Coding and non-coding RNA abnormalities in bipolar disorder

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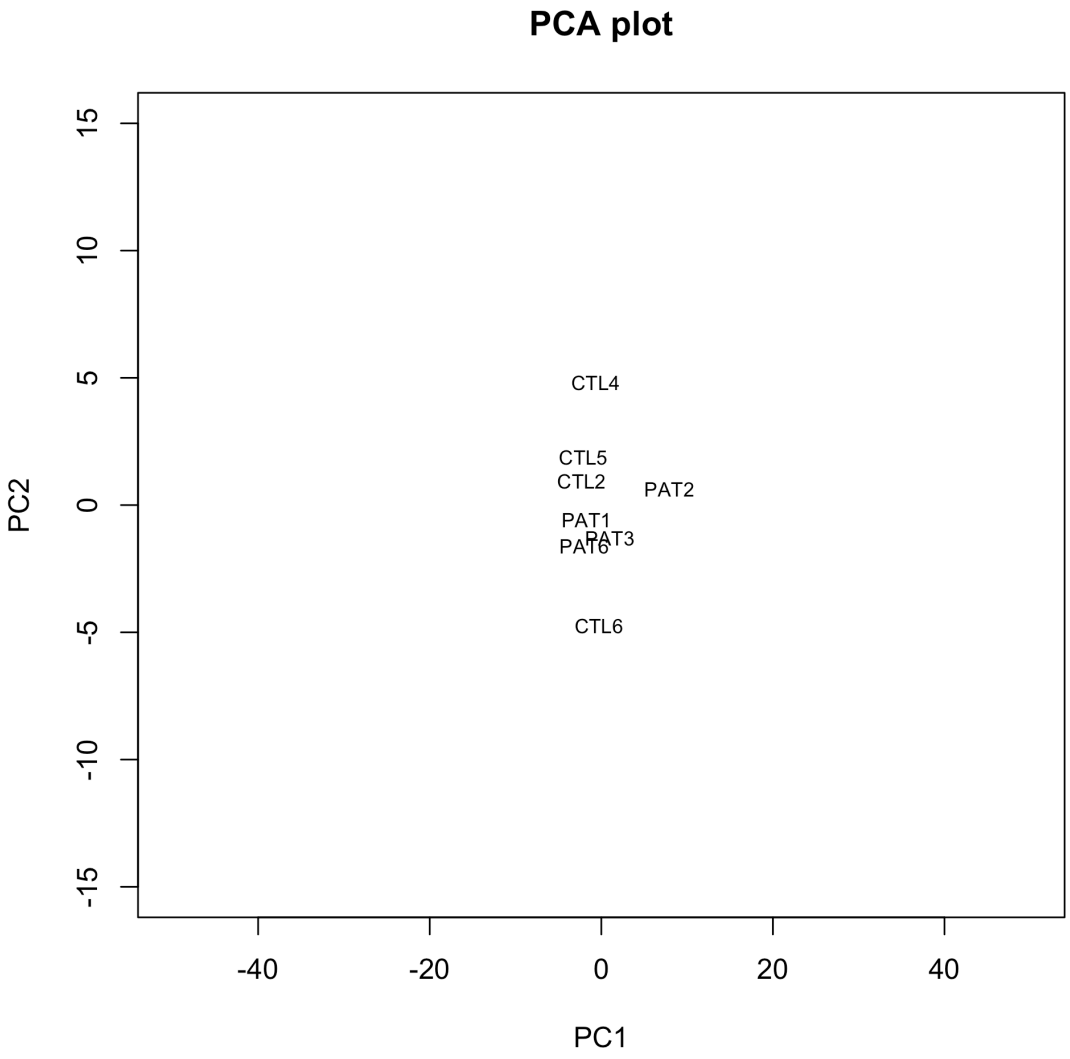
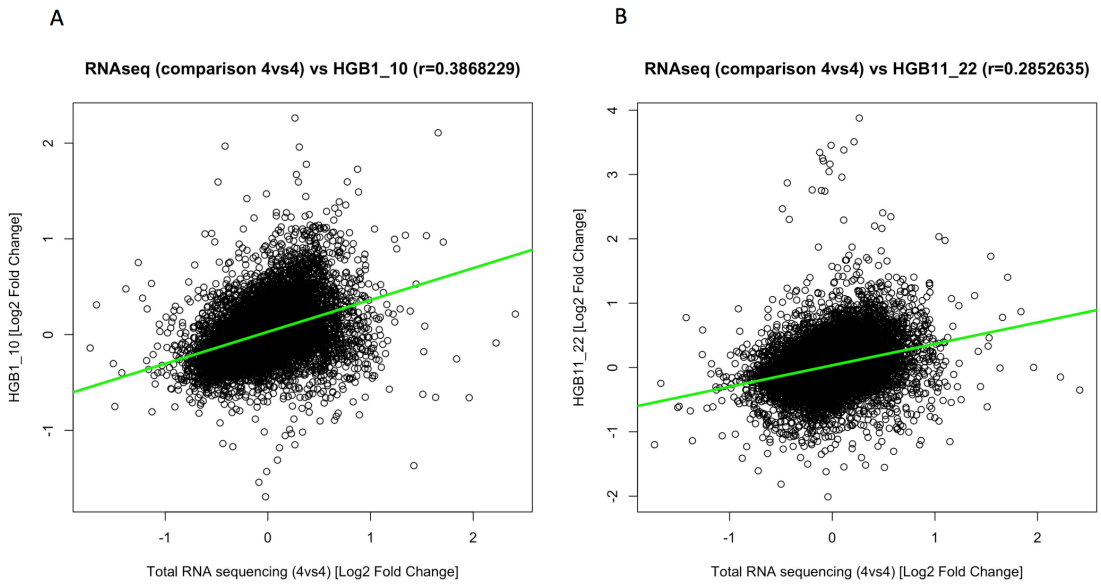
Supplemental Results

Supplemental Figure S1. Principal components analysis of all four cases (PAT1, PAT2, PAT3 and PAT6) and the controls (CTL2, CTL4, CTL5, and CTL6) used for sequencing. The clustering indicates no gross differences in clusering between cases and controls, rendering any case-control differences other than disease status driving our results unlikely.



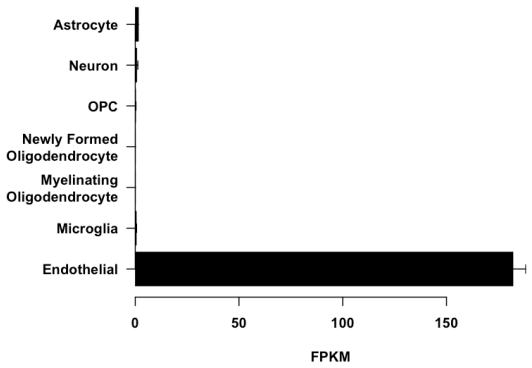
Supplemental Figure S2. Correlation analyses between our dataset and two independent sets of dorsolateral prefrontal cortex (DLPFC) brain samples published by Akula and colleagues.

Significant correlation in log2-fold change values (log2[mean(BPD)/mean(CTL)]) of genes from our dataset with the HGB1_10 set ($r=0.38$, $p < 2.2x10^{-16;}$ A). Significant correlation in log2-fold change values of genes from our dataset with the HGB11_22 set ($r=0.28$, $p < 2.2x10^{-16}$; B).

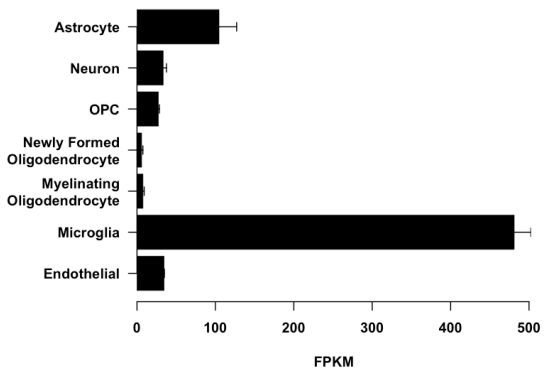


Supplemental Figure S3 A-E. Cell-type specificity of each of the top five differentially expressed genes between bipolar disorder cases and controls: of the genes *Cd93* (A); *Socs3* (B); *Bcl6b* (C); *Tnfrs11b* (D); and *Podxl* (E).
All but one gene (*Socs3*) were found to have more than 10-fold higher expression levels in endothelial tissue than any other cell type; FPKM, fragments per kilobase of transcript sequence per million mapped fragments.

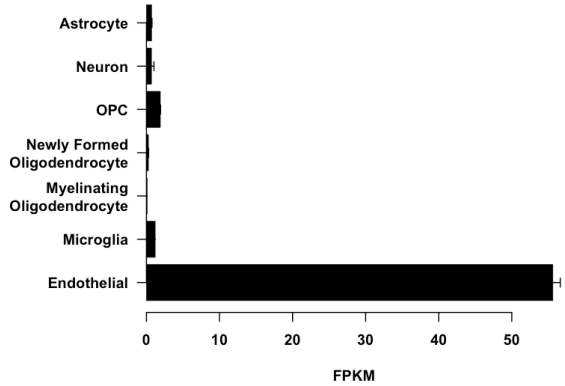
A



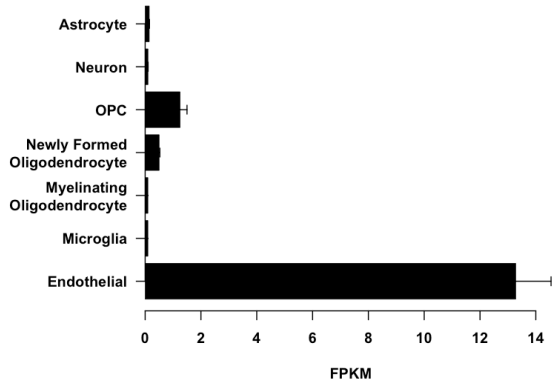
B



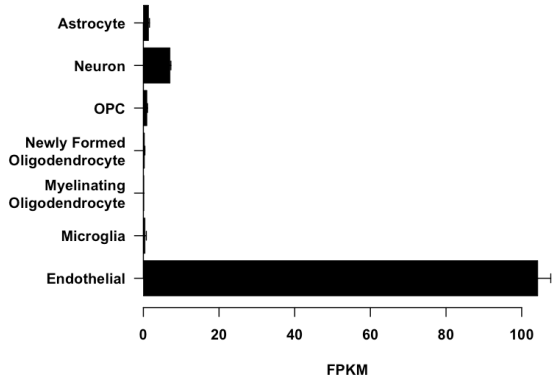
C


Astrocyte
Neuron
OPC
Newly Formed Oligodendrocyte
Myelinating Oligodendrocyte
Microglia
Endothelial
0
10
20
30
40
50
FPKM

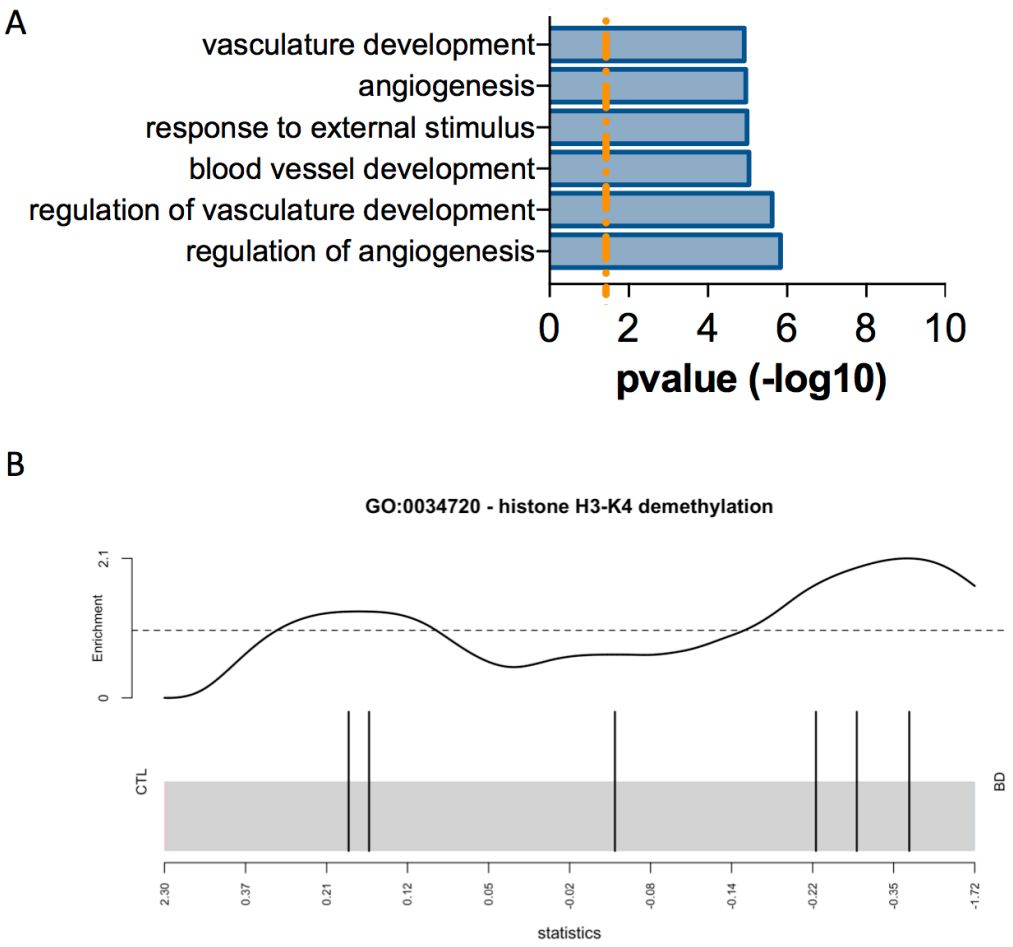
D


Astrocyte
Neuron
OPC
Newly Formed Oligodendrocyte
Myelinating Oligodendrocyte
Microglia
Endothelial
0
2
4
6
8
10
12
14
FPKM

E


Astrocyte
Neuron
OPC
Newly Formed Oligodendrocyte
Myelinating Oligodendrocyte
Microglia
Endothelial
0
20
40
60
80
100
FPKM

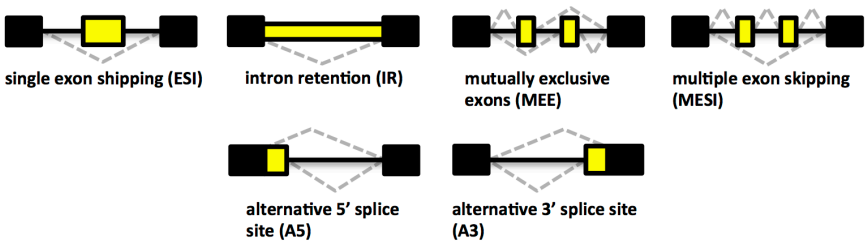
Supplemental Figure S4. Functional characterization of dysregulated pathways in BPD brain. Enrichments for GO biological processes among the 36 differentially expressed genes (DEGs) at FDR<0.1 (A). Gene-set enrichment analysis for BPD-associated gene sets showing enrichment for "Histone H3@K4 demethylation" (GO:0034720) term (adjusted p-value= 0.00017; B). BD=bipolar disorder.



Supplemental Figure S5. Alternative splicing (AS) case-control comparisons. The six most thoroughly described AS events are depicted (A). AS events were more abundant in the medial frontal gyrus of BPD patients than controls (B). Bar plots summarizing the relevant GO term categories of BPD-specific events for each AS class (C).

Abbreviations and explanations: BPD, bipolar disorder; Shared, events found in both controls and BPD brains; Total number of alternative splicing in Controls, total number of events found in control brains; Total number in BPD, total number of events found in BPD brains; Percentage (%), the increase in numbers of events in BPD compared to healthy controls. In parentheses, the number genes carrying AS events in BPD and control brains.

A

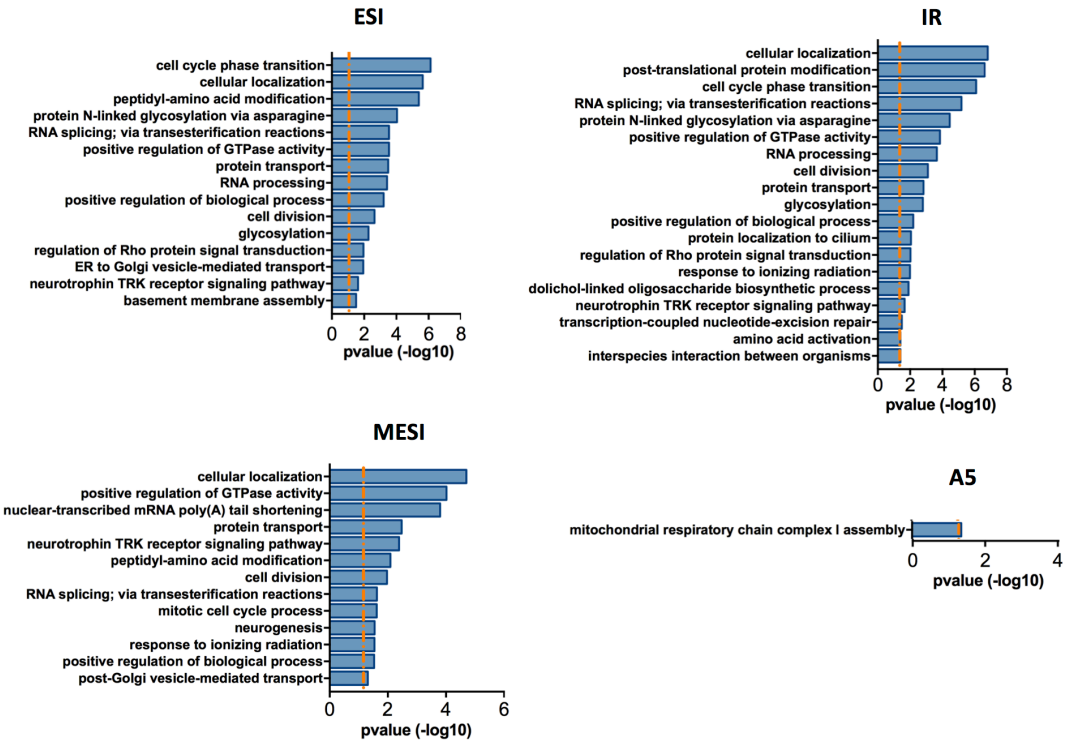


B

Number of events per AS class

AS class	Controls	Shared	BPD	Total number in Controls	Total number in BPD	Percentage (%)
ESI	10330 (4756)	21957 (9167)	11752 (5229)	32287 (13923)	33709 (14396)	4.4 (3.4)
MESI	8016 (3566)	13739 (6602)	9293 (3989)	21755 (10168)	23032 (10591)	5.9 (4.1)
ISI	19699 (5827)	42308 (10793)	23614 (6660)	62007 (16620)	65922 (17453)	6.3 (5)
A5	1409 (1168)	6115 (4325)	2032 (1563)	7524 (5493)	8147 (5888)	8.3 (7.2)
A3	1121 (939)	4313 (3117)	1605 (1276)	5434 (4056)	5918 (4393)	8.9 (8.3)
MEE	82 (40)	205 (102)	84 (41)	287 (142)	289 (143)	0.7 (0.7)

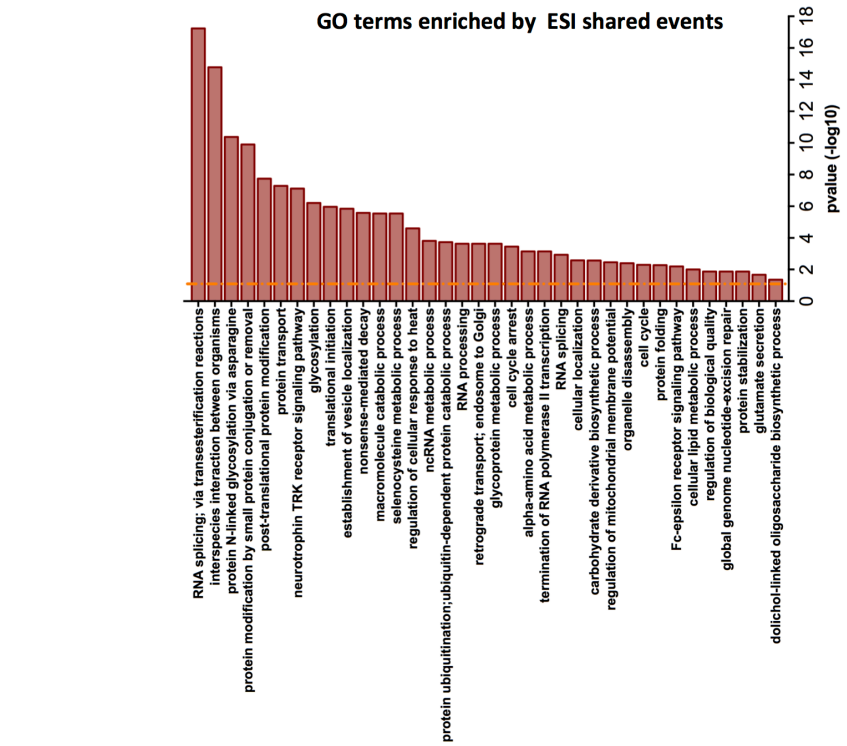
C

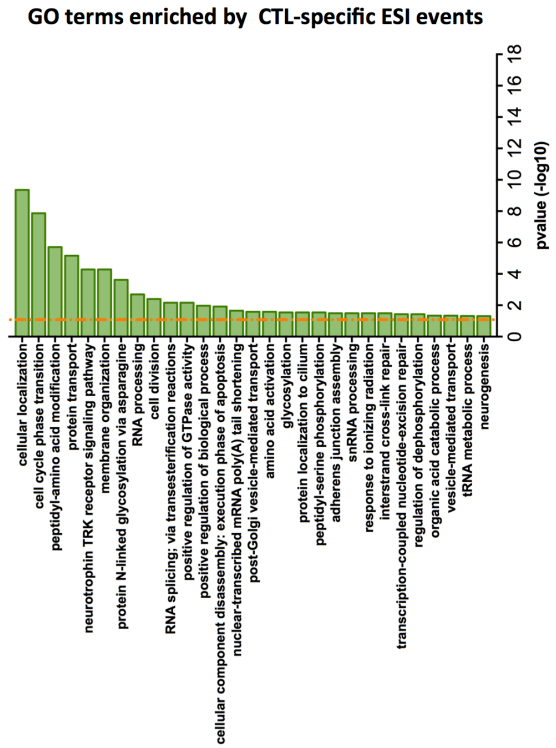


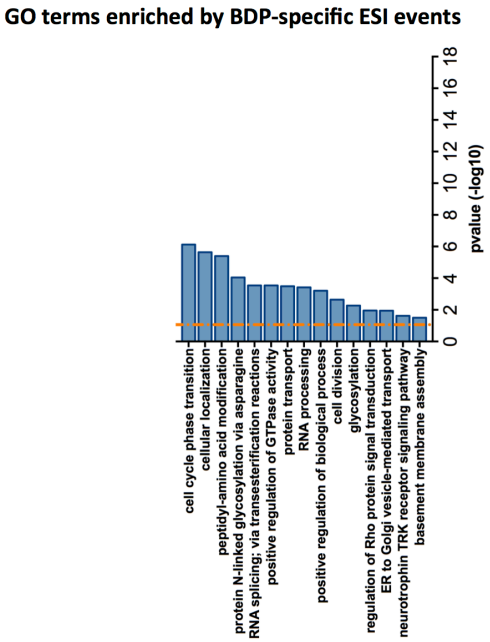
Supplemental Figure S6. GO term enrichment analysis of the unique and common events in BPD and controls for each of the AS classes.

Barplot graphs summary of gene ontology analysis of unique and common ESI (A), MESI (B), IRI (C), A5 (D), and A3 (E) events identified in control and BPD samples. No enrichment was found for genes carrying MEE events. Red, blue and green bars represent biological processes enriched by common, BPD-specific and control-specific AS events, respectively.

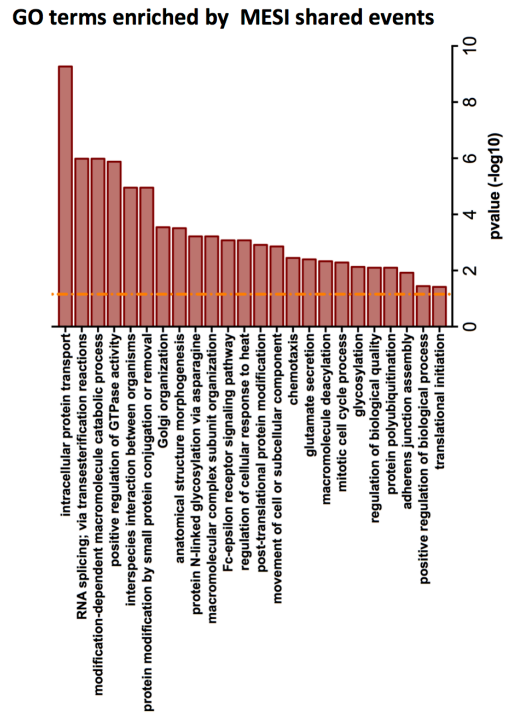
A.

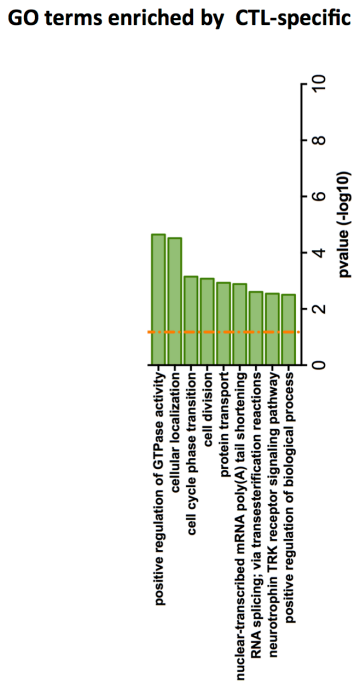

GO terms enriched by ESI shared events
RNA splicing; via transesterification reactions
interspecies interaction between organisms
protein N-linked glycosylation via asparagine
protein modification by small protein conjugation or removal
post-translational protein modification
protein transport
neurotrophin TRK receptor signaling pathway
glycosylation
translational initiation
establishment of vesicle localization
nonsense-mediated decay
macromolecule catabolic process
selenocysteine metabolic process
regulation of cellular response to heat
ncRNA metabolic process
protein ubiquitination;ubiquitin-dependent protein catabolic process
RNA processing
retrograde transport; endosome to Golgi
glycoprotein metabolic process
cell cycle arrest
alpha-amino acid metabolic process
termination of RNA polymerase II transcription
RNA splicing
cellular localization
carbohydrate derivative biosynthetic process
regulation of mitochondrial membrane potential
organelle disassembly
cell cycle
protein folding
Fc-epsilon receptor signaling pathway
cellular lipid metabolic process
regulation of biological quality
global genome nucleotide-excision repair
protein stabilization
glutamate secretion
dolichol-linked oligosaccharide biosynthetic process
0 2 4 6 8 10 12 14 16 18
pvalue (-log10)

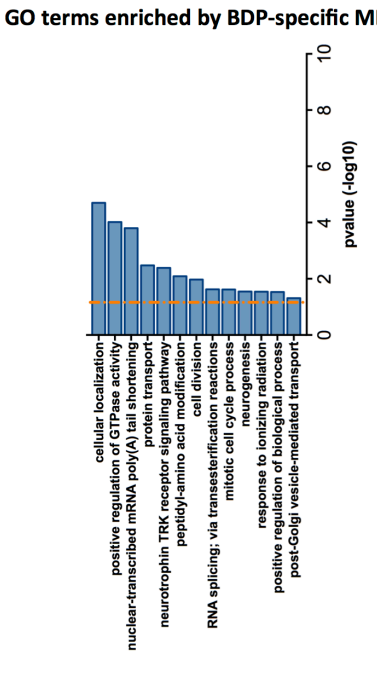

GO terms enriched by CTL-specific ESI events
cellular localization
cell cycle phase transition
peptidyl-amino acid modification
protein transport
neurotrophin TRK receptor signaling pathway
membrane organization
protein N-linked glycosylation via asparagine
RNA processing
cell division
RNA splicing; via transesterification reactions
positive regulation of GTPase activity
positive regulation of biological process
cellular component disassembly; execution phase of apoptosis
nuclear-transcribed mRNA poly(A) tail shortening
post-Golgi vesicle-mediated transport
amino acid activation
glycosylation
protein localization to cilium
peptidyl-serine phosphorylation
adherens junction assembly
snRNA processing
response to ionizing radiation
interstrand cross-link repair
transcription-coupled nucleotide-excision repair
regulation of dephosphorylation
organic acid catabolic process
vesicle-mediated transport
tRNA metabolic process
neurogenesis
0 2 4 6 8 10 12 14 16 18
pvalue (-log10)


GO terms enriched by BDP-specific ESI events
cell cycle phase transition
cellular localization
peptidyl-amino acid modification
protein N-linked glycosylation via asparagine
RNA splicing; via transesterification reactions
positive regulation of GTPase activity
protein transport
RNA processing
positive regulation of biological process
cell division
glycosylation
regulation of Rho protein signal transduction
ER to Golgi vesicle-mediated transport
neurotrophin TRK receptor signaling pathway
basement membrane assembly
0 2 4 6 8 10 12 14 16 18
pvalue (-log10)

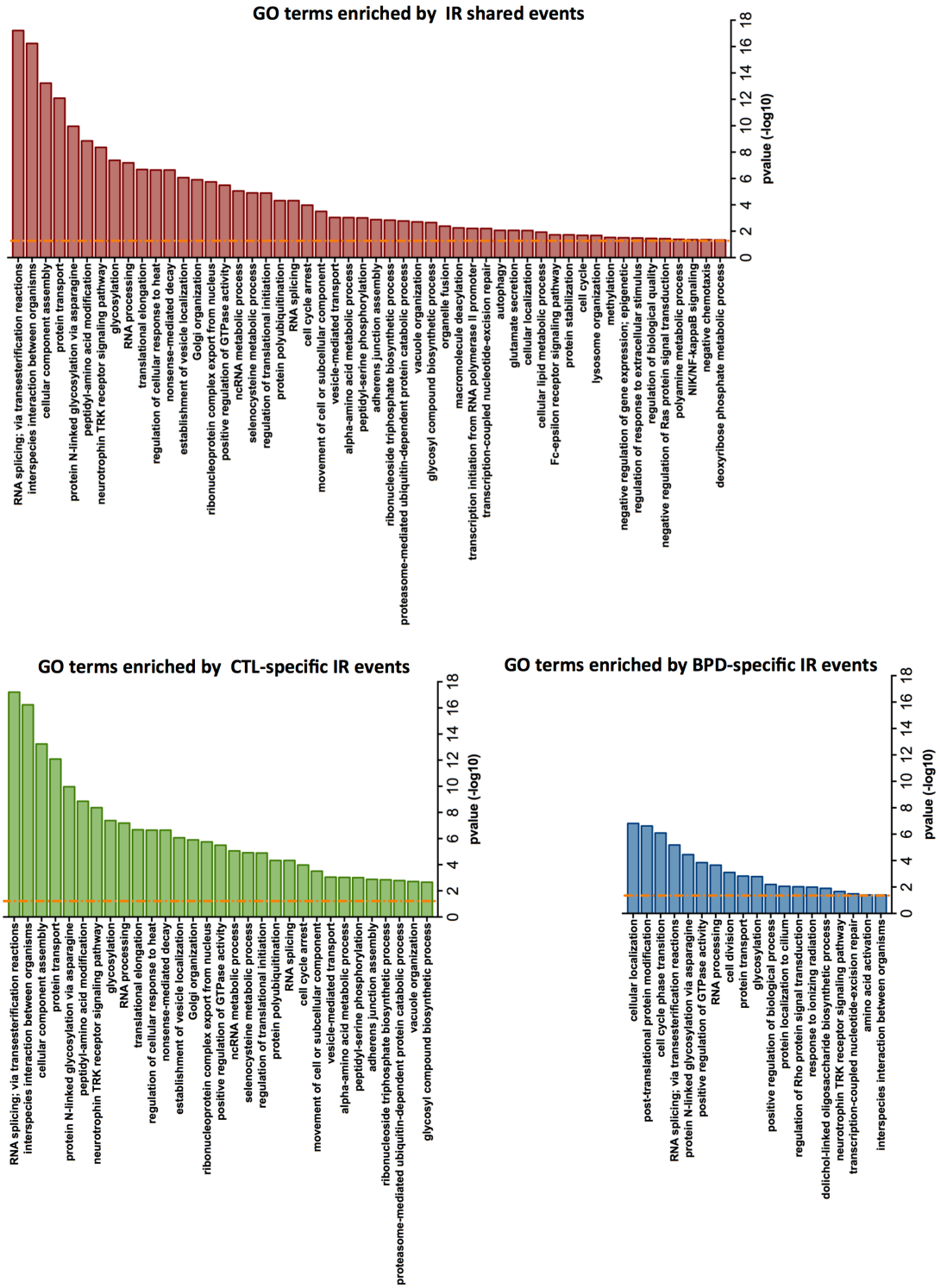
B.


GO terms enriched by MESI shared events
intracellular protein transport
RNA splicing; via transesterification reactions
modification-dependent macromolecule catabolic process
positive regulation of GTPase activity
interspecies interaction between organisms
protein modification by small protein conjugation or removal
Golgi organization
anatomical structure morphogenesis
protein N-linked glycosylation via asparagine
macromolecular complex subunit organization
Fc-epsilon receptor signaling pathway
regulation of cellular response to heat
post-translational protein modification
movement of cell or subcellular component
chemotaxis
glutamate secretion
macromolecule deacylation
mitotic cell cycle process
glycosylation
regulation of biological quality
protein polyubiquitination
adherens junction assembly
positive regulation of biological process
translational initiation
0
2
4
6
8
10
pvalue (-log10)

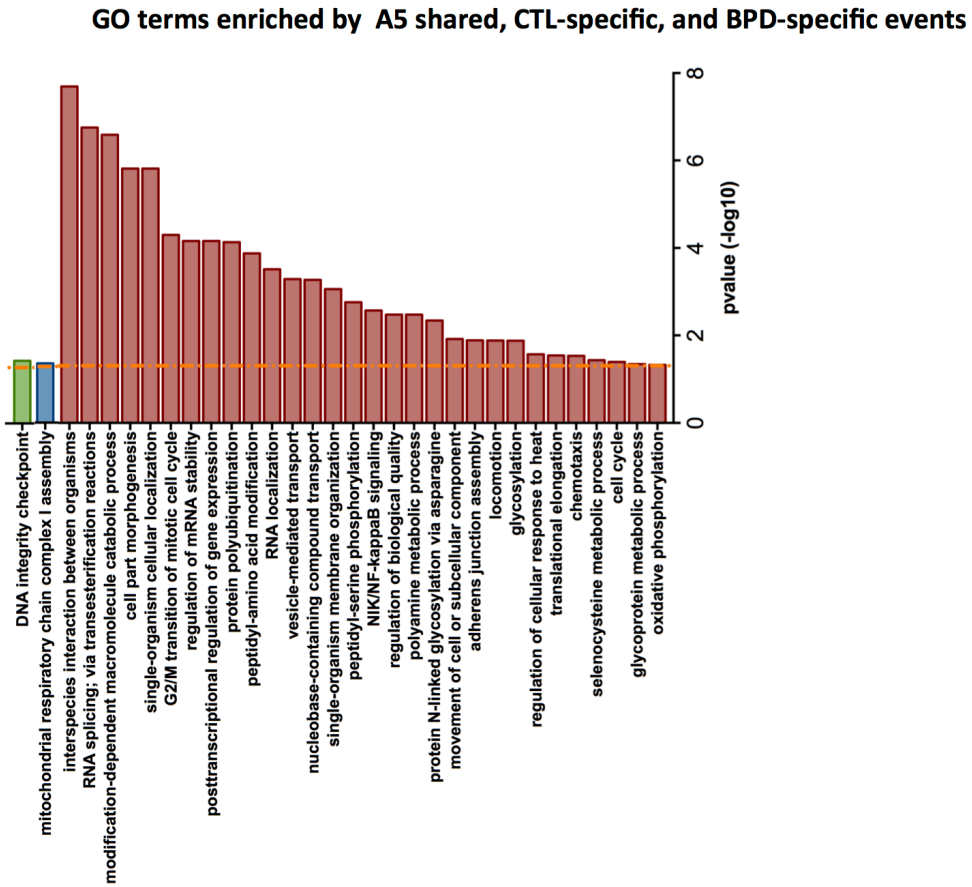

GO terms enriched by CTL-specific MESI events
positive regulation of GTPase activity
cellular localization
cell cycle phase transition
cell division
protein transport
nuclear-transcribed mRNA poly(A) tail shortening
RNA splicing; via transesterification reactions
neurotrophin TRK receptor signaling pathway
positive regulation of biological process
0
2
4
6
8
10
pvalue (-log10)


GO terms enriched by BDP-specific MESI events
cellular localization
positive regulation of GTPase activity
nuclear-transcribed mRNA poly(A) tail shortening
protein transport
neurotrophin TRK receptor signaling pathway
peptidyl-amino acid modification
cell division
RNA splicing; via transesterification reactions
mitotic cell cycle process
neurogenesis
response to ionizing radiation
positive regulation of biological process
post-Golgi vesicle-mediated transport
0
2
4
6
8
10
pvalue (-log10)

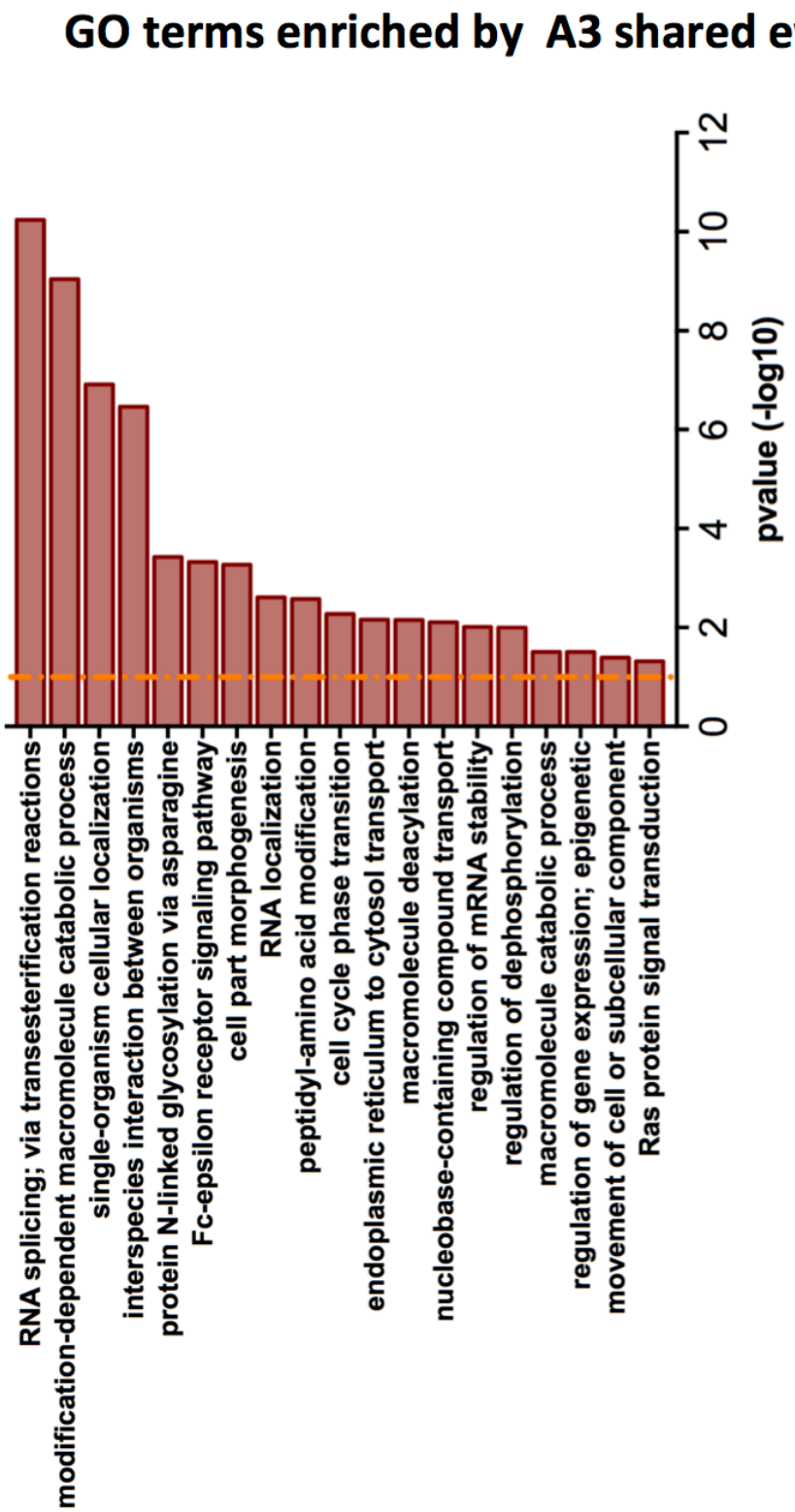
C.


GO terms enriched by IR shared events
RNA splicing; via transesterification reactions
interspecies interaction between organisms
cellular component assembly
protein transport
protein N-linked glycosylation via asparagine
peptidyl-amino acid modification
neurotrophin TRK receptor signaling pathway
glycosylation
RNA processing
translational elongation
regulation of cellular response to heat
nonsense-mediated decay
establishment of vesicle localization
Golgi organization
ribonucleoprotein complex export from nucleus
positive regulation of GTPase activity
ncRNA metabolic process
selenocysteine metabolic process
regulation of translational initiation
protein polyubiquitination
RNA splicing
cell cycle arrest
movement of cell or subcellular component
vesicle-mediated transport
alpha-amino acid metabolic process
peptidyl-serine phosphorylation
adherens junction assembly
ribonucleoside triphosphate biosynthetic process
proteasome-mediated ubiquitin-dependent protein catabolic process
vacuole organization
glycosyl compound biosynthetic process
organelle fusion
macromolecule deacylation
transcription initiation from RNA polymerase II promoter
transcription-coupled nucleotide-excision repair
autophagy
glutamate secretion
cellular localization
cellular lipid metabolic process
Fc-epsilon receptor signaling pathway
protein stabilization
cell cycle
lysosome organization
methylation
negative regulation of gene expression; epigenetic
regulation of response to extracellular stimulus
regulation of biological quality
negative regulation of Ras protein signal transduction
polyamine metabolic process
NIK/NF-kappaB signaling
negative chemotaxis
deoxyribose phosphate metabolic process
0 2 4 6 8 10 12 14 16 18
pvalue (-log10)
GO terms enriched by CTL-specific IR events
RNA splicing; via transesterification reactions
interspecies interaction between organisms
cellular component assembly
protein transport
protein N-linked glycosylation via asparagine
peptidyl-amino acid modification
neurotrophin TRK receptor signaling pathway
glycosylation
RNA processing
translational elongation
regulation of cellular response to heat
nonsense-mediated decay
establishment of vesicle localization
Golgi organization
ribonucleoprotein complex export from nucleus
positive regulation of GTPase activity
ncRNA metabolic process
selenocysteine metabolic process
regulation of translational initiation
protein polyubiquitination
RNA splicing
cell cycle arrest
movement of cell or subcellular component
vesicle-mediated transport
alpha-amino acid metabolic process
peptidyl-serine phosphorylation
adherens junction assembly
ribonucleoside triphosphate biosynthetic process
proteasome-mediated ubiquitin-dependent protein catabolic process
vacuole organization
glycosyl compound biosynthetic process
0 2 4 6 8 10 12 14 16 18
pvalue (-log10)
GO terms enriched by BPD-specific IR events
cellular localization
post-translational protein modification
cell cycle phase transition
RNA splicing; via transesterification reactions
protein N-linked glycosylation via asparagine
positive regulation of GTPase activity
RNA processing
cell division
protein transport
glycosylation
positive regulation of biological process
protein localization to cilium
regulation of Rho protein signal transduction
response to ionizing radiation
dolichol-linked oligosaccharide biosynthetic process
neurotrophin TRK receptor signaling pathway
transcription-coupled nucleotide-excision repair
amino acid activation
interspecies interaction between organisms
0 2 4 6 8 10 12 14 16 18
pvalue (-log10)

D.


GO terms enriched by A5 shared, CTL-specific, and BPD-specific events
pvalue (-log10)
0
2
4
6
8
DNA integrity checkpoint
mitochondrial respiratory chain complex I assembly
interspecies interaction between organisms
RNA splicing; via transesterification reactions
modification-dependent macromolecule catabolic process
cell part morphogenesis
single-organism cellular localization
G2/M transition of mitotic cell cycle
regulation of mRNA stability
posttranscriptional regulation of gene expression
protein polyubiquitination
peptidyl-amino acid modification
RNA localization
vesicle-mediated transport
nucleobase-containing compound transport
single-organism membrane organization
peptidyl-serine phosphorylation
NIK/NF-kappaB signaling
regulation of biological quality
polyamine metabolic process
protein N-linked glycosylation via asparagine
movement of cell or subcellular component
adherens junction assembly
locomotion
glycosylation
regulation of cellular response to heat
translational elongation
chemotaxis
selenocysteine metabolic process
cell cycle
glycoprotein metabolic process
oxidative phosphorylation

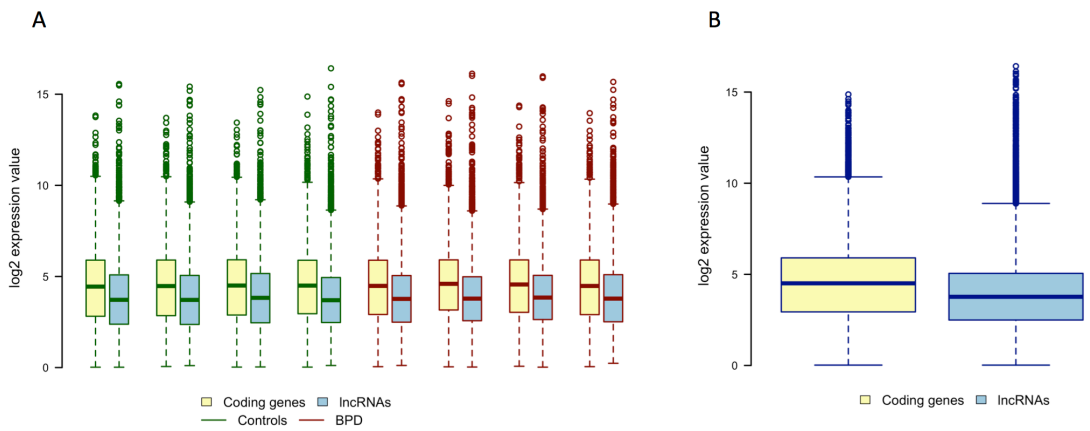
E.


GO terms enriched by A3 shared events
RNA splicing; via transesterification reactions
modification-dependent macromolecule catabolic process
single-organism cellular localization
interspecies interaction between organisms
protein N-linked glycosylation via asparagine
Fc-epsilon receptor signaling pathway
cell part morphogenesis
RNA localization
peptidyl-amino acid modification
cell cycle phase transition
endoplasmic reticulum to cytosol transport
macromolecule deacylation
nucleobase-containing compound transport
regulation of mRNA stability
regulation of dephosphorylation
macromolecule catabolic process
regulation of gene expression; epigenetic
movement of cell or subcellular component
Ras protein signal transduction
0
2
4
6
8
10
12
pvalue (-log10)

Supplemental Figure S7. lncRNAs.

Boxplots of normalized log2 expression values of protein coding genes and lncRNAs in every sequenced sample (left four are controls and right four are BPD patients; A). Boxplots of normalized log2 expression values of protein coding genes and lncRNAs (B). lncRNAs displayed lower overall expression compared to protein coding genes ($p = 1.08x10^{-9}$).

The bottom and top of the boxes represent the first and third quartiles, respectively, and the band inside the box is the median; the ends of the whiskers represent the lowest value within 1.5 interquartile range (IQR) of the lower quartile and the highest value within 1.5 IQR of the upper quartile.



Supplemental Table:

- Sheets 1 and 2: Detailed demographic information of the study population. Abbreviations: BPD, bipolar disorder; F, female; M, male; NA, not available; PMI, postmortem interval; Side, brain side; L, left; Mood stabilizers at death, use of mood stabilizers in the last 24 hours of life; Psychoactive drugs at death, use of psychotropic medications in the last 24 hours of life; Psychoactive drugs, ever-reported use of psychotropic medications; ECT, history of electroconvulsive therapy; 'Y' represents if there is a record of an individual having taken at least one medication/therapy and 'N' if there is no such record.
- Sheet 3: Postmortem brain tissue information. Outlined are the postmortem brain conditions per subject.
- Sheet 4: Differential regulation of gene expression in medial frontal gyrus in bipolar disorder. Summary table of the differentially expressed genes in BPD medial frontal gyrus at FDR<0.05 (bold) and FDR<0.1.
- Sheet 5: Results of the validation and replication analyses. Shown are the summary statistics for the 5 genes in the validation and replication steps.
- Sheet 6: Summary statistics for GO term enrichment analysis on the 36 differentially expressed genes at FDR<0.1 (A); and gene-set enrichment analysis (GSEA) for BPD-associated gene sets (B).
- Sheet 7: Overview of chi-squared test results for each of the AS (alternative splicing) classes and the total number of AS events. Also shown are the numbers of genes carrying such AS events.
- Sheet 8: Overlap between AS events and DEGs.
- Sheet 9: AS events in BPD susceptibility genes.
- Sheet 10: Summary table of the differentially expressed lncRNA transcripts.
- Sheet 11: Overview of circular RNAs populating the medial frontal gyrus (first 2000 rows).
- Sheet 12: When applying a log fold change cutoff of ≥ 1.5 or ≤ -1.5 irrespective of statistical significance, 689 circRNAs were either upregulated or downregulated in BPD
- Sheet 13: This is the full list of circRNA toptags from the DE analysis, where all the logFC and the FDR of all circRNA transcripts included in the DE analysis can be found.
- Sheet 14: Three additional circular transcripts generate the ZDHHC11 locus (whose expression in logFC is between 0,04 and 0,49).
- Sheet 15: Of the 104 known BPD-associated loci described in ref7-9, 43 gave rise to a number of circular transcripts. Although all of them are not significant, five of these circRNAs exhibit high logFC (green = up; red = down).
- Sheet 16: There is no significant difference in expression for linear NEBL and EPHA3 transcripts between the controls and BPD brains.