Differential HspBP1 expression accounts for the greater vulnerability of neurons than astrocytes to misfolded proteins

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Although it is well known that astrocytes are less vulnerable than neurons in neurodegenerative diseases, the mechanism behind this differential vulnerability is unclear. Here we report that neurons and astrocytes show markedly different activities in C terminus of Hsp70-interacting protein (CHIP), a cochaperone of Hsp70. In astrocytes, CHIP is more actively monoubiquitinated and binds to mutant huntingtin (mHtt), the Huntington's disease protein, more avidly, facilitating its K48-linked polyubiquitination and degradation. Astrocytes also show the higher level and heat-shock induction of Hsp70 and faster CHIP-mediated degradation of various misfolded proteins than neurons. In contrast to astrocytes, neurons express abundant HspBP1, a CHIP inhibitory protein, resulting in the low activity of CHIP. Silencing HspBP1 expression via CRISPR-Cas9 in neurons ameliorated mHtt aggregation and neuropathology in HD knockin mouse brains. Our findings indicate a critical role of HspBP1 in differential CHIP/Hsp70 activities in neuronal and glial cells and the greater neuronal vulnerability to misfolded proteins in neurodegenerative diseases.

polyglutamine | Huntington | chaperone | misfolding | neurodegeneration

Protein misfolding causes a wide range of neurodegenerative diseases, including Alzheimer's, Parkinson's, and polyglutamine diseases, such as Huntington's disease (HD) (1). Although all these disease proteins are ubiquitously expressed in different types of cells, they preferentially accumulate in neuronal cells and cause neurotoxicity (2). In the brain, more than 90% of cells are glial cells, which are affected to a much lesser extent than neurons in neurodegenerative diseases (3). A challenging issue in the pathogenesis of neurodegenerative diseases is why misfolded proteins preferentially kill neurons rather than glial cells in the brain. Understanding the mechanism for the selective neurodegeneration is important for developing effective treatments of neurodegenerative diseases.

It is established that the accumulation of misfolded proteins in neuronal cells is a prerequisite for their toxicity (4, 5). Preventing the accumulation of misfolded proteins in cells relies highly on molecular chaperones, which refold misfolded proteins, and the ubiquitin–proteasome system (UPS) and autophagy, which degrade misfolded proteins. C terminus of Hsp70-interacting protein (CHIP), a cochaperone of Hsp70 with ubiquitin E3 ligase, links chaperones to the UPS and plays a critical role in protein triage decisions. Mice with CHIP deficiency show increased levels of toxic oligomer proteins and decreased proteasomal activity (6). CHIP is recruited to chaperone–substrate complexes and consequently polyubiquitinates the chaperone-bound substrates, which are then degraded by the proteasome (7–9). Hsp70-binding protein 1 (HspBP1), a cochaperone of Hsp70 that inhibits the activity of Hsp70 ATPase (10), also associates with CHIP and prevents CHIP-mediated ubiquitination on substrates (11). However, it remains unclear whether CHIP/Hsp70 activities are differentially regulated in neuronal and glial cells and whether differential CHIP/Hsp70 activities account for the preferential neuronal vulnerability to misfolded proteins in neurodegenerative diseases.

In the current study, we investigated the mechanism underlying the differential vulnerability of neurons and glia to misfolded proteins. We found that the higher CHIP activity in astrocytes than neurons accounts for the higher Hsp70 activity and faster degradation of misfolded proteins in astrocytes. Neurons express much more abundant HspBP1, which inhibits CHIP activity, and knocking down HspBP1 in neurons rescued neuropathology in a HD knockin (KI) mouse model. Our findings provide mechanistic insight into the differential vulnerabilities of neuronal and glial cells to misfolded proteins and also offer a therapeutic target in neurodegenerative diseases.

Results

Increased Monoubiquitinated CHIP Indicates Active CHIP in Astrocytes. Our recent study showed that astrocytes degraded mutant huntingtin (mHtt), the HD protein, faster than neurons (12). Since CHIP is a cochaperone of Hsp70 with ubiquitin E3 ligase activity for degradation of misfolded proteins (7–9), we examined its expression in cultured astrocytes and neurons and found more abundant CHIP with a high molecular weight (MW) in astrocytes than neurons (Fig. 1*A*). This high-MW CHIP is reminiscent of monoubiquitinated CHIP (Ub1-CHIP) as reported previously (9) and consistently, is decreased by CHIP knockdown (KD) (Fig. 1*B*). To provide further evidence for the ubiquitination of the high-MW CHIP, we transfected CHIP-myc in cultured astrocytes and immunoprecipitated CHIP with anti-myc antibody.

Significance

It remains unclear why astrocytes are affected to a lesser extent than neurons in a variety of neurodegenerative diseases. We report the higher activity of C terminus of Hsp70-interacting protein (CHIP), cochaperone of Hsp70, in astrocytes than in neurons, which not only promotes the degradation of misfolded proteins, but also upregulates levels of basal and stress-induced Hsp70 in astrocytes. Furthermore, the low activity of CHIP in neurons is caused by the abundant expression of HspBP1, an inhibitor of CHIP. Knocking down HspBP1 in neurons prevents the accumulation and aggregation of the Huntington's disease (HD) protein and ameliorates neuropathology in a HD knockin mouse model. These findings suggest that HspBP1 accounts for differential vulnerabilities of neurons and glia to misfolded proteins.

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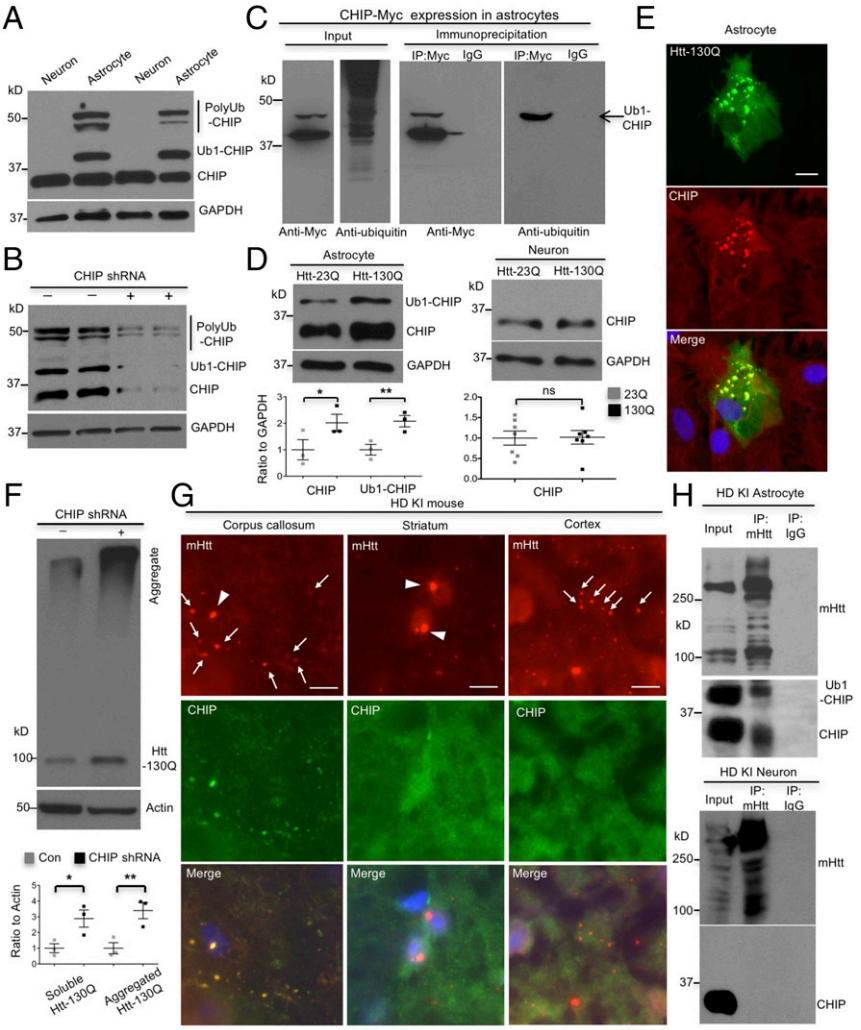

Fig. 1. CHIP is more actively involved in mHtt degradation in astrocytes. (*A*) More abundant monoubiquitinated CHIP (Ub1-CHIP) is present in cultured astrocytes than in neurons. (*B*) CHIP knockdown decreased Ub1-CHIP in astrocytes ($n = 3$ independent experiments). (*C*) Transfected CHIP-myc in cultured astrocytes was immunoprecipitated by anti-myc. Immunoblotting of the immunoprecipitates with anti-ubiqutitin indicated the presence of mono-ubiquitinated CHIP in astrocytes. (*D*) Expression of transfected mHtt (Htt-130Q) selectively increases the levels of CHIP and Ub1-CHIP in astrocytes but not in neurons [$n = 3$ independent experiments (astrocytes) and $n = 5$ independent experiments (neurons)]. (*E*) Immunofluorescent staining shows the colocalization of transfected mHtt (Htt-130Q) with endogenous CHIP in cultured astrocytes. (Scale bar, 5 μm.) (*F*) CHIP knockdown increases both soluble and aggregated mHtt (Htt-130Q) in astrocytes ($n = 3$ independent experiments). (*G*) Colocalization of CHIP with mHtt aggregates was detected in glial cells in the corpus callosum, but not in neuronal cells in the striatum and cortex, in HD140Q KI mice at the age of 12 mo. Arrowheads indicate nuclear mHtt aggregates; arrows indicate cytoplasmic aggregates in glial cells or neuropil mHtt aggregates in neurons. (Scale bar, 10 μm.) (*H*) Association of mHtt with CHIP and Ub1-CHIP was detected in HD140Q KI astrocytes but not in KI neurons. Quantitative data beneath the blots are represented as mean ± SEM (error bar). $^*P < 0.05$, $^{**}P < 0.01$ (unpaired two-tailed Student's *t* test); ns, not significant.

Immunoblotting with anti-ubiquitin to detect endogenous ubiquitin in immunoprecipitates clearly showed that the high-MW band represents monoubiquitinated CHIP in astrocytes (Fig. 1*C*).

Because CHIP is monoubiquitinated when it polyubiquitinates misfolded proteins (9), increased Ub1-CHIP suggests that CHIP is more active in astrocytes than neurons tackling misfolded proteins. To test this idea, we transfected N-terminal Htt (1–230 amino acids), which contained normal 23 (Htt-23Q) or expanded 130 (Htt-130Q) glutamine repeats conjugated with the photoswitchable fluorescent protein Dendra2 (12, 13) into astrocytes and neurons. Compared with Htt-23Q, Htt-130Q increased the expression of CHIP and Ub1-CHIP in astrocytes but not in neurons (Fig. 1*D* and Fig. S1*A*), suggesting that CHIP plays a specific role in coping with mHtt in astrocytes. Indeed, we found that CHIP colocalizes with transfected Htt-130Q (Fig. 1*E*), and CHIP knockdown stabilized Htt-130Q in astrocytes (Fig. 1*F*). Furthermore, we found that in HD 140Q KI mouse model in which full-length mHtt is expressed at endogenous levels, CHIP is colocalized with mHtt aggregates in the corpus callosum (CC) where astrocytes are enriched (Fig. 1*G*). It has been reported that in the brains of HD patients and KI mice, large and round aggregates are localized in neuronal nuclei, and small, bead-like aggregates are localized in neuropils (14, 15). However, there is absence of CHIP in neuronal aggregates (Fig. 1*G*), suggesting that CHIP does not associate with mHtt in neurons. Double immunofluorescent staining was also unable to reveal CHIP aggregates in neuronal cells in KI mice (Fig. S1*B*). Furthermore, by immunoprecipitating endogenous mHtt from cultured neurons and astrocytes of KI mice, we also saw an association of mHtt with both CHIP and Ub1-CHIP in astrocytes, but not in neurons (Fig. 1*H*). Collectively, these data suggest that CHIP is more active to clear mHtt in astrocytes.

Active CHIP Promotes Ubiquitination of mHtt in Astrocytes. CHIP has ubiquitin E3 ligase activity and polyubiquitinates substrates for proteasomal degradation (7, 8). Indeed, our results indicated that astrocyte-specific association between CHIP and mHtt increased K48-linked polyubiquitinated mHtt in HD KI astrocytes (Fig. 2 *A* and *B*), which is corroborated by elimination of the enhanced K48-polyubiquitinated mHtt via CHIP knockdown (Fig. 2 *C* and *D*). Using in vitro proteasome activity assay, we ruled out the possibility that the increased K48-linked polyubiquitination of mHtt in astrocytes is due to mHtt-causing proteasomal dysfunction (Fig. 2*E*). Given that K48 polyubiquitination targets substrates for proteasomal degradation, increased K48-linked polyubiquitinated mHtt explains the faster clearance of mHtt in astrocytes (12).

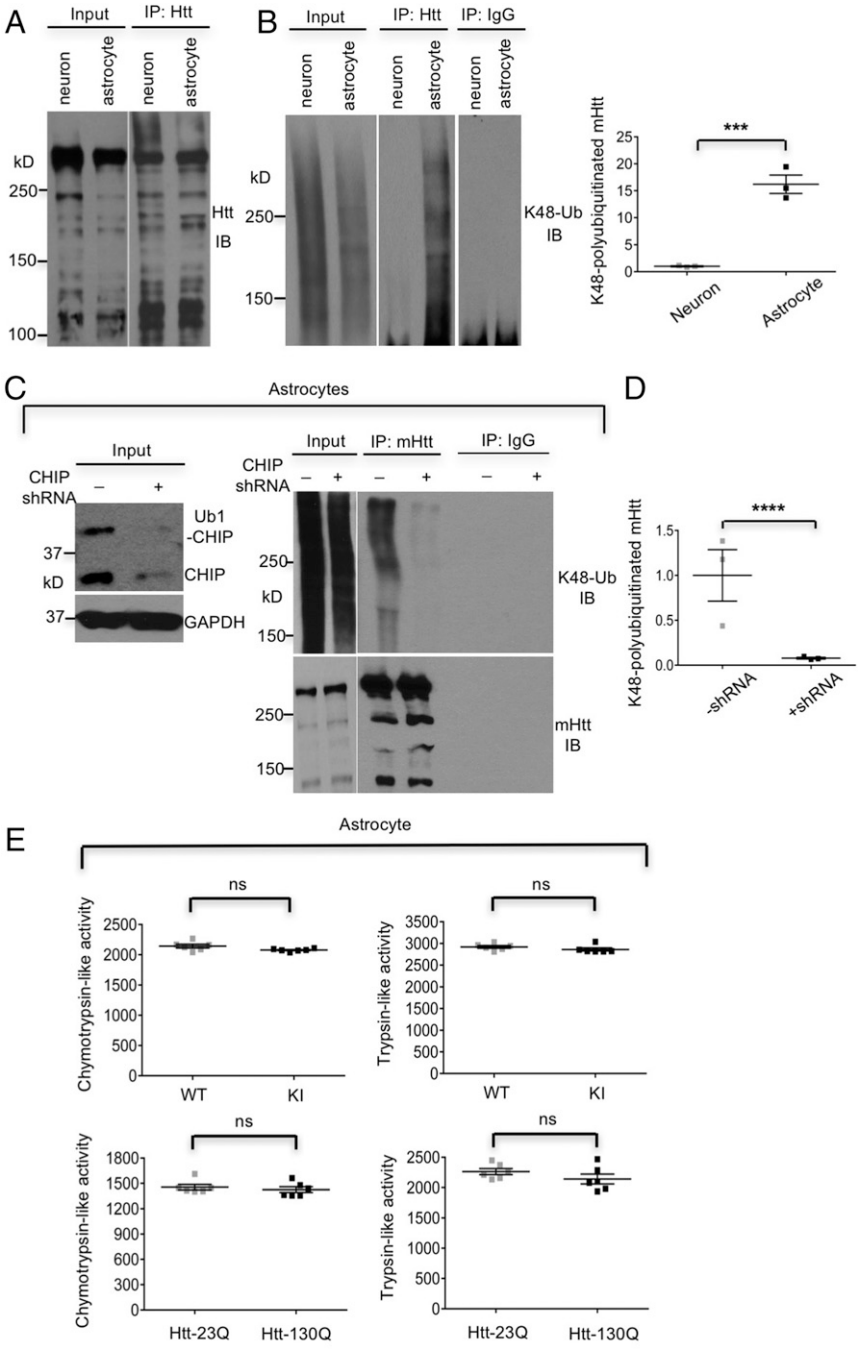

Fig. 2. CHIP enhances K48-linked polyubiquitination on mHtt in astrocytes. (*A* and *B*) There are more K48-polyubiquitinated mHtt in KI cortical astrocytes than KI neurons. Ratios of K48-polyubiquitinated mHtt to total immunoprecipitated mHtt are from three independent experiments. (*C* and *D*) CHIP knockdown suppressed K48-linked polyubiquitination on endogenous mHtt in HD140Q KI cortical astrocytes. Ratios of K48-polyubiquitinated mHtt to total immunoprecipitated mHtt are from three independent experiments. (*E*) Expression of mHtt in Htt-130Q transfected astrocytes and HD140Q KI astrocytes did not affect proteasomal function compared with Htt-23Q transfected or wild-type (WT) astrocytes ($n = 3$ independent experiments). In *A*–*D*, cultured cells were treated with lactacystin (10 μM for 6 h) to inhibit proteasomal degradation. ***$P < 0.001$, ****$P < 0.0001$. ns, not significant. Data are represented as mean ± SEM. Error bars indicate SEM.

Active CHIP Increases Hsp70 and Facilitates Degradation of Various Misfolded Proteins in Astrocytes. Although the binding of CHIP to substrates depends on Hsp70 (16, 17) and CHIP is able to enhance Hsp70 expression in cultured fibroblast cells in response to heat shock (18), its relation with Hsp70 in neuronal and glial cells remained elusive. By inducing Hsp70 in HEK293 cells with PU-H71, a compound that up-regulates Hsp70 transcription (19), we found that increased Hsp70 promoted the association of CHIP with transfected mHtt (Htt-73Q) and that this association was suppressed by Hsp70 knockdown (Fig. S2). Importantly, we also found that basal Hsp70 was more abundant in cultured astrocytes than neurons (Fig. S3*A*). To validate this difference in the brain, we isolated the CC, a brain region enriched in astrocytes, and the cerebral cortex (CTX) with less abundant astrocytes to examine Hsp70 levels. Western blotting also showed a higher level of basal Hsp70 in the astrocyte-enriched corpus callosum (Fig. S3*B*). Consistently, double immunostaining confirmed the higher level of Hsp70 in astrocytes than neurons in the mouse brain (Fig. S3*C*).

We then compared heat-shock responses in wild-type neurons and astrocytes under hyperthermia conditions and found robustly increased Hsp70 in astrocytes but not in neurons (Fig. 3*A*). In addition, expression of mHtt did not have an impact on these differential responses (Fig. S4). Strikingly, both CHIP and Ub1-CHIP were increased in wild-type and HD 140Q KI astrocytes, whereas CHIP declined in neurons after heat shock (Fig. 3*A* and Fig. S4). To verify that the different Hsp70 responses in cultured neurons and astrocytes also occur in brain tissues, we isolated brain slices from wild-type and HD140Q KI mice. The brain slices consisting of the cerebral cortex and corpus callosum were incubated under normal physiological or heat-shock stress conditions. The cortex and corpus callosum were then dissected for Western blotting. Consistently, hyperthermia induced Hsp70 in glia- but not neuron-enriched regions in both wild-type and HD140Q KI mouse brains (Fig. 3*B*). The differential heat-shock response found here is consistent with previous findings that heat-shock response is absent in neurons, but robust in astrocytes (20–22).

The exact mechanism underlying distinct heat-shock responses from astrocytes and neurons is unknown. We postulated that differential activities of CHIP in astrocytes and neurons cause different heat-shock responses. Indeed, CHIP knockdown abolished heat-shock induction of Hsp70 in astrocytes (Fig. 3 *C* and *E*), confirming that the specific induction of Hsp70 by heat-shock stress in astrocytes is CHIP mediated. Furthermore, CHIP

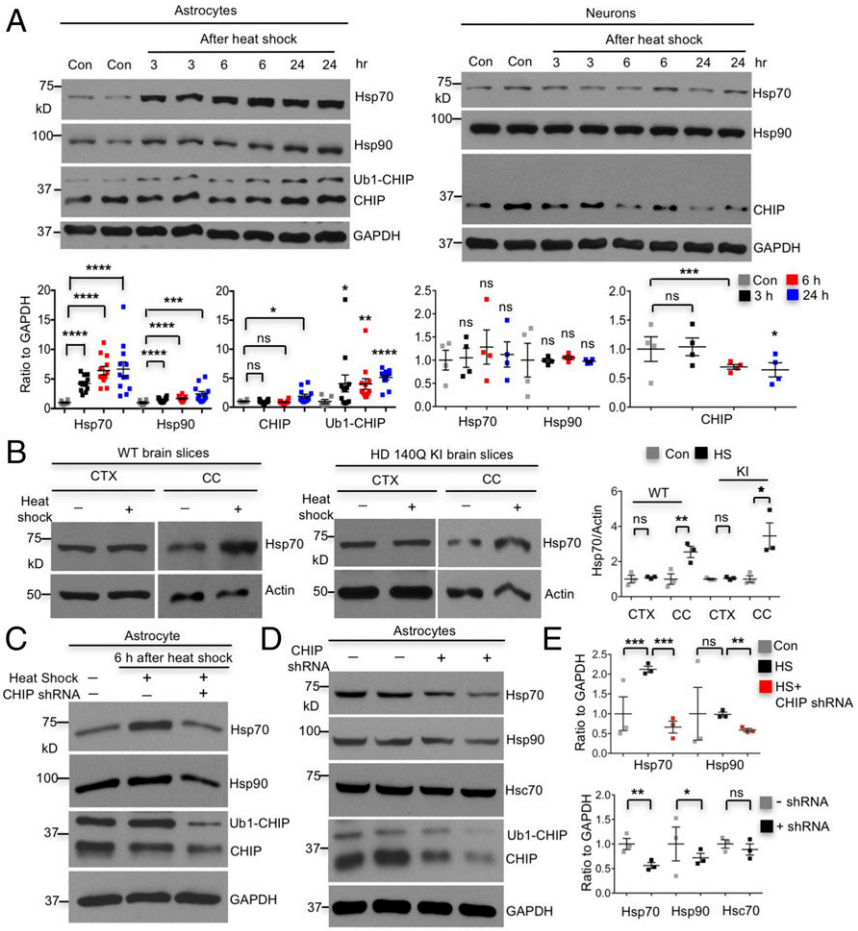

Fig. 3. Differential CHIP activity causes distinct heat-shock responses in astrocytes and neurons. (*A*) Typical heat shock (42 °C for 1 h) induced Hsp70, Hsp90, CHIP, and Ub1-CHIP specifically in wild-type cortical astrocytes around 30 days in vitro (DIV 30) (*Left*), but not in wild-type cortical neurons at DIV 10–14 (*Right*) (at least three independent experiments). (*B*) Heat-shock stress increased Hsp70 in the corpus callosum, but not in the cortex, of brain slices from wild-type (WT) and HD140Q KI mice at the age of 3 mo ($n = 3$ independent experiments). CC, corpus callosum; CTX, cortex; HS, heat shock. (*C*) Induction of Hsp70 by heat shock (42 °C, 1 h) was completely inhibited by CHIP knockdown, and no Hsp90 was induced in wild-type astrocytes at DIV 45–60. (*D*) CHIP knockdown decreased the expression of Hsp70/Hsp90, but did not affect levels of Hsc70 in DIV 45–60 wild-type astrocytes. (*E*) Ratios of indicated proteins to GAPDH are from three independent experiments (*Upper* for *C* and *Lower* for *D*). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ (unpaired two-tailed Student's *t* test). ns, not significant. Data are represented as mean ± SEM (error bar).

knockdown also decreases the basal levels of Hsp70/Hsp90 but not Hsc70 in astrocytes (Fig. 3 *D* and *E*), which is the opposite of previous findings that CHIP knockdown enhanced the basal Hsp70 in immortal cell lines (18). The basal levels of Hsp70 may be various in different types of cells. The finding that CHIP is more active in astrocytes also explains why astrocytes have the higher basal levels of Hsp70 than neurons. Together, our results suggest that CHIP-dependent up-regulation of Hsp70 in astrocytes contributes to the preferential and Hsp70-dependent binding of CHIP to mHtt in astrocytes.

To investigate whether CHIP also promotes the clearance of other misfolded proteins in astrocytes, we transfected mutant TDP-43 (M337V), polyQ expanded TATA box binding protein (TBP-105Q), or mutant α-synuclein (A53T), in astrocytes and neurons, as these mutant proteins cause neurodegenerative diseases (23, 24). Cycloheximide (CHX) half-life assay demonstrated that all these mutant proteins were removed faster by astrocytes than neurons, which was abolished by CHIP knockdown (Fig. 4). These results suggest that astrocytes use CHIP to accelerate the degradation of misfolded proteins, which protects astrocytes from misfolded proteins in neurodegenerative diseases.

We further investigated the role of CHIP in viability of astrocytes under severe heat-shock stress (45 °C for 30 min). Astrocytes were resistant to heat-shock stress, which was indicated by no difference in viability and morphology between untreated and hyperthermia-treated astrocytes (Fig. 5 *A* and *C*). In contrast, ∼86% neurons were killed by the same hyperthermia condition (Fig. 5 *A* and *C*). However, the lethality of astrocytes after hyperthermia was increased when CHIP was knocked down (Fig. 5 *B–E*), indicating that the high activity of CHIP is involved not only in the prompt clearance of mHtt, but also in protection of cell viability.

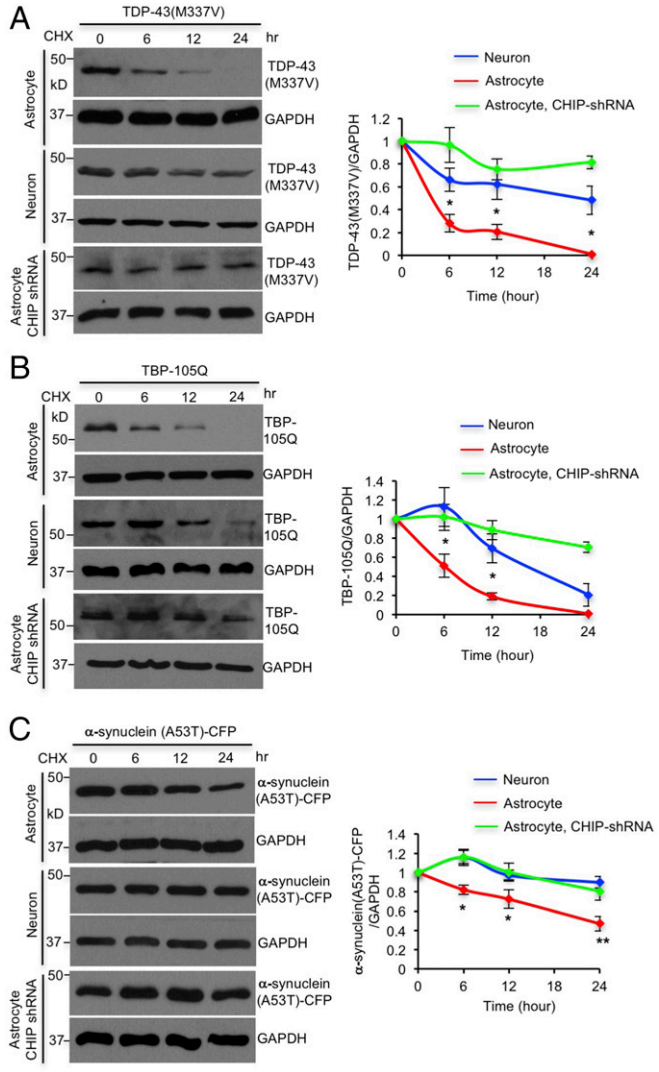

Fig. 4. CHIP mediates faster degradation of misfolded proteins in astrocytes. (*A–C*) Mutant TDP-43 (M337V), TBP-105Q, and α-synuclein (A53T)-cyan fluorescent protein (CFP) were degraded faster by astrocytes than neurons, which was eliminated by CHIP knockdown in astrocytes. $*P < 0.05$, $**P < 0.01$ (unpaired two-tailed Student's *t* test). ns, not significant. Data are represented as mean ± SEM (error bar).

Differential HspBP1 Expression Results in Distinct CHIP Activity Between Astrocytes and Neurons. Our findings raise a key question of why CHIP is more active in astrocytes. Using RNA-seq database to compare the profiles of gene expression between neurons and astrocytes (25), we found that HspBP1, a cochaperone of Hsp70 that inhibits CHIP E3 ligase activity (11), shows approximately fivefold increase at the transcriptional level in neurons compared with astrocytes. The different expression level of HspBP1 was first confirmed with Western blotting, showing the abundant level of HspBP1 in cultured neurons but the almost undetectable level in cultured astrocytes (Fig. 6*A*). In addition, HspBP1 is distributed throughout the neurons, and its cellular localization is quite consistent with that of CHIP (Fig. 6*B*). In contrast, only faint or background staining of HspBP1 was seen in astrocytes (Fig. 6*B*). Since Bag2 has been reported to be another CHIP inhibitor, we also examined the level of Bag2 and found there was no difference between neurons and astrocytes, suggesting that Bag2 is not the determinant of the high activity of CHIP in astrocytes (Fig. S5). Furthermore, we performed HspBP1 immunostaining of mouse brains, in which neurons and astrocytes were labeled respectively with antibodies to cell-specific markers, NeuN and glial fibrillary acidic protein (GFAP). Indeed, there was more HspBP1 staining in neurons, whereas HspBP1 was almost undetectable in astrocytes (Fig. 6 *C* and *E*). Consistently, in the brain section of human basal ganglia, human neurons exhibited a considerable amount of HspBP1, whereas human astrocytes lacked HspBP1 staining (Fig. 6 *D* and *E*). Together, our results indicate intrinsic and significant difference of HspBP1 expression in neurons and astrocytes, which may account for differential CHIP activities in astrocytes and neurons.

To investigate whether expression of HspBP1 could inhibit CHIP activation and CHIP-mediated degradation of mHtt in astrocytes, we transfected HspBP1 into cultured astrocytes. Overexpression of HspBP1 reduced Ub1-CHIP and increased the unmodified form of CHIP correspondingly (Fig. 7*A*). This blockade of conversion of CHIP to Ub1-CHIP suggests an inhibition of CHIP activity by HspBP1 in astrocytes. In addition, increased unmodified CHIP was not caused by alteration in CHIP expression since HspBP1 overexpression did not affect the transcription of CHIP in astrocytes (Fig. 7*B*). Furthermore, cotransfection of HspBP1 and mHtt (Htt-130Q) into cultured astrocytes stabilized mHtt, suggesting that CHIP-mediated degradation of mHtt was inhibited by HspBP1. In addition, HspBP1 suppressed mHtt-induced increases in CHIP and Ub1-CHIP, which is evidenced by decreased Ub1-CHIP and CHIP in cotransfected astrocytes (Fig. 7*C*).

Given that CHIP is required for heat-shock response in astrocytes (Fig. 3*C*), we also investigated whether expression of HspBP1 could inhibit heat-shock response via suppressing CHIP activity. Indeed, HspBP1 suppressed heat-shock induction of Hsp70, CHIP, and Ub1-CHIP in astrocytes (Fig. 7*D*). Moreover, HspBP1 reduced Hsp70, but not Hsc70, under physiological

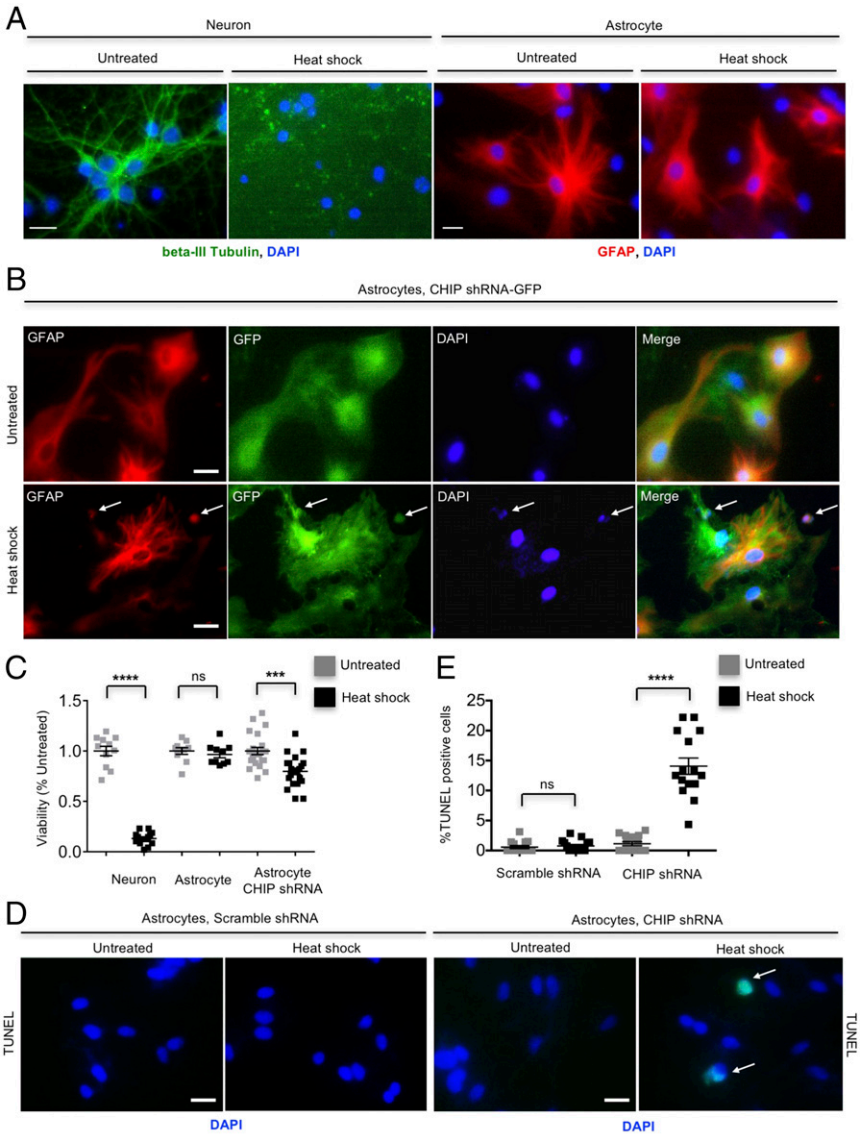

Fig. 5. High activity of CHIP contributes to astrocytic resistance to hyperthermia. (*A*) Apparent demise of cultured cortical neurons at DIV 10–12 after heat-shock stress (45 °C, 30 min). The same heat-shock stress (45 °C, 30 min) did not affect viability of cultured cortical astrocytes at DIV 40–45. Neurons and astrocytes were fixed and immunostained at 24 h after heat shock. (*B*) CHIP knockdown caused death of a portion of CHIP shRNA-transfected astrocytes, which was characterized by shrunken nucleus, at 24 h after heat shock (45 °C, 30 min). Arrows indicate corpse of astrocytes. (*C*) Quantitative results of Fig. 5 *A* and *B*. At least 10 visual fields were randomly picked up for each group, and the live cells in each visual field were quantified. (*D* and *E*) TUNEL assay showing increased susceptibility of CHIP knockdown astrocytes to heat-shock stress (45 °C, 30 min). Arrows indicate apoptotic astrocytes (TUNEL-positive cells indicated by green fluorescence). ***$P < 0.001$, ****$P < 0.0001$ (unpaired two-tailed Student's *t* test); ns, not significant. Data are represented as mean ± SEM. Error bars indicate SEM. (Scale bar, 10 μm.)

conditions (Fig. 7*E*). Taken together, the inhibitory effect of HspBP1 on CHIP-mediated protective cellular processes in astrocytes supports our conclusion that lack of HspBP1 accounts for the high activity of CHIP in astrocytes.

Silencing HspBP1 Expression Activated CHIP in Neurons and Ameliorated Neuropathology. If the expression of HspBP1 in neuronal cells is responsible for the increased accumulation of misfolded proteins, knocking down HspBP1 in neurons should alleviate misfolded proteins causing neuropathology. Thus, we used CRISPR-Cas9 to target the HspBP1 gene (Fig. S6*A*) and silence the expression of HspBP1 in the striatum of HD 140Q KI mice via stereotaxic injection of adeno-associated viruses (AAVs) expressing Cas9 and HspBP1 sgRNA. T7E1 assay and Western blotting validated the genome editing of HspBP1 sgRNA/Cas9 and efficient reduction of HspBP1 at the protein level in N2a cells, respectively (Fig. S6 *B* and *C*). The targeted HspBP1 DNA was also subcloned for sequencing, which verified Cas9/gRNA-mediated mutations in the HspBP1 gene (Fig. S6*D*). Furthermore, HspBP1 KD induced the expression of Ub1-CHIP in cultured neurons, suggesting increased activity of CHIP (Fig. 8*A*). In addition, Hsp70/Hsp90 were induced by heat shock in HspBP1 knockdown neurons (Fig. 8*B*). To knock down HspBP1 in the HD KI mouse brain, both HspBP1 sgRNA and Cas9 were transduced into the mouse brain striatal region via stereotaxic injection (Fig. S7*A*). Four to 6 wk after injection of AAV-HspBP1 sgRNA and AAV-Cas9, HspBP1 was markedly decreased in the striatum of HD KI mice compared with the control injected only with AAV-HspBP1 sgRNA (Fig. S7*B*). In this experiment, cells expressing AAV-HspBP1 sgRNA could be identified as red fluorescent

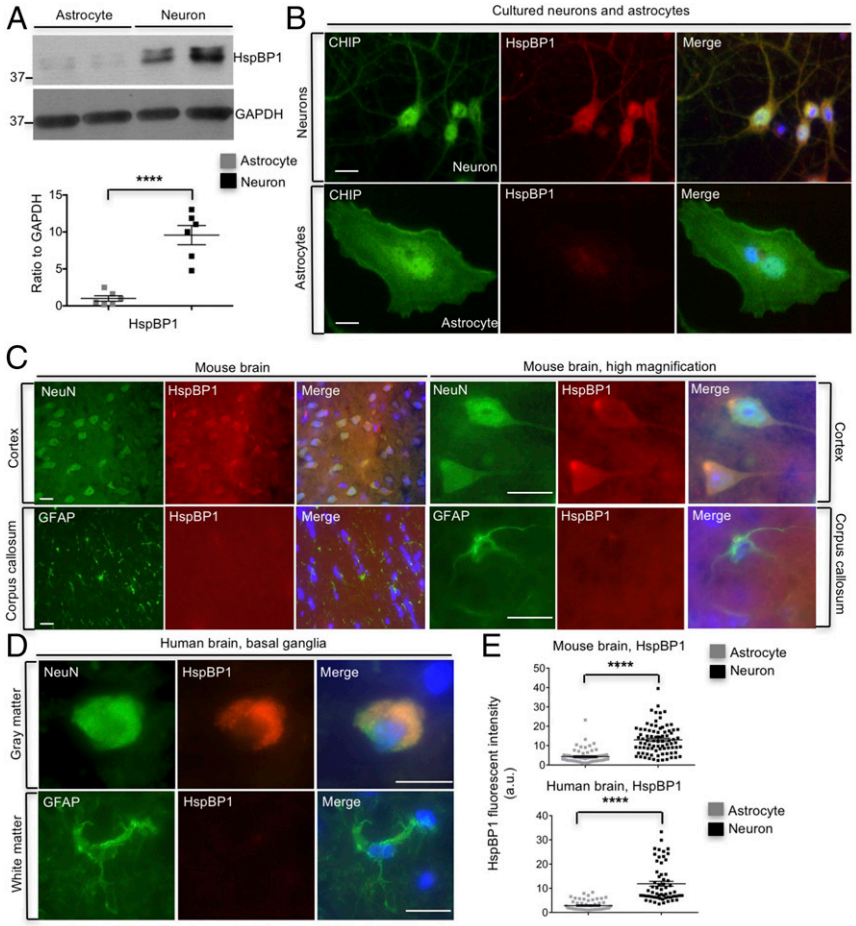

Fig. 6. Deficient expression of HspBP1 in astrocytes. (*A*) Higher levels of HspBP1 in cultured cortical neurons than astrocytes were shown by Western blotting ($n = 3$ independent experiments). (*B*) Representative images of immunostaining showed differential expression levels of HspBP1 between cultured neurons and astrocytes. (Scale bar, 5 μm.) (*C*) Fluorescent immunostaining revealing greater abundance of HspBP1 in neurons than astrocytes in mouse brain. (Scale bar, 20 μm.) (*D*) Immunostaining demonstrated more HspBP1 expression in neurons in the gray matter of human basal ganglia than that in astrocytes in the white matter of basal ganglia. (Scale bar, 20 μm.) (*E*) Statistical results for *C* and *D* [mice $n = 3$, human $n = 2$ (57 y)]. $^{****}P < 0.0001$ (unpaired two-tailed Student's *t* test). Data are represented as mean ± SEM (error bar).

protein (RFP) cells. Consequently, Cas9-mediated HspBP1 knockdown markedly increased Hsp70 in RFP-positive cells (Fig. S7*C*) and reduced mHtt aggregates in the striatum (Fig. 8 *C* and *D*). Astrogliosis is characterized by increased GFAP staining and is an early neuropathological event caused by neuronal injury in HD KI mouse brains (26). Reducing mHtt accumulation in neuronal cells via knocking down HspBP1 attenuated the extent of reactive astrocytes (Fig. 8 *E* and *F*). Synaptophysin is a presynaptic marker protein whose loss is also found in HD KI mice (27). HspBP1 knockdown restored the intensity of immunostaining of synaptophysin in the striatum of HD KI mice (Fig. 8 *G* and *H*). All these results suggest that knocking down HspBP1 in neuronal cells could increase CHIP activity, enhance the clearance of mHtt, and subsequently reduce mHtt-mediated neuropathology. Based on our findings, we propose that the high-level expression of HspBP1 in neurons accounts for the preferential accumulation of misfolded proteins in neurons and their greater vulnerability than astrocytes to misfolded proteins (Fig. 8*I*).

Discussion

Although misfolded proteins are ubiquitously expressed in neuronal and glial cells in the brain, they are more readily to accumulate in neurons and kill neuronal cells (2). This differential vulnerability to misfolded proteins is likely due to distinct capacities of maintaining protein homeostasis between neuronal and glial cells. However, whether and how neuronal and glial cells have differential capacities to maintain protein folding and to prevent protein misfolding have not been rigorously investigated. Our findings demonstrate for the first time that the higher activity of CHIP in astrocytes than neurons is related to monoubiquitinated CHIP. Additional new finding is that the differential HspBP1 expression accounts for different CHIP/Hsp70 activities in neurons and astrocytes.

Our results also revealed two major functional consequences of increased CHIP activity in astrocytes, both of which account for their faster degradation of misfolded proteins. First, CHIP associates with mHtt specifically in astrocytes, resulting in polyubiquitination and subsequent proteasomal degradation of mHtt. The increased mHtt ubiquitination in astrocytes explains why astrocytes clear mHtt more efficiently than neurons (12). In support of this idea, clearance of several other neurodegeneration-related misfolded proteins is also CHIP dependent in astrocytes. Second, active CHIP enhances both basal and inducible levels of Hsp70 in astrocytes, and increased Hsp70 prevents aggregation of misfolded proteins and facilitates the binding of CHIP to misfolded proteins.

Heat-shock response plays a vital role in maintaining cellular homeostasis. Chaperone network is extremely sophisticated and well coordinated. For example, CHIP activates HSF1 (28) but is

inhibited by HspBP1 (11). Our findings provide compelling evidence that HspBP1 is differentially expressed in neurons and astrocytes, which accounts for cell-type-dependent regulation of CHIP and Hsp70 activities. Consistent with this idea, expression of HspBP1 attenuates CHIP-dependent protective effects in astrocytes. Conversely, silencing HspBP1 expression with CRISPR-Cas9 in neurons activated CHIP and alleviated neuropathology in the HD knockin mouse model.

Our findings offer a mechanistic insight into the phenomenon that astrocytes, the largest cell population in the brains, are able to clear misfolded protein more efficiently so that they are affected to a much lesser extent than neurons in a variety of neurodegenerative diseases (3). Since CHIP is an ubiquitin E3 ligase that bridges the chaperone system and the UPS (7, 8), the increased CHIP activity may also contribute to the higher proteasomal activity in astrocytes than neurons (29). The finding that inhibition of HspBP1 in HD140Q KI mouse brain can diminish mHtt aggregation and neuropathology also suggests the therapeutic benefits by removing endogenous inhibition of CHIP activity in neuronal cells, which can be an alternative approach to treat a wide range of neurodegenerative diseases that are caused by the accumulation of misfolded proteins in neuronal cells.

Materials and Methods

Animals. Full-length mHtt CAG140Q (HD KI) mice were kindly provided by Michael Levine, University of California Los Angeles (30) and maintained at the Emory University animal facility. This study was carried out in strict accordance with the recommendations in the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee of Emory University (permit number: 2003631).

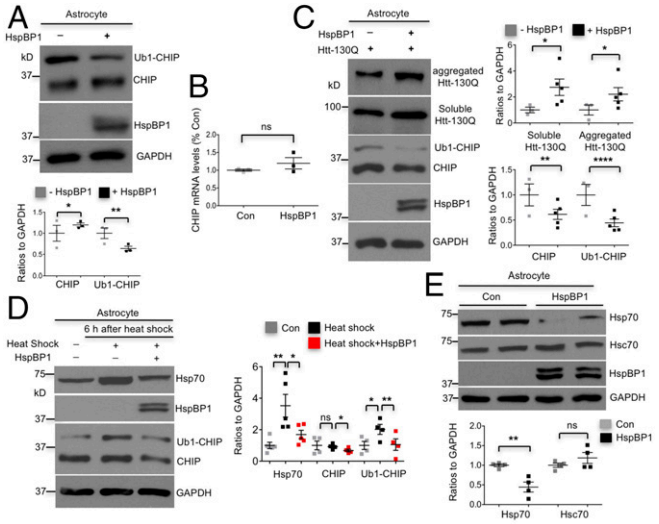

Fig. 7. Deficient expression of HspBP1 in astrocytes contributes to high activity of CHIP in astrocytes. (*A*) Expression of HspBP1 in cultured astrocytes reduced the level of Ub1-CHIP, and major form of CHIP increased correspondingly ($n = 3$ independent experiments). (*B*) qRT-PCR showed that overexpression of HspBP1 did not influence transcription of CHIP in cultured astrocytes ($n = 3$ independent experiments). (*C*) HspBP1 expression in cultured astrocytes stabilized aggregated and soluble mHtt and suppressed induction of CHIP and Ub1-CHIP by mHtt, which was seen in Fig. 1*D* ($n = 3$ independent experiments). (*D*) Induction of Hsp70, CHIP, and Ub1-CHIP by heat-shock stress at 42 °C for 1 h was significantly inhibited by HspBP1 expression in DIV 30–50 astrocytes ($n = 4$ independent experiments). (*E*) Expression of HspBP1 decreased basal levels of Hsp70 in astrocytes ($n = 3$ independent experiments). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$ (unpaired two-tailed Student's *t* test). Data are represented as mean ± SEM (error bar).

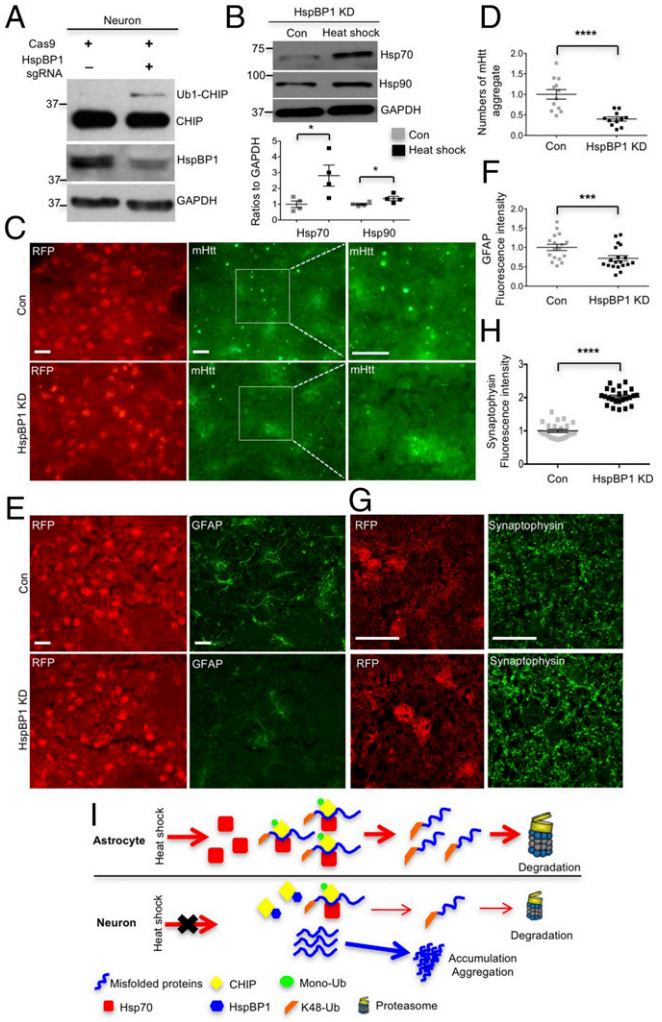

Fig. 8. Knocking down HspBP1 is neuroprotective in HD 140Q KI mice. (*A*) HspBP1 knockdown (KD) in cultured neurons induced Ub1-CHIP expression. (*B*) Hsp70/90 were induced 6 h after heat shock (42 °C for 1 h) in HspBP1 knockdown neurons ($n = 3$ independent experiment). (*C–H*) HspBP1 KD decreased mHtt aggregates and reactive astrocytes, rescued the loss of synaptophysin in the striatum of HD140Q KI mice at the age of 13–14 mo, compared with the injection with HspBP1 sgRNA alone [control (Con)]. $^{*}P < 0.05$, $^{***}P < 0.001$, $^{****}P < 0.0001$ (unpaired two-tailed Student's *t* test). (Scale bar, 20 μm.) Data are represented as mean ± SEM (error bar). (*I*) A proposed model for the differential accumulation and toxicity of misfolded proteins in neurons and astrocytes: CHIP is more active and monoubiquitinated in astrocytes due to deficient HspBP1 expression. Activated CHIP preferentially binds to and polyubiquitinates misfolded proteins and confers higher Hsp70, resulting in the prompt proteasomal degradation of misfolded proteins in astrocytes.

Stereotaxic Injection of Viral Vectors. Mice were anesthetized with an i.p. injection of Avertin (0.5 mg/g). Their heads were placed and fixed in a Kopf stereotaxic frame (model 1900) equipped with a digital manipulator, and a UMP3-1 Ultra pump. Mice were kept deeply anesthetized as assessed by monitoring pinch withdrawal and respiration rate. Viral vector injections were given in the striatum (0.6 mm anterior to bregma, 2.0 mm lateral to the midline, and 3.5 mm ventral to dura). The injections were performed at a rate of 0.2 μL/min. The needle was left in place for 10 min after each injection to minimize upward flow of viral solution after raising the needle.

Additional information is provided in *SI Materials and Methods*.

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