Differential Transcriptional Changes in Mice Exposed to Chemically Distinct Diesel Samples

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Abstract: Epidemiological studies have linked exposure to ambient particulate matter (PM) with increased asthmatic symptoms. Diesel exhaust particles (DEP) are a predominant source of vehicle derived ambient PM, and experimental studies have demonstrated that they may have adjuvant potential when given with an antigen. We previously compared 3 DEP samples: N-DEP, A-DEP, and C-DEP in a murine ovalbumin (OVA) mucosal sensitization model and reported the adjuvant activity to be: C-DEP \( \approx \) A-DEP \( \approx \) N-DEP. The present study analyzed gene expression changes from the lungs of these mice. Transcription profiling demonstrated that all the DEP samples altered cytokine and toll-like receptor pathways regardless of type, with or without antigen sensitization. Further analysis of DEP exposure with OVA showed that all DEP treatments altered networks involved in immune and inflammatory responses. The A- and C-DEP/OVA treatments induced differential expression of apoptosis pathways in association with stronger adjuvant responses, while expression of cell cycle control and DNA damage pathways were also altered in the C-DEP/OVA treatment. This comprehensive approach using gene expression analysis to examine changes at a pathway level provides detailed information on events occurring in the lung after DEP exposure, and confirms that the most bioactive sample induced many more individual genes and changes in immuno-regulatory and homeostatic pathways.

Keywords: diesel exhaust particles, allergy, inflammation, lung, genomics, mouse model
Introduction

Epidemiology studies have reported an association between increased ambient particle matter (PM) levels and hospital admission rates due to respiratory illnesses including asthma. Diesel exhaust particles (DEP) are an important contributor to ambient PM and many studies have focused on DEP as a model anthropogenic pollutant. DEP consist of a carbon core surrounded by various amounts of adsorbed organic compounds, including polycyclic aromatic hydrocarbons (PAHs), quinones, and nitro-PAHs. Human and rodent studies have shown that DEP augments the induction of allergic lung disease when given with an antigen. Although the chemical composition and biologic mechanisms associated with the adjuvant effects of DEP are not well understood, the organic components of DEP and resulting oxidative stress responses are thought to skew the immune system towards a T-helper 2 (TH2) response.

The composition of DEP varies greatly depending on the type of engine, load, and method of collection, which in turn can alter its biological function. Singh et al. investigated the chemical characteristics and pulmonary toxicity of two different particles, an automobile derived DEP (A-DEP) and National Institute of Standard Technology standard reference material 2975 (N-DEP) generated from a heavy forklift. The two particle samples exhibited disparate pulmonary toxicity and mutagenic activity, which reflected their dissimilar chemical composition. Recently, we assessed the effects of N-DEP, A-DEP, and a newer particle termed C-DEP (generated from a diesel engine used to power a compressor) in a murine ovalbumin (OVA) mucosal sensitization model. These samples differed in their percentage of dichloromethane (DCM) extractable organic material (EOM) and PAHs with N-DEP, C-DEP, and A-DEP containing 1.5%, 18.9%, and 67% EOM, and 47, 431, and 522 µg of PAHs per gram of DEP, respectively.

Materials and Methods

Animals

Female BALB/C mice (8–10 weeks old) were obtained from Charles River Laboratories (Raleigh, NC) and allowed to acclimate for a minimum of one week prior to dosing. Mice were randomly assigned to treatment groups and housed in an AAALAC-approved animal facility at the US-EPA. All animal procedures were reviewed and approved by the US-EPA’s Institutional Animal Care and Use Committee. Housing environment conditions include a 12-h light/dark cycle at an ambient temperature of 22 ± 1 °C and relative humidity of 55 ± 5 °C. Mice were provided water and mouse chow ad libitum. Additional mice from each facility were routinely monitored serologically for Sendai, mouse pneumonia, mouse hepatitis, and other murine viruses, as well as mycoplasma.

Production of T_{H2} cytokines, while reducing levels of the T_{H1} cytokine IL-12 in the BALF. In contrast, the elemental carbon rich N-DEP/OVA induced a milder T_{H2} phenotype post-sensitization while the A-DEP did not alter measured post-sensitization values, but displayed strong adjuvant activities post-challenge.

We have previously demonstrated that inhalation exposure to fresh diesel exhaust (DE) from the same engine that produced the C-DEP sample at an occupationally relevant dose, caused mild adjuvant effects compared to air controls. Global gene expression analysis showed that the DE in combination with OVA sensitization altered oxidative stress and metabolism pathways, whereas DE in the absence of immunization modulated cell cycle control, growth and differentiation, G-proteins, and cell adhesion pathways. To understand more precisely which pathways and cellular signaling events were amplified by the three chemically distinct DEP samples during sensitization, we initiated a similar global genomic approach. Microarray analysis of whole-lung RNA was used to elucidate the pathways and networks involved in the effects of N-DEP, A-DEP, and C-DEP with or without allergen sensitization in BALB/C mice. The design of the study permitted direct comparison of early global gene expression changes with the previously reported pulmonary immune and inflammatory effects of DEP alone and in combination with OVA.
Particle samples
Standard Reference Material (SRM) 2975 diesel exhaust particle sample (N-DEP) was purchased from National Institute of Standard Technology (NIST) (Gaithersburg, MD). The reported mean diameter of these particles was \(11.2 \pm 0.1 \mu m\) by area distribution, and the surface area, as determined by nitrogen adsorption, was \(91 \mu m^2/g\). The certified analysis contains 11 certified concentrations and 28 reference concentrations for selected PAHs found in the DEPs. The DEP was generated by a heavy-duty forklift diesel engine and collected under “hot” conditions without a dilution tunnel.

Automobile DEP (A-DEP; courtesy of T. Kobayashi, NIES, Japan) was generated and collected under conditions previously described. Briefly, the sample was generated by a light-duty (2740 cc), 4-cylinder Isuzu diesel engine. DEP was collected under “cold” (50 °C) conditions onto glass-fiber filters and on steel duct walls in a constant-volume sampling system fitted at the end of a dilution tunnel.

Compressor DEP (C-DEP) was generated in-house as described by Cao et al at the EPA using a 30 kW (40 hp) 4-cylinder Deutz BF4M1008 diesel engine connected to a 22.3 kW Saylor Bell air compressor to provide 20% load. The generated particles were collected under “hot” conditions in a baghouse.

Experimental design
DEP samples (N-, C-, A-DEP) were suspended at a concentration of 3 mg/ml in saline alone or with 0.4 mg/ml of OVA. Particles were sonicated using a Microson Ultrasonic Cell Disruptor (Micromix) for 10 min. Mice were randomly divided into 8 treatment groups containing 5 mice each, anesthetized with isoflurane, and exposed to saline, 20 µg OVA, 150 µg DEP, or DEP + OVA by intranasal instillation on Days 0 and 13 and necropsied 18 hrs later. Additional groups of animals were held for subsequent phenotypic analysis post-challenge.

Necropsy and RNA isolation
Mice were euthanized with sodium pentobarbital and bled by cardiac puncture. The chest wall was opened and the left lung lobe was removed, quick frozen in liquid nitrogen, and stored at −80 °C. RNA from frozen lung tissue was isolated using RNeasy (Qiagen, Valencia, CA) following manufacturer’s protocol. Quantity and quality of the RNA was measured using a Nanospect and Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA), respectively. Three mice were randomly selected from each group of five animals for microarray analysis.

Microarray
RNA samples were selected prepared, processed, and hybridized to the Affymetrix Mouse 430 A gene chip at Expression Analysis (Durham, NC), as described in the GeneChip Expression Analysis Manual (Affymetrix; Santa Clara, CA). The hybridized probe array was stained with streptavidin phycoerythrin conjugate and scanned by the GeneChip® Scanner 3000 (Affymetrix; Santa Clara, CA). The amount of light emitted at 570 nm is proportional to the bound target at each location on the probe array.

The Mouse 430 A Genome chip contains over 22,000 probe sets representing over 14,000 well-characterized mouse genes. A detailed description can be found at http://www.affymetrix.com/products/arrays/specific/mouse430.affx. A total of 24 gene chips representing lung samples from 24 individual mice (8 treatments, \(N = 3\)) were used in this study. The microarray data have been deposited at Genome Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO series accession number GSE22357.

Overall data analysis strategy
The analysis approach of this data set, consisting of 2 controls and 6 treatment groups, was to use a binary comparison approach of each treatment group compared to its respective control: N-DEP and saline, A-DEP and saline, C-DEP and saline, N-DEP/OVA and OVA, A-DEP/OVA and OVA, and C-DEP/OVA and OVA. The analysis of these data sets consisted of: 1) evaluating the data quality; 2) performing principal components analysis (PCA) for a global inspection of within group sample correspondence and to examine model and dose effects; 3) performing Gene Set Enrichment Analysis (GSEA) to determine differentially expressed gene sets between a treatment group and its control; 4) extracting core genes responsible for a particular gene set identified as significant from the
GSEA analysis; 5) determining common differentially expressed genes across treatment groups; 6) mapping core genes to functional pathways using KEGG pathways and MetaCore GENEGO® to identify altered pathways and networks unique or in common among the treatments.

Principal component analysis (PCA)
PCA transforms microarray data from all gene chips to a new coordinate system using an orthogonal linear transformation, which identifies a lower dimensional coordinate system that accounts for most of the variance in the data set. This analysis was employed to survey the data for within-group outliers and model and dose effects using Rosetta Resolver (Rosetta Inpharmatics, Agilent Technologies, Palo Alto, CA) following linear weighting normalization ($P < 0.001$). Each individual gene chip or gene expression profile was represented by a single data point and the variance between a pair of gene chips was comparable to the distance between the data points. The closer the data points the greater the similarity of their gene expression profiles. This analysis was employed as a visual tool to initially inspect the data for within group and across group similarities and dissimilarities.

Gene set enrichment analysis (GSEA)
GSEA is a powerful computational method that utilizes an a priori defined set of genes to determine statistically significant, concordant differences between two phenotypes. For this analysis, probe-level data from 24 gene chips were imported into Gene Pattern (www.genepattern.org) and preprocessed using the Robust Multichip Average (RMA) method, which uses background correction, quantile normalization, and median scaling to generate estimated expression summaries. The RMA generated values were imported into GSEA to determine gene sets associated with each diesel treatment group compared to its respective control. The molecular signature database (MSigDB) C2 provided on the website http://www.broad.mit.edu/gsea/msigdb/msigdbindex.html, which contains 1687 gene sets, was queried for association with a particular treatment in each pairwise comparison (N-DEP/OVA and OVA, A-DEP/OVA and OVA, C-DEP/OVA and OVA, N-DEP and saline, A-DEP and saline, and C-DEP and saline). Only gene sets with a minimal gene set size of 15 genes per pathway and a maximum of 90 were queried. We acknowledge our use of GSEA software and MSigDB (http://www.broad.mit.edu/gsea/).17

Pathway level analysis
The gene sets with a false discovery rate (FDR) q-value of <0.01 were used to create a core gene list. The core gene list is comprised of genes responsible for a gene set being considered significant. These genes were exported and then applied to two pathway analysis programs, KEGG Pathway Analysis (http://gather.genome.duke.edu/) and MetaCore GENEGO® (http://www.genego.com/metacore), which maps genes to pathways and determines pathway significance. All pathways with a $P$-value of <0.001 and at least 5 or more differentially expressed genes were reported.

Results
Principal component analysis (PCA)
PCA was applied to provide a multidimensional gene expression profile of each gene chip in a 3 dimensional space to reveal clusters in the experimental data. All data from the 24 gene chips were analyzed with each dot representing a gene chip (Fig. 1a). After analysis the gene chips were then highlighted in either blue (OVA treatment) or red (saline control). Good separation of the two groups was observed, illustrating a model effect between antigen and saline. The saline group appeared to be more tightly clustered than OVA indicating lower within group variability. To determine if exposure to chemically different DEP samples induces diverse genetic profiles, the gene chips were highlighted according to diesel sample (purple- A-DEP and A-DEP/OVA, blue- C-DEP and C-DEP/OVA, green- N-DEP and N-DEP/OVA, and yellow- saline and OVA) (Fig. 1b). The plot revealed separation of the saline and OVA treated C-DEP and N-DEP groups, while the saline and OVA A-DEP exposed mice clustered together.

Gene set enrichment analysis (GSEA)
GSEA was developed to overcome the limitations of relatively small individual differential gene expression changes among biologically related genes and small sample size. In contrast to conventional microarray analysis programs, the algorithm employed by GSEA derives its power by focusing on gene sets with biological relevance rather than individual genes.17,18
To test for sets of related genes that were altered in the lungs of mice exposed to the various treatments, we employed GSEA. The arrays were separated into 6 binary groups; N-DEP/saline and saline, A-DEP/saline and saline, C-DEP/saline and saline, N-DEP/OVA and OVA, A-DEP/OVA and OVA, and C-DEP/OVA and OVA. The C2 collection of curated gene sets from the MSigDB were queried and a detailed description of each gene set can be found on the website http://www.broad.mit.edu/gsea/msigdb/msigdb_zindex.html. Gene sets with an FDR q-value of <0.01 were considered significant. The number of significant gene sets associated with N-DEP, A-DEP, and C-DEP, as determined by the pairwise comparisons (DEP exposure and saline control), was 101, 90, and 98, respectively. In the context of antigen,
60, 68, and 113 gene sets were associated with N-, A-, and C-DEP/OVA, respectively. The complete list of the significant gene sets is found in Appendices 1–6.

**Venn analyses**

Venn analyses were performed to identify the common genes to all DEP exposures. The core genes (those genes responsible for a gene set being considered significant with an FDR q-values of \(<0.01\)) were extracted from the significant gene sets associated with each diesel exposure identified by GSEA. A Venn diagram was constructed to identify genes common among the 3 DEP/saline exposure pairwise comparisons (Fig. 2a). A-DEP/saline exposure resulted in the greatest number of differentially expressed genes (545). 200 genes were common among all 3 DEP treatments. Similarly a Venn diagram was constructed for the genes associated with each DEP/OVA exposure (Fig. 2b). C-DEP/OVA exposure resulted in greatest number of differentially expressed genes (800). 236 genes were found common to all DEP/OVA exposures. The two sets of common genes were applied to another Venn diagram to identify the 117 common genes among all DEP exposures (Fig. 2c).

**KEGG pathway analyses**

To understand the biological significance of the common genes associated with the 3 DEP/saline exposures, the 200 genes were imported into the gene annotation tool, Gather (http://gather.genome.duke.edu/), and the genes were mapped to KEGG pathways, using the criteria that pathways must have 5 or more differentially expressed genes and be over-represented based on a hypergeometric test with a \(P\)-value <0.001. Cytokine-cytokine receptor interaction and toll-like receptor signaling pathways were common to all DEP/saline exposures (Table 1) with the majority of the genes being associated with neutrophil signaling. The 236 genes common among the 3 DEP/OVA exposures also significantly populated the cytokine-cytokine receptor interaction and toll-like receptor (TLR) signaling pathway (Table 2), but with better coverage of these pathways (40 and 17 genes for DEP/OVA versus 28 and 11 genes for DEP/saline, respectively). In addition the DEP/OVA treatments also significantly altered 11 genes associated with apoptosis pathways (Table 2).

![Figure 2. Venn analyses.](image)

To understand the effects of the individual DEP/OVA exposures, the extracted core genes were mapped to KEGG pathways and the results represented in Tables 4–6. All 3 exposures populated the cytokine-cytokine receptor pathway similarly with 56, 56, and 51 genes for N-DEP/OVA, A-DEP/OVA, and C-DEP/OVA, respectively. Additionally, the TLR pathway...
Table 1. KEGG pathways mapped from the 200 common genes associated with DEP/saline exposure.

| KEGG pathway | # Genes | P value |
|--------------|---------|---------|
| Cytokine-cytokine receptor interaction | 28 | <0.0001 |
| Toll-like receptor signaling pathway | 11 | <0.0001 |

Table 2. KEGG pathways mapped from the 236 genes common to all DEP/OVA exposure.

| KEGG pathway | # Genes | P value |
|--------------|---------|---------|
| Cytokine-cytokine receptor interaction | 40 | <0.0001 |
| Toll-like receptor signaling pathway | 17 | <0.0001 |
| Apoptosis | 11 | 0.0002 |

Table 3. KEGG pathways mapped from the 117 genes common to both DEP/OVA and DEP/saline exposure.

| KEGG pathway | # Genes | P value |
|--------------|---------|---------|
| Cytokine-cytokine receptor interaction | 26 | <0.0001 |
| Toll-like receptor signaling pathway | 10 | <0.0001 |

Table 4. KEGG pathway mapped from the 526 genes associated with N-DEP/OVA exposure.

| KEGG pathway | # Genes | P value |
|--------------|---------|---------|
| Cytokine-cytokine receptor interaction | 56 | <0.0001 |
| Toll-like receptor signaling pathway | 23 | <0.0001 |
| Neuroactive ligand-receptor interaction | 6 | 0.0002 |
contained similar amounts of genes with 23, 26, and
28 genes for N-DEP/OVA, A-DEP/OVA, and C-DEP/
OVA, respectively. This pathway contained TLRs as
well as many pro-inflammatory cytokines and tran-
scription factors. N-DEP/OVA and C-DEP/OVA
altered the expression of genes in the neuroactive
ligand-receptor interaction pathway. A-DEP/OVA
and C-DEP/OVA also altered the apoptosis pathway,
while additional pathways for pyrimidine metabolism
and aminoacyl-tRNA biosynthesis were unique to the
C-DEP/OVA exposures.

**GeneGo analysis**
The C-DEP/OVA exposure gave the highest tran-
scriptional changes based on the numbers of signifi-
cant gene sets, extracted core genes, and the KEGG

| KEGG pathway                                         | # Genes |  P  value |
|------------------------------------------------------|---------|-----------|
| Cytokine-cytokine receptor interaction               |         | <0.0001   |
| Ccl11 Ccl17 Ccl2 Ccl22 Ccl3 Ccl4 Ccl6 Ccl7 Ccl8 Ccl9 Ccr1 Ccr2 Ccr4 Ccr5 Csf1 Csf2 Csf2ra Csf2rb1 Csf2rb2 Csf3r Cxcl1 Cxcl10 Cxcl11 Cxcl13 Cxcl2 Cxcl5 Cxcl9 Ifnar2 Ifnb1 Ifng Ifngr2 Il1a Il1b Il1r1 Il1r2 Il2 Il2rg Il4 Il5 Il6 Il8rb Inhba Osrm Tgfb1 Tgfb1 Tnf Tnfrsf1a Tnfrsf1b Tnfrsf5 Tnfrsf9 Tnfrsf10 Tnfrsf13 Tnfrsf13b | 56      | <0.0001   |
| Toll-like receptor signaling pathway                  |         | <0.0001   |
| Ccl3 Ccl4 Cd14 Cxcl10 Cxcl11 Cxcl9 Ifnar2 Ifnb1 Ikbke Il1a Il12a Il12b Il1b Il6 Lbp Map3k7ip1 Mapk13 Myd88 Nfk1 Nfk2 Nfkbia Pik3cd Rac2 Stat1 Tlr1 Tlr2 Tnf | 26      | <0.0001   |
| Apoptosis                                            |         | <0.0001   |
| Bax Birc3 Capn1 Casp3 Cflar Csf2rb1 Csf2rb2 Il1a Il1b Il1r1 Myd88 Nfk1 Nfk2 Nfkbia Pik3cd Ripk1 Tnf Tnfrsf1a Tnfrsf10 | 19      | <0.0001   |

**Table 5.** KEGG pathways mapped from the 483 genes associated with A-DEP/OVA exposure.

| KEGG pathway                                         | # Genes |  P  value |
|------------------------------------------------------|---------|-----------|
| Cytokine-cytokine receptor interaction               |         | <0.0001   |
| Ccl11 Ccl17 Ccl2 Ccl22 Ccl3 Ccl4 Ccl6 Ccl7 Ccl8 Ccl9 Ccr1 Ccr2 Ccr5 Csf1 Csf2 Csf2ra Csf2rb1 Csf2rb2 Csf3r Cxcl1 Cxcl10 Cxcl11 Cxcl13 Cxcl2 Cxcl5 Cxcl9 Ifngr2 Il10a Il1a Il1b Il1r1 Il1r2 Il2 Il2rg Il3a Il6 Il7r Il8rb Inhba Llb Osrm Tgfb1 Tgfb1 Tnf Tnfrsf1a Tnfrsf1b Tnfrsf5 Tnfrsf9 Tnfrsf13b | 51      | <0.0001   |
| Aminoacyl-tRNA biosynthesis                          |         | <0.0001   |
| Aars Cars Fars1 Farslb Gars lars Kars Nars Rars Tars Vars2 Wars Yars | 13      | <0.0001   |
| Toll-like receptor signaling pathway                 |         | <0.0001   |
| Casp8 Ccl3 Ccl4 Cd14 Chuk Cxcl10 Cxcl11 Cxcl9 Ikbkb Ikbke Il1b Il6 Ikrkap1 Lbp Ly96 Map2k4 Map3k7 Mapk13 Myd88 Nfk1 Nfk2 Nfkbia Pik3cd Rac2 Stat1 Tlr2 Tlr4 Tlr7 Tnf | 28      | <0.0001   |
| Pyrimidine metabolism                                |         | <0.0001   |
| Ctps Dck Dlymk Dut Ecgf1 Nme1 Nme2 Pola2 Pold1 Pold2 Pole2 Polr2 g Polr2h Polr3k Prim1 Rrm2 Tnrd1 Umpk Umpk Ump Upp1 | 21      | <0.0001   |
| Neuroactive ligand-receptor interaction              |         | <0.0001   |
| Adora2b Bzrp C3ar1 Grik5 Gzma P2ry6 Ptg4er4 | 7       | <0.0001   |

**Table 6.** KEGG pathways mapped from the 800 genes associated with C-DEP/OVA exposure.
pathways, however, these analyses were not specific enough to allow an inference as to why or how C-DEP was able to elicit a stronger T\textsubscript{H2} response post-sensitization. We therefore mapped the 3 sets of genes to GeneGo curated databases and presented the results as networks and pathways. Figure 3 depicts the significance (-\log(p)values) of the top 20 differentially affected GeneGo process networks for all 3 DEP/OVA exposures (N-DEP/OVA-blue; A-DEP/OVA-red; C-DEP/OVA-orange). Using this approach the similarities and differences of the groups can be clearly discerned. All groups significantly altered networks related to antigen presentation, inflammation, and cell adhesion. In addition the C-DEP/OVA exposure also altered networks related to cell cycle control, DNA damage, and protein degradation.

GeneGo pathway analysis revealed that the pathways common to all treatments were associated with MHC class I antigen presentation, inflammation, and other pathways related to the innate immune response. The A-DEP/OVA and C-DEP/OVA common pathways were involved with TNF-\(\alpha\) mediated apoptosis pathways whereas C-DEP/OVA alone also induced altered expression of Fas, inhibitor of apoptosis (IAP), and mitochondrial mediated apoptosis and cell cycle regulation pathways (Fig. 4).

**Discussion**

DEP have been reported to act as adjuvants to upregulate immune responses resulting in increased allergic lung disease; however, there is still a lack of understanding as to what DEP component or components are responsible for these effects, and the underlying mechanisms through which they act. The present study examined global transcriptional changes in the lungs of mice after exposure to three chemically distinct DEP samples (N-, A- and C-DEP) with or without antigen sensitization. Overall, all the DEP and DEP/OVA samples significantly altered cytokine and TLR pathways compared to their respective controls, however, the DEP/OVA samples induced a greater number of genes in these pathways. In addition,
A- and C-DEP/OVA samples altered apoptosis pathways while the most biologically active DEP sample, C-DEP/OVA, also altered cell cycle and DNA repair networks.

We recently reported the inflammatory and adjuvant effects of the above three diesels, N-, A-, and C-DEP.\(^1\) Although it is important to identify individual genes associated with adverse biological effects, the resulting phenotypic changes likely occur through interactions of multiple genes. Therefore, to associate transcriptional responses with the allergic phenotype we utilized a global approach to a) characterize the genomic signature of DEP as a class and b) identify pathways common and unique to the DEP/OVA exposures.

Our genomic signature response to DEP as a class compound is well supported by the literature. DEP exposure has been shown to induce lung inflammation as manifested by neutrophil infiltration and elevated levels of total protein, albumin, LDH, and reactive oxygen species (ROS) in the lung as well as up-regulation of inflammatory cytokines.\(^3,11,12,19,20\) Furthermore, DEP and other particles including ambient PM have been shown to induce TLR4 expression in the lung,\(^21,22\) while TLR4 deficient mice developed less airway inflammation in response to DEP compared to wild type controls.\(^23\) In agreement with these findings, our results demonstrated the cytokine-cytokine receptor and the TLR interaction KEGG pathways were significantly altered in all DEP/saline exposures. These findings not only provide further evidence for the TLR pathway involvement in DEP induced inflammatory response, but also confirmed a common genomic signature response of DEP, regardless of chemical composition.

It has been established that while DEP alone can induce an inflammatory response, when given with an antigen they also act as an immunologic adjuvant.\(^3-6\) The DEP/OVA exposures did not alter KEGG pathways such as antigen processing and presentation or T cell receptor signaling. Instead, in a similar fashion to DEP alone, genomic analysis of all the DEP/OVA exposed samples displayed common alterations in the cytokine-cytokine receptor and TLR KEGG pathways.

![Figure 4. Results of GeneGo mapping of differentially affected pathways. The core genes from significantly altered gene sets associated with each DEP/OVA exposure (N-DEP/OVA-blue; A-DEP/OVA-red; C-DEP/OVA-orange) was imported into GeneGo to generate a list of the top 20 pathways.](image-url)
Diesel-enhanced pulmonary allergy

This is a likely response because under this analysis all DEP/OVA samples were compared to OVA exposure. However, compared to DEP/saline, DEP/OVA exposed samples showed a more pronounced increase in the number of genes enriched in these pathways. This provides further evidence, on the genomic level, that DEP and OVA interacted synergistically to produce an immuno-modulatory effect.

Li et al.24 proposed a hierarchical oxidative stress model to explain DEP induced effects whereby low levels of oxidative stress induce antioxidant defense mechanisms to restore redox balance in the cell (tier 1). Intermediate levels of oxidative stress (tier 2) activate MAPK and NF-κB cascades, which induce inflammation, while high levels of oxidative stress (tier 3) activate apoptosis and apoptosis/necrosis pathways.24 In agreement with this model, the study presented here demonstrates similar effects in vivo. Antioxidant transcription factors and enzymes such as Nrf2, heme oxygenase 1 (HO-1), and superoxide dismutase 2 (SOD2) were up-regulated in response to all three DEP/OVA exposures, indicative of low levels of oxidative stress (tier 1). The tier 2 responses, MAPKs, NF-κB, as well as inflammatory, T1, and T12 cytokines and chemokines, were also up-regulated in all three DEP/OVA exposures. In addition, A- and C-DEP/OVA exposures altered apoptosis (tier 3) pathways with C-DEP/OVA having altered the greatest number of these pathways. From this analysis it would appear that these early changes were predictive of the previously reported post-challenge phenotype.11

It has been established that DEP organic compounds can generate ROS25 and excessive ROS production can lead to a variety of cellular responses including DNA damage.26 In fact, oxidative DNA damage (8-hydroxydeoxyguanosine) has been detected in mouse lung DNA after DEP exposure.27 Although the A-DEP sample contained the greatest amount of DCM EOM, C-DEP contained the greatest amount of PAHs11 and the C-DEP/OVA exposure group was unique in significantly altering cell cycle and DNA damage pathways. Global transcriptional analysis of lung tissue revealed up-regulation of cell cycle control genes including 6 cyclin genes, 7 cell division cycle genes, 7 members of the family of MAP kinases, 2 cyclin-dependent kinases, RAS p21 protein activator 3 (Rasa3), and 5 other RAS related proteins. Although we can not say with certainty that these changes were due to the PAH content, this evidence suggests the newer C-DEP sample generated more oxidative damage.

Conclusion

In conclusion, mice exposed to all three DEP samples with or without OVA had altered cytokine and toll-like receptor pathways suggesting these responses are common to all DEPs regardless of chemical profile. All DEP/OVA exposures increased transcription of genes involved in tier 1 and 2 of the hierarchical stress response model described by Li et al.24,28,29 Additionally, A- and C-DEP/OVA exposures significantly altered the most number of apoptosis pathways (tier 3). The C-DEP/OVA also altered cell cycle and DNA damage pathways suggesting it is the most bioactive sample. While the C-DEP sample also contained the largest amount of PAHs, these studies were not designed to address whether these components were causal to the effect. Nevertheless, it is known that PAHs can induce a pro-allergic effect.30–32 This comprehensive approach using gene expression analysis to examine pathway changes at a transcriptional level provides a clearer picture of the events occurring in the lung after DEP exposure in the presence or absence of antigen. The results illustrate a wide range of altered pathways suggesting this method may be more sensitive and can be used for identifying mechanisms involved in induction of immune responses that lead to increased severity of allergic lung disease.

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## Appendix

### Appendix 1. Significantly altered gene sets by N-DEP/saline compared to saline.

| Name                                         | Size | ES  | NES | FDR q-val   |
|----------------------------------------------|------|-----|-----|-------------|
| CARIES_PULP_HIGH_UP                           | 68   | 0.81| 2.85| <1.0E-06    |
| LAL_KO_3MO_UP                                 | 46   | 0.84| 2.80| <1.0E-06    |
| FLECHNER_KIDNEY_TRANSPLANT_REJECTION_UP       | 72   | 0.78| 2.79| <1.0E-06    |
| LINDSTEDT_DENDB_8H_VS_48H_UP                   | 58   | 0.81| 2.78| <1.0E-06    |
| LAL_KO_6MO_UP                                 | 58   | 0.80| 2.75| <1.0E-06    |
| WIELAND_HEPATITIS_B_INDUCED                   | 71   | 0.77| 2.71| <1.0E-06    |
| GALINDO_ACT_UP                                | 75   | 0.74| 2.66| <1.0E-06    |
| YANG_OSTEOLASTS_SIG                           | 39   | 0.84| 2.61| <1.0E-06    |
| NAKAJIMA_MCS_UP                               | 85   | 0.70| 2.55| <1.0E-06    |
| BLEO_HUMAN_LYMHP_HIGH_24HRS_UP                | 86   | 0.69| 2.54| <1.0E-06    |
| BASSO_GERMINAL_CENTER_CD40_UP                 | 82   | 0.69| 2.54| <1.0E-06    |
| HINATA_NFKB_UP                                | 89   | 0.69| 2.52| <1.0E-06    |
| NADLER_OBESITY_UP                             | 57   | 0.73| 2.51| <1.0E-06    |
| SANA_TNFA_ENDOTHELIAL_UP                      | 61   | 0.72| 2.50| <1.0E-06    |
| NEMETH_TNF_UP                                 | 82   | 0.69| 2.49| <1.0E-06    |
| MUNSHI_MM_VS_PCS_UP                           | 64   | 0.67| 2.34| <1.0E-06    |
| MUNSHI_MM_UP                                  | 57   | 0.69| 2.34| 7.18E-05    |
| NI2_MOUSE_UP                                  | 40   | 0.74| 2.33| 6.78E-05    |
| TNFA_NFKB_DEP_UP                              | 17   | 0.86| 2.33| 6.43E-05    |
| SHIPP_FL_VS_DLBCOL_DN                        | 30   | 0.74| 2.24| 6.11E-05    |
| LIN DSTEDT_ENDEND_UP                           | 44   | 0.69| 2.23| 5.81E-05    |
| TAVOR_CEBP_UP                                 | 42   | 0.69| 2.22| 5.55E-05    |
| HOUSTIS_ROS                                   | 32   | 0.73| 2.21| 5.31E-05    |
| MARTINELLI_IFNS_DIFF                          | 16   | 0.83| 2.19| 5.09E-05    |
| ZUCCHI_EPITHELIAL_DN                          | 36   | 0.68| 2.19| 4.88E-05    |
| TPA_SENS_MIDDLE_UP                            | 55   | 0.64| 2.17| 1.39E-04    |
| ROSS_CBF_MYH                                  | 38   | 0.70| 2.17| 2.21E-04    |
| CROONQUIST_IL6_RAS_UP                         | 18   | 0.81| 2.15| 2.99E-04    |
| ZHAN_MULTIPLE_MYELOMA_VS_NORMAL_DN            | 33   | 0.71| 2.15| 2.89E-04    |
| NAKAJIMA_MCSMBP_MAST                          | 37   | 0.69| 2.14| 3.19E-04    |
| ABBUD_LIF_UP                                  | 45   | 0.66| 2.14| 3.09E-04    |
| KNUDSEN_PMNS_UP                               | 65   | 0.62| 2.14| 2.99E-04    |
| JEGHLINGER_EMT_UP                             | 56   | 0.63| 2.12| 4.73E-04    |
| DAC_BLADDER_UP                                | 23   | 0.76| 2.12| 4.59E-04    |
| DAC_IFN_BLADDER_UP                            | 16   | 0.82| 2.12| 4.46E-04    |
| PEART_HISTONE_DN                              | 63   | 0.61| 2.11| 4.34E-04    |
| RADAEVA_IFNA_UP                               | 38   | 0.67| 2.10| 4.22E-04    |
| ZELLER_MYC_UP                                 | 23   | 0.73| 2.10| 4.11E-04    |
| CANCER_UNDIFFERENTIATED_META_UP               | 62   | 0.61| 2.10| 4.62E-04    |
| MARSHALL_SPLEEN_BAL                           | 25   | 0.74| 2.09| 4.50E-04    |
| PROTEOSOMEPATHWAY                             | 21   | 0.73| 2.09| 4.69E-04    |
| TNFALPHA_ALL_UP                               | 66   | 0.59| 2.08| 5.41E-04    |
| EMT_UP                                       | 55   | 0.60| 2.08| 5.29E-04    |
| SCHUMACHER_MYC_UP                              | 47   | 0.63| 2.07| 5.17E-04    |
| PASSERINI_INFLAMMATION                        | 23   | 0.73| 2.07| 5.05E-04    |
| APPEL_IMATINIB_UP                             | 29   | 0.70| 2.07| 4.94E-04    |
| MYC_TARGETS                                   | 39   | 0.65| 2.07| 4.84E-04    |
| ADIP_DIFF_CLUSTER4                            | 31   | 0.68| 2.07| 4.99E-04    |
| CMV_24HRS_UP                                  | 61   | 0.61| 2.06| 4.89E-04    |
| OXIDATIVE_PHOSPHORYLATION                     | 55   | 0.61| 2.06| 5.28E-04    |
| CMV_ALL_UP                                    | 81   | 0.57| 2.05| 6.36E-04    |

(Continued)
## Appendix 1. (Continued)

| Name                                          | Size | ES  | NES | FDR q-val |
|-----------------------------------------------|------|-----|-----|-----------|
| HOFMANN_MDS_CD34_LOW_AND_HIGH_RISK            | 31   | 0.68| 2.05| 7.14E-04 |
| FERRANDO_MLL_T_ALL_DN                        | 71   | 0.57| 2.03| 9.73E-04 |
| IFNALPHA_NL_UP                               | 19   | 0.74| 2.01| 1.16E-03 |
| ERM_KO_SERTOLI_DN                            | 17   | 0.77| 2.01| 1.18E-03 |
| AGED_MOUSE_NEOCORTEX_UP                      | 60   | 0.59| 2.01| 1.16E-03 |
| INOS_ALL_UP                                  | 47   | 0.61| 2.01| 1.16E-03 |
| CANTHARIDIN_DN                               | 45   | 0.61| 2.00| 1.22E-03 |
| COLLER_MYC_UP                                | 17   | 0.77| 2.00| 1.43E-03 |
| ROS_MOUSE_AORTA_DN                           | 68   | 0.57| 2.00| 1.40E-03 |
| PROTEASOME                                   | 17   | 0.75| 2.00| 1.38E-03 |
| NKTPATHWAY                                   | 28   | 0.67| 1.99| 1.53E-03 |
| HADDAD_CD45CD7_PLUS_VS_MINUS_UP               | 52   | 0.58| 1.99| 1.51E-03 |
| IL6_FIBRO_UP                                 | 35   | 0.63| 1.99| 1.48E-03 |
| HEARTFAILURE_VENTRICLE_DN                    | 56   | 0.59| 1.99| 1.55E-03 |
| APOPTOSIS                                    | 64   | 0.56| 1.98| 1.84E-03 |
| TAKEDA_NUP8_HOXA9_3D_DN                      | 20   | 0.71| 1.97| 1.85E-03 |
| LIAN_MYELOID_DIFF_GRANULE                    | 28   | 0.67| 1.96| 2.33E-03 |
| HADDAD_HSC_CD7_UP                            | 52   | 0.58| 1.96| 2.39E-03 |
| ST_TUMOR_NECROSIS_FACTOR_PATHWAY             | 28   | 0.67| 1.96| 2.56E-03 |
| MOOTHA_VOXPHOS                               | 73   | 0.55| 1.95| 2.71E-03 |
| CHAUHAN_2ME2                                 | 42   | 0.60| 1.95| 2.76E-03 |
| RIBOSOMAL_PROTEINS                           | 78   | 0.54| 1.95| 2.77E-03 |
| TNFALPHA_4HRS_UP                             | 34   | 0.63| 1.94| 2.91E-03 |
| LEE_MYC_TGFA_UP                              | 54   | 0.57| 1.94| 2.89E-03 |
| MOREAUX_TACI_HI_IN_PPC_UP                    | 43   | 0.59| 1.94| 2.97E-03 |
| BHATTACHARYA_ESC_UP                          | 57   | 0.57| 1.94| 3.07E-03 |
| BRCA_BRCA1_POS                               | 68   | 0.56| 1.94| 3.09E-03 |
| IFNALPHA_HCC_UP                              | 23   | 0.70| 1.94| 3.13E-03 |
| DSRNA_UP                                     | 32   | 0.64| 1.94| 3.09E-03 |
| PROTEASOME_DEGRADATION                       | 32   | 0.64| 1.93| 3.26E-03 |
| BENNETT_SLE_UP                               | 19   | 0.70| 1.93| 3.39E-03 |
| TARTE_PC                                     | 65   | 0.55| 1.93| 3.58E-03 |
| CMV_HCMV_TIMECOURSE_12HRS_UP                  | 21   | 0.69| 1.93| 3.58E-03 |
| TNFALPHA_30MIN_UP                            | 37   | 0.61| 1.92| 3.71E-03 |
| UVB_NHEK3_C0                                 | 73   | 0.54| 1.92| 3.89E-03 |
| GOLDRATH_CYTOLYTIC                           | 24   | 0.67| 1.92| 3.84E-03 |
| LIAN_MYELOID_DIFF_RECEPTORS                  | 33   | 0.61| 1.92| 3.84E-03 |
| PARK_RARALPHA_UP                             | 34   | 0.61| 1.91| 4.34E-03 |
| IL2PATHWAY                                   | 21   | 0.68| 1.90| 4.71E-03 |
| STEMCELL_COMMON_DN                           | 54   | 0.56| 1.90| 4.66E-03 |
| AGED_MOUSE_CEREBELLUM_UP                     | 58   | 0.55| 1.89| 5.69E-03 |
| DER_IFNG_UP                                  | 54   | 0.56| 1.88| 6.10E-03 |
| CCR5PATHWAY                                  | 18   | 0.70| 1.87| 7.21E-03 |
| HALMOS_OEBP_UP                               | 41   | 0.57| 1.86| 8.29E-03 |
| ZHAN_MM_CD138_MF_VS_REST                     | 30   | 0.62| 1.85| 8.56E-03 |
| HOHENKIRK_MONOCYTE_DEND_UP                   | 85   | 0.51| 1.85| 8.52E-03 |
| ST_GAQ_PATHWAY                               | 24   | 0.65| 1.85| 8.95E-03 |
| PHOTOSYNTHESIS                               | 21   | 0.67| 1.85| 9.02E-03 |
| IFN_GAMMA_UP                                 | 35   | 0.59| 1.84| 9.97E-03 |
| IFNA_UV-CMV_COMMON_HCMV_6HRS_UP              | 20   | 0.67| 1.84| 9.88E-03 |

**Abbreviations:** ES, enrichment score; NES, normalized enrichment score; FDR, false discovery rate.
Appendix 2. Significantly altered gene sets by A-DEP/saline compared to saline.

| Name                                      | Size | ES  | NES  | FDR q-val   |
|-------------------------------------------|------|-----|------|-------------|
| CARIES_PULP_HIGH_UP                       | 68   | 0.77| 2.89 | <1.00E-06   |
| LAL_KO_3MO_UP                             | 46   | 0.78| 2.73 | <1.00E-06   |
| LAL_KO_6MO_UP                             | 58   | 0.73| 2.65 | <1.00E-06   |
| DNA_REPLICATION_REACTOME                  | 41   | 0.75| 2.55 | <1.00E-06   |
| ELECTRON_TRANSPORT_CHAIN                  | 86   | 0.65| 2.55 | <1.00E-06   |
| CANCER_UNDIFFERENTIATED_META_UP          | 62   | 0.69| 2.51 | <1.00E-06   |
| MOOTHA_VOXPHOS                            | 73   | 0.67| 2.48 | <1.00E-06   |
| YANG_OSTECLASTS_SIG                       | 39   | 0.74| 2.45 | <1.00E-06   |
| MANALO_HYPOXIA_DN                         | 73   | 0.65| 2.44 | <1.00E-06   |
| CANCER_NEOPlastic_META_UP                 | 59   | 0.68| 2.44 | <1.00E-06   |
| YU_CMYC_UP                                | 37   | 0.72| 2.38 | <1.00E-06   |
| GALINDO_ACT_UP                            | 75   | 0.62| 2.37 | <1.00E-06   |
| OXIDATIVE_PHOSPHORYLATION                 | 55   | 0.66| 2.36 | <1.00E-06   |
| FLECHNER_KIDNEY_TRANSPLANT_REJECTION_UP   | 72   | 0.62| 2.34 | <1.00E-06   |
| LINDSTEDT_DEND_BH_VS_48H_UP               | 58   | 0.65| 2.32 | <1.00E-06   |
| CANTHARIDIN_DN                            | 45   | 0.66| 2.31 | <1.00E-06   |
| SERUM_FIBROBLAST_CELLCYCLE                | 88   | 0.58| 2.30 | <1.00E-06   |
| FERRANDO_MLL_T_ALL_DN                     | 71   | 0.61| 2.30 | <1.00E-06   |
| SCHUMACHER_MYC_UP                         | 47   | 0.66| 2.29 | <1.00E-06   |
| WIELAND_HEPATITIS_B_INDUCED               | 71   | 0.62| 2.29 | <1.00E-06   |
| ADIP_DIFF_CLUSTER4                        | 31   | 0.72| 2.28 | <1.00E-06   |
| P21_ANY_DN                                | 27   | 0.74| 2.28 | <1.00E-06   |
| HOUSTIS_ROS                               | 32   | 0.71| 2.27 | <1.00E-06   |
| PEART_HISTONE_DN                          | 63   | 0.62| 2.27 | <1.00E-06   |
| IDX_TSA_UP_CLUSTER3                       | 81   | 0.60| 2.26 | 4.59E-05    |
| NEMETH_TNF_UP                             | 82   | 0.59| 2.25 | 4.42E-05    |
| CMV_IE86_UP                               | 42   | 0.67| 2.24 | 4.25E-05    |
| BLEO_HUMAN_LYMPH_HIGH_24HRS_UP            | 86   | 0.57| 2.21 | 4.10E-05    |
| INOS_ALL_UP                               | 47   | 0.64| 2.21 | 3.96E-05    |
| MENSSEN_MYC_UP                            | 30   | 0.70| 2.21 | 3.83E-05    |
| UVB_NHEK2_UP                              | 55   | 0.61| 2.17 | 2.29E-04    |
| N12_MOUSE_UP                              | 40   | 0.64| 2.16 | 2.97E-04    |
| PROTEASOME_DEGRADATION                    | 32   | 0.67| 2.15 | 4.35E-04    |
| RIBOSOMAL_PROTEINS                        | 78   | 0.56| 2.15 | 4.57E-04    |
| BHATTACHARYA_ESC_UP                       | 57   | 0.59| 2.12 | 5.44E-04    |
| HG_PROGERIA_DN                            | 24   | 0.71| 2.12 | 5.29E-04    |
| NAKAJIMA_MCS_UP                           | 85   | 0.55| 2.12 | 5.47E-04    |
| COLLER_MYC_UP                             | 17   | 0.76| 2.10 | 6.62E-04    |
| HEARTFAILURE_VENTRICLE_DN                 | 56   | 0.58| 2.09 | 6.74E-04    |
| OLDAGE_DN                                 | 45   | 0.61| 2.09 | 6.58E-04    |
| BASSO_GERMINAL_CENTER_CD40_UP             | 82   | 0.54| 2.07 | 9.37E-04    |
| DOX_RESIST_GASTRIC_UP                     | 30   | 0.65| 2.06 | 1.26E-03    |
| ZELLER_MYC_UP                             | 23   | 0.69| 2.04 | 1.70E-03    |
| UVB_NHEK1_C1                              | 41   | 0.60| 2.03 | 1.69E-03    |
| MOREAUX_TACI_HI_IN_PPC_UP                 | 43   | 0.60| 2.03 | 1.70E-03    |
| RIBAVIRIN_RSV_UP                          | 18   | 0.72| 2.01 | 2.22E-03    |
| P21_P53_ANY_DN                            | 35   | 0.62| 2.01 | 2.17E-03    |
| BREAST_DUCTAL_CARCINOMA_GENES             | 19   | 0.71| 2.01 | 2.30E-03    |
| CMV_24HRS_UP                              | 61   | 0.55| 2.00 | 2.42E-03    |
| REN_E2F1_TARGETS                          | 37   | 0.60| 2.00 | 2.52E-03    |

(Continued)
### Appendix 2. (Continued)

| Name                                      | Size | ES  | NES  | FDR q-val |
|-------------------------------------------|------|-----|------|-----------|
| IDX_TSA_UP_CLUSTER5                       | 82   | 0.52| 2.00 | 2.49E-03  |
| CROONQUIST_IL6_STARVE_UP                  | 32   | 0.62| 1.99 | 2.51E-03  |
| TNFALPHA_ALL_UP                           | 66   | 0.53| 1.99 | 2.60E-03  |
| TSA_CD4_UP                                | 24   | 0.65| 1.99 | 2.55E-03  |
| PYRIMIDINE_METABOLISM                     | 55   | 0.55| 1.98 | 2.90E-03  |
| HINATA_NFKB_UP                            | 89   | 0.51| 1.97 | 3.15E-03  |
| MYC_TARGETS                               | 39   | 0.59| 1.96 | 3.66E-03  |
| ADIP_DIFF_CLUSTER5                        | 34   | 0.59| 1.95 | 4.09E-03  |
| SHIPP_FL_VS_DLBCL_DN                      | 30   | 0.61| 1.95 | 4.06E-03  |
| TNFA_NFKB_DEP_UP                          | 17   | 0.71| 1.95 | 4.23E-03  |
| ATP_SYNTHESIS                             | 20   | 0.67| 1.95 | 4.18E-03  |
| TNFALPHA_30MIN_UP                         | 37   | 0.58| 1.95 | 4.12E-03  |
| HPV31_DN                                  | 37   | 0.58| 1.94 | 4.28E-03  |
| P21_P53_MIDDLE_DN                         | 17   | 0.71| 1.94 | 4.23E-03  |
| TAVOR_CEBP_UP                             | 42   | 0.57| 1.93 | 4.64E-03  |
| PHOTOSYNTHESIS                            | 21   | 0.67| 1.93 | 4.74E-03  |
| PROTEASOMEPATHWAY                         | 21   | 0.65| 1.92 | 5.43E-03  |
| TARTE_PC                                  | 65   | 0.52| 1.91 | 5.70E-03  |
| TYPE_III_SECRETION_SYSTEM                 | 20   | 0.67| 1.91 | 5.66E-03  |
| CMV_ALL_UP                                | 81   | 0.50| 1.91 | 5.88E-03  |
| UEDA_MOUSE_SCN                            | 86   | 0.49| 1.91 | 5.82E-03  |
| G1_TO_S_CELL_CYCLE.REACTOME               | 65   | 0.51| 1.90 | 6.40E-03  |
| STRESS_TPA_SPECIFIC_UP                    | 34   | 0.59| 1.90 | 6.36E-03  |
| CELL_CYCLE                                | 71   | 0.50| 1.90 | 6.42E-03  |
| FLAGELLAR_ASSEMBLY                        | 20   | 0.67| 1.89 | 6.60E-03  |
| ABBUD_LIF_UP                              | 45   | 0.54| 1.89 | 7.01E-03  |
| KNUDSEN_PMNS_UP                           | 65   | 0.51| 1.88 | 7.46E-03  |
| TPA_SENS_MIDDLE_UP                        | 55   | 0.53| 1.88 | 7.62E-03  |
| CARBON_FIXATION                          | 18   | 0.66| 1.88 | 7.67E-03  |
| MMS_HUMAN_LYMPH_HIGH_24HRS_UP             | 18   | 0.69| 1.88 | 7.58E-03  |
| ZHAN_MM_CD138_PR_VS_REST                  | 28   | 0.62| 1.87 | 7.97E-03  |
| ZUCCHI_EPITHELIAL_DN                      | 36   | 0.58| 1.87 | 8.21E-03  |
| TIS7_OVEREXP_DN                           | 17   | 0.67| 1.87 | 8.23E-03  |
| ZHAN_MULTIPLE_MYELOMA_VS_NORMAL_DN        | 33   | 0.58| 1.87 | 8.23E-03  |
| ROS_MOUSE_AORTA_DN                        | 68   | 0.50| 1.87 | 8.17E-03  |
| BRENTANI_DNA_METHYLATION_AND_MODIFICATION | 23   | 0.63| 1.86 | 8.83E-03  |
| NADLER_OBESITY_UP                         | 57   | 0.52| 1.86 | 8.73E-03  |
| BLEO_MOUSE_LYMPH_LOW_24HRS_DN             | 24   | 0.63| 1.86 | 8.70E-03  |
| ET743_SARCOMA_UP                          | 56   | 0.51| 1.86 | 8.63E-03  |
| UVB_NHEK3_C6                              | 25   | 0.61| 1.85 | 9.32E-03  |

**Abbreviations:** ES, enrichment score; NES, normalized enrichment score; FDR, false discovery rate.
### Appendix 3. Significantly altered gene sets by C-DEP/saline compared to saline.

| Name                                                  | Size | ES   | NES  | FDR q-val   |
|-------------------------------------------------------|------|------|------|-------------|
| CARIES_PULP_HIGH_UP                                   | 68   | 0.77 | 2.90 | <1.00E-06  |
| GALINDO_ACT_UP                                        | 75   | 0.72 | 2.79 | <1.00E-06  |
| YANG_OSTECLASTS_SIG                                   | 39   | 0.79 | 2.74 | <1.00E-06  |
| LINDSTEDT_DEND_8H_VS_48H_UP                           | 58   | 0.70 | 2.69 | <1.00E-06  |
| HINATA_NFKB_UP                                        | 89   | 0.65 | 2.64 | <1.00E-06  |
| NAKAJIMA_MCS_UP                                       | 85   | 0.65 | 2.58 | <1.00E-06  |
| NEMETH_TNF_UP                                         | 82   | 0.64 | 2.57 | <1.00E-06  |
| LAL_KO_06MO_UP                                        | 58   | 0.68 | 2.57 | <1.00E-06  |
| WIELAND_HEPATITIS_B_INDUCED                           | 71   | 0.66 | 2.53 | <1.00E-06  |
| LAL_KO_3MO_UP                                         | 46   | 0.73 | 2.52 | <1.00E-06  |
| HOUSTIS_ros                                          | 32   | 0.75 | 2.49 | <1.00E-06  |
| SANA_TNFA_ENDOTHELIAL_UP                              | 61   | 0.66 | 2.48 | <1.00E-06  |
| FLECHNER_KIDNEY_TRANSPRENT_REJECTION_UP               | 72   | 0.64 | 2.47 | <1.00E-06  |
| BLEO_HUMAN_LYMHP_HIG24HRS_UP                           | 86   | 0.61 | 2.45 | <1.00E-06  |
| NADLER_OBESITY_UP                                     | 57   | 0.66 | 2.45 | <1.00E-06  |
| ADIP_DIFF_CLUSTER4                                    | 31   | 0.74 | 2.44 | <1.00E-06  |
| MYC_TARGETS                                           | 39   | 0.71 | 2.44 | <1.00E-06  |
| TNFA_NFKB_DEP_UP                                      | 17   | 0.86 | 2.42 | <1.00E-06  |
| NI2_MOUSE_UP                                          | 40   | 0.71 | 2.41 | <1.00E-06  |
| INOS_ALL_UP                                           | 47   | 0.68 | 2.39 | <1.00E-06  |
| TPA_SENS_MIDDLE_UP                                    | 55   | 0.66 | 2.38 | <1.00E-06  |
| TSA_CD4_UP                                            | 24   | 0.76 | 2.35 | <1.00E-06  |
| CANCER_UNDIFFERENTIATED_META_UP                       | 62   | 0.62 | 2.35 | <1.00E-06  |
| ZELLER_MYC_UP                                         | 23   | 0.76 | 2.33 | <1.00E-06  |
| ZUCCHI_EPITHELIAL_UP                                  | 36   | 0.70 | 2.33 | <1.00E-06  |
| CMV_IE86_UP                                           | 42   | 0.66 | 2.26 | 5.58E-05   |
| CMV_24HRS_UP                                          | 61   | 0.59 | 2.26 | 5.37E-05   |
| MUNSHI_MM_UP                                          | 57   | 0.59 | 2.24 | 1.03E-04   |
| DAC_BLADDER_UP                                        | 23   | 0.74 | 2.23 | 1.47E-04   |
| PASSERINI_INFLAMMATION                                | 23   | 0.71 | 2.19 | 3.21E-04   |
| BASSO GERMINAL_CENTER_CD40_UP                         | 82   | 0.56 | 2.19 | 3.11E-04   |
| IDX_TSA_UP_CLUSTER3                                   | 81   | 0.56 | 2.18 | 4.32E-04   |
| MARTINELLI_IFNS_DIFF                                  | 16   | 0.79 | 2.18 | 4.19E-04   |
| CMV_ALL_UP                                            | 81   | 0.54 | 2.17 | 4.47E-04   |
| P21_ANY_DN                                            | 27   | 0.69 | 2.16 | 4.73E-04   |
| MANALO_HYPOXIA_DN                                     | 73   | 0.55 | 2.16 | 4.97E-04   |
| MUNSHI_MM_vs_PCS_UP                                   | 64   | 0.58 | 2.15 | 5.20E-04   |
| FERRANDO_MLL_T_ALL_DN                                 | 71   | 0.56 | 2.15 | 5.06E-04   |
| SHIPP_FL_VS_DLBCN_DN                                   | 30   | 0.66 | 2.14 | 5.30E-04   |
| MMS_HUMAN_LYMHP_HIG24HRS_UP                            | 18   | 0.76 | 2.14 | 5.16E-04   |
| DAC_IFN_BLADDER_UP                                    | 16   | 0.78 | 2.14 | 5.37E-04   |
| DNA_REPLICATION_REACTOME                              | 41   | 0.62 | 2.13 | 5.24E-04   |
| PROTEASOME_DEGRADATION                                | 32   | 0.66 | 2.13 | 5.43E-04   |
| ROSS_CBFX_MHY                                         | 38   | 0.61 | 2.12 | 7.18E-04   |
| HG_PROGERIA_DN                                        | 24   | 0.70 | 2.11 | 7.33E-04   |
| ROS_MOUSE_AORTA_DN                                    | 68   | 0.55 | 2.10 | 8.96E-04   |
| OLDAGE_DN                                             | 45   | 0.60 | 2.09 | 1.05E-03   |
| COLLER_MYC_UP                                         | 17   | 0.76 | 2.09 | 1.14E-03   |
| KNUDSEN_PMNS_UP                                       | 65   | 0.55 | 2.08 | 1.29E-03   |
| TARTIE_PC                                             | 65   | 0.54 | 2.07 | 1.37E-03   |

(Continued)
### Appendix 3. (Continued)

| Name                              | Size | ES  | NES  | FDR q-val |
|-----------------------------------|------|-----|------|-----------|
| PROTEASOME                        | 17   | 0.75| 2.07 | 1.37E-03  |
| DER_IFNG_UP                       | 54   | 0.56| 2.06 | 1.37E-03  |
| CANCER NEOPLASTIC META_UP         | 59   | 0.55| 2.06 | 1.40E-03  |
| CHAUHAN_2ME2                      | 42   | 0.60| 2.06 | 1.48E-03  |
| SCHUMACHER MYC_UP                 | 47   | 0.60| 2.06 | 1.45E-03  |
| UVB_NHEK2_UP                      | 55   | 0.57| 2.06 | 1.50E-03  |
| NKTPATHWAY                        | 28   | 0.65| 2.06 | 1.50E-03  |
| MENSSEN MYC_UP                    | 30   | 0.64| 2.06 | 1.52E-03  |
| SERUM_FIBROBLAST_CELLCYCLE        | 88   | 0.52| 2.05 | 1.54E-03  |
| TAVOR_CEBP_UP                     | 42   | 0.59| 2.05 | 1.58E-03  |
| PROTEASOMEPATHWAY                 | 21   | 0.69| 2.04 | 1.71E-03  |
| IFNALPHA_NL_UP                    | 19   | 0.71| 2.04 | 1.68E-03  |
| JECHLINGER EMT_UP                 | 56   | 0.55| 2.04 | 1.70E-03  |
| ABBUD_LIF_UP                      | 45   | 0.59| 2.04 | 1.76E-03  |
| IL6_FIBRO_UP                      | 35   | 0.60| 2.03 | 1.79E-03  |
| PEART_HISTONE_DN                  | 63   | 0.53| 2.02 | 2.33E-03  |
| AS3_FIBRO_DN                      | 26   | 0.65| 2.03 | 2.34E-03  |
| YU_CMyc_UP                        | 37   | 0.59| 2.01 | 2.34E-03  |
| P21_P53 MIDDLE_DN                 | 17   | 0.71| 2.01 | 2.31E-03  |
| STRESS_TPA_SPECIFIC_UP            | 34   | 0.61| 2.01 | 2.40E-03  |
| DSRNA_UP                          | 32   | 0.60| 2.00 | 2.60E-03  |
| ERM_KO_SERTOLI_DN                 | 17   | 0.71| 1.99 | 2.64E-03  |
| RADAEA_IFNA_UP                    | 38   | 0.59| 1.99 | 2.73E-03  |
| TNFALPHA_4HRS_UP                  | 34   | 0.60| 1.99 | 2.90E-03  |
| CANTHARIDIN_DN                    | 45   | 0.55| 1.98 | 2.97E-03  |
| IFN_GAMMA_UP                      | 35   | 0.59| 1.98 | 3.04E-03  |
| HOFMANN_MDS_CD34_LOW_AND_HIGH_RISK| 31   | 0.61| 1.97 | 3.31E-03  |
| ADIP_DIFF_CLUSTERS5               | 34   | 0.59| 1.97 | 3.27E-03  |
| TIS7_OVEREXP_DN                   | 17   | 0.69| 1.97 | 3.22E-03  |
| LIAN_MYELOID_DIFF_RECEPTORS       | 33   | 0.59| 1.97 | 3.36E-03  |
| MARSHALL_SPLEEN_BAL               | 25   | 0.63| 1.95 | 4.34E-03  |
| NAKAJIMA_MCSMBP_MAST              | 37   | 0.58| 1.94 | 4.80E-03  |
| TAKEDA_NUP8 HOX A9 3D_DN           | 20   | 0.67| 1.93 | 5.19E-03  |
| PYRIMIDINE METABOLISM             | 55   | 0.52| 1.93 | 5.34E-03  |
| TNFALPHA_ALL_UP                   | 66   | 0.51| 1.92 | 6.17E-03  |
| CMV_HCMV TIMECOURSE 12HRS_UP      | 21   | 0.65| 1.92 | 6.12E-03  |
| HEARTFAILURE VENTRICLE_DN         | 56   | 0.52| 1.92 | 6.14E-03  |
| IFNALPHA_HCC_UP                   | 23   | 0.64| 1.91 | 6.44E-03  |
| HYPOXIA REVIEW                    | 68   | 0.50| 1.91 | 6.64E-03  |
| LINDSTEDT DEND UP                 | 44   | 0.54| 1.91 | 6.87E-03  |
| CROONQUIST IL6_RAS_UP             | 18   | 0.67| 1.90 | 7.79E-03  |
| LEE MYC TGFA_UP                   | 54   | 0.51| 1.89 | 7.81E-03  |
| EMT_UP                            | 55   | 0.51| 1.89 | 8.20E-03  |
| LEE ACOX1_UP                      | 58   | 0.51| 1.89 | 8.21E-03  |
| BENNETT_SLE_UP                    | 19   | 0.65| 1.88 | 8.45E-03  |
| ZHAN MMPC SIMAL                   | 41   | 0.55| 1.88 | 8.40E-03  |
| P21 P53 ANY_DN                    | 35   | 0.57| 1.88 | 8.63E-03  |
| BREAST DUCTAL CARCINOMA GENES     | 19   | 0.65| 1.88 | 8.74E-03  |

**Abbreviations:** ES, enrichment score; NES, normalized enrichment score; FDR, false discovery rate.
Appendix 4. Significantly altered gene sets by N-DEP/OVA compared to OVA.

| Name                                           | Size | ES | NES | FDR q-val          |
|------------------------------------------------|------|----|-----|--------------------|
| CARIES_PULP_HIGH_UP                            | 68   | 0.81 | 2.76 | <1.00E-06          |
| LAL_KO_3MO_UP                                  | 46   | 0.83 | 2.61 | <1.00E-06          |
| LAL_KO_6MO_UP                                  | 58   | 0.79 | 2.60 | <1.00E-06          |
| NEMETH_TNF_UP                                  | 82   | 0.74 | 2.59 | <1.00E-06          |
| WIELAND_HEPATITIS_B_INDUCED                    | 71   | 0.74 | 2.54 | <1.00E-06          |
| NADLER_OBESITY_UP                              | 57   | 0.76 | 2.51 | <1.00E-06          |
| YANG_OSTEOLASTS_SIG                            | 39   | 0.82 | 2.50 | <1.00E-06          |
| LINDSTEDT_DEND_6H_VS_48H_UP                    | 58   | 0.73 | 2.45 | <1.00E-06          |
| NAKAJIMA_MCS_UP                                | 85   | 0.69 | 2.44 | <1.00E-06          |
| SANA_TNF_ENDOTHELIAL_UP                        | 61   | 0.71 | 2.41 | <1.00E-06          |
| BASSO_GERMINAL_CENTER_CD40_UP                  | 82   | 0.69 | 2.40 | <1.00E-06          |
| GALINDO_ACT_UP                                 | 75   | 0.69 | 2.40 | <1.00E-06          |
| FLECHNER_KIDNEY_TRANPLANT_REJECTION_UP         | 72   | 0.69 | 2.39 | <1.00E-06          |
| HINATA_NFKB_UP                                 | 89   | 0.64 | 2.26 | <1.00E-06          |
| NI2_MOUSE_UP                                   | 40   | 0.70 | 2.16 | 3.02E-04           |
| BLEO_HUMAN_LYMPH_HIGH_24HRS_UP                 | 86   | 0.60 | 2.14 | 2.83E-04           |
| GOLDRATH_CYTOLYTIC                            | 24   | 0.77 | 2.14 | 3.32E-04           |
| BRENTANI_IMMUNE_FUNCTION                      | 42   | 0.68 | 2.13 | 3.75E-04           |
| JECHELINGER_EMT_UP                             | 56   | 0.64 | 2.11 | 5.30E-04           |
| ADIP_DIFF_CLUSTER3                             | 28   | 0.73 | 2.10 | 5.03E-04           |
| TNFA_NFKB_DEP_UP                               | 17   | 0.80 | 2.09 | 5.83E-04           |
| YU_CMYC_DN                                     | 53   | 0.65 | 2.09 | 5.57E-04           |
| YAGI_AML_PROGNOSIS                            | 31   | 0.70 | 2.07 | 7.26E-04           |
| ROSS_MLL_FUSION                                | 60   | 0.62 | 2.05 | 1.12E-03           |
| LIAN_MYELOID_DIFF_RECEPTORS                   | 33   | 0.69 | 2.03 | 1.47E-03           |
| EMT_UP                                        | 55   | 0.61 | 2.02 | 1.50E-03           |
| PASSERINI_INFLAMMATION                         | 23   | 0.74 | 2.01 | 1.69E-03           |
| APPEL_IMATINIB_UP                              | 29   | 0.70 | 2.01 | 1.96E-03           |
| ABBUD_LIF_UP                                   | 45   | 0.64 | 2.01 | 1.89E-03           |
| TAKEDA_NUP8_HOXA9_3D_DN                        | 20   | 0.74 | 2.01 | 1.86E-03           |
| LINDSTEDT_DEND_DN                              | 53   | 0.61 | 2.01 | 1.87E-03           |
| CMV_ALL_UP                                     | 81   | 0.56 | 2.01 | 1.81E-03           |
| TAVOR_CEBP_UP                                  | 42   | 0.63 | 1.99 | 2.34E-03           |
| STEMCELL_COMMON_DN                             | 54   | 0.60 | 1.98 | 2.89E-03           |
| SCHUMACHER_MYC_UP                              | 47   | 0.61 | 1.97 | 3.54E-03           |
| ERM_KO_SERTOLI_DN                              | 17   | 0.75 | 1.96 | 3.66E-03           |
| DAC_IFN_BLADDER_UP                             | 16   | 0.77 | 1.95 | 4.26E-03           |
| SANA_IFNG_ENDOTHELIAL_UP                       | 45   | 0.62 | 1.95 | 4.26E-03           |
| WANG_HOXA9_VS_MEIS1_UP                         | 25   | 0.68 | 1.94 | 4.75E-03           |
| LIAN_MYELOID_DIFF_GRANULE                      | 28   | 0.68 | 1.93 | 5.19E-03           |
| CMV_24HRS_UP                                   | 61   | 0.58 | 1.93 | 5.58E-03           |
| MARTINELLI_IFNS_DIFF                           | 16   | 0.75 | 1.93 | 5.45E-03           |
| TARTE_PC                                       | 65   | 0.56 | 1.92 | 5.48E-03           |
| MYC_TARGETS                                    | 39   | 0.63 | 1.92 | 5.46E-03           |
| WANG_MLL_CBP_VS_GMP_UP                         | 42   | 0.61 | 1.91 | 6.10E-03           |
| XU_CBP_UP                                      | 25   | 0.64 | 1.91 | 6.53E-03           |
| LOTEM_LEUKEMIA_UP                              | 22   | 0.69 | 1.91 | 6.39E-03           |
| LINDSTEDT_DEND_DN                              | 44   | 0.61 | 1.90 | 6.60E-03           |
| LU_IL4BCELL                                    | 62   | 0.56 | 1.90 | 6.79E-03           |
| ZELLER_MYC_UP                                  | 23   | 0.69 | 1.90 | 6.99E-03           |
| CASPASEPATHWAY                                 | 20   | 0.69 | 1.90 | 6.98E-03           |
| ROSS_CBF_MYH                                   | 38   | 0.62 | 1.90 | 6.76E-03           |

(Continued)
### Appendix 4. (Continued)

| Name                                  | Size | ES  | NES  | FDR q-val     |
|----------------------------------------|------|-----|------|---------------|
| CMV_8HRS_UP                            | 27   | 0.66| 1.88 | 8.38E-03      |
| TPA_SENS_MIDDLE_UP                     | 55   | 0.57| 1.88 | 8.27E-03      |
| DSRNA_UP                              | 32   | 0.63| 1.87 | 8.52E-03      |
| NAKAJIMA_MCSMBP_MAST                  | 37   | 0.61| 1.87 | 8.37E-03      |
| CROONQUIST_IL6_RAS_UP                 | 18   | 0.72| 1.87 | 8.38E-03      |
| GENOTOXINS_ALL_24HRS_REG              | 22   | 0.68| 1.87 | 8.26E-03      |
| HOHENKIRK_MONOCYTE_DEND_UP            | 85   | 0.53| 1.87 | 8.40E-03      |
| SHIPP_FL_VS_DLBCCL_DN                 | 30   | 0.63| 1.87 | 8.34E-03      |

**Abbreviations:** ES, enrichment score; NES, normalized enrichment score; FDR, false discovery rate.

### Appendix 5. Significantly altered gene sets by A-DEP/OVA compared to OVA.

| Name                                      | Size | ES  | NES  | FDR q-val     |
|-------------------------------------------|------|-----|------|---------------|
| YANG_OSTECLASTS_SIG                       | 39   | 0.86| 2.84 | <1.00E-06     |
| LAL KO_6MO_UP                            | 58   | 0.74| 2.59 | <1.00E-06     |
| NAKAJIMA_MCS_UP                           | 85   | 0.68| 2.58 | <1.00E-06     |
| LINDSTEDT_DEND_8H_VS_48H_UP               | 58   | 0.73| 2.58 | <1.00E-06     |
| LAL KO_3MO_UP                            | 46   | 0.76| 2.56 | <1.00E-06     |
| NADLER_OBESITY_UP                        | 57   | 0.72| 2.56 | <1.00E-06     |
| GALINDO_ACT_UP                           | 75   | 0.70| 2.54 | <1.00E-06     |
| SANA_TNF_ENDOTHELIAL_UP                   | 61   | 0.71| 2.52 | <1.00E-06     |
| CARIES_PULP_HIGH_UP                      | 68   | 0.70| 2.50 | <1.00E-06     |
| NEMETH_TNF_UP                            | 82   | 0.66| 2.43 | <1.00E-06     |
| JECHLINGER_EM_T_UP                      | 56   | 0.67 | 2.32 | <1.00E-06    |
| HINATA_NFKB_UP                           | 89   | 0.60| 2.29 | <1.00E-06     |
| TNFA_NFKB_DEP_UP                        | 17   | 0.85| 2.29 | <1.00E-06     |
| NI2_MOUSE_UP                             | 40   | 0.70| 2.26 | 7.14E-05      |
| ERM KO_SERTOLI_DN                       | 17   | 0.83| 2.25 | 6.67E-05      |
| BASSO_GERMINAL_CENTER_CD40_UP            | 82   | 0.61| 2.24 | 6.25E-05      |
| WIELAND_HEPATITIS_B_INDUCED             | 71   | 0.62| 2.22 | 5.88E-05      |
| EMT_UP                                  | 55   | 0.63| 2.21 | 5.56E-05      |
| PASSERINI_INFLAMMATION                   | 23   | 0.75| 2.19 | 5.26E-05      |
| NAKAJIMA_MCSMBP_MAST                    | 37   | 0.68| 2.17 | 5.00E-05      |
| TPA_SENS_MIDDLE_UP                      | 55   | 0.62| 2.16 | 4.76E-05      |
| LIAN_MYELOID_DIFF RECEPTORS             | 33   | 0.69| 2.14 | 1.40E-04      |
| BENNETT_SLE_UP                          | 19   | 0.77| 2.13 | 2.24E-04      |
| TGFBETA_C2_UP                           | 17   | 0.79| 2.12 | 2.14E-04      |
| TAKEDA_NUP8_HOXA9_3D_DN                 | 20   | 0.76| 2.12 | 2.48E-04      |
| RADAeva_IFNA_UP                         | 38   | 0.65| 2.10 | 3.19E-04      |
| IL1_CORNEA_UP                           | 53   | 0.60| 2.08 | 5.72E-04      |
| DAC_BLADDER_UP                          | 23   | 0.72| 2.08 | 5.89E-04      |
| DAC_IFN_BLADDER_UP                      | 16   | 0.79| 2.08 | 6.03E-04      |
| MARTINELLI_IFNS_DIFF                    | 16   | 0.77| 2.07 | 6.89E-04      |
| TAVOR_CEBP_UP                           | 42   | 0.62| 2.06 | 7.32E-04      |
| AS3_FIBRO_DN                            | 26   | 0.70| 2.05 | 7.74E-04      |
| HEARTFAILURE_VENTRICLE_DN               | 56   | 0.59| 2.03 | 1.03E-03      |
| CANCER_UNDIFFERENTIATED_META_UP         | 62   | 0.56| 2.01 | 1.43E-03      |
| FLECHNER_KIDNEY_TRANSPLANT_REJECTION_UP | 72   | 0.55| 2.01 | 1.39E-03      |
| ADIP_DIFF_CLUSTER4                      | 31   | 0.64| 2.01 | 1.38E-03      |
| KNUDSEN_PMNS_UP                         | 65   | 0.56| 2.00 | 1.39E-03      |

(Continued)
### Appendix 5. (Continued)

| Name                                      | Size | ES   | NES  | FDR    |
|--------------------------------------------|------|------|------|--------|
| AGED_MOUSE_CEREBELLUM_UP                   | 58   | 0.58 | 1.99 | 1.66E-03 |
| CMV_ALL_UP                                 | 81   | 0.54 | 1.98 | 1.90E-03 |
| BLEO_HUMAN_LYMPH_HIGH_24HRS_UP             | 86   | 0.52 | 1.98 | 2.06E-03 |
| MATRIX_METALLOPROTEINASES                  | 24   | 0.69 | 1.98 | 2.04E-03 |
| DORSEY_DOXYCYCLINE_UP                      | 23   | 0.68 | 1.96 | 2.53E-03 |
| PASSERINI_EM                               | 34   | 0.62 | 1.96 | 2.56E-03 |
| TSA_CD4_UP                                 | 24   | 0.66 | 1.95 | 2.62E-03 |
| CROONQUIST_IL6_RAS_UP                      | 18   | 0.70 | 1.95 | 2.70E-03 |
| ZUCCHI_EPITHELIAL_DN                       | 36   | 0.60 | 1.94 | 3.52E-03 |
| CMV_8HRS_UP                                | 27   | 0.64 | 1.93 | 4.18E-03 |
| IFNALPHA_HCC_UP                            | 23   | 0.66 | 1.92 | 4.21E-03 |
| SANA_IFNG_ENDOTHELIAL_UP                   | 45   | 0.58 | 1.92 | 4.35E-03 |
| APPEL_IMATINIB_UP                          | 29   | 0.63 | 1.92 | 4.30E-03 |
| SHEPARD_POS_REG_OF_CELL_PROLIFERATION      | 85   | 0.52 | 1.92 | 4.22E-03 |
| CROONQUIST_IL6_STROMA_UP                   | 34   | 0.60 | 1.92 | 4.34E-03 |
| SERUM_FIBROBLAST_CELLCYCLE                 | 88   | 0.50 | 1.91 | 4.61E-03 |
| DSRNA_UP                                   | 32   | 0.61 | 1.91 | 4.91E-03 |
| MYC_TARGETS                                | 39   | 0.58 | 1.90 | 5.89E-03 |
| INSULIN_NIH3T3_UP                          | 15   | 0.72 | 1.89 | 6.12E-03 |
| CHAUHAN_2ME2                                | 42   | 0.56 | 1.89 | 6.17E-03 |
| CAMPTOTECIN_PROBCELL_DN                    | 21   | 0.66 | 1.89 | 6.08E-03 |
| LINDESTEDT_DEND_UP                         | 53   | 0.55 | 1.89 | 6.21E-03 |
| LEE_DEN_A_UP                               | 55   | 0.54 | 1.88 | 6.31E-03 |
| ROSS_MLL_FUSION                            | 60   | 0.53 | 1.88 | 6.51E-03 |
| CCR5PATHWAY                                | 18   | 0.68 | 1.88 | 6.64E-03 |
| PEART_HISTONE_DN                           | 63   | 0.52 | 1.88 | 6.61E-03 |
| IRITANI_ADPROX_DN                          | 45   | 0.55 | 1.87 | 7.03E-03 |
| ABBUD_LIF_UP                               | 45   | 0.56 | 1.87 | 7.16E-03 |
| MUNSHI/MM_UP                               | 57   | 0.53 | 1.87 | 7.10E-03 |
| ZHAN_MULTIPLE_MYELOMA_VS_NORMAL_DN         | 33   | 0.61 | 1.87 | 7.10E-03 |
| NKPATHWAY                                  | 28   | 0.62 | 1.85 | 9.28E-03 |

**Abbreviations:** ES, enrichment score; NES, normalized enrichment score; FDR, false discovery rate.

### Appendix 6. Significantly altered gene sets by C-DEP/OVA compared to OVA.

| Name                                      | Size | ES   | NES  | FDR    |
|--------------------------------------------|------|------|------|--------|
| CARIES_PULP_HIGH_UP                        | 68   | 0.80 | 2.94 | <1.00E-06 |
| WIELAND_HEPATITIS_B_INDUCED                | 71   | 0.76 | 2.83 | <1.00E-06 |
| NEMETH_TNF_UP                              | 82   | 0.73 | 2.77 | <1.00E-06 |
| YANG_OSTEOLASTS_SIG                        | 39   | 0.82 | 2.73 | <1.00E-06 |
| LINDESTEDT_DEND_6H_VS_48H_UP               | 58   | 0.76 | 2.73 | <1.00E-06 |
| FLECHNER_KIDNEY_TRANSPLANT_REJECTION_UP    | 72   | 0.73 | 2.71 | <1.00E-06 |
| IFNA_HCMV_6HRS_UP                          | 38   | 0.80 | 2.64 | <1.00E-06 |
| LAL_KO_6MO_UP                              | 58   | 0.76 | 2.64 | <1.00E-06 |
| GALINDO_ACT_UP                             | 75   | 0.71 | 2.62 | <1.00E-06 |
| LAL_KO_3MO_UP                              | 46   | 0.77 | 2.60 | <1.00E-06 |
| SANA_TNF_EODOTHELIAL_UP                    | 61   | 0.73 | 2.59 | <1.00E-06 |
| CMV_ALL_UP                                 | 81   | 0.67 | 2.50 | <1.00E-06 |
| SANA_IFNG_ENDOTHELIAL_UP                   | 45   | 0.74 | 2.50 | <1.00E-06 |
| RADAeva_IFNA_UP                            | 38   | 0.76 | 2.49 | <1.00E-06 |
| CMV_8HRS_UP                                | 27   | 0.81 | 2.48 | <1.00E-06 |

(Continued)
| Name                                                            | Size | ES | NES | FDR q-val |
|-----------------------------------------------------------------|------|----|-----|-----------|
| BASSO_GERMINAL_CENTER_CD40_UP                                    | 82   | 0.66 | 2.48 | <1.00E-06 |
| NAKAIMA_MCS_UP                                                   | 85   | 0.65 | 2.46 | <1.00E-06 |
| DER_IFNA_UP                                                     | 53   | 0.69 | 2.45 | <1.00E-06 |
| CANCER_NEoplastIC_META_UP                                       | 59   | 0.69 | 2.45 | <1.00E-06 |
| DER_IFNB_UP                                                     | 76   | 0.65 | 2.41 | <1.00E-06 |
| HINATA_NFKB_UP                                                  | 89   | 0.63 | 2.41 | <1.00E-06 |
| CMV_HCMV_TIMECOURSe_12HRS_UP                                    | 21   | 0.82 | 2.39 | <1.00E-06 |
| CANCER_UNDIFFERENTIATED_META_UP                                 | 62   | 0.66 | 2.34 | <1.00E-06 |
| CMV_24HRS_UP                                                    | 61   | 0.66 | 2.34 | <1.00E-06 |
| MANALO_HYPOXIA_DN                                               | 73   | 0.62 | 2.33 | <1.00E-06 |
| NADLER_OBESITY_UP                                               | 57   | 0.66 | 2.32 | <1.00E-06 |
| IFNA_UV-CMV_COMMON_HCMV_6HRS_UP                                 | 20   | 0.81 | 2.32 | <1.00E-06 |
| SCHUMACHER_MYC_UP                                               | 47   | 0.68 | 2.32 | <1.00E-06 |
| DER_IFNG_UP                                                     | 54   | 0.66 | 2.28 | <1.00E-06 |
| IFN_ANY_UP                                                      | 71   | 0.61 | 2.27 | <1.00E-06 |
| PEART_HISTONE_DN                                                | 63   | 0.63 | 2.26 | <1.00E-06 |
| IFN_BETA_UP                                                     | 55   | 0.64 | 2.26 | <1.00E-06 |
| ADIP_DIFF_CLUSTER4                                               | 31   | 0.72 | 2.25 | <1.00E-06 |
| IFNALPHA_NL_UP                                                  | 19   | 0.80 | 2.25 | <1.00E-06 |
| IFN_GAMMA_UP                                                    | 35   | 0.69 | 2.23 | <1.00E-06 |
| GOLDRATH_CYTOLYTIC                                             | 24   | 0.75 | 2.21 | <1.00E-06 |
| IFNALPHA_HCC_UP                                                 | 23   | 0.74 | 2.20 | <1.00E-06 |
| CANTHARIDIN_DN                                                  | 45   | 0.65 | 2.20 | <1.00E-06 |
| MOREAUX_TACI_HI_IN_PPC_UP                                       | 43   | 0.66 | 2.19 | <1.00E-06 |
| DAC_IFN_BLADErr_UP                                              | 16   | 0.82 | 2.19 | <1.00E-06 |
| BENNETT_SLE_UP                                                  | 19   | 0.79 | 2.18 | <1.00E-06 |
| STRESS_TPA_SPECIFIC_UP                                          | 34   | 0.69 | 2.17 | 2.91E-05  |
| TNFA_NFKB_DEP_UP                                                | 17   | 0.81 | 2.17 | 2.84E-05  |
| TARTE_PC                                                        | 65   | 0.60 | 2.17 | 2.77E-05  |
| BLEO_HUMAN_LYMPh_HIGH_24HRS_UP                                   | 86   | 0.57 | 2.17 | 2.71E-05  |
| DSRNA_UP                                                        | 32   | 0.69 | 2.16 | 2.65E-05  |
| TSA_CD4_UP                                                      | 24   | 0.73 | 2.15 | 5.32E-05  |
| JECBLINGER_EMT_UP                                               | 56   | 0.61 | 2.14 | 1.30E-04  |
| INOS_ALL_UP                                                     | 47   | 0.62 | 2.14 | 1.27E-04  |
| IFN_ALL_UP                                                      | 16   | 0.79 | 2.14 | 1.25E-04  |
| IFN_ALPHA_UP                                                    | 34   | 0.67 | 2.13 | 1.95E-04  |
| LIAN_MYeLOID_DIFF_RECEPTORS                                     | 33   | 0.67 | 2.13 | 1.91E-04  |
| BRCA_BRCA1_POS                                                  | 68   | 0.58 | 2.13 | 2.11E-04  |
| ERM_KO_SERTOLI_DN                                               | 17   | 0.78 | 2.12 | 2.07E-04  |
| COLLER_MYC_UP                                                   | 17   | 0.78 | 2.12 | 2.03E-04  |
| AMINOACYL_TRNA_BIOSYNTHESIS                                     | 18   | 0.75 | 2.10 | 4.86E-04  |
| PROTEASOMEPATHWAY                                               | 21   | 0.73 | 2.09 | 5.20E-04  |
| HSC_INTERMEDIATEPROGENITORS_ADULT                               | 88   | 0.54 | 2.09 | 5.11E-04  |
| PASSERINI_INFLAMMATION                                          | 23   | 0.70 | 2.08 | 6.52E-04  |
| LINDESTEDT_DEND_DN                                              | 53   | 0.59 | 2.07 | 6.83E-04  |
| ROSS_CBF_MYH                                                    | 38   | 0.63 | 2.07 | 7.52E-04  |
| LU_IL4BCELL                                                     | 62   | 0.57 | 2.06 | 7.81E-04  |
| DNA_REPLICATION_REACTOME                                         | 41   | 0.64 | 2.06 | 7.68E-04  |
| STRESS_GENOTOXIC_SPECIFIC_DN                                    | 36   | 0.63 | 2.06 | 9.11E-04  |
| SERUM_FIBROBLAST_CELLCYCLE                                     | 88   | 0.54 | 2.05 | 9.35E-04  |
| YAGI_AML_PROGNOSIS                                             | 31   | 0.65 | 2.05 | 9.21E-04  |
| EMT_UP                                                          | 55   | 0.58 | 2.05 | 9.07E-04  |
### Appendix 6. (Continued)

| Name                                                   | Size | ES   | NES   | FDR q-val |
|--------------------------------------------------------|------|------|-------|-----------|
| PROTEASOME_DEGRADATION                                  | 32   | 0.65 | 2.05  | 9.12E-04  |
| NI2_MOUSE_UP                                           | 40   | 0.62 | 2.05  | 9.88E-04  |
| HSC_INTERMEDIATEPROGENIORS_SHARED                      | 80   | 0.54 | 2.05  | 9.74E-04  |
| LINDSTEDT_DEND_UP                                      | 44   | 0.61 | 2.04  | 1.05E-03  |
| TRNA_SYNTHETASES                                       | 17   | 0.75 | 2.03  | 1.19E-03  |
| SHIPP_FL_VS_DLBCL_DN                                    | 30   | 0.66 | 2.02  | 1.29E-03  |
| INSULIN_ADIP_INSSENS_UP                                | 17   | 0.73 | 2.02  | 1.36E-03  |
| PROTEASOME                                             | 17   | 0.74 | 2.01  | 1.46E-03  |
| GOLDRATH_CELL_CYCLE                                    | 28   | 0.65 | 2.01  | 1.52E-03  |
| ZHAN_MULTIPLY_MYELOMA_SUBCLASSES_DIFF                   | 26   | 0.67 | 2.01  | 1.68E-03  |
| YU_CMYC_DN                                             | 53   | 0.58 | 2.00  | 1.72E-03  |
| UV-CMV_UNIQUE_HCMV_6HRS_UP                              | 83   | 0.53 | 2.00  | 1.72E-03  |
| CHOLESTEROL_BIOSYNTHESIS                               | 15   | 0.76 | 2.00  | 1.69E-03  |
| TAKEDA_NUP8_HOXA9_3D_DN                                 | 20   | 0.70 | 2.00  | 1.69E-03  |
| YU_CMYC_UP                                             | 37   | 0.61 | 1.99  | 1.77E-03  |
| UEDA_MOUSE_SCN                                          | 86   | 0.53 | 1.98  | 2.20E-03  |
| APOPTOSIS                                              | 64   | 0.55 | 1.98  | 2.34E-03  |
| ZELLER_MYC_UP                                          | 23   | 0.68 | 1.96  | 3.06E-03  |
| DAC_BLADDER_UP                                         | 23   | 0.67 | 1.96  | 3.11E-03  |
| MYC_TARGETS                                            | 39   | 0.58 | 1.94  | 3.99E-03  |
| BLEO_MOUSE_LYMPH_HIGH_24HRS_DN                         | 32   | 0.62 | 1.94  | 3.97E-03  |
| CMV_HCMV_6HRS_UP                                       | 19   | 0.70 | 1.94  | 3.99E-03  |
| HDAC1_COLON_CUR24HRS_UP                                | 28   | 0.63 | 1.94  | 3.96E-03  |
| UNDERHILL_PROLIFERATION                                | 18   | 0.69 | 1.93  | 4.03E-03  |
| MENSSEN_MYC_UP                                         | 30   | 0.63 | 1.93  | 4.16E-03  |
| IL1_CORNEA_UP                                          | 53   | 0.55 | 1.93  | 4.15E-03  |
| FASPATHWAY                                             | 25   | 0.65 | 1.93  | 4.18E-03  |
| MUNSHI_MM_UP                                           | 57   | 0.54 | 1.92  | 5.04E-03  |
| IDX_TSA_UP_CLUSTER3                                     | 81   | 0.51 | 1.92  | 5.09E-03  |
| ST_TUMOR_NECROSIS_FACTOR_PATHWAY                       | 28   | 0.64 | 1.91  | 5.10E-03  |
| PYRIMIDINE_METABOLISM                                  | 55   | 0.54 | 1.91  | 5.39E-03  |
| KNUDSEN_PMNS_UP                                        | 65   | 0.53 | 1.91  | 5.40E-03  |
| NFKBPATHWAY                                            | 23   | 0.65 | 1.90  | 5.53E-03  |
| HEARTFAILURE_VENTRICLE_DN                              | 56   | 0.53 | 1.90  | 5.62E-03  |
| CMV_UV-CMV_COMMON_HCMV_6HRS_UP                         | 17   | 0.70 | 1.90  | 6.00E-03  |
| ABBUD_LIF_UP                                           | 45   | 0.56 | 1.88  | 7.06E-03  |
| HPV31_DN                                               | 37   | 0.57 | 1.88  | 7.03E-03  |
| LOTEM_LEUKEMIA_UP                                      | 22   | 0.65 | 1.88  | 7.17E-03  |
| TPA_SENS_MIDDLE_UP                                     | 55   | 0.54 | 1.88  | 7.59E-03  |
| CASPASEPATHWAY                                         | 20   | 0.67 | 1.88  | 7.62E-03  |
| UVC_HIGH_D3_DN                                         | 35   | 0.59 | 1.87  | 7.75E-03  |
| WANG_MLL_CBP_VS_GMP_UP                                  | 42   | 0.56 | 1.87  | 8.33E-03  |
| MARSHALL_SPLEEN_BAL                                    | 25   | 0.62 | 1.86  | 9.11E-03  |
| XU_CBP_UP                                              | 25   | 0.61 | 1.86  | 9.64E-03  |
| MUNSHI_MM_VS_PCS_UP                                    | 64   | 0.51 | 1.86  | 9.70E-03  |
| GENOTOXINS_ALL_24HRS_REG                                | 22   | 0.64 | 1.86  | 9.65E-03  |

**Abbreviations:** ES, enrichment score; NES, normalized enrichment score; FDR, false discovery rate.
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