PNAS
www.pnas.org

Supplementary Information for

Maternal cannabis use is associated with suppression of immune gene networks in placenta and increased anxiety phenotypes in offspring

Gregory Rompala, PhD*[1], Yoko Nomura, PhD*[2,3,4], Yasmin Hurd, PhD[1]

*GR and YN contributed equally

Yasmin Hurd, PhD.
Email: **Yasmin.Hurd@mssm.edu**

This PDF file includes:

Figures S1 to S5
Tables S1 to S4
Legends for Datasets S1 to S7

Other supplementary materials for this manuscript include the following:

Datasets S1 to S7

Supplementary Figures

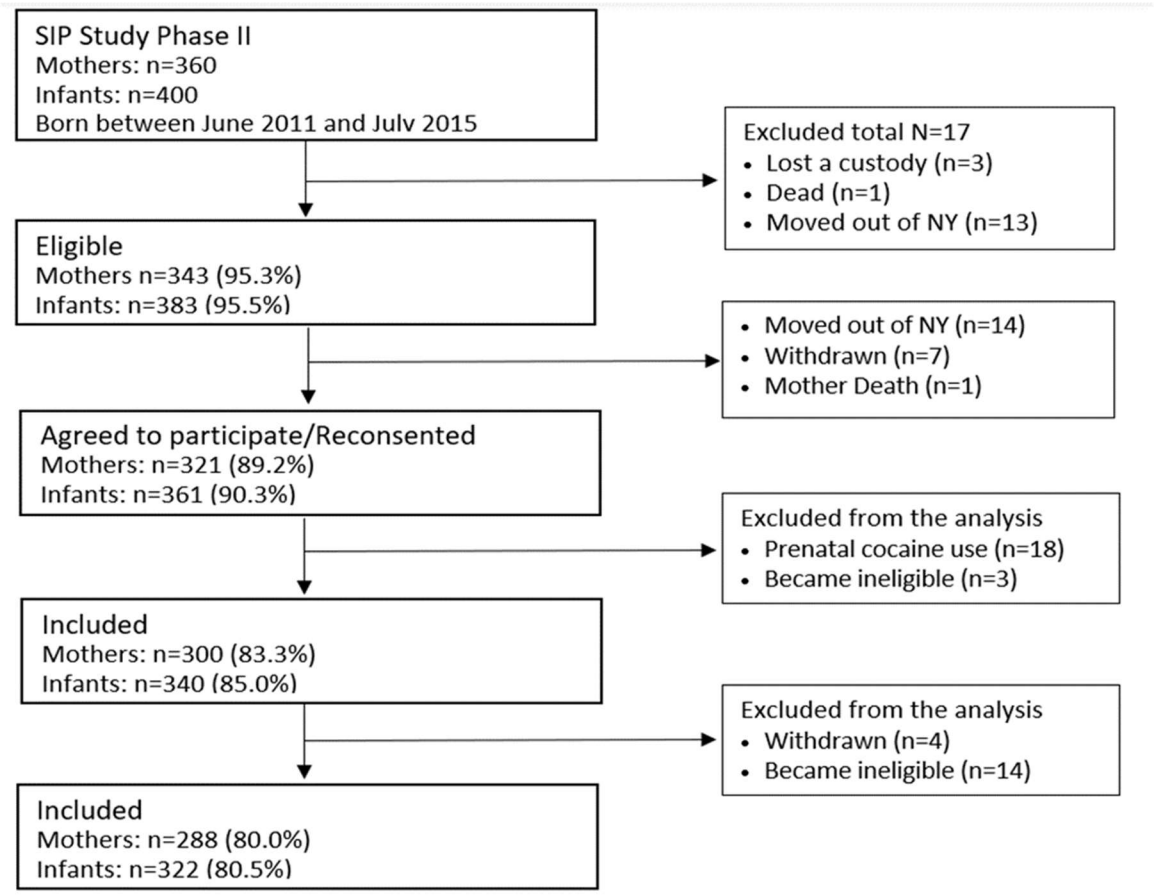

Figure S1. Consort flow chart. Diagram showing the participants who met the inclusion/exclusion criteria for the current study.

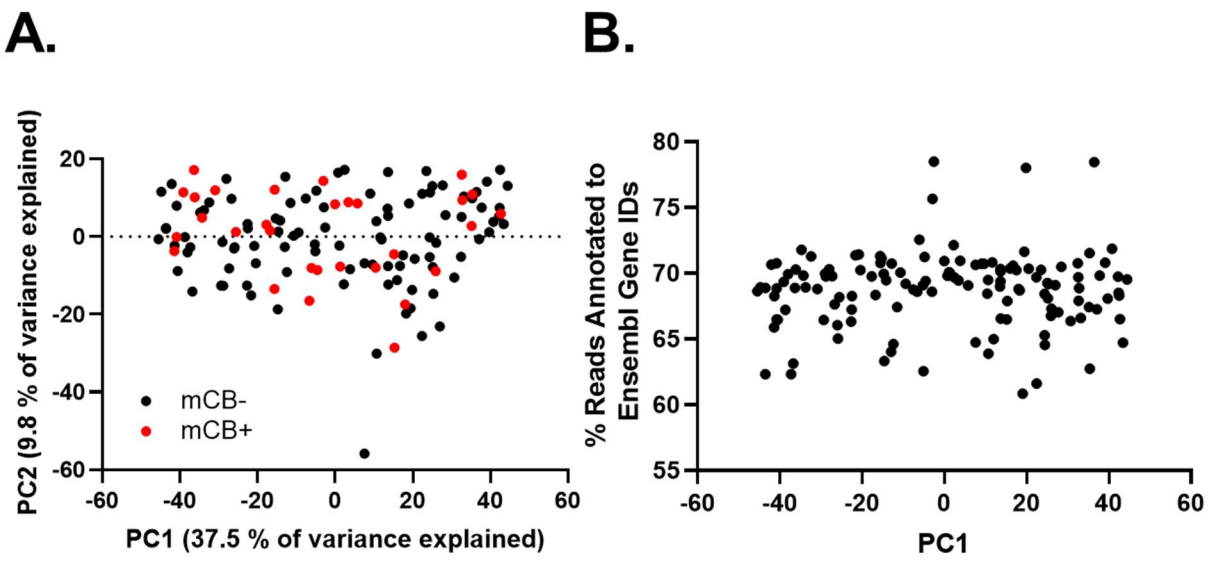

Figure S2. Principal component (PC) analysis of placenta RNA-seq dataset. (A) Scatterplot representing the first and second PC for each maternal cannabis use (mCB) and non-maternal cannabis use (mCB-) sample determined from normalized gene expression in placenta. **(B)** Scatter plot showing no relationship between PC1 and gene annotation efficiency.

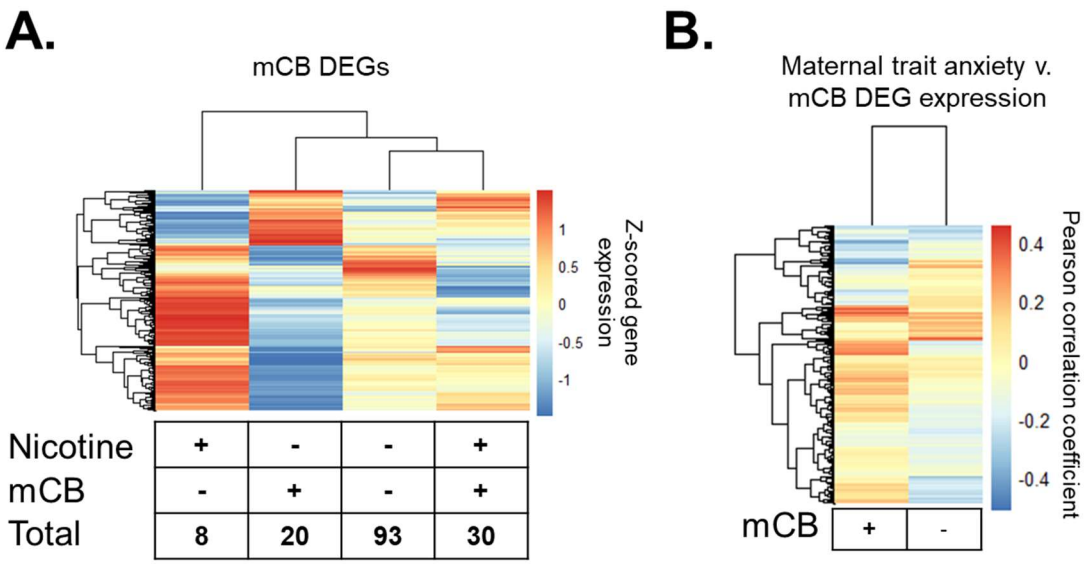

Figure S3. Evaluating relationship between covariates and placental gene expression associated with maternal cannabis use. (A) Hierarchical clustering of heatmap separating mCB-DEGs by maternal cigarette smoking (nicotine) and cannabis use. Although cigarette smokers were overrepresented in the maternal cannabis use cohort, the impact of maternal cannabis on DEGs is more pronounced in the subcohort without cigarette smoking. **(B)** Heatmap showing correlation strength of mCB-DEGs with maternal trait anxiety scores. While maternal cannabis use was associated with increased trait anxiety (see Figure 1), the relationship between trait anxiety and mCB DEGs was distinct between the mCB+ and mCB- populations.

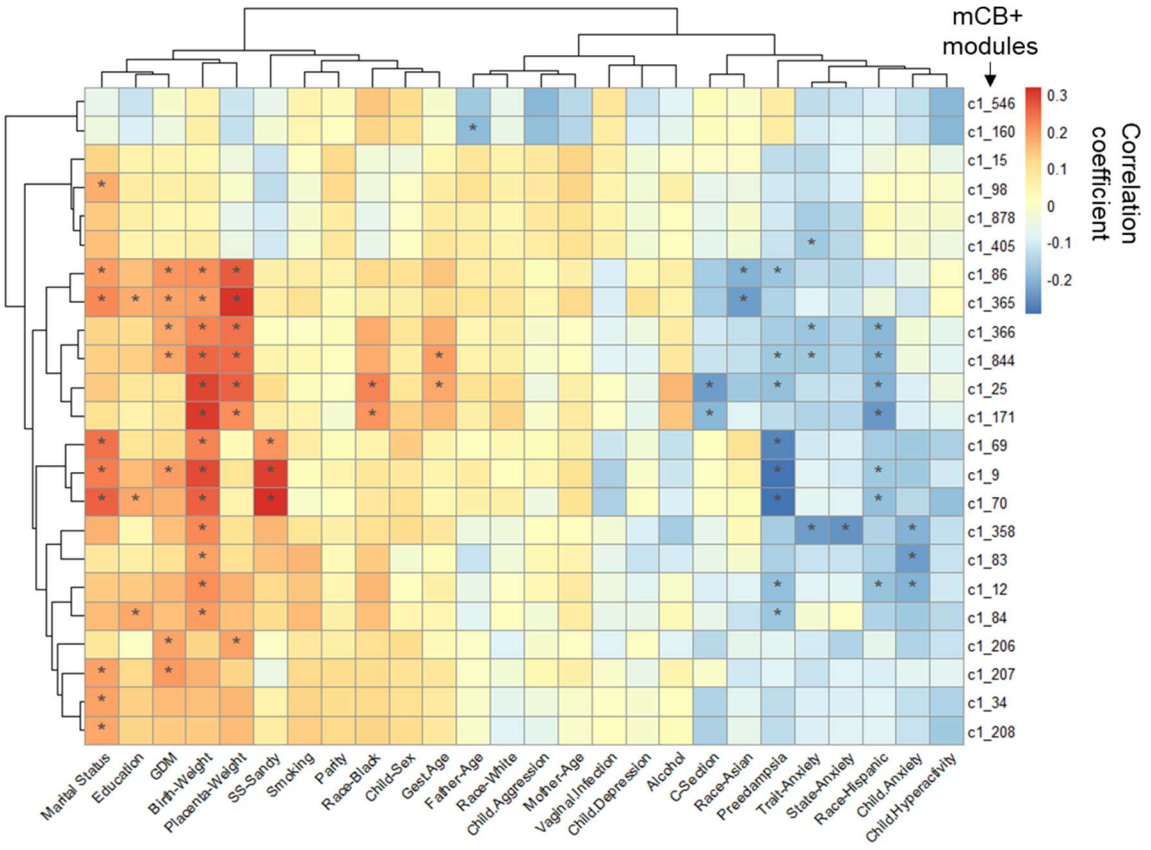

Figure S4. Relationship between mCB-linked placental gene networks and demographic factors. Heatmap depicting correlation coefficients between eigengenes for networks enriched with mCB differentially expressed genes and maternal demographic information (also included are the child neurobehavioral traits from Figure 2C). * = $p < 0.05$. Several of the networks enriched for mCB+ DEGs had eigengenes significantly correlated with demographic factors, most prominently birth weight, preeclampsia, and marital status. Of the three modules significantly associated with child anxiety (c1_358, c1_83, c1_12), all three were associated with birth weight with c1_358 also linked to maternal trait and state anxiety during pregnancy. In addition, the c1_12 eigengene was correlated with Hispanic race and preeclampsia. Overall, this analysis indicates that, in addition to maternal cannabis use, several prenatal factors may impact immune-related placental

gene expression networks, including those linked with anxiety in young children. Featured dicotomous measures (e.g., 0 or 1): Marital Status (married/common-law), SS-Sandy= pregnancy during Superstorm Sandy, Smoking, Race, Child-Sex = female (0) or male (1). Alcohol = self-reported use during pregnancy, GDM = gestational diabetes mellitus, Preeclampsia, Vaginal Infection, C-section (C-section birth). Continuous measures: Trait-Anxiety, State-Anxiety, Education = educational attainment from 0 (some elementary) to 6 (graduate degree), Birth-Weight, Placenta-Weight, gestational age (Gest-Age), Father-Age, Mother-Age, Parity.

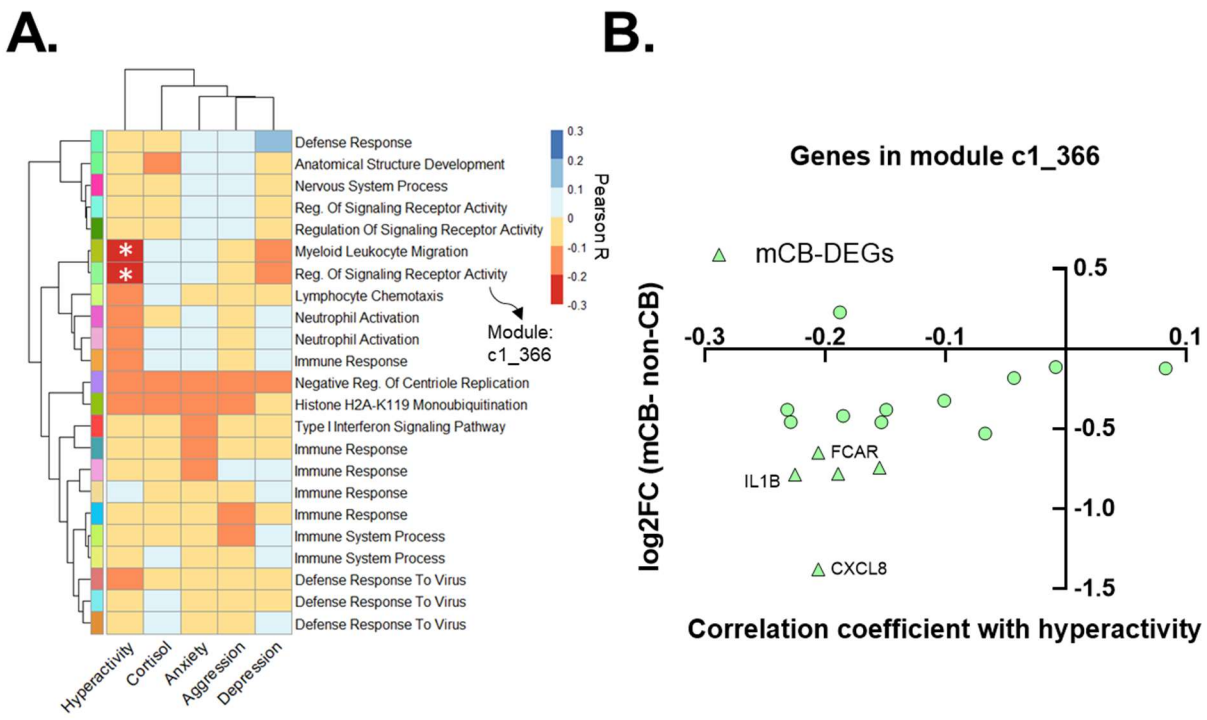

Figure S5. Relationship between mCB-linked placental gene networks and neurobehavioral trait scores in young children. (A) Heatmap depicting correlation coefficients between eigengenes for networks enriched with mCB differentially expressed genes (rows) and neurobehavioral traits (columns). * = p<0.05. Each network is labelled with a unique color and its most significant gene ontology term. **(B)** Relationship between the genes in module c1_366 and hyperactivity levels. Differentially expressed genes for mCB are represented as triangles.

Supplementary Tables

Table S1. Clinically significant neurobehavioral traits in young children with mCB.

Clinically significant behavioral problems	Non-CB (N=236)		mCB (N=68)					
	N	%total	N	%total	OR (95% CI)	pval	AOR (95% CI)	pval
Aggression	18	7.6	11	16.7	2.42 (1.08 – 5.43)	0.028	4.04 (1.31- 12.43)	0.015
Anxiety	51	21.8	24	36.4	2.05 (1.14 – 3.70)	0.016	2.47 (1.13 – 5.43)	0.024
Attention problem	41	17.4	18	26.9	1.74 (0.92 – 3.29)	0.089	1.79 (0.83 – 3.85)	0.135
Atypicality	39	16.6	20	30.3	2.35 (1.26 – 4.37)	0.006	2.11 (1.00 – 4.53)	0.05
Depression	34	14.5	16	23.9	1.85 (0.95 – 3.60)	0.07	1.67 (.71 – 3.94)	0.242
Hyperactivity	25	10.7	13	19.1	1.98 (0.95 – 4.11)	0.065	2.48 (1.02 – 6.07)	0.042
Somatization	33	14	10	14.9	1.07 (0.50 – 2.31)	0.855	0.51 (0.18 – 1.42)	0.196
Withdrawal	36	15.3	12	17.6	1.19 (0.58 – 2.44)	0.634	0.86 (0.35 – 2.11)	0.75

Table S2. Interaction between mCB and child sex for neurobehavioral traits in young children.

	Non-CB				mCB				mCB x Child Sex	
	Males		Females		Males		Females			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	**F-test$_{(1,296)}$**	**p-value**
Aggression	47.47	8.41	45.76	8.3	49.25	9.47	52.25	13.45	4.96	**0.027**
Anxiety	49.35	10.9	52.34	10.87	54.37	11.8	56.34	13.37	0.005	0.942
Attention Problems	52.2	8.26	47.82	9.3	51.88	10.75	49.84	10.29	1.201	0.274
Atypicality	51.04	10.9	48.85	8.29	53.59	9.13	55.78	13.96	2.353	0.126
Depression	49.24	10.3	47.85	10.07	50.41	10.8	52.81	12.1	1.87	0.173
Hypeactivity	50.22	9.22	46.77	8.83	53.41	9.33	50.59	12.08	0.165	0.685
Somatization	49.5	9.61	48.98	9.07	49.41	9.69	49.16	11	0.348	0.556
Withdrawal	49.42	8.86	48.86	9.01	49.69	11.02	50.5	9.913	0.34	0.56

Table S3. Correlation between heart rate variability metrics and neurobehavioral traits in young children.

	Pre-startle		Startle		Post-startle	
	HF-HRV	**nHF-HRV**	**HF-HRV**	**nHF-HRV**	**HF-HRV**	**nHF-HRV**
Aggression	0.09	0.15	0.02	0.11	0.03	0.06
Anxiety	0.05	0.10	0.03	0.08	0.01	0.08
Attention Problems	0.08	0.08	-0.04	0.04	0.05	0.10
Atypicality	0.14	0.12	-0.04	0.05	-0.05	0.09
Depression	0.10	0.13	-0.02	0.08	0.05	0.11
Hyperactivity	0.08	0.05	-0.10	-0.06	0.02	0.14
Somatization	-0.01	-0.01	-0.02	-0.03	-0.06	0.01
Withdrawal	-0.10	0.02	-0.05	-0.07	-0.16	-0.15
values= Pearson's coefficient						

Table S4. Demographics for subset of cohort included in placenta RNA-seq analysis.

Demographics for RNA-seq		Non-CB N=101		mCB N=30	
		Mean	**SD**	**Mean**	**SD**
Maternal Age		27.94	0.62	25.57	1.05
Paternal Age		30.08	0.69	28.21	1.18
Parity		2.74	0.21	3.83	0.62
Gestational Age (weeks)		39.13	0.23	39.32	0.37
Birth Weight (g)		3391	70.41	3195	136.1
Placenta Weight (g)		657.11	166.6	578.18	110.19
Prenatal Trait Anxiety		36.36	1.11	40.62	2.12
Prenatal State Anxiety		36.42	1.11	42.53	2.11
		N		**N**	
Prenatal Depression		31		11	
Child Sex: Male		49		12	
Child Sex: Female		52		18	
Marital Status	Married	46		5	
	Common Law	7		5	
	Single	46		24	
	Widowed	0		1	
	Divorced	2		0	
Race	Asian	7		2	
	Black	59		14	
	Hispanic	19		11	
	White	11		2	
	Other	5		1	
Educational Attainment	Less than elementary	1		0	
	Some high school	11		4	
	High school graduate	23		11	
	Some college	34		7	
	Associate Degree	17		5	
	Bachelor's Degree	6		3	
	Graduate Degree	3		0	
Prenatal Alcohol Use		7		3	
Prenatal Cigarette Smoking		8		10	
Pregnant during Superstorm Sandy		32		6	
C-section		34		8	
Vaginal Infection		35		11	
Preeclampsia		13		7	
Gestational Diabetes		12		3	

Legend for Supplementary Datasets.

Dataset S1. RNA-sequencing quality control metrics.

Dataset S2. Differential expression analysis for placenta RNA-seq dataset.

Dataset S3. Gene ontology analysis for mCB differentially expressed genes.

Dataset S4. Reference list of placental cell-type marker genes.

Dataset S5. Placenta gene co-expression modules with gene-module assignments and hub genes.

Dataset S6. Placenta gene co-expression networks and eigengene relationships with neurobehavioral traits in young children as well as with cohort demographics.

Dataset S7. Examining the differentially expressed genes in co-expression networks associated with neurobehavioral traits in young children.