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Investigation of Huntingtin's Role in DNA Repair and Transcription

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Investigation of Huntingtin's Role in DNA Repair and Transcription

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Investigation of Huntingtin's Role in DNA Repair and Transcription

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Huntington's disease (HD) is a devastating and fatal neurodegenerative disease, caused by the expansion of a CAG repeat tract in the mutant Huntingtin gene (*mHTT*). The mechanism of toxicity imparted by mHTT has yet to be fully elucidated, despite decades of research since its description. Research into the normal cellular function of wild type HTT has also been hindered due to its size, complexity, and promiscuity. This dissertation presents a novel function of HTT in the cell as a member of the nuclear Transcription-Coupled DNA Repair (TCR) complex. Both wild type and mHTT were shown to interact with several nuclear DNA repair and transcription proteins, including PNKP, RNA Polymerase, DNA ligase III, CREB, and Ataxin3 (Atxn3). HTT is directly implicated in regulating the DNA repair activities of PNKP, and deubiquitinase activity of Atxn3. This finding is supported by accumulation of DNA damage in transcriptionally active regions of the genome, compared to silenced regions, in HD cell and animal models. Furthermore, mHTT induced cell toxicity is causally linked to prolonged DNA damage response and ATM activation, and inhibition of ATM activity can ameliorate this deleterious response. This dissertation presents additional functions for HTT in mitochondria which parallels its role in the nucleus, as a part of the mitochondrial transcription and DNA repair pathways. HTT interacts with mitochondrial RNA polymerase, DNA polymerase gamma,

mitochondrial transcription factors, PNKP, and Atxn3. Chromatin immunoprecipitation confirms binding of HTT with the mitochondrial DNA. As observed in the nucleus, mHTT impairs the activity of mitochondrial PNKP and Atxn3, leading to accumulated mitochondrial DNA damage. Collectively, these findings provide new insights into how mHTT-mediated abrogation of PNKP and Atxn3 activities simultaneously impairs both nuclear and mitochondrial DNA repair; disrupting transcription, inducing DNA strand breaks, and activating the pro-apoptotic DDR-ATM→p53 signaling pathway to trigger neurotoxicity. Our results also suggest potential targets for developing therapeutic modalities to combat neurodegeneration and neuronal dysfunction in HD.

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List of Abbreviations

AD- Alzheimer's Disease
ALS- Amyotrophic Lateral Sclerosis
APE1- Apurinic endonuclease 1
ATM- Ataxia and Telangiectasia Mutated Kinase
Atxn3- Ataxin3
BDNF- Brain derived neurotrophic factor
BER- Base excision repair
BIFC- Bimolecular Fluorescence Complementation
CBP- CREB binding protein
cDNA- Complementary DNA
ChIP- Chromatin immunoprecipitation
CRB- Cerebellum
CSA- Cockayne syndrome A
CSB- Cockayne syndrome B
CTX- Cortex
DDR- DNA damage response pathway
DNAligIII- DNA ligase III
DSB- Double strand DNA break
FL- Full length
GABA- Gama aminobutyric acid
GFP- Green fluorescent protein
HAP1- Huntingtin associated protein 1
HEAT- Huntingtin, elongation factor 3, protein phosphatase 2A, and TOR1domain
HD- Huntington's disease
HTT- Huntingtin
Htt-Q25- WT huntingtin
Htt-Q97- Expanded Huntingtin
LC3- Microtubule-associated protein 1A/1B-light chain 3
IC- Immunocomplex
IKK- Inhibitor of NFKB Kinase
IP- Immunoprecipitation
ME- Mitochondrial extract
Mt- Mitochondria
NE- Nuclear extract
NER- Nucleotide excision repair
NFKB- Nuclear factor-κB
NHEJ- Nonhomologous end-joining
PD- Parkinson's Disease
PGC1-a- Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PNKP- Polynucleotide Kinsase 3'-Phosphatase
PolyQ- Poly-glutamine repeat
RNAPol- RNAPolB

ROS- Reactive Oxygen Species
SCA3- Spinocerebellar ataxia type 3
siRNA- small interfering RNA
SSB- Single strand DNA break
STR- Striatum
SUMO- Small Ubiquitin-like MOdifier
TBP- TATA binding protein
TCR- Transcription-coupled DNA repair
TF- Transcription factor
TFAM- Mitochondrial transcription factor A
TFB1/2M- Mitochondrial transcription factor B 1 or 2
TFIIS- Transcription Factor II S
UV- Ultraviolet
WB- western blot
WT- wild type

Chapter 1: Introduction

HUNTINGTON'S DISEASE

HD is a devastating, autosomal dominant neurodegenerative disease (Ross and Tabrizi 2011). HD manifests with a characteristic triad of symptoms including motor dysfunction, behavioral/emotional disruption, and cognitive decline (Ross and Tabrizi 2011). Clinical diagnosis often occurs following the development of uncontrolled, uncoordinated movements, and tremors; collectively referred to as chorea. The age at onset of symptoms is highly variable, with an approximate average of 45 years of age (Brackenridge 1971; Jones and Phillips 1970). However, cognitive and behavioral changes have been observed long before onset of motor symptoms. The average life expectancy of HD patients is 10-20yrs post diagnosis, but is also highly variable(Brackenridge 1971). Pneumonia, heart failure, and suicide are the most common causes of death among HD patients. HD is incurable and no therapeutic approach has been successful in slowing the progression of the disease. Current treatments options revolve around managing symptoms and improving quality of life, such as tetrabenazine for the management of chorea (Dalby 1969).

The pathological hallmark of HD is the death of GABAergic neurons in the substantia nigra, and glutaminergic cortical neurons (Ross and Tabrizi 2011). Loss of these and other neurons results in substantial reduction in overall brain mass and volume. HD is a systemic disease, and beyond neurological defects, many peripheral tissues are affected, and patients suffer from severe metabolic dysfunctions, including weight loss resulting from wasting of skeletal and cardiac muscles. Mitochondrial dysfunction is another important hallmark of HD, which contributes significantly to pathogenesis.

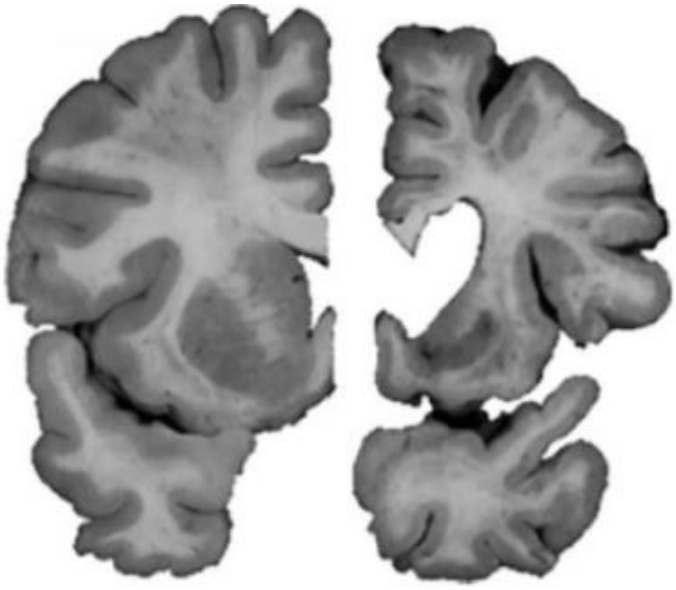


Figure 1.1: Normal vs HD brain

Right: Control brain; Left: HD brain. (Vonsattel, Keller, and Pilar Amaya 2008)

HUNTINGTIN GENETICS

Historical records dating back centuries describe motor diseases called "chorea," from the Greek *khoreia*, referring to the dance-like motions caused by uncontrolled and uncoordinated muscle movements. HD and its hereditary nature were formally described in 1872 by American physician George Huntington, in his publication "*On Chorea*." Genetic markers for HD were eventually linked to chromosome 4 in 1983 (Gusella et al. 1983); throughout the 1980s and early 90s, a collaborative research effort continued narrowing down the location of the HD locus to the short arm (Wasmuth et al. 1988; Whaley et al. 1988; Bates et al. 1991), and further haplotype analysis identified a 100kb region of chr4p16.3 (Macdonald et al. 1992). The huntingtin gene was finally described in 1993; initially termed IT15, the 210kb gene was identified to contain an polymorphic CAG trinucleotide repeat within exon 1 (S. H. Li et al. 1993). This CAG repeat was shown to be far longer in HD patients, compared to controls. Three other trinucleotide repeat expansion disorders had been described prior to HTT: Fragile X

Syndrome (Verkerk et al. 1991), Spinal Bulbar Muscular Atrophy (Spada et al. 1991), and Myotonic Dystrophy Type 1 (Brook et al. 1992). To date, a total of 17 trinucleotide repeat expansion disorders have been identified, 10 of which are caused by CAG repeats.

In HD, the length of the HTT CAG repeat carries a positive association with the age at onset and symptom severity, longer repeats resulting in a younger age of onset. The CAG repeat length is highly heterogeneous within populations, with normal individuals carrying repeats between 10 and 30. Repeats beyond 35 begin showing variable disease penetrance, with full penetrance observed at 40 repeats and above (McNeil et al. 1997). The CAG repeat is a highly unstable motif with a tendency to expand or contract during DNA replication; this results in a phenomenon known as anticipation, wherein the expanded allele grows longer with each successive generation resulting in progressively earlier diagnosis (VLIS, VOLKERS, and WENT 1976). Very long repeats, greater than 60CAG, result in childhood onset juvenile HD which progresses quickly and severely, with death following less than 10 years post diagnosis (Trottier, Biancalana, and Mandel 1994). Life expectancy and patient prognosis are also largely attributable to repeat length; however, while CAG length is the best determinant of age of onset, genetic studies have shown this only accounts for 50-75% of observed variability (McNeil et al. 1997).

The unstable nature of CAG repeats also results in somatic instability, as the repeat expands or contracts during cell division. This instability has been suggested to contribute to age of onset, and may be a factor in the tissue specificity of HD and other trinucleotide repeat expansion disorders (Swami et al. 2009).

THE HUNTINGTIN PROTEIN

Huntingtin (HTT) is a large 350kDa protein, with the characteristic polyQ tract located at the N-terminal, following a short 17 amino acid regulatory domain (Shi Hua Li and Li 1998; Deguire et al. 2018). The polyQ region takes on an unstable alpha-helical structure, which is hypothesized to be a potential protein-interaction domain (M. W. Kim

et al. 2009). HTT contains several other protein-protein interaction domains including multiple HEAT (huntingtin, elongation factor 3, protein phosphatase 2A, and TOR1) domains (S. H. Li et al. 1993; W. Li et al. 2006). HTT also contains regulatory domains which are targets for various post-translational modifications including phosphorylation, ubiquitination, SUMOylation, acetylation, and palmitoylation (X. Cong et al. 2011; Steffan et al. 2004; Thompson et al. 2009; Yanai et al. 2006). Nuclear/cytoplasmic transport of HTT is regulated by an N-terminal nuclear localization sequence, and C-terminal nuclear export sequence (Desmond et al. 2012; Desmond, Maiuri, and Truant 2013; Xia et al. 2003).

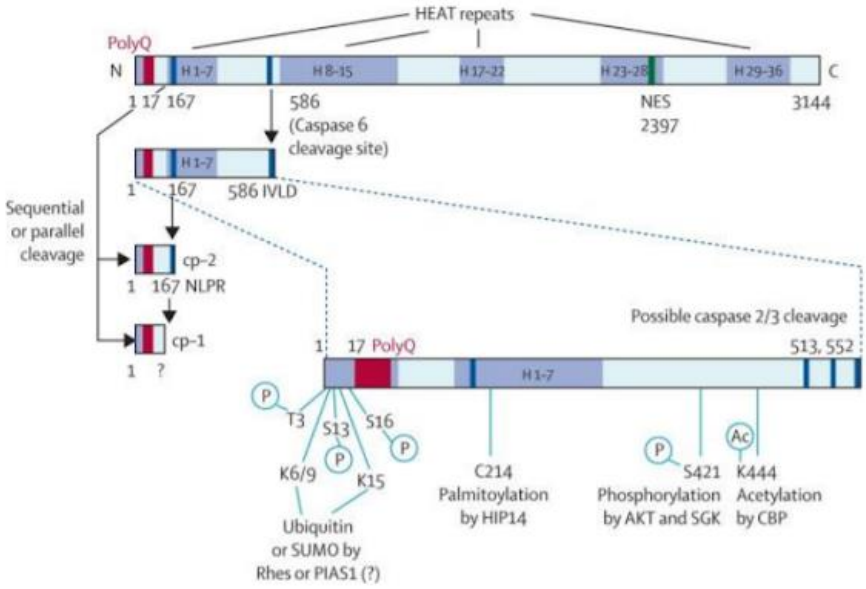


Figure 1.2: Huntingtin protein domains, post-translational modification sites, and cleavage sites.

(Ross and Tabrizi 2011)

Many studies have sought to determine its normal cellular function, and it has been associated with several important cellular pathways. However, the precise role of wtHTT in those pathways remains elusive. Knockout of HTT is embryonic lethal in mice, suggesting a critical role in development (Zeitlin et al. 1995). HTT is also required post

development as knockout of HTT in adult mice is also lethal, resulting in widespread apoptosis and eventual death (Wang et al. 2016; Zeitlin et al. 1995). Conversely, upregulation of HTT has been shown to impart a protective anti-apoptotic effect on striatal neurons in HD mouse models (Leavitt et al. 2006). HTT is expressed in all tissues, and expression is relatively equal in most tissues with upregulation in the testis and central nervous system, and is particularly enriched in corticostriatal neurons (DiFiglia et al. 1995; Fusco et al. 1999). HTT seems to act as a scaffolding protein in its interaction with B-tubulin, microtubules, and the dynein/dynactin complex, and furthermore may be required for mitotic spindle formation (Caviston et al. 2007; Godin et al. 2010; Hoffner, Kahlem, and Djian 2002; Tukamoto et al. 1997). The interaction of HTT with autophagy proteins p62 and LC3 indicates that HTT scaffolding plays a role in autophagosome formation and cargo recruitment (Rui et al. 2015). HTT is also involved in golgi-mediated protein transport (Strehlow, Li, and Myers 2007). Such interactions indicate HTT may serve an important role in cellular trafficking pathways such as endocytosis, and vesicular and organelle transfer pathways (Caviston et al. 2007; Velier et al. 1998). The presence of multiple HEAT repeats supports this, as such motifs are common in proteins which are involved in intracellular trafficking (Neuwald and Hirano 2000; Takano and Gusella 2002). Similarly, HTT has been associated with synaptic vesicles and in both the presynaptic and postsynaptic terminals (DiFiglia et al. 1995; Marcora and Kennedy 2010). Many HTT associated proteins have also been studied in the context of subcellular trafficking. HTT associated protein 1 (HAP1) associates with the kinesin motor complex, binding directly to the kinesin light chain (Caviston et al. 2007; McGuire et al. 2006). HAP1 is also implicated in regulating endocytosis and vesicular trafficking with HTT itself, as well as synaptic vesicle exocytosis (Mackenzie et al. 2016; 2017). Similarly, HTT and HAP40 together have been proposed to regulate association of vesicles with actin (Pal et al., 2006). Association of HTT with the mitochondrial membrane has further implicated it in mitochondrial energy production and/or motility (Pal et al. 2006; Damiano

et al. 2010). HTT is also involved in gene regulation, as suggested by its presence in the nucleus and interactions with numerous transcription factors (Kegel et al. 2002).

MECHANISMS OF HD

The CAG repeat of the HTT gene is translated into an N-terminal poly-glutamine repeat in the protein. Precisely how CAG expansion causes Huntington's disease has yet to be fully elucidated; however, it is known that expression of expanded HTT results in increased DNA damage, transcriptional dysregulation, and mitochondrial impairment (Ross and Tabrizi 2011). The prevailing hypothesis of pathogenesis is that expanded HTT misfolds, causing inactivation of key transcription factors and coactivators. Reduced DNA binding by these factors results in reduced transcription of their target genes and disruption of many vital cellular pathways, resulting in further metabolic dysfunction.

Misfolded HTT aggregates and accumulates in intracellular inclusions, which are a common post-mortem hallmark of HD and many other neurodegenerative diseases. An N-terminal fragment of mHTT contributes to the formation of these aggregates, as it takes on a beta-sheet conformation that is particularly prone to aggregation, and extremely resistant to degradation (Perutz et al. 1994). This mechanism of formation is similar to other protein misfolding and amyloid diseases (Chen et al. 2002; McGowan et al. 2000). This fragment appears to be of particular importance to HD pathogenesis, as expression of the mutant N-terminal fragment has been shown to be sufficient to induce HD symptoms in cell and animal models. The N-terminal fragment results from cleavage of full length HTT by calpains and other proteases, and both WT and mHTT are subject to this cleavage (Bizat et al. 2003; Graham et al. 2006; Hermel et al. 2004; Y. J. Kim et al. 2001; M. Kim et al. 2003; Wellington et al. 1998). Aberrant alternative splicing may also account for production of the N-terminal mHTT fragment (Sathasivam et al. 2013). Accumulation of mHTT aggregates overstresses the autophagy and ubiquitin proteasome systems of affected cells, the two main protein degradation pathways. Autophagy and proteasomal proteins

make up a substantial portion of HD aggregates, and it has been proposed that sequestration of these factors results in further disruptions to cellular homeostasis (Davies and Scherzinger 1997; DiFiglia et al. 1997; Waelter et al. 2001). Similarly, HD aggregates are highly ubiquitinated, and depletion of the ubiquitin pool may indirectly impact many signaling pathways which depend on ubiquitin ligation. These aggregates have also been suggested to impair cytoskeletal dynamics, vesicle and organelle transport, and mitochondrial motility. However, the mechanistic link between these aggregates and cellular dysfunction is tenuous, as aggregates have been observed many years prior to clinical diagnosis, but also significantly after the point at which cellular dysfunctions can be observed. Similarly, the presence of aggregates does not strongly correlate with cell death in HD brain tissues (Vonsattel, Keller, and Pilar Amaya 2008). This has led to competing hypotheses which postulate that the aggregates are a protective mechanism to sequester more toxic soluble forms of mHTT (Arrasate et al. 2004; M. Kim et al. 1999; Saudou et al. 1998). Studies supporting this hypothesis have observed that impairment of the Ubiquitin-Proteasome System (UPS) is reversed after aggregate formation in cell and mouse models (Mitra, Tsvetkov, and Finkbeiner 2009; Ortega et al. 2010). Other recent studies have suggested that the normal autophagy system is in fact capable of resolving mHTT aggregates (Juenemann et al. 2013; Schipper-Krom et al. 2014).

Mutant HTT negatively impacts vesicular trafficking and microtubule formation through its interaction with B-tubulin. This disruption has been shown to reduce insulin secretion, which likely contributes to metabolic dysfunction in HD (Smith et al. 2009). Impaired exocytosis has been proposed to explain reduced secretion of Brain-Derived Neuroprotective Factor (BDNF) by cortical neurons, which is critical to survival of striatal neurons (Anthony Altar et al. 1997; Ferrer et al. 2000; Gauthier et al. 2004)

Mitochondrial dysfunction in HD leads to overall reduction in ATP synthesis and cellular metabolism, and results in a cascade of pro-apoptotic signaling (Ross and Tabrizi 2011). Several mechanisms have been proposed to explain this dysfunction. PGC-1a is a

transcription factor responsible for regulating nuclear genes critical to mitochondrial biogenesis and respiration; this regulation is disrupted in HD and contributes to mitochondrial defects(Johri, Chandra, and Beal 2013). Mitochondrial dynamics have also been shown to be impaired in HD. Axonal transport of mitochondria is reduced in both the anterograde and retrograde directions in cell and mouse models of HD (Trushina et al. 2004). Furthermore, HD cells have highly fragmented mitochondria. HTT interacts with Drp1, which is an important factor regulating mitochondrial fission; mHTT has been shown to increase Drp1 activity, thereby increasing fragmentation rate (J. Kim et al. 2010; U. Shirendeb et al. 2011; U. P. Shirendeb et al. 2012). Dysfunctional mitochondria release excessive amounts of reactive oxygen species (ROS) produced by the impaired electron transport chain. This ROS causes further oxidative cellular damage to mitochondria, which HD cells struggle to repair, resulting in a positive feedback loop of increasing ROS production and damage.

Transcriptional dysregulation is an important factor in HD pathogenesis, and expression profiles of many genes are substantially altered in HD. HTT has been shown to directly interact with a number of transcription factors (TFs) including p53, PGC-1a, TBP, SP1, and CREB binding protein (CBP) (Zhai et al. 2005). Many transcription factors contain polyglutamine rich regions which may facilitate direct interactions with HTT through its polyQ tract, and therefore the expanded polyQ of mHTT may negatively impact gene regulation by those factors. Of particular note, HTT has been implicated in the transcriptional regulation of BDNF, and mHTT has been shown to reduce expression of this critical neuronal survival factor (Zuccato et al. 2001). Furthermore, HTT has been shown to bind to DNA at promoter regions either directly or in complex with TFs, in order to modulate transcription (Benn et al. 2008). DNA binding by HTT was also observed to be polyQ dependent, with increased interactions between mHTT and DNA imparting an abnormal conformation to the helix (Benn et al. 2008). Depletion of the ubiquitin pool due to sequestration in ubiquitin-rich aggregates may also directly impact transcription, as

ubiquitin signaling is vital to transcriptional regulation. Mutant HTT also affects NFKB signaling, increasing NFKB activation through an interaction of mHTT with IKK (Khoshnan et al. 2004).

Recently, more focus has been put on investigating the contributions of the DNA repair pathway, as accumulations of DNA damage and chronic DNA damage response pathway activation have become implicated in HD pathogenesis.

HD transgenic mouse models and human patients suffer from extensive oxidative DNA damage (Bogdanov et al. 2001). The first 17 amino acids in HTT, referred to as the N17 domain, act as an important regulatory domain that can act as a direct ROS sensor through phosphorylation of the M8 residue(X. Gu et al. 2015). Phosphorylation of serines 13 and 16 initiates translocation of HTT from the cytoplasm to the nucleus. In the nucleus, HTT colocalizes with several proteins which are heavily involved in the nucleotide excision repair pathway (NER); including APE1, XRCC1, and Poly-ADP-Ribose (PAR) (Maiuri et al. 2016). HTT has also been shown to interact with a number of transcription factors, including CREB binding protein (CBP), TATA binding protein (TBP), TFIIF, and TFIID (Huang et al. 1998; Zhai et al. 2005a). Mutant HTT has also been shown to inhibit formation of Ku70/80 on DNA double strand break ends, and the subsequent recruitment of DNA-PK proteins, which are important initiating steps in the nonhomologous end joining pathway (Enokido et al. 2010). The 2017 publication by *Maiuri et al* established that HTT is translocated to the nucleus and localizes to regions of DNA damage, in an ATM-dependent manner (Maiuri et al. 2017). Furthermore, our own recent publication supports this newer DNA damage centered outlook on HD mechanisms. We reported in 2015 and 2019 that HTT associates with a number of transcription and DNA repair factors, including Ataxin3, RNA polymerase, DNA ligase, and PNKP (R. Gao et al. 2015; 2019). We also showed that the presence of mHTT inhibits PNKPs DNA end processing activities and ATXN3s deubiquitinase activity; thereby impairing transcription-coupled DNA repair (TCR). Disruption of TCR by the incorporation of mHTT results in

accumulation of DNA damage and reduced transcriptional activity, which resulted in aberrant activation of the ATM-p53 pathway. Increasing PNKP activity, via overexpression, reduced ATM-p53 activation and resulted in improved survival in HD cell models.

DNA DAMAGE AND REPAIR

DNA damage is strongly linked to age related cognitive decline, and likewise, increased DNA damage is a common pathologic feature of neurodegenerative diseases, including HD, Parkinson's disease, Alzheimer's disease, and the Spinocerebellar Ataxias. Similarly, dysfunctional DNA repair mechanisms have been shown to cause a number of neurodevelopmental and neurodegenerative diseases (Borgesius et al. 2011; Compe and Egly 2012; McKinnon 2009).

Cells are subject to several types of DNA damage; and contain a suite of distinct DNA repair mechanisms that act on specific types of damage (Anttinen et al. 2008; Chapman, Taylor, and Boulton 2012; Compe and Egly 2012; Deans and West 2011; Jiricny 2006; Lieber 2010). DNA can suffer damage from numerous endogenous and exogenous sources. Exogenous sources include UV radiation, and cellular and environmental toxins. The primary source of endogenous DNA damage is reactive oxygen species (ROS) produced via the mitochondrial electron transport chain and other metabolic processes. Other endogenous sources include reactive nitrogen species, reactive carbonyl species, alkylating agents, and lipid peroxidation products. The brain is protected from common exogenous sources of DNA damage by the skull and blood brain barrier. However, the brain is an oxygen hungry organ, consuming upwards of 20% of the body's oxygen, and this high metabolic demand results in the production of significant levels of ROS and other endogenous toxins (Raichle and Gusnard 2002). Therefore, the majority of neuronal DNA damage is induced by ROS generated through the mitochondrial electron transport chain and other metabolic processes (Raichle and Gusnard 2002).

Manipulation of DNA itself during processes such as replication, transcription, and DNA repair is another common cause of DNA damage (Pourquier and Pommier 2001). During these processes, single stranded DNA is transiently cleaved by DNA topoisomerases to facilitate topological changes to supercoiling and release torsional stress applied to the DNA during helicase unwinding. Other topoisomerases produce double strand breaks to facilitate release of concatenated DNA during chromosomal replication (Ashour, Atteya, and El-Khamisy 2015). Disruption of these processes can result in the transient cleavage of DNA becoming more permanent and difficult to resolve. Topoisomerase inhibitors are toxic to replicating cell populations, as a result they key to many anti-cancer therapies (Delgado et al. 2018).

Accumulation of DNA damage activates the DNA Damage Response pathway (DDR), initiated by activation of Ataxia Telangiectasia Mutated kinase (ATM), and ATM Related Kinase (ATR) (Cimprich and Cortez 2008; Shiloh and Ziv 2013). Activation of ATM/ATR signaling stimulates the phosphorylation of p53 and Chk2 kinase, resulting in a halt of the cell cycle and initiation of DNA repair mechanisms (Cimprich and Cortez 2008; Shiloh and Ziv 2013). If damage is not repaired in a timely manner, chronic activation of the p53 pathway by ATM/ATR signaling induces apoptosis (Cimprich and Cortez 2008; Shiloh and Ziv 2013).

Double Strand Break Repair

In proliferating cells, DNA strand breaks result in halt of replication forks, and cell cycle arrest; culminating in apoptosis if damage is unresolved (Iyama and Wilson 2013; McKinnon 2013). Dividing cells are capable of repairing double strand breaks (DSBs) via homologous recombination when sister chromatids are accessible, limiting this repair pathway to the S and G2 phases of the cell cycle (Iyama and Wilson 2013; McKinnon 2013). However, the chromatids of non-proliferating cells, such as neurons, are inaccessible and double strand breaks must be repaired via nonhomologous end joining

(NHEJ) (Iyama and Wilson 2013; McKinnon 2013; Nijhawan, Honarpour, and Wang 2000). NHEJ is and inherently mutagenic pathway, which facilitates the ligation of DSBs without regard for sequence fidelity. As a result of their reliance on this imperfect process, nondividing cell populations are at significant risk from such damage. NHEJ is initiated by the recognition and binding of DSB ends by the Ku70/80 heterodimer (Meek, Dang, and Lees-Miller 2008). Ku70/80 then recruits the catalytic subunits of DNA protein kinase (DNA-PKcs), and end processing enzymes to prepare the DNA ends for ligation (Ma et al. 2004; Meek, Dang, and Lees-Miller 2008). DSBs frequently require extensive end processing due to the presence of bulky lesions, or mismatched overhangs; these are resected by the Artemis endonuclease to provide a region of microhomology to permit annealing of the disparate strands (Chang, Watanabe, and Lieber 2015; Goodarzi et al. 2006; J. Gu et al. 2010). Additional end processing may be required by PNKP(Karimi-Busheri et al. 1999). Ligation is carried out by the XRCC4/LIG4 complex with assistance from factors such as XLF (Dai et al. 2003; Lieber 2010).

As a result of their reliance on NHEJ, neurons are particularly susceptible to double strand break; indeed a number of neurodegenerative diseases such as HD, AD, and ALS; have been associated with accumulation of DSBs or dysfunction of the requisite NHEJ proteins (Enokido et al. 2010; Lieber 2010; Mosbach, Poggi, and Richard 2019). Depletion of Ku70/80, XRCC4, or LIG4 in mouse brain tissues results in extensive apoptosis of adult neurons (Chechlacz, Vemuri, and Naegele 2001; Gatz et al. 2011; Y. Gao et al. 1998).

Base Excision Repair

Base Excision Repair (BER) is responsible for removal of inappropriate DNA base modifications, such as alkylation, oxidation, deamination, depurination, single strand breaks, and others (David, O'Shea, and Kundu 2007; Krokan and Bjørås 2013; Wallace 2014). As a testament to the importance of the BER pathway, many of the DNA glycosylases are highly conserved from prokaryotes to eukaryotes (David, O'Shea, and

Kundu 2007; Prakash, Doublié, and Wallace 2012). The offending base is removed by a base specific DNA glycosylase, and the abasic site is excised by an apurinic endonuclease (APE1) (Wiederhold et al. 2004). DNA polymerase beta (Polβ) then fills the gap for short patch BER. Alternatively, the DNAPolβ can continue processing for long patch BER; resulting in strand displacement of the 5' end of the gap, which is then removed prior to ligation by flapases such as FEN1 (Prasad et al. 2000). PNKP is also critical for processing the nicked DNA ends in order to permit ligation (David, O'Shea, and Kundu 2007; Karimi-Busheri et al. 1999; Whitehouse et al. 2001). Short patch BER concludes with ligation by XRCC1 or DNAligI, while long patch repair relies preferentially on DNAligI to close the nicked DNA (David, O'Shea, and Kundu 2007; Hanssen-Bauer et al. 2011; Sallmyr et al. 2020; Whitehouse et al. 2001).

Several neurodegenerative diseases have been associated with deficiencies in BER. DNA strand slipping during BER has even been directly implicated in CAG repeat expansion (Goold et al. 2019; Liu and Wilson 2012; Madabhushi, Pan, and Tsai 2014). Mutations in PNKP and other end processing enzymes are associated with several neurodegenerative and developmental disorders (Chatterjee et al. 2015; Chechlacz, Vemuri, and Naegele 2001; Dumitrache and McKinnon 2017; Poulton et al. 2013). XRCC1 and other ligases are similarly associated with neurodegenerative disorders (Kalasova et al. 2020; Y. Lee et al. 2009).

Nucleotide Excision Repair

Nucleotide Excision Repair (NER) is required for resolving larger, bulky DNA lesions which distort the DNA double helix. Global genomic NER (GG-NER) follows a similar path to BER, with excision of the damaged area via endonucleases, and subsequent filling and ligation by DNAPol and DNAlig, respectively. GG-NER is initiated by recognition of a lesion by the protein Xeroderma Pigmentosum Group C (XPC), then XPC in complex with RAD23b and CETN2 recruit TFIIH (Spivak 2015).

One of the most important mechanisms of DNA repair is a subset of the NER pathway, known as Transcription Coupled DNA Repair (TCR) (Hanawalt and Spivak 2008). TCR allows for rapid and preferential repair of DNA lesions in actively transcribed regions of the genome, thereby maintaining transcriptional activity and fidelity. This method of repair is particularly important for maintaining genomic integrity in nondividing cells such as neurons. In this pathway, RNAPolII stalls at sites of unresolved DNA damage; stalling of the polymerase initiates recruitment of DNA repair proteins, which repair lesions via nucleotide excision repair (Hanawalt and Spivak 2008). Blocked RNAPolII complexes must be resolved before transcription can resume, and unresolved polymerase complexes can initiate strong p53-dependent apoptosis signaling (Yamaizumi and Sugano 1994). A number of proteins are known to be involved in the TCR pathway, including Cockayne Syndrome Group A and B proteins (CSA/CSB), Transcription Factor IIS (TFIIS), p300, and NEDD4 (Fousteri et al. 2006). CSA and CSB are associate with stalled RNAPolII; while their precise functions are not fully elucidated, CSB is an ATPase of the SWI2/SNF2 family of chromatin remodelers (Eisen, Sweder, and Hanawalt 1995; Fousteri et al. 2006; Kettenberger, Armache, and Cramer 2003; Venema et al. 1990). Mutations in these proteins cause Cockayne Syndrome, which manifests with severe UV sensitivity, neurodegeneration, premature aging, and a number of other severe defects (Venema et al. 1990). TFIIS stimulates the intrinsic nuclease activity of RNAPolII, allowing for cleavage of the 3' end of the mRNA, after Pol backtracking (Kettenberger, Armache, and Cramer 2003). P300 is a histone acetyltransferase, which frees the chromatin behind the stalled polymerase (Cazzalini et al. 2008). NEDD4 is an E3 ubiquitin ligase, which can ubiquitinate RNAPolII, targeting it for proteasomal degradation and freeing up access to the damaged region (Anindya, Aygün, and Svejstrup 2007). Arrested polymerases can be resolved by three different mechanisms, backtracking, dissociation and proteasomal degradation, or lesion bypass (Hanawalt and Spivak 2008; Vermeulen and Fousteri 2013). Inhibition of TCR results in accumulation of DNA damage in actively

transcribed regions of the genome, and global reduction of transcription. Chronic ATM signaling, DNA damage accumulation, and decreased transcription are all features of the neurodegenerative diseases described above.

Chapter 2: Mutant huntingtin impairs PNKP and ATXN3, disrupting DNA repair and transcription

INTRODUCTION

In the decades following the identification of the expanded polyQ of mHTT, much effort has been made to understand how this mutation can cause such various, multifactorial cellular dysfunctions. Recently, in the past decade, more research has focused on determining how mHTT expression leads to increased DNA damage, reduced DNA repair, and transcriptional dysregulation; as observed in HD patients and models (Bertoni et al. 2011; Giuliano et al. 2003; Illuzzi et al. 2009; Jimenez-Sanchez et al. 2017; Lu et al. 2014; Ross and Tabrizi 2011). Aberrant activation of the DNA damage response pathway, activated by ATM-p53 signaling, is a key factor of HD pathogenesis. Persistent DNA damage and subsequent ATM activation result in pro-apoptotic p53 signaling, and genetic or pharmacological ablation of ATM activity has been shown to ameliorate this pathway and reduce neuronal cell death in HD cell and animal models (Lu et al. 2014). Recent genome wide association studies, seeking mutations which act as genetic modifiers of HD age of onset, identified many loci coding for various DDR components (Bettencourt et al. 2016; J. M. Lee et al. 2015). Many of those modifiers have further been implicated in similar trinucleotide repeat expansion disorders (Bettencourt et al. 2016). It remains unclear if aberrant DDR pathway activation is mechanistically interlinked with the transcriptional dysregulation observed in HD.

Recent publications from our own lab and collaborators have significant implications for this DNA repair centered hypothesis. We recently reported that Ataxin-3, regulates the activity of the vital DNA repair protein polynucleotide kinase-3'-phosphatase (PNKP). PNKP is a DNA repair enzyme necessary for the repair of non-ligatable DNA strand break ends (Karimi-Busheri et al. 1999; Shen et al. 2010). PNKP catalyzes the

repair of aberrant 3'-P, and 5'-OH strand break ends; activities which are necessary for repair of single strand breaks by the DNAligIII/XRCC1 complex (Karimi-Busheri et al. 1999). PNKP activity is particularly important for repairing the damage caused by ROS, as repair intermediates require end processing. ATM dependent phosphorylation of PNKP is required for its activation, and acts to protect PNKP from ubiquitin-dependent proteasomal degradation (Parsons et al. 2012). Mutations in PNKP have been linked to neurological defects including microcephaly, seizures, developmental delay, and cerebellar atrophy (Shen et al. 2010; Poulton et al. 2013). Ataxin3 is polyQ protein, and expansions of the polyQ tract in excess of 60aa causes autosomal dominant Spinocerebellar Ataxia Type 3 (SCA3), the most commonly inherited ataxia and the most second most commonly inherited polyQ disease after HD (Takiyama et al. 1993).

Our 2015 publication established the novel interaction between PNKP and Atxn3 (R. Gao et al. 2015). Our studies show that Atxn3 is capable of stimulating PNKPs 5'-phosphatase activity, and conversely, pathologically expanded Atxn3 inhibits this same activity. Furthermore, we also reported that PNKP plays a key role in transcription-coupled base excision repair (TC-BER) and transcription-coupled double strand break repair (TC-DSBR) (Chakraborty et al. 2015; 2016).

Because HD is extremely similar to SCA3, these findings lead our group to investigate if HTT interacts with this same complex and functions in a similar capacity.

Aims:

1. Confirm interaction of HTT with Atxn3, PNKP, transcriptional machinery, and other DNA repair factors
2. Investigate the impact of HTT polyQ expansion on PNKP and ATXN3 activities
3. Determine how mHTT effects ATM-p53 signaling and the DNA damage response

RESULTS

HTT IS PART OF A TCR COMPLEX

Both wtHTT and mHTT interact with transcription factors and co-activators including CBP (McCampbell et al. 2000; Nucifora et al. 2001; Steffan et al. 2000), TATA-binding protein (TBP) (Huang et al. 1998), p53 (Bae et al. 2005; Steffan et al. 2000), the general transcription factors TFIID and TFIIF (Zhai et al. 2005b), and specificity protein 1 (Sp1) (Dunah et al. 2002). POLR2A also interacts with HTT and is detected in nuclear inclusions in the HD brain (Huang et al. 1998; Suhr et al. 2001). It is hypothesized that wtHTT, which shuttles into the nucleus, assists in the assembly of transcription factor and co-activator complexes to regulate target gene expression, and that polyQ expansion perturbs the functional integrity of these complexes (Kumar, Vaish, and Ratan 2014; Luthi-Carter and Cha 2003; Ross and Tabrizi 2011). How mHTT disrupts the activities of specific promoters and whether mHTT-mediated transcriptional dysregulation is linked to DNA damage accumulation and aberrant DDR pathway activation remains unknown.

Given that HTT interacts with huntingtin-associated protein 1 (HAP-1) (X. J. Li et al. 1995), while ATXN3 interacts with HAP-1 (Takeshita et al. 2011) and PNKP (Chatterjee et al. 2015; R. Gao et al. 2015), we asked whether ATXN3 and PNKP might interact with HTT to form a TCR complex and if this is affected by polyQ expansion. We isolated nuclear protein extract (NE) and cytosolic protein extract (CE) from SH-SY5Y cells and the fractions were analyzed by western blot (WB) to determine purity of nuclear protein fractions (Figure 1A). We immunoprecipitated (IP'd) endogenous wtHTT from the NE of SH-SY5Y cells, and WBs of the immunocomplexes (ICs) showed the presence of HAP-1, ATXN3, CBP, TAFII 130 (TAF4), POLR2A, PNKP, and LIG 3 (Figures 1B). Similarly, IP of endogenous ATXN3 from NEs revealed these proteins in the ATXN3-IC (Figures 1C). Finally, IP of PNKP from NEs confirmed that they were also present in the PNKP-IC (Figures 1D). To verify the specificity of these interactions *in vivo*, we analyzed the ICs for the presence of apurinic-apyrimidinic endonuclease 1 (APE1), another critical

DNA base excision repair (BER) enzyme that works independently of PNKP-mediated BER pathways (Wiederhold et al. 2004). APE1 was not detected (Figures 1B to D), suggesting interaction specificity and selectivity. Finally, IP of POLR2A again revealed these proteins in the IC (Figures 1E). For further confirmation, we IP'd Myc-tagged HTT from the NEs of PC12 cells expressing Myc-tagged FL-wtHTT-Q23 or FL-mHTT-Q148. WB confirmed the presence of ATXN3, PNKP, POLR2A, CBP, and LIG 3 but not APE1 in the Myc-IC (Figures 1F, 1G), suggesting that HTT, POLR2A, CBP, ATXN3, LIG 3, and PNKP form a multiprotein TCR complex. Proximity ligation assays (PLAs) were then performed to verify interaction specificity (R. Gao et al. 2015). The reconstitution of fluorescence in neuronal cells (Figures 1H to M) and postmortem human brain sections (Supplemental Figure 1) suggested substantial interaction among these proteins. Importantly, the majority of the PLA signals was from the nuclei but substantial amount of signals were from the periphery or cytoplasm. Immunostaining the cells with mitochondrial markers suggested that HTT forms similar complexes in the mitochondria (data not shown). Importantly, about 60-70% of the PLA signal was nuclear in control brain, while the complexes were predominantly in the perinuclei or cytoplasm of HD brain sections (Supplemental Figure 1). Since PNKP and HTT are present in the mitochondria (Mandal et al. 2012; Orr et al. 2008), the extranuclear signals detected in the control subjects are presumably from mitochondrial HTT-ATXN3-PNKP complexes. WB analysis of subcellular protein fractions from neuronal cells show the presence of HTT, ATXN3 and PNKP in mitochondria (data not shown). Moreover, co-staining the cells or brain sections with mitochondrial markers suggested presence of HTT, ATXN3 and PNKP in mitochondria (data not shown). These findings indicate that HTT may form a similar complex in mitochondria regulating mtDNA repair and transcription.

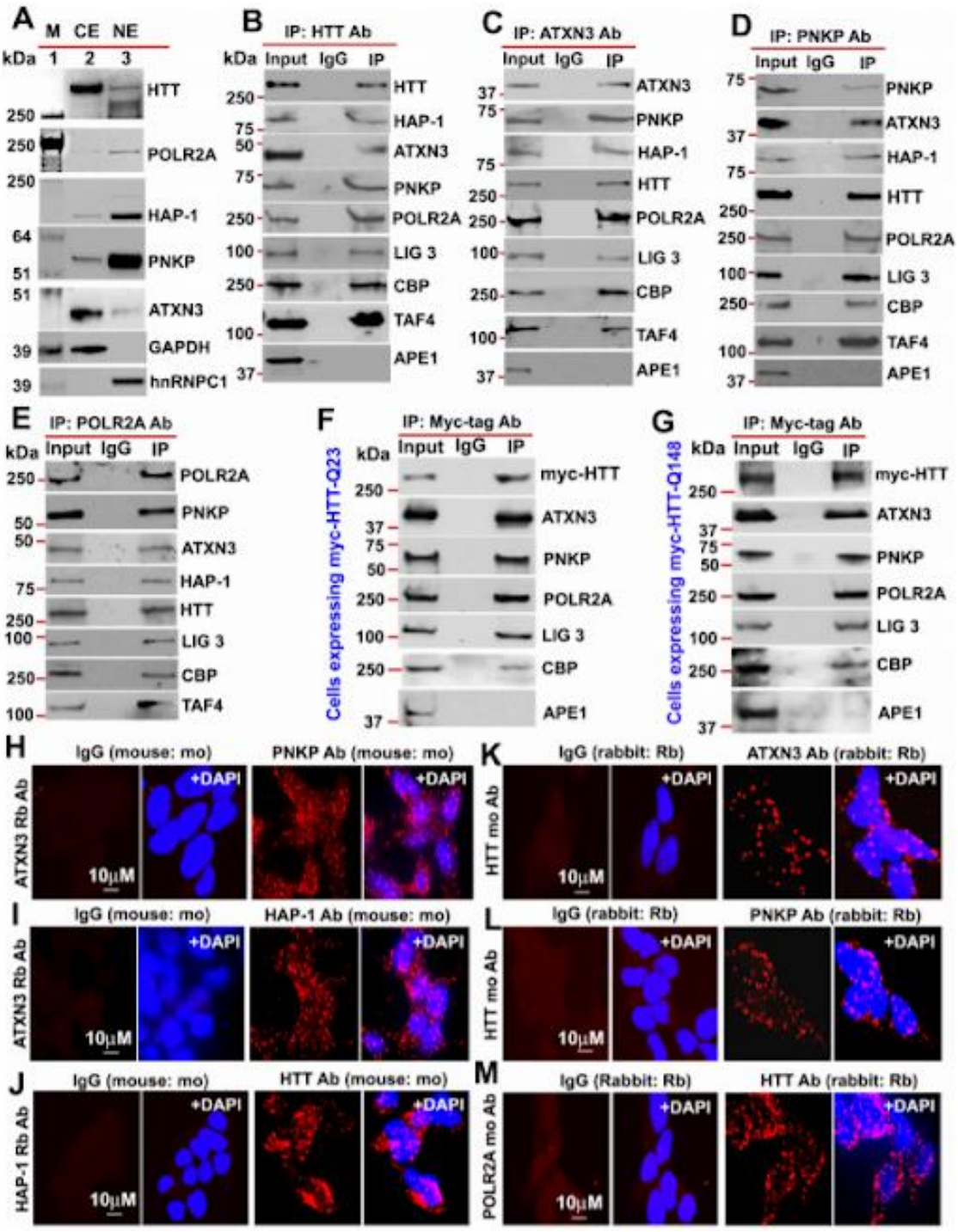


Figure 2.1. HTT is a part of the TCR complex.

(A) Nuclear extract (NE), and cytosolic extract (CE) were purified from human neuroblastoma SH-SY5Y cells and the protein fractions were analyzed by western blots (WBs) to detect HTT, ATXN3, PNKP, and HAP1 levels in these sub-cellular fractions. GAPDH and hnRNPC1/C2 were used as cytosolic and nuclear markers respectively.

APE1 was used as a negative control in panels A to D. (B) Endogenous HTT was immunoprecipitated (IP'd) from NEs of SH-SY5Y cells and immunocomplex (IC) were analyzed by western blot (WB) to examine the TCR proteins(HAP-1, ATXN3, PNKP, POLR2A, LIG 3, CBP, and TAFII 130 (TAF4). (C) Endogenous ATXN3 was IP'd from NEs of SH-SY5Y cells and IC was subjected to WB to detect associated TCR complex components with respective antibodies. (D) Endogenous PNKP was IP'd from NEs of SH-SY5Y cells and IC was analyzed by WB to examine associated TCR components. (E) Endogenous POLR2A was IP'd from NEs of SH-SY5Y cells and IC was analyzed by WB to detect associated TCR proteins. (F) NEs was isolated from PC12 cells ectopically expressing a Myc-tagged full-length normal wild type HTT (FL-wtHTTQ23) for assessing the possible interaction of HTT with POLR2A and associated TCR proteins. Exogenous Myc-wtHTT-Q23 was IP'd with an anti-Myc antibody, and the Myc immunocomplex was subjected to WBs with respective antibodies. APE1 was used as a negative control in panels F and G. (G) NEs was isolated from PC12 cells ectopically expressing a Myc-tagged full-length mutant HTT (FL-mHTT-Q148). Exogenous Myc-wtHTT-Q148 was IP'd with an anti-Myc antibody, and the Myc immunocomplex was subjected to WBs with respective antibodies. Proximity Ligation Assay (PLA) in SH-SY5Y cells to examine the protein-protein interaction using the following antibody pairs. Red fluorescence indicates positive PLA signals for protein-protein interactions. Nuclei were stained with DAPI. (H) ATXN3 (rabbit: Rb) and IgG (mouse: mo) or PNKP (mouse: mo) antibodies. (I) ATXN3 (rabbit: Rb) with IgG (mouse: mo) or HAP-1 (mouse: mo) antibodies. (J) HAP-1 (rabbit: Rb) and IgG (mouse: mo) or HTT (mouse: mo) antibodies. (K) HTT (mouse: mo) with IgG (rabbit: Rb) or ATXN3 (rabbit: Rb) antibodies. (L) HTT (mouse: mo) with IgG (rabbit: Rb) or PNKP (rabbit: Rb) antibodies, and. (M) POLR2A (mouse: mo) with IgG (rabbit: Rb) or HTT (rabbit: Rb) antibodies.

The possible *in vivo* association of these proteins was further assessed by immunostaining HTT, PNKP, and ATXN3 in postmortem brain tissue from patients with HD and control subjects. Confocal microscopy revealed colocalization of HTT with PNKP and ATXN3 in HD and control brain (Figures 2A & B; arrows). Colocalization of ATXN3 with PNKP was observed in both groups (Figure 2C; arrows). Marked HTT/PNKP colocalization was also observed in brain sections from HD knock-in (zQ175) (Menalled et al. 2012) and WT control mouse brain tissue (data not shown).

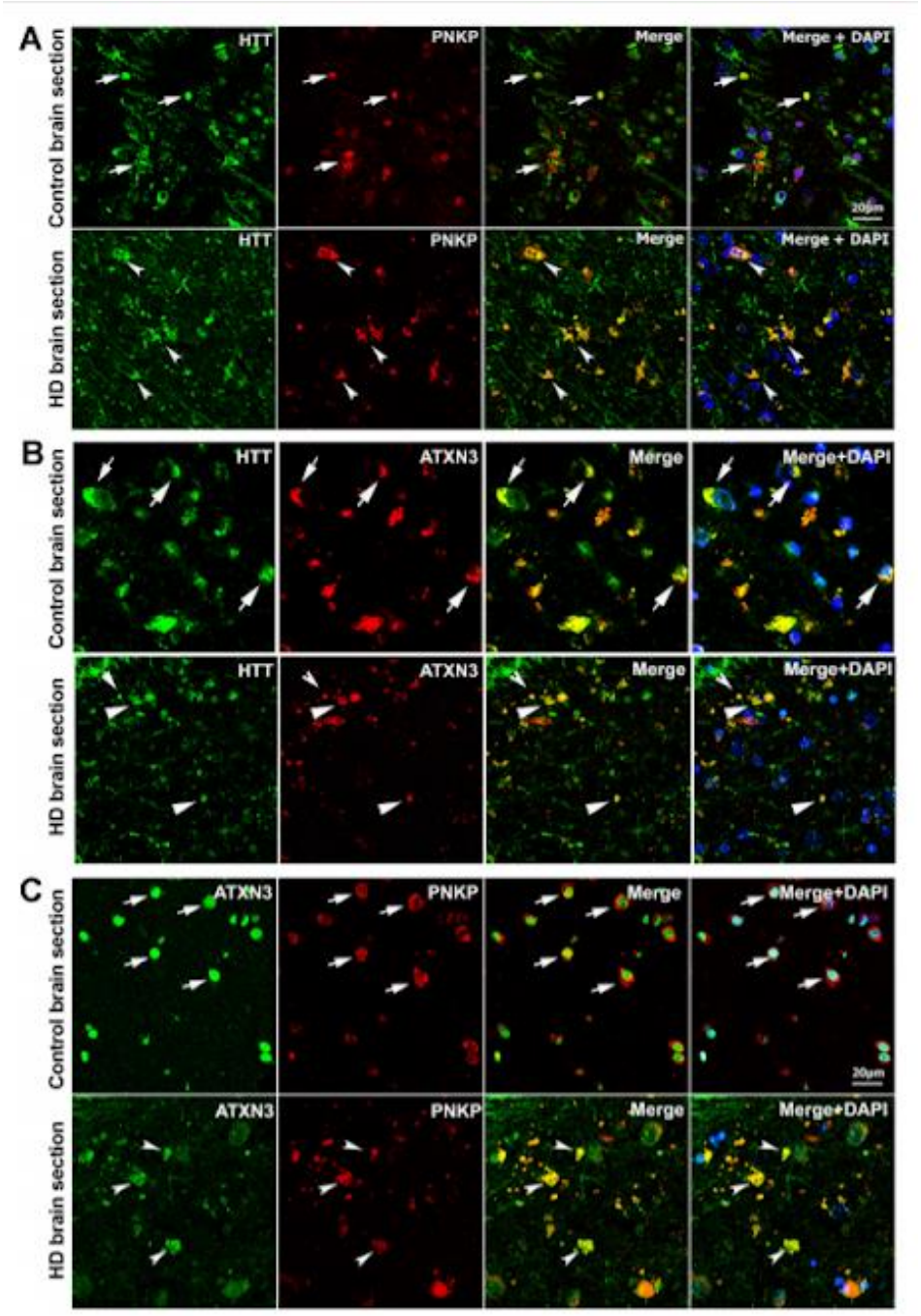


Figure 2.2. HTT colocalizes with PNKP and ATXN3 in postmortem human brain sections.

(A) Normal and HD postmortem brain (mHTT-Q94) sections were analyzed by double immunolabeling with antibodies against HTT (green) and PNKP (red) to assess their in vivo colocalization and possible interactions (representative colocalization of HTT and PNKP are shown by arrows). For panels A and B, merge of red and green fluorescence appears as yellow/orange, and nuclei were stained with DAPI (blue). (B) Normal and HD

brain (mHTT-Q82; early onset HD patients, disease grade 4/4, manifestingFigure 2. HTT colocalizes with PNKP and ATXN3 in postmortem human brain sections. (A) Normal and HD postmortem brain (mHTT-Q94) sections were analyzed by double immunolabeling with antibodies against HTT (green) and PNKP (red) to assess their in vivo colocalization and possible interactions (representative colocalization of HTT and PNKP are shown by arrows). For panels A and B, merge of red and green fluorescence appears as yellow/orange, and nuclei were stained with DAPI (blue). (B) Normal and HD brain (mHTT-Q82; early onset HD patients, disease grade 4/4, manifesting severe phenotype) sections analyzed by double immunolabeling with antibodies against HTT (green) and ATXN3 (red) to assess their in vivo colocalization and possible interaction (arrows). (C) Normal and HD brain (mHTT-Q94; early onset HD patients, disease grade 4/4, manifesting severe phenotype) sections were analyzed by double immunolabeling with antibodies against ATXN3 (green) and PNKP (red) to assess their in vivo colocalization and possible interaction (arrows).

THE C-TERMINAL CATALYTIC DOMAIN OF PNKP INTERACTS WITH HTT

PNKP contains an N-terminal fork head-associated (FHA) domain, C-terminal fused 3'-phosphatase (PHOS) domain, and 5'-kinase (KIN) domain. The PHOS domain hydrolyzes 3'-phosphate groups, while the KIN domain promotes addition of a phosphate group to the 5'-OH at damaged sites for error-free repair (Karimi-Busheri et al. 1999). To identify the specific PNKP domain(s) that interact with HTT, full-length PNKP (FL-PNKP); the FHA, PHOS, and KIN domains; the FHA+PHOS domains; or the PHOS+KIN domains were expressed as a FLAG-tagged peptide, as illustrated in Figure 3A. We individually expressed these domains in SH-SY5Y cells (Figure 3B; upper panel) and isolated the NEs. IPs of these domains with a FLAG antibody and subsequent WB analysis of the IC showed the presence of HTT in the FLAG-(FL-PNKP)-IC and FLAG-(PHOS+KIN)-IC (Figure 3B; Lower panel, lanes 1 & 6, arrow). HTT was not detected in FLAG-ICs when the individual FHA, PHOS, or KIN domains were IP'd (Figure 3B; lanes 2-5, arrow). This suggests that the C-terminal catalytic domain of PNKP interacts with HTT, but the individual FHA, PHOS, and KIN domains are not sufficient. We separately expressed the PNKP domains in cells, isolated the NEs, and IP'd endogenous HTT. WBs revealed the presence of full-length and PHOS+KIN domains in the HTT-IC (Figure 3C; Lower panel, lanes 1 & 6). When we expressed the PNKP domains in PC12 cells

expressing Myc-wtHTT-Q23 or Myc-mHTT-Q148 (Figures 3D & E; upper panels), IP of the Myc-HTT and WB revealed the full-length protein or PHOS+KIN domain (Figures 3D & E, Lower panels, lanes 1 & 6). These data suggest that both wtHTT and mHTT interact with the C-terminal catalytic domain of PNKP.

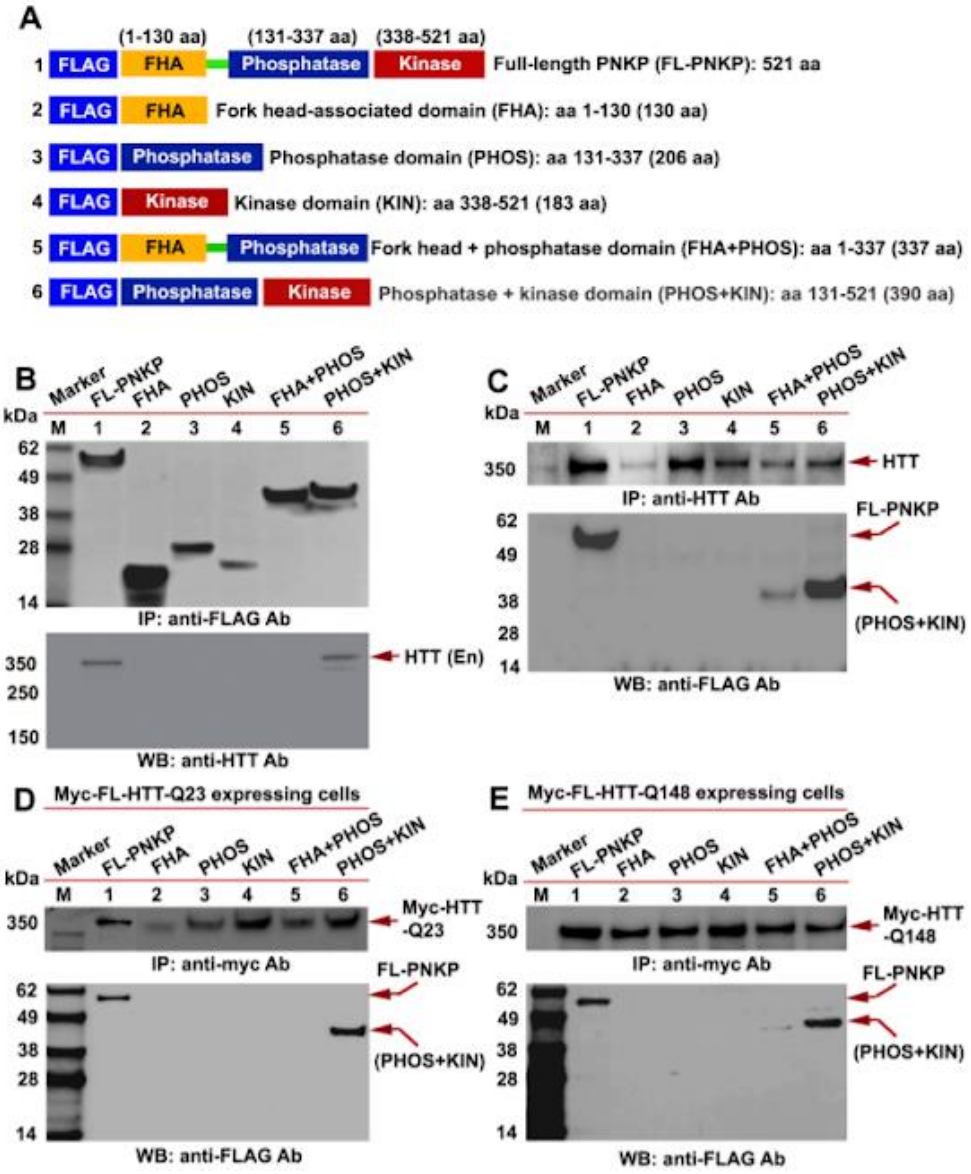


Figure 2.3. HTT interacts with the C-terminal catalytic domain of PNKP.

(A) Schematic illustrating various functional domains of PNKP expressed as FLAG-tagged peptides: (1) full-length PNKP containing N-terminal fork-head-associated (FHA)

domain, central phosphatase (PHOS) and C-terminal kinase (KIN) domains; (2) FHA domain (1–130 amino acids); (3) PHOS domain (131–337 amino acids); (4) KIN domain (338–521 amino acids); (5) FHA and PHOS domains (1–137 amino acids); and (6) PHOS and KIN domains (131–521 amino acids). (B) Plasmids encoding full-length PNKP (FL-PNKP) or various PNKP domains were separately transfected into SH-SY5Y cells (lanes 1 to 6) and NEs isolated 48 hr post-transfection. Lanes 1 to 6 in the WB (upper panel) shows the pull-down of full-length PNKP (FL-PNKP) and various PNKP domains that were IP'd with an anti-FLAG Ab. The WB in the lower panel shows the presence of endogenous HTT (arrow) in the FLAG-IC. M: protein molecular weight marker. (C) Plasmids encoding full-length PNKP (FLPNKP) and various PNKP domains were separately transfected into SH-SY5Y cells (lanes 1 to 6), NEs isolated, and HTT was IP'd with an anti-HTT antibody. The pull-down of endogenous HTT is shown in the upper panel (arrow). The HTT-IC was analyzed by WB (lower panel) to detect FL-PNKP or various PNKP domains with an anti-FLAG Ab (arrows). (D) Plasmids encoding FLAG-tagged full-length PNKP (FL-PNKP) or various PNKP domains were transfected separately into PC12 cells expressing full-length Myc-tagged normal HTT encoding 23Qs (Myc-FL-wtHTT-Q23) (lanes 1 to 6), NEs were isolated, and Myc-HTT IP'd with Myc tag antibody. Upper panel is the WB showing the IP of HTT with an anti-Myc tag antibody. The Myc-IC was analyzed by WB to assess interaction of various PNKP domains with HTT with an anti-FLAG antibody (lower panel, arrows). (E) Plasmids encoding the full-length PNKP (FL-PNKP) or various PNKP domains were transfected into PC12 cells expressing Myc-tagged full-length mutant HTT encoding 148Qs (Myc-FL-mHTT-Q148) (lanes 1 to 6), NEs isolated, and Myc-tagged HTT was IP'd with an anti-Myc-tag antibody. Upper panel is the WB showing IP of Myc-HTT with anti-Myc tag antibody. Interactions of FL-PNKP or various PNKP domains with FL-HTT were analyzed by WB with an anti-FLAG antibody (lower panel, arrows).

N-terminal-truncated HTT fragments interact with the catalytic domain of PNKP

The N-terminal-truncated fragment of mHTT (NT-mHTT) containing the polyQ expansion is encoded by exon 1 of the HTT gene. Transgenic mice expressing exon 1 or a truncated fragment extending beyond the first exon (N171) with NT-mHTT recapitulate HD-like neurological and behavioral abnormalities (Mangiarini et al. 1996; Schilling et al. 1999). To test whether this fragment interacts with PNKP, we expressed NT-wtHTT-Q23 or NT-mHTT-Q97 (1-586 base pairs) as a GFP-tagged peptide in SH-SY5Y cells (Figure 4A; upper panel), isolated the NEs, and IP'd the GFP-NT-HTT fusion peptide with a GFP antibody. WBs showed the presence of PNKP, ATXN3, and HTT in the GFP-IC (Figure 4A; lower panel, lanes 4 & 6). We next IP'd this fragment from PC12 cells expressing Myc-NT-wtHTT-Q23 or NT-mHTT-Q148 and found ATXN3, PNKP, POLR2A, CBP,

and LIG 3 in the Myc-IC. Importantly, APE1 was not detected in the IC, again suggesting interaction specificity (Figure 4B). To identify which PNKP domain interacts with NT-HTT, we expressed various domains as FLAG-tagged peptides in SH-SY5Y cells expressing either Myc-NT-HTT-Q23 or Myc-NT-HTT-Q97 (Figures 4C & D; upper panels) and IP'd Myc-tagged fragments from the NEs. WBs revealed FL-PNKP and PNKP-(PHOS+KIN) domains in the Myc immunocomplex (Figures 4C & D; lanes 1 & 6), suggesting that the N-terminal fragment of HTT interacts with the C-terminal catalytic domain of PNKP. However, from the WB analyses we could not establish whether the interaction of the mutant HTT fragment (NT-mHTT) with PNKP-(PHOS+KIN) domain is stronger than the interaction with the N-terminal fragment of WT HTT (NT-wtHTT; Figures 4C & D; lanes 6). The PNKP-(FHA+PHOS) domain also showed a relatively weaker interaction with the N-terminal fragment of HTT (Figures 4C & D; lanes 5) indicating that the FHA-PHOS domain of PNKP alone interacts with the N-terminal fragment of HTT.

To further assess these possible interactions, we performed bi-molecular fluorescence complementation (BiFC) assays as we previously reported (R. Gao et al. 2015). We cloned either the full-length or C-terminal catalytic domain of PNKP at the N-terminus of cyan fluorescent protein (CFP) into plasmid pBiFC-VN173 to construct plasmids pVN-PNKP and pVN-(PHOS+KIN), respectively. We also cloned the N-terminal fragment of wtHTT and mHTT cDNA (encoding 23 and 97 glutamines, respectively) at the C-terminus of CFP in plasmid pBiFC-VC155 to construct pVC-NT-HTT-Q23 and pVC-NT-HTT-Q97, respectively (detailed descriptions of these plasmids are provided in the STAR Methods). Cotransfection of plasmid pVN-PNKP with the parent plasmid pBIFC-VC155 did not reconstitute fluorescence (Figure 4E; Panel 1), whereas cotransfection of pVN-PNKP with either pVC-NT-HTT-Q23 or pVC-NT-HTT-Q97 did (Figure 4E; Panels 2 & 3). Similarly, cotransfection of pVN-(PHOS+KIN) with pBIFC-VC155 did not produce fluorescence (Figure 4E; Panel 4), whereas cotransfection of pVN-

(PHOS+KIN) with either pVC-NT-HTT-Q23 or pVC-NT-HTT-Q97 robustly reconstituted fluorescence (Figure 4E, Panels 5 & 6). Although these data suggest that the N-terminal of mHTT interacts with PNKP, these experiments do not inform the relative strengths of interaction between these peptides. Nonetheless, the IP and BIFC studies suggest that the truncated-N-terminal fragments of both WT and mHTT interact with the C-terminal catalytic domain of PNKP. The interaction of these peptides with the PHOS-KIN domain of PNKP is relatively stronger than with the PHOS domain alone. However, more rigorous structural and biophysical measurements using purified proteins/peptides will be required to understand the true nature of these protein-protein interactions, the relative binding efficacies and to identify the direct interacting partners in this complex. Moreover, since the HTT-TCR complex is not fully characterized, the presence of additional unidentified components of the complex could significantly alter these interactions *in vivo*.

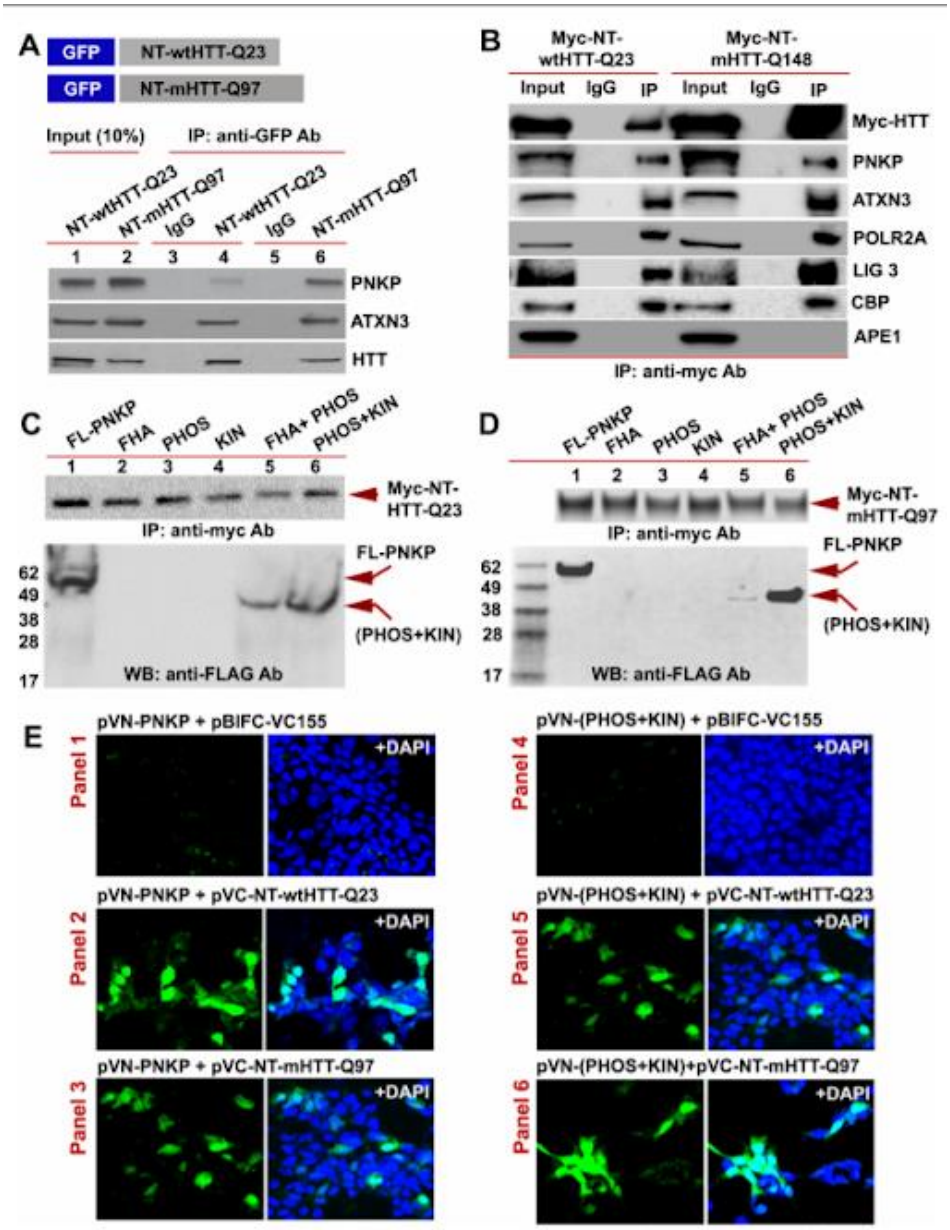


Figure 2.4. The N-terminus of HTT interacts with the C-terminal catalytic domain of PNKP.

(A) Schematic showing GFP-tagged N-terminal fragment of wild-type normal HTT encoding 23Qs or mutant HTT encoding 97Qs (NT-wtHTT-Q23 and NT-mHTT-Q97 plasmid vectors respectively; upper panel). SH-SY5Y cells were transfected with NT-wtHTT-Q23 or NT-mHTT-Q97, NEs isolated, fusion peptides IP'd from NE with an anti-GFP antibody, and WBs performed with respective antibodies to detect endogenous PNKP, ATXN3, or HTT in the GFP-IC (lower panel). (B) NEs from PC12 cells expressing Myc-tagged N-terminal fragment of wild-type normal HTT encoding 23Qs or mutant HTT encoding 148Qs (NT-wtHTT-Q23 or NT-mHTT-Q148, respectively) were isolated and the Myc-HTT was IP'd with an anti-Myc tag Ab and Myc-IC was analyzed

by WBs to detect various TCR complex components with respective antibodies. (C) Plasmids encoding full-length PNKP (FL-PNKP) or various PNKP domains (lanes 1 to 6) were separately transfected into SH-SY5Y cells expressing the N-terminal fragment of HTT encoding 23Qs (Myc-NT-wtHTT-Q23), NEs were isolated and the NT-HTT was IP'd with an anti-Myc tag Ab. The upper panel shows pull down of Myc-NT-HTT-Q23. The Myc-IC was analyzed by WBs with an anti-FLAG Ab to detect FL-PNKP or PNKP domains (lower panel; arrows). (D) Plasmids encoding full-length PNKP (FL-PNKP) or various domains (lanes 1 to 6) were separately transfected into SH-SY5Y cells expressing the N-terminal fragment of mutant HTT encoding 97Qs (Myc-mHTT-Q97) and NEs were isolated and the Myc-NT-HTT-Q97 was IP'd with an anti-Myc tag Ab and the Myc-IC was analyzed by WBs to detect FL-PNKP or PNKP domains (lower panel; arrows). (E) BiFC assay of SH-SY5Y cells cotransfected with plasmids: Panel 1) pVN173-PNKP and pVC-BIFC-155, Panel 2) pVN-PNKP and pVC-NTwtHTT-Q23, Panel 3) pVN-PNKP and pVC-NT-mHTT-Q97, Panel 4) pVN (PHOS + KIN) and pVC-BIFC-155, Panel 5) pVN-(PHOS + KIN) and pVC-NTwtHTT-Q23, and panel 6) pVN-(PHOS + KIN) and pVC-NT-mHTT-Q97. Reconstitution of fluorescence was monitored via fluorescence microscopy. Nuclei were stained with DAPI (blue).

mHTT abrogates PNKP activity to induce DNA damage and trigger DDR signaling

Given that PNKP interacts with mHTT, we measured the 3'-phosphatase activity of PNKP in induced pluripotent stem cells (iPSCs) differentiated to neurons enriched for medium striatal neuronal populations from HD and unaffected control subjects using a modification of Telezhkin et al. (Telezhkin et al. 2016a). HD iPSC-derived neurons (mHTT-109Qs) were compared to control neurons (wtHTT-33Q; HD iPSC Consortium, 2017) and activity was found to be 70-80% lower in the NE of HD neurons, while PNKP protein levels did not change (representative experiment, Figures 5A to C). Similar differences were found for neurons with adult onset alleles (Q50 and Q53) compared to controls (Q18 and Q28). In these comparisons there was substantially reduced (70 to 80%) PNKP activity in HD neurons (Q50 and Q53) compared with control neurons (Q18 or Q28) (Supplemental Figure 2), supporting an impairment in human neurons in the presence of mHTT.

We next measured PNKP activity in PC12 cells expressing exogenous full-length wtHTT (FL-wtHTT-Q23) and full-length mHTT (FL-mHTT-Q148) (Igarashi et al. 2003; Tanaka et al. 2006). We found that it was about 30-40% higher in the NE of PC12 cells

expressing wtHTT, and about 70% lower in the NE of PC12 cells expressing FL-mHTT-Q148 compared to control cells, while PNKP protein levels did not change (Supplemental Figure 3). These data suggest that wtHTT and mHTT stimulate and abrogate PNKP activity, respectively. Since wtHTT interacts with and stimulates PNKP activity, we examined the extent to which HTT depletion alters PNKP activity. We found that in HTT-depleted cells, PNKP activity was reduced by >70% (Supplemental Figure 3), suggesting that wtHTT plays key roles in stimulating PNKP activity, maintaining the functional integrity of the TCR complex, and repairing DNA damage. PNKP activity was 80-90% decreased in the striatum (STR) and cortex (CTX), and marginally (5%) decreased in the cerebellum (CRBL) of male heterozygous asymptomatic zQ175 mice at 7 weeks, whereas PNKP protein levels were not different from WT (Figures 5D to F). An identical trend was observed in female littermates (data not shown).

Because the N-terminal of mHTT interacts with PNKP, we investigated whether N-terminal truncated fragment of mHTT interferes with PNKP activity in PC12 cells or N171-82Q mice (Schilling et al. 1999; Tanaka et al. 2006). PNKP activity was ~30% higher in PC12 cells expressing NT-wtHTT-Q23 and >80% lower in cells expressing NT-mHTT-Q148 (Supplemental Figure 4). Similar to full-length HTT, PNKP activity was decreased in SH-SY5Y cells expressing NT-mHTT with variable glutamine expansions (Supplemental Figure 4). Moreover, PNKP activity was >80% decreased in the STR and CTX of N171-82Q brain compared to control (Supplemental Figure 4). To test if mHTT specifically blocks PNKP activity rather than interfering with DNA repair *per se*, we examined how it modulated the repair of two nicked DNA duplexes: one without a 3'-phosphate end that requires DNA polymerase and ligase activities but not PNKP activity for repair, and another duplex with a 3'-phosphate end that requires PNKP and DNA polymerase and ligase activities for complete repair. We observed that NEs from cells expressing mHTT or from zQ175 mouse brain did not hamper repair of the duplex that required DNA polymerase and ligase activities but did not require PNKP activity. In

contrast, NEs from these cells and mice did abrogate repair of the duplex that requires PNKP (Figures 5G to J), suggesting that mHTT specifically blocks PNKP activity but does not interfere with the activities of other repair enzymes in the TCR complex. In response to DNA strand break accumulations, ATM is activated by phosphorylation which phosphorylates p53, which in turn activates pro-apoptotic gene transcription (Chipuk et al. 2004; Nakano and Vousden 2001; Oda et al. 2000). Consistently, we found chronic activation of the DDR-ATM-p53 pathway in HD neurons (Supplemental Figure 5) and in zQ175 CTX (Supplemental Figure 5) compared with respective controls. mHTT expression has been shown to activate p53 in HD, whereas deleting p53 in the HD transgenic brain rescues behavioral abnormalities (Bae et al. 2005). Consistently, markedly increased mRNA expression of p53 target genes (e.g., Bcl2L11, Pmaip1, Bid, Pidd1 and Apaf1) were observed in the STR but not in CRBL of zQ175 mice compared to controls (Supplemental Figure 5).

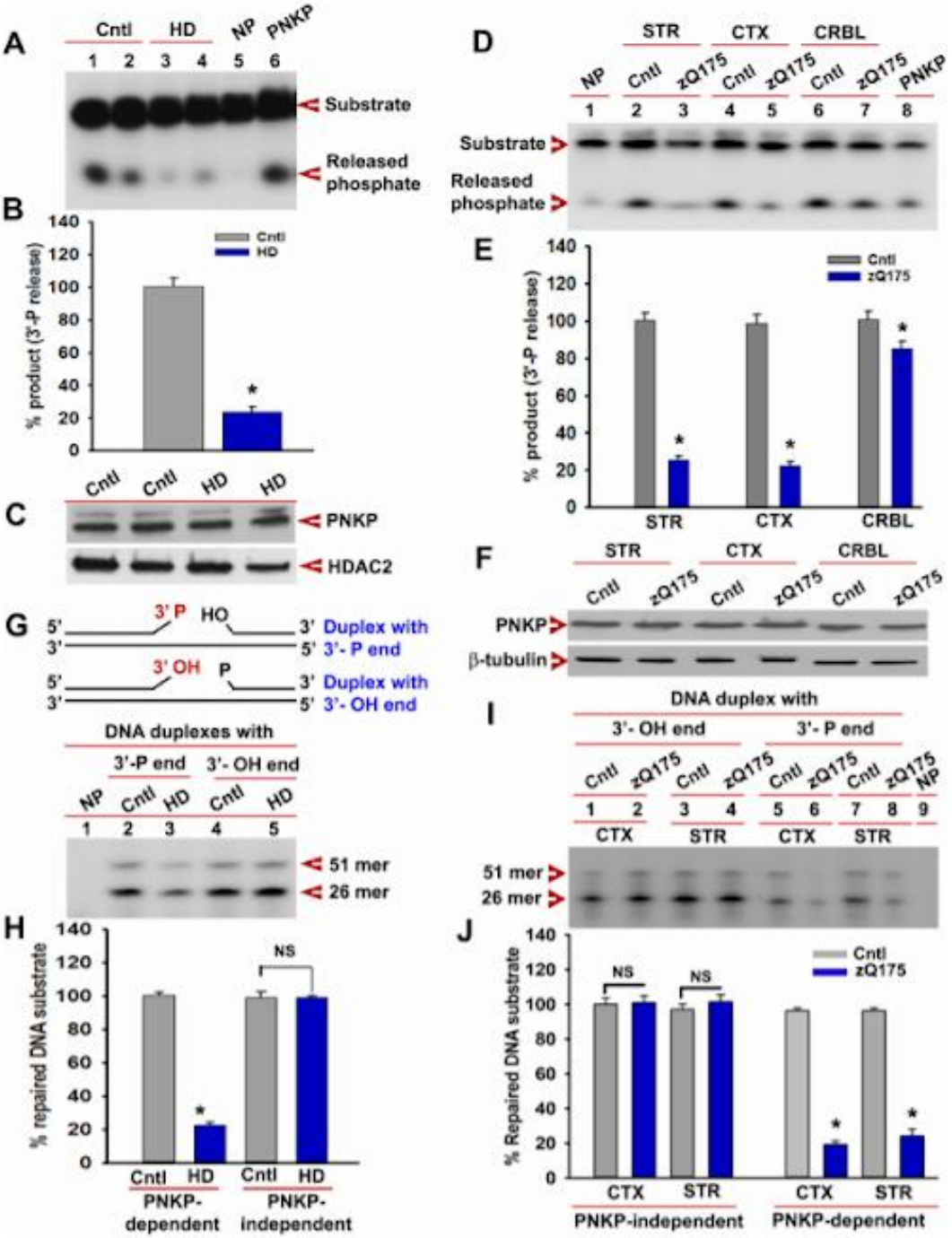


Figure 2.5. mHTT abrogates PNKP activity in vitro and in vivo.

(A) The 3'-phosphatase activities of PNKP in the NE (250 ng each) of control (lanes 1 and 2, differentiation replicates of Q33 iPSCs), and HD neurons (lanes 3 and 4, differentiation replicates of Q109 iPSCs) were determined by amount of phosphate release from the DNA substrate (arrows). No protein extract was added to the substrate in lane 5 (NP), and purified PNKP (25 fmol) was added as a positive control (lane 6). (B)

Relative 3'-phosphatase activities (in terms of % product) of PNKP in control (Q33) and HD (Q109) neurons. Data represent mean ± SD, *p<0.001 when compared with control. The quantification was measured by taking into account two biological replicates and three technical replicates. (C) NEs from control (Q33) and HD neurons (Q109) were analyzed by WB to determine PNKP protein levels (upper panel); HDAC2 was used as a loading control (lower panel). (D) PNKP activities in the NEs from the striatum (STR), cortex (CTX) and cerebellum (CRBL) of 7 weeks old WT control and zQ175 transgenic mice (n = 5; STR or CTX or CRBL were pooled from five littermate mice); no protein was added to the substrate in lane 1 (NP), and purified PNKP was added as a positive control (lane 8). (E) Relative PNKP activities (in terms of % product) in the STR, CTX, and CRBL of 7 weeks old zQ175 transgenic (n = 5) and age-matched control (n = 5) mice. Five biological replicates and three technical replicates were used in this study. Data represent mean ± SD, *p<0.001 when compared with control. (F) NEs from the STR, CTX, and CRBL of zQ175 (n = 5) and agematched wild type control (n = 5) mice were analyzed by WB to determine PNKP levels (upper panel); b-tubulin was used as a loading control (lower panel). (G) mHTT specifically abrogates PNKP activity without interfering with DNA polymerase or ligase activities. Total DNA repair was assessed with NE (2.5 mg) from control (Q33) and HD (Q109) neurons added to two nicked DNA duplexes (upper panel): one with 3'-phosphate ends that require PNKP activity (lanes 2 and 3, lower panel), and the other with clean 3'-OH termini that do not require PNKP activity but need DNA polymerase and ligase activities (lanes 4 and 5, lower panel) for effective repair. The 51-mer DNA band (arrow) represents repaired DNA duplexes in G and I. (H) Relative PNKP and PNKP-independent DNA repair efficiencies in HD (Q109) and control (Q33) primary neurons. NS denotes not significant difference in H and J. Two biological replicates and three technical replicates were used in this study. Data represent mean ± SD. (I) NEs from zQ175 transgenic (n = 5) and control (n = 5) mice CTX and STR were added to nicked DNA substrates as described above, and total DNA repair was assessed. (J) PNKPdependent or -independent repair of the DNA duplexes by NEs from control and zQ175 transgenic mouse brain tissue. Data represent mean ± SD, *p<0.001 for E, H, and J. Three biological replicates and three technical replicates were used in this assay.

We next expressed the N-terminal truncated fragment of mHTT encoding Q97 (NT-mHTT-Q97) in SH-SY5Y cells overexpressing PNKP and carried out a comet assay (Olive and Banath, 2006). Analysis of mutant cells showed more strand breaks, which were substantially rescued after PNKP overexpression (Supplemental Figure 6), suggesting that mHTT-mediated ablation of PNKP activity contributes to increased DNA strand breaks. Consistently, we noted activation of ATM-p53 signaling in cells expressing NT-mHTT-Q97 (Supplemental Figure 6), and PNKP overexpression reduced mHTT-mediated DDR-ATM pathway activation (Supplemental Figure 6). PC12 cells expressing the full-length

mHTT encoding 148Qs (FL-mHTT-Q148) showed increased caspase-3 activity and PNKP overexpression reduced caspase-3 activation (Supplemental Figure 6). Consistently, PC12 cells expressing FL-mHTT-Q148 also showed higher cell toxicity and PNKP overexpression significantly rescued cell toxicity (Supplemental Figure 6). Collectively, these results suggest that mHTT-mediated activation of the ATM-p53 pathway and associated cell toxicity is at least partially due to PNKP inactivation by mHTT.

mHTT preferentially induces DNA breaks in the transcriptionally active genome

Emerging evidence suggests that the TCR complex plays a pivotal role in editing strand breaks in actively transcribing template DNA to maintain genome integrity and cell survival, and its inactivation leads to preferential accumulation of DNA breaks in the transcriptionally active genome (Chakraborty et al. 2016; 2015; Hanawalt and Spivak 2008). Since mHTT abrogates the activity of PNKP, a key component of the TCR complex (Chakraborty et al. 2016), we compared the associations of HTT and TCR proteins with transcriptionally active versus inactive genomes and asked whether the former accumulates more strand breaks in the HD brain. Chromatin immunoprecipitation (ChIP) revealed significantly higher HTT occupancy on actively transcribing genes in the brain (e.g., neuronal differentiation factor 1 and 2 [Neurod1 and Neurod2], neurogenic basic-helix-loop-helix protein neurogenin 1 [Neurog1], tubulin beta 3 class III [Tubb3], neuron-specific enolase 2 [Eno2γ], and DNA polymerase beta [Pol b]) over genes that are not transcribed in the brain but actively transcribed in skeletal or cardiac muscle (e.g., myogenic differentiation factor 1 [Myod1]; myogenic factor 4; myogenin [Myog]; and myosin heavy chain 2, 4, 6, or 7 [Myh2, Myh4, Myh6, or Myh7]; (Figures 6A & B). Increased association between HTT with the transcriptionally active genome and mHTT-mediated abrogation of PNKP activity indicate that the wtHTT-TCR complex repairs lesions during transcriptional elongation, but polyQ expansion might impair the TCR and facilitate DNA damage accumulation. To test this theory, we performed Long-amplicon

quantitative polymerase chain reaction (LA-qPCR) analysis, a versatile technique to measure nuclear and mitochondrial DNA damage (S. Y. Cong et al. 2005; Haibing et al. 2003) to assess DNA strand breakage in actively transcribing and non-transcribing genes in the transgenic mouse cortex (CTX). The results revealed 60-70% lower PCR-amplification of actively transcribing genes in asymptomatic (7 wks) zQ175 mouse CTX compared to age-matched WT controls (Figures 5C & D). In contrast, the amplification efficacy for non-transcribing genes in the zQ175 CTX was only marginally (10-15%) reduced (Figures 6E & F), indicating less DNA damage accumulation. Consistent with the levels of PNKP activities observed in the striatum (STR) and cerebellum (CRBL), the LA-qPCR analysis revealed substantial DNA damage accumulation in STR but negligible DNA damage accumulations in the CRBL (Supplemental Figure 7). Moreover, immunostaining of the HD patients' brain and HD transgenic mouse brain sections with anti-phospho-53BP1 antibody, a DNA damage marker, showed increased presence of DNA damage as compared to control (Supplemental Figure 8). Consistently, preferential accumulation of DNA strand breaks was observed in iPSC-derived HD primary neurons (Q50 and Q53) than controls (Q18 and Q28) (Supplemental Figure 9). Increased DNA break accumulation was also observed in actively transcribing genes vs. non-transcribing genes in the N171-82Q transgenic CTX than the age-matched controls (Supplemental Figure 10). These data support our hypothesis that the HTT-TCR complex repairs strand breaks during transcription, and that this function is impaired by polyQ expansion, resulting in persistent strand break accumulation predominantly affecting actively transcribing genes in HD.

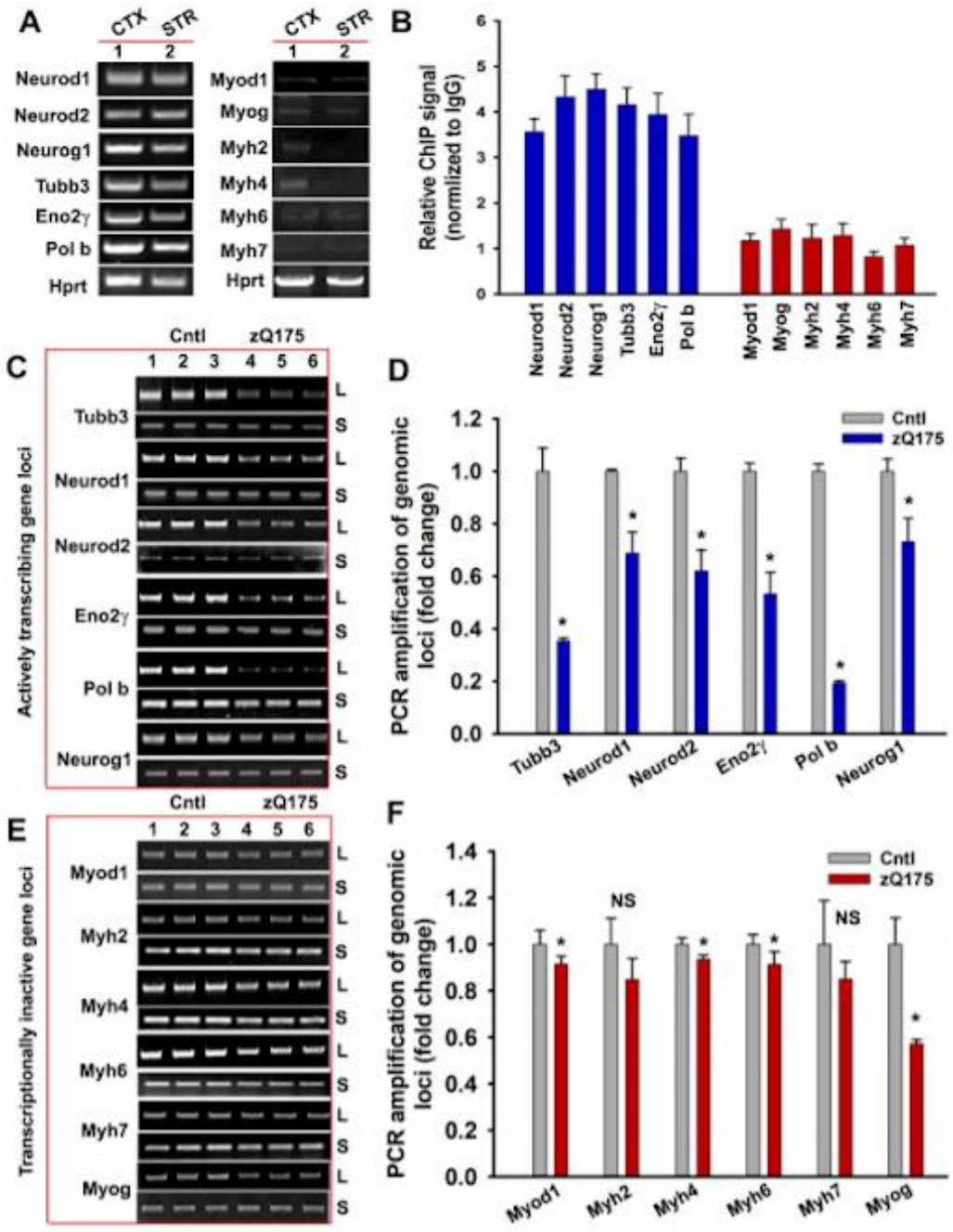


Figure 2.6. mHTT preferentially induces DNA damage/strand breaks in the transcriptionally active genome.

(A) Tissue from CTX and STR of 5 WT mouse brain (7 weeks old) was pooled and total RNA was isolated and expression levels of various genes were measured using qRT-PCR analysis. Left panel shows amplified product of transcribing genes and the right panel for non-transcribing genes. (B) ChIP analysis showing relative occupancy of wtHTT on the actively transcribing (blue) vs. transcriptionally inactive (red) genome loci in 7 weeks old WT mouse STR. Three biological replicates and three technical replicates were used in

this assay. Data represent mean ± SD. (C) Genomic DNA was isolated from the CTX of asymptomatic (7 weeks old) zQ175 transgenic (CTX from five transgenic mice were pooled) and age-matched WT control (CTX from 5 WT control mice were pooled) mice. Various transcriptionally active gene loci (Neurod1, Neurod2, Neurog1, Tubb3, Eno2g, and Pol b) were PCR-amplified from the genomic DNA and analyzed on agarose gels; L: long amplicon (6 to 12 kb product), S: short amplicon (200–300 bp). (D) Relative PCR amplification efficacies of various actively transcribing gene loci in 7 weeks old WT control and zQ175 mouse brains (CTX). Data represent mean ± SD, *p<0.001. Five biological replicates each with three technical replicates were used in this assay. (E) PCR amplification of genomic DNA isolated from the CTX of asymptomatic (7 weeks) zQ175 and WT control mice and various loci that are transcriptionally inactive in brain (Myod1, Myog, Myh2, Myh4, Myh6, and Myh7) were PCR amplified. PCR products from WT control (lanes 1–3) and zQ175 mice (lanes 4 to 6) were analyzed on agarose gels. L: long amplicon (6 to 12 kb product), S: short amplicon (200–300 bp). (F) Relative amounts of PCR products from the transcriptionally inactive genomic loci in the CTX of WT control and zQ175 mice. Data represent mean ± SD; *p<0.001. Five biological replicates each with three technical replicates were used in this assay.

HTT facilitates CBP degradation by inactivating ATXN3

Given that the deubiquitinase ATXN3 is present in the TCR complex, interacts with mHTT, and is sequestered in polyQ aggregates in HD brain, we postulated that compromised ATXN3 activity might increase ubiquitination and decrease levels of TCR components, adversely impacting complex functionality and transcription. To explore this possibility, we examined whether mHTT stimulates ubiquitination and degradation of specific TCR complex proteins. WB analyses of NEs from HD and control iPSC-derived primary neurons revealed a significant decrease in soluble CBP protein levels in HD neurons, whereas ATXN3, PNKP, POLR2A, and CREB levels were not affected (Figures 7A & B). Quantitative reverse transcription PCR analyses did not show a significant change in CBP mRNA levels upon mHTT expression (data not shown), suggesting that mHTT does not interfere with the expression of CBP in HD. This finding indicate that CBP might be degraded more in HD. However, an alternative possibility is that CBP becomes insoluble when post-translationally modified. Substantially reduced levels of CBP was also observed in the soluble fraction of proteins from cells expressing exogenous mHTT (data

not shown). To determine whether abrogating ATXN3 activity causes reduced CBP levels, we measured TCR protein levels in ATXN3-depleted cells. Similar to HD iPSC-derived primary neurons, markedly lower CBP levels were observed in the soluble protein extract from the ATXN3-depleted cells (Figures 7C & D). Consistent with a previous report (Giralt et al. 2012), CBP levels were dramatically (~80%) reduced in the zQ175 CTX but only marginally (~20%) decreased in the CRBL (Figures 7E & F). To test whether ATXN3 interacts with CBP, we co-expressed Myc-ATXN3 and FLAG-CBP and IP'd Myc-ATXN3 from the NEs. WBs showed CBP in the Myc-IC (Figure 7G). Conversely, IP of the FLAG-CBP and subsequent WB revealed ATXN3 (Figure 7H). The PLA results also suggested intracellular interaction between ATXN3 and CBP (Figure 7I). Confocal microscopy showed distinct colocalization of ATXN3 and CBP in HD and control brain sections (Figure 7J, arrows). A recent study also showed significantly increased ubiquitination and reduced level of CBP in $Hdh^{Q7/Q111}$ HD transgenic mouse brain (Giuliano et al. 2003; Bae et al. 2005; Illuzzi et al. 2009; Bertoni et al. 2011; Lu et al. 2014). To test whether mHTT expression increases CBP ubiquitination in zQ175 mouse brain, we IP'd CBP from the NE of zQ175 and control mouse brain. Consistent with a previous report (Haibing et al. 2003; Giralt et al. 2012), WBs of the CBP IC showed increased CBP ubiquitination in the transgenic brain (Figure 7K; lower panel). These data suggest that decreased ATXN3 activity due to its interaction with mHTT in the TCR complex may increase ubiquitination, and increased ubiquitination of CBP may cause aberrant localization of CBP, negatively impacting its solubility in HD. It is also possible that increased ubiquitination may facilitate degradation of CBP in HD.

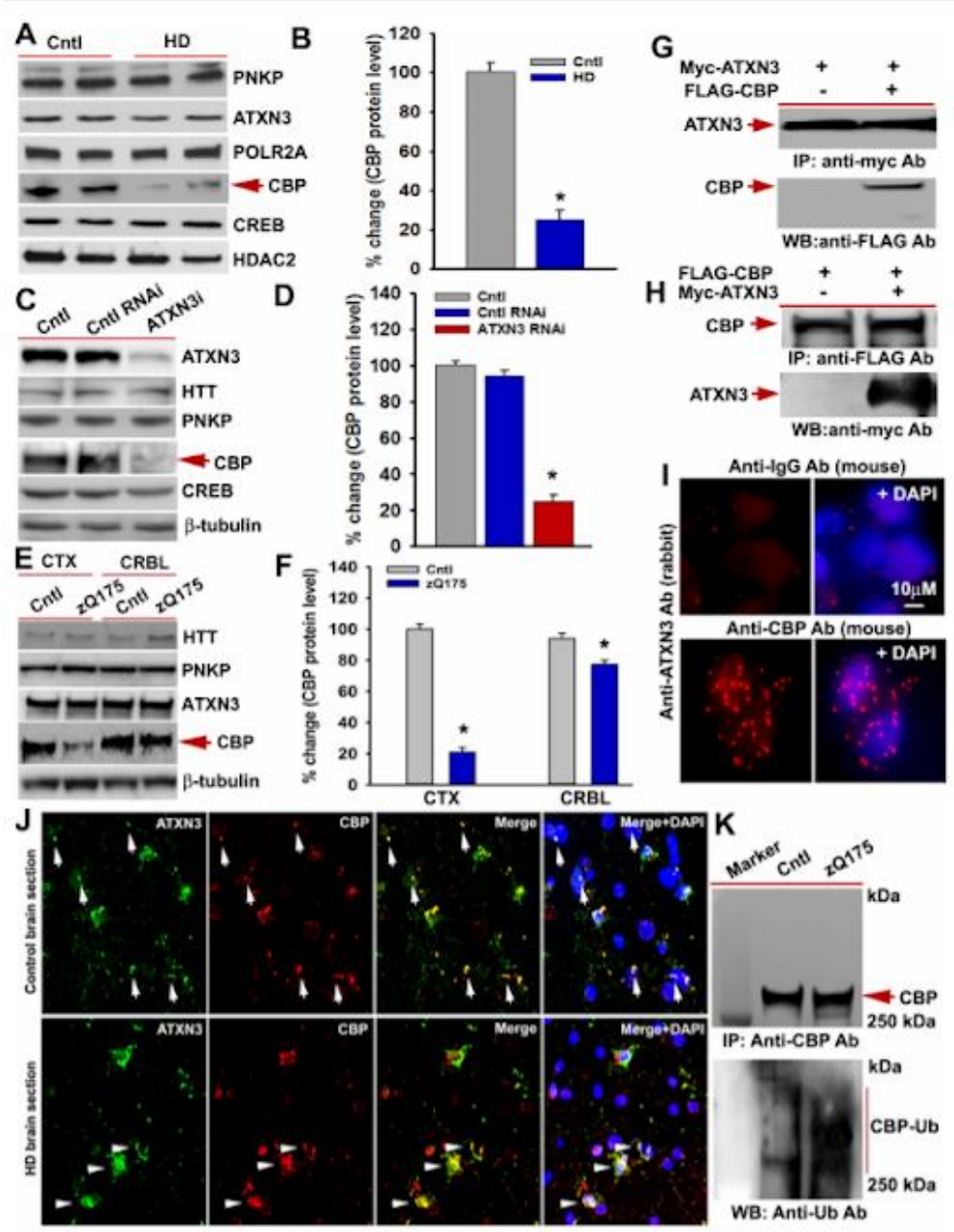


Figure 2.7. mHTT facilitates CBP degradation by inactivating ATXN3.

(A) Nuclear extracts (NEs) isolated from control primary neurons (Q18 and Q28) and HD neurons (Q53 and Q109) were analyzed by WBs to measure PNKP, ATXN3, POLR2A, CBP, and CREB levels; HDAC2 was the loading control. (B) Relative CBP levels in control and HD neurons normalized to HDAC2. Two biological replicates and three technical replicates were used in this assay. Data represent mean ± SD; *p<0.001. (C) NEs isolated from SH-SY5Y cells expressing control shRNA or ATXN3 shRNA were subjected to WB to determine ATXN3, HTT, PNKP, CBP, and CREB levels; b-tubulin was used as loading control. (D) Relative CBP levels (normalized to b-actin) in WT control cells, cells expressing control RNAi or ATXN3 RNAi. Data represent mean ±

SD; *p<0.001. (E) NEs isolated from CTX and CRBL of zQ175 and WT control mice, and analyzed by WB to determine HTT, PNKP, ATXN3, POLR2A and CBP levels; b-tubulin was the loading control. (F) Relative CBP levels in the CTX and CRBL in WT control and zQ175 mice. CBP levels were normalized to b-tubulin. Data represent mean ± SD; *p<0.001. (G) HEK293 cells were cotransfected with plasmids expressing Myc-ATXN3 and FLAG-CBP, Myc-ATXN3 IP'd with a Myc antibody, analyzed by WB to detect CBP in the Myc-IC (arrow). (H) HEK293 cells cotransfected with plasmids expressing Myc-ATXN3 and FLAG-CBP, NEs isolated and CBP IP'd with a FLAG antibody, ICs were subjected to WB to detect ATXN3 (arrow). (I) SH-SY5Y cells were analyzed by PLA to examine interactions between CBP and ATXN3. Nuclei were stained with DAPI (upper panel). Reconstitution of red fluorescence indicates interaction of CBP with ATXN3 (lower panel). (J) Control (upper panel) and HD (lower panel) patient brain sections were analyzed by immunostaining with antibodies against ATXN3 (green) and CBP (red) to assess their in vivo interactions. Merged red and green fluorescence appears as yellow/orange; nuclei stained with DAPI (blue). Arrow indicates the respective colocalization. (K) Total protein was isolated from control and zQ175 mice CTX, and CBP was IP'd with a CBP antibody (upper panel), and the IC analyzed with anti-ubiquitin antibody to detect CBP ubiquitination (lower panel).

DISCUSSION

Our findings reveal a critical proximal event by which polyQ expansions in mHTT induce DNA damage to activate the DDR ATM→p53 pro-apoptotic signaling cascade and disrupt tissue-specific transcriptional activity – key pathogenic features consistently described in HD (Chakraborty et al. 2016). A significant association of wtHTT with PNKP, ATXN3, POLR2A and associated transcription factors suggest that wtHTT may act as a scaffold factor to assemble various core components of the TCR complex. Our data suggest that formation of this functional TCR complex with wtHTT is essential for sensing and editing DNA lesions in the template strand during transcriptional elongation in post-mitotic differentiated neurons and may contribute in maintaining genome integrity and neuronal survival. Our results further indicate that interaction of PNKP with wtHTT stimulates its DNA end-processing activity to facilitate neuronal DNA repair. The role of wtHTT in maintaining TCR complex functionality and genome integrity is further validated by the fact that depletion of endogenous wtHTT protein dramatically depletes PNKP activity with a concurrent increase in DNA damage accumulation. In contrast, mHTT with polyQ

expansions interacts with several key components of the complex but abrogates the activities of PNKP and ATXN3, thereby disrupting DNA repair and transcription leading to a possible early trigger for neurotoxicity and functional decline in HD (Figure 8).

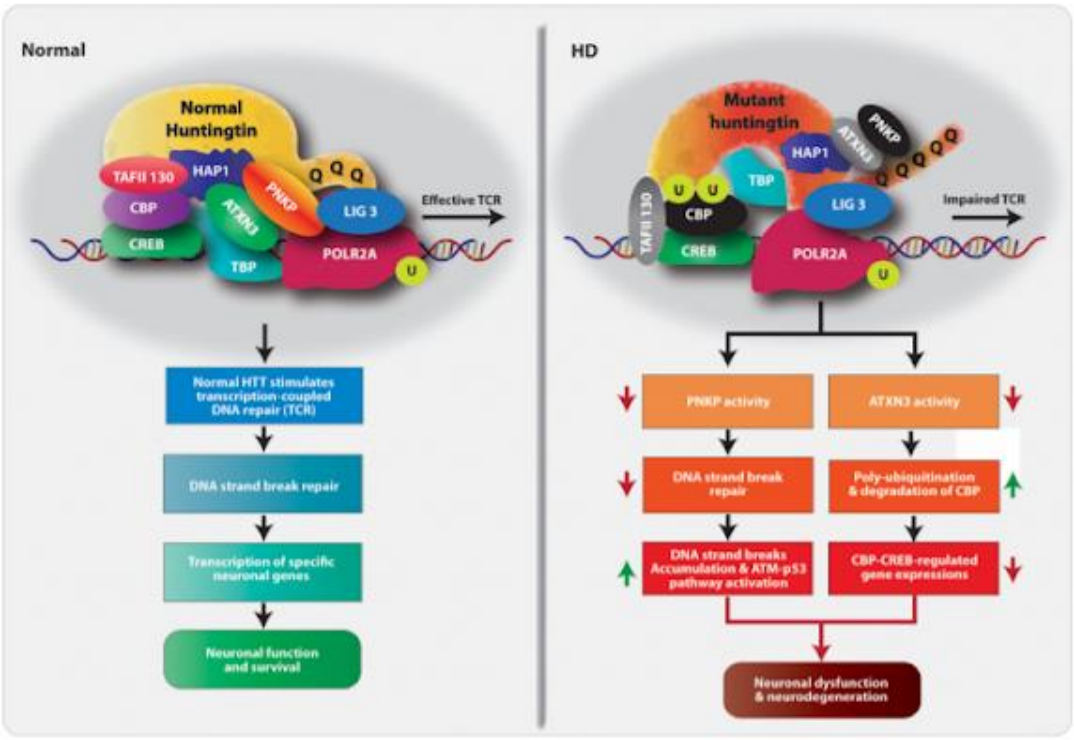


Figure 2.8. Proposed mechanism by which mHTT triggers neurotoxicity in HD.

Schematic diagram of our hypothesized mechanism by which polyQ expansions in mHTT compromise the functional integrity of the TCR complex. Normal HTT forms a multiprotein TCR complex with POLR2A, ATXN3, PNKP, CBP, and additional DNA repair enzymes, and this structure monitors and edits DNA strand breaks/damage during transcriptional elongation, preserving genome integrity, transcription and neuronal survival. In HD, polyQ expansions in mHTT impair the normal function of the TCR complex; mHTT-mediated inactivation of PNKP activity impairs DNA repair, which leads to the persistence of DNA strand breaks and chronic activation of ATMp53 pro-apoptotic signaling. Additionally, mHTT-mediated inactivation of ATXN3's deubiquitinating activity facilitates ubiquitination and degradation of CBP, impairing CBP-CREB-regulated gene transcription and further amplifying pro-degenerative output in the HD brain. PolyQ expansion in mHTT thus adversely impacts DNA repair and transcription and neural function and survival, triggering neurotoxicity and functional decline in HD.

Our data demonstrate that mHTT interaction with PNKP and the resultant decline in PNKP's enzymatic activity was evident in CTX and STR of HD transgenic mouse models but insignificant in the CRBL. Consistent with these findings, CTX and STR, the most affected brain regions in HD displayed extensive DNA strand breaks with impaired DNA repair capacity. In contrast, PNKP activity and genome integrity was marginally affected in CRBL, the brain region that is reported to be relatively unaffected in HD. However, no alterations in the steady-state levels of PNKP, ATXN3 and other key TCR components were observed between CRBL vs. CTX or STR. Our present data does not explain why mHTT expression specifically impacts PNKP activity and genome integrity in the CTX and STR but spares CRBL. Further investigation is required to understand region-specific decreases in PNKP activity and DNA break accumulation in the HD brain. Complete characterization of the TCR complex in different brain regions may provide valuable insight into the selective neuronal vulnerability to mHTT-mediated toxicity. It will be interesting to understand the mechanism that imparts protection to CRBL against mHTT and could provide another node in the signaling pathway that could potentially be developed as a therapeutic target. Furthermore, more rigorous biophysical and structural studies are necessary with purified peptides/proteins to characterize the true nature of the protein-protein interactions and interacting partners to understand how this putative HTT-RNA polymerase complex maintains neuronal genome integrity and survival.

It is notable that the HTT-TCR complex preferentially associates only with the transcriptionally active genome both *in vitro* and *in vivo*. This suggests that the complex could be actively involved in repairing lesions in the template DNA strand during transcription and thus maintaining sequence integrity of the transcriptionally active genomes over the non-transcribing genome. This HTT-TCR complex may provide an additional layer of protective mechanism to maintain the sequence integrity of highly transcriptionally active genes in post-mitotic neurons. Depletion of PNKP activity by mHTT and subsequent accumulation of DNA strand breaks in the transcriptionally active

genome extends our previous report (Burnett, Li, and Pittman 2003; Chai et al. 2004). Collectively, these data support our hypothesis that polyQ expansions in HTT result in the preferential accumulation of strand breaks in the transcriptionally active genome. Persistent DNA strand breaks in the actively transcribing genes may stall or impair transcription elongation, preventing adequate expression of a wide variety of neuronal genes and may contribute to the complexity and variability of HD pathology. Moreover, unrepaired lesions may further induce chronic activation of ATM→p53 signaling, as evidenced by increased phosphorylation of ATM, H2AX, and p53 in the HD brain (Supplemental Figure 2). Chronic ATM-p53 pathway activation resulted in an increased expression of several of the p53 target genes may facilitate neuronal apoptosis in HD. These data support the hypothesis that decreased PNKP activity could be an important proximal event that triggers early neurotoxicity in HD; however, it remains to be tested whether restoration of PNKP activity and DNA repair efficiency can rescue genome integrity and structural and behavioral defects in HD models.

Our results provide evidence that ATXN3 is another key regulatory component of the TCR complex. Our data suggest that the mHTT-mediated decrease in ATXN3 activity either enhances degradation of specific TCR complex components or prevents appropriate formation of the TCR complex in HD. We propose that abrogating ATXN3 activity is a potential mechanism by which mHTT decreases CBP activity and thus adversely impacts the transcription of CREB-dependent genes in HD. ATXN3 binds and deubiquitinates both mono- and polyubiquitin chains in target proteins (Schmitt et al. 2007). ATXN3 inactivation in mice increases protein ubiquitination (Wyttenbach et al. 2001), so diminished ATXN3 activity could influence CREB-regulated gene expression as described in HD (Mantamadiotis et al. 2002). Since disruption of CREB activity in the brain triggers neurodegeneration, mHTT-mediated decreases in ATXN3, CBP, and CREB activities might compromise neuronal function and trigger neurotoxicity, further amplifying pro-degenerative output in HD. The identification of HTT, CBP, PNKP, and ATXN3 as key

regulatory components of the TCR complex and our description of how polyQ expansions disrupt the complex's functional integrity provide important insight into how mHTT could coordinately disrupts CREB-mediated transcription, increases DNA strand breaks, and activates ATM→p53 signaling. Collectively, these events compromise neuronal survival in HD. We hypothesize that POLR2A-mediated transcription might temporarily pause at DNA lesions, leading to mono-ubiquitination of specific TCR complex components, which signals complex assembly to stimulate and/or coordinate lesion repair in normal cells. ATXN3 deubiquitinates the components after repair, and normal transcription resumes. In contrast, the TCR complex stalls at strand breaks in mHTT-carrying cells, and due to reduced ATXN3 activity, specific complex component (s) are polyubiquitinated and accumulate aberrantly in polyQ inclusions (Figure 8). We propose that mHTT-mediated ATXN3 inactivation might impair CBP/CREB-dependent transcription, while reduced PNKP activity might result in DNA break accumulation and DDR pathway activation. The combination of chronic DDR signaling and dysregulation of CREB-dependent genes could trigger selective neuronal degeneration, a hallmark of HD. Defective DNA repair in post-mitotic neurons is an emerging causative factor of cognitive decline in neurodegenerative diseases (Madabhushi, Pan, and Tsai 2014b; Madabhushi et al. 2015; Rass, Ahel, and West 2007). Consistent with our findings, point mutations in PNKP result in microcephaly and seizures (Shen et al. 2010), whereas a frame-shift mutation in the PNKP gene was identified in a neurodegenerative disorder characterized by epilepsy (Poulton et al. 2013). Therefore, mHTT-mediated ablation of PNKP activity could lead to impaired DNA repair, persistent accumulation of DNA strand breaks that may in part contribute to neurotoxicity and neuronal dysfunction in HD.

This study provides multiple lines of evidence suggesting that mHTT-mediated loss of DNA repair and deubiquitinating activity could possibly be critical proximal events that impair the TCR. This could provide a mechanistic link between transcriptional dysregulation leading to aberrant activation of ATM-dependent pro-degenerative pathways

and early neurotoxicity in HD. Although the final biological output triggered by impaired TCR and unrepaired DNA strand breaks in HD remains to be fully described, the present data indicate a potential mechanism by which polyQ expansions in mHTT could disrupt the functional integrity of TCR complex and compromises transcriptional regulation and genomic integrity in post-mitotic neurons. Molecular strategies that interfere with the interaction of mHTT with the TCR complex could reduce neurotoxicity and slow functional decline in HD. Alternatively, molecular approaches to stimulate PNKP activity could be a reasonable way to combat transcriptional dysregulation and inappropriate activation of pro-apoptotic signaling in HD. Our findings could help elucidate the cell type-specific pattern of pathology in HD. We propose the possibility that the compromised TCR efficiency in the basal ganglia or cortex could render these neuronal populations more vulnerable. Collectively, our findings suggest an intriguing molecular mechanism that could explain how mHTT expression in HD could compromise genome integrity and neuronal survival.

Chapter 3: Mutant huntingtin impairs mitochondrial DNA repair, replication and transcription

INTRODUCTION

Mitochondrial dysfunction was first proposed to be the underlying pathogenic origin in 1993, and subsequent work confirmed that HD patients suffered from disrupted mitochondrial morphology, reduced number and size of mitochondria, as well as disruption of the oxidative phosphorylation chain (Beal, Hyman, and Koroshetz 1993; Johri, Chandra, and Beal 2013; Tabrizi et al. 1999). Compromised mitochondrial DNA (mtDNA) integrity and energetics are major pathogenic factors driving energy dyshomeostasis, neurotoxicity, and neurological decline in HD (Ayala-Peña 2013; Bossy-Wetzel, Petrilli, and Knott 2008; Browne and Beal 2004; Damiano et al. 2010; Horton et al. 1995; J. Kim et al. 2010; Reddy, Mao, and Manczak 2009; Schapira 1997; Siddiqui et al. 2012; U. Shirendeb et al. 2011). Functional inactivation of wtHTT (Ismailoglu et al. 2014) or expression of mHTT (Acevedo-Torres et al. 2009; Ayala-Peña 2013; U. Shirendeb et al. 2011; Siddiqui et al. 2012) disrupts structural and functional mt integrity, and mHTT depletes mtDNA copy numbers (J. Kim et al. 2010; Petersen et al. 2014). However, how mHTT induces mtDNA damage, reduces mtDNA copy number, and triggers functional mitochondrial decline remains largely unknown.

mtDNA is subjected to significant levels of ROS due to its proximity to the site of oxidative phosphorylation, and due to its inherent lack of nucleosomes which protect nuclear DNA from similar insults (Shokolenko et al. 2009). The postmitotic nature of a neuron demands a robust nuclear DNA damage response to prevent apoptosis of these critical cells, and the same is true for mitochondria within neurons. Base excision repair (BER) is the primary mechanism for repairing the oxidized bases in mtDNA; however, evidence suggests that nucleotide excision repair (NER) also occurs (Weissman et al.

2007). Still, it is unclear whether mtDNA is repaired during transcription. Mitochondria have additional mechanisms to maintain their genomic integrity; there are hundreds of copies of the mt genome in a given cell, and the constant fission and fusion of the mitochondria, and disposal of dysfunctional mitochondria through autophagy help to prevent accumulation of damaged mtDNA (J. Kim et al. 2010; Reddy, Mao, and Manczak 2009). However, all such mechanisms are disrupted in HD, and the resulting unresolved mtDNA damage may be a key mechanism of neuronal dysfunction.

Previous studies reported the presence of both wtHTT and mHTT in mitochondria (Orr et al. 2008; Petrasch-Parwez et al. 2007). Further studies showed that expression of mHTT induces mtDNA damage and degeneration (Acevedo-Torres et al. 2009; Ayala-Peña 2013; U. Shirendeb et al. 2011; Siddiqui et al. 2012). Similarly, wtHTT depletion also triggers mt degeneration (Ismailoglu et al. 2014). Together these studies indicate that HTT plays a critical role in mitochondrial biology.

As a result of our recently published findings, described in Chapter 2, we sought to determine if HTT has a similar direct involvement in mitochondrial DNA repair. Here we found that wtHTT forms a TCR complex in mitochondria with the mitochondria-specific RNA polymerase (POLRMT), mtDNA polymerase γ (POLGA), mt transcription factors (TFAM, TFB1M, and TFB2M), ATXN3, and the DNA strand break repair enzyme PNKP. This novel complex synchronously regulates mtDNA repair, replication, and transcription. PolyQ expansion in mHTT impairs the functional integrity of this complex, resulting in persistent accumulation of mtDNA damage, reduced mtDNA copy number, and aberrant mtDNA gene transcription. These results suggest how HTT synchronously regulates mtDNA repair, transcription and replication, and demonstrate how polyQ expansion in mHTT interferes with these key molecular events disrupting the structural and functional integrity of mitochondria.

Aim 1: Establish that HTT forms a TCR complex within the mitochondria

Aim 2: Establish that mHTT inhibits Mt TCR and induces mtDNA damage.

Aim 3: To test the hypothesis that accumulation of mitochondrial DNA damage triggers synapse dysfunction and loss.

RESULTS

HTT IS A COMPONENT OF A MT TCR COMPLEX

To clarify the functional role of wtHTT in mitochondria, we measured HTT, ATXN3, and PNKP levels in cytosolic, nuclear, and mt proteins fractions from mutant and control cells. Western blot (WBs) confirmed the mt presence of all three proteins (Figure 1A). Chromatin immunoprecipitation (ChIP) analyses of WT mouse brain tissue showed substantial interactions among HTT, ATXN3, PNKP, and mtDNA (Figure 1B), suggesting that the three proteins associate with mtDNA *in vivo*. We next investigated if ATXN3 and PNKP form an mt TCR complex with HTT. We purified the mt protein extract (ME) from neuronal cells and immunoprecipitated (IP'd) endogenous POLRMT. WBs of the immunocomplexes (ICs) confirmed the presence of HTT, ATXN3, PNKP, mtDNA polymerase γ (POLG), and TFAM in the POLRMT IC (Figure 1C). We also observed Cockayne syndrome protein B (CSB) (Figure 1C), which regulates mtDNA repair and function (Aamann et al. 2010) in POLRMT IC. IP of TFAM from the ME revealed the same proteins (Figure 1D), and IP of mt HTT from the ME confirmed the presence of these proteins in the HTT IC (Figure 1E). Similarly, IP of mt ATXN3 from the ME demonstrated that these proteins were in the ATXN3 IC (Figure 1F). We analyzed ICs for the presence of the outer mitochondrial membrane receptor subunit TOM20, but it was not detectable (Figures 1C to E), indicating that these interactions are specific to the novel complex. For further confirmation, Myc-POLRMT was IP'd from the MEs of neuronal cells expressing the protein. WB confirmed the presence of endogenous HTT, TFAM, ATXN3, and PNKP

in the IC (Figure 1G). Conversely IP of Myc-TFAM from the ME revealed the putative TCR proteins in the IC (Figure 1H), substantiating our interpretation that wtHTT is present in mitochondria where it forms a multiprotein mt TCR complex with POLRMT, POLG, ATXN3, TFAM and PNKP.

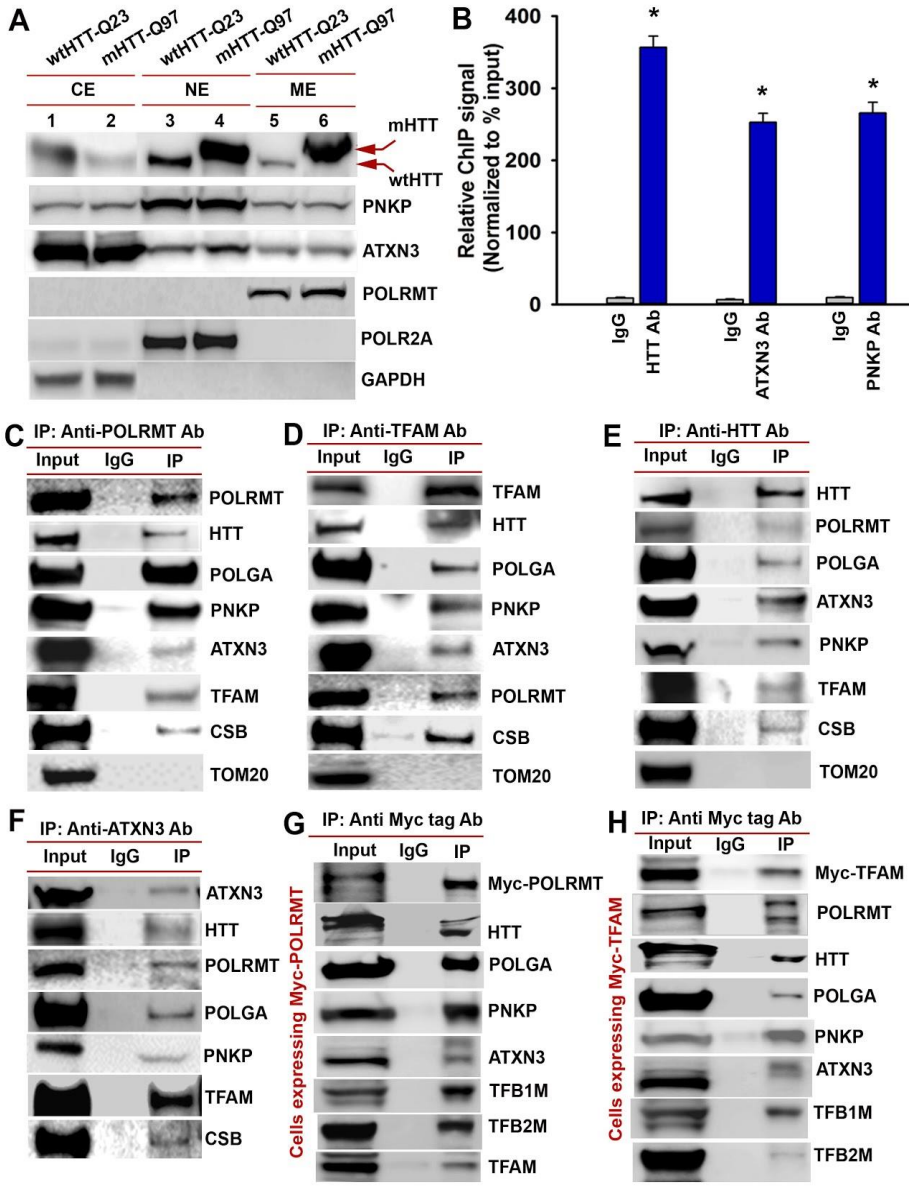


Figure 3.1: HTT is part of a Mt-TCR complex.

(A) Cytosolic, nuclear, and mitochondrial fractions were purified from human neuroblastoma SH-SY5Y cells and the protein fractions were analyzed by western blots (WBs) to detect HTT, ATXN3, PNKP, and POLRMT. (B) MtDNA was isolated from mouse brain tissue, CHip was used to identify interactions of HTT, ATXN3, and PNKP with the mtDNA. (C) Endogenous POLRMT was IP'd from MEs of SH-SY5Y cells and IC was subjected to WB to detect associated TCR complex components with respective antibodies. (D) Endogenous TFAM was IP'd from MEs of SH-SY5Y cells and IC was subjected to WB to detect associated TCR complex components with respective antibodies. (E) Endogenous wtHTT was IP'd from MEs of SH-SY5Y cells and IC was subjected to WB to detect associated TCR complex components with respective antibodies. (F) Endogenous ATXN3 was IP'd from MEs of SH-SY5Y cells and IC was subjected to WB to detect associated TCR complex components with respective antibodies. (G) Myc-tagged POLRMT was IP'd from MEs of SH-SY5Y cells and IC was subjected to WB to detect associated TCR complex components with respective antibodies. (H) Myc-tagged TFAM was IP'd from MEs of SH-SY5Y cells and IC was subjected to WB to detect associated TCR complex components with respective antibodies.

Proximity ligation assays (PLAs) in neuronal cells (R. Gao et al. 2015; 2019) were then performed to further verify interaction specificity among POLRMT, HTT, PNKP, ATXN3 and TFAM. Robust fluorescence reconstitutions (green, arrows) suggested substantial mt interactions (red) (Figure 2), supporting our hypothesis that wtHTT forms a multiprotein mt TCR complex with POLRMT, mt transcription factors (TFAM, TFB1M and TFB2M), POLG, PNKP, and ATXN3.

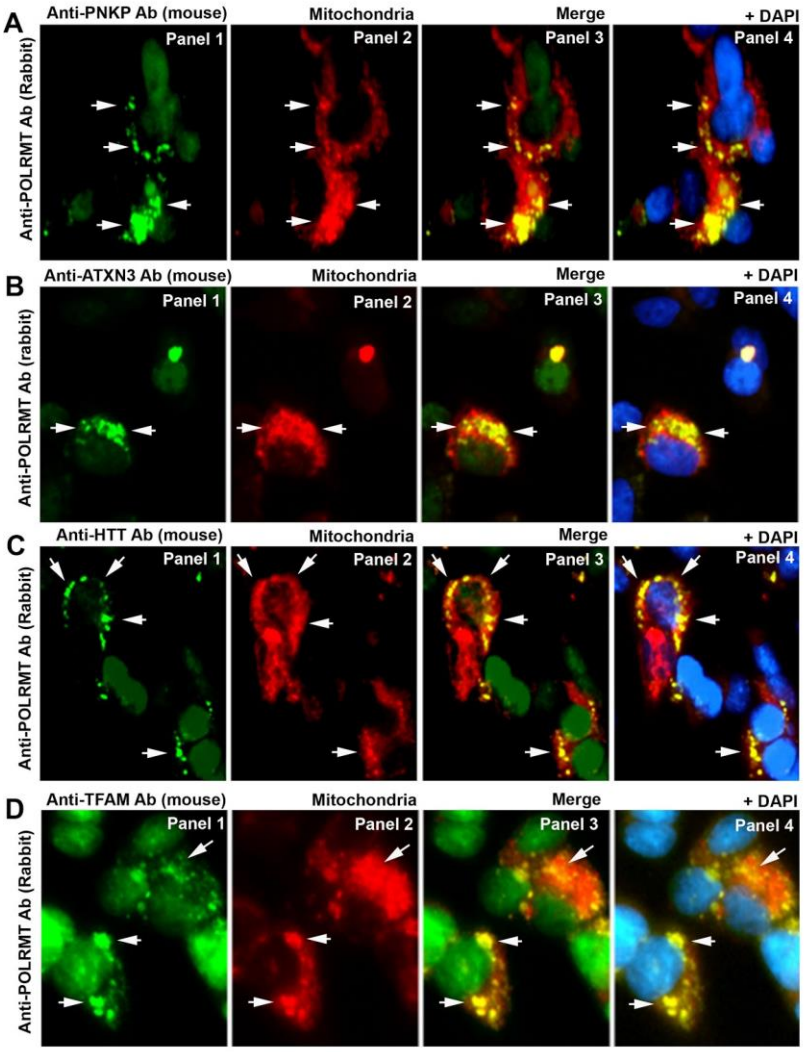


Figure 3.2: POLRMT interacts with PNKP, ATXN3, HTT, and TFAM together with mitochondria in SH-SY5Y cells.

(A) SH-SY5Y cells were analyzed by PLA to show interaction of POLRMT and PNKP (green), and colocalizes with mitochondria (mitotrackerRED, arrows), nuclei were stained with DAPI (blue). (B) SH-SY5Y cells were analyzed by PLA to show interaction of POLRMT and ATXN3 (green), and colocalizes with mitochondria (mitotrackerRED, arrows), nuclei were stained with DAPI (blue). (C) SH-SY5Y cells were analyzed by PLA to show interaction of POLRMT and HTT (green), and colocalizes with mitochondria (mitotrackerRED, arrows), nuclei were stained with DAPI (blue). (D) SH-SY5Y cells were analyzed by PLA to show interaction of POLRMT and TFAM (green), and colocalizes with mitochondria (mitotrackerRED, arrows), nuclei were stained with DAPI (blue).

MHTT DECREASES MT PNKP ACTIVITY TO CAUSE MTDNA DAMAGE ACCUMULATION IN CELLS

Accumulation of mtDNA damage and compromised mtDNA integrity are hallmark features of HD, and it is hypothesized that mtDNA lesion accumulation plays a pivotal driving role in mt pathology, neurotoxicity, and progressive neurological decline (Browne and Beal 2004; Damiano et al. 2010; Horton et al. 1995; J. Kim et al. 2010; Reddy, Mao, and Manczak 2009; Siddiqui et al. 2012; U. Shirendeb et al. 2011). However, how mHTT induces mtDNA damage in HD remains unknown. We recently showed that mHTT interacts with PNKP in the nucleus, where it impairs its activity and decreases DNA repair efficacy, leading to persistent accumulation of damage/strand breaks in nuclear DNA (R. Gao et al. 2019). Since wtHTT, mHTT and PNKP are present in mitochondria, and PNKP interacts with mHTT, we measured mt PNKP activity in primary neurons derived from HD and control subjects. We differentiated induced pluripotent stem cells (iPSCs) from HD and unaffected control subjects (Lim et al. 2017; Telezhkin et al. 2016b) to neurons enriched for a medium striatal neuronal populations, isolated mt protein extract (ME) and measured PNKP activities in ME of differentiated neurons. The PNKP activity in ME from HD iPSC-derived neurons (Q18, Q28 and 109Qs) was found to be 60-70% lower in the ME of HD neurons compared with control neurons (Q18, Q28 and 33Q; Figures 3A to C), suggesting an impairment of mt PNKP activity in human neurons in the presence of mHTT. Since mt PNKP activity was diminished in mutant cells, we assessed mtDNA damages/strand break frequencies in mutant and control cells using long-amplicon quantitative polymerase chain reaction (LA-qPCR), a versatile method for assessing nuclear and mtDNA damage (Santos et al. 2006; R. Gao et al. 2019). Compared with control neurons, there was significantly more mtDNA damage in HD neurons (Q50, Q58 and Q109) compared with control neurons (Q28, Q18 & Q33; Figure 3B). To test whether expression of mHTT *per se* suppresses mt PNKP activity, and disrupts mtDNA repair and maintenance, we purified mitochondria from SH-SY5Y cells expressing exogenous mHTT

or wtHTT and measured PNKP 3'-phosphatase activity in ME. Compared to control cells, it was ~30% higher in ME of cells expressing wtHTT-Q23 and 70 to 80% lower in cells expressing mHTT-Q97 (Figure 3C), suggesting that wtHTT and mHTT stimulate and suppress mt PNKP activity, respectively. Consistently, mtDNA damage was also significantly more in cells expressing mHTT compared with cells expressing wtHTT (Figure 3D). Since the N-terminal fragment of mHTT (NT-mHTT) interacts with PNKP (R. Gao et al. 2019), and NT-mHTT fragments are present in mitochondria where they trigger degeneration (Orr et al. 2008), we examined if NT-mHTT interferes with mt PNKP activity. PNKP 3'-phosphatase activity was reduced by over 80% in MEs from SH-SY5Y cells expressing NT-mHTT-Q97 (Figure 3E), and mtDNA damage was also significantly higher in SH-SY5Y cells expressing NT-mHTT-Q97 (Figure 3F).

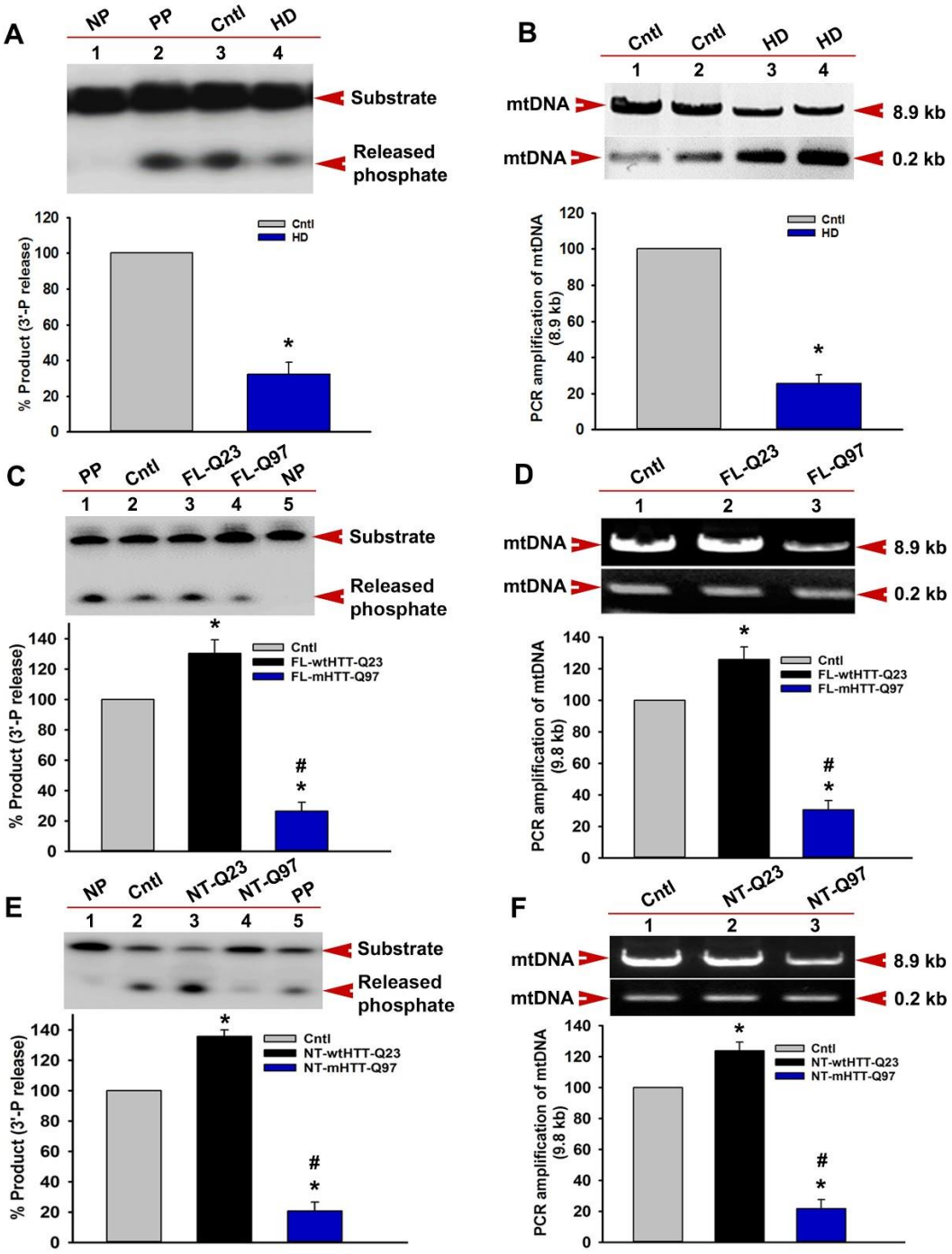


Figure 3.3: mHTT impairs mtDNA repair.

(A) The 3'-phosphatase activities of PNKP in ME isolated from normal (lane 3) and HD IPSC-derived neurons (lane 4). (B) LA-qPCR analysis of mtDNA from normal and HD IPSC-derived neurons. (C) The 3'-phosphatase activities of PNKP in ME isolated from SH-SY5Y control cells expressing full length HTT-Q23 (lane 3) and cells expressing FL-HTT-Q97 (lane 4). (D) LA-qPCR analysis of mtDNA from control SH-SY5Y cells, and cells expressing FL-HTT-Q23 or FL-HTT-Q97. (E) The 3'-phosphatase activities of

PNKP in ME isolated from SH-SY5Y control cells expressing N-terminal HTT-Q23 (lane 3) and cells expressing NT-HTT-Q97 (lane 4). (F) LA-qPCR analysis of mtDNA from control SH-SY5Y cells, and cells expressing NT-HTT-Q23 or NT-HTT-Q97. No protein (NP) and purified PNKP (PP) were used as negative and positive controls, respectively, for A,C, and E.

We next measured 3'-phosphatase activity of PNKP in PC12 cells expressing the full-length mHTT (Igarashi et al. 2003). PC12 cells expressing the full-length mHTT (FL-mHTT-Q148) showed significantly reduced mt PNKP activities and increased accumulation of mtDNA damage compared with control, PNKP overexpression substantially rescued this effect (Figures 4A-E). Moreover, we found that functional disruption of the TCR complex (siRNA knockdown of HTT) reduced mt PNKP activity and increased mtDNA damage (Figure 4F-K). Further, overexpression of wtHTT significantly increased mt PNKP activity improves mtDNA integrity compared to controls (Figure 4L-O). These findings suggest that HTT-TCR complex plays an important role maintaining mt genome integrity and mHTT with polyQ expansion disrupts mtDNA maintenance in HD.

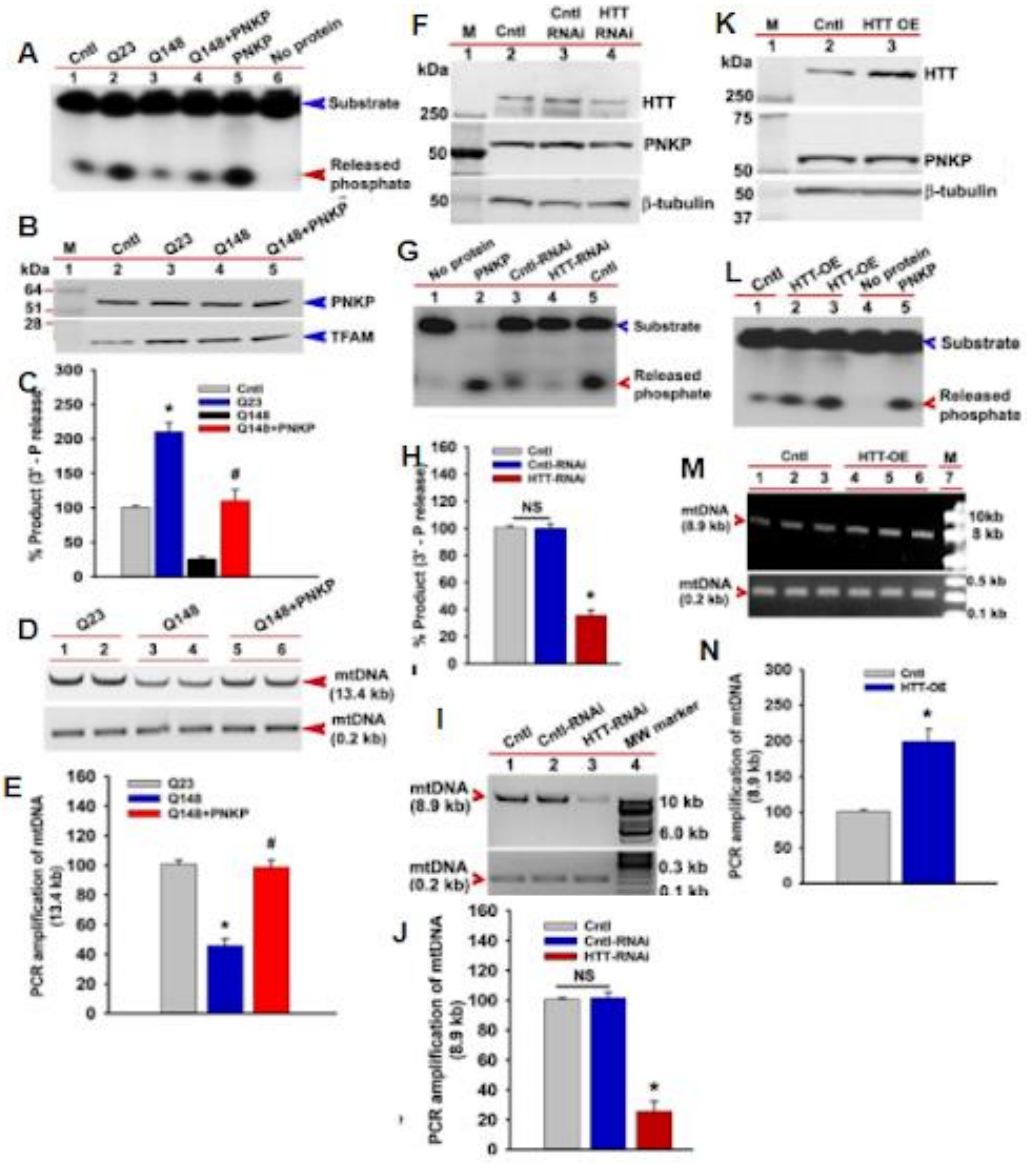


Figure 3.4: PNKP overexpression rescues mtDNA repair in HD cell models.

(A) The 3'-phosphatase activities of PNKP in ME isolated from PC12 cells expressing full length wt (lane2), mHTT (lane3), or expressing both mHTT and PNKP (lane3). (B) WB showing levels of PNKP in mitochondria from PC12 cells in A. (C) Relative mitochondrial PNKP activity, shown in A. (D) LA-qPCR analysis of mtDNA from PC12 cells expressing full length wt (lanes 1 and 2), mHTT (lanes 3 and 4), or expressing both mHTT and PNKP (lanes 5 and 6). (E) Relative mtDNA amplification, shown in D. (F) WB showing shRNA mediated HTT knockdown in SH-SY5Y cells. (G) 3'-phosphatase activities of PNKP in ME isolated from ctrl and HTT-KD SH-SY5Y cells. (H) Relative mt PNKP activity, shown in G. (I) LA-qPCR analysis of mtDNA from ctrl and HTT-KD SH-SY5Y cells. (J) Relative mtDNA amplification, shown in I. (K) WB showing overexpression of wtHTT in SH-SY5Y cells. (L) 3'-phosphatase activities of PNKP in

ME isolated from ctrl and HTT-OE SH-SY5Y cells. (M) LA-qPCR analysis of mtDNA from ctrl and HTT-OE SH-SY5Y cells. (O) Relative mtDNA amplification, shown in M.

TRANSGENIC EXPRESSION OF MHTT IN MOUSE BRAIN DECREASES MT PNKP ACTIVITY AND CAUSES MTDNA DAMAGE ACCUMULATION.

To examine whether mHTT expression perturbs mt PNKP activity *in vivo*, we measured PNKP 3'-phosphatase activity in the zQ175 transgenic mouse brain expressing full-length mHTT (zQ175 transgenic mice) (Menalled et al. 2012). In asymptomatic, 7-week-old heterozygous zQ175 mice, mt PNKP activity was decreased by 60-70% in the striatum (STR) and cortex (CTX) and by 5% in the cerebellum (CRBL) (Figures 5A-C). LA-qPCR revealed greater mtDNA damage in the CTX and STR of zQ175 mouse brains, whereas substantially less mtDNA damage was observed in the CRBL (Figures 5D and E). We next measured PNKP activities in the N171-82Q transgenic mouse brain expressing the N-terminal of mHTT with 82 glutamines (Schilling et al. 1999). We observed a >70% decreased PNKP activity in MEs from the CTX and STR of asymptomatic (7-week-old) heterozygous N171-82Q mice and marginally lower PNKP activity in the CRBL (Figures 5F and G). Consistently, the N171-82Q CTX and STR from 7-week-old mice showed substantially more mtDNA damage (Figures 5H and I). These findings suggest that both full-length and NT-mHTT are present in mitochondria, where they interact with and abrogate mt PNKP activity both in cells and *in vivo*. The subsequent decrease in PNKP activity impairs mtDNA repair, leading to persistent accumulation of mtDNA strand breaks/damage in HD.

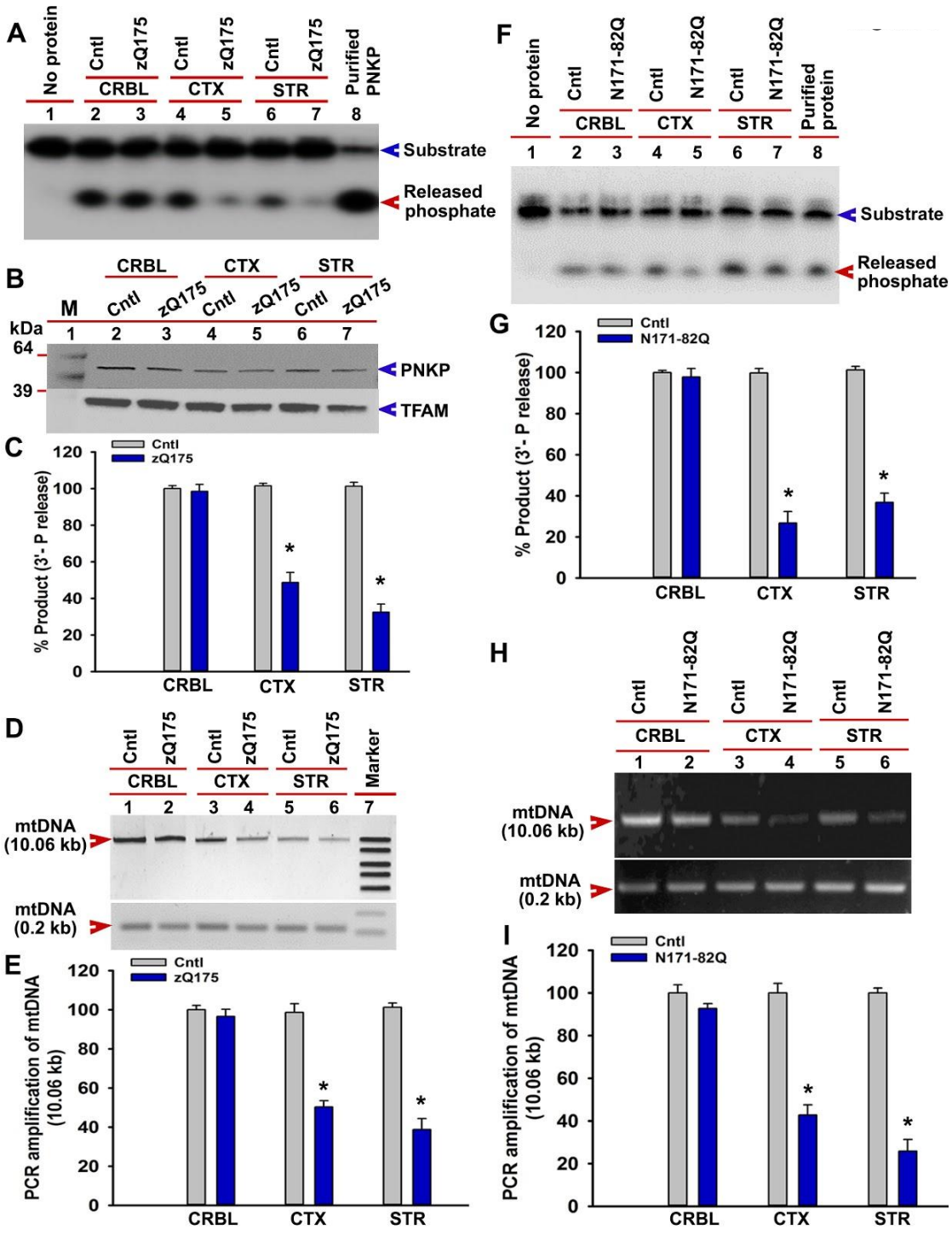


Figure 3.5: HD mouse models show reduced PNKP activity, and increased mtDNA damage.

(A) The 3'-phosphatase activities of PNKP in ME isolated from cerebellum, cortex, and striatum of ctrl and zQ175 mice. (B) WB of PNKP levels in cerebellum, cortex, and striatum of ctrl and zQ175 mice. (C) Relative PNKP activity, shown in A. (D) LA-

qPCR analysis of mtDNA from cerebellum, cortex, and striatum of ctrl and zQ175 mice. (E) Relative mtDNA amplification, shown in C. (F) The 3'-phosphatase activities of PNKP in ME isolated from cerebellum, cortex, and striatum of ctrl and N171-82Q mice. (G) Relative PNKP activity, shown in E. (H) LA-qPCR analysis of mtDNA from cerebellum, cortex, and striatum of ctrl and N171-82Q mice. (I) Relative mtDNA amplification, shown in H.

HTT preferentially associates with transcriptionally active mt genome sequences and polyQ expansions enhances its association with mtDNA

The translocating RNA polymerase complex senses DNA damage during transcriptional elongation and initiates TCR prior to resuming transcription (Hanawalt 1994). TCR plays an important role in preferentially repairing damage in the actively transcribing template DNA strand to maintain genome integrity and cell survival, and its inactivation results in DNA damage accumulation in the transcriptionally active genome (Chatterjee et al. 2015; Chakraborty et al. 2016; R. Gao et al. 2019). However, whether damage is repaired by the mt POLRMT complex during mtDNA transcription remains unknown. Since HTT is present in the mt TCR complex and mHTT diminishes mt PNKP activity, we performed ChIP analyses to compare the relative association of HTT and TCR proteins with transcriptionally active versus inactive mtDNA sequences. We observed a higher association of wtHTT with actively transcribing mtDNA sequences in the mouse brain (e.g., mt NADH dehydrogenase subunit 2, cytochrome c oxidase 1 and 2, and cytochrome B [MTND2, MTCO1, MTCO2, and MTCytB]) over the non-transcribing D-loop sequences (Figure 6A). We next examined whether mHTT with polyQ expansion shows altered association with mtDNA *in vivo*. The ChIP analysis revealed a stronger association of mHTT with mtDNA compared with wtHTT (Figure 6B). We also observed greater association of PNKP with actively transcribing mtDNA sequences compared with the D-loop sequences (Figures 6C). These results suggest that the HTT-TCR complex preferentially associates with the actively transcribing mtDNA and repairs mtDNA lesions during transcription elongation, and mHTT impairs the activity of the HTT-TCR complex

and thus decreases mt PNKP activity, resulting in the persistent mtDNA lesion accumulation.

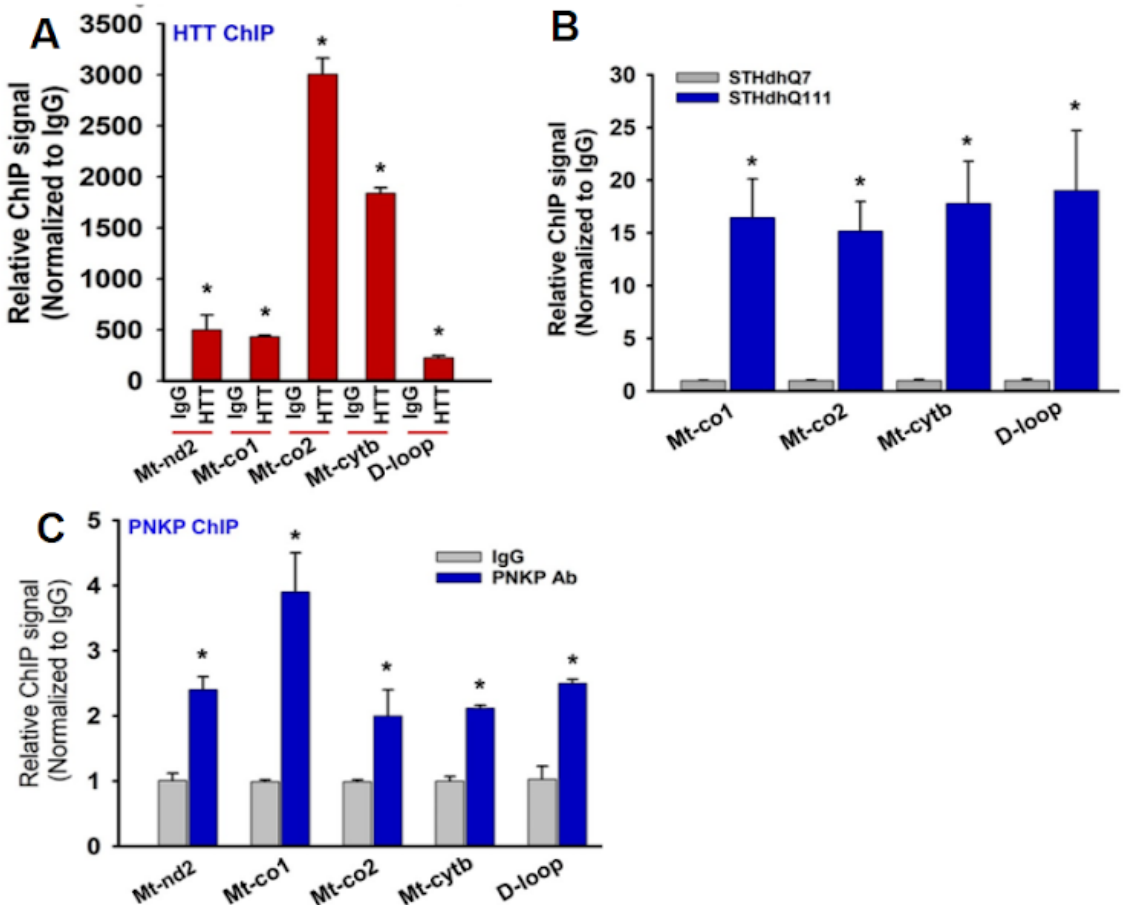


Figure 3.6: HTT occupies actively transcribed regions of the mtDNA.

(A) ChIP analysis showing relative occupancy of wtHTT on transcriptionally active and inactive mtDNA loci, in SH-SY5Y cells. (B) ChIP analysis showing relative occupancy of wtHTT and mHTT on transcriptionally active and inactive mtDNA loci, in ctrl (STHdhQ7) and HD (STHdhQ111) cells, respectively. (C) ChIP analysis showing relative occupancy of PNKP on transcriptionally active and inactive mtDNA loci, in SH-SY5Y cells.

DISCUSSION

Findings in the last two decades have shown that mt dysfunction and metabolic deficiencies precede neurological phenotypes and overt neurodegeneration in HD. Patients

with HD exhibit profound weight loss despite sustained caloric intake, suggesting marked mt and energetic impairments (Browne and Beal 2004; Sawa 2001). Positron emission tomography revealed that reduced striatal glucose utilization precedes atrophy, suggesting that energy dyshomeostasis occurs prior to clinical symptom onset (Berent et al. 1988; Kuwert et al. 1990; Kuhl et al. 1984; Leenders et al. 1986; Martin et al. 1992; Young et al. 1986). Abnormal mt morphology (Tellez-Nagel, Johnson, and Terry 1974) and decreased ETC complex activity (Browne et al. 1997; M. Gu et al. 1996) are reported in patients with HD. Mt respiration is also impaired in a cell model of HD (Gines et al. 2003; Milakovic and Johnson 2005). Abnormal mt ETC complexes and ultrastructure have been also described in HD mouse models (Aidt et al. 2013; SJ et al. 2000). These findings suggest that mHTT disrupts structural and functional integrity of mitochondria. However, there is still no direct causative mechanism by which mHTT drives mt decline in HD.

Data presented in this chapter demonstrate for the first time that HTT is a part of a novel transcription-coupled DNA repair complex within mitochondria, and that this multi-protein complex is critical in mediating mtDNA repair and maintaining mtDNA integrity. This discovery of this complex, supported by numerous protein-protein interaction assays presented in this chapter, is a significant finding, as transcription-coupled DNA repair has not been previously identified in mitochondria. ChIP analysis showing the preferential occupancy of HTT and PNKP on transcriptionally active regions of the mitochondrial genome further support the classification of this complex as being involved in TCR. Of particular note, it appears that mHTT shows much greater occupancy of the mtDNA; several possibilities may account for this finding: it may be the result of overactivation of the mitochondrial DNA damage response to accumulating genomic damage, or mHTT may disrupt the release of the TCR complex from the mtDNA in the event of a failed repair attempt. Expression of mHTT reduced PNKP activity within the mitochondria, and resulted in increased mtDNA damage. Similarly, RNAi knockdown of HTT in neuronal cells perturbed mt PNKP activity, and increased mtDNA damage. In

contrast, cells overexpressing wtHTT show increased mtDNA repair activity and PNKP activity. Together these data indicate that HTT fulfills an important role in mtDNA repair by mediating the activity of PNKP, mirroring the role of nuclear HTT as described in Chapter 2 (R. Gao et al. 2019). These results from cells were confirmed using two different HD mouse models, which showed similar reductions in PNKP activity and increases in mtDNA damage, which were pronounced in the cortex and striatum, and less severe in the cerebellum. This coincides with the brain region specific neurodegeneration observed in HD patients. Further investigation will be required to explain why mHTT preferentially affects mitochondrial DNA repair in these regions of the brain.

These findings strongly indicate that HTT plays a critical role in mitochondrial health, at least in part, by maintaining the rate of mtDNA repair. Chapter 3 provides further evidence that polyQ expansion in HTT disrupts the complex's function, resulting in impaired mtDNA repair and persistent accumulation of mtDNA lesions. It is tempting to speculate that inefficient mtDNA repair may be the primary contributor to the progressive accumulation of mtDNA lesions and mt dysfunction- hallmark of HD (Ayala-Peña 2013; Browne et al. 1997). Based on this evidence, we propose that in HD a combination of reduced normal HTT level and presence of mHTT, which is dysfunctional together cause mt dysfunction, which in turn triggers the progressive loss of neurons.

Human mtDNA is a double-stranded circular molecule of 16,569 base pair and encodes 37 genes; 2 ribosomal RNA (12S and 16S rRNA), 22 transfer RNA (tRNA) and 13 polypeptides, all of them are essential components of oxidative phosphorylation, ATP production and mt energetics. Impaired mitochondrial TCR would severely reduce the rate of transcription of these vital genes, resulting in depletion of the translated protein pool. It is reasonable to hypothesize that overtime, this scarcity of proteins which make up the electron transport chain and other critical pathways would result in overall mitochondrial impairment. mtDNA transcription requires a combination of POLRMT, TFAM, and mt transcription factor TFB1M or TFB2M (Falkenberg et al. 2002). TFAM and TFB1M or

TFB2M directly interact with POLRMT to form a heterodimer that initiates transcription. In addition to regulating transcription, TFAM regulates mtDNA replication and copy number. In support of this notion, TFAM-null mice have reduced mtDNA copy numbers and develop respiratory chain deficiency (Larsson et al. 1998). On the other hand, overexpression of TFAM upregulates mtDNA copy number in mice (Ekstrand et al. 2004), suggesting that it regulates mtDNA replication and energy metabolism. Decreased TFAM activity and mtDNA copy number are also reported in HD (J. Kim et al. 2010; Petersen et al. 2014). Since TFAM is present in the mt TCR complex, mHTT-mediated inactivation of the mt TCR complex may reduce TFAM activity, with the overall effect of reducing mtDNA transcription and copy number in HD. Moreover, since ATXN3 is present in the mt TCR complex, inactivation of mtATXN3 may also impact deubiquitination and degradation of mt TCR complex components, which may further diminish mtDNA repair and transcription in HD.

In the context of neurodegenerative diseases and HD in particular, it is notable that mitochondria are the central components of synapses where they provide the energy required for synaptic potentiation and activity (Ly and Verstreken 2006), subsequently, damaged mitochondria have been shown to perturb synaptic activity. Mt dysfunction negatively impacts synapse-strengthening tetanic stimulation, and electrical stimulation promotes rapid delivery of mitochondria to the synapses, facilitating potentiation (Sheng and Cai 2012; Tong 2007). It is likely that dysfunctional mitochondria contributes significantly to synaptic dysfunction as described in HD (J. Y. Li, Plomann, and Brundin 2003; Orr et al. 2008; U. Shirendeb et al. 2011; Trushina et al. 2004). More interventional studies are required to establish whether impaired mt dynamics and synaptic function can be attributed to dysfunctional TCR and whether synaptic defects might be ameliorated by restoring PNKP activity in HD.

In conclusion, we report that mHTT-mediated disruption of mt TCR is an early event that synchronously impairs mtDNA repair, transcription, and replication to cause mt

dysfunction and neurotoxicity in HD. Developing strategies to prevent the aberrant interaction of mHTT with PNKP might help block or slow functional decline and possibly restore normal physiological functions. Approaches to stimulate PNKP activity may be an alternative approach to alleviate neurotoxicity in HD. Together with our previous studies (R. Gao et al. 2015; 2019), these findings substantially expand our understanding of how mHTT disrupts mt function and triggers neurotoxicity and neuronal dysfunction in HD.

Chapter 4: Materials and Methods

PLASMID CONSTRUCTION

The construction of plasmids expressing the N-terminal fragment of HTT (exon1: NT-HTT-Q23 and NT-HTT-Q148) and full-length HTT (FL-HTT-Q23 and FL-HTT-Q148) was described previously (Tanaka et al. 2006). The N-terminal fragments of wtHTT and mHTT were sub-cloned in pAcGFPC1 (Clontech, USA) to construct pGFP-NT-HTT-Q23 and pGFP-NT-HTT-Q97, respectively. The number of CAG repeats contracted to 97 after propagation in *Escherichia coli*. The plasmids pGFP-NT-HTT-Q23 and pGFP-NT-HTT-Q97 were digested with NheI and MluI, and the fragments containing GFP-NT-HTT-Q23 and GFP-NT-HTT-Q97 were sub-cloned into the TET-inducible responder plasmid pTRE3G (Clontech, USA) using appropriate linkers. The plasmid pTet-ON (Clontech, USA) and responder plasmids (pTRE-GFP-NT-HTT-Q97 or pTRE-GFP-NT-HTT-Q23) were transfected into SH-SY5Y cells, and clones were selected with neomycin. The stable inducible clones expressing GFP-NT-HTT-Q97 or GFP-NT-HTT-Q23 were incubated with medium containing doxycycline (500 ng/mL), and transgene expression was assessed by WB using anti-GFP antibodies. The PNKP cDNA was cloned into pcDNA3.1/hygro (Invitrogen, USA) to construct pRPS-PNKP, which was transfected into SH-SY5Y cells encoding inducible GFP-NT-HTT-Q23 and GFP-NT-HTT-Q97. The clones were selected for hygromycin resistance. PNKP expression was examined by WB, and PNKP activity was assessed as described previously (Chatterjee et al. 2015). To express PNKP and its functional domains as FLAG-tagged peptides, the full-length cDNA and FHA domain (1-300 amino acids), kinase domain (131-337 amino acids), phosphatase domain (338-521 amino acids), FHA and kinase domain (1-337 amino acids), and kinase and phosphatase domain (131-521 amino acids) were PCR-amplified using specific primers and cloned into plasmid pCMV-DYKDDDDK (Clontech, USA).

PLASMIDS FOR THE BIMOLECULAR FLUORESCENCE COMPLEMENTATION ASSAY

Plasmids pBiFC-VN173 (encoding 1 to 172 N-terminal amino acids of cyan fluorescent protein, CFP) and pBIFC-VC155 (encoding 155 to 238 C-terminal amino acids of CFP) were kindly provided by Dr. Chang-Deng Hu (Addgene plasmids 22011 and 22010). The N-terminal fragments of HTT cDNA (encoding 23 or 97 glutamines) were cloned in-frame with the C-terminal amino acids of CFP in plasmid pBiFC-VC155 to construct pVC-NT-HTT-Q23 and pVC-NT-HTT-Q97, respectively. Full-length PNKP or its catalytic domain (phosphatase and kinase domains, 131-521 amino acids) was cloned in plasmid pBIFC-VN173 to construct pVN-PNKP or pVN-(PHOS+KIN)-PNKP, respectively. SH-SY5Y cells (2×10^5 cells) were grown on chamber slides and transfected 24 hours later. Plasmids pVN-(PHOS+KIN)-PNKP and pVC-HTT-Q23 or pVN-(PHOS+KIN)-PNKP and pVC-HTT-Q97 were cotransfected, and reconstitution of the green/yellow fluorescence of CFP was monitored by fluorescence microscopy.

CELL CULTURE AND PLASMID TRANSFECTION

Human neuroblastoma SH-SY5Y cells were purchased from ATCC (Cat # CRL-2266) and cultured in Dulbecco's minimum essential medium (DMEM) containing 15% fetal bovine serum (FBS), and 1% B-27 (Invitrogen, USA). SH-SY5Y cells stably encoding inducible GFP-NT-HTT-Q23 or GFP-NT-HTT-Q97 were cultured in DMEM, and transgene expression was induced by adding doxycycline to the medium to a final concentration of 500 ng/mL. PC12 cells carrying full-length wtHTT-Q23 or mHTT-Q148 were cultured in DMEM containing 15% FBS and doxycycline (500 ng/mL). HTT expression was induced by withdrawing doxycycline from the media for 5-7 days, and transgene expression was verified by WB. Plasmids expressing the RNAi targeting ATXN3 were from Dharmacon, USA. SH-SY5Y cells were transfected with the ATXN3-RNAi plasmids using Lipofectamine RNAi-MAX reagent (Invitrogen, USA); stable cells were selected for puromycin resistance and differentiated in DMEM containing 5 μM

retinoic acid. All the cell lines were authenticated by short tandem repeat analysis in the UTMB Molecular Genomics Core. The possible mycoplasma contaminations in all the cell lines were tested using GeM Mycoplasma Detection Kit (SIGMA, Cat# MP0025) using a PCR based screening method and cells were found to be free from mycoplasma contamination.

ANALYSIS OF HTT-ASSOCIATED TCR PROTEINS BY CO-IMMUNOPRECIPITATION (CO-IP)

Co-IP from NEs: NEs from SH-SY5Y cells were isolated and treated with benzonase to remove DNA and RNA to avoid nucleic acid-mediated Co-IP. Specific target proteins were IP'd, and the IC was washed extensively with cold Tris-buffered saline (50 mM Tris-HCl [pH 7.5] 200 mM NaCl) containing 1 mM EDTA, 1% Triton-X100, and 10% glycerol. The complexes were eluted from the beads with 25 mM Tris-HCl (pH 7.5) and 500 mM NaCl and analyzed by WB.

Co-IP from tissue: Approximately 250 mg of cortex from freshly sacrificed WT mice was harvested and homogenized with 4 volumes of ice-cold buffer (0.25 M sucrose, 15 mM Tris-HCl [pH 7.9], 60 mM KCl, 15 mM NaCl, 5 mM EDTA, 1 mM EGTA, 0.15 mM spermine, 0.5 mM spermidine, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride [PMSF], and protease inhibitors [Roche Applied Science, Germany]) with ∼20 strokes to disrupt tissues (Chakraborty et al. 2015). Homogenization was continued until a single-cell slurry was obtained, incubated on ice for 15 min, and centrifuged at 1,000 × g to obtain the cell pellet. NEs were then prepared from the cell pellet for co-IP analysis. The ICs were analyzed by WB to identify interacting protein partners.

HD AUTOPSY BRAIN TISSUE SAMPLES

Human autopsy specimens were obtained in accordance with local legislation and ethical rules. Control brain samples were collected from age-matched individuals without neurodegenerative disorders. The HD brain tissue samples were obtained from patients with HD who were clinically characterized based on the presence of chorea and motor, mood, and cognitive impairment. The molecular diagnosis of HD was established by analyzing genomic DNA extracted from peripheral blood using a combination of PCR and Southern blotting. HTT CAG repeat lengths were established by sequencing the expansion loci of the mutant allele. All brain autopsies were immediately frozen in liquid nitrogen and stored at -80 ºC until further analysis.

HD iPSC DIFFERENTIATION

Products purchased from Thermo Fisher Scientific (Waltham, MA USA), unless otherwise specified. Three control: CS25iCTR18n6, CS14iCTR28n6, CS83iCTR33n1 and three HD: CS87iHD50n7, CS03iHD53n3 and CS09iHD109n1 iPSC lines were derived and cultured as previously described on hESC-qualified Matrigel® (HD iPSC Consortium, 2017). Once at 70% confluency, neural induction and further differentiation of neural progenitors with the addition of Activin A (Peprotech, USA), was performed as previously described in (Telezhkin et al. 2016a). Neuronal maturation was performed as previously described (Telezhkin et al. 2016a) on Nunc™ 6 well plates. After 3 weeks of maturation, medium was removed and cells were washed once with PBS pH 7.4, without Mg^{2+} and Ca^{2+}. Subsequently, cells were washed with 4°C PBS pH 7.4, without Mg^{2+} and Ca^{2+}, and scraped using a cell scraper, pipetted into a centrifuge tube and centrifuged at 250 *x g* for 3 minutes. PBS was removed and samples were flash frozen in liquid nitrogen.

HD TRANSGENIC MICE

The HD knock-in mouse model zQ175 expresses full-length mHTT from the endogenous mouse HTT promoter (Menalled et al. 2012). The N171-82Q transgenic mouse

line expresses the truncated N-terminus of human HTT cDNA with a polyQ repeat length of 82 under control of the mouse prion promoter (Schilling et al. 1999). Heterozygous transgenic mice and control non-transgenic littermates (n = 4-5 pools of two animals per genotype) were sacrificed, and fresh brain tissues were used for enzyme assays, isolating genomic DNA, and obtaining protein for WB analyses. For immunofluorescence assays, transgenic and control littermate mice were deeply anesthetized and transcardially perfused with sterile phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in PBS. Brains were post-fixed overnight in fixative solution and embedded in OCT and stored in liquid nitrogen. Slides with 4-μm-thick frozen sections were processed for immunostaining with appropriate antibodies. All animal studies were performed according to the standard approved procedure of the respective university (University of California Irvine, or University of California San Diego), and national guidelines and animal usage protocols from the Animal Care and Use Committee were followed.

ALKALINE COMET ASSAYS

Alkaline comet assays were performed using a Comet Assay Kit (Trevigen, USA). Cells were suspended in 85 μL ice-cold PBS and gently mixed with an equal volume of 1% low-melting-point agarose. The cell suspension was dropped onto an agarose layer and incubated in lysis buffer for 1 h. After lysis, slides were incubated in buffer containing 0.3 M NaOH, 1 mM EDTA (pH 13) for 40 min and electrophoresed for 1 h. After neutralization, slides were stained and analyzed with a fluorescence microscope.

ANTIBODIES AND WB ANALYSIS

Cell pellets or brain tissues were homogenized, and total protein was isolated using a protein extraction kit (Millipore, USA). The cytosolic and nuclear fractions were isolated from cells/tissue using a NE-PER protein extraction kit (Thermo Scientific, USA). WBs were performed according to the standard procedure, and each experiment was performed

at least three times to ensure statistically significant results. The antibodies for p53 (Cat #9282), p53-S15 (Cat #9286), p53-S20 (Cat #9287), p53-S46 (Cat #2521), Chk2 (Cat #2662), Chk2-T68 (Cat #2661), CBP (Cat #7389) and APE1 (Cat #4128) were from Cell Signaling, USA; anti-H2AX (Cat #ab11175) and γH2AX-S139 (Cat #ab11174) were from Abcam, UK; anti-ATM (Cat #1549-1) and ATM-S1981 (Cat #2152-1) were from Epitomics, USA, or anti-ATM from Santa Cruz (sc-23921), anti-ataxin-3 monoclonal antibody (Cat #MAB 5360), monoclonal anti-HTT antibody (MAB 2170) and 5TF1-1C2 (Mab1574) were from Millipore, USA. Rabbit polyclonal HAP-1 (Cat #TA306425) was from Origene, USA, and mouse monoclonal HAP-1 (MA1-46412) was from Thermo Scientific, USA. RNA pol II (sc-899) and DNA ligase 3 (sc-135883) were from Santa Cruz Biotechnology, USA. PNKP rabbit polyclonal antibody (Cat #MBP-1-A7257) was from Novus Biologicals, USA, and BioBharati Life Science (Cat# BB-AB0105), India, and PNKP mouse monoclonal antibody was a kind gift from Dr. Michael Weinfeld (University of Alberta, Canada).

IMMUNOHISTOCHEMICAL ANALYSIS

SH-SY5Y cells or frozen brain sections were immunostained with anti-PNKP, HTT, CBP, POLR2A, ATXN3, and anti-polyQ 5T1-1C2 antibodies. Nuclei were stained with DAPI (Molecular Probe, USA) and imaged under a confocal microscope.

CELL TOXICITY ASSAY

Expression of mHTT or wtHTT was induced in PC12 cells by removing doxycycline from the culture medium for 4-7 days. Induced cells were dissociated with Accutase (Gibco), and collected by centrifugation. Cell toxicity was assayed using a commercially available Annexin-V Cell Toxicity Assay kit (4830-01-K, Trevigen, USA). $1x10^6$ Cells were incubated at room temperature with 1µl Annexin-V-FITC (1µg/ml) and 5ul Propidium Iodide, in the provided binding buffer, for 15min, before diluting with

binding buffer. FITC fluorescence was analyzed by flow cytometry using a Cytoflex (Beckman Coulter), measuring 10,000 events per sample. Gating on main cell population was performed by FSC/SSC gating. Positive thresholds determined with unstained negative control, and H_2O_2 treated positive control samples. Identical thresholds applied to all samples. Data was analyzed using CytExpert software (Beckman Coulter).

IMAGE COLLECTION

Images were collected using a Zeiss LSM-510 META confocal microscope with 40× or 60× 1.2 numerical aperture water immersion objectives. Images were obtained using two excitation wavelengths (488 and 543 nm) by sequential acquisition. Images were collected using 4-frame-Kallman-averaging with a pixel time of 1.26 μs, a pixel size of 110 nm, and optical slices of 1.0 μm. Z-stack acquisition was performed at 0.8-μm steps. Orthogonal views were processed with LSM 510 software.

CASPASE-3 ACTIVITY MEASUREMENTS

Caspase-3 activities were measured using a Caspase-3 assay kit (BD Biosciences, USA) based on hydrolysis of the substrate acetyl-Asp-Glu-Val-Asp p-nitroanilide (Ac-DEVD-pNA), resulting in release of the p-nitroaniline (pNA) moiety. Released pNA is detected at 405 nm. Comparison of pNA absorbances from the sample and control allows determination of the fold increase in caspase-3 activity (relative caspase-3 activity is expressed in arbitrary units).

IN SITU PROXIMITY LIGATION ASSAY (PLA)

SH-SY5Y cells were plated on chamber slides and cultured in DMEM for 24 h. SH-SY5Y cells or brain sections were fixed with 4% paraformaldehyde, permeabilized with 0.2% Tween-20, washed with 1× PBS, incubated with primary antibodies for PNKP (mouse monoclonal), HTT (rabbit polyclonal and mouse monoclonal), PNKP (mouse

monoclonal), POLR2A (rabbit polyclonal), HAP-1 (rabbit polyclonal and mouse monoclonal), ATXN3 (rabbit polyclonal and mouse monoclonal), and DNA ligase 3 (rabbit polyclonal). These samples were subjected to PLAs using the Duolink PLA kit (O-Link Biosciences, Sweden). Nuclei were stained with DAPI, and PLA signals were visualized under a fluorescence microscope at 20× magnification.

PNKP ACTIVITY MEASUREMENTS

The 3'-phosphatase activity of PNKP in the nuclear extract (250--500 ng) of cells/ mouse brains or with purified recombinant His-tagged PNKP (25 fmol) was conducted as we described previously (Wiederhold et al. 2004; Mandal et al. 2012; Chatterjee et al. 2015). Nuclear extracts for the 3' phosphatase assay was prepared following standard protocols from cells (Chakraborty et al., 2016) or mouse brains tissues (Chakraborty et al. 2015). A ^{32}P-labeled 3'-phosphate-containing 51-mer oligo substrate with a strand break in the middle (5 pmol) was incubated at 37°C for 15 min in buffer A (25 mM Tris-HCl, pH 7.5, 100 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 10% glycerol and 0.1 µg/µl acetylated BSA) with 5 pmol of unlabeled (cold) substrate. The reaction was stopped by adding buffer B (80% formamide, 10 mM NaOH) and the reaction products were electrophoresed on a 20% Urea-PAGE to measure the amount of 3' phosphate release from the radio-labelled substrate. The radioactive bands were visualized in PhosphorImager (GE Healthcare, USA). The data were represented as % of the phosphate release (% product) with the total radiolabeled substrate as 100.

TOTAL DNA REPAIR ASSAY

Total DNA repair assays were carried out according to the protocol of Wiederhold et al. (Wiederhold et al. 2004). Briefly, 10 pmol DNA substrate (a 51-mer DNA-oligo) annealed to two shorter DNA duplexes, one containing 3'-P and the other with 5'-P with a 4-nt gap in the middle was used to assess total repair activity (DNA end cleaning + gap

filling through polymerization + ligation to fill the ends) in NEs (2.5 μg) from wtHTT- and mHTT-expressing neuronal cells and zQ175 and control mouse brain samples. Total repair activity was also assessed with the same substrate with DNA oligos containing 3'-OH (clean DNA ends). In both cases, the 20-μL reaction mixture contained 1 mM ATP, 50 μM unlabeled dNTPs, and 0.5 pmol [α-32P]-dCTP (the concentration of cold dCTP was lowered to 5 μM) in BER buffer and incubated for 45 min at 30 °C. The reaction products were analyzed with 20% urea-polyacrylamide gel electrophoresis, and the radioactive bands were detected in a Phosphorimager (GE Life Sciences, USA).

GENE EXPRESSION ANALYSIS BY REAL-TIME QUANTITATIVE RT-PCR

Freshly dissected brain tissue from transgenic and age-matched control mice was homogenized in TRIzol (Thermo Scientific, USA), and total RNA was extracted using an RNA extraction kit (Qiagen, USA) and purified using a DNA-free DNAse Kit (Ambion, USA). Next, 1 μg of total RNA was reverse-transcribed using an RT-PCR kit (Clontech, USA). A cDNA aliquot from each reaction was quantified, and 500 ng of cDNA from each reaction was used for qRT-PCR. 18S rRNA was used as control for the qRT-PCR analysis. The reactions were repeated three times using the following primers.

Neurod1:

F: AGCCCTGATCTGGTCTCCTT;

R: CTGGTGCAGTCAGTTAGGGG

Neurod2:

F: AAGCCAGTGTCTCTTCGTGG;

R: TTGGACAGCTTCTGCGTCTT

Neurog1:

F: CCAGGACGAAGAGCAGGAAC;

R: GGTCAGAGAGTGGTGATGCC

Tubb3:

F: TGAGGCCTCCTCTCACAAGT;

R: ACCACGCTGAAGGTGTTCAT

Eno2 γ:

F: CCCAGGATGGGGATTTTGCT;

R: CCTCCCCTGATCTGCTACCT

Pol b:

F: TTCCACCGGTAAGACCCAGG;

R: GCCAGTAACTCGAGTCAGGA

Myod1:

F: AGCATAGTGGAGCGCATCTC;

R: TTGGGGCTGGATCTAGGACA

Myog:

F: GAGGAAGTCTGTGTCGGTGG;

R: CCACGATGGACGTAAGGGAG

Myh2:

F: CGAGAGACGAGTGAAGGAGC;

R: GAATCACACAGGCGCATGAC

Myh4:

F: AGCGCAGAGTGAAGGAACTC;

R: TCTCCTGTCACCTCTCAACAGA

Myh6:

F: ATAAAGGGGCTGGAGCACTG;

R: TCGAACTTGGGTGGGTTCTG

Myh7:

F: CCTTACTTGCTACCCTCAGGTG;

R: GGCCATGTCCTCGATCTTGT

Gapdh

F: ATGAGAGAGGCCCAGCTACT;

R: TTTGCCGTGAGTGGAGTCAT

Bcl2L11:

F: TTGGATTCACACCACCTCCG;

R: CGGGATTACCTTGCGGTTCT

Pmaip 1:

F: CTCGCTTGCTTTTGGTTCCC;

R: ACGACTGCCCCCATACAATG

Bid:

F: CCACAACATTGCCAGACATCTCG:

R: TCACCTCATCAAGGGCTTTGGC

Pidd:

F: ACAGAAGAGCCTCGGCAAGTCT:

R: GAAAGGCACAGCAGAGGGCTTA

Apaf1:

F: CACGAGTTCGTGGCATATAGGC:

R: GGAAATGGCTGTCGTCCAAGGA

CHROMATIN IMMUNOPRECIPITATION (CHIP)

ChIP assays were performed using fresh brain tissue of WT mice as previously described (Chakraborty et al. 2016; Sailaja, Takizawa, and Meshorer 2012). Briefly, 80-100 mg of freshly harvested CTX was chopped into small pieces and fixed in 1% formaldehyde for 15 min. The samples were centrifuged at 440 × g for 5 min at room temperature, and 0.125 M glycine was added to terminate cross-linking. The samples were washed two to three times with ice-cold PBS (containing protease inhibitors) and

centrifuged each time at 440 × g for 4 min at 4 °C. The pellet was resuspended in 1 mL ice-cold lysis buffer (10 mM EDTA, 1% [w/v] SDS, 50 mM Tris-HCl [pH 7.5]) with protease inhibitors and PMSF for 15 min and homogenized to produce a single-cell suspension. The samples were then transferred to pre-cooled 1.5-mL tubes and centrifuged at 2260 × g for 5 min. The pellet was resuspended in lysis buffer and sonicated to generate ~500-bp DNA fragments. The samples were centrifuged at 20,780 × g for 30 min at 4 °C, and supernatants were collected for ChIP. The sheared chromatin was IP'd for 6 h at 4 °C with 10 μg isotype control IgG (Santa Cruz Biotechnology, USA: sc-2027) or anti-HTT antibody. After DNA recovery with proteinase K treatment followed by phenol extraction and ethanol precipitation, 1% of input chromatin and the precipitated DNA were analyzed by qPCR with the following primers. ChIP data are presented as percent binding relative to the input value.

Neurod1:

F: CTGCAAAGGTTTGTCCCAAGC;

R: CTGGTGCAGTCAGTTAGGGG

Neurod2:

F: CAGGCCCTCCCAAGAGACTT;

R: TCGTGTTAGGGTGAAGGCGT

Neurog1:

F: GCTTGCTCCAGGAAGAACCT;

R: AGAGACACCGCTACTAGGCA

Tubb3:

F: GTGGGGCTCTCCCCTAAAAC;

R: TTGGGAGCGCACAGTTAGAG

Eno2 γ:

F: TAGGGGTGCCTAGTCCTGTC;

R: GAGTGCTGGATGTGTGGTCA

Myod1:

F: ATCTGACACTGGAGTCGCTTT;

R: TTAGTCTCAGCTGCTGGTTCC

Myog:

F: GGCCACCAGAGCTAGAACAG;

R: ATGAAGGCTGTGGACTTGGG

Myh2:

F: TCAGTGAGCAGTGGGAGCTA;

R: GTACAAACACGGGGACACCC

Myh4:

F: AGGTGTACAACTCCGTGGGT;

R: GCTCTAGCAAGACCAGTCACG

Myh6:

F: TCGTGCCTGATGACAAGGAG;

R: CTTTCTGGCAAGCGAGCATC

Myh7:

F: ATTGGTGCCAAGGTGGGTTT;

R: CCTGGGGTTCCCAGAATCAC

LA-qPCR ANALYSIS TO ASSESS DNA STRAND BREAKS

LA-qPCR assays were carried out following an existing protocol (Santos et al., 2006). Briefly, tissues were harvested from the cortex (CTX), striatum (STR), and cerebellum (CRBL) of control and HD transgenic mice, and genomic DNA was extracted using the genomic-tip 20/G kit (Qiagen, Germany). Genomic DNA was quantified, and gene-specific LA-qPCR analyses were performed using Long Amp Taq DNA polymerase (NEB, USA). Various genomic loci were PCR-amplified from actively transcribing genes

in brain (e.g., neuronal differentiation factor 1 and 2 [Neurod1 and Neurod2], neurogenic basic-helix-loop-helix protein neurogenin 1 [Neurog1], tubulin beta 3 class III [Tubb3], neuron-specific enolase 2 [Eno2γ], and DNA polymerase β [Pol b]). Non-transcribing loci (e.g., myogenic differentiation factor 1 [Myod1]; myogenic factor 4; myogenin [Myog]; and myosin heavy chain 2, 4, 6, or 7 [Myh2, Myh4, Myh6, or Myh7]) were amplified using the primers listed below. Loci from genomic DNA isolated from iPSC-derived control and HD primary neurons were PCR-amplified with the primers listed below. The cycle numbers and DNA concentrations were standardized before each final reaction so that the reaction remained within the linear amplification range (Santos et al. 2006). The final PCR conditions were optimized at 94 °C for 30 s (94 °C for 30 s, 55–60 °C for 30 s depending on the oligo annealing temperature, 65 °C for 10 min) for 25 cycles and 65 °C for 10 min. Each reaction used 15 ng of DNA template, and the LA-qPCRs for all studied genes used the same stock of diluted DNA samples to avoid amplification variations due to sample preparation. A small DNA fragment for each gene was amplified to normalize large fragment amplification. The PCR conditions were 94 °C for 30 s, 54 °C for 20 s, 68 °C for 30 s for 25 cycles, and 68 °C for 5 min. Short PCR used 15 ng of the template from the same DNA aliquot. The amplified products were visualized on gels and quantified with the ImageJ software based on three independent replicate PCRs. The extent of damage was calculated according to our previously described method (Chakraborty et al., 2016).

Mouse neurod1

Long:

F: CTCGCAGGTGCAATATGAATC;

R: GCAACTGCATGGGAGTTTTCT

Short:

F: CTGCAAAGGTTTGTCCCAAGC;

R: CTGGTGCAGTCAGTTAGGGG

Mouse neurod2:

Long:

F: GGCAGTGGTTGGGATGGTAT;

R: CTCACTCTGTGCTGTCTGTCTC

Short:

F: CAGGCCCTCCCAAGAGACTT;

R: TCGTGTTAGGGTGAAGGCGT

Mouse neurog1

Long:

F: GATGAGCCCCTGAAGACGAG;

R: GCCAATCTTGCTTCTTGCGT

Short:

F: GCTTGCTCCAGGAAGAACCT;

R: AGAGACACCGCTACTAGGCA

Mouse tubb3

Long:

F: GGTACAGGGGATGTGGTTGG;

R: GAGTCTCCTGCCTGTCCCTA

Short:

F: GTGGGGCTCTCCCCTAAAAC;

R: TTGGGAGCGCACAGTTAGAG

Mouse eno2 γ

Long:

F: CTTGTTCTTCGGGGACCCTC;

R: CATCCGTGTGCTTAAGGGGT

Short:

F: TAGGGGTGCCTAGTCCTGTC;

R: GAGTGCTGGATGTGTGGTCA

Mouse pol b

Long:

F: TATCTCTCTTCCTCTTCACTT;

R: GTGATGCCGCCGTTGAGGGTCTCCTG

Short:

F: TATGGACCCCCATGAGGAACA;

R: AACCGTCGGCTAAAGACGTG

Mouse myod1

Long:

F: ATAGACTTGACAGGCCCCGA;

R: GGACCGTTTCACCTGCATTG

Short:

F: ATCTGACACTGGAGTCGCTTT;

R: TTAGTCTCAGCTGCTGGTTCC

Mouse myog

Long:

F: ACAAGCCTTTTCCGACCTGA;

R: CCATGGCCAAGGCGACTTAT

Short:

F: GGCCACCAGAGCTAGAACAG;

R: ATGAAGGCTGTGGACTTGGG

Mouse myh2

Long:

F: ATCTCAGGAGCACCCATCCT;

R: GAAAAGGGTGTGCCAAGCAG

Short:

F: TCAGTGAGCAGTGGGAGCTA;

R: GTACAAACACGGGGACACCC

Mouse myh4

Long:

F: GACGTGGAACTGTTAGGCCA;

R: AAGCCAGAGTCTTCAACCCG

Short:

F: AGGTGTACAACTCCGTGGGT;

R: GCTCTAGCAAGACCAGTCACG

Mouse myh6

Long:

F: GACAAGGGGCATTGTAGCCT;

R: TCTGCCTACCTTATGGGGCT

Short:

F: TCGTGCCTGATGACAAGGAG;

R: CTTTCTGGCAAGCGAGCATC

Mouse myh7

Long:

F: TTTGGGTTGGCCTGTCAGTT;

R: ATCCCTAGCTGGGGCTTGTA

Short:

F: ATTGGTGCCAAGGTGGGTTT;

R: CCTGGGGTTCCCAGAATCAC

Human TUBB3

Long:

F: TGCTTCTCATGCTTGCTACCAC;

R: TCTGTCCCTGTAGGAGGATGT

Short:

F: CCTGTCCCTTTGTTGGAGGG;

R: CGAGGTGGGCTAACAATGGA

Human NEUROD1

Long:

F: CCGCGCTTAGCATCACTAAC;

R: TGGCACTGGTTCTGTGGTATT

Short:

F: TGCCTCTCCCTTGTTGAATGTAG;

R: TTCTTTTTGGGGCCGCGTCT

Human POLB

Long:

F: CATGTCACCACTGGACTCTGCAC

R: CCTGGAGTAGGAACAAAAATTGCT

Short:

F: AGTGGGCTGGATGTAACCTG

R: CCAGTAGATGTGCTGCCAGA

Human ENO2 γ

Long:

F: ACGTGTGCTGCAAGCAATTT;

R: CCTGAAACTCCCCTGACACC

Short:

F: GGTGAGCAATAAGCCAGCCT;

R: CAGCTTGTTGCCAGCATGAG

STATISTICAL ANALYSIS

Data reported as mean ± SD and the statistical analysis was performed using Sigma Plot (SYSTAT Software). Differences between two experimental groups were analyzed by Student's t test (2-tail, assuming unequal variances). When comparing multiple groups, One-way ANOVA was performed followed by Tukey's post-hoc test to determine significance. In all cases, $p < 0.05$ was considered significant.

Chapter 5: Concluding Remarks and Future Directions

Results presented in this thesis substantially advance the hypothesis that the wtHTT protein is involved in the DNA repair process, and that disruption of this repair by mHTT underlies the pathogenesis of Huntington's disease. This work improves our understanding of the wild-type role of HTT, and how CAG repeat expansion perturbs that function. The ultimate goal of this research is to improve our understanding of this tragic disease so that researchers might home-in on possible routes of treatment. If the hypothesis presented here is correct, finding ways to increase DNA repair through other mechanisms to compensate for disruptions to TCR by mHTT may offer a viable treatment. By doing so, it may be possible to delay the onset of HD symptoms. However, it is likely that there is a certain "point of no return," at which point neurons would no longer be capable of recovery and any such treatment would be of little or no use. Determining exactly when this "point of no return" occurs during HD pathogenesis, will be critical in implementing any treatment regime focused on rescuing genomic integrity.

HTT IN THE NUCLEUS

The study presented in Chapter 2 is the first to show the interaction of HTT with TCR proteins, including RNAPol2, DNALigIII, CSB, and PNKP. These interactions were identified by co-IP, colocalization, and proximity ligation assay; and this protein complex was shown to interact directly with the DNA by ChIP assays. It also shows that HTT is somehow involved in regulating the activities of PNKP. LA-qPCR assays show that disruption of those activities by mHTT is sufficient to impair TCR, and lead to an accumulation of DNA damage. This unresolved damage results in subsequent chronic

activation of the ATM-p53 pathway, ultimately leading to cell death. However, the mechanism behind the impaired DNA repair remains unclear; it is possible that HTT may be directly regulating the activity of PNKP, or alternatively it may be necessary for the formation of the proper tertiary or quaternary protein structure. HTT is commonly hypothesized to act as a scaffolding protein, and contemporary publications have suggested HTT acts as a scaffold for various DNA damage response and repair proteins. Similarly, our lab has previously published findings showing that Atxn3 is involved in regulating PNKP activity, which is perturbed by polyQ expansion of Atxn3. Results presented in Chapter 2 also suggest that mHTT disrupts the deubiquitinase activity of Atxn3. Together, these results imply that HTT may indirectly regulate PNKP, with Atxn3 as the intermediary. If HTT directly regulates either PNKP or Atxn3, it may be possible to develop targeted therapeutics to specifically activate them in spite of the perturbations imparted by mHTT. The TCR complex is a large multiprotein complex and it is likely that mHTT disrupts activities of other proteins within, beside those identified here, and further work will be required to identify such factors. It would be particularly revealing if a group could produce a structure of HTT and mHTT within the TCR complex on the DNA.

It is also worth deeper investigations into HTT's involvement in any other DNA repair or transcriptional processes, to determine if its role is specific to the TCR pathway. TCR, of course, is a convergence point between transcription and DNA repair, and it may be possible that HTT has a role in transcription beyond its involvement in coupled DNA repair. Furthermore, if HTT is confirmed to interact with other DNA repair factors, such a finding may be evidence that HTT acts as a universal scaffold for organizing the components of many different DNA repair pathways. Such a finding seems probable and coincides nicely with HTTs hypothesized role as a major scaffolding protein.

Data from Chapter 2 provide important insights into one of the most poorly understood aspects of HD and other neurodegenerative diseases: brain region specificity. DNA repair assays using samples from HD mice, show drastically reduced DNA repair potential in the striatum and cortex, as compared to the cerebellum. These results mirror the brain region specificity observed in HD patients. This result is highly suggestive of an alternative or compensatory DNA repair mechanism in unaffected regions such as the cerebellum; a mechanism that is independent of HTT and therefore not directly affected in HD. If such a mechanism could be identified, and activated in the most vulnerable cell populations, it could lead to the discovery of a technical "cure" for the disease.

HTT IN THE MITOCHONDRIA

The discovery of a novel mitochondrial TCR complex, outlined in Chapter 3, merits significant investigation in its own right. Mitochondrial DNA repair is poorly understood compared to the various nuclear repair pathways. It is not clear based on the results of Chapter 3, which DNA repair mechanism is transcription-coupled in mitochondria. In the nucleus, TCR is canonically considered to be a subset of the larger NER pathway. However, it is not clear to what extent NER is employed by the mitochondria, and TCR has never been reported in mitochondria. Therefore, these findings open intriguing lines of inquiry regarding the full repertoire of DNA repair pathways in mitochondria.

The interaction of HTT with the mtDNA, confirmed by ChIP assays, is another novel finding presented in Chapter 3. Previously it had been assumed that HTT interacted at the surface of the mitochondria, and was involved in processes such as motility, fusion/fission, and autophagy. This may indeed be the case, however it does not exclude

the possibility that HTT is also involved in internal mitochondrial processes such as transcription or repair. Similar to the nucleus, much further characterization of this mitochondrial complex will be required to understand what function HTT is fulfilling and how mHTT manages to disrupt that function.

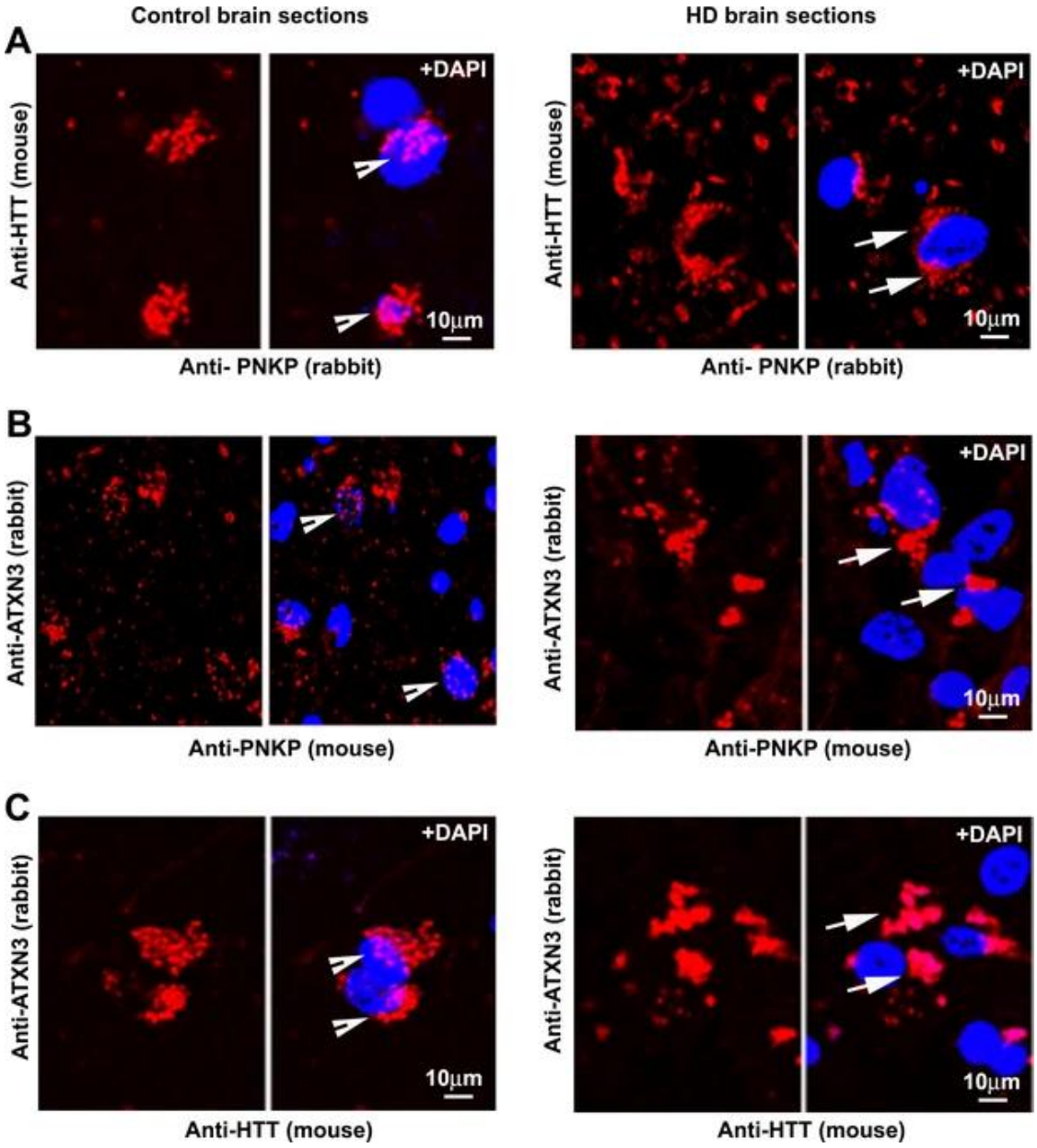
RELATION TO OTHER NUCLEOTIDE REPEAT EXPANSION DISORDERS

CAG repeat expansions are the genetic cause of 10 diseases including HD, and other polynucleotide repeat expansions are responsible for numerous other neurodegenerative and neurodevelopmental disorders. It is important to note that disease causing expansions are not exclusive to the translated regions of a gene. Polynucleotide repeats are common motifs located in introns, exons, 3' and 5' UTRs, or in promoter regions of genes. Therefore, some component of all of these diseases must occur at the genetic level, and does not necessitate the translation of a repeat containing protein. Additionally, these repeats are evolutionarily conserved at the codon level. For example, replacement of the HTT CAG tract with a tract composed of non-repetitive glutamine codons (a mix of CAG, CAA, CAC, and CAT) serves to eliminate spontaneous somatic expansions in cell models. There must be some evolutionary advantage to maintaining these specific repeats, or they would have long ago been replaced with non-deleterious sequences which code for identical proteins or produce identical DNA and RNA secondary structures. However, despite their clear importance, little progress has been made in investigating the function of these repeats, outside of a disease context. Many nucleotide repeat expansion disorders, particularly the polyQ diseases, manifest with very similar symptoms and disease progression. It is particularly intriguing that several polyQ proteins besides HTT and Atxn3, are associated with either transcription or DNA repair. Specifically, the Androgen Receptor which is responsible for SpinalBulbar Muscular Atrophy, DRPLA which is responsible for Dentatorubropallidoluysian Atrophy,

Atxn1 and Atxn2 which are responsible for SCA1 and SCA2 respectively, are all suspected transcription factors. TATA Binding Protein is another polyQ protein, responsible for SCA17, and it has long been identified as a key eukaryotic transcriptional initiator. Therefore, understanding the native role of these sequences in gene regulation or protein function should be a key focus of future research for all of the nucleotide repeat expansion disorders, as findings in any one case may be relevant to the other diseases as well.

Appendices

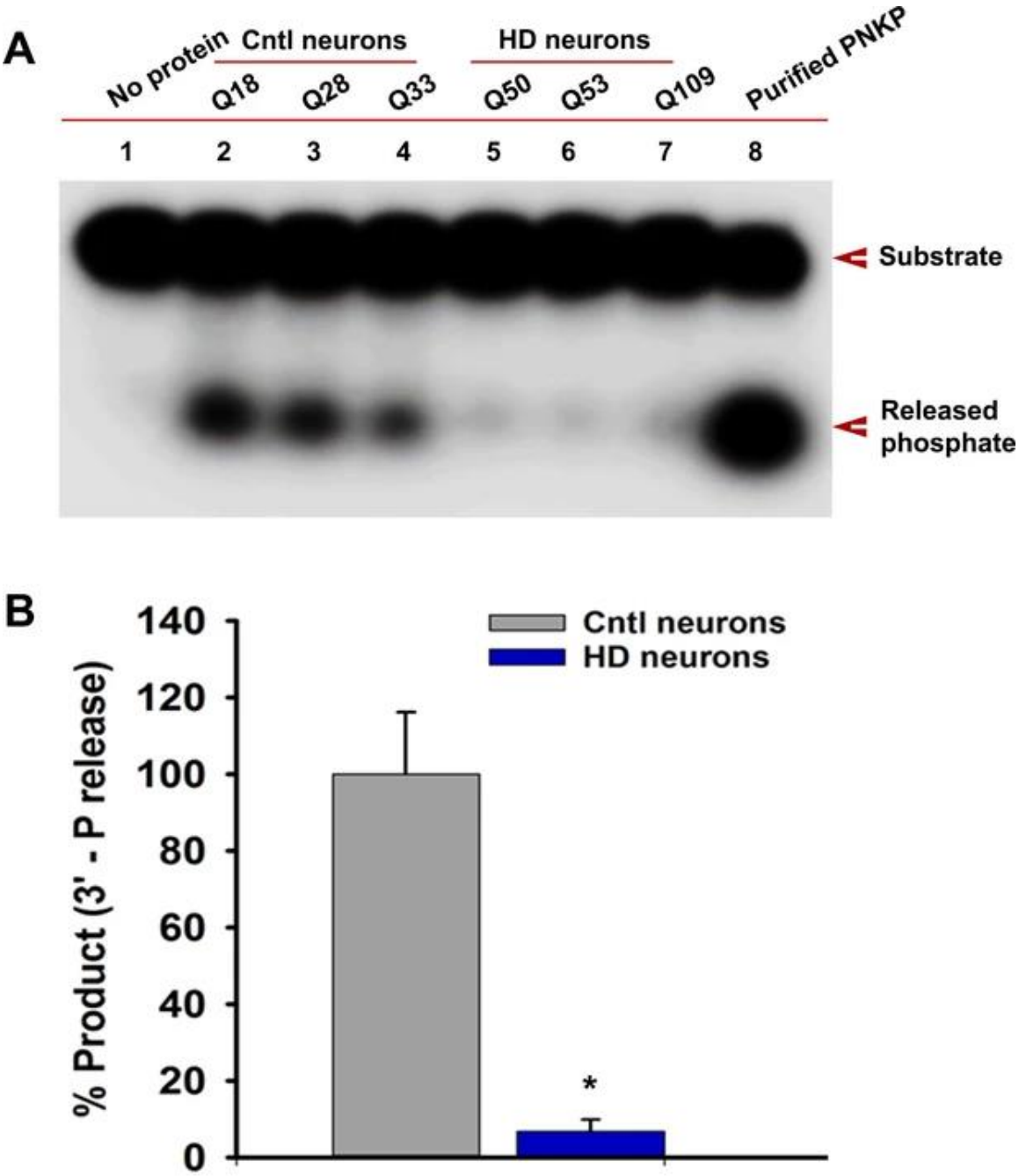
Supplemental Figure 1: HTT is a component of the TCR complex in vivo



(A) Proximity Ligation Analysis (PLA) was performed on normal (left) and HD (expressing mHTT-Q58, adult onset HD patient, disease grade 4/4 and manifested severe phenotypes) patient brain sections (right) with anti-HTT (mouse) and anti-PNKP (rabbit) antibodies, and confocal image analysis was performed to assess possible in vivo interaction of HTT and PNKP. Arrows indicate the representative colocalization of the HTT and PNKP proteins in the nucleus. Nuclei were stained by DAPI (blue). Bar = 10 μm. (B) PLA on control

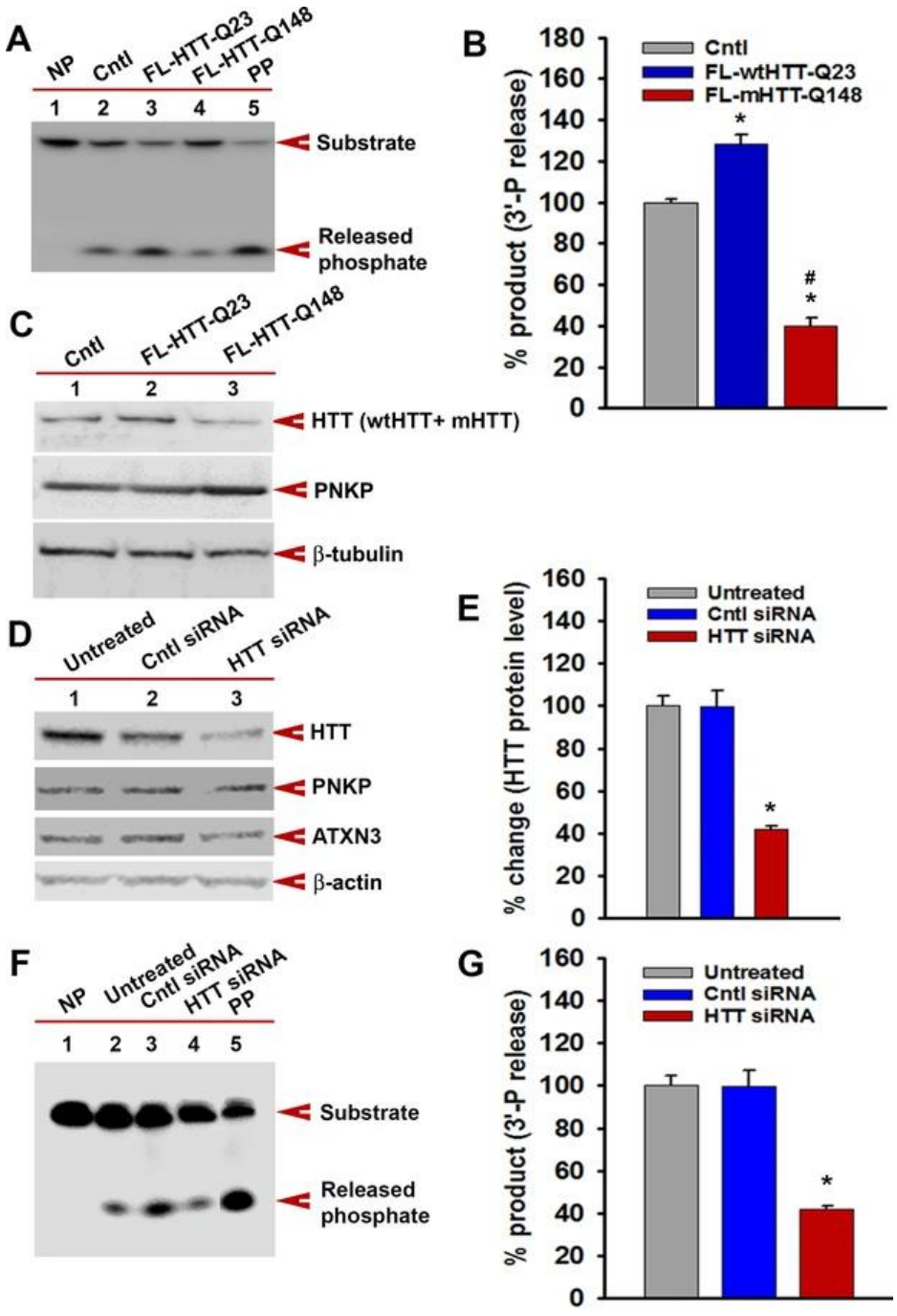
and HD brain section was performed with anti-ATXN3 (rabbit) and anti-PNKP (mouse) antibodies to assess possible in vivo interaction. (C) ATXN3 (rabbit) and HTT (mouse) antibodies to assess the in vivo protein association of the two proteins. Red fluorescence indicates positive protein-protein interactions (arrows indicate positive PLA signals).

Supplemental Figure 2: Endogenous level of mHTT is sufficient to deplete nuclear PNKP activity in iPSC-derived HD primary striatal neurons.



(A) The 3'-phosphatase activities of PNKP in the NE (250 ng each) of control (lanes 2, 3 and 4; differentiated iPSCs expressing Q18, Q28 and Q33), and HD neurons (lanes 5, 6 and 7; differentiated iPSCs expressing Q50, Q53 and Q109) were determined by measuring phosphate release from the DNA substrate (arrows). No protein extract was added to the substrate in lane one and purified PNKP (25 fmol) was added as a positive control (lane 8). (B) Relative 3'-phosphatase activities (in terms of % product) of PNKP in control (Q18, Q28 and Q33) and HD (Q50, Q53 and Q109) neurons. Data represent mean ± SD, *$p<0.001$ when compared with control. Three biological replicates and four technical replicates were used in measuring the phosphatase activities of PNKP.

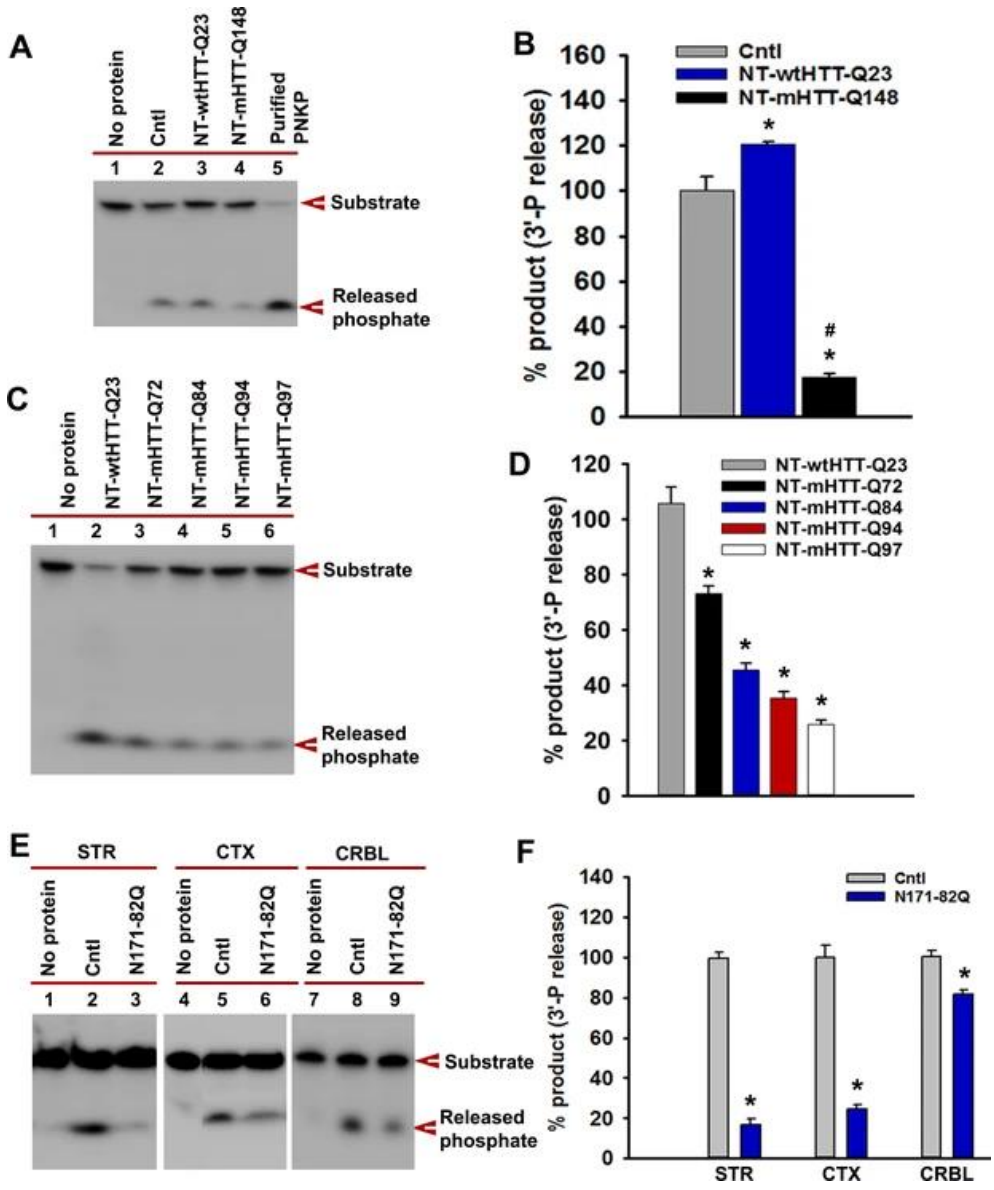
Supplemental Figure 3: mHTT-mediated inactivation of the TCR complex abrogates PNKP activity.



(A) The 3'-phosphatase activities of PNKP in the NE (250 ng each) of WT (Cntl: lane 2), wtHTT-Q23-expressing (lane 3), and mHTT-Q148-expressing (lane 4) PC12 cells were determined by measuring phosphate release from the DNA substrate (arrows). No protein extract was added to the substrate in lane 1 (NP), and purified PNKP (PP; 25 fmol) was added as a positive control (lane 5). (B) Relative 3'-phosphatase activities (in terms of %

product) of PNKP in WT control cells and cells expressing wtHTT-Q23 or mHTT-Q148. Data represent mean ± SD, *p<0.001 when compared with control and #p<0.001 when compared with FL-HTT-Q148. (C) NEs from WT control, wtHTT-Q23, or mHTT-Q148-expressing PC12 cells were analyzed by WB to determine PNKP protein levels; β-tubulin was the loading control. (D) NEs were isolated from untreated SH-SY5Y cells (lane 1), cells transfected with control siRNA (lane 2), or HTT siRNA (lane 3), and analyzed by WBs to detect HTT, PNKP, and ATXN3; β-actin was the loading control. (E) Relative levels of HTT in untreated SH-SY5Y cells, cells transfected with control siRNA, and HTT siRNA. Data represent mean ± SD; *p<0.001. (F) The 3'-phosphatase activities of PNKP in the NE of SH-SY5Y cells (lane 2), cells transfected with control siRNA (lane 3) and HTT siRNA (lane 4). No protein extract was added to the substrate in lane 1 (NP), and purified PNKP (PP; 25 fmol) was added as a positive control (lane 5). (G) Relative 3'-phosphatase activities (in terms of % product) of PNKP in untreated SH-SY5Y cells, transfected with control siRNA or HTT siRNA. Data represent mean ± SD; *p<0.001.

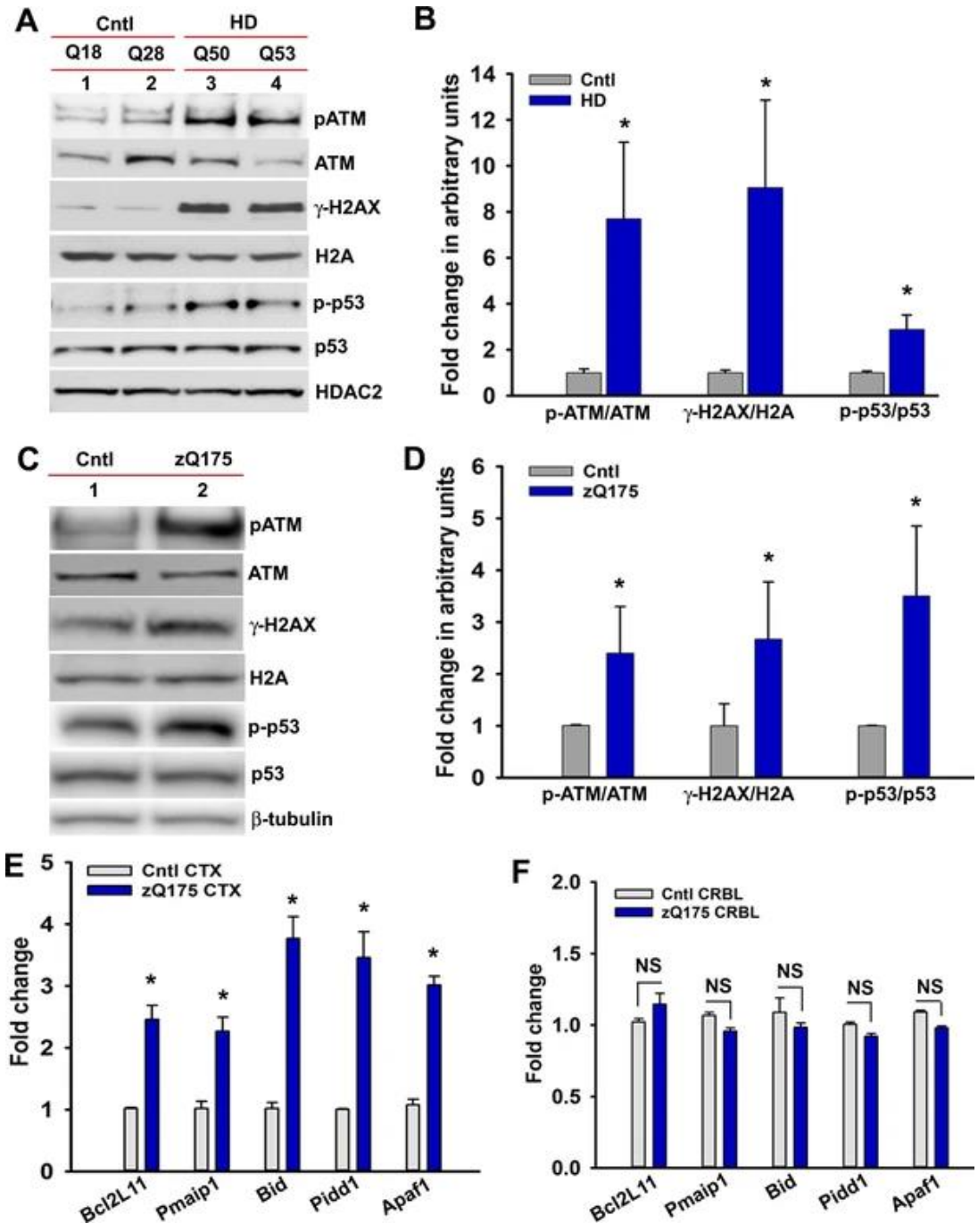
Supplemental Figure 4: Expression of the N-terminus of mHTT abrogates PNKP activity.



(A) NEs were isolated from WT PC12 cells (Cntl; lane 2) or cells expressing NT-wtHTT-Q23 (lane 3) or NT-mHTT-Q148 (lane 4), and PNKP activity was assessed. (B) Relative amounts of phosphate release from WT control PC12 cells and cells expressing NT-wtHTT-Q23 and NT-mHTT-Q148. Data represent mean ± SD, *$p<0.001$ when phosphate release in NT-wtHTT-Q23 and NT-mHTT-Q148 was compared with control PC12 cells and #$p<0.001$ when the phosphate release in NT-mHTT-Q148 was compared with NT-wtHTT-Q23. (C) NEs were isolated from SH-SY5Y cells expressing the N-terminus of HTT encoding Q23, Q72, Q84, Q94, and Q97, and PNKP activity was assessed. The products were analyzed to determine how much phosphate was released from the substrate (arrow). (D) The amounts of phosphate released from the substrate (arrow) when NEs of SH-SY5Y cells expressing NT-HTT encoding Q23, Q72,

Q84, Q94, and Q97 were added. Data represents mean ± SD, *p<0.001. Three technical replicates were used in this assay. (E) PNKP activities measured in NEs from STR, CTX, or CRBL of N171-82Q and control mice. (F) Relative PNKP activities in control and N171-82Q transgenic mice STR, CTX, and CRBL. Data represents mean ± SD, *p<0.001.

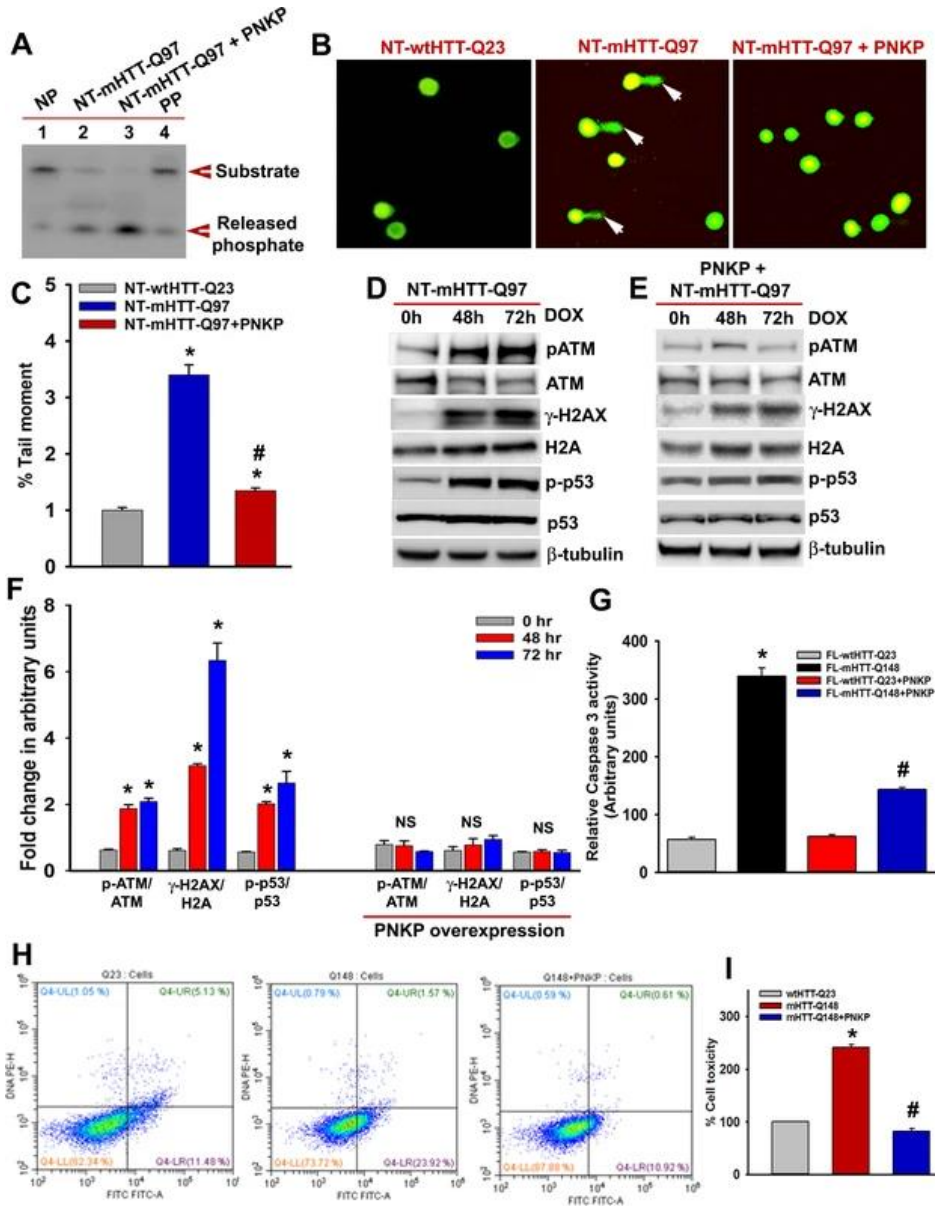
Supplemental Figure 5: mHTT triggers DNA damage response (DDR)-ATM signaling.



(A) Total protein from the induced pluripotent stem cell (iPSC)-derived primary control neurons (Q18 and Q28) and HD primary neurons (Q50 and Q53) was isolated and analyzed by WBs to detect p-ATM-S1981, total ATM, p-γH2AX-S139, total H2AX, p-p53-S15, and total p53 levels; HDAC2 was the loading control. (B) Relative levels of p-ATM-S1981, p-γH2AX, and p-p53-S15 in iPSC-derived control and HD neurons with respect to the corresponding total proteins.

Data represent mean ± SEM. (C) Total protein from the STR of zQ175 and age-matched control WT mice (n = 4) was isolated and the protein extracts were pooled together and analyzed by WBs to detect p-ATM-S1981, total ATM, p-γH2AX-S139, total H2AX, p-p53-S15, and total p53 levels; β-tubulin was the loading control. (D) Relative levels of p-ATM-S1981, p-γH2AX-S139, and p-p53-S15 in control and zQ175 mice STR with respect to the corresponding total proteins. Data represent mean ± SEM. (E) Total RNA was isolated from the CTX of 36 weeks old zQ175 and age-matched control WT mice, and mRNA levels of the p53 target genes analyzed by q-RT-PCR. 18 s rRNA gene was used as control. Data represent mean ± SD; *$p<0.001$ in B, D, and E. (F) Total RNA was isolated from the CRBL of 36 weeks old zQ175 and age-matched control WT mice, and mRNA levels of the p53 target genes analyzed by q-RT-PCR. NS = not significant.

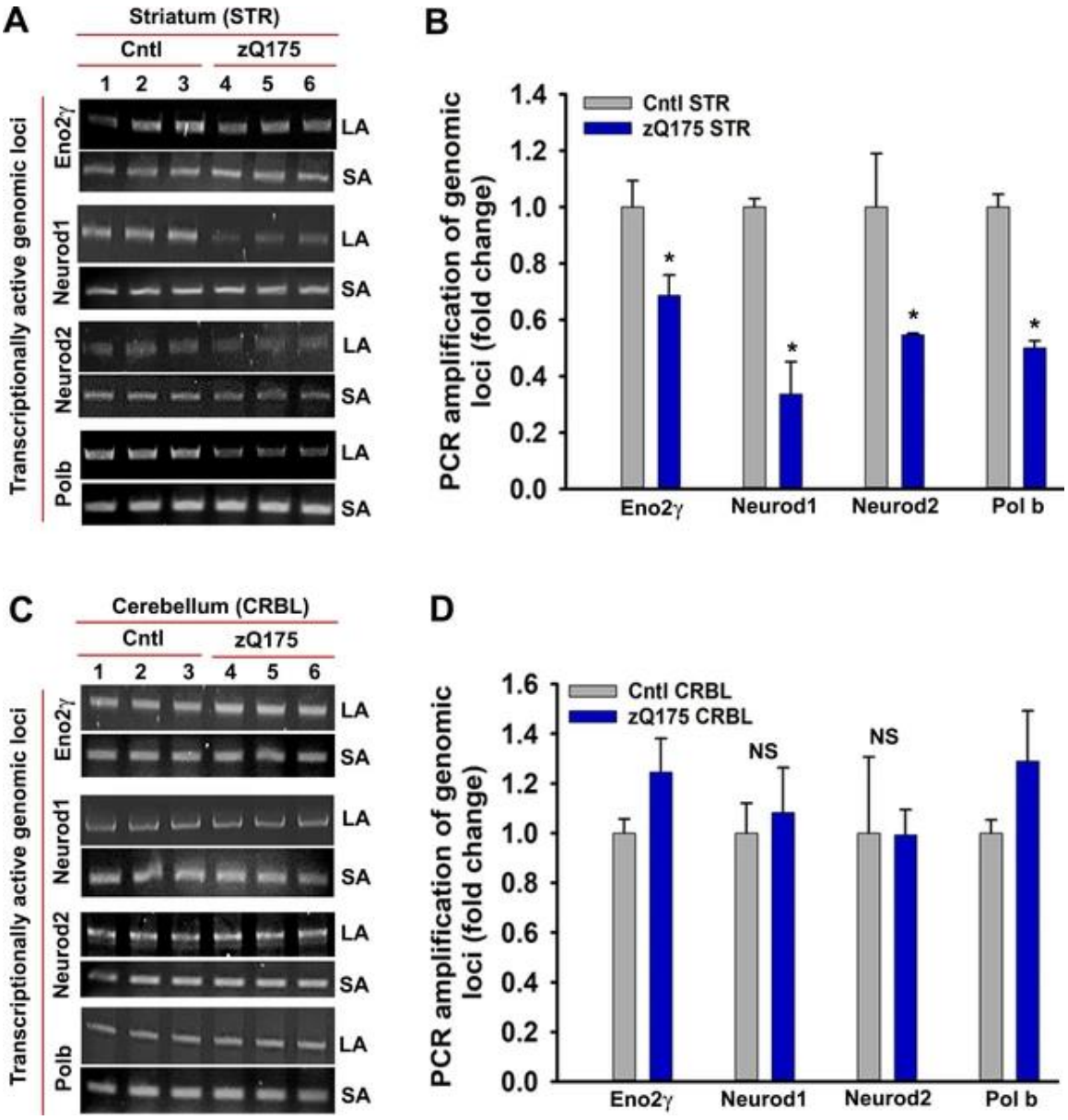
Supplemental Figure 6: PNKP overexpression in mutant cells rescues cell toxicity.



(A) NEs isolated from the mutant SH-SY5Y cells (NT-mHTT-Q97) (lane 2) and mutant cells with PNKP overexpression (lane 3), and PNKP activity was measured. No protein was added in lane 1 (NP) and purified PNKP was added in lane 4 (PP). DNA substrate and the released phosphate are shown by arrows. (B) Alkaline comet analyses of cells expressing NT-wtHTT-Q23 (left panel), NT-mHTT-Q97 (center panel), and co-expressing NT-mHTT-Q97 and PNKP (right panel). Comet tails (DNA strand breaks/damages) are shown by arrows. Nuclei are stained with SYBR green. (C) Tail moments representing strand breaks in cells expressing NT-wtHTT-Q23, NT-mHTT-Q97, and co-expressing NT-mHTT-Q97 and PNKP. Data represent mean ± SEM; *p<0.001 compared with NT-wtHTTQ23 and # p<0.001 compared with NT-mHTT-Q97 in C, E, G and I.

(D) Expression of NT-mHTT-Q97 was induced (by adding doxycycline to the medium) in differentiated SH-SY5Y cells that were harvested 0, 48, and 72 hr post-induction, and NEs were analyzed by WBs to detect p-ATM-S1981, total ATM, p-p53-S15, total p53, p-γH2AX-S139, and total H2A levels; β-tubulin is the loading control. (E) Expression of NT-mHTT-Q97 was induced in differentiated SH-SY5Y cells overexpressing PNKP. NEs were analyzed to determine the levels of p-ATM-S1981, total ATM, p-γH2AX-S139, total H2A, p-p53-S15, and total p53; β-tubulin is the loading control. (F) Relative levels of p-ATM-S1981, p-γH2AX, and p-p53-S15 with respect to the corresponding total proteins. Cells were harvested 0 (gray), 48 (red), and 72 (blue) h after NT-mHTT-Q97 expression; Data represent mean ± SEM. (G) Caspase-3 activities in PC12 cells expressing FL-wtHTT-Q23, FL-mHTT-Q148, co-expressing FL-wtHTT-Q23 and PNKP, or co-expressing FL-mHTT-Q148 and PNKP. (H) Representative flow cytometry analysis showing cell toxicity in PC12 cells expressing FL-wtHTT-Q23, FL-mHTT-Q148, and co-expressing FL-mHTT-Q148 + PNKP. X-axis: Annexin-V-FITC signal. Y-axis: Propidium Iodide signal. Healthy cells are represented in the lower left quadrant, early apoptotic cells in the lower right quadrant, late apoptotic cells in the upper right quadrant, and necrotic cells in the upper left quadrant. (I) Relative levels of cell toxicity of cells expressing FL-wtHTT-Q23, FL-mHTT-Q148 and co-expressing FL-mHTT-Q148 and PNKP. Data represent mean ± SD; *$p<0.001$ when compared wtHTT-Q23 vs. mHTT-Q148, and #$p<0.001$ when compared mHTT-Q148 +PNKP with mHTT-Q148.

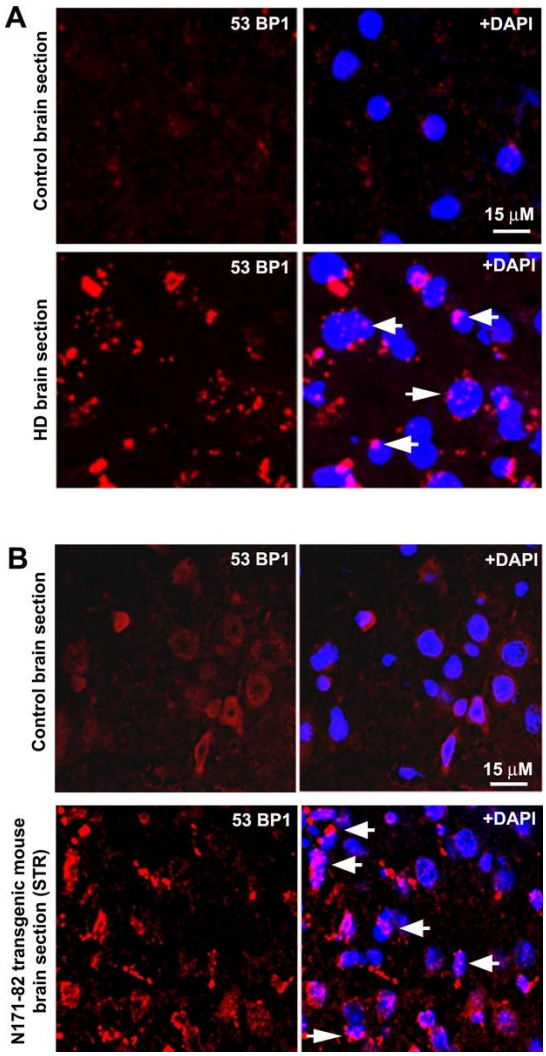
Supplemental Figure 7: mHTT expression induces DNA strand breaks in STR but not in CRBL.



(A) Genomic DNA was isolated from the STR of asymptomatic zQ175 transgenic (STR from three transgenic mice were pooled) and age-matched WT control (STR from three control mice were pooled) mice. Various transcriptionally active gene loci (Eno2γ, Neurod1, Neurod2 and Pol b) were PCR-amplified from the genomic DNA and analyzed on agarose gels; the lanes in figure represents a technical replicate. LA: long amplicon (6- to 12 kb product), SA: short amplicon (200–300 bp). PCR products from WT control mice (lanes 1–3) and zQ175 mice (lanes 4 to 6) were analyzed on agarose gels. (B) Relative PCR amplification efficacies of various gene loci in WT control and zQ175 transgenic mouse STR. Data represent mean ± SD; *$p<0.001$ compared with control. (C) Genomic DNA

was isolated from the CRBL of asymptomatic zQ175 transgenic (CRBL from three transgenic mice were pooled) and age-matched WT control (CRBL from 3 WT control mice were pooled) mice. Various gene loci (Eno2γ, Neurod1, Neurod2 and Pol b) were PCR-amplified from the genomic DNA and analyzed on agarose gels; LA: long amplicon (6- to 12 kb product), SA: short amplicon (200–300 bp). PCR products from WT control mice (lanes 1–3) and zQ175 mice (lanes 4 to 6) were analyzed on agarose gels. (D) Relative PCR amplification efficacies of various gene loci in WT control and zQ175 transgenic mouse CRBL. NS = not significant.

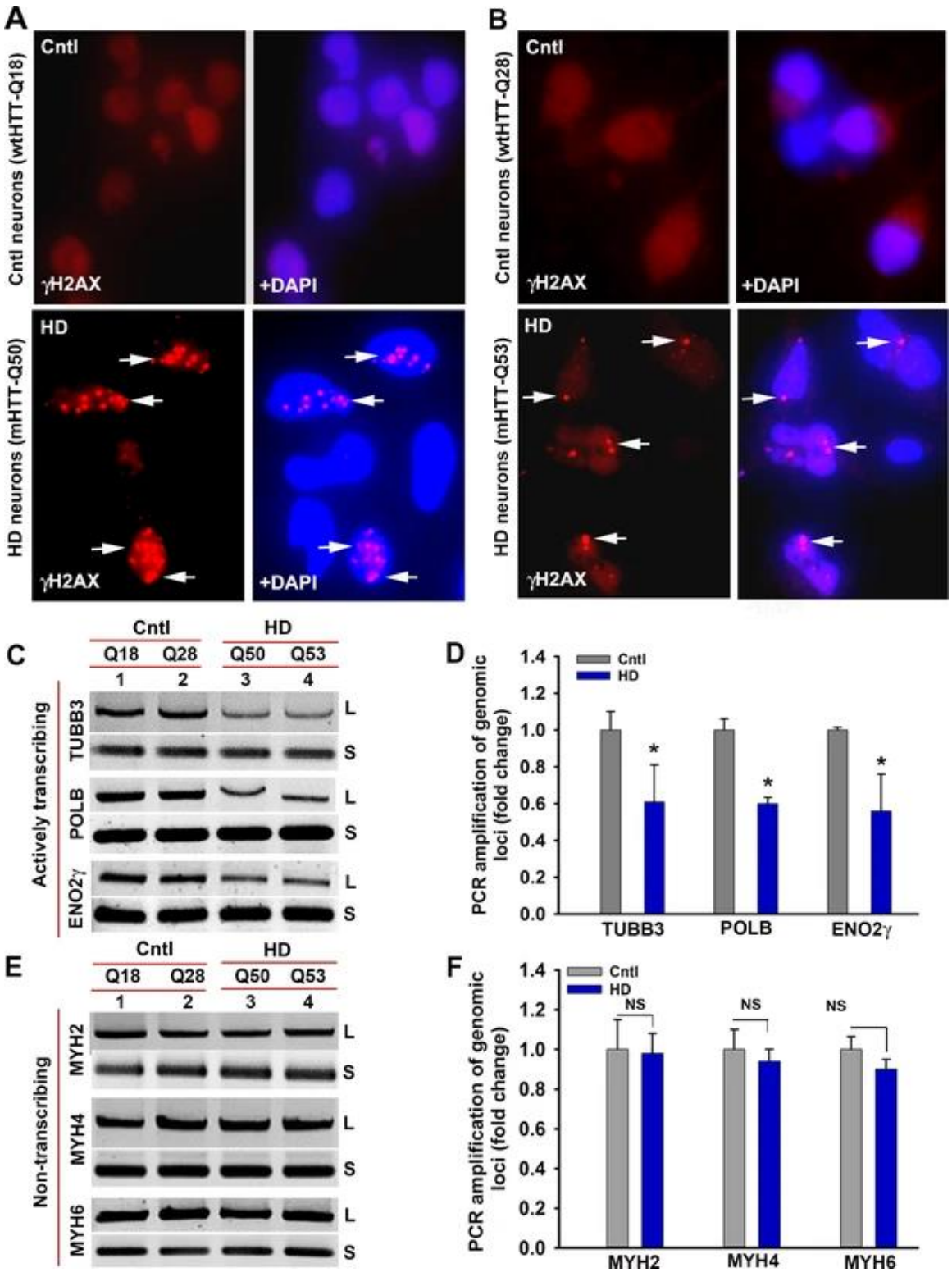
Supplemental Figure 8: HD patients' brain and HD transgenic mouse brain accumulate DNA damages.



(A) A representative confocal image showing immunostaining of HD patients' brain section expressing mHTT-Q58 (lower panel) and age-matched normal control

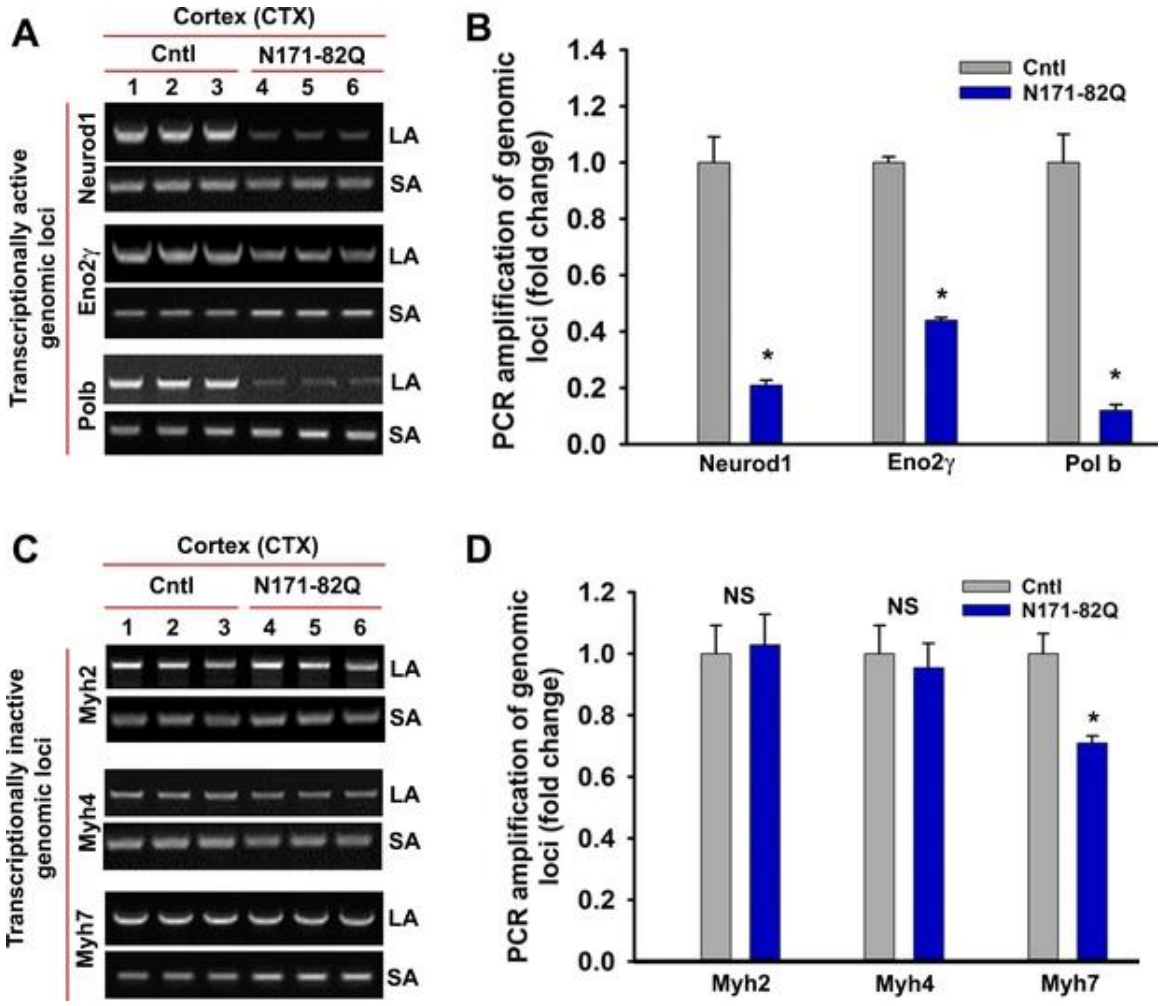
brain section (upper panel) with anti-phospho-53BP1 (p53-binding protein) antibody. Arrows show 53BP1 foci formation in HD brain section indicating DNA strand break accumulations. (B) A representative confocal image showing immunostaining of N171-82Q transgenic mouse brain (STR) section (lower panel) and age-matched normal control brain (STR) section (upper panel) with anti-phospho-53BP1 antibody. Arrows show 53BP1 foci formation in HD brain section indicating DNA strand break accumulations.

Supplemental Figure 9: HD primary neurons accumulate DNA breaks preferentially in the actively transcribing genome.



(A) Control and HD primary neurons expressing Q18 and Q50 respectively were immunostained with anti-γH2AX antibody to detect DNA strand breaks (arrows). (B) Control and HD primary neurons expressing Q28 and Q53 respectively were immunostained with anti-γH2AX antibody to detect DNA strand breaks (arrows). (C) LA-qPCR analysis. Genomic DNA was isolated from the control (Q18 and Q28) and HD (Q50 and Q53) primary neurons, genomic loci were PCR amplified, and the PCR products analyzed on agarose gels. Relative intensity of the PCR product indicates the PCR amplification efficacy of TUBB3, POLB and ENO2γ genomic locus that is actively transcribed in HD (lanes 3 and 4) vs. control neurons (lanes 1 and 2). L = long amplicon, and S = short amplicon in C and E. (D) Relative PCR amplification efficacy of TUBB3, POLB, and ENO2γ in control and HD neurons. Data represent mean ± SD; *$p<0.001$. (E) LA-qPCR analysis. Agarose gel showing PCR amplification of MYH2, MYH4 and MYH6, genomic loci that are not actively transcribed in control (lanes 1 and 2) and HD neurons (lanes 3 and 4). (F) Relative PCR amplification efficacy of MYH2, MYH4 and MYH6 loci in control vs. HD neurons. NS = not significant. Two biological replicates for control as well as for mutant subjects with three technical replicates were used in the LA-qPCR analyses.

Supplemental Figure 10: N171-82Q transgenic mouse brain predominantly accumulates strand breaks in the transcriptionally active genome.



LA-qPCR analysis. Genomic DNA was isolated from the CTX of symptomatic (16 wks old) N171-82Q transgenic (n = 3, pooled) and age-matched control mice (n = 3, pooled), various genomic loci were PCR amplified, and the PCR products were analyzed on agarose gels (left panels), and the PCR products were quantified (right panels). (A) The relative intensity of the PCR product indicates the PCR amplification efficacy of Neurod1, Eno2γ and Pol b, actively transcribing genomic loci in N171-82Q transgenic (lanes 4 to 6) vs. control CTX (lanes 1 to 3). LA = long amplicon, and SA = short amplicon in A and C. (B) Relative PCR amplification efficacy of Neurod1, Eno2γ and pol b in the CTX. Data represent mean± SD; *p<0.001. (C) LA-qPCR showing PCR amplification efficacy of Myh2, Myh4 and Myh7 in the CTX of N171-82Q transgenic (lanes 4 to 6) vs. age-matched controls (lanes 1 to 3). (D) Relative PCR amplification efficacy of Myh2, Myh4, and Myh7 loci in the CTX of N171-82Q transgenic and age-matched control mice. NS = not significant. Three biological replicates and three technical replicates were used for the LA-qPCR analyses.

References

Aamann, Maria D, Martin M Sorensen, Christina Hvitby, Brian R Berquist, Meltem Muftuoglu, Jingyan Tian, Nadja C de Souza-Pinto, et al. 2010. "Cockayne Syndrome Group B Protein Promotes Mitochondrial DNA Stability by Supporting the DNA Repair Association with the Mitochondrial Membrane." *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology* 24 (7): 2334–46. https://doi.org/10.1096/fj.09-147991.

Acevedo-Torres, Karina, Lexsy Berríos, Nydia Rosario, Vanessa Dufault, Serguei Skatchkov, Misty J. Eaton, Carlos A. Torres-Ramos, and Sylvette Ayala-Torres. 2009. "Mitochondrial DNA Damage Is a Hallmark of Chemically Induced and the R6/2 Transgenic Model of Huntington's Disease." *DNA Repair* 8 (1): 126–36. https://doi.org/10.1016/j.dnarep.2008.09.004.

Aidt, Frederik Heurlin, Signe Marie Borch Nielsen, Jørgen Kanters, Dominik Pesta, Troels Tolstrup Nielsen, Anne Nørremølle, Lis Hasholt, Michael Christiansen, and Christian Munch Hagen. 2013. "Dysfunctional Mitochondrial Respiration in the Striatum of the Huntington's Disease Transgenic R6/2 Mouse Model." *PLoS Currents* 5 (APR 2013). https://doi.org/10.1371/currents.hd.d8917b4862929772c5a2f2a34ef1c201.

Anindya, Roy, Ozan Aygün, and Jesper Q Svejstrup. 2007. "Damage-Induced Ubiquitylation of Human RNA Polymerase II by the Ubiquitin Ligase Nedd4, but Not Cockayne Syndrome Proteins or BRCA1." *Molecular Cell* 28 (3): 386–97. https://doi.org/10.1016/j.molcel.2007.10.008.

Anthony Altar, C., Ning Cai, Tricia Bliven, Melissa Juhasz, James M. Conner, Ann L. Acheson, Ronald M. Lindsay, and Stanley J. Wiegand. 1997. "Anterograde Transport of Brain-Derived Neurotrophic Factor and Its Role in the Brain." *Nature* 389 (6653): 856–60. https://doi.org/10.1038/39885.

Anttinen, Anu, Leena Koulu, Eeva Nikoskelainen, Raija Portin, Timo Kurki, Matti Erkinjuntti, Nicolaas G J Jaspers, et al. 2008. "Neurological Symptoms and Natural Course of Xeroderma Pigmentosum." *Brain : A Journal of Neurology* 131 (Pt 8): 1979–89. https://doi.org/10.1093/brain/awn126.

Arrasate, Montserrat, Siddhartha Mitra, Erik S. Schweitzer, Mark R. Segal, and Steven Finkbeiner. 2004. "Inclusion Body Formation Reduces Levels of Mutant Huntingtin and the Risk of Neuronal Death." *Nature*. https://doi.org/10.1038/nature02998.

Ashour, Mohamed E., Reham Atteya, and Sherif F. El-Khamisy. 2015. "Topoisomerase-Mediated Chromosomal Break Repair: An Emerging Player in Many Games." *Nature Reviews Cancer*. https://doi.org/10.1038/nrc3892.

Ayala-Peña, Sylvette. 2013. "Role of Oxidative DNA Damage in Mitochondrial Dysfunction and Huntington's Disease Pathogenesis." *Free Radical Biology and Medicine*. Elsevier Inc. https://doi.org/10.1016/j.freeradbiomed.2013.04.017.

Bae, Byoung il, Hong Xu, Shuichi Igarashi, Masahiro Fujimuro, Nishant Agrawal, Yoichi Taya, S. Diane Hayward, et al. 2005. "P53 Mediates Cellular Dysfunction and Behavioral Abnormalities in Huntington's Disease." *Neuron* 47 (1): 29–41. https://doi.org/10.1016/j.neuron.2005.06.005.

Bates, G. P., M. E. MacDonald, S. Baxendale, S. Youngman, C. Lin, W. L. Whaley, J. J. Wasmuth, J. F. Gusella, and H. Lehrach. 1991. “Defined Physical Limits of the Huntington Disease Gene Candidate Region.” *American Journal of Human Genetics* 49 (1): 7–16. https://pubmed.ncbi.nlm.nih.gov/1829581/.

Beal, M. Flint, Bradley T. Hyman, and Walter Koroshetz. 1993. “Do Defecs in Mitochondrial Energy Metabolism Underlie the Pathology of Neurodegenerative Diseases?” *Trends in Neurosciences* 16 (4): 125–31. https://doi.org/10.1016/0166-2236(93)90117-5.

Benn, Caroline L, Tingting Sun, Ghazaleh Sadri-Vakili, Karen N McFarland, Derek P DiRocco, George J Yohrling, Timothy W Clark, Bérengère Bouzou, and Jang-Ho J Cha. 2008. “Huntingtin Modulates Transcription, Occupies Gene Promoters in Vivo, and Binds Directly to DNA in a Polyglutamine-Dependent Manner.” *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 28 (42): 10720–33. https://doi.org/10.1523/JNEUROSCI.2126-08.2008.

Berent, Stanley, Bruno Giordani, Shirley Lehtinen, Dorene Markel, John B. Penney, H. A. Buchtel, Simon Starosta-Rubinstein, Richard Hichwa, and Anne B. Young. 1988. “Positron Emission Tomographic Scan Investigations of Huntington’s Disease: Cerebral Metabolic Correlates of Cognitive Function.” *Annals of Neurology* 23 (6): 541–46. https://doi.org/10.1002/ana.410230603.

Bertoni, Alessandra, Paola Giuliano, Mario Galgani, Deborah Rotoli, Luca Ulianich, Annagrazia Adornetto, Maria Rosaria Santillo, Antonio Porcellini, and Vittorio Enrico Avvedimento. 2011. “Early and Late Events Induced by PolyQ-Expanded Proteins: Identification of a Common Pathogenic Property of PolyQ-Expanded Proteins.” *Journal of Biological Chemistry* 286 (6): 4727–41. https://doi.org/10.1074/jbc.M110.156521.

Bettencourt, Conceição, Davina Hensman-Moss, Michael Flower, Sarah Wiethoff, Alexis Brice, Cyril Goizet, Giovanni Stevanin, et al. 2016. “DNA Repair Pathways Underlie a Common Genetic Mechanism Modulating Onset in Polyglutamine Diseases.” *Annals of Neurology* 79 (6): 983–90. https://doi.org/10.1002/ana.24656.

Bizat, Nicolas, Jean Michel Hermel, Frédéric Boyer, Carine Jacquard, Christophe Créminon, Stéphane Ouary, Carole Escartin, Philippe Hantraye, Stan Krajewski, and Emmanuel Brouillet. 2003. “Calpain Is a Major Cell Death Effector in Selective Striatal Degeneration Induced in Vivo by 3-Nitropropionate: Implications for Huntington’s Disease.” *Journal of Neuroscience* 23 (12): 5020–30. https://doi.org/10.1523/jneurosci.23-12-05020.2003.

Bogdanov, Misha B., Ole A. Andreassen, Alpaslan Dedeoglu, Robert J. Ferrante, and M. Flint Beal. 2001. “Increased Oxidative Damage to DNA in a Transgenic Mouse Model of Huntington’s Disease.” *Journal of Neurochemistry* 79 (6): 1246–49. https://doi.org/10.1046/j.1471-4159.2001.00689.x.

Borgesius, Nils Z, Monique C de Waard, Ingrid van der Pluijm, Azar Omrani, Gerben C M Zondag, Gijsbertus T J van der Horst, David W Melton, Jan H J Hoeijmakers, Dick Jaarsma, and Ype Elgersma. 2011. “Accelerated Age-Related Cognitive Decline and Neurodegeneration, Caused by Deficient DNA Repair.” *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 31 (35): 12543–53. https://doi.org/10.1523/JNEUROSCI.1589-11.2011.

Bossy-Wetzel, Ella, Alejandra Petrilli, and Andrew B. Knott. 2008. “Mutant Huntingtin and Mitochondrial Dysfunction.” *Trends in Neurosciences* 31 (12): 609–16. https://doi.org/10.1016/j.tins.2008.09.004.

Brackenridge, C. J. 1971. “The Relation of Type of Initial Symptoms and Line of Transmission to Ages at Onset and Death in Huntington’s Disease.” *Clinical Genetics* 2 (5): 287–97. https://doi.org/10.1111/j.1399-0004.1971.tb00289.x.

Brook, J. David, Mila E. McCurrach, Helen G. Harley, Alan J. Buckler, Deanna Church, Hiroyuki Aburatani, Kent Hunter, et al. 1992. “Molecular Basis of Myotonic Dystrophy: Expansion of a Trinucleotide (CTG) Repeat at the 3′ End of a Transcript Encoding a Protein Kinase Family Member.” *Cell* 68 (4): 799–808. https://doi.org/10.1016/0092-8674(92)90154-5.

Browne, Susan E., and M. Flint Beal. 2004. “The Energetics of Huntington’s Disease.” *Neurochemical Research*. Neurochem Res. https://doi.org/10.1023/B:NERE.0000014824.04728.dd.

Browne, Susan E., Allen C. Bowling, Usha MacGarvey, M. Jay Baik, Stephanie C. Berger, Miratul M.K. Muqit, Edward D. Bird, and M. Flint Beal. 1997. “Oxidative Damage and Metabolic Dysfunction in Huntington’s Disease: Selective Vulnerability of the Basal Ganglia.” *Annals of Neurology* 41 (5): 646–53. https://doi.org/10.1002/ana.410410514.

Burnett, Barrington, Fusheng Li, and Randall N. Pittman. 2003. “The Polyglutamine Neurodegenerative Protein Ataxin-3 Binds Polyubiquitylated Proteins and Has Ubiquitin Protease Activity.” *Human Molecular Genetics* 12 (23): 3195–3205. https://doi.org/10.1093/hmg/ddg344.

Caviston, Juliane P., Jennifer L. Ross, Sheila M. Antony, Mariko Tokito, and Erika L.F. Holzbaur. 2007. “Huntingtin Facilitates Dynein/Dynactin-Mediated Vesicle Transport.” *Proceedings of the National Academy of Sciences of the United States of America* 104 (24): 10045–50. https://doi.org/10.1073/pnas.0610628104.

Cazzalini, Ornella, Paola Perucca, Monica Savio, Daniela Necchi, Livia Bianchi, Lucia A Stivala, Bernard Ducommun, A Ivana Scovassi, and Ennio Prosperi. 2008. “Interaction of P21(CDKN1A) with PCNA Regulates the Histone Acetyltransferase Activity of P300 in Nucleotide Excision Repair.” *Nucleic Acids Research* 36 (5): 1713–22. https://doi.org/10.1093/nar/gkn014.

Chai, Yaohui, Sarah Shoesmith Berke, Robert E. Cohen, and Henry L. Paulson. 2004. “Poly-Ubiquitin Binding by the Polyglutamine Disease Protein Ataxin-3 Links Its Normal Function to Protein Surveillance Pathways.” *Journal of Biological Chemistry* 279 (5): 3605–11. https://doi.org/10.1074/jbc.M310939200.

Chakraborty, Anirban, Nisha Tapryal, Tatiana Venkova, Nobuo Horikoshi, Raj K. Pandita, Altaf H. Sarker, Partha S. Sarkar, Tej K. Pandita, and Tapas K. Hazra. 2016. “Classical Non-Homologous End-Joining Pathway Utilizes Nascent RNA for Error-Free Double-Strand Break Repair of Transcribed Genes.” *Nature Communications* 7 (October). https://doi.org/10.1038/ncomms13049.

Chakraborty, Anirban, Maki Wakamiya, Tatiana Venkova-Canova, Raj K. Pandita, Leopoldo Aguilera-Aguirre, Altaf H. Sarker, Dharmendra Kumar Singh, et al. 2015. “Neil2-Null Mice Accumulate Oxidized DNA Bases in the Transcriptionally Active Sequences of the Genome and Are Susceptible to Innate Inflammation.” *Journal of*

Biological Chemistry 290 (41): 24636–48. https://doi.org/10.1074/jbc.M115.658146.

Chang, Howard H.Y., Go Watanabe, and Michael R. Lieber. 2015. "Unifying the DNA End-Processing Roles of the Artemis Nuclease: Ku-Dependentartemis Resection Atbluntdnaends." *Journal of Biological Chemistry* 290 (40): 24036–50. https://doi.org/10.1074/jbc.M115.680900.

Chapman, J Ross, Martin R G Taylor, and Simon J Boulton. 2012. "Playing the End Game: DNA Double-Strand Break Repair Pathway Choice." *Molecular Cell* 47 (4): 497–510. https://doi.org/10.1016/j.molcel.2012.07.029.

Chatterjee, Arpita, Saikat Saha, Anirban Chakraborty, Anabela Silva-Fernandes, Santi M. Mandal, Andreia Neves-Carvalho, Yongping Liu, et al. 2015. "The Role of the Mammalian DNA End-Processing Enzyme Polynucleotide Kinase 3'-Phosphatase in Spinocerebellar Ataxia Type 3 Pathogenesis." *PLoS Genetics* 11 (1). https://doi.org/10.1371/journal.pgen.1004749.

Chechlacz, Magdalena, Mohan C. Vemuri, and Janice R. Naegele. 2001. "Role of DNA-Dependent Protein Kinase in Neuronal Survival." *Journal of Neurochemistry* 78 (1): 141–54. https://doi.org/10.1046/j.1471-4159.2001.00380.x.

Chen, Songming, Valerie Berthelier, J. Bradley Hamilton, Brian O'Nuallain, and Ronald Wetzel. 2002. "Amyloid-like Features of Polyglutamine Aggregates and Their Assembly Kinetics." *Biochemistry* 41 (23): 7391–99. https://doi.org/10.1021/bi011772q.

Chipuk, Jerry E., Tomomi Kuwana, Lisa Bouchier-Hayes, Nathalie M. Droin, Donald D. Newmeyer, Martin Schuler, and Douglas R. Green. 2004. "Direct Activation of Bax by P53 Mediates Mitochondrial Membrane Permeabilization and Apoptosis." *Science* 303 (5660): 1010–14. https://doi.org/10.1126/science.1092734.

Cimprich, Karlene A., and David Cortez. 2008. "ATR: An Essential Regulator of Genome Integrity." *Nature Reviews Molecular Cell Biology* 9 (8): 616–27. https://doi.org/10.1038/nrm2450.

Compe, Emmanuel, and Jean-Marc Egly. 2012. "TFIIH: When Transcription Met DNA Repair." *Nature Reviews Molecular Cell Biology* 13 (6): 343–54. https://doi.org/10.1038/nrm3350.

Cong, Shu Yan, Barry A. Pepers, Bernd O. Evert, David C. Rubinsztein, Raymund A.C. Roos, Gert Jan B. van Ommen, and Josephine C. Dorsman. 2005. "Mutant Huntingtin Represses CBP, but Not P300, by Binding and Protein Degradation." *Molecular and Cellular Neuroscience* 30 (1): 12–23. https://doi.org/10.1016/j.mcn.2005.05.003.

Cong, Xin, Jason M. Held, Francesco DeGiacomo, Akilah Bonner, Jan Marie Chen, Birgit Schilling, Gregg A. Czerwieniec, Bradford W. Gibson, and Lisa M. Ellerby. 2011. "Mass Spectrometric Identification of Novel Lysine Acetylation Sites in Huntingtin." *Molecular and Cellular Proteomics* 10 (10). https://doi.org/10.1074/mcp.M111.009829.

Dai, Y., B. Kysela, L. A. Hanakahi, K. Manolis, E. Riballo, M. Stumm, T. O. Harville, S. C. West, M. A. Oettinger, and P. A. Jeggo. 2003. "Nonhomologous End Joining and V(D)J Recombination Require an Additional Factor." *Proceedings of the National Academy of Sciences of the United States of America* 100 (5): 2462–67. https://doi.org/10.1073/pnas.0437964100.

Dalby, M. A. 1969. “Effect of Tetrabenazine on Extrapyramidal Movement Disorders.” *British Medical Journal* 2 (5654): 422–23. https://doi.org/10.1136/bmj.2.5654.422.
Damiano, Maria, Laurie Galvan, Nicole Déglon, and Emmanuel Brouillet. 2010. “Mitochondria in Huntington’s Disease.” *Biochimica et Biophysica Acta - Molecular Basis of Disease*. Biochim Biophys Acta. https://doi.org/10.1016/j.bbadis.2009.07.012.
David, Sheila S., Valerie L. O’Shea, and Sucharita Kundu. 2007. “Base-Excision Repair of Oxidative DNA Damage.” *Nature*. Nature Publishing Group. https://doi.org/10.1038/nature05978.
Davies, S.W., and E. Scherzinger. 1997. “Nuclear Inclusions in Huntington’s Disease.” *Trends in Cell Biology* 7 (11): 422. https://doi.org/10.1016/s0962-8924(97)88136-6.
Deans, Andrew J., and Stephen C. West. 2011. “DNA Interstrand Crosslink Repair and Cancer.” *Nature Reviews Cancer* 11 (7): 467–80. https://doi.org/10.1038/nrc3088.
Deguire, Sean M, Francesco S Ruggeri, Mohamed-Bilal Fares, Anass Chiki, Urszula Cendrowska, Giovanni Dietler, and Hilal A Lashuel. 2018. “N-Terminal Huntingtin (Htt) Phosphorylation Is a Molecular Switch Regulating Htt Aggregation, Helical Conformation, Internalization, and Nuclear Targeting.” https://doi.org/10.1074/jbc.RA118.004621.
Delgado, Justine L., Chao Ming Hsieh, Nei Li Chan, and Hiroshi Hiasa. 2018. “Topoisomerases as Anticancer Targets.” *Biochemical Journal*. Portland Press Ltd. https://doi.org/10.1042/BCJ20160583.
Desmond, Randy Singh Atwal, Jianrun Xia, and Ray Truant. 2012. “Identification of a Karyopherin B1/B2 Proline-Tyrosine Nuclear Localization Signal in Huntingtin Protein.” *Journal of Biological Chemistry* 287 (47): 39626–33. https://doi.org/10.1074/jbc.M112.412379.
Desmond, Carly R, Tamara Maiuri, and Ray Truant. 2013. “A Multifunctional, Multi-Pathway Intracellular Localization Signal in Huntingtin.” *Communicative & Integrative Biology* 6 (2): e23318. https://doi.org/10.4161/cib.23318.
DiFiglia, Marian, Ellen Sapp, Kathryn O. Chase, Stephen W. Davies, Gillian P. Bates, J. P. Vonsattel, and Neil Aronin. 1997. “Aggregation of Huntingtin in Neuronal Intranuclear Inclusions and Dystrophic Neurites in Brain.” *Science* 277 (5334): 1990–93. https://doi.org/10.1126/science.277.5334.1990.
DiFiglia, Marian, Ellen Sapp, Kathryn Chase, Cordula Schwarz, Alison Meloni, Christine Young, Eileen Martin, et al. 1995. “Huntingtin Is a Cytoplasmic Protein Associated with Vesicles in Human and Rat Brain Neurons.” *Neuron* 14 (5): 1075–81. https://doi.org/10.1016/0896-6273(95)90346-1.
Dumitrache, Lavinia C, and Peter J McKinnon. 2017. “Polynucleotide Kinase-Phosphatase (PNKP) Mutations and Neurologic Disease.” *Mechanisms of Ageing and Development* 161 (Pt A): 121–29. https://doi.org/10.1016/j.mad.2016.04.009.
Dunah, Anthone W., Hyunkyung Jeong, April Griffin, Yong Man Kim, David G. Standaert, Steven M. Hersch, M. Maral Mouradian, Anne B. Young, Naoko Tanese, and Dimitri Krainc. 2002. “Sp1 and TAFII130 Transcriptional Activity Disrupted in Early Huntington’s Disease.” *Science* 296 (5576): 2238–43. https://doi.org/10.1126/science.1072613.

Eisen, J A, K S Sweder, and P C Hanawalt. 1995. “Evolution of the SNF2 Family of Proteins: Subfamilies with Distinct Sequences and Functions.” *Nucleic Acids Research* 23 (14): 2715–23. http://www.ncbi.nlm.nih.gov/pubmed/7651832.

Ekstrand, Mats I., Maria Falkenberg, Anja Rantanen, Chan Bae Park, Martina Gaspari, Kjell Hultenby, Pierre Rustin, Claes M. Gustafsson, and Nils Göran Larsson. 2004. “Mitochondrial Transcription Factor A Regulates MtDNA Copy Number in Mammals.” *Human Molecular Genetics* 13 (9): 935–44. https://doi.org/10.1093/hmg/ddh109.

Enokido, Yasushi, Takuya Tamura, Hikaru Ito, Anup Arumughan, Akihiko Komuro, Hiroki Shiwaku, Masaki Sone, et al. 2010. “Mutant Huntingtin Impairs Ku70-Mediated DNA Repair.” *Journal of Cell Biology* 189 (3): 425–43. https://doi.org/10.1083/jcb.200905138.

Falkenberg, Maria, Martina Gaspari, Anja Rantanen, Aleksandra Trifunovic, Nils Göran Larsson, and Claes M. Gustafsson. 2002. “Mitochondrial Transcription Factors B1 and B2 Activate Transcription of Human MtDNA.” *Nature Genetics* 31 (3): 289–94. https://doi.org/10.1038/ng909.

Ferrer, I., E. Goutan, C. Marín, M. J. Rey, and T. Ribalta. 2000. “Brain-Derived Neurotrophic Factor in Huntington Disease.” *Brain Research* 866 (1–2): 257–61. https://doi.org/10.1016/S0006-8993(00)02237-X.

Fousteri, Maria, Wim Vermeulen, Albert A van Zeeland, and Leon H F Mullenders. 2006. “Cockayne Syndrome A and B Proteins Differentially Regulate Recruitment of Chromatin Remodeling and Repair Factors to Stalled RNA Polymerase II in Vivo.” *Molecular Cell* 23 (4): 471–82. https://doi.org/10.1016/j.molcel.2006.06.029.

Fusco, Francesca R., Quan Chen, William J. Lamoreaux, Griselle Figueredo-Cardenas, Yun Jiao, Jonathan A. Coffman, D. James Surmeier, Marcia G. Honig, Leon R. Carlock, and Anton Reiner. 1999. “Cellular Localization of Huntingtin in Striatal and Cortical Neurons in Rats: Lack of Correlation with Neuronal Vulnerability in Huntington’s Disease.” *Journal of Neuroscience* 19 (4): 1189–1202. https://doi.org/10.1523/jneurosci.19-04-01189.1999.

Gao, Rui, Anirban Chakraborty, Charlene Geater, Subrata Pradhan, Kara L. Gordon, Jeffrey Snowden, Subo Yuan, et al. 2019. “Mutant Huntingtin Impairs PNKP and ATXN3, Disrupting DNA Repair and Transcription.” *ELife* 8 (April). https://doi.org/10.7554/eLife.42988.

Gao, Rui, Yongping Liu, Anabela Silva-Fernandes, Xiang Fang, Adriana Paulucci-Holthauzen, Arpita Chatterjee, Hang L Zhang, et al. 2015. “Inactivation of PNKP by Mutant ATXN3 Triggers Apoptosis by Activating the DNA Damage-Response Pathway in SCA3.” *PLoS Genetics* 11 (1): e1004834. https://doi.org/10.1371/journal.pgen.1004834.

Gao, Yijie, Yi Sun, Karen M. Frank, Pieter Dikkes, Yuko Fujiwara, Katherine J. Seidl, Jo Ann M. Sekiguchi, et al. 1998. “A Critical Role for DNA End-Joining Proteins in Both Lymphogenesis and Neurogenesis.” *Cell* 95 (7): 891–902. https://doi.org/10.1016/S0092-8674(00)81714-6.

Gatz, Susanne A., Limei Ju, Ralph Gruber, Eva Hoffmann, Antony M. Carr, Zhao Qi Wang, Cong Liu, and Penny A. Jeggo. 2011. “Requirement for DNA Ligase IV during Embryonic Neuronal Development.” *Journal of Neuroscience* 31 (27): 10088–100. https://doi.org/10.1523/JNEUROSCI.1324-11.2011.

Gauthier, Laurent R., Bénédicte C. Charrin, Maria Borrell-Pagès, Jim P. Dompierre, Hélène Rangone, Fabrice P. Cordelières, Jan de Mey, et al. 2004. "Huntingtin Controls Neurotrophic Support and Survival of Neurons by Enhancing BDNF Vesicular Transport along Microtubules." *Cell* 118 (1): 127–38. https://doi.org/10.1016/j.cell.2004.06.018.

Gines, Silvia, Ihn Sik Seong, Elisa Fossale, Elena Ivanova, Flavia Trettel, James F. Gusella, Vanessa C. Wheeler, Francesca Persichetti, and Marcy E. MacDonald. 2003. "Specific Progressive CAMP Reduction Implicates Energy Deficit in Presymptomatic Huntington's Disease Knock-in Mice." *Human Molecular Genetics* 12 (5): 497–508. https://doi.org/10.1093/hmg/ddg046.

Giralt, A., M. Puigdellívol, O. Carretón, P. Paoletti, J. Valero, A. Parra-damas, C. A. Saura, J. Alberch, and S. Ginés. 2012. "Long-Term Memory Deficits in Huntington's Disease Are Associated with Reduced CBP Histone Acetylase Activity." *Human Molecular Genetics* 21 (6): 1203–16. https://doi.org/10.1093/hmg/ddr552.

Giuliano, Paola, Tiziana de Cristofaro, Adelina Affaitati, Grazia M. Pizzulo, Antonio Feliciello, Chiara Criscuolo, Giuseppe de Michele, Alessandro Filla, Enrico v. Avvedimento, and Stelio Varrone. 2003. "DNA Damage Induced by Polyglutamine-Expanded Proteins." *Human Molecular Genetics*. Hum Mol Genet. https://doi.org/10.1093/hmg/ddg242.

Godin, Juliette D., Kelly Colombo, Maria Molina-Calavita, Guy Keryer, Diana Zala, Béé Edicte C. Charrin, Paula Dietrich, et al. 2010. "Huntingtin Is Required for Mitotic Spindle Orientation and Mammalian Neurogenesis." *Neuron* 67 (3): 392–406. https://doi.org/10.1016/j.neuron.2010.06.027.

Goodarzi, Aaron A., Yaping Yu, Enriqueta Riballo, Pauline Douglas, Sarah A. Walker, Ruiqiong Ye, Christine Härer, et al. 2006. "DNA-PK Autophosphorylation Facilitates Artemis Endonuclease Activity." *EMBO Journal* 25 (16): 3880–89. https://doi.org/10.1038/sj.emboj.7601255.

Goold, Robert, Michael Flower, Davina Hensman Moss, Chris Medway, Alison Wood-Kaczmar, Ralph Andre, Pamela Farshim, et al. 2019. "FAN1 Modifies Huntington's Disease Progression by Stabilizing the Expanded HTT CAG Repeat." *Human Molecular Genetics* 28 (4). https://doi.org/10.1093/hmg/ddy375.

Graham, Rona K., Yu Deng, Elizabeth J. Slow, Brendan Haigh, Nagat Bissada, Ge Lu, Jacqueline Pearson, et al. 2006. "Cleavage at the Caspase-6 Site Is Required for Neuronal Dysfunction and Degeneration Due to Mutant Huntingtin." *Cell* 125 (6): 1179–91. https://doi.org/10.1016/j.cell.2006.04.026.

Gu, Jiafeng, Sicong Li, Xiaoshan Zhang, Ling Chi Wang, Doris Niewolik, Klaus Schwarz, Randy J. Legerski, Ebrahim Zandi, and Michael R. Lieber. 2010. "DNA-PKcs Regulates a Single-Stranded DNA Endonuclease Activity of Artemis." *DNA Repair* 9 (4): 429–37. https://doi.org/10.1016/j.dnarep.2010.01.001.

Gu, M., M. T. Gash, V. M. Mann, F. Javoy-Agid, J. M. Cooper, and A. H.V. Schapira. 1996. "Mitochondrial Defect in Huntington's Disease Caudate Nucleus." *Annals of Neurology* 39 (3): 385–89. https://doi.org/10.1002/ana.410390317.

Gu, Xiaofeng, Jeffrey P. Cantle, Erin R. Greiner, C. Y.Daniel Lee, Albert M. Barth, Fuying Gao, Chang Sin Park, et al. 2015. "N17 Modifies Mutant Huntingtin Nuclear

Pathogenesis and Severity of Disease in HD BAC Transgenic Mice." *Neuron*. https://doi.org/10.1016/j.neuron.2015.01.008.
Gusella, James F., Nancy S. Wexler, P. Michael Conneally, Susan L. Naylor, Mary Anne Anderson, Rudolph E. Tanzi, Paul C. Watkins, et al. 1983. "A Polymorphic DNA Marker Genetically Linked to Huntington's Disease." *Nature* 306 (5940): 234–38. https://doi.org/10.1038/306234a0.
Haibing, Jiang, Frederick C. Nucifora, Christopher A. Ross, and Donald B. DeFranco. 2003. "Cell Death Triggered by Polyglutamine-Expanded Huntingtin in a Neuronal Cell Line Is Associated with Degradation of CREB-Binding Protein." *Human Molecular Genetics*. Hum Mol Genet. https://doi.org/10.1093/hmg/ddg002.
Hanawalt, Philip C. 1994. "Transcription-Coupled Repair and Human Disease." *Science*. Science. https://doi.org/10.1126/science.7801121.
Hanawalt, Philip C., and Graciela Spivak. 2008. "Transcription-Coupled DNA Repair: Two Decades of Progress and Surprises." *Nature Reviews Molecular Cell Biology*. Nat Rev Mol Cell Biol. https://doi.org/10.1038/nrm2549.
Hanssen-Bauer, Audun, Karin Solvang-Garten, Ottar Sundheim, Javier Peña-Diaz, Sonja Andersen, Geir Slupphaug, Hans E. Krokan, David M. Wilson, Mansour Akbari, and Marit Otterlei. 2011. "XRCC1 Coordinates Disparate Responses and Multiprotein Repair Complexes Depending on the Nature and Context of the DNA Damage." *Environmental and Molecular Mutagenesis* 52 (8): 623–35. https://doi.org/10.1002/em.20663.
Hermel, E., J. Gafni, S. S. Propp, B. R. Leavitt, C. L. Wellington, J. E. Young, A. S. Hackam, et al. 2004. "Specific Caspase Interactions and Amplification Are Involved in Selective Neuronal Vulnerability in Huntington's Disease." *Cell Death and Differentiation* 11 (4): 424–38. https://doi.org/10.1038/sj.cdd.4401358.
Hoffner, Guylaine, Pascal Kahlem, and Philippe Djian. 2002. "Perinuclear Localization of Huntingtin as a Consequence of Its Binding to Microtubules through an Interaction with β-Tubulin: Relevance to Huntington's Disease." *Journal of Cell Science* 115 (5): 941–48.
Horton, T. M., B. H. Graham, M. Corral-Debrinski, J. M. Shoffner, A. E. Kaufman, M. F. Beal, and D. C. Wallace. 1995. "Marked Increase in Mitochondrial Dna Deletion Levels in the Cerebral Cortex of Huntington's Disease Patients." *Neurology* 45 (10): 1879–83. https://doi.org/10.1212/WNL.45.10.1879.
Huang, C C, P W Faber, F Persichetti, V Mittal, J P Vonsattel, M E MacDonald, and J F Gusella. 1998. "Amyloid Formation by Mutant Huntingtin: Threshold, Progressivity and Recruitment of Normal Polyglutamine Proteins." *Somatic Cell and Molecular Genetics* 24 (4): 217–33. http://www.ncbi.nlm.nih.gov/pubmed/10410676.
Igarashi, Shuichi, Hokuto Morita, Kyla M. Bennett, Yuji Tanaka, Simone Engelender, Matthew F. Peters, Jillian K. Cooper, Jonathan D. Wood, Akira Sawa, and Christopher A. Ross. 2003. "Inducible PC12 Cell Model of Huntington's Disease Shows Toxicity and Decreased Histone Acetylation." *NeuroReport* 14 (4): 565–68. https://doi.org/10.1097/00001756-200303240-00007.
Illuzzi, Jennifer, Sarah Yerkes, Hetal Parekh-Olmedo, and Eric B. Kmiec. 2009. "DNA Breakage and Induction of DNA Damage Response Proteins Precede the Appearance of Visible Mutant Huntingtin Aggregates." *Journal of Neuroscience Research* 87 (3): 733–47. https://doi.org/10.1002/jnr.21881.

Ismailoglu, Ismail, Qiuying Chen, Melissa Popowski, Lili Yang, Steven S. Gross, and Ali H. Brivanlou. 2014. "Huntingtin Protein Is Essential for Mitochondrial Metabolism, Bioenergetics and Structure in Murine Embryonic Stem Cells." *Developmental Biology* 391 (2): 230–40. https://doi.org/10.1016/j.ydbio.2014.04.005.

Iyama, Teruaki, and David M. Wilson. 2013. "DNA Repair Mechanisms in Dividing and Non-Dividing Cells." *DNA Repair* 12 (8): 620–36. https://doi.org/10.1016/j.dnarep.2013.04.015.

Jimenez-Sanchez, Maria, Floriana Licitra, Benjamin R. Underwood, and David C. Rubinsztein. 2017. "Huntington's Disease: Mechanisms of Pathogenesis and Therapeutic Strategies." *Cold Spring Harbor Perspectives in Medicine* 7 (7): 1–22. https://doi.org/10.1101/cshperspect.a024240.

Jiricny, Josef. 2006. "The Multifaceted Mismatch-Repair System." *Nature Reviews. Molecular Cell Biology* 7 (5): 335–46. https://doi.org/10.1038/nrm1907.

Johri, Ashu, Abhishek Chandra, and M. Flint Beal. 2013. "PGC-1α, Mitochondrial Dysfunction, and Huntington's Disease." *Free Radical Biology and Medicine*. Elsevier Inc. https://doi.org/10.1016/j.freeradbiomed.2013.04.016.

Jones, M. B., and C. R. Phillips. 1970. "Affected Parent and Age of Onset in Huntington's Chorea." *Journal of Medical Genetics* 7 (1): 20–21. https://doi.org/10.1136/jmg.7.1.20.

Juenemann, Katrin, Sabine Schipper-Krom, Anne Wiemhoefer, Alexander Kloss, Alicia Sanz Sanz, and Eric A.J. Reits. 2013. "Expanded Polyglutamine-Containing N-Terminal Huntingtin Fragments Are Entirely Degraded by Mammalian Proteasomes." *Journal of Biological Chemistry* 288 (38): 27068–84. https://doi.org/10.1074/jbc.M113.486076.

Kalasova, Ilona, Richard Hailstone, Janin Bublitz, Jovel Bogantes, Winfried Hofmann, Alejandro Leal, Hana Hanzlikova, and Keith W. Caldecott. 2020. "Pathological Mutations in PNKP Trigger Defects in DNA Single-Strand Break Repair but Not DNA Double-Strand Break Repair." *Nucleic Acids Research* 48 (12): 6672–84. https://doi.org/10.1093/nar/gkaa489.

Karimi-Busheri, Feridoun, Graham Daly, Peter Robins, Benito Canas, Darryl J.C. Pappin, John Sgouros, Gerald G. Miller, et al. 1999. "Molecular Characterization of a Human DNA Kinase." *Journal of Biological Chemistry* 274 (34): 24187–94. https://doi.org/10.1074/jbc.274.34.24187.

Kegel, Kimberly B., Alison R. Meloni, Yong Yi, Yun J. Kim, Erin Doyle, Benjamin G. Cuiffo, Ellen Sapp, et al. 2002. "Huntingtin Is Present in the Nucleus, Interacts with the Transcriptional Corepressor C-Terminal Binding Protein, and Represses Transcription." *Journal of Biological Chemistry* 277 (9): 7466–76. https://doi.org/10.1074/jbc.M103946200.

Kettenberger, Hubert, Karim-Jean Armache, and Patrick Cramer. 2003. "Architecture of the RNA Polymerase II-TFIIS Complex and Implications for MRNA Cleavage." *Cell* 114 (3): 347–57. http://www.ncbi.nlm.nih.gov/pubmed/12914699.

Khoshnan, Ali, Jan Ko, Erin E. Watkin, Lisa A. Paige, Peter H. Reinhart, and Paul H. Patterson. 2004. "Activation of the IκB Kinase Complex and Nuclear Factor-KB Contributes to Mutant Huntingtin Neurotoxicity." *Journal of Neuroscience* 24 (37): 7999–8008. https://doi.org/10.1523/JNEUROSCI.2675-04.2004.

Kim, Jinho, Jennifer P. Moody, Christina K. Edgerly, Olivia L. Bordiuk, Kerry Cormier, Karen Smith, M. Flint Beal, and Robert J. Ferrante. 2010. “Mitochondrial Loss, Dysfunction and Altered Dynamics in Huntington’s Disease.” *Human Molecular Genetics* 19 (20): 3919–35. https://doi.org/10.1093/hmg/ddq306.

Kim, Manho, H. S. Lee, Genevieve LaForet, Charmian McIntyre, Eileen J. Martin, Patrick Chang, Tae Wan Kim, et al. 1999. “Mutant Huntingtin Expression in Clonal Striatal Cells: Dissociation of Inclusion Formation and Neuronal Survival by Caspase Inhibition.” *Journal of Neuroscience* 19 (3): 964–73. https://doi.org/10.1523/jneurosci.19-03-00964.1999.

Kim, Manho, Jae Kyu Roh, Byung Woo Yoon, Lami Kang, Yun J. Kim, Neil Aronin, and Marian DiFiglia. 2003. “Huntingtin Is Degraded to Small Fragments by Calpain after Ischemic Injury.” *Experimental Neurology* 183 (1): 109–15. https://doi.org/10.1016/S0014-4886(03)00132-8.

Kim, Mee Whi, Yogarany Chelliah, Sang Woo Kim, Zbyszek Otwinowski, and Ilya Bezprozvanny. 2009. “Secondary Structure of Huntingtin Amino-Terminal Region.” *Structure* 17 (9): 1205–12. https://doi.org/10.1016/j.str.2009.08.002.

Kim, Yun J., Yong Yi, Ellen Sapp, Yumei Wang, Ben Cuiffo, Kimberly B. Kegel, Zheng Hong Qin, Neil Aronin, and Marian DiFiglia. 2001. “Caspase 3-Cleaved N-Terminal Fragments of Wild-Type and Mutant Huntingtin Are Present in Normal and Huntington’s Disease Brains, Associate with Membranes, and Undergo Calpaindependent Proteolysis.” *Proceedings of the National Academy of Sciences of the United States of America* 98 (22): 12784–89. https://doi.org/10.1073/pnas.221451398.

Krokan, Hans E., and Magnar Bjørås. 2013. “Base Excision Repair.” *Cold Spring Harbor Perspectives in Biology* 5 (4): 1–22. https://doi.org/10.1101/cshperspect.a012583.

Kuhl, David E., E. Jeffrey Metter, Walter H. Riege, and Charles H. Markham. 1984. “Patterns of Cerebral Glucose Utilization in Parkinson’s Disease and Huntington’s Disease.” *Annals of Neurology* 15 (1 S): 119–25. https://doi.org/10.1002/ana.410150723.

Kumar, Amit, Manisha Vaish, and Rajiv R. Ratan. 2014. “Transcriptional Dysregulation in Huntington’s Disease: A Failure of Adaptive Transcriptional Homeostasis.” *Drug Discovery Today*. Elsevier Ltd. https://doi.org/10.1016/j.drudis.2014.03.016.

Kuwert, Torsten, Herwig W. Lange, Karl Josef Langen, Hans Herzog, Albrecht Aulich, and Ludwig Emil Feinendegen. 1990. “Cortical and Subcortical Glucose Consumption Measured by Pet in Patients with Huntington’s Disease.” *Brain* 113 (5): 1405–23. https://doi.org/10.1093/brain/113.5.1405.

Larsson, Nils Göran, Jianming Wang, Hans Wilhelmsson, Anders Oldfors, Pierre Rustin, Mark Lewandoski, Gregory S. Barsh, and David A. Clayton. 1998. “Mitochondrial Transcription Factor A Is Necessary for MtDNA Maintenance and Embryogenesis in Mice.” *Nature Genetics* 18 (3): 231–36. https://doi.org/10.1038/ng0398-231.

Leavitt, Blair R., Jeremy M. van Raamsdonk, Jacqueline Shehadeh, Herman Fernandes, Zoe Murphy, Rona K. Graham, Cheryl L. Wellington, Lynn A. Raymond, and Michael R. Hayden. 2006. “Wild-Type Huntingtin Protects Neurons from Excitotoxicity.” *Journal of Neurochemistry* 96 (4): 1121–29. https://doi.org/10.1111/j.1471-4159.2005.03605.x.

Lee, Jong Min, Vanessa C. Wheeler, Michael J. Chao, Jean Paul G. Vonsattel, Ricardo Mouro Pinto, Diane Lucente, Kawther Abu-Elneel, et al. 2015. “Identification of Genetic Factors That Modify Clinical Onset of Huntington’s Disease.” *Cell* 162 (3): 516–26. https://doi.org/10.1016/j.cell.2015.07.003.

Lee, Youngsoo, Sachin Katyal, Yang Li, Sherif F. El-Khamisy, Helen R. Russell, Keith W. Caldecott, and Peter J. McKinnon. 2009. “The Genesis of Cerebellar Interneurons and the Prevention of Neural DNA Damage Require XRCC1.” *Nature Neuroscience* 12 (8): 973–80. https://doi.org/10.1038/nn.2375.

Leenders, K. L., R. S.J. Frackowiak, N. Quinn, and C. D. Marsden. 1986. “Brain Energy Metabolism and Dopaminergic Function in Huntington’s Disease Measured in Vivo Using Positron Emission Tomography.” *Movement Disorders* 1 (1): 69–77. https://doi.org/10.1002/mds.870010110.

Li, Jia Yi, Markus Plomann, and Patrik Brundin. 2003. “Huntington’s Disease: A Synaptopathy?” *Trends in Molecular Medicine* 9 (10): 414–20. https://doi.org/10.1016/j.molmed.2003.08.006.

Li, S. H., G. Schilling, W. S. Young, X. Li, R. L. Margolis, O. C. Stine, M. v. Wagster, et al. 1993. “Huntington’s Disease Gene (IT15) Is Widely Expressed in Human and Rat Tissues.” *Neuron* 11 (5): 985–93. https://doi.org/10.1016/0896-6273(93)90127-D.

Li, Shi Hua, and Xiao Jiang Li. 1998. “Aggregation of N-Terminal Huntingtin Is Dependent on the Length of Its Glutamine Repeats.” *Human Molecular Genetics* 7 (5): 777–82. https://doi.org/10.1093/hmg/7.5.777.

Li, Wei, Louise C. Serpell, Wendy J. Carter, David C. Rubinsztein, and James A. Huntington. 2006. “Expression and Characterization of Full-Length Human Huntingtin, an Elongated HEAT Repeat Protein.” *Journal of Biological Chemistry* 281 (23): 15916–22. https://doi.org/10.1074/jbc.M511007200.

Li, Xiao Jiang, Shi Hua Li, Alan H. Sharp, Frederick C. Nucifora, Gabriele Schilling, Anthony Lanahan, Paul Worley, Solomon H. Snydert, and Christopher A. Ross. 1995. “A Huntingtin-Associated Protein Enriched in Brain with Implications for Pathology.” *Nature* 378 (6555): 398–402. https://doi.org/10.1038/378398a0.

Lieber, Michael R. 2010. “The Mechanism of Double-Strand DNA Break Repair by the Nonhomologous DNA End-Joining Pathway.” *Annual Review of Biochemistry* 79 (1): 181–211. https://doi.org/10.1146/annurev.biochem.052308.093131.

Lim, Ryan G., Lisa L. Salazar, Daniel K. Wilton, Alvin R. King, Jennifer T. Stocksdale, Delaram Sharifabad, Alice L. Lau, et al. 2017. “Developmental Alterations in Huntington’s Disease Neural Cells and Pharmacological Rescue in Cells and Mice.” *Nature Neuroscience* 20 (5): 648–60. https://doi.org/10.1038/nn.4532.

Liu, Yuan, and Samuel H. Wilson. 2012. “DNA Base Excision Repair: A Mechanism of Trinucleotide Repeat Expansion.” *Trends in Biochemical Sciences*. NIH Public Access. https://doi.org/10.1016/j.tibs.2011.12.002.

Lu, Xiao Hong, Virginia B. Mattis, Nan Wang, Ismael Al-Ramahi, Nick van den Berg, Silvina A. Fratantoni, Henry Waldvogel, et al. 2014. “Targeting ATM Ameliorates Mutant Huntingtin Toxicity in Cell and Animal Models of Huntington’s Disease.” *Science Translational Medicine* 6 (268): 268ra178. https://doi.org/10.1126/scitranslmed.3010523.

Luthi-Carter, Ruth, and Jang Ho J. Cha. 2003. “Mechanisms of Transcriptional Dysregulation in Huntington’s Disease.” *Clinical Neuroscience Research* 3 (3): 165–77. https://doi.org/10.1016/S1566-2772(03)00059-8.

Ly, Cindy v., and Patrik Verstreken. 2006. “Mitochondria at the Synapse.” *Neuroscientist*. Neuroscientist. https://doi.org/10.1177/1073858406287661.

Ma, Yunmei, Haihui Lu, Brigette Tippin, Myron F. Goodman, Noriko Shimazaki, Osamu Koiwai, Chih Lin Hsieh, Klaus Schwarz, and Michael R. Lieber. 2004. “A Biochemically Defined System for Mammalian Nonhomologous DNA End Joining.” *Molecular Cell* 16 (5): 701–13. https://doi.org/10.1016/j.molcel.2004.11.017.

Macdonald, Marcy E., Andrea Novelletto, Carol Lin, Dan Tagle, Glenn Barnes, Gillian Bates, Sherry Taylor, et al. 1992. “The Huntington’s Disease Candidate Region Exhibits Many Different Haplotypes.” *Nature Genetics* 1 (2): 99–103. https://doi.org/10.1038/ng0592-99.

Mackenzie, Kimberly D., Yoon Lim, Michael D. Duffield, Timothy Chataway, Xin Fu Zhou, and Damien J. Keating. 2017. “Huntingtin-Associated Protein-1 (HAP1) Regulates Endocytosis and Interacts with Multiple Trafficking-Related Proteins.” *Cellular Signalling* 35 (July): 176–87. https://doi.org/10.1016/j.cellsig.2017.02.023.

Mackenzie, Kimberly D., Amanda L. Lumsden, Feng Guo, Michael D. Duffield, Timothy Chataway, Yoon Lim, Xin Fu Zhou, and Damien J. Keating. 2016. “Huntingtin-Associated Protein-1 Is a Synapsin I-Binding Protein Regulating Synaptic Vesicle Exocytosis and Synapsin I Trafficking.” *Journal of Neurochemistry*, September, 710–21. https://doi.org/10.1111/jnc.13703.

Madabhushi, Ram, Fan Gao, Andreas R. Pfenning, Ling Pan, Satoko Yamakawa, Jinsoo Seo, Richard Rueda, et al. 2015. “Activity-Induced DNA Breaks Govern the Expression of Neuronal Early-Response Genes.” *Cell* 161 (7): 1592–1605. https://doi.org/10.1016/j.cell.2015.05.032.

Madabhushi, Ram, Ling Pan, and Li Huei Tsai. 2014. “DNA Damage and Its Links to Neurodegeneration.” *Neuron*. Cell Press. https://doi.org/10.1016/j.neuron.2014.06.034.

Maiuri, Tamara, Andrew J. Mocle, Claudia L. Hung, Jianrun Xia, Willeke M. C. van Roon-Mom, and Ray Truant. 2016. “Huntingtin Is a Scaffolding Protein in the ATM Oxidative DNA Damage Response Complex.” *Human Molecular Genetics* 26 (2): ddw395. https://doi.org/10.1093/hmg/ddw395.

Maiuri, Tamara, Andrew J Mocle, Claudia L Hung, Jianrun Xia, Willeke M C van Roon-Mom, and Ray Truant. 2017. “Huntingtin Is a Scaffolding Protein in the ATM Oxidative DNA Damage Response Complex.” *Human Molecular Genetics* 26 (2): 395–406. https://doi.org/10.1093/hmg/ddw395.

Mandal, Santi M., Muralidhar L. Hegde, Arpita Chatterjee, Pavana M. Hegde, Bartosz Szczesny, Dibyendu Banerjee, Istvan Boldogh, et al. 2012. “Role of Human DNA Glycosylase Nei-like 2 (NEIL2) and Single Strand Break Repair Protein Polynucleotide Kinase 3′-Phosphatase in Maintenance of Mitochondrial Genome.” *Journal of Biological Chemistry* 287 (4): 2819–29. https://doi.org/10.1074/jbc.M111.272179.

Mangiarini, Laura, Kirupa Sathasivam, Mary Seller, Barbara Cozens, Alex Harper, Colin Hetherington, Martin Lawton, et al. 1996. “Exon I of the HD Gene with an

Expanded CAG Repeat Is Sufficient to Cause a Progressive Neurological Phenotype in Transgenic Mice." *Cell* 87 (3): 493–506. https://doi.org/10.1016/S0092-8674(00)81369-0.

Mantamadiotis, Theo, Thomas Lemberger, Susanne C. Bleckmann, Heidrun Kern, Oliver Kretz, Ana Martin Villalba, François Tronche, et al. 2002. "Disruption of CREB Function in Brain Leads to Neurodegeneration." *Nature Genetics* 31 (1): 47–54. https://doi.org/10.1038/ng882.

Marcora, Edoardo, and Mary B. Kennedy. 2010. "The Huntington's Disease Mutation Impairs Huntingtin's Role in the Transport of NF-KB from the Synapse to the Nucleus." *Human Molecular Genetics* 19 (22): 4373–84. https://doi.org/10.1093/hmg/ddq358.

Martin, W. R.W., C. Clark, W. Ammann, A. J. Stoessl, W. Shtybel, and M. R. Hayden. 1992. "Cortical Glucose Metabolism in Huntington's Disease." *Neurology* 42 (1): 223–29. https://doi.org/10.1212/wnl.42.1.223.

McCampbell, Alexander, J. Paul Taylor, Addis A. Taye, Jon Robitschek, Mei Li, Jessica Walcott, Diane Merry, et al. 2000. "CREB-Binding Protein Sequestration by Expanded Polyglutamine." *Human Molecular Genetics* 9 (14): 2197–2202. https://doi.org/10.1093/hmg/9.14.2197.

McGowan, D. P., W. van Roon-Mom, H. Holloway, G. P. Bates, L. Mangiarini, G. J.S. Cooper, R. L.M. Faull, and R. G. Snell. 2000. "Amyloid-like Inclusions in Huntington's Disease." *Neuroscience* 100 (4): 677–80. https://doi.org/10.1016/S0306-4522(00)00391-2.

McGuire, John Russel, Juan Rong, Shi Hua Li, and Xiao Jiang Li. 2006. "Interaction of Huntingtin-Associated Protein-1 with Kinesin Light Chain: Implications in Intracellular Trafficking in Neurons." *Journal of Biological Chemistry* 281 (6): 3552–59. https://doi.org/10.1074/jbc.M509806200.

McKinnon, Peter J. 2009. "DNA Repair Deficiency and Neurological Disease." *Nature Reviews Neuroscience* 10 (2): 100–112. https://doi.org/10.1038/nrn2559.

McKinnon, Peter J. 2013. "Maintaining Genome Stability in the Nervous System." *Nature Neuroscience* 16 (11): 1523–29. https://doi.org/10.1038/nn.3537.

McNeil, Sandra M., Andrea Novelletto, Jayalakshmi Srinidhi, Glenn Barnes, Ira Kornbluth, Michael R. Altherr, John J. Wasmuth, James F. Gusella, Marcy E. MacDonald, and Richard H. Myers. 1997. "Reduced Penetrance of the Huntington's Disease Mutation." *Human Molecular Genetics* 6 (5): 775–79. https://doi.org/10.1093/hmg/6.5.775.

Meek, Katheryn, Van Dang, and Susan P. Lees-Miller. 2008. "Chapter 2 DNA-PK. The Means to Justify the Ends?" *Advances in Immunology*. Adv Immunol. https://doi.org/10.1016/S0065-2776(08)00602-0.

Menalled, Liliana B., Andrea E. Kudwa, Sam Miller, Jon Fitzpatrick, Judy Watson-Johnson, Nicole Keating, Melinda Ruiz, et al. 2012. "Comprehensive Behavioral and Molecular Characterization of a New Knock-In Mouse Model of Huntington's Disease: ZQ175." *PLoS ONE* 7 (12). https://doi.org/10.1371/journal.pone.0049838.

Milakovic, Tamara, and Gail V.W. Johnson. 2005. "Mitochondrial Respiration and ATP Production Are Significantly Impaired in Striatal Cells Expressing Mutant Huntingtin." *Journal of Biological Chemistry* 280 (35): 30773–82. https://doi.org/10.1074/jbc.M504749200.

Mitra, Siddhartha, Andrey S. Tsvetkov, and Steven Finkbeiner. 2009. “Protein Turnover and Inclusion Body Formation.” *Autophagy*. Taylor and Francis Inc. https://doi.org/10.4161/auto.5.7.9291.

Mosbach, Valentine, Lucie Poggi, and Guy-Franck Richard. 2019. “Trinucleotide Repeat Instability during Double-Strand Break Repair: From Mechanisms to Gene Therapy.” *Current Genetics* 65: 17–28. https://doi.org/10.1007/s00294-018-0865-1.

Nakano, Katsunori, and Karen H. Vousden. 2001. “PUMA, a Novel Proapoptotic Gene, Is Induced by P53.” *Molecular Cell* 7 (3): 683–94. https://doi.org/10.1016/S1097-2765(01)00214-3.

Neuwald, A. F., and T. Hirano. 2000. “HEAT Repeats Associated with Condensins, Cohesins, and Other Complexes Involved in Chromosome-Related Functions.” *Genome Research* 10 (10): 1445–52. https://doi.org/10.1101/gr.147400.

Nijhawan, D, N Honarpour, and X Wang. 2000. “Apoptosis in Neural Development and Disease.” *Annual Review of Neuroscience* 23 (1): 73–87. https://doi.org/10.1146/annurev.neuro.23.1.73.

Nucifora, Jr, M. Sasaki, M. F. Peters, H. Huang, J. K. Cooper, M. Yamada, H. Takahashi, et al. 2001. “Interference by Huntingtin and Atrophin-1 with CBP-Mediated Transcription Leading to Cellular Toxicity.” *Science* 291 (5512): 2423–28. https://doi.org/10.1126/science.1056784.

Oda, Eri, Rieko Ohki, Hideki Murasawa, Jiro Nemoto, Tsukasa Shibue, Toshiharu Yamashita, Takashi Tokino, Tadatsugu Taniguchi, and Nobuyuki Tanaka. 2000. “Noxa, a BH3-Only Member of the Bcl-2 Family and Candidate Mediator of P53-Induced Apoptosis.” *Science* 288 (5468): 1053–58. https://doi.org/10.1126/science.288.5468.1053.

Orr, Adam L., Shihua Li, Chuan En Wang, He Li, Jianjun Wang, Juan Rong, Xingshun Xu, Pier Giorgio Mastroberardino, J. Timothy Greenamyre, and Xiao Jiang Li. 2008. “N-Terminal Mutant Huntingtin Associates with Mitochondria and Impairs Mitochondrial Trafficking.” *Journal of Neuroscience* 28 (11): 2783–92. https://doi.org/10.1523/JNEUROSCI.0106-08.2008.

Ortega, Zaira, Miguel Díaz-Hernández, Christa J. Maynard, Félix Hernández, Nico P. Dantuma, and José J. Lucas. 2010. “Acute Polyglutamine Expression in Inducible Mouse Model Unravels Ubiquitin/Proteasome System Impairment and Permanent Recovery Attributable to Aggregate Formation.” *Journal of Neuroscience* 30 (10): 3675–88. https://doi.org/10.1523/JNEUROSCI.5673-09.2010.

Pal, Arun, Fedor Severin, Barbara Lommer, Anna Shevchenko, and Marino Zerial. 2006. “Huntingtin-HAP40 Complex Is a Novel Rab5 Effector That Regulates Early Endosome Motility and Is up-Regulated in Huntington’s Disease.” *Journal of Cell Biology* 172 (4): 605–18. https://doi.org/10.1083/jcb.200509091.

Parsons, Jason L, Svetlana v Khoronenkova, Irina I Dianova, Nicola Ternette, Benedikt M Kessler, Pran K Datta, and Grigory L Dianov. 2012. “Phosphorylation of PNKP by ATM Prevents Its Proteasomal Degradation and Enhances Resistance to Oxidative Stress.” *Nucleic Acids Research* 40 (22): 11404–15. https://doi.org/10.1093/nar/gks909.

Perutz, Max F., Tony Johnson, Masashi Suzuki, and John T. Finch. 1994. “Glutamine Repeats as Polar Zippers: Their Possible Role in Inherited Neurodegenerative

Diseases." *Proceedings of the National Academy of Sciences of the United States of America* 91 (12): 5355–58. https://doi.org/10.1073/pnas.91.12.5355.
Petersen, Maria Hvidberg, Esben Budtz-Jørgensen, Sven Asger Sørensen, Jørgen Erik Nielsen, Lena Elisabeth Hjermind, Tua Vinther-Jensen, Signe Marie Borch Nielsen, and Anne Nørremølle. 2014. "Reduction in Mitochondrial DNA Copy Number in Peripheral Leukocytes after Onset of Huntington's Disease." *Mitochondrion* 17: 14–21. https://doi.org/10.1016/j.mito.2014.05.001.
Petrasch-Parwez, Elisabeth, Huu Phuc Nguyen, Marlen Löbbecke-Schumacher, Hans Werner Habbes, Stefan Wieczorek, Olaf Riess, Karl Hermann Andres, Rolf Dermietzel, and Stephan von Hörsten. 2007. "Cellular and Subcellular Localization of Huntington Aggregates in the Brain of a Rat Transgenic for Huntington Disease." *Journal of Comparative Neurology* 501 (5): 716–30. https://doi.org/10.1002/cne.21272.
Poulton, Cathryn, Renske Oegema, Daphne Heijsman, Jeannette Hoogeboom, Rachel Schot, Hans Stroink, Michèl A. Willemsen, et al. 2013. "Progressive Cerebellar Atrophy and Polyneuropathy: Expanding the Spectrum of PNKP Mutations." *Neurogenetics* 14 (1): 43–51. https://doi.org/10.1007/s10048-012-0351-8.
Pourquier, Philippe, and Yves Pommier. 2001. "Topoisomerase I-Mediated DNA Damage." *Advances in Cancer Research*. Academic Press Inc. https://doi.org/10.1016/s0065-230x(01)80016-6.
Prakash, Aishwarya, Sylvie Doublié, and Susan S. Wallace. 2012. "The Fpg/Nei Family of DNA Glycosylases: Substrates, Structures, and Search for Damage." In *Progress in Molecular Biology and Translational Science*, 110:71–91. Elsevier B.V. https://doi.org/10.1016/B978-0-12-387665-2.00004-3.
Prasad, Rajendra, Grigory L. Dianov, Vilhelm A. Bohr, and Samuel H. Wilson. 2000. "FEN1 Stimulation of DNA Polymerase β Mediates an Excision Step in Mammalian Long Patch Base Excision Repair." *Journal of Biological Chemistry* 275 (6): 4460–66. https://doi.org/10.1074/jbc.275.6.4460.
Raichle, Marcus E, and Debra A Gusnard. 2002. "Appraising the Brain's Energy Budget." *Proceedings of the National Academy of Sciences of the United States of America* 99 (16): 10237–39. https://doi.org/10.1073/pnas.172399499.
Rass, Ulrich, Ivan Ahel, and Stephen C. West. 2007. "Defective DNA Repair and Neurodegenerative Disease." *Cell*. Cell. https://doi.org/10.1016/j.cell.2007.08.043.
Reddy, P. Hemachandra, Peizhong Mao, and Maria Manczak. 2009. "Mitochondrial Structural and Functional Dynamics in Huntington's Disease." *Brain Research Reviews*. Brain Res Rev. https://doi.org/10.1016/j.brainresrev.2009.04.001.
Ross, Christopher A, and Sarah J Tabrizi. 2011. "Huntington's Disease: From Molecular Pathogenesis to Clinical Treatment." *The Lancet Neurology* 10 (1): 83–98. https://doi.org/10.1016/S1474-4422(10)70245-3.
Rui, Yan Ning, Zhen Xu, Bindi Patel, Zhihua Chen, Dongsheng Chen, Antonio Tito, Gabriela David, et al. 2015. "Huntingtin Functions as a Scaffold for Selective Macroautophagy." *Nature Cell Biology* 17 (3): 262–75. https://doi.org/10.1038/ncb3101.
Sailaja, Badi Sri, Takumi Takizawa, and Eran Meshorer. 2012. "Chromatin Immunoprecipitation in Mouse Hippocampal Cells and Tissues." *Methods in Molecular Biology* 809: 353–64. https://doi.org/10.1007/978-1-61779-376-9_24.

Sallmyr, Annahita, Ishtiaque Rashid, Seema Khattri Bhandari, Tasmin Naila, and Alan E. Tomkinson. 2020. “Human DNA Ligases in Replication and Repair.” *DNA Repair*. Elsevier B.V. https://doi.org/10.1016/j.dnarep.2020.102908.

Santos, Janine H., Joel N. Meyer, Bhaskar S. Mandavilli, and Bennett van Houten. 2006. “Quantitative PCR-Based Measurement of Nuclear and Mitochondrial DNA Damage and Repair in Mammalian Cells.” *Methods in Molecular Biology (Clifton, N.J.)* 314: 183–99. https://doi.org/10.1385/1-59259-973-7:183.

Sathasivam, Kirupa, Andreas Neueder, Theresa A. Gipson, Christian Landles, Agnesska C. Benjamin, Marie K. Bondulich, Donna L. Smith, et al. 2013. “Aberrant Splicing of HTT Generates the Pathogenic Exon 1 Protein in Huntington Disease.” *Proceedings of the National Academy of Sciences of the United States of America* 110 (6): 2366–70. https://doi.org/10.1073/pnas.1221891110.

Saudou, Frédéric, Steven Finkbeiner, Didier Devys, and Michael E. Greenberg. 1998. “Huntingtin Acts in the Nucleus to Induce Apoptosis but Death Does Not Correlate with the Formation of Intranuclear Inclusions.” *Cell* 95 (1): 55–66. https://doi.org/10.1016/S0092-8674(00)81782-1.

Sawa, Akira. 2001. “Mechanisms for Neuronal Cell Death and Dysfunction in Huntington’s Disease: Pathological Cross-Talk between the Nucleus and the Mitochondria?” *Journal of Molecular Medicine*. J Mol Med (Berl). https://doi.org/10.1007/s001090100223.

Schapira, A. H.V. 1997. “Mitochondrial Function in Huntington’s Disease: Clues for Pathogenesis and Prospects for Treatment.” *Annals of Neurology*. Ann Neurol. https://doi.org/10.1002/ana.410410203.

Schilling, Gabriele, Mark W. Becher, Alan H. Sharp, Hyder A. Jinnah, Kui Duan, Joyce A. Kotzuk, Hilda H. Slunt, et al. 1999. “Intranuclear Inclusions and Neuritic Aggregates in Transgenic Mice Expressing a Mutant N-Terminal Fragment of Huntingtin.” *Human Molecular Genetics* 8 (3): 397–407. https://doi.org/10.1093/hmg/8.3.397.

Schipper-Krom, Sabine, Katrin Juenemann, Anne H. Jansen, Anne Wiemhoefer, Rianne van den Nieuwendijk, Donna L. Smith, Mark A. Hink, et al. 2014. “Dynamic Recruitment of Active Proteasomes into Polyglutamine Initiated Inclusion Bodies.” *FEBS Letters* 588 (1): 151–59. https://doi.org/10.1016/j.febslet.2013.11.023.

Schmitt, Ina, Marion Linden, Hassan Khazneh, Bernd O. Evert, Peter Breuer, Thomas Klockgether, and Ullrich Wuellner. 2007. “Inactivation of the Mouse Atxn3 (Ataxin-3) Gene Increases Protein Ubiquitination.” *Biochemical and Biophysical Research Communications* 362 (3): 734–39. https://doi.org/10.1016/j.bbrc.2007.08.062.

Shen, Jun, Edward C. Gilmore, Christine A. Marshall, Mary Haddadin, John J. Reynolds, Wafaa Eyaid, Adria Bodell, et al. 2010. “Mutations in PNKP Cause Microcephaly, Seizures and Defects in DNA Repair.” *Nature Genetics* 42 (3): 245–49. https://doi.org/10.1038/ng.526.

Sheng, Zu Hang, and Qian Cai. 2012. “Mitochondrial Transport in Neurons: Impact on Synaptic Homeostasis and Neurodegeneration.” *Nature Reviews Neuroscience*. Nat Rev Neurosci. https://doi.org/10.1038/nrn3156.

Shiloh, Yosef, and Yael Ziv. 2013. “The ATM Protein Kinase: Regulating the Cellular Response to Genotoxic Stress, and More.” *Nature Reviews Molecular Cell Biology* 14 (4): 197–210. https://doi.org/10.1038/nrm3546.

Shirendeb, Ulziibat P., Marcus J. Calkins, Maria Manczak, Vishwanath Anekonda, Brett Dufour, Jodi L. McBride, Peizhong Mao, and P. Hemachandra Reddy. 2012. “Mutant Huntingtin’s Interaction with Mitochondrial Protein Drp1 Impairs Mitochondrial Biogenesis and Causes Defective Axonal Transport and Synaptic Degeneration in Huntington’s Disease.” *Human Molecular Genetics* 21 (2): 406–20. https://doi.org/10.1093/hmg/ddr475.

Shirendeb, Ulziibat, Arubala P. Reddy, Maria Manczak, Marcus J. Calkins, Peizhong Mao, Danilo A. Tagle, and P. Hemachandra Reddy. 2011. “Abnormal Mitochondrial Dynamics, Mitochondrial Loss and Mutant Huntingtin Oligomers in Huntington’s Disease: Implications for Selective Neuronal Damage.” *Human Molecular Genetics* 20 (7): 1438–55. https://doi.org/10.1093/hmg/ddr024.

Shokolenko, Inna, Natalia Venediktova, Alexandra Bochkareva, Glenn I. Wilson, and Mikhail F. Alexeyev. 2009. “Oxidative Stress Induces Degradation of Mitochondrial DNA.” *Nucleic Acids Research* 37 (8): 2539–48. https://doi.org/10.1093/nar/gkp100.

Siddiqui, Almas, Sulay Rivera-Sánchez, María Del R. Castro, Karina Acevedo-Torres, Anand Rane, Carlos A. Torres-Ramos, David G. Nicholls, Julie K. Andersen, and Sylvette Ayala-Torres. 2012. “Mitochondrial DNA Damage Is Associated with Reduced Mitochondrial Bioenergetics in Huntington’s Disease.” *Free Radical Biology and Medicine* 53 (7): 1478–88. https://doi.org/10.1016/j.freeradbiomed.2012.06.008.

SJ, Tabrizi, Workman J, Hart PE, Mangiarini L, Mahal A, Bates G, Cooper JM, and Schapira AH. 2000. “Mitochondrial Dysfunction and Free Radical Damage in the Huntington R6/2 Transgenic Mouse.” *Annals of Neurology* 47 (1). https://doi.org/10.1002/1531-8249(200001)47:1<80::AID-ANA13>3.3.CO;2-B.

Smith, Ruben, Karl Bacos, Valentina Fedele, Denis Soulet, Helena A. Walz, Stefanie Obermüller, Anders Lindqvist, et al. 2009. “Mutant Huntingtin Interacts with β-Tubulin and Disrupts Vesicular Transport and Insulin Secretion.” *Human Molecular Genetics* 18 (20): 3942–54. https://doi.org/10.1093/hmg/ddp336.

Spada, Albert R.La, Elizabeth M. Wilson, Dennis B. Lubahn, A. E. Harding, and Kenneth H. Fischbeck. 1991. “Androgen Receptor Gene Mutations in X-Linked Spinal and Bulbar Muscular Atrophy.” *Nature* 352 (6330): 77–79. https://doi.org/10.1038/352077a0.

Spivak, Graciela. 2015. “Nucleotide Excision Repair in Humans.” *DNA Repair*. Elsevier B.V. https://doi.org/10.1016/j.dnarep.2015.09.003.

Steffan, Joan S., Namita Agrawal, Judit Pallos, Erica Rockabrand, Lloyd C. Trotman, Natalia Slepko, Katalin Illes, et al. 2004. “SUMO Modification of Huntingtin and Huntington’s Disease Pathology.” *Science* 304 (5667): 100–104. https://doi.org/10.1126/science.1092194.

Steffan, Joan S., Aleksey Kazantsev, Olivera Spasic-Boskovic, Marilee Greenwald, Ya Zhen Zhu, Heike Gohler, Erich E. Wanker, Gillian P. Bates, David E. Housman, and Leslie M. Thompson. 2000. “The Huntington’s Disease Protein Interacts with P53 and CREB-Binding Protein and Represses Transcription.” *Proceedings of the*

National Academy of Sciences of the United States of America 97 (12): 6763–68. https://doi.org/10.1073/pnas.100110097.

Strehlow, Anne N.T., Jun Z. Li, and Richard M. Myers. 2007. "Wild-Type Huntingtin Participates in Protein Trafficking between the Golgi and the Extracellular Space." *Human Molecular Genetics* 16 (4): 391–409. https://doi.org/10.1093/hmg/ddl467.

Suhr, Steven T., Marie Claude Senut, Julian P. Whitelegge, Kym F. Faull, Denise B. Cuizon, and Fred H. Gage. 2001. "Identities of Sequestered Proteins in Aggregates from Cells with Induced Polyglutamine Expression." *Journal of Cell Biology* 153 (2): 283–94. https://doi.org/10.1083/jcb.153.2.283.

Swami, Meera, Audrey E. Hendricks, Tammy Gillis, Tiffany Massood, Jayalakshmi Mysore, Richard H. Myers, and Vanessa C. Wheeler. 2009. "Somatic Expansion of the Huntington's Disease CAG Repeat in the Brain Is Associated with an Earlier Age of Disease Onset." *Human Molecular Genetics* 18 (16): 3039–47. https://doi.org/10.1093/hmg/ddp242.

Tabrizi, S. J., M. W.J. Cleeter, J. Xuereb, J. W. Taanman, J. M. Cooper, and A. H.V. Schapira. 1999. "Biochemical Abnormalities and Excitotoxicity in Huntington's Disease Brain." *Annals of Neurology* 45 (1): 25–32. https://doi.org/10.1002/1531-8249(199901)45:1<25::AID-ART6>3.0.CO;2-E.

Takano, Hiroki, and James F. Gusella. 2002. "The Predominantly HEAT-like Motif Structure of Huntingtin and Its Association and Coincident Nuclear Entry with Dorsal, an NF-KB/Rel/Dorsal Family Transcription Factor." *BMC Neuroscience* 3 (October). https://doi.org/10.1186/1471-2202-3-15.

Takeshita, Yukio, Ryutaro Fujinaga, Keiji Kokubu, Md Nabiul Islam, Mir Rubayet Jahan, Akie Yanai, Akira Kakizuka, and Koh Shinoda. 2011. "Interaction of Ataxin-3 with Huntingtin-Associated Protein 1 through Josephin Domain." *NeuroReport* 22 (5): 232–38. https://doi.org/10.1097/WNR.0b013e32834505f4.

Takiyama, Y., M. Nishizawa, H. Tanaka, S. Kawashima, H. Sakamoto, Y. Karube, H. Shimazaki, et al. 1993. "The Gene for Machado–Joseph Disease Maps to Human Chromosome 14q." *Nature Genetics* 4 (3): 300–304. https://doi.org/10.1038/ng0793-300.

Tanaka, Yuji, Shuichi Igarashi, Masayuki Nakamura, Juliette Gafni, Cameron Torcassi, Gabrielle Schilling, Danielle Crippen, et al. 2006. "Progressive Phenotype and Nuclear Accumulation of an Amino-Terminal Cleavage Fragment in a Transgenic Mouse Model with Inducible Expression of Full-Length Mutant Huntingtin." *Neurobiology of Disease* 21 (2): 381–91. https://doi.org/10.1016/j.nbd.2005.07.014.

Telezhkin, Vsevolod, Christian Schnell, Polina Yarova, Sun Yung, Emma Cope, Alis Hughes, Belinda A. Thompson, et al. 2016. "Forced Cell Cycle Exit and Modulation of GABAA, CREB, and GSK3β Signaling Promote Functional Maturation of Induced Pluripotent Stem Cell-Derived Neurons." *American Journal of Physiology - Cell Physiology* 310 (7): C520–41. https://doi.org/10.1152/ajpcell.00166.2015.

Tellez-Nagel, Isabel, Anne B. Johnson, and Robert D. Terry. 1974. "Studies on Brain Biopsies of Patients with Huntington's Chorea." *Journal of Neuropathology and Experimental Neurology* 33 (2): 308–32. https://doi.org/10.1097/00005072-197404000-00008.

Thompson, Leslie Michels, Charity T. Aiken, Linda S. Kaltenbach, Namita Agrawal, Katalin Illes, Ali Khoshnan, Marta Martinez-Vincente, et al. 2009. "IKK

Phosphorylates Huntingtin and Targets It for Degradation by the Proteasome and Lysosome." *Journal of Cell Biology* 187 (7): 1083–99. https://doi.org/10.1083/jcb.200909067.
Tong, James Jiayuan. 2007. "Mitochondrial Delivery Is Essential for Synaptic Potentiation." *Biological Bulletin* 212 (2): 169–75. https://doi.org/10.2307/25066594.
Trottier, Yvon, Valérie Biancalana, and Jean Louis Mandel. 1994. "Instability of CAG Repeats in Huntington's Disease: Relation to Parental Transmission and Age of Onset." *Journal of Medical Genetics* 31 (5): 377–82. https://doi.org/10.1136/jmg.31.5.377.
Trushina, Eugenia, Roy B. Dyer, John D. Badger, Daren Ure, Lars Eide, David D. Tran, Brent T. Vrieze, et al. 2004. "Mutant Huntingtin Impairs Axonal Trafficking in Mammalian Neurons In Vivo and In Vitro." *Molecular and Cellular Biology* 24 (18): 8195–8209. https://doi.org/10.1128/mcb.24.18.8195-8209.2004.
Tukamoto, Tadashi, Nobuyuki Nukina, Keiko Ide, and Ichiro Kanazawa. 1997. "Huntington's Disease Gene Product, Huntingtin, Associates with Microtubules in Vitro." *Molecular Brain Research* 51 (1–2): 8–14. https://doi.org/10.1016/S0169-328X(97)00205-2.
Velier, James, Manho Kim, Cordula Schwarz, Tae Wan Kim, Ellen Sapp, Kathryn Chase, Neil Aronin, and Marian DiFiglia. 1998. "Wild-Type and Mutant Huntingtins Function in Vesicle Trafficking in the Secretory and Endocytic Pathways." *Experimental Neurology* 152 (1): 34–40. https://doi.org/10.1006/exnr.1998.6832.
Venema, J, L H Mullenders, A T Natarajan, A A van Zeeland, and L v Mayne. 1990. "The Genetic Defect in Cockayne Syndrome Is Associated with a Defect in Repair of UV-Induced DNA Damage in Transcriptionally Active DNA." *Proceedings of the National Academy of Sciences of the United States of America* 87 (12): 4707–11. http://www.ncbi.nlm.nih.gov/pubmed/2352945.
Verkerk, Annemiske J.M.H., Maura Pieretti, James S. Sutcliffe, Ying Hui Fu, Derek P.A. Kuhl, Antonio Pizzuti, Orly Reiner, et al. 1991. "Identification of a Gene (FMR-1) Containing a CGG Repeat Coincident with a Breakpoint Cluster Region Exhibiting Length Variation in Fragile X Syndrome." *Cell* 65 (5): 905–14. https://doi.org/10.1016/0092-8674(91)90397-H.
Vermeulen, W., and M. Fousteri. 2013. "Mammalian Transcription-Coupled Excision Repair." *Cold Spring Harbor Perspectives in Biology* 5 (8): a012625–a012625. https://doi.org/10.1101/cshperspect.a012625.
VLIS, M. VEGTER-VAN der, W. S. VOLKERS, and L. N. WENT. 1976. "Ages of Death of Children with Huntington's Chorea and of Their Affected Parents." *Annals of Human Genetics* 39 (3): 329–34. https://doi.org/10.1111/j.1469-1809.1976.tb00137.x.
Vonsattel, Jean Paul G., Christian Keller, and Maria del Pilar Amaya. 2008. "Neuropathology of Huntington's Disease." *Handbook of Clinical Neurology*. Handb Clin Neurol. https://doi.org/10.1016/S0072-9752(07)01256-0.
Waelter, S., A. Boeddrich, R. Lurz, E. Scherzinger, G. Lueder, H. Lehrach, and E. E. Wanker. 2001. "Accumulation of Mutant Huntingtin Fragments in Aggresome-like Inclusion Bodies as a Result of Insufficient Protein Degradation." *Molecular Biology of the Cell* 12 (5): 1393–1407. https://doi.org/10.1091/mbc.12.5.1393.

Wallace, Susan S. 2014. “Base Excision Repair: A Critical Player in Many Games.” *DNA Repair* 19: 14–26. https://doi.org/10.1016/j.dnarep.2014.03.030.

Wang, Xuejuan, Huanyu Chu, Mengjuan Lv, Zhihui Zhang, Shuwan Qiu, Haiyan Liu, Xuetong Shen, Weiwu Wang, and Gang Cai. 2016. “ARTICLE Structure of the Intact ATM/Tel1 Kinase.” *Nature Communications* 7. https://doi.org/10.1038/ncomms11655.

Wasmuth, John J., Jeffrey Hewitt, Barbara Smith, Denis Allard, Jonathan L. Haines, Douglas Skarecky, Eric Partlow, and Michael R. Hayden. 1988. “A Highly Polymorphic Locus Very Tightly Linked to the Huntington’s Disease Gene.” *Nature* 332 (6166): 734–36. https://doi.org/10.1038/332734a0.

Weissman, L., N. C. de Souza-Pinto, T. Stevnsner, and V. A. Bohr. 2007. “DNA Repair, Mitochondria, and Neurodegeneration.” *Neuroscience*. Neuroscience. https://doi.org/10.1016/j.neuroscience.2006.08.061.

Wellington, Cheryl L., Lisa M. Ellerby, Abigail S. Hackam, Russell L. Margolis, Mark A. Trifiro, Roshni Singaraja, Krista McCutcheon, et al. 1998. “Caspase Cleavage of Gene Products Associated with Triplet Expansion Disorders Generates Truncated Fragments Containing the Polyglutamine Tract.” *Journal of Biological Chemistry* 273 (15): 9158–67. https://doi.org/10.1074/jbc.273.15.9158.

Whaley, William L., Frank Michiels, Marcy E. Macdonald, Donna Romano, Michael Zimmer, Barbara Smith, Julie Leavitt, et al. 1988. “Mapping of D4S98/S114/S113 Confines the Huntington’s Defect to a Reduced Physical Region at the Telomere of Chromosome 4.” *Nucleic Acids Research* 16 (24): 11769–80. https://doi.org/10.1093/nar/16.24.11769.

Whitehouse, C J, R M Taylor, A Thistlethwaite, H Zhang, F Karimi-Busheri, D D Lasko, M Weinfeld, and K W Caldecott. 2001. “XRCC1 Stimulates Human Polynucleotide Kinase Activity at Damaged DNA Termini and Accelerates DNA Single-Strand Break Repair.” *Cell* 104 (1): 107–17. http://www.ncbi.nlm.nih.gov/pubmed/11163244.

Wiederhold, Lee, John B. Leppard, Padmini Kedar, Feridoun Karimi-Busheri, Aghdass Rasouli-Nia, Michael Weinfeld, Alan E. Tomkinson, et al. 2004. “AP Endonuclease-Independent DNA Base Excision Repair in Human Cells.” *Molecular Cell* 15 (2): 209–20. https://doi.org/10.1016/j.molcel.2004.06.003.

Wyttenbach, Andreas, Jina Swartz, Hiroko Kita, Thomas Thykjaer, Jenny Carmichael, Jane Bradley, Rosemary Brown, et al. 2001. “Polyglutamine Expansions Cause Decreased CRE-Mediated Transcription and Early Gene Expression Changes Prior to Cell Death in an Inducible Cell Model of Hungtington’s Disease.” *Human Molecular Genetics* 10 (17): 1829–45. https://doi.org/10.1093/hmg/10.17.1829.

Xia, Jianrun, Denise H. Lee, Jillian Taylor, Mark Vandelft, and Ray Truant. 2003. “Huntingtin Contains a Highly Conserved Nuclear Export Signal.” *Human Molecular Genetics* 12 (12): 1393–1403. https://doi.org/10.1093/hmg/ddg156.

Yamaizumi, M, and T Sugano. 1994. “U.v.-Induced Nuclear Accumulation of P53 Is Evoked through DNA Damage of Actively Transcribed Genes Independent of the Cell Cycle.” *Oncogene* 9 (10): 2775–84. http://www.ncbi.nlm.nih.gov/pubmed/8084582.

Yanai, Anat, Kun Huang, Rujun Kang, Roshni R. Singaraja, Pamela Arstikaitis, Lu Gan, Paul C. Orban, et al. 2006. “Palmitoylation of Huntingtin by HIP14is Essential for

Its Trafficking and Function." *Nature Neuroscience* 9 (6): 824–31. https://doi.org/10.1038/nn1702.
Young, Anne B., John B. Penney, Simon Starosta-Rubinstein, Dorene S. Markel, Stanley Berent, Bruno Giordani, Richard Ehrenkaufer, Douglas Jewett, and Richard Hichwa. 1986. "PET Scan Investigations of Huntington's Disease: Cerebral Metabolic Correlates of Neurological Features and Functional Decline." *Annals of Neurology* 20 (3): 296–303. https://doi.org/10.1002/ana.410200305.
Zeitlin, Scott, Jeh Ping Liu, Deborah L. Chapman, Virginia E. Papaioannou, and Argiris Efstratiadis. 1995. "Increased Apoptosis and Early Embryonic Lethality in Mice Nullizygous for the Huntington's Disease Gene Homologue." *Nature Genetics* 11 (2): 155–63. https://doi.org/10.1038/ng1095-155.
Zhai, Weiguo, Hyunkyung Jeong, Libin Cui, Dimitri Krainc, and Robert Tjian. 2005a. "In Vitro Analysis of Huntingtin-Mediated Transcriptional Repression Reveals Multiple Transcription Factor Targets." *Cell* 123 (7): 1241–53. https://doi.org/10.1016/j.cell.2005.10.030.
Zuccato, C., A. Ciammola, D. Rigamonti, B. R. Leavitt, D. Goffredo, L. Conti, M. E. MacDonald, et al. 2001. "Loss of Huntingtin-Mediated BDNF Gene Transcription in Huntington's Disease." *Science* 293 (5529): 493–98. https://doi.org/10.1126/science.1059581.

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3) Kongari R, **Snowden J**, Berry JD, Young R. 2018. Localization and Regulation of the T1 Unimolecular Spanin. J Virol pii: e00380-18 dio: 10.1128/JCI.00380-18.

4) Guan J, **Snowden JD**, Cahill JL, Rasche ES, Kuty Everett GF. 2015. Complete genome sequence of *Citrobacter freundii* myophage Mordin. Genome Announc 3(5):e01203-15. doi:10.1128/genomeA.01203-15.

5) **Snowden JD**, Vega Gonzalez AE, Maroun JW, Hernandez AC, Kuty Everett GF. 2015. Complete genome sequence of *Bacillus megaterium* podophage Pascal. Genome Announc 3(1):e01429-14. doi:10.1128/genomeA.01429-14.

Abstracts

1) Mutant Huntingtin Induces DNA Damage by Inactivating DNA Repair Enzyme PNKP; **Jeffrey Snowden**, Rui Gao, Anirban Chakraborty, Charlene Geater, Subrata Pradhan, Kara L. Gordon, Subo Yuan, Audrey S. Dickey, Sanjeev Choudhary, Tetsuo Ashizawa, Lisa M. Ellerby, Albert R. La Spada, Leslie M.

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2) Mutant Huntingtin Preferentially Induces DNA Damage in the Actively Transcribing Genome by Inactivating DNA Strand Break Repair Enzyme PNKP; **Jeffrey Snowden,** Rui Gao, Anirban Chakraborty, Kara Gordon, Albert La Spada, Tapas K. Hazra, Partha S. Sarkar. UTMB 23rd Annual Forum on Aging, 2020

3) Mutant Huntingtin Preferentially Induces DNA Damage in the Actively Transcribing Genome by Inactivating DNA Strand Break Repair Enzyme PNKP. **Jeffrey Snowden**, Rui Gao, Anirban Chakraborty, Subrata Pradhan, Kara L. Gordon, Tetsuo Ashizawa, Albert R. La Spada, Tapas K. Hazra, Partha S. Sarkar. UTMB 6th Annual Cell Biology Student Symposium, 2019

4) Mutant Huntingtin Preferentially Induces DNA Damage in the Actively Transcribing Genome by Inactivating DNA Strand Break Repair Enzyme PNKP. **Jeffrey Snowden**, Rui Gao, Anirban Chakraborty, Kara L. Gordon, Tetsuo Ashizawa, Albert R. La Spada, Tapas K. Hazra, Partha S. Sarkar. Huntington Study Group 2018

5) Mutant Huntingtin impairs transcription-coupled DNA repair by impairing DNA repair enzyme PNKP; **Jeffrey Snowden**, Rui Gao, Anirban Chakraborty, Kara L. Gordon, Tetsuo Ashizawa, Albert R. La Spada, Tapas K. Hazra, Partha S. Sarkar. UTMB 5th Annual Cell Biology Student Symposium, 2018

6) Pathologically Expanded Huntingtin Impairs the Activity of Polynucleotide Kinase 5'-Phosphatase, a DNA Repair Enzyme; **Jeffrey Snowden**, Rui Gao, Anirban Chakraborty, Kara Gordon, Albert La Spada, Tapas K. Hazra, Partha S. Sarkar. UTMB 5th Annual Cell Biology Student Symposium, 2017

7) Mutant Huntingtin Impairs Transcription-Coupled DNA Repair by Inactivating DNA Strand Break Repair Enzyme PNKP; **Jeffrey Snowden**, Rui Gao, Anirban Chakraborty, Kara Gordon, Albert La Spada, Tapas K. Hazra, Partha S. Sarkar. Gordon Research Conference: CAG Repeat Expansion Disorders 2017