**Supplementary Material**

**Longitudinal Biochemical Assay Analysis of Mutant Huntingtin Exon 1 Protein in R6/2 Mice**

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| --- | --- | --- | --- |
| **Animal** | **Age (weeks)** | **(CAG)n Genomic DNA (Tail)** | **(CAG)n cDNA (Striatum)** |
| 1 | 5 | 125 | 125 |
| 2 | 5 | 129 | 129 |
| 3 | 5 | 123 | 123 |
| 4 | 7 | 128 | 128 |
| 5 | 7 | 131 | 131 |
| 6 | 7 | 131 | 130 |
| 7 | 9 | 126 | 126 |
| 8 | 9 | 126 | 126 |
| 9 | 9 | 126 | 126 |
| 10 | 11 | 129 | 129 |
| 11 | 11 | 126 | 127 |
| 12 | 11 | 128 | 127 |

**Supplementary Table 1.** CAG repeats from genomic tail DNA comparable to counts obtained from striatal cDNA from the same animal showing lack of somatic expansion in these animals. cDNA generated from RNA harvested from striatum. CAG repeat sizing of cDNA was performed by Laragen.



**Supplementary Figure 1.** Detection of mHTTex1p is highly variable using TRIzol reagent. A) Striatal and C) Cortical protein samples recovered from TRIzol preparations show highly variable detection of both soluble monomeric and soluble HMW mHTTex1p as analyzed by PAGE and western blot. AGE analysis of B) Striatal and samples show a significant change in oligomer levels (F3,8=16.02, p<0.001) while D) Cortical samples do not (F3,8=0.95, p>0.05). Western blots quantified by mean pixel value and normalized to actin. AGE blots were quantified by mean pixel value. Data analyzed by 1-way ANOVA followed by Tukey’s multiple comparison test. \*p<0.05, \*\*p<0.01, values represent means ± SEM. n=3 for all time points. HTT antibody MAB5492 used to detect mHTTex1p.



**Supplementary Figure 2.** Detection of mHTTex1p in hippocampus and cerebellum of R6/2 mice varies depending on break method. Insoluble fraction in A) hippocampus reveals a significant increase in a HMW species of mHTTex1p throughout disease progression (F3,8=21.05, p<0.001). Soluble fraction shows an inverse, significantly decreased monomeric form of mHTTex1p throughout disease progression (F3,8=142.2, p<0.0001). B) T-PER processed hippocampal tissue samples did not show decrease in soluble monomeric mHTTex1p (F3,8=0.13, p>0.05) or HMW accumulated mHTTex1p (F3,8=5.41, p<0.05). Insoluble fraction in C) cerebellum reveals a significant increase in HMW species of mHTTex1p throughout disease progression (F3,8=29.11, p<0.0001). Soluble fraction did not detect a significant decreased monomeric form of mHTTex1p (F3,8= 2.51, p>0.05). However, soluble monomeric mHTTex1p decrease was detected in D) T-PER processed cerebellar tissue samples (HMW: F3,8=6.32, p<0.05, Monomer: F3,8=4.25, p<0.05). Fluctuations in soluble, monomeric mHTTex1p correspond to varying CAG repeats in R6/2 mice. Western blots quantified by mean pixel value. Soluble fraction normalized to actin and analyzed by 1-way ANOVA followed by Tukey’s multiple comparison test. \*p<0.05, \*\*p<0.01, values represent means ± SEM. n=3 for all time points. HTT antibody MAB5492 used to detect mHTTex1p.



**Supplementary Figure 3.** Detection of mHTTex1p in peripheral tissue varies depending on break method. A) Insoluble HMW mHTTex1p increases significantly throughout disease progression in Liver (F3,8=83.34, p<0.0001) but Soluble, monomeric mHTTex1p is not detectable. B) Liver tissue broken in T-PER resolves soluble monomer revealing no change in detectable protein abundance (F3,8=2.95, p>0.05) while HMW mHTTex1p showed a significant increase (F3,8=12.79, p<0.01). C) Skeletal muscle also reveals a significant increase in insoluble HMW mHTTex1p (F3,8=7.53, p<0.05). Soluble monomer is not detected in either Soluble/Insoluble fractionated samples. Western blots quantified by mean pixel value. Soluble fraction normalized to actin (liver) or GAPDH (Skeletal Muscle) and analyzed by 1-way ANOVA followed by Tukey’s multiple comparison test. \*p<0.05, \*\*p<0.01, values represent means ± SEM. n=3 for all time points. HTT antibody MAB5492 used to detect mHTTex1p.

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**Supplementary Figure 4.** Delta CT (dCT) values used to analyze transcriptional alterations detected by qPCR in R6/2 mice. A) Striatal gene dCT values relative to NT week 5 shows a significant progressive increase in full length murine *Htt* (Genotype: F1,16=5.22, p<0.05) and decrease in *Darpp-32* (Genotype: F1,16=58.54, p<0.0001). Striatal gene dCT values relative to R6/2 week 5 shows a progressive increase of R6/2 Transgene expression (F3,8=4.41, p<0.05). B) Cortical gene dCT values relative to NT week 5 shows significant changes in *Darpp-32* (Genotype: F1,16=160.1, p<0.0001) but not *Htt* (Age: F1,16=2.69, p>0.05, Genotype: F1,16=0.02, p>0.05) or R6/2 transgene relative to week 5 R6/2 animals (F3,8=0.62, p>0.05). \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001, values represent mean fold change. n=3 for each gene and timepoint. Statistical analysis was completed using 2-way ANOVAs for comparing to NT animals followed by Sidak’s multiple comparison’s test and 1-way ANOVAs comparing R6/2 mice at different ages followed by Tukey’s multiple comparison test.