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Supplementary Information for

IKKβ slows Huntington's Disease progression in R6/1 mice

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References for SI reference citations

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Supplemental Figures:

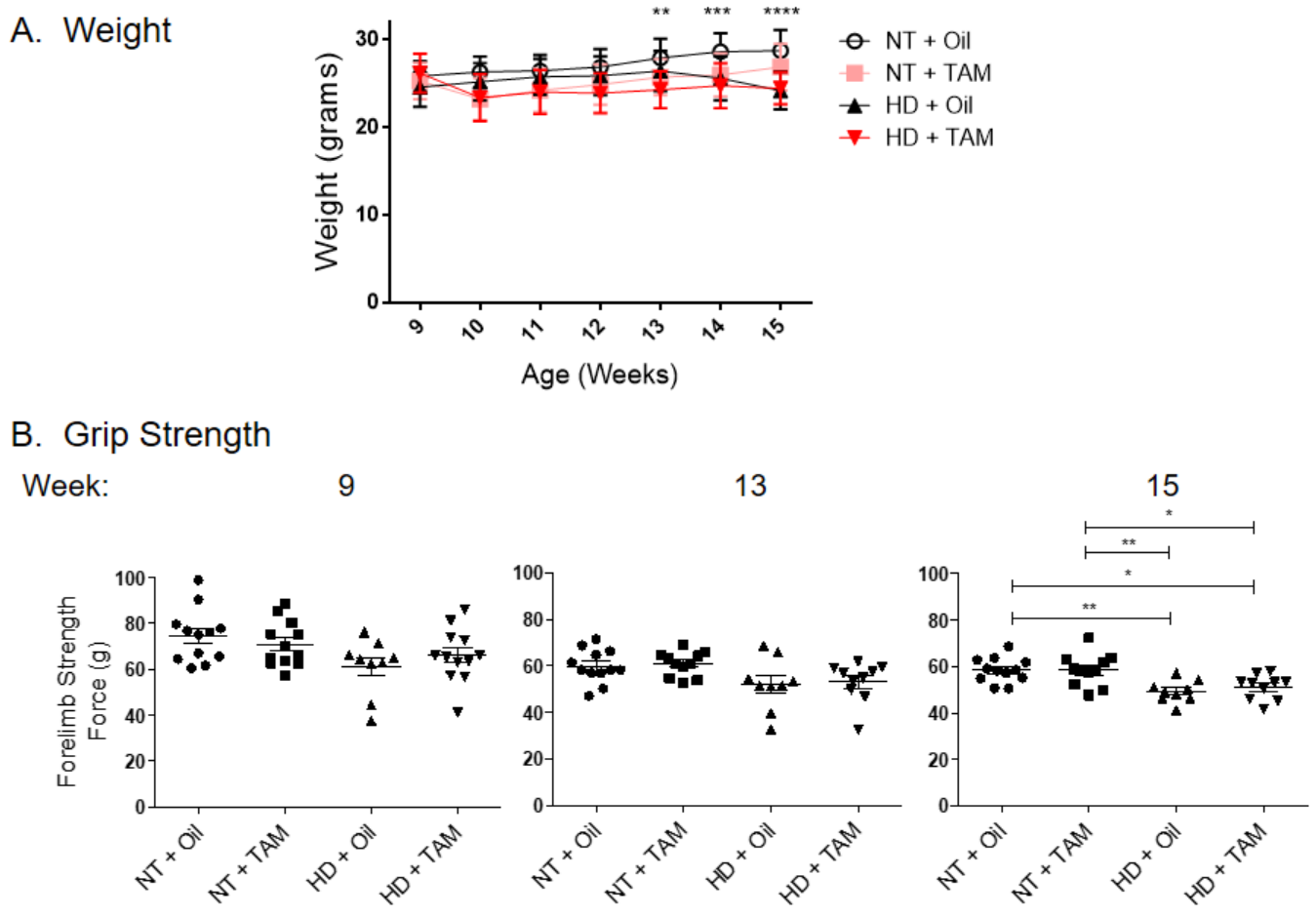

Fig. S1. Weight measurements and grip strength behavior are unaffected for male tamoxifen- vs. oil-treated R6/1 and non-transgenic WT controls. Tamoxifen-induced IKKβ knockout did not impact animal weight (A) or grip strength (B) in R6/1 (HD) or non-transgenic (NT) WT control male mice (n = NT-Oil: 12; NT-Tamoxifen: 12; HD-Oil: 9; HD-Tamoxifen: 12 at week 9, and NT-Oil: 12; NT-Tamoxifen: 11; HD-Oil: 9; HD-Tamoxifen: 10 following injections). Weight loss from week 13-15 was significant for HD-Oil vs. NT-Oil, but was unaffected by tamoxifen treatment. A significant difference in grip strength was observed between HD and NT controls at 15 weeks, which was not impacted by tamoxifen-induced IKKβ knockout. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ values represent means ± SEM. Statistical significance was determined by one-way ANOVA with Tukey post-testing for A and Bonferroni post-testing for B.

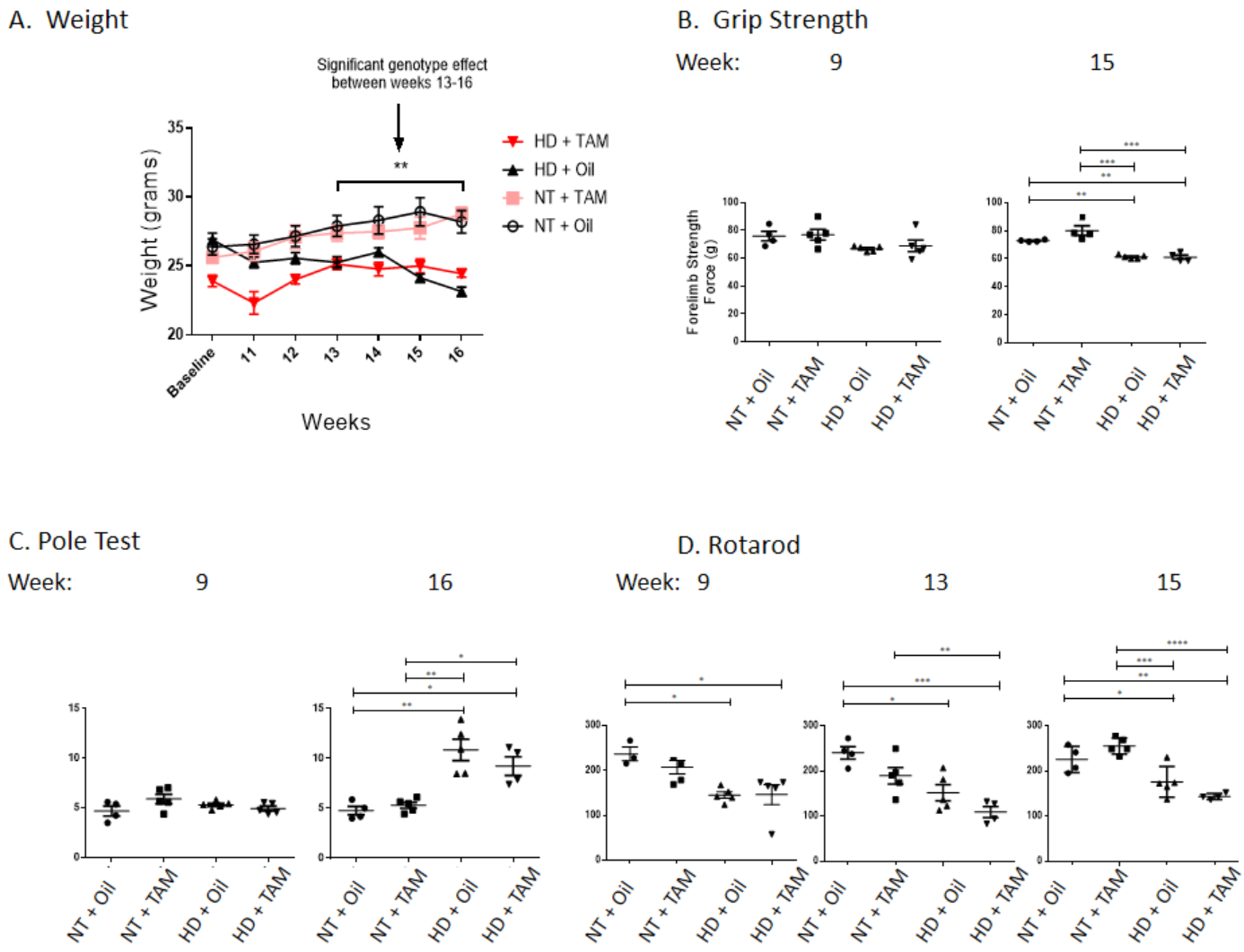

Fig. S2. Male R6/1 (HD) and non-transgenic (NT) WT control weight and behavior are not significantly affected by tamoxifen treatment in the absence of the Cre and floxed alleles of IKKβ. Standard R6/1 (HD) and non-transgenic (NT) WT controls, without the Cre and floxed alleles of IKKβ, were treated with tamoxifen or oil control and were assessed for weight (A), grip strength (B), pole test (C) and rotarod (D). Tamoxifen- or oil- treatment without IKKβ knockout did not impact HD or NT control weight, or behavioral tasks (n = 5 males/ all groups at week 8; HD-Tamoxifen = 4 males/group, all other groups n=5 males/group following injections). *p < 0.05, **p < 0.01, ***p < 0.001 values represent means ± SEM. Statistical significance was determined by one-way ANOVA with Bonferroni post-testing.

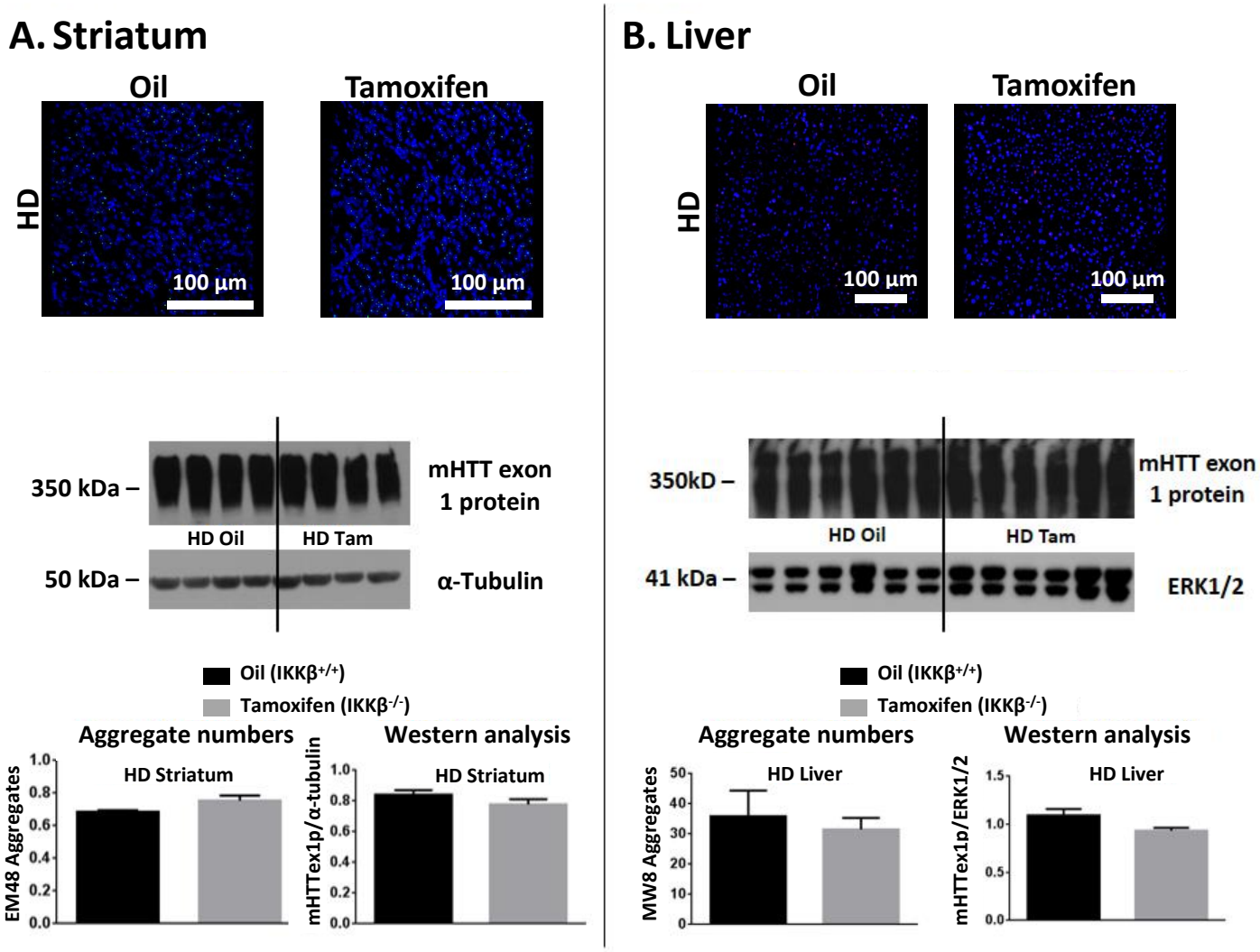

Fig. S3. Levels of transgenic mutant HTT exon 1 protein and aggregates are not altered by IKKβ knockout in striatum and liver. Male R6/1 (HD) mice containing the tamoxifen-inducible Cre and floxed alleles of IKKβ were treated with tamoxifen or oil vehicle control. Transgenic mutant HTT exon 1 protein aggregates from 16 week-old male mice were counted using Imaris in striatum (A) and in liver (B), and mutant HTT protein levels were quantitated by western analysis using an antibody that specifically detects only the transgenic human mutant HTT exon 1 protein, MAB5492. Relative aggregate numbers, and protein levels normalized to loading controls, revealed no significant differences with IKKβ knockout in striatum or in liver. Aggregate numbers in striatum, detected with anti-HTT EM48 (A), were much higher in general than in liver, detected with anti-HTT MW8 (B).

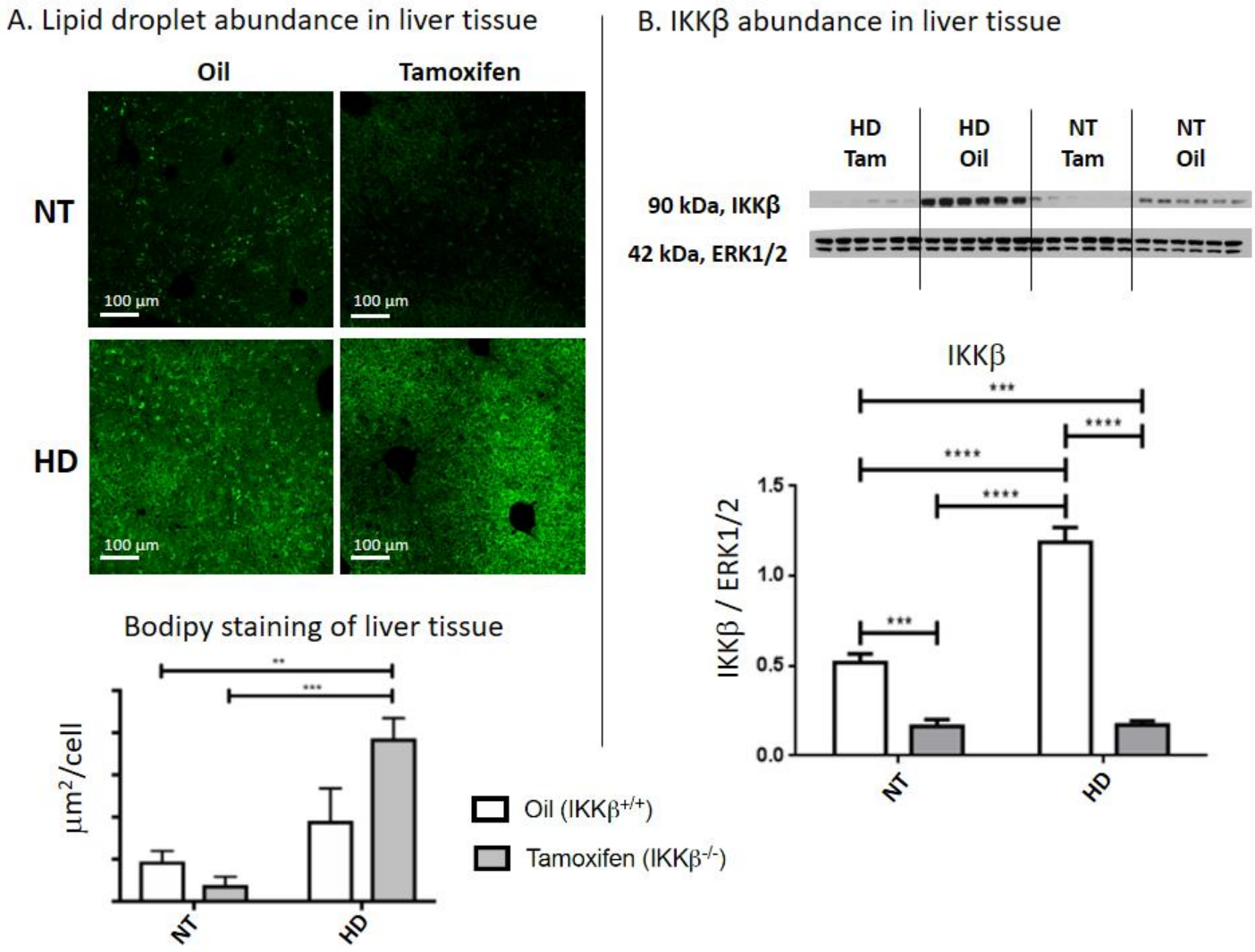

Fig. S4. Lipid droplet levels and IKKβ protein abundance are increased in R6/1 liver. (A) Bodipy staining of liver tissue sections to quantitate lipid droplet levels was done on tamoxifen vs. oil treated R6/1 (HD) and non-transgenic mice (NT) containing the tamoxifen-inducible Cre and floxed alleles of IKKβ. Levels of lipid droplets quantitated by Imaris were elevated in HD liver vs. non-transgenic controls, and highest in IKKβ knockout HD liver, suggestive of liver dyshomeostasis in R6/1 mice. n = 4 male animals/group. $^{**}p < 0.01$, $^{***}p < 0.001$ values represent means ± SEM. Statistical significance was determined by one-way ANOVA followed by post-hoc unpaired student's t tests. (B) IKKβ levels are significantly elevated in oil-treated male 16 week HD liver soluble tissue fraction vs. NT control, and are significantly reduced with tamoxifen treatment in HD and NT liver, as assessed by western analysis. $^{***}p < 0.001$, $^{****}p < 0.0001$ values represent means ± SEM. Statistical significance was determined by one-way ANOVA with Bonferroni post-testing.

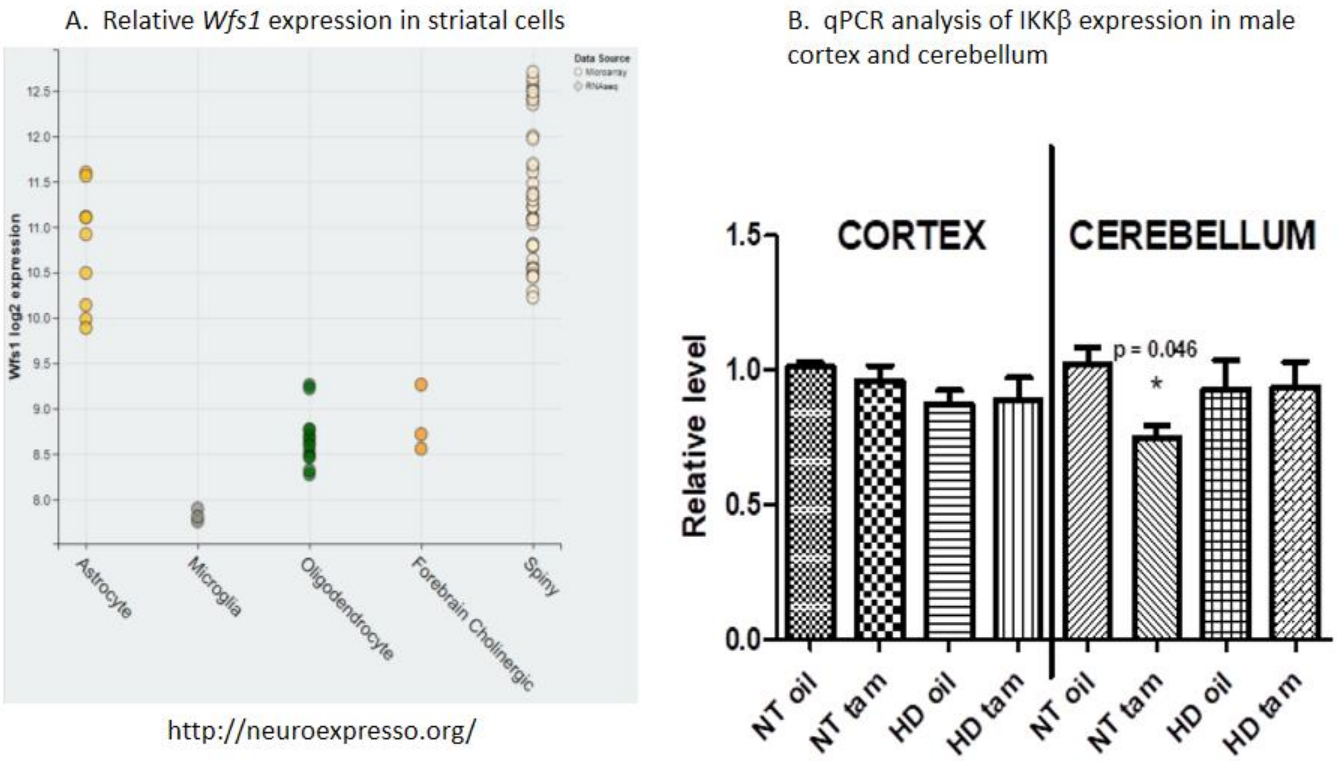

Fig. S5. A. The *Wfs1* promoter, used in this study to drive expression of the tamoxifen-inducible Cre, is highly expressed in medium spiny neurons but not expressed well in microglia. A graph copied from the Neuroexpresso.org website (1) depicts striatal-specific cell type expression of *Wfs1* determined from microarray analysis. Tamoxifen-treatment of R6/1 (HD) and non-transgenic (NT) controls, containing floxed alleles of IKKβ and the tamoxifen-inducible Cre driven by the *Wfs1* promoter, would be expected to yield better knockout of IKKβ in medium spiny neurons than in microglia because of the relative expression of *Wfs1* in these two cell types. **B. Relative IKKβ gene expression levels in cortex and cerebellum of tamoxifen vs. oil treated HD and NT male mice.** Relative levels of IKKβ ddCt values normalized to RPLPO were calculated for male 16 week-old cortex and cerebellum. For cortex, n=3 males/group for NT-Oil, NT-Tamoxifen and HD-Tamoxifen, and n=5 males/group for HD-Oil. For cerebellum, n=6 males/group for NT-Oil and NT-Tamoxifen, and n=5 males/group for HD-Tamoxifen and HD-Oil. For statistical analysis, ddCT values for each gene of interest were compared in a two-way ANOVA with posthoc analyses using Tukey's HSD with Bonferroni correction, and adjusted p values reported: *$p < 0.05$.

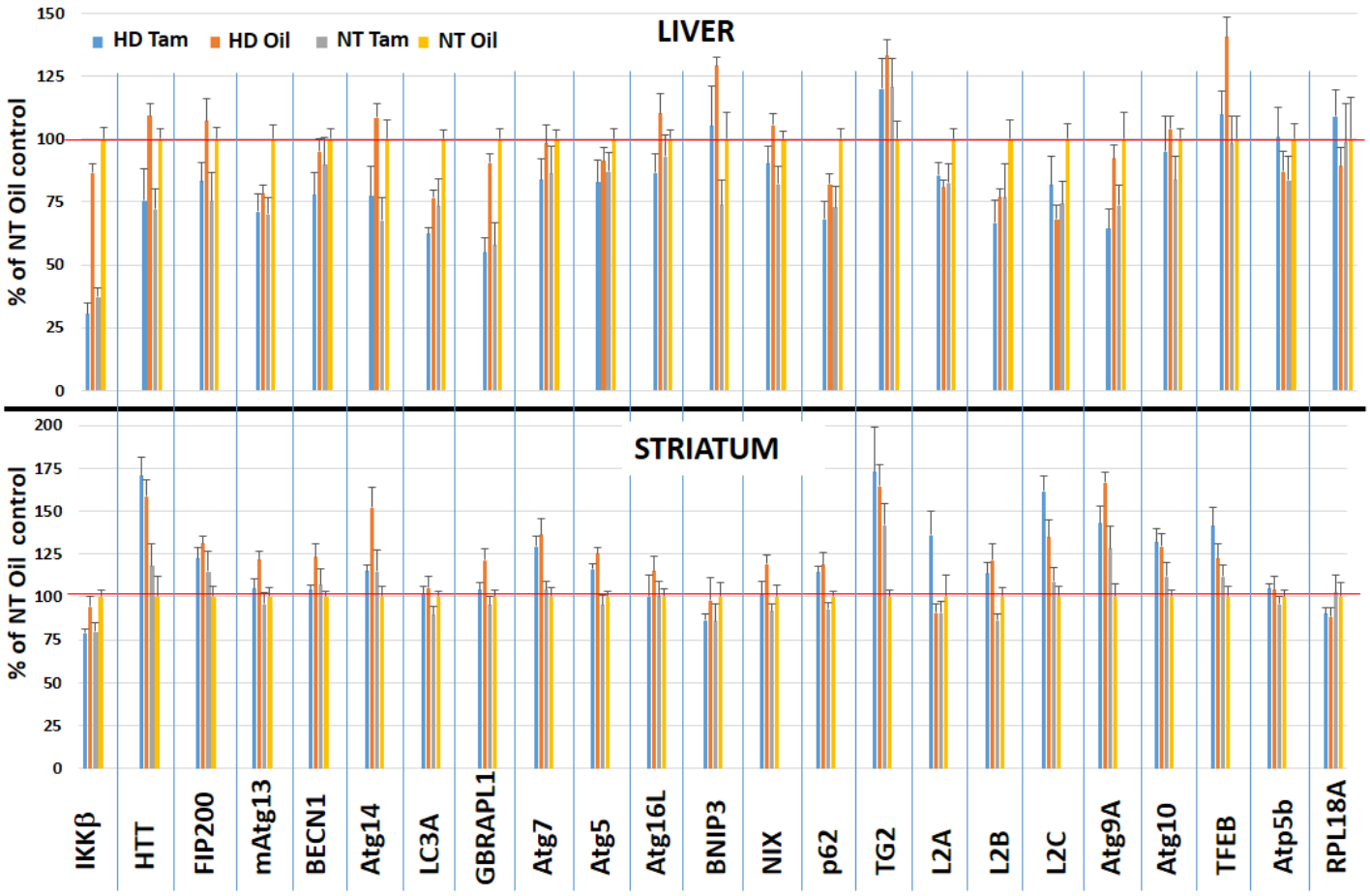

Fig. S6. qRT-PCR relative levels of the expression of autophagy genes in liver and striatum of R6/1 and non-transgenic mice with and without IKKβ knockout. qRT-PCR was used to determine the relative expression of select autophagy genes in liver and striatum in 16 week male tissue collected at the end of the study. Samples from R6/1 (HD) treated with tamoxifen, R6/1 (HD) treated with oil, and non-transgenic WT (NT) treated with tamoxifen (tam) were compared with non-transgenic WT (NT) oil-treated controls (set as 100%). ddCt was calculated relative to RPLPO for IKKβ, HTT, and 19 other selected autophagy-related genes, as well as 2 unaffected controls Atp5b and RPL18A. Liver, n=8 males/group. Striatum, n=5 males/group for HD-Tamoxifen, HD-Oil, and NT-Tamoxifen, n=6 for NT-Oil. Graphed values represent the mean +/- SEM.

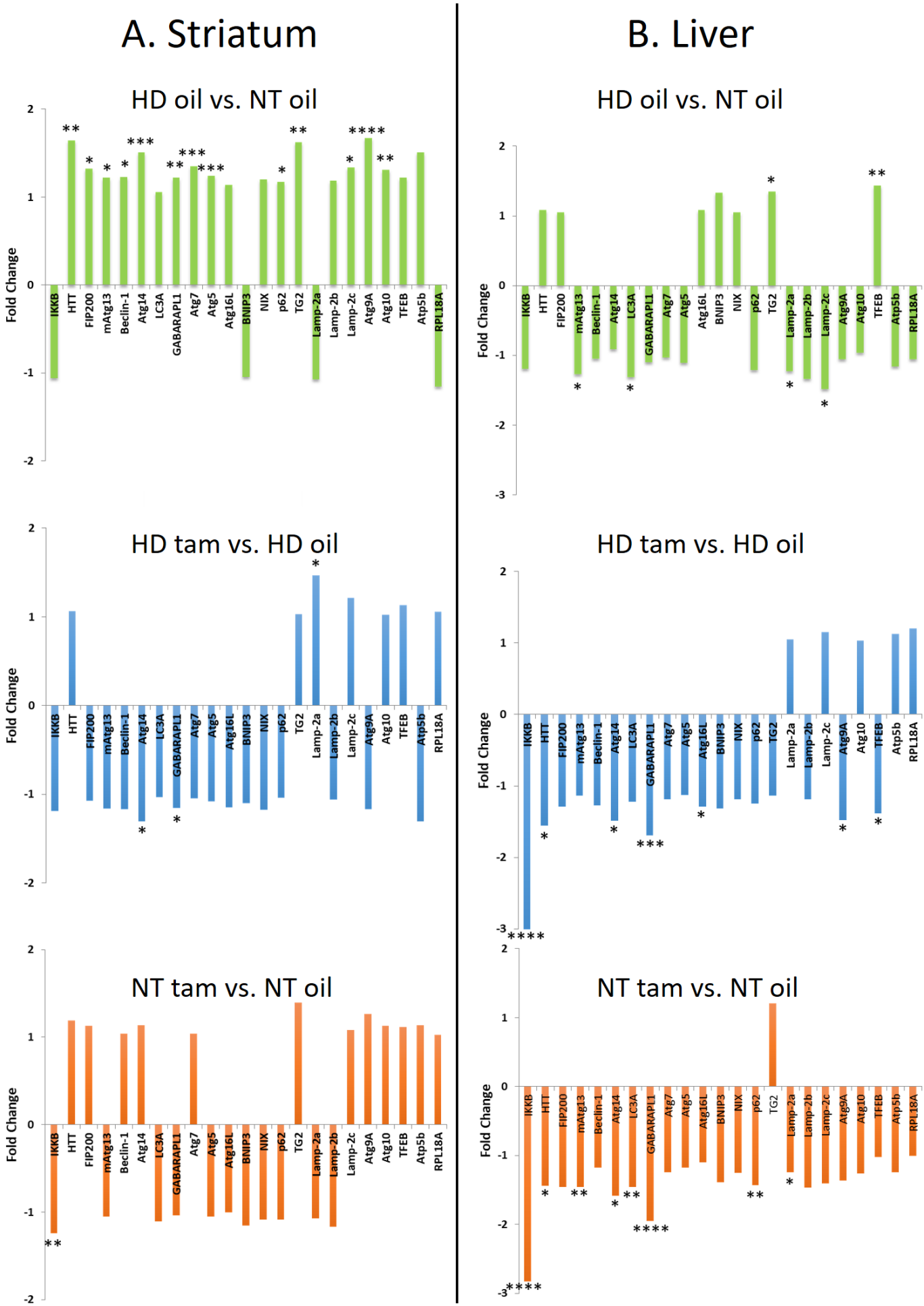
A. Striatum
B. Liver
HD oil vs. NT oil
HD tam vs. HD oil
NT tam vs. NT oil
Fold Change
IKKB
HTT
FIP200
mAtg13
Beclin-1
Atg14
LC3A
GABARAPL1
Atg7
Atg5
Atg16L
BNIP3
NIX
p62
TG2
Lamp-2a
Lamp-2b
Lamp-2c
Atg9A
Atg10
TFEB
Atp5b
RPL18A

Fig. S7. qRT-PCR analysis of autophagy gene expression with tamoxifen-induced IKKβ knockout in striatum and liver. Fold change in ddCt values normalized to RPLPO was calculated for male 16 week-old R6/1 (HD) oil-treated vs. non-transgenic WT (NT) oil-treated, HD tamoxifen-treated vs. HD oil-treated, and NT tamoxifen-treated vs. NT oil-treated striatum (A) and liver (B). Liver, n=8 males/group. Striatum, n=5 males/group for HD tam, HD oil, and NT tam, n=6 for NT oil. For statistical analysis, ddCT values for each gene of interest were compared in a two-way ANOVA with posthoc analyses using Tukey's HSD with Bonferroni correction, and adjusted p values reported: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

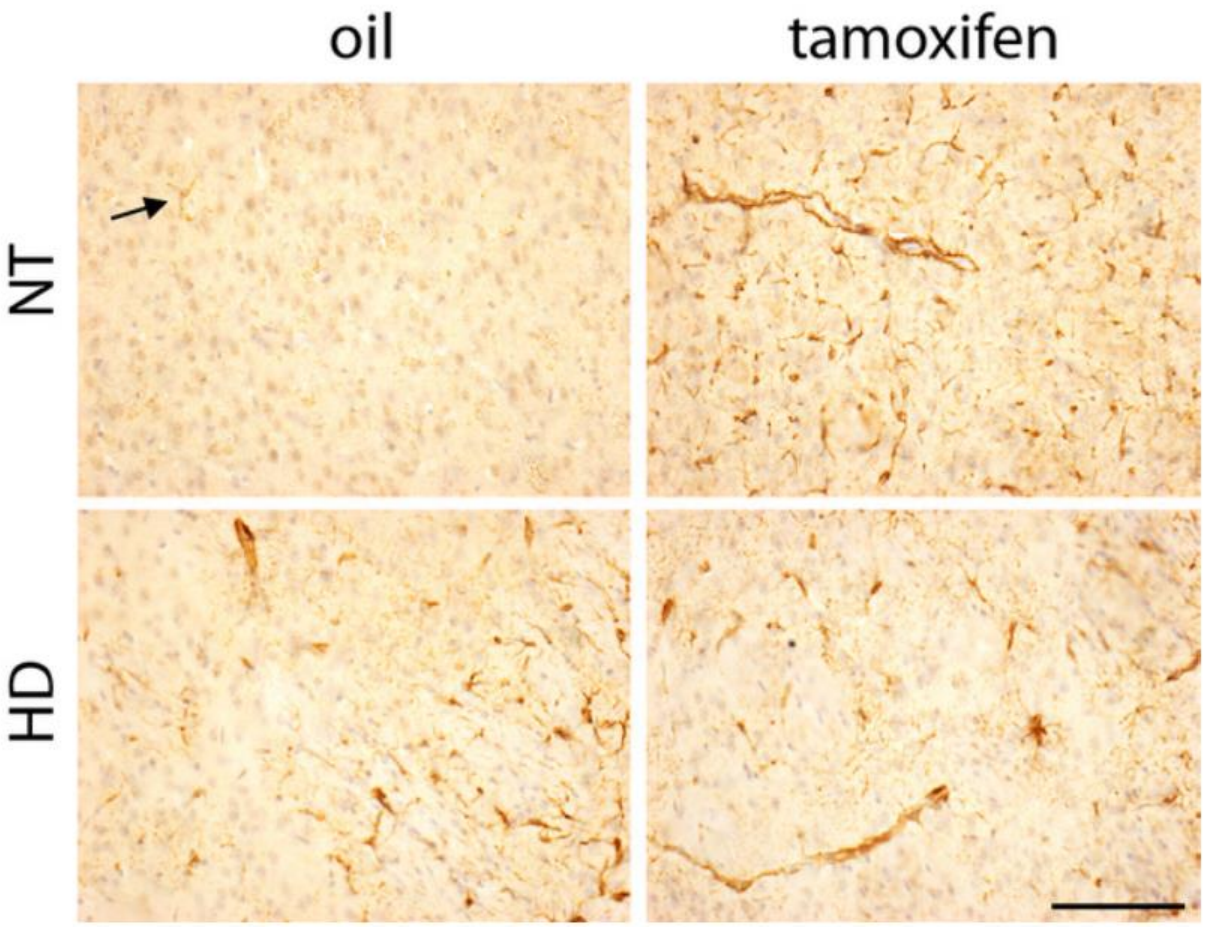

Fig. S8. GFAP-positive astrocytes are increased in NT striatum with IKKβ knockout and increased in general in HD striatum. WT control non-transgenic (NT) oil striatum shows relatively few GFAP+ astrocytes (arrowhead). NT tamoxifen control with IKKβ knockout shows many GFAP+ astrocytes, including prominent perivascular end feet. Compared with NT oil, both HD oil and HD tamoxifen striatal sections also have increased GFAP+ astrocytes. Background staining of pencil fibers is also present, confirming striatal location. Scale bar, 100 microns.

References:

1. Mancarci BO, *et al.* (2017) Cross-Laboratory Analysis of Brain Cell Type Transcriptomes with Applications to Interpretation of Bulk Tissue Data. *eNeuro* 4(6).