Site-Specific Ubiquitination of Pathogenic Huntingtin Attenuates its Deleterious Effects

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Supplementary Information:

- Supplementary Materials and Methods
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Supplementary Information.

Materials and methods:

Cell lines and culture conditions: HEK293 cells were grown at 37^0C and 5% CO_2 in DMEM (Biological Industries, Bet HaEmek, Israel) supplemented with 10% FBS (Gibco), sodium pyruvate (1 mM; Biological Industries), L-glutamine (2 mM; Biological Industries), and penicillin/streptomycin ((100 U/ml)/(0.1 mg/ml); Biological Industries). SH-SY5Y cells were grown at 37^0C and 5% CO_2 in a 1:1 mixture of ATCC-formulated EMEM and F12 Nutrient Mixture, supplemented with 20% FBS, sodium pyruvate (1 mM), L-glutamine (2 mM), and penicillin/streptomycin ((100 U/ml)/(0.1 mg/ml).

Ethics: All experiments were approved by the local ethics committee at Regierungspraesidium Tuebingen (approval ID: HG11/12), and by the "Technion-Israel Institute of Technology Committee for the Supervision of Animal Experiments" (ethics approval number IL-151-00-32). All animal-related experiments were carried out in accordance with the German Animal Welfare Act and the guidelines of the Federation of European Laboratory Animal Science Associations, based on European Union legislation (Directive 2010/63/EU).

DNA constructs: The DNA constructs used to express Htt17Q:EGFP and Htt134Q:EGFP were generated as follows: Large scale gene synthesis was used to synthesize the first exon of the *HTT* gene containing either 17 or 134 CAG repeats, fused to EGFP and flanked by AgeI (5') and BsrGI (3'). This segment was inserted into FUGWm upstream to EGFP, using the AgeI and BsrGI sites, to create FU-Htt17Q:EGFP-Wm (encoded protein is designated as 'Htt17Q') and FU-Htt134Q:EGFP-Wm (encoded protein is designated as 'Htt134Q'). All cloning and gene syntheses were carried out by GenScript (Piscataway NJ, USA). Lys 6 and Lys 9 in FU-Htt134Q:EGFP-Wm were replaced by Arg residues to create FU-Htt134Qm:EGFP-Wm (m stands for mutated; see *Site-directed mutagenesis* below; encoded protein is designated as 'Htt134Qm'). All DNA constructs were introduced into neurons using 3rd generation a Lentiviral expression vector based on the FUGWm backbone (43), and into cultured cell lines using standard transfection reagents (see under *Lentivirus production and transduction* and *Cell transfection* below).

Site directed mutagenesis: FU-Htt134Q:EGFP-Wm was used as template for site directed mutagenesis to substitute Lys residues 6 and 9 with Arg. Mutagenesis was performed using the appropriate oligonucleotides and the QuickChange II XL-Site directed mutagenesis kit (Agilent Technologies) according to the manufacturer's instructions.

Lentivirus production and transduction: Lentiviral particles were produced by transfecting HEK293T cells with a mixture of three packaging plasmids: pLP1, pLP2, and pLP\VSVG (packaging vector mix of the ViraPower four-plasmid Lentiviral expression system; Invitrogen), and the appropriate expression vector (either FU-Htt17Q:EGFP-Wm, FU-Htt134Q:EGFP-Wm, or FU-Htt134Qm:EGFP-Wm). HEK293T cell transfection was performed using Lipofectamine 2000 (Invitrogen) in 10 cm plates in cells that had reached 80% confluence. Supernatant was collected after 48 hours, passed through 0.45 μm filters, aliquoted, and stored at -80°C. Transduction of cortical neuronal cultures was performed at 14 days *in vitro* (DIV), by adding different amounts of the filtered supernatant to each dish as determined real time PCR analysis (Fig S6A).

Cell transfection: Cultured cell lines were transfected with either FU-Htt17Q:EGFP-Wm, FU-Htt134Q:EGFP-Wm, or FU-Htt134Qm:EGFP-Wm. HEK293 cells were transfected using CalFectin transfection reagent (Bio Consult), and SH-SY5Y cells were transfected using Lipofectamine 3000 (ThermoFisher). Transfections were carried out with equal amounts of DNA (which resulted in similar expression levels; Fig. S6B), according to the manufacturers' instructions.

Real Time PCR: (i) RNA extraction - cortical neurons were plated on a 6 well plate ($2x10^6$ cells per well), and kept to develop for 14 days. At DIV 14, an equal amount of FU-Htt134Q:EGFP-Wm or FU-Htt134Qm:EGFP-Wm viral particles was added to each well for periods of 24 and 48 hours. RNA was extracted from each well by using NucleoSpin® RNA kit (Macherey-Nagel). (ii) cDNA preparation – 1 μg RNA was used to prepare 1 μg of cDNA by using RevertAID H minus First Strand cDNA Synthesis Kit (ThermoFisher) according to the manufacturer's instructions with random hexamer as primers. (iii) RT PCR - TaqMan® fast universal PCR master mix (Applied Biosystems) was used. Each reaction was carried out in triplicates using Taqman primers for EGFP gene (Applied Biosystems). HPRT gene was used as a control (Applied Biosystems). StepOnePlus™ Real Time PCR System apparatus (Applied Biosystems) was used with a comparative C_T ($\Delta\Delta C_T$) platform.

40 cycles of amplification were used. The results were analyzed using StepOne™ software v2.3.

Proteomic profile of HEK293 cells overexpressing Htt proteins: HEK293 cells grown on 10 cm plates were transfected (in triplicates) with 3 µg cDNA coding for either FU-Htt17Q:EGFP-Wm, FU-Htt134Q:EGFP-Wm or FU-Htt134Qm:EGFP-Wm. Four days after transfection, cells were counted and $20x10^6$ cells from each triplicate were pelleted by centrifugation (300 x g, 5 min, 4^oC), washed twice with PBS, and the pellet was frozen by liquid N_2 and stored at -80^0C. The cellular proteomes were analyzed in two different methods: (i) Sequential lysis – from each pellet, soluble and insoluble fractions were prepared. (a) Soluble material extraction – cell pellet was incubated for 30 min at 4^oC in lysis buffer (50 mM Tris-HCl pH 8.8, 100 mM NaCl, 5 mM MgCl2, 0.5% (w/v) Nonident P-40 (NP-40), 1 mM EDTA, and 1:100 protease inhibitor cocktail; Roche). Soluble material was removed by centrifugation for 5 min at 14,000 RPM at 4^oC. (b) Insoluble material extraction - the pellet containing the insoluble material was resuspended in 300 µl DNase I buffer (20 mM Tris-HCl, pH 8.0, 15 mM MgCl2, and DNase I (Boehringer Mannheim) at a final concentration of 0.5 mg/ml), and incubated for 1 hour at 37^oC. After DNA digestion, the samples were centrifuged for 5 min at 13,300 RPM at room temperature. The supernatant was removed, and the remaining pellet was resuspended in a buffer containing 100 mM Tris-HCl pH 7.9 and 10% SDS, followed by boiling at 95^oC for 5 min, and labeled as '10% SDS fraction'. Under these conditions, the entire pellet was solubilized. (ii) Gel analysis – each pellet was resuspended in a lysis buffer containing 100 mM Tris-HCl pH 7.9 and 10% SDS, followed by one cycle of sonication (using a probe) for 30 seconds, and boiling at 95^oC for 10 min (until the entire pellet was solubilized). Protein concentration was determined by the BCA protein assay (ThermoFisher), and 50 µg protein were resolved by SDS-PAGE (10%). Insoluble material was extracted from the stacking gel and the soluble material was extracted from the resolving gel.

Proteolysis and MS analysis of soluble and insoluble fractions: The proteins from the different fractions were precipitated in 80% acetone, washed, and the protein pellets were resuspended in 9 M Urea, 400 mM ABC and 10 mM DTT, with a cycle of sonication. Proteins quantities were determined by a Bradford Test. 20 µg protein from each sample were reduced with 3 mM DTT (60^oC for 30 min),

modified with 12 mM IAA in 400 mM ABC (in the dark, room temperature for 30 min) and digested in 1 M Urea, and 50 mM ABC with modified trypsin (Promega) at a 1:50 enzyme-to-substrate ratio (overnight at 37ºC). An additional second trypsinization was carried out for 4 hours. The tryptic peptides were desalted using C18 tips (Top tip, Glygen), dried and re-suspended in 0.1% Formic acid. 1 µg peptides from each fraction were resolved by reverse-phase chromatography on 0.075 X 180 mm fused silica capillaries packed with Reprosil reversed phase material. The peptides were eluted with linear 60 minutes gradient of 5 to 28%, 15 minutes gradient of 28 to 95%, and 25 minutes at 95% acetonitrile, with 0.1% formic acid in water at flow rates of 0.15 µl/min. MS was performed by Q Exactive HFX mass spectrometer (ThermoFisher) in a positive mode using repetitively full MS scan followed by collision-induced dissociation (HCD) of the 18 most dominant ions selected from the first MS scan. The MS data from three biological repeats was analyzed using the MaxQuant software 1.5.2.8 (Mathias Mann's group) vs. the human proteome from the Uniprot database with 1% FDR. The data were quantified by label free analysis using the same software. Statistical analyses of the identification and quantization results were done using Perseus 1.6.10.43 software (Mathias Mann's group). Stringent criteria were used for the identification of proteins with increased or decreased abundance: (i) The minimum acceptable change was a factor of ~1.75. (ii) P≤0.05, T-test assuming unequal variance, for comparisons between cells expressing Htt134Q, Htt134Qm or Htt17Q.

Proteolysis and MS analysis of gel-extracted proteins: Following SDS-PAGE, proteins from the stacking gel, including the well, were reduced with 3 mM DTT (60ºC for 30 min), modified with 10 mM IAA in 100 mM ABC (in the dark, room temperature for 30 min) and digested in 10% acetonitrile and 10 mM ABC with modified trypsin (Promega) at a 1:10 enzyme-to-substrate ratio (overnight at 37ºC). The resulting peptides were desalted using C18 tips (Homemade stage tips), dried and re-suspended in 0.1% Formic acid. The resolving part of the gel was sliced to 2 (in the middle), and the proteins were trypsinized as described for the stacking gel. MS and data analyses were carried out done similar to the methodology that is described above.

Imaging data analysis: All imaging data analyses were performed using custom written software ('OpenView') which includes features for automated/manual tracking of individual aggregates and measurements of fluorescent intensities over

time (described in detail in (41)) (i) Aggregates appearance calculation - aggregates appearance was measured by the time the first 5 aggregates were detected for each Lentiviral vector; (ii) Measurement of aggregates intensities - areas were placed programmatically at each time step using identical parameters on the centers of fluorescent aggregates, and mean pixel intensities within these areas were obtained from maximal intensity projections of Z section stacks. (iii) Aggregate formation rates - individual aggregates were identified and tracked semi-automatically. Areas were placed initially over all aggregates and then a smaller subset was tracked backward in time.

Western blot analyses: (i) Htt134Q:EGFP and Htt134Qm:EGFP expression level - HEK293 cells were grown and lysed as described under *Proteomic profile of HEK293 cells overexpressing Htt proteins* (in the sub-section *gel analysis*). Protein concentration was determined by the BCA protein assay (ThermoFisher), and 35 µg protein were resolved by SDS-PAGE (10%) and transferred to nitrocellulose membrane (0.2 µm; Bio-Rad). (ii) Dot blot - HEK293 cells were grown and lysed as described under *Proteomic profile of HEK293 cells overexpressing Htt proteins* (in the sub-section *sequential lysis*). Aliquots corresponding to 2.5%, 5%, and 10% of the 10% SDS fraction were diluted into 200 µl 10% SDS in PBS. The samples were then filtered on a BRL dot-blot filtration device through a cellulose acetate membrane (Schleicher and Schuell, Keene, NH, USA, 0.2 µm pore size) that was pre-equilibrated with TBS (20 mM Tris-Hcl, pH 7.6, 150 mM NaCl) and 2% SDS. The membrane was then washed twice with 200 µl TBS and 0.1% SDS. All membranes were blocked for 1 hour in TBST (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.1% Tween20) with 5% non-fat dried milk. Staining was performed using anti-Htt (H7540, Sigma, 1:1000) and anti-Vinculin (V9131, Sigma, 1:1000) or anti-EGFP (2955S, Cell signaling, 1:1000) and anti-tubulin (T9026, Millipore, 1:10,000) as primary antibodies, and peroxidase-conjugated anti rabbit or mouse (as needed) (ImmunoResearch Laboratories, 1:10,000) as a secondary antibody. Enhanced chemiluminescence (ECL) (Pierce) was used for immune detection.

Calculation of aggregates numbers per cell: HEK293 cells were transfected with FU-Htt134Q:EGFP-Wm or FU-Htt134Qm:EGFP-Wm. One day following transfection, cells were counted by a Vi Cell XR cell counter (Beckman Coulter), and 7,500 cells were seeded in wells of a 96 well plate (µClear, Black, Greiner). Three days later, cells were supplemented with NucBlue® Live ReadyProbes

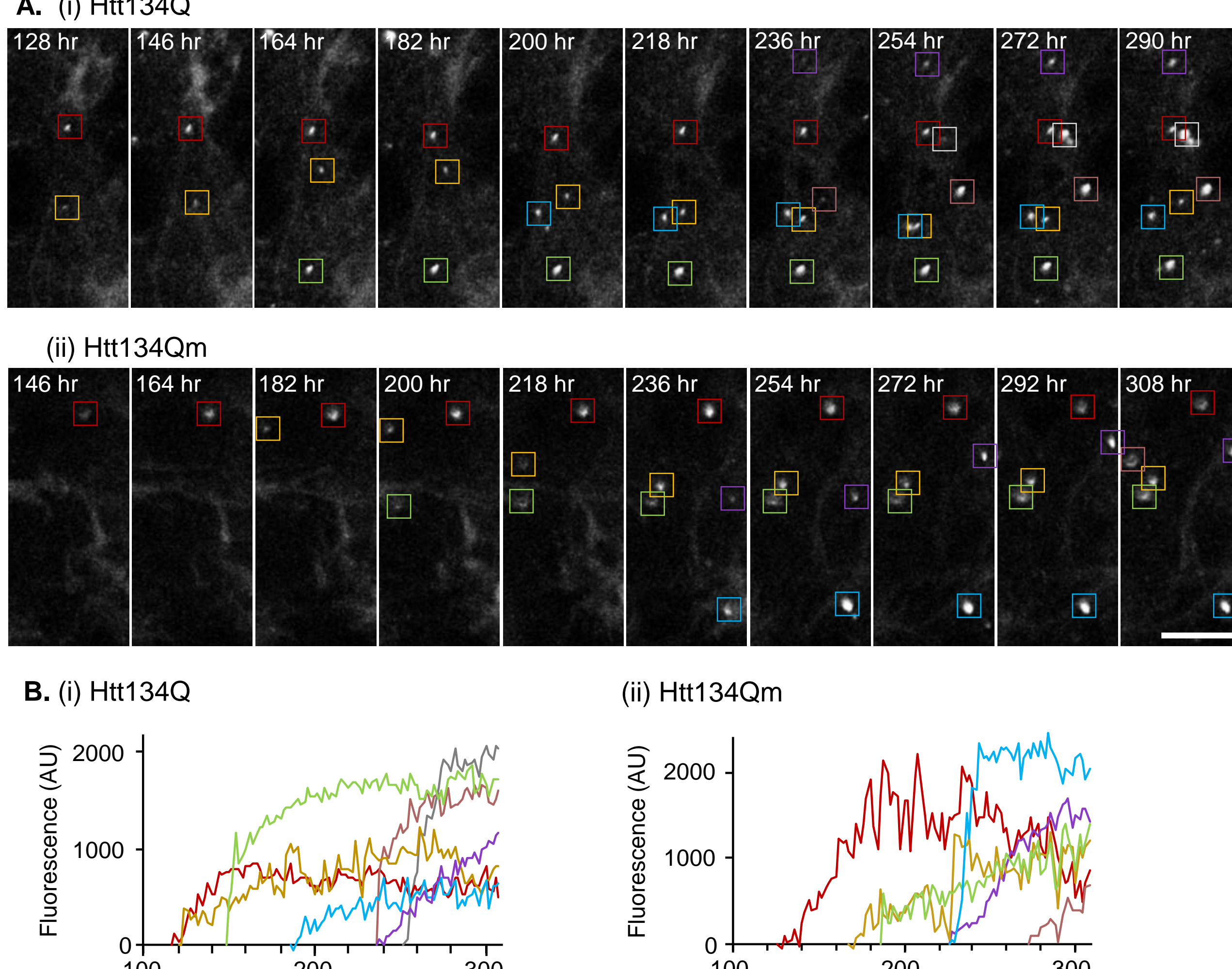
Reagent according to the manufacture's protocol (ThermoFisher) and scanned by ImageXpress® Micro Confocal system (Molecular Devices). Aggregates number per cell was determined by the MetaXpress software (Molecular Devices).

Cell viability assays: (i) Trypan Blue dye-exclusion method: HEK293 cells were transfected with either FU-Htt134Q:EGFP-Wm, or FU-Htt134Qm:EGFP-Wm. One day following transfection, 2.5 x 10^6 cells were seeded on three 10 cm plates. Cells from each plate were collected together with the growth medium at days 3-5 following transfection. Cell counter was used to quantify live cells, based on the trypan blue-exclusion method. (ii) Propidium iodide (PI) incorporation: HEK293 and SH-SY5Y cells were transfected with either FU-Htt17Q:EGFP-Wm, FU-Htt134Q:EGFP-Wm, or FU-Htt134Qm:EGFP-Wm (non-transfected cells - 'NT' - were also grown and used as a control). One day following transfection, cells were counted and 7,500 cells were seeded in wells of a 96 well plate (µClear, Black, Greiner). The percentage of dead cells was evaluated three days later using the ReadyProbes® Cell Viability Imaging Kit (Blue/Red) (ThermoFisher) according to the manufacturer's protocol. In short, 2 drops each of NucBlue® reagent and PI/1ml of cell growth medium were added. Cells were then scanned by ImageXpress® Micro Confocal system (Molecular Devices), and cell viability was determined by counting total (marked by NucBlue®) and dead (marked by PI) cells as measured by the MetaXpress software (Molecular Devices).

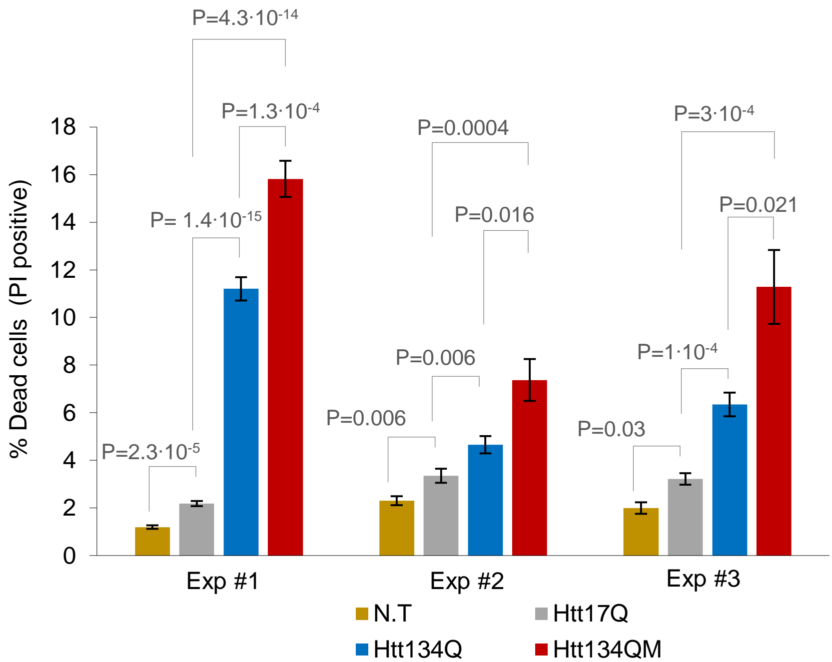
Cell cycle analysis: HEK293 cells were transfected with either FU-Htt17Q:EGFP-Wm, FU-Htt134Q:EGFP-Wm, or FU-Htt134Qm:EGFP-Wm. Two and four days after transfection, cells were counted and 2x10^6 cells were pelleted by centrifugation (300 x g, 5 min, 4^0C), washed once with PBS, and the pellet was resuspended in 1 ml cold PBS. The cells' suspension was added slowly into 4 ml cold ethanol (100%), vortexed thoroughly, and stored at -20^0C for at least 15 min. Ethanol was removed by centrifugation (300 x g, 5 min, 4^0C). Cells were resuspended in 1 ml PBS, and left to rehydrate in room temperature for 15 min. The cells were then incubated for 30 min in 37^0C with 10 mg/ml RNase A (Macherey-Nagel), followed by centrifugation (300 x g, 5 min, 4^0C) and resuspension in 1 ml staining buffer (100 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, and 0.1% NP-40). Extracted cells' suspension was transferred to FACS tubes with cell strainer snap cap mesh. PI was added (at a final concentration of 0.05 mg/ml; Sigma) to each sample immediately before

FACS analysis, that was performed using High Throughput LSR Fortessa. Data were analyzed by the FCS Express 5 software.

Hakim et al., Figure S1

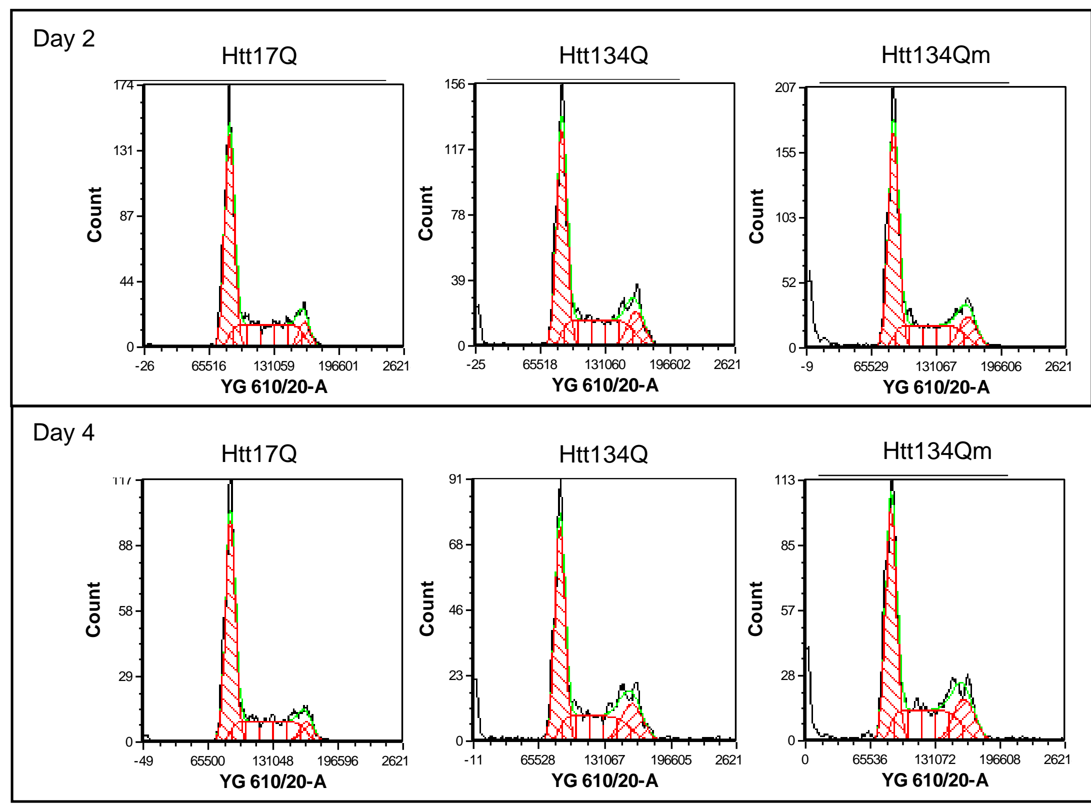


Hakim et al., Figure S2

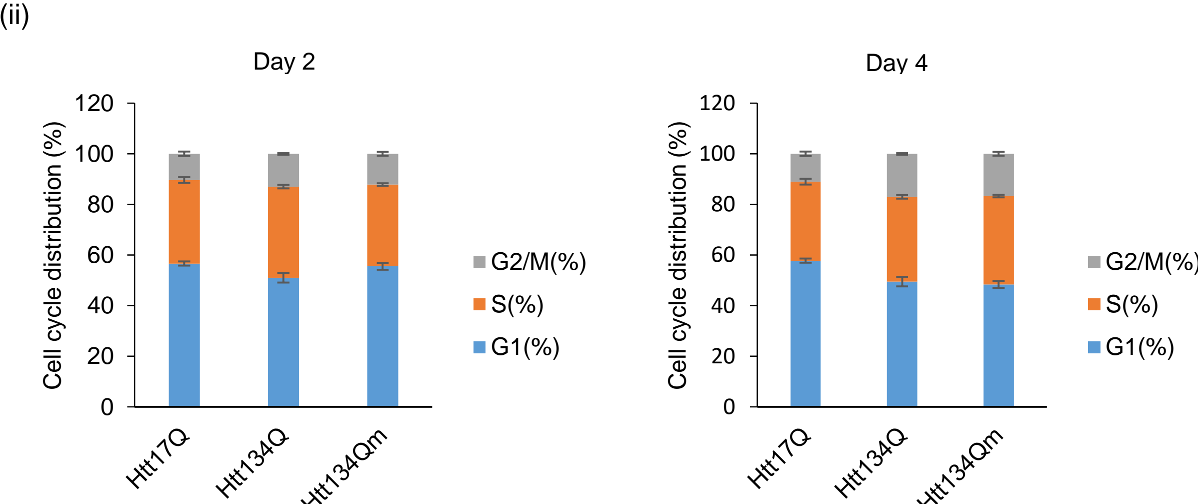


Hakim et al., Figure S3

A. (i)



(ii)



Hakim et al., Figure S4

HUNTINGTON DISEASE

Gene Names
- AP2A1
- CASP3
- NDUFA4
- NDUFA8
- NDUFA9
- NDUFB7
- NDUFV2
- POLR2F
- POLR2G
- POLR2H
- POLR2J
- RCOR1
- SDHC
- SP1
- TRAF2
- UQCRFS1

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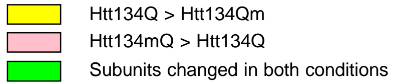
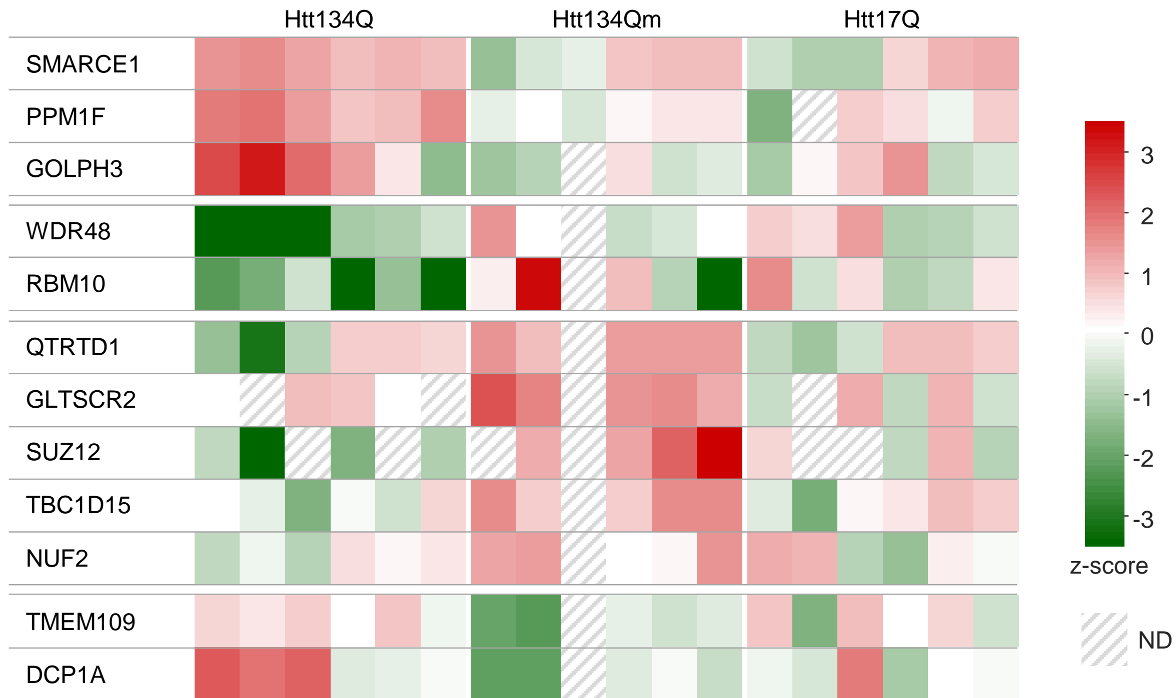
(c) Kanehisa Laboratories

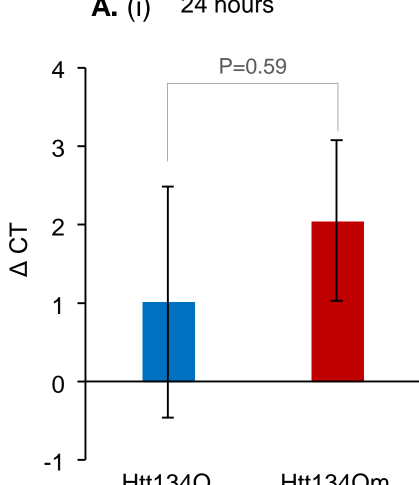


Hakim et al., Figure S5

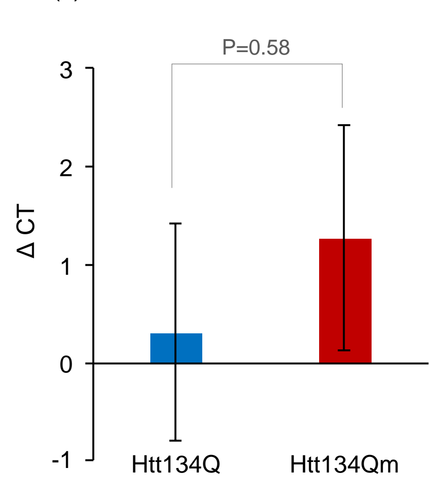


Hakim et al., Figure S6

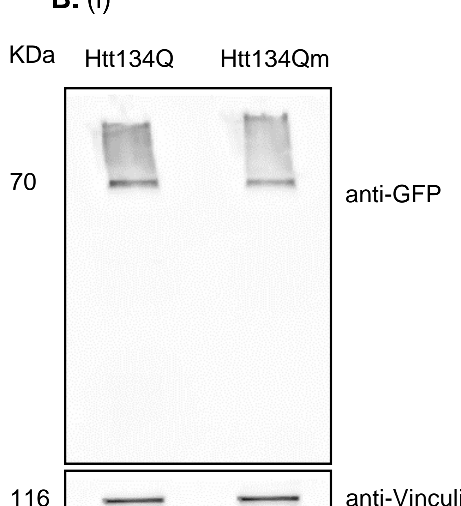
A. (i) 24 hours



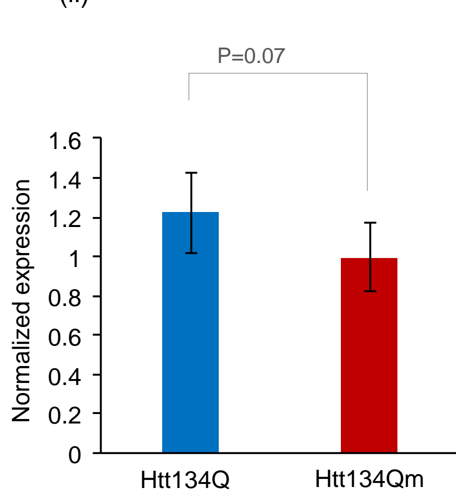
(ii) 48 hours



B. (i)



(ii)



Legends to SI Appendix Figures:

Movies S1. Long term imaging of Htt134Q:EGFP and Htt134Qm:EGFP expressed in primary cultures of rat cortical cells. Cultured cortical neurons were transduced with FU-Htt134Q:EGFP-Wm or FU-Htt134Qm:EGFP-Wm viral particles at DIV 14. Imaging was initiated at DIV 15, and was carried out by automated multisite time-lapse confocal microscopy, at 2-hours intervals (3 sections at each location), for a period of 2 weeks. (**A**) Htt134Q:EGFP (**B**) Htt134Qm:EGFP.

Fig. S1. Htt134Q:EGFP ubiquitination does not affect aggregate formation rates. Cortical neurons in culture were transduced with FU-Htt134Q:EGFP-Wm or FU-Htt134Qm:EGFP-Wm viral particles at DIV 14. Imaging was carried out as described under *Movies S1*. (**A**) Individual aggregates were identified and tracked semi-automatically. (i) Htt134Q:EGFP; (ii) Htt134Qm:EGFP. (**B**) Time course of aggregates' formation for the aggregates shown in (A) of Htt134Q:EGFP (i) and Htt134Qm:EGFP (ii). Curves color coded according to colored boxes in A. Times indicate hours from transduction. Bar, 10µm.

Fig. S2. Htt134Q:EGFP ubiquitination alleviates the pathological effects of polyQ expansion. HEK293 cells were transfected with either FU-Htt17Q:EGFP-Wm, FU-Htt134Q:EGFP-Wm or FU-Htt134Qm:EGFP-Wm. Non-transfected (N.T.) cells were used as controls. Four days following transfection, the cells were treated with PI and NucBlue® and visualized using a high content imaging system (ImageXpress® Micro Confocal system). The percentage of dead cells was calculated as the number of PI-positive cells relative to NucBlue®-positive cells (25 fields of view per well, N.T: 60 wells, 17Q: 55 wells, 134Q: 59 wells and 134Qm: 50 wells, three independent experiments; Error bars = SEM; P values from two-tailed t-Tests assuming unequal variances are shown).

Fig. S3. Htt134Q:EGFP ubiquitination does not affect cell division rates. FACS analysis for cell cycle stage distribution of HEK293 cells overexpressing either FU-Htt17Q:EGFP-Wm, FU-Htt134Q:EGFP-Wm or FU-Htt134Qm:EGFP-Wm harvested two or four days following transfection. Cells were fixed and stained with PI (Sigma) and relative DNA content was analyzed using high throughput cell cytometry. (i) Representative histograms from each cell population from a single experiment; (ii) Quantification of cell cycle stage distributions (three independent experiments; Error bars = SEM).

Fig. S4. Differentially expressed proteins indicated on KEGG mapping of Huntington's disease. Proteins whose abundance was higher in cells expressing Htt134Q:EGFP are indicated in yellow; Proteins whose abundance was higher in cells expressing Htt134Qm:EGFP are indicated in pink; Subunit changes in both conditions are indicated in green.

Fig. S5. Heat map for 12 proteins whose abundance was selectively altered in cells expressing either Htt134Q:EGFP or Htt134Qm:EGFP (see also Table S3). Shown are data derived from six replicates from two separate experiments. Values are expressed as Z-scores according to the bar on the right. Samples in which a protein was not detected (ND) are marked as hatched rectangles.

Fig. S6. Htt134Q:EGFP and Htt134Qm:EGFP have similar expression levels. (A, B) Measurements of Htt134Q:EGFP and Htt134Qm:EGFP expression levels by RT-PCR and western blots. (**A**) RT-PCR analysis of cortical neurons transfected with FU-Htt134Q:EGFP-Wm or FU-Htt134Qm:EGFP-Wm 24 hours (i) or 48 hours post transfection (ii). GFP primers were used as the target primers and HPRT as the endogenous gene primers. (**B**) Western blot analysis of HEK293 cells transfected with FU-Htt134Q:EGFP-Wm or FU-Htt134Qm:EGFP-Wm four days post transfection, using a-GFP antibody (i) A representative blot from a single experiment. (ii) Quantification of data shown in (i) from three independent experiments. Error bars: SEM. P values from Two tailed paired t-Test assuming unequal variances are shown.

Hakim et al., Table S1

Table S1. Up-regulated proteins: comparison between Htt134Q and 134Qm-overexpressing cells. Gel. (A) Htt134Q > Htt134Qm; (B) Htt134Qm > Htt134Q

A.

Gene name	Protein ID	Fold change	Gene name	Protein ID	Fold change
MYBL2	P10244	70.85	RBM20	Q5T481	2.12
ZNF292	J3KNV1	64.75	MTIF3	Q9H2K0	2.11
NT5C	J3KRC4	32.29	WASF1	Q92558	2.11
MKRN2	C9J494	26.56	LIAS	O43766	2.05
FUNDC1	Q8IVP5	25.37	MRPL41	Q8IXM3	2.04
WDR87	E7ESW6	23.90	L2HGDH	C9JVN9	2.04
PITX2	D6RFI4	21.74	POLR2J	P52435	2.04
HIST1H4F	J3KNC0	16.06	FAM98C	Q17RN3	2.01
RAB39B	Q96DA2	8.14	SDHC	Q99643	2.00
SENP1	Q9P0U3	7.79	GBE1	Q04446	2.00
CYFIP2	E7EVJ5	6.25	TAMM41	Q96BW9	1.99
MAP2K4	P45985	5.03	COMMD3-BMI1	R4GMX3	1.99
HSPB11	Q9Y547	4.95	CRTAP	O75718	1.98
AACS	Q86V21	2.97	NDUFA9	Q16795	1.98
CTNNAL1	Q9UBT7	2.89	RPL7L1	Q6DKI1	1.98
MOCS1	Q9NZB8	2.88	NDUFAF5	Q5TEU4	1.97
NEDD8-MDP1	E9PL57	2.84	SDR39U1	Q9NRG7	1.96
ALB	A0A0C4DGB6	2.80	LPGAT1	Q92604	1.95
GNA13	Q14344	2.68	SLC25A19	Q9HC21	1.95
PLSCR1	C9J7K9	2.67	GNPNAT1	Q96EK6	1.94
FAM177A1	G3V583	2.65	AK4	P27144	1.93
LMAN2L	Q9H0V9	2.64	RAB4A	P20338	1.91
HSD17B7	P56937	2.63	NSUN4	Q96CB9	1.90
GNPDA2	Q8TDQ7	2.62	DFFA	O00273	1.89
NME7	Q9Y5B8	2.48	SKA3	Q8IX90	1.89
NIP7	Q9Y221	2.45	C1QBP	Q07021	1.89
NDNL2	Q96MG7	2.42	BRI3BP	Q8WY22	1.88
TWF2	Q6IBS0	2.42	MTX2	C9JAZ1	1.87
DDB2	Q92466	2.41	THOC6	Q86W42	1.87
CDC34	P49427	2.33	PPCDC	H3BRQ0	1.86
B3GAT3	G3V150	2.30	ARGLU1	Q9NWB6	1.86
TMCO1	Q9UM00	2.27	GDPD1	Q8N9F7	1.85
RRP36	Q96EU6	2.27	TTI1	O43156	1.85
SPR	P35270	2.27	HSPBP1	Q9NZL4	1.85
SIX1	Q15475	2.26	TCEAL4	Q96EI5	1.84
RFT1	B5MDE0	2.26	SNRPA1	P09661	1.84
GTF2E2	P29084	2.25	SFT2D3	Q587I9	1.84
NDUFA8	P51970	2.23	RRP7A	Q9Y3A4	1.83
ARMC10	Q8N2F6	2.23	BANF1	O75531	1.82
REEP6	Q96HR9	2.22	UQCRFS1	P47985	1.80
TIMM17B	O60830	2.21	SORBS3	H0YAZ3	1.79
PRDX4	Q13162	2.19	FAHD2A	Q96GK7	1.78
SEC11A	H0YNG3	2.19	BRE	Q9NXR7	1.77
MRPS34	C9JJ19	2.18	FAM103A1	A0A3B3IU46	1.76
GFP	CON__Q9U6Y5	2.18	LRRC59	Q96AG4	1.75
NDUFAF1	H0YL22	2.17	NIT1	Q86X76	1.74
HIST1H2AC	Q93077	2.15	JUN	P05412	1.74
GNB1L	Q9BYB4	2.13			

B.

Gene name	Protein ID	Fold change	Gene name	Protein ID	Fold change
PIWIL3	E9PIP6	82.97	DLGAP5	Q15398	2.95
ANKLE2	Q86XL3	81.42	EML1	F8W717	2.86
CTDSP1	H7C3E0	30.72	FSIP2	Q5CZC0	2.78
GYG1	C9JQ42	9.43	ZNF768	H3BS42	2.06
GTSE1	Q9NYZ3	4.26	SMG7	B1ALB4	2.02
HIST1H1E	P10412	3.27	SP1	P08047	1.94
GTF2A2	P52657	3.26	ASND1	L0R819	1.92

Table S2. Up-regulated proteins: comparison between Htt134Q and 134Qm-overexpressing cells. NP-40. (A) Htt134Q > Htt134Qm; (B) Htt134Qm > Htt134Q

A.

Gene name	Protein ID	Fold change	Gene name	Protein ID	Fold change	Gene name	Protein ID	Fold change
CLN6	A0A1B0GW73	284.10	NUP133	Q8WUM0	2.79	AP1S1	A0A2R8YGH5	1.96
PAIP2	Q9BPZ3	241.09	HEBP1	F5GWX2	2.77	SPIN1	Q9Y657	1.96
RAD23A	P54725	162.59	ADNP	Q9H2P0	2.76	HSPA6	P17066	1.96
LARP4	Q96J85	90.92	COPS6	E7EM64	2.72	LSM2	Q9Y333	1.96
SLU7	O95391	85.72	S100A10	P60903	2.72	WDR43	Q15061	1.95
CD276	A0A0C4DGH0	83.33	MDC1	Q14676	2.71	XPO1	O14980	1.95
PCGF6	Q9BYE7	79.19	DBI	P07108	2.71	WRAP53	Q9BUR4	1.95
POLA2	Q14181	71.75	PPM1F	P49593	2.66	SERPINB6	A0A2R8YD12	1.95
POLR1D	P0DPB6	71.23	SPRYD4	Q8WW59	2.62	RPRD1A	Q96P16	1.94
CENPV	Q7Z7K6	69.25	NUP35	Q8NFH5	2.61	PRMT1	E9PKG1	1.94
PCBP2	Q15366	68.69	ABHD10	Q9NUJ1	2.61	ATP5F1	P24539	1.94
RNPC3	Q96LT9	58.14	NDUFA4	O00483	2.59	PRRC1	Q96M27	1.93
DNAJC3	X6R9L0	54.44	TOMM22	Q9NS69	2.55	EIF2B2	P49770	1.93
FAM120A	Q9NZB2	46.32	NUP37	Q8NFH4	2.55	DTYMK	P23919	1.93
PI4K2A	Q9BTU6	45.74	TMED2	E7EQ72	2.54	GNA11	A0A087WVZ3	1.92
SSR1	C9J3L8	43.52	C20orf27	Q9GZN8	2.48	TFG	Q92734	1.92
CDK11A	A0A0D9SEI3	33.31	TOMM5	F8W8Z9	2.48	EIF2B4	A0A087WTA5	1.92
NIP7	Q9Y221	33.00	PCID2	Q5JVF3	2.46	MRPS5	P82675	1.92
CBX5	P45973	20.89	RCN1	Q15293	2.46	COA3	Q9Y2R0	1.91
SCAF11	F8VXG7	20.70	NDUFAF3	Q9BU61	2.45	SPRYD4	Q8WW59	1.91
SCML2	Q9UQR0	14.81	VPRBP	Q9Y4B6	2.43	EIF4G2	P78344	1.90
POLR2G	P62487	14.44	PSMF1	Q5QPM7	2.43	PRPF38B	Q5VTL8	1.90
RCOR1	Q9UKL0	12.90	SGPL1	O95470	2.42	LSM5	B8ZZF8	1.89
NUDT16L1	Q9BRJ7	10.84	RTN4	F8W914	2.42	IPO9	Q96P70	1.89
UBXN7	O94888	10.14	RAD23B	P54727	2.40	NUP107	P57740	1.89
KIAA1429	Q69YN4	9.82	ARFIP2	A0A087X1E4	2.40	ROMO1	P60602	1.88
CSTB	P04080	9.25	AP2A1	O95782	2.37	SYMPK	Q92797	1.88
ANAPC5	F5GY68	8.50	WDR26	Q9H7D7	2.33	USMG5	Q96IX5	1.87
DCP1A	Q9NPI6	8.48	NECAP2	D6RB24	2.31	RBFOX1	A0A0G2JSB3	1.87
BROX	Q5VW32	6.82	GFP	CON__Q9U6Y5	2.30	MRPL21	Q7Z2W9	1.87
DPM3	Q9P2X0	6.35	SEC63	Q9UGP8	2.28	DDX56	Q9NY93	1.87
CWC15	Q9P013	6.13	BPTF	Q12830	2.28	RPLP2	P05387	1.86
FAM98A	Q8NCA5	6.03	EIF4A2	Q14240	2.27	APMAP	Q9HDC9	1.86
AS3MT	A0A087WVD4	5.48	ATAD2	Q6PL18	2.26	EXOC5	O00471	1.86
FAM114A2	Q9NRY5	5.34	GLG1	Q92896	2.26	AKAP12	Q02952	1.86
GORASP2	Q9H8Y8	5.25	GTF3C3	Q9Y5Q9	2.26	IGF2BP3	O00425	1.85
TNKS1BP1	Q9C0C2	4.81	ARL8A;ARL8B	Q96BM9	2.23	TIPRL	O75663	1.85
SF3B4	Q15427	4.51	AQR	O60306	2.22	CCDC51	Q96ER9	1.85
RSRC2	Q7L4I2	4.16	NT5C2	P49902	2.21	SAR1A	Q9NR31	1.85
CRK	P46108	3.93	ALDOC	P09972	2.20	CFDP1	Q9UEE9	1.84
RAB5A	P20339	3.86	CASP3	P42574	2.18	MOV10	Q9HCE1	1.84
AAAS	Q9NRG9	3.73	PSMA4	P25789	2.18	ATP5J	P18859	1.84
POLR2H	C9JLU1	3.66	SAAL1	J3KND1	2.18	RDH11	Q8TC12	1.83
NUP98	P52948	3.62	ERO1L	Q96HE7	2.17	ANKFY1	Q9P2R3	1.82
PPIL3	B8ZZ77	3.59	UBTF	P17480	2.16	KDM3B	Q7LBC6	1.81
AURKB	Q96GD4	3.47	PSMD8	K7EJC1	2.16	U2AF1	P0DN76	1.81
SMARCE1	A0A2R8Y855	3.44	DYNLT1	P63172	2.16	ARFGAP1	E5RHT6	1.81
NLE1	Q9NVX2	3.44	HSP90AB4P	Q58FF6	2.15	SCRIB	A0A0G2JNZ2	1.81
CUL4B	K4DI93	3.40	MPC2	Q5R3B4	2.15	TRAF2	Q12933	1.81
RHOB	P62745	3.35	HMOX2	A0A087WT44	2.15	NUDT1	Q9BRJ7	1.80
DDX10	Q13206	3.35	ATXN10	Q9UBB4	2.12	ACP1	P24666	1.80
MARCKS	P29966	3.35	SNRNP27	Q8WVK2	2.11	NDRG3	Q9UGV2	1.80
PRPF4B	Q13523	3.34	GNAI3	P08754	2.09	TFRC	P02786	1.80
PPIG	Q13427	3.27	EMG1	Q92979	2.09	BLOC1S1	A0A087WSV2	1.80
CAPN2	P17655	3.24	PPIH	O43447	2.07	NUDT21	O43809	1.79
BRD2	P25440	3.21	WDR12	Q9GZL7	2.05	MLLT11	Q13015	1.79
AGK	E9PC15	3.20	NDUFV2	E7EPT4	2.05	PCBP3	E9PFP8	1.79
RTN3	B7Z4M1	3.19	LSM14A	Q8ND56	2.05	GPATCH4	Q5T3I0	1.79
RNMT	O43148	3.17	C1QBP	Q07021	2.02	UBAP2L	F8W726	1.78
IKBKAP	O95163	3.12	BASP1	P80723	2.02	YTHDF2	Q9Y5A9	1.78
SNAPIN	O95295	3.12	MMTAG2	Q9BU76	2.02	PWP1	Q13610	1.78
ASAH1	A0A1B0GV06	3.05	NDUFB7	P17568	2.01	TNPO3	C9J7E5	1.78
MRPL52	G3V3U6	3.05	VPS29	F8VXU5	2.01	SURF6	O75683	1.77
PRC1	O43663	3.03	DHX8	Q14562	2.01	SCCPDH	Q8NBX0	1.77
FANCI	Q9NVI1	2.97	SFXN4	Q6P4A7	2.01	ATF7IP	Q6VMQ6	1.76
SPC25	Q9HBM1	2.92	YBX3	P16989	2.00	COPB1	P53618	1.75
C6orf211	Q9H993	2.90	GNPDA1	P46926	1.98	STT3A	P46977	1.75
DPF2	J3KMZ8	2.85	M6PR	P20645	1.97	OSTC	A0A087WUD3	1.74
TMEM109	Q9BVC6	2.80	HEATR3	Q7Z4Q2	1.97	RFC5	P40937	1.74

B.

Gene name	Protein ID	Fold change	Gene name	Protein ID	Fold change
SACS	438.15	Q9NZJ4	WDR48	2.59	Q8TAF3
SUMO4	346.72	Q6EEV6	CC2D1A	2.58	Q6P1N0
TRPT1	157.22	Q86TN4	NUF2	2.43	E9PQC4
H2AFY2	74.11	Q9P0M6	SREK1IP1	2.20	Q8N9Q2
HIRIP3	67.50	Q9BW71	FDX1	2.16	P10109
WDR76	48.85	A0A0C4DFX7	HMGA1	2.13	P17096
RPL3L	41.37	Q92901	H3F3B	2.12	K7EK07
KAT8	34.85	Q9H7Z6	THYN1	2.03	Q9P016
NIPBL	29.11	Q6KC79	MAP1B	1.97	P46821
FLOT1	21.81	O75955	POLR2F	1.94	U3KPY1
CENPK	19.10	D6RHD3	DNAJB12	1.91	J3KPS0
PPHLN1	16.40	F8W0Q9	LIG1	1.91	F5GZ28
CHAMP1	5.29	Q96JM3	EIF2AK2	1.86	P19525
MBD1	5.24	A0A0A0MS90	GLDC	1.84	P23378
MRPL57	4.29	Q9BQC6	TRAFD1	1.81	O14545
PRPF31	4.25	Q8WWY3	RBM10	1.78	P98175
QTRTD1	3.80	Q9H974	LSM3	1.78	P62310
SRRM1	3.40	A9Z1X7	IGF2BP2	1.75	F8W930
RRP9	2.99	O43818	TXNDC12	1.75	O95881
DSG2	2.68	Q14126		-	

Table S3: Proteins with significant and consistent changes in cells expressing Htt134Q, Htt134Qm, or Htt17Q

Protein ID	Protein name	Protein expression level	State
A0A2R8Y855	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1 SMARCE1	134Q 134Qm 17Q	Up Regulated Htt134Q
P49593	Protein phosphatase 1F PPM1F	134Q 134Qm 17Q	
Q9H4A6	Golgi phosphoprotein 3 GOLPH3	134Q 134Qm 17Q	
Q8TAF3	WD repeat-containing protein 48 WDR48	134Q 134Qm 17Q	Down Regulated Htt134Q
P98175	RNA-binding protein 10 RBM10	134Q 134Qm 17Q	
Q9H974	Queuine tRNA-ribosyltransferase subunit QTRTD1 QTRTD1	134Q 134Qm 17Q	Up regulated 134Qm
Q9NZM5	Glioma tumor suppressor candidate region gene 2 protein GLTSCR2	134Q 134Qm 17Q	
J3QQW9	Polycomb protein SUZ12 SUZ12	134Q 134Qm 17Q	
Q8TC07	TBC1 domain family member 15 TBC1D15	134Q 134Qm 17Q	
E9PQC4	Kinetochore protein Nuf2 NUF2	134Q 134Qm 17Q	
Q9BVC6	Transmembrane protein 109 TMEM109	134Q 134Qm 17Q	Down Regulated 134Qm
Q9NPI6	mRNA-decapping enzyme 1A DCP1A	134Q 134Qm 17Q	