ONLINE SUPPLEMENTARY FIGURES

IRS2 increases mitochondrial dysfunction and oxidative stress in the mouse model of Huntington's disease

[1]Marianna Sadagurski, [1]Zhiyong Cheng, [1]Aldo Rozzo, [2]Isabella Palazzolo,

[1]Xiaocheng Dong, [2]Dimitri Krainc and [1]Morris F. White

[1]Howard Hughes Medical Institute
Division of Endocrinology
Children's Hospital Boston,
[2]Massachusetts General Hospital,
Harvard Medical School
Boston, Massachusetts, USA.

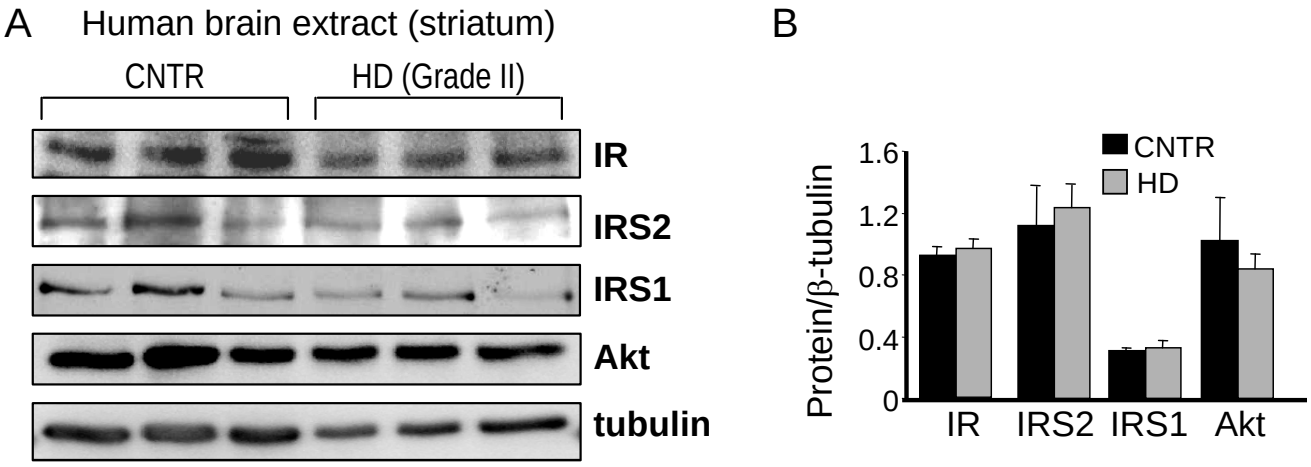
Corresponding author:
Morris F. White
Division of Endocrinology
Children's Hospital Boston
Howard Hughes Medical Institute
Harvard Medical School
Karp Family Research Laboratories, Rm 4210
300 Longwood Avenue
Boston, Massachusetts 02115, USA
Phone: (617) 919-2846
Fax: (617) 730-0244
Email: morris.white@childrens.harvard.edu

GENOTYPE	FEMALE MICE						MALE MICE					
	N	Mean Age	SE	Sig	95% CI Low	Hi	N	Mean Age	SE	Sig	95% CI Low	Hi
		weeks			*weeks*			*weeks*			*weeks*	
(Glc < 200 mg/dl)												
R6/2	29	16.0	0.5	—	15.1	16.9	32	15.1	0.5		14.1	16.3
R6/2•Irs2^{ntg}	21	11.0	0.7	<0.00	9.6	12.2	17	11.9	0.7	<0.001	10.5	13.2
R6/2•Irs2^{btg}	8	16.1	0.7	0.94	14.7	17.4	8	15.7	0.9	0.76	13.9	17.5
R6/2•Irs2$^{+/-}$	45	22.4	0.4	<0.00	21.6	23.1	0	—	—		—	—
R6/2•Irs2$^{+/-}$•Irs2^{btg}	12	21.9	0.9	<0.00	20.2	23.6	13	21.5	0.8	<0.00	19.9	23.1
(Glc > 200 mg/dl)												
R6/2•Irs2$^{+/-}$	3	17.5	0.5	—	16.5	18.4	21	19.5	1	—	17.5	21.4

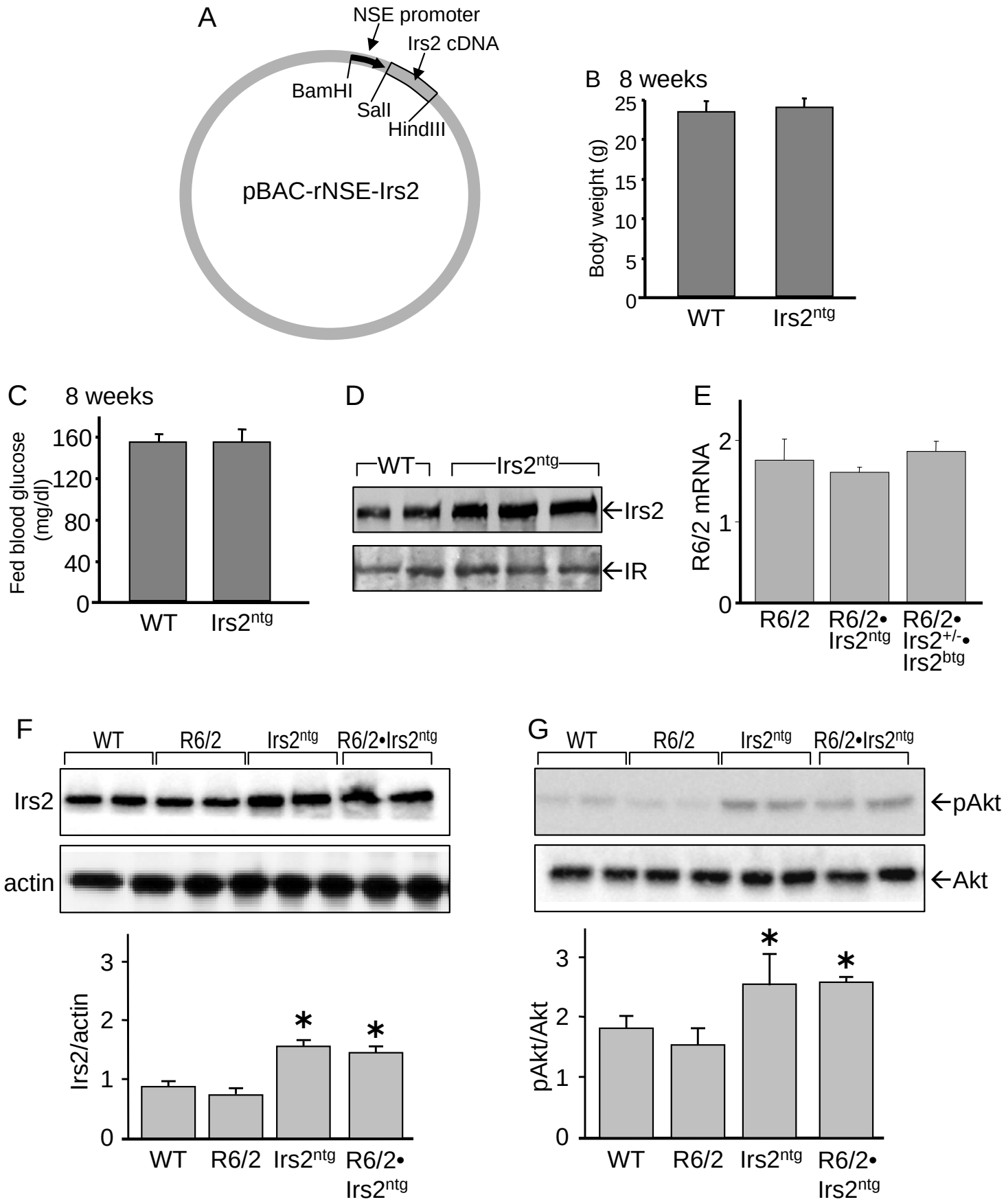
Supplementary Table 1. Kaplan Meier analysis. Survival data was stratified into mice that never developed diabetes or mice that developed diabetes (fed Glc > 200 mg/dl) before death. Male and female mice were analyzed together because gender was not a significant covariate. The mean survival times (weeks) for each genotype was determined by Kaplan Meier analysis (SPSS v18.), and the standard error (SE) and 95% confidence interval (CI) are reported. Mean age of survival for male or female mice was compared by log rank test for each genotype against R6/2, and he p-value (Sig.) is provided where it can be calculated.

	B	SE	SIG	Risk of death	95.0% CI	
					low	Hi
R6/2				1.0		
R6/2•Irs2^{ntg}	1.27	0.2	<0.005	3.6	2.3	5.6
R6/2•Irs2$^{+/-}$	-2.6	0.4	<0.005	-13.4	-27	-7
R6/2•Irs2$^{\beta tg}$	-0.1	0.3	0.85	-1.1	0.5	1.8
R6/2•Irs2$^{+/-}$ •Irs2$^{\beta tg}$	-2.4	0.4	<0.05	-11.3	-23	-6
Sex	0.08	0.2	0.68	1.1	0.7	1.6
Glc > 200 mg/dl	0.89	0.4	0.02	2.4	1.1	5.2

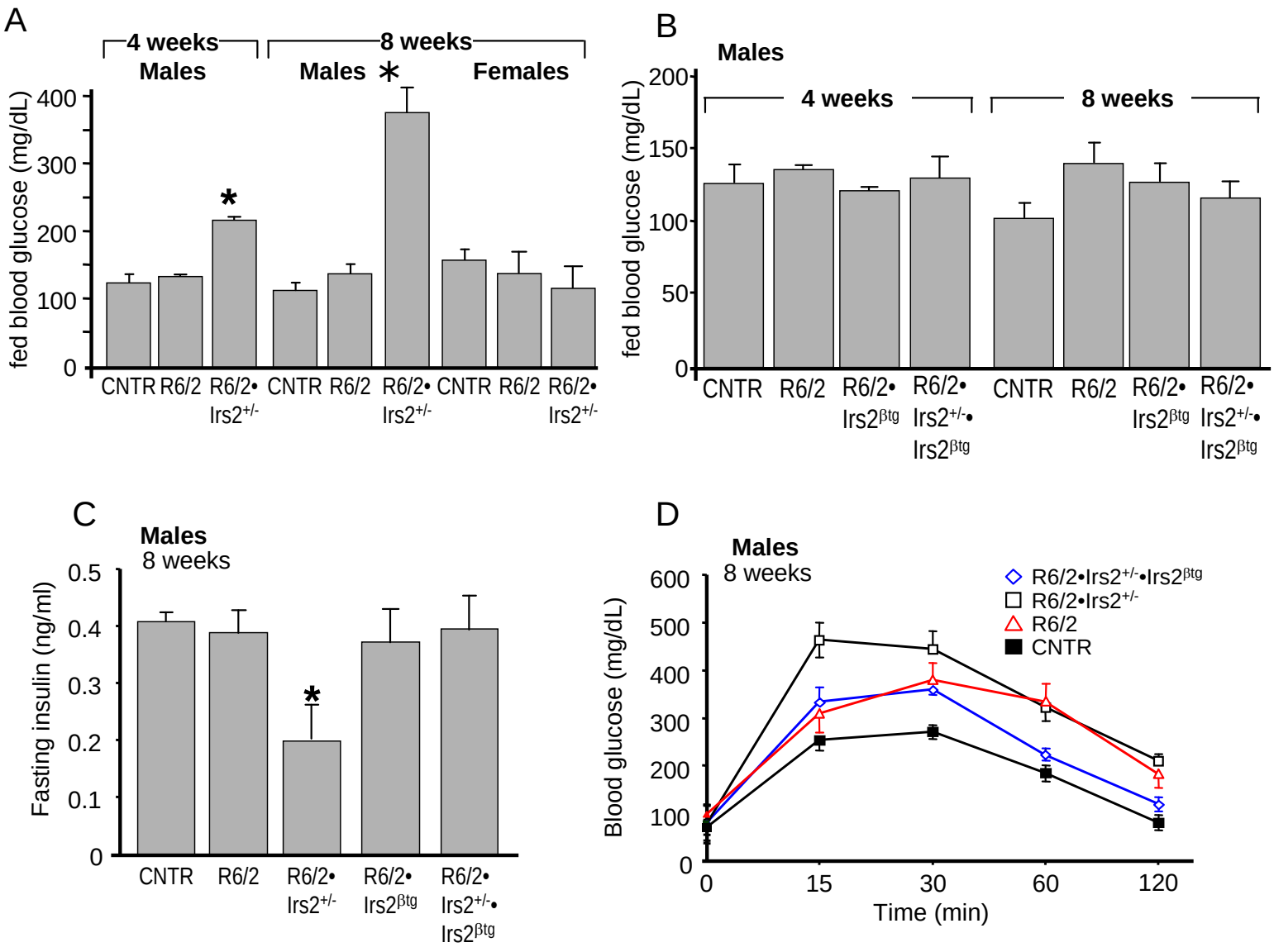
Supplementary Table 2. Cox regression. The time until death was analyzed using Cox regression (SPSS, v18). The independent variables—Genotype, Sex, and glucose>200—were replaced with a set of indicator variables to indicate the presence or absence of category membership. B is the unstandardized regression coefficient and its standard error (SE). The significance of each variable (Sig.) tests the null hypothesis to determine whether the coefficient is zero when the other covariates were set to their mean values. Exp(B) for the categorical covariates of interest is the "risk of death" relative to the control case (R6/2-mice): When Exp(B) < 1, the negative reciprocal -1/Exp(B) was calculated to show the magnitude of "risk of death" on a linear scale.



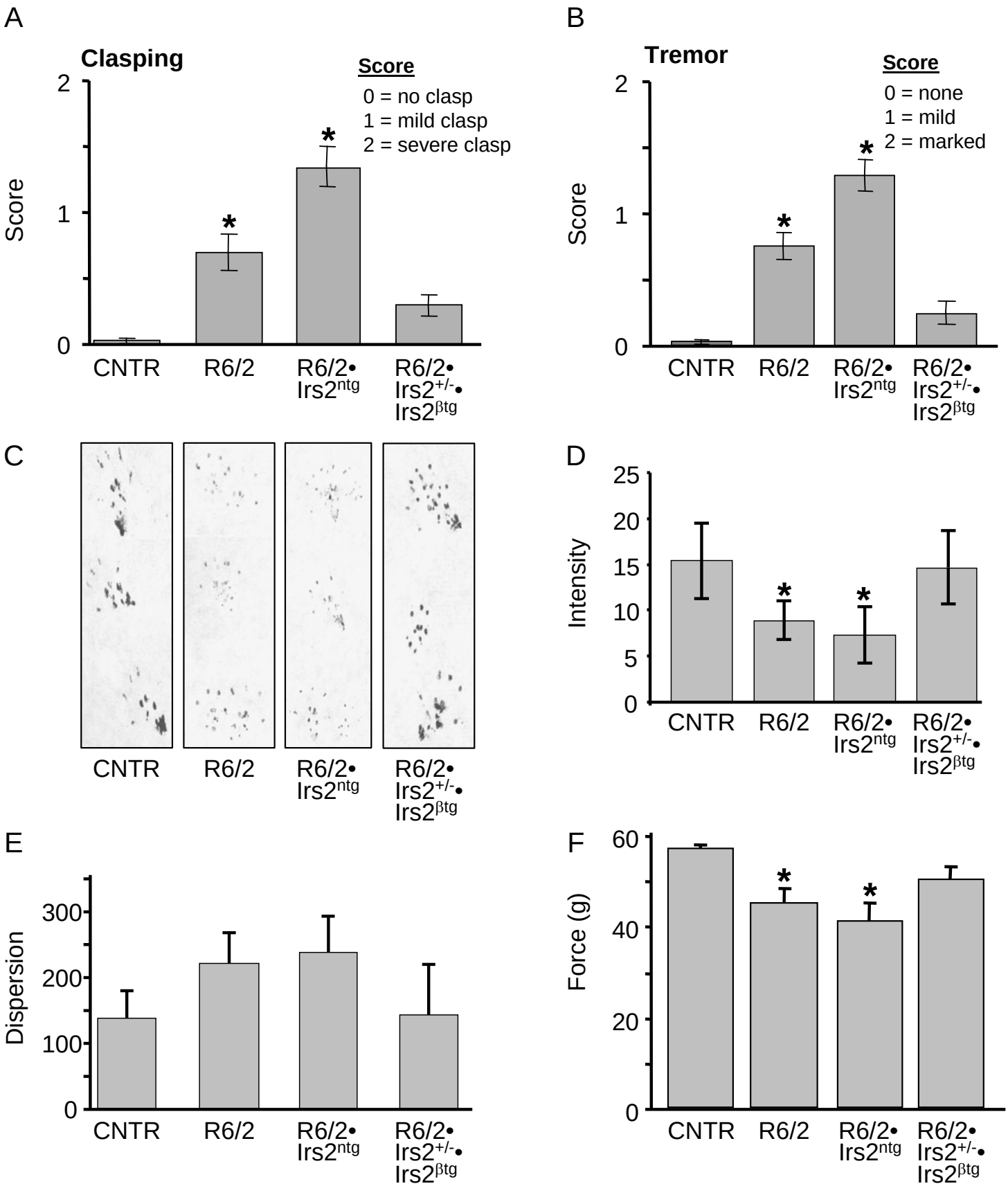
Supplementary Figure 1. (A) Western blot of IR, Irs2, Irs1 and Akt from control and HD patients (Grade II). (B) The bar graphs present the mean ± SD (n=3) values normalized against β-tubulin. Images were analyzed and quantified with Kodak MI software.



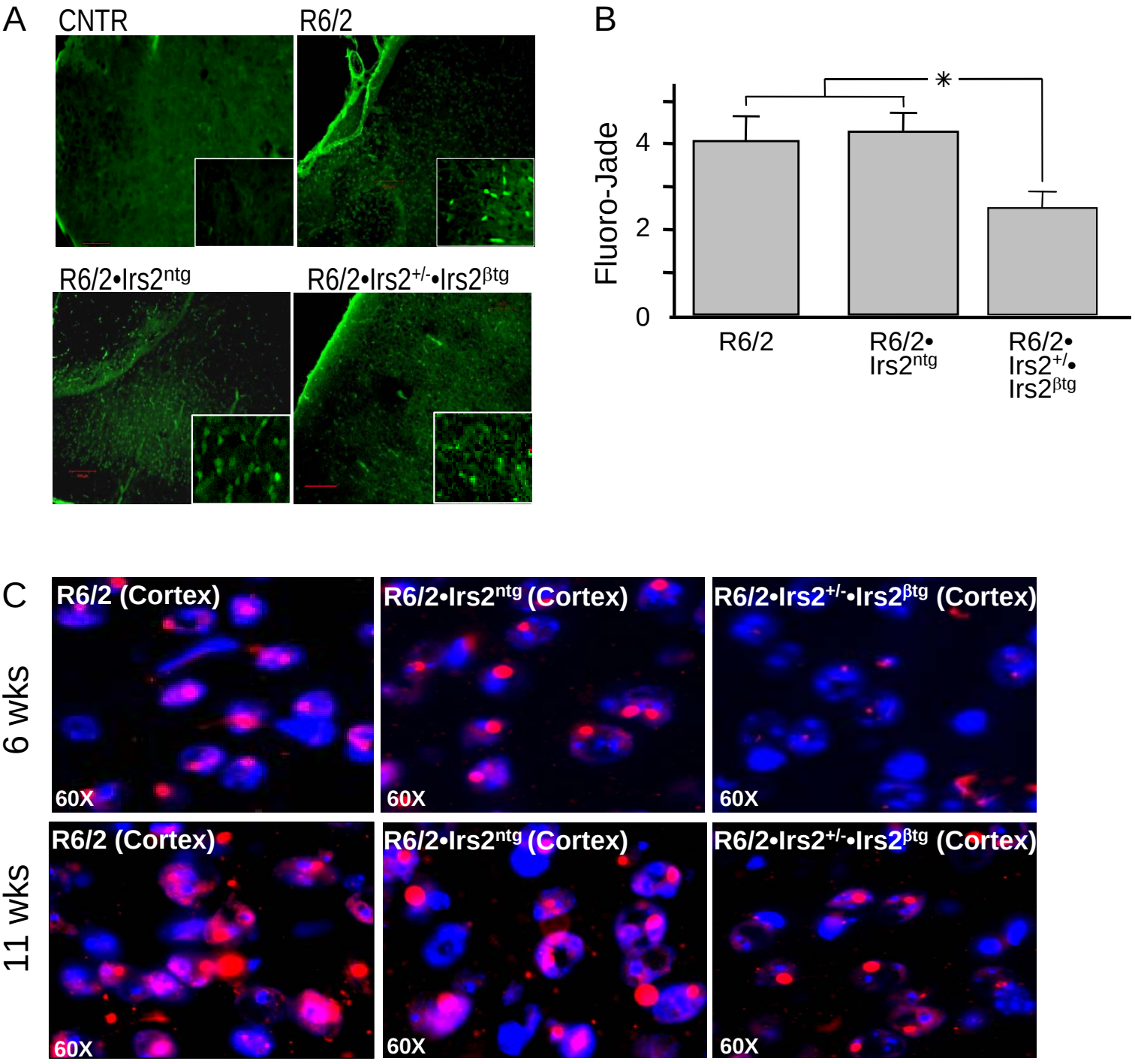
Supplementary Figure 2. Characterization of Irs2^{ntg} mice. (A) Generation of Irs2^{ntg} mice. (B) Body weight and (C) Blood glucose measurements of WT and Irs2^{ntg} mice (D) Western blot of Irs2 immunoprecipitates and insulin receptor (IR) from WT and Irs2^{ntg} brain. (E) R6/2 transgene mRNA expression levels in brains of all genotypes. (F and G) Western blot of Irs2 and phosphorylated AKT in brain extracts from WT, Irs2^{ntg}, R6/2 and R6/2●Irs2^{ntg} mice. The bar graphs present the mean ± SD (n=10) values normalized against actin (Irs2) or Akt (pAkt). *, p< 0.05 compared to the WT.



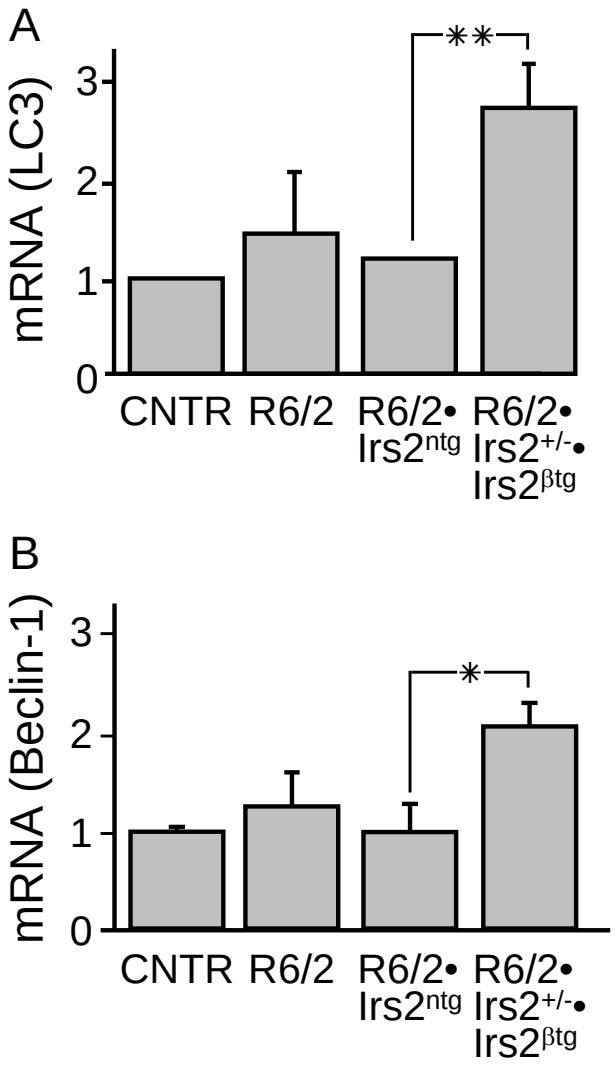
Supplementary Figure 3. Metabolic parameters of the experimental animals. (A) Fed blood glucose of 4 and 8 weeks old male CNTR, R6/2 and R6/2●Irs2$^{+/-}$ mice and 8 weeks old female mice (B) Fed blood glucose of 4 and 8 weeks old male CNTR, R6/2, R6/2●Irs2$^{+/-}$ and R6/2●Irs2$^{+/-}$●Irs2$^{\beta tg}$-male mice (C) Fasting insulin levels of 8 weeks old male mice of all genotypes (D) Glucose tolerance test of 8 weeks old male mice of all genotypes.The results in bar graphs were presented as mean ± SD (n=10). *, p< 0.05 compared with the control.



Supplementary Figure 4. Behavior analysis of experimental animals. The severity of (A) Clasping, (B) Tremor is demonstrated. Abnormal movements are shown in (C) Hind paw print patterns during walking. Quantification analysis of (D) Intensity of paws print that represents the abnormal posture and (E) Paw print dispersion that reflects the degree of tremor and abnormal movements. A decrease in the dispersion of the footprints indicates that mice have less tremor and abnormal movements. (F) Grip strength analysis of 11-weeks-old experimental groups. See Methods for experimental details. The results in bar graphs were presented as mean ± SD (n=10). *, $p < 0.05$ compared with the control.



Supplementary Figure 5. (A) Fluoro-Jade B immunostaining of cortex from 11-week-old Cntr, R6/2, R6/2•Irs2^{ntg} and R6/2•Irs2$^{+/-}$•Irs2$^{\beta tg}$ --mice; the Fluoro-Jade B signal is quantified in (B). All bar graphs depict the mean SD of 4 sections from 5 mice per genotype; *, p< 0.05; **, p<0.01. (C) Cortex from 6 and 11-week-old R6/2, R6/2•Irs2^{ntg} and R6/2•Irs2$^{+/-}$•Irs2$^{\beta tg}$ mice is stained with anti-Htt antibody (red), and nuclei are stained with DAPI (blue). Representative images of cortical neurons are shown, demonstrating the size of inclusion bodies (60X magnification).



Supplementary Figure 6. The mRNA concentration of (A) LC3b and (B) Beclin in brain extracts from 11-week-old CNTR, R6/2, R6/2•Irs2^{ntg} and R6/2•Irs2$^{+/-}$•Irs2$^{\beta tg}$-mice