

1 Post-mortem molecular profiling of three psychiatric disorders

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3 Kevin M. Bowling^{1,*}, Ph.D., Ryne C. Ramaker^{1,2,*}, B.S., Brittany N. Lasseigne^{1*}, Ph.D.,
4 Megan H. Hagenauer³, Ph.D., Andrew A. Hardigan^{1,2}, B.S., Nick S. Davis^{1,#}, B.S., Jason
5 Gertz^{1,%}, Ph.D., Preston M. Cartagena⁴, Psy.D., David M. Walsh⁴, Psy.D., Marquis P.
6 Vawter⁴, Ph.D., Edward G. Jones (deceased), M.D., Ph.D., Alan F. Schatzberg⁵, M.D.,
7 Jack D. Barchas⁶, M.D., Ph.D., Stan J. Watson³, M.D., Ph.D., Blynn G. Bunney⁴, Ph.D.,
8 Huda Akil³, Ph.D., William E. Bunney⁴, M.D., Jun Z. Li⁷, Ph.D., Sara J. Cooper¹, Ph.D.,
9 and Richard M. Myers¹, Ph.D.

10 *These authors contributed equally to this study

11 ¹HudsonAlpha Institute for Biotechnology, Huntsville, AL, USA

12 ²Department of Genetics, The University of Alabama at Birmingham, Birmingham, AL,
13 USA

14 ³Mental Health Research Institute, University of Michigan, Ann Arbor, MI, USA

15 ⁴Department of Psychiatry and Human Behavior, College of Medicine, University of
16 California, Irvine, Irvine, CA, USA

17 ⁵Department of Psychiatry, Stanford University School of Medicine, Stanford, CA, USA

18 ⁶Psychiatry, Weill Cornell Medical College, New York, NY, USA

19 ⁷Department of Human Genetics, University of Michigan, Ann Arbor, MI, USA

20

21 Present address:

22 [#]Duke University, Durham, NC, USA

23 [%]University of Utah School of Medicine, Salt Lake City, UT, USA

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2 To whom correspondence should be addressed:

3 Richard M. Myers, Ph.D.

4 HudsonAlpha Institute for Biotechnology

5 601 Genome Way

6 Huntsville, AL 35806

7 Telephone: 256-327-0431

8 FAX: 256-327-0978

9 rmyers@hudsonalpha.org

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1 **Abstract**

2 **Background**

3 Psychiatric disorders are multigenic diseases with complex etiology contributing
4 significantly to human morbidity and mortality. Although clinically distinct, several
5 disorders share many symptoms suggesting common underlying molecular changes
6 exist that may implicate important regulators of pathogenesis and new therapeutic
7 targets.

8 **Results**

9 We compared molecular signatures across brain regions and disorders in the
10 transcriptomes of postmortem human brain samples. We performed RNA sequencing
11 on tissue from the anterior cingulate cortex, dorsolateral prefrontal cortex, and nucleus
12 accumbens from three groups of 24 patients each diagnosed with schizophrenia, bipolar
13 disorder, or major depressive disorder, and from 24 control subjects, and validated the
14 results in an independent cohort. The most significant disease differences were in the
15 anterior cingulate cortex of schizophrenia samples compared to controls. Transcriptional
16 changes were assessed in an independent cohort, revealing the transcription factor
17 *EGR1* as significantly down regulated in both cohorts and as a potential regulator of
18 broader transcription changes observed in schizophrenia patients. Additionally, broad
19 down regulation of genes specific to neurons and concordant up regulation of genes
20 specific to astrocytes was observed in SZ and BPD patients relative to controls. We also
21 assessed the biochemical consequences of gene expression changes with untargeted
22 metabolomic profiling and identified disruption of GABA levels in schizophrenia patients.

1 **Conclusions**

2 We provide a comprehensive post-mortem transcriptome profile of three psychiatric
3 disorders across three brain regions. We highlight a high-confidence set of
4 independently validated genes differentially expressed between schizophrenia and
5 control patients in the anterior cingulate cortex and integrate transcriptional changes
6 with untargeted metabolite profiling.

7 **Keywords**

8 Schizophrenia, Bipolar Disorder, Major Depressive Disorder, RNA sequencing,
9 metabolomics, *EGR1*

10 **Background**

11 Schizophrenia (SZ), bipolar disorder (BPD), and major depressive disorder (MDD) are
12 multigenic diseases with complex etiology and are large sources of morbidity and
13 mortality in the population. All three disorders are associated with high rates of suicide,
14 with ~90% of the ~41,000 people who commit suicide each year in the U.S. having a
15 diagnosable psychiatric disorder [2]. Notably, while clinically distinct, these disorders
16 also share many symptoms, including psychosis, suicidal ideation, sleep disturbances
17 and cognitive deficits [3–5]. This phenotypic overlap suggests potential common genetic
18 etiology, which is supported by recent large-scale genome-wide association studies [6–
19 9]. However, this overlap has not been fully characterized with functional genomic
20 approaches. Current therapies for these psychiatric disorders are ineffective in many
21 patients and often only treat a subset of an individual patient’s symptoms [10].
22 Approaches targeting the underlying molecular pathologies within and across these

1 types of disorders are necessary to address the immense burden of psychiatric disease
2 around the world and improve care for the millions of people diagnosed with these
3 conditions.

4 Previous studies [11–15] analyzed brain tissue with RNA sequencing (RNA-seq) in SZ
5 and BPD, and identified altered expression of GABA-related genes in the superior
6 temporal gyrus and hippocampus, as well as differentially expressed genes related to
7 neuroplasticity and mammalian circadian rhythms. Our study focused on the anterior
8 cingulate cortex (AnCg), dorsolateral prefrontal cortex (DLPFC), and nucleus
9 accumbens (nAcc), regions which are often associated with mood alterations, cognition,
10 impulse control, motivation, reward, and pleasure – all behaviors known to be altered in
11 psychiatric disorders [16,17]. To assess gene expression changes associated with
12 psychiatric disease in these three brain regions, we performed RNA-seq on macro-
13 dissected post-mortem tissues in four well-documented cohorts of 24 patients each with
14 SZ, BPD, MDD and 24 controls (CTL) (96 individuals total). Additionally, we conducted
15 metabolomic profiling of AnCg tissue from the same subjects. RNA-seq analysis
16 revealed common expression profiles in SZ and BPD patients supporting the notion that
17 these disorders share a common molecular etiology. Transcriptional changes were most
18 pronounced in the AnCg with SZ and BPD exhibiting strongly correlated differences
19 from CTL samples. Differentially expressed genes were associated with cell-type
20 composition with BPD and SZ samples showing decreased expression of neuron-
21 specific transcripts. We validated this result with RNA-seq data from an independent
22 cohort of 35 cases each of SZ, BPD, and CTL post-mortem cingulate cortex samples
23 from the Stanley Neuropathology Consortium Integrative Database (SNCID;

1 <http://sncid.stanleyresearch.org>) Array Collection. We present a set of validated genes
2 differentially expressed between SZ and CTL patients, perform an integrated analysis of
3 metabolic pathway disruptions, and highlight a role for the transcription factor, *EGR1*,
4 whose down-regulation in SZ patients may drive a large portion of observed
5 transcription changes.

6 **Methods**

7 See Supplemental Methods for additional detail.

8 **Patient Sample Collection and Preparation**

9 Sample collection, including human subject recruitment and characterization, tissue
10 dissection, and RNA extraction, was described previously [18,19] as part of the Brain
11 Donor Program at the University of California, Irvine, Department of Psychiatry and
12 Human Behavior (Pritzker Neuropsychiatric Disorders Research Consortium) under IRB
13 approval. In brief, coronal slices of the brain were rapidly frozen on aluminum plates that
14 were pre-frozen to -120°C and dissected as described previously [20]. All samples were
15 diagnosed by psychological autopsy, which included collection and analyses of medical
16 and psychiatric records, toxicology, medical examiners' reports, and 141-item family
17 interviews. Agonal state scores were assigned based on a previously published scale
18 [21]. Controls were selected based upon absence of severe psychiatric disturbance and
19 mental illness within first-degree relatives.

20 We obtained fastq files from RNA-seq experiments for our validation cohort from the
21 Stanley Neuropathology Consortium Integrative Database (SNCID;
22 <http://sncid.stanleyresearch.org>) Array Collection comprising 35 cases each of SZ, BPD,

1 and CTL of post-mortem cingulate cortex with permission on June 30, 2015. For our
2 analysis, we included the 27 SZ, 26 CTL, and 25 BPD SNCID samples that were
3 successfully downloaded and represented unique samples. SNCID RNA-seq
4 methodology and data processing is described in detail in a previous publication that
5 makes use of the data [11].

6 **RNA-seq and Data Processing**

7 To extract nucleic acid, 20 mg of post-mortem brain tissue was homogenized in Qiagen
8 RLT buffer + 1% BME using an MP FastPrep-24 and Lysing Matrix D beads for three
9 rounds of 45 seconds at 6.5 m/s (FastPrep homogenizer, lysing matrix D, MP Bio). Total
10 RNA was isolated from 350 μ L tissue homogenate using the Norgen Animal Tissue
11 RNA Purification Kit (Norgen Biotek Corporation). We made RNA-seq libraries from 250
12 ng total RNA using polyA selection (Dynabeads mRNA DIRECT kit, Life Technologies)
13 and transposase-based non-stranded library construction (Tn-RNA-seq) as described
14 previously [22]. To mitigate potentially confounding batch effects in sample preparation
15 we randomly assigned samples from all brain regions and disorders into batches of 24
16 samples. We used KAPA to quantitate the library concentrations and pooled 4 samples
17 in order to achieve equal concentration of the four libraries in each lane. Pools were
18 determined by random from the 291 samples. Samples were also randomly selected for
19 pooling in an effort to limit potentially confounding sequencing batch effects. The pooled
20 libraries were sequenced on an Illumina HiSeq 2000 sequencing machine using paired-
21 end 50 bp reads and a 6 bp index read, resulting in an average of 48.2 million reads per
22 library. To quantify the expression of each gene in both Pritzker and SNCID datasets,
23 RNA-seq reads were processed with aRNApipe v1.1 using default settings [23]. Briefly,

1 reads were aligned and counted with STAR v2.4.2a to GRCh37_E75 [24]. All alignment
2 quality metrics were obtained from the picard tools module
3 (<http://broadinstitute.github.io/picard/>) available in aRNApipe. Transcripts expressed
4 from the X and Y chromosomes were omitted from the study.

5 Quantitative PCR (qPCR) was performed on 10 SZ and 10 CTL patients to validate
6 *EGR1* RNA-seq measurements. RNA was extracted as described above from tissue
7 lysates a second time. Reverse transcription was performed on 250ng of input RNA with
8 the Applied Biosystems high capacity cDNA reverse transcription kit. Validated Taqman
9 assays for *EGR1* (Hs00152928_m1) and the housekeeper genes *GAPDH*
10 (Hs02758991_g1) and *ACTB* (Hs01060665_g1) were used for qPCR. cDNA was
11 diluted by a factor of 10 before use as input for the Taqman assay. The qPCR
12 reaction was performed on an Applied Biosystems Quant Studio 6 Flex system
13 using the recommended amplification protocol for Taqman assays.

14 **Sequencing Data Analysis**

15 All data analysis in R was performed with version 3.1.2.

16 *Differential Expression Analysis and Normalization*

17 To examine gene expression changes, we employed the R package DESeq2 [1]
18 (version 1.6.3), using default settings, but employing likelihood ratio test (LRT)
19 hypothesis testing, and removing non-convergent transcripts from subsequent analysis.
20 Genes differentially expressed between each disorder and CTL samples, by brain
21 region, were identified with DESeq2 (adjusted p-value<0.05), including age, brain pH,
22 PMI, and percentage of reads uniquely aligned (PRUA) as covariates (Full Model:

1 ~Age+PMI+pH+PRUA+Disorder, Reduced Model: ~ Age+PMI+pH+PRUA). For
2 downstream heatmap visualization, PCA, and cell-type analysis, transcripts were
3 underwent a log-like normalization using DESeq2's varianceStabilizingTransformation
4 function and were corrected for PRUA by computing residuals to a linear model
5 regressing PRUA on normalized transcript amount with the R lm function unless
6 otherwise specified.

7 *PCA and Hierarchical Clustering*

8 PCA analysis was performed in R on normalized data using the prcomp() command.
9 Hierarchical clustering of normalized transcript data was done in R with the hclust
10 command (method="ward", distance="Euclidean")

11 *Pathway Enrichment Analysis*

12 Pathway analysis was conducted using the web-based tool LRPath [25] using all GO
13 term annotations, adjusting to transcript read count with RNA-Enrich, including
14 directionality and limiting maximum GO term size to 500 genes. GO term visualization
15 was performed using the Cytoscape Enrichment Map plug-in [26]. The Genesetfile
16 (.gmt) GO annotations from February 1, 2017 were downloaded from
17 http://download.baderlab.org/EM_Genesets/. The LRPath output was parsed and used
18 as an enrichment file with all upregulated pathways colored red and all downregulated
19 pathways colored blue, regardless of degree of upregulation. Mapping parameters
20 were; p-value cutoff = 0.005, FDR cutoff = 0.1 and Jaccard coefficient > 0.3. Resulting
21 networks were exported as PDFs. Summary terms were added to the plot based on the
22 GO terms in those clusters. In order to assess overlap between significant GO terms
23 and our analysis and the GWAS study described by the Psychiatric Genomics

1 Consortium, we downloaded the p-values reported for Schizophrenia hits from
2 Supplemental Table 4, which contained 424 significant GO terms. We used a chi-
3 squared test to assess significant overlap between the two groups. Supplemental Table
4 X reports the p-values measured in SZ based on this study along with those calculated
5 in our analysis.

6 *EGR1 ChIP-seq peak analysis*

7 Narrow peak bed files from optimal IDR thresholded peaks were obtained from the
8 ENCODE data portal (www.encodeproject.org) for *EGR1* ChIP-seq data in GM12878,
9 H1-hESC, and K562 cell lines (ENCODE file IDs: ENCFF002CIV, ENCFF002CGW,
10 ENCFF002CLV). Consensus *EGR1* peaks were identified by intersecting peaks from all
11 three cell lines, which resulted in a final list of 4,121 peaks that were present in each cell
12 line (with a minimum overlap of 1bp). The distance from each annotated transcription
13 start site (TSS) to the nearest consensus *EGR1* peak was computed using TSSs
14 annotated in the ENSEMBL gene transfer format (GTF) file used for aligning RNA-seq
15 reads (GRCh37_E75).

16 *Cell-Specific Enrichment Analysis*

17 Sets of transcripts uniquely expressed by several brain cell-types were obtained from
18 figure 1B in Darmanis et. al [27]. An index for each cell-type was created by finding the
19 median normalized expression value for each cell-type associated transcript set. Index
20 values were compared across patient clusters by non-parametric rank sum tests and
21 spearman correlation with top principal components. To validate our method, we
22 calculated cell-type specific indices from an independent cohort of previously published
23 purified brain cells [28,29]. FPKM-normalized transcript data was obtained from

1 supplemental table 4 of Zhang et. al. (2014) and cell-type indexes were calculated as
2 described above. To examine index performance in mixed cell populations, we obtained
3 fastq files for neuron and astrocyte-purified brain samples from GEO accession
4 GSE73721 and generated raw count files as described above. We next mixed
5 expression profiles *in silico* by performing random down-sampling of neuron and
6 astrocyte count levels and summing the results such that mixed populations containing
7 specific proportions of counts from neuron- and astrocyte-purified tissue were
8 generated. For example, to generate an 80/20 neuron to astrocyte mixture, neuron and
9 astrocyte count columns (which started at an equivalent number of 5,759,178 aligned
10 reads) were randomly down-sampled to 4,607,342 and 1,151,836 counts respectively
11 and summed across each gene to result in a proportionately mixed population of
12 aligned count data simulating heterogeneous tissue. Then we calculated a
13 neuron/astrocyte index ratio capable of predicting the *in silico* mixing weights. Briefly,
14 we assumed index values for mixed cell populations were directly proportional to mixing
15 weights of their respective purified tissue, thus the predicted cell proportion for a given
16 cell type was simply calculated as:

17
$$\text{predicted cell proportion} = \text{observed index value} / \text{purified tissue index value}$$

18 To insure cell-type predictive power was unique to indices derived from Darmanis et. al
19 transcripts, we generated indices from 10,000 randomly sampled transcript sets of
20 equivalent size and examined their performance in predicting *in silico* mixing weights.
21 Mean squared prediction errors (MSE) were calculated for each of the 10,000 null
22 indices and compared to the MSE of Darmanis et. al.-derived indices.

23 **Metabolomics**

1 *Sample preparation*

2 Sections of approximately 100mg of frozen tissue were weighed and homogenized for
3 45 seconds at 6.5M/s with ceramic beads in 1mL of 50% methanol using the MP
4 FastPrep-24 homogenizer (MP Biomedicals). A sample volume equivalent to 10mg of
5 initial tissue weight was dried down at 55°C for 60 minutes using a vacuum concentrator
6 system (Labconco). Derivatization by methoximation and trimethylsilylation was done as
7 previously described [30].

8 We analyzed technical replicates of each tissue sample, in randomized order.

9 *GCxGC-TOFMS analysis*

10 All derivatized samples were analyzed on a Leco Pegasus 4D system (GCxGC-
11 TOFMS), controlled by the ChromaTof software (Leco, St. Joseph, MI). Samples were
12 analyzed as described previously [30] with minor modifications in temperature ramp.

13 *Data analysis and metabolite identification*

14 Peak calling, deconvolution and library spectral matching were done using ChromaTOF
15 4.5 software. Peaks were identified by spectral match using the NIST, GOLM [31], and
16 Fiehn libraries (Leco), and confirmed by running derivatized standards (Sigma). We
17 used Guineu for multiple sample alignment [32].

18 *Integrated Pathway Analysis*

19 Altered metabolites and transcripts were analyzed for enrichment in KEGG pathways
20 containing both metabolite and gene features. A non-parametric, threshold free pathway
21 analysis similar to that of a previously described method [33] was first performed on
22 metabolite and transcript data separately. Our method builds on the principle described

1 by Subramanian that implements a one-tailed Wilcox test to identify pathways enriched
2 for low p-values. Instead of just accounting for enrichment at the gene level, we use
3 metabolite or transcript p-value ranks within each pathway compared to remaining non-
4 pathway metabolites or transcripts with a one-tailed Wilcox test to test the hypothesis
5 that elements of a given pathway may be enriched for lower p-value ranks than
6 background elements. Metabolite and transcript p-values were subsequently combined
7 to provide an integrated enrichment significance p-value using Fisher's method.
8 Pathways had to contain greater than 5 genes and 1 metabolite measured in our
9 dataset to be included in the analysis. Supplemental table 10 lists p-values for enriched
10 pathways based on genes, metabolites or combined.

11 **Results**

12 **Region-specific gene expression in control and psychiatric brain tissue**

13 We collected post-mortem human brain tissue, associated clinical data, including age,
14 sex, brain pH, and post-mortem interval (PMI), and cytotoxicology results (Tables S1-2)
15 for matched cohorts of 24 patients each diagnosed with SZ, BPD, or MDD, as well as
16 24 control individuals with no personal history of, or first-degree relatives diagnosed
17 with, psychiatric disorders. Importantly, to limit the effect of acute patient stress at the
18 time of death as a potential confounder we included only patients with an agonal factor
19 score of zero and a minimum brain pH of 6.5 [19]. Using RNA-seq [22], we profiled gene
20 expression in three macro-dissected brain regions (AnCg, DLPFC, nAcc). After quality
21 control, we analyzed 57,905 ENSEMBL transcripts in a total of 281 brain samples
22 (Table S3).

1 To examine heterogeneity across brain regions and subjects, we performed a principal
2 component analysis (PCA; Figure S1A) of all transcripts. The first principal component
3 (PC1, 21.8% of the variation) separates cortical AnCg and DLPFC samples from
4 subcortical nAcc samples. Examination of the first and second principal components for
5 disorder associations reveals a separation of some SZ and BPD samples from all other
6 samples (Figures S1B and S2A-C). However, in agreement with several previously
7 reported post-mortem brain RNA sequencing studies [15], we found several principal
8 components to be highly correlated with quality metrics including the percentage of
9 reads uniquely aligned and percentage of reads aligned to mitochondrial sequence
10 (absolute $Rho > 0.5$, $FDR < 1E-16$, Table S4). To reduce the potentially confounding
11 effects of sample quality, we repeated the PCA on expression data normalized to the
12 percentage of reads uniquely aligned for each sample and found that global disease-
13 specific expression differences were significantly reduced (Figures S1C and S2D-I).

14 **Disease-specific gene expression in control and psychiatric brains**

15 We next applied DESeq2 [1], a method for differential analysis of sequence read count
16 data, to identify genes differentially expressed across disorders within each brain region
17 after correcting for biological and technical covariates. The largest number of significant
18 expression changes occurred in AnCg between SZ and CTL individuals (87 transcripts,
19 $FDR < 0.05$, Figure 1A). Pathway enrichment analysis of differentially expressed genes
20 between SZ and CTL patients revealed 935 gene ontology (GO) terms with an
21 $FDR < 0.05$ (Table S5) (122 GOCC, 159 GOMF, and 654 GOBP). Significant GO terms
22 fall into the broad categories of synaptic function and signaling (e.g. neurotransmitter
23 transport, ion transport, calcium signaling) (Figure S3). These terms overlap significantly

1 with those identified by the Psychiatric Genomics Consortium in their analysis of GWAS
2 implicated genes [34] with 68 GO terms meeting a p-value cutoff of <0.05 in both
3 datasets ($p < 0.0001$, Chi-square test). Additionally, nine genes were differentially
4 expressed between SZ and CTL individuals in DLPFC. Three of these were also
5 identified in AnCg: *SST*, *PDPK2P* and *KLHL14*. No transcripts had an $FDR < 0.05$ when
6 comparing BPD or MDD samples to CTLs in any brain region, or comparing SZ and
7 CTL tissues in nAcc (Table S6). To examine potential common gene expression
8 patterns between the psychiatric disorders, we performed pair-wise correlation
9 calculations of all transcript \log_2 fold changes for each disorder versus controls in each
10 brain region. Of the nine case-control comparisons (for three regions and three
11 diseases), a particularly strong correlation is observed between BPD and SZ compared
12 to either SZ or BPD and MDD in each brain region (Figure 1B). In the AnCg, BPD and
13 SZ share 1,020 common genes differentially expressed at an uncorrected DESeq2 P -
14 value < 0.05 compared to only 248 and 143 genes shared between MDD and SZ or BPD
15 respectively (Figure 1C). This strong overlap between BPD and SZ (Fisher's exact p -
16 value $< 1E-16$) indicates that although expression changes are weaker in BPD they
17 follow a trend similar to those identified in SZ.

18 Because previous post-mortem analyses have been limited by, and are particularly
19 vulnerable to, biases inherent to examining a single patient cohort, we sought to
20 generate a robust set of SZ associated transcripts by validating our observed
21 expression changes in an independent cohort. To accomplish this, we examined gene
22 expression differences in the AnCg between SZ and CTL samples in the SNCID RNA-
23 seq Array dataset [14], revealing 1,003 genes differentially regulated (DESeq2

1 uncorrected $P < 0.05$) in both datasets (Fisher's $P < 1E-16$, Table S7). The magnitude and
2 direction of change in significant transcripts in the Pritzker dataset were highly
3 correlated with the SNCID dataset ($Rho = 0.202$, $P < 1E-16$), particularly in transcripts that
4 met an $FDR < 0.05$ cutoff ($Rho = 0.812$, $P < 1E-16$; Figure 1D). We performed hierarchical
5 clustering of SZ and CTL samples in the SNCID validation cohort using the 1,003
6 transcripts differentially expressed between SZ and CTL in the Pritzker dataset ($P < 0.05$,
7 Figure 1E), and found these transcripts successfully distinguished the two disease
8 groups with only 5 out of 27 SZ and 2 out of 26 CTL samples misclassified.

9 Of particular interest are a group of 5 genes significant at a $FDR < 0.05$ in both cohorts
10 that includes a nearly 2-fold decrease in expression of the transcription factor *EGR1*
11 (Table S7A, Figure 2A). Quantitative PCR (qPCR) validation of the transcript confirmed
12 reduced *EGR1* expression in SZ samples (Figure 2B). *EGR1*, a zinc finger transcription
13 factor, has been recently implicated in SZ by a GWAS study [6], thus we sought to
14 investigate its role as a potential driver of the transcriptional changes observed in the
15 AnCg of SZ patients using publicly available genome-wide occupancy data from the
16 ENCODE consortium (<https://www.encodeproject.org>). To obtain high confidence *EGR1*
17 binding sites we intersected chromatin immunoprecipitation sequencing (ChIP-Seq)
18 peaks derived from the H1-hESC, K562, and GM12878 cell lines. We found that
19 transcripts whose transcription start sites (TSSs) were within 1kb of an *EGR1* binding
20 site had significantly lower DESeq2 P -values (Wilcox $P = 9.68E-5$) and significantly more
21 negative \log_2 fold changes (SZ/CTL, Wilcox $P = 7.69E-15$) than transcripts whose TSSs
22 were greater than 1kb from an *EGR1* binding site. A monotonic decrease in this effect

1 was observed as the distance threshold used for this comparison was increased from
2 1kb to 50kb (Figure 2C).

3 **Cell type specific changes**

4 In addition to dysregulation of broadly acting transcription factors, another mechanism
5 that can drive large-scale transcriptional changes in bulk tissue is alterations in
6 constituent cell type proportions. Previous studies have observed decreases in neuron
7 density and increased glial scarring in psychiatric disorders [35,36]. To test for signs of
8 these alterations in our data set we examined the expression of cell type-specific
9 transcripts identified using data from a single cell RNA sequencing study that identified
10 transcripts capable of classifying individual cells into the major neuronal, glial, and
11 vascular cell-types in the brain. We generated cell type indices using the median of
12 normalized counts for each cell type-specific transcript set. Examining cell type indices
13 in a previously published RNA-seq analysis of purified brain cells reveals high specificity
14 of each index to the appropriate cell type and accurate deconvolution of transcriptomes
15 mixed *in silico* [29,28] (Figure S4A-F). Moreover, median values from 10,000 randomly
16 sampled transcript sets are not able to deconvolute mixed cell transcriptomes,
17 demonstrating that predictive power is relatively unique to the Darmanis et al. transcript
18 sets (Figure S4G-I).

19 Application of the cell type indices to patient AnCg expression data revealed a
20 significant decrease in neuron specific expression (Wilcox $P<0.05$) and a significant
21 increase in astrocyte specific expression (Wilcox $P<0.05$) in SZ and BPD patients
22 compared to controls (Figures 3A-B). Other cell-type indices were not significantly
23 different between psychiatric patients and controls (Figure S5). Further supporting a

1 decrease in neuronal gene expression, we found a significant negative correlation
2 between transcript expression in patient brains relative to control brains and the degree
3 of neuron specificity (fold enrichment of neuron expression over other cell types) in SZ
4 and BPD (ρ -0.50 and -0.41, $P < 1E-16$, SZ shown in Figure 3C).

5 **Transcriptomic changes reflected in altered metabolomic profiles**

6 To assess the biochemical consequences of expression changes, we used 2D-GCMS
7 to measure metabolite levels in 86 of the AnCg samples (sufficient tissue was
8 unavailable for 10 samples). We measured and identified 141 unique metabolites (Table
9 S8). Similar to our transcript analysis, metabolite levels (Table S9) successfully
10 differentiated SZ and BPD patients from CTLs (Figures 4A-B), while MDD metabolite
11 profiles were very similar to CTLs (Figure S6). Several of the most significant
12 metabolites, including GABA, are known to be relevant to BPD and SZ [37].
13 Furthermore, GABA/glutamate metabolite ratios correlate strongly with average *GAD1*
14 and *GAD2* expression levels ($Rho = 0.413$, $P = 0.007$, Figures 4C-D). This metabolite-
15 gene relationship is consistent with previous multi-level phenomic analyses [38] and
16 demonstrates realized biochemical consequences from altered gene expression.
17 Notably, reductions in GABA could coincide with loss of neurons suggested by the gene
18 expression data. Integrated pathway analyses of KEGG pathways enriched for both
19 altered metabolites and transcripts between SZ and CTL patients revealed disruption of
20 synaptic and neurotransmitter signaling (Figure S7, Table S10).

21 **Discussion**

1 Here, we describe a large transcriptomic dataset across three brain regions (DLPFC,
2 AnCg, and nAcc) in SZ, BPD, and MDD patients, as well as CTL samples matched for
3 agonal state and brain pH. In MDD, we do not identify any transcripts that meet
4 genome-wide significance for differential expression between cases and controls in any
5 brain region. This finding agrees with previous post-mortem RNA-seq studies [39],
6 however sample size and the choice of brain regions examined likely contributed to our
7 inability to replicate results from previous non-transcriptome wide sequencing based
8 approaches comparing MDD to CTL in post-mortem brain [40]. One limitation of our
9 study is that females are underrepresented at a rate of about 5:1. This reflects the
10 increased chance of accidental death among males [41], but limits us in our ability to
11 make more general conclusions about these disorders and to address known
12 differences between the sexes as they relate to these disorders. We also do not have
13 information on the smoking status for our cohort, which is an important covariate as
14 smoking rates are higher among patients with psychiatric disorders and smoking has
15 been demonstrated to effect gene expression [42,43]. Another potential limitation
16 inherent to post-mortem cohort analyses is accounting for patient drug use. As detailed
17 in supplemental table 2, patient toxicology reports were positive for several prescribed
18 and illicit drugs that were not present in CTL samples. As this is a bias inherent to
19 psychiatric patients it is impossible to disentangle from non-treatment related disease
20 patterns in a post-mortem analysis.

21 Another important limitation of post-mortem RNA-sequencing studies is RNA quality.
22 We found a significant proportion of variation in our data to be associated with multiple
23 alignment quality metrics. Significant effort went into controlling for potential sources of

1 bias due to differences in RNA quality. We only included tissue from patients with an
2 agonal score of 0 and who had a brain pH of 6.5 or greater. We also controlled for brain
3 pH, post-mortem interval, and alignment quality in all differential expression analyses.
4 Our study, as well as future post-mortem studies, could be improved by directly
5 measuring RNA quality at the time of sample preparation (e.g. RNA integrity number
6 (RIN)). Despite these caveats, we believe our data do yield new insights that contribute
7 to our growing understanding of these disorders.

8 The most dramatic gene expression signals we observed were brain region-specific.
9 The majority of disease-associated expression differences were seen in the AnCg of SZ
10 compared to CTL patients. The AnCg has been associated with multiple disease-
11 relevant functions, including cognition, error detection, conflict resolution, motivation,
12 and modulation of emotion [44–46]. We observed a striking overlap in SZ- and BPD-
13 associated expression changes consistent with previous findings [37,47].

14 One of the more intriguing transcripts significantly down regulated ($FDR < 0.05$) in both
15 cohorts of SZ patients was the zinc finger transcription factor, *EGR1*. We provide
16 evidence that this factor may be driving a large proportion of variation in SZ patients as
17 transcripts near consensus *EGR1* binding sites tend to have decreased expression in
18 SZ patients. Down regulation of *EGR1* has been previously described in the prefrontal
19 cortex of post-mortem brain samples from SZ patients [48,49]. *EGR1* has also
20 previously been associated with several phenotypes relevant to psychiatric disorder
21 including neural differentiation [50], emotional memory formation [51], response to
22 antipsychotics [52], and has recently been described as part of a transcription factor-
23 miRNA co-regulatory network capable of acting as a biomarker in peripheral blood cells

1 (PBCs) for SZ [53]. In mice, loss of *EGR1* has linked to neuronal loss in a model of
2 Alzheimer's Disease [54]. *EGR1* is also important for regulation of the NMDA Receptor
3 pathway, which is critical for synaptic plasticity and memory formation and has been
4 implicated in SZ in humans [55]. We believe a more detailed examination of genome-
5 wide *EGR1* occupancy in post-mortem brain tissue or cultured neurons could yield
6 additional information and assessment of the functional consequences of *EGR1*
7 perturbation is required to confirm this factor's role in SZ pathogenesis.

8 We also see evidence for depletion of neuron-specific transcripts and increased levels
9 of astrocyte-specific transcripts in SZ and BPD patients. This observation is further
10 supported by metabolomic analysis of the AnCg, which found a concordant decrease in
11 GABA levels in BPD and SZ individuals. Neuronal depletion has been previously
12 described in SZ [35,36]. Insufficient tissue remains from our patient cohort to validate
13 computational cell type predictions immunohistochemically, however our data strongly
14 suggests that future post-mortem studies should be cognizant of cell type heterogeneity
15 across patient samples. The method for cell type composition estimation is limited in its
16 accuracy to estimating only the major classes of cells present. Transcripts represented
17 in cell types present at only a small minority could be over or under-represented using
18 this technique. Based on these results, future studies should consider using robust
19 techniques for assessing tissue composition to examine potential cell type proportion
20 differences between disease cohorts and to identify which transcriptional changes occur
21 in conjunction with, and independent of, those differences.

22 We observed greatly reduced or no significant expression differences in the DLPFC and
23 nAcc, which contradicts several previous studies [56,57]. We do not intend to claim that

1 no transcriptional changes occur in these brain regions as our study was designed to
2 broadly compare transcriptional alterations across multiple brain regions in multiple
3 psychiatric disorders, thereby sacrificing exceptional sample sizes in any single disorder
4 in any specific brain region. However, our data does suggest that of the regions we
5 tested, the strongest transcriptional changes occur in the AnCg of SZ patients.
6 Moreover, this data provides a useful resource for future studies facilitating the testing
7 of preliminary hypotheses or validation of significant findings.

8 **Conclusions**

9 Our study provides several meaningful and novel contributions to the understanding of
10 psychiatric disease. We provide a well-annotated data set that has the potential to act
11 as a broadly applicable resource to investigators interested in molecular changes in
12 multiple psychiatric disorders across multiple brain regions. We have conducted an
13 extensive characterization of the molecular overlap between SZ and BPD at the
14 transcript and metabolite level across multiple brain regions. We provide a high
15 confidence set of genes differentially expressed between SZ and CTL patients utilizing
16 two independent cohorts and highlight down regulation of *EGR1* as a potential driver of
17 broader scale transcription changes. We also establish that a significant proportion of
18 transcriptome variation within SZ and BPD cohorts is correlated with expression
19 changes in previously identified cell type-specific transcripts.

20 **List of abbreviations**

21 RNA-seq – RNA sequencing
22 GABA – gamma-Aminobutyric acid

- 1 GWAS – genome-wide association study
- 2 SZ – schizophrenia
- 3 BPD – bipolar disorder
- 4 MDD – major depression disorder
- 5 CTL – control
- 6 AnCg – anterior cingulate gyrus
- 7 DLPFC – dorsolateral prefrontal cortex
- 8 nAcc – nucleus accumbens
- 9 GO – gene ontology
- 10 ChIP-seq – chromatin immunoprecipitation with DNA sequencing
- 11 PCA – principal component analysis

12 **Declarations**

13 **Ethics approval and consent to participate**

14 Sample collection, including human subject recruitment and characterization, was
15 conducted as part of the Brain Donor Program at the University of California, Irvine,
16 Department of Psychiatry and Human Behavior (Pritzker Neuropsychiatric Disorders
17 Research Consortium) under IRB approval (UCI 88-041, UCI 97-74).

18 **Consent for publication**

19 Not applicable

20 **Availability of data and materials**

1 The datasets supporting the conclusions of this article are available in the GEO
2 repository, GSE80655.

3 **Competing interests**

4 The authors declare that they have no competing interests.

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9 **Author's Contributions**

10 HA, SJW, AFS, WEB, JDB, HK, SJC and RMM conceived of study
11 KMB, RCR, BNL, SJC, AAH, MH, JZL and RMM designed the experiments
12 EGJ performed brain dissections
13 PMC procured the brain tissue samples
14 MPV analyzed pH on all cases and matched the 4 cohorts
15 DWM obtained demographic and clinical data on all subjects through analyses of
16 medical records and next-of-kin interviews
17 NSD, JG, and KMB collected RNAs and performed Tn-RNA-seq library construction
18 RCR and BNL analyzed the RNA-seq data
19 RCR and SJC performed and analyzed metabolomics experiments
20 KMB, RCR, and BNL wrote the first draft of the paper

1 JZL, BGB, WEB, SJW, SJC, HA and RMM contributed to the writing of the paper

2 All authors read and approved the final manuscript.

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10 **Figure Legends**

11 **Figure 1.** (A) Histograms of case vs. control differential expression (DESeq2 P -values)
12 for SZ (red), BPD (blue), and MDD (green) in each brain region assayed. (B) Pairwise
13 spearman correlations of \log_2 fold gene expression changes between each disorder and
14 CTL in each brain region. Circle sizes are scaled to reflect Spearman correlations. (C)
15 Venn diagram showing overlap of genes differentially expressed between SZ (red), BPD
16 (blue), MDD (green) vs. CTL at a p -value <0.05 . (D) \log_2 fold expression change
17 correlation of 87 genes with $FDR<0.05$ comparing SZ and CTL (AnCg) in the Pritzker
18 dataset with the SNCID dataset (Spearman coefficient=0.812, p -value <0.0001).
19 Transcripts differentially expressed at an $FDR<0.05$ in both cohorts are identified with
20 red circles. (E) Hierarchical clustering 27 SZ and 26 CTL tissues in the SNCID dataset
21 using variance-stabilized expression of 87 significant genes between SZ and CTL in the
22 AnCg identified by DESeq2 ($FDR<0.05$) in the Pritzker dataset. CTL (black), SZ (red),
23 lowly expressed genes (blue pixels), highly expressed genes (yellow pixels).

1 **Figure 2.** (A) Boxplots indicating relative expression of *EGR1* in the AnCg of SZ (red),
2 BPD (blue), MDD (green), and CTL (gray). (B) Correlation plot comparing RNA-seq
3 measured expression level of *EGR1* to qPCR measured expression in 10 SZ (red) and
4 10 CTL (black) patients. (C) Wilcoxon *P*-values resulting from comparing the degree of
5 differential expression (based on DESeq2 *P*-values) of genes whose TSSs neighbor
6 *EGR1* binding sites to genes whose TSSs are greater than a range of distance
7 thresholds.

8 **Figure 3.** Boxplots indicating neuron- (A) and astrocyte- (B) specific expression indices
9 in the AnCg for SZ (red), BPD (blue), MDD (green), and CTL (gray) individuals. (C)
10 Correlation plot comparing the \log_2 expression fold change between SZ and CTL
11 patients in the AnCg and the \log_2 expression fold change between dissected neurons
12 and all other dissected brain cell types (astrocytes, oligodendrocytes, endothelial cells,
13 and microglia).

14 **Figure 4.** Hierarchical clustering of (A) 25 metabolites that differ most between SZ (red)
15 and CTL (black) individuals, and (B) 25 metabolites that differ most between BPD (blue)
16 and CTL (black) individuals. (C) Boxplots indicating relative expression of GAD1 and
17 GAD2 enzymes in the AnCg of SZ (red) and CTL (gray) patients. (D) Correlation plot
18 comparing average GAD1 and GAD2 expression and the GABA/Glutamate metabolite
19 level ratio in the AnCg of SZ (red) and CTL (black) individuals.

20 **Supplementary Figure Legends**

21 **Figure S1.** A) Principal components analysis of all 281 brain tissues. AnCg (red
22 squares), DLPFC (blue triangles), nAcc (green circles). B) Principal components
23 analysis of all 281 brain tissues. CTL (gray squares), BPD (blue triangles), MDD (green

1 circles), SZ (red triangles). (C) Principal components analysis of all 281 brain tissues
2 after correcting RNA-seq data for alignment quality. CTL (gray squares), BPD (blue
3 triangles), MDD (green circles), SZ (red triangles).

4 **Figure S2.** Principal components analysis of all AnCg (A,D), DLPFC (B,E), and nAcc
5 (C,F) samples before and after correction for RNA-seq alignment quality. (G-I) PC1
6 values in CTL (gray), BPD (blue), MDD (green), and SZ (red) patients pre- and post-
7 RNA-seq alignment quality correction in the AnCg (G), DLPFC (H), and nAcc (I).

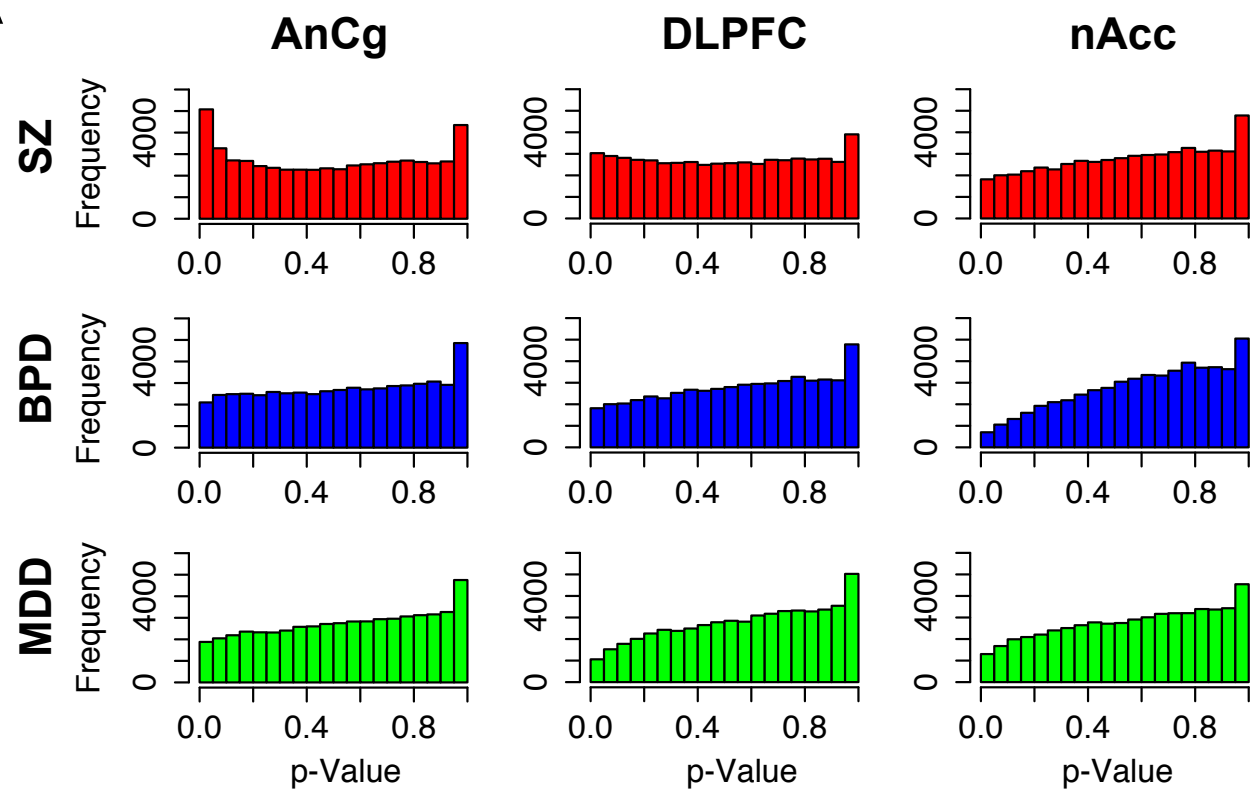
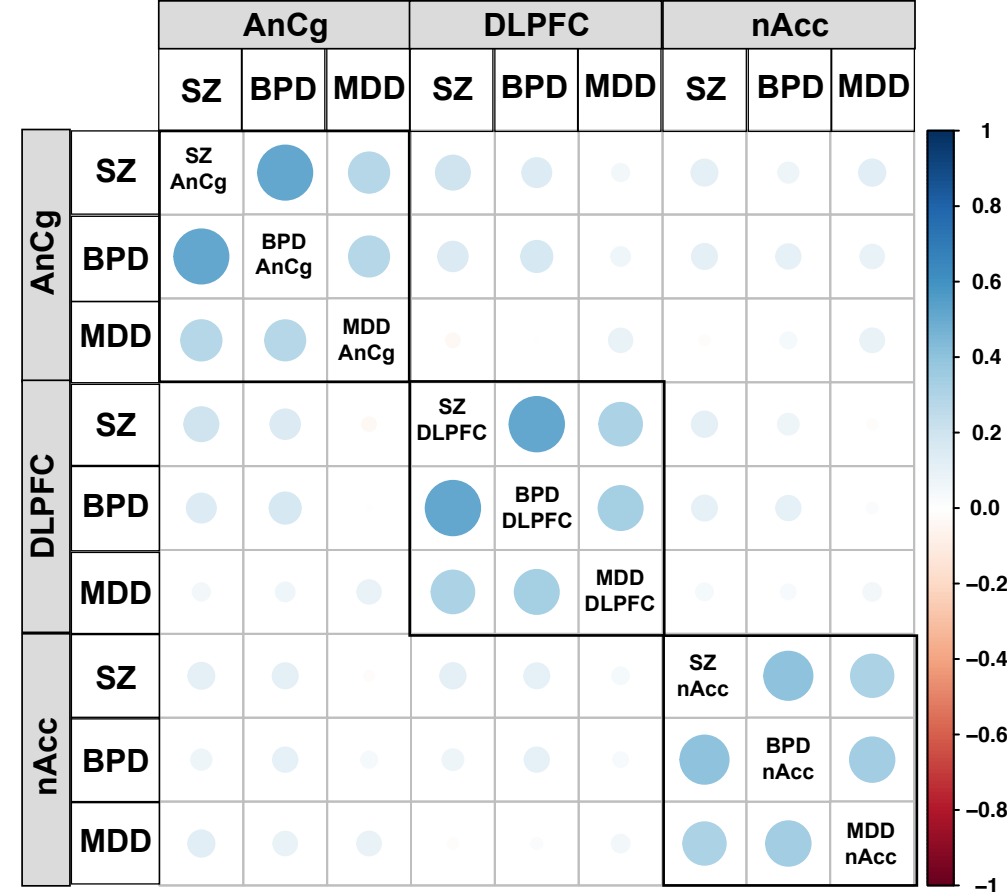
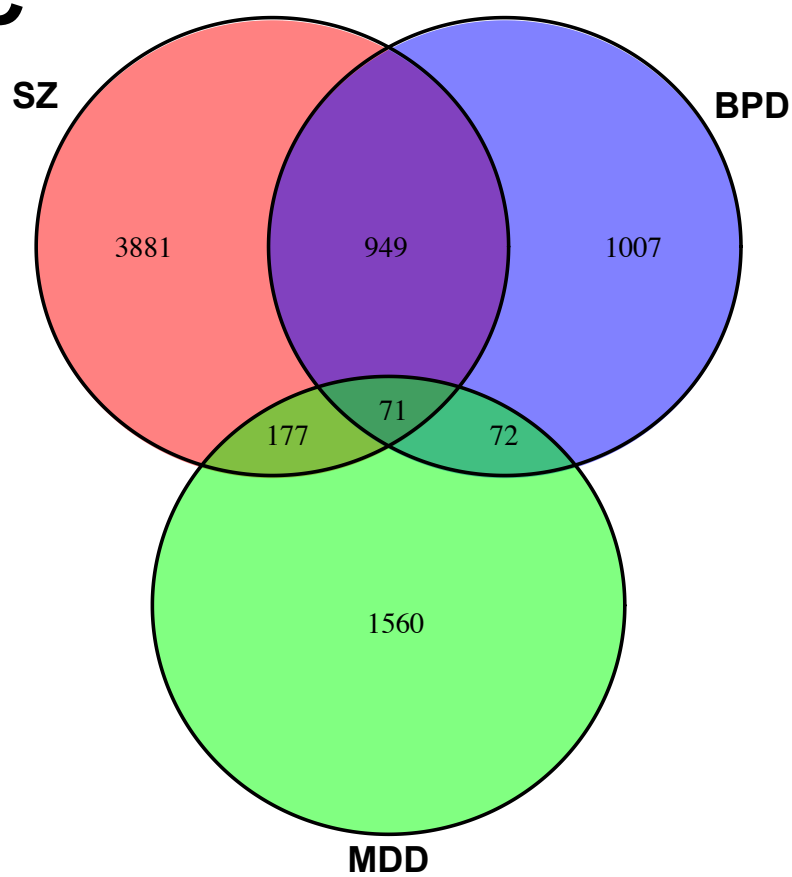
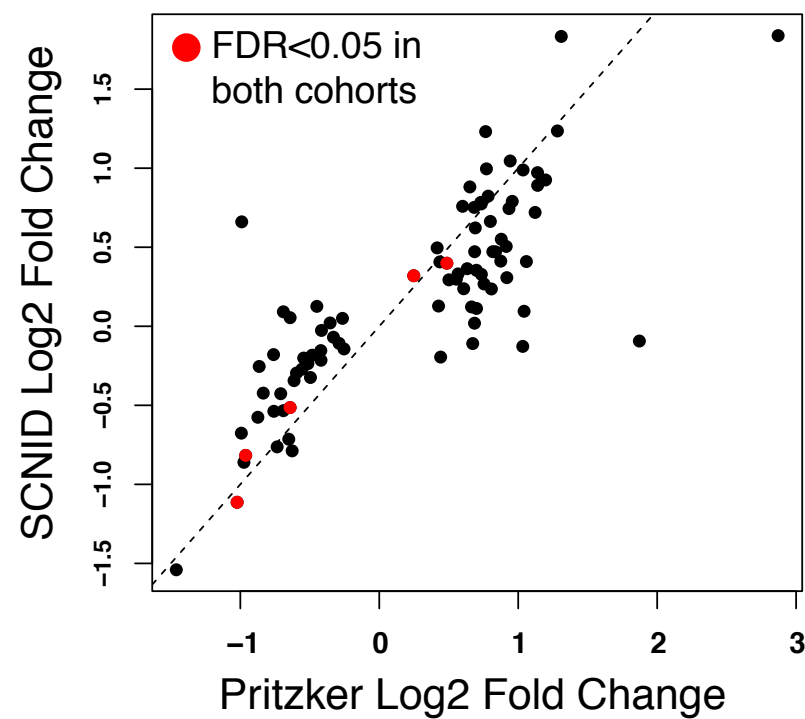
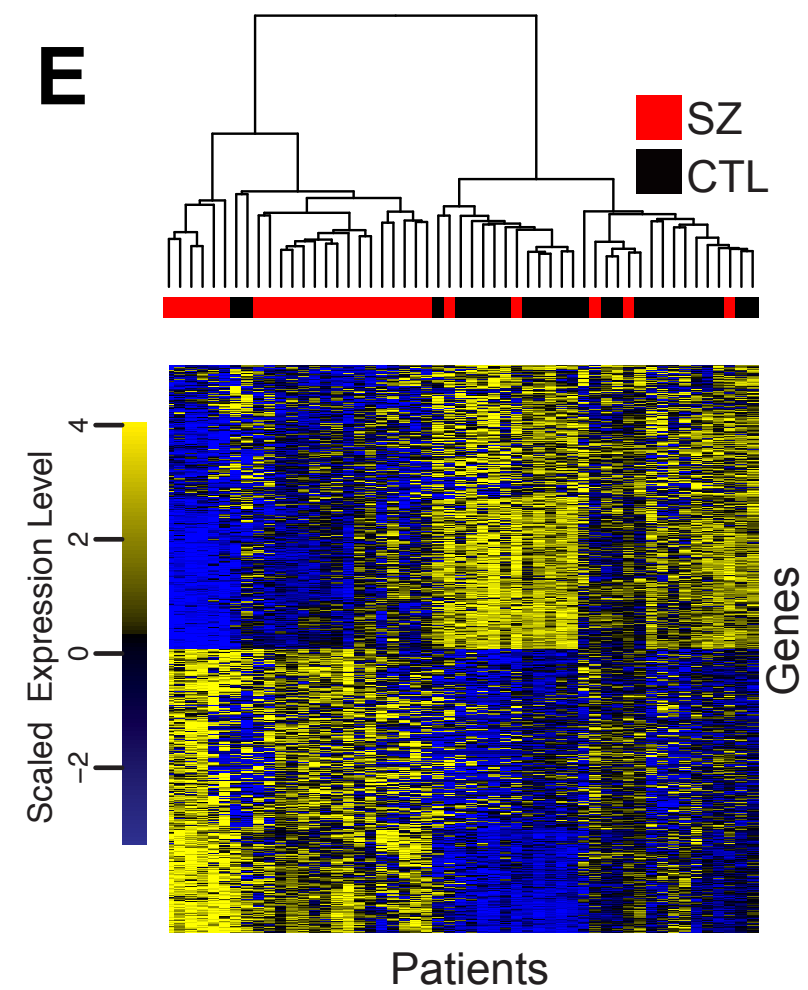
8 **Figure S3.** GO-term analysis for transcripts differentially expressed in SZ vs. CTL in
9 AnCg (FDR<0.05). Up-regulation (red circles), down-regulation (blue circles).

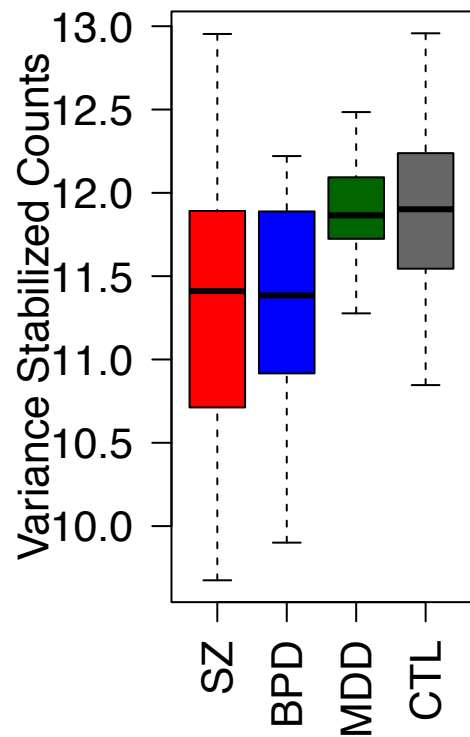
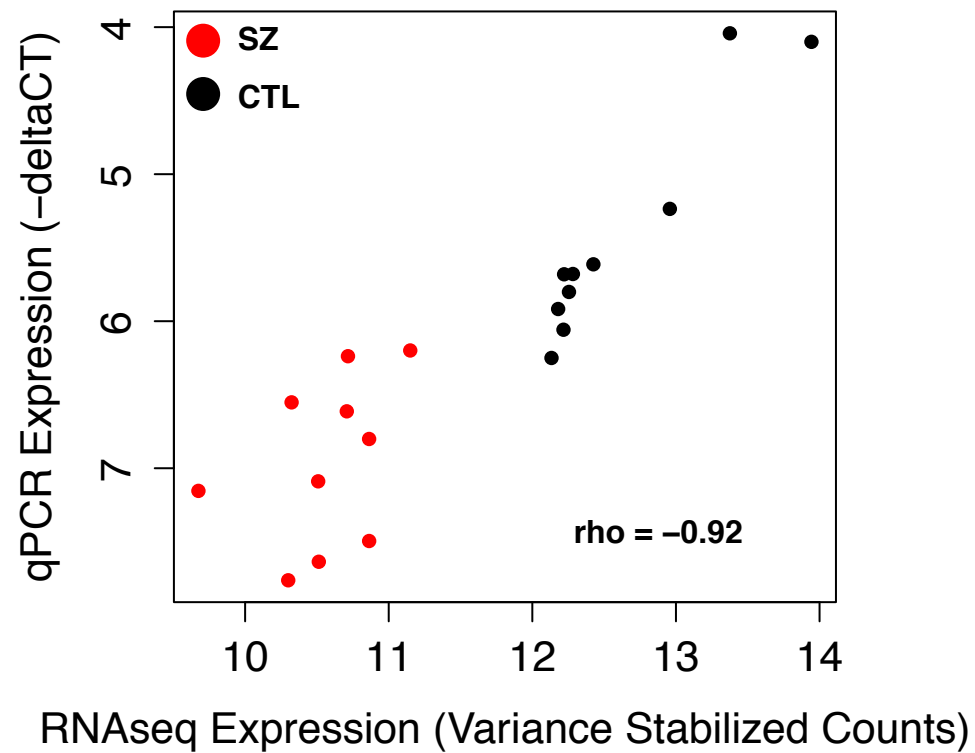
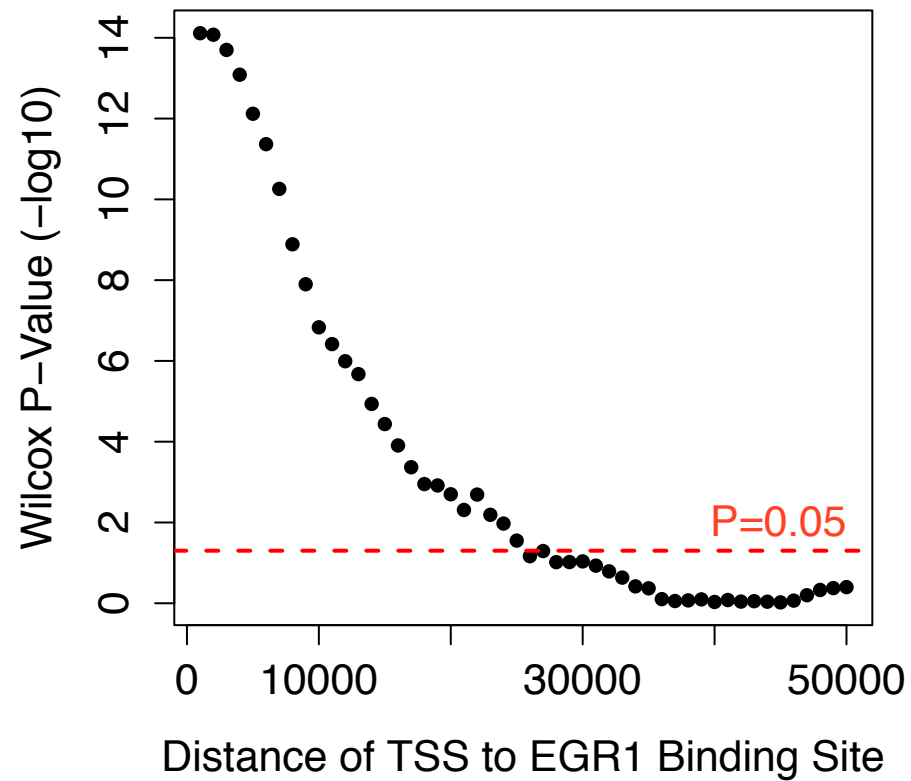
10 **Figure S4.** Examination of cell-type specific index in purified (A) neuron, (B) astrocytes,
11 (C) oligodendrocytes, (D) microglia, and (E) endothelial cells from brain tissue. (F)
12 Neuron and astrocyte indices are capable of predicting *in silico* mixed cell-type
13 proportions. (G) Mean values with standard deviation for predictions of indices
14 generated on 10,000 randomly sampled, null transcript sets. (H, I) Histogram of mean
15 squared error of null index cell type proportion predictions for mixed neuron and
16 astrocyte transcriptomes with Darmanis et al. transcript performance indicated in red.

17 **Figure S5.** Boxplots of endothelial (A), microglia (B), and oligodendrocyte (C) cell type
18 indices in SZ (red), BPD (blue), MDD (green), and CTL (gray) individuals.

19 **Figure S6.** Hierarchical clustering of 25 metabolites with levels that differ most between
20 MDD (green) and CTL (black) individuals.

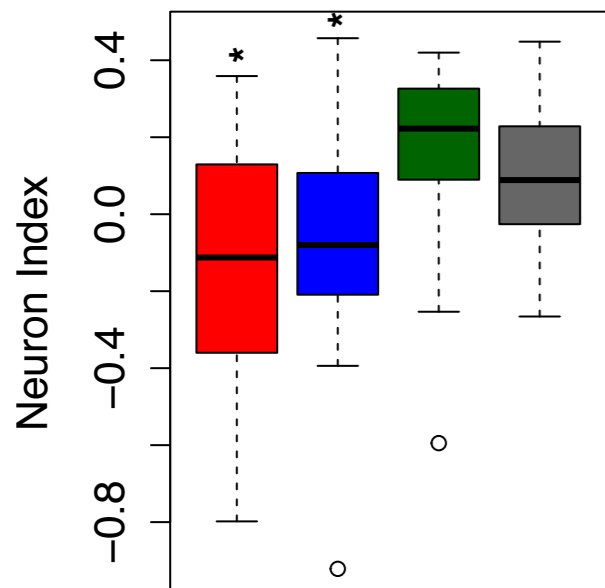
21 **Figure S7.** Integrated KEGG pathway analysis of metabolite and RNAseq differences
22 between SZ and CTL patients. Top 10 pathways shown for metabolite, transcript and
23 combined analysis.

A**B****C****D****E**

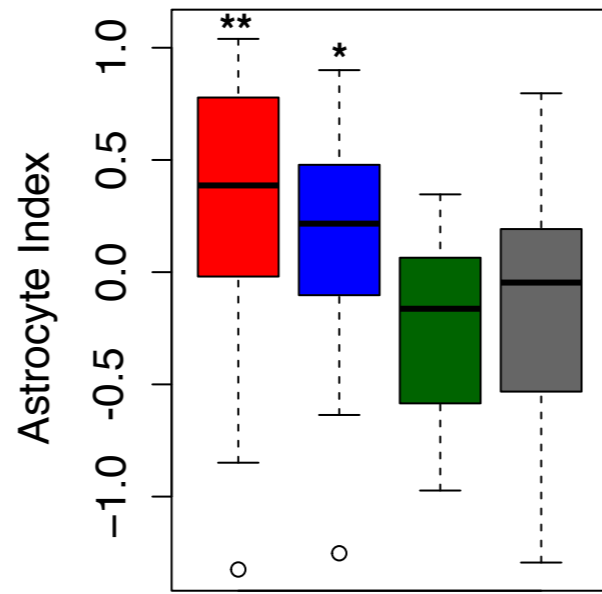
A**B****C**

A

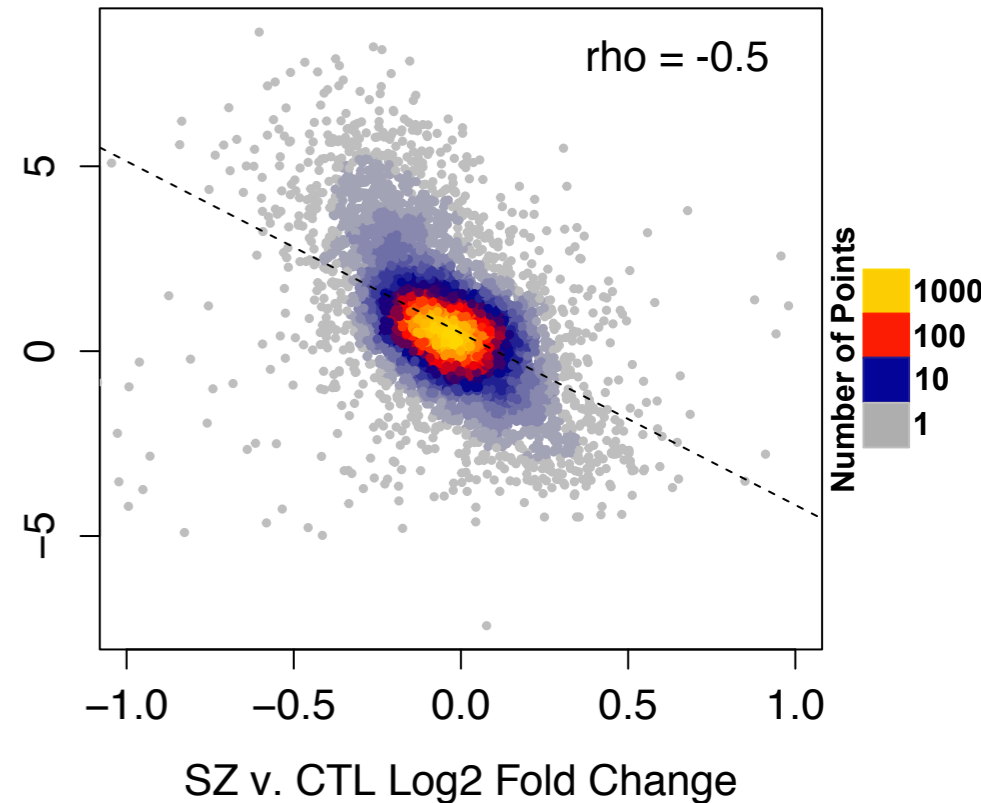
Neuron Specific Expression

**B**

Astrocyte Specific Expression

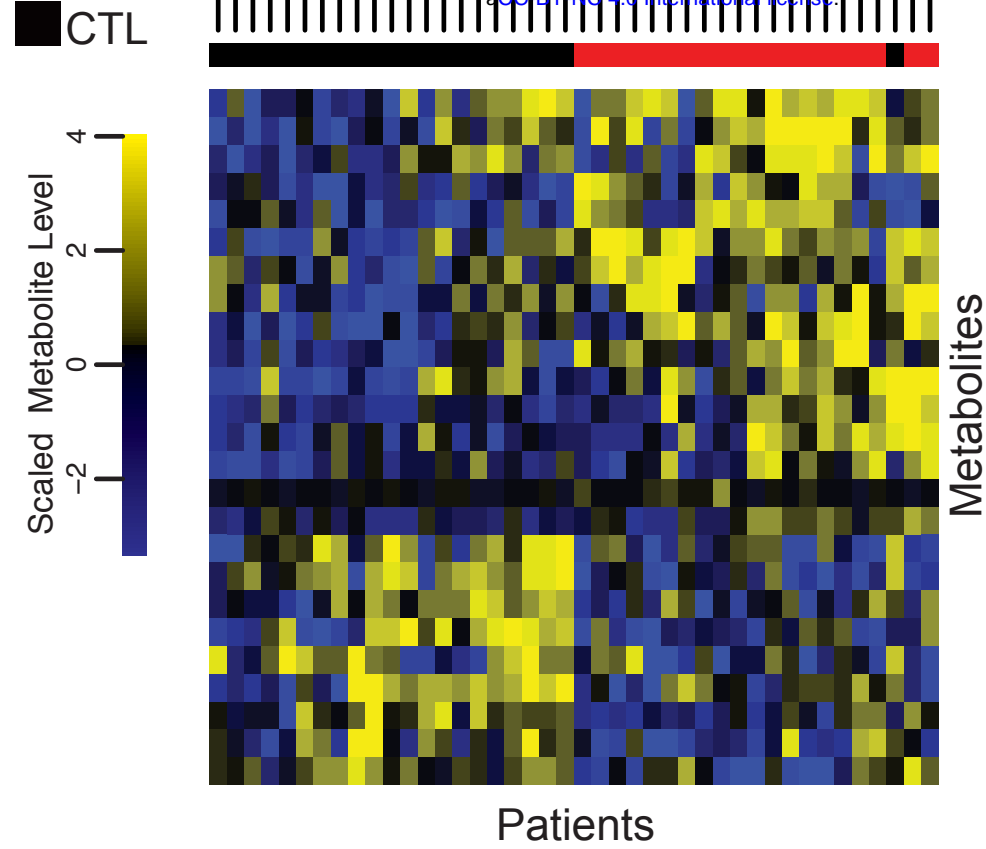
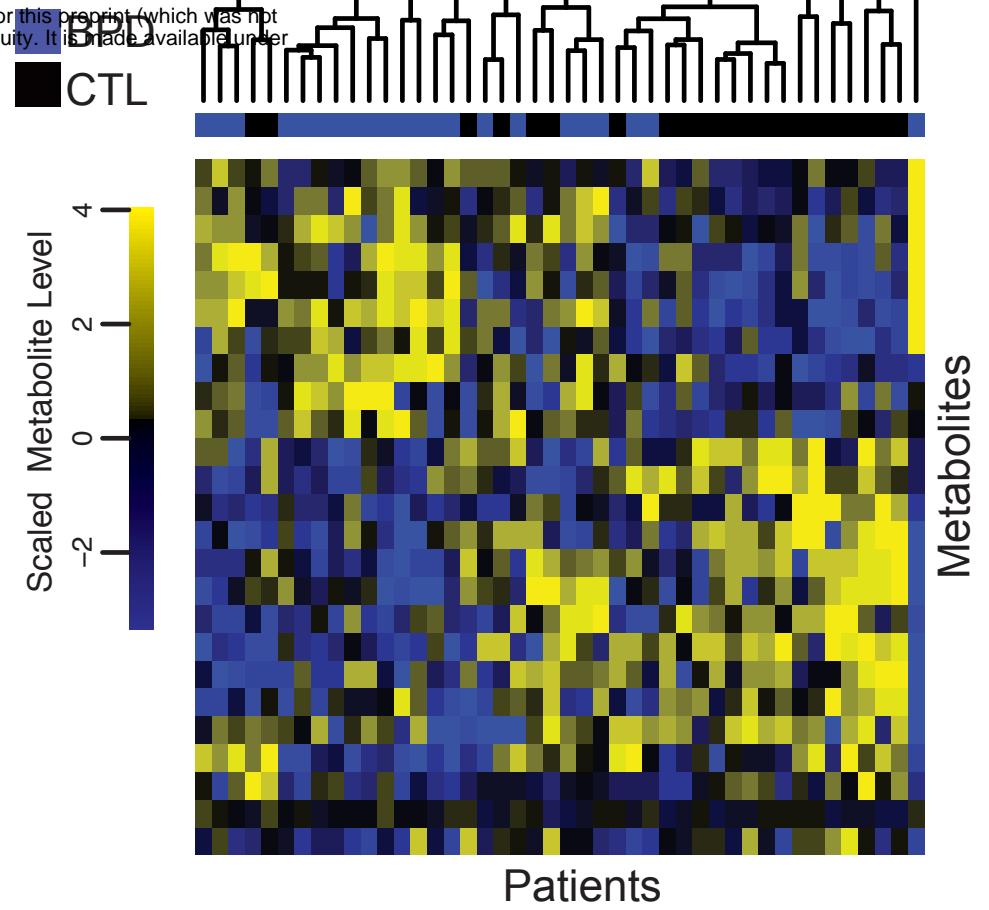
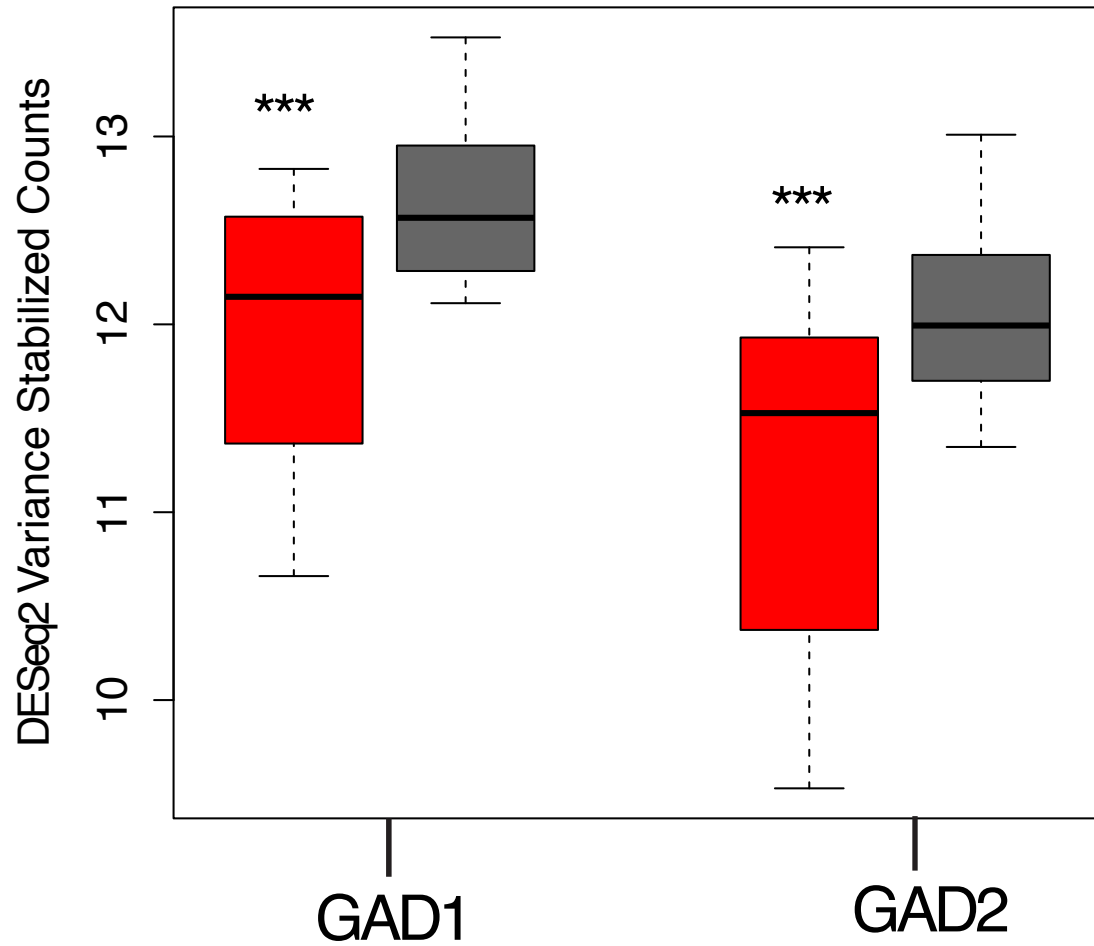
**C**

Log2 Fold Enrichment in Neurons

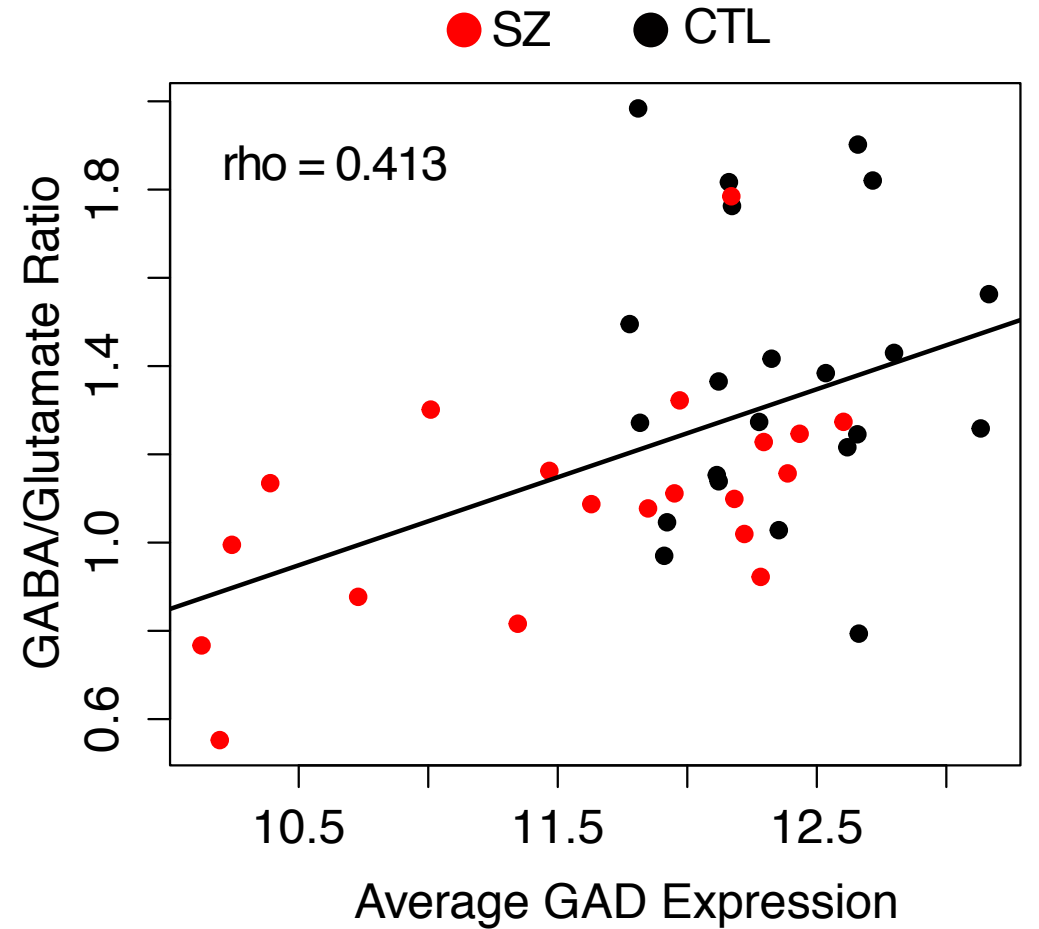


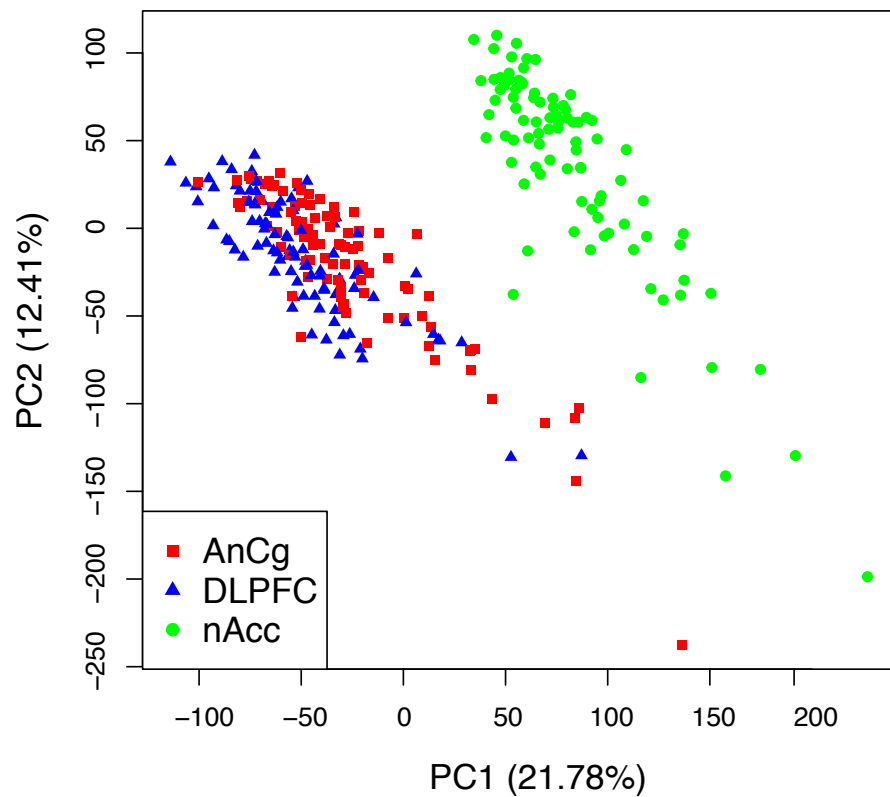
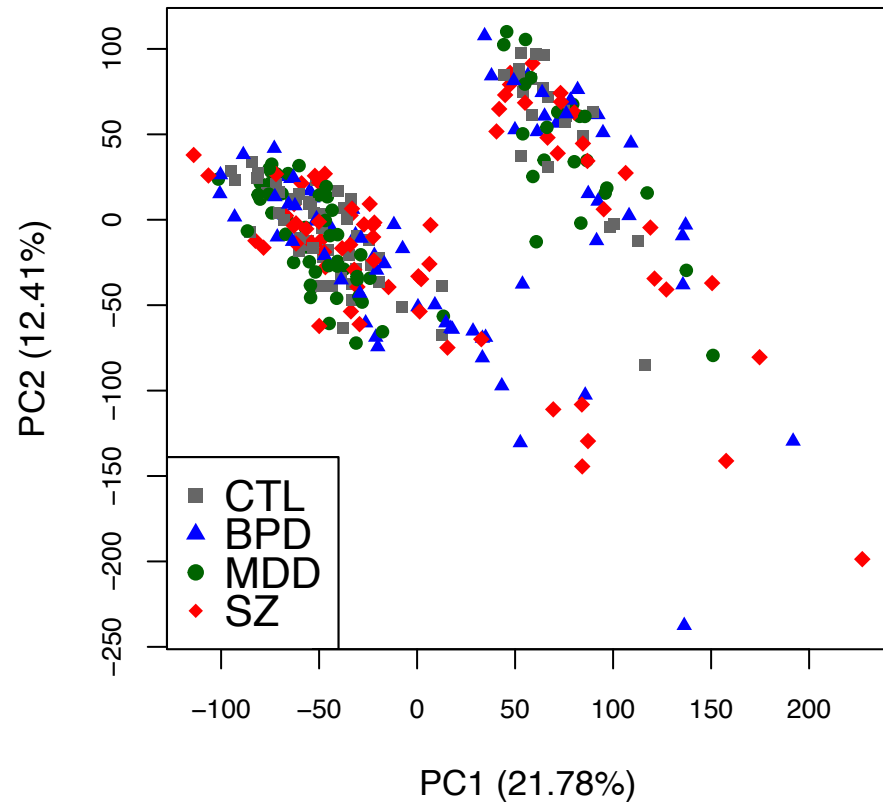
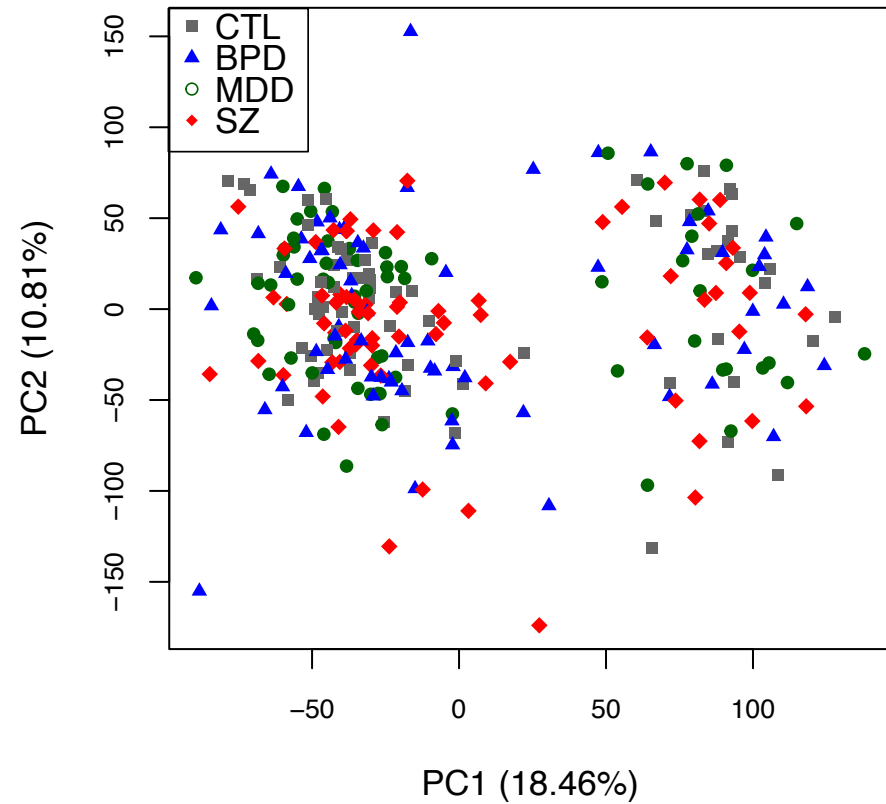
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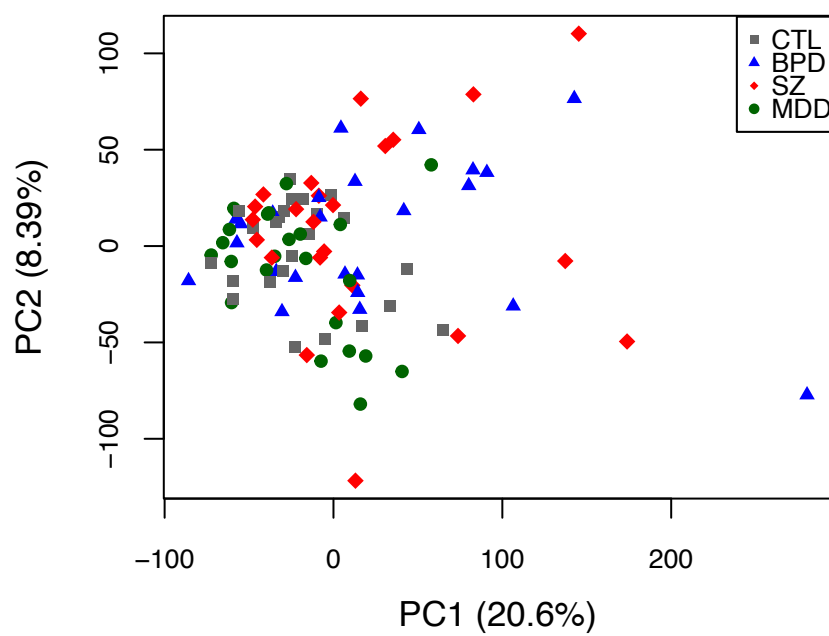
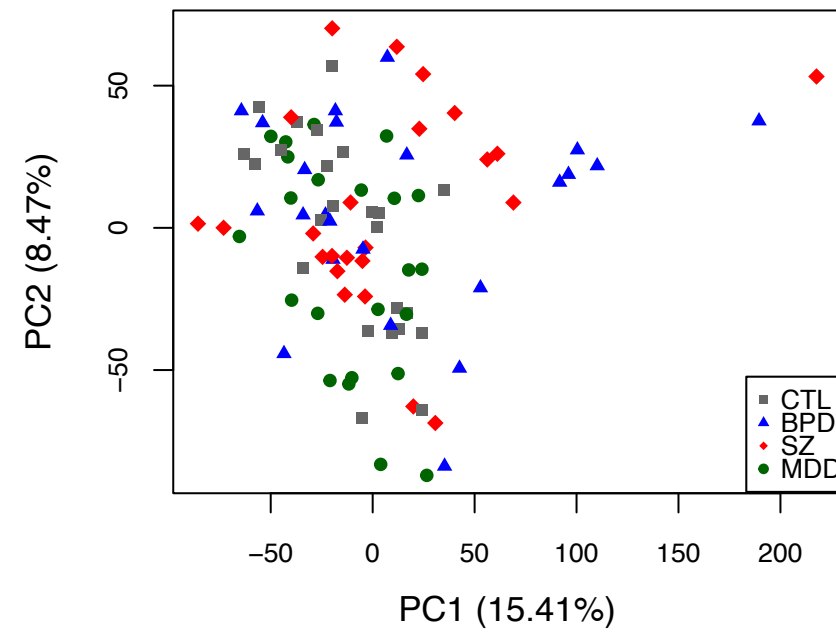
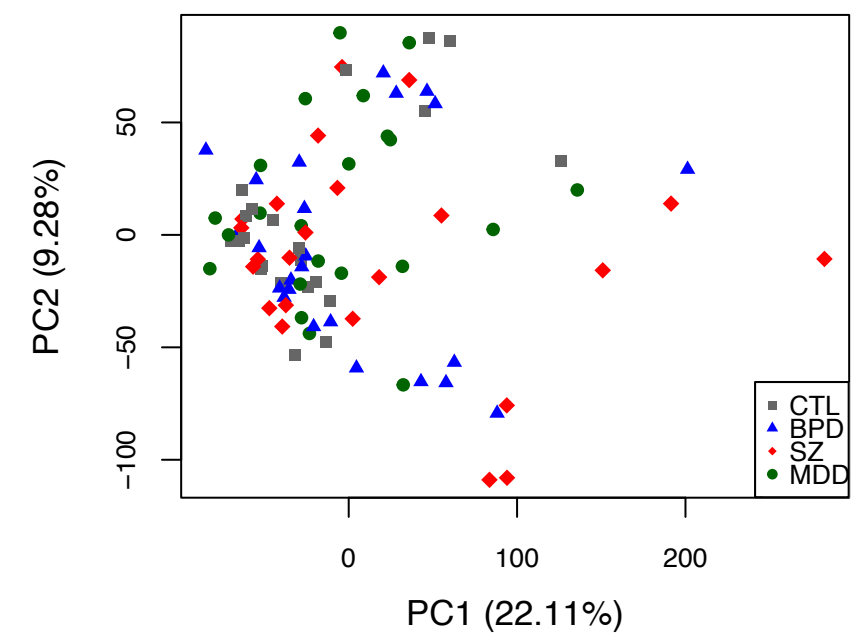
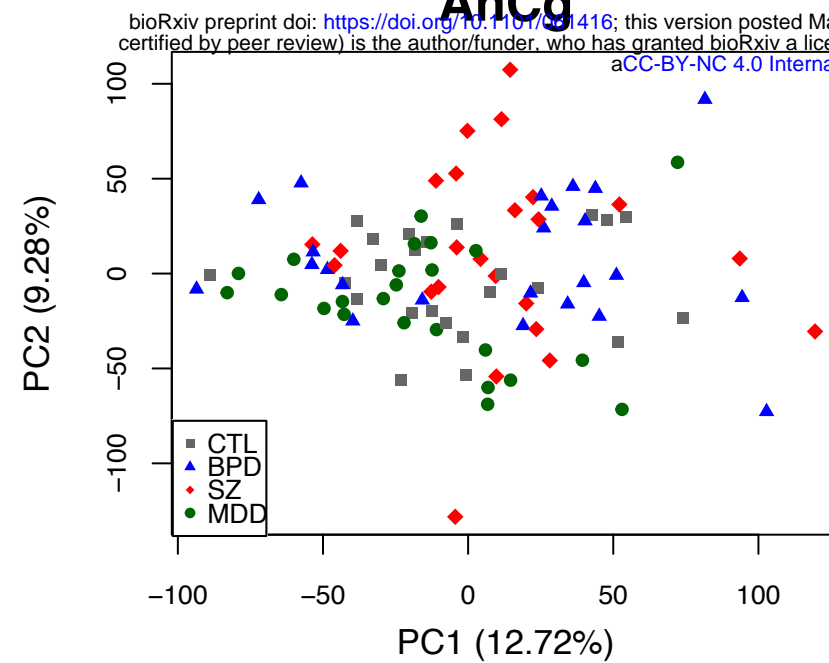
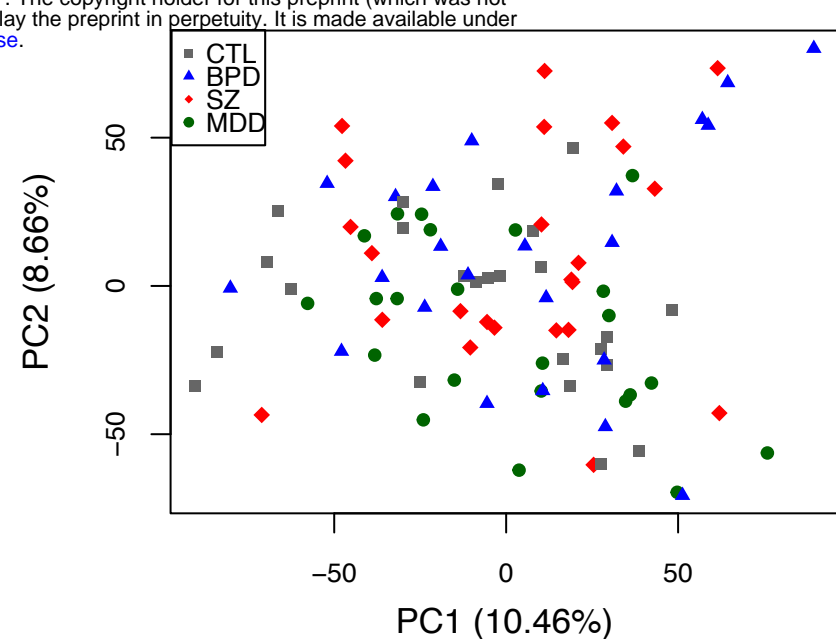
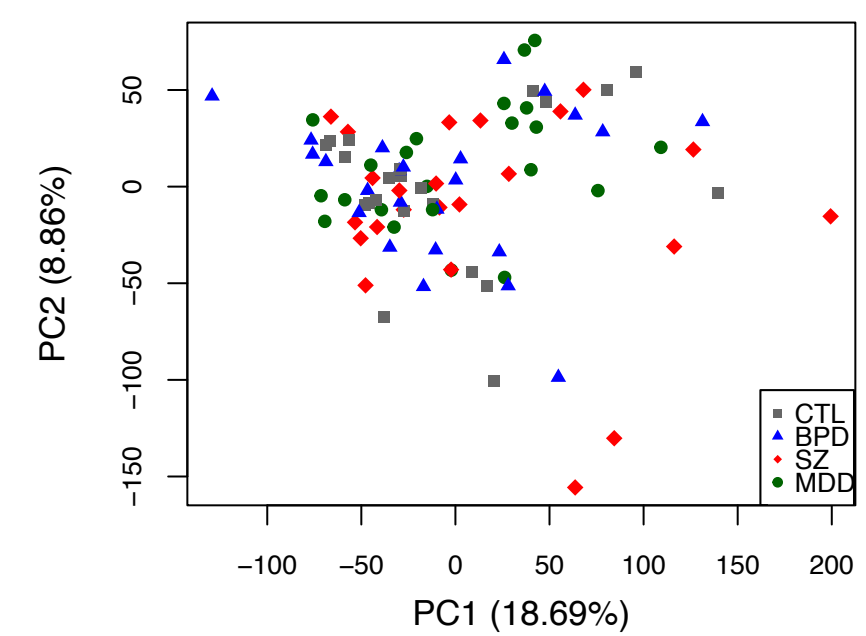
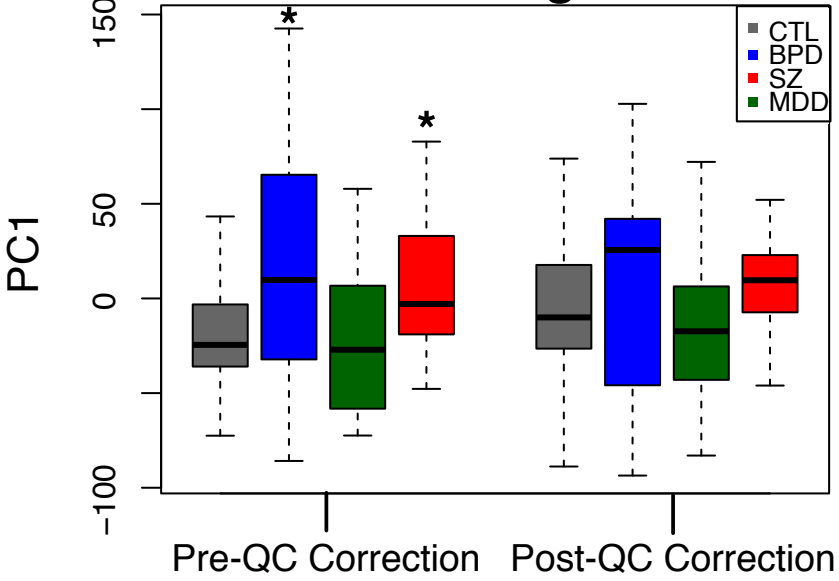
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**B****C**

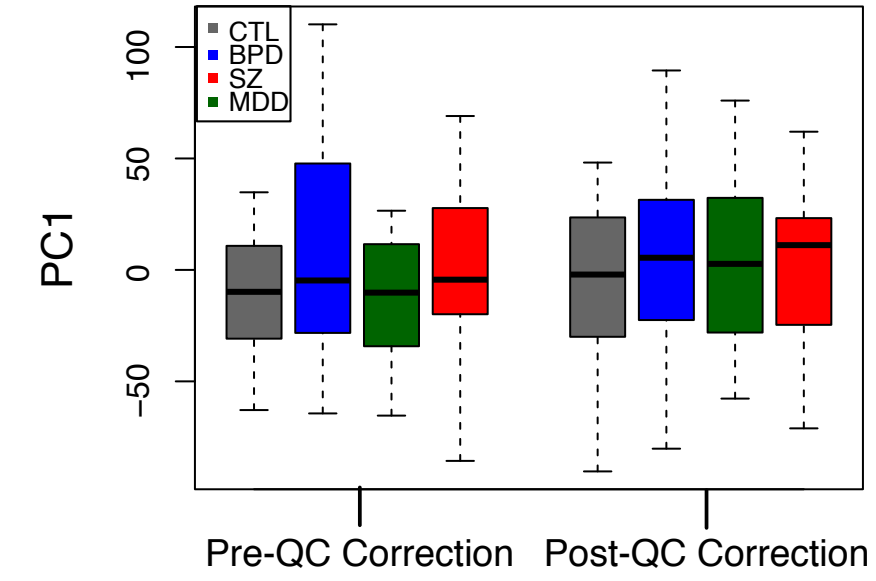
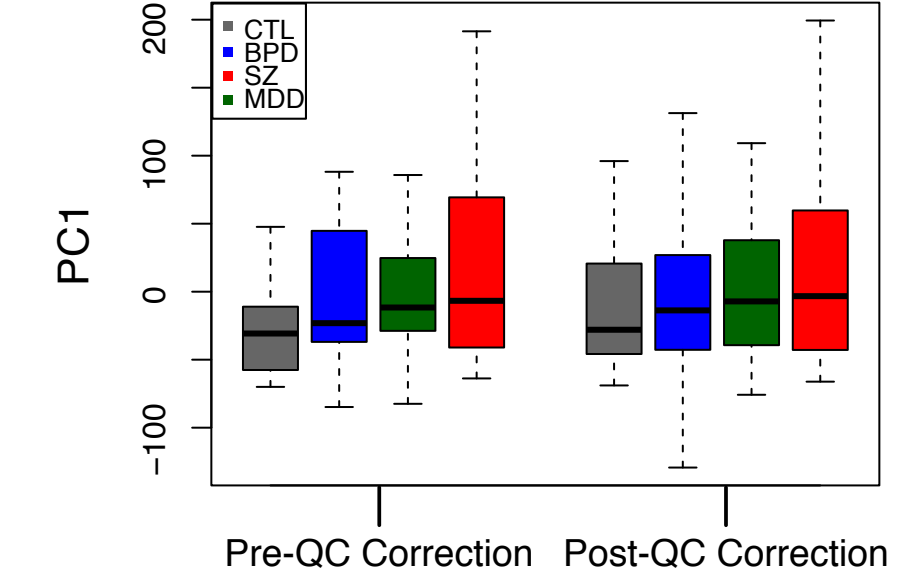
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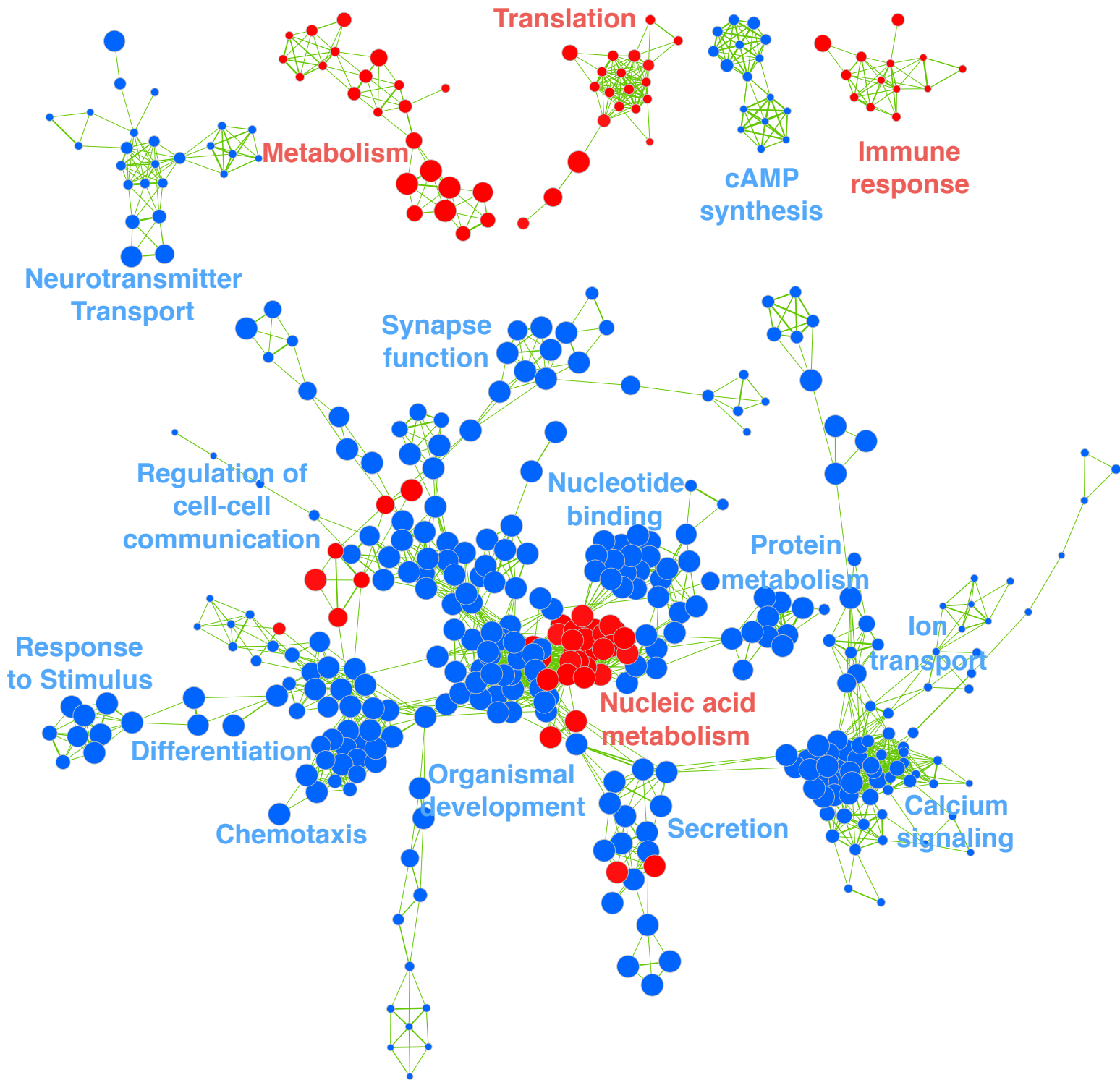
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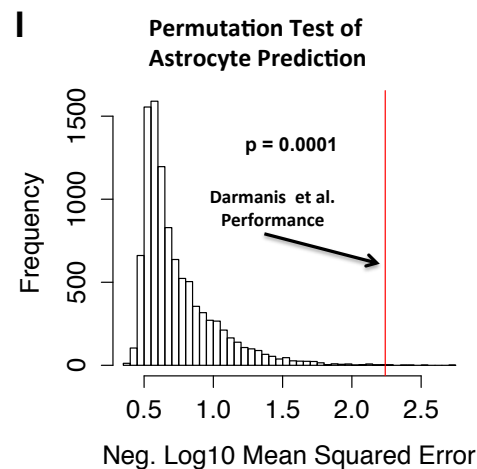
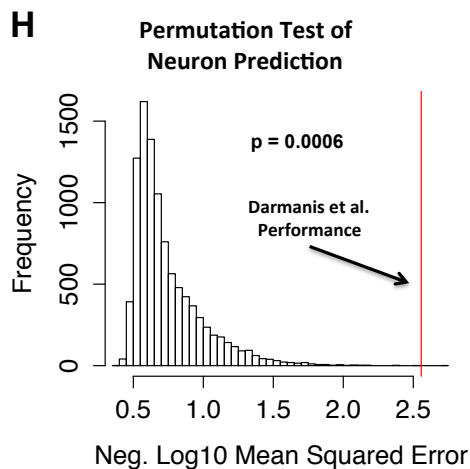
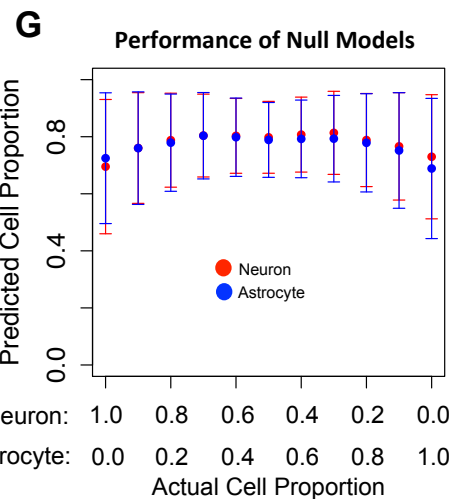
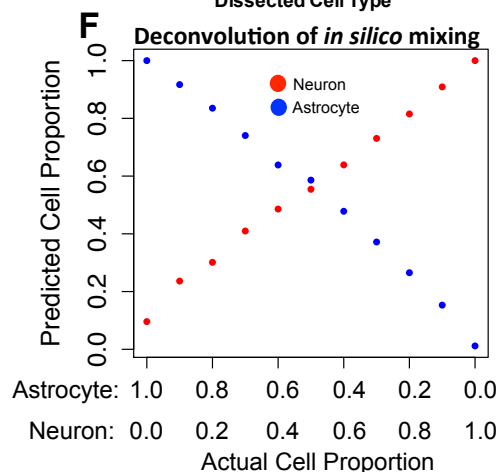
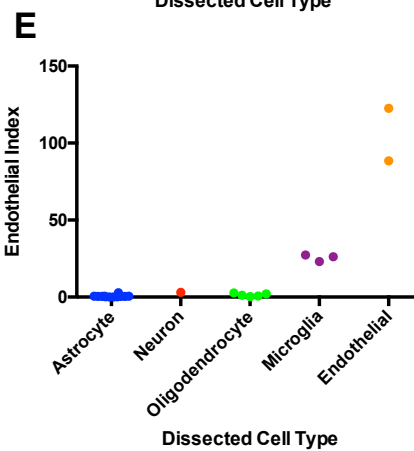
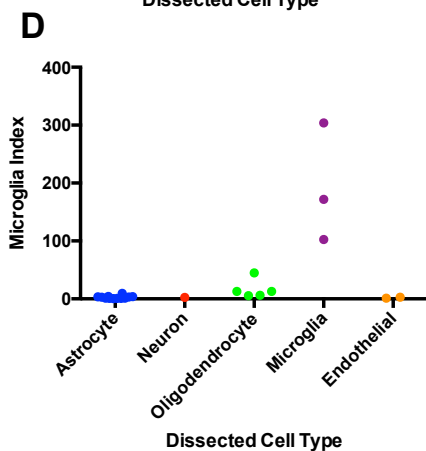
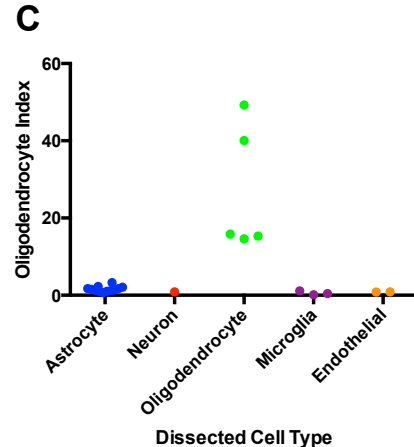
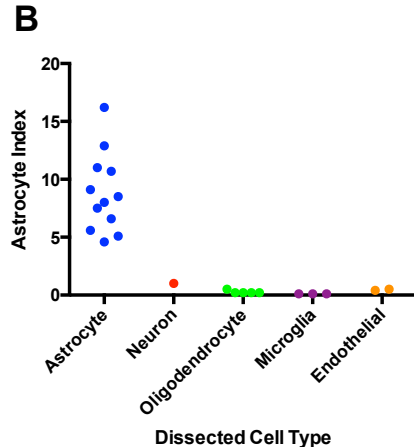
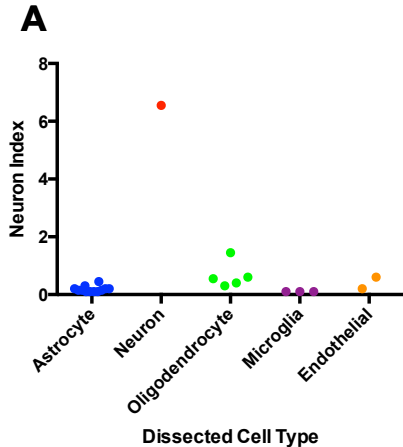
A**Colored by Brain Region****B****Colored by Disorder****C****Corrected for RNA Quality**

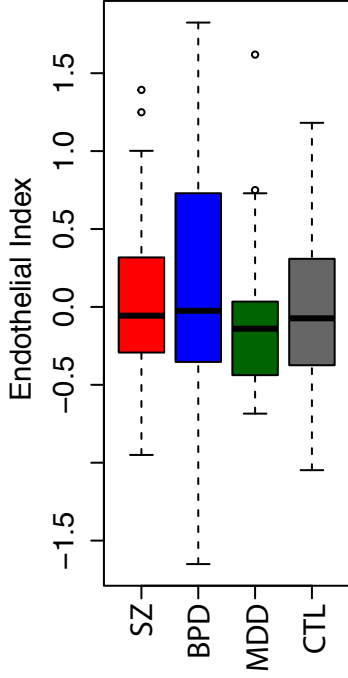
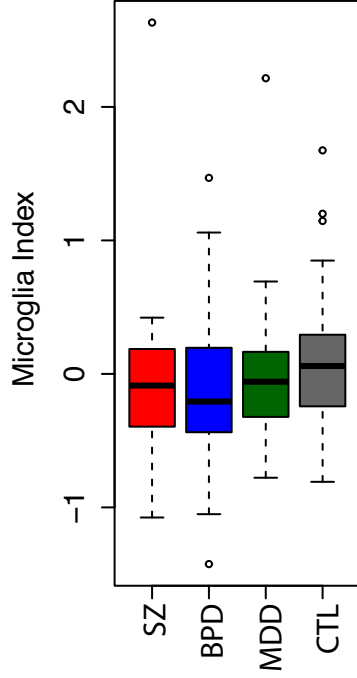
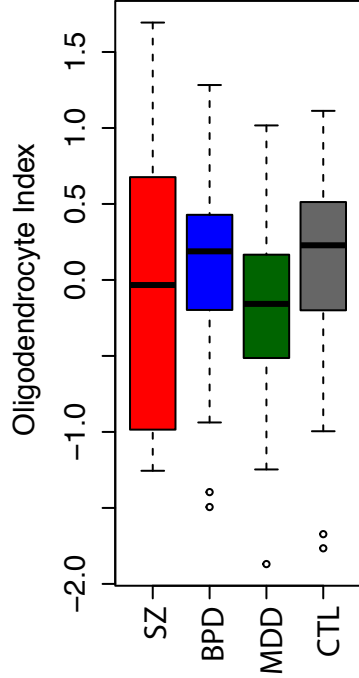
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*Wilcox P-Value < 0.05

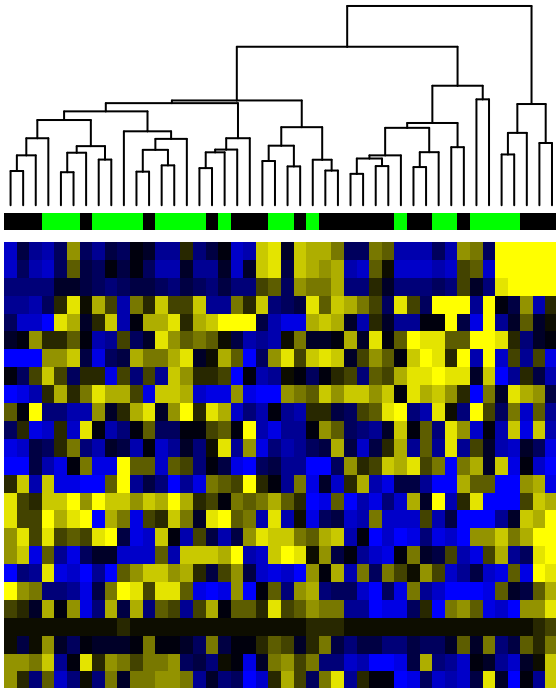
H**DLPFC****I****nAcc**



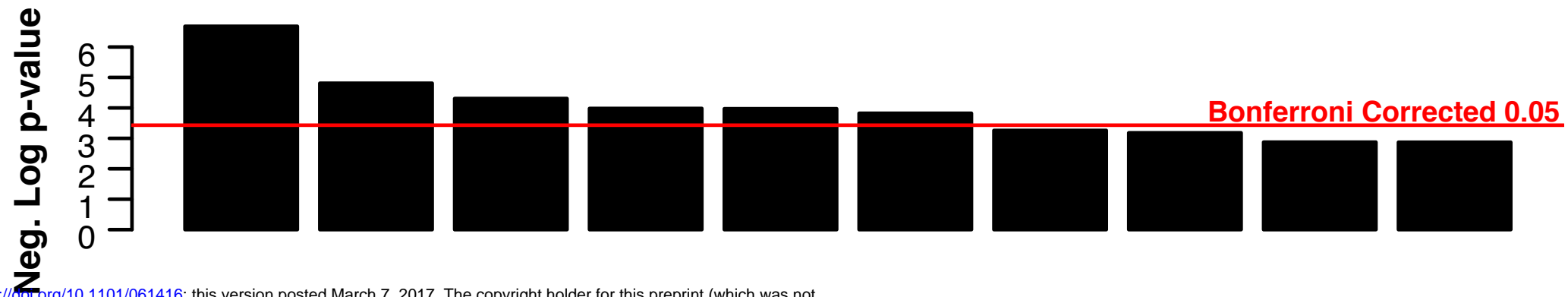


A**B****C**

MDD
CTL



Combined RNAseq and Metabolomics Pathway Enrichment



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RNAseq Pathway Enrichment



Metabolite Pathway Enrichment

