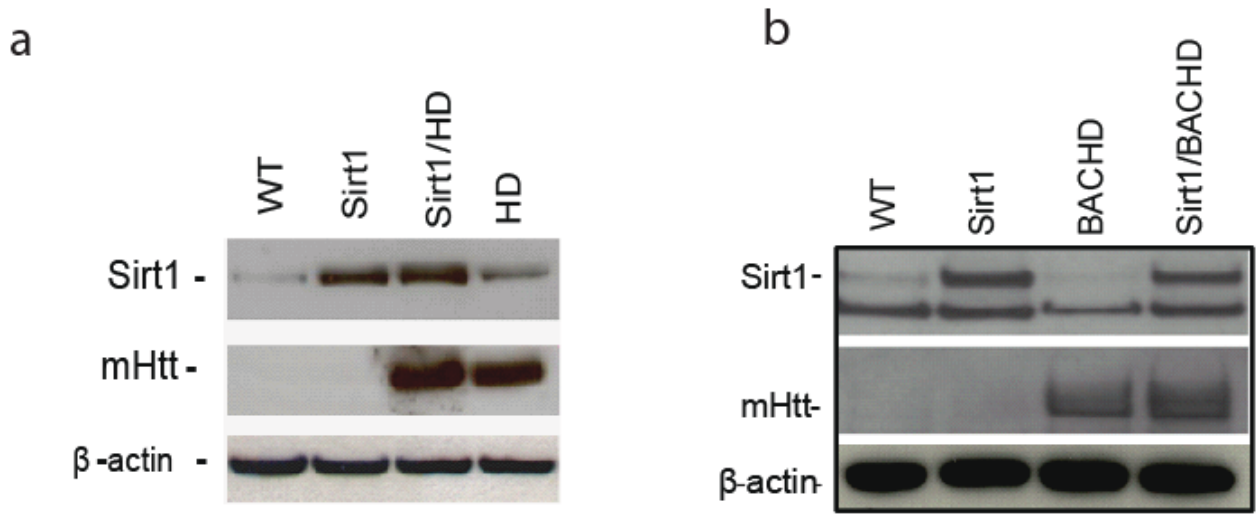
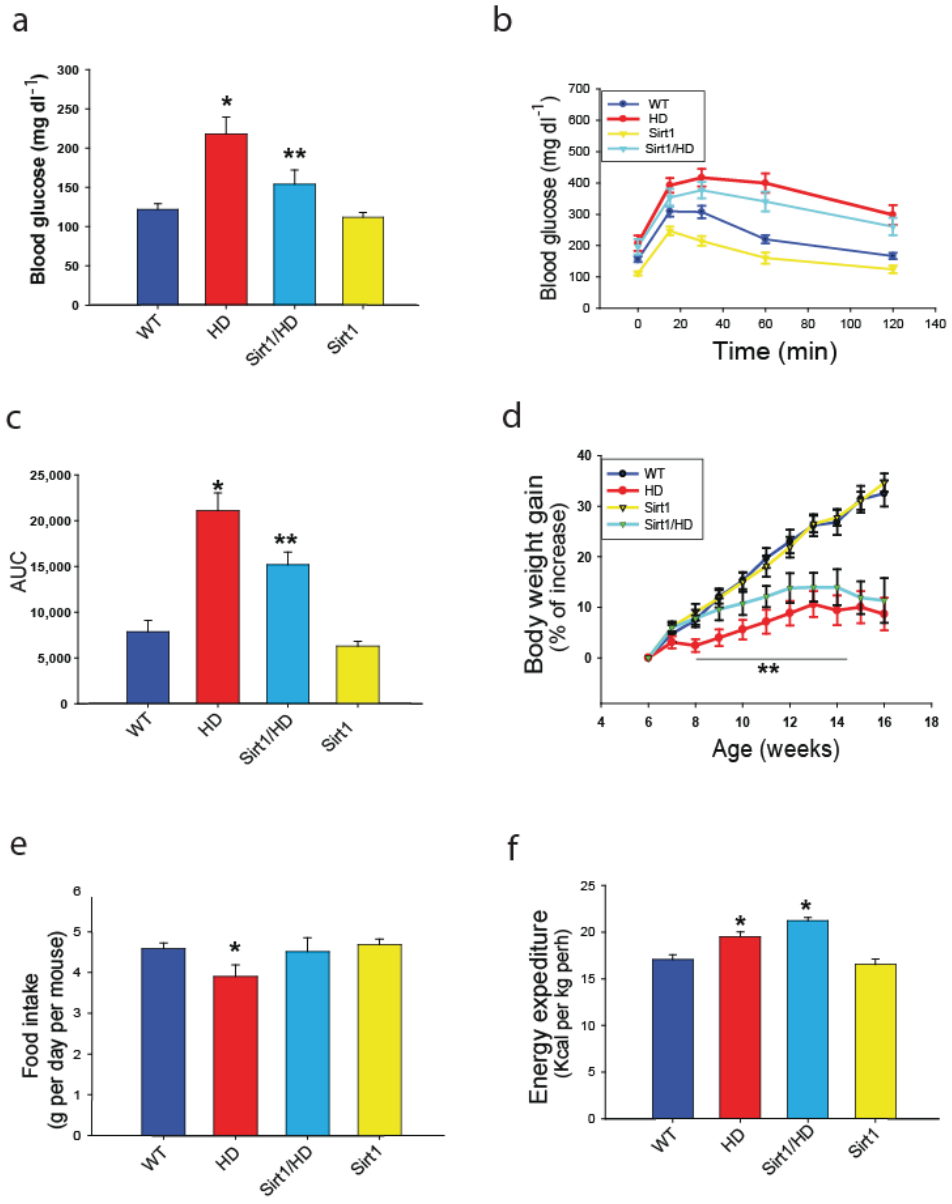
Supplementary Fig. 1



Supplementary. Fig. 1. (**a**) Sirt1 and mutant HTT (detected by HTT 81-90 antibody) protein levels were detected by Western blotting in cerebral cortex of N171-82Q mice. (**b**) Sirt1 and mutant HTT (detected by 1C2 antibody) protein levels were detected by Western blotting in cerebral cortex of BACHD mice.

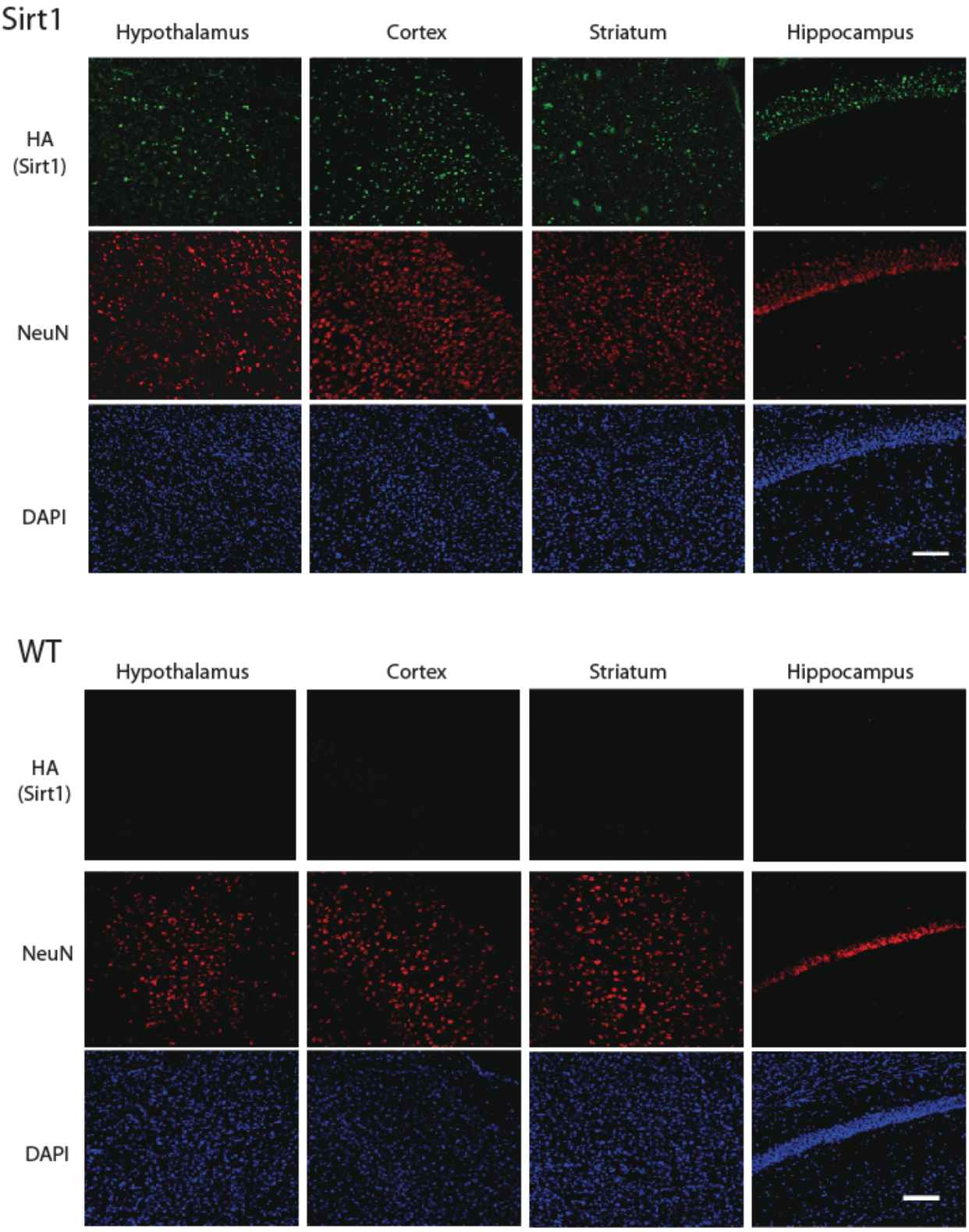
Supplementary Fig. 2



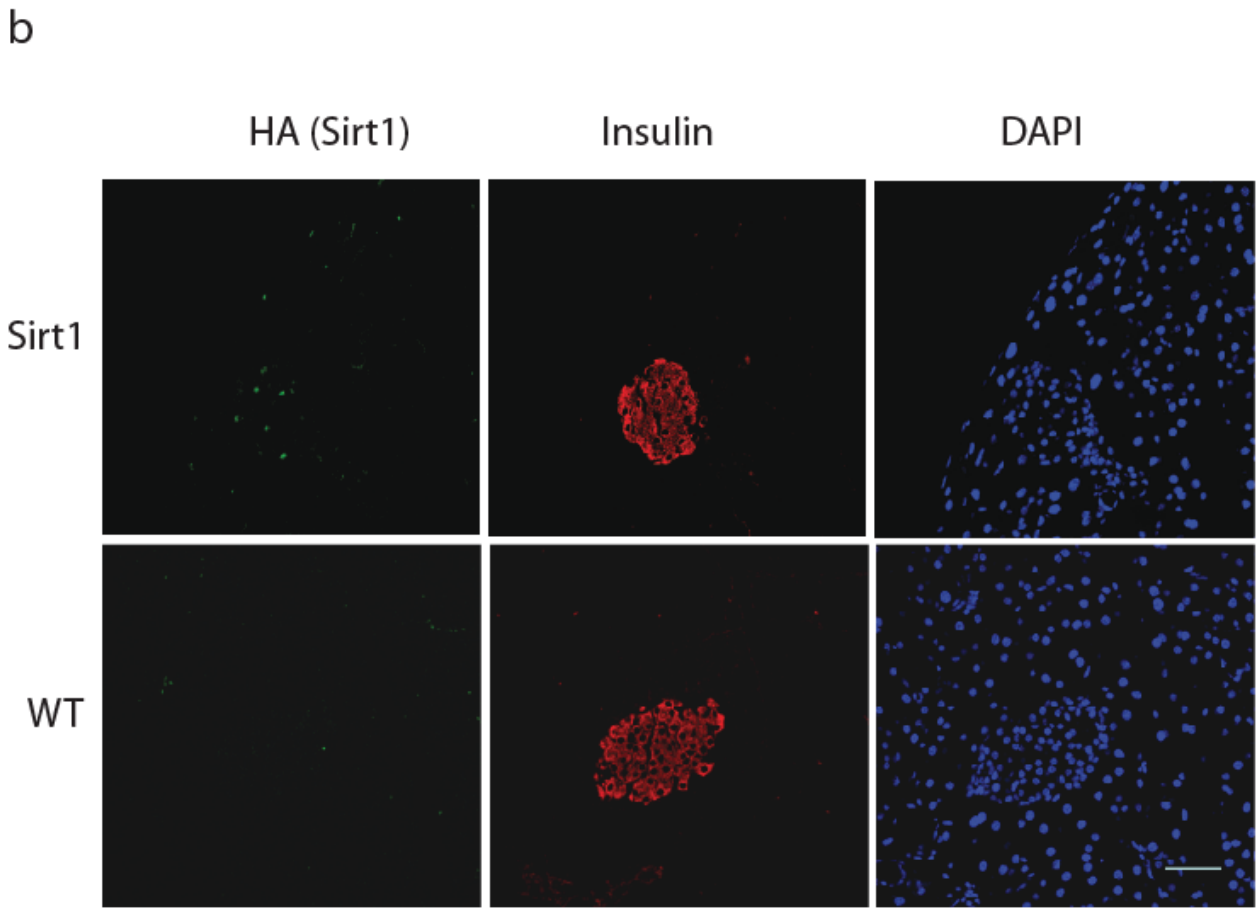
Supplementary Fig. 2. Sirt1 improves glucose tolerance and regulates energy metabolism in N171-82Q HD mice. (**a**) Blood glucose levels were measured after an overnight fast in 14-week-old mice. Mean ± S.E.M., *n*=10. (**b-c**) Mice were fasted overnight and then administered D-glucose (1g/kg, i.p); blood samples were taken before and at indicated times after glucose administration. The absolute values (b) and area under curve (AUC) (c). *n*=12. Mean ± S.E.M. (**d-f**) Effects of Sirt1 overexpression on body weight (d), food intake (e), energy expenditure (f) measured by an Oxymax metabolic system at 16 weeks old mice. Mean ± S.E.M.. *n*=12. *p<0.01 compared with the value of wild type (WT); **p<0.05 compared with the value of HD.

Supplementary Fig. 3

a

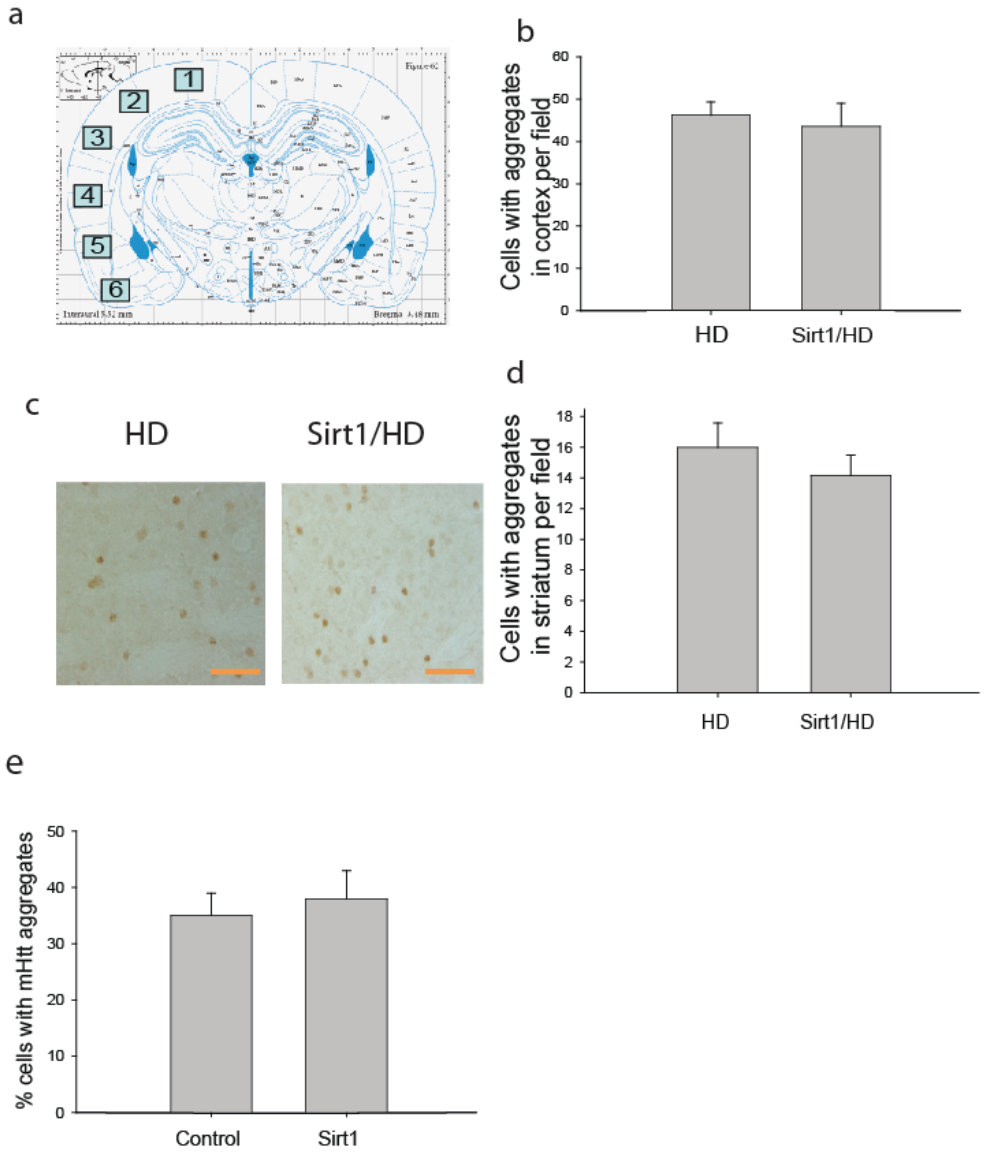


Nature Medicine doi:10.1038/nm.2558



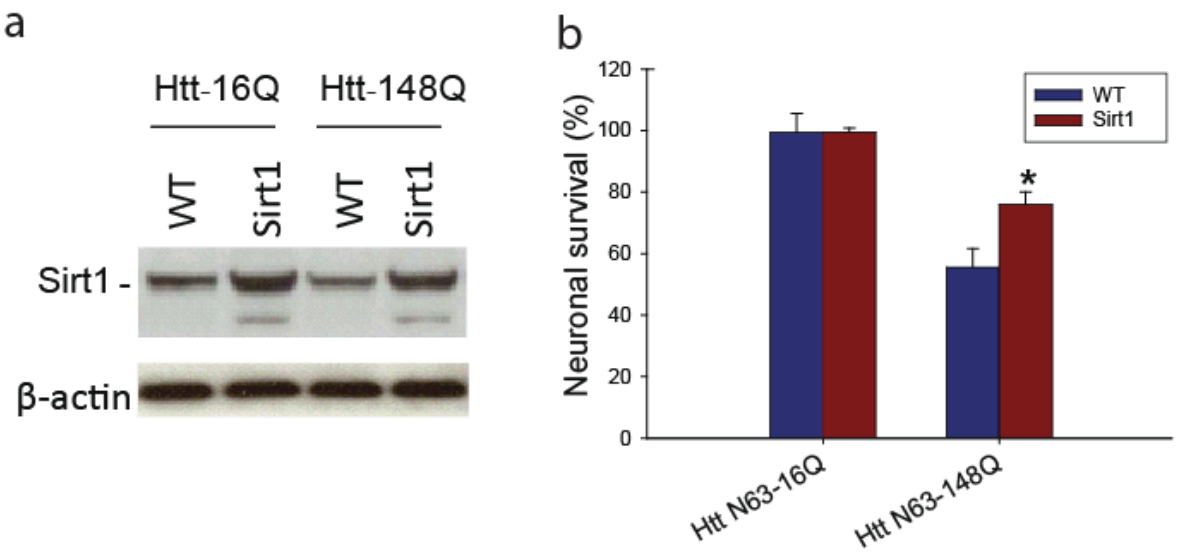
Supplementary Fig. 3 Transgene Sirt1 is highly expressed in the brain and barely detectable in the pancreas by immunofluorescent staining in Sirt1 transgenic mice. (**a**) Representative pictures of immunofluorescent staining of transgene Sirt1 (HA-tagged, green), NeuN staining (red), and DAPI in different brain regions from a Sirt1 trangenic mouse (Sirt1) and a wild type control mouse (WT). Scale bar 100 μm. (**b**) Representative pictures of immunofluorescent staining of transgene Sirt1 (HA, green), insulin (red), and DAPI (blue) in pancreatic islets of Langerhans from a Sirt1 transgenic mouse (Sirt1) and a wild type littermate control (WT). Scale bar 50 μm.

Supplementary Fig. 4



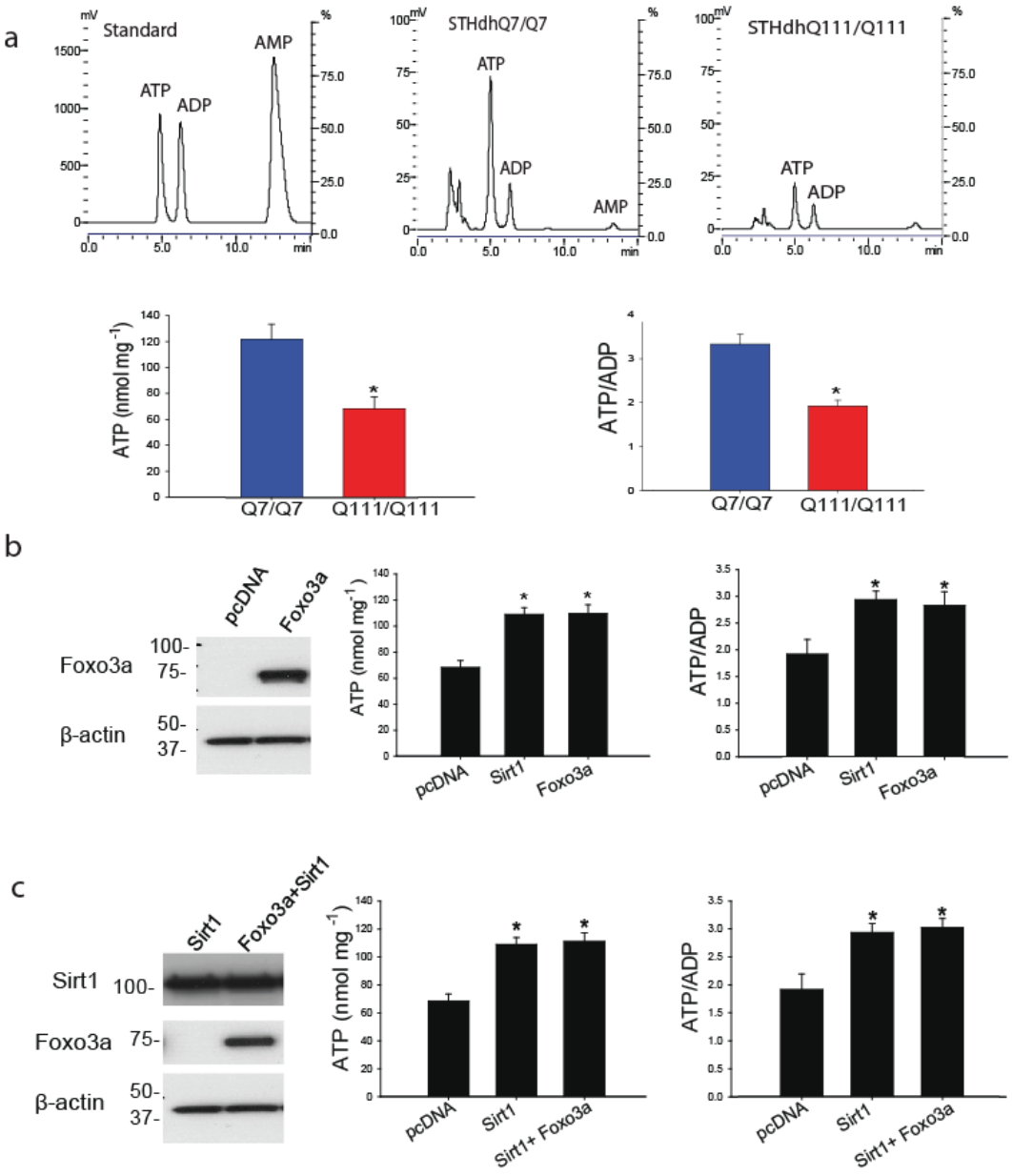
Supplementary Fig. 4. Sirt1 has no effects on mutant HTT aggregates in N171-82Q HD mice. (**a**) Diagram shows the areas of the cortex that were evaluated for HTT aggregates. (**b**) Average number of cells with mutant HTT aggregates in the cerebral cortex of mice in each microscope field. (**c**) Representative images from striatum immunostained with EM48 antibody. Scale bars 50 µm. (**d**) Average number of cells with mutant HTT aggregates in the striatum of mice in each microscope field. Mean ± S.E.M. from 5 mice per group. (**e**) N-terminal mutant HTT (N63-148Q-myc) expression was induced by withdrawal of doxycycline from the medium, and Sirt1 overexpression was introduced by retrovirus at the same time as mutant HTT induction. Cells were fixed for immunofluorescent staining at 48 h after induction of mutant HTT expression. Mutant HTT was labeled with myc antibody (myc-tagged N63-148Q). Mean ± S.E.M. from three independent experiments.

Supplementary Fig. 5



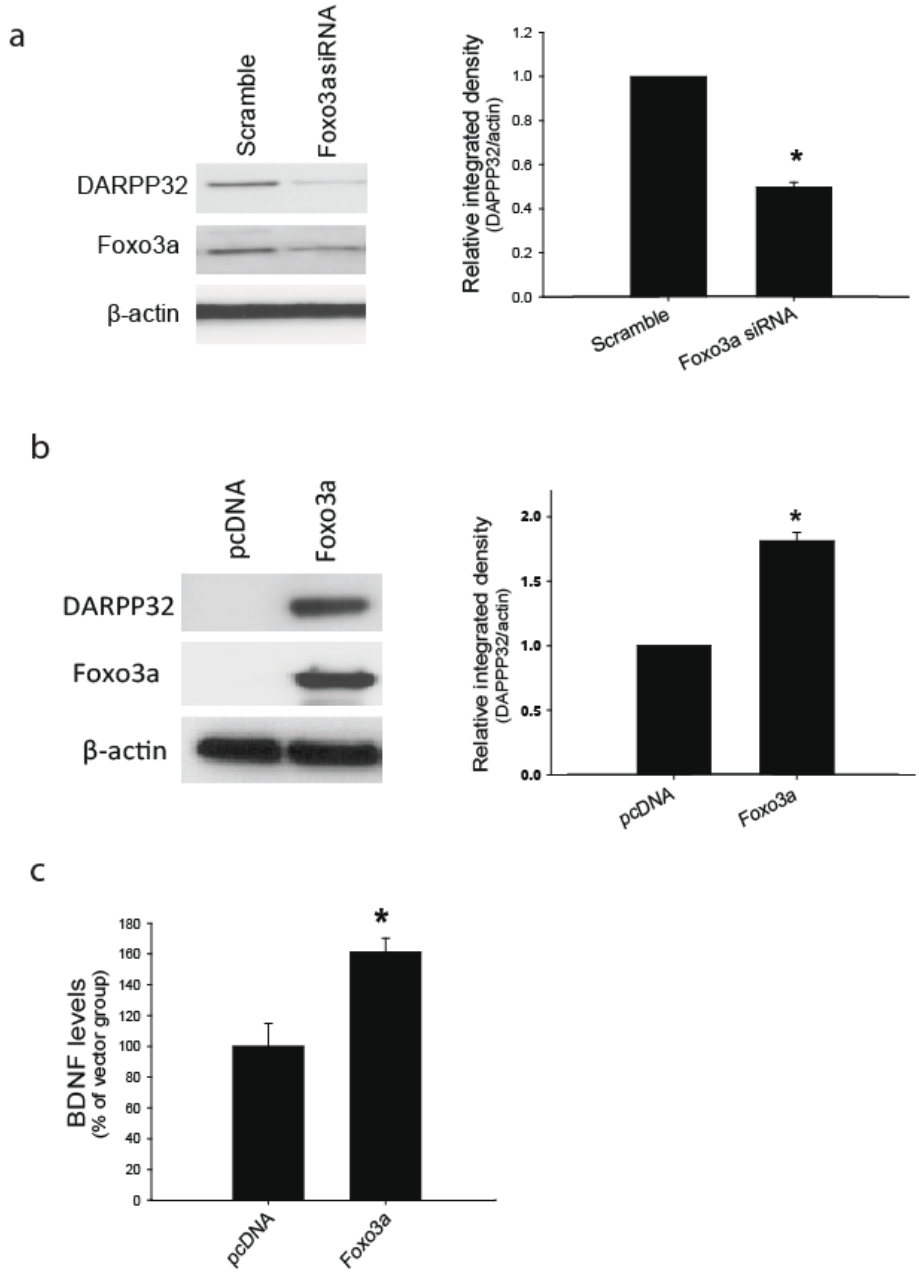
Supplementary Fig. 5. **Sirt1 increases neuronal resistance to mutant HTT toxicity**. (**a**) Primary cortical neurons were cultured from Sirt1 transgenic mice or wild type (WT) control mice. Levels of Sirt1 in primary cortical neurons were detected by Western blotting. (**b**) Neurons overexpressing Sirt1 are more resistant to mutant HTT-induced neurotoxicity. Cortical neurons were cultured from Sirt1 transgenic mice or wild type control mice, and transfected with mutant *HTT* (Htt-N63-148Q) or normal *HTT* (Htt-N63-16Q) fragment at DIV5, and neuronal survival was assessed at 48 h after transfection by nuclear DNA morphology. The numbers of surviving neurons in transfected neurons were measured. *$p<0.05$ *vs* the value of WT neurons transfected with HTT-N63-148Q.

Supplementary Fig. 6



Supplementary Fig. 6. Effects of Foxo3a and Sirt1 on mutant HTT-induced ATP deficits in STHdh $^{Q111/Q111}$ cells. **(a)** HPLC chromatograms showing adenosine nucleotides ATP, ADP, and AMP in wild-type STHdh $^{Q7/Q7}$ and quantification data showing decreased ATP levels as well as ATP/ADP ratio in mutant STHdh $^{Q111/Q111}$ cells. (**b**) Increase in Foxo3a had similar protective effects as Sirt1 on mutant HTT-induced ATP deficits in STHdh $^{Q111/Q111}$ cells. (**c**) Effects of overexpression of Foxo3a and Sirt1 on mutant HTT-induced energy deficits in STHdh $^{Q111/Q111}$ cells. Mean ± S.E.M from three independent experiments. *p<0.05 *vs* the values of pcDNA vector-transfected group by Student's *t*-tests.

Supplementary Fig. 7



Supplementary Fig. 7. Foxo3a regulates levels of DARPP32 and BDNF levels in cells expressing mutant HTT (STHdh $^{Q111/Q111}$ cells). (**a**) Knockdown of Foxo3a by siRNA decreased DARPP32 levels in STHdh $^{Q111/Q111}$ cells. Foxo3a siRNA or scramble controls were transfected into STHdh $^{Q111/Q111}$ cells and DARPP32 levels were determined at 24 h after transfection. *p< 0.01 *vs* scramble RNA transfected group. (**b-c**) Overexpression of Foxo3a increased levels of DARPP32 (b) and BDNF (c) in STHdh $^{Q111/Q111}$ cells. Foxo3a cDNA or vector control (pc DNA) was transfected into cells, and levels of DARPP32 and BDNF were determined at 48 h after transfection. *p<0.05 *vs* pc DNA vector group by Student's *t*-tests.

Supplementary Methods

Mutant HTT aggregates immunostaining Brain sections were cut at 40 μm and immunostained with an EM48 antibody (mouse monoclonal antibody 1:100. a gift from S. Li and X. Li at Emory University), which preferentially recognizes mutant HTT aggregates. The cell numbers with mutant HTT inclusions in the striatum and cortex were counted by a person blinded to the groups. N63-148Q PC12 cells were transduced with Sirt1 retrovirus, 48 h after transduction, cells were fixed by 4% paraformaldehyde and immunofluorescent staining was carried out with following antibodies to c-myc (for mutant HTT, as N63-148Q is myc tagged, 1:300, Oncogene), Sirt1 (1:1000, Upstate Inc.). Pictures were taken and percentage of cells with mHTT aggregates was calculated.

Foxo3a overexpress/siRNA STHdh cells were planted in 6-well plates at the day before transfection. Cells were then transfected with Lipofectamine TM 2000 (Invitrogen) according to the manufacturer's instructions. Briefly, 2 μg Foxo3a pcDNA plasmid (Addgene) and 4 μl Lipofectamine TM 2000 were diluted in 250 μl of Opti-MEM medium respectively. For Foxo3a siRNA (Sigma), 200 pmol/well RNA and 4 μl/well Lipofectamine TM 2000 were diluted in 250 μl of Opti-MEM medium, respectively. After incubation for 5 min, DNA or siRNA and Lipofectamine TM 2000 were mixed and incubated for 20 min at room temperature. 500 μL of the mixture of DNA or RNA and LipofectamineTM2000 were added to cultures. The maintenance medium DMEM containing FBS was replaced at 4 h after transfection.

Coimmunoprecipitation for detecting mutant HTT and Sirt1 interaction BACHD mouse brain tissues were lysed in RIPA buffer (cell signaling) containing protease inhibitors (Sigma) on ice for 30 min. After adding 2 times volume of PBS, tissue lysates were immunoprecipitated using antibody to Sirt1 (1:1000, Upstate,) overnight, then washed with RIPA/PBS (1:2) buffer, and membranes were blotted with MW1 antibody (1:10000, gift from Dr. Paul H. Patterson) and antibody to Sirt1 (1:10000, Upstate).

Immunofluorescent staining in pancreas Animals were anesthetized using inhalation isoflurane (~1ml/430cm^3) before euthanization by decapitation. The pancreas was collected from each animal and fixed in formalin for 8-10 hours and then transferred into phosphate buffered saline (PBS). Subsequently, the pancreata were embedded in paraffin and sectioned at 5μm thickness. Pancreas sections were immunostained as described previously (Martin et al., 2009). Briefly, after antigen retrieval with citrate buffer (Invitrogen, Carlsbad, CA) at 98°C for 20 minutes, sections were blocked in 5% bovine serum albumin (BSA; Sigma, St. Louis, MO) for 1 hour, followed by incubation with insulin (1:500; Sigma) and SIRT(HA) (1:1000) antibodies diluted in 1% BSA overnight at 4°C. Subsequently, after washing in PBS, sections were incubated for 1 hour with fluorescent secondary antibodies (Alexa 488, Alexa 568, 1:1000, Invitrogen), and nuclei were counterstained with DAPI. No fluorescent staining was observed in any sections when primary antibodies were omitted. Images were collected using a confocal microscope LSM-710 (Carl Zeiss).

Blood glucose and insulin levels Mice were fasted overnight and blood samples were collected by venipuncture. Blood glucose concentrations were measured with a glucometer (Lifescan Inc, Milpitas, CA). The glucose tolerance test was performed on mice following an overnight fast: mice were given D-glucose (1g/kg, i.p), and the blood glucose concentration was measured in samples taken at 0, 15, 30, and 120 min after glucose administration. Serum insulin levels were measured by ELISA (Crystal Chem).

Energy expenditure Mice were initially housed (up to 1 week) in ventilated caging (Innovive) adapted to the local light:dark cycle. Mice were then weighed and moved to individual chambers of an Oxymax indirect calorimeter (Columbus Instruments). The chambers (4 x 8 x 7) are Lucite, with stainless steel or plastic floors. Daily body weight and food intake were monitored while the mice remained in the Oxymax. Energy production can be estimated by measuring the amount of O_2 consumed (i.e., oxidative phosphorylation). For fat and carbohydrates, this information comes from the respiratory exchange ratio (RER). RER is the ratio of VCO_2/VO_2 per unit of time.

Behavioral tests and Survival study Survival was monitored daily by two experienced operators (M. Jiang and Q. Peng). The mice were considered to be at the end of life when HD mice were unable to right themselves after being placed on their backs and initiate movement after being gently prodded for 30 sec. Motor behavioral performance was assessed with a rotarod apparatus (Columbus Instruments). The open field box was divided into 25 squares (5 × 5 cm each, San Diego Instrument). The mice were allowed to habituate for 10 min and then their activity was recorded for the following 10 min. The number of squares that the mice entered during the last 2 min of the recording session was determined. This short time at the end of the session was chosen in order to increase the habituation period, during which the activity is increased in normal animals because of their exploratory behavior. This increase in activity is the greatest during the first 20 min in a new environment.