

March 24, 2019

Supplemental Information for:

Epigenetic Changes in Alveolar Type II Lung Cells of A/J

Mice Following Intranasal Treatment with

Lipopolysaccharide

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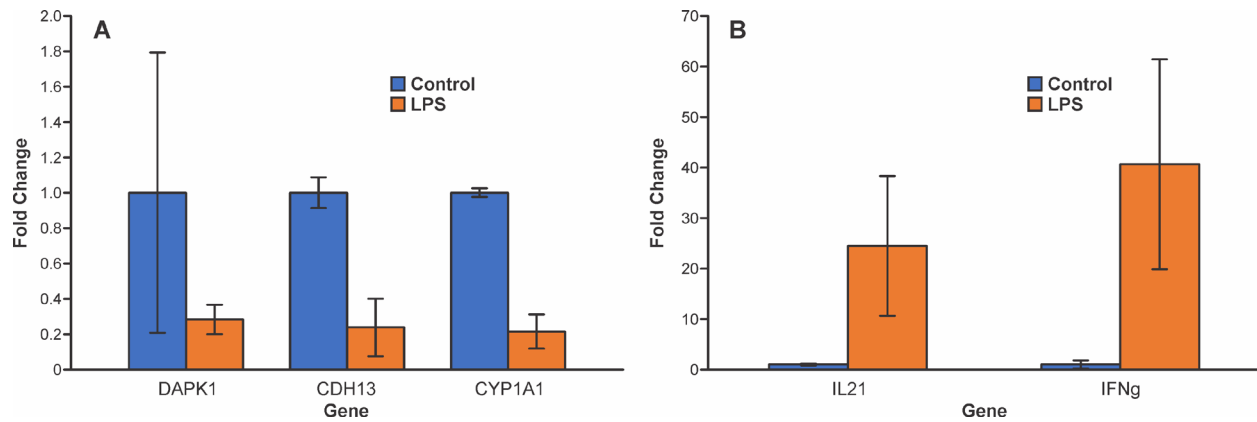
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Table S1. Primer sequences used for validation of RNA-Seq results.

Gene	Forward (5' - 3')	Reverse (5' - 3')
mCDH13	CCTGACAAGCCATCTCCTAAC	GACATCCAATCCTGCCATATCT
mDAPK1	CCGCTGTCAACTACGACTTT	GTCCTGGATTGTCATCCTCTTC
mCYP1A1	ACATCACAGACAGCCTCATT	CTCCACGAGATAGCAGTTGT
mIL21	TCTATCAGCTCCACAAGATGT	TGTGCTTCTGTTTCTTTCCT
mIFN γ	CTGATGGGAGGAGATGTC	ATTCGGGTGTAGTCACAGTT
m β -actin	ACTCTTCCAGCCTTCCTTCC	GTACTIONGCGCTCAGGAGGAG

Figure S1. Validation of RNA-Seq results for 5 genes: CDH13, DAPK1, CYP1A1, IL21, and IFN γ . RT-PCR confirms our RNA-Seq results for selected genes with decreased expression (**A**) and increased expression (**B**).



Supplement S-2. Summary of Pathway Analysis of RNA-seq data



Name

Value

Analysis Settings

Reference set: Ingenuity Knowledge Base (Genes Only)

Relationship to include: Direct and Indirect

Does not Include Endogenous Chemicals

Optional Analyses: My Pathways My List

Filter Summary:

Consider only molecules and/or relationships where

(species = Rat OR Uncategorized OR Mouse OR Human) AND

(confidence = Experimentally Observed) AND

(tissues/cell lines = Lens OR Melanoma Cell Lines not otherwise specified OR Monocyte-derived dendritic cells not otherwise specified OR Melanocytes OR Myeloma Cell Lines not otherwise specified OR Beta islet cells OR HCT-15 OR Other Stem cells OR INS-1 OR H460 OR Osteosarcoma Cell Lines not otherwise specified OR Bone marrow cells not otherwise specified OR NCI-H226 OR Nervous System not

otherwise specified OR Caudate Nucleus OR Embryonic stem cells OR A2780 OR Naive helper T cells OR P19 OR Other Memory T lymphocytes OR Immune cells not otherwise specified OR MALME-3M OR Bone marrow-derived macrophages OR SR OR DU-145 OR SK-OV-3 OR UACC-257 OR Other Pancreatic Cancer Cell Lines OR THP-1 OR Brainstem OR M14 OR J774 OR Other Lung Cancer Cell Lines OR Ventricular Zone OR Spinal Cord OR Olfactory Bulb OR Sertoli cells OR U2OS OR Granulosa cells OR Skeletal Muscle OR SK-MEL-28 OR Other Monocyte-derived dendritic cells OR MDA-MB-361 OR SW-480 OR PANC-1 OR CCRF-CEM OR HCT-116 OR Other Macrophage Cancer Cell Lines OR Lymph node OR Th17 cells OR Prostate Cancer Cell Lines not otherwise specified OR Hepatocytes OR Organ Systems not otherwise specified OR Other Granulocytes OR Immature monocyte-derived dendritic cells OR Thymocytes OR Other Endothelial cells OR Leukemia Cell Lines not otherwise specified OR Granule cells OR Other Myeloma Cell Lines OR Lymphocytes not otherwise specified OR Other Ovarian Cancer Cell Lines OR RKO OR Epithelial cells not otherwise specified OR Vd2 Gamma-delta T cells OR Hypothalamus OR MDA-MB-231 OR Lung OR Hippocampus OR HL-60 OR HuH7 OR Adipocytes OR HUVEC cells OR Immune cell lines not otherwise specified OR Gray Matter OR Stromal cells OR HCC-2998 OR Splenocytes OR Neutrophils OR Other Cells OR A498 OR SK-N-SH OR Bladder OR Macrophages not otherwise specified OR BT-474 OR Other Osteosarcoma Cell Lines OR RAW 264.7 OR Testis OR SNB-75 OR WEHI-231 OR Peritoneal macrophages OR RXF-393 OR Trachea OR Lung Cancer Cell Lines not otherwise specified OR Monocytes not otherwise specified OR Other Breast Cancer Cell Lines OR Heart OR U266 OR HT29 OR Other Colon Cancer Cell Lines OR Min6 OR Amygdala OR Small Intestine OR Astrocytes OR Hematopoietic progenitor cells OR CD4+ T-lymphocytes OR Central memory cytotoxic T cells OR Prostate Gland OR Other Smooth muscle cells OR ACHN OR Cortical neurons OR Jurkat OR Pro-B lymphocytes OR NCI-H23 OR White Matter OR 786-0 OR Bone marrow-derived dendritic cells OR Chondrocytes OR Sciatic Nerve OR Other NK cells OR Cartilage Tissue OR Neurons not otherwise specified OR SK-MEL-5 OR Other Peripheral blood leukocytes OR Hep3B OR Other Bone marrow cells OR Other Immune cell lines OR Skin OR NCI-ADR-RES OR Other Nervous System OR Salivary Gland OR Other CNS Cell Lines OR RPMI-8266 OR Macrophage Cancer Cell Lines not otherwise specified OR Brain OR Other Prostate Cancer Cell Lines OR Choroid Plexus OR Granule Cell Layer OR LOX IMVI OR Medulla Oblongata OR Th2 cells OR Activated Vd1 Gamma-delta T cells OR MDA-MB-468 OR HS 578T OR U937 OR T lymphocytes not otherwise specified OR Peripheral blood monocytes OR Peripheral blood leukocytes not otherwise specified OR Cardiomyocytes OR Cytotoxic T cells OR TK-10 OR Parietal Lobe OR B lymphocytes not otherwise specified OR Uterus OR Breast Cancer Cell Lines not otherwise specified OR Activated Vd2 Gamma-delta T cells OR CD56bright NK cells OR COLO205 OR EKVX OR Vd1 Gamma-delta T cells OR Cerebral Cortex OR CAKI-1 OR K-562 OR Vascular smooth muscle cells OR Stem cells not otherwise specified OR MOLT-4 OR Langerhans cells OR Megakaryocytes OR Pituitary Gland OR Other Teratocarcinoma Cell Lines OR Retina OR RBL-2H3 OR Purkinje cells OR Plasmacytoid dendritic cells OR Mononuclear leukocytes not otherwise specified OR Other Lymphocytes OR MG-63 OR MDA-MB-435 OR Other Epithelial cells OR Microvascular endothelial cells OR Tissues and Primary Cells not otherwise specified OR Intraepithelial T lymphocytes OR A375 OR SK-MEL-2 OR Cell Line not otherwise specified OR BDCA-3+ dendritic cells OR Cerebral Ventricles OR A549-ATCC OR PC-3 OR Smooth muscle cells not

otherwise specified OR NB4 OR Stomach OR Dendritic cells not otherwise specified OR OVCAR-4 OR Thalamus OR BDCA-1+ dendritic cells OR Activated CD56dim NK cells OR BA/F3 OR SF-268 OR Natural T-regulatory cells OR Other T lymphocytes OR Ovary OR OVCAR-8 OR Pancreatic Cancer Cell Lines not otherwise specified OR Keratinocytes OR Pre-B lymphocytes OR HEL OR Other Cell Line OR Effector memory RA+ cytotoxic T cells OR Oocytes OR CNS Cell Lines not otherwise specified OR SN12C OR Mammary Gland OR Esophagus OR Liver OR Activated CD56bright NK cells OR NCI-H522 OR NT2/D1 OR Other B lymphocytes OR Other Neuroblastoma Cell Lines OR Cerebellum OR BT-549 OR UACC-62 OR HOP-92 OR Eosinophils OR Kidney OR Calvaria OR Memory B cells OR Other Neurons OR U251 OR MCF7 OR Other Hepatoma Cell Lines OR Other Tissues and Primary Cells OR NK cells not otherwise specified OR Colon Cancer Cell Lines not otherwise specified OR Kidney Cancer Cell Lines not otherwise specified OR Fibroblasts OR Striatum OR SF-295 OR Adrenal Gland OR Other Organ Systems OR SF-539 OR Forestomach OR Smooth Muscle OR Th1 cells OR Activated helper T cells OR HepG2 OR Other Monocytes OR Memory T lymphocytes not otherwise specified OR Monocyte-derived macrophage OR Lymphoma Cell Lines not otherwise specified OR Neuroblastoma Cell Lines not otherwise specified OR Other Dendritic cells OR Subventricular Zone OR Blood platelets OR Cells not otherwise specified OR Central memory helper T cells OR Effector memory helper T cells OR Hepatoma Cell Lines not otherwise specified OR NCI-H332M OR Plasma cells OR Peripheral blood lymphocytes OR HOP-62 OR Other Lymphoma Cell Lines OR Corpus Callosum OR Dorsal Root Ganglion OR Mesenchymal stem cells OR J-774A.1 OR Adipose OR Other Kidney Cancer Cell Lines OR Teratocarcinoma Cell Lines not otherwise specified OR Cervical cancer cell line not otherwise specified OR PBMCs OR Putamen OR KM-12 OR Cornea OR Other Immune cells OR Placenta OR T47-D OR Thyroid Gland OR Other Melanoma Cell Lines OR HMC-1 OR Crypt OR UO-31 OR Trigeminal Ganglion OR HeLa OR IGROV1 OR Other Leukemia Cell Lines OR Large Intestine OR Ovarian Cancer Cell Lines not otherwise specified OR Endothelial cells not otherwise specified OR Nucleus Accumbens OR Osteoblasts OR Effector memory cytotoxic T cells OR Pancreas OR Murine NKT cells OR Naive B cells OR SW-620 OR Granulocytes not otherwise specified OR Microglia OR Myeloid dendritic cells OR Other Macrophages OR Spleen OR Mature monocyte-derived dendritic cells OR U87MG OR OVCAR-3 OR Pyramidal neurons OR OVCAR-5 OR CD56dim NK cells OR MDA-N OR Other Cervical cancer cell line OR Epidermis OR Substantia Nigra OR Thymus OR Other Mononuclear leukocytes OR Dermis OR Effector T cells OR CD34+ cells OR Mast cells) AND

(data sources = An Open Access Database of Genome-wide Association Results OR BIND OR BioGRID OR Catalogue Of Somatic Mutations In Cancer (COSMIC) OR Chemical Carcinogenesis Research Information System (CCRIS) OR ClinicalTrials.gov OR ClinVar OR Cognia OR DIP OR DrugBank OR Gene Ontology (GO) OR GVK Biosciences OR Hazardous Substances Data Bank (HSDB) OR HumanCyc OR Ingenuity Expert Findings OR Ingenuity ExpertAssist Findings OR IntAct OR Interactome studies OR MIPS OR miRBase OR miRecords OR Mouse Genome Database (MGD) OR Obesity Gene Map Database OR Online Mendelian Inheritance in Man (OMIM) OR TarBase OR TargetScan Human)

Top Canonical Pathways

Name	p-value	Overlap
Hepatic Fibrosis / Hepatic Stellate Cell Activation	2.61E-16	21.3 % 39/183
Granulocyte Adhesion and Diapedesis	1.71E-14	20.3 % 36/177
Agranulocyte Adhesion and Diapedesis	1.41E-13	19.0 % 36/189
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	6.97E-11	19.7 % 27/137
LXR/RXR Activation	1.22E-10	20.7 % 25/121

Top Upstream Regulators

Name	p-value	Predicted Activation
lipopolysaccharide	5.03E-92	Activated
IFNG	7.27E-71	Activated
TNF	1.05E-58	Activated
IL10RA	5.24E-58	Inhibited
STAT3	8.21E-53	

Top Diseases and Bio Functions

Diseases and Disorders

Name	p-value range	# Molecules
Immunological Disease	7.33E-10 - 8.47E-52	476
Endocrine System Disorders	5.51E-10 - 4.88E-45	331
Gastrointestinal Disease	5.87E-10 - 4.88E-45	804
Metabolic Disease	3.41E-11 - 4.88E-45	217
Organismal Injury and Abnormalities	7.26E-10 - 4.88E-45	895

Molecular and Cellular Functions

Name	p-value range	# Molecules
Cellular Movement	7.10E-10 - 1.35E-59	353
Cell-To-Cell Signaling and Interaction	7.18E-10 - 1.38E-32	252
Cellular Growth and Proliferation	3.37E-10 - 1.99E-32	469
Cellular Development	3.37E-10 - 1.38E-25	396
Cell Death and Survival	5.74E-10 - 3.94E-23	377

Physiological System Development and Function

Name	p-value range	# Molecules
Immune Cell Trafficking	3.65E-10 - 4.04E-50	223
Hematological System Development and Function	3.65E-10 - 1.05E-39	317
Cardiovascular System Development and Function	5.10E-10 - 5.47E-33	240
Organismal Development	5.64E-10 - 2.45E-31	349
Organismal Survival	9.62E-22 - 1.97E-30	317

Top Tox Functions

Assays: Clinical Chemistry and Hematology

Name	p-value range	# Molecules
Increased Levels of Creatinine	4.74E-01 - 3.38E-04	9
Decreased Levels of Albumin	1.68E-01 - 8.38E-04	6
Increased Levels of AST	3.68E-03 - 3.68E-03	4
Increased Levels of Alkaline Phosphatase	4.74E-03 - 4.74E-03	9
Increased Levels of ALT	2.75E-01 - 1.30E-02	5

Cardiotoxicity

Name	p-value range	# Molecules
Cardiac Infarction	1.75E-01 - 4.21E-14	46
Cardiac Hypertrophy	2.93E-01 - 8.13E-08	45
Cardiac Arteriopathy	3.07E-01 - 9.13E-08	39
Heart Failure	3.68E-01 - 1.67E-07	36
Cardiac Dysfunction	3.68E-01 - 6.90E-07	28

Hepatotoxicity

Name	p-value range	# Molecules
Liver Damage	3.07E-01 - 3.03E-09	45

Liver Cirrhosis	1.68E-01 - 7.45E-09	36
Liver Inflammation/Hepatitis	3.07E-01 - 1.47E-07	41
Liver Proliferation	5.62E-01 - 4.11E-07	29
Liver Fibrosis	3.07E-01 - 1.85E-06	35

Nephrotoxicity

Name	p-value range	# Molecules
Renal Damage	8.76E-02 - 9.32E-13	43
Kidney Failure	4.74E-01 - 2.04E-10	38
Renal Proliferation	2.75E-01 - 3.98E-08	34
Renal Tubule Injury	4.48E-02 - 5.80E-07	25
Renal Inflammation	1.00E00 - 8.71E-07	41

Top Regulator Effect Networks

ID	Regulators	Disease & Functions	Consistency Score
1	TNFSF12	inflammatory response	3.75
2	SOCS1	infection of mammalia	3.357
3	IFNA1/IFNA13	replication of virus	3.328
4	IL2	inhibition of cells	3.328
5	TNFSF12	migration of phagocytes	3.328

Top Networks

ID	Associated Network Functions	Score
1	Organismal Injury and Abnormalities, Drug Metabolism, Protein Synthesis	41
2	Antimicrobial Response, Inflammatory Response, Endocrine System Disorders	39
3	Cancer, Connective Tissue Disorders, Organismal Injury and Abnormalities	34
4	Infectious Diseases, Cellular Movement, Immune Cell Trafficking	34
5	Cellular Assembly and Organization, Protein Synthesis, Cellular Function and Maintenance	32

Top Tox Lists

Name	p-value	Overlap
Hepatic Fibrosis	2.33E-13	25.7 % 26/101
Increases Liver Damage	9.08E-12	22.2 % 26/117
LXR/RXR Activation	1.76E-10	20.3 % 25/123
Cardiac Hypertrophy	1.46E-08	10.9 % 48/442

Liver Proliferation

4.65E-07

12.7 % 29/229

Top My Lists

Top My Pathways

Top Analysis-Ready Molecules

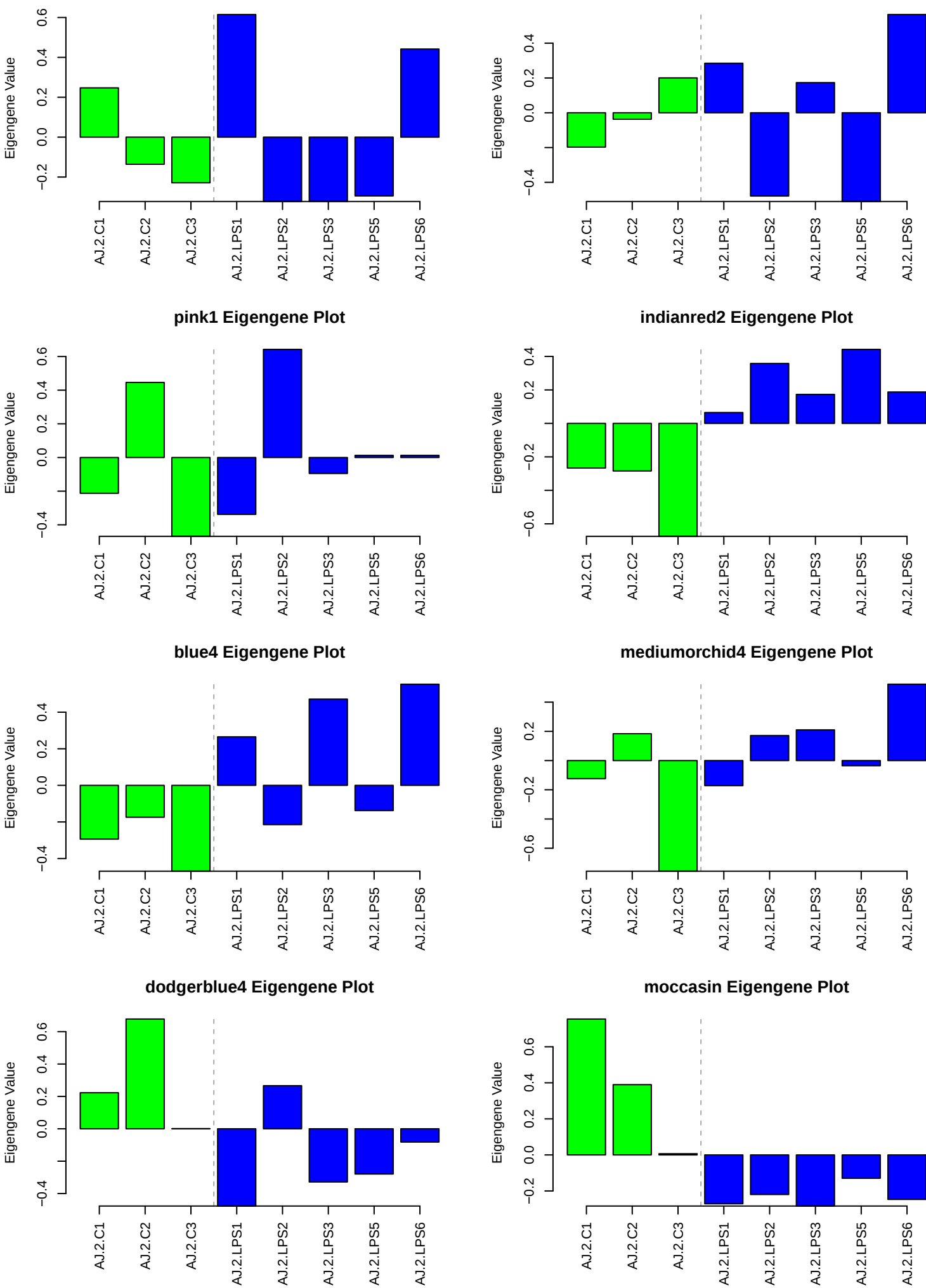
Expr Fold Change

Molecules	Expr. Value	Chart
Stfa3	↑ 524.140	
Gm15056	↑ 444.252	
Stfa2/Stfa2l1*	↑ 354.613	
Saa3	↑ 344.774	
Ly6a (includes others)*	↑ 249.958	
Stfa1 (includes others)*	↑ 232.727	
CSTA*	↑ 159.700	
Gm5416	↑ 139.399	
TARM1	↑ 85.214	
SAA1	↑ 81.904	

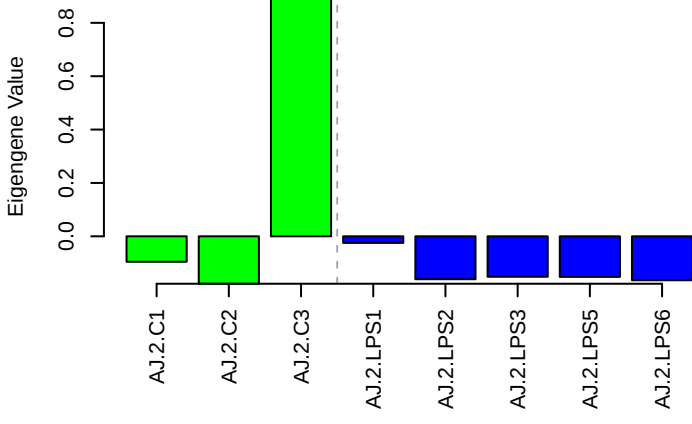
Expr Fold Change

Molecules	Expr. Value	Chart
Chil3/Chil4	↓ -287.303	
Nxf7	↓ -86.820	
FABP1	↓ -31.470	
CDH4	↓ -28.783	
PCDHAC2	↓ -22.282	
CD207	↓ -21.868	
CPNE5	↓ -21.777	
GRIN2B	↓ -21.363	
ESR2	↓ -20.996	
CYP2A6 (includes others)	↓ -20.395	

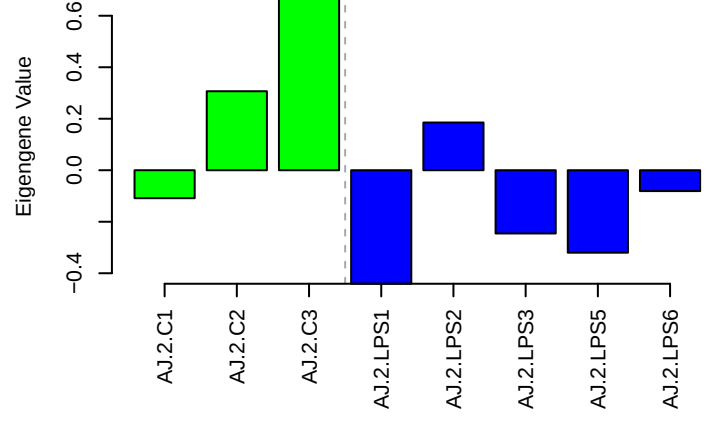
Figure S3. Weighted gene co-expression network analysis (WGCNA) analysis of gene expression data from RNA-Seq.



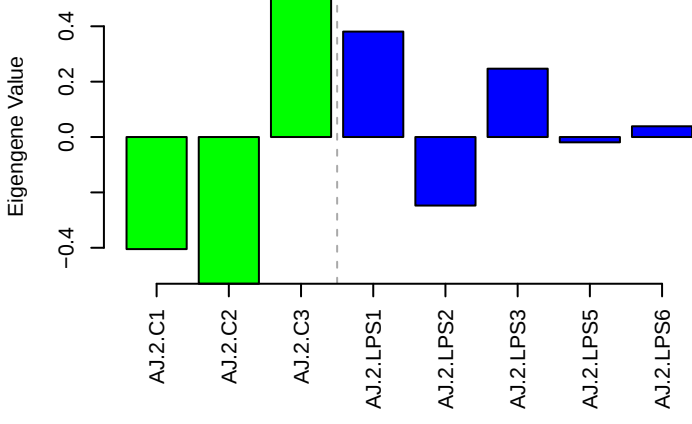
brown3 Eigengene Plot



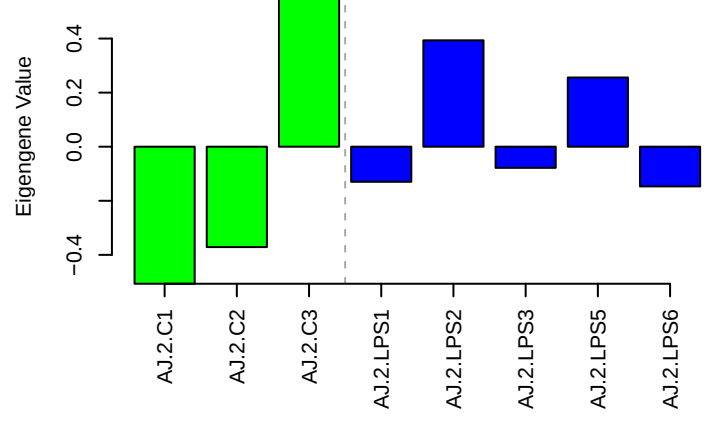
darkseagreen Eigengene Plot



magenta2 Eigengene Plot



tan Eigengene Plot



grey Eigengene Plot

