Mutant huntingtin impairs PNKP and ATXN3, disrupting DNA repair and transcription


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How huntingtin (HTT) triggers neurotoxicity in Huntington’s disease (HD) remains unclear. We report that HTT forms a transcription-coupled DNA repair (TCR) complex with RNA polymerase II subunit A (POLR2A), ataxin-3, the DNA repair enzyme polynucleotide-kinase-3'-phosphatase (PNKP), and cyclic AMP-response element-binding (CREB) protein (CBP). This complex senses and facilitates DNA damage repair during transcriptional elongation, but its functional integrity is impaired by mutant HTT. Abrogated PNKP activity results in persistent DNA break accumulation, preferentially in actively transcribed genes, and aberrant activation of DNA damage-response ataxia telangiectasia-mutated (ATM) signaling in HD transgenic mouse and cell models. A concomitant decrease in Ataxin-3 activity facilitates CBP ubiquitination and degradation, adversely impacting transcription and DNA repair. Increasing PNKP activity in mutant cells improves genome integrity and cell survival. These findings suggest a potential molecular mechanism of how mutant HTT activates DNA damage-response pro-degenerative pathways and impairs transcription, triggering neurotoxicity and functional decline in HD.

**Keywords:** Huntington’s disease, CAG repeats, polyglutamine, transcription-coupled DNA repair, HTT, ATXN3, PNKP, DNA damage, ATM, apoptosis

**HIGHLIGHTS**

- Huntingtin forms a transcription-coupled DNA repair complex with ATXN3 and PNKP
- Mutant huntingtin impairs PNKP activity, inducing DNA strand-break accumulation
- Persistent strand breakage activates DNA damage-response signaling
- Mutant huntingtin impairs ATXN3 activity and facilitates TCR complex degradation
INTRODUCTION

Huntington’s disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG triplet repeat expansion in exon 1 of the HTT gene that is translated into polyglutamine (polyQ) sequences in the huntingtin (HTT) protein which leads to progressive deterioration of cognitive and motor functions (The Huntington’s Disease Collaborative Research Group, 1993; Ross and Tabrizi, 2011, Vonsattel and DiFiglia, 1998). The polyQ expansion in the mHTT protein leads to progressive degeneration most overly affecting γ-aminobutyric acid (GABA)-releasing striatal neurons and glutamatergic cortical neurons, although neuronal dysfunction and tissue atrophy in other brain regions is also present (Vonsattel and DiFiglia, 1998; Ross and Tabrizi, 2011). Altered conformation of the mutant protein is reported to reduce normal function of the protein as well as facilitate aberrant protein-protein interactions or subcellular localization, leading to neurotoxicity. Among the numerous molecular interactions and signaling pathways implicated in HD pathomechanism, transcriptional dysregulation (Jimenez-Sanchez M et al, 2017; Ross and Tabrizi, 2011; Valor, 2015), mitochondrial (mt) dysfunction (Shirendeb et al., 2011; Siddiqui et al., 2012), DNA strand break accumulation, and atypical ataxia telangiectasia-mutated (ATM) pathway activation, involved in the DNA damage response (Bertoni et al., 2011; Giuliano et al., 2003; Illuzzi et al., 2009; Lu et al, 2014), have emerged as key players in HD-related neuronal dysfunction. Genetic or pharmacological ablation of ATM activity to ameliorate the consequence of aberrant ATM activation decreased neurotoxicity in HD animal models and HD induced pluripotent stem cells, respectively (Lu et al., 2014), supporting the emerging view that inappropriate and chronic DNA damage-response (DDR) pathway activation is a critical contributor to HD pathogenesis. Although, recent genome-wide association (GWA) studies and genetic data from other sources
suggest that DNA damage and repair pathways are central to the pathogenesis of HD and other
diseases associated with CAG repeat expansion (Bettencourt et al., 2016; GeM-HD Consortium,
2015), the perplexing questions that remain to be elucidated include how polyQ expansion
induces DNA strand breaks, activates the DDR pathway, and disrupts transcription. It is also
unclear whether transcriptional dysregulation and atypical ATM activation are mechanistically
interconnected. We recently reported that the wild-type (wt) form of the deubiquitinating enzyme
ataxin-3 (wtATXN3) enhances the activity of polynucleotide kinase-3'-phosphatase (PNKP), a
bifunctional DNA repair enzyme with both 3'-phosphatase and 5'-kinase activities that processes
unligatable DNA ends to maintain genome integrity and promote neuronal survival. In contrast,
mutant ATXN3 (mATXN3) abrogates PNKP activity to induce DNA strand breaks and activate
the DDR-ATM→p53 pathway, as observed in spinocerebellar ataxia 3 (SCA3; Chatterjee et al.,
2015; Gao et al., 2015). Furthermore, we recently 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).

Here our data demonstrate that wtHTT is a part of a transcription-coupled DNA repair
(TCR) complex formed by RNA polymerase II subunit A (POLR2A), basic transcription factors,
PNKP, ATXN3, DNA ligase 3 (LIG 3), cyclic AMP response element-binding (CREB) protein
(CBP, histone acetyltransferase), and this complex identifies lesions in the template DNA strand
and mediates their repair during transcriptional elongation. The polyQ expansion in mHTT
impairs PNKP and ATXN3 activities, disrupting the functional integrity of the TCR complex to
adversely impact both transcription and DNA repair. Low PNKP activity leads to persistent
accumulation of DNA lesions, predominantly in actively transcribing genes, resulting in unusual
activation of the ATM-dependent p53 signaling pathway. Increased PNKP activity in mutant
cells improved cell survival by substantially reducing DNA strand breaks and restricting ATM→p53 pathway activation. Likewise, low ATXN3 activity increases CBP ubiquitination and degradation thereby negatively influencing CREB-dependent transcription. These findings provide important mechanistic insights that could explain how mHTT may trigger neurotoxicity in HD.

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., 2005), 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 et al., 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; Li et al., 1995), while ATXN3 interacts with HAP-1 (Takeshita et al., 2011) and PNKP (Chatterjee et al., 2015; 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 and Figure 1-figure supplement 1). Similarly, IP of endogenous ATXN3 from NEs revealed these proteins in the ATXN3-IC (Figures 1C and Figure 1-figure supplement 2). Finally, IP of PNKP from NEs confirmed that they were also present in the PNKP-IC (Figures 1D and Figure 1-figure supplement 3). 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 and Figure 1-figure supplement 4). 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, Figure 1-figure supplement 5 & 6), 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 (Gao et al., 2015). The reconstitution of fluorescence in neuronal cells (Figures 1H to M) and postmortem human brain sections (Figure 1-figure supplement 7) 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 (Figure 1-figure supplement 7). 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.

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).

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.

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 (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.

**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. (2016). 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) (Figure 5-figure supplement 1), 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 (Figure 5-figure supplement 2A to C). 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% (Figure 5-figure supplement 2D to G), 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 (Figure 5-
Similar to full-length HTT, PNKP activity was decreased in SH-SY5Y cells expressing NT-mHTT with variable glutamine expansions (Figure 5-figure supplement 3C & D). Moreover, PNKP activity was >80% decreased in the STR and CTX of N171-82Q brain compared to control (Figure 5-figure supplement 3E & F). 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 et al., 2001; Oda et al., 2000). Consistently, we found chronic activation of the DDR-ATM-p53 pathway in HD neurons Figure 5-figure supplement 4A & B) and in zQ175 CTX (Figure 5-figure supplement 4C & D) 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 (Figure 5-figure supplement 4E & F).
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 (Figure 5-figure supplement 5A to C), 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 (Figure 5-figure supplement 5D & E), and PNKP overexpression reduced mHTT-mediated DDR-ATM pathway activation (Figure 5-figure supplement 5F). PC12 cells expressing the full-length mHTT encoding 148Qs (FL-mHTT-Q148) showed increased caspase-3 activity and PNKP overexpression reduced caspase-3 activation Figure 5-figure supplement 5G). Consistently, PC12 cells expressing FL-mHTT-Q148 also showed higher cell toxicity and PNKP overexpression significantly rescued cell toxicity (Figure 5-figure supplement 5H & I).

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., 2015, 2016; 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 (Gao et al., 2015; Santos et al., 2006) 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 6C & 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 (Figure 6-figure supplement 1). 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 (Figure 6-figure supplement 2). Consistently, preferential accumulation of DNA strand
breaks was observed in iPSC-derived HD primary neurons (Q50 and Q53) than controls (Q18 and Q28) (Figure 6-figure supplement 3). 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 Figure 6-figure supplement 4). 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.

**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 (Cong et al., 2005; Jiang et al., 2003), 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<sup>Q7/Q111</sup> HD transgenic mouse brain (Giralt et al., 2012). 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 (Jiang 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.
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 (Giuliano et al., 2003; Bae et al., 2005; Illuzzi et al., 2009; Bertoni et al., 2011; Lu et al., 2014). 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).

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 (Chakraborty et al., 2016). 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 (Figure S8). 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 (Burnett et al., 2003; Chai et al., 2004). ATXN3 inactivation in mice increases protein ubiquitination (Schmitt et al., 2007), so diminished ATXN3 activity could influence CREB-regulated gene expression as described in HD (Wyttenbach et al., 2001). Since disruption of CREB activity in the brain triggers neurodegeneration (Mantamadiotis et al., 2002), 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 et al., 2014; 2015; Rass et al., 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.
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-PTT-Q23 and pVC-NT-PTT-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., 2016). Neuronal maturation was performed as previously described (Telezhkin et al., 2016) on Nunc™ 6 well plates. After 3 weeks of maturation, medium was removed and cells were washed once with PBS pH 7.4, without Mg²⁺ and Ca²⁺. 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 procedures involving animals were in accordance with the National Institutes of Health Guide for the care and use of Laboratory Animals, and approved by the Institutional Animal Care and Use Committee of University of California Irivine (protocol #: AUP-18-155); and Duke University (protocol #: A225-17-09).

**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. (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 [$\alpha$-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: CTGGTGCAGTCAAGTTAGGGG
Neurod2: F: AAGCCAGTGTCTTCTGCGTGG; R: TTGGACAGCTTCTGCGTCTT
Neurog1: F: CCAGGACGAAGAGCAGGAAC; R: GGTCAGAGAGGTGGATGCC
Tubb3: F: TGAGGCGCTCCTCCTCACAAGT; R: ACCACGCTGAAGGTGTTTCAT
Eno2 γ: F: CCCAGGATGGGGATTTTGCT; R: CCTCCCCTGATCTGCTACCT
Pol b: F: TTCCACCGTGTAAGACCCAGG; R: GCCAGTAACCTCGAGTCAAGGA
Myod1: F: AGCATAGTGGAGCGCATCTC; R: TTGGGGCTGGATCTAGGACA
Myog: F: GAGGAAGTCTGTGTCGGTGG; R: CCACGATGGACGTAAGGGAG
Myh2: F: CGAGAGACGAGTGAAGGAC; R: GAATCACACAGGCGCATGAC
Myh4: F: AGCGCAGAGGAGAACTC; R: TCTCCTGTCACCTCTCAACAGA
Myh6: F: ATAAAGGGGCTGGAGCAGCT; R: TCGAACCTGGGTTGGTTCTG
Myh7: F: CCTTACTTGCTACCCCTCAGGT; R: GGCCATGTGCTCGATCTTGT
Gapdh F: ATGAGAGAGCGGGCTACT; R: TTTGCCGAGTGAGTGCAT
Chromatin immunoprecipitation (ChIP)

ChIP assays were performed using fresh brain tissue of WT mice as previously described (Chakraborty et al, 2016; Sailaja et al., 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: CTGGTGCAATCGTCAATAGGGG
Neurod2: F: CAGGCCCTCCCAAGAGACTT; R: TCGTGTTAGGGTGAAGGCGT

Neurog1: F: GCTTGCTCCAGGAAGAACCT; R: AGAGACACCGCTACTAAGCA

Tubb3: F: GTGGGGCTCTCCCCTAAAAC; R: TTGGGAGCGCACAAGTGTTCAG

Eno2 γ: F: TAGGGGTGCCTAGTCCTGTC; R: GAGTGCTGGATGTGTGGTCA

Myod1: F: ATCTGACAAGTGGGAGCTTT; R: TTAGTCTCAGGTGGCTGAG

Myog: F: GGCCACCAGCTAGAACAG; R: ATGAAGGCTGTGGACTTGGG

Myh2: F: TCAGTGAGCAGTGGGAGCTA; R: GTACAAACACGGGGACACCC

Myh4: F: AGGTGTACAACTCCGTGGGT; R: GCTCTAGCAAGACCAGTCACG

Myh6: F: TCGTGCCTGTGACAAGGAG; R: CTTTCTGGCAAGCGAGCATC

Myh7: F: ATTTGTGCCAAGGTGGGT; R: CCTGGGGTTCCCCAGAATCAC

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: CTGCAAAGGTTTTGTCCCAAGC; R: CTGGTGCACTCAGTTAGGG

**Mouse neurod2:**

Long: F: GGCAGTGGTTGGGATGGTAT; R: CTCACTCTGTGCTGTCTCTC
Short: F: CAGGCCCTCCCAAGAGACTT; R: TCGTGTAGGGTGAAGGGC

**Mouse neurog1**

Long: F: GATGAGGCCCTGAAGACGAG; R: GCCAATCTTGTCTTCTGCG
Short: F: GCTTGCTCCAGGAAGAACCT; R: AGAGACACCGCTACTAGGCA

**Mouse tubb3**

Long: F: GGTACAGGGGATGTGGTTGG; R: GAGTCTCCTGCCTGTCCCTA
Short: F: GTGGGGCTCTCCCCTAAAAC; R: TTGGGAGCGCAGTGTTAGA

**Mouse eno2 γ**
Mouse pol b
Long: F: TATCTCTCTCTCTTCCTTTA; R: GTGATGCGCCTTGGGAGGGTCTCTCT
Short: F: TATGGACCTCCATGAGGAACA; R: AACCCTGGCTAAAGACGTG

Mouse myod1
Long: F: ATAGACTTGACAGCAGCCCGAG; R: GGACCCTTCTACCTGCAATT
Short: F: ATCTGACACTGGAGTCGCTTT; R: TTAGTCTCAGCTGCTGGTTCC

Mouse myog
Long: F: ACAAGCCTTTTCCGACCTGA; R: CCATGGCCAAGGCGACTTAT
Short: F: GGCCACCAGAGCTAGAACAG; R: ATGAAGGCTGTGGACTTGGG

Mouse myh2
Long: F: ATCTCAGGAGCACCACACTTT; R: GAAAAGGGTGTGGGCAAGCAC
Short: F: TCAGTGAGCAGTGGGAGCTA; R: GTACAAACACGGGGACACCC

Mouse myh4
Long: F: GACGTGGAAGACTGTTAGGGA; R: AAGCCAGAGCTTTCAACCCCG
Short: F: AGGTGTACAACTCCGAGGA; R: GCTCTAGCAAGACCAGTCACG

Mouse myh6
Long: F: GACAAGGGGCATTGTAGCCT; R: TCTGCTACCTATTAGGGGT
Short: F: ATACGTGGGCTTGGGCTTT; R: CCTGGGGTTCCCAGAATCAC

Human TUBB3
Long: F: TGCTTCTCTAGGCTGTACCC; R: TCTGTCCCTCTTGGAGGATGT
Short: F: CCTGTCCCTTTTGGAGG; R: CGAGGTGGGCTAAAGATGGA

Human NEUROD1
Long: F: CCGCGCTTACAGCTCAGTTA; R: TGGCAGCTTGAGGAGCTTT
Short: F: TGCTTCTCTCTTTGGGAATAGT; R: TCTTTTTGGGGCGCAGCT

Human POLB
Long: F: CATGTCACCACCTGACTCTGCAC  R: CCTGGAGTAGGAAACAAAAATTGCT
Short: F: AGTGGGCTGGATGTAACCTG  R: CCAGTAGATGTGCTGCTGAGCAGA

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.

ACKNOWLEDGMENTS
This research was supported by a Mitchell Center Grant (University of Texas Medical Branch, Galveston TX) and National Institutes of Health grants RO1 NS079541-01 to P.S.S. and T.K.H. and R01 EY026089-01A1 to P.S.S.; 2R01 NS073976 grant to T.K.H., R01-NS100529 to L.M.E.; R01s AG033082 and R01 NS065874 to A.R.L, R01s NS089076 and NS090390 to L.M.T and Hereditary Disease Foundation Fellowship to C.G. We thank Dr. Jean-Paul Vonsattel at the New York Brain Bank, Columbia University for providing HD patient brain samples. We sincerely thank Drs. Harry Orr and David Konkel for their critical comments. The manuscript was edited by Dr. Lindsay Reese.

DECLARATION OF INTERESTS: The authors declare no conflict of interest
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FIGURES AND FIGURE LEGENDS

Figure 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-wtHTT-Q23) 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.

Figure 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).

**Figure 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 h 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 (FL-PNKP) 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).

Figure 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-NT-wtHTT-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-NT-wtHTT-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).

**Figure 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 & 2, differentiation replicates of Q33 iPSCs), and HD neurons (lanes 3 & 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 means ± 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 5 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 means ± SD, *p<0.001 when compared with control.

(F) NEs from the STR, CTX, and CRBL of zQ175 (n=5) and age-matched wild type control (n=5) mice were analyzed by WB to determine PNKP levels (upper panel); β-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 µg) 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 means ± 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) PNKP-dependent or -independent repair of the DNA duplexes by NEs from control and zQ175 transgenic mouse brain tissue. Data represent means ± SD, *p<0.001 for E, H, and J. Three biological replicates and three technical replicates were used in this assay.

Figure 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 5 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, Eno2γ, 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 means ± 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 means ± SD; *p<0.001. Five biological replicates each with three technical replicates were used in this assay.

**Figure 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 means ± 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, & CREB levels; β-tubulin was used as loading control.

(D) Relative CBP levels (normalized to β-actin) in WT control cells, cells expressing control RNAi or ATXN3 RNAi. Data represent means ± 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; β-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 β-tubulin. Data represent means ± 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 \textit{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).

\textbf{Figure 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 ATM-p53 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.
SUPPLEMENTARY FIGURES AND LEGENDS

Figure 1-figure supplement 1: Huntingtin is a part of the transcription-coupled DNA repair (TCR) complex.

Nuclear extracts (NEs) from human neuroblastoma SH-SY5Y cells was isolated and the endogenous HTT was immunoprecipitated (IP’d) with anti-HTT antibody. The HTT immunocomplex (HTT-IC) was analyzed by western blots (WBs) to examine the presence of various TCR proteins with respective antibodies (HAP-1, ATXN3, PNKP, POLR2A, LIG 3, CBP, and TAFII 130 (TAF4) shown in the figures. The original WBs show the protein bands and molecular weight markers.

Figure 1-figure supplement 2: ATXN3 is present in the HTT-TCR complex.

Nuclear extracts (NEs) from human neuroblastoma SH-SY5Y cells was isolated and the endogenous ataxin-3 was immunoprecipitated (IP’d) with anti-ataxin-3 antibody. The atxin-3 immunocomplex (ATXN3-IC) was analyzed by western blots (WBs) to examine the presence of various TCR proteins with respective antibodies. The original WBs show the protein bands and molecular weight markers.

Figure 1-figure supplement 3: DNA strand break repair enzyme PNKP is present in the TCR complex.

Endogenous PNKP was IP’d from NEs of SH-SY5Y cells and PNKP IC was analyzed by WBs to examine the presence of PNKP-associated TCR components. The original WBs show the protein bands and molecular weight markers.
**Figure 1-figure supplement 4:** RNA polymerase large subunit (POLR2A) is present in the HTT-TCR complex.

POLR2A was IP’d from NEs of SH-SY5Y cells and IC was analyzed by WBs to detect the POLR2A-associated TCR proteins. Presence of HAP-1, ATXN3, PNKP, POLR2A, LIG 3, CBP, and TAFII 130 (TAF4) are shown in figures. The original WBs show the protein bands and molecular weight markers.

**Figure 1-figure supplement 5:** Wild-type normal HTT is a part of the multiprotein TCR complex *in vitro.*

Nuclear extract (NEs) was isolated from PC12 cells exogenously expressing Myc-tagged full-length wild-type normal HTT encoding 23Qs (FL-HTT-Q23), and the Myc-HTT-Q23 protein was immunoprecipitated (IP’d) with anti-Myc-tag Ab, and the Myc immunocomplex (Myc-IC) was analyzed by western blots (WBs) for assessing the possible interaction of HTT with POLR2A and associated TCR proteins (ATXN3, PNKP, POLR2A, LIG 3, and CBP).

**Figure 1-figure supplement 6:** Mutant HTT is a part of the multiprotein TCR complex *in vitro.*

Nuclear extract (NEs) was isolated from PC12 cells ectopically expressing a Myc-tagged full-length mutant HTT encoding 148Qs (FL-mHTT-Q148) for assessing the possible interaction of mutant HTT with POLR2A and associated TCR proteins. Myc-FL-mHTT-Q148 was IP’d with an anti-Myc antibody, and the Myc immunocomplex was subjected to WBs with respective antibodies.
**Figure 1-figure supplement 7: 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).

**Figure 5-figure supplement 1: 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 & 4; differentiated iPSCs expressing Q18, Q28 and Q33), and HD neurons (lanes 5, 6 & 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 1 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 means ± SD,
*p<0.001 when compared with control. Three biological replicates and four technical replicates were used in measuring the phosphatase activities of PNKP.

**Figure 5-figure supplement 2: 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 means ± 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.

**Figure 5-figure supplement 3: 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 means ± 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.
Relative PNKP activities in control and N171-82Q transgenic mice STR, CTX, and CRBL. Data represents mean ± SD, \(*p<0.001\). Three biological replicates and three technical replicates were used in figure S10E and F.

**Figure 5-figure supplement 4: 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. 18s rRNA gene was used as control. Data represent mean ± SD; \(*p<0.001\) in B, D, & E.
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.

Figure 5-figure supplement 5: 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 is 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 means ± SEM; * p<0.001 compared with NT-wtHTTQ23 and # p<0.001 compared with NT-mHTT-Q97 in C, E, G & 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 hours 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), & 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 means ± SD; * p<0.001 when compared wtHTT-Q23 vs. mHTT-Q148, and #p<0.001 when compared mHTT-Q148+PNKP with mHTT-Q148.

Figure 6-figure supplement 1: 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 3 transgenic mice were pooled) and age-matched WT control (STR from 3 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 means ± SD; * p<0.001 compared with control.

(C) Genomic DNA was isolated from the CRBL of asymptomatic zQ175 transgenic (CRBL from 3 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.

Figure 6-figure supplement 2. 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)
Figure 6-figure supplement 3: 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) & 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 & ENO2γ genomic locus that is actively transcribed in HD (lanes 3 & 4) vs. control neurons (lanes 1 & 2). L = long amplicon, and S = short amplicon in C & E.

(D) Relative PCR amplification efficacy of TUBB3, POLB, & ENO2γ in control and HD neurons. Data represent means ± SD; *p<0.001.

(E) LA-qPCR analysis. Agarose gel showing PCR amplification of MYH2, MYH4 & MYH6, genomic loci that are not actively transcribed in control (lanes 1 & 2) and HD neurons (lanes 3 & 4).

(F) Relative PCR amplification efficacy of MYH2, MYH4 & 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.
Figure 6-figure supplement 4: 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 means ± 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.
Figure 1

(A) MCE NE

(B) IP: HTT Ab

(C) IP: ATXN3 Ab

(D) IP: PNKP Ab

(E) IP: POLR2A Ab

(F) IP: Myc-tag Ab

(G) IP: Myc-tag Ab

(H) IgG (mouse: mo) PNKP Ab (mouse: mo)

(I) IgG (mouse: mo) HAP-1 Ab (mouse: mo)

(J) IgG (mouse: mo) HTT Ab (mouse: mo)

(K) IgG (rabbit: Rb) ATXN3 Ab (rabbit: Rb)

(L) IgG (rabbit: Rb) PNKP Ab (rabbit: Rb)

(M) IgG (Rabbit: Rb) HTT Ab (rabbit: Rb) POLR2A mo Ab
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Cells expressing Myc-FL-HTT-Q23

- PNKP
- POLR2A
- LIG 3
- CBP
- APE1

Myc-HTT

ATXN3
**Figure 3**

**A**

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**B**

![western blot result](image)

**C**

![western blot result](image)

**D**

**Myc-FL-HTT-Q23 expressing cells**

![western blot result](image)

**E**

**Myc-FL-HTT-Q148 expressing cells**

![western blot result](image)
Normal Huntington in Normal

- Normal HTT stimulates transcription-coupled DNA repair (TCR)
- DNA strand break repair
- Transcription of specific neuronal genes
- Neuronal function and survival

Effective TCR

HD

Mutant huntingtin

- PNKP activity
- ATXN3 activity
- DNA strand break repair
- Poly-ubiquitination & degradation of CBP
- DNA strand breaks Accumulation & ATM-p53 pathway activation
- CBP-CREB-regulated gene expressions
- Neuronal dysfunction & neurodegeneration

Impaired TCR