Supporting Information

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SI Text

**Stereotactic Injection.** During stereotactic surgery, mice were anesthetized with isoflurane (1-chloro-2,2,2-trifluoroethyl-difluoromethylether) (Inibsa) diluted in a 50% O2-50% NO2 mixture. Intrastriatal administration of 1 μL lactacystin at 20 μg/μL (right side) and 1 μL H2O (left side) was performed on anesthetised mice using a 10-μL Hamilton syringe at the following stereotaxic coordinates (medial-lateral: 1.5 mm; antero-posterior: 0.5 mm; dorso-ventral: 1.2 mm). Lactacystin and H2O were infused at a rate of approximately 1 μL/min, and the needle slowly withdrawn 2 min after the infusion was complete. Mice were sacrificed by CO2 24 h after the surgery. For histological analysis, brains were removed after decapitation and halved sagittally. Hemispheres were fixed with 4% paraformaldehyde in PBS at 4 °C for 24 h, and embedded in 3.5% bacteriological agar in dH2O. Serial 30-μm slices were cut using a vibratome and placed in a 30% glycerol; 30% ethylene-glycol, 30% dH2O; and 10% phosphate buffer solution, pH 7.2. For proteasome activity or Western blot analysis, a 0.5-mm thick coronal slice was excised surrounding the injection site, and an approximate 300 μm3 tissue piece was subsequently excised surrounding the injection trajectory for analysis.

**In Situ Hybridization.** Oligonucleotides were 3' end-labeled with (α35S)dATP (Amersham Pharmacia Biotech) using terminal deoxynucleotidyltransferase (Amersham) at 37 °C for 2 h. Labeled oligonucleotides were purified using Chroma spin+ Te-10 columns (Clontech). In situ hybridization and imaging were performed on 16 μm sagittal brain sections as previously described (1). Slides were exposed to phosphoimager plates to obtain images for quantification purposes, followed by exposure to autoradiographic film (β-max, Amersham) for higher-resolution images.

Fig. S1. Accumulation of reporters in PC12 cell lines after proteasome inhibitor treatment. N-mutHtt-CFP PC12 cell lines expressing Ub\textsuperscript{G76V}-YFP (Upper) and YFP-CL1 (Lower) had been left untreated (Left) or had been treated for 6 h with 10 \mu M of the proteasome inhibitor MG132 (Right).
Fig. S2. UFD reporter expression in the brains of UbGFP mice. Quantitation of relative in situ hybridization signal intensity in different brain regions of UbGFP lines 1 and 2: Cortex (Ctx), striatum (Str), hippocampal pyramidal neuron layer (Hc), and whole cerebellum (Cb). Numbers above bars indicate ratio of in situ hybridization signal for line 1: line 2. SEM n = 3 age matched UbGFP/1 and UbGFP/2 mouse pairs aged 6, 16, and 30 weeks.
Fig. S3. Low levels of GFP reporter are detectable in neuronal cells in brains of UbGFP/1 mice. Double immunostaining of GFP and neuron-specific marker NeuN in 12-week UbGFP/1 cortex. (Scale bar, 20 μm.)
Fig. S4. Identification of cell types accumulating reporter upon proteasome inhibition. Chymotrypsin-like (A) and caspase-like (B) activity was measured in striatal tissue 24 h after injection with 20 μg lactacystin or vehicle control using the substrates suc-LLVY-aminomethylcoumarin and z-LLE-β-2-naphthylamine, respectively. Sections from lactacystin treated UbGFP/1 (C) and UbGFP/2 (D) mice were probed with antibodies specific for neurons (NeuN), microglia (Iba1), astrocytes (GFAP), and oligodendrocytes (CNPase). Left in green show native GFP fluorescence, Right in red show the immunostainings. (Scale bar, 20 μm.) Arrows indicate double-fluorescent cells. (E) Western blot analysis of UbGFP and GFP reporter products in striatal tissue of UbGFP/1 mice 24 h after injection with 20 μg lactacystin or vehicle control. Far right lane, non-reporter mouse control (C).
Fig. S5. Characterization of GFP fragment. (A) The low molecular weight fragment corresponds in size with GFP, not with deubiquitylated Ub\textsuperscript{G76V}.GFP which would possess an additional 13 amino acid linker sequence, similar to the rapidly deubiquitylated Ub-R-YFP reporter (labeled X-YFP). Brain tissue lysates from NTg or UbGFP/1 mice were spiked with cell extracts from cells expressing GFP, or Ub-R-YFP. A doublet band was detected in Ub\textsuperscript{G76V}.GFP/1 tissue spiked with Ub-R-YFP (lane 6), but not with GFP (lane 4). (B) Clearance rates of Ub\textsuperscript{G76V}.GFP and GFP products after blockage of protein synthesis with cycloheximide (100 μg/mL) in primary fibroblast cultures from a UbGFP/1 mouse. Cells were harvested at the indicated time points and analyzed by Western blotting with a polyclonal GFP antibody (Upper) or monoclonal GAPDH antibody as a loading control (Lower). The full length Ub\textsuperscript{G76V}.GFP (UbGFP), and the truncated GFP fragment are indicated. NTg represents fibroblasts from a non-transgenic mouse.
Fig. S6. Slight elevation in reporter expression due to N-mutHtt expression. (A) In situ hybridization of UbGFP-GFP reporter in mice co-expressing mutHtt shows no gross alterations in expression pattern. Representative sections of UbGFP/1, 31-week; UbGFP/1:R6/1, 31-week; and UbGFP/1:R6/2, 14-week brain. (B) Quantitation of in situ hybridization signal in different brain regions of UbGFP/1:R6/1 mice compared with UbGFP/1 controls, matched for age and genetic background (UbGFP n = 3 and UbGFP/1:R6/1 n = 3; 2 mice of 31 weeks and 4 mice of 40 weeks). Cortex (Ctx), striatum (Str), cerebellum (Cb).
Fig. S7. Absence of UPS impairment in R6/1 mice. (A) Representative cryosections from brains of UbGFP/1 (positive control), UbGFP/2 and UbGFP/2:R6/1 (36 week) mice, co-immunostained for Htt and GFP. Nuclei were counterstained with Hoechst. Cortex (Ctx) and striatum (Str). (B) Representative cryosections from brains of 19 week non-transgenic (NTg), UbGFP/1, and UbGFP/1:R6/1, mice co-immunostained for Htt and GFP. (Scale bars, 20 μm.)
Fig. S8. Titration of proteasome inhibition with accumulation of UbG76V-GFP and polyubiquitin conjugates. Primary UbGFP/1 fibroblast cultures were treated with 0 to 200 nM epoxomycin for 6 h and harvested for (A) Western blot analysis of UbGFP and polyubiquitin conjugate accumulation, or (B) measurement of chymotryptic-like proteasome activity using succinyl-LLVY-aminoethylcoumarin. (C) Western blotting of lysates of HeLa cells that had been left untreated (left lanes) or that had been treated for 6 h with 10 μM proteasome inhibitor epoxomycin. Right lane, 14-week R6/2 brain homogenate. Samples were run on an SDS/PAGE gel with a 1% agarose stacking gel.
Fig. 59. No alterations in proteases TPP-II and PSA in R6/2 mice. Brain homogenates from 12-week-old R6/2 mice and non-transgenic (NTg) littermate controls were analyzed by Western blotting for detection of puromycin sensitive aminopeptidase (PSA) and tripeptidyl peptidase II (TPP-II) (A). Quantitation of band intensities corrected for GAPDH loading control show no significant alterations in either protease in R6/2 brain B.