Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Neurodevelopmental regression is associated with chromatin, transcriptional and immune dysregulation which can be reversed by HDAC inhibition

Russell Dale

russell.dale@sydney.edu.au

University of Sydney https://orcid.org/0000-0002-2495-1826

Hiroya Nishida

Velda Han

Brooke Keating

Katherine Zyner

Jessica Hayes

Brian Gloss

Nader Aryamanesh

Pinki Dey

Lee L Marshall

Erica Tsang

Sarah Alshammery

Xianzhong Lau

https://orcid.org/0000-0001-7845-8933

Ruwani Dissanayake

https://orcid.org/0000-0003-1422-2117

Shekeeb Mohammad

https://orcid.org/0000-0003-1219-4781

Mark Graham

Shrujna Patel

The Children's Hospital at Westmead https://orcid.org/0000-0003-4928-692X

Article

Keywords: neurodevelopmental disorders, autism spectrum disorder, developmental regression, epigenetics, omics

Posted Date: May 21st, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9529401/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: The authors have declared there is **NO** conflict of interest to disclose

Abstract

Neurodevelopmental regression in children, including autistic regression, is often associated with inconclusive diagnostic investigations, leaving the underlying pathophysiology poorly understood. We conducted peripheral blood multi-omic profiling in 15 children with neurodevelopmental regression (median age 9 (4-14) years, 60% males, 7 had developmental issues prior to regression), and 18 healthy controls (median age 12 (7-16) years, 56% males). Our comprehensive analysis included peripheral blood bulk RNA sequencing (8 regression vs. 8 controls), single-cell RNA sequencing (two cohorts of 4 regression vs. 4 controls), bulk proteomics (4 regression vs. 4 controls), phosphoproteomics (4 regression vs. 4 controls), and analysis of transposase-accessible chromatin (ATAC) sequencing (8 regression vs. 6 controls). Despite clinical heterogeneity, we identified convergent pathophysiological processes, including dysregulated epigenetic pathways (chromatin remodelling, histone modifications, transcription factors) and immune responses, as well as downregulated ribosomal and translational pathways. Our data supports a predisposition to a heterochromatin (closed) state in patients. We therefore treated patient PBMCs with *in vitro*histone deacetylase inhibitor, givinostat, to modulate RNA expression (1 regression vs. 1 control). Through single-cell RNA sequencing, we showed that *in vitro* givinostat reversed the dysregulated ribosomal and translational pathways, and downregulated immune pathways in patient PBMCs. Disruptions in epigenetic, immune and translational processes may arise from complex genetic and environmental interactions in children with neurodevelopmental regression. Therapies that target immune dysregulation or epigenetic modifications may represent effective treatment options for these individuals.

Introduction

Neurodevelopmental disorders (NDDs) affect 1 in 6 children with an increasing prevalence[1], and arise from disruptions in brain development during critical prenatal and postnatal developmental windows[2]. Key developmental processes, including neurogenesis, synaptogenesis, myelination, and synaptic pruning by microglia actively shape brain architecture during this period[3]. While genetic factors play a critical role in these processes, the current diagnostic yield of exome sequencing in cases of autistic regression is only 9%[4] which highlights the contribution of common, as well as rare, gene variants[5]. These genes have been found to be involved in chromatin modification, transcriptional control, synaptic organisation, and immune regulation[6–8]. Additionally, environmental triggers also significantly impact these developmental processes. The interplay between genetic and environmental factors is proposed to operate through epigenetic modifications leading to changes in immune function, brain structure, cognitive functions, and behaviour[2].

Developmental trajectories in children with NDDs are classically characterized by gradual and steady progress. However, some children experience regression, defined by the loss of previously acquired skills. This regression can be abrupt, triggered by specific events, or gradual in nature. Autistic regression, observed in up to one third of individuals with autism spectrum disorders (ASD), typically

manifests in the second year of life[9–13], although sometimes occurs at an older age, referred to as "childhood disintegrative disorder"[14]. Autistic regression involves losing previously acquired skills in social interaction, language, cognition, or behavioural changes[9–13]. Additionally, some children lose developmental skills, such as cognitive decline, memory loss or executive dysfunction, without fulfilling the criteria for autism or a definable childhood dementia syndrome[15,16]. Unfortunately, treatment in neurodevelopmental regression remains limited, primarily focused on treating comorbid behaviours, and providing developmental therapies.

We hypothesize that epigenetic and immune dysregulation in both the blood and brain, occurring during critical developmental periods, plays a key role in neurodevelopmental regression. In this study, we used a multi-omic approach in peripheral blood to explore the pathophysiology of neurodevelopmental regression and used *in vitro* pharmacotranscriptomics to explore potential future therapeutics.

Methods

Participant selection

Children (<18 years of age) were recruited into this study if they met criteria for neurodevelopmental (autistic or cognitive) regression.

1. Neurodevelopmental regression was defined as one or more episodes of losing previously established skills; the loss of spoken language (such as a child who was regularly speaking phrases who stops speaking) and/or loss of social and communication abilities (such as a child previously using gestures and making regular eye contact who loses such social responsiveness[17]), or cognitive decline/memory loss. These observations were associated with functional decline, with corroboration of the parental observations from teachers and other family members.
2. Children underwent standard clinical investigations using routine application of neuroimaging, and cerebrospinal fluid and genomic testing (as defined by Australian Medicare supported genomic testing).
3. All children had ongoing symptoms and were in the 'chronic phase' of their illness. No patient had an infectious disease in the preceding two weeks prior to blood sampling.
4. The patients who had single-cell RNA sequencing (scRNAseq), proteomics/phosphoproteomics (n=8) and assay for transposase-accessible chromatin sequencing (ATACseq, n=8) were all screened and were negative for rare pathogenic variants that cause ASD (trio exome n=3, and autism gene panel n=5).

Details of the omics analyses performed for each patient are summarised in Table S1.

Control selection

The inclusion criterion for healthy controls was the absence of neurodevelopmental disorders, autoimmune diseases, and severe allergic conditions, as well as the absence of infection during the two weeks before blood draw. Controls were sex- matched to patients, and there was no significant age difference between patients and controls.

Semi-structured clinical interview

Clinicians recorded the child's history using a semi-structured clinical review for all participants, using DSM-5 criteria[18,19]. Details around the regression episodes were collated including development prior to regression, timings of regression, and developmental domains affected, including loss of social communication, loss of language/meaningful words, loss of imaginative play/restricted play, and restricted/repetitive patterns of behavioural interest. Videos were used to corroborate observations. Patient family history, birth history, past medical histories, and prior medications trialled were recorded.

Sample collection

After written parental/caregiver informed consent was obtained, venous blood samples were collected for testing in PAXgene™ blood RNA tubes (Qiagen, Hilden, Germany) and Acid Citric Dextrose tubes (BD). Peripheral blood mononuclear cells (PBMCs) were isolated from the whole blood and stored as previously described[20,21].

Bulk RNA sequencing

RNAseq was performed in 8 children with neurodevelopmental regression compared to 8 healthy controls (Table S1). This workflow includes RNA extraction from PAXgene™ blood RNA tubes, depletion of ribosomal RNA via hybrid capture (Illumina Ribo-Zero), and Illumina TruSeq Stranded Total RNA Library Preparation (input 200-1,000 ng of Total RNA). The stranded RNA samples were sequenced on the Illumina NovaSeq X next generation sequencing platform (2 x 150 base pairs) for a depth of 50 million paired end reads. The cleaned sequence reads were aligned against the *Homo sapiens* genome (Build version hg38), and the STAR aligner (v2.5.3a) was used to map unique reads to the genomic sequences[22].

HIVE™ *Single-cell RNA sequencing*

scRNAseq was performed in 8 children with neurodevelopmental regression compared to 8 healthy controls (two cohorts of 4 patients vs 4 healthy controls). Red blood cells (RBCs) were depleted from whole blood by immunomagnetic negative selection, preserving all leukocyte populations, including granulocytes[23]. Two cohorts of 8 HIVE devices (4 patients and 4 controls for each cohort) were each loaded with 30,000 cells in 1 mL of DPBS + 1% FBS, followed by 3mL of cell media (DPBS + 1% FBS), according to the manufacturer's protocol, as previously described[23].

HIVE devices were processed according to the manufacturer's instructions including bead capture with transcripts, followed by extraction by centrifugation. Subsequent HIVE library preparation steps were performed in a 96-well plate format. HIVE scRNAseq libraries were paired-end sequenced (2 × 150 bp) with custom primers (provided in the HIVE single-cell RNA seq processing kit) on an Illumina NovaSeq X platform[23].

Proteomic analysis

Proteomics and phosphoproteomics were performed in 4 children with neurodevelopmental regression and 4 controls (second scRNAseq cohort), as previously described[24]. Following isolation, PBMCs were lysed in 200 µL lysis buffer, heated at 85°C for 10 minutes in a heating block and stored at -80°C. Further methods of LC-MS/MS are described in Supplementary Methods. We identified 5,961 proteins suitable for analysis.

Phosphoproteomics analysis

Phosphopeptides were enriched using a previously described method[25]. Hydrophilic interaction liquid chromatography was used to fractionate the phosphopeptides, as described for proteomics. Further details of liquid chromatography-tandem mass spectrometry (LC-MS/MS) are described in Supplementary Methods. We identified 7,550 phosphopeptides suitable for analysis. Proteomics and phosphoproteomics database searching used MaxQuant v1.6.7.0, using the settings provided in Supplementary Methods.

Assay for transposase-accessible chromatin sequencing

Frozen PBMC aliquots from 8 patients and 6 controls were thawed rapidly in a 37 °C water bath, followed by washing with thawing medium, and incubated in culture medium at 37 °C in a 5% CO2 atmosphere for 3 hours (further details are provided in Supplementary Methods). Following the incubation, the cells were washed and stained with DAPI (1:100). Live cells (DAPI-negative) were sorted using the BD FACSAriaTM III Cell Sorter at the Westmead Cytometry Core Facility, Westmead Research Hub. ATACseq libraries were generated from FACS-isolated PBMCs using the Active Motif ATAC-Seq Kit (Active Motif, Cat# 53150) according to the manufacturer's instructions. Libraries were multiplexed and sequenced on an Illumina NextSeq 2000 platform (2 × 50 bp), achieving ~50 million paired end reads per sample.

In vitro givinostat and monomethylfumarate treatment of PBMCs for single-cell RNA sequencing

Frozen PBMC aliquots from a patient and a control were thawed and incubated using the same procedure as described for ATACseq. Following the incubation, the cells were treated with 90 ng/mL givinostat and 1700 ng/mL monomethylfumarate (MMF) or media (0.08% ethanol) in culture media (RPMI 1640 + 10% FBS + 1% glutamax + 1% penicillin-streptomycin) for 24 hours at 37°C in a 5% CO2 atmosphere. These two drugs were selected due to their known anti-inflammatory properties.

Concentrations of givinostat and MMF were determined by peak plasma concentrations following standard dosing[26-29].

Bioinformatic analysis

Omics data were analysed in the R statistical environment (R.4.5.1)[30] with tidyverse[31]. For bulk RNAseq, proteomics, phosphoproteomics and ATACseq, filtering and normalization steps were first performed. Subsequently, normalization with removal of unwanted variation, using the remove unwanted variation *(RUV)* R package was performed[32], using 500 negative control genes/proteins with little or no change in RNA expression across samples from an initial ANOVA test. For bulk RNA-seq (k = 10), proteomics (k = 4), phosphoproteomics (k = 4), and ATACseq (k = 5), the specified numbers of factors of unwanted variation were used, respectively. For linear modelling, the limma R package was used and the p-values were calculated using the empirical Bayes method 'eBayes' function[33]. The false discovery rate correction (FDR) was applied to the p-values by calculating the adjusted p-values. Significant differentially expressed genes and differentially abundant proteins (DEGs and DAPs, respectively) were defined as those with adjusted p-values/ FDR < 0.05.

For scRNAseq, the data was analysed using the Seurat package[34]. Cells with a high mitochondrial transcript ratio (>0.15) were excluded. Experiments were integrated using the *FindIntegrationAnchors* function in Seurat then immune cell types were assigned using *scPred*[35]. Merged data were then split by cell type and separately normalized using SingleCellTransform, then UMAP (uniform manifold approximation and *projection)* projections were made using the first 30 dimensions. DEGs with significant FDR values <0.05 were identified using *FindMarkers*.

Pathway enrichment analysis

Gene set enrichment analysis (GSEA) for bulk RNAseq, scRNAseq and proteomics: The genes and proteins were ranked based on their sign(logFC) x log10Pvalue scores [36,37]. Enriched gene sets were identified based on a running sum statistic (normalized enrichment score (NES)) and statistical significance, based on the FDR. Significant GSEA Gene Ontology (GO) pathways (FDR <0.05) were further simplified using the *simplify* function in clusterProfiler[38].

Over representation enrichment analysis (ORA) for phosphoproteomics and ATACseq: For phosphoproteomics, the number of significant pathways was deliberately reduced by (a) limiting the background to only proteins detectable by mass spectrometry and (b) using the ranked list method within gProfiler, which weights enrichment toward highly ranked genes. Up- and down-regulated phosphorylation were analysed separately, and the maximum positive and negative quantitative values for phospho-regulation were assigned to each protein. Proteins were then ranked based on both the quantitative value and significance of maximal change. Significant ORA GO pathways (FDR <0.05) were further simplified using the *simplify* function in clusterProfiler[38].

For ATACseq, up- and down-regulated transposase-accessible regions were analysed separately. Each accessible region was assigned to the gene whose promoter was closest to that region. Enriched gene sets were identified based on statistical significance, based on the FDR. Significant ORA GO pathways (FDR <0.05) were further simplified using the *simplify* function in clusterProfiler[38].

Bar and dot plots were plotted using *ggplot2*[39]package, and heatmaps using the *pheatmap* package[40]. Connectivity network (CNET) plots were created using *enrichplot* package where the enriched pathways are represented by their respective colours, and corresponding genes' adjusted p value.

Ethics approval

Ethical approval was granted by the Sydney Children's Hospitals Network Human Research Ethics Committee (HREC/18/SCHN/227, 2021/ETH00356). Informed written consent was obtained from all participants (parents/caregivers).

Results

Clinical data

The samples and patient details used for the multi-omic studies are presented in Figure S1 and Table S1. A total of 15 children with a history of neurodevelopmental regression (median age 9 (4–14) years at time of sampling, 60% males, with a median time from first regression to sampling of 5 (0.5–11) years) and a total of 15 controls (median age 12 (7–16) years, 60% males) were recruited for this study.

Family history of neurodevelopmental, neuropsychiatric or autoimmune disorders, and pregnancy related complications are summarized in Table S2. For the 15 patients in this study, 7 had some developmental issues prior to the onset of regression, including ADHD (n = 4), ASD (n = 2), and language delay (n = 1), whereas the remaining 8 children had neurotypical development before regression.

The first regression occurred at a median age of 4 (1.5–12) years, and 10 children were reported to have triggers associated with the regression event, including infections (n = 9, impetigo, sore throat, and urinary tract infection), and influenza vaccination (n = 1). During the autistic and cognitive regression, symptoms included new onset emotional lability/depression (n = 15), new onset or exacerbation of ritualistic and compulsive behaviour (n = 15), academic and/or cognitive decline (n = 13), loss of language or meaningful words (n = 12), behavioural developmental regression or loss in self-help skills (n = 12), loss of social skills (n = 11), sensory or motor abnormalities (n = 11), tics (n = 10), loss of imaginative play (n = 9), sleep disturbance (n = 8) and restricted food intake (n = 7). The first regression lasted an average of 11.7 weeks (median 7 weeks, range 2–52 weeks) followed by partial but incomplete recovery, excluding the two individuals who showed no recovery and continued to decline (Table S2).

The regression symptoms were impairing, requiring visits to a hospital emergency department (n = 8), and long school absence (> 3 months) (n = 4). The majority (n = 13) reported subsequent recurrent

regressions (occurring 1–6 times a year). The current conventional diagnostics at last follow-up are presented in Table S2 and include ASD (n = 12), ADHD (n = 12), OCD (n = 12), and intellectual disability (n = 5).

Whole leukocytes bulk RNA sequencing

Bulk RNAseq was performed in 8 children with neurodevelopmental regression compared to 8 healthy controls (Table S1, Figure S2). There was no significant difference in blood cell type composition between regression versus control samples (Table S1). Unsupervised principal component analysis (PCA, Fig. 1A) showed clear discrimination between groups. A total of 3,675 upregulated and 3,766 downregulated DEGs (FDR < 0.05) were found. Top 10 upregulated GSEA GO pathways were involved in immune, epigenetic and signaling functions (Fig. 1B, red). Top 10 downregulated GSEA GO pathways were involved in ribosomal, translation, and mitochondrial functions (Fig. 1B, blue) (full pathway list in Table S3).

Whole leukocytes single-cell RNA sequencing

To build on the bulk RNA seq findings and explore gene expression across different cell types, we performed two independent cohorts of scRNAseq (each cohort composed of 4 regression patients versus 4 controls). All 8 children with neurodevelopmental regression were negative for rare pathogenic gene variants associated with ASD. Cells were clustered into 9 different cell types based on cell markers (Fig. 1C).

scRNAseq cohort 1 was performed in 4 children (mean age 9.5 (7–14) years, all males, Table S1, Figure S3) with neurodevelopmental regression versus 4 healthy controls (11.3 (7–14) years, all males). A total of 7,240 cells from patients and 6,761 cells from controls were sequenced. DEGs per cell type ranged from 0 to 868. Upregulated GSEA GO pathways were involved in immune, transcription and signaling functions (Fig. 1D, red). Downregulated GSEA GO pathways were predominantly in ribosome and translational pathways across most cell types (Fig. 1D, in blue) (full pathway list in Table S4).

scRNAseq in cohort 2 was similar to cohort 1 (Table S1, full pathway list in Table S5, Figure S4): Upregulated GSEA GO pathways included immune pathways. Downregulated GSEA GO pathways included ribosome/translation pathways across most cell types.

PBMC proteomics

Blood proteomics was performed in 4 children with neurodevelopmental regression compared to 4 healthy controls (Table S1, Figure S5). Unsupervised PCA of blood proteomics showed clear discrimination between groups (Fig. 1E). A total of 432 upregulated and 720 downregulated DAPs (FDR < 0.05) were found. Upregulated GSEA GO pathways were involved in endocytosis and epigenetic (histone) function (Fig. 1F, red). Downregulated GSEA GO pathways were involved in ribosomal function (Fig. 1F, blue) (full pathway list in Table S6).

Blood RNA-protein correlation

We analysed concordance between DEGs that were upregulated and DAPs that were upregulated: The top 5 upregulated ORA GO pathways were predominantly immune-related (granule-associated and phagolysosomal) (Figure S6A, Table S7). Likewise, we analysed significant downregulated DEGs and downregulated DAPs: the top 5 downregulated ORA GO pathways were ribosome and translation pathways (Figure S6B). DEGs and DAPs in the most downregulated pathway (cytoplasmic translation) were ribosomal protein, eukaryotic translation initiation and elongation factor genes, and RNA-binding or RNA-modifying genes/proteins (Table S7).

PBMC phosphoproteomics

Using the same cohort and controls as the proteomic analysis, we examined phosphopeptide abundance (Figure S7). A total of 2,109 upregulated and 2,396 downregulated differentially abundant phosphopeptides (FDR < 0.05) were identified. The top 5 upregulated ORA GO pathways were GTPase and actin pathways (Figure S7D). The top 5 downregulated ORA GO pathways were transcriptional and chromatin pathways (Figure S7E, full pathway list in Table S8).

PBMC chromatin availability (ATAC sequencing)

The main findings from the RNA and proteomic analyses were dysregulated immune, epigenetic (histone and chromatin) and ribosomal pathways. We hypothesized that the primary driver of this signature was ‘transcriptional regulation’ due to chromatin availability. ATACseq allows examination of ‘open chromatin’ by coupling transposase enzymatic cleavage of DNA that is available for transcription (Fig. 2A, Figure S8).

ATACseq was performed in 8 children with neurodevelopmental regression compared to 6 healthy controls (Table S1). Unsupervised PCA (Figure S8A) and heatmap analysis (Figure S8B) showed clear discrimination between groups. A total of 4,763 upregulated and 7,315 downregulated differentially accessible chromatin regions were identified, and volcano plot showed a predominance of downregulated accessible chromatin regions, indicating a tendency toward chromatin closure (Fig. 2B). All of the top 10 upregulated ORA GO pathways were related with immune function (Fig. 2C), whereas the top 10 ORA GO downregulated pathways were predominantly involved in transcription, RNA and chromatin regulation (Fig. 2D, full pathway list in Table S9).

Blood RNA-ATAC sequencing correlation

We explored the correlation between chromatin accessibility and RNA expression and found 644 overlapping genes between the upregulated DEGs from the bulk RNAseq and upregulated genes from ATAC seq, as well as 1,113 overlapping genes between downregulated DEGs from bulk RNAseq and downregulated genes from ATACseq (Fig. 2E and 2F). Overlapping upregulated genes were enriched in

immune-related pathways (Fig. 2G, S9A), whereas overlapping downregulated genes were enriched in ribosomal and RNA-related function (Fig. 2H, S9B; full pathway list in Table S10).

RNA signature is reversible with in vitro HDAC inhibitor incubation

Our data suggested patient cells are predisposed to a heterochromatin (closed) state. Therefore, we tried to modify the RNA expression by incubating patient PBMCs with givinostat, a histone deacetylase (HDAC) inhibitor which promotes an euchromatin (open) state (Figure S10). Through scRNAseq of developmental regression-media versus control-media, we found that the baseline signature revealed dysregulated (predominantly upregulated) immune pathways and downregulated ribosomal/translation pathways (Fig. 3A, left column).

In vitro givinostat incubation for 24 hours resulted in a reversal of these baseline findings, with upregulation of ribosomal/translation pathways in givinostat treated developmental regression cells compared to developmental regression cells treated with media only, and downregulation of immune pathways (Fig. 3A, middle column). By contrast, incubation of developmental regression cells with MMF, an immune modulatory therapy used to treat multiple sclerosis, resulted in only minor modification of dysregulated pathways (Fig. 3A, right column; full pathway list in Table S11).

We further analysed genes associated with the ‘antigen binding’ pathway, which was the most upregulated immune pathway at baseline in the bulk analysis and was reversed following givinostat treatment. Genes that were upregulated at baseline and subsequently downregulated by givinostat were primarily related to *HLA/MHC* genes (*B2M, HLA* genes) and antigen processing (*TAP1, TAPBP*) (Fig. 3B).

Discussion

We conducted in-depth peripheral blood multi-omics profiling and *in vitro* HDAC inhibitor treatment in a cohort of children with neurodevelopmental regression. Neurodevelopmental regression significantly impairs school functioning and quality of life. Most of the children in our studies experienced regression compatible with "autistic regression", although some did not exhibit symptoms that fully met the criteria for ASD. Despite heterogenous clinical presentations, we identified convergent pathophysiological processes underlying these cases. Through peripheral blood bulk RNAseq, scRNAseq, bulk proteomics and phosphoproteomics, and ATACseq, we identified common pathways. These include dysregulated epigenetic (chromatin remodelling, histone modifications, transcription factors) and immune responses, as well as downregulated ribosomal and translational pathways. Epigenetic and immune processes are increasingly recognized to play critical roles in the pathogenesis of ASD[41]. Highly penetrant chromatin, histone-related and immune genes are well known contributors to ASD[6–8]. However, genetic testing in our cohort yielded negative results for pathogenic variants associated with ASD. We hypothesize that the abnormal epigenetic and immune signature found in our cohort of children with neurodevelopmental

regression could arise from susceptibility genes missed with current screening, environmental factors, or an interplay of both genes and environment.

Firstly, dysregulated ribosomal and translational pathways were consistently identified through the multi-omics profiling in our study. The translation of mRNA into functional proteins occurs in ribosomes, which play critical roles in brain development. Disruptions in the translational machinery have been linked to NDDs[42,43]. Additionally, environmental exposures, such as infections and stressors, can impair ribosomal biogenesis through epigenetic processes[44–46]. During stress conditions, such as proteostatic imbalance, nutrient deprivation, and oxidative stress, cells have been shown to assemble heterochromatin in rDNA, leading to transcriptional repression[47,48]. Perturbations in ribosomal biogenesis can reduce protein synthesis crucial for dendritic and synaptic function, contributing to changes in neuronal connectivity associated with NDDs. In our study, the consistent downregulation of ribosomal and translational pathways observed across multi-omics analyses highlights the importance of these pathways, and align with previous transcriptomic studies of NDDs[49].

Secondly, we identified a prominent signal of epigenetic dysregulation, involving chromatin organization, histone modifications, and transcription factors. Epigenetic modifications can disrupt critical brain developmental processes, such as neuronal differentiation, migration, synaptic function, and brain connectivity[50]. Modifications such as histone acetylation, methylation, phosphorylation, and ubiquitination play a pivotal role in shaping chromatin structure, directly impacting DNA accessibility to transcriptional machinery. These modifications influence the tightness of nucleosome packaging within chromatin; for instance, increased histone acetylation or reduced histone deacetylation leads to a more relaxed chromatin configuration, which enhances DNA accessibility and promotes gene transcription. Dysregulation of histone acetylation has been reported in both genetic and non-genetic animal models of ASD[51–56]. HDAC inhibitors have been shown to improve social and behavioural functions in animal models exhibiting abnormally low levels of histone acetylation in the prefrontal cortex, such as those with 16p11.2 deletion or *Shank3* deficiency[53,54,56]. In our study, the ATACseq indicated a predisposition to a closed chromatin state in children with neurodevelopmental regression, and *in vitro* treatment with an HDAC inhibitor reversed the baseline RNA signature.

Thirdly, we identified dysregulated immune pathways in RNA, proteome and ATACseq in our cohort. scRNAseq revealed heterogenous patterns of immune regulation across cell types, with a mix of up- and downregulation instead of a uniform trend. Immune dysregulation has also been observed in various NDDs, through studies of peripheral blood and brain samples in humans[57–59]. Several molecular signaling pathways, including those involving cytokines, major histocompatibility complex molecules, complement factors, and microglia, have been identified as links between peripheral and central immune activation, and the development of NDD phenotypes[57].

Epigenetic changes and immune dysregulation in NDDs may be exacerbated by environmental factors, which affect brain development from the pre-natal stage through postnatal life. Animal models and

human epidemiological data support an association between maternal inflammation during pregnancy and offspring NDDs[2,60]. In our study, there was high prevalence of maternal autoimmunity, psychiatric disorders (e.g. anxiety, depression) and pregnancy related complications (e.g. gestational diabetes, infections). A meta-analysis of two high-risk prospective human cohort studies involving umbilical cord blood revealed transcriptional changes to chromatin, autoimmune, and environmental response genes in children with NDDs[61]. This suggests that epigenetic factors and immune processes play important roles at the gene-environment interface early in the development of NDDs. Chromatin genes have high expression in the central nervous system during gestation and are downregulated after birth supporting the concept of increased vulnerability to epigenetic-environmental interaction during pregnancy[62,63]. Additionally, animal models show that immune insults during pregnancy can prime microglia via epigenetic reprogramming, heightening susceptibility to postnatal “second hit” events in offspring, such as infections or stress, which can trigger neurobehavioral abnormalities[64,65]. In our cohort, infection provoked regressions were highly prevalent, often recurrent, and had significant lasting effects on child development. While some children were able to partially recover between regressions (but rarely back to previous baseline), others experienced progressive symptoms without return to developmental baseline.

The strengths of our study include an in-depth multi-omics analysis in children with autistic and neurodevelopmental regression, revealing epigenetic, transcriptional, immune, and ribosomal abnormalities. The ATACseq supported the hypothesis of a ‘heterochromatin’ state, and our *in vitro* pharmacotranscriptomics revealed the immune-ribosomal RNA signature was reversed by an HDAC inhibitor. Limitations of this study include small sample size and heterogeneity in clinical symptomatology. Although extensive efforts were made to corroborate the reports of regression with video evidence and multiple observers, there remains no ‘standardized’ approach to demonstrating regression[15] and recall bias from observers is possible. Secondly, omics analyses were performed many years after initial regression event, and the majority of the children were taking psychiatric medications (including anti-depressants) during this study, which can theoretically influence gene and protein expression. Thirdly, this study focused on children with loss of skills or developmental regression and did not include more classical (non-regressive) presentations of NDDs for comparison. Fourthly, it is important to highlight that bulk and HIVE™ scRNAseq of whole blood included all leukocyte populations, whereas proteomics, phosphoproteomics, ATACseq and the givinostat assay were performed on PBMCs (excluding neutrophils). In clinical cohorts like this, omics analysis of brain tissues is not feasible, making investigations using peripheral blood a more practical and accessible approach. Finally, we only examined epigenetic state with ATACseq, and other epigenetic mechanisms and omic methodologies may add understanding.

Future studies involving larger, longitudinal study of treatment-naïve cohorts of NDDs, together with in depth evaluation of genetic backgrounds and environmental exposures, will enable further genotype-phenotype correlations. While our study highlights epigenetic mechanisms, the timing and triggers of epigenetic modifications in these children remain unclear. Epigenetic dysregulation could stem from heritable DNA variation in genes involved in epigenetic machinery (including the non-coding RNA),

epigenetic marks inherited or transgenerationally passed from their parents, or may be secondary to environmentally induced epigenetic marks during pregnancy or early life.

To date, treatments for NDDs primarily focus on managing symptoms and providing developmental support. Our findings highlight a role for epigenetic modifications in neurodevelopment through pathways involved in chromatin remodeling and gene regulation, contributing to disrupted brain development in children with developmental regression. These results suggest that histone modifications may regulate chromatin accessibility in these children and support the potential of epigenetic regulators, such as butyrate or histone deacetylase inhibitors, as therapeutic options[66,67].

Resource availability

Anonymized data not published within this article and R code will be made available by request from any qualified investigator. The proteomics data are available via ProteomeXchange with identifier PXD060771.

Declarations

Acknowledgements

We would like to thank the patients and their families for participating in this study. We would like to thank the doctors and nurses in the Department of Paediatric Endocrinology at The Children's Hospital at Westmead, Sydney for their help and support to recruit controls. We would like to thank the Children's Medical Research Institute for access to the Biomedical Proteomics Facility and bioinformatic support and thank the Australian Genome Research Facility for providing expertise in sequencing.

Author contributions

Conceptualization: HN, VXH, SP, RCD; Methodology: HN, VXH, JPH, SP, RCD; Investigation: HN, VXH, BAK, KGZ, JPH, SA, SP; Formal analysis: HN, VXH, KGZ, BG, NA, PD, LLM, XL, RD, MG, SP, RCD, Writing – Original Draft: HN, VXH, RCD; Writing – Review & Editing: HN, VXH, BAK, KGZ, JPH, BG, NA, ET, SA, RD, SM, MG, SP, RCD; Funding Acquisition, RCD; Supervision: SP and RCD.

Conflicts of interest

The authors declare no competing interests.

Funding

Financial support for the study was granted by the Dale NHMRC Investigator Grant AP1193648, Petre Foundation, Cerebral Palsy Alliance, Bioplatforms Australia, PANS Australia, PANDAS Network and International OCD Foundation. VXH was supported by ExxonMobil NUS Research Scholarship and

National University Hospital Singapore (NUHS) Clinician-Scientist Program during the course of this study.

References

1. Zablotsky, B. *et al.* Prevalence and Trends of Developmental Disabilities among Children in the United States: 2009-2017. *Pediatrics* **144** (2019). https://doi.org/10.1542/peds.2019-0811
2. Han, V. X., Patel, S., Jones, H. F. & Dale, R. C. Maternal immune activation and neuroinflammation in human neurodevelopmental disorders. *Nature Reviews Neurology* **17**, 564-579 (2021). https://doi.org/10.1038/s41582-021-00530-8
3. Schafer, D. P. *et al.* Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* **74**, 691-705 (2012). https://doi.org/10.1016/j.neuron.2012.03.026
4. Goin-Kochel, R. P., Trinh, S., Barber, S. & Bernier, R. Gene Disrupting Mutations Associated with Regression in Autism Spectrum Disorder. *J Autism Dev Disord* **47**, 3600-3607 (2017). https://doi.org/10.1007/s10803-017-3256-4
5. Anttila, V. *et al.* Analysis of shared heritability in common disorders of the brain. *Science* **360** (2018). https://doi.org/10.1126/science.aap8757
6. De Rubeis, S. *et al.* Synaptic, transcriptional and chromatin genes disrupted in autism. *Nature* **515**, 209-215 (2014). https://doi.org/10.1038/nature13772
7. Gabriele, M., Lopez Tobon, A., D'Agostino, G. & Testa, G. The chromatin basis of neurodevelopmental disorders: Rethinking dysfunction along the molecular and temporal axes. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **84**, 306-327 (2018). https://doi.org/10.1016/j.pnpbp.2017.12.013
8. Lasalle, J. Autism genes keep turning up chromatin. *OA Autism* **1** (2013). https://doi.org/10.13172/2052-7810-1-2-610
9. Al Backer, N. B. Developmental regression in autism spectrum disorder. *Sudan J Paediatr* **15**, 21-26 (2015).
10. Boterberg, S., Charman, T., Marschik, P. B., Bölte, S. & Roeyers, H. Regression in autism spectrum disorder: A critical overview of retrospective findings and recommendations for future research. *Neuroscience & Biobehavioral Reviews* **102**, 24-55 (2019). https://doi.org/https://doi.org/10.1016/j.neubiorev.2019.03.013
11. Richler, J. *et al.* Is there a 'regressive phenotype' of Autism Spectrum Disorder associated with the measles-mumps-rubella vaccine? A CPEA Study. *J Autism Dev Disord* **36**, 299-316 (2006). https://doi.org/10.1007/s10803-005-0070-1
12. Mouridsen, S. E. Childhood disintegrative disorder. *Brain and Development* **25**, 225-228 (2003).
13. Rosman, N. P. & Bergia, B. M. Childhood disintegrative disorder: distinction from autistic disorder and predictors of outcome. *Journal of Child Neurology* **28**, 1587-1598 (2013).

14. Association, A. P. *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision*. (2000).
15. Elvidge, K. L. *et al.* The collective burden of childhood dementia: a scoping review. *Brain* **146**, 4446-4455 (2023). https://doi.org/10.1093/brain/awad242
16. Djafar, J. V., Johnson, A. M., Elvidge, K. L. & Farrar, M. A. Childhood Dementia: A Collective Clinical Approach to Advance Therapeutic Development and Care. *Pediatr Neurol* **139**, 76-85 (2023). https://doi.org/10.1016/j.pediatrneurol.2022.11.015
17. Furley, K. *et al.* Developmental regression in children: Current and future directions. *Cortex* **169**, 5-17 (2023). https://doi.org/https://doi.org/10.1016/j.cortex.2023.09.001
18. Association, A. P. *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition*. 5th edn, (American Psychiatric Publishing, 2013).
19. Patel, S. *et al.* NDD-ECHO: A standardised digital assessment tool to capture early life environmental and inflammatory factors for children with neurodevelopmental disorders. *Brain Behav Immun Health* **46**, 101011 (2025). https://doi.org/10.1016/j.bbih.2025.101011
20. Hayes, J. P. *et al.* Butyrate modifies epigenetic and immune pathways in peripheral mononuclear cells from children with neurodevelopmental disorders associated with chromatin dysregulation. *Neurotherapeutics*, e00792 (2025). https://doi.org/10.1016/j.neurot.2025.e00792
21. Han, V. X. *et al.* Epigenetic, ribosomal, and immune dysregulation in paediatric acute-onset neuropsychiatric syndrome. *Mol Psychiatry* **30**, 5389-5404 (2025). https://doi.org/10.1038/s41380-025-03127-5
22. Dobin, A. *et al.* STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15-21 (2013). https://doi.org/10.1093/bioinformatics/bts635
23. Han, V. X. *et al.* IV Immunoglobulin Is Associated With Epigenetic, Ribosomal, and Immune Changes in Pediatric Acute-Onset Neuropsychiatric Syndrome. *Neurol Neuroimmunol Neuroinflamm* **12**, e200467 (2025). https://doi.org/10.1212/nxi.0000000000200467
24. Tsang, E. *et al.* Ketogenic diet modifies ribosomal protein dysregulation in KMT2D Kabuki syndrome. *EBioMedicine* **104**, 105156 (2024). https://doi.org/10.1016/j.ebiom.2024.105156
25. Engholm-Keller, K. & Larsen, M. R. Improving the Phosphoproteome Coverage for Limited Sample Amounts Using TiO2-SIMAC-HILIC (TiSH) Phosphopeptide Enrichment and Fractionation. *Methods Mol Biol* **1355**, 161-177 (2016). https://doi.org/10.1007/978-1-4939-3049-4_11
26. Lamb, Y. N. Givinostat: First Approval. *Drugs* **84**, 849-856 (2024). https://doi.org/10.1007/s40265-024-02052-1
27. Fiorentini, F. *et al.* Population pharmacokinetic-pharmacodynamic analysis of givinostat. *Expert Opin Drug Metab Toxicol* **19**, 229-238 (2023). https://doi.org/10.1080/17425255.2023.2219839
28. Drug Label: TECFIDERA. (U.S. Food and Drug Administration, 2013).
29. Lategan, T. W., Wang, L., Sprague, T. N. & Rousseau, F. S. Pharmacokinetics and Bioavailability of Monomethyl Fumarate Following a Single Oral Dose of Bafiertam™ (Monomethyl Fumarate) or

Tecfidera(®) (Dimethyl Fumarate). *CNS Drugs* **35**, 567-574 (2021). https://doi.org/10.1007/s40263-021-00799-9

30. R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/ . (2013).
31. Wickham, H. *et al.* Welcome to the Tidyverse. *Journal of open source software* **4**, 1686 (2019).
32. Risso, D., Ngai, J., Speed, T. P. & Dudoit, S. Normalization of RNA-seq data using factor analysis of control genes or samples. *Nat Biotechnol* **32**, 896-902 (2014). https://doi.org/10.1038/nbt.2931
33. Ritchie, M. E. *et al.* limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* **43**, e47 (2015). https://doi.org/10.1093/nar/gkv007
34. Hao, Y. *et al.* Integrated analysis of multimodal single-cell data. *Cell* **184**, 3573-3587.e3529 (2021). https://doi.org/10.1016/j.cell.2021.04.048
35. Alquicira-Hernandez, J., Sathe, A., Ji, H. P., Nguyen, Q. & Powell, J. E. scPred: accurate supervised method for cell-type classification from single-cell RNA-seq data. *Genome Biology* **20**, 264 (2019). https://doi.org/10.1186/s13059-019-1862-5
36. Subramanian, A. *et al.* Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* **102**, 15545-15550 (2005). https://doi.org/10.1073/pnas.0506580102
37. Reimand, J. *et al.* Pathway enrichment analysis and visualization of omics data using g:Profiler, GSEA, Cytoscape and EnrichmentMap. *Nature Protocols* **14**, 482-517 (2019). https://doi.org/10.1038/s41596-018-0103-9
38. Wu, T. *et al.* clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *Innovation (Camb)* **2**, 100141 (2021). https://doi.org/10.1016/j.xinn.2021.100141
39. Ginestet, C. (Oxford University Press, 2011).
40. pheatmap: Pretty Heatmaps (2025).
41. Moreno, R. J., Rose, D. & Ashwood, P. Altered phenotype and gene expression of regulatory T cells (Tregs) in children with Autism, and the relationship with comorbid gastrointestinal symptoms. *J Neuroinflammation* **23** (2026). https://doi.org/10.1186/s12974-026-03701-w
42. Narla, A. & Ebert, B. L. Ribosomopathies: human disorders of ribosome dysfunction. *Blood* **115**, 3196-3205 (2010). https://doi.org/10.1182/blood-2009-10-178129
43. Paul, M. S. *et al.* Rare EIF4A2 variants are associated with a neurodevelopmental disorder characterized by intellectual disability, hypotonia, and epilepsy. *Am J Hum Genet* **110**, 120-145 (2023). https://doi.org/10.1016/j.ajhg.2022.11.011
44. Vind, A. C., Genzor, A. V. & Bekker-Jensen, S. Ribosomal stress-surveillance: three pathways is a magic number. *Nucleic Acids Res* **48**, 10648-10661 (2020). https://doi.org/10.1093/nar/gkaa757
45. Terhorst, A. *et al.* The environmental stress response regulates ribosome content in cell cycle-arrested S. cerevisiae. *Front Cell Dev Biol* **11**, 1118766 (2023). https://doi.org/10.3389/fcell.2023.1118766

46. Szaflarski, W. *et al.* Early rRNA processing is a stress-dependent regulatory event whose inhibition maintains nucleolar integrity. *Nucleic Acids Res* **50**, 1033-1051 (2022). https://doi.org/10.1093/nar/gkab1231
47. Hirai, H., Takemata, N., Tamura, M. & Ohta, K. Facultative heterochromatin formation in rDNA is essential for cell survival during nutritional starvation. *Nucleic Acids Research* **50**, 3727-3744 (2022). https://doi.org/10.1093/nar/gkac175
48. Lorenz, D. R., Meyer, L. F., Grady, P. J., Meyer, M. M. & Cam, H. P. Heterochromatin assembly and transcriptome repression by Set1 in coordination with a class II histone deacetylase. *Elife* **3**, e04506 (2014). https://doi.org/10.7554/eLife.04506
49. Pramparo, T. *et al.* Prediction of Autism by Translation and Immune/Inflammation Coexpressed Genes in Toddlers From Pediatric Community Practices. *JAMA Psychiatry* **72**, 386-394 (2015). https://doi.org/10.1001/jamapsychiatry.2014.3008
50. Zahir, F. R. & Brown, C. J. Epigenetic Impacts on Neurodevelopment: Pathophysiological Mechanisms and Genetic Modes of Action. *Pediatric Research* **69**, 92-100 (2011). https://doi.org/10.1203/PDR.0b013e318213565e
51. Kumari, D. & Usdin, K. The distribution of repressive histone modifications on silenced FMR1 alleles provides clues to the mechanism of gene silencing in fragile X syndrome. *Hum Mol Genet* **19**, 4634-4642 (2010). https://doi.org/10.1093/hmg/ddq394
52. Li, Y. *et al.* Reducing histone acetylation rescues cognitive deficits in a mouse model of Fragile X syndrome. *Nat Commun* **9**, 2494 (2018). https://doi.org/10.1038/s41467-018-04869-3
53. Ma, K. *et al.* Histone deacetylase inhibitor MS-275 restores social and synaptic function in a Shank3-deficient mouse model of autism. *Neuropsychopharmacology* **43**, 1779-1788 (2018). https://doi.org/10.1038/s41386-018-0073-1
54. Qin, L. *et al.* Social deficits in Shank3-deficient mouse models of autism are rescued by histone deacetylase (HDAC) inhibition. *Nat Neurosci* **21**, 564-575 (2018). https://doi.org/10.1038/s41593-018-0110-8
55. Liu, H. *et al.* Valproic Acid Induces Autism-Like Synaptic and Behavioral Deficits by Disrupting Histone Acetylation of Prefrontal Cortex ALDH1A1 in Rats. *Front Neurosci* **15**, 641284 (2021). https://doi.org/10.3389/fnins.2021.641284
56. Wang, W. *et al.* Histone Deacetylase Inhibition Restores Behavioral and Synaptic Function in a Mouse Model of 16p11.2 Deletion. *Int J Neuropsychopharmacol* **25**, 877-889 (2022). https://doi.org/10.1093/ijnp/pyac048
57. Estes, M. L. & McAllister, A. K. Immune mediators in the brain and peripheral tissues in autism spectrum disorder. *Nat. Rev. Neurosci.* **16**, 469-486 (2015). https://doi.org/10.1038/nrn3978
58. Hughes, H. K., Mills Ko, E., Rose, D. & Ashwood, P. Immune dysfunction and autoimmunity as pathological mechanisms in autism spectrum disorders. *Front. Cell. Neurosci.* **12**, 405 (2018). https://doi.org/10.3389/fncel.2018.00405

59. Martino, D., Zis, P. & Buttiglione, M. The role of immune mechanisms in Tourette syndrome. *Brain Res* **1617**, 126-143 (2015). https://doi.org/10.1016/j.brainres.2014.04.027
60. Osman, H. C. *et al.* Impact of maternal immune activation and sex on placental and fetal brain cytokine and gene expression profiles in a preclinical model of neurodevelopmental disorders. *J Neuroinflammation* **21**, 118 (2024). https://doi.org/10.1186/s12974-024-03106-7
61. Mordaunt, C. E. *et al.* A meta-analysis of two high-risk prospective cohort studies reveals autism-specific transcriptional changes to chromatin, autoimmune, and environmental response genes in umbilical cord blood. *Mol Autism* **10**, 36 (2019). https://doi.org/10.1186/s13229-019-0287-z
62. Herring, C. A. *et al.* Human prefrontal cortex gene regulatory dynamics from gestation to adulthood at single-cell resolution. *Cell* **185**, 4428-4447.e4428 (2022). https://doi.org/10.1016/j.cell.2022.09.039
63. Weng, M. K. *et al.* Extensive transcriptional regulation of chromatin modifiers during human neurodevelopment. *PLoS One* **7**, e36708 (2012). https://doi.org/10.1371/journal.pone.0036708
64. Martins-Ferreira, R., Leal, B., Costa, P. P. & Ballestar, E. Microglial innate memory and epigenetic reprogramming in neurological disorders. *Progress in Neurobiology* **200**, 101971 (2021). https://doi.org/https://doi.org/10.1016/j.pneurobio.2020.101971
65. Cheray, M. & Joseph, B. Epigenetics Control Microglia Plasticity. *Front Cell Neurosci* **12**, 243 (2018). https://doi.org/10.3389/fncel.2018.00243
66. Berni Canani, R., Di Costanzo, M. & Leone, L. The epigenetic effects of butyrate: potential therapeutic implications for clinical practice. *Clinical Epigenetics* **4**, 4 (2012). https://doi.org/10.1186/1868-7083-4-4
67. Xie, A. *et al.* Bacterial Butyrate in Parkinson's Disease Is Linked to Epigenetic Changes and Depressive Symptoms. *Movement Disorders* **37**, 1644-1653 (2022). https://doi.org/https://doi.org/10.1002/mds.29128

Figures

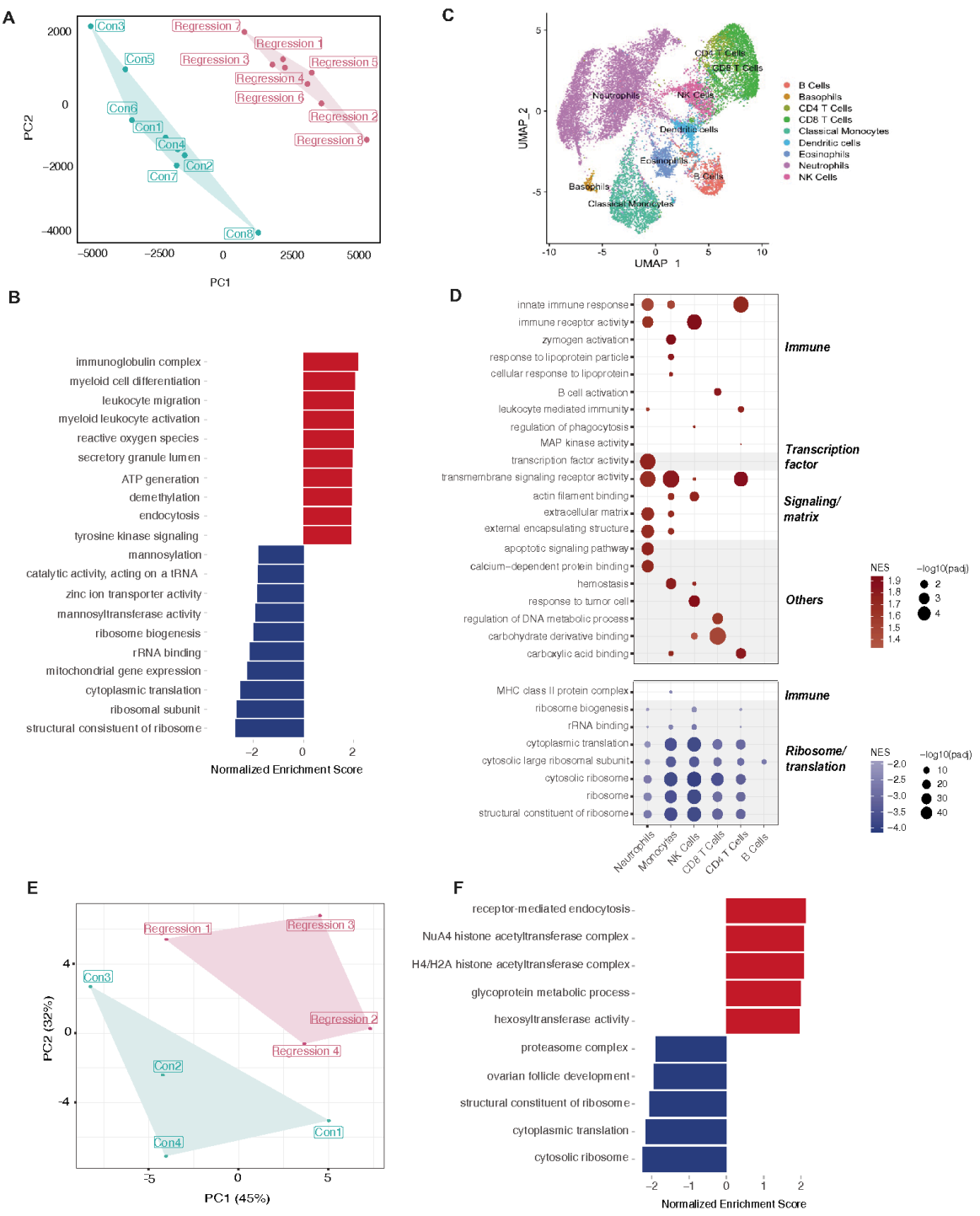


Figure 1

Blood bulk RNA sequencing, single-cell RNA sequencing and proteomics of children with neurodevelopmental regression versus healthy controls

(A) Principal component analysis (PCA) of peripheral blood bulk RNA sequencing performed in children with neurodevelopmental regression and controls. The x-axis represents Principal Component 1 (PC1),

while the y-axis represents Principal Component 2 (PC2). Unbiased hierarchical clustering of gene expression between children with neurodevelopmental regression and controls showed separation of data indicating strong group discrimination post RUV.

(B) Bar plot of Top 10 up- and down-regulated Gene Set Enrichment Analysis (GSEA) Gene Ontology (GO) bulk RNA sequencing pathways in children with developmental regression compared to the controls. The top 10 up-regulated GSEA GO pathways (red) were mainly involved in immune function (immunoglobulin complex, myeloid cell differentiation/activation, leukocyte migration), epigenetic mechanisms (demethylation), and signaling transduction (secretory granule lumen, endocytosis, tyrosine kinase signaling). The top 10 down-regulated GSEA GO pathways (blue) included ribosomal biogenesis, translation, and mitochondrial expression pathways.

(C) Uniform manifold approximation and projection (UMAP) of cohort 1 including 4 children with neurodevelopmental regression and 4 control samples in single-cell transcriptomics identified 9 unique cell types based on cell markers, including neutrophils, classical monocytes, natural killer (NK) cells, dendritic cells, basophils, eosinophils, B cells, CD8 T cells and CD4 T cells.

(D) Dot plot visualizing the top 5 GSEA pathways in scRNA seq across all cell types within cohort 1. The dot colour represents the normalized enrichment score (NES), with red indicating upregulation and blue indicating downregulation in neurodevelopmental regressive patients compared to controls, while white represents zero. The size of each dot corresponds to the -log10(padj) of the pathway. Upregulated GSEA GO pathways (FDR <0.05) were clustered in functions involving innate immune (immune response, immune receptor activity), transcription factor, signaling/matrix pathway (transmembrane signaling receptor activity, and actin filament binding). The upregulated innate immune pathways occurred predominantly in neutrophils, monocytes, NK cells and CD4 T cells. The upregulated transcription factor pathways occurred in neutrophils. Downregulated GSEA GO pathways (FDR <0.05) included immune pathways (MHC class II protein complex) and ribosome/translational pathways. Downregulated ribosome/translation pathways were present across most cell types.

(E) PCA performed on peripheral blood proteomics in children with neurodevelopmental regression and controls. The x-axis represents PC1, while the y-axis represents PC2. Unbiased hierarchical clustering of protein abundance between children with neurodevelopmental regression and controls showed separation of data indicating strong group discrimination post RUV.

(F) Bar plot of the top 5 up- and down-regulated GSEA GO proteome pathways (FDR <0.05) in children with developmental regression compared to the controls. The top 2 up-regulated pathways (red) include regulation of receptor-mediated endocytosis, and histone acetyltransferase complex. The top 5 down-regulated pathways (blue) were predominantly ribosomal and translational pathways.

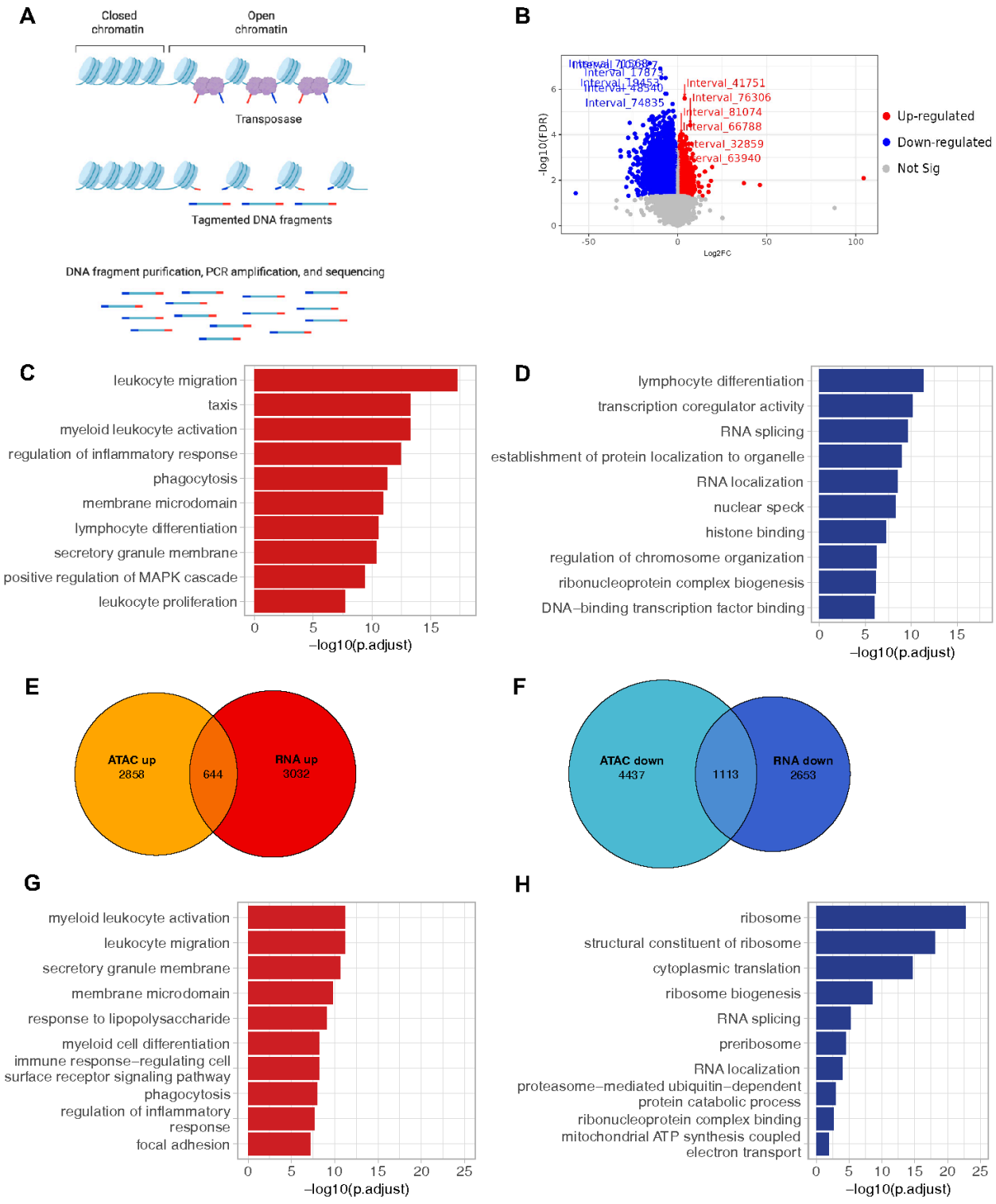


Figure 2

Blood assay of transposase-accessible chromatin sequencing (ATACseq) in children with neurodevelopmental regression versus controls

(A) Schematic overview of the principle of ATACseq. The transposase preferentially accesses open chromatin and inserts sequencing adapters into accessible regions, generating tagmented DNA

fragments. These fragments are then purified, PCR-amplified, and sequenced.

(B) Volcano plot of differentially accessible chromatin regions showing more downregulated than upregulated regions, indicating a predisposition toward a closed chromatin state in patient cells.

(C) Bar plot of the top 10 upregulated ORA GO ATACseq pathways (FDR <0.05) in children with developmental regression compared to the controls. All of the top 10 pathways were related with immune function.

(D) Bar plot of the top 10 downregulated ORA GO ATACseq pathways (FDR <0.05) in children with developmental regression compared to the controls. Top 10 downregulated pathways were involved in transcription, RNA and chromatin regulation.

(E) Venn diagram showing the number of genes upregulated in ATACseq, bulk RNA sequencing, or both. A total of 644 overlapping genes were upregulated at both the RNA level and the chromatin accessibility level.

(F) Venn diagram showing the number of genes downregulated in ATACseq, bulk RNA sequencing, or both. A total of 1,113 overlapping genes were downregulated at both the RNA level and the chromatin accessibility level.

(G) Bar plot of the top 10 upregulated ORA GO pathways (FDR <0.05) generated from overlapping DEG from both bulk RNA sequencing and ATACseq. All of the top 10 pathways were related with immune function.

(H) Bar plot of the top 10 downregulated ORA GO pathways (FDR <0.05)generated from overlapping DEG from both bulk RNA sequencing and ATACseq. The top 10 pathways were predominantly involved in ribosomal and RNA function.

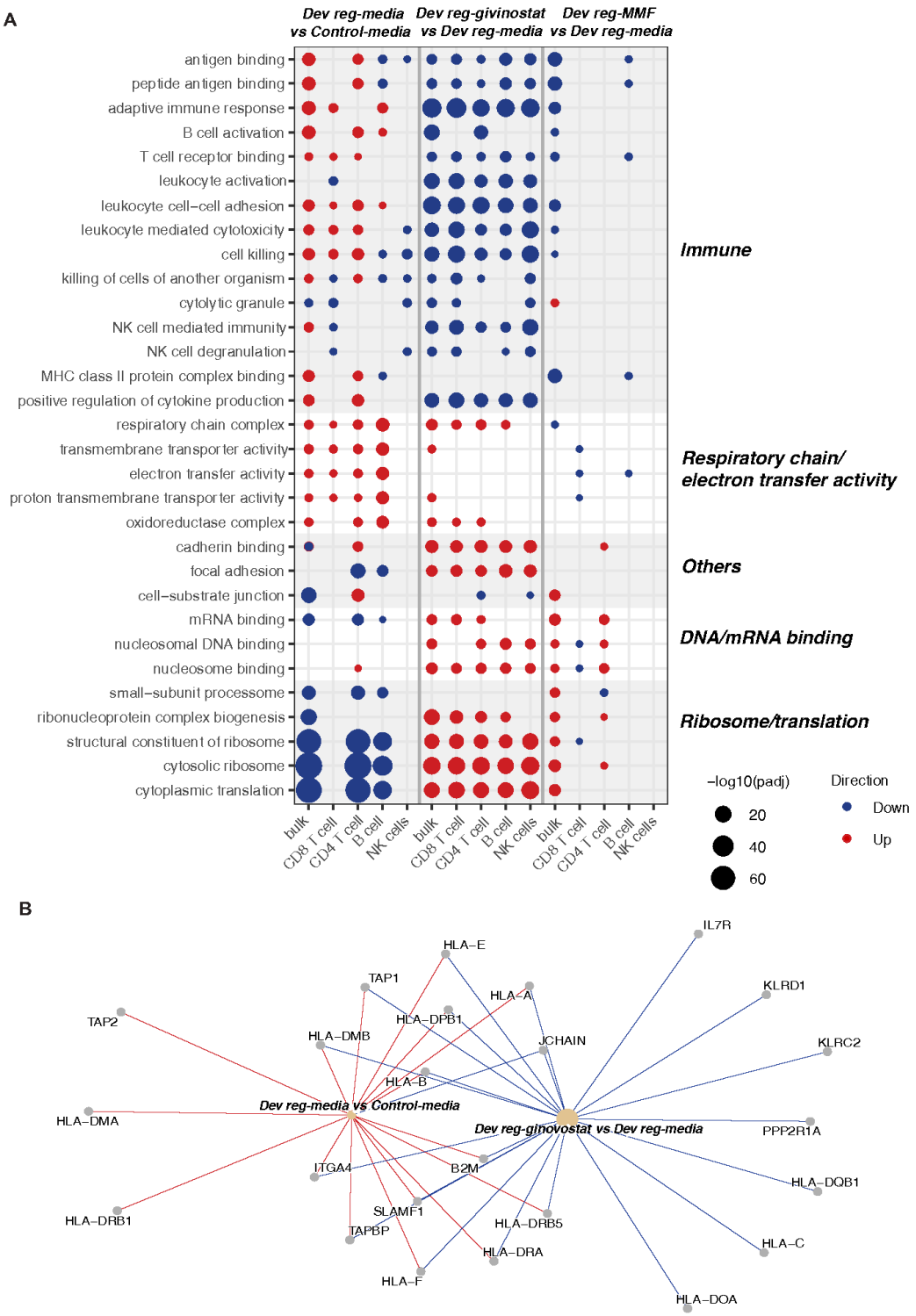


Figure 3

Effects of *in vitro* givinostat on peripheral blood immune cells assessed by single-cell RNA sequencing (scRNAseq)

(A) Dot plot of GSEA GO pathways ranked in the top 5 across all cell types and present in 5 or more cell types across comparisons. The dot colour represents the normalized enrichment score (NES), with red

indicating upregulation and blue indicating downregulation for each comparison, while white represents no significant difference. The size of each dot corresponds to the -log10(padj) of the pathway. In the dev reg-media vs control-media comparison (left), pathways related to respiratory chain/electron transfer activity were upregulated, whereas ribosome/translation pathways were downregulated. Immune pathways also showed dysregulation, although the direction varied by cell type and pathway. Following givinostat treatment (middle), ribosome/translation pathways were upregulated, whereas immune pathways were downregulated. Monomethylfumarate treatment (right) resulted in only minor modification of dysregulated pathways.

(B) Genes associated with the 'antigen binding' pathway that were upregulated at baseline and subsequently downregulated by givinostat were primarily related to HLA/MHC (*B2M, HLA*genes) and antigen processing (*TAP1, TAPBP*).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS4Devregcohort1scRNAseqpostsimplify.xlsx
- TableS3DevregbulkRNAGSEAGOpostsimplify.xlsx
- TableS8DevregORAGOphospathwaylist20250124.xlsx
- TableS12DevregSuppTables23Marrd.docx
- TableS9DevregATACORAGOsimple.xlsx
- TableS7DevregORAGORNAprotein.xlsx
- Devregsmethod310326RDBrookeHN.docx
- TableS10DevregATACRNAORAGOsimplecombined.xlsx
- TableS5DevregscRNAseqsimplifyHIVE220241202.xlsx
- TableS11Devreggivinostatcombined.xlsx
- FigS110DevRegSuppFig200426.pdf
- TableS6DevregProteomicsGSEAGOsimplifyHIVE220250210.xlsx
- Supplementalinformation.docx