

Supporting Online Material

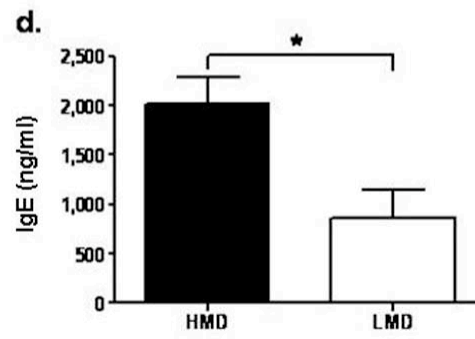
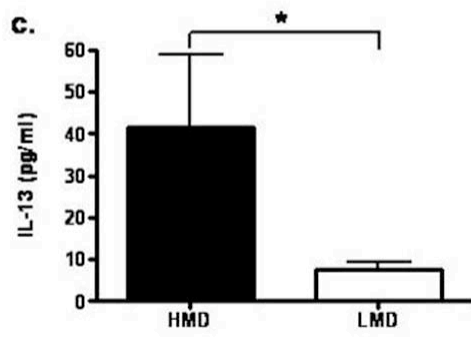
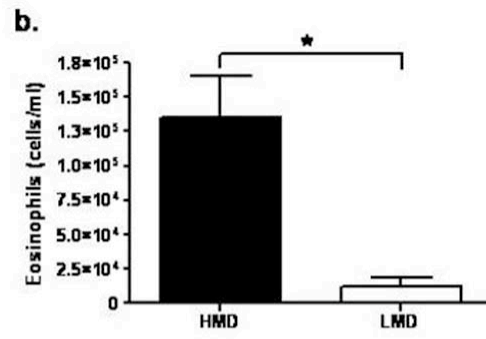
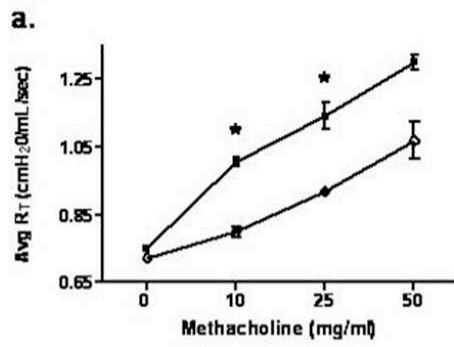


Figure S2.

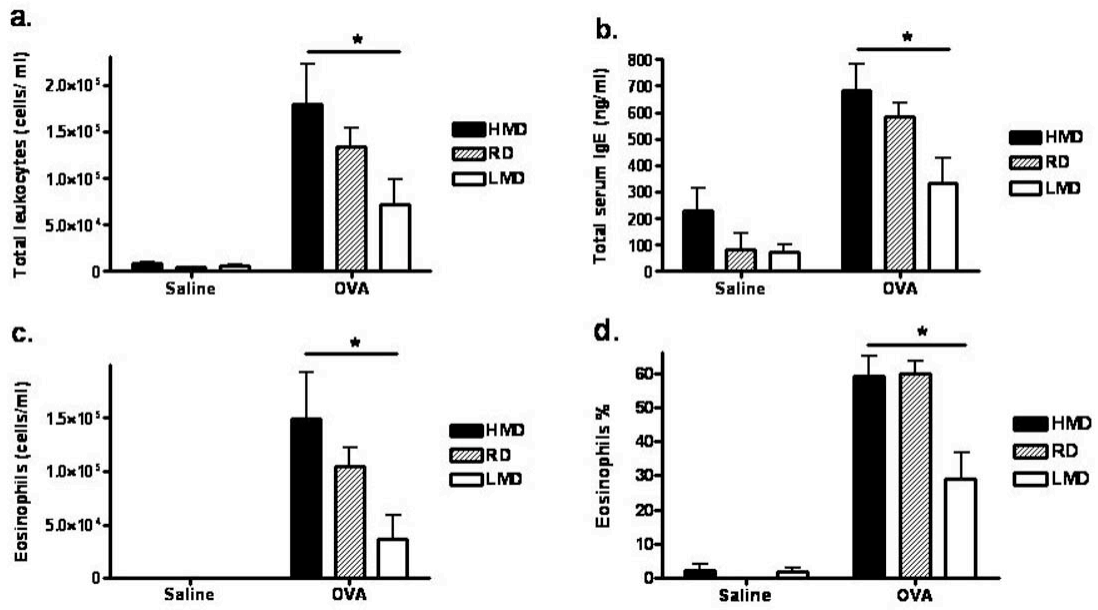


Figure S3.

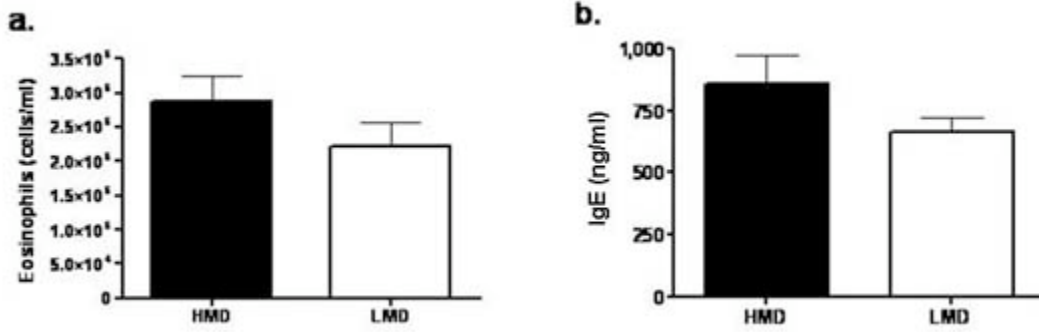


Figure S4.

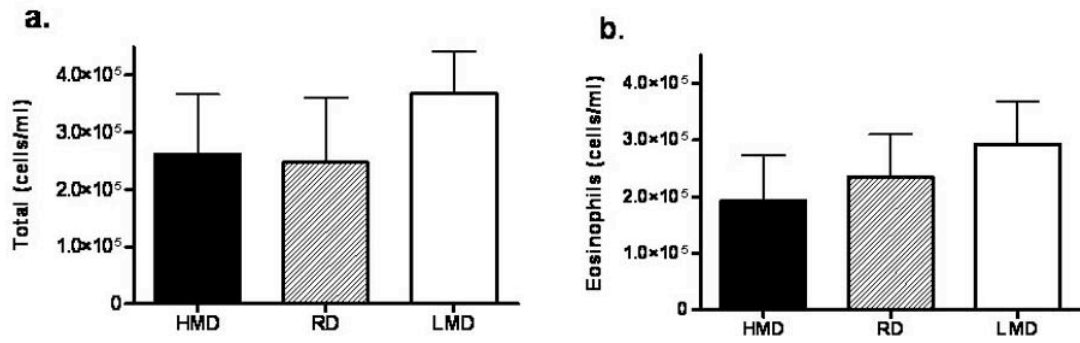


Figure S5.

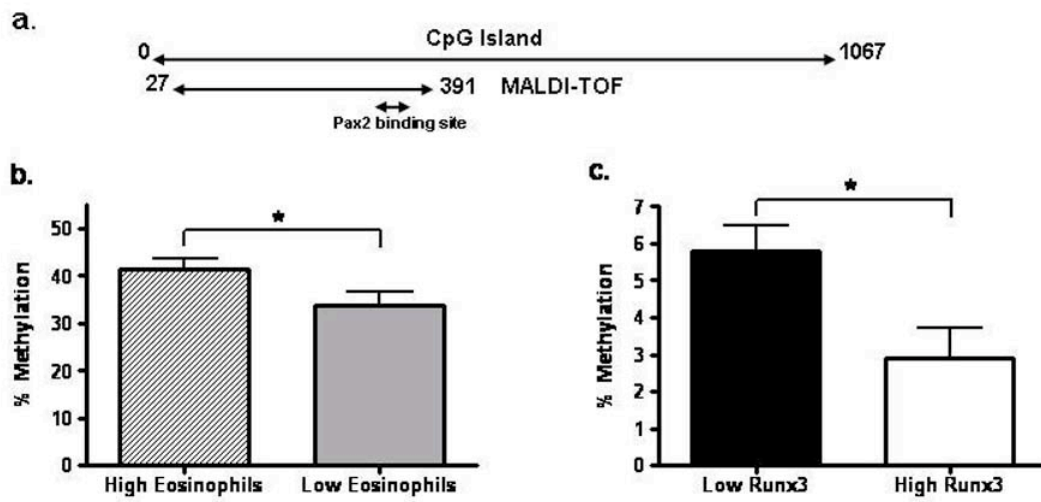


Table. S1. Composition of Methyl donor diets

	High Methyl-donor	Low Methyl-donor
L-methionine	12.7g	5.2
Choline	16g	1g
Betaine	15g	0
Cyanocobalamin	175ug	25ug
Folic acid	17mg	2mg
Zinc	180mg	30mg
Genistein	300mg	0
Oil	70g Soybean oil	70g Corn oil

Table S2. List of genomic sequence tags with a P-value ≤ 0.05 . The genomic sequences, tag counts in the low methylation diet/non-asthmatic (LMD) and high methylation diet/asthmatic (HMD) MSDK libraries, chromosomal location (chr), p-values based on the Z-scores as the statistical significance of the observed differences, the position of each genomic tag relative to the nearest gene, the gene symbol and gene name are indicated. Blue highlighting indicates that additional genes near the tag are listed. Genes selected for further down-stream analysis are highlighted in yellow. MSDK library construction and data analysis was performed as indicated in the Materials and Methods.

Genomic tag sequence	LMD	HMD	Chr	Position	p value	pos relative to nearest gene	Symbol	Description
GAATCTGAAATCTGTCA	11	1	19	27283105	0.001603	1k bp upstream of start	Vldlr	very low density lipoprotein receptor
CTCGCCCTGCAACCCGG	9	1	2	68271869	0.005526	83k bp upstream of start	Stk39	Serine/threonine kinase 39
CTGTCCCTGCCCATCTC	9	1	4	120147333	0.005526	16k bp upstream of start	Cited4	STE20/SPS1 homolog Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 4
TGCTCTGCCTTCCCCGT	9	1	X	17133328	0.005526	63k bp upstream of start		Tag sequence surrounded by repeat elements
CTCCAAACCCAGCCTTC	8	1	19	43493500	0.010307	1k bp upstream of start	Cnm1	cyclin M1
GAAAACCTTTTCGGCCGA	8	1	12	79668652	0.010307	888 bp downstream of start	Mpp5	membrane protein, palmitoylated 5 (MAGUK p55 subfamily member 5)
GCCGTGCCACGGTCCCC	8	1	6	83421861	0.010307	14k bp downstream of stop	AK218083	Mus musculus cDNA, clone: Y2G0143P06. Alternative Splicing Library L13
GCCGTGCCACGGTCCCC	8	1	6	83421861	0.010307		Dguok	Deoxyguanosine kinase
AATCATTGTTTACCGGA	8	0	4	128308015	0.002296	32k bp upstream of start	A3galt2	alpha 1 3-galactosyltransferase 2
ACCTACCCAGGCAGCCT	8	0	14	20774293	0.002296	853 bp upstream of stop	Zfp503	zinc-finger protein NOLZ1
ATAAGCAGGGGTCCGGG	8	0	19	5085417	0.002296	3k bp upstream of start	Tmem151	Hypothetical protein LOC381199
GTAGAGGAGGGGGAGAG	8	0	1	193838624	0.002296	927 bp downstream of start	Rcor3	REST corepressor 3
CTGCCGTGTAGGAGGA	7	1	2	167922644	0.019292	798 bp upstream of stop	Dpm1	Dolichol-phosphate(beta-D)mannosyltransferase
TTTTGACCTTTTCAGTC	7	1	4	140819726	0.019292	1k bp upstream of stop	B30016D10Rik	hypothetical protein LOC320456
CAATCTTGAGCTGGGAA	7	1	14	54047354	0.019292	1k bp upstream of start	Zfx2	zinc finger homeobox 2
CCTAGCCACACTCCCT	7	1	2	167970651	0.019292	17k bp upstream of start	Dpm1	dolichol-phosphate (beta-D) mannosyltransferase
CTCAGACCCGCGCAGTC	7	1	1	84163695	0.019292	85k bp downstream of stop	Dner	delta/notch-like EGF-related receptor
CTCAGACCCGCGCAGTC	7	1	1	84163695	0.019292		5033414K04Rik	hypothetical protein LOC98496
CGTGCCGCGGGGATGC	7	1	10	7353490	0.019292	4k bp upstream of start	Pcmt1	protein-L-isoaspartate (D-aspartate)
CAAAGCAGCCCGACGC	7	1	8	111593479	0.019292	10k bp upstream of start	Atbf1	AT motif binding factor 1
CGCGCTCCCGCCCTCC	7	1	2	181522175	0.019292	2k bp downstream of stop	Samd10	sterile alpha motif domain containing 10
CGCGCTCCCGCCCTCC	7	1	2	181522175	0.019292		Prpf6	U5 snRNP-associated 102 kDa protein
CGCGCTCCCGCCCTCC	7	1	2	181522175	0.019292		UckL1	uridine-cytidine kinase 1-like 1
TCCCTATGTGGTACCT	7	1	2	118594615	0.019292	1k bp downstream of start	Bahd1	bromo adjacent homology domain containing 1
TCTCCCAAGACTCGC	7	1	7	114205995	0.019292	467 bp upstream of start	Pde3b	phosphodiesterase 3B, cGMP-inhibited
GTAGATGATCTGTTCC	7	1	9	20726841	0.019292	10k bp upstream of start	Edg5	endothelial differentiation, sphingolipid G-protein-coupled receptor, 5
GGTGTACCAGCCATCT	7	1	2	50969874	0.019292	17k bp upstream of start	Rnd3	Rho family GTPase 3
CCAAGCAGATCCCTCT	7	0	3	88110325	0.004344	2k bp upstream of stop	Hapl2	hyaluronan and proteoglycan link protein 2
ATCACACAGACCTTTTG	6	1	13	46739837	0.036266	23k bp downstream of stop	Nup153	nucleoporin 153
ATCACACAGACCTTTTG	6	1	13	46739837	0.036266	23k bp downstream of stop	Kif13a	kinesin family member 13A

CGTATTCCCGTAGACCC	6	1	4	134424851	0.036266	32k bp upstream of stop	Runx3	runt related transcription factor 3 (CpG island in the center of the gene)
GAGTACGCTGCGAAGGC	6	1	5	123275857	0.036266	166 bp downstream of start	Tmem142a	transmembrane protein 142A
AGTGGCCACAGTGAGGC	6	1	15	78671291	0.036266	1k bp downstream of start	Cdc42ep1	CDC42 effector protein (Rho GTPase binding) 1
CATCTATCAGATCACCC	6	1	X	13467876	0.036266			tag sequence surrounded by repeat elements
GCAGCTGCGGTGCAGCT	6	1	6	47978066	0.036266		BC066046	Mus musculus RIKEN cDNA 2500002G23 gene
CAGCTGGCCTCGGCGCA	6	1	15	93423692	0.036266	96k bp upstream of start	Prickle1	prickle like 1 (Drosophila)
CCGGCCGCCGCGCGTT	6	1	19	47307020	0.036266	13k bp downstream of stop	Sh3pxd2a	SH3 and PX domains 2A
CCGGCCGCCGCGCGTT	6	1	19	47307020	0.036266		NeurL	neuralized homolog
CGGAGTGACAGGGAGG	6	1	11	49745130	0.036266	126 bp upstream of start	Rasgef1c	RasGEF domain family, member 1C
CGGCGTGCAGCGCGGCC	6	1	5	118430960	0.036266	166 bp downstream of start	Hrk	harakiri, BCL2 interacting protein (contains only BH3 domain)
ATCTCTGAGAAACAGCC	6	1	5	28401520	0.036266	634 bp upstream of start	Insig1	insulin induced gene 1
GTTAATTCCTCAAGTGCA	6	1	4	33360710	0.036266	672 bp downstream of start	Ube2j1	ubiquitin-conjugating enzyme E2, J1
TGGCTGATTGCCTGTTC	6	1	13	84696543	0.036266	1k bp downstream of start	Tmem161b	Hypothetical protein LOC72745
TGTAGGGACACTTTGTG	6	1	7	65249206	0.036266	72k bp upstream of start	Tjp1	tight junction protein 1
AACCGAAGAGGTGAGCC	6	1	13	46940532	0.036266	51k bp upstream of start	Kif13a	kinesin family member 13A
GGACAAGCTGCACTCCC	6	1	1	158078808	0.036266	17k bp downstream of stop	Tdrd5	tudor domain containing 5 (no CpG island)
GCGCAGCGCACCCGGGG	6	1	5	117618653	0.036266	318 bp downstream of start	Wsb2	WD repeat and SOCS box-containing 2
GCGCGCCGGTGGGGGC	6	1	11	102986958	0.036266	445 bp upstream of start	Fmn1	lymphocyte specific formin related protein
GCGGATGGCCCCAGGTG	6	1	18	5591911	0.036266	39 bp downstream of start	Zfx1a	zinc finger homeobox 1a
GCGGCGCCTGCTGCTCC	6	1	6	140586083	0.036266	1k bp downstream of start	Aebp2	AE binding protein 2 isoform 1
GGACAGAGGGCGTGGA	6	1	15	73576164	0.036266	11k bp downstream of stop	Ptp4a3	protein tyrosine phosphatase 4a3
GGCCGGGCCGGCGCTT	6	1	7	24190862	0.036266	3 bp downstream of start	Igsf4c	immunoglobulin superfamily, member 4C
GGGAGGGGCAGTAGGCG	6	1	4	141511597	0.036266	137k bp upstream of start	9030409G11 Rik	RIKEN cDNA 9030409G11 gene (9030409G11Rik), mRNA no gene
GGGCCACCCGCAGTGCA	6	1	2	149521559	0.036266			
GTAACCGGCACAGAAGA	6	1	1	36491167	0.036266		Ankrd39	ankyrin repeat domain 39
CAGAATGGGTGCTGCCT	6	0	19	29317014	0.008280	1k bp upstream of start	Jak2	Janus kinase 2
AGCGCTGCCTTCGGG	6	0	8	12397131	0.008280		Sox1	SRY (sex determining region Y)-box 1
GCACTGCCCCGCTATT	6	0	14	30324020	0.008280	132 bp upstream of start	Eaf1	ELL associated factor 1
GCACTGCCCCGCTATT	6	0	14	30324020	0.008280		Mett 16	methyltransferase like 6
TGTTCTGTCTTGCCTTC	6	0	7	142077009	0.008280	12k bp downstream of stop	Krtap5-1	keratin associated protein 5-1
GCAGCGTCCCGGTCGG	6	0	17	45036480	0.008280	4k bp upstream of start	Hsp90ab1	heat shock protein 90kDa alpha (cytosolic), class B member 1
CATTTCCGAGTGGCGTC	6	0	13	31818630	0.008280	4k bp downstream of stop	Foxc1	forkhead box C1
GAGTTGGCACAAGTAGG	6	0	5	143783929	0.008280		Rac1	RAS-related C3 botulinum substrate 1
CTTAGGTTGGTCTCTG	6	0	11	11737408	0.008280	23k bp upstream of stop	Ddc	dopa decarboxylase (CpG island in middle of gene)
AAAAATCTAAGTTCAGA	6	0	1	121663976	0.008280		Ptpn4	4 days neonate male adipose cDNA, RIKEN full-length enriched library, clone:B430311G24 product:protein tyrosine phosphatase, non-receptor type 4, full insert sequence
CTGGCGGCGACCTGCAA	6	0	2	73074000	0.008280	1k bp downstream of start	Sp9	trans-acting transcription factor 9
CGACAGAGGGCCGGGGG	6	0	18	80870439	0.008280		Nfatc1	nuclear factor of activated T-cells
CCGACGTCATCCTCCAG	6	0	8	73014094	0.008280	685 bp downstream of start	Hapln4	hyaluronan and proteoglycan link protein 4 (brain link protein 2)
GAGCACTGGCTGGTGG	15	3	2	84515311	0.001603	8k bp downstream of stop	Zdhhc5	Zinc finger DHHC domain containing 5
CTCTAGGGCGCACGCT	10	2	10	59502264	0.009987	439 bp downstream of start	Spock2	sparc/osteonectin, cwcv and kazal-like domains proteoglycan 2
AACAGTGGCGCGCGCGG	5	0	5	147740932	0.015936	306 bp upstream of start	Pan3	PABP-dependent poly(A) nuclease 3
CCGCTCTCTGCTGCC	5	0	8	9771455	0.015936	92k bp upstream of start	Tmem28	transmembrane protein 28
AGCGCAGCCGACTGCAC	5	0	4	99149882	0.015936	1k bp upstream of stop	Foxd3	forkhead box D3
ATCCGCGTGGGGGCCG	5	0	5	32413267	0.015936	152 bp downstream of start	Fosl2	fos-like antigen 2
GCGGCGGCGCGGGGCG	5	0	13	48979767	0.015936	68k bp downstream of stop	AK172909	mRNA for mKIAA0183

GCGGCGGCGGCGACGAC	5	0	11	87864016	0.015936	136 bp downstream of start	Sfrs1	splicing factor, arginine/serine-rich 1 (ASF/SF2)
TTCAGGCTGAGGCTTAA	5	0	15	79632365	0.015936	18k bp upstream of stop	Nptr	neuronal pentraxin receptor
TTCGCCACTTGGGCCCC	5	0	10	79519355	0.015936	24 bp downstream of start	Stk11	serine/threonine kinase 11
TTGGTCTCATTGTGCCT	5	0	9	16253054	0.015936			
TTTATATTTTGGGAAGA	5	0	17	26572054	0.015936	5k bp upstream of start	Nkx2-5	NK2 transcription factor related, locus 5 (Drosophila)
TCAGCCGCTCACCCAGC	5	0	12	71371756	0.015936	15 bp downstream of start	Txndc1	thioredoxin domain containing 1
GAGGCGCCAAGGACAGT	5	0	17	4951893	0.015936	53k bp downstream of stop	BC052557	AT rich interactive domain 1B (Swi like) mRNA clone IMAGE:5257371
CGCCAGGCTGTAGTCGC	5	0	12	51570762	0.015936	22k bp downstream of stop	Prkcn	protein kinase C
CGGCAGCTGTAGTAACC	5	0	2	74469536	0.015936	387 bp downstream of start	Hoxd13	homeo box D13
CGGGCGCACGGGTAGTA	5	0	6	52189815	0.015936	1k bp upstream of start	Hoxa13	homeo box A13
CTGTTTGCAGAGCCTGA	5	0	1	193891215	0.015936	381 bp upstream of start	Kcnh1	potassium voltage-gated channel, subfamily H (eag-related), member 1
GCTCGGCGCTCAGCCCG	5	0	17	25841032	0.015936	115 bp downstream of start	Tmem8	transmembrane protein 8 (five membrane-spanning domains)
GCAGGGACTCAAGGGGG	5	0	12	77741159	0.015936	15k bp upstream of start	Rab15	RAB 15 member RAS oncogene family
GCCGCTCGGCCTCCCTC	5	0	9	8134757	0.015936	8k bp upstream of start	AK129341	hypothetical protein LOC234915

Supplemental Figure Legends

Figure S1. Diet can regulate the severity of allergic airways disease in mice.

C57BL/6J mice placed on either high methylation or low methylation diets during breeding. F1 progeny remained on modified diets. Eight to ten weeks old F1 progeny were immunized and challenged to ovalbumin. Dietary changes modified the severity of allergic asthma in these animals [HMD (N=45) or LMD (N=12)]. Animals continuously exposed to high methyl donors demonstrate enhanced allergic airways disease, including: **(A)** airway hyper-reactivity (HMD; filled boxes, N=4, LMD; open diamonds, N=4), **(B)** absolute eosinophils in lavage fluid, **(C)** IL-13 levels in lavage fluid, **(D)** serum IgE level.

Figure S2. Gestational exposure to dietary methyl donors regulates severity of allergic airways disease.

Adult C57BL/6J progeny exposed to high methyl-donor (HMD) diet *in utero* demonstrate enhanced allergic inflammation, when compared to low methyl-donor (LMD) diet. C57BL/6J breeding pairs were fed HMD, regular diet (RD=NIH-31), or LMD beginning 2 weeks prior to mating and during pregnancy. Experimental diets were continued until the time of weaning, when F1 progeny were placed on RD (regular diet=NIH-31). At 6-10 weeks of age, F1 mice were immunized and challenged with ovalbumin. When compared to LMD, perinatal exposure to HMD was associated with enhanced **(A)** total cells in the lung lavage (*P<0.05, N=31-63), **(B)** total serum IgE (N=15-26, *P<0.05), **(C)** concentration of lung lavage eosinophils (N=31-63, * P<0.05), and **(D)** percentage of lung lavage eosinophils, N=31-63, * P<0.05).

Figure. S3. Dietary changes during adulthood do *not* significantly alter severity of allergic airways disease.

Three week old C57BL/6J mice were placed on HMD (N=20)

or LMD (N=19) for 7 weeks. Exposure to dietary modification during adulthood did not significantly alter the severity of allergic airways disease in mice (OVA) as measured by either **(A)** absolute eosinophils in lavage fluid or **(B)** total serum IgE level.

Figure S4. Dietary changes during lactation do *not* significantly alter severity of allergic airways disease. Breeding dams were placed on regular diet. Upon delivery of pups, dams were placed on HMD, RD, or LMD throughout lactation. All pups were placed on RD at the time of weaning and are identified by exposures during lactation (HMD; N=11, RD; N=11, LMD; N=19). Methyl exposure during breast feeding did not significantly alter the severity of allergic airways disease (OVA) in pups as measured by (A) total cells in the lavage or (B) absolute eosinophils.

Figure S5. Quantitative DNA methylation measurements from Maldivof mass-spectrometry analysis and Runx3. (A) Schematic diagram of the CpG Island studied within the mouse Runx3 gene. Size and location of the fragments analyzed by MALDI TOF are indicated. **(B)** Comparison of the average methylation level of 12 CpG dinucleotides between high responders (n = 30) and low responders (N = 12) in challenged C57/BL6 mice (*P=0.05). **(C)** Association between the Runx3 relative expression and the level of methylation in one CpG dinucleotide, which is located within a predicted Pax2 transcription factor binding site [CCGGGACG, located at nucleotide 282 of CpG (mouse chr4:134424152134425178)]. These results suggest that lower expression correlates with an increase in methylation level (*P= 0.01).