

Neuron, Volume 107

Supplemental Information

**Cell Type-Specific Transcriptomics Reveals
that Mutant Huntingtin Leads to Mitochondrial RNA
Release and Neuronal Innate Immune Activation**

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SUPPLEMENTAL INFORMATION

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Figure S2. WGCNA analysis of R6/2 model TRAP data, related to Figure 2.

Figure S3. Shared predicted regulators in cell types assessed in TRAP, related to Figures 2 and 3.

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Figure S16. Comparison of human HD snRNA-Seq data to the zQ175DN and R6/2 mouse model snRNA-Seq data, related to Figures 4 and 5.

Figure S17. WGCNA analysis of iSPN 3- and 6-months of age allelic series TRAP data, related to Figures 3, 5, and 7.

Figure S18. mHTT promotes the accumulation of cytosolic mtDNA, and striatal enrichment of TOMM70A related to Figure 7.

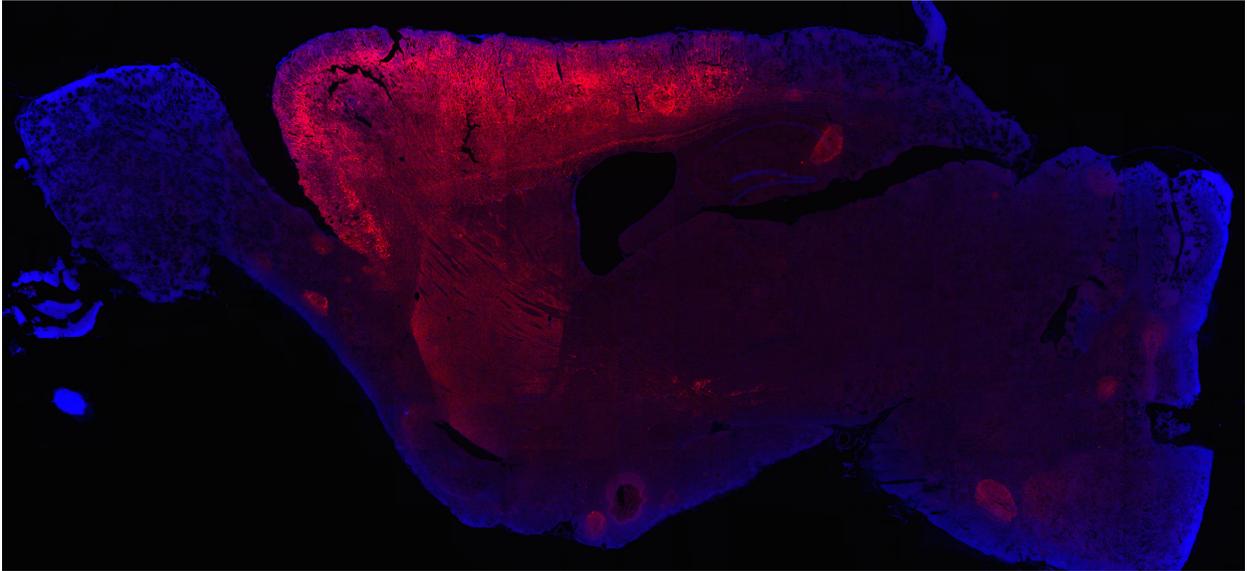
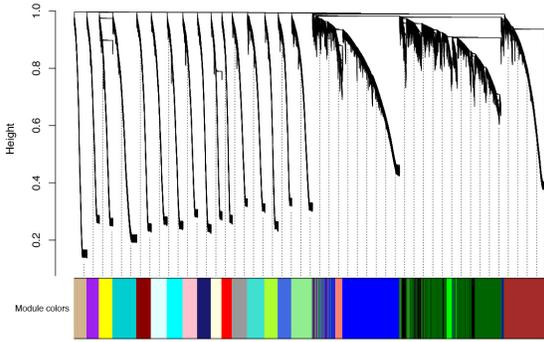
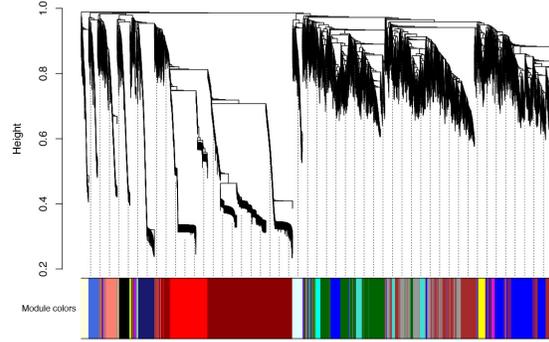


Figure S1. Retro-TRAP labeling of corticostriatal projection neurons, related to Figure 2. A tiled scan stack of a sagittal mouse brain section showing corticostriatal projection neurons (in red pseudocolor, note the labeled corticostriatal projection neuron axons extending into the striatum; neuropil, not cellular signal in the striatum) targeted by striatal injection of a Retro-TRAP virus (STAR Methods).

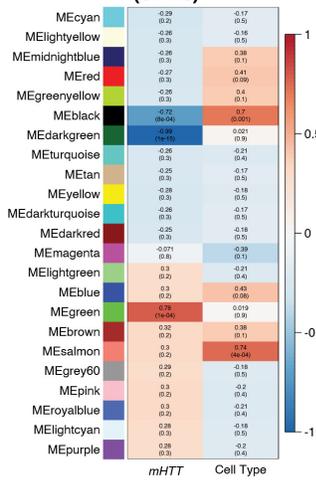
A Cluster Dendrogram of R6/2 TRAP for dSPN and iSPN



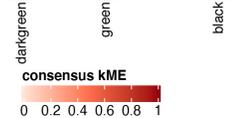
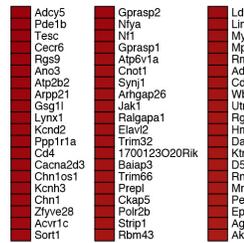
E Cluster Dendrogram of R6/2 TRAP for CStrPN



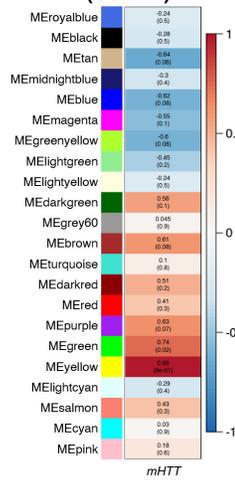
B Module-Traits Relationships (SPNs)



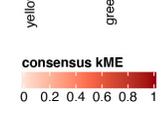
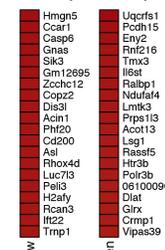
C Hub Genes (SPNs)



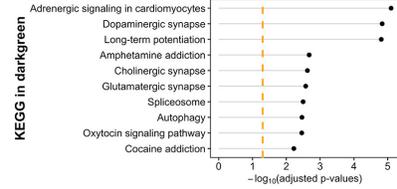
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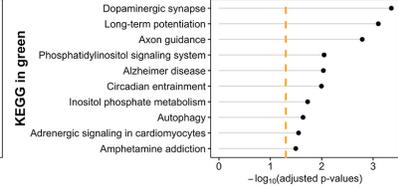
G Hub Genes (CStrPN)



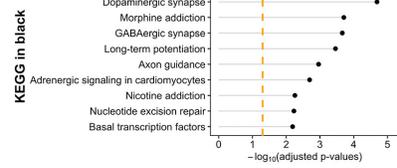
D KEGG in darkgreen



H KEGG in yellow



D KEGG in black



H KEGG in green

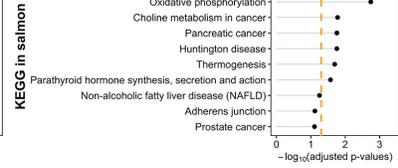


Figure S2. WGCNA analysis of R6/2 model TRAP data, related to Figure 2. Consensus coexpression network analysis of the dSPN and iSPN R6/2 TRAP data reveals multiple *mHTT*- and SPN cell type-associated modules, each labeled by a unique color in the clustering dendrogram (A) and in the module-trait table that represents the Pearson correlation of each module eigengene with the specified trait (either the presence of *mHTT* or Cell Type) (B). Also shown are the top module hub genes (C) and the top ten KEGG enriched pathways (D) in selected modules from B. Similar consensus coexpression network analysis of the CStrPN R6/2 TRAP data, revealing top *mHTT*-associated modules in the clustering dendrogram (E) and module-trait table (F), as well as the top module hub genes (G) and the top ten KEGG enriched pathways (H) in selected modules from F. **B and F:** red signifies a strong positive correlation and blue signifies a strong negative correlation, and Fisher's asymptotic test *p*-values for the module-trait relationships are listed below each correlation. **C and G:** Hub genes in each consensus module were those defined as having expression values that were highly correlated with that module's eigengene values (consensus kME). **D and H:** KEGG pathway analysis results are represented with Fisher's exact test $-\log_{10}$ -adjusted *p*-value and the yellow dashed line notes the significance threshold.

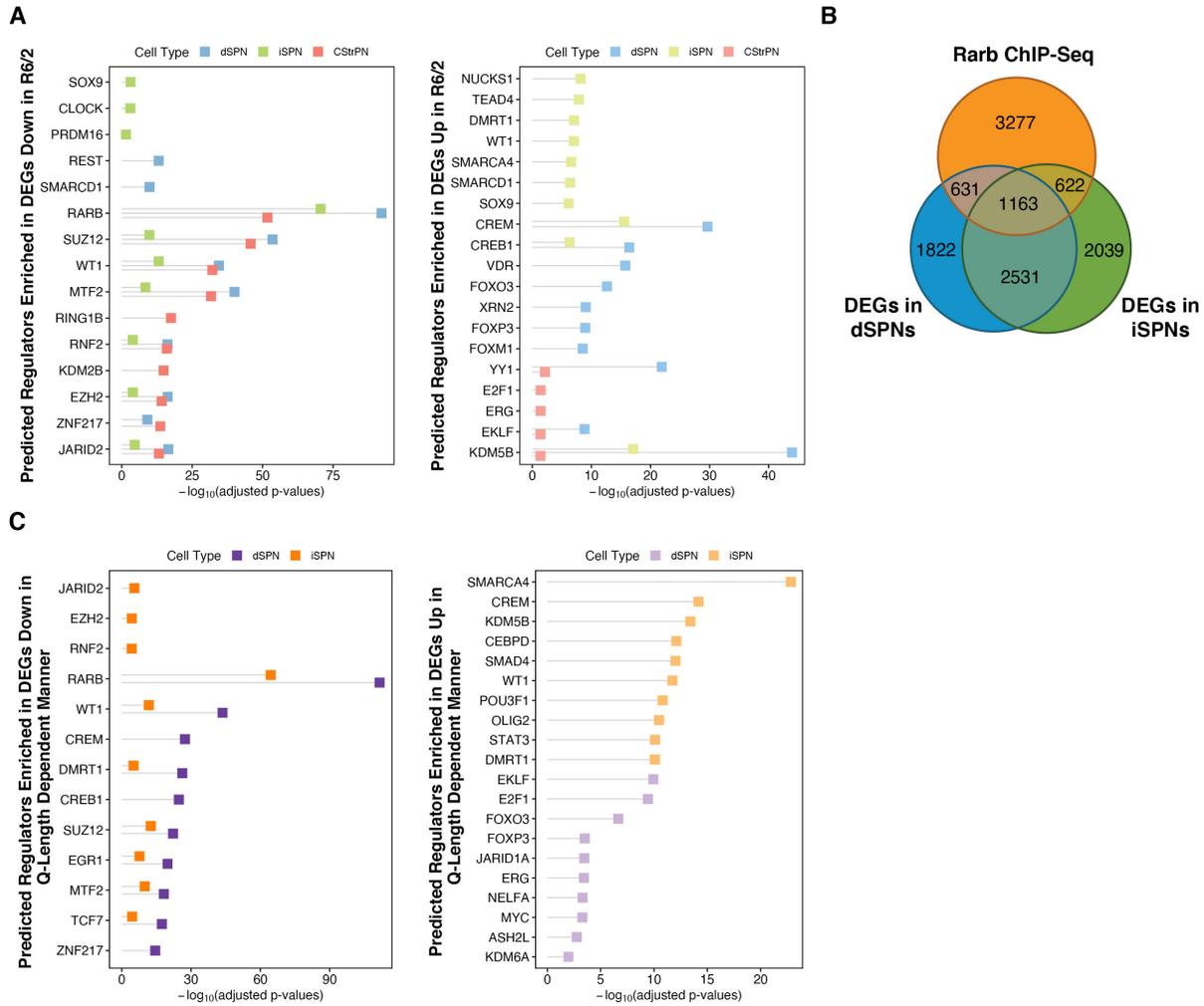


Figure S3. Shared predicted regulators in cell types assessed in TRAP, related to Figures 2 and 3. (A) Predicted transcriptional regulators, by ChEA analysis, of genes that were downregulated (left panel) and upregulated (right panel) across the three cell types profiled in the R6/2 model, represented with Fisher's exact test $-\log_{10}$ -adjusted p -value. (B) Venn overlap analysis of overlap between published striatal Rarb ChIP-Seq data, as well as genes dysregulated in either dSPNs or iSPNs in the R6/2 model. (C) Geneset enrichment analysis using ChEA annotations to predict transcriptional regulators of genes that were downregulated (left panel) and upregulated (right panel) across dSPNs and iSPNs in a CAG-length dependent manner in the full-length *mHTT* knockin models, represented with Fisher's exact test $-\log_{10}$ -adjusted p -value.

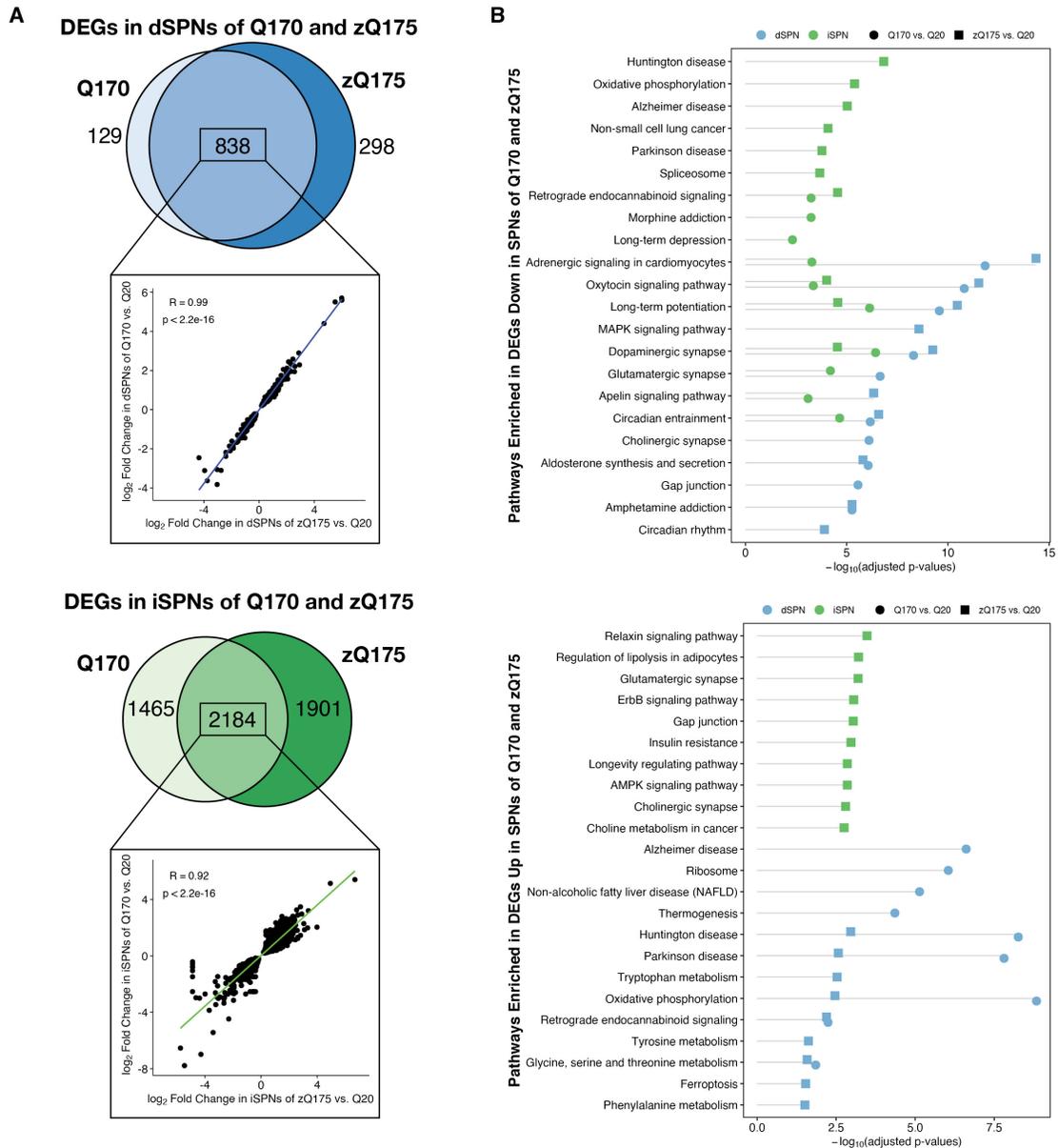


Figure S4. The Q170 and zQ175DN (zQ175) knockin models display similar gene expression changes in SPNs at 6-months of age, as reported by TRAP, related to Figure 3. (A) Venn diagram overlap analysis of differentially expressed genes in dSPNs (top panel) and iSPNs (bottom panel) of the Q170 and zQ175DN knockin models (each compared to Q20 control). A high degree of overlap was observed in dSPNs, and to a lesser extent, iSPNs. **(B)** KEGG pathway analysis of genes downregulated (top panel) and upregulated (bottom panel) in dSPNs and iSPNs of the Q170 and zQ175DN knockin models (each compared to Q20 control), represented with Fisher's exact test $-\log_{10}$ -adjusted p -value.

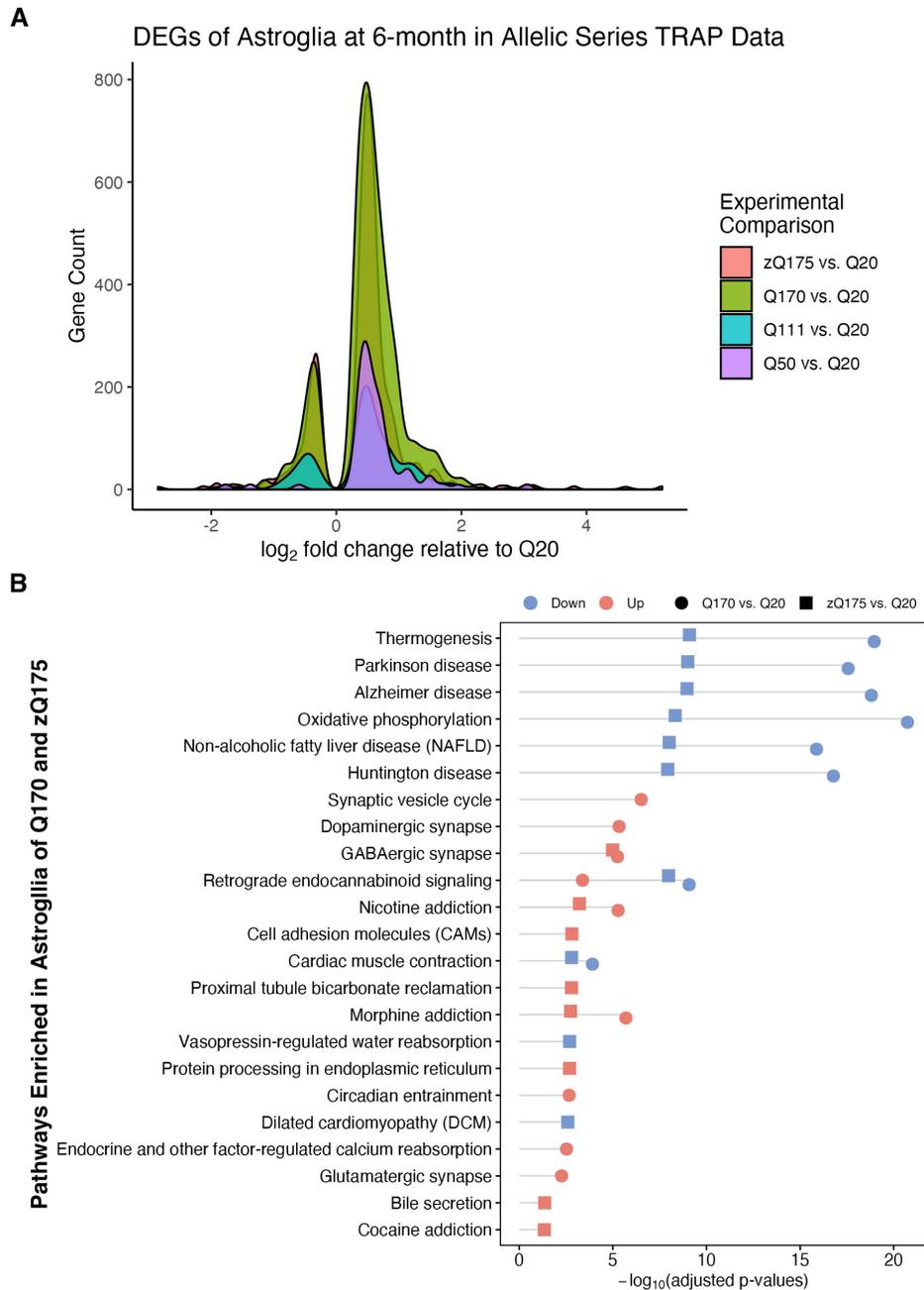


Figure S5. Differentially expressed genes in astroglia from the striatum of 6-month old CAG knockin allele model tissue, as reported by TRAP, related to Figure 3.

(A) Number of differentially expressed genes for each knockin allele studied in the astroglial study (each comparison vs. Q20). (B) KEGG pathway analysis of genes downregulated and upregulated in astroglia of the Q170 and zQ175DN knockin models (each compared to Q20 control), represented with Fisher's exact test $-\log_{10}$ -adjusted p -value.

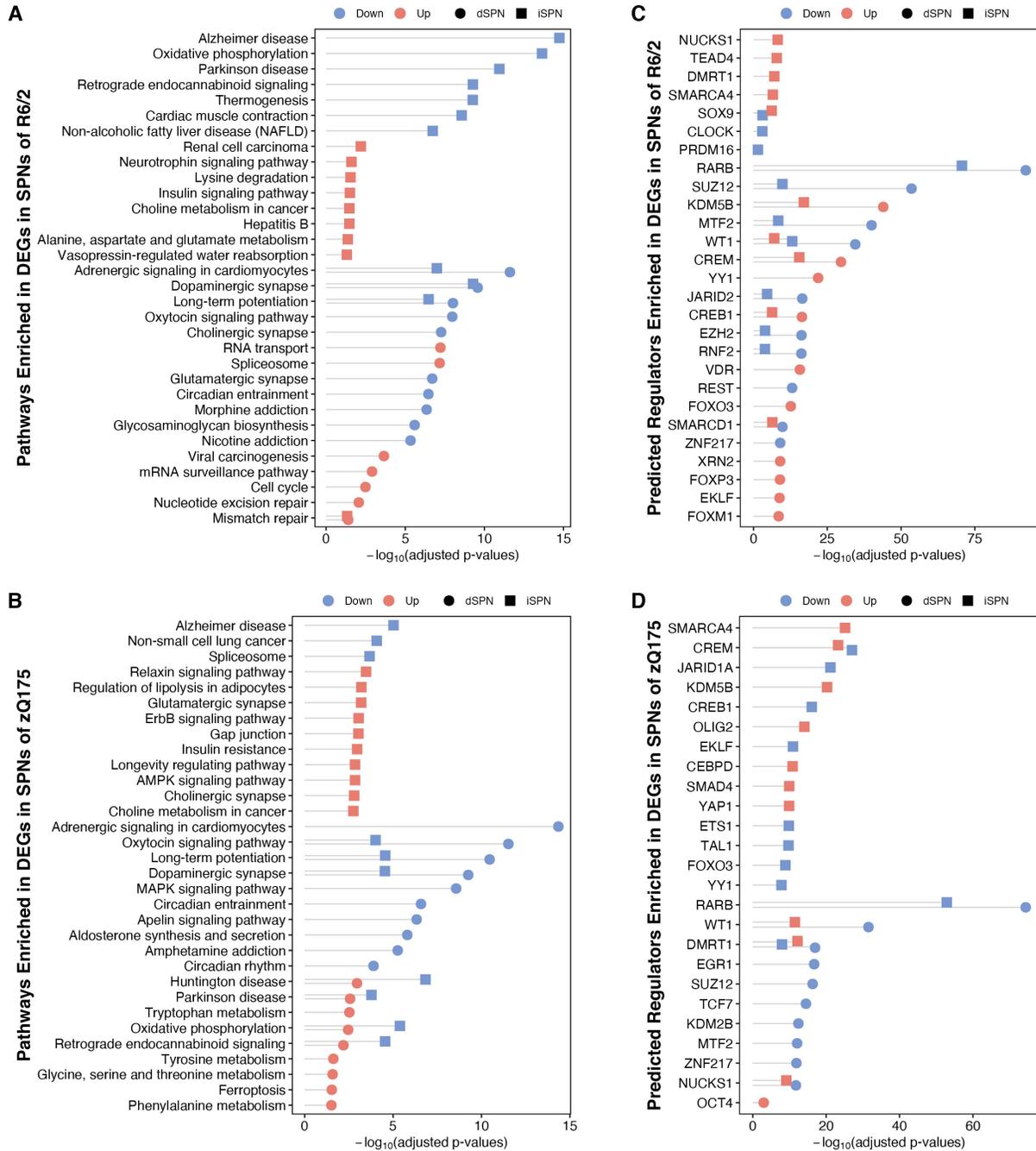


Figure S6. Comparison of dSPN and iSPN differentially expressed genes across the R6/2 and the zQ175DN models, as reported by TRAP, related to Figures 2 and 3.

KEGG pathway analysis (A, B) and predicted regulators (C, D) of genes downregulated and upregulated in dSPNs and iSPNs of the R6/2 and zQ175DN knockin models, represented with Fisher's exact test $-\log_{10}$ -adjusted p -value.

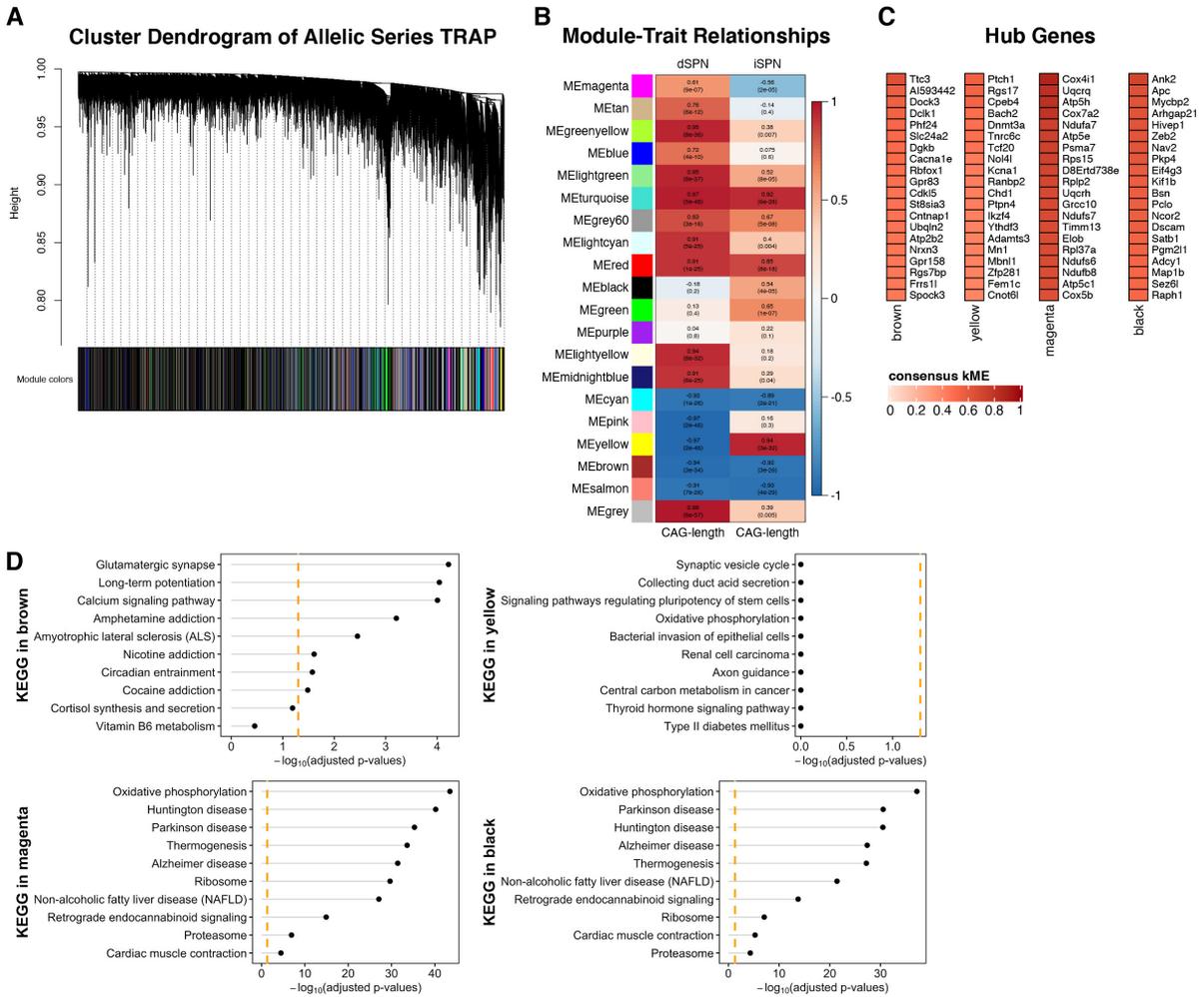


Figure S7. WGCNA analysis of dSPN and iSPN 6-months of age allelic series TRAP data, related to Figure 3. Consensus coexpression network analysis of the dSPN and iSPN 6-months of age allelic series TRAP data reveals multiple CAG-length-associated modules, each labeled by a unique color in the clustering dendrogram (A) and in the module-trait table that represents the Pearson correlation of each module eigengene with CAG-length (B). Also shown are the top module hub genes (C) and the top ten KEGG enriched pathways (D) in selected modules from B. B: red signifies a strong positive correlation and blue signifies a strong negative correlation, and Fisher's asymptotic test p -values for the module-trait relationships are listed below each correlation. C: Hub genes in each consensus module were those defined as having expression values that were highly correlated with that module's eigengene values (consensus kME). D: KEGG pathway analysis results are represented with Fisher's exact test $-\log_{10}$ -adjusted p -value and the yellow dashed line notes the significance threshold.

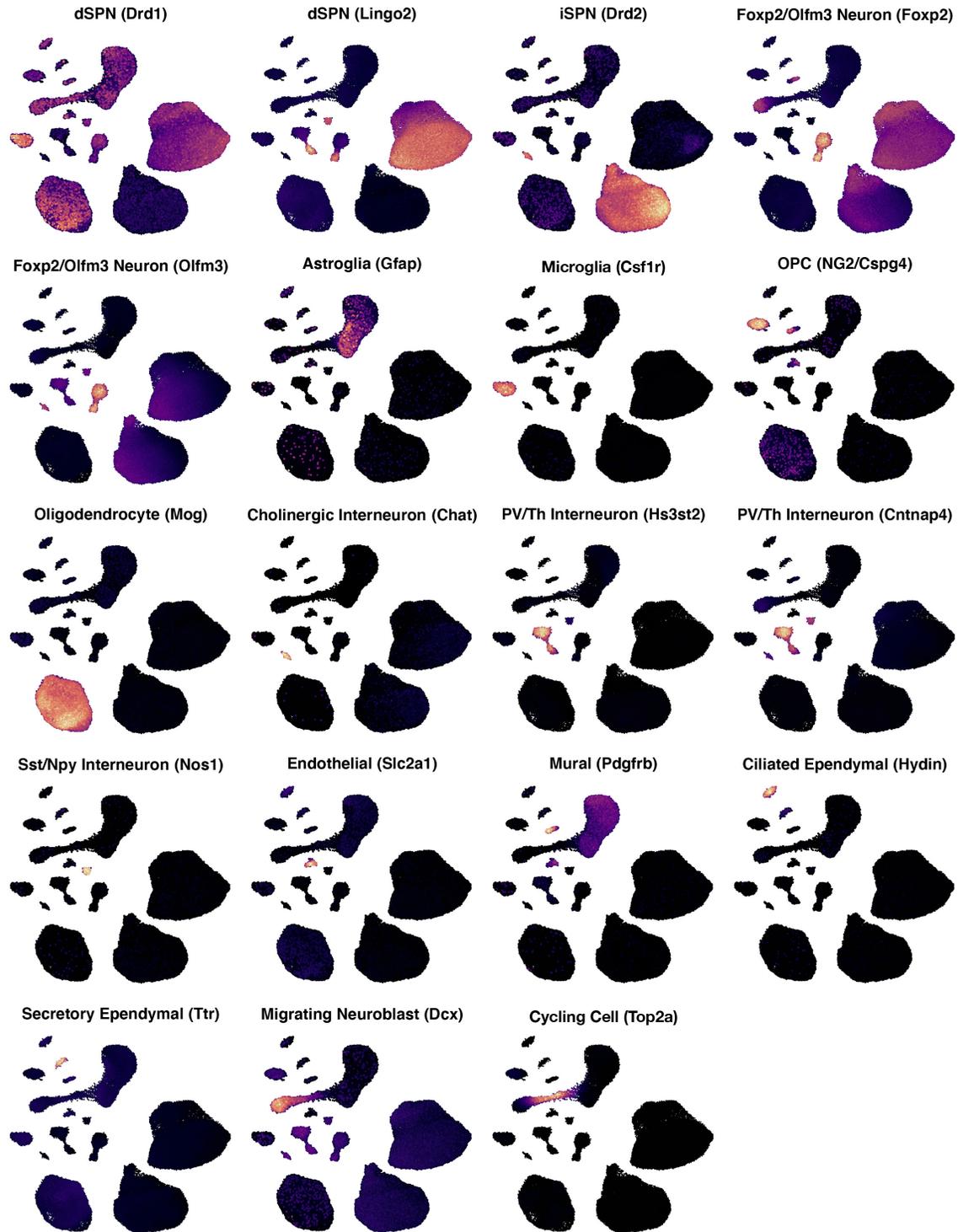


Figure S8. ACTIONet plots of all snRNA-Seq clustered cell types in mouse striatal tissue, with marked expression of known cell type marker genes, related to Figure 4. From the control and R6/2 model groups, same cluster identities as in Figure 4A.

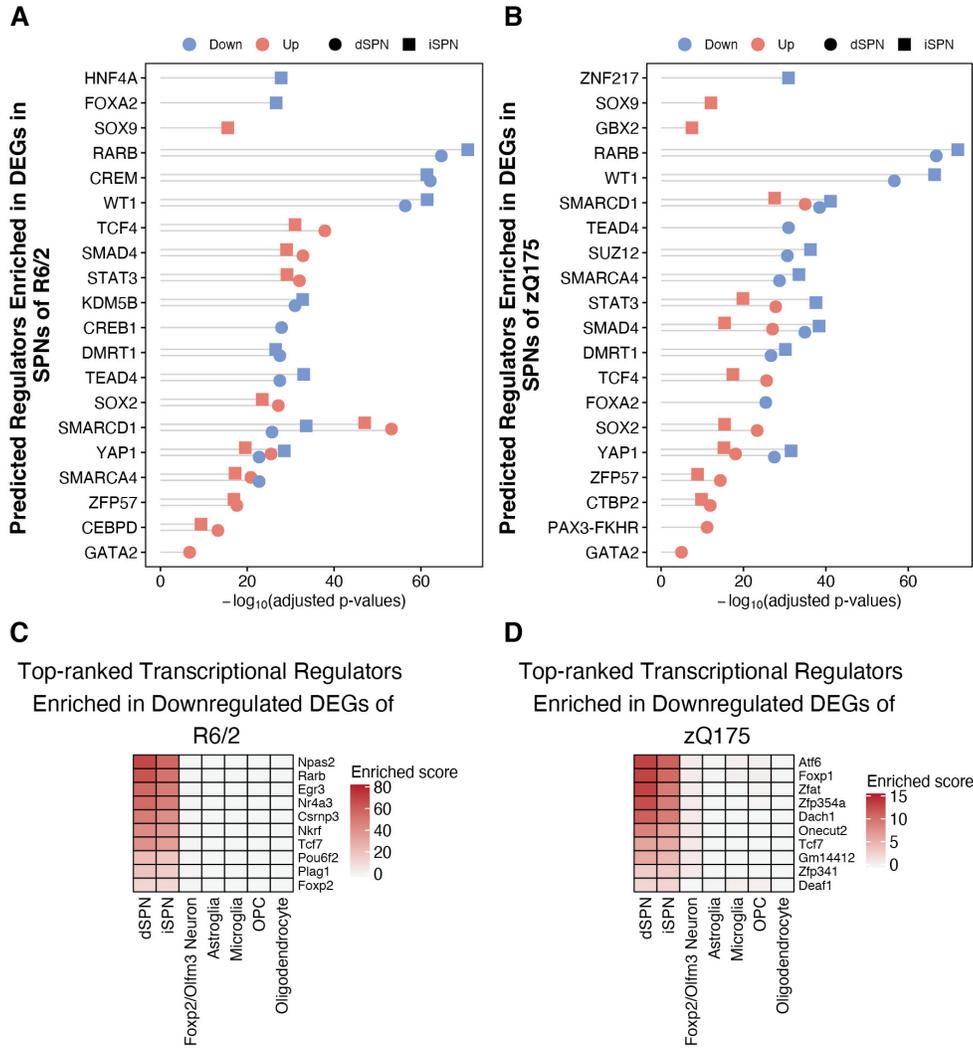
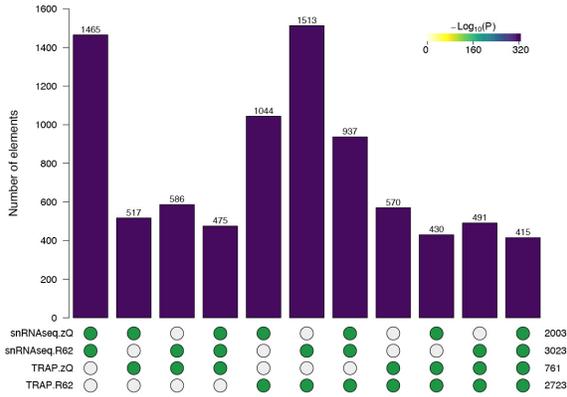
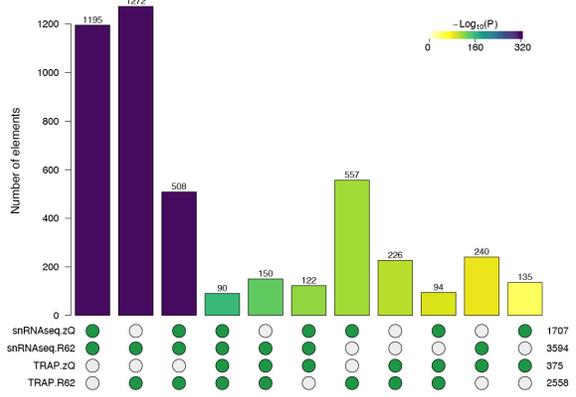


Figure S10. Predicted regulators of dSPN and iSPN gene expression changes in the R6/2 and zQ175DN models, as reported by snRNA-Seq, related to Figure 4. (A-B) Comparison of dSPN and iSPN differentially expressed gene predicted regulators across the R6/2 (**A**) and zQ175DN (**B**) models as predicted by Chromatin Enrichment Analysis of the snRNA-Seq data. Predicted top-ranked transcriptional regulators of downregulated genes in R6/2 (**C**) and in zQ175DN (**D**) estimated using the ACTIONet framework based on the meta-analysis of the activity of their downstream targets (additional cell types here shown for comparison). Reported values are the $-\log_{10}$ of meta- p -values. This ACTIONet framework analysis is only shown for the downregulated genes, as there were no significantly ranked predicted transcriptional regulators for the upregulated genes.

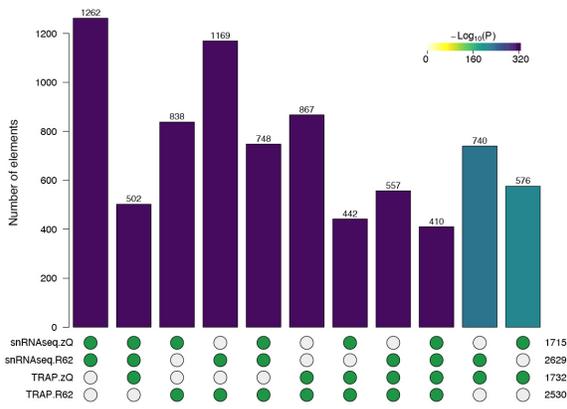
A Downregulated DEGs in dSPNs of R6/2 and zQ175



B Upregulated DEGs in dSPNs of R6/2 and zQ175



C Downregulated DEGs in iSPNs of R6/2 and zQ175



D Upregulated DEGs in iSPNs of R6/2 and zQ175

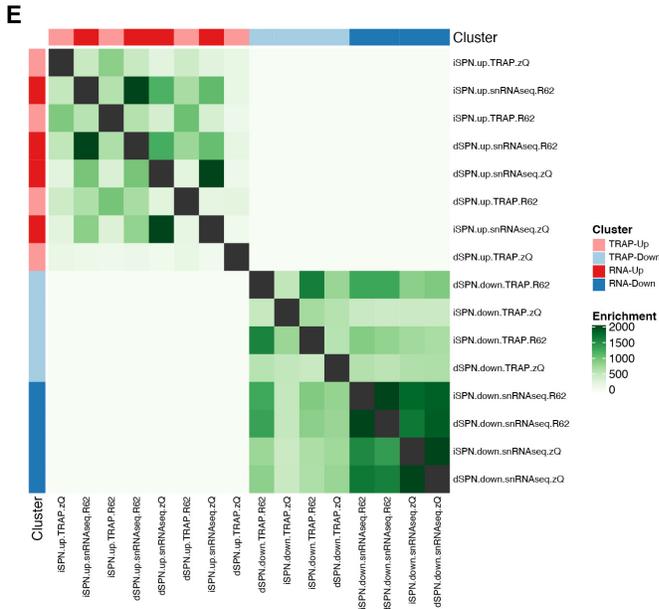
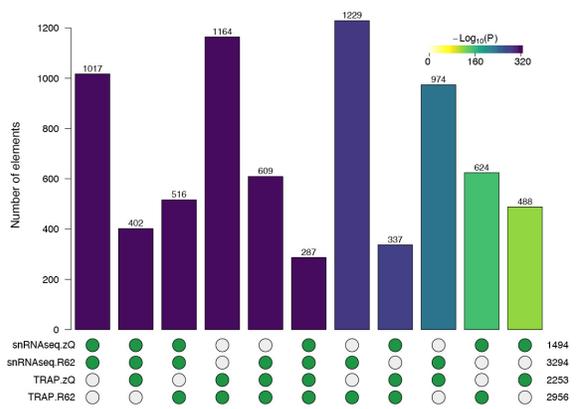


Figure S11. Comparison of TRAP and snRNA-Seq reported gene expression changes across models and SPN subtypes, related to Figures 2-4. Differentially expressed genes from the snRNA-Seq and TRAP studies were identified using a one-sided Welch *t*-test or a negative binomial model test (DESeq2), respectively (STAR Methods). Each mouse model/technology platform combination was independently thresholded to identify a set of perturbed upregulated and downregulated genes. Finally, a statistical test of multi-set intersections between perturbed genes was performed using the SuperExactTest (Wang et al., 2015) and reported for dSPN (**A**) downregulated genes, and (**B**) upregulated genes, as well as iSPN (**C**) downregulated genes, and (**D**) upregulated genes. A-D: The number above each bar represents the overlap in genes per comparison. Below each bar, green circles indicate the gene sets that were considered in each comparison, and numbers to the right indicate the total size of each of these individual gene sets. Bars are colored according to their statistical significance as noted in the insert. Panel **E** provides an alternative view in which pairwise set overlaps are computed, individually, to construct a similarity graph. An adjacency matrix heatmap is shown while gene-set groups (4 clusters) with similar behavior are marked.

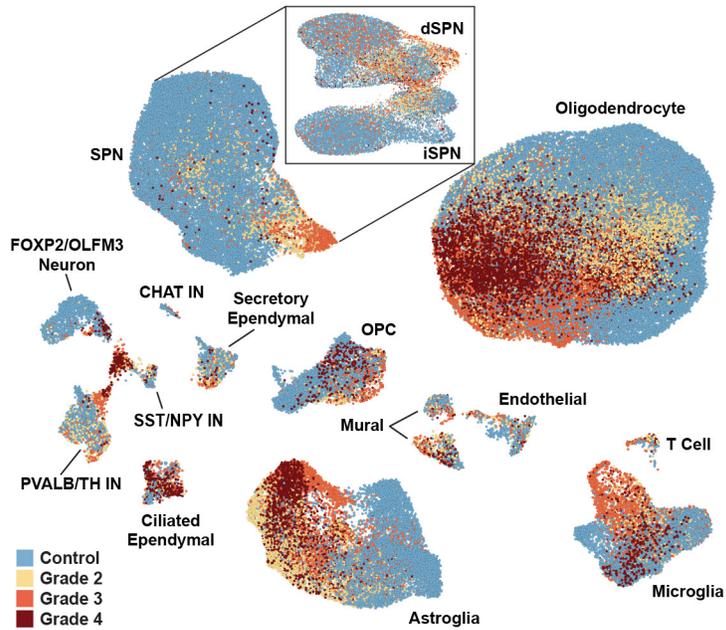


Figure S12. Contribution, by HD grade, of nuclei to the final HD cell type groups used for snRNA-Seq differential gene expression analysis, related to Figure 5. ACTIONNet plots graphically demonstrating the contribution of each HD grade to each cell type identified in the pooled analysis HD human tissue samples that were used for calling differentially expressed genes. Table S2 provides numbers in addition to this graphical demonstration.

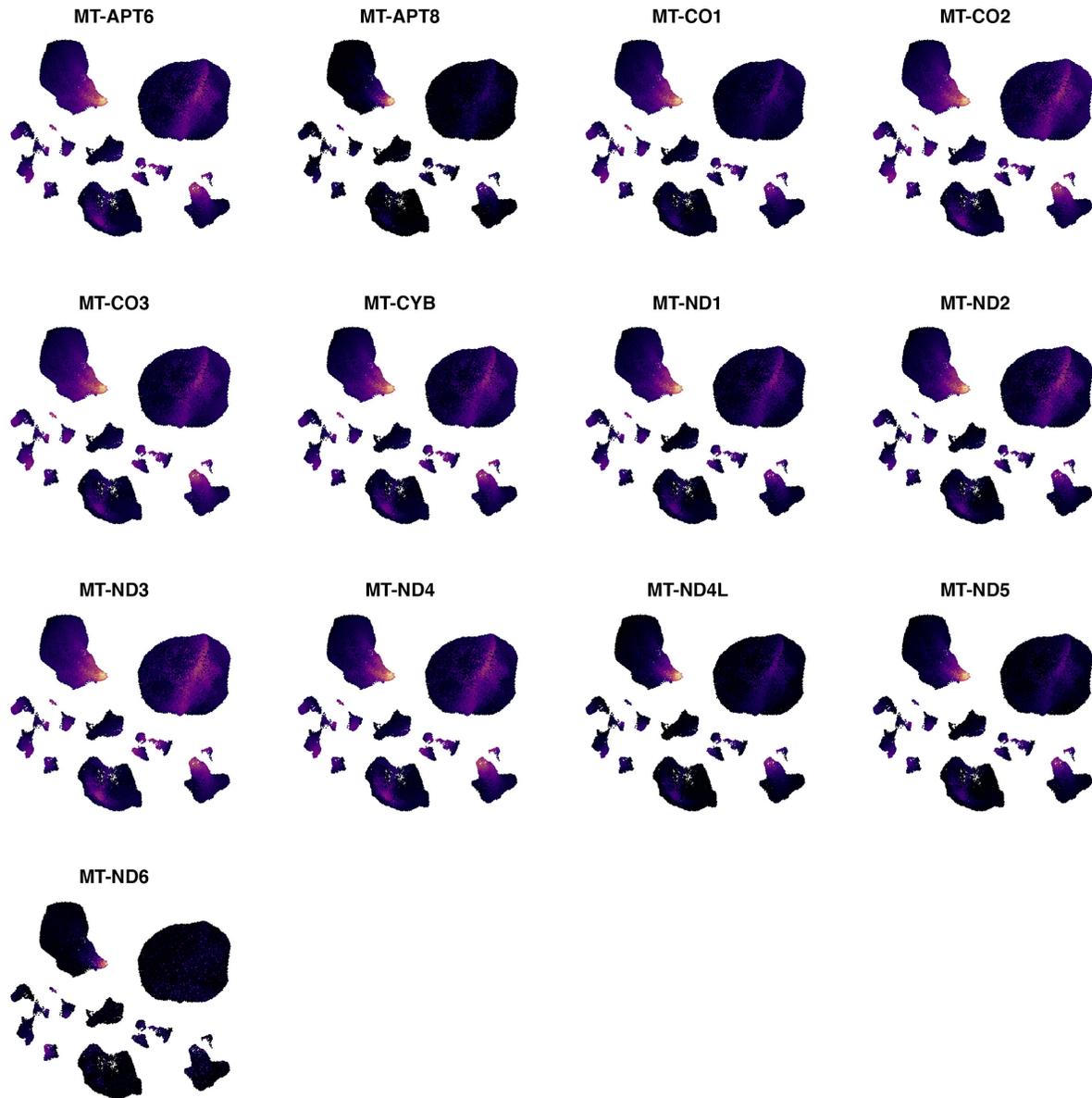


Figure S13. Expression heatmaps of mtRNAs projected onto ACTIONet plot for human cell types, related to Figure 5. mtRNA expression in HD caudate and putamen samples (same cluster identities as in Figures 5 and S12).

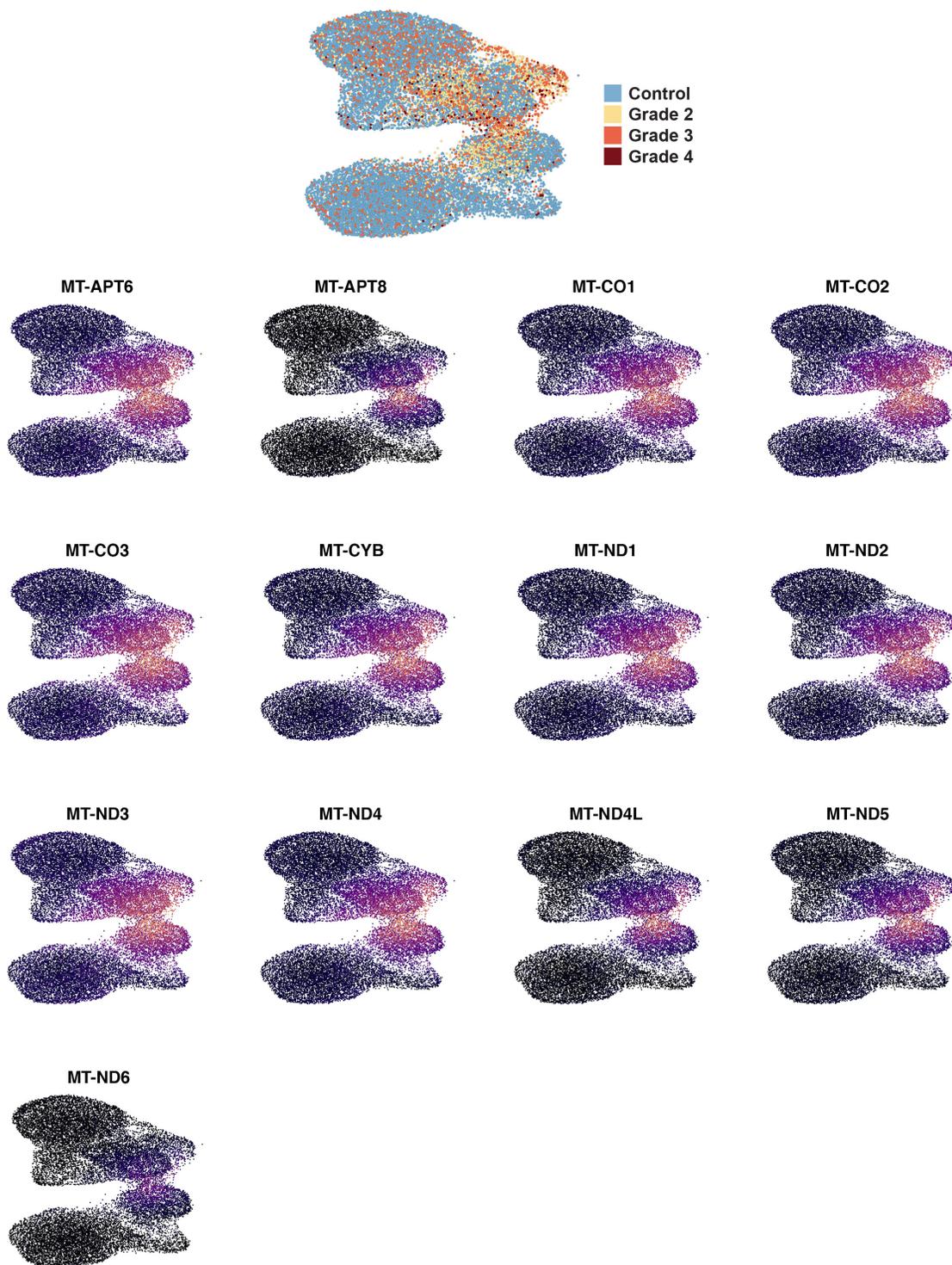


Figure S14. Contribution of SPNs by HD grade and expression heatmaps of mtRNAs projected onto ACTIONet plots for SPNs, related to Figure 5.

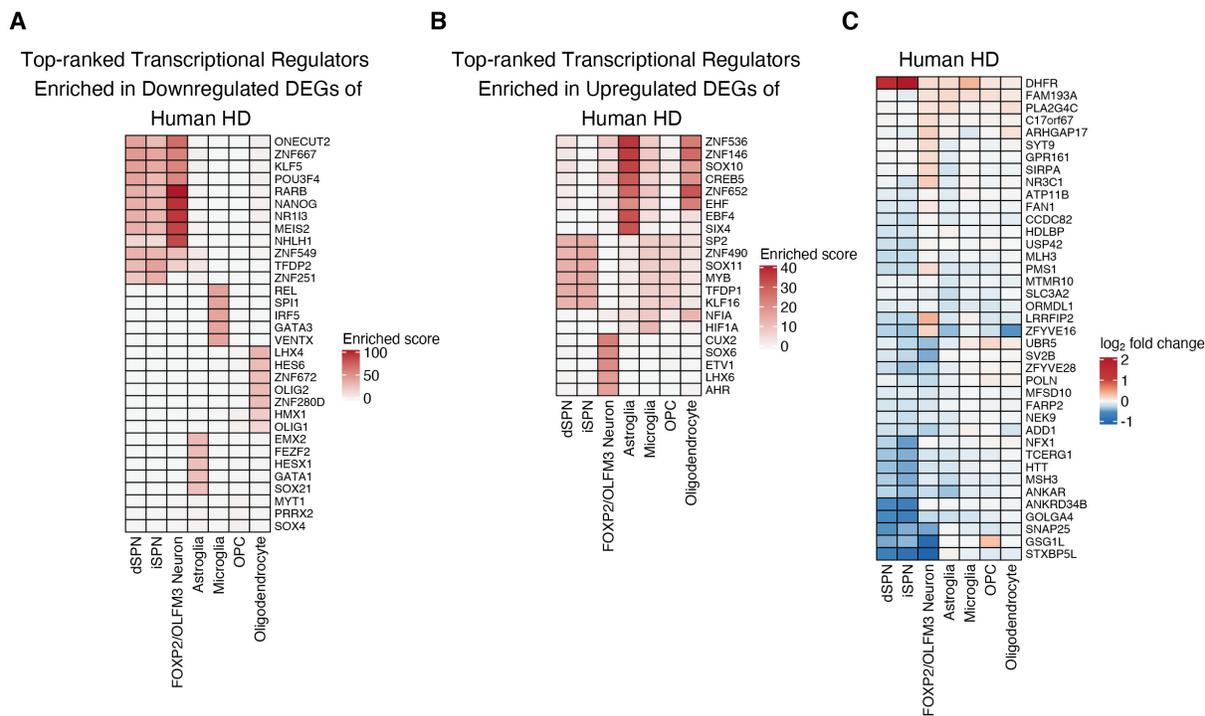


Figure S15. Predicted top-ranked transcriptional regulators of perturbed genes across all cell types in the human HD snRNA-Seq data and comparison of human HD snRNA-Seq data to GeM-HD study data, related to Figure 5. Predicted top-ranked transcriptional regulators of downregulated (A) and upregulated (B) genes in the human HD snRNA-Seq data, estimated using meta-analysis of the activity of their downstream targets [STAR Methods, (Keenan et al., 2019)]. Reported values are the $-\log_{10}$ of meta- p -values. (C) Reported values are \log_2 -fold change of the overlapped genes between the GeM-HD GWAS modifiers with $p_{GWA12345-3510} < 0.0005$ and the DEGs in the human HD snRNA-Seq data.

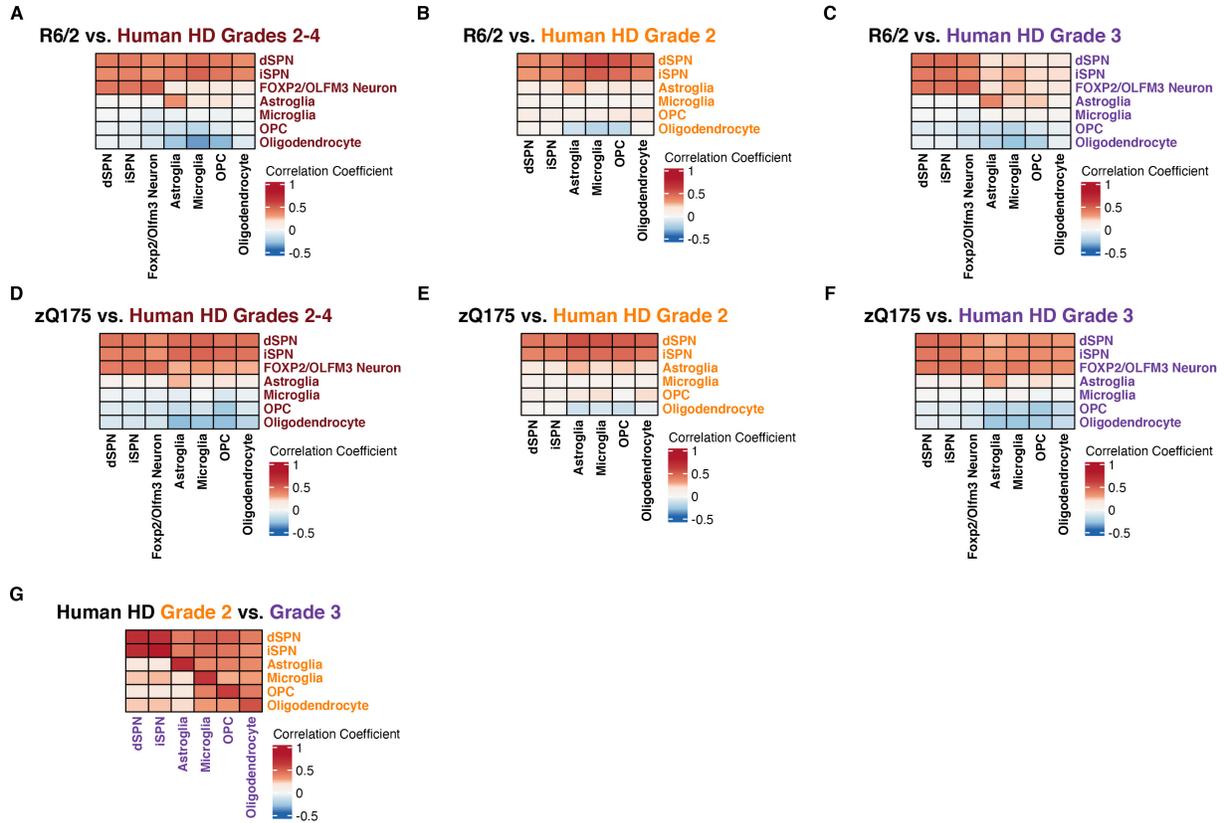


Figure S16. Comparison of human HD snRNA-Seq data to the zQ175DN and R6/2 mouse model snRNA-Seq data, related to Figures 4 and 5. Differentially expressed log₂-fold-change of genes in case-vs.-control comparisons were computed and used as a quantitative measure of gene perturbation in each cell type/model. Values were batch-corrected and Pearson’s correlation between profiles are reported for the comparisons between the human snRNA-Seq data and the R6/2 (A-C) and zQ175DN (D-F) model snRNA-Seq data by all HD grade (A+D), HD grade 2 only (B+E), or HD grade 3 only (C+F) comparisons. As a comparison, grade 2 HD human vs. grade 3 HD human snRNA-Seq data were highly correlated for all cell types (G).

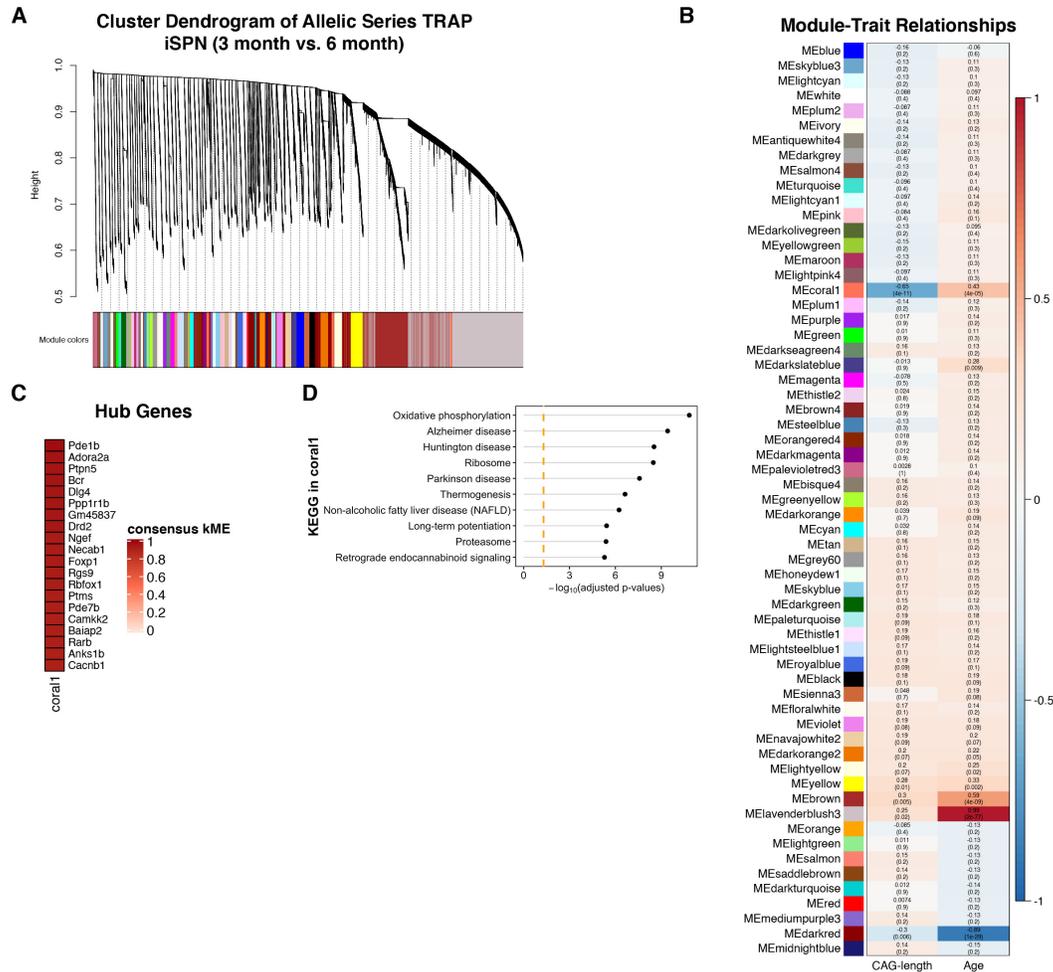


Figure S17. WGCNA analysis of iSPN 3- and 6-months of age allelic series TRAP data, related to Figures 3, 5, and 7. Consensus coexpression network analysis of the iSPN 3- and 6-months of age allelic series TRAP data reveals multiple CAG-length- and age-associated modules, each labeled by a unique color in the clustering dendrogram (A) and in the module-trait correlation table that represents the Pearson correlation of the module eigengene with the specified trait (CAG-length or Age) (B). Also shown are the top module hub genes (C) and the top ten KEGG enriched pathways (D) in selected modules from B. B: red signifies a strong positive correlation and blue signifies a strong negative correlation, and Fisher’s asymptotic test *p*-values for the module-trait relationships are listed below each correlation. C: Hub genes in each consensus module were those defined as having expression values that were highly correlated with that module’s eigengene values (consensus kME). D: KEGG pathway analysis results are represented with Fisher’s exact test $-\log_{10}$ -adjusted *p*-value and the yellow dashed line notes the significance threshold.

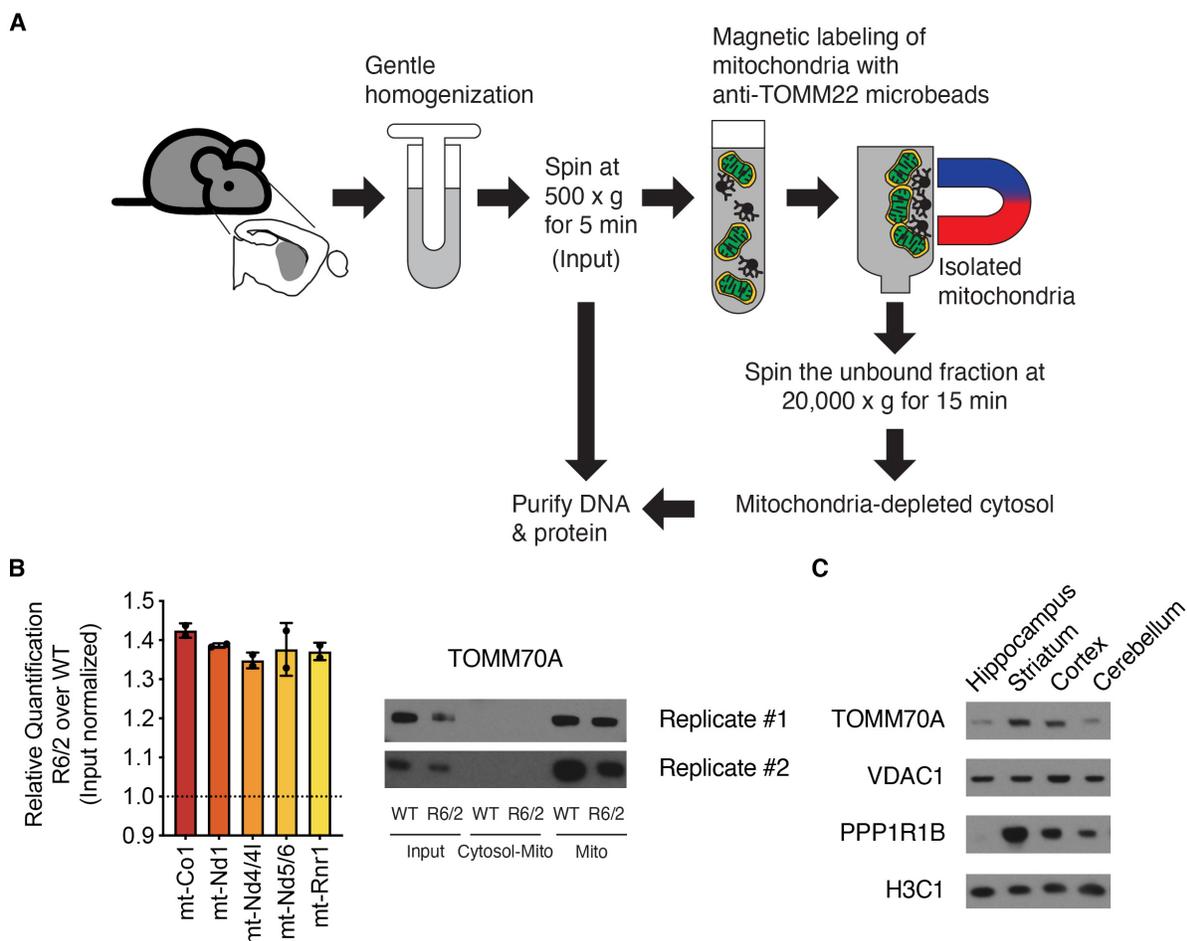


Figure S18. mHTT promotes the accumulation of cytosolic mtDNA, and striatal enrichment of TOMM70A related to Figure 7. (A) Schematic of the subcellular fractionation approach: after gentle dounce homogenization and clearing of unbroken cells by centrifugation for 5 min at 500 x g (input fraction), mitochondria are gently removed from the input fraction by immunomagnetic purification using anti-TOMM22 antibody-coated magnetic beads (which are then collected as the ‘bound’ mitochondrial fraction). The flow-through ‘unbound’ fraction was further depleted of any residual mitochondria with a second fractionation step, by centrifugation at 20,000 x g for 15 min. The resulting supernatant from this centrifugation was the “cytosol - mitochondria” fraction used for studies presented in panel B. (B) Left panel: qPCR analysis of the “cytosol –mitochondria” fractions of R6/2 and isogenic control mouse striatal tissue revealed an increase in cytosolic mtDNA content for DNAs encoding mt-Co1, mt-Nd1, mt-Nd4, mt-Nd5,

and mt-Rnr1 in R6/2 as compared to isogenic control mice. Right panel: Western blot analysis of TOMM70A distribution in the subcellular fractionation samples revealed the presence of this mitochondrial marker only in the input and mitochondrial fractions, but not in the “cytosol - mitochondria fraction.” (C) Western blot analysis of TOMM70A (the mouse ortholog of human TOMM70) expression across the indicated mouse brain regions, demonstrating that TOMM70A displays an expression pattern similar to the striatal-enriched protein DARPP-32 (PPP1R1B). VDAC is a control mitochondrial protein loaded for comparison, and histone protein H3C1 is a loading control.

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