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Complete suppression of Htt fibrilization and disaggregation of Htt fibrils by a trimeric chaperone complex

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 19 July 2017

Thank you for submitting your manuscript to The EMBO journal and my apologies for the unusually long duration of the review process in this case. We have now finally received reports from three referees and these are included below.

As you will see from the reports, the referees all express interest in the findings reported in your manuscript and support publication in The EMBO Journal, following adequate revision.

For the revised manuscript I would particularly ask you to focus your efforts on the following points:

-> Please extend the analysis of disaggregation as suggested by ref #1

-> Please provide additional data on the interaction between the three 'complex' members in mammalian cells; this should also help you address the minor points from ref #1 regarding the terminology used.

-> Ref #2 raises a number of technical points about the Htt constructs used and the generality of the disaggregase effect. From my side, these are valid points that should help improve the overall conclusiveness and impact of the revised manuscript.

Given the referees' overall positive recommendations, I would like to invite you to submit a revised version of the manuscript, addressing the comments of all three reviewers.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

REFEREE REPORTS

Referee #1:

In this manuscript, the authors describe a tripartite chaperoning pathway that suppresses aggregation of Htt both in vitro and in vivo. Using an in vitro assay that uses proteolytic release of Htt to initiate aggregation, the authors demonstrate that a chaperone network comprising constitutive Hsc70, DNAJB1, and Hsp110 inhibit Htt aggregation in an ATP-dependent mechanism. Furthermore, the authors show that this same complex can induce dissociation of fibrils as well. The authors demonstrate that for inhibition of aggregation the Hsc70 ATPase activity is required, but not the ATPase activity of Hsp110. In contrast, fibril dissociation requires both ATPase activities. Using other Hsp70s and DNAJs the authors show that there preferential networks between these chaperones and co-chaperones for the inhibition of Htt aggregation. Notably, the inducible Hsp70s are less effective at inhibiting Htt aggregation due to the reduced ATPase activity. The authors then go on to demonstrate that the optimal chaperoning pathway appears to function in vivo using C. elegans, demonstrating that depletion of components of this pathway increase aggregation of multiple polyQ containing proteins in worms. Furthermore, they show that RNAi depletion of DNAJB1 and Hsp110 in iPSC neuronal lineages increases Htt aggregation, indicating that the pathway is important for regulating aggregation in mammals. Finally, they show overexoression of DNAJB1 (the rate limiting chaperone in this pathway) inhibits Htt aggregation in cell culture models.

Overall, this is an interesting study and the experiments are all well performed. I do have a few comments included below that I think should be addressed prior to acceptance, but this is a strong candidate for publication in EMBO. Notably, I'd like to see a little more regarding the aggregate dissolution by the chaperoning network in vitro. That aspect of the work seems less developed than the inhibition of aggregation. It would also be good to perform a couple more experiments regarding the 'preference' for chaperoning networks to inhibit Htt aggregation in cell culture models. Finally, I'd recommend the author be a little careful with some of their language, notably the use of 'complex' and 'complete suppression'. I understand what they are saying, and I don't necessarily disagree, but I think that the use of these terms somewhat overstates the findings of the manuscript (see minor comments below).

Major Comments.

1. The data showing dissociation of aggregates seems less well developed than the inhibition of aggregation. It would be nice to show the dissociation using another assay. Notably, the use of the YPet/CyPET assay is not employed for disaggregation, and it would be good to show that disaggregation works using this (or a similar) assay.
2. The YPet/CyPet assay used throughout this manuscript dependence on the cleavage of a GST to initiate the aggregation reaction. It would be good to confirm that the addition of the various chaperones does not influence this cleavage event, thus inhibiting aggregation by affecting initiation. I don't believe this to be the case, but it is important to show for the data shown in Figure 1.
3. The preference for specific chaperone networks to inhibit Htt aggregation was really interesting. I do wonder if the same preference plays out in mammalian cells. The authors show that knockdown of DNAJB1 and HSP110 in iPSC neurons increases Htt aggregation and overexpression of DNAJB1 suppresses aggregation in mammalian cells. However, it would be great to show a direct interaction. For example, IP HttExon1 and blot for HSPA8, DNAJB1, and HSP110 (and other components of this pathway) to show that these factors are preferentially binding to Htt.

4. The difference between constitutive and inducible Hsp70 is interesting. I'd like to see some discussion on how these two Hsp70s have different efficacies in regulating Htt aggregation from a molecular perspective.

Minor Comments.

1. I'm not sure I'd refer to this as complex. The HSP70 chaperoning pathway doesn't generally function as a complex but as more of a pathway. Considering that a complex is not shown, I recommend reconsidering that use of the term complex. Alternatively, the authors could perform some IP experiments to determine if such a complex does form.
2. Similar to the above, I'd be somewhat careful with the term 'complete' suppression. It is definitely true that you observe inhibition up to ~24 h. However, it is possible it is just delayed (similar to what is observed with incubations in the absence of HSP110. I recommend including a caveat with this phrase. Something like complete suppression for 24 h.
3. I think some panels in Figure 4 are mislabeled in the text or figure. Notably, I think Figure 4e is mislabeled.

Referee #2:

This work reports on the modulation of HTT-48Q aggregates by a mixture of molecular chaperones that is composed of HspA8, Hsp110 and DnaJB1. The bulk of the data presented is from a FRET assay that monitors the aggregation of GST-HTTExon1Q48-CyPET and GST-HTTExon1Q48-YPET. Additional experiments are conducted in C. elegans or differentiated patient fibroblasts. The finding from in vitro assays is that HspA8, Hsp110 and DnaJB1 reduce HTT48Q aggregation and also dissolve preformed aggregates.

This work would add to a growing body of literature which shows that Hsp70 and its co-factors cooperate to modulate protein aggregate biology. What appears to be new is the use of Htt48 and HspA8, Hsp110, and DnaJB1 in assays that monitor protein aggregation.

There is a history to the study of HTT aggregation that raises questions on the broad impact of the data reported that might be addressed through experimentation. HTT aggregation occurs in a manner that is dependent upon the length of polyQ chains. Htt23Q is not thought to aggregate and is used as a control in assays where aggregation of longer polyQ repeats is monitored.

Since the constructs of Exon148Q contain a GST and Cypher or YPET motif, it is critical to show that the aggregation of Htt48 reported is not due to misfolding/aggregation of reporter domains attached to Htt48Q. Data from experiments from controls with Htt23Q would address this concern.

Experiments from members of the field are often conducted with Htt53, Htt96, and Htt103Q. Does extending the polyQ domain on HTT impact is rate of aggregation in FRET assays. Is the trio of HspA8, Hsp110 and DnaJB1 able to suppress formation or dissolve aggregates of forms of HttExon that contain polyQ tracts that are long than 48Q?

Htt exon1 contains a 17 amino acid peptide, a polyQ tract, and a C-terimal polyproline rich stretch. It appears that the proline domain of Htt is not present in the forms of Htt48Q used in this study. The presence of the proline domain of Htt Exon1 strongly enhances HttpolyQ aggregation. It is therefore important for the reader to know if HSCA8, HSP110 and DNAJB1 can also suppress the aggregation of form of HttExon1polyQ that contains a proline domain.

There is concern about the data presented in Figure 6. It looks like Htt44Q encoded by the patient fibroblast does not form amyloid-like aggregates where cells are differentiated. Yet, it does form aggregates if DNAJB1 is depleted. It is hard to interpret these data because it is not clear why a 50% loss of DNAJB1 leads Htt to now be detected by filter trap assays? Can Htt be detected by western bolt in these cells? Do levels of total Htt increase when DNAJB1 is partially depleted? DNAJB1 is required for degradation of short-live proteins, so how do the authors rule out the possibility that

loss of DNAJB1 hinders the degradation of Htt versus failing to suppress its aggregation? Data from work with C. elegans and patient derived fibroblasts are not done in sufficient detail for the reader to know why levels of HttpolyQ change when activity of chaperones are modulated.

Referee #3:

The manuscript by Scior, et al., Wanker, Prigione and Kirstein entitled "Complete Suppression of Htt fibrilization and disaggregation of Htt fibrils by a trimeric chaperone complex" merits serious consideration for publication in the EMBO Journal, as this papers goes significantly beyond the work of Bukau and Kampinga-the pioneers who showed that the 40-70-nucleotide exchange factor proteostasis network pathway can accomplish disaggregation. The authors use a powerful FRET-based HttExon1 aggregation assay, wherein equimolar amounts of GST-HttExon1Q48=CyPet and GST-HttExon1Q48=YPet are mixed and FRET is observed proportional to the extent of aggregation. PreCission protease removes the solubilizing GST tag triggering Htt Q48 aggregation. Transmission Electron Microscopy (TEM) analysis confirms aggregation and demonstrates that the FRET tags do not interfere with amyloidogenesis-only fibrilized Htt can be sedimented by ultracentrifugation. Bukau and others have recently demonstrated that a Hsp70, along with an Hsp40 and an Hsp110 can disaggregate kinetically labile aggregates, including Asyn amyloid fibrils, but not kinetically stable amyloids like Abeta, however the ability of this proteostasis network pathway to completely suppress amyloidogenesis has not yet been demonstrated, which these authors demonstrated with poly Q expanded Htt. Moreover the authors provide evidence for the idea that distinct 70-40-110 combinations exhibit optimal suppression vs disaggregase activity-however, this reviewer hypothesizes that if the suppression activity is modest and disaggregation dominant, then aggregation would be minimal?

Suppression was first probed with recombinant Hsc70, the Hsp110 Apg2, and the Hsp40 DNAJB1. At a 7 fold excess of Hsc70 over HttExon1Q48, with the 70-40-110 ratio being 2:1:1, complete suppression of amyloidogenesis based on FRET, TEM and sedimentation experiments was observed. ATP was required for this effect. As shown previously, this combination was able to disaggregate the HttExon1Q48 fibrils within 20 h. Both suppression of amyloidogenesis and disaggregation required ATP. Strictly analogous data were generated using the isolated 70, 40 and 110 chaperones from C.elegans. The class B J protein appears to be very important, and an increase in its concentration can mask the effects of low 70 or 110 concentrations.

Apyrase addition, which rapidly depletes ATP, destroys the ability of the 70-40-110 system to prevent Htt aggregation. Hsp70s ATPase activity appears to be critical for Htt aggregation suppression based on mutagenesis. The nucleotide exchange factor function of 110 is also critical for aggregation suppression. While amorphous citrate synthase aggregates didn't influence Htt aggregation, the presence of these aggregates titrated away critical components of the 40-70-110 proteostasis network pathway, interfering with the ability of the proteostasis pathway to suppress Htt aggregation. At equal concentrations, Hsc70 was a more potent suppressor of aggregation than the inducible Hsp70 from C. elegans. Multiple Hsp40 proteins can suppress Htt aggregation, with the class B J's being most potent with the matched 70 and 110. The presence of the 40-70-110 system can also suppress seeded Htt aggregation. Using the human and the C.elegans chaperone combinations, the authors showed that the Hsc70, HSP-110 and a type B j protein constitutes the most active disaggregase for the resolubilization of Htt fibrils. The Hsp40 and to a lesser extent the Hsp70 protein were bound to the fibrils, unlike 110, but in nematodes expressing Q128Htt, the 40-70-and 110 were observed on the fibrils. In C. elegans , Q15 aggregates if the 70-40-110 pathway is suppressed by RNAi.

These results were then validated in neuronal progenitor cells from an HD HttQ44 patient. While no Htt aggregates have been reported in these cells, knockdown of DNAJB1 and HSPA4 leads to pronounced HttQ44 aggregation, in complete agreement with analogous experiments in the nematode Htt models reported by these authors.

The authors provide compelling evidence that the Hsp40-70-110 pathway can be protective in Huntington's disease, and there data suggest that Hsp40 upregulation could be very powerful for suppressing Htt expanded Q aggregation from becoming pathogenic.

It isn't clear that the statement that the "J-protein is the rate-limiting chaperone in the suppression of Htt fibrilization"-if there was kinetic evidence for this I missed it?

The sentence "C. elegans encodes for four HSP-70 proteins, only one HSP110 protein.....needs rewriting.

Less is more in the discussion, the discussion of this paper goes on and on.....I would encourage the authors to hit the key take home messages in 5 pages without too much redundancy with the results and introduction sections.

Overall, a pleasure to read, and upon revision will be a nice EMBO paper.

1st Revision - authors' response 18 September 2017

Thank you very much for your answer and we would like to extend our appreciation to all reviewers for their time to assess our work, their positive feedback and constructive critical comments. As outlined in our point-by-point response we could experimentally address all comments raised by the reviewers.
We have performed the following additional experiments to address the concerns of the reviewers:

1. We included an additional experimental method to study disaggregation of HttExon1Q_{48} fibrils. We employed a filter retardation assay to demonstrate the disaggregation of Htt fibrils by human and nematode chaperones in a time-resolved manner (Fig 4E).
2. To demonstrate the physical interaction of human chaperones with HttExon1Q_{97} aggregates we performed an *ex vivo* analysis where we isolated the Htt aggregates and demonstrated the association of the chaperones Hsc70, DNAJB1 and Apg2 with the Htt aggregates using filter retardation analysis (Fig 1H).
3. We included a control demonstrating that the chaperones (HSP-1, DNJ-13, HSP-110) do not inhibit or delay the cleavage reaction between GST and HttExon1Q_{48} construct by the PreSP protease (Fig EV1C).
4. We added an additional control for the employed HttExon1Q_{48} constructs by using a construct that harbors a polyQ stretch below the pathogenic threshold of 35Q residues, HttExon1Q_{23}-CyPet/YPet. This control does not form fibrils and expectantly does not exhibit FRET (Fig 1C).
5. As suggested by reviewer 2 we also studied if the chaperone complex could suppress the fibrilization of HttExon1 harboring a longer polyQ stretch (>Q_{48}). We analyzed the suppression of fibrilization of HttExon1Q_{75} and demonstrate that HSP-1, DNJ-13 and HSP-110 could indeed almost completely suppress the aggregation of HttExon1Q_{75} (Fig 2D). Interestingly, the chaperones could not disaggregate HttExon1Q_{75} fibrils (Fig EV2E) suggesting that the amyloid fibrils formed by HttExon1Q_{75} are more rigid and inert towards chaperone-mediated resolubilisation.
6. To address the concern that depletion of chaperones could lead to an accumulation of Htt by e.g. inhibiting proteolytic pathways, we analyzed the Htt levels in our chaperone knockdown experiments. We can show that the overall levels of HttExon1Q_{97} do not change, but that depletion of chaperones lead to a redistribution from the soluble to the insoluble fraction (Figure EV2F+G).
7. We also addressed the concern that the employed Htt constructs might lack the poly proline region, by pointing out that HttExon1 includes the polyP region that follows the polyQ stretch. We have included the protein sequence of the construct in the rebuttal letter.

With the additional experiments (points 1-6) we believe we addressed the open questions you pointed out and asked us to focus on:

“For the revised manuscript I would particularly ask you to focus your efforts on the following points:

-> Please extend the analysis of disaggregation as suggested by ref #1

-> Please provide additional data on the interaction between the three 'complex' members in mammalian cells; this should also help you address the minor points from ref #1 regarding the terminology used.

-> Ref #2 raises a number of technical points about the Htt constructs used and the generality of the disaggregase effect. From my side, these are valid points that should help improve the overall conclusiveness and impact of the revised manuscript.“

For better readability we have indicated our response to the individual comments by the reviewers *italicized*.

Referee #1:

In this manuscript, the authors describe a tripartite chaperoning pathway that suppresses aggregation of Htt both in vitro and in vivo. Using an in vitro assay that uses proteolytic release of Htt to initiate aggregation, the authors demonstrate that a chaperone network comprising constitutive Hsc70, DNAJB1, and Hsp110 inhibit Htt aggregation in an ATP-dependent mechanism. Furthermore, the authors show that this same complex can induce dissociation of fibrils as well. The authors demonstrate that for inhibition of aggregation the Hsc70 ATPase activity is required, but not the ATPase activity of Hsp110. In contrast, fibril dissociation requires both ATPase activities. Using other Hsp70s and DNAJs the authors show that there preferential networks between these chaperones and co-chaperones for the inhibition of Htt aggregation. Notably, the inducible Hsp70s are less effective at inhibiting Htt aggregation due to the reduced ATPase activity. The authors then go on to demonstrate that the optimal chaperoning pathway appears to function in vivo using C. elegans, demonstrating that depletion of components of this pathway increase aggregation of multiple polyQ containing proteins in worms. Furthermore, they show that RNAi depletion of DNAJB1 and Hsp110 in iPSC neuronal lineages increases Htt aggregation, indicating that the pathway is important for regulating aggregation in mammals. Finally, they show overexoression of DNAJB1 (the rate limiting chaperone in this pathway) inhibits Htt aggregation in cell culture models.

Overall, this is an interesting study and the experiments are all well performed. I do have a few comments included below that I think should be addressed prior to acceptance, but this is a strong candidate for publication in EMBO. Notably, I'd like to see a little more regarding the aggregate dissolution by the chaperoning network in vitro. That aspect of the work seems less developed than the inhibition of aggregation. It would also be good to perform a couple more experiments regarding the 'preference' for chaperoning networks to inhibit Htt aggregation in cell culture models. Finally, I'd recommend the author be a little careful with some of their language, notably the use of 'complex' and 'complete suppression'. I understand what they are saying, and I don't necessarily disagree, but I think that the use of these terms somewhat overstates the findings of the manuscript (see minor comments below).

We thank the reviewer for the positive feedback and also for the constructive criticism.

Major Comments.

1. The data showing dissociation of aggregates seems less well developed than the inhibition of aggregation. It would be nice to show the dissociation using another assay. Notably, the use of the YPet/CyPET assay is not employed for disaggregation, and it would be good to show that disaggregation works using this (or a similar) assay.

The reviewer asks for an additional assay to analyze the disaggregation of Htt fibrils by the chaperone complex. First, we would like to state that we analyzed the disaggregation of Htt fibrils already by two independent methods:

1. *using EM as read-out to visualize the resulting Htt protein moieties as depicted in figures 1G and 4D*
2. *using a sedimentation analysis that employs an ultracentrifugation step to separate the soluble and insoluble fraction upon disaggregation by the chaperones and a subsequent analysis of the fluorescence intensity as read-out for the abundance of the HttExon1Q_{48}-CFP moiety in both fractions as depicted in figures 1F, 4F and EV1I*

In this revised version of the manuscript we added a third analysis of disaggregation that employs a filter trap assay. We analyzed the disaggregation of HttExon1Q_{48} fibrils by the human (Hsc70, DNAJB1 and Apg2) as well as nematode chaperones (HSP-1, DNJ-13 and HSP-110) in a time course experiment and included this new data set in figure 4E.

Taken together we have now demonstrated disaggregation activity by three independent assays for the human as well as nematode chaperones to allow us to conclude that these chaperones exhibit disaggregation activity for HttExon1Q_{48} fibrils.

2. The YPet/CyPet assay used throughout this manuscript dependence on the cleavage of a GST to initiate the aggregation reaction. It would be good to confirm that the addition of the various chaperones does not influence this cleavage event, thus inhibiting aggregation by affecting initiation. I don't believe this to be the case, but it is important to show for the data shown in Figure 1.

The reviewer is absolutely right that it is an important control to show that the presence of chaperones does not affect the cleavage reaction of the PreScission (PreSP) protease that cleaves off the GST tag. We performed this analysis and could indeed show that the chaperones (HSP-1, DNJ-13 and HSP-110 + ATP) do not inhibit the cleavage reaction and included these data in figure EV1C as suggested by the reviewer.

3. The preference for specific chaperone networks to inhibit Htt aggregation was really interesting. I do wonder if the same preference plays out in mammalian cells. The authors show that knockdown of DNAJB1 and HSP110 in iPSC neurons increases Htt aggregation and overexpression of DNAJB1 suppresses aggregation in mammalian cells. However, it would be great to show a direct interaction. For example, IP HttExon1 and blot for HSPA8, DNAJB1, and HSP110 (and other components of this pathway) to show that these factors are preferentially binding to Htt.

The reviewer asks for an analysis of the interaction of the chaperones HSPA8 (Hsc70), DNAJB1 and Apg2 (Hsp110) with HttExon1 in mammalian cells. We have employed a filter trap assay to analyze the physical interaction of Hsc70, DNAJB1 and Apg2 with HttExon1Q_{97} aggregates and could indeed observe that these three chaperones associate with the Htt aggregates. We have added these data in figure 1H.

Our data validate previous findings of an interaction of these chaperones with Htt as shown in the HDNetDB database (http://hdnetdb.sysbiolab.eu) that summarizes all interactions of Htt proteins and Hsc70, Hsp110 and DNAJB1 were among the identified interaction partners. This database also identified other Hsp70 members besides HSPA8 (HSPA1B, HSPA1L, HSPA2, HSPA5, HSPA9 and HSPA12A) and other J-proteins as Htt interaction partners besides DNAJB1 (DNAJB2, DNAJ1, DNAJA2, DNAJA3, DNAJC1, DNAJC4, DNAJC5, DNAJC11 and DNAJC21). These interactions however do not discriminate between discrete chaperone activities such as suppression of fibrilization or disaggregation and of course also not between direct and indirect interactions.

However, these data suggest that indeed different chaperone complexes could form that interact and remodel Htt proteins in mammalian cells. We have emphasized this point also in the discussion.

4. The difference between constitutive and inducible Hsp70 is interesting. I'd like to see some discussion on how these two Hsp70s have different efficacies in regulating Htt aggregation from a molecular perspective.

The reviewer asks for some discussion on the differences in Htt remodeling capacity exhibited by the constitutive Hsc70 chaperone vs the inducible Hsp70s. It is striking that the constitutive Hsc70 is together with the J-Protein partner and Hsp110 much more powerful in Htt suppression and disaggregation activities compared to chaperone mixtures of the inducible Hsp70s. We could show that although the basal ATPase activities among all Hsp70s (including Hsc70) are similar, the constitutive Hsc70 (HSP-1 in C. elegans) can be stimulated two-fold higher by the partner J-protein and the NEF, HSP-110, compared to the inducible Hsp70s (figure EV1H). Thus the constitutive Hsc70 can rely on twice the energy for its chaperone activity. This could be at least a contributing factor for the higher remodeling capacity exhibited by Hsc70 chaperone complexes. We have discussed this point in the discussion.

Minor Comments.

1. I'm not sure I'd refer to this as complex. The HSP70 chaperoning pathway doesn't generally function as a complex but as more of a pathway. Considering that a complex is not shown, I recommend reconsidering that use of the term complex. Alternatively, the authors could perform some IP experiments to determine if such a complex does form.

The reviewer is right, we do not yet know whether the involved chaperones act sequentially or indeed form a physical complex. We observed however that the chaperones functionally cooperate with each other to suppress Htt fibrilization and disaggregate Htt fibrils. In addition, we could demonstrate a physical interaction of all three chaperones with the Htt aggregates in vivo (Fig 4H) as well as ex vivo (Fig 1H).

In the manuscript we want to emphasize that it is not a single chaperone, but a combination or complex of chaperones from three chaperone families that exert those activities. For clarity we refer to them as chaperone complex yet are aware of the open question regarding their mode of interaction and chaperone activity.
In the manuscript (result section) we stated the following to clarify this point: "The three chaperones functionally cooperate to suppress the HttExon1Q_{48} fibrilization and are from now on referred to as chaperone complex."
And in the discussion section: "Further studies are required to gain more mechanistic insight into the mode of action of the chaperone-mediated suppression of Htt fibrilization and disaggregation of these amyloid fibrils. Do Hsp70, Hsp110 and the J-protein form a physical complex at any time of their course of action or do they bind and act sequentially."

2. Similar to the above, I'd be somewhat careful with the term 'complete' suppression. It is definitely true that you observe inhibition up to ~24 h. However, it is possible it is just delayed (similar to what is observed with incubations in the absence of HSP110. I recommend including a caveat with this phrase. Something like complete suppression for 24 h.

The reviewer is correct. We used the term "complete suppression" to distinguish between a full suppression and a delay of aggregation within the experimental time period. We are aware however that we can only make this statement for the duration of our experiments and have rephrased the statement to "We refer from now on only to a complete suppression if the chaperones fully inhibit

any FRET signal of the HttExon1Q_{48}-CyPet/YPet pair over the complete time period of the experiment that lasts usually between 20 and 30 h."

3. I think some panels in Figure 4 are mislabeled in the text or figure. Notably, I think Figure 4e is mislabeled.

We thank the reviewer for pointing out that figures 4 E, F and G were mixed up in the result section. We have corrected this in the revised manuscript.

Referee #2:

This work reports on the modulation of HTT-48Q aggregates by a mixture of molecular chaperones that is composed of HspA8, Hsp110 and DnaJB1. The bulk of the data presented is from a FRET assay that monitors the aggregation of GST-HTTExon1Q48-CyPET and GST-HTTExon1Q48-YPET. Additional experiments are conducted in C. elegans or differentiated patient fibroblasts. The finding from in vitro assays is that HspA8, Hsp110 and DnaJB1 reduce HTT48Q aggregation and also dissolve preformed aggregates.

This work would add to a growing body of literature which shows that Hsp70 and its co-factors cooperate to modulate protein aggregate biology. What appears to be new is the use of Htt48 and HspA8, Hsp110, and DnaJB1 in assays that monitor protein aggregation.

There is a history to the study of HTT aggregation that raises questions on the broad impact of the data reported that might be addressed through experimentation. HTT aggregation occurs in a manner that is dependent upon the length of polyQ chains. Htt23Q is not thought to aggregate and is used as a control in assays where aggregation of longer polyQ repeats is monitored.

Since the constructs of Exon148Q contain a GST and Cypher or YPET motif, it is critical to show that the aggregation of Htt48 reported is not due to misfolding/aggregation of reporter domains attached to Htt48Q. Data from experiments from controls with Htt23Q would address this concern.

The reviewer asks for a control that the fluorescence fusion partners, CyPet and YPet do not affect the aggregation propensity of HttExon1Q_{48} by using HttExon1Q_{23} that harbors a polyQ stretch below the pathogenic glutamine length.
We have performed this analysis and can show that HttExon1Q_{23}-CyPet/YPet does not form fibrils and can thus demonstrate that the fluorescence partner proteins do not affect the aggregation propensity of the Htt proteins. In fact, even doubling the concentration of HttExon1Q_{23} does not lead to a fibrilization (compare dark blue with turquoise curves in Fig 1C). We have added this control experiment to figure 1C and thank the reviewer for pointing out this missing control experiment.

Experiments from members of the field are often conducted with Htt53, Htt96, and Htt103Q. Does extending the polyQ domain on HTT impact is rate of aggregation in FRET assays. Is the trio of HspA8, Hsp110 and DnaJB1 able to suppress formation or dissolve aggregates of forms of HttExon that contain polyQ tracts that are long than 48Q?

The reviewer is interested to see whether the chaperones can suppress the fibrilisation of HttExon1 constructs with longer polyQ stretches. This is indeed a very interesting question. We have generated HttExon1Q_{75} constructs and analyzed the activity of the chaperones and could observe that the chaperones, Hsp70, J-protein and HSP-110 could indeed almost completely suppress the aggregation of this HttExon1 construct with a longer (Q_{75}) polyQ stretch. We have added this data

set as Fig 2D in the revised version of the manuscript. A disaggregation of pre-formed HttExon1Q_{75} fibrils was not observed within a time frame of 12 hours suggesting that the fibrils formed by longer polyQ stretches are more stable and resistant towards chaperone-mediated remodeling (Fig EV2E). In addition the in vivo work in our study uses multiple polyQ lengths e.g. the mammalian cell lines we analyzed upon modulation of the chaperones express HttExon1Q_{97} and HttQ_{44}; and the nematode lines express Htt513$Q_{15/128}$, Q_{35}-YFP and AT3CT$Q_{45/63}$. Thus we believe we have analyzed the chaperone capacity for a broad range of different polyQ lengths and flanking regions.

Htt exon1 contains a 17 amino acid peptide, a polyQ tract, and a C-terimal polyproline rich stretch. It appears that the proline domain of Htt is not present in the forms of Htt48Q used in this study. The presence of the proline domain of Htt Exon1 strongly enhances HttpolyQ aggregation. It is therefore important for the reader to know if HSCA8, HSP110 and DNAJB1 can also suppress the aggregation of form of HttExon1polyQ that contains a proline domain.

The reviewer asks if the poly-proline stretch is present in the constructs used in this study as it has been shown that the proline stretch also affects the aggregation of Htt. The poly-proline stretch is encoded by Exon1 of Htt and is present in the HttExon1Q_{48} constructs used in all experiments of our study. The complete sequence of the HttExon1Q_{48} is as follows:

MATLEKLMKAFESLKSFQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQ
QQQQQQQPPPPPPPPPPPQLPQPPPQAQPLLPQLQPPPPPPPPPPPGPAAAEEPLHRP

There is concern about the data presented in Figure 6. It looks like Htt44Q encoded by the patient fibroblast does not form amyloid-like aggregates where cells are differentiated.

Indeed, the absence of the detection of Htt aggregates in neural cells differentiated from HD patient-derived iPSCs is known. iPSC-derived neural cells have been found to exhibit several abnormalities, including impaired bioenergetics, but no evidence of aggregates has been reported so far (The HD Consortium, Cell Stem Cell 2012; Xu et al., Stem Cell Report, 2017). The lack of aggregates in the iPSC-derived neural cells is believed to be due to the detection limitation of the available assays and the low abundance of endogenous Htt protein, and possibly also to the limited maturation of iPSC-derived neural progenitors and post-mitotic neurons, which still resemble cells of the fetal human brain rather than cells of the adult human brain (reviewed in Tao and Zhang, Cell Stem Cell, 2016, or Inak et al., Stem Cells, 2017).

Yet, it does form aggregates if DNAJB1 is depleted. It is hard to interpret these data because it is not clear why a 50% loss of DNAJB1 leads Htt to now be detected by filter trap assays? Can Htt be detected by western bolt in these cells? Do levels of total Htt increase when DNAJB1 is partially depleted? DNAJB1 is required for degradation of short-live proteins, so how do the authors rule out the possibility that loss of DNAJB1 hinders the degradation of Htt versus failing to suppress its aggregation?

The reviewer wonders if the increase in Htt aggregation upon depletion of particularly DNAJB1 can also be explained by an overall higher level off Htt e.g. due to impaired proteolysis upon knockdown of DNAJB1. This is a very valid concern. We have analyzed the levels of HttExon1Q_{97} in HEK cells upon knockdown of not only DNAJB1, but also Hsc70 (HSPA8) and Apg2 (Hsp110). We observed that upon knockdown of the chaperones only the distribution between soluble and insoluble Htt protein levels changes, but not the overall level. We have included a typical western blot in the revised manuscript in figure EV2F and a quantification of 3 experiments in figure EV2G. For the quantification, we combined the signal intensities of both soluble and insoluble fraction of the respective sample and normalized them to tubulin. As can be seen in figure EV2F+G, knockdown of

the chaperones leads to a shift of the signal intensities of Htt from the soluble to the insoluble levels, the overall protein levels however do not change significantly compared to the control.

Data from work with C. elegans and patient derived fibroblasts are not done in sufficient detail for the reader to know why levels of HttpolyQ change when activity of chaperones are modulated.

The reviewer addresses the relationship of the depletion of chaperones and the protein levels of HttpolyQ in C. elegans and in cell culture. We would like to point out that not the overall levels of Htt change (see question and answer above), but that the distribution from soluble to insoluble fraction changes for Htt upon modulation of chaperones (see new data shown in figure EV2F+G). Analogous, we also did not observe a change in the overall levels of Q40-YFP upon depletion of the chaperones in the nematode model (data not shown).

Referee #3:

The manuscript by Scior, et al., Wanker, Prigione and Kirstein entitled "Complete Suppression of Htt fibrilization and disaggregation of Htt fibrils by a trimeric chaperone complex" merits serious consideration for publication in the EMBO Journal, as this papers goes significantly beyond the work of Bukau and Kampinga-the pioneers who showed that the 40-70-nucleotide exchange factor proteostasis network pathway can accomplish disaggregation. The authors use a powerful FRET-based HttExon1 aggregation assay, wherein equimolar amounts of GST-HttExon1Q48=CyPet and GST-HttExon1Q48=YPet are mixed and FRET is observed proportional to the extent of aggregation. PreCission protease removes the solubilizing GST tag triggering Htt Q48 aggregation. Transmission Electron Microscopy (TEM) analysis confirms aggregation and demonstrates that the FRET tags do not interfere with amyloidogenesis-only fibrilized Htt can be sedimented by ultracentrifugation. Bukau and others have recently demonstrated that a Hsp70, along with an Hsp40 and an Hsp110 can disaggregate kinetically labile aggregates, including Asyn amyloid fibrils, but not kinetically stable amyloids like Abeta, however the ability of this proteostasis network pathway to completely suppress amyloidogenesis has not yet been demonstrated, which these authors demonstrated with poly Q expanded Htt. Moreover the authors provide evidence for the idea that distinct 70-40-110 combinations exhibit optimal suppression vs disaggregase activity-however, this reviewer hypothesizes that if the suppression activity is modest and disaggregation dominant, then aggregation would be minimal?

We thank the reviewer for the very positive feedback to our work. And of course the reviewer is right that both activities, suppression of fibrilization and disaggregation of fibrils could take place at the same time – especially in vivo and the chaperones may shift the equilibrium of the fibrilization state of Htt towards the soluble fraction by either suppressing the fibrilization or disaggregation or both activities at the same time.

Suppression was first probed with recombinant Hsc70, the Hsp110 Apg2, and the Hsp40 DNAJB1. At a 7 fold excess of Hsc70 over HttExon1Q48, with the 70-40-110 ratio being 2:1:1, complete suppression of amyloidogenesis based on FRET, TEM and sedimentation experiments was observed. ATP was required for this effect. As shown previously, this combination was able to disaggregate the HttExon1Q48 fibrils within 20 h.

We do not exactly know to which previous work the reviewer refers to, but would like to point out that disaggregation of HttExon1Q_{48} fibrils has not been shown before.

Both suppression of amyloidogenesis and disaggregation required ATP. Strictly analogous data were generated using the isolated 70, 40 and 110 chaperones from C.elegans. The class B J protein appears to be very important, and an increase in its concentration can mask the effects of low 70 or 110 concentrations.
Apyrase addition, which rapidly depletes ATP, destroys the ability of the 70-40-110 system to prevent Htt aggregation. Hsp70s ATPase activity appears to be critical for Htt aggregation suppression based on mutagenesis. The nucleotide exchange factor function of 110 is also critical for aggregation suppression. While amorphous citrate synthase aggregates didn't influence Htt aggregation, the presence of these aggregates titrated away critical components of the 40-70-110 proteostasis network pathway, interfering with the ability of the proteostasis pathway to suppress Htt aggregation. At equal concentrations, Hsc70 was a more potent suppressor of aggregation than the inducible Hsp70 from C. elegans. Multiple Hsp40 proteins can suppress Htt aggregation, with the class B J's being most potent with the matched 70 and 110. The presence of the 40-70-110 system can also suppress seeded Htt aggregation. Using the human and the C.elegans chaperone combinations, the authors showed that the Hsc70, HSP-110 and a type B j protein constitutes the most active disaggregase for the resolubilization of Htt fibrils. The Hsp40 and to a lesser extent the Hsp70 protein were bound to the fibrils, unlike 110, but in nematodes expressing Q128Htt, the 40-70-and 110 were observed on the fibrils. In C. elegans , Q15 aggregates if the 70-40-110 pathway is suppressed by RNAi.
These results were then validated in neuronal progenitor cells from an HD HttQ44 patient. While no Htt aggregates have been reported in these cells, knockdown of DNAJB1 and HSPA4 leads to pronounced HttQ44 aggregation, in complete agreement with analogous experiments in the nematode Htt models reported by these authors.
The authors provide compelling evidence that the Hsp40-70-110 pathway can be protective in Huntington's disease, and there data suggest that Hsp40 upregulation could be very powerful for suppressing Htt expanded Q aggregation from becoming pathogenic.

We thank this reviewer for the very positive evaluation of our work.

It isn't clear that the statement that the "J-protein is the rate-limiting chaperone in the suppression of Htt fibrilization"-if there was kinetic evidence for this I missed it?

This reviewer is correct. We did not perform a kinetic analysis. We however observed in our in vitro FRET assays that reducing levels of either Hsp70 or Hsp110 protein can be compensated by higher J-protein levels, to support the suppression of Htt fibrilization activity. On the other hand, a reduction of the amount of the J-protein abrogates the suppression activity that cannot be rescued by higher levels of Hsp70 and / or Hsp110 (figure 2B). We rephrased the statements to clarify this point throughout the manuscript.

The sentence "C. elegans encodes for four HSP-70 proteins, only one HSP110 protein.....needs rewriting.

We have rephrased this sentence to: "The genome of C. elegans harbors genes encoding for four HSP-70, one HSP-110, but 25 J-proteins for expression in the cytosol alone (Table EV1)."

Less is more in the discussion, the discussion of this paper goes on and on.....I would encourage the authors to hit the key take home messages in 5 pages without too much redundancy with the results and introduction sections.

We have made a considerable effort to avoid redundancy and to shorten the discussion.

Overall, a pleasure to read, and upon revision will be a nice EMBO paper.

We thank the reviewer for the very positive feedback.

2nd Editorial Decision 18 October 2017

Thank you for submitting a revised version of your manuscript to The EMBO Journal. It has now been seen by two of the original referees and their comments are shown below.

As you will see they both find that all criticisms have been sufficiently addressed and recommend the manuscript for publication. However, before we can officially accept the manuscript there are a few editorial issues concerning text and figures that I need you to address in a final revision:

-> Please include a conflict of interest statement at the end of the manuscript

-> Please update the literature callouts in the text to fit with the EMBO Journal format (1 author + et al)

-> For Table EV1, please include a brief legend in an individual tab.

-> We noticed that the manuscript text has become very long in the revised manuscript (94000 cts not counting EV legends and references). We abandoned our 55.000 characters limit years ago and do not enforce a strict control of manuscript length, but for the sake of clarity to the reader I would encourage you to shorten/streamline the manuscript in some parts. Furthermore, I would suggest that you move some of the more detailed materials and methods (eg primer sequences, reagent information etc) to an Appendix file.

-> We noticed that the images in fig 1D and 1G are also shown in fig 2C and 4D. I realise that they are used in comparison with different things but I would still ask you to acknowledge in the legend that these are indeed the same images.

-> Please include a scale bar in figure 6A

-> Please specific the nature of the error bars (standard deviation vs standard error) for figures 5 and 6

-> We generally encourage the publication of source data, particularly for electrophoretic gels and blots, with the aim of making primary data more accessible and transparent to the reader. We would need 1 file per figure (which can be a composite of source data from several panels) in jpg, gif or PDF format, uploaded as "Source data files". The gels should be labelled with the appropriate figure/panel number, and should have molecular weight markers; further annotation would clearly be useful but is not essential. These files will be published online with the article as a supplementary "Source Data". Please let me know if you have any questions about this policy.

-> Papers published in The EMBO Journal include a 'Synopsis' to further enhance discoverability. Synopses are displayed on the html version of the paper and are freely accessible to all readers. The synopsis includes a short standfirst - written by the handling editor - as well as 2-5 one sentence bullet points that summarise the paper and are provided by the authors. I would therefore ask you to include your suggestions for bullet points.

-> In addition, I would encourage you to provide an image for the synopsis. This image should provide a rapid overview of the question addressed in the study but still needs to be kept fairly modest since the image size cannot exceed 550x400 pixels.

Thank you again for giving us the chance to consider your manuscript for The EMBO Journal, I look forward to receiving your final revision.

REFEREE REPORTS

Referee #1:

In the revised manuscript, the authors have sufficiently addressed all of my initial concerns. They have shown the disaggregation activity through an alternative assay (although not the FRET assay) and have provided additional insights into the relationship between the different components of the HSP70 chaperoning pathway in this activity. While I'm still not sure I'd refer to this as a 'complex', I understand the authors' argument for the use of this term. I think that the manuscript has been improved by the review process and is now appropriate for publication in EMBO.

Referee #2:

The authors submitted an extensively revised version of a manuscript that describes a role for cytosolic chaperone complex in suppression of Htt aggregation.

The FRET assay utilized is novel and the data on the tripartite action of an Hsc70, Hsp110 and J-protein in suppression of HTT aggregation is of broad interest.

The authors did a nice job addressing concerns expressed reviewers and no additional concerns were identified.

A suggestion is to include Hsp70 and possibly the other chaperones in the title as this will increase interest in the work from folks who study protein homeostasis.

2nd Revision - authors' response 19 October 2017

Thank you very much for your email. We have provided the missing information and I just uploaded all files and resubmitted them via the online submission platform.

-> Please include a conflict of interest statement at the end of the manuscript

We have included a statement that the authors have no conflict of interest.

-> Please update the literature callouts in the text to fit with the EMBO Journal format (1 author + et al)

We have formatted the citation style accordingly via Endnote for the EMBO format.

-> For Table EV1, please include a brief legend in an individual tab.

We have provided the missing legend

-> We noticed that the manuscript text has become very long in the revised manuscript (94000 cts not counting EV legends and references). We abandoned our 55.000 characters limit years ago and do not enforce a strict control of manuscript length, but for the sake of clarity to the reader I would encourage you to shorten/streamline the manuscript in some parts. Furthermore, I would suggest that you move some of the more detailed materials and methods (eg primer sequences, reagent information etc) to an Appendix file.

We have moved a substantial part of the Methods section to an appendix that lists the more general method information and all oligonucleotide sequences. This change considerably shortened the length of the overall manuscript.

-> We noticed that the images in fig 1D and 1G are also shown in fig 2C and 4D. I realise that they are used in comparison with different things but I would still ask you to acknowledge in the legend that these are indeed the same images.

We have added the information that these images are the same in the respective figure legends.

-> Please include a scale bar in figure 6A

We have added scale bars to figure 6A.

-> Please specific the nature of the error bars (standard deviation vs standard error) for figures 5 and 6

We have added the information that the error depicts the standard deviation.

-> We generally encourage the publication of source data, particularly for electrophoretic gels and blots, with the aim of making primary data more accessible and transparent to the reader. We would need 1 file per figure (which can be a composite of source data from several panels) in jpg, gif or PDF format, uploaded as "Source data files". The gels should be labelled with the appropriate figure/panel number, and should have molecular weight markers; further annotation would clearly be useful but is not essential. These files will be published online with the article as a supplementary "Source Data". Please let me know if you have any questions about this policy.

As we provide already a lot of the original files in the Expanded View figures (e.g. gel showing the purification of all proteins used in this study, EM controls etc) there is no need from our point of view for more source data.

-> Papers published in The EMBO Journal include a 'Synopsis' to further enhance discoverability. Synopses are displayed on the html version of the paper and are freely accessible to all readers. The synopsis includes a short standfirst - written by the handling editor - as well as 2-5 one sentence bullet points that summarise the paper and are provided by the authors. I would therefore ask you to include your suggestions for bullet points.

-> In addition, I would encourage you to provide an image for the synopsis. This image should provide a rapid overview of the question addressed in the study but still needs to be kept fairly modest since the image size cannot exceed 550x400 pixels.

We have provided a bullet point list and a cover art for the synopsis.

Thank you again for giving us the chance to consider your manuscript for The EMBO Journal, I look forward to receiving your final revision.

I thank you for giving us the opportunity to provide you with the final editorial corrections and I thank both reviewers for their time and positive feedback for the evaluation of the revision.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Janine Kirstein
Journal Submitted to: EMBO Journal
Manuscript Number: EMBOJ-2017-97212R

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	The sample size for each experiment was chosen based on common procedures for the respective method. It should ensure an unbiased analysis.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	No animal studies were carried out.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	see above
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Randomization is a common procedure and has been used in this study for e.g. selecting specimen for imaging analysis.
For animal studies, include a statement about randomization even if no randomization was used.	No animal studies were carried out.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	N/A
4.b. For animal studies, include a statement about blinding even if no blinding was done	No animal studies were carried out.
5. For every figure, are statistical tests justified as appropriate?	Standard deviation (SD) was used and indicated in the figures that display error bars.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	N/A
Is there an estimate of variation within each group of data?	N/A
Is the variance similar between the groups that are being statistically compared?	N/A

C- Reagents

USEFUL LINKS FOR COMPLETING THIS FORM

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http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

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http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Rabbit polyclonal antibodies against: HSP-1, HSP-110, C12C8.1, DNJ-12, DNJ-13, DNJ-19, reference: this study, Charles River (France); Guinea Pig polyclonal antibody against F44E5.4, reference: this study, Pineda (Berlin); Rabbit monoclonal anti-Htt antibody: source: abcam, catalog number: ab109115, clone number: EPR5526; Rabbit polyclonal anti DNAJB1 antibody: source: proteintech, catalog number: 13174-1-AP; Rabbit polyclonal anti HSPA8(Hsc70) antibody: source: proteintech, catalog number: 10654-1-AP; Mouse monoclonal anti β-Actin antibody: source: Santa Cruz Biotechnology, catalog number: sc-47778; Mouse monoclonalanti GFP antibody: source: Enzo, catalog number: ADI-SAB-500-E; Mouse monoclonal anti α-tubulin antibody: source: Sigma-Aldrich, catalog number: T5168; Mouse monoclonal anti-HA antibody: source: Sigma-Aldrich, catalog number: H3663, clone: HA-7; Goat Anti-Rabbit Cyanine3 conjugated antibody: source: ThermoFisher Scientific, catalog number: A10520; Donkey anti-Guinea pig CF555 conjugated antibody: source: Sigma-Aldrich, catalog number: SAB4600297; Goat anti mouse HRP conjugated antibody: source: Thermo Fisher scientific; catalog number: 31430; Goat anti rabbit HRP conjugated antibody: source: Thermo Fisher scientific; catalog number: 31462; Goat anti-rabbit IRDye800CW conjugated: source: Licor, catalog number: 925-32211
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	HEK293, Source: ATCC, Number:CRL-1573, and have been tested for mycoplasma in our lab (negative result); CH4 fibroblasts were obtained from the skin biopsy of a HD patient (male, age 25-38, Q length 44Q). Human embryonic stem cell (hESC) line H9 (WA09) was purchased from WiCell and employed according to the German law (license to Dr. Prigione).

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	N/A
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	N/A
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	N/A

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	N/A
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	N/A
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	N/A
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	N/A

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	No data that qualify to be deposited in a public repository have been denerated in this study.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	N/A
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	N/A
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N/A

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	N/A