

Introduction

Neurodegenerative disorders (ND) are increasingly common conditions, due to aging populations, with no cure. Huntington's Disease (HD) is a ND caused by CAG repeat expansions in the Huntingtin (*HTT*), leading to an expanded polyglutamine tract in HTT (mHTT), resulting in atrophy of the striatum. Researchers do not fully understand how *HTT* mutation causes HD.

RNA processing encompasses multiple molecular events that modify RNA molecules, altering the proteins' encoding sequences, by splicing introns and exons and by terminating at different sites via alternative polyadenylation (APA) (Figure 1).

Altered RNA processing in HD has previously been detected:

- Widespread dysregulation of alternative splicing (AS) detected in HD¹.
- Genes with altered APA has been associated with HD, supporting APA's pathogenesis².

The cause and effects of RNA processing changes to disease pathology are unknown.

We hypothesize mutant *HTT* and mHTT disrupts RNA processing across many levels, contributing to HD pathology.

We aim to analyse multiple RNA processing levels' changes, integrated together, to inform on dysregulated molecular processes in HD.

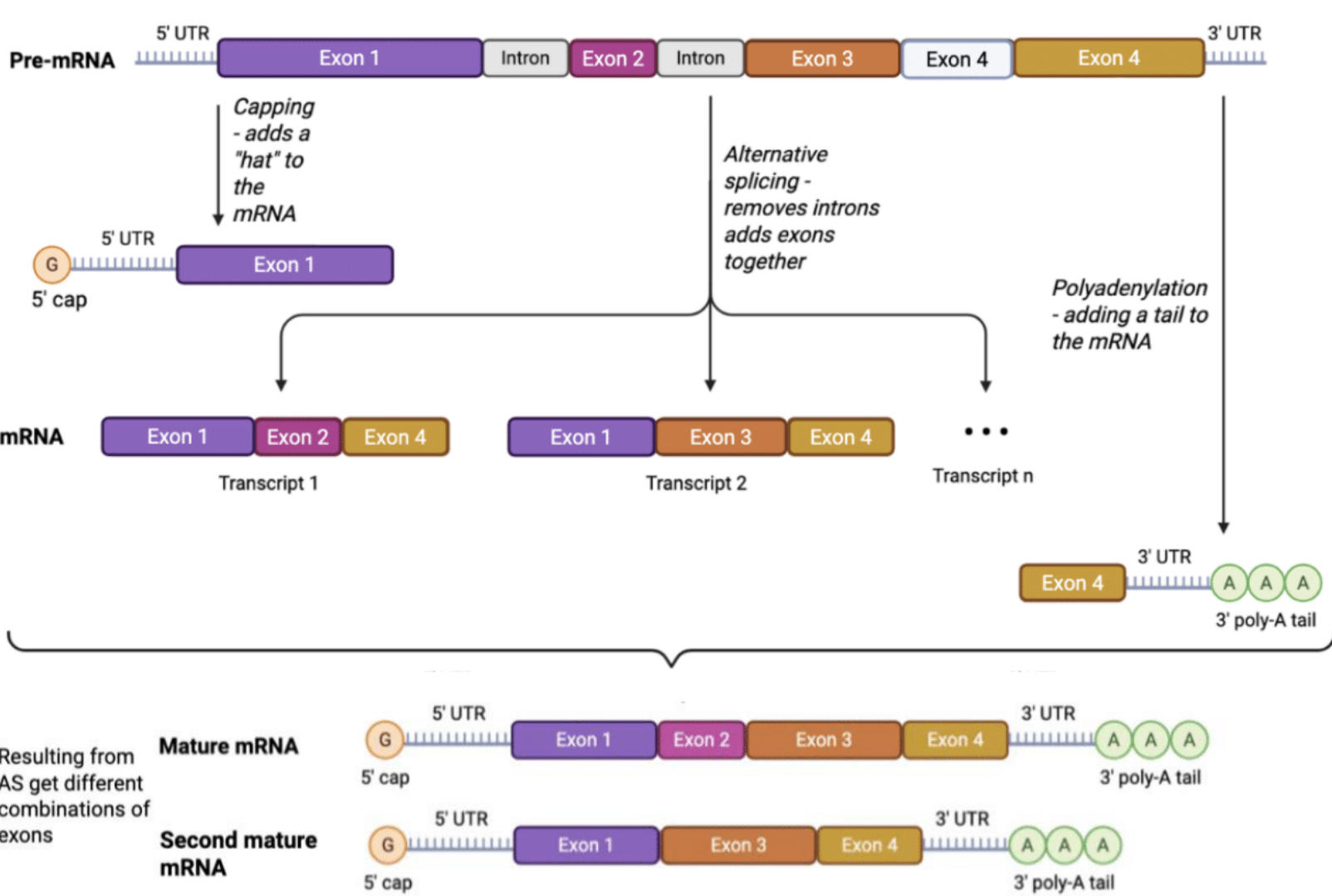


Figure 1. RNA Processing mechanism. APA leads to varying mRNA strand lengths as polyadenylation can occur at multiple sites in an mRNA molecule³.

Methods & Materials

We performed a comprehensive global RNA processing events analysis including:

- AS analysis, APA analyses, RNA binding protein motif enrichment analysis

Their contributions to HD pathology, across onset ages and developmental stages, was investigated using RNA-seq data from an established human isogenic system (Figure 2).

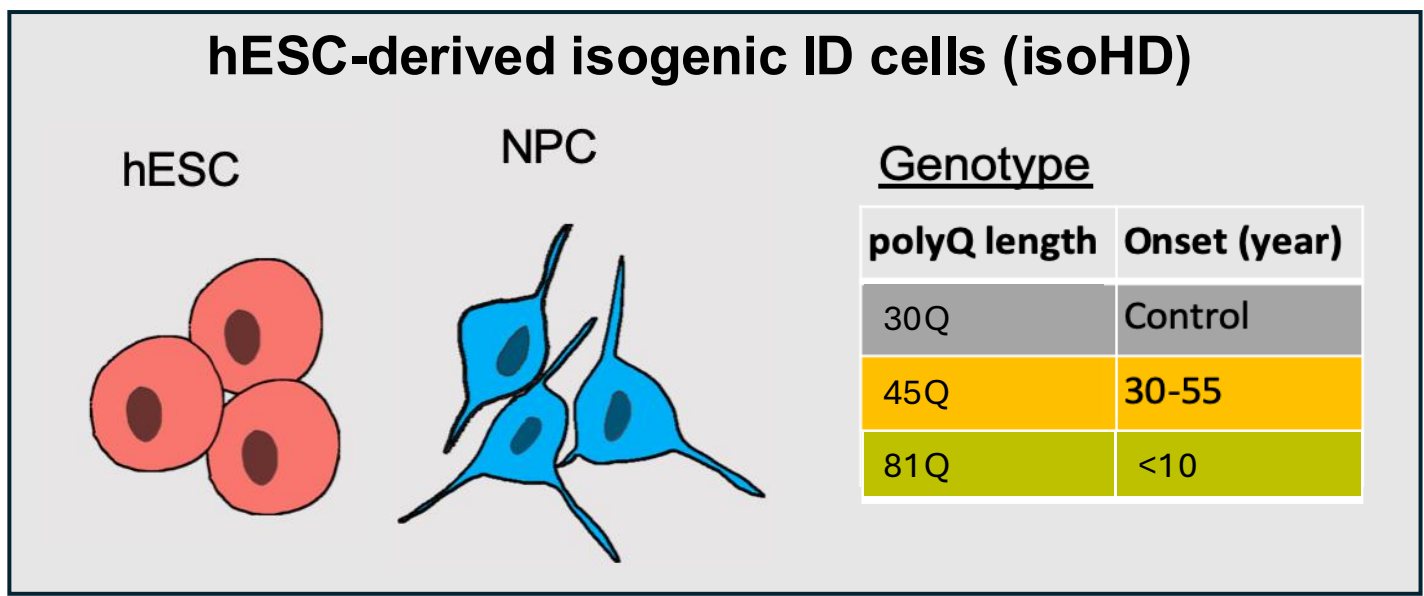


Figure 2. Experimental Design. Differentiation into various cell types models different development stages. 3 CAG repeat lengths, encoding amino acid Q (glutamine). Key: hESC–Human Embryonic Stem Cells, NPC–Neural Progenitor Cells.

Results

We performed APA analysis, comparing across expanded CAG lengths to control CAG lengths within each cell type. For each pairwise comparison we note **widespread, significant, APA changes**. When comparing between 81Q and controls for both differentiation stages, we found an **enrichment of mRNA processing and RNA splicing in genes with significant APA** (Figure 3).

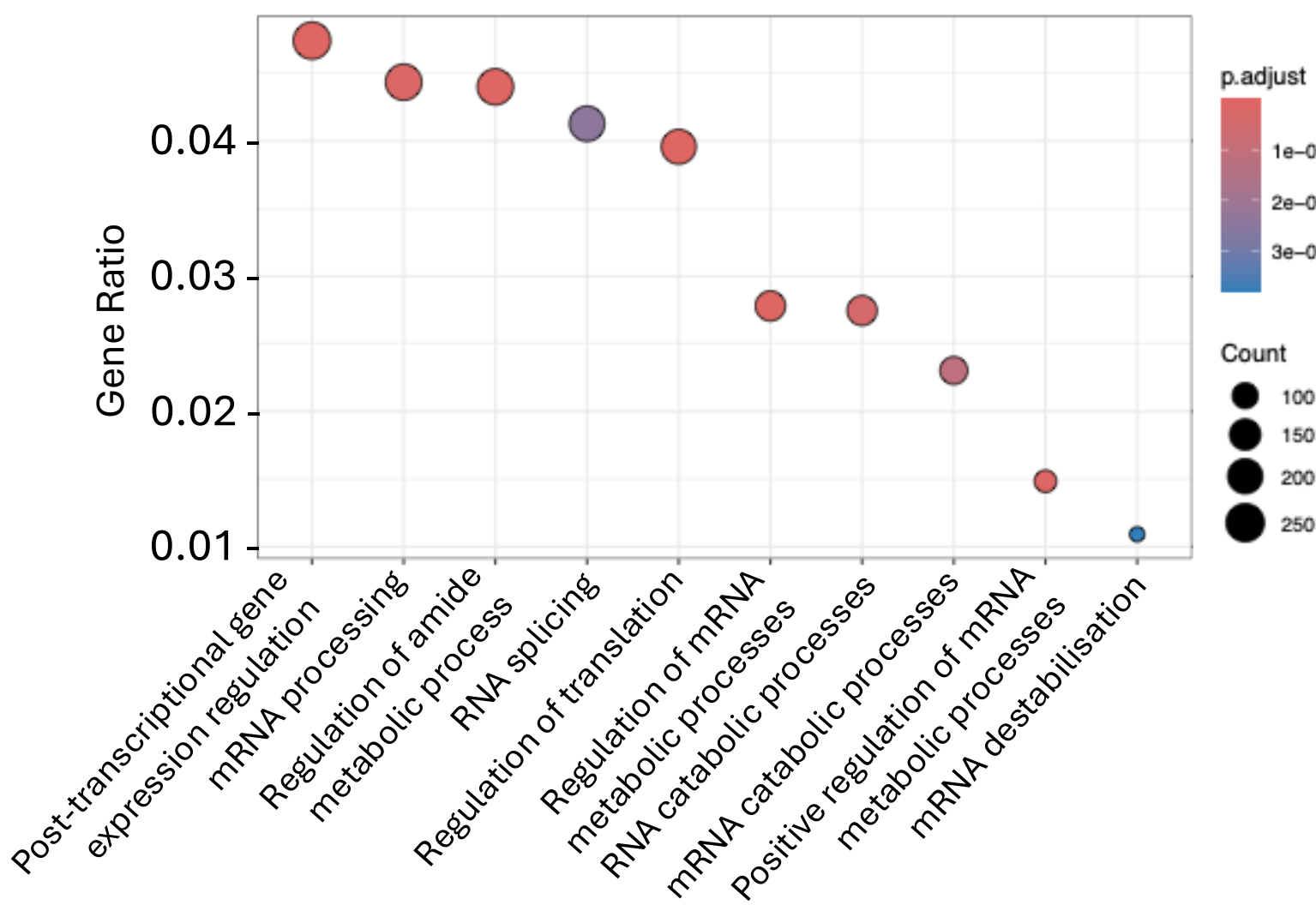


Figure 3. NPC 45Q vs 30Q APA genes associated pathways. APA genes were found by MAAPER⁴ and APATrap⁵ software. Differential biological processes pathway associations were detected by ClusterProfiler⁶.

Using the results from previously published AS analysis¹, we performed an RNA binding protein motif enrichment analysis on the AS sites and found **CPEB2 motifs were enriched within alternatively spliced regions** in early and late onset ESC and NPCs compared to controls (Table 1). **CPEB2 is a known regulator of cytoplasmic polyadenylation**.

Table 1. CPEB2 and 4 RBP motifs. AS was detected by Leafcutter software^{1,7}. Motifs in AS regions were found by HOMER software^{8,9}.

Motif	Name
	CPEB2
	CPEB4

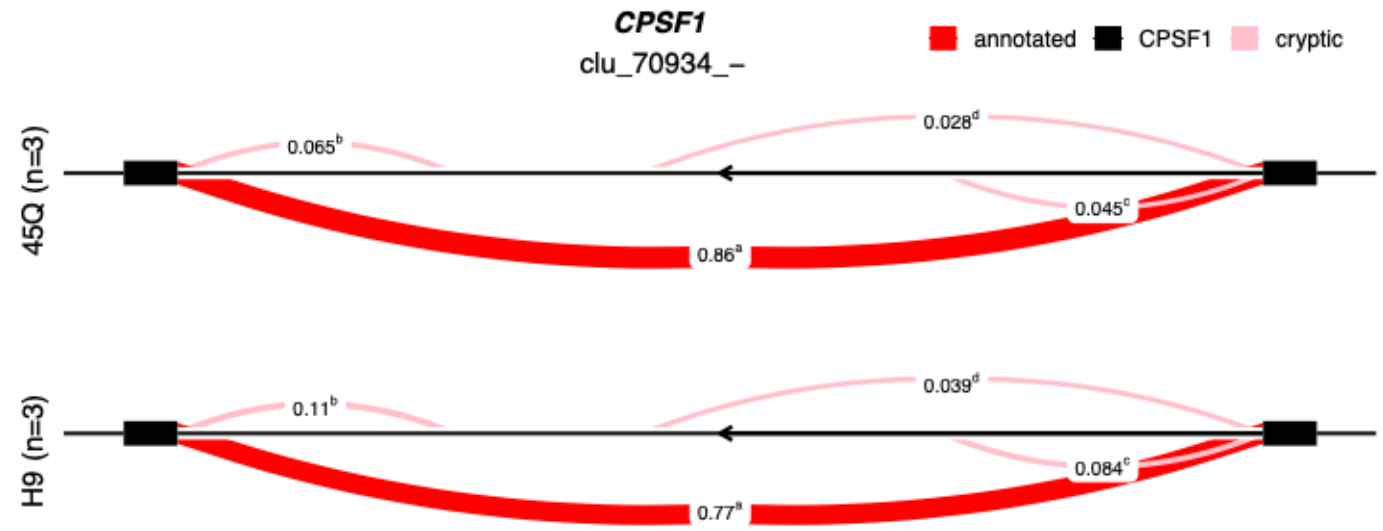


Figure 4. AS of CPSF1 in ESC 45Q vs 30Q. AS was found by Leafcutter^{1,7} and plot was made using Leafviz⁷ software.

Furthermore, upon revisiting the previously published AS analysis¹, we also noted **AS of genes that regulate APA**, such as cleavage and polyadenylation specificity factor complex subunit 1 (CPSF1);(Figure 4). CPSF1's complex recognises signals for where to add the polyA tail, as well as aiding cleavage of the pre-mRNA to allow for polyA tail addition.

Our findings indicate a link between RNA processing events and support our integrative approach.

AS genes were associated with actin binding, cell-cell adhesion, and GTPase activity across cells modelling early development stages of HD for early and late onset disease (Figure 5).

Figure 5. NPC 81Q vs 30Q molecular function APA associated pathways. APA genes were found with MAAPER⁴ and APATrap⁵ software. ClusterProfiler plotted differential pathway associations⁶.

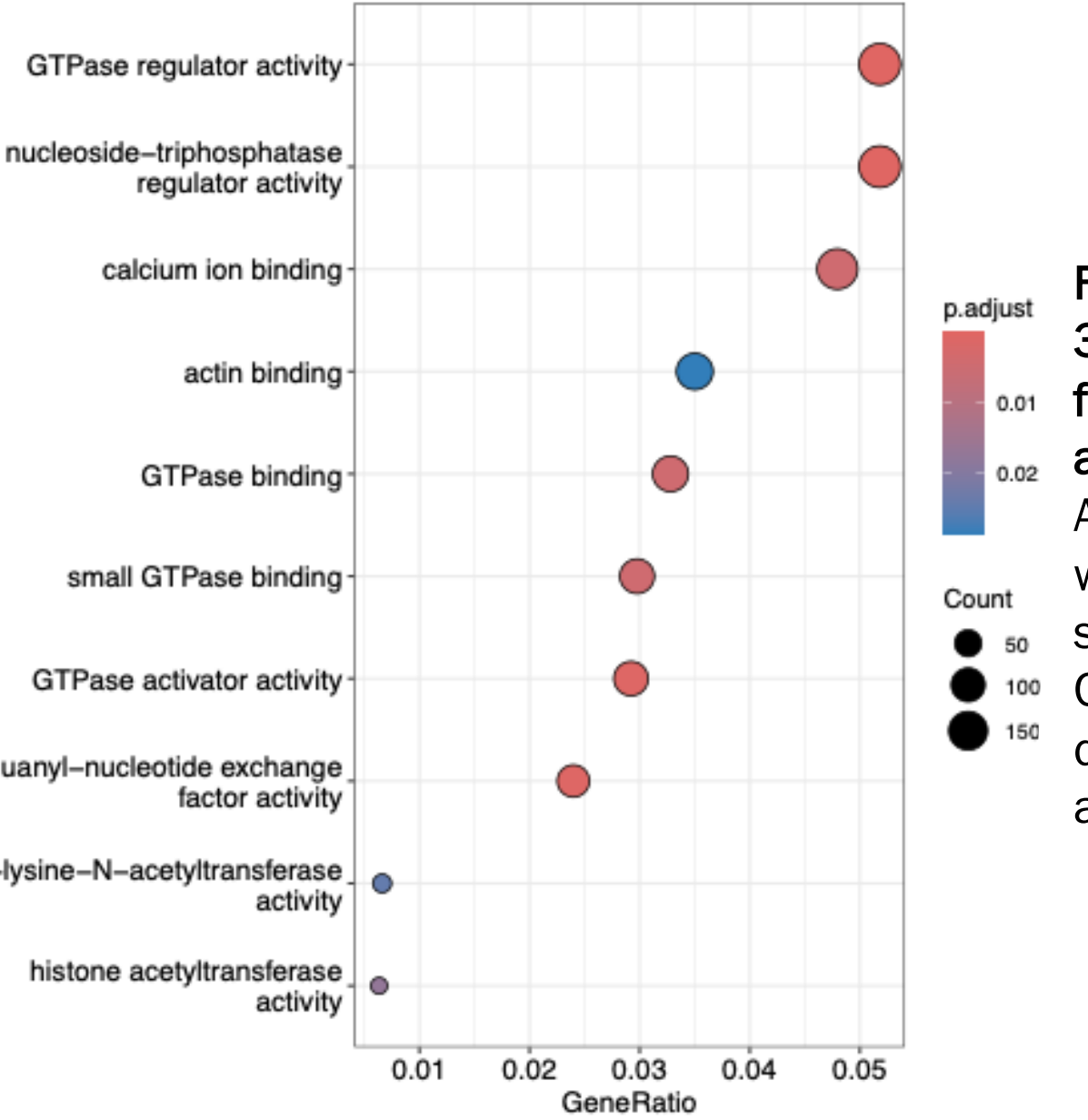
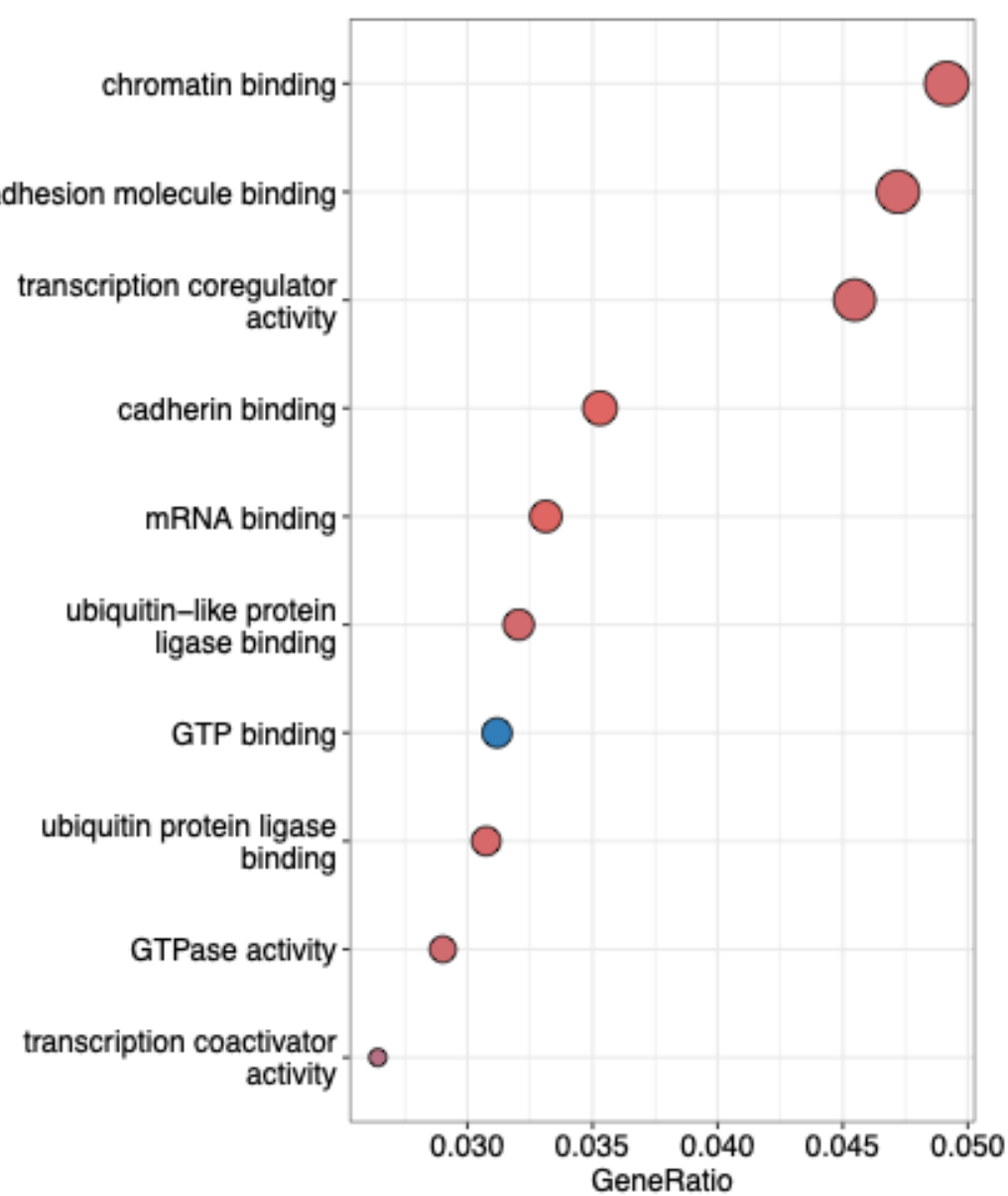


Figure 6. NPC 81Q vs 30Q molecular function AS associated pathways. AS genes were found with Leafcutter^{1,7} software. ClusterProfiler plotted differential pathway associations⁶.

APA genes were also associated with GTPase binding, and cell adhesion molecule binding (Figure 6). While these **pathways are known to be dysregulated in HD**, our work indicates that RNA processing, through several mechanisms, may be an underlying contributor across these pathways.

Conclusion & Future Work

1. We see widespread APA changes in isoHD cell lines, across CAG lengths and developmental stages.
2. We see interplay of AS and APA RNA processing events.
3. RNA processing, via several mechanisms, may be an underlying contributor to pathways known to be dysregulated in HD. Future work will incorporate transcript usage and expression data to elucidate putative downstream functional dysregulation. Finally, validating findings with RNA-seq data from post-mortem sources.