Description of Additional Supplementary Files

Supplementary Data 1. Isoform Atlas and Annotation. Related to Figure 1. This table is derived from classification.txt produced by pigeon classify, which is based on SQANTI3 QC. It is a tab-separated file where isoforms are rows and QC features computed by pigeon classify are columns. For a full glossary of columns and their meaning, see https://github.com/ConesaLab/SQANTI3/wiki/Understanding-the-output-of-SQANTI3-QC#classifcols. It contains 182,371 high-confidence mRNA isoforms identified across the t00, t04, and t30 time points. The table also includes an additional column that indicates the results of the overlap comparison with the external fetal brain dataset [13], where Ensembl or TALON IDs are listed if the isoform identified in our dataset overlaps with one in the Patowary dataset.

Supplementary Data 2. Catalog of Microexons identified in human iPSC-derived neurons. This table lists the genomic coordinates and classification of 1,930 microexons in 8,008 transcripts (defined as exons < 28 nucleotides) detected in the long-read RNAseq dataset.

- **seqnames, start, end, strand**: Genomic location of the microexon (GRCh38).
- **width**: Length of the microexon in nucleotides.
- **gene_id/transcript_id**: Unique identifier for the PacBio gene ID and transcript isoform containing the microexon.
- **MIC_coord**: Combined genomic coordinates (chr:start-end).
- **GENCODE_v47**: Logical column indicating whether the microexon is found in GENCODE v47 (TRUE), or not (FALSE).
- **vastDB:** Logical column indicating whether the microexon is found in vastDB (TRUE), or not (FALSE).
- **EVENT:** vastDB exon ID.

Supplementary Data 3. Proteogenomic Validation of Novel Isoforms. Related to Figure 2. This table in .csv file format, lists novel peptides identified by mass spectrometry that provide direct experimental evidence for the translation of protein-coding sequences. It provides the amino acid sequence for each peptide, the corresponding isoform ID(s), and the parent gene.

Supplementary Data 4. Gene and Protein Expression Dynamics. Related to Figure 3. This table contains the complete gene-level differential expression results and functional enrichment analyses underlying the mRNA-protein dynamics shown in Figure 3.

- **Differential Gene Expression:** Full statistical results from DESeq2 for mRNA-level differential expression between time points (t00, t04, t30).
- **Differential Protein Expression:** Full statistical results from MSstatsTMT for protein-level differential expression.
- **Clusters:** The nine-category expression trajectory assignment (e.g., 'UU', 'D-') for each of the 8,493 genes with both mRNA and protein data.
- **SFARI Gene Enrichment:** Statistical results (odds ratio, *P*-value; One-sided Fisher's exact test with Bonferroni correction) for the enrichment of SFARI ASD risk genes in each protein and mRNA cluster, as shown in Figure 3B.
- **GO Terms - Protein:** Complete Gene Ontology (GO) enrichment analysis results for each of the nine protein expression trajectories.
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Supplementary Data 5. Isoform Switching Dynamics and Functional Consequences. Related to Figures 4 & 5. This table provides the complete isoform-level differential analysis results and the characterization of their functional consequences, as summarized in Figure 4 & 5.

- **DTE (Differential Transcript Expression):** Statistical results (log2FC, FDR) for all isoforms with significant changes in abundance (~49,000 events).

- **DTU (Differential Transcript Usage):** Statistical results (change in isoform fraction 'dIF', FDR) for all isoforms with significant changes in relative usage (~24,000 events).
- **Summarized/Detailed switch conseq/AS:** Gene-level and isoform switch-level characterization of the alternative splicing (e.g., microexon inclusion, intron retention) and functional consequences (e.g., NMD status, protein domain gain/loss) of isoform switching. Significance determined by binomial test.
- **SFARI Gene Enrichment:** Statistical results (odds ratio, *P*-value) for the enrichment of SFARI ASD risk genes among genes undergoing each class of switching event, as shown in Figure 5C. One-sided Fisher's exact test with Bonferroni correction.
- **GO Term Enrichment:** Complete Gene Ontology (GO) enrichment analysis results for genes regulated by specific switching events (e.g., intron retention), as shown in Figure 5B. One-sided Fisher's exact test, Benjamini-Hochberg (BH)-adjusted *P* values.

Supplementary Data 6. Coordination of alternative transcription start sites (ATSS) and alternative splicing (AS). Related to Figure 6. This table provides the complete statistical results and supporting data for the analysis of ATSS-AS coordination.

- **Quasi - Results & EMMs:** Contains the full statistical output from the primary quasi-binomial generalized linear model approach. This includes the 2,467 significant coordination events, their FDR values for 'global' and 'interaction' effects, and the estimated marginal means (EMMs) values. Model comparisons using F-tests on ANOVA tables were used to compute likelihood ratio test *P*-values, corrected using the Benjamini-Hochberg (FDR) method.
- **Fishers - Results:** Contains the complete results from the validation analysis using the contingency table-based Fisher's exact test method, for comparison. Two-sided Fisher's exact tests with Benjamini-Hochberg (BH) false discovery rate correction.

Supplementary Data 7. Coordination of Alternative Splicing (AS) and Alternative Polyadenylation (APA). Related to Figure 7 and Figure S16. This table provides the complete statistical results and supporting data for the analysis of AS-APA coordination and 3'UTR dynamics.

- **Quasi - Results & EMMs:** Contains the full statistical output from the primary quasi-binomial generalized linear model approach. This includes the 2,789 significant coordination events, their FDR values for 'global' and 'interaction' effects, and the estimated marginal means (EMMs) values. Model comparisons using F-tests on ANOVA tables were used to compute likelihood ratio test *P*-values, corrected using the Benjamini-Hochberg (FDR) method.
- **Fishers - Results:** Contains the complete results from the validation analysis using the contingency table-based Fisher's exact test method, for comparison. Two-sided Fisher's exact tests with Benjamini-Hochberg (BH) false discovery rate correction.
- **QAPA & APA:** Includes the output from the QAPA analysis and the custom long-read APA analysis, detailing proximal or distal polyA site usage (PPAU/DPAU) and the comparison of dPPAU/dDPAU to gene expression (log2FC) used to generate **Figure S16**.

Supplementary Data 8. Burden of Disruptive *de novo* Mutations in ASD Cohorts. Related to Figure 8. This table provides QC attributes related to all variants used for the *de novo* variant (DNV) burden analysis, as summarized in Fig. 8B. The data is stratified by cohort (MSSNG, SSC, SPARK_WGS, and SPARK_WES) in the "data_cohort" column, and by variant category in the "is_known" column, which is a column that is "True" when the isoform is known, "False" when the isoform is novel.

- **Known:** Variants classified as disruptive using the GENCODE V47 reference.
- **Novel:** Variants reclassified as disruptive *only* by this study's novel transcriptome. This data is used to calculate the odds ratios (OR) and *P*-values (Fisher's exact test)