SUPPLEMENTAL METHODS

Human postmortem tissue

Human tissue processing was approved by the Institutional Review Board of the participating institutions. Specimens from rostral dorsolateral PFC from 17 subjects diagnosed with SCZ, matched to 17 controls of similar age, gender, autolysis time and tissue pH were included in this study (Supplemental Table 3). Procedures for tissue collection, neuropathologic examination (to rule out degenerative and neurological disease), and diagnosing SCZ using DSM IV-based diagnostic criteria were described previously 1,2.

Differentiation of hiPSC-derived neurons

All patient (2 male, 1 female) and control (2 male, 1 female) hiPSCs were reprogrammed with tetracycline-inducible lentiviruses from fibroblasts obtained from ATCC (control: BJ(CRL-2522)) or Coriell (controls: GM03440, GM03651; SCZ: GM02038, GM01792, GM01835); all available clinical information is described3, (Topol et al. 2015). Control and SCZ forebrain neural progenitor cells (NPCs) were differentiated and validated from hiPSCs3, and grown on Matrigel-coated plates in NPC media (DMEM/F12, 1x N2, 1x B27-RA (Invitrogen), 1 µg/ml Laminin (Invitrogen) and 20 ng/ml FGF2 (Invitrogen). For neuronal differentiations, NPCs were dissociated with Accutase (Millipore), plated onto poly-ornithine/Laminin-coated plates in neural differentiation media (DMEM/F12, 1x N2, 1X B27-RA, 20 ng/ml BDNF (Peprotech), 20 ng/ml GDNF (Peprotech), 1 mm dibutyrl-cyclicAMP (Sigma), 200 nm ascorbic acid (Sigma)) and differentiated for 6 weeks.

ChIP-seq (Chromatin Immunoprecipitation sequencing)

Procedures for extraction and sorting of NeuN+ neuronal nuclei from the cortical gray matter, and subsequent chromatin immunoprecipitation with anti-H3K4me3 antibody and ChIP-seq library preparation were recently described 4-7. To avoid batch effects, cases were processed together with controls. ChIP-seq data from a subset of the controls have been presented in previous studies.
(Supplemental Table 3). Cross-immunoreactivity of the anti-H3K4me3 antibody (Cell Signaling, 9751BC) with other histone methylation forms, including mono- and di-H3K4 (H3K4me1/2) was controlled by dot blots and synthetic blocking peptides as described. ChIPseq data were aligned to HG19 using Bowtie2 allowing for two mismatches, duplicates were removed using SAMtools, and peaks called using MACS using MACS version 2.0.10.20131216 which uses a dynamic Poisson distribution with Benjamini-Hochberg false discovery correction p<0.01. To maximize the input for the intended regulatory motif analyses further downstream, we did not normalize samples to an input sample, resulting in a total of 86,450 peaks in controls and 78,852 peaks in disease cases, totaling 137,373 unique peaks. Disease-related peaks were defined as >1.5-fold difference between controls and schizophrenia cases, with a cumulative poisson P < 0.0001 and functionally annotated using hypergeometric optimization of the motif enrichment analysis suite (HOMER) (Supplemental Tables 5, 6). All ChIP-seq data will be deposited into GEO/NCBI.

**MEF2C knockdown by DsiRNA**

MEF2C expression was knocked down in HEK293 cells using 20 uM TriFecta predesigned diser-substrate siRNA (DsiRNA) against human MEF2C (Integrated DNA Technologies). Cells were transfected with MEF2C (MEF2C.13.1, MEF2C.13.2, MEF2C.13.3) N=3 or control (control DsiRNA negative control, DsiRNA TYE 563 transfection control, or DsiRNA HPRT positive control) siRNA N=3. TriFECTa guarantees a 70% knockdown from a 90% transfection. H3K4me3 chromatin immunoprecipitation (ChIP) quantitative PCR (ChIP-PCR) in knock-down and control cells was performed similar to the ChIP-seq studies described above, but sequence-specific PCR-based H3K4me3 quantification using Power SYBR Green (Life Technologies).

**Gene Ontology Analysis**

Gene ontology enrichment analysis was performed for gene ontology, drug response, and phenotype groups for SCZ-up and SCZ-down peaks using WebGestalt. Filters to call a group
included (i) minimum of 10 genes called and (ii) hypergeometric test with Bonferroni adjusted p<0.001 (*Supplemental Table 9*).

**Superenhancer Enrichment**

Superenhancer enrichment among the overall pool of disease-affected H3K4me3 sequences was determined against the total set of enhancers among the disease-affected H3K4me3 tag sequences as background, and the proportion of dysregulated H3K4me3 sequences versus all H3K4me3 sequence, using hypergeometric test p<0.05 (*Supplemental Tables 10,11*).

**Motif analyses**

Motif binding sites were predicted from the data using RSAT *de novo* motif finding software, which determines over-represented motifs using a right-tailed binomial significance test corrected by the total number of words predicted (evalue) and reported as a weight significance value \(-\log_{10}(\text{evalue})\). All motifs with a weight > 7.5 are reported\(^{12}\). Significant motifs associated with significant SCZ-up sequences were assessed using RSAT *de novo* motifs by comparing significantly up-regulated H3K4me3 sequence to all total H3K4me3 sequence. Similarly, significant motifs associated with significant SCZ-down sequence was used by comparing all downregulated H3K4me3 sequence to all H3K4me3 sequence (*Supplemental Tables 13, 14*). Significant motifs within the genetic risk map for schizophrenia were determined, by using the 108 risk regions from the Psychiatric Genomics Consortium\(^{13}\), with a 2kb window centered on the single nucleotide polymorphism with the highest P-value using the human genome as background (*Supplemental Table 1*). Furthermore, using the total set of N=342 brain-specific super-enhancers\(^{14}\) as background, *de novo* motifs were predicted from disease-affected superenhancers, with enrichment in the SCZ-up peaks defined by RSAT *de novo* motifs, P<0.05, right-tailed binomial significance test, corrected by the total number of motifs predicted=evalue, and reported as weight significance \(-\log_{10}(\text{evalue})>7.5\) (*Supplemental Table 12*)\(^{12}\). Motifs were clustered using a neighbor joining tree with the canonical MEF2C motif using Clustal Omega\(^{15,16}\).
Gel shift assays

100-300 mg cortical gray matter from PFC was dounced in Buffer A (300mM sucrose, 2mM magnesium acetate, 3mM calcium chloride, 10mN Tris.Cl (pH 8.0), 0.1% Triton X-100, 0.5 mM DTT), then mixed with an equal volume of Buffer B (2M sucrose, 5mM magensium acetate, 10mM Tris.CL (pH 8.0), 0.5 mM DTT), overlaid on 6 mL of Buffer B and ultracentrifuged (14,500 g, 30 min, 4°C). Nuclear extracts were prepared by suspending the pelleted nuclei in 2 volumes of Buffer C (25mM HEPES containing 25% glycerol, 0.42M NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM DTT and protease inhibitor cocktail (Sigma). Native gel electrophoretic mobility-shift assays with 32P end-labeled 27bp DNA probes (1X or 50 nM) and nuclear extracts were run for three adult PFC specimens (50† g/sample), after 20 minute incubation at 22°C in binding buffer containing 10mM Tris-HCl (pH7.5), 50mM NaCl, 0.5mM DTT. To control for specificity, unlabeled (cold) probe in 100-fold excess over 32P end-labeled DNA probes (Supplemental Table 15). All gels were run at 150 V for 150 min at 4°C in the presence of 6% glycerol, dried for 2 hours at 80°C and exposed to X-ray film overnight at -70°C.

CommonMind Consortium and Brain Cloud

Gene expression data were obtained from publically accessible RNAseq datasets of the CommonMind Consortium (CMC), including PFC transcriptomes from N=256 schizophrenia cases and N=281 controls (www.synapse.com/cm; freeze1.v9.). Variance modeling at the observational level (VOOM) of individual observations was to estimate the mean-variance relationship of the log-counts and generate a precision weight for each observation to unlock linear model analysis for the RNA-seq data17. Furthermore, RNA-seq data underwent surrogate variable analysis (SVA) adjusted for batch affects. Data was downloaded from CMC as VOOM normalized with SVA adjustment. We asked if TFs with a de novo predicted motif from the PGC2 data were dysregulated in the CMC dataset using unpaired t test with Benjamini-Hochberg False Discovery Rate correction 18. Developmental trajectories of specific transcripts were downloaded from the BrainCloud microarray dataset, including N= 272 control specimens from PFC19 (Figure 1C).
Animal work

All animal work was approved by the Institutional Animal Care and Use Committee of Icahn School of Medicine at Mount Sinai.

Genetic engineering in PFC

Adeno-associated virus, serotype 8 (AAV) (UNC Vector Core) for expression of mouse 1399 bp Mef2c green fluorescent protein (GFP) (Origene MG226868, NM_001170537.1) under the control of the 486 bp human SYNAPSIN I promoter was bilaterally injected into the PFC of ten P27 wild-type C57/6BL mice using stereotactic coordinates -1.5 mm anterior/posterior, 0.4 mm medial/lateral, and 1.5 dorsal/ventral. As a control Cre-GFP under the control of the human SYNAPSIN I promoter was bilaterally injected into the PFC of ten age-matched C57/6BL mice. 1 µl of virus for each hemisphere (~4.7 X 10^9 genomic copies) was injected at a rate of 0.25 µl /min using a Hamilton syringe, micro pump, and stereotactic frame (Stoelting). In a separate cohort of mice, full length mouse Mef2c fused to the cDNA of the bacterial DNA adenine methyltransferase (Dam) was expressed at P90 via injection of a herpes simplex virus (HSV) driven by the IE 4/5 promoter into the PFC of eight male wild-type C57/6BL mice. Finally, spine density measurements were conducted in a third cohort of mice, conditional transgenic line TLG498, Thy1-STOP-GFP-F20 expressing membrane-bound GFP-F. Mice were injected with a mixture of AAV8-GFP-Cre/AAV8-Cre-mCherry (1.0/0.1uL, respectively) for control, or AAV8-Mef2cGFP/AAV8-Cre-mCherry (1.0/0.1uL, respectively) (n=3 per group). Littermates were randomly assigned to each group. The sample sizes employed were similar to those in previous studies reporting alterations in working memory performance after genetic engineering in PFC^{21,22}.

Antipsychotic Treatment

Experiments were performed on adult (8–20 weeks old) male mice on a C57BL/6 background. Animals were housed at 12 h light/dark cycle at 23°C with food and water ad libitum. Mice were
treated chronically (21 days) with clozapine (R&D Systems; 10 mg/kg) N=5 or vehicle N=5, and sacrificed one day after the last injection, bilateral frontal cortex was dissected and frozen at –80ºC. Protein from cortical gray matter from PFC was obtained by douncing in Buffer A (300mM sucrose, 2mM magnesium acetate, 3mM calcium chloride, 10mN Tris.Cl (pH 8.0), 0.1% Triton X-100, 0.5 mM DTT). A western blot for MEF2C (sc-13268 X, Santa Cruz, CA) and control nuclear histone 3 (H3, 4499P, Cell Signaling, Danvers, MA) was obtained using a 1:1000 concentration of MEF2C and 1:1000 concentration of H3, and a 1:5000 dilution of anti-rabbit IgG (7074, Cell Signaling, Danvers, MA).

**Behavioral Studies**

*Eight-arm radial maze.* The maze consisted of eight arms (7.5 X 35 cm, 17.5 cm high walls) assembled radially around a circular starting platform. Mice were placed onto the starting platform and were free to enter the arms. Mice were tested until all eight arms were visited once. Each repeat entry was counted as an error.

*Open Field.* The open field chamber consisted of a white Plexiglas box (40 x 40 cm, 30 cm high), illuminated with bright white light (350 lux). Mice were placed individually into the box for 20 minutes. Time spent in an imaginary center square (15 x 15 cm) of the open field was recorded using Fusion 5.0 Superflex system.

*Light-dark box.* An open field chamber described above was used for this test. A black plastic box (20 x 40 cm, 30 cm high) was placed in the field. The box had a black plastic lid and an opening on the bottom (5 cm diameter) to allow the mouse to enter the surrounding arena. Mice were placed in the black box with the lid closed and allowed to move freely ten minutes. Behaviors (distance moved and stereotypy) were recorded using Fusion 5.0 Superflex system.

*Object recognition.* An open field chamber described above was used for all object recognition tests. On day one mouse were allowed to explore the empty chamber for ten minutes (habituation). On day two white blocks were introduced and the time each mouse spent interacting with his nose with the
objects were recorded for ten minutes (same object). On day three one white block and one purple circular vial were placed in the same positions of the objects from day two. The time each mouse spent interacting with his nose with the objects was recorded for ten minutes (novel object). Interactions were recorded using the video-based EthoVision tracking system (Noldus).

*Tail suspension.* Mice were suspended by their tails using duct tape from a 45 cm height for five minutes. Video was recorded and time spent immobile was measured.

*Forced swim.* Mice were placed in five liter Kimax beakers filled with three liters of water and allowed to swim five minutes. Video was recorded and total immobility time was measured.

*Elevated Plus Maze.* Mice were placed in the center of a plus maze elevated 65 cm from the ground with two 35 cm open and two 35 cm closed arms with a 5 x 5 cm center. Time spent in open and closed arms was video-tracked for ten minutes using the EthoVision tracking system (Noldus).

*Motor skills / rotarod.* Rotarod (ENV-575M, Med Associated) was used to measure motor coordination in mice. The rotarod was set up with a slow acceleration mode, in which the rotation speed increased from 3 to 40 rpm in five minutes. Mice were trained for ten consecutive five minute trials with a five minute intertrial interval rest. Twenty-four hours later, mice were retested for three trails at five minute intervals. Locomotor coordination performance was defined as mean latency to fall off.

*Social Novelty.* A three chamber apparatus was used to determine social novelty preference. Test mice were habituated to the center chamber for 10 minutes and the full chamber for 10 minutes the day before testing. On testing day mice were habituated to the center chamber for 5 minutes and the full chamber for 5 minutes. At the start of the test a stimulus mouse was placed in a coral in one of the chambers and an object was placed in a coral of the other chambers and sociability was tested for 10 minutes. During the next phase a novel mouse was introduced in place of the novel object and social novelty was tested for 10 minutes. A subset of behavioral studies were conducted by blind observers.
Dendritic Spine Analysis and Imaging

Imaging was performed at least 4 weeks after surgery. Mice were anesthetized with a terminal intraperitoneal injection of a ketamine/xylazine mixture (IP: 200 and 30 mg/kg, respectively). Intracardial perfusion was performed with 100ml of 10% sucrose followed by 200ml of 4% paraformaldehyde in PBS. Brains were removed and placed in 4% paraformaldehyde overnight at 4°C, followed by incubation in 30% sucrose until isotonic. Brains were cut on a freezing microtome (Leica SM2010 R) into coronal sections (40 µm) then mounted using DAPI Fluoromount-G (0100-20, SouthernBiotech).

All spines were on secondary and tertiary basal dendrites within 150um of the soma of Layer III pyramidal neurons. Distance from soma was measured using the scale bar function of ZEN 2012 software (Carl Zeiss). At least one dendrite was analyzed per neuron with a minimum of 5 neurons analyzed per hemisphere. To confirm that spines were captured from desired regions, images at x10 were analyzed. If a dendrite bifurcated, only the longest branch was counted. The minimum dendritic length quantified was 20um. If more than one dendrite was analyzed from a single neuron, the data was averaged. Images were taken with a Carl Zeiss CLSM780 microscope (laser λ = 488 nm).

Deconvolution and image processing were done with AutoDeblur (Media Cybernetics) and ImageJ software (NIH), respectively. NeuronStudio was used for semi-automated 3D spine detection, quantification and classification. Spines were classified as one of three types (thin, mushroom, stubby) based on head-to-neck diameter and length ratios. A thin spine was defined as having a maximum head diameter of <0.6 µm, and a total length that exceeded twice the head diameter. Mushroom and stubby spines were detected by setting the minimum head diameter to 0.6 µm23, 24. Stubby spines were differentiated from mushroom by the absence of a neck. All protrusions ≤3 µm from the dendrite, and satisfying the defined parameters on NeuronStudio, were counted.

AAV-Mef2c-GFP Nuclei Sorting and qPCR
AAV injected mouse PFC was isolated from each mouse (N=7/group). Nuclei were isolated via ultracentrifugation and neurons were stained using Alexa 544 NeuN+. Nuclei were sorted based on NeuN and GFP and frozen in Qiazol. NeuN+/GFP+ nuclei were used for qPCR. Nuclei were lysed in Qiazol and RNA isolated via an equal volume of chloroform followed by isopropanol precipitation. cDNA was made using the SuperVilo kit and transcripts were pre-amplified using the ssoPreAmp kit. qPCR was performed using the Power Sybr Green kit (ABI). qPCR primers were designed at Mef2c (Supplemental Table 15). Genes within 2000 base pairs of the canonical Mef2c motif, motif 2, and a control motif for human and mouse samples. qPCR was performed using Power Sybr Green (ABI) and fold change and significance analyzed between NeuN+/GFP+ versus NeuN+/GFP- nuclei.

**HSV-Mef2c-Dam PCR**

HSV injected left mouse PFC was assayed for Mef2c binding using adenine methylation quantification. PFC was homogenized, lysed, and proteinase K treated overnight. Phenol:choloform clean-up was performed and DNA was precipitated overnight using one tenth the volume sodium acetate pH 5.4 and two times the volume 100% ethanol. DNA was incubated DpnII which selectively cuts unmethylated but not methylated GATC tetramers. Predicted adenine methylation sites at Mef2c binding motifs were amplified by primers spanning the GATC site and run on 2% agarose gels (Supplemental Table 15). HSV-Mef2c-Dam mice were compared to wild type mice (N=5/group).

**Chromosome Conformation Capture (3C) Assays**

Chromosome conformation capture (3C) assays were performed using 200 mg postmortem PFC tissue (Supplementary Table 15) or 1.2 million neurons differentiated from hiPSCs. PFC tissue N=7 disease cases and N=4 control brains) or IPSC-derived neurons (from donors other than our postmortem brains) N=3/group, were homogenized and crosslinked for 10 minutes at 25°C in 1% formaldehyde, 1X protease inhibitor (Sigma) and 2 mL lysis buffer (10 mmol/L Tris hydrogen chloride pH 8.0 / 10 mmol/L sodium chloride / 0.2% IPEGAL CA-630 (Sigma Aldrich, St. Louis, Missouri)). A final concentration of 0.125 mol/L glycine was added for 10 minutes to stop crosslinking. The
homogenate was incubated for another 25 minutes at 4°C. Cells were lysed by pipetting > 50 times and spun at 5000 rpm. Supernatant was removed and the pellet was washed twice with 1X New England Buffer 4 (NEB4) (New England Biolabs, Boston, Massachusetts). Samples were resuspended in 200 µl of 1X NEB4 and divided into four 50 µl aliquots. An additional 312 µl of 1X NEB4 and 38 µl of 1% SDS were added to each aliquot and the samples were incubated at 65°C for 10 minutes. To quench the SDS 10% of Triton X-100 was added to each sample and the samples were digested with \textit{HindIII}-HF (NEB) at 37°C overnight with vigorous shaking.

\textit{HindIII}-HF was inactivated by the addition 86 µl of 10% SDS incubated for 30 minutes at 65°C. Ligation mixture (7.61 ml) was added to each sample. The ligation mixture consisted of 745 µl of 10% Triton X-100, 745 µl of 10X ligation buffer (1 M Tris HCl pH 7.5, 1 M MgCl\textsubscript{2}, 1 M DTT dithiothreitol (Bio-Rad)), 80 µl of 10 mg/ml bovine serum albumin (NEB), 80 µl of 100 mM ATP (Sigma) and 5960 µl of autoclaved water. 50 µl of T4 DNA ligase (1 U/µl, Invitrogen) was added to three aliquots and one sample was used as a no ligase control. Ligation proceeded for five hours at 16°C and samples were reverse cross-linked at 65°C overnight with 50 uL of 10 mg/ml of proteinase K (Sigma). For improved ligated DNA recovery another 50 µl of proteinase K was added and incubated at 65°C for two hours. DNA was extracted with phenol (pH 8.0, Fisher), and phenol-chloroform (1:1) (pH 8, Fisher). DNA was precipitated using 1/10 the volume of 3M sodium acetate (pH. 5.4) and 2.5 the volume of ice-cold ethanol overnight. The samples were centrifuged at 8000 RPM for 30 minutes and washed with 70% ethanol. The final DNA pellet was dissolved in 1X TE buffer (pH 8.0). Phenol and phenol-chloroform extraction and ethanol precipitation was repeated. The final 3C library was washed five times with 70% ethanol. Ligase and no ligase reactions were dissolved in 100 µl and 33 µl of TE buffer (pH 8.0) respectively\textsuperscript{25}. Ligase and no ligases libraries alone were run on a 2% agarose gel to visualize ligation efficiency. Samples ran at a higher molecular weight after ligation, indicated by an upward shift on the gels\textsuperscript{26, 27}.

Physical looping interactions were quantified using PCR. Primers were designed less than 120 bp from a \textit{HindIII} restriction site (Table S12). The PCR products were resolved on a 2% agarose gel
and the level of interaction between two primers was measured semiquantitatively using band intensities normalized with the background (raw 3C interaction) with ImageJ. Library input was adjusted for each library according the interaction between two neighboring primers (<5000 bp apart) and two distant primers (<30,000 apart) control primer 1 (CCTGGATCATCAGACAGAACTAAAGCTCTT) located at chr13:99113854 and control primer 2 (CTTCAACTGAAAACACACGACAGGAAGAA) located at chr13:99109553 (Supplemental Table 15). Specificity of 3C PCR products was confirmed by sequencing. Negative controls included 3C assays exactly as described above but with no ligase added.

Statistical tests were done under proper checks for normalization, and if indicated, non-parametric tests were applied.

References

1. Akbarian S, Ruehl MG, Bliven E, Luiz LA, Peranelli AC, Baker SP et al. Chromatin alterations associated with down-regulated metabolic gene expression in the prefrontal cortex of subjects with schizophrenia. Arch Gen Psychiatry 2005; 62(8): 829-840.

2. Huang HS, Matevossian A, Whittle C, Kim SY, Schumacher A, Baker SP et al. Prefrontal dysfunction in schizophrenia involves mixed-lineage leukemia 1-regulated histone methylation at GABAergic gene promoters. The Journal of neuroscience: the official journal of the Society for Neuroscience 2007; 27(42): 11254-11262.

3. Brennand KJ, Simone A, Jou J, Gelboin-Burkhart C, Tran N, Sangar S et al. Modelling schizophrenia using human induced pluripotent stem cells. Nature 2011; 473(7346): 221-225.

4. Cheung I, Shulha HP, Jiang Y, Matevossian A, Wang J, Weng Z et al. Developmental regulation and individual differences of neuronal H3K4me3 epigenomes in the prefrontal cortex. Proceedings of the National Academy of Sciences of the United States of America 2010; 107(19): 8824-8829.

5. Connor C, Cheung I, Simon A, Jakovcevski M, Weng Z, Akbarian S. A simple method for improving the specificity of anti-methyl histone antibodies. Epigenetics 2010; 5(5): 392-395.
6. Jiang Y, Matevosian A, Huang HS, Straubhaar J, Akbarian S. Isolation of neuronal chromatin from brain tissue. *BMC neuroscience* 2008; 9: 42.

7. Matevosian A, Akbarian S. Neuronal nuclei isolation from human postmortem brain tissue. *J Vis Exp* 2008; (20).

8. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 2009; 25(16): 2078-2079.

9. Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE et al. Model-based analysis of ChIP-Seq (MACS). *Genome biology* 2008; 9(9): R137.

10. Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P et al. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell* 2010; 38(4): 576-589.

11. Wang J, Duncan D, Shi Z, Zhang B. WEB-based GEne SeT AnaLysis Toolkit (WebGestalt): update 2013. *Nucleic acids research* 2013; 41(Web Server issue): W77-83.

12. Thomas-Chollier M, Herrmann C, Defrance M, Sand O, Thieffry D, van Helden J. RSAT peak-motifs: motif analysis in full-size ChIP-seq datasets. *Nucleic acids research* 2012; 40(4): e31.

13. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 2014; 511(7510): 421-427.

14. Hnisz D, Abraham BJ, Lee TI, Lau A, Saint-Andre V, Sigova AA et al. Super-enhancers in the control of cell identity and disease. *Cell* 2013; 155(4): 934-947.

15. Goujon M, McWilliam H, Li W, Valentin F, Squizzato S, Paern J et al. A new bioinformatics analysis tools framework at EMBL-EBI. *Nucleic acids research* 2010; 38(Web Server issue): W695-699.

16. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* 2011; 7: 539.

17. Law CW, Chen Y, Shi W, Smyth GK. voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome biology* 2014; 15(2): R29.
18. Hochberg Y, Benjamini Y. More powerful procedures for multiple significance testing. *Stat Med* 1990; 9(7): 811-818.

19. Colantuoni C, Lipska BK, Ye T, Hyde TM, Tao R, Leek JT *et al.* Temporal dynamics and genetic control of transcription in the human prefrontal cortex. *Nature* 2011; 478(7370): 519-523.

20. Chakravarthy S, Keck T, Roelandse M, Hartman R, Jeromin A, Perry S *et al.* Cre-dependent expression of multiple transgenes in isolated neurons of the adult forebrain. *PLoS One* 2008; 3(8): e3059.

21. Jakovcevski M, Ruan H, Shen EY, Dincer A, Javidfar B, Ma Q *et al.* Neuronal Kmt2a/Mll1 histone methyltransferase is essential for prefrontal synaptic plasticity and working memory. *J Neurosci* 2015; 35(13): 5097-5108.

22. Jakovcevski M, Bharadwaj R, Straubhaar J, Gao G, Gavin DP, Jakovcevski I *et al.* Prefrontal cortical dysfunction after overexpression of histone deacetylase 1. *Biol Psychiatry* 2013; 74(9): 696-705.

23. Rodriguez A, Ehlenberger DB, Dickstein DL, Hof PR, Wearne SL. Automated three-dimensional detection and shape classification of dendritic spines from fluorescence microscopy images. *PLoS One* 2008; 3(4): e1997.

24. Dumitriu D, Hao J, Hara Y, Kaufmann J, Janssen WG, Lou W *et al.* Selective changes in thin spine density and morphology in monkey prefrontal cortex correlate with aging-related cognitive impairment. *The Journal of Neuroscience: the official journal of the Society for Neuroscience* 2010; 30(22): 7507-7515.

25. Mitchell AC, Bharadwaj R, Whittle C, Krueger W, Mirnics K, Hurd Y *et al.* The Genome in Three Dimensions: A New Frontier in Human Brain Research. *Biological psychiatry* 2013.

26. Dekker J. The three 'C' s of chromosome conformation capture: controls, controls, controls. *Nat Meth* 2006; 3(1): 17-21.

27. Dekker J, Rippe K, Dekker M, Kleckner N. Capturing Chromosome Conformation. *Science* 2002; 295(5558): 1306-1311.

28. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nature methods* 2012; 9(7): 671-675.
Supplemental Figures

Supplemental Figure 1: MEF2C immunoreactivity in frontal cortex of mice treated with saline and clozapine (10mg/kg) for 21 days (N=5/group). Histone H3 loading control. There is no consistent difference between clozapine and saline animals.

Supplemental Figure 2: MEF2C siRNA knockdown up-regulates H3K4me3 levels at Mef2c target sequences (A) Image from TYE563 transfected HEK cells to confirm efficient transfection. The scale bar represents 100 µm. (B) H3K4me3 ChIP qPCR, with quantification (Chip-to-input) for three MEF2C target sequences at MEF2C, MEF2A and TBLIXR promoters. MEF2A promoter-bound H3K4me3 is significantly increased after MEF2C siRNA knockdown (p=0.0426, t=2.931, df=4).

Supplemental Figure 3: No change in anxiety or depression after neuronal overexpression of Mef2c (AAV^hSYN1-Mef2cGfp) in mouse PFC (N=10/group) (related to Figure 2). (A,B) Open field: Mice were placed in an open field chamber for 20 minutes. Significant differences between AAV^hSYN1-Mef2cGfp and AAV^hSYN1-CreGfp control are limited to the first five minutes of test. 2-way anova (F=13.58, p=0.0004), Bonferroni post-hoc t=3.311, p<0.05. Time center vs. periphery in open field. 2-way Anova (F=1.525, p=0.0002. No significant difference at specific time points by posthoc Bonferroni. (C) Elevated plus maze. Mice were placed on an elevated plus maze for ten minutes. There was no significant difference between control and Mef2c overexpressing mice (D) Light dark box: Mice were placed in a chamber with a light and dark covered portion for 10 minutes. (E) 5 min forced swim. There are no significant differences between control animals and Mef2c overexpressing mice.
**Supplemental Figure 4:** Behavioral assessment of the Cbfβ transcription factor. (A) CBFB survives statistical filtering of the CMC dataset with Benjamini-Hochberg False Discovery Rate of p=0.025, with a subtle 1% increase in CMC cases versus controls. Mice with prefrontal HSV^Cbfb^ injections, in comparison with HSV^Gfp^ controls, do not show significant differences in (B) object recognition or (C) social novelty, or (D) open field and (F) elevated plus maze, (G) light dark box and (H) forced swim.

**Supplemental Figure 5:** Summary of radial arm maze errors, assessed after 5 weeks of bilateral PFC injection for hSynapsin1 promoter-driven Mef2c, or as CreGfp control. Animal received daily doses of MK-801 (0.2mg/kg) 30 min prior to test and training sessions. The AAV8^hSYN1-Mef2cGfp^ mice significantly outperformed AAV8^hSYN1-CreGfp^ mice on the test day (Day 3, unpaired two-tailed t-test, P=0.0176 (t=3.087, df=7).

**Supplemental Figure 6:** (A) Bar graph, (mean±S.E.M.) showing changes in 3C interaction frequencies of MEF2C TSS/promoter DNA with sequences positioned 500kb downstream, at risk SNP rs16867576. Data shown for neurons differentiated from human induced pluripotent stem cells (hiPSCs). All data expressed as levels in disease cases, relative to the controls. N=3 disease cases, N=3 controls. P<0.05 paired t-test (case-control) for neuronal cultures. (B) Representative image form neuronal cultures. All cases and control cultures robustly express βIII-tubulin (red) and the dendritic marker MAP2 (green). DAPI counterstain (blue). Scale bar 100 μm.
SUPPLEMENTAL FIGURE 1

Mouse Cortex Western Blot

Lane# → 1 2 3 4 5 6 7 8 9 10
Saline Clozapine

MEF2C 55 kD
H3 17 kD
SUPPLEMENTAL FIGURE 2

A

B

% Input

H3K4me3 loci

Control

MEF2C siRNA

0 20 40 60 80 100

MEF2C  MEF2A  TBLIXR1
SUPPLEMENTAL FIGURE 5

Control + MK-801
AAV-Mef2c + MK-801

Radial Arm Maze

Errors (n)

Day

*
SUPPLEMENTAL FIGURE 6

A

B

SCZ-CON

3C Primer #12

0.0

0.5

1.0

*