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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒ Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	All data collection softwares came with the equipment utilized for experiments, including IncuCyte 2011A, MO. Control (NT.115), PerkinElmer EnVision Manager Version 1.13, HKL2000 V719, Image Lab Version 3.0, ZEN 2.3
Data analysis	The softwares utilized for analysis were all commercially available or could be downloaded from open source, including GraphPad Prism 7, ImageJ 1.52a, Origin8, Microsoft Excel 2016, PASS 16, Nanotemper analysis (1.5.41) , PyMOL 2.2, HKL2000, Phaser 2.8, Mascot 2.3, PASS 16, IncuCyte 2011A, MO.Affinity Analysis (NT.115).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The protein structure data has been uploaded to the PDB database with entry number 6J04. The source data in excel files have been provided for all essential plots in the figures. The full gel blots and the proteomics datasets have been provided as supplementary tables. All the other data are available from the authors upon request.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	To ensure to reach a statistical power>0.8, power analyses were performed for each assay based on estimated values by PASS 16 (https://www.ncss.com/software/pass/) before experiments. Estimation was based on our previously published results on similar experiments and preliminary experiments. The power analysis suggested n > =3 for mHTT level measurements and n > =5 for behavioral experiments. In all the experiments we performed, we have used a larger n than these numbers, and we also performed post-experiment power analyses to ensure that power > 0.8 for all the significant differences.
Data exclusions	Data were not excluded unless clear experimental failures occurred, including cell contamination, gel transfer failures and lack of signals in positive controls. The exclusion criteria were pre-established.
Replication	All experimental data was reliably reproduced in multiple independent experiments as indicated in the figure legends. The protein-compound interaction experiments and the HTT-lowering experiments have been replicated by at least two independent researchers.
Randomization	For the in vivo experiments in the mouse, randomization was performed by assigning random numbers. For the Drosophila experiments, the flies were randomly distributed into the vials after anesthesia.
Blinding	As indicated in the figure legends, the investigators were blinded during data collection and analysis where possible, included immunofluorescence experiments, drug treatment experiments in mouse and fly models for HTT level measurements and behavioral assays.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	All unique materials (some of the patient fibroblast lines) are readily available from the authors.

Antibodies

Antibodies used

Anti-β-tubulin (Abcam, cat. no. ab6046, lot no. GR3209100-1, 1:10000)
Anti-TUBB3 (Biolegends (Covance), cat. no. 801202, clone TUJ1, lot no. B2335555, 1:500)
Anti-ATXN3 (Millipore, cat. no. MAB5360, clone no. 1H9, lot no. 3096481,1:1000):
Anti-Gapdh (Proteintech, cat. no. 60004-1, lot no. 10004129, 1:5000)
Anti-NBR1 (ThermoFisher Scientific, cat. no. PA5-54660, lot no. SJ2462971A, 0.4 μg/mL)
Anti-β-actin (Beyotime, cat.no. AA128, clone no.AC-74, lot no. 031918180821, 1:1000)
Anti-TBP antibody (Abcam, cat. no. ab818, clone no.1TBP18, lot no. GR315577-3, 1:1000)
Anti-SQSTM1 antibody (ThermoFisher Scientific, cat.no. PA5-27247, lot no. SC2360851N, 1:1000)
Anti-spectrin antibody (Millipore, cat.no. MAB1622, clone no. AA6, lot no. 2943221, 1:2000)
Anti-Ncoa4 antibody (Santa cruz, cat.no. sc-373739, clone no. C-4, 1:200)
Anti-GST antibody (ProteinTech, cat.no. HRP-66001, clone no. 3G12B10, lot no. 20000091, 1:2000)
anti-MBP antibody (ProteinTech, cat.no. 15089-1-AP, lot no. 00058716, 1:3000)
Anti-His antibody (Beyotime, cat. no. AH367, clone no.AD1.1.10, lot no.011018180312, 1:100)
Anti-LC3B antibody (ThermoFisher Scientific, cat.no.PA1-16930, lot no.T12629311, 1:1000; ThermoFisher Scientific, cat.no. 700712, clone no. 2H30L32, lot no. 2086347, 1:200)
Phospho-Erk1/2 Pathway Sampler Kit (Cell Signaling Technology cat.no.9911, 1:1000; Phospho-MEK1/2, clone no.41G9, lot no.18, Phospho-p44/42 MAPK, clone no.D13.14.4E, lot no.24)
Anti-GFP (Cell Signaling Technologies, cat. no. 2956, clone no. D5.1, lot no. 4, 1:1000)
Anti-BubR1 antibody (BD Transduction, cat. no. 612503, clone no. 9/BUBR1, 1:1000)
Anti-Huntingtin Protein antibody 2166 (Millipore, cat. no. MAB2166, clone no.1HU-4C8, lot no. 2943221, 1:1000)
Anti-polyQ antibody 3B5H10 (Sigma, cat. no. P1874, clone no. 3B5H10, lot no. 047M4820V, 1:1000)
Anti-HTT antibody (D7F7)XP (Cell Signaling Technologies, cat. no. 5656s, clone no. D7F7, lot no. 4, 1:1000)
The other HTT antibodies including 2B7, ab1 and MW1 were previously published and characterized by other groups, and they originally obtained from those groups. They were diluted at 1:1000 for Western-blots.

Validation

The anti-β-tubulin antibody has been validated for Western-blots of both human and mouse samples by many previous publications (e.g. PMID: 28869595). The anti-TUBB3 antibody has been validated for immunocytochemistry of human sample by many previous publications (e.g. PMID: 30545851). The anti-ATXN3 antibody has been validated for Western-blots of both human and mouse samples by many several publications (e.g. PMID: 28180282). The anti-Gapdh antibody has been validated for Western-blots of mouse sample by many previous publications (e.g. PMID: 31091447). The anti-NBR1 antibody has been validated for Western-blots of mouse in antibodypedia （https://www.antibodypedia.com/gene/8116/NBR1/antibody/3592759/PA5-54660). Anti-β-actin antibody has been validated for Western-blots of both human and mouse samples by many previous publications (e.g. PMID: 30459625). The anti-TBP antibody has been validated for Western-blots of both human and mouse samples by many previous publications (e.g. PMID: 28280206). The anti-SQSTM1 antibody has been validated for Western-blots of mouse sample by many previous publications (e.g. PMID: 28869595). The anti-spectrin antibody has been validated for Western-blots of both human and mouse samples by many previous publications (e.g. PMID: 28869595). The anti-Ncoa4 antibody has been validated for Western-blots of both human and mouse samples by many previous publications (e.g. PMID: 30630985). The anti-GST antibody and the anti-MBP antibody has been validated for in vitro pull-down and Western-blot by experimental data in this study (Fig 4a-b). Anti-His antibody has been validated for immunostaining by experimental data in this study (Fig 4c). The anti-LC3B antibody (ThermoFisher Scientific, cat.no. PA1-16930) has been validated for Western-blots of mouse sample by vender (https://www.thermofisher.com/cn/zh/antibody/product/LC3B-Antibody-Polyclonal/PA1-16930). The anti-LC3B antibody (ThermoFisher Scientific, ThermoFisher Scientific, cat.no. 700712) has been validated by previous publications for immunostaining in human and mouse cells (PMID: 29151587, 22622129). The anti-GFP antibody has been validated by previous literature (PMID: 31112137; PMID: 31067454; PMID: 30996031). The Phospho-Erk1/2 Pathway Sampler Kit have been validated for Western-blots of mouse sample by several previous publications (e.g. PMID: 29636449). The anti-BubR1 antibody has been validated for Western-blots of both human and mouse samples by many previous publications (e.g. PMID: 27528194). The anti-HTT antibody (D7F7)XP have been validated for Western-blots of both human and mouse samples by many previous publications.(e.g. PMID: 26863614, PMID: 23575829). The other HTT antibodies including 2166, 3B5H10, 2B7, ab1 and MW1 have been validated for Western-blots of both human and mouse samples by many previous publications (e.g. PMID: 25738228). The antibody 2166 has also been validated for immunostaining experiments in mouse samples by this study (Fig. 4d).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Some of the primary patient fibroblasts were obtained from HD patients (Q47, Q49, Q55) and healthy sibling (WT, Q19) controls in a Mongolian Huntington's disease family. The HD Q68 fibroblast line was obtained from Coriell Cell Repositories (Camden, NJ, USA). The PD line was obtained from an idiopathic Parkinson's disease patient, and the SCA3 line was obtained from a SCA3 patient with the ATXN3 expansion mutation (Q74). The studies were approved by The Ethic Community of Institutes of Biomedical Sciences at Fudan University (#28) for obtaining the HD and wild-type patient fibroblasts, and by Huashan Hospital Institutional Review Board at Fudan University (#174) for obtaining the PD and SCA3 patient fibroblasts. Verbal and written consent was obtained from patients. The procedures were in compliance with all relevant ethical regulations. The immortalized fibroblasts were generated by infection of lentivirus expressing SV40T. For generation of iPS cells (iPSCs), the primary fibroblasts were transduced with the retroviral STEMCCA polycistronic reprogramming system (Millipore, cat. no. SCR548). The iPSCs were confirmed positive for Tra-1-81, Tra-1-60, SSEA-4 and Nanog by immunofluorescence and flow-cytometry. All four vector-encoded transgenes were found to be silenced and the karyotype was normal. iPSC were cultured in E8 medium (ThermoFisher Scientific, cat. no. A1517001) on Matrigel (Corning, cat. no. 354277) surface. iPSCs were differentiated to Pax6-expressing primitive neuroepithelia (NE) for 10-12 days in a neural induction medium. Sonic hedgehog (SHH, 200 ng/ml) was added at days 10-25 to induce ventral progenitors. For neuronal differentiation, neural progenitor clusters were dissociated and placed onto poly-ornithine/laminin-coated coverslips at day

	26 in Neurobasal medium (ThermoFisher Scientific, cat. no. 21103049), with 1× B-27 (ThermoFisher Scientific, cat. no. 17504044), 1× N-2 (ThermoFisher Scientific, cat. no. 17504048), brain derived neurotrophic factor (BDNF, 20 ng/ml, Protech, cat. no. 450-02), glial-derived neurotrophic factor (GDNF, 10 ng/ml, Protech, cat. no. 450-10), insulin-like growth factor 1 (IGF1, 10 ng/ml, Protech, cat. no. 100-11) and Vitamin C (Sigma cat. no. D-0260, 200 ng/ml). The mouse striatal cells (STHdh) were obtained from Coriell Cell Repositories (Camden, NJ, USA). The HEK293T cells and the HeLa cells were originally obtained from American Type Culture Collection (ATCC). Atg5 WT and KO MEFs were from N. Mizushima.
Authentication	The HEK293T and HeLa cell lines were authenticated by Short Tandem Repeat (STR) profiling methods. The Atg5 WT and KO MEFs were obtained directly from the laboratory which generated these cell lines (N. Mizushima), and they were further authenticated by Short Tandem Repeat (STR) profiling methods comparing with primary cultured MEFs. The patient fibroblasts were obtained and cultured from patients, and they were not authenticated.
Mycoplasma contamination	The cells were tested every two months by a TransDetect PCR Mycroplasma Detection Kit (Transgen Biotech, cat. no. FM311-01) to ensure that they are mycoplasma free.
Commonly misidentified lines (See ICLAC register)	HeLa cells were used in the HTT-LC3 colocalization experiments, because it is commonly used cell line for autophagy experiments and it showed more distinct LC3 puncta than other cells that we have tested. In addition, it has high transfection efficiency.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	The fruitfly experiments used Drosophila Melanogaster, and adult virgin female flies were used for experiments at indicated days of age (ranging from 0 to 50 days old after eclosion). The mouse experiments used the C57BL/6 strain including both male and female of the desired genotype. For icv experiments, the age was 3 months +/- 0 days old; for ip-injection followed by testing of cortical HTT, the age was 5 months +/- 0 days old; for ip-injection followed by testing of striatal HTT, the age was 10 months +/- 3 days old; for ip-injection followed by behavioral analysis, the age was 10 months +/- 3 days old.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.

Human research participants

Policy information about studies involving human research participants

Population characteristics	Since we are testing the compounds in patient cells rather than patient groups, we do not have population characteristics of human participants. For each patient, we cultured many cells from them and treat different group of cells with different compounds to test their effects within each patient cell lines. In general, the patients were diagnosed base on symptoms and genetic testings, and they received no treatment at the time they provided dermal fibroblasts. The HD patients (Q47/Q19, 46-year-old female; Q49/Q19, 44-year-old male; Q55/Q19, 39-year-old male) and the healthy sibling control (Q19/Q19, 39-year-old female) were from a Mongolian family. The The SCA3 patient was a 32 years old female patient when providing the dermal fibroblasts. She first came to the clinic complaining with clumsy and slowness in the lower limbs and left upper limb for 1 year. Her father and grandfather had the same symptoms but had passed away. After a one year follow up, she developed unstable walking. She was diagnosed as spinocerebellar ataxia and confirmed by genetic testing with the repeat number of Q74/Q27 in ATXN3. The PD patient was a 74 years old male patient when providing the dermal fibroblasts. He developed tremor, rigidity and bradykinesia for 6 years. The symptoms started at age 68 and he was diagnosed as PD at age 69 and followed up in our center for 5 years. A panel containing 254 PD and related genes and PD MLPA were carried out in the patient but did not find any known mutations related to PD. DAT-PET CT found the decreased DAT binding in the right caudate nucleus and putamen.
Recruitment	The HD and SCA3 patients were recruited by clinical symptoms and confirmed with genetic testing. The PD patients were recruited by clinical symptoms and confirmed by follow-up visits of more than 5 years and DAT PECT-CT. The recruitment could be biased because only a few patients who see the collaborating doctor and want to donate dermal fibroblasts for potential future research were selected. This is typical for preclinical studies, and our study is comparing different compound treated groups within each of the cell line, and thus not influenced by patient-to-patient variations. Nonetheless, while we have tested multiple patient cells and obtained consistent results, it is still possible that some of the other patient cells show different results.