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RESEARCH ARTICLE | *Nervous System Pathophysiology*

Caveolin-1 regulation of *disrupted-in-schizophrenia*-1 as a potential therapeutic target for schizophrenia

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Kassan A, Egawa J, Zhang Z, Almenar-Queralt A, Nguyen QM, Lajevardi Y, Kim K, Posadas E, Jeste DV, Roth DM, Patel PM, Patel HH, Head BP. Caveolin-1 regulation of *disrupted-in-schizophrenia*-1 as a potential therapeutic target for schizophrenia. *J Neurophysiol* 117: 436–444, 2017. First published November 2, 2016; doi:10.1152/jn.00481.2016.—Schizophrenia is a debilitating psychiatric disorder manifested in early adulthood. *Disrupted-in-schizophrenia-1* (*DISC1*) is a susceptible gene for schizophrenia (Hodgkinson et al. 2004; Millar et al. 2000; St Clair et al. 1990) implicated in neuronal development, brain maturation, and neuroplasticity (Brandon and Sawa 2011; Chubb et al. 2008). Therefore, *DISC1* is a promising candidate gene for schizophrenia, but the molecular mechanisms underlying its role in the pathogenesis of the disease are still poorly understood. Interestingly, caveolin-1 (Cav-1), a cholesterol binding and scaffolding protein, regulates neuronal signal transduction and promotes neuroplasticity. In this study we examined the role of Cav-1 in mediating DISC1 expression in neurons in vitro and the hippocampus in vivo. Overexpressing Cav-1 specifically in neurons using a neuron-specific synapsin promoter (*SynCav1*) increased expression of DISC1 and proteins involved in synaptic plasticity (PSD95, synaptobrevin, synaptophysin, neurexin, and syntaxin 1). Similarly, *SynCav1*-transfected differentiated human neurons derived from induced pluripotent stem cells (hiPSCs) exhibited increased expression of DISC1 and markers of synaptic plasticity. Conversely, hippocampi from Cav-1 knockout (KO) exhibited decreased expression of DISC1 and proteins involved in synaptic plasticity. Finally, *SynCav1* delivery to the hippocampus of Cav-1 KO mice and Cav-1 KO neurons in culture restored expression of DISC1 and markers of synaptic plasticity. Furthermore, we found that Cav-1 coimmunoprecipitated with DISC1 in brain tissue. These findings suggest an important role by which neuron-targeted Cav-1 regulates DISC1 neurobiology with implications for synaptic plasticity. Therefore, *SynCav1* might be a potential therapeutic target for restoring neuronal function in schizophrenia.

NEW & NOTEWORTHY The present study is the first to demonstrate that caveolin-1 can regulate DISC1 expression in neuronal models. Furthermore, the findings are consistent across three separate neuronal models that include rodent neurons (*in vitro* and *in vivo*) and human differentiated neurons derived from induced pluripotent stem cells. These findings justify further investigation regarding the modulatory role by caveolin on synaptic function and as a potential therapeutic target for the treatment of schizophrenia.

SCHIZOPHRENIA IS TYPICALLY manifested in late adolescence or early adulthood with an estimated prevalence of ~1% (Saha et al. 2005). Schizophrenia is partly a genetic disorder, although it likely involves multiple recessive genes and environmental factors such as physical or psychological abuse and birth complications (Jaaro-Peled et al. 2009; Schmitt et al. 2014; Vilain et al. 2013). Although pharmacological treatments such as antipsychotics are available for schizophrenia, these classes of drugs show poor efficacy for most patients (Lieberman et al. 2005), especially in reversing cognitive abnormalities (Brown and McGrath 2011; Buchanan et al. 2007).

DISC1 is a schizophrenia-associated gene originally identified in a Scottish family (Millar et al. 2000; Muir et al. 2008; St Clair et al. 1990), and later studies have shown an increasing amount of evidence that supports the possibility that *DISC1* may be a candidate gene for schizophrenia (Callicott et al. 2005; Ekelund et al. 2001; Hamshere et al. 2005; Harrison and Weinberger 2005; Hennah et al. 2003; Hodgkinson et al. 2004; Qu et al. 2007; Song et al. 2008). DISC1 protein is highly expressed in the developing brain (Schurov et al. 2004) and in the dentate gyrus of the adult hippocampus (Austin et al. 2004); it is a multifunctional protein involved in neuritogenesis and neuronal signaling (Brandon et al. 2009; Ishizuka et al. 2006; Narayan et al. 2013). DISC1 is located in multiple intracellular locations [i.e., the nucleus (Sawamura et al. 2005), mitochondria (Millar et al. 2005), and axons and synapses (Kirkpatrick et al. 2006; Miyoshi et al. 2003)]. Loss of DISC1 function causes deficits in neural development, neuronal proliferation, axonal growth, and cytoskeleton modulation, which are consistent with abnormal neural development in schizophrenia (Harrison 1997; Lewis and Levitt 2002; Lewis and Moghaddam 2006).

Proper neuronal growth (i.e., dendritic arborization, axonal guidance, and formation of synaptic contacts) and neurotrans-

mission are dependent on a polarized membrane platform that organizes key membrane receptors, which in turn transduce extracellular cues. A necessary organizer of neuronal signaling components is the scaffolding protein caveolin-1 (Cav-1) (Head et al. 2008, 2011). Cav-1 is widely expressed in the central and peripheral nervous systems (Boulware et al. 2007; Heiman et al. 2008). Within neurons, Cav-1 regulates membrane/lipid raft (MLR) formation and neurotransmitter and neurotrophin signaling (Head et al. 2008), promotes dendritic growth and arborization (Head et al. 2011), and, when overexpressed in hippocampal neurons in vivo, augments functional neuroplasticity and improves learning and memory (Mandyam et al. 2015). To date no relevant functional role of Cav-1 in the pathogenesis of schizophrenia has been described, although *CAV1* gene disruption was recently identified in some patients suffering from schizophrenia (Walsh et al. 2008). Because Cav-1 organizes and regulates neurotransmitter and neurotrophic receptor signaling pathways (Bilderback et al. 1999; Gaudreault et al. 2005; Suzuki et al. 2004) and G protein-coupled neurotransmitter receptors (Allen et al. 2007; Bhatnagar et al. 2004; Francesconi et al. 2009) necessary for proper dendritic growth and arborization (Head et al. 2011; Mandyam et al. 2015), disruption of *CAV1* would likely impair neuronal signaling, leading to a schizophrenia-like phenotype. Interestingly, recent findings support the involvement of caveolin in schizophrenia; for instance, central nervous system pathologies in Cav-1 knockout (Cav-1 KO) mice are similar to those exhibited with schizophrenia (Head et al. 2008; Trushina et al. 2006), because Cav-1 KO mice demonstrate increased sensitivity to the psychotomimetic effects of *N*-methyl-D-aspartate receptor (NMDAR) antagonist phencyclidine (PCP) (Allen et al. 2011), a phenomenon also observed in schizophrenic patients (Lahti et al. 1995; Luby et al. 1959). Additionally, Cav-1 interacts with the serotonin receptor 5-HT$_{2A}$ (Bhatnagar et al. 2004), a target for atypical antipsychotic drugs (Meltzer et al. 1989). Interestingly, Cav-1 KO mice showed attenuated biochemical and behavioral actions of atypical antipsychotic drugs (Allen et al. 2011). These findings provide evidence suggesting a link between Cav-1 and schizophrenia.

In the present study, our goal was to examine if Cav-1 modulates expression of DISC1 in various neuronal systems. Interestingly, treatment of primary neurons with *SynCav1* lentivirus significantly enhanced the expression of DISC1. Furthermore, human differentiated neuronal progenitor cells (NPCs) derived from induced pluripotent stem cells (hiPSCs) transfected with *SynCav1* showed higher DISC1 protein expression. In addition, hippocampal homogenates from Cav-1 KO vs. wild-type (WT) mice showed a significant reduction in DISC1, and synaptic proteins such as PSD95, synaptophysin, synaptobrevin, and syntaxin 1 were also significantly reduced; however, *AAV9-SynCav1* delivery to the hippocampus in vivo restored expression of these synaptic proteins.

MATERIALS AND METHODS

Animals. All animals [C57BL/6 mice, Cav-1 KO mice (Cav^{tm1Mls}), and rats (Jackson Laboratories, Bar Harbor, ME)] were treated in compliance with the *Guide for the Care and Use of Laboratory Animals* (Washington, DC: National Academy of Sciences, 2011). All animal use protocols were approved by the VA San Diego Healthcare System Institutional Animal Care and Use Committee (San Diego,

CA) before any procedures were performed. Adult male mice (2–3 mo old) were housed under normal conditions with ad libitum access to food and water. The use of human cells was approved under the University of California San Diego Human Research Protection Institutional Review Board 150071.

Stereotactic injection. Mice were anesthetized and prepared for surgery with a protocol modified from a previously described study (Mandyam et al. 2015). Hippocampus-targeted injections were controlled using Injectomate software (Neurostar, Berlin, Germany). Injections were made using a 33-gauge, 10-μl Hamilton gas-tight syringe (Hamilton, Reno, NV). At each coordinate, the needle was lowered at a rate of 0.32 mm/s. After 60 s, 0.5 μl of adeno-associated virus serotype 9 (*AAV9*) containing synapsin-red fluorescent protein (RFP; *SynRFP*) or synapsin-caveolin-1 (*SynCav1*) was injected over 60 s [0.5 μl/min injection rate at a viral titer of 10^9 genome copies/μl] at three locations (rostral to caudal) in each hippocampal hemisphere with an indwelling time of 1 min. Sagittal brain sections were stained to confirm location and spread of RFP (data not shown). Sections were also stained for hematoxylin and eosin, and histopathologic analysis did not reveal any gross morphology or cell death in the hippocampal sections (data not shown).

Primary neuron isolation and culture. Neonatal rat neurons were isolated from hippocampi using a papain dissociation kit (Worthington Biochemical, Lakewood, NJ) as previously described (Head et al. 2008, 2011). Neurons were cultured in neurobasal A medium supplemented with B27 (2%), 250 mM GLUTMax1, and penicillin-streptomycin (1%). Cells were cultured on poly-D-lysine/laminin (2 μg/cm^2)-coated plates at 37°C in 5% CO$_2$ for 4 days before transfection with lentiviral vectors containing the synapsin promoter upstream of the *CAV1* gene (*SynCav1*). Synapsin-green fluorescent protein (*SynGFP*) was used as control vector. Titer for both vectors was 10^9 infectious units per milliliter.

The human neurons were differentiated from the Craig Venter 4a (CV4a) neuronal progenitor cells (NPCs) derived from hiPSCs as previously described (Woodruff et al. 2013). Cells were first grown on poly-L-ornithine/laminin-coated plates. Cells were maintained in NPC base media (DMEM/F12, N2, B27, and penicillin-streptomycin) supplemented with bFGF (20 ng/ml). Cells were then differentiated for 3–4 wk in differentiating media (NPC base media supplemented with bone-derived neurotropic factor, glial cell-derived neurotropic factor, and dibutyryl cAMP). Neurons were then infected with *SynCav1* or *SynGFP* as control.

Sample preparation and immunoblot analysis. Mice were anesthetized with pentobarbital sodium (60 mg/kg intraperitoneally), followed by decapitation. The brain was removed and placed in ice-cold lysis buffer (150 mM Na$_2$CO$_3$; pH 11), minced, and homogenized with a Tissuemiser (Fisher Scientific, Waltham, MA). Homogenates were further centrifuged at 600 *g* to clear debris. Cell lysates were prepared in buffer (50 mM Tris·HCl, 150 mM NaCl, 1% Triton X-100; pH 7.4) supplemented with protease and phosphatase inhibitors cocktail (Cell Signaling, Beverly, MA). After 30-min incubation on ice, the cells were homogenized by a 23-gauge needle, and the lysates were cleared of debris and unbroken cells by centrifugation (800 *g*, 5 min at 4°C). Protein concentrations were determined using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA). Equal amounts of cell lysates (10 μg) were loaded to determine expression of Cav-1, PSD95, neurexin (BD Biosciences, Franklin Lakes, NJ), syntaxin 1, synaptobrevin, synaptophysin (Abcam, Cambridge, MA), and DISC1 (Thermo Fisher Scientific, Waltham, MA). All protein expression was normalized to GAPDH (Cell Signaling Technology, Danvers, MA). Horseradish peroxidase-conjugated secondary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Immunoblots were subsequently detected by Lumigen ECL Ultra (Lumigen, Southfield, MI). The densitometry of the different bands was further quantified using ImageJ software (National Institutes of Health, Bethesda, MD) with normalization to GAPDH.

Immunoprecipitation. Immunoprecipitation (IP) of Cav-1 and DISC1 was performed using protein A agarose (Roche, Life Science) according to the manufacturer's protocol. In brief, brain samples were homogenized in lysis buffer. The supernatants were collected by centrifugation at 10,000 *g* for 10 min at 4°C. Lysates were then incubated with antibodies for either Cav-1 or DISC1 at 4°C for 3 h, followed by overnight incubation in agarose A. The immunoprecipitates were analyzed for the presence of Cav-1 and DISC1 by immunoblot assay.

Statistical analysis. Results are means ± SE and were analyzed using GraphPad Prism 6 (GraphPad Software, San Diego, CA). We used *t*-tests and one-way ANOVA to compare certain paired parameters. Values of $P < 0.05$ were considered significant.

RESULTS

Cav-1 coimmunoprecipitates with DISC1 in brain tissue and human neurons. At present, there are no reports that Cav-1 and DISC1 localize to the same protein complex in neuronal tissue; thus we sought to determine if Cav-1 localizes with DISC1 in brain homogenates. Our data are the first to demonstrate that Cav-1 coimmunoprecipitated with DISC1 (Fig. 1, *A* and *B*). The DISC1 antibody specifically only detects a band at ~95 kDa. Quantification of IP bands was normalized to the total input (Fig. 1*C*). To strengthen our data, we also studied the interaction between Cav-1 and DISC1 in differentiated human neuron (i.e., differentiated NPC) derived from hiPSCs. Similar to what we observed in brain homogenates, the data show that Cav-1 and DISC1 coimmunoprecipitated in human differentiated neurons (Fig. 1, *D* and *E*). Quantification of IP bands was normalized to the total input (Fig. 1*F*).

Neuron-targeted overexpression of Cav-1 (SynCav1) enhances expression of DISC1 and synaptic proteins in primary neurons. To explore the implication of Cav-1 and DISC1 on the synaptic integrity, we overexpressed Cav-1 specifically in neurons by using a synapsin promoter as previously described (Head et al. 2011). Primary neurons isolated from hippocampi of postnatal *day 1–3* rats were transfected with lentiviral *SynCav1*, followed by immunoblot analysis. The data show that *SynCav1* increased protein expression of Cav-1, DISC1, and membrane proteins necessary for synaptic plasticity and maintenance (PSD95, synaptobrevin, neurexin, and syntaxin 1). However, there was no significant change in synaptophysin expression level. (Fig. 2).

Hippocampi from Cav-1 KO mice exhibit decreased expression of DISC1 and synaptic proteins. Because data in Fig. 1 showed that *SynCav1* increased expression of DISC1 and synaptic proteins, we tested whether the opposite oc-
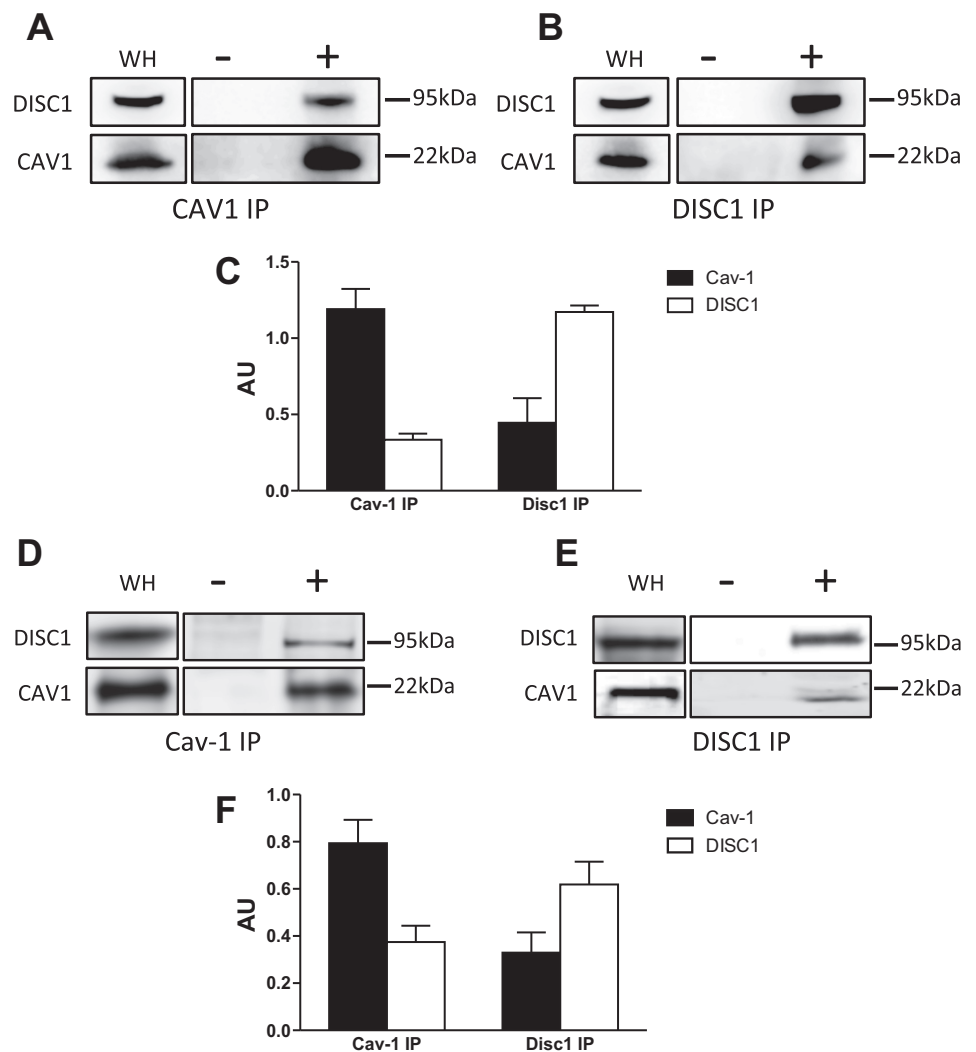

Fig. 1. Caveolin-1 (Cav-1) is an interaction partner of disrupted-in-schizophrenia-1 (DISC1). Brain homogenates were immunoprecipitated with a Cav-1 or DISC1 antibodies. Immunoprecipitates were then probed for the presence of Cav-1 and DISC1 by Western blotting (representative blots from *n* = 3 experiments). Negative controls are incubated without antibodies (*A* and *B*). Quantification of immunoblots was normalized to the total input (*C*). In parallel, induced pluripotent stem cell (iPSC) homogenates were also immunoprecipitated with Cav-1 or DISC1 antibodies. IP were then probed for the presence of Cav-1 and DISC1 by Western blotting (representative blots from *n* = 3 experiments). Negative controls are incubated without antibodies (*D* and *E*). Quantification of immunoblots was normalized to the total input (*F*). AU, arbitrary units; WH, whole homogenate.
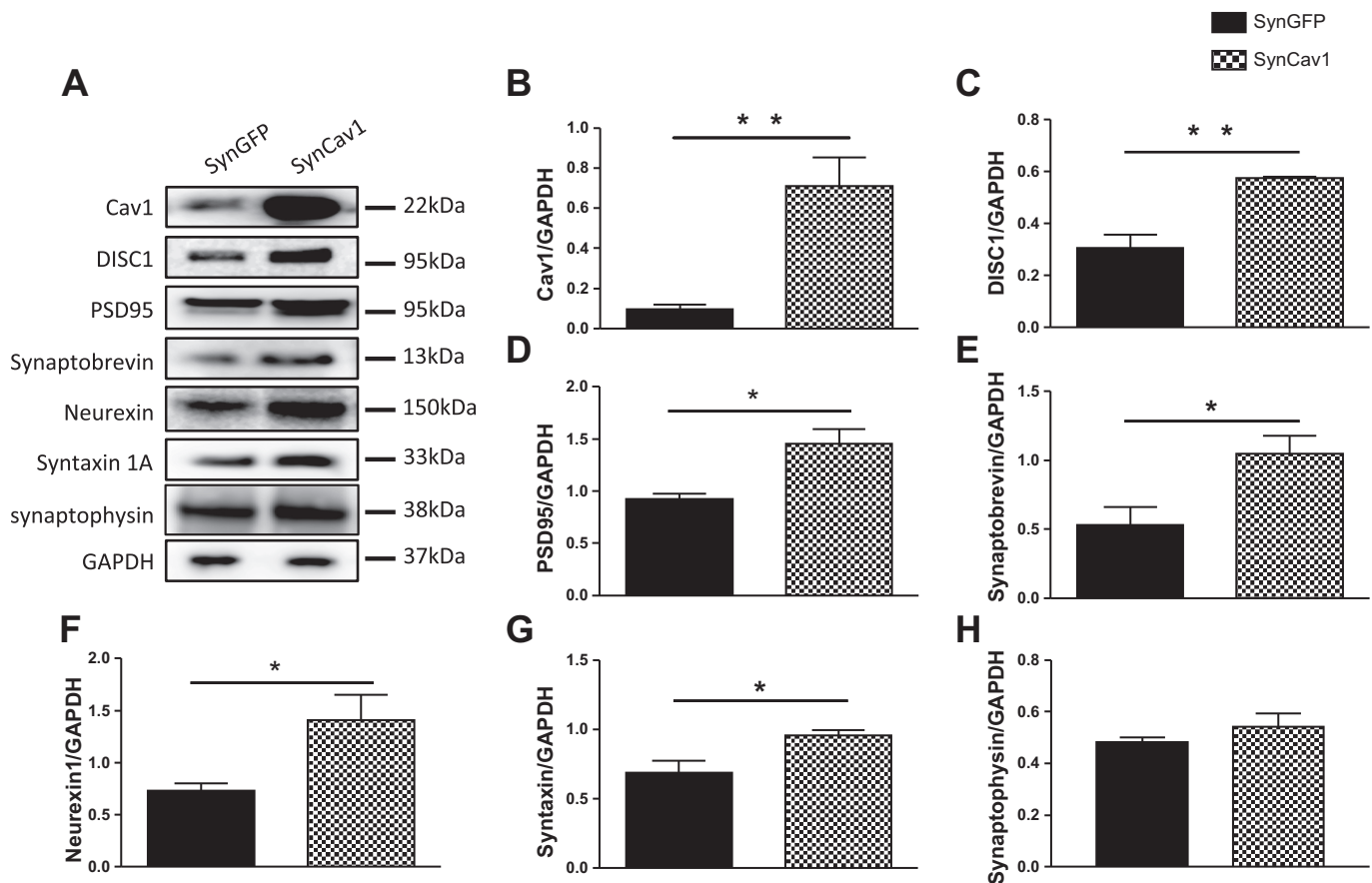
Fig. 2. Neuron-targeted expression of caveolin-1 (Cav-1) enhances expression of disrupted-in-schizophrenia-1 (DISC1) and synaptic proteins in wild-type (WT) primary neurons. Primary rodent neurons were isolated from rats' neonatal hippocampi. Neurons were grown in culture for 4 days and infected with a lentivirus containing *SynCav1* or synapsin-green fluorescent protein (*SynGFP*) as control (2×10^9 viral particles) for 72 h. Homogenates were immunoblotted for Cav-1, DISC1, PSD95, synaptobrevin, neurexin 1, syntaxin 1A, synaptophysin, synaptophysin, and GAPDH (*A*). Quantification of Western blots showed overexpression of Cav-1 protein (*B*). *SynCav1* also significantly enhanced the protein expression of DISC1 (*C*) and other synaptic proteins: PSD95 (*D*), synaptobrevin (*E*), neurexin 1 (*F*), and syntaxin 1A (*G*). No significant difference was detected in synaptophysin (*H*). Representative blots are from $n = 4$ experiments. $*P < 0.05$; $**P < 0.01$, *SynCav1* vs. *SynGFP*.

curred in brain tissue from Cav-1 KO mice. Indeed, immunoblot data showed that loss of Cav-1 was associated with decreased protein expression of DISC1 (Fig. 3, *A* and *B*) and the synaptic proteins PSD95, synaptobrevin, neurexin 1, syntaxin, and synaptophysin (Fig. 3, *A* and *C–G*). Interestingly, loss of Cav-1 seemed to affect both post- and presynaptic proteins, suggesting decreased synaptic strength and plasticity.

Re-expressing Cav-1 in Cav-1 KO mice increases DISC1 and synaptic proteins. To elucidate whether reintroducing Cav-1 could reverse the effect seen with loss of Cav-1, Cav-1 KO mice underwent stereotactic injections of *AAV9-SynCav1* as previously described (Mandyam et al. 2015). Successful overexpression of Cav-1 in the hippocampus was confirmed by immunoblot analysis (Fig. 4, *A* and *B*). Interestingly, re-expression of Cav-1 in Cav-1 KO hippocampi significantly increased the levels of DISC1 expression (Fig. 4, *A* and *C*) as well as expression of pre- and postsynaptic proteins such as PSD95, synaptobrevin, neurexin 1, and synaptophysin (Fig. 4, *A*, *D–F*, and *H*). However, there was no significant difference in protein expression levels of syntaxin (Fig. 4*G*). All protein expression levels were compared with control without injection.

SynCav1 enhances expression of DISC1 and synaptic proteins in differentiated human neurons derived from hiPSCs. To investigate whether the effect from Cav-1 on DISC1 and synaptic protein could be recapitulated in a human neuronal cell model, primary human fibroblasts were reprogrammed into hiPSCs and subsequently differentiated into neurons as previously described (Woodruff et al. 2013). Interestingly, our data indicate that *SynCav1*-transfected human neurons (Fig. 5, *A* and *B*) exhibited increased protein expression of DISC1 (Fig. 5, *A* and *C*), as well as synaptic proteins such as PSD95, synaptophysin, neurexin, syntaxin 1A, and synaptobrevin (Fig. 5, *A* and *D–H*), results akin to what we observed in primary rodent neurons and rodent brains following *SynCav1* transfection.

DISCUSSION

Our study has demonstrated the importance of Cav-1 in regulating DISC1 and synaptic proteins. *SynCav1* enhanced DISC1 expression and synaptic proteins in primary rodent neurons as well as in rodent brains. Furthermore, Cav-1 KO mice showed a reduction in the expression levels of DISC1 and synaptic proteins. This reduced expression of synaptic proteins was reversed after *AAV9-SynCav1* delivery to the hippocampus
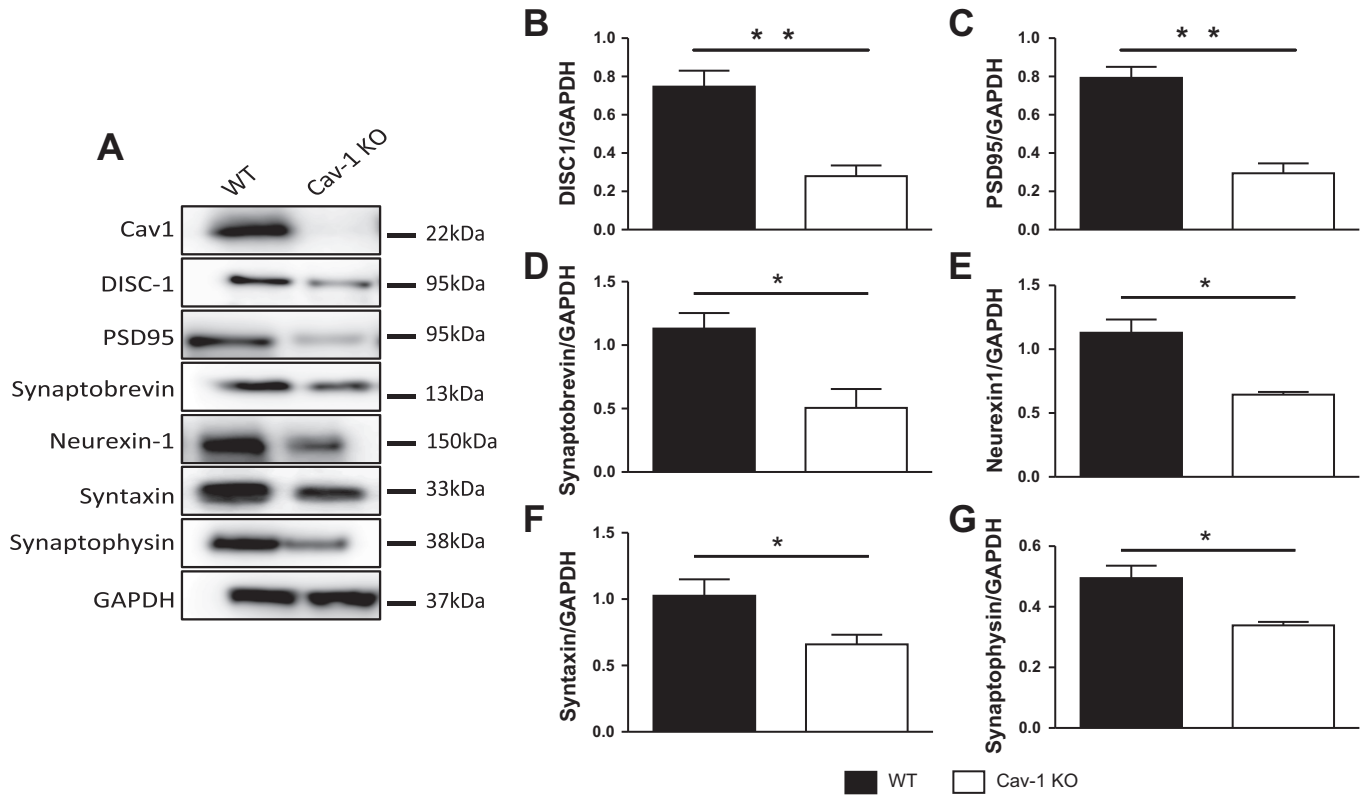
Fig. 3. Hippocampal homogenates show a caveolin-dependent reduction in disrupted-in-schizophrenia-1 (DISC1) and synaptic proteins. Hippocampi were isolated from the brains of wild-type (WT) and caveolin-1 knockout (Cav-1 KO) mice (2–3 mo). Homogenates were immunoblotted for Cav-1, DISC1, PSD95, synaptobrevin, neurexin 1, syntaxin 1A, synaptophysin, and GAPDH (*A*). Quantification of Western blots showed a significant decrease in the protein expression level of DISC1 (*B*) and other synaptic proteins: PSD95 (*C*), synaptobrevin (*D*), neurexin 1 (*E*), syntaxin 1A (*F*), and synaptophysin (*G*). Representative blots are from $n = 4$ experiments. $*P < 0.05$; $**P < 0.01$, WT vs. Cav-1 KO.

of adult mice. The most important part of our study came when we were able to reproduce the data obtained in rodent brains and primary rodent neurons with the use of differentiated human neurons derived from hiPSCs.

We have previously shown that Cav-1 is essential for maintaining and stabilizing proper synaptic signaling (Head et al. 2011) and improving learning and memory in aged rodents (Mandyam et al. 2015). Several studies from our group have shown that neuron-targeted overexpression of Cav-1 promotes neuronal survival and dendritic arborization in vitro (Head et al. 2011). Accordingly, our data showed that *SynCav1* enhances expression of synaptic proteins (PSD95, neurexin, synaptobrevin, syntaxin 1, synaptophysin), important for maintaining synaptic plasticity (Calabrese et al. 2006; Guirland and Zheng 2007; Hotulainen and Hoogenraad 2010). On the other hand, loss of Cav-1 accelerates neurodegeneration (Head et al. 2010). Interestingly, our data show that loss of Cav-1 is associated with a reduction in synaptic protein expression. In fact, alteration in synaptic plasticity is responsible for many neurological and neuropsychiatric diseases (Arguello and Gogos 2012; Brennan et al. 2013; Lawrie et al. 2002; Nestler et al. 2002; Selkoe et al. 2012; Uhlhaas 2013). Taken together, our data indicate a positive role of Cav-1 in regulation of synaptic proteins and plasticity.

Risk genes and genetic mutations identified in patients with schizophrenia are involved in synaptic function (Drew et al. 2011; Frankle et al. 2003; Gogos and Gerber 2006; Lisman et

al. 2008; Xu et al. 2012). Similar studies on putative mouse models of schizophrenia have strongly suggested synaptic dysfunction (Frankle et al. 2003; Fromer et al. 2014; Hall et al. 2015; Kirov et al. 2012; Xu et al. 2012). A significant amount of studies support the possibility that DISC1 may be one of the candidate genes for schizophrenia (Callicott et al. 2005; Ekelund et al. 2001; Hamshere et al. 2005; Harrison and Weinberger 2005; Hennah et al. 2003; Hodgkinson et al. 2004; Qu et al. 2007; Song et al. 2008). The importance of DISC1 in synaptic function comes from its interaction with many proteins enriched in the synapses that regulate synaptic maturation, function, and plasticity (Brandon 2007; Camargo et al. 2007). Furthermore, DISC1 mouse models display synaptic pathologies (Lee et al. 2011) and show cognitive deficits reflecting those found in schizophrenia, such as impaired working memory (Koike et al. 2006; Kvajo et al. 2008). Additionally, it has been shown that Cav-1 KO mice present with behavioral deficits similar to those seen in many schizophrenia-like symptoms such as altered motor function and altered emotion, as well as memory deficits (Colao et al. 1998; Gioiosa et al. 2008; Head et al. 2008; Trushina et al. 2006). Because there has been much interest in understanding the neurobiology of schizophrenia over the past decade (Harrison and Weinberger 2005; Owen et al. 2005; Sawa and Snyder 2002), in the present study we investigated a potential interaction between DISC1 and Cav-1 on synapse biology. Interestingly, our data indicate that DISC1 is downregulated in Cav-1 KO models and that it localizes to the same protein complex
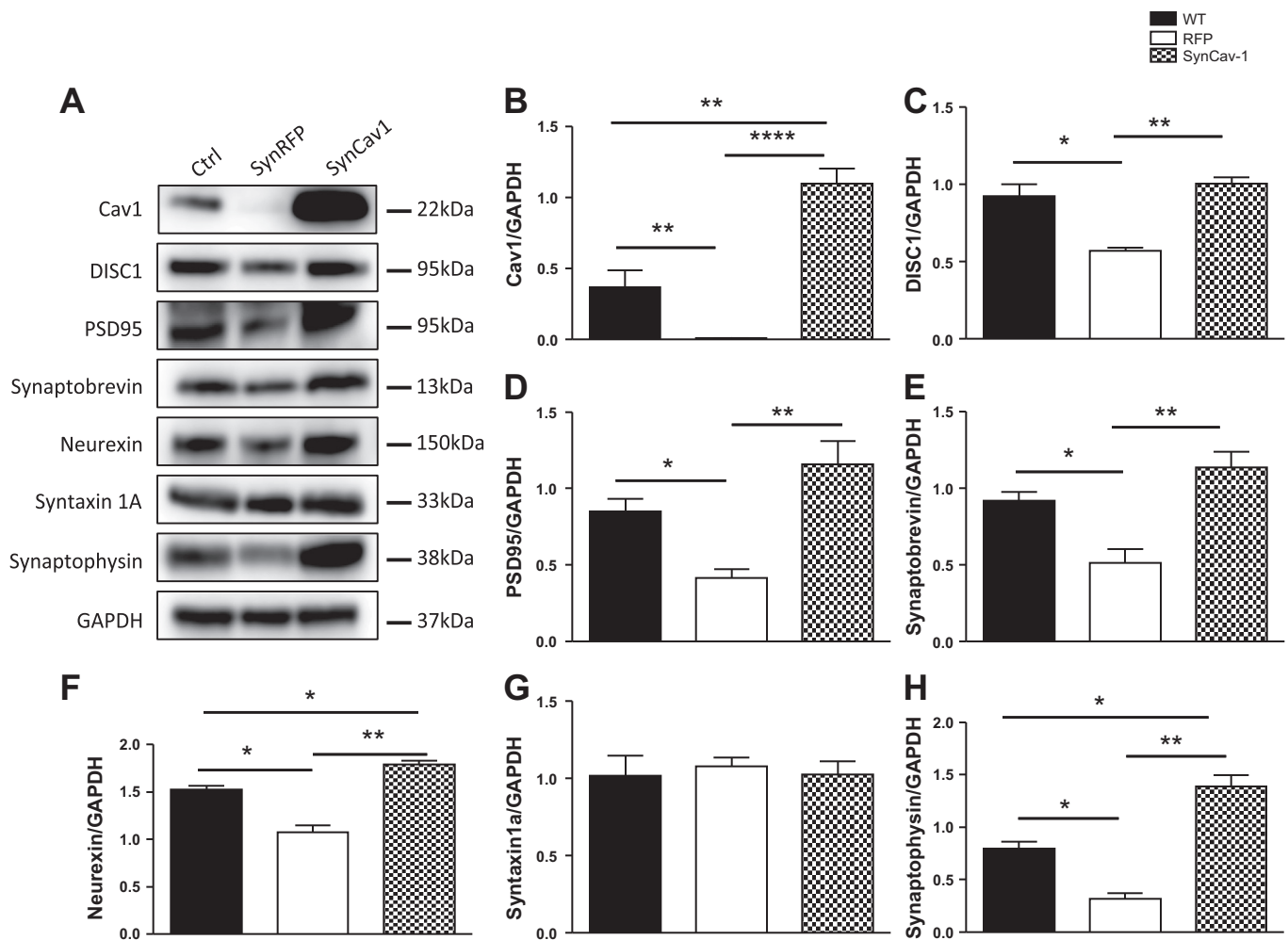
Fig. 4. Neuron-targeted expression of caveolin-1 (Cav-1) enhances expression of disrupted-in-schizophrenia-1 (DISC1) and synaptic proteins in caveolin-1 knockout (Cav-1 KO) mice hippocampi. Cav-1 KO mice (2 mo) were subjected to stereotactic injection of adeno-associated virus serotype 9 (AAV9) containing *SynCav1* or synapsin-red fluorescent protein (SynRFP; as control). Mice were killed 1 mo later and hippocampi were collected and homogenized. Homogenates were immunoblotted for Cav-1, DISC1, PSD95, synaptobrevin, neurexin 1, syntaxin 1A, syntaxin, synaptophysin, and GAPDH (*A*). Western blot quantification showed that *SynCav1* injection restored the Cav-1 expression (*B*) and significantly increased the expression levels of DISC1 (*C*) and other synaptic proteins: PSD95 (*D*), synaptobrevin (*E*), neurexin 1 (*F*) and synaptobrevin (*H*). No significant difference was detected in syntaxin 1A (*G*). Representative blots are from $n = 4$ experiments. $*P < 0.05$; $**P < 0.01$; $****P < 0.0001$, *SynCav1* vs. *SynRFP*.

with Cav-1. Additionally, *SynCav1* led to upregulation in DISC1 protein expression.

A limitation of the present study is a lack of understanding of the cellular mechanism through which Cav-1 regulates DISC1 expression. Previous work from our group has shown that Cav-1 colocalizes and coimmunoprecipitates with NMDARs and that loss of Cav-1 disrupts NMDAR-mediated signaling, NMDAR-mediated cAMP production, and NMDAR-mediated neuroprotection against oxygen-glucose deprivation (Head et al. 2008, 2011). Interestingly, others have shown in certain mouse models involving reduced NMDAR expression that these mice also have decreased DISC1 levels and exhibit schizophrenia-like mental disorders such as increased motor activity and deficits in social and sexual interactions (Mohn et al. 1999). The modulation of DISC1 by Cav-1 could also involve cAMP signaling. Previous studies have shown that DISC1 regulates cAMP production through its interaction with certain phosphodiesterases (e.g., PDE4) in postsynaptic densities (Bradshaw et al. 2011; Gamo et al. 2013; Soda et al. 2013; Wang et al. 2008). Based on our

past and current findings that *SynCav1* increases NMDAR and DISC1 expression and augments NMDAR-mediated cAMP production (Head et al. 2011), it is conceivable that *SynCav1* could potentially reverse the schizophrenia-like behavioral phenotype in a DISC1/cAMP-dependent signaling pathway. More experiments are needed to confirm this hypothesis.

Pharmacological agents intended to treat schizophrenia are limited to antipsychotic drugs, which exert their effects through blockade of the type 2 dopaminergic receptor (Carlsson and Lindqvist 1963). However, these agents show little efficacy, suggesting a need to discover novel molecular targets and approaches to develop and improve delivery of more effective therapies. Interestingly, Cav-1 KO mice are resistant to atypical antipsychotic drugs (Allen et al. 2011). In this context, Cav-1 is a scaffold for D_2-dopamine (Somkuwar et al. 2016) and 5-HT_{2A} receptors, which represent canonical targets for typical and atypical antipsychotic drugs (Bhatnagar et al. 2004; Genedani et al. 2005; Meltzer et al. 1989; Roth et al. 2004). Cav-1 KO mice also exhibit increased sensitivity to psychomimetic effects of phencyclidine (PCP), a phenomenon
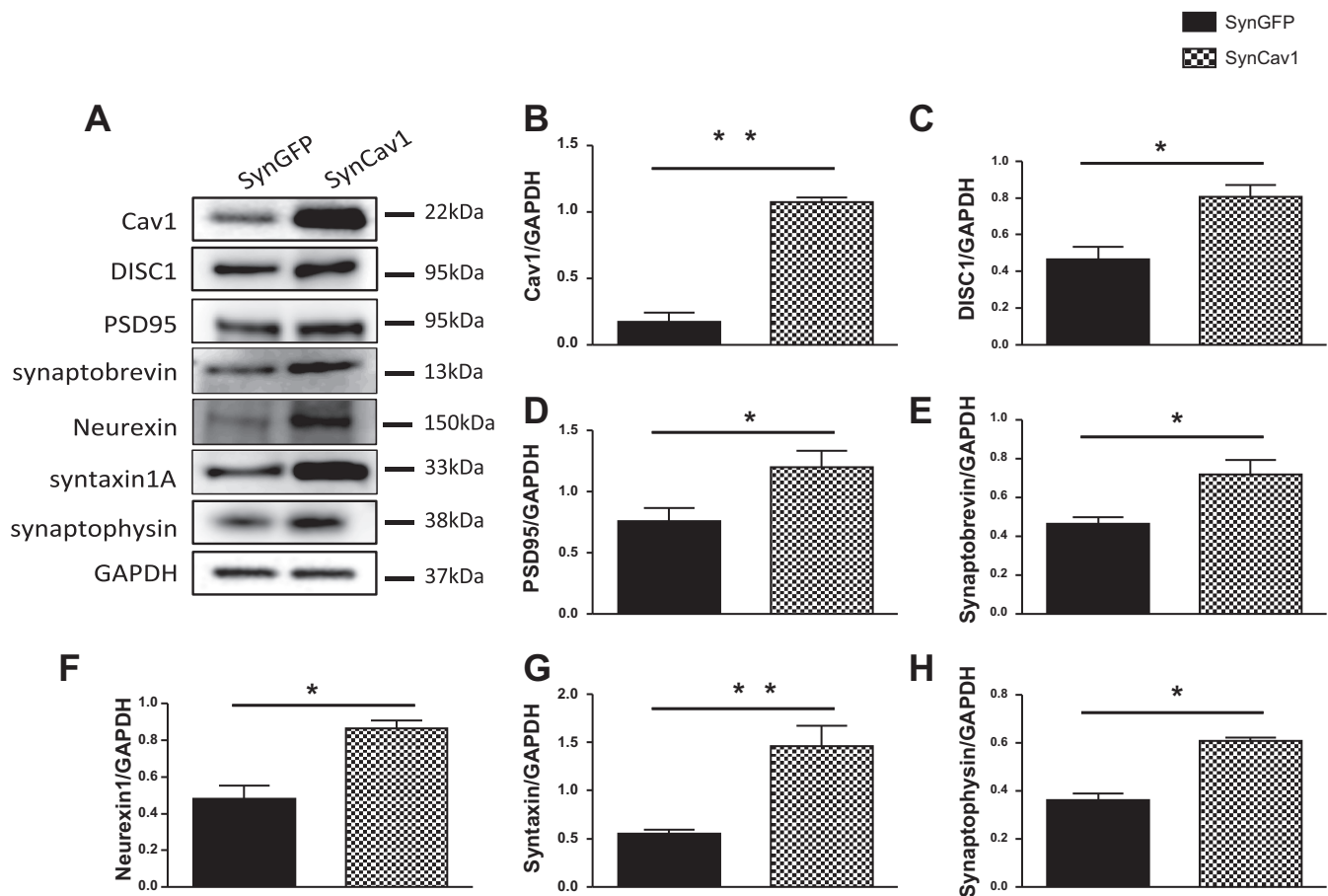
Fig. 5. Neuron-targeted expression of caveolin-1 (Cav-1) enhances expression of disrupted-in-schizophrenia-1 (DISC1) and synaptic proteins in human differentiated primary neurons. The human neurons were differentiated from the Craig Venter 4a neuronal stem cells, which are derived from human induced pluripotent stem cells. Neurons were differentiated in differentiating media for 3–4 wk. After differentiation, neurons were infected by a lentivirus containing the Cav-1 driven by a synapsin promoter (HIV-synCAV1, or *SynCav1*) for 72 h. *SynGFP* served as control vector (10^9 viral particle from both vectors). Homogenates were immunoblotted for Cav-1, DISC1, PSD95, synaptobrevin, neurexin 1, syntaxin 1A, synaptophysin, and GAPDH (*A*). Quantification of Western blots showed overexpression of Cav-1 protein (*B*). *SynCav1* also significantly enhanced the protein expression of DISC1 (*C*) and other synaptic proteins: PSD95 (*D*), synaptobrevin (*E*), neurexin 1 (*F*), syntaxin 1A(*G*), and synaptophysin (*H*). Representative blots are from $n = 4$ experiments. *$P < 0.05$; **$P < 0.01$, *SynCav1* vs. *SynGFP*.

observed in patients with schizophrenia (Lahti et al. 1995; Luby et al. 1959). For instance, PCP significantly disrupted prepulse inhibition (PPI; the magnitude of response as a function of repeated stimuli) in Cav-1 KO mice and increased locomotor activity (Allen et al. 2011). These findings suggest the possibility that restoring Cav-1 could in one way reduce nonresponsiveness to antipsychotics through modulation of certain molecular targets such as DISC1 or synaptic proteins. Therefore, we believe that maintaining the neuronal function by specifically targeting membrane microdomains (i.e., MLR) and associated synaptic proteins may be potential treatment for this psychiatric disorder.

In summary, the present findings are the first to demonstrate that genetic manipulation of the scaffolding protein Cav-1 specifically in neurons both in vitro and in vivo directly regulates expression of DISC1 and maintains synaptic protein expression essential for neuronal function, synapse formation, and plasticity. Neuronal Cav-1 maybe a control point for neurotransmission and neuromodulation, which are otherwise impaired in those afflicted with schizophrenia. Further understanding of how Cav-1 modulates DISC1 to maintain and organize neuronal growth, signaling, and proper function is of

utmost importance to better understand and identify potential molecular targets for treating schizophrenia.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

A.K., J.E., Z.Z., A.A.-Q., Q.M.N., Y.L., K.K., and E.P. performed experiments; A.K. analyzed data; A.K. interpreted results of experiments; A.K. prepared figures; A.K. drafted manuscript; A.K., D.V.J., D.M.R., P.M.P., H.H.P., and B.P.H. edited and revised manuscript; A.K. and B.P.H. approved final version of manuscript.

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