Supplementary Information

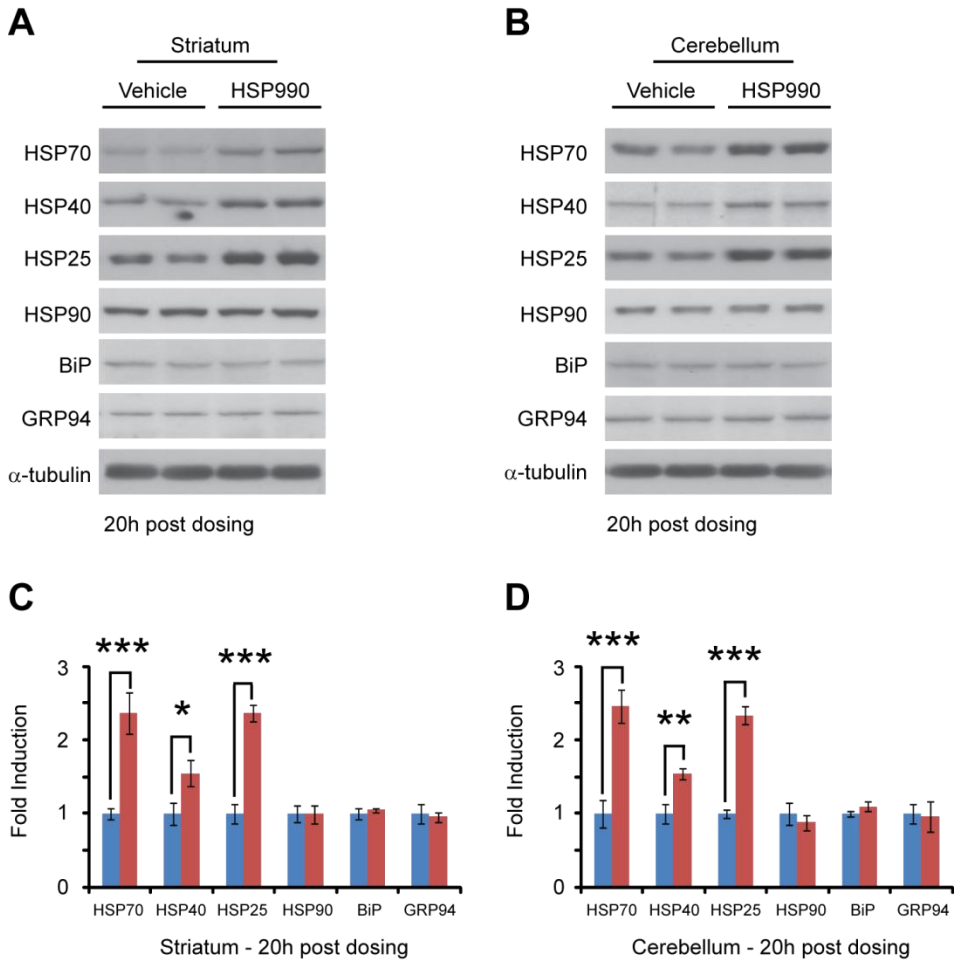


Figure S1. HSP990 up-regulates major heat shock proteins in mouse striatum and cerebellum

Representative western blots of chaperone and α-tubulin levels in (A) striatum and (B) cerebellum of wild type mice 20 hours post dosing with vehicle or HSP990 (12 mg/kg). Densitometry was used to calculate chaperone expression relative to levels of α-tubulin for each sample. The mean fold induction of each chaperone after HSP990 treatment was then calculated relative to chaperone levels after vehicle treatment and plotted +/- SEM for (C) striatum and (D) cerebellum ($n \geq 5$ per treatment group). Student's *t*-test was used to determine statistical significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

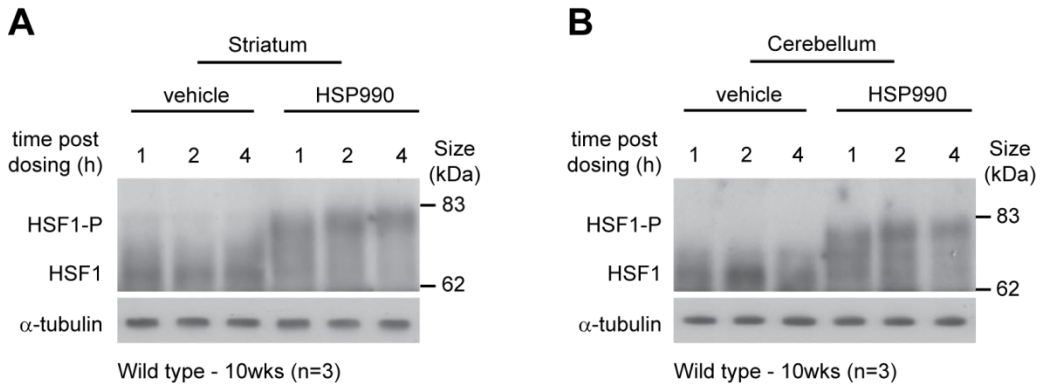


Figure S2. HSP990 treatment leads to HSF1 hyper-phosphorylation in mouse brain tissues

Representative western blots of HSF1 and α-tubulin in 10 week WT (A) striatum and (B) cerebellum 1, 2, and 4 hours after treatment with vehicle or HSP990 (12 mg/kg) (n = 3 per treatment group; HSF1-P = hyper-phosphorylated HSF1).

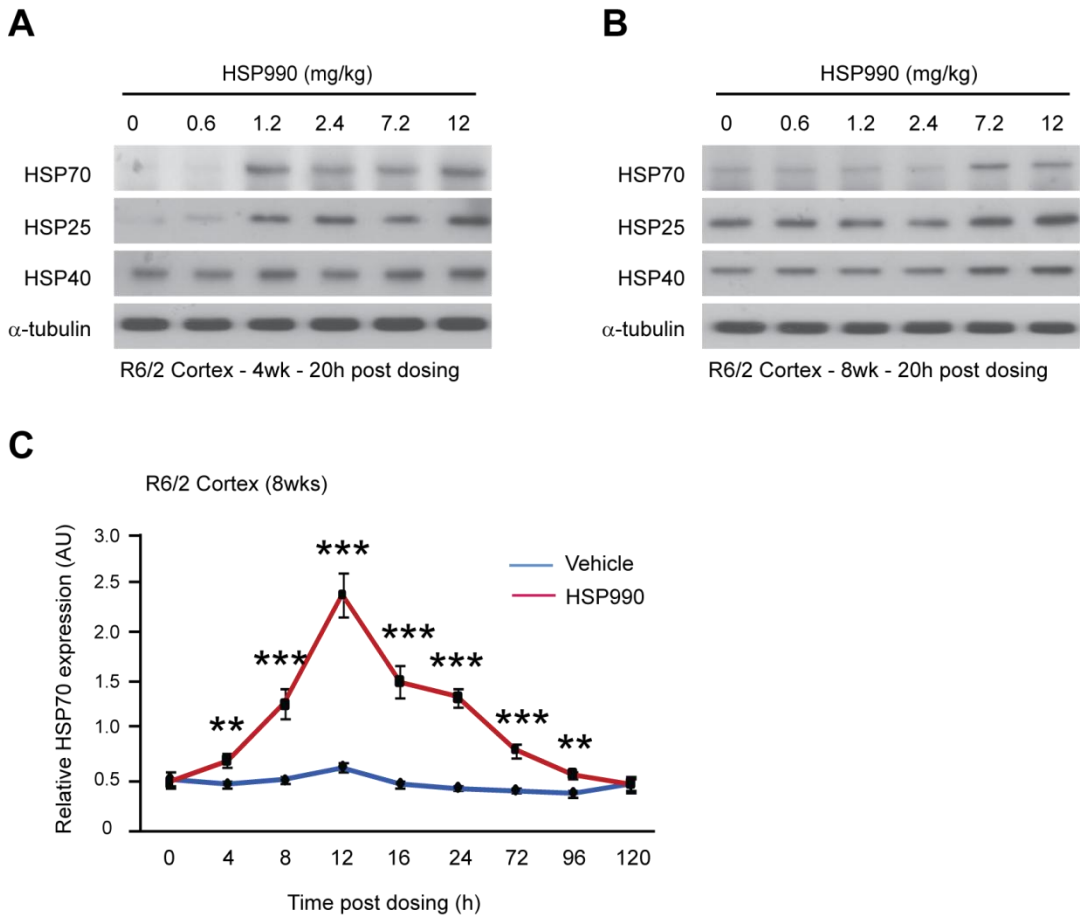


Figure S3. HSP990 treatment provides prolonged up-regulation of HSP70 after acute oral administration at non-toxic doses

Representative western blots of HSP70, HSP25, HSP40 and α-tubulin in (A) 4 and (B) 8 week R6/2 brain tissue 20 hours after treatment with vehicle alone or HSP990 at doses of 0, 0.6, 1.2, 2.4, 7.2, or 12 mg/kg (n = 4 per treatment group). (C) HSP70 ELISA in cortex harvested from 8 week old R6/2 mice 0, 4, 8, 12, 24, 72, 96, and 120 hours post treatment with vehicle or HSP990 (7.2 mg/kg). Values were plotted as the mean ± SEM for each treatment group (n = 4). Student's t-test was used to determine statistical significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

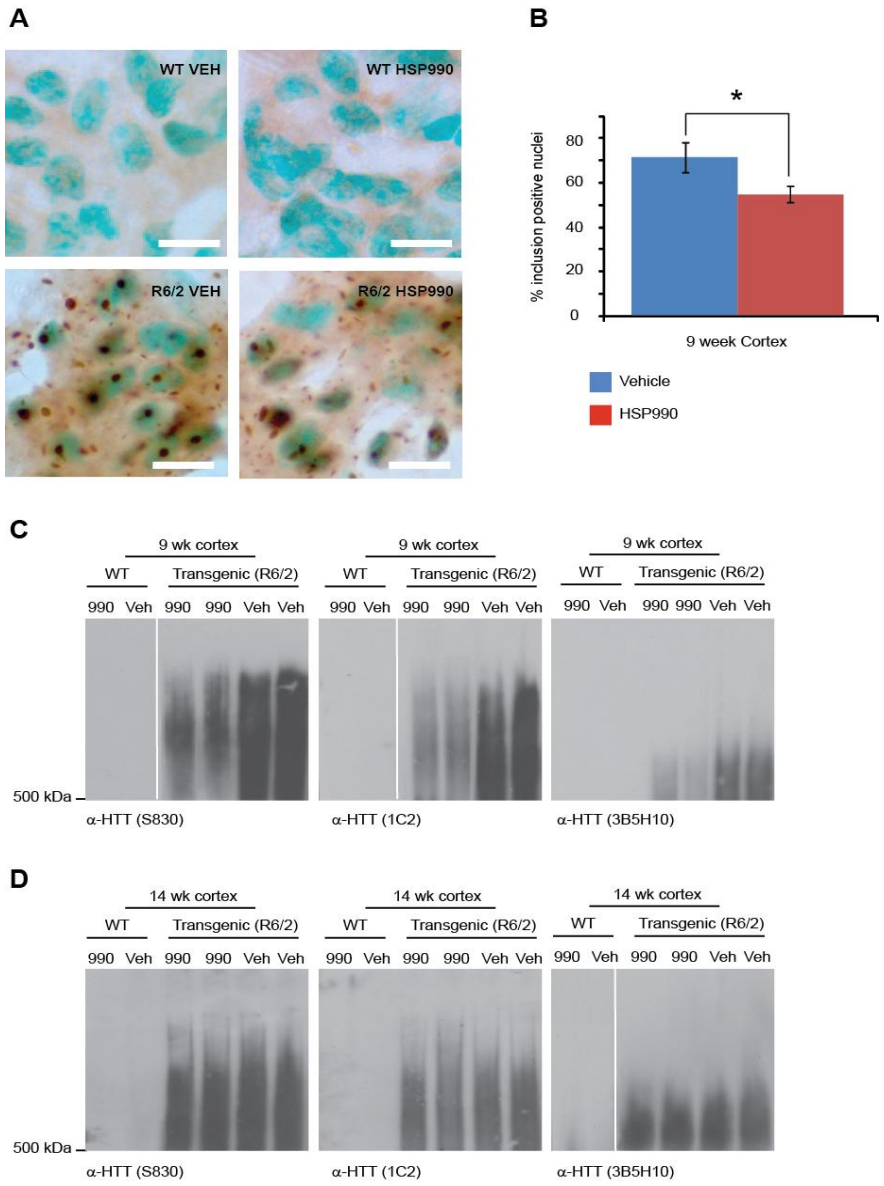


Figure S4. HSP990 treatment reduces aggregate load in R6/2 cortex

(A) Representative images from 9 week old mouse cortices after staining with an anti-huntingtin exon 1 antibody (S830) and methyl green (nuclear counterstain). Mouse brains were harvested at PD study completion (24 hours after last dose), snap frozen in isopentane and sectioned (15 µm thickness). Scale bars represent 10 µm. (B) The percentage of inclusion positive nuclei (≥ 250 nuclei across 3 sections) was then determined blindly for each mouse and the mean was plotted ± SEM for each treatment group (n = 3). Student's *t*-test was used to determine statistical significance (* $p < 0.05$). Representative western blots (n = 4) of agarose gel electrophoresis and anti-huntingtin (HTT) immunodetection (S830, 1C2, and 3B5H10) of cortical protein lysates isolated from wild type (WT) and R6/2 mice at (C) 9 weeks (end of pharmaco-dynamic study) and (D) 14 weeks of age (end of trial). White lines indicate that samples were from the same blot but lanes were non-contiguous.

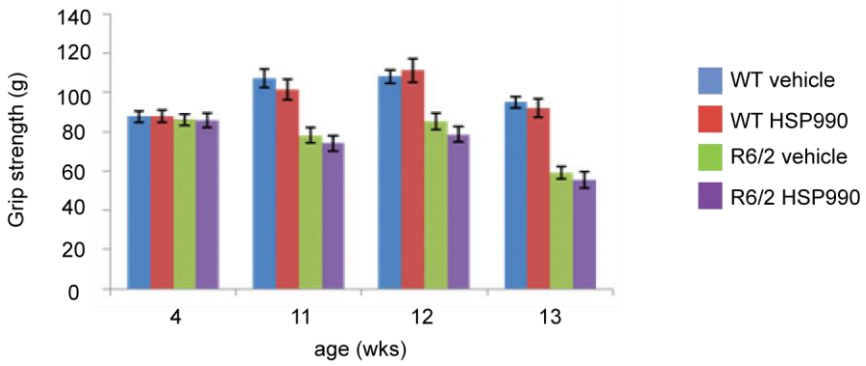


Figure S5. HSP990 treatment does not alter grip strength in WT or R6/2 mice

Mouse forelimb grip strength was assessed at 4, 11, 12, and 13 weeks of age using a San Diego instruments grip strength metre. Mean grip strength was then plotted for each treatment group ± SEM ($n \geq 12$).

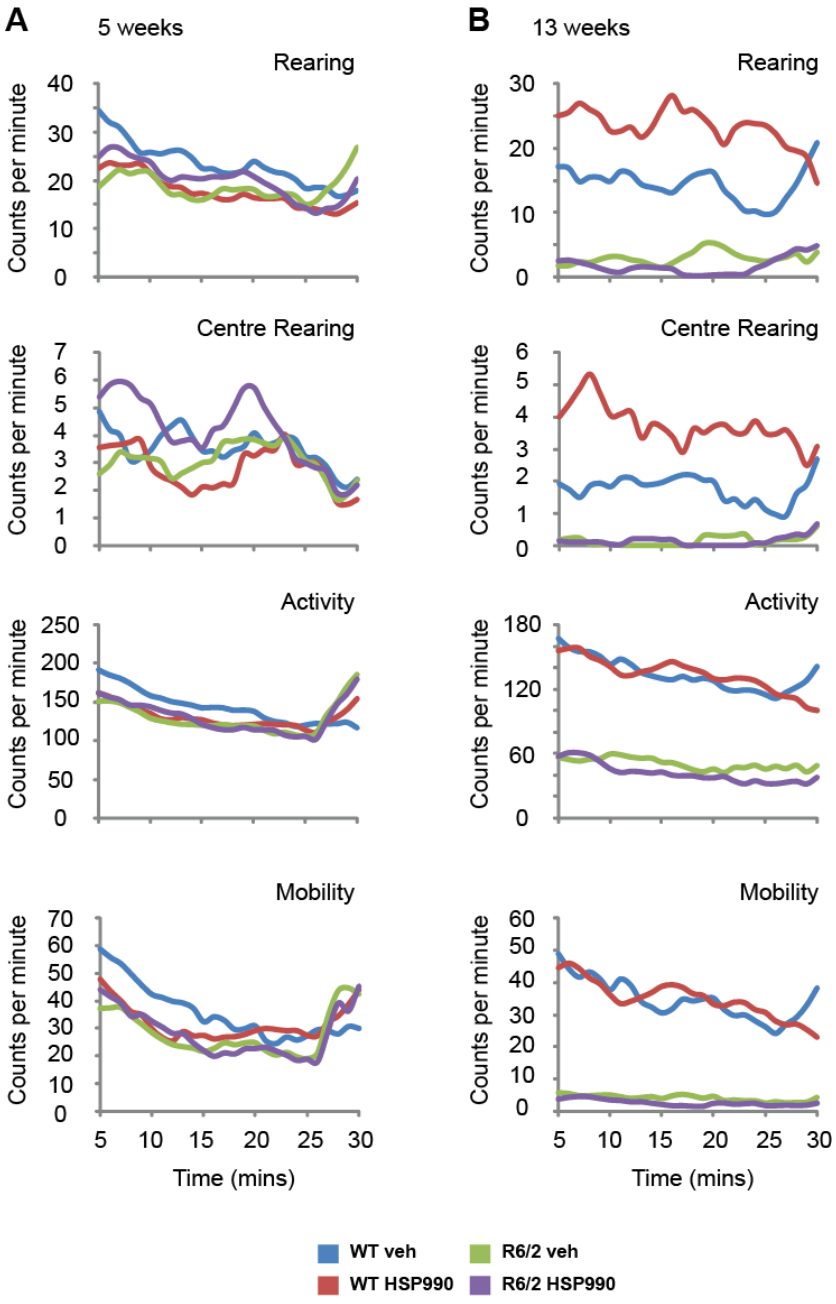


Figure S6. HSP990 treatment does not have any significant effect on a variety of activity parameters in WT or R6/2 mice

Five minute moving average graphs depicting the performance of WT and R6/2 mice across a range of open field activity parameters after dosing with vehicle or HSP990 at 5 (A) or 13 (B) weeks of age. Moving averages were calculated using Microsoft Excel and mean number of counts of each group was plotted for each unit of assessment. Blue, red, green, and purple bars represent WT vehicle, WT HSP990, R6/2 vehicle and R6/2 HSP990 groups respectively ($n \geq 12$ per treatment group).

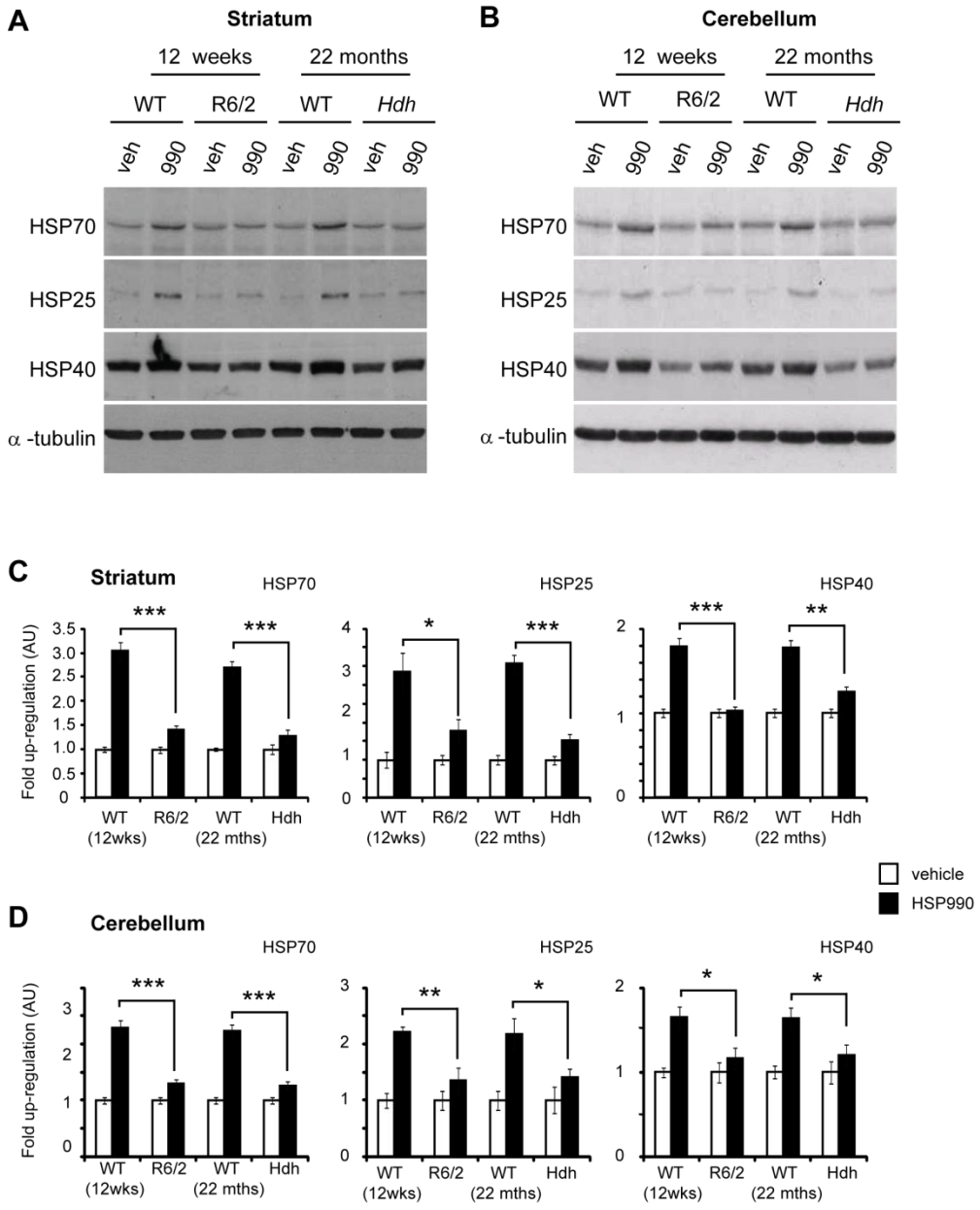


Figure S7. HSP up-regulation is impaired in striatum and cerebellum of HD mouse models

Representative western blots of HSP70, 25, 40, and α-tubulin in WT, R6/2 or *Hdh*$^{Q150/Q150}$ (Hdh) (A) striatum and (B) cerebellum 20 hours post treatment with vehicle or HSP990 (12 mg/kg). Densitometry was used to calculate the expression levels of HSP70, HSP25 and HSP40 in mouse striatum (C) and cerebellum (D) relative to levels of α-tubulin. The fold expression of each chaperone was then calculated for HSP990 treatment groups (black bars) relative to vehicle groups (white bars). Values are plotted as the mean fold +/- SEM (n = 4 per treatment group). Student's *t*-test was used to determine statistical significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

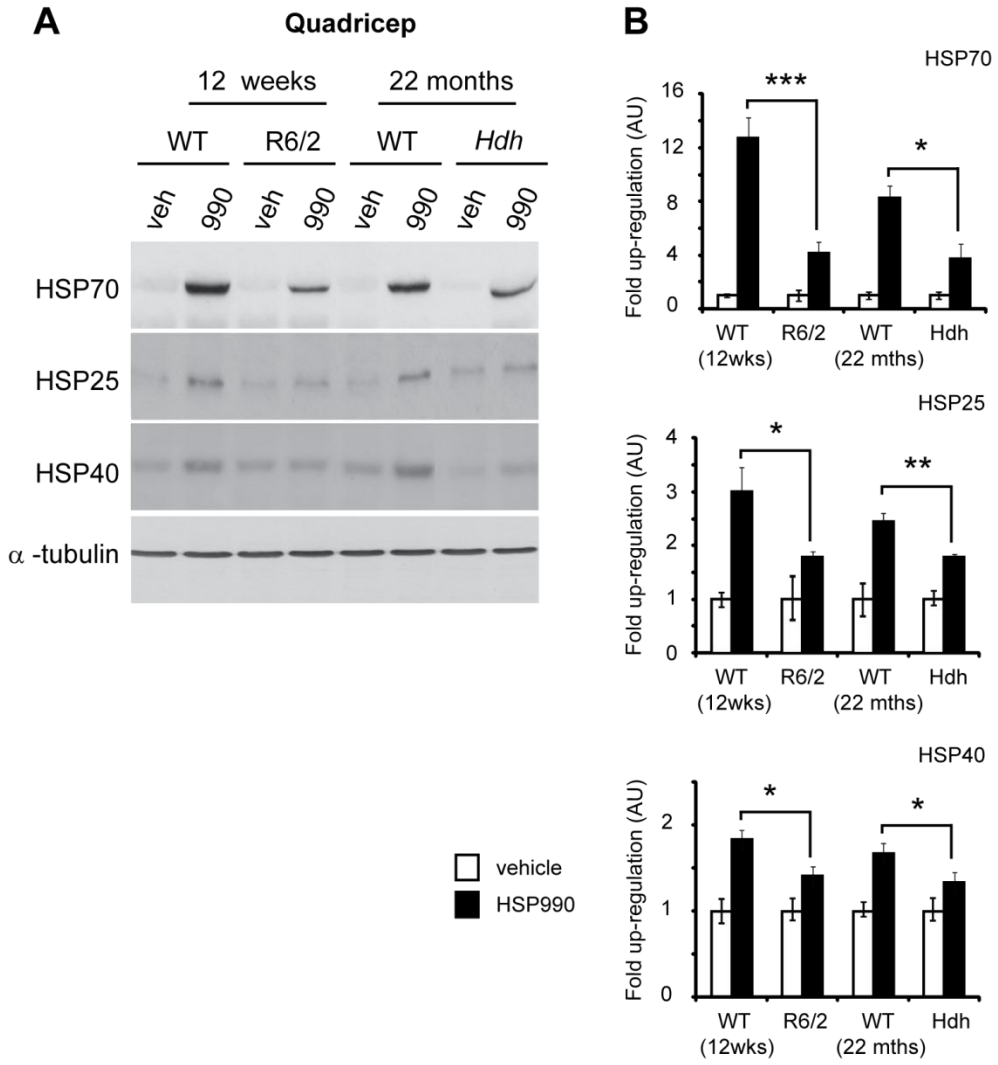


Figure S8. HSP up-regulation is impaired in quadriceps muscle of HD mouse models

(A) Representative western blots of HSP70, 25, 40, and α-tubulin in WT, R6/2 or *Hdh*$^{Q150/Q150}$ (Hdh) quadricep muscle 20 hours post treatment with vehicle or HSP990 (12 mg/kg). (B) Densitometry was used to calculate the expression levels of HSP70, HSP25 and HSP40 relative to levels of α-tubulin. The fold expression of each chaperone was then calculated for HSP990 treatment groups (black bars) relative to vehicle groups (white bars). Values are plotted as the mean fold ± SEM (n = 4 per treatment group). Student's *t*-test was used to determine statistical significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

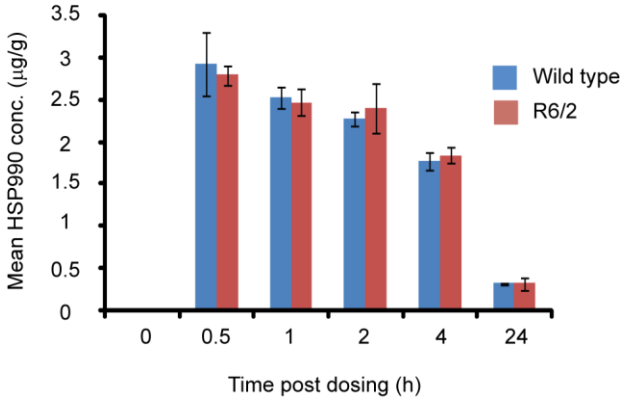


Figure S9. PK analysis of HSP990 in WT and R6/2 brain tissue at 12 weeks of age

WT and R6/2 half brains were harvested 0, 0.5, 1, 2, 4, and 24 hours post dosing with HSP990 (12 mg/kg). The level of HSP990 present in mouse brain tissue was then determined by mass spectrometry and mean HSP990 (µg/g) was plotted ± SEM for each treatment group (n = 3).

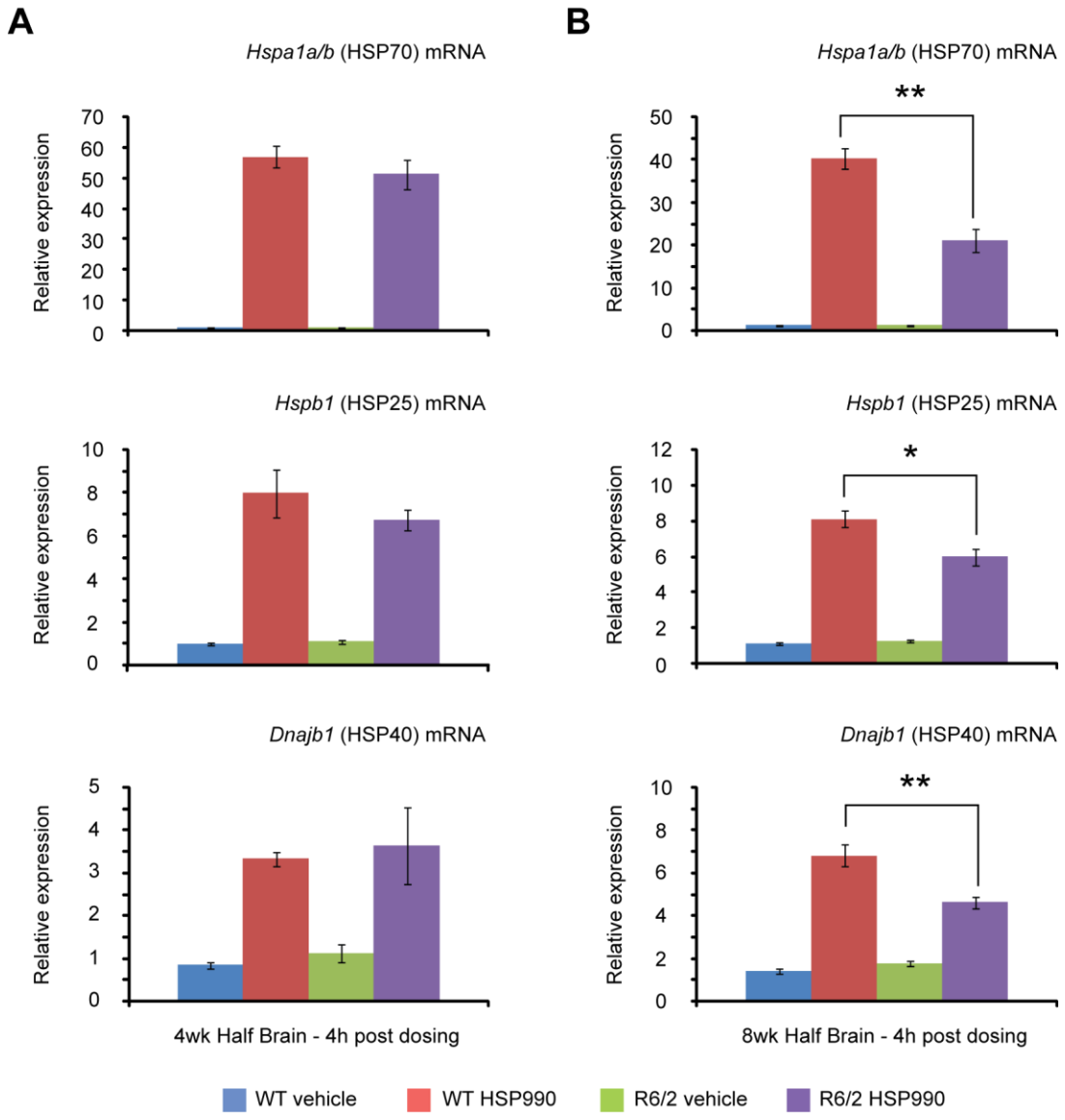
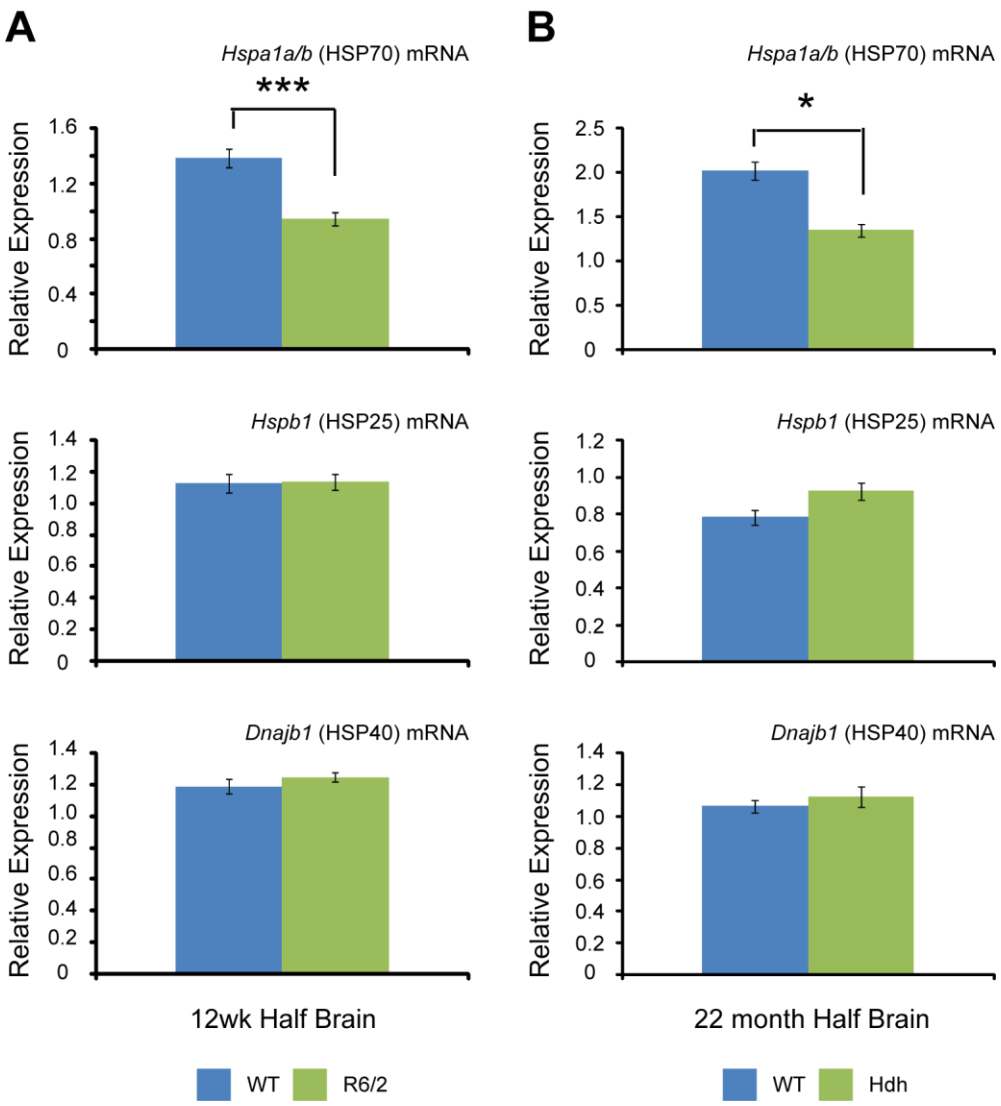


Figure S10. HS mRNA induction after HSP990 treatment becomes impaired in R6/2 mice at 8 weeks of age

Taqman qPCR of *Hspa1a/b* (HSP70) *Hspb1* (HSP25) and *Dnajb1* (HSP40) was performed on half brains of WT and R6/2 mice harvested 4 hours post treatment with vehicle or HSP990 (12 mg/kg) at (A) 4 or (B) 8 weeks of age. Chaperone mRNA expression levels were normalized to the housekeeping gene *Atp5b* and the ΔCt method was used to calculate relative levels of HS mRNA between WT and R6/2 treatment groups ($n \geq 5$). Values for each group were plotted as mean ± SEM. Student's *t*-test was used to determine statistical significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).



\

Figure S11. *Hspa1a/b* is transcriptionally dysregulated with disease progression in mouse models of HD but *Hspb1* (HSP25) and *Dnajb1* (HSP40) are not

Taqman qPCR of *Hspa1a/b* (HSP70) *Hspb1* (HSP25) and *Dnajb1* (HSP40) was performed on half brains of WT and R6/2 mice (12 weeks) 0, 4, or 8 hours post treatment with vehicle or HSP990 (12 mg/kg) and on half brains from WT and $Hdh^{Q150/Q150}$ mice (22 months) 2 hours post treatment with vehicle or HSP990. Chaperone mRNA expression levels were normalized to the housekeeping gene *Atp5b* and the ΔCt method was used to calculate relative levels of HS mRNA between WT and R6/2 (A) or WT and $Hdh^{Q150/Q150}$ mice (B) from vehicle treated groups only. Values for each group were plotted as mean +/- SEM (n = 12 for 12 wk WT and R6/2 treatment groups; n = 4 for 22 month WT and Hdh treatment groups). Blue bars represent WT (12 wk or 22 month) and green bars represent 12 wk R6/2 or 22 month $Hdh^{Q150/Q150}$ (Hdh). Student's *t*-test was used to determine statistical significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

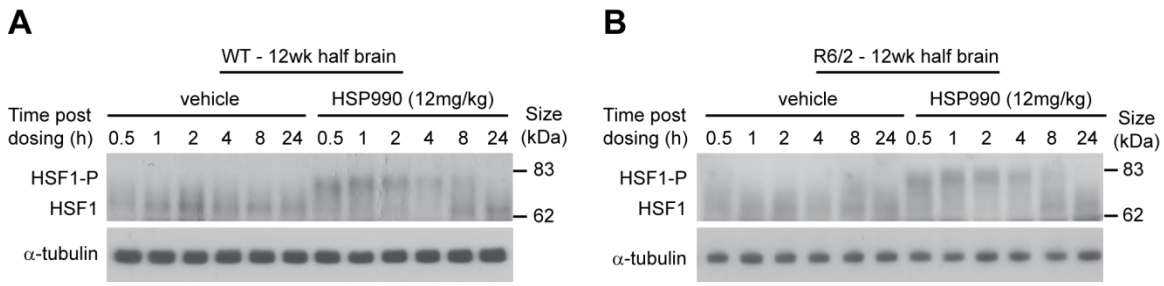


Figure S12. Dynamics of HSF1 hyper-phosphorylation in WT and R6/2 Half brain after treatment with vehicle or HSP990 (12 mg/kg)

Representative western blots of HSF1 in 12 week (A) wild type and (B) R6/2 half brain tissue 0.5, 1, 2, 4, 8, and 24 hours post treatment with vehicle or HSP990 (12 mg/kg) (n = 3 per treatment group). HSF1-P = hyper-phosphorylated HSF1.

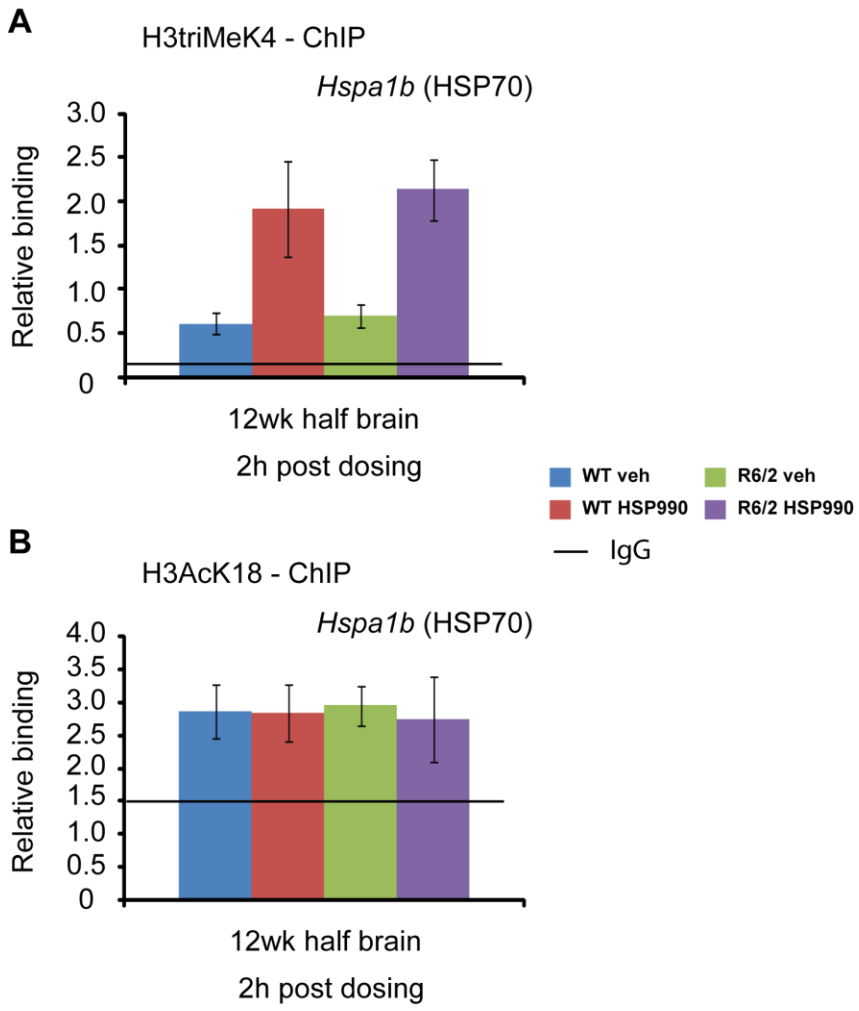


Figure S13. Levels of H3triMeK4 and H3AcK18 are not significantly different between wild type and R6/2 mice 2 hours post treatment with vehicle or HSP9990

The amount of H3triMeK4 (A) or H3AcK18 (B) bound to HS promoters 2 hours after treatment with vehicle or HSP990 (12 mg/kg) was determined by ChIP in 12 week WT and R6/2 half brains. SYBR green qPCR was performed on immunoprecipitated chromatin using primers specific for the *Hspa1b* (HSP70) promoter. Signal after pull down was normalized to 10% of the input for each sample. Values were plotted as means +/- SEM for all treatment groups (n = 4). In all graphs, blue, red, green, and purple bars represent WT vehicle, WT HSP990, R6/2 vehicle, and R6/2 HSP990 treatment groups respectively. The black line on graphs A and B denotes the mean amount of signal obtained after pull-down with rabbit IgG alone (n=2).

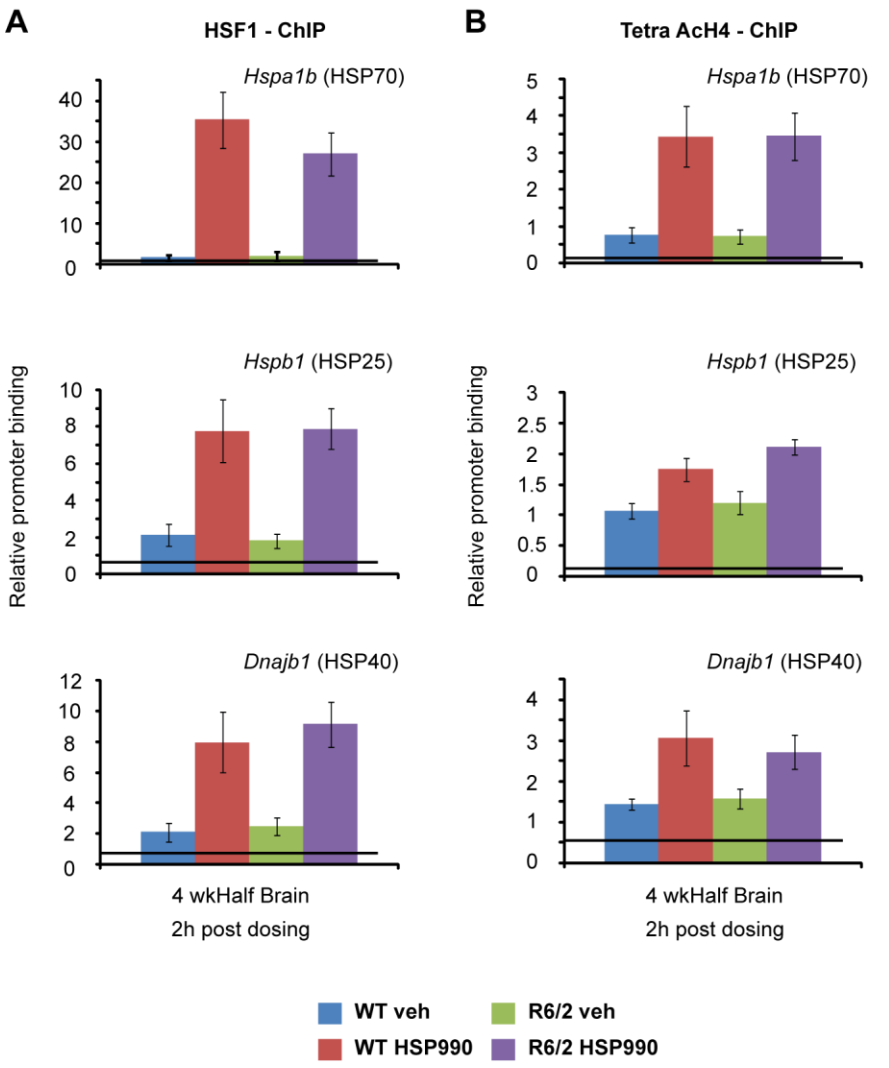


Figure S14. Levels of HSF1 and tetra-acetylated H4 at heat shock promoters is not significantly different between wild type and R6/2 mice at 4 weeks of age in vehicle or HSP990 treatment groups

The amount of HSF1 (A) and Tetra-AcH4 (B) bound to HS promoters 2 hours after treatment with vehicle or HSP990 (12 mg/kg) was determined by ChIP in 4 week WT and R6/2 half brains. SYBR green qPCR was performed on immuno-precipitated chromatin using antibodies specific for each factor and primers specific for the promoters of *Hspa1b* (Hsp70), *Hspb1*(Hsp25), and *Dnajb1*(Hsp40). Signal after pull-down was normalized to 10% of the input for each sample. Values were plotted as the mean relative signal +/- SEM for each treatment group (n = 4) were blue, red, green, and purple bars represent WT vehicle, WT HSP990, R6/2 vehicle, and R6/2 HSP990 treatment groups respectively. The black line on graphs denotes the mean amount of signal obtained after pull-down with rabbit IgG alone (n=2).

Table S1: Statistical analysis of activity measures

Mice were assessed for a period of 30 min for total activity, mobility, rearing and unsupported rearing (Figure S6) and data were analysed by GLM ANOVA. Mice of all treatment groups exhibit most activity during the first 15 minutes of the assessment period (1). R6/2 mice show an overall hypoactivity relative to WT mice from 9 weeks (Table S1, genotype) although the pattern of activity over the course of the 30 min period was significantly different between R6/2 and WT mice (genotype*time) by 9 weeks of age. HSP990 treatment had no effect on WT mice, both in overall activity (treatment) and in the 30 minute pattern of activity (treatment*time). There was no overall improvement in R6/2 hypoactivity through HSP990 treatment (genotype*treatment), nor was the R6/2 pattern of hypoactivity changed (genotype*treatment*time). Key: Pink $p < 0.001$, Orange $p < 0.01$, Yellow $p < 0.05$.

Comparison	Week	Activity	Mobility	Rearing	Center Rearing
Time					
	5	< 0.001	< 0.001	< 0.001	0.036
	7	< 0.001	< 0.001	< 0.001	0.003
	9	< 0.001	< 0.001	0.253	0.14
	11	< 0.001	< 0.001	0.35	0.185
	13	< 0.001	< 0.001	0.266	0.197
Genotype	5	0.506	0.374	0.179	0.857
	7	0.439	0.115	0.323	0.924
	9	0.018	0.035	0.899	0.853
	11	0.003	< 0.001	0.092	0.218
	13	< 0.001	< 0.001	< 0.001	0.002
Genotype *Time	5	0.9	0.751	0.757	0.91
	7	0.202	0.191	0.417	0.584
	9	0.04	0.121	0.033	0.102
	11	0.009	0.044	0.348	0.088
	13	0.007	0.016	0.001	0.016
Treatment	5	0.515	0.482	0.162	0.854
	7	0.822	0.533	0.229	0.263
	9	0.276	0.713	0.454	0.12
	11	0.843	0.984	0.514	0.226
	13	0.208	0.452	0.827	0.334
Treatment *Time	5	0.061	0.073	0.728	0.745
	7	0.035	0.068	0.794	0.813
	9	0.226	0.362	0.169	0.528
	11	0.619	0.734	0.445	0.323
	13	0.033	0.143	0.241	0.427
Treatment *Genotype	5	0.631	0.746	0.121	0.095
	7	0.803	0.988	0.469	0.986
	9	0.551	0.571	0.492	0.745
	11	0.562	0.467	0.149	0.265
	13	0.692	0.893	0.506	0.408
Treatment *Genotype *Time	5	0.533	0.317	0.764	0.612
	7	0.8	0.59	0.094	0.139
	9	0.271	0.265	0.118	0.303
	11	0.65	0.368	0.204	0.424
	13	0.223	0.76	0.311	0.359

Table S2: Antibodies used in this study

Antibody	Catalogue number	Source	Dilution /amount	Application	Reference
HSF1	N/A	R.Morimoto	1 in 500	WB	(2)
HSF1	H-311	Santa Cruz	2ug	IP	N/A
HSF1	H-311X	Santa Cruz	2ug	ChIP	N/A
HSF1	ab61382	Abcam	1 in 1000	WB	N/A
Hsp25	SPA-801	Stressgen	1 in 1000	WB	N/A
Hsp40	SPA-400	Stressgen	1 in 5000	WB	N/A
Hsp70	SPA-810	Stressgen	1 in 1000	WB	N/A
Hsp90	SPA-835	Stressgen	1 in 5000	WB	N/A
BiP	SPA-826	Stressgen	1 in 500	WB	N/A
Grp94	ab2791	Abcam	1 in 5000	WB	N/A
S830	N/A	In House	1 in 1000	WB	(3)
MW8	N/A	P.Patterson	1 in 2000	SEPRION	(4)
MW1	N/A	P.Patterson	1ng in 6ul	TR-FRET	(4)
2B7	N/A	Novartis	10ng in 6ul	TR-FRET	(5)
RNApolII	ab817	Abcam	2ug	ChIP	N/A
H3AcK9	ab10812	Abcam	2ug	ChIP	N/A
H3AcK18	ab1191	Abcam	2ug	ChIP	N/A
H3AcK27	ab4729	Abcam	2ug	ChIP	N/A
H3triMeK4	ab8580	Abcam	2ug	ChIP	N/A
Tetra AcH4	06-866	Millipore	2ug	ChIP	N/A
α-tubulin	T9026	Sigma	1 in 30,000	WB	N/A
Histone H3	ab1791	abcam	1 in 30,000	WB	N/A
anti-Goat	P044901	Dako	1 in 5000	WB	N/A
anti-Mouse	P0260	Dako	1 in 5000	WB	N/A
anti-Rat	P0450	Dako	1 in 5000	WB	N/A
anti-Rabbit	32460	Pierce	1 in 20,000	WB	N/A
1C2	MAB1574	Sigma	1 in 1000	WB	N/A
3B5H10	P1874	Sigma	1 in 1000	WB	N/A

Key: WB = Western blotting, IP = immmunoprecipitation, ChIP = chromatin immunoprecipitation, SEPRION = SEPRION ligand ELISA for aggregated huntingtin, TR-FRET = time resolved forster resonance energy transfer for soluble huntingtin.

Table S3: Primer and probe sequences for RTqPCR

Name	Application	Forward	Reverse	Probe
Hspa1a/b	Taqman	GGT GGT GCA GTC CGA CAT G	TTG GGC TTG TCG CCG T	CAC TGG CCC TTC CAG GTG GTG AA
Hspb1	Taqman	CAC TGG CAA GCA CGA AGA AAG	GCG TGT ATT TCC GGG TGA AG	ACC GAG AGA TGT AGC CAT GTT CGT CCT G
Dnajb1	Taqman	CCCCATGCCATGTTTGCT	GCGCTGCCCAAAAAAGG	TCT TCG GTG GCA GAA ACC CCT TTG A
Human Htt exon1	Taqman	GCT GCA CCG ACC GAG T	CGC AGG CTG CAG TTA C	CAG CTC CCT GTC CCG GCG G
Hspa1b promoter	SYBR green	CAC CAG CAC TTC CCC A	CGC CCT GCG CCT TTA AG	N/A
Hspb1 promoter	SYBR green	GGC TCC AGT CCG GCA CTT CT	GGC GCT CGG TCA TGT TCT TG	N/A
Dnajb1 promoter	SYBR green	CGC CGG ACG GGT ATA TAG AG	GGC CCA GCG TCT GAT AGT AG	N/A
Hspa1b (-250)	SYBR green	GCC GGT GAA GAC TCC TTA AA	GCT TGT CTC TGG ATG GAA CC	N/A
Hspa1b (0)	SYBR green	AGC CTT CCA GAA GCA GAG C	GAG TAG GTG GTG CCC AGG T	N/A
Hspa1b (+350)	SYBR green	GGT GAA CTA CAA GGG CGA GA	CTT CAT CTT CGT CAG CAC CA	N/A
Hspa1b (+450)	SYBR green	CCG CCT ACT TCA ACG ACT CT	GAT CCG CAG CAC GTT TAG A	N/A
Hspa1b (+700)	SYBR green	GAG GGG AGG ACT TCG ACA AC	TGG CTG ATG TCC TTC TTG TG	N/A
Hspa1b (+1050)	SYBR green	CGA CCT GAA CAA GAG CAT CA	CTG CAC GTT CTC CGA CTT GT	N/A
Hspa1b (+1900)	SYBR green	CCA TCG AGG AGG TGG ATT AG	GAC AGT AAT CGG TGC CCA AG	N/A

Atp5b primer and probe mix for Taqman qPCR was purchased from Primer design.

References

1. Hockly, E., Tse, J., Barker, A.L., Moolman, D.L., Beunard, J.L., Revington, A.P., Holt, K., Sunshine, S., Moffitt, H., Sathasivam, K., et al. 2006. Evaluation of the benzothiazole aggregation inhibitors riluzole and PGL-135 as therapeutics for Huntington's disease. *Neurobiol Dis* 21:228-236.
2. Westerheide, S.D., Bosman, J.D., Mbadugha, B.N., Kawahara, T.L., Matsumoto, G., Kim, S., Gu, W., Devlin, J.P., Silverman, R.B., and Morimoto, R.I. 2004. Celastrols as inducers of the heat shock response and cytoprotection. *J Biol Chem* 279:56053-56060.
3. Sathasivam, K., Woodman, B., Mahal, A., Bertaux, F., Wanker, E.E., Shima, D.T., and Bates, G.P. 2001. Centrosome disorganization in fibroblast cultures derived from R6/2 Huntington's disease (HD) transgenic mice and HD patients. *Hum Mol Genet* 10:2425-2435.
4. Ko, J., Ou, S., and Patterson, P.H. 2001. New anti-huntingtin monoclonal antibodies: implications for huntingtin conformation and its binding proteins. *Brain Res Bull* 56:319-329.
5. Weiss, A., Roscic, A., and Paganetti, P. 2009. Inducible mutant huntingtin expression in HN10 cells reproduces Huntington's disease-like neuronal dysfunction. *Mol Neurodegener* 4:11.