Mass Spectrometric Identification of Novel Lysine Acetylation Sites in Huntingtin*

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Huntingtin (Htt) is a protein with a polyglutamine stretch in the N-terminus and expansion of the polyglutamine stretch causes Huntington’s disease (HD). Htt is a multiple domain protein whose function has not been well characterized. Previous reports have shown, however, that post-translational modifications of Htt such as phosphorylation and acetylation modulate mutant Htt toxicity, localization, and vesicular trafficking. Lysine acetylation of Htt is of particular importance in HD as this modification regulates disease progression and toxicity. Treatment of mouse models with histone deacetylase inhibitors ameliorates HD-like symptoms and alterations in acetylation of Htt promotes clearance of the protein. Given the importance of acetylation in HD and other diseases, we focused on the systematic identification of lysine acetylation sites in Htt23Q (1–612) in a cell culture model using mass spectrometry. Myc-tagged Htt23Q (1–612) overexpressed in the HEK 293T cell line was immunoprecipitated, separated by SDS-PAGE, digested and subjected to high performance liquid chromatography tandem MS analysis. Five lysine acetylation sites were identified, including three novel sites Lys-178, Lys-236, Lys-345 and two previously described sites Lys-9 and Lys-444. Antibodies specific to three of the Htt acetylation sites were produced and confirmed the acetylation sites in Htt. A multiple reaction monitoring MS assay was developed to compare quantitatively the Lys-178 acetylation level between wild-type Htt23Q and mutant Htt148Q (1–612). This report represents the first comprehensive mapping of lysine acetylation sites in N-terminal region of Htt. Molecular & Cellular Proteomics 10: 10.1074/mcp.M111.009829, 1–13, 2011.

Huntington’s disease (HD)1 is a hereditary neurodegenerative disorder characterized by unrestrained movements, emotional disturbances, and psychological deterioration (1). HD patients suffer neuronal degeneration in the striatum and frontal and temporal cortex (2). HD is caused by the expansion of a polyglutamine (polyQ) stretch within the huntingtin protein (Htt). Under normal conditions, Htt is a protein with a polyQ stretch containing 2–34 repeats whereas the disease form of Htt has a polyQ repeat length longer than 36–37. Mechanisms of toxicity for mutant Htt include proteolytic cleavage by proteases such as caspase to produce toxic fragments, impaired vesicular transport, and altered transcription by binding with specific transcription coactivators and transcription factors (3–13).

Previous studies show that Htt undergoes post-translational modifications (PTMs), which are associated with alterations in localization or conformation of Htt and regulate mutant Htt toxicity (14–17). For example, phosphorylation of Htt at Ser-421 by protein kinase Akt1 can protect striatal neurons against mutant Htt-induced toxicity (18). We previously completed an analysis by MS of the phosphorylation of full-length Htt identifying numerous phosphorylation sites throughout the 3144 amino acid sequence of Htt. One of the sites was the phosphorylation at Ser-536, which blocked Htt calpain proteolysis and toxicity (14, 19, 20). In addition, lack of phosphorylation at serine-1181 Ser-1181 and Ser-1201 and serine-1201 by cyclin-dependent kinase 5 (Cdk5) leads to toxicity and accelerated neuron death (21, 22). Other PTMs such as SUMOylation (23) and ubiquitination (24) occur in Htt and alter cellular toxicity and turnover.

Acetylation is a covalent reaction in which an acetyl group is introduced to the free amino group of the protein N-terminus or the e-amino group of lysine residues. Similar to phosphorylation, acetylation is reversible and highly regulated by histone acetyltransferases and histone deacetylases (HDACs) (25–28). Because lysine acetylation in proteins is involved in many biological functions including transcriptional regulation (29–31), apoptosis (32–34), energy metabolism (35), and DNA-related activities (36–44), there is increasing interest in exploring protein acetylation in vitro and in vivo. As compared with phosphorylation, lysine acetylation of substrates in mammalian systems generally have less specificity for the residues surrounding acetyllysine residues making predictions of acetylation sites less reliable (35, 45). To date the only estab-

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lished preferences are that mitochondrial substrates with histidine and tyrosine at the +1 position and sites found on protein surfaces (35, 45). Thus, the identification of acetylation sites in proteins requires analytical techniques such as MS.

Imbalance in protein acetylation is particularly relevant to a wide range of neurodegenerative diseases including HD and modulating acetylation levels represents a therapeutic target for treatment of these diseases. For example, treatment with HDAC inhibitors improves neurological performance and attenuates disease phenotypes in mouse models of polyglutamine expansion diseases including HD, amyotrophic lateral sclerosis, and Parkinson disease (46–51). The proposed mechanism for this therapeutic benefit is through correction of transcriptional dysregulation via altered histone acetylation. However, additional mechanisms such as changes in the acetylation status of the Huntington’s disease protein Htt itself or other protein targets may be relevant.

Different techniques have been applied to the discovery of lysine acetylation in proteins. Radiolabeling and immunodetection are among the most popular methods and have facilitated identification of many acetylated proteins (52–55). However, neither approach provides information on the number and exact site of acetylation. In recent years, mass spectrometry has become a powerful tool in detecting protein PTMs including acetylation (56–62). Acetylated peptides can be distinguished from their unmodified forms by a characteristic mass shift of +42.016 Da, although trimethylation of lysines has a similar mass shift of +42.047 Da. Tandem mass spectrometry provides sequence information and can locate the acetylation sites. Moreover, tandem mass spectra of acetylated peptides reveal highly specific ion fragments at m/z 143.1 and 126.1 that can be used as “reporter” ions indicating acetylated lysine residues (8, 63–65) as well as differentiating this modification from trimethylation (66). The application of mass spectrometry can also accelerate the analysis of lysine acetylation. Previous mass spectrometric analyses have in part focused on specific target proteins, such as histones (67, 68) and p53 (69). But recently lysine acetylation studies have been extended to investigate diverse cellular proteins (45, 70) and whole organism proteomes (35).

Here we report mass spectrometric analysis of acetyllysine in a disease-relevant truncated N-terminal form of Htt. Myc-tagged Htt23Q (with a polyQ length of 23) and Htt148Q (with an expanded polyQ length of 148) containing amino acids 1–612 were immunoprecipitated from mammalian cell lysates, and subjected to nano-liquid chromatography-electrospray ionization tandem MS (LC-ESI-MS/MS) analyses after in-solution digestion with several proteases. In all, five lysine acetylation sites of Htt23Q (1–612) and Htt148Q (1–612) were identified by MS/MS in this study and antibodies against these sites were generated. Further, a multiple reaction monitoring (MRM) MS method was developed to compare the Lys-178 acetylation levels between wild-type Htt23Q and mutant Htt148Q (1–612).

**EXPERIMENTAL PROCEDURES**

**Site-directed Mutagenesis—**Site-directed mutagenesis of the Htt constructs pTet-c-myc-Htt23Q and pTet-c-myc-Htt148Q (20) was performed using the QuickChange kit (Stratagene, La Jolla, CA) using the following primers: a double stop was inserted after amino acid 612, forward, 5’-GCAAGGAT-TGTTCCCTTAGAGC-TCGGAGG-GGCTTCAAG-3’, reverse, 5’-CCTGAAATGCC-TCGAGGCCCTAC-TAA-GGAAGATACCCCGG3’. PCR was performed using 15 or 25 ng of DNA, 5 μl of 10X Pfu buffer (Stratagene), 2.5 μl of dimethyl sulfoxide, 0.2 mM dNTPs (Roche Molecular Biochemicals), 125 ng each of forward and reverse primers (Integrated DNA Technologies, Coralville, IA), 3 μl of Quick Change Solution (Stratagene), and 1 μl Pfu Turbo Polymerase (Stratagene) at 95 °C for 1 min, 16 cycles at 96 °C for 50s, 55 °C for 1 min, and 65 or 68 °C for 45 min, and 68 °C for 10 min. Plasmids were DpnI (Stratagene)-treated, transformed into XL1-Blue Supercompetent cells (Stratagene), and purified using the Qiagen Plasmid Mini Kit. Mutations, CAG repeat length, and construct integrity were confirmed by DNA sequencing. Mutated constructs of pTet-c-myc-Htt23Q (1–612) and pTet-c-myc-Htt148Q (1–612) with the stop codon after amino acid 612 were used for these studies.

**Cell Culture and Transfection—**HEK 293T cells were grown in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum. Htt constructs pTet-c-myc-Htt23Q (1–612), pTet-c-myc-Htt148Q (1–612), Htt15Q (1–1212), Htt138Q (1–1212), or vector control were transfected into HEK 293T cells with Superfect (Qiagen, Valencia, CA) according to the manufacturer’s instructions (4). Serum was withdrawn from the medium 24 h before harvesting to reduce protein contamination for mass spectrometric analysis. The cells were treated with a HDAC inhibitor mixture containing 50 μM trichostatin A (from a 50 mM stock solution in ethanol, Sigma); 30 μM sodium butyrate (from a 55 mM aqueous stock solution, Sigma) and 30 μM nicotinamide (from a 1 mM aqueous solution, Sigma) for 4 h before harvesting. The HDAC treated cells were harvested 48 h after transfection.

**Immunoprecipitation and Western Blot Analysis—**Harvested cells were lysed with Mammalian Protein Extraction Reagent (M-PER, Pierce) lysis buffer with protease inhibitors (Mini Complete, Roche) and HDAC inhibitors as described above. Lysates were sonicated 5 times with a 3-s pulse and spun at 16000 × g for 20 min to remove debris. Protein concentration was determined with BCA Protein Assay Kit (Pierce). HEK 293T cell lysates with either c-myc-Htt23Q (1–612), pTet-c-myc-Htt148Q (1–612), or vector control were incubated with immobilized anti-c-Myc agarose beads (Pierce) overnight. The beads were washed with Tris-buffered saline/Tween 20 (TBST) (25 mM Tris, 0.15 mM NaCl, pH 7.2 ± 0.05% Tween-20, Pierce) and 10 mM Tris, pH 7.4, respectively. Htt was eluted with M-PER buffer containing 10 mg/ml c-Myc peptide (Anaspec), 200 mM NaCl and 0.1% Nonidet P-40 (Sigma). Immunoprecipitated samples were dialyzed in TBST and 25 mM NH4HCO3 sequentially, and then applied to a clean-up procedure using a two-dimensional clean-up kit (Amersham Biosciences). The resulting pellet was dissolved in appropriate amount of SDS-PAGE loading buffer, depending on the size of the pellet, and run on a NuPAGE 4–12% BisTris gel (Invitrogen, Carlsbad, CA) in 3-(N-morpholino)propanesulfonic acid (MOPS) running buffer (Invitrogen) for 80 min at 200V. Typically 10% of the sample was used for Western blotting and the rest of the sample was used for mass spectrometric analysis.

Custom antibodies (Open Biosystems) were produced by injecting synthesized peptides (supplemental Table S1) corresponding to the acetylated lysine sites into rabbits. The day 58 postimmunization bleeds were collected. Serum was tested for signal by Western blots. Those with positive results were purified by affinity binding using immobilized peptides (supplemental Table S1) and stored for use.

**In-gel Proteolytic Digestion—**Bands containing Htt were excised from the one-dimensional SDS-PAGE gels and subsequently digested in-gel with trypsin and trypsin-like enzymes. The resulting peptides were purified by reverse phase high performance liquid chromatography (RP-HPLC) and desalted by precolumns containing aminobisphosphonate (36). Peptides were then analyzed by MS and MS/MS as described above.
destained and dehydrated with acetonitrile. For trypsin and Asp-N digestion, proteins were reduced with 10 mM dithiothreitoll (Sigma) in 25 mM NH₄HCO₃ at 56 °C for 1 h and alkylated with 55 mM iodoacetamide (Sigma) in 25 mM NH₄HCO₃ at room temperature for 45 min. The samples were then incubated with 100–200 ng sequencing grade trypsin (Promega) or Asp-N (Roche) at 37 °C for 16–18 h. For the chymotrypsin (Roche) digestion, dithiothreitol was dissolved in a 25 mM NH₄HCO₃ solution containing 4 M guanidine hydrochloride (Sigma) in the reduction/denaturation step. After alkylation, the samples were incubated with 100–125 ng chymotrypsin at 25 °C for 16–18 h. The peptides were subjected to aqueous extraction (H₂O) and hydrophobic extraction (50% acetonitrile, 5% formic acid) and concentrated under vacuum to a 10–15 μl final volume.

**Quadrupole Time-of-Flight Mass Spectrometric Analysis of Peptides**—Mass spectra of peptides generated after proteolytic digestion of Htt were acquired by reverse-phase nano-high performance liquid chromatography (HPLC)-ESI-MS/MS using an Eksigent nano-LC two-dimensional HPLC system (Eksigent, Dublin, CA) that was directly connected to a quadrupole time-of-flight (QqTOF) QSTAR Elite mass spectrometer (AB SCIEX, Concord, Canada). Briefly, an aliquot of digested peptide mixture (~2 μl at a concentration of 1 μg/μl) was loaded onto a guard column (C18 Acclaim PepMap100, 300 μm I.D. × 5 mm, 5 μm particle size, 100 Å pore size; Dionex, Sunnyvale, CA) and eluted with the loading solvent (98% solvent A, 2% solvent B, flow rate: 20 μl/min for 5 min where solvent A consisted of 0.1% formic acid in 98% H₂O, 2% acetonitrile and solvent B consisted of 0.1% formic acid in 98% acetonitrile, 2% H₂O). Subsequently, samples were transferred onto the C18-nanocapillary HPLC column (C18 Acclaim PepMap100, 75 μm I.D. × 15 cm, 3 μm particle size, 100 Å pore size; Dionex) and eluted at a flow rate of 300 nL/min using the following gradient: 2–40% solvent B in A (from 0 to 35 min), 40–80% solvent B in A (from 35 to 45 min) and at 80% solvent B in A (from 45 to 55 min), with a total run time of 95 min (including mobile phase equilibration). In some experiments, another gradient was applied: 2–30% solvent B in A (from 0 to 30 min), 30–80% solvent B in A (from 30 to 35 min) and at 80% solvent B in A (from 35 to 39 min), with a total runtime of 60 min (including mobile phase equilibration). Mass spectra (ESI-MS) and tandem mass spectra (ESI-MS/MS) were recorded in positive-ion mode with a resolution of 12,000–15,000 full-width at half-maximum. Data acquisition was performed with an ion spray voltage of 2300 V, curtain gas of 20 psi, ion source gas of 17 psi, and an interface heater temperature of 100°C. A declustering potential of 65 and focusing potential of 275. For collision induced dissociation tandem mass spectrometry (CID-MS/MS), the mass window for precursor ion selection of the quadrupole mass analyzer was set to ±1 m/z. The precursor ions were fragmented in a collision cell using nitrogen as the collision gas. A CAD gas setting of 5 was applied. Information dependent acquisition was used for MS/MS data sets were also searched using the Mascot in-house search parameters were used: enzyme specificity was defined as trypsin with two possible missed cleavages, carbamidomethyl (Cys) was chosen as fixed modification, acetyl (Protein N-terminal), Glu->pyro-Glu (N-terminal Glu), and oxidation (Met) were chosen as variable modifications, mass tolerance for precursor ions was 50 ppm, mass tolerance for fragment ions was 0.4 Da, mass tolerance for fragment ions was 0.4 Da.

**RESULTS**

**Immunoprecipitation of Htt**—To purify Htt for MS analysis, myc-Htt23Q (1–612) and myc-Htt148Q (1–612) was overexpressed in 293T cells and immunoprecipitated in the presence of HDAC inhibitors. As shown in Fig. 1A, Western blot analysis of lysates (input before incubation with immobilized anti-c-Myc antibody) and flow through (supernatant after incubation with immobilized anti-c-Myc antibody) demonstrate depletion of Htt with c-Myc beads. Complete depletion of Htt from the
cell lysates was not observed because of the fact that the c-myc tag of Htt became acetylated under these conditions. c-Myc-tagged Htt in the lysates bound to the immobilized anti-c-Myc antibody. As shown in Fig. 1 B, Western blot analysis demonstrates that Htt23Q (1–612) and Htt148Q (1–612) elutes after immunoprecipitation from 293T cells. The eluted proteins were dialyzed and precipitated to remove excess c-Myc peptide and salt. The resulting proteins were separated by one-dimensional SDS-PAGE and visualized with Sypro Ruby protein gel stain (Fig. 1 C). Although some co-immunoprecipitated proteins have similar molecular weight as Htt, their presence did not interfere with mass spectrometry analysis.

**Identification of Htt by Mass Spectrometry**—The gel band containing Htt23Q (1–612) was excised and digested with trypsin, Asp-N, and chymotrypsin, respectively. The resulting peptides were separated and analyzed by nano-HPLC (reversed-phase C18 chromatography) coupled to a hybrid quadrupole time-of-flight mass spectrometer (QSTAR Elite). Peptide sequence information was provided by CID.

supplemental Table S5 shows a list of all Htt peptides sequenced by nano-HPLC-ESI-MS/MS.

**Acetylated Lysines Identified by Nano-HPLC-ESI-MS/MS**—The ESI-MS/MS data were searched against a customized Htt (1–612) database by Mascot to identify lysine acetylation sites. Rigorous criteria were used to identify acetyllysine containing peptides to avoid false positive assignments. This became relevant after Stevens et al. (72) described reports of other groups that incorrectly assigned post-translationally modified peptides. Our criteria for identifying acetyl lysine modifications consisted of the following: (1) pairs of unmodified peptides and acetyllysine peptides were observed that showed similar ion series, except in a few cases where the unmodified peptides was not observed because of further cleavage at the unmodified lysine by trypsin; (2) peptides containing acetyllysine showed a increased retention time shift of 2–4 min relative to the unmodified peptide because acetylated peptides are more hydrophobic and elute later (Table I); (3) mass accuracy of the molecular ion was <50 ppm.
for both the unmodified and acetyl lysine modified peptides; (4) MS/MS fragmentation spectra of the acetyl lysine containing peptides required the presence of y- or b- fragments that flanked the acetyl lysine residue (for more details see below), and finally; (5) peptides containing acetyllysine must show an acetyllysine immonium ions (m/z 126.1 and/or 143.1). These criteria ensure the accuracy of acetyllysine peptide identifications.

As shown in Table I, we identified three novel sites of lysine acetylation (Lys-178, Lys-236, and Lys-345) and two previously published sites (Lys-9 and Lys-444) (73, 74) in human Htt (1–612) from their MS/MS spectra. We found that only five of the 27 possible lysine sites in Htt23Q (1–612) were detected as acetylated in the MS/MS spectra of the Htt peptides.
was also observed (as listed in supplemental Table S5). The pair was chromatographically separated by nearly 3 min, the hydrophobic acetylated peptide eluted at 23.40 min and the unmodified peptide at 20.67 min.

The tandem mass spectrum of acetyl lysine peptide EIKKAcNGAPR (residue 175–183) generated from trypsin digestion of Htt23Q (1–612) is shown in Fig. 2B. The b-ion series and an almost complete y-ion series that flanked the acetyl lysine site was consistent with the presence of acetyl group at Lys-178. As before, the acetyl lysine immonium ion at m/z 126.1 was detected as well. However, the corresponding unmodified peptide was not observed likely because of further cleavage of one of the two internal lysine residues.

Fig. 2C shows the ESI-MS/MS tandem mass spectra of lysine-acetylated peptide AAAVPKAcIM (residue 231–238) generated after chymotrypsin digestion. As in the two previous examples, several y-ions flanked the acetyl lysine site at Lys-236 and an acetyl lysine immonium ion at m/z 126.1 was detected. In addition, the corresponding unmodified peptide AAAVPKAcIM (residue 231–238) was detected in the experiment (as listed in supplemental Table S5) and also separated by nearly 3 min, with the more hydrophobic acetylated peptide eluting at 24.3 min and the unmodified peptide at 21.1 min.

Two other previously reported lysine acetylation sites, Lys-9 and Lys-444 (73, 74), were also identified using the same approach as described above (73, 74). The tandem mass spectrum of the corresponding acetyl lysine peptides encompassing these two known sites are provided in Fig. 3A and supplemental Fig. S2. Both b- and y-ion series flanked the acetyl lysine at Lys-9 in the chymotryptic peptide EKLMKAcAF (residues 5–11). The acetyllysine-specific immonium ions at m/z 126.1 and 143 under Q2 transmission setting 1 described in supplemental Table S2.

![Fig. 3. ESI-MS/MS tandem mass spectra of the acetylated lysine peptide EKLMKAcAF (residue 5–11) obtained after Asp-N digestion of immunoprecipitated Htt23Q (1–612). A, The molecular ion [M+2H]2+ at m/z 454.74 (M = 907.47 Da) was selected for CID. K* refers to the two immonium ions for acetyllysine at m/z 126.1 and 143 under Q2 transmission setting 1 described in supplemental Table S2. B, The molecular ion [M+2H]2+ at m/z 454.74 (M = 907.47 Da) was selected for CID. K* refers to the two immonium ions for acetyllysine at m/z 126.1 and 143 under Q2 transmission setting 2 described in supplemental Table S2.](https://www.asbmb.org/molecular-cellular-proteomics/article-content/10.1074/mcp.M111.009829-6)
no +42 Da mass shift for the b_x-b_y fragments ions eliminates the possibility of acetylation at Lys-6. Last, manipulating Q2 transmission parameters when acquiring tandem mass spectra had a predictable effect on spectra quality and overall confidence in both identification of the lysine acetyl group as a PTM and the specific site of lysine acetylation.

Fig. 3 also shows the MS/MS spectra of the same peptide EKLMK_{Ac}AF obtained with the mass spectrometric Q2 transmission set to enhance low mass ions (Fig. 3A) in comparison to the Q2 transmission set to focus on medium and high mass fragment ions (Fig. 3B). Details for the Q2 transmission window settings can be found in supplemental Table S2. The peptide follows similar fragmentation patterns under both conditions. However, as shown in Fig. 3B, the most intense ions are b- and y-ion series in the medium to high mass range (above m/z 400). With the Q2 transmission parameter setting adjusted to favor the low mass ion region, more and higher abundance peaks were observed in the mass range below m/z 300 (Fig. 3A) which enhances the appearance of highly selective acetyl lysine immonium ions at m/z 126.1 and 143.1. Under these altered transmission conditions, some MS/MS spectra of acetyl lysine containing peptides contain immonium ions that are among the most intense peaks in the spectra, whereas few b- and y-ions were observed (data not shown).

Acetylation Antibodies to Lys-236, Lys-345 and Lys-444 Confirm Site of Acetylation—Our experiments allowed new modifications to be uncovered in Htt. To confirm the three novel acetyl lysine sites identified in our MS studies of Htt, site-specific polyclonal anti-acetyl lysine antibodies were generated to four (Lys-178, Lys-236, Lys-345, and Lys-444) of the identified Htt acetylation sites. The sera from rabbits to acetyl lysine antibody to the Htt Lys-178 site were of low titer. Western blot analysis for acetylation of Htt revealed that both wild-type and mutant Htt were acetylated at Lys-236, Lys-345, and Lys-444 (Fig. 4). As previously reported we found that mutant Htt has higher levels of acetylation at Lys-444 when compared with wild-type Htt (73). Interestingly, we observed that the level of acetylation of Htt varied in the wild-type and mutant Htt. Generally, the levels of acetylation appear to be higher in Htt138Q (1–1212) at Lys-236, Lys-345 and Lys-444 when compared with Htt15Q (1–1212). We used these constructs because they do not contain an artificial tag in the Htt protein. The production of acetyl lysine to Lys-9 was not pursued because this has been previously published (75).

Comparison of Lysine Acetylation Levels at Lys-178 in Htt23Q to Htt148Q—To study the progressive lysine acetylation in Htt, a mass spectrometry-based semiquantitative method was employed using peak area to evaluate the relative abundance of Lys-178 acetylation in wild-type and mutant Htt. We focused on this particular acetylation site for several reasons: (1) it is near the polyglutamine stretch and an identified protease cleavage site (near the polyglutamine stretch and a76, 77); (2) antibodies were not successfully generated to this site to allow quantification; and (3) to establish mass spectrometry methods to monitor changes in acetylation quantitatively. For this analysis, Htt23Q (1–612) and Htt148Q (1–612) were immunoprecipitated from 293T cells and lysates were probed with anti-acetyllysine-236, 345, 444 polyclonal antibodies and N-terminal Htt antibody 2166.

For this targeted lysine-containing tryptic peptide EIKK_{Ac}NGAP183R, lack of acetylation causes further cleavage by trypsin and generates shorter peptides than the acetylated peptide and, therefore, were not observed. In this case, MRM transitions to six nonmodified peptides throughout the Htt sequence were used to normalize for quantitation supplemental Fig. S5. A heavy isotope-labeled synthetic peptide corresponding to the targeted acetyl lysine-containing peptide was employed to validate the method. When spiked...
in the digested Htt samples, the synthetic peptide EIKKAcNGAP*R (where P* is 13C5 15N1-proline) elutes at the same retention time as the endogenous peptide 175EIKKAcNGAP183R. (supplemental Fig. S3). Moreover, the MS/MS spectrum of peptide 175EIKKAcNGAP183R obtained by the QqTOF mass spectrometer and the hybrid triple quadrupole/linear ion trap mass spectrometer (4000 QTRAP) show very similar fragmentation patterns (supplemental Fig. S4). A preparation of six biological replicates were purified for both Htt23Q (1–612) and Htt148Q (1–612), and these were analyzed using the semiquantitative LC-MRM-MS assay described above, targeting specifically the novel Lysine Acetylation Sites in Huntingtin.

| Biological replicates | Peak area 175EIKKAcNGAP183R 23Q | Peak area 175EIKKAcNGAP183R 148Q | Htt 23Q/148Q ratio | Fold change of acetyl K178 | Total Htt | Normalized fold change of acetyl K178 |
|-----------------------|---------------------------------|---------------------------------|---------------------|-------------------------|----------|-------------------------------------|
| 1                     | 182527                           | 62700                           | 2.9                 | 0.89                    | 3.3      | 4.8 ± 4.0/   |
| 2                     | 166802                           | 51498                           | 3.2                 | 0.89                    | 3.6      |                     |
| 3                     | 114802                           | 32163                           | 3.6                 | 0.97                    | 3.7      |                     |
| 4                     | 79483                            | 27854                           | 2.9                 | 0.96                    | 3.0      |                     |
| 5                     | 384157                           | 235079                          | 1.6                 | 0.65                    | 2.5      |                     |
| 6                     | 665288                           | 51899                           | 12.8                | 0.99                    | 12.9     | 4.8 ± 4.0/e |

a Extracted ion chromatogram peak area of MRM transition 352.2(Q1)/272.2(Q3) for the acetyl peptide 175EIKKAcNGAP183R.

b The relative Htt levels were obtained from non-K178-containing peptides. More detail is displayed in supplemental methods.

c The fold change in 23Q/148Q fold normalized of total Htt.

d Using Wilcoxon signed rank test with a two-tailed test to evaluated statistical significance we found a p* < 0.03 for Htt23Q compared to Htt148Q.

e Normalization is described in detail in the supplemental materials.

Fig. 5. Representative MRM extracted ion chromatograms (XIC) for wild type (Htt23Q) and mutant (Htt148Q) peptide. A, MRM extracted ion chromatograms (XIC) for wild type (Htt23Q) and mutant (Htt148Q) peptide 175EIKKAcNGAP183R containing Lys-178 for each transition. Three transitions were selected for our analysis as depicted by the three panels. This was used in our quantitative analysis of the ratio of Htt23Q to Htt148Q. B, MRM extracted ion chromatograms (XIC) for wild-type (Htt23Q) and mutant (Htt148Q) peptide 175EIKKAcNGAP183R containing Lys-178 for each transition in the presence of HDAC inhibitors.
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Table III

| Biological replicates | Peak area | Htt 23Q/148Q ratio |
|-----------------------|-----------|-------------------|
|                       | 23Q | 148Q   | Fold change of acetyl K178 | Total Htt | Normalized fold change of acetyl K178 |
| 1                     | 393530 | 218286 | 1.8 | 1.3 | 1.4 |
| 2                     | 164565 | 109927 | 1.5 | 1.3 | 1.1 |
| 3                     | 306265 | 138232 | 2.2 | 1.5 | 1.5 |

1.3 ± 0.18<sup>d</sup> Average ± standard deviation

- <sup>a</sup> Extracted ion chromatogram peak area of MRM transition 352.2(Q1)/272.2(Q3) for the acetyllysine peptide 175EIKK<sub>Ac</sub>NGAP<sub>183R</sub>.
- <sup>b</sup> The relative Htt levels were obtained from non-K178-containing peptides. More detail is displayed in supplemental methods.
- <sup>c</sup> The fold change in 23Q/148Q fold normalized of total Htt.
- <sup>d</sup> Using Wilcoxon signed rank test with a two-tailed test to evaluated statistical significance we found a p* < 0.1443 for Htt23Q compared to Htt148Q.
- <sup>e</sup> Normalization is described in detail in the supplemental materials.

Fig. 6. **Summary scheme of Htt PTMs.** A, Schematic of Htt structure with four HEAT repeat domains (HH) and caspase/calpain domain. Red arrows indicate lysine acetylation sites identified by MS/MS in this study. B, N-terminal fragment of mutant Htt has enhanced toxicity.

175EIKK<sub>Ac</sub>NGAP<sub>183R</sub>. Lys-178 acetylation levels from Htt23Q and Htt148Q and comparison after normalization to overall Htt protein abundance as shown in Table II.

In Fig. 5A, the representative extracted ion chromatograms (XIC) of the purified Htt23Q (1–612) and Htt148Q (1–612) are shown for three MRM transitions. Using MRM analysis, the level of acetylated lysine at Lys-178 in Htt23Q (1–612) is 4.83-fold higher than in the Htt148Q protein (Table II). Using the Wilcoxon signed rank test with a two-tailed test to evaluated statistical significance we found a p* < 0.03. We conclude the increase in acetylated Lys-178 in the wild-type Htt is statistically significant when compared with the disease form of Htt. Consistent with our hypothesis that HDAC inhibition may act through modification of Htt, we found that the levels of Htt acetylation increased when HEK293 were cultured in the presence of HDAC inhibitors (trichostatin A, sodium butyrate, nicotinamide) (Fig. 5B). During this treatment the level of acetylation of Htt23Q when compared with Htt148Q was
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normalized (Table III). Our results suggest HDAC treatment corrects altered acetylation levels between normal and the disease form of Htt.

**DISCUSSION**

As shown in Fig. 6, Htt is a very large protein with 3144 amino acids and the polyQ region is located at the N terminus. Proteolysis, localization, and clearance events for Htt play a key role in HD pathogenesis (10, 11, 20, 77). Htt is susceptible to proteolytic cleavage at Asp-513 and -552 by caspase-3/7, at Asp-586 by caspase-6 and at aspartic acid 552 by caspase-2 (Fig. 6A). The resulting N-terminal fragments are toxic (10, 78, 79). Htt is a substrate of calpains as well, cleaving at threonine Thr-469 and Ser-536 (11, 80). In addition, aspartyl endopeptidases cleave Htt within the amino acid region 104–114 (cpA) and 146–214 (cpB). The cpA fragments form nuclear aggregates (76, 77). Because the N-terminal 612 amino acids contain many of the proteolytic cleavage sites involved in the pathogenesis of HD (Fig. 6B summarizes the cascade) and contains the pathogenic polyQ stretch, we focused our study on the N-terminus and generated a construct containing the first 612 amino acids of Htt.

Acetylation has been recognized as a regulatory modification analogous to phosphorylation (27). Therefore, modification of mutant Htt acetylation is likely to control disease progression and toxicity analogous to that found for phosphorylation. Recent studies reveal that certain HDAC inhibitors have therapeutic results in HD transgenic mouse models (49, 81). In those studies, researchers focused on histone and attributed the neuroprotective effect to increased histone acetylation and resulting increased gene transcription. However, HDACs and HDAC inhibitors not only act on histones, but also on diverse range of proteins. Htt is one such candidate.

As summarized, five lysine acetylation sites were identified in our analysis. Among them, Lys-9 is in amino acids 1–17 of Htt flanking the polyQ tract (See Fig. 6). The first 17 amino acids of Htt modulate the cytoplasmic localization of Htt exon 1 protein (Httex1p), Htt aggregation and its effects on calcium homeostasis (74). Thompson et al. found acetylation of Lys-9 was detected on exogenous IKK overexpression and was regulated by phosphorylation of Ser-13 (82). Jeong et al. demonstrate that increased acetylation at Lys-444 facilitates trafficking of mutant Htt into autophagosomes and reduces the toxicity of mutant Htt (73). Post-translational modifications, including acetylation, can also effect Htt’s interaction with associated proteins. The other three novel Htt acetyllysine sites (Lys-178, Lys-236, Lys-345) identified in our study reside either close to sites of proteolysis, protein interactions domains, or in the lipid-binding domain of Htt (75, 83). Therefore, they may have an impact on the cleavage of Htt or association of Htt with lipids.

The Htt lysine acetylation sites in this study were identified in cells expressing Htt23Q. A MRM-based quantitative study was conducted comparing the acetylation of Lys-178 in the wild type and mutant Htt. Our results indicate that wild-type Htt tends to be more acetylated at Lys-178 than the disease causing form of Htt. The site of acetylation is near the polyQ tract. Jeong et al. (74) demonstrated for Lys-444 that the acetylation levels are higher in mutant Htt when compared with wild-type using Western blot analysis with an antibody directed to the acetylated Lys-444 peptide. Our Western blot probed with a custom antibody for acetylated Lys-444 showed similar trends for truncated mutant Htt proteins (Htt 1–612 and Htt 1–1212) consistent with this earlier study. The level of acetylation in the wild-type versus mutant Htt was not reported for the acetylation of Lys-9 (82). However, the Lys-9-acetylation regulating Ser-13 phosphorylation is more efficient for the wild type than for the expanded polyQ truncated Htt protein. Considering the relative positions of Lys-9, Lys-178, Lys-236, Lys-345, and Lys-444 to the N-terminus, and the polyQ region, there seems to be a pattern showing more wild-type Htt lysine acetylation at the N-terminal and/or close to the polyQ region. On the other hand, there is also a trend toward a higher level of lysine acetylation in mutant Htt at the C-terminus and/or away from the polyQ region.

In conclusion, our study confirmed that Htt is a highly acetylated protein and identified three novel acetylation sites. Further, we found quantitatively that the level of acetylation of mutant Htt was different than wild-type Htt. Consistent with our hypothesis that HDAC inhibition can directly target the disease protein, we found that HDAC inhibition corrected the altered levels of acetylation of mutant Htt relative to wild-type Htt. Given the increasing importance of acetylation in regulating neurodegenerative diseases, the new acetylation sites identified here will allow further functional exploration of the role of these PTMs in Huntington’s disease.

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This article contains supplemental Figs. S1 to S5 and Tables S1 to S5.

The data associated with this manuscript may be downloaded from ProteomeCommons.org Tranche using the following hash: cbC GgLS4Ld6E7TbQUaAXE7xEHxMHuJD2LA4/Qwxx4kdtISouGLc 8ST9J99aCIOMmz0SaAhCLfLN7921ApIGEUUmwAAAAAAAbhQ

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**REFERENCES**

1. The Huntington’s Disease Research Group, H. D. C. R. (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington’s disease chromosomes. Cell 72, 971–983

2. Vonsattel, J. P., Myers, R. H., Stevens, T. J., Ferrante, R. J., Bird, E. D., and
Richardson, E. P., Jr. (1985) Neuropathological classification of Huntington's disease. J. Neuropathol. Exp. Neurol. 44, 559–577.

3. Zuccato, C., Belayev, N., Conforti, P., Ooi, L., Tartari, M., Papadimou, E., MacDonald, M., Fossale, E., Zeilin, S., Buckley, N., and Cattaneo, E. (2007) Widespread disruption of repressor element-1 silencing transcription factor-neuron-restrictive silencer factor occupancy at its target genes in Huntington's disease. J. Neurosci. 27, 6972–6983.

4. Gafni, J., Hermel, E., Young, J. E., Wellington, C. L., Hayden, M. R., and Ellery, L. M. (2004) Inhibition of calpain cleavage of huntingtin reduces toxicity: accumulation of calpain/caspase fragments in the nucleus. J. Biol. Chem. 279, 20211–20220.

5. Cha, J. H. (2000) Transcriptional dysregulation in Huntington's disease. Trends Neurosci. 23, 387–392.

6. Arrasate, M., Mitra, S., Schweitzer, E. S., Segal, M. R., and Finkbeiner, S. (2004) Independent body formation reduces levels of mutant huntingtin and the risk of neuronal death. Nature 431, 805–810.

7. Bae, B. I., Xu, H., Igarashi, S., Fujimoro, M., Agrawal, N., Taya, Y., Hayward, S. D., Moran, T. H., Montell, C., Ross, C. A., Snyder, S. H., and Sawa, A. (2005) p53 mediates cellular dysfunction and behavioral abnormalities in Huntington's disease. Neuron 47, 39–41.

8. Dunah, A. W., Jeong, H., Griffin, A., Kim, Y. M., Standaert, D. G., Hersch, S. M., Marder, M. D., Young, J. E., Wellington, C. L., Hayden, M. R., and Ellery, L. M. (2006) Huntingtin phosphorylation sites mapped by Akt.

14. Rangone, H., Poizat, G., Troncoso, J., Ross, C. A., MacDonald, M. E., and Cha, J. H. (2006) Decreased association of the nuclear transcription factor Sp1 with genes downregulated in Huntington's disease.

15. Luo, S., Vacher, C., Davies, J. E., and Rubinsztein, D. C. (2005) Cdk5 phosphorylation of huntingtin reduces its cleavage by caspases: implications for mutant huntingtin toxicity. J. Cell Biol. 169, 647–656.

16. Luo, S., Saudou, F., and Humbert, S. (2007) Phosphorylation of huntingtin by cyclin-dependent kinase 5 is induced by DNA damage and regulates wild-type and mutant huntingtin toxicity in neurons. J. Neurochem. 97, 2718–2728.

17. Steffan, J. S., Agrawal, N., Pallos, J., Rockabrand, E., Trotman, L. C., Slepek, N., Ilies, K., Lukacsovich, T., Zhu, Y. Z., Cattaneo, E., Pandolfi, P. P., Thompson, L. M., and Marsh, J. L. (2004) SUMO modification of Huntingtin and Huntington's disease pathology. Science 304, 100–104.

18. Saudou, F., Finkbeiner, S., Devys, D., and Greenberg, M. E. (1998) Huntingtin phosphorylation by Akt.

19. Humbert, S., Bryson, E. A., Cordelie` re, F. P., Connors, N. C., Datta, S. R., Finkbeiner, S., Greenberg, M. E., and Saudou, F. (2002) The IGF-1/Akt survival and inhibitory complex as revealed by lysine acetylation and mass spectrometry.

20. Kuo, M. H., and Allis, C. D. (1998) Roles of histone acetyltransferases and deacetylases in gene regulation. Bioessays 20, 615–626.

21. Shahbazian, M. D., and Grunstein, M. (2007) Functions of site-specific histone lysine acetylation and deacetylation. Annu. Rev. Biochem. 76, 75–100.

22. Williams, S. K., Truong, D., and Tyler, J. K. (2008) Acetylation in the global core of histone H3 on lysine-56 promotes chromatin disassembly during transcriptional activation. Proc. Natl. Acad. Sci. U.S.A. 105, 9000–9005.

23. Yang, X. J., and Seto, E. (2007) HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. Oncogene 26, 5310–5318.

24. Kuo, M. H., and Allis, C. D. (1998) Roles of histone acetyltransferases and deacetylases in gene regulation. Bioessays 20, 615–626.

25. Wang, K., and Seto, E. (2007) HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. Oncogene 26, 5310–5318.

26. Wang, K., and Seto, E. (2007) HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. Oncogene 26, 5310–5318.

27. Kouzarides, T. (2000) Acetylation: a regulatory modification to rival phosphorylation? EMBO J. 19, 1179–1191.

28. Zhong, S., Yang, C., Zhang, X., and Pestell, R. G. (2004) Acetylation of nuclear receptors in cellular growth and apoptosis. Biochem. Pharmacol. 68, 1199–1208.

29. Roh, M. S., Kim, C. W., Park, B. S., Kim, G. C., Kwon, H. C., Su, J. H., Cho, K. H., Yee, S. B., and Yoo, Y. H. (2004) Mechanism of histone deacetylase inhibitor Trichostatin A induced apoptosis in human osteosarcoma cells. Apoptosis 9, 583–589.

30. Balakin, K. V., Ivanenkov, Y. A., Kiselyov, A. S., and Tkachenko, S. E. (2007) Histone deacetylase inhibitors in cancer therapy: latest developments, trends and medicinal chemistry perspective. Anticancer Agents Med. Chem. 7, 576–591.

31. Williams, S. K., Truong, D., and Tyler, J. K. (2008) Acetylation in the global core of histone H3 on lysine-56 promotes chromatin disassembly during transcriptional activation. Proc. Natl. Acad. Sci. U.S.A. 105, 9000–9005.

32. Mohammadi, M., Young, J. E., Wellington, C. L., Hayden, M. R., and Ellery, L. M. (2006) Huntingtin is ubiquitinated and interacts with a specific ubiquitin-conjugating enzyme. J. Biol. Chem. 271, 13985–13994.

33. Davies, J. R., and Spencer, V. A. Control of histone modifications. J. Cell. Biochem. Suppl 32–33:141–148, 1999.

34. Spencer, V. A., and Davies, J. R. (1999) Role of covalent modifications of histones in regulating gene expression. Gene 240, 1–12.

35. Escargueil, A. E., Soares, D. G., Salvador, M., Larsen, A. K., and Henriques, P. P., Thompson, L. M., and Marsh, J. L. (2004) SUMO modification of Huntington and Huntington’s disease pathology. Science 304, 100–104.

36. Kalchic, M. A., Graham, R. K., Xia, G., Koide, H. B., Hodgson, J. G., Graham, K. C., Goldberg, Y. P., Getitz, R. D., Pickart, C. M., and Hayden, M. R. (2006) Huntingtin is ubiquitinated and interacts with a specific ubiquitin-conjugating enzyme. J. Biol. Chem. 271, 13985–13994.

37. Chang, J., and Seto, E. (2007) HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. Oncogene 26, 5310–5318.

38. Koh, M. H., and Allis, C. D. (1998) Roles of histone acetyltransferases and deacetylases in gene regulation. Bioessays 20, 615–626.

39. Wang, K., and Seto, E. (2007) HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. Oncogene 26, 5310–5318.

40. Wang, K., and Seto, E. (2007) HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. Oncogene 26, 5310–5318.

41. Wang, K., and Seto, E. (2007) HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. Oncogene 26, 5310–5318.

42. Wang, K., and Seto, E. (2007) HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. Oncogene 26, 5310–5318.
Novel Lysine Acetylation Sites in Huntingtin

45. Chiang, M. C., Chen, H. M., Lee, Y. H., Chang, H. H., Wu, Y. C., Soong, B. W., Chen, C. M., Wu, Y. R., Liu, C. S., Niu, D. M., Wu, J. Y., Chen, Y. T., and Chen, Y. (2007) Dysregulation of CEBPalpha by mutant Huntingtin causes the urea cycle deficiency in Huntington’s disease. Hum. Mol. Genet. 16, 483–498

46. Kim, S. G., Sprung, R., Chen, Y., Xu, Y., Ball, H., Pel, J., Cheng, T., Kho, Y., Xiao, H., Xiao, L., Grishin, N. V., White, M., Yang, X. J., and Zhao, Y. (2006) Substrate and functional diversity of lysine acetylation revealed by a proteomics approach. Mol. Cell 23, 607–616

47. Monti, B., Gatta, V., Piretti, F., Raffaelli, S. S., Virgili, M., and Contestabile, A. (2006) p38 MAP kinase is neuroprotective in the rotenone rat model of Parkinson’s disease: involvement of alpha-synuclein. Neurotox. Res. 17, 130–141

48. Kilgore, M., Miller, C. A., Fass, D. M., Henning, K. M., Haggarty, S. J., Sweett, J. (2006) Inhibitors of class 1 histone deacetylases reverse context-dependent deficits in a mouse model of Alzheimer’s disease. Neuropsychopharmacology 35, 870–880

49. Hockly, E., Richon, V. M., Woodman, B., Smith, D. L., Zhou, X., Rosa, E., Sathasivam, K., Ghazi-Noori, S., Mahal, A., Lowden, P. A., Steffan, J. S., Marsh, J. L., Thompson, L. M., Lewis, C. M., Marks, P. A., and Bates, G. P. (2003) Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor down-regulates p53 and p21 in A549 cells. Nucl. Acids Res. 31, 1074–1082

50. Ferrante, R. J., Kubilus, J. K., Lee, J., Ryu, H., Beesen, A., Zucker, B., Smith, K., Kowall, N. W., Ratan, R. R., Luthi-Carter, R., and Hersch, S. M. (2003) Histone deacetylation inhibition by sodium butyrate chemotherapy ameliorates the neurodegenerative phenotype in Huntington’s disease. Proc. Natl. Acad. Sci. U.S.A. 100, 2041–2046

51. Feng, H. L., Leng, Y., Ma, C. H., Zhang, J., Ren, M., and Chuang, D. M. (2000) Combined lithium and valproate treatment delays disease onset, reduces neurological deficits and prolongs survival in an amyotrophic lateral sclerosis mouse model. Neuroscience 155, 567–572

52. Butler, R., and Bates, G. P. (2006) Histone deacetylase inhibitors as therapeutics for polyglutamine disorders. Nat. Rev. Neurosci. 7, 784–796

53. Bridges, K. R., Schmidt, G. J., Jensen, M., Cerami, A., and Bunn, H. F. (1975) The acetylation of hemoglobin by aspirin. In vitro and in vivo. J. Biol. Chem. 250, 4683–4689

54. Schmitt, M., and Matthies, H. (1979) [Biochemical studies on histones of the central nervous system. I. Characterization and partial identification of the label of histones from rat brain following intraventricular administration of 1-14C-acetate]. Acta Biol. Med. Ger. 38, 673–676

55. Li, Y., Xiao, H., Campos, E. I., Ho, V. C., and Li, G. (2005) Development of a PAN-specific, affinity-purified anti-acetylated lysine antibody for identification of acetylated protein. J. Immunol. Meth. 301, 201–207

56. Schmitt, M., and Matthies, H. (1979) [Biochemical studies on histones of the central nervous system. II. Acetylation of lysine amino group in isolated rat brain histones]. Acta Biol. Med. Ger. 38, 677–685

57. Li, Y., Xiao, H., Campos, E. I., Ho, V. C., and Li, G. (2005) Development of a PAN-specific, affinity-purified anti-acetylated lysine antibody for identification of acetylated protein. J. Immunol. Meth. 301, 201–207

58. Schmitt, M., and Matthies, H. (1979) [Biochemical studies on histones of the central nervous system. III. Application of a PAN-specific, affinity-purified anti-acetylated lysine antibody for identification of acetylated protein]. J. Immunol. Meth. 301, 201–207

59. Li, Y., Xiao, H., Campos, E. I., Ho, V. C., and Li, G. (2005) Development of a PAN-specific, affinity-purified anti-acetylated lysine antibody for identification of acetylated protein. J. Immunol. Meth. 301, 201–207

60. Li, Y., Xiao, H., Campos, E. I., Ho, V. C., and Li, G. (2005) Development of a PAN-specific, affinity-purified anti-acetylated lysine antibody for identification of acetylated protein. J. Immunol. Meth. 301, 201–207
82. Thompson, L. M., Aiken, C. T., Kaltenbach, L. S., Agrawal, N., Illes, K., Khoshnan, A., Martinez-Vincente, M., Arrasate, M., O'Rourke, J. G., Khashwji, H., Lukacsovich, T., Zhu, Y. Z., Lau, A. L., Massey, A., Hayden, M. R., Zeitlin, S. O., Finkbeiner, S., Green, K. N., LaFerla, F. M., Bates, G., Huang, L., Patterson, P. H., Lo, D. C., Cuervo, A. M., Marsh, J. L., and Steffan, J. S. (2009) IKK phosphorylates Huntingtin and targets it for degradation by the proteasome and lysosome. J. Cell Biol. 187, 1083–1099.

83. Kegel, K. B., Sapp, E., Yoder, J., Cuiffo, B., Sobin, L., Kim, Y. J., Qin, Z. H., Hayden, M. R., Aronin, N., Scott, D. L., Isenberg, G., Goldmann, W. H., and DiFiglia, M. (2005) Huntingtin associates with acidic phospholipids at the plasma membrane. J. Biol. Chem. 280, 36464–36473.

84. DiFiglia, M., Sena-Esteves, M., Chase, K., Sapp, E., Pfister, E., Sass, M., Yoder, J., Reeves, P., Pandey, R. K., Rajeev, K. G., Manoharan, M., Sah, D. W., Zamore, P. D., and Aronin, N. (2007) Therapeutic silencing of mutant huntingtin with siRNA attenuates striatal and cortical neuropathology and behavioral deficits. Proc. Natl. Acad. Sci. U.S.A. 104, 17204–17209.

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