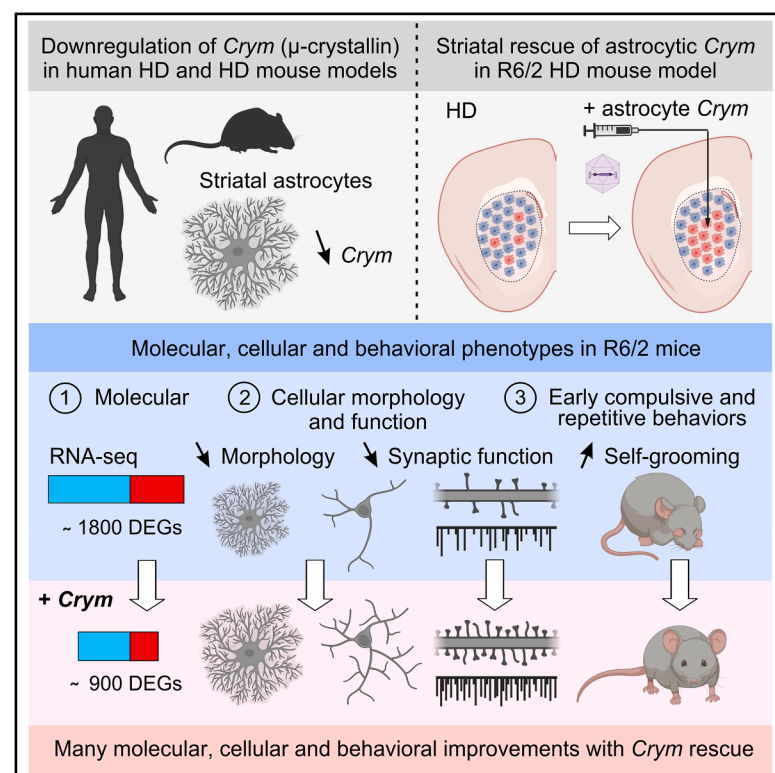


Striatal astrocyte μ -crystallin (*Crym*) loss contributes to early-stage Huntington's disease pathophysiology

Graphical abstract



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In brief

Crym-positive astrocytes represent a subpopulation within the striatum. This study identifies μ -crystallin-expressing *Crym*-positive astrocytes as modulators of Huntington's disease pathophysiology and suggests that restoring μ -crystallin or targeting *Crym*-positive astrocytes could be beneficial in early disease stages.

Highlights

- Striatal astrocyte *Crym* expression is reduced in Huntington's disease
- Rescue of astrocyte *Crym* improves phenotypes of HD model mice
- Targeting *Crym*+ astrocytes in HD may be beneficial
- Astrocyte subpopulations may be exploitable in disease

Article

Striatal astrocyte μ -crystallin (*Crym*) loss contributes to early-stage Huntington's disease pathophysiology

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SUMMARY

A striatal astrocyte subpopulation expressing μ -crystallin (gene: *Crym*) regulates perseverative behaviors, but its role in disease is unclear. We found that astrocytic *Crym* and μ -crystallin protein expression levels are reduced in human Huntington's disease (HD) tissue, cerebrospinal fluid, and HD mouse models. Selective astrocytic μ -crystallin restoration in a HD mouse model improved astrocyte and neuronal morphology, electrophysiology, gene expression, and early-stage compulsive and repetitive behaviors. Late-stage motor deficits remained mostly unchanged. Approximately 32% of the μ -crystallin rescued astrocytic genes overlapped with those dysregulated in human HD, indicating conserved molecular corrections. These results identify μ -crystallin-expressing astrocytes as modulators of HD pathophysiology and suggest that restoring μ -crystallin or targeting *Crym*-positive astrocytes could be beneficial in early disease stages.

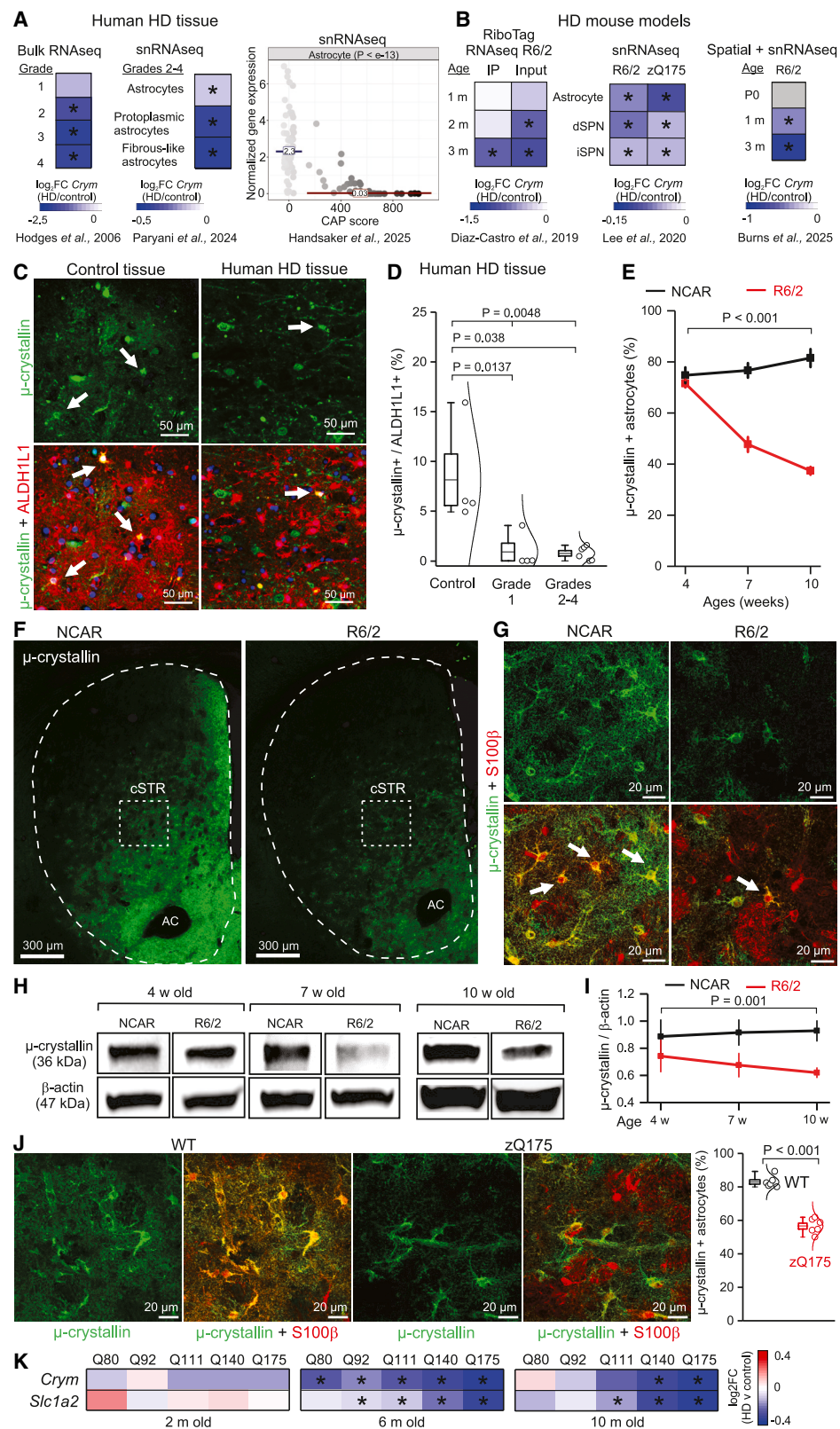
INTRODUCTION

Huntington's disease (HD) is a progressive neurodegenerative disorder characterized by early psychiatric and cognitive symptoms and later-stage motor deficits.¹ The disease is caused by polyglutamine repeats in the Huntingtin (HTT) protein, leading to the production of a mutant protein (mHTT) that has harmful effects.¹ The striatum is particularly vulnerable and exhibits atrophy. In both HD mouse models and human HD tissue, striatal neurons and astrocytes express mHTT,^{2,3} and RNA sequencing (RNA-seq) of postmortem HD samples reveals that astrocytes and neurons are altered.^{4–8} Astrocytes display functional deficits in HD,^{8,9} but how they contribute to stages of HD pathogenesis remains largely unexplored.

Astrocytes¹⁰ are vital components of neural circuits^{11,12} and are involved in brain disorders.^{8,13–15} Since astrocytes interact extensively with neurons, understanding how astrocytes contribute to physiological and pathological processes in the

central nervous system (CNS) is important.^{15,16} For example, an important recent finding has been that astrocytes are diverse, comprising populations displaying brain region-specific properties.^{17–20} The functions of such region-enriched astrocytes are being explored for plasticity, physiology, behavior, and disease,¹⁹ including for HD.^{9,21,22}

Recent work provided evidence for anatomically allocated *Crym*-positive striatal astrocytes^{23,24} and their roles in regulating behavior.²⁵ Such striatal astrocytes expressed *Crym* and its gene product protein, μ -crystallin.²⁶ μ -crystallin is a poorly understood cytosolic protein that is proposed to function as a ketimine reductase²⁷ or to bind NADPH to buffer thyroid hormone (T3), thus controlling T3 effects.^{26,28} In the CNS, *Crym* is found in populations of cortical, hippocampal, and amygdala neurons^{29,30} and is highly expressed in the striatum of humans.⁴ Little was known about μ -crystallin within *Crym*+ striatal astrocytes until recently when it was found that *Crym*-positive astrocytes regulate striatal neurons to control perseverative behaviors.²⁵



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We explored *Crym*⁺ and μ -crystallin-expressing astrocytes²⁵ mainly from the central striatum (cSTR) and show that they make a measurable contribution to early-stage HD pathophysiology in mice, extending early studies.³¹ In humans, early-stage HD symptoms such as compulsions can be severe,³² but the underlying mechanisms remain obscure. Our findings show that *Crym*⁺ astrocytes contribute to early-stage phenotypes of compulsions and repetitive behaviors, although not to later-stage motor deficits in mice. Our findings show that identifying and targeting populations of astrocytes,^{17–19} such as the *Crym*⁺ subpopulation, may be useful in HD and other brain diseases.

RESULTS

Crym mRNA and μ -crystallin protein levels are decreased in striatal astrocytes in HD

Strong evidence for astrocyte diversity comes from studies of different regions of the adult mouse CNS.^{17,18,20,23,24,33–35} Such studies show that striatal astrocytes express a gene called *Crym*^{23,24,36} encoding for the protein μ -crystallin.²⁶ Since astrocytes are known to contribute to HD pathogenesis,⁹ we explored if *Crym* was altered in published HD RNA-seq studies.

By assessing published RNA-seq studies of human HD, we found that *Crym* levels were decreased in bulk striatal tissue⁶ and within striatal astrocytes,^{4,5,7} including with increasing disease severity assessed with the CAG-age product (CAP), a surrogate measure of disease burden based on CAG repeat length and age (Figure 1A). Similarly, *Crym* levels decreased in striatal astrocytes from R6/2 and zQ175 HD mouse models^{37–39} assessed using RiboTag RNA-seq, single-nucleus RNA-seq (snRNA-seq),⁵ and snRNA-seq with spatial transcriptomics⁴⁰ (Figure 1B). To further characterize *CRYM* expression in human astrocytes, we analyzed single-cell RNA-seq data from control and HD human caudate samples, including both protoplasmic and fibrous astrocytes⁴ (Figure S1). *CRYM* expression was enriched in protoplasmic astrocyte clusters P1 and P3 and was reduced in HD.⁴ In fibrous astrocytes, *CRYM* expression was limited to a subset of cells and was decreased in HD

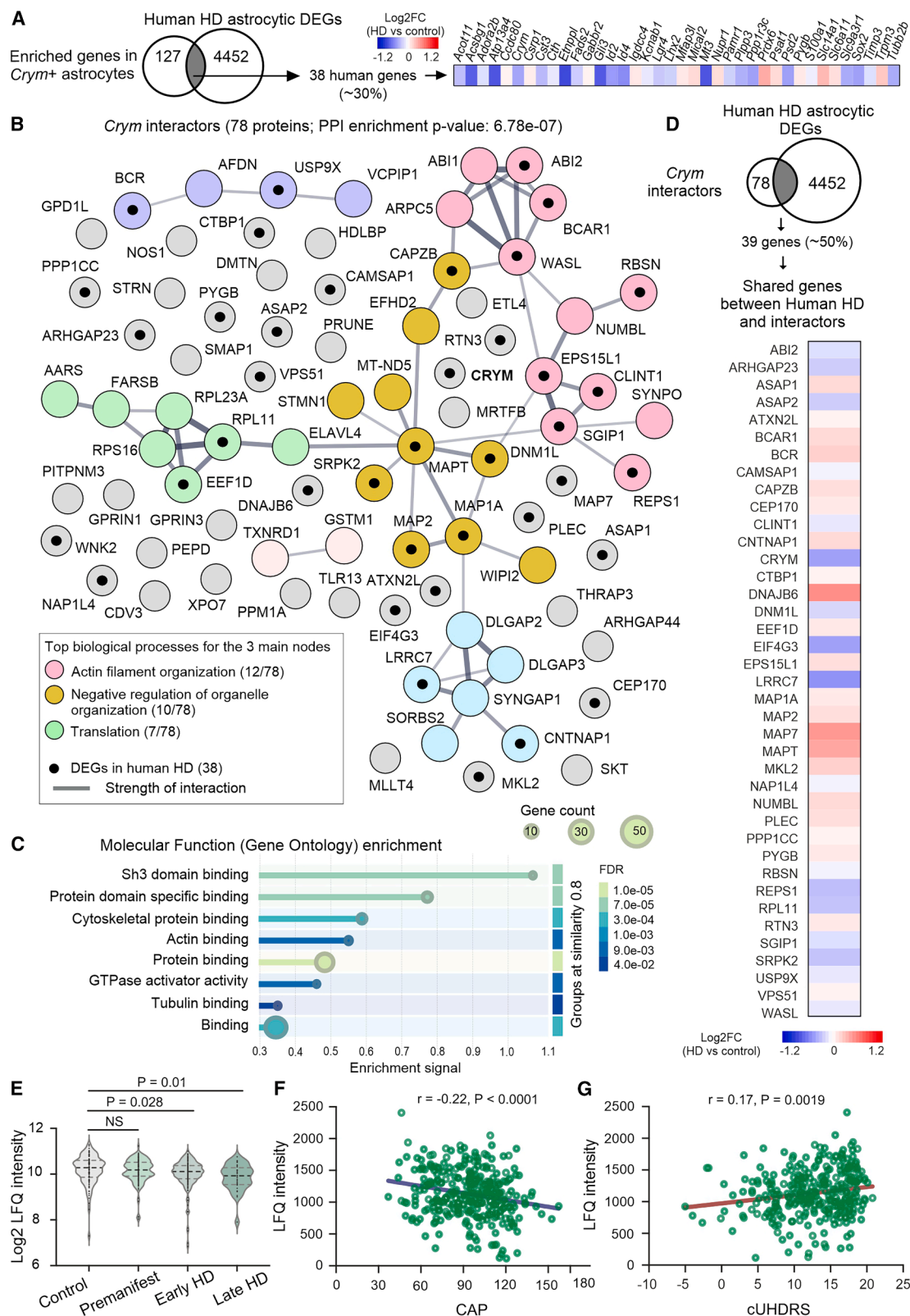
(Figure S1). The concordance across studies showing *Crym* levels decrease in HD and in HD model mice, specifically in caudate protoplasmic astrocytes,⁴ was striking (Figures 1A and 1B; Figure S1).

To examine protein expression,⁴² we performed μ -crystallin immunohistochemistry (IHC) on postmortem HD caudate nucleus tissue at early stages (Vonsattel grade 1) and advanced grades (Vonsattel grades 2, 3, and 4) relative to controls. We identified a μ -crystallin⁺ astrocyte population and found a significant decrease in the number of ALDH1L1⁺ astrocytes colocalized with μ -crystallin in HD (Figures 1C and 1D; $n = 10$ HD samples and $n = 4$ control samples). This reduction was detected in HD grade 1 brains, suggesting that μ -crystallin loss represents an early change. Similarly, compared with non-carrier (NCAR) controls, R6/2 HD mice exhibited reduced numbers of S100 β ⁺ astrocytes^{23–25,43} colocalizing with μ -crystallin in the cSTR and ventral striatum (vSTR) (Figures 1E–1G; Figures S2A–S2C; $n = 8$ mice). Interestingly, in mice, the reduced expression of μ -crystallin was age-dependent, being normal at 4 weeks and decreasing significantly by $\sim 50\%$ and $\sim 25\%$ thereafter (Figures 1E, 1H, and 1I; Figures S2 and S4; $n = 8$ mice). We also evaluated heterozygous zQ175 knockin HD model mice and their wild-type (WT) controls at 12 months of age.³⁸ In zQ175 mice we detected significantly reduced numbers of μ -crystallin-expressing astrocytes (Figure 1J; $n = 6$ mice) and decreased immunostaining for μ -crystallin (Figure S2F).

Because the decrease in μ -crystallin expression was greater with age in R6/2 mice (Figure 1E), we explored this trend using data from the allelic series of knockin HD mice that carry polyglutamine lengths between 80 and 175 and that have been assessed by bulk striatal RNA-seq at 2, 6, and 10 months of age⁴⁴ (relative to a polyglutamine length of 20 as control). In these comparisons, we used astrocytic *Slc1a2* as a reference because it is known to decrease.⁴⁵ At 2 months of age, we found no decrease in either *Crym* or *Slc1a2* at any polyglutamine length (Figure 1K). However, at 6 and 10 months of age, *Crym* and *Slc1a2* decreased equivalently in a polyglutamine length-dependent manner (Figure 1K).

Figure 1. *Crym* mRNA and μ -crystallin protein levels are decreased in striatal astrocytes in HD

- (A) *Crym* expression in human caudate tissue from HD patients vs. controls using bulk RNA-seq (Hodges et al.⁶) and astrocyte snRNA-seq (Lee et al.⁵ Paryani et al.⁴ and Handsaker et al.⁷).
- (B) Striatal *Crym* expression in HD mouse models vs. NCAR controls (Diaz-Castro et al.,⁴¹ Lee et al.,⁵ and Burns et al.⁴⁰). Asterisks indicate statistically significant differences reported in the corresponding studies, whereas the heatmaps show the trend.
- (C) μ -crystallin immunostaining (green) in ALDH1L1-positive astrocytes (red) from control and HD human caudate tissue. White arrows indicate examples of co-localization.
- (D) μ -crystallin⁺ astrocytes in control, grade 1 HD, and grades 2–4 HD caudate tissue ($n = 4$ for control, $n = 4$ for grade 1, and $n = 6$ across grades 2, 3, and 4; Table S1). A Kruskal-Wallis t test (the upper p value) was performed to compare the proportion of μ -crystallin-positive astrocytes in controls ($n = 4$) to HD ($n = 10$) with a non-parametric ANOVA. The other p values represent comparisons of controls to grade 1 HD ($n = 4$) and to HD grades 2–4 ($n = 6$) with Dunn's post hoc tests.
- (E) μ -crystallin⁺ astrocytes in NCAR and R6/2 mice at 4, 7, and 10 weeks ($n = 8$ brain slices, 4 mice per group, two-way ANOVA followed by Tukey's post hoc test).
- (F) Images showing striatal μ -crystallin expression in NCAR and R6/2 mice at 10 weeks.
- (G) μ -crystallin (green) and S100 β (red) in the central striatum (cSTR) of NCAR and R6/2 mice at 10 weeks. White arrows indicate co-localization.
- (H) μ -crystallin and β -actin from striatal tissue of NCAR and R6/2 mice at 4, 7, and 10 weeks.
- (I) μ -crystallin protein levels normalized to β -actin ($n = 8$ mice per group, two-way ANOVA followed by Tukey's post hoc test).
- (J) μ -crystallin (green) and S100 β (red) in the striatum of WT and zQ175 mice at 12 months. Quantification (right) shows a decrease in the percentage of μ -crystallin⁺ astrocytes in zQ175 mice ($n = 6$ mice per group, two-sample t test).
- (K) *Crym* and *Slc1a2* expression in knockin HD mice carrying different polyglutamine (CAG) repeat lengths (80–175) compared with control mice with 20 CAGs at 2, 6, and 10 months. Asterisks indicate statistically significant changes.
- In this figure, box plots are presented with a mean $\pm$ SEM, and the whiskers show the 10th–90th percentile range along with the individual data points and the distribution.



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There were differences in the numbers of *Crym*⁺ astrocytes in human and mouse striatum (Figures 1D and 1E), and the age of humans relative to mice may explain this, but nonetheless the data clearly show that μ -crystallin protein expression is reduced in HD and in HD model mice (Figures 1C–1I), supporting gene expression findings (Figures 1A, 1B, and 1F; Figures S2A and S2C).

In mice, *Crym* was also found in striatal neurons adjacent to the ventricle²⁵ and some populations of cortical and hippocampal neurons.^{29,30,46} In these regions we found that μ -crystallin expression was also reduced in R6/2 HD model mice (Figures S2B, S2D, and S2E). In accord, in human RNA-seq analyses⁴ *CRYM* expression in both caudate direct and indirect pathway medium spiny neurons (MSNs) was markedly reduced in HD, particularly in *FOXP2*-enriched cluster 2 (Figures S3A–S3C). In human tissue, IHC for NeuN and μ -crystallin confirmed reduction of μ -crystallin protein expression in neurons of the ventral anterior striatum in HD samples (Figures S3D and S3E).

Genes associated with *Crym*⁺ astrocytes and the μ -crystallin interactome in HD

We next evaluated 127 genes enriched within *Crym*⁺ astrocytes²⁵ relative to other striatal astrocytes and using hypergeometric analysis found that ~30% (38/127 genes) overlapped with differentially expressed genes (DEGs) identified in human HD⁴ (Figure 2A). To explore how reduced μ -crystallin expression in HD may contribute to pathogenesis, we performed STRING analysis⁴⁷ on 78 μ -crystallin interactors^{43,48} within striatal astrocytes²⁵ to identify known and predicted protein-protein interactions (PPIs) and their major biological processes (Figures 2B and 2C). The μ -crystallin interactome was involved in functions related to actin filament organization, cytoskeletal protein binding, and actin binding (Figures 2B and 2C). Using hypergeometric analysis, we found that 50% (39/78 proteins) were altered in human HD tissue (Figure 2D). In Figure 2B, the μ -crystallin interacting proteins altered in human gene expression studies are shown with black dots.

We next explored if μ -crystallin was reduced in cerebrospinal fluid (CSF) from individuals with HD using mass spectrometry (Table S2). We found decreased protein expression by 12%

and 21%, respectively, when comparing healthy controls with HD gene carriers during early or advanced HD (Figure 2E). Correlating μ -crystallin expression level with the CAP score revealed a significant negative association ($r = -0.22$, $p < 0.0001$), consistent with group-wise differences (Figure 2F). When compared with the composite unified HD rating scale (cUHDRS), a score integrating four clinical measures where higher values reflect better motor, cognitive, and functional performance, μ -crystallin levels showed a significant positive correlation ($r = 0.17$, $p = 0.0019$), consistent with a decline of this protein as disease progresses (Figure 2G).

AAV2/5-mediated striatal astrocyte μ -crystallin restoration in HD model mice

To explore the consequences of μ -crystallin loss in HD, we used a transgenic Exon 1 mHTT model (R6/2) of HD^{37,49} (Figure 1). In this model, astrocytes can be genetically targeted^{50–56} and endpoints assessed. While no model recapitulates the human disease, R6/2 mice exhibit consistent signatures of transcriptional dysregulation between knockin full-length mouse models and human HD postmortem tissue evaluations.^{5,41,44,57} Advantages of R6/2 mice over knockin mouse models for electrophysiology and behavioral phenotyping were also a consideration.^{58–63}

In WT mice,²⁵ striatal *Crym*⁺ astrocytes were explored by using a loss-of-function approach in the cSTR. However, since we found that μ -crystallin levels were decreased in the cSTR and vSTR of HD model mice (Figure 1), we generated AAVs to restore μ -crystallin expression with local AAV2/5-mediated⁶⁴ delivery of *Crym*-P2A-mScarlet. For the controls, we used AAVs to express mScarlet alone (Figure 3A). AAVs were delivered to both R6/2 and NCAR mice (Figures 3A and 3B).

We used AAVs to deliver *Crym*-P2A-mScarlet or mScarlet to astrocytes at 4 weeks of age and performed evaluations at 7 and 10 weeks, followed by molecular studies that included IHC and RNA-seq (Figure 3A). We found that AAV2/5 *Crym*-P2A-mScarlet restored μ -crystallin expression levels in R6/2 mice to levels in NCAR mice (Figures 3B and 3C; $n = 9$ mice). No ectopic expression of μ -crystallin was detected in the dorsolateral striatum or in the subventricular zone (SVZ).²⁵ Furthermore, restored expression of μ -crystallin within the

Figure 2. Genes associated with *Crym*⁺ astrocytes and the μ -crystallin interactome are dysregulated in HD

- (A) Left, overlap between 127 genes enriched in *Crym*⁺ astrocytes identified by single-cell RNA-seq (Ollivier et al.²⁵) and astrocytic DEGs in human caudate HD tissue (from Paryani et al.⁴; 4,452 genes; p value < 0.05). Hypergeometric tests were performed for these comparisons: p value = 0 for human HD. Right, the corresponding heatmap shows the 38 shared genes in human HD caudate tissue (bottom).
- (B) Scale-free STRING protein-protein association network of the 78 μ -crystallin interactors identified²⁵ by *Crym*-BioID2 expressed in striatal astrocytes. Node colors indicate association with specific biological processes, and the thickness of gray edges reflects interaction strength. Black dots mark proteins that are DEGs in human HD.⁴
- (C) Functional enrichment analysis of the 78 μ -crystallin interactors using “Molecular Function” categories from STRING. The x axis shows the enrichment signal, which reflects enrichment magnitude and statistical significance (FDR) in STRING.
- (D) Top, Venn diagrams showing the overlap between the 78 μ -crystallin interactors in astrocytes (Ollivier et al.²⁵) and astrocytic DEGs in human HD caudate tissue (from Paryani et al.⁴; 4,452 genes; p value < 0.05). Hypergeometric tests were performed for these comparisons: p value = 0 for human HD. Bottom, heatmaps show the 39 shared genes in human HD caudate tissue.
- (E) Group-wise comparisons of μ -crystallin protein, expressed as log2 label-free quantification (LFQ) values between healthy controls and HD gene carriers in CSF ($n = 93$ controls, $n = 131$ premanifest HD, $n = 161$ early HD, and $n = 32$ late HD). Healthy control vs. premanifest HD corresponds to 3% decrease (non-significant), control vs. early HD to a 12% decrease ($p = 0.028$), and control vs. late HD to a 21% decrease ($p = 0.01$) of μ -crystallin protein CSF levels. Violin plot shows the median and quartiles.
- (F) Correlation between the CAP score (increases with HD disease state) and μ -crystallin protein expression ($r = -0.22$, $p < 0.0001$).
- (G) Correlation between the cUHDRS (decreases with HD disease state) and μ -crystallin expression ($r = 0.17$, $p = 0.0019$) in CSF. Pearson's correlation coefficient and p values are presented in panels (F)–(G).

cSTR in R6/2 mice was not significantly different from that observed in NCAR mice, providing no evidence for expression above normal levels (Figures 3B–3D). However, although we targeted the AAV microinjections to the cSTR, some expression was also seen in vSTR due to unavoidable spread of the AAV away from the injection site. Within the cSTR, the percentage of S100 β + astrocytes that expressed μ -crystallin ($\sim$ 80%) in R6/2 mice following AAV2/5 *Crym*-P2A-mScarlet was like NCAR mice for the cSTR (Figure 3D; $n = 9$ mice). We observed no expression of μ -crystallin in neurons in the cSTR²⁵ (Figure 3E), supporting the astrocyte specificity of the AAVs.^{23,65,66}

Astrocyte properties after μ -crystallin restoration in HD model mice

As expected, no mHTT puncta were found in NCAR mice (Figures 4A and 4B; $n = 3$ mice). By contrast, $\sim$ 40% of STR astrocytes contained mHTT puncta in R6/2 mice,^{51–54} and this percentage was unaltered by μ -crystallin expression (Figures 4A and 4B).

Astrocyte cellular assessments were performed at 7 weeks of age (Figure 3A) because we observed improved behaviors at this age⁶⁷ (see later). We used LifeAct-GFP to image the astrocyte actin cytoskeleton and territories^{43,68} and found that astrocyte territories were smaller in R6/2 mice^{53,56,69,70} when compared with NCAR mice; this deficit was rescued by astrocytic μ -crystallin expression (Figures 4D and 4F; $n = 5$ mice). Astrocytes also display loss of K⁺ channels in HD, which results in higher membrane resistance.⁵⁴ In accord, membrane resistance and conductance changes in R6/2 mice were recovered by astrocytic μ -crystallin expression (Figures 4G–4I; $n = 4$ mice). Although HD is associated with a reduction in several K⁺ channels,⁷¹ one K⁺ channel that displays reduced expression in R6/2 mice is Kir4.1.⁵⁴ We found that Ba²⁺-sensitive Kir4.1 currents were smaller in R6/2 mice and were recovered by astrocytic expression of μ -crystallin (Figures 4J and 4K; $n = 4$ mice). In accord, loss of Kir4.1 in R6/2 mice was recovered at the protein level (Figures 4L and 4M; $n = 5$ mice). The resting membrane potential of astrocytes does not change between groups (Figure 4G), because it is clamped near the K⁺ equilibrium potential⁷² (Figure 4H). Deficits in gap junctional coupling were not rescued by μ -crystallin restoration (Figures S5A and S5B; $n = 4$ –5 mice), despite the finding that morphology was recovered. Loss of Glut-1 expression^{9,21} seen in R6/2 mice was recovered by μ -crystallin expression (Figures S5C and S5D; $n = 5$ mice).

Astrocytic *Crym* restoration rescues neuronal properties and engages the frontal cortex

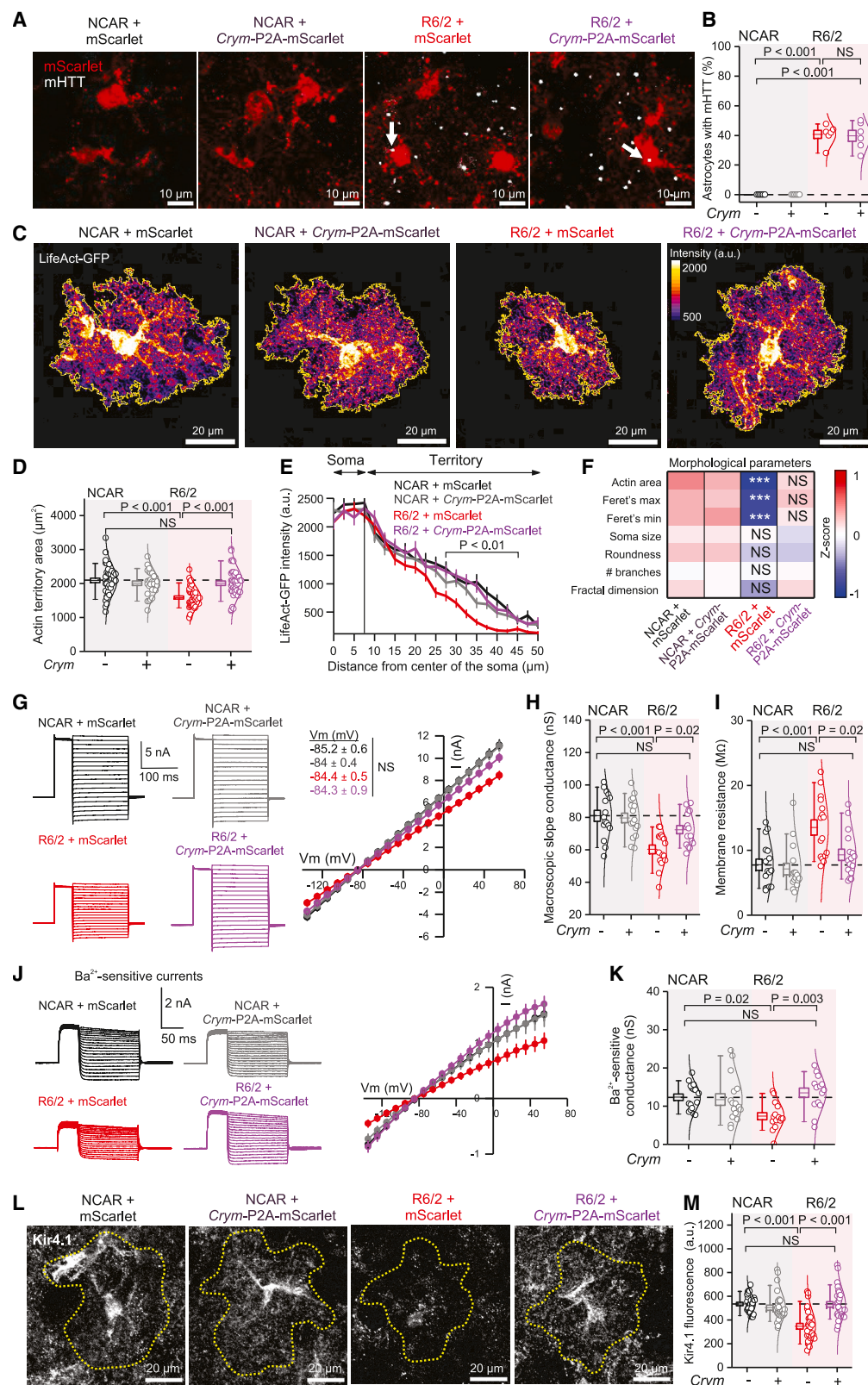
MSNs are altered and contribute to HD pathophysiology,^{73,74} prompting us to evaluate if astrocytic μ -crystallin restoration affected HD-associated MSN malfunctions. We found no mHTT puncta in NCAR mice (Figures 5A and 5B; $n = 3$ mice) and no change in the density of mHTT+ neurons in R6/2 mice after astrocytic μ -crystallin expression (Figures 5A and 5B). We found that MSNs from R6/2 mice were more excitable than those from NCAR mice (Figures 5C–5G; $n = 5$ mice). However, μ -crystallin rescue with *Crym*-P2A-mScarlet did not remedy this excitability (Figures 5E and 5F; $n = 5$ mice) or the increased membrane resistance⁷⁵ (Figure 5G; $n = 5$ mice). During these experiments, we employed intracellular biocytin dialysis⁷⁶ and with IHC found that MSN dendritic morphology/arborization and spine density were reduced in MSNs from R6/2 mice⁷⁵ and rescued significantly by *Crym*-P2A-mScarlet AAVs (Figures 5H–5K; $n = 5$ mice in each condition). This recalls findings that astrocytic mitochondrial deficits in HD contribute to reduced MSN dendritic branching.⁷⁷ There was also a trend for MSN somata sizes to be smaller in R6/2 mice relative to NCARs (Figure 5H), but this did not reach significance, and there was no significant effect of *Crym*-P2A-mScarlet (180 ± 14 , 160 ± 16 , 124 ± 9 , and $129 \pm 15 \mu\text{m}^2$ for NCAR + mScarlet, NCAR + *Crym*-P2A-mScarlet, R6/2 + mScarlet, and R6/2 + *Crym*-P2A-mScarlet, respectively; Figure 5H).

The striatum is part of the basal ganglia cortico-striatal-thalamo-cortical loop,⁷⁸ and the cSTR receives glutamatergic input from the orbitofrontal and motor cortex.²⁵ We recorded spontaneous excitatory postsynaptic currents (sEPSCs) arriving onto MSNs in the cSTR (Figures S6A–S6C; $n = 9$ mice) and found no differences in the frequency or amplitude between the four experimental groups (Figures S6A–S6C). We next repeated these evaluations for action potential independent miniature excitatory postsynaptic currents (mEPSCs in 0.5 μM tetrodotoxin, TTX; Figures 6A–6C; $n = 4$ mice). In these experiments, we found that mEPSC frequency was significantly lower in the R6/2 plus mScarlet group relative to NCAR (Figures 6B and 6C). Furthermore, this deficit was recovered when μ -crystallin was restored in astrocytes in the R6/2 *Crym*-P2A-mScarlet group (Figures 6B and 6C). The loss and recovery of mEPSCs is concordant with the spine density data reported above. The clear differences between sEPSCs and mEPSCs suggested that in R6/2 mice a larger fraction of the sEPSCs were due to action potential firing, likely from cortical neuron activity.⁷⁹ To explore this possibility, we assessed how TTX (0.5 μM) affected

Figure 3. AAV2/5-mediated striatal astrocyte μ -crystallin restoration in HD model mice

(A) AAV strategy used to restore astrocytic μ -crystallin expression in the central striatum (cSTR), followed by behavioral, cellular, and molecular assessments. (B and C) Images showing striatal μ -crystallin expression in the four experimental groups: NCAR + mScarlet, NCAR + *Crym*-P2A-mScarlet, R6/2 + mScarlet, and R6/2 + *Crym*-P2A-mScarlet at 10 weeks. White squares indicate areas of the striatum analyzed: cSTR, ventral striatum (vSTR), dorsolateral striatum (dlSTR), and SVZ. μ -crystallin expression (green) was restored in cSTR and vSTR. mScarlet signals (red) show the injection site in each condition. Scale bars, 500 μm ; (C) μ -crystallin expression intensity in cSTR, vSTR, dlSTR, and SVZ (from left to right) across the four experimental groups ($n = 9$ mice per group, two-way ANOVAs followed by Tukey's post hoc tests). (D and E) Left, μ -crystallin (green) with S100 β (red) or NeuN (white) in the four experimental groups in cSTR. Right, the percentage of μ -crystallin+ astrocytes (top) and the percentage of μ -crystallin+ neurons (bottom) across the four experimental groups ($n = 9$ mice per group, two-way ANOVAs followed by Tukey's post hoc tests).

In this figure, box plots are presented with a mean $\pm$ SEM, and the whiskers show the 10th–90th percentile range along with the individual data points and the distribution.



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EPSC frequency and amplitude (Figures 6D and 6E; Figures S6D and S6E) and found that TTX significantly reduced EPSC frequency in the R6/2 plus mScarlet group and that this effect was not observed in the R6/2 plus *Crym*-P2A-mScarlet group (Figures 6D and 6E; $n = 4$ mice). These data show that in R6/2 mice, a greater fraction of the EPSCs onto MSNs are the result of action potential firing and that this fraction was corrected by astrocyte μ -crystallin. The effects of TTX on EPSC amplitudes were not related to HD or to μ -crystallin (Figures S6D and S6E).

To investigate involvement of cortical regions, we assessed cFOS as a marker of neuronal activity²⁵ in the orbitofrontal cortex (OFC) and motor cortex (M1) (Figures 6F and 6G; Figures S6F and S6G). In both regions, cFOS was significantly reduced in R6/2 mice. Interestingly, restoration of *Crym* in striatal astrocytes rescued cFOS in OFC but not in M1, suggesting a circuit effect.²⁵ To explore the molecular changes associated with this rescue, we performed bulk RNA-seq of the frontal cortex following restoration of *Crym* in striatal astrocytes. We compared the number of DEGs (false discovery rate [FDR] < 0.05, $\log_2$ fold change > 0.3, FPKM > 1) between NCAR and R6/2 mice under control conditions (NCAR + mScarlet vs. R6/2 + mScarlet) and following μ -crystallin restoration (NCAR + *Crym* vs. R6/2 + *Crym*). Astrocytic μ -crystallin restoration reduced the number of DEGs in the frontal cortex of R6/2 mice from 1,128 to 911 (19% reduction, Figures 6H and 6I; top 20 in Figure 6K). Pathway enrichment analysis revealed significant enrichment of Gene Ontology (GO) biological processes related to synaptic function and neurotransmission, as well as components associated with neuronal projections and axonal structures (Figure 6J). These findings suggest improvement in frontal circuit function following *Crym* restoration in the cSTR of R6/2 mice.

Improvement of early-stage behaviors after astrocytic μ -crystallin restoration in HD mice

We next examined behavioral phenotypes in R6/2 mice^{37,51,56,67} and assessed how they were influenced by astrocytic μ -crystallin restoration. We focused on early-stage behaviors at 7 weeks of age, which correspond to the onset of compulsive

and repetitive behaviors in R6/2 mice, and on motor behaviors at 10 weeks⁵¹ (Figure 3A). These time points were selected to allow sufficient time for AAV-mediated μ -crystallin expression and to avoid late-stage morbidity.

All four experimental groups—NCAR control, R6/2, and their respective AAV-treated cohorts—showed comparable body weights at 4 and 7 weeks (Figure 7A; $n = 11$ –12 mice). By 10 weeks, R6/2 mice exhibited expected weight loss relative to NCAR controls. R6/2 mice receiving astrocytic μ -crystallin displayed a modest upward trend in body weight such that they were no different from NCAR (Figure 7A; $n = 11$ –12 mice). General health and appearance were consistent with prior observations for NCAR and R6/2 mice.^{51,56}

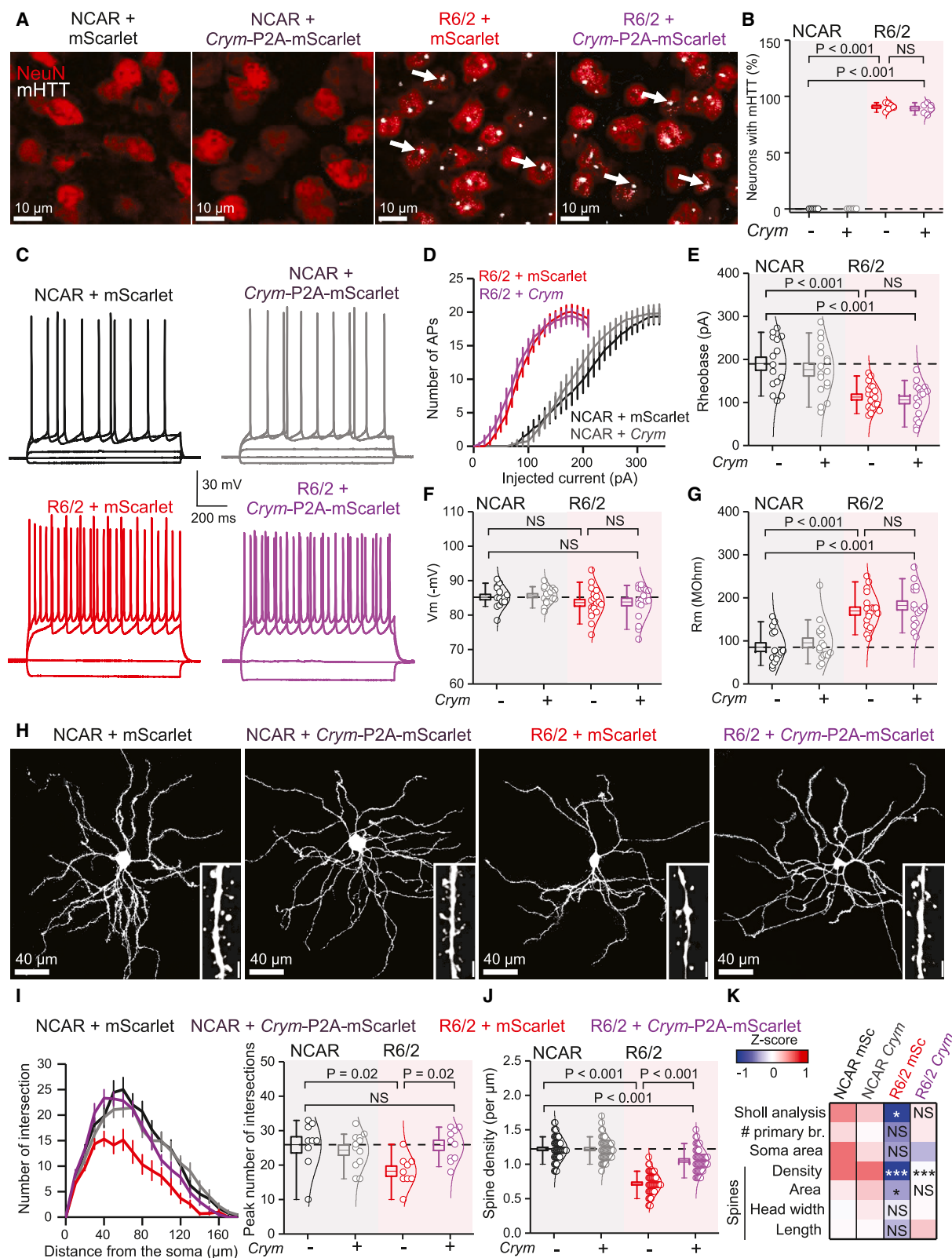
At 7 weeks, R6/2 mice displayed increased grooming, indicative of compulsive-like behavior, which was normalized by astrocytic μ -crystallin restoration (Figure 7B; $n = 17$ –18 mice). Conversely, rearing frequency, an index of exploratory behavior, was reduced in R6/2 mice but partially rescued by μ -crystallin (Figure 7B; $n = 17$ –18 mice). We also used the novel object recognition (NOR) test because it involves the striatum⁸⁰ and because *Crym* knockout from striatal astrocytes results in NOR deficits.²⁵ Importantly, during the training phase, all groups spent a similar amount of time exploring the two objects, indicating that exploration deficits do not affect this test (Figure S7A). During the testing phase, however, R6/2 mice spent more time exploring the familiar object,²⁵ resulting in a reduced recognition index compared with NCAR controls. Both the recognition index and the time spent with the familiar object were restored to control levels following astrocytic μ -crystallin expression (Figure 7C; $n = 9$ mice).

At 10 weeks, R6/2 mice exhibited robust motor deficits relative to NCAR controls, and most of these impairments were not improved by astrocytic μ -crystallin. The lack of rescue was consistent across multiple assays, including hindlimb clasping, forelimb grip strength, and footprint stride length and width—although open-field ambulation was partially rescued (Figure 7D; $n = 15$ –18 mice). Time spent in the center of the open field and ambulatory speed, two parameters commonly used to

Figure 4. Recovery of astrocyte properties after μ -crystallin restoration in HD model mice

(A) Images showing mHTT aggregates (white) in astrocytes (mScarlet⁺ in red) within the cSTR of 7-week-old NCAR or R6/2 mice injected with mScarlet control AAV or *Crym*-mScarlet AAV.
(B) Percentage of S100 β ⁺ cells that were also mHTT⁺ (6 fields of view per mouse, $n = 3$ mice per group, two-way ANOVA followed by Tukey's post hoc test).
(C) Thresholded images of LifeAct-GFP expression in the cSTR of 7-week-old NCAR or R6/2 mice injected with mScarlet control AAV or *Crym*-mScarlet AAV.
(D) Actin territory area (μm^2) across the four experimental groups ($n = 40$ astrocytes per group from 5 mice, two-way ANOVA followed by Tukey's post hoc test).
(E) LifeAct-GFP mean intensity. Each point represents the mean intensity from 40 cells per group (5 mice per group, two-way repeated-measures ANOVA followed by Tukey's post hoc test; p value < 0.05 at 25–45 μm).
(F) Astrocyte morphology Z scores for each experimental group. Statistics indicate comparison with the control group (NCAR + mScarlet) ($n = 40$ astrocytes per group from 5 mice, two-way ANOVA followed by Tukey's post hoc test).
(G–I) Voltage-clamp recordings from astrocytes in each experimental group at 7 weeks.
(G) Traces, current-voltage relationships, and resting membrane potential (V_m).
(H and I) Macroscopic slope conductance (nS) (H) and membrane resistance (M Ω) (I) ($n = 16$ cells per group from 4 mice, two-way ANOVA followed by Tukey's post hoc test or Kruskal-Wallis ANOVA, followed by Dunn's post hoc tests).
(J and K) Traces and current-voltage plots of Ba²⁺-sensitive currents (J). Ba²⁺-sensitive conductance (nS) in the four experimental groups (K) ($n = 16$ cells per group from 4 mice, two-way ANOVA followed by Tukey's post hoc test).
(L and M) IHC images (L) and average data (M) showing that Kir4.1 expression was reduced in R6/2 mice and recovered by *Crym*-P2A-mScarlet. The yellow outlines were based on the mScarlet fluorescence and were used to define a single astrocyte ($n = 30$ astrocytes from 5 mice per group, Kruskal-Wallis ANOVA, followed by Dunn's post hoc tests).

In this figure, box plots are presented with a mean $\pm$ SEM, and the whiskers show the 10th–90th percentile range, along with the individual data points and the distribution.



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assess anxiety-like behavior, revealed no evidence of increased anxiety-like phenotypes in R6/2 mice (Figure S7B), in accord with previous data in *Crym* knockout mice.²⁵ A Z score heatmap summarizing behavioral outcomes across ages (Figure 7E) highlights that astrocytic μ -crystallin restoration rescued early-stage compulsive/repetitive behaviors, whereas motor deficits at later stages remained unaffected.

Astrocytic transcriptomic improvements following astrocyte μ -crystallin rescue in HD mice

To investigate mechanisms that accompany improvements after astrocytic μ -crystallin restoration in R6/2 mice, we performed astrocyte transcriptomic profiling using RiboTag AAVs⁸¹ (Figures 8A and S8; $n = 5$ mice). We compared the number of DEGs (FDR < 0.05, $\log_2$ fold change > 0.3, FPKM > 1) between NCAR and R6/2 mice under control conditions (NCAR + mScarlet vs. R6/2 + mScarlet) and following μ -crystallin restoration (NCAR + *Crym* vs. R6/2 + *Crym*). Transcriptomic rescue was assessed by comparing the change of DEGs between these two conditions. Astrocytes in HD were predominantly characterized by downregulated^{41,51,56} DEGs (Figure 8B), and μ -crystallin expression reduced the number of DEGs in R6/2 mice, from 1,817 to 933 (~50% rescue, Figures 8B and 8C).

From these analyses, we identified the top rescued DEGs, i.e., those most strongly up- or downregulated in R6/2 astrocytes and normalized by μ -crystallin expression (top 30 in Figure 8D). Pathway analysis revealed that astrocytic pathophysiology in R6/2 mice was dominated by disruptions in cholesterol homeostasis, metabolic regulation, peroxisomal membrane protein transport, and Rho-GTPase signaling (Figure 8E). Following μ -crystallin restoration, many of these pathways were partially normalized, with additional enrichment in processes such as actin nucleation by the ARP/WASP complex (Figure 8E). Collectively, the RNA-seq data demonstrate that astrocytes in R6/2 mice exhibit widespread transcriptional dysregulation and that μ -crystallin restoration reverses some of these molecular abnormalities. Several rescued pathways align with the morphological improvements observed in astrocytes (Figure 4).

We also examined candidate genes related to astrogliosis, neuroinflammation, and core astrocytic functions (Figure 8F; Figures S9A–S9D). R6/2 mice displayed dysregulation of genes

associated with these processes,⁴¹ and μ -crystallin restoration partially normalized many (Figure 8F). Cholesterol biosynthesis pathways, which were strongly dysregulated in R6/2 mice, showed some (14%) recovery following *Crym*. By contrast, genes related to potassium homeostasis, synaptic organization, cell adhesion, and extracellular matrix remodeling were markedly rescued, with some categories showing recovery of 50% (Figure 8F). These transcriptomic changes are consistent with the cellular improvements observed in astrocytes following *Crym* restoration (Figure 4). We also examined genes involved in thyroid hormone signaling and ketimine reductase activity, given that μ -crystallin is described as a thyroid hormone-binding protein and ketimine reductase.^{26–28} Of the 21 genes analyzed, only two were altered in R6/2 mice, and neither was rescued by μ -crystallin (Figure S9). For validations, we evaluated NRCAM³³ at the protein level and found that it was reduced in R6/2 mice and restored by *Crym* expression (Figures 8G and 8H).

Bulk RNA-seq reveals non-cell-autonomous effects of astrocytic μ -crystallin restoration

To define how astrocytic μ -crystallin restoration influences the striatum, we performed bulk RNA-seq (Figures S10A–S10E; $n = 5$ mice). This analysis identified 3,138 DEGs in R6/2 mice relative to controls. Following μ -crystallin restoration, the number of DEGs was reduced to 2,788, representing an ~11% rescue (Figures S10A and S10B). To infer the cellular origins of these transcriptional changes, we integrated bulk RNA-seq data with published single-cell RNA-seq datasets³⁵ (Figure S10C). The majority of DEGs in R6/2 mice mapped to neurons, oligodendrocytes, and endothelial cells, indicating widespread molecular disruption across cells. The rescued DEGs following μ -crystallin expression also mapped to similar populations, suggesting astrocytic μ -crystallin restoration mitigates HD-associated molecular pathology across the striatal cellular network (Figures S10C and S10D). Pathway analysis of all rescued DEGs revealed normalization of several pathways disrupted in HD, including those related to lysosomal function, protein repair, cilium biogenesis, STAT3 signaling, GPCR signaling, and thrombospondin-mediated interactions (Figure S10E). These transcriptomic findings indicate that μ -crystallin acts within astrocytes to correct HD-associated

Figure 5. Recovery of neuronal morphology, but not hyperexcitability, after astrocyte μ -crystallin restoration in HD model mice

(A) mHTT aggregates (white) in medium spiny neurons (MSNs; NeuN⁺ in red) within the cSTR of 7-week-old NCAR or R6/2 mice injected with mScarlet control AAV or *Crym*-mScarlet AAV.
(B) Percentage of mScarlet⁺ cells that were also mHTT⁺ (6 fields of view per mouse, $n = 3$ mice per group, two-way ANOVA followed by Tukey's post hoc test).
(C and D) MSN membrane responses to current injection (C) and plots showing the relationship between injected current and the number of action potentials (APs) (D) across the four experimental groups.
(E–G) MSN rheobase (E), resting membrane potential (F), and membrane resistance (G) ($n = 15$ –19 cells per group from 5 mice, two-way ANOVA followed by Tukey's post hoc test or Kruskal-Wallis ANOVA, followed by Dunn's post hoc tests).
(H) Biocytin-filled MSNs showing dendritic morphology and dendritic spines in the four experimental groups (scale bar, 2 μ m for inset).
(I) Sholl analysis of MSN dendritic arborization performed at 10 μ m increments (left) and maximal number of intersections per cell (right) ($n = 9$ –12 MSNs from 5 mice, two-way ANOVA followed by Tukey's post hoc test).
(J) Spine density quantification across the four experimental groups ($n = 52$ –54 dendrites from 8–9 MSNs from 5 mice, two-way ANOVA followed by Tukey's post hoc test).
(K) MSN morphological Z scores for each experimental group. Statistics indicate comparisons with the control group (NCAR + mScarlet) ($n = 9$ –12 MSNs per group from 5 mice, two-way ANOVA followed by Tukey's post hoc test or Kruskal-Wallis ANOVA, followed by Dunn's post hoc tests).
In this figure, box plots are presented with a mean $\pm$ SEM, and the whiskers show the 10th–90th percentile range along with the individual data points and the distribution.

molecular abnormalities (Figures 8B–8F) while exerting non-cell-autonomous effects that improve neighboring striatal cells (Figures S10A–S10E).

Comparison of transcriptomic data following μ -crystallin restoration to human postmortem data

To determine whether the transcriptomic improvements observed in astrocytes following μ -crystallin restoration in R6/2 mice were relevant to human HD, we compared the rescued DEGs from our mouse datasets with human postmortem caudate RNA-seq data from astrocyte-enriched⁴ tissue samples (Figure 8I). This cross-species comparison revealed that 32% of the astrocytic DEGs rescued by μ -crystallin (280 genes in total) overlapped with astrocyte-specific DEGs identified in human HD (Figure 8I). These shared DEGs included both upregulated and downregulated transcripts, with the majority exhibiting directionally consistent changes between mouse and human datasets (Figure 8I). To further explore this comparison, we validated HTRA1 at the protein level, a serine protease downregulated in R6/2 mice and HD human and rescued after *Crym* restoration. Consistent with the RNA-seq data, HTRA1 expression was reduced in R6/2 mice and restored following *Crym* expression (Figures 8J and 8K). These findings demonstrate that astrocytic μ -crystallin restoration in R6/2 mice normalizes molecular pathways disrupted in murine HD and recapitulates transcriptional corrections relevant to human disease.

DISCUSSION

We identified a molecularly and anatomically defined population of *Crym*-expressing astrocytes in the cSTR and vSTR^{23–25,36} as contributors to early HD pathophysiology in mice, extending past studies showing that loss of *Crym* expression renders neurons more vulnerable to mHTT.³¹ By combining human postmortem and CSF analyses, assessments in R6/2 and zQ175 HD mouse models, and targeted gene restoration in the R6/2 model, we demonstrate that *Crym* mRNA and its encoded protein, μ -crystallin, are reduced in astrocytes during HD progression. This reduction occurs

across experiments, indicating conserved vulnerability or malfunction of *Crym*⁺ astrocytes. Analysis in human HD shows *CRYM* was reduced in protoplasmic astrocytes in the caudate nucleus but not in other parts of the brain.⁴ Therefore, evaluations of past data support findings reported here at the protein level for ventral caudate and putamen, which is consistent with the expression of *Crym* in the cSTR and vSTR in mice.^{23–25} The loss of μ -crystallin coincides with other widespread transcriptional and functional impairments^{4–7} in astrocytes, including dysregulated cholesterol biosynthesis, altered potassium channel function, and reduced morphological complexity.⁹ These findings strengthen the concept that astrocyte subpopulations^{18,19} play distinct roles in disease-related mechanisms rather than acting as a uniform population. μ -crystallin measurements in CSF may capture astrocyte dysfunction and offer prognostic insights for clinical trials.

Astrocytic μ -crystallin recovery normalized aspects of astrocyte biology,^{41,52–54,56} including actin organization, expanded astrocyte territories, and restored electrophysiological properties such as membrane conductance and Kir4.1-mediated potassium currents. These effects suggest that μ -crystallin expression supports astrocytic structural integrity and ion homeostasis, both of which are essential for maintaining neuronal excitability and synaptic stability and known to be altered in HD.⁸ μ -crystallin expression did not alter the presence of mHTT inclusions, consistent with its cytosolic localization. Instead, its influence appears to be mediated through cytoplasmic and membrane-associated pathways that regulate astrocytic morphology, homeostasis, and signaling.

The recovery of astrocyte functions had measurable consequences for neuronal physiology and parts of the cortico-striatal circuit. Although μ -crystallin restoration did not correct the intrinsic hyperexcitability of MSNs, it significantly improved dendritic arborization and spine density. These improvements likely arise from restored astrocyte-neuron interactions, including improved potassium buffering, metabolic support, cell-adhesion molecules, and synaptogenic factors. The normalization of mEPSCs further indicates that μ -crystallin restoration stabilizes synaptic communication within cortico-striatal circuits.

Figure 6. Recovery of striatal excitatory synaptic and frontal cortex properties after striatal astrocyte μ -crystallin restoration in HD model mice

(A) Miniature excitatory postsynaptic currents (mEPSCs) recorded from one MSN in 7-week-old NCAR or R6/2 mice injected with mScarlet control AAV or *Crym*-mScarlet AAV.
(B and C) mEPSC inter-event intervals (B) and amplitudes (C). Insets show pooled data across the four experimental groups ($n = 17$ MSNs per group from 4 mice, two-way ANOVA followed by Tukey's post hoc test).
(D) EPSC frequency before and after TTX application across groups ($n = 17$ MSNs per group from 4 mice, paired-sample t tests).
(E) Percent change in EPSC frequency after TTX application ($n = 17$ MSNs per group from 4 mice, two-way ANOVA followed by Tukey's post hoc test).
(F) cFOS and NeuN expression in the frontal cortex (OFC). Scale bars, 20 μ m.
(G) Percent of cFOS neurons across the four conditions ($n = 10$ brain slices per group from 5 mice, two-way ANOVA followed by Tukey's post hoc test).
(H) DEG analysis (FDR < 0.05) showing numbers of up- and downregulated genes in R6/2 mice (R6/2 + mScarlet vs. NCAR + mScarlet, denoted as "R6/2") and after *Crym* restoration (R6/2 + *Crym* vs. NCAR + *Crym*, denoted as "R6/2 + *Crym*") in the frontal cortex. Analysis was restricted to genes with >0.3-fold change in disease (R6/2 vs. NCAR) and FPKM > 1.
(I) DEGs in disease condition (R6/2, left) and after *Crym* restoration (R6/2 + *Crym*, right). Approximately 19% of the frontal cortex DEGs were rescued following *Crym* restoration in the cSTR (1,128 DEGs in R6/2 vs. 911 DEGs in R6/2 + *Crym*).
(J) Top 5 rescued GO biological process (top) and GO cellular components (bottom) identified with Enrichr. Circle size represents p value.
(K) Rescued genes in the frontal cortex that were from the list of the top 20 downregulated (left) or top 20 upregulated (right) in R6/2 mice. In this figure, box plots are presented with a mean $\pm$ SEM, and the whiskers show the 10th–90th percentile range along with the individual data points and the distribution.

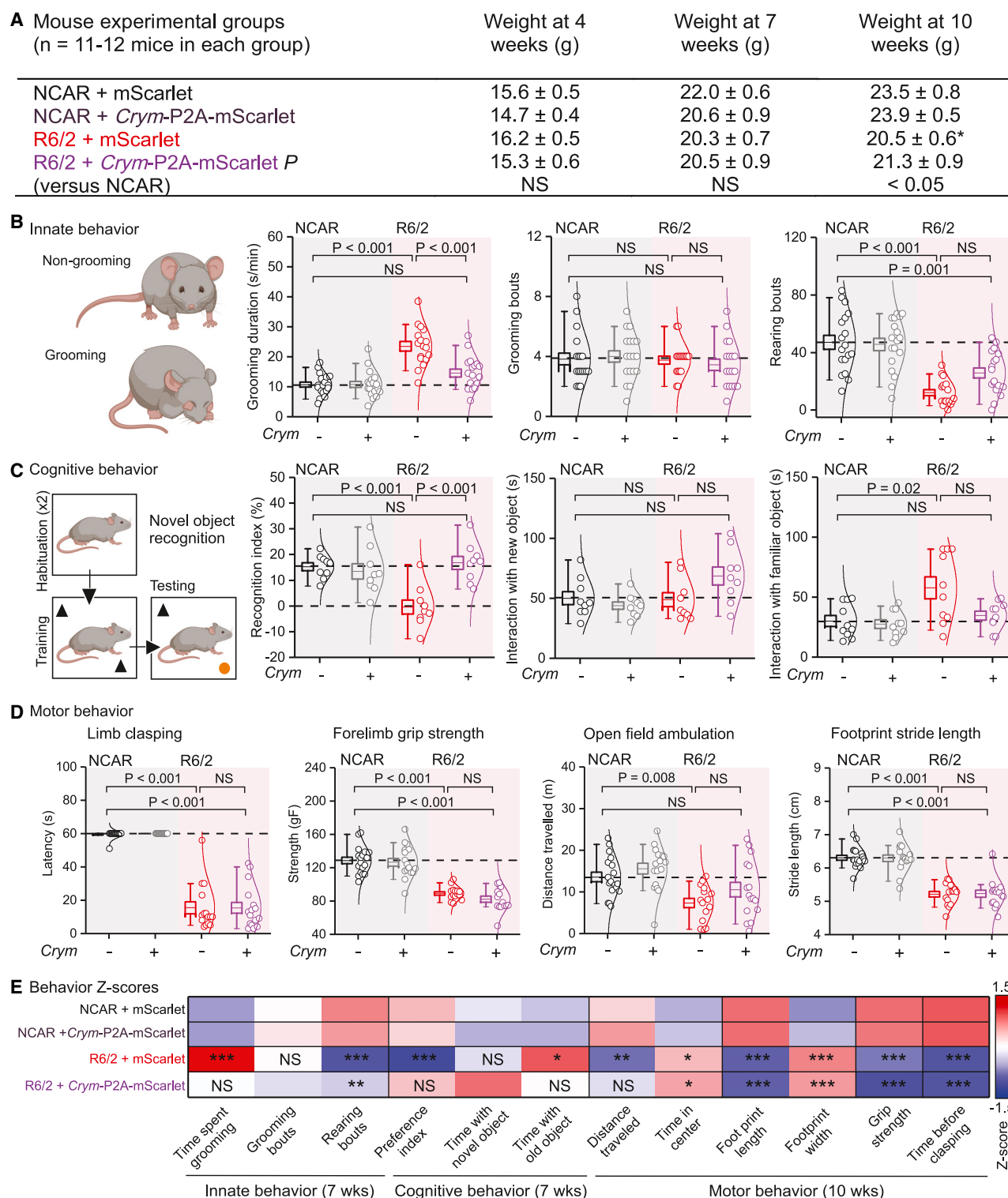


Figure 7. Recovery of early-stage behaviors after astrocytic μ -crystallin restoration in HD model mice

(A) Body weight of 4-, 7-, and 10-week-old NCAR or R6/2 mice injected with mScarlet control AAV or *Crym*-mScarlet AAV (n = 11-12 mice per group, two-way ANOVA followed by Tukey's post hoc test).

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Behavioral analyses revealed that astrocytic μ -crystallin restoration selectively improved early-stage compulsive and repetitive phenotypes in R6/2 mice,⁶⁷ while later-stage motor deficits remained unaffected. This stage-specific rescue suggests that *Crym*+ astrocytes primarily influence neural circuit dysfunctions underlying early behavioral abnormalities rather than the more severe processes driving motor decline that are associated with striatal degeneration. The temporal dissociation between early and late phenotypes implies that distinct astrocyte-dependent mechanisms operate across disease stage contexts^{56,84} and that interventions targeting astrocytic dysfunction may be most effective before extensive neuronal damage or striatal volume loss occurs.

It is often tempting to seek a primary mechanism by which astrocytes regulate neurons, but since there are many thousands of DEGs in HD,^{4–6,40,41} because astrocytes regulate neurons in many ways,^{13,16,85} and because both astrocytes and neurons display cell-autonomous and non-cell-autonomous roles in HD,⁵¹ it is unlikely that any single mechanism can explain observations in HD. Instead, our findings indicate improvements that are likely to involve multiple mechanisms that commence by restoring μ -crystallin expression within astrocytes. Astrocyte-specific RNA-seq revealed that μ -crystallin restoration rescued almost half of the HD-associated astrocyte transcriptional abnormalities. Rescued genes were enriched in pathways related to transcriptional regulation, cell adhesion, extracellular matrix organization, and Rho-GTPase signaling. These pathways are central to astrocytic morphology and homeostasis and are disrupted in HD⁹ and reflect molecular functions predicted from the μ -crystallin interactome.²⁵ Such transcriptomic changes were accompanied by a restoration of astrocytic morphology and astrocytic proteins.^{9,54,86–88} Together, these findings support a model in which *Crym* acts as a regulator of astrocytic programs rather than through a single molecular target. Bulk RNA-seq demonstrated that astrocytic μ -crystallin expression exerted broader non-cell-autonomous effects, improving gene expression across cell types, including neurons. Such mechanisms could reflect improved tissue homeostasis and/or cell-cell interactions subserved by cell-surface proteomes shared by astrocytes and neurons.⁵⁵

Crym was consistently reduced in expression in human post-mortem striatal tissue. Moreover, approximately one-third of the astrocytic genes rescued by μ -crystallin in R6/2 mice overlapped with those dysregulated in human HD astrocytes, and a subset of bulk striatal genes exhibited similar directionality between species. This cross-species concordance implies that the molecular effects of μ -crystallin restoration in mice reflect processes

relevant to human HD pathophysiology. More generally, μ -crystallin is implicated in demyelinating disease,⁸⁹ neuroinflammation,⁹⁰ and the effects of social isolation on drug seeking.³⁰ It is therefore possible that μ -crystallin contributes more broadly to brain disorders. To our knowledge, μ -crystallin protein levels in the CNS or body fluids have not previously been assessed as potential HD biomarkers. CSF proteomic findings in HD individuals indicate that μ -crystallin warrants further investigation as a complementary biomarker candidate.

Our data show *Crym*+ astrocytes as a specialized and functionally relevant subpopulation whose malfunctions contribute to early HD pathology. Our findings support a model in which loss of μ -crystallin compromises astrocytic structure and function, leading to secondary neuronal, circuit, and behavioral dysfunctions. Restoring μ -crystallin expression reestablishes some astrocyte functions, improves neuronal morphology, and rescues early behavioral phenotypes. Our study highlights the therapeutic potential of targeting molecularly defined astrocyte subpopulations.¹⁴

RESOURCE AVAILABILITY

Lead contact

Requests for further information, resources, and reagents should be directed to and will be fulfilled by the lead contact, Baljit S. Khakh (bkhakh@mednet.ucla.edu).

Materials availability

Plasmids generated in this study have been deposited at Addgene ID: 249539 and 249540.

Data and code availability

- RNA-seq data have been deposited at GEO: GSE310059 and are publicly available as of the date of publication.
- CSF proteomic data for μ -crystallin are available from Enroll-HD Data. Enroll-HD clinical data are shared with the research community through specified datasets. Access to data may be obtained through request, subject to approval by the Scientific Review Committee that weighs the scientific merit of the proposed project against the increased risk for participant identification. Any researcher employed by a recognized research organization can open an Enroll-HD access account to obtain data from Enroll-HD, subject to the researcher's employer/institution signing the appropriate data use agreement(s). More detailed access information is available at <https://enroll-hd.org/for-researchers/data-from-human-samples/> and <https://enroll-hd.org/for-researchers/access-data/>. The specific dataset "HDClarity Proteomics Data for CSF – Mass Spec Data" can be requested here: <https://enroll-hd.org/for-researchers/access-data-biosamples/>.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

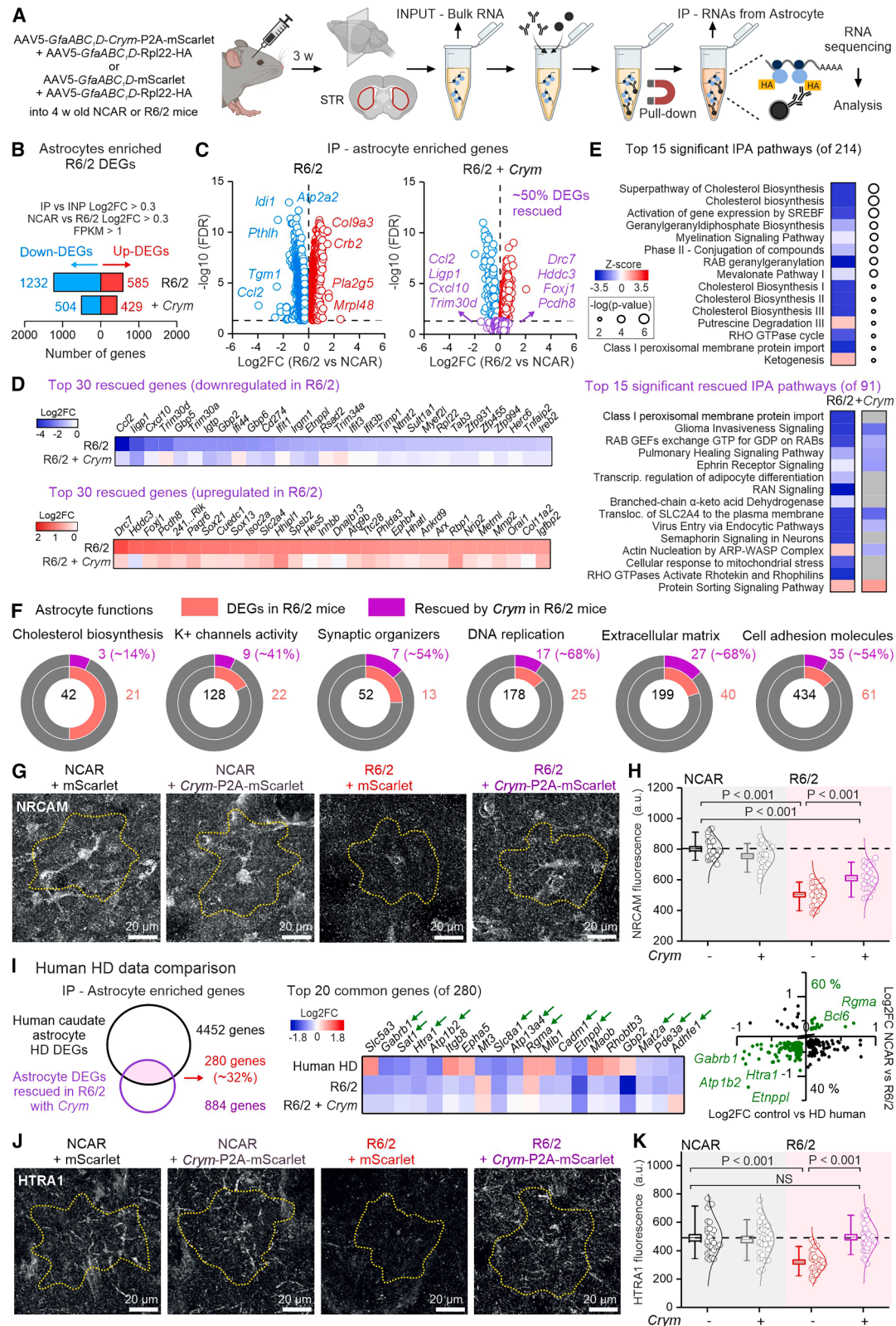
(B) Representation of self-grooming behavior showing disengaged and engaged phases. Right, quantification of self-grooming duration, grooming bouts, and rearing bouts across the four experimental groups ($n = 17$ – 18 mice per group, two-way ANOVA followed by Tukey's post hoc test or Kruskal-Wallis ANOVA, followed by Dunn's post hoc tests).

(C) Schematic of the NOR test. Right, recognition index (%), interaction time with the novel object, and interaction time with the familiar object ($n = 9$ mice per group, two-way ANOVA followed by Tukey's post hoc test).

(D) Motor behaviors. From left to right: latency to clasp hindlimbs in the tail suspension test, forelimb grip strength measured over five consecutive trials, total distance traveled in the open-field test (10 min), and stride length in the footprint test ($n = 15$ – 18 mice per group, two-way ANOVA followed by Tukey's post hoc test or Kruskal-Wallis ANOVA, followed by Dunn's post hoc tests).

(E) Behavioral test Z scores for each experimental group. Statistical comparisons were performed against the control group (NCAR + mScarlet) ($n = 9$ – 18 mice per group, two-way ANOVA followed by Tukey's post hoc test or Kruskal-Wallis ANOVA, followed by Dunn's post hoc tests).

In this figure, box plots are presented with a mean $\pm$ SEM, and the whiskers show the 10th–90th percentile range along with the individual data points and the distribution.



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AUTHOR CONTRIBUTIONS

M.O. performed most of the experiments and analyzed the data. S.G.P. performed bulk RNA-seq data mapping, as shown in Figure 8H and Figure S3D, and provided guidance on RNA-seq analysis. V.H.C. and C.N. performed the AAV injections and sample preparations for the frontal cortex experiments (Figures 6H–6K). S.X. and O.A.-D. performed and analyzed human tissue IHC shown in Figures 1C and 1D. F.L.Q., N.C., and N.H.S. performed proteomic analysis of human CSF for μ -crystallin as shown in Figures 2E, 2F, and 2G. R.K. helped guide RNA-seq analyses. B.S.K. directed the experiments/study, recruited collaborators, and planned the figures with M.O. B.S.K. wrote the paper with help from M.O. and the other authors.

DECLARATION OF INTERESTS

B.S.K. is a member of the *Neuron* editorial advisory board.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this manuscript, ChatGpt4.1 was sometimes used in order to improve the readability of some subsections. After using this tool, the authors reviewed and edited the content and take(s) full responsibility for the content of the publication.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- **EXPERIMENTAL MODEL AND PARTICIPANT DETAILS**
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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.neuron.2026.08.027>.

Figure 8. Astrocyte transcriptomic improvements following astrocyte μ -crystallin rescue in HD mice

(A) Astrocyte-specific RiboTag RNA-seq in 7-week-old NCAR and R6/2 mice injected with mScarlet control AAV or *Crym*-mScarlet AAV.
 (B) DEG analysis ($FDR < 0.05$) showing numbers of up- and downregulated astrocyte genes in R6/2 mice (R6/2 + mScarlet vs. NCAR + mScarlet, denoted as "R6/2") and after *Crym* restoration (R6/2 + *Crym* vs. NCAR + *Crym*, denoted as "R6/2 + *Crym*"). Analysis was restricted to genes with > 0.3 -fold enrichment in IP vs. input, > 0.3 -fold change in disease (R6/2 vs. NCAR), and FPKM > 1 .
 (C) DEGs in disease condition (R6/2, left) and after *Crym* restoration (R6/2 + *Crym*, right). Approximately half of the DEGs were rescued following *Crym* restoration (1,817 DEGs in R6/2 vs. 933 DEGs in R6/2 + *Crym*).
 (D) Rescued astrocytic genes that were from the list of the top 30 downregulated (top) or top 30 upregulated (bottom) in R6/2.
 (E) Pathways identified from astrocyte-enriched DEGs ($FDR < 0.05$). Top: 15 of 214 significant pathways in R6/2 astrocytes. Bottom: 15 of 91 significant pathways rescued after *Crym* restoration (pathways significant in R6/2 but not in R6/2 + *Crym*). Circle size represents scaled- $\log(p)$ value; color indicates Z score. DEG analysis was restricted to genes with > 0.3 -fold change in disease (R6/2 vs. NCAR) and FPKM > 1 .
 (F) DEGs in R6/2 mice (pink) and number of *Crym*-rescued genes (purple) within several astrocyte function. Gene sets are derived from *The Gene Ontology Resource*.⁸²
 (G and H) IHC images (G) and average data (H) showing that NRCAM expression was reduced in R6/2 mice and recovered by *Crym*-P2A-mScarlet.
 (I) Left, overlap between 884 rescued astrocytic genes and 4,125 astrocytic human DEGs (p value < 0.05) identified by snRNA-seq.⁴ The hypergeometric test (R function *phyper*) was used to assess the association between mouse rescued genes and human HD astrocyte gene sets. The FDR was highly significant at 0. Middle, heatmap of the top 20 shared genes between human HD and R6/2 + *Crym* (ranked by significance in human HD). Right, correlation plot of $\log_2FC$ values for shared genes between human HD and R6/2 mice. Green arrows indicate genes changing in the same direction in both species (60%), whereas the black dots are those that change in opposite directions.
 (J and K) Images (J) and average data (K) showing that HTRA1 expression was reduced in R6/2 mice and recovered by *Crym*-P2A-mScarlet. The yellow outlines were based on the mScarlet fluorescence signal and were used to define a single astrocyte ($n = 24$ –30 astrocytes from 5 mice per group two-way ANOVA followed by Tukey's post hoc test). In this figure, box plots are presented with a mean $\pm$ SEM, and the whiskers show the 10th–90th percentile range along with the individual data points and the distribution.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
mouse anti- μ -crystallin (F-11)	Santa Cruz	Cat# SC376687 RRID:AB_11150103
rabbit anti-ALDH1L1	EnCor	# RPCA-ALDH1L1 RRID: AB_2572222
chicken anti-GFAP	Abcam	Cat# ab4674 RRID:AB_304558
rabbit anti-S100 β	Abcam	Cat# ab52642 RRID: AB_882426
rabbit anti-Sox9	Millipore	Cat# ab5535 RRID: AB_2239761
rabbit anti-NeuN	Cell Signaling Technology	Cat# 12943 RRID: AB_2630395
rabbit anti-NeuN	Millipore	Cat#: ABN78 RPID: AB_10807945
guinea pig anti-NeuN	Synaptic system	Cat# 266004 RRID:AB_2619988
guinea pig anti-RFP	Synaptic System	Cat# 390-004 RRID: AB_2737052
rabbit anti-RFP	Rockland	Cat# 600-401-379 RRID:AB_2209751
chicken anti-GFP	Abcam	Cat# ab13970 RRID: AB_300798
mouse anti-mHTT	Millipore Sigma	Cat# MAB5492 RRID: AB_347723
rabbit anti-KIR4.1	Alomone Labs	Cat# APC-035 RRID:AB_2040120
rabbit anti-GLT1	Synaptic Systems	Cat# 250 203 RRID:AB_11042312
mouse anti-HA	Biologend	Cat# 901514 RRID: AB_2565335
rabbit anti-NRCAM	Proteintech	Cat# 21608-1-AP-RRID:AB_10859786
Rabbit anti-HTRA1	Thermo Fisher Scientific	Cat# 55011-1-AP RRID:AB_10859830
Alexa Fluor 488 goat anti-mouse	Thermo Scientific	Cat# A11001 RRID: AB_2534069
Alexa Fluor 488 goat anti-rabbit	Thermo Scientific	Cat# A11008 RRID:AB_143165
Alexa Fluor 488 goat anti-chicken	Thermo Scientific	Cat# A11039 RRID:AB_2534096
Alexa Fluor 546 goat anti-guinea pig	Thermo Scientific	Cat# A11074 RRID: AB_2534118
Alexa Fluor 546 goat anti-rabbit	Thermo Scientific	Cat# A11035 RRID: AB_2534093
Alexa Fluor 546 goat anti-chicken	Thermo Scientific	Cat# A-11040 RRID:AB_2534097
Alexa Fluor 647 goat anti-mouse	Thermo Scientific	Cat# A21235 RRID:AB_2535804

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Alexa Fluor 647 goat anti-rabbit	Thermo Scientific	Cat# A21244 RRID:AB_2535812
Alexa Fluor 647 goat anti-guinea pig	Thermo Scientific	Cat# A21450 RRID:AB_2535867
Alexa Fluor 405 goat anti-mouse	Thermo Scientific	Cat# A31553 RRID:AB_221604
Alexa Fluor 405 goat anti-rabbit	Thermo Scientific	Cat# A31556 RRID:AB_221605
Alexa Fluor 405 goat anti-guinea pig	Thermo Scientific	Cat# ab175678 RRID:AB_2827755
Alexa Fluor 647 donkey anti-chicken	Thermo Scientific	Cat# A78952 RRID:AB_2921074
Alexa Fluor 488 donkey anti-mouse	Thermo Scientific	Cat# A21202 RRID:AB_141607
Alexa Fluor 546 donkey anti-rabbit	Thermo Scientific	Cat# A10040 RRID:AB_2534016
streptavidin conjugated Alexa 488	Thermo Scientific	Cat# S21374 RRID: AB_2336066

Mounting solution

Everbright TrueBlack Hardset Mounting Medium with DAPI	Biotium	Cat# 23018 RRID: N/A
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Bacterial and virus strains

AAV2/5-GfaABC ₁ D-Crym-P2A-mScarlet-4X6T	This manuscript	Addgene ID: 249539
AAV2/5-GfaABC ₁ D-mScarlet-4X6T	This manuscript	Addgene ID: 249540
AAV2/5-GfaABC ₁ D-Rpl22HA	Diaz-Castro et al. ⁴¹	Addgene ID: 111811
AAV2/5-GfaABC ₁ D-LifeAct-GFP	Soto et al. ⁴³	Addgene ID: 190199

Chemicals, peptides, and recombinant proteins

Formalin (buffered 10%)	Fisher Chemical	Cat# SF100-20
Normal goat serum	Vector Laboratories	Cat# S-1000-20
Donkey serum	Sigma-Aldrich	Cat# D9663
Phosphate buffered saline	Sigma-Aldrich	Cat# P4417-100TAB

Experimental models: Organisms/strains

Mouse: B6CBA-Tg(HDexon1)62Gpb/3J	Jackson Laboratory	Stock# 006494; RRID:IMSR_JAX:006494
Mouse: B6J.129S1-Httm1Mfc/190Chdij	Jackson Laboratory	Stock# 027410 RRID:IMSR_JAX:027410
Mouse: C57Bl/6NTac inbred mice	Taconic	Stock# B6; RRID:IMSR_TAC:b6

Deposited data

Source data used to generate figures	This manuscript	Data S1
CSF proteomic data for μ -crystallin	This manuscript	Data S2 : see Note 1 below
Raw and normalized RNA-seq data	This manuscript	GSE310059 and Data S3

Software and algorithms

OriginPro 2017	Origin Lab Corporation	RRID:SCR_014212
OriginPro 2022	Origin Lab Corporation	RRID:SCR_014212
CorelDraw 2019 v21	Corel Corporation	RRID:SCR_014235
pCLAMP10.4	Molecular Devices	RRID:SCR_011323
ClampFit10.4	Molecular Devices	RRID:SCR_011323
Fluoview FV31S-SW	Olympus	RRID:SCR_028793
ImageJ v1.53t	NIH	RRID:SCR_003070

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
ANY-maze	Stoelting Co.	RRID:SCR_014289
STRING database	https://string-db.org	RRID:SCR_005223
LimmaVoom	Rdrr.io	https://rdrr.io/bioc/limma/man/voom.html

Enroll-HD data availability

Enroll-HD clinical data are shared with the research community through specified datasets. Access to data may be obtained through request, subject to approval by the Scientific Review Committee that weighs the scientific merit of the proposed project against the increased risk for participant identification. Any researcher employed by a recognized research organization can open an Enroll-HD access account to obtain data from Enroll-HD, subject to the researcher's employer/institution signing the appropriate data use agreement(s). More detailed access information is available at <https://enroll-hd.org/for-researchers/data-from-human-samples/> and <https://enroll-hd.org/for-researchers/access-data/>. The specific dataset "HDClarity Proteomics Data for CSF – Mass Spec Data" can be requested here <https://enroll-hd.org/for-researchers/access-data-biosamples/>.

EXPERIMENTAL MODEL AND PARTICIPANT DETAILS

Human subjects

For the human data in Figure 1, all study protocols were approved by the Columbia University Irving Medical Center Institutional Review Board (protocol numbers AAV4161 and AAAT2895). Postmortem striatal specimens obtained during autopsy from control (individuals whose brains did not show significant neuropathology) and HD donors were obtained from the New York Brain Bank. Consent from the next of kin was obtained for all brain donations. The tissue was dissected by a board-certified neuropathologist (OAD) or under the supervision of a board-certified neuropathologist. The demographic data is provided in Table S1).

For the human data in Figures 2E–2G, the CSF samples were obtained from the ongoing HDClarity study (NCT02855476), which is a multi-site CSF collection initiative to facilitate therapeutic development for HD.⁹¹ The study is designed to collect biofluid samples from individuals with HD across the spectrum of the disease, from early pre-manifest to advanced manifest HD as well as healthy controls. Participant medical history and clinical measures are collected through the Enroll-HD clinical platform.⁹² Both HDClarity and Enroll-HD are conducted in accordance with the Declaration of Helsinki; all patients provide written informed consent for participation for future analyses based on their data. The HDClarity study was approved centrally by Camberwell St Giles Research Ethics Committee (IRAS 185506) and each site's ethics board. The demographic data is provided in Table S2.

Mouse models

All animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Chancellor's Animal Research Committee at the University of California, Los Angeles. All mice were housed with food and water available *ad libitum* in a 12-hour light/dark environment at temperatures of 20–22°C with 40%–60% humidity. All animals were healthy with no obvious behavioral phenotype (other than the HD model mice), were not involved in previous studies, and were sacrificed during the light cycle. R6/2 and non-carrier (NCAR) control mice at 2–4 weeks were purchased from the Jackson Laboratory (Strain B6CBA-Tg (HDexon1) 62Gpb/3J; JAX #006494); the R6/2 mice had an average CAG length of 120. Heterozygous zQ175 and wild-type (WT) control mice at 12 months were obtained from CHDI (Strain B6J.129S1-*Htt*^{tm1Mfc}/190Chd1J; JAX #027410) with average CAG length of 190. Studies on R6/2 and control NCAR mice were conducted when they were 7 or 10 weeks of age. In keeping with past work,⁵¹ only male mice were used, and we thus did not assess sex differences. Therefore, this should be considered as a limitation of the generalizability of our research.

METHOD DETAILS

Cloning and AAV production

Cloning was performed using the In-fusion kit (Takara). Briefly, P2A-mScarlet and mScarlet sequences were amplified and inserted into pAAV-*GfaABC₁D*-MCS-4x6T-WPRE (Addgene #196417) to create pAAV-*GfaABC₁D*-mScarlet-4x6T-WPRE and pAAV-*GfaABC₁D*-P2A-mScarlet-4x6T-WPRE. For the control plasmid, P2A-mScarlet-4x6T were amplified and inserted into pZac2.1-*GfaABC₁D*-BioID2-HA (Addgene #176740) instead of the BioID2-HA to create pZac2.1-*GfaABC₁D*-mScarlet-4x6T-WPRE (Addgene #249540). Next, *Crym* (from *GfaABC₁D*-*Crym*-BioID2, Addgene #200070) and P2A-mScarlet-4x6T were amplified and both fragments were inserted in pZac2.1-*GfaABC₁D*-BioID2-HA (Addgene #176740) instead of the BioID2-HA to create pZac2.1-*GfaABC₁D*-*Crym*-P2A-mScarlet-4x6T-WPRE (Addgene #249539). The two final plasmids were sent for sequencing and to VectorBuilder (Chicago, IL) for AAV2/5 production.

Stereotaxic microinjections of adeno-associated viruses

All surgical procedures were conducted under general anesthesia using continuous isoflurane (induction at 5%, maintenance at 1% vol/vol–2% vol/vol). Depth of anesthesia was monitored continuously and adjusted when necessary. Following the induction of anesthesia, the mice were fitted into a stereotaxic frame with their heads secured by blunt ear bars and their noses placed into a veterinary grade anesthesia and ventilation system (VetEquip). Mice were administered with 0.1 mg/kg of buprenorphine (Buprenex, 0.1 mg/ml) subcutaneously before surgery. The surgical incision site was then cleaned three times with 10% povidone iodine and 70% ethanol (vol/vol). Skin incisions were made, followed by craniotomies of 2–3 mm in diameter above the frontal cortex using a small steel burr (Fine Science Tools) powered by a high-speed drill (K.1070, Freedom). Saline (0.9%) was applied onto the skull to reduce heating caused by drilling. Bilateral viral injections were carried out by using a stereotaxic apparatus (David Kopf Instruments) to guide the placement of beveled glass pipettes (1B100-4, World Precision Instruments). For the central striatum (cSTR) the coordinates were 0.8 mm anterior to bregma, 1.85 mm lateral to midline, and 2.9 mm from the pial surface. Adeno-associated viruses (AAVs) were injected at 200 nL/min by using a syringe pump (Pump11 PicoPlus Elite, Harvard Apparatus). Following AAV microinjections, glass pipettes were left in place for at least 10 min prior to slow withdrawal. Surgical wounds were closed with external 5–0 nylon sutures. Following surgery, animals were allowed to recover overnight in cages placed partially on a low voltage heating pad. Buprenorphine (Buprenex; 0.1 mg/kg) was administered subcutaneously before surgery and two times per day for up to 2 days after surgery. AAV injected mice were used for experiments three weeks post-surgery. Viruses used were: 0.3–0.5 μ L AAV2/5 *GfaABC₇D-Crym*-P2A-mScarlet (2×10^{13} gc/ml; Addgene #249539), 0.3–0.5 μ L AAV2/5 *GfaABC₇D*-mScarlet (2×10^{13} gc/ml; Addgene #249540), 0.3 μ L AAV2/5-*GfaABC₇D*-Rpl22-HA (2×10^{13} gc/ml; Addgene #111811), 0.3 μ L AAV2/5 *GfaABC₇D*-LifeAct-GFP (2×10^{11} gc/ml; Addgene #190199). All AAVs are listed in the Key Resources Table.

Human postmortem tissue immunohistochemistry (IHC) and analysis

Formalin-fixed paraffin-embedded human brain sections were cut at 5–7 micrometer thickness from the basal ganglia at the level of caudate head, rostral putamen, and nucleus accumbens. The sections were processed on a Leica® Bond RXm autostainer according to the manufacturer's instructions. The following antibodies were used: Primary antibodies – μ -crystallin (mouse, 1:500, Santa Cruz #SC376687), GFAP (chicken, 1:1000, Abcam #ab4674), and ALDH1L1 (rabbit, 1:100, Encor, #RPCA-ALDH1L1), NeuN (rabbit, 1:300, Millipore cat#: ABN78). Secondary antibodies – donkey anti-chicken 647, donkey anti-mouse 488, and donkey anti-rabbit 546- all at 1:500 – Invitrogen. Standard deparaffinization and rehydration steps preceded antigen retrieval in Leica ER2 (cat# AR9640) antigen retrieval buffer for 20 minutes. Next, a one-hour incubation in a blocking in 10% donkey serum containing PBS-based buffer preceded labeling with primary antibody incubation for 1 hour at room temperature. Three washes with Leica wash buffer (Ref#AR9590) followed. Next, labeling with species appropriate Alexa fluor conjugated secondary antibodies for 30 minutes at room temperature. After three washes, a DAPI containing mounting solution (Everbright TrueBlack Hardset Mounting Medium with DAPI, #23018) was used to label nuclei and quench autofluorescence prior to coverslipping. This is a mounting solution that diminishes all autofluorescence in lipofuscin.

All immunofluorescent images were taken on the Leica Thunder Imager DMI8. Images were acquired at 40x oil immersion objectives using a Leica K5 camera. 5 tile scans were taken from each section to label approximately 350–450 ALDH1L1+GFAP+ astrocytes per individual. Images were taken from the ventral caudate and putamen. Leica Biosystems LAS X software was used for image capture. Leica Thunder instant computational clearing was used to remove out-of-focus light. For image analysis, all observers were blind to experimental conditions. Two observers analyzed the data. Tiff files were used for analysis in Qupath 0.4.2. Annotations were created in each image to cover the tissue. To detect cells, we used the “cell detection” function (analysis tab), and the DAPI channel was set as the Detection Channel. Next, we trained an object classifier to classify the detections for the different channels. Training data was created from each image, for each channel, to mark the cells positive for a specific antigen. The classifier was tuned such that the classification results matched the impression of the observer. The proportions of ALDH1L1+CRYM+/ALDH1L1+ astrocytes and CRYM+NeuN+/NeuN+ neurons were determined for each image, and the average proportion was calculated.

Mouse immunohistochemistry (IHC)

Frozen sections

For transcardial perfusion, mice were euthanized with isoflurane and perfused with 0.1 M phosphate buffered saline followed by 10% buffered formalin (Fisher #SF100-20). Heparin (50 units) was injected into the heart to prevent blood clotting. After gentle removal from the skull, the brain was postfixed in 10% buffered formalin overnight at 4°C. For the characterization of striatal μ -crystallin expression during development, P0 and P7 brains were directly postfixed in 10% buffered formalin without trans cardiac perfusion. The tissue was cryoprotected in 30% sucrose PBS solution for at least 48 hours at 4°C until use. 40 μ m coronal sections were prepared using a cryostat microtome (Leica) at -20°C and processed for immunohistochemistry. Sections were washed 3 times in 0.1 M PBS for 10 min each, and then incubated in a blocking solution containing 10% NGS in 0.1 M PBS with 0.2% Triton-X 100 for 1 hour at RT with agitation. Sections were then incubated with agitation in primary antibodies diluted blocking solution overnight at 4°C. The following primary antibodies were used: mouse anti- μ -crystallin (1:250; Santa Cruz, #sc-376687), chicken anti-GFP (1:1000; Abcam #ab13970), mouse anti-NeuN (1:500; Millipore #MAB377), guinea pig anti-NeuN (1:500; Synaptic system, #266004), rabbit anti-S100 β (1:1000; Abcam #ab41548), guinea pig anti-RFP (1:1000; Synaptic System, #390-004), rabbit anti-RFP (1:1000; Rockland #600-401-379), rabbit anti-KIR4.1 (1:500; Alomone Labs #APC-035), rabbit anti-GLT1 (1:500; Synaptic Systems #250203), rabbit

anti-NRCAM (1:500; Proteintech #21608-1-AP), rabbit anti-HTRA1 (1:500; Proteintech #55011-1-AP). The next day the sections were washed 3 times in 0.1 M PBS for 10 min each before incubation at RT for 2 hours with secondary antibodies diluted in 0.1 M PBS. Alexa conjugated (Molecular Probes) secondary antibodies were used at 1:1000 dilution: Alexa Fluor 488 goat anti-mouse (A11010), Alexa Fluor 405 goat anti-mouse (A31553), Alexa Fluor 647 goat anti-mouse (A-21235), Alexa Fluor 488 goat anti-rabbit (A11008), Alexa Fluor 546 goat anti-rabbit (A11001), Alexa Fluor 647 goat anti-rabbit (A21244), Alexa Fluor 405 goat anti-rabbit (A-31556) Alexa Fluor 488 goat anti-chicken (A11039), Alexa Fluor 546 goat anti-chicken (A-11040), Alexa Fluor 647 goat anti-guinea pig (A-21450), Alexa Fluor 546 goat anti-guinea pig (A-11074), Alexa Fluor 405 goat anti-guinea pig (ab175678) Streptavidin, Alexa Fluor 488 conjugate (S11223). The sections were rinsed 3 times in 0.1 M PBS for 10 min each before being mounted on microscope slides and sealed in fluoromount-G. Fluorescence images were taken using UplanSApo 10X 0.4 NA, UplanFLN 40X 1.30 NA oil immersion or PlanApo N 60X 1.45 NA oil immersion objective lens on a confocal laser-scanning microscope (Fluoview FV3000; Olympus). Laser settings were kept the same within each experiment. Images represent maximum intensity projections of optical sections with a step size of 1 μ m or 5 μ m for the entire brain/striatum images. Images were processed with ImageJ (NIH v2.1). Cell counting was done on maximum intensity projections using the Cell Counter plugin; only cells with soma completely within the region of interest (ROI) were counted.

Acute sections

Acute sections were used for biocytin staining to assess medium spiny neuron (MSN) morphology, spine density, spine head width, spine area and spine length. 300 μ m fresh brain slices were placed into 10% buffered formalin overnight at 4°C and processed as followed for IHC. Sections were washed 3 times in 0.1 M PBS with 2% Triton-X 100 for 5 min each, and then incubated in a blocking solution containing 10% NGS in 0.1 M PBS with 1% Triton-X 100 for 1 hour at RT with agitation. Sections were then incubated at RT with streptavidin conjugated Alexa 488 (1:250, S11223) diluted in 0.1 M PBS with 0.4% Triton-X 100 for 3 hours. The sections were rinsed 3 times in 0.1 M PBS for 10 min each before being mounted on microscope slides in fluoromount-G. Images were obtained in the same way as IHC for frozen sections except with a step size of 0.33 μ m. For quantification of spine density, we only analyzed spines on dendritic shafts that are parallel to the imaging plane to minimize the possibility of rotational artifacts. Spine density was calculated by dividing the number of spines by the length of the dendritic segment. MSN morphology was determined using a Sholl analysis plug in with ImageJ (NIH, v2.1).

Astrocyte morphology

R6/2 and NCAR mice (4 weeks-old) were bilaterally injected in the central striatum with a low titer of astrocytic Life-Act-GFP (AAV2/5 *GfaABC₁D*-LifeAct-GFP at 2×10^{11} gc/mL) in conjunction with AAVs expressing *Crym* (AAV2/5 *GfaABC₁D*-*Crym*-P2A-mScarlet) or mScarlet (AAV2/5 *GfaABC₁D*-mScarlet) at 2×10^{13} gc/mL. Low titer allowed us to obtain a sparse labelling of astrocyte to facilitate the morphology analysis. Analysis of astrocyte morphology parameters including astrocyte territory measurements and fractal dimension analysis was conducted on maximum intensity projections as previously described.^{24,66}

Western blots

4, 7, and 10-weeks-old R6/2 and NCAR mice were decapitated and striata were dissected and homogenized with a dounce and pestle in ice-cold RIPA buffer (150 mM NaCl, 50 mM Tris pH 8.0, 1% Triton-X, 0.5% sodium deoxycholate, 0.1% SDS and Halt protease inhibitor (Thermo Scientific, #78429)). The homogenate was incubated at 4 °C while spinning for 1 h. The homogenate was sonicated and then centrifuged at 4 °C for 10 min at 15,600g. The clarified lysate was collected and the protein concentration was measured using a BCA protein assay kit (Thermo). The samples were then mixed with 2 $\times$ Laemmli solution (Bio-Rad) containing β -mercaptoethanol. The samples were boiled at 95 °C for 10 min before being electrophoretically separated by 10% SDS-PAGE (30 μ g protein per lane) and transferred onto a nitrocellulose membrane (0.45 μ m). The membrane was incubated with agitation in a solution containing 5% BSA, 0.1% Tween-20 and 0.1 M PBS for 1 h. The membrane was probed with anti- μ -crystallin (Santa Cruz, #sc-376687) at 1:250 for 2 h. The membrane was then treated with Pierce chemiluminescence solution for 1 min and imaged. The blot was incubated overnight at 4 °C with rabbit anti- β -actin (1:1,000; Abcam, ab8227). IRDye 800CW anti-rabbit (1:10,000; Li-Cor) was used as the secondary antibody, and images were acquired on a Li-Cor odyssey infrared imager. Signal intensities at expected molecular weights were quantified using ImageJ. The streptavidin signal levels were normalized to β -actin by dividing the μ -crystallin signal intensity by the β -actin signal intensity.

Behavioral tests

Behavioral tests were performed during the light cycle between 9 AM and 6 PM, 3 to 5 weeks after the AAV injection. Males were used in behavioral tests. All the experimental mice were transferred to the behavior testing room at least 30 min before the tests to acclimatize to the environment and to reduce stress. Temperature and humidity of the experimental rooms were kept at 23 $\pm$ 2°C and 55 $\pm$ 5%, respectively. Background noise (65 $\pm$ 2 dB) was generated by a white noise generator (San Diego Instruments). The brightness of the experimental room was kept < 20 lux.

Open field test

The open field chamber consisted of a square arena (28.5 $\times$ 30 cm) enclosed by walls made of translucent polyethylene (15 cm tall). Locomotor activity of mice was recorded for 10 or 30 min using a camera located above the open field chamber. The open field behaviors were analyzed with an automated video tracking software ANY-maze (v6.3 Stoelting).

Footprint test

A 1 m-long runway (8 cm wide) was lined with paper. Each mouse had its hind paws painted with non-toxic ink and was placed at an open end of the runway. The mice were allowed to walk to the other end with a darkened box. For the gait analysis, stride length and width were measured and averaged for both left and right hindlimbs over five steps.

Self-grooming behavior

Mice were placed individually into plastic cylinders (15 cm in diameter and 35 cm tall), and allowed to habituate for 20 min. Self-grooming behavior was recorded for 10 min. A timer was used to assess the cumulative time spent in self-grooming behavior which included paw licking, unilateral and bilateral strokes around the nose, mouth, and face, paw movement over the head and behind the ears, body fur licking, body scratching with hind paws, tail licking, and genital cleaning. The number of self-grooming and rearing bouts was also counted. Separate grooming bouts were considered when the pause was more than 5 sec or if behaviors other than self-grooming occurred. The self-grooming microstructure was not assessed.

Novel object recognition

On day 1 and day 2, mice were placed in an empty open chamber (28.5 × 30 cm) for 10 minutes for habituation. On day 3 (training day), mice were placed in the same open chamber containing two identical objects evenly spaced apart; the trial was video recorded for 10 minutes. For the novel object recognition test, day 4 (testing day), 24 hours after training, mice were placed in the same open chamber, with one of the two objects replaced with a novel object. The trial was video recorded for 10 minutes. Time exploring around the objects was manually measured. Recognition index was calculated as follows: (time exploring the novel object – time exploring the familiar object) / (time exploring both objects) – 50.

Hindlimb clasping test

Each mouse was suspended by holding the tail at a height of approximately 10 inches above the home cage. Time taken (in seconds) by the mouse to move both forelimbs close to the body and for both paws to clasp was recorded. Maximum allotted time for clasping was 60 s and the mice that did not clasp in that time were given a data value of 60 s. Mice that visibly clasped in under 60 s were immediately lowered into the home cage to avoid any distress.

Forelimb grip strength

Forelimb strength was measured using a customized grip strength meter (Columbia instruments) with an adjustable wire mesh grip designed for mice. Both the grip strength meter and the mesh grip were positioned horizontally. Each mouse was held by the base of the tail and lowered to grasp the mesh with both forelimbs. A steady horizontal force was then applied to extend the mouse away from the mesh until it relinquished its grip on the mesh. Maximum pulling force required to separate the mouse from the mesh grip was recorded by the meter. Each mouse was tested in five trials with a 5 min inter trial interval and average maximum pulling force (per mouse) was used to assess grip strength

Brain slice preparation and electrophysiological recordings in the striatal slices

Coronal striatal slices were prepared from 7–8 weeks old R6/2 or NCAR mice injected with mScarlet control AAV or *Crym*-mScarlet AAV. Briefly, animals were deeply anesthetized with isoflurane and decapitated with sharp shears. The brains were placed and sliced in ice-cold modified artificial CSF (aCSF) containing the following: 194 mM sucrose, 30 mM NaCl, 4.5 mM KCl, 1 mM MgCl₂, 26 mM NaHCO₃, 1.2 mM NaH₂PO₄, 10 mM D-glucose, and saturated with 95% O₂ and 5% CO₂. A vibratome (DSK Microslicer; Ted Pella, Inc.) was used to cut 300 μ m brain sections. The slices were allowed to equilibrate for 30 min at 33°C in normal aCSF containing: 124 mM NaCl, 4.5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 26 mM NaHCO₃, 1.2 mM NaH₂PO₄, and 10 mM D-glucose continuously bubbled with 95% O₂ and 5% CO₂. Slices were then stored at 21–23°C in the same buffer until use. All slices were used within 6 hours of slicing. The brain slice recordings were performed at RT (21–23 °C). Whole-cell patch-clamp recordings were made from astrocytes or medium spiny neurons in the central striatum using patch pipettes with a typical resistance of 4–6 M Ω . MSNs were morphologically and electrophysiologically identified. Astrocytes were identified with mScarlet expression. The intracellular solution for MSN membrane properties, EPSCs, and astrocyte recordings comprised the following: 135 mM potassium gluconate, 3 mM KCl, 10 mM HEPES, 1 mM EGTA, 0.3 mM Na-ATP, 4 mM Mg-ATP, 0.1 mM CaCl₂, 8 mM Na₂-phosphocreatine, with pH adjusted to 7.3. To isolate mEPSCs, MSNs were voltage-clamped at -70 mV and pre-incubated with 0.3 μ M TTX (abcam, #1069) before recording. In some cases, 1 mg/ml biocytin (Tocris, #3349) was added to the intracellular solution to subsequently visualize patched neuron. All recordings were performed at RT, using pCLAMP11.2 (Axon Instruments, Molecular Devices) and a MultiClamp 700B amplifier (Axon Instruments, Molecular Devices). Cells with Ra that exceeded 25 M Ω were excluded from analysis. Analysis for membrane properties was performed using ClampFit 10.7 software. Analysis for mEPSCs and sEPSCs was performed using Mini Analysis Program (Synaptosoft, V6.0.8).

RNA-sequencing experiments using RiboTag

R6/2 and NCAR mice (4 weeks-old) were bilaterally injected in the central striatum with astrocytic RiboTag (AAV-2/5-*GfaABC₁D*-Rpl22-HA) in conjunction with AAVs expressing *Crym* (AAV2/5 *GfaABC₁D*-*Crym*-P2A-mScarlet) or mScarlet (AAV2/5 *GfaABC₁D*-mScarlet). RNA was immunoprecipitated (IP) from astrocytes at 7–8 weeks old as previously described.⁵⁶ Briefly, freshly dissected striatum tissues were collected and individually homogenized in 1 ml of homogenization buffer (50 mM Tris pH 7.4, 100 mM KCl, 12 mM MgCl₂, 1% NP-40, 1 mM Dithiothreitol (DTT), 1X Protease inhibitors, 200 U/ml RNasin, 100 μ g/ml Cyclohexamide, 1 mg/ml Heparin). RNA was extracted from 20% of cleared lysate as Input (200 μ l). The remaining lysate (800 μ l) was incubated with 5 μ l of mouse anti-HA antibody

(Biolegend #901514) with rocking for 4 hours at 4°C followed by addition of 200 μ l of magnetic beads (Pierce #88803) and overnight incubation with rocking at 4°C. The beads were washed three times in high salt solution (50 mM Tris pH 7.4, 300 mM KCl, 12 mM $MgCl_2$, 1% NP-40, 1 mM Dithiothreitol (DTT), 100 μ g/ml Cyclohexamide). RNA was purified from the IP and corresponding Input samples (RNeasy Plus Micro QIAGEN #74034). For bulk RNAseq of OFC tissue a similar procedure was followed as that used for input samples. RNA concentration and quality were assessed with Agilent 2100 Bioanalyzer. RNA samples with RNA integrity number (RIN) greater than seven was processed with Ribo-Zero Gold kit (Epicentre, WI) to remove ribosomal RNA. Sequencing libraries were prepared using Illumina TruSeq stranded RNA sample prep kit or ABClone FAST RNA library preparation kit (following the manufacturer's protocol). After library preparation, amplified double-stranded cDNA was fragmented into 125 bp (Covaris-S2, Woburn, MA) DNA fragments, which were (200 ng) end-repaired to generate blunt ends with 5'- phosphates and 3'- hydroxyls and adapters ligated. The purified cDNA library products were evaluated using the Agilent Bioanalyzer (Santa Rosa, CA) and diluted to 10 nM. Samples in each experiment were multiplexed into a single pool to avoid batch effects, and 150 bp-paired-end sequencing was performed using an NovaSeq X plus (Illumina, San Diego, CA). A yield between 59 and 115 million reads (average 87.8M) was obtained per sample. Quality control was performed on base qualities and nucleotide composition of sequences. Alignment to the *M. musculus* GRCm39 (mm39) reference gene annotation was performed using the STAR spliced read aligner (v2.7.5c) with default parameters. Additional QC was performed after the alignment to examine: the degree of mismatch rate, mapping rate to the whole genome, repeats, chromosomes, key transcriptomic regions (exons, introns, UTRs, genes), insert sizes, AT/GC dropout, transcript coverage and GC bias. Between 75% and 93% (average 87.0%) of the reads mapped uniquely to the mouse genome. Total counts of read-fragments aligned to candidate gene regions were derived using HTSeq program (v0.12.4; www.huber.embl.de/users/anders/HTSeq/doc/overview.html) with mouse GRCm39.112 as a reference and used as a basis for the quantification of gene expression. Only uniquely mapped reads were used for subsequent analyses. Differential expression analysis was conducted with R-project and the Bioconductor package limma-voom (3.50.3). RNA-seq data has been deposited within the Gene Expression Omnibus (GEO # GSE310059) repository (www.ncbi.nlm.nih.gov/geo). For pathways analysis, QIAGEN ingenuity pathway analysis (IPA) or Enrichr GO analysis were performed to identify canonical pathways associated with these genes (made available by QIAGEN Digital Insights).

Mapping of DEGs from bulk RNAseq to striatal cell types

Cell type enriched genes of the mouse striatum based on scRNAseq data were obtained as described previously.⁵⁵ Genes with log2 FC > 0.25 and adj. P-value < 0.05 were considered cell type enriched and used to map bulk RNAseq DEGs onto neurons, OPCs, oligodendrocytes, astrocytes, endothelial cells, microglia, smooth muscle cells or pericytes. Genes that did not pass the log2FC and FDR filter cut off were not considered as cell type identifying genes and were not categorized. The analysis of bulk RNAseq data in this way assumes that the proportions of the cells do not change and should be interpreted with this caveat in mind.

Comparisons to published RNAseq studies for humans and mice

Comparisons made in Figures 2 and 8 were made from databases published in past studies. The 127 enriched genes in *Crym*+ astrocytes were identified by scRNA-seq analysis as described²⁵ (GSE225741). The human HD DEGs used in Figures 2A, 2B, 7K, and 7L are from caudate bulk RNA-sequencing and caudate astrocyte single nucleus RNA-sequencing⁴ (GSE242198). Significant DEGs only were used for the comparisons (p-value < 0.05). In Figure 2B, the 78 Astrocyte *Crym* interactors were obtained with astrocyte-specific *Crym*-BioID2 and previously published.²⁵ The protein network graph, the clustering analysis, the biological processes and molecular functions pathways were all generated using STRING (V12.0). Node colors indicate association with specific biological process and the thickness of gray edges between proteins reflects the predicted strength of interaction.

CSF mass spectrometry and bioinformatic analysis

Human CSF samples were processed in an SDC buffer (1% w/v sodium deoxycholate, "SDC", Sigma-Aldrich; 40 mM 2-Chloroacetamide, "CAA", Sigma-Aldrich; 10 mM Tris(2-carboxyethyl)phosphine hydrochloride, "TCEP", Sigma-Aldrich; 100 mM Tris, Sigma-Aldrich; MS grade water, VWR) and boiled at 95°C for 10 minutes at 1200 rpm to denature proteins. A master mix of Trypsin (T6567; Sigma-Aldrich) and LysC (125-02543; Fujifilm, WAKO) enzyme was added at 1:100 (w/w) to digest proteins for 2 hours at 37°C and 800 rpm before the reaction was quenched by acidification using quenching buffer (MS grade isopropanol, Fisher Scientific; 1% w/w trifluoroacetic acid, "TFA", Sigma-Aldrich). Samples were then transferred to and spun through StageTips containing two layers of SDB-RPS material (Empore) in a plate centrifuge. The captured peptides were cleaned up on the StageTips through two organic buffer wash steps (quenching buffer, described above) and two inorganic buffer wash steps (0.2% TFA, Sigma-Aldrich; MS grade water, VWR), and peptides were then eluted using a basic elution buffer (1% ammonia, Merck; 80% MS grade acetonitrile, Merck; MS grade water, VWR) and vacuum-dried to complete dryness at 45°C. Dry peptides were then stored at -80°C until shortly before the samples were scheduled for mass spectrometry analysis, at which time they were resuspended in a mass spectrometry compatible buffer (0.1% TFA, Sigma-Aldrich; 5% MS grade acetonitrile, Merck; MS grade water, VWR) and measured in duplicate by Nanodrop 2000. 400 ng peptides per sample were loaded onto Evotips according to the manufacturer's instructions (Evotip Quick Guide, 2022 version) using isopropanol (MS grade isopropanol, Fisher Scientific), Evosep solvent A (0.1% formic acid, Merck; milliQ water) and Evosep solvent B (0.1% formic acid, Merck; MS grade acetonitrile, Merck). Samples were injected on an Orbitrap Astral mass spectrometer (Thermo Fisher Scientific) connected to an Evosep One HPLC system (Evosep Biosystems) by an 80mm analytical column (EV1115 Performance column, Evosep Biosystems) kept at 55°C and attached to an

EASY-Spray ion source. Peptides were eluted using a standardized 21-minute gradient (corresponding to 60 samples per day; '60SPD'), and all samples were run in DIA mode with the instrument operated at a full-MS resolution of 240,000 with a full scan range of 380 to 980 m/z with automatic gain control (AGC) target set to 500%, and a maximum injection time (IT) of 3ms. Fragment ion (MS/MS) scans ran with 200 3-Th windows with a scan range of 150 to 2000 m/z and a maximum IT of 5ms. Isolated ion fragmentation was conducted using HCD at 25% normalized collision energy (NCE). A standardized HeLa digest and a pooled CSF digest were used as technical and internal quality controls, respectively, and were acquired at regular intervals to ensure consistent instrument performance and sample reproducibility throughout. Raw files were processed in Spectronaut version 20.1 (Biognosys) using directDIA in a two-step process wherein a spectral library was created from multiple search archives and used to analyze the full dataset to ease computational load. Label-free quantification (LFQ) was used to estimate relative protein abundances across samples, as this provides broad proteome coverage without the use of isotopic or chemical labels as would be needed for absolute quantification. LFQ relies on stable instrument performance and appropriate normalization to mitigate variation between runs, as was done for this study, and is suitable for comparative studies. For analysis, enzyme specificity was set to Trypsin/P with peptides from 7 to 52 amino acid lengths and maximum 2 missed cleavages allowed, and carbamidomethyl (C) was permitted as a fixed modification, and oxidation (M) and acetyl (N-term) were permitted as variable modifications. The false discovery rate (FDR) was set to 0.01 on all levels, and cross-run normalization was run in global mode. Run-level protein scoring was performed by highest scoring observation. All bioinformatic analyses were conducted in Python using the Clinical Knowledge Graph (CKG).⁹³ As part of quality control, hemoglobin-related markers (HBA2, HBB, HBD) were evaluated across all samples using standardized z-scores to identify potential outliers and to assess whether extreme values ($|z\text{-score}| > 3$) influenced the total number of detected proteins, as previously described.⁹⁴ CRYM was detected in all samples, resulting in 100% data completeness for this protein across the entire cohort. Sample order was randomized and batch correction was performed using pyComBat⁹⁵ and correcting for sample plate distribution while factoring in the groupings as covariates as recommended by the developers. Post-data processing coefficients of variation (CVs) for CRYM were consistent and low across all study groups, ranging from 31% to 37%. Differential expression of CRYM between the experimental groups was identified using t-test with permutation-based threshold $FDR < 0.05$ at 250 randomizations and an $s0$ -value of 0.01. Protein correlation to CAP and cUHDS were evaluated for HD groups using Pearson's correlation coefficients on LFQ values in GraphPad Prism (version 10.4.2). For cUHDS correlations, 10 HD individuals had unavailable clinical score thus were excluded from the analysis.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical tests were run in OriginPro 2017 (OriginLab v9.4.2). Box plots are presented with a mean $\pm$ S.E.M. and the whiskers show the 10 percentile–90 percentile range along with the individual data points and the distribution line. Sample sizes were not determined in advance and were based on past studies that are cited at the relevant sections of the manuscript and methods. All mice were assigned to experimental groups at random. No data points were excluded from any experiment. Statistical tests were chosen as described below. All replicates were biological, not technical. Blinding was not done. For each set of data to be compared, we used OriginPro to determine whether the data were normally distributed or not. If they were normally distributed, we used parametric tests. If the data were not normally distributed, we used non-parametric tests. Paired or unpaired Student's T-test or Mann-Whitney tests were used for statistical analyses with two samples (as appropriate). Two-way ANOVA tests followed by Tukey's *post-hoc* test or Kruskal–Wallis ANOVA, followed by Dunn's *post hoc* tests were used for statistical analyses with more than three samples. For the human IHC data, a Kruskal–Wallis T-test was performed to compare the proportion of CRYM+ astrocytes in controls ($n = 4$) to HD ($n = 10$), and then a non-parametric ANOVA was performed in controls to grade 1 HD ($n = 4$), and to HD grades 2–4 ($n = 6$) with *post-hoc* tests. In all cases, a significant difference was declared at $P < 0.05$ and is indicated as such throughout. Findings that were non-significant are indicated as NS. The numbers of mice or independent samples for each experiment are indicated in the results section.