2018

A Potential Role For Sap97 In Psychiatric Disorders

Preetika Gupta
University of Pennsylvania, preetika@pennmedicine.upenn.edu

A Potential Role For Sap97 In Psychiatric Disorders

Abstract
The goal of this dissertation is to further understand the genetic architecture of neuropsychiatric disorders, such as autism spectrum disorder (ASD) and schizophrenia (SCZ). We attempt to understand the functional significance of the gene synapse associated protein of 97KDa (SAP97) and identify a novel role for SAP97 in the etiology of neuropsychiatric disorders.

SAP97 belongs to a family of scaffolding proteins, the membrane-associated guanylate kinases (MAGUKs), that are highly enriched in the postsynaptic density of synapses and play an important role in organizing protein complexes necessary for synaptic development and plasticity. Large-scale genetic studies have implicated MAGUKs in neuropsychiatric disorders such as intellectual disability, ASD, and SCZ, but knock-out mice have been impossible to study because the Sap97 null mice die soon after birth due to a craniofacial defect. In Chapter 2, we studied the transcriptomic and behavioral consequences of a viable, brain-specific conditional knockout of Sap97 (SAP97-cKO). RNA sequencing (RNAseq) from hippocampi from control and SAP97-cKO male animals identified 67 differentially expressed transcripts, which were specifically enriched for SCZ-related genes. Subjecting SAP97-cKO mice to a battery of behavioral tests revealed a subtle anxiety-like phenotype present in both male and female SAP97-cKO animals, as well as a mild male-specific cognitive deficit and female-specific motor learning deficit. Collectively, this work suggests that loss of Sap97 alters behavior, and may contribute to some of the endophenotypes present in SCZ. In Chapter 3, we discuss how the SAP97-cKO mouse may serve as a novel model system for interrogating aspects of the cellular and molecular defects underlying SCZ and other related neuropsychiatric disorders.

Degree Type
Dissertation

Degree Name
Doctor of Philosophy (PhD)

Graduate Group
Neuroscience

First Advisor
Robert G. Kalb

Keywords
Autism, MAGUK, mouse model, SAP97, Schizophrenia

Subject Categories
Neuroscience and Neurobiology

A POTENTIAL ROLE FOR SAP97 IN PSYCHIATRIC DISORDERS

Preetika Gupta

A DISSERTATION

in

Neuroscience

Presented to the Faculties of the University of Pennsylvania

in

Partial Fulfillment of the Requirements for the

Degree of Doctor of Philosophy

2018

Supervisor of Dissertation

_____

Dr. Robert G. Kalb, M.D.

Professor of Neurology, Northwestern University Feinberg School of Medicine

Graduate Group Chairperson

_____

Dr. Joshua I. Gold, Ph.D.

Professor of Neuroscience, Perelman School of Medicine at the University of Pennsylvania

Dissertation Committee

Dr. Minghong Ma, Ph.D.—Professor of Neuroscience

Dr. Marc Fuccillo, M.D., Ph.D.—Assistant Professor of Neuroscience

Dr. Zhaolan Zhou, Ph.D.—Associate Professor of Genetics

Dr. Matthew Dalva, Ph.D.—Professor of Neuroscience and Vice Chair

DEDICATION

This thesis is dedicated to my family, Sunil and Seema Gupta, Utsav Gupta, Rebecca Neff, Ethan Neff (dog), and Rajni Gupta (cat). Thank you for always helping me become a better version of myself, while standing by my side throughout the entire process.

“most importantly love
like it’s the only thing you know how
at the end of the day all this
means nothing
this page
where you’re sitting
your degree
your job
the money
nothing even matters
except love and human connection
who you loved
and how deeply you loved them
how you touched the people around you
and how much you gave them”
--Rupi Kaur, from *milk and honey*

ACKNOWLEDGMENT

To begin, I would like to thank my thesis advisor, Dr. Robert G. Kalb, for his guidance and mentorship throughout my graduate school career. Bob, thank you for providing a working environment where people are encouraged to pursue the questions they find most engaging. I am grateful to you for allowing me the independence to carry out research I found meaningful, and I am a better scientist as a result. Thank you for your positive perspective, encouragement, and partaking in and tying the "Who has the messiest lab bench?" competition with me.

This dissertation project would not have been possible without many collaborators. I would like to acknowledge Soumyashant Nayak, Gregory Grant, Komal Rathi, and Deanne Taylor for their hard work, contributions, and bioinformatics expertise. I would also like to thank the members of my thesis committee, Minghong Ma, Marc Fuccillo, Joe Zhou, Ted Abel, and Matthew Dalva for their time and insight. Thank you for shaping this project into the final product that it is today.

I'd also like to thank all past and current members of the Kalb Lab for making it a fun place to come to every day. Special thanks to Ogul E. Uner (now a medical student at Emory University), for his hard work and contribution to this thesis project, and his never-faltering rosy outlook on life. I would also like to extend special thanks to the Kalb Lab "stalwarts", Shachee Doshi and Heather Bennett, who stayed behind in Philadelphia after the lab relocated to Northwestern University. Thank you for your support, teamwork, and humor through the peaks and valleys of science.

I am also incredibly grateful to the Neuroscience Graduate Group (NGG), including the administrators, faculty, and students. Special thanks to Josh Gold, for creating a graduate training program that encourages students to voice their thoughts

and pursue opportunities outside of the lab. I would also like to thank the NGG Coordinator and Assistant Coordinator, Christine Clay and Thomas Hindman, for their superhuman organizational skills. You made the nitty-grittiness of this process feel flawless, and I am incredibly grateful for your genuine care for all NGG students.

My passion for science was born thanks to my alma matter, the University of California, San Diego. Thank you to the Roberto Malinow lab for accepting me as a naïve undergraduate student and allowing me the opportunity to learn and grow. Special thanks to my direct advisor in the lab, Dr. Christophe Proulx. Christophe, thank you for teaching me how to think like a scientist. Thank you for trusting me with important work (even when I didn't know how to pipette), and providing me with life advice that I still use to this day.

Being on the opposite side of the country as my family has been incredibly challenging over the past six years. I found my east coast adoptive family in my taekwon-do school, Red Tiger. I am incredibly grateful to Masters Marcello, Mario, and Monica for inspiring me to train my hardest and achieve my 1st degree black belt. Master Marcello, thank you for providing a dojang where each student feels welcome and important. Thank you for dedicating your time and energy to the progress of your students. And lastly, thank you for teaching me that being a nice person does not equate to being a weak person. Red Tiger will always be my east coast family.

I would also like to thank my best friends, Ariel Badger and Carolyn Thickett. We've known each other for more than a decade, and in that time, you two have witnessed the days I've shined the brightest, and have also stood by me during some dark, not-so-pretty times. I am grateful for all the girls-nights, e-mails, texts, phone conversations, and belly-aching laughter. I am also incredibly grateful for your

constructive feedback and advice, never done in a judgmental way, but always in a way that made me feel loved and accepted.

Thank you to my family—my parents, Sunil and Seema Gupta, my older brother Utsav, and soon-to-be sister-in-law Rebecca Neff. Utsav, thank you for always letting me be the Player 2 to your Player 1 throughout our childhood years of gaming (even when you probably preferred one of the guys). Even though I hated it at the time, thank you for designing and making me complete extra homework worksheets to "make me smarter" (they probably helped). And lastly, thank you for never letting me feel sorry for myself and reminding me that I have the power to change my reality (Preetika: "I got a D on the midterm. I'm doomed!" Utsav: "So? You're making excuses. Just get 100% on the next exam." Preetika: "Oh, yeah, ok." *Preetika studies hard, gets 100% on next exam, and raises grade to an A, The Beginning*). Becky, thank you for being both an older sister and friend. I've felt nothing but warmth, kindness, and generosity from you. Thank you for looking out for me and sharing your big heart. Mom and Dad, I wouldn't be where I am today if it weren't for you. Thank you for always encouraging me to work hard and be the best version of myself. Thank you for always being there when I needed you most. Thank you for being my biggest cheerleaders, and believing in me when I wasn't able to believe in myself.

And last, but not least, I'd like to thank all the mice that sacrificed their lives to the data for this thesis work. None of this knowledge would be known without the help of the little guys.

ABSTRACT

A POTENTIAL ROLE FOR SAP97 IN PSYCHIATRIC DISORDERS

Preetika Gupta

Robert G. Kalb

The goal of this dissertation is to further understand the genetic architecture of neuropsychiatric disorders, such as autism spectrum disorder (ASD) and schizophrenia (SCZ). We attempt to understand the functional significance of the gene synapse associated protein of 97KDa (*SAP97*) and identify a novel role for *SAP97* in the etiology of neuropsychiatric disorders.

SAP97 belongs to a family of scaffolding proteins, the membrane-associated guanylate kinases (MAGUKs), that are highly enriched in the postsynaptic density of synapses and play an important role in organizing protein complexes necessary for synaptic development and plasticity. Large-scale genetic studies have implicated MAGUKs in neuropsychiatric disorders such as intellectual disability, ASD, and SCZ, but knock-out mice have been impossible to study because the *Sap97* null mice die soon after birth due to a craniofacial defect. In Chapter 2, we studied the transcriptomic and behavioral consequences of a viable, brain-specific conditional knockout of *Sap97* (SAP97-cKO). RNA sequencing (RNAseq) from hippocampi from control and SAP97-cKO male animals identified 67 differentially expressed transcripts, which were specifically enriched for SCZ-related genes. Subjecting SAP97-cKO mice to a battery of behavioral tests revealed a subtle anxiety-like phenotype present in both male and female SAP97-cKO animals, as well as a mild male-specific cognitive deficit and female-specific motor

learning deficit. Collectively, this work suggests that loss of *Sap97* alters behavior, and may contribute to some of the endophenotypes present in SCZ. In Chapter 3, we discuss how the SAP97-cKO mouse may serve as a novel model system for interrogating aspects of the cellular and molecular defects underlying SCZ and other related neuropsychiatric disorders.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

CHAPTER 1: INTRODUCTION

This thesis attempts to elucidate the potential contribution of *SAP97* to neuropsychiatric disorders, such as autism spectrum disorder and schizophrenia. In particular, we examine the effects of loss of *Sap97* to mouse behavior and alterations in the transcriptome. Here, I will outline the critical information needed to understand the progress made in this subject.

SYNAPTOPATHIES: DISORDERS OF THE SYNAPSE

The genomic revolution has transformed the field of neuroscience by providing a platform to decipher the brain and its disorders. Advancements in whole exome and deep sequencing technologies allow for examination of the genetic architecture of psychiatric disorders using large patient cohorts. These large-scale genetic studies have implicated key overlapping molecular and cellular pathways that are impacted across mental disorders, such as chromatin regulation and the post-synaptic density, with dysfunction of the synapse being a convergence point (Grant, 2012; De Rubeis et al., 2014; McCarthy et al., 2014; Ardiles et al., 2017; Luo et al., 2017). This observation coined the term “synaptopathies,” or diseases of the synapse, to collectively classify these disorders.

Human mutations in genes encoding synaptic proteins are increasingly identified in neurodevelopmental disorders such as epilepsy, intellectual disability, autism spectrum disorder, and schizophrenia. While these disorders each present with unique symptoms, there is increasing recognition of the overlapping comorbidities between these disorders, in addition to the large genetic heterogeneity within these disorders

(Luo et al., 2017). As a result, addressing the common underlying genetic mechanisms may be key for developing therapies across a broad range of behavioral symptoms.

In this thesis, we choose to focus on the potential contribution of *SAP97* to autism spectrum disorder and schizophrenia, two neuropsychiatric conditions that are prevalent in today's society and share a subset of risk-associated genes. Below, we provide a background for each disorder, and discuss both the importance and complications associated with each current genetic model.

AUTISM SPECTRUM DISORDERS

Epidemiology and clinical presentation

Autism spectrum disorder (ASD) is diagnosed in approximately 1 out of 110 children in the United States and is 4-5 times more common in male children than in females (The Autism Genome Project Consortium, 2007; Sungur et al., 2017). Newer prospective studies of younger siblings of children with ASD who are at elevated risk provide evidence that the age of onset for the majority of cases of ASD is the second year of life (Zwaigenbaum et al., 2006; Martínez-Pedraza and Carter, 2009). Several studies document that the mean age at which parents first report concerns to a medical professional is between 18 and 24 months of age (Sullivan et al., 2007; Martínez-Pedraza and Carter, 2009). Although ASD behaviors lie on a continuum in the general population, individuals with ASD are characterized by severe and pervasive impairments in reciprocal social interaction and communication and exhibit stereotyped behaviors, as well as restricted interests and activities (Martínez-Pedraza and Carter, 2009). Most parents first report concerns in the area of speech and language in addition to extreme sensory over-or underreactivity and disturbances in the acquisition of social

communication, play, and motor development (Young et al., 2003). Parents also report concerns regarding sleep and eating, which may be associated with sensory sensitivities or with the insistence on sameness (Goodlin-Jones et al., 2008; Sharp et al., 2013).

Neuropathologic profile of ASD

Neuropathologic explorations in human postmortem tissue allow for investigation at the cellular and cytoarchitectural levels from individuals with ASD. Global brain development abnormalities are seen in the archicortex, cerebellum, brainstem, and other subcortical structures, with region-specific severity of neuropathology in young children with ASD (Wegiel et al., 2014; 2015). Brain size as well as head circumference of a subset of subjects with ASD is increased compared to normal age-based values (Sacco et al., 2015). An assessment of the microarchitecture of cortical areas commonly implicated in ASD between subjects with ASD and controls reveals disorganization of gray and white matter, and disorganized cortical structure and nodules of misplaced neurons (Casanova et al., 2013; Stoner et al., 2014; Wegiel et al., 2015). These defects reflect alterations in neuronal maturation and migration processes in subjects with ASD. A study from one subject with ASD described finding pencil fibers consisting of oligodendrocytes, astrocytes, and glia that disrupted cortical lamination in the prefrontal cortex (Hashemi et al., 2016).

Young children with ASD also have significantly reduced neuronal and cytoplasmic volumes in the majority of examined areas compared to age-matched controls (Wegiel et al., 2014; 2015). The distribution of neuronal sizes becomes more comparable between control and ASD individuals in adulthood, which is likely a result of opposing developmental trajectories (Wegiel et al., 2014; 2015). Subjects with ASD also have a significant increase in neuropil, comprising the dendrites, non-myelinated axons,

synapses, vasculature, and glial cell processes present in between cell bodies (Varghese et al., 2017). Neurons show reduced dendritic branching in the hippocampus of ASD subjects as compared to controls (Raymond et al., 1996). Studies measuring spine density on apical dendrites in the cortex of ASD subjects report slower pruning of spines in the temporal lobe (Hutsler and Zhang, 2010; Tang et al., 2014). This results in the difference in spine densities between ASD and controls being greater in adolescence than in early childhood (Tang et al., 2014). These changes appear to be related to alterations that occur during early pregnancy, such as reduced programmed cell death and/or increased cell proliferation, altered cell migration, abnormal cell differentiation, abnormal neurite sprouting, and pruning that cause atypical wiring of the brain (Lacivita et al., 2017).

The biggest motivation for studying ASD is that the disorder has no known cure. Psychotropic medications currently available alleviate psychiatric and behavior problems, such as aggression, self-injury, hyperactivity, anxiety, and mood symptoms, but they do not have an effect on the core symptoms of ASD (Young and Findling, 2015). To date, the only approved drugs to treat symptoms of ASD are risperidone and aripiprazole, both used to treat aggression, self-injury, and severe tantrums (Lacivita et al., 2017). The lack of treatment is due in part to the multifactorial nature of the disorder.

Another motivation for studying ASD is that prevalence rates have dramatically increased in the past decade (Christensen et al., 2016). There are various reasons for this increase, including broadening of the spectrum to include milder forms of the disorder, improved clinical detection, and higher public awareness (Levy et al., 2009). As a result, ASD has recently emerged as a major public health issue worldwide. Due to the lack of promising treatment, there is an urgent need for ASD research. One of the obstacles in studying the disorder is that hundreds of risk genes have been identified,

with not one major causative gene. Rare variants have been identified that are highly penetrant, and common variants can contribute to small effect sizes (Lacivita et al., 2017). Below, we discuss what is currently known regarding the genetic underpinnings of ASD.

Genetic susceptibility to ASD

Interestingly, family genetic data supports a first-degree relative recurrence risk of approximately 5-10%, which points to disruption of genetic architecture as being a leading cause of the disease (Ritvo et al., 1989; Sumi et al., 2006). It is believed that the neurocognitive phenotype of ASD is the result of a complex and highly heterogeneous set of genetic and environmental causes (Lacivita et al., 2017). In some patients, the cause of the disorder is purely genetic (due to known chromosomal mutations), while in other patients, the disorder is more likely related to environmental causes such as prenatal exposure to chemical pollutants, toxins, viruses, or drugs (Persico and Merelli, 2014; Lacivita et al., 2017). For this thesis work, we have chosen to focus primarily on the genetic contribution to ASD and other related psychiatric disorders. The genetic abnormalities associated with ASD and other related psychiatric disorders may be grouped into three classes: 1) at least 5% are caused by single gene mutations, 2) approximately 10% are copy number variations including duplications, large deletions, inversions, and translocations of chromosomes, and 3) many are polygenic risk factors due to accumulation of common variants, each contributing to a portion of the risk (Varghese et al., 2017). Most research done in the laboratory to model and study the unique contribution of each risk-associated gene utilizes rodent models.

GENETIC MOUSE MODELS OF ASD

There are two types of animal models for ASD: environmentally induced (by exposure of the pregnant animals to certain toxins or infection/inflammation) and those that are induced by genetic manipulations. In this introduction, we have chosen to focus on genetic mouse models of ASD. More than a hundred *de novo* single gene mutations and copy-number variants have been implicated in ASD, each occurring in a small subset of cases (Kazdoba et al., 2015). Mutant mouse models with syntenic mutations offer investigators a tool for understanding the role of each gene in modulating biological and behavioral phenotypes relevant to ASD (Kazdoba et al., 2015). Investigations of ASD, schizophrenia, and other related psychiatric disorders indicate a highly polygenic architecture with small effect sizes of each implicated risk variant (Ebert and Greenberg, 2013; Fromer et al., 2014; Kato, 2014; Smoller et al., 2018). As a result, mouse modeling of these disorders by targeting one such risk variant typically demonstrates a moderate, or incomplete manifestation of the human disorder. Below, we have highlighted the most prominent genetic mouse models of ASD. Extensive characterization of these models demonstrates that while ASD is genetically complex, these studies are useful in describing the direct contribution of each gene.

As mentioned previously, a remarkable number of risk genes for ASD code for synaptic proteins, including cell adhesion proteins, neuroligins and neurexins, and postsynaptic scaffolding proteins such as the *PROSAP/SHANK* family. Mice with targeted mutations in many of these genes have been generated and characterized, as described below.

CNTNAP2

The contactin associated protein-like 2 (*CNTNAP2*) gene, a cell adhesion

molecule located on chromosome 7, encodes contactin-associated protein-like 2 (*CASPR2*), a member of the neurexin superfamily (Rodenas-Cuadrado et al., 2013). Several mutations in the *CNTNAP2* locus, including rare, common and deletion variants, have been associated with ASD (Alarcón et al., 2008; Arking et al., 2008; Rossi et al., 2008; Poot et al., 2009). A recessive nonsense mutation in *CNTNAP2* was shown to cause a syndromic form of ASD, cortical dysplasia, and focal epilepsy syndrome (Alarcón et al., 2008; Arking et al., 2008). The *CNTNAP2* variant that increases risk for the language endophenotype in ASD was shown to lead to abnormal functional brain connectivity in human subjects (Weinstein-Fudim and Ornoy, 2016). Knockout mice for the mutation show migration abnormalities, reduced number of interneurons, and abnormal neuronal network activity (Scott-Van Zeeland et al., 2010). Mice lacking *Cntnap2* also exhibit behavioral abnormalities such as reduced juvenile ultrasonic vocalizations, reduced social interaction time, and increased repetitive behaviors (Peñagarikano et al., 2011).

Neuroligins and Neurexins

Neuroligins are cell adhesion molecules located at the postsynaptic side of the synapse and interact with neurexins, their presynaptic partner protein (Bang and Owczarek, 2013). Neuroligins contribute to synaptic neurotransmission through their influence on synaptic formation (Hu et al., 2015). Neuroligin (NLGN) proteins encoded by X-linked genes, such as *NLGN3* and *NLGN4*, have been associated with ASD in large genome-wide studies (Auranen et al., 2002; Glessner et al., 2009). Using amino acid sequencing in linkage and proband case studies, deletions and frameshifts in *NLGN3* and *NLGN4* sequences have been identified in individuals with ASD (Laumonier et al., 2004; Lawson-Yuen et al., 2008). Knockout mouse models have been created for

four neuroligin isoforms—*Nlgn1*, *Nlgn2*, *Nlgn3*, and *Nlgn4*. *Nlgn1* KO mice show minimal social deficits, but have increased grooming and spatial learning impairments along with impaired hippocampal long-term potentiation (Blundell et al., 2010). *Nlgn2* KO mice show no social deficits, but display increased anxiety-like behavior, decreased pain sensitivity, and poor motor coordination (Blundell et al., 2010; Wöhr et al., 2013). In addition, *Nlgn2* KO mice had decreased inhibitory neurotransmission, as well as decreased immunostaining of inhibitory synapse markers (Blundell et al., 2010). *Nlgn3* knock-in (R451C) mice, with a ASD-related point mutation, did not display robust ASD-like behaviors, but rather had mild developmental differences, enhanced spatial learning, and reduced acoustic startle (Tabuchi et al., 2007; Chadman et al., 2008; Etherton et al., 2011). These results would suggest that this ASD-related point mutation delayed development, altered learning, and reduced sensitivity to stimuli. *Nlgn* knock-in mice also exhibited increased inhibitory neurotransmission in the barrel cortex, increased excitatory neurotransmission and enhanced long-term potentiation in the hippocampus, and increased dendritic branching in the hippocampus (Tabuchi et al., 2007; Etherton et al., 2011). *Nlgn3* KO mice show no social deficits, but are impaired in fear conditioning and olfaction, and are hyperactive. *Nlgn3* KO mice also show decreased total brain volume (Radyushkin et al., 2009). And lastly, *Nlgn4* KO mice show reduced sociability and ultrasonic vocalizations, as well as a reduction in total brain volume (Jamain et al., 2008; El-Kordi et al., 2013). Genetically modified mice have also been made for neurexins (NRXN), the neuroligin partner protein. Numerous association studies have identified mutations in the *NRXN1* gene, located on chromosome 2, in intellectual disabilities and ASD (Feng et al., 2006; Szatmari et al., 2007; Zahir et al., 2007; Glessner et al., 2009). *Nrxn1* KO mice display increased grooming, reduced locomotor activity, reduced sensorimotor gating, and increased aggression (Etherton et al., 2009;

Grayton et al., 2013). Together, these studies demonstrate that the *Nlgn* and *Nrxn* genes may not play a prominent role in social behavior, but may instead regulate anxiety and cognition.

SHANK/ProSAP2 Family

The *SHANK* family of genes, located on chromosome 22q, encodes scaffolding proteins that assist in the synaptic organization of excitatory glutamatergic neurons by binding to postsynaptic density proteins, signaling molecules, postsynaptic receptors, and cytoskeletal proteins (Grabrucker et al., 2014). Genetic studies have identified *de novo* and inherited mutations in *SHANK1*, *SHANK2*, and *SHANK3* (Berkel et al., 2010; Boccuto et al., 2012; Sato et al., 2012). 22q13 deletion syndrome, also known as Phelan-McDermid syndrome, is caused by a deletion on the distal part of the long arm of chromosome 22 and is associated with ASD-like behaviors (Phelan and McDermid, 2011; Kolevzon et al., 2015). *SHANK3* is one of the most commonly mutated genes within the Phelan-McDermid critical region (Phelan and McDermid, 2011). Genetically-modified mouse models have been generated and characterized for the three *Shank* isoforms. *Shank1* KO mice do not display robust social deficits, but emit fewer ultrasonic vocalizations and have motor impairments (Silverman et al., 2011; Wöhr et al., 2011). *Shank1* KO mice also display dendritic spine abnormalities, including weaker basal synaptic neurotransmission (Hung et al., 2008). *Shank2* KO mice, however, show reduced sociability in addition to abnormal ultrasonic vocalizations (Schmeisser et al., 2012). *Shank2* KO mice also had reduced number of hippocampal dendritic spines and reduced glutamatergic neurotransmission in the hippocampus (Schmeisser et al., 2012). Multiple transgenic mouse models of *Shank3*, with deletions in various domains of the gene, have also been generated and characterized. Reduced sociability, reduced

ultrasonic vocalizations, and high levels of repetitive self-grooming were dependent upon which isoform of *Shank3* was deleted (Peça et al., 2011; Wang et al., 2011b; Kouser et al., 2013). Reduced basal neurotransmission, as well as abnormalities in neuronal morphology (neuronal hypertrophy, dendritic spine deficits) have been identified in most of these models (Peça et al., 2011; Wang et al., 2011b; Kouser et al., 2013). Overall, these studies highlight that the *Shank* gene family may be responsible for normal social behavior, maintaining normal synaptic function and neuronal structure, and that complete or partial loss of *Shank* may also induce repetitive behaviors.

SCHIZOPHRENIA

Epidemiology and clinical presentation

In addition to ASD, we also chose to examine the potential contribution of *SAP97* to schizophrenia (SCZ). SCZ affects approximately 5 out of every 1000 individuals (Wu et al., 2006). The age of onset varies between men and women, where men tend to have a younger onset, with the peak incidence for men and women lies between 15-24 years of age (Wu et al., 2006). Men have about a 30-40% higher lifetime risk of developing SCZ. Like many other psychiatric disorders, SCZ is diagnosed by its symptoms, which fall into three main categories: positive, negative, and cognitive. Positive symptoms include hallucinations, delusions, and disorganized thinking (Lehman et al., 2006; Tandon et al., 2009). Negative symptoms include social withdrawal, blunted affect, and a decreased in incentive motivation (Lehman et al., 2006; Tandon et al., 2009). Cognitive symptoms encompass deficits in processing speed, working memory, attentional set-shifting, and verbal memory (Lehman et al., 2006; Tandon et al., 2009). Typically, the negative and cognitive symptoms are more predictive for the long-term prognosis of the disorder (Green et al., 2000). Similar to ASD, there is evidence

suggesting a strong genetic component of SCZ. Classical twin studies demonstrated a 50% concordance rate for SCZ among monozygotic twins and a reduced rate of 15% if the twins are dizygotic (Canetta and Kellendonk, 2018).

Neuropathologic profile of SCZ

Abnormalities in neurodevelopment might be responsible for the cognitive deficits in SCZ (Tripathi et al., 2018). In SCZ, abnormal brain development begins as early as prenatal life, which intensifies during childhood and continues until adulthood (Tripathi et al., 2018). Many brain areas are altered in SCZ, such as the third and lateral ventricles, prefrontal cortex, amygdala, medial temporal lobe, basal ganglia, thalamus, corpus collosum, and cerebellum (Tripathi et al., 2018). Abnormalities in neurotransmission, including the neurotransmitters dopamine, serotonin, and glutamate, have also provided the basis for theories on the pathophysiology of SCZ (Lavretsky et al., 2008). Other theories implicate aspartate, glycine, and GABA as part of the neurochemical imbalance of SCZ (Lavretsky et al., 2008). The core symptoms of SCZ, such as negative symptoms and executive dysfunction, are thought to result directly from altered neuroplasticity (Voineskos et al., 2013). SCZ alters brain derived neurotrophic factor (BDNF), which is associated with hippocampal neuroplasticity, attributing to the cognitive deficits present in the disorder (Nieto, 2013). Abnormal activity at dopamine receptor sites is also thought to be associated with many symptoms of SCZ. Low dopamine levels within the nigrostriatal pathway are thought to affect the extrapyramidal system, leading to motor symptoms (Lavretsky et al., 2008; Patel et al., 2014). The mesolimbic pathway may play a role in the positive symptoms of SCZ in the presence of excess dopamine (Lavretsky et al., 2008; Patel et al., 2014). Negative symptoms and cognitive deficits may also be due to low mesocortical dopamine levels (Lavretsky et al., 2008).

One of the leading motivations for studying SCZ is that is has no known cure. Current pharmacological agents, such as second-generation antipsychotics, are used to treat the symptoms of the disorder rather than the underlying cause (Lewis and Lieberman, 2018). However, similar to ASD and many other neuropsychiatric disorders, one obstacle in studying the genetic cause of SCZ is that whole genome studies have identified over 100 gene variants that are associated with the disorder (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). While few studies of common variants have produced important insight into possible biological mechanisms of SCZ, most common variants only minimally increase the risk for the disorder. In order to elucidate the contributions of each genetic variant to the etiology of SCZ, various genetic mouse models have been generated and characterized.

GENETIC MOUSE MODELS OF SCHIZOPHRENIA

SCZ has both genetic and environmental components, and an attempt has been made to model both aspects of the disorder. In this thesis introduction, we focus on describing genetic models of SCZ. Several mouse models have been useful for studying the behavioral consequences of specific synaptic gene alterations and the mechanisms potentially underlying the pathogenesis of SCZ.

Neuregulin

Neuregulin 1 (*NRG1*) is part of a family of growth and differentiation factors, and was first suggested as a potential candidate gene for SCZ in a study of the Icelandic population (Stefansson et al., 2002). This association between *NRG1* and SCZ was later confirmed in Scottish and Irish populations (Stefansson et al., 2002; Corvin et al., 2004). *NRG1* is essential for neurodevelopment, with key roles in synapse formation,

neuronal migration, synaptic plasticity, and regulation of neurotransmitter systems (Falls, 2003). While homozygous null mice for *Nrg1* die midgestation, heterozygous mutant mice are viable (Gerlai et al., 2000). *Nrg1* hypomorph epidermal growth factor-like domain models result in hyperactivity with impaired PPI (Gerlai et al., 2000; Duffy et al., 2008). Additionally, most NRG1 proteins are synthesized with a transmembrane (TM) domain, and *Nrg1* hypomorph TM models also result in hyperactivity and exhibit impaired PPI, altered habituation, increased aggression, and decreased functional NMDA receptors (Stefansson et al., 2002; Karl et al., 2007; O'Tuathaigh et al., 2008). *Nrg1* immunoglobulin-like domain mutant mice, while not hyperactive, are impaired in the latent inhibition task (Rimer et al., 2005). A more recent model focusing on the deletion of a specific *Nrg1* isoform (Type III) produces mice with a more pronounced PPI deficit, impaired performance on delayed alteration memory tasks, enlarged lateral ventricles, and decreased spine density (Chen et al., 2008). Collectively, these studies indicate that *Nrg1* may play a more prominent role in the sensorimotor gating phenotype of SCZ, while its effects on activity and memory remain unclear.

DISC1

Disrupted-in-schizophrenia-1 (*DISC1*) was identified as a candidate gene when it was found to be disrupted by a balanced translocation that cosegregates with SCZ and related psychopathologies (Millar et al., 2000; 2001). *DISC1* plays an important role in neurite outgrowth, cell migration, and cell signaling (Mackie et al., 2007). A mutant mouse model of *Disc1* carrying a deletion variant displayed impairments in working memory, deceased mPFC volume, altered synaptic transmission in the hippocampus, and reduced dendritic growth in the dentate gyrus (Koike et al., 2006; Kvajo et al., 2008). An inducible *Disc1* C-terminal fragment transgenic model exhibited abnormal spatial

working memory, deficits in social interaction, and decreased hippocampal dendritic complexity (Li et al., 2007). A model expressing the dominant negative C-terminal truncated *Disc1* exhibited hyperactivity, disrupted PPI, depressive-like symptoms, and enlarged lateral ventricles (Hikida et al., 2007). Inducible expression of mutant human *DISC1* also produced mice with enlarged lateral ventricles, deficits in spatial working memory, impaired social interaction, and hyperactivity (Pletnikov et al., 2007). And lastly, truncated *Disc1* transgenic mice exhibit enlarged lateral ventricles, decreased cortical neurogenesis, increased immobility and reduced vocalization in depression-related tests, as well as impairment in latent inhibition (Shen et al., 2008). These studies demonstrate that while *Disc1* clearly contributes to the pathophysiology of SCZ, the nature of the mutation has profound effects on the range of observed behavioral phenotypes.

Dysbindin

Several studies have implicated dysbindin (*DTNBP1*) as a SCZ candidate gene (Straub et al., 2002; Tang et al., 2003; Kirov et al., 2004). *DTNBP1* binds to dystrobrevins, components of the dystrophin-associated glycol-protein complex (DGC), and is thought to play a fundamental role in regulating synaptic structure and signaling (Benson et al., 2001). *Dtnbp1* deletion mice on a DBA/2J background strain were found to have increased anxiety and impaired social interaction, as well as deficits in working and recognition memory (Hattori et al., 2008; Takao et al., 2008). These mice also displayed increased freezing response to a conditioned stimulus, suggesting deficits in emotional and motivated learning and memory (Bhardwaj et al., 2009). These mice also had decreased dopamine levels, reduced steady state levels of snapin (synaptic priming regulator), and deficiencies in neurosecretion (Murotani et al., 2007; Feng et al., 2008;

Chen et al., 2008a). However, a study of *Dtnb1* KO mice on a C57Bl/6 strain showed no evidence of increased anxiety, although replicated the spatial learning and memory deficit (Cox et al., 2009). While *Dtnb1* partially contributes to a SCZ phenotype, the extent remains unclear due to complications with the background strain.

22q11.2 deletion

The 22q11.2 deletion is a rare chromosomal mutation spanning ~3 Mb that has also been associated with SCZ (Drew et al., 2011). 22q11.2 deletion syndrome (22q11DS) is characterized by a 25-fold increased risk for developing SCZ as well as cardiac and facial anomalies (Karayiorgou et al., 2010). This deletion syndrome also increases the risk of other psychiatric disorders, such as attention-deficit hyperactivity disorder, bipolar disorder, anxiety, and affective disorders (Murphy et al., 1999; Niklasson et al., 2001). 22q11.2 microdeletion carriers also show language delay, decreased full scale IQ, learning disabilities and mental retardation, and deficits in attention and working memory (Niklasson, 2001; Karayiorgou et al., 2010). Although this rare microdeletion is present in only 1-2% of patients with SCZ, its high penetrance for the disease makes 22q11DS genetic models an excellent opportunity to investigate the pathogenesis underlying certain behavioral abnormalities present in SCZ and other related disorders (Karayiorgou et al., 2010).

A region of mouse chromosome 16 is homologous to the 22q11.2 region in humans, containing murine versions to all genes except *CLTCL1*, with minimal reorganization of gene order (Paylor and Lindsay, 2006). Several mouse models have been generated and characterized with deletions that fall within or encompass the microdeletion (Drew et al., 2011). For example, the Df(16)A+/- mice show impairments in the acquisition of a delayed non-match to sample T-maze task that relies on spatial

working memory (Stark et al., 2008). These mice also display reduced synchronous activity between the dorsal hippocampus and medial prefrontal cortex during task acquisition, suggesting that deficits in communication between the hippocampus and prefrontal cortex may underlie working memory impairments (Sigurdsson et al., 2010). Another 22q11DS mouse model, Df(16)1+/-, showed weakened auditory thalamocortical connections in post-adolescent, but not pre-adolescent animals, mirroring the developmental timeline of behavioral impairments in PPI in these mice (Chun et al., 2014). This reduction in thalamocortical strength was specific to the auditory cortex, and was due to an unexpected increase in dopamine D2 receptors in the medial geniculate nucleus of the thalamus (Chun et al., 2014). Thalamocortical strength and PPI were normalized following acute administration of haloperidol, suggesting that the therapeutic effects of antipsychotic medications in SCZ could be due to targeting thalamic D2 receptors (Chun et al., 2014). Overall, the 22q11DS mouse models may highlight the SCZ-related behaviors, along with the associated mechanisms, linked to this chromosomal region.

SIGNIFICANCE OF CURRENT RODENT MODELS

The above discussion of current and popular rodent models for ASD and/or SCZ highlight the polygenic origin of these disorders. The literature suggests that, at least in the mouse, disruption of a single gene is typically not the cause of psychiatric disorder. However, study of these risk variants in genetically modified mice allow us the means to elucidate which behavioral domains are regulated by each candidate gene. Furthermore, we can investigate the specific molecular and cellular pathways regulated by each candidate gene that underlie the behavioral domain(s) in question. In this thesis work, we make the first attempt to understand the contribution of candidate gene *SAP97*

to psychiatric disorders such as ASD and SCZ. Below, we discuss the background of *SAP97's* gene family, the membrane associated guanylate kinases. We discuss their significance in synapse biology and the current evidence suggesting their role in psychiatric disorders.

MEMBRANE ASSOCIATED GUANYLATE KINASES

In this section of the introduction, we discuss the membrane associated guanylate kinases (MAGUKs), an integral group of synaptic genes known to play a prominent role in a broad range of psychiatric disorders, including ASD and SCZ. We choose to focus in depth on the MAGUK family as it is extensively expressed in the brain and well conserved throughout evolution. Below, we provide a general background and rationale for focusing on the contribution of MAGUKs to ASD, SCZ, and other related psychiatric disorders.

MAGUK Subfamily Classification

The MAGUK protein family is classified phylogenetically in 10 subfamilies by comparison of the genomic sequences of the core PDZ-SH3-GUK region and by the supplemental domains they possess (Oliva et al., 2011). Of the ten MAGUK subfamilies, members of the subfamilies DLG, CASK, MPP, CACNB, and MAGI are expressed in the central nervous system (CNS) where they play various roles in the formation and function of synapses (Laura et al., 2002; Jing-Ping et al., 2005; Deng et al., 2006). Members of the ZO family are not expressed in neurons, but are present in the brain where they play an import role in the formation and maintenance of the blood-brain barrier (Wolburg and Lippoldt, 2002). The DLG and CASK subfamilies are the most well-studied MAGUKs due to their clear role in synapse formation and function

(Oliva et al., 2011). While both subfamilies are also expressed in epithelial tissues and the peripheral nervous system, previous groups have focused on their function in the CNS (Oliva et al., 2011). For this body of work, we have chosen to focus on the DLG subfamily of MAGUKs.

Expression Pattern of the DLG MAGUK Subfamily

The DLG subfamily of MAGUKs (Dlg-MAGUK) is expressed in the CNS at all stages of development (Kim and Sheng, 2004; Funke et al., 2005). All members can be found presynaptically and postysnaptically, however some are mainly found in the postsynaptic compartment of excitatory synapses and restricted to the postsynaptic density (Kim and Sheng, 2004; Funke et al., 2005). The Dlg-MAGUK family also differs in their temporal and spatial expression. PSD-95 is expressed at low levels during embryonic and early postnatal development, but is enhanced during postnatal development, and reaches maximum expression at adulthood (Hsueh and Sheng, 1998; Al-Hallaq et al., 2001). PSD-93 shows a similar expression profile to PSD-95 in the hippocampus (Sans et al., 2000). SAP102, however, is highly expressed in the hippocampus during the first postnatal week, remains stable by postnatal day P35, and decreases into adulthood (Muller et al., 1996; Sans et al., 2000). SAP97 displays an expression pattern opposite to PSD-95 and PSD-93 in the hippocampus and other brain tissues, where expression levels decrease from embryo to adult stages (Cai et al., 2008). This observation suggests that SAP97 participates in developmental processes of the nervous system (Cai et al., 2008).

The role of Dlg-MAGUKs in Synapse Formation and Function

The expression pattern of Dlg-MAGUKs during development suggests that they are involved in the regulation of synaptogenesis, a highly regulated process of building neural circuits. Studies done in mammals as well as *Drosophila* neuromuscular junction suggest that Dlg-MAGUKs are necessary for the clustering and stabilization of glutamate receptors once the pre- and postsynaptic sites have been contacted and stabilized by adhesion proteins (Chen and Featherstone, 2005; Waites et al., 2009). The participation of Dlg-MAGUKs in the maturation of mammalian synapses has been shown via overexpression experiments. Overexpression of PSD-95 and SAP97 increases the size of spines and the formation of multi-innervated spines in hippocampal neurons (Nikonenko et al., 2008; Poglia et al., 2010). Additionally, overexpression of SAP97 promotes dendritic growth and requires the binding to the AMPA receptor subunit (AMPAR), GLUA1 (Zhou et al., 2008). SAP97, PSD-95, and SAP102 overexpression also enhance the expression of presynaptic proteins such as synatophisin, synapsin, and bassoon (Regalado, 2006). The overexpression of several PSD-95 interacting proteins also has an effect in spine morphogenesis (Lee et al., 2008).

While Dlg-MAGUK overexpression studies have been informative, loss of function experiments have not produced consistent results. Knockout mice for *Psd-95*, *Psd-93*, or *Sap102* do not have defects in synapse development (Miguad et al., 1998; McGee et al., 2001; Cuthbert et al., 2007). *Psd-95* mice carrying a targeted mutation that introduces a stop codon in the third PDZ domain show only altered dendritic spine density in the hippocampus (Vickers et al., 2006). Moreover, acute knockdown of *Psd-95* using shRNA does not produce defects in dendritic spine density in primary hippocampal cultures (Elias et al., 2006). *Sap97* knockout animals have not been possibly to study, as the mutant mice display a cleft palate and die prematurely

(Caruana and Bernstein, 2001). However, studies conducted on neuronal cultures from *Sap97* knockout animals do not show any defect in glutamate receptor distribution or AMPAR mediated currents (Howard et al., 2010). As it has been demonstrated that PSD-95, PSD-93, and SAP102 can compensate for each other, the lack of defects observed in the knockout mice can be explained by functional redundancy (Elias et al., 2006; Howard et al., 2010).

The Dlg-MAGUK family also plays an integral role in glutamate receptor clustering and trafficking, both of which are essential processes for the efficiency and plasticity of glutamatergic synapses. PSD-95 is the most well-studied Dlg-MAGUK for its role in clustering and trafficking glutamate receptors, especially NMDARs (Elias and Nicoll, 2007). PSD-95 is also implicated in the trafficking of AMPARs, although indirectly via transmembrane AMPAR regulatory proteins (TARPs) (Chen et al., 2000).

SAP97 has been implicated in receptor trafficking by various studies. *Sap97* occurs as two splice variants (α and β). SAP97α is mostly found at the postsynaptic density, while SAP97β is found in the perisynaptic region (Oliva et al., 2011). Both splice variants are able to bind the GLUA1 AMPAR (Oliva et al., 2011). Current evidence suggests that the ratio between these two isoforms can regulate the distribution of GLUA1, and as a result, synaptic strength (Waites et al., 2009). Acute overexpression of SAP97β promotes trafficking of AMPARs and NMDARs to the synapse in immature pyramidal neurons but not in mature neurons (Howard et al., 2010). However, chronic overexpression *in vivo* during development enhances synaptic transmission in mature neurons (Howard et al., 2010). These findings suggest that SAP97β plays a role in receptor trafficking during development rather than in adult plasticity.

THE CONTRIBUTION OF MAGUKs TO PSYCHIATRIC DISORDERS

The prominent role of Dlg-MAGUKs at glutamatergic synapses and in synaptic plasticity suggests that mutations in MAGUK genes would be involved in synaptic-related disorders, notably ASD and SCZ. Below, we outline the evidence implicating the Dlg-MAGUKs in ASD, SCZ, and related neuropsychiatric disorders, and discuss the current genetic models available.

Sequencing techniques and analytics have been used to identify *PSD-95* mutations in ASD and SCZ patients. Whole-exome sequencing studies of SCZ and ASD patients show disrupted mutations of proteins located in excitatory synapses of the PSD, such as NMDAR and *PSD-95* (Fromer et al., 2014; Purcell et al., 2014). Studies from postmortem SCZ patients reveal a significant decrease in *PSD-95* mRNA and protein expression levels in the dorsolateral and dorsomedial prefrontal cortex, suggesting an association between *PSD-95* dysfunction and SCZ (Ohnuma et al., 2000; Catts et al., 2016). *PSD-95* is also involved in a network of interactions with high-risk ASD genes that include *SHANK*, *HOMER*, *NLGN*, and *FMR1* (Gilman et al., 2011; Tsai et al., 2012; De Rubeis et al., 2014). Furthermore, *PSD-95* is a candidate gene disrupted in intellectual disability, a cognitive disorder characterized by a reduction of dendritic spines (Lelieveld et al., 2016). *PSD-95* has direct interactions with intellectual disability-related proteins within the excitatory PSD that include *ARC* and *IL1RAPL1*, which are responsible for regulating spine density and function (Pavlowsky et al., 2010; Valnegri et al., 2011; Fernández et al., 2017). To model with in mice, null animals have been made and characterized. Feyder et al. characterized the *Psd-95* knockout mice and the mice exhibit increased repetitive behaviors, abnormal communication, hyper-social behavior, impaired motor coordination, and increased stress-reactivity and anxiety-related

responses (Feyder et al., 2010). The extent to which the *Psd-95* null mice faithfully report on the contribution of *Psd-95* to psychiatric disease is an open question.

Mutations in the gene *SAP102* are found in patients with X-linked mental retardation (Tarpey et al., 2004). The mutations identified introduce premature stop codons within or before the third PDZ domain, and it is likely that this impairs the ability of SAP102 to interact with NMDAR and other proteins involved downstream of NMDAR signaling pathways (Tarpey et al., 2004; Zanni et al., 2009). The disruption of the ability to bind NMDARs may lead to altered synaptic plasticity and explain the intellectual impairment observed in individuals with *SAP102* mutations (Tarpey et al., 2004; Zanni et al., 2009). Cuthbert et al. report the first characterization of *Sap102* KO mice, and find that *Sap102* mutant mice display cognitive deficits with a specific spatial learning deficit (Cuthbert et al., 2007).

A variety of evidence implicates *SAP97* in the etiology of ASD and SCZ. Single nucleotide polymorphisms in *SAP97* have been linked to an increased risk of SCZ in males, which supports the possible involvement of *SAP97* gene variation in the susceptibility to SCZ and in the genetic basis for sex differences in the disorder (Uezato et al., 2012). The human *SAP97* gene resides in the chromosomal region 3q29, where multiple genome-wide analyses on copy number variations found an excess of microdeletions in SCZ (Kirov et al., 2011; Levinson et al., 2011; Kushima et al., 2016; Marshall et al., 2016). A meta-analysis demonstrated the 3q29 deletion confers a 40-fold increased risk for SCZ (Mulle, 2015). Additionally, individuals with 3q29 microdeletions spanning the *SAP97* locus display autism and intellectual disability (Quintero-Rivera et al., 2010). In another study of the expression levels of multiple postsynaptic density proteins, including PSD-95, PSD-93, and SAP102, the authors found a specific decrement in the level of SAP97 in post mortem frontal lobe from

schizophrenic patients (Toyooka et al., 2002). SAP97 levels were decreased to less than half that of control levels, and concordantly, its binding partner GLUA1 was similarly decreased in the same brain region (Toyooka et al., 2002). SAP97 is also the only member of the Dlg-MAGUK family that directly binds to the extreme C-terminus of the GLUA1 AMPAR, a subunit that promotes dendritic growth and patterned synaptic innervation (Zhou et al., 2008; Zhang et al., 2017). Thus, it is plausible that defects in these *SAP97*-dependent mechanisms contribute to a ASD and SCZ phenotype. However, unlike the other members of the Dlg-MAGUK family, the issue has been difficult to study because *Sap97* knockout mice die a few days after birth from a craniofacial defect (Caruana and Bernstein, 2001).

STATEMENT OF MOTIVATION AND HYPOTHESIS

The goal of this thesis is to understand the direct contribution of *SAP97* to neuropsychiatric disorders such as ASD and SCZ. While mouse models for *Psd-95*, *Psd-93*, and *Sap102* have been previously generated and characterized behaviorally, *Sap97* null animals have been impossible study. In Chapter 2, we determine whether *Sap97* directly contributes to the pathophysiology of ASD and SCZ, and in what capacity, by generating mice that have a conditional knockout of *Sap97* targeted to neurons using the Cre-loxP system. Given the substantial evidence supporting the involvement of the Dlg-MAGUK family in neuropsychiatric disorders, we hypothesized that loss of *Sap97* would contribute partially to the endophenotypes of ASD and/or SCZ-like phenotype. In order to test this hypothesis, we subjected the *Sap97* conditional knockout mice to a battery of behavioral tests and biochemical studies to screen for an ASD or SCZ-like phenotype. We report that loss of *Sap97* results in subtle sex-specific behavioral abnormalities and alters the expression of SCZ risk-associated gene

transcripts in the hippocampus. This thesis works provides the first broad behavioral and transcriptomic characterization of *Sap97* deficient animals, and provides a stepping-stone for understanding the molecular mechanism by which *SAP97* contributes for neuropsychiatric disorders.

CHAPTER 2: SAP97 REGULATES BEHAVIOR AND EXPRESSION OF SCHIZOPHRENIA RISK ENRICHED GENE SETS IN MOUSE HIPPOCAMPUS

SUMMARY

Synapse associated protein of 97KDa (SAP97) belongs to a family of scaffolding proteins, the membrane-associated guanylate kinases (MAGUKs), that are highly enriched in the postsynaptic density of synapses and play an important role in organizing protein complexes necessary for synaptic development and plasticity (Cai, 2006; Elias and Nicoll, 2007; Zhou et al., 2008; Chen et al., 2015; Zeng et al., 2016). Large-scale genetic studies have implicated MAGUKs in neuropsychiatric disorders such as intellectual disability, autism spectrum disorders (ASD), and schizophrenia (SCZ), but knock-out mice have been impossible to study because the *Sap97* null mice die soon after birth due to a craniofacial defect. We studied the transcriptomic and behavioral consequences of a brain-specific conditional knockout of *Sap97* (SAP97-cKO). RNA sequencing (RNAseq) from hippocampi from control and SAP97-cKO male animals identified 67 differentially expressed transcripts, which were specifically enriched for SCZ-related genes. Subjecting SAP97-cKO mice to a battery of behavioral tests revealed an anxiety-like phenotype present in both male and female SAP97-cKO animals, as well as a male-specific cognitive deficit and female-specific motor learning deficit. These data suggest that loss of *SAP97* regulates behavior, and may contribute to some of the endophenotypes present in SCZ. The SAP97-cKO mouse serves as a novel model system for interrogating aspects of the cellular and molecular defects underlying SCZ and other related neuropsychiatric disorders.

INTRODUCTION

Intellectual disabilities and neuropsychiatric behavioral disorders affect about 17.9% of individuals over their lifetime and interfere with the ability of people to experience a fulfilling and productive life (nimh.nih.gov). Some of these disorders are clearly developmental. For example, autism spectrum disorders (ASD) are characterized by impairments in social interaction and communication, and by restricted, repetitive behaviors and about 1% of children show signs and symptoms that lead to the diagnosis of ASD (Ebert and Greenberg, 2013; Uchino and Waga, 2013). Schizophrenia (SCZ) is another mental disorder that is characterized by disordered thought processes and disturbed emotional responsiveness (Grabrucker et al., 2014). The symptoms of SCZ usually appear during young adulthood, with an overall prevalence of about 0.7% (Fromer et al., 2014; Grabrucker et al., 2014). Technological advances have brought unprecedented insights into the genetic architecture of these and many other neuropsychiatric disorders (De Rubeis et al., 2014; Fromer et al., 2014; Zhao et al., 2014; Xing et al., 2016).

Exome-sequencing technology has allowed us to systematically scan genes for *de novo* mutations at the single-base resolution, potentially offering insights into risk-determining genes (Ghosh et al., 2013; Fromer et al., 2014). Whole-exome sequencing results from patients with ASD or SCZ reveal significantly enriched copy number variant (CNV) mutations in the synaptic gene set (Fromer et al., 2014). Among the most prevalent synaptic genes that have been uncovered in large-scale genomic studies have been alterations in the neurexins/neurolignins along with the *PROSAP/SHANK* family. Various genetically manipulated mice of these gene families recapitulate some of the behavioral features of ASD, SCZ, and intellectual disability (Peça et al., 2011; Wang et al., 2011b; Kouser et al., 2013; Han et al., 2014). However, none of these models

completely phenocopy disease in humans, consistent with the polygenic origin of these disorders.

Another important group of synaptic genes that has been implicated to be involved in ASD or SCZ is the Discs-large (Dlg) family of membrane associated guanylate kinases (MAGUKs) (Kristiansen et al., 2006; Funk et al., 2009; Feyder et al., 2010; Xing et al., 2016; Winkler et al., 2017). The Dlg family is the most comprehensively studied family of MAGUKs, and is comprised of *PSD-95*, *PSD-93*, *SAP102*, and *SAP97*. They share a common domain structure comprised of three PDZ domains, along with an SH3 and GUK domain. The Dlg-MAGUK family directly binds to many proteins in the postsynaptic density (i.e. glutamate receptor subunits, TARPS, and neurexin/neuroligin clusters), and regulates synaptic nanoscale structure and synaptic transmission (Bats et al., 2007; Mondin et al., 2011; Giannone et al., 2013) . Mice with a targeted deletion of *Psd-95*, *Psd-93*, and *Sap102* show a range of phenotypes also displayed by individuals with psychiatric disorders (Cuthbert et al., 2007; Feyder et al., 2010; Winkler et al., 2017).

A variety of evidence implicates *SAP97* in the etiology of ASD and SCZ: 1) single nucleotide polymorphisms in *SAP97* have been linked to an increased risk of schizophrenia in males (Uezato et al., 2012), 2) individuals with microdeletions spanning the *SAP97* locus display autism and intellectual disability (Quintero-Rivera et al., 2010), and 3) a study of expression levels of multiple postsynaptic density proteins found a specific decrement in the level of SAP97 in post mortem frontal lobe from schizophrenic patients (Toyooka et al., 2002). SAP97 is also the only member of the Dlg-MAGUK family that directly binds to the extreme C-terminus of the GLUA1 AMPA receptor (AMPAR), a subunit that promotes dendritic growth and patterned synaptic innervation (Zhou et al., 2008; Zhang et al., 2017). Thus, it is plausible that defects in these *SAP97*-

dependent mechanisms contribute to a ASD and SCZ phenotype. While these findings advocate for the participation of *SAP97* in the etiology of neuropsychiatric disorders, the issue has been difficult to study because *Sap97* knockout mice die a few days after birth from a craniofacial defect (Caruana and Bernstein, 2001).

In order to determine whether *SAP97* directly contributes to the pathophysiology of ASD and SCZ, we generated mice that have a conditional knockout of *Sap97* targeted to neurons using the Cre-loxP system. We then subjected these mice to a battery of behavioral tests and biochemical studies to screen for an ASD or SCZ-like phenotype. Overall, our results suggest that loss of *Sap97* results in sex-specific behavioral abnormalities as well as regulates transcripts of SCZ risk-related genes.

MATERIALS AND METHODS

Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee. The Cre-loxP system was used to generate a *Sap97* conditional knockout (cKO) mouse. SAP97fl/- mice were generated as previously described (RRID: IMSR_JAX:013097). Nestin-cre+/- mice on a C57Bl/6 background were purchased from Jackson Labs (stock number 003771, RRID: IMSR_JAX:003771). Nestin-cre+/-; SAP97fl/- mice were generated by crossing male Nestin-cre+/- with female SAP97fl/- mice. Nestin-cre+/-; SAP97fl/- male mice were then crossed with female SAP97fl/- mice to generate Nestin-cre+/-; SAP97fl/fl (SAP97-cKO) and littermate control animals. Littermate control animals (genotype: Nes-cre+/-, SAP97fl/fl, and wild-type) were averaged and compared to cKO animals. Genomic DNA was extracted from tail snips using the Phenolcholoroform acetate method to confirm genotypes. The primers used for genotyping were as follows: *Sap97* flox fwd-

AGAGTATGCTCTATGTGATGTTGTGTG rev-TAAGAAGGATCAACTGGCAAAGGTG; *CRE* fwd- ACCTGATGGACATGTTCAGG rev-CGAGTTGATAGCTGGCTGG

Behavioral Experiments

Open Field

Assessment of general exploratory behavior and anxiety were evaluated using the open field paradigm. Mice were placed in a white, opaque plexiglass box (40cm x 40cm) and were given 15 minutes to explore the apparatus. Exploratory locomotor activity (total distance traveled, average speed, and mean distance from border) was scored using the Any-MAZE tracking software (San Diego Instruments, San Diego, CA, RRID:SCR_014289).

Elevated Plus Maze

Assessment of anxiety-like behaviors was evaluated using an elevated plus maze (Coulbourn Instruments, Whitehall, PA). The mouse was initially placed in the center “free zone”, and was allowed to freely explore the apparatus for the 5-minute trial time. Time in the open arms versus the closed arms, as well as number of entries to these arms, was measured using the Any-MAZE tracking software.

Accelerating Rotarod

Assessment of motor learning and motor coordination was evaluated using the accelerating rotarod (Ugo Basile, Varese, Italy). The starting acceleration was 4 rpm, and accelerated to 40 rpm over a 5-minute trial time. Mice underwent 3 trials per day for 4 consecutive days, for a total of 12 trials. Latency to fall from the rod was manually measured and compared across the 4 days.

Novel Object Recognition (NOR)

Assessment of cognition was evaluated using the NOR paradigm. The testing apparatus was a white, opaque plexiglass box (40cm x 40cm). On day 1, mice were habituated to the testing apparatus for 15 minutes. On day 2, the mice were reintroduced to the testing apparatus and allowed to explore two identical objects equally spaced from the walls of the apparatus (objects A and A') for 5 minutes and the animal was then removed. Any-MAZE tracking software was used to measure the time spent investigating each object, and a preference index (PI) was calculated by dividing time spent investigating A' by time spent investigating A (A'/A). One hour later after identical object exploration, the mouse was placed back in the testing apparatus where one of the identical objects had been replaced with a novel object that differed in shape, color, and texture (object B). Again, the mouse was given 5 minutes to explore the two objects, and preference for the novel object was calculated by dividing time spent investigating B by time spent investigating A (B/A). Significant preference for the novel object was assessed by comparing the PI from the training phase to the PI from the testing phase.

Three Chambered Social Choice

Assessment of sociability was evaluated using the standard three-chambered social choice paradigm. A white, opaque plexiglass rectangular box was used, with three partitions (each 20cm x 40cm). The mouse was first given 5 minutes to habituate to the empty apparatus. After habituation, into the left and right compartments was placed either with an inanimate object (non-social zone) or an age and sex-matched C57Bl/6 mouse (social zone). The object and mouse were placed under clear, plexiglass cylinders with perforations to allow odor detection. During the testing phase, the test mouse was allowed five minutes to explore either zone. Time in each zone was

measured using the Any-MAZE tracking software and sniffing time of either the inanimate or social target was manually scored. Social zone preference was calculated by dividing social zone time by total zone exploration time, and social sniffing preference was calculated by dividing time spent sniffing the social target by total sniffing time.

Cued Fear Conditioning

Assessment of amygdala dependent fear learning was evaluated using the cued fear conditioning paradigm. Fear conditioning paradigms pair an emotionally neutral stimulus, such as light/tone (conditioned stimulus or CS) with an aversive stimulus, such as footshock (unconditioned stimulus or US), leading to the expression of a threat response (freezing) to presentation of the neutral CS alone. For these experiments, the context was altered between training (context A) and testing (context B) to isolate the light/tone (CS) cued response from the hippocampal dependent contextual response.

The cued fear conditioning paradigm used in this study was modified from experiments described in (Newton et al., 2004; Wolff et al., 2014). The CS consisted of simultaneous auditory (75dB, white noise, 20s) and light stimuli (yellow light pulses, 20s, flickering at 4 Hz) generated by built in audio and light stimuli generators (Med Associates, Fairfax, VT). The US consisted of a footshock (1.05mA, 1.5s) delivered through the metal grid floor. During CS-US pairings, the US was delivered immediately following the cessation of the CS. On day 1 of this paradigm, animals underwent fear conditioning training in context A, a rectangular conditioning chamber (21.6 cm × 17.8 cm × 12.7 cm) with Plexiglas and metal walls, and a metal grid floor (Med Associates, Fairfax, VT). Animals were allowed to freely explore the chamber for 1 min before experiencing 3 CS-US pairings (60s interstimulus interval).

One min after the final CS-US pairing (5 min total), mice were removed from context A and placed back in their home cage. Twenty-four hours later (day 2) animals underwent behavioral testing to measure freezing in context B, a custom made triangular conditioning chamber with black striped Plexiglas walls and a smooth, opaque black plastic floor, scented with organic vanilla extract. Mice were allowed to freely explore the chamber for 1 min before experiencing 3 presentations of the CS alone (60s, interstimulus interval). Again, animals were removed from context B after a total of 5min. During testing, freezing behavior was scan sampled every 5th second from the onset of the first CS presentation to the end of the trial (4 min total). Freezing was defined as a total lack of movement aside from respiration at the instant of every 5th second. The total number of freezing spells was then divided by total observations to generate a freezing percentage per animal.

Biochemistry

Mice were anesthetized with a pentobarbital solution and decapitated. The brain was removed, and each hemisphere of the cerebellum, cerebral cortex, and hippocampus was dissected. One hemisphere was rapidly transferred to a mortar and pestle prechilled on dry ice, and ground into a fine powder to be processed for RNA extraction by the RNeasy mini kit (Qiagen, Catalogue #74134) according to the manufacturer's instructions. Once RNA extraction was complete, conversion to cDNA was done using the iScript Supermix (Bio-Rad, Catalogue #1708841, Hercules, CA). The other hemisphere was transferred to a dounce prechilled on ice, and lysed in 1% Triton-X lysis buffer with protease and phosphatase inhibitors for generation of protein lysates.

Antibodies

The following antibodies were used in this study as follows: immunoblotting of SAP97 (Thermo Fisher Scientific, catalogue # PA1-741, RRID:AB_2092020); immunoblotting of PSD95 (NeuroMab, catalogue # 75-348, RRID:AB_2315909); immunoblotting of PSD93 (NeuroMab, catalogue # 75-284, RRID:AB_11001825); immunoblotting of SAP102 (NeuroMab, catalogue # 75-058, RRID:AB_2261666); immunoblotting beta-actin (Cell Signaling Technology, catalogue # 3700 (mouse), RRID:AB_2242334 or Sigma-Aldrich, catalogue # A2066 (rabbit), RRID:AB_476693). Secondary antibodies for immunoblots (IRDye) were purchased from Li-COR (Catalogue # 925-32210, RRID: AB_2687825 and Catalogue # 925-68021, RRID: AB_2713919).

Western Blot

Western blot was performed according to standard procedures (David and Kalb, 2005; Kim et al., 2005; Mojsilovic-Petrovic, 2006).

Quantitative PCR

Quantitative real-time PCR (qPCR) was carried out as previously described using the delta delta Ct method to calculate relative gene expression levels (Livak and Schmittgen, 2001). Ribosomal S17 and S18 (RS17, RS18) were used as reference genes. Each reaction consisted of cDNA, primers, and Power SYBR Green PCR Master Mix (Applied Biosystems, Catalogue # 4367659, Waltham, MA) with a total 25uL reaction volume. Melting curve analysis of the target sequences showed that all primers used in this study generated amplification of a single peak, without primer-dimer artifacts. Primer and cDNA concentrations were optimized prior to use in qPCR experiments. Each qPCR experiment consisted of 4-6 biological replicates, as well as

three technical replicates per sample. The primers used for qPCR included: *Glua1* fwd-CCCTGAGAGGTCCCGTAAAC rev- GCTCAGAGCACTGGTCTTGT; *Glua3* fwd-CCATGCTCTTGTCAGCTTCG rev- AGTCCACCTATGCTGATGGT; *Glua4* fwd-TGAATGAACAAGGCCTCTTGGA rev- AGGCACTCGTCTTGTCCTTG; *Nrcam* fwd-AAGACCCGCTGGACTTTGAA rev- GGCTTGCCATTGCCTTCTTA; *Huwe1* fwd-GTTGGGATTTCCCACCAGGA rev- CAGTCTGCAGGAGCTTCAGT; *Pten* fwd-CCTGCAGAAAGACTTGAAGGTG rev- CTGTGCAACTCTGCAGTTAAA; *Adam10* fwd-GGCTGGGAGGTCAGTATGGA rev- CTCGTGTGAGACTGCTCGTT; *Was* fwd-TCAGCTGAACAAGACCCCTG rev- CATGCATCAGGGCACCTACT; *Erbb4* fwd-ACCCAGGGGTGTAACGGT rev- TGGTAAAGTGGAATGGCCCG; *Sema4C* fwd-GGTGGCCGGAGTCAAACG rev- TTCAGTCCAGCAGCCCTCTTT; *Kcna3* fwd-TCCGAAAAGCCCGGAGTAAC rev- CTGTGGAGTTGCCCGTTTTG; *Kcna4* fwd-CACTTGCTGGGAATGGTGAAGT rev- GAGAAGGTGGTAGACGCAGT; *Kcna5* fwd-TAGGACACTGGCTGACCCAT rev- ACGCACAAGCAGCTCAAAAG; *Gng13* fwd-TTGCTGTCTCCTCCAAAACCTC rev- TCCCTCTTGAAGGCCAGTTG; *Fzd7* fwd-AGAACCTCGGCTACAACGTG rev- ACCGAACAAAGGAAGAACTGC; *Dlgap4* fwd-TTTGCTTCTCTGCCCGATCC rev- TGATGAACATTGCTTCAAGAGC; *Ctnna1* fwd-CAGTTCGCTGCAGAAATGAC rev- ACCTGTGTAACAAGAGGCTCC; *Calm3* fwd-GAGTAACCTCGATCCCCGAG rev- GAAGGCTTCCTTGAACTCTGC; *Kcnc1* fwd-CTACGCGCGGTATGTGGC rev- TCGGTCTTGTTCACGATGGG; *Axin2* fwd-CAGCCCAAGAACCGGGAAAT rev- AGCCTCCTCTCTTTTACAGCA; *Lef1* fwd-GTCGACTTCAGGTGGTAAGAGA rev- TGCTGTCAGTGTTCCTTGGG; *β-catenin* fwd-GTCAGTGCAGGAGGCCG rev- CAGGTCAGCTTGAGTAGCCA; *Runx2* fwd-GCCTTCAAGGTTGTAGCCCT rev- GTTCTCATCATTCCCGGCCA; *Kalirin* fwd-GAGTTCAGGGTGGGATGACG rev- CCATCATTCCGAAAAGATCCTCG; *Nudel1* fwd-

TTTCTTCCATAAAGGGGCAGT rev- ACACTGAGAGGCAGCATACC; *Fez1* fwd-ATCCCAGGCAGATTCAGTCC rev- TCTCAGCCCTTCATAGGACCA; *Tnik* fwd-TGCCGAACATGAGCAGGAAT rev- AGTAGAGCTTGCTCATGCAGT; *Citron* fwd-GAAGGAACACAAGGCCGAGA rev- TCCAGGTCGTTGAGCTTGTC; *Girdin* fwd-CACCACCTACTGCTGGTAGC rev- CTTTTCTCTCCCAGGCCCAC; *Grb2* fwd-CAGTGGAATTAAAAAGGGTGGCA rev- GGGAATCTTCCCTGCTGAAGAG; *S18* fwd-CAGCTCCAAGCGTTCCTGG rev-GGCCTTCAATTACAGTCGTCTTC; *S17* fwd-GATTCAGAGAGGGCCTGTGAG rev-CTGAGACCTCAGGAACGTAGT

RNA Sequencing

RNA was isolated from four control and four SAP97-cKO male hippocampi, quality evaluated by Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA), and sequenced with an Illumina HiSeq 4000 High-Throughput Sequencing System. The RNA-seq reads were aligned to the mouse genome mm10.GRCm38.p5 using STAR version 2.5.3a (Dobin et al., 2012). Next, normalization and quantification were performed with the PORT version 0.8.2a-beta pipeline (http://github.com/itmat/Normalization) which first removes reads that map to ribosomal RNA sequences or mitochondrial DNA and then uses a read re-sampling strategy for normalization to account for batch effects and differences in sequencing depth among the samples. After the normalization procedure, the gene level quantification was done by PORT with respect to the Ensemblv90 annotation. The normalized count of reads mapping to exon 10 of *Sap97* showed almost 30-fold reduction from an average of 242 in the control samples to an average of 8 in the SAP97-cKO samples affirming the efficacy of the knockdown procedure. The differential expression analysis was performed using the R Bioconductor package limma-voom (Law et al., 2014; Ritchie et

al., 2015). The top 566 genes with FDR < 0.5 and fold-change of greater than 1.6 were used for general pathway enrichment analyses, which were performed using Ingenuity IPA.

Analysis of overlap between differentially expressed genes and risk-associated disease genes

The significance of overlap between the set of differentially expressed genes (DEGs) and risk-associated ASD, SCZ, and other neuronal disorder genes was analyzed using non-parametric analysis. The ASD gene list was chosen from research by Silvia De Rubeis et al. (De Rubeis, et al., 2014), while the SCZ and ataxia gene lists were chosen from online resources (szdb.org, genedx.com). Details of the lists chosen and overlap analysis are discussed in results section. The mean and variance of the corresponding hypergeometric distribution were calculated. The *p* value of the significance of the overlap was estimated using the hypergeometric probability test.

Statistics

Data were analyzed using Prism (GraphPad Software, La Jolla, CA, RRID:SCR_015807). Significant differences within groups were determined using either Student's t-test, one-way ANOVA followed by Tukey's test for multiple comparisons, or repeated-measures two-way ANOVA followed by Tukey's test for multiple comparisons. For all tests except for RNAseq, the significance threshold was set to $p<0.05$. The significance threshold for DEGs in the RNAseq experiment was set to FDR <0.25.

RESULTS

Targeted deletion of SAP97 to neurons

Global *Sap97* knockout mice have been generated, but die soon after birth due to a craniofacial defect. In order to study the effect of loss of *Sap97* on neuronal development and behavior, we conditionally knocked out *Sap97* by crossing Nestin-cre mice with *Sap97* floxed mice (see Methods). SAP97-cKO mice were born at Mendelian ratios and were grossly normal. At two months of age, we harvested tissue from the cerebellum, hippocampus, and cortex from control and SAP97-cKO animals of both sexes and prepared protein lysates for western blot analysis. In all three brain regions, the abundance of SAP97 was significantly reduced (Cerebellum: Ctrl 3.998 ± 0.9833, n=4; SAP97-cKO 1.373 ± 0.348, n=5, p=0.0279; Hippocampus: Ctrl 4.252 ± 0.6751, n=5; SAP97-cKO 1.215 ± 0.4314, n=5, p=0.0053; Cortex: Ctrl 0.287 ± 0.08557, n=5; SAP97-cKO 0.06179 ± 0.01072, n=5, p=0.0311), indicating that we successfully generated cKO animals (Figure 1A-D). Additionally, in order to ensure male and female animals had comparable expression of SAP97, we harvested tissue from the cerebellum, hippocampus, and cortex from male and female C57Bl/6 animals and prepared protein lysates for western blot analysis. In all three brain regions, the abundance of SAP97 was not significantly different between male and female animals (Cerebellum: Male 0.3029 ± 0.03097, n=4; Female 0.3162 ± 0.03791, n=4; Hippocampus: Male 0.3296 ± 0.03764, n=4; Female 0.3135 ± 0.03483, n=4; Cortex: Male 0.3272 ± 0.0134, n=4; Female 0.257 ± 0.04237, n=4) (Figure 1E-F).

No apparent compensation by other DLG-MAGUK family members

Previous work shows that the Dlg-MAGUKs (PSD-95, PSD-93, SAP102, and SAP97) can have redundant functions in electrophysiological assays. In order to examine whether loss of SAP97 led to compensatory changes in the abundance of the other Dlg-MAGUK family members, we measured total protein levels in the cerebellum,

hippocampus, and cortex. There was no significant difference in the abundance of any other Dlg-MAGUK at the protein level in control versus SAP97-cKO male or female animals (Figure 2). These data suggest that if members of the Dlg-MAGUK family compensate for the lack of SAP97, they do so without a change in overall abundance.

No changes in gene expression level of known SAP97 binding partners or interactors

SAP97 is a scaffolding protein that allows for a large number of protein-protein interactions. Thus, the absence of SAP97 could potentially affect the expression level of numerous proteins. To determine whether loss of *Sap97* contributes to changes in expression levels of other identified members of the postsynaptic density, we conducted a directed qPCR screen. We measured mRNA levels firstly of all AMPAR subunits, as SAP97 is known to be the only Dlg-MAGUK to directly bind GLUA1. mRNA levels of *Glua1*, *Glua3*, and *Glua4* remained unchanged in the three brain regions that were probed (cerebellum, hippocampus, and cortex) (Figure 3A). Results from *Glua2* were highly variable and thus removed from the study.

We next sought to determine whether the levels of other genes known to interact with *Sap97* were affected by loss of *Sap97*. We measured mRNA levels of 16 genes in the hippocampus. From our selection of 16 genes, we observed no differences in the mRNA expression level between control and SAP97-cKO animals (Figure 3B). We also measured mRNA levels of 4 of these 16 genes in the cerebellum and cortex and observed no differences in the mRNA expression level between control and SAP97-cKO animals (Figure 3B). These results would suggest that the abundance of genes from our selection is not significantly regulated by *Sap97* expression.

SAP97 also shares direct and indirect binding partners with *DISC1*, a gene with strong association with neuropsychiatric disorders. Our lab has previously shown that SAP97 and DISC1 contribute to maintaining Wnt/β-catenin signaling within a homeostatic range. In order to address whether loss of *Sap97* has an effect on the Wnt/β-catenin pathway in the SAP97-cKO mice, we measured mRNA levels of 4 pathway-related genes in the hippocampus, and 2 of these genes in the cortex. In both brain regions, we observed no differences in the mRNA expression level between control and SAP97-cKO animals (Figure 3C). We also measured mRNA levels of 7 *Disc1* pathway-related genes in the cortex and observed no differences in expression between control and SAP97-cKO animals (Figure 3D). These results would suggest that the abundance of genes from our selection is not significantly regulated by *Sap97* expression.

Identification of SAP97-regulated transcripts in the hippocampus

Given that we observed no group differences in our directed qPCR screen, we sought a broader, unbiased approach by performing RNAseq analysis on hippocampi from SAP97-cKO and control mice (n = 4 per group). For each animal, we verified the presence or absence of SAP97 by western blot on brain tissue before submitting hippocampal samples for sequencing.

A total of 66 genes were found to be significantly downregulated in the SAP97-cKO animals as compared to control hippocampi (FDR < 0.25) (Figure 4B, Table 1). In contrast, only one gene was upregulated in the hippocampi of SAP97-cKO animals as compared to control hippocampi. Gene ontology analysis of the DEGs revealed enrichment for numerous cellular and molecular functional categories, including those related to “Cell Morphology,” “Cellular Development,” and “Cell-To-Cell Signaling and

Interaction" (Table 2A-B). Additionally, the top enriched ID Associated Network Functions included "Cellular Development, Cellular Growth and Proliferation, Hematological System Development and Function," and "Developmental Disorder, Embryonic Development, Organ Development" (Table 2C). Gene ontology terms to describe gene products known to be associated with ASD or the neurexin-neuroligin-SHANK complex in mice frequently include "Cell Communication" and "Nervous System Development", which overlaps with the findings in our RNAseq study (Patel et al., 2015). Previous studies that have conducted RNAseq on SCZ patients and performed gene ontology analysis on the resulting DEGs have identified regulation of the actin cytoskeleton as a key pathway (Zhao et al., 2014). While the actin cytoskeleton was not directly implicated by our RNAseq study, it is essential for many of the gene ontology analysis terms listed in our data set. As proper arrangement of the actin cytoskeleton is essential for neuronal cell maturation and migration, neurite outgrowth, and maintenance of synaptic density and plasticity, dysregulation of these pathways in the nervous system could have severe consequences in psychiatric disorders such as SCZ.

Schizophrenia risk enrichment in DEG set

In order to determine whether the DEG set had a significant overlap with genes implicated in psychiatric disorders such as ASD and SCZ, we compared our DEG set with disease-related gene databases. For determining overlap with ASD-related genes, we used the gene set previously generated from the transmission and *de novo* association test (TADA), which consists of 107 genes. When we matched our DEG list to the TADA ASD gene list, we did not find the match percentage to be significant based on the hypergeometric distribution (Distribution mean = 0.30, standard deviation = 0.30; SAP97-cKO DEG 0.0) (Figure 4C). We next chose to compare our DEG list to SCZ risk-

related genes found from SZDB: A Database for Schizophrenia Genetic Research (szdb.org). The distilled list of genes from this database gives a score for each gene based on criteria such as convergent functional genomics, copy number variation, differential expression, genome wide association study, and linkage and association studies. The more categories a certain gene is implicated in, the higher the score for that gene. Based on this model, we chose the top 1,000 genes from this database to match to our SAP97-cKO DEG list. Interestingly, we found the SAP97-cKO DEG list to have a significant amount of overlap to the SZDB list based on the hypergeometric test (Distribution mean = 2.79, standard deviation = 1.63; SAP97-cKO DEG 13.43, p=0.0018) (Figure 4C). Finally, we matched our SAP97-cKO DEG list to ataxia risk-related genes as a negative control, as ataxia is not classified as a neuropsychiatric disorder and *SAP97* has not previously been implicated in ataxia. We used a list of ataxia risk-related genes compiled from GeneDx, whose clinical team compiled using multiple sources, including Online Mendelian Inheritance in Man (OMIM), Human Gene Mutation Database (HGMD), and Human Phenotype Ontology (HPO) terms. The total number of genes in this list was 993, which would also allow us to control for the size of the SCZ gene list used. When we compared our SAP97-cKO DEG list to the GeneDx ataxia gene set, we found no significant match percentage (Distribution mean = 2.77, standard deviation = 2.65; SAP97-cKO DEG 4.48) (Figure 4C). Together, these results suggest that SAP97-cKO DEGs are specifically enriched for SCZ risk-related genes.

Behavioral analysis of SAP97-cKO mice

Next, we performed a battery of behavioral tests to screen for behavioral deficits in the SAP97-cKO mice.

Anxiety-like phenotype in SAP97-cKO mice

We first performed the open field test to examine general ambulation and center exploration behavior. In the males, we observed no change in the total distance traveled (Ctrl 64.8 ± 2.102, n = 29; SAP97-cKO 61.76 ± 3.763, n = 19) (Figure 5A) or the speed of the animals (Ctrl 0.07214 ± 0.002259, n = 29; SAP97-cKO 0.06847 ± 0.004183, n = 19) (Figure 5B), but saw a reduction in the distance from the border (Ctrl 0.06531 ± 0.001558, n = 29; SAP97-cKO 0.05939 ± 0.00237, n = 19, p=0.0346) (Figure 5C). This indicates that the male SAP97-cKO mice stay closer to the perimeter of the apparatus as compared to littermate control, implying that while SAP97-cKO animals do not have a basic impairment in movement, they may have an anxiety-like phenotype. Female SAP97-cKO animals exhibited decreases in total distance traveled (Ctrl 64.39 ± 3.495, n = 24; SAP97-cKO 52.1 ± 4.519, n = 12, p=0.0446) (Figure 5A) and speed (Ctrl 0.0717 ± 0.004071, n = 24; SAP97-cKO 0.058 ± 0.005053, n = 12, p=0.0497) (Figure 5B) in addition to a reduction in distance from the border (Ctrl 0.06063 ± 0.001486, n = 24; SAP97-cKO 0.05369 ± 0.001995, n = 12, p=0.0088) (Figure 5C). Overall, these observations indicate an anxiety-like phenotype present in both male and female SAP97-cKO animals.

Anxiety is a common comorbidity associated with various psychiatric disorders. In order to further gauge whether SAP97-cKO mice had alterations in anxiety-like behavior, we performed the standard elevated plus maze. When comparing time spent in open arms versus the closed arms, we saw no significant differences between genotypes in both males (open arms: Ctrl 69.75 ± 4.098, n = 33; SAP97-cKO 62.47 ± 9.331, n = 15; closed arms: Ctrl 160.8 ± 5.339, n = 33; SAP97-cKO 179.3 ± 8.893, n = 15) and females (open arms: Ctrl 68.17 ± 6.734, n = 20; SAP97-cKO 64.85 ± 7.72, n = 13; closed arms: Ctrl 156.6 ± 8.515, n = 20; SAP97-cKO 172.5 ± 8.559, n = 13) (Figure

6A). Likewise, the number of entries into the open versus closed arms was similar between genotypes of both sexes (Figure 6B). Total distance traveled in the maze was also measured and compared between Ctrl and SAP97-cKO animals to ensure no significant differences in overall exploration of the maze (Figure 6C). Together with the open field results, these observations indicate an anxiety-like phenotype in both male and female SAP97-cKO animals that is particular to specific behavioral tasks.

No changes in cued fear conditioning behavior in male SAP97-cKO mice

Amygdala circuitry is key for regulating anxiety-like responses in mice, and amygdala neuronal activity has been shown to be increased in the open field paradigm (Wang et al., 2011a). Given the observed increase in anxiety-like behavior in the SAP97-cKO mice and the wide expression pattern of *Sap97*, we were interested to know whether dysfunction in the amygdala might contribute to these observations. We decided to test the mice in the standard cued fear-conditioning paradigm (see Methods), which is thought to be an amygdala specific behavior. Both control and SAP97-cKO male and female mice exhibited normal freezing behavior (Male: Ctrl 46.96 ± 3.652, n = 15; SAP97-cKO 58.53 ± 6.029, n = 10; Female: Ctrl 35.89 ± 5.249, n=3; SAP97-cKO 40.1 ± 3.932, n=4) (Figure 7). These results suggest that the SAP97-cKO mice have no deficit in cued fear conditioning behavior and the described anxiety phenotype may be independent of amygdala circuitry.

Male-specific cognitive deficit in SAP97-cKO mice

Cognitive deficits are another endophenotype observed in various psychiatric conditions. Given that cognitive deficits are also present in several mouse models of human psychiatric disorders, we examined SAP97-cKO mice for this behavior. The

novel object recognition task is a standard test for cognition that measures ability to recall an object previously observed, as indicated by preference for a novel object (see Methods). During the training phase of this task, we observed no significant differences between the ratio of time spent investigating the two identical objects A and A' for both males and females (Male: Ctrl 1.364 ± 0.1438, n = 29; SAP97-cKO 1.441 ± 0.1578, n = 15; Female: Ctrl 1.3 ± 0.2506, n = 22; SAP97-cKO 1.301 ± 0.2775, n = 10) indicating that the animals had no prior bias. During the testing phase, control male mice displayed a marked increase in the preference index for the novel object, while SAP97-cKO male mice showed no significant increase in novel object preference index (Ctrl A-A 1.364, Ctrl A-B 2.457; SAP97-cKO A-A 1.441, SAP97-cKO A-B 1.941, $F_{(3, 79)} = 5.311$, p=0.0022) (Figure 8). When we examined this behavior in the females, we observed a trending, but not significant, increase in preference index for the novel object in both control and SAP97-cKO animals (Ctrl A-A 1.3, Ctrl A-B 2.255; SAP97-cKO A-A 1.301, SAP97-cKO A-B 2.575, $F_{(3, 57)} = 2.59$, p=0.0616) (Figure 8). These findings suggest a male-specific cognitive deficit in the SAP97-cKO animals.

Female-specific motor learning deficit in SAP97-cKO mice

Alterations in motor learning and motor coordination have also been observed in mouse models of ASD. In order to determine whether this behavioral change is present in the SAP97-cKO mice, we performed the standard rotarod task (see Methods). Analysis of both sexes showed significant time effects (Male: $F_{(3, 6)} = 12.31$, p=0.0057; Female: $F_{(3, 6)} = 9.126$, p=0.0118), while only female animals showed a trend for genotype effects and significant time and genotype interaction effects (genotype effect: $F_{(1, 2)} = 11.53$, p=0.0769; time x genotype effect: $F_{(3, 6)} = 5.099$, p=0.0434) (Figure 9A). Control animals of both sexes showed a significant increase in latency to fall from the

rod from day 1 to day 4 (Male: Day 1 157.5 ± 19.12, Day 4 211.1 ± 2.074, n = 30, p=0.0010; Female: Day 1 171.6 ± 16.53, Day 4 237.3 ± 3.439, n = 21, p=0.0021), indicating learning of the task (Figure 9A-B). However, while male SAP97-cKO mice showed no learning impairment (Day 1 154.4 ± 16.64, Day 4 229.4 ± 0.8372, n = 18, p=0.0002), female SAP97-cKO mice showed no significant learning over the timecourse of this task (Day 1 186 ± 7.927, Day 4 213.3 ± 9.988, n = 16) (Figure 9A-B). In order to determine whether this female motor learning impairment was dependent on age, we tested a subset of aged animals (8-9 months) on the rotarod task. While both aged control and SAP97-cKO female animals did not display significant learning over the 4-day task (Control: Day 1 146.6 ± 15.06, Day 4 185.3 ± 3.153, n = 7; SAP97-cKO: Day 1 95.07 ± 8.834, Day 4 129.2 ± 7.136, n = 5), female SAP97-cKO animals performed worse overall as compared to littermate controls. These results suggest that there is a female-specific motor learning deficit present in the SAP97-cKO mice that persists with age.

No social deficits present in SAP97-cKO mice

Problems with socialization are often seen in patients with ASD, and many genetic mouse models of ASD have been able to mimic this behavioral deficit. We looked for this endophenotype in the SAP97-cKO mice using the three-chambered social choice paradigm (see Methods). During the testing phase of this paradigm, we measured preference for the social target zone versus the nonsocial target zone. Control and SAP97-cKO animals of both sexes exhibited a strong preference for spending time in the social target zone (Male: Ctrl-Nonsocial 0.334, Ctrl-Social 0.666, n = 15; SAP97-cKO-Nonsocial 0.4164, SAP97-cKO-Social 0.5836, n = 11, $F_{(3, 48)} = 24.67$, p<0.0001; Female: Ctrl-Nonsocial 0.3641, Ctrl-Social 0.6359, n = 8; SAP97-cKO-

Nonsocial 0.3045, SAP97-cKO-Social 0.6955, n = 8, $F_{(3, 20)} = 14.53$, $p<0.0001$) (Figure 10A). Manual scoring of sniffing preference for the social target versus the nonsocial target was also measured for the male animals. Control and SAP97-cKO male animals also exhibited a strong preference for sniffing/investigating the social target (Ctrl-Nonsocial 0.2663, Ctrl-Social 0.7337, n = 8; SAP97-cKO-Nonsocial 0.3513, SAP97-cKO-Social 0.6487, n = 8; $F_{(3,28)} = 54.84$, $p<0.0001$). These results suggest no social deficit in the SAP97-cKO mice.

DISCUSSION

SAP97 is a member of the Dlg-MAGUK family that has repeatedly been implicated in neuropsychiatric disorders (Quintero-Rivera et al., 2010; Uezato et al., 2012; 2015; Xing et al., 2016), although its direct role in contributing to pathology has been unexplored. We generated and studied mice that were null for *Sap97* in the nervous system and make three principal observations. First, there are no compensatory changes in expression levels of other Dlg-MAGUKS or AMPARs in the SAP97-cKO versus controls. Second, loss of *Sap97* is associated with changes in gene transcripts related to SCZ. And third, SAP97-cKO animals of both sexes display an anxiety-like phenotype, as well as a male-specific cognitive deficit and female-specific motor learning deficit. Our results argue that *Sap97* is required for normal brain function and its absence leads to specific behavioral deficits and transcriptomic changes associated with SCZ.

ASD and SCZ as polygenic disorders

Investigations of ASD, SCZ, and other related psychiatric disorders indicate a highly polygenic architecture with small effect sizes of each implicated risk variant.

Mouse modeling of these disorders by targeting one such risk variant typically demonstrates a moderate, or incomplete manifestation of the human disorder. This is well illustrated by human and mouse studies of the *PROSAP/SHANK* family member *SHANK3*. Human genetic studies link mutations in *SHANK3* to a broad range of neuropsychiatric disorders. For example, deletions of exons 1-9 or exons 1-17 of *SHANK3* have been found in patients exhibiting severe language delay and significant intellectual disability. Mice generated to mimic these deletions were generated by Peca et al. and the main behavioral effects were repetitive grooming and deficits in social interaction (Peça et al., 2011). Jiang et al. used a different targeting strategy to mimic the human deletions and the mice displayed repetitive behaviors, deficits in social interaction, abnormal ultrasonic communication patterns and learning and memory deficits (Jiang and Ehlers, 2013). In a second well-studied family, affected individuals displayed ASD-features and this was linked to a deletion of *SHANK3* exon 21 (an exon that included the Homer binding domain). Mice generated to mimic this genetic lesion were created by Kouser and Speed et al. and ~2.5 month old animals exhibit defects in spatial learning and memory, motor-coordination deficits, hypersensitivity to heat, novelty avoidance, but minimal social abnormalities and no repetitive grooming behavior (Kouser et al., 2013). Together, this work demonstrates that creating a mouse with a genetic lesion that closely mimics, or is identical, to the gene defect in humans with neuropsychiatric disease only partially recapitulates the human behavioral phenotypes.

This disparity between genetic lesions associated with psychiatric phenotypes and mice created to mimic the human condition also extends to the Dlg-MAGUK family. Nonsynonymous missense mutations in the Dlg-MAGUK family members have been found in ASD and SCZ patients, and decreased protein expression of PSD-95, PSD-93, and SAP97 has been observed in the cortex of postmortem SCZ patients. To model

with in mice, null alleles of *Psd-95, Psd-93*, and *Sap102* have been created—*Psd-95* and *Sap102* knockout animals share spatial learning memory deficits (Migaud et al., 1998; Cuthbert et al., 2007), while animals null for *Psd-95* or *Psd-93* share a hyper-social phenotype (Winkler et al., 2017). *Psd-95* and *Sap102* knockout animals display a mild, and *Psd-93* knockout animals display a severe, motor function defect (Cuthbert et al., 2007; Winkler et al., 2017). The *Psd-95* null mouse has been the most extensively investigated animal. Feyder et al. characterized the *Psd-95* knockout mice and the mice exhibit increased repetitive behaviors, abnormal communication, hyper-social behavior, impaired motor coordination, and increased stress-reactivity and anxiety-related responses (Feyder et al., 2010). The extent to which the *Psd-95* null mice faithfully report on the contribution of *PSD-95* to psychiatric disease is an open question.

SAP97 splice variants and their differing roles in the nervous system

SAP97 has wide molecular diversity, which is created by extensive alternative splicing. The two most well-studied *Sap97* splice variants are *Sap97α and Sap97β*. In *Sap97α,* the prototypic N-terminal L27 domain is replaced with a putative palmitoylation motif. Overexpression of SAP97α (but not SAP97β) was shown to enhance the synaptic levels of AMPARs and to compensate for the shRNA-mediated loss of PSD-95 in organotypic slices (Waites et al., 2009). *SAP97* isoform-specific biology may also extend into human SCZ data. Uezato and colleagues identified a new *SAP97* splicing variant that is transcribed from a previously unreported 95-base-pair exon (exon 3b). In post-mortem prefrontal cortices of patients with SCZ, mRNA expression of exon 3b was significantly reduced, specifically in patients with early-onset SCZ (Uezato et al., 2015). How reduced levels of the *SAP97* 3b transcript may be involved in the susceptibility and pathophysiology of early-onset SCZ is unknown. While our study provides a broad, all-

around characterization of the effect of *Sap97* on brain function, it will be necessary to conduct future studies aimed at addressing the individual roles of prominent splice variants.

The role of the Serpin family as a molecular module in SCZ

The RNAseq study we conducted on the hippocampi of SAP97-cKO animals indicated 67 DEGs, which were specifically enriched for SCZ-related risk genes. The specific SCZ-related risk genes we identify in our data are *Serping1*, *Runx3*, *Clec7A*, *Serpinh1, Cdh1, Ap1S2*, *Xbp1*, *Serpind1*, and *C4b*. These observations lead us to hypothesize that *Sap97* is a component of a "molecular module" of gene products that together subserve aspects of normal behavior. Further, we hypothesize that abnormalities in the operation of this molecular module give rise to select behavioral alterations. Defects in many molecular modules in aggregate manifest as the complex psychiatric disorder we recognize as SCZ. The components of this module may interact physically, functionally, developmentally, or in terms of localization. Future work will be required to elucidate: 1) how the components of this hypothesized molecular module mechanistically interact, and 2) how this impacts brain function and behavior.

Our attention is drawn to three genes that were differentially expressed in the hippocampus of SAP97-cKO mice versus controls—serine peptidase inhibitors (serpins), as this group of genes has previously been reported in the literature to be associated with SCZ (Madani et al., 2003; Hoogendoorn et al., 2004; Saetre et al., 2007; Allswede et al., 2017; Chang et al., 2017; Reumann et al., 2017). *SERPING1* was found to be upregulated in postmortem brain tissue from SCZ patients (Saetre et al., 2007; Chang et al., 2017). Additionally, a study of adult Swedish twins enriched for SCZ showed an association between gene expression level of *SERPING1* and thickness across the

cortex, a characteristic that is potentially involved in the pathogenesis of SCZ (Allswede et al., 2017). Polymorphisms in the promoter regions of genes on 22q11, a chromosomal region that has been associated with various psychiatric illnesses including SCZ, resulted in activity differences in the gene *SERPIND1* (Hoogendoorn et al., 2004). Another well-studied member of the serpin family previously implicated in SCZ, but not directly by our RNAseq data, is neuroserpin (*SERPINI1*). *SERPINI1* is restricted to regions in the brain where synaptic changes are associated with learning and memory (cortex, hippocampus, amygdala, and olfactory bulb) (Reumann et al., 2017). *SERPINI1* has also been implicated in dendrite growth, as overexpression studies in primary neurons leads to increased dendritic arborization and altered dendritic spine shape (Borges et al., 2010). Additionally, mice with dysregulated expression of *Serpini1* show selective reduction of locomotor activity in novel environments, anxiety-like responses, and neophobic response to novel objects (Madani et al., 2003). These behavioral phenotypes in the *Serpini1* deficient mice are reminiscent of the defects we see in the SAP97-cKO animals. *Serpini1* is also a known inhibitor of the extracellular protease tissue-type plasminogen activator (tPA). Conditions that affect the activity of tPA have consistently been described in drug-naïve cases of SCZ (Halacheva et al., 2009; Delluc et al., 2013; Song et al., 2014; Gris et al., 2015). Interestingly, psychotic patients on chronic warfarin therapy for deep-vein thrombosis showed remission of psychotic symptoms, indicating that defective modulation of the coagulation pathway might contribute to the pathogenesis of SCZ (Hoirisch-Clapauch et al., 2015). *C4B*, or complement component b, is another gene directly listed from our RNAseq study that has known roles in the coagulation pathway and is an important cofactor to the serine protease family. The strongest genetic association of SCZ at a population level involves variation in the Major Histocompatibility Complex (MHC) locus, where the association of

SCZ with the MHC locus arises substantially from many diverse alleles of the *C4* genes (Rezende, 2003; Schizophrenia Working Group of the Psychiatric Genomics Consortium et al., 2016; Allswede et al., 2017). These prior observations along with our findings from the SAP97-cKO RNAseq study may highlight the mechanism by which *SAP97* contributes to the etiology of SCZ.

Sex-specific differences in psychiatric disease

Psychiatric disorders are characterized by substantial sex-differences in their prevalence, symptomology, and treatment response (Kokras et al., 2014). Women are more likely than men to develop dementia, panic disorder, post-traumatic stress disorder, and major depression (Kessler et al., 2008; Wittchen et al., 2011). Conversely, the incidence of neurodevelopmental disorders such as ASD and SCZ is higher in males (Fombonne, 2003; Häfner, 2003). In our study, we conducted RNAseq on male hippocampal tissue from SAP97-cKO tissue and found the resulting DEGs to be specifically enriched for SCZ risk-related gene sets. However, our behavioral screen was undertaken on both male and female SAP97-cKO animals and identified interesting sex-specific differences. This raises the possibility that the RNAseq profile of female SAP97-cKO mice may be at least partially distinct from the male SAP97-cKO dataset.

One potential limitation of our study of female behavior is the lack of assessment of the estrous cycle. Female mice in distinct stages of the estrous cycle have been previously shown to perform differently in behavioral tasks related to anxiety and cognition. Furthermore, it is thought that oestrogens play a protective role against SCZ (Kulkarni et al., 2013). It will be vital to perform behavioral testing at different stages of the female estrous cycle, as well as corroborate behavioral findings with RNAseq data in order to have a complete understanding of the role of *SAP97* in the female brain.

Conclusion

Our study provides the first broad behavioral and transcriptomic characterization of *Sap97* in the mouse nervous system. Despite study limitations, we show that loss of *Sap97* contributes to enrichment of SCZ related genes, as well as behavioral abnormalities in both male and female animals. Our findings are congruous with previous literature of monogenic mouse models of psychiatric disorders reporting a partial manifestation of the disease phenotype and thus are a first step to understanding the molecular mechanism by which *SAP97* contributes to neuropsychiatric disorders.

FIGURE LEGENDS

Figure 2.1. SAP97 protein is sufficiently knocked down in SAP97-cKO animals. (A) Western blots showing reduced SAP97 band intensity in male SAP97-cKO cerebellum, hippocampus, and cortex. (B) Quantification of male western blot analysis. (C) Western blots showing reduced SAP97 band intensity in female SAP97-cKO hippocampus and cortex. (D) Quantification of female western blot analysis. (E) Western blots showing no significant changes in SAP97 band intensity between male and female C57Bl/6 animals. (F) Quantification of male versus female C57B/6 western blot analysis. *P<.05, **P<.01 (two-tailed Student's *t* test). Data are presented as mean ± SEM.

Figure 2.2 No compensation by Dlg-MAGUK family abundance in SAP97-cKO animals. (A) Western blots and quantification showing no significant change in abundance of PSD-95 in cerebellum, hippocampus, and cortex of either male or female SAP97-cKO animals. (B) Western blots and quantification showing no significant change in abundance of PSD-93 in cerebellum, hippocampus, and cortex of either male or female SAP97-cKO animals. (C) Western blots and quantification showing no significant change in abundance of SAP102 in cerebellum, hippocampus, and cortex of either male or female SAP97-cKO animals. n.s., no significance (two-tailed Student's *t* test). Data are presented as mean ± SEM.

Figure 2.3. No change in mRNA expression level of AMPAR subunits, selected *SAP97* interactor genes, selected *Wnt/β-catenin* pathway targets, and selected *DISC1* pathway targets in SAP97-cKO animals. (A) qPCR results showing no significant change

in abundance of *Glua1*, *Glua3*, or *Glua4* mRNA transcripts in selected brain regions. (B) qPCR results showing no significant change in abundance of mRNA levels of selected *Sap97* interactor genes in selected brain regions. (C) qPCR results showing no significant change in abundance of Wnt/β-catenin pathway targets in selected brain regions. (D) qPCR results showing no significant change in abundance of *DISC1* pathway targets in cortex. n.s., no significance (two-tailed Student's *t* test). Data are presented as mean ± SEM.

Figure 2.4. Loss of SAP97 leads to downregulation of DEGs and enrichment of SCZ risk-related genes. (A) qPCR verification of top DEG (*Sgk1*) from RNAseq study. (B) Heat map representation of downregulation of DEGs in SAP97-cKO hippocampus. (C) DEGs are specifically enriched for SCZ risk-related genes. *P<.05 (two-tailed Student's *t* test), **P<.01 (hypergeometric probability test).

Figure 2.5. Comparison of open field behavior indicates anxiety-like phenotype in both male and female SAP97-cKO animals. (A) No group differences seen in average distance traveled in male animals. Female SAP97-cKO animals display significantly less distance traveled. (B) No group differences seen in average speed in male animals, while female SAP97-cKO show decreased speed. (C) Both male and female SAP97-cKO animals show decreased average distance from border of apparatus. n.s., no significance, *P<.05, **P<.01 (two-tailed Student's *t* test). Data are presented as mean ± SEM.

Figure 2.6. Comparison of elevated plus maze behavior between control and SAP97-cKO animals. (A) No group differences seen in average total time spent in open arms vs closed arms of maze. (B) No group differences seen in total open arm entries or closed arm entries. (C) No group differences seen in average distance traveled in elevated plus maze

apparatus. n.s., no significance (two-tailed Student's *t* test). Data are presented as mean ± SEM.

Figure 2.7. Comparison of cued fear conditioning behavior between control and SAP97-cKO animals. (A) Freezing behavior during habituation phase. Both control and SAP97-cKO male and female animals exhibit low levels of freezing with no significant differences between groups during habituation. (B) Freezing behavior during testing phase. Both male and female SAP97-cKO animals show no differences in freezing behavior compared to littermate controls. n.s., no significance (two-tailed Student's *t* test). Data are presented as mean ± SEM.

Figure 2.8. Comparison of novel object recognition behavior indicates male-specific cognitive deficit. Control male animals exhibit preference for novel object (Ctrl A-A vs Ctrl A-B), while SAP97-cKO male animals do not show preference. Both control and SAP97-cKO female animals show trend for preference of novel object, but did not reach significance. n.s., no significance, **P<.01 (ordinary one-way ANOVA with Tukey's test for multiple comparisons). Data are presented as mean ± SEM.

Figure 2.9. Comparison of rotarod behavior indicates female-specific motor learning deficit. (A) Both control and SAP97-cKO male animals show learning over the 4-day course of rotarod paradigm. Control female animals show increased motor learning over course of 4 days, while female SAP97-cKO show no significant increase in motor learning. (B) Plots showing comparison of Day 1 versus Day 4 rotarod data for control and SAP97-cKO animals indicates female-specific motor learning deficit. (C) Aged SAP97-cKO males show learning impairment over the 4-day course of rotarod. Both aged control and SAP97-

cKO female animals show no significant increase in motor learning, however, aged female SAP97-cKO animals have significantly worse performance on the task as compared to aged littermate controls. (D) Plots showing comparison of Day 1 versus Day 4 rotarod data for aged control and SAP97-cKO animals. n.s., no significance, **P<.01, ***P<.001 (repeated-measures two-way ANOVA with Tukey's test for multiple comparisons). Data are presented as mean ± SEM.

Figure 2.10. Comparison of social choice behavior between control and SAP97-cKO animals. (A) No significant differences observed between control and SAP97-cKO male or female animals in preference for social target. (B) No significant differences observed between control and SAP97-cKO male animals in preference for sniffing/investigating social target. **P<.01, ***P<.001, ****P<.0001 (ordinary one-way ANOVA with Tukey's test for multiple comparisons). Data are presented as mean ± SEM.

Table 2.1. List of genes with significant expression differences between control and SAP97-cKO mice.

Ensemble Genome ID	Gene Symbol	Log Fold Change
ENSMUSG00000019970	Sgk1	-0.604982801
ENSMUSG00000022770	Dlg1	-0.920172279
ENSMUSG00000023224	Serping1	-1.092300119
ENSMUSG00000029304	Spp1	-2.086028102
ENSMUSG00000031431	Tsc22d3	-0.662945543
ENSMUSG00000021390	Ogn	-1.36634343
ENSMUSG00000026728	Vim	-0.73795218
ENSMUSG00000022769	Sdf2l1	-0.813450163
ENSMUSG00000020467	Efemp1	-0.785266079
ENSMUSG00000033227	Wnt6	-1.860240362
ENSMUSG00000055128	Cgrrf1	-0.544558433
ENSMUSG00000037254	Itih2	-1.44816667
ENSMUSG00000070691	Runx3	-2.433336132
ENSMUSG00000032575	Manf	-0.581728345
ENSMUSG00000031289	Il13ra2	-1.998579379
ENSMUSG00000029661	Col1a2	-1.062558267
ENSMUSG00000067038	Rps12-ps3	2.467941625
ENSMUSG00000054619	Mettl7a1	-0.39870621
ENSMUSG00000024650	Slc22a6	-1.704915114
ENSMUSG00000015090	Ptgds	-1.253057418
ENSMUSG00000026043	Col3a1	-1.300397001
ENSMUSG00000030357	Fkbp4	-0.343944084
ENSMUSG00000071005	Ccl19	-2.363646432
ENSMUSG00000105843	Gm42644	-1.52239332
ENSMUSG00000030711	Sult1a1	-0.875568486

ENSMUSG00000079293	Clec7a	-2.007730582
ENSMUSG00000070436	Serpinh1	-0.658564574
ENSMUSG00000030154	Klrb1f	-2.174662835
ENSMUSG00000004951	Hspb1	-1.133328217
ENSMUSG00000036777	Anln	-0.750523147
ENSMUSG00000027248	Pdia3	-0.350126593
ENSMUSG00000030218	Mgp	-1.053034845
ENSMUSG00000030108	Slc6a13	-1.258734628
ENSMUSG00000057836	Xlr3a	-2.154616401
ENSMUSG00000000303	Cdh1	-1.987938463
ENSMUSG00000024087	Cyp1b1	-0.95956574
ENSMUSG00000032231	Anxa2	-0.831693618
ENSMUSG00000060591	Ifitm2	-0.82147731
ENSMUSG00000049241	Hcar1	-1.730353275
ENSMUSG00000032060	Cryab	-0.561687665
ENSMUSG00000026638	Irf6	-1.238846117
ENSMUSG00000022548	Apod	-0.868596841
ENSMUSG00000013584	Aldh1a2	-1.291353747
ENSMUSG00000019539	Rcn3	-0.615255839
ENSMUSG00000040055	Gjb6	-0.57564983
ENSMUSG00000040310	Alx4	-1.225356858
ENSMUSG00000031367	Ap1s2	-0.377838987
ENSMUSG00000023272	Creld2	-0.51269519
ENSMUSG00000038155	Gstp2	-1.96171475
ENSMUSG00000107215	Gm43197	-2.106413397
ENSMUSG00000074896	Ifit3	-0.830338737
ENSMUSG00000020484	Xbp1	-0.420933711
ENSMUSG00000034435	Tmem30b	-2.348757156
ENSMUSG00000022766	Serpind1	-1.521272379

ENSMUSG00000005125	Ndrg1	-0.558146377
ENSMUSG00000038393	Txnip	-0.584362558
ENSMUSG00000041548	Hspb8	-0.559621993
ENSMUSG00000034165	Ccnd3	-0.399017781
ENSMUSG00000027048	Abcb11	-2.19248413
ENSMUSG00000073418	C4b	-0.516645555
ENSMUSG00000025823	Pdia4	-0.55418986
ENSMUSG00000031070	Mrgprf	-1.766073987
ENSMUSG00000032179	Bmp5	-1.738362368
ENSMUSG00000043795	Prr33	-2.024615333
ENSMUSG00000048368	Omd	-2.520720092
ENSMUSG00000066861	Oas1g	-2.341011247
ENSMUSG00000024371	C2	-1.517189655

Table 2.2A. List of top diseases identified through IPA that were affected in hippocampus of SAP97-cKO animals.

Name	p-value	Genes Affected
Organismal Injury and Abnormalities	2.58E-02 - 1.30E-05	37
Respiratory Disease	2.52E-02 - 1.30E-05	10
Endocrine System Disorders	2.52E-02 - 1.42E-05	16
Gastrointestinal Disease	2.21E-02 - 1.42E-05	21
Immunological Disease	2.52E-02 - 1.42E-05	14

Table 2.2B. List of top molecular and cellular functions identified through IPA that were affected in hippocampus of SAP97-cKO animals.

Name	p-value	Genes Affected
Cell Death and Survival	2.83E-02 - 5.79E-05	21
Cellular Movement	2.52E-02 - 6.58E-05	18
Cell Morphology	2.52E-02 - 2.70E-04	19
Cellular Development	2.52E-02 - 2.70E-04	25
Cell-To-Cell Signaling and Interaction	2.47E-02 - 3.32E-04	17

Table 2.2C. List of top networks identified through IPA that were affected in hippocampus of SAP97-cKO animals

ID Associated Network Functions	Score
Organismal Injury and Abnormalities, Respiratory Disease, Cellular Movement	35
Cellular Development, Cellular Growth and Proliferation, Hematological System Development and Function	22
Ophthalmic Disease, Organismal Injury and Abnormalities, Hereditary Disorder	22
Cell Cycle, Gene Expression, Skeletal and Muscular System Development and Function	5
Developmental Disorder, Embryonic Development, Organ Development	2

Figure 2.1

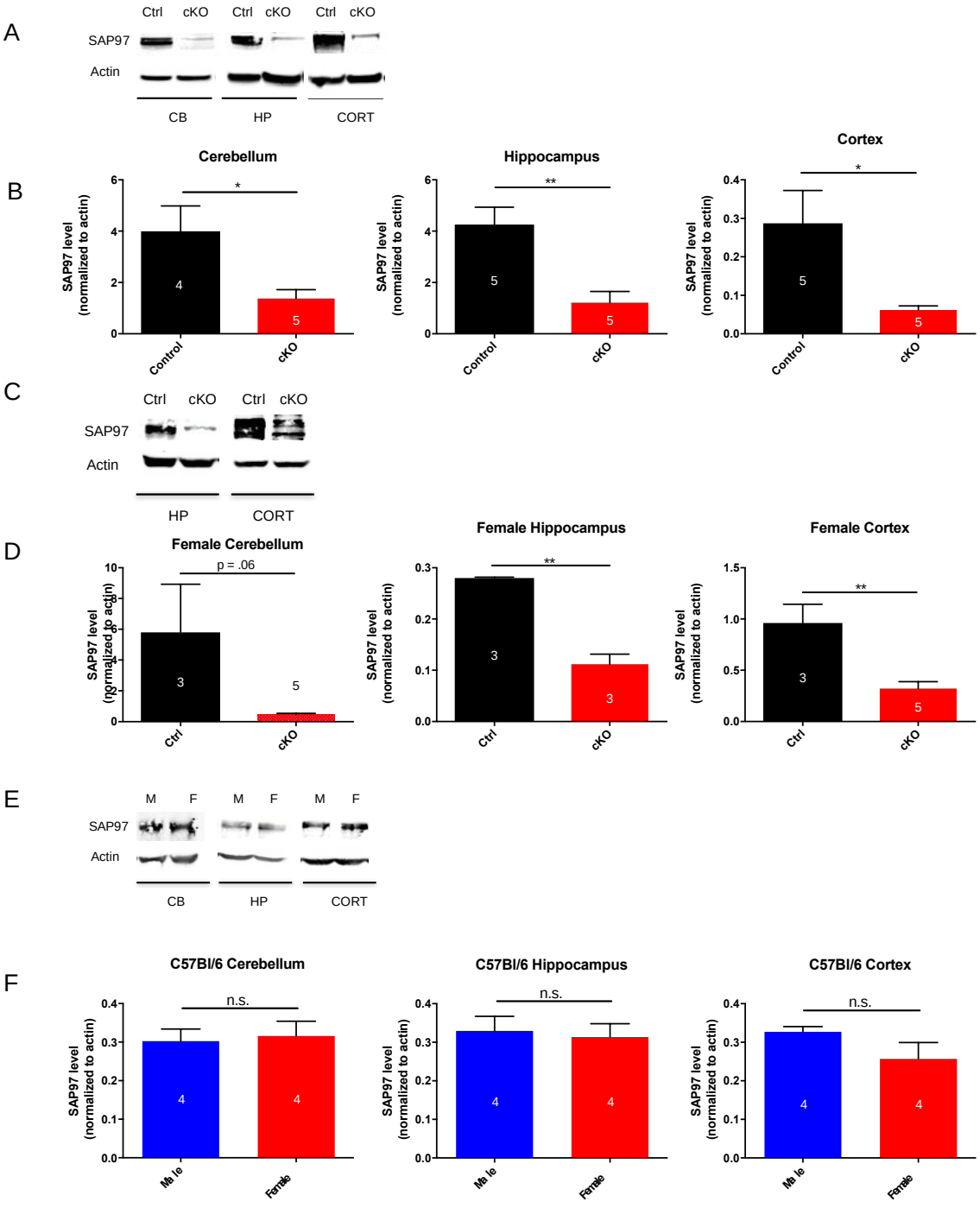

A
Ctrl cKO Ctrl cKO Ctrl cKO
SAP97
Actin
CB HP CORT
B
Cerebellum
Hippocampus
Cortex
SAP97 level (normalized to actin)
Control cKO
C
Ctrl cKO Ctrl cKO
SAP97
Actin
HP CORT
D
Female Cerebellum
Female Hippocampus
Female Cortex
p = .06
Ctrl cKO
E
M F M F M F
SAP97
Actin
CB HP CORT
F
C57Bl/6 Cerebellum
C57Bl/6 Hippocampus
C57Bl/6 Cortex
n.s.
Male Female

Figure 2.2

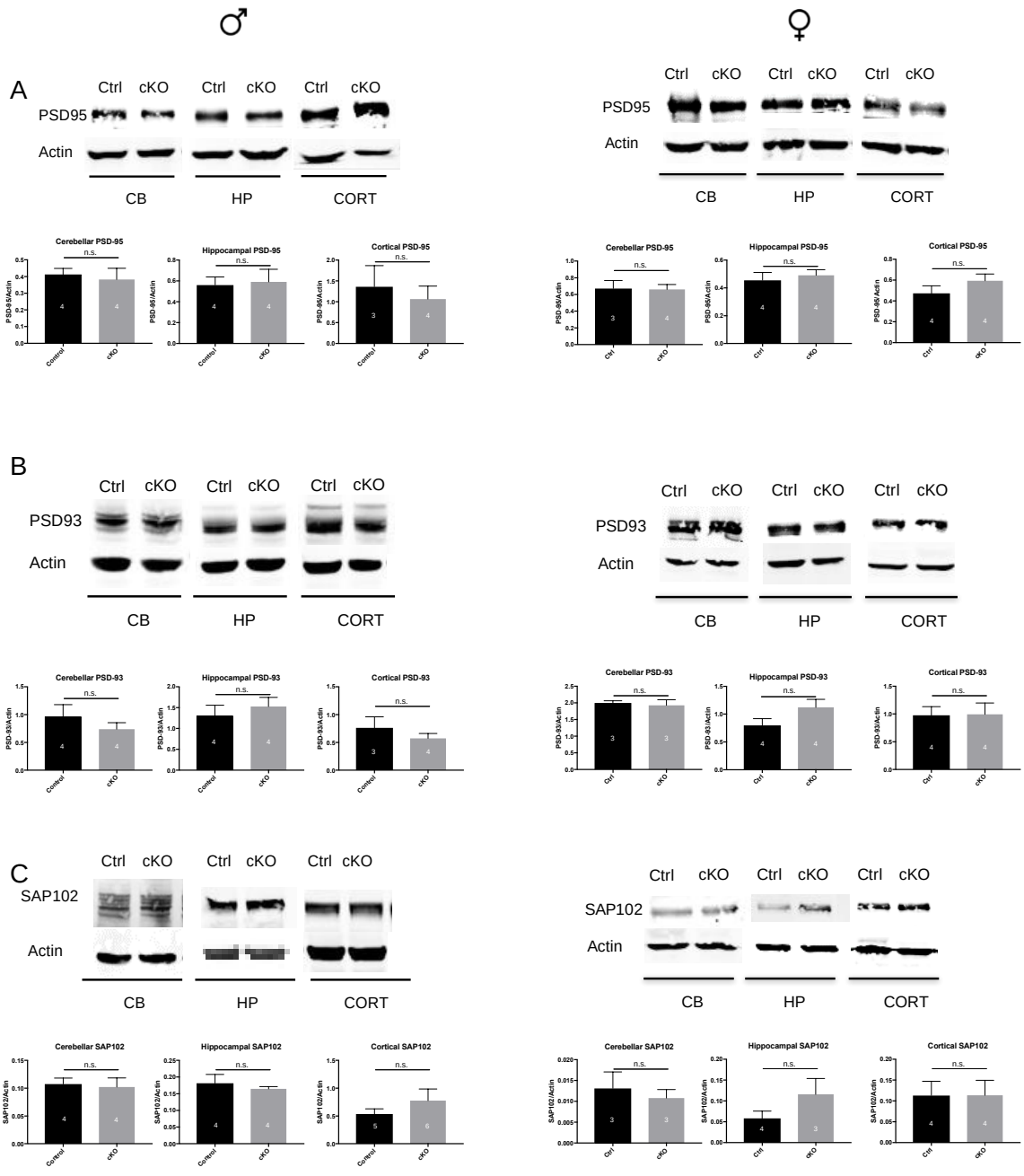

♂
♀
A
Ctrl
cKO
PSD95
Actin
CB
HP
CORT
Cerebellar PSD-95
Hippocampal PSD-95
Cortical PSD-95
n.s.
B
PSD93
Cerebellar PSD-93
Hippocampal PSD-93
Cortical PSD-93
C
SAP102
Cerebellar SAP102
Hippocampal SAP102
Cortical SAP102

Figure 2.3

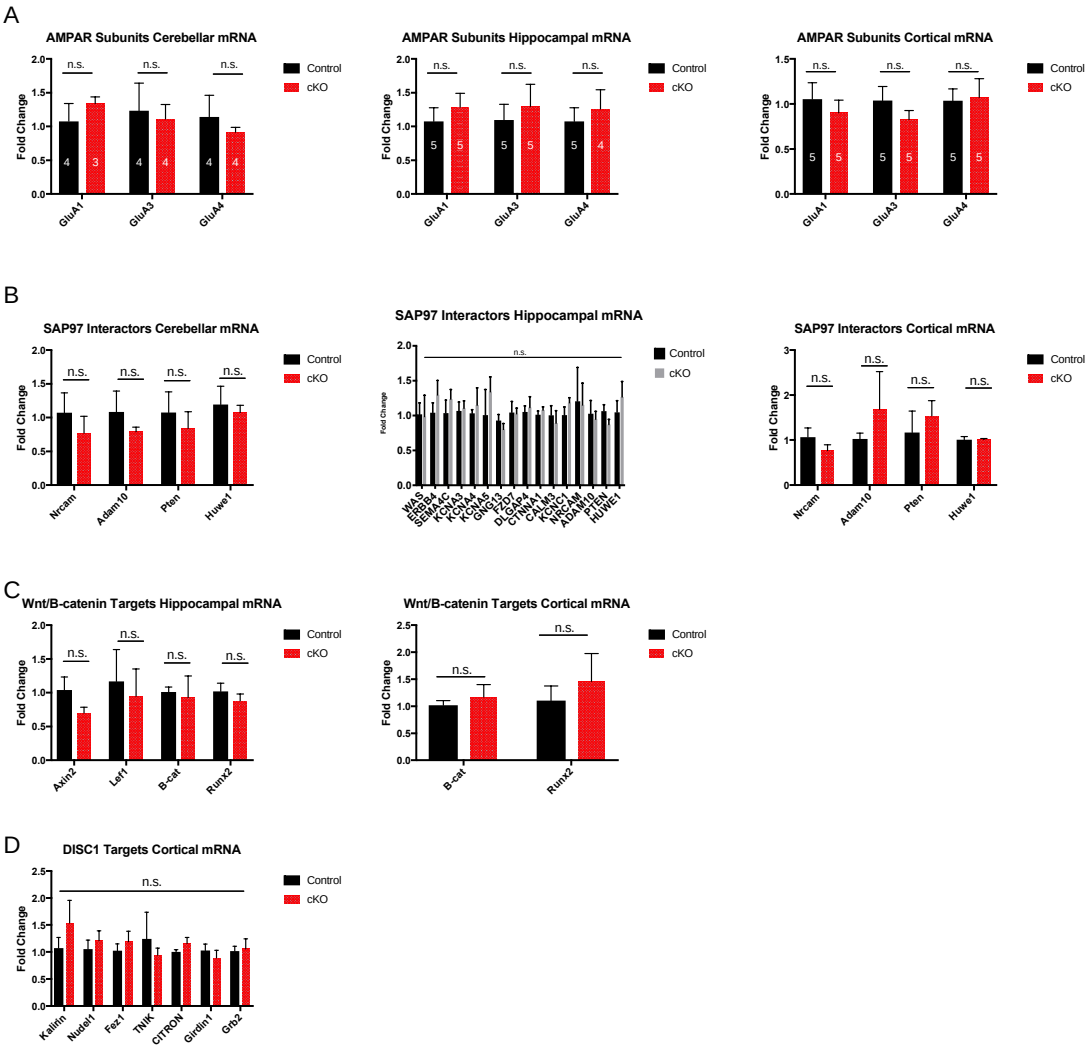

A
AMPAR Subunits Cerebellar mRNA
AMPAR Subunits Hippocampal mRNA
AMPAR Subunits Cortical mRNA
Fold Change
Control
cKO
n.s.
GluA1
GluA3
GluA4
B
SAP97 Interactors Cerebellar mRNA
SAP97 Interactors Hippocampal mRNA
SAP97 Interactors Cortical mRNA
Nrcam
Adam10
Pten
Huwe1
C
Wnt/B-catenin Targets Hippocampal mRNA
Wnt/B-catenin Targets Cortical mRNA
Axin2
Lef1
B-cat
Runx2
D
DISC1 Targets Cortical mRNA
Kalirin
Nudel1
Fez1
TNIK
CITRON
Girdin1
Grb2

Figure 2.4

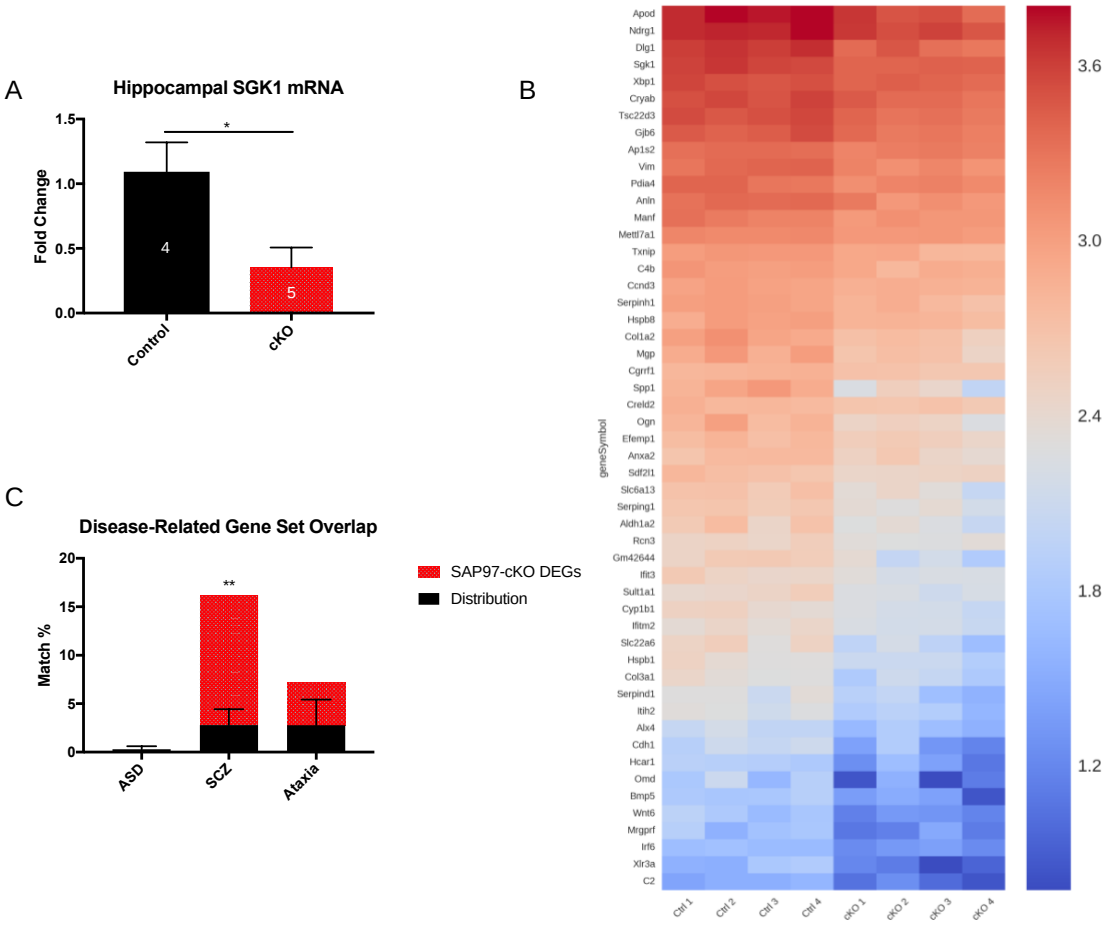

A
Hippocampal SGK1 mRNA
Fold Change
1.5
1.0
0.5
0.0
*
4
5
Control
cKO
B
Apod
Ndrg1
Dlg1
Sgk1
Xbp1
Cryab
Tsc22d3
Gjb6
Ap1s2
Vim
Pdia4
Anln
Manf
Mettl7a1
Txnip
C4b
Ccnd3
Serpinh1
Hspb8
Col1a2
Mgp
Cgnrf1
Spp1
Creld2
Ogn
Efemp1
Anxa2
Sdf2l1
Slc6a13
Serping1
Aldh1a2
Rcn3
Gm42644
Ifit3
Sult1a1
Cyp1b1
Ifitm2
Slc22a6
Hspb1
Col3a1
Serpind1
Itih2
Alx4
Cdh1
Hcar1
Omd
Bmp5
Wnt6
Mrgprf
Irf6
Xlr3a
C2
geneSymbol
Ctrl 1
Ctrl 2
Ctrl 3
Ctrl 4
cKO 1
cKO 2
cKO 3
cKO 4
3.6
3.0
2.4
1.8
1.2
C
Disease-Related Gene Set Overlap
Match %
20
15
10
5
0
**
ASD
SCZ
Ataxia
SAP97-cKO DEGs
Distribution

Figure 2.5

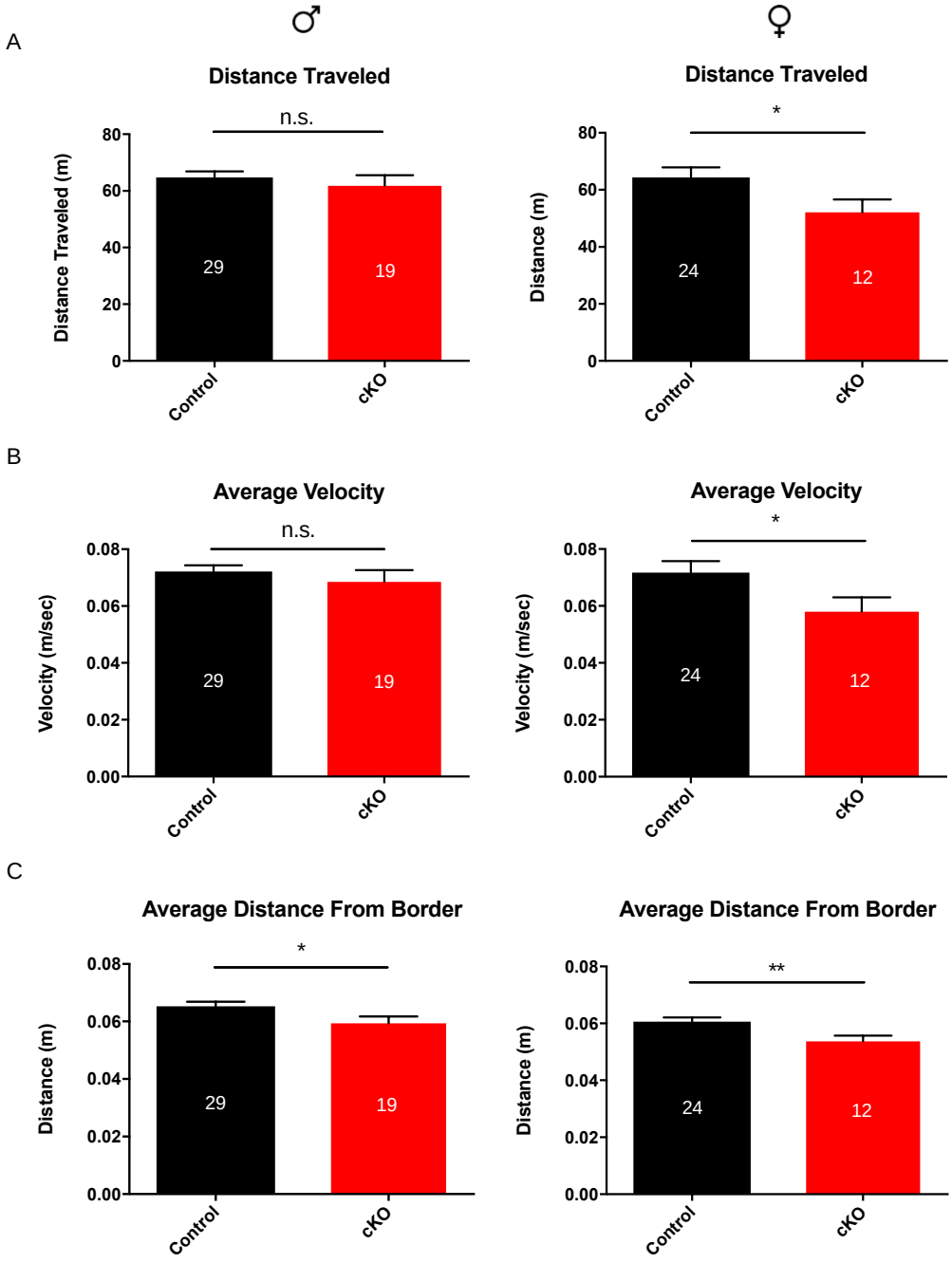

A
♂
Distance Traveled
n.s.
Distance Traveled (m)
80
60
40
20
0
29
19
Control
cKO
♀
Distance Traveled
*
Distance (m)
24
12
B
Average Velocity
n.s.
Velocity (m/sec)
0.08
0.06
0.04
0.02
0.00
Average Velocity
*
C
Average Distance From Border
*
Distance (m)
Average Distance From Border
**

Figure 2.6

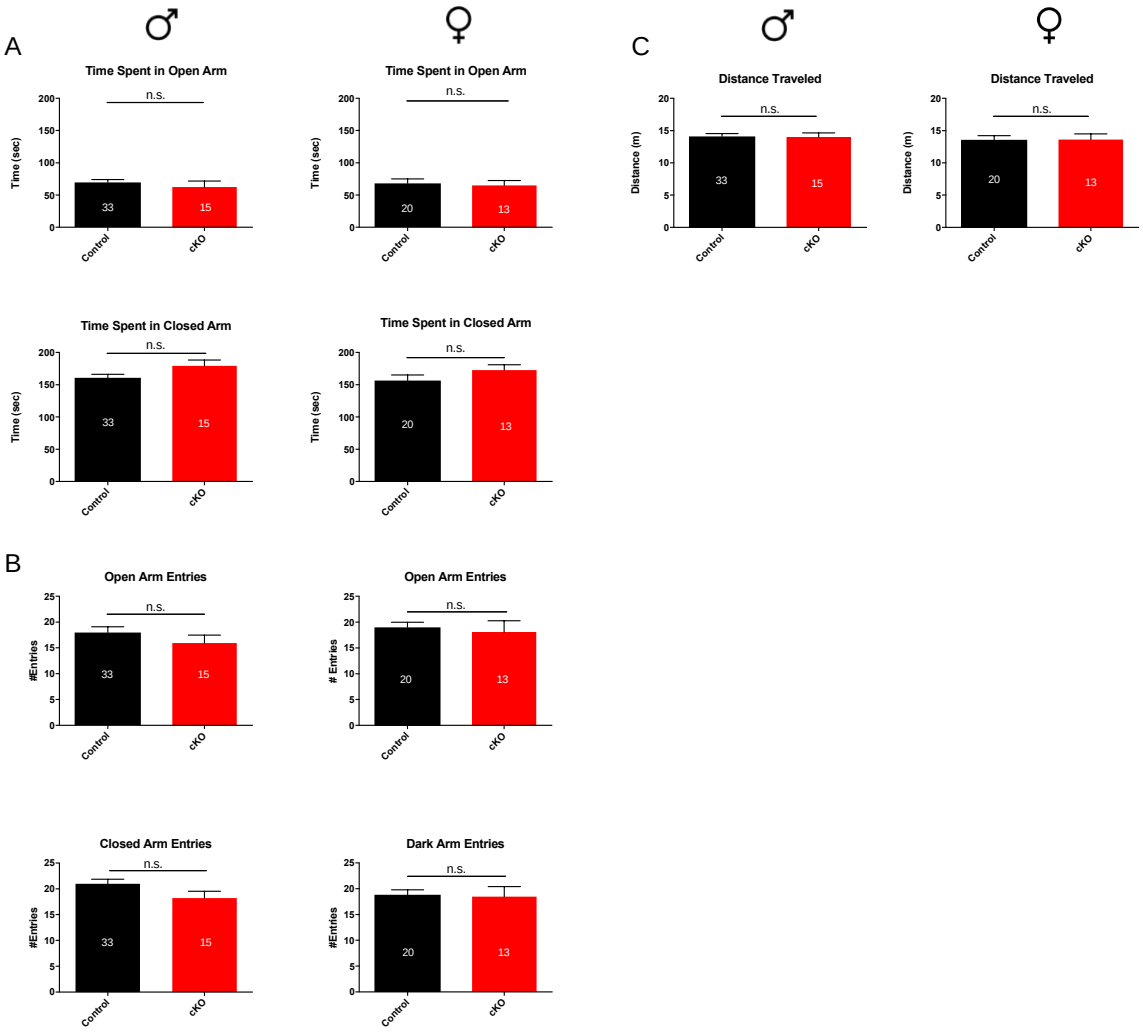

A
♂
♀
Time Spent in Open Arm
Time Spent in Closed Arm
Time (sec)
n.s.
33
15
20
13
Control
cKO
C
Distance Traveled
Distance (m)
B
Open Arm Entries
Closed Arm Entries
Dark Arm Entries
#Entries
Entries

Figure 2.7

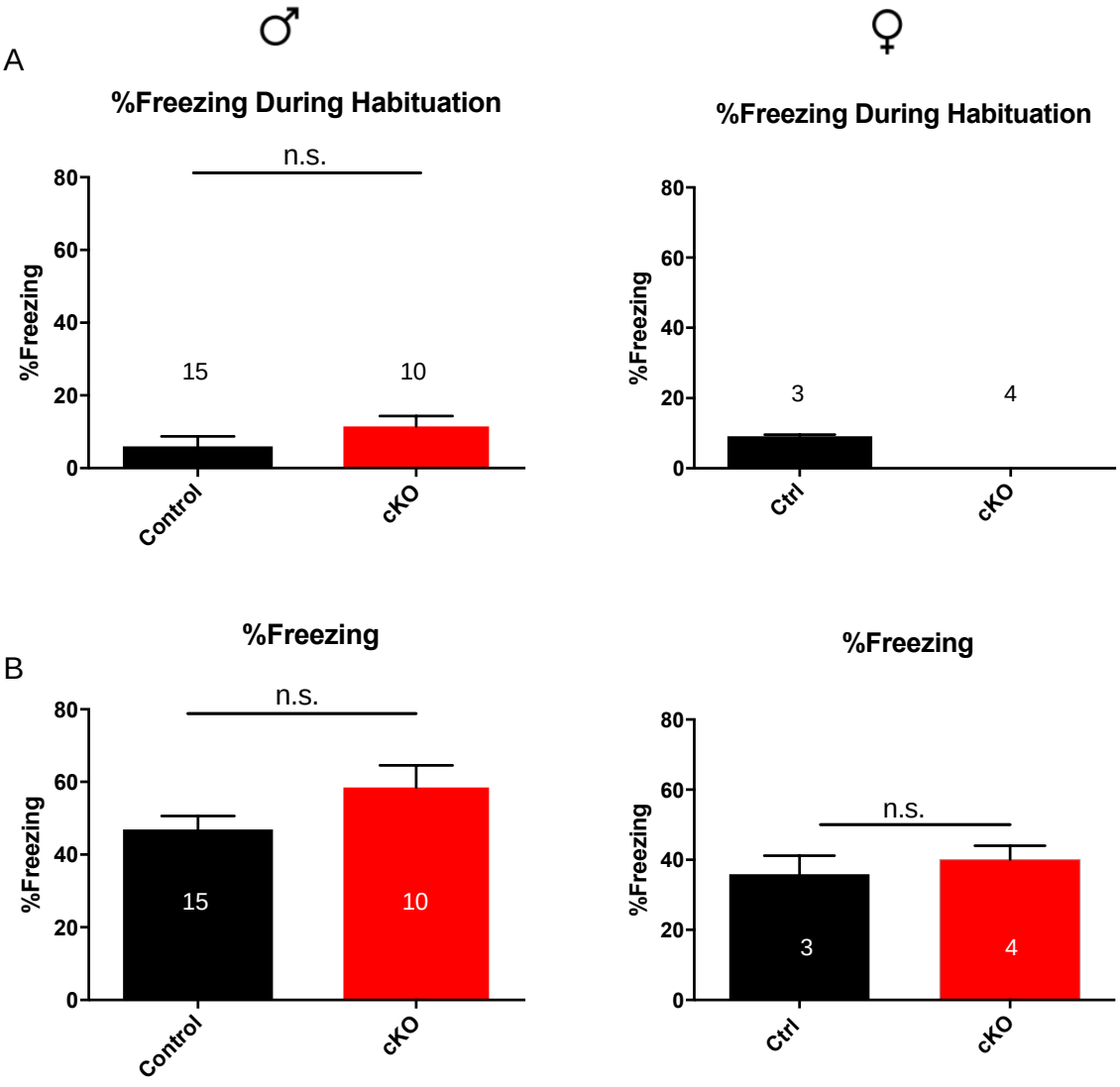

A
♂
%Freezing During Habituation
n.s.
%Freezing
80
60
40
20
0
15
10
Control
cKO
♀
%Freezing During Habituation
%Freezing
80
60
40
20
0
3
4
Ctrl
cKO
B
%Freezing
n.s.
%Freezing
80
60
40
20
0
15
10
Control
cKO
%Freezing
n.s.
%Freezing
80
60
40
20
0
3
4
Ctrl
cKO

Figure 2.8

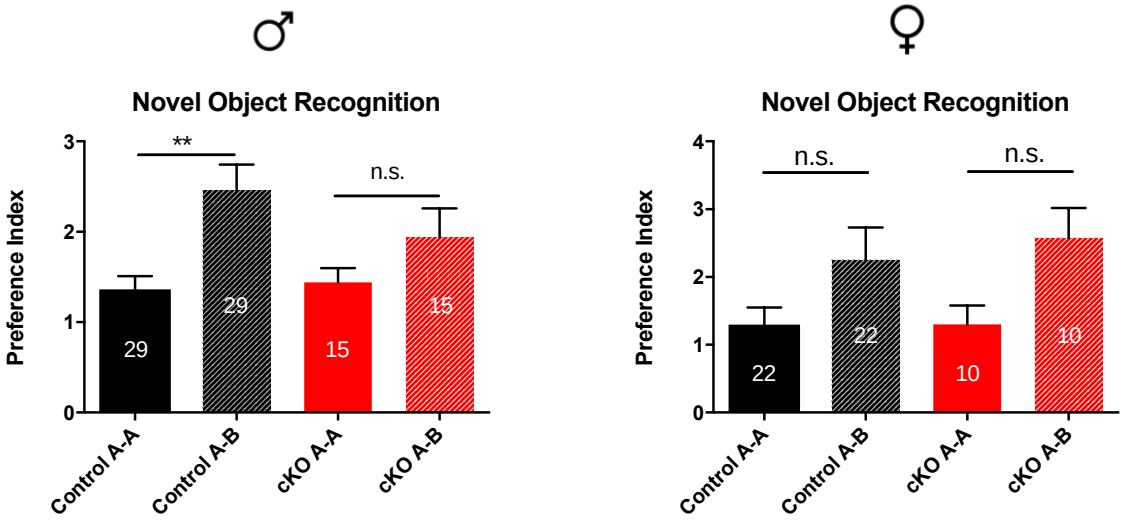
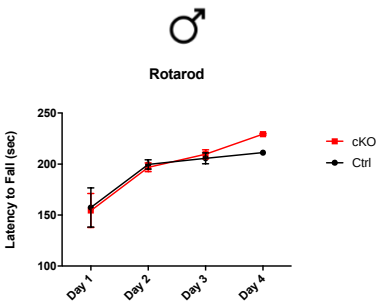
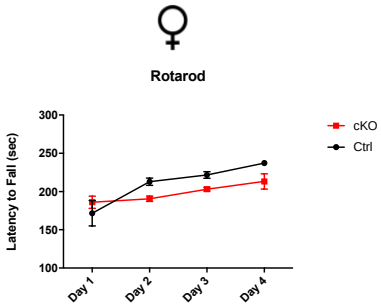

♂
Novel Object Recognition
Preference Index
**
n.s.
29
29
15
15
Control A-A
Control A-B
cKO A-A
cKO A-B
♀
Novel Object Recognition
Preference Index
n.s.
n.s.
22
22
10
10
Control A-A
Control A-B
cKO A-A
cKO A-B

Figure 2.9

A

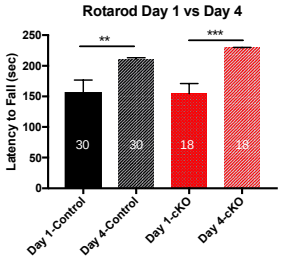


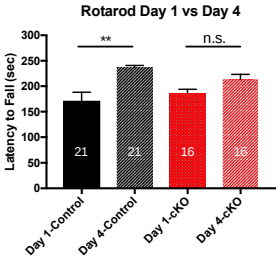
male control n = 30
male cKO n = 18



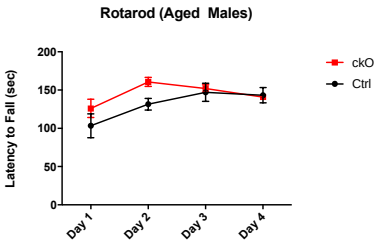
female control n = 21
female cKO n = 16

B

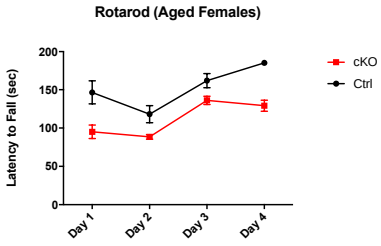




C

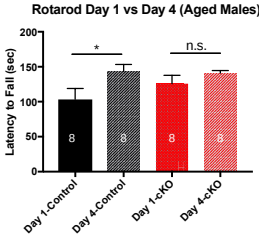


male control n = 8
male cKO n = 8



female control n = 7
female cKO n = 5

D



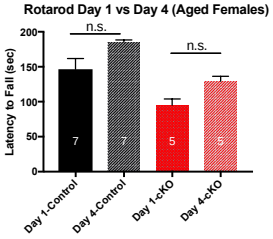
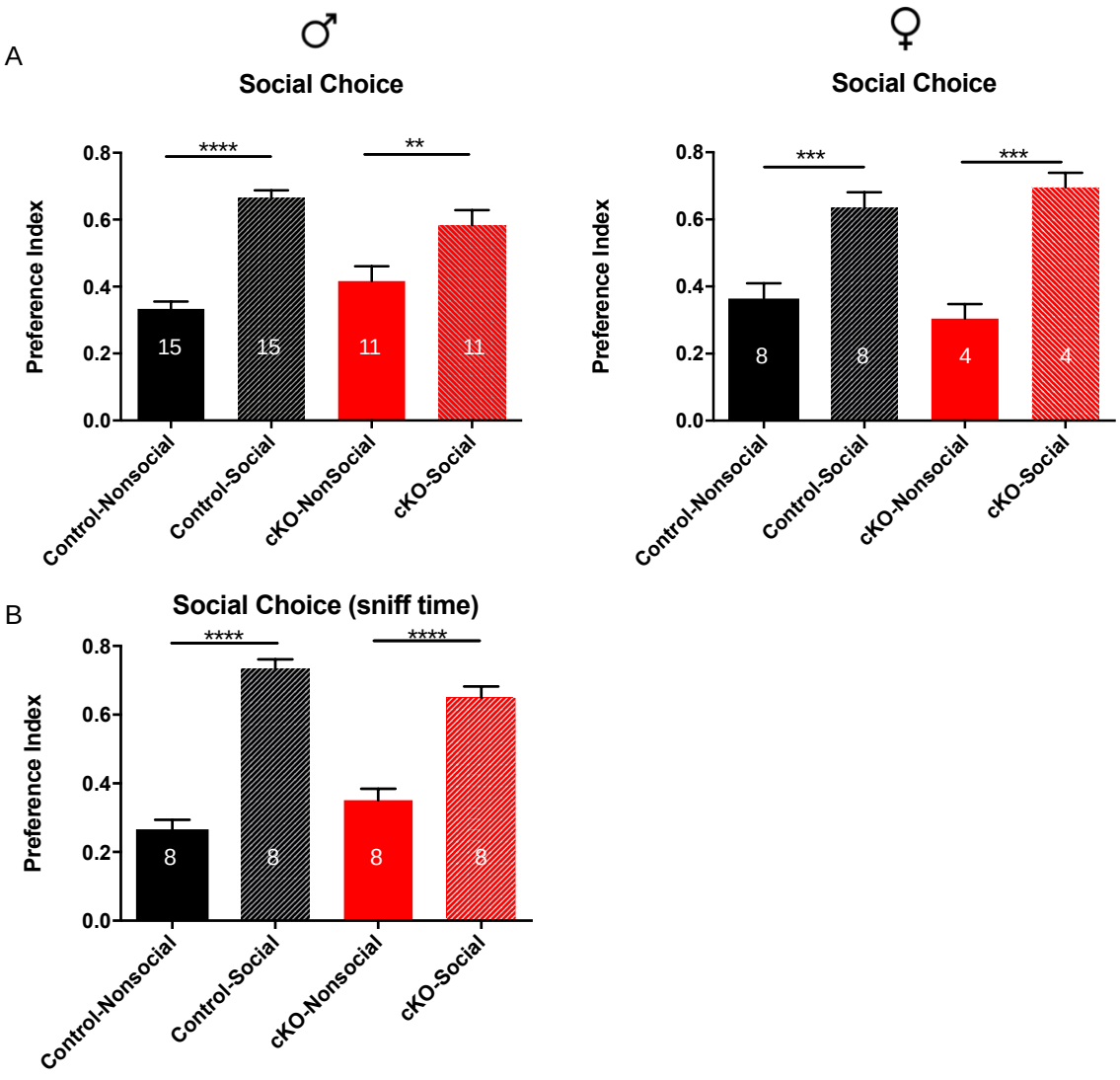


Figure 2.10


A
♂
Social Choice
Preference Index
0.0
0.2
0.4
0.6
0.8
****
**
15
15
11
11
Control-Nonsocial
Control-Social
cKO-NonSocial
cKO-Social
♀
Social Choice
***
***
8
8
4
4
Control-Nonsocial
Control-Social
cKO-Nonsocial
cKO-Social
B
Social Choice (sniff time)
****
****
8
8
8
8
Control-Nonsocial
Control-Social
cKO-Nonsocial
cKO-Social

CHAPTER 3: GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

In this thesis work, I have attempted to further understand the direct role of *SAP97* in psychiatric disorders, such as ASD and SCZ. In Chapter 2, I characterize mice conditionally lacking *Sap97* in the nervous system at the behavioral and transcriptomic level. I find that while SAP97-cKO mice have relatively subtle behavioral deficits, loss of *Sap97* results in an enrichment of SCZ risk-related DEGs. Below, I will describe the implications for this work and discuss remaining questions for future work.

THE MOLECULAR MODULE HYPOTHESIS

Investigations of ASD, SCZ, and other related psychiatric disorders indicate a highly polygenic architecture with small effect sizes of each implicated risk variant. Mouse modeling of these disorders by targeting one such risk variant typically demonstrates a moderate, or incomplete manifestation of the human disorder, as discussed in the Introduction of this thesis. Results from this thesis work coincide with previous results indicating psychiatric disorders to be polygenic in nature. While SAP97-cKO mice did not show robust changes across the behavioral domains related to ASD and/or SCZ, we observed subtle, sex-specific abnormalities. Likewise, loss of *Sap97* resulted in mild changes at the transcriptomic level as well, with 67 DEGs. However, this DEG set was enriched for SCZ risk-related genes, where 4 of 9 of these SCZ risk genes play a role in common pathways. These genes are *Serping1*, *Serpinh1*, *Serpind1*, and *C4b*.

This first three of these genes (*Serping1*, *Serpinh1*, *Serpind1*) are known as serine protease inhibitors, or serpins. Protease inhibition by serpins controls an array of biological processes, including coagulation and inflammation, and this family of genes

has previously been reported in the literature to be associated with SCZ (Madani et al., 2003; Saetre et al., 2007; Borges et al., 2010; Chang et al., 2017; Reumann et al., 2017; Weickert et al., 2018). *C4B*, or complement component b, has known roles in the coagulation pathway and is an important cofactor to the serine protease family. The strongest genetic association of SCZ at a population level involves variation in the Major Histocompatibility Complex (MHC) locus, where the association of SCZ with the MHC locus arises substantially from many diverse alleles of the *C4* genes (Rezende, 2003; Hoirisch-Clapauch et al., 2015; Schizophrenia Working Group of the Psychiatric Genomics Consortium et al., 2016).

These findings suggest that *Sap97* is a member of a tight cluster of genes, or "molecular module" that interacts and subserves aspects of normal behavior. Defects in many molecular modules in aggregate may result in the complete manifestation of the disorder. The components of this module may interact physically, functionally, developmentally, or in terms of localization. In order to elucidate how the components of this hypothesized molecular module interact, and how this impacts organismal behavior, a number of experiments can be done. Below, I outline a few potential future experiments to address these questions.

Interaction within the *SAP97* molecular module

One question to be addressed is whether *Sap97*, *Serping1*, *Serpinh1*, *Serpind1*, and *C4b* physically interact. One method to address this question would be by coimmunoprecipitation (CoIP) experiments. We can begin by overexpressing *Sap97* and one or a combination of SCZ risk genes in HEK cells, and subsequently perform CoIP. We may additionally examine whether this molecular module interacts

endogenously by performing CoIP experiments on brain tissue lysate generated from control and SAP97-cKO animals.

It is also plausible that the *SAP97* molecular module interacts in the region-specific manner. For example, members of the serpin family, such as *SERPINI1*, are restricted to regions in the brain where synaptic changes are associated with learning and memory (i.e. cortex, hippocampus, amygdala). One could argue that as a scaffolding protein with multiple protein-protein binding domains, *SAP97* may aid in tethering members of the molecular module to synaptic regions, where they act together to ensure proper synapse formation, function, and behavioral output. To address this question, we can prepare synaptosomes from control and SAP97-cKO animals and examine physical interaction within this proposed molecular module.

Previous evidence from the literature also suggests that members of the *SAP97* molecular module may interact functionally as well. For example, the serpin family of genes, namely *Serpini1,* has also been implicated in dendrite growth (Borges et al., 2010; Reumann et al., 2017). And similar to SAP97, overexpression studies in primary neurons leads to increased dendritic arborization (Borges et al., 2010). Additionally, characterization of mice lacking Schnurri-2, or MHC-binding protein 2, show immature dendritic spine morphology characterized by increases in spine length and decreases in spine diameter (Nakao et al., 2017). Schnurri-2 knockout mice also exhibited increases in *C4b* gene, which is thought to mediate synapse elimination during postnatal development, and show SCZ-like behaviors (Takao et al., 2013). Other observed changes in these mice included a significant reduction in GLUA1 and a trend for decreased expression of PSD-95, both of which have strong association with SAP97 (Nakao et al., 2017). Overall, these results suggest that the *SAP97* molecular module we have identified may possibly function at the synapse and play a role in dendrite and

spine morphology, and ultimately, proper behavioral output. We can attempt to address this question in the future by conducting co-knockdown experiments (i.e. knockdown of serpin family/*C4B* in addition to *SAP97*) and using dendritic growth as a readout. We can also employ the use of targeted viral vectors to further the knockdown level of other module members within the SAP97-cKO mice. It is plausible that a more severe knockdown effect of this module will result in a larger behavioral defect compared to what we observed in our studies.

Neuroinflammation and psychiatric disorders

Results from previous studies also indicate that our identified *SAP97* molecular module may also serve as a potential interface between inflammation and synaptic dysfunctions. Inflammation has been posited as a potential mechanism underlying the development and progression of SCZ and other related neuropsychiatric disorders, and meta-analyses have demonstrated that patients with SCZ reliably exhibit increased markers of inflammation (Potvin et al., 2008; Miller et al., 2011; Goldsmith et al., 2016; Miller and Goldsmith, 2016; Schizophrenia Working Group of the Psychiatric Genomics Consortium et al., 2016). Furthermore, there is growing literature showing that increased inflammatory cytokines may be linked to negative symptoms in patients with SCZ (Garcia-Rizo et al., 2012; Liu et al., 2012; Asevedo et al., 2014; Kissi et al., 2018). In particular, TNFα and interleukin-6 were found to be associated with deficit syndrome, a distinct subtype of SCZ characterized by primary and enduring negative symptoms (Goldsmith et al., 2018). A previous study identified TNFα as a critical molecule involved in the synaptic alterations seen in mice with experimental autoimmune encephalomyelitis (EAE), an animal model of brain inflammation (Centonze et al., 2009). TNFα has the potential to promote dendritic spine loss in EAE brains through an excitotoxic

mechanism (Centonze et al., 2009). *SAP97* also binds to TNFα converting enzyme (TACE) via the *SAP97* PDZ3 domain (Peiretti, 2003). Interestingly, overexpression of *SAP97* reduced the release of three different TACE-processed substrates, including TNFα (Peiretti, 2003). This suggests that *SAP97* participates in regulating the inflammatory response. Given that the serpin family of genes and *C4B* are also known to play important roles in the inhibition of the inflammatory response, an interesting potential future experiment would be to examine inflammation in the SAP97-cKO animals by measuring levels of cytokines such as TNFα and interleukin-6 in plasma collected from control and SAP97-cKO animals. As the *SAP97* molecular module is downregulated in the SAP97-cKO animals, we would expect these animals to have a heightened immune response. This increased immune response could be a potential mechanism underlying the specific behavioral deficits observed in the SAP97-cKO mice, and may serve as a target for therapeutic intervention.

ENVIRONMENTAL MODELS OF PSYCHIATRIC DISORDERS IMPLICATE IMMUNE RESPONSE

In this thesis work, we have chosen to focus predominately on examining and modeling the genetic etiology of psychiatric disorders. However, genetics do not account for all patient cases, and environmental factors are thought to contribute significantly to disease risk. Multiple environmental rodent models of psychiatric disorders implicate a heightened immune response.

A commonly-used environmentally induced model of ASD is the propionic acid (PPA) model. PPA is a short chain fatty acid, a metabolic end-product of enteric bacteria in the gut, and a common food preservative. Various studies have indicated that PPA causes ASD-like behaviors and neuroinflammatory response in rats (MacFabe

et al., 2007; Shultz et al., 2008; MacFabe et al., 2011). For example, Shultz et al. reported that exposure to PPA resulted in impaired social behavior measured as distance apart, proximity, and play behavior in rats (Shultz et al., 2008). MacFabe et al. demonstrated that rats treated with PPA showed restricted behavioral interest to a specific object among a group of objects, impaired social behavior, and impaired reversal in a T-maze task compared to controls, in addition to reactive astrogliosis and activated microglia in the brain (MacFabe et al., 2011).

As SCZ is considered a neurodevelopmental disorder, early environmental factors potentially play a role in the etiology of the disease. One early life factor associated with SCZ is maternal infection during pregnancy (Brown and Derkits, 2010). Early epidemiological studies found an increased rate of SCZ among offspring who were *in utero* during major influenza epidemics as compared to non-epidemic periods (Brown and Derkits, 2010). This association was replicated in several geographic populations (Brown and Derkits, 2010). Additional studies found an increased risk of SCZ among offspring of mothers who received diagnosis of influenza, toxoplasmosis, rubella, or bacterial infection during pregnancy (Brown et al., 2000; Brown et al., 2004; Brown et al., 2005; Sorensen et al., 2009). High levels of pro-inflammatory cytokines in the maternal serum during pregnancy were also found to increase risk of SCZ in *in utero* offspring (Canetta et al., 2014). Maternal immune activation (MIA), is not specific to schizophrenia, but may also increase the risk for ASD, bipolar disorder, and depression (Canetta et al., 2014).

Several groups have studied prenatal infection in rodent models. Both direct viral infection of the fetus, as well as abnormal activation of the maternal immune system, resulted in behavioral impairments relevant to SCZ (Shi et al., 2002; Shi et al., 2005; Meyer and Feldon, 2012). The maternal immune system can be activated with a

synthetic double-stranded RNA, polyinosinic-polycytidylic acid (PolyIC). The PolyIC model of SCZ has gained wide recognition in the scientific community as it successfully accounts for several aspects of SCZ: epidemiology, pathophysiology, symptomology, and treatment (Meyer and Feldon, 2012).

PolyIC is a commercially available synthetic analog of double-stranded RNA. Double-stranded RNA is generated during viral infection as a replication intermediate for single-stranded RNA or as a byproduct of symmetrical transcription in DNA viruses (Takeuchi and Akira, 2007). It is recognized as a foreign by the mammalian immune system through the transmembrane protein toll-like receptor 3 (TLR3) (Alexopoulou et al., 2001). Upon binding to TLRs, double stranded RNA, or the synthetic analog PolyIC, stimulates production and release of many pro-inflammatory cytokines, including interleukin-1B, interleukin-6, and TNFα (Fortier et al., 2004; Cunningham et al., 2007). PolyIC is also a potent inducer of the type 1 interferons INF-a and INF-b (Kimura et al., 1994; Traynor et al., 2004). Administering PolyIC can therefore mimic the acute phase response to viral infection. Maternal exposure to PolyIC is capable of altering pro- and anti-inflammatory cytokine levels in the three relevant compartments of the maternal-fetal interface of rodents, namely the placenta, amniotic fluid, and the fetus (Meyer et al., 2006). This allows the model to include aspects of maternal/fetal inflammation, taking into account one of the most relevant immunological mechanisms suggested to be crucial for mediating the long-term effects of prenatal infection on brain and behavioral development (Patterson, 2009; Meyer et al., 2016). The PolyIC mouse model of SCZ recapitulates behavioral phenotypes such as sensorimotor gating deficits, impaired working memory, and reduced social behavior (Ibi et al., 2009).

Increased susceptibility to environmental stress remains an open question in the SAP97-cKO animals. As discussed earlier, the literature supports the idea that inhibition

of the *SAP97* molecular module may induce a heightened immune response in the SAP97-cKO animals. However, as the behavioral deficits observed were moderate, it is plausible to assume that the potential immune response in these animals was not sufficient to induce a large-scale behavioral effect. A complementary experiment to using targeted viral vectors to further the knockdown level of other *SAP97* molecular module members would be to expose SAP97-cKO animals to environmental stress (i.e. maternal immune activation), and measure whether we obtain a more complete manifestation of SCZ as compared to the environmental stress or SAP97-cKO model alone. Results from these proposed experiments would elucidate the mechanism by which loss of *SAP97* contributes to the etiology of SCZ.

LIMITATIONS OF RODENT MODELS

How useful are rodent models, in general, for understanding human psychiatric disorders? Animal models aim to recapitulate behavioral symptoms, but this approach has limitations as some of the behavioral symptoms are distinctive for humans and are not measurable in animals (i.e. delusions or hallucinations) (Canetta and Kellendonk, 2018). Additionally, compensatory mechanisms present in mice may not have the same effects in humans (Deconinck et al., 1997).

One classical example is the *mdx* mouse model for Duchenne muscular dystrophy (DMD). DMD in humans is caused by lack of dystrophin, a large membrane-associated protein expressed in muscle and the brain (Tinsley et al., 1994). The *mdx* mouse model lacks dystrophin due to a mutation that results in a premature stop codon, but presents with a much milder form of the disease than in humans (Bulfield et al., 1984). Compensation for lack of dystrophin by structurally related proteins such as utrophin may also be more successful in the mouse, leading to a milder phenotype than

in humans (Deconinck et al., 1997). Dystrophin/utrophin double knockout mice have been generated and present with many more clinical signs of DMD than *mdx* mice (Deconinck et al., 1997). Nevertheless, the *mdx* mouse model is a popular model for studying DMD and has proven useful for examining potential therapeutics and molecular mechanisms underlying the disorder.

The story of the *mdx* mouse model may also be true for the SAP97-cKO model. *SAP97* has wide molecular diversity, which is created by extensive alternative splicing. Uezato and colleagues identified a new *SAP97* splicing variant that is transcribed from a previously unreported 95-base-pair exon (exon 3b) (Uezato et al., 2015). In post-mortem prefrontal cortices of patients with SCZ, mRNA expression of exon 3b was significantly reduced, specifically in patients with early-onset SCZ (Uezato et al., 2015). However, this exon is primate-specific (Uezato et al., 2017). It is plausible that this primate-specific exon of *SAP97* is responsible for contributing to a more severe manifestation of SCZ in humans, while loss of mouse *SAP97* is more easily compensated for by other genes. In this thesis work, we have shown that loss of *SAP97* is not compensated for by change in overall abundance of the other Dlg-MAGUK family members. This does not rule out compensation in terms of localization (i.e. at the synapse versus whole tissue) or by activity. These open questions should be addressed in the future to further understand *SAP97's* role in disease in humans versus the mouse.

Difficulty in using DSM criteria for rodent models of psychiatric disorders

An additional complication with using rodents to model psychiatric disorders is determining how symptoms in an animal model add up to a recognized human disorder. The Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IVTR) contains scant knowledge of the pathophysiology underlying these disorders (Nestler

and Hyman, 2010). Diagnoses are based solely on phenomenology, such as symptoms, signs, and course of illness. As a result, the boundaries between DSM-IVTR disorders, and the boundaries between disorder and normal variation, are unclear (Hyman, 2010). For example, two patients with major depression may exhibit no overlap of the 9 symptoms listed in the DSM criteria for Major Depressive Episode. The multiple symptom combination, in addition to the inability to assess certain symptoms in mice, means that different mouse models of depression would have little in common (Nestler and Hyman, 2010). This issue is extended to a variety of psychiatric disorders, including ASD and SCZ.

Additionally, DSM-IVTR diagnoses do not currently map onto abnormalities of molecules, synapses, cells, or neural circuits for psychiatric disorders. There are no molecular or cellular abnormalities in the human disease which could validate potential phenomenology in an animal (Nestler and Hyman, 2010). Individual symptoms observed in animal models may not have a simple, straightforward correspondence to human symptoms. As a result, animal models are unlikely to mirror the full extent of a given psychiatric disorder. While animal models of disease are useful, it is necessary to keep in mind the imperfections of rodent models when interpreting results.

CONCLUSIONS

This thesis work provides the first broad behavioral and transcriptomic characterization of *SAP97* in the mouse nervous system. Despite study limitations, we show that loss of *SAP97* contributes to enrichment of SCZ related genes, as well as moderate sex-specific behavioral abnormalities. We have potentially identified a module of genes where *SAP97* and the serpin/*C4B* family are participants, whose role is to regulate inflammatory response in the nervous system. While we have taken the first

steps to elucidate the contribution of *SAP97* to psychiatric disorders, further investigation is needed to validate the “molecular module” hypothesis and fully understand the downstream pathways and behaviors affected by this module. Further understanding of the role of *SAP97* in regulating inflammatory response and behavior may identify new targets for therapeutic intervention.

BIBLIOGRAPHY

Alarcón M, Abrahams BS, Stone JL, Duvall JA, Perederiy JV, Bomar JM, Sebat J, Wigler M, Martin CL, Ledbetter DH, Nelson SF, Cantor RM, Geschwind DH (2008) Linkage, Association, and Gene-Expression Analyses Identify CNTNAP2 as an Autism-Susceptibility Gene. The American Journal of Human Genetics 82:150–159.

Alexopoulou L, Holt AC, Medzhitov R, Flavell RA (2001) Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature 413(6857):732-8.

Al-Hallaq RA, Yasuda RP, Wolfe BB (2001) Enrichment of N-methyl-d-aspartate NR1 splice variants and synaptic proteins in rat postsynaptic densities. J Neurochem 77(1):110-9.

Allswede DM, Zheutlin AB, Chung Y, Anderson K, Hultman CM, Ingvar M, Cannon TD (2017) Complement Gene Expression Correlates with Superior Frontal Cortical Thickness in Humans. Nature Publishing Group:1–9.

Auranen M, Vanhala R, Varilo T, Ayers K, Kempas E, Ylisaukko-Oja T, Sinsheimer JS, Peltonen L, Järvelä I (2002) A Genomewide Screen for Autism-Spectrum Disorders: Evidence for a Major Susceptibility Locus on Chromosome 3q25-27. Am J Hum Genet 71(4):777-90.

Ardiles AO, Grabrucker AM, Scholl FG, Rudenko G, Borsello T (2017) Editorial Molecular and Cellular Mechanisms of Synaptopathies. Neural Plasticity:1–3.

Arking DE, Cutler DJ, Brune CW, Teslovich TM, West K, Ikeda M, Rea A, Guy M, Lin S, Cook EH Jr., Chakravarti A (2008) A Common Genetic Variant in the Neurexin Superfamily Member CNTNAP2 Increases Familial Risk of Autism. The American Journal of Human Genetics 82:160–164.

Asevedo E, Rizzo LB, Gadelha A, Mansur RB, Ota VK, Berberian AA, Scarpato BS, Teixeira AL, Bressan RA, Brietzke E (2014) Peripheral interleukin-2 level is associated with negative symptoms and cognitive performance in schizophrenia. Physiology & Behavior 129:194–198.

Bang ML, Owczarek S (2013) A Matter of Balance: Role of Neurexin and Neuroligin at the Synapse. Neurochem Res 38:1174–1189.

Bats C, Groc L, Choquet D (2007) The Interaction between Stargazin and PSD-95 Regulates AMPA Receptor Surface Trafficking. Neuron 53:719–734.

Benson MA, Newey SE, Martin-Rendon E, Hawkes R, Blake DJ (2001) Dysbindin, a Novel Coiled-coil-containing Protein That Interacts with the Dystrobrevins in Muscle and Brain. Journal of Biological Chemistry 276:24232–24241.

Berkel S, Marshall CR, Weiss B, Howe J, Roeth R, Moog U, Endris V, Roberts W, Szatmari P, Pinto D, Bonin M, Riess A, Engels H, Sprengel R, Scherer SW, Rappold GA

(2010) Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation. Nat Genet 42:489–491.

Bhardwaj SK, Baharnoori M, Sharif-Askari B, Kamath A, Williams S, Srivastava LK (2009) Behavioral characterization of dysbindin-1 deficient sandy mice. Behavioural Brain Research 197:435–441.

Blundell J, Blaiss CA, Etherton MR, Espinosa F, Tabuchi K, Walz C, Bolliger MF, Sudhof TC, Powell CM (2010) Neuroligin-1 Deletion Results in Impaired Spatial Memory and Increased Repetitive Behavior. Journal of Neuroscience 30:2115–2129.

Boccuto L, Lauri M, Sarasua SM, Skinner CD, Buccella D, Dwivedi A, Orteschi D, Collins JS, Zollino M, Visconti P, DuPont B, Tiziano D, Schroer RJ, Neri G, Stevenson RE, Gurrieri F, Schwartz CE (2012) Prevalence of SHANK3 variants in patients with different subtypes of autism spectrum disorders. 21:310–316.

Borges VM, Lee TW, Christie DL, Birch NP (2010) Neuroserpin regulates the density of dendritic protrusions and dendritic spine shape in cultured hippocampal neurons. J Neurosci Res 88:n/a–n/a.

Brown AS, Begg MD, Gravenstein S, Schaefer CA, Wyatt RJ, Bresnahan M, Babulas VP, Susser ES (2004) Serologic Evidence of Prenatal Influenza in the Etiology of Schizophrenia. Arch Gen Psychiatry 61(8):774-80.

Brown AS, Cohen P, Greenwald S, Susser E (2000) Nonaffective Psychosis After Prenatal Exposure to Rubella. Am J Psychiatry 157(3):438-43.

Brown AS, Derkits EJ (2010) Prenatal Infection and Schizophrenia: A Review of Epidemiologic and Translational Studies. AJP 167:261–280.

Brown AS, Schaefer CA, Quesenberry CP Jr, Liu L, Babulas VP, Susser ES (2005) Maternal Exposure to Toxoplasmosis and Risk of Schizophrenia in Adult Offspring. Am J Psychiatry 162(4):767-73.

Bulfield G, Siller WG, Wight PA, Moore KJ (1984) X chromosome-linked muscular dystrophy (mdx) in the mouse. Proc Natl Acad Sci USA 81(4):1189-92.

Cai C (2006) Interaction between SAP97 and PSD-95, Two Maguk Proteins Involved in Synaptic Trafficking of AMPA Receptors. Journal of Biological Chemistry 281:4267–4273.

Cai C, Li H, Kangasniemi A, Pihlajamaa T, Ossowski Von L, Kerkelä K, Schulz S, Rivera C, Keinänen K (2008) Somatostatin receptor subtype 1 is a PDZ ligand for synapse-associated protein 97 and a potential regulator of growth cone dynamics. NSC 157:833–843.

Canetta S, Kellendonk C (2018) Can we use mice to study schizophrenia? Philos Trans R Soc Lond B Biol Sci 373(1742).

Canetta S, Sourander A, Surcel H-M, Hinkka-Yli-Salomäki S, Leiviskä J, Kellendonk C, McKeague IW, Brown AS (2014) Elevated Maternal C-Reactive Protein and Increased Risk of Schizophrenia in a National Birth Cohort. AJP 171:960–968.

Canetta SE, Bao Y, Co MDT, Ennis FA, Cruz J, Terajima M, Shen L, Kellendonk C, Schaefer CA, Brown AS (2014b) Serological Documentation of Maternal Influenza Exposure and Bipolar Disorder in Adult Offspring. AJP 171:557–563.

Caruana G, Bernstein A (2001) Craniofacial Dysmorphogenesis Including Cleft Palate in Mice with an Insertional Mutation in the discs large Gene. Molecular and Cellular Biology 21:1475–1483.

Casanova MF, El-Baz AS, Kamat SS, Dombroski BA, Khalifa F, Elnakib A, Soliman A, Allison-McNutt A, Switala AE (2013) Focal cortical dysplasias in autism spectrum disorders. 1:1–1.

Catts VS, Lai YL, Weickert CS, Weickert TW, Catts SV (2016) A quantitative review of the postmortem evidence for decreased cortical N-methyl-d-aspartate receptor expression levels in schizophrenia: How can we link molecular abnormalities to mismatch negativity deficits? Biological Psychology 116:57–67.

Centonze D et al. (2009) Inflammation Triggers Synaptic Alteration and Degeneration in Experimental Autoimmune Encephalomyelitis. Journal of Neuroscience 29:3442–3452.

Chadman KK, Gong S, Scattoni ML, Boltuck SE, Gandhy SU, Heintz N, Crawley JN (2008) Minimal aberrant behavioral phenotypes of neuroligin-3 R451C knockin mice. Autism Research 1:147–158.

Chang X, Liu Y, Hahn C-G, Gur RE, Sleiman PMA, Hakonarson H (2017) RNA-seq analysis of amygdala tissue reveals characteristic expression profiles in schizophrenia. Nature Publishing Group 7:1–8.

Chen K, Featherstone DE (2005) BMC Biology. BMC Biol 3:1–13.

Chen L, Chetkovich DM, Petralia RS, Sweeney NT, Kawasaki Y, Wenthold RJ, Bredt DS, Nicoll RA (2000) Stargazin regulates synaptic targeting of AMPA receptors by two distinct mechanisms. Nature 21-28;408(6815):936-43.

Chen X, Levy JM, Hou A, Winters C, Azzam R, Sousa AA, Leapman RD, Nicoll RA, Reese TS (2015) PSD-95 family MAGUKs are essential for anchoring AMPA and NMDA receptor complexes at the postsynaptic density. Proc Natl Acad Sci USA 112:E6983–E6992.

Chen X-W, Feng Y-Q, Hao C-J, Guo X-L, He X, Zhou Z-Y, Guo N, Huang H-P, Xiong W, Zheng H, Zuo P-L, Zhang CX, Li W, Zhou Z (2008) DTNBP1, a schizophrenia susceptibility gene, affects kinetics of transmitter release. J Cell Biol 181:791–801.

Chen YJJ, Johnson MA, Lieberman MD, Goodchild RE, Schobel S, Lewandowski N, Rosoklija G, Liu RC, Gingrich JA, Small S, Moore H, Dwork AJ, Talmage DA, Role LW

(2008) Type III Neuregulin-1 Is Required for Normal Sensorimotor Gating, Memory-Related Behaviors, and Corticostriatal Circuit Components. Journal of Neuroscience 28:6872–6883.

Chun S, Westmoreland JJ, Bayazitov IT, Eddins D, Pani AK, Smeyne RJ, Yu J, Blundon JA, Zakharenko SS (2014) Specific disruption of thalamic inputs to the auditory cortex in schizophrenia models. Science 344:1178–1182.

Corvin AP, Morris DW, McGhee K, Schwaiger S, Scully P, Quinn J, Meagher D, St Clair D, Waddington JL, Gill M (2004) Confirmation and refinement of an “at-risk” haplotype for schizophrenia suggests the EST cluster, Hs.97362, as a potential susceptibility gene at the Neuregulin-1 locus. Mol Psychiatry 9:208–212.

Cox MM, Tucker AM, Tang J, Talbot K, Richer DC, Yeh L, Arnold SE (2009) Neurobehavioral abnormalities in the dysbindin-1 mutant, sandy, on a C57BL/6J genetic background. Genes, Brain and Behavior 8:390–397.

Cunningham C, Campion S, Teeling J, Felton L, Perry VH (2007) The sickness behaviour and CNS inflammatory mediator profile induced by systemic challenge of mice with synthetic double-stranded RNA (poly I:C). Brain, Behavior, and Immunity 21:490–502.

Cuthbert PC, Stanford LE, Coba MP, Ainge JA, Fink AE, Opazo P, Delgado JY, Komiyama NH, O'Dell TJ, Grant SGN (2007) Synapse-Associated Protein 102/dlgh3 Couples the NMDA Receptor to Specific Plasticity Pathways and Learning Strategies. Journal of Neuroscience 27:2673–2682.

David S, Kalb RG (2005) Serum/glucocorticoid-inducible kinase can phosphorylate the cyclic AMP response element binding protein, CREB. FEBS Letters 579:1534–1538.

De Rubeis S et al. (2014) Synaptic, transcriptional and chromatin genes disrupted in autism. Nature 515:209–215.

Deconinck AE, Rafael JA, Skinner JA, Brown SC, Potter AC, Metzinger L, Watt DJ, Dickson JG, Tinsley JM, Davies KE (1997) Utrophin-Dystrophin-Deficient Mice as a Model for Duchenne Muscular Dystrophy. Cell 90(4):717-27.

Delluc A, Rousseau A, Le Galudec M, Canceil O, Woodhams B, Etienne S, Walter M, Mottier D, Van Dreden P, Lacut K (2013) Prevalence of antiphospholipid antibodies in psychiatric patients users and non-users of antipsychotics. Br J Haematol 164:272–279.

Deng F, Price MG, Davis CF, Mori M, Burgess DL (2006) Stargazin and Other Transmembrane AMPA Receptor Regulating Proteins Interact with Synaptic Scaffolding Protein MAGI-2 in Brain. Journal of Neuroscience 26:7875–7884.

Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR (2012) STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29:15–21.

Drew LJ, Crabtree GW, Markx S, Stark KL, Chaverneff F, Xu B, Mukai J, Fenelon K, Hsu P-K, Gogos JA, Karayiorgou M (2011) The 22q11.2 microdeletion: Fifteen years of insights into the genetic and neural complexity of psychiatric disorders. International Journal of Developmental Neuroscience 29:259–281.

Duffy L, Cappas E, Scimone A, Schofield PR, Karl T (2008) Behavioral profile of a heterozygous mutant mouse model for EGF-like domain neuregulin 1. Behavioral Neuroscience 122:748–759.

Ebert DH, Greenberg ME (2013) Activity-dependent neuronal signalling and autism spectrum disorder. Nature 493:327–337.

El-Kordi A, Winkler D, Hammerschmidt K, Kästner A, Krueger D, Ronnenberg A, Ritter C, Jatho J, Radyushkin K, Bourgeron T, Fischer J, Brose N, Ehrenreich H (2013) Development of an autism severity score for mice using Nlgn4 null mutants as a construct-valid model of heritable monogenic autism. Behavioural Brain Research 251:41–49.

Elias GM, Funke L, Stein V, Grant SG, Bredt DS, Nicoll RA (2006) Synapse-Specific and Developmentally Regulated Targeting of AMPA Receptors by a Family of MAGUK Scaffolding Proteins. Neuron 52:307–320.

Elias GM, Nicoll RA (2007) Synaptic trafficking of glutamate receptors by MAGUK scaffolding proteins. Trends in Cell Biology 17:343–352.

Etherton MR, Blaiss CA, Powell CM, Südhof TC (2009) Mouse neurexin-1 deletion causes correlated electrophysiological and behavioral changes consistent with cognitive impairments. Proc Natl Acad Sci USA 106(42):17998-8003.

Etherton M, Földy C, Sharma M, Tabuchi K, Liu X, Shamloo M, Malenka RC, Südhof TC (2011) Autism-linked neuroligin-3 R451C mutation differentially alters hippocampal and cortical synaptic function. Proc Natl Acad Sci USA 108(33):13764-9.

Falls D (2003) Neuregulins: functions, forms, and signaling strategies. Experimental Cell Research 284:14–30.

Feng J, Schroer R, Yan J, Song W, Yang C, Bockholt A, Cook EH Jr., Skinner C, Schwartz CE, Sommer SS (2006) High frequency of neurexin 1β signal peptide structural variants in patients with autism. Neuroscience Letters 409:10–13.

Feng Y-Q, Zhou Z-Y, He X, Wang H, Guo X-L, Hao C-J, Guo Y, Zhen X-C, Li W (2008) Dysbindin deficiency in sandy mice causes reduction of snapin and displays behaviors related to schizophrenia. Schizophrenia Research 106:218–228.

Fernández E et al. (2017) Arc Requires PSD95 for Assembly into Postsynaptic Complexes Involved with Neural Dysfunction and Intelligence. CellReports 21:679–691.

Feyder M et al. (2010) Association of Mouse Dlg4(PSD-95) Gene Deletion and Human DLG4Gene Variation With Phenotypes Relevant to Autism Spectrum Disorders and

Williams' Syndrome. AJP 167:1508–1517.

Fombonne E (2003) The Prevalence of Autism. JAMA 289(1):87-9.

Fortier M-E, Kent S, Ashdown H, Poole S, Boksa P, Luheshi GN (2004) The viral mimic, polyinosinic:polycytidylic acid, induces fever in rats via an interleukin-1-dependent mechanism. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 287:R759–R766.

Fromer M et al. (2014) De novo mutations in schizophrenia implicate synaptic networks. Nature 506:179–184.

Funk AJ, Rumbaugh G, Harotunian V, McCullumsmith RE, Meador-Woodruff JH (2009) Decreased expression of NMDA receptor-associated proteins in frontal cortex of elderly patients with schizophrenia. NeuroReport 20:1019–1022.

Funke L, Dakoji S, Bredt DS (2005) Membrane-associated guanylate kinases regulate adhesion and plasticity at cell junctions. Annu Rev Biochem 74:219–245.

Garcia-Rizo C, Fernandez-Egea E, Oliveira C, Justicia A, Bernardo M, Kirkpatrick B (2012) Inflammatory markers in antipsychotic-naïve patients with nonaffective psychosis and deficit vs. nondeficit features. Psychiatry Research 198:212–215.

Gerlai R, Pisacane P, Erickson S (2000) Heregulin, but not ErbB2 or ErbB3, heterozygous mutant mice exhibit hyperactivity in multiple behavioral tasks. Behav Brain Res 109(2):219-27.

Ghosh A, Michalon A, Lindemann L, Fontoura P, Santarelli L (2013) Drug discovery for autism spectrum disorder: challenges and opportunities. Nat Rev Drug Discov 12:777–790.

Giannone G, Mondin M, Grillo-Bosch D, Tessier B, Saint-Michel E, Czöndör K, Sainlos M, Choquet D, Thoumine O (2013) Neurexin-1β Binding to Neuroligin-1 Triggers the Preferential Recruitment of PSD-95 versus Gephyrin through Tyrosine Phosphorylation of Neuroligin-1. CellReports 3:1996–2007.

Gilman SR, Iossifov I, Levy D, Ronemus M, Wigler M, Vitkup D (2011) Rare De Novo Variants Associated with Autism Implicate a Large Functional Network of Genes Involved in Formation and Function of Synapses. Neuron 70:898–907.

Glessner JT et al. (2009) Autism genome-wide copy number variation reveals ubiquitin and neuronal genes. Nature 459:569–573.

Goldsmith DR, Haroon E, Miller AH, Strauss GP, Buckley PF, Miller BJ (2018) TNF-α and IL-6 are associated with the deficit syndrome and negative symptoms in patients with chronic schizophrenia. Schizophrenia Research:1–4.

Goldsmith DR, Rapaport MH, Miller BJ (2016) A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder

and depression. Mol Psychiatry 21:1696–1709.

Goodlin-Jones BL, Tang K, Liu J, Anders TF (2008) Sleep Patterns in Preschool-Age Children With Autism, Developmental Delay, and Typical Development. Journal of the American Academy of Child & Adolescent Psychiatry 47:930–938.

Grabrucker S, Proepper C, Mangus K, Eckert M, Chhabra R, Schmeisser MJ, Boeckers TM, Grabrucker AM (2014) The PSD protein ProSAP2/Shank3 displays synapto-nuclear shuttling which is deregulated in a schizophrenia-associated mutation. Experimental Neurology 253:126–137.

Grant SG (2012) Synaptopathies: diseases of the synaptome. Current Opinion in Neurobiology 22:522–529.

Gray KM, Tonge BJ (2001) Are there early features of autism in infants and preschool children. J Paediatr Child Health 37(3):221-6.

Grayton HM, Missler M, Collier DA, Fernandes C (2013) Altered Social Behaviours in Neurexin 1α Knockout Mice Resemble Core Symptoms in Neurodevelopmental Disorders Trezza V, ed. PLoS ONE 8:e67114–13.

Green MF, Kern RS, Braff DL, Mintz J (2000) Neurocognitive Deficits and Functional Outcome in Schizophrenia: Are We Measuring the "Right Stuff"? Schizophr Bull 26(1):119-36.

Gris J-C, Nobile B, Bouvier S (2015) Neuropsychiatric presentations of antiphospholipid antibodies. Thromb Res 135:S56–S59.

Halacheva K, Dimova S, Tolev T, Dimov D, Nikolova M (2009) Elevated anticardiolipin antibodies in schizophrenic patients before and during neuroleptic medication. Psychiatry Research 169:51–55.

Han K, Holder JL, Jr, Schaaf CP, Lu H, Chen H, Kang H, Tang J, Wu Z, Hao S, Cheung SW, Yu P, Sun H, Breman AM, Patel A, Lu H-C, Zoghbi HY (2014) SHANK3 overexpression causesmanic-like behaviour with uniquepharmacogenetic properties. Nature 503:72–77.

Hashemi E, Ariza J, Lechpammer M, Noctor SC, Martínez-Cerdeño V (2016) Abnormal white matter tracts resembling pencil fibers involving prefrontal cortex (Brodmann area 47) in autism: a case report. Journal of Medical Case Reports:1–4.

Hattori S, Murotani T, Matsuzaki S, Ishizuka T, Kumamoto N, Takeda M, Tohyama M, Yamatodani A, Kunugi H, Hashimoto R (2008) Behavioral abnormalities and dopamine reductions in sdy mutant mice with a deletion in Dtnbp1, a susceptibility gene for schizophrenia. Biochemical and Biophysical Research Communications 373:298–302.

Häfner H (2003) Gender differences in schizophrenia. Psychoneuroendocrinology 28:17–54.

Hikida T, Jaaro-Peled H, Seshadri S, Oishi K, Hookway C, Kong S, Wu D, Xue R, Andradé M, Tankou S, Mori S, Gallagher M, Ishizuka K, Pletnikov M, Kida S, Sawa A (2007) Dominant-negative DISC1 transgenic mice display schizophrenia-associated phenotypes detected by measures translatable to humans. Proc Natl Acad Sci USA 104(36):14501-6.

Hoirisch-Clapauch S, Amaral OB, Mezzasalma MAU, Panizzutti R, Nardi AE (2015) Dysfunction in the coagulation system and schizophrenia. 6:e704–e708.

Hoogendoorn B, Coleman SL, Guy CA, Smith SK, O'Donovan MC, Buckland PR (2004) Functional analysis of polymorphisms in the promoter regions of genes on 22q11. Hum Mutat 24:35–42.

Howard MA, Elias GM, Elias LAB, Swat W, Nicoll RA (2010) The role of SAP97 in synaptic glutamate receptor dynamics. Proc Natl Acad Sci USA 107:3805–3810.

Hsueh YP, Sheng M (1999) Requirement of N-terminal Cysteines of PSD-95 for PSD-95 Multimerization and Ternary Complex Formation, but Not for Binding to Potassium Channel Kv1.4. J Biol Chem 274(1):532-6.

Hu X, Luo J-H, Xu J (2015) The Interplay between Synaptic Activity and Neuroligin Function in the CNS. BioMed Research International:1–13.

Hung AY, Futai K, Sala C, Valtschanoff JG, Ryu J, Woodworth MA, Kidd FL, Sung CC, Miyakawa T, Bear MF, Weinberg RJ, Sheng M (2008) Smaller Dendritic Spines, Weaker Synaptic Transmission, but Enhanced Spatial Learning in Mice Lacking Shank1. Journal of Neuroscience 28:1697–1708.

Hutsler JJ, Zhang H (2010) Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. Brain Research 1309:83–94.

Hyman SE (2010) The diagnosis of mental disorders: the problem of reification. Annu Rev Clin Psychol 6:155-79.

Ibi D, Nagai T, Kitahara Y, Mizoguchi H, Koike H, Shiraki A, Takuma K, Kamei H, Noda Y, Nitta A, Nabeshima T, Yoneda Y, Yamada K (2009) Neonatal polyI:C treatment in mice results in schizophrenia-like behavioral and neurochemical abnormalities in adulthood. Neuroscience Research 64:297–305.

Jamain S, Radyushkin K, Hammerschmidt K, Granon S, Boretius S, Varoqueaux F, Ramanantsoa N, Gallego J, Ronnenberg A, Winter D, Frahm J, Fischer J, Bourgeron T, Ehrenreich H, Brose N (2008) Reduced social interaction and ultrasonic communication in a mouse model of monogenic heritable autism. Proc Natl Acad Sci USA 105(5):1710-5.

Jiang Y-H, Ehlers MD (2013) Modeling Autism by SHANK Gene Mutations in Mice. Neuron 78:8–27.

Jing-Ping Z, Tian Q-B, Sakagami H, Kondo H, Endo S, Suzuki T (2005) p55 protein is a

member of PSD scaffold proteins in the rat brain and interacts with various PSD proteins. Molecular Brain Research 135:204–216.

Karayiorgou M, Simon TJ, Gogos JA (2010) 22q11.2 microdeletions: linking DNA structural variation to brain dysfunction and schizophrenia. Nat Rev Neurosci 11:402–416.

Karl T, Duffy L, Scimone A, Harvey RP, Schofield PR (2007) Altered motor activity, exploration and anxiety in heterozygous neuregulin 1 mutant mice: implications for understanding schizophrenia. Genes, Brain and Behavior 6:677–687.

Kato T (2014) Whole genome/exome sequencing in mood and psychotic disorders. Psychiatry Clin Neurosci 69:65–76.

Kazdoba TM, Leach PT, Crawley JN (2015) Behavioral phenotypes of genetic mouse models of autism. Genes, Brain and Behavior 15:7–26.

Kessler RC (2007) The global burden of anxiety and mood disorders: putting the European Study of the Epidemiology of Mental Disorders (ESEMed) findings into perspective. J Clin Psychiatry 68 Suppl 2:10-9.

Kim C-H, Takamiya K, Petralia RS, Sattler R, Yu S, Zhou W, Kalb R, Wenthold R, Huganir R (2005) Persistent hippocampal CA1 LTP in mice lacking the C-terminal PDZ ligand of GluR1. Nat Neurosci 8:985–987.

Kim E, Sheng M (2004) PDZ domain proteins of synapses. Nat Rev Neurosci 5:771–781.

Kimura M, Toth LA, Agostini H, Cady AB, Majde JA, Krueger JM (1994) Comparison of acute phase responses induced in rabbits by lipopolysaccharide and double-stranded RNA. Am J Physiol 267(6 Pt 2):R1596-605.

Kirov G et al. (2011) De novo CNV analysis implicates specific abnormalities of postsynaptic signalling complexes in the pathogenesis of schizophrenia. Mol Psychiatry:1–12.

Kirov G, Ivanov D, Williams NM, Preece A, Nikolov I, Milev R, Koleva S, Dimitrova A, Toncheva D, O'Donovan MC, Owen MJ (2004) Strong evidence for association between the dystrobrevin binding protein 1 gene (DTNBP1) and schizophrenia in 488 parent-offspring trios from Bulgaria. Biological Psychiatry 55:971–975.

Kissi El Y, Samoud S, Mtiraoui A, Letaief L, Hannachi N, Ayachi M, Ben Hadj Ali B, Boukadida J (2018) Increased Interleukin-17 and decreased BAFF serum levels in drug-free acute schizophrenia. :1–6.

Koike H, Arguello PA, Kvajo M, Karayiorgou M, Gogos JA (2006) Disc1 is mutated in the 129S6 SvEv strain and modulates working memory in mice. Proc Natl Acad Sci USA 103(10):3693-7.

Kokras N, Dalla C (2014) Sex differences in animal models of psychiatric disorders. Br J Pharmacol 171:1–25.

Kolevzon A, Bush L, Wang AT, Halpern D, Frank Y, Grodberg D, Rapaport R, Tavassoli T, Chaplin W, Soorya L, Buxbaum JD (2015) A pilot controlled trial of insulin-like growth factor-1 in children with Phelan-McDermid syndrome. Mol Autism 5(1):54.

Kouser M, Speed HE, Dewey CM, Reimers JM, Widman AJ, Gupta N, Liu S, Jaramillo TC, Bangash M, Xiao B, Worley PF, Powell CM (2013) Loss of Predominant Shank3 Isoforms Results in Hippocampus-Dependent Impairments in Behavior and Synaptic Transmission. Journal of Neuroscience 33:18448–18468.

Kristiansen LV, Beneyto M, Haroutunian V, Meador-Woodruff JH (2006) Changes in NMDA receptor subunits and interacting PSD proteins in dorsolateral prefrontal and anterior cingulate cortex indicate abnormal regional expression in schizophrenia. Mol Psychiatry 11:737–747.

Kulkarni J, Gavrilidis E, Worsley R, Van Rheenen T, Hayes E (2013) The Role of Estrogen in the Treatment of Men with Schizophrenia. Int J Endocrinol Metab 11:1–8.

Kushima I et al. (2016) High-resolution copy number variation analysis of schizophrenia in Japan. 22:430–440.

Kvajo M, McKellar H, Arguello PA, Drew LJ, Moore H, MacDermott AB, Karayiorgou M, Gogos JA (2008) A mutation in mouse Disc1 that models a schizophrenia risk allele leads to specific alterations in neuronal architecture and cognition. Proc Natl Acad Sci USA 105(19):7076-81.

Lacivita E, Perrone R, Margari L, Leopoldo M (2017) Targets for Drug Therapy for Autism Spectrum Disorder: Challenges and Future Directions. J Med Chem 60:9114–9141.

Laumonnier F, Bonnet-Brilhault F, Gomot M, Blanc R, David A, Moizard MP, Raynaud M, Ronce N, Lemonnier E, Calvas P, Laudier B, Chelly J, Fryns JP, Ropers HH, Hamel BC, Andres C, Barthélémy C, Moraine C, Briault S (2004) X-Linked Mental Retardation and Autism Are Associated with a Mutation in the NLGN4 Gene, a Member of the Neuroligin Family. Am J Hum Genet 74(3):552-7.

Laura RP, Ross S, Koeppen H, Lasky LA (2002) MAGI-1: A Widely Expressed, Alternatively Spliced Tight Junction Protein. Experimental Cell Research 275:155–170.

Lavretsy H (2008) History of schizophrenia as a psychiatric disorder. Clinical Handbook of Schizophrenia :1–11.

Law CW, Chen Y, Shi W, Smyth GK (2014) voom: precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biol 15(2):R29.

Lawson-Yuen A, Saldivar J-S, Sommer S, Picker J (2008) Familial deletion within NLGN4 associated with autism and Tourette syndrome. European Journal of Human

Genetics 16:614–618.

Lee HW, Choi J, Shin H, Kim K, Yang J, Na M, Choi SY, Kang GB, Eom SH, Kim H, Kim E (2008) Preso, A Novel PSD-95-Interacting FERM and PDZ Domain Protein That Regulates Dendritic Spine Morphogenesis. Journal of Neuroscience 28:14546–14556.

Lehman AF, Lieberman JA, Dixon LB, McGlashan TH, Miller AL, Perkins DO, Kreyenbuhl J (2006) Treatment of Patients With Schizophrenia Second Edition. :1–184.

Lelieveld SH et al. (2016) Meta-analysis of 2,104 trios provides support for 10 new genes for intellectual disability. Nat Neurosci 19:1194–1196.

Levinson DF et al. (2011) Copy Number Variants in Schizophrenia: Confirmation of Five Previous Findings and New Evidence for 3q29 Microdeletions and VIPR2 Duplications. AJP 168:302–316.

Levy SE, Mandell DS, Schultz RT (2009) Autism. The Lancet 374:1627–1638.

Lewis S, Lieberman J (2018) CATIE and CUtLASS: can we handle the truth? Br J Psychiatry 192:161–163.

Li W, Zhou Y, Jentsch JD, Brown RA, Tian X, Ehninger D, Hennah W, Peltonen L, Lönnqvist J, Huttunen MO, Kaprio J, Trachtenberg JT, Silva AJ, Cannon TD (2007) Specific developmental disruption of disrupted-in-schizophrenia-1 function results in schizophrenia-related phenotypes in mice. Proc Natl Acad Sci USA 104(46):18280-5.

Liu H, Kang Y, Liang J, Li C, Xiu M, Chen D, Yang F, Wang F, Wu G, Haile CN, Kosten TA, Kosten TR, Zhang XY (2012) Lower serum interleukin-2 levels in schizophrenic patients with tardive dyskinesia. Psychiatry Research 198:329–331.

Livak KJ, Schmittgen TD (2001) Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods 25:402–408.

Luo J, Norris RH, Gordon SL, Nithianantharajah J (2017) Neurodevelopmental synaptopathies_ Insights from behaviour in rodent models of synapse gene mutations. Progress in Neuropsychopharmacology & Biological Psychiatry:1–0.

MacFabe D, Cain D, Rodriguez Capote K, Franklin A, Hoffman J, Boon F, Taylor A, Kavaliers M, Ossenkopp K (2007) Neurobiological effects of intraventricular propionic acid in rats: Possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders. Behavioural Brain Research 176:149–169.

MacFabe DF, Cain NE, Boon F, Ossenkopp K-P, Cain DP (2011) Effects of the enteric bacterial metabolic product propionic acid on object-directed behavior, social behavior, cognition, and neuroinflammation in adolescent rats: Relevance to autism spectrum disorder. Behavioural Brain Research 217:47–54.

Mackie S, Millar JK, Porteous DJ (2007) Role of DISC1 in neural development and schizophrenia. Current Opinion in Neurobiology 17:95–102.

Madani R, Kozlov S, Akhmedov A, Cinelli P, Kinter J, Lipp H-P, Sonderegger P, Wolfer DP (2003) Impaired explorative behavior and neophobia in genetically modified mice lacking or overexpressing the extracellular serine protease inhibitor neuroserpin. Molecular and Cellular Neuroscience 23:473–494.

Martínez-Pedraza F de L, Carter AS (2009) Autism Spectrum Disorders in Young Children. Child and Adolescent Psychiatric Clinics of North America 18:645–663.

McCarthy SE, Gillis J, Kramer M, Lihm J, Yoon S, Berstein Y, Mistry M, Pavlidis P, Solomon R, Ghiban E, Antoniou E, Kelleher E, O'Brien C, Donohoe G, Gill M, Morris DW, McCombie WR, Corvin A (2014) De novo mutations in schizophrenia implicate chromatin remodeling and support a genetic overlap with autism and intellectual disability. Mol Psychiatry 19:652–658.

McGee AW, Topinka JR, Hashimoto K, Petralia RS, Kakizawa S, Kauer FW, Aguilera-Moreno A, Wenthold RJ, Kano M, Bredt DS (2001) PSD-93 Knock-Out Mice Reveal That Neuronal MAGUKs Are Not Required for Development or Function of Parallel Fiber Synapses in Cerebellum. J Neurosci 21(9):3085-91.

Meyer U, Feldon J (2012) To poly(I:C) or not to poly(I:C): Advancing preclinical schizophrenia research through the use of prenatal immune activation models. Neuropharmacology 62:1308–1321.

Meyer U, Feldon J, Schedlowski M, Yee BK (2006) Immunological stress at the maternal–foetal interface: A link between neurodevelopment and adult psychopathology. Brain, Behavior, and Immunity 20:378–388.

Meyer U, Yee BK, Feldon J (2016) The Neurodevelopmental Impact of Prenatal Infections at Different Times of Pregnancy: The Earlier the Worse? Neuroscientist 13:241–256.

Migaud M, Charlesworth P, Dempster M, Webster LC, Watabe AM, Makhinson M, He Y, Ramsay MF, Morris RG, Morrison JH, O'Dell TJ, Grant SG (1999) Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. Nature 396(6710):433-9.

Millar JK (2000) Disruption of two novel genes by a translocation co-segregating with schizophrenia. Human Molecular Genetics 9:1415–1423.

Millar JK, Christie S, Anderson S, Lawson D, Hsiao-Wei Loh D, Devon RS, Arveiler B, Muir WJ, Blackwood DH, Porteous DJ (2001) Genomic structure and localisation within a linkage hotspot of Disrupted In Schizophrenia 1, a gene disrupted by a translocation segregating with schizophrenia. Mol Psychiatry 6(2):173-8.

Miller BJ, Buckley P, Seabolt W, Mellor A, Kirkpatrick B (2011) Meta-Analysis of Cytokine Alterations in Schizophrenia: Clinical Status and Antipsychotic Effects. Biological Psychiatry 70:663–671.

Miller BJ, Goldsmith DR (2016) Towards an Immunophenotype of Schizophrenia:

Progress, Potential Mechanisms, and Future Directions. 42:299–317.

Mojsilovic-Petrovic J (2006) Protecting Motor Neurons from Toxic Insult by Antagonism of Adenosine A2a and Trk Receptors. Journal of Neuroscience 26:9250–9263.

Mondin M, Labrousse V, Hosy E, Heine M, Tessier B, Levet F, Poujol C, Blanchet C, Choquet D, Thoumine O (2011) Neurexin-Neuroligin Adhesions Capture Surface-Diffusing AMPA Receptors through PSD-95 Scaffolds. Journal of Neuroscience 31:13500–13515.

Mulle JG (2015) The 3q29 deletion confers >40-fold increase in risk for schizophrenia. 20:1028–1029.

Müller BM, Kistner U, Kindler S, Chung WJ, Kuhlendahl S, Fenster SD, Lau LF, Veh RW, Huganir RL, Gundelfinger ED, Garner CC (1996) SAP102, a Novel Postsynaptic Protein That Interacts with NMDA Receptor Complexes In Vivo. Neuron 17(2):255-65.

Murotani T, Ishizuka T, Hattori S, Hashimoto R, Matsuzaki S, Yamatodani A (2007) High dopamine turnover in the brains of Sandy mice. Neuroscience Letters 421:47–51.

Murphy KC, Jones LA, Owen MJ (1999) High Rates of Schizophrenia in Adults With Velo-Cardio-Facial Syndrome. Arch Gen Psychiatry 56(10):940-5.

Nakao A, Miyazaki N, Ohira K, Hagihara H, Takagi T, Usuda N, Ishii S, Murata K, Miyakawa T (2017) Immature morphological properties in subcellular-scale structures in the dentate gyrus of Schnurri-2 knockout mice: a model for schizophrenia and intellectual disability. :1–11.

Nestler EJ, Hyman SE (2010) Animal Models of Neuropsychiatric Disorders. Nat Neurosci 13(10): 1161-1169.

Newton JR, Ellsworth C, Miyakawa T, Tonegawa S, Sur M (2004) Acceleration of visually cued conditioned fear through the auditory pathway. Nat Neurosci 7:968–973.

Nieto R (2013) BDNF and schizophrenia: from neurodevelopment to neuronal plasticity, learning, and memory. :1–11.

Niklasson L, Rasmussen P, Oskarsdóttir S, Gillberg C (2001) Neuropsychiatric disorders in the 22q11 deletion syndrome. Genet Med 3(1):79-84.

Nikonenko I, Boda B, Steen S, Knott G, Welker E, Muller D (2008) PSD-95 promotes synaptogenesis and multiinnervated spine formation through nitric oxide signaling. J Cell Biol 183:1115–1127.

Ohnuma T, Kato H, Arai H, Faull RL, McKenna PJ, Emson PC (2000) Gene expression of PSD95 in prefrontal cortex and hippocampus in schizophrenia. Neuroreport 28;11(14):3133-7.

O'Tuathaigh CMP, O'Connor A-M, O'Sullivan GJ, Lai D, Harvey R, Croke DT,

Waddington JL (2008) Disruption to social dyadic interactions but not emotional/anxiety-related behaviour in mice with heterozygous “knockout” of the schizophrenia risk gene neuregulin-1. Progress in Neuro-Psychopharmacology and Biological Psychiatry 32:462–466.

Oliva C, Escobedo P, Astorga C, Molina C, Sierralta J (2011) Role of the maguk protein family in synapse formation and function Ferrús A, ed. Devel Neurobio 72:57–72.

Patel KR, Cherian J, Gohil K, Atkinson D (2014) Schizophrenia: Overview and Treatment Options. PT 39(9):638-45.

Patel S, Roncaglia P, Lovering RC (2015) Using Gene Ontology to describe the role of the neurexin-neuroligin-SHANK complex in human, mouse and rat and its relevance to autism. BMC Bioinformatics:1–18.

Patterson PH (2009) Immune involvement in schizophrenia and autism: Etiology, pathology and animal models. Behavioural Brain Research 204:313–321.

Pavlowsky A, Gianfelice A, Pallotto M, Zanchi A, Vara H, Khelfaoui M, Valnegri P, Rezai X, Bassani S, Brambilla D, Kumpost J, Blahos J, Roux MJ, Humeau Y, Chelly J, Passafaro M, Giustetto M, Billuart P, Sala C (2010) A Postsynaptic Signaling Pathway that May Account for the Cognitive Defect Due to IL1RAPL1 Mutation. Current Biology 20:103–115.

Paylor R, Lindsay E (2006) Mouse Models of 22q11 Deletion Syndrome. Biological Psychiatry 59:1172–1179.

Peça J, Feliciano C, Ting JT, Wang W, Wells MF, Venkatraman TN, Lascola CD, Fu Z, Feng G (2011) Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. Nature 472:437–442.

Peiretti F (2003) Identification of SAP97 as an intracellular binding partner of TACE. Journal of Cell Science 116:1949–1957.

Peñagarikano O, Abrahams BS, Herman EI, Winden KD, Gdalyahu A, Dong H, Sonnenblick LI, Gruver R, Almajano J, Bragin A, Golshani P, Trachtenberg JT, Peles E, Geschwind DH (2011) Absence of CNTNAP2 Leads to Epilepsy, Neuronal Migration Abnormalities, and Core Autism-Related Deficits. Cell 147:235–246.

Persico AM, Merelli S (2014) Environmental Factors in the Onset of Autism Spectrum Disorder. Curr Dev Disord Rep 1:8–19.

Phelan K, McDermid HE (2011) The 22q13.3 Deletion Syndrome (Phelan-McDermid Syndrome). Molecular Syndromology:1–16.

Pletnikov MV, Ayhan Y, Nikolskaia O, Xu Y, Ovanesov MV, Huang H, Mori S, Moran TH, Ross CA (2007) Inducible expression of mutant human DISC1 in mice is associated with brain and behavioral abnormalities reminiscent of schizophrenia. Mol Psychiatry 13:173–186.

Poglia L, Muller D, Nikonenko I (2010) Ultrastructural modifications of spine and synapse morphology by SAP97. Hippocampus 17:n/a–n/a.

Poot M, Beyer V, Schwaab I, Damatova N, van't Slot R, Prothero J, Holder SE, Haaf T (2009) Disruption of CNTNAP2 and additional structural genome changes in a boy with speech delay and autism spectrum disorder. Neurogenetics 11:81–89.

Potvin S, Stip E, Sepehry AA, Gendron A, Bah R, Kouassi E (2008) Inflammatory Cytokine Alterations in Schizophrenia: A Systematic Quantitative Review. Biological Psychiatry 63:801–808.

Psychosis Endophenotypes International Consortium et al. (2016) Contribution of copy number variants to schizophrenia from a genome-wide study of 41,321 subjects. Nat Genet 49:27–35.

Purcell SM et al. (2014) A polygenic burden of rare disruptive mutations in schizophrenia. Nature 506:185–190.

Quintero-Rivera F, Sharifi-Hannauer P, Martinez-Agosto JA (2010) Autistic and psychiatric findings associated with the 3q29 microdeletion syndrome: Case report and review. Am J Med Genet 152A:2459–2467.

Radyushkin K, Hammerschmidt K, Boretius S, Varoqueaux F, El-Kordi A, Ronnenberg A, Winter D, Frahm J, Fischer J, Brose N, Ehrenreich H (2009) Neuroligin-3-deficient mice: model of a monogenic heritable form of autism with an olfactory deficit. Genes, Brain and Behavior 8:416–425.

Regalado MP (2006) Transsynaptic Signaling by Postsynaptic Synapse-Associated Protein 97. Journal of Neuroscience 26:2343–2357.

Reumann R, Vierk R, Zhou L, Gries F, Kraus V, Mienert J, Romswinkel E, Morellini F, Ferrer I, Nicolini C, Fahnestock M, Rune G, Glatzel M, Galliciotti G (2017) The serine protease inhibitor neuroserpin is required for normal synaptic plasticity and regulates learning and social behavior. Learn Mem 24:650–659.

Rezende SM (2003) Coagulation, inflammation, and apoptosis: different roles for protein S and the protein S-C4b binding protein complex. Blood 103:1192–1201.

Rimer M, Barrett DW, Maldonado MA, Vock VM, Gonzalez-Lima F (2005) Neuregulin-1 immunoglobulin-like domain mutant mice: clozapine sensitivity and impaired latent inhibition. Neuroreport 16(3):271-5.

Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK (2015) limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Research 43:e47–e47.

Ritvo ER, Jorde LB, Mason-Brothers A, Freeman BJ, Pingree C, Jones MB, McMahon WM, Petersen PB, Jenson WR, Mo A (1989) The UCLA-University of Utah

epidemiologic survey of autism: recurrence risk estimates and genetic counseling. Am J Psychiatry 146(8):1032-6.

Rodenas-Cuadrado P, Ho J, Vernes SC (2013) Shining a light on CNTNAP2: complex functions to complex disorders. European Journal of Human Genetics 22:171–178.

Rossi E, Verri AP, Patricelli MG, Destefani V, Ricca I, Vetro A, Ciccone R, Giorda R, Toniolo D, Maraschio P, Zuffardi O (2008) A 12 Mb deletion at 7q33eq35 associated with autism spectrum disorders and primary amenorrhea. European Journal of Medical Genetics 51:631–638.

Sacco R, Gabriele S, Persico AM (2015) Head circumference and brain size in autism spectrum disorder_ A systematic review and meta-analysis. Psychiatry Research: Neuroimaging 234:239–251.

Saetre P, Emilsson L, Axelsson E, Kreuger J, Lindholm E, Jazin E (2007) Inflammation-related genes up-regulated in schizophrenia brains. BMC Psychiatry 7:551–10.

Sans N, Petralia RS, Wang YX, Blahos J 2nd, Hell JW, Wenthold RJ (2000) A developmental change in NMDA receptor-associated proteins at hippocampal synapses. J Neurosci 20(3):1260-71.

Sato D et al. (2012) SHANK1 Deletions in Males with Autism Spectrum Disorder. The American Journal of Human Genetics 90:879–887.

Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014) Biological insights from 108 schizophrenia-associated genetic loci. Nature 511:421–427.

Schizophrenia Working Group of the Psychiatric Genomics Consortium, Sekar A, Bialas AR, de Rivera H, Davis A, Hammond TR, Kamitaki N, Tooley K, Presumey J, Baum M, Van Doren V, Genovese G, Rose SA, Handsaker RE, Daly MJ, Carroll MC, Stevens B, McCarroll SA (2016) Schizophrenia risk from complex variation of complement component 4. Nature 530:177–183.

Schmeisser MJ et al. (2012) Autistic-like behaviours and hyperactivity in mice lacking ProSAP1/Shank2. Nature 486:256–260.

Scott-Van Zeeland AA, Abrahams BS, Alvarez-Retuerto AI, Sonnenblick LI, Rudie JD, Ghahremani D, Mumford JA, Poldrack RA, Dapretto M, Geschwind DH, Bookheimer SY (2010) Altered Functional Connectivity in Frontal Lobe Circuits Is Associated with Variation in the Autism Risk Gene CNTNAP2. Science Translational Medicine 2:56ra80–56ra80.

Sharp WG, Berry RC, McCracken C, Nuhu NN, Marvel E, Saulnier CA, Klin A, Jones W, Jaquess DL (2013) Feeding Problems and Nutrient Intake in Children with Autism Spectrum Disorders: A Meta-analysis and Comprehensive Review of the Literature. J Autism Dev Disord 43:2159–2173.

Shen S, Lang B, Nakamoto C, Zhang F, Pu J, Kuan SL, Chatzi C, He S, Mackie I,

Brandon NJ, Marquis KL, Day M, Hurko O, McCaig CD, Riedel G, St Clair D (2008) Schizophrenia-Related Neural and Behavioral Phenotypes in Transgenic Mice Expressing Truncated Disc1. Journal of Neuroscience 28:10893–10904.

Shi L, Fatemi SH, Sidwell RW, Patterson PH (2002) Maternal Influenza Infection Causes Marked Behavioral and Pharmacological Changes in the Offspring. J Neurosci 23(1):297-302.

Shi L, Tu N, Patterson PH (2005) Maternal influenza infection is likely to alter fetal brain development indirectly: the virus is not detected in the fetus. International Journal of Developmental Neuroscience 23:299–305.

Shultz SR, MacFabe DF, Ossenkopp K-P, Scratch S, Whelan J, Taylor R, Cain DP (2008) Intracerebroventricular injection of propionic acid, an enteric bacterial metabolic end-product, impairs social behavior in the rat: Implications for an animal model of autism. Neuropharmacology 54:901–911.

Sigurdsson T, Stark KL, Karayiorgou M, Gogos JA, Gordon JA (2010) Impaired hippocampal–prefrontal synchrony in a genetic mouse model of schizophrenia. Nature 464:763–767.

Silverman JL, Turner SM, Barkan CL, Tolu SS, Saxena R, Hung AY, Sheng M, Crawley JN (2011) Sociability and motor functions in Shank1 mutant mice. Brain Research 1380:120–137.

Smoller JW, Andreassen OA, Edenberg HJ, Faraone SV, Glatt SJ, Kendler KS (2018) Psychiatric genetics and the structure of psychopathology. Mol Psychiatry:1–12.

Song X, Fan X, Li X, Kennedy D, Pang L, Quan M, Chen X, Gao J, Zhang W, Zhang J, Lv L (2014) Serum levels of BDNF, folate and homocysteine: In relation to hippocampal volume and psychopathology in drug naïve, first episode schizophrenia. Schizophrenia Research 159:51–55.

Sorensen HJ, Mortensen EL, Reinisch JM, Mednick SA (2009) Association Between Prenatal Exposure to Bacterial Infection and Risk of Schizophrenia. Schizophrenia Bulletin 35:631–637.

Stark KL, Xu B, Bagchi A, Lai W-S, Liu H, Hsu R, Wan X, Pavlidis P, Mills AA, Karayiorgou M, Gogos JA (2008) Altered brain microRNA biogenesis contributes to phenotypic deficits in a 22q11-deletion mouse model. Nat Genet 40:751–760.

Stefansson H et al. (2002) Neuregulin 1 and Susceptibility to Schizophrenia. The American Journal of Human Genetics 71:877–892.

Stefansson H, Sarginson J, Kong A, Yates P, Steinthorsdottir V, Gudfinnsson E, Gunnarsdottir S, Walker N, Petursson H, Crombie C, Ingason A, Gulcher JR, Stefansson K, St Clair D (2003) Association of Neuregulin 1 with Schizophrenia Confirmed in a Scottish Population. Am J Hum Genet 72(1):83-7.

Stoner R, Chow ML, Boyle MP, Sunkin SM, Mouton PR, Roy S, Wynshaw-Boris A, Colamarino SA, Lein ES, Courchesne E (2014) Patches of Disorganization in the Neocortex of Children with Autism. N Engl J Med 370:1209–1219.

Straub RE, Jiang Y, MacLean CJ, Ma Y, Webb BT, Myakishev MV, Harris-Kerr C, Wormley B, Sadek H, Kadambi B, Cesare AJ, Gibberman A, Wang X, O'Neill FA, Walsh D, Kendler KS (2002) Genetic variation in the 6p22.3 gene DTNBP1, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. Am J Hum Genet 71(2):337-48.

Sullivan M, Finelli J, Marvin A, Garrett-Mayer E, Bauman M, Landa R (2007) Response to Joint Attention in Toddlers at Risk for Autism Spectrum Disorder: A Prospective Study. J Autism Dev Disord 37:37–48.

Sumi S, Taniai H, Miyachi T, Tanemura M (2006) Sibling risk of pervasive developmental disorder estimated by means of an epidemiologic survey in Nagoya, Japan. J Hum Genet 51:518–522.

Sungur AÖ, Schwarting RKW, Wöhr M (2017) Behavioral phenotypes and neurobiological mechanisms in the Shank1 mouse model for autism spectrum disorder_ A translational perspective. Behavioural Brain Research:1–0.

Szatmari P, Maziade M, Zwaigenbaum L, Mérette C, Roy M-A, Joober R, Palmour R (2007) Informative phenotypes for genetic studies of psychiatric disorders. Am J Med Genet 144B:581–588.

Tabuchi K, Blundell J, Etherton MR, Hammer RE, Liu X, Powell CM, Sudhof TC (2007) A Neuroligin-3 Mutation Implicated in Autism Increases Inhibitory Synaptic Transmission in Mice. Science 318:71–76.

Takeuchi O, Akira S (2007) Recognition of viruses by innate immunity. Immunol Rev 220:214-24.

Takao K et al. (2013) Deficiency of Schnurri-2, an MHC Enhancer Binding Protein, Induces Mild Chronic Inflammation in the Brain and Confers Molecular, Neuronal, and Behavioral Phenotypes Related to Schizophrenia. Neuropsychopharmacology 38:1409–1425.

Takao K, Toyama K, Nakanishi K, Hattori S, Takamura H, Takeda M, Miyakawa T, Hashimoto R (2008) Impaired long-term memory retention and working memory in sdy mutant mice with a deletion in Dtnbp1, a susceptibility gene for schizophrenia. Mol Brain 1:11–12.

Tandon R, Nasrallah HA, Keshavan MS (2009) Schizophrenia, “just the facts” 4. Clinical features and conceptualization. Schizophrenia Research 110:1–23.

Tang G, Gudsnuk K, Kuo S-H, Cotrina ML, Rosoklija G, Sosunov A, Sonders MS, Kanter E, Castagna C, Yamamoto A, Yue Z, Arancio O, Peterson BS, Champagne F, Dwork AJ, Goldman J, Sulzer D (2014) Loss of mTOR-Dependent Macroautophagy Causes

Autistic-like Synaptic Pruning Deficits. Neuron 83:1131–1143.

Tang JX, Zhou J, Fan JB, Li XW, Shi YY, Gu NF, Feng GY, Xing YL, Shi JG, He L (2003) Family-based association study of DTNBP1 in 6p22.3 and schizophrenia. Mol Psychiatry 8(8):717-8:1–2.

Tarpey P et al. (2004) Mutations in the DLG3 Gene Cause Nonsyndromic X-Linked Mental Retardation. The American Journal of Human Genetics 75:318–324.

The Autism Genome Project Consortium (2007) Mapping autism risk loci using genetic linkage and chromosomal rearrangements. Nat Genet 39:319–328.

Tinsley JM, Blake DJ, Zuellig RA, Davies KE (1994) Increasing complexity of the dystrophin-associated protein complex. Proc Natl Acad Sci USA 91(18):8307-13.

Toyooka K, Iritani S, Makifuchi T, Shirakawa O, Kitamura N, Maeda K, Nakamura R, Niizato K, Watanabe M, Kakita A, Takahashi H, Someya T, Nawa H (2002) Selective reduction of a PDZ protein, SAP-97, in the prefrontal cortex of patients with chronic schizophrenia. J Neurochem 83(4):797-806.

Traynor TR, Majde JA, Bohnet SG, Krueger JM (2004) Intratracheal double-stranded RNA plus interferon-γ: A model for analysis of the acute phase response to respiratory viral infections. Life Sciences 74:2563–2576.

Tripathi A, Kar SK, Shukla R (2018) Cognitive Deficits in Schizophrenia: Understanding the Biological Correlates and Remediation Strategies. Clin Psychopharmacol Neurosci 16:7–17.

Tsai N-P, Wilkerson JR, Guo W, Maksimova MA, DeMartino GN, Cowan CW, Huber KM (2012) Multiple Autism-Linked Genes Mediate Synapse Elimination via Proteasomal Degradation of a Synaptic Scaffold PSD-95. Cell 151:1581–1594.

Uchino S, Waga C (2013) SHANK3 as an autism spectrum disorder-associated gene. Brain and Development 35:106–110.

Uezato A, Kimura-Sato J, Yamamoto N, Iijima Y, Kunugi H, Nishikawa T (2012) Further evidence for a male-selective genetic association of synapse-associated protein 97 (SAP97) gene with schizophrenia. Behavioral and Brain Functions 8:2.

Uezato A, Yamamoto N, Iwayama Y, Hiraoka S, Hiraaki E, Umino A, Haramo E, Umino M, Yoshikawa T, Nishikawa T (2015) Reduced cortical expression of a newly identified splicing variant of the DLG1 gene in patients with early-onset schizophrenia. 5:e654–e658.

Uezato A, Yamamoto N, Jitoku D, Haramo E, Hiraaki E, Iwayama Y, Toyota T, Umino M, Umino A, Iwata Y, Suzuki K, Kikuchi M, Hashimoto T, Kanahara N, Kurumaji A, Yoshikawa T, Nishikawa T (2017) Genetic and molecular risk factors within the newly identified primate-specific exon of the SAP97/DLG1gene in the 3q29 schizophrenia-associated locus. Am J Med Genet 174:798–807.

Valnegri P, Montrasio C, Brambilla D, Ko J, Passafaro M, Sala C (2011) The X-linked intellectual disability protein IL1RAPL1 regulates excitatory synapse formation by binding PTPδ and RhoGAP2. Human Molecular Genetics 20:4797–4809.

Varghese M, Keshav N, Jacot-Descombes S, Warda T, Wicinski B, Dickstein DL, Harony-Nicolas H, Rubeis S, Drapeau E, Buxbaum JD, Hof PR (2017) Autism spectrum disorder: neuropathology and animal models. Acta Neuropathologica 134:537–566.

Vickers CA, Stephens B, Bowen J, Arbuthnott GW, Grant SGN, Ingham CA (2006) Neurone specific regulation of dendritic spines in vivo by post synaptic density 95 protein (PSD-95). Brain Research 1090:89–98.

Voineskos D, Rogasch NC, Rajji TK, Fitzgerald PB, Daskalakis ZJ (2013) A Review of Evidence Linking Disrupted Neural Plasticity to Schizophrenia. Can J Psychiatry 58:86–92.

Waites CL, Specht CG, Hartel K, Leal-Ortiz S, Genoux D, Li D, Drisdel RC, Jeyifous O, Cheyne JE, Green WN, Montgomery JM, Garner CC (2009) Synaptic SAP97 Isoforms Regulate AMPA Receptor Dynamics and Access to Presynaptic Glutamate. Journal of Neuroscience 29:4332–4345.

Wang DV, Wang F, Liu J, Zhang L, Wang Z, Lin L (2011) Neurons in the Amygdala with Response-Selectivity for Anxiety in Two Ethologically Based Tests. PLoS ONE 6:e18739–7.

Wang X, McCoy PA, Rodriguiz RM, Pan Y, Je HS, Roberts AC, Kim CJ, Berrios J, Colvin JS, Bousquet-Moore D, Lorenzo I, Wu G, Weinberg RJ, Ehlers MD, Philpot BD, Beaudet AL, Wetsel WC, Jiang Y-H (2011) Synaptic dysfunction and abnormal behaviors in mice lacking major isoforms of Shank3. Human Molecular Genetics 20:3093–3108.

Wolburg H, Lippoldt A (2002) Tight junctions of the blood–brain barrier: Development, composition and regulation. Vascul Pharmacol 38(6):323-37.

Wegiel J, Flory M, Kuchna I, Nowicki K, Ma SY, Imaki H, Wegiel J, Cohen IL, London E, Brown WT, Wisniewski T (2014) Brain-region specific alterations of the trajectories of neuronal volume growth throughout the lifespan in autism. 2:1–18.

Wegiel J, Flory M, Kuchna I, Nowicki K, Ma SY, Imaki H, Wegiel J, Frackowiak J, Kolecka BM, Wierzba-Bobrowicz T, London E, Wisniewski T, Hof PR, Brown WT (2015) Neuronal nucleus and cytoplasm volume deficit in children with autism and volume increase in adolescents and adults. acta neuropathol commun 3:6897–17.

Weickert CS, Rothmond DA, Purves-Tyson TD (2018) Considerations for optimal use of postmortem human brains for molecular psychiatry: lessons from schizophrenia. Handb Clin Neurol 150:221-235.

Weinstein-Fudim L, Ornoy A (2016) Genetic and non-genetic animal models for autism spectrum disorders (ASD). Reproductive Toxicology 64:116–140.

Winkler D, Daher F, Wüstefeld L, Hammerschmidt K, Poggi G, Seelbach A, Krueger-Burg D, Vafadari B, Ronnenberg A, Liu Y, Kaczmarek L, Schlüter OM, Ehrenreich H, Dere E (2017) Hypersocial behavior and biological redundancy in mice with reduced expression of PSD95 or PSD93. Behavioural Brain Research:1–11.

Wittchen HU, Jacobi F, Rehm J, Gustavsson A, Svensson M, Jönsson B, Olesen J, Allgulander C, Alonso J, Faravelli C, Fratiglioni L, Jennum P, Lieb R, Maercker A, van Os J, Preisig M, Salvador-Carulla L, Simon R, Steinhausen HC (2011) The size and burden of mental disorders and other disorders of the brain in Europe 2010. European Neuropsychopharmacology 21:655–679.

Wolburg H, Lippoldt A (2002) Tight junctions of the blood–brain barrier: Development, composition and regulation. Vascul Pharmacol 38(6):323-37.

Wolff SBE, Gründemann J, Tovote P, Krabbe S, Jacobson GA, Müller C, Herry C, Ehrlich I, Friedrich RW, Letzkus JJ, Lüthi A (2014) Amygdala interneuron subtypes control fear learning through disinhibition. Nature 509:453–458.

Wöhr M, Roullet FI, Hung AY, Sheng M, Crawley JN (2011) Communication Impairments in Mice Lacking Shank1: Reduced Levels of Ultrasonic Vocalizations and Scent Marking Behavior Crusio WE, ed. PLoS ONE 6:e20631–18.

Wöhr M, Silverman JL, Scattoni ML, Turner SM, Harris MJ, Saxena R, Crawley JN (2013) Developmental delays and reduced pup ultrasonic vocalizations but normal sociability in mice lacking the postsynaptic cell adhesion protein neuroligin2. Behavioural Brain Research 251:50–64.

Wu EQ, Shi L, Birnbaum H, Hudson T, Kessler R (2006) Annual prevalence of diagnosed schizophrenia in the USA: a claims data analysis approach. Psychol Med 36:1535–1536.

Xing J, Kimura H, Wang C, Ishizuka K, Kushima I, Arioka Y, Yoshimi A, Nakamura Y, Shiino T, Oya-Ito T, Takasaki Y, Uno Y, Okada T, Iidaka T, Aleksic B, Mori D, Ozaki N (2016) Resequencing and Association Analysis of Six PSD-95-Related Genes as Possible Susceptibility Genes for Schizophrenia and Autism Spectrum Disorders. Nature Publishing Group:1–8.

Young N Ji, Findling RL (2015) An update on pharmacotherapy for autism spectrum disorder in children and adolescents. Current Opinion in Psychiatry:1–11.

Young RL, Brewer N, Pattison C (2003) Parental identification of early behavioural abnormalities in children with autistic disorder. Autism 7(2):125-43.

Zahir FR, Baross A, Delaney AD, Eydoux P, Fernandes ND, Pugh T, Marra MA, Friedman JM (2007) A patient with vertebral, cognitive and behavioural abnormalities and a de novo deletion of NRXN1. Journal of Medical Genetics 45:239–243.

Zanni G, van Esch H, Bensalem A, Saillour Y, Poirier K, Castelnau L, Ropers HH, de Brouwer APM, Laumonnier F, Fryns J-P, Chelly J (2009) A novel mutation in the DLG3

gene encoding the synapse-associated protein 102 (SAP102) causes non-syndromic mental retardation. Neurogenetics 11:251–255.

Zeng M, Shang Y, Araki Y, Guo T, Huganir RL, Zhang M (2016) Phase Transition in Postsynaptic Densities Underlies Formation of Synaptic Complexes and Synaptic Plasticity. Cell 166:1163–1175.e12.

Zhang L, Jablonski AM, Mojsilovic-Petrovic J, Ding H, Seeholzer S, Newton IP, Nathke I, Neve R, Zhai J, Shang Y, Zhang M, Kalb RG (2017) SAP97 Binding Partner CRIPT Promotes Dendrite Growth in Vitroand in Vivo. eNeuro:ENEURO.0175–17.2017–61.

Zhao Z, Xu J, Chen J, Kim S, Reimers M, Bacanu S-A, Yu H, Liu C, Sun J, Wang Q, Jia P, Xu F, Zhang Y, Kendler KS, Peng Z, Chen X (2014) Transcriptome sequencing and genome-wide association analyses reveal lysosomal function and actin cytoskeleton remodeling in schizophrenia and bipolar disorder. Mol Psychiatry 20:563–572.

Zhou W, Zhang L, Guoxiang X, Mojsilovic-Petrovic J, Takamaya K, Sattler R, Huganir R, Kalb R (2008) GluR1 Controls Dendrite Growth through Its Binding Partner, SAP97. Journal of Neuroscience 28:10220–10233.

Zwaigenbaum L, Thurm A, Stone W, Baranek G, Bryson S, Iverson J, Kau A, Klin A, Lord C, Landa R, Rogers S, Sigman M (2006) Studying the Emergence of Autism Spectrum Disorders in High-risk Infants: Methodological and Practical Issues. J Autism Dev Disord 37:466–480.