The proteomic architecture of schizophrenia iPSC-derived cerebral organoids reveals alterations in GWAS and neuronal development factors

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Schizophrenia (Scz) is a brain disorder that has a typical onset in early adulthood but otherwise maintains unknown disease origins. Unfortunately, little progress has been made in understanding the molecular mechanisms underlying neurodevelopment of Scz due to ethical and technical limitations in accessing developing human brain tissue. To overcome this challenge, we have previously utilized patient-derived induced pluripotent stem cells (iPSCs) to generate self-developing, self-maturing, and self-organizing 3D brain-like tissue known as cerebral organoids. As a continuation of this prior work, here we provide an architectural map of the developing Scz organoid proteome. Utilizing iPSCs from n = 25 human donors (n = 8 healthy Ctrl donors, and n = 17 Scz patients), we generated 3D cerebral organoids, employed 16-plex isobaric sample-barcoding chemistry, and simultaneously subjected samples to comprehensive high-throughput liquid-chromatography/mass-spectrometry (LC/MS) quantitative proteomics. Of 3,705 proteins identified by high-throughput proteomic profiling, we identified that just ~2.62% of the organoid global proteomic landscape was differentially regulated in Scz organoids. In sum, just 43 proteins were up-regulated and 54 were down-regulated in Scz patient-derived organoids. Notably, a range of neuronal factors were depleted in Scz organoids (e.g., MAP2, TUBB3, SV2A, GAP43, CRABP1, NCAM1 etc.). Based on global enrichment analysis, alterations in key pathways that regulate nervous system development (e.g., axonogenesis, axon development, axon guidance, morphogenesis pathways regulating neuronal differentiation, as well as substantia nigra development) were perturbed in Scz patient-derived organoids. We also identified prominent alterations in two novel GWAS factors, Pleiotrophin (PTN) and Podocalyxin (PODXL), in Scz organoids. In sum, this work serves as both a report and a resource that researchers can leverage to compare, contrast, or orthogonally validate Scz factors and pathways identified in observational clinical studies and other model systems.

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INTRODUCTION

Schizophrenia (Scz) is a debilitating brain disorder that occurs in approximately ~1% of the population [1]. While Scz onset typically occurs in early adulthood, subtle brain changes and symptoms often begin emerging years prior to onset during the so-called “prodromal period” [2, 3]. In spite of this, it has remained unclear when Scz neuropathology actually begins to unfold in the brain [1]. For instance, does Scz neuropathology begin a couple of years prior to onset in adolescence when prodromal features progressively emerge? Or does Scz neuropathology begin much earlier in neurodevelopment at a scale that is not yet resolvable? Following decades of investigation, there is now strong epidemiological evidence that indicates risk of Scz may begin to accumulate during in utero brain development [4–7]. This includes data from numerous, independent, large-scale population studies [4–7]. Critically, it remains unclear if in utero risk factors for later Scz onset, such as maternal immune activation, famine, or hormonal/steroid factors, elicit risk by inducing neurodevelopmental alterations or promoting rates of de novo mutation [8]. While the latter can’t be ruled out as a potential etiological contributor, the former hypothesis holds strong merit given the highly-regulated nature of cortical development in utero and the fact that innumerous Scz risk factors exhibit known roles in central nervous system development. Indeed, some novel biological intermediaries are starting to be discovered which link in utero environmental risk factors to potential genetic factors, alterations, and/or vulnerabilities [9]. However, resolving these neurodevelopmental hypotheses of Scz has been difficult. Critically, ethical and technical constraints in accessing human primary brain tissue have arrested progress in delineating the neurodevelopmental trajectory of Scz. These ethical and technical limitations are further compounded by our inability to identify prospective cases of Scz, which has further sequestered our understanding of neurodevelopmental mechanisms of psychosis and has caused a rift between the known epidemiology and the presumed neurobiology of Scz. For instance, in the largest GWAS conducted to date a total of

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RESULTS
To study the molecular architecture of developing human brain-like tissue, we generated 3D cerebral organoids from human iPSCs from banked by the NIMH. In sum, biologics from \( n = 25 \) human donors were sampled comprising \( n = 8 \) healthy Ctrls and \( n = 17 \) Scz patients. Briefly, iPSCs from human donors were grown in 2D culture atop vitronectin-coated plates before being dissociated with Accutase to yield single-cell iPSC suspensions. Stem cell suspensions were correspondingly cultured into 3D aggregates, known as embryoid bodies, before being subjected to a chemically minimalist neural induction media for up to 7 days in vitro (DIV). After exhibiting evidence of neuroepithelial expansions and/or other morphological evidence of neural induction, tissue was impregnated into a matrigel droplet as a scaffold for further tissue expansion. Developing organoids were then matured under constant agitation atop an orbital shaker. Following this, at approximately 35-40 DIV, organoids from all 25 human donors were sampled for TMT quantitative proteomics. Briefly, this involved dissociating organoids, preparing peptide suspensions (digestion, reduction, and alkylation), barcoding samples with isobaric TMTpro 16-plex chemistry, and then multiplexing samples for simultaneous detection and analysis via nano high-sensitivity proteome profiling (for a simplified schematic of our experimental pipeline, see Fig. 1).

Analysis of organoid proteomes revealed sufficient peptide coverage for high-confidence quantitative analysis of 3705 proteins (peptide >1; intensity >0) across all 25 human donor samples. Based on Log2 transformed protein intensities, the Coefficient of Variation (CV) of Scz and Ctrl proteome groups was highly stringent; Median CV for Ctrls was 1.07% and for Scz 1.23%. This provided confidence in both the degree of neural induction achieved between samples, and that organoids were overall of a very similar and thus comparable composition between iPSC donors and within groups.

To gain insight into differences between Scz and Ctrl organoids, we next sought to determine which proteins (based on their expression) differed between these groups. Further analysis revealed the significant differential expression of peptide fragments belonging to 97 proteins in Scz organoids, of which 43 were up-regulated (\( p \text{ value} < 0.05, \text{Log2FC} > 0.05 \)) and 54 were down-regulated (\( p \text{ value} < 0.05, \text{Log2FC} < -0.05 \)). Thus, in sum, ~2.62% of the total organoid proteome was differentially expressed in Scz organoids, with equivalent (\( \pm 1.16\% \) vs. \( \pm 1.46\%) \) proportions of differentially expressed proteins being up- and down-regulated, respectively.

Deeper examination of significantly down-regulated proteins in Scz organoids, sorted by Log2FC values (see Table 1), revealed several important changes. Notably, we detected a depletion of factors that support neuronal development, differentiation, identity and/or function. Down-regulated neuronal development factors in Scz organoids comprised Neuromodulin (GAP43; Log2FC = −1.183, \( p = 0.010 \)), Cellular Retinoic Acid-Binding Protein 1 (CRABP1; Log2FC = −1.018, \( p = 0.016 \)), Neural Cell Adhesion Molecule (NCAM1; Log2FC = −0.854, \( p = 0.014 \)), expression of the myelin-modulating factor Myelin Expression Factor 2 (MYEF2; Log2FC = −0.537, \( p < 0.001 \)). Likewise, down-regulated expression of several other neuronal factors – involved in both neuronal identity and prototypic function – included Microtubule-Associated Protein 2 (MAP2), Tubulin Beta-3 Chain (TUBB3, or β3), Sypatonic Vesicle Glycoprotein 2 A (SV2A), and other neuron-specific markers (see Fig. 2). In addition to these changes, we also screened our dataset against novel, yet statistically prominent, Scz GWAS factors identified in the largest population genetic dataset reported to date [33]. One important Scz GWAS factor to emerge from our analysis of down-regulated proteins in Scz organoids was Pleiotrophin (PTN). In our prior work [1], we also detected the differential expression of PTN at both the protein and RNA level in Scz organoids, including in
Similar to our review of down-regulated proteins, we also identified a number of biologically interesting observations in our up-regulated Scz protein set list (see Table 2). This included up-regulation of numerous fibrinogens (FGG, FGB, FGA; Log2FC = 0.749-0.768, p = 0.008–0.010) and apolipoproteins (APOM, APOA1, APOE, APOC3, APOB; Log2FC = 0.562–0.771, p = 0.001–0.015). However, one of the most notable up-regulated protein was another Scz GWAS factor [33] that (like PTN) we had also identified in our prior Scz patient-derived organoid work [1]; namely, Podocalyxin (PODXL; Log2FC = 0.939, p < 0.001). Therefore, similar to our replication of down-regulated PTN expression in Scz organoids, this analysis in a larger pool of patients confirms that PODXL is another high-confidence candidate that may play a role in modulating Scz risk during early brain development.

We next sought to understand the potential functionality of our differentially expressed protein targets by parsing these factors into pathways, which may also unveil broader changes in regulatory networks underscoring disease-related phenotypes. We principally examined Gene Ontology (GO) pathways, parsed by annotations belonging to biological (Tables 3–4) and molecular (Tables 5–6) function of differentially expressed proteins. We first considered down-regulated GO biological pathways. Down-regulated GO biological pathways essential for normative brain assembly, development, and maturation overwhelmingly defined Scz patient-derived organoids. This included down-regulated expression of factors that map to axonogenesis, axon development, axon guidance, morphogenesis pathways regulating neuronal differentiation, and, broadly speaking, central nervous system development (due the sheer number of pathways involved here, please refer to Table 3 for statistical values).

Another interesting down-regulated GO biological process pathway in Scz organoids was specific enrichment for factors regulating substantia nigra development (GO:0021762, adjusted p = 0.0182, Neg Log10 = 1.74), which is of interest given that this midbrain region belongs to the basal ganglia which holds broad relevance to Scz neuropathology and its treatment (e.g., dopamine and monoamine hypotheses of Scz development and symptoms). Contrary to down-regulated GO biological pathways, up-regulated pathways in Scz organoids broadly reflected pathway involvement in cellular metabolism, chylomicron assembly and remodeling, steroid and steroid pathways, as well as lipoprotein remodeling and metabolism-related pathways (refer to Table 4 for statistical values).

Broadly speaking, these changes were also reflected in our analysis of GO pathways annotated for molecular functionality. Specifically, down-regulated GO molecular functions in Scz organoids comprised cytoskeletal structural, binding, and activity, as well as metabolic pathways relevant to neurodevelopment such GTP binding and GTPase activity (see Table 5; also identified in our prior prenatal drug modeling organoid work [11]). Similarly, up-regulated GO molecular function pathways in Scz organoids were typically related to sterol activity, cell adhesion, and lipoprotein binding/transfer/activity (see Table 6). In sum, these data provide additional veracity to the idea that there are metabolic functions underscoring the depletion of neuronal development factors in Scz organoids.

Lastly, we also considered whether Reactome pathways might unveil other novel biology in Scz organoids. Overall, an analysis of down-regulated (Table 7) and up-regulated (Table 8) Reactome pathways in Scz organoids revealed broadly similar pathway enrichment to those identified via GO analysis, with some notable exceptions. First, in our down-regulated Reactome pathway analysis, we noted that there were numerous significant pathways involved in NMDA receptor activation and assembly, ER to Golgi transport, as well as synaptic transmission (see Table 7 for a comprehensive list and statistical values). Contrary to this, and in addition to a convergent detection of lipoprotein-related metabolism pathways, unique Reactome
Table 1. 54 Down-regulated proteins in Scz organoids (<−0.5 Log2FC, \(p < 0.05\)).

| Gene Name | Protein Name | Uniprot ID | Log2FC | P Value |
|-----------|--------------|------------|--------|---------|
| GAP43     | Neuromodulin  | P17677     | −1.183 | 0.010   |
| CRABP1    | Cellular retinoic acid-binding protein 1 | P29762 | −1.018 | 0.016   |
| TUBB3     | Tubulin beta-3 chain | Q13509 | −1.015 | 0.001   |
| MAP2      | Microtubule-associated protein 2 | P11137-3 | −0.969 | 0.009   |
| BASP1     | Brain acid soluble protein 1 | P80723 | −0.939 | 0.006   |
| INA       | Alpha-internexin | Q16352 | −0.921 | 0.035   |
| FABP7     | Fatty acid-binding protein, brain | Q15540 | −0.903 | 0.025   |
| SV2A      | Synaptic vesicle glycoprotein 2A | Q7LOJ3-2 | −0.899 | 0.037   |
| PKM       | Pyruvate kinase PKM | P14618-2 | −0.866 | 0.004   |
| NCAM1     | Neural Cell Adhesion Molecule | A0A087WTF6 | −0.854 | 0.014   |
| CALM3     | Calmodulin-3 | P0DP25 | −0.847 | 0.002   |
| TUBB2B    | Tubulin beta-2B chain | Q9BVA1 | −0.840 | 0.019   |
| TNN1      | Troponin I 1 | G3V489 | −0.827 | 0.037   |
| ATP1A3    | Sodium/potassium-transporting ATPase subunit alpha-3 | P13637 | −0.817 | 0.042   |
| CRMP1     | Dihydropyrimidinase-related protein 1 | Q14194 | −0.789 | 0.019   |
| RUFY3     | Protein RUFY3 | Q7LO99 | −0.774 | 0.014   |
| ATAT1     | Alpha-tubulin N-acetyltransferase 1 | QSSQI0-7 | −0.773 | 0.023   |
| PEA15     | Astrocytic phosphoprotein PEA-15 | Q15121 | −0.764 | 0.030   |
| H1-0      | Histone H1.0 | P07305-2 | −0.760 | 0.009   |
| NCALD     | Neurocalcin-delta | P61601 | −0.738 | 0.000   |
| PPM1B     | Protein phosphatase 1B | O75688 | −0.714 | 0.030   |
| TAGLN3    | Transgelin-3 | Q9UJ15 | −0.705 | 0.001   |
| PTN       | Pleiotrophin   | P21246 | −0.700 | 0.030   |
| CRIP2     | Cysteine-rich protein 2 | P52943 | −0.690 | 0.005   |
| RAB6B     | Ras-related protein Rab-6B | Q9NRW1 | −0.684 | 0.010   |
| ENO2      | Gamma-enolase | P09104-2 | −0.682 | 0.027   |
| TUBB4A    | Tubulin beta-4A chain | P04350 | −0.676 | 0.008   |
| DPYSL5    | Dihydropyrimidinase-related protein 5 | Q9BP6U6 | −0.673 | 0.001   |
| SEPTIN3   | Neuronal-specific septin-3 | Q9UH30-3 | −0.667 | 0.015   |
| GDI1      | Rab GDP dissociation inhibitor alpha | P31150 | −0.659 | 0.011   |
| FHL1      | Four and a half LIM domains protein 1 | Q13642-1 | −0.658 | 0.010   |
| TUBA1A    | Tubulin alpha-1A chain | Q7U36-2 | −0.653 | 0.019   |
| MARCKS    | Myristoylated alanine-rich C-kinase substrate | P29966 | −0.650 | 0.002   |
| UCHL1     | Ubiquitin carboxyl-terminal hydrolase isozyme L1 | P09936 | −0.629 | 0.033   |
| LAMA4     | Laminin subunit alpha-4 | Q16363-2 | −0.619 | 0.016   |
| TCEAL3    | Transcription elongation factor A protein-like 3 | Q969E4 | −0.614 | 0.044   |
| TUBB4B    | Tubulin beta-4B chain | P68371 | −0.595 | 0.014   |
| H3-2      | Histone HIST2H3PS2 | Q5TEC6 | −0.574 | 0.001   |
| PTM5      | Parathymosin | P20962 | −0.565 | 0.008   |
| PALM      | Paralemmin-1 | O75781-2 | −0.552 | 0.000   |
| RTN1      | Reticulon-1 | Q16799-3 | −0.551 | 0.038   |
| FBN3      | Fibrillin-3 | Q7SN90 | −0.538 | 0.010   |
| MYEF2     | Myelin Expression Factor 2 | A0A087WUT0 | −0.537 | 0.001   |
| H2AC20    | Histone H2A type 2-C | Q16777 | −0.531 | 0.008   |
| DPYSL2    | Dihydropyrimidinase-related protein 2 | Q16555 | −0.529 | 0.014   |
| MAP1B     | Microtubule-associated protein 1B | P46821 | −0.527 | 0.020   |
| HDGFJ3    | Hepatoma-derived growth factor-related protein 3 | Q9Y3E1 | −0.517 | 0.002   |
| CKB       | Creatine kinase B-type | P12277 | −0.513 | 0.037   |
| KIF5C     | Kinesin heavy chain isoform 5C | O60282 | −0.512 | 0.014   |
| SCRN1     | Secemin-1 | Q12765 | −0.510 | 0.004   |
| HP1BP3    | Heterochromatin protein 1-binding protein 3 | Q5SSJ5 | −0.509 | 0.000   |
pathways that were up-regulated in Scz organoids comprised post-translational protein phosphorylation, pathways related to MAPK signaling, and IGF-related pathways. Overall, these data suggest that yin-and-yang alterations in Scz organoids exist, whereby the disruption of neuronal-development factors and pathways yields enrichment for pathways presumably involved in either compensation or other disease-related neuropathology including phenotypes that have possibly not yet been articulated in human-derived tissue (e.g. specific metabolic changes).

| Gene Name | Protein Name | Uniprot ID | Log2FC | P Value |
|-----------|--------------|------------|--------|---------|
| H3C1      | Histone H3.1 | P68431     | −0.502 | 0.010   |
| CPE       | Carboxypeptidase E | D6RF88 | −0.501 | 0.040   |
| HSDL1     | Inactive hydroxysteroid dehydrogenase-like protein 1 | Q3SXMS-2 | −0.501 | 0.021   |

**Fig. 2** Differential expression in the Scz cerebral organoid proteome. Principal component analysis of the cerebral organoid proteome indicated data grouping based on phenotype, and protein expression distributions indicated data correlation across all samples. This statistical baseline allowed us to consider the differentially expressed proteins present in Scz patient-derived cerebral organoids, which are shown here as a volcano plot split by log2 fold change and −log10 adjusted p values. In sum, ~2.62% of 3705 proteins (peptide >1; intensity >0) identified exhibited differential expression. Significantly up-regulated proteins that surpassed log2 fold change thresholding are depicted to the right in red (p value <0.05, Log2FC > 0.05), whereas down-regulated proteins (p value < 0.05, Log2FC < -0.05) are presented to the left of the plot in blue. Notable Scz GWAS factors (see 108 loci identified in [33]) included the up-regulation of PODXL and down-regulation of PTN, which replicated our previous findings in a smaller cohort [1]. Note also the down-regulation of the neural stem cell proliferation factor CRABP1 [93] as well as canonical neuronal development markers (e.g. NCAM1 [94], NCALD [95], and CPE [78]), neuronal markers (e.g. MAP2, TUBB3, MAP1B), synaptic markers (e.g., SV2A). Conversely, a range of apolipoproteins (APOE, APOA1, APOB, APOC3) were found to be up-regulated in Scz patient-derived cerebral organoids.
DISCUSSION
The aim of the current study was to further our knowledge of Scz by providing a deep, unbiased, analysis of molecular factors regulating central nervous system development in human-derived 3D tissue. To circumvent ethical and technical limitations in being able to access developing neural tissue from Scz patients [11], we generated 3D iPSC-derived cerebral organoids from \( n = 25 \) human donors (\( n = 8 \) Ctrl donors and \( n = 17 \) Scz donors). This approach allowed us to generate a theoretically limitless supply of self-regulating 3D neural tissue that recapitulated hallmark features of early brain assembly and corticogenesis [34, 35]. Samples were correspondingly subjected to cutting-edge isobaric barcoding chemistry that allowed up to 15 human donor samples (+ 1 pool for normalization) to be condensed into a single tube that could then be deconstructed via high-sensitivity, online, nano liquid-chromatography/mass-spectrometry proteomics. This allowed us to generate a posttranslational molecular map of factors in Scz patient-derived tissue/organoid samples.

Table 2. 43 Up-regulated proteins in Scz organoids (>0.5 Log2FC, \( p < 0.05 \)).

| Gene Name       | Protein Name                  | Uniprot ID | Log2FC | P-value |
|-----------------|--------------------------------|------------|--------|---------|
| SLC2A3          | Solute carrier family 2, facilitated glucose transporter member 3 | P11169     | 1.019  | 0.003   |
| GSTA2           | Glutathione S-transferase A2   | P09210     | 0.954  | 0.030   |
| PODXL           | Podocalyxin                    | O00592-2   | 0.939  | 0.000   |
| KRT18           | Keratin, type I cytoskeletal 18 | P05783     | 0.884  | 0.000   |
| AFP             | Alpha-fetoprotein              | P02771     | 0.868  | 0.027   |
| S100A10         | Protein S100-A10               | P60903     | 0.861  | 0.032   |
| AHSG            | Alpha-2-HS-glycoprotein        | P02765     | 0.843  | 0.001   |
| APOM            | Apolipoprotein M               | O95445-2   | 0.771  | 0.002   |
| FGG             | Fibrinogen gamma chain         | P02679-2   | 0.768  | 0.010   |
| FGB             | Fibrinogen beta chain          | P02675     | 0.753  | 0.001   |
| FGA             | Fibrinogen alpha chain         | P02671-2   | 0.749  | 0.008   |
| LIN28A          | Protein lin-28 homolog A       | Q9H922     | 0.731  | 0.001   |
| SLC9A3R1        | Na(+)/H( + ) exchange regulatory cofactor NHE-RF1 | Q14745     | 0.726  | 0.001   |
| APOA1           | Apolipoprotein A-I             | P02647     | 0.715  | 0.015   |
| SERPINB9        | Serpin B9                      | P50453     | 0.712  | 0.001   |
| SERPINA1        | Alpha-1-antitrypsin            | P01009     | 0.705  | 0.009   |
| APOE            | Apolipoprotein E               | P02649     | 0.698  | 0.006   |
| TF              | Serotransferrin                | P02787     | 0.687  | 0.005   |
| S100A11         | Protein S100-A11               | P31949     | 0.685  | 0.012   |
| APOC3           | Apolipoprotein C-III           | P02656     | 0.678  | 0.027   |
| EPCAM           | Epithelial cell adhesion molecule | P16422     | 0.677  | 0.041   |
| FN1             | Fibronectin                    | P02751-5   | 0.650  | 0.010   |
| APOA4           | Apolipoprotein A-IV            | P06727     | 0.634  | 0.009   |
| PDLIM1          | PDZ and LIM domain protein 1   | O00151     | 0.624  | 0.000   |
| LCP1            | Plastin-2                      | P13796     | 0.611  | 0.005   |
| TINAGL1         | Tubulointerstitial nephritis antigen-like | Q9GZM7-3  | 0.591  | 0.043   |
| TJ2P            | Tight junction protein ZO-2    | Q9UDY2-5   | 0.591  | 0.000   |
| SULT2A1         | Sulphotransferase 2A1          | Q06520     | 0.588  | 0.001   |
| HMGCS2          | Hydroxymethylglutaryl-CoA synthase, mitochondrial | P54868-2  | 0.580  | 0.009   |
| SNAP23          | Synaptosomal-associated protein 23 | O00161     | 0.563  | 0.000   |
| DSP             | Desmoplakin                    | P15924     | 0.562  | 0.000   |
| APOB            | Apolipoprotein B-100           | P04114     | 0.562  | 0.015   |
| ELOA            | Elongin-A                      | Q14241     | 0.560  | 0.011   |
| UTP14A          | U3 small nucleolar RNA-associated protein 14 homolog A | Q9BV6J-3  | 0.536  | 0.029   |
| FKBP11          | Peptidyl-prolyl cis-trans isomerase FKBP11 | Q9NYL4-2   | 0.534  | 0.021   |
| F11R            | Junctional adhesion molecule A | Q9Y624     | 0.534  | 0.001   |
| ARID3A          | AT-rich interactive domain-containing protein 3A | Q99856    | 0.532  | 0.001   |
| OSBP9L          | Oxysterol-binding protein-related protein 9 | Q96SU4-7  | 0.531  | 0.001   |
| REEP6           | Receptor expression-enhancing protein 6 | Q96HR9-2  | 0.530  | 0.006   |
| ECHDC1          | Ethylmalonyl-CoA decarboxylase | Q9NTX5-2   | 0.524  | 0.007   |
| SCD             | Acyl-CoA desaturase             | O00767     | 0.509  | 0.001   |
| METTL7B         | Methyltransferase-like protein 7B | Q6UX53    | 0.505  | 0.031   |
| DPP4            | Dipeptidyl peptidase 4         | P27487     | 0.500  | 0.031   |
Consequently, we were able to identify that Scz organoids principally differed from healthy Ctrls due to differences in the total quantity of molecular factors (rather than their diversity), the altered expression of an ensemble of neuronal factors, and the differential regulation of specific GWAS-implicated [33] disease candidates (namely, PTN and PODXL).

**Convergence upon depletion of neuronal factors in Scz organoids**

The first phenotype to arise in our molecular mapping of Scz organoids was the extent to which canonical neuron identity and development factors were depleted in Scz patient-derived organoids. For several decades, numerous theories have emerged which link neuronal and synaptic function with Scz [36–38], particularly as it relates to cortical dysfunction [39–41] and the cognitive symptoms [42, 43] observed in clinical cases [44]. Recently, progress has been made in understandingly early-arising changes within the developing brain that may influence novel neurodevelopmental factors with putative links to Scz [45]. This has led to numerous investigations of early-arising biological phenomenon in various model systems. Human-derived models, usually leveraging the power of gene edited or patient-derived iPSCs, have consequently revealed alterations in neuronal differentiation [46], mitochondrial metabolic function [47, 48], catecholamine levels [49], neuron-glia interactions [50], synaptogenesis [51], and synaptic function [52]. Thus, patient-derived iPSCs have proven to be a powerful tool in tracing early neurodevelopmental features of Scz [53]. However, iPSCs can be further exploited if used to generate human-derived organoids, a model system of human brain development which recapitulates endogenous self-regulatory mechanisms associated with cortical patterning [11]. Building upon prior Scz organoid work [1, 27–29], here we report lower levels of an ensemble of neuron-related development factors comprising GAP43, CRABP1, NCAM1, and MYEF2 as well as identity factors comprising MAP2, TUBB3, and SV2A. Broadly speaking, these molecular findlings are consistent with our prior work which reported disrupted neurogenesis and lower total neuron numbers within Scz cerebral organoids [1, 54, 55] – a phenotype which has also been independently reported by other groups [28]. Thus, fewer neurons will result in less MAP2, TUBB3, and SV2A expression, which is consistent with the molecular outcomes of this independent investigation. Our detection of lower NCAM1 protein levels in Scz organoids is also consistent with a prior report that found decreased NCAM1 expression in Scz neural progenitor cells [56].

### Table 3.

Down-regulated GO biological processes in Scz organoids (p < 0.05).

| Biological process                                      | GO:BP Term_ID | Adjusted p-value | Neg Log10 adjusted p |
|---------------------------------------------------------|---------------|------------------|----------------------|
| Axon Development                                        | 0061564       | 1.88E–07         | 6.725                |
| Nervous System Development                              | 0007399       | 2.98E–07         | 6.525                |
| Plasma Membrane Bounded Cell Projection Organization    | 0120036       | 7.32E–07         | 6.136                |
| Axonogenesis                                             | 0007409       | 8.26E–07         | 6.083                |
| Cell Projection Organization                            | 0030030       | 1.16E–06         | 5.937                |
| Cell Morphogenesis Involved in Neuron Differentation    | 0048667       | 1.29E–06         | 5.889                |
| Neuron Projection Morphogenesis                         | 0048812       | 4.63E–06         | 5.335                |
| Plasma Membrane Bounded Cell Projection Morphogenesis   | 0120039       | 6.03E–06         | 5.219                |
| Cell Projection Morphogenesis                           | 0048858       | 6.50E–06         | 5.187                |
| Cell Part Morphogenesis                                 | 0032990       | 8.89E–06         | 5.051                |
| Cell Morphogenesis Involved in Differentiation          | 00000904      | 2.09E–05         | 4.680                |
| Neuron Differentiation                                  | 0030182       | 4.18E–05         | 4.379                |
| Cellular Component Morphogenesis                        | 0032989       | 4.21E–05         | 4.375                |
| Neuron Development                                      | 0048666       | 9.53E–05         | 4.021                |
| Neuron Projection Development                           | 0031175       | 0.000125503      | 3.901                |
| Cell Morphogenesis                                      | 0000902       | 0.00170319       | 3.769                |
| Generation of Neurons                                  | 0048699       | 0.00192896       | 3.715                |
| System Development                                      | 0048731       | 0.00289587       | 3.538                |
| Neurogenesis                                            | 0022008       | 0.0059592         | 3.225                |
| Multicellular Organism Development                      | 0007275       | 0.0099934        | 3.000                |
| Anatomical Structure Development                        | 0048856       | 0.00263881       | 2.685                |
| Axon Guidance                                           | 0007411       | 0.00217016       | 2.674                |
| Neuron Projection Guidance                              | 0097485       | 0.00217362       | 2.663                |
| Negative Regulation of Microtubule Polymerization or Depolymerization | 0031111 | 0.011544881     | 1.938                |
| Microtubule-Based Process                               | 0007017       | 0.01300057       | 1.886                |
| Cytoskeleton Organization                               | 0007010       | 0.01330451       | 1.876                |
| Anatomical Structure Morphogenesis                      | 0009653       | 0.014459529      | 1.840                |
| Regulation of Axon Extension                            | 0030516       | 0.015068023      | 1.822                |
| Developmental Process                                   | 0032502       | 0.01628204       | 1.788                |
| Substantia Nigra Development                            | 0021762       | 0.018211464      | 1.740                |
| Microtubule Cytoskeleton Organization                   | 0000226       | 0.023833988      | 1.623                |
| Regulation of Extent of Cell Growth                     | 0061387       | 0.027856692      | 1.555                |
| Axon Extension                                          | 0048675       | 0.047873583      | 1.320                |
| Biological process                                                                 | GO:BP Term_ID | Adjusted p value | Neg Log10 adjusted p |
|-----------------------------------------------------------------------------------|---------------|------------------|----------------------|
| Chylomicron Remodeling                                                            | GO:0034371    | 1.03E−08         | 7.988                |
| Chylomicron Assembly                                                              | GO:0034378    | 3.76E−08         | 7.425                |
| Plasma Lipoprotein Particle Assembly                                              | GO:0034377    | 1.15E−07         | 6.938                |
| Triglyceride-Rich Lipoprotein Particle Remodeling                                 | GO:0034370    | 1.62E−07         | 6.790                |
| Plasma Lipoprotein Particle Remodeling                                            | GO:0034369    | 1.73E−07         | 6.762                |
| Protein-Lipid Complex Remodeling                                                  | GO:0034368    | 1.73E−07         | 6.762                |
| Protein-Containing Complex Remodeling                                             | GO:0034367    | 2.53E−07         | 6.598                |
| Protein-Lipid Complex Assembly                                                    | GO:0065005    | 2.53E−07         | 6.598                |
| High-Density Lipoprotein Particle Remodeling                                      | GO:0034375    | 6.89E−07         | 6.162                |
| Reverse Cholesterol Transport                                                     | GO:0043691    | 2.10E−06         | 5.677                |
| Plasma Lipoprotein Particle Organization                                          | GO:0071827    | 3.08E−06         | 5.511                |
| Protein-Lipid Complex Subunit Organization                                        | GO:0071825    | 4.88E−06         | 5.312                |
| Cholesterol Efflux                                                                | GO:0033344    | 1.47E−05         | 4.832                |
| Terpenoid Metabolic Process                                                       | GO:0006721    | 1.85E−05         | 4.733                |
| Very-Low-Density Lipoprotein Particle Remodeling                                  | GO:0034372    | 1.97E−05         | 4.706                |
| Platelet Degranulation                                                            | GO:0002576    | 2.31E−05         | 4.636                |
| Sterol Transport                                                                  | GO:0015918    | 2.44E−05         | 4.612                |
| Phospholipid Efflux                                                               | GO:0003370    | 2.84E−05         | 4.547                |
| Isoprenoid Metabolic Process                                                      | GO:0006720    | 5.53E−05         | 4.257                |
| Positive Regulation of Substrate Adhesion-Dependent Cell Spreading                | GO:1900026    | 6.58E−05         | 4.182                |
| High-Density Lipoprotein Particle Assembly                                        | GO:0034380    | 7.18E−05         | 4.144                |
| Cell-Cell Adhesion                                                                | GO:0098609    | 8.60E−05         | 4.066                |
| High-Density Lipoprotein Particle Clearance                                       | GO:0034384    | 0.000120368      | 3.919                |
| Cholesterol Homeostasis                                                           | GO:0042632    | 0.000156831      | 3.805                |
| Post-Translational Protein Modification                                           | GO:0043687    | 0.000163138      | 3.787                |
| Sterol Homeostasis                                                                | GO:0055092    | 0.000166565      | 3.778                |
| Retinoid Metabolic Process                                                        | GO:0001523    | 0.00024961       | 3.603                |
| Regulation of Plasma Lipoprotein Particle Levels                                  | GO:0097006    | 0.000263855      | 3.579                |
| Regulation of Substrate Adhesion-Dependent Cell Spreading                         | GO:1900024    | 0.000344919      | 3.462                |
| Diterpenoid Metabolic Process                                                     | GO:0016101    | 0.000345502      | 3.462                |
| Cholesterol Transport                                                             | GO:0030301    | 0.000383496      | 3.416                |
| Heterotypic Cell-Cell Adhesion                                                    | GO:0034113    | 0.000409755      | 3.387                |
| Cholesterol Biosynthetic Process                                                  | GO:0006695    | 0.000568257      | 3.245                |
| Secondary Alcohol Biosynthetic Process                                            | GO:1902653    | 0.000568257      | 3.245                |
| Regulation of Heterotypic Cell-Cell Adhesion                                      | GO:0034114    | 0.000580431      | 3.236                |
| Regulation of Cdc42 Protein Signal Transduction                                   | GO:0032489    | 0.000667476      | 3.176                |
| Sterol Biosynthetic Process                                                       | GO:0016126    | 0.000893117      | 3.049                |
| Plasma Lipoprotein Particle Clearance                                             | GO:0034381    | 0.000959187      | 3.018                |
| Lipoprotein Metabolic Process                                                     | GO:0042157    | 0.001034387      | 2.985                |
| Chylomicron Remnant Clearance                                                     | GO:0034382    | 0.001066283      | 2.972                |
| Triglyceride-Rich Lipoprotein Particle Clearance                                  | GO:0071830    | 0.001066283      | 2.972                |
| Steroid Metabolic Process                                                         | GO:0008202    | 0.00122924       | 2.910                |
| Cholesterol Metabolic Process                                                     | GO:0008203    | 0.001569372      | 2.804                |
| Positive Regulation of Cholesterol Esterification                                | GO:0010873    | 0.001596909      | 2.797                |
| Regulated Exocytosis                                                              | GO:0045055    | 0.00171245       | 2.766                |
| Positive Regulation of Cell Morphogenesis Involved in Differentiation            | GO:0010770    | 0.00174483       | 2.758                |
| Very-Low-Density Lipoprotein Particle Clearance                                   | GO:0034447    | 0.002277712      | 2.643                |
| Secondary Alcohol Metabolic Process                                               | GO:1902652    | 0.002312476      | 2.636                |
| Homotypic Cell-Cell Adhesion                                                     | GO:0034109    | 0.002650901      | 2.577                |
| Triglyceride Catabolic Process                                                    | GO:0019433    | 0.002810211      | 2.551                |
| Sterol Metabolic Process                                                         | GO:0016125    | 0.002987054      | 2.525                |
| Biological process                                           | GO:BP Term_ID | Adjusted p value | Neg Log10 adjusted p |
|--------------------------------------------------------------|---------------|------------------|---------------------|
| Acylglycerol Homeostasis                                     | GO:0055090    | 0.003467959      | 2.460               |
| Triglyceride Homeostasis                                     | GO:0070328    | 0.003467959      | 2.460               |
| Lipid Homeostasis                                            | GO:0055088    | 0.003686054      | 2.433               |
| Vesicle-Mediated Transport                                   | GO:0016192    | 0.003686287      | 2.433               |
| Regulation of Triglyceride Metabolic Process                 | GO:0090207    | 0.004233532      | 2.373               |
| Regulation of Cell Morphogenesis Involved in Differentiation | GO:0010769    | 0.004547582      | 2.342               |
| Secretion                                                   | GO:0046903    | 0.004648136      | 2.333               |
| Cell Adhesion                                                | GO:0007155    | 0.00503037       | 2.298               |
| Biological Adhesion                                          | GO:0022610    | 0.005314483      | 2.275               |
| Organic Hydroxy Compound Transport                           | GO:0015850    | 0.005357541      | 2.271               |
| Intermembrane Lipid Transfer                                 | GO:0120009    | 0.006131955      | 2.212               |
| Exocytosis                                                   | GO:0006587    | 0.006499796      | 2.187               |
| Steroid Biosynthetic Process                                 | GO:0006694    | 0.006623642      | 2.179               |
| Cdc42 Protein Signal Transduction                            | GO:0032488    | 0.006865718      | 2.163               |
| Regulation of Cholesterol Sстерification                    | GO:0010872    | 0.006865718      | 2.163               |
| Regulation of Triglyceride Catabolic Process                 | GO:0046464    | 0.007287829      | 2.137               |
| Acylglycerol Catabolic Process                               | GO:0046461    | 0.007287829      | 2.137               |
| Neutral Lipid Catabolic Process                              | GO:0046461    | 0.007287829      | 2.137               |
| Substrate Adhesion-Dependent Cell Spreading                  | GO:0034446    | 0.008081591      | 2.093               |
| Negative Regulation of Plasma Lipoprotein Oxidation          | GO:0034445    | 0.008917017      | 2.050               |
| Regulation of Plasma Lipoprotein Oxidation                   | GO:0034444    | 0.008917017      | 2.050               |
| Secretion by Cell                                           | GO:0032940    | 0.009039373      | 2.044               |
| Triglyceride Metabolic Process                               | GO:0006641    | 0.00964557       | 2.016               |
| Positive Regulation of Cell Adhesion                         | GO:0045785    | 0.009890818      | 2.005               |
| Regulation of Cell Morphogenesis                             | GO:0026204    | 0.01018014       | 1.992               |
| Positive Regulation of Heterotypic Cell-Cell Adhesion        | GO:0034116    | 0.010529465      | 1.978               |
| Regulation of Cell-Cell Adhesion                             | GO:0022407    | 0.011281044      | 1.948               |
| Negative Regulation of Blood Coagulation                     | GO:0030195    | 0.01262872       | 1.899               |
| Negative Regulation of Hemostasis                           | GO:0190047    | 0.013577968      | 1.867               |
| Export from Cell                                             | GO:0140352    | 0.014773845      | 1.831               |
| Cholesterol Esterification                                   | GO:0034435    | 0.015294749      | 1.815               |
| Steroid Esterification                                       | GO:0034433    | 0.015294749      | 1.815               |
| Sterol Esterification                                        | GO:0034434    | 0.015294749      | 1.815               |
| Positive Regulation of Cell-Substrate Adhesion               | GO:0010811    | 0.01579924       | 1.801               |
| Negative Regulation of Coagulation                           | GO:0050819    | 0.019135052      | 1.718               |
| Lipid Catabolic Process                                      | GO:0016042    | 0.020745205      | 1.683               |
| Platelet Aggregation                                         | GO:0070527    | 0.023183729      | 1.635               |
| Plasma Lipoprotein Particle Oxidation                        | GO:0034441    | 0.026712669      | 1.573               |
| Acylglycerol Metabolic Process                               | GO:0006639    | 0.028359777      | 1.547               |
| Neutral Lipid Metabolic Process                              | GO:0006638    | 0.029346144      | 1.532               |
| Supramolecular Fiber Organization                            | GO:0097435    | 0.029625         | 1.528               |
| Cell Activation                                              | GO:0001775    | 0.029657104      | 1.528               |
| Macromolecule Localization                                   | GO:0033036    | 0.029741556      | 1.527               |
| Transport                                                   | GO:0006810    | 0.030983148      | 1.509               |
| Organic Hydroxy Compound Biosynthetic Process                | GO:1901617    | 0.031015934      | 1.508               |
| Regulation of Blood Coagulation                              | GO:0030193    | 0.033129732      | 1.480               |
| Alcohol Biosynthetic Process                                 | GO:0046165    | 0.035839906      | 1.446               |
| Regulation of Hemostasis                                     | GO:1900046    | 0.037051615      | 1.431               |
| Plasminogen Activation                                       | GO:0031639    | 0.037580747      | 1.425               |
| Regulation of Lipoprotein Lipase Activity                    | GO:0051004    | 0.037580747      | 1.425               |
| Regulation of Localization                                  | GO:0032879    | 0.04005842       | 1.397               |
growth-associated factor GAP43 have also been observed across multiple brain regions and independent studies that have evaluated postmortem Scz patient tissue [57–61]. When combined, these data support the idea [1, 28] that a loss of factors which support neuronal development yields an upstream depletion of neurons within Scz patient-derived organoids [1, 28].

**Table 4 continued**

| Biological process                                                | GO:BP Term_ID | Adjusted p-value | Neg Log10 adjusted p |
|-------------------------------------------------------------------|---------------|------------------|----------------------|
| Glycerolipid Catabolic Process                                    | GO:0046503    | 0.041304441      | 1.384                |
| Vascular Process in Circulatory System                           | GO:0003018    | 0.041522396      | 1.382                |
| Regulation of Vesicle-Mediated Transport                         | GO:0060627    | 0.04457903       | 1.351                |
| Regulation of Cholesterol Transport                              | GO:0032374    | 0.045905316      | 1.338                |
| Regulation of Sterol Transport                                    | GO:0032371    | 0.045905316      | 1.338                |
| Fibrinolysis                                                      | GO:0042730    | 0.048124122      | 1.318                |
| Regulation of Coagulation                                         | GO:0050818    | 0.048341707      | 1.316                |

**Table 5.** Down-regulated GO molecular functions in Scz organoids (p < 0.05).

| Molecular function                                                | GO:MF Term_ID | Adjusted p-value | Neg Log10 adjusted p |
|-------------------------------------------------------------------|---------------|------------------|----------------------|
| Structural Constituent of Cytoskeleton                            | GO:0005200    | 0.000173652      | 3.760                |
| Cytoskeletal Protein Binding                                      | GO:0008092    | 0.005488124      | 2.261                |
| GTPase Activity                                                   | GO:0003924    | 0.007451524      | 2.128                |
| Nucleoside-Triphosphatase Activity                               | GO:0017111    | 0.008195516      | 2.086                |
| Pyrophosphatase Activity                                          | GO:0016462    | 0.020438022      | 1.690                |
| Hydrolyase Activity, Acting on Acid Anhydrides, in Phosphorus-Containing Anhydrides | GO:0016818 | 0.023962071      | 1.620                |
| Hydrolyase Activity, Acting on Acid Anhydrides                    | GO:0016817    | 0.024306171      | 1.614                |
| Tubulin Binding                                                   | GO:0015631    | 0.034081605      | 1.467                |
| GTP Binding                                                       | GO:0005525    | 0.034081605      | 1.467                |
| Microtubule Binding                                               | GO:0008017    | 0.043893561      | 1.358                |
| Structural Molecule Activity                                     | GO:0005198    | 0.047624863      | 1.322                |
| Guanyl Ribonucleotide Binding                                     | GO:0032561    | 0.047641356      | 1.322                |
| Guanyl Nucleotide Binding                                         | GO:0019001    | 0.047641356      | 1.322                |

**Table 6.** Up-regulated GO molecular functions in Scz organoids (p < 0.05).

| Molecular function                                                | GO:MF Term_ID | Adjusted p-value | Neg Log10 adjusted p |
|-------------------------------------------------------------------|---------------|------------------|----------------------|
| Sterol Transporter Activity                                       | GO:0015248    | 4.01E−06         | 5.397                |
| Cadherin Binding Involved in Cell-Cell Adhesion                  | GO:0098641    | 2.28E−05         | 4.642                |
| Cell-Cell Adhesion Mediator Activity                             | GO:0098632    | 2.68E−05         | 4.571                |
| Cholesterol Transfer Activity                                    | GO:0120020    | 5.42E−05         | 4.266                |
| Cell Adhesion Mediator Activity                                  | GO:0098631    | 6.36E−05         | 4.197                |
| Sterol Transfer Activity                                          | GO:0120015    | 6.35E−05         | 4.184                |
| Phosphatidylcholine-Sterol O-Acyltransferase Activator Activity  | GO:0060228    | 7.46E−05         | 4.127                |
| Cell Adhesion Molecule Binding                                   | GO:0050839    | 7.67E−05         | 4.115                |
| Lipoprotein Particle Receptor Binding                            | GO:0070325    | 0.000150248      | 3.823                |
| Lipid Transporter Activity                                       | GO:0005319    | 0.000343266      | 3.464                |
| Lipid Transfer Activity                                           | GO:0120013    | 0.001163791      | 2.934                |
| Sterol Binding                                                    | GO:0032934    | 0.003401382      | 2.468                |
| High-Density Lipoprotein Particle Receptor Binding                | GO:0070653    | 0.005374955      | 2.270                |
| Steroid Binding                                                   | GO:0005496    | 0.025672825      | 1.591                |
| Signaling Receptor Binding                                        | GO:0005102    | 0.031882878      | 1.496                |

Regulation of novel GWAS factors (PTN & PODXL) in Scz organoids
The other major phenotype identified in our molecular mapping of Scz cerebral organoids was the differential expression of two novel GWAS factors, namely PTN and PODXL. This analysis comprised us cross-referencing the highest-confident GWAS factors.
| Reactome pathway                                                                 | Reactome Term_ID        | Adjusted p-value | Neg Log10 adjusted p |
|---------------------------------------------------------------------------------|------------------------|------------------|---------------------|
| L1CAM Interactions                                                             | REAC-R-HSA-373760      | 4.04E−07         | 6.393               |
| Microtubule-Dependent Trafficking of Connexons from Golgi to the Plasma Membrane | REAC-R-HSA-190840      | 7.37E−07         | 6.133               |
| Transport of Connexons to the Plasma Membrane                                  | REAC-R-HSA-190872      | 9.65E−07         | 6.015               |
| Recycling Pathway of L1                                                          | REAC-R-HSA-437239      | 1.35E−06         | 5.869               |
| Post-Chaperonin Tubulin Folding Pathway                                         | REAC-R-HSA-389977      | 2.00E−06         | 5.698               |
| COPI-Independent Golgi-to-ER Retrograde Traffic                                 | REAC-R-HSA-6811436     | 2.51E−06         | 5.601               |
| Formation of Tubulin Folding Intermediates by CCT/TriC                         | REAC-R-HSA-389960      | 3.09E−06         | 5.510               |
| Activation of AMPK Downstream of NMDARs                                         | REAC-R-HSA-9619483     | 4.59E−06         | 5.338               |
| Prefoldin Mediated Transfer of Substrate to CCT/TriC                            | REAC-R-HSA-389957      | 4.59E−06         | 5.338               |
| Sealing of the Nuclear Envelope (NE) by ESCRT-III                              | REAC-R-HSA-9668328     | 9.34E−06         | 5.030               |
| RHO GTPases Activate IQGAPs                                                     | REAC-R-HSA-5626467     | 9.34E−06         | 5.030               |
| Cooperation of Prefoldin and TriC/CCT in Actin and Tubulin Folding             | REAC-R-HSA-389958      | 1.10E−05         | 4.959               |
| Gap Junction Assembly                                                           | REAC-R-HSA-190861      | 2.30E−05         | 4.638               |
| HCMV Early Events                                                               | REAC-R-HSA-9609690     | 3.03E−05         | 4.518               |
| Assembly and Cell Surface Presentation of NMDA Receptors                        | REAC-R-HSA-9609736     | 4.36E−05         | 4.360               |
| Aggrephagy                                                                      | REAC-R-HSA-9646399     | 4.91E−05         | 4.309               |
| Carboxyterminal Post-Translational Modifications of Tubulin                    | REAC-R-HSA-8955332     | 6.18E−05         | 4.209               |
| Gap Junction Trafficking                                                        | REAC-R-HSA-190828      | 8.54E−05         | 4.069               |
| HCMV Infection                                                                 | REAC-R-HSA-9609646     | 9.31E−05         | 4.031               |
| Gap Junction Trafficking and Regulation                                         | REAC-R-HSA-157858      | 9.47E−05         | 4.024               |
| Intraflagellar Transport                                                        | REAC-R-HSA-5620924     | 0.000140206      | 3.853               |
| HSP90 Chaperone Cycle for Steroid Hormone Receptors (SHR)                       | REAC-R-HSA-3371497     | 0.000184495      | 3.734               |
| Kinesins                                                                        | REAC-R-HSA-983189      | 0.000259999      | 3.585               |
| Nuclear Envelope (NE) Reassembly                                                | REAC-R-HSA-2995410     | 0.000519297      | 3.285               |
| Translocation of SLC2A4 (GLUT4) to the Plasma Membrane                          | REAC-R-HSA-1445148     | 0.000597876      | 3.223               |
| Golgi-to-ER Retrograde Transport                                                | REAC-R-HSA-8856688     | 0.000664661      | 3.177               |
| Axon Guidance                                                                   | REAC-R-HSA-422475      | 0.00067359       | 3.172               |
| Post NMDA Receptor Activation Events                                            | REAC-R-HSA-438064      | 0.000783019      | 3.106               |
| The Role of GTSE1 in G2/M Progression after G2 Checkpoint                       | REAC-R-HSA-8852276     | 0.000949362      | 3.023               |
| Nervous System Development                                                      | REAC-R-HSA-9675108     | 0.001007002      | 2.997               |
| Selective Autophagy                                                            | REAC-R-HSA-9663891     | 0.001010583      | 2.995               |
| Activation of NMDA Receptors and Postsynaptic Events                           | REAC-R-HSA-442755      | 0.00171304       | 2.766               |
| Recruitment of NuMA to Mitotic Centrosomes                                      | REAC-R-HSA-380320      | 0.002242316      | 2.649               |
| Chaperonin-Mediated Protein Folding                                            | REAC-R-HSA-390466      | 0.002362049      | 2.627               |
| Factors Involved in Megakaryocyte Development and Platelet Production           | REAC-R-HSA-983231      | 0.002639065      | 2.579               |
| COPI-Dependent Golgi-to-ER Retrograde Traffic                                   | REAC-R-HSA-6811434     | 0.00307982       | 2.517               |
| COPI-Mediated Anterograde Transport                                            | REAC-R-HSA-6807878     | 0.00347233       | 2.475               |
| Protein Folding                                                                 | REAC-R-HSA-391251      | 0.00347233       | 2.475               |
| CRMPs in Sema3A Signaling                                                       | REAC-R-HSA-399956      | 0.003988868      | 2.399               |
| Hedgehog 'off' State                                                           | REAC-R-HSA-5610787     | 0.005515212      | 2.258               |
| Neurotransmitter Receptors and Postsynaptic Signal Transmission                 | REAC-R-HSA-112314      | 0.005949918      | 2.225               |
| EML4 and NUDC in Mitotic Spindle Formation                                      | REAC-R-HSA-9648025     | 0.00605753       | 2.221               |
| Cilium Assembly                                                                 | REAC-R-HSA-5617833     | 0.006863941      | 2.163               |
| Intra-Golgi and Retrograde Golgi-to-ER traffic                                  | REAC-R-HSA-6811442     | 0.007259898      | 2.139               |
| Resolution of Sister Chromatid Cohesion                                          | REAC-R-HSA-2500257     | 0.008317829      | 2.080               |
| MHC Class II Antigen Presentation                                               | REAC-R-HSA-2132295     | 0.008317829      | 2.080               |
| RHO GTPase Effectors                                                           | REAC-R-HSA-195258      | 0.0094632        | 2.024               |
| Developmental Biology                                                           | REAC-R-HSA-1266738     | 0.011666055      | 1.933               |
| Macropathphy                                                                    | REAC-R-HSA-1632852     | 0.012565891      | 1.901               |
| RHO GTPases Activate Formins                                                    | REAC-R-HSA-5663220     | 0.013491876      | 1.870               |
| Signaling by Hedgehog                                                          | REAC-R-HSA-5358351     | 0.02087328       | 1.680               |
factors identified in unbiased clinical samples (see [33]) with our complete list of differentially expressed proteins. In our prior report utilizing a smaller TMT-LC/MS cohort design [1], we identified the differential expression of four GWAS candidates in Scz cerebral organoids at the protein level (PTN, COMT, PLCL1, and PODXL). Of these candidates, we were able to detect and replicate the differential expression of two of these factors in our much larger sample of \( n = 25 \) reported here. This specifically comprised alterations in PTN (down-regulated) and PODXL (up-regulated). These factors represent high-confidence GWAS factors associated with Scz, but otherwise have relatively unknown disease relevance. PTN has also been reported to be depleted in neural

| Reactome pathway                                      | Reactome Term_ID | Adjusted p-value | Neg Log10 adjusted p |
|-------------------------------------------------------|-----------------|------------------|----------------------|
| Autophagy                                             | REAC:R-HSA-9612973 | 0.02087328       | 1.680                |
| ER to Golgi Anterograde Transport                    | REAC:R-HSA-199977 | 0.025184333      | 1.599                |
| Transmission across Chemical Synapses                | REAC:R-HSA-112315 | 0.028395357      | 1.547                |
| M Phase                                               | REAC:R-HSA-68886 | 0.044247896      | 1.354                |

| Reactome pathway                                      | Reactome Term_ID | Adjusted p-value | Neg Log10 adjusted p |
|-------------------------------------------------------|-----------------|------------------|----------------------|
| Post-Translational Protein Phosphorylation            | REAC:R-HSA-8957275 | 8.31E-09         | 8.080                |
| Chylomicron Assembly                                  | REAC:R-HSA-8963888 | 1.82E-08         | 7.739                |
| Chylomicron Remodeling                                | REAC:R-HSA-8963901 | 1.82E-08         | 7.739                |
| Regulation of Insulin-like Growth Factor (IGF) Transport and Uptake by Insulin-like Growth Factor Binding Proteins (IGFBPs) | REAC:R-HSA-381426 | 3.18E-08         | 7.498                |
| Plasma Lipoprotein Assembly                           | REAC:R-HSA-8963898 | 6.10E-07         | 6.215                |
| Retinoid Metabolism and Transport                     | REAC:R-HSA-975634 | 1.25E-06         | 5.902                |
| Metabolism of Fat-Soluble Vitamins                    | REAC:R-HSA-6806667 | 2.16E-06         | 5.665                |
| Plasma Lipoprotein Remodeling                         | REAC:R-HSA-8963899 | 9.87E-06         | 5.006                |
| Platelet Degranulation                                | REAC:R-HSA-114608 | 3.04E-05         | 4.517                |
| Response to Elevated Platelet Cytosolic Ca2+          | REAC:R-HSA-76005 | 3.98E-05         | 4.400                |
| Regulation of TLR by Endogenous Ligand                | REAC:R-HSA-5686938 | 0.000100533       | 3.998                |
| Visual Phototransduction                              | REAC:R-HSA-2187338 | 0.000156361       | 3.806                |
| Metabolism of Vitamins and Cofactors                  | REAC:R-HSA-196854 | 0.000455116       | 3.342                |
| Plasma Lipoprotein Assembly, Remodeling, and Clearance| REAC:R-HSA-174824 | 0.000616778       | 3.210                |
| HDL remodeling                                         | REAC:R-HSA-8964058 | 0.000786847       | 3.104                |
| Hemostasis                                            | REAC:R-HSA-109582 | 0.001130796       | 2.947                |
| GRB2:SOS Provides Linkage to MAPK Signaling for Integrins | REAC:R-HSA-354194 | 0.003373717       | 2.472                |
| Platelet Activation, Signaling and Aggregation         | REAC:R-HSA-76002 | 0.003972412       | 2.401                |
| p130Cas Linkage to MAPK Signaling for Integrins       | REAC:R-HSA-372708 | 0.004208218       | 2.376                |
| Scavenging by Class A Receptors                       | REAC:R-HSA-3000480 | 0.008886469       | 2.051                |
| Common Pathway of Fibrin Clot Formation               | REAC:R-HSA-140875 | 0.014033493       | 1.853                |
| Integrin Signaling                                    | REAC:R-HSA-354192 | 0.023493017       | 1.629                |
| Chylomicron Clearance                                 | REAC:R-HSA-8964026 | 0.032311883       | 1.491                |
| Scavenging by Class B Receptors                       | REAC:R-HSA-3000471 | 0.032311883       | 1.491                |
| Integrin Cell Surface Interactions                    | REAC:R-HSA-216083 | 0.043417684       | 1.362                |
| Plasma Lipoprotein Clearance                          | REAC:R-HSA-8964043 | 0.044251662       | 1.354                |
progenitors and shown to regulate both neurogenesis and survival phenotypes in Szc cerebral organoids [1], providing the first functional molecular data related to this candidate within the Scz literature. Other groups have also recently identified that PTN secreted from neural stem cells supports the maturation of newborn neurons [62], and can function as a neurotrophic growth factor in vivo to modulate neuronal loss [63] and long-term potentiation induction [64]; PTN has also since been implicated in a novel amphetamine-model of relevance to Scz [65], a recent computational protein-network analysis underlying Scz [66], as well as at least one nascent Scz gene-association study (n = 1,823 humans) [67]. On the other hand, little work has been completed on the role of PODXL in Scz, probably because PODXL is a renal-enriched factor most often associated with kidney podocytes and mesothelial cells [68]. Of note, PODXL has recently been shown to play a role in neurite outgrowth, branching, axonal fascilitation, and synapse number [69], supporting a potential role for this factor in synaptic plasticity. Additionally, PODXL was recently shown to be an apical determinant that may alter lumen size of neural progenitor cell rosettes during morphogenesis [70]. Thus, PODXL may be a fruitful target for future investigations seeking to deconvolute the role of novel S Zw GAS factors within the developing brain.

Other novel differentially expressed candidates in Scz organoids
Lastly, it is worth emphasizing several other differentially expressed molecular candidates observed in Scz cerebral organoids hold biological interest. First and foremost, we identified that Carbboxypeptidase E (CPE) was downregulated in Scz cerebral organoids. CPE is a prohormone-processing enzyme [71] and regulated secretory pathway receptor [72], possibly best known for regulating the sorting and activity-dependent secretion of BDNF [73, 74] as well as TrkB surface insertion [75] in neurons. However, CPE was recently suggested to also function as a growth factor independently of its enzymatic and sorting activities [76]. Indeed, amongst other reports suggesting a role in neuroprotection [77], it has recently been shown that CPE regulates cortical neuron migration and dendritic morphology [78]. However, the degree to which these effects is dependent upon its cargo, which includes other growth factors (e.g. BDNF), remains unclear. Lastly, the other notable differentially expressed candidates worthy of discussion comprised alterations within the apolipoprotein family, specifically APOM, APOA1, APOE, AP0C3, and APOB. Apolipoproteins have been previously investigated as potential metabolic-related biomarkers [79] in peripherally accessible biological fluids (e.g. CSF [80] or plasma [81]). This specifically includes alterations in AP0E and APOA1 in Scz patients [82]. These findings are broadly related to cholesterol [83], fatty acid [84], phospholipid metabolism [85], as well as other membrane-related [86] hypotheses of Scz (which are all somewhat related and/or derived from similar evidence pools). Nonetheless, it is interesting that evidence related to these hypotheses was detectable and reproducible across our sample of patients, and may indicate that further work on potential metabolic factors may also be a further avenue of fruitful research.

3D cerebral organoid tissue generation
We adapted the same undirected-differentiation organoid system that we used in our previous, more extensive, analysis of neurodevelopmental mechanisms [1], which had been previously published by Lancaster et al. in Nature [17] and Nature Protocols [87]. Briefly, 2D iPSC colonies were dissociated and cultured into 3D embryoid bodies in ultra-low attachment plates (Corning; CAT#: 3474). Rock inhibitor (1:100; Stem Cell Tech, CAT#: 72304) and basic fibroblast growth factor (Pepro Tech, CAT#: 100-188) are included in media for the first 2-4 days of embryoid body culturing to promote stem cell aggregation and survival. Following this, healthy embryoid bodies are isolated and transferred to Nunclon Sphera 24 well plates (Thermo Scientific, CAT#: 174930) for neural fate specification, using neural induction media. Successful early ‘organoids’ were embedded in a 30 µl Matrigel (Corning, CAT#: 354234) spheroid-droplet and polymerized at 37 °C for 20-30 min which provided a matrix for subsequent neural induction. Successful early ‘organoids’ were embedded in a 30 µl Matrigel (Corning, CAT#: 354234) spheroid-droplet and polymerized at 37 °C for 20-30 min which provided a matrix for subsequent neural induction. Successful early ‘organoids’ were embedded in a 30 µl Matrigel (Corning, CAT#: 354234) spheroid-droplet and polymerized at 37 °C for 20-30 min which provided a matrix for subsequent neural induction.

Proteomics sample preparation, TMT labeling and LC/MS
Isobaric stable isotope labeling was achieved viaTandem Mass Tag pro (TMTpro) chemistry and Liquid-Chromatography/Mass-Spectrometry (LC/ MS) proteomics as previously described [1, 11, 65]. Briefly, intact organoids were reduced with dithiothreitol and underwent alkylation with iodoacetamide before tryptic digestion at 37 °C overnight. For barcoding chemistry, we employed TMTpro 16-plex labeling according to the manufacturer’s instructions (Thermo Fisher Scientific, CAT#: A44521). Each multi-plex experiment contained relevant organoid samples with an additional pooled isobaric reference label made up of the same peptide digest from the pooled mix of organoids (for data normalization between runs; TMT Tag 134 N for both TMT-LC/MS runs). A list of sample labeling strategies and replicates is available in the PRIDE proteomics exchange repository. TMT-labeled peptides were desalted using C18 stage-tips prior to LC-MS analysis. An EASY-nLC 1200, which was coupled to a Fusion Lumos mass spectrometer, (Thermo Fisher Scientific) was utilized in positive, data-dependent acquisition mode, with samples analyzed in technical duplicate. Buffer A (0.1% FA in water) and buffer B (0.1% FA in 80% ACN) were used as mobile phases for gradient separation. TMT-labeled peptides were analyzed on a 75 µm I.D. column (ReproSil-Pur C18-AQ, 3µm, Dr. Maisch GmbH, Germany) was packed in-house. A separation gradient of 5–10% buffer B over 1 min, 10%-35% buffer B over 229 min, and 35%-100% B over 5 min at a flow rate of 300 nL/min was adapted. An Orbitrap mass analyzer acquired Full MS scans over a range of 350-1500 m/z with resolution 120,000 at m/z 200. The top 20 most-abundant precursors were selected with an isolation
window of 0.7 Thomsons and fragmented by high-energy collisional dissociation with normalized collision energy of 40. The Orbitrap mass analyzer was also used to acquire MS/MS scans. The automatic gain control target value was 166 for full scans and 54e4 for MS/MS scans respectively, and the maximum ion injection time was 54 ms for both.

**Data processing and bioinformatics pipeline for quantitative analysis**

Mass spectra were pre-processed as described [1, 11, 65] and processed using MaxQuant [88] (1.5.5.1). Spectra were searched against the full set of human protein sequences annotated in UniProt (sequence database Sep-2017) using Andromeda. Data was searched as described [1, 11] as a separate and single (combined) batches, with fixed modification, cysteine carbamidomethylation and variable modifications, N-acetylation and methionine oxidation. Searches were performed using a 20 ppm precursor ion tolerance for total protein level analysis. Further modifications included TMT tags on peptide N termini/lysine residues (+229.16293 Da) set as static modifications. Data was processed using trypsin/P as the proteolytic enzyme with up to 2 missed cleavage sites allowed. Peptides less than seven amino acids were not considered for further analysis because of lack of uniqueness, and a 1% False-Discovery Rate (FDR) was used to filter at peptide and protein levels. Protein identification required at least two unique or razor peptides per protein group. Contaminants, and reverse identification were excluded from further data analysis. Quantification was performed with the reporter ion quantification normalization in MaxQuant. Protein intensities were log2 transformed using Perseus [89] (1.x:10). The violin plots of log2 transformed protein intensity distribution and the boxplot of coefficient of variations per sample group were visualized using R package ggplot2. Proteins quantified in at least 70% of samples in a minimum of one sample group were subjected to downstream visualization (principal component analysis, volcano plot) and statistical analysis using Perseus. For principal component analysis, missing values were imputed from normal distribution (downshift 1.8, width 0.3) using Perseus. For differential expression analysis proteins were subjected to Welch t-test; p-value < 0.05 and |log2FC| >0.5 visualized in volcano plot and subjected to downstream functional enrichment analysis using gProfiler, including Gene Ontology, KEGG and Reactome databases [as described, [90, 91]].

**DATA AVAILABILITY**

The MS proteomics raw data and MaxQuant search parameters have been deposited to the ProteomeXchange Consortium (http://www.proteomexchange.org/) via the PRIDE partner repository [92] with the data set identifier PXD027812.

**REFERENCES**

1. Notaras, M, et al. Schizophrenia is defined by cell-specific neuropathology and multiple neurodevelopmental mechanisms in patient-derived cerebral organoids. Mol Psychiatry. 2021.
2. Kleistkörter J, Hellmich M, Steinmeyer EM, Schulze-Lutter F. Diagnosing schizophrenia in the initial prodromal phase. Arch Gen Psychiatry. 2001;58:158–164.
3. Comblat B, Lencz T, Smith CW, Cornell CU, Auher MM, Nakayama E. The schizophrenia prodrome revisited: a neurodevelopmental perspective. Schizophr Bull. 2003;29:633–651.
4. Brown AS, Susser ES. In utero infection and adult schizophrenia. Ment Retard Dev Disabil Res Rev. 2002;8:51–57.
5. Kunugi H, et al. Schizophrenia following in utero exposure to the 1957 influenza epidemics in Japan. Am J Psychiatry. 1995;3:450–452.
6. Takei N, Mortensen PB, Klaening U, Murray RM, Sham PC, O’Callaghan E, et al. Relationship between in utero exposure to influenza epidemics and risk of schizophrenia in Denmark. Biol Psychiatry. 1996;40:817–824.
7. Procopio M, Davies RJ, Marrriott P. The hormonal environment in utero as a potential etiological agent for schizophrenia. Eur Arch Psychiatry Neurol Sci. 2006;256:77–81.
8. McClellan JM, Susser E, King M-C. Maternal famine, de novo mutations, and schizophrenia. JAMA. 2006;296:582–584.
9. Boks MP, Houtepen LC, Xu Z, He Y, Ursini G, Majozer AX, et al. Genetic vulnerability to DUSP22 promoter hypermethylation is involved in the relation between in utero famine exposure and schizophrenia. NPJ Schizoph. 2018;4:1–8.
10. Hyman SE. The daunting polygenicity of mental illness: making a new map. Philos Trans R Soc B. Biol Sci. 2018;373:20170031.
11. Notaras, M, et al., Neurodevelopmental signatures of narcotic and neuropsychiatric risk factors in 3D human-derived forebrain organoids. Mol Psychiatry, 2021, 1–24.
36. Mehta UM, Thirhattil J, Aneelraj D, Jadhav P, Gangadhar BN, Keshavan MS. Mirror neuron dysfunction in schizophrenia and its functional implications: a systematic review. Schizophr Res. 2014;160:9–19.

37. Freedman R, Waldo M, Bickford-Winner P, Nagamoto H. Elementory neuronal dysfunctions in schizophrenia. Schizophr Res. 1991;4:233–243.

38. Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia. Arch Gen Psychiatry. 1995;52:591–597.

39. Gonzalez-Burgos G, Lewis DA. GABA neurons and the mechanisms of network oscillations: implications for understanding cortical dysfunction in schizophrenia. Schizophr Bull. 2008;34:944–961.

40. Lewis DA. GABAergic local circuit neurons and prefrontal cortical dysfunction in schizophrenia. Brain Res Rev. 2000;31:270–276.

41. Curley AA, Lewis DA. Cortical basket cell dysfunction in schizophrenia. J Physiol. 2012;590:715–724.

42. Lewis DA. Inhibitory neurons in human cortical circuits: substrate for cognitive dysfunction in schizophrenia. Curr Opin Neurol. 2014;26:22–26.

43. Mukherjee A, Carvalho F, Eliez S, Caroni P. Long-lasting rescue of network dysfunction. Proc Natl Acad Sci USA. 2002;99:330–342.

44. Freedman R, Waldo M, Bickford-Wimer P, Nagamoto H. Elementary neuronal dysfunctions in schizophrenia. Schizophr Res. 1991;4:233–243.

45. Lewis DA. Inhibitory neurons in human cortical circuits: substrate for cognitive dysfunction in schizophrenia. Curr Opin Neurol. 2014;26:22–26.

46. M. Notaras et al.
88. Cox J, Mann M. MaxQuant enables high peptide identification rates, individualized ppb-range mass accuracies and proteome-wide protein quantification. Nat Biotechnol. 2008;26:1367–1372.
89. Tyanova S, Temu T, Sinitcyn P, Carlson A, Hein MY, Geiger T, et al. The Perseus computational platform for comprehensive analysis of (prote) omics data. Nat Methods. 2016;13:731–740.
90. Carli A, Afshar-Sterle S, Rai A, Fang H, O’Keefe R, Tse J, et al. Cancer stem cell marker DCLK1 reprograms small extracellular vesicles toward migratory phenotype in gastric cancer cells. Proteomics. 2021;21:2000098.
91. Kompa AR, Greening DW, Kong AM, McMillan PJ, Fang H, Saxena R, et al. Sustained subcutaneous delivery of secretome of human cardiac stem cells promotes cardiac repair following myocardial infarction. Cardiovasc Res. 2021;117:918–929.
92. Perez-Riverol Y, Ciordas A, Bai J, Bernal-Llinares M, Hewapathirana S, Kundu DJ, et al. The PRIDE database and related tools and resources in 2019: improving support for quantification data. Nuc Acids Res. 2019;47:D442–D450.
93. Lin Y-L, Persaud SD, Nhieu J, Wei LN. Cellular retinoic acid–binding protein 1 modulates stem cell proliferation to affect learning and memory in male mice. Endocrinol. 2017;158:3004–3014.
94. Frese CK, Mikhaylova M, Stucchi R, Gautier V, Liu Q, Mohammed S, et al. Quantitative map of proteome dynamics during neuronal differentiation. Cell Rep. 2017;18:1527–1542.
95. Upadhyay A, Hosseinibarkooie S, Schneider S, Kaczmarek A, Torres-Benito L, Mendoza-Ferreira N, et al. Neurocalcin delta knockout impairs adult neurogenesis whereas half reduction is not pathological. Front Mol Neurosci. 2019;12:1–15.

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AUTHOR CONTRIBUTIONS
MN and DC conceived the project and designed experiments. MN generated all 3D tissue from human stem cells, supervised biological interrogation of datasets, and wrote the manuscript. Our technician, AL, provided important logistical support by assisting with the generation and processing of 3D human-derived tissue. Lastly, HF and DG completed all computational analysis, with DG serving as the senior author overseeing bioinformatics analyses.

COMPETING INTERESTS
The authors declare no competing interests.

ADDITIONAL INFORMATION
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