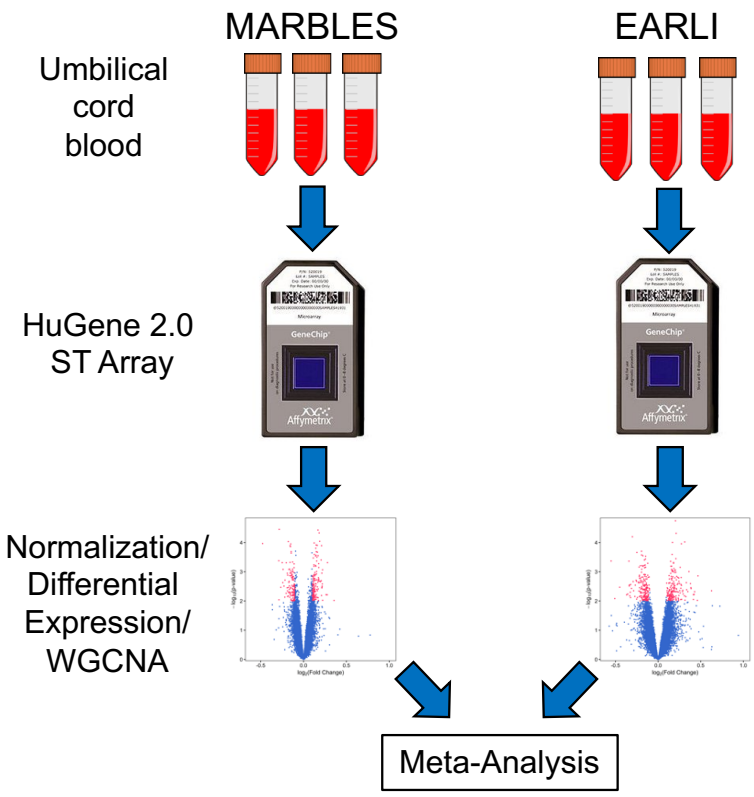
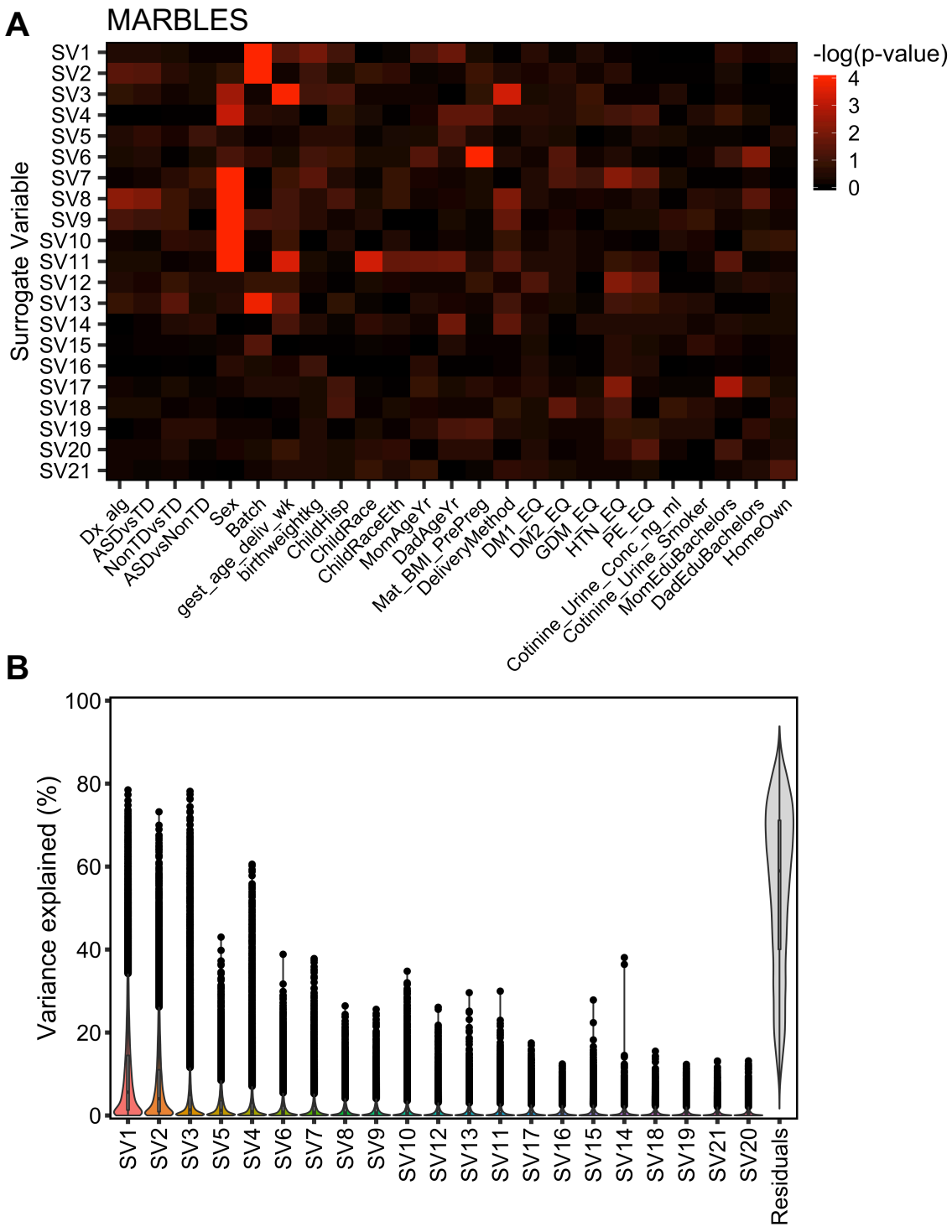
Supplemental Figures



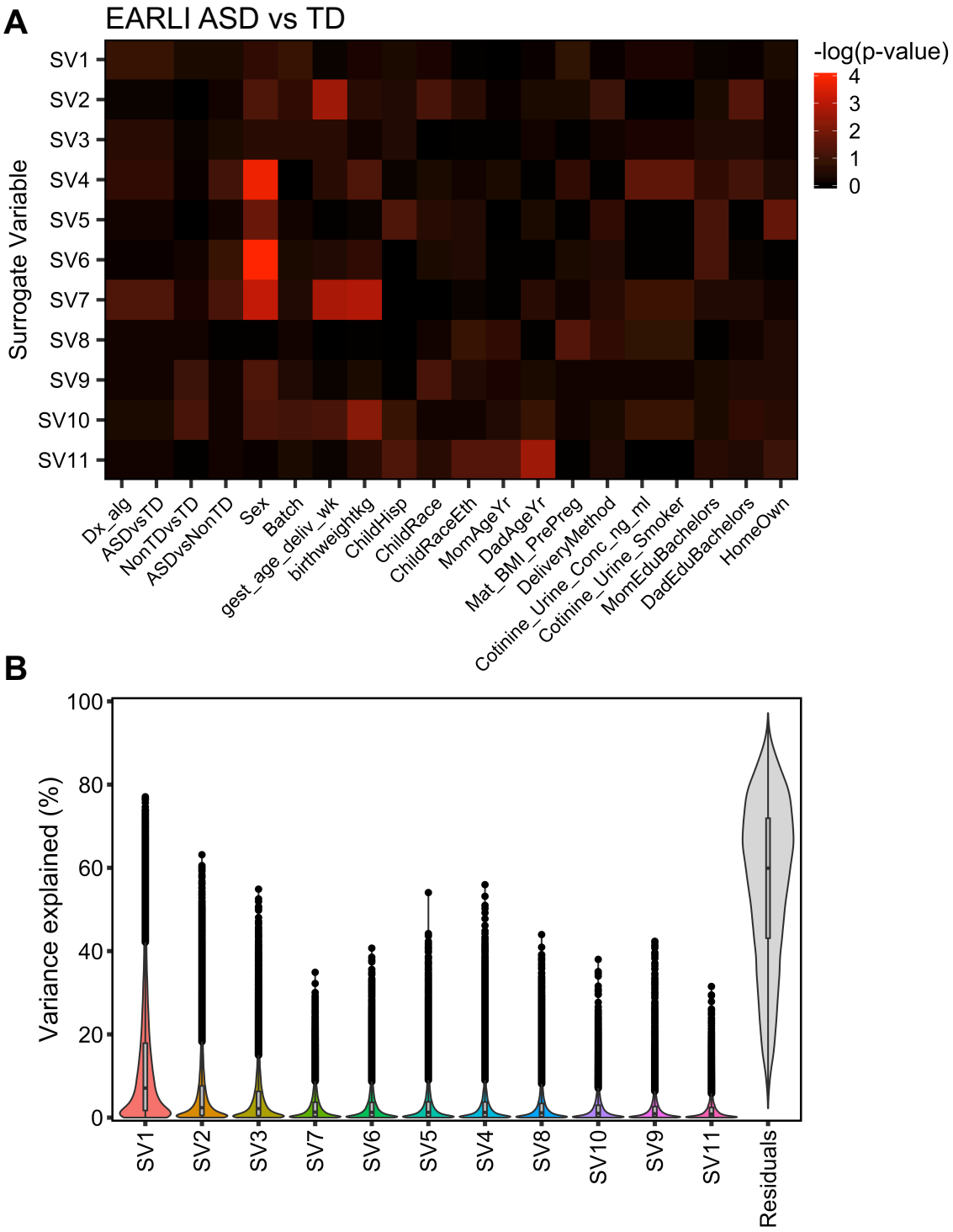
Supplemental Figure 1. Overview of study design. Umbilical cord blood was collected independently from participants in the high-risk prospective MARBLES and EARLI studies and frozen. After behavioral assessment at 36 months, RNA was extracted and assessed for gene expression with the HuGene 2.0 ST Array (Affymetrix). Probe intensities were normalized and analyzed for differential expression within each study. Differential expression analysis included SVA to control for technical and biological variables including array batch and sex. Probe fold change and standard error were combined across studies in the meta-analysis. Expression data was adjusted for batch before WGCNA. Consensus modules were identified and correlation z-scores were combined across studies in the meta-analysis.



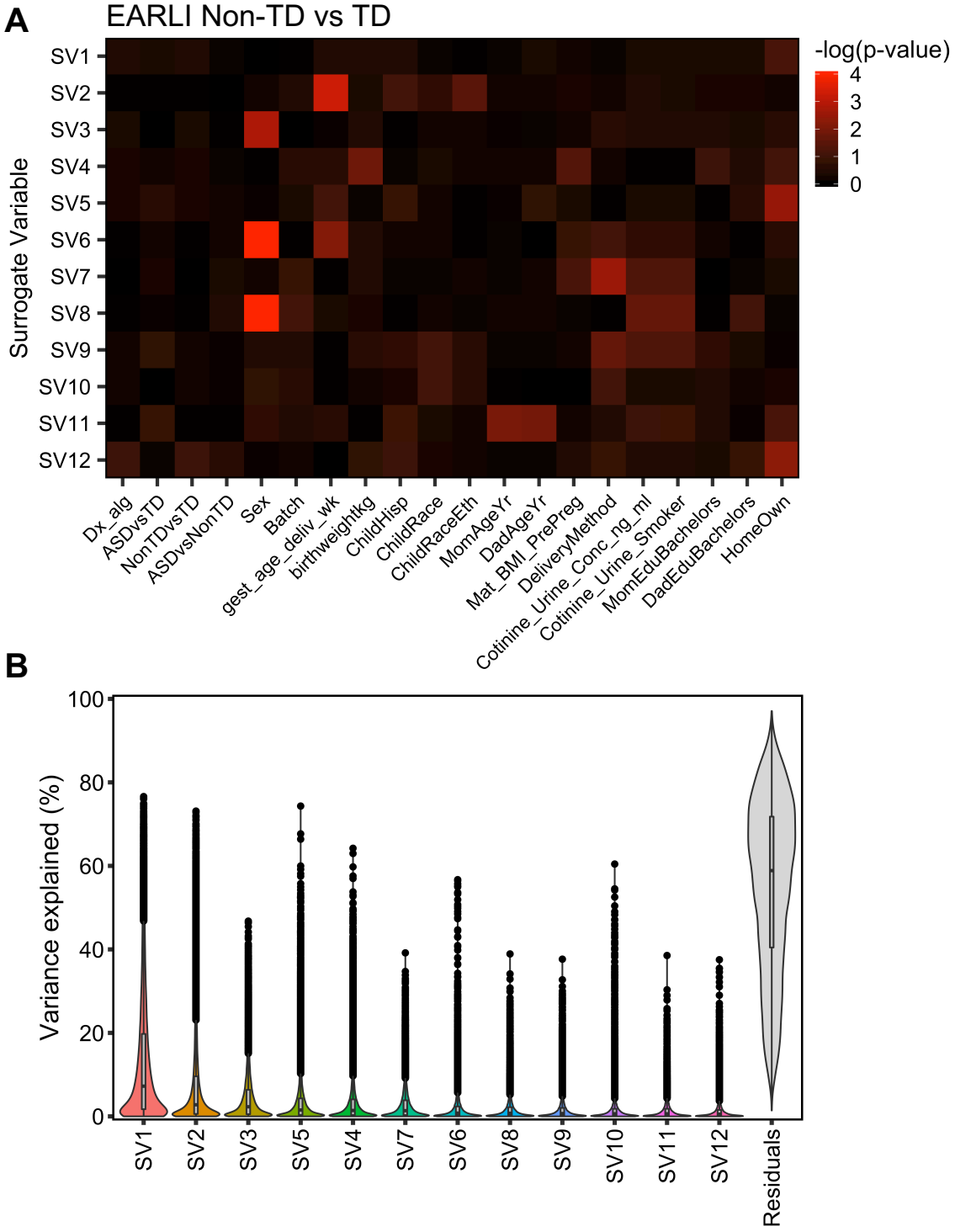
Supplemental Figure 2. Surrogate variable analysis in MARBLES subjects.

(A) Association of surrogate variables with covariates using linear regression. (B) Proportion

of variance in expression of each probe explained by each surrogate variable, sorted by median variance explained.

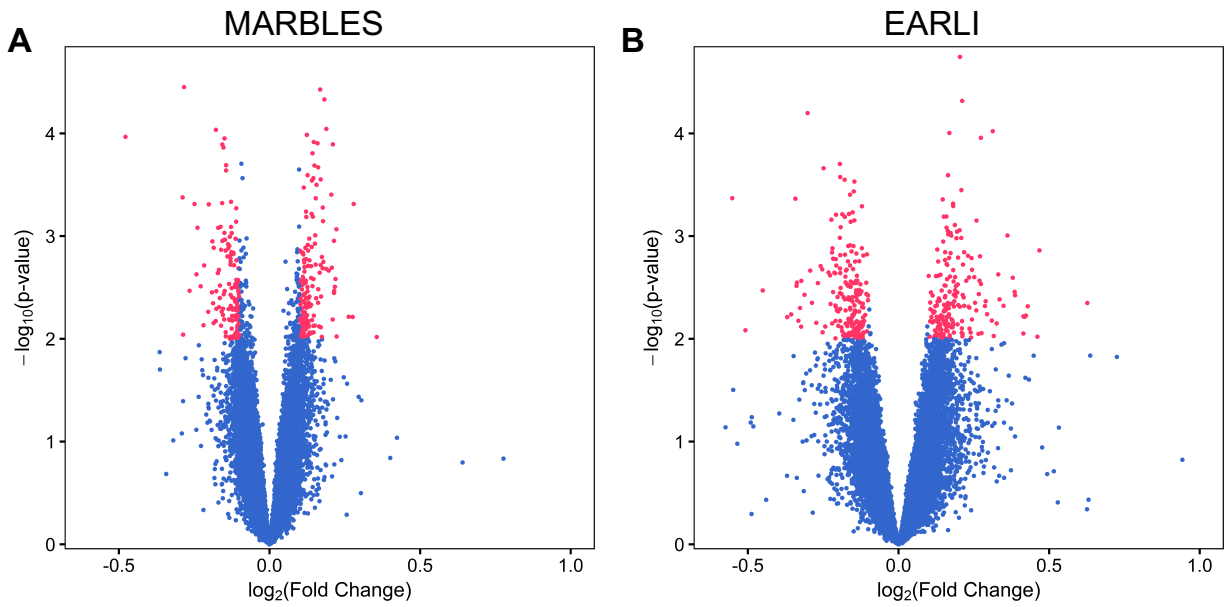


Supplemental Figure 3. Surrogate variable analysis in EARLI subjects for the ASD versus TD comparison. (A) Association of surrogate variables with covariates using linear regression. (B) Proportion of variance in expression of each probe explained by each surrogate variable, sorted by median variance explained.

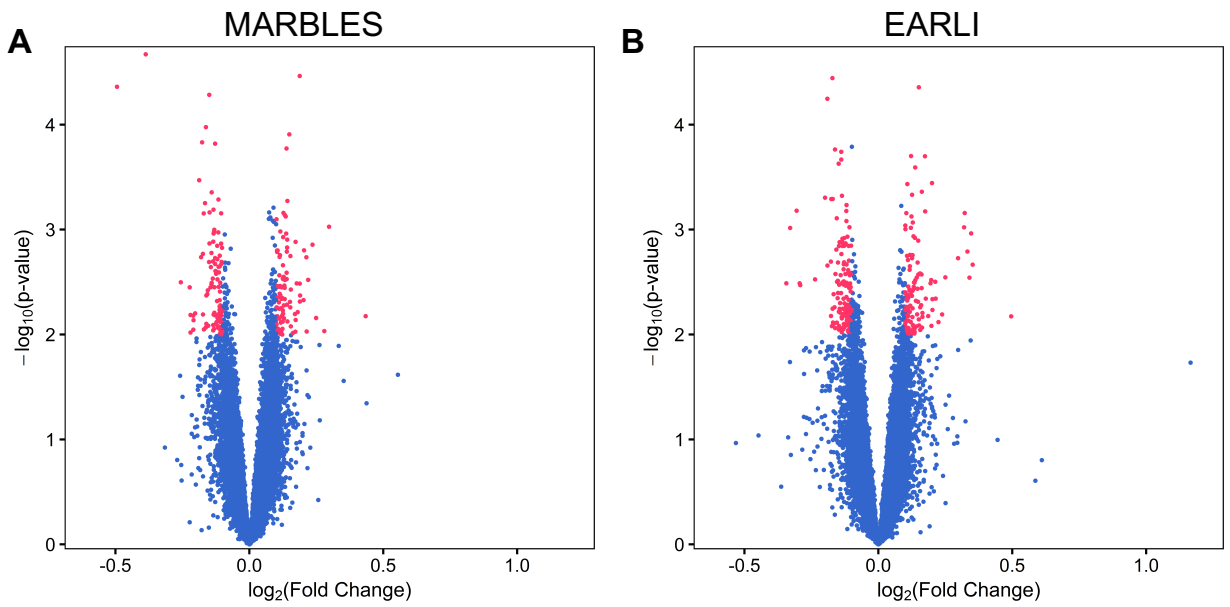


Supplemental Figure 4. Surrogate variable analysis in EARLI subjects for the Non-TD versus TD comparison. (A) Association of surrogate variables with covariates using linear

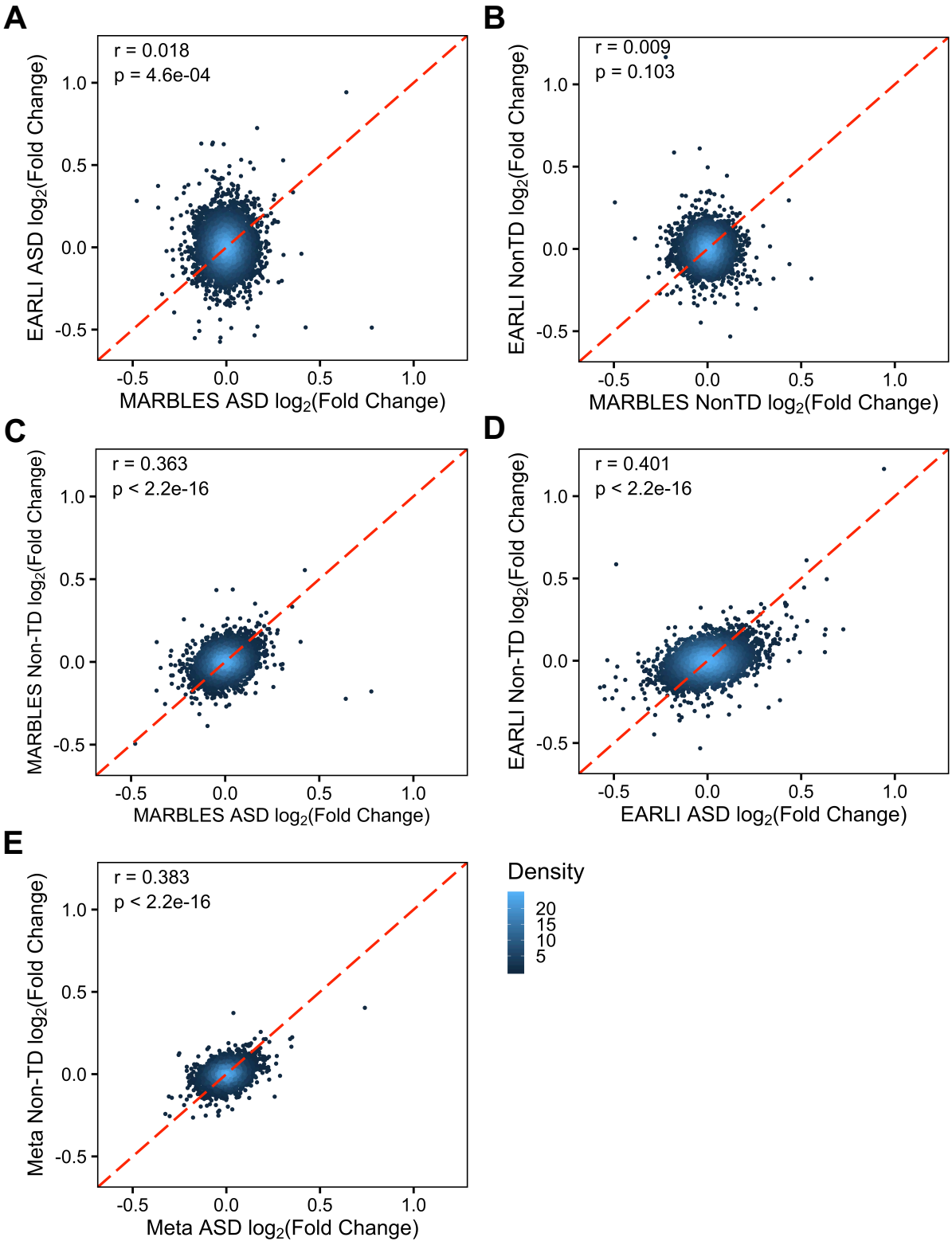
regression. (B) Proportion of variance in expression of each probe explained by each surrogate variable, sorted by median variance explained.



Supplemental Figure 5. Identification of ASD-associated differentially-expressed genes in cord blood within each study. Gene expression in umbilical cord blood samples from subjects with typical development or those diagnosed with ASD at age 3 was assessed by expression microarray. SVA was performed to control for technical and biological variables including sex and array batch. (A) Identification of 291 differentially-expressed genes in the MARBLES study (295 probes; $\log_2$(fold change) > 0.1, $p < 0.01$; TD n = 77, 40 male/37 female; ASD n = 41, 30 male/11 female). (B) Identification of 386 differentially-expressed genes in the EARLI study (392 probes; $\log_2$(fold change) > 0.1, $p < 0.01$; TD n = 43, 19 male/24 female; ASD n = 18, 13 male/5 female).

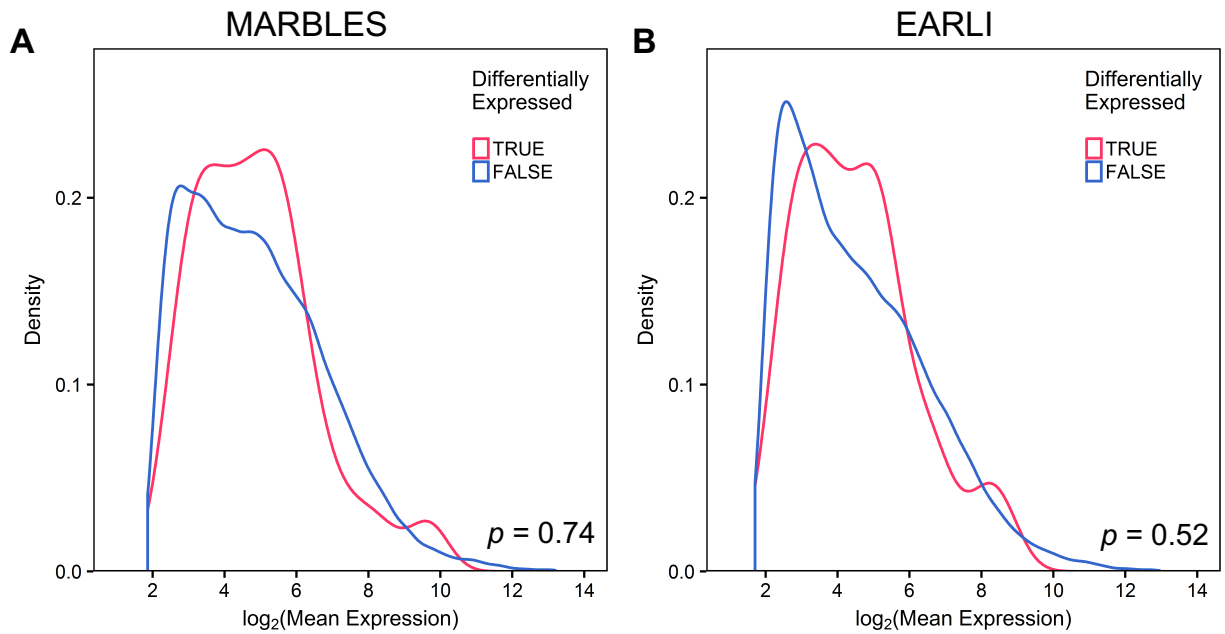


Supplemental Figure 6. Identification of Non-TD-associated differentially-expressed genes in cord blood within each study. Gene expression in umbilical cord blood samples from subjects with typical development or those diagnosed with Non-TD at age 3 was assessed by expression microarray. SVA was performed to control for technical and biological variables including sex and array batch. (A) Identification of 201 differentially-expressed genes in the MARBLES study (208 probes; $\log_2$(fold change) > 0.1, $p < 0.01$; TD $n = 77$, 40 male/37 female; Non-TD $n = 44$, 27 male/17 female). (B) Identification of 386 differentially-expressed genes in the EARLI study (392 probes; $\log_2$(fold change) > 0.1, $p < 0.01$; TD $n = 43$, 19 male/24 female; Non-TD $n = 48$, 23 male/25 female).

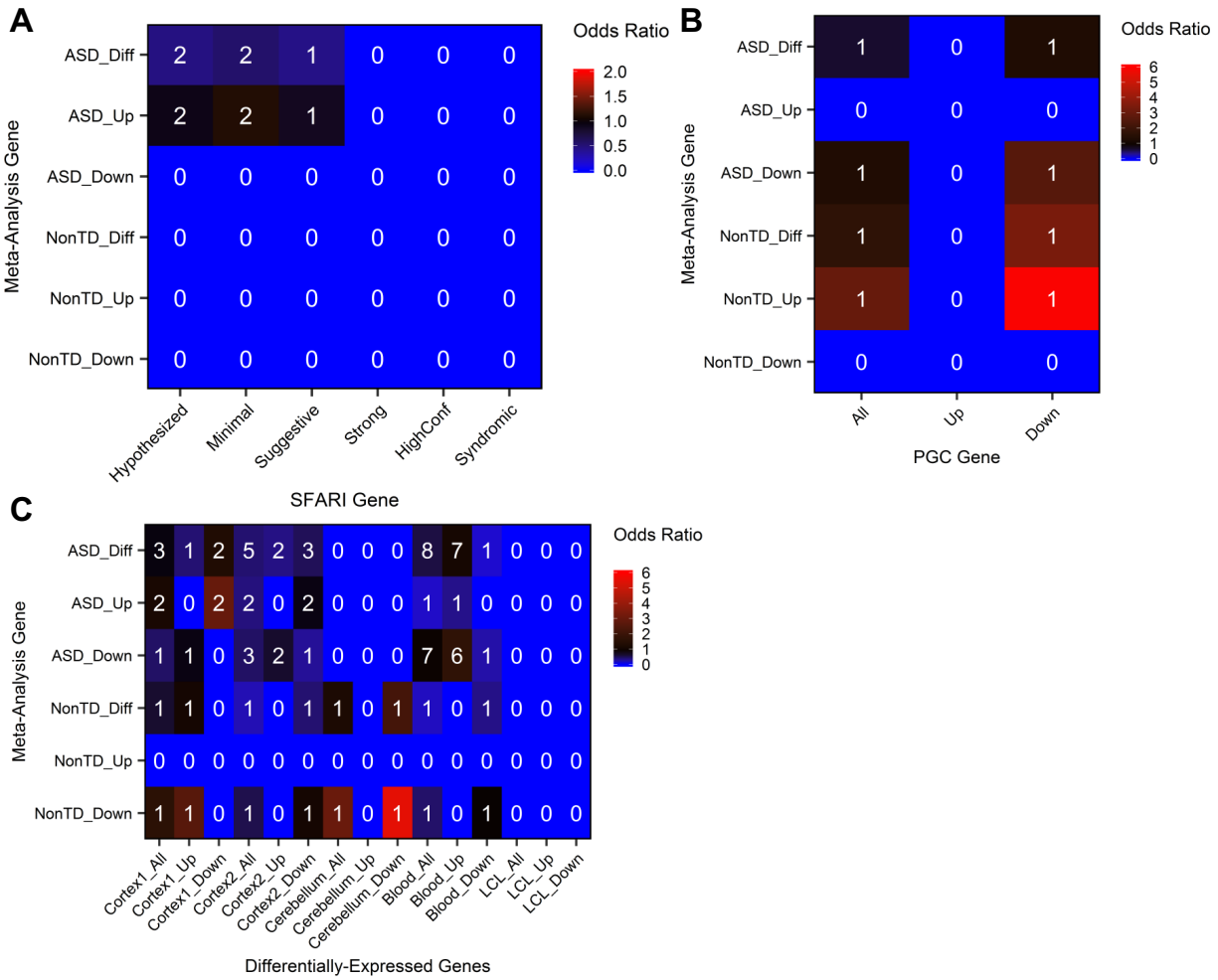


Supplemental Figure 7. Correlations between ASD and Non-TD expression differences in MARBLES and EARLI subjects. $\log_2$(Fold Change) relative to TD expression is plotted for

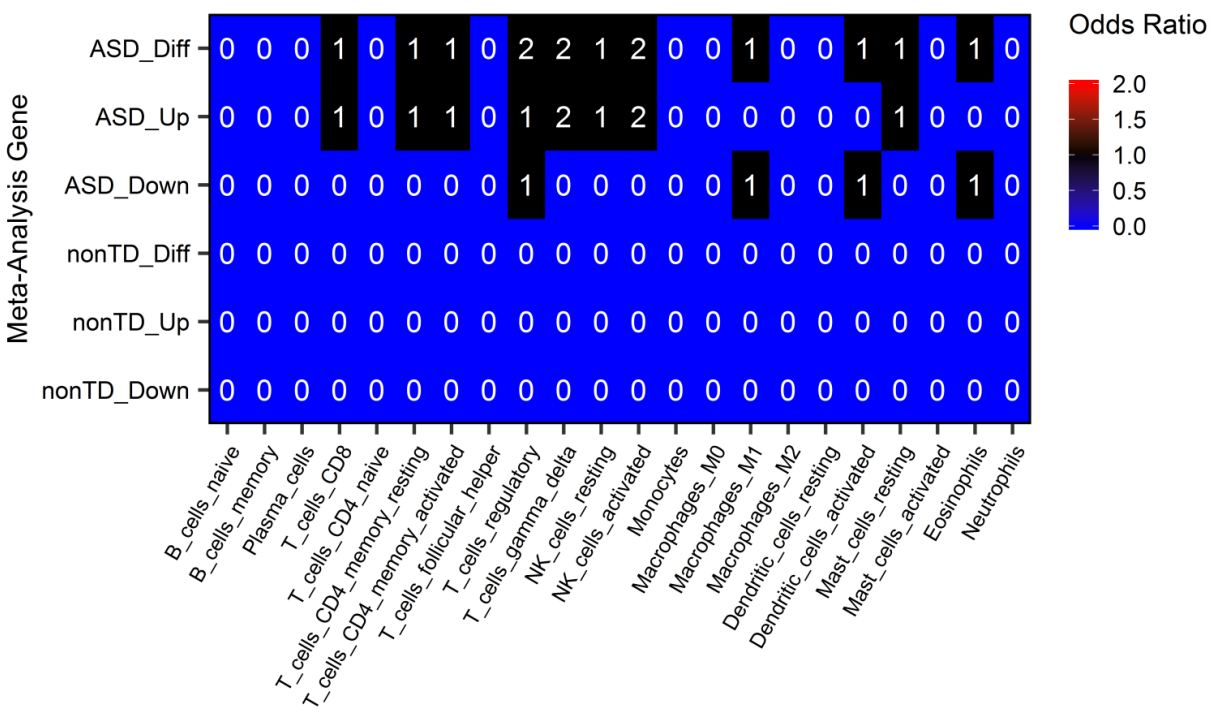
each probe, along with Pearson's correlation coefficients and p-values, for (A) ASD in MARBLES vs EARLI, (B) Non-TD in MARBLES vs EARLI, (C) ASD vs Non-TD in MARBLES, (D) ASD vs Non-TD in EARLI, or (E) ASD vs Non-TD in meta-analysis. Points are colored by density.



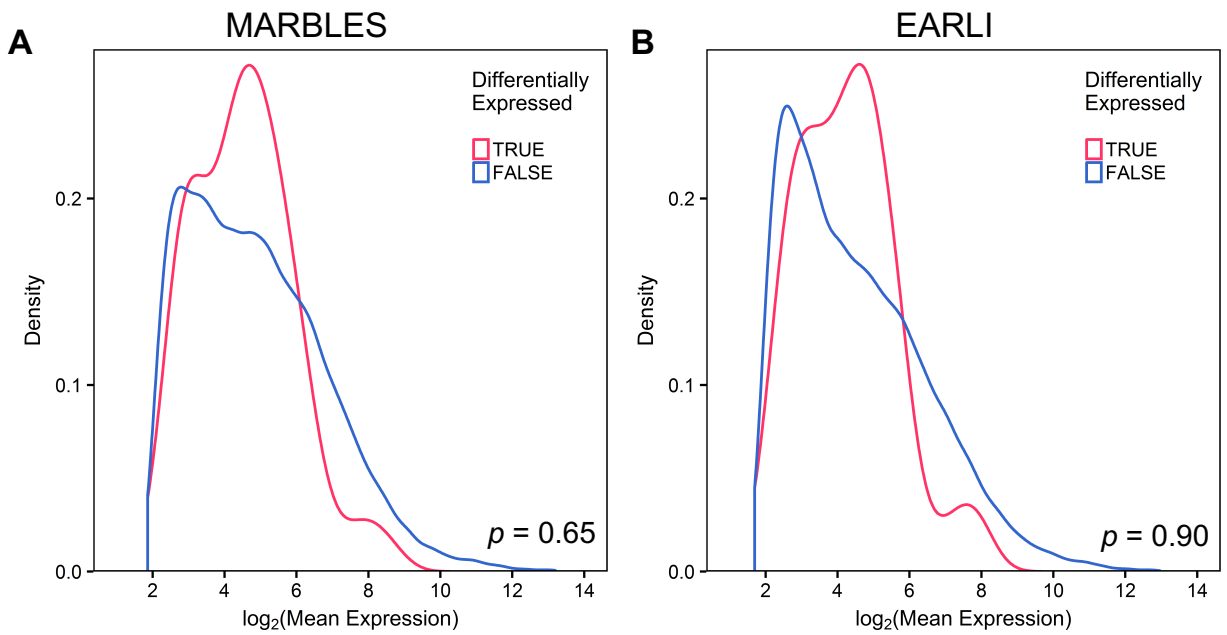
Supplemental Figure 8. Expression level distribution of meta-analysis ASD versus TD differential probes is similar to non-differential probes. Mean expression of meta-analysis ASD-associated probes was compared to probes with no difference in samples from the (A) MARBLES and (B) EARLI studies. Significance was assessed by comparing the median expression of differentially-expressed probes to the distribution of median expression of 10,000 equal-sized sets of randomly-sampled probes (MARBLES: differential = 4.70, non-differential = 4.64, $p = 0.74$; EARLI: differential = 4.34, non-differential = 4.19, $p = 0.52$).



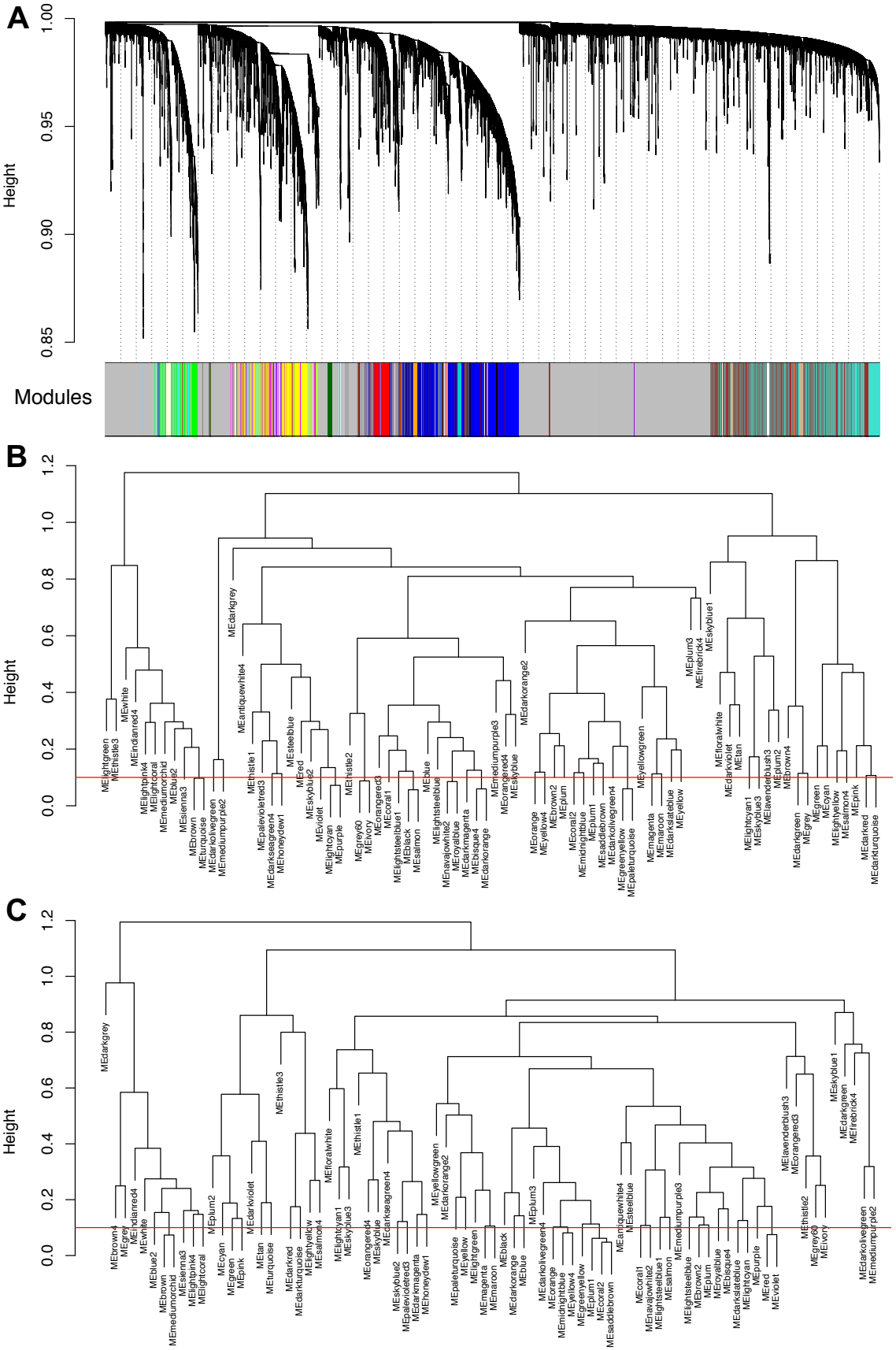
Supplemental Figure 9. Cord blood differentially-expressed genes are not enriched for ASD-associated gene sets. Differentially-expressed genes were overlapped with (A) putative ASD risk genes from SFARI gene by evidence level, (B) genes near ASD-associated SNPs from the psychiatric genomics consortium (PGC) by risk direction, or (C) differentially-expressed genes identified in ASD patients by tissue type. Heatmaps show number of overlapping genes and are colored by enrichment odds ratio. Significance was determined with Fisher's exact test (* FDR *q*-value < 0.05).



Supplemental Figure 10. Cord blood differentially-expressed genes are depleted for blood cell-specific genes. Differentially-expressed genes were overlapped with genes upregulated in purified cell types from peripheral blood (Newman et al. 2015). Heatmaps show number of overlapping genes and are colored by enrichment odds ratio. Significance was determined with Fisher's exact test (* FDR *q*-value < 0.05).



Supplemental Figure 11. Expression level distribution of meta-analysis Non-TD versus TD differential probes is similar to non-differential probes. Mean expression of meta-analysis Non-TD-associated probes was compared to probes with no difference in samples from the (A) MARBLES and (B) EARLI studies. Significance was assessed by comparing the median expression of differentially-expressed probes to the distribution of median expression of 10,000 equal-sized sets of randomly-sampled probes (MARBLES: differential = 4.48, non-differential = 4.64, $p = 0.65$; EARLI: differential = 4.15, non-differential = 4.20, $p = 0.90$).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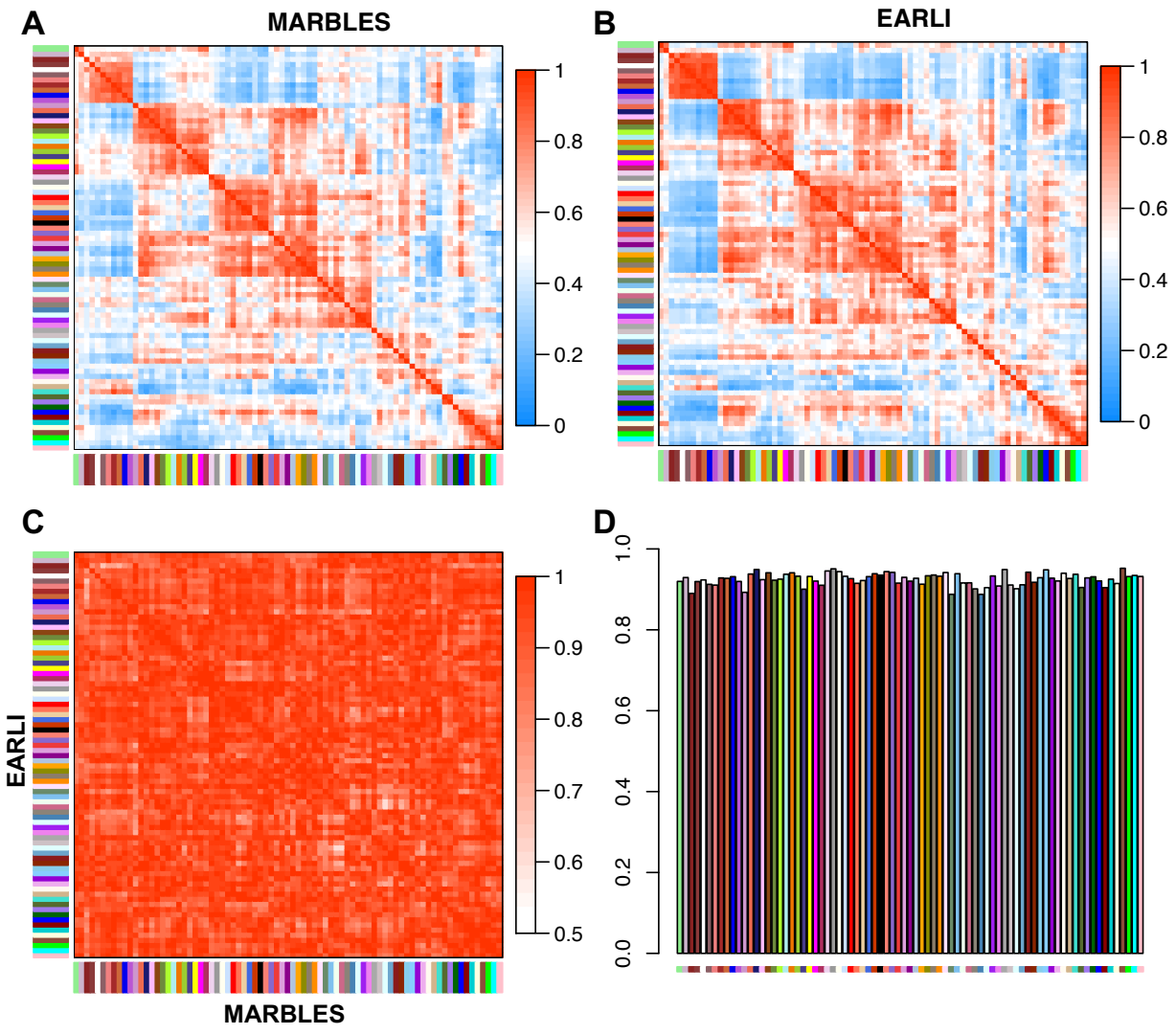
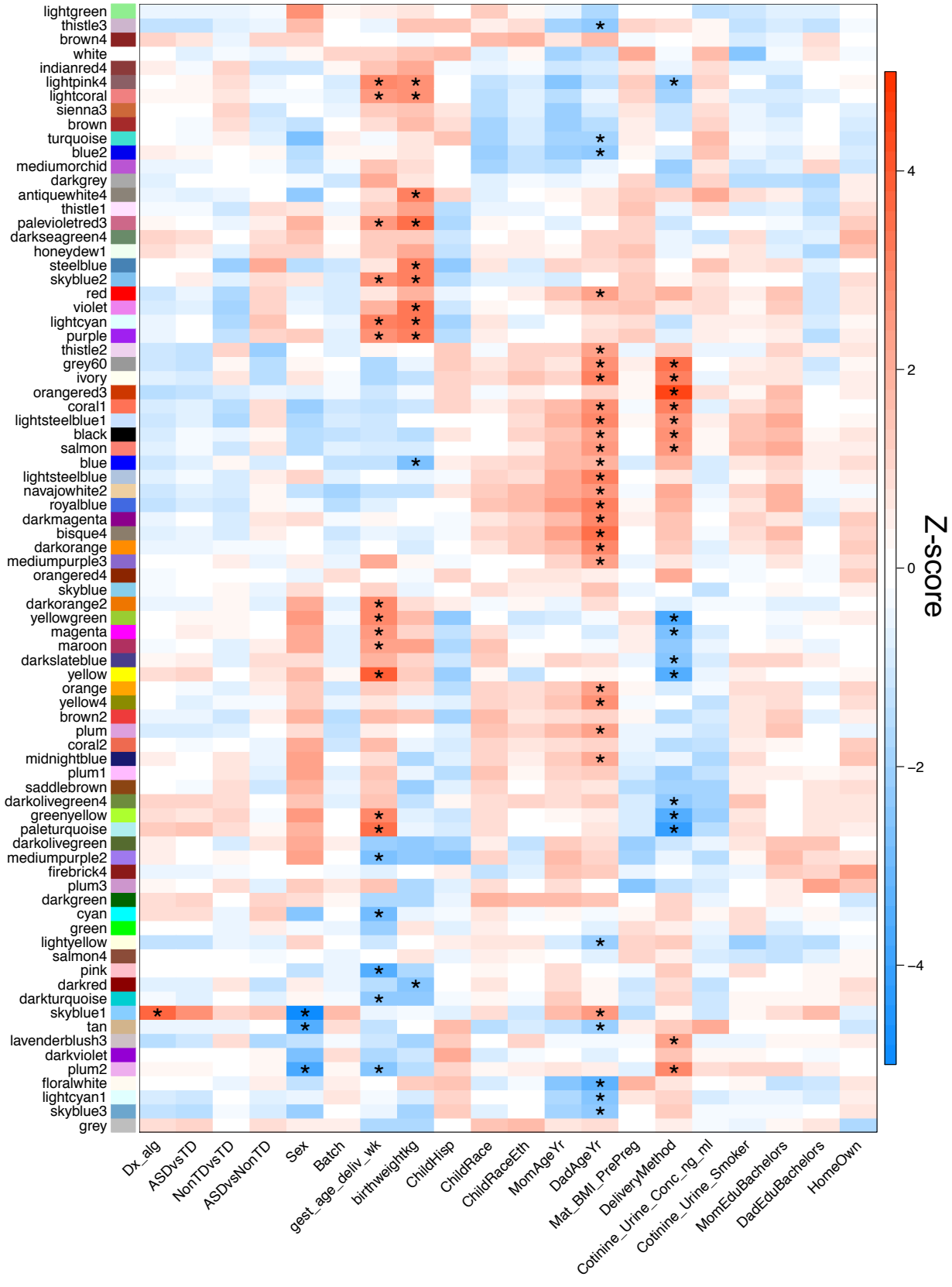
Supplemental Figure 12. Consensus coexpression modules identified in MARBLES and EARLI. (A) Gene dendrogram clustered by normalized intensity with identified consensus modules. (B) Module dendrogram clustered by consensus module eigengenes in MARBLES subjects. (C) Module dendrogram clustered by consensus module eigengenes in EARLI subjects.



Supplemental Figure 13. Consensus module eigengene networks are preserved between MARBLES and EARLI subjects. (A) Correlation between consensus module eigengenes in

MARBLES subjects. (B) Correlation between consensus module eigengenes in EARLI subjects. (C) Pairwise preservation of consensus module eigengene correlations between MARBLES and EARLI subjects. (D) Mean preservation of consensus module eigengene correlations between MARBLES and EARLI subjects. Overall preservation = 0.93.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ChildRace
ChildRaceEth
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DadAgeYr
Mat_BMI_PrePreg
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Cotinine_Urine_Smoker
MomEduBachelors
DadEduBachelors
HomeOwn
Z-score
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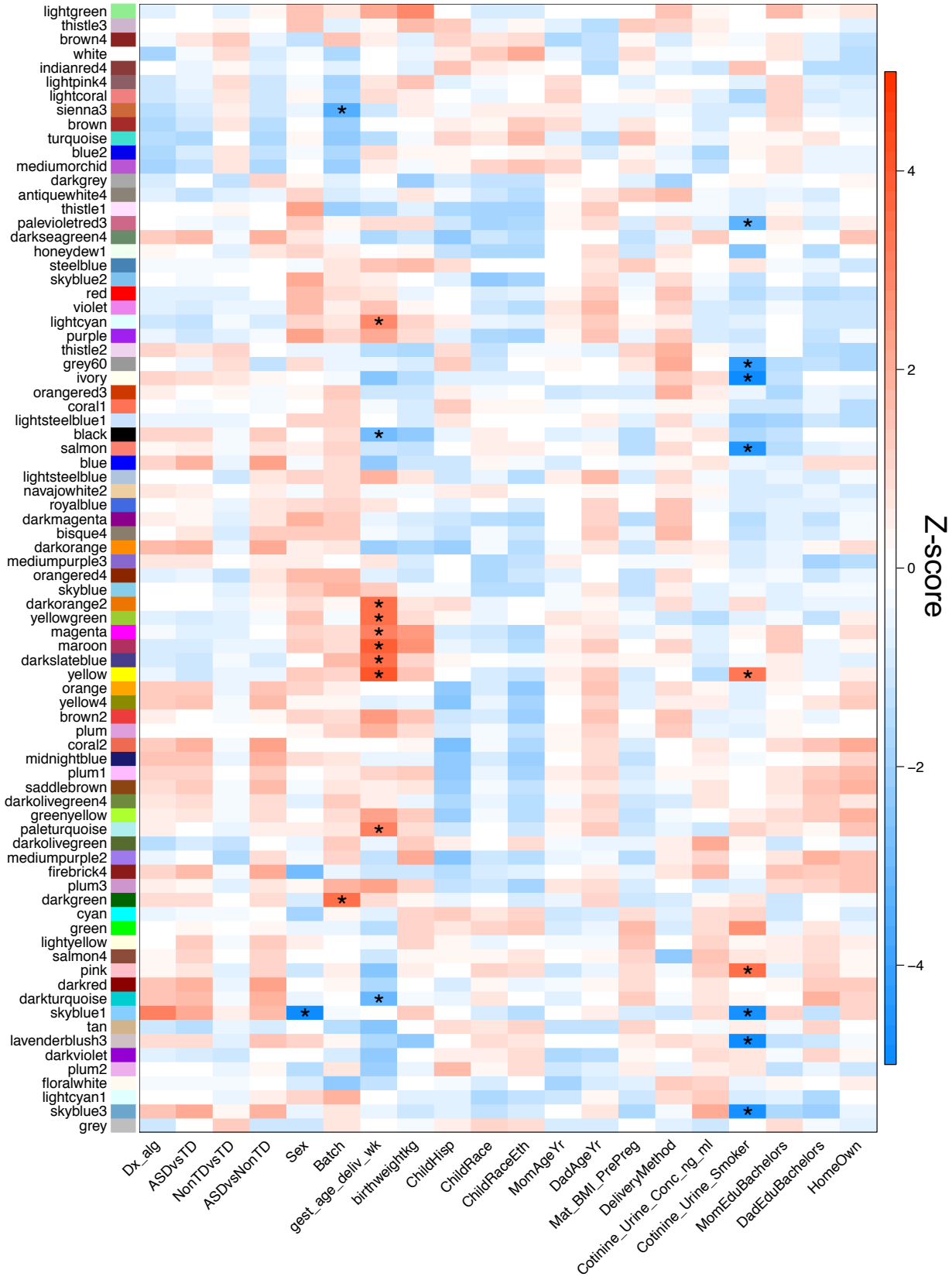
Supplemental Figure 14. Consensus modules are correlated with diagnosis and demographic factors in MARBLES subjects. Heatmap of biweight midcorrelation Z-scores of module eigengenes with sample covariates (ASD $n = 41$, Non-TD $n = 44$, TD $n = 76$). P-values were adjusted for all comparisons using the FDR method (* $q < 0.05$).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ChildRaceEth
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DadAgeYr
Mat_BMI_PrePreg
DeliveryMethod
Cotinine_Urine_Conc_ng_ml
Cotinine_Urine_Smoker
MomEduBachelors
DadEduBachelors
HomeOwn
Z-score
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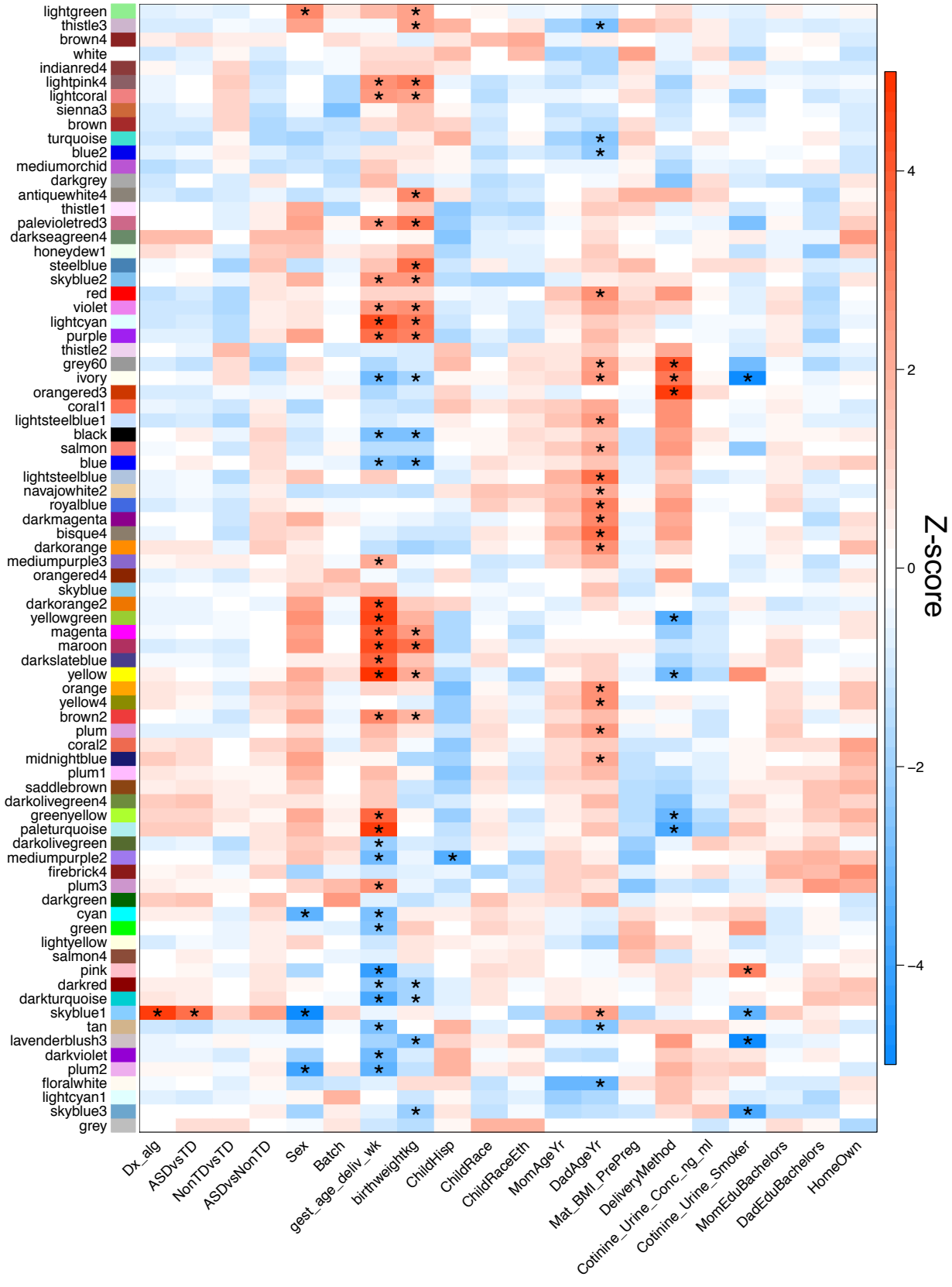
Supplemental Figure 15. Consensus modules are correlated with demographic factors in EARLI subjects. Heatmap of biweight midcorrelation Z-scores of module eigengenes with sample covariates (ASD *n* = 19, Non-TD *n* = 47, TD *n* = 43). P-values were adjusted for all comparisons using the FDR method (* $q < 0.05$).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HomeOwn
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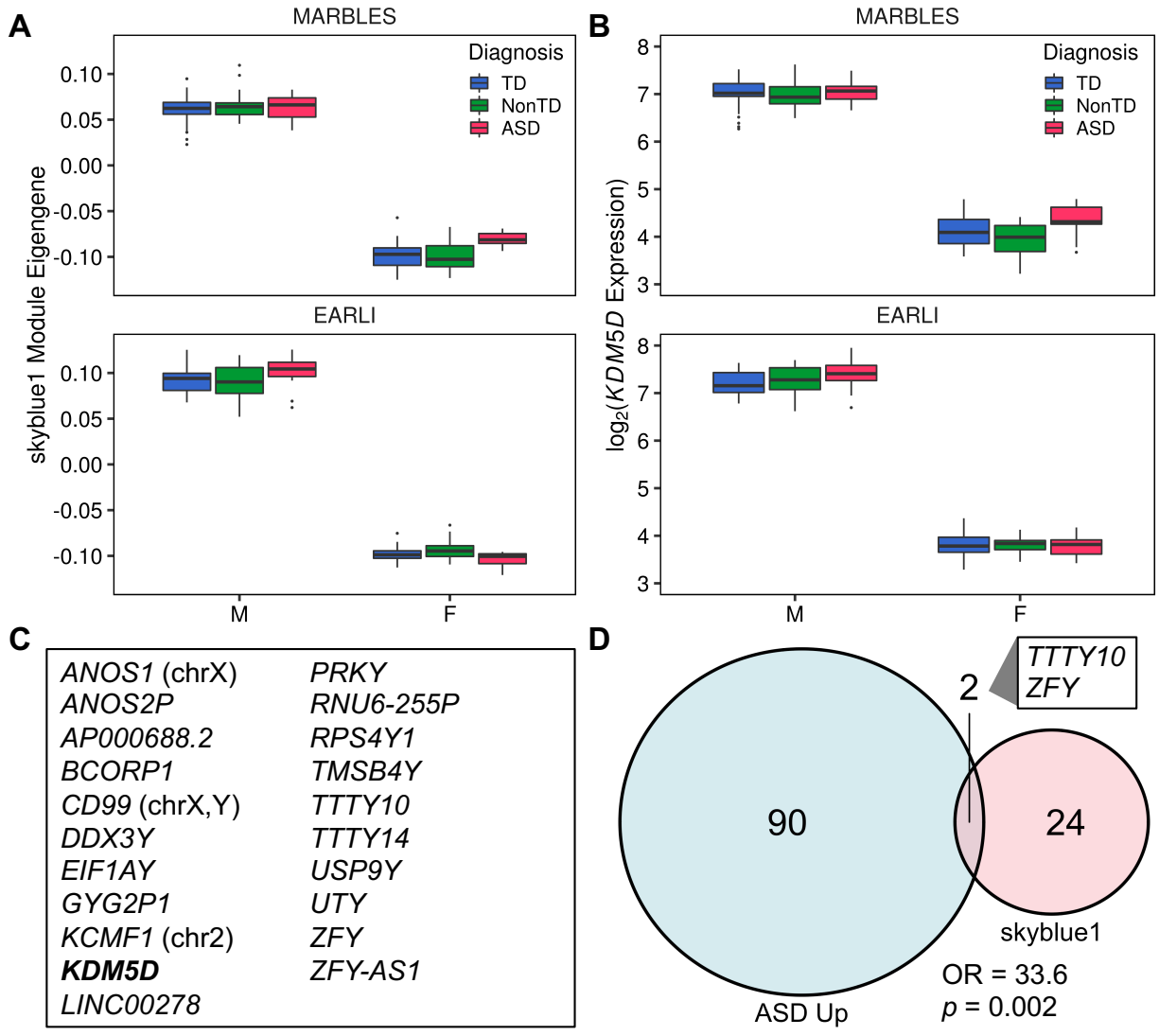
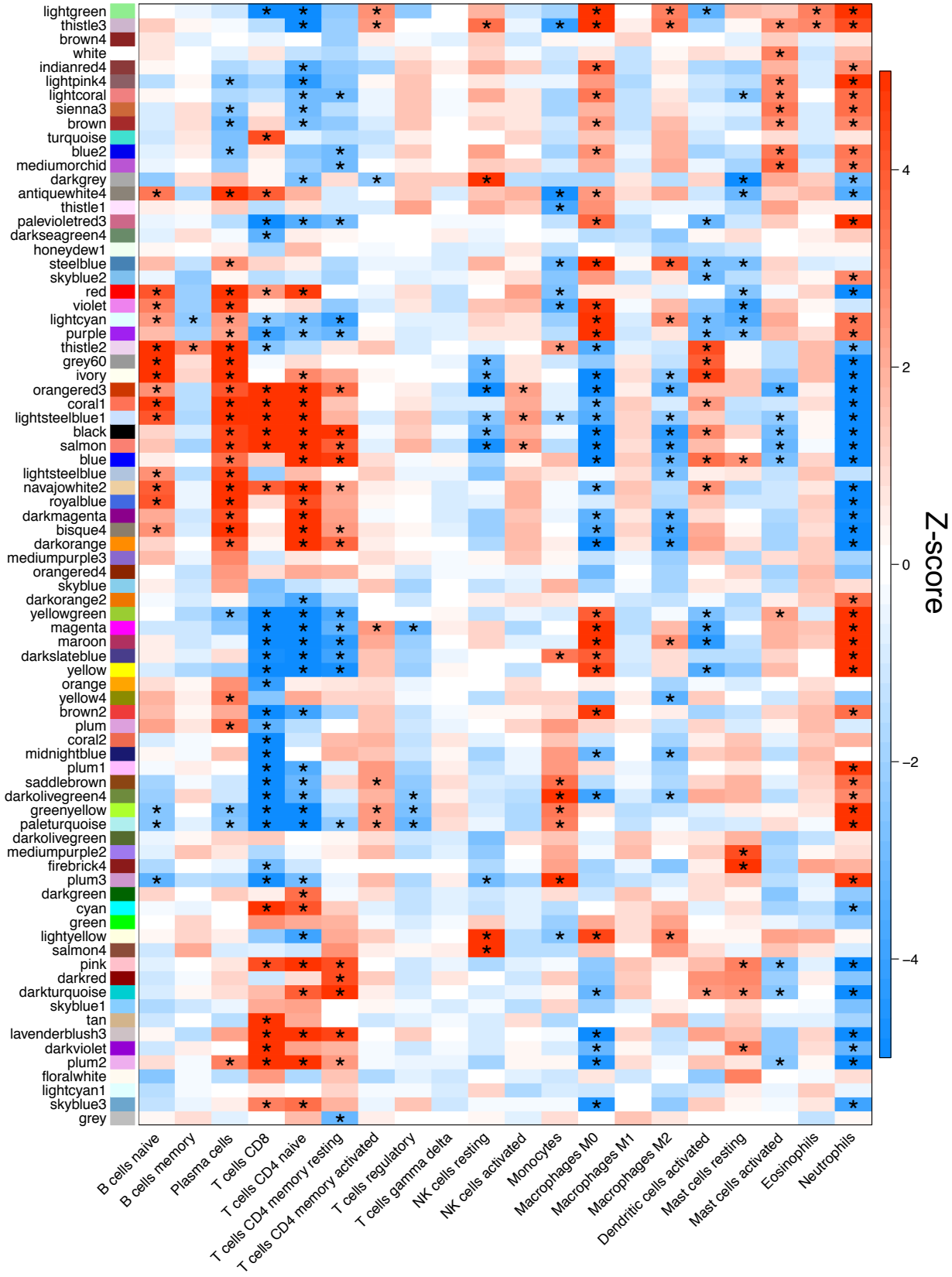
Supplemental Figure 16. Consensus modules are correlated with diagnosis and demographic factors in meta-analysis. Heatmap of meta-analysis biweight midcorrelation Z-scores of module eigengenes with sample covariates (MARBLES: ASD *n* = 41, Non-TD *n* = 44, TD *n* = 76; EARLI: ASD *n* = 19, Non-TD *n* = 47, TD *n* = 43). Z-scores from the individual studies were combined using Stouffer's method with weights given by the square root of sample *n.* P-values were adjusted for all comparisons using the FDR method (* $q < 0.05$).



Supplemental Figure 17. Skyblue1 module is specifically expressed in males and is enriched for genes upregulated in ASD. Boxplots of (A) skyblue1 module eigengene or (B) Normalized $\log_2$(expression) of skyblue1 hub gene *KDM5D* by diagnosis and sex. P-values for the skyblue1 module eigengene were adjusted for the total number of modules using the FDR method. (Meta-analysis ME ~ Diagnosis q = 1.3E-4, ME ~ Sex q = 3.4E-172; Meta-analysis *KDM5D* ~ Diagnosis p = 1.2E-6, *KDM5D* ~ Sex p = 6.4E-164). (C) Genes annotated to probes in the skyblue1 module. Genes are located on the Y chromosome unless otherwise indicated. (D) Overlap of skyblue1 module probes with probes upregulated in ASD meta-analysis. Overlap significance was assessed using Fisher's exact test.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B cells memory
Plasma cells
T cells CD8
T cells CD4 naive
T cells CD4 memory resting
T cells CD4 memory activated
T cells regulatory
T cells gamma delta
NK cells resting
NK cells activated
Monocytes
Macrophages M0
Macrophages M1
Macrophages M2
Dendritic cells activated
Mast cells resting
Mast cells activated
Eosinophils
Neutrophils
Z-score
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−4

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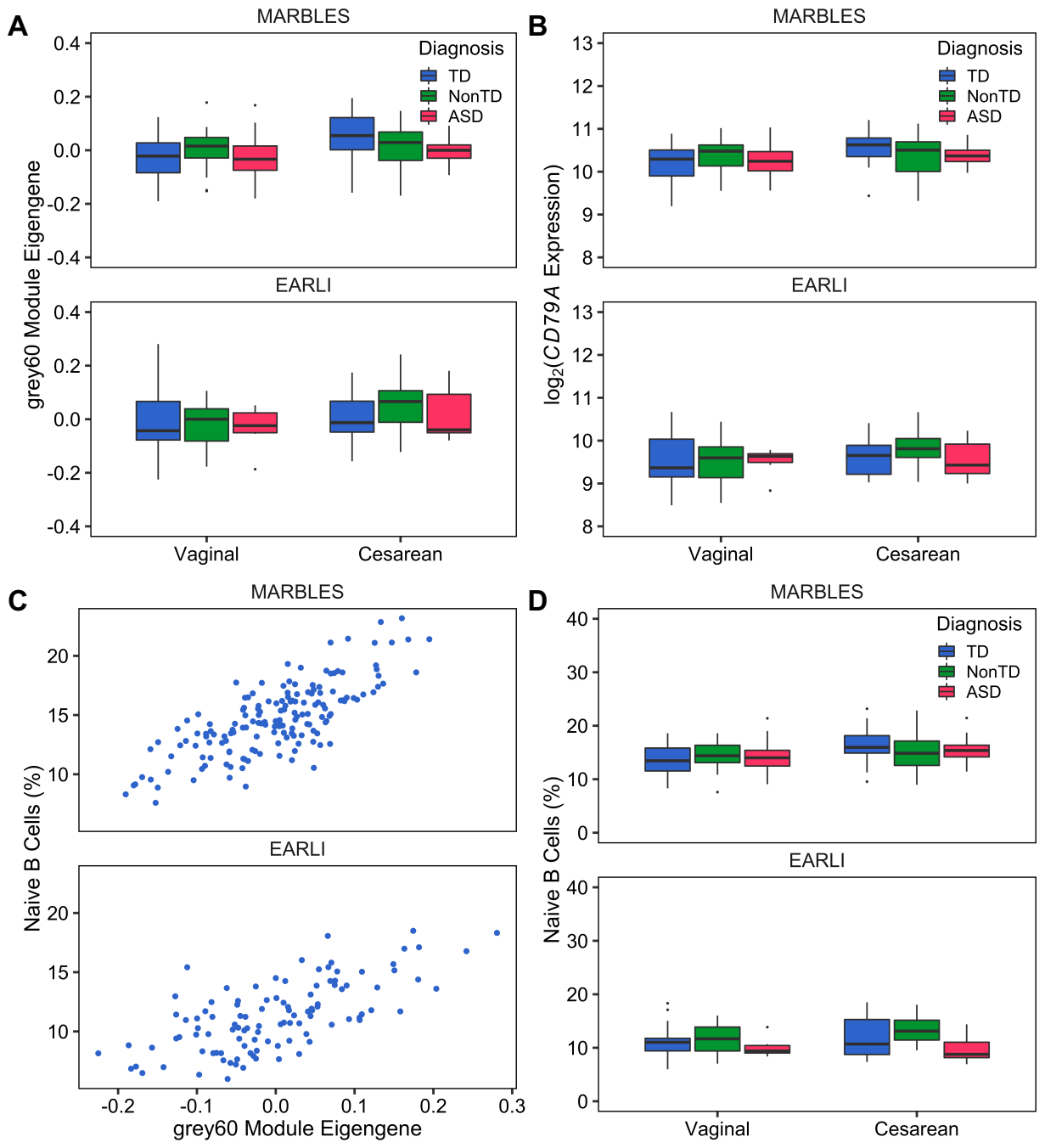
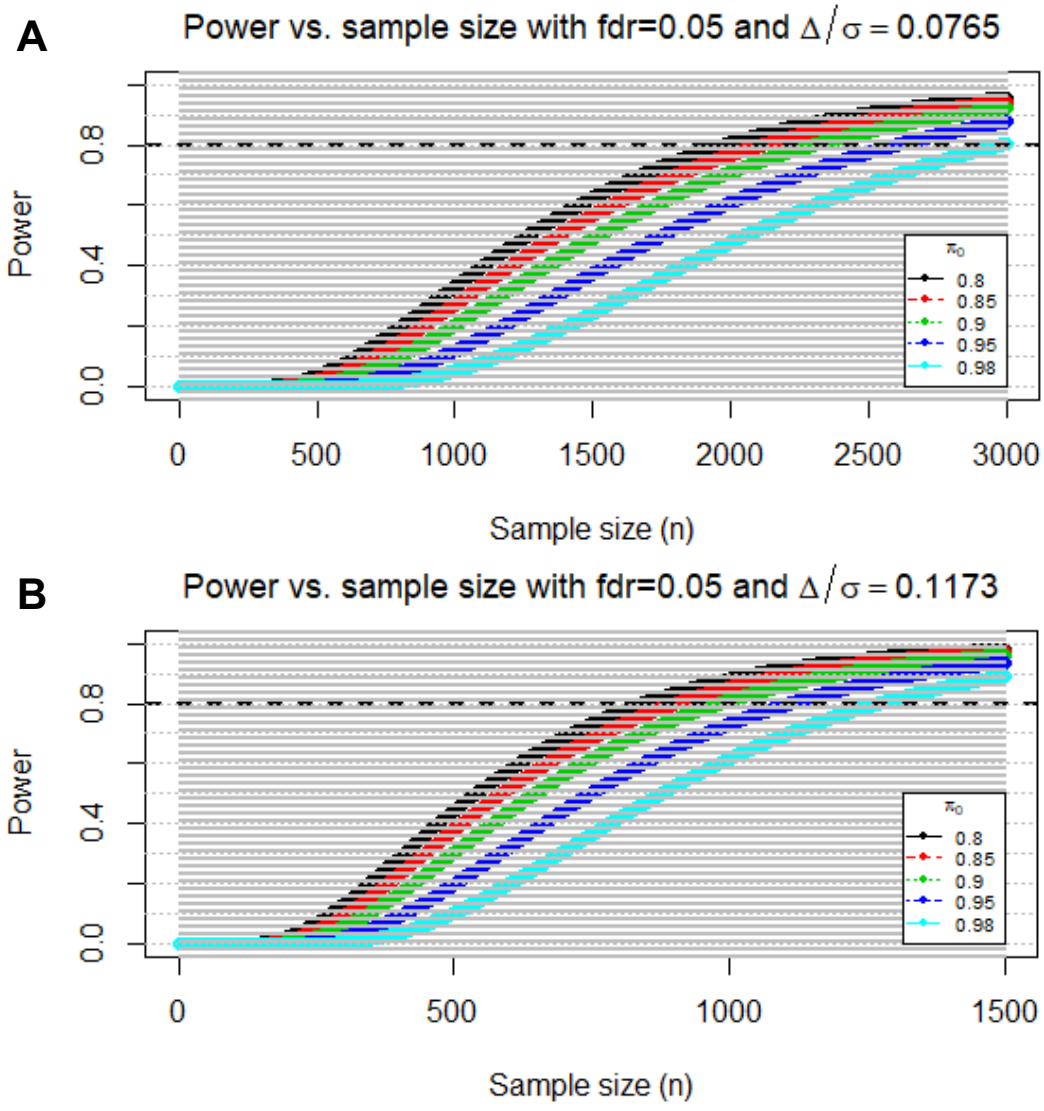
Supplemental Figure 18. Consensus modules are strongly correlated with cell type proportions in meta-analysis. Heatmap of meta-analysis biweight midcorrelation Z-scores of module eigengenes with cell type proporitions (MARBLES: ASD *n* = 41, Non-TD *n* = 44, TD *n* = 76; EARLI: ASD *n* = 19, Non-TD *n* = 47, TD *n* = 43). Z-scores from the individual studies were combined using Stouffer's method with weights given by the square root of sample *n.* P-values were adjusted for all comparisons using the FDR method (* $q < 0.05$).



Supplemental Figure 19. The B cell-associated grey60 module is upregulated during cesarean delivery and is downregulated in ASD. Boxplots of (A) grey60 module eigengene or (B) Normalized $\log_2$(expression) of grey60 hub gene *CD79A* by diagnosis, delivery method, and study. (Meta-analysis ME ~ ASD vs Non-TD p = 0.048, ME ~ Delivery Method p = 4.7E-5; Meta-analysis *CD79A* ~ ASD vs Non-TD p = 0.047, *CD79A* ~ Delivery

Method *p* =4.2E-4). The grey60 module contains the genes *IGLV1-40* which is upregulated in both ASD and Non-TD compared to TD, and *IGLC2* which is upregulated in ASD vs TD. (C) Scatterplot of grey60 module eigengene compared to estimated proportion of naïve B cells (Meta-analysis B cells ~ ME *p* = 8.8E-46). (D) Boxplot of naïve B cells by diagnosis, delivery method, and study (Meta-analysis B cells ~ ASD vs Non-TD *p* = 0.020, B cells ~ Delivery Method *p* = 1.7E-4).



Supplemental Figure 20. Anticipated power curve calculated by *ssize.fdr* R package with projected proportions of non-differentially expressed genes as 0.8, 0.85, 0.9, 0.95 and 0.98. False discovery rate was set as 0.05 and standard deviation for all genes (sigma) was set as 1.96. Common effect size for all genes (delta) was estimated **A** based on common effect size for all genes or **B** based on common effect size of top 10 differential genes.