Abnormal degradation of the neuronal stress-protective transcription factor HSF1 in Huntington’s disease

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Huntington’s Disease (HD) is a neurodegenerative disease caused by poly-glutamine expansion in the Htt protein, resulting in Htt misfolding and cell death. Expression of the cellular protein folding and pro-survival machinery by heat shock transcription factor 1 (HSF1) ameliorates biochemical and neurobiological defects caused by protein misfolding. We report that HSF1 is degraded in cells and mice expressing mutant Htt, in medium spiny neurons derived from human HD iPSCs and in brain samples from patients with HD. Mutant Htt increases CK2α′ kinase and Fbxw7 E3 ligase levels, phosphorylating HSF1 and promoting its proteasomal degradation. An HD mouse model heterozygous for CK2α′ shows increased HSF1 and chaperone levels, maintenance of striatal excitatory synapses, clearance of Htt aggregates and preserves body mass compared with HD mice homozygous for CK2α′. These results reveal a pathway that could be modulated to prevent neuronal dysfunction and muscle wasting caused by protein misfolding in HD.

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Huntington’s Disease (HD) is a genetically encoded autosomal dominant neurodegenerative disease caused by a polyglutamine (Q) expansion (CAG trinucleotide repeat) within exon 1 of the Huntingtin (HTT) gene. The encoded Htt-polyQ protein is expressed in almost all cells, which leads to defects in transcription, autophagy, mitochondrial function, signalling and apoptosis. Although HD preferentially affects neuronal function and the survival of striatal and cortical neurons, defects are observed in peripheral tissues in mouse models and in patients that include skeletal muscle wasting and cardiac atrophy, perhaps reflecting toxicity and apoptosis due to the ubiquitous expression of Htt-polyQ (ref. 4).

The presence of a pathogenic polyQ expansion causes Htt to misfold and aggregate, driving inappropriate interactions with transcription factors, signalling and cell integrity proteins and other key cellular regulatory factors in both the cytosol and nucleus. The protein quality control machinery, including chaperones, the ubiquitin proteasome, autophagy and other factors play critical roles in the folding, trafficking, modification and degradation of both newly synthesized and misfolded proteins (polyQ) disease. Accordingly, increased expression of chaperones such as Hsp104, Hsp70, Hsp40 and Hsp27, or critical components in the autophagy pathway, ameliorates protein aggregation and cell death in cellular, fly, worm and mouse polyQ expansion disease models. As chaperones function in obligate hetero-multimeric complexes, the coordinate expression of distinct chaperones synergize in the amelioration of polyQ protein aggregation and cellular stress protection in polyQ-expansion models.

Heat shock transcription factor 1 (HSF1) is a stress-responsive transcription factor that protects cells from protein misfolding, aggregation and apoptosis by expressing genes involved in protein quality control, stress adaptation and cell survival. HSF1 is activated in response to elevated temperature, oxidant exposure, metals and other conditions that cause protein misfolding. Under normal cell growth conditions, HSF1 is present as an inactive monomer repressed by Hsp40, Hsp70, Hsp90 and TRiC, protein chaperones involved in the folding and maturation of hundreds of cellular client proteins. In response to proteotoxic stress HSF1 assembles as a multimer, binds heat shock elements in target gene promoters and activates expression of stress-protective genes. HSF1 undergoes many post-translational modifications including both basal and stress-induced phosphorylation, sumoylation, ubiquitylation and acetylation that mediate repressive or activating regulatory roles.

Consistent with HSF1 activating protein folding and stress-protective pathways, hsf1−/− mice in the context of an R6/2 HD model show increased brain Htt aggregation and a shortened lifespan, while expression of a constitutively active form of HSF1 inhibited Htt-polyQ aggregation and prolonged lifespan. Moreover, a heterozygous HSF1 mouse model of spinal and bulbar muscular atrophy with a pathogenic polyQ repeat in the androgen receptor (AR), exhibited increased AR-polyQ aggregates in neurons and non-neuronal tissues and enhanced neurodegeneration.

While there is strong evidence for beneficial effects of HSF1 in polyQ expansion models, HSF1 target gene expression is compromised in the presence of disease-associated polyQ-expansion proteins. Pharmacological activation of HSF1 with a blood–brain barrier-penetrant Hsp90 inhibitor increased HSF1 target gene expression and was initially effective in disease amelioration. However, this beneficial effect was observed only at early stages and was proposed to be due to the inability of HSF1 to bind target genes in the altered chromatin environment found in the R6/2 mouse model. Other reports suggest that HSF1 protein levels may affect the expression of the protein folding machinery components in HD models. Given the therapeutic potential for HSF1 activation in protein misfolding disease, it is important to clarify our understanding of the mechanisms by which HSF1 activation is defective in HD.

Here we demonstrate that HSF1 protein levels are strongly decreased in HD models, in differentiated human inducible pluripotent stem cells and in HD patient striatum and cortex, with a concomitant defect in target gene expression. This defect is due to inappropriate degradation of HSF1 via phosphorylation-stimulated ubiquitin-dependent degradation induced by abnormally high levels of the casein kinase 2α (CK2α)-prime (CK2α′) kinase and the Fbxw7 E3 ligase. Diminution of CK2α′ expression in the zQK1175 mouse HD model restores HSF1 levels and activity and prevents mutant Htt aggregation, striatal excitatory synapse loss and cachexia. These studies elucidate a critical molecular mechanism for inappropriate degradation of the protein misfolding stress-protective transcription factor HSF1 and suggests a novel therapeutic target (CK2α′) that is potentially amenable to pharmacological intervention for the treatment of HD.

Results

Decreased HSF1 correlates with elevated HSF1-P-S303/307. To explore whether the defect in HSF1 activation lies in changes in HSF1 abundance in the presence of misfolded polyQ-Htt, we compared HSF1 protein levels, target promoter occupancy and gene expression in PC12 cells with Tetracycline inducible cassettes expressing human Htt exon 1 with non-pathogenic Q repeats (Htt-Q23-green fluorescent protein (GFP)) versus pathogenic repeats (Htt-Q74-GFP). We performed these studies under control (37 °C) and heat shock conditions (42 °C), allowing us to both analyse the defect in HSF1 under polyQ protein expressing conditions and explore the expression of inducible HSF1 target genes (Fig. 1a). The HSF1 targets Hsp70, Hsp25, Hspb5 and Bag3 are strongly expressed in response to heat shock activation in Htt-Q23 cells and in Htt-Q74 cells in the absence of tetracycline (− Dox), while Htt-Q74-expressing cells (+ Dox) showed a strong decrease in their expression in response to heat shock (Fig. 1b; Supplementary Fig. 1A,B). We also analysed Hsp70 and Hsp25 transcript levels over time after heat shock in Htt-Q74 cells (Fig. 1c). The decrease in HSF1 target gene expression in Htt-Q74-expressing cells (Fig. 1b and Supplementary Fig. 1A) correlated with decreased Hsp70 and Hsp25 messenger RNA (mRNA) levels (Fig. 1c) and a parallel reduction in HSF1 occupancy of the Hsp70 promoter (Fig. 1d). A similar defect in HSF1 activation was observed in response to azetidine 2-carboxylic acid, a proline analogue that causes protein misfolding and HSF1 activation (Supplementary Fig. 1C). These results demonstrate a clear defect in HSF1 DNA binding and target gene activation under protein misfolding stress conditions in Htt-Q74 cells, but not Htt-Q23 cells.

A reduction in HSF1 protein levels in Htt-Q74-expressing cells was observed under control conditions that was exacerbated in response to proteotoxic heat shock conditions (Fig. 1b). A similar reduction was observed using whole-cell extracts prepared with urea or acetone precipitation, ruling out the possibility of HSF1 partitioning into the insoluble fraction (Supplementary Fig. 1D,E). However, no significant changes in HSF1 mRNA were observed (Supplementary Fig. 1F). This reduction in HSF1 levels was accompanied by increased HSF1 phosphorylation at Ser303 and Ser307, located within the central regulatory domain. Induction of Htt-Q74 expression greatly elevated S303/307 phosphorylation, further enhanced in response to heat shock (Fig. 1b), and occurred in a time-dependent fashion.
Figure 1 | HSF1 levels are decreased and P-Ser303/307 increased in HD. (a) Diagram of the PC12-HttQ23 and Htt-Q74 proteins and experimental design. (b) Cells expressing either Htt-Q23 or Htt-Q74 were cultivated at 37 °C (c) in the presence of tetracycline (Dox) for 3 days to induce expression, exposed to heat shock 1 h at 42 °C, allowed to recover at 37 °C for 7 h (HS) and protein extracts immunoblotted with the indicated antibodies. HSF1 bands were quantitated using Quantity One image software (BioRad) and values normalized using GAPDH as loading control and referenced to control at 37 °C (c) in the absence of −Dox. (c) qRT-PCR analysis of the Hsp70 and Hsp25 genes in Htt-Q74-expressing cells as in B. HS (+Dox) group was compared with HS (−Dox) group. Error bars represent means ± s.e.m., n = 4. Unpaired t-test, ***P<0.001. (d) Analysis of Hsp70 promoter occupancy by HSF1. Error bars represent means ± s.e.m., n = 3. Unpaired t-test, **P<0.01. (e) Mouse-derived striatal STHdhQ7 and STHdhQ111 cells were cultured at 33 °C in the non-pathogenic STHdhQ7. (f) Dorsal striatal sections from WT and KIQ175 mice (n = 3) at 6 months assayed by immunohistochemistry (IHC) for mHtt aggregation (1C2), HSF1 and HSF1-S303 phosphorylation using DAPI staining as control. Scale bar: 10 μm. (g) Gastrocnemius muscle extracts from WT and KI175 mice (n = 4) of the indicated age, immunoblotted for HSF1, Hsp70 and GAPDH. (h) Protein extracts from HD patient striatum (Supplementary Table 1) and controls immunoblotted with indicated antibodies. (i) HSF1 phosphoproteomic analysis under non-pathogenic (−Dox) and pathogenic (+Dox) conditions in hsf1−/− MEF inducible cell line expressing GFP-Htt-Q74 expressing HSF1. See Supplementary Fig. 8 for uncropped immunoblots. HSF1 represented by the Regulatory domain, DBD; DNA binding domain; LZ1-3 and LZ4; leucine zipper domains.
These same changes were observed in the striatal neuron-derived cells from STHdhQ111/Q111 knock-in mice when compared with the non-pathogenic STHdhQ7/Q7 cells (Fig. 1e). A spinal and bulbar muscular atrophy PC12 cell model (AR-Q112), also showed decreased HSF1 and Hsp25/Hsp70 expression levels and increased HSF1-S303 phosphorylation compared with non-pathogenic cells (AR-Q10; Supplementary Fig. 1H). Together, distinct cellular models of pathogenic polyQ-expansion protein misfolding disease show defective HSF1 activation, increased HSF1-S303/307 phosphorylation and decreased HSF1 protein levels.

To ascertain if these observations made in cellular models of HD are found in animal models of HD, HSF1 levels were evaluated in wild-type (WT) C57Bl/6 mice and in the zQ175 (herein KIQ175) heterozygous knock-in HD model. This mouse model exhibits striatal and cortical excitatory synaptic defects, abnormal gait and motor defects, loss of body weight, reduced levels of striatal marker genes and other defects similar to human HD patients. Although striatal tissue from 2-month-old KI175 mice showed no difference in the levels of HSF1 compared with wild-type littermates, a strong reduction in HSF1 levels was observed at both 6 and 12 months, as was a reduction in Hsp70 (Fig. 1f). This correlates with the appearance of Htt-polyQ aggregates, increased HSF1 S303 phosphorylation in the striatum (Fig. 1g and Supplementary Fig. 1l) and the age of onset of motor deficits in this HD model. Similar results were observed in the cortex and gastrocnemius muscle, where HSF1 and Hsp70 dramatically decreased at 6 and 12 months of age (Fig. 1h and Supplementary Fig. 1j). Interestingly, Hsp70 levels in the gastrocnemius muscle of KI175 HD mice also showed a strong diminution from that found in wild-type mice at 2 months, even before a strong reduction in HSF1 levels (Fig. 1h).

These results demonstrate that HSF1 levels are reduced in striatal and cortical tissues, and in skeletal muscle in the heterozygous KI175 HD model, even before the reported onset of motor or cognitive deficits.

To evaluate HSF1 levels in patients with HD, post-mortem age- and sex-matched HD and control striatal and cortex samples were assessed using a total of 14 independent striatal samples and 7 cortex samples (Fig. 1i; Supplementary Fig. 2A–C,F,G and Supplementary Table 1). In 10 of 14 samples analysed from the striatum of HD patients, and 5 of 7 samples analysed from the cortex, a strong reduction in HSF1 levels was evident. Moreover, HD protein extracts displayed reduced HSF1 electrophoretic mobility, irrespective of residual protein levels and tissue sample source, and an increase in HSF1 S303 phosphorylation (Fig. 1i). Accordingly, the levels of Hsp70 and Hsp25 were decreased in 7 out of 10 HD samples that exhibited decreased HSF1 relative to controls (Fig. 1i; Supplementary Fig. 2A,B,F), consistent with a previous transcriptome analyses of human HD brain.

To explore the role of S303 and S307 phosphorylation in HSF1 regulation in HD, HSF1 phosphoproteomics was performed in hsf1Δ/C0 MEFs inducibly expressing human Htt-Q74 or Htt-Q23 and transfected with an HSF1 expression vector (Fig. 1j and Supplementary Data 1). Out of 95 total serine and threonine residues present in HSF1, only four were detected as phosphorylated in Htt-Q23 cells corresponding to S303, S314, T357 and S363. In Htt-Q74 cells HSF1 was phosphorylated at S303, S307 and S363, highlighting the increased occurrence of HSF1 S307 phosphorylation under pathogenic polyQ conditions. Although this analysis does not quantify the abundance of phosphorylation events, these and other results (Fig. 1b,e,g and Supplementary Fig. 1G) demonstrate that HSF1 S303 and S307 phosphorylation are specifically increased in the presence of a pathogenic polyQ protein in parallel with decreased HSF1 protein abundance and activity.

HSF1 activation, increased HSF1 S303/307 phosphorylation and polyQ-expansion protein misfolding disease show defective HSF1 activation, increased HSF1-S303 phosphorylation and decreased HSF1 protein levels.

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Figure 2 | Proteasomal HSF1 degradation in HD is mediated by Phospho-S303/S307. (a,b) PC12 cells expressing Htt-Q74 for 1, 2 or 3 days followed by Heat Shock (HS) and recovery as indicated. Control and HS in the absence of Dox correspond to cells incubated during 3 days at 37 °C. (c) Htt-Q74 cells Dox-induced or not for 3 days and exposed to 2 μM 17-AAG and/or MG132 (5 μM) for 6 h and extracts immunoblotted with the indicated antibodies. (d) Diagram of the effects of 17-AAG and MG132 treatment and Htt-Q74 expression in HSF1. (e) Htt-Q74 cells transfected with human influenza haemagglutinin-ubiquitin (HA-Ub) plasmid Dox induced or not and treated with 5 μM MG132 treatment for 6 h. Whole-cell extract and HA immunoprecipitated (IP:HA) and HSF1 immunoprecipitated samples (IP:HSF1) were immunoblotted as indicated. (f) Transcript levels for indicated E3 ligases evaluated by qRT-PCR from striatum of WT and KIQ175 mice at 6 months. Error bars represent ± s.e.m., (n = 3). Unpaired t-test, *P < 0.05. (g) Human striatum samples from HD patients and controls and (h) Mouse striatum from 12 months old WT and KIQ175 mice were immunoblotted for HSF1 and Fbxw7. (i) Fbxw7 siRNA in STHdhQ7 and STHdhQ111 cells using scrambled RNA (Scr) as control. HSF1 was quantified using Quantity One image software and normalized using GAPDH as loading control and referenced to control at 37 °C in the non-pathogenic STHdhQ7. (j) hsf1−/− MEFs transfected with WT HSF1 or S303A mutant and Fbxw7-FLAG; samples immunoprecipitated with anti-FLAG and HSF1 detected. (k) hsf1−/− MEFs expressing Dox-inducible Htt-Q74-GFP transfected with WT HSF1 or HSF1-S303A HS and 1 h recovery at 37 °C for 7 h. HSF1 was quantified using Quantity One image software and normalized using GAPDH as loading control and referenced to control (–Dox) expressing WT HSF1. See Supplementary Fig. 9 for uncropped immunoblots.
Htt-polyQ-dependent HSF1 phosphorylation by Casein Kinase 2.

To identify protein kinases involved in controlling HSF1 phosphorylation and degradation in HD, we used a previously developed screen for regulators of human HSF1 expressed in yeast hsf1Δ cells (Fig. 3a). The basis of the screen is that human HSF1 (hHSF1) exists in an inactive form in yeast and does not complement the viability defect of hsf1Δ cells. Yeast hsf1Δ cells harbouring both a galactose-inducible, glucose-repressible yHSF1 plasmid and a constitutively expressed human HSF1 plasmid were cultured in galactose conditions to allow yHSF–dependent growth. In glucose medium human HSF1 is insufficient to promote viability unless small molecules activate HSF1 and promote cell growth (Fig. 3a). A library of commercial Ser/Thr and Tyr protein kinase inhibitors was evaluated for molecules able to support cell viability in a human HSF1-dependent manner (Fig. 3b). All five hits from this screen have the common feature that, among other kinases, they inhibit CK2, a kinase in which the holoenzyme is composed of 2 regulatory and 2 catalytic subunits (Fig. 3c). These results suggested an inhibitory role for CK2 in human HSF1 regulation in yeast. Because of the limited specificity of these kinase inhibitors, the modulation of human HSF1 by yeast CK2 was tested by deletion of the CKB1 gene, encoding a CK2 stimulatory subunit in S. cerevisiae (Fig. 3c). When the yeast HSF1 expression plasmid was evicted from cells expressing human HSF1, isogenic ckb1Δ cells were viable and exhibited increased human HSF1 protein levels compared with WT cells (Fig. 3d–f).

In vitro phosphorylation assays were conducted using purified recombinant human glutathione S-transferase (GST)- CK2α or CK2α’ catalytic subunits, or commercial CK2 holoenzyme, and recombinant human HSF1 (Fig. 3g). Mass spectrometry analysis of HSF1 revealed that 11 Ser and 6 Thr residues were phosphorylated in vitro by CK2 (Fig. 3g and Supplementary Data 2) with most located in the regulatory domain. Although some residues were phosphorylated by all three CK2 preparations, other phosphorylation sites were unique. This is particularly relevant since the catalytic subunits CK2α and CK2α’ can function in vivo in the absence of the CKBβ regulatory subunit43,44. Intriguingly, HSF1-S303-P was identified only in the presence of CK2 holoenzyme and HSF1-S307-P was identified solely in the presence of CK2α’. Of the HSF1 residues phosphorylated in vitro by CK2, 8 different Ser and Thr residues were mutated to encode Ala (Supplementary Fig. 3A) but only the HSF1-S303A and/or S307A mutants promoted robust human HSF1-dependent yeast growth (Fig. 3h and Supplementary Fig. 3B) consistent with previous studies of these mutants showing elevated HSF1 protein levels in yeast32. The HSF1-S303A and S303A/S307A mutants were further evaluated in the humanized yeast assay in the presence of the CK2 inhibitor TID43. While TID43 administration allowed WT human HSF1-dependent yeast growth and potentiated S303A mutant growth, no further growth stimulation was observed for the S303A/S307A double mutant, showing insensitivity to pharmacological CK2 inhibition (Fig. 3h). Taken together these results demonstrate that CK2 directly phosphorylates human HSF1 at S303 and S307 in vitro and pharmacological or genetic inhibition of these events increases human HSF1 activity and abundance in yeast.

To investigate a role for mammalian CK2 (Fig. 4a) in HSF1 degradation in HD, Htt-Q74 expressing cells were incubated with two CK2 inhibitors that activate human HSF1 in the yeast assay, TID43 and Emodin (Fig. 3b). Both molecules facilitated Hsp70 expression in non-pathogenic (Htt-Q73) and pathogenic (Htt-Q74) conditions in a dose-dependent manner (Fig. 4b,c) and activated HSF1 in human ARPE cells (Supplementary Fig. 4A,B). Incubation of Htt-Q74 cells with TID43 or Emodin before or after Htt-Q74 induction (Supplementary Fig. 4C) decreased HSF1-S303 phosphorylation, increased HSF1 and Hsp70 levels (Fig. 4d and Supplementary Fig. 4D), decreased Htt-Q74 aggregates (Fig. 4e,f; Supplementary Fig. 4E) and increased Hsp70 expression and cell viability in an HSF1-dependent manner (Fig. 4g and Supplementary Fig. 4F,G). While Gsk3β phosphorylates HSF1 S303 and drives HSF1 degradation in cancer cells36,41, a GSK3 inhibitor or GSK3 RNAi knockdown moderately stimulated Hsp70 expression (Supplementary Fig. 4E,H) and did not impact Htt-Q74 aggregation (Supplementary Fig. 4E). Furthermore, knockdown of Gsk3z and/or Gsk3β had a detrimental effect on HSF1 protein stability and did not change the HSF1-S303 phosphorylation state (Supplementary Fig. 4H).

While yeast CK2 catalytic activity is positively regulated by the CKB subunits (Fig. 3c), mammalian CK2 activity can be either activated or inhibited by the CK2β regulatory subunit (Fig. 4a) thus modulating activity towards specific substrates43,44. Consistent with CK2β functioning as a negative regulator of HSF1 phosphorylation by CK2, silencing of CK2β in the presence of Htt-Q74 decreased HSF1 protein levels and Hsp70 expression (Fig. 4b). However, short interfering RNA (siRNA)-mediated knockdown of the catalytic CK2α’ subunit alone, or in combination with the CK2α subunit, reduced HSF1 S303 and S307 phosphorylation (Fig. 4i), increased HSF1 protein levels and Hsp70 expression and increased cell viability in the presence of Htt-Q74 (Fig. 4i; Supplementary Fig. 4I–K). A knockdown of kin-3, the sole C. elegans CK2 catalytic subunit (Supplementary Fig. 4L), resulted in higher expression of hsp-25 in an HSF1-dependent manner, suggesting a conservation of function, but it did not suppress protein aggregation or toxicity in animals overexpressing polyQ-YFP fusion proteins in body wall muscle cells, perhaps due to the potent toxicity of the Q37::YFP model (Supplementary Fig. 4M).

CK2α’ is highly abundant in HD medium spiny neurons. The abundance of the CK2 catalytic and regulatory subunits was evaluated in cell and mouse HD models and in striatum from patients with HD (Fig. 5). In Htt-Q74 expressing PC12 cells CK2α’ showed increased abundance that was further increased under HS conditions (Fig. 5a), correlating with increased HSF1 S303/307 phosphorylation and decreased HSF1 levels (Fig. 1b). In the KIQ175 HD mouse model CK2α’ mRNA (Fig. 5b) and protein levels were markedly increased in striatum and gastrocnemius muscle (Fig. 5c,d). Immuno-histochemical analysis of the dorsal striatum also revealed that CK2α’ expression was increased in KIQ175 mice (Fig. 5e) while no signal was detected in CK2α’−/− mice (Supplementary Fig. 5A). CK2α’ expression co-localized with the neuronal marker (NeuN) and the medium spiny neuron (MSN) markers DARPp32, Ctip2 and Fox1P (refs 45,46) in both WT and KIQ175 mice (Fig. 5e and Supplementary Fig. 5B,C). The analysis of the GS and CD68 markers showed no co-localization with astrocytes or reactive microglia, respectively (Supplementary Fig. 5B,D). These results demonstrated that MSNs express increased CK2α’ in the presence of mHtt (Supplementary Fig. 5E). Strikingly, striatal tissue from patients with HD also exhibited elevated CK2α’ mRNA and protein levels (Fig. 5f,g). Nine of 14 patients with HD showed increased CK2α’ expression in the striatum compared with control samples and among all 10 patients that showed reduced HSF1, 7 showed increased CK2α’ (Supplementary Fig. 2A,B,D).

HSF1 is degraded in mouse and human MSN-like HD cells. Our findings demonstrate a correlation between decreased HSF1 abundance and activity, and increased expression of the HSF1 degradation machinery components CK2α’ and Fbxw7. These
findings were corroborated in the striatal-derived mouse HD STHdhQ111 cell-line compared with STHdh Q7 cells (Fig. 6a). Importantly, we tested the expression levels of HSF1, Hsp70, CK2α′ and Fbxw7 in human HD induced pluripotent stem cells (iPSC)-derived MSNs from 33Q (Control) and 60Q (HD) containing allele individuals in the absence or presence of brain-derived neurotrophic factor (BDNF; Fig. 6b). Although no differences in the expression profile of these proteins were observed under BDNF-withdrawal stress conditions, we found that HSF1 abundance and Hsp70 expression were reduced, and CK2α′ and Fbxw7 increased, in the 60Q cell line.

**CK2α′ lowering increases HSF1 and decreases mHtt aggregation.** To explore a physiological role for CK2α′ in regulating HSF1 abundance and activity in vivo, the consequences of CK2α′ knock-out was evaluated in otherwise WT mice. CK2α′−/− mice...
CK2α reduction preserves MSNs and muscle mass. Previous studies demonstrated decreased abundance of dendritic spines exhibited higher HSF1 protein levels and Hsp70 expression compared to CK2xα+/− littersmates, suggesting a physiological role for CK2 in regulating HSF1 abundance and activity (Supplementary Fig. 6A). Given the dramatic elevation in CK2xα levels in HD models and patients, mice heterozygous for CK2xα (CK2xα+/−) were generated in the KIQ175 background to evaluate the impact on HSF1 levels, activity and on the neuro-pathology observed in this HD model (Fig. 7a and Supplementary Fig. 6B). KIQ175/CK2xα+/− mice exhibited ~2.5-fold decreased CK2xα, and ~1.5-fold increased CK2α striatal mRNA levels compared to KIQ175 littersmates (Fig. 7b and Supplementary Fig. 6C). Heterozygosity of CK2xα increased expression of the HSF1 targets Hsp70 and Hsp25 (Fig. 7c and Supplementary Fig. 6D).

The co-activator peroxisome proliferator-activated receptor gamma (PGC-1α) is an HSF1 target that is a key regulator of mitochondrial biogenesis and energy metabolism. Both PGC-1α and its target genes, CYCS (cytochrome c), TFAM (mitochondrial transcription factor) and NDUFS3 (NADH dehydrogenase) are reduced in the striatum of the N171-28Q HD mouse model and in human patients, in part underlying the mitochondrial dysfunction observed in HD (ref. 48). KIQ175/CK2xα+/− mice showed elevated expression of PGC-1α and target genes compared to KIQ175 littersmates (Fig. 7d and Supplementary Fig. 6E). Importantly, HSF1 and Hsp70 protein levels were increased, mutant Htt aggregates decreased and soluble mutant Htt protein increased in the striatum of KIQ175/CK2xα+/− mice compared to KI175 littersmates (Fig. 7e,f; Supplementary Fig. 6F,G).
**Figure 5 | CK2α' abundance is elevated in Huntington's disease.** (a) CK2α, CK2α' and CK2β protein levels in Htt-Q74 expressing cells under control (−Dox) or heat shock conditions at 42°C for 1 h (HS). CK2 subunit abundance was quantified using Quantity One image software normalized using GAPDH as loading control. CK2α' ratio is shown and referenced to control (−Dox) cells. (b) CK2α, CK2α' and CK2β striatal mRNA levels from WT and KIQ175 mice at 6 months of age. The value given for the amount of mRNA in the control group (WT) was set as 1. Error bars represent mean ± s.e.m., (n = 4 animals). Values for the KIQ175 group were compared to the WT group. Statistical significance was measured by two-tailed unpaired t-test *P < 0.05, **P < 0.05, n.s, no significant. Values for the Huntington's disease (HD) group were compared to the control (C) group. CK2α' bands from immunoblots were quantified using Quantity One image software (BioRad) and the protein values were normalized using GAPDH as loading control and referenced to the corresponding age-sex-matched control patient. See Supplementary Fig. 11 for uncropped immunoblots.
and changes in spine morphology in HD patients and mouse HD models. These phenotypes correlate with reduced expression of vital HSF1 targets in HD, such as Hsp70, which functions in dendritic maturation and neuronal communication. Significant alterations in MSN morphology and composition were detected between WT and KIQ175 mice, with increased immature MSNs and decreased mature MSNs in KIQ175 mice (Fig. 8a,b). Striatal degeneration can be detected by alterations in excitatory synapse formation in KIQ175 mice at an early stage (P21) that may precede neurodegeneration. Striatal inputs from the cortex and thalamus can be distinguished by the differential expression of the presynaptic proteins VGlut1 (cortico-striatal) and VGlut2 (thalamo-striatal) and their co-localization with post-synaptic presynaptic proteins VGlut1 (cortico-striatal) and VGlut2 (thalamo-striatal) and their co-localization with post-synaptic vesicle protein 2 and synaptotagmin I and attenuates presynaptic signalling pathways and neuronal communication. Moreover, Hsp70 stabilizes presynaptic proteins such as syntaxin I, synaptic vesicle protein 2 and synaptotagmin I and attenuates hypoxia-induced motor and sensory impairment. HSF1 also activates BDNF expression, which contributes to the survival of peripheral and CNS neurons and that is markedly depleted in HD brain. Interestingly, amyloid peptide (Aβ) transgenic mice treated with the HSF1 activator 17-AAG had increased BDNF expression in the hippocampal region and displayed attenuated Aβ-induced synaptic toxicity and memory impairment. Our data suggests that reduced expression of HSF1 target genes in the striatum of HD mice and patients with HD is associated with decreased levels of HSF1. Prevention of HSF1 degradation by CK2 inhibition increases the expression of chaperones in the striatum. Indeed, striatal and cortical neurons, which are primary postmortem HD patient samples, and studies by others in flies and mice, clearly demonstrate a strong diminution in HSF1 protein abundance.

Moreover, we find that the levels of both HSF1 and HSF1 target genes, are strongly reduced in gastrocnemius muscle of the KIQ175 HD mouse. This is consistent with the accumulation of nuclear mutant Htt inclusions in muscle fibres, and perhaps with the cachexia observed in the periphery in this mouse model and HD patients. These studies suggest an intrinsic link between the periphery and the central nervous system (CNS) in HD with respect to HSF1 abundance and activity. It has also been demonstrated that α-synuclein A53T mutant expressing cells, but not WT α-synuclein cells show HSF1 depletion; similarly, in tissue of patients with diffuse Lewy body disease HSF1 depletion is observed. Rodent models of Alzheimer’s disease also exhibit low HSF1 levels in the cerebellum, suggesting that Parkinson’s disease (PD), Alzheimer’s Disease (AD) and perhaps other neurodegenerative diseases associated with protein misfolding may exhibit inappropriate degradation of HSF1.

Here we demonstrate that one mechanism for defective HSF1 activation in HD is pathogenic polyQ-Htt-dependent targeting of HSF1 for proteasomal degradation, mediated by the action of CK2 kinase and the phosphorylation of HSF1 at S303/S307. Our observations that CK2α catalytic subunit levels are dramatically elevated in MSNs in rodent HD models, in differentiated human iPSCs derived from patients with HD and in the striatum from patients with HD suggest that mutant Htt elevates the expression or stability of this kinase through as yet unidentified mechanisms. Consistent with our findings, others have shown increased expression of CK2 in striatal tissue of yeast artificial chromosome (YAC) transgenic mice expressing full-length mutant Htt (ref. 56). Moreover, AD models and patients with Alzheimer’s Disease exhibit low levels of HSF1 and elevated CK2 (refs 55,57,58), and increasing HSF1 activity in an AD murine model reverses cerebellar Purkinje cell deficiency. Here, we have demonstrated that CK2α is increased in MSNs in HD, the neuronal sub-type most susceptible to mHtt aggregation and neurodegeneration, whereas it is known that CK2α/α’ is increased in the astrocytes in the hippocampus and temporal cortex of AD patients, the most affected tissue in AD (ref. 58). Our data demonstrate that CK2α and mHtt are found in MSNs in HD mice, while in AD CK2 co-localizes with amyloid deposits in astrocytes. However, it is currently unclear if this causes the HSF1 protein depletion that has been reported in AD. Notably, pharmacological inhibition of CK2 also affects the phosphorylation state of Htt at Ser13 and Ser16, which alters WT Htt protein aggregation and localization. It is currently unknown what the relative contributions of these or other CK2 targets are to HD phenotype amelioration when CK2 is inhibited.

HSF1 target genes also encode proteins that function within the synaptic environment controlling dendritic maturation, presynaptic signalling pathways and neuronal communication. Moreover, Hsp70 stabilizes presynaptic proteins such as syntaxin I, synaptic vesicle protein 2 and synaptotagmin I and attenuates hypoxia-induced motor and sensory impairment. HSF1 also activates BDNF expression, which contributes to the survival of peripheral and CNS neurons and that is markedly depleted in HD brain. Interestingly, amyloid peptide (Aβ) transgenic mice treated with the HSF1 activator 17-AAG had increased BDNF expression in the hippocampal region and displayed attenuated Aβ-induced synaptic toxicity and memory impairment. Our data suggests that reduced expression of HSF1 target genes in the striatum of HD mice and patients with HD is associated with decreased levels of HSF1. Prevention of HSF1 degradation by CK2α inhibition increases the expression of chaperones in the striatum. Indeed, striatal and cortical neurons, which are primary postmortem HD patient samples, and studies by others in flies and mice, clearly demonstrate a strong diminution in HSF1 protein abundance.
Figure 7 | CK2α heterozygosity increases HSF1 abundance and activity in HD mice. (a) Mouse breeding scheme to generate WT, KIQ175, CK2α+/- and KIQ175/CK2α+/-/+ mice. (b) CK2α, CK2α and CK2β mRNA levels from KIQ175 and KIQ175/CK2α+/-/+ striatum at 6 months. Data was normalized to GAPDH and referenced to WT. (c) Chaperones Hsp70 and Hsp25 and (d) Mitochondrial activity-related genes PGC-1α, CYCS, NDUF3 and TFAM mRNA levels from the striatum of KIQ175 and KIQ175/CK2α+/-/+ at 6 months. (e) Immunoblots of striatum from KIQ175, WT, KIQ175/CK2α+/-/+ and CK2α+/-/+ at 6 months. (f) WT and mutant Htt immunoblot analysis from striatum of KIQ175, WT, KIQ175/CK2α+/-/+ and CK2α+/-/+ at 6 months. Long and Short exposures are presented. mRNA data shows fold change compared to the control group (WT; Supplementary Fig. 6) which values were set as 1. Error bars represent mean ± s.e.m., (n = 3 animals). Unpaired t-test *P<0.05, **P<0.05. Values for the KIQ175/CK2α+/-/+ group were compared to the KIQ175 group. See Supplementary Fig. 12 for uncropped immunoblots.

sites of neurodegeneration in HD, have reduced levels of Hsp70 expression as compared with other neurons which are more resistant to mHtt aggregation. Transcriptomic analysis of brain from patients with HD revealed reductions in chaperone family members and other HSF1 target genes with metabolic and synaptic functions (Supplementary Data 3). Although we demonstrate that HSF1 is responsible for the decrease in chaperone expression in the striatum, other transcription factors such as p53 and NF-Y may activate Hsp70 in other areas of the brain.

The depletion of HSF1 in HD is associated with increased phosphorylation of S303 and S307 within the HSF1 central regulatory domain. This region has been previously associated with negative regulation of HSF1 through poorly understood mechanisms. Nevertheless, R6/2 transgenic mice expressing a constitutively active form of HSF1 (lacking a 94 amino-acid of the central regulatory region encompassing S303 and S307), inhibited Htt-polyQ aggregate formation and prolonged lifespan. Interestingly, in cancer cells phosphorylation of S303 and S307 by distinct protein kinases is required for the interaction of HSF1 with the Fbxw7 E3 ligase, responsible for ubiquitin-dependent degradation of nuclear HSF1 in melanoma cancer cells. While cancer cells show decreased levels of Fbxw7 and elevated HSF1 protein levels, pathogenic polyQ-expressing cells and tissues show the opposite: increased Fbxw7 levels and increased CK2-dependent S303/307 phosphorylation of HSF1, which together drives HSF1 degradation (Fig. 9). The importance of maintaining appropriate levels of HSF1 may provide an avenue for therapeutic intervention for aging or diseased cells that have a decreased capacity to maintain protein homeostasis. However,
Figure 8 | CK2α⁺ heterozygosity ameliorates biochemical and neurobiological defects in KI175 HD mice. (a, b) Dendrite spine number and morphology of MSNs in the dorsal-striatum of KI175 and KI175/CK2α⁺/⁻ at 6 months. Scale bar, 10 μm. Error bars indicate mean ± s.e.m., (n = 12 cells/animal, 3 animals/genotype). Unpaired t-test *P<0.05, **P<0.01. (c) Excitatory synapse input in the dorsal striatum. (d) Immunostaining of the cortico-striatal pre-synaptic marker (VGlut1, red), the thalamo-striatal pre-synaptic marker (VGlut2, red) and the post-synaptic marker PSD95 (green) in the dorsal-striatum of KI175, WT, KI175/CK2α⁺/⁻, and CK2α⁺/⁻ at 6 months. Scale bar, 10 μM. (e) Quantification of VGlut1-PSD95 and (f) VGlut2-PSD95 co-localized synaptic puncta from B. Error bars indicate mean ± s.e.m., (n = 3 animals per genotype, 3 sections per animal, 15 sections per scan). Unpaired t-test *P<0.05, **P<0.01. (g, h) Size and body weight of KI175 (n = 6), WT (n = 8), KI175/CK2α⁺/⁻ (n = 11) and CK2α⁺/⁻ (n = 11) at 6 months. Error bars indicate ± s.e.m. Unpaired t-test *P<0.05.
mHtt expression increases the abundance of CK2α kinase and the Fbxw7 Fbox protein. CK2α phosphorylates HSF1 S303 and S307, inactivating HSF1 transcriptional activity and recruiting the Fbxw7 E3 ligase. The E3 ligase complex ubiquitinylates HSF1, targeting the protein for proteasomal degradation. Decreased HSF1 levels compromise the expression of HSF1 target genes that are essential for coping with misfolded and aggregated mHtt in Huntington’s Disease and for neuronal function and survival.

Further studies will be required to better understand the molecular mechanism by which poly-glutamine expanded Htt drives increased expression of the HSF1 degradation components CK2α and Fbxw7.

Methods
Cell culture and cell lines. Mammalian cell lines used in this study were the inducible rat PC12 cells expressing HttQ23-GFP and HttQ74-GFP (a gift from Dr. Rubinstein, University of Cambridge), the inducible rat PC12 cells expressing AR-Q10 and AR-Q12 (a gift from Dr. Merry, Thomas Jefferson University), the mouse-derived striatal cells STHdhQ7 and STHdhQ211 (Coriell Cell Repositories), the hsf1−/− MEFs expressing Htt-Q23-GFP and Htt-Q74-GFP (prepared in this work), human HEK-293T cells (ATCC CRL-1573) and human retinal pigment epithelial cell line ARPE-19 (ATCC CRL-2302). Human HD iPSC-derived from 33Q and 60Q individuals were cultured as precursors and further differentiated into MNSi66. On day 42 of differentiation, cell aggregates were plated into 24-well plates coated with Matrigel (BD). Two weeks later cells were transferred to neural induction medium to induce MN formation.

PC12 cells lines were maintained on Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 5% Tet-approved foetal bovine serum (FBS), 10% horse serum, 100 μg/ml G418, 75 μg/ml Hygromycin B and 100 μM penicillin/streptomycin grown at 37 °C. MEFs were maintained in high-glucose DMEM supplemented with 10% (FBS), 0.1 mM nonessential amino acids, 100 μM penicillin/streptomycin, and 55 μM 2-mercaptoethanol and grown at 37 °C. Striatal cells were grown at 33 °C in DMEM supplemented with 10% FBS and 100 μM penicillin/streptomycin. HEK-293 T cells were maintained on DMEM supplemented with 10% (FBS) and 100 μM penicillin/streptomycin grown at 37 °C. ARPE cells were grown in DMEM-F12 supplemented with 10% FBS and 100 μM penicillin/streptomycin. Doxycycline induction treatments were performed using 10 μg/ml for 2–3 days. All culture media were supplemented with MycoZap-Plus-PR (Lonza) for mycoplasma elimination.

PC12 cells were authenticated by immunoblotting analysis on doxycycline induction for the presence of Htt-polyQ constructs with anti-GFP antibody, AR-polyQ cells were authenticated for the presence of AR-polyQ with anti-N20 antibody. The mouse-derived striatal cells STHdhQ7 and STHdhQ211 (Coriell Cell Repositories) were authenticated by immunoblotting for the presence of mutant Htt with anti-Htt (Mab2166). The hsf1−/− MEF cells were authenticated by immunoblotting for HSF1 with anti-HSF1 (Enzo 10H8). No misidentified cells maintained by ICLAC were used in this study.
**Viability assays.** Cell viability was assessed using the XTT Cell viability assay kit (Roche) following manufacturers’ recommendations.

**RNA preparation and RT-PCR.** RNA was extracted from PC12 Htt-Q74 cells and human tissue by using RNeasy extraction kit (Qiagen) according to manufacturers’ instructions. For worm RNA extraction, 50 worms were incubated in a thermo-cycler at 4°C for 10 min and RNA extraction was performed using phenol-chloroform, precipitated with one volume of 100% isopropanol and 20 µg glycogen and resuspended in water. cDNA for all samples was prepared from 1 µg RNA using the SuperScript First Strand Synthesis System for RT-PCR kit (Invitrogen) according to manufacturer’s instructions. SYBR green based PCR was performed with SYBR mix (BioRad).

**Chromatin immunoprecipitation.** For chromatin immunoprecipitation assays Htt-Q74 cells were grown in 10 cm plates to 75% confluency, HttQ74 expression was subjected to immunoblot analysis to validate protein levels used for immunoprecipitation cell lysates were prepared in 500 µl lysis buffer (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100) and sonicated three times for 30 sec. Samples were centrifuged at 12,000 r.p.m. during 10 min. An aliquot (30 µl) was saved for input control DNA. 5 µg affinity purified anti-HSF1 antibody (Bethyl)12 was added and samples were incubated overnight at 4°C with constant rocking. Protein G agrose beads were added and incubated for 4 h at 4°C. Immunocomplexes were washed, eluted using elution buffer (10 mM Tris-HCl pH 7.5, 65% glycerol, 25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100), incubated on ice during 10 min and centrifuged at 14,000 rpm for 10 min at 4°C. Anti-FLAG M2 magnetic beads (Sigma) were incubated with protein samples overnight at 4°C. Elution was carried out in 1× TE, 10% SDS at 65°C for 10 min.

**Mouse strains.** For this study we used a full-length knock-in mouse model of HD known as zQ175, which harbours a chimeric human/mouse exon 1 carrying an expansion of ~175 CAG repeats and the human poly-proline region 28, at 2, 6 and 12 months of age comparing to WT (C57BL/6) animals at the same age. Two females and two males were used for each genotype and age. Breeding and genotyping conditions are detailed in Supplemental Experiments. For analysis of the effect on the absence of CK2z′ in the HSF1 protein abundance we obtained CK2z′ heterozygous mice (CK2z′+/−) and females from Dr. Seldin. For our analyses on the effect of the absence of CK2z′ in the Kq175 background (C57BL/6) we crossed males CK2z′+/− and females Htp4075/a generating the four genotype pairs: WT (CK2z′+/+), CK2z′+/− (CK2z′+/− Ht+), Kq175 (CK2z′+/+ Ht0/2+), Kq175 (CK2z′+/− Ht0/2+). Animals were analysed at 5 weeks and 6 months of age. Sample size was set to a minimum of three animals per genotype for every analysis. For body weight monitoring only males were evaluated. No randomization of animals was used in this study. The IACUC of Center of Animal Care and Use at Duke University approved the animal protocol used in this project (approval number: A173-14-07).

**Immunohistochemistry.** Mice were perfused intra-cardially with tris-buffered saline (TBS) (25 mM Tris-base, 135 mM NaCl, 3 mM KCl, pH 7.6) supplemented with 7.5 µl heparin followed with 4% PFA in TBS as previously described in ref. 49. Brains were dissected, fixed with 4% PFA in TBS at 4°C overnight, cryoprotected with 30% sucrose in TBS overnight and embedded in a 2:1 mixture of 30% sucrose in TBS/OCT (Tissue-Tek). Brains were cryo-sectioned at 20 µm using a Leica CM3505S, washed and permeabilized in TBS with 0.2% Triton X-100 (TBST). Sections were blocked in 5% normal goat serum (NGS) in TBST for 1 h at room temperature. Primary antibodies were diluted in 5% NGS in TBS: mouse anti-icC2 (mHtt) 1:500 (Millipore), rat anti-HSFl-1:500 (Enzo SPA-950-R), rabbit anti-HSFl-1:1000 (Ab47369), rabbit anti-CK2z′ (EAP0505) (Elabscience) 1:200, rat anti-Darpp32 (BD Biosciences) 1:1,000, mouse anti-Glutamine Synthetase (BD Biosciences) 1:1,000, rat anti-CD68 (clone FA-11) (BioLegend) 1:1,000, mouse anti-FOX1P (IC12) 1:500, rat anti-Ctip2 (Abcam) 1:500. Sections were incubated overnight at 4°C with primary antibodies. Secondary Alexa-fluorophore-conjugated antibodies (Invitrogen) were added (1:200 in TBST with 5% NGS) for 2 h at room temperature. Slides were mounted in Vectashield with DAPI (Vector laboratories) and images acquired on a confocal laser-scanning microscope (Leica SP5).

**Synapse quantification in mouse brain sections.** Three independent experiments were performed for each mouse, containing the dorsal striatum (bregma 0.5–1.1 mm) and were stained with presynaptic VGlut1 or VGlut2 (Chemicon, anti-guinea pig, 1:500) and postsynaptic PSD95 (Zymed, Rabbit, 1:500) markers as described previously 49. Secondary Alexa-fluorophore-conjugated markers were used. The Puncta Analyzer Plugin for ImageJ (available upon request; e.corugli@cellbio.duke.edu) was used to enumerate co-localized synaptic puncta. Maximum projections of three consecutive optical sections (corresponding to 1 µm depth) were generated. The Puncta Analyzer Plugin for ImageJ (available upon request; e.corugli@cellbio.duke.edu) was used to enumerate co-localized synaptic puncta. This assay takes the advantage of the fact that presynaptic and postsynaptic protein partners reside in separate cells (see Figure 6). We used one z-tile per animal. Sections were generated from piglet Alexa 488 (VGlut1/2) dilution 1:1200 and goat anti-rabbit Alexa 594 (PSD95) dilution 1:1200. Three mice for each genotype: WT, CK2z′+/−, Kq175 and Kq175/CK2z′+/− were evaluated in a double-blinded fashion. The 5-µm-thick confocal scans (optical section depth 0.35 µm, 15 sections per scan, image area per scan = 20.945 µm²) of the synaptic zone in dorsal striatum were performed at 63× magnification on a Leica SP5 confocal laser-scanning microscope. Maximum projections of three consecutive optical sections (corresponding to 1 µm depth) were generated. The Puncta Analyzer Plugin for ImageJ (available upon request; e.corugli@cellbio.duke.edu) was used to enumerate co-localized synaptic puncta. This assay takes the advantage of the fact that presynaptic and postsynaptic protein partners reside in separate cells (see Figure 6). We used one z-tile per animal. Sections were generated from piglet Alexa 488 (VGlut1/2) dilution 1:1200 and goat anti-rabbit Alexa 594 (PSD95) dilution 1:1200. Three mice for each genotype: WT, CK2z′+/−, Kq175 and Kq175/CK2z′+/− were evaluated in a double-blinded fashion. The 5-µm-thick confocal scans (optical section depth 0.35 µm, 15 sections per scan, image area per scan = 20.945 µm²) of the synaptic zone in dorsal striatum were performed at 63× magnification on a Leica SP5 confocal laser-scanning microscope.
the striatum were identified by their morphology. Secondary and tertiary apical dendrites were imaged for spine analysis as follows: z-stacks (30 μm total on z-axis, single section thickness = 0.5 μm) of Golgi-stained dendrites were taken at 63× magnification on a Zeiss AxioImager M1 microscope. A series of TIFF files corresponding to each image stack were loaded into the Reconstruct programme (http://synapses.clm.utexas.edu) and spine analyses performed as previously described.25,26 The classification of spines is based on width, length, and length/width ratio measurements taken using the Reconstruct software (http://synapses.clm.utexas.edu; RRID:iff:0000-23420) designed and validated.27 Spines were identified and classified by choosing 10 μm segments of dendrites and identified on selected dendritic stretches. The z-length (spine length) and spine head width were measured for each spine. Measurements were exported to a custom Microsoft Excel macro that was used to classify spines based on the width, length, and length/width ratio measurements taken in Reconstruct. Spines were categorized based on the following hierarchical criteria: (1) more than one spine head = ‘branched spine,’ (2) head width > 0.7 μm = ‘mushroom spine,’ (3) length > 2 μm = ‘filopodia,’ (4) length/width > 1 = ‘thin spine,’ and (5) length/width < 1 = ‘stubby spine.’ Branched and mushroom spines were identified as mature spines, thin and stubby spines were categorized as intermediate spines, and filopodia were classified as immature spines. Statistical analyses of changes in spine density, length, width and spine type were conducted in the R software programme (StataSoft). A total of 7 animals per genotype were analyzed as MSNs in the dorsal striatum in a blinded fashion. The number of spines analyzed per neuron type per age per genotype exceeded 1,000.

**Human samples.** HD Brain tissues were obtained from three independent sources; from Banc de Teixits Neurologics Biobanc Hospital Clinic-DIBAPS (Barcelona Brain Bank) via Dr Isidre Ferrer28 followed by the guidelines of the local ethics committee (ref. 09/07), and commercial Brain Tissue from Brain Tissue Resource Center. Cases with and without clinical neurological disease were processed in the same way following the same sampling protocols. Control and HD cases were compared pairwise for sex, age and postmortem time (Supplementary Table 1). Brain tissue was homogenized in 25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 1% Triton X-100. Extra SDS was added to the suspension to a final concentration of 2% (w per v) and heated at 95°C for 10 min and protein concentration determined using the BCA protein quantitation assay (Piecer). A total of 60 μg protein was loaded onto SDS–PAGE for immunoblotting.

**mHtt expression and aggregation analysis.** Protein samples from mouse striatum at 6 months were prepared in cell lysis buffer (25 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100 and 0.1% SDS). Extra SDS was added to the suspension to a final concentration of 2% (w per v) and lysates heated at 95°C for 5 min to solubilize tissue. Total tissue homogenate was then sonicated three times 45 s on ice, centrifuged at 12,000 r.p.m. for 10 min and protein concentration determined using the BCA protein quantitation assay (Piecer). A total of 60 μg protein was loaded onto SDS–PAGE for immunoblotting.

**Immunoblot analysis.** Protein samples were separated on 4–20% SDS Criterion TGX Stain-Free gel (BioRad) at 110 V. Proteins were transferred to nitrocellulose membrane (BioRad 0.2 μm) in Tris–Glycine Buffer (25 mM Tris-Base, 200 mM glycine) at 25 V for 30 min in Trans Turbo Transfer system (BioRad). The membrane was blocked with 5% non-fat dry milk in TBS containing 0.25% Tween-20 (TBST) for 1 h at room temperature, incubated with primary antibodies: 1:1000 in TBST containing 2.5% milk overnight at 4°C, and then washed 3 times with TBST for 10 min each in TBST followed by incubation with secondary Amersham ECL HRP Conjugated Antibodies 1:5000 in TBST containing 2.5% milk (GE Healthcare) for 1 h. After washing three times for 15 min each in TBST bands were detected with SuperSignal Chemiluminescent substrate (Thermo Scientific). Primary antibodies used in this study were used at 1:1,000 dilution: anti-CK2α (M2, Signet), anti-panGsk3 (IH8, Santa Cruz), anti-Erdj3 (C-7, Santa Cruz), anti-Hspb5 (Abcam), anti-CK2 (Ab75309, Abcam), anti-CK2α (Ab6025, Abcam), anti-FBXW7 (Ab109617, Abcam), anti-Ht (Mab2166, Chemicon) and (Mab1574, Millipore). Anti-Hsp60 (SPA-807, Stressgen), anti-Hsp90 (AI-P9-846F, Enzo), anti-Erkd3 (C-7, Santa Cruz), anti-Hsp70 (pDB6 #27083) and GST-CK2α expression vectors were purchased from Addgene (pDB1-27083 and pDB6-27084, respectively). Vectors were transformed into E. coli strain BL21 (DE3). Overnight cultures were diluted 1:100, grown to OD600 = 0.6 at 37°C and induced with 1 mM isopropyl 1-thio-β-d-galactopyranoside for 5 h at 37°C. Cell pellets were lysed in GST equilibration buffer (30 mM Tris, 150 mM sodium chloride, pH 8.0) supplemented, using sonication three times with 30 s bursts. Lysates were cleared by centrifugation at 20,000g for 30 min and incubated with 2 ml (bed volume) of Glutathione agarose beads (Pierce) per liter of culture. Beads were washed twice with NB +/− 40 mM Im-HCl, twice with NB supplemented with 40 mM Im-HCl, 5 mM ATP, and 20 mM MgCl2, and once with NB +/− 40 mM Im-HCl. Bound protein was eluted with a Sephacryl S400 (GE Healthcare) gel filtration column using an AKTA FPLC (GE Healthcare) at a flow rate of 1.3 ml per min in 25 mM HEPES, pH 7.5, and 150 mM NaCl at 4°C. Fractions corresponding to HSF1 monomer and HSF1 trimer were collected, pooled, concentrated and aliquoted at 10 μM for future use.

**In vitro phosphorylation of HSF1 by CK2.** Quantification of the specific activity (U per ml) of GST-Ck2α (Addgene pDB1-27083) and GST-Ck2α (Addgene pDB6-27084) purified recombinant enzymes was performed using casein kinase assay kit (Promega) and commercial CK2 (New England Biolabs) as positive control for phosphorylation. Quantification was carried out following per manufacture's instructions. Purified recombinant human HSF1 trimer (1 μg) was incubated with recombinant CK2 (Holoenzyme or GST purified subunits CK2α or CK2γ) with 5,000 U ml−1 as negative controls for the assay we used samples without ATP or samples without enzyme. The reaction was carried out at 37°C during 30 min (control conditions) or at 37°C during 20 min followed by 10 min at 42°C (Heat shock conditions). 6 × SDS was added and boiled for 2 min to terminate the reaction. Samples were analysed by phosphoproteomics.

**C. elegans experiments.** All Caenorhabditis elegans strains were maintained on normal growth media seeded with E. coli OP50 until required for experiments. To perform RNAi against kin-3, E. coli HT115 expressing kin-3 double-stranded RNA or the vector L4440 (control) was cultured in LB broth containing ampicillin (50 μg ml−1) for 8 h. Cultures were concentrated (×10−5) and seeded onto normal growth media plates containing isopropyl-1-thio-β-d-galactopyranoside (1 mM) and ampicillin (50 μg ml−1). Plates were incubated overnight at 37°C and allowed to cool before plating animals. For each strain, Q37:YP7 (rnl1225[Punc-54:q37: ypl110]Cb) and Q37;YFP hsfs1 (isy4411) (from Dr, Morimoto, Northwestern University), L1s were plated on RNAi plates and grown for a total of 20 days at 20°C. To prevent progeny from contaminating plates, animals were transferred every two days.

**Phosphoproteomic analysis.** Phosphoproteomic analysis was performed by the Proteomics and Metabolomics Shared Resource at Duke University. Protein samples were mixed with loading buffer and reduced with 10 mM DTT at 70°C for 10 min before SDS–PAGE separation on a 4–12% bis-tris acrylamide gel...
(NuPAGE, Invitrogen) with colloidal coomassie staining. Bands corresponding to HSF1 were excised and subjected to standardized in-gel trypsin digestion (http://www.bio.duke.edu/core/protomic/sample-preparation/documents/In-gelDigestionProtocolrevised.pdf). Extracted peptides were lyophilized to dryness and resuspended in 12 μl of 0.2% formic acid/2% acetonitrile. Phosphopeptides were enriched using GL Biosciences TiO2-derivatized tips according to the manufacturer’s protocol. Each sample was subjected to chromatographic separa- tion on a Waters NanoAcquity UPLC equipped with a 1.7 μm BEH130 C18, 75 μm I.D. X 250 mm reversed-phase column. The mobile phase consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. Following a 4 μl injection, peptides were trapped for 5 min on a 5 μm Symmetry C18, 180 μm I.D. X 20 mm column at 5 μl/min, followed by a linear elution gradient of 5% B to 40% B was performed over 30 min at 400 nl per min. The analytical column was connected to a fused silica PicoTip emitter (New Objective, Cambridge, MA) with a 10 μm tip orifice and coupled to a QExactive Plus mass spectrometer through an electrospray interface operating in a data-dependent mode of acquisition. The instrument was set to acquire a precursor MS scan from m/z 375–1765 with MS/MS spectra acquired for the ten most abundant precursor ions. For all experiments higher-energy collisional dissociation (HCD) energy settings were 27 eV and a 120 s dynamic exclusion was employed for previously fragmented precursor ions.

Raw LC-MS/MS data files were processed in Proteome Discoverer (Thermo Scientific) and then submitted to independent Mascot searches (Matrix Science) against a SwissProt database (Human taxonomy) containing both forward and reverse entries of each protein (20,322 forward entries). Search tolerances were 5 ppm for precursor ions and 0.02 Da for product ions using trypsin specificity with up to two missed cleavages. Carbamidomethylation (+57.0214 Da on C) was set as a fixed modification, whereas oxidation (+15.9949 Da on M), deamidation (+0.98 Da on NQ), and phosphorylation (+7.99 Da on STY) were considered dynamic mass modifications. All searched spectra were imported into Scaffold (v4.3, Proteome Software) and scoring thresholds were set to achieve a peptide false discovery rate of 1% using the peptideProphet algorithm. Raw data can be found in Supplementary Data 1 and 2.

Statistical analysis. The t-test was used to compare mean values in groups of samples for all experiments. Error bars were calculated using mean ± s.e.m. with a group size n ≥ 3. All reported P-values were calculated for groups with unequal variance using the Excel software programme (Microsoft) and a two-tailed unequal-tailed t-test. Statistical significance of CK2 subunits mRNA expression between groups with unequal variances was set as a fixed modification, whereas oxidation (+15.9949 Da on M), deamidation (+0.98 Da on NQ), and phosphorylation (+7.99 Da on STY) were considered dynamic mass modifications. All searched spectra were imported into Scaffold (v4.3, Proteome Software) and scoring thresholds were set to achieve a peptide false discovery rate of 1% using the peptideProphet algorithm. Raw data can be found in Supplementary Data 1 and 2.

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Data availability. All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

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Authors contributions

R.G.P., E.T.B., D.W.N., A.M.J., A.D., S.U.M. and S.S.A. contributed to the study conception, design, conducted experiments and participated in data interpretation. E.C. provided human protein extracts and participated in data analysis. D.C.L., A.A., C.A.R., C.E. and D.J.T. contributed to the conception and design of experiments and data interpretation. All authors contributed to writing and editing the manuscript.

Additional information

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: D.J.T. is a founder of Chaperone Therapeutics and a member of the SAB.

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