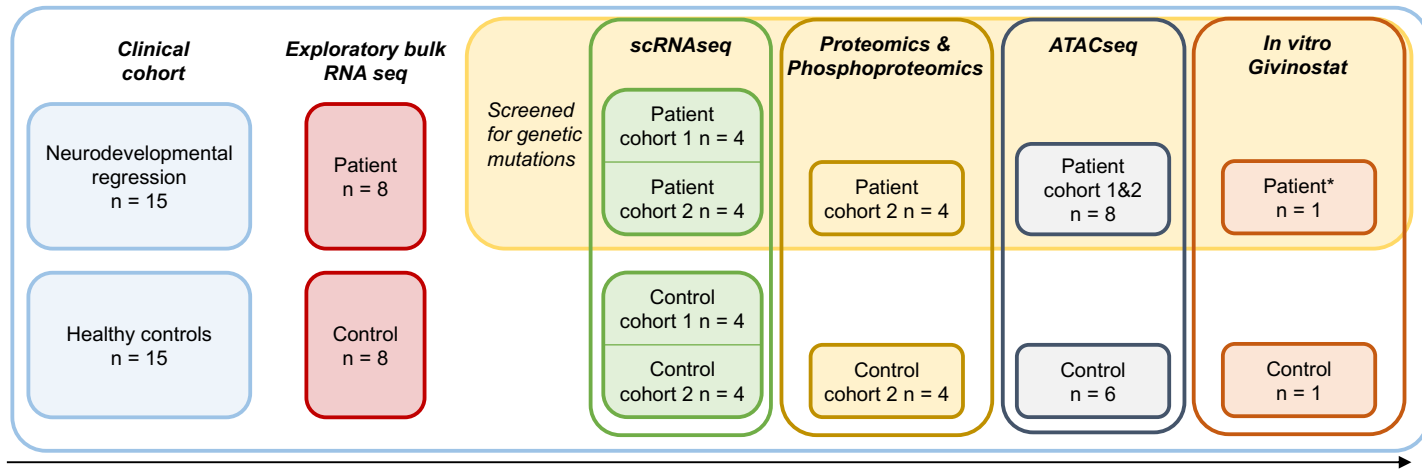


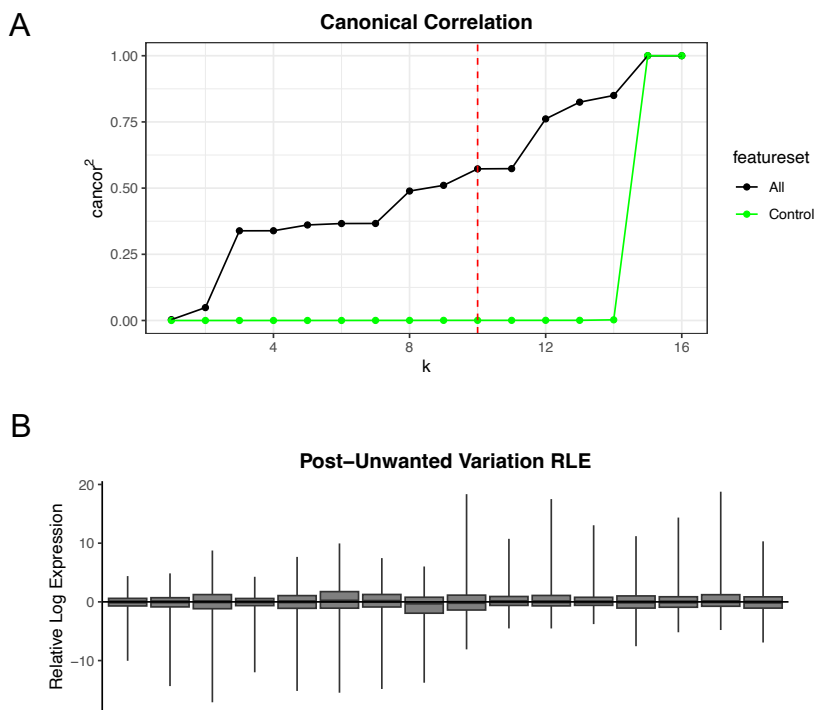
Supplementary Figures

Figure S1: Study Workflow



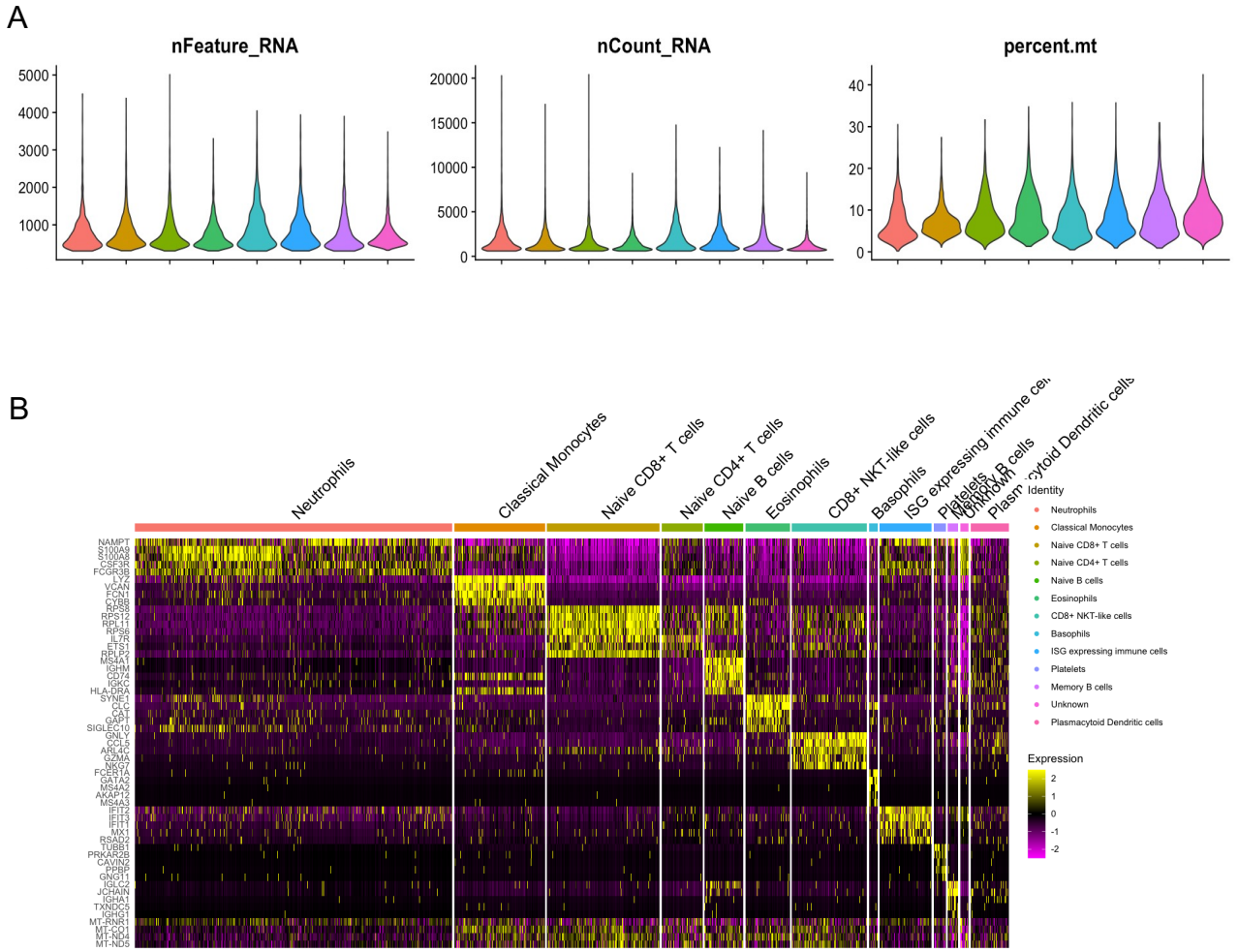
15 children with neurodevelopmental regression were recruited for this study and underwent comprehensive clinical assessments. Bulk RNA sequencing was performed, comparing samples from 8 affected children to 8 healthy controls. Genetic screening of eight children with neurodevelopmental regression included in subsequent studies (1 overlap with the initial group) revealed no pathogenic mutations. These children were analysed by single-cell RNA sequencing in two separate cohorts, each comprising four affected children and four matched controls. Additionally, the second cohort (n=4) underwent proteomic and phosphoproteomic analyses, all eight children underwent ATAC sequencing and one child was subjected to an in vitro givinostat assay to further investigate the underlying molecular mechanisms.

Figure S2: Bulk RNA seq



- (A) In the bulk RNA sequencing, $k=10$ (factors of unwanted variation) were used to remove genes that had minimal differential expression, compared to negative control genes
- (B) Box and whiskers relative log expression plot of samples after normalization

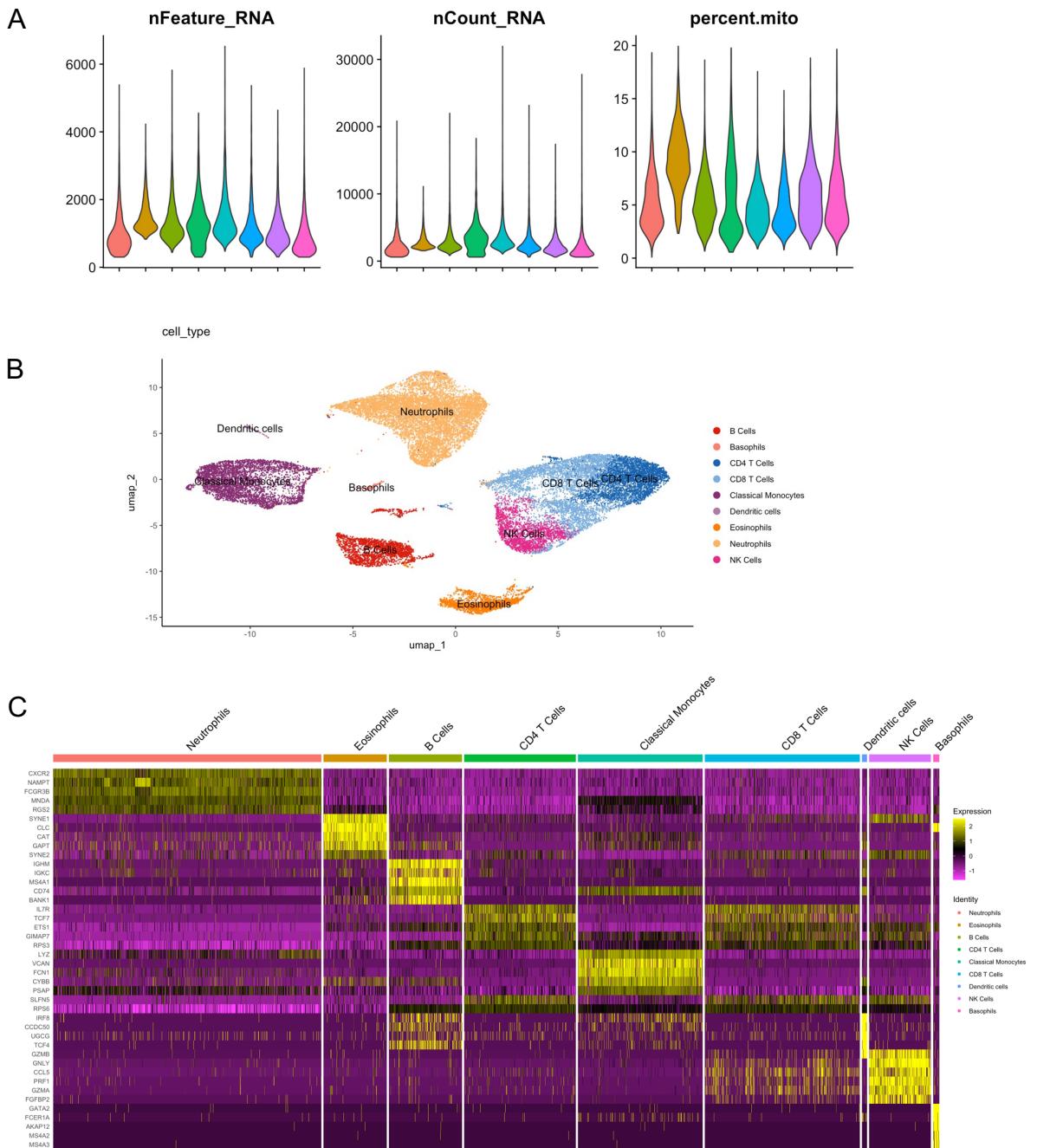
Figure S3: Single cell RNA seq analysis – Cohort 1



(A) Number of features, counts and percentage of mitochondrial genes per sample

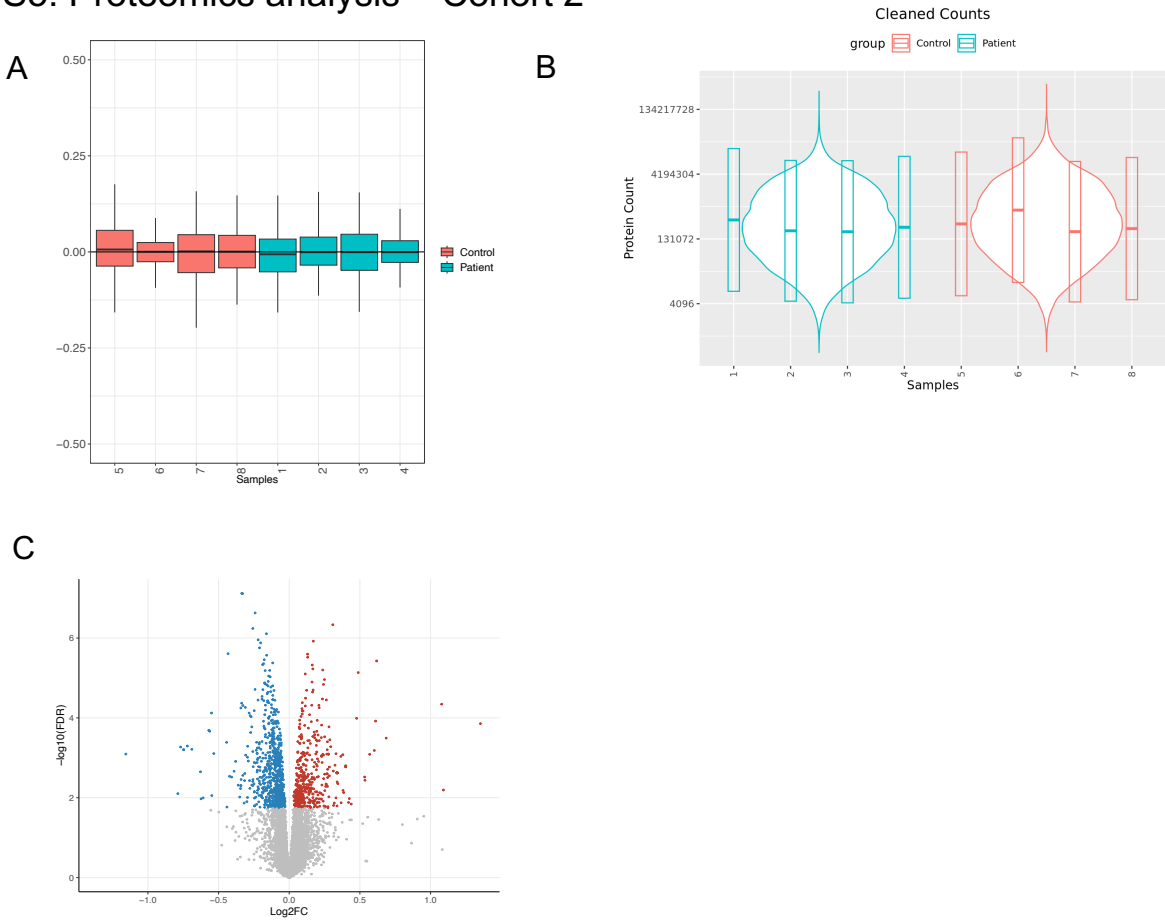
(B) Heat map of top differentially expressed genes based on cell type: genes are the top 5 markers of differential analysis between cell types (FDR <0.05) ranked by the difference in proportion of cells expressing the respective gene

Figure S4: Single cell RNA seq analysis – Cohort 2



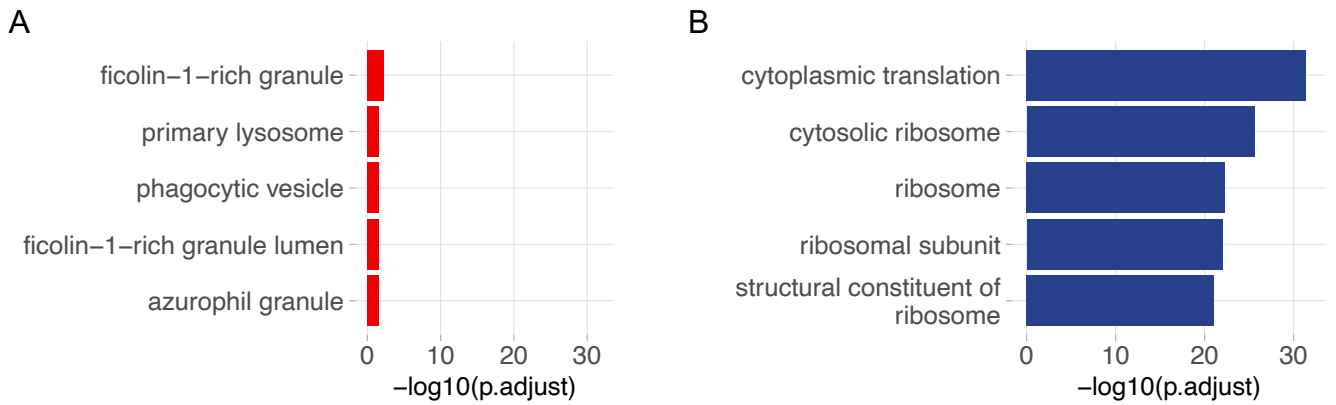
- (A) Number of features, counts and percentage of mitochondrial genes per sample
- (B) Uniform manifold approximation and projection (UMAP) of single cells from single cell RNA sequencing.
- (C) Heat map of top differentially expressed genes based on cell type: genes are the top 5 markers of differential analysis between cell types (FDR <0.05) ranked by the difference in proportion of cells expressing the respective gene

Figure S5: Proteomics analysis – Cohort 2



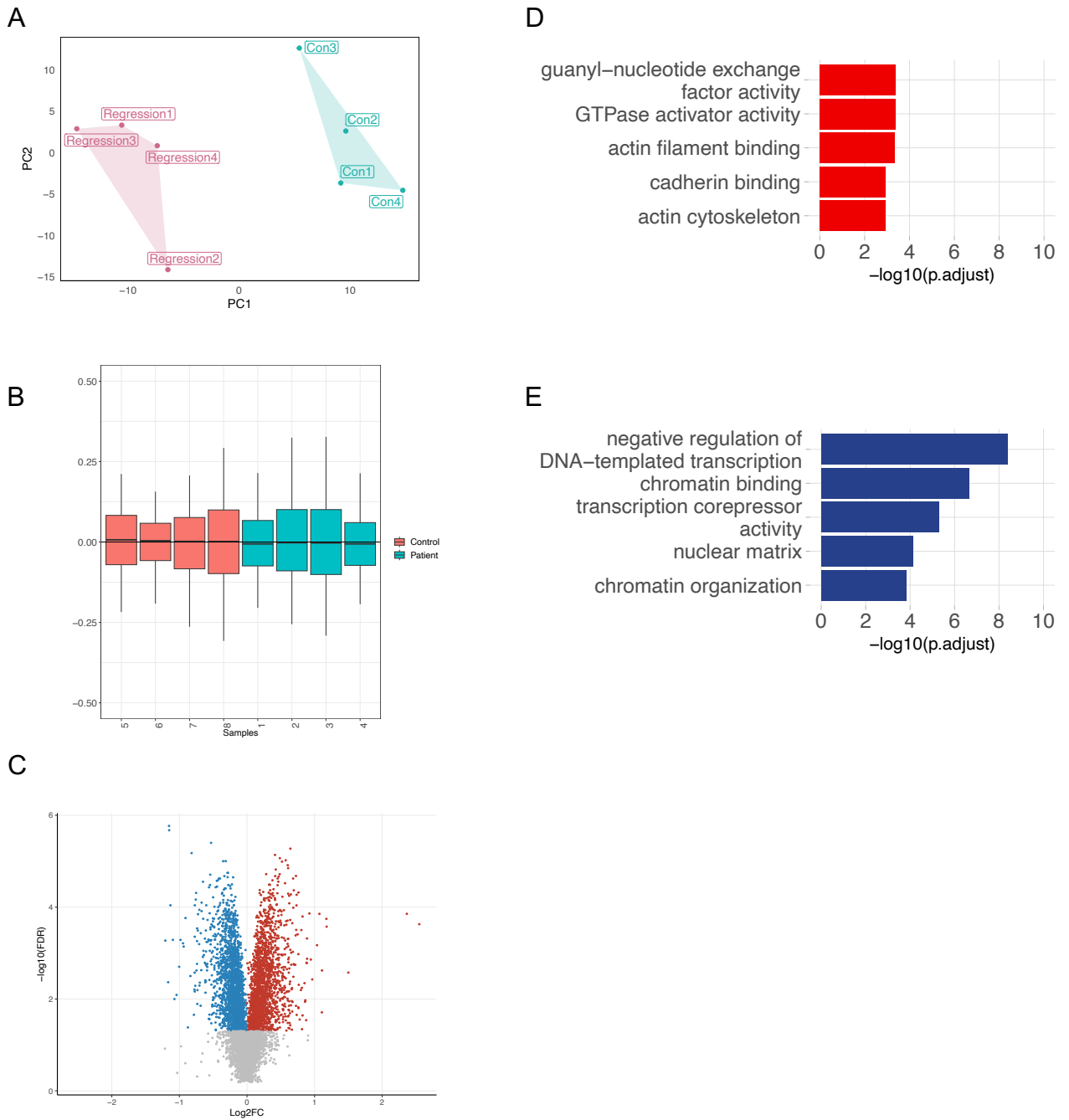
- (A) Box and whiskers relative log expression plot of samples after normalization
- (B) Violin plot of protein counts of samples after normalization
- (C) Volcano plot of differentially expressed proteins between children with neurodevelopmental regression compared to controls

Figure S6: Bulk RNA and proteomics pathway analysis



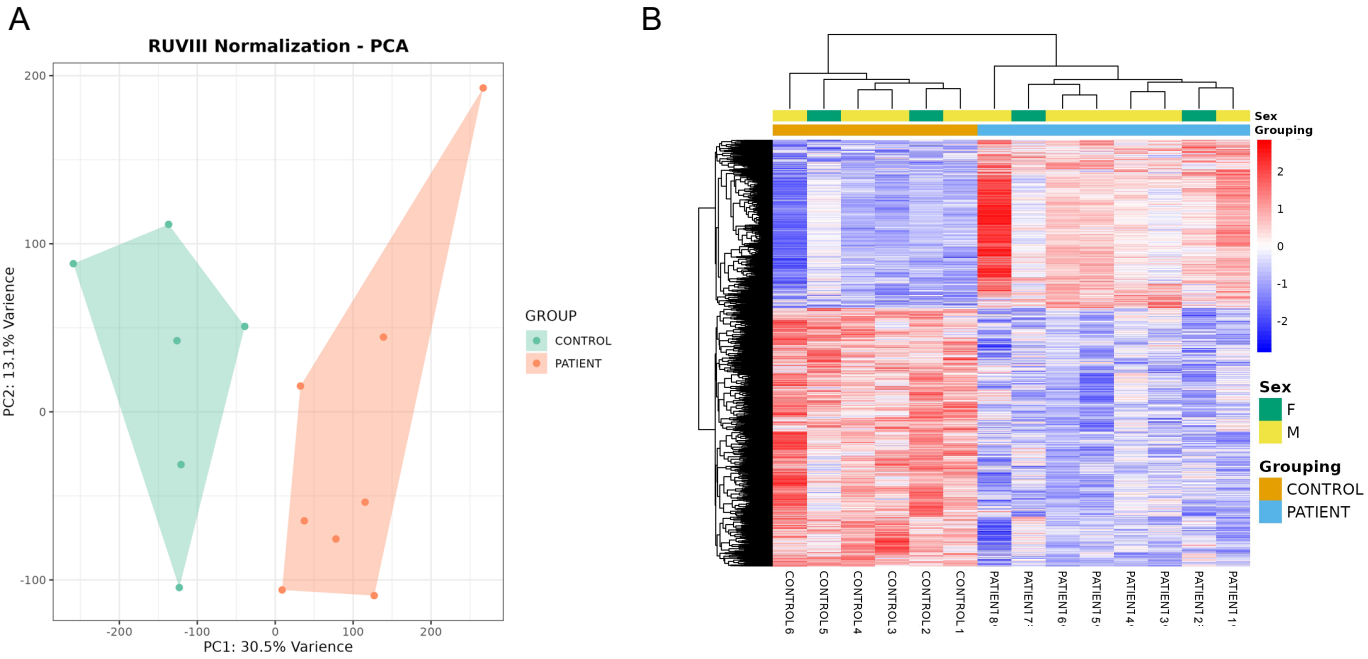
- (A) Top 5 ORA GO pathways consistently upregulated in both bulk RNA sequencing and proteomics were predominantly immune-related (granule-associated and phagolysosomal)
- (B) Top 5 ORA GO pathways consistently downregulated in both bulk RNA sequencing and proteomics were ribosome and translation pathways

Figure S7: Phosphoproteomics analysis – Cohort 2



- (A) Principal component analysis of phosphoproteomics shows good discrimination between children with neurodevelopmental regression versus controls
- (B) Box and whiskers relative log expression plot of samples after normalization
- (C) Volcano plot of differentially expressed phosphopeptides between children with neurodevelopmental regression compared to controls
- (D) Top 5 upregulated ORA GO pathways in phosphoproteomics were GTPase and actin pathways
- (E) Top 5 downregulated ORA GO pathways in phosphoproteomics were transcriptional and chromatin pathways

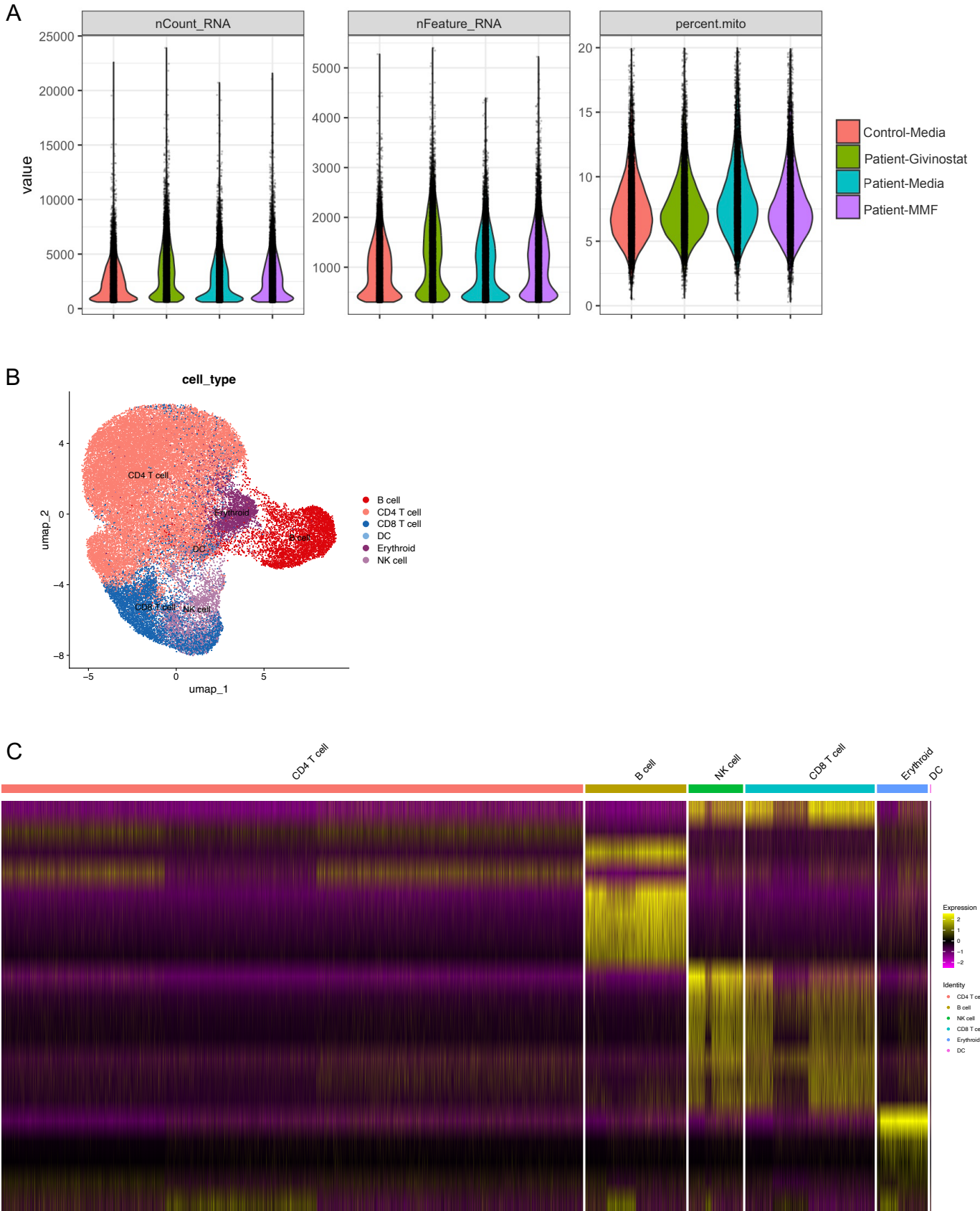
Figure S8: ATAC sequencing pathway analysis



(A) Principal component analysis of phosphoproteomics shows good discrimination between children with neurodevelopmental regression versus controls

(B) Heatmap also shows good discrimination between the two groups

Figure S10: In vitro givinostat pathways analysis



(A) Number of features, counts and percentage of mitochondrial genes per sample
 (B) Uniform manifold approximation and projection (UMAP) of single cells from single cell RNA sequencing of in vitro givinostat assay
 (C) Heat map of top differentially expressed genes based on cell type