Supplementary Materials for

DNA methylation in lung cells is a key modulator of asthma endotypes and genetic risk

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This PDF files includes:

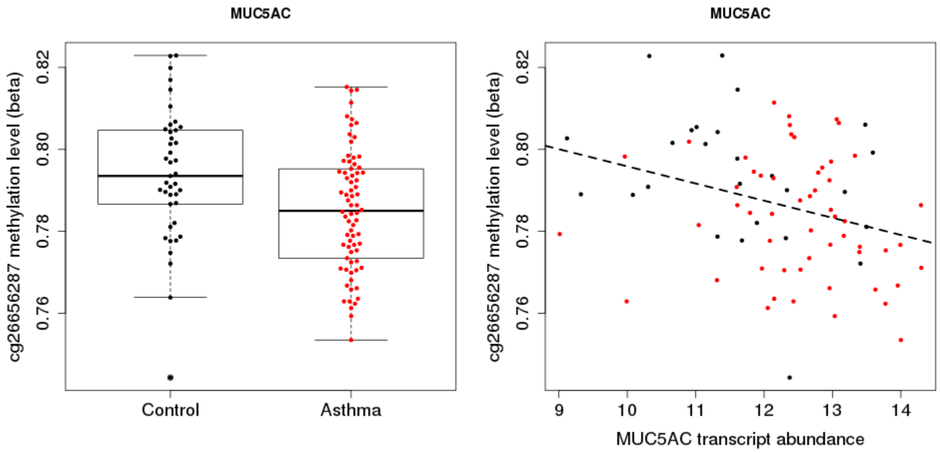
- Figs. S1 to S7
- Table S1
- Captions for Table S1

Other Supplementary Materials:

Additional Data Tables S1-S6

Supplementary Figures

Figure S1. Differentially methylated CpGs (DMCs) at asthma candidate genes. (a) Boxplots showing methylation levels at a CpG site (cg26656287) upstream of the transcription start site of *MUC5AC* (P = 0.0018), a mucin required for airway defense against foreign particulates and pathogens. (b) Scatter plot showing the correlation between *MUC5AC* transcript abundance and cg11303839 methylation levels (x-axis) (P = 0.0038; r = -0.32). (c) Boxplots showing methylation levels at a CpG site (cg04757093) and *RUNX3*, a transcription factor induced in airway epithelial cells by viral exposure (P = $1.67\text{x}10^{-5}$). (d) Scatter plot showing the correlation between *RUNX3* transcript abundance and cg04757093 methylation levels (x-axis) (P = $3.76\text{x}10^{-7}$; r = 0.53).

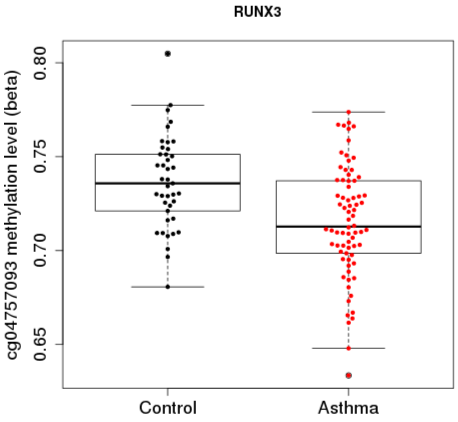


a.

b.

c.

d.


RUNX3
cg04757093 methylation level (beta)
0.80
0.75
0.70
0.65
Control
Asthma

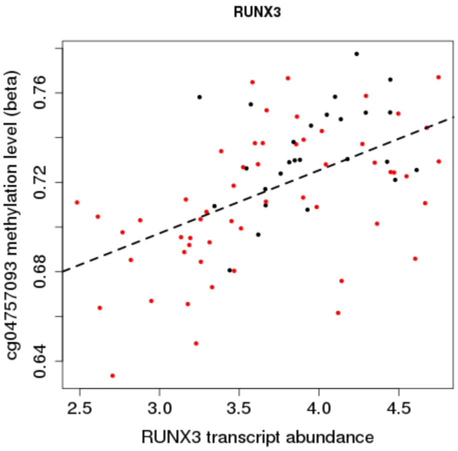
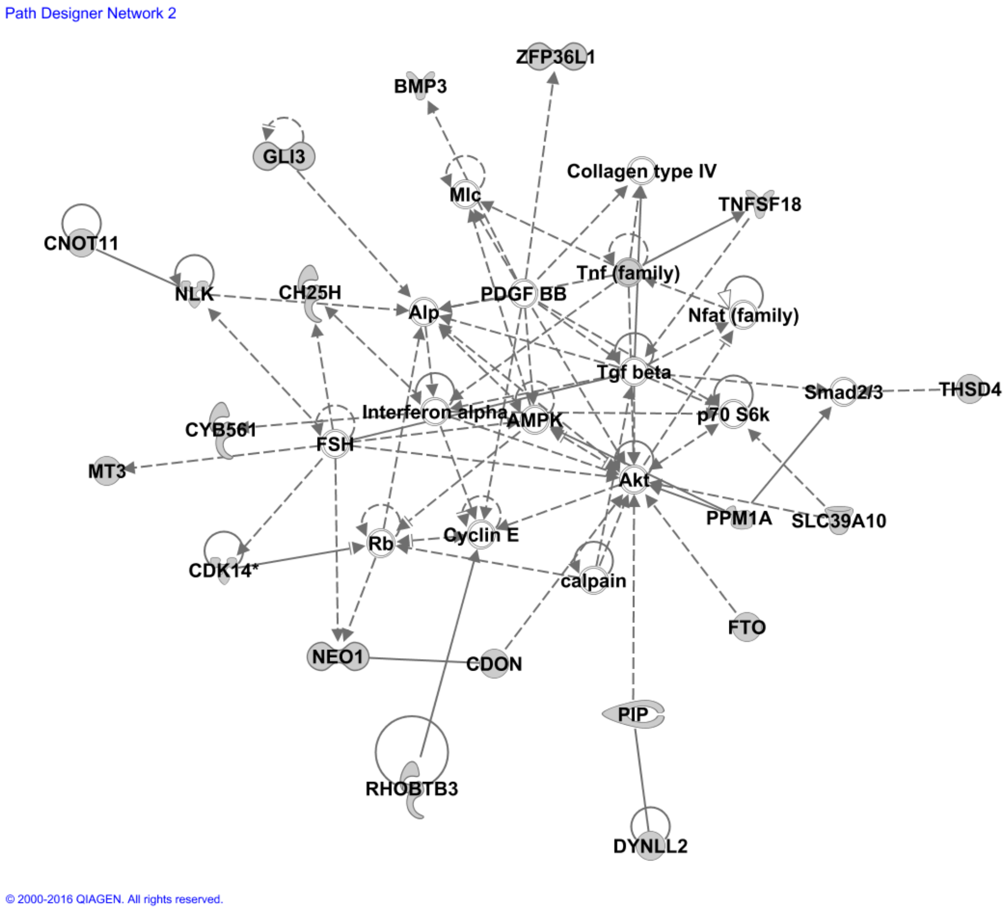
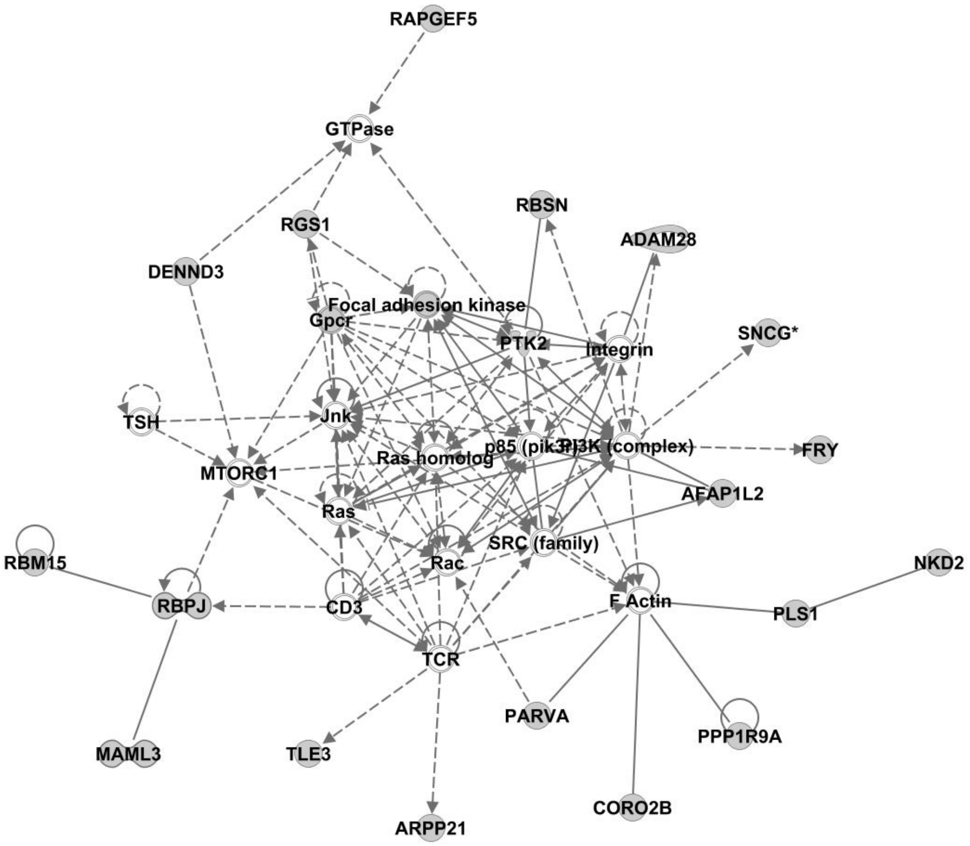

RUNX3
cg04757093 methylation level (beta)
0.76
0.72
0.68
0.64
2.5
3.0
3.5
4.0
4.5
RUNX3 transcript abundance

Figure S2. Ingenuity Pathway analysis networks generated from genes correlated with CpGs in Weighted Gene Co-expression Network Analysis (WGCNA) module 1 (see Table 1). Genes included in this network were correlated with methylation levels at nearby differentially methylated CpGs (DMCs) (r > 0.15). There were five networks with a score ≥ 25. The fifth network is shown in Figure 5a. (**a)** network score = 33, (**b**) network score = 33, (**c**) network score = 28, (**d**) network score = 26. Genes that were associated with a differentially methylated CpG are shown in grey. Molecule shapes: oval = transcriptional regulator, diamond = enzyme, dashed line rectangle = channel, up triangle = phosphatase, down triangle = kinase, trapezoid = transporter, circle = other.

a

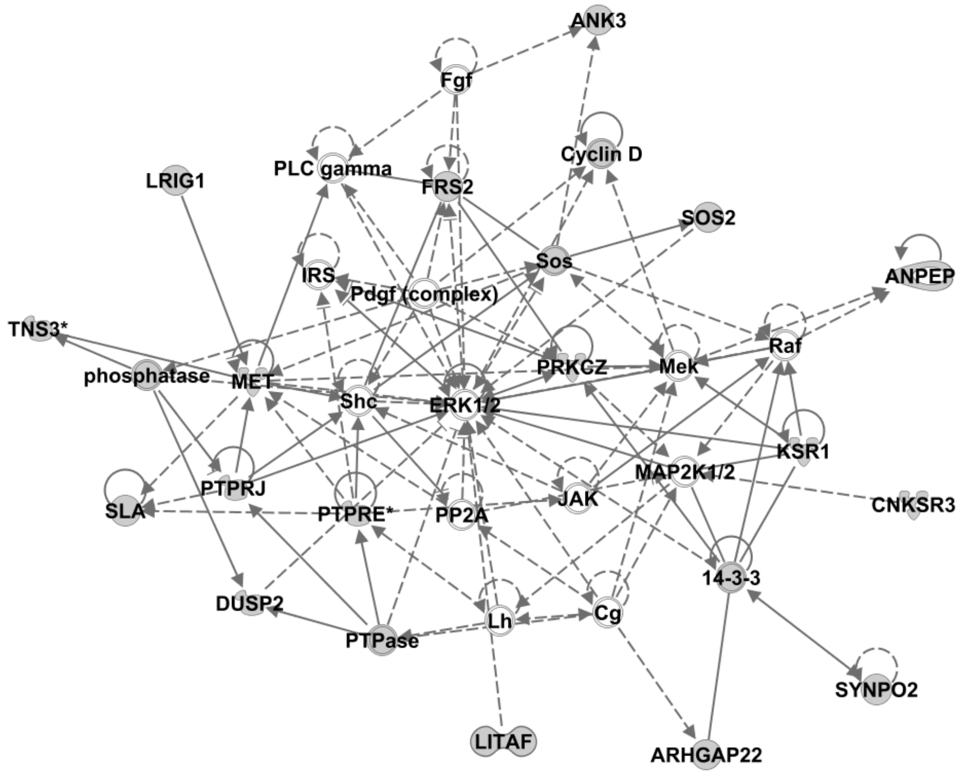


b.


Path Designer Network 3

c.

Path Designer Network 4



d.

Path Designer Network 5

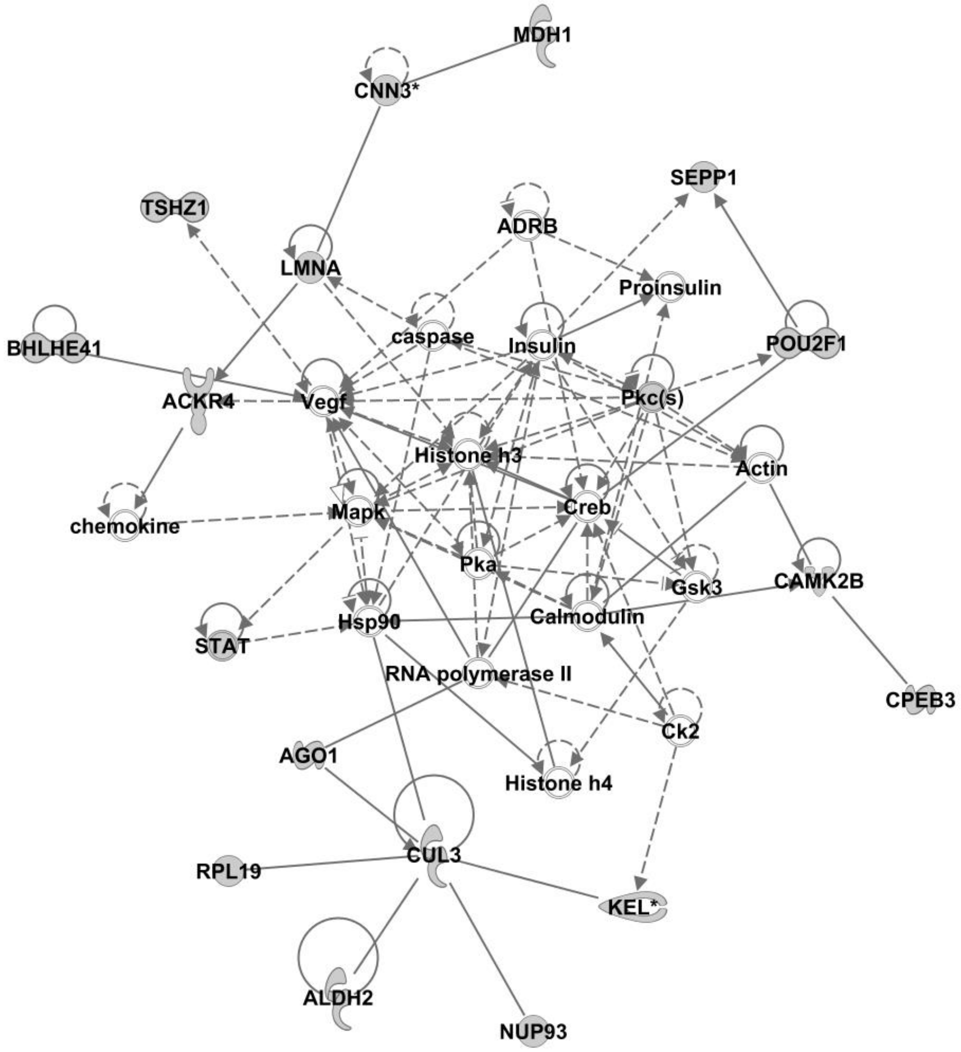


Figure S3. Ingenuity Pathway analysis networks generated from genes near CpGs in Weighted Gene Co-expression Network Analysis (WGCNA) module 2. Genes included in this network were correlated with nearby differentially methylated CpGs (DMCs) methylation levels (p-value < 0.2) network score = 25. Genes that were associated with a differentially methylated CpG are shown in grey. Molecule shapes: oval = transcriptional regulator, diamond = enzyme, dashed line rectangle = channel, up triangle = phosphatase, down triangle = kinase, trapezoid = transporter, circle = other.

Path Designer Network 1

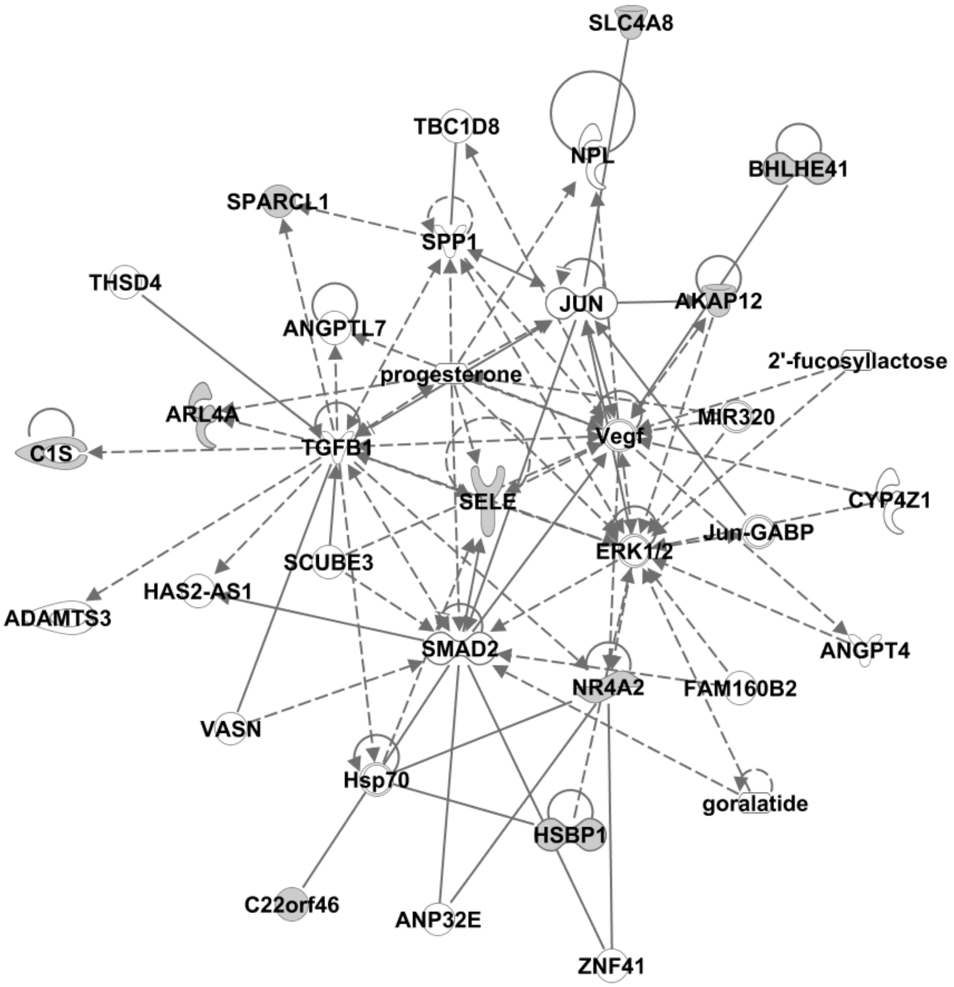


Figure S4. Ingenuity Pathway analysis networks generated from genes near CpGs in Weighted Gene Co-expression Network Analysis (WGCNA) module 3. Genes included in this network were correlated with nearby differentially methylated CpGs (DMCs) methylation levels (r > 0.15); network score = 35. Genes that were associated with a differentially methylated CpG are shown in grey. Molecule shapes: oval = transcriptional regulator, diamond = enzyme, dashed line rectangle = channel, up triangle =phosphatase, down triangle = kinase, trapezoid = transporter, circle = other.

Path Designer Network 1

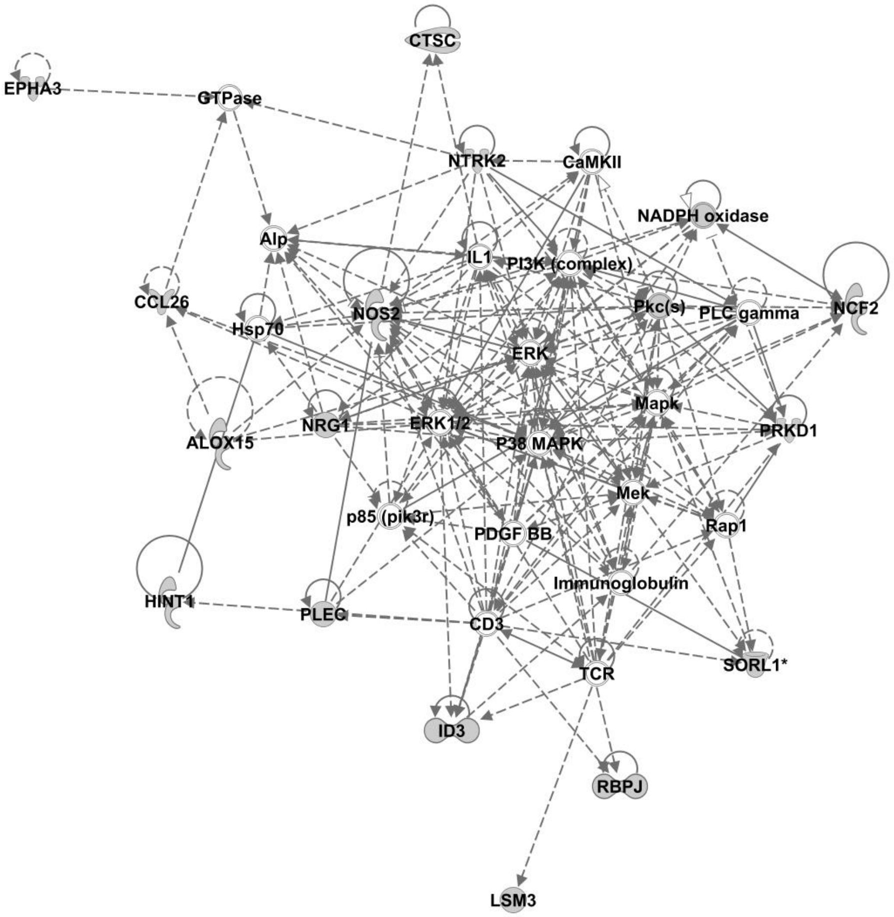
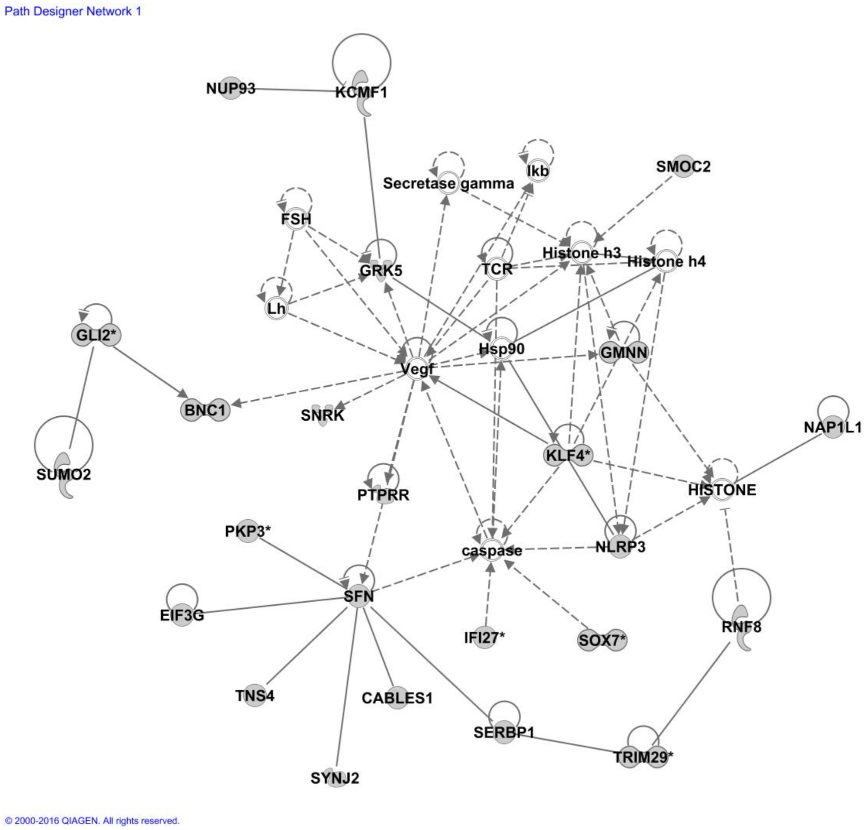


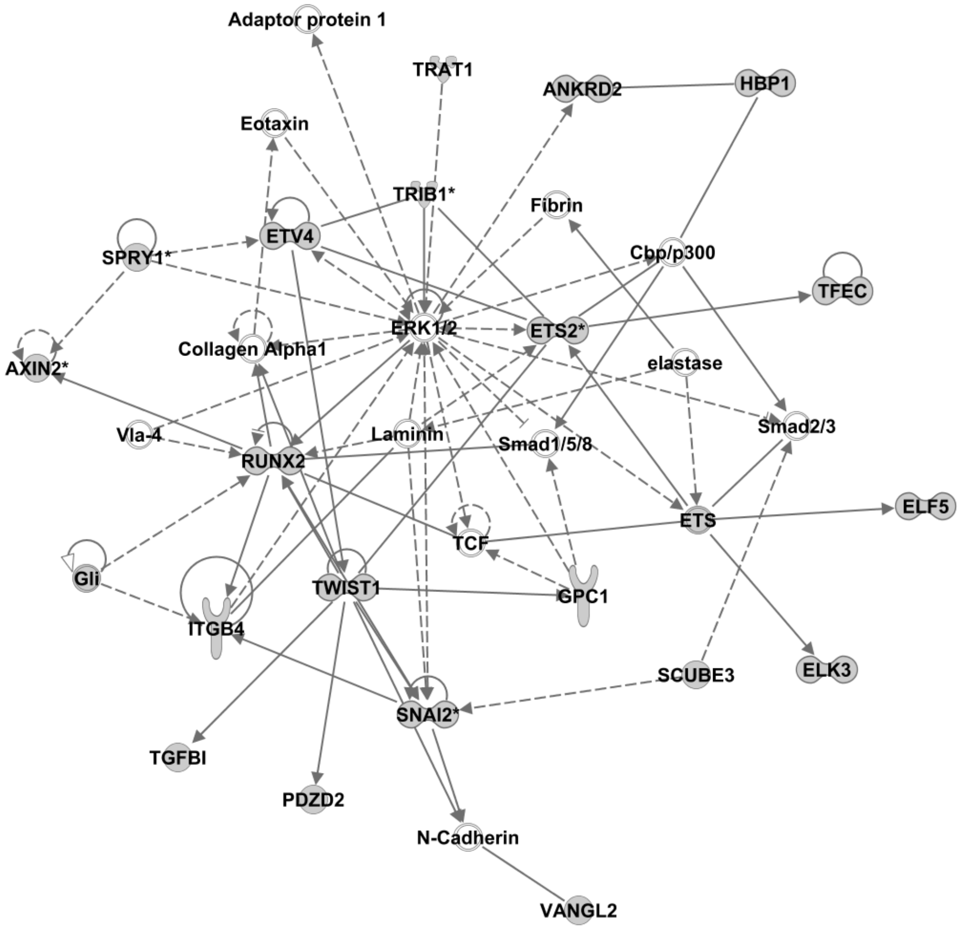
Figure S5. Ingenuity Pathway analysis networks generated from genes near CpGs in Weighted Gene Co-expression Network Analysis (wGCNA) module 4. Genes included in this network were correlated with nearby differentially methylated CpG (DMCs) methylation levels (r > 0.15) There were seven networks: **(a)** network 1, score of 38; **(b)** network 2, score of 29; **(c)** network 4, score of 29; **(d)** network 5, score of 25, **(e)** network 6, score of 25. Genes that were associated with a differentially methylated CpG are shown in grey. The seventh network is shown in Figure 5b. Molecule shapes: oval = transcriptional regulator, diamond = enzyme, dashed line rectangle = channel, up triangle =phosphatase, down triangle = kinase, trapezoid = transporter, circle = other.

a.

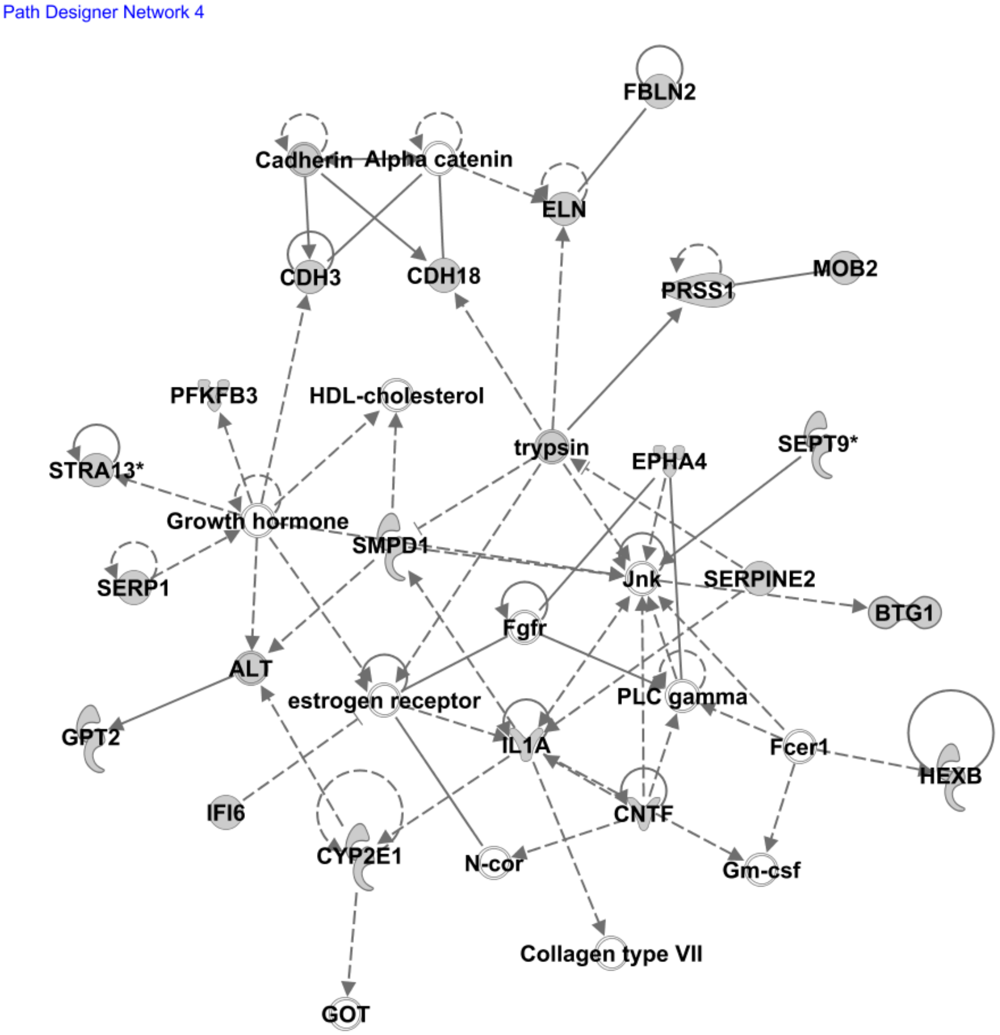


b.

Path Designer Network 2

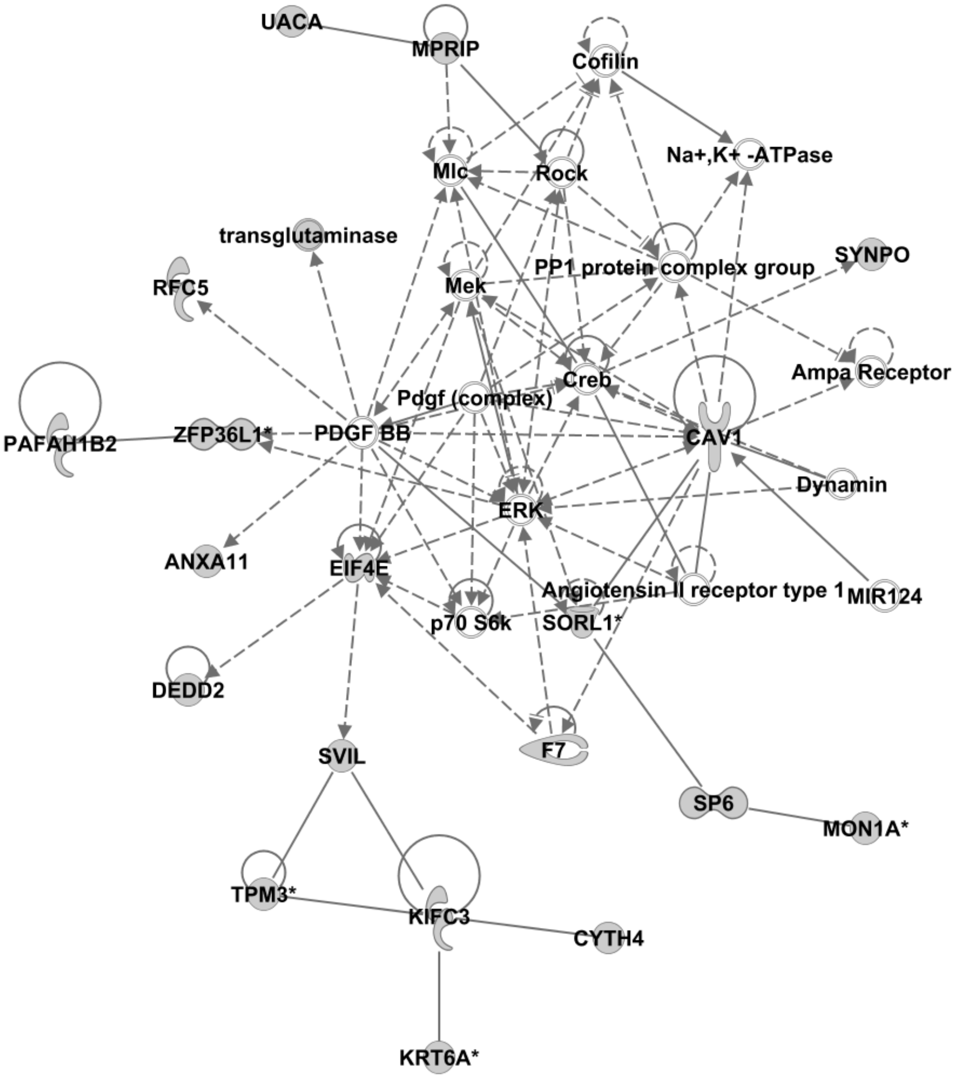


c.


Path Designer Network 4
FBLN2
Cadherin
Alpha catenin
ELN
CDH3
CDH18
PRSS1
MOB2
PFKFB3
HDL-cholesterol
trypsin
EPHA4
SEPT9*
STRA13*
Growth hormone
SMPD1
Jnk
SERPINE2
BTG1
SERP1
Fgfr
ALT
estrogen receptor
PLC gamma
GPT2
IL1A
Fcer1
HEXB
IFI6
CNTF
CYP2E1
N-cor
Gm-csf
Collagen type VII
GOT

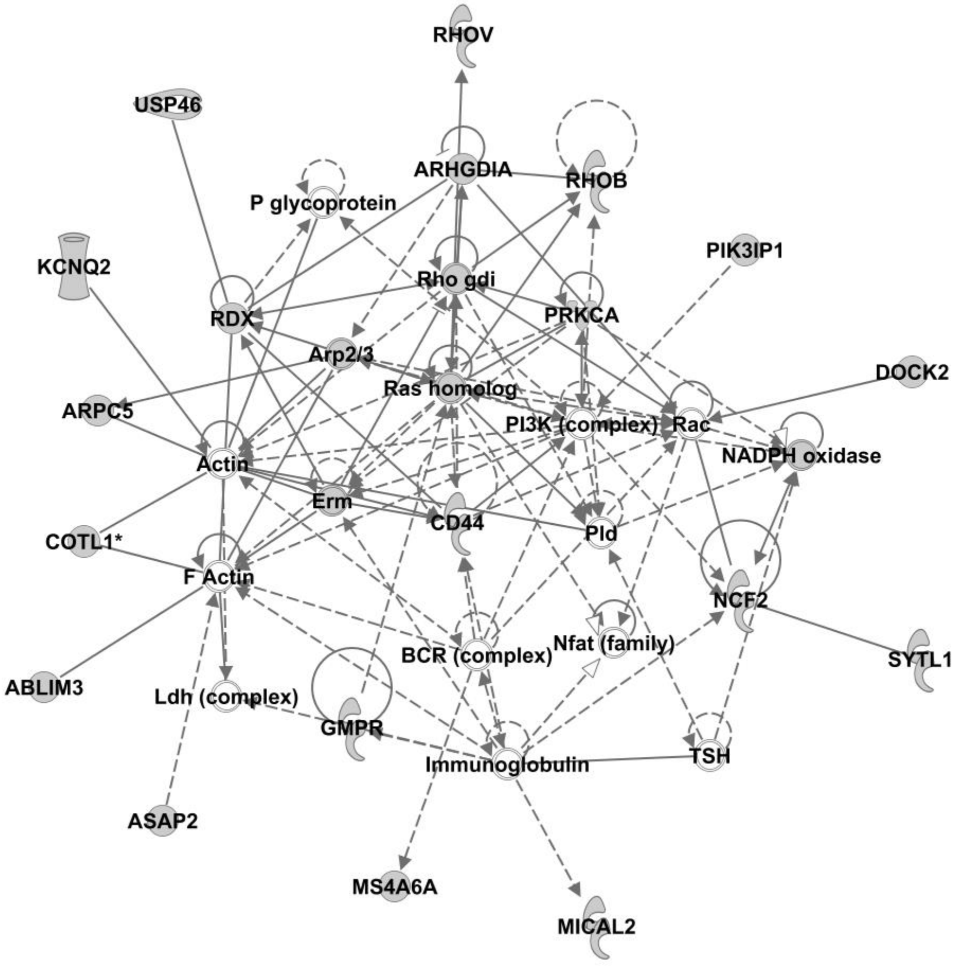
d.

Path Designer Network 5



e.

Path Designer Network 6



f.

Path Designer Network 7

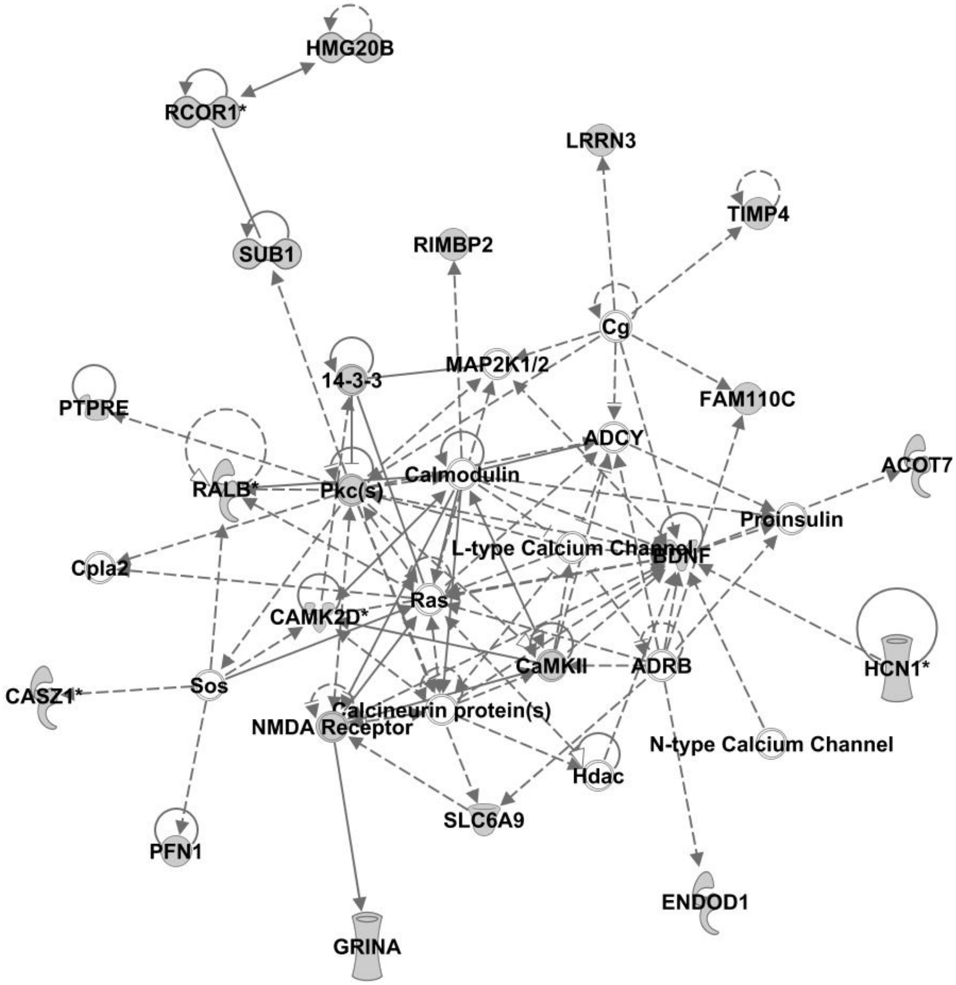


Figure S6. Ingenuity Pathway analysis upstream regulator analysis network generated from genes nearest each CpG in Weighted Gene Co-expression Network Analysis (WGCNA) module 4. Genes included in this analysis were correlated with nearby differentially methylated CpG (DMCs) methylation levels ($r > 0.15$). The most significant upstream regulator was TGFB1 ($P = 7.74 \times 10^{-9}$). The mechanistic network for this molecule contained additional upstream regulators: TNF ($P = 1.87 \times 10^{-7}$), IFN-gamma ($P = 1.65 \times 10^{-6}$), and JUN ($P = 9.64 \times 10\text{-}^{6}$).

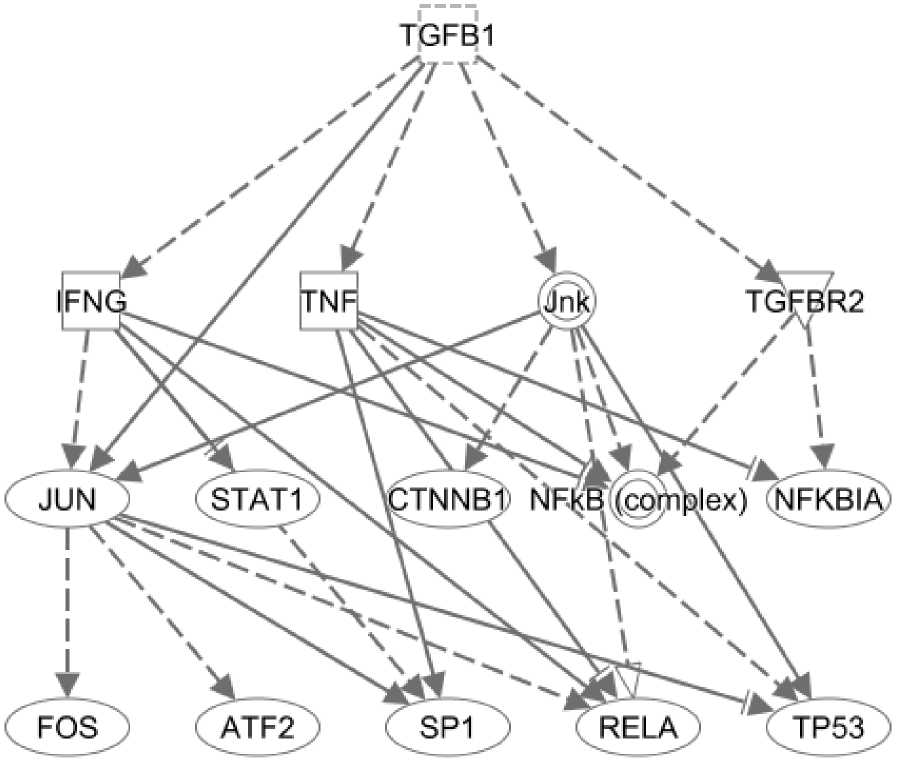
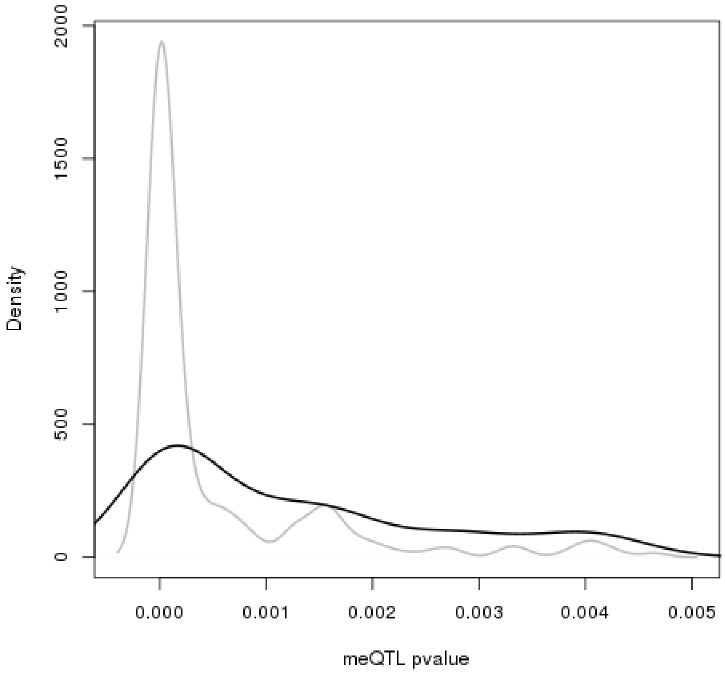


Figure S7. Density plot of meQTL p-value distributions. meQTLs for uncorrelated CpG sites not assigned to a WGCNA module are shown in grey; meQTLs for correlated CpG sites assigned to a module are shown in black. There is an enrichment of small (significant) p-values for meQTLs corresponding to uncorrelated CpG sites ($P > 2.2x10^{-16}$).



Supplementary Tables.

Supplemental Table S5. Enrichments of meQTL and eQTLs in airway epithelial cells for SNPs associated with asthma in the EVE or GABRIEL GWAS

	Odds Ratio	**95% CI (lower)**	**95% CI (upper)**	**Enrichment P**
a) meQTL				
EVE	1.32	1.08	1.60	0.0064
GABRIEL	1.25	0.94	1.65	0.12
Meta Analysis	**1.32**	**1.08**	**1.61**	**0.0017**
b) eQTL				
EVE	1.48	0.84	2.45	0.13
GABRIEL	2.42	1.34	4.11	0.0027
Meta Analysis	**1.86**	**1.27**	**2.76**	**0.0015**