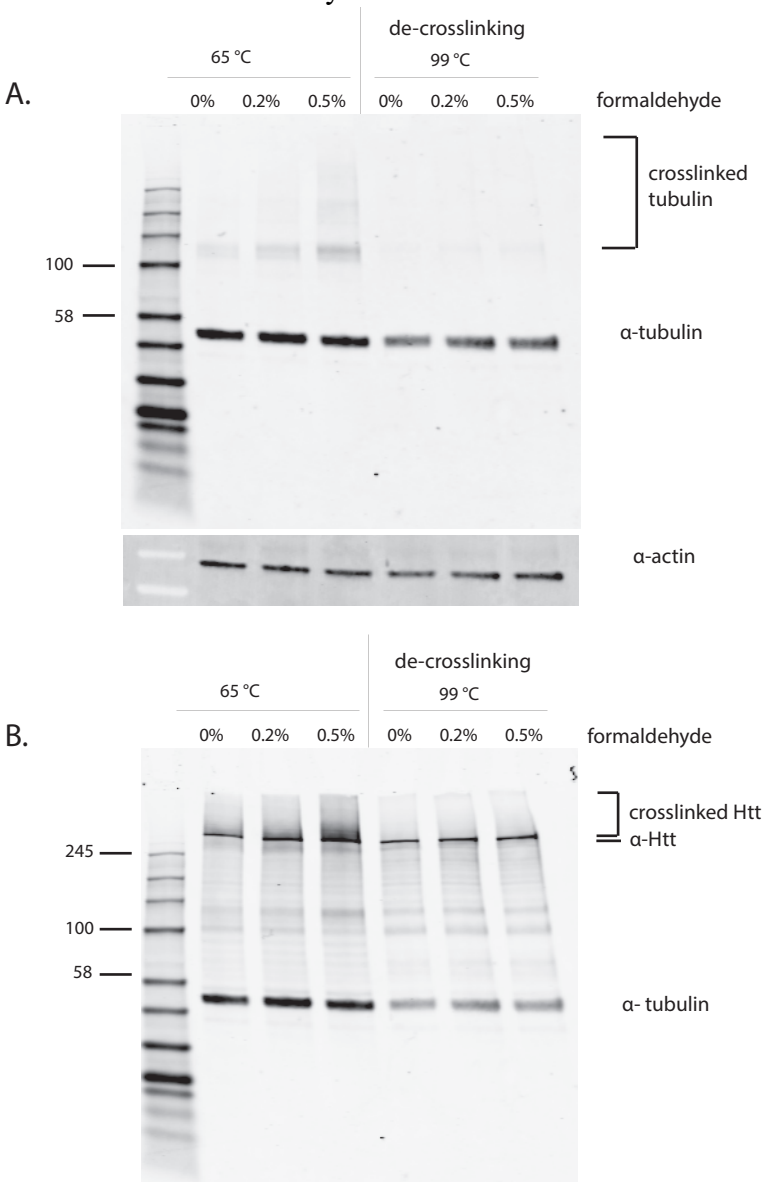
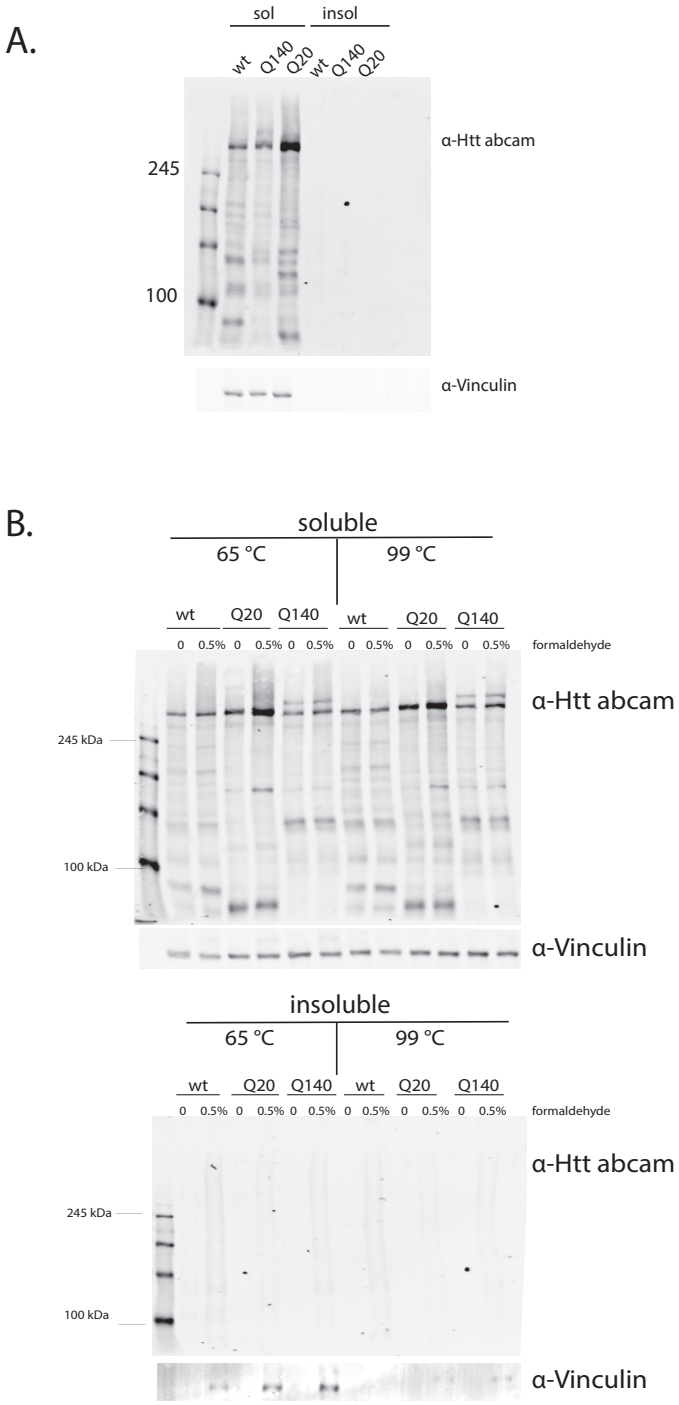
Supplementary Material

Identification of Full-Length Wild-Type and Mutant Huntingtin Interacting Proteins by Crosslinking Immunoprecipitation in Mice Brain Cortex

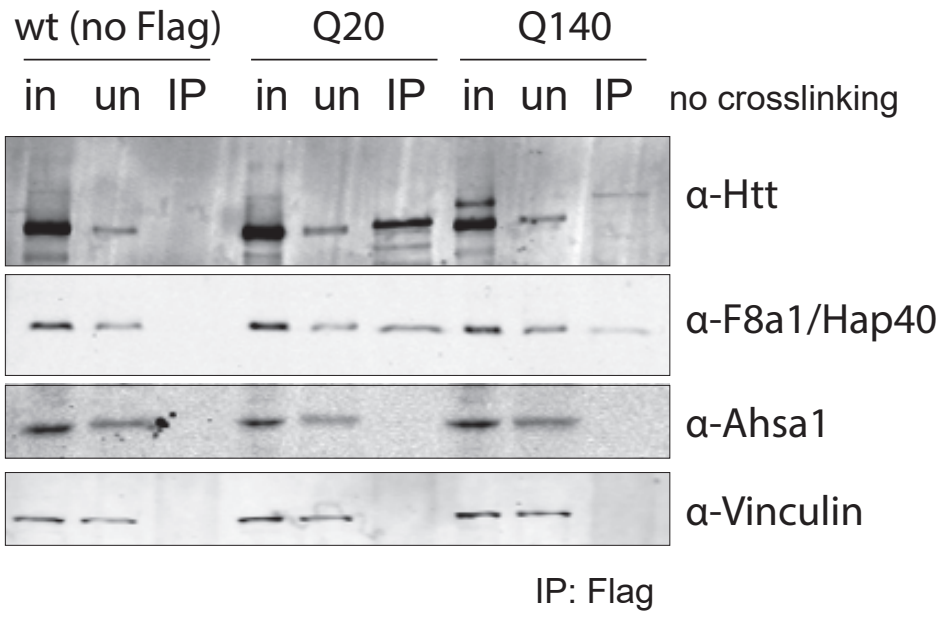
Supplementary Figure 1. Optimalization formaldehyde crosslinking in mice brain tissue. SDS-PAGE WB of wtHtt mice brain tissue treated with 0%, 0.2% or 0.5% formaldehyde for 10 min. Half of the samples were boiled in sample loading buffer for 5 min at 65°C for 5 min. Formaldehyde crosslinks remain intact under these conditions. Other half of the samples were boiled in sample loading buffer for 20 min at 99°C. Formaldehyde crosslinks were reversed under these conditions. A. SDS-PAGE WB for tubulin, a protein that is often used to study protein crosslinking. B. SDS-PAGE WB for Htt, our protein of interest. The membrane used for tubulin staining was re-incubated with α-Htt antibody.



Supplementary Figure 2. Levels of Htt protein in Triton X-100 soluble and insoluble fraction. A) SDS-PAGE WB of Htt in the Triton X-100 soluble and insoluble fraction of mice brain lysates. The Triton X-100 pellet was dissolved by 40 min incubation in 100% formic acid at 37°C, dried by speed vac and dissolved in 8M urea-based lysis buffer, as described previously [33]. A. Soluble and insoluble fraction of mice cortex brain lysates. B) Soluble and insoluble fractions of formaldehyde crosslinked (65°C) and de-crosslinked (99°C) mice cortex brain lysates.



Supplementary Figure 3. IP of Flag-tagged Htt without formaldehyde crosslinking. SDS-PAGE WB of input, unbound fraction and IP eluates of IP directed against Flag-tagged wtHtt and mHtt in mice cortex lysates. Non-Flag-tagged wtHtt mice were used as a negative control. Western blot staining performed for Htt, Hap40, Ahsa1 and Vinculin (negative control).



The supplementary tables have been uploaded to GitHub:
https://github.com/ReitsGroup/FlagIP_JHD

Supplementary Table 1. Quantitative data before and after imputation.
Tab 1 'before_imputation': This table shows the list of all the proteins identified and their log2 transformed LFQ intensity values. The reverse decoys, potential contaminants, proteins only identified by one peptide, and proteins identified by only PTM are excluded.

Tab 2 'after_imputation': This is the table used for comparisons. The missing LFQ intensity values denoted by NAs in the before_imputation table are imputed by taking a random value from the 1st quartile of the whole distribution.

Supplementary Table 2. Quantitative comparison of Q20 and Q140 with negative control.
Tab 1 'Q20_vs_control': Proteins that had non-NA LFQ intensities in at least 3 out of the 4 Q20 replicates in before_imputation table were compared with the control using two-sided, unpaired t-test. A p-value cut-off of <0.05 and a log2 fold change cut-off of >1 were used to select the significantly abundant proteins in Q20. The statistical comparison was done on the imputed values given in the after_imputation table, for the selected proteins.

Tab 2 'Q140_vs_control': Proteins that had non-NA LFQ intensities in at least 3 out of the 4 Q140 replicates in before_imputation table were compared with the control using two-sided, unpaired t-test. A p-value cut-off of <0.05 and a log2 fold change cut-off of >1 were used to select the significantly abundant proteins in Q140. The statistical comparison was done on the imputed values given in the after_imputation table, for the selected proteins.

Supplementary Table 3. Enriched biological processes in Q20 and Q140 mice brain cortex.
The biological processes that are enriched for Q20 and Q140 are given in separate tabs. Each table contains the names of the biological processes, associated p-values, enrichment scores and the genes names that are associated with each biological process. GOrilla calculates an enrichment score E obtained from (number of genes in the intersection (b) / number of genes in the target set (n)) / (total numbers of genes associated with a GO term (B) / total number of genes (N)).