Supporting Information

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SI Materials and Methods

Reagents. We obtained 2-Acetylphenothiazine (ML171), Apocynin, *tert*-Butylhydroquinone (tBHQ), glutathione (GSH), cyclohexamide (CHX), Hydrogen peroxide (H_2O_2), dihydroethidium (DHE), and MG132 from Sigma.

ROS Measurements. Cells were supplemented with 10 μM chloromethyl-dichlorodihydrofluorescein diacetate (CM-H2DCFDA; Invitrogen) for 30 min in full media, washed with PBS, trypsinized, and fluorescence was measured using FACS. To measure reactive oxygen species (ROS) in mouse tissues, snap-frozen organs were cut into 20 μm sections on a cryostat at −20 °C, washed with ice-cold buffer, mounted on precooled cover slides, and stained with 10 mM dihydroethidium (DHE) for 30 min. Representative sections from independent tissues were imaged with a Zeiss Axioplan2 microscope and analyzed using ImageJ mean-density calculation.

Mouse Strains, Cell Culture, and Transfection. Mouse strains were as described (1). HEK293, HEK293T, and mouse embryonic fibroblasts (MEF) cells were maintained as described (1). ST*hdh* cells were maintained at 33 degrees as described (2). ST*hdh*^{Q111}–Homologous to the E6-AP Carboxyl Terminus (HECT) domain and Ankyrin repeat containing E3 ubiquitin–protein ligase 1 (HACE1) or ST*hdh*^{Q111}–HACE1(CS) or murine stem cell virus (MSCV) cells were generated using retroviral infection as described (1). HACE1 knockdown (kd) in HEK293T was performed using RNAiMAX (Invitrogen) according to the manufacturer's instructions using Hs_HACE1_1 and Hs_HACE1_2 (FlexiTube, Qiagen). Nuclear factor erythroid 2-related factor 2 (*Nrf2*) kd in ST*Hdh* cells was performed with Lipofectamine2000 (Invitrogen) according to the manufacturer's instructions using ACUCAAAUCCCACCUUAAA and CAUGUUACGUGAUGAGGAU (Dharmacon). Neon (Invitrogen) was used for transfection of ST*Hdh* and for transfection of MEFs. Lipofectamine2000 (Invitrogen) was used for transfection of antioxidative stress response element (ARE)–luciferase and pRenilla (RL) vectors in all cell lines.

Vectors. GFP–HACE1 and HACE1–promoter–Luciferase vector were as described (3). HA–HACE1, HA–HACE1(C876S), HA–HACE1ΔANK, and HA–HACE1ΔHECT were as described (4). NQO1–ARE–luciferase was as described (5). MYC–NRF2 expression vector was as described (6). For *NQO1–ARE*, activity cells were plated and transfected in 12-well plates. Following treatments, cells were lysed and firefly and renilla luciferase activity was measured using Dual luciferase assay (Promega).

Western Blot and Cell Fractionation. Following treatment, cells grown in a 10 cm dish were washed twice with ice-cold PBS and then lysed with 500 μL 1% Nonidet P-40 buffer supplemented with phosphatase and protease inhibitors (Roche) and benzonase (Sigma). Samples were centrifuged for 15 min at maximum speed, and the supernatant was collected and analyzed by Western blot using anti-HSC70 (stress gene), anti-HA (Covance), anti-HACE1 (Sigma), and anti-eukaryotic Elongation Factor 2 (eEF2) (Cell Signaling) antibodies. The following antibodies were obtained from Santa Cruz: anti-GRB2, anti-NRF2, anti-Actin, anti-NQO1, anti-GCLC, anti–c-Jun, anti–c-Myc, and anti-GSH synthetase (GSS). Cell fractionation was performed using NE-PER kit (Thermo Scientific) according to the manufacturer's instructions.

Immunofluorescence. MEF cells were plated in coverslips and grown for 24 h. Cells were treated, washed with cold PBS, fixed with PFA 4% (wt/vol) for 20 min, permebilized with 0.1% Triton ×100 in PBS, blocked with 5% milk (wt/vol), incubated with anti-NRF2 (1:200) for 1 h, washed extensively with PBS, incubated with FITC-conjugated goat anti-rabbit (Molecular probes) (1:200) for 1 h, and washed extensively. Slides were mounted on coverslips and imaged using Axiovision epi-fluorescent microscope (Zeiss).

Cell Death Assays. Treated attached and detached cells were collected, stained with annexin V or annexin V and propidium iodide (PI) as indicated using FITC Annexin V Apoptosis Detection Kit I (BD pharmagen) according to the manufacturer's instructions. Cells were analyzed using FACS, and cell death was normalized to control. For comparing survival in serum starvation between ST*Hdh*^{Q7} and ST*Hdh*^{Q111} cells, we used the MultiTox assay (Promega) according to the manufacturer's instructions.

qRT-PCR. RNA was extracted from cells and analyzed as in ref. 1, and quantitative RT-PCR was performed and quantified as described (7). The following primers were used: *HACE1*, forward AGTTGCCCGAGGATAATGAAAC and reverse TCCACCGATCCACAATTTGCT; *NQO1*, forward GAAGAGCACTGATCGTACTGGC and reverse GGATACTGAAAGTTCGCAGGG; *Hace1*, forward TAAAGCAGGGGATTGCTGTACG and reverse ATTCACGCACAACGCCTTGA; *Nqo1*, forward AGGATGGGAGGTACTCGAATC and reverse TGCTAGAGATGACTCGGAAGG; and *Hmox1*, forward AGGTACACATCCAAGCCGAGA and reverse CATCACCAGCTTAAAGCCTTCT.

NQO1 and DNA Oxidation Staining. Paraffin embedded tissues were deparaffinized and rehydrated, followed by antigen retrieval in boiling citrate buffer. Primary rabbit polyclonal antibody for NQO1 (Abcam #Ab34173) was used at a concentration of 4.9 μg/mL and stained with horseradish peroxidase-based detection reagents. DNA oxidation was performed on formalin-fixed, paraffin-embedded tissues after deparaffinization and antigen retrieval. Immunohistochemistry staining was performed with antibodies against 8-oxo-dG (R&D Systems) using the alkaline–phosphatase–labeled streptavidin–biotin ABC kit (Vector Labs). Sections were counterstained with hematoxylin (Vector Labs). Images were acquired with an Axioplan2 microscope (Zeiss).

Clinical Material. Snap-frozen matched tissue specimens of human normal brain and Huntington disease (HD) brain were obtained from the Huntington Disease Biobank at the University of British Columbia. Details of genotype, age, sex, and postmortem interval are provided in Table S1.

Analysis of Hace1 in Mouse Tissue. Striatal and cortical tissues were microdissected from the brains of 3-, 6-, 9-, or 12-mo-old YAC128 and WT littermates and snap frozen. mRNA analysis was performed by qRT-PCR as in ref. 8. Protein analysis was performed as in ref. 3.

Statistics. Bar graphs represent mean and error bars represent SEM. Significant differences between samples were determined using Student *t* test.

1. Zhang L, et al. (2007) The E3 ligase HACE1 is a critical chromosome 6q21 tumor suppressor involved in multiple cancers. *Nat Med* 13(9):1060–1069.
2. Trettel F, et al. (2000) Dominant phenotypes produced by the HD mutation in STHdh (Q111) striatal cells. *Hum Mol Genet* 9(19):2799–2809.
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7. Malhotra D, et al. (2010) Global mapping of binding sites for Nrf2 identifies novel targets in cell survival response through ChIP-Seq profiling and network analysis. *Nucleic Acids Res* 38(17):5718–5734.
8. Ehrnhoefer DE, et al. (2013) p53 increases caspase-6 expression and activation in muscle tissue expressing mutant huntingtin. *Hum Mol Genet* 23(3):717–729.

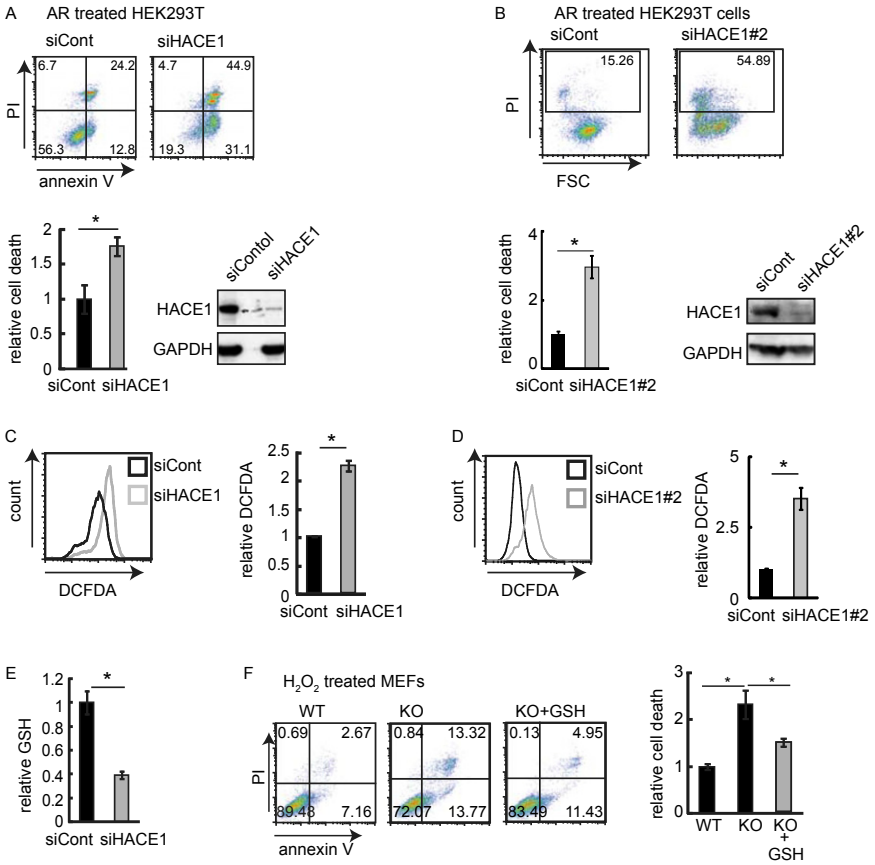

Fig. S1. HACE1 loss reduces antioxidative stress responses. (*A*) HEK293T cells were transfected with siRNA targeting HACE1 or control siRNA (siCont). Cells were treated with arsenite (AR) (10 μM) for 8 h, and cell death was determined with annexin V and PI staining. (*Upper*) Typical FACS plots. (*Lower Left*) Cell death relative to control. $n = 3$. $^*P < 0.05$. (*Lower Right*) Efficiency of siRNA was determined by Western blot. (*B*) Validation of experiment in Fig. S1*A*. HEK293T cells were transfected with an independent siRNA targeting HACE1 (siHACE1#2) or control siRNA as indicated. Cells were treated with AR (10 μM) for 8 h, and cell death was determined by PI staining. (*Upper*) Typical FACS plots. (*Lower Left*) Cell death relative to control. $n = 3$. $^*P < 0.05$. (*Lower Right*) Efficiency of siRNA was determined by Western blot. (*C*) HEK293T cells were transfected as in *A*. ROS levels were determined using DCFDA (10 μM; 45 min incubation in full media) and measured by FACS. (*Left*) Typical FACS histograms. (*Right*) Geometric means relative to siControl. $n = 3$; $^*P < 0.05$. (*D*) Validation of experiment in Fig. S1*C*. HEK293T cells were transfected with an independent siRNA targeting HACE1 (siHACE1#2) or control siRNA as indicated. ROS levels were determined using DCFDA (10 μM; 45 min incubation in full media) and measured by FACS. (*Left*) Typical FACS histograms. (*Right*) Geometric means relative to siControl. $n = 3$; $^*P < 0.05$. (*E*) HEK293T cells were transfected with siRNA targeting HACE1 or control siRNA (siCont). GSH levels were measured in lysates and presented relative to control. $n = 3$; $^*P < 0.05$. (*F*) Cells were pretreated with GSH or left untreated for 16 h. GSH media was replaced with normal media, and cells were challenged with H_2O_2 (400 μM; 8 h). Cell death was measured by annexin V and PI staining. (*Left*) Typical FACS plots. (*Right*) Relative cell death. $n = 3$; $^*P < 0.05$.

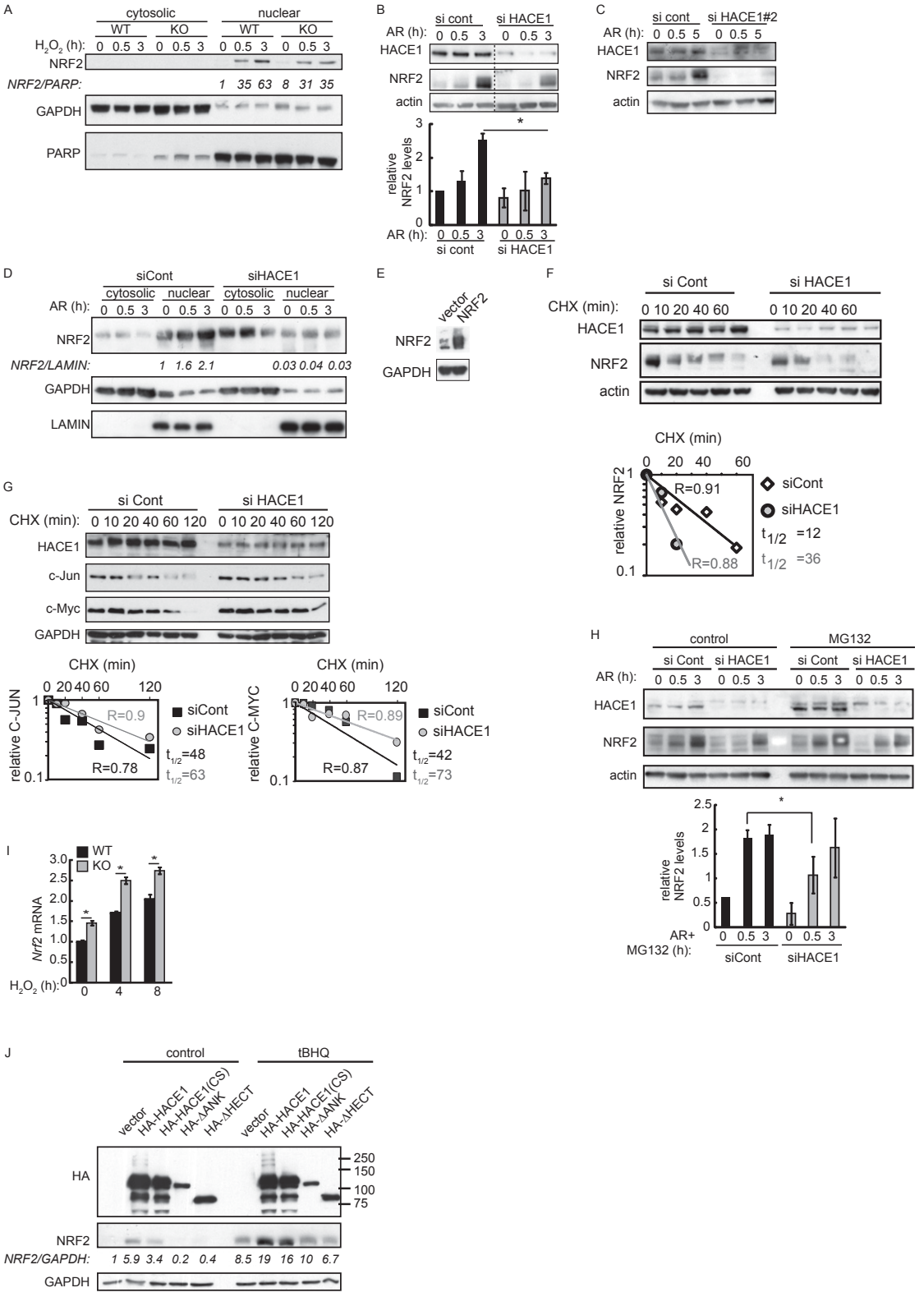

Fig. S2. HACE1 is important for stress-induced NRF2 accumulation and localization. (*A*) *Hace1* WT or KO MEFs were treated with H_2O_2 (200 μM) for the indicated time points. Cells were lysed and fractionated to isolate nuclear and cytosolic fractions as indicated. Fractions were confirmed using Western blot with GAPDH as a marker for cytosolic fractions and PARP for nuclear fractions. NRF2 localization was determined using anti-NRF2 antibodies and quantified relative to Poly(ADP-ribose) Polymerase (PARP). (*B*) HEK293T cells were transfected with siRNA targeting HACE1 or control siRNA as indicated. Cells were treated with AR (1 μM) for the indicated time points. Cells were lysed and NRF2 protein levels were determined using Western blot. NRF2 levels were normalized to actin. $n = 3$; $^*P < 0.05$. (*C*) Validation of experiment in S2B with an independent siRNA targeting HACE1 (siHACE1#2). (*D*) HEK293T cells were transfected with anti-HACE1 or control siRNA for 72 h. Cells were treated and analyzed as in *A*. Nuclear fractions were determined using anti-LAMIN A/C (LAMIN) antibodies and cytosolic fractions using anti-GAPDH antibodies. NRF2 levels were normalized to LAMIN. (*E*) Supporting Fig. 2*E*. *Hace1* WT or KO MEFs

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were transfected with a vector encoding MYC–NRF2 or empty vector. Western blot demonstrates NRF2 expression in transfected cells. (*F*) HEK293T cells were transfected with control siRNA (siCont) or HACE1-targeting siRNA (siHACE1) for 72 h. Cells were pretreated with AR (1 μM) for 15 min and then treated with CHX (10 μM). Cell lysates were collected at the indicated time points, and NRF2 levels were determined using Western blot (*Upper*) and normalized to actin levels and time 0 (*Lower*). (*G*) HEK293T cells were transfected with siRNAs targeting HACE1 or with control siRNAs. Cells were treated with AR (1 μM; 15 min). Cells were pulsed with CHX (10 μM) for the indicated times. c-JUN and c-MYC levels were determined using Western blotting (*Upper*), and indicated protein levels were quantified and normalized to GAPDH (*Lower*). (*H*) HEK293T cells were transfected with siRNA targeting HACE1 or control siRNA as indicated. Cells were treated with AR (1 μM) or AR and MG132 (10 μM) for the indicated time points. Cells were lysed and NRF2 protein levels were determined using Western blot. NRF2 levels were normalized to actin. $n = 3$; $^*P < 0.05$. (*I*) *Hace1* WT or KO MEFs were treated with H_2O_2 (200 μM) for the indicated time points. *Nrf2* mRNA levels were measured using qRT-PCR. (*J*) HEK293 cells were transfected with the indicated vectors for 48 h. Cells were treated with tBHQ (10 μM) for 3 h as indicated. Cell lysates were analyzed by Western blot using the indicated antibodies.

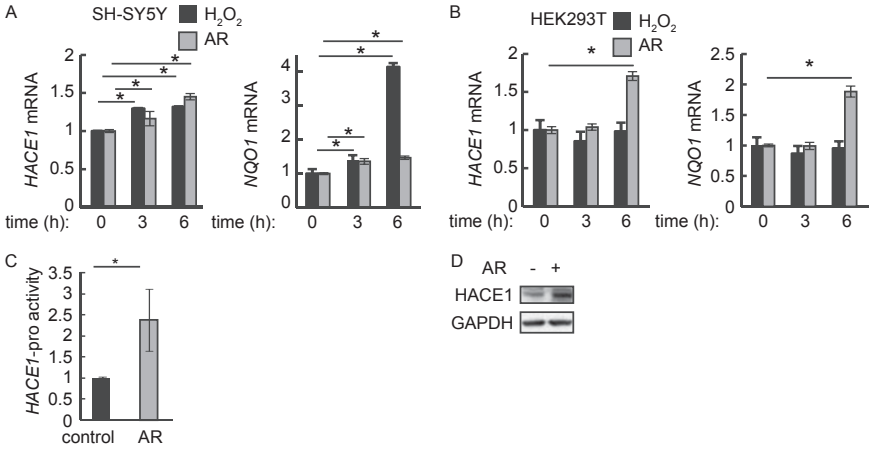

Fig. S3. *HACE1* is an oxidative stress response gene. (*A*) SH-SY5Y cells were treated with H_2O_2 (200 μM) or AR (1 μM) for the indicated time points, and *HACE1* and *NQO1* mRNA expression was determined using qRT-PCR. $n = 3$; $^*P < 0.05$. (*B*) HEK293T cells were treated with H_2O_2 (200 μM) or AR (1 μM) for the indicated time points, and *HACE1* and *NQO1* mRNA expression was determined using qRT-PCR. $n = 3$ $P < 0.05$. (*C*) HEK293T cells cotransfected with the *HACE1*–LUC vector along with pRenilla vector as transfection control and were treated with AR (1 μM) for 8 h as indicated. Luciferase activity was determined using a plate reader. $n = 3$. $^*P < 0.01$. (*D*) HEK293T cells were treated with AR (1 μM) for 8 h as indicted. HACE1 protein levels were determined using Western blot.

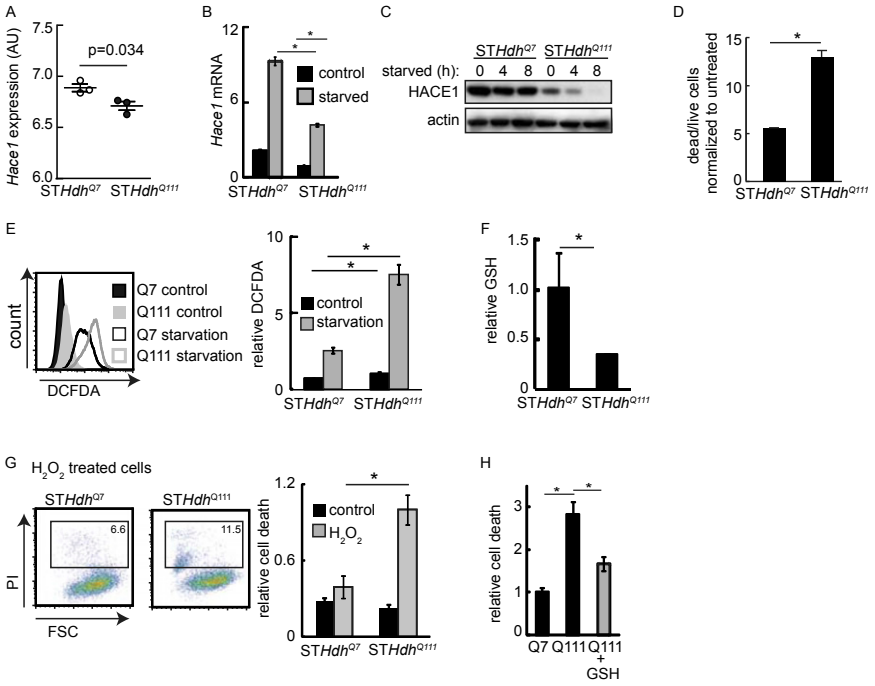

Fig. S4. Mutant Huntingtin (mHTT)-associated oxidative stress and toxicity is correlated with reduced HACE1 levels. (*A*) *Hace1* mRNA expression in ST*Hdh* cells obtained from published microarray experiment Gene Expression Omnibus Accession 2911 (1). (*B*) ST*Hdh* cells were serum starved for 6 h. *Hace1* mRNA levels were determined by qRT-PCR. Values were normalized to untreated ST*Hdh*^{Q7} cells. $n = 3$; $^*P < 0.01$. (*C*) ST*Hdh* cells were treated with serum starvation for the indicated time points. HACE1 protein levels were determined using Western blot. Actin was used as a loading control. (*D*) ST*Hdh* cells were left untreated or treated with serum starvation for 24 h. Cell death was measured using DualTox assay. $n = 3$; $^*P < 0.05$. (*E*) The indicated ST*Hdh* cells were treated with serum starvation or left untreated for 6 h. ROS were measured using DCFDA. (*Left*) Typical FACS histograms. (*Right*) Average geometric means relative to untreated *STHdh*^{Q7}. $n = 3$; $^*P < 0.05$. (*F*) GSH levels were determined in the indicated ST*Hdh* cell lines and normalized to *STHdh*^{Q7} values. $n = 3$; $^*P < 0.05$. (*G*) The indicated ST*Hdh* cells were treated with H_2O_2 (600 μM; 8 h) or left untreated. Cell death was measured using PI. (*Left*) Typical FACS plots. (*Right*) Cell death relative to treated *STHdh*^{Q111}. $n = 3$; $^*P < 0.05$. (*H*) ST*Hdh*^{Q111} cells were pretreated with GSH (1 mM) for 24 h. The media was replaced and the cells were challenged with H_2O_2 (600 μM; 8 h) or left untreated. Cell death was measured using PI. $n = 3$; $^*P < 0.05$.

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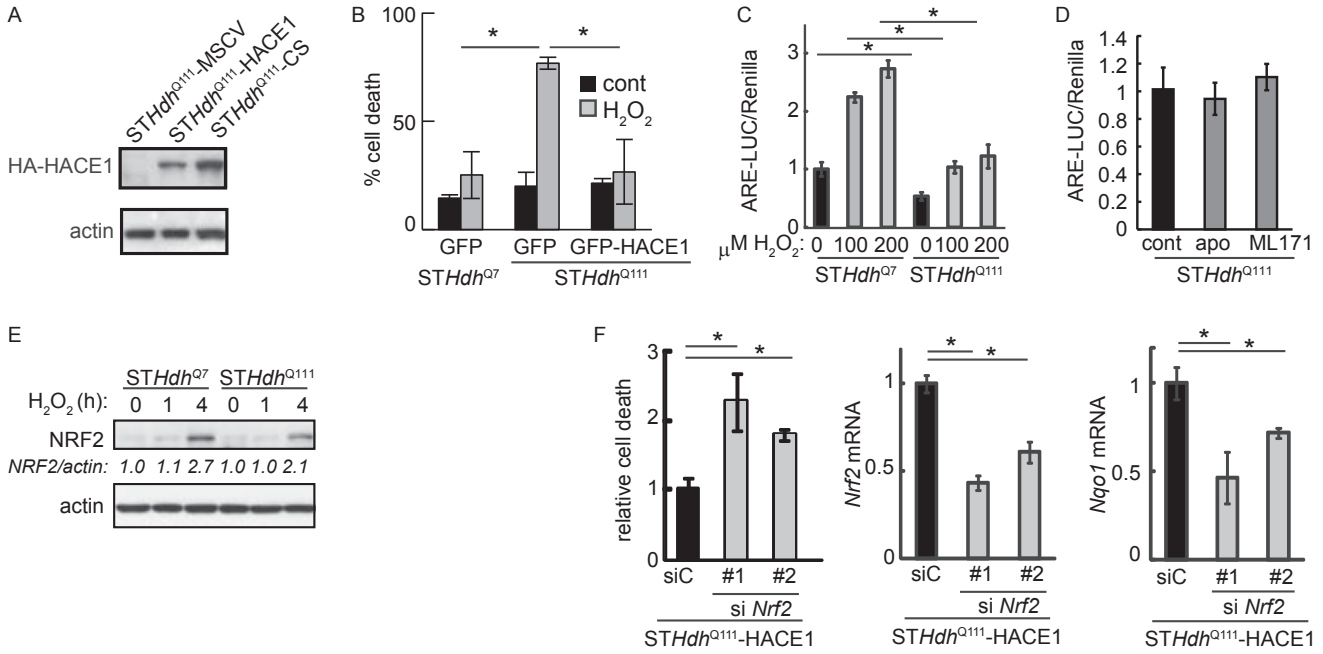

Fig. S5. HACE1 expression rescues ST*Hdh* cells from mHTT toxicity. (*A*) Stable HA–HACE1 or E3 ligase dead mutant HA–HACE1(C876S) (CS) expression was confirmed using Western blot. (*B*) The indicated ST*Hdh* cell lines were transfected with GFP or GFP–HACE1 vector as indicated for 24 h. The cells were treated with H_2O_2 (600 μM; 24 h), and cell death of the GFP-expressing cells was measured using PI. $n = 3$; $^*P < 0.05$. (*C*) The indicated ST*Hdh* cell lines were transfected with the NRF2 activity reporter ARE–LUC and pRL transfection control vectors for 24 h. The cells were treated with the indicated concentrations of H_2O_2 (8 h), and luciferase activity was measured using a plate reader. $n = 3$; $^*P < 0.05$. (*D*) STHdh^{Q111} cells were transfected as in *C* and were treated with 10 μM of the NADPH oxidase inhibitors 2-Acetylphenothiazine (ML171) or Apocynin (APO) for 3 h as indicated. NRF2 activity was determined using a plate reader. $n = 3$. (*E*) The indicated ST*Hdh* cells were treated with H_2O_2 (200 μM) for the indicated time points. NRF2 protein levels were determined using Western blot and normalized to actin. (*F*) STHdh^{Q111}–HACE1 cells transfected with control siRNA (siC) or NRF2-targeting siRNAs (si *Nrf2*#1 and *Nrf2*#2) were treated with H_2O_2 (400 μM; 24 h). (*Left*) Cell death was measured using PI staining and normalized to siC. (*Center*) NRF2 KD was confirmed by qRT-PCR. (*Right*) Functional validation of NRF2 KD was performed by analyzing mRNA levels of the NRF2 target *Nqo1* using qRT-PCR. $n = 3$; $^*P < 0.01$.

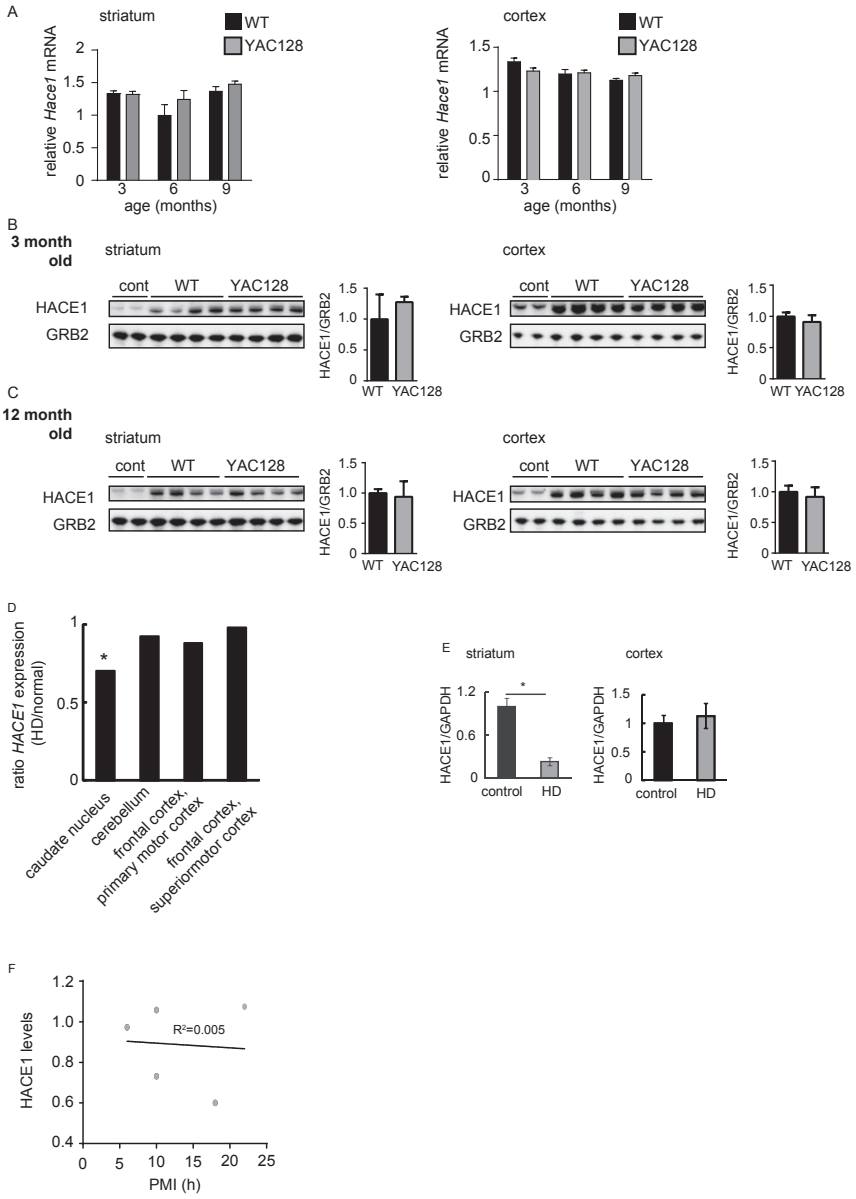

Fig. S6. HACE1 expression in HD brain. (*A*) *Hace1* mRNA expression in the indicated brain regions obtained from YAC128 (HD) and FVB (control) mice at the indicated ages. (*B*) Hace1 protein levels in the indicated brain regions obtained from 3-mo-old YAC128 and WT mice were determined using Western blot. Brain lysates from Hace1 KO mice were used as controls. Hace1 levels were normalized to GRB2. (*C*) Hace1 protein levels in the indicated brain regions obtained form 12-mo-old YAC128 and FVB mice were determined using Western blot. Brain lysates from Hace1 KO mice were used as controls. Hace1 levels were normalized to GRB2. (*D*) *HACE1* mRNA expression in human HD/control brain regions. Published microarray experiment (1). $*P < 10^{-6}$. (*E*) The levels of HACE1 protein in the Western blots presented in Fig. 5 were quantified and normalized to GAPDH. $*P < 0.01$. (*F*) The levels of HACE1 protein in the striatal samples obtained from control brains were plotted against their postmortem intervals and analyzed for correlation. There is no significant correlation; $P = 0.9$.

1. Hodges A, et al. (2006) Regional and cellular gene expression changes in human Huntington's disease brain. *Hum Mol Genet* 15(6):965–977.

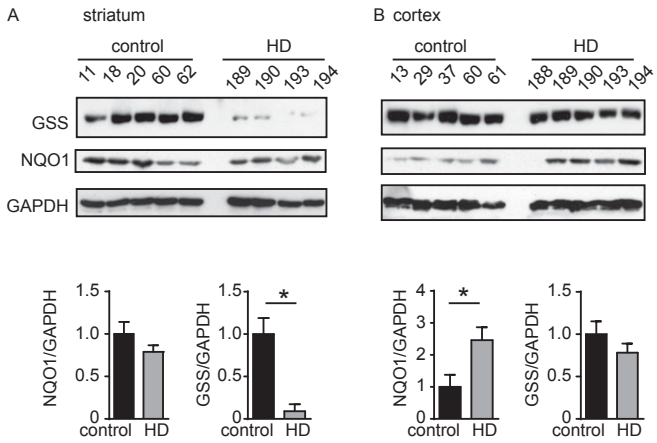

Fig. S7. Expression of NRF2 targets in HD striatum and cortex. The levels of the indicated proteins in human brain samples obtained from HD patients or controls were determined by Western blot and normalized to the loading control, GAPDH. (*A*) Striatum. (*B*) Cortex. *$P < 0.05$.

Table S1. Summary of frozen human postmortem striatal and cortical samples

Sample	HD grade	Htt CAG length, short/long	Age at death	Sex	PMI, h
HD-188	3	21/49	43	F	3.5
HD-189	3	23/44	63	F	5.5
HD-190	3	17/42	64	M	10
HD-193	3	17/53	53	F	9
HD-194	3	23/47	59	F	7
Mean ± SD			56.4 ± 8.6		7 ± 2.6
CTRL-11			65	M	22
CTRL-13			50	M	5
CTRL-18			76	M	18
CTRL-20			74	M	6
CTRL-29			74	M	2
CTRL-37			68	M	7
CTRL-60			46	M	10
CTRL-61			36	F	10
CTRL-62			29	F	4.5
Mean ± SD			57.6 ± 17.7		9.4 ± 6.6

Details of the human tissue used in this study including HD grade, HTT GAC repeat length, age at death, sex, and postmortem intervals (PMIs). Mean ages at death and PMI ± SD are shown and are not significantly different between HD and controls.