

Endogenous Cell Type–Specific Disrupted in Schizophrenia 1 Interactomes Reveal Protein Networks Associated With Neurodevelopmental Disorders

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ABSTRACT

BACKGROUND: Disrupted in schizophrenia 1 (DISC1) has been implicated in a number of psychiatric diseases along with neurodevelopmental phenotypes such as the proliferation and differentiation of neural progenitor cells. While there has been significant effort directed toward understanding the function of DISC1 through the determination of its protein-protein interactions within an in vitro setting, endogenous interactions involving DISC1 within a cell type–specific setting relevant to neural development remain unclear.

METHODS: Using CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9) genome engineering technology, we inserted an endogenous 3X-FLAG tag at the C-terminus of the canonical *DISC1* gene in human induced pluripotent stem cells (iPSCs). We further differentiated these cells and used affinity purification to determine protein-protein interactions involving DISC1 in iPSC-derived neural progenitor cells and astrocytes.

RESULTS: We were able to determine 151 novel cell type–specific proteins present in DISC1 endogenous interactomes. The DISC1 interactomes can be clustered into several subcomplexes that suggest novel DISC1 cell-specific functions. In addition, the DISC1 interactome in iPSC-derived neural progenitor cells associates in a connected network containing proteins found to harbor de novo mutations in patients affected by schizophrenia and contains a subset of novel interactions that are known to harbor syndromic mutations in neurodevelopmental disorders.

CONCLUSIONS: Endogenous DISC1 interactomes within iPSC-derived human neural progenitor cells and astrocytes are able to provide context to DISC1 function in a cell type–specific setting relevant to neural development and enables the integration of psychiatric disease risk factors within a set of defined molecular functions.

Keywords: CRISPR, DISC1, Neural development, Neurodevelopmental disorders, Proteomics, Schizophrenia

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Since its discovery, disrupted in schizophrenia 1 (DISC1) has been implicated in contributing to multiple psychiatric disorders (1–11) and functional studies have shown DISC1 to play critical roles in several cell types throughout brain development (12,13). Induced pluripotent stem cells (iPSCs) with clinically relevant mutations in DISC1 have been shown to exhibit neurodevelopmental phenotypes following differentiation into human neural progenitor cells (hNPCs) such as abnormalities in proliferation and altered cell fate (14–16). The proliferation of primary NPCs has also been shown to be affected by astrocytes overexpressing a dominant negative form of DISC1 (17). DISC1 does not have an enzymatic function and has been considered a scaffold protein (18), with its function usually characterized in association with modulation of activity and/or localization of its binding partners (19–21). However, the

identification of endogenous DISC1 protein interactions has not been straightforward.

The large-scale identification of DISC1 protein interactions has been largely composed of in vitro systems such as yeast-two hybrid–based assays (22–28). These studies have identified dozens of proteins with the potential to directly interact with DISC1 in in vivo interactions. In addition, a relatively low number of interactions have also been observed in low-throughput studies using recombinant expression systems (7,19,20). However, these assays usually do not account for tissue or cell specificity and the relative stoichiometry between interacting partners. The investigation of endogenous DISC1 protein-protein interactions has been limited to low-throughput assays (29,30).

To determine DISC1 protein complexes at endogenous expression levels in cell types representing early brain

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development and to eliminate nonspecific interactions, we inserted a 3X-FLAG (31) coding sequence at the C-terminal end of the endogenous *DISC1* gene using CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9) genome engineering in human iPSCs. *DISC1*^{FLAG} iPSCs were differentiated into hNPCs and astrocytes, and endogenous *DISC1* binding partners were determined by immunoprecipitation of *DISC1*-FLAG followed by high-performance liquid chromatography coupled to tandem mass spectrometry in cell type-specific settings. We identified 163 nonredundant proteins in *DISC1* protein complexes associated with different subcellular compartments. We confirmed the involvement of *DISC1* in centrosomal dynamics (32) and messenger RNA (mRNA) transport (33) along with novel cell type-specific functions such as regulation of transcription in hNPCs and cytoskeleton processes in astrocytes. We also showed that *DISC1* is expressed at substoichiometric levels when compared with its binding partners. This indicates that *DISC1* may associate with a discrete subset of its interacting molecules involved in specific molecular functions. Finally, with *DISC1* being implicated in a variety of psychiatric disorders, we also showed a novel set of protein interactors contributing to complex brain disorders such as schizophrenia and intellectual disability (ID) in protein interaction networks identified in hNPCs, but not in astrocytes or recombinant immunoprecipitation experiments.

METHODS AND MATERIALS

Cell Culture and Neural Differentiation

The control 03231 iPSC line was generated from a lymphoblastoid cell line derived from a healthy 56-year-old man (National Institute of Neurological Disorders and Stroke repository, ND03231) as previously described (34). For gene editing, guide RNAs were cloned into pSpCas9(BB)-2A-Puro (PX459) V2.0 and the homologous recombination donor vector was constructed using the pFETCH_Donor plasmid (35,36). Following nucleofection (Lonza, Basel, Switzerland) and puromycin selection, individual colonies were genotyped to confirm insertion of the 3X-FLAG sequence in the *DISC1* gene. Predicted off-target regions were identified using CRISPOR (37), polymerase chain reaction (PCR) amplified, and Sanger sequenced. Subsequent differentiation of iPSCs was carried out as previously described, with slight modifications (38–41). Full details are available in the [Supplemental Methods](#).

Biochemistry

Immunoprecipitation, Western blotting, and mass spectrometry were carried out as previously described (42,43). All primary and secondary antibodies used along with their dilutions can be found in [Supplemental Table S16](#). Full details are available in the [Supplemental Methods](#).

Data Analysis

Mass spectrometry data analysis was carried out as previously described (42,43). The datasets generated and/or analyzed during the current study have been deposited to the PRIDE (ProteomeXchange Consortium via the Proteomics IDentifications) partner repository under the dataset

identifier PXD007103 (44). Full details for all analyses are available in the [Supplemental Methods](#).

RESULTS

Generation of *DISC1* FLAG Tag Lines

To detect *DISC1* interactions and avoid nonspecific interactions, we utilized the CRISPR/Cas9 genome engineering system to insert a 3X-FLAG sequence (31) at the C-terminal region of the canonical *DISC1* sequence in a previously characterized control iPSC line derived from a healthy 56-year-old man with no known psychiatric disorders (34) (Figure 1A, Supplemental Figure S1A). It should be noted that the particular iPSC line used in this study carries the common missense mutation of R264Q (rs3738401). However, this is the most common missense mutation in *DISC1* with an allele frequency of 29.23% (45), and recent studies have shown no link between this mutation and schizophrenia (46,47). Thus, it is highly unlikely that this common missense mutation will have an effect in the protein interactions reported. PCR-based genotyping of individual iPSC colonies was able to show the successful homozygous insertion of the 3X-FLAG tag (designated *DISC1*^{FLAG}), which was confirmed via Sanger sequencing (Figure 1B, C). The top predicted off-target sequences of the guide RNA used for gene editing were also sequenced to confirm that no undesired mutagenesis occurred within these regions (Supplemental Table S1). The newly derived *DISC1*^{FLAG} iPSC line maintained expression of pluripotency markers such as octamer-binding transcription factor 4 and stage-specific embryonic antigen 4 (Figure 1D, Supplemental Figure S1B, C).

We then differentiated the *DISC1*^{FLAG} iPSCs along with the wild-type (WT) parental iPSCs into hNPCs using a dual SMAD inhibition based differentiation scheme (38,39). The iPSC-derived NPCs were positive for the NPC markers paired box protein 6, SRY (sex determining region Y)-box 2, and nestin in both the *DISC1*^{FLAG} and WT cell lines (Figure 1E, Supplemental Figure S1D, E). Further, owing to the ability of *DISC1* to influence gliogenesis and proliferation of NPCs through astrocytes (17,48), hNPCs were then differentiated into S100 β /glial fibrillary acidic protein-positive astrocytes over a 30-day period (41) and were confirmed to be multipotent (Figure 1F, Supplemental Figure S1F–H).

To visualize *DISC1* hNPCs and astrocytes, we performed immunoprecipitation of *DISC1*-FLAG followed by immunoblotting with anti-FLAG antibodies (Figure 1G, H). Previous studies have shown over 50 different mRNA transcript splice variants of *DISC1* in the human brain (49), and numerous molecular weights of *DISC1* have been observed in Western blot assays (50). Western blot analysis shows that both cell types express a FLAG-*DISC1* isoform corresponding to the commonly reported long isoform of 98 kDa (isoform L, National Center for Biotechnology Information reference sequence NP001158009.1) (50). We also observed additional bands between 100 and 75 kDa, which may correspond to the smaller predicted molecular weight isoform (isoform a, National Center for Biotechnology Information reference sequence NP_001158012.1). Thus, the insertion of the 3X-FLAG tag labeled multiple isoforms that encompass the canonical C-terminal region of *DISC1*.

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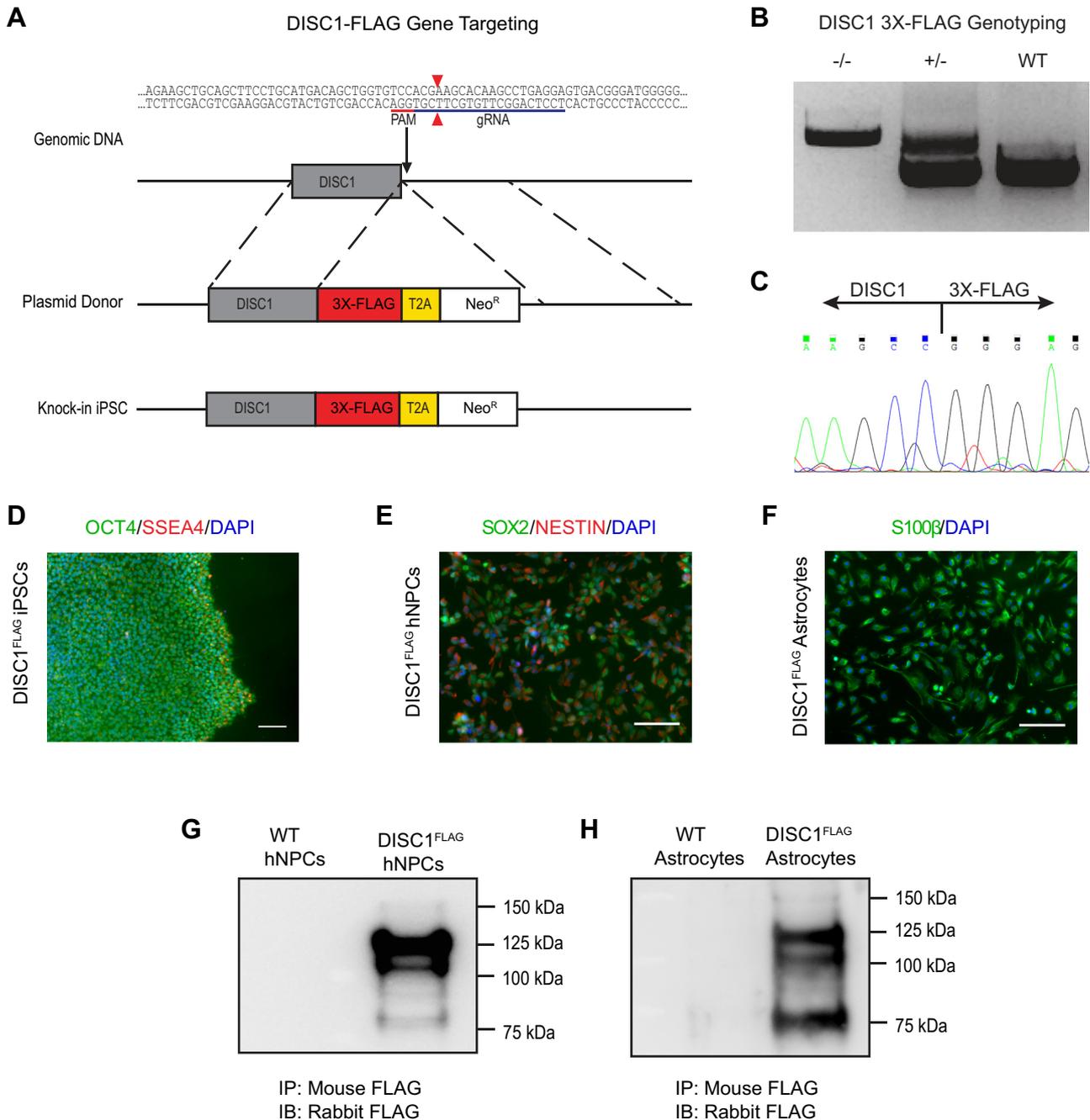


Figure 1. Generation of disrupted in schizophrenia 1 FLAG ($DISC1^{FLAG}$) cell lines. **(A)** Gene targeting scheme illustrating the insertion of a 3X-FLAG sequence at the C-terminal region of DISC1 in induced pluripotent stem cells (iPSCs) using the CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9) nuclease genome engineering system. The site of double-strand break induction is annotated by the red triangles. **(B)** Agarose gel showing polymerase chain reaction–based screening for the insertion of the 3X-FLAG tag sequence. **(C)** Sanger sequencing conformation of the 3X-FLAG tag sequence. **(D)** $DISC1^{FLAG}$ iPSCs express markers of pluripotency such as octamer-binding transcription factor 4 (OCT4) and stage-specific embryonic antigen 4 (SSEA4). **(E)** $DISC1^{FLAG}$ iPSC-derived neural progenitors express the neural progenitor markers SRY (sex determining region Y)-box 2 (SOX2) and nestin. **(F)** $DISC1^{FLAG}$ astrocytes express the astrocyte marker S100 β . **(G)** Immunoprecipitation (IP) followed by immunoblot (IB) of DISC1 using anti-FLAG antibodies in wild-type (WT) and $DISC1^{FLAG}$ human neural progenitor cells (hNPCs). **(H)** IP followed by IB of DISC1 using anti-FLAG antibodies in WT and $DISC1^{FLAG}$ astrocytes. All bands corresponding to DISC1 isoforms were absent in WT controls and include a shift of 3.92 kDa in their molecular weight due to the insertion of the 3X-FLAG sequence. Scale bars in **(D)**, **(E)**, and **(F)** = 100 μ m. gRNA, guide RNA; PAM, protospacer adjacent motif.

Incorporation of the 3X-FLAG Tag Does Not Alter Expression or Function of DISC1

Decreased expression of DISC1 has been observed in whole blood from patients affected by schizophrenia (21,51) and has been reported to modulate neural progenitor proliferation (12,13). Therefore, we wanted to verify that the insertion of the 3X-FLAG tag did not alter DISC1 expression or hNPC proliferation. Quantification of *DISC1* mRNA levels via quantitative PCR using primers that were specific to either a large majority of the reported *DISC1* isoforms (exons 2–4) or only those isoforms containing the conical C-terminal sequence (exons 12–13) (Supplemental Figure S2A) was able to show no significant difference in DISC1 expression between WT and DISC1^{FLAG} hNPC lines, regardless of the primer set used for quantitative PCR analysis (Figure 2A). In addition, quantification of bromodeoxyuridine incorporation was able to show no significant difference in proliferation between DISC1^{FLAG} and parental hNPCs (Figure 2B, C).

In addition to effects on expression and proliferation, we wanted to ensure the 3X-FLAG tag did not alter the localization of DISC1. Multiple studies have determined that DISC1 is localized to the centrosome (23,32,52,53) and the perinuclear region (53). These studies have indicated that the C-terminal region of DISC1 is essential for its subcellular localization, as the lack of the C-terminal region can disrupt localization and cause DISC1 to be diffusely spread throughout the cytoplasm (52,53). However, we found that traditional immunofluorescence methods using an anti-FLAG antibody were unable to visualize the endogenous-tagged DISC1 (Supplemental Figure S2B). This may be a result of the relatively low expression of DISC1, as it has been previously reported that DISC1 is a low-abundance protein in mouse brain (54) and we were unable to detect endogenous DISC1 in total cell lysate without the use of immunoprecipitation enrichment assays (data not shown).

Owing to its low abundance, we utilized the CRISPR/VP64-p65-Rta activation system to upregulate the expression of DISC1 in hNPCs for the sole purpose of visualizing DISC1-FLAG (55). Here, we employed four single-guide RNAs spread throughout the first 250 bp of the DISC1 promoter region (Figure 2D). With an approximate 34% transfection efficiency, this resulted in an approximate twofold increase in gene expression and allowed us to detect FLAG-DISC1 localization in hNPCs (Figure 2E, F; Supplemental Figure SC, D). Costaining with the centrosomal marker and DISC1 interactor, A-kinase anchoring protein 9 (AKAP9) (22), along with DAPI, was able to show DISC1 localization at both the centrosome and perinuclear region in hNPCs, coinciding with previous reports (23,32,56) (Figure 2F). This suggests that the 3X-FLAG tag does not impair DISC1 localization.

Identification of Endogenous DISC1 Interactomes

We co-immunopurified endogenous DISC1 and its binding partners in hNPCs using an anti-FLAG-M2 antibody (57) attached to magnetic beads and performed sequential elutions using an anti-3X-FLAG peptide, followed by a final release of all bound protein via boiling of the magnetic beads. Each step was subject to immunoblotting to measure the efficiency, yield, and specificity of DISC1 protein elution

(Figure 3A, right panel). We observed that more than 90% of DISC1 could be released within a single elution. This was followed by identification of DISC1 interactors via high-performance liquid chromatography coupled to tandem mass spectrometry.

DISC1 hNPC interactors were considered if they were identified with multiple unique peptides in duplicate assays and no peptides in WT controls (36 interactors). However, because of the low expression level of DISC1, this stringent criterion might impair the identification of true DISC1 interactors. Thus, we also considered interactors with different levels of confidence, bringing the number of interactors identified via mass spectrometry to a total of 107 interactors (Figure 3A, left panel; Supplemental Table S2). To confirm DISC1 interactions and the validity of our approach, we performed DISC1 immunoprecipitation followed by Western blot analysis from DISC1 interactors that we identified with different levels of confidence (Figure 3B). Moreover, we were also able to confirm several previously reported DISC1 protein interactors, including TRAF2 and NCK interacting kinase (NIK), phosphodiesterase 4D interacting protein, centrosomal protein 170 (CEP170), and AKAP9, bringing the total number of interactors to 112 (Figure 3A, B).

Inference of hNPC DISC1 localization through its interactome indicates that DISC1 associates with proteins in different subcellular structures such as the nucleus, centrosome, endoplasmic reticulum, and cytoplasm. Gene ontology enrichment analysis of DISC1 interactors provides further evidence for this, as terms such as “cytosol,” “ER,” and “nucleoplasm” are significantly enriched ($p < .05$ for all, Bonferroni corrected) (Figure 3E, Supplemental Table S3). This corresponds with previous reports of DISC1 at the endoplasmic reticulum and nucleus in neuronal systems (58,59).

We then identified DISC1-interacting partners in astrocytes differentiated from hNPCs. Here, we were able to identify 51 interactors that were categorized according to the various confidence levels as was completed for hNPCs (Figure 3C, Supplemental Table S4). Western blot analysis of DISC1 interactors was able to confirm a number of the identified interactors including nudE neurodevelopment protein 1 (NDE1), GRIP1-associated protein 1, and gelsolin. In addition, we were again able to confirm reported DISC1 interactors including centrosomal proteins platelet-activating factor acetylhydrolase IB subunit alpha (also known as lissencephaly-1) and NDE1 like 1, bringing the total number of interactors to 57 (Figure 3D). This indicates that a core DISC1 function, such as modulation of centrosomal dynamics, is conserved in astrocytes. The astrocyte interactome also shows an increased number of cytoskeletal proteins, which is corroborated through gene ontology enrichment analysis of DISC1 astrocyte interactors. Here, terms such as “actin filament binding,” “focal adhesion,” and “actin cytoskeleton” are significantly enriched ($p < .05$ for all, Bonferroni corrected) (Figure 3F, Supplemental Table S5), indicating that DISC1 may also play a role in astrocyte cytoskeletal organization and, therefore, astrocyte vesicle mobility and trafficking.

While the enrichment of cytoskeletal proteins may indicate a cell type-specific function or localization of DISC1 in astrocytes, poly(A) RNA binding is significantly enriched in both cell types ($p < .05$ for all, Bonferroni corrected) (Figure 3E, F; Supplemental Tables S3 and S5). A recent report characterized

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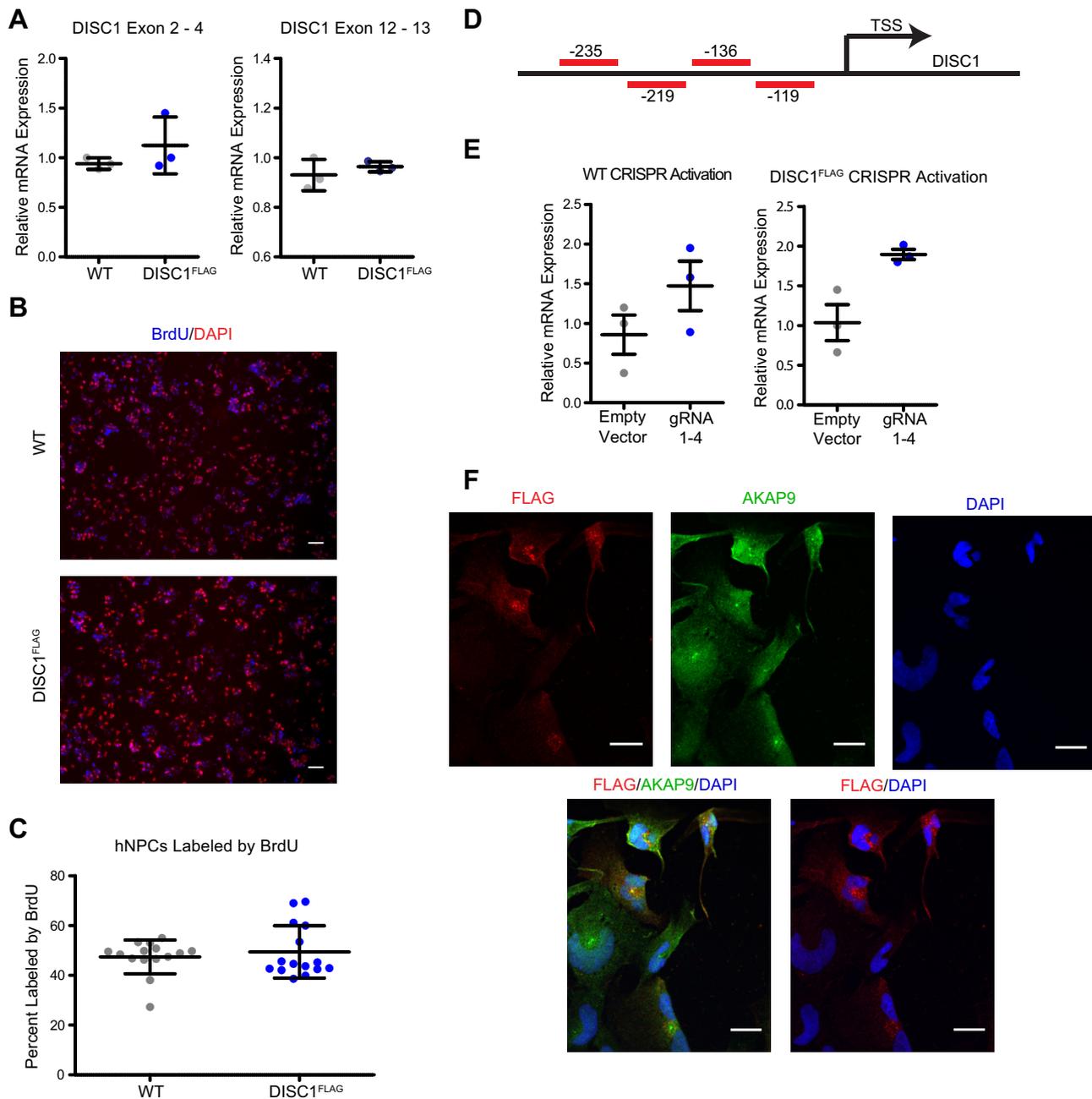
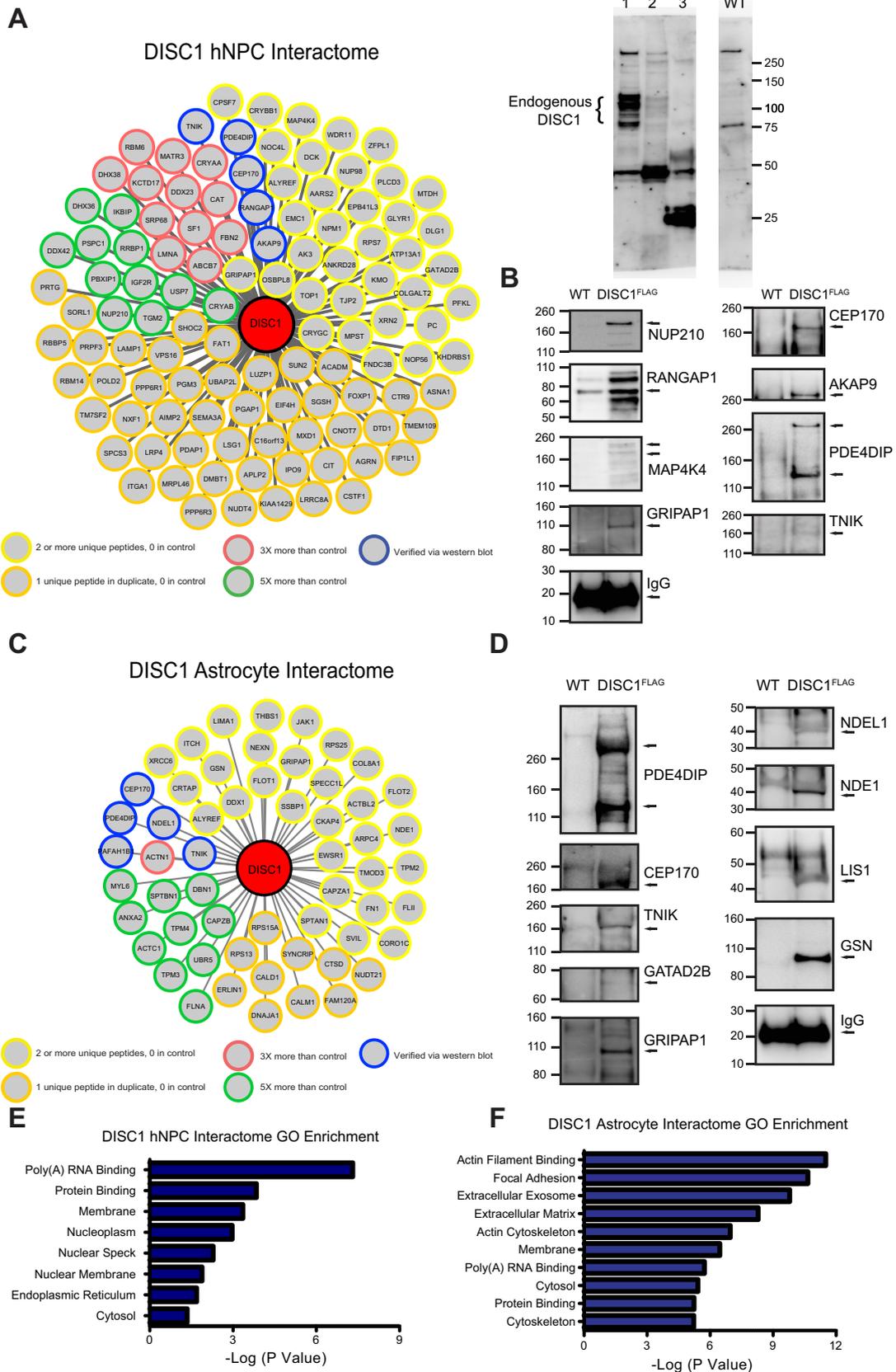


Figure 2. Insertion of 3X-FLAG at the C-terminus of disrupted in schizophrenia 1 (DISC1) does not alter expression or function of DISC1. **(A)** DISC1 expression levels showed no significant difference between DISC1^{FLAG} human neural progenitor cells (hNPCs) and the wild-type (WT) hNPC line as measured by quantitative polymerase chain reaction ($n = 3$ biological replicates). **(B)** Representative immunofluorescence images of bromodeoxyuridine (BrdU) incorporation in WT and DISC1^{FLAG} hNPCs. **(C)** Quantification of BrdU incorporation in both WT and DISC1^{FLAG} hNPC lines shows no significant difference in proliferation ($n = 3$ biological replicates with five random fields imaged and quantified for each). **(D)** Schematic illustrating the position of guide RNAs (gRNAs) used to upregulate expression of DISC1 relative to the transcription start site of DISC1. **(E)** Quantitative polymerase chain reaction demonstrating upregulation of DISC1 following CRISPR (clustered regularly interspaced short palindromic repeats) activation. **(F)** Immunofluorescence following upregulation of DISC1 using the CRISPR activation system in WT and DISC1^{FLAG} hNPCs. Images display co-staining of DAPI and the centrosomal marker, A-kinase anchoring protein 9 (AKAP9), along with FLAG (DISC1). Data in panels **(A)**, **(C)**, and **(E)** are plotted as mean \pm SD. Scale bar in **(B)** = 100 μ m. Scale bar in **(F)** = 20 μ m. mRNA, messenger RNA; TSS, transcription start site.

DISC1 as an RNA binding protein and showed its involvement in RNA transport in adult hippocampal dendrites (33), suggesting a common regulatory function in hNPCs, astrocytes,

and neurons. This is exemplified by the association of DISC1 with RNA binding proteins, including members of the nuclear pore complex (e.g., nucleoporin 210 [NUP210], nucleoporin 98



[NUP98], Ran GTPase-activating protein 1) in hNPCs, along with Aly/REF export factor, which is involved in nuclear mRNA transport and is a common DISC1 interactor in both hNPCs and astrocytes.

We identified multiple unique DISC1 peptides accounting for 21.66% and 34.13% coverage of the DISC1 canonical (L) isoform in hNPCs and astrocytes, respectively (Supplemental Table S6). This relatively low coverage, along with the inability to detect DISC1 above background with traditional immunofluorescence or immunoblotting assays, is in agreement with low expression levels as previously reported (54). Characterization of the expression of *DISC1* in hNPCs and astrocytes relative to the identified DISC1 interactors using publicly available data that directly compares the expression of genes in both iPSC-derived hNPCs and astrocytes (41) was able to show that over 90% of DISC1-interacting proteins have higher expression levels than *DISC1* in both cell types (Supplemental Figure S3, Supplemental Table S7). While transcript levels may not always be directly correlated to protein levels, our mass spectrometry results are in agreement with DISC1 low expression levels in hNPCs and astrocytes. This suggests that for a particular interactor, only a small fraction of the overall protein levels may be interacting with DISC1 at any given time, indicating that DISC1 may interact at substoichiometric ratios.

Endogenous DISC1 Interactome and Previously Reported Protein-Protein Interactions

With this being the first report of endogenous DISC1 protein-protein interactions in iPSC-derived hNPCs and astrocytes, we wanted to compare our data with what has been previously reported. Here, we curated a list of previously reported DISC1 interactions (Supplemental Table S8) and were able to identify a total of nine proteins in hNPCs that we confirmed either by mass spectrometry or via immunoprecipitation followed by Western blot, and nine proteins in astrocytes (Supplemental Table S10). We also observed additional proteins derived from yeast-two hybrid assays present in at least one sample in hNPCs (seven proteins) and astrocytes (three proteins) (Supplemental Table S10).

As a large number of protein interactors in hNPCs and astrocytes were novel, we hypothesized that this may be due to cell type-specific interactions and DISC1 stoichiometry ratios. To test this, we performed immunoprecipitation of the full-length recombinant DISC1 in adult mouse cortex. This is comparable to previous studies that have characterized DISC1 interactions with respect to both nonstoichiometric conditions and developmental context. As opposed to the 20% to 30% coverage obtained under endogenous conditions, we obtained close to 100% using the recombinant protein. Under these conditions, we identified 148 proteins in DISC1 immunoprecipitation

experiments and absent in corresponding WT controls, several of which were verified via Western blot (Supplemental Figure S4A, B; Supplemental Table S9). As opposed to the nine proteins identified in NPCs or astrocytes, we identified a total of 42 previously reported interactions using recombinant DISC1 in a tissue similar to what has been used previously (Supplemental Table S10).

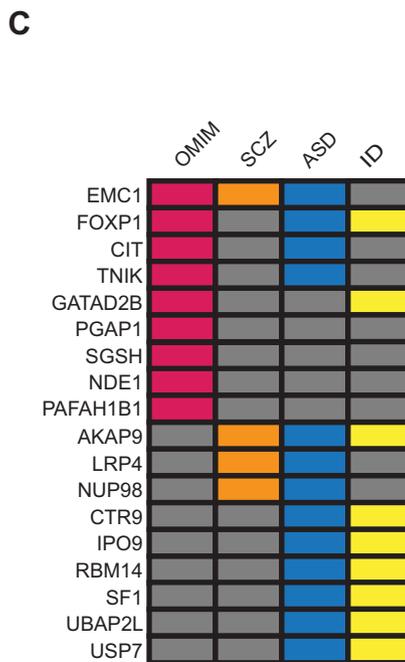
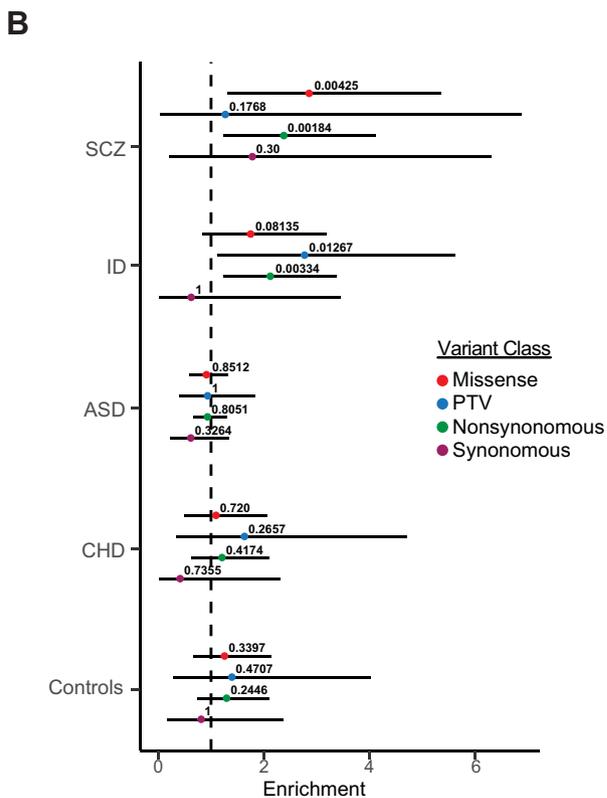
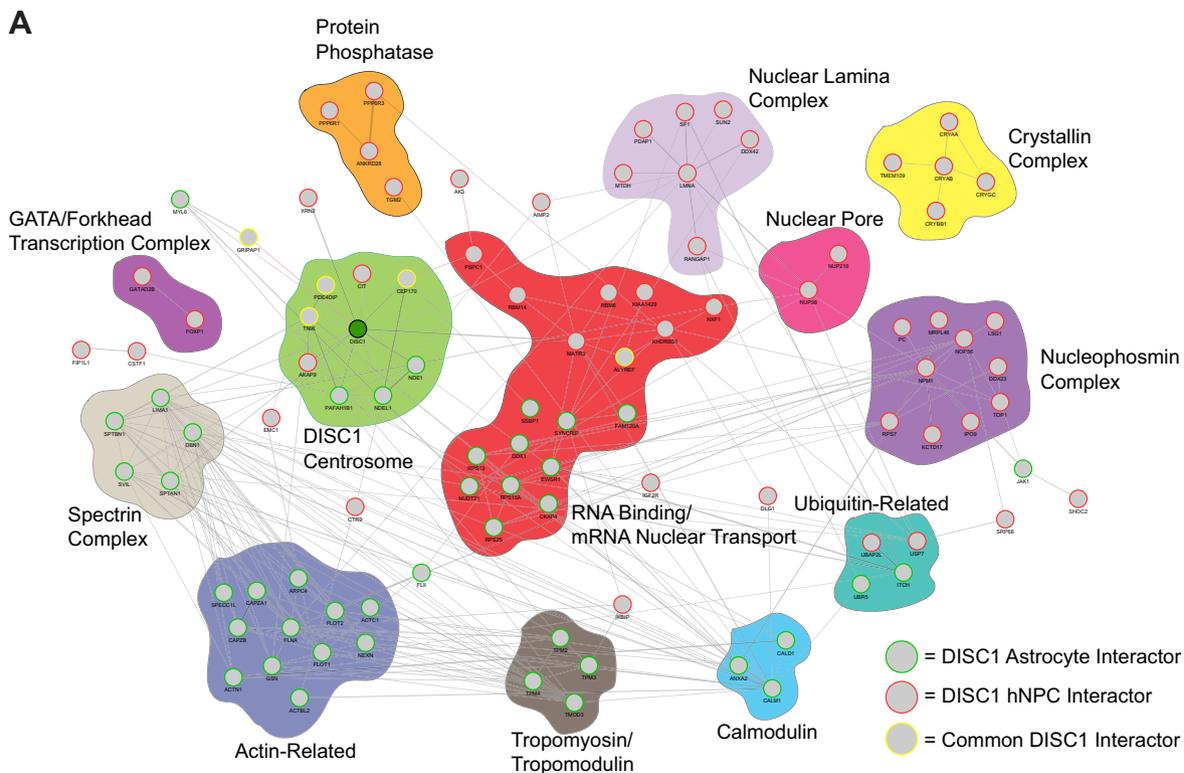
We also found a number of related proteins to previously reported interactions in both structure and function (Supplemental Table S11). For example, while the nuclear pore protein, nucleoporin 160 (NUP160), was previously characterized via yeast-two hybrid and detected in the DISC1 recombinant immunoprecipitation, we identified NUP210 and NUP98 as interactors of DISC1 in hNPCs. This highlights the role cellular context plays in determining protein interactions.

Clustering of DISC1 Interactors and Psychiatric Disease

Next, we asked if the endogenous DISC1 interactomes could cluster in subsets of protein interactions that can indicate both protein functions and complexes that DISC1 may associate with. Here, we extracted reported protein interactions for each DISC1 interactor identified in our screens and clustered them in a hNPC- or astrocyte-DISC1 protein interaction network. We identified a number of molecular functions and cellular locations where DISC1 interactors associated in interconnected clusters of protein interactions. Both hNPCs and astrocyte interactomes contain the centrosomal interactome, including TNIK, phosphodiesterase 4D interacting protein, and CEP170, along with proteins that clustered into functions such as RNA binding and transport (Figure 4A, Supplemental Table S12). hNPCs interactions also clustered in the perinuclear region including the nuclear pore and nuclear lamina complexes. In contrast, DISC1 interactors identified in astrocytes clustered into complexes involving cytoskeletal processes such as actin-related proteins or the spectrin complex (Figure 4A, Supplemental Table S12). While clusters of proteins involved in centrosome dynamics have been previously described, a majority of the clusters uncovered are novel and include proteins characterized as contributing to psychiatric disorders, such as GATA zinc finger domain containing 2B and forkhead box protein 1, which were identified in hNPCs (60,61).

DISC1 has been associated with numerous psychiatric disorders (9,11,62,63). However, the lack of association with schizophrenia through both genome-wide association and exome sequencing studies has brought the applicability of DISC1 to psychiatric disorders into question (64,65). Irrespective of this, proteins may be able to regulate disease-relevant pathways through the modulation of protein interactions and function in protein networks where disease

Figure 3. Identification of the endogenous disrupted in schizophrenia 1 (DISC1) interactome in human neural progenitor cells (hNPCs) and astrocytes. **(A)** Right panel: Western blot following Immunoprecipitation and elution of DISC1 from DISC1^{FLAG} and wild-type (WT) hNPCs using an anti-3X-FLAG peptide. Comparing elutions shows that a majority of DISC1 is eluted within the first elution. Lane 1 = first elution, Lane 2 = second elution, Lane 3 = boiling of magnetic beads postelutions. Left panel: DISC1 interactome in hNPCs as characterized via immunoprecipitation followed by mass spectrometry or Western blotting. Criteria for inclusion is indicated by colored border of nodes. **(B)** Western blots in hNPCs showing conformation of proteins identified via immunoprecipitation followed by mass spectrometry or previously identified protein-protein interactions involving DISC1. **(C)** DISC1 interactome in astrocytes as characterized via immunoprecipitation followed by mass spectrometry or Western blotting. **(D)** Western blots in astrocytes showing conformation of proteins identified via immunoprecipitation followed by mass spectrometry or previously identified protein-protein interactions involving DISC1. **(E)** Gene ontology (GO) analysis of the identified DISC1 interactome in hNPCs. **(F)** GO analysis of the identified DISC1 interactome in astrocytes.



risk factors are associated, as the case has been made for DISC1 (20). Therefore, we tested the hNPC, astrocyte, and recombinant DISC1 interactomes for enrichment in proteins affected by de novo mutations, separated by mutation class, in schizophrenia, autism spectrum disorder, ID, congenital heart disease, and an aggregate of all controls in denovo-db (66) (Figure 4C, Supplemental Table S13). For both the astrocyte and recombinant DISC1 interactomes, there was no statistically significant enrichment in any category tested with the exception of nonsynonymous mutations associated with ID in the DISC1 recombinant interactome. However, in the hNPC DISC1 interactome, we did find enrichment of nonsynonymous mutations in schizophrenia following correcting for multiple comparisons (nominal p value = 1.84×10^{-3} , Bonferroni threshold $p < 2.40 \times 10^{-3}$). ID also presented enrichment in nonsynonymous mutations, but did not pass the Bonferroni threshold (nominal p value = 3.34×10^{-3}) (Figure 4B, Supplemental Table S13). While the schizophrenia enrichment was driven by a diverse set of proteins, the nonsignificant enrichment in ID was driven by proteins that were found to have recurrent mutations including forkhead box protein 1, GATA zinc finger domain containing 2B, and ubiquitin specific peptidase 7 (Figure 4B, C; Supplemental Table S13). This suggests that DISC1 function within hNPCs may contribute more to the risk of disease when compared with DISC1 function in astrocytes or in the adult cortex.

In addition to proteins that have been implicated through exome-sequencing studies, we also focused on proteins known to be syndromic for neurodevelopmental disorders as classified by the Online Mendelian Inheritance in Man database (Figure 4C). Here, DISC1 interactors included the previously described DISC1 interacting kinases citron rho-interacting serine/threonine kinase (microcephaly) and TNIK (ID), along with NDE1 (microhydranencephaly) and platelet-activating factor acetylhydrolase IB subunit alpha (lissencephaly). Novel interactors that are also known to be syndromic include the transcription factors forkhead box protein 1 (ID and autism spectrum disorder) and GATA zinc finger domain containing 2B (ID), ubiquitin specific peptidase 7 (ID and autism spectrum disorder), post-GPI attachment to proteins 1 (ID), N-sulfoglucosamine sulfohydrolase (mucopolysaccharidosis type IIIA), and ER membrane protein complex subunit 1 (developmental delay). This is in accordance with our previous findings, showing that the interactomes of molecules involved in developmental disorders, such as TNIK (67), show an enrichment in de novo mutations in a variety of complex brain disorders (43).

Mutations in DISC1 Interactors Regulate Shared Cellular Functions

Proteins associated in protein interaction networks are usually involved in the regulation of shared cellular processes and mutations in genes encoding for these proteins are more likely

to be associated to the same disease (68–70). We observed that *DISC1* mutations alter the expression levels of many novel DISC1 interactors, with a significant overlap in differentially expressed genes found in iPSC-derived neurons from patients with a frameshift mutation that affects the isoforms selectively tagged here (14) (see Supplemental Analyses). In addition, with DISC1 interacting with several centrosomal proteins in hNPCs, we hypothesized that similarly to DISC1, they might also affect hNPC proliferation. One of the proteins, the TNIK, has also been involved in a number of psychiatric disorders and ID (67,71–73). This suggests that TNIK mutations may also be associated with alterations in cellular proliferation in hNPCs. Thus, we introduced a truncating mutation at the Citron homology domain of TNIK, which resulted in a TNIK knockout cell line (Supplemental Figure S4A). Compared with WT cell lines, we found a marked decrease of proliferation in TNIK knockout hNPCs, as measured by a 40% decrease in phosphohistone H3-positive cells ($p < .001$, Student's t test) (Supplemental Figure S4B, C). Interestingly, it has been reported that DISC1 may inhibit TNIK function in mature neurons in vitro (6). However, our results indicate a synergistic interaction of DISC1 and TNIK in which both molecules are needed to regulate hNPC proliferation, highlighting the importance of the identification of cell-specific protein networks.

DISCUSSION

Through the combination of genome engineering and mass spectrometry, we are able to report the first endogenous interactome of DISC1 in human iPSC-derived NPCs and astrocytes. While we ensured that there were no transcriptional changes at the mRNA level due to the insertion of the endogenous tag, we should note the unlikely possibility of an endogenous tag's interfering with protein expression levels, distribution, or protein interactions. Nonetheless, the novel interactions identified here can be categorized into numerous functional modules that are in line with previously reported functions of DISC1 and expand on them. For example, DISC1 is associated with a number of proteins that are known to reside at the centrosome, including AKAP9, CEP170, NDE1 like 1, and platelet-activating factor acetylhydrolase IB subunit alpha. Our results are able to include molecules such as phosphodiesterase 4D interacting protein into the DISC1/centrosome protein interaction network in both hNPCs and astrocytes.

Using the DISC1 interactions determined in hNPCs, astrocytes, the recombinant protein, or all previously reported interactions, we were able to show that only the DISC1 hNPC interactome is enriched in nonsynonymous mutations as a whole identified in sporadic cases of schizophrenia. Determining DISC1 interactors in hNPCs is also able to provide context to neurodevelopmental phenotypes observed in

Figure 4. Clustering and enrichment of disrupted in schizophrenia 1 (DISC1) interactome in psychiatric disease risk factors. **(A)** Clustering of the endogenous DISC1 human neural progenitor cell (hNPC) and astrocyte interactomes based on protein-protein interactions derived from public databases. **(B)** Enrichment of the DISC1 hNPC interactome in proteins found to have de novo mutations in patients affected by schizophrenia (SCZ), intellectual disability (ID), autism spectrum disorder (ASD), and congenital heart disease (CHD), along with all controls listed in denovo-db. Variant classes are split between missense mutations, protein-truncating variants (PTVs), nonsynonymous mutations (includes missense mutations and PTVs), and synonymous mutations. Error bars represent the 95% confidence interval and nominal p values are displayed in bold for each test. **(C)** Heat map representing proteins implicated in contributing to neurological disorders via Online Mendelian Inheritance in Man (OMIM) along with proteins containing nonsynonymous de novo mutations in SCZ, ASD, or ID when found in two or more gene lists. mRNA, messenger RNA.

previous studies. For example, clinically relevant mutations in *DISC1* created via genome engineering in iPSCs were shown to result in differential subpopulations of neural progenitors following differentiation (16). Here, we show that *DISC1* binds to nuclear pore proteins such as NUP210 and NUP98, both of which have been shown to influence neural differentiation (74,75). While relatively little is known concerning the function of *DISC1* in astrocytes, it has been shown to influence gliogenesis of primary NPCs (48), indicating a common function of influencing differentiation potential. Here, the common functional module of regulatory RNA-binding proteins between hNPCs and astrocytes may play a role in this process.

While *DISC1* has been regularly described as a scaffold protein, the expression of *DISC1* appears to be extremely low which is corroborated through both transcriptomic and proteomic data. Mass spectrometry data derived from public repositories such as PeptideAtlas do not contain any *DISC1* peptides observed in any neural cell types or brain tissue as a whole (76). Thus, the substoichiometric characteristics of *DISC1* protein interactions suggest that *DISC1* binds and affects only a specific subset of its interactors at any given time.

Our results are also able to provide the first confirmation via immunoprecipitation followed by Western blotting for *DISC1* interactors identified in yeast-two hybrid screens including AKAP9, CEP170, and GRIP1-associated protein 1. However, comparison of the endogenous interactome of *DISC1* in iPSC-derived hNPCs and astrocytes to the multiple *DISC1* yeast-two hybrid screens is able to show that a vast majority of the interactions detected here were not apparent in those studies. While protein interactions derived from affinity purification may include secondary or tertiary interactions compared with binary interactions identified via yeast-two hybrid assays, these assays do not take context or stoichiometric ratios into account. In contrast to the endogenous interactions, when we performed immunoprecipitation of recombinant *DISC1* in a non-stoichiometric fashion and in an experimental setting resembling what has been previously published, we found an abundance of previously reported interactions. This underscores the importance of defining endogenous protein-protein interactions within a particular cellular context of interest with the correct stoichiometric ratios between interacting proteins.

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