

ONLINE DATA SUPPLEMENT

Alterations in Gene Expression and DNA Methylation During Murine and Human Lung Alveolar Septation

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METHODS

Lung tissue samples

Mouse lung tissue samples: C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME) and bred in-house. Pups were born naturally and lungs were isolated by manual dissection at postnatal day 3 (P3) and at 6 weeks of age (P42) to allow comparison between newborn lungs before initiation and adult lungs after completion of alveolar septation, which generally begins after P4 and is essentially complete by P28 (1). We used young adult (6 weeks) mice to ensure that alveolar septation was indeed completed, as there is some evidence that alveolar septation continues for at least as long as 4 weeks in mice, although most is completed by 14 days. All animal procedures were carried out according to the protocol approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Alabama at Birmingham.

Human lung tissue samples: Formalin-fixed, paraffin embedded (FFPE) de-identified lung tissue samples were obtained from the University of Alabama at Birmingham and Children's of Alabama Department of Pathology, following Institutional Review Board (IRB) approval. The study group consisted of preterm infants with BPD (BPD, postmenstrual age 28 to 42 weeks, n=6), without evidence of pulmonary/systemic infection. All infants with BPD had some evidence of pulmonary vascular remodeling. For comparison, preterm stillbirths (Preterm, 24 to 26 weeks gestation, n=5) and term stillbirths (Term, 36 to 40 weeks, n=6) were used as control groups. These stillbirths were not caused by infection or conditions affecting lung development.

DNA isolation and DNA methylation profiling

Mouse: Mouse genomic DNA was extracted from three whole lung tissue samples per time point using Qiagen DNeasy Blood and Tissue Kit (Qiagen, California USA). Using the methylated DNA immunoprecipitation protocol by Mohn et al. (2), isolated DNA was sheared by sonication and treated with anti-5-methylcytosine antibodies to immunoprecipitate methylated DNA fragments (Anti-5-methylcytosine, MABE146, Millipore). High-throughput sequencing was then performed using the enriched methylated fragments (MeDIP-Seq) using HiSeq2000 (Illumina, California USA) following the manufacturer's protocol.

Human: Human genomic DNA was isolated from FFPE lung tissue samples using the QIAmp FFPE Tissue Kit (Qiagen, California USA). Because DNA from FFPE samples can be highly degraded, quality of isolated DNA was assessed using the Illumina FFPE QC Kit (Illumina, California USA) and only samples that passed quality control underwent restoration using Illumina FFPE DNA Restore Kit (Illumina, California USA). The methylation profile of each restored sample was then assayed using the Illumina HumanMethylation450 DNA Analysis BeadChip.

RNA isolation and gene expression profiling

Mouse: Total RNA from five mouse whole lung tissue samples per time point was isolated using the Qiagen RNeasy Mini kit (Qiagen, California USA). The isolated RNA was run on an Agilent Bioanalyzer 2100 for quality and quantity assessment. RNA samples were then profiled using the Agilent Whole-Mouse Genome Microarrays Kit (Agilent, G4122F) following the manufacturer's protocol (Agilent, California USA).

Validation was performed using specific primers by TaqMan Gene Expression assays (Life Technologies, Grand Island, NY) using RNA isolated from different mouse lungs for MMP3 (Mm00440295_m1), TNC (Mm00495662_m1), and ZFP536 (Mm00552423_m1).

Human: RNA was isolated using Qiagen RNeasy FFPE kit (Qiagen, Valencia, CA) from paraffin-embedded formalin-fixed samples of human preterm, term, and BPD lungs (n=4/group). Selected genes (the ones with greatest fold-change in the study by Bhattacharya et al.(3)) were further evaluated by TaqMan Gene Expression assays (Life Technologies) from RNA isolated using Qiagen RNeasy FFPE kit (Qiagen, Valencia, CA) from the unrelated FFPE samples at UAB (n=4/group) that were also used for the DNA methylation analysis. These genes were: CPA3 (Taqman Hs00157019_m1), CCL18 (Hs00268113_m1), IGHA1 (Hs00733892_m1), and TPSB2(TPSAB1) (Hs02576518_gH), which were normalized to 18S expression (Eukaryotic 18S rRNA Endogenous Control (VIC®/TAMRA™ probe, primer limited); 4310893E) or GAPDH (GAPDH Hs02758991_g1) in the same sample. Additional validation to confirm microarray data for genes potentially regulated by DNA methylation was done for KIAA1217 (Hs01594940_m1) and ZNF438 (Hs00330222_m1).

Data analyses

DNA methylation data

Mouse: MeDIP-Seq data was mapped to the latest mouse reference genome using Bowtie 2 (<http://bowtie-bio.sourceforge.net/bowtie2/>). Duplicates were removed, and the Model-based Analysis of ChIP-Seq (MACS) software program

(<http://liulab.dfci.harvard.edu/MACS/>) was used to identify qualified peak regions with P value of <0.05. Genes meeting this criterion were identified as being differentially methylated between newborn and adult lungs.

DNA methylation of TNC and MMP3 was confirmed as follows: Genomic DNA was bisulfite converted with Qiagen EpiTect Fast DNA Bisulfite Kit, PCR products were produced with Qiagen Pyromark PCR kit, and gel extracted with the Qiagen Qiaquick Gel Extraction Kit. Purified PCR products were cloned into the p-Drive cloning vector via the Qiagen PCR Cloning Plus Kit. White clones (blue-white screening on LB/Amp/X-gal/IPTG agar plates) were inoculated into LB-Amp broth and incubated overnight with shaking at 37°C and recombinant plasmid was isolated with Qiagen QiaPrep Spin Miniprep Kit. All recombinant plasmids were Sanger sequenced with the T7 promoter primer (ABI 3730 DNA Analyzer with Applied Biosystems Big Dye Terminator v3.1 Ready Reaction kit protocol). Pyrosequencing was performed with a Qiagen Pyromark Q24 with Pyromark Q24 Gold Reagents. (Forward and reverse primers for TNC: AGAGAATTTGATAGTTTTTGAATAGG and AAAAAACCCAAAATAAAACCTTTCTC and MMP3: TTATTTGATAATATGTGAAAAGGAATAAA and CCTAAATAAAAAATAATTCTCCTTAAAAA). DNA methylation of DCTD and TMCC3 was confirmed by pyrosequencing using commercially available primers from Qiagen (Pyromark CpG Assays, Qiagen) and the Pyromark HS-96 pyrosequencing instrument from Qiagen.

Human: HumanMethylation450 data were analyzed using the “minfi” package in Bioconductor (<http://www.bioconductor.org/packages/release/bioc/html/minfi.html>). Data were normalized using the subset-quantile within array normalization (SWAN) method (4) for better integration of the two types of probes on the array. F test was used to compare the three groups and t-tests were used for pair-wise contrasts to identify differential methylation at each CpG site based on M values (logit

transformation of the beta values). False discovery rate (FDR) < 0.05 was used to select significant CpG sites in BPD compared to Preterm and Term groups (5). CpG sites were localized to either promoter CpG islands, or shores (0-2 kb from promoter CpG islands) or shelves (2-4 kb from promoter CpG islands).

Gene expression data

Mouse: Gene expression data were analyzed using GeneSpring GX version 11 (Agilent, California USA) software. A stringent FDR with a cutoff of 0.01 and Bonferroni correction for multiple comparisons was used and genes with a fold change ≥ 2 were identified as being differentially expressed between newborn and adult lungs. The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo [accession nos. GSE41412 (time series expression data)] and previously reported in relation to regulation by microRNA during alveolar septation (6) .

Human: Gene expression data were extracted from a previously published dataset by Bhattacharya et al. describing genome-wide expression in lung tissue obtained from BPD cases (n=11) or controls (n=9) (3). Probe sets were designed to interrogate the identified differentially methylated genes in BPD using the Affymetrix HG-U133 plus array and matched using Gene Symbols and NetAffx (www.netaffx.com). Robust multiarray averaging (RMA) normalized data were used to assess for gene differential expression using a non-corrected student's t-test, with a p-value of less than 0.05 defined as statistically significant. Ingenuity Pathway Analysis (IPA) (Ingenuity Systems, California USA) was used to identify canonical pathways significantly over-represented by gene demonstrating changes in methylation or expression. Gene Set Enrichment Analysis (GSEA) was performed using RMA normalized

expression intensities of all probe sets. Threshold size for individual gene sets was limited 5 to 50 genes for GSEA. Canonical Pathways and Gene Sets with a p-value of less than 0.05 were considered as significantly dysregulated.

Integrating gene expression and DNA methylation

Data obtained from gene expression analysis was compared with DNA methylation analysis to identify genes that were significantly altered in both DNA methylation as well as gene expression. Genes demonstrating inverse relationship between methylation status and gene expression were of interest, as these indicated genes likely to be epigenetically altered by DNA methylation. Analysis was not restricted to known promoter regions and included methylation sites not currently known to regulate gene expression in order to provide an unbiased approach. Pathway analysis of these genes was performed with IPA.

Table S1: Genes with increased DNA methylation and increased mRNA expression in newborn mouse lung compared to adult mouse lung (arranged in order of increasing p-value; * Indicates genes with methylation in more than one locus).

Gene symbol	ProbeName	Gene title	Entrez Gene	Adj. P-value	Fold change in gene expression	Fold enrichment in methylation
Slc27a6	A_55_P2039379	Solute carrier family 27 (fatty acid transporter), member 6	225579	1.35E-06	18.4	10.3
Cenpn	A_52_P304947	Centromere protein N	72155	2.02E-06	10.4	27.0
Zfp618	A_55_P2012478	Zinc fingerprotein 618	72701	2.23E-06	6.7	5.8
Tiam2	A_51_P256066	T-cell lymphoma invasion and metastasis 2	24001	4.06E-06	3.8	14.3
Lrrn1	A_51_P412966	Leucine rich repeat protein 1, neuronal	16979	8.34E-06	22.7	25.23
Apol8	A_51_P115471	Apolipoprotein L 8	239552	1.10E-05	2.6	45.07
Gypa	A_52_P77080	Glycophorin A	14934	1.34E-05	11.6	73.94
Fbn2*	A_51_P103850	Fibrillin 2	14119	1.37E-05	51.5	10.3
Dbc1	A_55_P2124941	Deleted in bladder cancer 1 (human)	56710	1.71E-05	7.3	10.35

Nid2	A_51_P31 5666	Nidogen 2	18074	2.08E-05	4.5	16.72
Thrap3*	A_55_P19 89865	Thyroid hormone receptor associated protein 3	230753	2.35E-05	2.8	9.53
Lmnb1	A_51_P12 0717	Lamin B1	16906	2.52E-05	7.2	31.73
Fbn2*	A_55_P21 21096	Fibrillin 2	14119	2.93E-05	26.8	10.3
E2f7	A_55_P20 62598	E2F transcription factor 7	52679	3.16E-05	10.8	16.31
Cd109	A_51_P34 9546	CD109 antigen	235505	3.19E-05	9.2	7.06
E2f8	A_55_P20 00833	E2F transcription factor 8	108961	3.30E-05	10.3	81.13
Wif1	A_51_P48 4526	Wnt inhibitory factor 1	24117	3.90E-05	7.6	33.11
Dlk1*	A_55_P21 05371	Delta-like 1 homolog (Drosophila)	13386	4.09E-05	241.2	12.85
Lrr1*	A_55_P20 76941	Leucine rich repeat protein 1	69706	4.59E-05	9.8	21.015
Ccdc36	A_52_P55 7430	Coiled-coil domain containing 36	434438	5.23E-05	13.9	45.07
Mcm3*	A_55_P19 53087	Minichromosome maintenance	17215	5.45E-05	3.6	20.69

		deficient 3 (S. cerevisiae)				
Auts2	A_51_P39 8191	Autism susceptibility candidate 2	319974	5.66E-05	2.8	31.53667
Zdhhc2 2	A_55_P20 97518	Zinc finger, DHHC-type containing 22	238331	5.90E-05	3.3	72.12
Frem2	A_55_P20 97869	Fras1 related extracellular matrix protein 2	242022	6.89E-05	6.7	8.72
Ska1	A_52_P13 9650	RIKEN cDNA 2810433K01 gene	66468	6.96E-05	14.8	45.07
Mcm3*	A_51_P32 4934	Minichromosome maintenance deficient 3 (S. cerevisiae)	17215	7.11E-05	3.7	20.69
Mex3a	A_52_P70 6060	Mex3 homolog A (C. elegans)	72640	7.17E-05	14.1	24.83
Maged2	A_55_P20 93994	Melanoma antigen, family D, 2	80884	7.51E-05	4.1	54.09
D43004 1D05Rik	A_55_P20 62926	RIKEN cDNA D430041D05 gene	241589	9.37E-05	3.0	45.07
Igsf10	A_55_P21 57902	Immunoglobulin superfamily, member 10	242050	1.07E-04	2.5	18.365
Irak1bp 1	A_55_P20 27361	Interleukin-1 receptor-associated	65099	1.14E-04	2.1	35.13667

		kinase 1 binding protein 1				
Fech	A_55_P20 42146	Ferrochelatase	14151	1.35E-04	2.7	45.07
Unc5c	A_66_P12 9566	Unc-5 homolog C (C. elegans)	22253	1.61E-04	7.1	33.11
Fanci	A_55_P20 25790	Fanconi anemia, complementatio n group I	208836	1.65E-04	6.3	49.66
Kif2c*	A_66_P12 5209	Kinesin family member 2C	73804	1.74E-04	13.1	9.6
Snca	A_51_P50 9643	Synuclein, alpha	20617	2.08E-04	4.0	17.12
Tcf21	A_51_P45 9944	Transcription factor 21	21412	2.12E-04	2.4	45.07
Obsl1	A_51_P47 9583	Obscurin-like 1	98733	2.27E-04	5.4	27.44
Dlk1*	A_66_P11 7477	Delta-like 1 homolog (Drosophila)	13386	2.67E-04	186.3	12.85
Dctd*	A_52_P18 3826	DCMP deaminase	320685	2.70E-04	6.2	45.07
Mfap4	A_55_P20 05470	Microfibrillar- associated protein 4	76293	2.78E-04	2.2	7.77
Kif2c*	A_55_P21 00209	Kinesin family member 2C	73804	2.98E-04	12.0	9.6
Id4	A_52_P44 9871	Inhibitor of DNA binding 4	15904	3.45E-04	2.2	45.07
Ppp2r5 c	A_66_P13 9664	Protein phosphatase 2,	26931	3.50E-04	2.1	72.12

		regulatory subunit B (B56), gamma isoform				
Armxc2	A_55_P2012221	Armadillo repeat containing, X-linked 2	67416	3.54E-04	2.3	14.78
Kcnma1	A_55_P2378486	Potassium large conductance calcium-activated channel, subfamily M, alpha member 1	16531	3.67E-04	2.7	33.11
Tnc*	A_55_P2421597	Tenascin C	21923	3.95E-04	26.7	31.85667
Prdx4	A_55_P1979457	Peroxiredoxin 4	53381	4.72E-04	2.4	17.15
Dctd*	A_52_P301085	DCMP deaminase	320685	5.16E-04	13.0	45.07
Stfa1	A_55_P1963017	Stefin A1	20861	6.13E-04	51.5	72.12
Shh	A_52_P49014	Sonic hedgehog	20423	6.47E-04	3.9	7.33
Gli3	A_51_P171288	GLI-Kruppel family member GLI3	14634	6.59E-04	4.7	45.07
Pank1	A_51_P123320	Pantothenate kinase 1	75735	9.19E-04	5.6	18.35
Chn1	A_51_P335758	Chimerin (chimaerin) 1	108699	9.33E-04	2.4	10.12
Lrr1*	A_51_P34	Leucine rich	69706	9.38E-04	14.6	21.015

	7240	repeat protein 1				
Ccnj1	A_52_P650387	Cyclin J-like	380694	1.17E-03	3.2	7.76
Cit	A_55_P1999633	Citron	12704	1.20E-03	6.5	68.97
Prdm8	A_55_P2024525	PR domain containing 8	77630	1.23E-03	2.4	47.735
Shisa3	A_55_P2181104	Shisa homolog 3		1.44E-03	14.8	5.52
Lama1	A_66_P118600	Laminin, alpha 1	16772	1.53E-03	4.5	7.02
Hs6st3	A_55_P2070379	Heparan sulfate 6-O-sulfotransferase 3	50787	1.56E-03	8.4	41.38
Dennd2a	A_51_P155085	DENN/MADD domain containing 2A	209773	2.04E-03	2.5	54.09
Shisa6	A_55_P2154749	Gene model 879, (NCBI)	380702	2.22E-03	5.2	26.575
Egln3*	A_52_P387009	EGL nine homolog 3 (C. elegans)	112407	2.23E-03	10.7	19.63
Brd3	A_52_P652104	Bromodomain containing 3	67382	2.28E-03	2.1	57.94
Fancd2	A_52_P556462	Fanconi anemia, complementation group D2	211651	2.50E-03	5.9	45.07
Ctnna2*	A_55_P2075726	Catenin (cadherin associated	12386	2.61E-03	3.6	33.11

		protein), alpha 2				
Maged1	A_51_P17 4854	Melanoma antigen, family D, 1	94275	2.62E-03	2.3	45.07
Thrap3 *	A_55_P20 90535	Thyroid hormone receptor associated protein 3	230753	3.05E-03	2.5	9.53
Pa2g4	A_55_P21 17146	Proliferation- associated 2G4	18813	3.15E-03	2.4	45.07
Robo1	A_55_P19 85070	Roundabout homolog 1 (Drosophila)	19876	3.55E-03	3.0	17.335
Aif1l	A_51_P47 2829	RIKEN cDNA 2810003C17 gene	108897	3.83E-03	4.4	45.07
Lrrtm3	A_66_P13 4355	Leucine rich repeat transmembrane neuronal 3	216028	4.04E-03	3.5	21.19833
Cdk5ra p2	A_55_P21 12240	CDK5 regulatory subunit associated protein 2	214444	4.11E-03	2.3	45.07
Lhfpl2	A_55_P21 52771	Lipoma HMGIC fusion partner-like 2	218454	4.15E-03	4.8	8.39
Sox9	A_52_P21 4630	SRY-box containing gene 9	20682	4.42E-03	6.8	27.26

Tnc*	A_52_P35 5169	Tenascin C	21923	4.60E-03	54.3	31.85667
Fat4	A_52_P67 5530	FAT tumor suppressor homolog 4 (Drosophila)	329628	4.83E-03	2.5	24.83
Pfdn1	A_52_P45 6059	Prefoldin 1	67199	4.84E-03	2.1	7.28
Ctnna2 *	A_51_P43 8841	Catenin (cadherin associated protein), alpha 2	12386	4.89E-03	17.0	33.11
Tubb5	A_52_P16 3795	Tubulin, beta 5	22154	4.92E-03	3.0	42.01667
Ptpla	A_55_P21 03105	Protein tyrosine phosphatase- like (proline instead of catalytic arginine), member a	30963	5.07E-03	2.8	9.02
Adam1 2	A_51_P51 0882	A disintegrin and metallopeptidas e domain 12 (meltrin alpha)	11489	5.38E-03	13.6	9.94
150000 9L16Ri k	A_55_P21 26790	RIKEN cDNA 1500009L16 gene	69784	5.53E-03	4.5	9.93
Fbln5	A_55_P20 88615	Fibulin 5	23876	5.71E-03	2.5	63.1
Btnl9	A_55_P21	Butyrophilin-	237754	6.28E-03	4.8	63.1

	45125	like 9				
Egln3*	A_55_P19 60916	EGL nine homolog 3 (C. elegans)	112407	6.71E-03	12.2	19.63
Ncam1	A_51_P38 0309	Neural cell adhesion molecule 1	17967	7.47E-03	3.1	25.87
Scml2	A_66_P10 2256	Sex comb on midleg-like 2 (Drosophila)	107815	7.80E-03	6.4	24.67
Col22a 1	A_52_P43 4306	Collagen, type XXII, alpha 1	69700	7.95E-03	9.4	20.69
Ckap4	A_66_P10 4422	Cytoskeleton- associated protein 4	216197	8.36E-03	3.7	18.97
Rhou	A_51_P22 7392	Ras homolog gene family, member U	69581	8.67E-03	2.0	8.204286
Arhgap 28	A_65_P11 853	Rho GTPase activating protein 28	268970	8.80E-03	2.0	28.667

Table S2: Genes with increased DNA methylation and increased mRNA expression in adult mouse lung compared to newborn mouse lung (arranged in order of increasing p-value)

Gene Symbol	Probe Name	Gene Title	Entrez Gene	Adj. P-value	Fold change in gene expression	Fold enrichment in methylation
Nrn1	A_51_P308844	Neuritin 1	68404	1.69E-07	11.4	43.98
Aox3	A_52_P16752	Aldehyde oxidase 3	71724	2.78E-06	8.3	43.98
Ifit3	A_51_P359570	Interferon-induced protein with tetratricopeptide repeats 3	15959	3.05E-06	12.8	16.13
Oas2	A_55_P2019719	2'-5' oligoadenylate synthetase 2	246728	2.23E-05	8.1	32.26
Sp110	A_55_P2152566	Sp110 nuclear body protein	109032	2.82E-05	3.2	43.98
Ly6c1	A_55_P2064771	Lymphocyte antigen 6 complex, locus C1	17067	4.93E-05	4.2	10.02
Aim1	A_55_P2116059	Absent in melanoma 1	11630	5.87E-05	3.3	6.31
Slco3a1	A_66_P135106	Solute carrier organic anion transporter family, member 3a1	108116	6.81E-05	2.5	52.78
Flt3	A_66_P116412	FMS-like tyrosine kinase 3	14255	9.65E-05	6.4	13.46
St3gal1	A_65_P03728	ST3 beta-galactoside alpha-	20442	1.23E-04	2.1	

		2,3-sialyltransferase 1				35.19
Scd1	A_52_P68 2382	Stearoyl-Coenzyme A desaturase 1	20249	2.40E-04	6.2	43.98
Zbtb16	A_55_P20 24155	Zinc finger and BTB domain containing 16	235320	2.82E-04	3.8	40.32
Scml4	A_55_P20 20338	Sex comb on midleg-like 4 (Drosophila)	268297	3.28E-04	4.3	43.98
Il6st	A_51_P47 3888	Interleukin 6 signal transducer	16195	3.32E-04	2.4	43.98
Glb1l3	A_52_P29 5140	Galactosidase, beta 1 like 3	70893	5.71E-04	6.7	33.6
Gria1	A_55_P21 13758	Glutamate receptor, ionotropic, AMPA1 (alpha 1)	14799	7.84E-04	7.8	46.55
Grem2	A_51_P15 2990	Gremlin 2 homolog, cysteine knot superfamily (Xenopus laevis)	23893	8.26E-04	3.1	43.98
Lrg1	A_51_P34 6938	Leucine-rich alpha-2-glycoprotein 1	76905	8.32E-04	10.3	52.78
Kcnj15	A_55_P21 64090	Potassium inwardly-rectifying channel, subfamily J, member 15	16516	8.44E-04	2.3	70.37
Ly6a	A_51_P26 5495	Lymphocyte antigen 6 complex,	110454	9.56E-04	5.1	18.82

		locus A				
Serping 1	A_55_P19 92889	Serine (or cysteine) peptidase inhibitor, clade G, member 1	12258	1.53E-03	2.3	52.78
Adamts 12	A_51_P36 7780	ADAMTS-like 2	77794	1.65E-03	2.9	43.98
Grap2	A_52_P15 0683	GRB2-related adaptor protein 2	17444	1.99E-03	4.7	61.57
Wdly4	A_52_P19 9614	WD repeat and FYVE domain containing 4	545030	2.43E-03	3.0	43.98
Ifngr1	A_51_P39 1445	Interferon gamma receptor 1	15979	2.48E-03	2.3	52.78
Tmem2 8	A_55_P21 85178	Transmembrane protein 28	270028	2.89E-03	20.9	32.26
Sp140	A_55_P20 86811	Sp140 nuclear body protein	434484	3.38E-03	2.6	43.98
Stat4	A_51_P17 7092	Signal transducer and activator of transcription 4	20849	6.23E-03	5.7	30.235
Rnf213	A_51_P15 9503	Ring finger protein 213	629974	6.37E-03	2.2	35.19
Pitpnc1	A_52_P60 9109	Phosphatidylinosit ol transfer protein, cytoplasmic 1	71795	7.76E-03	2.8	43.98
Mtmr7	A_66_P11 7578	Myotubularin related protein 7	54384	7.80E-03	2.6	32.26

Table S3: Networks associated with genes differentially methylated in newborn mouse lung with decreased expression in newborn mouse lung.

Molecules in Network	Score	Focus Molecules	Top Functions
Actin, Akt, ANGPT2, Ap1, Calcineurin protein(s), CaMKII, caspase, CD3, Cg, Collagen type I, EFEMP1, ERK, Fibrinogen, GGCT, GRIA1, Histone h3, IGFBP3, Jnk, Klr1c (includes others), Mapk, MMP3, Mmp, P38 MAPK, PHF15, Pkc(s), PRDX6, PRKCQ, Rac, SCML4, SH3BP5, SOD3, SRC (family), TCR, Vegf, WISP2	30	14	Cardiovascular system development and function, cardiovascular disease, developmental disorder
B2M, BCL6, BCR (complex), BLNK, CCL5, CCR6, CD19, ERK1/2, HELZ2, Ifn, IFN type 1, Iga, Ige, IgG1, Igg3, IgG, IgG2a, IgG2b, Igm, IL1, IL12 (complex), IL12 (family), Immunoglobulin, Interferon alpha, LDL, LILRB3, LRP2, NFAT (complex), PLC gamma, Ras, RASGRP1, SH2D1A, STAT4, Tgf beta, VAV	25	12	Inflammatory response, cellular development, cellular growth and proliferation
AMPD3, caffeic acid, CCL1, CD44, Cd209a, CEACAM1, CEBPB, COBL, Collagen type II, Collagen type III, cyclooxygenase, D-glucose, ELF3, ELK3, FPR2, GBP5, HAPLN1, HLA-DOB, HSF1, IL-1R, IL17RA, INMT, ITGB6, LGALS3BP, LIMS2,	17	9	Cell-to-cell signaling and interaction, cellular function and maintenance, inflammatory response

MAPK1, MHC Class II (complex), MMP10, SAA, SCUBE2, SFTPD, SP110, Tenascin, TIMP4, TNF			
26s Proteasome, ADIPOR1, ADRA1A, ALDH1A1, AXL, CCL1, Chi3l3/Chi3l4, CUL2, CYR61, Fabp5, FPR2, glucosamine, Hdac, IGFBP3, IL17F, Insulin, KCNJ15, KISS1, KNG1, LEP, melatonin, MTMR7, NFkB (complex), NR1H4, phosphatidylinositol, PI3K (complex), Pka, PLC, PTGER2, RNA polymerase II, SAA, SFRP1, SFTPD, ST3GAL1, TMCC3	15	8	Cellular movement, carbohydrate metabolism, hematological system development and function
ACTN4, ADAM2, Adam3, ADIPOR1, Aox3, AXL, BSCL2, CANX, CARM1, CHP1, CREBL2, DCN, ELAVL1, ELF3, EPAS1, ETS2, ETV6, FOSL2, HEATR1, HILPDA, HTRA2, KRT17, MTPN, MYBBP1A, NRN1, OCLN, PGK1, SERPINA1, SLC25A13, SLC9A4, SUMO2, TCF20, TMEM167B, UBC, ZBTB4	13	7	Cell-to-cell signaling and interaction, cellular assembly and organization, reproductive system development and function
ERG, SPTBN5, TRPC4	2	1	Cardiovascular system development and function, cell morphology, cellular development

Table S4: Networks associated with genes differentially methylated in adult mouse lung with decreased expression in adult mouse lung.

Molecules in Network	Score	Focus Molecules	Top Functions
Akt, Alpha catenin, CDH11, COL6A3, Collagen(s), CTNNA2, DGKI, E2F7, E2F8, ELN, ERK1/2, ERMAP, EXT1, KCNMA1, KIAA0101, Mbp, MMP19, NFkB (complex), PI3K (complex), PLAC1, PRDM8, PRRX1, PTPRB, PTX3, RNA polymerase II, RRM1, RRM2, SHISA2, SKP2, SOX9, Tmsb4x (includes others), TNC, Vegf, WIF1, Wnt	57	22	Organ morphology, reproductive system development and function, cancer
AHR, APP, AUTS2, BUD31, C1orf35, Ca ²⁺ , Camk, CaMK I, CAMK1G, CAMKK1, CCNB1IP1, CD8A, COL12A1, COL27A1, DCTD, DIDO1, EDNRA, EME1, GRB2, IFNG, MMP19, nitrate, Pdlim3, PTK2, RFC5, ROBO1, SETD3, SLC27A6, SPAG5, TTF2, UBC, UNC5C, ZC3HAV1L, ZFYVE26, ZNF536	31	14	Cellular development, cellular growth and proliferation, cell-to-cell signaling and interaction
CDKL5, LRRC4B, Ptprd	2	1	Cell-to-cell signaling and interaction, system development and function, hereditary disorder
HLA-B, JUNB, Raet1d/Raet1e	2	1	Cell death and survival, cell-to-cell signaling and interaction, cellular function and maintenance

Table S5: Genes with decreased DNA methylation and decreased mRNA expression in BPD lung compared to Preterm lung. (arranged in order of decreasing fold change; † Indicates genes with differential expression identified by multiple probe sets; % Me indicate methylation level of CpG locus)

Gene Symbol	Probe Name	Gene Title	Entrez Gene	Adj. P-value	Fold change	Location	% Me Preterm	% Me Term	% Me BPD	Location of CpG site
BAIAP2 †	207832_at	BAI1-associated protein 2	10458	3.96 E-02	0.87	cg00026327	0.72	0.58	0.59	S_Shelf
BAIAP2 †	1556145_at	BAI1-associated protein 2	10458	2.53 E-03	0.79	cg00026327	0.72	0.58	0.59	S_Shelf

Table S6: Genes with increased DNA methylation and increased mRNA expression in BPD lung compared to Preterm lung. (arranged in order of decreasing fold change; * Indicates genes with methylation in more than one locus; % Me values indicate methylation level of CpG locus)

Gene Symbol	Probe Name	Gene Title	Entrez Gene	Adj. P-value	Fold change	Location	% Me Preterm	% Me Term	% Me BPD	Location of CpG site
GFI1	206589_at	growth factor independent 1	2672	4.34 E-02	1.87	cg01766941	0.57	0.69	0.69	S_Shore
ANKRD55	220112_at	ankyrin repeat domain 55	79722	4.90 E-02	1.42	cg22709202	0.58	0.62	0.66	Body
TP53RK	225402_at	TP53 regulating kinase	112858	3.63 E-02	1.39	cg20598650	0.24	0.38	0.37	Body
NRP2*	223510_at	neuropilin 2	8828	3.85 E-02	1.32	cg10307632	0.57	0.69	0.69	Enhancer
NRP2*	223510_at	neuropilin 2	8828	3.85 E-02	1.32	cg19795793	0.54	0.73	0.73	S_Shore
CDH23	232845_at	cadherin-like	64072	1.98 E-02	1.30	cg15077792	0.36	0.47	0.48	Enhancer

		23								
TRP S1*	2242 18_s _at	tricho rhinop halan geal syndr ome I	7227	4.03 E-02	1.21	cg06 3685 90	0.06	0.15	0.16	Island
TRP S1*	2242 18_s _at	tricho rhinop halan geal syndr ome I	7227	4.03 E-02	1.21	cg16 8219 92	0.12	0.27	0.27	Island
TRP S1*	2242 18_s _at	tricho rhinop halan geal syndr ome I	7227	4.03 E-02	1.21	cg12 3965 23	0.07	0.13	0.11	N_Sho re
MY BPC 1	2140 87_s _at	myosi n bindin g protei n C, slow type	4604	6.98 E-03	1.14	cg21 7266 18	0.32	0.41	0.42	S_Shor e
PRD M16	2379 65_a t	PR domai n contai ning 16	6397 6	2.10 E-02	1.11	cg17 0332 87	0.22	0.33	0.33	Island

Table S7: Summary of CpG location site in relation to genes in BPD lung compared to Preterm lung

	Enhancer	N Shore	S Shore	Island	Body	S Shelf
Inverse direction of methylation and expression	CASZ1 DSCAML1 FLJ22536 KIAA1217 MYOM2 ZNF438	ACHE BMP7 GSTM3 KCNC3 KCNH2 NBEA NCALD NRG2 PHC1	ADCY1 EYA4 RYBP	BTC STXBP6	C13ORF26 PLEKHB1	EHD4
Same direction of methylation and expression	NRP2	TRPS1	GFI1 MYBPC1 NRP2	PRDM16 TRPS1	ANKRD55 TP53RK	BAIAP2

Table S8: Pathways associated with genes differentially methylated in BPD lung compared to Preterm or Term lung

Canonical Pathways (Methylated; 149)	-log(p-value)	Ratio	Molecules
Basal Cell Carcinoma Signaling	3.71E00	6.76E-02	PTCH1,BMP7,CTNNB1,BMP10,FZD7
Role of NANOG in Mammalian Embryonic Stem Cell Pluripotency	2.84E00	4.31E-02	BMP7,CTNNB1,BMP10,SALL4,FZD7
Human Embryonic Stem Cell Pluripotency	2.49E00	3.29E-02	BMP7,CTNNB1,BMP10,SALL4,FZD7
Factors Promoting Cardiogenesis in Vertebrates	2.38E00	4.3E-02	BMP7,CTNNB1,BMP10,FZD7
Cardiomyocyte Differentiation via BMP Receptors	2.06E00	9.52E-02	BMP7,BMP10
Wnt/ β -catenin Signaling	2.04E00	2.87E-02	CDH3,PPP2R2B,SOX11,CTNNB1,FZD7
Molecular Mechanisms of Cancer	1.86E00	1.89E-02	PTCH1,ADCY1,CDK6,BMP7,CTNNB1,BMP10,FZD7
Glioblastoma Multiforme Signaling	1.62E00	2.52E-02	IGF1R,CDK6,CTNNB1,FZD7
Role of Oct4 in Mammalian Embryonic Stem Cell Pluripotency	1.36E00	4.44E-02	PHC1,SALL4

Table S9: Gene sets significantly upregulated in BPD lung compared to Term or Preterm lung

Name	Size	P-value
HELLER_HDAC_TARGETS_SILENCED_BY_METHYLATION_UP	5	1.16E-02
KYNG_DNA_DAMAGE_UP	6	1.47E-02
FEVR_CTNNB1_TARGETS_UP	9	1.54E-02
HORIUCHI_WTAP_TARGETS_UP	5	2.00E-02
YOSHIMURA_MAPK8_TARGETS_DN	5	4.72E-02

Table S10: Gene sets significantly downregulated in BPD lung compared to Term or Preterm lung

Name	Size	P-val
CUI_TCF21_TARGETS_2_DN	7	2.91E-02
DURAND_STROMA_MAX_UP	5	2.73E-02
HELLER_HDAC_TARGETS_DN	5	7.65E-03
DUTERTRE ESTRADIOL_RESPONSE_24HR_DN	11	1.55E-02
RODRIGUES_THYROID_CARCINOMA_POORLY_DIFFERENTIATED_DN	7	3.21E-02
MIKKELSEN_MCV6_HCP_WITH_H3K27ME3	5	2.71E-02
SHETH_LIVER_CANCER_VS_TXNIP_LOSS_PAM4	6	1.31E-02

Table S11: Genes with increased methylation in Preterm lung compared to Term lung (arranged in order of decreasing P value); % Me indicates methylation level of CpG locus

Gene Symbol	Gene Title	Entrez Gene	P-value	FDR	Location	% Me Preterm	% Me Term
KCNIP1	Kv channel interacting protein 1	30820	6.87E-07	0.05597698	cg10931884	0.80	0.52
BAIAP2	BAI1-associated protein 2	10458	3.51E-06	0.079804137	cg00026327	0.72	0.58
SLC2A9	Solute carrier family 2 (facilitated glucose transporter), member 9	56606	2.60E-05	0.090852935	cg16785938	0.65	0.56
FRMD8	FERM domain containing 8	83786	3.12E-05	0.090852935	cg26186665	0.32	0.24
KRTAP5-8	keratin associated protein 5-8	57830	3.24E-05	0.090852935	cg00153942	0.53	0.47
C11orf94	chromosome 11 open reading frame 94	143678	3.30E-05	0.090852935	cg03135577	0.68	0.61
MYOM2	Myomesin (M-protein) 2, 165kDa	9172	3.47E-05	0.090852935	cg00295418	0.83	0.55
CARD14	Caspase recruitment domain family, member 14	79092	3.67E-05	0.090852935	cg21336650	0.73	0.62
ZNF438	Zinc finger protein 438	220929	5.00E-05	0.090852935	cg01656216	0.71	0.57
WDR86	WD repeat domain 86	349136	5.71E-05	0.090852935	cg01545109	0.88	0.81
CFHR5	Complement factor	81494	6.29E-05	0.09085293	cg26049187	0.89	0.83

	H-related 5			5			
GNRH1	Gonadotropin-releasing hormone 1 (luteinizing-releasing hormone)	2796	6.85E-05	0.090852935	cg17647884	0.91	0.89
NLRP5	NLR family, pyrin domain containing 5	126206	7.78E-05	0.090852935	cg15419294	0.90	0.87
TWF2	Twinfilin, actin-binding protein, homolog 2 (Drosophila)	11344	8.36E-05	0.090852935	cg16457413	0.81	0.77
MIR541	microRNA 541	100126308	9.29E-05	0.090852935	cg04638655	0.83	0.75
SNAP47	synaptosomal-associated protein, 47kDa	116841	9.88E-05	0.090852935	cg05007495	0.64	0.61
NEAT1	nuclear paraspeckle assembly transcript 1	283131	0.000101504	0.090852935	cg23463310	0.19	0.10
PSD	Pleckstrin and Sec7 domain containing	5662	0.000106512	0.090852935	cg14562559	0.60	0.55
KLHL29	Kelch-like 29 (Drosophila)	114818	0.000107379	0.090852935	cg17209692	0.92	0.89
KRCC1	Lysine-rich coiled-coil 1	51315	0.000119549	0.090852935	cg12691004	0.29	0.19
ZFYVE19	Zinc finger, FYVE domain containing 19	84936	0.000126975	0.090852935	cg03012280	0.91	0.89
CUEDC2	CUE domain containing 2	79004	0.000135044	0.092239781	cg10103942	0.88	0.85

PLEKHM 2	Pleckstrin homology domain containing, family M (with RUN domain) member 2	23207	0.000138 142	0.09304622 5	cg03339674	0.91	0.86
C13orf16	Chromosome 13 open reading frame 16	121793	0.000145 859	0.09428923 1	cg21308062	0.81	0.74
CPNE9	Copine family member IX	151835	0.000153 664	0.09520962 9	cg27185267	0.80	0.77
RHOBTB 1	Rho-related BTB domain containing 1	9886	0.000160 92	0.09520962 9	cg10157558	0.90	0.86
ASAP2	ArfGAP with SH3 domain, ankyrin repeat and PH domain 2	8853	0.000170 95	0.09520962 9	cg14019348	0.93	0.90
MS4A3	Membrane- spanning 4- domains, subfamily A, member 3 (hematopoietic cell-specific)	932	0.000172 114	0.09520962 9	cg14328641	0.75	0.71
MIR596	microRNA 596	693181	0.000174 242	0.09520962 9	cg11539066	0.89	0.72
CD6	CD6 molecule	923	0.000174 305	0.09520962 9	cg07380416	0.90	0.85
OC90	Otoconin 90	729330	0.000176 567	0.09520962 9	cg23399286	0.73	0.69
FRMD1	FERM domain containing 1	79981	0.000176 914	0.09520962 9	cg10107241	0.71	0.62
LPCAT1	lysophosphatidylch	79888	0.000179	0.09571874	cg11468635	0.78	0.69

	oline acyltransferase 1		985				
ALPI	Alkaline phosphatase, intestinal	248	0.000196 663	0.09712132 8	cg05007992	0.86	0.81
SH3BP4	SH3-domain binding protein 4	23677	0.000198 393	0.09734616	cg27577781	0.78	0.72

Table S12: Top 50 genes with increased methylation in Term lung compared to Preterm lung (arranged in order of decreasing P value); % Me indicates methylation level of CpG locus

Gene Symbol	Gene Title	Entrez Gene	P-value	FDR	Location	% Me Preterm	% Me Term
LRRC2	Leucine rich repeat containing 2	79442	4.74E-07	0.05597698	cg25821245	0.09	0.16
PPM1B	Protein phosphatase 1B (formerly 2C), magnesium-dependent, beta isoform	5495	6.06E-07	0.05597698	cg19769080	0.15	0.20
FAIM	Fas apoptotic inhibitory molecule	55179	1.11E-06	0.057558422	cg09429345	0.10	0.13
EYA4	Eyes absent homolog 4 (Drosophila)	2070	1.14E-06	0.057558422	cg08917489	0.21	0.27
DUSP5P	Dual specificity phosphatase 5 pseudogene	574029	1.41E-06	0.057558422	cg26859841	0.15	0.19
PRDM16	PR domain containing 16	63976	1.88E-06	0.068207794	cg08642716	0.51	0.60
HDAC4	Histone deacetylase 4	9759	2.63E-06	0.079804137	cg18121901	0.44	0.74
PLAT	Plasminogen activator, tissue	5327	2.93E-06	0.079804137	cg13880167	0.78	0.87
NTNG1	Netrin G1	22854	2.98E-06	0.079804137	cg07005523	0.03	0.06
MXRA7	Matrix-remodelling associated 7	439921	4.33E-06	0.079804137	cg15052447	0.14	0.23
NFE2L3	Nuclear factor (erythroid-derived 2)-like 3	9603	4.96E-06	0.079804137	cg21699330	0.32	0.53
SOX10	SRY (sex determining	6663	5.04E-	0.079804137	cg06614002	0.64	0.72

	region Y)-box 10		06				
GABBR1	Gamma-aminobutyric acid (GABA) B receptor, 1	2550	5.30E-06	0.079804137	cg08093277	0.25	0.35
RASA3	RAS p21 protein activator 3	22821	5.34E-06	0.079804137	cg27086157	0.32	0.73
CCDC42B	coiled-coil domain containing 42B	387885	6.39E-06	0.084276744	cg15033653	0.04	0.09
MIRLET7BHG	MIRLET7B host gene	400931	6.46E-06	0.084276744	cg03984375	0.18	0.30
NR4A2	Nuclear receptor subfamily 4, group A, member 2	4929	6.73E-06	0.084276744	cg00558219	0.76	0.86
ZNF665	Zinc finger protein 665	79788	8.20E-06	0.084435167	cg12360029	0.10	0.14
MYBPC1	Myosin binding protein C, slow type	4604	9.12E-06	0.084435167	cg21726618	0.32	0.41
LECT1	Leukocyte cell derived chemotaxin 1	11061	9.19E-06	0.084435167	cg00345862	0.19	0.24
IQSEC1	IQ motif and Sec7 domain 1	9922	9.62E-06	0.084435167	cg17696731	0.34	0.52
MSI1	Musashi homolog 1 (Drosophila)	4440	9.62E-06	0.084435167	cg26008908	0.07	0.15
PRR5	Proline rich 5 (renal)	55615	9.67E-06	0.084435167	cg22778120	0.42	0.59
FGF11	Fibroblast growth factor 11	2256	9.82E-06	0.084435167	cg03613649	0.33	0.45
PTCH1	Patched homolog 1 (Drosophila)	5727	9.85E-06	0.084435167	cg14323293	0.19	0.29
TMEM51	Transmembrane protein 51	55092	1.11E-05	0.086651287	cg25469418	0.45	0.58
GLI3	GLI-Kruppel family member GLI3 (Greig	2737	1.14E-05	0.086651287	cg24497732	0.23	0.34

	cephalopolysyndactyly syndrome)						
SCG5	Secretogranin V (7B2 protein)	6447	1.17E-05	0.086651287	cg12134633	0.06	0.19
C1QTNF6	C1q and tumor necrosis factor related protein 6	114904	1.19E-05	0.086651287	cg20979921	0.20	0.30
GPR98	G protein-coupled receptor 98	84059	1.26E-05	0.086651287	cg21998208	0.18	0.23
PCDHB10	Protocadherin beta 10	56126	1.31E-05	0.086651287	cg18131851	0.05	0.07
FHL2	Four and a half LIM domains 2	2274	1.31E-05	0.086651287	cg26344233	0.03	0.06
TRIO	Triple functional domain (PTPRF interacting)	7204	1.41E-05	0.086651287	cg09086037	0.73	0.86
UBAP2L	Ubiquitin associated protein 2-like	9898	1.42E-05	0.086651287	cg14859874	0.04	0.56
GAS2	growth arrest-specific 2 [Homo sapiens	2620	1.43E-05	0.086651287	cg12575928	0.22	0.30
NRXN2	Neurexin 2	9379	1.44E-05	0.086651287	cg17491228	0.65	0.75
PLEKHB1	Pleckstrin homology domain containing, family B (evectins) member 1	58473	1.44E-05	0.086651287	cg25288155	0.27	0.36
GPHA2	Glycoprotein hormone alpha 2	170589	1.47E-05	0.087107329	cg12374682	0.17	0.26
IL17D	Interleukin 17D	53342	1.52E-05	0.087421955	cg12081946	0.09	0.14
ZIC1	Zic family member 1 (odd-paired homolog, Drosophila)	7545	1.56E-05	0.087421955	cg21571166	0.14	0.19

ALS2CR4	Amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 4	65062	1.58E-05	0.087421955	cg12544106	0.11	0.15
ANKRD39	ankyrin repeat domain 39	51239	1.63E-05	0.088424968	cg20091689	0.12	0.23
KDM2B	lysine (K)-specific demethylase 2B	84678	1.66E-05	0.088424968	cg07793808	0.13	0.22
KSR2	Kinase suppressor of ras 2	283455	1.69E-05	0.089018757	cg11009362	0.11	0.18
BTBD11	BTB (POZ) domain containing 11	121551	1.77E-05	0.089834719	cg01478234	0.08	0.26
TRIM31	Tripartite motif-containing 31	11074	1.83E-05	0.089834719	cg19940194	0.82	0.88
TRMU	TRNA 5-methylaminomethyl-2-thiouridylate methyltransferase	55687	1.85E-05	0.089834719	cg27032184	0.13	0.20
MAD1L1	MAD1 mitotic arrest deficient-like 1 (yeast)	8379	1.93E-05	0.090852935	cg22460466	0.65	0.75
PBX1	Pre-B-cell leukemia homeobox 1	5087	2.02E-05	0.090852935	cg20682146	0.19	0.29
LARS2	Leucyl-tRNA synthetase 2, mitochondrial	23395	2.05E-05	0.090852935	cg03621566	0.05	0.06

Table S13: Genes differentially methylated in Preterm compared to Term lung with methylation in more than one locus. * indicates gene with locus that had increased methylation in Preterm compared to Term lung. % Me indicates methylation level of CpG locus

Gene symbol	Gene title	Entrez ID	p EvsL	fdr EvsL	Location	% Me Preterm	% Me Term
ANGPT2	Angiopoietin 2	285	2.30E-05	0.090853	cg20490392	0.39	0.53
ANGPT2	Angiopoietin 2	285	2.61E-05	0.090853	cg04017131	0.31	0.45
ANGPT2	Angiopoietin 2	285	6.54E-05	0.090853	cg20460488	0.36	0.45
C10orf41	Chromosome 10 open reading frame 41	283065	6.42E-05	0.090853	cg19057830	0.17	0.28
C10orf41	Chromosome 10 open reading frame 41	283065	0.000117	0.090853	cg15182635	0.05	0.10
C13orf26	Chromosome 13 open reading frame 26	122046	0.000106	0.090853	cg13614409	0.07	0.14
C13orf26 (aka TEX26)	Chromosome 13 open reading frame 26	122046	0.000205	0.099216	cg16290689	0.18	0.29
C22orf9	Chromosome 22 open reading frame 9	23313	7.10E-05	0.090853	cg04223044	0.21	0.34
C22orf9	Chromosome 22 open reading frame 9	23313	0.000103	0.090853	cg22488367	0.18	0.35
CCDC42 B	coiled-coil domain containing 42B	387885	6.39E-06	0.084277	cg15033653	0.04	0.09
CCDC42 B	coiled-coil domain containing 42B	387885	8.86E-05	0.090853	cg03393040	0.07	0.16
CPNE9*	Copine family member IX	151835	0.000154	0.09521	cg27185267	0.80	0.77
CPNE9	Copine family member IX	151835	0.000129691	0.090852935	cg16452224	0.08	0.13
GABBR1	Gamma-aminobutyric acid (GABA) B receptor, 1	2550	5.30E-06	0.079804	cg08093277	0.25	0.35
GABBR1	Gamma-aminobutyric	2550	3.64E-05	0.090853	cg08862148	0.19	0.26

	acid (GABA) B receptor, 1						
GAS2	growth arrest-specific 2	2620	1.43E-05	0.086651	cg12575928	0.22	0.30
GAS2	growth arrest-specific 2	2620	9.26E-05	0.090853	cg15585294	0.15	0.25
GLI3	GLI-Kruppel family member GLI3 (Greig cephalopolysyndactyly syndrome)	2737	1.14E-05	0.086651	cg24497732	0.23	0.34
GLI3	GLI-Kruppel family member GLI3 (Greig cephalopolysyndactyly syndrome)	2737	8.85E-05	0.090853	cg17588800	0.23	0.35
HLA-DPB1	Major histocompatibility complex, class II, DP beta 1	3115	0.000112	0.090853	cg10850215	0.09	0.26
HLA-DPB1	Major histocompatibility complex, class II, DP beta 1	3115	0.000113	0.090853	cg17588455	0.14	0.28
HMGA2	High mobility group AT-hook 2	8091	3.35E-05	0.090853	cg20088964	0.12	0.17
HMGA2	High mobility group AT-hook 2	8091	0.000193	0.096635	cg25161912	0.55	0.72
IL17REL	interleukin 17 receptor E-like	400935	2.82E-05	0.090853	cg03721641	0.25	0.41
IL17REL	interleukin 17 receptor E-like	400935	7.93E-05	0.090853	cg26206185	0.20	0.30
IQSEC1	IQ motif and Sec7 domain 1	9922	9.62E-06	0.084435	cg17696731	0.34	0.52
IQSEC1	IQ motif and Sec7	9922	0.00011	0.090853	cg08102294	0.46	0.59

	domain 1		9				
IQSEC1	IQ motif and Sec7 domain 1	9922	0.000173	0.09521	cg26766694	0.27	0.40
KDM2B	lysine (K)-specific demethylase 2B	84678	1.66E-05	0.088425	cg07793808	0.13	0.22
KDM2B	lysine (K)-specific demethylase 2B	84678	0.00012	0.090853	cg15234492	0.25	0.38
LOC100302652 aka GPR75	GPR75-ASB3 readthrough	100302652	3.82E-05	0.090853	cg13356253	0.47	0.63
LOC100302652 aka GPR75	GPR75-ASB3 readthrough	100302652	6.88E-05	0.090853	cg19878627	0.52	0.68
LOC100302652 aka GPR75	GPR75-ASB3 readthrough	100302652	0.000136	0.092271	cg00701946	0.31	0.51
LOC153684	Hypothetical protein LOC153684	153684	7.19E-05	0.090853	cg23435915	0.24	0.39
LOC153684	Hypothetical protein LOC153684	153684	8.62E-05	0.090853	cg18254356	0.21	0.38
LOC401097	Similar to LOC166075	401097	4.19E-05	0.090853	cg13036381	0.12	0.26
LOC401097	Similar to LOC166075	401097	8.60E-05	0.090853	cg03281561	0.11	0.17
MBP	Myelin basic protein	4155	5.10E-05	0.090853	cg07807210	0.53	0.67
MBP	myelin basic protein	4155	9.82E-05	0.090853	cg11598403	0.24	0.43
MBP	myelin basic protein	4155	0.000142	0.093556	cg17266581	0.20	0.40
MYOM2 *	Myomesin (M-protein) 2, 165kDa	9172	3.47E-05	0.090853	cg00295418	0.83	0.55

MYOM2	Myomesin (M-protein) 2, 165kDa	9172	4.32E-05	0.090853	cg05241134	0.18	0.28
NR4A2	Nuclear receptor subfamily 4, group A, member 2	4929	6.73E-06	0.084277	cg00558219	0.76	0.86
NR4A2	Nuclear receptor subfamily 4, group A, member 2	4929	4.64E-05	0.090853	cg00194126	0.12	0.30
NR4A2	Nuclear receptor subfamily 4, group A, member 2	4929	5.73E-05	0.090853	cg03339537	0.48	0.66
NR4A2	Nuclear receptor subfamily 4, group A, member 2	4929	7.47E-05	0.090853	cg00426720	0.37	0.48
NR4A2	Nuclear receptor subfamily 4, group A, member 2	4929	0.000146	0.094289	cg10089963	0.47	0.65
NR4A2	Nuclear receptor subfamily 4, group A, member 2	4929	0.000167	0.09521	cg07516970	0.73	0.85
NR4A2	Nuclear receptor subfamily 4, group A, member 2	4929	0.000181	0.095719	cg00240195	0.41	0.60
NRG2	Neuregulin 2	9542	3.68E-05	0.090853	cg19583819	0.27	0.41
NRG2	Neuregulin 2	9542	9.34E-05	0.090853	cg24897141	0.16	0.27
PLEKHB1	Pleckstrin homology domain containing, family B (evectins) member 1	58473	1.44E-05	0.086651	cg25288155	0.27	0.36
PLEKHB1	Pleckstrin homology domain containing, family B (evectins) member 1	58473	0.00012	0.090853	cg11512608	0.34	0.46

PRPH	Peripherin	5630	7.32E-05	0.090853	cg09595479	0.10	0.20
PRPH	Peripherin	5630	0.00012 1	0.090853	cg16205352	0.45	0.62
RPH3AL	Rabphilin 3A-like (without C2 domains)	9501	4.11E-05	0.090853	cg13532547	0.16	0.24
RPH3AL	Rabphilin 3A-like (without C2 domains)	9501	9.22E-05	0.090853	cg25513659	0.06	0.11
RUNX3	Runt-related transcription factor 3	864	5.03E-05	0.090853	cg24006721	0.14	0.24
RUNX3	Runt-related transcription factor 3	864	9.66E-05	0.090853	cg24019564	0.15	0.20
RUNX3	runt-related transcription factor 3	864	0.00012 6	0.090853	cg05162523	0.31	0.51
RYBP	RING1 and YY1 binding protein	23429	3.90E-05	0.090853	cg06759518	0.17	0.26
RYBP	RING1 and YY1 binding protein	23429	5.39E-05	0.090853	cg16990174	0.20	0.28
SALL4	Sal-like 4 (Drosophila)	57167	0.00016 9	0.09521	cg19871388	0.10	0.19
SALL4	Sal-like 4 (Drosophila)	57167	0.00017 6	0.09521	cg18900591	0.26	0.44
SKAP1	Src kinase associated phosphoprotein 1	8631	4.85E-05	0.090853	cg24636368	0.13	0.27
SKAP1	Src kinase associated phosphoprotein 1	8631	6.44E-05	0.090853	cg00582562	0.16	0.29
SLC43A 1	Solute carrier family 43, member 1	8501	2.78E-05	0.090853	cg17330838	0.09	0.24
SLC43A 1	Solute carrier family 43, member 1	8501	8.31E-05	0.090853	cg23075364	0.07	0.18
SPEF2	sperm flagellar 2	79925	0.00015 3	0.09521	cg24675098	0.06	0.14
SPEF2	sperm flagellar 2	79925	0.00016 4	0.09521	cg24892139	0.05	0.17

TET1	tet methylcytosine dioxygenase 1	80312	3.90E-05	0.090853	cg19127638	0.48	0.66
TET1	tet methylcytosine dioxygenase 1	80312	0.000168	0.09521	cg17817532	0.24	0.39
TLE3	Transducin-like enhancer of split 3 (E(sp1) homolog, Drosophila)	7090	8.20E-05	0.090853	cg21245875	0.24	0.41
TLE3	transducin-like enhancer of split 3	7090	0.000171	0.09521	cg22537334	0.36	0.59
TRIM15	Tripartite motif-containing 15	89870	6.58E-05	0.090853	cg15671996	0.40	0.54
TRIM15	Tripartite motif-containing 15	89870	0.000113	0.090853	cg05563595	0.13	0.19
TRIM31	Tripartite motif-containing 31	11074	1.83E-05	0.089835	cg19940194	0.82	0.88
TRIM31	Tripartite motif-containing 31	11074	0.000171	0.09521	cg00787996	0.42	0.64
TRIO	Triple functional domain (PTPRF interacting)	7204	1.41E-05	0.086651	cg09086037	0.73	0.86
TRIO	Triple functional domain (PTPRF interacting)	7204	0.000127	0.090853	cg16694455	0.88	0.90
WDFY4	WDFY family member 4	57705	5.70E-05	0.090853	cg23837220	0.16	0.28
WDFY4	WDFY family member 4	57705	9.48E-05	0.090852935	cg11509088	0.19	0.31

Table S14: Pathway analysis for differentially methylated genes, and overlap between mouse and human lung. Ingenuity Pathway Analysis (IPA) identified (at $p < 0.05$) 16 pathways involving 231 unique genes differentially methylated in mouse lung, and 7 pathways involving 462 unique genes differentially methylated in human lung. Three pathways were common to both mouse and human lung DNA methylation analyses (marked by *).

Mouse Pathway Analysis	Human Pathway Analysis
Hepatic Fibrosis / Hepatic Stellate Cell Activation	Basal Cell Carcinoma Signaling*
T Helper Cell Differentiation	Wnt/a-catenin Signaling
Molecular Mechanisms of Cancer	Sonic Hedgehog Signaling*
Interferon Signaling	Axonal Guidance Signaling*
Basal Cell Carcinoma Signaling*	Glycine Biosynthesis III
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	GDP-L-fucose Biosynthesis I (from GDP-D-mannose)
Heparan Sulfate Biosynthesis (Late Stages)	RhoA Signaling
Axonal Guidance Signaling*	
S-methyl-5-thio-b-D-ribose 1-phosphate Degradation	
Pyrimidine Deoxyribonucleotides De Novo Biosynthesis I	
Heparan Sulfate Biosynthesis	
Role of JAK1, JAK2 and TYK2 in Interferon Signaling	
Sonic Hedgehog Signaling*	
RAR Activation	
Heme Biosynthesis from Uroporphyrinogen-III I	
Retinoate Biosynthesis I	

Figure Legends:

Figure S1: Validation of DNA methylation of MMP3 (increased in newborn compared to adult) and TNC (increased in adult compared to newborn) by bisulfite conversion, PCR, and sequencing.

Figure S2: Validation of DNA methylation of TMCC3 (increased in newborn compared to adult) and DCTD (increased in adult compared to newborn) by pyrosequencing using Qiagen's Pyromark CpG Assay

Figure S3: Graphical representation of Network 1 from Table S3 associated with genes differentially methylated in newborn mouse lung with decreased expression in newborn mouse lung (Direct interaction: solid line; Indirect interaction: dashed line; magnitude of change in expression corresponds to intensity of shading of molecule).

Figure S4: Graphical representation of Network 2 from Table S3 associated with genes differentially methylated in newborn mouse lung with decreased expression in newborn mouse lung (Direct interaction: solid line; Indirect interaction: dashed line; magnitude of change in expression corresponds to intensity of shading of molecule).

Figure S5: Graphical representation of Network 3 from Table S3 associated with genes differentially methylated in newborn mouse lung with decreased expression in newborn mouse lung (Direct interaction: solid line; Indirect interaction: dashed line; magnitude of change in expression corresponds to intensity of shading of molecule).

Figure S6: Graphical representation of Network 4 from Table S3 associated with genes differentially methylated in newborn mouse lung with decreased expression in newborn mouse lung (Direct interaction: solid line; Indirect interaction: dashed line; magnitude of change in expression corresponds to intensity of shading of molecule).

Figure S7: Graphical representation of Network 5 from Table S3 associated with genes differentially methylated in newborn mouse lung with decreased expression in newborn mouse lung (Direct interaction: solid line; Indirect interaction: dashed line; magnitude of change in expression corresponds to intensity of shading of molecule).

Figure S8: Graphical representation of Network 6 from Table S3 associated with genes differentially methylated in newborn mouse lung with decreased expression in newborn mouse lung (Direct interaction: solid line; Indirect interaction: dashed line; magnitude of change in expression corresponds to intensity of shading of molecule).

Figure S9: Graphical representation of Network 1 from Table S4 associated with genes differentially methylated in adult mouse lung with decreased expression in adult mouse lung. (Direct interaction: solid line; Indirect interaction: dashed line; magnitude of change in expression corresponds to intensity of shading of molecule).

Figure S10: Graphical representation of Network 2 from Table S4 associated with genes differentially methylated in adult mouse lung with decreased expression in adult mouse lung. (Direct interaction: solid line; Indirect interaction: dashed line; magnitude of change in expression corresponds to intensity of shading of molecule).

Figure S11: Graphical representation of Network 3 from Table S4 associated with genes differentially methylated in adult mouse lung with decreased expression in adult mouse lung. (Direct interaction: solid line; Indirect interaction: dashed line; magnitude of change in expression corresponds to intensity of shading of molecule).

Figure S12: Graphical representation of Network 4 from Table S4 associated with genes differentially methylated in adult mouse lung with decreased expression in adult mouse lung. (Direct interaction: solid line; Indirect interaction: dashed line; magnitude of change in expression corresponds to intensity of shading of molecule).

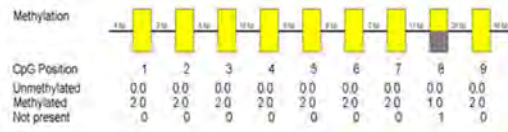
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- E6. Schulz MH, Pandit KV, Lino Cardenas CL, Ambalavanan N, Kaminski N, Bar-Joseph Z. Reconstructing dynamic microRNA-regulated interaction networks. *Proc Natl Acad Sci U S A* 2013;110:15686-15691.

Bisulfite conversion, PCR, and sequencing

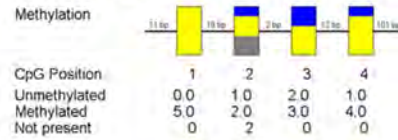
Increased methylation in newborn

MMP3 Newborn

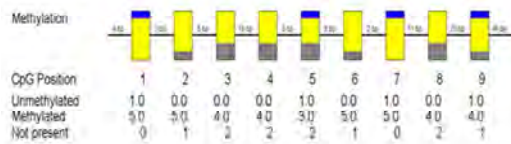


Increased methylation in adult

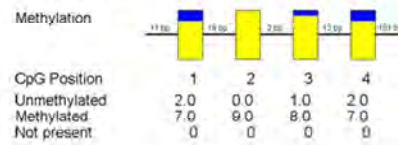
TNC Newborn



MMP3 Adult

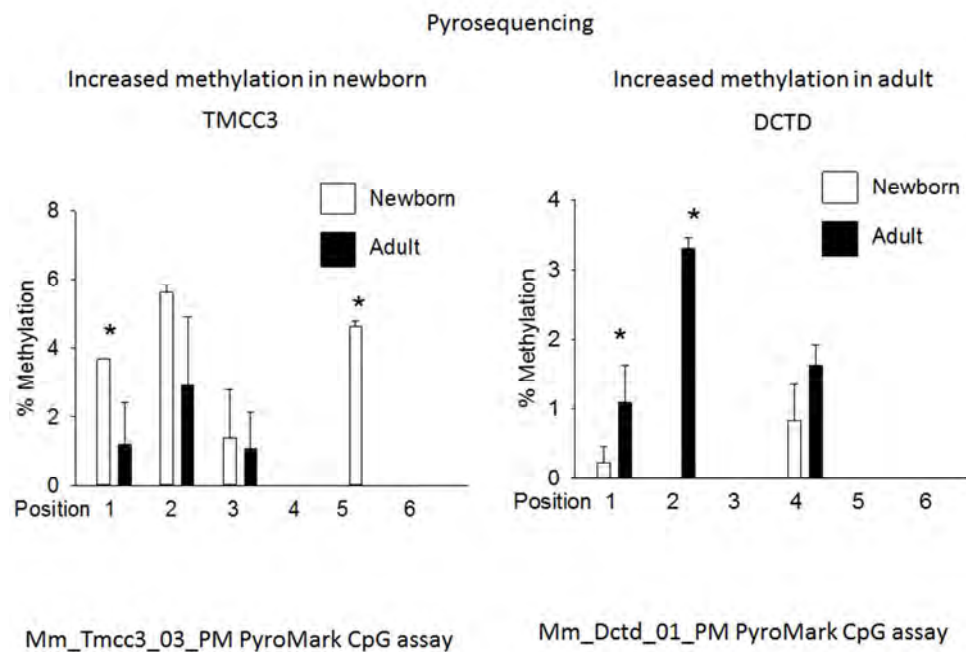


TNC Adult

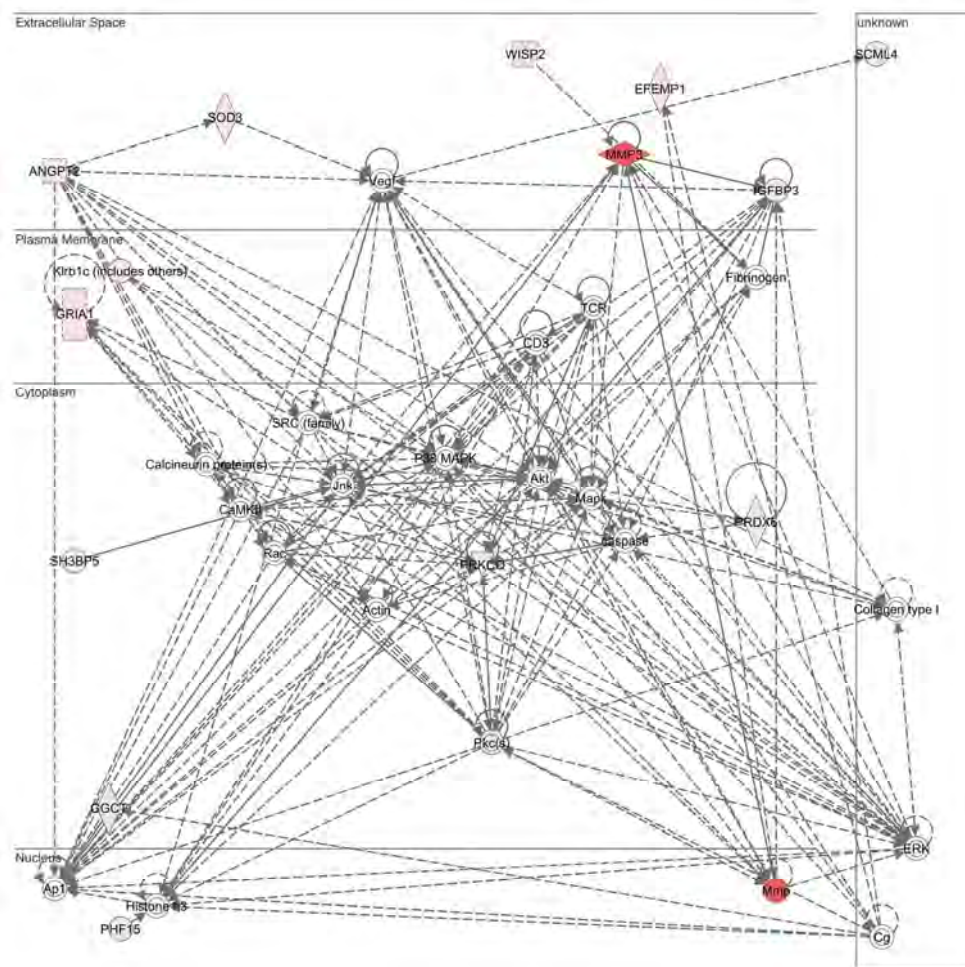


Legend: ■ unmethylated ■ methylated ■ not present

Validation of DNA methylation of MMP3 (increased in newborn compared to adult) and TNC (increased in adult compared to newborn) by bisulfite conversion, PCR, and sequencing
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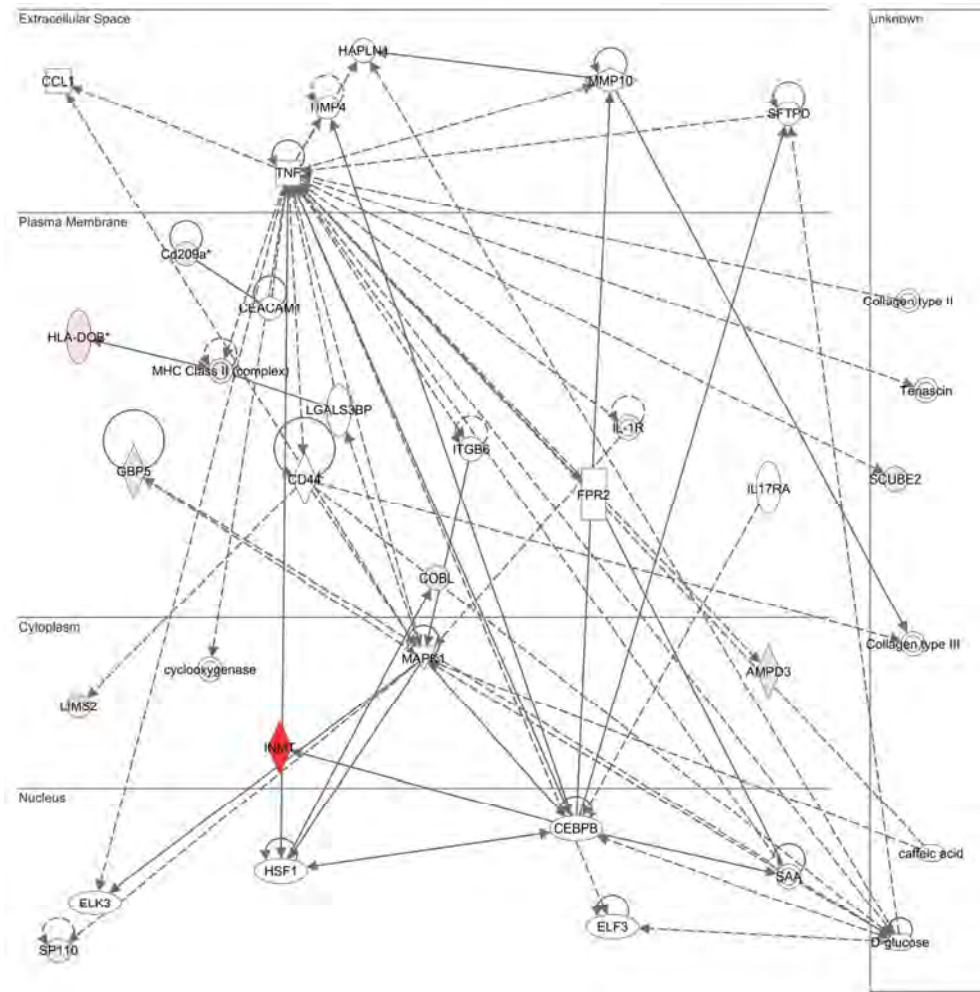


Validation of DNA methylation of TMCC3 (increased in newborn compared to adult) and DCTD (increased in adult compared to newborn) by pyrosequencing using Qiagen's Pyromark CpG Assay
78x53mm (300 x 300 DPI)



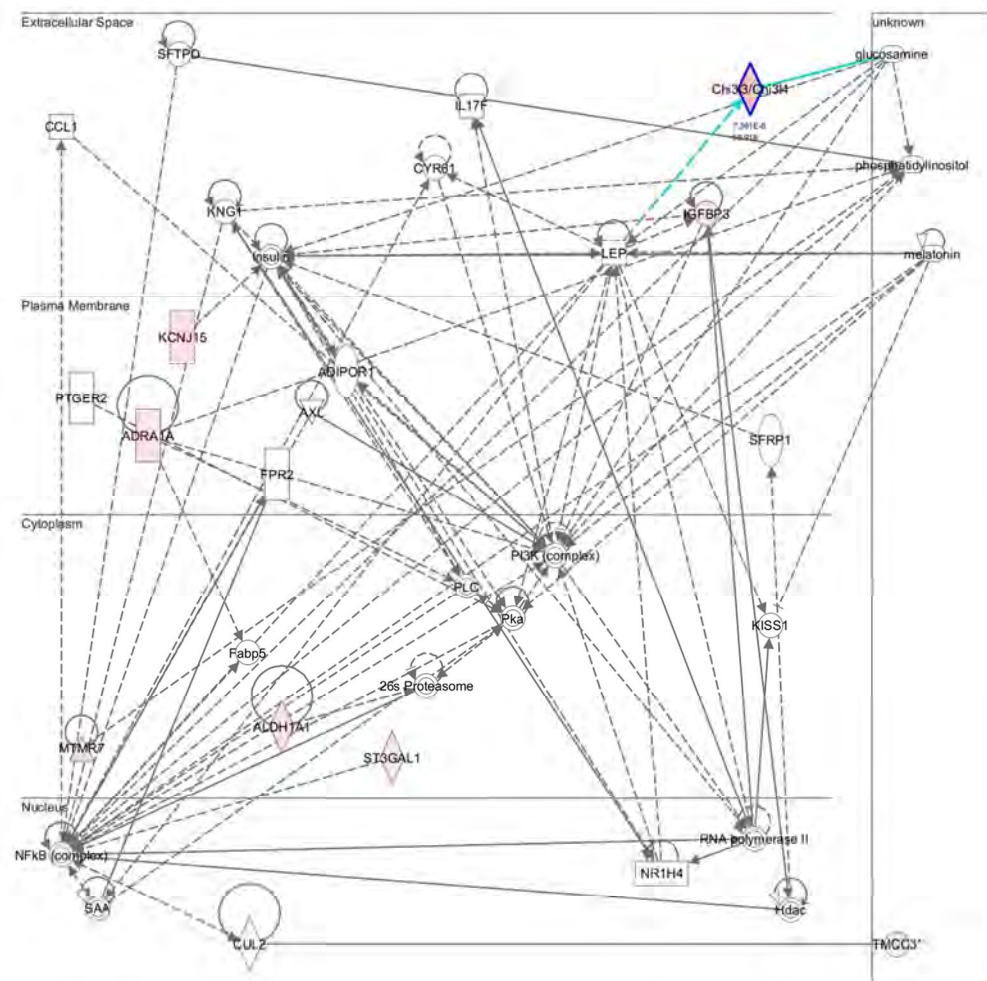
Graphical representation of Network 1 from Table S3 associated with genes differentially methylated in newborn mouse lung with decreased expression in newborn mouse lung (Direct interaction: solid line; Indirect interaction: dashed line; magnitude of change in expression corresponds to intensity of shading of molecule)
135x132mm (300 x 300 DPI)

135x133mm (300 x 300 DPI)



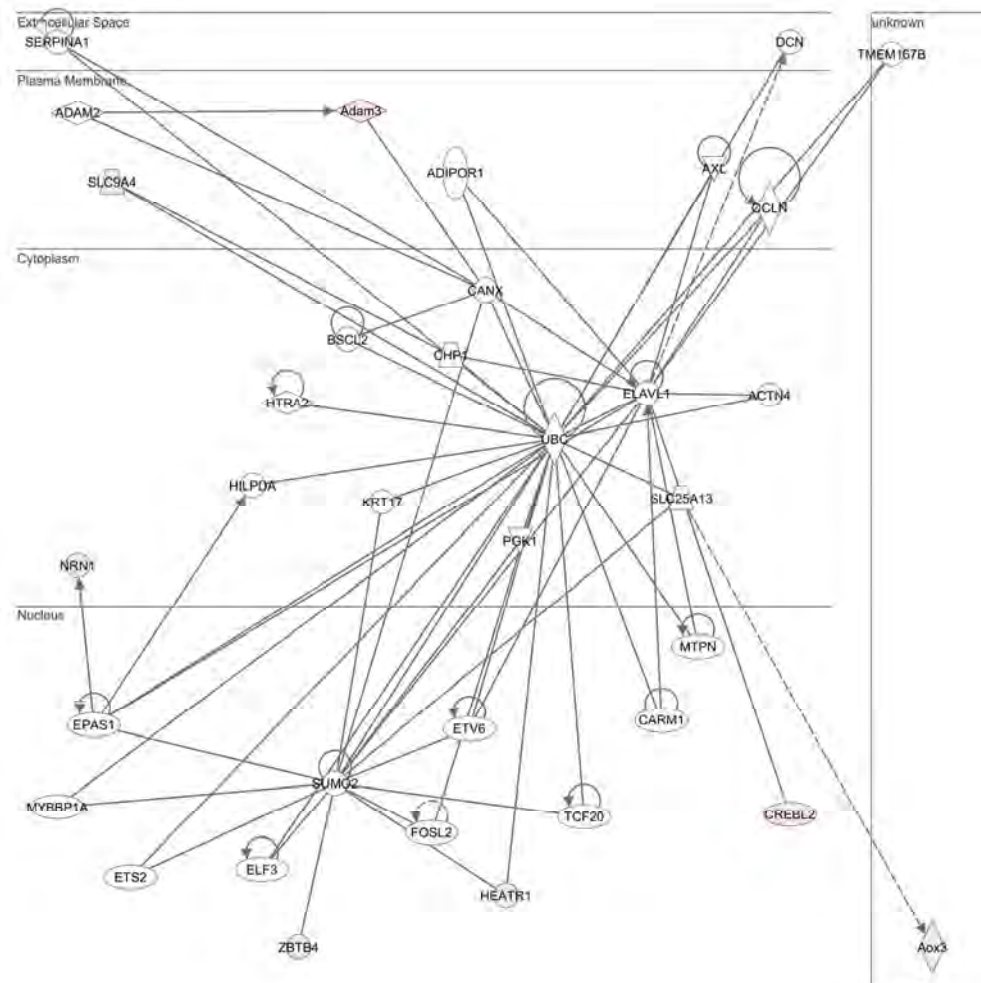
Graphical representation of Network 3 from Table S3 associated with genes differentially methylated in newborn mouse lung with decreased expression in newborn mouse lung (Direct interaction: solid line; Indirect interaction: dashed line; magnitude of change in expression corresponds to intensity of shading of molecule).

135x135mm (300 x 300 DPI)



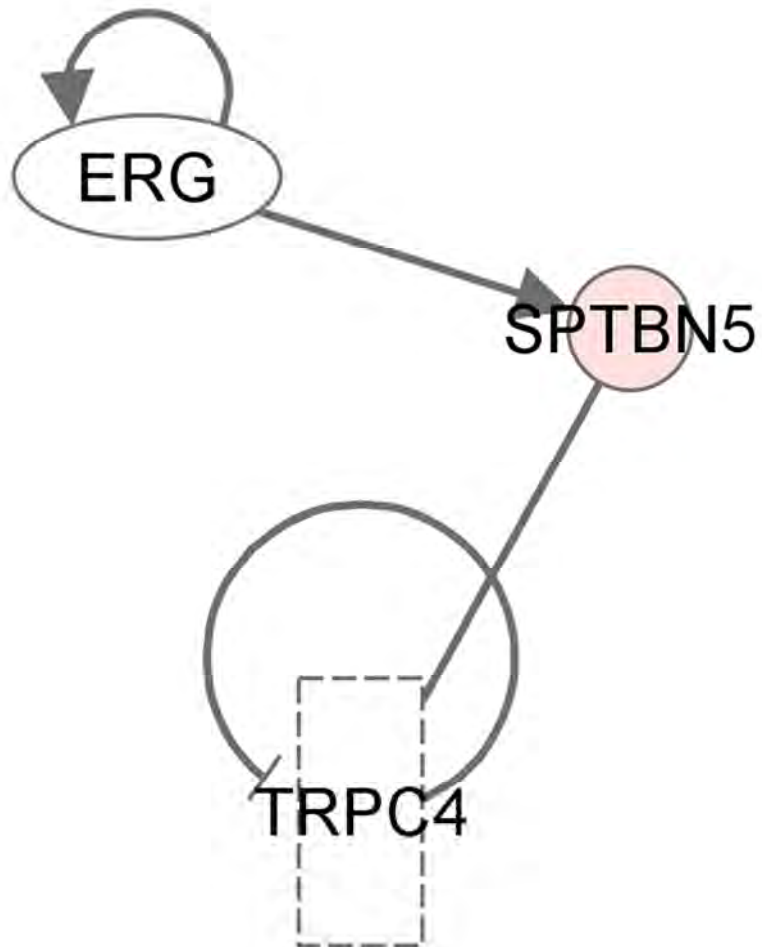
Graphical representation of Network 4 from Table S3 associated with genes differentially methylated in newborn mouse lung with decreased expression in newborn mouse lung (Direct interaction: solid line; Indirect interaction: dashed line; magnitude of change in expression corresponds to intensity of shading of molecule).

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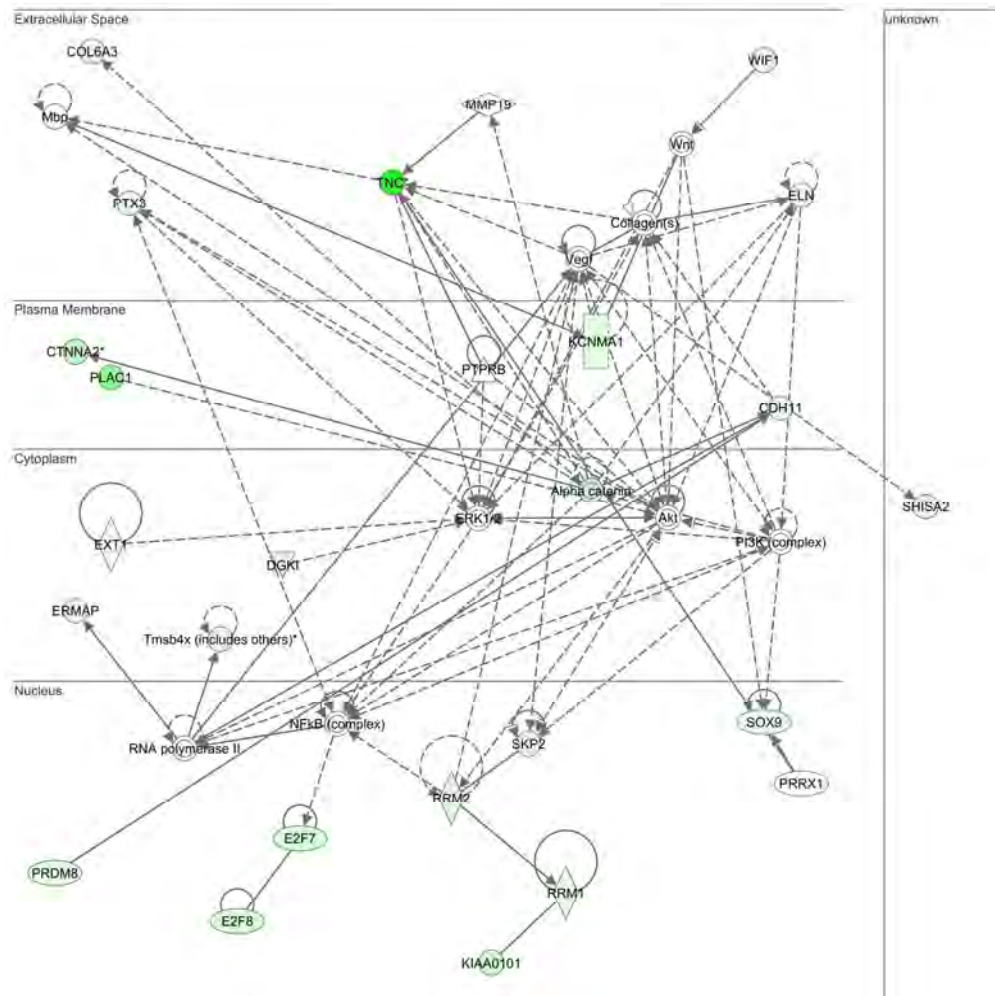
Graphical representation of Network 5 from Table S3 associated with genes differentially methylated in newborn mouse lung with decreased expression in newborn mouse lung (Direct interaction: solid line; Indirect interaction: dashed line; magnitude of change in expression corresponds to intensity of shading of molecule).

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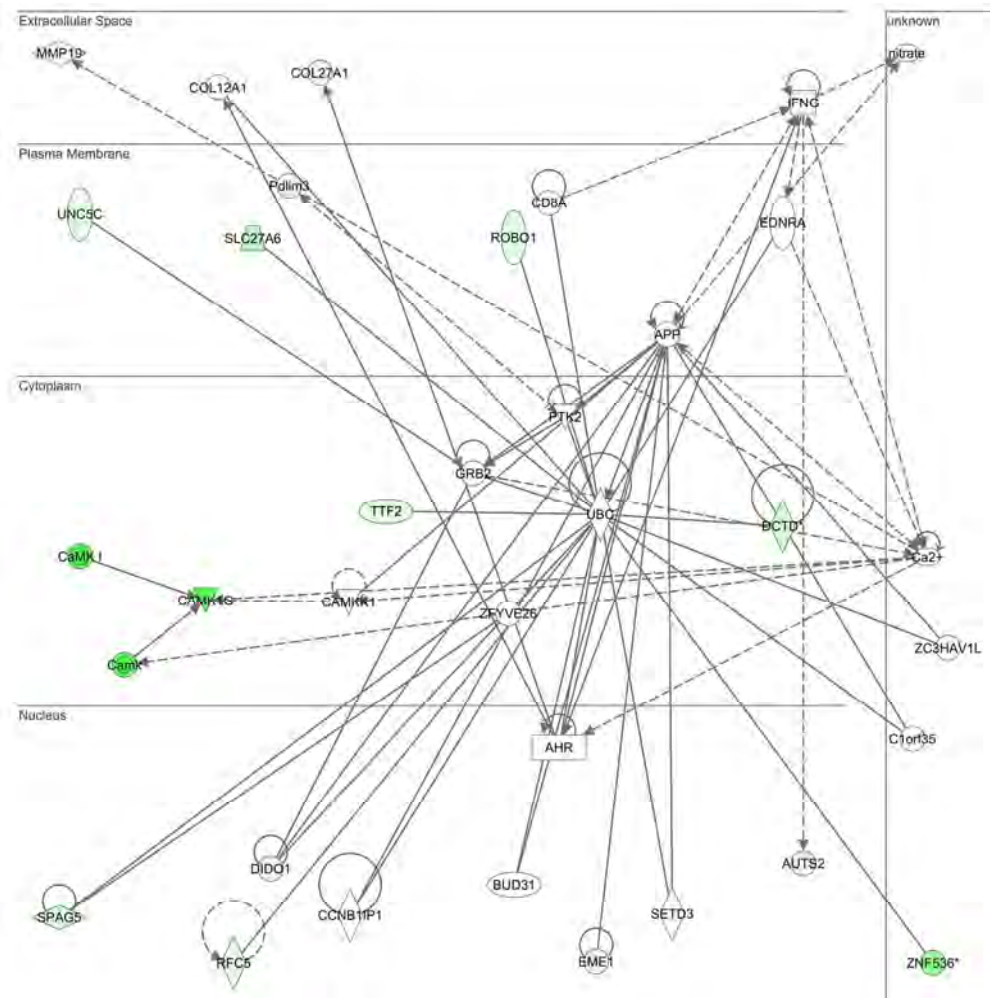
Graphical representation of Network 6 from Table S3 associated with genes differentially methylated in newborn mouse lung with decreased expression in newborn mouse lung (Direct interaction: solid line; Indirect interaction: dashed line; magnitude of change in expression corresponds to intensity of shading of molecule).

135x144mm (300 x 300 DPI)



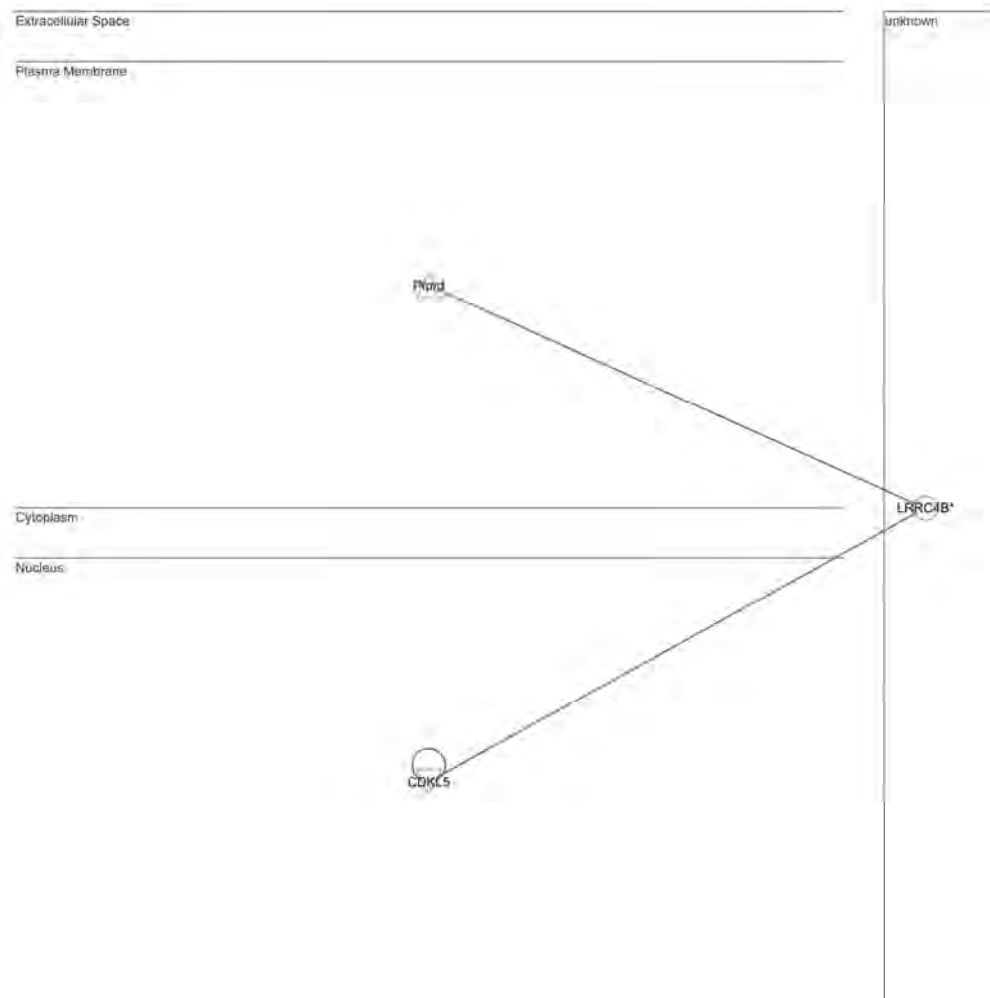
Graphical representation of Network 1 from Table S4 associated with genes differentially methylated in adult mouse lung with decreased expression in adult mouse lung. (Direct interaction: solid line; Indirect interaction: dashed line; magnitude of change in expression corresponds to intensity of shading of molecule).

135x134mm (300 x 300 DPI)



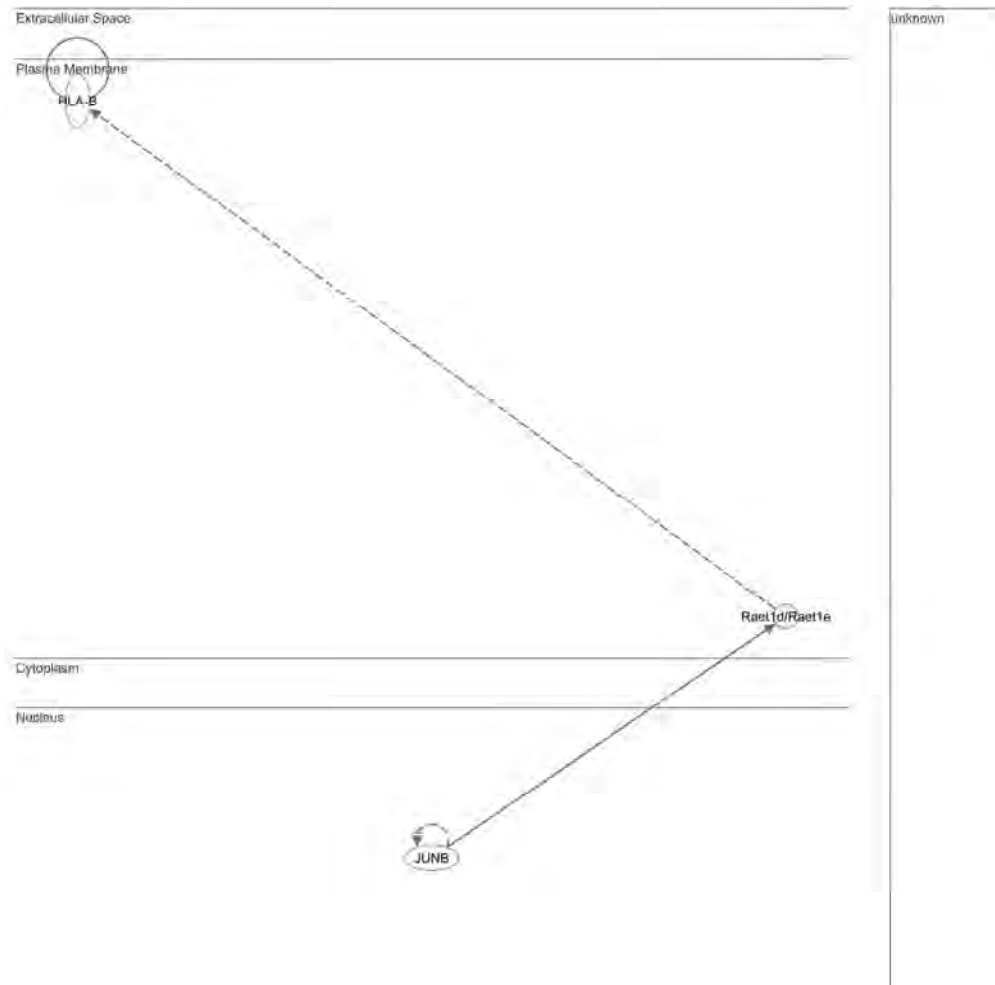
Graphical representation of Network 2 from Table S4 associated with genes differentially methylated in adult mouse lung with decreased expression in adult mouse lung. (Direct interaction: solid line; Indirect interaction: dashed line; magnitude of change in expression corresponds to intensity of shading of molecule).

135x135mm (300 x 300 DPI)



Graphical representation of Network 3 from Table S4 associated with genes differentially methylated in adult mouse lung with decreased expression in adult mouse lung. (Direct interaction: solid line; Indirect interaction: dashed line; magnitude of change in expression corresponds to intensity of shading of molecule).

135x135mm (300 x 300 DPI)



Graphical representation of Network 4 from Table S4 associated with genes differentially methylated in adult mouse lung with decreased expression in adult mouse lung. (Direct interaction: solid line; Indirect interaction: dashed line; magnitude of change in expression corresponds to intensity of shading of molecule).

135x133mm (300 x 300 DPI)