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Archival Report

MEF2C Hypofunction in Neuronal and Neuroimmune Populations Produces *MEF2C* Haploinsufficiency Syndrome–like Behaviors in Mice

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ABSTRACT

BACKGROUND: Microdeletions of the *MEF2C* gene are linked to a syndromic form of autism termed *MEF2C* haploinsufficiency syndrome (MCHS). MEF2C hypofunction in neurons is presumed to underlie most of the symptoms of MCHS. However, it is unclear in which cell populations *MEF2C* functions to regulate neurotypical development.

METHODS: Multiple biochemical, molecular, electrophysiological, behavioral, and transgenic mouse approaches were used to characterize MCHS-relevant synaptic, behavioral, and gene expression changes in mouse models of MCHS.

RESULTS: We showed that MCHS-associated missense mutations cluster in the conserved DNA binding domain and disrupt *MEF2C* DNA binding. DNA binding–deficient global *Mef2c* heterozygous mice (*Mef2c*-Het) displayed numerous MCHS-related behaviors, including autism-related behaviors, changes in cortical gene expression, and deficits in cortical excitatory synaptic transmission. We detected hundreds of dysregulated genes in *Mef2c*-Het cortex, including significant enrichments of autism risk and excitatory neuron genes. In addition, we observed an enrichment of upregulated microglial genes, but this was not due to neuroinflammation in the *Mef2c*-Het cortex. Importantly, conditional *Mef2c* heterozygosity in forebrain excitatory neurons reproduced a subset of the *Mef2c*-Het phenotypes, while conditional *Mef2c* heterozygosity in microglia reproduced social deficits and repetitive behavior.

CONCLUSIONS: Taken together, our findings show that mutations found in individuals with MCHS disrupt the DNA-binding function of MEF2C, and DNA binding–deficient *Mef2c* global heterozygous mice display numerous MCHS-related phenotypes, including excitatory neuron and microglia gene expression changes. Our findings suggest that MEF2C regulates typical brain development and function through multiple cell types, including excitatory neuronal and neuroimmune populations.

Keywords: Autism, *Mef2c*, Microglia, Mouse, Neurodevelopmental disorder, Neuron

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MEF2 (myocyte enhancer factor 2) proteins are members of the MADS family of transcription factors that regulate gene expression during development and adulthood. In the brain, MEF2C is important for neuronal differentiation and synapse development (1). MEF2 proteins regulate numerous genes associated with synapse formation and function as well as multiple genes linked to neurodevelopmental disorders, including autism spectrum disorder (ASD) (2–4). Constitutively active MEF2C can promote glutamatergic synapse elimination, a process requiring the RNA-binding function of FMRP (fragile X mental retardation protein) (5–8). Conditional knockout (cKO) of *Mef2c* in neuronal populations within the mouse brain produces myriad severe behavioral and synaptic phenotypes, which emphasizes the importance of this gene in healthy brain development (2,9–12).

MEF2C in the developing and mature brain is also expressed in microglia (13–15)—a population of macrophage-like cells throughout the brain that regulates synapse formation and pruning during early brain development (16–18). Microglia influence a number of brain functions, including synapse elimination, synapse formation, fasciculation of the corpus callosum, survival of oligodendrocyte precursor cells, and phagocytosis of other brain

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cells (16,19–24). Microglia are recognized not only as responding to infection or injury, but also as important regulators of brain development and function (25). In addition, microglial dysfunction might play an important role in disease pathology for other neurodevelopmental disorders, including Rett syndrome (26–29).

Microdeletions on chromosome 5q14.3 that include the *MEF2C* gene or point mutations within the protein-coding region of *MEF2C* are linked to a recently described neurodevelopmental disorder, termed *MEF2C* haploinsufficiency syndrome (MCHS) (30–40). Common symptoms of MCHS include ASD, absence of speech, stereotypical behaviors, hyperactivity, intellectual disability, hypotonia and motor abnormalities, high pain tolerance, sleep disturbances, and epilepsy. Individuals with *MEF2C* point mutations typically present with fewer and/or milder symptoms (30–40). Owing to the abundance of neurological symptoms and neuronal-enriched expression of MEF2C, *MEF2C* haploinsufficiency within neurons is presumed to underlie most, if not all, MCHS symptoms. Interestingly, single-cell genomic profiling from cortical tissue of patients with idiopathic autism revealed that upper-layer excitatory neurons and microglia are preferentially affected in autism (41), and as both neurons and microglia express MEF2C, we sought to explore the possible cell type–specific effects of MEF2C hypofunction in MCHS-related behaviors in a construct-valid mouse model of human MCHS.

METHODS AND MATERIALS

Patients

Patients with developmental delay and a significant variant in the *MEF2C* gene were selected for this study. These patients were seen for clinical genetics evaluations at the Greenwood Genetic Center (Greenwood, SC), and data from these visits were gathered from records review. Internal informed consent to review and publish the data was obtained for each subject.

Animals

Mef2c$^{+/-}$ (*Mef2c*-Het) mice were generated by crossing *Mef2c-flox* mice (RRID:MGI:3719006) to Prm-Cre mice (The Jackson Laboratory, Bar Harbor, ME). The Prm-Cre allele was subsequently removed during repeated backcrossing to C57BL/6J wild-type mice. *Mef2c* conditional heterozygous (*Mef2c*-cHet) mice were generated by crossing *Mef2c-flox* mice with cell type–selective Cre-expressing transgenic mice [*Emx1-Cre* (42), *PV-Cre*, *Pcp2-Cre*, or *Cx3Cr1*$^{creER/creER}$ (20) (all from The Jackson Laboratory)] to generate *Mef2c*$^{fl/+}$; *Cre*$^{+}$ *Mef2c*-cHet mice that were compared with their Cre-negative or flox-negative littermates (control mice). Experimenters were blinded to the mouse genotype during data acquisition and analysis. All procedures were conducted in accordance with the Medical University of South Carolina Institutional Animal Care and Use Committee and National Institutes of Health guidelines.

Detailed Methods are in Supplement 1.

RESULTS

Patient *MEF2C* Missense Mutations Cluster in DNA Binding and Dimerization Domains and Disrupt DNA Binding

Deletions or mutations in *MEF2C* are assumed to create loss-of-function alleles that cause the symptoms of MCHS (30–40). Given that microdeletions of 5q14.3 often include additional genes beyond *MEF2C*, we identified individuals with mutations within the *MEF2C* protein-coding region, including an intragenic duplication (i.e., p.D40_C41dup) and two missense variants (i.e., p.K30N and p.I46T) (Table S1 in Supplement 1). We compared their clinical histories with those associated with two previously reported missense variants in the *MEF2C* gene (31). All 5 patients presented with global developmental delay and seizures. Common features of these individuals included absence of speech, repetitive movements, hypotonia, varied but inconsistent abnormalities on brain magnetic resonance imaging, and breathing disturbances. High pain tolerance was noted in two of the patients. There were some minor facial dysmorphisms noted, though there did not seem to be a consistently recognizable gestalt. When a list of additional MCHS mutations was assembled (C.W.C. and S.A.S., personal communications, October 2019), several frameshift and premature stop codon mutations were identified, all of which, if stable, are predicted to produce a truncated MEF2C protein lacking its C-terminal nuclear localization sequence. We noted that all of the *MEF2C* missense (or small duplication) mutations were clustered within the highly conserved MADS (DNA binding) or MEF2 (dimerization) domains (Figure 1A). In an MEF2 response element DNA binding assay, all 5 of the MADS domain patient mutations caused a loss of MEF2C DNA binding (Figure 1B, C and Figure S1A in Supplement 1), and they did not appear to interfere with wild-type MEF2C DNA binding (Figure S1B in Supplement 1), suggesting a loss-of-function phenotype.

Mef2c Heterozygous Mouse Model

To model the genetics of MCHS in mice, we generated a global heterozygous *Mef2c* mutant mouse lacking exon 2 (*Mef2c*$^{+/\Delta Ex2}$ or *Mef2c*-Het) (Figure 1D), which encodes a large portion of the MADS/MEF2 domains. The near full-length MEF2C$^{\Delta Ex2}$ protein had no detectable DNA binding affinity and did not reduce DNA binding affinity of wild-type MEF2C (Figure 1E, F and Figure S1C in Supplement 1). We observed a non-Mendelian frequency of *Mef2c*-Het mice, suggesting a partial embryonic lethality (Figure S1D in Supplement 1), similar to a previous report (43). To assess for gross morphological changes in *Mef2c*-Het brains, we measured the cortical thickness of the barrel cortex. We did not observe differences in cortical thickness of the barrel cortex between *Mef2c*-Het and control mice (Figure S1E in Supplement 1).

We examined whether male and female *Mef2c*-Het mice showed behavior phenotypes reminiscent of MCHS symptoms. Using a 3-chamber social interaction test, we observed that *Mef2c*-Het mice had a lack of social preference with a novel same-sex mouse (Figure 2A). We also found that *Mef2c*-Het male and female pups (postnatal day 7–10) produced significantly fewer ultrasonic vocalization calls during

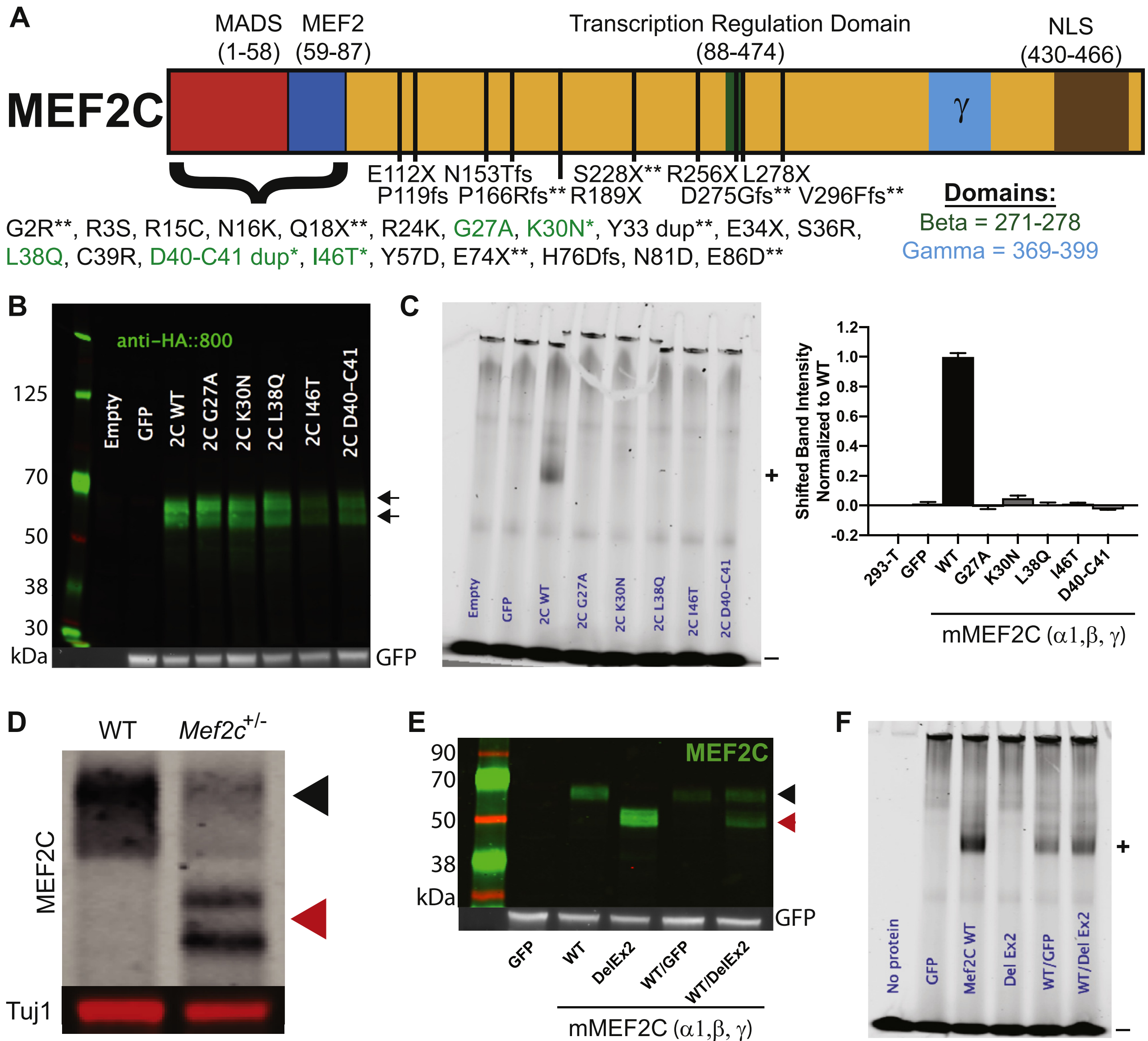

Figure 1. MCHS-associated mutations in MEF2C disrupt DNA binding. **(A)** Schematic of the MEF2C protein with locations of MCHS mutations. MCHS mutations in green are further characterized **(B, C)**. MCHS mutations that are newly described in this article are denoted with a single asterisk. MCHS mutations not previously reported (C.W.C. and S.A.S., personal communications, October 2019) are denoted by 2 asterisks. The alternatively spliced beta (green) and gamma (blue) domains are shown. All MEF2C transcripts contain a C-terminal NLS that is predicted to be absent in all of the indicated frameshift (fs) mutations. **(B)** Western blot of MEF2C WT and MCHS mutations in 293-T cells show that all MCHS mutations lead to protein expression. Arrows denote MEF2C WT and mutant protein bands. **(C)** Electrophoretic mobility shift assay using fluorescently labeled MRE probe and MEF2C protein lysates from 293-T cells containing MEF2C mutations. MEF2C bound probe is shifted in the gel (denoted by plus sign). Unbound fluorescent probe is denoted with a minus sign. Only MEF2C WT binds to the fluorescently labeled MRE, while MCHS mutant proteins fail to bind the MRE probe **(C)**. Quantification of bound probe is included **(C)**. **(D)** Western blot of MEF2C from cortical lysates of control and *Mef2c*$^{+/-}$ mice. The black arrow denotes MEF2C WT, and the red arrow denotes MEF2C DelEx2 **(D, E)**. **(E)** Western blot of MEF2C WT and MEF2C DelEx2 from 293-T cells. **(F)** MEF2C DelEx2 fails to bind the MRE probe and does not interfere with MEF2C WT binding to MRE probes. +, bound probe; −, unbound probe. Data are reported as mean ± SEM. Also see Figure S1 in Supplement 1. GFP, green fluorescent protein; MCHS, *MEF2C* haploinsufficiency syndrome; MRE, MEF2 response element; NLS, nuclear localization sequence; WT, wild-type.

maternal separation (Figure 2B), and young adult *Mef2c*-Het male mice produced significantly fewer ultrasonic vocalization calls (Figure 2C) in the presence of a female in estrus, suggesting that *Mef2c*-Het mice have deficits in a putative species-appropriate form of social communication. Male *Mef2c*-Het mice were hyperactive in a novel environment (Figure 2E) and displayed an increase in jumping (Figure 2F), a repetitive-type motor behavior; however, young adult *Mef2c*-Het mice displayed normal performance on the accelerating rotarod test of motor coordination (Figure 2D). In addition,

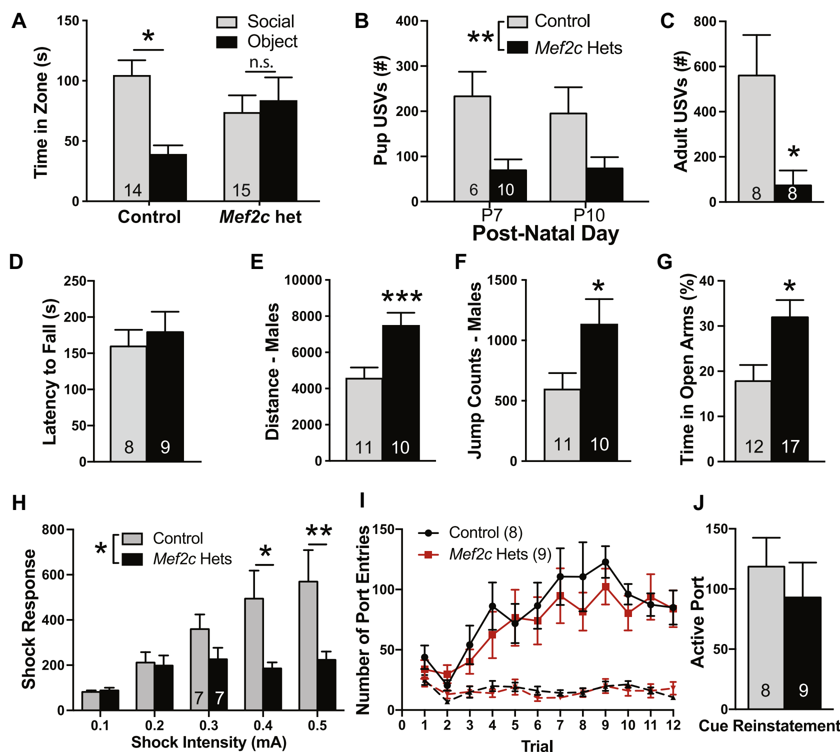

Figure 2. *Mef2c*$^{+/-}$ (*Mef2c*-Het) mice display multiple *MEF2C* haploinsufficiency syndrome–relevant behaviors. **(A)** Three-chamber social interaction test. Control mice spent significantly more time interacting with a novel animal over a novel object, while *Mef2c*-Het mice showed no preference for the novel object or the novel animal. **(B)** *Mef2c*-Het pups emitted fewer USVs during maternal separation in early postnatal development. **(C)** Adult male *Mef2c*-Het mice produced fewer USVs than control mice in the presence of a female mouse in estrus. **(D)** Control and *Mef2c*-Het mice had similar latencies to fall on an accelerating rotarod. **(E, F)** Male *Mef2c*-Het mice were hyperactive **(E)** and showed increased jump counts **(F)**. **(G)** *Mef2c*-Het mice spent significantly more time on the open arms of the elevated plus maze. **(H)** *Mef2c*-Het mice had reduced response to shock. **(I)** Both control and *Mef2c*-Het mice increased the number of active port entries (solid line) during sucrose self-administration. Dashed line represents inactive port entries. **(J)** Both control and *Mef2c*-Het mice showed similar active port entries during cue-induced reinstatement of sucrose seeking. Data are reported as mean ± SEM. Statistical significance was determined by 2-way analysis of variance **(A, B, H, I)** or unpaired *t* test **(C–G, J)**. Number of animals are reported in each graph. **p* < .05, ***p* < .01, ****p* < .005. n.s., not significant; P7, postnatal day 7; P10, postnatal day 10; USV, ultrasonic vocalizations. Also see Figure S2 in Supplement 1.

Mef2c-Het mice showed increased exploration of the open, unprotected arm of the elevated plus maze (Figure 2G). Interestingly, *Mef2c*-Het mice showed a reduction in startle response to electrical foot-shocks (Figure 2H). This phenotype might reflect reduced pain sensitivity, similar to subjects with MCHS (31,33), as startle responses to multiple white-noise intensities were indistinguishable from control mice (Figure S2A in Supplement 1).

Despite a common occurrence of intellectual disability in MCHS, we failed to detect any clear learning and memory-related deficits in Pavlovian fear conditioning tests (Figures S2B–D in Supplement 1), the Barnes maze test for spatial learning and memory (Figure S2E in Supplement 1), and the Y-maze test for spatial working memory (Figure S2F in Supplement 1) in the *Mef2c*-Het mice. These mice also showed a strong preference for the novel object in the novel object recognition test (Figure S2G in Supplement 1) and normal sucrose preference in a two-bottle choice test (Figure S2H in Supplement 1). In the cognitively demanding operant sucrose self-administration assay, the *Mef2c*-Het mice displayed wild-type levels of operant learning, operant discrimination (active vs. inactive port), context-related sucrose seeking after abstinence, extinction learning, and cue-induced reinstatement (Figure 2I, J and Figure S2I–L in Supplement 1). Taken together, our findings suggest that in contrast to the cKO of *Mef2c* in Emx1-lineage cells (2) or a related study (43), the global loss of one functional copy of *Mef2c* in mice is not sufficient to produce detectable deficits in learning and memory in the C57BL6/J genetic background.

Mef2c-Het Mice Display Input-Selective Reductions in Cortical Excitatory Synaptic Transmission

In young *Mef2c*-Het mice (postnatal day 35–40), gross structural organization of barrel fields within cortical layer 4 of the somatosensory cortex appeared normal (Figure 3A), and in somatosensory cortex layer 2/3 pyramidal neurons, we detected no significant differences by genotype for intrinsic excitability (Figure S3A in Supplement 1), apical or basal dendritic spine density, dendritic spine head diameter (Figure S3B in Supplement 1), or GABA-mediated inhibitory synaptic transmission (miniature inhibitory postsynaptic currents) (Figure 3B). However, patch-clamp recordings of layer 2/3 neurons revealed an input-selective deficit in glutamatergic synaptic transmission. Electrical stimulation of horizontal fibers in layer 2/3 of a neighboring cortical column produced a significant reduction in the amplitude of evoked excitatory postsynaptic currents (eEPSCs) (Figure 3C), suggesting a reduction in presynaptic and/or postsynaptic transmission. Paired-pulse facilitation analysis (50-ms interstimulus interval) of local horizontal inputs revealed a significant increase in paired-pulse facilitation ratio (Figure 3C), indicating a decrease in presynaptic release probability (44). These effects were input-selective given that electrical stimulation of layer 4 (within the same cortical column) produced eEPSC and paired-pulse facilitation responses in layer 2/3 neurons that were indistinguishable from control mice (Figure 3D). To examine if reductions in AMPA-mediated postsynaptic strength might also contribute to the reduced horizontal eEPSCs (Figure 3C), we measured miniature EPSCs (mEPSCs) under conditions in

which action potentials are blocked pharmacologically. In layer 2/3 cells from *Mef2c*-Het mice, we observed a significant reduction in mEPSCs amplitude (Figure 3E), suggesting an overall reduction in AMPA-mediated postsynaptic strength. Similar to layer 2/3, we also observed a significant reduction of mEPSC amplitude in somatosensory cortex layer 5 pyramidal neurons of *Mef2c*-Het mice (Figure 3F), suggesting that the reduction in glutamatergic postsynaptic strength is not limited to a specific cortical layer. Consistent with layer 2/3 pyramidal neurons, we did not observe any differences in dendritic spine density or dendritic spine head diameter in basal dendrites from layer 5 pyramidal neurons (Figure S3C in Supplement 1). There was no effect of genotype on layer 5 mEPSC frequency (Figure 3F), but we observed a significant increase in layer 2/3 mEPSC frequency (Figure 3E) that was not explained by an increase in dendritic spine density (Figure S3B in Supplement 1) or effects on presynaptic functions of local inputs (Figure 3C, D) and might represent a compensatory effect of long-range connections (11).

Mef2c-Het Mice Display Dysregulation of Cortical Genes Associated With ASD Risk, Excitatory Neurons, and Microglia

Using an unbiased RNA sequencing (RNA-seq) approach, we examined gene expression from whole cortex in control and *Mef2c*-Het mice (postnatal day 35–40), and we identified 490 genes that were significantly dysregulated (false discovery rate < 0.05) (Figure 4A; Figure S4A in Supplement 1; Tables S2 and S3 in Supplement 2). We confirmed select *Mef2c*-Het differentially expressed genes (DEGs) that are associated with ASD risk, microglia, and other cellular functions by quantitative reverse transcriptase polymerase chain reaction (Figure 4D). We also investigated the association of *Mef2c*-Het DEGs with sequencing data from various brain disorders. We found that the *Mef2c*-Het DEGs, particularly the downregulated genes, were overrepresented in genes associated with ASD risk and FMRP binding (Figure 4B, D and Table S2 in Supplement 2). We also assessed enrichment for *Mef2c*-Het DEGs in genes that were dysregulated in a meta-analysis of transcriptomic data across neuropsychiatric disorders (45). Interestingly, *Mef2c*-Het DEGs, particularly the downregulated genes, were significantly enriched for a PsychENCODE Consortium excitatory neuron module of genes that are downregulated in ASD (vs. other neuropsychiatric disorders) brains (geneM1) (Figure 4C and Table S2 in Supplement 2). *Mef2c*-Het DEGs, particularly the upregulated genes, were enriched in PsychENCODE module 6, which is a microglia module of genes upregulated in ASD but downregulated in schizophrenia and bipolar disorder (geneM6) (Figure 4C and Table S2 in Supplement 2). Using single-cell RNA sequencing data from mouse cortex (46), we observed that *Mef2c*-Het DEGs were strongly enriched for cortical excitatory neuron genes and microglia genes (Figure S4B in Supplement 1 and Table S3 in Supplement 2), further supporting the importance of MEF2C in regulating gene expression in the two key brain populations with high MEF2C expression. Interestingly, enrichment for microglia genes was not detected on DEGs from Emx1-Cre *Mef2c* cKO mice (*Mef2c* cKO$^{Emx1\text{-}cre}$) (Figure S4C in Supplement 1) (2), underscoring the specific association between microglia and *Mef2c*-Het mice. To further support the role of *Mef2c* in regulation of DEGs, we analyzed MEF2C chromatin immunoprecipitation sequencing data from an independent study (47). Notably, we found enrichment of *Mef2c*-Het DEGs in genes bound by MEF2C in multiple genomic regions (Table S2 in Supplement 2 and Figure S4D in Supplement 1). This result further validates the key role of MEF2C in regulating genes associated with microglia and synaptic etiologies.

Gene ontology analysis of *Mef2c*-Het DEGs revealed significant enrichment of microglia proliferation genes, cell metabolism genes, and genes in a microglia subpopulation in the developing brain that is restricted to unmyelinated axon tracts (Figure S5D in Supplement 1). As *Mef2c*-Het brains showed significant dysregulation of microglial genes (Figure 4C, D), and MEF2C is expressed in microglia in the developing and mature brain (Figure S5A, B in Supplement 1) (13–15), we analyzed the *Mef2c*-Het brain for possible upregulation of the microglia cell type and neuroimmune activation marker Iba1 (ionized calcium-binding adapter molecule 1) (48,49). In both the cortex and the hippocampus, we observed a significant increase in Iba1 expression (Figure 5A–C, E) without a change in the density of microglia (Figure S5C in Supplement 1), suggesting possible microglial activation in the *Mef2c*-Het brain. This increase in Iba1 was present without an obvious change in microglial cell morphology or microglial cell soma volume (Figure 5A, B, D, F). In addition, in the *Mef2c*-Het cortex, we observed no changes in classical and alternative pathway inflammatory genes, including *Cd68*, *Il6*, *Tnf*, *Il10*, and several others (Figure 5G). Many cytokines in control and *Mef2c*-Het mice were undetectable by cytokine antibody array, and there was no difference in the level of interferon-gamma between genotypes (Figure S5E in Supplement 1). However, we did note a significant increase in the expression of several complement-related genes linked previously to synaptic pruning and/or ASD risk, including *C1qb*, *C1qc*, and *C4b* (Figure 4D) (16,19,50–52). Moreover, we observed significant enrichments of upregulated *Mef2c*-Het DEGs in single-cell RNA-seq gene clusters associated with embryonic-like microglia, postnatal immature microglia, and homeostatic microglia (Figure 5H). Taken together, these results reveal that the reduction of MEF2C levels has significant impacts on microglia gene expression programs.

MEF2C Contributes to Neurotypical Behaviors Through Key Roles in Forebrain Excitatory Neurons and Microglia

In the mouse brain, MEF2C is expressed in several cell types, including cortical excitatory pyramidal cells, parvalbumin-positive GABAergic inhibitory neurons, cerebellar Purkinje cells, and microglia (2,9,13–15,53–56). As the *Mef2c*-Het mouse cortex showed robust changes in both excitatory neurons and microglia gene expression (Figure 4 and Figure S4 in Supplement 1), we generated cell type–specific conditional *Mef2c*-Het mice to explore the contribution of neurons versus microglia for the development of MCHS-like phenotypes. We first generated mice heterozygous for *Mef2c* in Emx1-lineage cells (*Mef2c*-cHet$^{Emx1\text{-}cre}$) (42), which represents approximately 85% of forebrain excitatory neurons throughout the

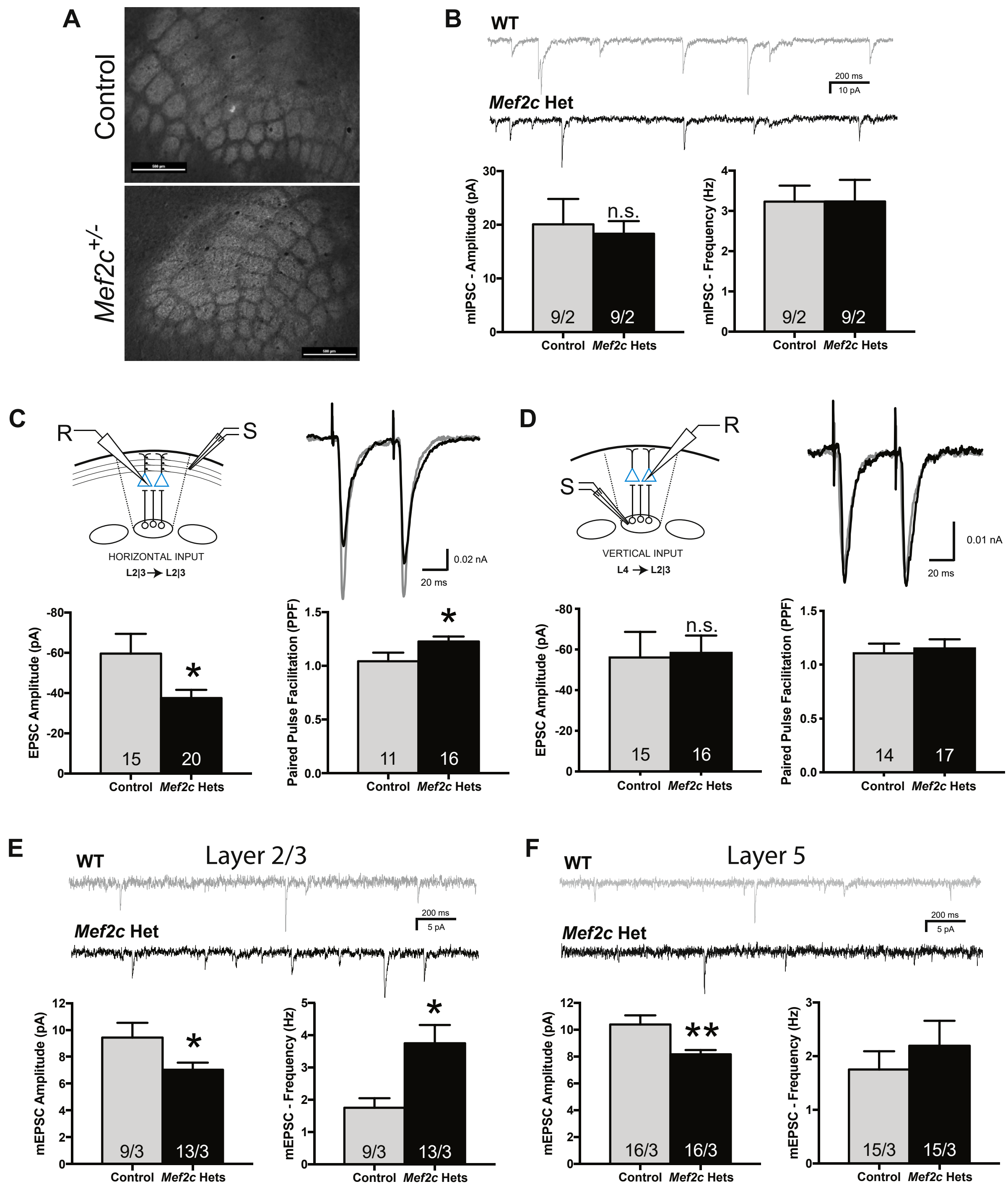

Figure 3. *Mef2c*$^{+/-}$ (*Mef2c*-Het) mice have alterations in cortical synaptic transmission. **(A)** Both control and *Mef2c*-Het mice had normal barrel fields in the somatosensory cortex, as reflected by VGlut2 staining. Scale bar = 500 μm. **(B–F)** Ex vivo recordings from organotypic slices were collected from pyramidal neurons within the barrel cortex field. **(B)** No changes were observed in mIPSC amplitude or frequency in the *Mef2c*-Het layer 2/3 pyramidal neurons. **(C)** Reduced EPSC amplitude and increased PPF were observed in layer 2/3 *Mef2c*-Het neurons after stimulating input neurons from neighboring layer 2/3 neurons in adjacent barrel fields (horizontal inputs). **(D)** No changes in evoked EPSC amplitude or PPF were observed in layer 2/3 pyramidal neurons after stimulating input neurons from layer 4 (vertical inputs). **(E, F)** *Mef2c*-Het cortical pyramidal neurons had reduced mEPSC amplitude in layer 2/3 **(E)** and layer 5 **(F)** and increased mEPSC frequency in layer 2/3 **(E)**. Data are reported as mean ± SEM. Statistical significance was determined by unpaired *t* test. Number of cells and animals, respectively, are reported in each graph. *$p < .05$, **$p < .01$. EPSC, excitatory postsynaptic current; mIPSC, miniature inhibitory post-synaptic current; n.s., not significant; PPF, paired-pulse facilitation; R, recording electrode; S, stimulating electrode; WT, wild-type. Also see Figure S3 in Supplement 1.

cortex and hippocampus. Similar to global *Mef2c*-Het mice, *Mef2c*-cHet$^{Emx1\text{-}cre}$ mice displayed altered anxiety-like behavior and male-selective increases in locomotion and repetitive jumping (Figure 6A–C), but they showed no changes in social behavior or shock sensitivity (Figure 6D and Figure S6A in Supplement 1). Interestingly, similar to global *Mef2c*-Het mice (Figure 3E) and *Mef2c* cKO$^{Emx1\text{-}cre}$ mice (2), we observed a reduction of mEPSC amplitude in layer 2/3 pyramidal neurons from *Mef2c*-cHet$^{Emx1\text{-}cre}$ mice (Figure S6N in Supplement 1). These findings suggest that Emx1-lineage excitatory forebrain neurons contribute to the development of some, but not all, of the behavior phenotypes observed in the global *Mef2c*-Het mice. Interestingly, not all MEF2C-expressing populations are critical for MCHS-related behaviors, as mice with MEF2C hypofunction in parvalbumin-positive GABAergic interneurons (*Mef2c*-cHet$^{PV\text{-}cre}$) or in cerebellar Purkinje cells (*Mef2c*-cHet$^{Pcp2\text{-}cre}$) showed behaviors indistinguishable from control mice (Figure S6B–K in Supplement 1).

We next generated microglia-selective *Mef2c*-Het mice (*Mef2c*-cHet$^{Cx3cr1\text{-}cre}$) (Figure S6L in Supplement 1). The conditional mutant mice displayed social impairments in the 3-chamber social interaction test (Figure 6H), similar to global *Mef2c*-Het mice. In addition, *Mef2c*-cHet$^{Cx3cr1\text{-}cre}$ mice showed a significant increase in male-specific repetitive jumping (Figure 6G), but with no discernable effects on exploratory activity (Figure 6F), anxiety-like behavior, or shock sensitivity (Figure 6E and Figure S6M in Supplement 1). To investigate the possible influence of microglial MEF2C hypofunction on neuronal function, we recorded horizontally evoked EPSCs in layer 2/3 of the somatosensory cortex of *Mef2c*-cHet$^{Cx3cr1\text{-}cre}$ mice and control mice. Similar to global *Mef2c*-Het mice, we observed a decrease in eEPSC amplitude in the *Mef2c*-cHet$^{Cx3cr1\text{-}cre}$ mice (Figure 6I). Interestingly, if we analyze by sex, the decrease in eEPSC amplitude is driven by males (Figure S6P, Q in Supplement 1). However, in contrast to the global *Mef2c*-Het mice, no deficits in presynaptic function were detected by paired-pulse ratio analysis in the *Mef2c*-cHet$^{Cx3cr1\text{-}cre}$ mice (Figure 6J). Taken together, our results suggest that 1) *Mef2c* haploinsufficiency in early postnatal microglia is sufficient to produce autism-related behaviors, 2) MEF2C-deficient microglia can produce a non–cell autonomous influence on excitatory synaptic transmission of layer 2/3 pyramidal neurons, and 3) the majority of MCHS-like phenotypes in the global *Mef2c*-Het mice can be recapitulated by MEF2C hypofunction in both forebrain excitatory neurons and microglia.

DISCUSSION

We report here three new *MEF2C* mutations in individuals with MCHS-related symptoms, and all three mutations disrupted MEF2C DNA binding. Interestingly, all of the known MCHS missense or duplication mutations cluster within the highly conserved DNA binding and dimerization domains (Figure 1A) (57). DNA binding-deficient *Mef2c*-Het mice displayed numerous behavioral phenotypes reminiscent of MCHS, including deficits in social interaction and communication (ultrasonic vocalizations), motor hyperactivity, repetitive behavior, anxiety-related behavior, and reduced sensitivity to a painful stimulus (foot-shock). In contrast to a previous study (43), the *Mef2c*-Het mice did not show any clear learning and memory deficits, which might support a unique, primate-specific role of MEF2C (58) or reflect a background strain interaction. The *Mef2c*-Het mice also possessed input-selective, presynaptic and postsynaptic deficits in glutamatergic excitatory synaptic transmission in the somatosensory cortex. Gene expression analysis of cortical tissue from *Mef2c*-Het mice revealed significant enrichment of DEGs linked to ASD risk, excitatory neurons, and microglia, which is notable considering the enrichment of dysregulated genes linked to cortical excitatory neurons and microglia in brains of individuals with idiopathic ASD (41). Conditional *Mef2c*-Het mice in Emx1-lineage cells, which represent predominantly forebrain excitatory neurons, reproduced several of the global *Mef2c*-Het behaviors and cortical synaptic phenotypes. Consistent with the dysregulation of microglial genes in *Mef2c*-Het mice, early postnatal conditional *Mef2c* heterozygosity in *Cx3cr1*-lineage cells, which are almost exclusively microglia in the brain (48,49,59), produced offspring with social deficits, increased repetitive behavior, and reduced cortical glutamatergic synaptic transmission, suggesting a critical role for MEF2C in microglia during neurotypical development and behavior and supporting the growing view that microglial dysfunction in the developing brain can underlie ASD symptoms.

Interestingly, we observed male-selective effects of *Mef2c* heterozygosity on hyperactivity and/or jumping behavior in *Mef2c*-Het and *Mef2c*-cHet mice (Figures 2E, F and 6B, C, G), suggesting an interaction between sex-based mechanisms and MEF2C functions. Indeed, numerous studies show that both neuron and microglia functions can be differentially regulated in males and females (60–63). It is also interesting to note that *Mef2c*-Het DEGs linked to excitatory neurons show a preferential downregulation, whereas *Mef2c*-Het DEGs linked to microglia display a preferential upregulation. MEF2C is reported to function as both a transcriptional activator and a repressor, and there are cell type–specific signaling mechanisms that regulate MEF2C activity (2,64,65).

MEF2 proteins can regulate activity-dependent glutamatergic synapse elimination (5–7,66), and MEF2C can function in cortical pyramidal neurons as a cell-autonomous transcriptional repressor to regulate dendritic spine density, synapse number, and AMPA-mediated postsynaptic strength (2,11). cKO of both *Mef2c* alleles in forebrain excitatory neurons produces mice with dramatic changes in cortical synapse functions, including decreased glutamatergic synaptic transmission, numerous alterations in typical mouse behaviors, and differential gene expression (2,9,10,12). In the present study, we detected an input-selective reduction in glutamatergic synaptic strength in layer 2/3 pyramidal neurons from *Mef2c*-Het mice as well as a reduction in presynaptic release from local layer 2/3 inputs (Figure 3C). Interestingly, we also observed an increase in mEPSC frequency in these neurons (Figure 3E) that is possibly due to an increase in synaptic inputs from long-range corticocortical inputs that was observed in sparse cell-autonomous *Mef2c* cKO in layer 2/3 cortical neurons (11). *Mef2c*-Het mice showed similar changes in basal glutamatergic synaptic transmission (i.e., reduced mEPSC amplitude and increased mEPSC frequency) in hippocampal dentate gyrus granule neurons, and pharmacological

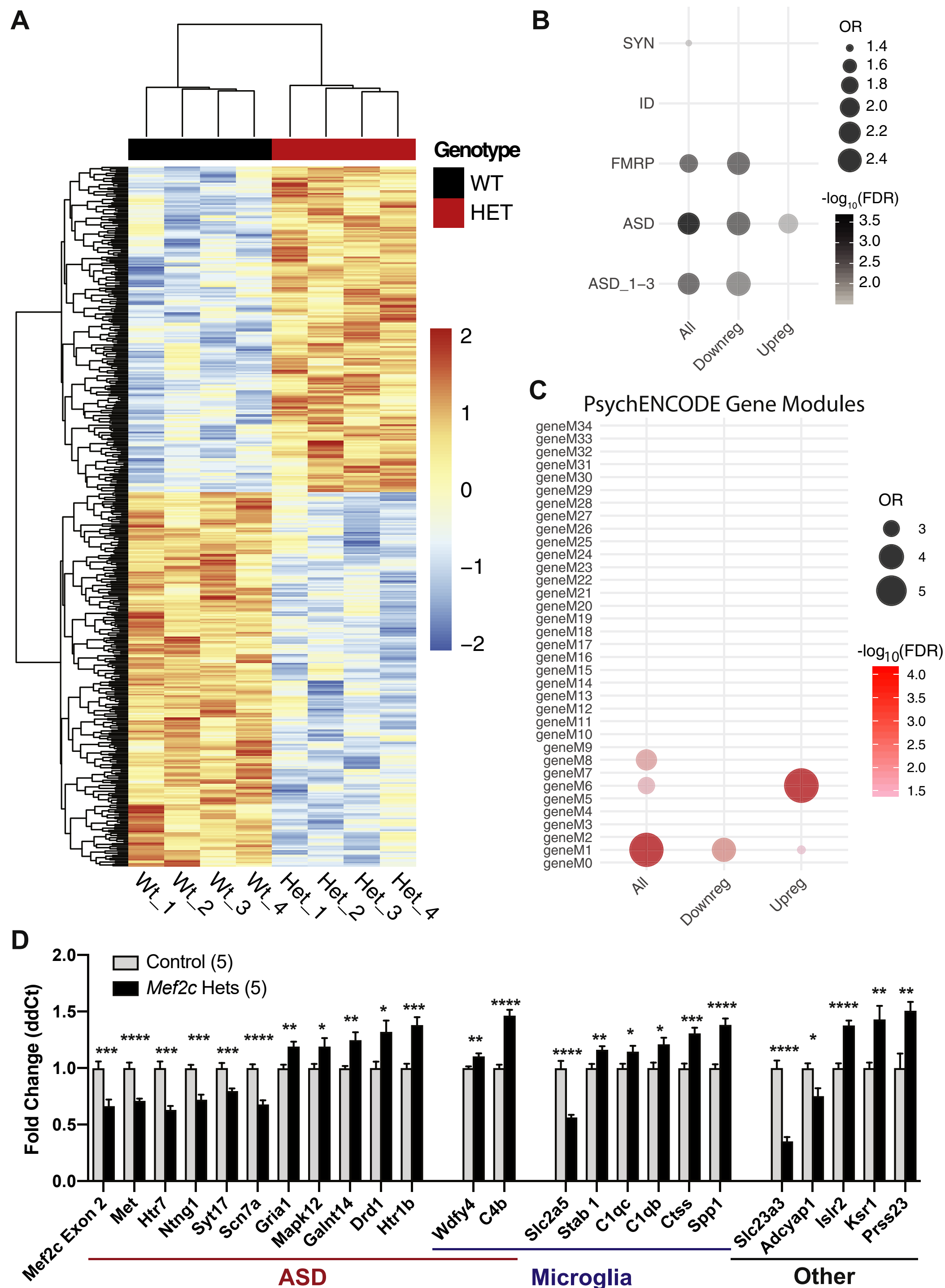

Figure 4. DEGs in *Mef2c*$^{+/-}$ (*Mef2c*-Het) cortex. **(A)** Heatmap showing DEGs in *Mef2c*-Het cortex (postnatal day 35–40) compared with control mice. Genes with higher expression are in red; genes with lower expression are in blue. **(B)** *Mef2c*-Het DEGs are significantly enriched in genes associated with FMRP, ASD, or scored ASD (ASD_1–3; high-confidence ASD genes) (see Methods and Materials). **(C)** *Mef2c* DEGs are enriched in gene modules dysregulated in neuropsychiatric disorders, specifically the M1 and M6 modules. **(D)** Quantitative polymerase chain reaction validation of select *Mef2c*-Het DEGs associated with ASD, microglia, or other cellular functions. Data are reported as mean ± SEM **(D)**. Statistical significance was determined by unpaired *t* test **(D)**. *$p < .05$, **$p < .01$, ***$p < .005$, ****$p < .0005$. See Methods and Materials for statistical analysis of panels **(A–C)**. Number of animals is 4/genotype for RNA sequencing and 5/genotype for quantitative polymerase chain reaction validation. ASD, autism spectrum disorder; DEGs, differentially expressed genes; Downreg, downregulated; FDR, false discovery rate; OR, odds ratio; Upreg, upregulated; WT, wild-type. Also see Figure S4 in Supplement 1.

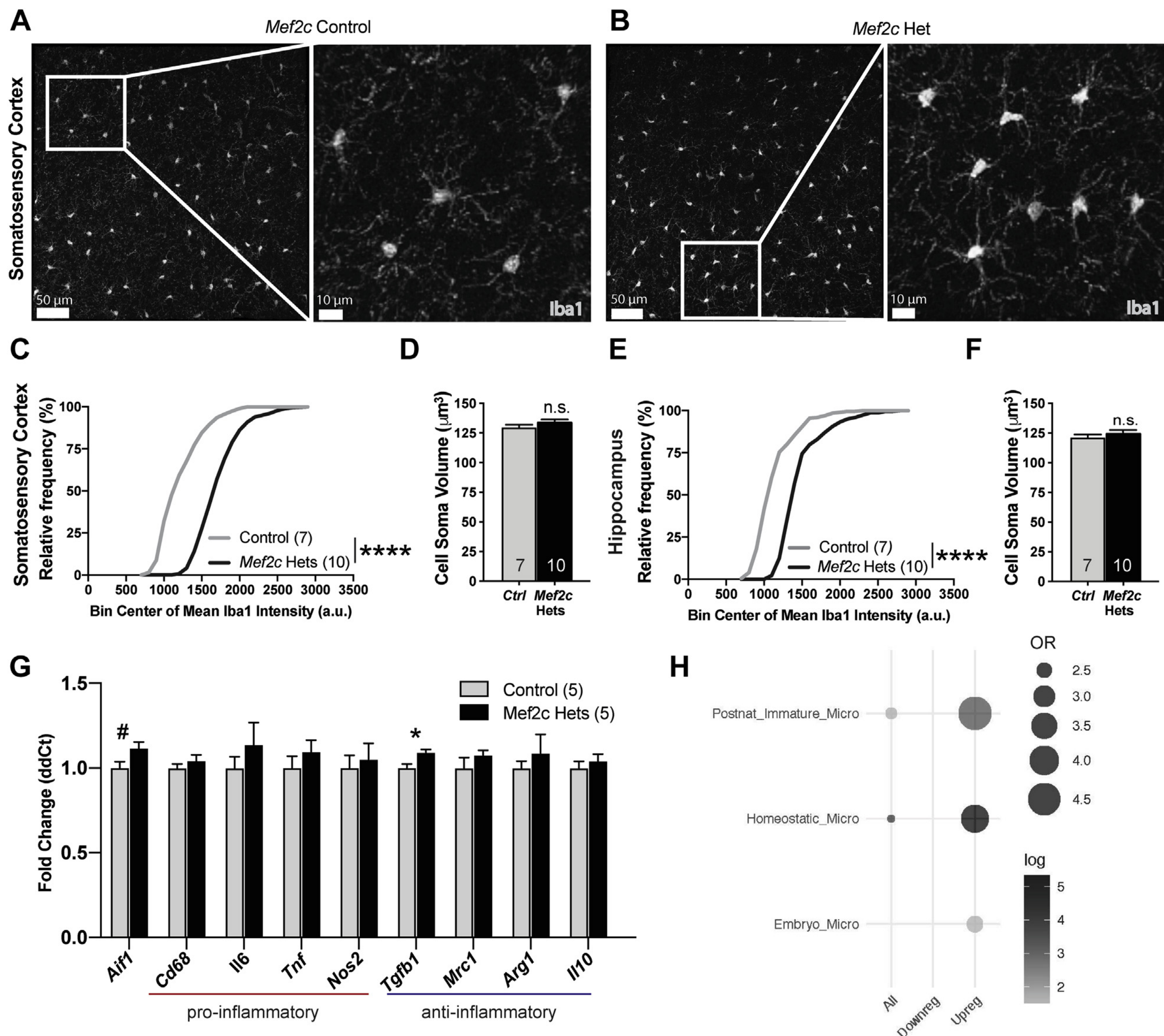

Figure 5. *Mef2c*$^{+/-}$ (*Mef2c*-Het) mice exhibit increased Iba1 expression levels. **(A, B)** Representative images of Iba1-positive microglia in the somatosensory cortex in control **(A)** and *Mef2c*-Het **(B)** mice. **(C, E)** *Mef2c*-Het mice have a right-shifted cumulative frequency distribution of mean Iba1 intensities in Iba1-positive cells (microglia) in the somatosensory cortex **(C)** and hippocampus **(E)** compared with control mice. Gray line represents distribution of control cells, and black line represents distribution of *Mef2c*-Het cells. **(D, F)** There is no difference in the cell soma volume of Iba1-positive cells (microglia) in the somatosensory cortex **(D)** or hippocampus **(F)** between control and *Mef2c*-Het mice. **(G)** Fold changes of genes associated with microglial activation in control and *Mef2c*-Het mice. **(H)** *Mef2c*-Het mice have an upregulation of genes expressed in postnatal immature, homeostatic, and embryonic microglia. Unless specified, data are reported as mean ± SEM. Statistical significance determined by Kolmogorov-Smirnov test **(C, E)**, unpaired 2-tailed nested *t* test **(D, F)**, or unpaired 2-tailed *t* test **(G)**. $\#p < .1$, $^{*}p < .05$, $^{****}p < .0001$. Sample sizes for each genotype are denoted on bars of or above each graph unless otherwise specified. Images **(A, B)** have contrast and brightness enhanced for ease of viewing. Images are modified equally for both genotypes. Downreg, downregulated; Embryo, embryonic; Micro, microglia; n.s., not significant; OR, odds ratio; Postnat, postnatal; Upreg, upregulated. Also see Figure S5 in Supplement 1.

manipulation of NMDA receptors rescued numerous phenotypes in the mutant mice (43). We also found that disruption of a single copy of *Mef2c* in microglia (*Mef2c*-cHet$^{Cx3Cr1\text{-}cre}$) (Figure 6I) or in excitatory pyramidal neurons (*Mef2c*-cHet$^{Emx1\text{-}cre}$) (Figure S6N, O in Supplement 1) is sufficient to reduce glutamatergic strength in layer 2/3 pyramidal neurons, suggesting that MEF2C functions in both neuronal and non-neuronal populations to regulate glutamatergic synaptic development and transmission.

As MCHS symptoms are reported predominantly from macrodeletions and microdeletions that disrupt *MEF2C* and multiple neighboring genes, we sought to identify possible loss-of-function *MEF2C* mutations within its protein coding region to better understand the relationship between symptoms and

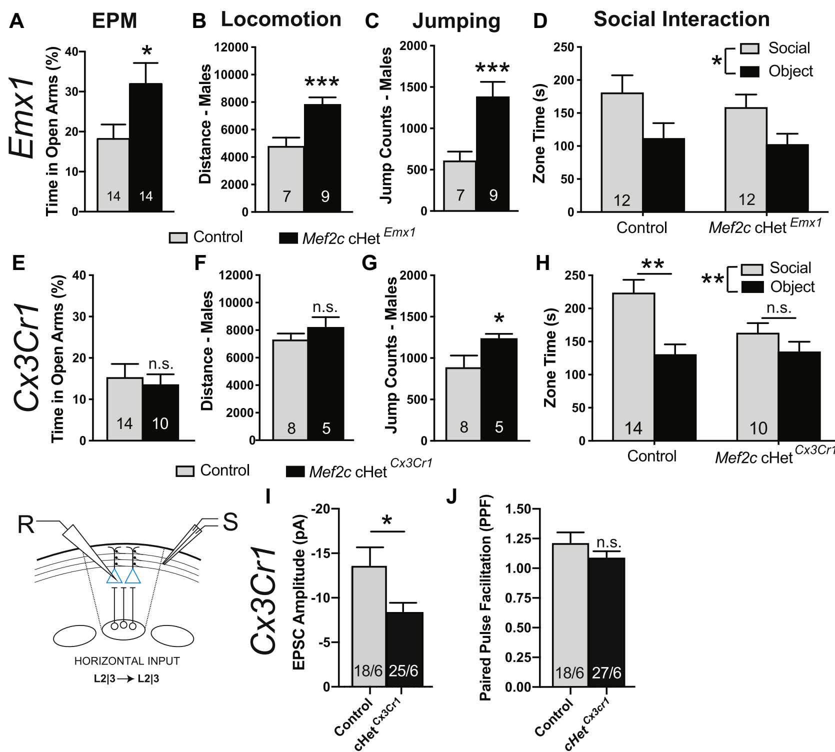

Figure 6. Cell type–selective phenotypes in *Mef2c* conditional heterozygous (*Mef2c*-cHet) mice. **(A–D)** Behaviors in *Mef2c* cHet^{Emx1} mice. **(A)** *Mef2c* cHet^{Emx1} mice spent more time on the open arms of the EPM. **(B, C)** Male *Mef2c*-cHet^{Emx1} mice were hyperactive **(B)** and showed increased jump counts **(C)**. **(D)** *Mef2c* cHet^{Emx1} mice had normal social interaction. **(E–H)** Behaviors in *Mef2c* cHet^{Cx3cr1} mice. **(E)** *Mef2c* cHet^{Cx3cr1} mice were similar to control mice in the EPM. **(F, G)** Male *Mef2c* cHet^{Cx3cr1} mice had normal activity **(F)** but showed increased jump counts **(G)** compared with control mice. **(H)** *Mef2c* cHet^{Cx3cr1} mice showed a lack of preference for interacting with a novel mouse (social) over the novel object. **(I, J)** *Mef2c* cHet^{Cx3cr1} mouse layer 2/3 pyramidal neurons had decreased evoked EPSC amplitude **(I)** without a change in PPF **(J)**. Data are reported as mean ± SEM. Statistical significance was determined by unpaired *t* test **(A–C, E–G, I–J)** or two-way analysis of variance **(D, H)**. $^*p < .05$, $^{**}p < .01$, $^{***}p < .005$. Number of animals **(A–H)** or cells/animals **(I–J)**, respectively, are reported in each graph. EPM, elevated plus maze; EPSC, excitatory postsynaptic current; n.s., not significant; PPF, paired-pulse facilitation; R, recording electrode; S, stimulating electrode. Also see Figure S6 in Supplement 1.

MEF2C. By comparing multiple new *MEF2C*-related mutations from individuals with developmental delay and other MCHS-associated symptoms, we observed that all of the missense mutations concentrated within the MEF2C DNA binding and dimerization domains (MADS/MEF2). All tested mutations dramatically reduced MEF2C DNA binding (Figure 1), suggesting loss-of-function mutant alleles; however, it remains possible that the mutated MEF2C proteins could influence cell function by titrating required MEF2C cofactors. In addition, multiple mutations produced a premature stop codon or a frameshift predicted to produce a truncated MEF2C lacking the C-terminal nuclear localization sequence, again presumably causing a nuclear loss-of-function or a dominant-interfering form of the protein.

Most individuals with MCHS (30–40) have robust physical and behavioral abnormalities, and some have abnormal brain magnetic resonance imaging scans, but no consistent effects are observed between subjects. Reported abnormalities are broad, including periventricular heterotopia, changes in corpus callosal thickness (thinned or thickened), ventricular changes (asymmetrical or enlarged), and changes in the gyral pattern of the cerebrum (30,33,34). *Mef2c*-Het mice did not have obvious changes in gross brain structures including cortical thickness (Figure S1E in Supplement 1), highlighting potential differences in brain development between humans and mice. Detailed analysis of *Mef2c*-Het brain structures will be important to determine if subtle morphological differences exist.

Developing and mature microglia play important roles in brain development, including synaptic phagocytosis (16,17). Microglia also mediate synapse patterning, neurogenesis, myelinogenesis, and cellular phagocytosis (18,20,24). MEF2C is expressed in both human and mouse microglia, and MEF2 proteins regulate microglia development (15). Microglia-enriched RNAs are dysregulated in human cortex from idiopathic ASD brains (41) and in the *Mef2c*-Het mouse cortex (Figure 4 and Figure S4 in Supplement 1), and we found that MEF2C hypofunction in microglia is sufficient to produce autism-like behaviors in mice (Figure 6G, H) and alter cortical glutamatergic signaling (Figure 6I, J). Surprisingly, despite a strong increase in the *Mef2c*-Het brain of the microglia cell type and activation marker Iba1, (Figure 5) as well as other microglia genes, including several complement genes (e.g., *C1qb*, *C1qc*, and *C4b*), osteopontin (*Spp1*), and cathepsin S (*Ctss*) (Figure 4C, D), we failed to detect a clear signature of basal neuroinflammation in *Mef2c*-Het brains (Figure 5G). Our findings suggest that loss of one *Mef2c* allele does not produce classic microglial activation, but rather that microglial development, function, and/or maturation might be perturbed. Of note, *Mef2c*-Het DEGs showed enrichment for a single-cell RNA-seq cluster of genes associated with embryonic and immature postnatal microglia, suggesting a possible delay in microglia maturation in *Mef2c*-Het mice. Future studies will be important to determine the precise roles of MEF2C in microglial development and function and whether *Mef2c* heterozygosity alters one or more of the numerous reported roles for microglia in brain development.

Taken together, our findings reveal that MEF2C hypofunction throughout development produces numerous complex changes in cortical synaptic transmission, gene expression, and behaviors reminiscent of MCHS and ASD. Specifically, the behaviors of *Mef2c*-Het mice are associated with robust, input-selective deficits in cortical excitatory

synaptic transmission and disruption of excitatory neuronal and microglial gene expression. Importantly, our cell type–selective manipulations strongly suggest that MEF2C contributes to neurotypical development through critical roles in both neuron and neuroimmune subpopulations, including forebrain excitatory neurons (Emx1-lineage) and microglia (CX3CR1-lineage). Understanding the role of MEF2C in these cell populations in the body are likely to provide important new insights into effective treatment strategies for symptoms of MCHS.

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AJH, CMB, AA, and CWC designed experiments, performed data analysis, and wrote the manuscript. HWM, DBE, and SAS collected MCHS patient data. AJH, CMB, KB, JYC, and AA performed behavior tests and analyzed data. SB and GK analyzed RNA-seq data. ET performed electrophysiology and data analysis. BMS and MDS performed dendritic spine morphology experiments. AJH, CMB, KB, JYC, and AT performed molecular/biochemical experiments and data analysis. AJH and CMB performed statistical analyses.

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ARTICLE INFORMATION

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REFERENCES

1. Assali A, Harrington AJ, Cowan CW (2019): Emerging roles for MEF2 in brain development and mental disorders. Curr Opin Neurobiol 59:49–58.
2. Harrington AJ, Raissi A, Rajkovich K, Berto S, Kumar J, Molinaro G, *et al.* (2016): MEF2C regulates cortical inhibitory and excitatory synapses and behaviors relevant to neurodevelopmental disorders. Elife 5:e20059.
3. Morrow EM, Yoo SY, Flavell SW, Kim TK, Lin Y, Hill RS, *et al.* (2008): Identifying autism loci and genes by tracing recent shared ancestry. Science 321:218–223.
4. Flavell SW, Kim TK, Gray JM, Harmin DA, Hemberg M, Hong EJ, *et al.* (2008): Genome-wide analysis of MEF2 transcriptional program reveals synaptic target genes and neuronal activity-dependent polyadenylation site selection. Neuron 60:1022–1038.
5. Flavell SW, Cowan CW, Kim TK, Greer PL, Lin Y, Paradis S, *et al.* (2006): Activity-dependent regulation of MEF2 transcription factors suppresses excitatory synapse number. Science 311:1008–1012.
6. Pfeiffer BE, Zang T, Wilkerson JR, Taniguchi M, Maksimova MA, Smith LN, *et al.* (2010): Fragile X mental retardation protein is required for synapse elimination by the activity-dependent transcription factor MEF2. Neuron 66:191–197.
7. Tsai NP, Wilkerson JR, Guo W, Maksimova MA, DeMartino GN, Cowan CW, *et al.* (2012): Multiple autism-linked genes mediate synapse elimination via proteasomal degradation of a synaptic scaffold PSD-95. Cell 151:1581–1594.
8. Zang T, Maksimova MA, Cowan CW, Bassel-Duby R, Olson EN, Huber KM (2013): Postsynaptic FMRP bidirectionally regulates excitatory synapses as a function of developmental age and MEF2 activity. Mol Cell Neurosci 56:39–49.
9. Barbosa AC, Kim MS, Ertunc M, Adachi M, Nelson ED, McAnally J, *et al.* (2008): MEF2C, a transcription factor that facilitates learning and memory by negative regulation of synapse numbers and function. Proc Natl Acad Sci U S A 105:9391–9396.
10. Li H, Radford JC, Ragusa MJ, Shea KL, McKercher SR, Zaremba JD, *et al.* (2008): Transcription factor MEF2C influences neural stem/progenitor cell differentiation and maturation in vivo. Proc Natl Acad Sci U S A 105:9397–9402.
11. Rajkovich KE, Loerwald KW, Hale CF, Hess CT, Gibson JR, Huber KM (2017): Experience-dependent and differential regulation of local and long-range excitatory neocortical circuits by postsynaptic Mef2c. Neuron 93:48–56.
12. Adachi M, Lin P-Y, Pranav H, Monteggia LM (2016): Postnatal loss of *Mef2c* results in dissociation of effects on synapse number and learning and memory. Biol Psychiatry 80:140–148.
13. Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, Keeffe S, *et al.* (2014): An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J Neurosci 34:11929–11947.
14. Deczkowska A, Matcovitch-Natan O, Tsitsou-Kampeli A, Ben-Hamo S, Dvir-Szternfeld R, Spinrad A, *et al.* (2017): Mef2C restrains microglial inflammatory response and is lost in brain ageing in an IFN-I-dependent manner. Nat Commun 8:717.
15. Gosselin D, Skola D, Coufal NG, Holtman IR, Schlachetzki JCM, Sajti E, *et al.* (2017): An environment-dependent transcriptional network specifies human microglia identity. Science 356, eaal3222.
16. Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, Yamasaki R, *et al.* (2012): Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. Neuron 74:691–705.
17. Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, *et al.* (2011): Synaptic pruning by microglia is necessary for normal brain development. Science 333:1456.
18. Zhan Y, Paolicelli RC, Sforazzini F, Weinhard L, Bolasco G, Pagani F, *et al.* (2014): Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. Nat Neurosci 17:400–406.
19. Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, Nouri N, *et al.* (2007): The classical complement cascade mediates CNS synapse elimination. Cell 131:1164–1178.
20. Parkhurst CN, Yang G, Ninan I, Savas JN, Yates JR 3rd, Lafaille JJ, *et al.* (2013): Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. Cell 155:1596–1609.
21. Pont-Lezica L, Beumer W, Colasse S, Drexhage H, Versnel M, Bessis A (2014): Microglia shape corpus callosum axon tract fasciculation: Functional impact of prenatal inflammation. Eur J Neurosci 39:1551–1557.
22. Hagemeyer N, Hanft KM, Akriditou MA, Unger N, Park ES, Stanley ER, *et al.* (2017): Microglia contribute to normal myelinogenesis and to oligodendrocyte progenitor maintenance during adulthood. Acta Neuropathol 134:441–458.

23. Shigemoto-Mogami Y, Hoshikawa K, Goldman JE, Sekino Y, Sato K (2014): Microglia enhance neurogenesis and oligodendrogenesis in the early postnatal subventricular zone. J Neurosci 34:2231.
24. Sierra A, Encinas JM, Deudero JJP, Chancey JH, Enikolopov G, Overstreet-Wadiche LS, *et al.* (2010): Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. Cell Stem Cell 7:483–495.
25. Li Q, Barres BA (2018): Microglia and macrophages in brain homeostasis and disease. Nat Rev Immunol 18:225–242.
26. Schafer DP, Heller CT, Gunner G, Heller M, Gordon C, Hammond T, *et al.* (2016): Microglia contribute to circuit defects in Mecp2 null mice independent of microglia-specific loss of Mecp2 expression. Elife 5:e15224.
27. Derecki NC, Cronk JC, Lu Z, Xu E, Abbott SB, Guyenet PG, *et al.* (2012): Wild-type microglia arrest pathology in a mouse model of Rett syndrome. Nature 484:105–109.
28. Horiuchi M, Smith L, Maezawa I, Jin LW (2017): CX3CR1 ablation ameliorates motor and respiratory dysfunctions and improves survival of a Rett syndrome mouse model. Brain Behav Immun 60:106–116.
29. Wang J, Wegener JE, Huang TW, Sripathy S, De Jesus-Cortes H, Xu P, *et al.* (2015): Wild-type microglia do not reverse pathology in mouse models of Rett syndrome. Nature 521:E1.
30. Le Meur N, Holder-Espinasse M, Jaillard S, Goldenberg A, Joriot S, Amati-Bonneau P, *et al.* (2010): MEF2C haploinsufficiency caused by either microdeletion of the 5q14.3 region or mutation is responsible for severe mental retardation with stereotypic movements, epilepsy and/or cerebral malformations. J Med Genet 47:22–29.
31. Zweier M, Gregor A, Zweier C, Engels H, Sticht H, Wohlleber E, *et al.* (2010): Mutations in MEF2C from the 5q14.3q15 microdeletion syndrome region are a frequent cause of severe mental retardation and diminish MECP2 and CDKL5 expression. Hum Mutat 31:722–733.
32. Vrecar I, Innes J, Jones EA, Kingston H, Reardon W, Kerr B, *et al.* (2017): Further clinical delineation of the MEF2C haploinsufficiency syndrome: Report on new cases and literature review of severe neurodevelopmental disorders presenting with seizures, absent speech, and involuntary movements. J Pediatr Gene 6:129–141.
33. Paciorkowski AR, Traylor RN, Rosenfeld JA, Hoover JM, Harris CJ, Winter S, *et al.* (2013): MEF2C haploinsufficiency features consistent hyperkinesis, variable epilepsy, and has a role in dorsal and ventral neuronal developmental pathways. Neurogenetics 14:99–111.
34. Zweier M, Rauch A (2012): The MEF2C-related and 5q14.3q15 microdeletion syndrome. Mol Syndromol 2:164–170.
35. Mikhail FM, Lose EJ, Robin NH, Descartes MD, Rutledge KD, Rutledge SL, *et al.* (2011): Clinically relevant single gene or intragenic deletions encompassing critical neurodevelopmental genes in patients with developmental delay, mental retardation, and/or autism spectrum disorders. Am J Med Genet A 155A:2386–2396.
36. Novara F, Beri S, Giorda R, Ortibus E, Nageshappa S, Darra F, *et al.* (2010): Refining the phenotype associated with MEF2C haploinsufficiency. Clin Genet 78:471–477.
37. Engels H, Wohlleber E, Zink A, Hoyer J, Ludwig KU, Brockschmidt FF, *et al.* (2009): A novel microdeletion syndrome involving 5q14.3-q15: Clinical and molecular cytogenetic characterization of three patients. Eur J Hum Genet 17:1592–1599.
38. Berland S, Houge G (2010): Late-onset gain of skills and peculiar jugular pit in an 11-year-old girl with 5q14.3 microdeletion including MEF2C. Clin Dysmorphol 19:222–224.
39. Bienvenu T, Diebold B, Chelly J, Isidor B (2013): Refining the phenotype associated with MEF2C point mutations. Neurogenetics 14:71–75.
40. Tonk V, Kyhm JH, Gibson CE, Wilson GN (2011): Interstitial deletion 5q14.3q21.3 with MEF2C haploinsufficiency and mild phenotype: When more is less. Am J Med Genet A 155A:1437–1441.
41. Velmeshev D, Schirmer L, Jung D, Haeussler M, Perez Y, Mayer S, *et al.* (2019): Single-cell genomics identifies cell type-specific molecular changes in autism. Science 364:685–689.
42. Gorski JA, Talley T, Qiu M, Puelles L, Rubenstein JLR, Jones KR (2002): Cortical excitatory neurons and glia, but not GABAergic neurons, are produced in the Emx1-expressing lineage. J Neurosci 22:6309–6314.
43. Tu S, Akhtar MW, Escorihuela RM, Amador-Arjona A, Swarup V, Parker J, *et al.* (2017): NitroSynapsin therapy for a mouse MEF2C haploinsufficiency model of human autism. Nat Commun 8:1488.
44. Fioravante D, Regehr WG (2011): Short-term forms of presynaptic plasticity. Curr Opin Neurobiol 21:269–274.
45. Gandal MJ, Zhang P, Hadjimichael E, Walker RL, Chen C, Liu S, *et al.* (2018): Transcriptome-wide isoform-level dysregulation in ASD, schizophrenia, and bipolar disorder. Science 362:eaat8127.
46. Saunders A, Macosko EZ, Wysoker A, Goldman M, Krienen FM, de Rivera H, *et al.* (2018): Molecular diversity and specializations among the cells of the adult mouse brain. Cell 174:1015–1030.e1016.
47. Telese F, Ma Q, Perez PM, Notani D, Oh S, Li W, *et al.* (2015): LRP8-reelin-regulated neuronal enhancer signature underlying learning and memory formation. Neuron 86:696–710.
48. Ito D, Imai Y, Ohsawa K, Nakajima K, Fukuuchi Y, Kohsaka S (1998): Microglia-specific localisation of a novel calcium binding protein. Iba1. Mol Brain Res 57:1–9.
49. Ito D, Tanaka K, Suzuki S, Dembo T, Fukuuchi Y (2001): Enhanced expression of Iba1, ionized calcium-binding adapter molecule 1, after transient focal cerebral ischemia in rat brain. Stroke 32:1208–1215.
50. Bialas AR, Stevens B (2013): TGF-β signaling regulates neuronal C1q expression and developmental synaptic refinement. Nat Neurosci 16:1773.
51. Odell D, Maciulis A, Cutler A, Warren L, McMahon WM, Coon H, *et al.* (2005): Confirmation of the association of the C4B null allele in autism. Hum Immunol 66:140–145.
52. Sekar A, Bialas AR, de Rivera H, Davis A, Hammond TR, Kamitaki N, *et al.* (2016): Schizophrenia risk from complex variation of complement component 4. Nature 530:177.
53. Kamath SP, Chen AI (2019): Myocyte enhancer factor 2c regulates dendritic complexity and connectivity of cerebellar purkinje cells. Mol Neurobiol 56:4102–4119.
54. Mayer C, Hafemeister C, Bandler RC, Machold R, Batista Brito R, Jaglin X, *et al.* (2018): Developmental diversification of cortical inhibitory interneurons. Nature 555:457–462.
55. Li Q, Cheng Z, Zhou L, Darmanis S, Neff NF, Okamoto J, *et al.* (2019): Developmental heterogeneity of microglia and brain myeloid cells revealed by deep single-cell RNA sequencing. Neuron 101:207–223.e210.
56. Hammond TR, Dufort C, Dissing-Olesen L, Giera S, Young A, Wysoker A, *et al.* (2019): Single-cell RNA sequencing of microglia throughout the mouse lifespan and in the injured brain reveals complex cell-state changes. Immunity 50:253–271.e256.
57. McKinsey TA, Zhang CL, Olson EN (2002): MEF2: A calcium-dependent regulator of cell division, differentiation and death. Trends Biochem Sci 27:40–47.
58. Ataman B, Boulting GL, Harmin DA, Yang MG, Baker-Salisbury M, Yap EL, *et al.* (2016): Evolution of Osteocrin as an activity-regulated factor in the primate brain. Nature 539:242–247.
59. Hoogland IC, Houbolt C, van Westerloo DJ, van Gool WA, van de Beek D (2015): Systemic inflammation and microglial activation: Systematic review of animal experiments. J Neuroinflammation 12:114.
60. Wright-Jin EC, Gutmann DH (2019): Microglia as dynamic cellular mediators of brain function. Trends Mol Med 25:967–979.
61. Lenz KM, Nugent BM, Haliyur R, McCarthy MM (2013): Microglia are essential to masculinization of brain and behavior. J Neurosci 33:2761.
62. Smith CJ, Bilbo SD (2019): Microglia sculpt sex differences in social behavior. Neuron 102:275–277.
63. Villa A, Gelosa P, Castiglioni L, Cimino M, Rizzi N, Pepe G, *et al.* (2018): Sex-specific features of microglia from adult mice. Cell Rep 23:3501–3511.
64. Lyons MR, Schwarz CM, West AE (2012): Members of the myocyte enhancer factor 2 transcription factor family differentially regulate Bdnf transcription in response to neuronal depolarization. J Neurosci 32:12780–12785.
65. Kang J, Gocke CB, Yu H (2006): Phosphorylation-facilitated sumoylation of MEF2C negatively regulates its transcriptional activity. BMC Biochem 7:5.
66. Pulipparacharuvil S, Renthal W, Hale CF, Taniguchi M, Xiao G, Kumar A, *et al.* (2008): Cocaine regulates MEF2 to control synaptic and behavioral plasticity. Neuron 59:621–633.