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Whole genome response in guinea pigs infected with the high virulence strain *Mycobacterium tuberculosis* TT372

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SUMMARY

In this study we conducted a microarray-based whole genomic analysis of gene expression in the lungs after exposure of guinea pigs to a low dose aerosol of the Atypical Beijing Western Cape TT372 strain of *Mycobacterium tuberculosis*, after harvesting lung tissues three weeks after infection at a time that effector immunity is starting to peak. The infection resulted in a very large up-regulation of multiple genes at this time, particularly in the context of a “chemokine storm” in the lungs. Overall gene expression was considerably reduced in animals that had been vaccinated with BCG two months earlier, but in both cases strong signatures featuring gamma interferon [IFN γ] and tumor necrosis factor [TNF α] were observed indicating the potent TH1 response in these animals. Even though their effects are not seen until later in the infection, even at this early time point gene expression patterns associated with the potential emergence of regulatory T cells were observed. Genes involving lung repair, response to oxidative stress, and cell trafficking were strongly expressed, but interesting these gene patterns differed substantially between the infected and vaccinated/infected groups of animals. Given the importance of this species as a relevant and cost-effective small animal model of tuberculosis, this approach has the potential to provide new information regarding the effects of vaccination on control of the disease process.

Keywords

Tuberculosis; Microarray analysis; Vaccination; Guinea pig model

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Ethical Approval: The animal studies reported here were approved by the Animal Care and Usage Committee, and the Biosafety Committee, at Colorado State University.

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1. Introduction

There is a general consensus that the guinea pig [*Cavia porcellus*] is a useful small animal model of infection with *Mycobacterium tuberculosis*. Over several decades it has produced a large amount of information pertaining especially to vaccine effects on the growth of the bacterium in the lungs and other organs [1–6], and more recently detailed observations of the pathology of the disease process have reinforced its similarity to the pathogenesis of disease in humans [7–12]. A notable limitation of the model in previous years was the lack of ability to specifically measure elements of the host immune response, but this has now changed with the considerable development of PCR-based assays for multiple immune markers [7, 13–16], and a breakthrough in gating strategies that has allowed the development and application of flow cytometric analysis [17]. As a result, it is now possible to measure both the development of the TH1 protective immune response, as well as the emergence of regulatory elements including Foxp3, TGF β , and IL-17.

Despite this, the model still lags behind other models, and particularly the mouse, in which a vast number of reagents are available. One recent approach to try to alleviate this limitation in the guinea pig has been the development of microarray based analysis, which followed the publication of guinea pig gene sequences by the NCBI nucleotide database. Using this information as a starting point, Tree and her colleagues [18] developed a limited array to establish a gene signature in guinea pigs vaccinated with BCG twenty weeks earlier. This was then followed by a second study [19] using a more extended customized array and guinea pigs vaccinated 14 weeks earlier; in both cases evidence for a strong TH1 response as well as cytokine responses [IL-1 α , IL-1 β] and chemokine responses [CXCL8, CXCL1/2] were observed. In neither case was a challenge infection applied, but more recently a global gene array was developed by Jain and her colleagues [20] in which guinea pigs were infected with *M. tuberculosis*. Here, as before, the predominant TH1 response was confirmed.

In that study [20] the laboratory adapted H37Rv strain was used, with animals exposed to an extremely high infectious dose [500 CFU], and the microarray analysis conducted at a late time point [day 70] when many animals were presumably succumbing from such a high dose infection. In the study reported here, we elected to use a clinically relevant strain, recently isolated in the Western Cape of South Africa [21], and determine gene expression at a relatively early time point when effector immunity was peaking as well as determine potential differences in the lungs between infected animals and animals infected after first being vaccinated by BCG. The results show here that both sets of animals showed evidence of a strong TH1, in confirmation of earlier reports [18–20], but that the range of gene up-regulation was much reduced in the vaccinated animals, presumably due to the fact, as demonstrated here, that the TT372 infection was already substantially controlled by the vaccine at this time.

2. Materials and Methods

2.1. Animal model

Female outbred Hartley guinea pigs (~450–500 g in weight) were purchased from the Charles River Laboratories (North Wilmington, MA) and held under barrier conditions in a biosafety level III animal laboratory. The specific-pathogen-free nature of the guinea pig colonies was demonstrated by testing sentinel animals. All experimental protocols were approved by the Animal Care and Usage Committee of Colorado State University and comply with NIH guidelines.

Guinea pigs were vaccinated intradermally with 1×10^4 *Mycobacterium bovis* BCG, strain Pasteur, or mock vaccinated with saline. *M. bovis* BCG was grown in Proskauer-Beck broth. Animals were challenged by aerosol exposure as above 8 weeks later.

Strain TT372 is a virulent “atypical Beijing” strain isolated from the Western Cape of South Africa [21]. It was grown in 7H9 broth containing 0.05% Tween 80 and OADC. When the culture reached an OD₆₀₀ reading of >0.600 it was bottled, frozen, and then titered. Thawed aliquots of frozen cultures were diluted in sterile saline to the desired inoculum concentration of 1×10^6 cfu/ml. The infection inoculum was determined for all the bacterial strains tested by plating serial dilutions of inoculum on nutrient 7H11 agar containing 10 µg/ml cycloheximide and 50 µg/ml of carbenicillin to prevent contamination. Colonies were counted after 3 weeks incubation at 37°C in humidified air.

A Madison chamber aerosol generation device was used to expose the animals to the *M. tuberculosis* infection. This device was calibrated to deliver approximately 10 bacilli into the lungs. Twenty one days later, lung tissues were harvested [five guinea pigs per group]. The left lobe from each animal was removed and immediately stored in RNAlater. The following day each lobe was pooled together, homogenized, and RNA purified as described below. The bacterial load in the right cranial lung lobe was then determined by plating serial dilutions of tissue homogenates on nutrient 7H11 agar containing 10 µg/ml cycloheximide and 50 µg/ml of carbenicillin. Colonies were counted after 3 weeks of incubation at 37°C in humidified air and data expressed as log₁₀ CFU.

2.2. Whole genome analysis

Total RNA was purified by digesting contaminating DNA with DNase followed by isolation of RNA using a Qiagen RNeasy minikit. Total nucleic acids were suspended in nuclease free water to a final volume of 350 µl. An equal volume of 70% ethanol was added to the solution and transferred to an RNeasy column and centrifuged for 1 min at 8000 rpm. The bound material was washed with 350 µl of RW1 wash buffer was added and DNase (Qiagen) treated for 15 minutes at room temperature. Following DNA digestion, the bound total RNA was washed with 350 µl of RW1 wash buffer, and then washed twice in 500 µl of RPE buffer at 8000 rpm and 13000 rpm respectively. Dry spin was done for 1 minute at 13000 rpm. RNA was eluted with nuclease free water.

A customized guinea pig 8×60K (AMADID: 040961) microarray was designed using Genotypic Right Design Technology, and was based on up to date sequences currently

available at the NCBI & Ensembl databases, thus allowing the design of a unique custom guinea pig microarray. The 60-mer oligonucleotide probes designed were specific to guinea pig genes and EST sequences. Probes were distributed among 6759 genes and 33825 ESTs in the microarray. The final microarray designed consisted of 62976 genomic features including replicated probes for the 8×60k Agilent array format [see Table. 1].

The concentration and purity of the extracted RNA was evaluated using a Nanodrop Spectrophotometer (Thermo Scientific). The integrity of the extracted RNA was analysed using a 2100 Bioanalyzer (Agilent). RNA quality was assessed based on 260/280 values, rRNA 28S/18S ratios, and RNA integrity number. The samples were then labeled using Agilent Quick Amp Kit (Part number: 5190-0442) and then 500ng of total RNA was reverse transcribed using oligodT primer tagged to the T7 promoter sequence. cDNA thus obtained was converted to double stranded cDNA in the same reaction. Then, the cDNA was converted to cRNA in an in-vitro transcription step using T7 RNA polymerase enzyme, and then Cy3 dye was added into the reaction mix. During cRNA synthesis the Cy3 dye was incorporated into the newly synthesized strands. cRNA obtained was further purified using a Qiagen RNeasy column. The concentration and amount of dye incorporated was determined using Nanodrop. Samples that passed quality control measures for specific activity and yield were hybridized on the customized guinea pig 8×60k array designed in-house using an Agilent Gene Expression Hybridization kit at 65° C for 16 hours. Hybridized slides were then washed using Agilent Gene Expression wash buffers. The microarray slides were then scanned on an Agilent G2600D scanner. Data extraction from the obtained images was done using Agilent Feature Extraction software (Version 11.5).

Feature extracted data was analyzed using Agilent GeneSpring GX software. Normalization of data was done using the 75th percentile shift (percentile shift normalization is a global normalization, where the locations of all the spot intensities in an array are adjusted). Significant differentially regulated genes that were either up or down regulated showing one fold or above changes in expression with significant P-values (<0.05) within the group of samples were identified. Statistical P-values were calculated using Student's t-test method and the Hochberg method. Differentially regulated genes were clustered using hierarchical clustering based on the Pearson coefficient correlation algorithm to identify significant gene expression patterns. Differentially regulated genes were classified into different functions based on their Gene Ontology functional category.

3. Results

3.1. Effect of BCG vaccination on the course of infection with strain TT372

As shown in Fig. 1, vaccination of guinea pigs with BCG substantially reduced the bacterial load on 21-days of the TT372 infection in all three target organs [$P < 0.001$ in all cases]. The degree of protection in the lungs [2.24-log] is very much at the high limit of this model, and is similar to very strong protection recently observed [21] against Beijing strains from the Western Cape region.

3.2. Whole genome analysis

An initial indication that substantial differences between infected animals and animals vaccinated then infected, in comparison to uninfected non-vaccinated control guinea pigs, was evident from heat map analysis [Fig. 2].

Changes in gene expression on day-21 of the TT372 infection are shown in Table 2, and are broken down into relevant categories. [Genes were selected in Tables 2–4 based on at least a twofold change in expression]. A very substantial number of genes associated with immune systems, the inflammatory response, cytokine and chemokine responses, and up-regulation of proteins involved in cell trafficking and surface adhesion, were observed. These included the expression of proteins driving the inflammasome, and proteins including IFN γ and TNF α that are central to the expression of the TH1 immune response in tuberculosis. A major facet of the response in these infected animals was predictably in terms of the cytokine and chemokine responses. Most cytokines expressed were pro-inflammatory, and in addition an array of receptors were up-regulated, including IL-10R, IL-15R and IL-21R, presumably to help drive effector and memory T cells, IL-17R and IL-23R, to control the emerging TH17 response, and IL-28R to support the response at the inflammasome level. In concert, multiple chemokines, including CXC ligands 8, 10, and 11 were expressed, involved in cell migration of neutrophils and T cells, as well as the chemokines CCL2 and CCL7, involved in recruiting macrophages to sites of inflammation. The ligand CCL20, which has been implicated in attracting regulatory T cells, was also increased. In parallel, multiple receptors were up-regulated, including CXCR2 and CXCR3, as well as CXCR7 [the role of which in the context of tuberculosis has yet to be investigated]. A much smaller number of genes were down-regulated, including cell adhesion genes, presumably reflecting changes needed for appropriate cell trafficking.

A similar pattern of up-regulation was seen in guinea pigs previously vaccinated with BCG prior to infection [Table 3], but here the patterns were much reduced with evidence for a narrower spectrum of responses. While the expression of IFN γ and TNF α were retained, a more focused cytokine and chemokine response was seen, including CXCL1/2 [GRO; as seen before [18, 19]], CCL10 and 20, and CCR4, all of which have been implicated in mechanisms influencing neutrophil influx as well as control of the influx and activity of TH17 and regulatory T cells. Only IL-23R was up-regulated to any degree [to sustain the TH17 response] whereas the regulatory T cell associated receptor IL-27 was significantly down-regulated. Overall, these expression patterns reflected a much more controlled response to the infection, with far fewer genes involved [summarized in Table 4]. Tables 5 and 6 give the actual recorded mean values from three assays for the highest and lowest gene expression levels in the two groups, with illustrative heat maps [Fig. 3].

4. Discussion

The results of this study show that, in terms of immunological markers, the most predominant were the expression of genes encoding IFN γ and TNF α , as well as a host of associated genes associated with these two cytokines including CD27, indicating a strong TH1 response in the lungs, fully consistent with previous flow cytometric analysis in this animal model [17, 22]. Moreover, in both infected [INF] and vaccinated and then infected

[V/INF] groups genes expressing CD8 and β_2 microglobulin were also up-regulated, again consistent with data [17] indicating the peak of this response is around day-20 of the infection. Increases in superoxide dismutase and catalase gene expression were seen in the INF but not V/INF groups, probably reflecting the fact that neutrophil influx into the lungs is depressed in BCG-vaccinated animals [10, 12, 22]. Another difference was the expression in the INF group of genes for the Toll-like receptors 1, 2, and 4, all of which can recognize mycobacterial products, whereas in the V/INF these were replaced by the orphan receptor TLR10.

As well as being consistent with others studies in the guinea pig model using this approach, these findings are consistent with studies in mice. In a study [23] of chronically infected mice, microarray analysis also showed substantial upregulation of the IFN γ /TNF α axis, multiple adhesion/trafficking molecules, genes observed above such as IL-7R and IDO, and a veritable “cytokine storm”. Similar results were also seen in an intravenous mouse infection model [24].

A frequent consequence of infection with high virulence clinical isolates such as TT372 is the generation of regulatory T cells [16, 25], and while these usually do not peak until around day 30–60 in this model, we observed increased expression of Lag-3 and CD28 in the INF group, which are associated with regulatory T cell emergence [26]. This group also up-regulated the adhesion marker P-selectin, as well as its ligand, consistent with this being central to cell trafficking [as we reported several years ago [7], guinea pigs seem to lack L-selectin/CD62L].

As for the V/INF animals, we observed expression of several genes not seen in the INF group, including CD109 [a receptor on bronchial endothelium that dampens the effects of TGF β] [27], the nuclear hormone receptor families ROR, RAR and RXR involved in the TH17/regulatory T cell axis [28]; Sec61, a channel system that connects the endosomal and cytoplasmic antigen processing pathways and which we previously suggested [29] could explain CD8 T cell priming without the need for cross-presentation; CD166, a member of the Ig superfamily of cell adhesion markers, and finally, iNOS, which produces NO which has both antimicrobial properties and will keep blood vessels dilated to allow cellular traffic.

We also observed down-regulation of certain markers, including the eosinophil attractants CCL5 and eotaxin. Mice lacking CCL5 are not more susceptible to tuberculosis infection, but do show changes in cell trafficking [30]. One study [31] has suggested that CCL5 counterbalances the influx of activated T cells and dendritic macrophages, so reduced expression would facilitate influx of these cells into lesions. Down-regulation of CD99, also implicated in dendritic cell migration toward lymph nodes, would also facilitate their local accumulation [32].

Inflammasome triggering, featuring up-regulation of IL-1 α and IL-1 β , were features of both groups, as were the pro-inflammatory cytokines IL-6, IL-10, and IL-11. In the V/INF group only an increase in IL-11 was seen, as well as IL-7, no doubt produced to support the memory T cell response established by the BCG vaccination. As for cytokine receptors, several were up-regulated in the INF group, including receptors associated with the TH17

axis [IL-17R, IL-23R], with the expansion of CD8 memory [IL-15R], and support of the effector response [IL-21R]. Both neutrophil and regulatory T cell influx are characteristics of the response in non-vaccinated guinea pigs which are delayed or reduced in BCG vaccinated animals [22, 33], and this may explain depression of the genes for IL-17R and IL-27R in the V/INF group.

As predicted by the rapid and severe inflammation observed in the lungs of guinea pigs infected with newly emerging clinical strains, the gene expression profile in the INF group exhibited a veritable “chemokine storm”, as was earlier noted [20]. CCL2 and CCL7, as well as TGF β , were up-regulated in both groups, whereas genes for several proteins involved in T cell migration into lesions [CXCL10, CXCR3, CXCR7] as well as macrophages [CSF-1, CXCR2, CCR2] were also highly expressed. Proteins involved in neutrophil influx, a major feature in infected animals, including CXCL8 and CXCL1/2 were also observed. Of note also was the up-regulation of the gene encoding for indoleamine 2,3-dioxygenase, an enzyme involved in tryptophan metabolism which can drive regulatory T cell activity [34–36].

Large numbers of genes involved in other systems were also up-regulated, especially in the INF group. These included genes associated with cellular events [importin-9, dystrobrevin, cyclin-1, spectrin, pinin, etc], and those associated with actin to control cytoskeletal events in cell migration [tropomyosin-1, kinesin, formin, radixin, etc]. Interestingly, we observed quite different profiles between the two groups in certain categories. For example, in the context of cell trafficking we observed up-regulation of particular markers in the INF group [cingulin, fibrillin, FRAS1] but different ones [leupaxin, CD45, osteopontin, cytohesin-1, macrosialin] in the V/INF group. In the context of the response to oxidative stress, sulfiredoxin, peroxiredoxin-6, calpain, tuftelin, and cavelin-2 were expressed in the V/INF group, but genes encoding hexokinase-3 and the Ox-LDL receptor genes were raised in the INF animals. Finally, in terms of lung injury repair, thrombospondin was up-regulated in the V/INF group, whereas aminin, paxillin, platelet derived growth factor-C, and plasminogen activator inhibitor were raised in the INF group.

Several notable responses were also down-regulated in the V/INF group. TNF-associated apoptosis related mechanisms were depressed, including the expression of catenin [which drives the expression of TRAIL], and copine-9, DAP-1, galectin-1, and Sac protein-3, were also down-regulated. A known marker of regulatory T cell activity, heme oxygenase, was depressed, as were blood/lymphatic vessel repair systems [semaphorin 3E, tetraspanin, lactadherin, and peroxisomal biogenesis factor-11]. Finally, in reverse to events in the INF group, the expression of Ox-LDL receptor and plasminogen activator inhibitor was reduced in the V/INF animals.

Collectively, this “snap-shot” into events in the lungs relatively early during the course of the infection reveal complex systems involving the host immune response, cardiovascular events, and wholesale cellular activation and movement, in which homeostatic control of these systems must be maintained whilst still allowing expression of protective immunity and control and containment of pulmonary lesions. From a holistic perspective these data further confirm the central importance of the IFN γ /TNF α mediated TH1 response, at this

point in time hardly surprising, but also illustrate that the emergence of regulatory systems mediated by TH17 and regulatory T cells are already showing signs of becoming operational this early in the host response, as is being increasingly noted [37, 38]. If the animal was first vaccinated with BCG prior to challenge, there was a substantial reduction in the numbers of genes up-regulated, reflecting faster control of the infection and far less damage and inflammation in the lungs. In addition, damaging responses, particularly those mediated by regulatory T cells or neutrophils, were depressed [at least at this early stage of the infection] consistent with our previous observations [16].

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Fig. 1.

Effect of BCG vaccination on the course of infection with strain TT372 in target organs after low dose aerosol. Guinea pigs were harvested on day-20 of the infection. Data is shown as log-10 mean values [n=5; $\pm$ SEM].

Fig. 2.

Whole genomic response heat map for the three experimental groups, showing consistency between the three individual data sets for the infected, and vaccinated then infected, groups.

Fig. 3.
Heat maps illustrating the highest and lowest gene expression values for the experimental groups.

TABLE ONE

Guinea Pig Microarray Probe distribution in 8X60K format

Sequence Data Source	Number of Probes
NCBI-EST	33825
Ensembl-Gene	6759
Total replicated probes	21073
Agilent controls	1319
Total features on the array	62976

TABLE TWO

Changes in gene expression in infected animals.

Functions	No. Of Genes Up	Genes Up	No. Of Genes Down	Genes Down
Antigen processing and presentation	16	CD1B3, Ifng, CD1B2, AF058395.1, B2MG_CAVPO, TAP1, ENSCPOT00000014608, I-Mar, SEC24B, KIF23, HFE, ENSCPOT00000014924, KIF2C, ENSCPOT00000007732, ENSCPOT00000001316, TAPBPL	7	CD1E, CTSE, CD1C2, CD1C3, CD1B4, ENSCPOT00000004483, CCL21
B cell receptor signaling	8	PLCG2, MND, NCKAP1L, LPXN, ENSCPOT00000011368, ENSCPOT00000010504, ENSCPOT00000008915, ENSCPOT00000009730	0	
Cell adhesion	51	TNF, IL1B, S1PR1, COL5A1, ENSCPOT00000023481, TGFB1, TNFRSF12A, TNC, ENSCPOT00000002609, ENSCPOT00000007693, RND1, ICAM3, ITAM_CAVPO, ARVCF, PSCD1, ENSCPOT00000015670, C1orf38, ENSCPOT00000014750, ITGAL, COL15A1, ENSCPOT00000014374, ENSCPOT00000014924, CXCR3, DSC2, DDR1, PECAM1, TFE3, PKP2, SELP, ITGB7, NCKAP1L, AGGF1, IL8_CAVPO, ENSCPOT00000015705, ITGA4, LPXN, TNFAIP6, PLAUI, BYSL, ENSCPOT00000010504, HCK, GPR56, CLEC7A, ENSCPOT00000013558, PVR, PLEKHA7, CD300A, CXCR7, CTNNA2, LOXL2, PSCDBP	16	CCL5, TGFB1, CITED2, ENSCPOT00000010687, NDUFB7, ENSCPOT00000010761, ADAMDEC1, ANXA9, CADM1, CCL21, CDH10, ESAM, POSTN, ENSCPOT00000003199, TGFB1I1, FXYD5
Chemokine	25	TNF, Ifng, AF058395.1, Cxcr2, IL1B, CCL2_CAVPO, ENSCPOT00000023827, TLR2, CSF1R, ENSCPOT00000015254, ENSCPOT00000000490, CXCL11, C5, CXCR3, CXCL10, IL6, Q9Z318_CAVPO, ENSCPOT00000014469, IL1B_CAVPO, GROA_CAVPO, CXCR7, ENSCPOT00000009142, ENSCPOT00000009756, CCL20, ENSCPOT00000011244	7	CCL5, CMTM8, CXCL12, CCL21, ENSCPOT00000008926, TICAM1, CCL17
Coagulation	18	Q07E12_CAVPO, SHH, F2RL2, C4BPB, PLAUR, ENSCPOT0000000806, KIF23, F13A1, KIF9, RAD51C, CD48, TF_CAVPO, PLAUI, KIF2C, CAPZA1, SIN3A, ENSCPOT00000009756, KIFC1	2	F7, VKORC1
Complement	10	ENSCPOT00000021457, ENSCPOT00000022384, SERPING1, C4BPB, C1QC, C1QB, C5, MASP2, XPC, C3	3	A1CF, C8G, C1QTNF2
Cytokine	51	TNF, TGFb3, IL1B, DCTN3, Q07E12_CAVPO, KLF6, IL17RA, ANXA11, TLR2, BCL6, ENSCPOT00000008387, TYMS, DOCK10, ZFYVE26, NOD2, SEH1L, CLEC5A, ENSCPOT00000004812, CXorf9, BATF, TIA1, CRTAM, DOCK2, CXCL11, E2F7, KIF23, AURKC, NIPBL, LCP2, CXCL10, IL6, CKAP2, TLR10, NUSAP1, PGGT1B, PRPF40A, IL1B_CAVPO, TNFA_CAVPO, ENSCPOT00000002090, GROA_CAVPO, ENSCPOT00000010504, STAG2, IL15RA, RACGAP1, FLT3, PRC1, CLEC4E,	9	G6PD, TMEM102, CEBPE, GRN_CAVPO, MIF, IL17RC, CADM1, CCL21, CCL17

Functions	No. Of Genes Up	Genes Up	No. Of Genes Down	Genes Down
		MAP2K3, ENSCPOT00000010453, ENSCPOT00000009756, CCL20		
endothelial	23	AF289990.1, Ang-2, IL1B, Q07E12_CAVPO, ATP5A1, PROCR, NRP2, ENSCPOT00000001319, C5, ESM1, FGF1, ENSCPOT00000007819, ENSCPOT00000014924, STAT1, SERPINE1, PECAM1, SELP, AGGF1, LYVE1, FLT3, LOXL2, ENSCPOT00000009756, ENSCPOT00000011244	13	TGFB1, RAMP2, CCL11, HSPB1, RAMP2_CAVPO, FIGF, FGF10, ETV2, REM1, HYI, ESAM, ENSCPOT00000014520, FGF18
Immune Response	79	CCL2, Fth1, CD1B3, TNF, Ifng, CD1B2, CXCL1, AF058395.1, IL1A, Mcpt3, IL1B, B2MG_CAVPO, CCL2_CAVPO, IRF8, ENSCPOT00000022384, PRF1, GROA_CAVPO, ENSCPOT00000023827, TLR2, OSM, PRG4, BCL6, CBLB, ENSCPOT00000008266, I-Mar, CYLD, ENSCPOT00000015254, BCL10, PLCG2, CLEC5A, SAMHD1, C4BPB, C1QC, ICAM3, ZBP1, ENSCPOT00000011160, C1QB, CXCL11, C5, POLR3E, IL23R, PRKCD, RNF125, CLEC10A, ITPR2, HFE, GEM, ENSCPOT00000014924, TNFSF13B, TNRC6A, MS4A1, TFE3, ITGB7, CXCL10, MARCO, ABI1, Q9Z318_CAVPO, TLR10, ENSCPOT00000001875, ENSCPOT00000015705, IFIH1, IL1B_CAVPO, ENSCPOT00000008684, SBNO2, TNFA_CAVPO, RGS1, ENSCPOT00000010504, FYB, HCK, CAPZA1, IGJ, SIN3A, CLEC4E, ENSCPOT00000007732, TRIM5, ENSCPOT00000009142, ENSCPOT00000001316, ENSCPOT00000009756, ENSCPOT00000011244	18	CD1E, CD1C2, CD1C3, CD1B4, CCL11, ENSCPOT00000004483, ENPP2, ADAMDEC1, C8G, DAK, CCL21, DEFB1, ENSCPOT00000008926, ENSCPOT00000003199, TICAM1, SH2D1A, ENSCPOT00000008745, CCL17
Inflammatory	43	CCL2, TNF, CXCL1, TNFAIP8L2, CCL2_CAVPO, IL17RA, ENSCPOT00000022384, HNRNPA0, GROA_CAVPO, TLR2, ENSCPOT00000027466, BCL6, TNIP3, NOD2, ENSCPOT00000004812, NFKBIZ, CXCL11, C5, C1orf38, IL23R, ENSCPOT00000014798, ENSCPOT00000014374, ENSCPOT00000014924, CXCR3, NFRKB, SELP, CXCL10, AOA, TLR10, ENSCPOT00000015705, IL1B_CAVPO, SBNO2, TNFAIP6, HCK, NLRC4, TPST1, PLA2G2D, MAP2K3, CD28, ENSCPOT00000009142, NT5E, CCL20, ENSCPOT00000011244	11	CCL5, CCL11, FAM132A, CTNNBIP1, IL17RC, JMJD3, CCL21, DEFB1, MGLL, ALOX5, CCL17
Leukocyte	25	TNF, ENSCPOT00000023827, TLR2, PTPRC, S100A8, ITAM_CAVPO, C5, ITGAL, CXCR3, TNFSF13B, LCP2, SELP, ITGB7, CXCL10, ENSCPOT00000015705, ITGA4, CD48, IL1B_CAVPO, TNFA_CAVPO, ENSCPOT00000010504, HCK, SELPLG, FLT3, S100A9, ENSCPOT00000009756	3	CCL5, PODXL2, CCL21
Natural killer cell mediated	2	CRTAM, LAG3	1	SH2D1A
T cell receptor signaling	8	CBLB, TRAT1, BCL10, PLCG2, ENSCPOT00000000806, KIAA0748, FYB, C6orf190	1	ENSCPOT00000013771

Functions	No. Of Genes Up	Genes Up	No. Of Genes Down	Genes Down
Purine	12	gpr109A, GPR171, HMGCS1, MTAP, GPR18, ENSCPOT00000008023, GDA, ACPP, SLC28A3, ENSCPOT00000008684, ATIC, NT5E	3	P2RX2, P2RX2_CAVPO, ADSSL1
Pyrimidine	5	TYMS, POLH, SLC28A3, TK1, NT5E	1	ERCC1
Phosphorylation	64	TNF, Ifng, AF058395.1, TGFb3, CTDSPL, Q07E12_CAVPO, TLR2, DUSP11, CSF1R, CLK1, OSM, STAT3, PPP3CA, CTDP1, ENSCPOT00000015254, BCL10, ENSCPOT00000012369, DUSP6, ERC1, MAP4K1, PDGFC, CCNB1, ENSCPOT00000003058, CDC2, ENSCPOT00000006676, FAM129A, STK10, TRPM7, HCLS1, ADAM10, VRK1, IL23R, IKBKE, ENSCPOT00000014798, ADAR, ENSCPOT00000012968, ENSCPOT00000007525, DDR1, CAMK4, CDKN2D, ABI1, EIF2AK2, HK2, PIK3R1, UBASH3B, PDGFRB, CDKN2C, ACVR1B, ENSCPOT00000011871, TNFA_CAVPO, WNK1, SRC, ENSCPOT00000010504, FYB, HCK, CNKSR3, SPRED1, FLT3, CDC25A, CCNE1, ARRB2, IPPK, ENSCPOT00000004292, RARA	16	CCL5, TGFb1, DUSP23, MAP3K11, ENSCPOT00000000272, CDKN1B, ENSCPOT00000013771, ENSCPOT00000010630, UCP3, ENSCPOT00000012652, PPP2R4, ENSCPOT000000003199, ENSCPOT00000013112, MAPK15, IMPA2, ENSCPOT00000012184
tricarboxylic acid cycle	0		0	
Lipid	49	TNF, AF289990.1, PLA2G2A, IL1B, Q07E12_CAVPO, ATP5A1, ANXA1, ANXA11, ENSCPOT00000012911, HMGCS1, SNCA, BCL10, PLCG2, LIPH, ENSCPOT00000013639, C9orf100, TNFRSF21, DOCK2, NR1H3, ENSCPOT00000015420, FGD3, PLAUR, APBB1IP, PAFAH2, FBNP1, IL1RN, PAG1, ENSCPOT00000010206, GLIPR1, PLEKHH1, TIAM2, RASA3, MBOAT1, ATP11A, PA2GA_CAVPO, ENSCPOT00000015123, ENSCPOT00000008915, ASAH1, FA2H, SIN3A, B4GALT4, PLEKHA7, ATP10D, PLA2G2D, CDC42BPB, ARHGAP15, SNTB1, LASS2, ENSCPOT00000002492	26	CD1E, GP-GPx4, SIGMAR1, PRPF19, PLIN3, ISYNA1, ENSCPOT00000013217, S100A13, ENSCPOT00000014951, PLSCR3, ENSCPOT00000012624, RTKN, CETP, PNPLA2, ANXA9, LEP, ARSE, THRSP, PLEKHM2, PLA2G12A, ARHGEF9, MTTP, CENTB1, C14orf2, ATP5G1, MGLL
Gluconeogenesis	0		1	FAM132A
Oxidative	11	AY757362.1, NDUFS2, ADNP2, ENSCPOT00000007243, ENSCPOT00000009771, ENSCPOT00000006676, STC2, SODM_CAVPO, SIN3A, IDH1, GCLC	6	U39842.1, GP-GPx4, G6PD, GPX7, UCP3, NDUFS8
Glycolysis	4	HK3, ADPGK, PGK2, PGAM4	1	ENSCPOT00000013112
Metabolism	95	TNF, Ifng, SOD, AF058395.1, SOD2, PLA2G2A, Q07E12_CAVPO, GGT7, CATA_CAVPO, ATP5A1, ENSCPOT00000015497, CTGF, DUT, SHH, SLC5A3, SAFB, SODM_CAVPO, CSF1R, CEBPA, ASPG, ENSCPOT00000007121, HMGCS1, SNCA, MTAP, TYMS, PLCG2, SEHL, ABCD4, SEC24B, OAS3, LASS5, GPT2, PIP4K2A, HS2ST1, TNFRSF21, SAMHD1, ENSCPOT00000009771, DHRS9, HK3, KMO, SGPL1, ENSCPOT00000014947, CUL4A, NR1H3, ARRDC3, CA1, PAFAH2, PPA1, CSAD, AHCYL1, PDCD4, IL1RN, ITPR2, PLEK, GDA, FMO2, ENSCPOT00000002222, ACPP, ENSCPOT00000014924, MTRR, ENSCPOT00000006749, PARP1, AOA1,	45	DCXR, DDC, CYP1A1, AB010634.1, IGF2, KCNJ11, LHBeta, MAN2B1, AF193576.1, GANAB, 5HT1D_CAVPO, GSTM3, SDR39U1, G6PD, CRABP2, NDUFS3, CLN6, ACE, FN3K, G6PC, ENSCPOT00000014951, IDUA, NDUFB11, GSTT1, ZNRD1, CETP, DAK, PNPLA2, YIF1A, LEP, ARSE, THRSP, UCP3, PCYT2, ENSCPOT00000005419, FIG4, VKORC1, GNG8, PRLHR, APOM, MTTP, C20orf52, PON1, MGLL, ALOX5

Functions	No. Of Genes Up	Genes Up	No. Of Genes Down	Genes Down
		CHST14, ENSCPOT00000002157, HK2, ENSCPOT00000010206, GLIPR1, GLT6D1, RRM2, NSMAF, TNFA_CAVPO, PIK3R5, PISD, TK1, KYNU, SOAT1, PA2GA_CAVPO, ASAH1, ART4, FA2H, SIN3A, B4GALT4, CHSY1, ENSCPOT00000006517, IDH1, ALDH9A1, LASS2, GFPT2, IPPK, GCLC, ENSCPOT00000002492, ENSCPOT00000012380, NT5E, NPL		
Amino acid	23	TNF, CEBPB, ASPG, PCSK6, DUSP6, ENSCPOT00000006927, PDGFC, GPT2, SLC16A10, ENSCPOT00000007756, CDC2, TRPM7, ADAM10, IKBKE, CSAD, CAMK4, EIF2AK2, PIK3R1, ACVR1B, TNFA_CAVPO, WNK1, SRC, ARRB2	9	DDC, COL1A2, UBE2Z, MAP3K11, GLYAT, DAP, ENSCPOT00000005419, MTTTP, SLC1A6
MAPK	15	TNF, IL1B, Q07E12_CAVPO, OSM, MAP4K1, SPRED2, C5, ENSCPOT00000014924, MAP3K2, ENSCPOT00000015705, SPRED1, MAP2K3, TRIM5, MAP2K1, NA	10	CCL5, TGFB1, SEMA4C, ENSCPOT00000000272, ENSCPOT00000012652, C1QTNF2, ENSCPOT00000013112, LGALS1, MAPK15, NA
JAK	7	STAT3, IL23R, STAT1, CTR9, JAKMIP1, ENSCPOT00000011244, NA	0	
Calcium	58	Cd8a, SLC8A1, PLA2G2A, Q07E12_CAVPO, B0FJL6_CAVPO, FN1, ANXA1, ANXA11, SHH, PRF1, JUNB, ENSCPOT00000007121, ENSCPOT00000012911, TBC1D9, TRAT1, STAT3, PPP3CA, ENSCPOT00000005095, GTF2I, PLCG2, ENSCPOT00000013639, ENSCPOT00000000490, S100A8, ENSCPOT00000005147, MAN1C1, TRPM7, C5, ENSCPOT00000003610, ITGAL, SLC24A3, TMEM16A, ITPR2, PLEK, MASP2, SCUBE2, ENSCPOT00000014924, STAT1, CXCR3, FLVCR2, DSC2, SELP, CAMK4, ENSCPOT00000015705, GALNT3, STC2, MMP16, RASA3, ENSCPOT00000015760, ENSCPOT00000010504, PA2GA_CAVPO, ENSCPOT00000013558, PLA2G2D, FGG, ENSCPOT00000009142, S100A9, ENSCPOT00000009756, ENSCPOT00000011244, NA	51	CCL5, AB016287.2, RAMP2, RAMP1, CRTH2, OCM, Q56D28_CAVPO, ENPP2, JUND, PLN, RAMP1_CAVPO, CLEC3B, RHBDL1, PRKCSH, RAMP2_CAVPO, FKBP1B, CETN3, CAMK2N1, ENSCPOT00000010687, RCN3, S100A10, ENSCPOT00000014110, ENSCPOT00000013903, ENSCPOT00000010761, SPOCK2, ENSCPOT00000012946, CAPNS1, S100A13, TNNT2, ENSCPOT00000013771, ENSCPOT00000005926, PNPLA2, VSNL1, ANXA9, NECAB1, KCNMB1, CCL21, CDH10, MYL2, PPP2R4, ENSCPOT00000008926, PLA2G12A, MYL3, CLCA1, S100A1, ONCO_CAVPO, ITGB1BP2, CALN1, FXYS5, SLN, NA
Wnt	19	Q07E12_CAVPO, WLS, SHH, TLR2, G3BP1, CYLD, PLCG2, FAM123B, CCNY, TLE1, ENSCPOT00000014534, CTR9, HMG2L1, MITF, ENSCPOT00000008464, SIAH2, CCNE1, RNF146, NA	6	CTNBP1, FGF10, ETV2, ZNRF3, TGFB1I1, NA

TABLE THREE

Changes in gene expression in vaccinated and then infected animals.

Functions	No. of Genes Up	Genes Up	No. Of Genes Down	Genes Down
Antigen processing and presentation	15	CD1B3, Ifng, AF148875.1, AF058395.1, B2MG_CAVPO, TAP1, ENSCPOT00000014608, 1-Mar, PSME4, HFE, ENSCPOT00000014924, TRAF6, SEC61A2, ENSCPOT00000007732, NA	6	CD1E, AP1B1, KIF3C, ENSCPOT00000002723, PSMF1, AP2A1
B cell receptor signaling	3	MNDA, ENSCPOT00000011368, ENSCPOT00000008915	0	
Cell adhesion	22	TNF, IL1B, IGFBP7, PCDHAC1, ENSCPOT00000023481, RIC8A, PVRL3, ASAH3L, ENSCPOT00000003675, NEDD9, ENSCPOT00000014924, PCDH17, PPP1R12A, ITGA1, ITGA4, ENSCPOT00000010939, PLXNC1, B4GALT1, ALCAM, CLEC7A, PCDH19, PXN	19	TGFB1, SCARB1, JUP, KIAA1462, ENSCPOT00000000983, MFGE8, DCBLD1, PCDH12, ADAMDEC1, COL15A1, ENSCPOT00000014636, ESAM, ENSCPOT00000004694, LAMB3, ENSCPOT00000013558, TGFB11, SUSP5, DSP, SPON2
Chemokine	12	TNF, Ifng, AF058395.1, IL1B, CCL2_CAVPO, QXQLG2_CAVPO, CXCL11, IL6R, CXCL10, ENSCPOT00000014469, GROA_CAVPO, CCL20	1	ZFPM1
Coagulation	6	Q9Z0X7_CAVPO, Q07E12_CAVPO, C4BPB, F13A1, RAD51C, CD48	1	KIF3C
Complement	3	SERPING1, C4BPB, C1QB	3	ERCC2, A1CF, C1QTNF2
Cytokine	38	TNF, TGFb3, IL1B, SPG20, DCTN3, Q07E12_CAVPO, KLF6, SPTBN1, STAMBP, ANXA11, BCL6, TYMS, CLEC5A, ENSCPOT00000004812, CXorf9, TIA1, CRTAM, CXCL11, FABP4, ENSCPOT00000011704, TNFSF15, TRAF6, RAB35, CXCL10, TLR10, PGGT1B, ZCCHC11, ENSCPOT00000010939, PRPF40A, GROA_CAVPO, ZBTB32, REL, GIMAP5, CLEC4E, ENSCPOT00000010453, CEP55, CCL20, IL7	4	GAS6, GRN_CAVPO, IL17RC, IL27RA
endothelial	14	FGF2, IL1B, Q07E12_CAVPO, ATP5A1, CCL11_CAVPO, NRP2, LECT1, ENSCPOT00000009931, TNFSF15, ENSCPOT00000014924, STAT1, SERPINE1, ENSCPOT00000004538, ENSCPOT00000010939	12	NOS3, TGFB1, SCARB1, SOX18, HSPB1, TNIP2, FGF10, GAS6, PCDH12, REM1, ESAM, PLVAP
Immune Response	51	CCL2, Fth1, CD1B3, TNF, Ifng, CXCL1, AF148875.1, AF058395.1, IL1A, Mcpt3, IL1B, B2MG_CAVPO, NDFIP1, CCL2_CAVPO, CCL11_CAVPO, ENSCPOT00000020083, TNFSF10, TNFSF12-TNFSF13, TYROBP, ENSCPOT00000025562, PRG4, BCL6, CBLB, 1-Mar, CLEC5A, SAMHD1, C4BPB, ENSCPOT00000011160, C1QB, CXCL11, IL23R, HFE, GEM, ENSCPOT00000014924, IL6R, MS4A1, TRAF6, CXCL10, MARCO, TLR10, ENSCPOT0000001875, POLR3G, IFIH1, GROA_CAVPO, RGS1, FYB, GIMAP5, ENSCPOT00000013741, CLEC4E, ENSCPOT00000007732, EIF2C1	12	CD1E, TP53, ENSCPOT000000000163, ADAMDEC1, GTPBP1, DAK, POLR3B, CD276, ENSCPOT00000002723, IL27RA, SH2D1A, SPON2
Inflammatory	25	CCL2, TNF, CXCL1, TNFAIP8L2, NDFIP1, CCL2_CAVPO, CCL11_CAVPO, BCL6, ENSCPOT00000004812, CXCL11, FABP4, IL23R, ENSCPOT00000014798, ENSCPOT00000014924, CXCL10, ENSCPOT00000004538, AOA, TLR10, B4GALT1, PARP4, GROA_CAVPO, NLRC4, ENSCPOT00000000753, PLA2G2D, CCL20	7	IL17RC, GGTLA1, PXX, CD276, ALOX5, ENSCPOT00000009038, ENSCPOT00000000584

Functions	No. of Genes Up	Genes Up	No. Of Genes Down	Genes Down
Leukocyte	13	TNF, NDFIP1, LENG8, ENSCPOT00000020083, S100A8, LECT1, IL6R, CXCL10, ITGA4, CD48, B4GALT1, ALCAM, S100A9	1	PODXL2
Natural killer cell mediated	2	CRTAM, GIMAP5	1	SH2D1A
T cell receptor signaling	5	CBLB, TRAT1, TRAF6, CD3E, FYB	2	KCNN4, CACNB3
Purine	4	SUCNR1, GPR171, GDA, AMPD1	6	P2RX2, P2RX2_CAVPO, SLC29A1, AACs, ADSSL1, IMPDH1
Pyrimidine	2	TYMS, CPEB2	2	ERCC1, CAD
Phosphorylation	57	TNF, Ifng, AF058395.1, TGFb3, CTDSPL, THTPA, Q07E12_CAVPO, CLK2, SPTBN1, SDCBP, MAP3K3, GNPTAB, CCNT1, PHOSPHO2, YWHAB, DUSP11, CLK1, TAOK3, CLK3, MORC3, ENSCPOT00000008467, RAP2C, ENSCPOT00000003058, CDC2, MKNK1, ADAM10, PIK3CG, ENSCPOT00000006954, IL23R, ENSCPOT00000014798, DYRK1A, DNAJC6, MOBKL1A, MRE11A, ENSCPOT00000012727, IL6R, SMG7, CAMK4, ENSCPOT00000004538, TNFRSF14, PPP1R12A, GMFB, TLK2, ACVR1B, CD3E, MAK, ENSCPOT00000006629, WNK1, PRKG1, FYB, ENSCPOT00000008082, SMG6, CSNK1G1, LRRK2, BARD1, ENSCPOT00000010028, PXN	20	BDKRB2, TGFB1, ILK, TP53, EPHA1, ERCC2, EIF2AK3, PRKCZ, TBRG4, ENSCPOT00000000983, GAS6, TADA3L, ENSCPOT00000005265, ENSCPOT00000010630, ENSCPOT00000014636, CAD, MTMR14, AGK, RRGTT, ENSCPOT00000000584
tricarboxylic acid cycle	2	SDHA, MDH1	1	IDH1
Lipid	53	TNF, PLA2G2A, IL1B, SPG20, Q07E12_CAVPO, PEX3, SPTBN1, PRDX6, ATP5A1, ANXA1, ANXA11, ENSCPOT00000022500, PLIN2, KATNA1, RTKN2, ARHGEF2, A9QUC5_CAVPO, ENSCPOT00000024338, PLEKHO1, ENSCPOT00000012911, SNCA, ATP8A1, FARP1, ATP11C, NFYC, SPATA13, ENSCPOT00000014029, PHLDB3, FABP4, ENSCPOT00000015420, APBB1IP, HNRPK, C20orf114, NRIP1, LIPJ, ENSCPOT00000004538, SYTL3, PI4K2A, ENSCPOT00000001466, SORBS1, PLEKHA3, ENSCPOT00000003080, ENSCPOT00000015123, GIMAP5, ENSCPOT00000008915, ASAH1, CAV2, ARHGAP12, PLA2G2D, ARHGAP15, AADACL1, PA2GA_CAVPO, ENSCPOT00000002492	34	CD1E, KCNN4, SIGMAR1, SCARB1, NAGA, PLIN3, DOK1, ISYNA1, PLCL1, ENSCPOT00000001820, AFAPIL1, ENSCPOT00000013284, GBGT1, ALDH3B2, OSBPL5, PLSCR3, SPNS2, NR1H2, IDH1, CETP, PNPLA2, ARSE, DGKA, BBS4, ARHGEF1, GDDP5, AGK, ENSCPOT00000004694, ARHGEF9, MTPP, PLD3, LARGE, SREBF1, ATP9A
Gluconeogenesis	2	CLK2, ACN9	0	
Oxidative	10	CYP1A2, DIABLO, PRDX6, NQO1_CAVPO, NDUFS2, SRXN1, ENSCPOT00000007243, DHCR24, ENSCPOT00000004289, LRRK2	6	U39842.1, TP53, ERCC2, IDH1, ALKBH1, OSGIN1
Glycolysis	3	ALDOA, PKM2, HK3	0	
Metabolism	78	Gba3, TNF, Ifng, CYP1A2, SULT1E1, AF058395.1, DAO, PLA2G2A, ADAMTS-1, THTPA, Q07E12_CAVPO, PAIP1, GGT7, GUSB, NDUFS1, ALDOA, SDHA, EIF4G2, MDH1, CATA_CAVPO, ATP5A1, ENSCPOT00000015497, LCAT, CTGF, DUT, PLA2G15, HGD, A9QUC5_CAVPO, GSTA4, HAAO, PRDX5, FPGS, AHSP, ASPG, SNCA, TYMS, SPEF2, RORA, EXOC6, PIP4K2A, HS2ST1, SAMHD1, REXO2, HK3, NFYC, KMO, ENSCPOT00000002798, DEF1B_CAVPO, ENSCPOT00000014029, PSME4, ASAH3L, ASZ1, AHCYL1, COQ3, TNFSF15, GDA, FMO2, ENSCPOT00000002222, ENSCPOT00000014924, ENSCPOT00000006749, ETNK1, ENSCPOT00000004538, AOA, ENSCPOT00000010009, ENSCPOT00000010939, AMPD1, B4GALT1, RRM2, CA12, ENSCPOT00000011011, KYNU, SOAT1, ASAH1, ART4, COL11A1, SLC35D1, PA2GA_CAVPO, ENSCPOT00000002492	56	DCXR, AY149906.1, HSD11B1, CYP1A1, KCNJ11, LHBeta, TP53, MAN2B1, 5HT1D_CAVPO, CYP1A1_CAVPO, KLF2, CACNA1A, MOGS, RETSAT, PLCL1, CRABP2, ACAA1, GNPDA1, ACE, BPHL, CHST7, AACs, SLC2A5, ALDH3B2, CDIPT, OSBPL5, IDUA, PHKA2, DNPEP, NR1H2, IDH1, ACRYL, ZNRD1, CETP, DAK, PNPLA2, CAD, ARSE, SPHK2, BBS4,

Functions	No. of Genes Up	Genes Up	No. Of Genes Down	Genes Down
				FIG4, POFUT2, GDPD5, MTMR14, MAOB, APOM, MTP, ITPKA, PSMF1, PLD3, PHGDH, LARGE, ALOX5, SREBF1, GALK1, ENSCPOT00000000584
Amino acid	20	EHHADH, TNF, DAO, ENSCPOT00000021822, ASPG, RRAGB, ENSCPOT00000007756, CDC2, PSME4, ADAM10, PIK3CG, ENSCPOT00000012727, CAMK4, B4GALT1, ACVR1B, ENSCPOT00000006629, WNK1, PRKG1, TNRC15, SLC1A6	11	CACNA1A, ENSCPOT00000000163, EIF2AK3, AARS, PRKCZ, ALG12, BPHL, SLC43A1, DAP, MTP, PSMF1
MAPK	6	TGFB1, ENSCPOT0000001127, TRAF7, C1QTNF2, LGALS1, NA	13	TNF, FGF2, IL1B, Q07E12_CAVPO, TAOK3, NF2, ENSCPOT00000004289, ENSCPOT00000014924, IL6R, FRS2, LRRK2, PXN, NA
JAK	0		3	IL23R, STAT1, NA
Calcium	36	CRLR, KCNN4, CRTH2, OCM, Q56D28_CAVPO, CAPN1, DOC2B, WFS1, JUND, PLN, CACNA1A, PRKCSH, CETN3, RCN3, PLCL1, FBLN2, EIF2AK3, ENSCPOT00000014110, ENSCPOT00000005147, GAS6, GRIN3B, CAPNS1, PCDH12, ATP2A3, ENSCPOT00000005926, PNPLA2, VSNL1, TRAF7, ENSCPOT00000003072, ONCO_CAVPO, ENSCPOT00000013558, CACNB3, ENSCPOT00000008875, CALN1, FBLN5, NA	55	TRP1, AB016288.1, Cd8a, FGF2, PLA2G2A, NCX1, Trpc3, SPARCL1, Q07E12_CAVPO, PPP2R3C, B0FJL6_CAVPO, FN1, ANXA1, ENSCPOT00000019805, ENSCPOT00000020083, PCDHAC1, GNPTAB, ANXA11, DTNB, PLA2G15, Q0QLG2_CAVPO, DIAPH1, FBN3, ENSCPOT00000012911, HOMER1, TRAT1, ENSCPOT00000011402, UTS2, ENSCPOT00000011789, S100A8, CAPN9, ENSCPOT00000004029, ENSCPOT00000009931, ENSCPOT00000003675, ENSCPOT00000014924, STAT1, SEPN1, PCDH17, CAMK4, SYTL3, CD3E, CUL5, CCDC109A, ENSCPOT00000001466, EFHA2, NIN, GIMAP5, PLA2G2D, TPCN1, FGG, S100A9, PA2GA_CAVPO, PCDH19, SLC25A25, NA
Wnt	7	ILK, FGF10, WNT7A, ENSCPOT00000008464, SOX17, TGFB111, NA	10	Q07E12_CAVPO, RAB5A, ENSCPOT00000008161, EGR1, BICC1, ENSCPOT00000000305, MDFIC, ENSCPOT00000014534, CSNK1G1, NA

TABLE FOUR

Numbers of gene changes in relevant categories

Category	Up-regulated		Down-regulated	
	INF	VACC/INF	INF	VACC/INF
Antigen processing	16	15	7	6
Cell adhesion systems	51	22	16	19
Chemokines	25	12	7	1
Cytokines	51	38	9	4
Immune response associated	79	51	18	12
Inflammation	43	25	11	7
T cell receptor	8	5	1	2
Signalling	22	6	10	16

TABLE FIVE

Major changes in gene expression in infected animals.

Gene identity	log ₂
CXCL10 [IP-10]	7.17
IDO	6.42
IL-6	5.71
CXCL11	5.59
Interferon gamma	5.49
CCL7	5.03
CCL20	4.44
TLR1	4.22
Osteopontin	4.04
Oxidized-LDL receptor	3.95
TNF alpha	3.93
CCR2	3.54
IL-1 β	3.34
Peroxisomal biogenesis factor 11	-4.92
CD1-b	-3.68
Galectin-2	-2.79
Endothelin converting enzyme	-2.58
Catalase	-2.37
Annexin-7	-2.09

TABLE SIX

Major changes in gene expression in vaccinated then infected animals.

Gene identity	log ₂
CXCL10 [IP-10]	5.05
Interferon gamma	4.64
IDO	4.19
CCL7	3.89
Pinin	3.58
β ₂ -microglobulin	3.49
Caveolin	3.44
TLR1	3.32
Beta-spectrin	3.15
CD3 epsilon chain	3.03
CD109	2.81
Peroxisomal biogenesis factor 11	-5.19
Catenin	-3.91
CD1	-3.72
Heme oxygenase 2	-2.27
Catalase	-2.23
Thromboxane synthase	-2.15