Supplementary Materials

Supplementary Materials and Methods

Cell Culture and Cell Line Generation

STHdh cells (CH00097–Q7/Q7 and CH00096–Q7/Q111) from Coriell Cell Repositories (Coriell Institute for Medical Research, Camden, UK) were cultured in Dulbecco's Modified Eagle Medium (DMEM) (#11965, Thermo Fisher Scientific, MA) with 10% fetal bovine serum (FBS) (#10082, Thermo Fisher Scientific, MA). The generation and authentication of HD iPSC-derived neurons were described previously [1]. All the mammalian cell lines were maintained at 37°C in a 5% CO_2 incubator, except for STHdh cells, which were maintained at 33°C under 5% CO_2. The cells were tested for *Mycoplasma* contamination and were identified *via* short tandem repeats. Mouse primary neurons were isolated and cultured as described previously[1]. The mouse experiments were carried out following the general guidelines published by the Association for Assessment and Accreditation of Laboratory Animal Care. The Animal Care and Use Committee of the School of Medicine at Fudan University approved the protocol used in animal experiments (Approvals 20140904 and 20170223-005).

Protein Extraction and Western Blots

Cell pellets were collected and lysed on ice for 30 min in 1× PBS + 1% Triton X-100 + 1× complete protease inhibitor (4693159001, Sigma, MO), sonicated for 10 s, and centrifuged at >20,000 × g at 4°C for 10 min. The supernatants were then loaded and transferred onto nitrocellulose membranes for Western blots. The primary antibodies used were anti-β-tubulin (#ab6046, Abcam, Cambridge, UK), anti-P62 (#ab56416, Abcam), anti-LC3B (PA1-16930,Thermo Fisher), and anti-caspase3 (#9661, Cell

signaling Technology, MA). The membrane was probed by AffiniPure peroxidase-conjugated secondary antibodies goat anti-mouse IgG HRP (m21001L, Abmart, Shanghai, China) and goat anti-rabbit IgG HRP (m21002L, Abmart). The specificity of all antibodies has been validated by previous reports or our knockdown or knockout experiments.

Measurement of Neuronal Loss and Apoptosis

The methods were as previously published [1]. Briefly, the neurons were transferred from standard culture conditions to conditions without BDNF where they displayed mutant HTT protein (mHTT)-dependent neuronal shrinkage and death, which was quantitated by immunostaining for the neuron-specific marker Tuj1 [2]. To measure caspase-3 activity, NucView 488 caspase-3 dye (#30029, Biotium, CA) was used to assess caspase-3 activity as an indicator of apoptosis. The cells were immunostained with NucView 488 and DAPI, images were captured by a Carl Zeiss microscope, and analyzed blindly for caspase-3 and Tuj1 quantification by ImageJ (Tuj1, #MMS-435P, Covance, NJ). The AST487 compound was obtained from Chemexpress (#HY-15002, at the indicated concentrations, Shanghai, China). Images of caspase-3 dye-treated STHdh cells were automatically captured by IncuCyte (IncuCyte FLR, Essen Bioscience, Göttingen, Germany). The experimenters were blinded to the treatment conditions. Phase-contrast images were captured and analyzed by IncuCyte 2011A software (Essen Bioscience, Göttingen, Germany).

Time-resolved Fluorescence Resonance Energy Transfer (TR-FRET) Assay

The HTRF assays were performed as described previously [1,3], and the 2B7/3B5H10 antibodies targeting HTT were previously validated [3]. The signals were further linearly-transformed by

background subtraction and normalization.

cDNA and siRNA Transfection

Plasmid *HTT-exon1-Q72* and *HTT-exon1-Q25* transfection was performed according to the manufacturer's protocol. The siRNAs were reverse-transfected into the cells with Lipofectamine 2000 (#11668, Life Technologies, MA) and into the iPSC-derived neurons with Lipofectamine RNAiMAX (#13778, Life Technologies). All siRNAs were validated by qPCR for target knock-down [1]. The siRNA target sequences were:

Scrambled siRNA: 5'-UUCUCCGAACGUGUCACGUTT-3';

HTT siRNA: 5'-CAGGUUUAUGAACUGACGUUA-3';

HIPK3 siRNA: 5'-CGAUGUGGAUUGAAGCGCATT-3';

Hipk3 siRNA: 5'-CCGUGUACCUCAAGACCUATT-3'.

***Drosophila* Models and Behavioral Experiments**

The *Drosophila* nervous system driver line elav-GAL4 (C155) expresses an N-terminal fragment of mHTT with 128Q under the elavC155>GAL4 driver. For clock neuron staining, *Drosophila* with *Exon1-Q72* under elavC155>GAL4 was used. For HTRF assay, in addition to the N-terminal fragment of *mHTT* with 128Q, *Drosophila* with UAS-fl-*HTT-Q128* (expressing human full-length HTT with 128Q when crossed to the GAL4 line) was also used, UAS-fl-*HTT-Q16* served as control. The *Drosophila* lines were obtained the Bloomington *Drosophila* Stock Center at the University of Indiana (http://flystocks.bio.indiana.edu/) or generated in our laboratory.

In behavioral experiments, we placed 15 age-matched virgin females in an empty vial and tapped

them down. The percentage of flies that climbed past a 7-cm line after 10 s was recorded. The mean of 5 observations was plotted for each vial on each day, and data from multiple vials containing different batches of flies were plotted and analyzed by two-way ANOVA. Blinding was used to perform the experiments.

HIPK3 Kinase Activity Assessment

The relative kinase activity of HIPK3 was measured using Kinase Enzyme Systems (#V4164, Promega Corporation, WI), following the manufacturer's protocol. In the assay, 384 low-volume plates were used. First, enzyme, substrate, ATP, and inhibitors were diluted in kinase buffer, followed by the addition of inhibitor or 5% DMSO, enzyme, and substrate and incubation for 60 min at room temperature. After the kinase reaction incubation, ADP-Glo™ reagent (#V9101, Promega Corporation) was added to all wells, mixed for 2 min, and incubated at room temperature for 40 min. Then, Kinase Detection Reagent was added to each well and incubated at room temperature for 30–60 min. Finally, the luminescence was measured with a plate-reading luminometer or charge-coupled device camera. To calculate the percentage of enzyme activity, the signal of the negative control (no enzyme and no inhibitor) was subtracted from the signal of each sample. Then we used the mean relative light unit (RLU) values for the 0% kinase activity (no compound and no enzyme) and the 100% kinase activity (no compound) to calculate the other percent enzyme activities remaining in the presence of the different dilutions of inhibitor.

Statistics

Statistical comparisons between two groups used the two-tailed unpaired Student's *t*-test. Statistical

comparisons among multiple groups used ANOVA tests. Significance was established at $P < 0.05$. In all graphs, error bars indicate SEM, and the biological replicate numbers are indicated above each bar and/or as the *n* numbers in the legends.

Supplementary References

[1] Yu M, Fu YH, Liang YJ, Song HK, Yao Y, Wu P, *et al*. Suppression of MAPK11 or HIPK3 reduces mutant Huntingtin levels in Huntington's disease models. Cell Res 2017, 27: 1441–1465.

[2] Yao YW, Cui XT, Al-Ramahi I, Sun XL, Li B, Hou JP, *et al*. A striatal-enriched intronic GPCR modulates huntingtin levels and toxicity. Elife 2015, 4: e05449.

[3] Fu YH, Wu P, Pan YY, Sun XL, Yang HY, Difiglia M, *et al*. A toxic mutant huntingtin species is resistant to selective autophagy. Nat Chem Biol 2017, 13: 1152–1154.