UNIVERSITY OF CALIFORNIA SAN DIEGO

Juvenile Huntington's Disease Human Brain Proteomics Analyses Reveals Dysregulated Mitochondrial Systems with Alterations in Neuropeptides

A thesis submitted in partial satisfaction of the
requirements for the degree Master of Science

in

Biology

by

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Professor Yishi Jin, Co-Chair
Professor Steven P. Briggs

2021

The thesis of William Poon is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

University of California San Diego

2021

TABLE OF CONTENTS

List of Abbreviations

HD: Huntington's disease

HTT: Huntingtin Gene

BA4: Brodmann Area 4

BA6: Brodmann Area 6

CAG: glutamine

polyQ: polyglutamine

Htt: Huntingtin protein

GO: gene ontology

LIST OF FIGURES

LIST OF TABLES

LIST OF SUPPLEMENTAL FIGURES

LIST OF Supplemental Tables

Acknowledgments

I would like to acknowledge Dr.Vivian Hook, Dr. Sonia Podvin, Charles Mosier, Leigh-Ana Rossitto and members of the Hook laboratory for their guidance and assistance in helping me achieve the data for my project. Through multiple efforts of conducting collaborative experiments, valuable data was discovered for my thesis.

This research was supported by a grant from the HDSA foundation awarded to V. Hook.

The introduction, Figure 1-3, includes material, currently being prepared for submission for publication and will appear as Podvin S., Poon W., Mosier C., Rossitto L.-A., Wei E., and V. Hook. "Juvenile Huntington's Disease Human Brain Proteomics Reveals Dysregulated Mitochondrial Systems and Neuropeptide Regulation." William Poon was the co-author of this paper.

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Chapter 3, Figure 13, includes material, currently being prepared for submission for publication and will appear as Podvin S., Poon W., Mosier C., Rossitto L.-A., Wei E., and V. Hook. "Juvenile Huntington's Disease Human Brain Proteomics Reveals Dysregulated

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ABSTRACT OF THE THESIS

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By

William Poon

Master of Science in Biology

University of California San Diego, 2021

Professor Vivian Hook, Chair
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Huntington's disease is a heritable neurodegenerative disease caused by an autosomal dominant trinucleotide expansion of 35+ CAG repeats in the HTT gene while juvenile HD results from a trinucleotide expansion of 60+ CAG repeats. In both cases, an increased number

of CAG repeats can increase the severity and onset of the disease. Additionally, there is a substantial dysregulation in protein interactions and cellular pathways in the putamen as well as global brain degeneration. Previous findings in proteomic analysis of expanded allele HTT knock in mice have suggested mitochondrial toxicity and downregulated vesicle trafficking are some of the main causes of HD pathology, but this analysis has not been done with juvenile HD human brain tissue. To discover the dysregulated proteins in the cortex areas of Brodmann Area 4 and Brodmann Area 6 combined with the putamen, regions that regulate motor function, we performed mass spectrometry and proteomic analysis on trypsin-digested postmortem juvenile HD brain tissue and postmortem human control brain tissue to find dysregulated proteins in both conditions. Our findings showed: (1) proteins in juvenile HD only, (2) control only, (3) dysregulated proteins, (4) dysregulation of mitochondrial components in juvenile HD, (5) distinct neuropeptides in the juvenile HD and control proteome. The data suggests that mutant Htt causes gain and loss of expression of proteins in the human brain. In the cortex, the absence of mitochondrial pathway components indicates loss of function in this system. Furthermore, neuropeptides were found only in juvenile HD and control, shared, and dysregulated in juvenile HD.

Introduction

Huntington's disease (HD) is a neurodegenerative disorder that is caused by a range of polyglutamine (polyQ(n)) repeat expansion in the protein huntingtin (Htt).[1][25] It is theorized that the mutant huntingtin protein disrupts many protein-protein interactions within the cell and consequently alters the cell's functions and phenotypes.[2] In addition, it is also proposed that there is change in the synaptic function associated with Huntington disease. [4]There is a current gap of knowledge in understanding the disrupted cellular pathways that may induce synaptic dysfunction within 'human' HD. In this project, we will use systems mass spectrometry to identify proteins that may contribute to the synaptic dysfunction of human HD. This can help us to better understand the pathology of the human HD condition and tackle the disease.

Genetic Basis of Htt Mutations and CAG Repeats- Huntington's disease has age-correlated severities from juvenile to adult onset. These different severities of HD are due to the number of CAG (glutamine) repeats in the HTT gene of the individual (Figure 1).[5][11] Normal individuals have 35 or fewer CAG repeats within their genome while diseased individuals have 36 or more CAG repeats.[11] As the number of CAG repeats increase, the onset of HD comes at an earlier stage in the individuals' life.[11] Individuals with > 60 CAG repeats have disease onset as children. [11][13] The huntingtin (Htt) protein is expressed throughout the human brain in numerous regions at varying levels of expression (Figure 2). Notably, Htt is expressed in cortex regions of Brodmann 4 and 6, and in putamen (Figure 3), which control motor function that is disrupted in HD.

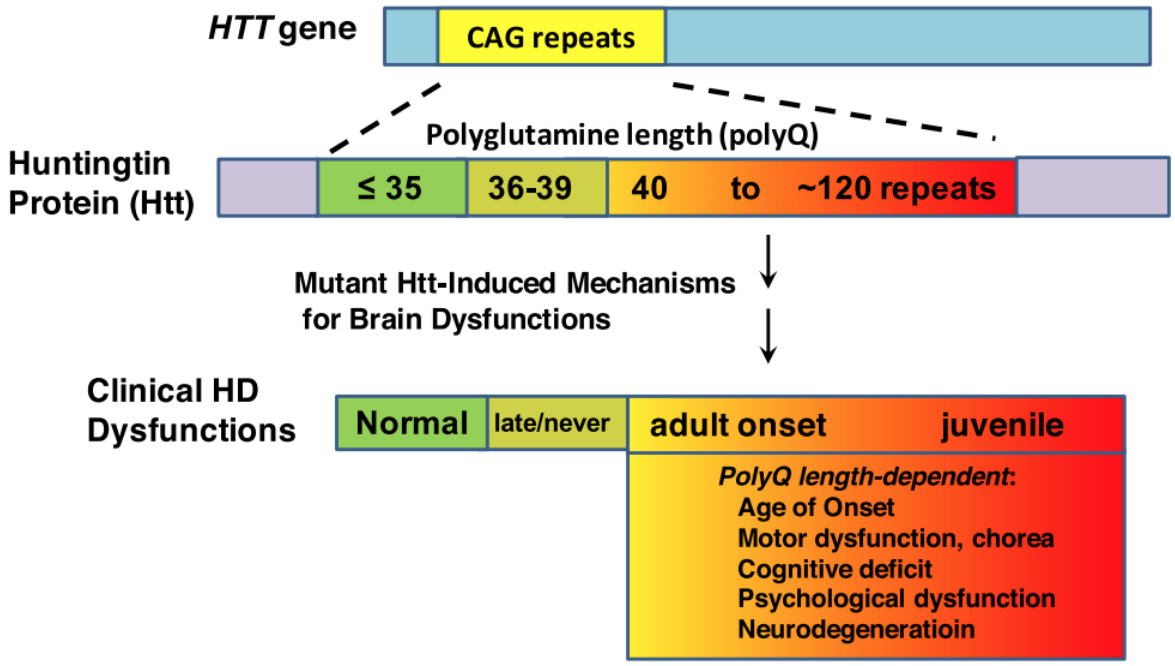

Figure 1: The Correlation of Number of CAG repeats with Onset of Disease and its Clinical Dysfunctions. The number of Htt gene CAG repeats correlates to the expression of Huntington's disease in HD patients. The link between age of onset and number of CAG repeats is also listed where normal phenotypes have ≤ 35 CAG repeats, 36-39 CAG repeats may or may not express HD, 40 to ~ 50 express the adult onset of HD, and ~60+ for the juvenile onset of HD. The clinical features of each dysfunctions of the disease are listed below. Adapted from "Multiple clinical features of Huntington's disease correlate with mutant HTT gene CAG repeat lengths and neurodegeneration," by V.Hook, H.Reardon, K.Yin, C.Mosier, and S. Podvin, 2018, *Journal of Neurology*, 266, p.551-564. © Springer-Verlag GmbH Germany, part of Springer Nature 2018[16]

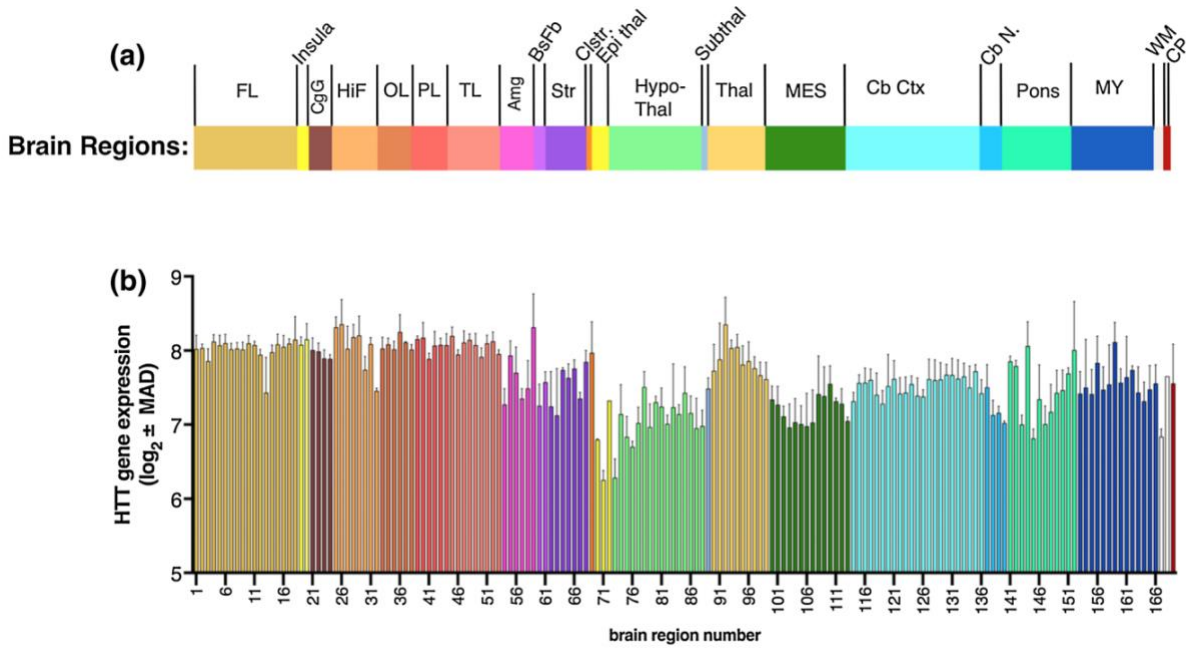

Figure 2: Varying Expressions of Huntingtin Protein in Different Parts of the Brain. Huntington gene expression was analyzed using microarray analysis of 169 brain regions from six donor brains. The Htt gene expression is associated with the (a) color of the top bar indicating the brain regions where FL frontal lobe, insula, CgG cingulate gyri, HiF hippocampal formation, OL occipital lobe, PL parietal lobe, TL temporal lobe, Amg amygdala, BsFb basal forebrain, Str striatum,Clstr claustrum, Epithal epithalamus; hypothalamus, Thal thalamus, Subthal subthalamus, MES mesencephalon, CbCtx cerebellar cortex,CbN cerebellar nuclei; pons, MY myelencephalon, WM white matter structures, CP choroid plexus of the lateral ventricles. (b) The HTT gene expression is shown in the 169 brain regions using $\log_2$ values from microarray analysis. Adapted from "Multiple clinical features of Huntington's disease correlate with mutant HTT gene CAG repeat lengths and neurodegeneration," by V.Hook, H.Reardon, K.Yin, C.Mosier, and S. Podvin, 2018, *Journal of Neurology*, 266, p.551-564. © Springer-Verlag GmbH Germany, part of Springer Nature 2018[16]

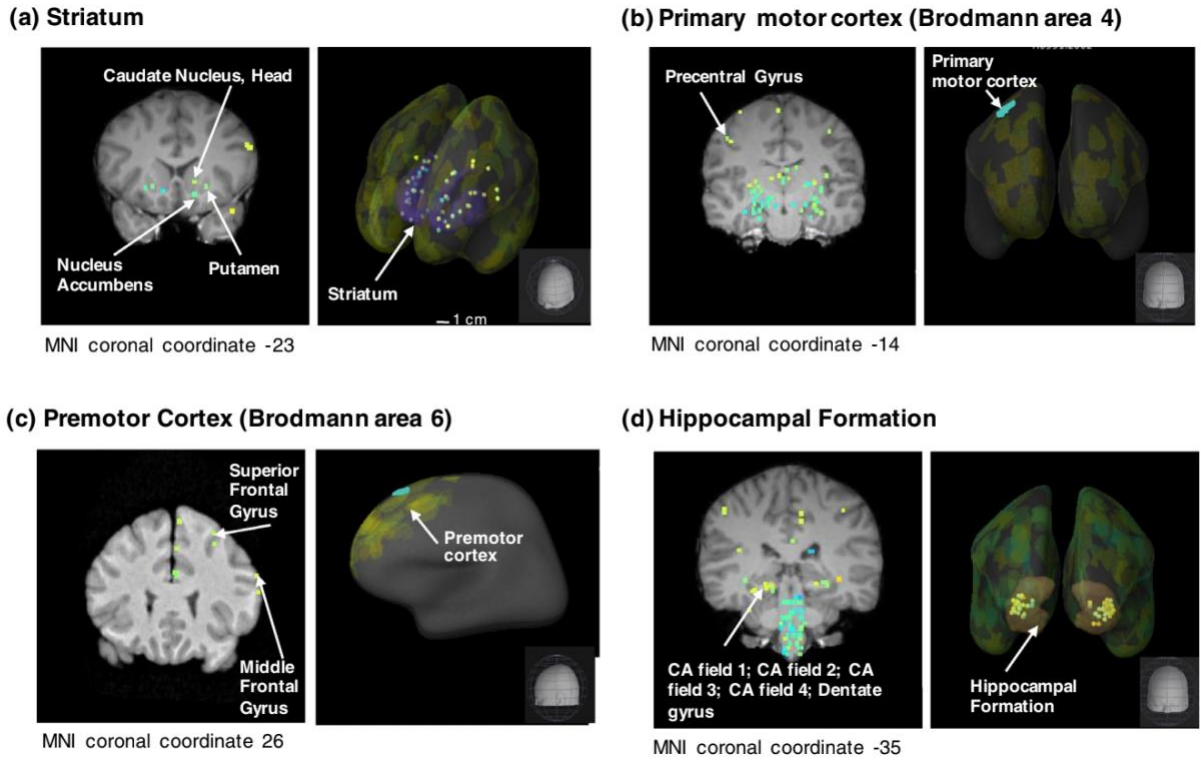

Figure 3: **3D Imaging of Huntington Protein Expression within the Striatum, Primary Motor Cortex, Premotor Cortex, and Hippocampus.** Htt gene expression localizations are shown in the different brain regions of the (a) striatum (b) primary motor cortex, Brodmann Area 4 (c) premotor cortex, Brodmann Area 6 (d) Hippocampus. The different colors represent varying gene expression levels based on $\log_2$ values. Adapted from "Multiple clinical features of Huntington's disease correlate with mutant HTT gene CAG repeat lengths and neurodegeneration," by V.Hook, H.Reardon, K.Yin, C.Mosier, and S. Podvin, 2018, *Journal of Neurology*, 266, p.551-564. © Springer-Verlag GmbH Germany, part of Springer Nature 2018[16]

Clinical Symptoms of Htt patients- Huntington disease can afflict many debilitating effects to people diagnosed with the disease. Some clinical features associated within both adult and juvenile onset HD include motor dysfunction and cognitive deficits.[20] Specifically, afflicted individuals experience involuntary movements, depression and dementia.[20] In addition to these symptoms, individuals may experience dysphagia, hypokinesia, akinesia, rigidity, and dystonia.[20]

Effects of Mutant Htt gene in iPSC neurons, embryonic stem cells, challenges towards understanding HD brain systems- The expanded polyQ(n) expressed in HD results in many

dysregulated molecular pathways. These dysregulated molecular pathways may cause debilitating symptoms of the disease. It is important to study these dysregulated molecular pathways to pinpoint specific proteins that may contribute to the development of the disease. In recent studies, there have been a lot of pathways studied in different non-human organisms and models, as well as human induced pluripotent stem cells (iPSC) from HD affected humans to embryonic stem cells. To identify these dysregulated proteins within iPSC, Chae and colleagues used mass spectrometry and GeneGo to examine what the dysregulated proteins were and their functions within iPSC cells.[10] As a result, Chae and colleagues identified 26 most significantly dysregulated proteins such as mitochondrial proteins and cytoplasmic proteins.[10] The mitochondrial and cytoplasmic proteins are significant because past research shows that mitochondrial dysregulation and cytoplasmic toxicity play a role in the pathology of HD.[17] However, the iPSC model is limited in identifying the dysregulated proteins within HD because there are some gene expression differences in iPSC cells vs normal HD cells since the environment the cells are grown in may affect the regulation of which genes are transcribed.[23] Another example of a model used to identify the dysregulated proteins within HD was using HD embryonic stem cells. To identify the dysregulated proteins within HD embryonic stem cell lines, McQuade and colleagues used mass spectrometry and bioinformatic programs to examine the proteins and their associated functions within these HD embryonic stem cell lines. [12] As a result, McQuade and colleagues discovered 27 significant proteins that are mitochondrial proteins and cytoskeletal proteins.[12] These proteins are significant because past research shows that mitochondrial dysregulation and alterations within the cytoskeleton play a role in the pathology of HD.[6][8][17] However, their model is limited in identifying the dysregulated proteins within HD because there are problems in the regulation of transcription when reprogramming cells back into

stem cells [15] and is entirely different from the intact human HD brain regions affected that involve in vivo neuronal circuits, multiple cell types, and human brain structure.

Effects of Mutant Htt Gene, challenges towards understanding HD brain systems- In addition to proteomics using iPSC neurons and embryonic stem cells models, proteomic studies of animal models and human models have been studied. To identify the distinct proteins of HD animal models, Shirazaki and his colleagues performed affinity purification mass spectrometry on mouse models and weighted gene correlation networks to form gene networks based on expression relationships.[21] He and his colleagues also performed gene ontology and ingenuity pathway analysis to annotate these gene networks with terms that describe their functions.[21] He and his colleagues discovered 747 significant proteins along with six gene networks with their functions annotated.[21] This is significant as terms relating to the mitochondria and cytoskeleton have been known to be associated with the disease.[6][8][17] However, the model is limited in identifying the molecular pathways and expression of these proteins since the mouse genome is different from the human genome.

In addition to studying molecular pathways of HD using the rat genome, proteomics studies on the human HD brain have been made to discover molecular pathways with HD individuals. To study the molecular pathways and proteins within HD human models, Ratovitski and his colleagues used isobaric labeling, mass spectrometry to label and quantify the proteins in the human HD brain sample.[19] He also used ingenuity pathway analysis and DAVID functional annotation tool to annotate the gene networks formed from the ingenuity pathway analysis.[19] They discovered significant terms such as Rho-mediated, integrin signaling, and protein transport.[19] However, this model only identified the proteins and molecular pathways within

adult onset of HD and not juvenile HD. As a result, a current gap of knowledge is the molecular pathways and proteins that are affected by juvenile HD.[19]

Synaptic Deficits in HD- In addition, it is known that synaptic function and plasticity is affected by Huntington's disease.[22] This can affect the way synapses communicate with each other via neuropeptides.[27] Specifically, synapses use dense core secretory vesicles to transport these neuropeptides and hormones to communicate with each other.[27] As a result, it is important to identify these neuropeptides to gain a better understanding of the molecular pathways of neuronal communication which may help us understand neurodegenerative diseases.[27] To identify these neuropeptides, Hook and her colleagues performed a proteomic analysis of bovine adrenal medulla to identify important neuropeptides and their associated terms to understand their functions within the neuronal communication system.[27] However, the bovine genome is different from the human genome.[27] Consequently, the molecular pathways and neuropeptide expression may be different within these two specimens. With these neuropeptides being found in bovine adrenal medulla, a current gap of knowledge is the identification and expression of neuropeptides in human systems.

Project Hypothesis and Specific Aims- Goal: In this project, our goal is to identify dysregulated cellular systems including synaptic peptide neurotransmitter systems in the neurodegeneration of human juvenile HD brain. The cortex and putamen were used for this study as these parts of the brain are hypothesized to be the most affected by the disease as shown by the symptoms. The cortex is responsible for responding to sensory information through motor movements while the putamen is responsible for motor control. ***Hypothesis:*** We hypothesize mutant Htt dysregulates many of the cell pathways, components and functions that results in (1) juvenile HD proteins that are not found in normal brain tissues (2) control only proteins that are

not found juvenile HD brain tissue (3) shared proteins found between the juvenile HD and control brain tissue that are downregulated and upregulated (4) neuropeptides found in the juvenile HD proteome, control only proteome, shared, and dysregulated in juvenile HD.

Specific Aims: Our specific aims are to (1) determine the cellular protein systems that are dysregulated in human juvenile HD brain cortex and putamen compared to age-matched controls, and (2) determine how the peptide neurotransmitter system of dense core secretory vesicles are dysregulated in human juvenile HD brain cortex and putamen compared to age-matched controls.

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Chapter 1: Experimental Procedures

Human Brain Tissue Preparation and Methanol Extraction: Juvenile HD brain tissue and Control Brain Tissue- Tissue samples from Brodman area 4 (BA4), Brodman area 6 (BA6) and putamen, were obtained from five human juvenile HD patients and seven human control individuals were obtained from NIH Neurobiobank in the United States. Each tissue sample was dissected into approximately 0.5 g pieces and weighed to assure masses were similar. A table of each subject's demographic information is shown in Table 1. To obtain a representative protein sample from each tissue piece, an approximate 1 mg sample of each dissected 0.5 g piece of each tissue was combined to prepare the protein homogenate for proteomics analyses. The combined approximate 1 mg samples from each tissue piece were placed in ice-cold buffer consisting of 100 mM Tris, pH 7.4, 50 mM NaCl, 1 mM EDTA, 10 uM Pepstatin A (Millipore), 10 uM Leupeptin (Millipore), 10 uM Chymostatin (Millipore), 10 uM E64c (Bachem), 100 uM AEBSF (Millipore) at a weight/volume ratio of 1:5. Samples were sonicated three times in 5 second bursts and placed on ice for 30 seconds between sonications. Next, the protein concentration of the samples was determined using the Lowry method (BioRad DC protein assay). To perform methanol precipitation of proteins, 600 ug of each sample is diluted using 100 µL of ice cold water, followed by 900 ul of ice cold methanol to form 90% methanol solution. The solution was incubated for fifteen minutes and centrifuged at 14,000g at 4C. The supernatant was removed and the resulting protein pellets were dried using a speed vac for three minutes.

Trypsin/LysC Digestion of Brain Samples and Preparation for Mass Spectrometry - The resulting protein pellets were suspended to sodium deoxycholate (SDC) trypsin digestion buffer to form a concentration of 2 mg/mL. To prepare the buffer, 687 µL of double-deionized water (ddH_2O), 100 µL of 10% sodium solution, 100 µL of 1M Tris- HCl, pH of 8, 13 µL of 775mM TCEP (define), 100 µL of 440mM chloroacetamide mixed with a 1mL solution of SDC buffer.

Each sample was incubated at 95℃ and cooled at room temperature for five minutes. The samples were subjected to a water bath sonicator for two minutes and diluted 1:2 using 100 µL of 100 mL Tris solution. Trypsin/Lys C solution was prepared by adding 40 µL of 50 mM acetic acid, for a final concentration of 0.5 µg/µL. The trypsin/Lys C solution was added in a 1:50 enzyme: protein ratio by adding 8 µL of the 0.5 µg/µL Trypsin/ Lys C solution to each 200µg protein sample. The protein samples were incubated at 37℃ for eighteen hours to digest proteins. The resulting tryptic peptide samples were quenched with 0.2% TFA (trifluoroacetic acid) by adding 4.5µL of 10% TFA. The peptide samples were frozen at -70℃.

To purify the peptide samples, C18 stage tip extraction was performed [18]. The stage tips were centrifuged at 3000 xg for three minutes. 100% acetonitrile (ACN) was added to the stage tips and centrifuged for four minutes at 2400 rpm. 150 µL of 0.1% trifluoroacetic acid (TFA) was added to the stage tip and centrifuged at 2400 rpm for four minutes, repeated three times. The peptide samples were loaded and centrifuged at 2000 rpm for five minutes. 150 µL of 0.1% TFA was added to the stage tips and centrifuged at 2400 rpm for four minutes twice. The following wash was discarded from the stage tips. Next, three elutions of 150 µL of 0.1% TFA and 40% ACN and 150 µL of 0.1% TFA and 70% ACN were performed on each peptide sample until all liquid has gone through the tip. The resulting samples were dried using speed vac for three hours.

Duplicate samples were each resuspended in 75 µL of LC grade water, then combined and mixed by pipetting, resulting in 150 µL. After resuspension of the samples, colorimetric peptide assays kit (Thermo Fisher) was performed to determine the concentration of each peptide sample. Next, the samples were diluted to 0.5 µg/ µL in 2% ACN, 0.1% TFA to be injected for LC-MS/MS.

LC-MS/MS Tandem Mass Spectrometry - Chromatography was performed on a Dionex UltiMate 3000 nano-LC (ThermoFisher Scientific), and tandem mass spectrometry (MS/MS) was performed on a Q-Exactive (ThermoFisher). Two ug of each sample was run in triplicate on a 1.7um ethylene bridged hybrid (BEH) column with a linear gradient of increasing ACN/ 0.1% formic acid (FA) from 5% - 80% for 25 minutes, followed by a linear gradient of 85% - 95% for 10 minutes, and finally 95% ACN for 15 minutes. Samples were injected in randomized order. A pooled universal reference sample of all JHD and control samples from all three brain regions was assayed between every six randomized samples to evaluate possible technical drift.

Mass spectra were acquired in positive ion mode with full data-dependent scans. MS1 scans ranged from 310 to 1200 m/z with resolution of 70,000 at 200 m/z, inject time of 100 ms. MS2 was acquired at a 1.5 m/z isolation window of 17,500, maximum injection time of 50 ms, automatic gain of 1×10^5, intensity threshold of 4×10^3, and HCD cell normalized collision energy of 27V.

Protein Identification- Protein identification and label-free quantitation (LFQ) were determined from extracted ion chromatography with PEAKs (v 8.5) bioinformatics software (BioInfor, Inc, Waterloo, ON, Canada).The raw files were searched against the UniprotKB/Swissprot human protein sequence database containing 71,783 entries. A decoy spectrum library of all proteins (randomized in sequence) was searched also of all proteins to determine false discoveries. The parameters were set with trypsin enzyme (cleaves Lys and Arg residues and allows for two missed/non-specific cleavages), PTMs (post-translational modifications carbamidomethylation on Cys, oxidation of Met, N-terminal acetylation, and phosphorylation on Ser, Thr, Tyr), precursor mass error tolerance of 20 ppm, mass tolerance for fragment ion of 0.01 Da, and threshold peptide score of $-\log_{10}P \geq 32$. The false detection rate (FDR)

was determined by PEAKs using the UniprotKB/Swissprot human protein sequence database. To obtain FDR of < 1%, threshold for protein identification was $-\log_{10}P \geq 55$ (add peptide identification -10lgP, PTM Score, etc).

Protein Quantification- LFQ of identified proteins was determined using PEAKs (v 8.5). The area under the curve (AUC) for each peak area in the ion chromatograph from MS2 data was calculated and added to estimate the quantity of the protein. AUCs from peptides were summed to determine LFQ of corresponding proteins. If a peptide sequence mapped to more than one protein, the peptide AUC was assigned to each protein. Parameters were set to peptide quality of >0.3 and abundance of 1×10^4 before LFQ was performed. Modifications were not considered when using label free quantitation. The intensity of each protein determined from LFQ was subjected to normalization to account for the minor instrumental differences of the mass spectrometer readings using LOESS-G using Normalyzer web application.The values were converted into log2 values. Any value that had no intensity data was imputed to include a value that was at the bottom 5% of all protein values. The average and standard deviation for JHD and control was calculated for each protein quantified. A Student's t-test was performed between the JHD only replicates and controlled only replicates to reveal significance ($p < 0.05$) between the two conditions. The Master Table includes all proteins that were identified and quantified and were separated between shared, control only condition and JHD only condition. The table also includes the quantification values associated with the p-values of each protein.

GO and STRING db Network Analysis- Gene ontology (GO) and KEGG pathways and proteins interaction networks were made using proteins from the JHD only condition, control only condition, and the shared conditions using STRING-db (https://string-db.org/), an open resource for GO and protein network analyses. The GO pathway analysis helps determine

significant terms that associate with each experimental condition. GO term enrichment was determined to be significant with FDR < 1% using Benjamini-Hochberg procedures.

Protein interacting networks were made using STRING-db (Version 11.0) which compares proteins to a database of known protein interactions. Parameters of the confidence score were set to 0.7 in the protein network map. Reference database types queried were experiments, databases, co-expression and co-occurrence.

Up and Downregulated Proteins in Juvenile HD and Control- For proteins that are shared in both the JHD and control condition, a $\log_2$ JHD/control ratio was calculated to determine the extremity of upregulation and downregulation of the proteins. The values were inputted into Heatmapper [3] to generate heatmaps. These bioinformatic processes were repeated for the three regions of Brodmann Area 4,6 and putamen. A general flowchart of our experimental procedures can be seen in Figure 4.

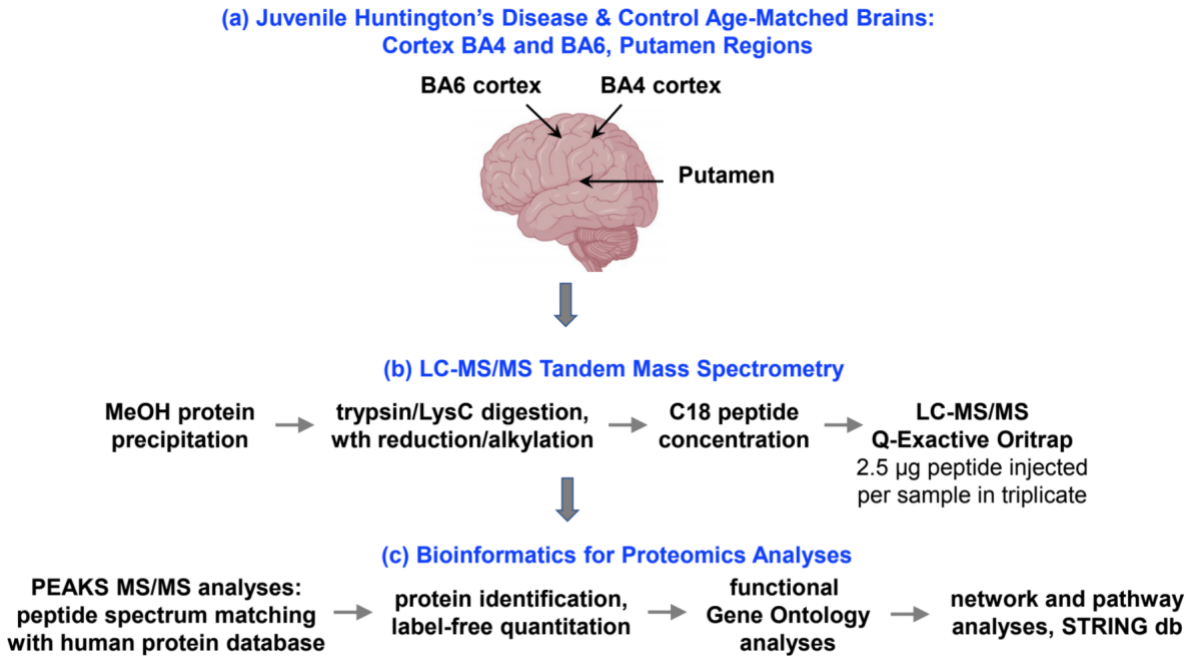

Figure 4: **Outline of Experimental Plan for Proteomics and Bioinformatics Analyses of Human Juvenile Huntington's Disease Brain Regions of Cortex (Brodmann areas 4 and 6) and Putamen.** (a) Juvenile H (J. HD) brain tissues were collected from the NIH Neurobank. (b) Samples were subjected to LC-MS/MS tandem mass spectrometry. (c) Data was analyzed by PEAKS for protein identification and quantitation, Gene Ontology (GO), and STRING db for network and pathway analyses.

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Table 1: Demographic Information of Juvenile HD samples and Aged-Match Control Brain Tissue Samples.

Demographic information of the sample subject obtained from NIH Neurobank are listed below. The demographic information includes the condition of the brain; age, gender, region, post mortem interval of the subject; and the number of CAG repeats in the HTT gene.

Condition	Age (subject letter)	Brain	Gender	PMI	Regions	#CAG Repeats In HTT gene
						Juvenile HD: CAG repeat numbers for each of two alleles of the HTT gene
Juvenile HD	29	a	M	16 hrs	Cortex: BA4, BA6 Putamen	16, 69
	26	b	F	12 hrs	Cortex: BA4, BA6 Putamen	19, 62
	20	c	F	6 hrs	Cortex: BA4, BA6 Putamen	17, 72
	26	d	F	26 hrs	Cortex: BA4, BA6	12, 97
	12	e	M	20 hrs	Cortex: BA4, BA6 Putamen	17, 120
	21	f	F	25 hrs	Putamen	17, 66
						Control: CAG repeat numbers likely representing both of the two HTT alleles
Control Age-Matched	23	a	M	11 hrs	Cortex: BA4, BA6 Putamen	18
	18	b	M	20 hrs	Cortex: BA4, BA6	17
	24	c	F	25 hrs	Cortex: BA4, BA6 Putamen	18
	20	d	M	15 hrs	Cortex: BA4, BA6, Putamen	17
	13	e	F	9 hrs	Cortex: BA4, BA6, Putamen	18
	16	f	F	9 hrs	Cortex: BA4, BA6, Putamen	12
	10	g	M	10 hrs	Cortex: BA4, BA6, Putamen	21
	20	h	M	15 hrs	Putamen	17

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Results

Chapter 2: Proteomic Analysis of Cortex, Brodmann Area 4 and Brodmann Area 6, in Human Juvenile HD brains

Protein Count and Proteomics Data Associating with Brodmann Area 4 and 6- There were 81 proteins associated with the juvenile HD only condition and 209 proteins associated with the control only condition in the Brodmann Area 4 of the cortex. There were 2877 proteins that were shared in both conditions in the Brodmann Area 4 of the cortex. There were 107 proteins associated with the juvenile HD only condition and 240 proteins associated with the control only condition in Brodmann Area 6 of the cortex. There were 3297 proteins shared in both conditions in the Brodmann Area 6.

Principal Component Analysis of Brodmann Area 4 and 6 Samples- After counting the number of proteins in the different regions for each condition, a principal component analysis was performed to visualize the extent of variation between our juvenile HD and control samples in both the Brodmann Area 4 and 6 as shown in Figure 5. In the principle component analysis of Brodmann Area 4 of the cortex, the data points representing control samples are clustered next to each other while juvenile HD data points are also clustered near each other. The clustering of the points in these two experimental conditions show low variability between samples in the juvenile HD condition and the control condition. The two experimental conditions are spread apart from each other in the graph which show that the juvenile HD data points are highly variable compared to the control condition which is to be expected. The control 1A sample is far from the juvenile HD and control only condition clusters which signifies that the sample is an outlier to the other control samples. As a result, the control 1A sample may vary significantly from the

other control samples. The same conclusions are seen in the principal component analysis of the Brodmann Area 6 of the cortex.

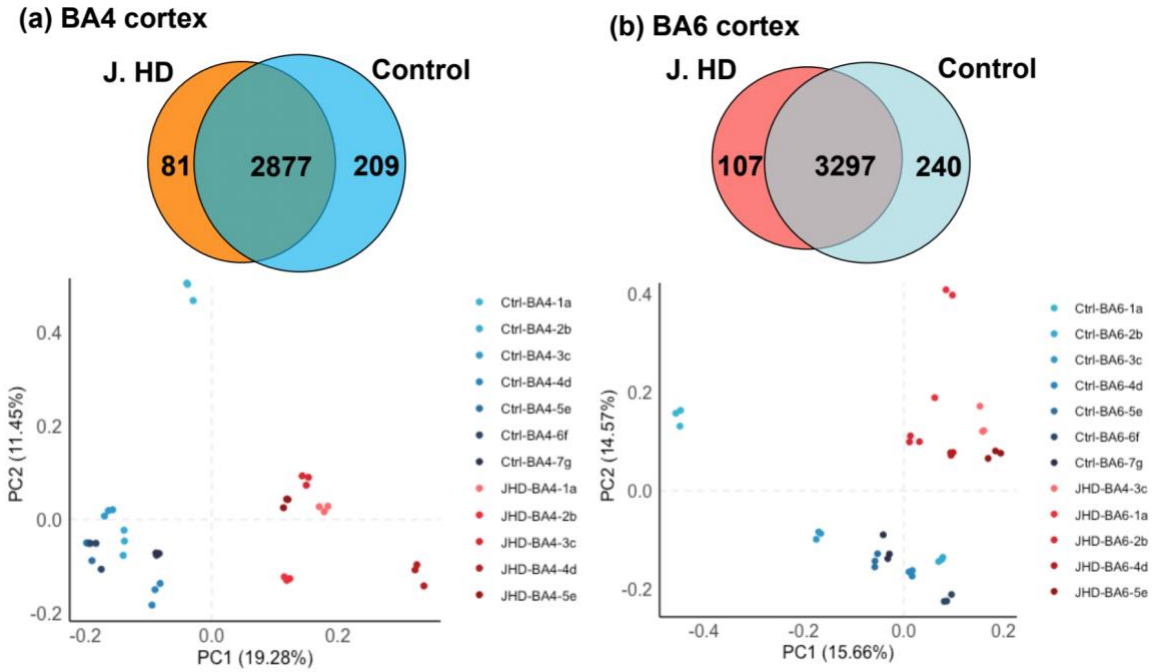

Figure 5: BA4 and BA6 Protein Identifications and Principal Component Analyses. The number of proteins identified in the juvenile HD condition, aged-match control condition, and shared in both (a) BA4 cortex and (b) BA6 cortex are shown in the Venn diagrams above. Both graphs represent a principal component analysis depicting the extent of variation between samples of one sample group compared to the other sample group.

Proteins Present only in Juvenile HD brain at Brodmann Area 4 and 6- Next, gene ontology (GO) using STRING-db was performed using the significant quantifiable proteins of the juvenile HD only conditions, control only condition, and shared between both conditions. The gene ontology analysis will annotate the gene sets within these three experimental conditions and identify significant functions of these gene sets. The significant functions were assessed in four categories: biological process, molecular function, cell component and KEGG pathways. Along with gene ontology analysis, a STRING network using STRING-db is formed using these gene sets to form a protein interaction network map to show how these proteins

interact with other proteins. The color-coded lines in between two different proteins indicate the gene ontology term associated with the interaction.

In the juvenile HD only STRING network for the Brodmann Area 4 of the cortex as shown in Figure 6, there were no GO terms that were significant. In the juvenile HD STRING network of Brodmann Area 6 of the cortex as shown in Figure 6, there were significant GO terms such as activation, catalysis and binding associated with the gene set. In addition to the STRING network analysis of these two regions, the top 20 most abundant quantifiable juvenile HD only proteins were gathered and put into the bar graph showcasing their intensities as shown in Figure 6.

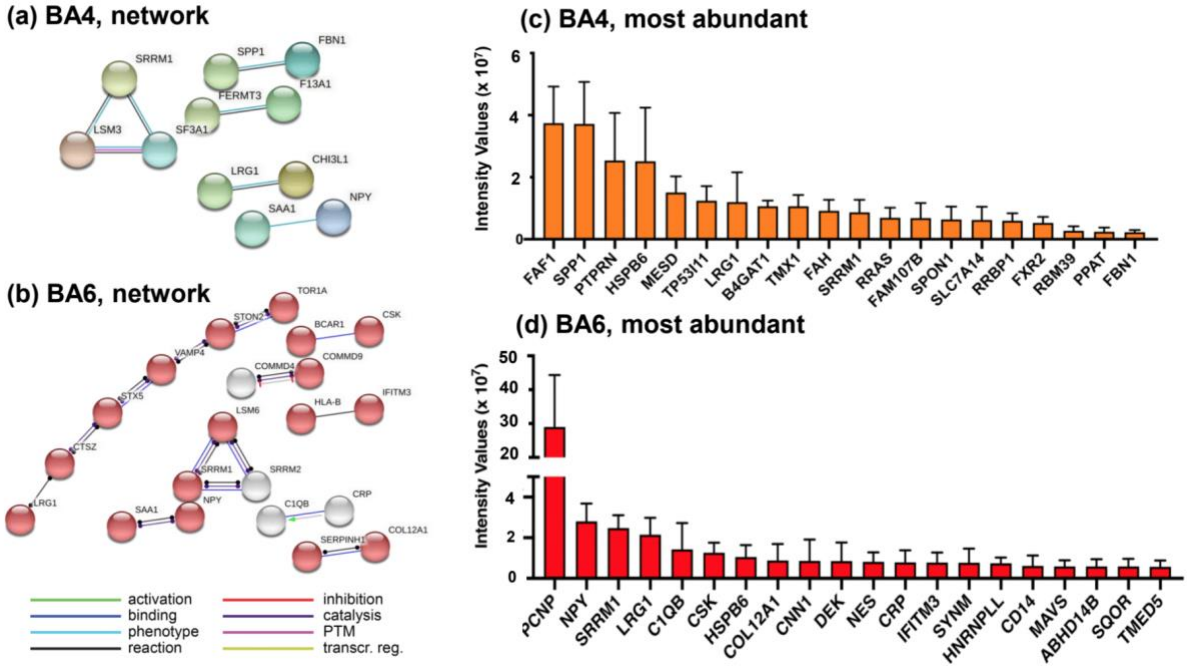

Figure 6: BA4 and BA6 proteins present in only Juvenile HD: Network and Abundant Components. STRING protein interaction networks for juvenile HD only proteins in (a) BA4 cortex and (b) BA6 cortex are shown to the left. The STRING protein interaction networks depict the name of the protein and the relationship of the interaction with other proteins. The color-coded lines represent the type of interaction between the two proteins. Intensity value graphs are shown on the right showing the top twenty abundant proteins and their intensity values for both juvenile HD only BA4 and BA6 proteins.

Proteins Present only in Control brain at Brodmann Area 4 and 6- Next, a gene ontology analysis was performed on the control only significant quantifiable proteins in both

Brodmann Area 4 of the cortex and Brodmann Area 6 of the cortex. The significant functions from the gene ontology analysis were organized from the lowest false detection rate (FDR) which showed the most significance to the highest false detection rate. The top 5 to 10 terms were gathered and formed into a table as shown in Figure 7. In the control only Brodmann Area 4 GO table, there were a lot of terms relating to the mitochondria in both biological processes and cell component categories. This signifies that the mitochondrial system is dysfunctional in the juvenile HD condition. This can be due to oxidative damage to the mitochondria causing neuronal stress and ultimately death to the neuron. There were some metabolic processes seen in the control only condition such as cellular amide metabolic processes. The same conclusions can be seen in the Brodmann Area 6 GO Table.

(a) BA4, Control Only

GO ID	GO Biological Process Pathway	Gene Count	FDR
GO: 0140053	mitochondrial gene expression	15 of 137	1.86E-07
GO: 0032543	mitochondrial translation	13 of 110	7.47E-07
GO: 0070125	mitochondrial translational elongation	12 of 89	7.47E-07
GO: 0070126	mitochondrial translational termination	12 of 91	7.47E-07
GO: 0044267	cellular protein metabolic process	62 of 3603	0.0109
GO: 0043603	cellular amide metabolic process	24 of 732	0.00049

GO ID	GO Cell Component Pathway	Gene Count	FDR
GO: 0005759	mitochondrial matrix	26 of 463	5.34E-09
GO: 0005739	mitochondrion	45 of 1531	1.02E-07
GO: 0005761	mitochondrial ribosome	12 of 86	1.02E-07
GO: 0098798	mitochondrial protein complex	17 of 251	3.25E-07
GO: 0005743	mitochondrial inner membrane	20 of 456	1.15E-05
GO: 0044391	mitochondrial envelope	25 of 722	1.62E-05
GO: 0031966	mitochondrial membrane	23 of 679	5.76E-05
GO: 0005762	mitochondrial large ribosomal subunit	7 of 53	9.68E-05
GO: 0005737	cytoplasm	156 of 11238	1.15E-05

(b) BA6, Control Only

GO ID	GO Biological Process Pathway	Gene Count	FDR
GO: 0032543	mitochondrial translation	12 of 110	3.87E-05
GO: 0070125	mitochondrial translational elongation	11 of 89	3.87E-05
GO: 0070126	mitochondrial translation termination	11 of 91	3.87E-05
GO: 0140053	mitochondrial gene expression	13 of 137	3.87E-05
GO: 0006518	peptide metabolic process	23 of 497	3.87E-05
GO: 0043603	cellular amide metabolic process	30 of 732	2.75E-05
GO: 0032984	protein complex disassembly	15 of 220	5.24E-05

GO ID	GO Cell Component Pathway	Gene Count	FDR
GO: 0005739	mitochondrion	48 of 1531	1.40E-07
GO: 0031966	mitochondrial membrane	30 of 679	1.63E-07
GO: 0005743	mitochondrial inner membrane	24 of 456	2.70E-07
GO: 0005761	mitochondrial ribosome	11 of 86	1.31E-06
GO: 0044429	mitochondrial part	34 of 1015	4.39E-06
GO: 0031090	organelle membrane	80 of 3337	5.16E-08
GO: 0005737	cytoplasm	192 of 11238	1.60E-12
GO: 0044424	Intracellular part	201 of 13996	1.99E-05

Figure 7: BA4 and BA6 Proteins Present in Only Aged- Matched Control Brains, Gene Ontology (GO) Analyses. A gene ontology table is depicted above showcasing the top five to ten terms for control only proteins in both (a) BA4 cortex and (b) BA6 cortex. The terms are organized by the false detection rate. The table also depicted the ID of the term and the gene count of the term.

A STRING protein interaction network also made along with the gene ontology analysis for the control only proteins in both regions as seen in Figure 8. The top term in each category of the gene ontology is used to color code the STRING protein interaction network. The color of the proteins represents the associated function in the STRING network. In Brodmann Area 4, there

were terms such as catalysis, mitochondrial matrix, and mitochondrial gene expression as the most significant terms of each category in the gene set. In Brodmann Area 6, there were terms such as cytoplasm and cell amide metabolism as the most significant terms of each category in the gene set.

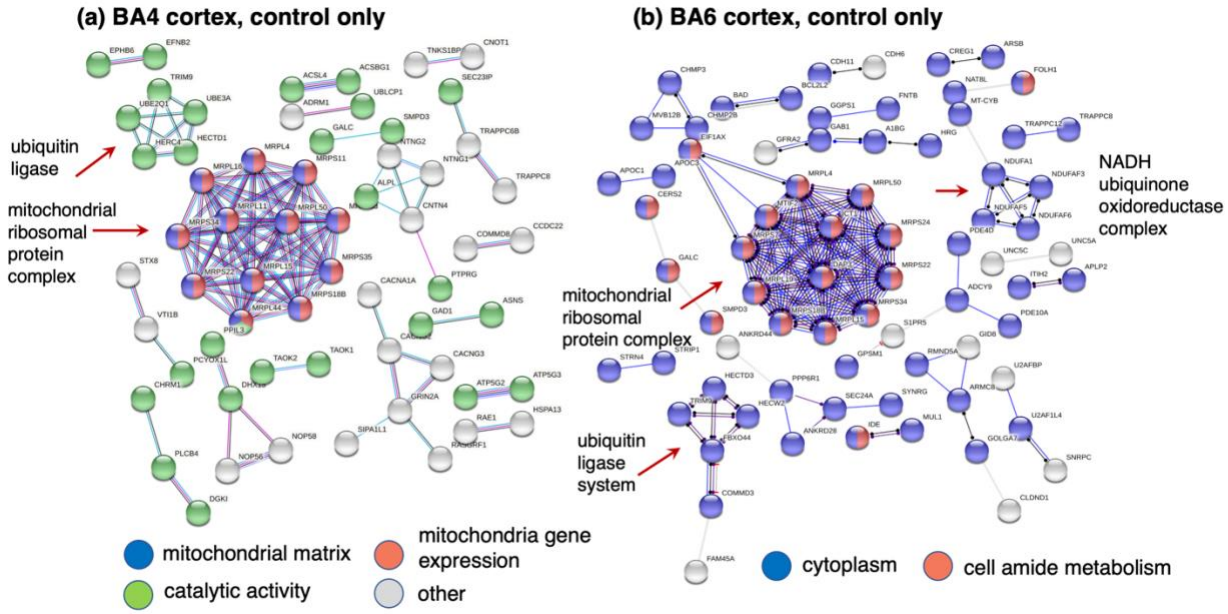

Figure 8: **BA4 and BA6 Proteins Present in Only Aged-Matched Control Brains, Network Analyses.** STRING protein interaction networks for aged-match only proteins in (a) BA4 cortex and (b) BA6 cortex are shown above. The STRING protein interaction networks depict the name of the protein and the relationship of the interaction with other proteins. The color of the proteins is associated with the colored circle next to the term shown in the legend. The red arrow represents particular clusters of proteins of interest.

Proteins Shared by Juvenile HD Brain Tissue and Control Brain Tissue; Up and Downregulated in Both Brodmann Area 4 and 6- After analyzing both the juvenile HD and control only conditions, the shared proteins were analyzed using heat maps, gene ontology analysis and STRING protein interaction networks. A heat map was created using the imputed logarithmic values ranging from greater than 2 and less than -2 of significant quantifiable proteins. The extent of upregulation shown in red and downregulation shown in blue can be seen in Figure 9. In the Brodmann Area 4 heat map, there is more upregulation than downregulation

in the juvenile HD condition and vice versa for the control condition. The same conclusions can be seen in the Brodmann Area 6 heat map.

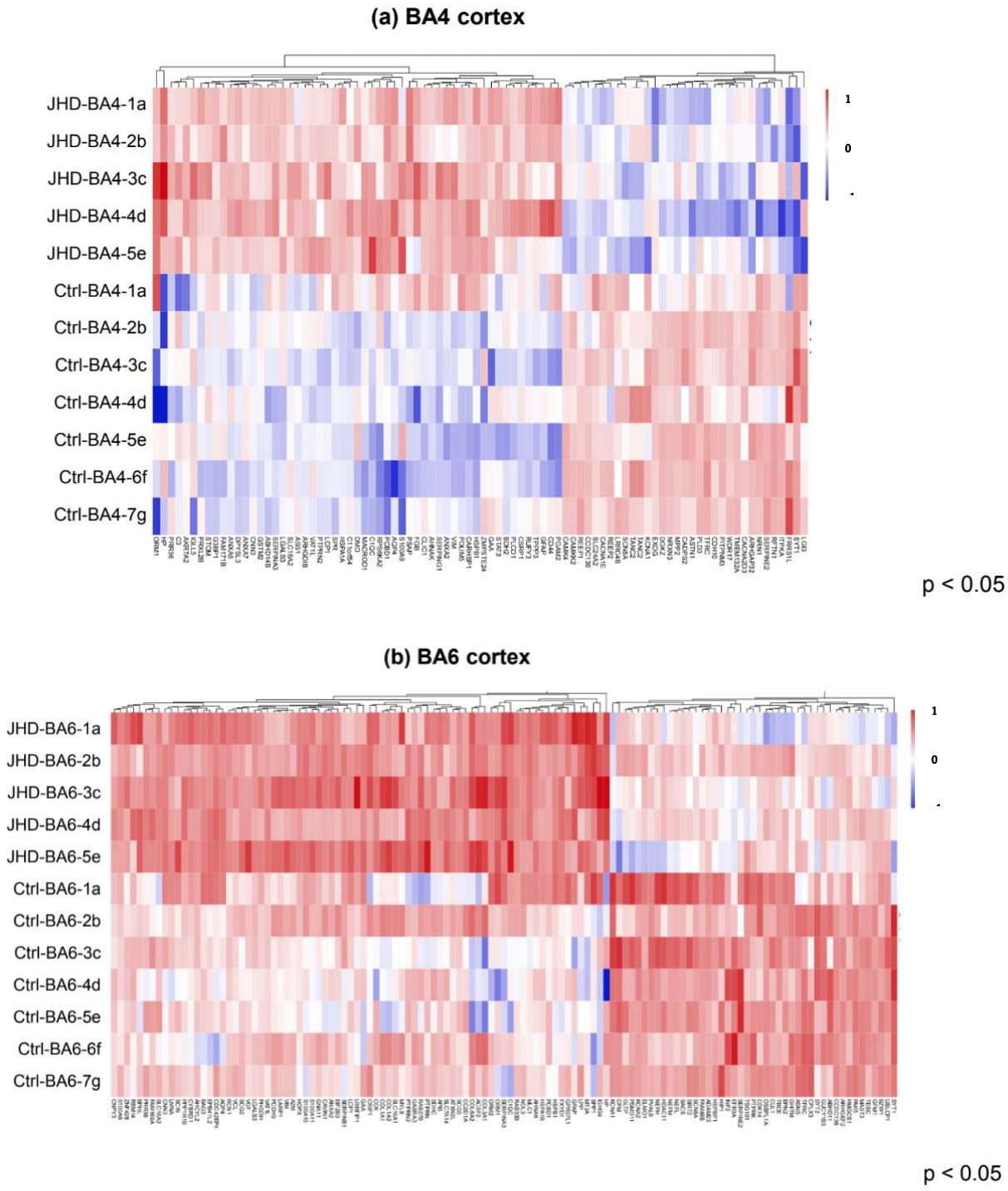

Figure 9: Expression of Shared BA4 and BA6 Proteins Illustrated by Heatmaps, $\log_2 > |1|$. A heatmap is shown above depicting the variation of expression of shared significant ($p < 0.05$) quantified and identified proteins (a) BA4 cortex and (b) BA6 cortex using $\log_2$ values. The sample name is represented on the left. The correlation of $\log_2$ values and color is shown on the right. The red represents the upregulation of proteins while the blue represents the down regulation of proteins.

Next, a gene ontology analysis was performed, and a STRING protein interaction network was made using the gene set of downregulated shared quantifiable proteins in both the Brodmann Area 4 and 6 of the cortex as shown in Figure 10. The top term in each category of the gene ontology is used to color code the STRING protein interaction network. The color of the proteins represents the associated function in the STRING network. In the STRING network consisting of downregulated Brodmann Area 4 proteins, there were significant terms associated with mitochondrial respiration and energy derivation by oxidation. In the STRING network consisting of downregulated Brodmann Area 6 proteins, there were significant terms associated with cellular process, protein binding and cytoplasm.

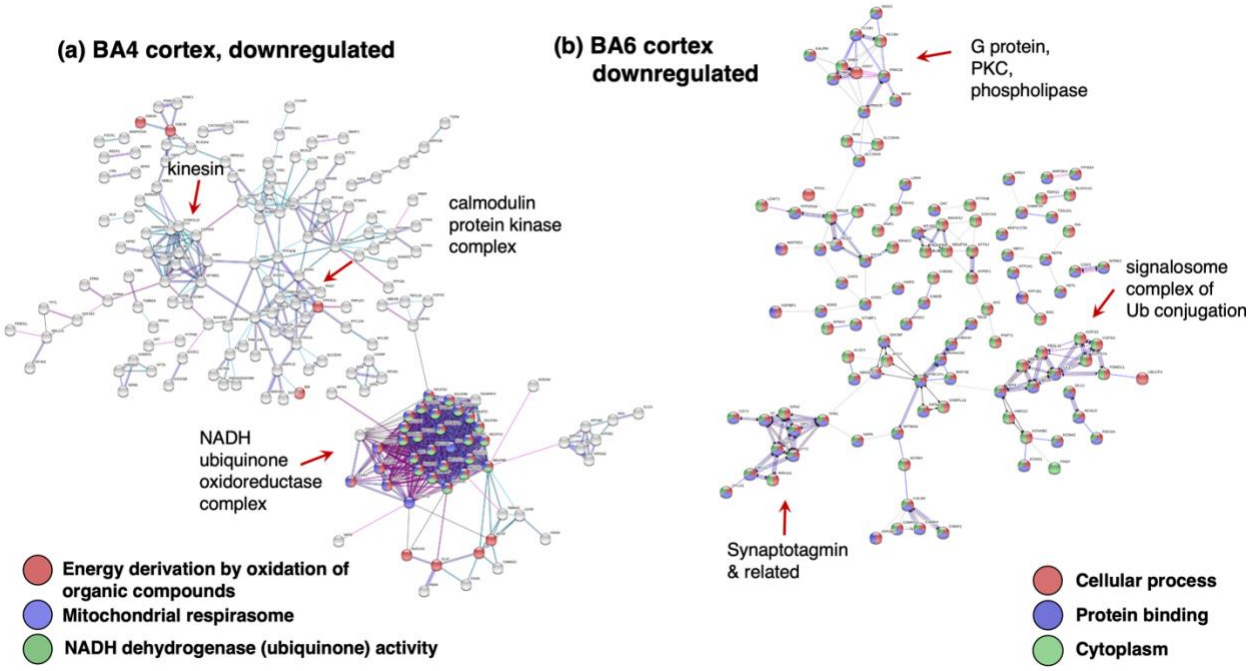

Figure 10: BA4 and BA6 Cortex Shared Downregulated Proteins, Network Analyses. STRING protein interaction networks for shared downregulated proteins in (a) BA4 cortex and (b) BA6 cortex are shown above. The STRING protein interaction networks depict the name of the protein and the relationship of the interaction with other proteins. The color of the proteins is associated with the colored circle next to the term shown in the legend. The red arrow represents particular clusters of proteins of interest.

In addition, a gene ontology analysis was performed, and a STRING protein interaction network was made using the gene set of upregulated shared quantifiable proteins in both Brodmann Area 4 and 6 of the cortex as shown in Figure 11. The top term in each category of the gene ontology is used to color code the STRING protein interaction network. The color of the proteins represents the associated function in the STRING network. In the STRING network consisting of upregulated Brodmann Area 4 proteins, there were significant terms associated with exocytosis, catalysis, and cytoplasm. In the STRING network consisting of upregulated Brodmann Area 6 proteins, there were significant terms associated with regulation biological, protein binding and cytoplasm.

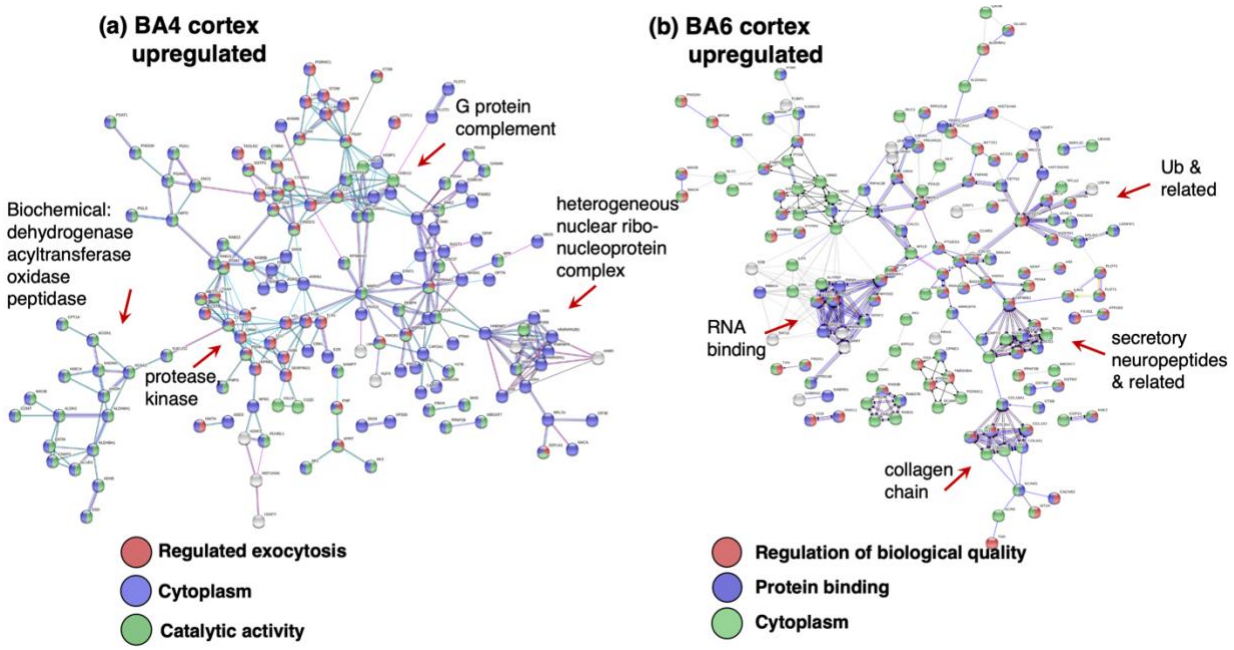

Figure 11: BA4 and BA6 Shared Upregulated Proteins, Network Analyses. STRING protein interaction networks for shared downregulated proteins in (a) BA4 cortex and (b) BA6 cortex are shown above. The STRING protein interaction networks depict the name of the protein and the relationship of the interaction with other proteins. The color of the proteins is associated with the colored circle next to the term shown in the legend. The red arrow represents particular clusters of proteins of interest.

Mitochondrial Gene Map Analysis of Brodmann Area 4 and 6- Due to the significant amounts of mitochondrial terms appearing in the downregulated or absent in juvenile HD, a mitochondrial map was created to showcase the genes and proteins downregulated or absent in juvenile HD as shown in Figure 12. In the mitochondria map, we can see the list of proteins affected by HD and what part of the mitochondrial system does it affect. This figure shows that NADH dehydrogenase was mostly affected by HD while other proteins such as cytochrome c oxidase, cytochrome c reductase, and F type ATPase were also affected by the diseases. As a result, HD affects the ATP synthesis pathway causing loss of energy to the cell and ultimately death.

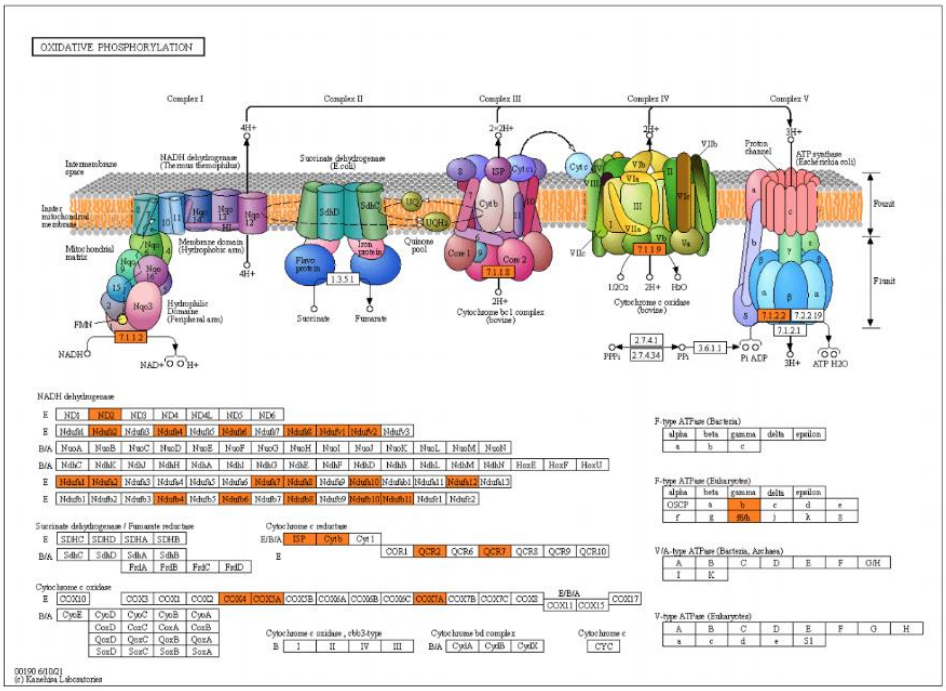

Figure 12: Mitochondria Map of Components Absent in Juvenile HD or Downregulated in Juvenile HD. The components that make up the complexes in the electron transport chain for oxidative phosphorylation are shown in the top of the figure. Affected mitochondrial components absent in juvenile HD or downregulated in juvenile HD are highlighted in the list of genes in each complex at the bottom of the figure.

Chapter 2, Figure 5, includes material, currently being prepared for submission for publication and will appear as Podvin S., Poon W., Mosier C., Rossitto L.-A., Wei E., and V. Hook.

"Juvenile Huntington's Disease Human Brain Proteomics Reveals Dysregulated Mitochondrial Systems and Neuropeptide Regulation." William Poon was the co-author of this paper.

Chapter 3: Proteomic Analysis of Putamen in Human Juvenile HD brain

Protein Count, Proteomics Data and Principal Component Analysis Associating with the Putamen- There were 167 proteins associated with the juvenile HD only condition and 471 proteins associated with the control only condition in the putamen. There were 3300 proteins that were shown between the juvenile HD and control condition in the putamen. Next, a principal component analysis was performed to visualize the extent of variation between our juvenile HD and control samples in the putamen as shown in Figure 13. The control only data points were clustered near each other and the juvenile HD data points were also clustered near each other. This shows that the control samples did not vary as much with the other control samples. The same can be concluded with the juvenile HD samples. The juvenile HD data points showed great separation to the control data points which signifies that the juvenile HD samples vary significantly to the control samples.

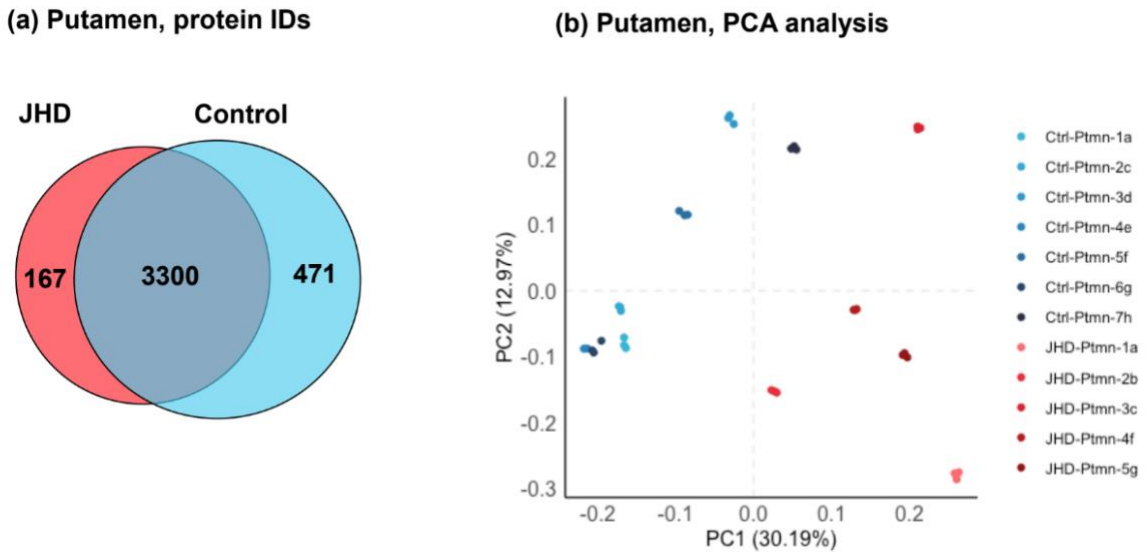

Figure 13: Putamen Protein identifications and Principal Component Analysis.
(a) The number of proteins identified in the juvenile HD condition, aged-match control condition, and shared in the putamen are shown in the Venn diagrams above. (b) The graph represents a principal component analysis depicting the extent of variation between samples of one sample group compared to the other sample group.

Proteins Present only in Juvenile HD brain at the Putamen- After a principal component analysis was performed on the putamen samples, gene ontology analysis was performed, and a STRING protein interaction network was made on the gene set of juvenile HD only proteins in the putamen. The significant functions from the gene ontology analysis were organized from the lowest false detection rate (FDR) which showed the most significance to the highest false detection rate. The top 5 to 10 terms were gathered and formed into a table as shown in Figure 14. In the juvenile HD only GO table, there were terms associated with the vesicle and cellular transport systems. The STRING protein interaction network consists of a map of proteins and how they interact with one another as shown in Figure 14. The top term in each category of the gene ontology is used to color code the STRING protein interaction network. The color of the proteins represents the associated function in the STRING network. In

the STRING network consisting of juvenile HD proteins in the putamen, there were proteins mostly associated with the endomembrane system.

(a) Putamen JHD Only GO

GO ID	GO Cell Component Pathway	Gene Count	FDR
GO:0041982	vesicle	41 of 2318	0.001
GO:0012505	endomembrane system	64 of 4347	0.00095
GO:0031410	cytoplasmic vesicle	39 of 2226	0.0016
GO:0005737	cytoplasm	122 of 11238	0.002
GO:0043227	membrane-bounded organelle	121 of 11244	0.0034
GO:0005793	endoplasmic reticulum-Golgi intermediate compartment	7 of 113	0.0046
GO:0035580	specific granule lumen	5 of 62	0.0115

(b) Putamen JHD Only STRING Network

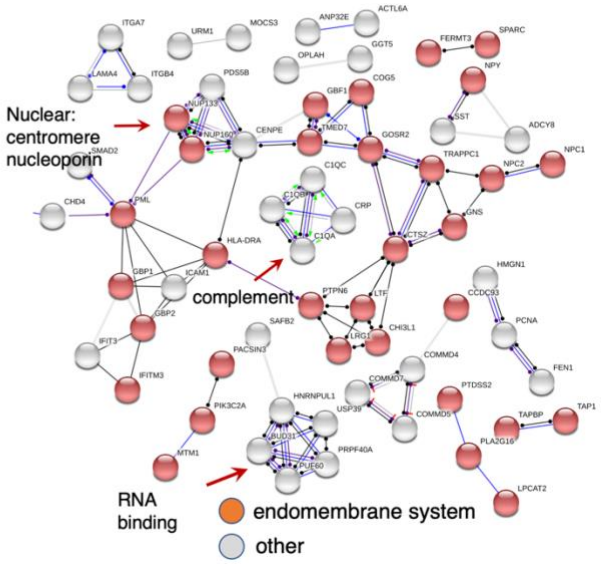

Figure 14: Putamen Proteins Present in Only Juvenile HD, GO and Network Analysis. (a) A gene ontology table is depicted on the left showcasing the top five to ten terms for juvenile HD only proteins in the putamen. The terms are organized by the false detection rate. The table also depicted the ID of the term and the gene count of the term. (b) A STRING protein interaction networks for juvenile HD proteins in the putamen are shown above. The STRING protein interaction networks depict the name of the protein and the relationship of the interaction with other proteins. The color of the proteins is associated with the colored circle next to the term shown in the legend. The red arrow represents particular clusters of proteins of interest.

Proteins Present only in Control Brain of the Putamen- In addition to the gene ontology analysis and STRING protein interaction network of juvenile HD proteins in the putamen, the same process was performed for control only proteins in the putamen as shown in Figure 15. In the GO table, the significant functions from the gene ontology analysis were organized from the lowest false detection rate (FDR) which showed the most significance to the highest false detection rate. The top 5 to 10 terms were gathered and formed into the table. In the control only GO table, there were terms associated with the mitochondria, metabolic processes and the

synapse. This infers that there may be synaptic dysfunction in the putamen since a lot of terms were associated with the synapse. In the STRING protein interaction network, the top term in each category of the gene ontology is used to color code the STRING protein interaction network. The color of the proteins represents the associated function in the STRING network. In the STRING network consisting of control only proteins in the putamen, there were proteins that were associated with phosphorus metabolism, catalysis and cytoplasm.

(a) Putamen Control Only, GO

GO ID	GO Biological Process Pathway	Gene Count	FDR
GO:0140053	mitochondrial gene expression	14 of 137	0.0043
GO:0006793	phosphorus metabolic process	91 of 2086	0.00011
GO:0006796	phosphate-containing metabolic process	90 of 2065	0.00011
GO:0006810	transport	141 of 4130	0.0047
GO:0048167	regulation of synaptic plasticity	16 of 164	0.0047
GO:0050804	modulation of chemical synaptic transmission	23 of 316	0.0047
GO:0051336	regulation of hydrolase activity	56 of 1238	0.0047

GO ID	GO Cell Component Pathway	Gene Count	FDR
GO:0045202	synapse	64 of 849	1.03E-12
GO:0014069	postsynaptic density	27 of 205	7.53E-10
GO:009894	postsynapse	36 of 435	2.90E-08
GO:0045211	postsynaptic membrane	26 of 237	3.47E-08
GO:0031966	mitochondrial membrane	46 of 679	4.35E-08
GO:0031090	organelle membrane	141 of 3337	6.91E-10

(b) Putamen Control Only, STRING Network

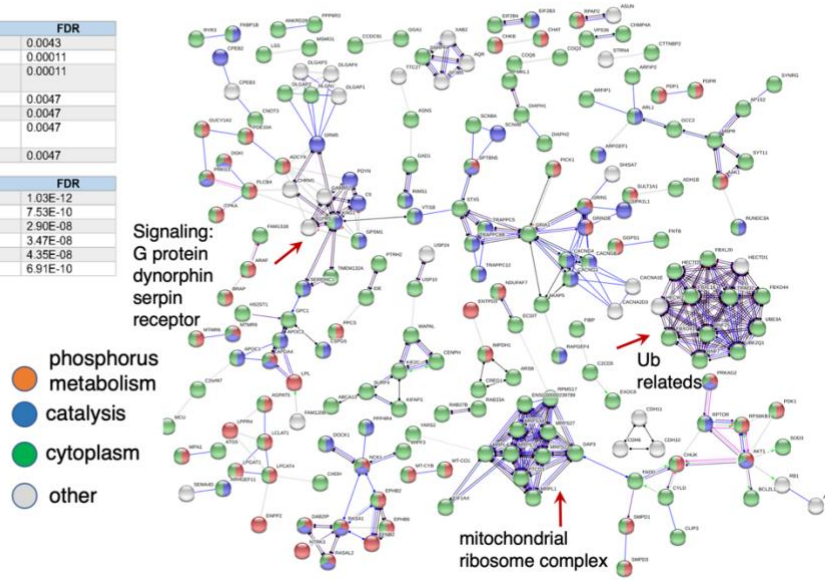

Figure 15: **Putamen Proteins Present in Aged-Match Control Only, GO and Network Analysis.** (a) A gene ontology table is depicted on the left showcasing the top five to ten terms for aged-match control only proteins in the putamen. The terms are organized by the false detection rate. The table also depicted the ID of the term and the gene count of the term. (b) A STRING protein interaction networks for aged-match control proteins in the putamen are shown above. The STRING protein interaction networks depict the name of the protein and the relationship of the interaction with other proteins. The color of the proteins is associated with the colored circle next to the term shown in the legend. The red arrow represents particular clusters of proteins of interest.

Proteins Shared by Juvenile HD Brain Tissue and Control Brain Tissue; Up and Downregulated in the Putamen- After analyzing both the juvenile HD and control only conditions, the shared proteins were analyzed using heat maps, gene ontology analysis and

STRING protein interaction networks. A heat map consisting of imputed logarithmic values ranging from greater than 2 and less than -2 of significant quantifiable proteins was used to showcase the extent of downregulation and upregulation of shared quantifiable proteins as shown in Figure 16. In the putamen heat map, there is more upregulation than downregulation in the juvenile HD condition and vice versa for the control condition.

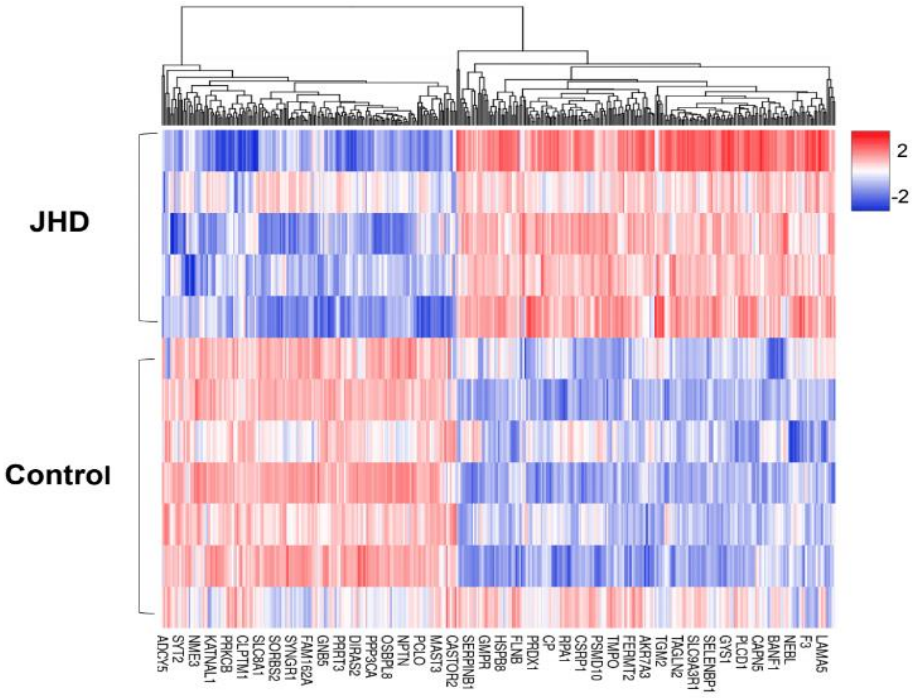

Figure 16: Expression of Shared Putamen Proteins Illustrated by Heatmaps, $\log_2 > |2|$.
A heatmap is shown above depicting the variation of expression of shared significant ($p < 0.05$) quantified and identified proteins putamen using $\log_2$ values. The sample condition is represented on the left. The correlation of $\log_2$ values and color is shown on the right. The red represents the upregulation of proteins while the blue represents the down regulation of proteins.

Using the gene set of the putamen upregulated shared proteins and downregulated shared proteins, gene ontology analysis was used to create GO tables as shown in Figure 17. The tables were organized from the lowest false detection rate (FDR) which showed the most significance to the highest false detection rate. The top 5 to 10 terms were gathered and formed into the table. In the downregulated shared proteins of the putamen GO table, there were terms mostly

associated with the mitochondria and ATP production. This infers that there is downregulation of mitochondrial functions in the putamen. In the upregulated shared proteins of the putamen GO table, there were terms mostly associated with the catabolic and metabolic processes.

(a) Putamen Downregulated GO Functions

GO ID	GO Biological Process	Gene Count	FDR
GO:0045333	cellular respiration	55 of 153	1.74E-38
GO:0006119	oxidative phosphorylation	47 of 100	2.53E-37
GO:0006119	generation of precursor metabolites and energy	72 of 388	1.58E-35
GO:0015980	energy derivation by oxidation of compounds	58 of 217	1.58E-35
GO:0009167	respiratory electron transport chain	56 of 230	1.53E-32
GO:0042775	mitochondrial ATP synthesis electron transport	39 of 78	7.84E-32
GO:0009205	purine ribonucleoside metabolic process	53 of 221	1.07E-30

GO ID	GO Cell Component Pathway	Gene Count	FDR
GO:0005737	inner mitochondrial membrane protein complex	50 of 128	2.83E-37
GO:0005746	mitochondria respirasome	43 of 85	9.99E-36
GO:0098803	respiratory chain complex	42 of 79	9.99D-36
GO:1990204	oxidoreductase complex	45 of 103	1.49E-35
GO:0005743	mitochondria inner membrane	74 of 456	3.94E-34
GO:0005739	mitochondrion	128 of 1531	4.23E-33
GO:0045202	synapse	97 of 849	5.42E-34
GO:0019866	organelle inner membrane	75 of 513	4.58E-32

(b) Putamen Upregulated GO Functions

GO ID	GO Biological Process	Gene Count	FDR
GO:0044248	cellular catabolic process	128 of 1646	1.56E-21
GO:0009056	catabolic process	133 of 1859	2.46 E-21
GO:0016071	mRNA metabolic process	72 of 667	3.72E-19
GO:0045055	regulated exocytosis	73 of 691	4.55E-19
GO:0044281	small molecule metabolic process	122 of 1779	2.39E-18

GO ID	GO Cell Component Pathway	Gene Count	FDR
GO:0005622	intracellular	506 of 14286	6.58E-39
GO:0005829	cytosol	302 of 4958	1.40E-49
GO:1990904	ribonucleoprotein complex	76 of 770	367E-19
GO:0043229	intracellular organelle	423 of 12193	3.95E-16
GO:0032991	protein-containing complex	222 of 4792	2.57E-16

Figure 17: GO Analyses of Shared Putamen Proteins that are Downregulated or Upregulated. A gene ontology table is depicted on the left showcasing the top five to ten terms for (a) downregulated and (b) upregulated shared proteins in the putamen. The terms are organized by the false detection rate. The table also depicted the ID of the term and the gene count of the term.

Along with the GO tables, a STRING protein interaction map was created using the gene sets of both upregulated and downregulated shared putamen proteins as shown in Figure 18. In the STRING protein interaction network, the top term in each category of the gene ontology is used to color code the STRING protein interaction network. The color of the proteins represents the associated function in the STRING network. In the STRING protein interaction network consisting of downregulated shared proteins of the putamen, there are proteins associated with respiration, dehydrogenase, and cytoplasm. In the STRING protein interaction network consisting of upregulated shared proteins of the putamen, there are proteins associated with cellular process, protein binding, and cytoplasm.

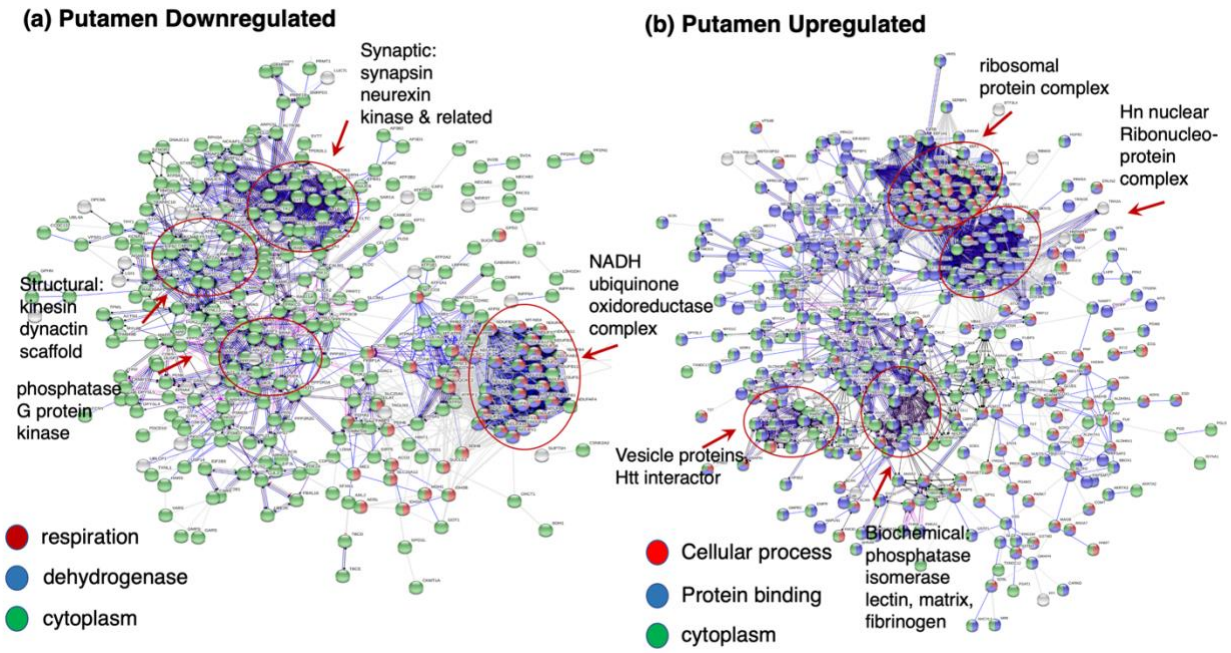

Figure 18: Network Analyses of Shared Putamen Proteins that are Downregulated or Upregulated. A STRING protein interaction networks for shared (a) downregulated or (b) upregulated in the putamen are shown above. The STRING protein interaction networks depict the name of the protein and the relationship of the interaction with other proteins. The color of the proteins is associated with the colored circle next to the term shown in the legend. The red arrow represents particular clusters of proteins of interest.

Neuropeptide Analysis of all Brain Regions- Since there were lots of GO terms associated with cellular transport, vesicles, and synapses, it would be important to look at the cell to cell communication of neurons. Specifically, neuropeptides are important to identify as they are the main methods of neuronal communication. To identify some of the neuropeptides in the juvenile HD only, control only, and shared within all three regions of the brain studied, our juvenile HD proteome data set was compared to the Neuropedia dataset of all known neuropeptides shown in Figure 19 and Supplemental Figure 7. In the juvenile HD only condition of the putamen, somatostatin and neuropeptide Y is only in juvenile HD condition while proenkephalin is only in the aged-match control. Six other neuropeptides were shared in both conditions such as

angiotensin and chromogranin A and B. Angiotensin is shown to be upregulated in juvenile HD and proenkephalin is shown to be downregulated in juvenile HD.

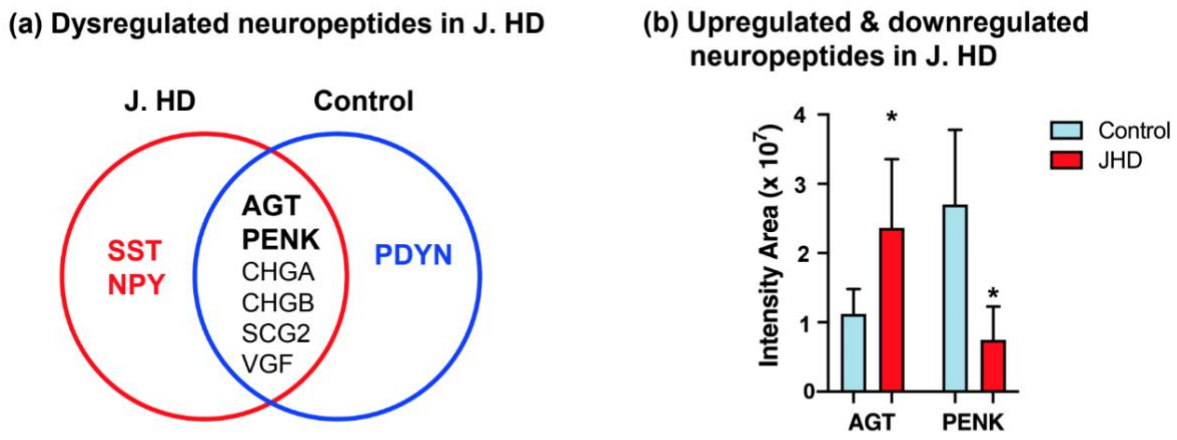

Figure 19: Neuropeptides Dysregulated in the Putamen in Juvenile HD.
(a) A Venn Diagram on the left is shown comparing neuropeptides that are juvenile HD only, shared, or in aged-match control in the putamen. (b) An intensity graph is shown on the right comparing angiotensin and proenkephalin levels between juvenile HD and aged-match control.

Chapter 3, Figure 13, includes material, currently being prepared for submission for publication and will appear as Podvin S., Poon W., Mosier C., Rossitto L.-A., Wei E., and V. Hook. “Juvenile Huntington’s Disease Human Brain Proteomics Reveals Dysregulated Mitochondrial Systems and Neuropeptide Regulation.” William Poon was the co-author of this paper.

Chapter 4: Discussion and Future Perspectives

Goal and Specific Aims of the Study - This study examined the (1) overall juvenile HD proteome in the Brodmann Areas 4 and 6, (2) overall juvenile HD proteome in the putamen, (3) neuropeptides identified in both control, shared and juvenile HD in all three regions of the brain studied. These results can help us understand (a) the cellular protein systems that are dysregulated in human juvenile HD brain cortex and putamen compared to age-matched controls (b) the peptide neurotransmitter system that are dysregulated in human juvenile HD brain cortex and putamen compared to age-matched controls.

Summary of Results - In the Brodmann Area 4 and 6, the data points were clustered near other points of the same group showing low variability between samples. There was also good separation between the JHD data points and control data points showing that both groups varied from each other. There was significant downregulation of the mitochondrial function or there was mitochondrial function absent in the juvenile HD condition. In the mitochondria map, the NADH dehydrogenase protein was mostly affected and other proteins such as cytochrome c oxidase, cytochrome c reductase, and F type ATPase were also affected. The ATP synthesis pathway was mostly affected in HD pathology.

In the putamen, there were terms associated with cytoplasm, vesicle, and cellular transport which indicated that cell transport may be upregulated in juvenile HD. There were terms relating to the synapse in the control only indicating that there may be synaptic dysfunction in the putamen. There were also lots of terms relating to the mitochondria that were downregulated or found in the control only condition indicating that the mitochondrial is dysfunctional in the putamen.

Since there were some GO terms found relating to cellular transport and synapse in the three regions, it is important to identify potential neuropeptides that may be affected by juvenile HD. In the study, there was neuropeptide Y identified in all three regions in the juvenile HD only condition suggesting neuropeptide Y could be one biomarker for the disease pathology. Somatostatin was found only in the putamen in the juvenile HD only condition. Dynorphin A was found only in the putamen of the control only condition.

Role of Cortical and Striatal Neurons in HD Pathology- In addition to neuropeptides being found to be affected in HD, it is important to address the location of where these neuropeptides are released. It is known that the neocortex and basal ganglia which includes the putamen has neurodegenerative properties in the HD pathology.[27] In the neocortex and basal ganglia, cortical neurons and striatal neurons are the main types of neurons found in both of these regions. These neurons are important for communication to other parts of the brain.[27] It is known that the cortex stimulates the striatal projection neurons via glutamatergic release.[27] The striatal neurons have tonic inhibition over corticostriatal neurons by increasing the number of dendritic spines on projection neurons.[27] In HD pathology, dopaminergic neurons are degenerated which can cause the imbalance of excitatory and inhibitory responses from other neurons.[27] If striatal dopaminergic neurons are decreased due to HD pathology, there is a loss of inhibitory control in glutamatergic release causing neurotoxicity to other parts of the brain.[27]

Future Directions Towards Neuropeptide Research Using Neuropeptidomics- In recent research, neuropeptidomics is becoming a booming field by identifying how pro neuropeptides become processed by being cleaved using proteases. As a result, diverse peptides are formed from their precursors using protease systems which generates neuropeptidomes. Using multiplex substrate profiling by mass spectrometry, cleavage profiles of neuropeptides can be discovered

which can help determine the neuropeptidome. In addition, other distinct neuropeptide forms may be discovered in the neuropeptidome. Future investigation of neuropeptides in more depth by neuropeptidomics should be performed for unbiased coverage of neuropeptide forms present in isolated nerve terminals of juvenile HD brain tissue.

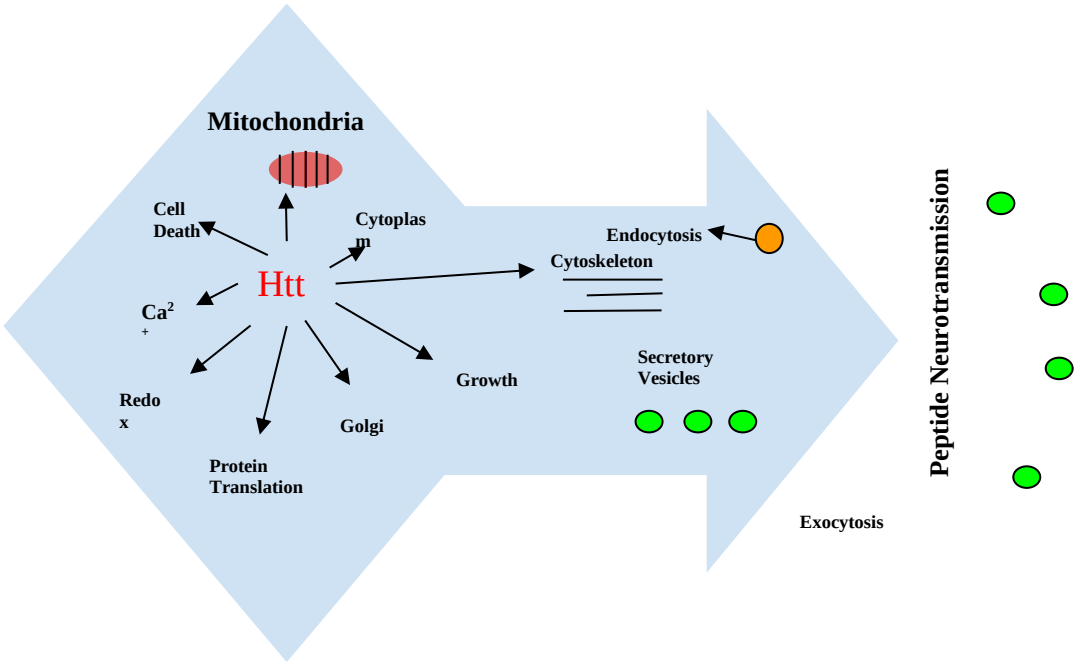

Figure 20: Cellular Pathways that Mutant Htt Can Regulate. A neuron is shown above with other pathways that the mutant Huntingtin protein can affect. Mutant Huntingtin protein can affect other pathways such as the Golgi system, secretory vesicle systems, protein translation system, cytoplasmic systems, cytoskeletal systems, cellular transport systems, and calcium signaling. The systems highlighted in our study are mitochondrial systems and peptide neurotransmission systems.

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Supplemental Information

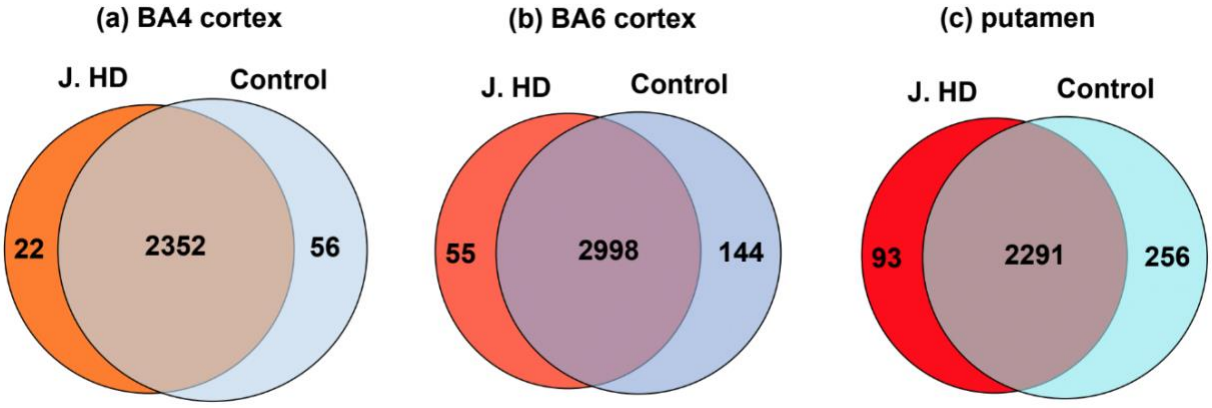

Figure S1: Quantifiable Proteins in BA4 and BA6 Cortex, and Putamen, from Juvenile HD and Aged-Match Control Brains. Venn diagrams containing the number of quantified proteins in juvenile HD, aged-match controls, and shared in the (a) BA4 cortex (b) BA6 cortex (c) putamen of the brain shown above.

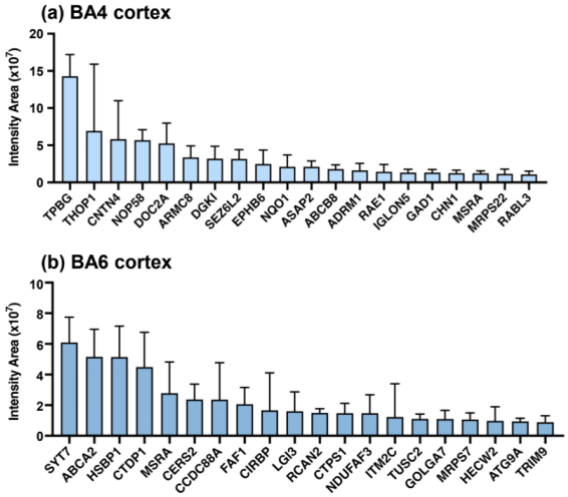

Figure S2: Most Abundant BA4 and BA6 proteins Present in Aged-Match Control Only Brains. The intensity graph shown above depicts the top twenty most abundant proteins in the aged-match control only condition in the (a) BA4 cortex and (b) BA6 cortex.

(a) BA4, downregulated, GO

GO ID	GO Biological Process	Gene Count	FDR
GO:0042775	mitochondrial ATP synthesis coupled electron transport	29 of 217	8.32E-15
GO:0032981	mitochondrial respiratory chain complex I assembly	17 of 65	8.46E-14
GO:0033108	mitochondrial respiratory chain complex assembly	18 of 98	1.28E-12
GO:0006120	mitochondrial electron transport, NADH to ubiquinone	14 of 44	1.99E-12
GO:0045333	cellular respiration	25 of 153	3.27E-16
GO:0006119	oxidative phosphorylation	20 of 100	1.89E-14
GO:0006091	generation of precursor metabolites and energy	31 o f 388	4.04E-13
GO:0046034	ATP metabolic process	23 of 190	7.04E-13
GO:0009205	purine ribonucleoside triphosphate metabolic process	24 of 221	1.29E-12

GO ID	GO Cell Component Pathway	Gene Count	FDR
GO:0005746	mitochondrial respirasome	22 of 85	2.96E-18
GO:009880	respiratory chain complex	21 of 79	5.14E-18
GO:199020	oxidoreductase complex	207 of 11238	5.28E-18
GO:0005747	mitochondrial respiratory chain complex I	16 of 46	2.16E-15
GO:0044455	mitochondrial membrane part	25 of 203	4.19E-15
GO:0098800	inner mitochondrial membrane protein complex	21 of 128	6.61E-15
GO:000573	cytoplasm	207 of 11238	5.28E-18

(b) BA6, downregulated, GO

GO ID	GO Biological Process	Gene Count	FDR
GO:0006796	phosphate-containing compound metabolic process	56 of 2065	4.57E-07
GO:0051234	establishment of localization	86 of 4248	2.10E-06
GO:1901564	organonitrogen compound metabolic process	99 of 5281	2.77E-06
GO:0016043	cellular component organization	97of 5163	3.44E-06
GO:0006810	transport	83 of 4130	3.87E-06
GO:0006996	organelle organization	69 of 3131	3.87E-06
GO:0051049	regulation of transport	45 of 1732	2.34E-05

GO ID	GO Cell Component Pathway	Gene Count	FDR
GO:0030424	axon	28 of 530	2.04E-09
GO:0045202	synapse	34 of 849	1.17E-08
GO:0043005	neuron projection	39 of 1142	3.51E-08
GO:0014069	postsynaptic density	14 of 205	8.12E-06
GO:0044304	main axon	9 of 67	8.12E-06
GO:0005829	cytosol	109 of 4958	1.29E-12
GO:0005622	intracellular	202 of 14286	1.04E-11

Figure S3: GO Analyses of Shared Downregulated BA4 and BA6 Proteins. A gene ontology table is depicted above showcasing the top five to ten terms for shared downregulated only proteins in both (a) BA4 cortex and (b) BA6 cortex. The terms are organized by the false detection rate. The table also depicted the ID of the term and the gene count of the term.

(a) BA4 Upregulated GO

GO ID	GO Biological Process Pathway	Gene Count	FDR
GO:0006887	exocytosis	48 of 774	2.27E-17
GO:0045055	regulated exocytosis	46 of 691	2.27E-17
GO:0032940	secretion by cell	52 of 959	7.86E-17
GO:0016192	vesicle-mediated transport	64 of 1699	1.52E-14
GO:0002443	leukocyte mediated immunity	43 of 632	7.86E-17
GO:0043312	neutrophil degranulation	38 of 485	1.05E-16
GO:0002274	myeloid leukocyte activation	39 of 574	1.11E-15
GO:0000225	immune effector process	47 of 927	1.35E-14

GO ID	GO Cell Component Pathway	Gene Count	FDR
GO:0005737	cytosol	207 of 11238	4.53E-24
GO:0031982	vesicle	79 of 2318	1.99E-16
GO:0030141	secretory granule	45 of 828	3.06E-15
GO:0099503	secretory vesicle	47 of 948	8.39E-15
GO:0031410	cytoplasmic vesicle	75 of 2226	3.06E-15
GO:0005622	intracellular	218 of 14286	4.64E-15
GO:0101002	ficolin-1-rich granules	19 of 186	1.56E-10

(b) BA6 Upregulated GO

GO ID	GO Biological Process Pathway	Gene Count	FDR
GO:0065008	regulation of biological quality	101 of 3559	2.24E-12
GO:0006810	transport	101 of 4130	8.98E-09
GO:0045055	regulated exocytosis	33 of 691	1.56E-07
GO:0002274	myeloid leukocyte activation	29 of 574	4.54E-07
GO:0036230	granulocyte activation	27 of 502	4.54-07
GO:0002275	myeloid cell immune response	27 of 519	7.01E-07

GO ID	GO Cell Component Pathway	Gene Count	FDR
GO:0031982	vesicle	76 of 2318	2.60E-12
GO:0099503	secretory vesicle	46 of 948	3.04E-12
GO:0005737	cytoplasm	216 of 11238	9.34E-20
GO:0030141	secretory granule	42 of 828	6.09E-12
GO:0005622	intracellular	229 of 14286	4.71E-11
GO:0012505	whole membrane	102 of 4347	3.97E-09

Figure S4: GO Analyses of Shared Upregulated BA4 and BA6 Proteins. A gene ontology table is depicted above showcasing the top five to ten terms for shared upregulated only proteins in both (a) BA4 cortex and (b) BA6 cortex. The terms are organized by the false detection rate. The table also depicted the ID of the term and the gene count of the term.

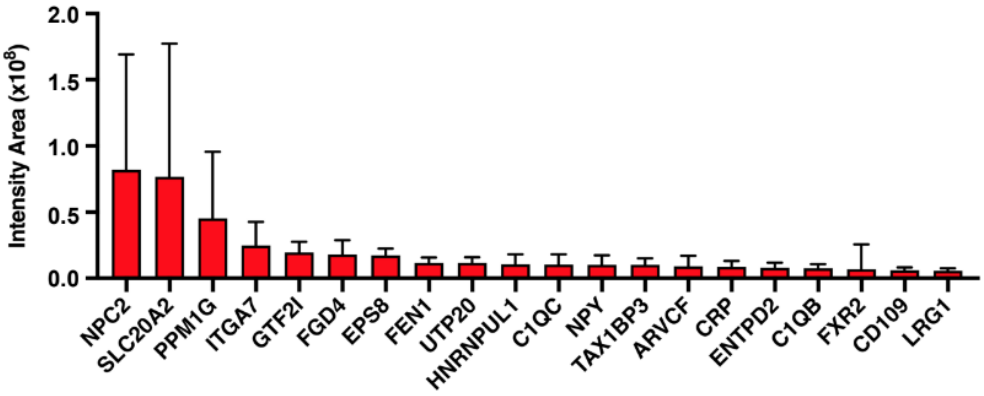

Figure S5: Most Abundant Putamen Proteins present Only in Juvenile HD. Intensity value graphs are shown above depicting the top twenty most abundant proteins and their intensity values for juvenile HD only proteins.

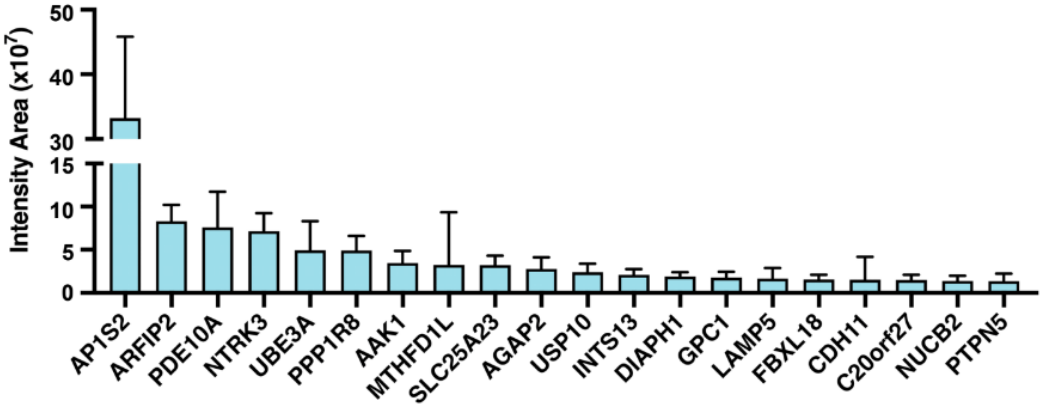

Figure S6: Most Abundant Putamen Proteins Present Only in Aged-Match Control. Intensity value graphs are shown above depicting the top twenty most abundant proteins and their intensity values for aged-match control only proteins.

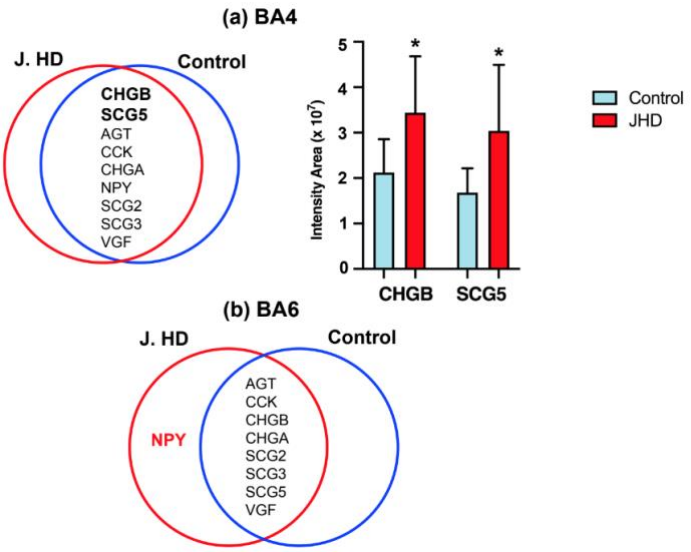

Figure S7: Neuropeptides Dysregulated in the BA4 and BA6 Cortex. A Venn Diagram on the left is shown comparing neuropeptides that are juvenile HD only, shared, or in aged-match control in the (a) BA4 cortex and (b) BA6 cortex. An intensity graph is shown on the right comparing chromogranin B and secretogranin 5 levels between juvenile HD and aged-match control.

SI Table #S1. BA4 and BA6 Proteins Present in only Controls. Hub Protein Functions of Network. A literature analysis was performed on BA4 and BA6 control hub proteins, which are proteins with the most interactions. The brain region, gene name, protein name, # of nodes (interactions), normal functions and the proteins' function in HD are listed in the table below.

Supplemental Table S1. BA4 and BA6 proteins present in only controls: hub protein functions of network
(Hub proteins from Figure 5)

Brain region	Gene name	Protein name	# nodes	Normal functions	Functions in HD
BA4 cortex	MRPL16	Mitochondrial Ribosomal Protein L16	11	Mitochondrial ribosome 39S subunit protein	possible ribosomal traffic jam in HD and other neurodegenerative disease[28]
	MRPL4	Mitochondrial Ribosomal Protein L4	11	Mitochondrial ribosome 39S subunit protein	"
	MRPS11	Mitochondrial Ribosomal Protein S11	11	Mitochondrial ribosome 28S subunit protein	"
	MRPS34	Mitochondrial Ribosomal Protein S34	11	Mitochondrial ribosome 28S subunit protein	"
	MRPL11	Mitochondrial Ribosomal Protein L11	11	Mitochondrial ribosome 39S subunit protein	"
	MRPL50	Mitochondrial Ribosomal Protein L50	11	Mitochondrial ribosome 39S subunit protein	"
	MRPS22	Mitochondrial Ribosomal Protein S22	11	Mitochondrial ribosome 28S subunit protein	"
	MRPL15	Mitochondrial Ribosomal Protein L15	11	Mitochondrial ribosome 39S subunit protein	"
	MRPS35	Mitochondrial Ribosomal Protein S35	11	Mitochondrial ribosome 28S subunit protein	"
	MRPL44	Mitochondrial Ribosomal Protein L44	11	Mitochondrial ribosome 39S subunit protein	"
	MRPS18B	Mitochondrial Ribosomal Protein S18B	11	Mitochondrial ribosome 28S subunit protein	"
	UBE2Q1	Ubiquitin Conjugating Enzyme E2 Q1	4	Ubiquitin. Catalyzes the covalent attachment of ubiquitin to other proteins	shown to have decreased activity in HD which causes synaptic dysfunction[29]
	UBE3A	Ubiquitin Protein Ligase E3A	4	Ubiquitin Ligase	loss of function due to bonding with mHtt aggregates[30]
	HERC4	HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase 4	4	Ubiquitin Ligase	UPS is impaired in mHtt which causes it to be unable to degrade mHtt which causes it to aggregate[31]
	HECTD1	HECT Domain E3 Ubiquitin Protein Ligase 1	4	E3 ubiquitin-protein ligase	UPS is impaired in mHtt which causes it to be unable to degrade mHtt which causes it to aggregate[31]
	TRIM9	Tripartite Motif Containing 9	4	Its function has not been identified. "Localizes to cytoplasmic bodies" "may also participate in the formation or breakdown of abnormal inclusions in neurodegenerative disorders."	n/a
BA6 cortex	MRPL4	Mitochondrial Ribosomal Protein L4	12	Mitochondrial ribosome 39S subunit protein	possible ribosomal traffic jam in HD and other neurodegenerative disease[28]
	MRPL50	Mitochondrial Ribosomal Protein L50	11	Mitochondrial ribosome 39S subunit protein	"
	MRPS24	Mitochondrial Ribosomal Protein S24	11	Mitochondrial ribosome 28S subunit protein	"
	MRPS7	Mitochondrial Ribosomal Protein S7	12	Mitochondrial ribosome 28S subunit protein	"
	MRPL19	Mitochondrial Ribosomal Protein L19	11	Mitochondrial ribosome 39S subunit protein	"
	MRPS22	Mitochondrial Ribosomal Protein S22	11	Mitochondrial ribosome 28S subunit protein	"
	MRPS18B	Mitochondrial Ribosomal Protein S18b	11	Mitochondrial Ribosomal 28S subunit protein	"
	MRPL15	Mitochondrial Ribosomal Protein L15	11	Mitochondrial Ribosomal 39S subunit protein	"
	MRPS34	Mitochondrial Ribosomal Protein S34	11	Mitochondrial Ribosomal 28S subunit protein	"
	MTIF2	Mitochondrial Translational Initiation Factor 2	11	Initiation Factor 2 protein	n/a
	ICT1	Mitochondrial Ribosomal Protein L58	11	A peptidyl-tRNA hydrolase and component of the large mitochondrial ribosome.	possible ribosomal traffic jam in HD and other neurodegenerative disease[28]
	DAP3	Death Associated Protein 3	11	28S subunit protein that also participates in apoptotic pathways which are initiated by tumor necrosis factor-alpha Involved in mediating interferon-gamma-induced cell death	activation of apoptotic cascade causing death to neurons over time[31]
	NDUFA1	NADH:Ubiquinone Oxidoreductase Subunit A1	4	component of complex I of the respiratory chain, which transfers electrons from NADH to ubiquinone	defect in this complex by mutation in one of its subunits which may cause decreased ATP production[32]
	NDUFAF3	NADH:Ubiquinone Oxidoreductase Complex Assembly Factor 3	3	mitochondrial complex I assembly protein that interacts with complex I subunits	"
	NDUFAF5	NADH:Ubiquinone Oxidoreductase Complex Assembly Factor 5	3	a mitochondrial protein that is associated with the matrix face of the mitochondrial inner membrane and is	"
	NEUFAF6	NADH:Ubiquinone Oxidoreductase Complex Assembly Factor 6	3	in the assembly of complex I (NADH-ubiquinone oxidoreductase) of the mitochondrial respiratory chain through regulation of subunit ND1 biogenesis	defect in this complex by mutation in one of its subunits which may cause decreased ATP production[32]
	HECTD3	HECT Domain E3 Ubiquitin Protein Ligase 3	3	transfers ubiquitin from an E2 ubiquitin-conjugating enzyme to targeted substrates	loss of function due to bonding with mHtt aggregates[30]
	HECW2	HECT, C2 And WW Domain Containing E3 Ubiquitin Protein	3	E3 ubiquitin-protein ligase that mediates ubiquitination of TP73	loss of function due to bonding with mHtt aggregates[30] involved with some connection to HACE1 protein[33]
	FBXO44	F-Box Protein 44	4	subunit of the ubiquitin protein ligase complex, functions in phosphorylation-dependent ubiquitination	n/a
	TRIM9	Tripartite Motif Containing 9	3	TRIM motif includes three zinc-binding domains, a RING, a B-box type 1 and a B-box type 2, and a coiled-coil region. Its function has not been identified.	n/a

SI Table #S2. BA4 Proteins Downregulated. Hub Protein Functions of Network. A literature analysis was performed on BA4 downregulated proteins, which are proteins with the most interactions. The brain region, gene name, protein name, # of nodes (interactions), normal functions and the proteins' function in HD are listed in the table below.

BA6 cortex	MRPL4	Mitochondrial Ribosomal Protein L4	12	Mitochondrial ribosome 39S subunit protein	possible ribosomal traffic jam in HD and other neurodegenerative disease[28]
	MRPL50	Mitochondrial Ribosomal Protein L50	11	Mitochondrial ribosome 39S subunit protein	"
	MRPS24	Mitochondrial Ribosomal Protein S24	11	Mitochondrial ribosome 28S subunit protein	"
	MRPS7	Mitochondrial Ribosomal Protein S7	12	Mitochondrial ribosome 28S subunit protein	"
	MRPL19	Mitochondrial Ribosomal Protein L19	11	Mitochondrial ribosome 39S subunit protein	"
	MRPS22	Mitochondrial Ribosomal Protein S22	11	Mitochondrial ribosome 28S subunit protein	"
	MRPS18B	Mitochondrial Ribosomal Protein S18b	11	Mitochondrial Ribosomal 28S subunit protein	"
	MRPL15	Mitochondrial Ribosomal Protein L15	11	Mitochondrial Ribosomal 39S subunit protein	"
	MRPS34	Mitochondrial Ribosomal Protein S34	11	Mitochondrial Ribosomal 28S subunit protein	"
	MTIF2	Mitochondrial Translational Initiation Factor 2	11	Initiation Factor 2 protein	n/a
	ICT1	Mitochondrial Ribosomal Protein L58	11	A peptidyl-tRNA hydrolase and component of the large mitochondrial ribosome.	possible ribosomal traffic jam in HD and other neurodegenerative disease[28]
	DAP3	Death Associated Protein 3	11	28S subunit protein that also participates in apoptotic pathways which are initiated by tumor necrosis factor-alpha Involved in mediating interferon-gamma-induced cell death	activation of apoptotic cascade causing death to neurons over time[31]
	NDUFA1	NADH:Ubiquinone Oxidoreductase Subunit A1	4	component of complex I of the respiratory chain, which transfers electrons from NADH to ubiquinone	defect in this complex by mutation in one of its subunits which may cause decreased ATP production[32]
	NDUFAF3	NADH:Ubiquinone Oxidoreductase Complex Assembly Factor 3	3	mitochondrial complex I assembly protein that interacts with complex I subunits	"
	NDUFAF5	NADH:Ubiquinone Oxidoreductase Complex Assembly Factor 5	3	a mitochondrial protein that is associated with the matrix face of the mitochondrial inner membrane and is required for complex I assembly.	"
	NDUFAF4	NADH:Ubiquinone Oxidoreductase Complex Assembly Factor 4	24	"	"
	NDUFC2	NADH:Ubiquinone Oxidoreductase Subunit C2	29	"	"
	NDUFS7	NADH:Ubiquinone Oxidoreductase Core Subunit S7	28	"	"
	NDUFB7	NADH:Ubiquinone Oxidoreductase Subunit B7	30	"	"
	NDUFAB1	NADH:Ubiquinone Oxidoreductase Subunit AB1	29	"	"
	NDUFS5	NADH:Ubiquinone Oxidoreductase Subunit S5	30	"	"
	NDUFB1	NADH:Ubiquinone Oxidoreductase Subunit B1	39	"	"
	NDUFV3	NADH:Ubiquinone Oxidoreductase Subunit V3	27	"	"
	NDUFA12	NADH:Ubiquinone Oxidoreductase Subunit A12	29	"	"
	UQCRB	Ubiquinol-Cytochrome C Reductase Binding Protein	27	Binds ubiquinone and participates in the transfer of electrons when ubiquinone is bound	complex III activity is reduced in HD since complex III levels are down[33]
	UQCRC2	Ubiquinol-Cytochrome C Reductase Core Protein 2	30	part of the ubiquinol-cytochrome c reductase complex (also known as complex III). This complex constitutes a part of the mitochondrial respiratory chain	complex III activity is reduced in HD since complex III levels are down[33]
	UQCRFS1	Ubiquinol-Cytochrome C Reductase, Rieske Iron-Sulfur Polypeptide 1	32	Component of the ubiquinol-cytochrome c oxidoreductase, a multisubunit transmembrane complex that is part of the mitochondrial electron transport chain which drives oxidative phosphorylation	complex III activity is reduced in HD since complex III levels are down[33]
	COX5A	Cytochrome C Oxidase Subunit 5A	28	Subunit of Cytochrome c oxidase (COX), terminal enzyme of the mitochondrial respiratory chain	allosteric inhibition of COX causing increased reactive oxygen species; thus leads to apoptosis[34]
	COX4I1	Cytochrome C Oxidase Subunit 4I1	10	"	"
	COX6C	Cytochrome C Oxidase Subunit 6C	18	Component of the cytochrome c oxidase, the last enzyme in the mitochondrial electron transport chain which drives oxidative phosphorylation.	"

	CAMK2A	Calcium/Calmodulin Dependent Protein Kinase II Alpha	4	Calmodulin-dependent kinases (CaMK) are a family of serine/threonine kinases that mediate many of the second messenger effects of Ca2+.	reduced expression of CAMK2 in HD[37]
	CAMK2B	Calcium/Calmodulin Dependent Protein Kinase II Beta	7	Calcium/calmodulin-dependent protein kinase that functions autonomously after Ca(2+)/calmodulin-binding and autophosphorylation, and is involved in dendritic spine and synapse formation, neuronal plasticity and regulation of sarcoplasmic reticulum Ca(2+) transport in skeletal muscle.	"
	CAMK2A	Calcium/Calmodulin Dependent Protein Kinase II Alpha	4	Calmodulin-dependent kinases (CaMK) are a family of serine/threonine kinases that mediate many of the second messenger effects of Ca2+.	"
	GNB5	G Protein Subunit Beta 5	5	Enhances GTPase-activating protein (GAP) activity of regulator of G protein signaling (RGS) proteins, hence involved in the termination of the signaling initiated by the G protein coupled receptors (GPCRs) by accelerating the GTP hydrolysis on the G-alpha subunits, thereby promoting their inactivation.	decreased expression or activity of GPCRs causes increase risk of neurodegenerative processes and changed neuronal plasticity [38]
	GNAI1	G Protein Subunit Alpha I1	6	Binds guanine nucleotide, can hydrolyze GTP, and can interact with other proteins. Alpha subunit of an inhibitory complex.	n/a
	PPP1CA	Protein Phosphatase 1 Catalytic Subunit Alpha	8	Protein phosphatase that associates with over 200 regulatory proteins to form highly specific holoenzymes which dephosphorylate hundreds of biological targets.	modulate Huntingtin exon 1 aggregation and toxicity[39]
	PPP3CB	Protein Phosphatase 3 Catalytic Subunit Beta	6	Calcium-dependent, calmodulin-stimulated protein phosphatase which plays an essential role in the transduction of intracellular Ca(2+)-mediated signals	n/a
	PRKCE	Protein Kinase C Epsilon	7	Protein kinase C (PKC) is a family of serine- and threonine-specific protein kinases that can be activated by calcium and the second messenger diacylglycerol.	n/a
	PRKCB	Protein Kinase C Beta	4	Calcium-activated, phospholipid- and diacylglycerol (DAG)-dependent serine/threonine-protein kinase involved in various cellular processes such as regulation of the B-cell receptor (BCR) signalosome, oxidative stress-induced apoptosis, androgen receptor-dependent transcription regulation, insulin signaling and endothelial cells proliferation	mRNA of PRKCB are decreased which may cause the loss of ability to store information [40]
	HRAS	HRas Proto-Oncogene, GTPase	13	Involved in the activation of Ras protein signal transduction	n/a
	GRIN2B	Glutamate Ionotropic Receptor NMDA Type Subunit 2B	4	Component of NMDA receptor complexes that function as heterotetrameric, ligand-gated ion channels with high calcium permeability and voltage-dependent sensitivity to magnesium	mHTT interacts with NMDA receptor causing excitotoxicity and neurodegeneration[41]
	RGS7	Regulator Of G Protein Signaling 7	2	Regulates G protein-coupled receptor signaling cascades. Inhibits signal transduction by increasing the GTPase activity of G protein alpha subunits, thereby driving them into their inactive GDP-bound form.	n/a
	SHOC2	SHOC2 Leucine Rich Repeat Scaffold Protein	3	Regulatory subunit of protein phosphatase 1 (PP1c) that acts as a M-Ras/MRAS effector and participates in MAPK pathway activation.	involved with SHOC2-Ras-Raf1 complex that has ATPase PSMC5 as component which can cause misfolding and aggregation of proteins in HD[42]
	KIF3B	Kinesin Family Member 3B	8	Acts as a heterodimer with kinesin family member 3A to aid in chromosome movement during mitosis and meiosis	mHTT may impair proteins for neuronal trafficking or organelle trafficking in neurons [43]
	KIF5A	Kinesin Family Member 5A	9	Subunit of complex that functions as a microtubule motor in intracellular organelle transport	"
	KLC2	Kinesin Light Chain 2	8	Light chain of kinesin, molecular motor responsible for moving vesicles and organelles along microtubules	mHTT may impair proteins for neuronal trafficking or organelle trafficking in neurons [43]
	DCTN1	Dynactin Subunit 1	12	Dynactin binds to both microtubules and cytoplasmic dynein. Subunit interacts with dynein and binds to microtubules	mHTT may impair proteins for neuronal trafficking or organelle trafficking in neurons [43]
	DYNC1LI2	Dynein Cytoplasmic 1 Light Intermediate Chain 2	12	Microtubule-associated motor protein. Non-catalytic accessory components of the cytoplasmic dynein 1 complex	mHTT may impair proteins for neuronal trafficking or organelle trafficking in neurons [43]
	SPTBN2	Spectrin Beta, Non-Erythrocytic 2	12	Regulates the glutamate signaling pathway by stabilizing the glutamate transporter EAAT4 at the surface of the plasma membrane	potential biomarker for neurodegenerative diseases[44]
	ANK3	Ankyrin 3	8	Believed to link the integral membrane proteins to the underlying spectrin-actin cytoskeleton and play key roles in activities such as cell motility, activation, proliferation, contact, and the maintenance of specialized membrane domains. originally found at the axonal initial segment and nodes of Ranvier of neurons in the central and peripheral nervous systems.	n/a
	CAPZA2	Capping Actin Protein Of Muscle Z-Line Subunit Alpha 2	11	Member of the F-actin capping protein alpha subunit family. It is the alpha subunit of the barbed-end actin binding protein Cap Z. By capping the barbed end of actin filaments, Cap Z regulates the growth of the actin filaments at the barbed end.	n/a
	RAB3GAP1	RAB3 GTPase Activating Protein Catalytic Subunit 1	4	Probable catalytic subunit of a GTPase activating protein that has specificity for Rab3 subfamily	mHtt prevents RAB3A from binding RAB3GAP1 preventing conversion of GTP-RAB3A to GDP-RAB3A, this prevents BDNF vesicle docking and secretion [45]
	SCN8A	Sodium Voltage-Gated Channel Alpha Subunit 8	3	Mediates the voltage-dependent sodium ion permeability of excitable membranes. Assuming opened or closed conformations in response to the voltage difference across the membrane, the protein forms a sodium-selective channel through which sodium ions may pass in accordance with their electrochemical gradient.	n/a

SI Table #S3. BA6 Proteins Downregulated. Hub Protein Functions of Network. A literature analysis was performed on BA6 downregulated hub proteins, which are proteins with the most interactions. The brain region, gene name, protein name, # of nodes (interactions), normal functions and the proteins' function in HD are listed in the table below.

Supplemental Table S3. BA6 proteins downregulated in J. HD compared to controls: hub protein functions of network
(from Figure 7b network analysis)

Brain region	Gene name	Protein name	# nodes	Normal functions	Functions in HD
BA6	GNB4	G Protein Subunit Beta 4	7	Beta subunit of G protein which integrates signals between receptors and effector proteins. Beta subunit regulates alpha subunit as well as certain signal transduction receptors and effectors.	decreased expression or activity of GPCRs causes increase risk of neurodegenerative processes and changed neuronal plasticity [38]
	GNB5	G Protein Subunit Beta 5	7	Enhances GTPase-activating protein (GAP) activity of regulator of G protein signaling (RGS) proteins, hence involved in the termination of the signaling initiated by the G protein coupled receptors (GPCRs) by accelerating the GTP hydrolysis on the G-alpha subunits.	decreased expression or activity of GPCRs causes increase risk of neurodegenerative processes and changed neuronal plasticity [38]
	GNG7	G Protein Subunit Gamma 7	7	Gamma chain of G protein required for GTPase activity, replacement of GDP by GTP and for G protein-effector interaction.	G protein transcription is interfered in HD and it is known that the transcription is downregulated. G protein trafficking molecules are known to be hidden away into mHtt aggregates[54]
	PLCB1	Phospholipase C Beta 1	6	Catalyzes the formation of inositol 1,4,5-trisphosphate and diacylglycerol from phosphatidylinositol 4,5-bisphosphate.	Phospholipid C is involved with BDNF-TrKB-PLC signaling which plays a key role to HD pathology[40]
	PLCB4	Phospholipase C Beta 4	6	Catalyzes the formation of inositol 1,4,5-trisphosphate and diacylglycerol from phosphatidylinositol 4,5-bisphosphate	"
	PRKCB	Protein Kinase C Beta	7	Calcium-activated, phospholipid- and diacylglycerol (DAG)-dependent serine/threonine-protein kinase involved in various cellular processes such as regulation of the B-cell receptor (BCR) signalosome, oxidative stress-induced apoptosis, androgen receptor-dependent transcription regulation, insulin signaling and endothelial cells proliferation	mRNA of PRKCB are decreased which may cause the loss of ability to store information[47]
	PRKCE	Protein Kinase C Epsilon	8	Calcium-independent, phospholipid- and diacylglycerol (DAG)-dependent serine/threonine-protein kinase that plays essential roles in the regulation of multiple cellular processes linked to cytoskeletal proteins, such as cell adhesion, motility, migration and cell cycle, functions in neuron growth and ion channel regulation, and is involved in immune response, cancer cell invasion and regulation of apoptosis	n/a
	COPS2	COP9 Signalosome Subunit 2	5	Essential component of the COP9 signalosome complex (CSN), a complex involved in various cellular and developmental processes. The CSN complex is an essential regulator of the ubiquitin (Ubl) conjugation pathway	involved with controlling dendrite branching, loss of control/regulation of dendrite branching causes neurological disease[48]
	COPS3	COP9 Signalosome Subunit 3	5	kinase activity that phosphorylates regulators involved in signal transduction. It phosphorylates I kappa-Balpha, p105, and c-Jun. It acts as a docking site for complex-mediated phosphorylation.	"
	COPS7A	COP9 Signalosome Subunit 7A	4	Component of the COP9 signalosome, an evolutionarily conserved multi-subunit protease that regulates the activity of the ubiquitin conjugation pathway.	"
	UBE2K	Ubiquitin Conjugating Enzyme E2 K	4	Interacts with RING finger proteins, and it can ubiquitinate huntingtin, the gene product for Huntington's disease. Known functions for this protein include a role in aggregate formation of expanded polyglutamine proteins and the suppression of apoptosis in polyglutamine diseases.	interact with huntingtin and control ubiquitination of neuronal intranuclear inclusions, this causes aggregation and toxicity[49]
	UBR4	Ubiquitin Protein Ligase E3 Component N-Recognin 4	7	An E3 ubiquitin-protein ligase that interacts with the retinoblastoma-associated protein in the nucleus and with calcium-bound calmodulin in the cytoplasm.	UPS is impaired in mHtt which causes it to be unable to degrade mHtt which causes it to aggregate[33]
	UBE3C	Ubiquitin Protein Ligase E3C	4	E3 ubiquitin-protein ligase that accepts ubiquitin from the E2	UPS is impaired in mHtt which causes it to be unable to degrade mHtt which causes it to aggregate[33]

	PRKCE	Protein Kinase C Epsilon	8	Calcium-independent, phospholipid- and diacylglycerol (DAG)-dependent serine/threonine-protein kinase that plays essential roles in the regulation of multiple cellular processes linked to cytoskeletal proteins, such as cell adhesion, motility, migration and cell cycle, functions in neuron growth and ion channel regulation, and is involved in immune response, cancer cell invasion and regulation of apoptosis	n/a
	COPS2	COP9 Signalosome Subunit 2	5	Essential component of the COP9 signalosome complex (CSN), a complex involved in various cellular and developmental processes. The CSN complex is an essential regulator of the ubiquitin (Ubl) conjugation pathway	involved with controlling dendrite branching, loss of control/regulation of dendrite branching causes neurological disease[48]
	COPS3	COP9 Signalosome Subunit 3	5	kinase activity that phosphorylates regulators involved in signal transduction. It phosphorylates I kappa-Balpha, p105, and c-Jun. It acts as a docking site for complex-mediated phosphorylation.	"
	COPS7A	COP9 Signalosome Subunit 7A	4	Component of the COP9 signalosome, an evolutionarily conserved multi-subunit protease that regulates the activity of the ubiquitin conjugation pathway.	"
	UBE2K	Ubiquitin Conjugating Enzyme E2 K	4	Interacts with RING finger proteins, and it can ubiquitinate huntingtin, the gene product for Huntington's disease. Known functions for this protein include a role in aggregate formation of expanded polyglutamine proteins and the suppression of apoptosis in polyglutamine diseases.	interact with huntingtin and control ubiquitination of neuronal intranuclear inclusions, this causes aggregation and toxicity[49]
	UBR4	Ubiquitin Protein Ligase E3 Component N-Recognin 4	7	An E3 ubiquitin-protein ligase that interacts with the retinoblastoma-associated protein in the nucleus and with calcium-bound calmodulin in the cytoplasm.	UPS is impaired in mHtt which causes it to be unable to degrade mHtt which causes it to aggregate[33]
	UBE3C	Ubiquitin Protein Ligase E3C	4	E3 ubiquitin-protein ligase that accepts ubiquitin from the E2	UPS is impaired in mHtt which causes it to be unable to degrade mHtt which causes it to aggregate[33]
	TFRC	Transferrin Receptor	7	Cell surface receptor necessary for cellular iron uptake by the process of receptor-mediated endocytosis. This receptor is required for erythropoiesis and neurologic development.	decreased uptake of iron with this receptor which causes accumulation of iron which can cause oxidative stress[52]
	CPLX1	Complexin 1	4	Positively regulates a late step in exocytosis of various cytoplasmic vesicles, such as synaptic vesicles and other secretory vesicles. the complexin/synaphin gene family are cytosolic proteins that function in synaptic vesicle exocytosis.	n/a
	EPN2	Epsin 2	5	Plays a role in the formation of clathrin-coated invaginations and endocytosis	n/a
	HIP1	Huntingtin Interacting Protein 1	5	Membrane-associated protein that functions in clathrin-mediated endocytosis and protein trafficking within the cell. The encoded protein binds to the huntingtin protein in the brain; this interaction is lost in Huntington's disease.	binds weakly to mHtt where it can diminish its function[53]
	PPFIA3	PTPRF Interacting Protein Alpha 3	2	Member of the LAR protein-tyrosine phosphatase-interacting protein (liprin) family	n/a

SI Table #S4. BA4 Proteins Upregulated. Hub Protein Functions of Network. A literature analysis was performed on BA4 upregulated hub proteins, which are proteins with the most interactions. The brain region, gene name, protein name, # of nodes (interactions), normal functions and the proteins' function in HD are listed in the table below.

Supplemental Table S4. BA4 proteins upregulated in J. HD compared to controls: hub protein functions of network
(from Figure 8a)

Brain region	Gene name	Protein name	# nodes	Normal functions	Functions in HD
BA4	HNRNPC	Heterogeneous Nuclear Ribonucleoprotein C	11	hnRNPs are RNA binding proteins and they are complex with heterogeneous nuclear RNA (hnRNA). These proteins are associated with pre-mRNAs in the nucleus and appear to influence pre-mRNA processing and other aspects of mRNA metabolism and transport.	abnormalities of hnRNP protein can cause disordered RNA metabolism[71] possibly may interact with TDP43 and interfere with alternative splicing [68]
	HNRNPA2B1	Heterogeneous Nuclear Ribonucleoprotein A2/B1	10	"	"
	HNRNPR	Heterogeneous Nuclear Ribonucleoprotein R	10	"	"
	HNRNPA1	Heterogeneous Nuclear Ribonucleoprotein A1	10	"	"
	DHX9	DExH-Box Helicase 9	9	Enzyme that catalyzes the ATP-dependent unwinding of double-stranded RNA and DNA-RNA complexes. This protein localizes to both the nucleus and the cytoplasm and functions as a transcriptional regulator.	n/a
	LSM6	LSM6 Homolog, U6 Small Nuclear RNA And MRNA Degradation Associated	7	Plays role in pre-mRNA splicing as component of the U4/U6-U5 tri-snRNP complex that is involved in spliceosome assembly, and as component of the pre catalytic spliceosome (spliceosome B complex)	n/a
	SNRNP200	Small Nuclear Ribonucleoprotein U5 Subunit 200	7	Plays role in pre-mRNA splicing as core component of pre catalytic, catalytic and postcatalytic spliceosomal complexes	mHtt binds to proteins that regulate the splicing pathway causing missplicing → causes RNA mediated neurotoxicity[70]
	ERH	ERH MRNA Splicing And Mitosis Factor	7	May have a role in the cell cycle	n/a
	SNRPA1	Small Nuclear Ribonucleoprotein Polypeptide A'	7	Involved in pre-mRNA splicing as component of the spliceosome	mHtt binds to proteins that regulate the splicing pathway causing missplicing → causes RNA mediated neurotoxicity[70]
	SSB	Small RNA Binding Exonuclease Protection Factor La	5	Binds to the 3' poly(U) terminus of nascent RNA polymerase III transcripts, protecting them from exonuclease digestion and facilitating their folding and maturation	n/a
	GNG10	G Protein Subunit Gamma 10	7	Involved as a modulator or transducer in various transmembrane signaling systems. The beta and gamma chains are required for the GTPase activity, for replacement of GDP by GTP, and for G protein-effector interaction.	n/a
	GNG12	G Protein Subunit Gamma 12	9	"	"
	GNAI2	G Protein Subunit Alpha I2	8	"	"
	GNB2	G Protein Subunit Beta 2	8	"	"
	ANXA2	Annexin A2	10	Calcium-regulated membrane-binding protein whose affinity for calcium is greatly enhanced by anionic phospholipids. It binds two calcium ions with high affinity. May be involved in heat-stress response	n/a
	C3	Complement C3	16	Plays a central role in the activation of the complement system. Its processing by C3 convertase is the central reaction in both classical and alternative complement pathways.	reactive microglia increases the amount of complement protein in HD which can cause neuronal cell death[69]
	HEBP1	Heme Binding Protein 1	8	The full-length protein encoded by this gene is an intracellular tetrapyrrole-binding protein. This protein includes a natural chemoattractant peptide of 21 amino acids at the N-terminus, which is a natural ligand for formyl peptide receptor-like receptor 2 (FPRL2) and promotes calcium mobilization and chemotaxis in monocytes and dendritic cells	n/a
	KTN1	Kinectin 1	3	Receptor for kinesin thus involved in kinesin-driven vesicle motility. Accumulates in integrin-based adhesion complexes (IAC) upon integrin aggregation by fibronectin	n/a
	CTSD	Cathepsin D	10	Acid protease active in intracellular protein breakdown. Plays a role in APP processing following cleavage and activation by ADAM30 which leads to APP degradation	can not efficiently break down mHtt protein, also mHtt disturbs vesicular trafficking from Golgi to endosome which decreases amount of cathepsin D[67]
	CLU	Clusterin	5	Secreted chaperones that can under some stress conditions can also be found in the cell cytosol. It has been suggested to be involved in several basic biological events such as cell death, tumor progression, and neurodegenerative disorders.	research suggests HD has abnormal immune activation which could be one cause of the disease[66]
	HP	Haptoglobin	10	Captures and combines with free plasma hemoglobin to allow hepatic recycling of heme iron and to prevent kidney damage.	function is not clear, but seen in elevated levels in CSF of HD patients[65]
	KPNB1	Karyopherin Subunit Beta 1	6	Functions in nuclear protein import, either in association with an adapter protein, like an importin-alpha subunit, which binds to nuclear localization signals (NLS) in cargo substrates, or by acting as an autonomous nuclear transport receptor.	can cause protein mislocalization and aggregation of proteins if this protein is abnormal[64]
	PGM1	Phosphoglucomutase 1	8	Isozyme of phosphoglucomutase (PGM) belongs to the phosphohexose mutase family that catalyzes the transfer of phosphate between the 1 and 6 positions of glucose.	n/a
	PDXK	Pyridoxal Kinase	6	Catalyzes the phosphorylation of the dietary vitamin B6 vitamers pyridoxal (PL), pyridoxine (PN) and pyridoxamine (PM) to form pyridoxal 5'-phosphate (PLP), pyridoxine 5'-phosphate (PNP) and pyridoxamine 5'-phosphate (PMP), respectively	decrease of pyridoxal kinase → decrease of PNP → decreased glutathione and decreases GABA and dopamine[63]
	ALDH6A1	Aldehyde Dehydrogenase 6 Family Member A1	4	Mitochondrial methylmalonate semialdehyde dehydrogenase that plays a role in the valine and pyrimidine catabolic pathways. This protein catalyzes the irreversible oxidative decarboxylation of malonate and	abnormalities of this protein can cause excessive aldehyde building causing mitochondrial dysfunction[62]

				methylmalonate semialdehydes to acetyl- and propionyl-CoA.	
	ALDH2	Aldehyde Dehydrogenase 2 Family Member	5	Second enzyme of the major oxidative pathway of alcohol metabolism	"
	ALDH9A1	Aldehyde Dehydrogenase 9 Family Member A1	6	Converts gamma-trimethyl aminobutyraldehyde into gamma-butyrobetaine with high efficiency (in vitro). Can catalyze the irreversible oxidation of a broad range of aldehydes to the corresponding acids in an NAD-dependent reaction, but with low efficiency.	"
	HADHA	Hydroxyacyl-CoA Dehydrogenase Trifunctional Multienzyme Complex Subunit Alpha	4	Mitochondrial trifunctional enzyme catalyzes the last three of the four reactions of the mitochondrial beta-oxidation pathway	n/a
	HADH	Hydroxyacyl-CoA Dehydrogenase	5	Mitochondrial fatty acid beta-oxidation enzyme that catalyzes the third step of the beta-oxidation cycle for medium and short-chain 3-hydroxy fatty acyl-CoAs (C4 to C10)	n/a
	ACAA2	Acetyl-CoA Acyltransferase 2	5	Catalyzes the last step of the mitochondrial beta-oxidation pathway, an aerobic process breaking down fatty acids into acetyl-CoA	n/a
	ACOX1	Acyl-CoA Oxidase 1	3	First enzyme of the fatty acid beta-oxidation pathway, which catalyzes the desaturation of acyl-CoAs to 2-trans-enoyl-CoAs. It donates electrons directly to molecular oxygen, thereby producing hydrogen peroxide	n/a
	CNDP2	Carnosine Dipeptidase 2	4	Hydrolyzes a variety of dipeptides including L-carnosine but has a strong preference for Cys-Gly	carnosine can reverse neurodegenerative effects from NMDA neurotoxicity [63]
	GATM	Glycine Amidinotransferase	4	Catalyzes the biosynthesis of guanidinoacetate, the immediate precursor of creatine	involved with creatine synthesis and impairment can lead to decreased energy production[64]

SI Table #S5. BA6 Proteins Upregulated. Hub Protein Functions of Network. A literature analysis was performed on BA6 upregulated hub proteins, which are proteins with the most interactions. The brain region, gene name, protein name, # of nodes (interactions), normal functions and the proteins' function in HD are listed in the table below.

Supplemental Table S5. BA6 proteins upregulated in J.HD compared to controls: hub protein functions of network
(from Figure 8b)

Brain region	Gene name	Protein name	# nodes	Normal functions	HD functions
BA6	COL1A1	Collagen Type I Alpha 1 Chain	6	"	n/a
	COL1A2	Collagen Type I Alpha 2 Chain	6	Subunit of Type I collagen	"
	COL3A1	Collagen Type III Alpha 1 Chain	6	"	"
	COL4A1	Collagen Type IV Alpha 2 Chain	7	Subunits of type IV collagen, the major structural component of basement membranes	"
	COL4A2	Collagen Type IV Alpha 2 Chain	7	"	"
	COL6A2	Collagen Type VI Alpha 2 Chain	7	"	"
	COL18A1	Collagen Type XVIII Alpha 1 Chain	8	"	"
	ALYREF	Aly/REF Export Factor	14	Heat stable, nuclear protein and functions as a molecular chaperone. It is thought to regulate dimerization and DNA binding	Aly/Ref is responsible for nuclear export, reduced nuclear export may cause mHtt accumulation in nucleus[67] [68]
	RBMX	RNA Binding Motif Protein X-Linked	13	Plays several role in the regulation of pre- and post-transcriptional processes. Implicated in tissue-specific regulation of gene transcription and alternative splicing of several pre-mRNAs.	n/a
	HNRNPU	Heterogeneous Nuclear Ribonucleoprotein U	12	DNA- and RNA-binding protein involved in several cellular processes such as nuclear chromatin organization, telomere-length regulation, transcription, mRNA alternative splicing and stability, Xist-mediated transcriptional silencing and mitotic cell progression	abnormalities of hnRNP protein can cause disordered RNA metabolism[69]
	HNRNPA3	Heterogeneous Nuclear Ribonucleoprotein A3	10	Plays a role in cytoplasmic trafficking of RNA. Binds to the cis-acting response element, A2RE. May be involved in pre-mRNA splicing.	"
	ELAVL1	ELAV Like RNA Binding Protein 1	14	Member of the ELAVL family of RNA-binding proteins that contain several RNA recognition motifs, and selectively bind AU-rich elements (AREs) found in the 3' untranslated regions of mRNA.	n/a
	MYO1C	Myosin IC	1	Member of the unconventional myosin protein family found in the cytoplasm, and one isoform with a unique N-terminus is also found in the nucleus.	may impair the secretory pathways causing HD pathology[68] loss of function may impair autophagosomes and cause protein aggregation [70]
	SRSF2	Serine And Arginine Rich Splicing Factor 2	8	Member of the serine/arginine (SR)-rich family of pre-mRNA splicing factors, which constitute part of the spliceosome	n/a
	SRSF7	Serine And Arginine Rich Splicing Factor 7	11	Required for pre-mRNA splicing. Can also modulate alternative splicing in vitro. Represses the splicing of MAPT/Tau exon 10	n/a
	SRRT	Serrate, RNA Effector Molecule	10	Acts as a mediator between the cap-binding complex (CBC) and the primary microRNAs (miRNAs) processing machinery during cell proliferation.	n/a
	TRA2B	Transformer 2 Beta Homolog	14	Nuclear protein which functions as sequence-specific serine/arginine splicing factor which plays a role in mRNA processing, splicing patterns, and gene expression	n/a
	SCG2	Secretogranin II	7	Neuroendocrine protein of the granin family that regulates the biogenesis of secretory granules	possibly related to abnormalities in vesicle drive processes[71]
	SCG3	Secretogranin III	7	Regulates the biogenesis of secretory granules	possibly related to abnormalities in vesicle drive processes[71]

	CHGB	Chromogranin B	7	Secretory protein abundant in peptidergic endocrine cells and neurons. This protein may serve as a precursor for regulatory peptides	n/a potential biomarker in many studies
	VGF	VGF Nerve Growth Factor Inducible	7	expressed in a subpopulation of neuroendocrine cells, and is upregulated by nerve growth factor	SUN N8075 blocks cell death by upregulation of VGF nerve growth factor[72]
	CNPY3	Canopy FGF Signaling Regulator 3	1	Binds members of the toll-like receptor protein family and functions as a chaperone to aid in folding and export of these proteins.	n/a
	HSP90B1	Heat Shock Protein 90 Beta Family Member 1	12	Molecular chaperone that functions in the processing and transport of secreted proteins	HSP90 interacts with N terminus of huntingtin protein with USP 90, [6]abnormalities of this protein may prevent mHtt degradation; [74] abnormalities in HSP90 have also known to cause protein aggregation[75]
	LAMC1	Laminin Subunit Gamma 1	9	Laminin is thought to mediate the attachment, migration and organization of cells into tissues during embryonic development by interacting with other extracellular matrix components.	n/a
	RCN1	Reticulocalbin 1	7	Calcium-binding protein located in the lumen of the ER. May regulate calcium-dependent activities.	n/a
	SPP1	Secreted Phosphoprotein 1	7	Involved in the attachment of osteoclasts to the mineralized bone matrix.	n/a
	UCHL1	Ubiquitin C-Terminal Hydrolase L1	2	Involved both in the processing of ubiquitin precursors and of ubiquitinated proteins	abnormalities of this protein can cause protein aggregation causing neurodegeneration[76]
	USP46	Ubiquitin Specific Peptidase 46	1	Deubiquitinating enzyme that plays a role in behavior, possibly by regulating GABA action. May act by mediating the deubiquitination of GAD1/GAD67	UPS is impaired in mHtt which causes it to be unable to degrade mHtt which causes it to aggregate[53]
	CETN2	Centrin 2	3	Plays a fundamental role in microtubule organizing center structure and function. Required for centriole duplication and correct spindle formation	possibly linked to poor efficiency of DNA repair mechanisms which can cause neurodegenerative diseases[76]
	HSPB1	Heat Shock Protein Family B (Small) Member 1	1	Small heat shock protein which functions as a molecular chaperone probably maintaining denatured proteins in a folding-competent state	abnormalities of heat shock proteins may cause proteotoxic stress, protein aggregations in neurodegenerative diseases[77]
	RPS27A	Ribosomal Protein S27a	12	Component of the 40S subunit of the ribosome	mHtt impairs ribosomal movement during translation elongation[78]
	SQSTM1	Sequestosome 1	1	Autophagy receptor required for selective macroautophagy (aggrephagy). Functions as a bridge between polyubiquitinated cargo and autophagosomes.	proteotoxic stress encourages p62 SQSTM1 complex formation caused by selective autophagy[79]
	SNCA	Synuclein Alpha	2	Alpha- and beta-synuclein inhibit phospholipase D2 selectively. SNCA peptides are a major component of amyloid plaques in the brains of patients with Alzheimer's disease. Defects in SNCA have been implicated in the pathogenesis of Parkinson disease.	alpha synuclein is related to Htt aggregation but no known mechanism for its toxicity and interaction with Htt[80]

SI Table #S6. Putamen Juvenile HD only Hubs. Hub Protein Functions of Network. A literature analysis was performed on putamen juvenile HD only hub proteins, which are proteins with the most interactions. The brain region, gene name, protein name, # of nodes (interactions), normal functions and the proteins' function in HD are listed in the table below.

Supplemental Table S6. Putamen proteins present in only J. HD: hub protein functions of network

Brain region	Gene name	Protein name	# nodes	Normal functions	Functions in HD
putamen	BUD31	BUD31 Homolog	4	Involved in the pre-mRNA splicing process	n/a
	HNRNPUL1	Heterogeneous Nuclear Ribonucleoprotein U Like 1	5	Acts as a basic transcriptional regulator. Represses basic transcription driven by several virus and cellular promoters.	possibly may interact with TDP43 and interfere with alternative splicing [88]
	PRPF40A	Pre-MRNA Processing Factor 40 Homolog A	4	Binds to WASL/N-WASP and suppresses its translocation from the nucleus to the cytoplasm, thereby inhibiting its cytoplasmic function	n/a
	PUF60	Poly(U) Binding Splicing Factor 60	4	Nucleic acid-binding protein that plays a role in a variety of nuclear processes, including pre-mRNA splicing and transcriptional regulation.	no known mechanism associated with HD, only know that abnormal RNA binding proteins may cause dysfunction in RNA regulation causing neurodegenerative diseases[91]
	USP39	Ubiquitin Specific Peptidase 39	4	Plays a role in pre-mRNA splicing as a component of the U4/U6-U5 tri-snRNP	n/a
	C1QA	Complement C1q A Chain	3	C-chain polypeptide of serum complement subcomponent C1q, which associates with C1r and C1s to yield the first component of the serum complement system	reactive microglia increases the amount of complement protein in HD which can cause neuronal cell death[92]
	C1QB	Complement C1q CB Chain	3	"	"
	C1QC	Complement C1q C Chain	3	"	"
	CRP	C-Reactive Protein	3	Belongs to the pentraxin family which also includes serum amyloid P component protein and pentraxin 3	"
	CENPE	Centromere Protein E	5	Kinesin-like motor protein that accumulates in the G2 phase of the cell cycle	n/a
	NUP133	Nucleoporin 133	4	Localizes to both sides of the nuclear pore complex at interphase, remains associated with the complex during mitosis and is targeted at early stages to the reforming nuclear envelope	nuclear pore complexes are abnormal in neurodegenerative diseases such as HD[93]
	NUP160	Nucleoporin 160	5	Mediates nucleoplasmic transport	nuclear pore complexes are abnormal in neurodegenerative diseases such as HD[93]
	PDS5B	PDS5 Cohesin Associated Factor B	3	Regulator of sister chromatid cohesion in mitosis which may stabilize cohesin complex association with chromatin.	n/a

SI Table #S7. Putamen Juvenile HD only Hubs. Hub Protein Functions of Network. A literature analysis was performed on putamen juvenile HD only hub proteins, which are proteins with the most interactions. The brain region, gene name, protein name, # of nodes (interactions), normal functions and the proteins' function in HD are listed in the table below.

Supplemental Table S7. Putamen proteins present in only controls: hub protein functions of network
(from Fig. 12 networks)

Brain region	Gene name	Protein name	# nodes	Normal functions	Functions in HD
Putamen	HECTD3	HECT Domain E3 Ubiquitin Protein Ligase 3	11	Accepts ubiquitin from an E2 ubiquitin-conjugating enzyme in the form of a thioester and then directly transfers the ubiquitin to targeted substrates	UPS is impaired in mHtt which causes it to be unable to degrade mHtt which causes it to aggregate[33]
	HECTD1	HECT Domain E3 Ubiquitin Protein Ligase 1	11	"	"
	HECW2	HECT, C2 And WW Domain Containing E3 Ubiquitin Protein	11	Mediates ubiquitination of TP73. Acts to stabilize TP73 and enhance activation of transcription by TP73	UPS is impaired in mHtt which causes it to be unable to degrade mHtt which causes it to aggregate[33]
	RNF7	Ring Finger Protein 7	11	Essential subunit of SKP1-cullin/CDC53-F box protein ubiquitin ligases	No known function in HD
	RNF25	Ring Finger Protein 25	11	RING finger motif. E3 ubiquitin-protein ligase that mediates ubiquitination and proteasomal degradation of NKD2	No known function in HD
	FBXL18	F-Box And Leucine Rich Repeat Protein 18	11	Substrate-recognition component of the SCF (SKP1-CUL1-F-box protein)-type E3 ubiquitin ligase complex.	No known function in HD
	FBXL20	F-Box And Leucine Rich Repeat Protein 20	11	See above	No known function in HD
	UBE3A	Ubiquitin Protein Ligase E3A	11	Essential subunit of SKP1-cullin/CDC53-F box protein ubiquitin ligases	Synaptic abnormalities due to UBE3A being strongly interacting with mHtt nuclear aggregates. This causes the protein to lose its function.[93]
	UBE2Q1	Ubiquitin Conjugating Enzyme E2 Q1	11	Catalyzes the covalent attachment of ubiquitin to other proteins	UPS is impaired in mHtt which causes it to be unable to degrade mHtt which causes it to aggregate[33]
	TRIM32	Tripartite Motif Containing 32	11	Has an E3 ubiquitin ligase activity. Ubiquitinates DTNBP1 (dysbindin) and promotes its degradation	UPS is impaired in mHtt which causes it to be unable to degrade mHtt which causes it to aggregate[33]
	MRPS34	Mitochondrial Ribosomal Protein S34	9	28S subunit protein	possible ribosomal traffic jam in HD and other neurodegenerative disease[28]
	MRPS27	Mitochondrial Ribosomal Protein S27	9	"	"
	MRPL4	Mitochondrial Ribosomal Protein L4	11	39S subunit protein	"
	MRPS27	Mitochondrial Ribosomal Protein S7	9	"	"
	MRPS22	Mitochondrial Ribosomal Protein S22	9	"	"
	MRPL1	Mitochondrial Ribosomal Protein L1	7	39S subunit protein	"
	DAP3	Death Associated Protein 3	10	28S subunit protein that also participates in apoptotic pathways	"
	ENSG00000239789	Mitochondrial Ribosomal Protein S17	9	28S subunit protein that belongs to the ribosomal protein S17P family	"
	PTCD3	Pentatricopeptide Repeat Domain 3	8	Mitochondrial RNA-binding protein that has a role in mitochondrial translation	Since PTCD3 is related to RNA processing of C1 complexes of mitochondria, may be related to mitochondrial function of complex I within HD[94]
	RPMS17	Mitochondrial Ribosomal Protein S17	9	28S subunit protein that belongs to the ribosomal protein S17P family.	possible ribosomal traffic jam in HD and other neurodegenerative disease[28]
	C5	Complement C5	6	component of the complement system, a part of the innate immune system that plays an important role in inflammation, host homeostasis, and host defense against pathogens	reactive microglia increases the amount of complement protein in HD which can cause neuronal cell death[83]
	GPSM1	G Protein Signaling Modulator 1	5	Receptor-independent activator of G protein signaling	G protein transcription is interfered in HD and it is known that the transcription is downregulated. G protein trafficking molecules are known to be hidden away into mHtt aggregates[84]
	KNG1	Kininogen 1	12	Bradykinin and related kinins are a family of small peptides which act as mediators of pain and inflammation.	No known function in HD
	PDYN	Prodynorphin	6	Preproprotein that is proteolytically processed to form the opioid peptides beta-neoendorphin, dynorphin, leu-enkephalin	Prodynorphin has been shown to be altered in HD but not sure if prodynorphin of proenkephalin derived peptides cause the pathophysiology of HD [85]
	S1PR5	Sphingosine-1-Phosphate Receptor 5	6	Regulates cell proliferation, apoptosis, motility, and neurite retraction	Has been shown the sphingosine metabolism and homeostasis is affected in HD but no specific mechanism has been known[86]
	SERPINC1	Serpin Family C Member 1	4	Serine protease inhibitor in plasma that regulates the blood coagulation cascade.	No known function in HD

SI Table #S8. Putamen Downregulated Hubs. Hub Protein Functions of Network. A literature analysis was performed on putamen downregulated hub proteins, which are proteins with the most interactions. The brain region, gene name, protein name, # of nodes (interactions), normal functions and the proteins' function in HD are listed in the table below.

Supplemental Table S8. Putamen downregulated proteins: hub protein functions of network

Brain region	Gene name	Protein name	# nodes	Normal functions	Functions in HD
Putamen	NDUFB10	NADH:Ubiquinone Oxidoreductase Subunit B10	47	Accessory subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I), that is believed not to be involved in catalysis.	HD patients have reduction in NADH Ubiquinone Oxidoreductase activity [87] [88]
	NDUFA7	NADH:Ubiquinone Oxidoreductase Subunit A7	40	This gene encodes a subunit of NADH:ubiquinone oxidoreductase (complex I), which is a multiprotein complex located in the inner mitochondrial membrane. Complex I functions in the transfer of electrons from NADH to the respiratory chain	"
	NDUFS7	NADH:Ubiquinone Oxidoreductase Subunit S7	43	"	"
	NDUFA11	NADH:Ubiquinone Oxidoreductase Subunit A11	43	"	"
	NDUFA12	NADH:Ubiquinone Oxidoreductase Subunit A12	45	"	"
	NDUFB3	NADH:Ubiquinone Oxidoreductase Subunit B3	42	"	"
	NDUFS6	NADH:Ubiquinone Oxidoreductase Subunit S6	33	"	"
	NDUFA4	NADH:Ubiquinone Oxidoreductase Subunit A4	46	"	"
	NDUFB7	NADH:Ubiquinone Oxidoreductase Subunit B7	49	"	"
	NDUFV2	NADH:Ubiquinone Oxidoreductase Subunit V2	44	"	"
	NDUFAB1	NADH:Ubiquinone Oxidoreductase Subunit AB1	45	"	"
	NDUFB4	NADH:Ubiquinone Oxidoreductase Subunit B4	38	"	"
	NDUFB11	NADH:Ubiquinone Oxidoreductase Subunit B11	42	"	"
	NDUFB9	NADH:Ubiquinone Oxidoreductase Subunit B9	48	"	"
	NDUFS1	NADH:Ubiquinone Oxidoreductase Subunit S1	41	"	"
	NDUFA5	NADH:Ubiquinone Oxidoreductase Subunit A5	43	"	"
	NDUFA13	NADH:Ubiquinone Oxidoreductase Subunit A13	41	"	"
	NDUFB5	NADH:Ubiquinone Oxidoreductase Subunit B5	48	"	"
	NDUFB8	NADH:Ubiquinone Oxidoreductase Subunit B8	51	"	"
	NDUFB5	NADH:Ubiquinone Oxidoreductase Subunit B5	48	"	"
	NDUFA5	NADH:Ubiquinone Oxidoreductase Subunit A5	43	"	"
	NDUFB8	NADH:Ubiquinone Oxidoreductase Subunit B8	51	"	"
	NDUFA2	NADH:Ubiquinone Oxidoreductase Subunit A2	43	"	"
	NDUFA6	NADH:Ubiquinone Oxidoreductase Subunit A6	47	"	"
	NDUFA8	NADH:Ubiquinone Oxidoreductase Subunit A8	46	"	"
	NDUFB1	NADH:Ubiquinone Oxidoreductase Subunit B1	38	"	"
	NDUFS2	NADH:Ubiquinone Oxidoreductase Subunit S2	52	"	"
	NDUFS4	NADH:Ubiquinone Oxidoreductase Subunit S4	49	"	"
	NDUFA10	NADH:Ubiquinone Oxidoreductase Subunit A10	44	"	"
	NDUFV1	NADH:Ubiquinone Oxidoreductase Subunit V1	43	"	"
	NDUFS3	NADH:Ubiquinone Oxidoreductase Subunit S3	47	"	"
	NDUFA9	NADH:Ubiquinone Oxidoreductase Subunit A9	44	"	"
	NDUFS5	NADH:Ubiquinone Oxidoreductase Subunit S5	43	"	"
	NDUFB6	NADH:Ubiquinone Oxidoreductase Subunit B6	43	"	"
	NDUFAF4	NADH:Ubiquinone Oxidoreductase Subunit AF4	32	"	"

	ATP5E	ATP Synthase F1 Subunit Epsilon	19	subunit of mitochondrial ATP synthase. produces ATP from ADP in the presence of a proton gradient across the membrane which is generated by electron transport complexes of the respiratory chain.	HD-iPSC cells show reversal of ATP synthase, producing ADP instead of ATP [10] Overexpression reduces mHtt aggregation and toxicity in cultured neuronal cells [69]
	MT-ND4	Mitochondrially Encoded NADH:Ubiquinone Oxidoreductase Core Subunit 4	40	Core subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I) that is believed to belong to the minimal assembly required for catalysis.	Age-associated and cachexia associated dysfunction in NADH:ubiquinone complex activity in HD patients [90]
	AP1G1	Adaptor Related Protein Complex 1 Subunit Gamma 1	13	Subunit of clathrin-associated adaptor protein complex 1 that plays a role in protein sorting in the late-Golgi/trans-Golgi network (TGN) and/or endosomes.	No reported role yet in HD.
	AP1B1	Adaptor Related Protein Complex 1 Subunit Beta 1	12	Found at the cytoplasmic face of coated vesicles located at the Golgi complex, where it mediates both the recruitment of clathrin to the membrane and the recognition of sorting signals within the cytosolic tails of transmembrane receptors	"
	AP2A1	Adaptor Related Protein Complex 2 Subunit Alpha 1	25	Part of the protein coat on the cytoplasmic face of coated vesicles which links clathrin to receptors in vesicles.	"
	ARPC2	Actin Related Protein 2/3 Complex Subunit 2	24	Mediates actin polymerization upon stimulation by nucleation-promoting factor.	No reported role yet in HD.
	ACTR2	Actin Related Protein 2	26	ATP binding component of the ARP2/3 complex.	"
	CAMK4	Calcium/Calmodulin Dependent Protein Kinase IV	3	Operates in the calcium-triggered CaMKK-CaMK4 signaling cascade and regulates, mainly by phosphorylation.	Increased expression in presymptomatic HD model mice, followed by decreased expression in symptomatic HD model mice [91]
	CAMKK2	Calcium/Calmodulin Dependent Protein Kinase Kinase 2	3	belongs to the Serine/Threonine protein kinase family, and to the Ca(2+)/calmodulin-dependent protein kinase subfamily. The major isoform of this gene plays a role in the calcium/calmodulin-dependent (CaM) kinase cascade.	"
	CALM1	Calmodulin 1	13	Calcium-induced activation of calmodulin regulates and modulates the function of cardiac ion channels	Regulated cross-linking of mHtt [92] Downregulation in cells expressing mHtt is associated with disrupted calcium homeostasis [93]
	CLTA	Clathrin Light Chain A	21	One of two clathrin light chain proteins which are believed to function as regulatory elements.	No reported role yet in HD.
	CLTB	Clathrin Light Chain B	21	"	"
	RAB3A	RAB3A, Member RAS Oncogene Family	15	Small GTP-binding protein that plays a central role in regulated exocytosis and secretion	Involved in impairment of BDNF release by astrocytes in HD model mice [94].
	RAB30	RAB30, Member RAS Oncogene Family	4	Rab are key regulators of intracellular membrane trafficking, from the formation of transport vesicles to their fusion with membrane	No reported role yet in HD.
	CASK	Calcium/Calmodulin Dependent Serine Protein Kinase	9	Multidomain scaffolding protein with a role in synaptic transmembrane protein anchoring and ion channel trafficking. Binds to cell-surface proteins, including amyloid precursor protein, neurexins and syndecans.	No reported role yet in HD
	CPLX1	Complexin 1	12	Binds to the SNAP receptor complex and disrupts it, allowing transmitter release	No reported role yet in HD
	GAK	Cyclin G Associated Kinase	19	Associates with cyclin G and CDK5. Seems to act as an auxilin homolog that is involved in the uncoating of clathrin-coated vesicles by Hsc70 in non-neuronal cells	No reported role yet in HD
	HSPA8	Heat Shock Protein Family A (Hsp70) Member 4	26	"Diseases associated include Vulvovaginitis and Babesiosis"	Loss of HSP70 proteins worsens neurodegeneration, HSP70 helps increase solubility of polyQ proteins which can cause anti protein aggregation effect[95]
	NRXN3	Neurexin 3	10	Functions in the nervous system as receptors and cell adhesion molecules	diminishing neurexin expression in glia can help HD pathology in neurons and

					glia but no known direct mechanism of how is known[54]
	PACSIN1	Protein Kinase C And Casein Kinase Substrate In Neurons 1	15	One of seven subunits of the human Arp2/3 protein complex. The Arp2/3 protein complex has been implicated in the control of actin polymerization in cells.	PACSIN 1 helps mediate domain flexibility of the huntingtin protein by binding to N17 of the Huntingtin protein. Expanded poly Q lengths can decrease the domain flexibility of huntingtin protein.[96]
	SGIP1	SH3GL Interacting Endocytic Adaptor 1	19	Endocytic protein that affects signaling by receptors in neuronal systems involved in energy homeostasis via its interaction with endophilins	No reported role yet in HD
	SYN3	Synapsin III	6	Member of the Wiskott-Aldrich syndrome (WAS) protein family. Shares similar domain structure, and associates with a variety of signaling molecules to alter the actin cytoskeleton.	decreased synapsin III can upregulate dopamine transmission in early stages of HD[99]
	SYT1	Synaptotagmin 1	24	Calcium sensor that participates in triggering neurotransmitter release at the synapse	increase of calcium may turn on calcium sensitive synaptotagmins permanently but will impair their physiological function; this will impair membrane trafficking and fusion[51]
	WASL	WASP Like Actin Nucleation Promoting Factor	21	Regulates actin polymerization by stimulating the actin-nucleating activity of the Arp2/3 complex.	No reported role yet in HD
	PPP1CC	Protein Phosphatase 1 Catalytic Subunit Gamma	17	Gamma isozyme of an ubiquitous serine/threonine phosphatase that regulates many cellular processes, including cell division.	Protein phosphatase 1 can control the rate of Htt exon 1 aggregation by controlling the amount of phosphorylation on its T3 residue[39]
	PPP2R1A	Protein Phosphatase 2 Scaffold Subunit Alpha	28	Serves as a scaffolding molecule to coordinate the assembly of the catalytic subunit and a variable regulatory B subunit	PP2A controls HTT phosphorylation at other residues on huntingtin protein and HTT toxicity [39]
	PPP2R5D	Protein Phosphatase 2 Regulatory Subunit B'Delta	19	The B regulatory subunit might modulate substrate selectivity and catalytic activity, and also might direct the localization of the catalytic enzyme to a particular subcellular compartment	"
	GNAO1	G Protein Subunit Alpha O1	5	Subunit of Go heterotrimeric G-protein signal-transducing complex	G protein transcription is interfered in HD and it is known that the transcription is downregulated.
					G protein trafficking molecules are known to be hidden away into mHtt aggregates[56]
	GNB5	G Protein Subunit Beta 5	15	Enhances GTPase-activating protein (GAP) activity of regulator of G protein signaling (RGS) proteins, hence involved in the termination of the signaling initiated by the G protein coupled receptors (GPCRs) by accelerating the GTP hydrolysis on the G-alpha subunits, thereby promoting their inactivation.	G protein transcription is interfered in HD and it is known that the transcription is downregulated. G protein trafficking molecules are known to be hidden away into mHtt aggregates[56]
	GNG7	G Protein Subunit Gamma 7	14	Plays a role in the regulation of adenylyl cyclase signaling in certain regions of the brain	G protein transcription is interfered in HD and it is known that the transcription is downregulated. G protein trafficking molecules are known to be hidden away into mHtt aggregates[56]
	MAPK10	Mitogen-Activated Protein Kinase 10	7	Serine/threonine-protein kinase involved in various processes such as neuronal proliferation, differentiation, migration and programmed cell death.	MAPK10 = JNK3; JNK3 phosphorylates kinesin 1 which lowers its binding efficiency on microtubules and transport cargo to fast axonal transport Usually JNK3 regulated membrane bounded organelle cargos transport with kinesin 1 but increase activation of JNK3 by polyQ- Htt causes decrease kinesin activity; this cause deficiency in axonal and synaptic function leading to neurodegeneration[97]
	MAP2K4	Mitogen-Activated Protein Kinase Kinase 4	7	Protein kinase which acts as an essential component of the MAP kinase signal transduction pathway	No reported role yet in HD
	BRAF	B-Raf Proto-Oncogene, Serine/Threonine Kinase	12	Plays a role in regulating the MAP kinase/ERK signaling pathway, which affects cell division, differentiation, and secretion.	No reported role yet in HD
	HRAS	HRas Proto-Oncogene, GTPase	22	Involved in the activation of Ras protein signal transduction	No reported role yet in HD
	KALRN	Kalirin RhoGEF Kinase (also Huntingtin-Associated Protein Interacting Protein)	5	Protein that interacts with the huntingtin-associated protein 1, which is a huntingtin binding protein that may function in vesicle trafficking	loss of Kalirin could lead to a decrease the amount of excitatory synapses in HD[100]

	MARK3	Microtubule Affinity Regulating Kinase 3	5	phosphorylation of microtubule-associated proteins for MAP2 and MAP4	No reported role yet in HD
	NRAS	NRAS Proto-Oncogene, GTPase	21	membrane protein that shuttles between the Golgi apparatus and the plasma membrane.	No reported role yet in HD
	PLCB1	Phospholipase C Beta 1	8	catalyzes the formation of inositol 1,4,5-trisphosphate and diacylglycerol from phosphatidylinositol 4,5-bisphosphate. plays an important role in the intracellular transduction of many extracellular signals.	Note: PLC Gamma has an association with HD No reported role yet in HD
	YWHAG	Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein Gamma	9	Belongs to the 14-3-3 family of proteins which mediate signal transduction by binding to phosphoserine-containing protein	No reported role yet in HD
	CAPZA2	Capping Actin Protein Of Muscle Z-Line Subunit Alpha 2	15	Alpha subunit of the barbed-end actin binding protein Cap Z. By capping the barbed end of actin filaments, Cap Z regulates the growth of the actin filaments at the barbed end.	"
	CAPZB	Capping Actin Protein Of Muscle Z-Line Subunit Beta	12	F-actin-capping proteins bind in a Ca(2+)-independent manner to the fast growing ends of actin filaments (barbed end) thereby blocking the exchange of subunits at these ends.	No reported role yet in HD
	KIF5A	Kinesin Family Member 5A	11	Microtubule-dependent motor required for slow axonal transport of neurofilament proteins	GABAAR/HAP1/KIF5 complex is impaired and dissociates from microtubules GABA trafficking impaired because KIF5 mediated transport of GABA receptors is impaired[101]
	KIF5C	Kinesin Family Member 5C	2	Kinesin heavy chain subunit involved in the transport of cargo within the central nervous system	"
	DLG3	Discs Large MAGUK Scaffold Protein 3	6	May play a role in clustering of NMDA receptors at excitatory synapses	PSD95= DLG4 NR2B/PSD95/htt complex increases excitotoxicity in medium sized spinal neurons[102]
	DLG4	Discs Large MAGUK Scaffold Protein 4	16	Postsynaptic scaffolding protein that plays a critical role in synaptogenesis and synaptic plasticity by providing a platform for the postsynaptic clustering of crucial synaptic proteins.	"
	APBA2	Amyloid Beta Precursor Protein Binding Family A Member 2	3	Stabilizes APP and inhibits production of proteolytic APP fragments including the A beta peptide that is deposited in the brains of Alzheimer's disease patients	No reported role yet in HD
	DCTN1	Dynactin Subunit 1	11	Largest subunit of dynactin, binds to both microtubules and cytoplasmic dynein	polyQ Htt impairs vesicular trafficking of BDNF specifically blocking HAP1 onto p150 of dynactin onto microtubules[103]
	HOMER1	Homer Scaffold Protein 1	3	Regulates group 1 metabotropic glutamate receptor function	HOMER1c and Hippi interaction play some role in neuronal death in HD[104] HOMER regulated mGlu5 ERK pathway activity, mGlu5 can cause neuronal toxicity by increasing calcium release from inside the cell[105]
	ITGAM	Integrin, Alpha M (Complement Component 3 Receptor 3 Subunit)	7	Important in the adherence of neutrophils and monocytes to stimulated endothelium, and also in the phagocytosis of complement coated particles.	deficiency of this protein does exacerbate the disease[106]
	RAB1B	RAB1B, Member RAS Oncogene Family	18	GTPases Rab are key regulators of intracellular membrane trafficking, from the formation of transport vesicles to their fusion with membranes.	No reported role yet in HD
	SNAP25	Synaptosome Associated Protein 25	21	presynaptic plasma membrane protein involved in the regulation of neurotransmitter release	possible defect in neurotransmitter release as there is downregulation of this protein in HD[107]

SI Table #S9. Putamen Upregulated Hubs. Hub Protein Functions of Network. A literature analysis was performed on putamen upregulated hub proteins, which are proteins with the most interactions. The brain region, gene name, protein name, # of nodes (interactions), normal functions and the proteins' function in HD are listed in the table below.

Supplemental Table S9. Putamen upregulated proteins: hub protein functions of network

Brain region	Gene name	Protein name	# nodes	Normal functions	Functions in HD
Putamen	RPL17	Ribosomal Protein L17	44	Ribosomal protein that is a component of the 60S subunit	mHtt blocks ribosomal translocation during elongation phase of translation in protein synthesis[78]
	RPS16	Ribosomal Protein S16	43	Ribosomal protein that is a component of the 40S subunit.	"
	RPS2	Ribosomal Protein S2	43	"	"
	RPS8	Ribosomal Protein S8	44	"	"
	RPS26	Ribosomal Protein S26	44	"	"
	RPS4X	Ribosomal Protein S4 X-Linked	44	"	"
	RPSA	Ribosomal Protein SA	45	"	"
	RPS12	Ribosomal Protein S12	44	"	"
	RPL11	Ribosomal Protein L11	46	"	"
	RPL9	Ribosomal Protein L9	45	"	"
	RPS27	Ribosomal Protein S27	46	"	"
	RPS19	Ribosomal Protein S19	44	"	"
	RPS5	Ribosomal Protein S5	44	"	"
	RPS3	Ribosomal Protein S3	47	"	"
	RPL10	Ribosomal Protein L10	42	"	"
	RPL8	Ribosomal Protein L8	44	"	"
	RPL4	Ribosomal Protein L4	43	"	"
	RPL6	Ribosomal Protein L6	44	"	"
	RPL7	Ribosomal Protein L7	43	"	"
	RPL15	Ribosomal Protein L15	44	"	"
	RPL27A	Ribosomal Protein L27A	43	"	"
	RPL38	Ribosomal Protein L38	42	"	"
	RPLP2	Ribosomal Protein LP2	41	"	"
	RPL13	Ribosomal Protein L13	45	"	"
	RPS17	Ribosomal Protein S17	43	"	"
	RPLP1	Ribosomal Protein LP1	41	"	"
	RPL19	Ribosomal Protein L19	43	"	"
	DDX17	DEAD-Box Helicase 17	2	RNA helicase, unwinds RNA and alters RNA structures through ATP binding and hydrolysis	No reported role yet in HD
	EEF2	Eukaryotic Translation Elongation Factor 2	35	essential factor for protein synthesis. It promotes the GTP-dependent translocation of the nascent protein chain from the A-site to the P-site of the ribosome	No reported role yet in HD
	FBL	Fibrillarin	27	Component of a nucleolar small nuclear ribonucleoprotein (snRNP) particle thought to participate in the first step in processing preribosomal RNA	No reported role yet in HD
	FAU	FAU Ubiquitin Like And Ribosomal Protein S30 Fusion	43	Fusion protein consisting of the ubiquitin-like protein fubi at the N terminus and ribosomal protein S30 at the C terminus.	UPS is impaired in mHtt which causes it to be unable to degrade mHtt which causes it to aggregate[33]
	IF4A1	Eukaryotic Translation Initiation Factor 4A1	38	ATP-dependent RNA helicase which is a subunit of the eIF4F complex involved in cap recognition and is required for mRNA binding to ribosome.	No reported role yet in HD
	HNRNPD	Heterogeneous Nuclear Ribonucleoprotein D	25	Binds with high affinity to RNA molecules that contain AU-rich elements (AREs) found within the 3'-UTR of many proto-oncogenes and cytokine mRNA	abnormalities of hnRNP protein can cause disordered RNA metabolism[66]
	HNRNPL	Heterogeneous Nuclear Ribonucleoprotein L	36	Splicing factor binding to exonic or intronic sites and acting as either an activator or repressor of exon inclusion. Exhibits a binding preference for CA-rich elements.	"
	HNRNPU	Heterogeneous Nuclear Ribonucleoprotein U	31	DNA- and RNA-binding protein involved in several cellular processes such as nuclear chromatin organization, telomere-length regulation, transcription, mRNA alternative splicing and stability, Xist-mediated transcriptional silencing and mitotic cell progression.	"
	HNRNPK	Heterogeneous Nuclear Ribonucleoprotein K	34	One of the major pre-mRNA-binding proteins. Binds tenaciously to poly(C) sequences. Likely to play a role in the nuclear metabolism of hnRNAs, particularly for pre-mRNAs that contain cytidine-rich sequences.	"
	HNRNPA2B1	Heterogeneous Nuclear Ribonucleoprotein A2/B1	35	Heterogeneous nuclear ribonucleoprotein (hnRNP) that associates with nascent pre-mRNAs, packaging them into hnRNP particles. The hnRNP particle arrangement on nascent hnRNA is non-random and	"

				sequence-dependent and serves to condense and stabilize the transcripts and minimize tangling and knotting.	
	SF3A3	Splicing Factor 3a Subunit 3	25	The splicing factor 3a heterotrimer includes subunits 1, 2 and 3 and is necessary for the in vitro conversion of 15S U2 snRNP into an active 17S particle that performs pre-mRNA splicing	No reported role yet in HD Note: SFSR6 is a splicing factor that contributes to HD
	SF3B1	Splicing Factor 3b Subunit 1	29	Involved in pre-mRNA splicing as a component of the splicing factor SF3B complex	No reported role yet in HD Note: SFSR6 is a splicing factor that contributes to HD
	SNRPA1	Small Nuclear Ribonucleoprotein Polypeptide A'	25	Involved in pre-mRNA splicing as component of the spliceosome	mHtt can impair splicing of mRNA by "capturing" proteins related to the splicing pathway[55]
	SNRPE	Small Nuclear Ribonucleoprotein Polypeptide E		Plays role in pre-mRNA splicing as core component of the SMN-Sm complex that mediates spliceosomal snRNP assembly and as component of the spliceosomal U1, U2, U4 and U5 small nuclear ribonucleoproteins (snRNPs), the building blocks of the spliceosome	mHtt can impair splicing of mRNA by "capturing" proteins related to the splicing pathway[55]
	SRSF9	Serine And Arginine Rich Splicing Factor 9	34	Plays a role in constitutive splicing and can modulate the selection of alternative splice sites. Represses the splicing of MAPT/Tau exon 10.	No reported role yet in HD
	SRSF7	Serine And Arginine Rich Splicing Factor 7	30	Required for pre-mRNA splicing. Can also modulate alternative splicing in vitro. Represses the splicing of MAPT/Tau exon 10. May function as export adapter involved in mRNA nuclear export such as of histone H2A.	"
	ALYREF	Aly/REF Export Factor	35	Export adapter involved in nuclear export of spliced and unspliced mRNA. Binds mRNA which is thought to be transferred to the NXF1-NXT1 heterodimer for export (TAP/NFX1 pathway)	Aly/Ref is responsible for nuclear export, reduced nuclear export may cause mHtt accumulation in nucleus[87][88]
	CCT5	Chaperonin Containing TCP1 Subunit 5	30	Component of the chaperonin-containing T-complex (TRiC), a molecular chaperone complex that assists the folding of proteins upon ATP hydrolysis.	overexpressing this protein can increase BDNF transport and augment the size of BACHD neurons decreasing the amount of mHtt [108]
	DHX9	DExH-Box Helicase 9	39	Multifunctional ATP-dependent nucleic acid helicase that unwinds DNA and RNA in a 3' to 5' direction and that plays important roles in many processes, such as DNA replication, transcriptional activation, post-transcriptional RNA regulation, mRNA translation and RNA-mediated gene silencing	No reported role yet in HD
	EFTUD2	Elongation Factor Tu GTP Binding Domain Containing 2	31	Required for pre-mRNA splicing as component of the spliceosome, including pre-catalytic, catalytic and post-catalytic spliceosomal complexes	No reported role yet in HD
	KHDRBS1	KH RNA Binding Domain Containing, Signal Transduction Associated 1	18	Recruited and tyrosine phosphorylated by several receptor systems, for example the T-cell, leptin and insulin receptors. Once phosphorylated, functions as an adapter protein in signal transduction cascades by binding to SH2 and SH3 domain-containing proteins	No reported role yet in HD
	PTBP1	Polypyrimidine Tract Binding Protein 1	37	Plays a role in pre-mRNA splicing and in the regulation of alternative splicing events. Activates exon skipping of its own pre-mRNA during muscle cell differentiation. Binds to the polypyrimidine tract of introns.	PTBP1, hNRP-K and nucleolin form a complex so that the protein TUNA can bind to Nanog, Sox2, and Fgf 4. In HD, dysregulation of TUNA protein can cause neuronal loss in HD[108]
	PTGDS	Prostaglandin D2 Synthase	2	Catalyzes the conversion of PGH2 to PGD2, a prostaglandin involved in smooth muscle contraction/relaxation and a potent inhibitor of platelet aggregation	No reported role yet in HD
	RNPS1	RNA Binding Protein With Serine Rich Domain 1	56	Part of pre- and post-splicing multiprotein mRNP complexes. Auxiliary component of the splicing-dependent multiprotein exon junction complex (EJC) deposited at splice junction on mRNAs	mutant CAG repeats can trap RNA binding proteins[55] ; RBPs can also form stress granules which are linked to neurodegeneration[113] but there is not known function of this protein in HD
	YBX1	Y-Box Binding Protein 1	25	DNA- and RNA-binding protein involved in various processes, such as translational repression, RNA stabilization, mRNA splicing, DNA repair and transcription regulation	No reported role yet in HD

	ANXA1	Annexin A1	6	Plays important roles in the innate immune response as effector of glucocorticoid-mediated responses and regulator of the inflammatory process. Has anti-inflammatory activity	
	CDH2	Cadherin 2	16	Calcium-dependent cell adhesion protein plays a role in the establishment of left-right asymmetry, development of the nervous system.	
	CKAP4	Cytoskeleton Associated Protein 4	21	Mediates the anchoring of the endoplasmic reticulum to microtubules. High-affinity epithelial cell surface receptor for the FZD8-related low molecular weight sialoglyco peptide APF/antiproliferative factor. Mediates the APF antiproliferative signaling within cells.	
	FGG	Fibrinogen Gamma Chain	25	Together with fibrinogen alpha (FGA) and fibrinogen beta (FGB), polymerizes to form an insoluble fibrin matrix. Has a major function in hemostasis as one of the primary components of blood clots.	
	KTN1	Kinectin 1	13	Receptor for kinesin thus involved in kinesin-driven vesicle motility. Accumulates in integrin-based adhesion complexes (IAC) upon integrin aggregation by fibronectin.	
	LGALS1	Galectin 1	13	Lectin that binds beta-galactoside and a wide array of complex carbohydrates. Plays a role in regulating apoptosis, cell proliferation and cell differentiation. Inhibits CD45 protein phosphatase activity and therefore the dephosphorylation of Lyn kinase. Strong inducer of T-cell apoptosis.	
	LMAN1	Lectin, Mannose Binding 1	1	Mannose-specific lectin. May recognize sugar residues of glycoproteins, glycolipids, or glycosylphosphatidyl inositol anchors and may be involved in the sorting or recycling of proteins, lipids, or both. The LMAN1-MCFD2 complex forms a specific cargo receptor for the ER-to-Golgi transport of selected proteins.	
	PDIA6	Protein Disulfide Isomerase Family A Member 6	17	May function as a chaperone that inhibits aggregation of misfolded proteins. Negatively regulates the unfolded protein response (UPR) through binding to UPR sensors such as ERN1, which in turn inactivates ERN1 signaling.	
	PPIB	Peptidylprolyl Isomerase B	2	PPIase that catalyzes the cis-trans isomerization of proline imidic peptide bonds in oligopeptides and may therefore assist protein folding.	
	PRKCSH	Protein Kinase C Substrate 80K-H	17	Regulatory subunit of glucosidase II that cleaves sequentially the 2 innermost alpha-1,3-linked glucose residues from the Glc(2)Man(9)GlcNAc(2) oligosaccharide precursor of immature glycoproteins	
	SPP1	Secreted Phosphoprotein 1	12	Binds tightly to hydroxyapatite. Appears to form an integral part of the mineralized matrix. Probably important to cell-matrix interaction. Acts as a cytokine involved in enhancing production of interferon-gamma and interleukin-12 and reducing production of interleukin-10 and is essential in the pathway that leads to type I immunity.	
	PTPRA	Protein Tyrosine Phosphatase Receptor Type A	1	Tyrosine protein phosphatase which is involved in integrin-mediated focal adhesion formation (By similarity). Following integrin engagement, specifically recruits BCAR3, BCAR1 and CRK to focal adhesions thereby promoting SRC-mediated phosphorylation of BRAC1 and the subsequent activation of PAK and small GTPase RAC1 and CDC42	
	TMED10	Transmembrane P24 Trafficking Protein 10	7	Cargo receptor involved in protein vesicular trafficking and quality control in the endoplasmic reticulum (ER) and Golgi	

	TNC	Tenascin C	14	Extracellular matrix protein implicated in guidance of migrating neurons as well as axons during development, synaptic plasticity as well as neuronal regeneration. Promotes neurite outgrowth from cortical neurons grown on a monolayer of astrocytes.	
	VCAN	Versican	15	May play a role in intercellular signaling and in connecting cells with the extracellular matrix. May take part in the regulation of cell motility, growth and differentiation. Binds hyaluronic acid.	
	CBL	Cbl Proto-Oncogene	10	Adapter protein that functions as a negative regulator of many signaling pathways that are triggered by activation of cell surface receptors. Acts as an E3 ubiquitin-protein ligase, which accepts ubiquitin from specific E2 ubiquitin-conjugating enzymes, and then transfers it to substrates promoting their degradation by the proteasome.	
	HIP1R	Huntingtin Interacting Protein 1 Related	10	Components of clathrin-coated pits and vesicles that may link the endocytic machinery to the actin cytoskeleton. Binds 3-phosphoinositides (via ENTH domain). May act through the ENTH domain to promote cell survival by stabilizing receptor tyrosine kinases following ligand-induced endocytosis.	
	MOSPD2	Motile Sperm Domain Containing 2	3	Endoplasmic reticulum-anchored receptor which modulates interorganelle contacts by interacting with other organelle-bound proteins via their FFAT motif	
	MSN	Moesin	3	Ezrin-radixin-moesin (ERM) family protein that connects the actin cytoskeleton to the plasma membrane and thereby regulates the structure and function of specific domains of the cell cortex. Tethers actin filaments by oscillating between a resting and an activated state providing transient interactions between moesin and the actin cytoskeleton.	
	NECAP2	NECAP Endocytosis Associated 2	10	Involved in endocytosis	
	RAB5C	RAB5C, Member RAS Oncogene Family	21	Protein transport. Probably involved in vesicular traffic	
	SNX18	Sorting Nexin 18	9	Involved in endocytosis and intracellular vesicle trafficking, both during interphase and at the end of mitosis. Required for efficient progress through mitosis and cytokinesis.	
	SYT5	Synaptotagmin 5	1	May be involved in Ca(2+)-dependent exocytosis of secretory vesicles through Ca(2+) and phospholipid binding to the C2 domain or may serve as Ca(2+) sensors in the process of vesicular trafficking and exocytosis.	
	VAMP3	Vesicle Associated Membrane Protein 3	11	SNARE involved in vesicular transport from the late endosomes to the trans-Golgi network.	
	VAMP7	Vesicle Associated Membrane Protein 7	10	Involved in the targeting and/or fusion of transport vesicles to their target membrane during transport of proteins from the early endosome to the lysosome. Required for heterotypic fusion of late endosomes with lysosomes and homotypic lysosomal fusion.	