Bacillus anthracis' lethal toxin induces broad transcriptional responses in human peripheral monocytes

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Abstract

Background: Anthrax lethal toxin (LT), produced by the Gram-positive bacterium Bacillus anthracis, is a highly effective zinc dependent metalloprotease that cleaves the N-terminus of mitogen-activated protein kinase kinases (MAPKKs or MEKs) and is known to play a role in impairing the host immune system during an inhalation anthrax infection. Here, we present the transcriptional responses of LT treated human monocytes in order to further elucidate the mechanisms of LT inhibition on the host immune system.

Results: Western Blot analysis demonstrated cleavage of endogenous MEK1 and MEK3 when human monocytes were treated with 500 ng/mL LT for four hours, proving their susceptibility to anthrax lethal toxin. Furthermore, staining with annexin V and propidium iodide revealed that LT treatment did not induce human peripheral monocyte apoptosis or necrosis. Using Affymetrix Human Genome U133 Plus 2.0 Arrays, we identified over 820 probe sets differentially regulated after LT treatment at the p < 0.001 significance level, interrupting the normal transduction of over 60 known pathways. As expected, the MAPKK signaling pathway was most drastically affected by LT, but numerous genes outside the well-recognized pathways were also influenced by LT including the IL-18 signaling pathway, Toll-like receptor pathway and the IFN alpha signaling pathway. Multiple genes involved in actin regulation, signal transduction, transcriptional regulation and cytokine signaling were identified after treatment with anthrax LT.

Conclusion: We conclude LT directly targets human peripheral monocytes and causes multiple aberrant gene responses that would be expected to be associated with defects in human monocyte's normal signaling transduction pathways and function. This study provides further insights into the mechanisms associated with the host immune system collapse during an anthrax infection, and suggests that anthrax LT may have additional downstream targets outside the well-known MAPK pathway.

Background

Bacillus anthracis, the causative agent of anthrax, is a gram-positive bacterium that is naturally found in the soil, and rarely affects the human population. Unfortunately, deliberate dissemination of anthrax spores is capable of delivering a highly potent and lethal air-borne bioterrorist agent, as documented in the 2001 U.S. anthrax attacks. Inhalation anthrax is a highly fatal, acute disease characterized by a rapid onset of systemic shock and ultimately death [1].

The most virulent strains of B. anthracis contain two plasmids, pXO2 and pXO1, encoding an antiphagocytic poly-D-glutamic acid capsule and three exotoxins: lethal factor, edema factor and protective antigen [2]. Protective antigen is an 83 kDa protein that is known to bind to two host cell receptors, TEM-8 and CMG-2, facilitating the entry of edema and/or lethal factor into host cells [3]. Lethal factor is a 90 kDa zinc-dependent metalloprotease that cleaves the N-terminus of mitogen-activated protein kinase kinases (MAPKKs or MEKs) [4,5]. Edema
factor is an 89 kDa adenylate cyclase that increases intracellular cAMP levels [6].

Previous studies using anthrax animal models have documented resistance to anthrax lethal toxin (LT) through depletion of host macrophages, suggesting that these cells play a critical role in anthrax LT induced lethality [7,8]. LT has also been shown to suppress cytokine responses by peripheral blood mononuclear cells, induce macrophage apoptosis, and prevent monocyte proliferation and differentiation [1,9,10]. Inhalation anthrax cases present clinical manifestations indicative of host immune collapse in humans and in nonhuman primate studies [11-13]. However, more recent studies investigating human monocytes and macrophages have suggested human alveolar macrophages are resistant to LT, and undifferentiated human monocytic cell lines are resistant to LT-induced death [10,14]. LT’s targeting of human monocytes/macrophages could help to explain the rapid onset of fatal symptoms and host demise during an inhalation anthrax infection, but the exact effects LT exerts on human peripheral monocytes, along with the mechanisms underlying the impairment of the host immune cell’s responses, have yet to be fully determined.

Previous studies investigating LT treated murine macrophages have shown a broad range in transcriptional effects induced by LT. These studies concluded LT-induced changes in macrophage inflammation, signaling, and transcription factors, along with changes in the immune response by macrophages. This study discovered the down regulation of CD-137 after LT treatment, shown to play a role in monocyte proliferation in response to LPS, and up regulation of plasminogen activator inhibitor type I, which results in fibrin deposits, massive imbalances in coagulation, and, in some instances, multi-organ failure [15,16]. Another study has measured the transcriptional responses of THP-1 cells after B. anthracis spore exposure, finding toxigenic B. anthracis strains suppress the cell signaling responses to infection [17].

Blood monocytes are mononuclear cells that play a major role in the host immune response through regulation of inflammatory responses, secretion of cytokine and antimicrobial factors, and direct pathogen clearance [18]. Monocytes are derived from monoblasts in the bone marrow, and circulate in the blood for 1-2 days before they migrate into tissues where they replenish the macrophage and dendritic pools [19-21]. Here, we determined human monocyte susceptibility to LT by demonstrating cleavage of MEKs, and utilized Affymetrix GeneChip® Human Genome U133 Plus 2.0 Arrays in order to identify additional mechanisms of LT impairment on the transcriptional responses of human peripheral monocytes. The arrays contained 54,675 probe sets representing over 22,000 of the best characterized human genes, providing extensive insights into the mechanisms behind LT induced dysfunction of human peripheral monocytes.

This study is the first to determine direct human monocyte susceptibility via cleavage of MEKs, along with the analysis of the transcriptional responses, to anthrax LT. The mechanisms of LT impairment on human peripheral monocytes will help elucidate the roles monocytes contribute during the host immune system collapse documented during an anthrax infection. The transcriptional analysis will serve to not only unravel the mechanisms behind the rapid onset of death in anthrax victims, but will also potentially provide new targets for controlling inflammation and enhancing host defense.

Results and discussion

Monocyte purity, apoptosis and susceptibility to anthrax LT

In order to first determine monocyte cell purity, isolated cells were analyzed using flow cytometry and gated using forward and side scatter, along with the monocytic marker, CD14. It was found that monocytes were isolated with a >85% purity (Figure 1A and 1B). Because previous reports have documented LT induced cell apoptosis, it was important to assure the transcriptional response of LT treated monocytes were independent of apoptosis. This was assured by the analysis of the necrosis and apoptosis markers, propidium iodide (PI) and annexin V, on human peripheral monocytes. Nearly all (99%) human peripheral monocytes showed no evidence of necrosis or apoptosis after a 4 h treatment of LT (Figure 1C and 1D). There has been some conflicting data suggesting monocytes, along with monocyte-derived cells, are not susceptible to the actions of anthrax LT. One study utilized human monocytic cell lines and found that undifferentiated monocytic cells did not undergo LT-induced cytotoxicity, while the differentiated cells were susceptible [10]. Another study investigating human alveolar macrophages (AM) found that these cells were relatively resistant to the actions of LT. It was ascertained that LT failed to suppress human AM cytokine responses, cleave MEKs, and induce apoptosis [14].

In order to explore the actions of LT on human peripheral monocytes, a Western Blot analysis was performed and MEK1, along with MEK3, cleavage was determined after a 4-hour treatment with LT. Human peripheral monocytes were found to be susceptible to the actions of LT as evidenced by cleavage of MEK1 and MEK3 (Figure 1E). HeLa cells were used as a positive control and β-actin was used to assure equal loading controls. We conclude that human peripheral monocytes are a direct target of anthrax LT.
Microarray analysis and results

Human peripheral monocytes were treated with LT or media alone, and microarray analysis was performed using four biological replicates from healthy volunteers. A total of 8 microarray hybridizations were employed and analyzed on Affymetrix Gene Chips® (HG U133 plus 2.0). The chips contained 54,675 probe sets and identified multiple differentially regulated pathways and genes by human peripheral monocytes after LT treatment. Unsupervised hierarchical analysis was used to assess the noise in the array experiments. First, probe sets whose signal intensity varied most in the data set were selected by applying a variation filter. Probes sets that displayed a coefficient of variation of greater than 0.5 were subjected to hierarchical analysis. The
clustering dendrogram showed the major node of separation between control and LT treated samples (Figure 2A).

To identify specific genes responsive to LT treatment, a paired t-test (by donor) was performed at a significance threshold of $p < 0.001$. Genes specified by 820 probe sets were found to be significant among the treatment groups (Table 1). The hierarchical cluster pattern of the significant probe sets is shown (Figure 3A). Of these probe sets, multiple gene products known to play a role in monocyte function were discovered (Figure 2B). The ability of probe sets significant at $p < 0.001$ to function as a classifier between treatment groups (LT treated vs. control) was established by leave-one-out-cross-validation and Monte Carlo simulations. Using 4 different prediction models, the classifier performed flawlessly. Of the significant genes identified, many are known to play a role in monocyte function (Figure 2C).

Using the Gene Set Expression Comparison Analysis, as implemented in BRB Array tools, the Biocarta pathways that were associated with the differentially regulated genes were identified. Over 60 differentially regulated pathways were discovered in monocytes in response to LT treatment. As expected, the most significant pathway affected by LT treatment was the MAPK signaling pathway, with the p38 MAPK signaling pathway being most impacted with 103 genes affected (Figure 3B). Additional pathways altered by LT at the $p < 0.001$ significance level included the IL-18, Toll-Like Receptor, IFN alpha, and G-Protein Family signaling pathways. It is interesting to note that a previous study measuring the transcriptional response of human alveolar macrophages to anthrax spores detected an activation of the TLR pathways [22], and our results indicated anthrax LT targets 87 genes within the TLR signaling pathway (Figure 3B).

RGS14 is a protein involved in the regulation of G-protein signaling through attenuation of G-protein heterotrimer signaling, thereby inactivating this signaling cascade. The Affymetrix microarrays revealed that RGS14 expression in LT treated monocytes showed a 6 fold increase in expression (Table 2). This is a potentially significant finding in that RGS14 inhibits G-proteins important for chemotaxis. Therefore LT could be impairing chemotaxis not only by blocking Hsp27 phosphorylation through disruption of the p38 pathway [23], but also by causing

**Figure 2** Unsupervised microarray analysis. A) Hierarchical clustering dendrogram showing similarities between expression patterns within each condition. Specimens were paired based on donor, using 4 separate donors as indicated in replica r1 through r4. B) Significant genes ($p < 0.001$) up or down regulated after LT treatment, along with their fold change, p-value and probe ID. C) Leave-one-out-cross validation was used to calculate mis-classification rate that yielded a 100% correct classification between pairs.
| Paramet. p-value | Geometric mean of intensities | Probe set | Gene symbol | Description |
|------------------|-----------------------------|----------|-------------|-------------|
| 1.78             | 222001_x_at                 | FAM91A2  | family with sequence similarity 91, member A2 |
| 1.25             | 218734_at                  | NAT11    | N-acetyltransferase 11 |
| 1.6              | 230350_at                  | NA       | NA          |
| 1.56             | 228930_at                  | NA       | NA          |
| 1.58             | 208661_s_at                | TTC3     | tetraicopeptide repeat domain 3 |
| 1.16             | 218716_x_at                | MTO1     | mitochondrial translation optimization 1 homolog (S. cerevisiae) |
| 1.15             | 238538_at                  | ANKRD11  | ankyrin repeat domain 11 |
| 2.92             | 225896_at                  | NA       | NA          |
| 3.87             | 227450_at                  | ERP27    | endoplasmic reticulum protein 27 kDa |
| 1.23             | 226602_s_at                | BCR      | breakpoint cluster region |
| 1.65             | 209123_at                  | QDPR     | quinoid dihydrorotidine reductase |
| 1.73             | 213934_s_at                | ZNF23    | zinc finger protein 23 (KOX 16) |
| 1.56             | 226419_s_at                | SFR51    | splicing factor, arginine/serine-rich 1 |
| 2.91             | 227946_at                  | OSBPL7   | oxysterol binding protein-like 7 |
| 1.84             | 242989_at                  | NA       | NA          |
| 1.92             | 242590_at                  | NA       | NA          |
| 1.36             | 204599_s_at                | LSM7     | LSM7 homolog, U6 small nuclear RNA associated (S. cerevisiae) |
| 1.68             | 225902_at                  | NA       | NA          |
| 1.29             | 220939_s_at                | DPP8     | dipetidyl-peptidase 8 |
| 1.2              | 218682_s_at                | SLC4A1AP | solute carrier family 4 (anion exchanger), member 1, adaptor protein |
| 2.21             | 212056_at                  | KIAA0182 | KIAA0182 |
| 2.87             | 222477_s_at                | TM7SF3   | transmembrane 7 superfamily member 3 |
| 2.01             | 202512_s_at                | ATG5     | ATG5 autophagy related 5 homolog (S. cerevisiae) |
| 1.52             | 209042_s_at                | UBE2G2   | ubiquitin-conjugating enzyme E2G 2 (UBC7 homolog, yeast) |
| 5.25             | 232181_at                  | LOC153346| hypothetical protein LOC153346 |
| 1.79             | 1554452_a_at               | HIG2     | hypoxia-inducible protein 2 |
| 2.11             | 228772_at                  | HNMT     | histamine N-methyltransferase |
| 1.3              | 221501_x_at                | LOC339047| hypothetical protein LOC339047 |
| 1.81             | 239038_at                  | C1orf52  | chromosome 1 open reading frame 52 |
| 1.95             | 203839_s_at                | TNK2     | tyrosine kinase, non-receptor, 2 |
| 1.89             | 227558_at                  | CBX4     | chromobox homolog 4 (Pc class homolog, Drosophila) |
| 1.4              | 214691_x_at                | FAM63B   | family with sequence similarity 63, member B |
| 1.32             | 228301_x_at                | NDUFB10  | NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10, 22kDa |
| 1.8              | 1556302_at                 | NA       | NA          |
| 3.27             | 229016_s_at                | TRERF1   | transcriptional regulating factor 1 |
| 3.85             | 223741_s_at                | TTYH2    | tweety homolog 2 (Drosophila) |
| 5.6              | 49306_at                   | RASSF4   | Ras association (RalGDS/AF-6) domain family member 4 |
| 1.49             | 32209_at                   | FAM89B   | family with sequence similarity 89, member B |
| 2.83             | 225208_at                  | PNKD     | paroxysmal nonkinesigenic dyskinesia |
| 1.59             | 228726_at                  | NA       | NA          |
| 1.17             | 1562984_at                 | NA       | NA          |
| 1.6              | 201639_s_at                | CPSF1    | cleavage and polyadenylation specific factor 1, 160kDa |
| 1.47             | 221649_s_at                | PPAN     | pet pan homolog (Drosophila) |
| 1.94             | 225360_at                  | TRABD    | TraB domain containing |
| 1.27             | 221005_s_at                | PTSD52   | phosphatidylycerine synthase 2 |
| 2.13             | 228914_at                  | NA       | NA          |
| Rank | P-value     | Fold Change | Gene ID | Description                                                                 |
|------|-------------|-------------|---------|-----------------------------------------------------------------------------|
| 47   | 6.47E-005   | 1.65        | 208206_s_at | RASGRP2 RAS guanyl releasing protein 2 (calcium and DAG-regulated)          |
| 48   | 6.49E-005   | 1.64        | 209198_s_at | SYT11 synaptotagmin XI                                                     |
| 49   | 6.55E-005   | 1.7         | 221575_s_at | SLY selenocysteine lyase                                                    |
| 50   | 6.74E-005   | 1.82        | 229969_at  | NA                                                                            |
| 51   | 6.76E-005   | 2.22        | 235513_at  | NA                                                                            |
| 52   | 6.77E-005   | 1.93        | 236922_at  | NA                                                                            |
| 53   | 6.78E-005   | 1.14        | 204364_s_at | RASGRP1 receptor accessory protein 1                                        |
| 54   | 6.87E-005   | 1.55        | 227025_at  | PHLN1 peripherin 1                                                          |
| 55   | 7.02E-005   | 1.78        | 227288_at  | SFRS12P1 SFRS12-interacting protein 1                                       |
| 56   | 7.13E-005   | 2.55        | 205075_at  | SERPINF2 serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 2 |
| 57   | 7.16E-005   | 2.29        | 229988_s_at | TMEM9 transmembrane protein 9                                              |
| 58   | 7.35E-005   | 1.39        | 231831_at  | COX19 COX19 cytochrome c oxidase assembly homolog (S. cerevisiae)           |
| 59   | 7.37E-005   | 1.88        | 221788_at  | NA                                                                            |
| 60   | 7.66E-005   | 1.61        | 236004_at  | NA                                                                            |
| 61   | 7.68E-005   | 1.85        | 219751_at  | SETD6 SET domain containing 6                                               |
| 62   | 7.92E-005   | 1.74        | 227273_at  | NA                                                                            |
| 63   | 8.67E-005   | 1.56        | 235787_at  | NA                                                                            |
| 64   | 8.91E-005   | 1.42        | 212888_at  | DICER1 dicer 1, ribonuclease type III                                        |
| 65   | 8.93E-005   | 1.93        | 1568593_a_at | NUDT16P nudix (nucleoside diphosphate linked moiety X)-type motif 16 pseudogene |
| 66   | 9.00E-005   | 1.92        | 226721_at  | DPY19L4 dpy-19-like 4 (C. elegans)                                          |
| 67   | 9.02E-005   | 1.39        | 227159_at  | GHDC GH3 domain containing                                                  |
| 68   | 9.14E-005   | 2.36        | 225982_at  | UBTF upstream binding transcription factor, RNA polymerase I                |
| 69   | 9.25E-005   | 1.62        | 220341_s_at | Csof4S chromosome 5 open reading frame 45                                  |
| 70   | 9.51E-005   | 1.36        | 214501_s_at | H2AFY H2A histone family, member Y                                          |
| 71   | 9.96E-005   | 7.6         | 226186_at  | NA                                                                            |
| 72   | 0.0001001   | 2.59        | 224946_s_at | CCDC115 coiled-coil domain containing 115                                  |
| 73   | 0.0001056   | 1.66        | 237059_at  | NA                                                                            |
| 74   | 0.0001076   | 3.11        | 38671_at   | PLXND1 plexin D1                                                            |
| 75   | 0.0001083   | 1.92        | 231912_s_at | DNF2P34B0335 DNF2P34B0335 protein                                           |
| 76   | 0.0001087   | 1.55        | 238492_at  | NA                                                                            |
| 77   | 0.0001088   | 2.05        | 228548_at  | NA                                                                            |
| 78   | 0.0001089   | 1.9         | 225757_s_at | CLMIN calmin (calponin-like, transmembrane)                                 |
| 79   | 0.0001093   | 1.15        | 203926_s_at | ATP5D ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit |
| 80   | 0.0001100   | 1.41        | 232520_s_at | NSFL1C NSFL1 (p97) cofactor (p47)                                            |
| 81   | 0.0001101   | 2.06        | 238012_at  | DPP7 dipeptidyl-peptidase 7                                                 |
| 82   | 0.0001101   | 1.38        | 211101_s_at | LILRA2 leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 2 |
| 83   | 0.0001109   | 1.35        | 210128_s_at | LTBP4 leukotriene B4 receptor                                               |
| 84   | 0.0001115   | 1.34        | 223393_s_at | TSHZ3 teashirt zinc finger homeobox 3                                       |
| 85   | 0.0001123   | 1.46        | 213628_at  | CLCC1 chloride channel CLIC-like 1                                          |
| 86   | 0.0001152   | 1.27        | 214870_s_at | LOC100132540 similar to LOC339047 protein                                  |
| 87   | 0.0001171   | 2.41        | 221756_at  | PFK3P1 phosphoinositide-3-kinase interacting protein 1                      |
| 88   | 0.0001171   | 1.62        | 222478_at  | VPS36 vacuolar protein sorting 36 homolog (S. cerevisiae)                   |
| 89   | 0.0001192   | 1.99        | 225719_s_at | MPRL55 mitochondrial ribosomal protein 15                                   |
| 90   | 0.0001206   | 1.87        | 212959_s_at | GNPTAB N-acetylglucosamine-1-phosphate transferase, alpha and beta subunits |
| 91   | 0.0001210   | 7.64        | 238520_at  | TRERF1 transcriptional regulating factor 1                                  |
| 92   | 0.0001235   | 3.04        | 226974_at  | NA                                                                            |
| Gene ID   | Fold Change | P-value   | Description                                                                 |
|-----------|-------------|-----------|------------------------------------------------------------------------------|
| 93        | 2.06        | 0.0001269 | farnesyltransferase, CAAX box, beta                                          |
| 94        | 2.04        | 0.0001288 | hypothetical protein LOC84792                                               |
| 95        | 1.46        | 0.0001306 | membrane protein, palmitoylated 5 (MAGUK p55 subfamily member 5)            |
| 96        | 1.5         | 0.0001312 | chromosome 1 open reading frame 85                                           |
| 97        | 1.19        | 0.0001336 | purine-rich element binding protein A                                        |
| 98        | 1.73        | 0.0001350 | spindlin family, member 3                                                   |
| 99        | 1.8         | 0.0001354 | phosphatidylinositol glycan anchor biosynthesis, class M                    |
| 100       | 2.61        | 0.0001371 | kelch repeat and BTB (POZ) domain containing 6                               |
| 101       | 1.44        | 0.0001408 | LOC154761                                                                    |
| 102       | 3.15        | 0.0001408 | v-myc myelocytomatosis viral oncogene homolog 1, lung carcinoma derived (avian) |
| 103       | 2.65        | 0.0001409 | protein phosphatase 1H (PP2C domain containing)                               |
| 104       | 1.61        | 0.0001414 | WD repeat domain 91                                                          |
| 105       | 2.07        | 0.0001418 | chromosome 9 open reading frame 140                                         |
| 106       | 1.92        | 0.0001426 | phosphoglucomutase 2                                                         |
| 107       | 1.74        | 0.0001451 | chromosome 2 open reading frame 68                                           |
| 108       | 1.49        | 0.0001454 | chromosome 5 open reading frame 44                                           |
| 109       | 1.8         | 0.0001462 | zinc finger protein 302                                                      |
| 110       | 1.17        | 0.0001537 | transducer of ERBB2, 1                                                      |
| 111       | 1.69        | 0.0001561 | golgi phosphoprotein 3-like                                                  |
| 112       | 1.32        | 0.0001565 | peroxiredoxin 5                                                             |
| 113       | 2.19        | 0.0001590 | interleukin 16 (lymphocyte chemoattractant factor)                          |
| 114       | 1.47        | 0.0001593 | protein phosphatase 2, regulatory subunit Bg#5%& beta isoform                |
| 115       | 1.48        | 0.0001599 | NA                                                                           |
| 116       | 1.14        | 0.0001599 | NA                                                                           |
| 117       | 1.54        | 0.0001646 | ARP1 actin-related protein 1 homolog B, centrinactin beta (yeast)            |
| 118       | 1.73        | 0.0001669 | NA                                                                           |
| 119       | 1.88        | 0.0001709 | chromosome 2 open reading frame 68                                           |
| 120       | 1.57        | 0.0001738 | anaphase promoting complex subunit 5                                         |
| 121       | 1.37        | 0.0001748 | mitogen-activated protein kinase kinase kinase 4                             |
| 122       | 2.81        | 0.0001762 | angiotensin II receptor-associated protein                                   |
| 123       | 3.66        | 0.0001767 | TBC1 domain family, member 4                                                |
| 124       | 2.06        | 0.0001784 | NOL1/NOP2/Sun domain family, member 5                                         |
| 125       | 1.69        | 0.0001787 | hematological and neurological expressed 1-like                              |
| 126       | 1.85        | 0.0001789 | zinc finger protein 589                                                      |
| 127       | 2.25        | 0.0001799 | tumor necrosis factor (ligand) superfamily, member 13                        |
| 128       | 2.07        | 0.0001836 | pre-B-cell leukemia homeobox interacting protein 1                           |
| 129       | 1.64        | 0.0001843 | DEAD (Asp-Glu-Ala-Asp) box polypeptide 51                                    |
| 130       | 1.99        | 0.0001852 | unc-119 homolog (C. elegans)                                                |
| 131       | 1.43        | 0.0001878 | fucokinase                                                                   |
| 132       | 3.32        | 0.0001878 | plexin D1                                                                    |
| 133       | 1.67        | 0.0001902 | small nuclear RNA activating complex, polypeptide 4, 190kDa                  |
| 134       | 1.84        | 0.0001983 | dehydrogenase/reductase (SDR family) member 4                                |
| 135       | 1.76        | 0.0001987 | family with sequence similarity 91, member A2                               |
| 136       | 1.27        | 0.0001990 | general transcription factor IIIC, polypeptide 2, beta 110kDa                |
| 137       | 1.81        | 0.0002007 | NA                                                                           |
| 138       | 1.31        | 0.0002010 | tripartite motif-containing 59                                               |
| 139       | 1.36        | 0.0002032 | NA                                                                           |
| Control vs Toxin corresponding P-value | Gene Expression | Description |
|--------------------------------------|-----------------|-------------|
| 0.0002081 4.03                      | LGR4            | leucine-rich repeat-containing G protein-coupled receptor 4 |
| 0.0002087 1.93                       | KSR1            | kinase suppressor of ras 1 |
| 0.0002093 1.45                       | NA              | NA          |
| 0.0002165 1.57                       | NA              | NA          |
| 0.0002167 1.4                        | AKR7A3          | aldo-keto reductase family 7, member A3 ( aflatoxin aldehyde reductase ) |
| 0.0002197 1.79                       | ANO8            | anoctamin 8 |
| 0.0002219 1.79                       | SLC19A1         | solute carrier family 19 (folate transporter), member 1 |
| 0.0002234 1.35                       | PIGC            | phosphatidylinositol glycan anchor biosynthesis, class C |
| 0.0002246 2.17                       | RUNX2           | runt-related transcription factor 2 |
| 0.0002281 1.24                       | VAMP4           | vesicle-associated membrane protein 4 |
| 0.0002334 1.35                       | ZNF763          | zinc finger protein 763 |
| 0.0002348 1.87                       | NA              | NA          |
| 0.0002362 1.53                       | ANAPC5          | anaphase promoting complex subunit 5 |
| 0.0002367 2.73                       | TBC1D4          | TBC1 domain family, member 4 |
| 0.0002416 1.35                       | NIP30           | NEFA-interacting nuclear protein NIP30 |
| 0.0002420 2.35                       | ATP2B4          | ATPase, Ca++ transporting, plasma membrane 4 |
| 0.0002425 1.4                        | BCS1L           | BCS1-like (yeast) |
| 0.0002450 1.85                       | NA              | NA          |
| 0.0002478 2.1                        | ANKDD1A         | ankyrin repeat and death domain containing 1A |
| 0.0002509 1.29                       | LOC399491       | LOC399491 protein |
| 0.0002517 1.66                       | NA              | NA          |
| 0.0002521 1.74                       | LOC100128223    | hypothetical protein LOC100128223 |
| 0.0002522 1.54                       | NA              | NA          |
| 0.0002531 2.4                        | CD47            | CD47 molecule |
| 0.0002559 1.53                       | NA              | NA          |
| 0.0002582 2.08                       | KCTD15          | potassium channel tetramerisation domain containing 1S |
| 0.0002606 4.49                       | CYP5A           | cytochrome b5 type A (microsomal) |
| 0.0002633 2.1                        | ROGDI           | rogdi homolog (Drosophila) |
| 0.0002634 1.86                       | NSUN5B          | NOL1/NOP2/Sun domain family, member 5B |
| 0.0002641 1.4                        | ABR             | active BCR-related gene |
| 0.0002648 2.01                       | DKFZP434B0335   | DKFZP434B0335 protein |
| 0.0002678 2.59                       | CP              | ceruloplasmin (ferrooxidase) |
| 0.0002697 1.78                       | NA              | NA          |
| 0.0002698 1.69                       | EPRS            | glutamyl-prolyl-tRNA synthetase |
| 0.0002699 2.67                       | IKZF1           | IKAROS family zinc finger 1 (Ikaros) |
| 0.0002715 1.44                       | LOC100137047-PLA2G4B | hypothetical protein LOC100137047-PLA2G4B |
| 0.0002762 1.68                       | SLCO3A1         | solute carrier organic anion transporter family, member 3A1 |
| 0.0002768 4.48                       | RASSF4          | Ras association (RalGDS/AF-6) domain family member 4 |
| 0.0002796 1.37                       | TMEM80          | transmembrane protein 80 |
| 0.0002806 1.49                       | TIMM51          | transmembrane 4 L six family member 19 |
| 0.0002817 1.66                       | NA              | NA          |
| 0.0002826 1.28                       | NA              | NA          |
| 0.0002838 2.77                       | NA              | NA          |
| 0.0002839 2.47                       | FJL20674        | hypothetical protein FJL20674 |
| 0.0002845 2.56                       | TOR3A           | torsin family 3, member A |
| 0.0002870 1.73                       | SFRS2B          | splicing factor, arginine/serine-rich 2B |
| Gene ID       | P-value | Fold Change | Description                                                                 |
|--------------|---------|-------------|------------------------------------------------------------------------------|
| 187          | 0.0002872 | 2.1         | 203317_at PSD4 pleckstrin and Sec7 domain containing 4                       |
| 188          | 0.0002969 | 1.32        | 238263_at LOC285965 hypothetical protein LOC285965                           |
| 189          | 0.0003005 | 2.64        | 235159_at NA NA                                                              |
| 190          | 0.0003043 | 1.24        | 218388_at PGLS 6-phosphogluconolactonase                                      |
| 191          | 0.0003057 | 1.3         | 206729_at TNFRSF8 tumor necrosis factor receptor superfamily, member 8       |
| 192          | 0.0003121 | 1.28        | 231130_at NA NA                                                              |
| 193          | 0.0003123 | 1.4         | 1552257_a_at TTLL12 tubulin tyrosine ligase-like family, member 12            |
| 194          | 0.0003129 | 1.29        | 219175_s_at SLC41A3 solute carrier family 41, member 3                       |
| 195          | 0.0003162 | 1.41        | 204786_s_at IFNAR2 interferon (alpha, beta and omega) receptor 2             |
| 196          | 0.0003216 | 1.35        | 227127_at TMEM110 transmembrane protein 110                                  |
| 197          | 0.0003219 | 3.64        | 202341_s_at TRIM2 tripartite motif-containing 2                              |
| 198          | 0.0003243 | 2.09        | 210731_s_at LGALS8 lectin, galactoside-binding, soluble, 8                   |
| 200          | 0.0003251 | 1.77        | 213374_s_at HIBCH 3-hydroxyisobutyryl-Coenzyme A hydrolase                   |
| 201          | 0.0003260 | 1.38        | 200931_s_at VCL vinculin                                                      |
| 202          | 0.0003264 | 1.45        | 230304_at NA NA                                                              |
| 203          | 0.0003271 | 2.03        | 235105_at FBXW2 F-box and WD repeat domain containing 2                      |
| 204          | 0.0003290 | 4.23        | 215726_s_at CYB5A cytochrome b5 type A (microsomal)                          |
| 205          | 0.0003300 | 2.01        | 242794_at MAML3 mastermind-like 3 (Drosophila)                               |
| 206          | 0.0003304 | 2.18        | 225961_at KCHDC5 kelch domain containing 5                                   |
| 207          | 0.0003309 | 1.48        | 212556_at SCRIB scribbled homolog (Drosophila)                               |
| 208          | 0.0003322 | 2.66        | 220494_s_at NA NA                                                            |
| 209          | 0.0003337 | 2.38        | 242297_at RREB1 ras responsive element binding protein 1                      |
| 210          | 0.0003349 | 1.9         | 228771_s_at ADRBK2 adrenergic, beta, receptor kinase 2                        |
| 211          | 0.0003390 | 1.19        | 227465_at KIAA0892 KIAA0892                                                  |
| 212          | 0.0003390 | 1.37        | 228667_at AGPAT4 1-acylglycerol-3-phosphate O-acyltransferase 4 (lysophosphatidic acid acyltransferase, delta) |
| 213          | 0.0003408 | 1.72        | 202161_at KPN1 protein kinase N1                                               |
| 214          | 0.0003417 | 1.32        | AFXX-LysX-M_at NA NA                                                          |
| 215          | 0.0003429 | 1.57        | 223314_at TSPAN14 tetraspanin 14                                              |
| 216          | 0.0003438 | 2.51        | 204610_s_at CCDC85B coiled-coil domain containing 85B                         |
| 217          | 0.0003438 | 2.03        | 218027_at MRPL15 mitochondrial ribosomal protein L15                           |
| 218          | 0.0003450 | 2.45        | 204718_at EPHB6 EPH receptor B6                                               |
| 219          | 0.0003454 | 2.06        | 227313_at CNPY4 canopoly 4 homolog (zebrafish)                                |
| 220          | 0.0003454 | 1.52        | 228600_s_at C7orf46 chromosome 7 open reading frame 46                       |
| 221          | 0.0003472 | 1.28        | 226335_at RPS5CA3 ribosomal protein 56 kinase, 90kDa, polypeptide 3           |
| 222          | 0.0003481 | 1.48        | 219147_s_at C9orf95 chromosome 9 open reading frame 95                        |
| 223          | 0.0003492 | 1.41        | 219801_s_at ZNF34 zinc finger protein 34                                      |
| 224          | 0.0003534 | 1.37        | 224865_s_at FAR1 fatty acyl CoA reductase 1                                   |
| 225          | 0.0003534 | 1.25        | 209450_at OSGEP O-sialoglycoprotein endopeptidase                             |
| 226          | 0.0003535 | 1.8         | 239016_s_at NA NA                                                            |
| 227          | 0.0003567 | 1.44        | 228670_at TEP1 telomerase-associated protein 1                                |
| 228          | 0.0003600 | 1.87        | 210580_s_at SULT1A3 sulfotransferase family, cytosolic, 1A, phenol-prefering, member 3 |
| 229          | 0.0003607 | 1.38        | 213945_s_at NUP210 nucleoporin 210kDa                                        |
| 230          | 0.0003644 | 1.32        | 218505_s_at WDR59 WD repeat domain 59                                        |
| 231          | 0.0003661 | 2.32        | 230343_at NA NA                                                              |
| 232          | 0.0003663 | 2.08        | 230888_at WDR91 WD repeat domain 91                                           |
| 233          | 0.0003691 | 1.87        | 226368_at CHST11 carbohydrate (chondroitin 4) sulfotransferase 11             |
| P-value | Gene Name | Description |
|---------|-----------|-------------|
| 2.34    | 213364_s_at | SNX1 sorting nexin 1 |
| 2.35    | 213626_at  | CBR4 carbonyl reductase 4 |
| 2.36    | AFFX-PheX-3_at | NA NA |
| 2.37    | 206567_s_at | PHF20 PHD finger protein 20 |
| 2.38    | 221090_s_at | OGFCO1 2-oxoglutarate and iron-dependent oxygenase domain containing 2 |
| 2.39    | 44040_at   | FBXO41 F-box protein 41 |
| 2.40    | 226238_at  | MCEE methylmalonyl CoA epimerase |
| 2.41    | 204562_at  | IRF4 interferon regulatory factor 4 |
| 2.42    | 226241_s_at | MRPL52 mitochondrial ribosomal protein L52 |
| 2.43    | 220178_at  | C19orf28 chromosome 19 open reading frame 28 |
| 2.44    | 209263_x_at | TSPAN4 tetraspanin 4 |
| 2.45    | 232228_at  | ZNF530 zinc finger protein 530 |
| 2.46    | 208760_at  | UBE2I ubiquitin-conjugating enzyme E2I (UBC9 homolog, yeast) |
| 2.47    | 224562_at  | WASF2 WAS protein family, member 2 |
| 2.48    | 213485_s_at | ABCC10 ATP-binding cassette, sub-family C (CFTR/MRP), member 10 |
| 2.49    | 202942_at  | ETFB electron-transfer-flavoprotein, beta polypeptide |
| 2.50    | AFFX-LysX-3_at | NA NA |
| 2.51    | 212135_s_at | ATP2B4 ATPase, Ca++ transporting, plasma membrane 4 |
| 2.52    | 217828_at  | SLTM SAFB-like, transcription modulator |
| 2.53    | 212875_s_at | C2CD2 C2 calcium-dependent domain containing 2 |
| 2.54    | 1557411_s_at | SLC25A43 solute carrier family 25, member 43 |
| 2.55    | 227117_at  | NA NA |
| 2.56    | 207124_s_at | GNB5 guanine nucleotide binding protein (G protein), beta 5 |
| 2.57    | 227607_at  | STAMBPL1 STAM binding protein-like 1 |
| 2.58    | 204538_x_at | NPIP nuclear pore complex interacting protein |
| 2.59    | 244619_at  | BCL10 B-cell CLL/lymphoma 10 |
| 2.60    | 223239_at  | C14orf129 chromosome 14 open reading frame 129 |
| 2.61    | 201087_at  | PXN paxillin |
| 2.62    | 219149_x_at | DBR1 debranching enzyme homolog 1 (S. cerevisiae) |
| 2.63    | 229905_at  | RAP1GDS1 RAP1, GTP-GDP dissociation stimulator 1 |
| 2.64    | 222111_at  | NA NA |
| 2.65    | 23052_s_at  | ZNF792 zinc finger protein 792 |
| 2.66    | 225748_at  | LTV1 LTV1 homolog (S. cerevisiae) |
| 2.67    | 241741_at  | CRLS1 cardiolipin synthase 1 |
| 2.68    | 221504_s_at | ATP6VIH ATPase, H+ transporting, lysosomal 50/57kDa, V1 subunit H |
| 2.69    | 213448_at  | NA NA |
| 2.70    | 201949_y_at | CAPZB capping protein (actin filament) muscle Z-line, beta |
| 2.71    | 234295_at  | DBR1 debranching enzyme homolog 1 (S. cerevisiae) |
| 2.72    | 217608_at  | SFRS12P1 SFRS12-interacting protein 1 |
| 2.73    | 215737_x_at | USF2 upstream transcription factor 2, c-fos interacting |
| 2.74    | 215873_s_at | ABCC10 ATP-binding cassette, sub-family C (CFTR/MRP), member 10 |
| 2.75    | 1552256_a_at | SCARB1 scavenger receptor class B, member 1 |
| 2.76    | 208657_s_at | 9-Sep septin 9 |
| 2.77    | 228066_at  | C1orf151 chromosome 1 open reading frame 151 |
| 2.78    | 222471_s_at | KCMF1 potassium channel modulatory factor 1 |
| 2.79    | 48808_at   | DHFR dihydrofolate reductase |
| 2.80    | 227228_s_at | CCDC88C coiled-coil domain containing 88C |
| 2.81    | 1558445_at | NA NA |
Table 1 Control vs Toxin corresponding P-value \( p < 0.001 \) (Continued)

| P-value | Gene ID/Description |
|---------|---------------------|
| 282 | 0.0004641 | 1.13 | 205540_s_at | RRAGB | Ras-related GTP binding B |
| 283 | 0.0004670 | 1.48 | 227239_at | FAM126A | family with sequence similarity 126, member A |
| 284 | 0.0004673 | 1.72 | 220246_at | CAMK1D | calcium/calmodulin-dependent protein kinase ID |
| 285 | 0.0004677 | 3.38 | 226478_at | NA | NA |
| 286 | 0.0004700 | 1.41 | 230235_at | NA | NA |
| 287 | 0.0004709 | 1.22 | 220790_s_at | LEPRE1 | leucine proline-enriched proteoglycan (leprecan) 1 |
| 288 | 0.0004727 | 2.69 | 223455_at | TCHP | trichoplein, keratin filament binding |
| 289 | 0.0004736 | 1.31 | 238552_at | NA | NA |
| 290 | 0.0004739 | 1.47 | 200827_at | PLOD1 | procollagen-lysine 1, 2-oxoglutarate 5-dioxygenase 1 |
| 291 | 0.0004751 | 1.28 | 227700_s_at | NA | NA |
| 292 | 0.0004785 | 4.77 | 238552_at | NA | NA |
| 293 | 0.0004788 | 1.88 | 242824_at | NA | NA |
| 294 | 0.0004793 | 1.08 | 215845_at | NA | NA |
| 295 | 0.0004795 | 1.92 | 211385_x_at | SULT1A2 | sulfotransferase family, cytosolic, 1A, phenol-prefering, member 2 |
| 296 | 0.0004800 | 1.38 | 226358_at | LOC145842 | hypothetical protein LOC145842 |
| 297 | 0.0004832 | 1.95 | 218854_s_at | PASK | PAS domain containing serine/threonine kinase |
| 298 | 0.0004841 | 5.65 | 205698_s_at | MAP2K6 | mitogen-activated protein kinase kinase 6 |
| 299 | 0.0004850 | 1.53 | 222611_at | AGGF1 | angiogenic factor with G patch and FHA domains 1 |
| 300 | 0.0004855 | 1.43 | 212036_s_at | PNN | pinin, desmosome associated protein |
| 301 | 0.0004858 | 1.6 | 244534_at | NA | NA |
| 302 | 0.0004876 | 1.58 | 1555751_a_at | GEMIN7 | gem (nuclear organelle) associated protein 7 |
| 303 | 0.0004879 | 1.87 | 203063_at | PPM1F | protein phosphatase 1F (PP2C domain containing) |
| 304 | 0.0004973 | 1.16 | 205922_at | VNN2 | vanin 2 |
| 305 | 0.0004975 | 1.23 | 202797_at | SACML1 | SAC1 suppressor of actin mutations 1-like (yeast) |
| 306 | 0.0005017 | 2.46 | 202826_at | SPINT1 | serine peptidase inhibitor, Kunitz type 1 |
| 307 | 0.0005059 | 1.73 | 226073_at | TMEM218 | transmembrane protein 218 |
| 308 | 0.0005077 | 1.55 | 238523_at | KLHL36 | kelch-like 36 (Drosophila) |
| 309 | 0.0005080 | 1.78 | 231843_at | DDX5S | DEAD (Asp-Glu-Ala-Asp) box polypeptide 55 |
| 310 | 0.0005094 | 2.26 | 219714_s_at | CACNA2D3 | calcium channel, voltage-dependent, alpha 2/delta subunit 3 |
| 311 | 0.0005097 | 2.02 | 229202_at | NA | NA |
| 312 | 0.0005114 | 3.54 | 290948_s_at | ZMYND8 | zinc finger, MYND-type containing 8 |
| 313 | 0.0005132 | 1.64 | 218473_s_at | GLT2D1 | glycosyltransferase 25 domain containing 1 |
| 314 | 0.0005172 | 1.71 | 65493_at | HEATR6 | HEAT repeat containing 6 |
| 315 | 0.0005179 | 2.03 | 236194_at | NA | NA |
| 316 | 0.0005179 | 2.28 | 225331_at | ORAI1 | ORAI calcium release-activated calcium modulator 1 |
| 317 | 0.0005201 | 1.58 | 219311_at | TRAPPC2 | trafficking protein complex 2 |
| 318 | 0.0005244 | 1.26 | 220036_s_at | LMBR1L | limb region 1 homolog (mouse)-like |
| 319 | 0.0005321 | 4.22 | 217997_at | TM7SF3 | transmembrane 7 superfamily member 3 |
| 320 | 0.0005335 | 1.26 | 211759_s_at | TBCB | tubulin folding cofactor B |
| 321 | 0.0005359 | 1.4 | 242155_s_at | NA | NA |
| 322 | 0.0005397 | 2 | 209377_s_at | HMGN3 | high mobility group nucleosomal binding domain 3 |
| 323 | 0.0005401 | 2.12 | 230653_at | LOC100132218 | hypothetical protein LOC100132218 |
| 324 | 0.0005504 | 1.77 | 224708_at | KIAA2013 | KIAA2013 |
| 325 | 0.0005504 | 1.9 | 204000_at | GNBS | guanine nucleotide binding protein (G protein), beta 5 |
| 326 | 0.0005559 | 1.32 | 244346_at | NA | NA |
| 327 | 0.0005568 | 1.9 | 225108_at | AGPS | alkylglycerone phosphate synthase |
| 328 | 0.0005599 | 1.85 | 236626_at | NA | NA |
| 329 | 0.0005615 | 1.85 | 228314_at | LRRRC8C | leucine rich repeat containing 8 family, member C |
Table 1 Control vs Toxin corresponding P-value p<0.001 (Continued)

| #  | P-value     | Gene Symbol | Description                       |
|----|-------------|-------------|-----------------------------------|
| 330 | 0.0005636   | ZNF763      | zinc finger protein 763           |
| 331 | 0.0005650   | FAM160B1    | family with sequence similarity 160, member B1 |
| 332 | 0.0005679   | NA          | NA                               |
| 333 | 0.0005686   | C9orf164    | chromosome 9 open reading frame 164 |
| 334 | 0.0005708   | C9orf25     | chromosome 9 open reading frame 25 |
| 335 | 0.0005724   | C12orf47    | chromosome 12 open reading frame 47 |
| 336 | 0.0005724   | ALKBH8      | alkB, alkylation repair homolog 8 (E. coli) |
| 337 | 0.0005728   | VKORC1      | vitamin K epoxide reductase complex, subunit 1 |
| 338 | 0.0005746   | DAPP1       | dual adaptor of phosphotyrosine and 3-phosphoinositides |
| 339 | 0.0005748   | GRB2        | growth factor receptor-bound protein 2 |
| 340 | 0.0005792   | FBX09       | F-box protein 9                   |
| 341 | 0.0005793   | ATR         | ataxia telangiectasia and Rad3 related |
| 342 | 0.0005805   | PSMC2       | proteasome (prosome, macropain) 265 subunit, ATPase, 2 |
| 343 | 0.0005806   | NHP2L1      | NH-P2 non-histone chromosome protein 2-like 1 (S. cerevisiae) |
| 344 | 0.0005811   | NA          | NA                               |
| 345 | 0.0005820   | DTD1        | D-tyrosyl-tRNA deacetylase 1 homolog (S. cerevisiae) |
| 346 | 0.0005858   | C12orf47    | chromosome 12 open reading frame 47 |
| 347 | 0.0005877   | SLC26A11    | solute carrier family 26, member 11 |
| 348 | 0.0005893   | TGLN2       | trans-golgi network protein 2     |
| 349 | 0.0005911   | SH3TC1      | SH3 domain and tetratricopeptide repeats 1 |
| 350 | 0.0005950   | NA          | NA                               |
| 351 | 0.0005992   | C21orf70    | chromosome 21 open reading frame 70 |
| 352 | 0.0006007   | ERAP2       | endoplasmic reticulum aminopeptidase 2 |
| 353 | 0.0006025   | ABCD4       | ATP-binding cassette, sub-family D (ALD), member 4 |
| 354 | 0.0006028   | DBI         | diazepam binding inhibitor (GABA receptor modulator, acyl-Coenzyme A binding protein) |
| 355 | 0.0006039   | CCDC69      | coiled-coil domain containing 69  |
| 356 | 0.0006041   | C2orf68     | chromosome 2 open reading frame 68 |
| 357 | 0.0006052   | TAF1        | TGFBI-induced anti-apoptotic factor 1 |
| 358 | 0.0006058   | LPCAT4      | lysophosphatidylcholine acyltransferase 4 |
| 359 | 0.0006135   | GSTK1       | glutathione S-transferase kappa 1 |
| 360 | 0.0006135   | GALNT6      | UDP-N-acetyl-alpha-D-galactosaminopolypeptide N-acetylgalactosaminyltransferase 6 (GalNAc-T6) |
| 361 | 0.0006147   | VEZF1       | vascular endothelial zinc finger 1 |
| 362 | 0.0006161   | C1orf85     | chromosome 1 open reading frame 85 |
| 363 | 0.0006190   | SULT1A2     | sulfotransferase family, cytosolic, 1A, phenol-prefering, member 2 |
| 364 | 0.0006205   | FLJ3046     | hypothetical gene supported by AK057608 |
| 365 | 0.0006228   | MICAL2      | microtubule associated monoxygenase, calponin and LIM domain containing 2 |
| 366 | 0.0006241   | C2orf64     | chromosome 2 open reading frame 64 |
| 367 | 0.0006246   | SULT1A1     | sulfotransferase family, cytosolic, 1A, phenol-prefering, member 1 |
| 368 | 0.0006275   | SULF2       | sulfatase 2                        |
| 369 | 0.0006277   | GOPC        | golgi associated PDZ and coiled-coil motif containing |
| 370 | 0.0006282   | USF2        | upstream transcription factor 2, c-fos interacting |
| 371 | 0.0006311   | FIGNL1      | fidgetin-like 1                    |
| 372 | 0.0006313   | MBP         | myelin basic protein               |
| 373 | 0.0006322   | FAM120C     | family with sequence similarity 120C |
| 374 | 0.0006326   | LONP1       | ion peptidase 1, mitochondrial     |
| 375 | 0.0006333   | NA          | NA                               |

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Table 1 Control vs Toxin corresponding P-value p<0.001 (Continued)

| s_at | 0.0006351 | 1.44 | 222294 | RAB27A, member RAS oncogene family |
|------|------------|------|--------|-----------------------------------|
| 376  | 0.0006411  | 3.49 | 210986_s | tropomyosin 1 (alpha) |
| 377  | 0.0006490  | 1.28 | 209932_s | deoxyuridine triphosphatase |
| 378  | 0.0006513  | 1.28 | 227656_s | chromosome 6 open reading frame 70 |
| 379  | 0.0006514  | 1.63 | 228131_at | excision repair cross-complementing rodent repair deficiency, complementation group 1 (includes overlapping antisense sequence) |
| 380  | 0.0006519  | 1.11 | 212848_s | chromosome 9 open reading frame 3 |
| 381  | 0.0006526  | 2.3  | 1552540_s | IQ motif containing D |
| 382  | 0.0006530  | 2.09 | 239698_at | NA |
| 383  | 0.0006586  | 1.62 | 1553102_a | coiled-coil domain containing 69 |
| 384  | 0.0006622  | 1.77 | 228542_at | MRS2 magnesium homeostasis factor homolog (S. cerevisiae) |
| 385  | 0.0006623  | 1.37 | 208956_s | deoxyuridine triphosphatase |
| 386  | 0.0006635  | 2.14 | 223528_s | METT11D1 methyltransferase 11 domain containing 1 |
| 387  | 0.0006636  | 1.38 | 201234_at | ILK integrin-linked kinase |
| 388  | 0.0006637  | 1.57 | 228694_at | NA |
| 389  | 0.0006659  | 1.36 | 225136_at | pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 2 |
| 390  | 0.0006723  | 1.55 | 212567_s | MAP4 microtubule-associated protein 4 |
| 391  | 0.0006726  | 1.52 | 219549_s | reticulin 3 |
| 392  | 0.0006730  | 1.89 | 232681_at | NA |
| 393  | 0.0006742  | 2.21 | 219627_at | ZNF767 zinc finger family member 767 |
| 394  | 0.0006762  | 2.7  | 231449_at | NA |
| 395  | 0.0006771  | 1.58 | 239035_at | MTHFR S,10-methyltetrahydrofolate reductase (NADPH) |
| 396  | 0.0006773  | 1.39 | 205256_at | ZBTB39 zinc finger and BTB domain containing 39 |
| 397  | 0.0006786  | 1.51 | 205945_at | IL6R interleukin 6 receptor |
| 398  | 0.0006802  | 4.26 | 230032_at | O-sialoglycoprotein endopeptidase-like 1 |
| 399  | 0.0006837  | 1.56 | 225888_at | chromosome 12 open reading frame 30 |
| 400  | 0.0006840  | 1.35 | 227767_at | casein kinase 1, gamma 3 |
| 401  | 0.0006879  | 1.76 | 205060_at | PARG poly (ADP-ribose) glycohydrolase |
| 402  | 0.0006921  | 1.37 | 239730_at | DiGeorge syndrome critical region gene 14 |
| 403  | 0.0006924  | 1.58 | 201029_s | CD99 CD99 molecule |
| 404  | 0.0006928  | 1.63 | 211709_s | CLEC11A C-type lectin domain family 11, member A |
| 405  | 0.0006952  | 1.95 | 201985_at | KIAA0196 KIAA0196 |
| 406  | 0.0006964  | 2.17 | 204995_at | cyclin-dependent kinase 5, regulatory subunit 1 (p35) |
| 407  | 0.0007029  | 1.52 | 217521_at | NA |
| 408  | 0.0007045  | 1.35 | 1558184_s | zinc finger protein 17 |
| 409  | 0.0007099  | 1.24 | 218167_at | archaelysin family metallopeptidase 2 |
| 410  | 0.0007119  | 1.52 | 226712_at | SSR1 signal sequence receptor, alpha |
| 411  | 0.0007129  | 1.22 | 238668_at | NA |
| 412  | 0.0007138  | 1.16 | 221651_s | immunoglobulin kappa constant |
| 413  | 0.0007143  | 1.85 | 64064_at | GIMAP5 GTPase, IMAP family member 5 |
| 414  | 0.0007160  | 1.24 | 234734_s | trinucleotide repeat containing 6A |
| 415  | 0.0007165  | 1.34 | 213582_at | ATP11A ATPase, class VI, type 11A |
| 416  | 0.0007176  | 1.34 | 226165_at | chromosome 8 open reading frame 59 |
| 417  | 0.0007186  | 2.61 | 205565_s | frataxin |
| 418  | 0.0007225  | 1.21 | 220251_at | chromosome 1 open reading frame 107 |
| 419  | 0.0007231  | 2.16 | 225980_at | chromosome 14 open reading frame 43 |
| 420  | 0.0007247  | 1.69 | 238379_s | NA |
| 421  | 0.0007275  | 1.63 | 213698_s | NA |
### Table 1 Control vs Toxin corresponding P-value $p<0.001$ (Continued)

| P-value   | Symbol   | Gene Symbol   | Description                                                                 |
|-----------|----------|---------------|-----------------------------------------------------------------------------|
| 0.0007266 | 1.72     | 1559034_at    | SIRPB2 signal-regulatory protein beta 2                                     |
| 0.0007273 | 1.21     | 201053_s_at   | PSMF1 proteasome (prosome, macropain) inhibitor subunit 1 (PI31)             |
| 0.0007318 | 1.1      | 40225_at      | GAK cyclin G associated kinase                                             |
| 0.0007329 | 2.14     | 209729_at     | GAS2L1 growth arrest-specific 2 like 1                                     |
| 0.0007344 | 1.55     | 221027_s_at   | PLA2G12A phospholipase A2, group XIIA                                        |
| 0.0007348 | 1.28     | 209724_s_at   | ZFP161 zinc finger protein 161 homolog (mouse)                              |
| 0.0007380 | 1.4      | 214494_s_at   | SPG7 spastic paraplegia 7 (pure and complicated autosomal recessive)       |
| 0.0007392 | 1.58     | 205131_x_at   | CLEC11A C-type lectin domain family 11, member A                           |
| 0.0007393 | 2.07     | 204019_s_at   | SH3YL1 SH3 domain containing, Ysc84-like 1 (S. cerevisiae)                 |
| 0.0007417 | 1.42     | 214861_at     | JMJD2C jumonji domain containing 2C                                         |
| 0.0007421 | 1.69     | 242965_at     | NA NA                                                                       |
| 0.0007485 | 1.99     | 228167_at     | KLHL6 kelch-like 6 (Drosophila)                                            |
| 0.0007547 | 2.15     | 209269_s_at   | SYK spleen tyrosine kinase                                                  |
| 0.0007563 | 1.5      | 244663_at     | NA NA                                                                       |
| 0.0007563 | 2.14     | 203802_x_at   | NSUN5 NOL1/NOP2/Sun domain family, member 5                                |
| 0.0007578 | 1.62     | 242108_at     | NA NA                                                                       |
| 0.0007655 | 1.46     | 205632_s_at   | PI3K1B phosphatidylinositol-4-phosphate 5-kinase, type I, beta              |
| 0.0007691 | 2.28     | 230925_at     | APBB1IP amyloid beta (A4) precursor protein-binding, family B, member 1     |
| 0.0007712 | 2.99     | 212757_s_at   | CAMK2G calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma    |
| 0.0007796 | 1.29     | 223716_s_at   | ZRANB2 zinc finger, RAN-binding domain containing 2                          |
| 0.0007811 | 1.3      | 206881_s_at   | LILRA3 leukocyte immunoglobulin-like receptor, subfamily A (without TM domain), member 3 |
| 0.0007841 | 2.01     | 214861_at     | NA NA                                                                       |
| 0.0007848 | 1.59     | 222622_at     | PGP phosphoglycolate phosphatase                                           |
| 0.0007851 | 1.62     | 218231_at     | NAGK N-acetylglucosamine kinase                                             |
| 0.0007878 | 1.79     | 1554544_a_at  | MBP myelin basic protein                                                    |
| 0.0007894 | 2.2      | 1554250_s_at  | TRIM73 tripartite motif-containing 73                                      |
| 0.0007896 | 2.19     | 216199_s_at   | MAP3K4 mitogen-activated protein kinase kinase 4                            |
| 0.0007925 | 1.3      | 223249_at     | TROVE2 TROVE domain family, member 2                                        |
| 0.0007966 | 1.65     | 226671_at     | PRR12 proline rich 12                                                       |
| 0.0007989 | 1.67     | 202534_x_at   | DHFR dihydrofolate reductase                                               |
| 0.0007995 | 2.43     | 202369_s_at   | TRAM2 translocation associated membrane protein 2                          |
| 0.0008009 | 2.59     | 218112_at     | MRPS34 mitochondrial ribosomal protein S34                                  |
| 0.0008035 | 1.48     | 230925_s_at   | APBB1IP amyloid beta (A4) precursor protein-binding, family B, member 1     |
| 0.0008086 | 1.16     | 213027_at     | TROVE2 TROVE domain family, member 2                                        |
| 0.0008124 | 2.99     | 1562289_at    | NA NA                                                                       |
| 0.0008148 | 1.41     | 202615_at     | GNAQ guanine nucleotide binding protein (G protein), q polypeptide          |
| 0.0008150 | 1.71     | 219151_s_at   | RABL2B RAB, member of RAS oncogene family-like 2B                           |
| 0.0008158 | 2.1      | 1559214_at    | NA NA                                                                       |
| 0.0008161 | 1.84     | 203711_s_at   | HIBCH 3-hydroxyisobutanyl-Coenzyme A hydrolase                             |
| 0.0008187 | 1.87     | 233955_x_at   | CXXCS CXXC finger 3                                                        |
| 0.0008205 | 1.26     | 201804_x_at   | TBCB tubulin folding cofactor B                                            |
| 0.0008207 | 1.44     | 211100_s_at   | LILRA2 leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 2 |
| 0.0008229 | 5.13     | 212757_s_at   | CAMK2G calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma    |
| 0.0008252 | 1.76     | 214202_at     | NA NA                                                                       |
### Table 1 Control vs Toxin corresponding P-value \( p < 0.001 \) (Continued)

| Gene ID | P-value | Fold Change | Description |
|---------|---------|-------------|-------------|
| 468     | 0.0008255 | 2.01 | UBL4A ubiquitin-like 4A |
| 469     | 0.0008277 | 1.35 | PRDX5 peroxiredoxin 5 |
| 470     | 0.0008278 | 1.41 | DBI diazepam binding inhibitor (GABA receptor modulator, acyl-Coenzyme A binding protein) |
| 471     | 0.0008279 | 1.53 | KCME1 potassium channel modulatory factor 1 |
| 472     | 0.0008283 | 1.29 | ANXA11 annexin A11 |
| 473     | 0.0008318 | 1.72 | INPP5F inositol polyphosphate-5-phosphatase F |
| 474     | 0.0008344 | 1.9 | LRP8 low density lipoprotein receptor-related protein 8, apolipoprotein e receptor |
| 475     | 0.0008347 | 1.42 | INSI2 insulin induced gene 2 |
| 476     | 0.0008361 | 1.54 | EBPL emopamil binding protein-like |
| 477     | 0.0008444 | 3.9 | TLR5 toll-like receptor 5 |
| 478     | 0.0008451 | 1.44 | ZNF512 zinc finger protein 512 |
| 479     | 0.0008463 | 1.9 | NA |
| 480     | 0.0008510 | 1.22 | HTRA2 HtrA serine peptidase 2 |
| 481     | 0.0008526 | 1.9 | ERMP1 endoplasmic reticulum metalloproteinase 1 |
| 482     | 0.0008590 | 1.42 | RPUSD1 RNA pseudouridylate synthase domain containing 1 |
| 483     | 0.0008602 | 1.89 | DDB2 damage-specific DNA binding protein 2, 48kDa |
| 484     | 0.0008607 | 1.79 | CXXC5 CXXC finger 5 |
| 485     | 0.0008619 | 1.42 | WDFY4 WDFY family member 4 |
| 486     | 0.0008625 | 1.21 | SMPD1 sphingomyelin phosphodiesterase 1, acid lysosomal |
| 487     | 0.0008648 | 2.16 | MDH2 malate dehydrogenase 2, NAD (mitochondrial) |
| 488     | 0.0008654 | 1.57 | ANAPC4 anaphase promoting complex subunit 4 |
| 489     | 0.0008674 | 1.63 | FLJ27365 FLJ27365 protein |
| 490     | 0.0008682 | 2.88 | PHF15 PHD finger protein 15 |
| 491     | 0.0008701 | 2.47 | SYT11 synaptotagmin XI |
| 492     | 0.0008755 | 1.49 | NOL5A nucleolar protein SA (56kDa with KRE/D repeat) |
| 493     | 0.0008868 | 5.04 | IL8RB interleukin 8 receptor, beta |
| 494     | 0.0008925 | 1.33 | HSPA1L heat shock 70kDa protein 1-like |
| 495     | 0.0008958 | 1.35 | C16orf80 chromosome 16 open reading frame 80 |
| 496     | 0.0008961 | 1.64 | PPP2R5E protein phosphatase 2, regulatory subunit 8g@#%&£, epsilon isoform |
| 497     | 0.0008967 | 1.56 | MOBKL1A MOB1, Mps One Binder kinase activator-like 1A (yeast) |
| 498     | 0.0009020 | 1.98 | KIAA1609 KIAA1609 |
| 499     | 0.0009056 | 1.82 | SIGIRR single immunoglobulin and toll-interleukin 1 receptor (TIR) domain |
| 500     | 0.0009103 | 1.06 | NA |
| 501     | 0.0009114 | 1.51 | NA |
| 502     | 0.0009136 | 1.47 | IL6R interleukin 6 receptor |
| 503     | 0.0009154 | 1.68 | LOC285965 hypothetical protein LOC285965 |
| 504     | 0.0009189 | 3.86 | KBTBD11 kelch repeat and BTB (POZ) domain containing 11 |
| 505     | 0.0009197 | 1.67 | RER1 retention in endoplasmic reticulum 1 homolog (S. cerevisiae) |
| 506     | 0.0009228 | 1.32 | C7orf42 chromosome 7 open reading frame 42 |
| 507     | 0.0009245 | 1.91 | KIAA1609 KIAA1609 |
| 508     | 0.0009272 | 1.24 | NA |
| 509     | 0.0009308 | 5.94 | RGS14 regulator of G-protein signaling 14 |
| 510     | 0.0009357 | 3.85 | ZNF362 zinc finger protein 362 |
| 511     | 0.0009370 | 1.35 | NA |
| 512     | 0.0009378 | 1.73 | PTCD3 Pentatricopeptide repeat domain 3 |
| 513     | 0.0009417 | 1.63 | PON2 paraoxonase 2 |
| ID   | log2foldchange | Log2_P_value | Gene Symbol | Gene Description |
|------|----------------|--------------|-------------|------------------|
| 514  | 1.44           | 0.0009436    | SHCBP1      | SHC SH2-domain binding protein 1 |
| 515  | 1.38           | 0.0009471    | MLLT10      | myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to 10 |
| 516  | 1.67           | 0.0009526    | NLRP1       | NLR family, pyrin domain containing 1 |
| 517  | 1.42           | 0.0009562    | SRPK1       | SFRS protein kinase 1 |
| 518  | 1.63           | 0.0009575    | PRKACB      | protein kinase, cAMP-dependent, catalytic, beta |
| 519  | 1.43           | 0.0009592    | NA          | NA |
| 520  | 1.25           | 0.0009614    | FXYD5       | FXYD domain containing ion transport regulator 5 |
| 521  | 1.45           | 0.0009626    | CUTA        | cutA divalent cation tolerance homolog (E. coli) |
| 522  | 1.7            | 0.0009634    | DBT         | dihydrolipoamide branched chain transacylase E2 |
| 523  | 1.17           | 0.0009679    | C7orf44     | chromosome 7 open reading frame 44 |
| 524  | 1.14           | 0.0009703    | NA          | NA |
| 525  | 1.7            | 0.0009717    | RN1F70      | ring finger protein 170 |
| 526  | 1.31           | 0.0009730    | GPSM2       | G-protein signaling regulator 2 (AGS3-like, C. elegans) |
| 527  | 1.73           | 0.0009732    | CTSD        | cathepsin D |
| 528  | 1.41           | 0.0009734    | MGC27345    | hypothetical protein MGC27345 |
| 529  | 1.29           | 0.0009738    | POLR2K      | polymerase (RNA) II (DNA directed) polypeptide K, 7.0kDa |
| 530  | 1.58           | 0.0009760    | NUFIP2      | nuclear fragile X mental retardation protein interacting protein 2 |
| 531  | 2.26           | 0.0009764    | MAP3K4      | mitogen-activated protein kinase kinase kinase 4 |
| 532  | 1.93           | 0.0009776    | CALM1       | calmodulin 1 (phosphorylase kinase, delta) |
| 533  | 2.65           | 0.0009778    | GARNL4      | GTPase activating Rap/RanGAP domain-like 4 |
| 534  | 1.48           | 0.0009805    | NA          | NA |
| 535  | 1.84           | 0.0009828    | NA          | NA |
| 536  | 1.58           | 0.0009900    | LDHB        | lactate dehydrogenase B |
| 537  | 1.81           | 0.0009925    | MBOAT1      | membrane bound O-acyltransferase domain containing 1 |
| 538  | -1.19          | 0.0009997    | NA          | NA |
| 539  | -1.6           | 0.0009983    | B9D2        | B9 protein domain 2 |
| 540  | -1.71          | 0.0009949    | KIAA0999    | KIAA0999 protein |
| 541  | -1.23          | 0.0009891    | SSBP1       | single-stranded DNA binding protein 1 |
| 542  | -1.47          | 0.0009868    | TME167A     | transmembrane protein 167A |
| 543  | -1.75          | 0.0009825    | IFIT5       | interferon-induced protein with tetraticopeptide repeats 5 |
| 544  | -1.67          | 0.0009805    | DDX60       | DEAD (Asp-Glu-Ala-Asp) box polypeptide 60 |
| 545  | -1.52          | 0.0009758    | HUS1        | HUS1 checkpoint homolog (S. pombe) |
| 546  | -2.4           | 0.0009746    | ETNK1       | ethanolamine kinase 1 |
| 547  | -1.24          | 0.0009745    | PPP1R12B    | protein phosphatase 1, regulatory (inhibitor) subunit 12B |
| 548  | -1.26          | 0.0009737    | NA          | NA |
| 549  | -1.33          | 0.0009681    | RAB27A      | RAB27A, member RAS oncogene family |
| 550  | -2.54          | 0.0009661    | RASAL2      | RAS protein activator like 2 |
| 551  | -1.49          | 0.0009592    | FCR2C       | Fc fragment of IgG, low affinity IIc, receptor for (CD32) |
| 552  | -3.72          | 0.0009568    | VRK2        | vaccinia related kinase 2 |
| 553  | -1.22          | 0.0009560    | LOC338809   | hypothetical protein LOC338809 |
| 554  | -2.66          | 0.0009506    | CCN5        | chemokine (C-C motif) receptor 5 |
| 555  | -1.47          | 0.0009500    | UBX7        | UBX domain protein 7 |
| 556  | -1.25          | 0.0009443    | NA          | NA |
| 557  | -1.21          | 0.0009406    | KIAA1632    | KIAA1632 |
| 558  | -1.12          | 0.0009366    | COL1         | collagen |
| 559  | -2.95          | 0.0009338    | LIPA        | lipase A, lysosomal acid, cholesterol esterase |
| Gene Symbol | P-value | Fold Change | Description |
|-------------|---------|-------------|-------------|
| MYST4       | 0.0009309 | -1.3 | MYST histone acetyltransferase (monocytic leukemia) 4 |
| HPSE        | 0.0009278 | -2.58 | heparanase |
| 2-Sep       | 0.0009220 | -1.58 | septin 2 |
| CNOT4       | 0.0009134 | -1.08 | CCR4-NOT transcription complex, subunit 4 |
| SDCCAG8     | 0.0009077 | -1.25 | serologically defined colon cancer antigen 8 |
| ITGA6       | 0.0009071 | -1.7 | integrin, alpha 6 |
| MYST4       | 0.0009063 | -3.06 | MYST histone acetyltransferase (monocytic leukemia) 4 |
| GSTZ1       | 0.0009031 | -1.27 | glutathione transferase zeta 1 |
| DDR1        | 0.0008974 | -1.42 | discoidin domain receptor tyrosine kinase 1 |
| FANCc       | 0.0008959 | -1.19 | Fanconi anemia, complementation group C |
| EML4        | 0.0008945 | -1.16 | echinoderm microtubule associated protein like 4 |
| RAB28       | 0.0008922 | -1.32 | RAB28, member RAS oncogene family |
| DHR9S       | 0.0008910 | -3.14 | dehydrogenase/reductase (SDR family) member 9 |
| N4BP1       | 0.0008877 | -1.67 | NEDD4 binding protein 1 |
| U2AF1       | 0.0008870 | -1.29 | U2 small nuclear RNA auxiliary factor 1 |
| GSTZ1       | 0.0008861 | -1.35 | glutathione transferase zeta 1 |
| SP2         | 0.0008869 | -1.32 | Sp2 transcription factor |
| ZNF1X       | 0.0008859 | -1.87 | zinc finger, NF1-type containing 1 |
| SIPATL2     | 0.0008850 | -1.13 | signal-induced proliferation-associated 1 like 2 |
| RGR         | 0.0008857 | -1.28 | retinoid G protein coupled receptor |
| KPN4A       | 0.0008853 | -1.22 | karyopherin alpha 4 (importin alpha 3) |
| GMF1        | 0.0008843 | -1.07 | G elongation factor, mitochondrial 1 |
| ARFGAP2     | 0.0008840 | -1.33 | ADP-ribosylation factor GTPase activating protein 2 |
| NA          | 0.0008835 | -1.24 | NA |
| SEC14L1     | 0.0008834 | -1.49 | SEC14-like 1 (S. cerevisiae) |
| ARL17       | 0.0008824 | -1.34 | ADP-ribosylation factor-like 17 |
| NA          | 0.0008819 | -1.33 | NA |
| EMP1        | 0.0008816 | -2.91 | epithelial membrane protein 1 |
| YPEL5       | 0.0008817 | -1.42 | yippee-like 5 (Drosophila) |
| SFQ         | 0.0008814 | -1.3 | splicing factor proline/glutamine-rich (polypyrimidine tract binding protein associated) |
| THEM4       | 0.0008810 | -1.35 | thioesterase superfamily member 4 |
| CDC42S1     | 0.0008803 | -1.53 | CDC42 small effector 1 |
| SEH1L       | 0.0007993 | -1.38 | SEH1-like (S. cerevisiae) |
| NA          | 0.0007951 | -1.4 | NA |
| TRIM25      | 0.0007921 | -3.08 | tripartite motif-containing 25 |
| ACTR2       | 0.0007824 | -1.55 | ARP2 actin-related protein 2 homolog (yeast) |
| PCTIK1      | 0.0007777 | -1.32 | PCTAIRE protein kinase 1 |
| KAT5        | 0.0007732 | -1.36 | K(lysine) acetyltransferase 5 |
| NAPA        | 0.0007729 | -1.47 | N-ethylmaleimide-sensitive factor attachment protein, alpha |
| DNAJC21     | 0.0007690 | -1.16 | DnaJ (Hsp40) homolog, subfamily C, member 21 |
| CACNA1G     | 0.0007673 | -1.31 | calcium channel, voltage-dependent, T type, alpha 1G subunit |
| SYNCRIP     | 0.0007657 | -1.53 | synaptotagmin binding, cytoplasmic RNA interacting protein |
| CD164       | 0.0007625 | -1.85 | CD164 molecule, sialomucin |
| SH3GLB1     | 0.0007608 | -1.45 | SH3-domain GRB2-like endophilin B1 |
| NA          | 0.0007426 | -1.25 | NA |
| XRN1        | 0.0007405 | -2.01 | 5'->3' exoribonuclease 1 |
Table 1 Control vs Toxin corresponding P-value p<0.001 (Continued)

| P-value       | log2FoldChange | probe_set_id | description                                                                 |
|---------------|----------------|--------------|-----------------------------------------------------------------------------|
| 0.00007371    | -1.41          | 236961_at    | NA                                                                          |
| 0.00007346    | -1.14          | 204080_at    | TOE1 target of EGR1, member 1 (nuclear)                                     |
| 0.00007341    | -1.29          | 243852_at    | LUC7L2 LUC7-like 2 (S. cerevisiae)                                          |
| 0.00007227    | -1.29          | 210317_s_at  | YWAHE tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide |
| 0.00007183    | -1.14          | 209284_s_at  | C3orf63 chromosome 3 open reading frame 63                                 |
| 0.00007193    | -1.29          | 243852_at    | LUC7L2 LUC7-like 2 (S. cerevisiae)                                          |
| 0.00007184    | -1.09          | 202814_s_at  | HEXIM1 hexamethylene bis-acetamide inducible 1                              |
| 0.00006943    | -1.35          | 226710_at    | C8orf82 chromosome 8 open reading frame 82                                  |
| 0.00006932    | -3.2           | 209969_s_at  | STAT1 signal transducer and activator of transcription 1, 91kDa             |
| 0.00006882    | -1.56          | 225242_s_at  | CCDC80 coiled-coil domain containing 80                                     |
| 0.00006875    | -1.5           | 214121_s_at  | PDIM7 PDZ and LIM domain 7 (enigma)                                         |
| 0.00006868    | -1.41          | 203916_at    | N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 2                   |
| 0.00006826    | -1.32          | 208901_s_at  | TOP1 topoisomerase (DNA) I                                                  |
| 0.00006769    | -2.95          | 200028_s_at  | MERTK c-mer proto-oncogene tyrosine kinase                                   |
| 0.00006749    | -1.35          | 205724_at    | PKP1 plakophilin 1 (ectodermal dysplasia/skin fragility syndrome)            |
| 0.00006737    | -1.23          | 228121_at    | NA                                                                          |
| 0.00006701    | -1.04          | 226928_s_at  | SLC25A37 solute carrier family 25, member 37                               |
| 0.00006670    | -1.37          | 1553301_at   | DIP2A DIP2 disco-interacting protein 2 homolog A (Drosophila)               |
| 0.00006616    | -1.27          | 1566301_at   | PPPR11 protein phosphatase 1, regulatory (inhibitor) subunit 11             |
| 0.00006570    | -1.46          | 234519_at    | NOBOX NOBOX oogenesis homeobox                                              |
| 0.00006553    | -1.24          | 218520_at    | TBK1 TANK-binding kinase 1                                                  |
| 0.00006552    | -1.55          | 201878_at    | ARIH1 ariadne homolog, ubiquitin-conjugating enzyme E2 binding protein, 1 (Drosophila) |
| 0.00006495    | -1.3           | 1564131_at   | NA                                                                          |
| 0.00006472    | -1.49          | 209102_s_at  | HBP1 HMG-box transcription factor 1                                         |
| 0.00006450    | -1.18          | 238586_at    | LOC731489 hypothetical protein LOC731489                                    |
| 0.00006425    | -1.03          | 216231_s_at  | B2M beta-2-microglobulin                                                    |
| 0.00006398    | -1.79          | 1552772_at   | CLEC4D C-type lectin domain family 4, member D                              |
| 0.00006384    | -1.42          | 201586_s_at  | SFPQ splicing factor proline/glutamine-rich (polypyrimidine tract binding protein associated) |
| 0.00006373    | -1.74          | 41644_at     | SASH1 SAM and SH3 domain containing 1                                        |
| 0.00006346    | -1.11          | 216652_s_at  | DR1 down-regulator of transcription 1, TBP-binding (negative cofactor 2)    |
| 0.00006313    | -1.23          | 212436_at    | TRIM33 tripartite motif-containing 33                                       |
| 0.00006284    | -1.57          | 212264_s_at  | WAPAL wings apart-like homolog (Drosophila)                                 |
| 0.00006259    | -1.2           | 226481_at    | VPRBP Vpr (HIV-1) binding protein                                           |
| 0.00006104    | -1.35          | 217490_at    | NA                                                                          |
| 0.00006091    | -1.29          | 1557463_at   | NA                                                                          |
| 0.00005929    | -1.35          | 238273_at    | PL-5283 PL-5283 protein                                                     |
| 0.00005927    | -1.77          | 203840_at    | BLZF1 basic leucine zipper nuclear factor 1                                 |
| 0.00005896    | -1.17          | 237604_at    | LOC415056 hypothetical gene LOC415056                                       |
| 0.00005883    | -1.9           | 222881_at    | HPSE hepanase                                                               |
| 0.00005855    | -1.25          | 220634_at    | TBPX4 T-box 4                                                               |
| 0.00005853    | -1.3           | 200669_s_at  | UBE2D3 ubiquitin-conjugating enzyme E2D 3 (UBC4/5 homolog, yeast)           |
| 0.00005801    | -1.05          | 232017_at    | TJP2 tight junction protein 2 (zona occludens 2)                            |
| 0.00005772    | -1.35          | 213918_s_at  | NIPBL Nipped-B homolog (Drosophila)                                         |
| 0.00005770    | -1.62          | 215357_s_at  | POLDIP3 polymerase (DNA-directed), delta interacting protein 3             |
| 0.00005750    | -1.4           | 1561354_at   | NA                                                                          |

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Table 1 Control vs Toxin corresponding P-value p<0.001 (Continued)

| P-value | Symbol   | Gene Name            |
|---------|----------|----------------------|
| 0.0005678 | 654     | 206516_at AMH        |
| 0.0005652 | 655     | 211030_s_at SLC6A6  |
| 0.0005634 | 656     | 222651_s_at TRPS1    |
| 0.0005618 | 657     | 57703_at SENPS      |
| 0.0005604 | 658     | 211372_s_at IL1R2   |
| 0.0005584 | 659     | 156724_at ORSH1     |
| 0.0005488 | 660     | 205003_at DOCK4     |
| 0.0005371 | 661     | 222262_s_at ETNK1   |
| 0.0005358 | 662     | 201684_s_at TOX4    |
| 0.0005357 | 663     | 206011_at CASP1     |
| 0.0005349 | 664     | 211505_s_at STAU1   |
| 0.0005342 | 665     | 1554049_s_at WDR42A |
| 0.0005321 | 666     | 225978_at FAM80B    |
| 0.0005228 | 667     | 215056_s_at NA      |
| 0.0005162 | 668     | 202066_at PPFIA1    |
| 0.0005087 | 669     | 226128_at NA        |
| 0.0005005 | 670     | 215056_s_at NA      |
| 0.0004997 | 671     | 217847_s_at THRAP3  |
| 0.0004992 | 672     | 229845_at MAPKAP1   |
| 0.0004955 | 673     | 211806_s_at KCN15   |
| 0.0004947 | 674     | 217503_s_at NA      |
| 0.0004946 | 675     | 221147_s_at WWOX    |
| 0.0004897 | 676     | 1552684_a_at SENP8  |
| 0.0004707 | 677     | 206251_s_at AVPR1A  |
| 0.0004609 | 678     | 223546_s_at LUC7L   |
| 0.0004595 | 679     | 208576_s_at HIST1H3B|
| 0.0004582 | 680     | 202684_s_at RNMT    |
| 0.0004567 | 681     | 201378_s_at UBP2L   |
| 0.0004553 | 682     | 202178_s_at PRKCI   |
| 0.0004545 | 683     | 1555139_a_at OTUD7B |
| 0.0004543 | 684     | 244595_s_at NA      |
| 0.0004511 | 685     | 210592_s_at 04/01/12|
| 0.0004444 | 686     | 1554327_s_at CANT1  |
| 0.0004435 | 687     | 223430_at SNF1LK2   |
| 0.0004430 | 688     | 223437_at CPSF3L    |
| 0.0004418 | 689     | 202230_s_at CHERP   |
| 0.0004358 | 690     | 211782_s_at IDS     |
| 0.0004288 | 691     | 208869_s_at GABARAP1|
| 0.0004258 | 692     | 1554646_at OSBPL1A  |
| 0.0004189 | 693     | 224410_s_at LMBR1   |
| 0.0004130 | 694     | 201698_s_at SFRS9   |
| 0.0004109 | 695     | 218578_s_at CDC73   |
| 0.0003904 | 696     | 1566456_s_at NA     |
| 0.0003884 | 697     | 226026_at DIRC2     |
| 0.0003884 | 698     | 222035_s_at PAPOLA  |
| 0.0003882 | 699     | 240313_at DMRTB1    |

Anti-Mullerian hormone
TRPS1, trichorhinophalangeal syndrome I
ORSI-H, olfactory receptor, family S, subfamily H, member 1
DOCK4, dedicator of cytokinesis 4
ETNK1, ethanolamine kinase 1
TOX high mobility group box family member 4
CASP1, caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)

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Table 1 Control vs Toxin corresponding P-value p<0.001 (Continued)

| Gene ID   | P-value | Log2 Fold Change | Description                                                                 |
|-----------|---------|------------------|-----------------------------------------------------------------------------|
| 700       | 0.0003880 | -1.65            | ST8SJ_A4 ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 4       |
| 701       | 0.0003868 | -2.87            | NA                                                                          |
| 702       | 0.0003850 | -1.43            | NXF2 nuclear RNA export factor 2                                             |
| 703       | 0.0003839 | -1.93            | CASP1 caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta,  |
|           |         |                  | convertase)                                                                |
| 704       | 0.0003823 | -1.24            | LYRM2 LYR motif containing 2                                                |
| 705       | 0.0003803 | -1.63            | UBAP2L ubiquitin associated protein 2-like                                   |
| 706       | 0.0003785 | -1.4              | TYMP thymidine phosphorylase                                                 |
| 707       | 0.0003695 | -1.53            | ATXN3 ataxin 3                                                              |
| 708       | 0.0003684 | -2.46            | SLC23A2 solute carrier family 23 (nucleobase transporters), member 2        |
| 709       | 0.0003661 | -1.25            | PPAT phosphoribosyl pyrophosphate amidotransferase                          |
| 710       | 0.0003575 | -1.29            | BMP7 bone morphogenetic protein 7                                           |
| 711       | 0.0003529 | -3.5             | GK3P glycerol kinase 3 pseudogene                                           |
| 712       | 0.0003527 | -1.22            | TOM1 target of myb1 (chicken)                                               |
| 713       | 0.0003455 | -1.18            | PSMD1 proteasome (prosome, macropain) 265 subunit, non-ATPase, 1            |
| 714       | 0.0003409 | -3.97            | LDLR low density lipoprotein receptor                                      |
| 715       | 0.0003389 | -1.07            | KTN1 kinectin 1 (kinesin receptor)                                          |
| 716       | 0.0003355 | -1.77            | FAM120AOS family with sequence similarity 120A opposite strand            |
| 717       | 0.0003300 | -1.17            | PTBP1 polyripyridine tract binding protein 1                                |
| 718       | 0.0003300 | -1.38            | EIF3J eukaryotic translation initiation factor 3, subunit J                 |
| 719       | 0.0003231 | -1.15            | NHLS2 NHS-like 2                                                            |
| 720       | 0.0003229 | -1.41            | FAS Fas (TNF receptor superfamily, member 6)                               |
| 721       | 0.0003210 | -2                | TOR1B torsin family 1, member B (torsin B)                                 |
| 722       | 0.0003206 | -1.3              | SORBS2 sorbin and SH3 domain containing 2                                   |
| 723       | 0.0003169 | -1.19            | VAPB VAMP (vesicle-associated membrane protein)-associated protein B and C  |
| 724       | 0.0003084 | -1.34            | MAPKAPK2 mitogen-activated protein kinase-activated protein kinase 2        |
| 725       | 0.0003068 | -1.11            | U18309_at NA                                                                 |
| 726       | 0.0002955 | -1.14            | L24F2 U2 small nuclear RNA auxiliary factor 2                               |
| 727       | 0.0002940 | -1.11            | PIP4K2A phosphatidylinositol-4-phosphate 4-kinase, type II, alpha           |
| 728       | 0.0002921 | -1.28            | MXRCC5 X-ray repair complementing defective repair in Chinese hamster cells  |
| 729       | 0.0002910 | -1.24            | PEX16 peroxisomal biogenesis factor 16                                      |
| 730       | 0.0002863 | -1.21            | LOC541469 hypothetical protein LOC541469                                    |
| 731       | 0.0002857 | -2.02            | ZCCHC2 zinc finger, CCHC domain containing 2                               |
| 732       | 0.0002808 | -1.2              | KBTBD4 kelch repeat and BTB (POZ) domain containing 4                       |
| 733       | 0.0002799 | -1.16            | FANCDD2 Fanconi anemia, complementation group D2                            |
| 734       | 0.0002764 | -1.34            | SUGT1P suppressor of G2 allele of SKP1 pseudogene (S. cerevisiae)           |
| 735       | 0.0002736 | -1.53            | FGR1OP2 FGR1 oncogene partner 2                                             |
| 736       | 0.0002703 | -1.66            | SASH1 SAM and SH3 domain containing 1                                       |
| 737       | 0.0002699 | -1.87            | SFT2D2 SFT2 domain containing 2                                             |
| 738       | 0.0002674 | -1.26            | FOXD1 forkhead box D1                                                      |
| 739       | 0.0002668 | -1.41            | AVPR2 arginine vasopressin receptor 2                                       |
| 740       | 0.0002632 | -1.21            | KNSL2 threonine synthase-like 2 (S. cerevisiae)                             |
| 741       | 0.0002619 | -1.76            | RBBM33 RNA binding motif protein 33                                         |
| 742       | 0.0002577 | -1.25            | NDUF51 NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75KDa (NADH-        |
|           |         |                  | coenzyme Q reductase)                                                       |
| 743       | 0.0002569 | -3.7             | SIGLEC9 sialic acid binding Ig-like lectin 9                               |
| 744       | 0.0002547 | -1.33            | CYBB cytochrome b-2,45, beta polypeptide                                    |
| 745       | 0.0002484 | -1.44            | CYBB cytochrome b-2,45, beta polypeptide                                    |
Table 1 Control vs Toxin corresponding P-value p<0.001 (Continued)

| Gene ID | Gene Symbol | Description | Fold Change | P-value |
|---------|-------------|-------------|-------------|---------|
| 746     | 220012_at   | ERO1LB     | -1.33       | 0.0002482 |
| 747     | 236106_at   | NA          | -2.41       | 0.0002373 |
| 748     | 242834_at   | NA          | -1.34       | 0.0002362 |
| 749     | 220498_at   | ACTL7B      | -1.2        | 0.0002316 |
| 750     | 240873_s_at | DAB2       | -1.44       | 0.0002303 |
| 751     | 221471_at   | SERINC3     | -1.19       | 0.0002296 |
| 752     | 213236_at   | SASH1       | -1.74       | 0.0002267 |
| 753     | 204098_s_at | NA          | -1.62       | 0.0002262 |
| 754     | 239372_at   | NA          | -1.2        | 0.0002220 |
| 755     | 240079_at   | ZNF81       | -1.35       | 0.0002182 |
| 756     | 205227_at   | IL1RAP      | -1.65       | 0.0002197 |
| 757     | 223751_x_at | TLR10       | -1.5        | 0.0002197 |
| 758     | 1553677_a_at| TIPRL       | -1.35       | 0.0002197 |
| 759     | AFFX-HUMISGF3A/M97935_5_at | STAT1 | -1.44       | 0.0002197 |
| 760     | 202240_at   | PLK1        | -1.11       | 0.0002197 |
| 761     | 1556281_at  | NA          | -1.28       | 0.0002197 |
| 762     | 222989_s_at | UBQLN1      | -1.53       | 0.0002197 |
| 763     | 204682_at   | LTBP2       | -1.16       | 0.0002197 |
| 764     | 225830_at   | PDZD8       | -1.37       | 0.0002197 |
| 765     | 229208_at   | CEP27       | -1.34       | 0.0002197 |
| 766     | 202211_at   | ARFGAP3     | -1.38       | 0.0002197 |
| 767     | 208696_at   | CCT5        | -1.21       | 0.0002197 |
| 768     | 219207_at   | EDC3        | -1.71       | 0.0002197 |
| 769     | 221348_s_at | WHSC1L1     | -1.31       | 0.0002197 |
| 770     | 211781_x_at | NA          | -1.59       | 0.0002197 |
| 771     | 226037_s_at | TAF9B       | -1.36       | 0.0002197 |
| 772     | 200901_s_at | M6PR        | -1.14       | 0.0002197 |
| 773     | 213173_s_at | PCNX        | -1.67       | 0.0002197 |
| 774     | 206038_s_at | NR2C2       | -2.06       | 0.0002197 |
| 775     | 225397_at   | C1Sorf57    | -1.24       | 0.0002197 |
| 776     | 211367_s_at | CASP1       | -2.14       | 0.0002197 |
| 777     | 222810_s_at | RASAL2      | -1.66       | 0.0002197 |
| 778     | 231718_s_at | SLU7        | -1.23       | 0.0002197 |
| 779     | 223905_at   | CCDC135     | -1.32       | 0.0002197 |
| 780     | 211672_s_at | ARPC4       | -1.28       | 0.0002197 |
| 781     | 200828_s_at | ZNF207      | -1.38       | 0.0002197 |
| 782     | 244211_at   | NA          | -1.29       | 0.0002197 |
| 783     | 205403_at   | IL1R2       | -1.22       | 0.0002197 |
| 784     | 209970_x_at | CASP1       | -1.83       | 0.0002197 |
| 785     | 217502_at   | IFIT2       | -6.35       | 0.0002197 |
| 786     | 237867_s_at | PID1        | -2.07       | 0.0002197 |
| 787     | 218516_s_at | IMPAD1      | -1.26       | 0.0002197 |
| 788     | 226312_at   | RICTOR      | -1.64       | 0.0002197 |
| 789     | 210940_s_at | GRM1        | -1.2        | 0.0002197 |
over-expression of RGS14, thereby inhibiting G-protein mediated signaling required for actin-based motility.

RGS14 expression is down-regulated during the maturation of monocytes to dendritic cells [24] and over-expression of this G-protein regulator would be expected to block monocyte maturation. RGS14 levels are also known to decrease in dendritic cells exposed to *Leishmania major* or *Toxoplasma gondii*, suggesting that RGS14 down-regulation may be an important step in a normal immune response, and up-regulation of RGS14 by LT could be contributing to LT’s immunosuppressive effects [25].

Three chemokine receptors were also altered after LT treatment, suggesting that LT may be inducing functional defects in monocyte response signaling. IL-8 receptor beta (CXCR2) was up-regulated after LT treatment (Table 2). CXCR2 transduces signaling through a G-protein activated second messenger system. This receptor is important for monocyte transendothelial migration, and up-regulation of CXCR2 could serve to enhance the delivery of monocytes to tissues. Anthrax spores must be phagocytosed by macrophages in order to germinate into viable bacteria. An increase in the macrophage pool may aid in a reservoir for increased germination of viable bacteria. IL-1 receptor type II (IL-1R2), was found to be markedly down-regulated. IL-1R2 is a decoy receptor for IL-1 that functions either at the cell surface or in a soluble form [26]. The decreased expression of the decoy receptor would presumably increase IL-1a levels and increase the febrile response of the host potentially at least in part explaining the high fever that commonly accompanies systemic anthrax [27].

CCR5 is a receptor for the monocyte chemokines

| Table 1 | Table 1 Control vs Toxin corresponding P-value p<0.001 (Continued) |
|---------|---------------------------------------------------------------|
| 790     | 9.02E-005          | -1.36 | 1554556_a_at | ATP11B | ATPase, class VI, type 11B |
| 791     | 8.89E-005          | -1.14 | 226735_at    | TAPT1 | transmembrane anterior posterior transformation 1 |
| 792     | 8.70E-005          | -3.84 | 213006_at    | CEBPD | CCAAT/enhancer binding protein (C/EBP), delta |
| 793     | 8.35E-005          | -1.31 | 207787_at    | KRT33B | keratin 33B |
| 794     | 8.34E-005          | -1.29 | 207410_s_at  | TLX2 | T-cell leukemia homeobox 2 |
| 795     | 8.21E-005          | -1.6 | 223596_at    | SLC12A6 | solute carrier family 12 (potassium/chloride transporters), member 6 |
| 796     | 7.98E-005          | -1.23 | 231859_at    | C14orf132 | chromosome 14 open reading frame 132 |
| 797     | 7.81E-005          | -1.25 | 228277_at    | FBX19 | F-box and leucine-rich repeat protein 19 |
| 798     | 7.75E-005          | -1.35 | 210470_x_at  | NONO | non-POU domain containing, octamer-binding |
| 799     | 7.52E-005          | -1.29 | 222432_s_at  | CCDC47 | coiled-coil domain containing 47 |
| 800     | 7.19E-005          | -1.8 | 238496_at    | NA | NA |
| 801     | 7.11E-005          | -1.38 | 208698_s_at  | NONO | non-POU domain containing, octamer-binding |
| 802     | 7.08E-005          | -4.66 | 203946_s_at  | ARG2 | arginase, type II |
| 803     | 7.03E-005          | -1.17 | 1559552_x_at | LOC100132923 | similar to hCG1993470 |
| 804     | 6.19E-005          | -2.35 | 220104_at    | ZC3HAV1 | zinc finger CCCH-type, antiviral 1 |
| 805     | 5.57E-005          | -2.43 | 203595_s_at  | IFIT5 | interferon-induced protein with tetratricopeptide repeats 5 |
| 806     | 5.53E-005          | -1.33 | 1569859_at   | NA | NA |
| 807     | 5.31E-005          | -1.5 | 224359_s_at  | HOOK3 | hook homolog 3 (Drosophila) |
| 808     | 4.19E-005          | -2.51 | 205921_s_at  | SLC6A6 | solute carrier family 6 (neurotransmitter transporter, taurine), member 6 |
| 809     | 3.18E-005          | -2.18 | 205749_at    | CYP1A1 | cytochrome P450, family 1, subfamily A, polypeptide 1 |
| 810     | 2.93E-005          | -1.38 | 1566136_at   | NA | NA |
| 811     | 2.02E-005          | -1.51 | 210992_x_at  | FCG2C | Fc fragment of IgG, low affinity IIc, receptor for (CD32) |
| 812     | 1.61E-005          | -1.22 | 207801_s_at  | RNF10 | ring finger protein 10 |
| 813     | 1.32E-005          | -2.03 | 222816_s_at  | ZCCHC2 | zinc finger, CCHC domain containing 2 |
| 814     | 1.29E-005          | -1.25 | 211884_s_at  | CIITA | class II, major histocompatibility complex, transactivator |
| 815     | 8.60E-006          | -1.68 | 212664_at    | TUBB4 | tubulin, beta 4 |
| 816     | 4.60E-006          | -1.16 | 212081_s_at  | BAT2 | HLA-B associated transcript 2 |
| 817     | 3.60E-006          | -1.21 | 1554177_s_at | ATP5S | ATP synthase, H+ transporting, mitochondrial F0 complex, subunit s (factor B) |
| 818     | 7.00E-007          | -1.82 | 224783_at    | FAM100B | family with sequence similarity 100, member B |
| 819     | 6.00E-007          | -1.57 | 208840_s_at  | G3BP2 | GTPase activating protein (SH3 domain) binding protein 2 |
| 820     | 4.00E-007          | -1.59 | 206717_at    | MYH8 | myosin, heavy chain 8, skeletal muscle, perinatal |
RANTES and MIP. The down-regulation of CCR5 by LT could reflect an inability of toxin-treated monocytes to differentiate into macrophages [28] (Table 2). During the early stages of infection, macrophages play a critical role in assisting *B. anthracis* pathogenesis by providing a place for bacteria germination from their spore form to viable bacteria. An increase in monocyte trafficking to allow an increase in spore uptake and subsequent germination would prove beneficial for *B. anthracis*. During later stages of infection, after release of viable bacteria, limiting monocyte differentiation to macrophages would assist in preventing clearance of viable bacteria.

In addition to an alteration in the chemokine response by LT, an additional enzyme, heparanase (HPSE), was found to be decreased in LT-treated human monocytes. This enzyme is an endoglycosidase that degrades heparin sulfate, resulting in disassembly of extracellular barriers required for cell migration [29]. Heparanase has also been postulated to play a role in inflammation [30] and our results showed a 2.6 fold decrease in heparanase gene expression (Table 2). One study has concluded that an *in vivo* siRNA against heparanase, along with an inhibitor of its enzymatic activity, results in a diminished inflammatory response [31]. Thus LT- mediated inhibition of heparanase expression could also contribute to the inhibition of the host immune response during an anthrax infection.

### Table 2 Predicted effects of LT on monocyte function.

| Gene      | Microarray | Effects                                                   |
|-----------|------------|-----------------------------------------------------------|
| RGS-14    | 5.61       | Blockade of monocyte maturation to dendritic cells, inhibition of chemotaxis |
| CXCR2     | 5.04       | Increased monocyte transendothelial migration into tissues |
| HPSE      | -2.58      | Diminished inflammatory response                           |
| CCR5      | -2.33      | Reduced responsiveness to the inflammatory mediators RANTES, MIP1 beta |
| ILIR2     | -12.5      | Increased IL1 alpha responsiveness and increased fever    |

1 Calculated fold changes compared to mock treated samples.

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**Figure 3** Supervised microarray analysis. A.) Hierarchical cluster analysis showing the 820 probe sets which were differentially expressed at the 0.001 significance level. The arrays clustering on the left are from control samples, whereas the cluster on the right shows the LT treated samples. Up-regulated genes are shown in red and down-regulated genes are shown in blue. B.) Biocarta pathway analysis showing the pathways most significantly affected by LT, along with the number of genes and p-value within each pathway that were affected. C.) Correlation of genes altered after treatment with anthrax LT using microarray analysis versus RT-PCR. Spearman correlation coefficient = 0.885.
An external verification method using quantitative real-time PCR was utilized to confirm the microarray data. The eight genes corresponding to RGS14, IL8RB, TLR5, PPM1H, CD47, SYK, CCR5, and IL1R2 were chosen for microarray confirmation in monocytes. CCR5 and IL1R2 were confirmed to be down-regulated at 4 h after LT treatment, reinforcing the microarray data, while the other six genes were up-regulated, again confirming the microarray data (Table 3). A correlation curve was plotted (Figure 3C) and analyzed, showing a linear relationship between the microarray results and RT-PCR with a correlation coefficient of 0.885. Results were performed in duplicates and fold values were normalized to GAPDH. To exclude the possibility the lymphocyte contamination might be contributing to our microarray findings, a higher purity monocyte population (98% purity), obtained by adherence followed by washing off non-adherent lymphocytes, was treated with 500 ng/mL LT for 4 h and gene expression was assessed using real-time PCR. These experiments verified 3 genes to be increased after LT treatment: RGS14, TLR5, and CD47 (1.21-1.70), as observed by the microarray of suspended cells. These findings suggest that the changes in messenger RNA observed are primarily contributed by monocytes, but we cannot entirely exclude a contribution by lymphocytes.

Conclusions
Our investigations show human peripheral monocytes are susceptible to the actions of anthrax LT and do not undergo LT-mediated cytotoxicity after a four hour toxin treatment. We also find that LT induces changes in several genes involved in previously unidentified pathways including the TLR pathway, IFN alpha pathway, and G-Protein family signaling pathways. The identification of several previously unappreciated gene products including RGS14, IL8 receptor beta, CD47, TNF ligand, IL-16, Syk, CCR5, and IL-1 receptor II adds to our understanding of how LT impacts the immune response. Our pathway analysis reveals that anthrax LT targets multiple normal immune-regulatory pathways that would be expected to protect the host against anthrax infection. The increase in RGS14 levels and decrease in CCR5, along with IL-1R2, would likely impair monocyte functions and help to facilitate bacteria survival. B. anthracis maintains a selective advantage by impairing the host immune responses, thereby allowing for invasion and dissemination of the highly fatal bacilli. Our findings encourage further investigations into how these pathways converge functionally to impair normal monocyte function, along with providing new insights into the regulation of the host defense system and inflammation.

Table 3 q-RTPCR confirmation of LT-induced genes.1

| Probe            | Microarray | q-RT-PCR | Gene name | Primer sequence           |
|------------------|------------|----------|-----------|---------------------------|
| 38290_at         | 5.61       | 7.40     | RGS14     | CAGGGATCTGTTAGAAGGCAGG    |
|                  |            |          | RGS14-R   | AGGTGATCCTGTTTCCAGC       |
| 207008_at        | 5.04       | 7.50     | IL8RB     | GTCTAAACAGCTCTGACTCACCAC  |
|                  |            |          | IL8RB-R   | TTAACCTCCGACTGTCGTCGC     |
| 210166_at        | 3.90       | 2.24     | TLR5      | TTTTCAAGAGCGGCGGCGCGG     |
|                  |            |          | TLR5-R    | AGCCGAGATGGTGTCAGCTG      |
| 212686_at        | 2.65       | 3.85     | PPM1H     | GAGTACAGAGAAAGGAGAGTTCG   |
|                  |            |          | PPM1H-R   | TCAATATGTCGCTTACCCGAC     |
| 226016_at        | 2.38       | 1.60     | CD47      | TTTGCTATCTCCTGTTCTGGG     |
|                  |            |          | CD47-R    | TGGGAGCAAAGAATGGGCTC      |
| 209269_at        | 2.15       | 1.60     | SYK       | CAAAGTTCAGCAAAAGGCG       |
|                  |            |          | SYK-R     | CATCCGCTTCCCTTCCTCAAC     |
| 206991_at        | -2.66      | -2.33    | CCR5      | CCAAAACGACATGCGCAAGCG     |
|                  |            |          | CCR5-R    | ACTGGGACGTGCGTCAAGCC      |
| 205403_at        | -1.25      | -2.80    | IL1R2     | TGGCAGCTACTGCTGACTACT     |
|                  |            |          | IL1R2-R   | TTGGGATATGAGATGAAACG      |

1 Calculated fold changes compared to mock treated samples.
20 min., centrifuged 1700 × g for 25 min at RT, no brake over Ficoll, re-suspended in 10 mL RPMI (Mediatech) complete media, centrifuged at 250 × g for 9 min. to remove platelets, and re-suspended to 7-9 × 10^5 cells/mL in RPMI. Monocytes were inverted at 37°C with 500 ng/mL LF and 500 ng/mL PA for 4 h. Additional qRT-PCR experiments were performed using higher monocyte purities (98%), obtained by first using a negative selection antibody cocktail (Stem Cell Technologies) isolation technique, followed by plastic adherence for 4 h, as described previously [32].

**Toxin purification**

LF and PA were purified as previously described [33]. Briefly, *Bacillus anthracis* culture media was filtered through a 0.22 uM filter, followed by diethylaminoethyl cellulose (DEAE) anion exchange chromatography. The toxins were then subjected to gel filtration and hydrophobic interaction fast protein liquid chromatography (FPLC) and highly purified toxin components were confirmed by Coomassie Blue staining.

**Monocyte purity and apoptosis analysis**

Monocytes were inverted at 37°C with 500 ng/mL LF and 500 ng/mL PA for 4 h, stained with CD-14 Pac Blue (BD Biosciences), Annexin-V-Fluorescein and propidium iodide (Roche). The cell population was gated first for CD14-Pac-Blue followed by analysis of the relative amount of Annexin (FL1) and PI (FL2) using flow cytometry FACScan (BD), and analyzed by FCS Express (De Novo).

**MEK cleavage**

Purified monocytes were incubated at 37°C with 500 ng/mL lethal toxin for 4 h. Cells were lysed, run on a 10% SDS-PAGE gel (Pierce), transferred to a PVDF membrane (Bio-rad) and probed for MEK1 (Upstate). Membranes were then stripped and probed for MEK3 (Santa Cruz). β-actin (Sigma) was used to check consistent loading amounts.

**RNA isolation**

Purified monocytes from 4 healthy volunteers were incubated at 37°C with media alone or with 500 ng/mL LT for 4 h. Total RNA was collected using RNAeasy mini kit (Qiagen) and RNA quantity and quality was assessed using NanoDrop (Thermo Scientific) technology.

**Microarray procedure**

100 ng total RNA was labeled using Affymetrix GeneChip® 3’ IVT Express Kit for each replicate. Amplified labeled RNA was purified, fragmented, then hybridized for 16 h on Affymetrix GeneChips® (HG U133 plus 2.0) representing approximately 22,000 well-characterized human genes. Arrays were washed using Affymetrix GeneChip® Fluidics Station FS450 and scanned using GeneChip® Scanner 3000 7 G.

**Microarray analysis**

Low-level analysis was performed using dChipmodeled-based expression matrix (dChip 2007 (DNA-Chip Analyzer), Build date: Jan 4, 2008). Unsupervised analysis - probes sets whose hybridization signal intensity exhibited a coefficient of variation of greater than 0.5 were analyzed by unsupervised hierarchical cluster analysis using algorithms implemented in dChip. Supervised analysis - significant probe sets between the treatment groups were identified using a paired t-test (by donor) at a significance threshold of p < 0.001. Leave-one-out-cross-validation using 4 prediction models was used to test the ability of probe sets significant at p < 0.001 to distinguish between the treatment groups. Microarray analyses were done using dCHIP and BRB-ArrayTools by Richard Simon (http://linus.nci.nih.gov/BRB-ArrayTools.html). The microarray data for this study was deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) [30] with accession numbersGSM848717 through GSM 848724. The microarray data is also available in a series with accession number GSE34407.

**Quantitative real time-PCR (qRT-PCR)**

RNA was collected using RNAeasy mini kit (Qiagen), quantitated using a Nanodrop system (Thermo Scientific), and 233 μg total RNA was used for cDNA synthesis using SuperScript III First-Strand Synthesis (Invitrogen). cDNA was quantitated using SYBR Green JumpStart TaqReady-Mix (Sigma) and 10 mM forward and 10 mM reverse primers were used for each indicated reaction. Primers used were as follows ACTB-F TCACCCGACGCGCCGT,ACTB-R TAATGTCAAGCAGATTCCC,GAPDH-F GGTCGGAGTCAACG, and GAPDH-R AGAGTTAAAA GCAGCCCTTGTT. All other primers are listed in Table 2. Reactions were run on the MJR Opticon Continuous Fluorescence detector (Bio-Rad) and analyzed with Opticon Monitor Software 1.08 (Bio-Rad).

**Authors’ contributions**

KC performed the experiments, analyzed data, interpreted study, drafted, and wrote the manuscript. CL performed the gene expression profiling, biostatistical analysis, and helped design the study. SZ participated in the study design and interpreted the study, as well as edited the manuscript. HB participated in the design and analyses of the study. CQ supplied the purified toxin and assisted in data interpretation. FS conceived the study and interpretation of the study, as well as edited the manuscript. All authors read and approved the final manuscript.

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