JClub

Jan 26, 2017

- Wu et al., *Nature Neurosci*, 2016
  Genome-wide, integrative analysis implicates microRNA dysregulation in autism spectrum disorder

- Parikshak et al., *Nature*, 2016
  Genome-wide changes in lncRNA, splicing, and regional gene expression patterns in autism

Autism Spectrum Disorders

- ASD now includes several conditions that used to be diagnosed separately: autistic disorder, pervasive developmental disorder not otherwise specified (PDD-NOS), Asperger syndrome, and childhood disintegrative disorder
- These disorders involve a wide range of cognitive and behavioral abnormalities: social deficits and communication difficulties, repetitive behaviors and interests, sensory issues, deviance in language development, poor motor skills' and in some cases, cognitive delays.
- About 1 percent of the world population has autism spectrum disorder. (CDC,2014)
- Prevalence in the United States is estimated at 1 in 68 births. (CDC, 2014)
- Prevalence of autism in U.S. children increased by 119.4 percent from 2000 (1 in 150) to 2010 (1 in 68). (CDC, 2014) Autism is the fastest-growing developmental disability. (CDC, 2008)
- Cost of lifelong care can be reduced by 2/3 with early diagnosis and intervention. (Autism Society estimate)
- ASD is almost 5 times more common among boys (1 in 42) than among girls (1 in 189)

http://www.autism-society.org/
http://www.cdc.gov/ncbddd/autism/

nature neuroscience

Genome-wide, integrative analysis implicates microRNA dysregulation in autism spectrum disorder

Ye E Wu[1,2], Neelroop N Parikshak[1,3,4], T Grant Belgard[1,2,6] & Daniel H Geschwind[1,2,4,5]

Genetic variants conferring risk for autism spectrum disorder (ASD) have been identified, but the role of post-transcriptional mechanisms in ASD is not well understood. We performed genome-wide microRNA (miRNA) expression profiling in post-mortem brains from individuals with ASD and controls and identified miRNAs and co-regulated modules that were perturbed in ASD. Putative targets of these ASD-affected miRNAs were enriched for genes that have been implicated in ASD risk. We confirmed regulatory relationships between several miRNAs and their putative target mRNAs in primary human neural progenitors. These include hsa-miR-21-3p, a miRNA of unknown CNS function that is upregulated in ASD and that targets neuronal genes downregulated in ASD, and hsa_can_1002-m, a previously unknown, primate-specific miRNA that is downregulated in ASD and that regulates the epidermal growth factor receptor and fibroblast growth factor receptor signaling pathways involved in neural development and immune function. Our findings support a role for miRNA dysregulation in ASD pathophysiology and provide a rich data set and framework for future analyses of miRNAs in neuropsychiatric diseases.

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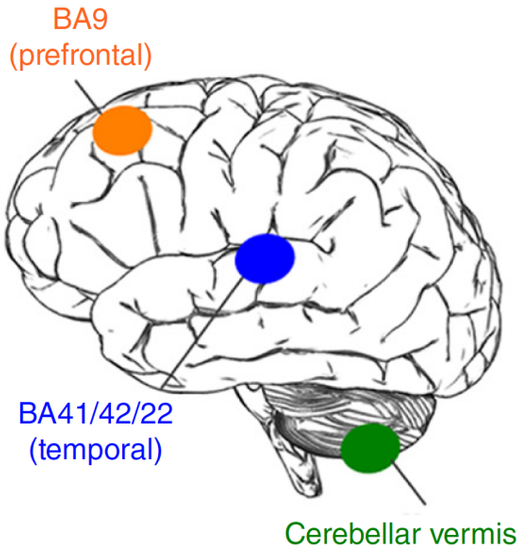
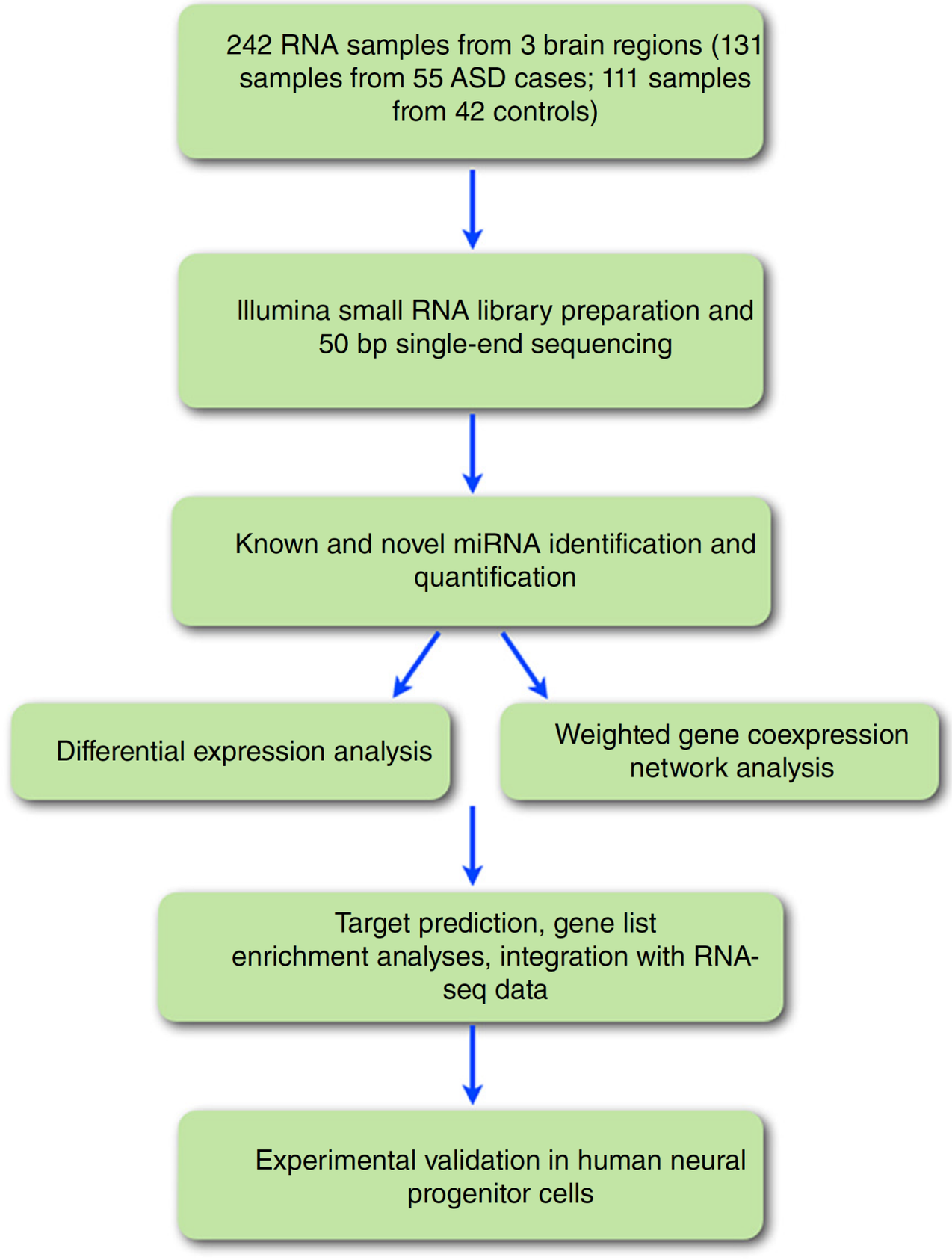
Flow chart of the overall approach

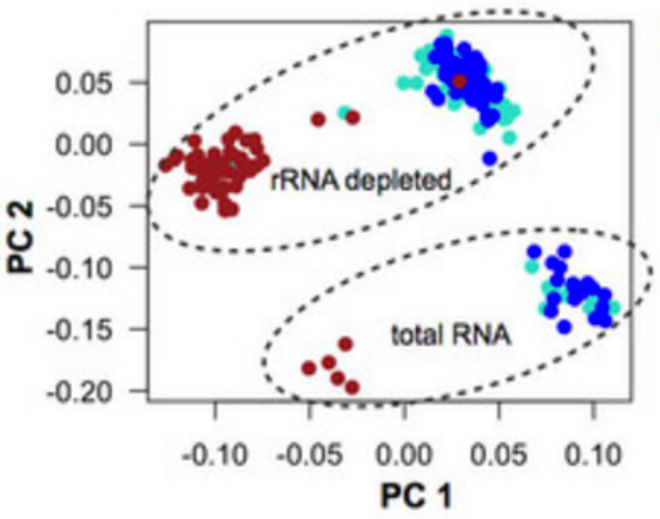


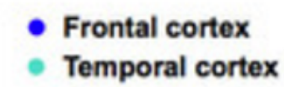
up to 3 brain regions (frontal cortex, temporal cortex, cerebellar vermis) implicated with ASD from **242** post-mortem brain samples

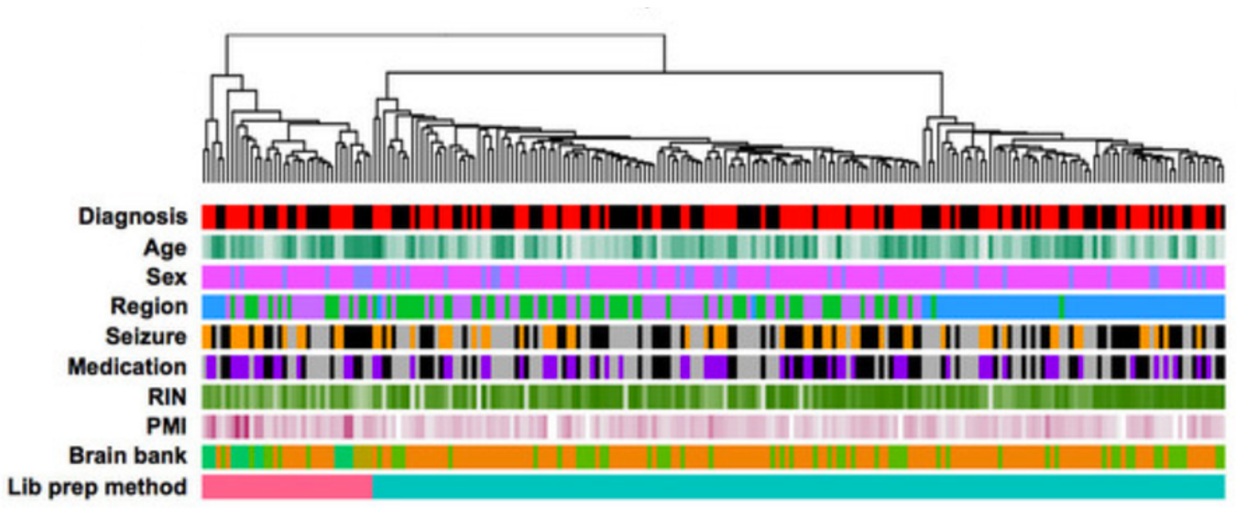
identified and quantified **699** miRNAs (552 documented in *miRBase* and 147 unknown)

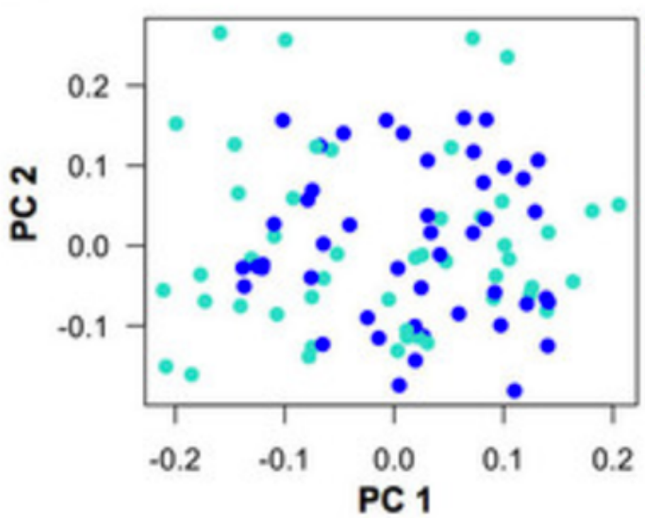


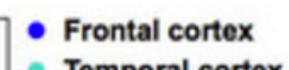
PCA and hierarchical sample clustering

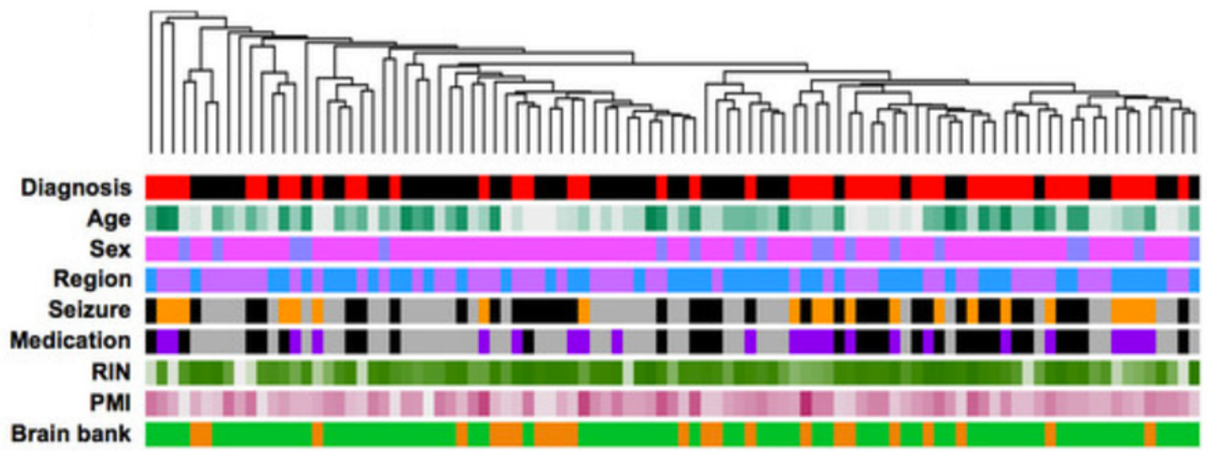






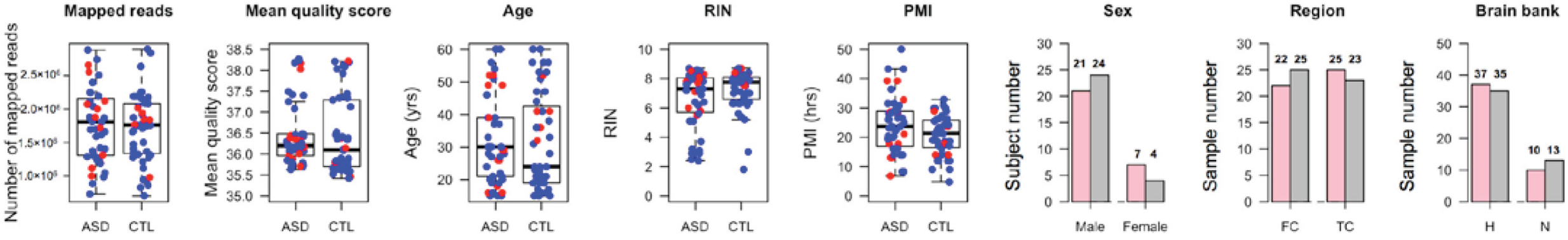




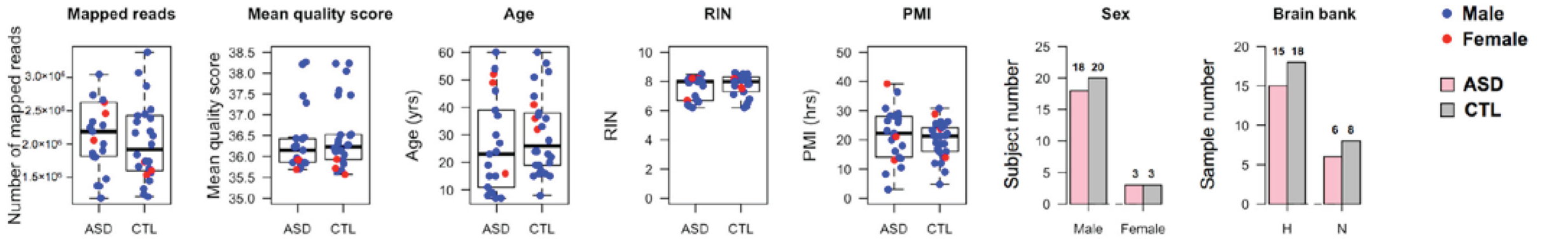


Characteristics of samples used in analyses

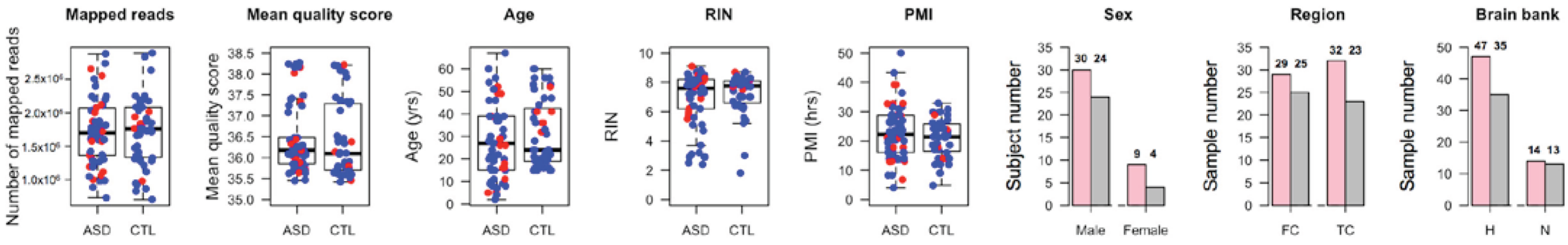
95 cortex samples for DGE analysis



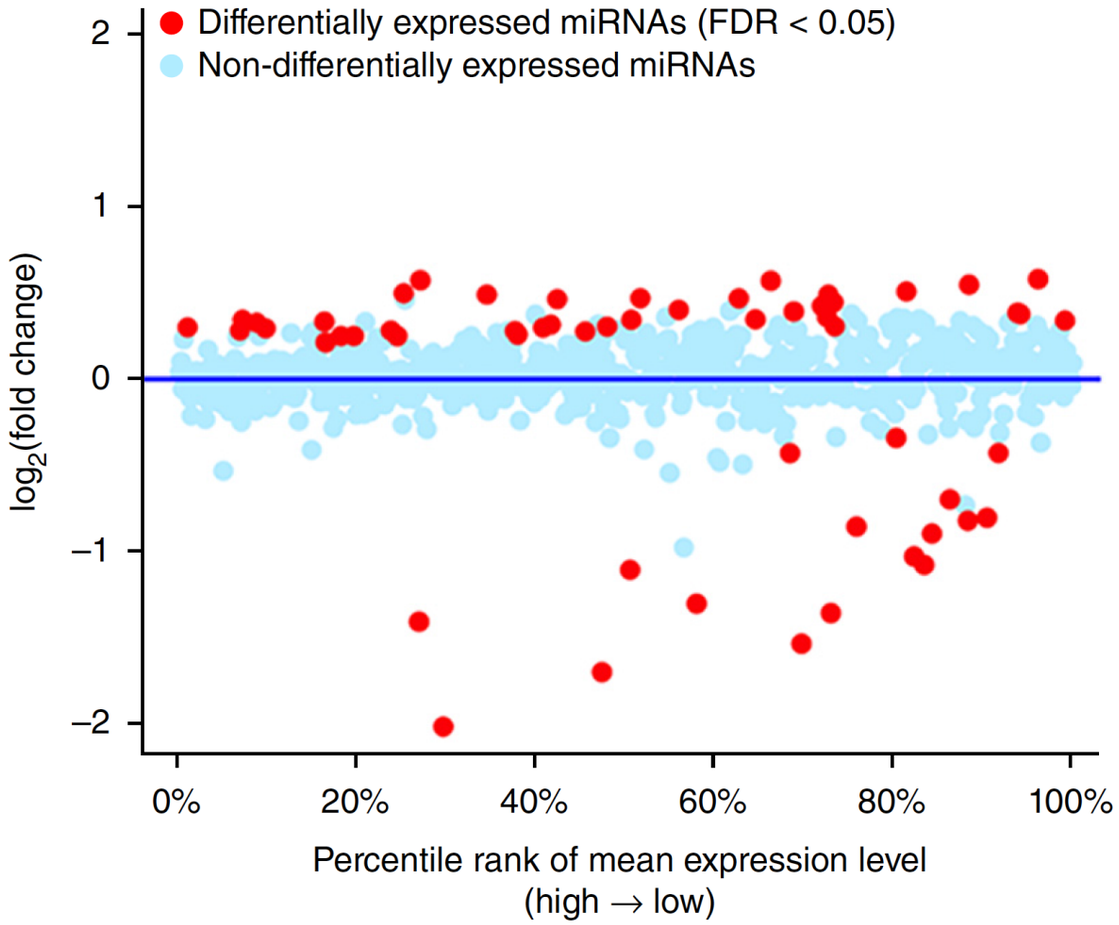
47 cerebellum samples for DGE analysis



109 cortex samples for WGCNA

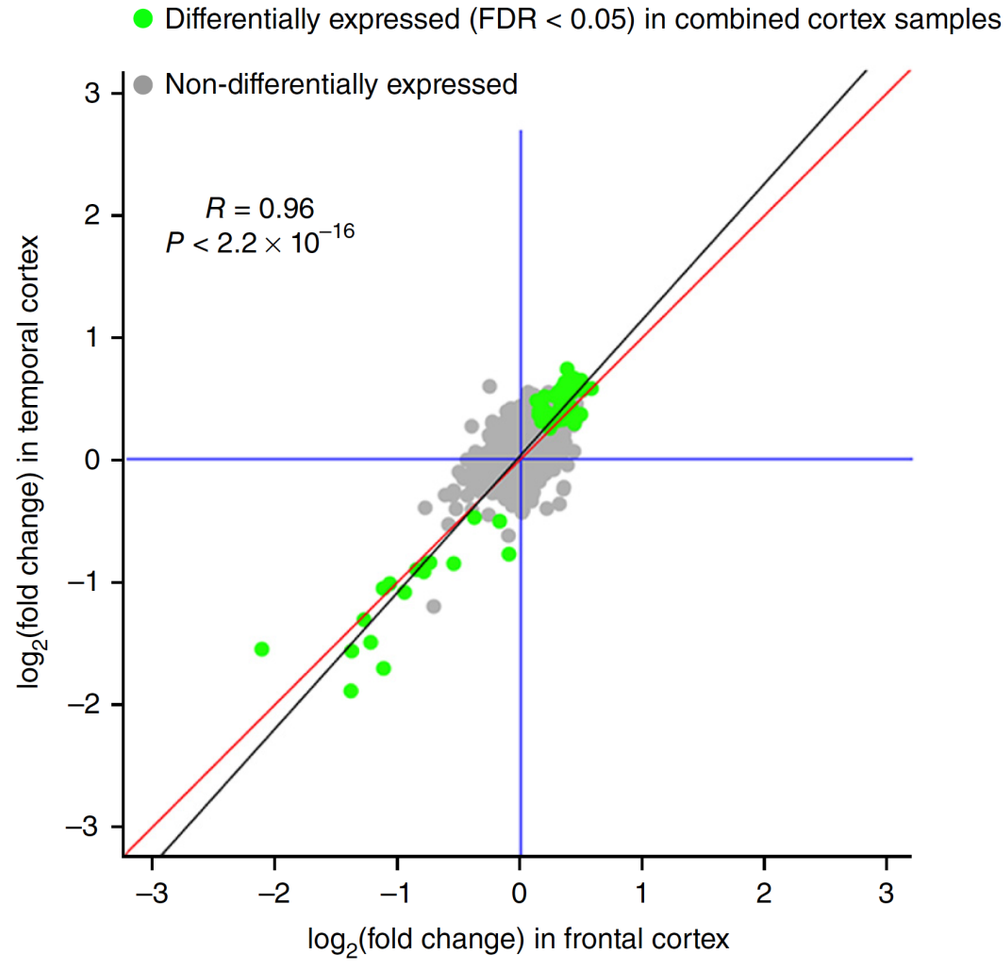


Differential expression analyses



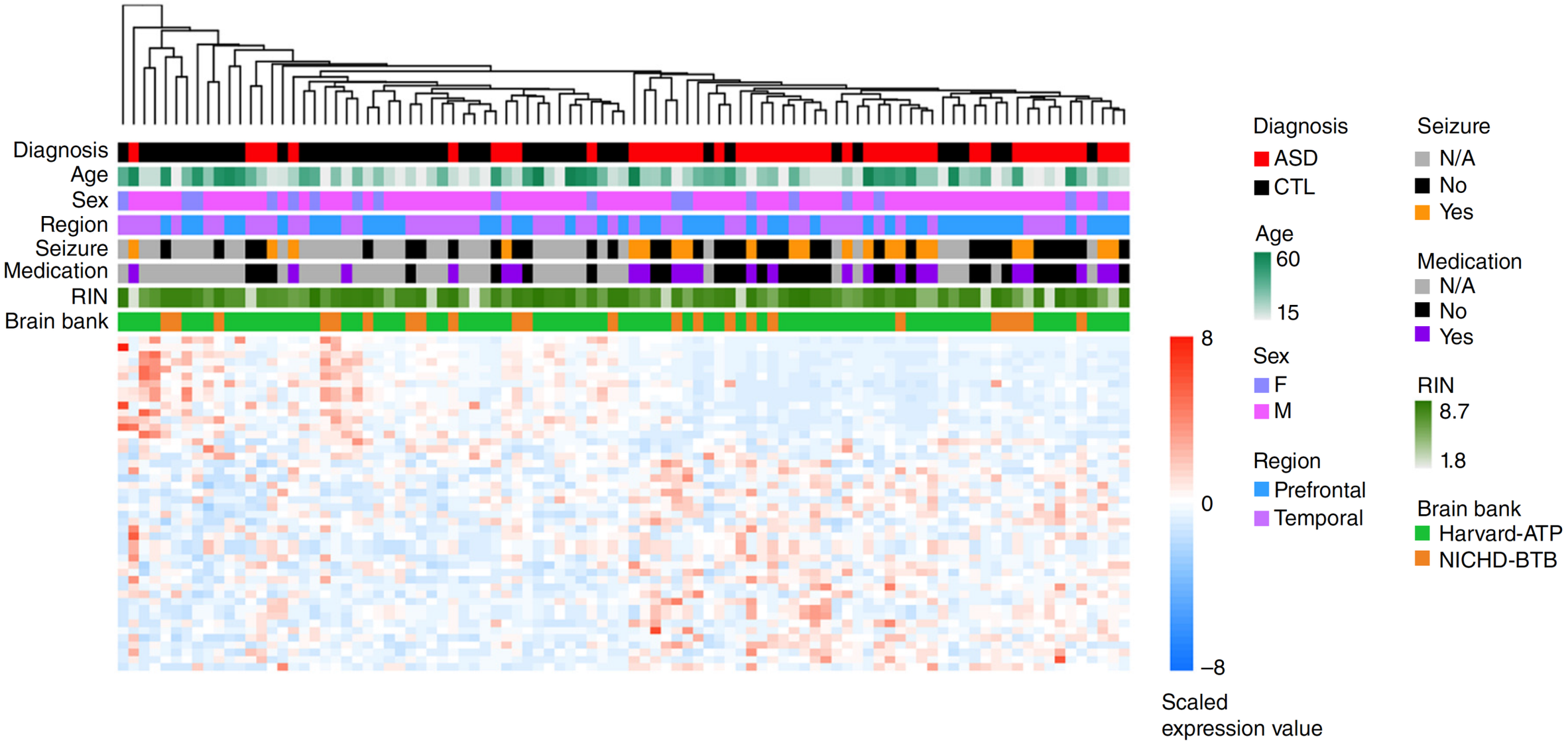
17 down-regulated and **41** up-regulated miRNA in ASD cortex compared to control

Concordance between FC and TC fold changes

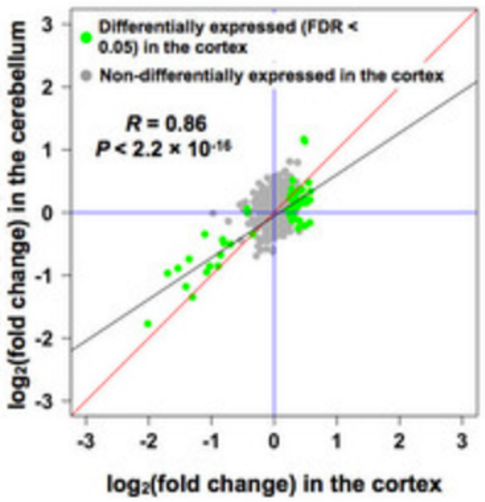


Hierarchical clustering of 95 cortex samples based on top DE miRNAs (FDR<0.05; abs(log2FC) > 0.3)

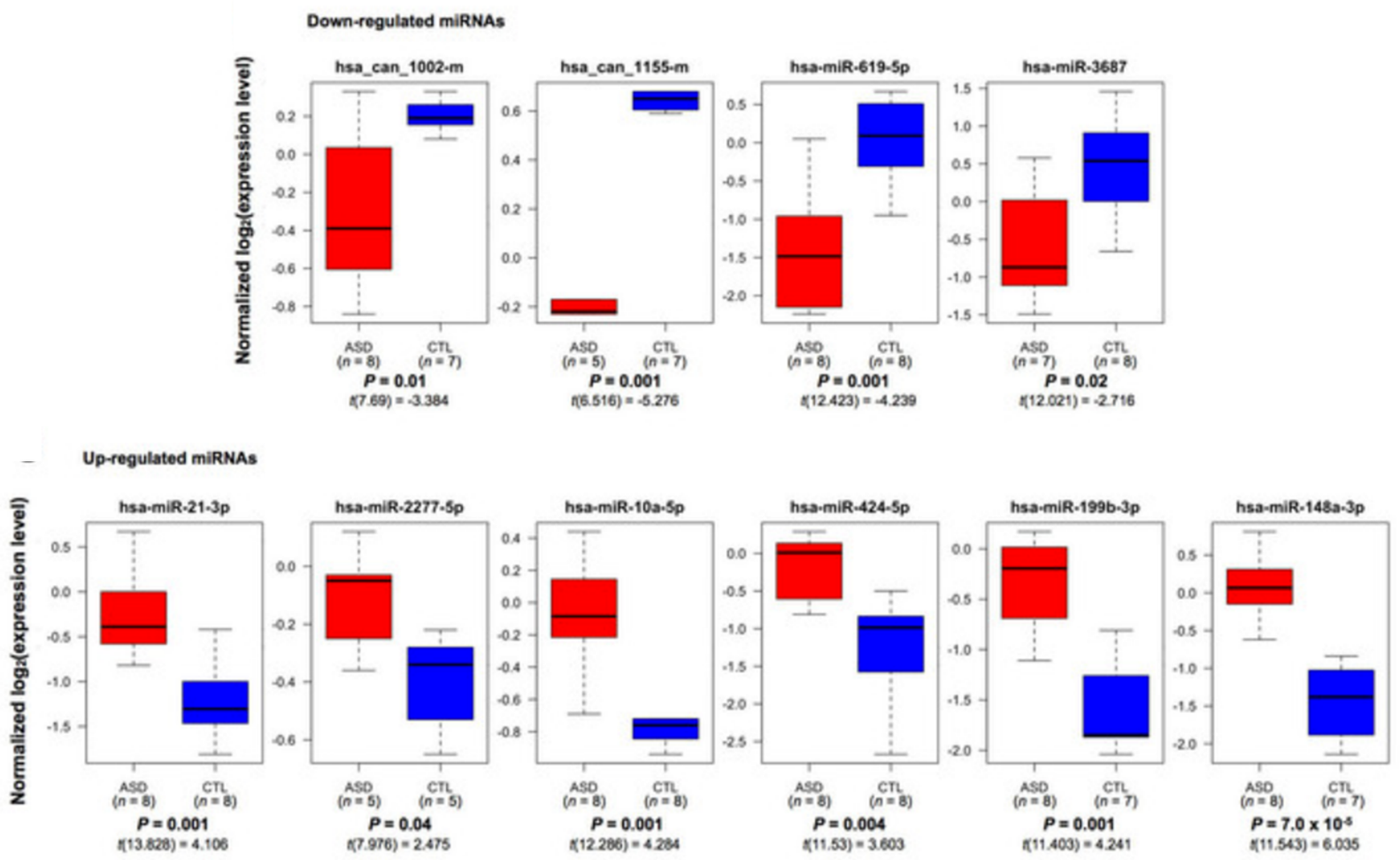
FDR<0.05; abs(log2FC) > 0.3



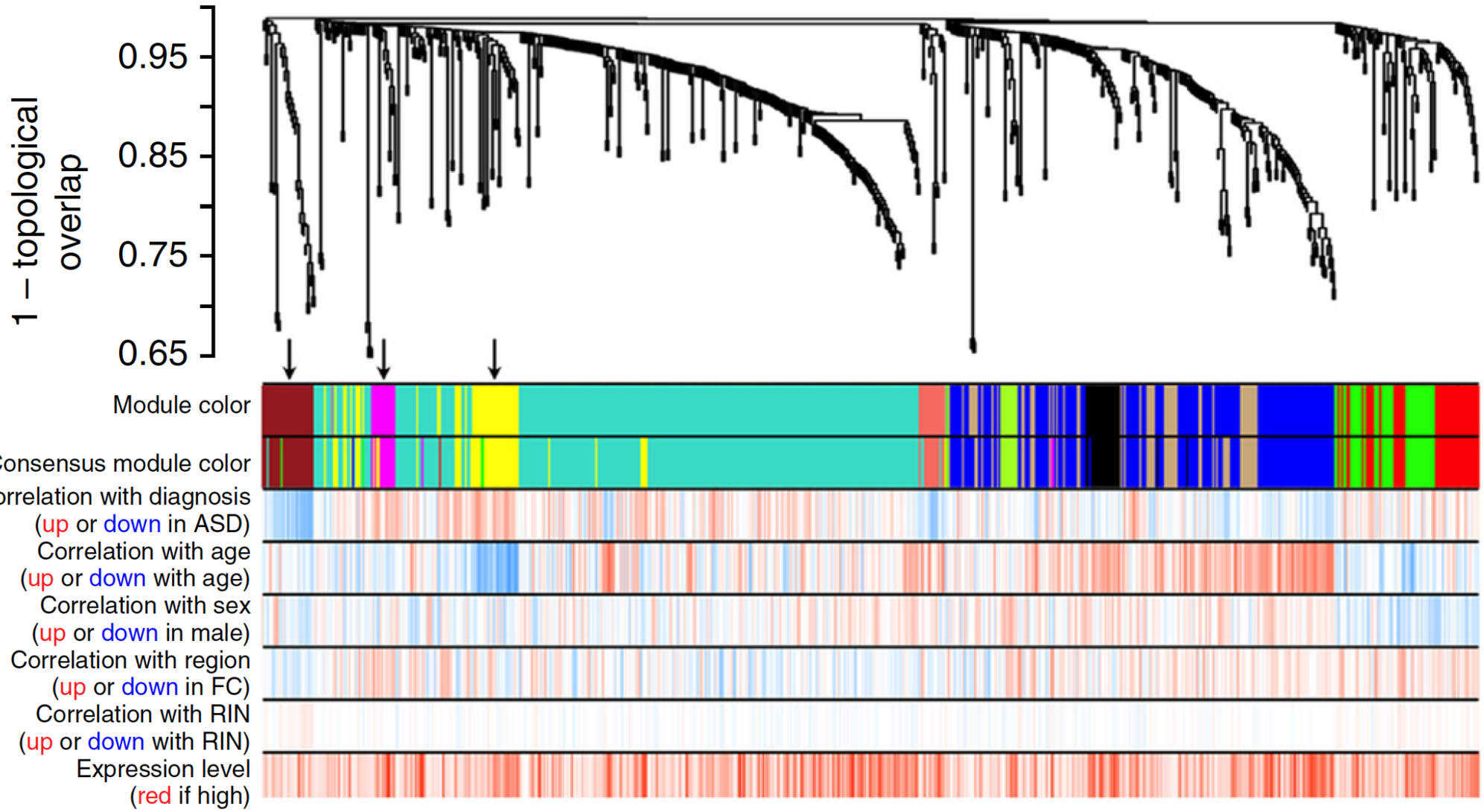
16 are miRNAs differentially expressed (at FDR<5%) in both cortex and cerebellum



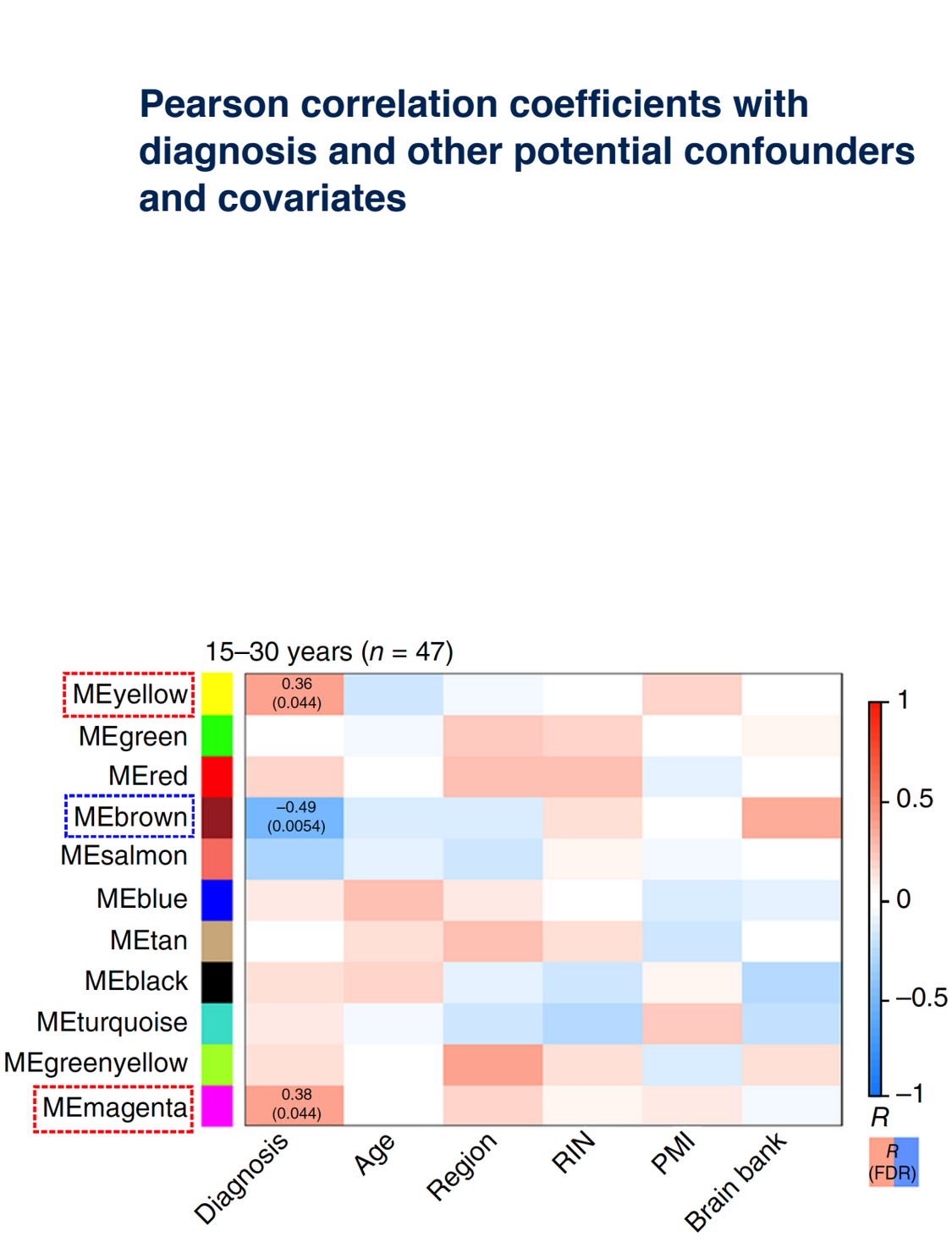
qRT-PCR validation of 10 differentially expressed miRNAs (*n*=5-8)

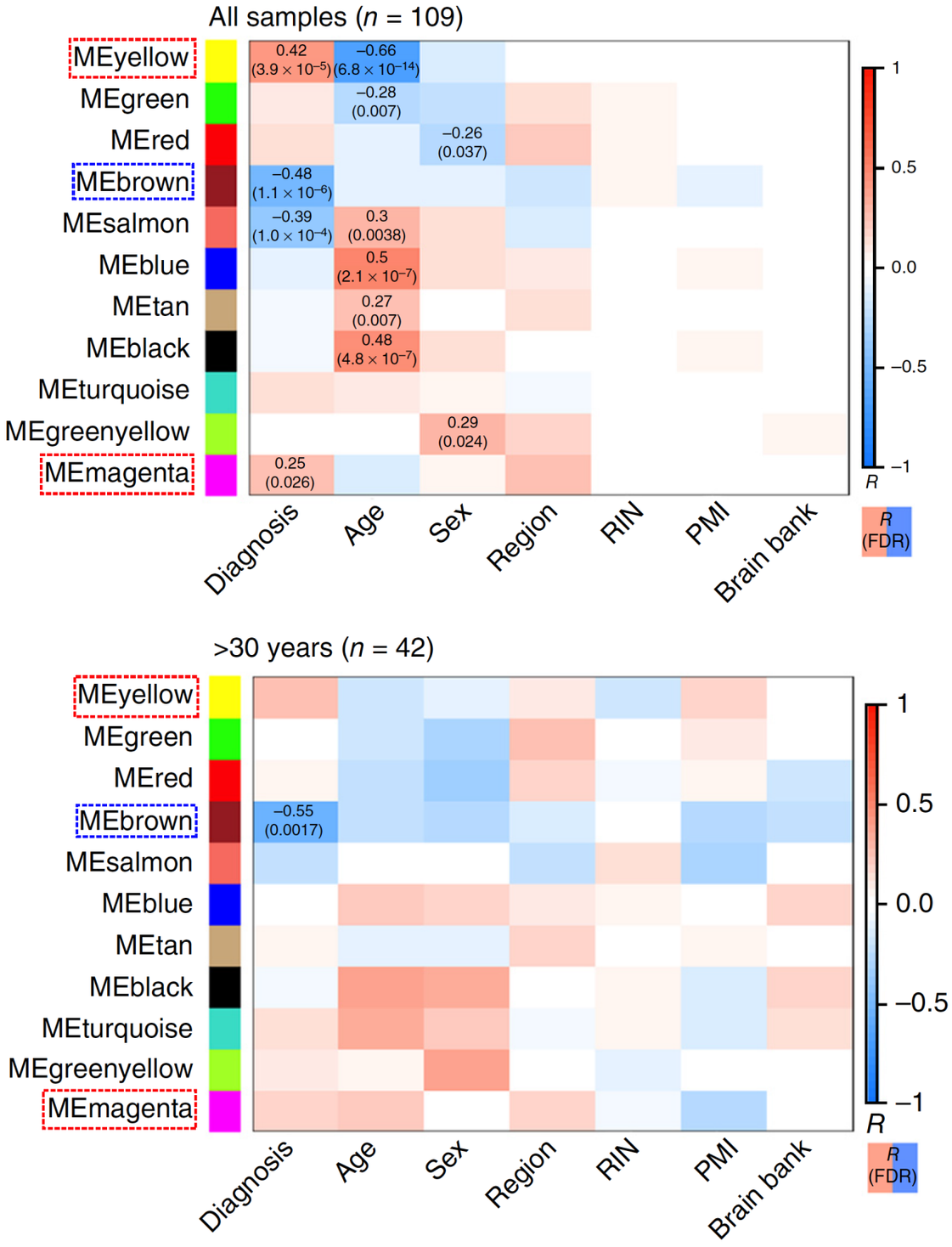


Weighted Gene Coexpression Network Analysis (WGCNA)

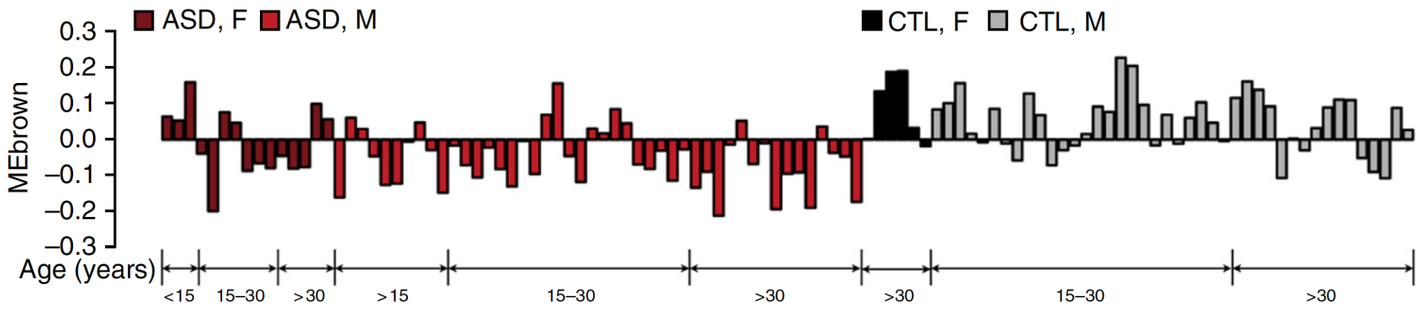


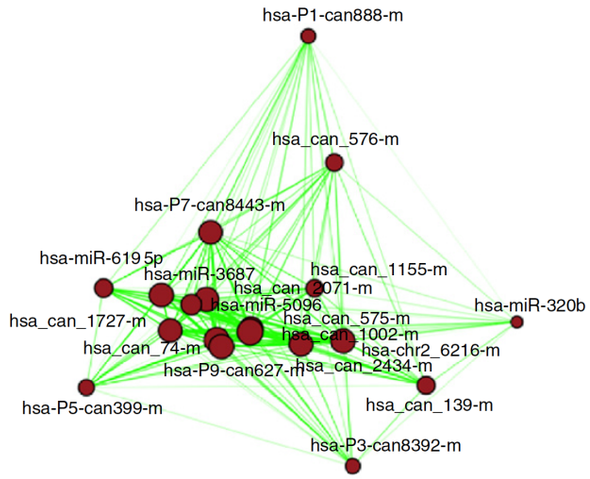
Pearson correlation coefficients with diagnosis and other potential confounders and covariates

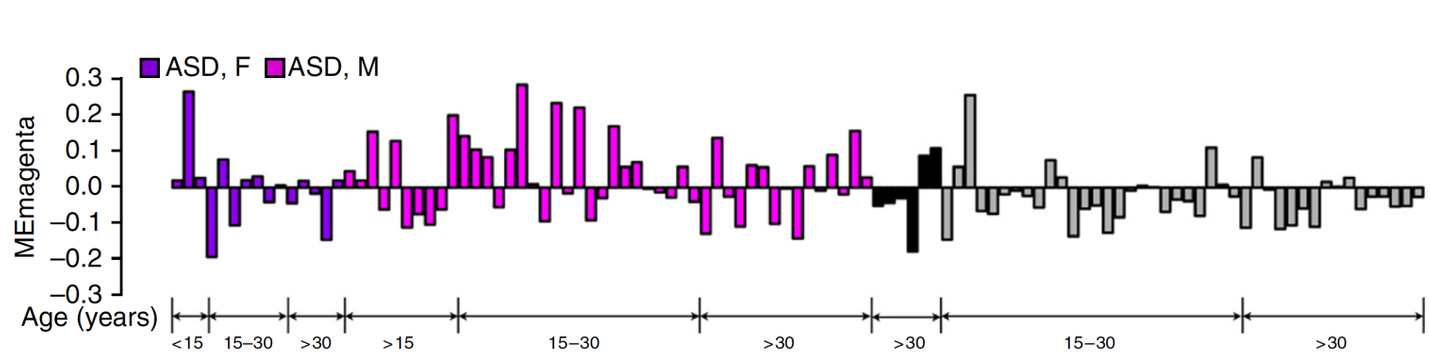


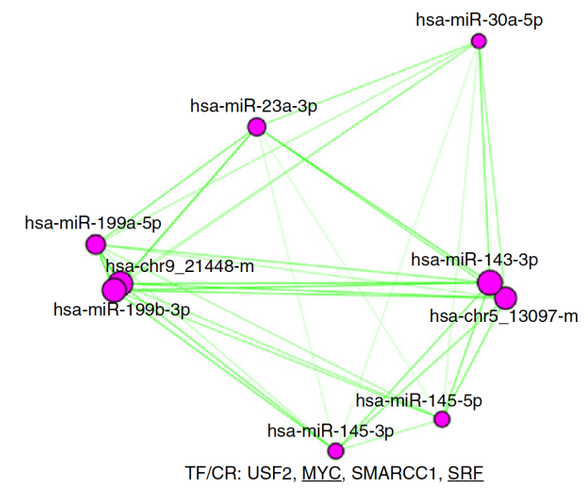


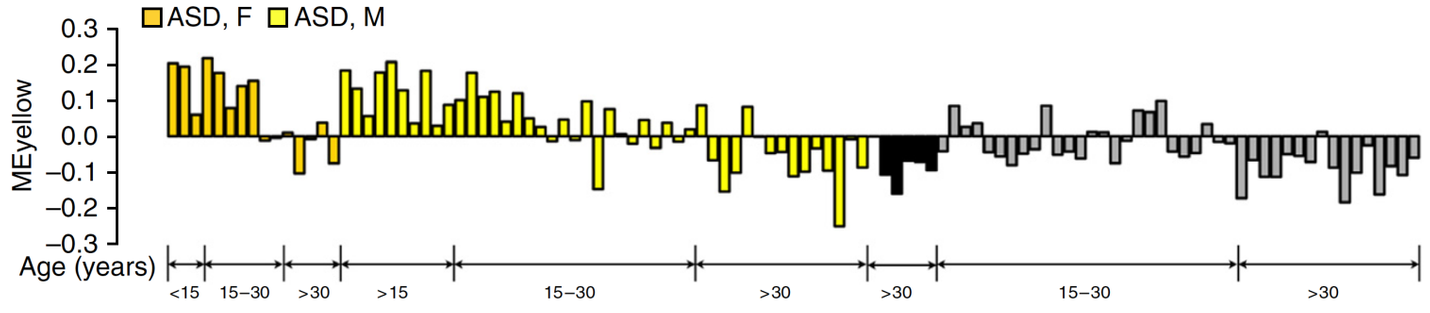
Scaled module eigengene values and network plots

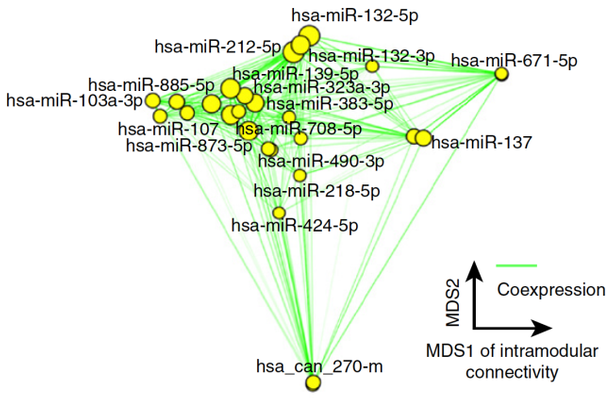








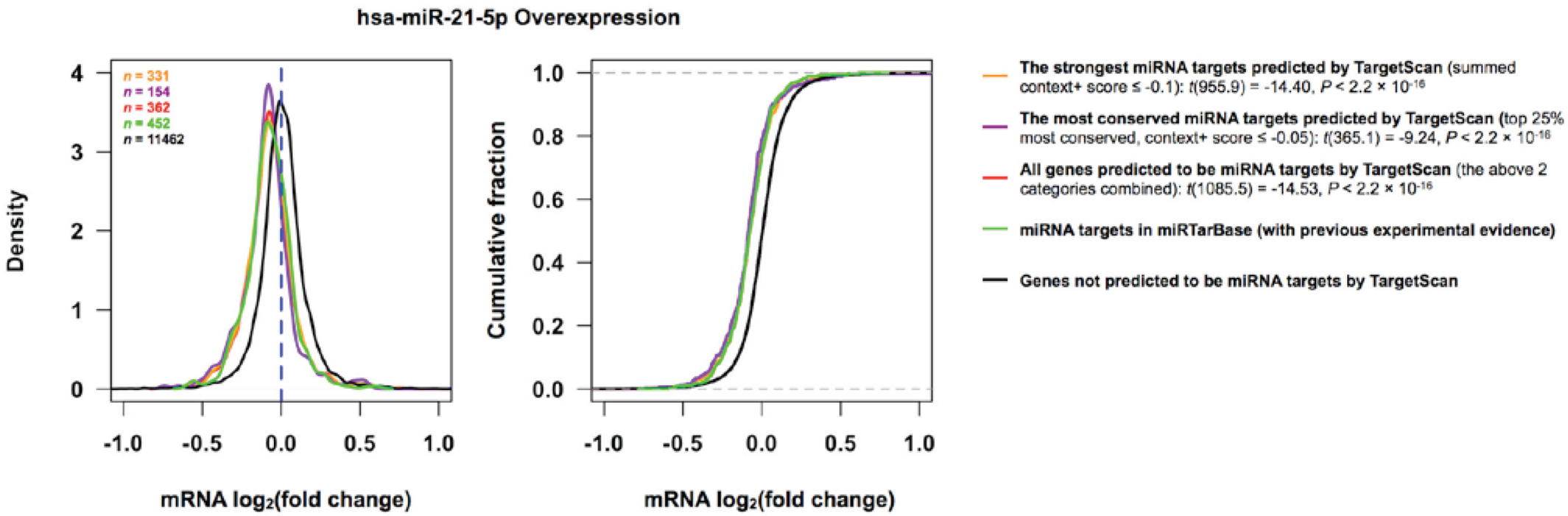




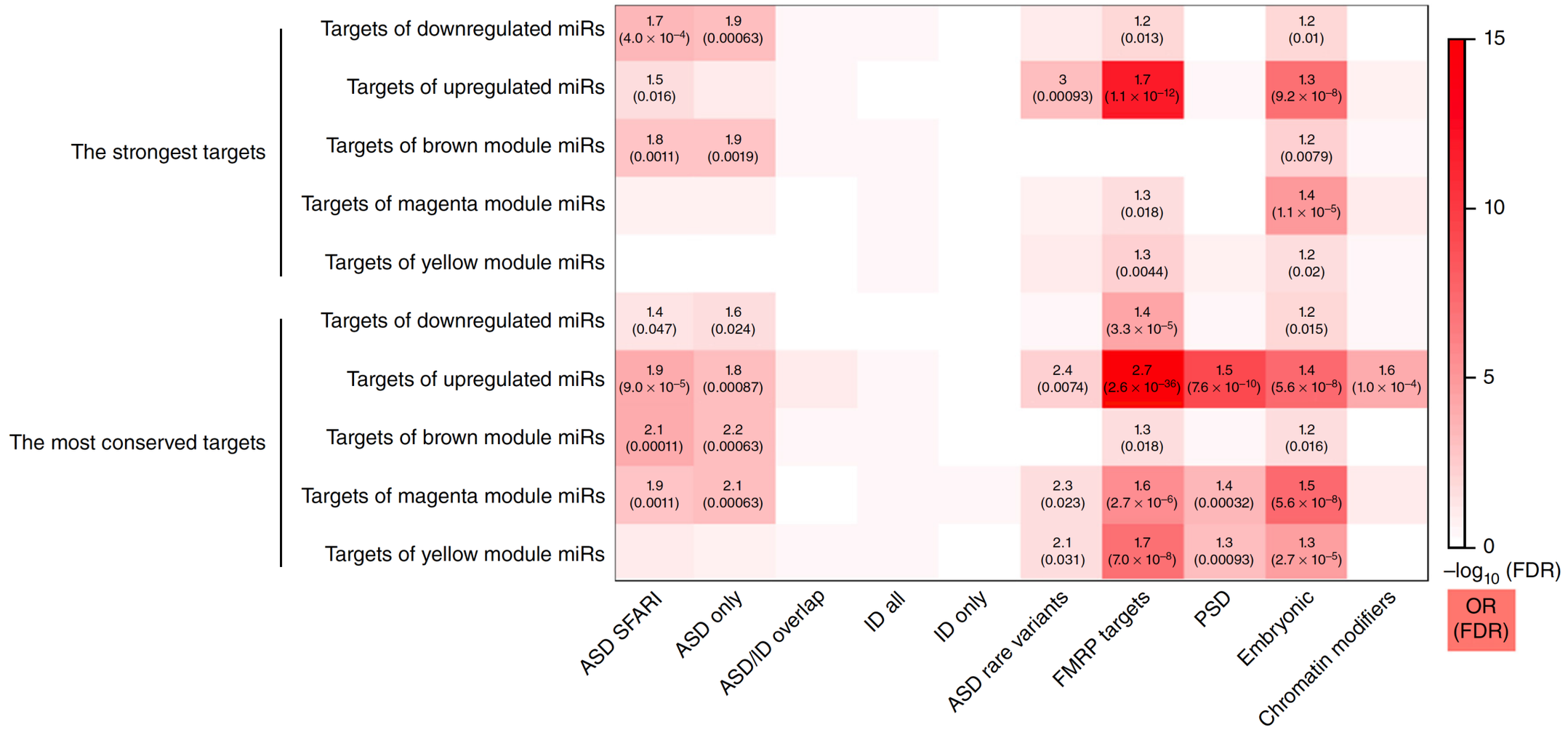
mRNA targets of top DE miRNAs and hub miRNAs in ASD-related modules

top targets that are expressed in the temporal and frontal cortex:
- targets with the highest predicted targeting efficacy and shared by two or more miRNA
- the most conserved target sites, which are more likely to have conserved physiological roles

experimentally validated hsa-miR-21-5p in human neural progenitor cells (hNPCs):

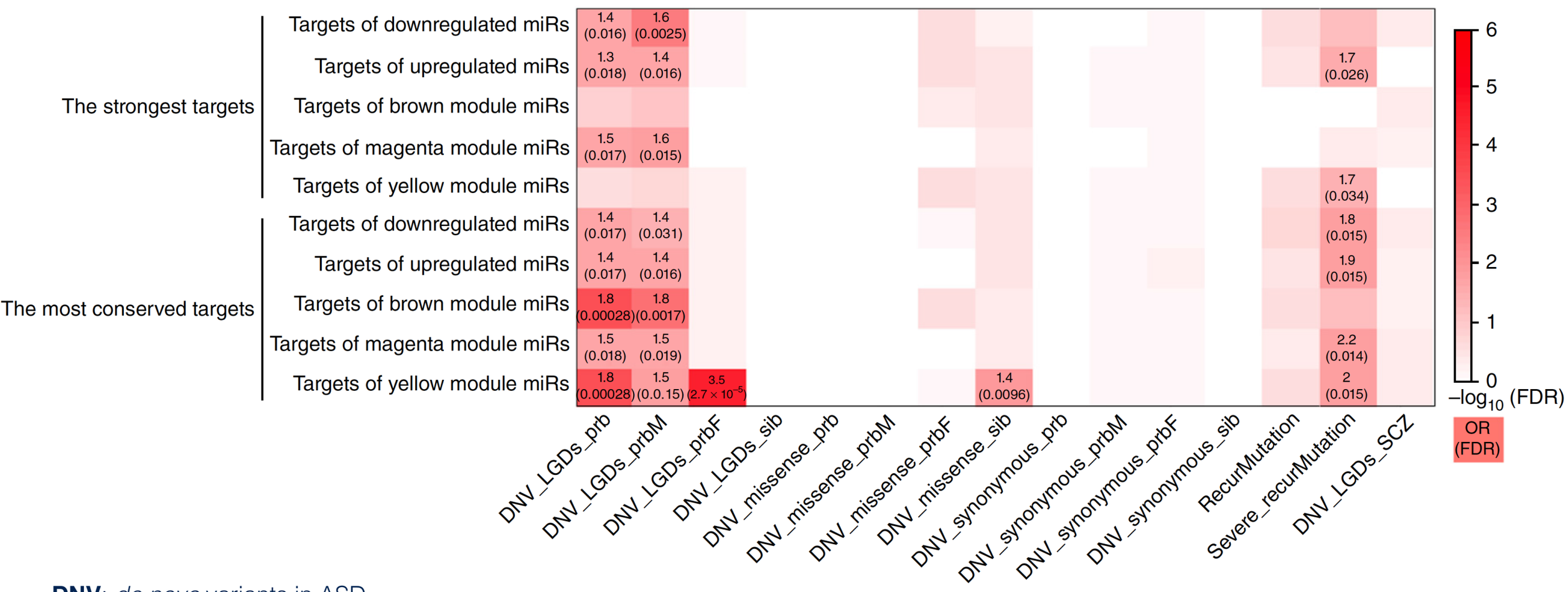


Enrichment of ASD risk genes among the top targets of ASD-affected miRNAs and miRNA modules

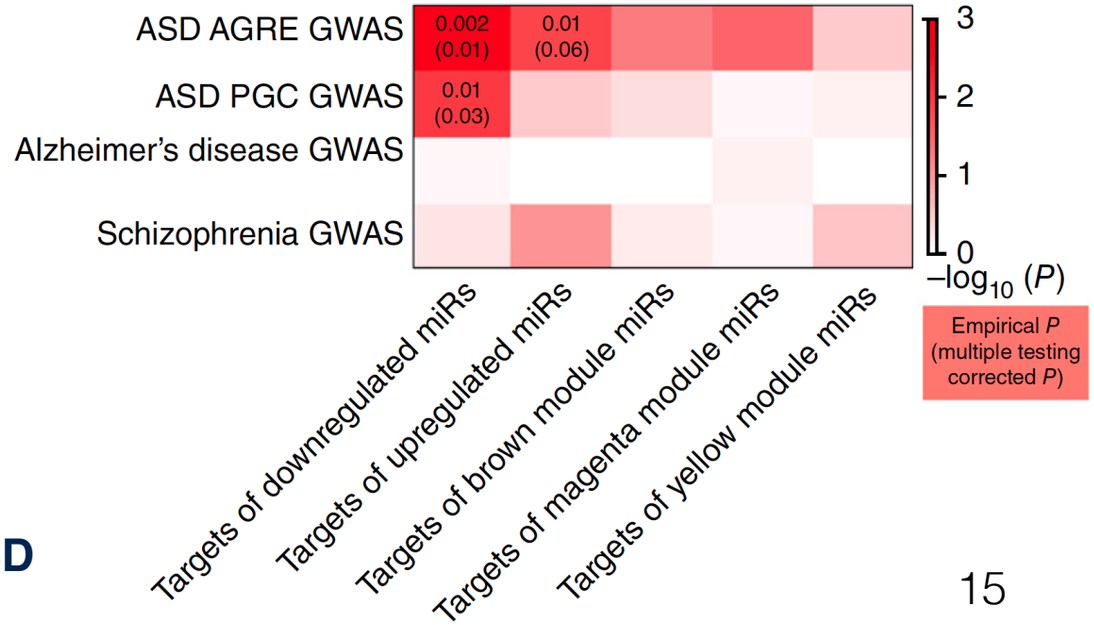


ASD SFARI: set of ASD risk genes from the Simons Foundation Autism Research Initiative (SFARI) AutDB database
ASD only: **ASD SFARI** genes not overlapping with **ID all** genes
ASD/ID overlap: **ASD SFARI** genes overlapping with **ID all** genes
ID all: genes implicated in monogenic form of intellectual disability
ID only: **ID all** genes not overlapping with **ASD SFARI** genes
ASD rare variants: ASD risk genes implicated by rare variants
FMRP targets: fragile X mental retardation protein transcripts
PSD: genes encoding postsynaptic density
Embryonic: genes expressed preferentially during embryonic brain development
Chromatin modifiers: genes encoding chromatin modifiers

Enrichment of genes affected by *de novo* ASD variants

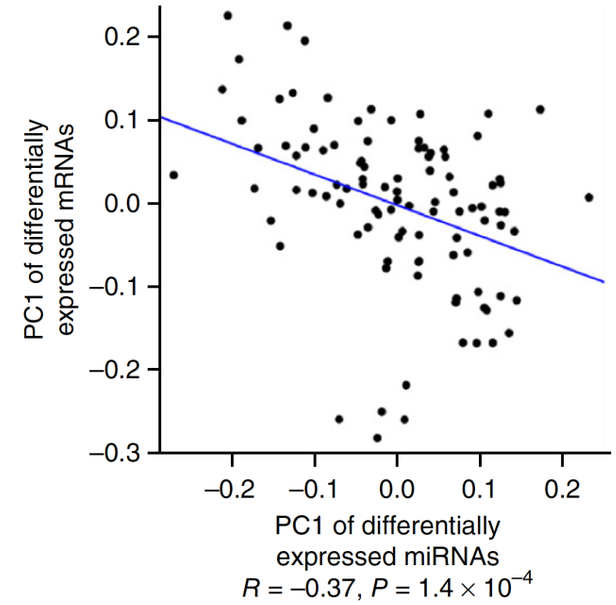


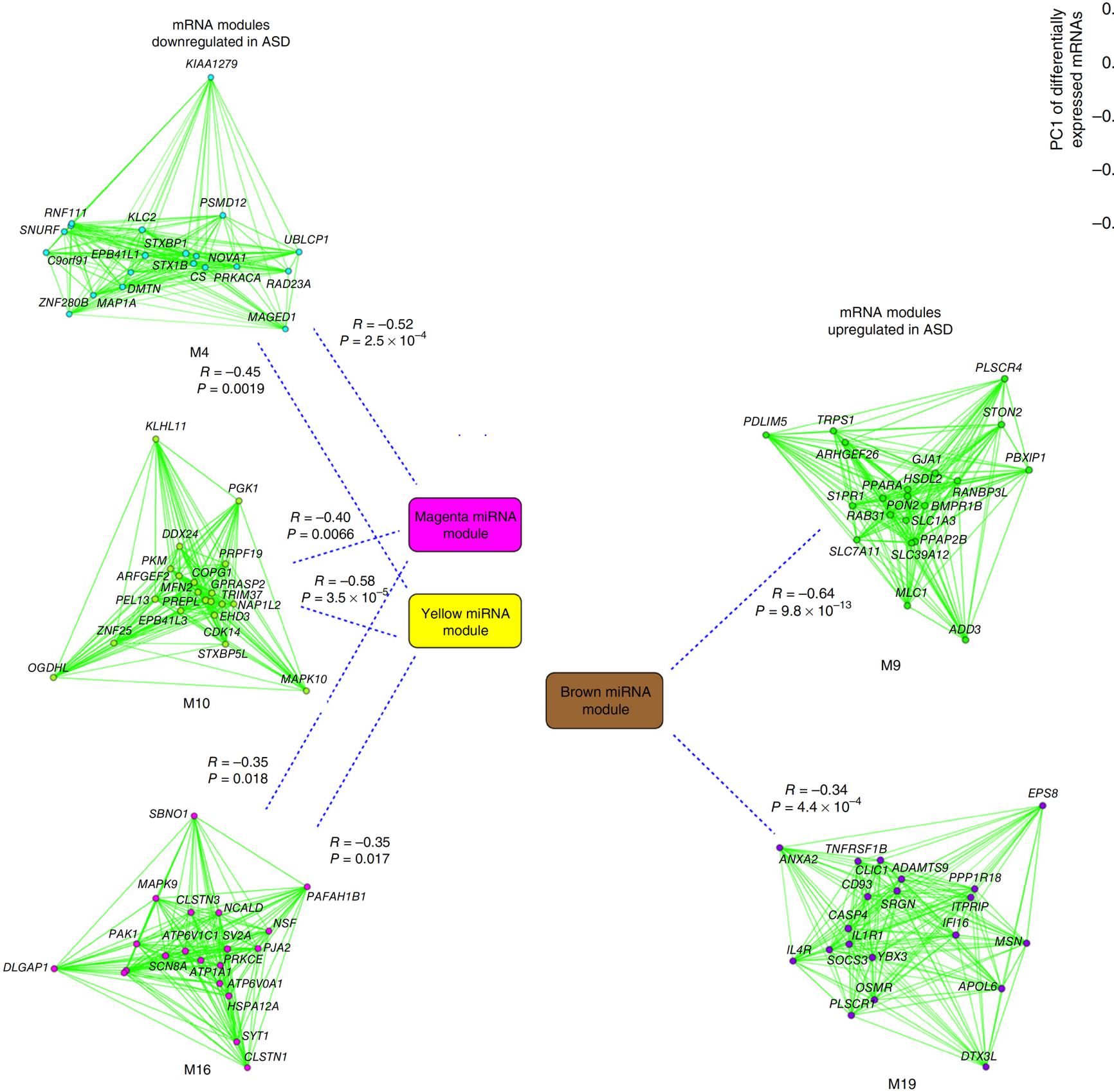
DNV: *de novo* variants in ASD
LGD: 'likely-gene-disrupting' (including nonsense, splice-site and frame shift)
prb, prbM, prbF: probants: all, male, female
sib: siblings
SCZ: individuals with schizophrenia



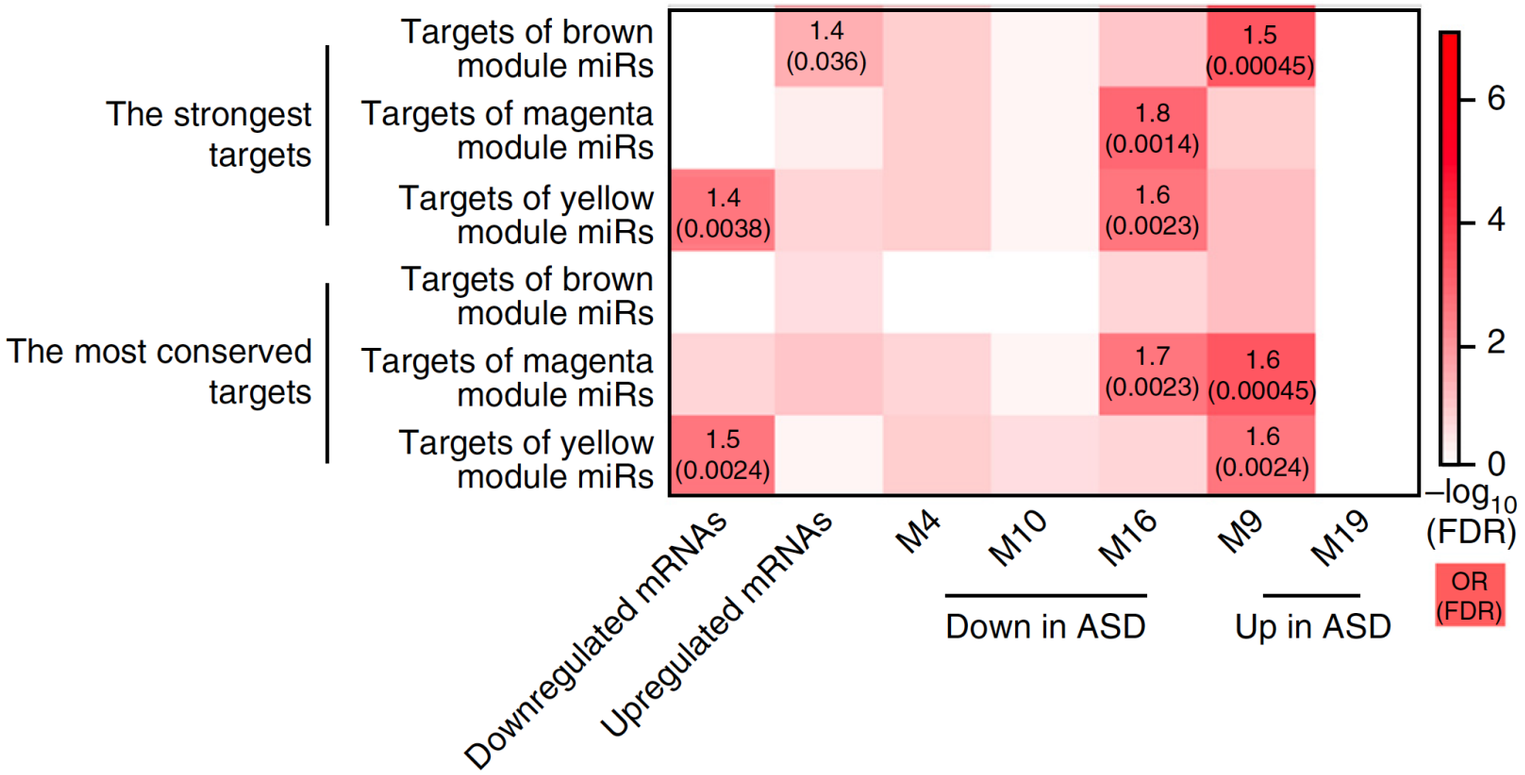
Enrichment of overlap with LD-based independent genomic regions associated with ASD

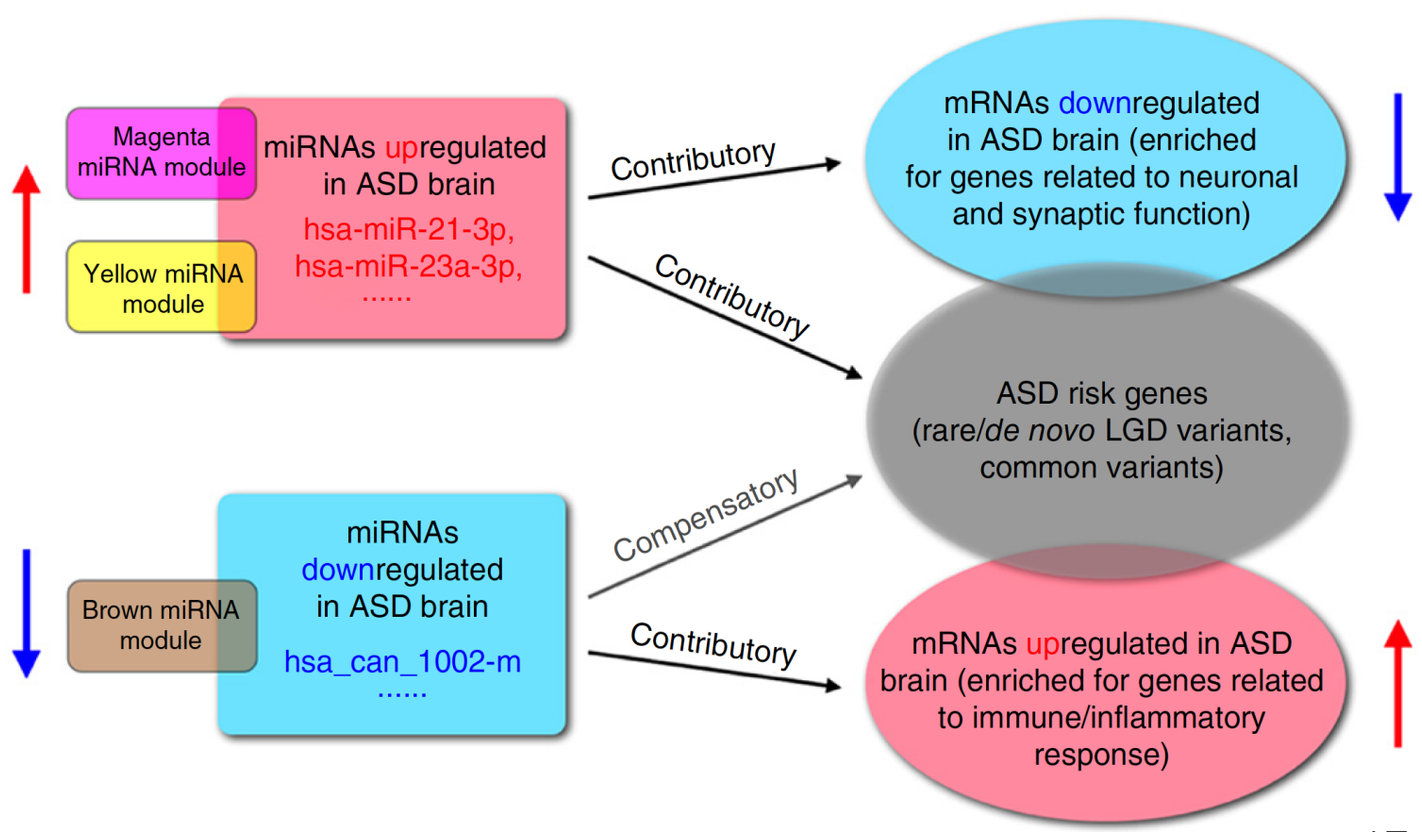
Relationship b/w miRNA and mRNA (101 matching samples in ‘unpublished observations’) expression changes





Enrichment for ASD-affected mRNAs and mRNA modules in the top targets of ASD-affected mRNAs



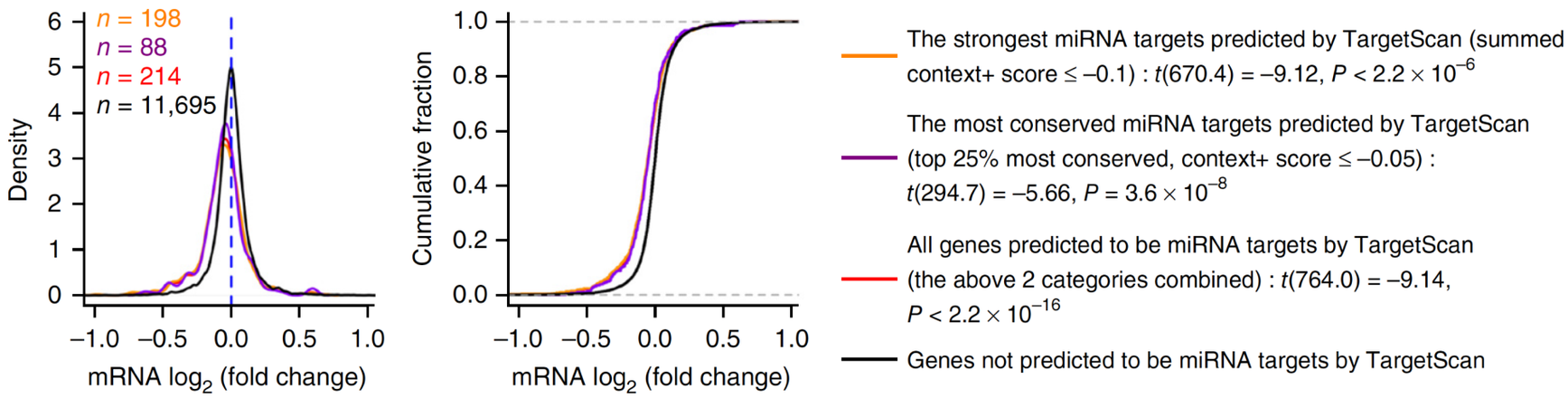


"The upregulated miRNAs and miRNA modules may have a contributory role by repressing ASD risk genes and neuronal/synaptic genes downregulated in ASD.

The downregulated miRNAs and miRNA module may contribute to the upregulation of immune/ inflammatory genes in ASD, but might also have a compensatory role given the enrichment of their targets for rare protein-disrupting and common genetic variants associated with ASD"

Experimental validation of hsa_can_1002-m

a



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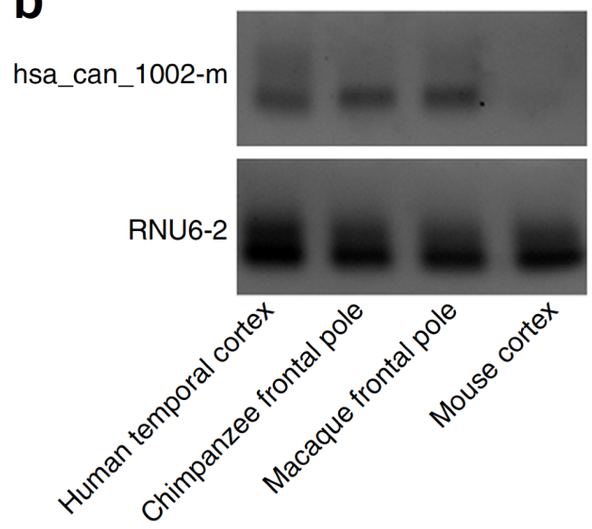
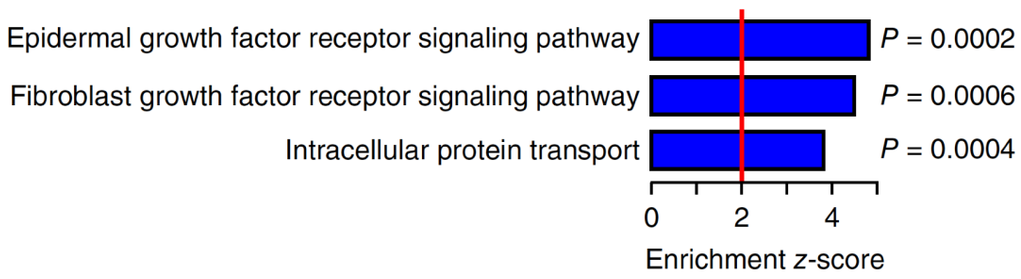
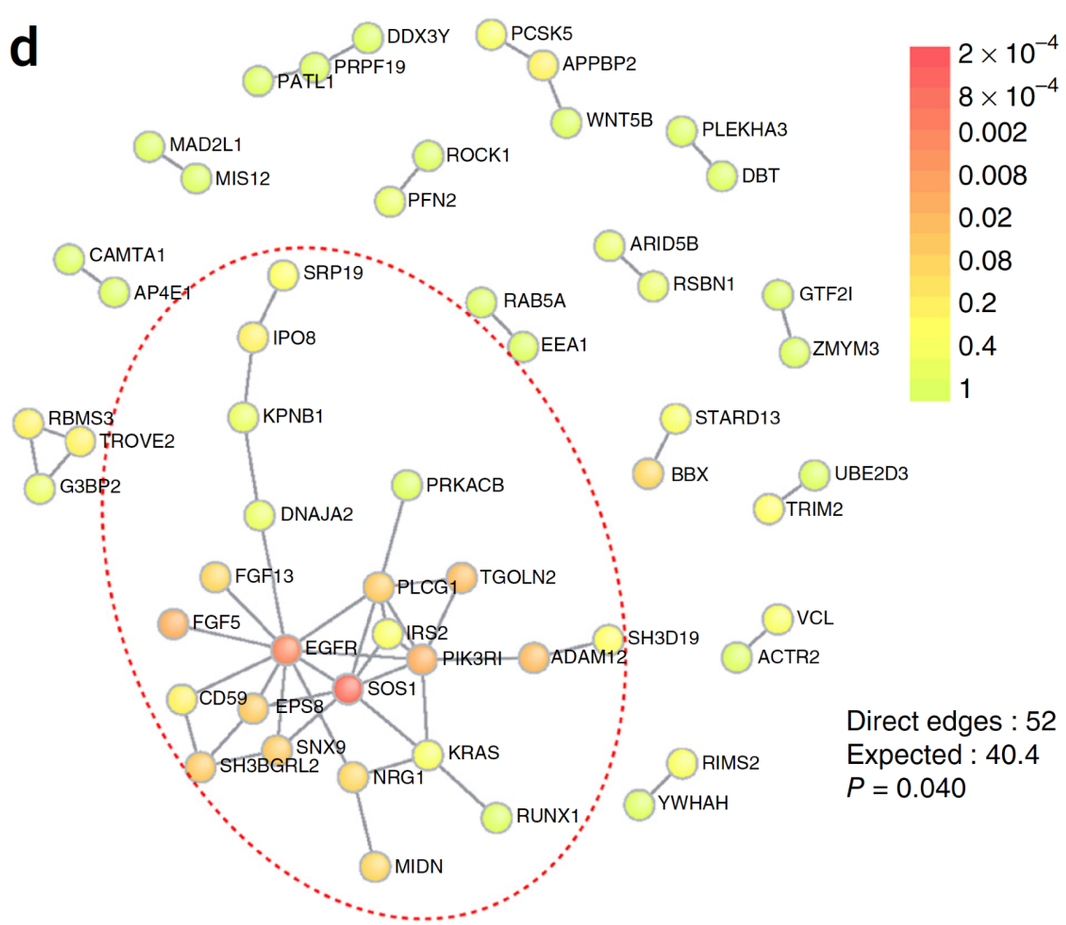


c

Go enrichment of hsa_can_1002-m targets



d



e

Target gene symbols	Description
EPS8, ADAM12, EGFR, CHUK, IRS2, FGF5, SOS1, PLCG1, KRAS, PRKACB, PIK3R1	Genes associated with EGFR and FGFR signaling pathways
DDAH1, CCNG1, MTM1, CD59, FEM1C	Genes in the M9 mRNA module (upregulated in ASD, related to immune/inflammatory response)
RUNX1, REST, ROCK1	Other genes upregulated in ASD cortex

LETTER

doi:10.1038/nature20612

Genome-wide changes in lncRNA, splicing, and regional gene expression patterns in autism

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Autism spectrum disorder (ASD) involves substantial genetic contributions. These contributions are profoundly heterogeneous but may converge on common pathways that are not yet well understood[1–3]. Here, through post-mortem genome-wide transcriptome analysis of the largest cohort of samples analysed so far, to our knowledge[4–7], we interrogate the noncoding transcriptome, alternative splicing, and upstream molecular regulators to broaden our understanding of molecular convergence in ASD. Our analysis reveals ASD-associated dysregulation of primate-specific long noncoding RNAs (lncRNAs), downregulation of the alternative splicing of activity-dependent neuron-specific exons, and attenuation of normal differences in gene expression between the frontal and temporal lobes. Our data suggest that SOX5, a transcription factor involved in neuron fate specification, contributes to this reduction in regional differences. We further demonstrate that a genetically defined subtype of ASD, chromosome 15q11.2-13.1 duplication syndrome (dup15q), shares the core transcriptomic signature observed in idiopathic ASD. Co-expression network analysis reveals that individuals with ASD show age-related changes in the trajectory of microglial and synaptic function over the first two decades, and suggests that genetic risk for ASD may influence changes in regional cortical gene expression. Our findings illustrate how diverse genetic perturbations can lead to phenotypic convergence at multiple biological levels in a complex neuropsychiatric disorder.

status (Extended Data Fig. 2a) and confirmed the technical quality of our data with qRT–PCR (Extended Data Fig. 2b, c). We next evaluated enrichment of the gene sets for pathways and cell types (Extended Data Fig. 2d, e), and found that the downregulated set was enriched in genes expressed in neurons and involved in neuronal pathways, including *PVALB* and *SYT2*, which are highly expressed in interneurons; by contrast, the upregulated gene set was enriched in genes expressed in microglia and astrocytes[8].

Although there was no significant DGE in the cerebellum (FDR < 0.05, *P* distributions in Fig. 1b), similar to observations in a smaller cohort[8], there was a replication signal in the cerebellum and overall concordance between ASD-related fold changes in the cortex and cerebellum (Extended Data Fig. 2f–h). The lack of significant DGE in the cerebellum is explained by the fact that changes in expression were consistently stronger in the cortex than in the cerebellum (Extended Data Fig. 2h), which suggests that the cortex is more selectively vulnerable to these transcriptomic alterations. We also compared our results to an RNA-seq study of protein coding genes in the occipital cortex of individuals with ASD and control subjects[4]. Despite significant technical differences that reduce power to detect DGE, and profiling of different brain regions in that study, there was a weak but significant correlation in fold changes, which was due mostly to upregulated genes in both studies ($P = 0.038$, Extended Data Fig. 2i, j).

We next explored lncRNAs, most of which have little functional annotation, and identified 60 lncRNAs in the DGE set (FDR < 0.05,

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*These authors contributed equally to this work.

RNA-seq workflow

Tissue dissection and RNA extraction
From frontal cortex (FC), temporal cortex (TC),
and cerebellar vermis (CB)

↓

Library preparation and RNA-seq
Unstranded, rRNA depleted libraries. 50bp paired end RNA-seq

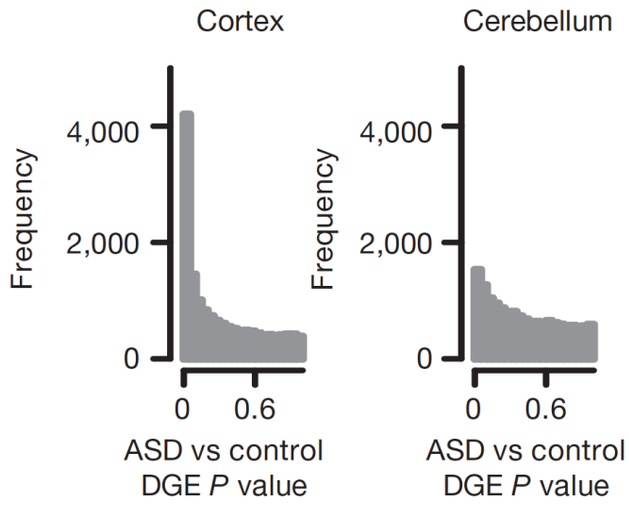
↓

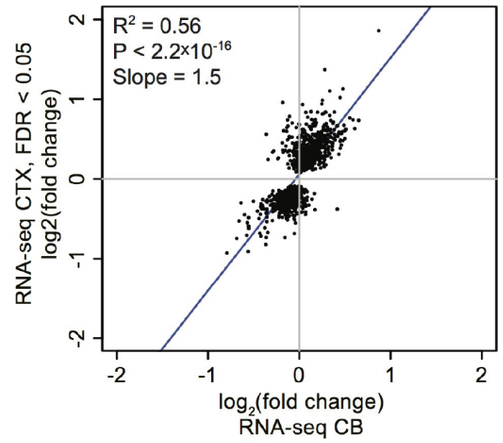
Sample and RNA-seq quality control
Genotyping from RNA-seq to remove poor quality samples (samtools)
Read alignment with TopHat v2
Quality control of mapped read files (samtools, PicardTools)
Quantification by HTSeq and Cufflinks
Removal of samples by exclusion criteria (Supplementary Information)

↓

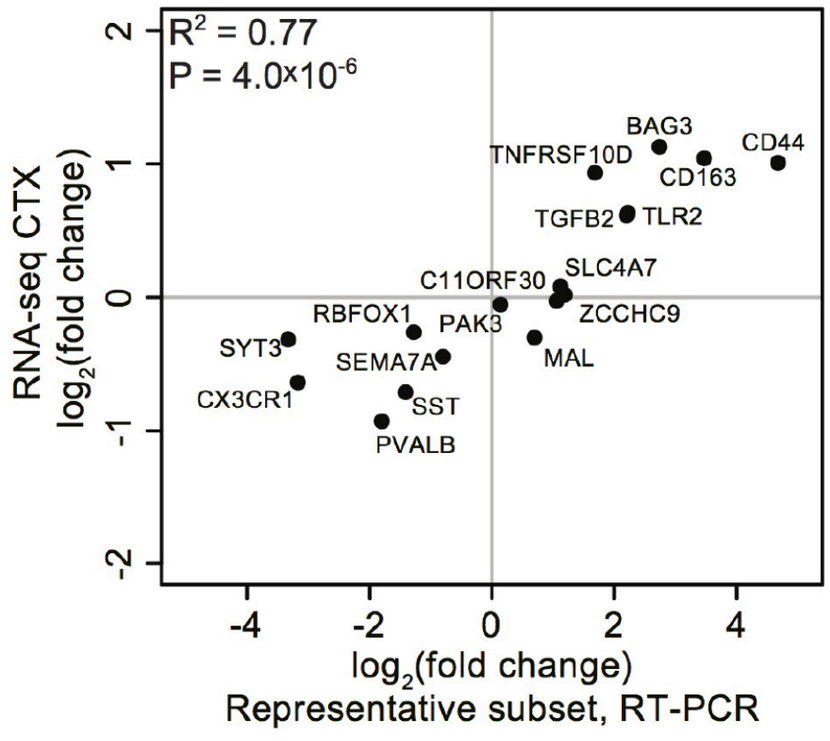
235* unique high quality samples for downstream analysis
Controls: 49 individuals (16 overlap Voineagu et al.)
42 FC, 32 TC, 41 CB
ASD: 39 individuals (14 overlap Voineagu et al.)
34 FC, 31 TC, 33 CB
Duplication 15q syndrome: 9 individuals (1 overlaps Voineagu et al.)
8 FC, 9 TC, 5 CB
*All counts correspond to unique samples,
251 total samples if counting replicates

DE for entire transcriptome and qRT-PCR validation



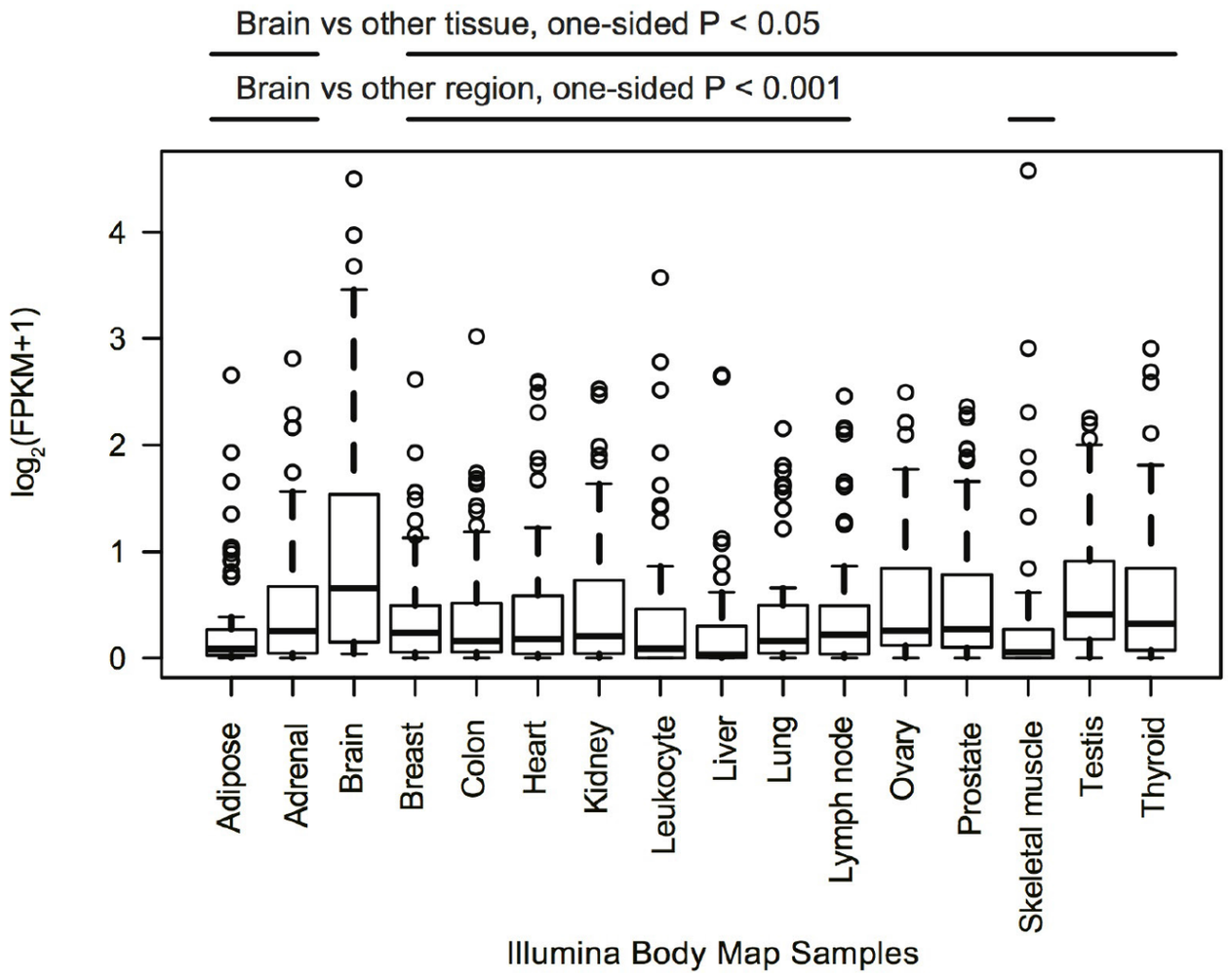


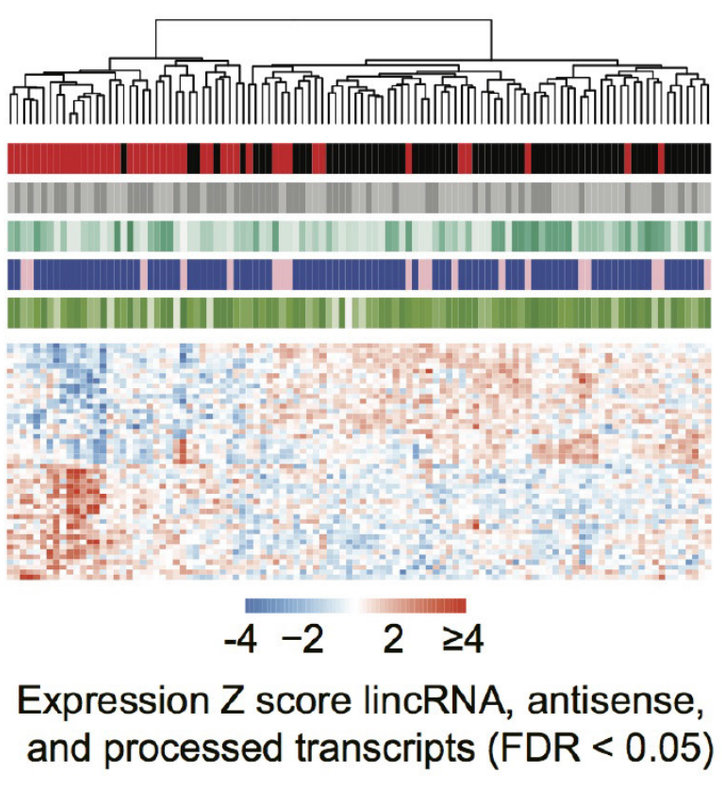
584 genes showed increased and **558** - decreased expression in idiopathic ASD cortex

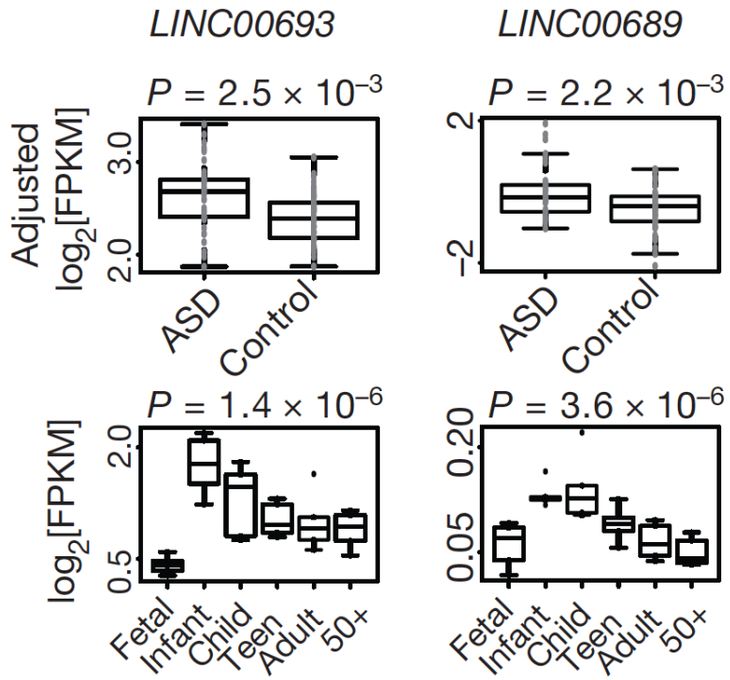


60 lncRNA in the DGE set: hierarchical clustering and enrichment in brain relative to other tissues

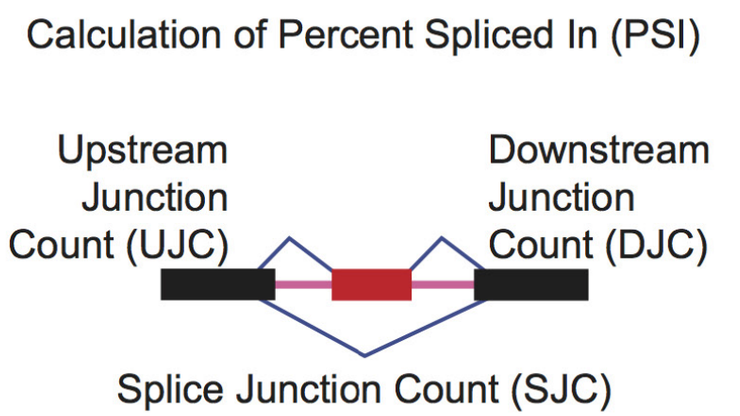
most exhibit primate-specific expression patterns in brain
20 interact with miRNA-protein complexes
9 - with FMRP
thus, predicted to affect protein expression through miRNA and FMRP interactions

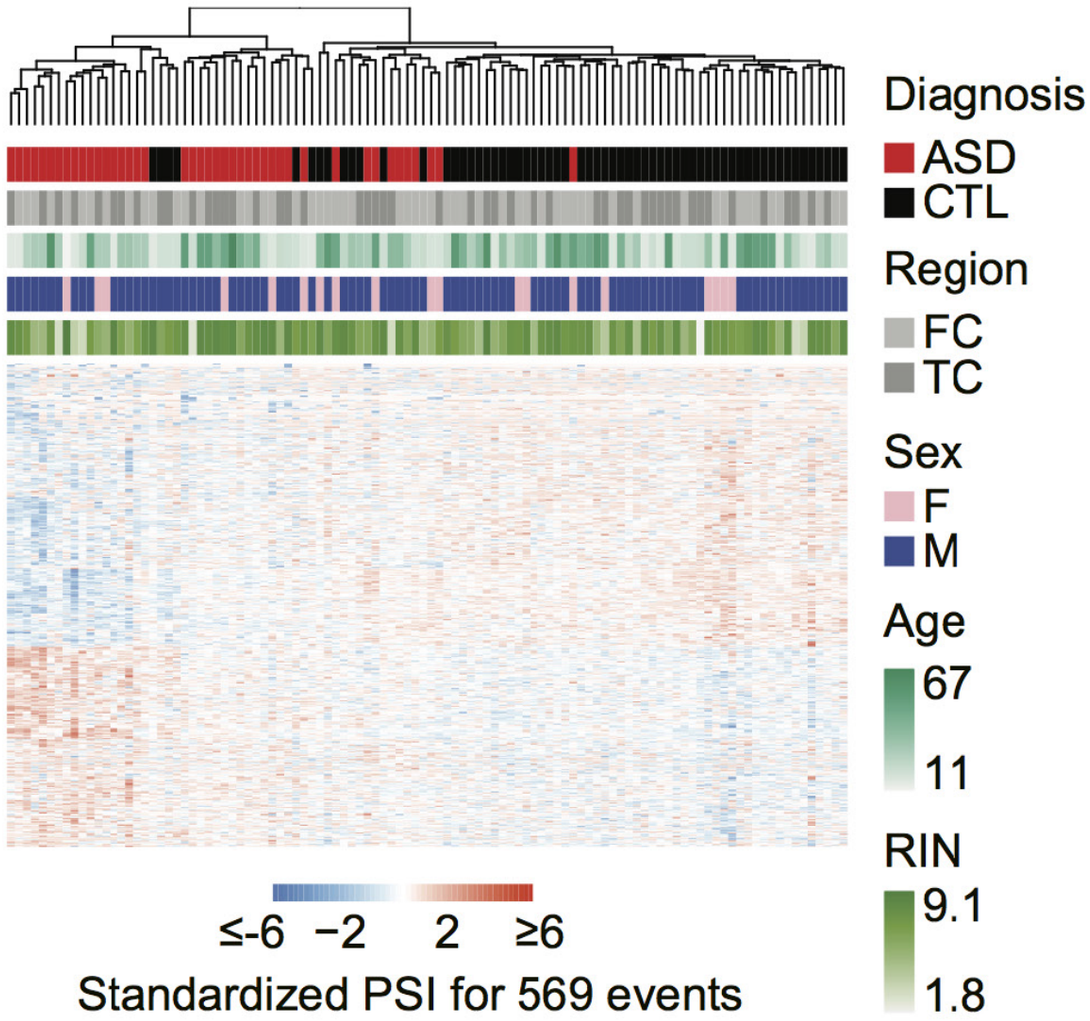




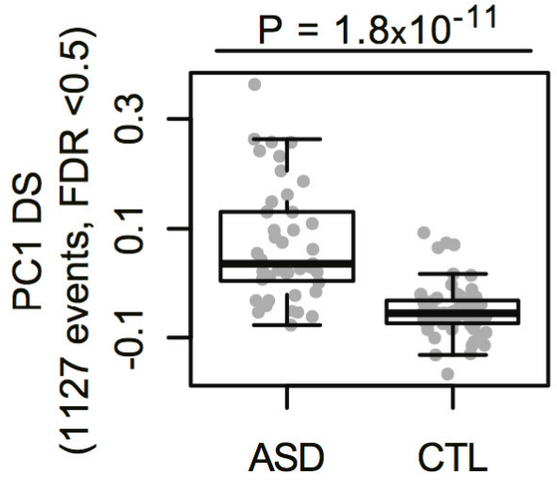


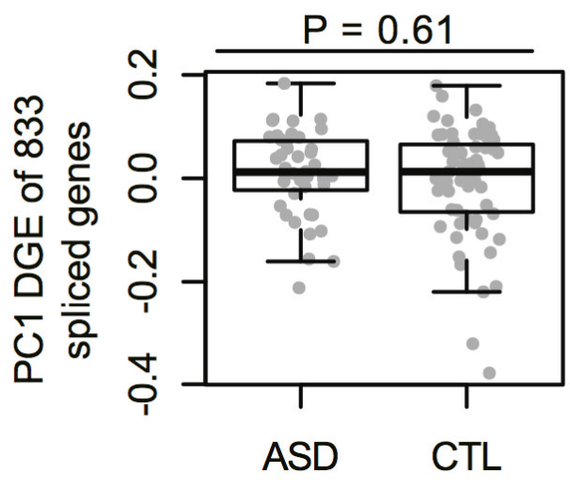
Splicing differential analysis and validation



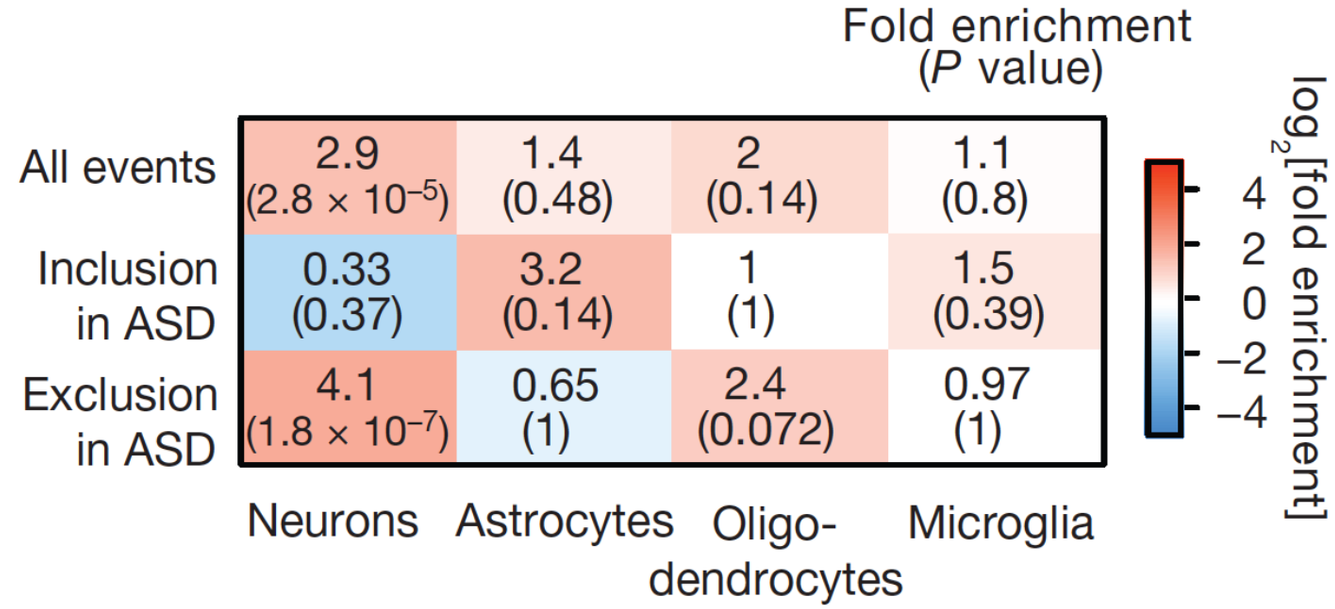


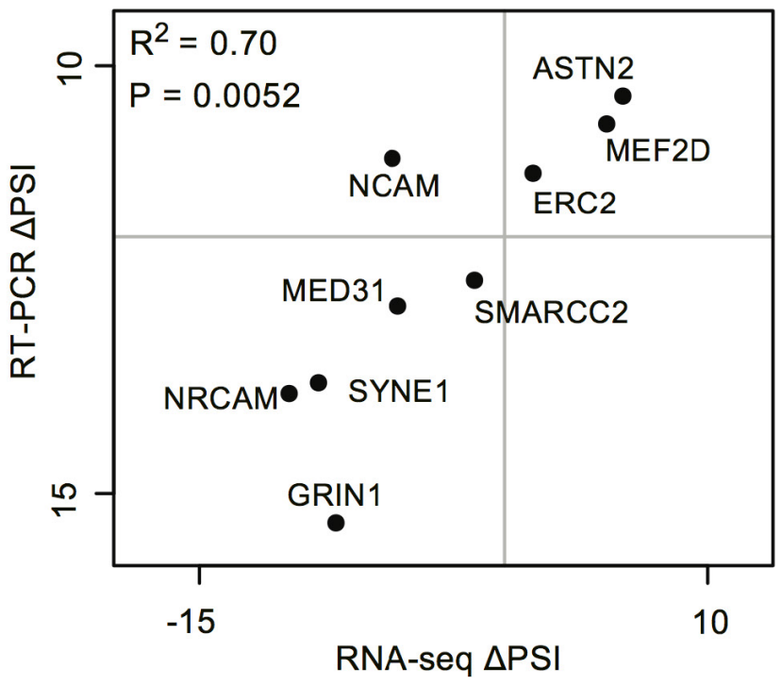
1127 differential splicing events in **833** genes in cortex

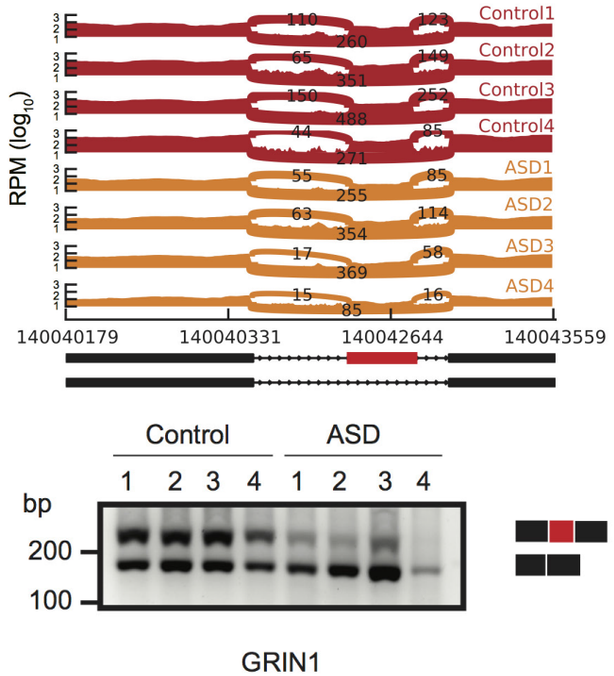


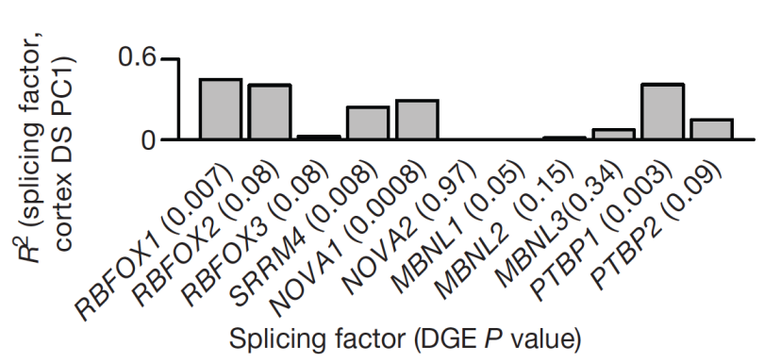


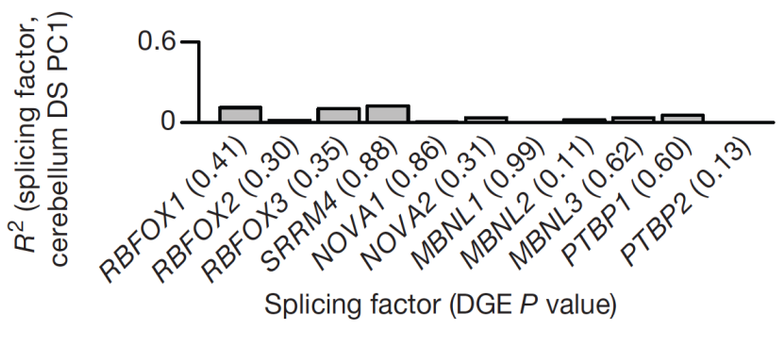
Splicing differential analysis and validation



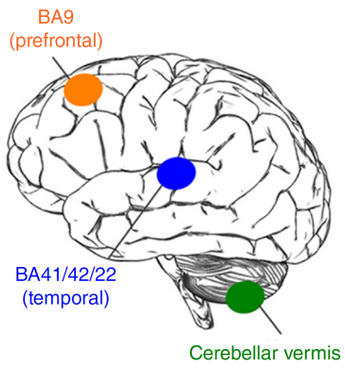


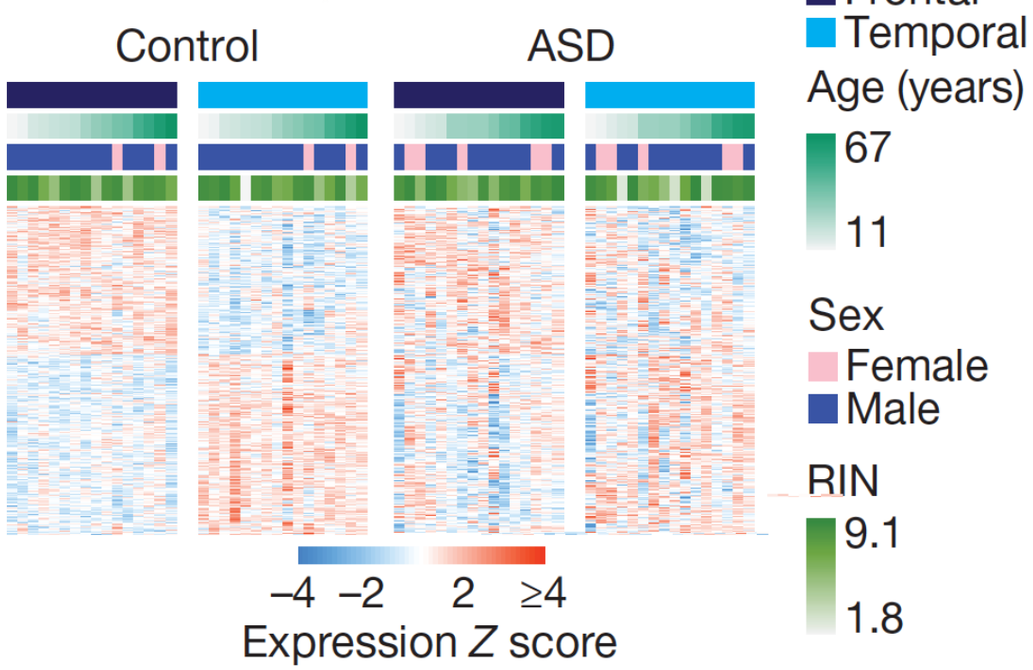






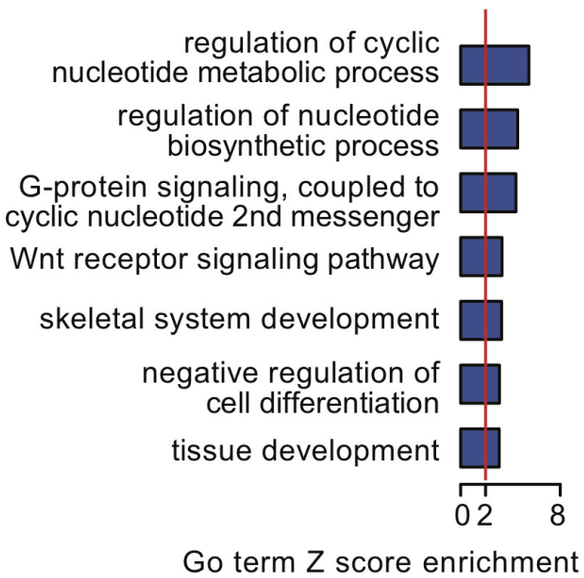
Attenuation of cortical patterning in ASD



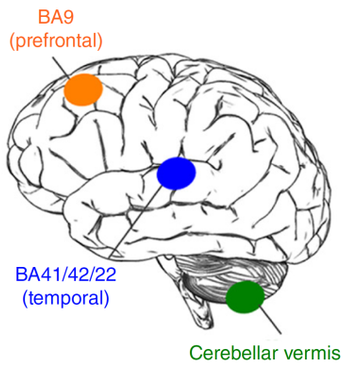


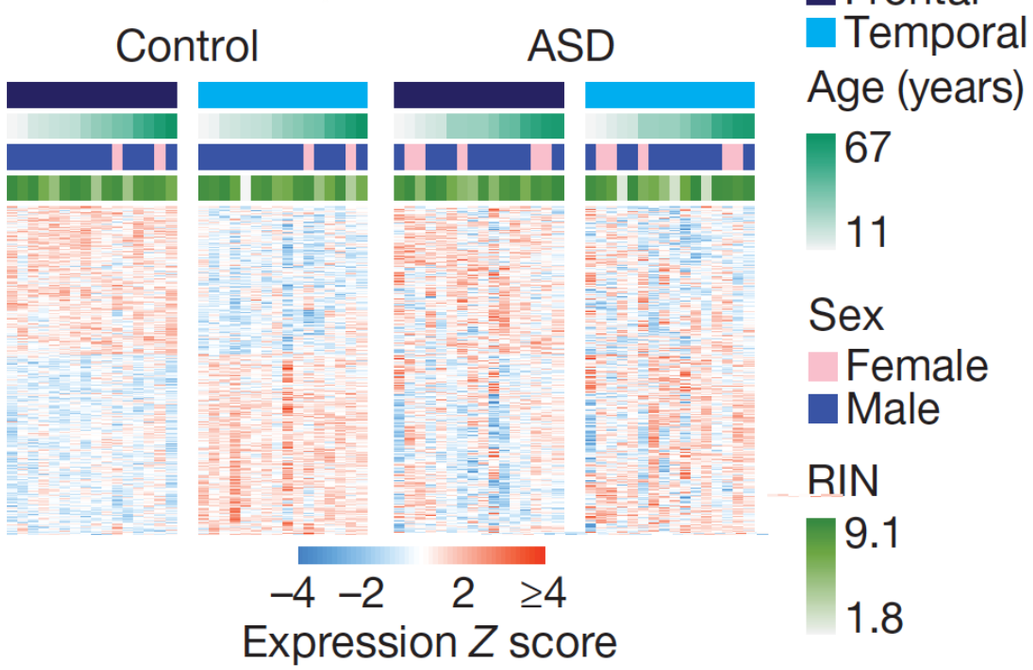
Number of genes DE b/w FC and TC:
551 in control samples
only **51** in ASD samples

Attenuated cortical patterning (ACP) set -
523 genes that show DE in control, but not ASD



Attenuation of cortical patterning in ASD





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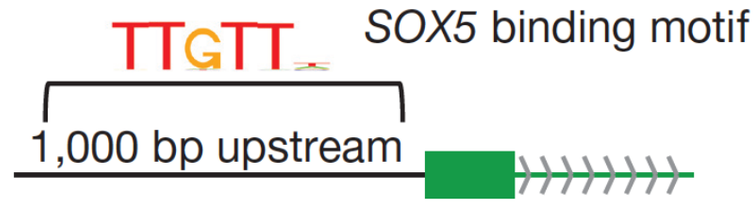
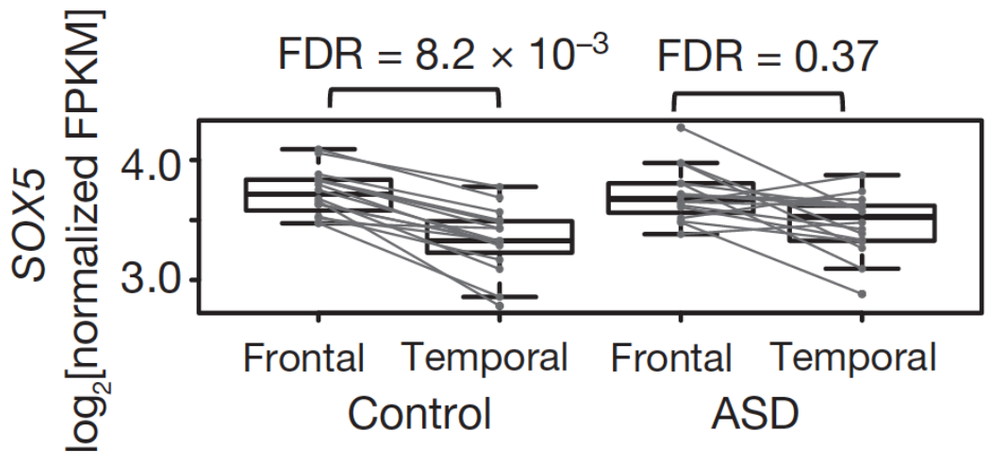
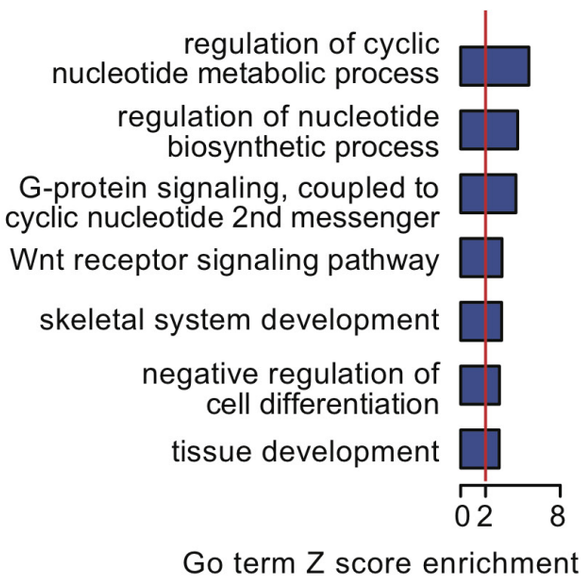
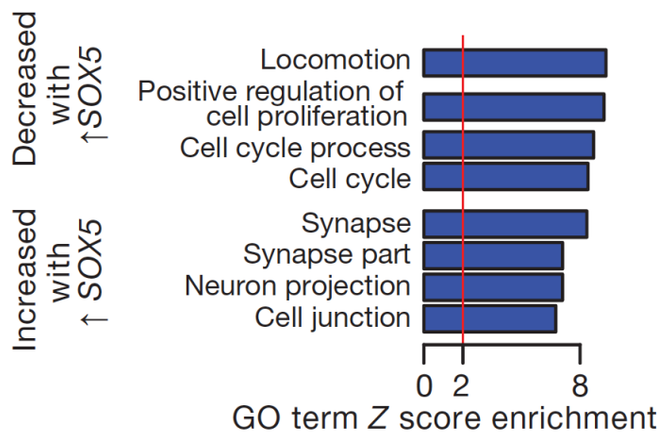
TF motif enrichment

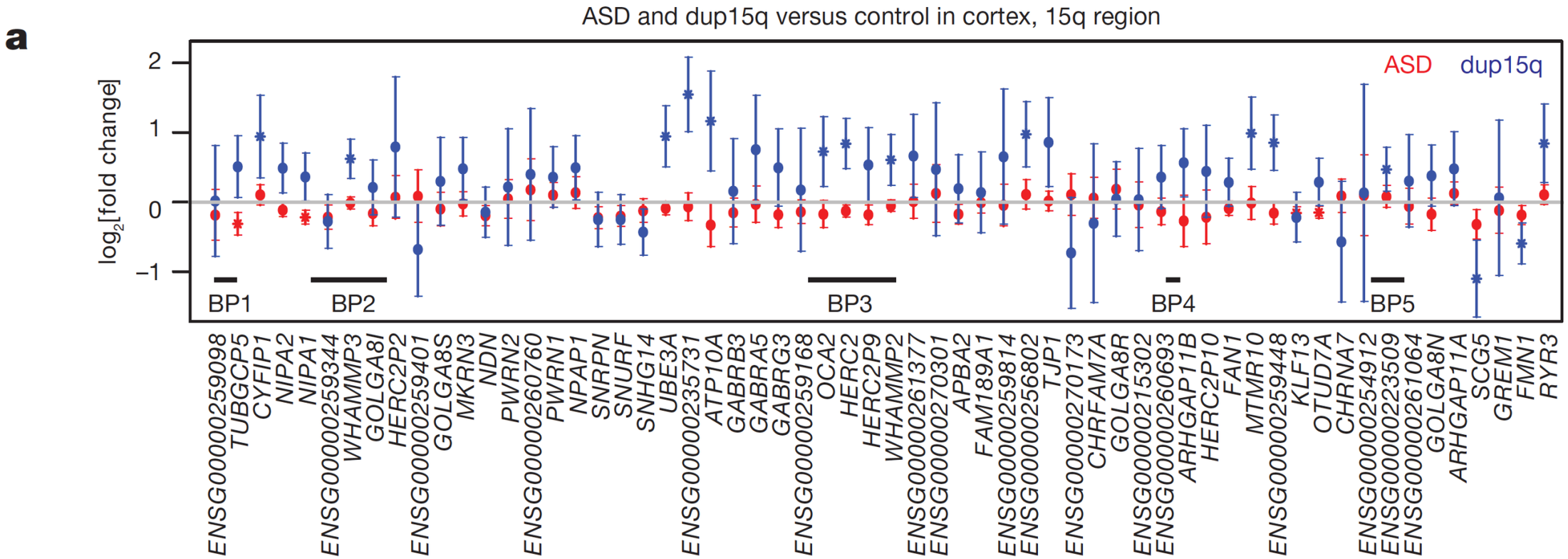








Duplication 15q syndrome recapitulates transcriptomic changes in idiopathic ASD



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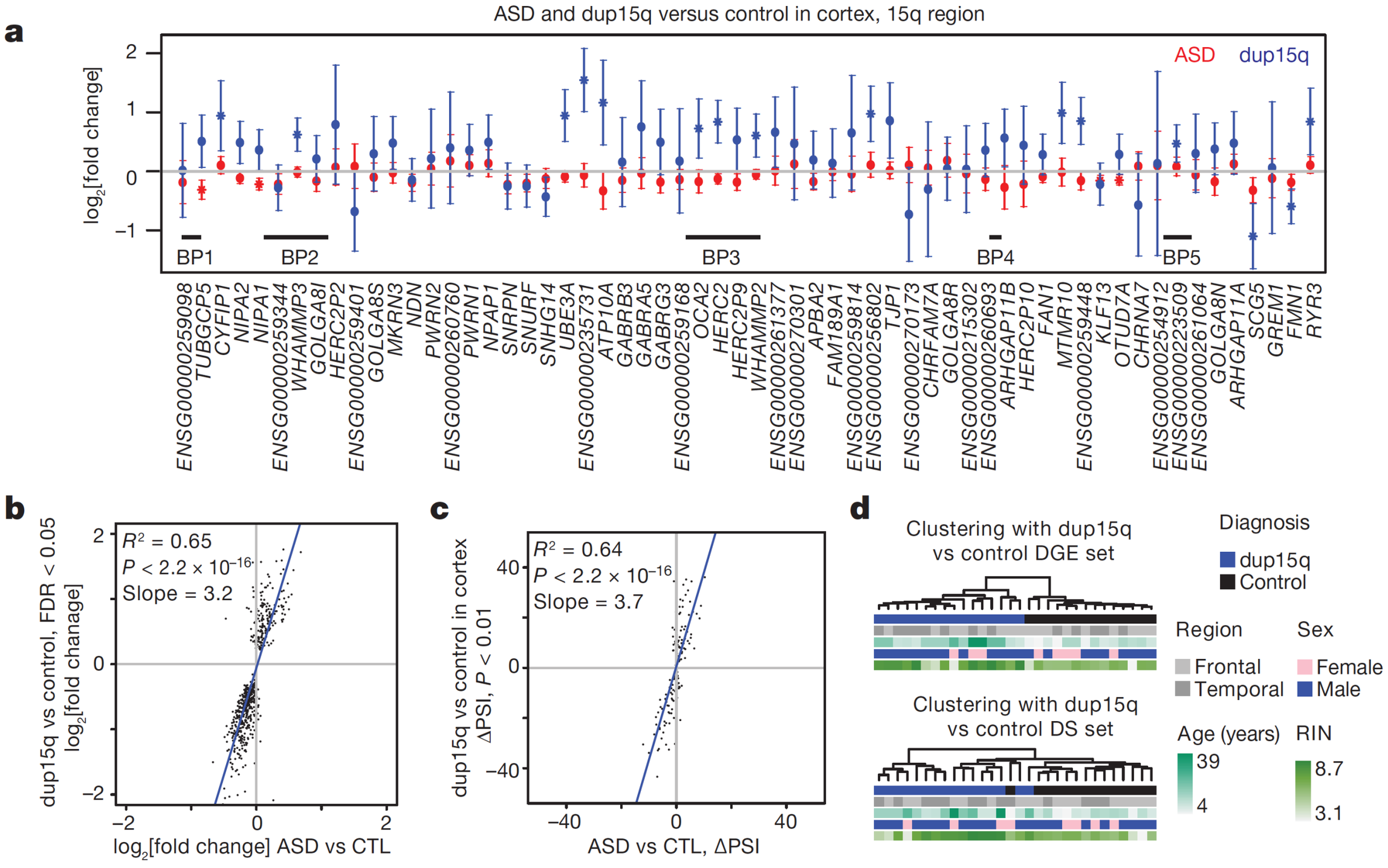
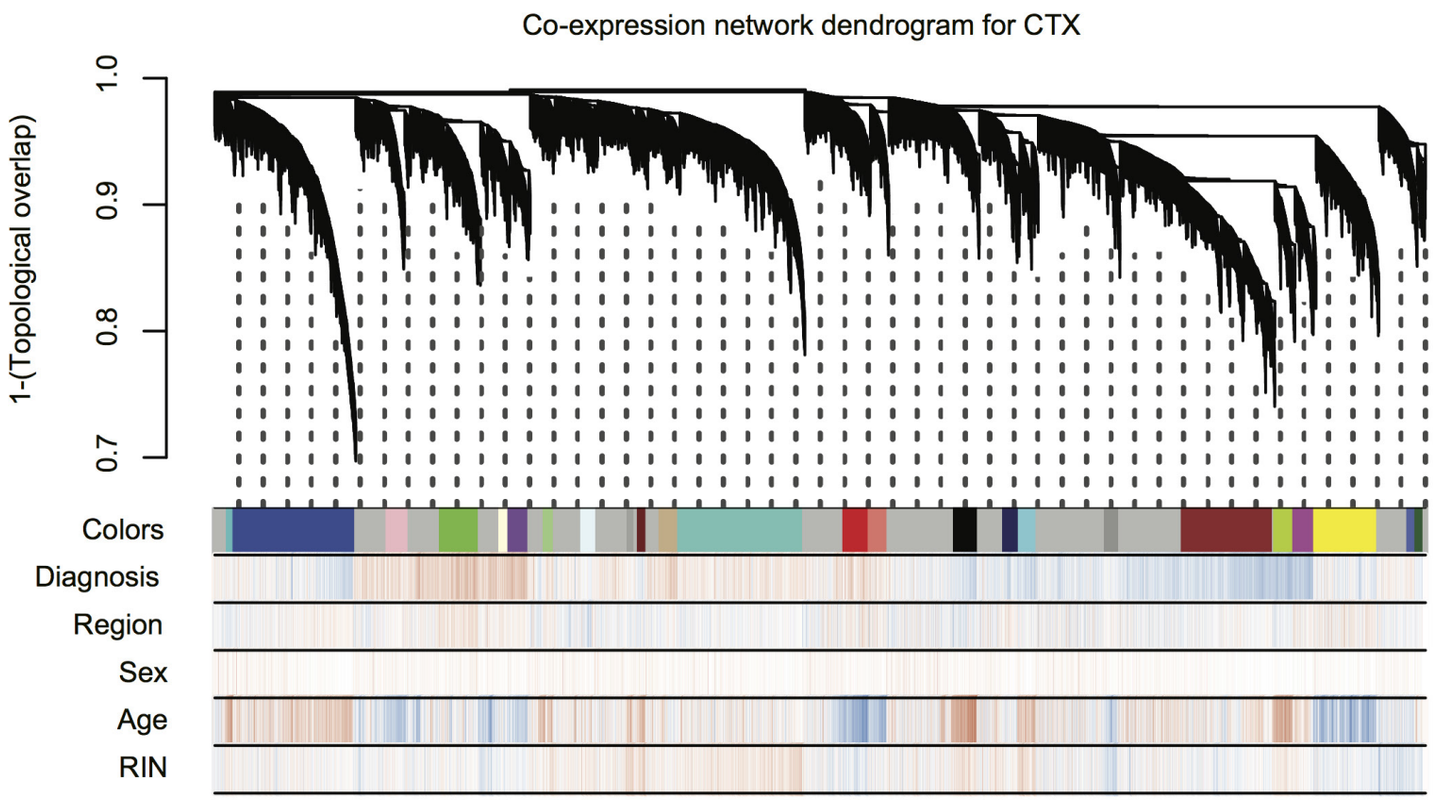
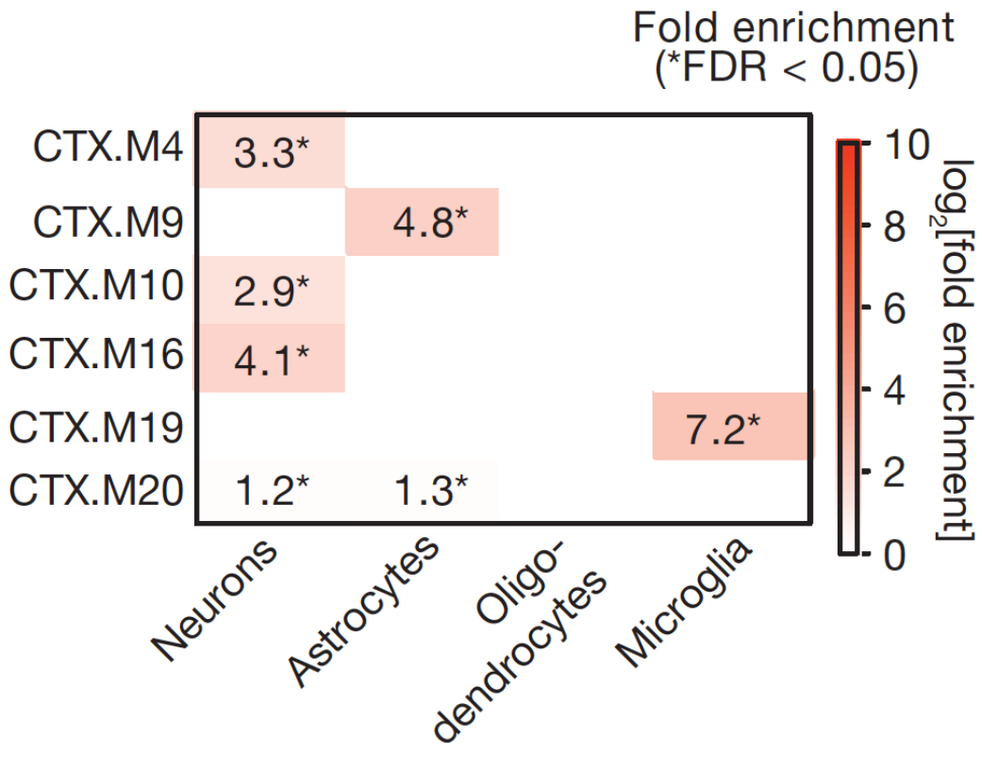
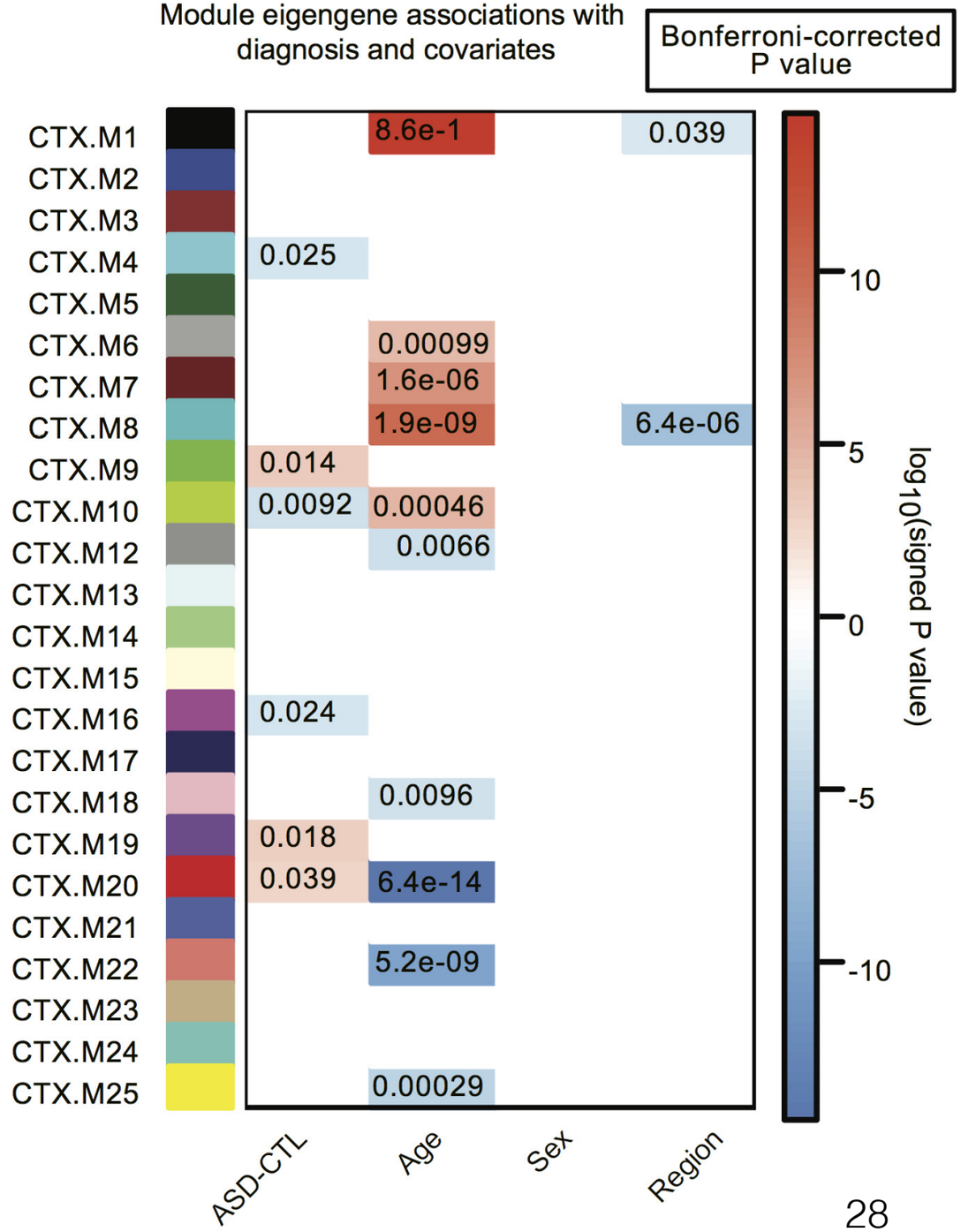


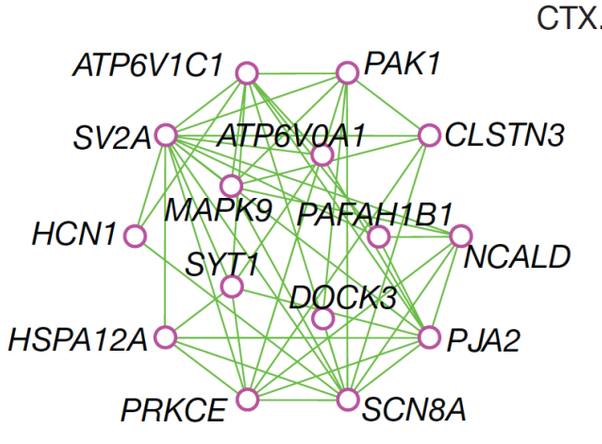
Co-expression network analysis

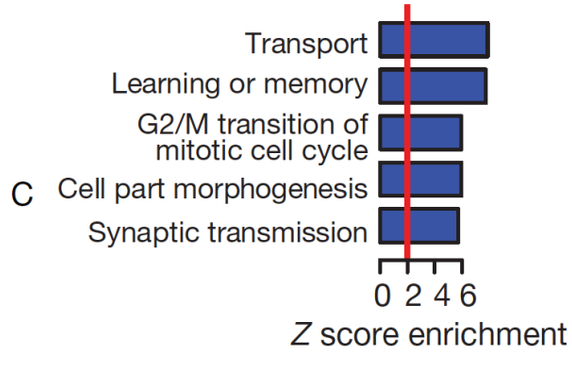


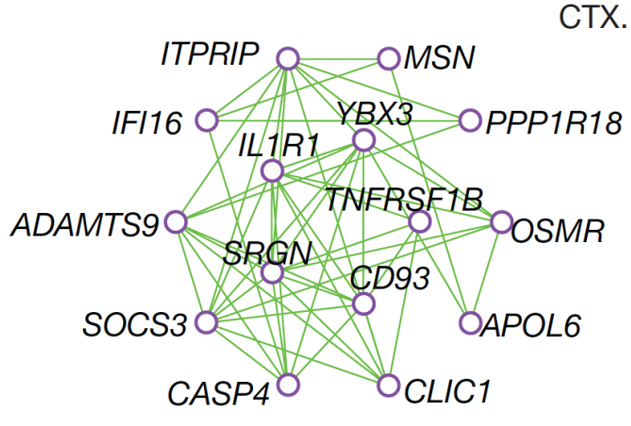


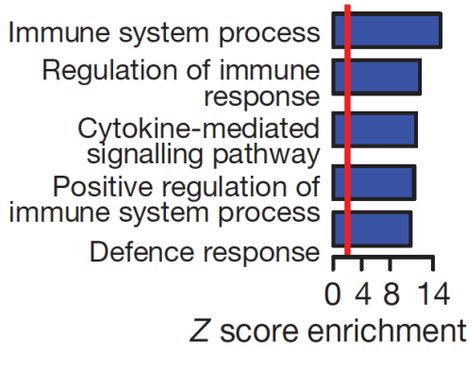


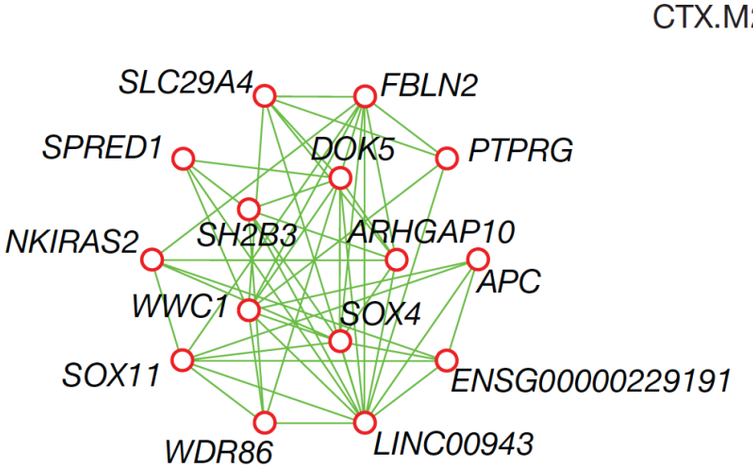
Co-expression network analysis

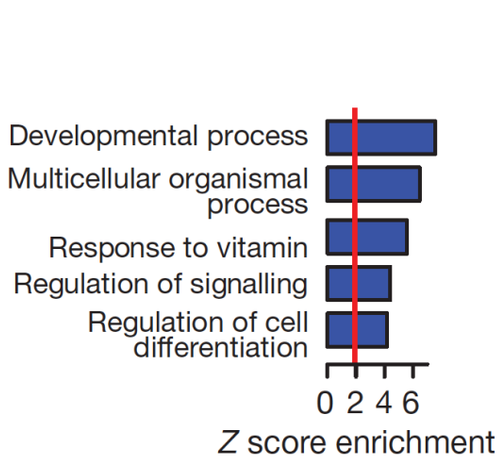


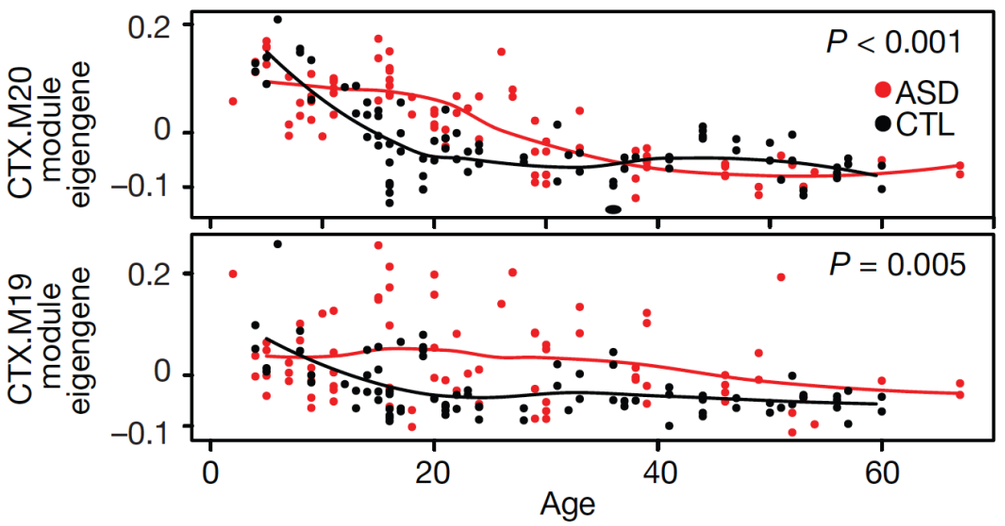












Related publications from the same lab

- Voineagu et al., *Nature* 2011
  Transcriptomic analysis of autistic brain reveals convergent molecular pathology

- Parikshak et al., *Cell* 2013
  Integrative Functional Genomic Analyses Implicate Specific Molecular Pathways and Circuits in Autism

- Parikshak et al., *Nature Rev Genet*, 2015
  Systems biology and gene networks in neurodevelopmental and neurodegenerative disorders

- de la Toree-Ubieta et al., *Nature Med,* 2016
  Advancing the understanding of autism disease mechanisms through genetics

- Gandal et al., *Nature Neurosci*, 2016
  The road to precision psychiatry: translating genetics into disease mechanisms

- Leppa et al., *Am J Hum Genet*, 2016
  Rare Inherited and De Novo CNVs Reveal Complex Contributions to ASD Risk in Multiplex Families

- Werling et al., *Nature Comm*, 2016
  Gene expression in human brain implicates sexually dimorphic pathways in autism spectrum disorders

- Sun et al., *Cell*, 2016
  Histone Acetylome-wide Association Study of Autism Spectrum Disorder

- Seyfried et al., *Cell Systems*, 2016
  A Multi-network Approach Identifies Protein-Specific Co-expression in Asymptomatic and Symptomatic Alzheimer's Disease

- Won et al., *Nature*, 2016
  Chromosome conformation elucidates regulatory relationships in developing human brain