**SUPPLEMENTAL DATA**

**EXPERIMENTAL PROCEDURES**

**Primary Antibodies**

| Antibody | Description | Source | WB dilution | IHC dilution |
|---|---|---|---|---|
| 2B7 | monoclonal | (1) | 1:750 | 1:100 |
| HD-1 | polyclonal (rabbit) | (2) | 1:1000 | - |
| 1C2 | monoclonal | Chemicon (MAB1574) | 1:1000 | - |
| 3B5H10 | monoclonal | Sigma (P1874) | - | - |
| 4H7H7 | monoclonal | this paper | - | - |
| MW1 | monoclonal | (3) | 1:1000 | 1:1000 |
| S830 | polyclonal (sheep) | (4) | 1:750 | 1:2000 |
| 4C9 | monoclonal | this paper† | - | - |
| MW8 | monoclonal | (3) | 1:750 | - |
| 1H6 | monoclonal | (5) | 1:1000 | 1:25 |
| HD-170 | polyclonal (rabbit) | (2) | - | 1:25 |
| HD-215 | polyclonal (rabbit) | (2) | - | 1:25 |
| HD-331 | polyclonal (rabbit) | (2) | 1:1000 | - |
| MAB2166 | monoclonal | Chemicon | 1:1000 | 1:50 |
| HD-494 | polyclonal (rabbit) | (2) | - | - |
| HD-654 | polyclonal (rabbit) | (2) | - | - |
| HD-A | polyclonal (rabbit) | (6) | - | - |
| MAB2170 | monoclonal | Chemicon | - | - |
| HD-C | polyclonal (rabbit) | (6) | - | - |
| β-Actin | monoclonal | Abcam (ab6276) | 1:10000 | - |
| Histone H3 | polyclonal (rabbit) | Upstate (07-690) | 1:25000 | - |

†monoclonal rose against QLPQPPPQAQPLLPQPPP

WB = western blotting, IHC = immunohistochemistry for confocal microscopy

**Secondary Antibodies**

| Antibody | Description | Source | WB dilution | IHC dilution |
|---|---|---|---|---|
| Anti-mouse HRP | polyclonal-rabbit | Dako (P0260) | 1:3000 | - |
| Anti-goat HRP | polyclonal-rabbit | Dako (P0449) | 1:3000 | - |
| Anti-rabbit-HRP | polyclonal-swine | Dako (P0217) | 1:3000 | - |
| Anti-mouse Alexa-488 | polyclonal-donkey | Molecular Probes A21202 | - | 1:2000 |
| Anti-rabbit Alexa-488 | polyclonal-donkey | Molecular Probes A21206 | - | 1:2000 |
| Anti-sheep Alexa-555 | polyclonal-donkey | Molecular Probes A21436 | - | 1:2000 |

WB = western blotting, IHC = immunohistochemistry for confocal microscopy

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## FIGURE LEGENDS

Supplemental Fig. 1. Proteolytic fragments of Htt can be detected on western blots of whole brain lysates but interpretation of the pattern is complex. Total brain lysates from WT and *Hdh*$^{Q150/Q150}$ mice at 2, 6 and 10 months of age were prepared in KCL buffer (7), fractionated by 8% SDS-PAGE and immunoprobed with the antibodies as indicated. Although many Htt fragments can be detected, the pattern is complicated by the presence of non-specific bands in both WT and *Hdh*$^{Q150/Q150}$ lanes. In the case of HD-331 and MAB2166, it is not possible to know whether the Htt bands represent N-terminal or internally generated fragments (see Fig. 1).

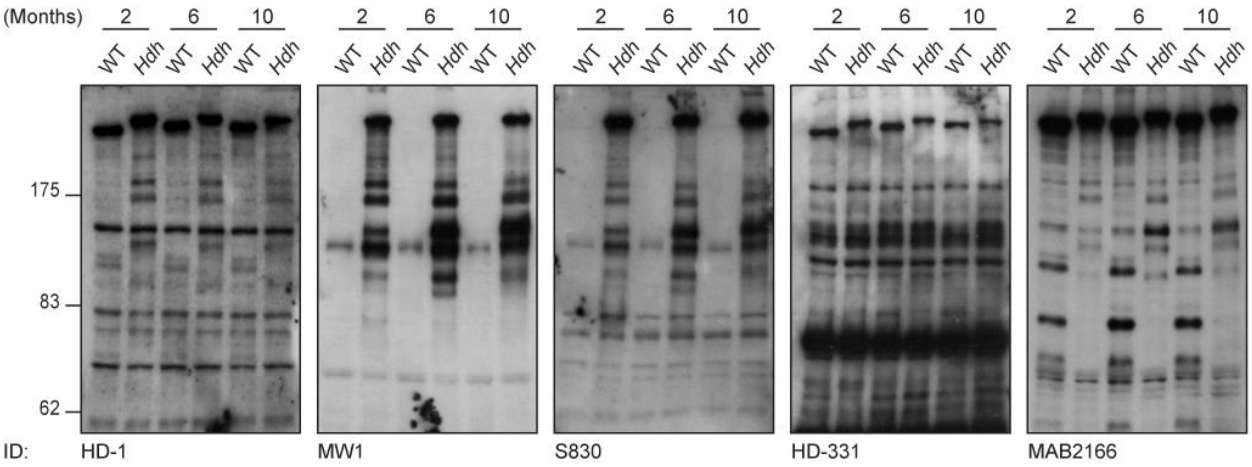
Supplemental Fig. 2. Optimisation of lysis buffers and sample treatment. It has been shown that the use of acidic lysis conditions in combination with high temperatures can result in cleavage of aspartyl-

proline peptide bonds (8). To avoid any possibility of this chemical cleavage occurring during sample preparation and processing, all lysis buffers used were at neutral pH. To test the effect of temperature, whole brains from *Hdh*$^{+/Q150}$ heterozygous mice were lysed in HEPES buffer and Htt fragments were immunoprecipited with 3B5H10. Prior to western blotting, samples were heated either to 75°C or 100°C. There was no difference in the pattern of fragments obtained and subsequently immunoprecipitated lysates were always heated to 75°C prior to SDS-PAGE fractionation.

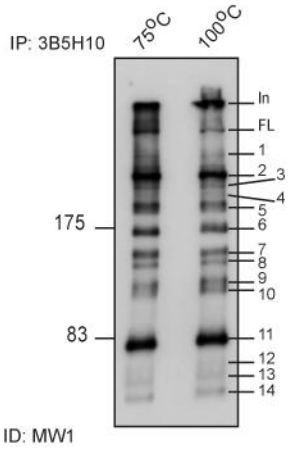
Supplemental Fig. 3. PolyQ specific antibodies did not immunopreciptate Htt or N-terminal fragments of Htt from WT brain lysates. WT lysate controls were included in all experiments performed in this study. The polyQ specific antibodies 3B5H10, MW1, 4H7H7 and 1C2 did not immunoprecipitate Htt or N-terminal fragments from WT lysates. Here we show the immunoprecipitation of Htt and N-terminal Htt fragments from *Hdh*$^{+/Q150}$ and WT brain lysates with 3B5H10 that were performed in parallel. Immunodetection was with MW1. In = interface between stacking and resolving gel; FL = full-length protein.

Supplemental Fig. 4. The pattern of fragments immunoprecipitated with MW8 is comparable to that immunoprecipitated with other antibodies that detect epitopes within the Htt exon 1 protein. Mutant Htt was immunoprecipitated from a 2 month *Hdh*$^{+/Q150}$ brain with MW8 and immunodetected with MW1. Immunoprecipitation of Htt fragments with 2B7 and S830 is shown for comparison. However, when used as a probe against western blots, MW8 behaves as a neo-epitope antibody and only recognizes fragment 13. In = interface between stacking and resolving gel; FL = full-length protein.

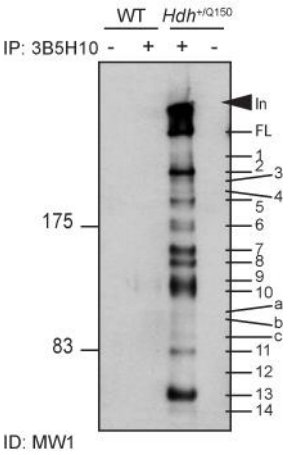
Supplemental Figure 1




Supplemental Figure 2



Supplemental Figure 3




Supplemental Figure 4