

Role of Alternative Polyadenylation in Driving Noradrenergic-to-Mesenchymal Transition in Neuroblastoma

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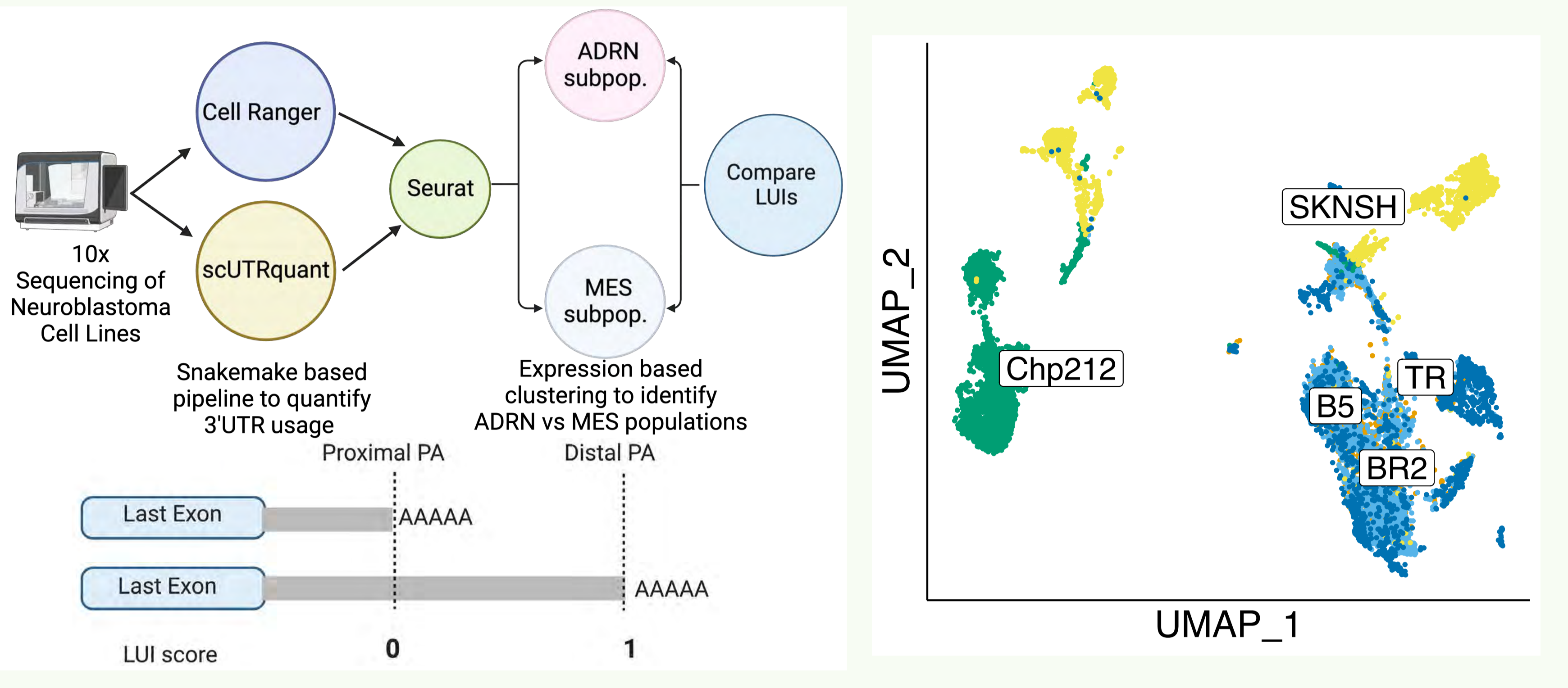
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Abstract

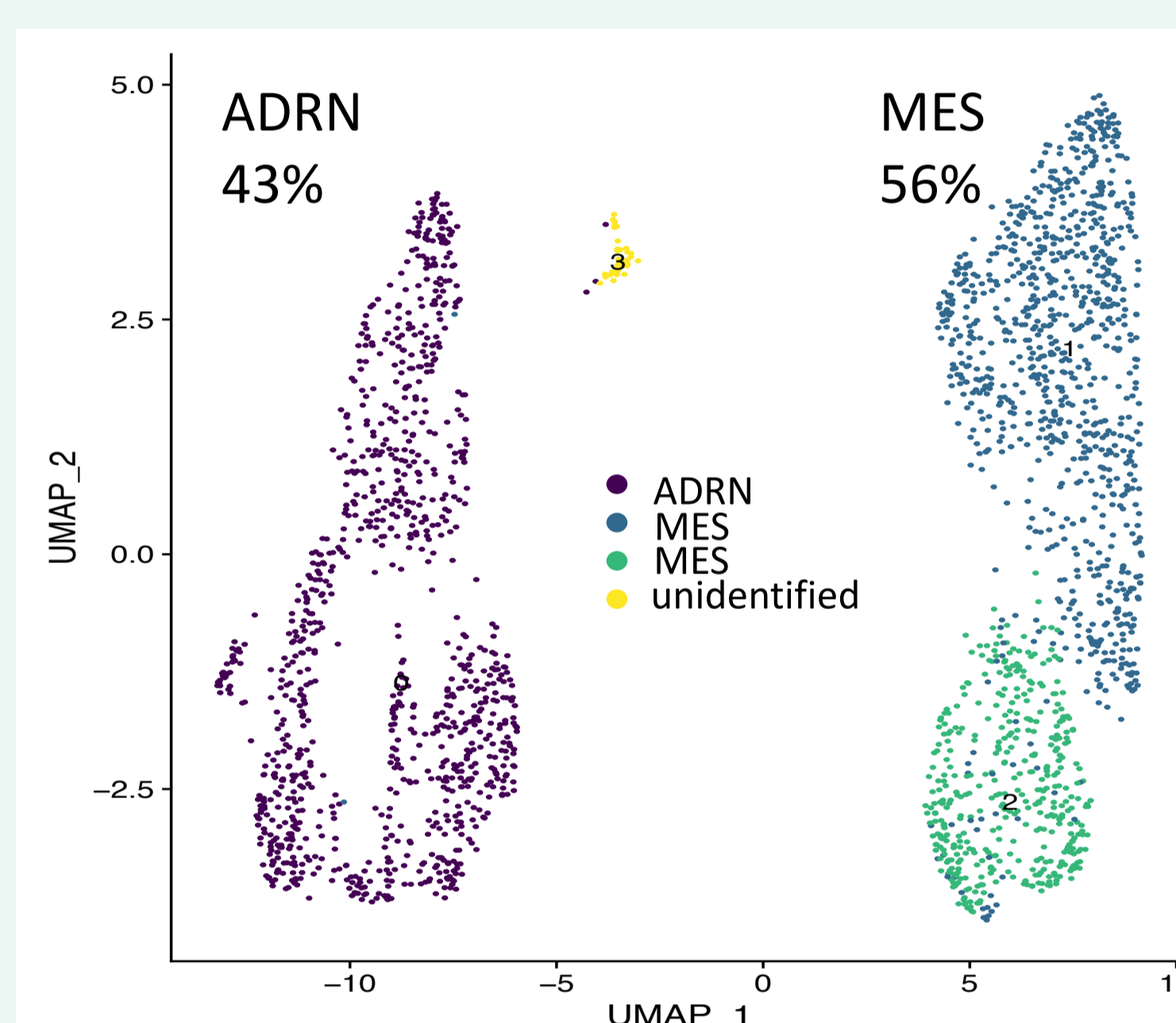
Neuroblastoma is the most common extracranial tumor in children; compared to adult cancers, neuroblastoma has a distinctly lower number of somatic mutations. Two distinct cell states in neuroblastoma, adrenergic (ADRN) and mesenchymal (MES), dynamically interconvert in the process of noradrenergic-to-mesenchymal transition (NMT). MES cells are implicated in conferring an additional level of pathogenicity due to their more migratory and therapy-resistant phenotype. Post-transcriptional regulation, known to be involved in neuroblastoma, is likely important in regulating NMT. A recent study has linked alternative polyadenylation (APA) to proliferation and neuronal differentiation in neuroblastoma.

Using an integrated computational and experimental approach, we explore if changes in APA affect NMT. With scRNAseq of five neuroblastoma cell lines, we identified distinct ADRN and MES populations and compared their usage of 3' untranslated regions (3'UTR) polyadenylation sites. Preliminary results show differential ADRN vs. MES 3'UTR usage in 180 genes that include transcription factors and chromatin modifiers. We are establishing an *in vitro* neuroblastoma APA model by biasing 3'UTR usage to shorter or longer extremes, which will enable us to study the effect of globally truncated or extended 3'UTRs on NMT. Elucidating the role of APA in NMT may reveal novel targetable vulnerabilities in neuroblastoma.

Pipeline for APA analysis using scRNAseq data

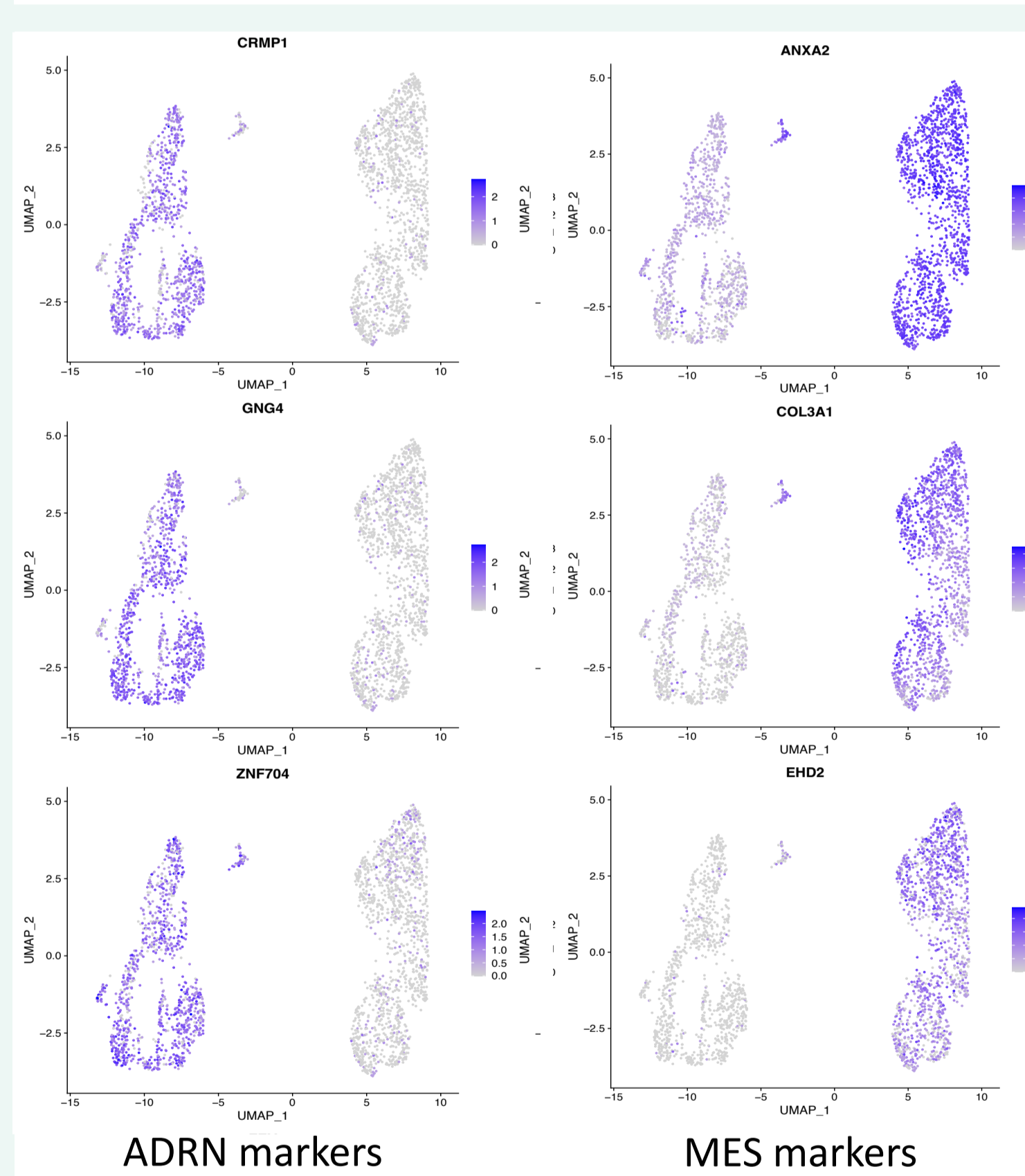


NB cell line, SK-N-SH, shows a clear distinction between MES and ADRN states



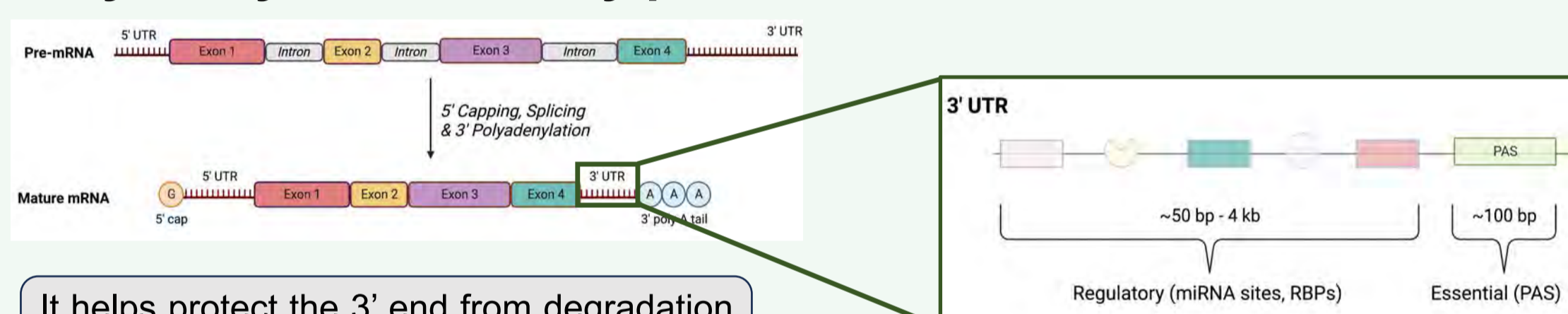
180 genes show differential UTR usage between ADRN and MES states in SK-N-SH cell line

Pathway enrichment analysis shows regulation of TGF- β pathway, methylation and acetylation and mRNA processing and transcription regulation as the most highly enriched terms. Additionally, 14 genes from this list are also a part of the ADRN/MES signature.



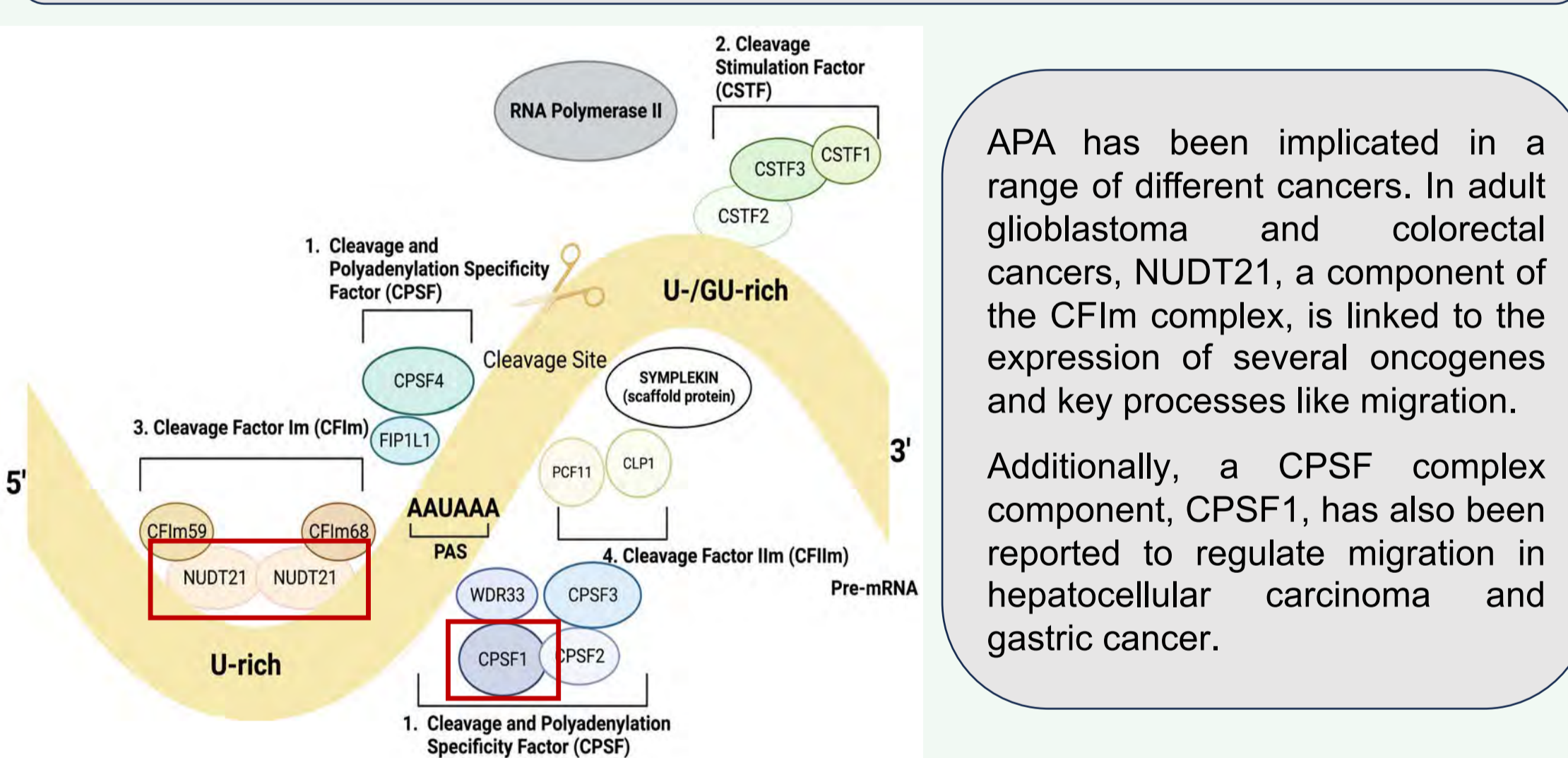
Markers from ADRN and MES signatures respectively, show a clean expression pattern in their respective cell types in SK-N-SH. It is also the only line where the two populations can be sorted and remain homogenous.

Polyadenylation is a key process in mRNA maturation



It helps protect the 3' end from degradation upon export from the nucleus.

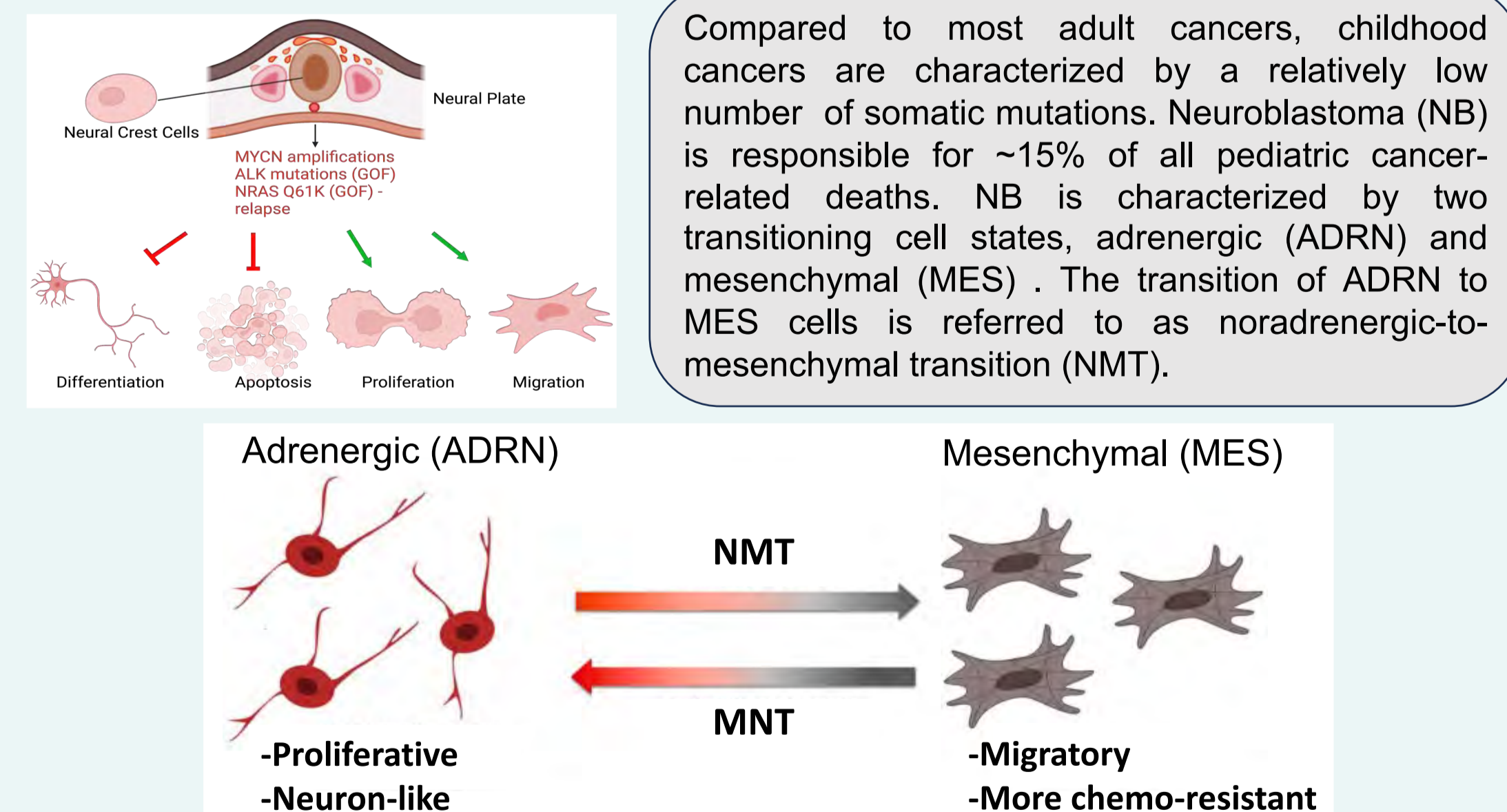
Over 60% of human genes are subject to APA due to the presence of multiple polyadenylation sites. APA results in transcripts that differ in either 3' untranslated regions (3'UTRs) only, or in both coding regions and 3'UTRs.



APA has been implicated in a range of different cancers. In adult glioblastoma and colorectal cancers, NUDT21, a component of the CFIm complex, is linked to the expression of several oncogenes and key processes like migration.

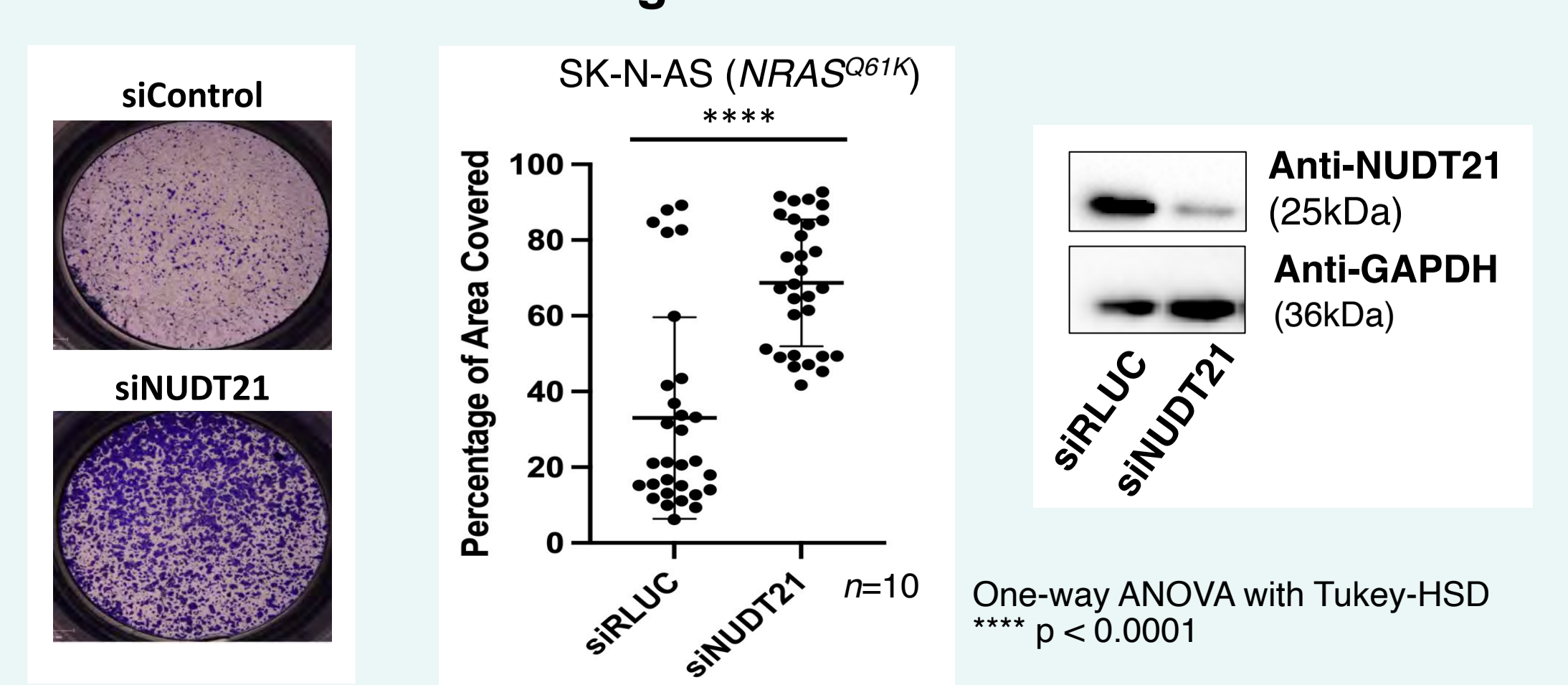
Additionally, a CPSF complex component, CPSF1, has also been reported to regulate migration in hepatocellular carcinoma and gastric cancer.

Neuroblastoma has two distinct cell states that dynamically interconvert



Compared to most adult cancers, childhood cancers are characterized by a relatively low number of somatic mutations. Neuroblastoma (NB) is responsible for ~15% of all pediatric cancer-related deaths. NB is characterized by two transitioning cell states, adrenergic (ADRN) and mesenchymal (MES). The transition of ADRN to MES cells is referred to as noradrenergic-to-mesenchymal transition (NMT).

NUDT21 KD increases migration in NRAS driven NB cells



Summary

- Established *in vitro* model to study APA in N-RAS driven NB cells.
- Increase in migration in N-RAS driven NB cells with NUDT21 KD.
- Increase in MES marker Vimentin with NUDT21 KD.
- Optimized pipeline to extract UTR isoform usage information from scRNAseq using NB cells.
- Identification of MES and ADRN subpopulations from NB cell lines using scRNAseq.
- Preliminary candidates from UTR analysis of MES and ADRN subpopulations from SK-N-SH cell line.

Future Directions

- Computationally and experimentally validate candidate genes from current 3'UTR analysis.
- Perform KD of APA factor CPSF1, reported to extend global 3'UTR length, and assay shifts in migration.
- Assay if changes in migration correspond to changes in NMT.
- Perform scRNAseq to ascertain if NUDT21 and CPSF1 leads to global shortening and lengthening of 3'UTRs in NB; as well as analyse pathways and genes affected.