# Inhalation of diesel exhaust and allergen alters human bronchial epithelium DNA methylation

Rachel L. Clifford Ph.D[a,1], Meaghan J. Jones Ph.D[a], Julia L MacIsaac Ph.D[a], Lisa M. McEwen BSc[a], Sarah J. Goodman BSc[a], Sara Mostafavi, Ph.D[a,b,c], Michael S. Kobor* Ph.D [a,d] and Chris Carlsten* MD[e]

[a] Centre for Molecular Medicine and Therapeutics, Child and Family Research Institute, University of British Columbia, 950 West 28th Avenue, Vancouver, V5Z 4H4, Canada

[b] Department of Statistics, University of British Columbia, 2329 West Mall, Vancouver, British Columbia, V6T 1Z4, Canada

[c] Canadian Institute for Advanced Research, Toronto, Ontario, Canada

[d] Human Early Learning Partnership, School of Population and Public Health, University of British Columbia, 2206 East Mall, Vancouver, British Columbia, V6T 1Z3, Canada.

[e] Air Pollution Exposure Laboratory, Chan-Yeung Centre for Occupational and Environmental Lung Disease, Division of Respiratory Medicine, Department of Medicine, University of British Columbia, 2275 Laurel St., Vancouver, British Columbia, V5Z 1M9, Canada

* Equal senior authors

**Corresponding author:** Dr Chris Carlsten, 2775 Laurel St., 7th Floor, Vancouver, British Columbia, V5Z 1M9, Canada. Phone 604-875-7229, Fax 604-875-4727, carlsten@mail.ubc.ca

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[1] Department of Respiratory Medicine and Nottingham Respiratory Research Unit, University of Nottingham, Clinical Sciences Building, City Hospital, Hucknall Road, Nottingham, NG5 1PB, UK

**Abstract**

**Background:** Allergic disease affects 30-40% of the world's population and its development is determined by interplay between environmental and inherited factors. Air pollution, primarily consisting of diesel exhaust emissions, has increased at a similar rate to allergic disease. Exposure to diesel exhaust may play a role in the development and progression of allergic disease, in particular allergic respiratory disease. One potential mechanism underlying the connection between air pollution and increased allergic disease incidence is DNA methylation, an epigenetic process with the capacity to integrate gene/environment interactions. **Objective**: To investigate the effect of allergen and diesel exhaust exposure on bronchial epithelial DNA methylation. **Methods**: We performed a randomized crossover-controlled exposure study to allergen and diesel exhaust in humans, and measured single site (CpG) resolution global DNA methylation in bronchial epithelial cells. **Results:** Exposure to allergen alone, diesel exhaust alone, or allergen and diesel exhaust together (co-exposure) led to significant changes in 7 CpG sites at 48 hours. However, when the same lung was exposed to allergen and diesel exhaust but separated by approximately four weeks, significant changes in over 500 sites were observed. Furthermore, sites of differential methylation differed depending on which exposure was experienced first. Functional analysis of differentially methylated CpG sites found genes involved in transcription factor activity, protein metabolism, cell adhesion and vascular development, among others. **Conclusion:** These findings suggest that specific exposures can prime the lung for changes in DNA methylation induced by a subsequent insult.

**Key Messages**

- Appreciation for the role of DNA methylation in mediating immunologic effects of environmental exposures is growing.
- We used a controlled human exposure to diesel exhaust and allergen, each alone or in combination, followed by airway (conducting airway epithelium) sampling and global assessment of DNA methylation, to interrogate epigenetic changes due to these acute exposures.
- Exposure to diesel exhaust and allergen each incur risk for DNA methylation changes upon subsequent exposure, and the order of such exposures determines both unique and overlapping epigenetic signals.

**Capsule summary**

Combined diesel exhaust and allergen is known to intensify the airway immune response, relative to either exposure alone. We demonstrated that DNA methylation of epithelial cells might be a mechanism underpinning such synergy.

**Keywords:** Allergen, particulate matter, diesel exhaust, air pollution, epigenetics, DNA methylation

**Abbreviations:**

DEP, diesel exhaust particles; Th2, T helper type 2; Th17, T helper type 17; IgE, Immunoglobulin E; DE, diesel exhaust.

## Introduction

Allergic disease is increasing worldwide with approximately 30-40% of the world's population now affected[1]. The development of allergic disease is determined by an interaction between genetic and environmental factors[2]. Air pollution is one of the major environmental factors impacting the development and exacerbation of allergic diseases, in particular respiratory diseases including asthma and allergic rhinitis[3].

Diesel exhaust (DE) contributes the majority of particulate matter present in urban air pollution[4], and diesel exhaust particles (DEP) reach the alveolar/gas exchange regions[5]. DE increases bronchial inflammation and airways resistance in healthy subjects, while short-term exposure to diesel traffic reduces airway function in asthmatic patients[6]. Exposure to DE plays a major role in the development and progression of allergies[4, 5]. For example, high DEP exposure is associated with more frequent asthma symptoms in children with allergic asthma[7]. Interestingly, co-exposure to allergen and DE(P) increases allergen specific IgE, asthma severity, air inflammation, and airway responsiveness in humans or in mouse models[7-9].

The molecular mechanisms responsible for the synergy between DE and allergen remain unclear. As mutations occur too slowly to explain the recent rapid increase in allergy associated disease, epigenetics is a potential mechanism by which gene-environment interactions may rapidly influence disease incidence and progression[10]. DNA methylation was among the first epigenetic mechanisms to be identified[11] and is the most extensively studied in relationship to disease[12] and human populations[13]. It is the potentially reversible addition of a methyl group to DNA cytosine residues, primarily where a cytosine is followed directly by a guanine (CpG sites)[14]. Consistent with epigenetics serving as a potential mediator between environment and genome, differential DNA methylation of a number of genes involved in multiple cellular processes including immune responses[15-17], nitric oxide synthesis[18, 19], and DNA binding[16], has been associated with air pollution exposure. We have previously shown controlled exposure to DE alters the methylation of CpG sites in circulating mononuclear cells[20]. Similarly, exposure to ambient pollution is associated with increased DNA methylation and decreased expression of the forkhead box protein 3 (Foxp3) and interferon gamma (IFNγ) genes in regulatory and effector T cells respectively, leading to impaired regulatory T cell function and associated increased asthma morbidity[15, 21].

Studies of mechanisms underlying the interaction between allergy and DE exposure have been limited to animals or performed as observational studies (rather than controlled exposure studies), investigation of candidate targets rather than global alterations, or of easily accessible tissues such as blood[20], rather than sampling the primary site of exposure, the lung. Here we tested the hypothesis that exposure to allergen and/or diesel exhaust would alter DNA methylation in bronchial epithelial cells by performing a randomized crossover controlled exposure study to allergen and DE in humans. We collected bronchial epithelial cells, the primary cell type exposed to inhalants, and assessed changes in global DNA methylation in response to exposure. We report that exposure to allergen, DE, or allergen and DE as a co-exposure had modest effects on DNA methylation when assessed at 48 hours post-exposure. However, exposure to both allergen and DE, with the exposures separated by four weeks, was strikingly different, significantly altering bronchial epithelial cell global DNA methylation. These results support the hypothesis that DNA methylation can serve as a molecular mechanism underlying the interaction between allergens and particulate air pollution on respiratory health.

## Methods

### Study Demographics

Seventeen Caucasian participants (See Table 1 and Table 2) were recruited to the Air Pollution Exposure Laboratory (APEL) in Vancouver, British Columbia, Canada. Written consent was obtained from all subjects, and the protocols were approved by the institutional review board for human studies at the University of British Columbia. Participants were 20 to 46 years old (median = 27, SD = 7.8), all non-smokers and 47% were asthmatic (Table 1). We excluded individuals with any of the following: (1) pregnancy/breastfeeding, (2) use of inhaled corticosteroids, (3) regular use of bronchodilator medication (*i.e.*, use of bronchodilators more than 3 times per week), (4) unstable asthma symptoms, (5) any use of vitamins A, C, E or other antioxidant supplements, (6) co-morbid conditions judged by the investigators to increase risk of dropout, or (7) work in an industrial setting or other setting of significant inhaled exposures. Males (n=7) and females (n=10) were included. Sensitization to birch, Timothy/Pacific grass and house dust mite (Dermatophagoides pteronyssinus group 1) (Table 2) was tested by skin prick, using standardized extracts. Medical grade allergen extracts (Hollister-Stier, Spokane, WA) in solutions were used. A wheal ≥3 mm to at least one of those allergens was required for inclusion in the study. Subjects withheld long-acting $\beta_2$-agonists for 48 hours, short-acting $\beta_2$-agonists for 6 hours, long-acting anti-histamines for 14 days, non-steroidal anti-inflammatories and aspirin for 7 days, and short-acting anti-histamines for 3 days prior to skin test. A series of 10-fold dilutions of the test allergen were used to determine the lowest skin prick dose needed to elicit a 3-mm wheal, based on the strong correlation between the concentration of allergen leading to skin test positivity and that prompting airway responsiveness[22]. In those whose test allergen was birch, we required that they avoid apple, pear, sweet cherry, peach, plum, apricot, almond, celery, carrot, potato, hazelnut, mango, and chili pepper to minimize concerns for oral allergy syndrome.

### Exposure design and procedures

Subjects entered a crossover experiment [23-25] (Figure 1) using two conditions (diesel exhaust [DE] ($300\mu g$ $PM_{2.5}/m^3$) or filtered air [FA]), the order of which was randomized and counter-balanced. DE exposure used a previously-described system that excludes potential contamination with lipopolysaccharide[26], except that in the present study we utilized a 2.5kW constant load. The Air Pollution Exposure Laboratory (APEL) was designed for the controlled inhalation of human subjects to aged and diluted DE to mimic "real-world" occupational and environmental conditions. Notably, the protocol allows for effective blinding to both the subject[27] and those analyzing all material and data.

One hour following each exposure to DE or FA, bronchoscopy was performed to deliver a saline-controlled segmental allergen challenge (SAC). A 5 mL solution of allergen extract in a concentration 10-fold lower than that minimal dose producing a positive wheal was instilled in a lower lobe bronchial segment, and 5 mL control (normal saline) was instilled in a contralateral lower lobe bronchial segment. Photographs were taken of the segments instilled, to guide subsequent bronchoscopy as needed. DE exposure preceded allergen instillation to avoid the possibility that starting with segmental allergen would lead to acute segmental bronchoconstriction and thus decreased deposition of diesel particulate matter within that segment. 48 hours after allergen challenge, bronchial brushes of airway epithelial cells (AECs) were obtained in the same segments. Side (right versus left) of lower lobe segmental challenge and sampling were reversed in the post-4 week period bronchoscopies (yielding samples 3,4,7 and 8 in Figure 1B) and the exact segment utilized for the pre-4 week period challenge and sampling was avoided in the post-4 week phase (to avoid artefact). Four samples did not generate sufficient DNA for analysis leaving 64 samples in total. All four samples were from the same individual (samples 5-8 of Figure 1A). 8 full sample sets remained for both groups and methylation analysis was not affected

### DNA isolation and DNA methylation arrays

Genomic DNA was isolated from bronchial brush samples, bisulfite converted and evaluated by Illumina Infinium HumanMethylation450 BeadChip array as described previously[28]. All procedures

were conducted using commercially available kits and done following the manufacturers' protocols. Bronchial brush samples were confirmed as >90% bronchial epithelial cells by light microscopy following cytospin and hematoxylin/eosin staining. Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Valancia, CA, USA). 750ng of the purified DNA was bisulfite-converted using the EZ-DNA methylation kit (Zymo Research, Orange, CA, USA), changing epigenetic data into sequence-based data by selective conversion of unmethylated cytosines to uracils. Bisulfite-converted DNA was assessed for concentration and quality using the NanoDrop, and 160ng of the conversion product was used for genome-wide DNA methylation evaluation at over 485,000 CpG sites using the Illumina Infinium HumanMethylation450 BeadChip array, as described previously[28].

**Data quality control and normalization**

Illumina GenomeStudio software was used to obtain raw signal intensities that were imported into R for quality control, normalisation, and statistical analysis. Any probes for which detection p-values were greater than 0.01, probes with missing beta values, and probes for which less than three beads contributed to the signal in 1 or more samples were excluded (29,587 probes removed). 11,648 probes residing on the X or Y chromosome were removed to control for gender-derived differences on the array. Finally, probes known to be polymorphic at the CpG or to cross-hybridize to somatic sites and sites on the X or Y chromosome[29], and those examining single nucleotide polymorphisms were removed (additional 28,211 probes removed). This left 416,066 probes for 64 samples from the 16 subjects for analysis. Colour correction, background adjustment and quantile normalisation were performed using the lumi R package and the data were normalised using Subset-quantile Within Array Normalization (SWAN) [30]. ComBat[31, 32] was used to remove any effects of batch (samples were run across two batches), chip (10 chips) and array location (12 arrays per chip). Average replicate correlations (mean ± SD of 6 replicates) increased from 0.9967 ± 0.0011 to 0.9976 ± 0.003 following ComBat. Two values of DNA methylation were calculated, beta-values (β-values) and M-values. β-values are the ratio of all methylated probe intensities over total signal intensities (methylated and unmethylated) and have a range from 0 to 1. They approximately represent percent methylation. M-values are log transformation of β-values and more statistically robust[33]. All statistical analyses were performed using M-values. Visualisation and discussion are presented as β-values.

**Differential methylation analysis**

All statistical analysis was performed using R statistical software. Probes were filtered to include only variable probes (probes which have a β-value standard deviation of greater than 0.05 across all samples; 43,485 probes). Probes with DNA methylation levels significantly different between the samples stated in the results section were identified using the R lm linear modelling function (paired where appropriate) followed by q-value calculation to control for false discovery rate (FDR) at 0.05. We did not control for participant demographics (for example age, sex, asthmatic status) as these were balanced between study group (Table 1 and 2) and our initial analysis ensured there was no difference in DNA methylation due solely to study group. A filtering step for potential biological effects was then performed to leave only probes that also had a β-value difference of greater than 0.1 (equivalent to a 10% change in methylation between groups).

**DAVID Functional Enrichment Analysis**

UCSC refgene accession-IDs corresponding to the 43,485 variable CpG probes were used as background for DAVID GO analysis[34]. UCSC refgene accession-IDs corresponding to our significant hits were input for DAVID GO analysis. Clusters with enrichment scores greater than 1.3 were considered significant.

**Bisulfite PCR-pyrosequencing**

Bisulfite PCR-pyrosequencing was used to validate differences in DNA methylation at select CpG sites. Bisulfite PCR-pyrosequencing assays were designed with PyroMark Assay Design 2.0 (Qiagen). The regions of interest were amplified by PCR using the HotstarTaq DNA polymerase kit (Qiagen) as follows: 15 minutes at 95°C (to activate the Taq polymerase), 45 cycles of 95°C for 30s, 58°C for 30s, and 72°C for 30s, and a 5 minute 72°C extension step. For pyrosequencing, a single-stranded DNA

was prepared from the PCR product with the Pyromark™ Vacuum Prep Workstation (Qiagen) and sequencing was performed using sequencing primers on a Pyromark™ Q96 MD pyrosequencer (Qiagen). The quantitative levels of methylation for each CpG dinucleotide were calculated with Pyro Q-CpG software (Qiagen). Primer sequences are listed in Supplementary Table 4.

## Results

### Study cohort and samples

To evaluate the effects of allergen and diesel exhaust exposure on DNA methylation in the human lung, we performed a rigorous randomized crossover controlled exposure study. Specifically, 17 individuals were randomized into two study groups (Group I and Group II), defined by whether filtered air (FA) or diesel exhaust (DE) was inhaled first (Figure 1A). Following inhalation of FA or DE, allergen was instilled into one lung and saline into the other. The process was repeated approximately 4 weeks later with opposite exposures. This resulted in 4 exposure conditions per subject: FAS (filtered air and saline), DES (diesel exhaust and saline), FAA (filtered air and allergen) and DEA (diesel exhaust and allergen). The resulting samples were numbered and are defined in Figure 1B. Importantly, in contrast to previous studies[12], we collected bronchial epithelial cells (BEC), the primary cell type exposed to inhalants. The average percentage of bronchial epithelial cells in the brushings was high and consistent across all conditions (97.3% for FAS, 96.9% for DES, 95.4% for FAA, 98.7% for DEA). We measured DNA methylation in genomic DNA extracted from BECs obtained from the four conditions at each time point, and in the majority of cases unpaired analyses were performed. However, where possible a paired comparison was performed and little difference identified (Figure S1 A and B and supplementary results section). Initial analysis revealed that no CpG sites were significantly differently methylated between Group I and Group II with an q-value< 0.05 (See Supplementary Results and Figure S2 for further detail).

### Initial exposure had minimal effect on DNA methylation

After determining that there were no DNA methylation differences between Group I and Group II (defined by whether filtered air (FA) or diesel exhaust (DE) was inhaled first (Figure 1A)), we tested the effect of isolated exposures and co-exposures. To do this, we investigated the effect of allergen exposure only (2.FAA vs 1.FAS), DE only (5.DES vs 1.FAS) and allergen and DE co-exposure (6.DEA vs 1.FAS) (Figure 1B). In these tests, no sample had received any prior study exposure. We did not identify any CpGs that had a significant change in methylation associated with allergen. Six CpGs were significantly altered by DE and seven CpGs by co-exposure. Of these, five CpGs were common between DE and co-exposure (Table S5). These data suggested that exposure of BECs to either allergen, DE, or both (co-exposure) had minimal effects on DNA methylation within the 48-hour time period that elapsed from exposure to sample collection.

### DNA methylation was significantly affected by allergen in lung previously exposed to DE

Given the limited effects of single exposures, we subsequently tested whether allergen and DE exposure altered DNA methylation when administered individually to the same lung but separated by 4-weeks. Specifically, we assessed differences in DNA methylation in BECs exposed to allergen following exposure to DE 4 weeks earlier (7.FAA) versus those completely unexposed (1.FAS), using linear modelling. The deviation of the raw p-value distributions from random suggested an association of allergen administration and DNA methylation in BrECs previously exposed to DE (Figure 2A). This was statistically significant as we identified 150 differentially methylated CpGs at a q-value <0.05 (red points Figure 2B). To assess the likelihood of statistically significant CpGs having biological impact the absolute difference between the means of the β values of the exposures were calculated and referred to as the delta beta (Δβ). We considered a Δβ of 0.1, corresponding to a 10% difference in CpG methylation, to be of biological interest. 75 probes had a q-value <0.05 and a minimum Δβ of 0.1 (green points Figure 2B and Figure 2C). Of these CpGs, 70 showed a decrease in DNA methylation in response to allergen after prior DE exposure, relative to the control (1.FAS) condition (Figure S3A). In contrast, only 5 probes displayed an increase in DNA methylation (Figure S3B).

Upon further investigation we found that, while not reaching statistical significance, the initial DE exposure (5.DES) caused an intermediate change in DNA methylation, in the same direction (Figure 2D, decrease and Figure 2E, increase) as that caused by sequential DE and allergen exposure. These data suggested that an initial DE exposure might prime DNA methylation of certain CpG sites within BECs and that upon subsequent allergen exposure these sites underwent further DNA methylation modification, resulting in a significant difference in DNA methylation relative to unexposed cells.

The 75 CpG sites differentially methylated in response to allergen following prior DE exposure were primarily in regions of low or intermediate CpG density (Figure 3A), significantly different from the distribution of the full analysis probe set (chi-squared p-value <0.05). Significant sites were within 0.5Mb of a transcription start site (TSS; red points Figure 3B), while the full analysis probe set fell in DNA regions up to 2Mb of a known TSS (black points Figure 3B), however this enrichment did not reach statistical significance (chi-squared p-value 0.3037). 40 probes were downstream of a TSS while 35 probes were upstream. The closest TSS to the 75 differentially methylated CpG sites were associated with 58 different genes (Supplementary Table 1). Several of these had more than one differentially methylated CpG associated with them, including 7 genes that had 2 or more (bold text Supplemental Table 1). Of particular note, we found a significant decrease in DNA methylation in response to allergen following prior DE exposure in 8 CpGs located upstream of the TSS of the TBX3 gene, a member of T-box family of transcription factors (Figure 3C). We confirmed these data using pyrosequencing of cg02824888 (Figure S4), which also showed significant (Mann-Whitney p<0.005) decrease in DNA methylation in response to allergen with prior DE exposure, with the difference in DNA methylation being comparable between the two technologies (10.7% by array versus 16.3% by pyrosequencing, Figure S5). Moving from single gene analysis to broader biological functions, we performed functional analysis using the DAVID Gene Ontology tool and identified two clusters above the recommended 1.3 enrichment score cutoff (1.58 and 1.55), which highlighted genes involved in protein metabolism and hormone/steroid stimulation among the 58 genes differentially methylated after subsequent exposure to DE and allergen (Figure S6).

**DNA methylation was significantly affected by DE exposure in lung previously exposed to allergen**

Having found significant changes in DNA methylation in lungs in response to DE exposure followed by allergen, we next asked whether the reverse order of exposures would result in similar effects. Specifically, we again used linear modelling to assess changes in DNA methylation in BECs exposed to DE following exposure to allergen 4 weeks earlier (4.DES) versus those completely unexposed (1.FAS). The deviation of the raw p-value distributions from random suggested an effect of DE on DNA methylation in BECs exposed to allergen (Figure 4A). Further, 22,904 CpGs showed a significant difference in methylation at a q-value <0.05 (red points Figure 4B), with 548 of those having a minimum $\Delta\beta$ of 0.1 (green points Figure 4B and Figure 4C). 10 CpGs were in common between the 75 differentially methylated in response to allergen after prior DE exposure (Supplemental Table 3). Of the CpGs with a minimum of 10% change in DNA methylation, 528 probes had a decrease in DNA methylation in response to DE after prior allergen exposure (Figure S6), relative to the control (1.FAS) condition whereas 20 probes had an increase in DNA methylation.

Again, while not sufficient to reach statistical significance, the initial allergen exposure (2.FAA) caused an intermediate change in DNA methylation, in the same direction as that caused by sequential allergen and DE exposure (Figure 4D, decrease and Figure 4E, increase). These data suggest that an initial allergen exposure primes DNA methylation of certain CpG sites (that differ from those altered by an initial DE exposure, as noted above) within BECs and that, upon subsequent DE exposure, these sites undergo further modification to DNA methylation, resulting in a statistical difference in DNA methylation compared to unexposed cells.

Of the 548 probes differentially methylated in response to DE after prior allergen exposure, close to 50% were in regions of low CpG density (Figure 5A), which differed from the distribution of the full analysis probe set (chi-squared p-value < 0.001). Furthermore, the differentially methylated CpG sites

were enriched within 0.5Mb of a TSS compared to the full data set (Chi-squared p-value 0.0002) (red points Figure 5A). 260 probes were downstream of a TSS and 288 probes were upstream. The closest TSS to the 548 differentially methylated sites were associated with 450 different genes (Supplementary Table 2). 63 of these had 2 or more differentially methylated CpGs associated with them (bold text Supplemental Table 2). In particular, we found a significant decrease in DNA methylation in response to DE following prior allergen exposure in 19 CpGs associated with genes of the Hox family. Five CpGs associated with HOXA3 (Figure 5C), three CpGs with HOXA4 (Figure 5D), 7 CpGs with HOXB1 (Figure 5E) and three CpGs with HOXB3 (Figure 5F). We confirmed these data using pyrosequencing of cg18680977 of the HOXA3 gene, cg11532431 of the HOXA4 gene, cg26634219 of the HOXB1 gene and cg11060532 of the HOXB3 gene (Figure S4). All showed significant decreases in DNA methylation in response to DE with prior allergen exposure, with the difference in DNA methylation being comparable between the two technologies (Figure S5). Functional analysis using the DAVID Gene Ontology tool identified 5 clusters (enrichment scores; 1.96, 1.43, 1.36, 1.35 and 1.34) and highlighted genes involved in biological/cell adhesion, protein localisation/transport, vascular development/angiogenesis, transcription factor activity/DNA binding and cell motion/migration among the 450 genes associated with the 548 CpGs differently methylated after subsequent exposure to allergen and DE (Figure S6B).

**Discussion**

The molecular mechanisms regulating the interaction between air pollution and allergic disease are poorly understood. Defining these mechanisms is critical for creating strategies to reduce susceptibility and severity of airways disease[8], especially given dynamics associated with both air pollution and allergens associated with global climate change[35]. DNA methylation may be one of the mechanisms by which environmental exposures, including DE, can exert lasting effects on gene expression, cell function, and human health at a rate consistent with the rapid increase in allergic disease in recent decades[8]. However, current evidence linking DNA methylation to the response to inhaled allergens and traffic pollution comes primarily from animal studies and observational human studies and has focused on repetitive DNA elements[36 37]. While we have previously shown controlled diesel exhaust exposure to alter CpG methylation in peripheral blood mononuclear cells[20], we are not aware of any previous controlled *in vivo* human study investigating global DNA methylation responses to DE in conjunction with allergen, and we pursued this given the commonality of these particular exposures and our desire to embrace the complexity beyond traditional single exposure models.

Here, we used a sequential exposure paradigm in combination with DNA methylation profiling of intact (*in vivo*) human lung cells to test whether multiple carefully characterized exposures over a period of weeks had compounding effects. Our major finding was that prior exposure to allergen or DE significantly altered global DNA methylation in the human epithelium upon subsequent exposure, approximately one month later, to the alternative exposure. Furthermore, the order of these exposures resulted in different patterns of altered DNA methylation. This finding suggests that DNA methylation was highly sensitive to differences in short-term exposure, which has implications for understanding epigenetic dynamics and moving towards preventive measures. Notably, while the methylation of thousands of CpG sites changed when DE and allergen were given sequentially and separated by 4 weeks, only a few had altered methylation 48 hours after initial exposure, suggesting a sequential insult on the lung was *required* to significantly alter DNA methylation. In agreement with this, samples collected from a lung exposed to allergen and diesel exhaust co-exposure 4 weeks previously but without additional exposure through a four week period (8.FAS, compared to 1.FAS) demonstrated no CpGs with significantly altered DNA methylation (Figure S7 vs Table S5) other than those already demonstrated after initial DE or DEA exposure (Table S5). We suggest that the initial exposure in the sequence did alter methylation of CpG sites to an intermediate (non-significant) level , and that the second exposure then caused further modification of methylation that reached our statistical thresholds. Of interest, in our study the sample used to assess the effect of DE in a lung previously exposed to allergen was taken from a location distinct to that where the allergen was earlier instilled. This suggests that local allergen instillation can prime the wider lung for future

exposure. In support of our observation, published studies instilled allergen in the right middle lung lobe and observed significant effects (eosinophil infiltration) in a separate saline-instilled (control) lobe[38].

Probing in detail, within the broader insights relating to the potential additive effects of DE and allergen, we identified particular genes whose DNA methylation pattern was associated with these agents in our sequential exposure paradigm. The gene associated with the greatest number of differentially methylated CpG sites in response to allergen following prior exposure to DE was TBX3. TBX3 and closely related TBX2 act as transcriptional repressors with roles in vertebrate development, cell fate, cell differentiation and cell cycle progression. Both are up-regulated in tumours and can cause bypass of cell senescence. *Tbx-2*-deficient mice display hypoplastic lungs with reduced branching morphogenesis and decreased mesenchymal and epithelial cell proliferation suggesting a critical role for these proteins in lung development[39]. Four of the Hox family of genes were associated with differentially methylated CpG sites in response to DE following prior exposure to allergen, HOXA3, HOXA4, HOXB1 and HOXB3. Hox genes encode homeodomain transcription factors that determine cell and tissue identities in the developing embryo and patterning of the developing mouse lung[40].

The majority of the changes in DNA methylation we observed were reductions after exposure. Air pollution induces oxidative stress which is thought to favour the demethylation process and cause lesions to DNA which prevent binding of DNA methyltransferases, thus potentially resulting in decreased methylation[10]. The detailed molecular mechanisms regulating the DNA methylation changes we observed should be investigated further via *in vitro* exposure of BECs to diesel exhaust and allergen.

Limitations of our study include the decision to recruit both asthmatic and non-asthmatic individuals knowing that we would not have statistical power to test the modifying effect of asthma status on changes in DNA methylation after allergen or DE exposure. Here we targeted those with atopy, but not necessarily asthma, as we were interested in the immune response in those sensitive to common aeroallergens. We knew that if we were to limit by asthmatic status then recruiting to a sample size with sufficient statistical power would be difficult given the demanding nature of the protocol. At inception we intended to restrict analysis to intra-subject comparison, given the crossover design, but when this became impossible we used other methods to check for demographic confounding. First we confirmed no statistical difference in methylation between the two groups of individuals in "unexposed" conditions. Second, we performed additional paired analyses (each subject serving as his/her own control) where possible (Figure S1). Importantly, the initial allergen alone exposure maintained a non-significant effect, and the DE with prior allergen exposure (4.DES vs 1.FAS) altered minimally, increasing statistically significant hits from 22904 to 23379; however the CpGs with a statistically significant change and a delta beta of >0.1 was maintained at 547 versus 548 in the unpaired analysis. This gives us confidence that individual characteristics, including asthmatic status, age and sex do not confound the data. This also applies to the fact we included subjects reactive to different allergens, primarily to increase our overall sample size. Furthermore, a recent examination of allergen effect on human airways showed no such effect modification on outcomes related to ours (sputum inflammatory cells or methacholine responsiveness)[41]. Furthermore, we recognize that the changes in DNA methylation after 4 weeks could be partially reflective of inflammation related to the stress of the prior bronchoscopy (independent of the exposures), but this concern is attenuated by the expectation that bronchoscopy-related inflammation would have resolved within the 4-week interval. Furthermore, the very high percentage of bronchial epithelial cells in the bronchial brushings is important in ensuring that observed changes in DNA methylation are not simply reflective of a changing distribution of cell types. Finally, it is important to consider the potential for previous exposure to external environmental inhalants to confound the results of our study. While the potential for such an effect cannot be eliminated entirely, all subjects were non-smokers and living in Metro Vancouver. Thus, it is unlikely that they are exposed to significant levels of air pollution (typical local exposures would be an order of magnitude below the level we use in our study). Also any undetected

incidental extra-experimental exposures would be non-differential and thus bias to null results, such that we might miss weaker effects but the significant findings would be then the stronger effects.

Since air pollution contributes to 6% of total mortality and 50% of mortality caused by air pollution is attributed to motorised traffic[42], the global relevance of these findings is already considerable. A recent study reporting related effect of sequential ultrafine particle and allergen exposure on bronchoalveolar lavage cell composition[43], adds further strength to the observations and warrants the initiation of larger studies to investigate this phenomenon in greater detail. There may be even more impact if this opens inquiries into whether similar additive effects occur with other exposures beyond air pollution. In spite of the complexity of epigenomics, we embraced the fact that epigenetic responses to environmental exposures are likely to depend on the timing, length and potency of the exposure and aim to understand these complexities so that we can leverage this knowledge towards preventive measures[44]. We enhanced the biological relevance of the current study by a) using a controlled exposure rather than an observational setting, which is prone to residual confounding; b) using inhalation of whole DE rather than DEP, providing a more realistic inhalant and route of exposure; c) using DNA from bronchial epithelial cells, the first line of defence for the lung in response to inhaled exposures rather than a surrogate tissue (i.e. blood).

In summary, we show that while a single short term exposure to either allergen, DE, or both as a co-exposure causes only minor changes in DNA methylation, sequential insults with allergen and DE separated by a 4-week period mediates significant changes in CpG site methylation. Although requiring further investigation, we suggest the initial insult acts to prime the methylome for the second resulting in an additive methylation change with potential biological relevance. Our data adds biological plausibility to evidence that air pollution and allergen have significant effects on cell biology and are relevant to policy makers who want evidence from models that embrace the complexity of the environment we inhale.

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**Figure Legends**

**Figure 1) Study design (A) and explanation of sample naming according to group status (B).** Individuals in Group I were first exposed to filtered air (FA); those in Group II were first exposed to diesel exhaust (DE). Post-exposure, allergen and saline wereinstilled 48 hours later bronchial brushings were taken for epithelial cell DNA isolation. 4 weeks later the process was repeated with opposing exposures. (filtered air/saline (FAS), filtered air/allergen (FAA), diesel exhaust/saline (DES) and diesel exhaust/allergen (DEA)).Samples 1-4 associated with Group I and samples 5-8 associated with Group II.

**Figure 2) CpG methylation was significantly affected by allergen in lung previously exposed to diesel exhaust.** A) p-value distribution for the effect of allergen in lung with prior DE exposure (7.FAA vs 1.FAS). Dashed line indicates distribution expected by chance; B) Plot of 43,485 variable CpG probes. Red=q-values < 0.05: green= q-value<0.05 *and* Δβ >0.1 C) Beta values of probes significantly different between control (1.FAS) and 7.FAA with a Δβ >0.1 (75 total probes). Each vertical band is a study subject. Blue: high methylation; yellow: low methylation; D/E) Reduction/increase in DNA methylation (Δβ), relative to 1.FAS, amongst 70/5 hits following the intermediate diesel exhaust exposure. Points are individual CpG sites and lines indicate mean ± SEM.

**Figure 3) Characteristics of the CpGs affected by allergen in lung previously exposed to diesel exhaust.** A) CpG island type for all analysis probes and the 75 top hits. LC = low CpG density, IC = intermediate CpG density, ICshore = intermediate CpG density overlapping with a high-density region, HC = high CpG density[29]; B) Relationship of Δβ and distance to the transcriptional start site. Red=q-value<0.05 and Δβ of >0.1; B) C) Individual CpG site plots of the 8 CpG hit sites associated with the TBX3 gene. Whiskers represent the minimum and maximum of all the data.

**Figure 4) CpG methylation was significantly affected by diesel exhaust in lung previously exposed to allergen.** A) p-value distribution for the effect of DE in lung with prior allergen exposure (4.DES Vs 1.FAS). Dashed line indicates distribution expected by chance; B) Plot of 43,485 variable CpG probes. Red=q-value < 0.05; green=q-value<0.05 *and* Δβ >0.1 : C) Heatmap of beta values of probes that were significantly different between control (1.FAS ) and 7.DES (DE with prior allergen) with a Δβ >0.1 (548 probes total). Each vertical band is a study subject. Blue: high methylation; yellow: low methylation; D/E) Reduction/increase in DNA methylation (Δβ), relative to 1.FAS, amongst 528/20 hits following the intermediate allergen exposure. Points are individual CpG sites and lines are mean ± SEM.

**Figure 5) Characteristics of the CpGs affected by diesel exhaust in lung previously exposed to allergen.** A) CpG island type for all analysis probes and the 548 top hits. LC = low CpG density, IC = intermediate CpG density, ICshore = intermediate CpG density overlapping with a high density region, HC = high CpG[29]; B) Relationship of Δβ and distance to the transcriptional start site. Red=q-value<0.05 and Δβ of >0.1; C-F) Individual CpG site plots of the CpG sites associated with 4 HOX genes (C) HOXA3, D) HOXA4, E) HOXB1, F) HOXB3). Whiskers represent the minimum and maximum of all the data.

## Supplementary Results

### Study group status had no effect on DNA methylation

As our analysis plan involved comparisons of samples that spanned our two study groups, our first analysis assessed significant differences in CpG-specific methylation due specifically to group assignment. We used linear modelling to compare two samples, one from each group, with the exact same study exposure; 3.DEA and 6.DEA. Both samples were collected from lungs 48 hours after co-exposure to DE and allergen with no prior allergen or DE exposure. The resulting unadjusted p-value distribution (Figure S1A) showed skewing towards high p-values, suggesting a low likelihood of differential CpG methylation by group assignment beyond than that expected by chance, thus allowing the subsequent between group comparisons of study exposure. Furthermore no sites were significantly different at an FDR of 0.05.

### Initial exposure had minimal effect on DNA methylation

Analyses inclusive of DE were unpaired to allow the same model to be run for all comparisons regardless of whether they were within or between groups. For allergen-only analysis, a paired model was possible and performed as both samples were taken from the same group of individuals, but we observed little such difference (Figure S2A).

### DNA methylation was significantly affected by DE in lung previously exposed to allergen.

As the samples used in the analysis of DE in lung previously exposed to allergen were all taken from Group I, i.e. from the same individuals, we also performed a paired analysis. This analysis found 23,379 CpGs that were significantly differentially methylated after BH correction between the control samples and the DE after prior allergen exposure samples, compared to the 22,904 identified by non-paired analysis (Figure S2B). When we applied the filter of a $\Delta\beta > 0.1$, 547 probes remained in comparison to 548 probes identified by non-paired analysis.

## Supplemental Figure Legends

**Supplemental Figure 1) Comparison of paired Vs non-paired analysis.** Paired vs unpaired p values for A) 2.FAA vs FAS and B) 4.DES vs 1.FAS.

**Supplemental Figure 2) CpGs methylation did not significantly differ between Group I and Group II.** A) p-value distribution for the effect of group population differences (3.DEA *versus* 6.DEA). The uniform distribution expected by chance is indicated with a horizontal dashed line

**Supplemental Figure 3) Individual CpG site methylation changes after exposure to allergen with prior diesel exhaust exposure.** A) 70 CpG sites that decrease in methylation; B) 5 CpG sites that increase in methylation

**Supplemental Figure 4) Bisulfite PCR-pyrosequencing of selected CpG site methylation.** A single CpG site per gene was selected for pyrosequencing analysis.. Data shown are mean ± SEM. *p < 0.05, **p < 0.01 for comparison of 1.FAS with 7.FAA/3.DES by unpaired Mann-Whitney test.

**Supplemental Figure 5) Assessment of the agreement between array and pyrosequencing analysis.** A single CpG site per gene was selected for pyrosequencing validation of methylation changes identified by array analysis.

**Supplemental Figure 6) David functional analysis.** A) DAVID functional annotation clusters for the 75 top hit probes after exposure to allergen with prior diesel exhaust exposure; C) DAVID functional annotation clusters for the 548 top hit probes after exposure to diesel exhaust with prior allergen exposure.

**Supplemental Figure 7) Individual CpG site methylation changes after exposure to allergen with prior diesel exhaust exposure.** A) 528 CpG sites that decrease in methylation; B) 20 CpG sites that increase in methylation.

**Supplemental Figure 8) Delayed effect of co-exposure on DNA methylation.** p-value distribution for the effect of delayed DEA exposure (1.FAS Vs 8.FAS). The uniform distribution expected by chance is indicated with a horizontal dashed line. Table shows the names and q-values of the CpGs that were significantly different following delayed co-exposure.

**Supplemental References**


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**Figure No.1**

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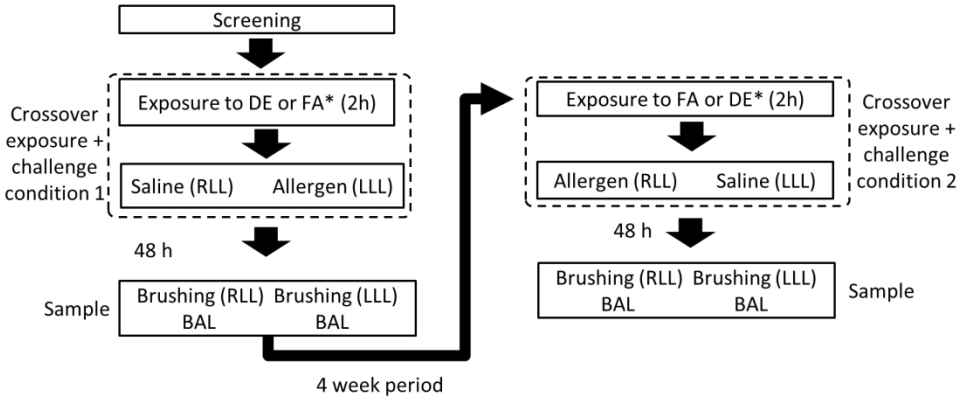
A)

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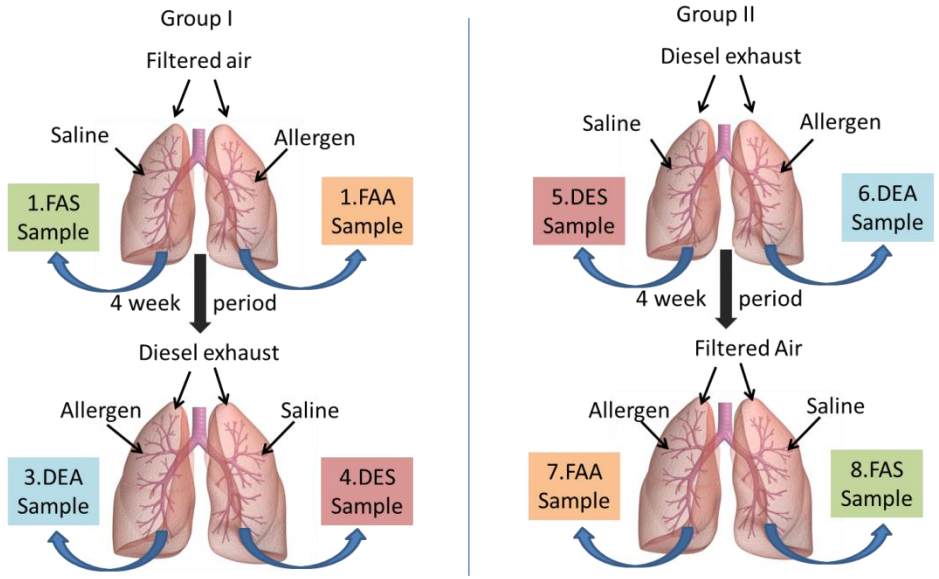

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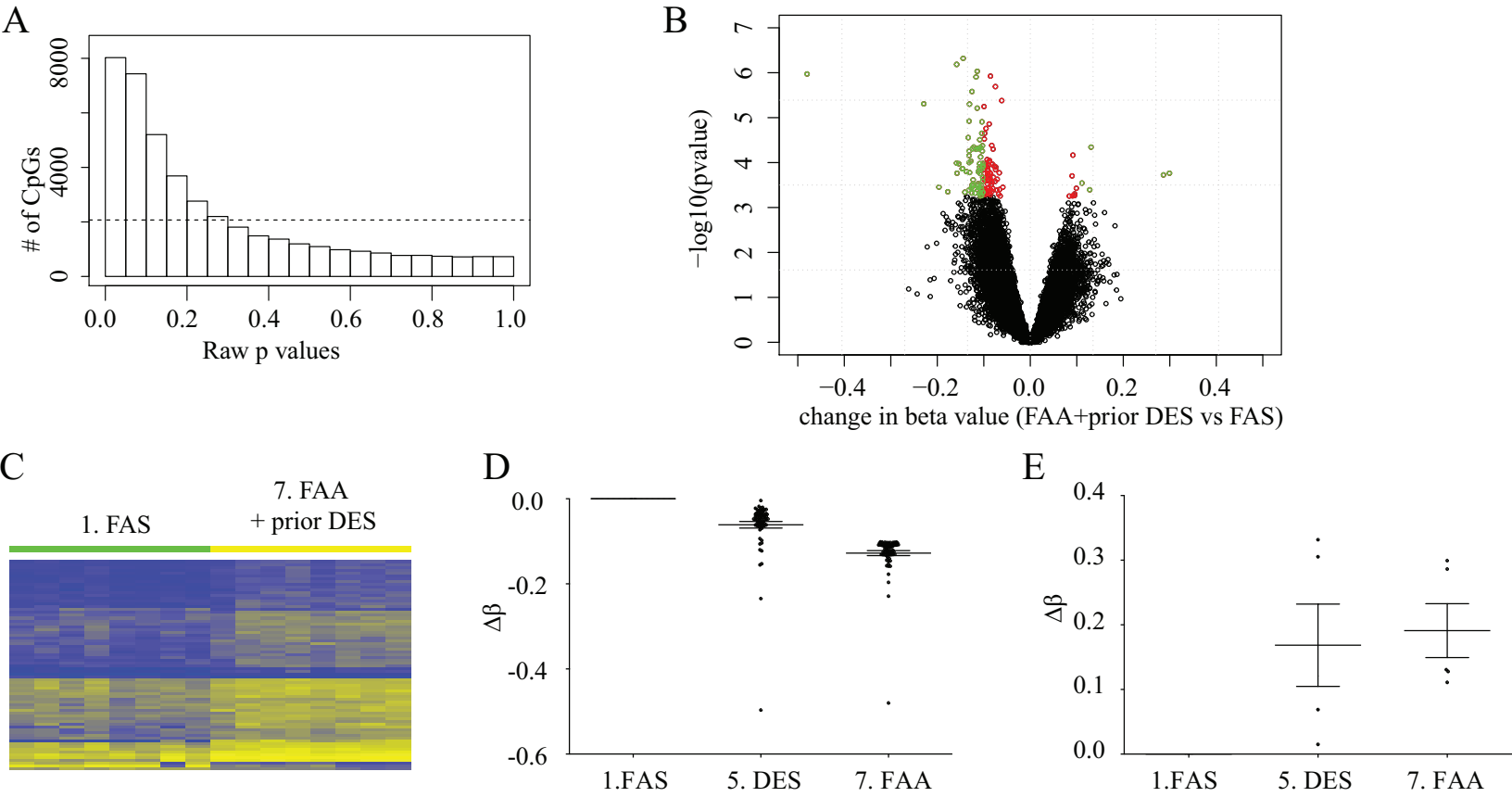

**Figure No.3**

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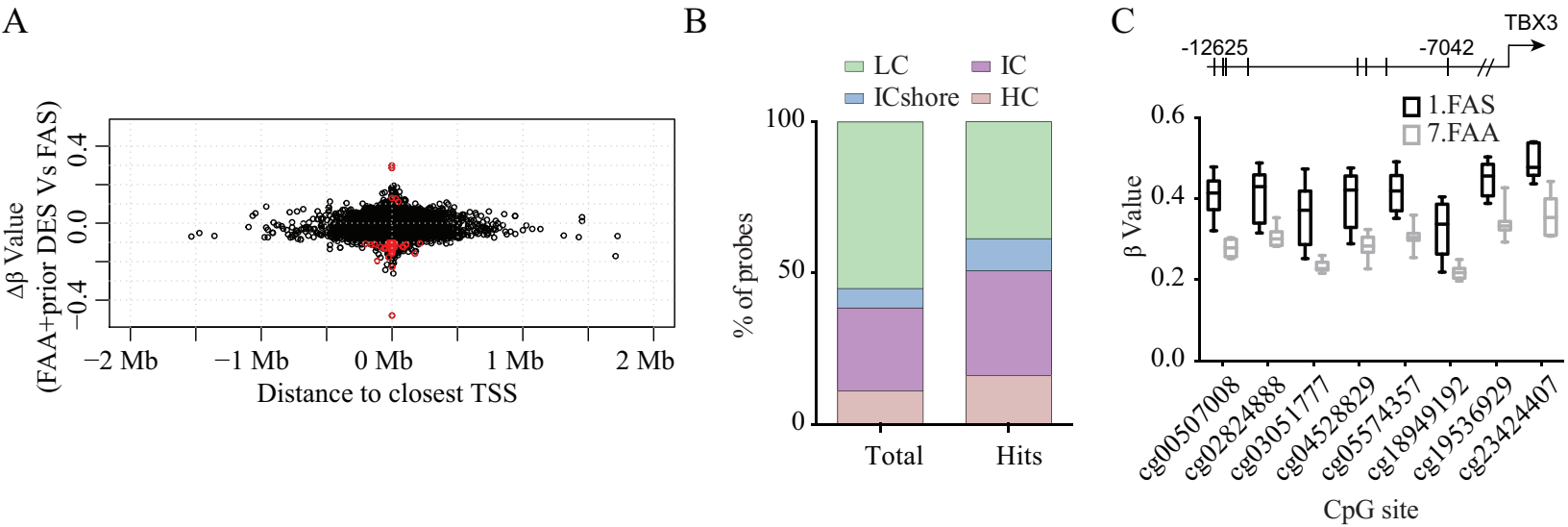

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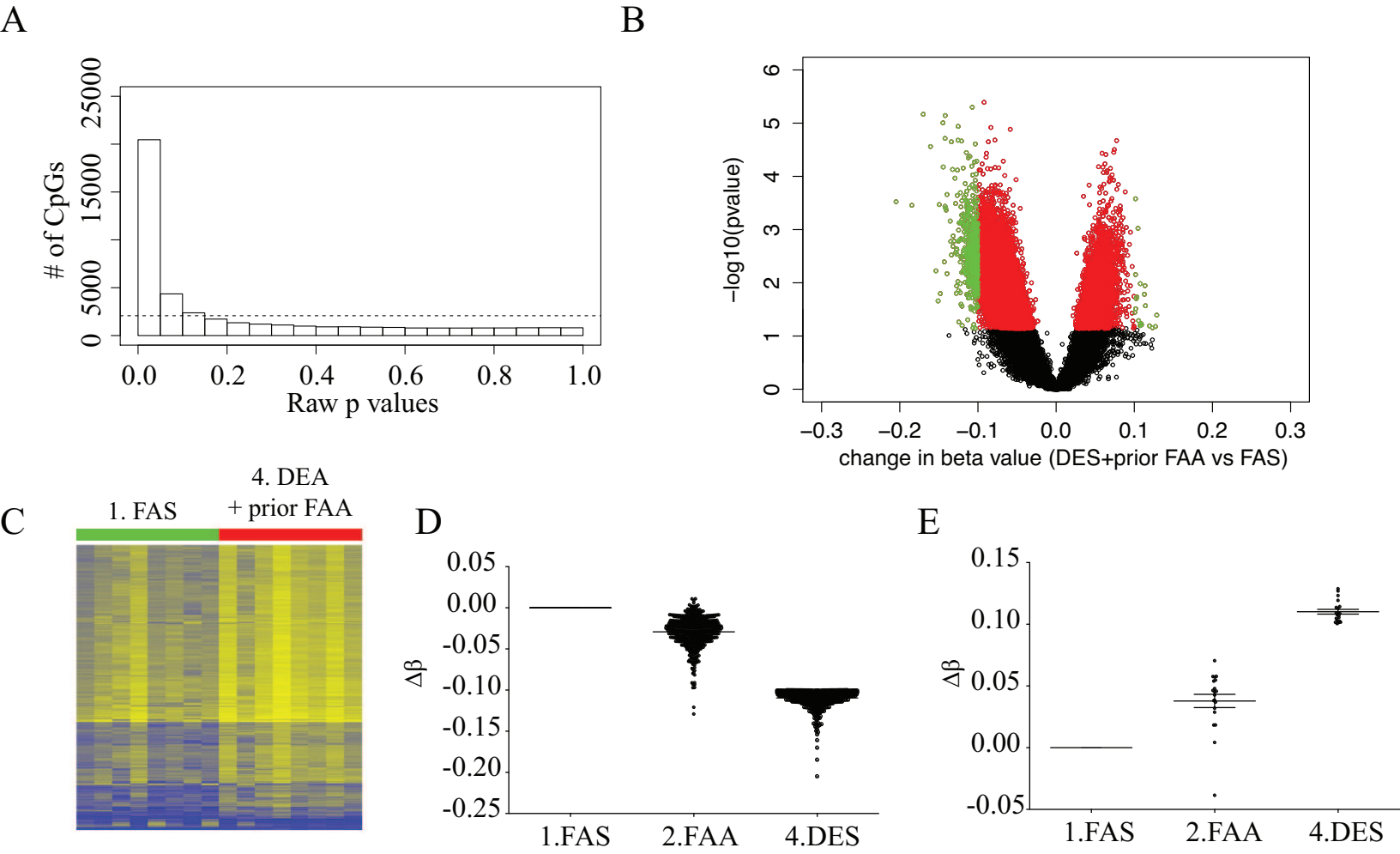

**Figure No.5**

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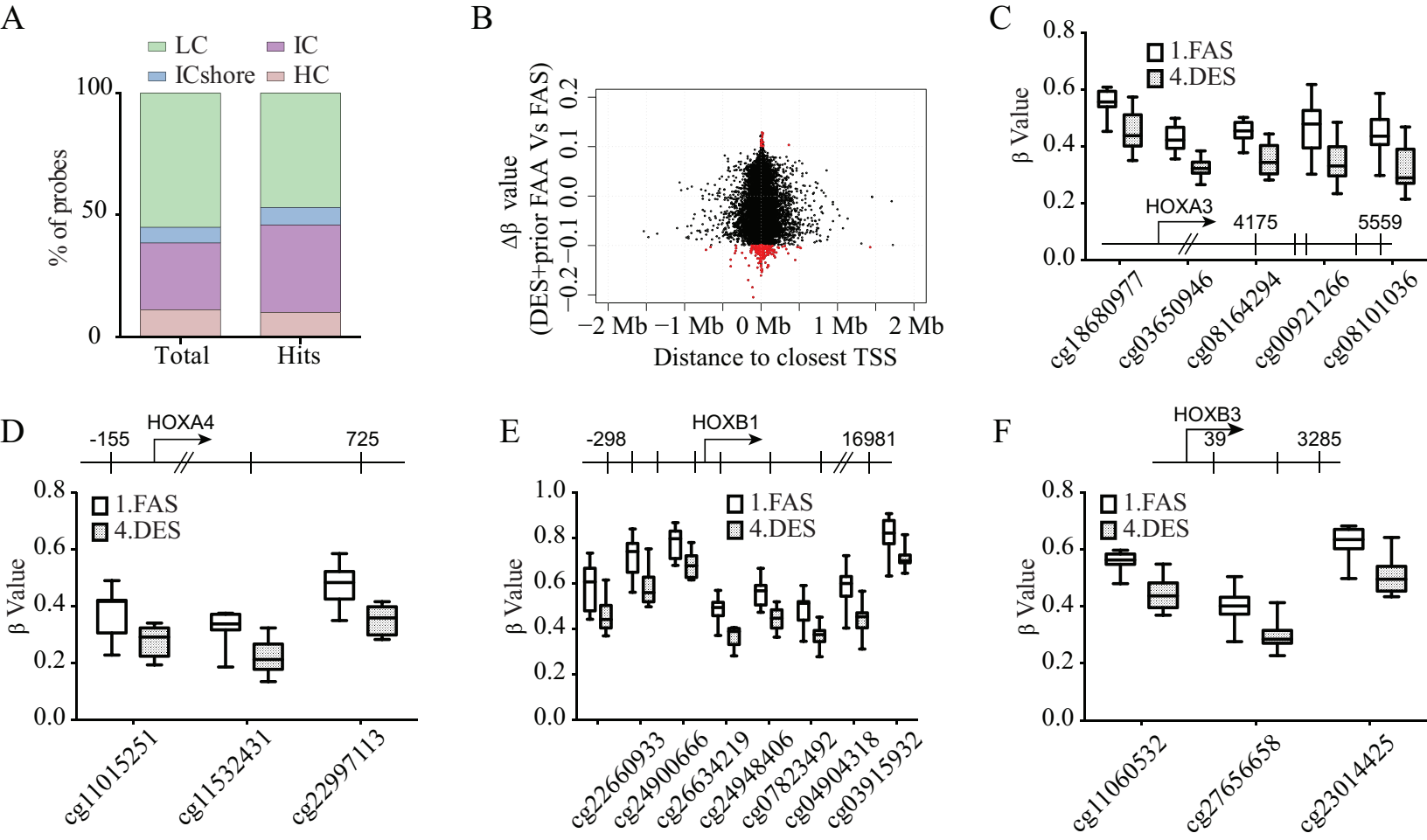

**Table 1**

Table 1: Grouped Participant Demographics

| | Group I (n=8) | Group II (n=9) | p-value |
|---|---|---|---|
| Age (years) | 29.50 ± 2.771 | 27.33 ± 2.718 | 0.5859 (t-test) |
| Sex | Male:3; Female:5 | Male:4; Female:5 | 1 (fishers exact) |
| **Asthmatic Status** | Asthmatic:3 ; Nonasthmatic:6 | Asthmatic:5; Nonasthmatic:4 | 0.6372 (fishers exact) |

**Table 2**

Table 2: Individual Participant Demographics

| Subject | Age | Gender | Asthmatic? | Allergen |
|---|---|---|---|---|
| **DE-53** | 20 | Female | Yes | D. pterinyssinus |
| **DE-58** | 31 | Female | No | D. pterinyssinus |
| **DE-70** | 24 | Female | Yes | D. pterinyssinus |
| **D2E-81** | 32 | Male | No | Timothy/Pacific Grass |
| **DE2-90** | 34 | Female | Yes | Timothy/Pacific Grass |
| **DE2-94** | 27 | Male | No | D. pterinyssinus |
| **DE2-96** | 25 | Female | No | D. pterinyssinus |
| **DE2-103** | 46 | Male | No | Birch |
| **DE2-105** | 27 | Male | Yes | Timothy/Pacific Grass |
| **DE2-106** | 46 | Female | Yes | D. pterinyssinus |
| **DE2-107** | 20 | Female | No | Timothy/Pacific Grass |
| **DE2-109** | 31 | Female | No | Birch |
| **DE2-112** | 28 | Male | Yes | Timothy/Pacific Grass |
| **DE2-114** | 23 | Male | Yes | Timothy/Pacific Grass |
| **DE2-115** | 23 | Female | Yes | D. pterinyssinus |
| **DE2-116** | 23 | Male | No | D. pterinyssinus |

**Supplemental Table 1**
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Supplementary Table 1: A list of the 58 genes represented by the closest TSS of the 75 differentially methylated sites between 7.FAA and 1.FAS. Bold text highlights genes with 2 or more differentially methylated CpGs associated with them.

| CpG | P-Value | Q-Value | Delta Beta (7.FAA-1.FAS) | Gene name associated with closest transcription start site |
|---|---|---|---|---|
| **cg06437703** | 9.28E-07 | 0.00278 | -0.114 | EIF4EBP1 |
| **cg04234412** | **1.07E-06** | **0.00278** | **-0.480** | **LOC391322** |
| **cg12419862** | **6.53E-07** | **0.00278** | **-0.158** | **LOC391322** |
| **cg20007245** | **1.24E-06** | **0.00278** | **-0.117** | **LOC391322** |
| **cg25703541** | **4.79E-07** | **0.00278** | **-0.144** | **LOC391322** |
| **cg04458567** | 2.62E-06 | 0.00439 | -0.125 | POLR1B |
| **cg09033563** | **4.93E-06** | **0.00610** | **-0.229** | **LOC391322** |
| **cg00507008** | **5.00E-06** | **0.00610** | **-0.131** | **TBX3** |
| **cg17130982** | 6.15E-06 | 0.00633 | -0.114 | AK054708 |
| **cg17745291** | 1.24E-05 | 0.01107 | -0.104 | ALS2CR8 |
| **cg11912202** | 1.19E-05 | 0.01107 | -0.132 | OLIG2 |
| **cg24864275** | 2.23E-05 | 0.01571 | -0.104 | FCGBP |
| **cg26767081** | 2.78E-05 | 0.01863 | -0.133 | BC061632 |
| **cg10156846** | 3.10E-05 | 0.01888 | -0.109 | DLX5 |
| **cg22996308** | 4.93E-05 | 0.02125 | -0.110 | 5S_rRNA |
| **cg04129308** | 4.53E-05 | 0.02125 | 0.131 | AX747860 |
| **cg18049328** | 4.21E-05 | 0.02125 | -0.103 | BRSK2 |
| **cg25861692** | 4.52E-05 | 0.02125 | -0.122 | CADM1 |
| **cg02888990** | 4.59E-05 | 0.02125 | -0.107 | FLJ43860 |
| **cg11542165** | **4.91E-05** | **0.02125** | **-0.124** | **HNF4A** |
| **cg24626554** | 5.08E-05 | 0.02125 | -0.117 | LOC389458 |
| **cg05574357** | **4.84E-05** | **0.02125** | **-0.113** | **TBX3** |
| **cg21041701** | 5.55E-05 | 0.02186 | -0.103 | H3F3C |
| **cg23424407** | **5.52E-05** | **0.02186** | **-0.131** | **TBX3** |
| **cg23615741** | **6.98E-05** | **0.02596** | **-0.132** | **NKX2-3** |
| **cg11676636** | 7.50E-05 | 0.02715 | -0.107 | ARHGAP22 |
| **cg25455724** | 1.04E-04 | 0.03016 | -0.102 | APOM |
| **cg05415871** | 1.03E-04 | 0.03016 | -0.158 | BC038783 |
| **cg18877361** | 1.16E-04 | 0.03016 | -0.103 | C14orf80 |
| **cg07064544** | **1.06E-04** | **0.03016** | **-0.154** | **HNF4A** |
| **cg17504394** | 9.25E-05 | 0.03016 | -0.128 | Mir_584 |
| **cg16597737** | 1.22E-04 | 0.03016 | -0.101 | SLC9A11 |
| **cg24820672** | **9.96E-05** | **0.03016** | **-0.131** | **SPACA7** |
| **cg19536929** | **1.06E-04** | **0.03016** | **-0.109** | **TBX3** |
| **cg16415457** | 1.26E-04 | 0.03016 | -0.109 | UBE2E1 |
| **cg02460314** | 1.38E-04 | 0.03139 | -0.146 | CPN1 |

| **cg21058973** | **1.37E-04** | **0.03139** | **-0.146** | **HNF4A** |
|---|---|---|---|---|
| **cg00622988** | 1.39E-04 | 0.03139 | -0.105 | IRX2 |
| **cg06703856** | 1.47E-04 | 0.03173 | -0.109 | LEAP2 |
| **cg15242686** | **1.72E-04** | **0.03179** | **0.299** | **GSTTP1** |
| **cg22389375** | 1.72E-04 | 0.03179 | -0.158 | KCNQ1DN |
| **cg04215055** | 1.60E-04 | 0.03179 | -0.112 | MGAT5B |
| **cg11480029** | 1.59E-04 | 0.03179 | -0.137 | OR9A2 |
| **cg03051777** | **1.67E-04** | **0.03179** | **-0.131** | **TBX3** |
| **cg08261841** | 1.76E-04 | 0.03179 | -0.108 | TMEM139 |
| **cg11141652** | **1.88E-04** | **0.03318** | **0.286** | **GSTTP1** |
| **cg07803284** | 2.16E-04 | 0.03530 | -0.102 | ETV5 |
| **cg07664999** | 2.42E-04 | 0.03774 | -0.123 | TM7SF4 |
| **cg08251704** | 2.60E-04 | 0.03852 | -0.102 | DGKD |
| **cg14962049** | 2.86E-04 | 0.04078 | 0.111 | PRELID2 |
| **cg16738929** | 2.96E-04 | 0.04133 | -0.112 | CUL2 |
| **cg23172828** | 3.08E-04 | 0.04191 | -0.122 | SMAD6 |
| **cg00005112** | 3.09E-04 | 0.04191 | -0.125 | WWP2 |
| **cg07091758** | 3.26E-04 | 0.04367 | -0.110 | PKD1L2 |
| **cg08076955** | 3.40E-04 | 0.04457 | -0.125 | HOXB1 |
| **cg04033390** | 3.43E-04 | 0.04457 | -0.110 | MIR3612 |
| **cg15671083** | **3.39E-04** | **0.04457** | **-0.105** | **UCN3** |
| **cg16611278** | 3.72E-04 | 0.04476 | -0.127 | DTX1 |
| **cg17479716** | 3.59E-04 | 0.04476 | -0.112 | IGSF21 |
| **cg11273702** | 3.52E-04 | 0.04476 | -0.196 | KBTBD8 |
| **cg00549574** | 3.58E-04 | 0.04476 | -0.112 | PRPSAP1 |
| **cg27349333** | **3.77E-04** | **0.04476** | **-0.121** | **UCN3** |
| **cg07659053** | 4.04E-04 | 0.04498 | 0.128 | BC036251 |
| **cg13424029** | **3.92E-04** | **0.04498** | **-0.105** | **NKX2-3** |
| **cg14972210** | 4.13E-04 | 0.04498 | -0.132 | SORCS3 |
| **cg26004771** | 4.45E-04 | 0.04673 | -0.177 | C1orf49 |
| **cg19103219** | 4.56E-04 | 0.04673 | -0.107 | chromosome 10 open reading frame 139 |
| **cg01463540** | 4.56E-04 | 0.04673 | -0.102 | DLGAP4 |
| **cg10528482** | 4.56E-04 | 0.04673 | -0.141 | SLC9A3 |
| **cg13609544** | 4.64E-04 | 0.04674 | -0.103 | HOXB3 |
| **cg22948337** | **4.76E-04** | **0.04692** | **-0.126** | **SPACA7** |
| **cg04528829** | **4.78E-04** | **0.04692** | **-0.119** | **TBX3** |
| **cg02824888** | **4.89E-04** | **0.04751** | **-0.108** | **TBX3** |
| **cg00190355** | 5.56E-04 | 0.04976 | -0.108 | BC013821 |
| **cg18949192** | **5.44E-04** | **0.04976** | **-0.104** | **TBX3** |

**Supplemental Table 2**
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Supplemental Table 2: A list of the 450 genes represented by the closest TSS of the 548 differentially methylated sites between 4.DES vs 1.FAS. Bold text highlights genes with 2 or more differentially methylated CpGs associated with them.

| CpG | P-Value | Q-Value | Delta Beta (4.DES-1.FAS) | Gene name assocated with closest transcription start site |
|---|---|---|---|---|
| **cg08960448** | 0.0040 | 0.0206 | -0.1066 | 41164 |
| **cg26944526** | 0.0003 | 0.0206 | -0.1125 | 5S_rRNA |
| **cg02258482** | 0.0009 | 0.0206 | -0.1070 | 7SK |
| **cg16350608** | 0.0004 | 0.0206 | -0.1413 | 7SK |
| **cg04661040** | 0.0008 | 0.0206 | -0.1139 | AAK1 |
| **cg12130225** | 0.0022 | 0.0206 | -0.1102 | ABCB10 |
| **cg02328793** | 0.0010 | 0.0206 | -0.1019 | ACOT7 |
| **cg20587996** | 0.0002 | 0.0206 | -0.1177 | ACTR3C |
| **cg12505085** | 0.0069 | 0.0206 | -0.1000 | ADK |
| **cg14043752** | 0.0014 | 0.0206 | -0.1152 | AFTPH |
| **cg26927232** | 0.0007 | 0.0206 | -0.1043 | AJ276246 |
| **cg18016148** | 0.0013 | 0.0206 | -0.1046 | AK025288 |
| **cg19307180** | 0.0008 | 0.0206 | -0.1159 | AK054970 |
| **cg27263068** | 0.0059 | 0.0206 | -0.1009 | AK055055 |
| **cg03985360** | 0.0010 | 0.0206 | -0.1070 | AK055368 |
| **cg01358966** | 0.0014 | 0.0206 | -0.1032 | AK075019 |
| **cg19510206** | 0.0017 | 0.0206 | -0.1042 | AK096230 |
| **cg23755884** | 0.0005 | 0.0206 | -0.1090 | AK128161 |
| **cg26049390** | 0.0030 | 0.0206 | -0.1003 | AK128216 |
| **cg08796240** | 0.0037 | 0.0206 | -0.1049 | AK128439 |
| **cg03991300** | 0.0021 | 0.0206 | -0.1022 | AK128777 |
| **cg26036018** | 0.0066 | 0.0206 | -0.1055 | AK4 |
| **cg10600964** | 0.0026 | 0.0206 | -0.1012 | AL050000 |
| **cg19765196** | **0.0022** | **0.0206** | **-0.1070** | **ALCAM** |
| **cg21832978** | **0.0062** | **0.0206** | **-0.1044** | **ALCAM** |
| **cg17665652** | 0.0005 | 0.0206 | -0.1133 | ANK2 |
| **cg19660531** | 0.0003 | 0.0206 | -0.1146 | ANO2 |
| **cg22222261** | 0.0016 | 0.0206 | -0.1030 | ANO5 |
| **cg07813214** | 0.0010 | 0.0206 | -0.1002 | AP1S3 |
| **cg16362173** | 0.0020 | 0.0206 | -0.1002 | ARF6 |
| **cg05816000** | 0.0036 | 0.0206 | -0.1008 | ARHGAP10 |
| **cg11310863** | 0.0065 | 0.0206 | -0.1010 | ARHGAP15 |
| **cg20892287** | 0.0012 | 0.0206 | -0.1082 | ARHGAP32 |
| **cg25422226** | 0.0014 | 0.0206 | -0.1073 | ARL4A |
| **cg27308445** | 0.0029 | 0.0206 | -0.1004 | ARSI |
| **cg03164112** | **0.0000** | **0.0206** | **-0.1419** | **ASB18** |
| **cg06473675** | **0.0000** | **0.0206** | **-0.1608** | **ASB18** |
| **cg02647835** | **0.0035** | **0.0206** | **-0.1023** | **ASPSCR1** |

| | | | | |
|---|---|---|---|---|
| **cg14419393** | **0.0067** | **0.0206** | **-0.1130** | **ASPSCR1** |
| **cg04819081** | 0.0003 | 0.0206 | -0.1010 | ATAD2B |
| **cg12194594** | 0.0027 | 0.0206 | -0.1183 | ATP11A |
| **cg18882091** | 0.0031 | 0.0206 | -0.1079 | ATP5J2 |
| **cg22795345** | 0.0019 | 0.0206 | -0.1083 | AX748212 |
| **cg11523350** | 0.0027 | 0.0206 | -0.1286 | BACE1 |
| **cg20446334** | 0.0005 | 0.0206 | -0.1148 | BAIAP2 |
| **cg05471602** | 0.0047 | 0.0206 | -0.1039 | BARX2 |
| **cg09831875** | 0.0040 | 0.0206 | -0.1090 | BC017209 |
| **cg04778178** | 0.0008 | 0.0206 | -0.1011 | BC035889 |
| **cg09576488** | 0.0004 | 0.0206 | -0.1148 | BC039545 |
| **cg23094620** | 0.0017 | 0.0206 | -0.1005 | BC040634 |
| **cg21594328** | 0.0003 | 0.0206 | -0.1095 | BC047484 |
| **cg12893852** | 0.0009 | 0.0206 | -0.1100 | BC047651 |
| **cg24361265** | 0.0057 | 0.0206 | -0.1183 | BC062349 |
| **cg06422039** | 0.0057 | 0.0206 | -0.1086 | BC084558 |
| **cg20477160** | **0.0004** | **0.0206** | **-0.1110** | **BC148242** |
| **cg25976672** | **0.0050** | **0.0206** | **-0.1044** | **BC148242** |
| **cg00498816** | **0.0068** | **0.0206** | **-0.1112** | **BC152380** |
| **cg18183817** | **0.0032** | **0.0206** | **-0.1137** | **BC152380** |
| **cg00953399** | 0.0001 | 0.0206 | -0.1080 | BET3L |
| **cg21510704** | 0.0008 | 0.0206 | -0.1043 | BX247991 |
| **cg08148252** | 0.0008 | 0.0206 | -0.1108 | BX647900 |
| **cg25134818** | 0.0021 | 0.0206 | -0.1058 | BZW2 |
| **cg01472043** | 0.0060 | 0.0206 | -0.1063 | C10orf47 |
| **cg22871947** | 0.0051 | 0.0206 | -0.1068 | C10orf71 |
| **cg01168833** | 0.0040 | 0.0206 | -0.1048 | C12orf75 |
| **cg18356785** | 0.0034 | 0.0206 | -0.1042 | C1QTNF4 |
| **cg01881444** | 0.0024 | 0.0206 | -0.1032 | C3orf64 |
| **cg07579997** | 0.0039 | 0.0206 | -0.1109 | C4orf49 |
| **cg06871919** | 0.0052 | 0.0206 | -0.1023 | C5orf22 |
| **cg17580613** | 0.0050 | 0.0206 | -0.1032 | C7orf30 |
| **cg05632631** | 0.0000 | 0.0206 | -0.1699 | CACNG3 |
| **cg27235315** | 0.0000 | 0.0206 | -0.1211 | CAPN8 |
| **cg00876266** | **0.0036** | **0.0206** | **-0.1003** | **CAPS** |
| **cg13545089** | **0.0002** | **0.0206** | **-0.1066** | **CAPS** |
| **cg16465280** | 0.0011 | 0.0206 | -0.1032 | CARD11 |
| **cg09118017** | 0.0060 | 0.0206 | -0.1008 | CASZ1 |
| **cg07790870** | 0.0020 | 0.0206 | -0.1049 | CBFA2T2 |
| **cg09405227** | 0.0039 | 0.0206 | -0.1044 | CCBP2 |
| **cg02901065** | 0.0022 | 0.0206 | -0.1072 | CCDC146 |
| **cg10370305** | 0.0035 | 0.0206 | -0.1025 | CCDC34 |
| **cg05233289** | 0.0023 | 0.0206 | -0.1271 | CCDC88B |

| | | | | |
|---|---|---|---|---|
| **cg17489451** | 0.0018 | 0.0206 | -0.1009 | CCDC91 |
| **cg17224401** | 0.0010 | 0.0206 | -0.1118 | CCR3 |
| **cg00606396** | 0.0008 | 0.0206 | -0.1160 | CDH18 |
| **cg26673975** | 0.0007 | 0.0206 | -0.1219 | CDH5 |
| **cg12864235** | 0.0013 | 0.0206 | -0.1036 | CDH9 |
| **cg08371050** | 0.0059 | 0.0206 | -0.1023 | CDK14 |
| **cg23235622** | 0.0053 | 0.0206 | -0.1080 | CEP250 |
| **cg01987516** | **0.0025** | **0.0206** | **-0.1067** | **chromosome 10 open reading frame 139** |
| **cg19103219** | **0.0004** | **0.0206** | **-0.1090** | **chromosome 10 open reading frame 139** |
| **cg14496909** | **0.0013** | **0.0206** | **-0.1317** | **CHSY1** |
| **cg18536607** | **0.0036** | **0.0206** | **-0.1180** | **CHSY1** |
| **cg10667102** | 0.0001 | 0.0206 | -0.1324 | CIRBP |
| **cg03339668** | **0.0075** | **0.0206** | **-0.1005** | **CIT** |
| **cg26097051** | **0.0030** | **0.0206** | **-0.1005** | **CIT** |
| **cg00804587** | 0.0032 | 0.0206 | -0.1017 | CLDN1 |
| **cg26442107** | 0.0017 | 0.0206 | -0.1347 | CLEC16A |
| **cg15016233** | 0.0065 | 0.0206 | -0.1053 | CMAS |
| **cg02366931** | **0.0045** | **0.0206** | **-0.1052** | **CMYA5** |
| **cg21928760** | 0.0016 | 0.0206 | -0.1032 | CNTN4 |
| **cg13879411** | **0.0010** | **0.0206** | **-0.1038** | **COL28A1** |
| **cg21450738** | 0.0000 | 0.0206 | -0.1421 | COX6C |
| **cg03951662** | 0.0043 | 0.0206 | -0.1010 | CPD |
| **cg02460314** | 0.0001 | 0.0206 | -0.1162 | CPN1 |
| **cg02483735** | 0.0003 | 0.0206 | -0.2047 | CSF3R |
| **cg05885484** | 0.0013 | 0.0206 | -0.1087 | CSNK1G3 |
| **cg00814909** | 0.0012 | 0.0206 | -0.1155 | CTBP1 |
| **cg09916651** | **0.0001** | **0.0206** | **-0.1444** | **CUX2** |
| **cg10535597** | **0.0066** | **0.0206** | **-0.1015** | **CUX2** |
| **cg11551879** | **0.0010** | **0.0206** | **-0.1023** | **CUX2** |
| **cg02971328** | 0.0044 | 0.0206 | -0.1013 | DAGLB |
| **cg12655375** | **0.0040** | **0.0206** | **-0.1044** | **DEF6** |
| **cg18486815** | **0.0019** | **0.0206** | **-0.1116** | **DEF6** |
| **cg10280963** | 0.0032 | 0.0206 | -0.1036 | DEK |
| **cg08379738** | 0.0078 | 0.0206 | -0.1008 | DENND1C |
| **cg10113526** | 0.0078 | 0.0206 | -0.1068 | DENND3 |
| **cg08251704** | 0.0002 | 0.0206 | -0.1046 | DGKD |
| **cg01379412** | 0.0048 | 0.0206 | -0.1050 | DIXDC1 |
| **cg11681321** | 0.0037 | 0.0206 | -0.1039 | DJ439531 |
| **cg11714341** | 0.0001 | 0.0206 | -0.1046 | DKFZp686M11215 |
| **cg03668763** | 0.0004 | 0.0206 | -0.1114 | DM376719 |
| **cg06776201** | 0.0010 | 0.0206 | -0.1247 | DPF3 |
| **cg05617002** | 0.0040 | 0.0206 | -0.1009 | DPP10 |

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|---|---|---|---|---|
| **cg08030922** | 0.0004 | 0.0206 | -0.1105 | DQ571524 |
| **cg01502353** | **0.0008** | **0.0206** | **-0.1086** | **DST** |
| **cg15750696** | **0.0029** | **0.0206** | **-0.1092** | **DST** |
| **cg01192077** | **0.0003** | **0.0206** | **-0.1495** | **EBF1** |
| **cg22586996** | **0.0040** | **0.0206** | **-0.1007** | **EBF1** |
| **cg24889827** | 0.0015 | 0.0206 | -0.1005 | EEA1 |
| **cg18454133** | 0.0041 | 0.0206 | -0.1225 | EFS |
| **cg00813343** | 0.0011 | 0.0206 | -0.1140 | EIF3IP1 |
| **cg24935217** | 0.0062 | 0.0206 | -0.1022 | ELF5 |
| **cg23208271** | 0.0069 | 0.0206 | -0.1003 | ELMO1 |
| **cg16058975** | 0.0004 | 0.0206 | -0.1088 | ELTD1 |
| **cg07899060** | 0.0057 | 0.0206 | -0.1011 | EMID2 |
| **cg14195925** | 0.0080 | 0.0206 | -0.1007 | ENGASE |
| **cg09313482** | 0.0002 | 0.0206 | -0.1017 | ENPP2 |
| **cg07331478** | 0.0003 | 0.0206 | -0.1032 | EPHA4 |
| **cg10231182** | 0.0042 | 0.0206 | -0.1011 | EPS8 |
| **cg02408480** | 0.0026 | 0.0206 | -0.1019 | ERCC8 |
| **cg00768179** | 0.0031 | 0.0206 | -0.1053 | ERRFI1 |
| **cg00944421** | 0.0034 | 0.0206 | -0.1034 | ESRP2 |
| **cg06429194** | **0.0025** | **0.0206** | **-0.1010** | **ETS1** |
| **cg21121082** | **0.0048** | **0.0206** | **-0.1138** | **ETS1** |
| **cg27078890** | **0.0041** | **0.0206** | **-0.1057** | **ETS1** |
| **cg01423643** | 0.0013 | 0.0206 | -0.1032 | F11R |
| **cg13563867** | 0.0007 | 0.0206 | -0.1087 | F3 |
| **cg01089602** | 0.0030 | 0.0206 | -0.1013 | F7 |
| **cg07213482** | 0.0021 | 0.0206 | -0.1040 | FAM13A |
| **cg11059712** | 0.0000 | 0.0206 | -0.1072 | FAM171A1 |
| **cg22973459** | 0.0010 | 0.0206 | -0.1033 | FAM178A |
| **cg19775206** | 0.0057 | 0.0206 | -0.1122 | FAM181A |
| **cg26815617** | 0.0034 | 0.0206 | -0.1010 | FAM183B |
| **cg13680876** | 0.0009 | 0.0206 | -0.1024 | FAM189A2 |
| **cg03003256** | 0.0020 | 0.0206 | -0.1116 | FAM26D |
| **cg00094158** | 0.0012 | 0.0206 | -0.1006 | FAM53B |
| **cg03501539** | 0.0007 | 0.0206 | -0.1240 | FAM55B |
| **cg12883629** | 0.0009 | 0.0206 | 0.1047 | FAM65B |
| **cg24052851** | 0.0016 | 0.0206 | -0.1054 | FAM75C2 |
| **cg18610569** | 0.0003 | 0.0206 | -0.1004 | FAT4 |
| **cg19674178** | 0.0013 | 0.0206 | -0.1024 | FGF7 |
| **cg02888990** | 0.0000 | 0.0206 | -0.1254 | FLJ43860 |
| **cg11223933** | **0.0044** | **0.0206** | **-0.1031** | **FMNL1** |
| **cg19735250** | **0.0071** | **0.0206** | **-0.1147** | **FMNL1** |
| **cg06980460** | 0.0044 | 0.0206 | -0.1082 | FOLH1 |
| **cg07929259** | 0.0068 | 0.0206 | -0.1029 | FOXA1 |

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|---|---|---|---|---|
| **cg19509778** | 0.0015 | 0.0206 | -0.1064 | FOXI2 |
| **cg24541672** | 0.0009 | 0.0206 | -0.1162 | FOXK2 |
| **cg25481160** | 0.0052 | 0.0206 | -0.1067 | FOXP1 |
| **cg14330460** | 0.0033 | 0.0206 | -0.1009 | FRMD1 |
| **cg22262233** | 0.0008 | 0.0206 | -0.1052 | GABRG3 |
| **cg25556008** | **0.0049** | **0.0206** | **-0.1025** | **GADD45B** |
| **cg17679980** | **0.0054** | **0.0206** | **-0.1015** | **GAS2** |
| **cg25982561** | **0.0052** | **0.0206** | **-0.1107** | **GAS2** |
| **cg21377950** | 0.0047 | 0.0206 | -0.1025 | GJD4 |
| **cg06007850** | 0.0040 | 0.0206 | -0.1005 | GMDS |
| **cg02479744** | 0.0021 | 0.0206 | -0.1068 | GMPR |
| **cg04206484** | 0.0061 | 0.0206 | -0.1161 | GPER |
| **cg21986821** | 0.0056 | 0.0206 | -0.1003 | GPR108 |
| **cg15720112** | 0.0012 | 0.0206 | -0.1032 | GRAMD3 |
| **cg08920233** | 0.0013 | 0.0206 | -0.1082 | GRIP1 |
| **cg09805403** | 0.0030 | 0.0206 | -0.1092 | GSPT1 |
| **cg03234702** | 0.0035 | 0.0206 | -0.1116 | HIST1H3E |
| **cg09515921** | 0.0057 | 0.0206 | -0.1028 | HLX |
| **cg25494605** | 0.0017 | 0.0206 | -0.1018 | HMP19 |
| **cg20848979** | 0.0031 | 0.0206 | -0.1129 | HNF4A |
| **cg03650946** | **0.0002** | **0.0206** | **-0.1034** | **HOXA3** |
| **cg08164294** | **0.0010** | **0.0206** | **-0.1004** | **HOXA3** |
| **cg18680977** | **0.0049** | **0.0206** | **-0.1021** | **HOXA3** |
| **cg11532431** | **0.0061** | **0.0206** | **-0.1044** | **HOXA4** |
| **cg22997113** | **0.0015** | **0.0206** | **-0.1218** | **HOXA4** |
| **cg04904318** | **0.0040** | **0.0206** | **-0.1431** | **HOXB1** |
| **cg07823492** | **0.0024** | **0.0206** | **-0.1182** | **HOXB1** |
| **cg22660933** | **0.0067** | **0.0206** | **-0.1395** | **HOXB1** |
| **cg24900666** | **0.0058** | **0.0206** | **-0.1021** | **HOXB1** |
| **cg24948406** | **0.0008** | **0.0206** | **-0.1201** | **HOXB1** |
| **cg26634219** | **0.0005** | **0.0206** | **-0.1188** | **HOXB1** |
| **cg11060532** | **0.0003** | **0.0206** | **-0.1149** | **HOXB3** |
| **cg23014425** | **0.0026** | **0.0206** | **-0.1143** | **HOXB3** |
| **cg27656658** | **0.0045** | **0.0206** | **-0.1006** | **HOXB3** |
| **cg14047130** | 0.0001 | 0.0206 | -0.1034 | HS3ST1 |
| **cg02332936** | 0.0011 | 0.0206 | -0.1175 | HSDL2 |
| **cg06127256** | 0.0032 | 0.0206 | -0.1003 | HTA |
| **cg22713469** | 0.0021 | 0.0206 | -0.1037 | HTR1A |
| **cg08269485** | 0.0014 | 0.0206 | -0.1035 | IFITM10 |
| **cg17479716** | 0.0004 | 0.0206 | -0.1407 | IGSF21 |
| **cg00796611** | 0.0006 | 0.0206 | -0.1022 | IKBKE |
| **cg09131332** | 0.0007 | 0.0206 | -0.1355 | IL17REL |
| **cg06658391** | 0.0055 | 0.0206 | -0.1069 | IL1RN |

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|---|---|---|---|---|
| **cg18145937** | 0.0004 | 0.0206 | -0.1029 | IL4 |
| **cg25861066** | 0.0001 | 0.0206 | -0.1021 | INHBA |
| **cg20783970** | 0.0006 | 0.0206 | -0.1150 | IRF2BP2 |
| **cg13327384** | 0.0039 | 0.0206 | -0.1036 | IRX1 |
| **cg06742021** | **0.0001** | **0.0206** | **-0.1189** | **IRX2** |
| **cg22986569** | **0.0002** | **0.0206** | **-0.1396** | **IRX2** |
| **cg04115185** | **0.0003** | **0.0206** | **-0.1286** | **IRX4** |
| **cg12924936** | **0.0004** | **0.0206** | **-0.1421** | **IRX4** |
| **cg15714227** | **0.0002** | **0.0206** | **-0.1156** | **IRX4** |
| **cg17006413** | **0.0022** | **0.0206** | **-0.1068** | **ISG15** |
| **cg21472517** | **0.0007** | **0.0206** | **-0.1206** | **ISG15** |
| **cg24655428** | 0.0065 | 0.0206 | -0.1073 | ISLR |
| **cg15638366** | **0.0006** | **0.0206** | **-0.1090** | **ITGA6** |
| **cg11273702** | 0.0003 | 0.0206 | -0.1846 | KBTBD8 |
| **cg15731035** | 0.0007 | 0.0206 | -0.1207 | KCNAB2 |
| **cg01323777** | **0.0035** | **0.0206** | **-0.1014** | **KCNAB3** |
| **cg13407335** | **0.0022** | **0.0206** | **-0.1168** | **KCNAB3** |
| **cg13675753** | **0.0016** | **0.0206** | **-0.1046** | **KCNJ1** |
| **cg25194720** | **0.0026** | **0.0206** | **-0.1069** | **KCNJ1** |
| **cg27056194** | **0.0002** | **0.0206** | **-0.1225** | **KCNJ1** |
| **cg23735602** | 0.0024 | 0.0206 | -0.1145 | KCNMB2 |
| **cg07148458** | 0.0023 | 0.0206 | -0.1119 | KCTD11 |
| **cg27221338** | **0.0026** | **0.0206** | **-0.1125** | **KDELR2** |
| **cg02479497** | **0.0047** | **0.0206** | **-0.1009** | **KIAA0182** |
| **cg27048684** | 0.0024 | 0.0206 | -0.1270 | KIAA0556 |
| **cg19593009** | 0.0069 | 0.0206 | -0.1017 | KIAA0825 |
| **cg07628631** | 0.0019 | 0.0206 | -0.1044 | KIAA1033 |
| **cg24814707** | 0.0000 | 0.0206 | -0.1256 | KIAA1211 |
| **cg24775327** | **0.0059** | **0.0206** | **-0.1161** | **KIAA1274** |
| **cg11035730** | 0.0049 | 0.0206 | -0.1057 | KIF26A |
| **cg26531076** | 0.0044 | 0.0206 | -0.1140 | KLF5 |
| **cg10982443** | 0.0034 | 0.0206 | -0.1085 | KLHL33 |
| **cg17667972** | 0.0046 | 0.0206 | -0.1034 | KRT4 |
| **cg20102877** | 0.0019 | 0.0206 | -0.1061 | KRTCAP3 |
| **cg05364691** | **0.0062** | **0.0206** | **-0.1006** | **LAMB4** |
| **cg16190209** | **0.0037** | **0.0206** | **-0.1009** | **LAMB4** |
| **cg26914392** | 0.0004 | 0.0206 | -0.1218 | LBR |
| **cg20926939** | 0.0046 | 0.0206 | -0.1067 | LDB2 |
| **cg25202503** | 0.0009 | 0.0206 | -0.1014 | LINC00346 |
| **cg16754346** | 0.0040 | 0.0206 | -0.1050 | LINC00426 |
| **cg03548062** | **0.0064** | **0.0206** | **-0.1208** | **LINC00523** |
| **cg09942166** | **0.0040** | **0.0206** | **-0.1048** | **LINC00523** |
| **cg11806439** | **0.0032** | **0.0206** | **-0.1179** | **LINC00523** |

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|---|---|---|---|---|
| **cg21869609** | 0.0058 | 0.0206 | -0.1002 | LINGO3 |
| **cg02346342** | 0.0015 | 0.0206 | -0.1023 | LOC100128126 |
| **cg01644640** | 0.0005 | 0.0206 | -0.1025 | LOC100128590 |
| **cg02473847** | **0.0012** | **0.0206** | **-0.1019** | **LOC100129534** |
| **cg09510202** | 0.0055 | 0.0206 | -0.1022 | LOC100130357 |
| **cg14845962** | 0.0032 | 0.0206 | -0.1199 | LOC100130776 |
| **cg17826530** | 0.0072 | 0.0206 | -0.1097 | LOC100131060 |
| **cg26402660** | 0.0024 | 0.0206 | -0.1025 | LOC100133311 |
| **cg27322699** | 0.0063 | 0.0206 | -0.1186 | LOC100507043 |
| **cg17014914** | 0.0012 | 0.0206 | -0.1054 | LOC100507466 |
| **cg21462633** | 0.0017 | 0.0206 | -0.1087 | LOC151171 |
| **cg14188346** | 0.0056 | 0.0206 | -0.1062 | LOC158435 |
| **cg03826463** | 0.0050 | 0.0206 | -0.1044 | LOC253573 |
| **cg16800228** | 0.0012 | 0.0206 | -0.1032 | LOC400456 |
| **cg24996154** | 0.0023 | 0.0206 | -0.1035 | LOC503519 |
| **cg09556515** | 0.0029 | 0.0206 | -0.1130 | LOC727710 |
| **cg09226986** | 0.0019 | 0.0206 | -0.1094 | LPAL2 |
| **cg01482958** | 0.0048 | 0.0206 | -0.1035 | LPAR3 |
| **cg25078649** | 0.0006 | 0.0206 | -0.1021 | LRRC73 |
| **cg08617354** | 0.0026 | 0.0206 | -0.1092 | LRRTM3 |
| **cg07395436** | 0.0001 | 0.0206 | -0.1071 | LYPLAL1 |
| **cg05258935** | 0.0072 | 0.0206 | -0.1139 | MAP4K1 |
| **cg16100355** | 0.0030 | 0.0206 | -0.1101 | MCF2L |
| **cg11602041** | 0.0047 | 0.0206 | -0.1017 | ME3 |
| **cg16558822** | **0.0011** | **0.0206** | **-0.1111** | **MECOM** |
| **cg18675617** | **0.0070** | **0.0206** | **0.1016** | **MEIS1** |
| **cg03260530** | 0.0016 | 0.0206 | -0.1011 | METRNL |
| **cg01218903** | 0.0002 | 0.0206 | -0.1265 | MGAT5B |
| **cg20731875** | 0.0032 | 0.0206 | -0.1223 | MGC12916 |
| **cg06553513** | **0.0003** | **0.0206** | **-0.1204** | **MGMT** |
| **cg17451493** | **0.0000** | **0.0206** | **-0.1104** | **MGMT** |
| **cg14576951** | 0.0022 | 0.0206 | -0.1020 | MICALL2 |
| **cg20288000** | 0.0054 | 0.0206 | -0.1032 | Mir_584 |
| **cg21935393** | 0.0001 | 0.0206 | -0.1293 | Mir_720 |
| **cg09574009** | 0.0006 | 0.0206 | -0.1056 | MIR1297 |
| **cg18792131** | 0.0016 | 0.0206 | -0.1237 | MIR200B |
| **cg11616411** | 0.0033 | 0.0206 | -0.1017 | MIR371A |
| **cg10418812** | 0.0029 | 0.0206 | -0.1270 | MIR4655 |
| **cg15077193** | 0.0032 | 0.0206 | -0.1014 | MIR4745 |
| **cg05778494** | 0.0019 | 0.0206 | -0.1043 | MIR92B |
| **cg08061524** | **0.0021** | **0.0206** | **-0.1321** | **MIRLET7BHG** |
| **cg13523819** | 0.0014 | 0.0206 | -0.1156 | MITF |
| **cg06308084** | 0.0049 | 0.0206 | -0.1070 | MLC1 |

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|---|---|---|---|---|
| **cg02016467** | 0.0067 | 0.0206 | -0.1064 | MSLN |
| **cg24404533** | 0.0055 | 0.0206 | -0.1007 | MUC6 |
| **cg04869379** | 0.0024 | 0.0206 | -0.1059 | MYO16 |
| **cg13432945** | 0.0021 | 0.0206 | -0.1014 | MYSM1 |
| **cg04784618** | 0.0003 | 0.0206 | -0.1141 | NAALADL2 |
| **cg19827875** | 0.0017 | 0.0206 | -0.1081 | NAV1 |
| **cg07613333** | 0.0013 | 0.0206 | -0.1003 | NAV2 |
| **cg13774505** | 0.0022 | 0.0206 | -0.1120 | NCS1 |
| **cg17147885** | 0.0057 | 0.0206 | -0.1075 | NEK10 |
| **cg03000585** | 0.0007 | 0.0206 | -0.1003 | NEXN |
| **cg00593773** | **0.0039** | **0.0206** | **-0.1044** | **NFATC1** |
| **cg25951288** | **0.0030** | **0.0206** | **-0.1023** | **NFATC1** |
| **cg09113483** | **0.0070** | **0.0206** | **-0.1007** | **NFIA** |
| **cg06807696** | 0.0062 | 0.0206 | -0.1149 | NFKBIA |
| **cg18980036** | 0.0004 | 0.0206 | -0.1018 | NKX6-2 |
| **cg14307471** | 0.0005 | 0.0206 | -0.1209 | NOL4 |
| **cg04969340** | 0.0000 | 0.0206 | -0.1340 | NOM1 |
| **cg22249735** | 0.0037 | 0.0206 | -0.1086 | NRP2 |
| **cg21660452** | 0.0030 | 0.0206 | -0.1338 | NRXN2 |
| **cg07657463** | 0.0059 | 0.0206 | -0.1009 | NSUN7 |
| **cg00540540** | 0.0075 | 0.0206 | -0.1055 | ODZ2 |
| **cg00376979** | **0.0001** | **0.0206** | **-0.1263** | **OLIG2** |
| **cg05183668** | **0.0000** | **0.0206** | **-0.1034** | **OLIG2** |
| **cg11912202** | **0.0000** | **0.0206** | **-0.1447** | **OLIG2** |
| **cg03895540** | 0.0027 | 0.0206 | -0.1070 | OR52N2 |
| **cg11480029** | 0.0000 | 0.0206 | -0.1123 | OR9A2 |
| **cg12927617** | 0.0037 | 0.0206 | -0.1119 | ORM1 |
| **cg00874605** | **0.0025** | **0.0206** | **-0.1175** | **PARN** |
| **cg04388123** | **0.0059** | **0.0206** | **-0.1054** | **PARN** |
| **cg14397402** | 0.0001 | 0.0206 | -0.1068 | PAX9 |
| **cg19637591** | 0.0009 | 0.0206 | -0.1046 | PCDH17 |
| **cg00817501** | 0.0020 | 0.0206 | -0.1009 | PCDH18 |
| **cg08548659** | 0.0008 | 0.0206 | -0.1035 | PCDH7 |
| **cg05148373** | 0.0003 | 0.0206 | 0.1015 | PDE1A |
| **cg18425032** | 0.0069 | 0.0206 | -0.1039 | PDE4D |
| **cg19350115** | 0.0026 | 0.0206 | -0.1148 | PDE7B |
| **cg05556923** | **0.0060** | **0.0206** | **-0.1537** | **PDGFA** |
| **cg09187695** | **0.0022** | **0.0206** | **-0.1024** | **PDGFA** |
| **cg02055988** | 0.0041 | 0.0206 | -0.1041 | PIK3IP1 |
| **cg00120783** | 0.0040 | 0.0206 | -0.1130 | PITPNA |
| **cg07091758** | 0.0001 | 0.0206 | -0.1074 | PKD1L2 |
| **cg12635662** | 0.0006 | 0.0206 | -0.1035 | PLAC1L |
| **cg24578857** | 0.0054 | 0.0206 | -0.1123 | PLD6 |

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|---|---|---|---|---|
| **cg22594071** | 0.0007 | 0.0206 | -0.1158 | PLSCR5 |
| **cg18180501** | 0.0011 | 0.0206 | -0.1014 | PP12613 |
| **cg00501542** | 0.0014 | 0.0206 | -0.1067 | PPFIA4 |
| **cg12069151** | 0.0036 | 0.0206 | -0.1015 | PPP1R10 |
| **cg20067575** | **0.0048** | **0.0206** | **-0.1040** | **PPP1R14C** |
| **cg26954245** | **0.0031** | **0.0206** | **-0.1077** | **PPP1R14C** |
| **cg07056967** | 0.0058 | 0.0206 | -0.1076 | PPP6R3 |
| **cg12466022** | 0.0074 | 0.0206 | -0.1014 | PPPDE1 |
| **cg11150222** | **0.0019** | **0.0206** | **-0.1006** | **PRDM1** |
| **cg21376090** | **0.0002** | **0.0206** | **-0.1109** | **PRDM1** |
| **cg23591302** | 0.0007 | 0.0206 | -0.1095 | PRICKLE1 |
| **cg27303421** | 0.0023 | 0.0206 | -0.1145 | PRL |
| **cg18235088** | **0.0030** | **0.0206** | **-0.1121** | **PRRT1** |
| **cg20151476** | 0.0005 | 0.0206 | -0.1008 | PSMG3 |
| **cg00908766** | 0.0013 | 0.0206 | -0.1086 | PSRC1 |
| **cg13049961** | 0.0024 | 0.0206 | -0.1090 | PURA |
| **cg15323253** | 0.0027 | 0.0206 | -0.1011 | PXDC1 |
| **cg11298446** | 0.0029 | 0.0206 | -0.1069 | RAB11FIP2 |
| **cg09772333** | 0.0035 | 0.0206 | -0.1054 | RABAC1 |
| **cg11526630** | 0.0016 | 0.0206 | -0.1177 | RADIL |
| **cg10846682** | 0.0017 | 0.0206 | -0.1250 | RAMP1 |
| **cg00730887** | 0.0022 | 0.0206 | -0.1046 | RAPGEF2 |
| **cg22906709** | 0.0016 | 0.0206 | -0.1134 | RASA3 |
| **cg01062942** | **0.0017** | **0.0206** | **-0.1304** | **RASAL3** |
| **cg21002957** | **0.0019** | **0.0206** | **-0.1096** | **RASAL3** |
| **cg22216196** | **0.0009** | **0.0206** | **-0.1063** | **RASAL3** |
| **cg01927373** | 0.0072 | 0.0206 | -0.1059 | RASGEF1B |
| **cg06389444** | 0.0019 | 0.0206 | -0.1062 | RBFOX3 |
| **cg08425628** | 0.0021 | 0.0206 | -0.1035 | RBM43 |
| **cg14864148** | 0.0052 | 0.0206 | -0.1047 | RELT |
| **cg15934776** | 0.0011 | 0.0206 | -0.1143 | REV1 |
| **cg04013650** | **0.0030** | **0.0206** | **-0.1042** | **RP1** |
| **cg13833632** | 0.0004 | 0.0206 | -0.1136 | RTKN2 |
| **cg00773370** | 0.0015 | 0.0206 | -0.1048 | SCNN1A |
| **cg09245302** | 0.0056 | 0.0206 | -0.1065 | SECTM1 |
| **cg19262563** | 0.0008 | 0.0206 | -0.1049 | SEMA3C |
| **cg06653026** | 0.0013 | 0.0206 | -0.1071 | SEMA3D |
| **cg21442419** | 0.0002 | 0.0206 | -0.1030 | SKI |
| **cg00655147** | 0.0058 | 0.0206 | -0.1042 | SLC16A12 |
| **cg18158015** | 0.0017 | 0.0206 | -0.1058 | SLC22A25 |
| **cg20127102** | 0.0022 | 0.0206 | -0.1006 | SLC43A3 |
| **cg27109043** | 0.0013 | 0.0206 | -0.1040 | SLC45A4 |
| **cg02344497** | 0.0023 | 0.0206 | -0.1026 | SLC4A7 |

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|---|---|---|---|---|
| **cg02102889** | 0.0006 | 0.0206 | -0.1106 | SLC7A11 |
| **cg22689016** | 0.0006 | 0.0206 | -0.1178 | SLC9A3 |
| **cg05906024** | **0.0044** | **0.0206** | **-0.1155** | **SLIT3** |
| **cg13908833** | **0.0064** | **0.0206** | **-0.1009** | **SLIT3** |
| **cg25984601** | **0.0013** | **0.0206** | **-0.1102** | **SLIT3** |
| **cg21718735** | 0.0025 | 0.0206 | -0.1093 | SMAD7 |
| **cg17648080** | 0.0015 | 0.0206 | -0.1058 | SMG6 |
| **cg25669593** | 0.0048 | 0.0206 | -0.1133 | SMOC2 |
| **cg05584602** | 0.0031 | 0.0206 | -0.1142 | SNX29 |
| **cg00572966** | 0.0020 | 0.0206 | -0.1083 | SP4 |
| **cg20290367** | 0.0023 | 0.0206 | -0.1008 | SPECC1 |
| **cg05694089** | 0.0022 | 0.0206 | -0.1013 | SPEN |
| **cg02813710** | 0.0049 | 0.0206 | -0.1174 | SRPK2 |
| **cg18180230** | 0.0001 | 0.0206 | -0.1017 | SRSF8 |
| **cg20018563** | 0.0000 | 0.0206 | -0.1148 | ST8SIA2 |
| **cg18481642** | 0.0034 | 0.0206 | -0.1098 | STAT1 |
| **cg18628646** | 0.0049 | 0.0206 | -0.1002 | STOX2 |
| **cg19847038** | **0.0005** | **0.0206** | **-0.1124** | **STX19** |
| **cg27200466** | **0.0023** | **0.0206** | **-0.1121** | **STX19** |
| **cg15173428** | 0.0054 | 0.0206 | -0.1056 | SYN3 |
| **cg04703974** | 0.0011 | 0.0206 | -0.1051 | SYNRG |
| **cg11171466** | 0.0008 | 0.0206 | -0.1126 | SYT8 |
| **cg07213771** | 0.0028 | 0.0206 | -0.1109 | TBX18 |
| **cg04370442** | 0.0016 | 0.0206 | -0.1229 | TEPP |
| **cg06784602** | 0.0045 | 0.0206 | -0.1037 | TGFBR2 |
| **cg01704739** | **0.0007** | **0.0206** | **-0.1054** | **TM4SF1** |
| **cg07664999** | 0.0017 | 0.0206 | -0.1047 | TM7SF4 |
| **cg10501093** | **0.0049** | **0.0206** | **-0.1120** | **TNFAIP2** |
| **cg18620571** | **0.0047** | **0.0206** | **-0.1173** | **TNFAIP2** |
| **cg01089914** | **0.0031** | **0.0206** | **-0.1070** | **TNS1** |
| **cg20225999** | **0.0035** | **0.0206** | **-0.1013** | **TNS1** |
| **cg08040824** | 0.0003 | 0.0206 | -0.1138 | TP63 |
| **cg23234149** | **0.0016** | **0.0206** | **-0.1096** | **TP73** |
| **cg18062333** | 0.0002 | 0.0206 | -0.1142 | TRNA |
| **cg22731383** | **0.0003** | **0.0206** | **-0.1049** | **TRNA_Pseudo** |
| **cg08638929** | 0.0054 | 0.0206 | -0.1028 | TSPAN1 |
| **cg25588348** | 0.0009 | 0.0206 | -0.1182 | TTLL5 |
| **cg07532576** | 0.0012 | 0.0206 | -0.1041 | TTN |
| **cg16689724** | 0.0006 | 0.0206 | -0.1166 | TUBE1 |
| **cg13974313** | 0.0012 | 0.0206 | -0.1039 | TXK |
| **cg16969274** | 0.0017 | 0.0206 | -0.1155 | U6 |
| **cg03668982** | 0.0008 | 0.0206 | -0.1002 | UBE2E1 |
| **cg06513139** | 0.0040 | 0.0206 | -0.1004 | UBXN6 |

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|---|---|---|---|---|
| **cg06634441** | **0.0003** | **0.0206** | **-0.1047** | **UGT2A1** |
| **cg23270582** | **0.0074** | **0.0206** | **-0.1006** | **UGT2A1** |
| **cg16807101** | 0.0008 | 0.0206 | -0.1001 | UHRF1BP1L |
| **cg01449677** | 0.0028 | 0.0206 | -0.1185 | USP36 |
| **cg14855367** | 0.0020 | 0.0206 | -0.1030 | UTS2D |
| **cg06795523** | 0.0032 | 0.0206 | -0.1036 | VRK2 |
| **cg24726965** | 0.0009 | 0.0206 | -0.1047 | WDPCP |
| **cg10090326** | 0.0021 | 0.0206 | -0.1028 | WDR25 |
| **cg00415665** | 0.0054 | 0.0206 | -0.1121 | ZHX2 |
| **cg17970299** | 0.0064 | 0.0206 | -0.1047 | ZNF385A |
| **cg22987448** | 0.0022 | 0.0206 | -0.1014 | ZNF414 |
| **cg06022562** | 0.0060 | 0.0206 | -0.1012 | ZNF750 |
| **cg05995260** | 0.0081 | 0.0206 | -0.1105 | AF075112 |
| **cg18820060** | 0.0082 | 0.0206 | -0.1014 | LOC285577 |
| **cg05881698** | 0.0081 | 0.0206 | -0.1085 | NRTN |
| **cg08485527** | 0.0083 | 0.0206 | -0.1022 | SEC14L1 |
| **cg14279856** | 0.0085 | 0.0206 | -0.1021 | DDR1 |
| **cg14582917** | 0.0084 | 0.0206 | -0.1027 | LOC100506190 |
| **cg13568106** | 0.0087 | 0.0207 | -0.1049 | FOXN1 |
| **cg01571001** | 0.0087 | 0.0207 | -0.1093 | GATA2 |
| **cg26150922** | 0.0087 | 0.0207 | -0.1017 | LONRF2 |
| **cg03346415** | 0.0089 | 0.0207 | -0.1122 | WWP2 |
| **cg17229698** | **0.0089** | **0.0207** | **-0.1116** | **SLIT3** |
| **cg02289739** | 0.0091 | 0.0207 | -0.1062 | AK057978 |
| **cg09378456** | 0.0092 | 0.0208 | -0.1042 | CFTR |
| **cg08018572** | 0.0092 | 0.0208 | -0.1033 | LMF1 |
| **cg14835484** | 0.0091 | 0.0208 | -0.1024 | TPM3 |
| **cg10628201** | 0.0092 | 0.0208 | -0.1116 | YDJC |
| **cg20344388** | **0.0093** | **0.0208** | **-0.1019** | **COL18A1** |
| **cg11585071** | **0.0094** | **0.0208** | **-0.1093** | **TP73** |
| **cg14316944** | 0.0099 | 0.0208 | -0.1109 | ACTG1 |
| **cg01446627** | 0.0096 | 0.0208 | -0.1090 | ADARB2-AS1 |
| **cg22641350** | 0.0095 | 0.0208 | -0.1135 | BC038783 |
| **cg06607764** | 0.0098 | 0.0208 | -0.1056 | CYTH1 |
| **cg07678266** | 0.0098 | 0.0208 | -0.1162 | GLT1D1 |
| **cg03915932** | **0.0097** | **0.0208** | **-0.1012** | **HOXB1** |
| **cg18812904** | 0.0094 | 0.0208 | -0.1071 | KCNG2 |
| **cg14496282** | **0.0095** | **0.0208** | **-0.1257** | **PDGFA** |
| **cg04757389** | 0.0094 | 0.0208 | -0.1030 | PTPRS |
| **cg24714094** | 0.0098 | 0.0208 | -0.1001 | RTDR1 |
| **cg15087376** | **0.0099** | **0.0208** | **-0.1004** | **TM4SF1** |
| **cg26427109** | 0.0104 | 0.0208 | 0.1077 | CD6 |
| **cg03044452** | 0.0107 | 0.0208 | -0.1020 | GGT6 |

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|---|---|---|---|---|
| **cg08101036** | **0.0104** | **0.0208** | **-0.1226** | **HOXA3** |
| **cg08622675** | **0.0106** | **0.0208** | **-0.1096** | **KDELR2** |
| **cg07474477** | 0.0103 | 0.0208 | -0.1020 | PKMYT1 |
| **cg03039843** | 0.0104 | 0.0208 | -0.1066 | SULT2B1 |
| **cg21665744** | 0.0108 | 0.0208 | -0.1017 | AK023033 |
| **cg05843596** | 0.0109 | 0.0208 | -0.1124 | AK123181 |
| **cg26313247** | 0.0110 | 0.0209 | -0.1076 | GPX5 |
| **cg08616951** | 0.0111 | 0.0209 | -0.1030 | PKP3 |
| **cg12937817** | 0.0112 | 0.0209 | -0.1023 | MAD1L1 |
| **cg04276301** | **0.0113** | **0.0209** | **0.1137** | **OTX1** |
| **cg09951047** | 0.0113 | 0.0209 | -0.1004 | MIR3074 |
| **cg03661817** | 0.0114 | 0.0210 | -0.1053 | KIFC3 |
| **cg25844471** | 0.0116 | 0.0210 | -0.1004 | C10orf67 |
| **cg23602690** | 0.0115 | 0.0210 | -0.1271 | DM119500 |
| **cg21478902** | 0.0118 | 0.0210 | -0.1015 | COL4A1 |
| **cg16397032** | 0.0118 | 0.0210 | -0.1118 | BC007399 |
| **cg03526459** | **0.0119** | **0.0210** | **-0.1114** | **TRNA_Pseudo** |
| **cg04148089** | **0.0123** | **0.0211** | **-0.1007** | **KIAA0182** |
| **cg10002850** | 0.0124 | 0.0211 | -0.1031 | EGFR |
| **cg09941601** | 0.0126 | 0.0212 | -0.1036 | FOXL1 |
| **cg24591913** | 0.0130 | 0.0213 | -0.1003 | PPYR1 |
| **cg12752420** | 0.0135 | 0.0215 | -0.1059 | C1orf210 |
| **cg04892182** | 0.0137 | 0.0216 | -0.1058 | KIAA1522 |
| **cg14052044** | 0.0137 | 0.0216 | -0.1000 | C19orf21 |
| **cg09655403** | **0.0140** | **0.0217** | **-0.1058** | **CMYA5** |
| **cg22009751** | 0.0140 | 0.0217 | -0.1247 | MIR2467 |
| **cg15744108** | 0.0143 | 0.0218 | -0.1015 | TRNA_Leu |
| **cg22615730** | 0.0147 | 0.0219 | -0.1027 | ANXA4 |
| **cg17342469** | **0.0152** | **0.0221** | **-0.1098** | **MIRLET7BHG** |
| **cg08407014** | **0.0153** | **0.0221** | **-0.1021** | **GADD45B** |
| **cg00921266** | **0.0153** | **0.0221** | **-0.1225** | **HOXA3** |
| **cg11015251** | **0.0156** | **0.0222** | **-0.1052** | **HOXA4** |
| **cg02944007** | 0.0157 | 0.0222 | -0.1002 | TSPAN10 |
| **cg02497558** | **0.0158** | **0.0223** | **-0.1276** | **HOXB1** |
| **cg11737879** | 0.0158 | 0.0223 | -0.1012 | PEX3 |
| **cg17239876** | **0.0159** | **0.0223** | **-0.1480** | **KIAA1274** |
| **cg27331292** | 0.0161 | 0.0224 | -0.1038 | SH2D3C |
| **cg18155888** | **0.0162** | **0.0224** | **-0.1002** | **LOC100129534** |
| **cg18451016** | 0.0163 | 0.0224 | -0.1120 | SF3A3 |
| **cg16595607** | **0.0164** | **0.0224** | **0.1129** | **OTX1** |
| **cg19825437** | **0.0166** | **0.0225** | **-0.1145** | **MECOM** |
| **cg12748890** | 0.0167 | 0.0225 | -0.1026 | SYTL1 |
| **cg11254532** | **0.0174** | **0.0228** | **-0.1028** | **NFIA** |

| | | | | |
|---|---|---|---|---|
| **cg14297340** | 0.0176 | 0.0229 | -0.1002 | LOC100507299 |
| **cg03903278** | 0.0185 | 0.0232 | 0.1033 | OTOL1 |
| **cg06463424** | **0.0186** | **0.0233** | **-0.1008** | **RP1** |
| **cg26209990** | **0.0188** | **0.0234** | **-0.1111** | **LEP** |
| **cg23792485** | 0.0190 | 0.0234 | -0.1077 | HNF4A |
| **cg13471599** | 0.0192 | 0.0235 | -0.1064 | MSI1 |
| **cg03003335** | 0.0194 | 0.0236 | -0.1123 | ACAP3 |
| **cg10271192** | **0.0199** | **0.0238** | **0.1025** | **OTX1** |
| **cg24812143** | **0.0219** | **0.0247** | **-0.1510** | **KIAA1274** |
| **cg09282336** | 0.0233 | 0.0254 | -0.1030 | CMIP |
| **cg22635491** | 0.0253 | 0.0263 | -0.1216 | TBX3 |
| **cg25979543** | 0.0258 | 0.0266 | -0.1012 | ARHGEF10L |
| **cg18290233** | **0.0271** | **0.0272** | **0.1143** | **NR2F1** |
| **cg12422539** | 0.0275 | 0.0274 | -0.1204 | AK091028 |
| **cg14721213** | 0.0281 | 0.0278 | -0.1003 | FMO2 |
| **cg08234689** | **0.0282** | **0.0278** | **-0.1093** | **LEP** |
| **cg08045906** | **0.0290** | **0.0282** | **-0.1013** | **PRRT1** |
| **cg01513157** | 0.0297 | 0.0285 | -0.1075 | SDR42E1 |
| **cg22022580** | 0.0297 | 0.0286 | -0.1024 | BC024306 |
| **cg00221709** | **0.0307** | **0.0290** | **0.1018** | **NR2F2** |
| **cg18524788** | 0.0316 | 0.0294 | -0.1087 | MIR148A |
| **cg02670123** | **0.0341** | **0.0306** | **-0.1044** | **ITGA6** |
| **cg01953658** | **0.0360** | **0.0316** | **0.1006** | **NR2F2** |
| **cg02081019** | **0.0405** | **0.0339** | **0.1288** | **NR2F1** |
| **cg01082798** | 0.0496 | 0.0386 | -0.1130 | AK024243 |
| **cg14457782** | **0.0515** | **0.0396** | **0.1055** | **WNK4** |
| **cg06528626** | **0.0547** | **0.0413** | **-0.1245** | **LMO3** |
| **cg11157253** | **0.0590** | **0.0435** | **0.1089** | **NR2F2** |
| **cg01223086** | **0.0601** | **0.0440** | **0.1089** | **NR2F2** |
| **cg27624826** | **0.0612** | **0.0446** | **-0.1053** | **TRNA_Pseudo** |
| **cg13851508** | **0.0619** | **0.0449** | **0.1091** | **MEIS1** |
| **cg16930572** | **0.0636** | **0.0458** | **-0.1203** | **LMO3** |
| **cg08409113** | **0.0639** | **0.0459** | **0.1072** | **WNK4** |
| **cg20933946** | **0.0644** | **0.0461** | **0.1193** | **NR2F2** |
| **cg21293562** | **0.0664** | **0.0472** | **0.1270** | **NR2F2** |
| **cg26267430** | **0.0699** | **0.0489** | **0.1233** | **NR2F2** |
| **cg01364755** | 0.0706 | 0.0493 | -0.1062 | ADARB2 |
| **cg04120686** | **0.0719** | **0.0499** | **-0.1031** | **MECOM** |

**Supplemental Table 3**

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Supplemental Table 3: CpGs differentially methylated in response to allergen after prior diesel exhaust exposure and in response to diesel exhaust after prior allergen exposure

| CpG | 1.FAS Vs 7.FAA | | 1.FAS Vs 4.DES | | Gene name of nearest TSS |
|---|---|---|---|---|---|
| | Δβ | Q value | Δβ | Q value | |
| **cg02460314** | -0.146 | 0.0314 | -0.116 | 0.0206 | CPN1 |
| **cg02888990** | -0.107 | 0.0213 | -0.125 | 0.0206 | FLJ43860 |
| **cg07091758** | -0.110 | 0.0437 | -0.107 | 0.0206 | PKD1L2 |
| **cg07664999** | -0.123 | 0.0377 | -0.105 | 0.0206 | TM7SF4 |
| **cg08251704** | -0.102 | 0.0385 | -0.105 | 0.0206 | DGKD |
| **cg11273702** | -0.196 | 0.0448 | -0.185 | 0.0206 | KBTBD8 |
| **cg11480029** | -0.137 | 0.0318 | -0.112 | 0.0206 | OR9A2 |
| **cg11912202** | -0.132 | 0.0111 | -0.145 | 0.0206 | OLIG2 |
| **cg17479716** | -0.112 | 0.0448 | -0.141 | 0.0206 | IGSF21 |
| **cg19103219** | -0.107 | 0.0467 | -0.109 | 0.0206 | chromosome 10 open reading frame 139 |

**Supplemental Table 4**

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Supplemental Table 4: Pyrosequencing Primers

| Primer Name | Primer Sequence |
|---|---|
| 3_TBX3 F1 | TTG TGT GGT TAT GGT ATA AAG TTA GT |
| 3_TBX3 R1* | /5BiodT/CA AAT ACA ATC CCT CCT ACT ATA ACA TAC |
| 3_TBX3 S1 | ATT TTG ATT TTG GTA GTA GAT |
| 4_HOXA3 F1 | GGA GAA AGT GAG GGT TGA TTA TTG AT |
| 4_HOXA3 R1* | /5BiodT/AA CCT CAT ACA TAC CAA TAA TTT TTA TCA |
| 4_HOXA3 S.2 | GGG TTT GAA GAG AAA ATG |
| 8_HOXA4 F1* | /5BiodT/GT TAG TGG TGT ATT TTT GGA TGA AGA AG |
| 8_HOXA4 R1 | CAC ATA CCC ACA TCT CAC C |
| 8_HOXA4 S1 | TCC CCT AAA CCT CTC |
| 9_HOXB1 F1 | TAG AGT GGG TAT TTT AAG AAG GAG TT |
| 9_HOXB1 R1* | /5BiodT/TA ACC CAT TAA CCT AAA AAA AAT CAC ATA T |
| 9_HOXB1 S1 | AAG AAG GAG TTT ATT TTA TTA |
| 12_HOXB3 F1* | /5BiodT/GA GAA ATG TTG GAT GTG GGA AGA TAG |
| 12_HOXB3 R1 | ACC CCA ACC CTA ACA ATA AAT CTC CCT A |
| 12_HOXB3 S1 | CAA TCT CCC CTT TCT CAC AAT AT |

**Supplemental Table 5**

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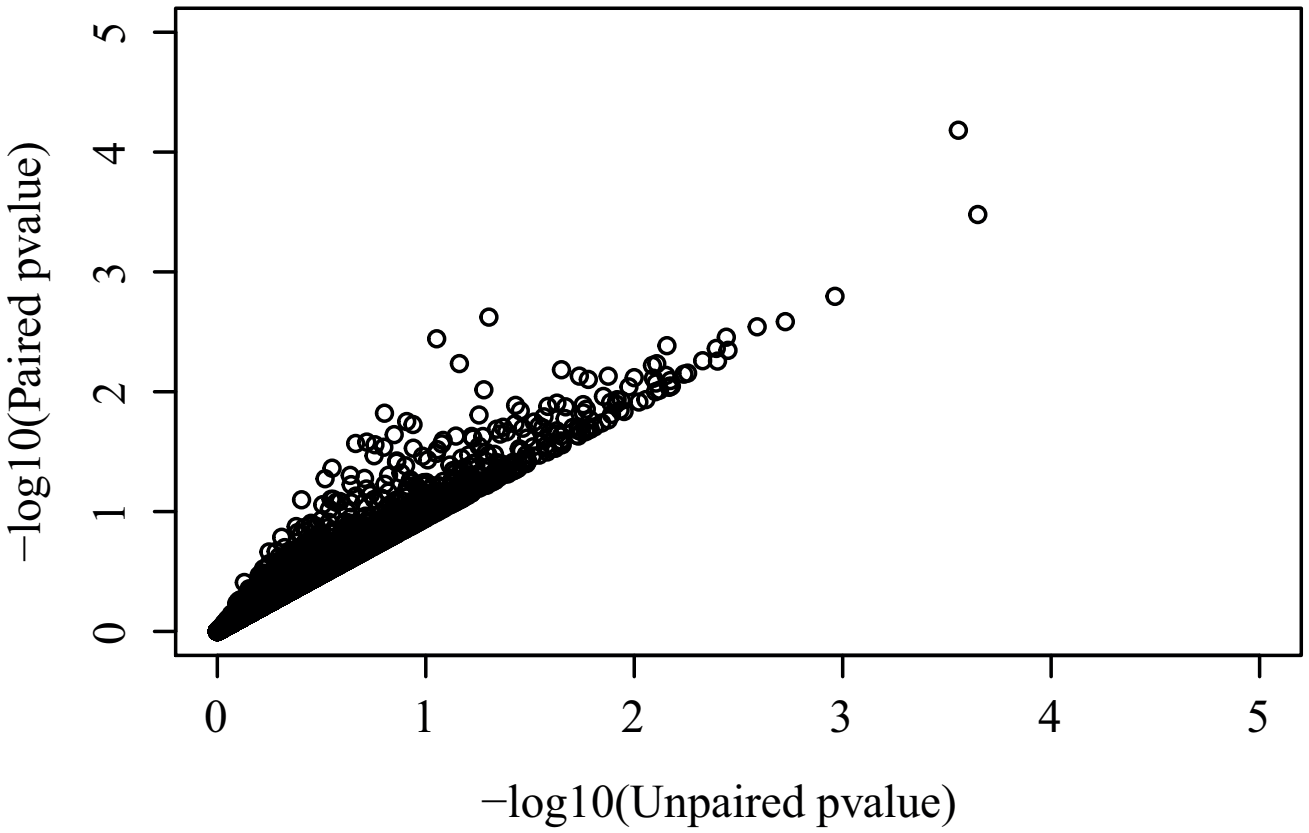
Supplemental Table 5: CpGs differentially methylated in response to diesel exhaust exposure or diesel exhaust and allergen co-exposure

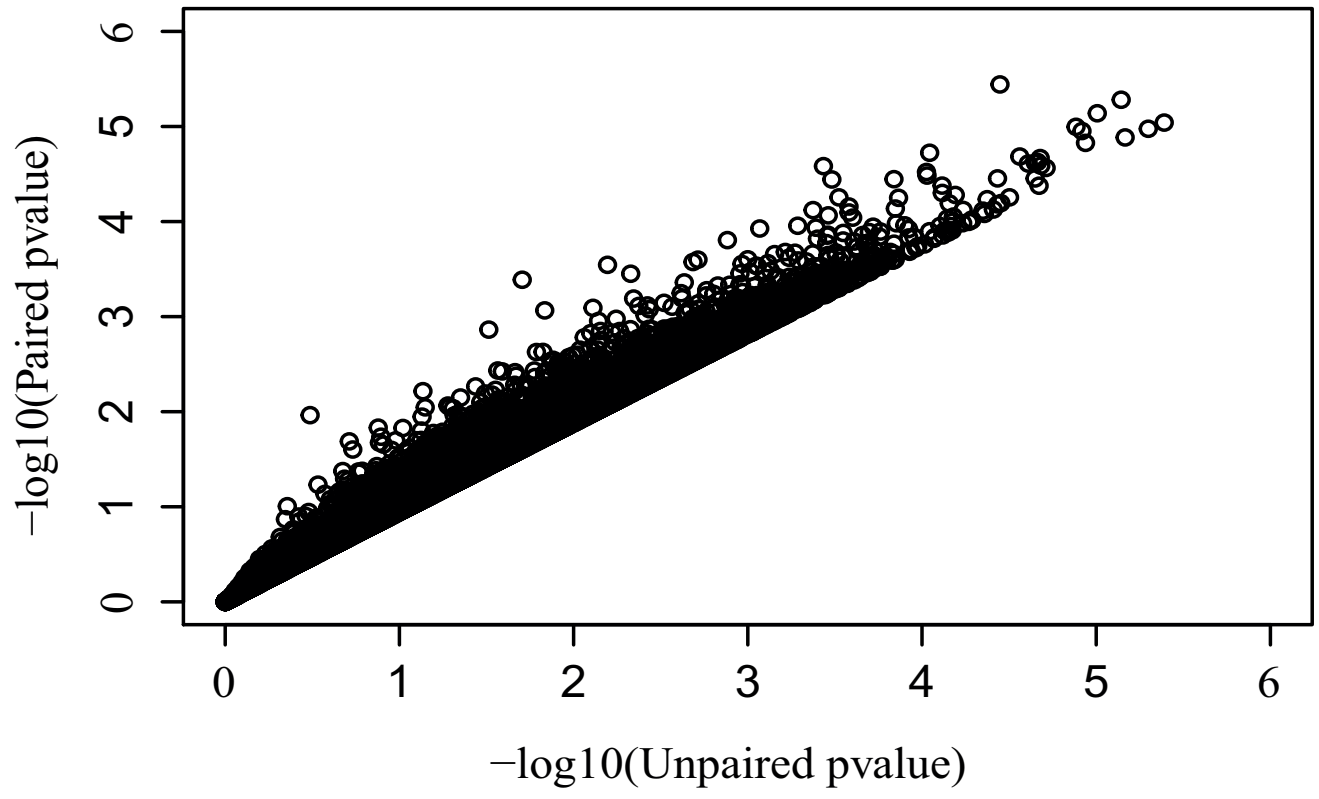
| Exposure | CpG | P value | Q value |
|---|---|---|---|
| **Diesel Exhaust** | cg04234412 | 5.66E-07 | 0.009 |
| | cg06437703 | 2.75E-06 | 0.017 |
| | cg09033563 | 1.97E-06 | 0.015 |
| | cg12419862 | 7.25E-07 | 0.009 |
| | cg20007245 | 9.43E-07 | 0.009 |
| | cg25703541 | 1.82E-07 | 0.007 |
| **Co-exposure** | cg00458681 | 2.09E-06 | 0.019 |
| | cg04234412 | 1.87E-06 | 0.019 |
| | cg09033563 | 2.73E-06 | 0.019 |
| | cg12419862 | 3.03E-06 | 0.019 |
| | cg20007245 | 6.04E-06 | 0.032 |
| | cg25703541 | 1.92E-06 | 0.019 |

**Supplemental Fig 1**

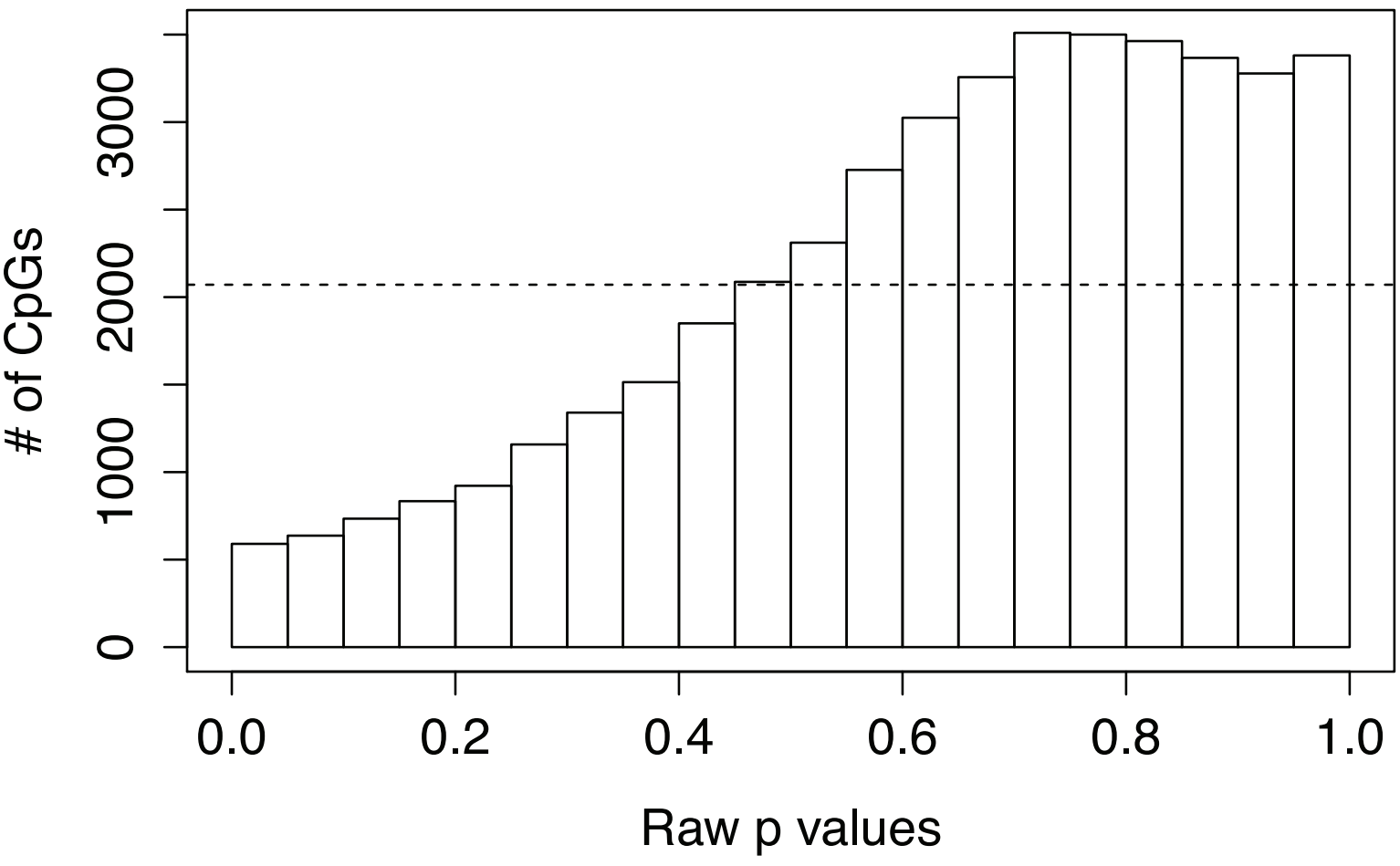
A



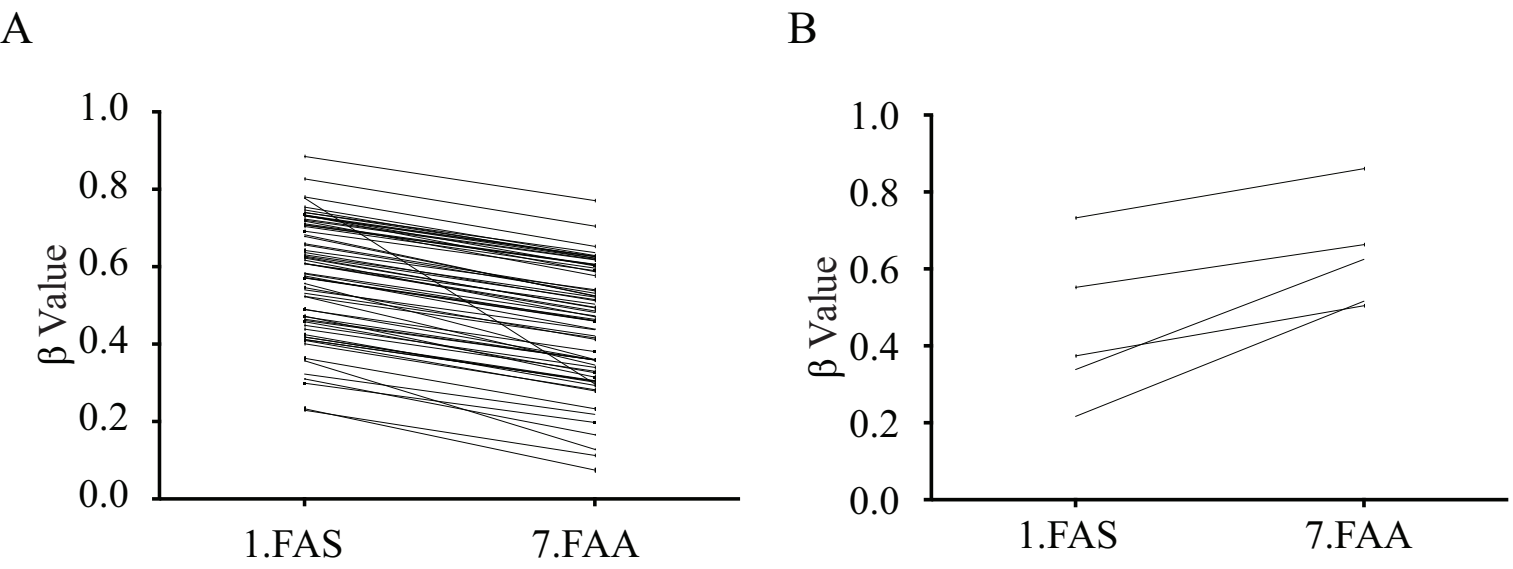

B



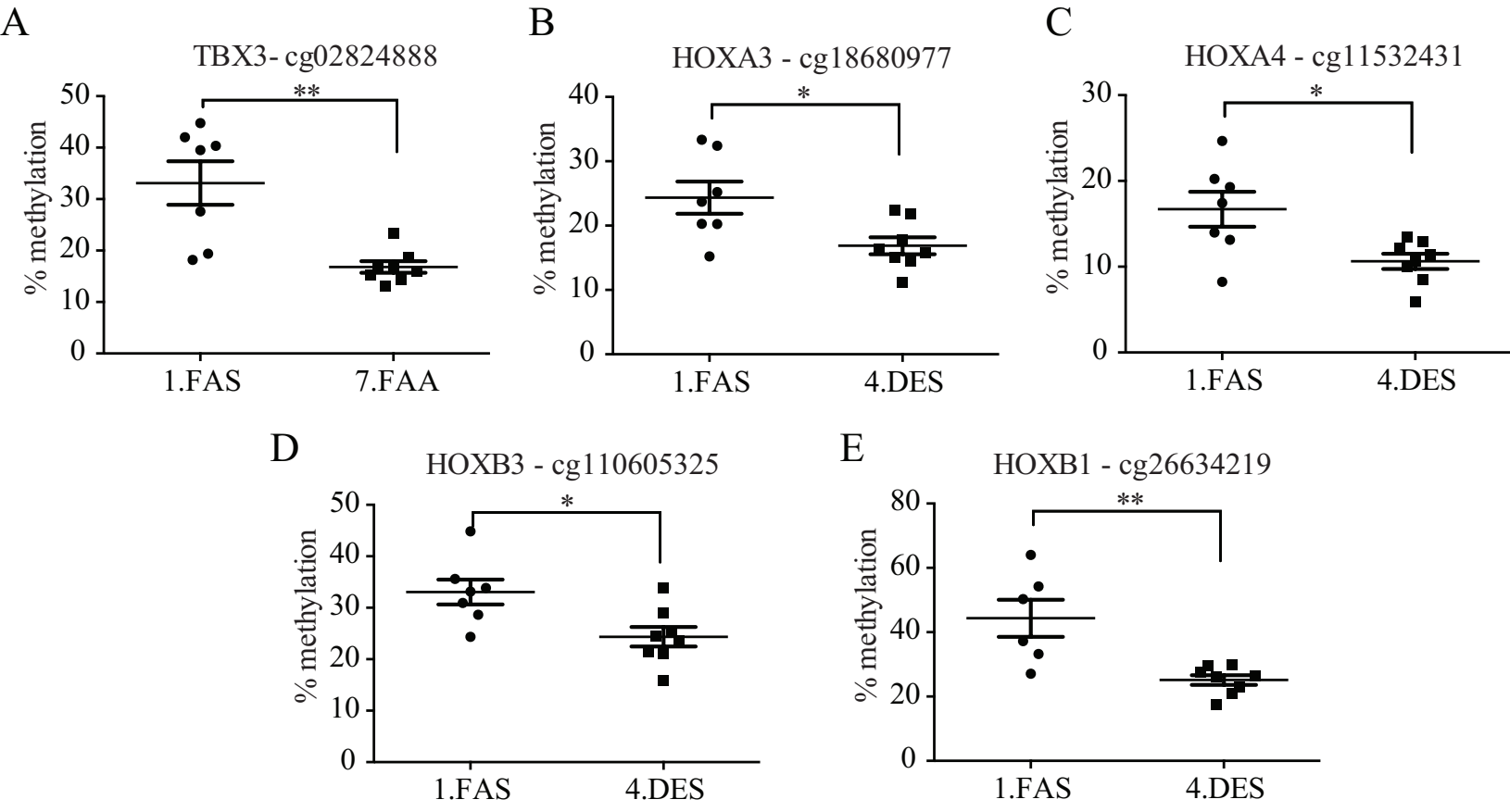
**Supplemental Fig 2**



**Supplemental Fig 3**



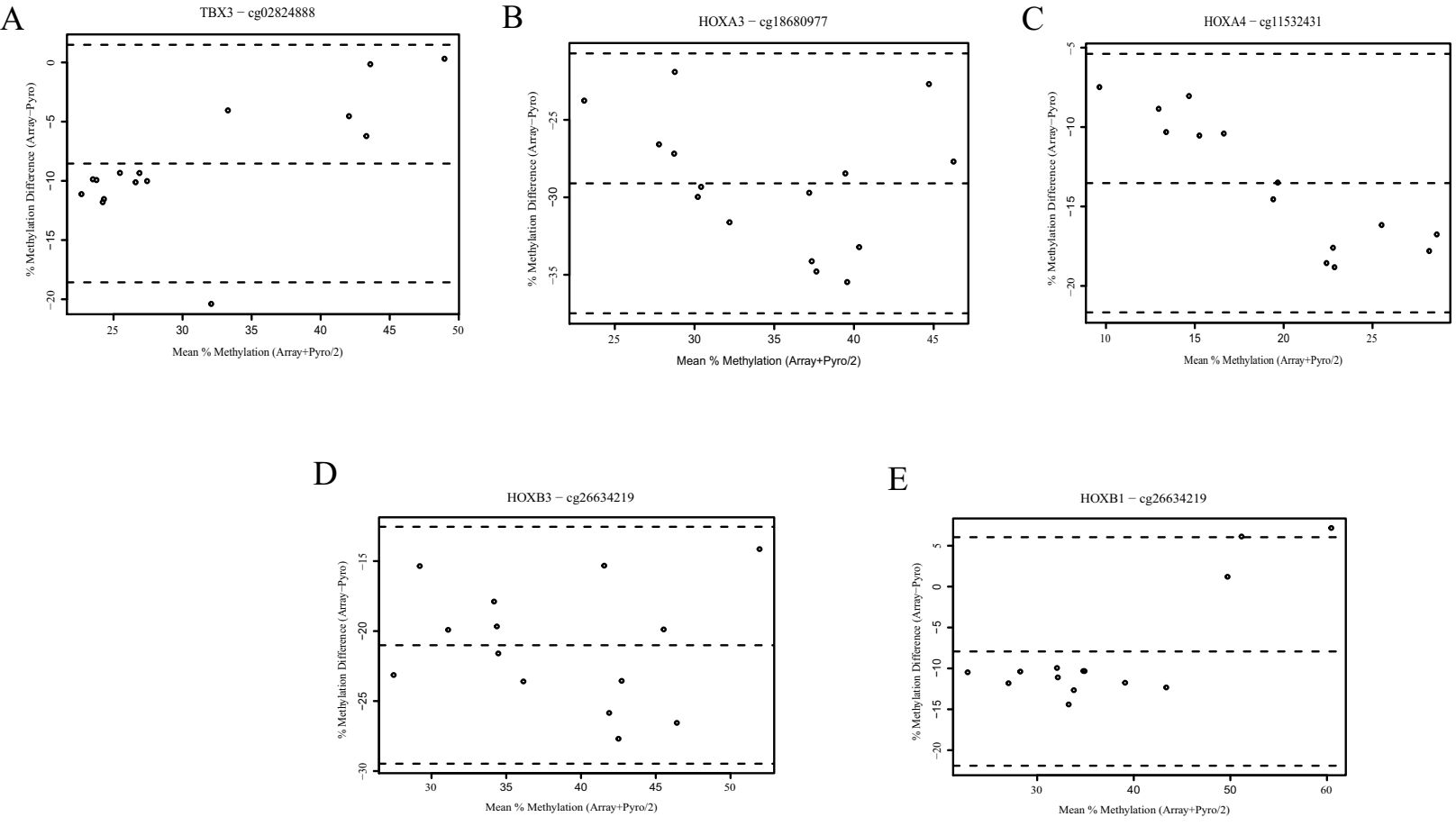
**Supplemental Fig 4**




F

| CpG | Δβ Array | Δβ Pyrosequencing | Pyrosequencing Mann - Whitney p value |
|---|---|---|---|
| cg18680977 | 10.2 | 7.5 | <0.05 |
| cg11532431 | 10.4 | 6.1 | <0.05 |
| cg26634219 | 11.8 | 19.1 | <0.005 |
| cg11060532 | 11.8 | 8.7 | <0.05 |

**Supplemental Fig 5**



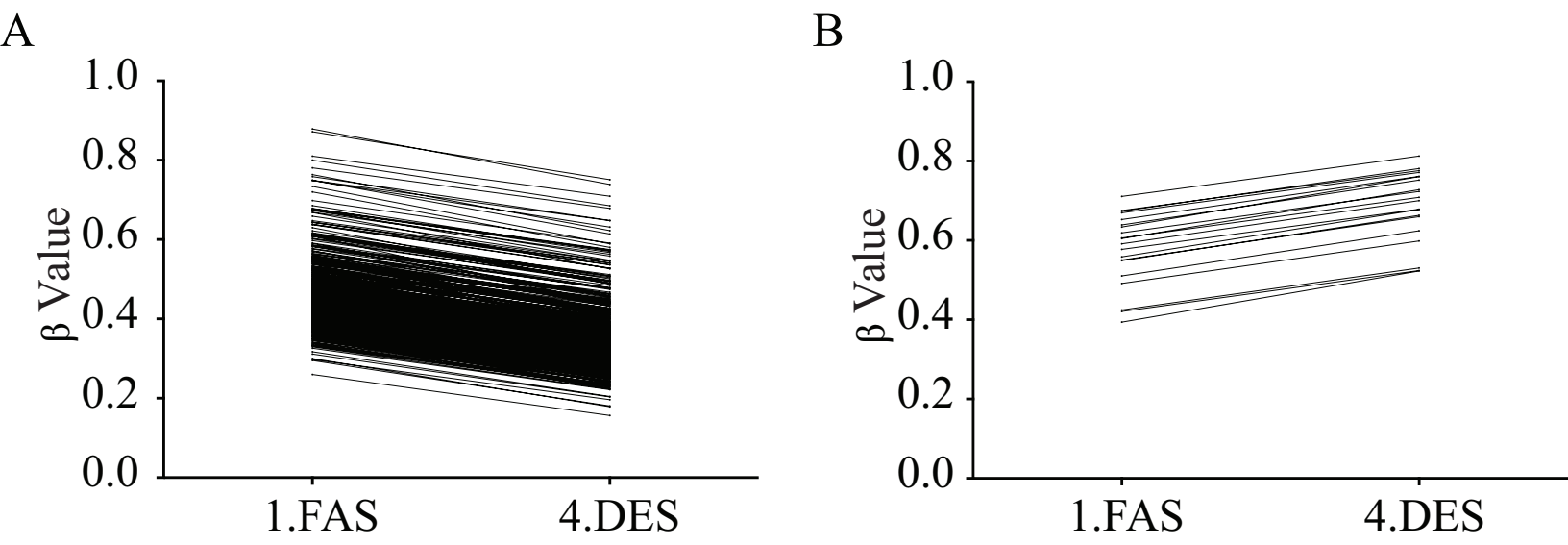
A **Supplemental Fig 6**

| Cluster | Enrichment Score | Term | Count | Benjamini P-value |
|---|---|---|---|---|
| 1 | 1.58 | GO:0032269 (negative regulation of cellular protein metabolic process) | 4 | 0.84 |
| | | GO:0051248 (negative regulation of protein metabolic process) | 4 | 0.64 |
| | | GO:0010605 (negative regulation of macromolecule metabolic process) | 6 | 0.83 |
| | | GO:0032268 (regulation of cellular protein metabolic process) | 4 | 0.95 |
| | | GO:0005829 (cytosol) | 3 | 0.99 |
| 2 | 1.55 | GO:0010033 (response to organic substance) | 7 | 0.61 |
| | | GO:0051384 (response to glucocorticoid stimulus) | 3 | 0.77 |
| | | GO:0031960 (response to corticosteroid stimulus) | 3 | 0.77 |
| | | GO:0009725 (response to hormone stimulus) | 4 | 0.96 |
| | | GO:0009719 (response to endogenous stimulus) | 4 | 0.96 |
| | | GO:0048545 (response to steroid hormone stimulus) | 3 | 0.96 |

B

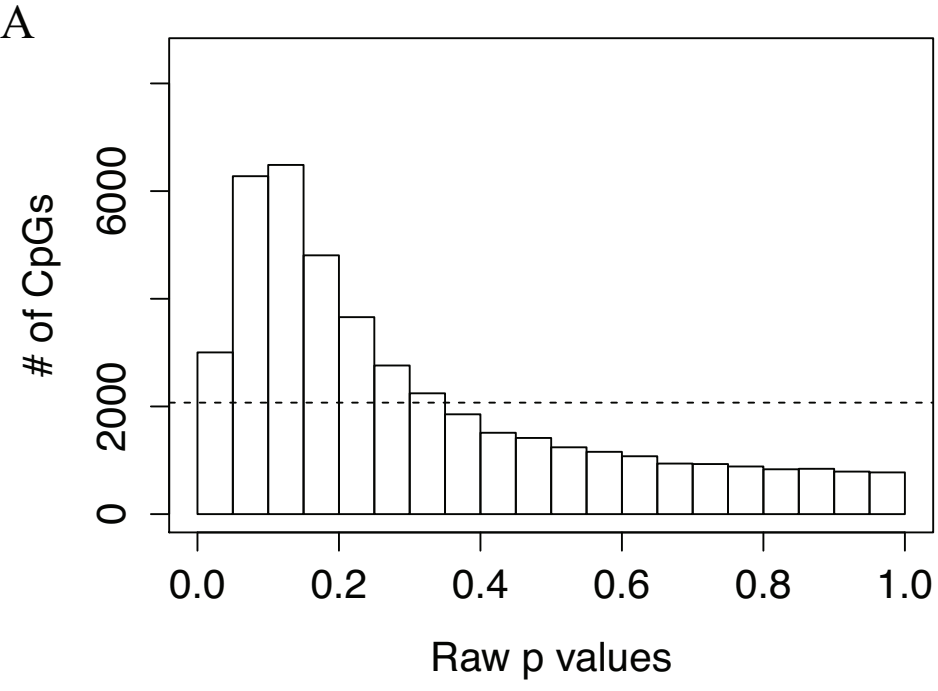
| Cluster | Enrichment Score | Term | Count | Benjamini P-value |
|---|---|---|---|---|
| 1 | 1.96 | GO:0022610 (biological adhesion) | 28 | 1 |
| | | GO:0007155 (cell adhesion) | 28 | 1 |
| | | GO:0016337 (cell-cell adhesion) | 11 | 0.95 |
| 2 | 1.43 | GO:0008104 (protein localization) | 23 | 0.93 |
| | | GO:0034613 (cellular protein localization) | 13 | 0.93 |
| | | GO:0070727 (cellular macromolecule localization) | 13 | 0.91 |
| | | GO:0015031 (protein transport) | 20 | 0.91 |
| | | GO:0006886 (intracellular protein transport) | 12 | 0.9 |
| | | GO:0045184 (establishment of protein localization) | 20 | 0.9 |
| | | GO:0046907 (intracellular transport) | 15 | 0.96 |
| 3 | 1.36 | GO:0001568 (blood vessel development) | 11 | 0.96 |
| | | GO:0001944 (vasculature development) | 11 | 0.97 |
| | | GO:0048514 (blood vessel morphogenesis) | 8 | 0.92 |
| | | GO:0001525 (angiogenesis) | 5 | 0.98 |
| 4 | 1.35 | GO:0043565 (sequence-specific DNA binding) | 23 | 0.13 |
| | | GO:0003700 (transcription factor activity) | 26 | 0.72 |
| | | GO:0051252 (regulation of RNA metabolic process) | 37 | 0.91 |
| | | GO:0006355 (regulation of transcription, DNA-dependent) | 36 | 0.91 |
| | | GO:0030528 (transcription regulator activity) | 34 | 0.95 |
| | | GO:0006357 (regulation of transcription from RNA | 20 | 0.92 |
| | | GO:0006350 (transcription) | 35 | 0.97 |
| | | GO:0045449 (regulation of transcription) | 43 | 0.97 |
| | | GO:0003677 (DNA binding) | 37 | 0.97 |
| 5 | 1.34 | GO:0040012 (regulation of locomotion) | 10 | 0.96 |
| | | GO:0051270 (regulation of cell motion) | 10 | 0.94 |
| | | GO:0030334 (regulation of cell migration) | 9 | 0.95 |
| | | GO:0051272 (positive regulation of cell motion) | 6 | 0.92 |
| | | GO:0030335 (positive regulation of cell migration) | 5 | 0.94 |
| | | GO:0040017 (positive regulation of locomotion) | 5 | 0.96 |

**Supplemental Fig 7**



**Supplemental Fig 8**

A




B

| CpG | Q Value |
|---|---|
| cg00458681 | 0.0436 |
| cg04234412 | 0.0436 |
| cg6437703 | 0.0436 |
| cg12419862 | 0.0459 |
| cg20007245 | 0.0436 |
| cg25703541 | 0.0436 |