

## **Supplementary methods**

### **Primary culture of mouse cortical neurons**

Mouse cortical neurons were prepared from E17~18 mouse embryo according to the method described in a text book (Goslin et al.) with slight modification. Briefly, isolated cortex from E17-18 of ICR mice was cut into small pieces with razors on a glass dish, transferred into a tube, and dissociated by shaking with 10 ml HBSS containing 0.25% trypsin, 0.5% glucose and 1.2 kunit/ml DNase I at 37°C for 7 minutes. After addition of 5 ml of FBS and centrifugation, cells were suspended with MEM containing 2 mM L-glutamine, 0.6% glucose, 1 mM sodium pyruvate, 10% FBS and penicillin-streptomycin, filtrated with cell strainer (70 µm) (BD Falcon), and subjected to centrifugation. After re-suspension of cells with same medium, live cells were counted by trypan blue staining and seeded on 12-well plates coated with polyethyleneimine. After 3 hours, medium was replaced to neurobasal medium containing 2% B27 supplement, 0.5 mM L-glutamine and 25 µm glutamate. Transfection was performed after 4 days incubation

### **Protein purification and In vitro binding assay**

Transformed E. coli (BL21) carrying pGEX-6P -Nhtt (Htt exon 1) containing 18Q, 42Q or 62Q grown in LB medium was incubated with 0.1 mM isopropyl-beta-D-thiogalactopyranoside (IPTG) at 20°C for two overnights. The cells were suspended with buffer A containing 50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.035% β- mercaptoethanol, 10% glycerol. After addition of Triton-X 100 (final conc. 1.2%) and 6 mg lysozyme, cells were sonicated and subjected to centrifugation at 12,000 rpm for 30 minutes. The supernatants were incubated with Glutathion Sepharose (Amersham) at 4 °C for 2 h. After washing the Sepharose with buffer A containing 5 mM Mg-ATP once and with buffer A twice, GST proteins were eluted with 16 mM glutathion / buffer A. The eluted proteins were dialyzed with buffer A for 2 h at 4 °C by using Slide-A-Lyzer Cassette (PIERCE). For purification of His-NF-YA, transformed E. coli (BL21) carrying pET-15b-NF-YA grown in LB medium was incubated with 1 mM IPTG at 37°C for 3 h, and lysed as described above. After centrifugation, pellets (inclusion bodies) containing His-NF-YA were resolved with buffer B

containing 8M urea, 50 mM Tris-HCl, pH 8.0, and incubated with TALON Metal Affinity Resin (Clontech) for 1 h at room temperature. The resin was washed twice with 10 mM imidazole / buffer B, and then incubated with 200 mM imidazole / buffer B to elute the protein. The eluted protein was subjected to rapid dilution with 10 times volume of buffer A, concentrated with Amicon Ultra (MILLIPORE), and dialyzed with buffer A as described above.

For the binding assay, 5  $\mu$ g of GST-Htt protein in 50  $\mu$ l buffer A was incubated with 1  $\mu$ l of HRV-3C protease (Novagen) at 4 °C for 2 h to cut off Nhtt from GST, and then placed at 37 °C for 18 h. During these processes, Nhtt containing 42Q or 62Q but not 18Q formed aggregates. These proteins were co-incubated with 2 or 5  $\mu$ g of His-NF-YA at 4 °C for 2 h. After addition of SDS-sample buffer, the samples were filtered by cellulose acetate membrane with 0.2  $\mu$ m pore size (ADVANTEC), and washed with buffer containing 2% SDS, 50 mM Tris-HCl, pH8.0, 10% glycerol. Trapped proteins were detected by incubation with anti-Htt or anti-NF-YA, followed by HRP-conjugated anti-mouse or rabbit IgG, respectively. For the co-aggregation assay, 10  $\mu$ g of GST-Nhtt protein was pre-incubated with HRV-3C at 4 °C for 2 h, then co-incubated with His-NF-YA at 37 °C for 0-18 h. The aggregated proteins were detected as above.

### **TaqMan RT-PCR**

TaqMan primer and probe sets were designed and synthesized based on Primer Express software (Applied Biosystems). The nucleotide sequences of the primers are shown in supplementary Table I. Preparations of total RNA and cDNAs and TaqMan RT-PCR was performed using four R6/2 mice and four control mice as described previously (Oyama et al., 2006). All values obtained were normalized with respect to levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA.

### **In situ hybridization**

cDNAs for HSP70 (*hspa1a*) (1309-1926 bp in CDS), HSP40 (*Hdj1*) (1-1023 bp; full CDS) or EGFP were used as a template for *in vitro* transcription of digoxigenin-labeled riboprobes, which were used for *in situ* hybridization using 20  $\mu$ m brain sections of 12 week-old R6/2 mouse fixed

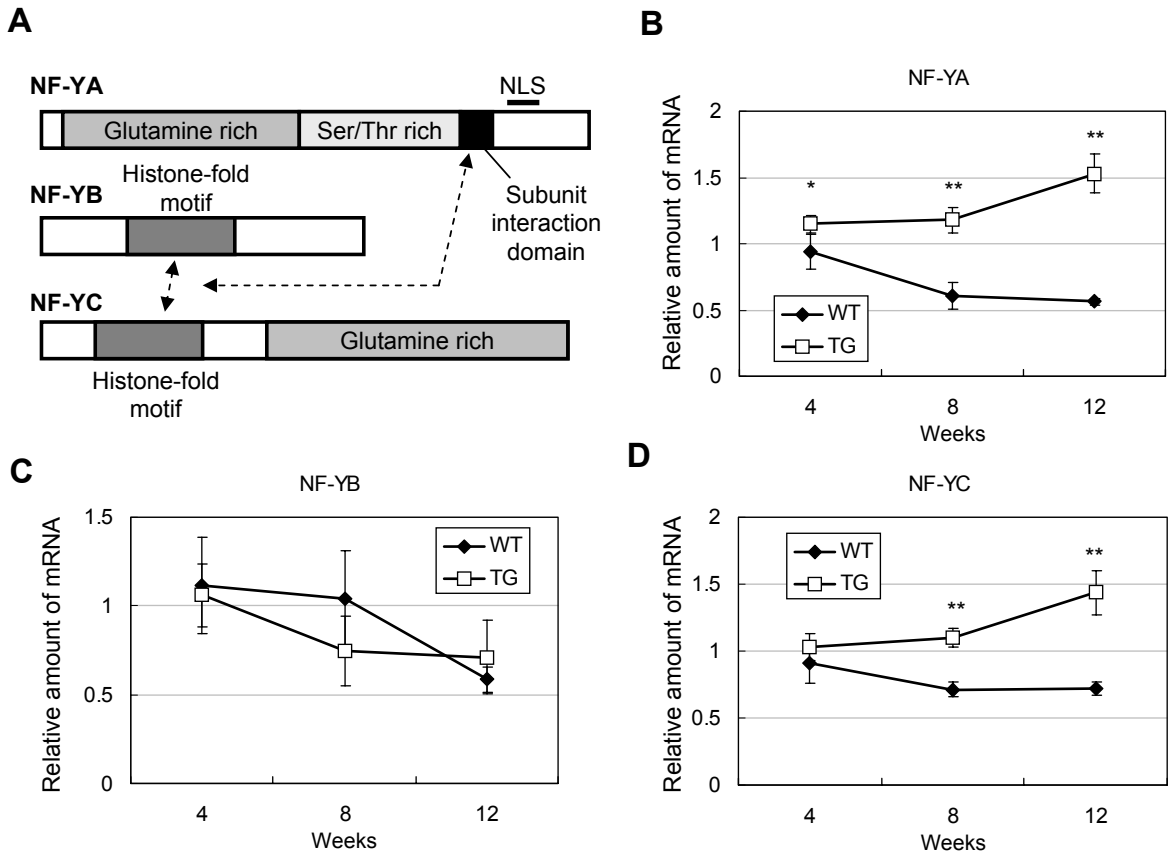
with 4% paraformaldehyde / PBS by perfusion as described previously (Kotliarova et al., 2005).

## **EMSA**

Isolated tissues (cortex and striatum) from mouse brains were homogenized with buffer C (20 mM Hepes, pH 7.9, 25% glycerol, 150 mM NaCl, 1.5 mM MgCl<sub>2</sub>, Complete protease inhibitor) using a glass homogenizer for ten strokes, after which NaCl was added to a final concentration of 420 mM. After incubation on ice for 20 minutes, the lysates were centrifuged at 800g for 5 minutes, and further clarified by centrifugation at 20,000g for 30 minutes. For probe preparation, sense oligonucleotides were labeled with <sup>32</sup>P by T4 DNA kinase and γ[<sup>32</sup>P]-ATP, purified with Sepharose G-50 spin column (Amersham), and were annealed with respective antisense oligonucleotides. Oligonucleotides used for this assay are listed in Table II. We incubated 10 or 20 μg of tissue lysate with <sup>32</sup>P-labeled probes at room temperature for 20 minutes. After adding loading dye, the reactants were subjected to native-PAGE using 4% polyacrylamide gel in 0.25 x TBE. The gel was dried, exposed to an imaging plate and analyzed by BAS-2500 (Fujifilm).

## **References**

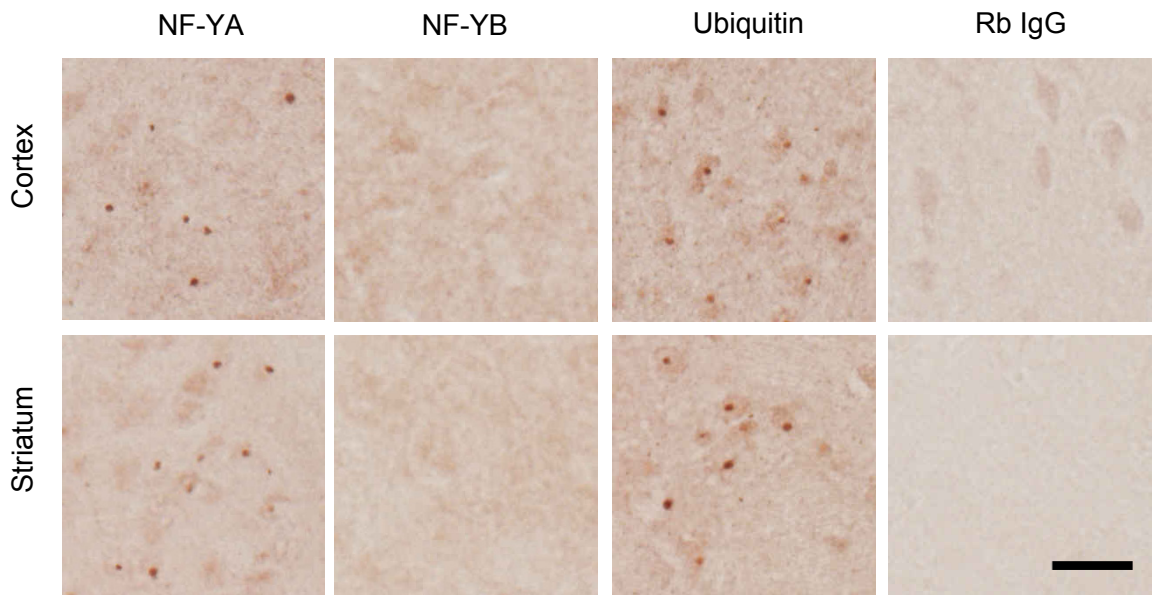
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### Supplementary Figure S1

#### Domain structures of NF-Y components and their mRNA expressions in R6/2 brain cerebrium

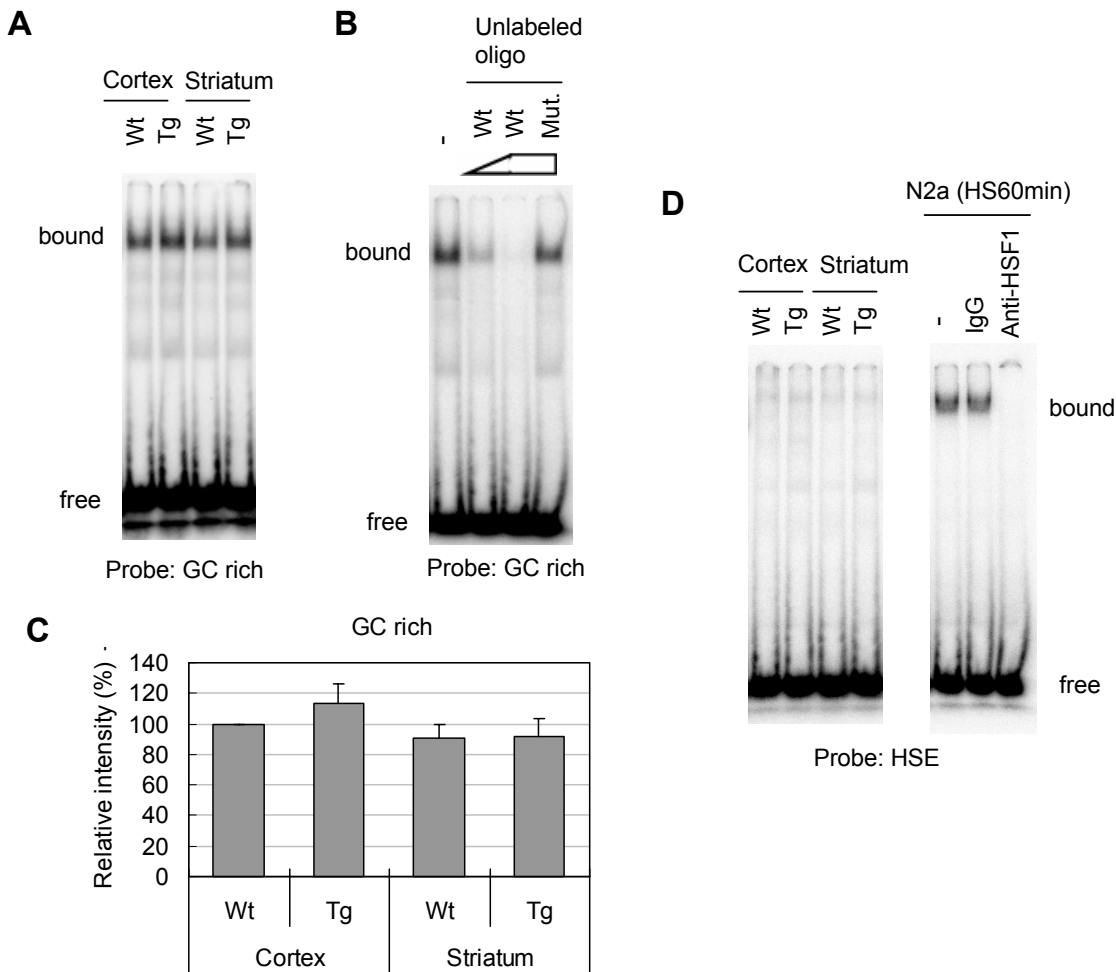
(A) Schematic representation of the structures of NF-Y components. NF-YB and NF-YC contain histone-like domains required for their dimerization. NF-YA binds to this heterodimer through its subunit interaction domain. Two large Q-rich domains are present in NF-YA and NF-YC, which are suggested to be important for transcriptional activity of NF-Y. (B-D) Quantitative RT-PCR analysis of NF-YA (B), NF-YB (C), or NF-YC (D) in cerebrium from 4, 8 or 12 week-old R6/2 or control mice. The expressions of all three genes were gradually decreased in control mice cerebrium (WT), whereas the expressions of NF-YA and NF-YC, but not NF-YB, were progressively increased in R6/2 mice (TG) during this period. Values are means ( $\pm$ SD) of four independent experiments (\* $p$ <0.05, \*\* $p$ <0.01).



### Supplementary Figure S2

#### Antibodies against NF-YB did not show dot-like stainings in R6/2 mouse brain

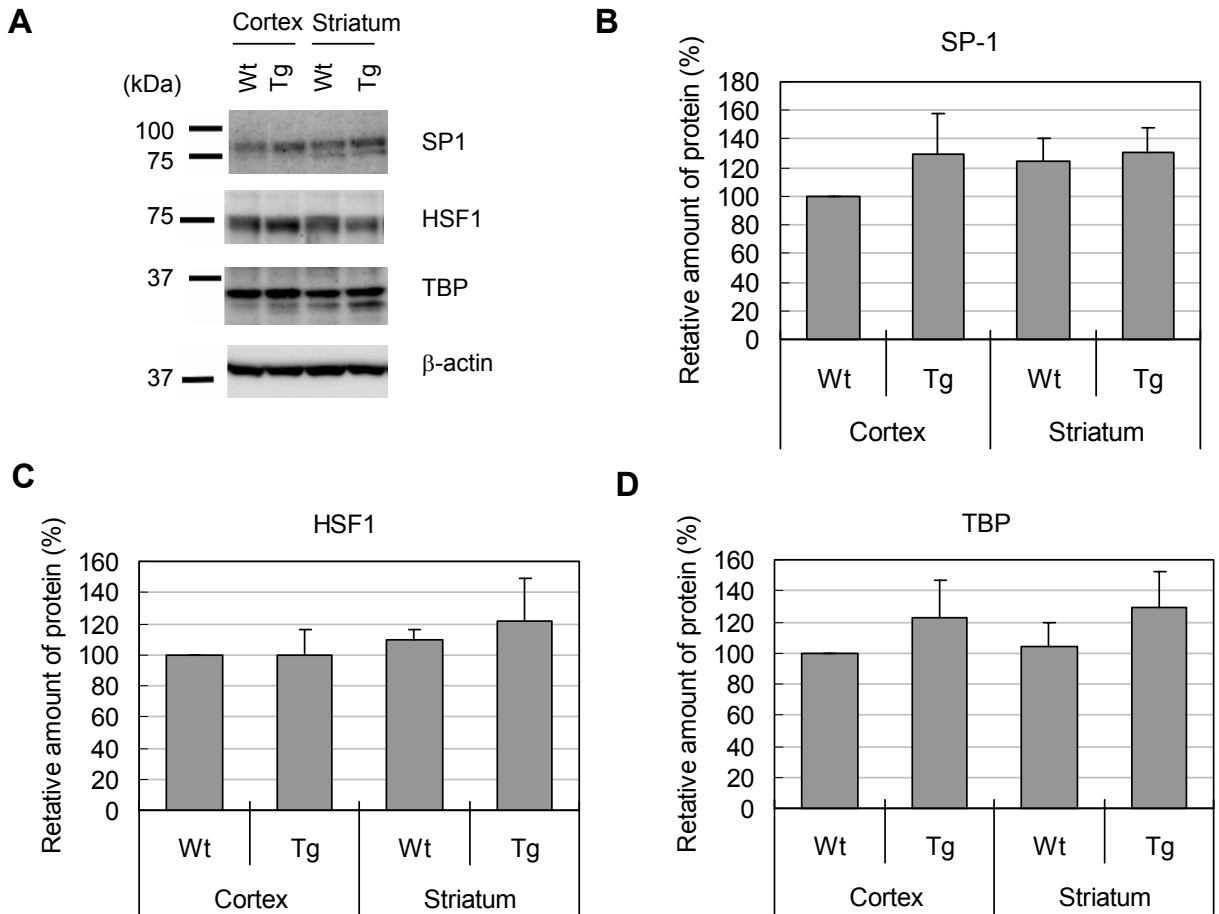
Coronal cryosections (20  $\mu\text{m}$ ) of brain prepared from perfusion fixed 12w R6/2 mice were stained with anti-NF-YA (sc-10779), anti-NF-YB, anti-ubiquitin (Z0458) or rabbit IgG. Dot-like stainings were observed by anti-NF-YA or anti-ubiquitin but not anti-NF-YB or rabbit IgG in cortex and striatum. Scale bar is 20  $\mu\text{m}$ .



### Supplementary Figure S3

#### EMSA using SP1 or HSE binding site as a probe

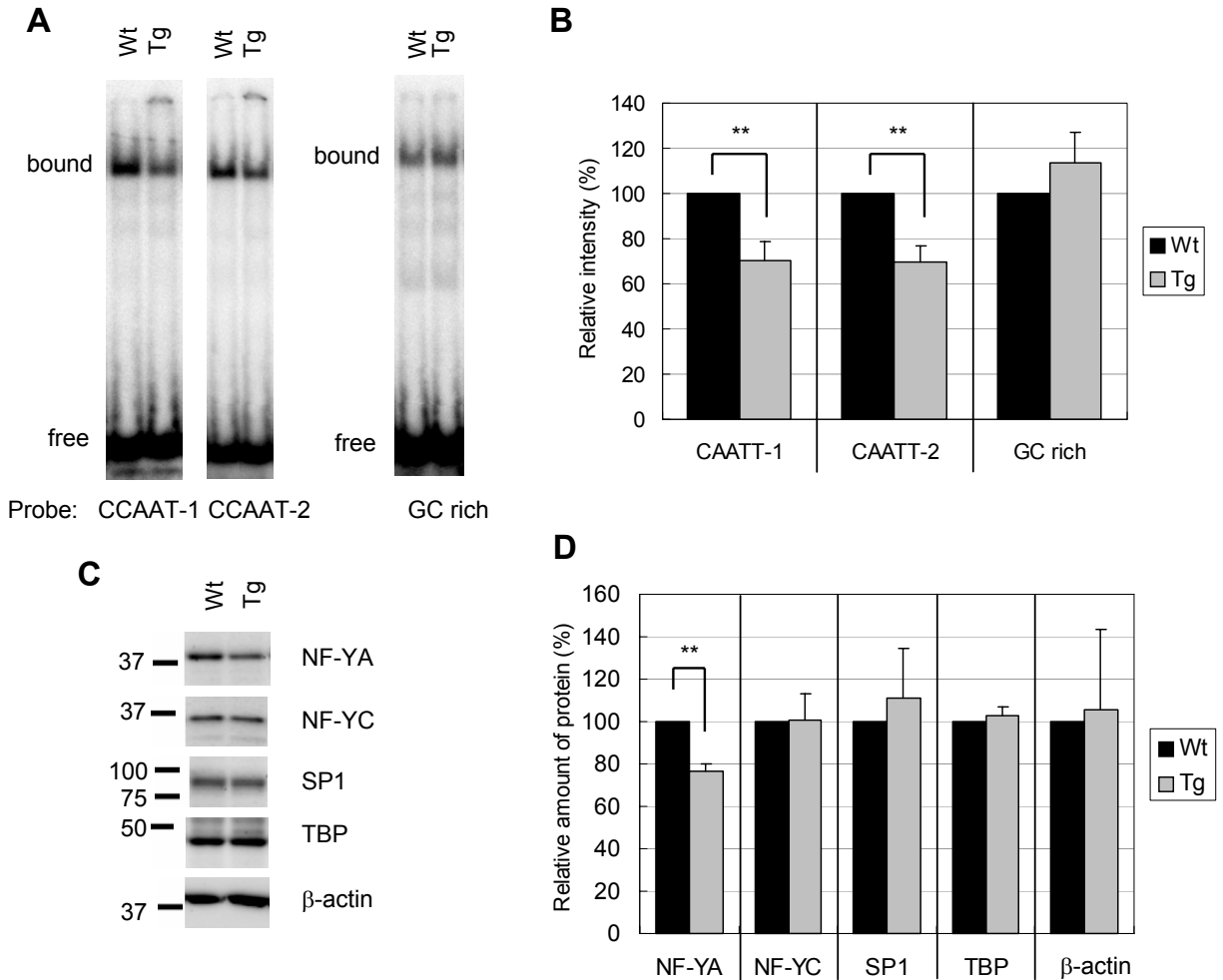
Cortex or striatum lysates prepared from the brains of 12 week-old R6/2 (Tg) or control mice (Wt) were subjected to EMSA. (A) EMSA using a GC rich region as a probe did not show any decrease in the amounts of DNA-protein complex in both lysates of R6/2 mice compared with those of control mice. (B) The amounts of these DNA-protein complexes were decreased by addition of 10 or 100-fold excess of unlabeled annealed oligo (Wt) but not 100-fold of excess of mutant oligo (Mut.). (C) Quantification of the amount of DNA-protein complex. Values are means ( $\pm$ SD) of three independent experiments. No significant difference in the amount of DNA-protein complex was observed between Tg lysates and Wt lysates. (D) EMSA using the HSE region as a probe did not show clear signals for DNA-protein complex in lysates from R6/2 or control mice (left panel). By using lysates from neuro2a cells treated with heat shock at 44°C for 60 minutes, a distinct band for the DNA-protein complex was observed, which disappeared by pre-incubation of lysates with anti-HSF1 but not control IgG.



### Supplementary Figure S4

#### Protein expressions of SP1, HSF1 and TBP in the lysates used for EMSA

(A) The lysates used for EMSA were subjected to Western blot analysis using anti-SP1, anti-HSF1, anti-TBP, and anti- $\beta$ -actin. (B-D) Quantification of the amount of SP1, HSF1 or TBP. Values are means ( $\pm$ SD) of three independent experiments. The protein expressions of these three proteins were not significantly changed in the lysates of R6/2 mice (Tg) compared with those of control mice (Wt).

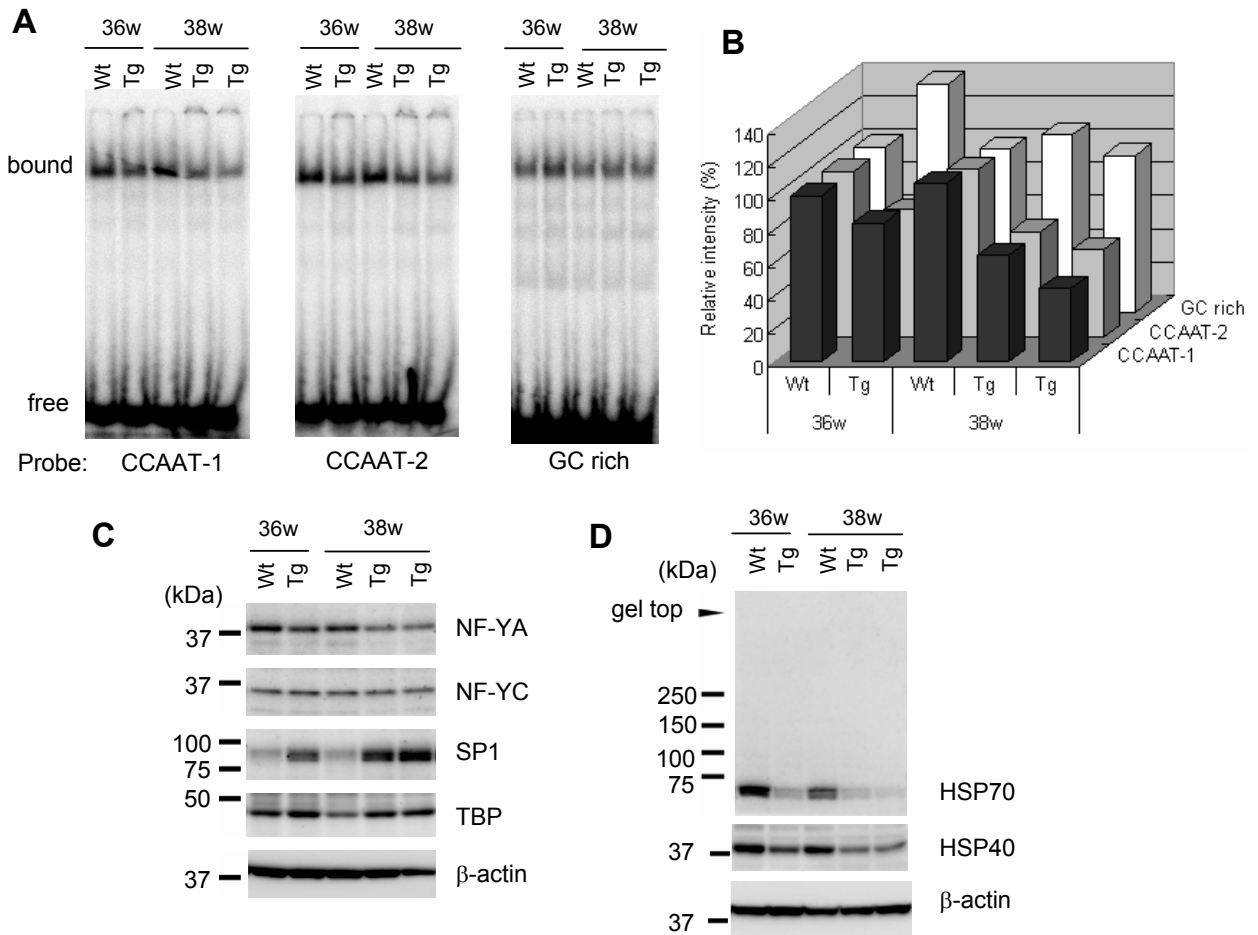


### Supplementary Figure S5

#### NF-Y binding to two CCAAT regions in HSP70 promoter was reduced in cortex of 8w R6/2 mouse brain

(A) Cortex lysates prepared from 8 week-old R6/2 (Tg) or control mouse (Wt) was subjected to EMSA using proximal CCAAT region (CCAAT-1), distal CCAAT region (CCAAT-2) or a GC rich region as a probe. (B) Quantification of the amount of DNA-protein complex. Note that NF-Y, but not SP1, binding to DNA was reduced in R6/2 cortex lysates. (C) The lysates used for EMSA were subjected to Western blot analysis using antibodies against NF-YA (YA1-3), NF-YC (YC5-3), anti-SP1, anti-TBP and  $\beta$ -actin. (D) Quantification of the amount of these proteins. The amount of NF-YA was specifically decreased in R6/2 cortex lysates. Values are means ( $\pm$ SD) of three independent experiments (\*\* $p$ <0.01).

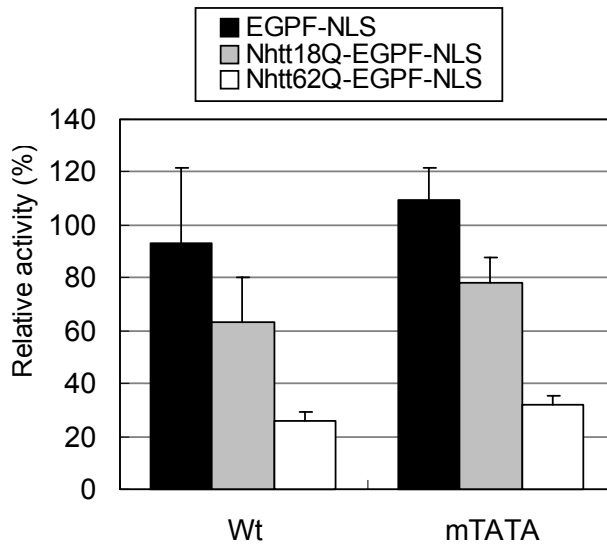




### Supplementary Figure S6

#### NF-Y binding to two CCAAT regions in HSP70 promoter was reduced in cortex of R6/1 mouse brain

(A) Cortex lysates prepared from 36 or 38 week-old R6/1 (Tg) or control mouse (Wt) was subjected to EMSA using proximal CCAAT region (CCAAT-1), distal CCAAT region (CCAAT-2) or a GC rich region as a probe. (B) Quantification of the amount of DNA-protein complex. Note that NF-Y, but not SP1, binding to DNA was reduced in R6/1 cortex lysates. (C) The lysates used for EMSA were subjected to Western blot analysis using antibodies against NF-YA (YA1-3), NF-YC (YC5-3), anti-SP1, anti-TBP and  $\beta$ -actin. The amount of NF-YA was decreased in R6/1 cortex lysates whereas the amounts of SP1 and TBP were increased in these lysates. (D) Total homogenates of cortexes from 36- or 38-week-old R6/1 or control mice were subjected to Western blot analysis using antibodies against HSP70 (sc-24), HSP40 or  $\beta$ -actin. Reductions of HSP70 and HSP40 proteins were observed in total lysates of R6/1 cortexes.



### Supplementary Figure S7

#### Effect of mutation in TBP binding site on mutant Htt-induced suppression of promoter activity of human HSP70 in primary cultured cortical neurons

*In vitro* cultured cortical neurons were transfected with indicated expression vectors together with reporter gene construct and CMV-LacZ vector. Six days after transfection, luciferase assay and  $\beta$ -galactosidase assay were performed. The values were luciferase activities normalized by  $\beta$ -galactosidase activities obtained from three independent experiments. Note that mutation of TBP binding site did not show obvious effect on mutant Htt-induced suppression of HSP70 promoter activity.

**Supplementary Table I. The nucleotide sequences of the primer for RT-PCR**

mRNA	Forward Primer	Reveres Primer	TaqMan Probe*
GAPDH	TGTGTCCGTCGTGGATCTGA	CCTGCTTCACCACCTTCTTGA	TGCCGCTGGAGAAACCTGCC
HSP70	GGTGGTGCAGTCCGACATG	TTGGGCTTGTGCGCCGT	CACTGGCCCTTCCAGGTGGTGAA
NF-YA	ACGAAGGAAATACCTCCATGAGTC	CTTCCCCACGCTTCCGT	CGGCATCGGCACGCCATG
NF-YB	CTTCATCACGTCGGAAGCAA	AGAATATCCTCCCATTGATTGTC	CGAAAGGTGCATCAGGAGAAGCGGA
NF-YC	AAAGCGCCAGGAGGAGGT	GCGTGAAGTAGTACTGGACAGGC	CGCCAGTCTGTGACTCCTGCGG

\*Probes were labeled with FAM and TAMRA

**Supplementary Table II. The nucleotide sequences of oligos used for probes of EMSA or constructions of mutants of reporter genes**

Oligos*	Sense	Antisense
CCAAT-1 Wt	ATCGAGCTCGGTGATTGGCTCAGAAGGGA	TCCCTTCTGAGCCAATCACCGAGCTCGAT
CCAAT-1 Mut.	ATCGAGCTCGGTGAT <u>AC</u> GCTCAGAAGGGA	TCCCTTCTGAG <u>CG</u> TATCACCGAGCTCGAT
CCAAT-2 Wt	GCACTCTGGCCTCTGATTGGTCCAAGGAAGGCT	AGCCTTCCTTGGACCAATCAGAGGCCAGAGTGC
CCAAT-2 Mut.	GCACTCTGGCCTCTG <u>C</u> T <u>C</u> GGTCCAAGGAAGGCT	AGCCTTCCTTGGAC <u>CG</u> AGCAGAGGCCAGAGTGC
GC rich Wt	TCAGAAGGGAAAAGGCGGGTCTCCGTGACGACT	AGTCGTCACGGAGACCCGCCTTTTCCCTTCTGA
GC rich Mut.	TCAGAAGGGAAAAG <u>T</u> <u>C</u> AGTCTCCGTGACGACT	AGTCGTCACGGAGACT <u>G</u> ACCTTTTCCCTTCTGA
HSE Wt	GAGGCGAAACCCCTGGAATATCCCGACCTGGC	GCCAGGTCGGGAATATCCAGGGGTTTCGCCTC
HSE Mut.	GAGGCGAAACCCCTG <u>C</u> TATATCCCGACCTGGC	GCCAGGTCGGGAATAT <u>A</u> GCAGGGGTTTCGCCTC
TATA Wt	TCTCCGTGACGACTTATAAAAGCCCAGGGGCAA	TTGCCCTGGGCTTTTATAAGTCGTCACGGAGA
TATA Mut.	TCTCCGTGACGACT <u>T</u> <u>C</u> GAAAGCCCAGGGGCAA	TTGCCCTGGGCTTT <u>G</u> <u>C</u> GAAAGTCGTCACGGAGA

\*Wt; wild type, Mut.; mutants. Mutated nucleotides were indicated by underlines.

**Supplementary Table III. Genes that contain CCAAT box in the proximal promoter region and are decreased in 8 week-old R6/2 brain cerebrum**

<b>Gene Symbol</b>	<b>Gene Title</b>	<b>Ratio</b>
1110008P14Rik*	RIKEN cDNA 1110008P14 gene	0.41834
Ankrd43	ankyrin repeat domain 43	0.685073
Arhgef7	Rho guanine nucleotide exchange factor (GEF7)	0.39874
Camk2b	calcium/calmodulin-dependent protein kinase II, beta	0.589485
Cck	cholecystokinin	0.448094
Ccl27	chemokine (C-C motif) ligand 27	0.674027
Ccng1	cyclin G1	0.551094
Ckmt1	creatine kinase, mitochondrial 1, ubiquitous	0.559079
Cldn11	claudin 11	0.646895
Dbp	D site albumin promoter binding protein	0.46068
Fabp5	fatty acid binding protein 5, epidermal	0.58336
Fdft1	farnesyl diphosphate farnesyl transferase 1	0.672385
Gpc1	glypican 1	0.657399
Hbb-b2	hemoglobin, beta adult minor chain	0.528184
Hmgcs1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	0.656488
Hsp90b1	heat shock protein 90kDa beta (Grp94), member 1	0.649137
Kalrn	kalirin, RhoGEF kinase	0.548081
Lgals1	lectin, galactose binding, soluble 1	0.643316
Napa	N-ethylmaleimide sensitive fusion protein attachment protein alpha	0.634084
Ncdn	neurochondrin	0.802563
Nptx1	neuronal pentraxin 1	0.800541
Npy	neuropeptide Y	0.363332
Pacsin1	protein kinase C and casein kinase substrate in neurons 1	0.561054
Penk1	preproenkephalin 1	0.376696
Plp1	proteolipid protein (myelin) 1	0.461637
Ppp1r1a	protein phosphatase 1, regulatory (inhibitor) subunit 1A	0.593286
Rab3b	RAB3B, member RAS oncogene family	0.550845
Rasl11b	RAS-like, family 11, member B	0.570419
Rbp4	retinol binding protein 4, plasma	0.521767
Sept5	septin 5	0.622104
Slc30a3	solute carrier family 30 (zinc transporter), member 3	0.49051
Srm	spermidine synthase	0.632395
St8sia5	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 5	0.498446
Tac1	tachykinin 1	0.70294
Tmem56	transmembrane protein 56	0.546252

\*Yellow indicates genes that contain multiple CCAAT boxes in putative promoter regions