Published in final edited form as:
J Immunol. 2009 February 1; 182(3): 1706–1729.

Gene Expression Patterns Induced by HPV-16 L1 VLP in Leukocytes from Vaccine Recipients

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Abstract

Human papilloma (HPV) virus-like particle (VLP) vaccines were recently licensed. Though neutralizing antibody titers are thought to be the main effectors of protection against infection, early predictors of long-term efficacy are not yet defined and a comprehensive understanding of innate and adaptive immune responses to vaccination is still lacking. Here, microarrays were used to compare the gene expression signature in HPV-16 L1 VLP-stimulated PBMC from 17 vaccine and 4 placebo recipients before vaccination, and 1 month after receiving the second immunization. Vaccination with a monovalent HPV-16 L1 VLP vaccine was associated with modulation of genes involved in the inflammatory/defense response, cytokine, interferon and cell cycle pathways in VLP-stimulated PBMC. Additionally, there was up-regulation of probesets associated with cytotoxic (GZMB, TNFSF10) and regulatory (INDO, CTLA4) activities. The strongest correlations with neutralizing antibody titers were found for cyclin d2 (CCND2) and galectin (LGALS2). Twenty-two differentially expressed probesets were selected for confirmation by RT-PCR in an independent sample set. Agreement with microarray data was seen for over two-thirds of these probesets. Up-regulation of immune/defense response genes by HPV-16 L1 VLP, in particular interferon-induced genes was observed in PBMC collected prior to vaccination, with many of these genes being further induced following vaccination. In conclusion, we identified important innate and adaptive response related- genes induced by vaccination with HPV-16 L1 VLP. Further studies are needed to identify gene expression signatures of immunogenicity and long-term protection with potential utility in prediction of long-term HPV vaccination outcomes in clinical trials.

Keywords

Human; Vaccination; Microarray

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Introduction

Cervical cancer is the second most common cancer among women worldwide (1,2). The recent development of prophylactic HPV vaccines provides an important new avenue for the prevention of this cancer and others linked to HPV infection.

The current HPV prophylactic vaccines consist of non-infectious, L1 recombinant HPV-like particles (VLP) (3,4). Vaccine clinical trials have shown near complete protection against cervical intraepithelial neoplasia (CIN) 2 or greater lesions (5–7). Neutralizing antibodies are believed to be the main effectors of protection. A robust humoral response has been observed after vaccination (8–10), and antibody levels against HPV-16 remain relatively high in the first few years after vaccination (11–13). However, length of protection afforded by vaccination is not currently known.

Studies by our group and others have shown that in addition to antibody responses, strong cell-mediated immune responses are observed after vaccination (14–17). It is known that cell-mediated immune responses are required for antibody production and for maintenance of antibody levels over time. Thus, it could be postulated that the patterns of innate and acquired cellular immune responses following initial vaccination might influence antibody responses and duration of protection afforded by vaccination (18,19). A better understanding of these early responses to vaccination might help elucidate mechanisms of vaccine protection via neutralizing antibodies, and assist in the identification of early markers of long-lasting vaccine responses.

Microarray analysis has been applied to determine gene expression patterns in studies of disease pathogenesis (20–23), immune response to infection (24–30), and immune response after immunization (31,32). However, few studies have looked at vaccine-induced responses in humans (33–35).

To characterize the immune response to HPV-16 L1VLP vaccination, we used microarray chips that cover 8638 verified sequences to investigate the gene profile associated with a recall response to the vaccine in leukocytes from vaccine recipients. We identified probesets that were differentially expressed in PBMC from vaccine recipients after *in vitro* stimulation with HPV-16 L1 VLP compared to pre-vaccination samples. We also analyzed the “primary” response to HPV-16 L1 VLP, independent of vaccination, by comparing the gene expression pattern of cells incubated with HPV-16 L1 VLP and unstimulated cells, using pre-vaccination samples.

In addition, the correlation of gene expression to neutralizing antibody titers developed after vaccination was examined to evaluate potential determinants of strong antibody responses. Key results were confirmed by RT-PCR in an independent set of vaccinated individuals.

This is the first study that evaluates the immune response to an HPV VLP vaccine using microarray technology. Our results contribute to a broader understanding of the effects of vaccination with a monovalent HPV-16 L1 VLP vaccine. The approach used here and the gene expression profile defined in this study may prove to be useful for future prediction of long-term HPV vaccination outcomes in ongoing clinical trials.

Materials and Methods

Study Design

Participants were selected from a double-blind, randomized, placebo-controlled phase II trial of a monovalent HPV-16 L1 VLP vaccine without adjuvant, which was conducted in a sample of 220 healthy, HIV seronegative adult female volunteers 18–25 years of age, as described previously (17). Briefly, subjects were enrolled at The Johns Hopkins University Center for Immunization Research (Baltimore). Pre-vaccination HPV-16 antibody or DNA status was not a criterion for eligibility into the trial. Subjects were determined by history to be at low risk for HPV16 exposure. Individuals were not eligible to participate if they had a history of more than four lifetime sexual partners or more than two sexual partners within the preceding 6 months. Additional exclusion criteria included history of abnormal cervical cytology, immunodeficiency, anaphylaxis to medicines or vaccines, receipt of blood products within 3 months of enrollment, current pregnancy or lactation, and any other condition that might interfere with the study objectives. Women were randomly assigned to receive three intramuscular doses of either 50 μg of HPV-16 L1 VLP vaccine without adjuvant, or placebo (0.5 mL saline). Blood specimens were collected before the initial dose (month 0), and 1 month after each of the subsequent vaccinations (months 2 and 7). The Johns Hopkins University Institutional Review Board approved the protocol for this study. Blood specimens were shipped to the HPV Immunology Laboratory (Frederick, MD), where PBMCs were cryopreserved.

Twenty-seven subjects (20 vaccine and 7 placebo recipients) were randomly selected for microarray measurements. Only month 0 and month 2 samples were selected for this study because earlier findings indicated that the largest increases in cytokine responses were typically observed at month 2 (17). Month 0 and 2 samples from 10 additional vaccine recipients were selected for a second, confirmatory phase of our study by RT-PCR.

HPV-16 L1 VLP vaccine

HPV-16 L1 VLPs were expressed in baculovirus-infected Sf9 insect cells (Novavax, Rockville, MD). Production of clinical lots of recombinant HPV-16 L1 VLP vaccines was performed in accordance with GMP guidelines as previously reported (36). The VLPs used to *in vitro* to stimulate PBMCs were similar to the ones used to vaccinate the subjects included in this study. VLPs were provided at 1 mg/mL, stored at −80°C and thawed immediately preceding *in vitro* stimulation, as previously described (16).

PBMC incubation and Microarray Analysis

Cryopreserved PBMCs were thawed and cultured (2.0×10^6 cells/mL) as previously described (17). 10×10^6 PBMCs were plated in each well of a 6-well plate (Costar) in RPMI-1640 supplemented with penicillin-streptomycin (100 μg/mL-100 U/mL; Gibco), L-glutamine (2mM), HEPES buffer (10 mM) and 10% heat-inactivated fetal calf serum (HyClone). Cells were cultured for 72 hours at 37°C with: media; HPV-16 L1 VLP (2.5 μg/mL); or Sf9/baculovirus insect cell lysate (0.1 μg/mL, Novavax) all diluted in cell culture media. Media was used as a background measurement for untreated cells. The Sf9/baculovirus insect cell lysate was used as a control antigen for the L1 VLP production system.

A total of eight incubations were setup on the same day for each subject. Incubations for two subjects were setup on the same day. The order of sample preparation was randomly defined from the list of selected subjects.

When harvesting, cultures were centrifuged and cell-free supernatants were aliquoted and frozen at −80°C. Total RNA extracts were performed using an RNeasy Total RNA isolation Kit (Qiagen). RLT lysis buffer was quickly added to the culture well and then to the cell pellet, in order to include both adherent and suspension cells. RNA purity and integrity were tested by microcapillary electrophoresis using the Agilent 2100 bioanalyzer (Agilent Technologies).

Microarray gene expression analysis was performed using the using the Human Genome Focus Array from the Affymetrix GeneChip system (Affymetrix, Inc.) that contains about 8700 probesets to 8638 characterized human genes. Total RNA preparation and labeled cRNA synthesis and hybridization were performed according to the manufacturer's recommended protocol (Affymetrix, Inc.). In short, 10 µg of total RNA were used for double-stranded cDNA (ds-cDNA) synthesis with the SuperScript™ II Reverse Transcriptase Kit (Invitrogen) and an oligo (dT) primer containing a T7 RNA polymerase promoter to prime the first-strand synthesis. Biotin-labeled cRNA was obtained by *in vitro* transcription after addition of T7 RNA polymerase and biotinylated nucleotides (Enzo Biochem) to the ds-cDNA. Labeled cRNA was fragmented and hybridized to the GeneChips, which were then washed and stained with streptavidin-conjugated PE by using the Affymetrix GeneChip Fluid Station 400 (Affymetrix, Inc.). To assess the quality of hybridization, the following quality control filters were used: scaling factor, background, percentage of present calls, noise, housekeeping genes (3'/5' ratios and intensity for GAPDH and Actin), RNA degradation slope and presence or absence of internal spike controls.

Gene expression levels were determined by laser scanning of the GeneChip at 570 nm and were log2 transformed following MAS5 probeset summarization. All results from a donor were excluded if one of the conditions (media and VLP cultures for the pre- and post-vaccination specimens) did not fulfill one of the previously mentioned quality control filters used to ensure that all chips were of comparable quality. These quality control procedures resulted in exclusion of two of the vaccine recipients and one placebo. One additional vaccine recipient was excluded due to a high degree of granulocyte contamination (61% versus 0.2–13.6% granulocytes for remaining samples), possibly due to poor PBMC Ficoll isolation, and because neutralizing antibodies against HPV-16 were detectable before vaccination. Two additional placebos were excluded because they had detectable antibodies in the course of our study. After all exclusions, 17 vaccine and 4 placebo recipients were used in subsequent analysis.

Data from probesets that were not reliably detected with a mean intensity> $\log_2 = 5$ in at least one experimental condition (media and VLP cultures for the pre- and post-vaccination specimens) were excluded before further analysis. A total of 7145 probesets remained after applying this criterion.

The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (37), and are accessible through GEO Series accession number GSE13587 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE13587).

Flow Cytometry

Major lymphocyte subsets for all samples were determined by flow cytometry (FC-500, Beckman-Coulter), as previously described (38). Flow cytometric analysis of main lymphocyte subsets indicated that overall levels of major lymphocyte subsets did not change considerably after vaccination (mean + SD pre *versus* post vaccination, were CD3+ cells: 78±7 *versus* 77 ±9, CD19+ cells: 10±6 *versus* 10±5, CD16+/56+ cells: 11±6 *versus* 11±6). In addition, placebo recipients had also similar overall levels of leukocyte subsets (mean ± SD month 0 versus month 2, were CD3: 77±4 versus 79±5, CD19: 11±3 versus 11±6, CD16+/CD56+ cells 9±1 versus 8±3).

Neutralizing antibodies

HPV-16 neutralizing antibody titers were determined using a pseudovirus-based neutralization assay performed as previously described (39). Serum neutralization titers were defined as the reciprocal of the highest dilution that caused at least 50% reduction in SEAP activity. A negative control using the Bovine Papillomavirus (BPV) pseudovirus was performed for all samples. Undetectable antibody levels were considered as 0. All individuals selected for the microarray analysis had no detectable antibodies prior to vaccination. After vaccination, all individuals developed antibody titers against HPV-16 (month 2 mean titer of 843±748, range of 78 to 2602; month 7 mean titer of 5209±7383, range of 111 to 25571; month 12 mean titer of 2366±4184, range of 148 to 15317).

Quantitative real-time PCR and confirmation of microarray results

Real-time PCR measurements were performed with samples from 10 independent vaccine recipients collected at month 0 and 2 post-vaccination within this same clinical trial. These individuals were selected randomly based on availability of cryopreserved PBMC at both time points and were not tested by microarray. One subject was excluded from analysis because anti-HPV-16 neutralizing antibodies were detectable before vaccination. All remaining nine donors, had no detectable neutralizing antibody titers before vaccination, and thus, a similar prevaccination antibody status as the individuals used for the microarray experiments. In addition, an induction in anti-HPV-16 titers was observed at month two after the first dose (mean titer at month 2 of 2675±2644, neutralizing titer range: 333–8508).

A total of 22 probesets (Table IV) were selected for confirmation. All selected probesets had an average fold induction of 2.0 or higher by microarray (range: 2.0–5.01), which was observed in at least 50% of subjects (range: 50–83%). Ten selected probesets had statistically significant differences before and after vaccination with significance levels of $p \leq 0.001$. These included GZMB, IFNG, HSD11B1, INDO, GGTLA1, KIF20A, GOLPH2, IL6, IL3 and AICDA. The remaining 12 probesets were selected either because their expression levels correlated with antibody titers at month 7 or 12 ($r > 0.52$; 5 probesets: CYP27B1, INHBA, MMP12, CD1B and CCL7) or they were cytokines/chemokines of potential interest (7 probesets: CSF2, IL2, IL5, CXCL13, LTA, LIF, CCL13).

PBMC incubation and total RNA extraction were performed as described above. One µg of total RNA extract was reverse-transcribed with random hexamer primers, using Super Script™ Reverse Transcriptase (Invitrogen). 1/20 of the first-strand cDNA synthesis reaction was used as template. Pre-designed primer and probe kits (TaqMan® Gene Expression Assays, Applied Biosystems) and the TaqMan® Universal PCR Master Mix (Applied Biosystems) were used according to manufacturer's recommendations. Briefly, stock reaction solutions were prepared by mixing 10 µL of PCR Master Mix (2X), 1 µL of Primer kit (20X) and 7.1 µL of DEPC-treated water per reaction. 18.1 µL of stock solution were dispensed to each well, and 1.9 µL of cDNA was then added (total volume per reaction: 20 µL). The following temperature program was used for all genes: 2 minutes at 52°C, 10 minutes at 95 °C and 50 cycles of 15 seconds at 95°C and 1 minute at 60°C. Fluorescence intensity was measured in real time during the extension step, using the iCycler IQ multicolor real time PCR detection system (Bio-Rad). The following formula was used to determine fold induction for each gene:

$$\text{FoldChange}=2^{-\{[(CtXVpost-CtRVpost)-(CtXMpost-CtRMpost)]-[(CtXVpre-CtRVpre)-(CtXMpre-CtRMpre)]\}}$$

Where CtXVpost = threshold cycle of the analyzed gene in month 2 cells treated with VLP; CtRVpost = threshold cycle of RPLPO (acidic ribosomal phosphoprotein P0 subunit) in month 2 cells treated with VLP; CtXMpost = threshold cycle of the analyzed gene in month 2 cells treated with media; CtRMpost = threshold cycle of RPLPO in month 2 cells treated with media; CtXVpre = threshold cycle of the analyzed gene in month 0 cells treated with VLP; CtRVpre = threshold cycle of RPLPO in month 0 cells treated with VLP; CtXMpre = threshold cycle of the analyzed gene in month 0 cells treated with media; CtRMpre = threshold cycle of RPLPO in month 0 cells treated with media.

To determine the cutoff value for PCR results, the fold change for RPLPO was calculated as follows:

$$\mathrm{FoldChange}=2^{-[(CtRVpost-CtRMpost)-(CtRVpre-CtRMpre)]}$$

This was used to determine variability of RPLPO for all subjects, and define the lowest fold change to consider a gene as up-regulated. Based on this, only genes with a fold change higher than the average plus two standard deviations of the value obtained for RPLPO were considered positive (i.e. 2.14-fold change).

Data analysis

To determine vaccination-induced changes, gene expression levels (presented as $\log_2$ of intensity) for each probeset were compared before and after vaccination. Media-incubated expression levels were subtracted from VLP-incubated samples to adjust for background noise. Paired t-tests comparing the mean before and after vaccination were used to determine statistical significance. To account for the evaluation of the large number of genes, p-values < 0.001 were considered significant. Further, only probesets with log2 expression change (*n*-fold) <–1.3 (downregulated) or >1.3 (upregulated) were considered. For the *a priori* analysis, changes in gene expression for a predefined set of probesets within pre-selected pathways (Defense, Inflammation, Cytokine, Interferon, Cell cycle, Signal transduction) were evaluated. The selection of probesets was based on the list of Gene Ontology pathways obtained for the probesets present in the Human Genome Focus Array. A p-value of less than 0.001 (and fold change>1.3) was used to define a significantly differentially expressed probeset. The probesets were then mapped to our *a priori* list of probesets in pathways of interest. The rates of differential expression (DE) in these pathways were compared to the rates of DE amongst non-pathway probesets. Gene-set analysis, a permutation-based approach was used to compute p-values for the pre-defined gene sets (25).

We compared the direction and magnitude of expression values from the vaccine recipients to that from four placebos to ensure that effects seen in vaccinated women were specific and not observed among placebo controls. Since the number of placebo recipients was small, we relied on the magnitude of effect rather than statistical significance to determine the specificity of findings among vaccinated women.

For the exploratory analyses, functional annotation and biological term enrichment analysis of regulated probesets were performed by using the DAVID knowledge base (40). This approach assumes independence under the null hypothesis.

Expression values for PBMCs incubated in culture media were evaluated before and after vaccination for background measurements. Gene expression values of VLP-treated cells were corrected for background by subtracting results from unstimulated cells. Paired t-tests were conducted to compare expression levels for each probeset before and after vaccination. All probesets that were differentially expressed at a significance level of $p<0.05$ (and fold change >1.3) were considered, including the 113 probesets that were significant at $p<0.001$ (and fold change >1.3). Since the purpose of this post-hoc evaluation was of exploratory nature, we used a high p-value threshold to define probesets of interest and did not adjust for multiple comparisons. Functional annotation of the differentially expressed probesets was performed using DAVID version 2.0 (41).

In order to determine if there is an association between gene expression and antibody levels for differentially expressed probesets, we calculated the Spearman correlation of the difference in expression levels post-pre vaccination (corrected for background, as summarized above) with neutralizing antibody titers at months 2, 7, and 12.

Results

Evaluation of Differential Response to HPV-16 L1 VLP Stimulation Induced by Vaccination

The main aim of our study was to determine changes in gene expression that are associated with HPV vaccination. To this end, we compared gene expression levels in PBMC from vaccine recipients obtained before vaccination and one month after the second vaccine dose (month 2). After filtering, we observed that overall 128 of 7145 probesets (1.8%) were found to be significantly differentially expressed after vaccination ($p<0.001$). Among these, 113 probesets met the fold-change criteria (fold-change >1.3). Of these, 63 (56%) were up-regulated and 50 (44%) were down-regulated. None of these probesets were significantly differentially expressed in baculovirus-treated cultures following vaccination, when compared with levels in baculovirus-treated pre-vaccination samples (all p-values were > 0.001, data not shown). Also, with the exception of probeset PLA2G4B, none of these probesets demonstrated evidence of differential expression of comparable magnitude among the placebo subjects evaluated (data not shown).

Forty-eight of the 113 significantly differentiated probesets mapped within the 2345 probesets identified within the six pathways targeted for our *a priori* analysis (cell cycle, cytokines, defense, inflammation, interferon response and cell signaling). As shown in Table I, the percentage of differentially expressed probesets was significantly increased in the cell cycle (1.7 fold, $p<0.001$), cytokine (2.3-fold, $p=0.002$), defense (2.7-fold, $p=0.002$), inflammation (3.0-fold, $p=0.004$) and interferon (4.6-fold, $p=0.037$) pathways, when compared to rates of differential expression among the 4800 probesets that did not map to probesets within *a priori* pathways (1.4%). The lists of significantly differentially expressed probesets ($p < 0.001$) within these *a priori* pathways are presented in Table II A–E. The most significant upregulated probesets were RRM2 (cell cycle pathway), CTSC (defense pathway), IFNG (cytokine and defense pathways), RFC3 (cell cycle pathway) and INDO (defense and interferon pathways), and the most significant downregulated probesets were TSC1 (cell cycle pathway), CD14 (defense and inflammation pathways), ADORA2B and DPP4 (defense pathway), as well as GRN (cytokine pathway) and DPP4 (defense pathway).

A similar list for the pathway that did not have significant evidence for enrichment in our *a priori* evaluation (cell signaling) is presented in Supplementary Table I. The list of significantly differentially expressed probesets not within one of our *a priori* pathways is shown in Supplementary Table II A–B. A summary of overall gene expression profiles induced by vaccination ($p<0.001$) is summarized in Figure 1. Biological term enrichment analysis was performed using DAVID. In addition to the above *a priori* pathways, the following were found to have evidence for enrichment. Upregulated pathways: Receptor binding (9 probesets), catabolism (9 probesets), hemopoietic or lymphoid cell development (4 probesets), cell death (8 probesets) and peptidase activity (8 probesets). Downregulated pathways: programmed cell death (7 probesets), phosphoric ester hydrolase (4 probesets) and alcohol metabolism (4 probesets).

For our exploratory analysis, we expanded the evaluation to probesets differentially expressed at a $p<0.05$ cutoff level, 624 of 7145 probesets (8.7%) were found to be differentially expressed (330 up-regulated, and 294 down-regulated; Table III).

Correlation of gene expression with neutralizing antibody titers after vaccination

Durability of protection after vaccination has not been established, but it is possible that individuals developing higher neutralizing titers upon immunization will have longer lasting protection against HPV infection. If so, understanding the immunological profile of individuals who develop higher neutralizing titers upon immunization might help elucidate the molecular mechanisms of long-term protection. With this in mind, we compared probeset expression levels induced by vaccination against neutralizing titers at one month after second vaccination (Month 2) and one or six months after the third vaccination (Month 7 or 12, respectively). Only individuals negative for neutralizing antibodies before vaccination were included. For this analysis, we considered the subset of 624 probesets that were differentially expressed at a $p < 0.05$.

Correlations were typically lower when vaccination-induced changes in probeset expression levels (measured at Month 2) were compared against neutralizing antibody levels at Month 2 (98% of correlations were between −0.50 and +0.50 at Month 2, compared to 87% when Month 7 and 84% when Month 12 neutralizing antibody levels were used). Table IV summarizes results for the subset of 22 probesets observed to have a correlation with neutralizing antibodies of >0.60 or <−0.60 at both Month 7 and Month 12 after vaccination. A complete list of results is provided in Supplementary Table III. The highest positive correlations at both month 7 and 12 after vaccination correlation (>0.70 at both time points) was observed for the following probesets: CCND2 (month 7 r=0.88; month 12 r=0.82), LGALS2 (month 7 r=0.86; month 12 r=0.74), and IL1RN (month 7=0.75; month 12= 0.73).

Confirmation of a subset of differentially expressed genes by quantitative RT-PCR

To confirm the microarray results, a subset of 22 differentially expressed genes (defined in Methods) was selected for confirmation by PCR, using samples from an independent group of vaccine recipients (n=9). As shown in Table V, the expression pattern of the selected genes concurred with the microarray data for 15 out of 22 of the analyzed probesets (GZMB, IFNG, HSD11B1, INDO, IL6, IL3, CSF2, CYP27B1, IL2, IL5, INHBA, MMP12, CD1B, CCL7 and LIF). For a smaller group of 6 probesets, the frequency of up-regulated probesets was lower using PCR than observed with microarray (GGTLA1, KIF20A, AICDA, CXCL13, LTA and CCL13). Finally, no up-regulation (mean fold change = 1.4) was detected by PCR for one of the selected probesets (GOLPH2), while 61% of samples showed an up-regulation by microarray analysis.

Analysis of pre-vaccination response to HPV-16 L1 VLP

Finally, we evaluated the direct effect of HPV-16 L1VLP on gene expression of PBMC obtained prior to vaccination by comparing levels of gene expression in PBMC incubated with HPV-16 L1 VLP against media-treated cells. This comparison allowed us to analyze the primary, vaccine-independent response to HPV-16 L1 VLP.

One-hundred thirty nine probesets were differentially expressed at a significance level of $p<0.001$ (106 up-regulated, 33 down-regulated) in pre-vaccination PBMC incubated with HPV-16 L1 VLP (Supplementary Table IV). An additional 258 probesets were differentially expressed at the $0.001<p<0.05$ level (154 up-regulated, 104 down-regulated). Pathway enrichment evaluation was suggestive of enrichment for probesets mapping to genes from inflammatory, immune response, chemotaxis, signal transduction and cell proliferation pathways, as summarized in Figure 2. Interestingly, at least 26 out of the 260 up-regulated probesets are interferon-induced genes, or play a role in interferon signaling. There was an overlap between the probesets directly induced by HPV-16 L1 VLP prior to vaccination and those differentially expressed in post-vaccination samples (n = 91 or 9 up-regulated probesets, and 45 or 7 down-regulated probesets when using $p<0.05$ or $p<0.001$, respectively; Figure 3).

The overlapped probesets belong to the most enriched pathways observed in HPV-16 L1 VLP-treated PBMC (for example, IFN-γ, IL-6, Granzyme B, INDO and several chemokines). The up-regulated, overlapping probesets significant at $p<0.001$ were INDO, LAMP3, TYMS, ALAS1, RRM2, CD38, IL6, IFNG and AKAP2. Down-regulated overlapping probesets significant at $p<0.001$ included CD9, HS3ST2, SORL1, ADFP, DFNA5, EPAS1 and ALDH3A2. A complete list of overlapping differentially expressed probesets is shown in supplementary Table IV.

Discussion

In this study, we characterized the gene expression pattern of recall immune responses to the HPV-16 L1 VLP by comparing pre- and post-vaccination gene expression patterns of PBMCs incubated with HPV-16 L1 VLP antigen to better characterize the cellular and innate immune responses in vaccination against HPV. Although the effector mechanism of protection for this vaccine is believed to be neutralizing antibodies, our results show that multiple pathways within the cellular and innate arms of the immune system are targeted upon vaccination. The exact role of these pathways in protection or duration of protection is still unknown but based on the results presented here deserves further investigation. The analysis of correlation between differentially expressed genes and neutralizing antibody levels allowed us to identify genes (in particular CCND2, LGALS2 and IL-1RN) that may be predictors of prolonged antibody responses. New studies are necessary to better determine which pre- and post-vaccination clinical, immunological and expression profiles are most associated with long-term protection against HPV.

We took two different approaches to data analysis: a conservative approach that takes into account the potential high false discovery rate that may accompany the high number of statistical comparisons performed, and an exploratory approach to define additional biological pathways and probesets altered in a recall immune response to the vaccine. Using the conservative approach, we pre-selected pathways based on overall knowledge of microbial immune responses. This reduced the number of comparisons made and focused our analysis on the strongest *a priori* pathways expected to be involved in immune responses to vaccination. Our results suggest that the response to HPV-16 L1 VLP in vaccine recipients involves modulation of genes within the cell cycle, cytokine, defense, inflammation and interferon pathways. This finding is consistent with the induction of various arms of the immune response (42,43) and with our previous data that a recall response to HPV-16 L1 VLP involves a broad and complex pattern of cytokines and chemokines (16,17).

No significant enrichment was observed for the signal transduction pathway in our conservative analysis despite differential expression in genes that belonged to this pathway. A possible explanation for this observation might be that the definition of this pathway is broad (1577 probesets), and that the differentially expressed probesets within the pathway represent a small subset of the total number. In our exploratory analysis, the signal transduction pathway was found to be over-represented. This information may be used for hypothesis generation in future studies.

Within the pathways evaluated, we identified up-regulation of genes involved in cytotoxic (CTSC, GZMA, GZMB, TNFSF10, FASLG) and important immuno-regulatory functions (INDO, HSD11B1, CTLA4, SOCS1). These results suggest that the immune response to HPV-16 L1 VLP induces a complex modulation of various components of the cell-mediated immune response, including cytokine secretion (T-helper cells), cytotoxic response, and activation of feedback mechanisms involving immunoregulatory genes. These results raise the need for future studies determining the contributing roles of these pathways in immunogenicity induced by VLPs. A better understanding of these pathways may be also useful in the design of improved vaccine strategies. In addition, a substantial fraction of the differentially expressed probesets was down-regulated after vaccination including genes that fell into cell death, immune response, signalling and metabolism pathways. Because we used PBMCs, the transcriptional change is representative of the changes from various cell types comprising the PBMC population and the cross-talk of extracellular signals, such as cytokines induced directly or indirectly by the VLPs. Interestingly, expression of a number of these down-regulated genes and corresponding proteins, such as CD9, CD14, ly86, LEF-1, CD26 have been shown to be down-modulated in the context of cell activation and/or inflammatory signals (44,45,46,47). Such changes may relate to the shutting down specific elements in order to better mount a protective immune response to HPV vaccine and avoid excessive inflammatory signals or limit feedback signaling. However, the underlying mechanisms and impact of these transcriptional changes in the development of an immune response to vaccination are not known.

When we examined the correlation of probeset expression levels with neutralizing antibody titers, more probesets correlated to antibody levels at months 7 and 12 post vaccination than at month 2. The highest correlations at month 7 and 12 were obtained for cyclin d2 (CCND2), which plays an essential role controlling the cell cycle at the G1/S transition (48); galectin 2 (LGALS2), a galactoside-binding lectin that has been shown to promote apoptosis in activated T-cells and to shift immune responses to a Th2 profile (49); and IL-1 receptor antagonist (IL_1RN) a regulator of IL-1 induced inflammatory response. In fact, cyclin D2 and AICDA are components of signaling cascades needed for B cell proliferation and antibody production (50,51). However, the potential role of galectin-2 and IL-1 receptor antagonist in B cell responses is unclear and could be of indirect nature since these are immune regulatory molecules. Confirmation of the role of the products of these genes in B cell memory responses to the vaccine would be informative in the future.

To confirm the microarray results, we selected PBMCs from an independent group of vaccine recipients and measured RNA expression for a subset of 22 probesets representing various biological pathways targeted by vaccination. We observed a comparable frequency of responders for 68% of analyzed genes (15 of 22). For an additional 27% (6 of 22) of analyzed genes, differential expression was similar to those observed by microarray testing, but at a lower frequency. We were unable to observe evidence of differential expression as noted by microarray for 1 of the 22 selected genes. These results validate our microarray findings, and suggest that the HPV-16 L1 VLP-specific response after vaccination involves activation of a complex cell-mediated immune response.

Our results demonstrate that the immune response to vaccination is heterogeneous among vaccine recipients at the gene expression level. For all the probesets analyzed, only a fraction of the participants were considered responders when looking at one gene at a time. The highest frequency was observed for HSD11B1 (15 of 18 donors in microarray, and 7 of 8 with RT-PCR). However when "responders" are defined by the global expression profile of multiple genes at one time the response rate is high (data not shown). Whether this heterogeneity of response will have an impact on long-term memory and quality of the immune responses over time needs to be addressed in further studies. It is possible that heterogeneity of response may be associated with previous exposures to HPV. Studies of comparison of expression profiles induced by vaccination in naturally infected *versus* naïve individuals are warranted. In addition, identification of gene signatures associated with infection or vaccination will also be important.

In addition to the evaluation of vaccine-specific immune responses, we characterized the vaccination-independent responses to VLP by comparing HPV-16 L1 VLP-incubated cells with media controls in pre-vaccination samples. Our analysis revealed that incubation with HPV-16 L1 VLP prior to vaccination induces up-regulation of the defense response, cytokine/inflammation, signal transduction, and cell proliferation pathways. If the responses elicited by

HPV-16 L1 VLP in the post and pre-vaccination responses are compared, about 30% of the probesets modulated by VLP (136 out of 397; $p<0.05$) prior to vaccination overlap with the HPV-16 L1 VLP specific responses induced by vaccination. It is interesting to note that for most of the overlapping probesets, responses observed after incubation with HPV-16 L1 VLP are stronger than before vaccination. This probably reflects an increase in the number of responding cells after vaccination. In contrast to the vaccination-specific responses, a strong induction of an interferon response was observed in the vaccination-independent responses. These findings are important considering the current potential applications of VLP as vectors for antigen delivery and as vaccines for various pathogens and tumors (52).

Peripheral blood leukocytes (PBMCs) are a valuable tool to study immune responses to various diseases (29,30,53). Use of PBMCs, however, has some limitations that need to be taken into account. PBMCs are heterogeneous in their cell composition. For this reason, an enhanced or reduced gene expression might be restricted to particular cell populations. In addition, the response to stimulation with HPV-16 L1 VLP in an *ex vivo* system might differ from responses that occur in disease-relevant tissues. Also, since the number of memory cells in PBMCs is low, a recall response could be diluted by the response from other cell populations. Thus, it is conceivable that large alterations in the expression of a unique gene from an underrepresented cell type may be overlooked upon examination of the PBMC population. Despite these considerations, we confirmed a group of genes using RT-PCR analysis, and a good correlation for a number of genes with neutralizing antibody levels was observed. Moreover, we found no correlation between levels of gene expression and the percentage of leukocyte subpopulations, suggesting that differences seen were not likely to be due to changes in cell populations. In fact, flow cytometric analysis of main lymphocyte subsets revealed no major cell subset differences between baseline (month 0) and month 2 samples.

An additional potential limitation of our study is the fact that results observed may not be directly extrapolated to patterns that will be obtained after vaccination with the commercially available HPV L1 VLP vaccines, because participants in our study were immunized with HPV-16 L1VLP without an adjuvant. In addition, the HPV-16 L1 VLP vaccine used in our study is monovalent and produced in a recombinant baculovirus system. However, our results provide interesting and unique information on HPV-16 L1 VLP-specific changes, independent of adjuvant effects.

In conclusion, we used gene expression profiling to identify important adaptive and innate immune responses induced by vaccination with HPV-16 L1 VLP vaccine. We also identified differentially expressed genes predictive of neutralizing antibody titers. Interestingly, the host transcriptional response to HPV-16 L1 VLPs following vaccination varies considerably among donors, raising the need for further studies to better understand the potential impact of those differences in APC function, T cell and B cell responses and how these gene signatures predict immunogenicity and long term outcome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Grant Support:

This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health (N01-CO-12400).

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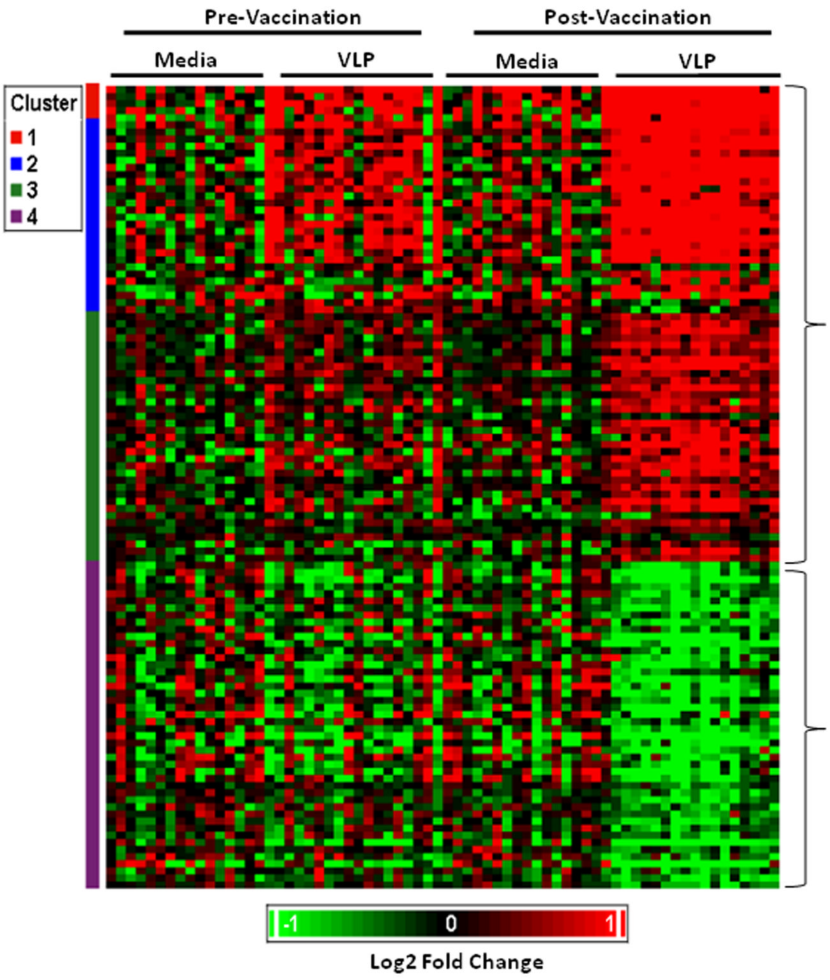
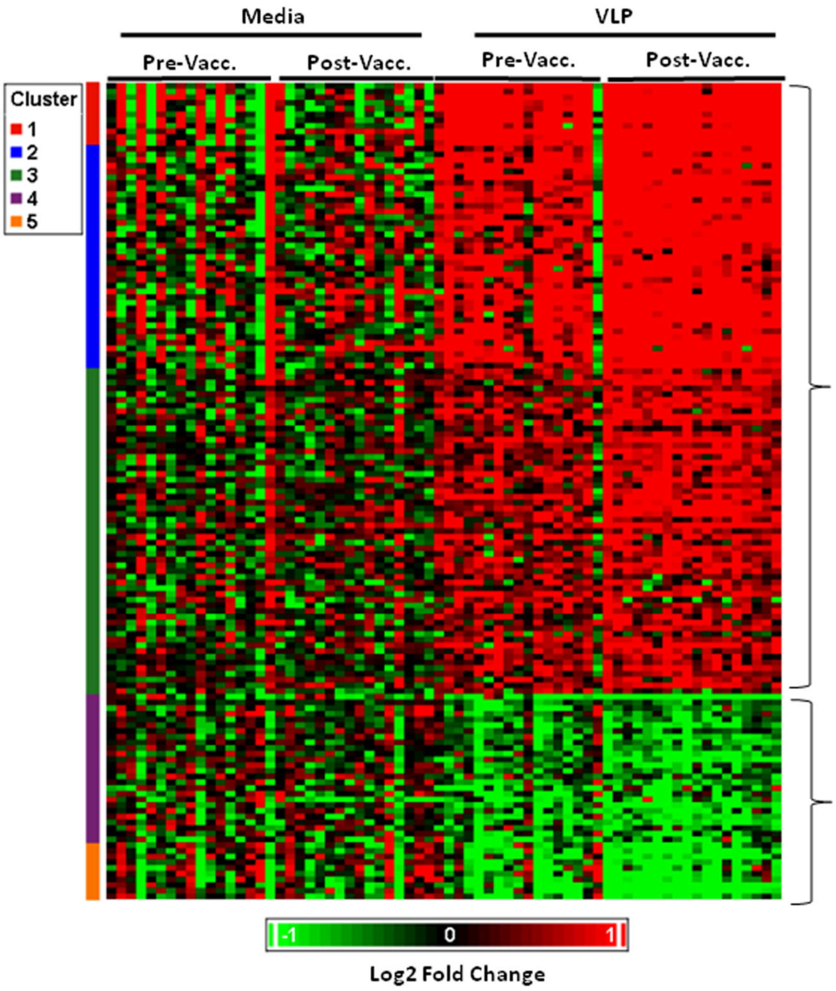


Annotation	# of Genes	Enrichment P-value
cytokine activity	7	3.7E-04
immune response	15	5.1E-04
defense response	15	1.3E-03
cell-cell signaling	9	3.0E-03
receptor binding	9	5.0E-03
catabolism	9	7.9E-03
hemopoietic or lymphoid organ development	4	8.3E-03
cell division	5	8.6E-03
response to pest, pathogen or parasite	8	1.4E-02
cell death	8	1.6E-02
peptidase activity	8	1.8E-02
response to other organism	8	1.9E-02
cell proliferation	7	3.6E-02

Annotation	# of Genes	Enrichment P-value
programmed cell death	7	3.7E-03
intracellular signaling cascade	9	9.2E-03
immune response	8	3.2E-02
positive regulation of cellular	5	3.4E-02
phosphoric ester hydrolase	4	4.3E-02
alcohol metabolism	4	4.4E-02

Figure 1. Expression of profiles of probesets significantly modulated by VLP in PBMC from vaccine recipients before and after vaccination with HPV-16 L1 VLP

PBMC from vaccine recipients were incubated with HPV-16 L1 VLP as indicated in Materials and Methods. Functional annotation of probesets differentially expressed (p<0.001) was done using DAVID. Upregulated genes are shown in red and downregulated genes in green. Each row represents a probset and each column represents an individual.



Annotation	# of Genes	Enrichment P-value
interferon induction	21	1.2E-36
defense response	46	9.9E-20
immune response	44	1.3E-19
response to pest, pathogen or parasite	30	1.7E-16
response to wounding	17	6.6E-08
antiviral defense	6	1.4E-06
cytokine	8	7.3E-05
cell-cell signaling	14	2.7E-04
inflammatory response	5	3.2E-04
chemotaxis	5	4.8E-04
G-protein-coupled receptor binding	5	8.1E-04
cytokine activity	8	2.1E-03
cell proliferation	11	9.1E-03
cell cycle	13	9.5E-03

Annotation	# of Genes	Enrichment P-value
lysosome	4	1.3E-03
protease	7	1.6E-03
endoplasmic reticulum	6	7.1E-03
peptidase activity	8	7.2E-03
lipid transport	3	8.3E-03
vldl	2	1.9E-02
membrane	18	2.5E-02
lipid metabolism	3	3.0E-02

Figure 2. Expression profiles of probesets significantly modulated following incubation of pre-vaccination PBMCs with HPV-16 L1 VLP

PBMC from pre-vaccination samples were incubated with HPV-16 L1 VLP as indicated in Materials and Methods. Functional annotation of probesets differentially expressed was done using DAVID. Upregulated genes are shown in red and downregulated genes in green.

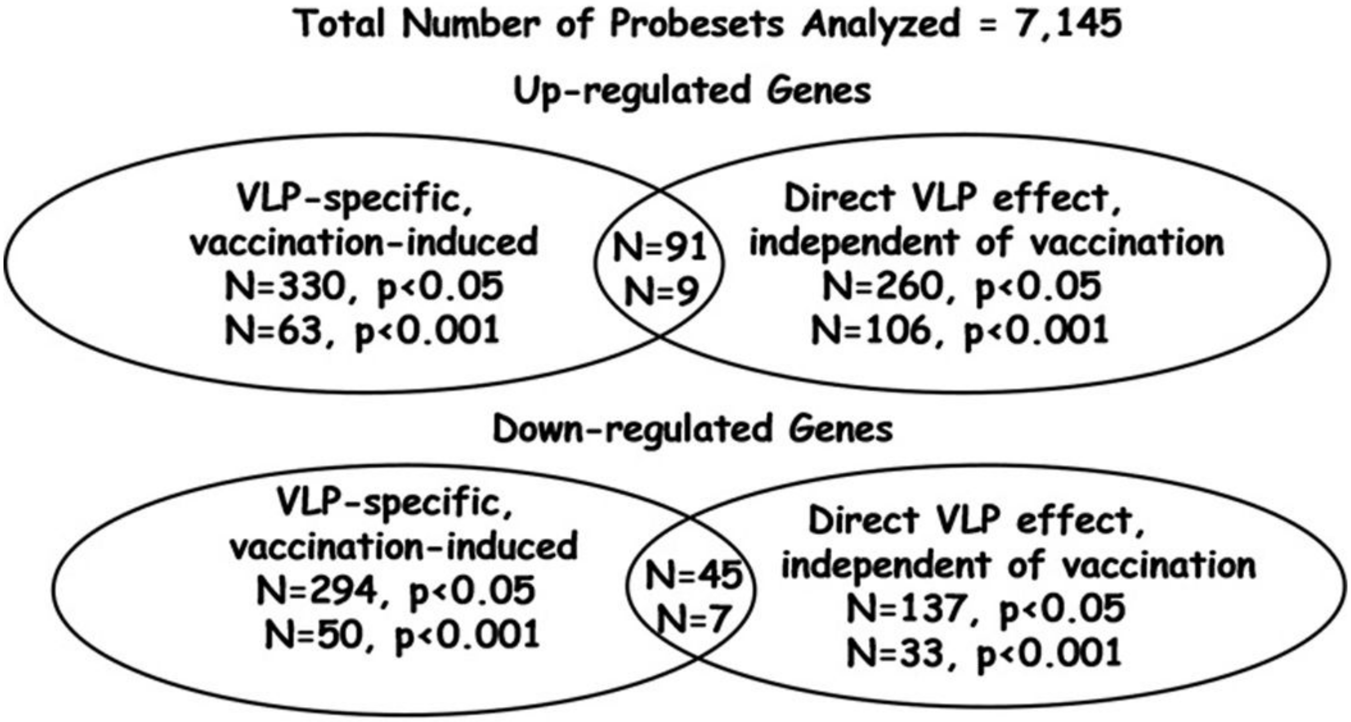


Figure 3. Number of probesets with increased or decreased expression in HPV-16 L1 VLP-treated PBMC from vaccine recipients induced by vaccination (vaccination-specific effect) or independent of vaccination (direct effect)

Number of probesets overlapping the lists of vaccination-specific probesets and the ones modulated by a direct HPV-16 L1VLP effect are also shown.

Table I

Frequency of Differentially Expressed (DE) Probe-Sets within A-Priori Defined Biological Pathways and Enrichment Ratio Compared to Probe-Sets not within A-Priori Defined Pathways

A-Priori Pathway	% DE probe-sets in pathway (# DE probe-sets/# total probe-sets in pathway)	DE enrichment ratio (a-priori pathway/not in a priori pathways[**])	p-value[*]	# Up-/down-regulated probe sets
Complete Array[**]	1.35 (65/4800)			34 / 30
Cell Cycle	2.29 (12/525)	1.69	<0.001	8 / 4
Cytokine	3.11 (9/289)	2.30	0.002	7 / 2
Defense	3.63 (22/606)	2.68	0.002	15 / 7
Inflammation	4.00 (6/150)	2.95	0.004	3 / 3
Interferon	6.25 (2/32)	4.62	0.037	2 / 0
Signal transduction	1.78 (28/1577)	1.31	0.920	11 / 17

[*] multiple comparison p-value computed using a bootstrap method as described in the Materials and Methods Section. $p<0.05$ is considered significant

[**] Excluding probe-sets mapping to a-priori pathways listed in the table. Note there is overlap of probe-sets across pathways.

Table II

Table II A. List of Genes Demonstrating Significant DE within the Cell Cycle Pathway

Gene Symbol	Gene Name	AffyID	Fold-Change	p-value
Up-regulated genes (expression higher post-vaccination than pre-vaccination)				
RRM2	ribonucleotide reductase M2 polypeptide	209773_s_at	2.06	0.000002
RFC3	replication factor C (activator 1) 3, 38kDa	204127_at	1.78	0.000022
PMS2 /// PMS2CL	PMS2 postmeiotic segregation increased 2 (S. cerevisiae)	209805_at	1.93	0.000257
PRC1	protein regulator of cytokinesis 1	218009_s_at	1.92	0.000321
CCNB2	cyclin B2	202705_at	1.66	0.000377
CCND2	cyclin D2	200953_s_at	1.42	0.000378
GINS2	DNA replication complex GINS protein PSF2	221521_s_at	3.50	0.000599
BUB1	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	209642_at	2.16	0.000754
Down-regulated genes (expression lower post-vaccination than pre-vaccination)				
TSC1	tuberous sclerosis 1	209390_at	−1.36	0.000023
LCK	lymphocyte-specific protein tyrosine kinase	204891_s_at	−1.31	0.000244
CDC25B	cell division cycle 25B	201853_s_at	−1.36	0.000428
ARHGEF1	Rho guanine nucleotide exchange factor (GEF) 1	203055_s_at	−1.36	0.000724

Table II B. List of Genes Demonstrating Significant DE within the Cytokine Pathway

Gene Symbol	Gene Name	AffyID	Fold-Change	p-value
Up-regulated genes (expression higher post-vaccination than pre-vaccination)				
IFNG	interferon, gamma	210354_at	2.57	0.000022
CXCL11	chemokine (C-X-C motif) ligand 11	210163_at	2.39	0.000086
IL6	interleukin 6 (interferon, beta 2)	205207_at	2.18	0.000295
IL3	interleukin 3 (colony-stimulating factor, multiple)	207906_at	4.03	0.000303
CXCL9	chemokine (C-X-C motif) ligand 9	203915_at	1.91	0.000507
TNF	tumor necrosis factor (TNF superfamily, member 2)	207113_s_at	1.81	0.000638
IL15	interleukin 15	205992_s_at	1.32	0.000979
Down-regulated genes (expression lower post-vaccination than pre-vaccination)				
GRN	granulin	211284_s_at	−1.52	0.000082
CSF3R	colony stimulating factor 3 receptor (granulocyte)	203591_s_at	−1.44	0.000355

Table II C. List of Genes Demonstrating Significant DE within the Defense Pathway

Gene Symbol	Gene Name	AffyID	Fold-Change	p-value
Up-regulated genes (expression higher post-vaccination than pre-vaccination)				
CTSC	cathepsin C	201487_at	1.52	0.000006
IFNG	interferon, gamma	210354_at	2.57	0.000022
INDO	indoleamine-pyrrole 2,3 dioxygenase	210029_at	2.47	0.000058
CXCL11	chemokine (C-X-C motif) ligand 11	210163_at	2.39	0.000086
KIR2DS5	killer cell immunoglobulin-like receptor, two domains, short cytoplasmic tail, 5	208203_x_at	2.11	0.000110
ADAR	adenosine deaminase, RNA-specific	201786_s_at	1.37	0.000249
IL6	interleukin 6 (interferon, beta 2)	205207_at	2.18	0.000295
IL3	interleukin 3 (colony-stimulating factor, multiple)	207906_at	4.03	0.000303

Table II C. List of Genes Demonstrating Significant DE within the Defense Pathway

Gene Symbol	Gene Name	AffyID	Fold-Change	p-value
AICDA	activation-induced cytidine deaminase	219841_at	2.91	0.000333
SLAMF1	signaling lymphocytic activation molecule family member 1	206181_at	1.42	0.000468
CXCL9	chemokine (C-X-C motif) ligand 9	203915_at	1.91	0.000507
TNF	tumor necrosis factor (TNF superfamily, member 2)	207113_s_at	1.81	0.000638
GZMA	granzyme A (granzyme 1, CTL-associated serine esterase 3)	205488_at	1.59	0.000755
C1QB	complement component 1, q subcomponent, beta polypeptide	202953_at	1.59	0.000775
IL15	interleukin 15	205992_s_at	1.32	0.000979
Down-regulated genes (expression lower post-vaccination than pre-vaccination)				
CD14	CD14 antigen	201743_at	−2.46	0.000031
ADORA2B	adenosine A2b receptor	205891_at	−1.69	0.000077
DPP4	dipeptidylpeptidase 4 (CD26, adenosine deaminase complexing protein 2)	203717_at	−1.35	0.000241
LY86	lymphocyte antigen 86	205859_at	−1.45	0.000340
CSF3R	colony stimulating factor 3 receptor (granulocyte)	203591_s_at	−1.44	0.000355
PLA2G4B	phospholipase A2, group IVB (cytosolic)	60528_at	−1.34	0.000546
FCGRT	Fc fragment of IgG, receptor, transporter, alpha	218831_s_at	−1.68	0.000678

Table II D. List of Genes Demonstrating Significant DE within the Inflammation Pathway

Gene Symbol	Gene Name	AffyID	Fold-Change	p-value
Up-regulated genes (expression higher post-vaccination than pre-vaccination)				
CXCL11	chemokine (C-X-C motif) ligand 11	210163_at	2.39	0.000086
CXCL9	chemokine (C-X-C motif) ligand 9	203915_at	1.91	0.000507
TNF	tumor necrosis factor (TNF superfamily, member 2)	207113_s_at	1.81	0.000638
Down-regulated genes (expression lower post-vaccination than pre-vaccination)				
CD14	CD14 antigen	201743_at	−2.46	0.000031
LY86	lymphocyte antigen 86	205859_at	−1.45	0.000340
PLA2G4B	phospholipase A2, group IVB (cytosolic)	60528_at	−1.34	0.000546

Table 2E. List of Genes Demonstrating Significant DE within the Interferon Pathway

Gene Symbol	Gene Name	AffyID	Fold-Change	p-value
Up-regulated genes (expression higher post-vaccination than pre-vaccination)				
INDO	indoleamine-pyrrole 2,3 dioxygenase	210029_at	2.47	0.0000584
CXCL9	chemokine (C-X-C motif) ligand 9	203915_at	1.91	0.0005074

[*] (VLP-Media) expression levels as defined in the Materials and Methods Section

Table III

List of DE Genes After Vaccination Using p<0.05 as Cutoff

Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name	Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name
Up-Regulated Genes					**Down-Regulated Genes**				
209773_s_at	2.06	0.000002	RRM2	ribonucleotide reductase m2 polypeptide	201005_at	−1.82	0.00000007	CD9	cd9 antigen (p24)
208921_s_at	1.48	0.000004	SRI	sorcin	201029_s_at	−1.30	0.000002	CD99	cd99 antigen
201487_at	1.52	0.000006	CTSC	cathepsin c	205695_at	−2.51	0.000009	SDS	serine dehydratase
200039_s_at	1.34	0.000010	PSMB2	proteasome (prosome, macropain) subunit, beta type, 2	203509_at	−1.71	0.000012	SORL1	sortilin-related receptor, l(dlr class) a repeats-containing
210164_at	2.33	0.000015	GZMB	granzyme b (granzyme 2, cytotoxic t-lymphocyte-associated serine esterase 1)	201591_s_at	−1.51	0.000020	NISCH	nischarin
210354_at	2.57	0.000022	IFNG	interferon, gamma	209390_at	−1.36	0.000023	TSC1	tuberous sclerosis 1
204127_at	1.78	0.000022	RFC3	replication factor c (activator 1) 3, 38kda	221558_s_at	−1.36	0.000026	LEF1	lymphoid enhancer-binding factor 1
204777_s_at	1.65	0.000030	MAL	mal, t-cell differentiation protein	201743_at	−2.46	0.000031	CD14	cd14 antigen
217848_s_at	1.86	0.000048	PPA1	pyrophosphatase (inorganic) 1	203979_at	−2.14	0.000032	CYP27A1	cytochrome p450, family 27, subfamily a, polypeptide 1
201274_at	1.39	0.000049	PSMA5	proteasome (prosome, macropain) subunit, alpha type, 5	204647_at	−1.99	0.000056	HOMER3	homer homolog 3 (drosophila)
205404_at	3.49	0.000052	HSD11B1	hydroxysteroid (11-beta) dehydrogenase 1	205891_at	−1.69	0.000077	ADORA2B	adenosine a2b receptor
210029_at	2.47	0.000058	INDO	indoleamine-pyrrole 2,3 dioxygenase	205960_at	−2.80	0.000082	PDK4	pyruvate dehydrogenase kinase, isozyme 4
219210_s_at	1.40	0.000063	RAB8B	rab8b, member ras oncogene family	211284_s_at	−1.52	0.000082	GRN	granulin
205582_s_at	3.90	0.000065	GGTLA1	gamma-glutamyltransferase-like activity 1	203695_s_at	−1.82	0.000089	DFNA5	deafness, autosomal dominant 5
216268_s_at	1.51	0.000077	JAG1	jagged 1 (alagille syndrome)	214198_s_at	−1.34	0.000101	DGCR2	kiaa0163 gene product
202760_s_at	1.58	0.000082	AKAP2 /// PALM2-AKAP2	a kinase (prka) anchor protein 2	204134_at	−2.87	0.000153	PDE2A	phosphodiesterase 2a, cgmp-stimulated
210163_at	2.39	0.000086	CXCL11	chemokine (c-x-c motif) ligand 11	222240_s_at	−1.36	0.000158	ISYNA1	myo-inositol 1-phosphate synthase a1
203471_s_at	1.40	0.000087	PLEK	pleckstrin	203382_s_at	−2.95	0.000183	APOE	apolipoprotein e
202941_at	1.34	0.000092	NDUFV2	nadh dehydrogenase (ubiquinone) flavoprotein 2, 24kda	204906_at	−1.36	0.000190	RPS6KA2	ribosomal protein s6 kinase, 90kda, polypeptide 2
209765_at	1.77	0.000096	ADAM19	adam metallopeptidase domain 19 (meltrin beta)	52940_at	−1.52	0.000204	SIGIRR	single immunoglobulin and toll-interleukin 1 receptor (tir) domain
208203_x_at	2.11	0.000110	KIR2DS5	killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 2	219889_at	−1.41	0.000207	FRAT1	frequently rearranged in advanced t-cell lymphomas
212581_x_at	1.34	0.000120	GAPDH	glyceraldehyde-3-phosphate dehydrogenase	209230_s_at	−2.44	0.000232	P8	p8 protein (candidate of metastasis 1)
202638_s_at	1.50	0.000145	ICAM1	intercellular adhesion molecule 1 (cd54), human rhinovirus receptor	209831_x_at	−1.35	0.000235	DNASE2	deoxyribonuclease ii, lysosomal
208799_at	1.34	0.000145	PSMB5	proteasome (prosome, macropain) subunit, beta type, 5	203717_at	−1.35	0.000241	DPP4	dipeptidyl-peptidase 4 (cd26, adenosine deaminase complexing protein 2)
218755_at	4.62	0.000152	KIF20A	kinesin family member 20a	204891_s_at	−1.31	0.000244	LCK	lymphocyte-specific protein tyrosine kinase
203217_s_at	1.35	0.000152	ST3GAL5	st3 beta-galactoside alpha-2,3-sialyltransferase 5	202054_s_at	−1.48	0.000256	ALDH3A2	aldehyde dehydrogenase 3 family, member a2
201037_at	1.52	0.000167	PFKP	phosphofructokinase, platelet	219697_at	−2.64	0.000260	HS3ST2	heparan sulfate (glucosamine) 3-o-sulfotransferase 2
205692_s_at	1.92	0.000181	CD38	cd38 antigen (p45)	208626_s_at	−1.30	0.000269	VAT1	vesicle amine transport protein 1 homolog (t californica)

Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name	Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name
Up-Regulated Genes					**Down-Regulated Genes**				
217771_at	3.13	0.000200	GOLPH2	golgi phosphoprotein 2	204466_s_at	−1.58	0.000286	SNCA	synuclein, alpha (non a4 component of amyloid precursor)
213011_s_at	1.32	0.000211	TPI1	triosephosphate isomerase 1	205859_at	−1.45	0.000340	LY86	lymphocyte antigen 86
201761_at	1.42	0.000226	MTHFD2	methylenetetrahydrofolate dehydrogenase (nadp+ dependent) 2, methenyltetrahydrofolate cyclohydrolase	203591_s_at	−1.44	0.000355	CSF3R	colony stimulating factor 3 receptor (granulocyte)
202589_at	1.75	0.000226	TYMS	thymidylate synthetase	221872_at	−2.32	0.000403	RARRES1	retinoic acid receptor responder (tazarotene induced) 1
202352_s_at	1.35	0.000231	PSMD12	proteasome (prosome, macropain) 26s subunit, non-atpase, 12	219445_at	−1.58	0.000404	GLTSCR1	glioma tumor suppressor candidate region gene 1
201323_at	1.43	0.000244	EBNA1BP2	ebna1 binding protein 2	204867_at	−1.75	0.000408	GCHFR	gtp cyclohydrolase i feedback regulator
209146_at	1.39	0.000247	SC4MOL	sterol-c4-methyl oxidase-like	201853_s_at	−1.36	0.000428	CDC25B	cell division cycle 25b
201786_s_at	1.37	0.000249	ADAR	adenosine deaminase, rna-specific	200878_at	−1.59	0.000443	EPAS1	endothelial pas domain protein 1
209805_at	1.93	0.000257	PMS2 /// PMS2CL	pms2 postmeiotic segregation increased 2 (s. cerevisiae), pms2-c terminal -like	220776_at	−1.89	0.000452	KCNJ14	potassium inwardly-rectifying channel, subfamily j, member 14
205569_at	1.66	0.000283	LAMP3	lysosomal-associated membrane protein 3	201278_at	−1.52	0.000496	DAB2	disabled homolog 2, mitogen-responsive phosphoprotein (drosophila)
205207_at	2.18	0.000295	IL6	interleukin 6 (interferon, beta 2)	209122_at	−1.60	0.000511	ADFP	adipose differentiation-related protein
207906_at	4.03	0.000303	IL3	interleukin 3 (colony-stimulating factor, multiple)	200766_at	−1.54	0.000544	CTSD	cathepsin d (lysosomal aspartyl peptidase)
218009_s_at	1.92	0.000321	PRC1	protein regulator of cytokinesis 1	60528_at	−1.34	0.000546	PLA2G4B	phospholipase a2, group ivb (cytosolic)
219841_at	2.91	0.000333	AICDA	activation-induced cytidine deaminase	203665_at	−1.61	0.000564	HMOX1	heme oxygenase (decycling) 1
214210_at	1.79	0.000349	SLC25A17	solute carrier family 25 (mitochondrial carrier; peroxisomal membrane protein, 34kda), member 17	218831_s_at	−1.68	0.000678	FCGRT	fc fragment of igg, receptor, transporter, alpha
202705_at	1.66	0.000377	CCNB2	cyclin b2	212774_at	−1.54	0.000688	ZNF238	zinc finger protein 238
200953_s_at	1.42	0.000378	CCND2	cyclin d2	202500_at	−1.50	0.000695	DNAJB2	dnaj (hsp40) homolog, subfamily b, member 2
201317_s_at	1.41	0.000395	PSMA2	proteasome (prosome, macropain) subunit, alpha type, 2	203055_s_at	−1.36	0.000724	ARHGEF1	rho guanine nucleotide exchange factor (gef) 1
203376_at	1.34	0.000400	CDC40	cell division cycle 40 homolog (yeast)	214780_s_at	−1.42	0.000801	MYO9B	myosin ixb
212185_x_at	1.72	0.000417	MT2A	metallothionein 2a	219113_x_at	−1.51	0.000846	DHRS10	dehydrogenase/reductase (sdr family) member 10
206181_at	1.42	0.000468	SLAMF1	signaling lymphocytic activation molecule family member 1	204638_at	−1.39	0.000861	ACP5	acid phosphatase 5, tartrate resistant
203915_at	1.91	0.000507	CXCL9	chemokine (c-x-c motif) ligand 9	218855_at	−1.51	0.000959	GPR175	seven transmembrane domain orphan receptor
208581_x_at	1.94	0.000511	MT1X	metallothionein 1x	204046_at	−1.42	0.001080	PLCB2	phospholipase c, beta 2
209803_s_at	1.84	0.000566	PHLDA2	pleckstrin homology-like domain, family a, member 2	221601_s_at	−1.41	0.001082	FAIM3	fas apoptotic inhibitory molecule 3
205633_s_at	1.60	0.000591	ALAS1	aminolevulinate, delta-, synthase 1	40225_at	−1.34	0.001110	GAK	cyclin g associated kinase
221521_s_at	3.50	0.000599	GINS2	dna replication complex gins protein psf2	201185_at	−1.53	0.001140	HTRA1	htra serine peptidase 1
204962_s_at	1.76	0.000638	CENPA	centromere protein a, 17kda	221579_s_at	−1.33	0.001161	NUDT3	diphosphoinositol polyphosphate phosphohydrolase
207113_s_at	1.81	0.000638	TNF	tumor necrosis factor (tnf superfamily, member 2)	221675_s_at	−1.48	0.001196	CHPT1	choline phosphotransferase 1
205126_at	1.45	0.000693	VRK2	vaccinia related kinase 2	217963_s_at	−1.63	0.001204	NGFRAP1	nerve growth factor receptor (tnfrsf16) associated protein 1

Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name	Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name
Up-Regulated Genes					**Down-Regulated Genes**				
209642_at	2.16	0.000754	BUB1	bub1 budding uninhibited by benzimidazoles 1 homolog (yeast)	218555_at	−1.47	0.001284	ANAPC2	anaphase promoting complex subunit 2
205488_at	1.59	0.000755	GZMA	granzyme a (granzyme 1, cytotoxic t-lymphocyte-associated serine esterase 3)	219452_at	−2.48	0.001417	DPEP2	dipeptidase 2
202953_at	1.59	0.000775	C1QB	complement component 1, q subcomponent, b chain	35626_at	−1.32	0.001587	SGSH	n-sulfoglucosamine sulfohydrolase (sulfamidase)
209267_s_at	1.68	0.000802	SLC39A8	solute carrier family 39 (zinc transporter), member 8	201819_at	−1.35	0.001596	SCARB1	scavenger receptor class b, member 1
204326_x_at	1.77	0.000952	MT1X	metallothionein 1×	211110_s_at	−2.00	0.001806	AR	androgen receptor (dihydrotestosterone receptor; testicular feminization; spinal and bulbar muscular atrophy; kennedy disease)
205992_s_at	1.32	0.000979	IL15	interleukin 15	209774_x_at	−1.76	0.002054	CXCL2	chemokine (c-x-c motif) ligand 2
205174_s_at	2.29	0.001056	QPCT	glutaminyl-peptide cyclotransferase (glutaminyl cyclase)	206608_s_at	−1.36	0.002175	RPGRIP1	retinitis pigmentosa gtpase regulator interacting protein 1
219960_s_at	1.30	0.001160	UCHL5	ubiquitin carboxyl-terminal hydrolase 15	204949_at	−1.34	0.002253	ICAM3	intercellular adhesion molecule 3
208864_s_at	1.34	0.001162	TXN	thioredoxin	214414_x_at	−1.59	0.002301	HBA2	hemoglobin, alpha 1
203213_at	2.50	0.001193	CDC2	cell division cycle 2, g1 to s and g2 to m	219549_s_at	−1.32	0.002316	RTN3	reticulon 3
210229_s_at	3.34	0.001253	CSF2	colony stimulating factor 2 (granulocyte-macrophage)	205382_s_at	−1.84	0.002559	CFD	complement factor d (adipsin)
205676_at	2.38	0.001331	CYP27B1	cytochrome p450, family 27, subfamily b, polypeptide 1	216092_s_at	−1.48	0.002565	SLC7A8	solute carrier family 7 (cationic amino acid transporter, y+ system), member 8
217979_at	1.51	0.001398	TSPAN13	tetraspanin 13	202449_s_at	−1.31	0.002571	RXRA	retinoid x receptor, alpha
202421_at	1.59	0.001460	IGSF3	immunoglobulin superfamily, member 3	205090_s_at	−1.30	0.002597	NAGPA	n-acetylglucosamine-1-phosphodiester alpha-n-acetylglucosaminidase
207277_at	1.65	0.001460	CD209	cd209 antigen	218427_at	−1.50	0.002685	SDCCAG3	serologically defined colon cancer antigen 3
205505_at	1.54	0.001515	GCNT1	glucosaminyl (n-acetyl) transferase 1, core 2 (beta-1,6-n-acetylglucosaminyltransferase)	207105_s_at	−1.49	0.002882	PIK3R2	phosphoinositide-3-kinase, regulatory subunit 2 (p85 beta)
211269_s_at	1.51	0.001562	IL2RA	interleukin 2 receptor, alpha	212346_s_at	−1.38	0.002913	MXD4	max dimerization protein 4
220358_at	2.21	0.001584	SNFT	jun dimerization protein p21snft	212552_at	−1.32	0.002917	HPCAL1	hippocalcin-like 1
209825_s_at	1.57	0.001612	UCK2	uridine-cytidine kinase 2	201427_s_at	−4.80	0.002990	SEPP1	selenoprotein p, plasma, 1
207900_at	2.43	0.001637	CCL17	chemokine (c-c motif) ligand 17	209409_at	−1.50	0.002998	GRB10	growth factor receptor-bound protein 10
205890_s_at	2.17	0.001649	GABBR1 /// UBD	ubiquitin d	207574_s_at	−1.31	0.003015	GADD45B	growth arrest and dna-damage-inducible, beta
201897_s_at	1.45	0.001660	CKS1B	cdc28 protein kinase regulatory subunit 1b	200710_at	−1.38	0.003071	ACADVL	acyl-coenzyme a dehydrogenase, very long chain
203200_s_at	1.36	0.001691	MTRR	5-methyltetrahydrofolate-homocysteine methyltransferase reductase	206471_s_at	−1.58	0.003112	PLXNC1	plexin c1
201013_s_at	1.33	0.001713	PAICS	phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase	205466_s_at	−2.39	0.003130	HS3ST1	heparan sulfate (glucosamine) 3-o-sulfotransferase 1
221331_x_at	2.49	0.001713	CTLA4	cytotoxic t-lymphocyte-associated protein 4	210980_s_at	−1.39	0.003134	ASAH1	n-acylsphingosine amidohydrolase (acid ceramidase) 1
37145_at	1.31	0.001870	GNLY	granulysin	200965_s_at	−1.37	0.003340	ABLIM1	actin binding lim protein 1
207849_at	3.91	0.001871	IL2	interleukin 2	204955_at	−1.40	0.003354	SRPX	sushi-repeat-containing protein, x-linked
202675_at	1.35	0.001876	SDHB	succinate dehydrogenase complex, subunit b, iron sulfur (ip)	202481_at	−1.31	0.003365	DHRS3	dehydrogenase/reductase (sdr family) member 3
202688_at	1.55	0.001887	TNFSF10	tumor necrosis factor (ligand) superfamily, member 10	205182_s_at	−1.86	0.003400	ZNF324	zinc finger protein 324

Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name	Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name
Up-Regulated Genes					**Down-Regulated Genes**				
204924_at	1.36	0.001944	TLR2	toll-like receptor 2	205683_x_at, 207741_x_at	−2.07	0.003403	TPSAB1	tryptase, alpha
201157_s_at	1.34	0.002035	NMT1	n-myristoyltransferase 1	207826_s_at	−1.69	0.003407	ID3	inhibitor of dna binding 3, dominant negative helix-loop-helix protein
209610_s_at	1.49	0.002047	SLC1A4	solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	221748_s_at	−1.41	0.003427	TNS1	tensin 1
210001_s_at	1.60	0.002354	SOCS1	suppressor of cytokine signaling 1	221210_s_at	−1.36	0.003450	NPL	n-acetylneuraminate pyruvate lyase (dihydrodipicolinate synthase)
33304_at	1.46	0.002364	ISG20	interferon stimulated exonuclease gene 20kda	204446_s_at	−1.45	0.003510	ALOX5	arachidonate 5-lipoxygenase
201625_s_at	1.57	0.002371	INSIG1	insulin induced gene 1	206368_at	−3.15	0.003566	CPLX2	complexin 2
203097_s_at	1.33	0.002475	RAPGEF2	rap guanine nucleotide exchange factor (gef) 2	206644_at	−2.17	0.003579	NR0B1	nuclear receptor subfamily 0, group b, member 1
204279_at	1.39	0.002485	PSMB9	proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2)	209782_s_at	−1.66	0.003585	DBP	d site of albumin promoter (albumin d-box) binding protein
204929_s_at	1.66	0.002546	VAMP5	vesicle-associated membrane protein 5 (myobrevin)	203803_at	−3.43	0.003661	PCYOX1	prenylcysteine oxidase 1
205159_at	1.40	0.002596	CSF2RB	colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)	205728_at	−2.76	0.003665	ODZ1	odz, odd oz/ten-m homolog 1(drosophila)
204033_at	1.61	0.002694	TRIP13	thyroid hormone receptor interactor 13	209355_s_at	−1.80	0.003724	PPAP2B	phosphatidic acid phosphatase type 2b
207500_at	2.31	0.002995	CASP5	caspase 5, apoptosis-related cysteine peptidase	202790_at	−2.17	0.003926	CLDN7	claudin 7
204103_at	1.47	0.002996	CCL4	chemokine (c-c motif) ligand 4	213553_x_at	−1.83	0.004021	APOC1	apolipoprotein c-i
205220_at	2.72	0.003027	GPR109B	g protein-coupled receptor 109b	220491_at	−1.57	0.004027	HAMP	hepcidin antimicrobial peptide
216834_at	2.24	0.003485	RGS1	regulator of g-protein signalling 1	204359_at	−1.83	0.004188	FLRT2	fibronectin leucine rich transmembrane protein 2
206745_at	1.80	0.003645	HOXC11	homeobox c11	217807_s_at	−1.48	0.004252	GLTSCR2	glioma tumor suppressor candidate region gene 2
204254_s_at	1.58	0.003671	VDR	vitamin d (1,25- dihydroxyvitamin d3) receptor	219952_s_at	−1.51	0.004269	MCOLN1	mucolipin 1
200054_at	1.34	0.003724	ZNF259	zinc finger protein 259	214369_s_at	−1.36	0.004357	RASGRP2	ras guanyl releasing protein 2 (calcium and dag-regulated)
202267_at	2.48	0.003954	LAMC2	laminin, gamma 2	209616_s_at	−1.96	0.004451	CES1	carboxylesterase 1 (monocyte/macrophage serine esterase 1)
207952_at	4.12	0.003994	IL5	interleukin 5 (colony-stimulating factor, eosinophil)	203167_at	−1.53	0.004531	TIMP2	timp metallopeptidase inhibitor 2
208450_at	3.03	0.004025	LGALS2	lectin, galactoside-binding, soluble, 2 (galectin 2)	218718_at	−1.74	0.004611	PDGFC	spinal cord-derived growth factor; secretory growth factor-like protein fallotein
218239_s_at	1.38	0.004091	GTPBP4	gtp binding protein 4	205222_at	−2.53	0.004683	EHHADH	enoyl-coenzyme a, hydratase/3-hydroxyacyl coenzyme a dehydrogenase
210511_s_at	5.01	0.004176	INHBA	inhibin, beta a (activin a, activin ab alpha polypeptide)	204394_at	−1.37	0.004684	SLC43A1	solute carrier family 43, member 1
210015_s_at	2.32	0.004275	MAP2	microtubule-associated protein 2	40569_at	−1.30	0.004743	ZNF42	zinc finger protein 42 (myeloid-specific retinoic acid-responsive)
201292_at	2.09	0.004316	TOP2A	topoisomerase (dna) ii alpha 170kda	219267_at	−1.41	0.004851	GLTP	glycolipid transfer protein
202847_at	1.30	0.004351	PCK2	phosphoenolpyruvate carboxykinase 2 (mitochondrial)	206339_at	−1.71	0.004861	CART	cocaine- and amphetamine-regulated transcript
206632_s_at	1.59	0.004372	APOBEC3B	apolipoprotein b mrna editing enzyme, catalytic polypeptide-like 3b	221364_at	−2.28	0.004933	GRID2	glutamate receptor, ionotropic, delta 2
208002_s_at	1.43	0.004425	ACOT7	acyl-coa thioesterase 7	214132_at	−1.36	0.004975	ATP5C1	atp synthase, h+ transporting, mitochondrial f1 complex, gamma polypeptide 1
202252_at	1.33	0.004492	RAB13	rab13, member ras oncogene family	202391_at	−1.37	0.005083	BASP1	brain abundant, membrane attached signal protein 1

Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name	Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name
Up-Regulated Genes					**Down-Regulated Genes**				
205242_at	3.37	0.004539	CXCL13	chemokine (c-x-c motif) ligand 13 (b-cell chemoattractant)	209990_s_at	−2.51	0.005271	GABBR2	gamma-aminobutyric acid (gaba) b receptor, 2
206513_at	1.37	0.004563	AIM2	absent in melanoma 2	207345_at	−1.94	0.005697	FST	follistatin
204580_at	2.69	0.004759	MMP12	matrix metallopeptidase 12 (macrophage elastase)	214743_at	−1.35	0.005728	CUTL1	cut-like 1, ccaat displacement protein (drosophila)
220865_s_at	1.37	0.004829	PDSS1	prenyl (decaprenyl) diphosphate synthase, subunit 1	213812_s_at	−1.38	0.006040	CAMKK2	calcium/calmodulin-dependent protein kinase kinase 2, beta
219761_at	2.14	0.004855	CLEC1A	c-type lectin domain family 1, member a	205486_at	−1.33	0.006059	TESK2	testis-specific kinase 2
206749_at	2.91	0.004909	CD1B	cd1b antigen	203136_at	−1.60	0.006081	RABAC1	rab acceptor 1 (prenylated)
201170_s_at	1.38	0.004946	BHLHB2	basic helix-loop-helix domain containing, class b, 2	204131_s_at	−1.51	0.006086	FOXO3A	forkhead box o3a
218866_s_at	1.42	0.004968	POLR3K	polymerase (rna) iii (dna directed) polypeptide k, 12.3 kda	209158_s_at	−1.35	0.006206	PSCD2	pleckstrin homology, sec7 and coiled-coil domains 2 (cytohesin–2)
201798_s_at	1.34	0.005042	FER1L3	fer-1-like 3, myoferlin (c. elegans)	221246_x_at, 221748_s_at	−1.42	0.006314	TNS1	tensin 1
200629_at	1.50	0.005046	WARS	interferon-induced protein 53	202108_at	−1.30	0.006356	PEPD	peptidase d
213415_at	1.90	0.005165	CLIC2	chloride intracellular channel 2	204360_s_at	−1.44	0.006377	NAGLU	n-acetylglucosaminidase, alpha- (sanfilippo disease iiib)
203275_at	1.32	0.005360	IRF2	interferon regulatory factor 2	217865_at	−1.32	0.006628	RNF130	ring finger protein 130
214512_s_at	1.38	0.005550	SUB1	sub1 homolog (s. cerevisiae)	219371_s_at	−1.53	0.006725	KLF2	kruppel-like factor 2 (lung)
206461_x_at	1.51	0.005684	MT1H	metallothionein 1h	202256_at	−1.46	0.007049	CD2BP2	cd2 antigen (cytoplasmic tail) binding protein 2
211333_s_at	1.48	0.005789	FASLG	fas ligand (tnf superfamily, member 6)	211145_x_at	−2.22	0.007309	IFNA21	interferon, alpha 21
210538_s_at	1.45	0.005790	BIRC3	baculoviral iap repeat-containing 3	221649_s_at	−1.35	0.007423	PPAN	peter pan homolog (drosophila)
204224_s_at	1.49	0.006013	GCH1	gtp cyclohydrolase 1 (dopa-responsive dystonia)	221378_at	−1.31	0.007478	CER1	cerberus 1, cysteine knot superfamily, homolog (xenopus laevis)
203564_at	1.52	0.006110	FANCG	fanconi anemia, complementation group g	206171_at	−1.51	0.007505	ADORA3	adenosine a3 receptor
207586_at	2.08	0.006245	SHH	sonic hedgehog homolog (drosophila)	205206_at	−1.72	0.007576	KAL1	kallmann syndrome 1 sequence
221463_at	2.63	0.006382	CCL24	chemokine (c-c motif) ligand 24	203414_at	−1.38	0.007979	MMD	monocyte to macrophage differentiation-associated
204748_at	2.60	0.006552	PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin g/h synthase and cyclooxygenase)	209541_at	−1.76	0.008232	IGF1	insulin-like growth factor 1 (somatomedin c)
202743_at	1.39	0.006584	PIK3R3	phosphoinositide-3-kinase, regulatory subunit 3 (p55, gamma)	221065_s_at	−1.81	0.008237	CHST8	carbohydrate (n-acetylgalactosamine 4−0) sulfotransferase 8
209785_s_at	1.35	0.006692	PLA2G4C	phospholipase a2, group ivc (cytosolic, calcium-independent)	208610_s_at	−1.41	0.008280	SRRM2	serine/arginine repetitive matrix 2
206975_at	3.99	0.006853	LTA	lymphotoxin alpha (tnf superfamily, member 1)	213592_at	−1.91	0.008299	AGTRL1	angiotensin ii receptor-like 1
202499_s_at	1.39	0.006958	SLC2A3	solute carrier family 2 (facilitated glucose transporter), member 3	201141_at	−1.44	0.008837	GPNMB	glycoprotein (transmembrane) nmb
201329_s_at	1.49	0.006989	ETS2	v-ets erythroblastosis virus e26 oncogene homolog 2 (avian)	220068_at	−2.11	0.009019	VPREB3	pre-b lymphocyte gene 3
204205_at	1.35	0.007064	APOBEC3G	apolipoprotein b mrna editing enzyme, catalytic polypeptide-like 3g	201186_at	−1.39	0.009581	LRPAP1	low density lipoprotein receptor-related protein associated protein 1
218039_at	1.35	0.007138	NUSAP1	nucleolar and spindle associated protein 1	204824_at	−1.56	0.009682	ENDOG	endonuclease g
214567_s_at	1.51	0.007306	XCL1 /// XCL2	chemokine (c motif) ligand 2, chemokine (c motif) ligand 1	220762_s_at	−1.46	0.009853	GNB1L	guanine nucleotide binding protein (g protein), beta polypeptide 1-like
206278_at	1.46	0.007320	PTAFR	platelet-activating factor receptor	209215_at	−1.33	0.009935	TETRAN	tetracycline transporter-like protein

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Up-Regulated Genes					**Down-Regulated Genes**				
204026_s_at	1.35	0.007406	ZWINT	zw10 interactor	219963_at	−1.60	0.010000	DUSP13	dual specificity phosphatase 13
211138_s_at	1.42	0.007457	KMO	kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)	202838_at	−2.05	0.010039	FUCA1	fucosidase, alpha-l- 1, tissue
201948_at	1.48	0.007689	GNL2	guanine nucleotide binding protein-like 2 (nucleolar)	202477_s_at	−1.36	0.010213	TUBGCP2	tubulin, gamma complex associated protein 2
202284_s_at	1.51	0.007786	CDKN1A	cyclin-dependent kinase inhibitor 1a (p21, cip1)	206739_at	−2.24	0.010378	HOXC5	homeobox c5
202357_s_at	1.82	0.007826	CFB	complement factor b	203113_s_at	−1.53	0.010380	EEF1D	eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein)
214022_s_at	1.35	0.007980	IFITM1	interferon induced transmembrane protein 1 (9–27)	210123_s_at	−2.12	0.010514	CHRNA7 /// CHRFAM7A /// LOC652740	cholinergic receptor, nicotinic, alpha 7
203975_s_at	1.54	0.008000	CHAF1A	chromatin assembly factor 1, subunit a (p150)	32837_at	−1.34	0.010565	AGPAT2	1-acylglycerol-3-phosphate o-acyltransferase 2 (lysophosphatidic acid acyltransferase, beta)
206547_s_at	2.70	0.008057	PPEF1	protein phosphatase, ef-hand calcium binding domain 1	217969_at	−1.34	0.010748	C11orf2	chromosome 11 open reading frame2
208075_s_at	2.35	0.008074	CCL7	chemokine (c-c motif) ligand 7	204862_s_at	−1.38	0.010803	NME3	non-metastatic cells 3, protein expressed in
206508_at	2.28	0.008142	TNFSF7	tumor necrosis factor (ligand) superfamily, member 7	210205_at	−1.35	0.010859	B3GALT4	udp-gal:betaglcnac beta 1,3-galactosyltransferase, polypeptide 4
35150_at	1.63	0.008147	CD40	cd40 antigen (tnf receptor superfamily member 5)	203980_at	−1.83	0.011023	FABP4	fatty acid binding protein 4, adipocyte
204070_at	1.33	0.008335	RARRES3	retinoic acid receptor responder (tazarotene induced) 3	202201_at	−1.44	0.011165	BLVRB	biliverdin reductase b (flavin reductase (nadph))
204440_at	1.31	0.008599	CD83	cd83 antigen (activated b lymphocytes, immunoglobulin superfamily)	217983_s_at	−1.53	0.011188	RNASET2	ribonuclease t2
209392_at	1.57	0.008806	ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)	209236_at	−1.34	0.011251	SLC23A2	solute carrier family 23 (nucleobase transporters), member 2
214933_at	1.36	0.008923	CACNA1A	calcium channel, voltage-dependent, p/q type, alpha 1a subunit	221116_at	−2.18	0.011341	PPARL	pparl
205266_at	2.00	0.009015	LIF	leukemia inhibitory factor (cholinergic differentiation factor)	207839_s_at	−1.33	0.012331	C9orf127	chromosome 9 open reading frame 127
201614_s_at	1.35	0.009016	RUVBL1	ruvb-like 1 (e. coli)	201820_at	−2.00	0.012345	KRT5	keratin 4
218662_s_at	1.65	0.009357	HCAP-G	chromosome condensation protein g	221830_at	−1.38	0.012420	RAP2A	rap2a, member of ras oncogene family
220665_at	2.32	0.009580	LUZP4	leucine zipper protein 4	204749_at	−2.44	0.012608	NAP1L3	nucleosome assembly protein 1-like 3
205570_at	1.31	0.009613	PIP5K2A	phosphatidylinositol-4-phosphate 5-kinase, type ii, alpha	219264_s_at	−1.60	0.012642	PPP2R3B	protein phosphatase 2 (formerly 2a), regulatory subunit b", beta
217892_s_at	1.31	0.009635	LIMA1	lim domain and actin binding 1	204595_s_at	−1.33	0.012951	STC1	stanniocalcin 1
204444_at	1.62	0.009826	KIF11	kinesin family member 11	208013_s_at	−1.42	0.013342	ACRV1	acrosomal vesicle protein 1
201263_at	1.35	0.009908	TARS	threonyl-trna synthetase	200665_s_at	−1.82	0.013513	SPARC	secreted protein, acidic, cysteine-rich (osteonectin)
202068_s_at	1.33	0.010149	LDLR	low density lipoprotein receptor (familial hypercholesterolemia)	207643_s_at	−1.32	0.013568	TNFRSF1A	tumor necrosis factor receptor superfamily, member 1a
212378_at	1.32	0.010160	GART	phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase	203126_at	−1.34	0.013930	IMPA2	inositol(myo)-1(or 4)-monophosphatase 2
204959_at	1.31	0.010219	MNDA	myeloid cell nuclear differentiation antigen	221666_s_at	−1.31	0.013942	PYCARD	pyd and card domain containing

Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name	Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name
Up-Regulated Genes					**Down-Regulated Genes**				
218350_s_at	1.30	0.010640	GMNN	geminin, dna replication inhibitor	217996_at	−1.44	0.014560	PHLDA1	pleckstrin homology-like domain, family a, member 1
219255_x_at	1.68	0.010644	IL17RB	interleukin 17 receptor b	219403_s_at	−1.44	0.014588	HPSE	heparanase
205476_at	2.47	0.011419	CCL20	chemokine (c-c motif) ligand 20	61874_at	−1.52	0.014677	C9orf7	chromosome 9 open reading frame 7
208892_s_at	1.38	0.011479	DUSP6	dual specificity phosphatase 6	208130_s_at	−1.78	0.014684	TBXAS1	thromboxane a synthase 1 (platelet, cytochrome p450, family 5, subfamily a)
204023_at	1.31	0.011734	RFC4	replication factor c (activator 1) 4, 37kda	200785_s_at	−1.47	0.014746	LRP1	low density lipoprotein-related protein 1 (alpha-2-macroglobulin receptor)
220957_at	1.33	0.012024	CTAGE1	cutaneous t-cell lymphoma-associated antigen 1	207117_at	−2.16	0.014850	H-plk	krueppel-related zinc finger protein
203105_s_at	1.34	0.012173	DNM1L	dynamin 1-like	206028_s_at	−1.50	0.014914	MERTK	c-mer proto-oncogene tyrosine kinase
220658_s_at	1.56	0.012531	ARNTL2	aryl hydrocarbon receptor nuclear translocator-like 2	209354_at	−1.32	0.014997	TNFRSF14	tumor necrosis factor receptor superfamily, member 14 (herpesvirus entry mediator)
204998_s_at	1.34	0.012554	ATF5	activating transcription factor 5	201064_s_at	−1.32	0.015387	PABPC4	poly(a) binding protein, cytoplasmic 4 (inducible form)
204170_s_at	1.44	0.013296	CKS2	cdc28 protein kinase regulatory subunit 2	204857_at	−1.39	0.015779	MAD1L1	mad1 mitotic arrest deficient-like 1 (yeast)
209726_at	1.56	0.013322	CA11	carbonic anhydrase xi	221287_at	−1.84	0.015826	RNASEL	ribonuclease l (2',5'-oligoisoadenylate synthetase-dependent)
202069_s_at	1.38	0.013436	IDH3A	isocitrate dehydrogenase 3 (nad+) alpha	209185_s_at	−1.38	0.016378	IRS2	insulin receptor substrate 2
204531_s_at	1.82	0.013582	BRCA1	breast cancer 1, early onset	220727_at	−1.49	0.016475	KCNK10	potassium channel, subfamily k, member 10
221209_s_at	1.90	0.013776	OTOR	otoraplin	201785_at	−1.97	0.016745	RNASE1	ribonuclease, rnase a family, 1 (pancreatic)
201315_x_at	1.34	0.014191	IFITM2	interferon induced transmembrane protein 2 (1–8d)	209458_x_at, 214414_x_at	−1.52	0.017735	HBA1 /// HBA2	hemoglobin, alpha 1
208881_x_at	1.33	0.014408	IDI1	isopentenyl-diphosphate delta isomerase 1	201876_at	−1.61	0.017903	PON2	paraoxonase 2
200886_s_at	1.30	0.014546	PGAM1 /// LOC642969 /// LOC643576	phosphoglycerate mutase 1 (brain)	216924_s_at	−1.97	0.018447	DRD2	dopamine receptor d2
203350_at	1.42	0.014818	AP1G1	adaptor-related protein complex 1, gamma 1 subunit	206196_s_at	−1.81	0.018469	RPIP8	rap2 interacting protein 8
204015_s_at	2.04	0.014931	DUSP4	dual specificity phosphatase 4	207922_s_at	−1.31	0.018875	MAEA	macrophage erythroblast attacher
203805_s_at	1.52	0.015213	FANCA	fanconi anemia, complementation group a	205911_at	−1.69	0.018983	PTHR1	parathyroid hormone receptor 1
203502_at	1.38	0.015219	BPGM	2,3-bisphosphoglycerate mutase	203910_at	−1.31	0.019419	ARHGAP29	rho gtpase activating protein 29
206254_at	1.76	0.015446	EGF	epidermal growth factor (beta-urogastrone)	204592_at	−1.49	0.019475	DLG4	discs, large homolog 4 (drosophila)
206096_at	1.39	0.015587	ZNF35	zinc finger protein 35 (clone hf.10)	205744_at	−2.07	0.019498	DOC2A	double c2-like domains, alpha
207904_s_at	2.23	0.015741	LNPEP	leucyl/cystinyl aminopeptidase	206623_at	−2.16	0.019526	PDE6A	phosphodiesterase 6a, cgmp-specific, rod, alpha
208393_s_at	1.31	0.015769	RAD50	rad50 homolog (s. cerevisiae)	212191_x_at	−1.37	0.019681	RPL13	ribosomal protein l13
203276_at	1.49	0.016097	LMNB1	lamin b1	204561_x_at	−1.44	0.019846	APOC2	apolipoprotein c-ii
206561_s_at	2.20	0.016231	AKR1B10	aldo-keto reductase family 1, member b11 (aldose reductase-like)	201212_at	−1.58	0.019878	LGMN	legumain
210176_at	1.38	0.016450	TLR1	toll-like receptor 1	202152_x_at	−1.38	0.020121	USF2	upstream transcription factor 2, c-fos interacting
219148_at	1.39	0.016529	PBK	pdz binding kinase	208982_at	−1.35	0.020229	PECAM1	platelet/endothelial cell adhesion molecule (cd31 antigen)
210367_s_at	1.40	0.016595	PTGES	prostaglandin e synthase	206816_s_at	−1.83	0.020456	SPAG8	sperm associated antigen 8

Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name	Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name
Up-Regulated Genes					**Down-Regulated Genes**				
205500_at	1.63	0.016907	C5	complement component 5	221123_x_at	−1.30	0.021205	ZNF395	hypothetical protein dkfzp434k1210
210007_s_at	1.45	0.017219	GPD2	glycerol-3-phosphate dehydrogenase 2 (mitochondrial)	220066_at	−1.33	0.021232	CARD15	caspase recruitment domain family, member 15
208204_s_at	1.83	0.017264	CAV3	caveolin 3	219752_at	−1.60	0.021342	RASAL1	ras protein activator like 1 (gap1 like)
205403_at	2.46	0.017286	IL1R2	interleukin 1 receptor, type ii	216860_s_at	−1.77	0.021418	GDF11	growth differentiation factor 11
218854_at	1.47	0.017922	SART2	squamous cell carcinoma antigen recognized by t cells 2	209030_s_at	−1.38	0.021664	IGSF4	immunoglobulin superfamily, member 4
210072_at	1.82	0.017991	CCL19	chemokine (c-c motif) ligand 19	202187_s_at	−1.36	0.021770	PPP2R5A	protein phosphatase 2, regulatory subunit b (b56), alpha isoform
203454_s_at	1.34	0.018216	ATOX1	atx1 antioxidant protein 1 homolog (yeast)	207421_at	−2.30	0.022313	CA5A	carbonic anhydrase va, mitochondrial
219866_at	2.10	0.018296	CLIC5	chloride intracellular channel 5	205131_x_at	−2.05	0.023117	CLEC11A	c-type lectin domain family 11, member a
214279_s_at	2.24	0.018713	NDRG2	ndrg family member 2	205498_at	−2.27	0.023139	GHR	growth hormone receptor
206682_at	1.46	0.018849	CLEC10A	c-type lectin domain family 10, member a	207741_x_at	−1.45	0.023415	TPSAB1 /// TPSB2 /// LOC652751	tryptase beta 2
209714_s_at	1.55	0.019322	CDKN3	cyclin-dependent kinase inhibitor 3 (cdk2-associated dual specificity phosphatase)	213213_at	−1.44	0.023500	DIDO1	death inducer-obliterator 1
202518_at	1.30	0.019575	BCL7B	b-cell cll/lymphoma 7b	219799_s_at	−1.42	0.023653	DHRS9	dehydrogenase/reductase (sdr family) member 9
211367_s_at	1.39	0.019698	CASP1	caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	220528_at	−2.33	0.023827	VNN3	vanin 3
212671_s_at	1.40	0.020228	HLA-DQA1 /// HLA-DQA2 /// LOC650946	major histocompatibility complex, class ii, dq alpha 1	219607_s_at	−1.86	0.024188	MS4A4A	membrane-spanning 4-domains, subfamily a, member 4
203409_at	1.56	0.020373	DDB2	lim homeobox 3, damage-specific dna binding protein 2, 48kda	208472_at	−1.42	0.024357	ZNFN1A4	zinc finger protein, subfamily 1a, 4 (eos)
200986_at	1.36	0.021457	SERPING1	serpin peptidase inhibitor, clade g (c1 inhibitor), member 1, (angioedema, hereditary)	214636_at	−1.55	0.024501	CALCB	calcitonin-related polypeptide, beta
220042_x_at	2.32	0.021477	HIVEP3	human immunodeficiency virus type i enhancer binding protein 3	203973_s_at	−1.40	0.024666	CEBPD	ccaat/enhancer binding protein (c/ebp), delta
204408_at	1.41	0.021502	APEX2	apex nuclease (apurinic/apyrimidinic endonuclease) 2	202812_at	−1.35	0.024799	GAA	glucosidase, alpha; acid (pompe disease, glycogen storage disease type ii)
218036_x_at	1.30	0.021537	NMD3	nmd3 homolog (s. cerevisiae)	204042_at	−1.32	0.025662	WASF3	was protein family, member 3
221034_s_at	1.44	0.021674	TEX13B	testis expressed sequence 13b	202450_s_at	−1.34	0.025802	CTSK	cathepsin k (pycnodysostosis)
205598_at	1.90	0.021893	TRAIP	traf interacting protein	201050_at	−1.43	0.025854	PLD3	phospholipase d family, member 3
205347_s_at	1.39	0.021921	TMSL8	thymosin-like 8	209978_s_at	−2.28	0.025937	LPA /// PLG	plasminogen, lipoprotein, lp(a)
220386_s_at	1.43	0.022175	EML4	echinoderm microtubule associated protein like 4	201753_s_at	−1.35	0.026385	ADD3	adducin 3 (gamma)
217738_at	1.35	0.022376	PBEF1 /// LOC646309 /// RP11-92J19.4	pre-b-cell colony enhancing factor 1	219892_at	−1.43	0.026459	TM6SF1	transmembrane 6 superfamily member 1
207662_at	1.64	0.023056	TBX1	t-box 1	205844_at	−1.74	0.026498	VNN1	vanin 1
203362_s_at	1.41	0.023308	MAD2L1	mad2 mitotic arrest deficient-like 1 (yeast)	207582_at	−2.12	0.027216	PIN1L	protein (peptidylprolyl cis/trans isomerase) nima-interacting 1-like

Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name	Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name
Up-Regulated Genes					**Down-Regulated Genes**				
206325_at	1.83	0.023449	SERPINA6	serpin peptidase inhibitor, clade a (alpha-1 antiproteinase, antitrypsin), member 6	203729_at	−1.41	0.027633	EMP3	epithelial membrane protein 3
220938_s_at	1.59	0.023571	GMEB1	glucocorticoid modulatory element binding protein 1	204079_at	−1.30	0.027635	TPST2	tyrosylprotein sulfotransferase 2
201202_at	1.40	0.023620	PCNA	proliferating cell nuclear antigen	208511_at	−2.68	0.027688	PTTG3	pituitary tumor-transforming 3
201507_at	1.38	0.023999	PFDN1	prefoldin subunit 1	209661_at	−1.90	0.027937	KIFC3	kinesin family member c3
37950_at	1.36	0.024052	PREP	prolyl endopeptidase	206106_at	−1.63	0.028087	MAPK12	mitogen-activated protein kinase 12
204835_at	1.66	0.024112	POLA	polymerase (dna directed), alpha	203028_s_at	−1.48	0.028274	CYBA	cytochrome b-245, alpha polypeptide
202780_at	1.40	0.024117	OXCT1	3-oxoacid coa transferase 1	219440_at	−2.54	0.028291	RAI2	retinoic acid induced 2
219424_at	1.97	0.024648	EBI3	epstein-barr virus induced gene 3	203996_s_at	−1.71	0.028734	C21orf2	chromosome 21 open reading frame 2
204769_s_at	1.35	0.025000	TAP2	transporter 2, atp-binding cassette, sub-family b (mdr/tap)	221061_at	−2.63	0.029166	PKD2L1	polycystic kidney disease 2-like 1
210119_at	2.62	0.026057	KCNJ15	potassium inwardly-rectifying channel, subfamily j, member 15	204367_at	−1.30	0.029203	SP2	sp2 transcription factor
207143_at	1.93	0.026156	CDK6	cyclin-dependent kinase 6	213436_at	−1.73	0.029349	CNR1	cannabinoid receptor 1 (brain)
218219_s_at	1.38	0.026250	LANCL2	lanc lantibiotic synthetase component c-like 2 (bacterial)	38157_at	−1.64	0.030123	DOM3Z	dom-3 homolog z (c. elegans)
219645_at	1.38	0.026851	CASQ1	calsequestrin 1 (fast-twitch, skeletal muscle)	210423_s_at	−1.56	0.030184	SLC11A1	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1
201710_at	1.85	0.027145	MYBL2	v-myb myeloblastosis viral oncogene homolog (avian)-like 2	201911_s_at	−2.04	0.031248	FARP1	ferm, rhogef (arhgef) and pleckstrin domain protein 1 (chondrocyte-derived)
203859_s_at	1.49	0.027287	PALM	paralemmin	220782_x_at	−1.82	0.031968	KLK12	kallikrein 12
204297_at	1.32	0.027612	PIK3C3	phosphoinositide-3-kinase, class 3	207482_at	−1.62	0.032061	C20orf10	chromosome 20 open reading frame 10
219971_at	1.37	0.028335	IL21R	interleukin 21 receptor	204697_s_at	−2.06	0.032591	CHGA	chromogranin a (parathyroid secretory protein 1)
211297_s_at	1.37	0.028415	CDK7	cyclin-dependent kinase 7 (mo15 homolog, xenopus laevis, cdk-activating kinase)	206873_at	−1.68	0.032847	CA6	carbonic anhydrase vi
204126_s_at	2.09	0.028496	CDC45L	cdc45 cell division cycle 45-like (s. cerevisiae)	207914_x_at	−1.54	0.033022	EVX1	eve, even-skipped homeobox homolog 1 (drosophila)
207455_at	1.46	0.028568	P2RY1	purinergic receptor p2y, g-protein coupled, 1	218206_x_at	−1.37	0.033390	SCAND1	scan domain containing 1
206765_at	1.33	0.028640	KCNJ2	potassium inwardly-rectifying channel, subfamily j, member 2	217975_at	−1.50	0.034679	WBP5	ww domain binding protein 5
206175_x_at	2.21	0.028671	ZNF222	zinc finger protein 222	219327_s_at	−1.60	0.035562	GPRC5C	g protein-coupled receptor, family c, group 5, member c
206173_x_at	2.11	0.028761	GABPB2	ga binding protein transcription factor, beta subunit 1, 53kda	206206_at	−1.39	0.035730	CD180	cd180 antigen
205463_s_at	1.84	0.029164	PDGFA	platelet-derived growth factor alpha polypeptide	222239_s_at	−1.31	0.035916	INTS6	dkfzp434b105 protein
206407_s_at	2.20	0.029186	CCL13	chemokine (c-c motif) ligand 13	220189_s_at	−1.44	0.036040	MGAT4B	mannosyl (alpha-1,3-)-glycoprotein beta-1,4-n-acetylglucosaminyltransferase, isozyme b
210772_at	1.37	0.029514	FPRL1	formyl peptide receptor-like 1	203476_at	−1.59	0.036505	TPBG	trophoblast glycoprotein
211200_s_at	2.47	0.029575	EFCAB2	ef-hand calcium binding domain 2	221005_s_at	−1.55	0.037185	PTDSS2	phosphatidylserine synthase 2
203592_s_at	1.54	0.029679	FSTL3	follistatin-like 3 (secreted glycoprotein)	209822_s_at	−1.37	0.037473	VLDLR	very low density lipoprotein receptor
203052_at	1.39	0.029744	C2	complement component 2	205638_at	−1.71	0.037898	BAI3	brain-specific angiogenesis inhibitor 3

Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name	Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name
Up-Regulated Genes					**Down-Regulated Genes**				
211499_s_at	1.67	0.029863	MAPK11	mitogen-activated protein kinase 11	220777_at	−1.74	0.038565	KIF13A	kinesin family member 13a
204162_at	1.99	0.030006	KNTC2	kinetochore associated 2	210139_s_at	−1.33	0.038607	PMP22	peripheral myelin protein 22
201737_s_at	1.35	0.030278	MARCH6	membrane-associated ring finger (c3hc4) 6	221324_at	−1.32	0.038629	TAS2R1	taste receptor, type 2, member 1
210549_s_at	2.57	0.030537	CCL23	chemokine (c-c motif) ligand 23	210884_s_at	−1.62	0.039295	LOC653423	sperm associated antigen 11
218931_at	1.90	0.030914	RAB17	rab17, member ras oncogene family	207739_s_at	−2.22	0.039456	GAGE1 /// GAGE2 /// GAGE3 /// GAGE4 /// GAGE5 /// GAGE6 /// GAGE7 /// GAGE7B /// GAGE8 /// LOC645009 /// LOC645037 /// LOC645073 /// LOC645093	g antigen 1, g antigen 3, g antigen 4, g antigen 2, g antigen 5, g antigen 8, g antigen 6
204304_s_at	1.48	0.030950	PROM1	prominin 1	209737_at	−1.42	0.039512	MAGI2	membrane associated guanylate kinase, ww and pdz domain containing 2
205450_at	2.53	0.030967	PHKA1	phosphorylase kinase, alpha 1 (muscle)	32094_at	−1.72	0.039607	CHST3	carbohydrate (chondroitin 6) sulfotransferase 3
204504_s_at	1.55	0.031187	HIRIP3	hira interacting protein 3	41660_at	−1.79	0.039999	CELSR1	cadherin, egf lag seven-pass g-type receptor 1 (flamingo homolog, drosophila)
209890_at	1.38	0.031529	TSPAN5	tetraspanin 5	206610_s_at	−1.92	0.040448	F11	coagulation factor xi (plasma thromboplastin antecedent)
34187_at	1.89	0.031861	RBMS2	rna binding motif, single stranded interacting protein 2	209988_s_at	−1.57	0.040611	ASCL1	achaete-scute complex-like 1 (drosophila)
218943_s_at	1.58	0.032067	DDX58	dead (asp-glu-ala-asp) box polypeptide 58	219922_s_at	−1.47	0.040966	LTBP3	latent transforming growth factor beta binding protein 3
202240_at	1.53	0.032437	PLK1	polo-like kinase 1 (drosophila)	205054_at	−1.78	0.041014	NEB	nebulin
205716_at	1.39	0.033296	MCFP	mitochondrial carrier family protein	206404_at	−1.45	0.041028	FGF9	fibroblast growth factor 9 (glia-activating factor)
208005_at	1.69	0.033662	NTN1	netrin 1	203305_at	−1.59	0.041286	F13A1	coagulation factor xiii, a1 polypeptide
220059_at	1.53	0.033861	BRDG1	bcr downstream signaling 1	201656_at	−1.38	0.041995	ITGA6	integrin, alpha 6
204767_s_at	1.39	0.034527	FEN1	flap structure-specific endonuclease 1	205384_at	−1.96	0.042828	FXYD1	fxyd domain containing ion transport regulator 1 (phospholemman)
210280_at	2.18	0.034875	MPZ	myelin protein zero (charcot-marie-tooth neuropathy 1b)	203618_at	−1.34	0.043195	FAIM2	fas apoptotic inhibitory molecule 2
213492_at	1.64	0.034876	COL2A1	collagen, type ii, alpha 1 (primary osteoarthritis, spondyloepiphyseal dysplasia, congenital)	210117_at	−2.03	0.044321	SPAG1	sperm associated antigen 1
212020_s_at	1.51	0.035187	MKI67	antigen identified by monoclonal antibody ki-67	206498_at	−1.85	0.044628	OCA2	oculocutaneous albinism ii (pink-eye dilution homolog, mouse)
206247_at	1.32	0.035337	MICB	mhc class i polypeptide-related sequence b	202621_at	−1.41	0.045125	IRF3	interferon regulatory factor 3
212657_s_at	1.61	0.035551	IL1RN	interleukin 1 receptor antagonist	205881_at	−2.10	0.045147	ZNF74	zinc finger protein 74 (cos52)

Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name	Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name
Up-Regulated Genes					**Down-Regulated Genes**				
215171_s_at	1.31	0.035701	TIMM17A	translocase of inner mitochondrial membrane 17 homolog a (yeast)	204449_at	−1.40	0.045296	PDCL	phosducin-like
210048_at	1.31	0.036177	NAPG	n-ethylmaleimide-sensitive factor attachment protein, gamma	209657_s_at	−1.38	0.045366	HSF2	heat shock transcription factor 2
221507_at	1.35	0.036194	TNPO2	transportin 2 (importin 3, karyopherin beta 2b)	208000_at	−1.41	0.045916	GML	gpi anchored molecule like protein
208118_x_at	1.31	0.036247	LAT1-3TM /// IMAA /// LOC440345 /// LOC440354 /// LOC595101 /// LOC641298 /// LOC646866	slc7a5 pseudogene, hypothetical gene loc283846, kiaa0220-like protein	206251_s_at	−2.18	0.046131	AVPR1A	arginine vasopressin receptor 1a
219125_s_at	1.35	0.036524	RAG1AP1	recombination activating gene 1 activating protein 1	207503_at	−2.22	0.046146	TCP10	t-complex 10 (mouse)
204092_s_at	1.49	0.037044	AURKA	aurora kinase a	202191_s_at	−1.33	0.046843	GAS7	growth arrest-specific 7
202627_s_at	1.79	0.037267	SERPINE1	serpin peptidase inhibitor, clade e (nexin, plasminogen activator inhibitor type 1), member 1	205200_at	−1.72	0.047002	CLEC3B	c-type lectin domain family 3, member b
208478_s_at	1.51	0.037303	BAX	bcl2-associated x protein	202178_at	−1.88	0.047241	PRKCZ	protein kinase c, zeta
207473_at	1.40	0.037435	MLN	motilin	217871_s_at	−1.31	0.047313	MIF	macrophage migration inhibitory factor (glycosylation-inhibiting factor)
207329_at	2.09	0.037908	MMP8	matrix metallopeptidase 8 (neutrophil collagenase)	221309_at	−1.90	0.047786	RBM17	rna binding motif protein 17
219199_at	1.64	0.037979	AFF4	af4/fmr2 family, member 4	205050_s_at	−1.54	0.047840	MAPK8IP2	mitogen-activated protein kinase 8 interacting protein 2
38037_at	1.51	0.038257	HBEGF	heparin-binding egf-like growth factor	203643_at	−1.55	0.048239	ERF	ets2 repressor factor
33767_at	1.39	0.038470	NEFH	neurofilament, heavy polypeptide 200kda	203422_at	−1.33	0.049132	POLD1	polymerase (dna directed), delta 1, catalytic subunit 125kda
207882_at	1.38	0.038622	HSAJ2425	p65 protein	204811_s_at	−1.35	0.049138	CACNA2D2	calcium channel, voltage-dependent, alpha 2/delta subunit 2
204864_s_at	1.55	0.038725	IL6ST	interleukin 6 signal transducer (gp130, oncostatin m receptor)	209264_s_at	−1.54	0.049162	TSPAN4	tetraspanin 4
204668_at	1.39	0.039002	RNF24	ring finger protein 24	207929_at	−1.44	0.049398	GRPR	gastrin-releasing peptide receptor
207016_s_at	1.50	0.039529	ALDH1A2	aldehyde dehydrogenase 1 family, member a2	219714_s_at	−1.90	0.049494	CACNA2D3	calcium channel, voltage-dependent, alpha 2/delta 3 subunit
207346_at	2.21	0.039649	STX2	epimorphin					
32128_at	1.50	0.039852	CCL18	chemokine (c-c motif) ligand 18 (pulmonary and activation-regulated)					
206932_at	1.67	0.040074	CH25H	cholesterol 25-hydroxylase					
205225_at	1.33	0.040378	ESR1	estrogen receptor 1					
202416_at	1.32	0.040835	DNAJC7	dnaj (hsp40) homolog, subfamily c, member 7					
220315_at	1.36	0.041177	PARP11	poly (adp-ribose) polymerase family, member 11					
206999_at	2.28	0.041871	IL12RB2	interleukin 12 receptor, beta 2					
220993_s_at	2.19	0.042266	GPR63	g protein-coupled receptor 63					
221170_at	1.60	0.042356	HRH4	histamine receptor h4					
207845_s_at	1.39	0.042369	ANAPC10	anaphase promoting complex subunit 10					
204401_at	1.40	0.042558	KCNN4	potassium intermediate/small conductance calcium-activated channel, subfamily n, member 4					

Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name	Affy ID	F.C. post vs. pre	p value	Gene Symbol	Gene Name
Up-Regulated Genes					**Down-Regulated Genes**				
207124_s_at	1.53	0.042696	GNB5	guanine nucleotide binding protein (g protein), beta 5					
219187_at	1.85	0.042908	FKBPL	fk506 binding protein like					
203952_at	1.76	0.042917	ATF6	activating transcription factor 6					
36742_at	1.57	0.043363	TRIM15	tripartite motif-containing 15					
218117_at	1.35	0.043508	RBX1	ring-box 1					
208592_s_at	1.49	0.043731	CD1E	cd1e antigen, e polypeptide					
221106_at	1.98	0.044473	SLC22A17	solute carrier family 22 (organic cation transporter), member 17					
207275_s_at	1.34	0.044487	ACSL1	fatty-acid-coenzyme a ligase, long-chain 1					
202403_s_at	2.21	0.044527	COL1A2	collagen, type i, alpha 2					
220633_s_at	1.30	0.044556	HP1BP3	heterochromatin protein 1, binding protein 3					
208916_at	1.36	0.044687	SLC1A5	solute carrier family 1 (neutral amino acid transporter), member 5					
203881_s_at	1.76	0.045235	DMD	dystrophin (muscular dystrophy, duchenne and becker types)					
203948_s_at	1.89	0.045239	MPO	myeloperoxidase					
203968_s_at	1.48	0.045435	CDC6	cdc6 cell division cycle 6 homolog (s. cerevisiae)					
209546_s_at	1.46	0.045614	APOL1	apolipoprotein l, 1					
217847_s_at	2.47	0.045686	THRAP3	thyroid hormone receptor associated protein 3					
206976_s_at	1.31	0.046433	HSPH1	heat shock 105kda/110kda protein 1					
210133_at	1.87	0.046451	CCL11	chemokine (c-c motif) ligand 11					
219540_at	1.31	0.046489	ZNF267	zinc finger protein 267					
203287_at	1.33	0.046497	LAD1	ladinin 1					
220091_at	1.49	0.047676	SLC2A6	solute carrier family 2 (facilitated glucose transporter), member 6					
213800_at	2.30	0.047921	CFH	complement factor h					
200793_s_at	1.37	0.048667	ACO2	aconitase 2, mitochondrial					
202732_at	1.34	0.048888	PKIG	protein kinase (camp-dependent, catalytic) inhibitor gamma					
39402_at	1.56	0.049565	IL1B	interleukin 1, beta					

[*] DE= differentially expressed

F.C.= fold change post versus pre vaccination

Table IV

Spearman Correlation of Gene Expression with Neutralizing Antibody Levels*

Affy ID	Gene Symbol	Gene name	Fold Change#	p-value-Microarray	Spearman's r		
					Month 2	Month 7**	Month 12**
200953_s_at	CCND2	cyclin d2	1.42	0.000378	**	0.88	0.82
208450_at	LGALS2	lectin, galactoside-binding, soluble, 2 (galectin 2)	3.03	0.0040252	**	0.86	0.74
212657_s_at	IL1RN	interleukin 1 receptor antagonist	1.61	0.035551	**	0.75	0.73
206749_at	CD1B	cd1b antigen	2.91	0.0049094	**	0.68	0.73
203564_at	FANCG	fanconi anemia, complementation group g	1.52	0.006110	**	0.68	0.65
221287_at	RNASEL	ribonuclease l (2',5'-oligoisoadenylate synthetase-dependent)	−1.84	0.0158257	**	0.64	0.84
206181_at	SLAMF1	signaling lymphocytic activation molecule family member 1	1.42	0.0004684	**	0.64	0.60
205638_at	BAI3	brain-specific angiogenesis inhibitor 3	−1.71	0.037898	**	0.63	0.71
205633_s_at	ALAS1	aminolevulinate, delta-, synthase 1	1.60	0.0005912	**	0.61	0.70
204092_s_at	AURKA	aurora kinase a	1.49	0.037044	**	0.61	0.63
204504_s_at	HIRIP3	hira interacting protein 3	1.55	0.031187	**	0.60	0.81
206206_at	CD180	cd180 antigen	−1.39	0.0357298	**	−0.61	−0.66
203126_at	IMPA2	inositol(myo)-1(or 4)-monophosphatase 2	−1.34	0.013930	**	−0.62	−0.62
34187_at	RBMS2	rna binding motif, single stranded interacting protein 2	1.89	0.0318612	**	−0.62	−0.65
202838_at	FUCA1	fucosidase, alpha-l- 1, tissue	−2.05	0.010039	**	−0.64	−0.76
201753_s_at	ADD3	adducin 3 (gamma)	−1.35	0.026385	**	−0.64	−0.64
209774_x_at	CXCL2	chemokine (c-x-c motif) ligand 2	−1.76	0.0020535	**	−0.69	−0.61
219697_at	HS3ST2	heparan sulfate (glucosamine) 3-o-sulfotransferase 2	−2.64	0.0002601	**	−0.69	−0.62
204394_at	SLC43A1	solute carrier family 43, member 1	−1.37	0.004684	**	−0.69	−0.64
221558_s_at	LEF1	lymphoid enhancer-binding factor 1	−1.36	0.0000261	**	−0.76	−0.69
212552_at	HPCAL1	hippocalcin-like 1	−1.32	0.0029172	**	−0.76	−0.75
201820_at	KRT5	keratin 4	−2.00	0.012345	**	−0.90	−0.73

[*] Neutralizing antibody titers [Median (range)]: Month 0=0 (0–0); Month 2=664 (78–4949); Month 7=2401 (111–25571); Month 12=986 (148–15316)

[**] Only probe sets with correlation coefficient values of r≥0.60 or r≤−0.60 at at both Month 7 and Month 12 are shown (p≤0.05 in all cases).

[#] (VLP-Media) expression levels as defined in the Materials and Methods Section

Table V

Confirmation of Microarray Results with Real-Time PCR in an independent sample (n=9)

Gene	Gene Name	Frequency (>2-fold)		Mean Fold Change		Mean Fold Change-Only Responders		p-value-Microarray
		Microarray	RT-PCR	Microarray	RT-PCR	Microarray	RT-PCR	
GZMB	granzyme b	12 of 18 (67%)	7 of 9 (78%)	2.3	3.0	3.0	3.6	0.00002
IFNG	interferon, gamma	12 of 18 (67%)	5 of 8 (62%)	2.6	5.0	4.0	7.0	0.00002
HSD11B1	hydroxysteroid (11-beta) dehydrogenase 1	15 of 18 (83%)	7 of 8 (88%)	3.5	4.0	4.8	4.4	0.00005
INDO	indoleamine-pyrrole 2,3 dioxygenase	11 of 18 (61%)	5 of 9 (56%)	2.5	2.4	3.5	3.5	0.00006
IL6	interleukin 6	9 of 18 (50%)	6 of 8 (75%)	2.2	4.5	3.5	5.7	0.00030
IL3	interleukin 3 (colony-stimulating factor, multiple)	11 of 18 (61%)	5 of 9 (56%)	4.0	2.9	9.3	4.2	0.00030
CSF2	colony stimulating factor 2 (granulocyte-macrophage)	12 of 18 (67%)	5 of 8 (62%)	3.3	3.5	6.6	4.7	0.00125
CYP27B1	cytochrome p450, family 27, subfamily b, polypeptide 1	13 of 18 (72%)	7 of 9 (78%)	2.4	3.2	3.8	3.8	0.00133
IL2	interleukin 2	11 of 18 (61%)	4 of 8 (50%)	3.9	3.7	9.0	6.4	0.00187
IL5	interleukin 5 (colony-stimulating factor, eosinophil)	11 of 18 (61%)	7 of 8 (88%)	4.1	67.5	10.8	77.0	0.00399
INHBA	inhibin, beta a (activin a, activin ab alpha polypeptide)	15 of 18 (83%)	7 of 9 (78%)	5.0	9.2	9.0	11.5	0.00418
MMP12	matrix metallopeptidase 12 (macrophage elastase)	12 of 18 (67%)	5 of 8 (62%)	2.7	39.7	6.5	62.8	0.00476
CD1B	cd1b antigen	11 of 18 (61%)	6 of 8 (75%)	2.9	5.7	5.7	7.3	0.00491
CCL7	chemokine (c-c motif) ligand 7	10 of 18 (56%)	7 of 9 (78%)	2.4	49.2	5.9	63.1	0.00807
LIF	leukemia inhibitory factor	10 of 18 (56%)	5 of 9 (56%)	2.0	2.6	4.0	3.4	0.00901
GGTLA1	gamma-glutamyltransferase-like activity 1	13 of 18 (72%)	3 of 8 (38%)	3.9	5.0	6.3	12.0	0.00006
KIF20A	kinesin family member 20a	13 of 18 (72%)	3 of 9 (33%)	4.6	1.7	7.8	2.9	0.00015
AICDA	activation-induced cytidine deaminase	10 of 18 (56%)	4 of 9 (44%)	2.9	2.2	5.3	3.6	0.00033

Gene	Gene Name	Frequency (>2-fold)		Mean Fold Change		Mean Fold Change-Only Responders		p-value-Microarray
		Microarray	RT-PCR	Microarray	RT-PCR	Microarray	RT-PCR	
CXCL13	chemokine (c-x-c motif) ligand 13 (b-cell chemoattractant)	10 of 18 (56%)	3 of 9 (33%)	3.4	2.3	9.4	3.9	0.00454
LTA	lymphotoxin alpha (tnf superfamily, member 1)	12 of 18 (67%)	4 of 9 (44%)	4.0	2.4	11.7	3.4	0.00685
CCL13	chemokine (c-c motif) ligand 13	13 of 18 (72%)	3 of 8 (38%)	2.2	10.0	4.1	24.8	0.02919
GOLPH2	golgi phosphoprotein 2	11 of 18 (61%)	0 of 8 (0%)	3.1	1.4	5.5	N/A	0.00020