

HAP40 protein levels are huntingtin-dependent and decrease in Huntington disease

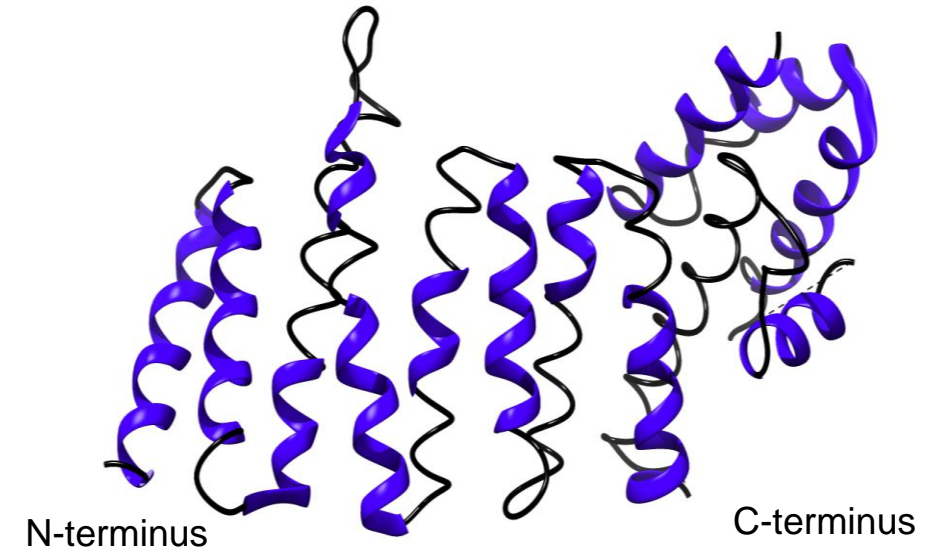
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The huntingtin-associated protein 40 (HAP40)

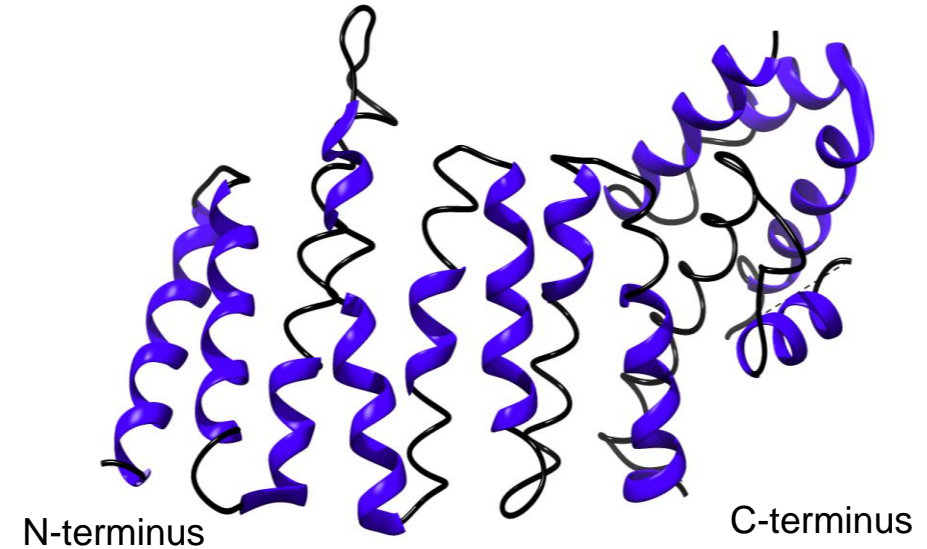
- **Most abundant interactor of HTT in human and mice**
(Shirasaki et al. 2012, Guo et al. 2018, Sap et al. 2021)
- **Tetratricopeptide-like helical domain (TPR) containing protein with centrally-located proline-rich region**
- **Both HAP40 and HTT are present in unikonts and absent in fungi → likely coevolution** (Seefelder et al. 2020)
- **Conserved interaction in fish** (Seefelder et al. 2020) **and fruit fly**
(Xu et al. 2020)
- **Unclear biological function and relevance in HD:** Interactor of Ras-related protein 5 (Pal et al. 2006, 2008)



Cryo-EM structure of HAP40. PDB file was retrieved from RCS protein data bank (Identifier 6EZ8.B, Guo et al. 2018). Visualization and image rendering were performed with Chimera and POV-ray respectively.

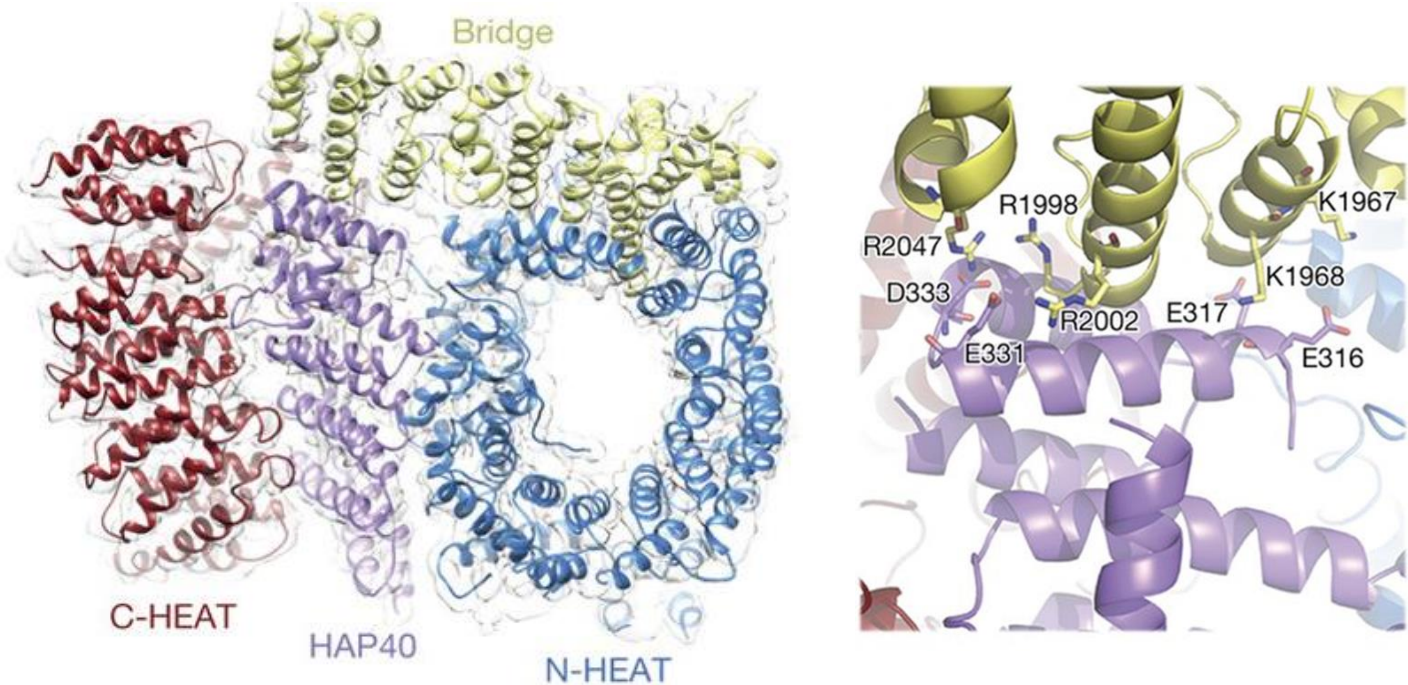
The huntingtin-associated protein 40 (HAP40)

- **Altered protein levels in HD patients?**
 - Elevated levels in murine brain tissue, human lymphocytes and fibroblasts (Pal et al. 2006)
 - Reduced levels in synaptosomes of HdH140Q/140Q mice (Valencia et al. 2013)

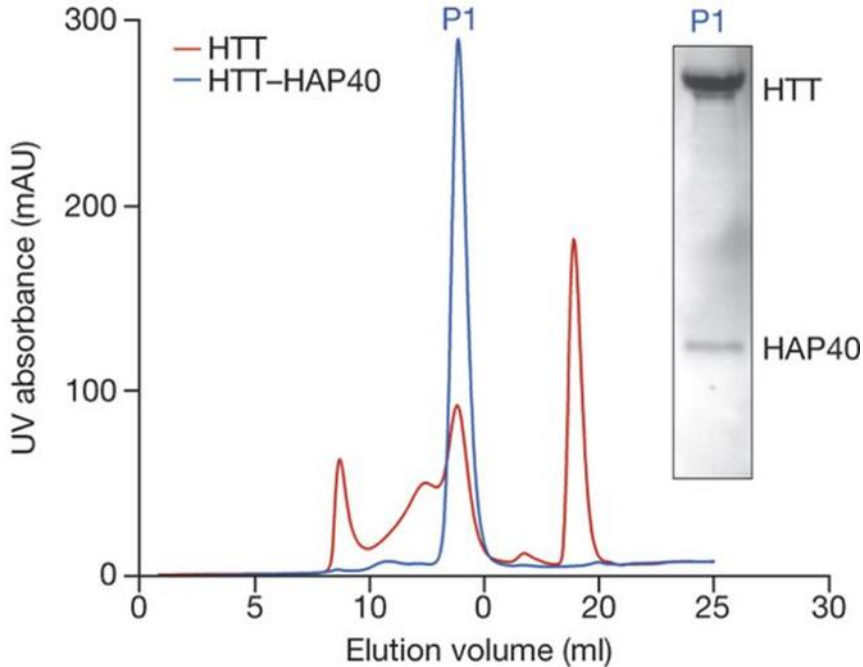


Cryo-EM structure of HAP40. PDB file was retrieved from RCS protein data bank (Identifier 6EZ8.B, Guo et al. 2018). Visualization and image rendering were performed with Chimera and POV-ray respectively.

HTT-HAP40 interaction involves all three HTT domains and shields HAP40

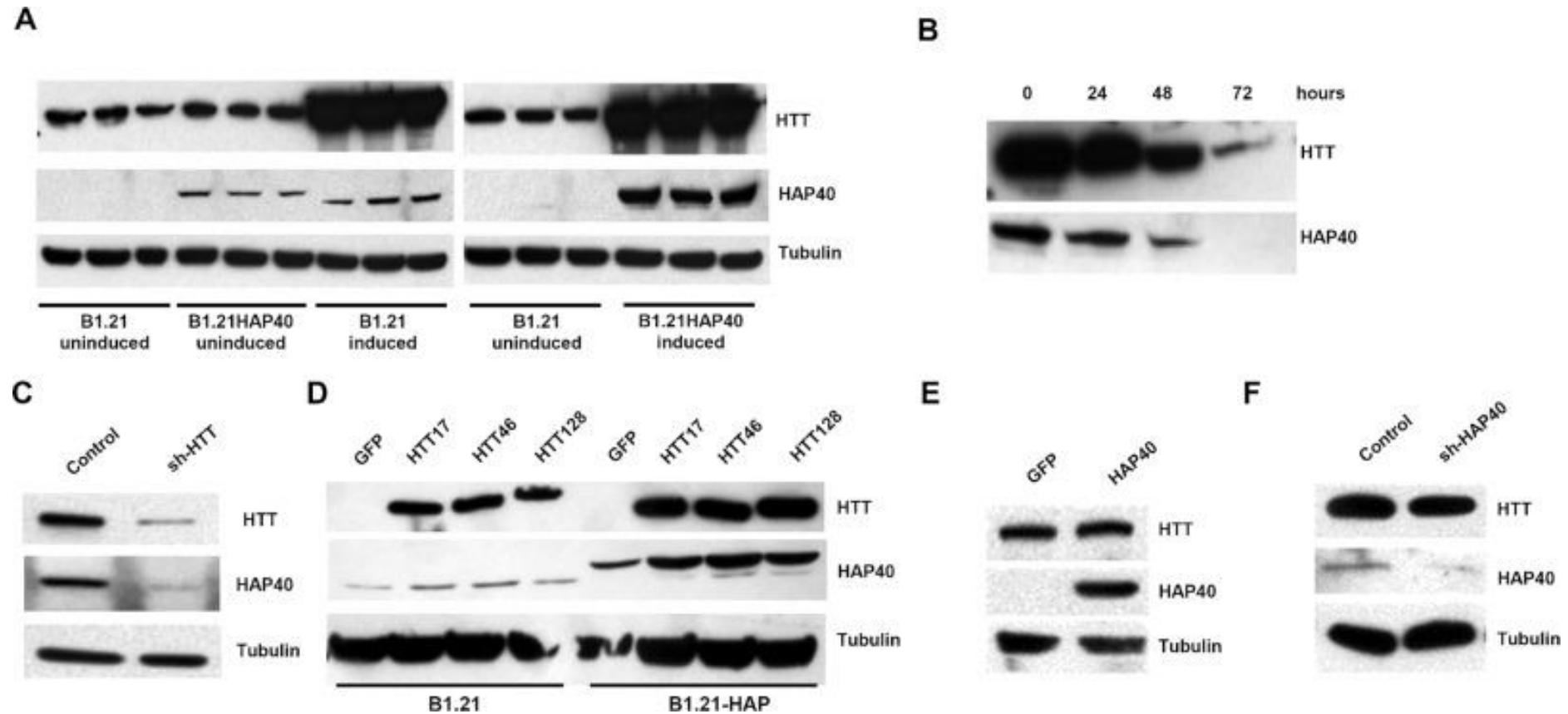


Unusual and extended interaction between HAP40 and HTT.
Figure taken from Guo et al. 2018.



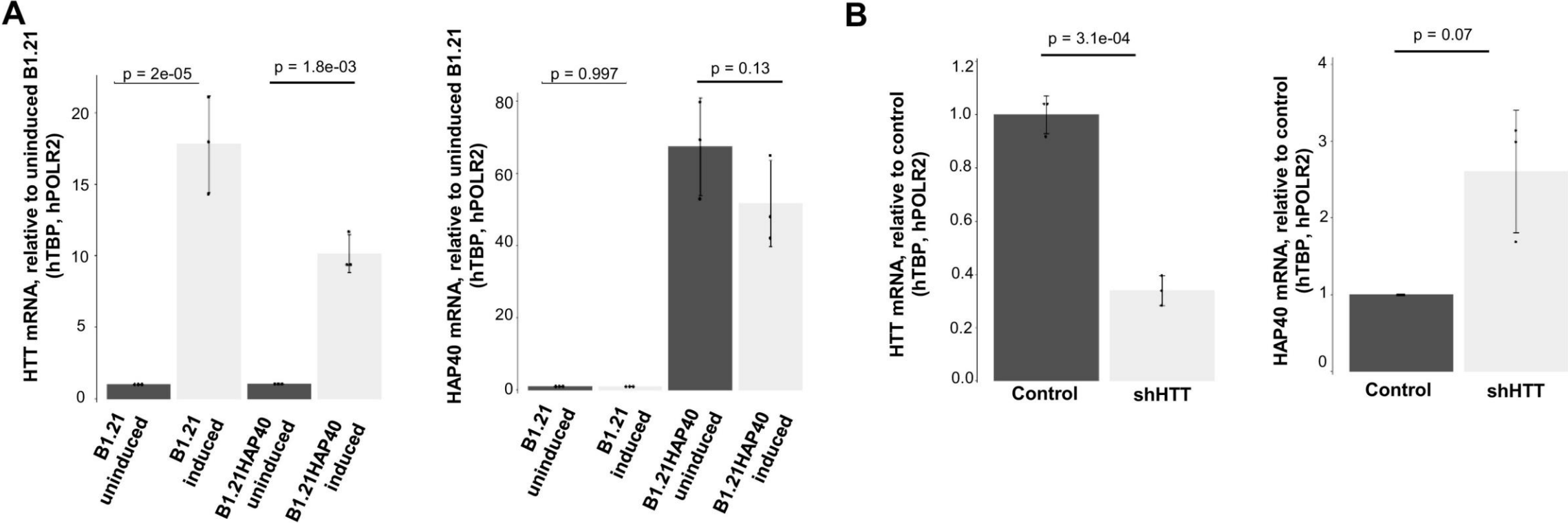
Interaction between HAP40 and HTT reduces HTT's inherent flexibility. Figure taken from Guo et al. 2018.

HTT expression affected cellular HAP40 protein levels



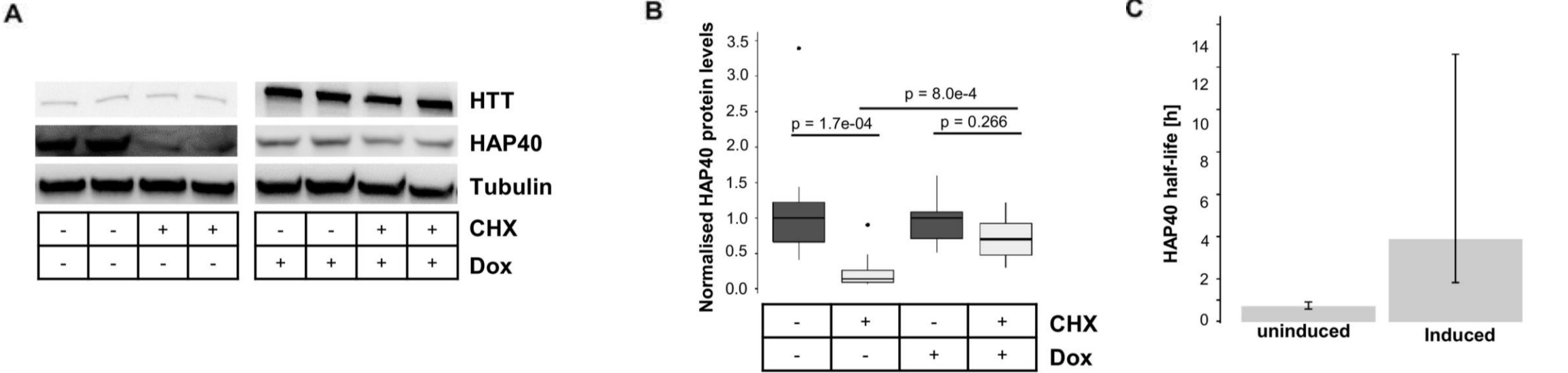
A HTT and HAP40 levels in B1.21 and B1.21-HAP40 cells after induction of HTT expression with doxycycline (Dox) **B** Time-lapse study of HTT and HAP40 in B1.21-HAP40 cells after removal of Dox. **C** HTT and HAP40 protein levels after stable shRNA-mediated HTT knock-down. **D** HTT and HAP40 levels following transfection of expression plasmids coding for EGFP (control) or HTT with different polyQ lengths, as indicated. **E** HTT and HAP40 levels after HAP40 overexpression. **F** HTT and HAP40 levels after shRNA-mediated HAP40 knock-down.

HTT expression did not affect HAP40 / F8A mRNA levels



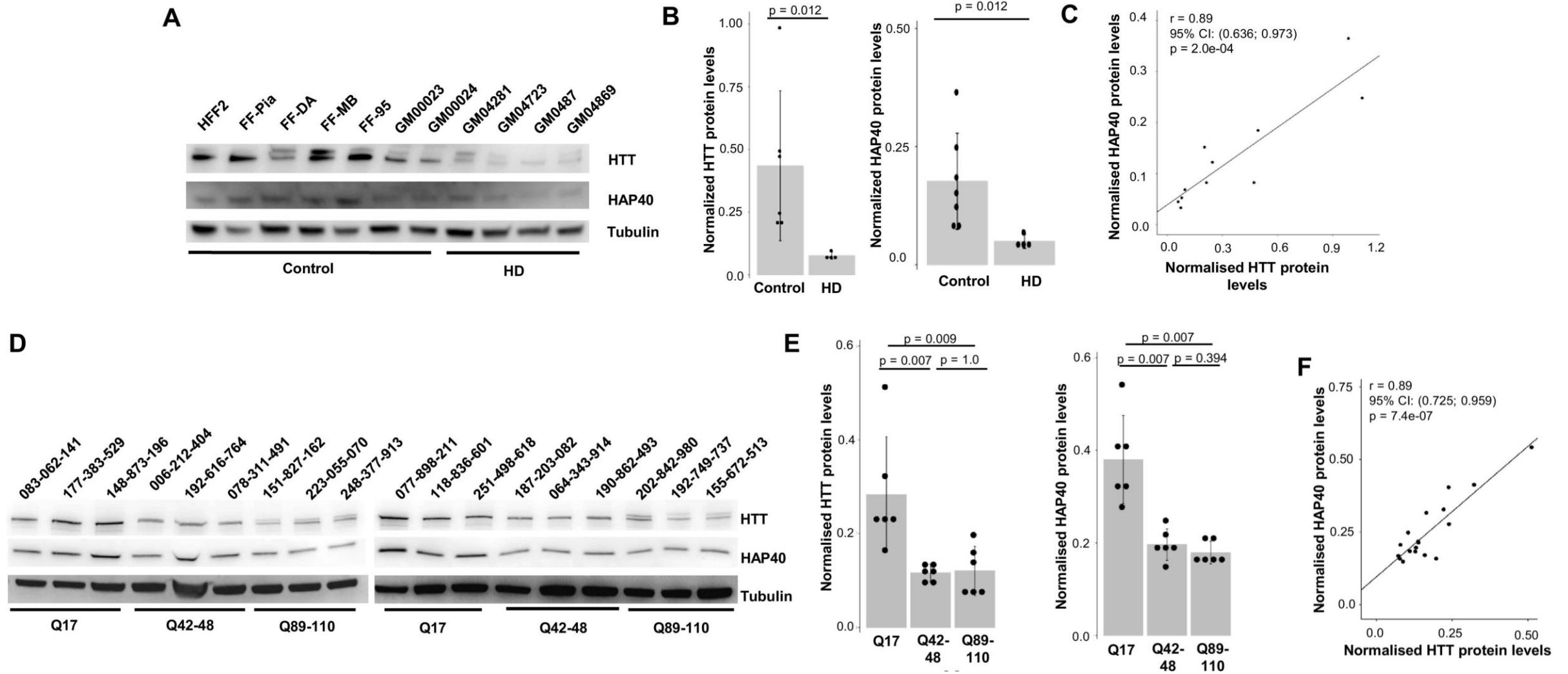
A RT-qPCR analysis of mRNA levels of HTT and HAP40 in B1.21 cells and B1.21-HAP40 cells in the presence or absence of HTT induction with Dox. **B** RT-qPCR analysis of mRNA levels of HTT and HAP40 in sh-HTT cells and their respective control cells.

HTT overexpression increased the intracellular stability of HAP40



A Stability of HAP40 in uninduced and induced B1.21-HAP40 cells as determined with the cycloheximide chase assay. **B** Quantification of the relative HAP40 levels from Western-blot analysis in the cycloheximide chase assay (n = 12). **C** HAP40 half-life in uninduced and induced B1.21-HAP40 cells. Half-life was estimated based on an exponential fit using the median HAP40 protein levels (n = 12).

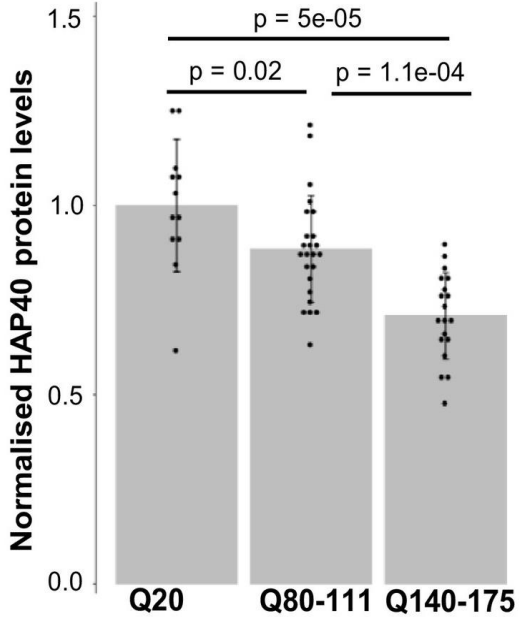
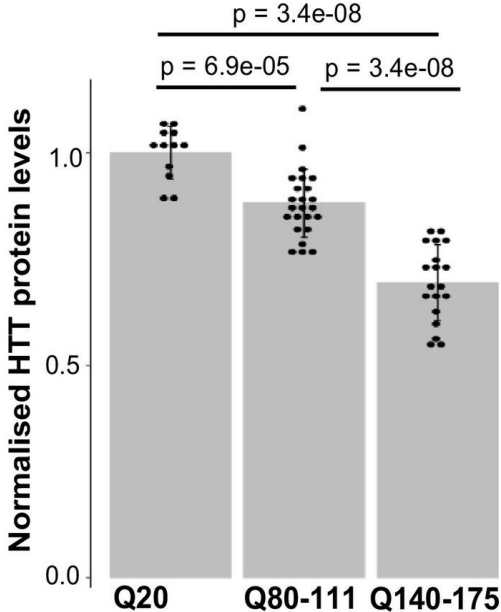
HAP40 and HTT levels were reduced in fibroblasts and lymphoblasts of HD patients



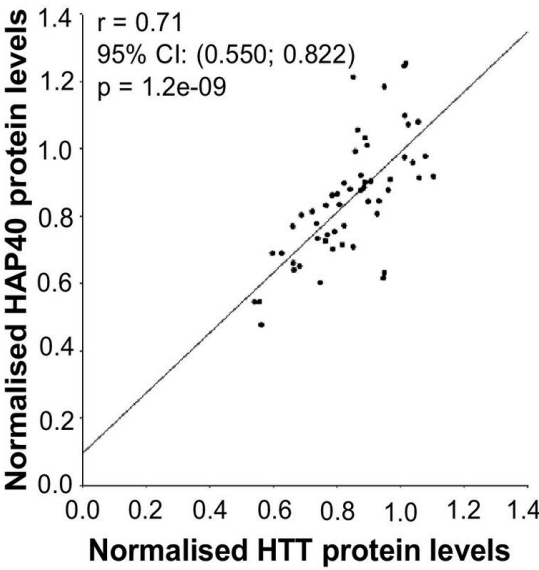
A-C HTT and HAP40 levels in fibroblasts from HD patients (n = 4) and healthy individuals (n = 7). **D-F** HTT (MAB2166) and HAP40 in lymphoblastoid cells from healthy donors (Q17, n = 6) or HD patients with middle (Q42–48, n = 6) and long polyQ expansions (Q89–110, n = 6).

HAP40 and HTT levels were reduced the striatum of HD knock-in mice

G



H



G Normalised HTT and HAP40 levels in the striatum of control mice (Q20, n = 12), and asymptomatic (Q80–111, n = 24) or symptomatic (Q140–175, n = 19) HD mice using mass spectrometry data that were retrieved from the HD Proteome base and normalised to the levels in control mice expressing Q20-HTT (Evotec AG; Langfelder et al., 2016). **H** Pearson's product-moment correlation between HTT and HAP40 levels in the striatum of mice.

Conclusion and open research questions

- **HAP40 protein levels strongly correlate with HTT levels:** potential application of HAP40 as HTT surrogate biomarker to measure reduced HTT levels
- **The HAP40 protein is stabilized upon HTT overexpression:**
 - Does HAP40 also stabilize HTT?
 - Does the interaction between HAP40 and HTT affects aggregation of mHTT?
- **HAP40 and HTT protein levels are reduced in Huntington disease patients and mice:**
 - Does a HAP40 loss-of-function contribute to the pathophysiology of HD?
 - What is the function of physiological function the HAP40-HTT interaction?

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