Supplemental Methods

Western blotting

After 10 DIV, neurons were lysed in RIPA (25 mM Tris pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS), supplemented by protease inhibitor (Roche 04693124001), phosphatase inhibitor (Roche 04906837001), 1mM DTT, 1mM PMSF. Lysates were mixed with 5X Loading Buffer (5% SDS, 0.3 M Tris pH 6.8, 1.1 mM Bromophenol blue, 37.5% glycerol), boiled for 10 minutes, sonicated for 5 minutes, and cooled on ice. Sample protein was resolved by 4-20% Tris-Glycine or 3-8% Tris-Acetate SDS-PAGE, followed by transfer to a 0.45μm PVDF membrane (Sigma Aldrich IPVH00010) for immunoblotting. Membranes were blocked for 1 hour in 5% BSA in TBST and probed with primary antibody overnight at 4C. Antibodies are shown in Supplemental Table 13. Quantification of western blots was performed in ImageJ software. Protein signal was quantified using mean grey values. Protein levels in samples infected by shRNA targeting one of the ASD-linked chromatin modifiers was quantified relative to the mean of samples infected by control shRNA.

Immunocytochemistry

Cells were fixed in 4% paraformaldehyde for 10 minutes, washed with PBS, and permeabilized in 0.5% Triton-X in PBS for 10 minutes. The cells were blocked (PBS, 3% BSA, 2% serum, 0.1% Triton-X) for at least 1 hour and stained with primary antibody overnight at 4°C. Coverslips were then washed 3X with PBS for 10 minutes, incubated with secondary antibodies for 1 hour at room temperature, stained with DAPI for 10 minutes, and washed again with PBS. Antibodies are included in Supplemental Table 13.

Cells were imaged on an upright Leica DM 6000, TCS SP8 laser scanning confocal microscope with 405 nm, 488, and 552 nm lasers. The microscope uses 2 HyD detectors and 3 PMT detectors. Objectives used were a 40x HC PL APO CS2 oil objective with a NA of 1.40. Type F immersion liquid (Leica) was used for oil objectives. Images were 175.91 x 171.91 microns, 1024 by 1024 pixels, and 16-bits per pixel.

Images were analyzed using ImageJ software. Quantification was performed blinded by first counting positively stained cells in each channel. The proportion of excitatory neurons was calculated to be one minus the fraction of total cells that were positive for the GABAergic marker, GAD67.

Statistical analyses

Overlap analysis:

R was used to calculate pairwise overlaps of differentially expressed gene lists and their corresponding significance values. For these comparisons using the narrow signatures, a background expressed gene list was defined for each DESeq2 comparison of an ASD-linked chromatin modifier versus Luciferase. Genes without an official gene symbol, a baseMean value greater than 5, or a defined adjusted p-value were filtered out of the background list. The intersection of these background lists was then used as a universal background gene list for hypergeometric tests on the narrow transcriptional signatures. For these comparisons using the broad signatures, a background expressed gene list was defined by an expression value greater than 5 using the filterByExpr function in edgeR (Robinson et al., 2010). InveractiveVenn was used to generate 5-way gene list Venn diagrams (Heberle et al., 2015). Multiple testing correction based on a 5% False Discovery Rate threshold using the Benjimini-Hochberg method (Benjamini and Hochberg, 1995). All published datasets used for analysis are described in Supplemental Table 11.

Gene Ontology analysis:

PANTHER (v17.0) was used to perform an overrepresentation test against the biological process complete ontology using default parameters. GeneWalk (v1.5.3) was run using Python 3.9 using default values. According default cutoffs, GeneWalk results were filtered for global_padj and gene_padj values less than 0.1 (Ietswaart et al., 2021). Revigo was used to remove redundant terms and to generate a 2-dimensional scatter based on semantic similarity of retained terms. Revigo input parameters used were: size of resulting list – tiny; remove obsolete GO terms – yes; species – *Mus Musculus*; semantic similarity measure – Resnik. Python 3.9 was used for processing of Revigo outputs with semantic similarity scatters clustered iteratively using Kmeans (sklearn.cluster.KMeans; default parameters; 2-15

clusters). Resulting clusters were assessed on the basis of silhouette scores and by manually judging the biological similarity of ontology terms placed within the same clusters.

ChromHMM analysis:

The chromatin state segmentation BED file containing coordinates of annotated chromatin states (Gorkin et al., 2020) was used in running the ChromHMM function OverlapEnrichment with parameters: java -mx4000M ChromHMM OverlapEnrichment inputsegment inputcoorddir outfileprefix. Fold enrichments were calculated as the ratio of the fraction of bases in a given chromatin state that were present in our input gene coordinates of interest to the fraction of the genome described by the input gene coordinates. Displayed heatmaps represent overlap enrichment output values range-normalized by column. Input coordinates for mm10 genic regions were generated via the UCSC Table Browser (group: Genes and gene predictions; track: GENCODE VM11 (Ensembl 86); table: basic). Mouse transcript names were converted to corresponding mouse Ensembl IDs using the R package biomaRt. Promoter coordinates were generated using the R package TxDb.Mmusculus.UCSC.mm10.knownGene ('knownGene'). The promoters function from the GenomicRanges (Lawrence et al., 2013) R package was used to generate coordinates 500 and 2000 base pairs upstream of TSS sites listed in knownGene.

GSEA:

The R package FGSEA (Korotkevich et al., 2016) was used to perform pre-ranked gene set enrichment analysis (GSEA) based on $\log_2$ fold changes obtained from DESeq2 differential expression analysis. Genes without a defined adjusted p-value were removed prior to running GSEA. The narrow and broad transcriptional signature lists were used as comparison gene sets.

Linear regression

Linear regression analysis was performed as described in Phan et al. 2020. Code was obtained from the Lieber Institute GitHub page (https://github.com/LieberInstitute/PTHS_mouse). Variance Stabilized Transformation (DESeq2 package) was used to scale gene expression values, which were used to compare read counts of transcriptional signature genes between human ASD and control samples. We

performed principal component analysis (PCA) on the genes homologous between mouse and human: 111 and 172 for the up and down-regulated *narrow* signatures, respectively; 960 and 1391 for the up and down-regulated *broad* signatures, respectively. Linear regression analysis was then performed on the first principal component (eigengene) for each transcriptional signature, modeling the effect of diagnosis while adjusting for sample metadata where available and a number of quality surrogate variables (qSVs), which help correct for additional confounds in the data and are determined by the num.sv() function in the sva R package (Jaffe et al., 2017). For Marchetto et al., 2017, the linear model adjusted for age, brain volume, and the top 6 qSVs; for Wright et al., 2017, the linear model adjusted for RIN, exonic mapping rate, sex, race, and the top 9 qSVs; for DeRosa et al. 2018, the subset of induced-neurons cultured for 35 DIV was used and the linear model adjusted for iPSC reprogramming method and the top 4 qSVs; for Velmeshev et al., 2020, the linear model was adjusted for sex, age, and the top 4 qSvs; for Velmeshev et al., 2019, bulk sequencing data was used and the linear model was adjusted for sex, age, and the top 4 qSVs. For Parikshak et al., 2016, linear regression was performed to the specifications of Phan et al., 2020.

Supplemental References

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