Legends to Supplementary Figures

Supplementary Figure 1. **Analysis of neuronal survival in the striatum of mHtt transgenic mice. (A)** Schema of the striatum region analyzed, where the red square corresponds to the neuronal population dissected and processed for further analysis. (**B**) Light microscopy images of EM-processed semi-thin sections of the striatum from 6 month-old mice, stained with Toluidine blue to visualize neurons (arrows), apoptotic cells (arrowheads), and myelinated axonal bundles (ax). **(C)** Electron micrographs of XBP1$^{Nes-/-}$-mHtt^{Q128} striatum used to monitor neuronal morphology. Examples of viable and apoptotic neurons are presented. See full quantification in Figure 1E,F. Scale bars, 20 μm (B) and 230 nm (C).

Supplementary Figure 2. **Analysis of XBP1 loss in striatum region. (A)** The mRNA level of *xbp1* was analyzed by real time PCR in total cDNA obtained from the brain striatum of XBP1$^{Nes-/-}$-mHtt^{Q128} or XBP1^{WT}-mHtt^{Q128} mice. All samples were normalized to β-actin levels. Average and SEM of the analysis of 3 animals per group are shown. **(B)** Motor performance was monitored with the rotarod assay in XBP1 wild-type or deficient animals bred onto the YAC128 HD mouse model (XBP1^{WT}-mHtt^{Q128} or XBP1$^{Nes-/-}$-mHtt^{Q128} respectively; 6-9 animals per group) over time. Rotarod values were normalized to the performance of XBP1^{WT} mice as a value of 1. Mean and SEM are presented. *:$p<0.05$ calculated with t-Student test.

Supplementary Figure 3. **Comparison of Huntingtin levels between male and female mice aged 6 months.** Htt expression was quantified from XBP1^{WT} (n=3; 1 female and 2 males), XBP1^{WT}-mHtt^{Q128} (n=8; 4 females and 4 males) and XBP1$^{Nes-/-}$-mHtt^{Q128} (n=13; 8 females and 5 males) mice. Mean and SEM are presented.

Supplementary Figure 4. **Knockdown of XBP1 in Neuro2A reduces the number of polyQ$_{79}$ inclusions. (A)** Neuro2A cells were stably transduced with lentiviral vectors expressing shRNA against XBP1 or control luciferase mRNA (shXBP1 and shCTR respectively). The expression of XBP1s or ATF4 (negative control) after treatment with tunicamycin (5 μg/ml, Tm) for 8 h was analyzed by Western blot. Levels of Hsp90 were used as loading control. **(B)** NSC34 cells were stably transduced with lentiviral vectors expressing shRNA against IRE1α or control luciferase mRNA (shIRE1α and shCTR respectively). Then IRE1α mRNA levels were monitored by real

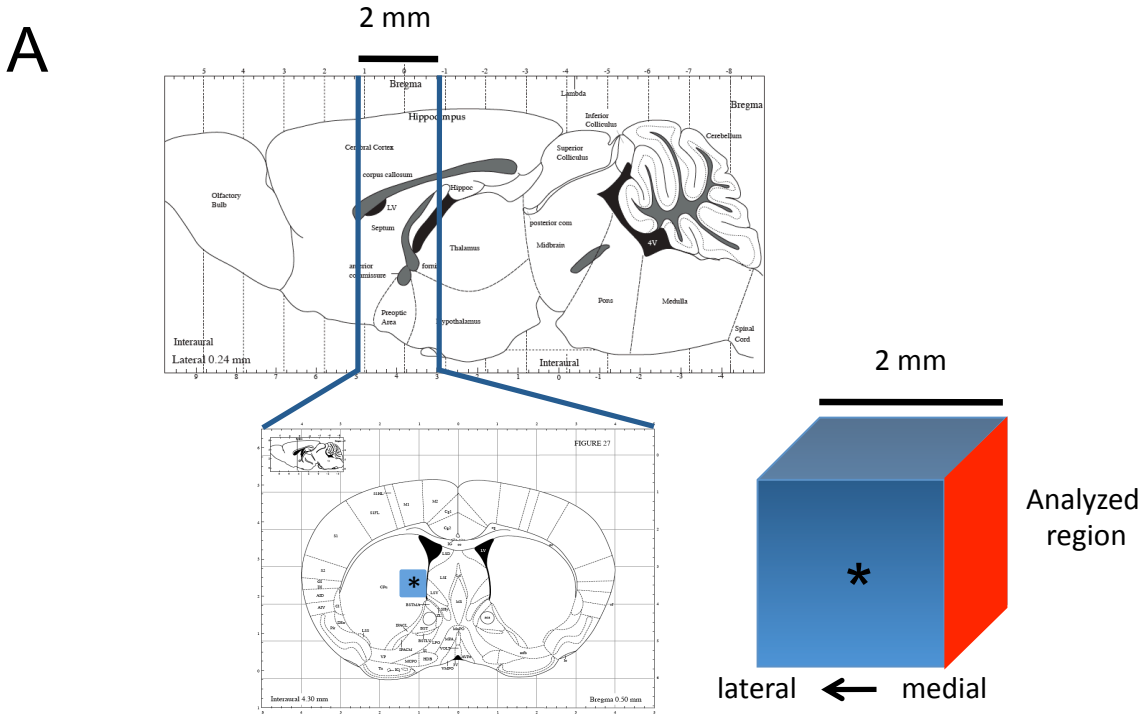
time PCR and normalized with actin levels. **(C)** Neuro2A shXBP1 or shCTR cells were transiently transfected with expression vectors for polyQ$_{79}$-EGFP. After 72h, polyQ$_{79}$-EGFP intracellular inclusions were quantified by fluorescent microscopy. The number of cells displaying intracellular inclusions was quantified in a total of at least 500 cells per experiment. Results are representative of three independent experiments performed. Average and standard deviation are presented. **(D)** Neuro2A cells were stably transduced with lentiviral vectors expressing shRNA against ATF4 or control luciferase mRNA (shATF4 and shCTR respectively). The expression of ATF4 after treatment with tunicamycin (5 μg/ml, Tm) for 8 h was analyzed by Western blot. Levels of Hsp90 were used as loading control. **(E)** LC3-II flux was monitored in Neuro2A shCTR or shATF4 cells. Cells were treated or not with a lysosome inhibitor cocktail (lys. inh.) containing 200 nM bafilomycin A$_1$, 10 μg/ml pepstatin and 10 μg/ml E64d for indicated time points and endogenous LC3 levels monitored by Western blot. Hsp90 levels served as loading control. **(F)** Neuro2A shATF4 or shCTR cells were transiently transfected with expression vectors for polyQ$_{79}$-EGFP. After 72 h, polyQ$_{79}$-EGFP aggregates were measured in cell extracts prepared in Triton X100 analyzed by Western blot and quantified of four independent experiments. Mean and SEM are presented.

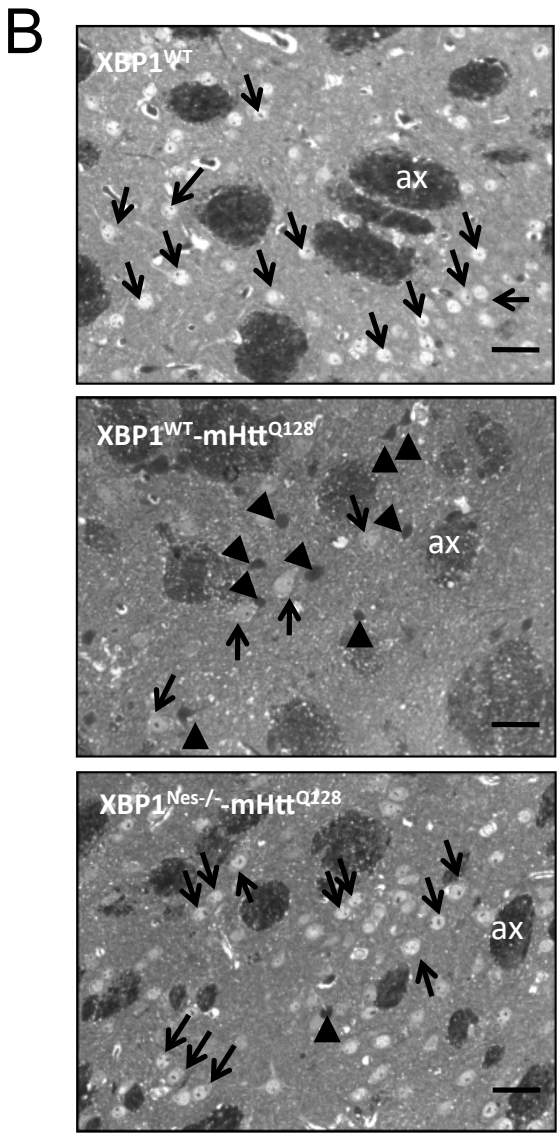
Supplementary Figure 5. **PolyQ$_{79}$-EGFP intracellular inclusions alter lysosomal content.** Neuro2A cells were transiently transfected with expression vector for polyQ$_{79}$-EGFP (green). After 24 h, cells were stained with lysotracker (red) and DAPI (blue), and the co-localization with polyQ$_{79}$-EGFP intracellular inclusions was determined by confocal microscopy. Scale bar, 10 μm.

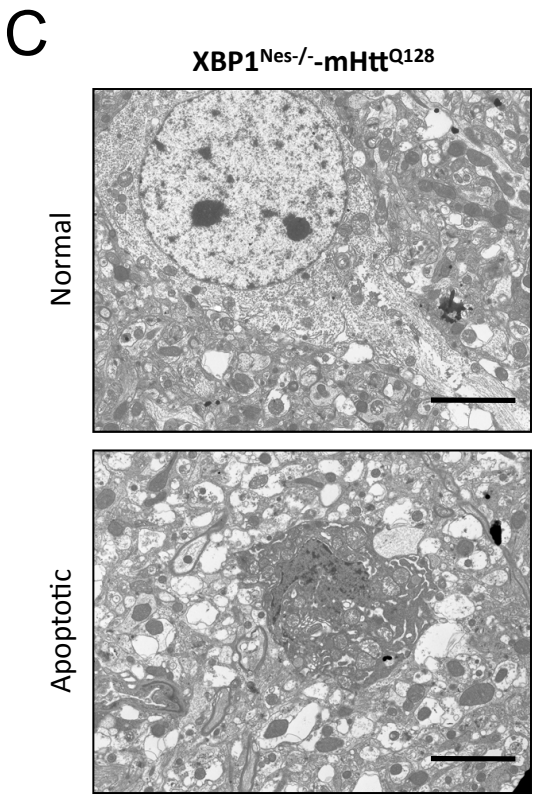
Supplementary Figure 6. **Lack of a global ER stress response in mHtt transgenic mice.** (**A**) The levels of XBP-1 mRNA splicing were monitored in striatum from three wild-type or YAC128 mice at 3 month of age. (**B**) The expression levels of PDI, BiP, Erp72, Grp58, ATF4 and CHOP were analyzed in the striatum of 6 months old mice by Western blot from XBP1^{WT} (n=2), XBP1^{WT}-mHtt^{Q128} (n=3) and XBP1$^{Nes-/-}$-mHtt^{Q128} (n=3) mice. Hsp90 was monitored as loading control. As positive control for ER stress, MEFs treated with 1 μg/ml tunicamycin (Tm) for 16 h is presented.

Supplementary Figure 7. **ATF4 deficiency does not alter FoxO1 levels in the striatum.** FoxO1 levels were analyzed in the striatum of indicated experimental groups by Western blot. γ-tubulin was used as loading control. Left panel: The relative levels of FoxO1 were quantified from $ATF4^{WT}$- $mHtt^{Q128}$ (n=6) and $ATF4^{-/-}$-$mHtt^{Q128}$ (n=5) mice and normalized with Hsp90 levels. Mean and SEM are presented.

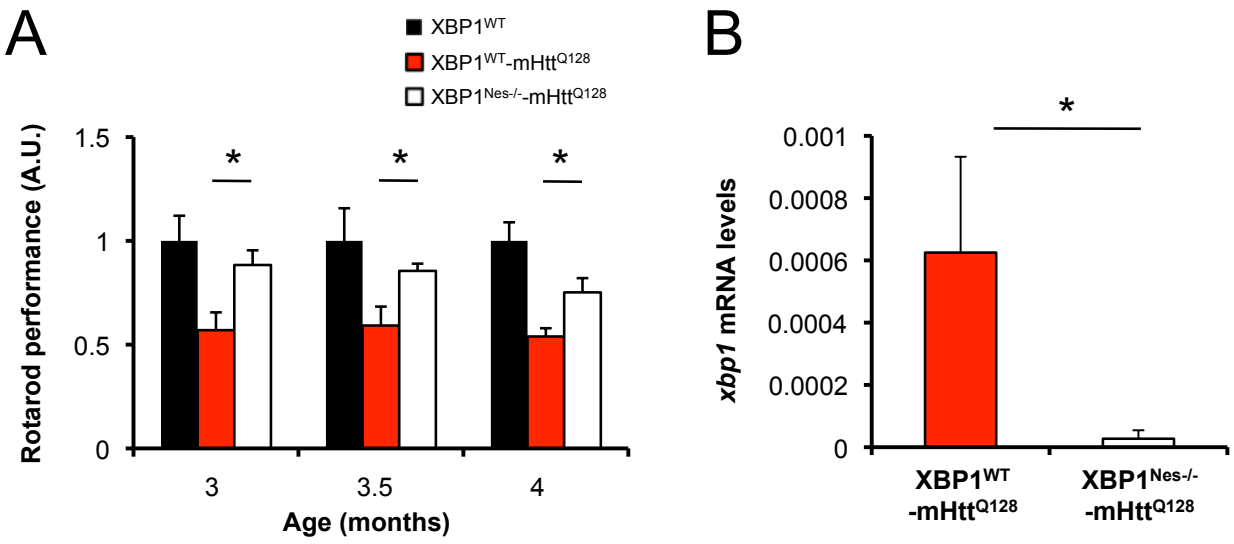
Supplementary Figure 1



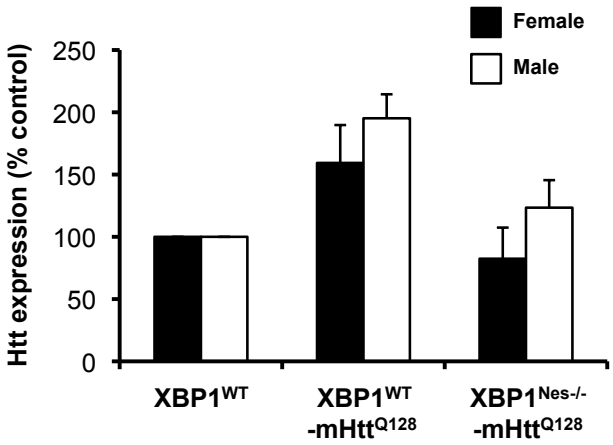




Supplementary Figure 2



Supplementary Figure 3



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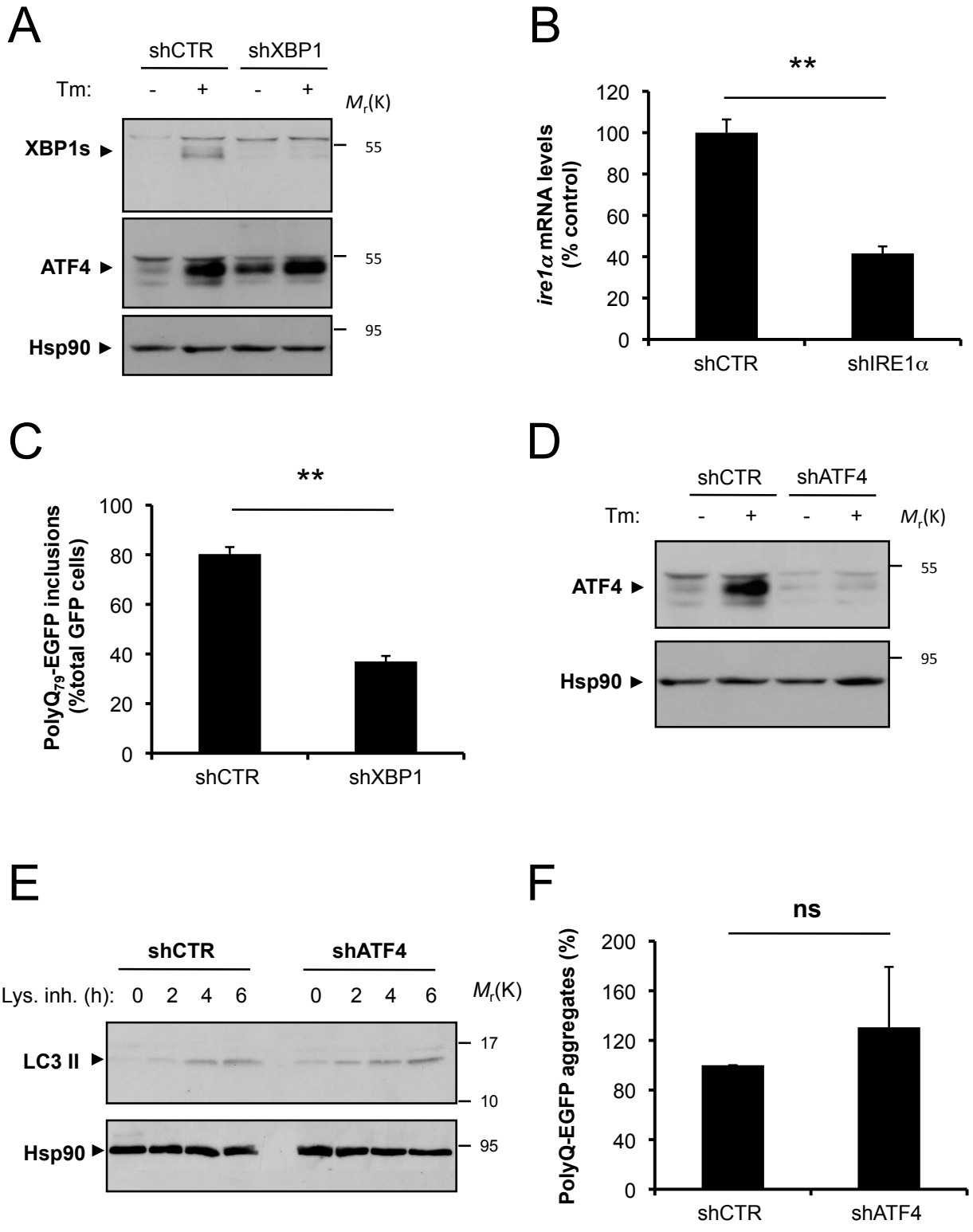

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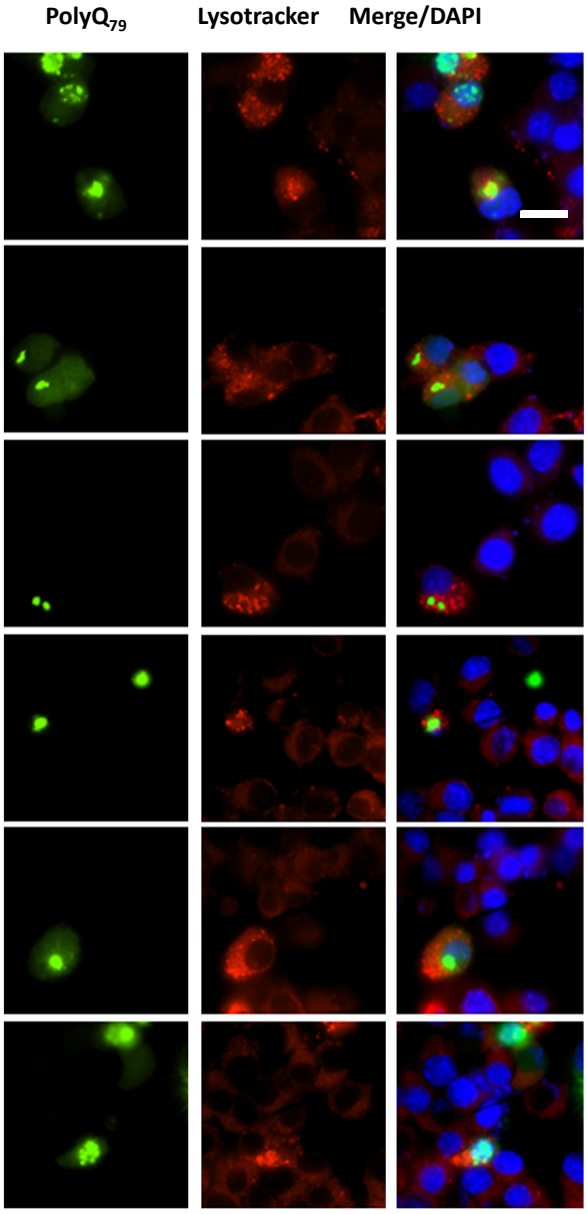
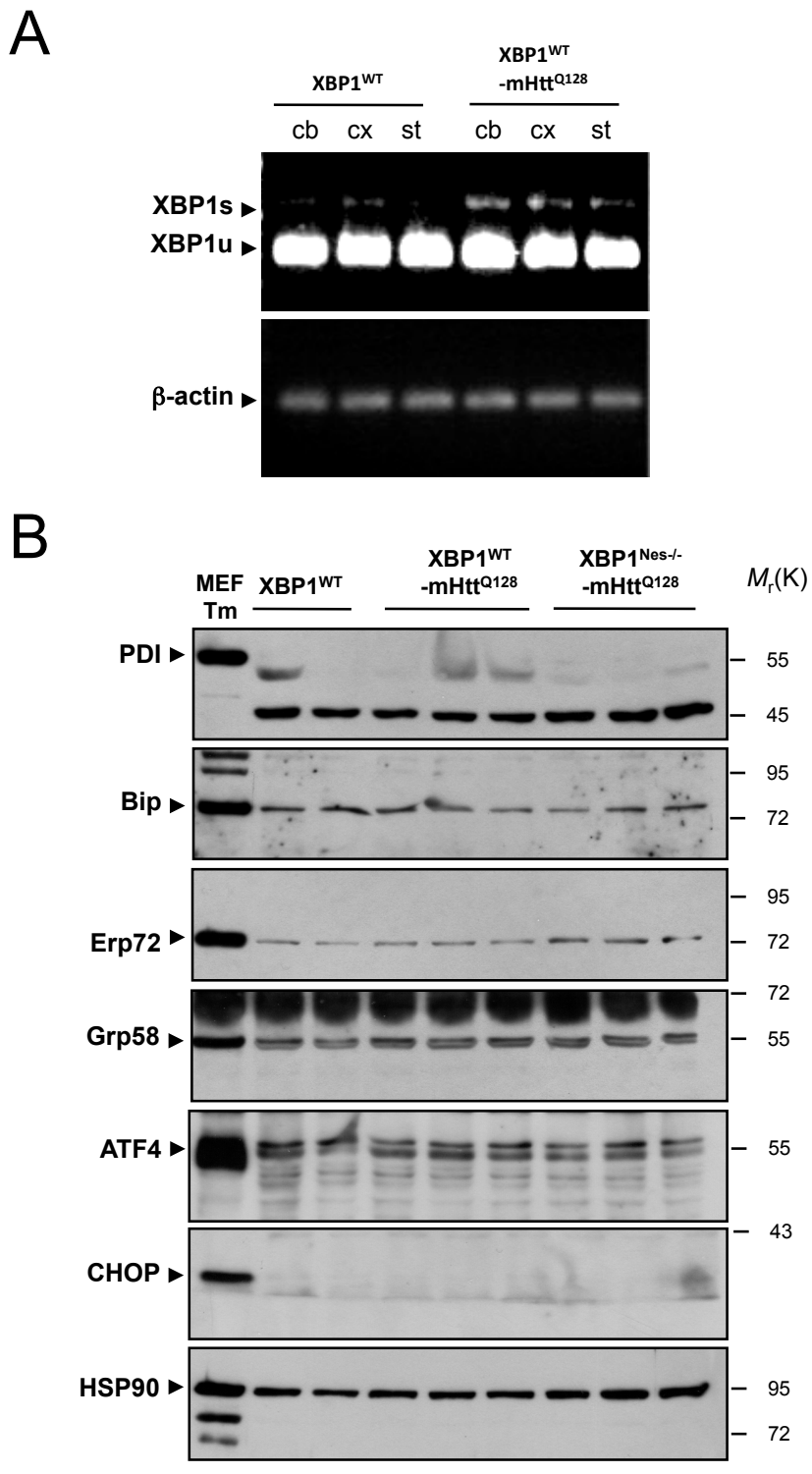
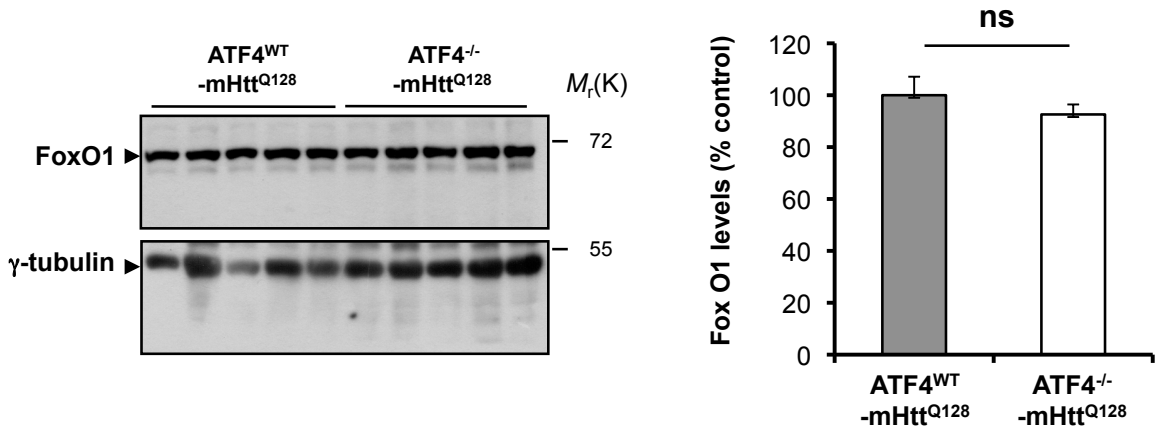


Supplementary Figure 6



Supplementary Figure 7



Supplementary Table 1

A

Genotype	Animal number	n. CAG repeats
$XBP1^{WT}$-$mHtt^{Q128}$	1	127
	2	127
	3	127
	4	127
	5	127
	6	127
$XBP1^{Nes-/-}$-$mHtt^{Q128}$	1	127
	2	127
	3	127
	4	127
	5	127
	6	127

B

Genotype	Animal Number	n. CAG repeats
$XBP1^{WT}$-$Hdh^{Q111/Q7}$	1	132
	2	132
	3	128
	4	130
	5	130
	6	127
	Average	**130**
$XBP1^{Nes-/-}$-$Hdh^{Q111/Q7}$	1	133
	2	131
	3	133
	4	133
	5	131
	6	128
	Average	**131**

Supplementary Table 2

ID	Tissue	Region	Age	Gender	n. CAG repeats allele1/2	Diagnosis
1	AN05543	BA7, CAP, Cerebellum	67	M	17/24	negative HD
2	AN01410	BA7, CAP, Cerebellum	41	M	17/27	negative HD
3	AN11114	BA7, CAP, Cerebellum	58	M	27/27	negative HD
4	AN09568	BA7, CAP, Cerebellum	57	M	17/21	negative HD
5	AN07875	BA7, CAP, Cerebellum	53	M	21/45	HD
6	AN08445	BA7, CAP, Cerebellum	68	M	26/45	HD
7	AN01682	BA7, CAP, Cerebellum	53	M	13/52	HD
8	AN18184	BA7, CAP, Cerebellum	40	M	26/45	HD