Supplementary Materials

Supplementary Experimental Procedures

Calibration of the Ratio\textsubscript{ASAP} using the Ratio\textsubscript{w}:

To calibrate the ratio detected by the ASAPRatio (Ratio\textsubscript{ASAP}) with the results of Western blot analysis, a logarithmic transformation was used. Let

\[ \log_{10}(\text{Ratio}_w) = \log_{10}(\text{Ratio}_{\text{ASAP}}) - x; \]

where Ratio\textsubscript{w} is the ratio detected by Western blot analysis, Ratio\textsubscript{ASAP} is the ratio detected by the ASAP ratio, and x is the difference between log\textsubscript{10}(Ratio\textsubscript{ASAP}) and log\textsubscript{10}(Ratio\textsubscript{w}).

According to equation (1), let

\[ D = \left[ \log_{10}(\text{Ratio}_{\text{ASAP}}) - x \right] - \log_{10}(\text{Ratio}_w), \quad \text{and} \]

\[ \sum D^2 = \sum \left\{ \left[ \log_{10}(\text{Ratio}_{\text{ASAP}}) - x \right] - \log_{10}(\text{Ratio}_w) \right\}^2, \]

where D is the difference between log\textsubscript{10}(Ratio\textsubscript{w}) and log\textsubscript{10}(Ratio\textsubscript{ASAP}) - x.

The value of x can be found by searching for the minimum of the sum of D\textsuperscript{2}. The x value was fitted from 1 to -1 in increments of 0.0001. Based on the calibration using Western blot analyses of proteins 1 to 6 in Table 1, the x values for Exp-I and Exp-II were -0.0696 and -0.0728, respectively. The fitting curves are illustrated below.
Fitting curve of experiment I.

\[ x = -0.0696 \text{ and } D^2 \text{ minimum } = 0.0069. \]

Fitting curve of experiment II.

\[ x = -0.0728 \text{ and } D^2 \text{ minimum } = 0.0186. \]
**Immunohistochemistry:**

In brief, 12-week-old WT mice were deeply anesthetized with sodium pentobarbital (50 mg/kg), and intracardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). Their brains were carefully removed, post-fixed with 4% paraformaldehyde/0.1 M PB for 3~5 h, and then immersed in 30% sucrose in 0.1 M PB for 2 days. Single immunostaining of Gβ1 was conducted as detailed elsewhere (1) with modifications. Briefly, striatal sections (20 µm) were first stained with an anti-Gβ1 antibody and visualized using a highly sensitive biotintyramide amplification system with Avidin-Alexa Fluor® 488. The sections were then incubated with DAPI for nuclei. Patterns of immunostaining were analyzed with the aid of a laser confocal microscope.

**Supplementary References**

Supplementary Tables

Table S1. Gene-specific primers used for real-time RT-PCR assays.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Unigene-Accession number</th>
<th>Primer pairs</th>
<th>Amplicon Size (bp)</th>
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<tr>
<td>14-3-3ζ</td>
<td>BAA11751</td>
<td>F, 5’-ATTTTCCCCCTCCTTCTCCTG-3’&lt;br&gt;R, 5’-ACTCCGGACACAGAAATATCC-3’</td>
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<td>FKBP12</td>
<td>NP_032045</td>
<td>F, 5’-ATGGAGTAGTGCAAGGTGGAGACC-3’&lt;br&gt;R, 5’-TTCCAGTTTTAGAAGCTCCACATCA-3’</td>
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<tr>
<td>Lim</td>
<td>NP_077185</td>
<td>F, 5’-AGCGCACCAGACCATGGCCT-3’&lt;br&gt;R, 5’-CTGAACGTGCTTCCTCCGGGT-3’</td>
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</table>

Table S2 (List of striatal proteins appearing in both ICAT experiments) and Table S3 (Product-Ion ion mass spectra of identified peptides) are submitted as separated files.
Supplementary Figures

Supplementary Fig. S1.

<table>
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<th>Mouse</th>
<th>D</th>
<th>C</th>
<th>N</th>
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Fig. S1. Expressions of lamin A/C (a nuclear marker) and α-tubulin (a cytosolic marker) in the nuclear-enriched and cytosolic fractions of the mouse striatum. Equal amount (25 μg per lane) of the debris (D), cytosolic (C), and nucleus-enriched (N) fractions from the striatum of 10.5-week-old HD or WT mice were analyzed using Western blot analyses. Representative images of 3 experiments are shown.
Fig. S2. The majority of Htt aggregates appeared in the nucleus-enriched fractions. Equal amount (25 μg per lane) of the debris (D), cytosolic (C), and nucleus-enriched (N) fractions from the striatum of 10.5-week-old HD or WT mice were analyzed by the filter assay. The insoluble Htt aggregates retained on the filter were detected using an anti-Htt antibody. Representative images of three experiments are shown. Representative pictures from a short (A, 20 s) and a long (B, 12 min) exposure to detect the immunoreactive Htt demonstrated that the majority of the Htt aggregates were found in the nucleus-enriched fractions. After long exposures, faint signals of Htt aggregates could also be detected in the debris fractions.
Supplementary Fig. S3.

Fig. S3. Expressions of mutant Htt aggregates, 14-3-3ζ, FKBP12, β-actin, PKCβ in the indicated fractions of the mouse striatum. Equal amount of the indicated fraction (25 μg per lane; debris, D; cytosolic, C; and nucleus-enriched, N) from the striatum of 10.5-week-old HD or WT mice were analyzed using Western blot analyses. Arrowheads mark the segregation lines between the stacking and separating gels. Only immunoreactive Htt (the thick arrow) could be detected in the stacking gels. Arrows indicate the expected sizes of the corresponding proteins in the separating gels. Reduced expression of 14-3-3ζ, FKBP12, β-actin, and PKCβ were observed in both the C and N fractions.
Supplementary Fig. S4.

Fig. S4. Peptide ratio distribution peak of a disease proteome might markedly deviate from a 1:1 unchanged, hypothetical proteome. (A) The dashed line represents the peptide ratio distribution of a 1:1 unchanged, hypothetical proteome. If the portion of the altered peptide ratio in an experimental proteome is small, the center of the ratio distribution peak of the experimental proteome (solid line) would be close to that of the 1:1 unchanged proteome. Based on this assumption, alignment of the centers to the 0 position of the x-axis would ensure that the potential error in protein quantitation of the two samples in comparison is corrected. (B) For severe degenerative diseases such as Huntington’s disease (HD), a significant portion of the disease proteome may have been changed. Thus, the center of the peptide ratio distribution peak of a disease proteome would significantly deviate from that of the 1:1 unchanged proteome. The larger the difference (Δ) is, the worse the normalization procedure becomes.
Fig. S5. Overexpression of Lim did not reduce the aggregation of mutant Htt in ST14A cells. ST14A cells were transfected with mutant Htt with polyQ expansion (Q109) plus an empty vector (pcDNA3) or an expression construct of V5-tagged Lim (Lim-V5) as indicated for 72 h. Cell lysates (50 μg per lane) collected from the indicated cells were subjected to Western blot analysis using the corresponding antibodies (A). Values of the relative intensities of total mutant Htt in the presence of Lim were determined by normalizing the signal of mutant Htt with that of the corresponding internal control (Actin), and are expressed as multiples of that of control cells expression an empty vector. Expression of Lim was illustrated in the bottom panel of (A). Cell lysates (30 μg) collected from the indicated cells were subjected to a filter retardation assay (B, D). The insoluble Htt aggregates retained on the filter were detected using the anti-Htt antibody. The relative amount of Htt aggregates was normalized with that of control cells in each experiment. A representative image of four independent experiments is shown. Data represent the mean ± SEM values from eight determinations of four independent experiments and are shown at the bottom of the corresponding lane.

* Specific comparison to control cells ($p < 0.001$; Student's $t$-test).
**Supplementary Fig. S6.**

**Fig. S6.** A G\(\beta\)1-positive signal found in striatal nuclei. Immunostaining of G\(\beta\)1 in the striatum of 12-week-old WT brains was performed. G\(\beta\)1 was visualized by the Alexa Fluor® 488 conjugated secondary antibody (green). The nucleus was visualized by DAPI (blue). The arrows mark the nuclei that contain G\(\beta\)1, while the arrowheads indicate the nuclei which harbor no G\(\beta\)1. The merged image is shown in the left panel.