy Mini Kit | Qiagen | 217004  |
| Commercial assay, kit             | Illumina TruSeq Stranded mRNA | Illumina | 20020595 |
| Commercial assay, kit             | Tyramide-Cy3 Plus kit | Perkin Elmer | NEL744001KT |

Continued on next page
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Commercial assay, kit             | PicoGreen   | Quant-iT PicoGreen dsDNA Assay Kit | P7589       |                        |
| Cell line (M. musculus)           | STHdh<sup>Q17/Q7</sup> Cells | Coriell Cell Repositories | CH00096     |                        |
| Strain, strain background (Drosophila) | White mutant, background genotype | Bloomington Drosophila Stock Center | 3605 | w<sup>T118</sup> |
| Genetic reagent (Drosophila)      | Non-targeting hpRNA | Vienna Drosophila Resource Center | 13974       |                        |
| Strain, strain background (Drosophila) | repo-Gal4 | Bloomington Drosophila Stock Center | 7415 | w<sup>T118</sup>, P<sup>w<sup>mt*<sup>=GAL4</sup></sup>repo/TM3, Sb<sup>1</sup> |
| Strain, strain background (Drosophila) | elav-Gal4 | Bloomington Drosophila Stock Center | 458 | P(GawB)elav<sup>C155</sup> |
| Genetic reagent (Drosophila)      | N-terminal HD model | Botas Laboratory | Branco et al., 2008 | UAS-HTT<sup>T231Q128</sup>/TM6B, tubulin-GAL80 (N-terminal) |
| Genetic reagent (Drosophila)      | Full-length HD model | Botas Laboratory | This paper | UAS-HTT<sup>P1200Q</sup>/CyO (full-length) |
| Genetic reagent (Drosophila)      | siRNA against human mutant HTT | Botas Laboratory | Kaltenbach et al., 2007 | UAS-siHTT |
| Genetic reagent (Drosophila)      | Classic CenG1A loss-of-function allele | Bloomington Drosophila Stock Center | 44301 | CenG1A<sup>LOF</sup> or y<sup>a</sup>W<sup>*<sup>=<sup>MI</sup>/CenG1A<sup>MI06024</sup> (Figure 3D) |
| Genetic reagent (Drosophila)      | Classic vlc loss-of-function allele | Bloomington Drosophila Stock Center | 10366 | vI<sup>109</sup>/lacW<sup>lacW109</sup>/CyO(Figure 3D) |
| Genetic reagent (Drosophila)      | Classic tm loss-of-function allele | Bloomington Drosophila Stock Center | 4550 | tm<sup>1</sup>or y<sup>a</sup>W<sup>723</sup> or P<sup>w<sup>mt*</sup>=<sup>lacW</sup>trn<sup>5064117</sup>/TM3, Sb<sup>1</sup>ser<sup>1</sup> (Figure 3D) |
| Genetic reagent (Drosophila)      | Classic cora loss-of-function allele | Bloomington Drosophila Stock Center | 9099 | cora<sup>LOF</sup> or P(y<sup>a</sup>Y<sup>T22=neoFRT</sup>3D cora<sup>4</sup>/CyO (Figure 3D) |
| Genetic reagent (Drosophila)      | RNAi against Sytbeta | Vienna Drosophila Resource Center | 10659 | UAS-Sytbeta<sup>RNAi</sup> (Figure 3D) |
| Genetic reagent (Drosophila)      | RNAi against mGluR | National Institute of Genetics, Japan | 11144 R-3 | UAS-mGluR<sup>RNAi</sup> (Figure 3D) |
| Genetic reagent (Drosophila)      | Neuronal mCD8::GFP reporter line | Bloomington Drosophila Stock Center | 5146 | P<sup>w<sup>10596</sup>/w<sup>GawB</sup>elav<sup>C155</sup>, P<sup>w<sup>mt*</sup>=<sup>UAS-mCD8::GFP.L</sup>P<sup>tp4E[L144], P<sup>y<sup>[a+7.2]=hsFLP]</sup>1, w<sup>*</sup> |
| Genetic reagent (Drosophila)      | Classical loss of function and overexpression alleles in Drosophila | Bloomington Drosophila Stock Center | Provided in Supplementary files 4 and 5 | |
| Genetic reagent (Drosophila)      | RNAi alleles in Drosophila | Vienna Drosophila Resource Center | Provided in Supplementary files 4 and 5 | |
| Genetic reagent (Drosophila)      | Cytological duplication alleles in Drosophila | GenetiVision | Provided in Supplementary files 4 and 5 | |
| Genetic reagent (M. musculus)     | Hdh<sup>Q175S</sup> Mice | Jackson Laboratories | 027410 | B6J.129S1-Htttm1Mfc/190ChdiJ |

Continued on next page
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Recombinant DNA reagent           | pMF3 Vector | Drosophila Genome Resource Center | 1237        |                        |
| Software, algorithm               | Adept Desktop | Omron                | N/A         |                        |
| Software, algorithm               | Video Savant  | IO Industries        | N/A         |                        |
| Software, algorithm               | MatLab with Image Processing Toolkit and Statistics Toolkit | MathWorks | https://www.mathworks.com/products/matlab.html |                        |
| Software, algorithm               | RSLogix      | Rockwell Automation | N/A         |                        |
| Software, algorithm               | Ultraware    | Rockwell Automation | N/A         |                        |
| Software, algorithm               | Assay Control | SRI International    | N/A         |                        |
| Software, algorithm               | FastPhenoTrack Vision Processing | SRI International | N/A         |                        |
| Software, algorithm               | TrackingServer Data Management | SRI International | N/A         |                        |
| Software, algorithm               | ScoringServer Behavioral Scoring | SRI International | N/A         |                        |
| Software, algorithm               | Trackviewer Visual Tracking and Viewing | SRI International | N/A         |                        |
| Software, algorithm               | Illustrator CC | Adobe                | https://www.adobe.com |                        |
| Software, algorithm               | R            | R Project for Statistical Computing | https://www.r-project.org/ |                        |
| Software, algorithm               | Fiji         | The Fiji Team        | https://fiji.sc/ |                        |
| Software, algorithm               | Image Studio Lite | LI-COR Biosciences | https://www.licor.com/bio/image-studio-lite/ |                        |
| Software, algorithm               | Bowtie       | Langmead and Salzberg, 2012 | http://bowtie-bio.sourceforge.net/index.shtml |                        |
| Software, algorithm               | RSEM         | Li and Dewey, 2011   | https://github.com/deweylab/RSEM |                        |
| Software, algorithm               | DESeq2       | Love et al., 2014    | https://bioconductor.org/packages/release/bioc/html/DESeq2.html |                        |
| Software, algorithm               | DIOPT        | Hu et al., 2011      | https://www.flyrnai.org/cgi-bin/DRSC_orthologs.pl |                        |
| Software, algorithm               | MGI          | The Mouse Genome Database | http://www.informatics.jax.org/genes.shtml |                        |
| Software, algorithm               | STRING       | Szklarczyk et al., 2015 | https://string-db.org/ |                        |
| Software, algorithm               | InfoMap      | Rosvall and Bergstrom, 2008 | https://cran.r-project.org/web/packages/igraph/index.html |                        |
| Software, algorithm               | Cytoscape    | The Cytoscape Consortium | https://cytoscape.org |                        |

Continued on next page
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|----------------------------------|-------------|---------------------|-------------|------------------------|
| Transfected construct (M. musculus) | AllStars Negative Control siRNA (Scramble) | Qiagen | 1027280 | |
| Transfected construct (M. musculus) | Htt SMARTPool siRNAs | Horizon Discovery Limited | L-040632-01-0005 | 5'- GAAUUAAGGUUCUGUUGA-3' 5'- GCGACUCAGCGCAACUAJA-3' 5'- GAUGAGCGGUUCUGAGUCG-3' 5'- UAACAUUGCUCAUUGUGA-3' |
| Transfected construct (M. musculus) | Serpina1a SMART Pool siRNAs | Horizon Discovery Limited | L-043380-01-0005 | 5'- GAAUUAACUUGAAGACAC-3' 5'-GGGCUGACCUCUCCGGAAU-3' 5'- UGGUAGAUCCCACACAUAA-3' 5'- GAAAGAUAGCUGAGGCGGU-3' |
| Sequence-based reagent | Primers for cloning human HTT | This paper | See experimental model detail | Forward 5'- gaattgcACCGCCAAGAC-3' Reverse 5'- tctagaGGCAGATTCACCAGGTA-3' |
| Sequence-based reagent | Primers for generating in situ probes for mouse Nrxn3 including RNA polymerase promoter sequences for T3 (forward) and T7 (reverse) | Allen Brain Atlas | https://portal.brain-map.org/ | Forward 5'- GCCAATTACCCCTCCTAAAGGCTTCCTCCCTTCTCTCTTAA-3' Reverse 5'- GCCGAATACCCCTCCTAAAGGCTTCCTCCCTTCTCTCTTAA-3' |

**Lead contact and material availability**

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

**Drosophila models**

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

In Figure 3D, we used the following mutants to assess the effect of reduced expression of synaptic genes in mHTT animals on behavior: UAS-non-targeting$^{ApRNA}$ (Vienna Drosophila Resource Center, ID: 13974), CenG1A$^{LOF}$ or $y^i$w*; Mi(Mi)CenG1A$^{Mi06024}$ (Bloomington Drosophila Stock Center, ID: 44301), vlc$^{LOF}$ or $y^i$w$^{67c23}$; P(w$^{rca}$ lacW)vlc$^{101109}$/CyO (Bloomington Drosophila Stock Center, ID: 10366), trr$^{LOF}$ or $y^i$w$^{67c23}$; P(w$^{rca}$ lacW)trr$^{506417}$/TM3, Sb$^1$ Ser$^1$ (Bloomington Drosophila Stock Center, ID: 4550), cora$^{LOF}$ or P($y^{17.2}$=neoFRT)43D cora$^{14}$/CyO (Bloomington Drosophila Stock Center, ID: 9099), UAS-Sytbeta$^{hpRNA}$ (Vienna Drosophila Resource Center, ID: 106559), and UAS-mGluR$^{RNAi}$ (National Institute of Genetics, Japan, ID: 11144-R3).

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

Forward 5'- gaattgcACCGCCAAGAAAGAAGAC-3'
We first digested the PCR product with EcoRI and ligated it with itself to obtain inverted repeats. We then digested the inverted repeat with XbaI and pasted the fragment into the pMF3 vector (also cut with XbaI); the resulting plasmid was injected into Drosophila embryos using standard methods (Dietzl et al., 2007). We validated that this line lowers mHTT levels.

**STHdh^Q111/Q7** mouse striatal cells

Immortalized mouse striatal cells heterozygous for mHTT (STHdh^Q111/Q7) were obtained from Coriell Cell Repositories (Camden, NJ) and cultured in DMEM (Life Technologies, cat. no. 11965) supplemented with 10% fetal bovine serum (Life Technologies, cat. no. 10082-147). The cells were tested every two months by a TransDetect PCR Mycoplasma Detection Kit (Transgen Biotech, cat. no. FM311-01) to ensure that they are mycoplasma free. The identity has not been authenticated by STR profiling, but has been validated by western blot, morphology, and phenotypic experiments.

**DEG identification in Drosophila HD models**

We performed RNA-seq on head tissue collected from Drosophila expressing N-terminal (UAS-HTT^NT231Q128) or full-length (UAS-HTT^FLQ200) human mHTT in neurons (elav-GAL4) or glia (repo-GAL4). For each combination of HD model and driver, RNA-seq was performed at three timepoints to capture the early, middle, and late phases of disease pathogenesis, corresponding to behavioral deficits caused by mHTT-induced neuronal or glial dysfunction. At each timepoint, samples for HD and age-matched controls were collected in triplicate. Drosophila expressing the N-terminal construct and corresponding controls were obtained at 7, 9, and 11 days post-eclosion for the neuronal driver, and at 5, 7, and 8 days post-eclosion for the glial driver. Drosophila expressing the full-length construct, samples were obtained at 18, 20, and 22 days post-eclosion for both the neuronal and glial driver. For RNA-seq, the neuronal N-terminal, glial N-terminal, and glial full-length model Drosophila were raised at 28°C. The neuronal full-length model Drosophila were raised at 29°C. For each genotype at each timepoint, we collected an equivalent number of control animals (elav-GAL4 or repo-GAL4) that were raised in the same conditions. Three replicates of 50 virgin females were collected for each genotype and timepoint. Animals were aged in the appropriate temperature and were transferred to fresh food daily until tissue was harvested. At the selected ages, animals were transferred to 1.5 mL tubes, flash frozen in liquid nitrogen, vigorously shaken, and then sieved to collect 50 heads/genotype/replica (~5 mg tissue/replica). Total RNA was extracted using the miRNeasy Mini Kit (Qiagen cat. no. 210074).

RNA-seq profiling and preprocessing was performed by Q2 Solutions (Morrisville, NC). Samples were converted into cDNA libraries using the Illumina TruSeq Stranded mRNA sample preparation kit (Illumina cat. no. 20020595) and were sequenced using HiSeq-Sequencing-2 × 50 bp-PE. Initial analysis was performed using Q2 Solution in-house mRNAv7 pipeline with a median of 49 million actual reads. After adapter sequences were removed, the reads were aligned to the Drosophila melananogaster transcriptome using Bowtie version 0.12.9 (Langmead and Salzberg, 2012). Expression was quantified using RSEM version 1.1.19, resulting in a median of 11,214 genes and 18,604 isoforms detected (Li and Dewey, 2011).

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

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

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

**PPI network and clustering**

To examine how the upregulated and downregulated core genes interact functionally, we used STRING v10.5 (Szklarczyk et al., 2015). Only high-confidence interactions (edge weight >0.7) were considered. Each node is converted from an ENSEMBL ID to human Entrez ID via the provided mapping file (v10, 04-28-2015). Four subgraphs of STRING were then induced on each core gene set separately. Nodes were further clustered with the InfoMap community detection algorithm (Rosvall and Bergstrom, 2008), implemented in the Python iGraph package, with the default settings (trials = 10) (Csardi and Nepusz, 2000).

**Drosophila behavioral assay**

We crossed female virgins that carried the mHTT transgene under the control of either the neuronal or glial driver, or the cell-specific driver alone, to males carrying the experimental allele. We introduced a heat-shock-induced lethality mutation on the Y chromosome (Y<sup>fn-hid</sup>) to the disease and cell-specific driver stocks to increase the efficiency of virgin collection (Starz-Gaiano et al., 2001). For crosses involving alleles that were lethal or sterile mutations on the X chromosome, this mating strategy was reversed. For behavioral assays, *elav>*HTTN<sup>NT231Q128</sup> and *repo>*HTTN<sup>NT231Q128</sup> animals were raised and maintained at 28.5˚C. *elav, repo>*HTTN<sup>NT231Q128</sup> animals were raised and maintained at 25˚C. Individual *Drosophila* in each genotype were randomly grouped into replicates of 10.

The negative geotaxis climbing assay was performed using a custom robotic system (SRI International, available in the Automated Behavioral Core at the Dan and Jan Duncan Neurological Research Institute). The robotic instrumentation elicited negative geotaxis by ‘tapping’ Drosophila housed in 96-vial arrays. After three taps, video cameras recorded and tracked the movement of animals at a rate of 30 frames per second for 7.5 s. For each genotype, we collected 4–6 replicates of 10 animals to be tested in parallel (biological replicates). Each trial was repeated three times (technical replicates). The automated, high-throughput system is capable of assaying 16 arrays (1536 total vials) in ~3.5 hr. To transform video recordings into quantifiable data, individual *Drosophila* were treated as an ellipse, and the software deconvoluted the movement of individuals by calculating the angle and distance that each ellipse moves between frames. Replicates were randomly assigned to positions throughout the plate and were blinded to users throughout the duration of experiments. The results of this analysis were used to compute more than two dozen individual and population metrics, including distance, speed, and stumbles.

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

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

mRNA ISH and immunofluorescence were performed on 25-µm-thick coronal brain sections cut from fresh-frozen brain harvested from a 6-month-old *Hdh<sup>Q175/+</sup>* mouse. We generated digoxigenin (DIG)-labeled mRNA antisense probes against *Nrxn3* using reverse-transcribed mouse cDNA as a template and an RNA DIG-labeling kit from Roche (Sigma). Primer and probe sequences for the *Nrxn3* probe are available in Allen Brain Atlas (http://www.brain-map.org). ISH was performed by the RNA In Situ Hybridization Core at Baylor College of Medicine using an automated robotic platform as previously described (Yaylaoglu et al., 2005) with modifications of the protocol for fluorescent ISH. In brief: after the described washes and blocking steps, the DIG-labeled probe was visualized using a tyramide-Cy3 Plus kit (1:50 dilution, 15 min incubation, Perkin Elmer). Following
washes in phosphate buffered saline (PBS), the slides were stained with 1:500 anti-GFAP rabbit polyclonal antibody (DAKO, Z0334) diluted in 1% blocking reagent in Tris buffered saline (Roche Applied Science, 11096176001) overnight at 4°C. After washing, slides were treated with 1:500 anti-rabbit IgG Alexa 488 secondary antibody for 30 min at room temperature (Invitrogen, A-11008). The slides were stained with DAPI and cover slipped using ProLong Diamond (Invitrogen, P36970). Images were taken at x63 magnification using a Leica SP8 confocal microscope.

For visualizing Serpina1, 8.5-month-old male zQ175 mice (four wildtype and three knock-in) were deeply anesthetized and transcardially perfused with 1× PBS. The tissues were then treated with 70% ethanol for 24 hr, 95% ethanol overnight, 100% ethanol for 4 hr, and chloroform overnight. Next tissues were treated with paraffin at room temperature overnight and again with paraffin at 65°C for 2 hr. Paraffin-embedded tissue blocks were coronally sectioned at the thickness of 8 μm, starting from Bregma 0.98 mm. Immunofluorescence for Serpina1 was conducted with rabbit anti-Serpina1a primary antibody (Invitrogen, PAS-16661), followed by the biotin labeled secondary antibody and detected by Alexa Fluor 488 conjugated streptavidin. Fluorescent imaging of the striatal region was performed on a Leica Sp8 confocal microscope.

**Immunoblot of Drosophila lysates**

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

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

**Knockdown of Serpina1a and HTRF in STHdh<sup>Q111/Q7</sup> cells**

STHdh<sup>Q111/Q7</sup> cells were reverse transfected with pooled siRNAs using Lipofectamine 2000 (Life Technologies, cat. no. 11668). Cells were treated with a pool of four small siRNAs per gene with the following sequences (Qiagen 1027280):

- **Htt**
  
  5’- GAAUAUACCGUUGUGUA-3’
  5’- CCACUCAGCCACUAUA-3’
  5’- GAUGAAGCUUUCGAUGG-3’
  5’- UGCAUCUGCUCAUGUGA-3’

- **Serpina1a**
  
  5’- GAAUAUAAACUAGAACCC-3’
  5’- GGGCUCCUCUGCAGGAA-3’
  5’- UGGUAGAUCCCCCACAAU-3’
  5’- GAAAGAUAGCUGAGGCGGU-3’

- **Scramble**

Following siRNA treatment, cell lysis buffer (1× PBS with 1% TritonX-100% and 1% EDTA-free protease inhibitor; Calbiochem, #539134) was added to each well and the plate was put on ice for 30 min. After incubation, cells were homogenized and lysates were extracted. Separately, HTRF assay buffer was prepared using 50 mM NaH<sub>2</sub>PO<sub>4</sub> (pH 7.4), 400 mM KF, 0.1% bovine serum albumin, 0.05% Tween-20, and Quant-ITTM PicoGreen (1:1500). The donor antibody, 2B7 conjugated to
terbium, was diluted in HTRF assay buffer to a concentration of 0.023 μg/mL, and the acceptor antibody, mAb2166 (SigmaAldrich) conjugated to fluorescent dye D2, was diluted to a final concentration of 1.4 μg/mL. 5 μL of the HTRF buffer was added to 5 μL of cell lysates (5 μL) in each well of a 384-well plate. Lysates were then incubated at 4°C overnight.

HTRF was performed in a Perkin Elmer EnVision multilabel plate reader (model #2104), measuring the 615 nM and 665 nM, as well as the PicoGreen signal at 485 nM. Each sample was measured following 30 cycles of the excitation at an interval of 16.6 ms.

**DEG identification in *Drosophila* HD models**

Differential expression analysis used the DESeq2 R package on a total of 12 comparisons (two HD models, two cell-specific drivers, and three timepoints) (Love et al., 2014). Outlier detection was performed using PCA on normalized gene expression data, resulting in one sample being removed. To establish a list of upregulated and downregulated DEGs in *Drosophila*, we examined the FDR at every timepoint in both genetic models. If the FDR was <0.05 at any data point in the HD models compared to control, we established that that gene was dysregulated due to the presence of mHTT in either neurons or glia. We did not take the magnitude of fold-change into account, only the direction (upregulated or downregulated) (Langfelder et al., 2016).

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

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

We established the lists of upregulated and downregulated DEGs in mice from RNA-seq data presented in Langfelder et al., 2016, where the authors profiled mRNA of an allelic series in a HD knock-in mouse model. We reanalyzed data from the striatum at 6 months, identifying gene expression alterations that were significant (FDR < 0.05) in the continuous-Q case, a summary regression variable derived from DESeq that tests the association of the expression profile with Q-length as a numeric variable (Love et al., 2014).

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

We randomly sampled 471 proteins (equivalent to the average number of input proteins in the mHTT Responding networks) 1000 times from 15,884 proteins that are expressed in the striatum. Implementing the same parameters that were used for the mHTT responding networks, we constructed clustered PPI networks with the random striatal protein lists as inputs. We calculated the average node degree and average node betweenness within each network of random genes and compiled a distribution using these results. A Z-score was calculated using the distribution compiled from the random striatal networks. These Z-scores were then used to calculate the p-values that are reported in Supplementary file 2. All simulations and statistical calculations were performed in R—this script can be found as Source code 2.

**Analysis of behavioral screen in *Drosophila***

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

**Statistical analysis for western blot and HTRF**

Images of western blots were analyzed using the Image Studio Lite software. We used an equivalent area to measure signal intensity across all replicates. We present proteins of interest as a ratio of the target protein to loading control \( (n = 5 \text{ immunoblots}) \). Experimental replicates were compared to controls using a one-sided Student’s t-test. For HTRF, levels of mHTT were calculated by taking the ratio of the fluorescence signals \( (665 \text{ nM}/615 \text{ nM}) \) and normalizing to the PicoGreen signal in experimental groups after subtracting the signal from wells containing only sample buffer and HTRF buffer, without protein lysates. Results are presented as the average and standard error of the mean of the \( \Delta F \% \) \( (\Delta F \% = (\text{Sample ratio} - \text{blank ratio})/\text{blank ratio} \times 100) \). Each treatment group consisted of nine replicates \( (n = 9) \). \( p \)-values were calculated using Fisher’s LSD test.

**Statistical analysis for immunohistochemistry**

The mean intensity for images of Serpina1a stained brain slices was measured using ImageJ. For each sample, five images were measured and the mean was calculated. The control group consisted of four samples \( (n = 4) \), and the HD group consisted of three samples \( (n = 3) \). Groups were compared using a two-tailed \( t \)-test assuming unequal variances.

**Acknowledgements**

We thank Vicky Brandt for critical input on the manuscript. We also thank Steve Goldman for sharing data cited in this work and his thoughtful insight. This work was supported by grants to JB from NIH/NIA (R01AG057339) and CHDI. BL is sponsored by Natural Science Foundation of China (31970747, 31601105, 81870990, 81925012). TO and MM were supported by the NIGMS Ruth L Kirschstein National Research Service Award (NRSA) Predoctoral Institutional Research Training Grant (T32 GM008307) provided to the Genetics and Genomics Graduate Program at Baylor College of Medicine. AL was supported by Baylor College of Medicine Medical Scientist Training Program and the NLM Training Program in Biomedical Informatics and Data Science (T15 LM007093) at the Gulf Coast Consortium. The High Throughput Behavioral Screening core at the Jan and Dan Duncan Neurological Research Institute was supported by generous philanthropy from the Hildebrand family foundation. The project was also supported by a shared instrumentation grant from the NIH (S10 OD016167) and Baylor College of Medicine IDDRC Grant Number P50HD103555 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development for use of the Microscopy Core facilities, the Cell and Tissue Pathogenesis Core, and the RNA In Situ Hybridization Core facility with the expert assistance of Dr. Cecilia Ljungberg. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Eunice Kennedy Shriver National Institute of Child Health and Human Development or the National Institutes of Health.

**Additional information**

| Funding | Grant reference number | Author |
|---------|-------------------------|--------|
| Natural Science Foundation of China | 31970747 | Boxun Lu |
| Natural Science Foundation of China | 31601105 | Boxun Lu |
| Natural Science Foundation of China | 81870990 | Boxun Lu |
| Natural Science Foundation of China | 81925012 | Boxun Lu |

Onur, Laitman, et al. eLife 2021;10:e64564. DOI: https://doi.org/10.7554/eLife.64564
The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Author contributions
Tarik Seref Onur, Data curation, Software, Formal analysis, Supervision, Validation, Investigation, Visualization, Methodology, Writing - original draft, Writing - review and editing; Andrew Laitman, Data curation, Software, Formal analysis, Funding acquisition, Validation, Investigation, Visualization, Methodology, Writing - original draft; He Zhao, Ryan Keyho, Hyemin Kim, Jennifer Wang, Huilan Wang, Data curation, Formal analysis, Investigation; Megan Mair, Data curation, Software, Formal analysis, Investigation; Lifang Li, Formal analysis, Investigation; Alma Perez, Investigation, Methodology; Maria de Haro, Resources, Investigation, Methodology; Ying-Wooi Wan, Data curation, Software, Formal analysis, Investigation, Visualization, Methodology; Genevera Allen, Software, Formal analysis, Visualization, Methodology; Boxun Lu, Conceptualization, Supervision, Investigation, Methodology; Ismael Al-Ramahi, Conceptualization, Resources, Investigation, Methodology, Zhandong Liu, Software, Supervision, Investigation, Methodology, Project administration; Juan Botas, Conceptualization, Resources, Supervision, Funding acquisition, Validation, Investigation, Methodology, Writing - original draft, Project administration, Writing - review and editing

Author ORCIDs
Tarik Seref Onur https://orcid.org/0000-0002-3234-6263
Juan Botas https://orcid.org/0000-0001-5476-5955

Decision letter and Author response
Decision letter https://doi.org/10.7554/eLife.64564.sa1
Author response https://doi.org/10.7554/eLife.64564.sa2

Additional files
Supplementary files
- Source code 1. R script for prefiltering differentially expressed genes (DEGs) in Drosophila Huntington’s disease (HD) models.
- Source code 2. R script for identification of differentially expressed genes (DEGs) in Drosophila Huntington’s disease (HD) models.
- Source code 3. Python code for identification of differentially expressed genes (DEGs) in postmortem human Huntington’s disease (HD) striata.
- Source code 4. Python code for identification of differentially expressed genes (DEGs) from Huntington’s disease (HD) mouse models.
- Source code 5. Python code for homology mappings across humans, mice, and Drosophila.
- Source code 6. Python code for comparing Huntington’s disease (HD) differentially expressed genes (DEGs) across species.
- Source code 7. Python code for clustering networks.
- Source code 8. R script for network analysis in a striatal background summarized in Supplementary file 2.
• Source code 9. R script for analyzing Drosophila behavioral assays.
• Supplementary file 1. Mappings of orthologous Huntington’s disease (HD) human, mouse, and Drosophila differentially expressed genes (DEGs).
• Supplementary file 2. Network connectivity of differentially expressed genes (DEGs) responding to glial or neuronal mutant Huntingtin (mHTT) expression.
• Supplementary file 3. Cluster membership and summary of cluster annotations for differentially expressed genes (DEGs) responding to glial or neuronal mutant Huntingtin (mHTT) expression.
• Supplementary file 4. Summary of statistical analysis for alleles in the Synapse Assembly cluster that modify glial mutant Huntingtin (mHTT)-induced behavioral impairments.
• Supplementary file 5. Summary of alleles screened and results for common modifiers of mutant Huntingtin (mHTT)-induced behavioral impairments in neurons and glia.
• Supplementary file 6. Summary of statistics for alleles that are common suppressors of neuronal and glial mutant Huntingtin (mHTT)-induced behavioral impairments.
• Transparent reporting form

Data availability
RNA-sequencing data produced by this study has been deposited in GEO under accession code GSE157287. We have provided source data for figures 2—6, and for figure 3-figure supplement 1, figure 5-figure supplement 1, and figure 6-figure supplements 1—3. Codes for analyzing gene expression, networks, and Drosophila behavior are provided.

The following dataset was generated:

| Author(s)          | Year | Dataset title                                                                 | Dataset URL                                                                 | Database and Identifier                      |
|--------------------|------|-------------------------------------------------------------------------------|------------------------------------------------------------------------------|-----------------------------------------------|
| Onur TS, Laitman A, Perez A, Wan YW, Al-Ramahi I, Liu Z, Botas J | 2020 | RNA-sequencing of Drosophila expressing mutant Huntington in neurons or glia | https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE157287                 | NCBI Gene Expression Omnibus, GSE157287       |

The following previously published datasets were used:

| Author(s)                   | Year | Dataset title                                                                 | Dataset URL                                                                 | Database and Identifier                      |
|-----------------------------|------|-------------------------------------------------------------------------------|------------------------------------------------------------------------------|-----------------------------------------------|
| Osipovitch M, Asenjo-Martinez A, Cornwell A, Dhaliwal S, Zou L, Chandler-Militello D, Wang S, Li X, Benraiss S-J, Lampp A, Benraiss A, Windrem M, Goldman SA | 2018 | hESC-based human glial chimeric mice reveal glial differentiation defects in Huntington disease | https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE105041                 | NCBI Gene Expression Omnibus, GSE105041       |
| Langfelder P, Gao F, Wang N, Howland D, Kwak S, Vogt TF, Aaronson JS, Rosinski J, Coppola G, Horvath S, Yang WX | 2016 | Transcriptome profiling in knock-in mouse models of Huntington’s disease [striatum; cortex; liver; tissue survey] | https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE65776                 | NCBI Gene Expression Omnibus, GSE65776       |
| Hodges A, Strand AD, Aragaki AK, Kuhn A, Sengstag T, Hughes G, Elliston LA, Hartog C, Goldstein DR, Thu D, Hollingsworth ZR, Collin F, Synk B, Holmans PA, Young | 2006 | Human cerebellum, frontal cortex [BA4, BA9] and caudate nucleus HD tissue experiment | https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE3790                 | NCBI Gene Expression Omnibus, GSE3790         |
References

Abu-Rumeileh S, Halbg beauer S, Steinacker P, Anderl-Straub S, Poli schi B, Ludolphi AC, Capell ari S, Parchi P, Otto M. 2020. CSF SerpinA1 in Creutzfeldt-Jakob disease and frontotemporal lobar degeneration. Annals of Clinical and Translational Neurology 7:191–199. DOI: https://doi.org/10.1016/j.yactn.2020.03.005, PMID: 31957347

Al-Dalhamah O, Sosunov AA, Shaik A, Ofori K, Liu Y, Vonsattel JP, Adorjan I, Menon V, Goldman JE. 2020. Single-nucleus RNA-seq identifies Huntington disease astrocyte states. Acta Neuropsycho logica Communications 8:19. DOI: https://doi.org/10.1186/s40478-020-0880-6, PMID: 32070434

Al-Ramahi I, Lu B, Di Paola S, Pang K, de Haro M, Peluso I, Gallego-Flores T, Malik NT, Erikson K, Bleiberg BA, Avalos M, Fan G, Rivers LE, Laitman AM, Diaz-García JR, Hild M, Palacino J, Liu Z, Medina DL, Botas J. 2018. High-Throughput functional analysis distinguishes pathogenic, Nonpathogenic, and compensatory transcriptional changes in neurodegeneration. Cell Systems 7:28–40. DOI: https://doi.org/10.1016/j.cels.2018.05.010, PMID: 29936182

Bardai FH, Ordonez DG, Bailey RM, Hamm M, Lewis J, Feany MB. 2018. Lrrk promotes tau neurotoxicity through dysregulation of actin and mitochondrial dynamics. PLOS Biology 16:e2006265. DOI: https://doi.org/10.1371/journal.pbio.2006265, PMID: 30571694

Barker RA, Fujimaki M, Rogers P, Rubinstein DC. 2020. Huntington-in lowering strategies for Huntington's disease. Expert Opinion on Investigational Drugs 29:1125–1132. DOI: https://doi.org/10.1080/13543784.2020.1804552, PMID: 32745442

Barnat M, Capizzi A, Aparicio E, Boluda S, Wennagel K, Kacher R, Lenoir S, Agasse F, Evers MM, Konstantinova P, Hayden MR. 2020. Potent and sustained huntingtin lowering via AAV5 encoding PAK1 regulates ATXN1 levels providing an opportunity to modify its toxicity in spinocerebellar ataxia type 1. Human Molecular Genetics 29:16005–160028. DOI: https://doi.org/10.1093/hmg/ddy200, PMID: 32675289

Bayraktar OA, Bartels T, Holmqvist S, Kleshchevnikov V, Martirosyan A, Polioudakis D, Ben Haim L, Young AMH, Batiuk MY, Prakash K, Brown A, Roberts K, Paredes MF, Kawaguchi R, Stockley JH, Sabeur K, Chang SM, Huang E, Hutchinson P, Ullian EM, et al. 2020. Astrocyte layers in the mammalian cerebral cortex revealed by a single-cell in situ transcriptomic map. Nature Neuroscience 23:500–509. DOI: https://doi.org/10.1038/s41593-020-0602-1, PMID: 32203496

Benraiss A, Wang S, Herrlinger S, Li X, Chandler-Militello D, Macaueri J, Burm HB, Toner M, Osipovitch M, Jim Xu Q, Ding F, Wang F, Kang N, Kang J, Curtin PC, Brunner D, Windrem MS, Munoz-Sanjuan I, Nedergaard M, Goldman SA. 2016. Human Glia can both induce and rescue aspects of disease phenotype in Huntington disease. Human Molecular Genetics 27:11758. DOI: https://doi.org/10.1093/hmg/ddv175, PMID: 27273432

Blake JA, Eppig JT, Kadin JA, Richardson JE, Smith CL, Bult CJ, the Mouse Genome Database Group. 2017. Mouse genome database (MGD)-2017: community knowledge resource for the laboratory mouse. Nucleic Acids Research 45:D723–D729. DOI: https://doi.org/10.1093/nar/gkw1040, PMID: 27899570

Bondar VV, Adamski CJ, Onur TS, Tan Q, Wang L, Diaz-García J, Park J, Orr HT, Botas J, Zoghbi HY. 2018. PAK1 regulates ATXN1 levels providing an opportunity to modify its toxicity in spinocerebellar ataxia type 1. Human Molecular Genetics 27:2863–2873. DOI: https://doi.org/10.1093/hmg/ddy200, PMID: 29680311

Bradford J, Shin J-Y, Roberts M, Wang C-E, Li X-J, Li S. 2009. Expression of mutant huntingtin in mouse brain astrocytes causes age-dependent neurological symptoms. PNAS 106:22480–22485. DOI: https://doi.org/10.1073/pnas.0911503106

Branco J, Al-Ramahi I, Ukani L, Pérez AM, Fernandez-Funez P, Rincón-Limas D, Botas J. 2008. Comparative analysis of genetic modifiers in Drosophila points to common and distinct mechanisms of pathogenesis among polyglutamine diseases. Human Molecular Genetics 17:376–390. DOI: https://doi.org/10.1093/hmg/ddm315, PMID: 17984172

Buscemi L, Genet V, Lopatar J, Monta na V, Pucci L, Spagnuolo P, Zehnder T, Grubišić V, Truttman AM, Sala C, Hirt L, Parpura V, Puyal J, Beppi P. 2017. Homer1 scaffold proteins govern Ca2+ dynamics in normal and reactive astrocytes. Cerebral Cortex 27:2365–2384. DOI: https://doi.org/10.1093/cercor/bhw078, PMID: 27075036

Cabezas-Llobet N, Campurbi S, García B, Alberch J, Xifró X. 2018. Human alpha 1-antitrypsin protects neurons and glial cells against oxygen and glucose deprivation through inhibition of interleukins expression. Biochimica Et Biophysica Acta (BBA) - General Subjects 1862:1852–1861. DOI: https://doi.org/10.1016/j.bbagen.2018.05.017

Caron NS, Southwell AL, Brouwers CC, Cengio LD, Xifro X, Black HF, Anderson LM, Ko S, Zhu X, van Deventer SJ, Evers MM, Konstantinova P, Hayden MR. 2020. Potent and sustained huntingtin lowering via AAV5 encoding miRNA preserves striatal volume and cognitive function in a humanized mouse model of Huntington disease. Nucleic Acids Research 48:36–54. DOI: https://doi.org/10.1093/nar/gkz976, PMID: 31745548

Chung HL, Wangler MF, Marcogliese PC, Jo J, Ravenscroft TA, Zuo Z, Duraine L, Salehjazadeh S, Li-Kroeger D, Schmidt RE, Pestronk A, Rosenfeld JA, Burrel L, Hendron MJ, Chen S, Shillington A, Vawter-Lee M, Hopkin R,
Onur, Laitman, et al. eLife 2021;10:e64564. DOI: https://doi.org/10.7554/eLife.64564
Jamain S, Quach H, Betancur C, Råstam M, Collineau C, Gilberg IC, Soderstrom H, Giros B, Leboyer M, Gilberg C, Bourgeron T. 2003. Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. Nature Genetics 34:27–29. DOI: https://doi.org/10.1038/ng1136, PMID: 12669065

Jiang R, Diaz-Castro B, Looger LL, Khakh BS. 2016. Dysfunctional calcium and glutamate signaling in striatal astrocytes from Huntington’s Disease Model Mice. Journal of Neuroscience 36:3453–3470. DOI: https://doi.org/10.1523/JNEUROSCI.3693-15.2016, PMID: 27013675

Kaltenbach LS, Romero E, Becklin RR, Chettier R, Bell R, Phansalkar A, Strand A, Torcassi C, Savage J, Hurlburt A, Cha GH, Ukanli L, Chepanoske CL, Zhen Y, Sahasarabudhe S, Olson J, Kurschner C, Ellerby LM, Peltier JM, Botas J, et al. 2007. Huntingtin interacting proteins are genetic modifiers of neurodegeneration. PLOS Genetics 3:e82. DOI: https://doi.org/10.1371/journal.pgen.0030082, PMID: 17500595

Kim YJ, Yi Y, Sapp E, Wang Y, Cuifo B, Kegel KB, Qin ZH, Aronin N, DiFiglia M. 2001. Caspase 3-cleaved N-terminal fragments of wild-type and mutant huntingtin are present in normal and Huntington’s disease brains, associate with membranes, and undergo calpain-dependent proteolysis. PNAS 98:12784–12789. DOI: https://doi.org/10.1073/pnas.221451398, PMID: 11675509

Langfelder P, Cantle JP, Chatzopoulou D, Wang N, Gao F, Al-Ramahi I, Lu XH, Ramos EM, El-Zein K, Zhao Y, Deveraseta S, Tebbe A, Schaab C, Lavery DJ, Howland D, Kwak S, Botas J, Aaronson JS, Rosinski J, Copolla G, et al. 2016. Integrated genomics and proteomics define huntingtin CAG length-dependent networks in mice. Nature neuroscience 19:623–633. DOI: https://doi.org/10.1038/nn.4256, PMID: 26909923

Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with bowtie 2. Nature Methods 9:357–359. DOI: https://doi.org/10.1038/nmeth.1923, PMID: 22382896

Li Z, Wang C, Wang Z, Zhu C, Li J, Sha T, Ma L, Gao C, Yang Y, Sun Y, Wang J, Sun X, Lu C, DiFiglia M, Yei, Ding C, Luo S, Dang Y, Ding Y, Fei Y, et al. 2019. Allele-selective lowering of mutant HTT protein by HTT-LC3 linker compounds. Nature 575:203–209. DOI: https://doi.org/10.1038/s41586-019-1722-1, PMID: 31666698

Li B, Dewey CN. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. Genome biology 12:323. DOI: https://doi.org/10.1186/1471-2105-12-323

Lian H, Yang L, Cole A, Sun L, Chiang AC, Fowler SW, Shim DJ, Rodriguez-Rivera J, Taglialetela G, Jankowsky JL, Lu HC, Zheng H. 2015. NFκB-activated astroglial release of complement C3 compromises neuronal morphology and function associated with Alzheimer’s disease. Neuron 85:101–115. DOI: https://doi.org/10.1016/j.neuron.2014.11.018, PMID: 25533482

Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, Bennett ML, Münch AE, Chung WS, Peterson TC, Wilton DK, Frouin A, Napier BA, Panicker N, Kumar M, Buckwalter MS, Rowitch DH, Dawson VL, Dawson TM, Stevens B, et al. 2017. Neuroactive reactive astrocytes are induced by activated microglia. Nature 541:481–487. DOI: https://doi.org/10.1038/nature21029, PMID: 28099414

Litvinchuk A, Wan YW, Swartzlander DB, Chen F, Cole A, Propson NE, Wang Q, Zhang B, Liu Z, Zheng H. 2018. Complement C3αR inactivation attenuates tau pathology and reverses an immune network deregulated in tauopathy models and Alzheimer’s Disease. Neuron 100:1337–1353. DOI: https://doi.org/10.1016/j.neuron.2018.03.031, PMID: 30415998

Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15:550. DOI: https://doi.org/10.1186/s13059-014-0505-0, PMID: 25516281

McInnes J, Wierda K, Snellina A, Boulti L, Wang YC, Stancu IC, Apo ´ stolo N, Gevaert K, Dewachter I, Spires-Jones TL, De Strooper B, De Wit J, Zhou L, Verstreken P. 2018. Synaptopotinin-3 mediates presynaptic dysfunction induced by tau. Neuron 97:823–835. DOI: https://doi.org/10.1016/j.neuron.2018.01.022, PMID: 29398363

McKinstry SU, Karadeniz YB, Worthington AK, Hayrapetyan YY, Ozlu MI, Serafin-Molina K, Risher WC, Ustunkaya T, Dragatis I, Zeitlin S, Yin HH, Eroglu C. 2014. Huntingtin is required for normal excitatory synapse development in cortical and striatal circuits. Journal of Neuroscience 34:9455–9472. DOI: https://doi.org/10.1523/JNEUROSCI.4699-13.2014

Miller JA, Menon V, Goldy J, Kayaks A, Lee CK, Smith KA, Shen EH, Phillips JW, Lein ES, Hawrylycz MJ. 2014. Improving reliability and absolute quantification of human brain microarray data by filtering and scaling probes using RNA-Seq. BMC genomics 15:154. DOI: https://doi.org/10.1186/1471-2164-15-154, PMID: 24564186

Nakanishi M, Nomura J, Ji X, Tamada K, Arai T, Takahashi E, Bucan M, Takumi T. 2017. Functional significance of rare neuroligin 1 variants found in autism. PLOS genetics 13:e1006940. DOI: https://doi.org/10.1371/journal.pgen.1006940, PMID: 28841651

Neu eder A, Landles C, Ghosh R, Howland D, Myers RH, Faull RLM, Tabrizi SJ, Bates GP. 2017. The pathogenic exon 1 HTT protein is produced by incomplete splicing in Huntington’s disease patients. Scientific Reports 7:e01510-z. DOI: https://doi.org/10.1038/s41598-017-01510-z

O’Rourke JG, Gareau JR, Ochaba J, Song W, Raskó T, Reverter D, Lee J, Montes AM, Palles J, Mee L, Vashishtha M, Apostol BL, Nicholson TP, Illes K, Zhu YZ, Dasso M, Bates GP, DiFiglia M, Davidson B, Wanker EE, et al. 2013. SUMO-2 and PIA51 modulate insoluble mutant huntingtin protein accumulation. Cell Reports 4:362–375. DOI: https://doi.org/10.1016/j.celrep.2013.06.034, PMID: 23871671

Ochaba J, Lukacsovich T, Csikos G, Zheng S, Margulis J, Salazar L, Mao K, Lau AL, Yeung SY, Humbert S, Saudou F, Klionsky DJ, Finkbeiner S, Zeitlin SO, Marsh JL, Housman DE, Thompson LM, Steffen JS. 2014. Potential function for the huntingtin protein as a scaffold for selective autophagy. PNAS 111:16889–16894. DOI: https://doi.org/10.1073/pnas.1420103111, PMID: 25385587
Onur, Laitman, et al. eLife 2021;10:e64564. DOI: https://doi.org/10.7554/eLife.64564
The Huntington’s Disease Collaborative Research Group. 1993. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington’s disease chromosomes. The Huntington’s Disease Collaborative Research Group. Cell 72:971–983. DOI: https://doi.org/10.1016/0092-8674(93)90585-e, PMID: 8458085

Tong X, Ao Y, Faas GC, Nwaobi SE, Xu J, Haustein MD, Anderson MA, Mody I, Olsen ML, Sofroniev MV, Khakh BS. 2014. Astrocyte KIR4.1 ion channel deficits contribute to neuronal dysfunction in Huntington’s disease model mice. Nature neuroscience 17:694–702. DOI: https://doi.org/10.1038/nn.3691, PMID: 24686787

Trajkovic K, Jeong H, Kraemer D. 2017. Mutant huntingtin is secreted via a late endosomal/Lysosomal unconventional secretory pathway. The Journal of Neuroscience 37:9000–9012. DOI: https://doi.org/10.1523/JNEUROSCI.0118-17.2017, PMID: 28821645

Trotter JH, Dargaei Z, Wohr M, Liakath-Ali K, Raja K, Essayan-Perez S, Nabet A, Liu X, Sudhof TC. 2020. Astrocytic Neurexin-1 Orchestrates functional synapse assembly. bioRxiv. DOI: https://doi.org/10.1101/2020.08.21.262097

Tsuang DW, Greenwood TA, Jayadev S, Davis M, Shutes-David A, Bird TD. 2018. A genetic study of psychosis in Huntington’s disease: evidence for the involvement of glutamate signaling. Journal of Neuroscience 7:51–59. DOI: https://doi.org/10.3233/JHD-170277

Ushkaryov YA, Petrenko AG, Geppert M, Sudhof TC. 1992. Neurexins: synaptic cell surface proteins related to the alpha-latrotoxin receptor and laminin. Science 257:50–56. DOI: https://doi.org/10.1126/science.1621094, PMID: 1621094

Vaags AK, Lionel AC, Sato D, Goodenberger M, Stein QP, Curran S, Ogilvie C, Ahn JW, Drmic I, Senman L, Chysler C, Thompson A, Russell C, Prasad A, Walker S, Pinto D, Marshall CR, Stavropoulos DJ, Zwaigenbaum L, Fernandez BA, et al. 2012. Rare deletions at the neurexin 3 locus in autism spectrum disorder. American journal of human genetics 90:133–141. DOI: https://doi.org/10.1016/j.ajhg.2011.11.025, PMID: 22209245

Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP. 1985. Neuropathological classification of Huntington’s disease. Journal of neuropathology and experimental neurology 44:559–577. DOI: https://doi.org/10.1097/00005072-198511000-00003, PMID: 2935239

Wang N, Gray M, Lu XH, Cantle JP, Holley SM, Greiner E, Gu X, Shirasaki D, Cepeda C, Li Y, Dong H, Levine MS, Yang XW. 2014. Neuronal targets for reducing mutant huntingtin expression to ameliorate disease in a mouse model of Huntington’s disease. Nature medicine 20:536–541. DOI: https://doi.org/10.1038/nm.3514, PMID: 24784230

Wang J, Gong J, Li L, Chen Y, Liu L, Gu H, Luo X, Hou F, Zhang J, Song R. 2018. Neurexin gene family variants as risk factors for autism spectrum disorder: genetic risk for autism spectrum disorder. Autism Research 11:37–43. DOI: https://doi.org/10.1002/aur.1881

Weiss S, Melon JE, Ormerod KG, Zhang YV, Littleton JT. 2019. Glial Ca	extsuperscript{2+} signaling links endocytosis to K	extsuperscript{+} buffering around neuronal somas to regulate excitability. eLife 8:e44186. DOI: https://doi.org/10.7554/eLife.44186, PMID: 31025939

Wellington CL, Ellerby LM, Gutekunst C-A, Rogers D, Warby S, Graham RK, Loubser O, van Raamsdonk J, Singaraja R, Yang Y-Z, Gafni J, Breeden D, Hersch SM, Leavitt BR, Roy S, Nicholson DW, Hayden MR. 2002. Caspase cleavage of mutant huntingtin precedes neurodegeneration in Huntington’s Disease. The Journal of Neuroscience 22:7862–7872. DOI: https://doi.org/10.1523/JNEUROSCI.21-08-07862.2002

Windrem MS, Otsipovitch M, Liu Z, Bates J, Chandler-Militello D, Zou L, Munir J, Schan Z, McCoy K, Miller RH, Wang S, Nedergaard M, Finding RL, Tesar PJ, Goldman SA. 2017. Human iPSC glial mouse chimeras reveal glial contributions to schizophrenia. Cell Stem Cell 21:195–208. DOI: https://doi.org/10.1016/j.stem.2017.06.012, PMID: 28736215

Wood TE, Barry J, Yang Z, Cepeda C, Levine MS, Gray M. 2018. Mutant huntingtin reduction in astrocytes slows disease progression in thebachd conditional Huntington’s disease mouse model. Human Molecular Genetics 28:487–500. DOI: https://doi.org/10.1093/hmg/ddy363

Yamamoto A, Lucas JJ, Hen R. 2000. Reversal of neuropathology and motor dysfunction in a conditional model of Huntington’s disease. Cell 101:57–66. DOI: https://doi.org/10.1016/S0092-8674(00)00623-6, PMID: 10778856

Yao Y, Cui X, Al-Ramahi I, Sun X, Li B, Hou J, Difiglio M, Palacino J, Wu Z-Y, Ma L, Botas J, Lu B. 2015. A striatal-enriched intronic GPCR modulates huntingtin levels and toxicity. eLife 4:e05449. DOI: https://doi.org/10.7554/eLife.05449

Yaylaoglu MB, Tittmus A, Visel A, Alvarez-Bolado G, Thaller C, Eichele G. 2005. Comprehensive expression atlas of fibroblast growth factors and their receptors generated by a novel robotic in situ hybridization platform. Developmental dynamics 234:371–386. DOI: https://doi.org/10.1002/dvdy.20441, PMID: 16123981

Yuva-Aydemir Y, Almeida S, Gao FB. 2018. Insights into C9ORF72-Related ALS/FTD from Drosophila and iPSC models. Trends in Neurosciences 41:457–469. DOI: https://doi.org/10.1016/j.tins.2018.04.002, PMID: 29729808

Zeng X, Sun M, Liu L, Chen F, Wei L, Xie W. 2007. Neurexin-1 is required for synapse formation and larval associative learning in Drosophila. FEBS letters 581:2509–2516. DOI: https://doi.org/10.1016/j.febslet.2007.04.068, PMID: 17498701

Ziegenfuss JS, Doherty J, Freeman MR. 2012. Distinct molecular pathways mediate glial activation and engulfment of axonal debris after axotomy. Nature neuroscience 15:979–987. DOI: https://doi.org/10.1038/nn.3135, PMID: 22706267