Reactive oxygen species mediate proinflammatory cytokine release and EGFR-dependent mucin secretion in airway epithelial cells exposed to *Pseudomonas pyocyanin*

Balázs Rada, Paul Gardina, Timothy G. Myers and Thomas L. Leto

**Online supplementary figures**

**Supplementary Figure 1.** Exposure to pyocyanin does not lead to STAT6 phosphorylation in H292 cells. H292 cells were exposed to 8 µM pyocyanin or 10 ng/mL interleukin (IL)-4 (used as a positive control for STAT6 activation) for 0-2 days. Cell lysates were subjected to Western blotting, phosphorylated and total STAT6 levels were detected by specific antibodies and compared to loading control (β-actin).

**Supplementary Figure 2.** Confirmation of gene expression data obtained from the microarray experiment by real-time RT-PCR for EGF, ADAM17 and MEK1. Changes in gene expression levels were followed over time for 2 days in H292 cells exposed to pyocyanin (8 µM) (mean +/- S.E.M., n=2-4).

**Supplementary Figure 3.** Microarray analysis of pyocyanin-exposed bronchial epithelial cells. The volcano plot shows the log₂ ratio of expression values in pyocyanin-treated relative to untreated cells (x-axis) and the -log(p-value) (y-axis) for each probe on the microarray. The dashed red line at 1.80 indicates the significance cutoff for multiple comparisons as determined by FDR of 0.05. Dashed blue lines represent selected thresholds at 4-fold difference (vertical lines) and an un-adjusted p-value of 10⁻³ (horizontal line). Probes exceeding the thresholds (upper left and right quadrants) were taken for further study. This set comprises 389 probes representing 302 unique transcripts and 268 unique Entrez gene identifiers.

**Supplementary Figure 4.** Network #1 was generated by Ingenuity Pathway Analysis (IPA) software as the most statistically significant set of connections derived from 268 pyocyanin-responsive genes identified by microarray. The resulting gene set is strongly associated with tumor necrosis factor alpha signaling. The top three functions associated with Network #1 (score = 52) were: Cell-mediated immune response, Cellular development, Cellular growth & proliferation. Color coding: red = up with pyocyanin; green = down with pyocyanin.

**Supplementary Figure 5.** Network #2 identified by Ingenuity Pathway Analysis (IPA) software contains pyocyanin-responsive genes associated with NF-κB signaling. The top three functions associated with Network #2 were: Cell death, Cancer, Cell-to-cell signaling.
Supplementary Figure 1.

Rada et al.

Untreated
Pyocyanin
IL-4

Phospho-STAT6

Untreated
Pyocyanin
IL-4

STAT6

Untreated
Pyocyanin
IL-4

Actin

Time: 0 0.25 0.5 1 2 8 24 60 hrs
Supplementary Figure 2.

Rada et al.
Supplementary Figure 3.
Supplementary Figure 4.

Red - up in pyocyanin
Green - down
(darker = higher fold difference)
Supplementary Figure 5.

Red - up in pyocyanin
Green - down
(Arrows = higher fold difference)
Reactive oxygen species mediate inflammatory cytokine release and EGFR-dependent mucin secretion in airway epithelial cells exposed to *Pseudomonas* pyocyanin

Balázs Rada, Paul Gardina, Timothy G. Myers and Thomas L. Leto

**Online supplementary Material**

**Supplementary Table 1.** List of up-regulated genes.

**Supplementary Table 2.** List of down-regulated genes.
## Supplementary Table 1.

**Genes Up-Regulated by Pyocyanin** *(p-value < 0.001, log₂ ratio > 2)*

| RefSeq    | Entrez Gene ID | Gene   | Description                                      | Log₂ Ratio | Neg. log₁₀ p-value |
|-----------|----------------|--------|--------------------------------------------------|------------|--------------------|
| NM_172219 | 1440           | CSF3   | colony stimulating factor 3, variant 2           | 7.88       | 4.32               |
| NM_181339 | 11009          | IL24   | interleukin 24, variant 2                        | 7.01       | 5.05               |
| NM_000600 | 3569           | IL6    | interleukin 6 (interferon, beta 2)               | 7.00       | 4.61               |
| NM_000600 | 3569           | IL6    | interleukin 6 (interferon, beta 2)               | 6.93       | 4.82               |
| NM_000600 | 3569           | IL6    | interleukin 6 (interferon, beta 2)               | 6.86       | 5.32               |
| NM_000600 | 3569           | IL6    | interleukin 6 (interferon, beta 2)               | 6.81       | 4.68               |
| NM_000600 | 3569           | IL6    | interleukin 6 (interferon, beta 2)               | 6.81       | 4.16               |
| NM_000600 | 3569           | IL6    | interleukin 6 (interferon, beta 2)               | 6.80       | 4.54               |
| NM_000600 | 3569           | IL6    | interleukin 6 (interferon, beta 2)               | 6.80       | 4.39               |
| NM_181339 | 11009          | IL24   | interleukin 24, variant 2                        | 6.74       | 4.78               |
| NM_000600 | 3569           | IL6    | interleukin 6 (interferon, beta 2)               | 6.74       | 4.97               |
| NM_000600 | 3569           | IL6    | interleukin 6 (interferon, beta 2)               | 6.74       | 4.73               |
| NM_000600 | 3569           | IL6    | interleukin 6 (interferon, beta 2)               | 6.66       | 4.00               |
| NM_004864 | 9518           | GDF15  | growth differentiation factor 15                 | 6.13       | 4.56               |
| NM_004591 | 6364           | CCL20  | chemokine (C-C motif) ligand 20, variant 1        | 5.88       | 4.43               |
| NM_000576 | 3553           | IL1B   | interleukin 1, beta                              | 5.77       | 6.32               |
| NM_000576 | 3553           | IL1B   | interleukin 1, beta                              | 5.77       | 6.15               |
| NM_000576 | 3553           | IL1B   | interleukin 1, beta                              | 5.73       | 6.01               |
| NM_000576 | 3553           | IL1B   | interleukin 1, beta                              | 5.71       | 5.90               |
| NM_000576 | 3553           | IL1B   | interleukin 1, beta                              | 5.70       | 5.71               |
| Accession | Gene ID | Description                                      | Expression | Functional Score |
|-----------|---------|--------------------------------------------------|------------|------------------|
| NM_000576| 3553    | interleukin 1, beta                              | 5.70       | 6.55             |
| NM_000576| 3553    | interleukin 1, beta                              | 5.70       | 7.00             |
| NM_000576| 3553    | interleukin 1, beta                              | 5.67       | 6.07             |
| NM_000576| 3553    | interleukin 1, beta                              | 5.67       | 6.25             |
| NM_002090| 2921    | chemokine (C-X-C motif) ligand 3                  | 5.67       | 5.46             |
| NM_000576| 3553    | interleukin 1, beta                              | 5.67       | 5.77             |
| NM_000575| 3552    | interleukin 1, alpha                             | 5.47       | 5.27             |
| NM_000575| 3552    | interleukin 1, alpha                             | 5.46       | 5.28             |
| NM_000575| 3552    | interleukin 1, alpha                             | 5.38       | 3.98             |
| NM_000575| 3552    | interleukin 1, alpha                             | 5.34       | 5.11             |
| NM_000575| 3552    | interleukin 1, alpha                             | 5.32       | 4.52             |
| NM_000575| 3552    | interleukin 1, alpha                             | 5.29       | 5.25             |
| NM_000575| 3552    | interleukin 1, alpha                             | 5.27       | 4.67             |
| NM_000575| 3552    | interleukin 1, alpha                             | 5.20       | 3.85             |
| NM_000575| 3552    | interleukin 1, alpha                             | 5.14       | 6.14             |
| NM_002089| 2920    | chemokine (C-X-C motif) ligand 2                  | 5.11       | 5.68             |
| MUC5A     | 9586    | CAMP responsive element binding protein 5        | 5.20       | 5.48             |
| NM_000575| 3552    | interleukin 1, alpha                             | 5.20       | 3.85             |
| NM_000575| 3552    | interleukin 1, alpha                             | 5.14       | 6.14             |
| NM_000575| 3552    | interleukin 1, alpha                             | 5.01       | 3.77             |
| NM_004165| 6236    | Ras-related associated with diabetes, variant 2  | 4.90       | 3.67             |
| NM_004165| 6236    | Ras-related associated with diabetes, variant 2  | 4.84       | 4.79             |
| NM_015714| 50486   | G0/G1switch 2                                    | 4.69       | 4.98             |
| Accession | Start | Gene | Description | R1 | R2 |
|-----------|-------|------|-------------|----|----|
| NM_000584 | 3576  | IL8  | interleukin 8 | 4.51 | 4.19 |
| NM_003467 | 7852  | CXCR4| chemokine (C-X-C motif) receptor 4, variant 2 | 4.46 | 4.52 |
| NM_003467 | 7852  | CXCR4| chemokine (C-X-C motif) receptor 4, variant 2 | 4.45 | 4.45 |
| NM_002090 | 2921  | CXCL3| chemokine (C-X-C motif) ligand 3 | 4.42 | 3.68 |
| NM_003467 | 7852  | CXCR4| chemokine (C-X-C motif) receptor 4, variant 2 | 4.41 | 5.11 |
| NM_003467 | 7852  | CXCR4| chemokine (C-X-C motif) receptor 4, variant 2 | 4.41 | 4.78 |
| SERPINB   | 5055  | 2    | Serpin peptidase inhibitor, clade B (ovalbumin), member 2 | 4.39 | 4.43 |
| NM_003467 | 7852  | CXCR4| chemokine (C-X-C motif) receptor 4, variant 2 | 4.39 | 3.94 |
| NM_003467 | 7852  | CXCR4| chemokine (C-X-C motif) receptor 4, variant 2 | 4.37 | 4.99 |
| NM_003467 | 7852  | CXCR4| chemokine (C-X-C motif) receptor 4, variant 2 | 4.35 | 4.37 |
| NM_003467 | 7852  | CXCR4| chemokine (C-X-C motif) receptor 4, variant 2 | 4.34 | 4.84 |
| NM_003467 | 7852  | CXCR4| chemokine (C-X-C motif) receptor 4, variant 2 | 4.28 | 4.45 |
| NM_002842 | 5794  | PTPRH| protein tyrosine phosphatase, receptor type | 4.25 | 4.68 |
| NM_003467 | 7852  | CXCR4| chemokine (C-X-C motif) receptor 4, variant 2 | 4.24 | 3.41 |
| NM_002421 | 4312  | MMP1 | matrix metalloproteinase 1 | 4.19 | 4.21 |
| HSD17B    | 3294  | 2    | hydroxysteroid (17-beta) dehydrogenase 2 | 4.17 | 4.52 |
| NM_002421 | 4312  | MMP1 | matrix metalloproteinase 1 (interstitial collagenase) | 4.16 | 4.66 |
| NM_002421 | 4312  | MMP1 | matrix metalloproteinase 1 (interstitial collagenase) | 4.14 | 4.16 |
| NM_002421 | 4312  | MMP1 | matrix metalloproteinase 1 (interstitial collagenase) | 4.13 | 5.31 |
| NM_002421 | 4312  | MMP1 | matrix metalloproteinase 1 (interstitial collagenase) | 4.13 | 5.35 |
| NM_002421 | 4312  | MMP1 | matrix metalloproteinase 1 (interstitial collagenase) | 4.11 | 4.40 |
| NM_002421 | 4312  | MMP1 | matrix metalloproteinase 1 (interstitial collagenase) | 4.09 | 4.03 |
| NM_007123 | 7399  | USH2A| Usher syndrome 2A (autosomal recessive, mild), variant 1 | 4.06 | 3.69 |
| Gene ID   | Accession | Symbol | Description                                                                 | Log2 Fold Change | Log2 Fold Change |
|-----------|-----------|--------|-----------------------------------------------------------------------------|------------------|------------------|
| NM_002421 | 4312      | MMP1   | matrix metalloproteinase 1 (interstitial collagenase)                        | 4.05             | 4.43             |
| NM_001009 | 991       | SYTL3  | synaptotagmin-like 3                                                        | 4.02             | 4.23             |
| NM_002089 | 2920      | CXCL2  | chemokine (C-X-C motif) ligand 2                                            | 4.01             | 4.69             |
| NM_000963 | 5743      | PTGS2  | prostaglandin-endoperoxide synthase 2                                       | 4.00             | 4.65             |
| NM_002421 | 4312      | MMP1   | matrix metalloproteinase 1 (interstitial collagenase)                        | 3.99             | 3.97             |
| NM_000963 | 5743      | PTGS2  | prostaglandin-endoperoxide synthase 2                                       | 3.98             | 5.01             |
| NM_000963 | 5743      | PTGS2  | prostaglandin-endoperoxide synthase 2                                       | 3.97             | 4.03             |
| NM_000963 | 5743      | PTGS2  | prostaglandin-endoperoxide synthase 2                                       | 3.96             | 5.17             |
| NM_002421 | 4312      | MMP1   | matrix metalloproteinase 1 (interstitial collagenase)                        | 3.95             | 4.21             |
| NM_000963 | 5743      | PTGS2  | prostaglandin-endoperoxide synthase 2                                       | 3.95             | 5.08             |
| NM_000963 | 5743      | PTGS2  | prostaglandin-endoperoxide synthase 2                                       | 3.95             | 4.67             |
| NM_001030 | 287       | ATF3   | activating transcription factor 3, variant 3                                 | 3.93             | 4.57             |
| NM_000963 | 5743      | PTGS2  | prostaglandin-endoperoxide synthase 2                                       | 3.93             | 4.88             |
| NM_000963 | 5743      | PTGS2  | prostaglandin-endoperoxide synthase 2                                       | 3.93             | 4.31             |
| NM_000963 | 5743      | PTGS2  | prostaglandin-endoperoxide synthase 2                                       | 3.91             | 4.48             |
| NM_000963 | 5743      | PTGS2  | prostaglandin-endoperoxide synthase 2                                       | 3.88             | 6.29             |
| NM_016584 | 51561     | IL23A  | interleukin 23, alpha subunit p19                                          | 3.85             | 4.59             |
| Gene Name     | Accession | Description                                                                 | Log2 Fold Change | Fold Change |
|--------------|-----------|-----------------------------------------------------------------------------|------------------|-------------|
| PTGS2        | NM_000963 | Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) | 3.81             | 3.84        |
| C15orf4      | NM_197955 | Chromosome 15 open reading frame 48, variant 1                              | 3.77             | 4.68        |
| MUC5A        | NM_173343 | Mucin 5AC, oligomeric mucus/gel-forming                                     | 3.74             | 4.24        |
| F10          | NM_000504 | Coagulation factor X                                                         | 3.66             | 3.70        |
| ELF3         | 1999      | E74-like factor 3 (ets domain transcription factor, epithelial-specific)     | 3.65             | 3.42        |
| CDKN1A       | NM_00389  | Cyclin-dependent kinase inhibitor 1A (p21, Cip1), variant 1                 | 3.54             | 3.97        |
| CDKN1A       | NM_00389  | Cyclin-dependent kinase inhibitor 1A (p21, Cip1), variant 1                 | 3.53             | 4.45        |
| MUC5A        | NM_020130 | Mucin 5AC, oligomeric mucus/gel-forming                                     | 3.50             | 4.84        |
| C8orf4       | NM_02130  | Chromosome 8 open reading frame 4                                             | 3.50             | 4.44        |
| CDKN1A       | NM_00389  | Cyclin-dependent kinase inhibitor 1A (p21, Cip1), variant 1                 | 3.49             | 4.02        |
| CDKN1A       | NM_00389  | Cyclin-dependent kinase inhibitor 1A (p21, Cip1), variant 1                 | 3.48             | 3.79        |
| PPBP         | NM_002704 | Pro-platelet basic protein (chemokine (C-X-C motif) ligand 7)               | 3.48             | 3.39        |
| CDKN1A       | NM_00389  | Cyclin-dependent kinase inhibitor 1A (p21, Cip1), variant 1                 | 3.46             | 4.88        |
| IL13RA2      | NM_000640 | Interleukin 13 receptor, alpha 2                                             | 3.46             | 4.65        |
| SAA2         | NM_030754 | Serum amyloid A2, variant 1                                                  | 3.45             | 3.66        |
| S100A7       | NM_002963 | S100 calcium binding protein A7                                              | 3.43             | 4.34        |
| Accession | Exon | Gene/Symbol | Description | Log2Ratio | Log2FoldChange |
|-----------|------|-------------|-------------|------------|---------------|
| NM_001030 | 287  | ATF3        | activating transcription factor 3, variant 3 | 3.42       | 4.91          |
|           |      |             | cyclin-dependent kinase inhibitor 1A (p21, Cip1), variant 1 |          |               |
| NM_000389 | 1026 | CDKN1A      | cyclin-dependent kinase inhibitor 1A (p21, Cip1), variant 1 | 3.42       | 3.93          |
| NM_182962 | 330  | BIRC3       | baculoviral IAP repeat-containing 3, variant 2 | 3.41       | 3.94          |
| NM_000389 | 1026 | CDKN1A      | cyclin-dependent kinase inhibitor 1A (p21, Cip1), variant 1 | 3.41       | 5.62          |
| NM_000389 | 1026 | CDKN1A      | cyclin-dependent kinase inhibitor 1A (p21, Cip1), variant 1 | 3.40       | 4.42          |
| NM_000805 | 2520 | GAST        | gastrin     | 3.40       | 3.70          |
| NM_00594  | 7124 | TNF         | tumor necrosis factor (TNF superfamily, member 2) | 3.38       | 4.02          |
| NM_014330 | 23645| PPP1R1      | protein phosphatase 1, regulatory (inhibitor) subunit 15A | 3.34       | 4.88          |
| NM_002619 | 5196 | PF4         | platelet factor 4 (chemokine (C-X-C motif) ligand 4) | 3.34       | 4.69          |
| NM_000389 | 1026 | CDKN1A      | cyclin-dependent kinase inhibitor 1A (p21, Cip1), variant 1 | 3.34       | 3.91          |
| NM_033413 | 90506| LRRC46      | leucine rich repeat containing 46 | 3.33       | 3.61          |
| XM_001716 | 387763| LOC387    | PREDICTED: hypothetical LOC387763 | 3.33       | 4.07          |
| NM_000389 | 1026 | CDKN1A      | cyclin-dependent kinase inhibitor 1A (p21, Cip1), variant 1 | 3.33       | 4.08          |
| NM_001009 | 991  | SYTL3       | synaptotagmin-like 3 | 3.32       | 3.62          |
| NM_001124 | 758  | SPNS2       | spinster homolog 2 (Drosophila) | 3.31       | 4.27          |
| NM_002565 | 5030 | P2RY4       | pyrimidinergic receptor P2Y, G-protein coupled, 4 | 3.27       | 3.70          |
|           |      |             | Unknown     | 3.25       | 3.92          |
| NM_199161 | 6288 | SAA1        | serum amyloid A1, variant 2 | 3.24       | 4.02          |
| Gene    | Unigene | Description                                                                 | Fold Change |
|---------|---------|-----------------------------------------------------------------------------|------------|
| NM_004433  | 1999 ELF3 | E74-like factor 3 (ets domain transcription factor, epithelial-specific), variant 1 | 3.24       |
| NM_004406  | 1755 DMBT1 | deleted in malignant brain tumors 1, variant 1                              | 3.21       |
|         |          | HIST1H2                                                                     |            |
| NM_080593  | 85236 BK | histone cluster 1, H2bk                                                       | 3.16       |
| NM_004079  | 1520 CTSS | cathepsin S                                                                  | 3.15       |
| NM_153840  | 266977 GPR110 | G protein-coupled receptor 110, variant 1                                    | 3.14       |
| NM_001945  | 1839 HBEGF | heparin-binding EGF-like growth factor                                        | 3.13       |
| NM_014467  | 27286 SRPX2 | sushi-repeat-containing protein, X-linked 2                                   | 3.13       |
| NM_017527  | 54742 LY6K | lymphocyte antigen 6 complex, locus K                                         | 3.13       |
| NM_000389  | 1026 CDKN1A | cyclin-dependent kinase inhibitor 1A (p21, Cip1), variant 1                  | 3.08       |
| GALNTL    |          | UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-like 2 | 3.06       |
| NM_054110  | 117248 2 | PR domain containing 1, with ZNF domain, variant 2                            | 3.05       |
| NM_182907  | 639 PRDM1 | serpin peptidase inhibitor, clade B (ovalbumin), member 2                   | 3.05       |
| NM_002575  | 5055 2 | protease, serine, 22                                                         | 3.03       |
| NM_032149  | 84103 C4orf17 | chromosome 4 open reading frame 17                                            | 3.04       |
| NM_006290  | 7128 TNFAIP3 | tumor necrosis factor, alpha-induced protein 3                               | 3.04       |
| NM_022119  | 64063 PRSS22 | leukemia inhibitory factor (cholinergic differentiation factor)               | 2.99       |
| NM_005987  | 6698 SPRR1A | small proline-rich protein 1A                                                | 2.97       |
| NM_020698  | 57458 TMCC3 | transmembrane and coiled-coil domain family 3                                | 2.97       |
| NM_002309  | 3976 LIF | predicted: misc_RNA                                                          | 2.94       |
| NM_006290  | 7128 TNFAIP3 | tumor necrosis factor, alpha-induced protein 3                               | 2.94       |
| LOC646     |          | PREDICTED: misc_RNA                                                          | 2.94       |
| Gene Symbol | Accession Number | Description | Log2 Fold Change | Expression Ratio |
|-------------|------------------|-------------|-----------------|-----------------|
| Homo sapiens, clone IMAGE:4445372 | 9 | HMOX1 heme oxygenase (decycling) 1 | 2.94 | 4.39 |
| NM_002133 | 3162 | HMOX1 heme oxygenase (decycling) 1 | 2.94 | 3.66 |
| NM_017527 | 54742 | LY6K lymphocyte antigen 6 complex, locus K | 2.92 | 4.53 |
| NM_002133 | 3162 | HMOX1 heme oxygenase (decycling) 1 | 2.92 | 3.93 |
| NM_005319 | 3006 | HIST1H1 histone cluster 1, H1c | 2.92 | 3.56 |
| NM_002133 | 3162 | HMOX1 heme oxygenase (decycling) 1 | 2.91 | 3.48 |
| NM_006988 | 9510 | ADAMT ADAM metallopeptidase with thrombospondin type S1 1 motif, 1 | 2.90 | 3.68 |
| NM_002133 | 3162 | HMOX1 heme oxygenase (decycling) 1 | 2.90 | 3.72 |
| NM_015589 | 23034 | SAMD4 sterile alpha motif domain containing 4A | 2.89 | 4.03 |
| NM_032572 | 84659 | RNASE7 ribonuclease, RNase A family, 7 | 2.89 | 3.95 |
| NM_001024 | 466 | SOD2 superoxide dismutase 2, mitochondrial, nuclear | 2.88 | 4.13 |
| NM_001945 | 6648 | HBEGF heparin-binding EGF-like growth factor | 2.88 | 4.73 |
| NM_002133 | 3162 | HMOX1 heme oxygenase (decycling) 1 | 2.87 | 3.82 |
| NM_001024 | 912 | CEACA carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein), variant 2 | 2.87 | 6.29 |
| NM_002133 | 3162 | HMOX1 heme oxygenase (decycling) 1 | 2.86 | 4.49 |
| NM_003897 | 8870 | IER3 immediate early response 3 | 2.86 | 3.26 |
| NM_001040 | 059 | 8334 AC Histone cluster 1, H2ac | 2.86 | 5.90 |
| 068 | CD68 CD68 molecule, variant 2 | 2.85 | 3.91 |
| NM_002133 | 3162 | HMOX1 heme oxygenase (decycling) 1 | 2.84 | 3.83 |
| NM_198447 | 127845 | GOLT1A golgi transport 1 homolog A (S. cerevisiae) | 2.84 | 3.84 |
| NM_006495 | 2124 | EVI2B ecotropic viral integration site 2B | 2.84 | 4.12 |
| Gene Accession | Gene Symbol | Description | Fold Change | Expression Level |
|---------------|-------------|-------------|-------------|-----------------|
| NM_001039     | ANGPTL4     | angiopoietin-like 4, variant 3 | 2.83 | 4.00 |
| NM_002133     | HMOX1       | heme oxygenase (decycling) 1 | 2.80 | 3.74 |
| NM_002133     | HMOX1       | heme oxygenase (decycling) 1 | 2.79 | 5.42 |
| NM_002133     | HMOX1       | heme oxygenase (decycling) 1 | 2.78 | 4.29 |
| NM_006851     | GLIPR1      | GLI pathogenesis-related 1 | 2.74 | 3.76 |
| NM_032935     | MT4         | metallothionein 4 | 2.73 | 3.09 |
| NM_006871     | RIPK3       | receptor-interacting serine-threonine kinase 3 | 2.73 | 3.54 |
| NM_004417     | DUSP1       | dual specificity phosphatase 1 | 2.73 | 3.95 |
| NM_153675     | FOXA2       | forkhead box A2, variant 2 | 2.72 | 3.04 |
| NM_002298     | LCP1        | lymphocyte cytosolic protein 1 (L-plastin) | 2.69 | 3.70 |
|               | HIST2H2     | histone cluster 2, H2aa3 | 2.67 | 3.89 |
| NM_003516     | AA3         | histone cluster 2, H2ac | 2.65 | 3.62 |
| NM_003512     | AC          | histone cluster 1, H2ac | 2.64 | 3.93 |
| NM_004751     | GCNT3       | glucosaminyl (N-acetyl) transferase 3, mucin type | 2.63 | 3.90 |
| NM_012304     | FBXL7       | F-box and leucine-rich repeat protein 7 | 2.62 | 4.11 |
| NM_080741     | NEU4        | sialidase 4 | 2.62 | 4.10 |
| NM_004419     | DUSP5       | dual specificity phosphatase 5 | 2.62 | 4.10 |
| NM_001097     | FUT3        | fucosyltransferase 3 (galactoside 3(4)-L-fucosyltransferase, Lewis blood group), variant 4 | 2.60 | 4.45 |
| NM_001109     | ADAM8       | ADAM metallopeptidase domain 8 | 2.60 | 3.26 |
| NM_003528     | BE          | histone cluster 2, H2be | 2.60 | 4.46 |
|               |             | Transcribed locus | 2.60 | 3.34 |
| NM_007115     | TNFAIP6     | tumor necrosis factor, alpha-induced protein 6 | 2.58 | 3.29 |
| NM_153840     | GPR110      | G protein-coupled receptor 110, variant 1 | 2.56 | 3.91 |
| Accession  | Value    | Description                                      | Fold Ch | Expression |
|------------|----------|--------------------------------------------------|---------|------------|
| NM_001257 | 1012     | CDH13 cadherin 13, H-cadherin (heart)             | 2.55    | 3.63       |
| NM_015364 | 2364     | LY96 lymphocyte antigen 96                       | 2.55    | 4.05       |
| NM_001039 | 667      | ANGPTL angiopoietin-like 4, variant 3             | 2.55    | 4.48       |
| NM_022664 | 1893     | ECM1 extracellular matrix protein 1, variant 2   | 2.54    | 3.62       |
| NM_003516 | 8337     | HIST2H2 histone cluster 2, H2aa3                 | 2.54    | 4.61       |
| NM_005658 | 7185     | TRAF1 TNF receptor-associated factor 1            | 2.53    | 5.02       |
| NM_004753 | 9249     | DHRS3 dehydrogenase/reductase (SDR family) member 3 | 2.53    | 4.98       |
| NM_002727 | 5552     | SRGN Serglycin                                    | 2.53    | 3.59       |
| NM_173842 | 3557     | IL1RN interleukin 1 receptor antagonist, variant 1| 2.49    | 4.08       |
| NM_004431 | 1969     | EPHA2 EPH receptor A2                             | 2.48    | 3.57       |
| NM_138788 | 120224   | TMEM4 transmembrane protein 45B                   | 2.48    | 4.74       |
| NM_016084 | 5165     | RASD1 RAS, dexamethasone-induced 1                | 2.47    | 4.45       |
|           | 9245     | GCNT3 Glucosaminyl (N-acetyl) transferase 3, mucin type | 2.47    | 4.52       |
| NM_15329  | 2       | TMEM9 transmembrane protein 92                    | 2.46    | 3.58       |
| NM_178450 | 115123   | MARCH membrane-associated ring finger (C3HC4) 3   | 2.46    | 3.38       |
| NM_002659 | 5329     | PLAUR plasminogen activator, urokinase receptor, variant 1 | 2.46    | 3.82       |
| NM_153675 | 3170     | FOXA2 forkhead box A2, variant 2                  | 2.45    | 3.56       |
| NM_006988 | 9510     | ADAMT ADAM metallopeptidase with thrombospondin type | 2.44    | 3.74       |
| NM_198593 | 114897   | C1QTNF C1q and tumor necrosis factor related protein 1, variant 2 | 2.44    | 3.27       |
| Accession   | Gene    | Description                                                                 | Fold Change | Expression   |
|------------|---------|------------------------------------------------------------------------------|-------------|-------------|
| NM_015685  | 27111   | SDCBP2 syndecan binding protein (syntenin) 2, variant 2                      | 2.44        | 3.48        |
| NM_144729  | 11221   | DUSP10 dual specificity phosphatase 10, variant 3                            | 2.44        | 4.14        |
| 207107     | SFTPF   | Surfactant associated protein F                                              | 2.44        | 4.16        |
| HIST2H2    |         |                                                                               |             |             |
| NM_003528  | 8349    | BE histone cluster 2, H2be                                                   | 2.44        | 4.96        |
| NM_000758  | 1437    | CSF2 colony stimulating factor 2 (granulocyte-macrophage)                   | 2.44        | 3.91        |
| NM_001570  | 3656    | IRAK2 interleukin-1 receptor-associated kinase 2                             | 2.43        | 3.34        |
| NM_000499  | 1543    | CYP1A1 cytochrome P450, family 1, subfamily A, polypeptide 1                | 2.43        | 3.07        |
| NM_001005  | 367     | TTYH1 tweety homolog 1 (Drosophila), variant 2                              | 2.43        | 3.82        |
| NM_024636  | 79689   | STEAP4 STEAP family member 4                                                | 2.42        | 3.50        |
|           |         |                                                                               |             |             |
| NM_001353  | 1645    | AKR1C1 aldo-keto reductase family 1, member C1 (dihydropyridine dehydrogenase 1; 20-alpha (3-alpha)-hydroxysteroid dehydrogenase) | 2.40        | 3.65        |
| NM_144975  | 162394  | SLFN5 schlafen family member 5                                              | 2.40        | 3.73        |
| NM_002638  | 5266    | PI3 peptidase inhibitor 3, skin-derived (SKALP)                              | 2.40        | 4.39        |
|           |         |                                                                               |             |             |
| NM_016084  | 51655   | RASD1 RAS, dexamethasone-induced 1                                           | 2.39        | 3.39        |
| NR_002786  | 152302  | CIDECP cell death-inducing DFFA-like effector c pseudogene on chromosome 3  | 2.38        | 3.84        |
| XM_001720  | 100130  | LOC100 PREDICTED: hypothetical protein LOC100                                 | 2.34        | 5.04        |
| 237        | 938     | 130938 PREDICTED: hypothetical protein LOC100                                 | 2.34        | 3.76        |
| NM_024780  | 79838   | TMC5 transmembrane channel-like 5, variant 3                                | 2.34        | 3.76        |
| ALDH1A     |         |                                                                               |             |             |
| NM_000693  | 220     | 3 aldehyde dehydrogenase 1 family, member A3                               | 2.34        | 3.90        |
| Accession | Entry | Description | Log2 Ratio | Log2 Fold Change |
|-----------|-------|-------------|------------|------------------|
| NM_004210 | 9148  | NEURL neuralized homolog (Drosophila) | 2.34 | 4.04 |
| NM_002965 | 6280  | S100A9 S100 calcium binding protein A9 | 2.34 | 3.52 |
|           |       | CDNA FLJ39181 fis, clone OCBBF2004235 | 2.33 | 3.46 |
| NM_020716 | 57476 | GRAMD GRAM domain containing 1B | 2.32 | 3.40 |
| NR_002834 | 574029| DUSP5P dual specificity phosphatase 5 pseudogene on chromosome 1 | 2.32 | 3.84 |
| NM_199335 | 2533  | FYB FYN binding protein (FYB-120/130), variant 2 | 2.32 | 4.17 |
| 266977    | GPR110| G protein-coupled receptor 110 | 2.31 | 4.70 |
| NM_004040 | 388   | RHOB ras homolog gene family, member B | 2.31 | 3.38 |
| NM_004822 | 9423  | NTN1 netrin 1 | 2.30 | 3.56 |
| NM_003364 | 7378  | UPP1 uridine phosphorylase 1, variant 1 | 2.29 | 3.49 |
| NM_000024 | 154   | ADRB2 adrenergic, beta-2-, receptor, surface | 2.29 | 5.30 |
|           | 100132| LOC100 Hypothetical protein | 2.29 | 3.56 |
|           | 244   | 132244 | Hypothetical protein | 2.29 | 3.56 |
| NM_058172 | 118429| ANTXR2 anthrax toxin receptor 2 | 2.29 | 4.28 |
| NM_003856 | 9173  | IL1RL1 interleukin 1 receptor-like 1, variant 2 | 2.29 | 3.44 |
| NM_016321 | 51458 | RHCG Rh family, C glycoprotein | 2.27 | 4.13 |
| NM_001248 | 956   | ENTPD3 ectonucleoside triphosphate diphosphohydrolase 3 | 2.27 | 4.13 |
| NM_002527 | 4908  | NTF3 neurotrophin 3, variant 2 | 2.27 | 3.16 |
| NM_002195 | 3641  | INSL4 insulin-like 4 (placenta) | 2.26 | 4.16 |
|           |       | interleukin 8 receptor, beta pseudogene on chromosome 2 | 2.26 | 3.27 |
| NR_002712 | 3580  | IL8RBP | 2.26 | 3.27 |
| NM_000024 | 154   | ADRB2 adrenergic, beta-2-, receptor, surface | 2.26 | 3.44 |
| NM_000024 | 154   | ADRB2 adrenergic, beta-2-, receptor, surface | 2.26 | 3.67 |
| NM_004083 | 1649  | DDIT3 DNA-damage-inducible 3 | 2.24 | 3.97 |

NM_004040 | 388   | RHOB ras homolog gene family, member B | 2.31 | 3.38 |
NM_004822 | 9423  | NTN1 netrin 1 | 2.30 | 3.56 |
NM_003364 | 7378  | UPP1 uridine phosphorylase 1, variant 1 | 2.29 | 3.49 |
NM_000024 | 154   | ADRB2 adrenergic, beta-2-, receptor, surface | 2.29 | 5.30 |
| Accession | ID   | Symbol | Description                                                                 | Fold | 
|-----------|------|--------|-----------------------------------------------------------------------------|------| 
| NM_000024 | 154  | ADRB2  | adrenergic, beta-2-, receptor, surface                                       | 2.24 | 
| LOC728    |      |        |                                                                              | 3.98 | 
| XR_036845 | 728364| 364    | PREDICTED: misc_RNA                                                          | 2.22 | 
|           |      |        |                                                                              | 3.33 | 
| NM_002034 | 2527 | FUT5   | fucosyltransferase 5 (alpha (1,3) fucosyltransferase)                       | 2.22 | 
|           |      |        |                                                                              | 5.51 | 
| NM_198174 | 57822| GRHL3  | grainyhead-like 3 (Drosophila), variant 3                                  | 2.22 | 
|           |      |        |                                                                              | 3.49 | 
| NM_000024 | 154  | ADRB2  | adrenergic, beta-2-, receptor, surface                                       | 2.21 | 
|           |      |        | oxidative stress induced growth inhibitor 1, variant                        |      | 
| NM_182981 | 29948| OSGIN1 |                                                                                | 2.21 | 
|           |      |        |                                                                               | 3.02 | 
| NM_000024 | 154  | ADRB2  | adrenergic, beta-2-, receptor, surface                                       | 2.21 | 
|           |      |        | six transmembrane epithelial antigen of the prostate 1                       | 2.20 | 
| NM_012449 | 26872| STEAP1 |                                                                                | 4.21 | 
|           |      |        |                                                                               |      | 
| NM_006350 | 10468| FST    | follistatin, variant FST317                                                  | 2.20 | 
|           |      |        |                                                                               | 3.80 | 
| NM_058172 | 118429| ANTXR2 | anthrax toxin receptor 2                                                      | 2.19 | 
|           |      |        |                                                                               | 4.80 | 
| NM_021181 | 57823| SLAMF7 | SLAM family member 7                                                          | 2.19 | 
|           |      |        |                                                                               | 3.63 | 
| HIST2H4   | 554313| B      | Histone cluster 2, H4b                                                        | 2.18 | 
|           |      |        |                                                                               | 3.78 | 
| NM_003155 | 6781 | STC1   | stanniocalcin 1                                                               | 2.17 | 
|           |      |        |                                                                               | 4.60 | 
| NM_000024 | 154  | ADRB2  | adrenergic, beta-2-, receptor, surface                                       | 2.17 | 
|           |      |        |                                                                               | 4.23 | 
| NM_001003 | 794  | MGLL   | monoglyceride lipase, variant 2                                               | 2.17 | 
|           |      |        |                                                                               | 3.31 | 
| NM_000024 | 154  | ADRB2  | adrenergic, beta-2-, receptor, surface                                       | 2.17 | 
|           |      |        |                                                                               | 4.14 | 
| NM_001248 | 956  | ENTPD3 | ectonucleoside triphosphate diphosphohydrolase 3                             | 2.16 | 
|           |      |        | dehydrogenase/reductase (SDR family) member 2, variant 2                     | 3.76 | 
| NM_005794 | 10202| DHRS2  |                                                                                | 2.16 | 
|           |      |        | variant 2                                                                    | 3.54 | 
| NM_021800 | 56521| DNAJC1 | DnaJ (Hsp40) homolog, subfamily C, member 12, variant 1                       | 2.16 | 
|           |      |        |                                                                               | 4.35 | 

Nature Publishing Group
| Accession  | Chromosome | Description                                                                 | Exp | Log2FDR |
|-----------|------------|------------------------------------------------------------------------------|-----|----------|
| NM_000024 | 154        | ADRB2 adrenergic, beta-2-, receptor, surface                                | 2.16| 3.79    |
| NM_002160 | 3371       | TNC tenascin C                                                              | 2.15| 3.41    |
| NM_002281 | 3887       | KRT81 keratin 81                                                            | 2.15| 3.76    |
| NM_000024 | 154        | ADRB2 adrenergic, beta-2-, receptor, surface                                | 2.14| 4.34    |
| NM_005723 | 10098      | TSPAN5 tetraspanin 5                                                        | 2.14| 3.56    |
| NM_144497 | 9590       | AKAP12 A kinase (PRKA) anchor protein (gravin) 12, variant                 | 2.14| 3.12    |
| NM_153685 | 196500     | chromosome 12 open reading frame 53                                         | 2.13| 4.07    |
| NM_004235 | 9314       | KLF4 Kruppel-like factor 4 (gut)                                             | 2.13| 3.54    |
| NM_002032 | 2495       | FTH1 ferritin, heavy polypeptide 1                                          | 2.13| 3.59    |
| NM_178863 | 253980     | KCTD13 potassium channel tetramerisation domain containing 13              | 2.12| 3.64    |
| LR_042007 | 728978     | LOC728 PREDICTED: misc_RNA                                                  | 2.12| 3.55    |
| NM_005379 | 4640       | MYO1A myosin IA                                                             | 2.12| 4.37    |
| NM_021065 | 3013       | AD histone cluster 1, H2ad                                                   | 2.12| 3.48    |
| NM_144497 | 9590       | AKAP12 A kinase (PRKA) anchor protein (gravin) 12, variant                 | 2.11| 3.36    |
| NM_001172 | 384        | ARG2 arginase, type II, nuclear gene encoding mitochondrial protein         | 2.11| 3.88    |
| NM_005764 | 10158      | PDZK1IP PDZK1 interacting protein 1                                         | 2.11| 3.22    |
| NM_022094 | 63924      | CIDE cell death-inducing DFFA-like effector c                              | 2.11| 3.56    |
| Gene ID       | Transcript ID | Description                                      | Log2 Fold   | p-Value  |
|--------------|---------------|--------------------------------------------------|-------------|----------|
| NM_017899    | 54997         | TESC tescalcin                                   | 2.10        | 4.04     |
| NM_021013    | 3885          | KRT34 keratin 34                                 | 2.10        | 3.65     |
| NM_001037    | 165           | FOXK1 forkhead box K1                            | 2.09        | 3.06     |
|             | 221937        | ALDO-KRT1 aldo-keto reductase family 1, member C1|             |          |
|             |               | (dihydrodiol dehydrogenase 1; 20-alpha (3-alpha)-|             |          |
|             |               | hydroxysteroid dehydrogenase)                   |             |          |
| NM_001353    | 1645          | AKR1C1 aldo-keto reductase family 1, member C1   | 2.09        | 3.58     |
| NM_001001    | 437           | CCL3L3 chemokine (C-C motif) ligand 3-like 3     | 2.08        | 3.14     |
| NM_001110    | 354           | ZP3 zona pellucida glycoprotein 3 (sperm receptor)|             |          |
|             | 7784          | PLAC8 placenta-specific 8, variant 2             | 2.08        | 3.48     |
|             |               | Transcribed locus                                | 2.07        | 3.36     |
| NM_175884    | 168455        | FLJ3603 hypothetical protein FLJ36031            | 2.08        | 4.94     |
| NM_016619    | 51316         | PLAC8 placenta-specific 8, variant 2             | 2.08        | 3.48     |
| NM_001097    | 600           | TMEM2 transmembrane protein 22, variant 3        | 2.07        | 3.12     |
| NM_003975    | 9047          | SH2D2A SH2 domain protein 2A                     | 2.07        | 3.69     |
| NM_182743    | 7296          | TXNRD1 thioredoxin reductase 1, variant 4        | 2.07        | 3.95     |
| NM_005555    | 3854          | KRT6B keratin 6B                                 | 2.07        | 3.48     |
| NM_001102    | 595           | DTX2 deltex homolog 2 (Drosophila), variant 3    | 2.07        | 3.58     |
|             | 113878        | GTP binding protein overexpressed in skeletal    |             |          |
|             |               | muscle, variant 2                                |             |          |
| NM_181702    | 2669          | GEM CDNA FLJ10586 fis, clone NT2RP2003986        | 2.06        | 3.46     |
| NM_001179    | 419           | ART3 ADP-riboseyltransferase 3, variant 2        | 2.06        | 3.69     |
| Accession | Gene ID | Gene Name                       | Expression Ratio |
|-----------|---------|---------------------------------|------------------|
| NM_001353 | 1645    | AKR1C1 (aldo-keto reductase)     | 2.06 3.59        |
| NM_005953 | 4502    | MT2A (metallothionein 2A)        | 2.05 3.53        |
| NM_002502 | 4791    | NFKB2 (nuclear factor of kappa)  | 2.05 3.12        |
|           |         |                                | mRNA; cDNA DKFZp434M054 | 2.04 4.71 |
| NM_001098 | 212     | HRH1 (histamine receptor H1)    | 2.03 4.45        |
| NM_022767 | 64782   | AEN (apoptosis enhancing nuclease)| 2.03 3.94       |
|           |         | SEC61A                          |                  |
| NM_018144 | 55176   | Sec61 alpha 2 subunit           | 2.03 4.01        |
|           |         | (S. cerevisiae)                 |                  |
| NM_182507 | 144501  | KRT80 (keratin 80)              | 2.02 3.52        |
| NM_021972 | 8877    | SPHK1 (sphingosine kinase)       | 2.02 3.01        |
| NM_033646 | 1005    | CDH7 (cadherin 7)               | 2.02 5.43        |
|           |         | (type 2, variant a)             |                  |
| NM_015657 | 26154   | ABCA12 (ATP-binding cassette)   | 2.01 4.00        |
|           |         | (sub-family A (ABC1), member)   |                  |
| NM_003841 | 8794    | TNFRSF10C (tumor necrosis factor)| 2.01 4.57        |
|           | 10C     | receptor superfamily, member 10c, decoy |              |
| NM_003965 | 9034    | CCRL2 (chemokine (C-C motif)    | 2.00 3.08        |
|           |         | receptor-like 2)                |                  |
| NM_001097 | 600     | TMEM2 (transmembrane protein)    | 2.00 3.36        |
|           | 80723   | 2 (variant 3)                   |                  |
Supplementary Table 2.

Genes Down-Regulated by Pyocyanin (p-value < 0.001, log2 ratio < -2)

| RefSeq   | Entrez Gene ID | Gene       | Description                                           | Log2 Ratio | Neg. log10 p-value |
|----------|----------------|------------|-------------------------------------------------------|------------|--------------------|
| NM_153000| 147495         | APCDD1     | adenomatosis polyposis coli down-regulated 1          | -4.76      | 4.25               |
| NM_002191| 3623           | INHA       | inhibin, alpha                                       | -4.22      | 4.83               |
| NM_002048| 2619           | GAS1       | growth arrest-specific 1                             | -4.10      | 4.97               |
| NM_001885| 1410           | CRYAB      | crystallin, alpha B                                  | -3.63      | 3.73               |
| NM_005218| 1672           | DEFB1      | defensin, beta 1                                     | -3.33      | 3.26               |
|          | 9340           | GLP2R      | Glucagon-like peptide 2 receptor                      | -3.31      | 3.92               |
| NM_002272| 3851           | KRT4       | keratin 4                                            | -3.25      | 4.11               |
| NM_001216| 768            | CA9        | carbonic anhydrase IX                                | -3.22      | 3.84               |
| NM_001122| 679            | ODZ2       | odz, odd Oz/ten-m homolog 2 (Drosophila)             | -3.18      | 4.12               |
| NM_177403| 338382         | RAB7B      | RAB7B, member RAS oncogene family                    | -3.15      | 3.67               |
| NM_001878| 1382           | CRABP2     | cellular retinoic acid binding protein 2             | -3.09      | 4.39               |
| NM_001312| 1397           | CRIP2      | cysteine-rich protein 2                               | -3.04      | 3.97               |
| NM_175068| 319101         | KRT73      | keratin 73                                           | -3.03      | 3.91               |
| NM_000093| 1289           | COL5A1     | collagen, type V, alpha 1                             | -2.93      | 5.13               |
| NM_001018| 057            | DKK3       | dickkopf homolog 3 (Xenopus laevis), variant 3       | -2.88      | 4.35               |
| NM_001018| 057            | DKK3       | dickkopf homolog 3 (Xenopus laevis), variant 3       | -2.86      | 3.36               |
| NM_181677| 5521 B         | PPP2R2     | protein phosphatase 2 (formerly 2A), regulatory subunit B, beta isoform, variant 5 | -2.78      | 4.17               |
|          | 10451           | VAV3       | Vav 3 guanine nucleotide exchange factor             | -2.77      | 3.26               |
| Accession   | Start | End   | Gene Description                                           | Log2 Fold Change | Log10 Fold Change |
|-------------|-------|-------|------------------------------------------------------------|------------------|-------------------|
| NM_001083   | 896   | 645832| SEBOX homeobox, variant 2                                  | -2.73            | 4.76              |
| NM_019601   | 56241 | SUSD2 | sushi domain containing 2                                   | -2.69            | 4.74              |
| NM_001079   | 874   | 10451 | vav 3 guanine nucleotide exchange factor, variant 2         | -2.68            | 3.46              |
| NM_003016   | 6427  | SFRS2 | splicing factor, arginine/serine-rich 2                     | -2.57            | 3.46              |
| NM_001111   | 89797 | NAV2  | neuron navigator 2, variant 4                              | -2.51            | 4.32              |
| NM_007173   | 11098 | PRSS23| protease, serine, 23                                      | -2.48            | 4.42              |
| NM_016571   | 51557 | GLULD1| domain containing 1                                        | -2.47            | 4.01              |
| NR_002196   | 283120| H19   | H19, imprinted maternally expressed (non-protein coding) on chromosome 11. | -2.45            | 4.58              |
| NM_000424   | 3852  | KRT5  | keratin 5                                                  | -2.44            | 4.34              |
| NM_000093   | 1289  | COL5A1| collagen, type V, alpha 1                                  | -2.37            | 3.55              |
| NM_024336   | 79191 | IRX3  | iroquois homeobox 3                                        | -2.32            | 4.00              |
| NM_016588   | 51299 | NRN1  | neuritin 1                                                 | -2.29            | 4.81              |
| NM_032229   | 84189 | SLITRK6| SLIT and NTRK-like family, member 6                        | -2.28            | 3.34              |
|             |       |       | Transcribed locus                                          | -2.28            | 4.38              |
| NM_017848   | 54954 | C     | family with sequence similarity 120C                      | -2.27            | 4.21              |
| NM_174959   | 136306| SVOPL | SVOP-like                                                  | -2.25            | 3.83              |
| NM_020686   | 18    | ABAT  | 4-aminobutyrate aminotransferase, nuclear gene encoding mitochondrial protein, variant 1 | -2.25            | 4.71              |
| NM_030806   | 81563 | C1orf21| chromosome 1 open reading frame 21                        | -2.22            | 4.42              |
|             | 79883 | PODNL1| Podocan-like 1                                             | -2.22            | 3.77              |
| NM_199512   | 151887| CCDC80| coiled-coil domain containing 80, variant 2               | -2.22            | 3.40              |
| Gene Symbol | Accession Number | Description | Log2 Fold Change |
|-------------|------------------|-------------|-----------------|
| NM_003621   | 8495             | PPFIBP2     | -2.21           |
| NM_030761   | 54361            | WNT4        | -2.20           |
| NM_001447   | 2196             | FAT2        | -2.18           |
| NM_005581   | 4059             | BCAM        | -2.18           |
| NM_014387   | 27040            | LAT         | -2.18           |
| NM_00435    | 4854             | NOTCH3      | -2.17           |
|             | 205              | AK3L1       | -2.16           |
| NM_016240   | 51435            | SCARA3      | -2.15           |
| NM_004772   | 9315             | C5orf13     | -2.14           |
|             | 244              | ANXA8L      | -2.14           |
| NM_145810   | 83879            | CDCA7       | -2.13           |
| NM_007308   | 6622             | SNCA        | -2.12           |
|             | 23363            | OBSL1       | -2.11           |
| NM_005669   | 7905             | REEP5       | -2.09           |
| NM_007308   | 6622             | SNCA        | -2.08           |
| NM_032812   | 8498             | PLXDC2      | -2.08           |
| NM_173061   | 831              | CAST        | -2.08           |
| NM_001333   | 1515             | CTSL2       | -2.08           |
| NM_181712   | 163782           | KANK4       | -2.07           |
| NM_016240   | 51435            | SCARA3      | -2.06           |
| NM_199512   | 151887           | CCDC80      | -2.06           |
| Gene ID    | Accession | Gene Name                  | Symbol     | Log2 Fold Change | P-Value |
|------------|-----------|----------------------------|------------|------------------|---------|
| NM_007308  | 6622      | SNCA synuclein, alpha      | SNCA       | -2.06            | 6.59    |
|            |           | (non A4 component of amyloid precursor), variant NACP112 |           |                  |         |
| NM_007308  | 6622      | SNCA synuclein, alpha      | SNCA       | -2.06            | 4.03    |
|            |           | (non A4 component of amyloid precursor), variant NACP112 |           |                  |         |
| NM_001111  | 019       | NAV2 neuron navigator      | NAV2       | -2.05            | 4.17    |
| NM_001964  | 1958      | EGR1 early growth response | EGR1       | -2.04            | 3.78    |
| NM_016731  | 2348      | FOLR1 folate receptor      | FOLR1      | -2.04            | 3.43    |
| NM_007308  | 6622      | SNCA synuclein, alpha      | SNCA       | -2.04            | 3.20    |
|            |           | (non A4 component of amyloid precursor), variant NACP112 |           |                  |         |
| NM_007308  | 6622      | SNCA synuclein, alpha      | SNCA       | -2.03            | 3.92    |
|            |           | (non A4 component of amyloid precursor), variant NACP112 |           |                  |         |
| NM_007308  | 6622      | SNCA synuclein, alpha      | SNCA       | -2.02            | 3.60    |
|            |           | (non A4 component of amyloid precursor), variant NACP112 |           |                  |         |
| NM_001127  | 464       | ZNF469 zinc finger protein | ZNF469     | -2.01            | 4.08    |
| NM_001719  | 655       | BMP7 bone morphogenetic    | BMP7       | -2.00            | 4.27    |
| NM_153000  | 147495    | APCDD1 adenomatosis polyposis coli down-regulated 1 | APCDD1     | -4.76            | 4.25    |
| NM_002191  | 3623      | INHA inhibin, alpha        | INHA       | -4.22            | 4.83    |
| NM_002048  | 2619      | GAS1 growth arrest-specific | GAS1       | -4.10            | 4.97    |
| NM_001885  | 1410      | CRYAB crystallin, alpha B  | CRYAB      | -3.63            | 3.73    |
| NM_005218  | 1672      | DEFB1 defensin, beta 1     | DEFB1      | -3.33            | 3.26    |
| NM_002272  | 3851      | KRT4 keratin 4             | KRT4       | -3.25            | 4.11    |
| NM_001216  | 768       | CA9 carbonic anhydrase IX  | CA9        | -3.22            | 3.84    |
| NM_001122  | 679       | ODZ2 odz, odd Oz/ten-m homolog 2 (Drosophila) | ODZ2       | -3.18            | 4.12    |
| Accession   | Gene ID | Gene Name                      | Description                                                                 | Expression Level |
|-------------|---------|---------------------------------|-----------------------------------------------------------------------------|------------------|
| NM_177403   | 338382  | RAB7B                           | RAB7B, member RAS oncogene family                                           | -3.15 3.67       |
| NM_001878   | 1382    | CRABP2                          | cellular retinoic acid binding protein 2                                   | -3.09 4.39       |
| NM_001312   | 1397    | CRIP2                           | cysteine-rich protein 2                                                   | -3.04 3.97       |
| NM_175068   | 319101  | KRT73                           | keratin 73                                                                  | -3.03 3.91       |
| NM_000093   | 1289    | COL5A1                          | collagen, type V, alpha 1                                                | -2.93 5.13       |
| NM_001018   | 27122   | DKK3                            | dickkopf homolog 3 (Xenopus laevis), variant 3                            | -2.88 4.35       |
| NM_001018   | 27122   | DKK3                            | dickkopf homolog 3 (Xenopus laevis), variant 3                            | -2.86 3.36       |
|             |         | PPP2R2                          | protein phosphatase 2 (formerly 2A), regulatory subunit B, beta isoform, variant 5 | -2.78 4.17       |
| NM_181677   | 5521    | VAV3                            | Vav 3 guanine nucleotide exchange factor                                   | -2.77 3.26       |
| NM_001083   | 645832  | SEBOX                           | SEBOX homeobox, variant 2                                                 | -2.73 4.76       |
| NM_019601   | 56241   | SUSD2                           | sushi domain containing 2                                                   | -2.69 4.74       |
| NM_001079   | 10451   | VAV3                            | vav 3 guanine nucleotide exchange factor, variant 2                        | -2.68 3.46       |
| NM_003016   | 6427    | SFRS2                           | splicing factor, arginine/serine-rich 2                                    | -2.57 3.46       |
| NM_007173   | 5087    | PBX1                            | Pre-B-cell leukemia homeobox 1                                             | -2.54 5.20       |
| NM_001111   | 89797   | NAV2                            | neuron navigator 2, variant 4                                             | -2.51 4.32       |
| NM_007173   | 11098   | PRSS23                          | protease, serine, 23                                                     | -2.48 4.42       |
| NM_016571   | 51557   | GLULD1                          | glutamate-ammonia ligase (glutamine synthetase) domain containing 1        | -2.47 4.01       |
| NR_002196   | 283120  | H19                             | H19, imprinted maternally expressed (non-protein coding) on chromosome 11. | -2.45 4.58       |
| NM_000424   | 3852    | KRT5                            | keratin 5                                                                   | -2.44 4.34       |
| NM_000093   | 1289    | COL5A1                          | collagen, type V, alpha 1                                                 | -2.37 3.55       |
| Accession  | Gene Symbol | Gene Description                              | Log2 Fold Change | q-value |
|------------|-------------|-----------------------------------------------|-----------------|---------|
| NM_024336  | IRX3        | iroquois homeobox 3                           | -2.32           | 4.00    |
| NM_016588  | NRN1        | neuritin 1                                     | -2.29           | 4.81    |
| NM_032229  | SLITRK6     | SLIT and NTRK-like family, member 6            | -2.28           | 3.34    |
|            | Trans       | Transcribed locus                              | -2.28           | 4.38    |
| NM_017848  | C           | family with sequence similarity 120C           | -2.27           | 4.21    |
| NM_174959  | SVOPL       | SVOP-like                                     | -2.25           | 3.83    |
| NM_020686  | ABAT        | 4-aminobutyrate aminotransferase, nuclear gene | -2.25           | 4.71    |
| NM_030806  | C1orf21     | chromosome 1 open reading frame 21            | -2.22           | 4.42    |
|            | PODNL1      | Podocan-like 1                                 | -2.22           | 3.77    |
| NM_199512  | CCDC80      | coiled-coil domain containing 80, variant 2   | -2.22           | 3.40    |
| NM_003621  | PPFIBP2     | PTPRF interacting protein, binding protein 2  | -2.21           | 3.31    |
|            |             | (liprin beta 2)                                |                 |         |
| NM_030761  | WNT4        | wingless-type MMTV integration site family,   | -2.20           | 3.60    |
|            |             | member 4                                      |                 |         |
| NM_001447  | FAT2        | FAT tumor suppressor homolog 2 (Drosophila)    | -2.18           | 3.82    |
| NM_005581  | BCAM        | basal cell adhesion molecule (Lutheran blood  | -2.18           | 4.25    |
|            |             | group), variant 1                             |                 |         |
| NM_014387  | LAT         | linker for activation of T cells, variant 1    | -2.18           | 3.66    |
| NM_00435   | NOTCH3      | Notch homolog 3 (Drosophila)                  | -2.17           | 3.53    |
|            | AK3L1       | Adenylate kinase 3-like 1                     | -2.16           | 3.84    |
| NM_016240  | SCARA3      | scavenger receptor class A, member 3, variant | -2.15           | 4.01    |
| NM_004772  | C5orf13     | chromosome 5 open reading frame 13            | -2.14           | 4.11    |
|            | ANXA8L      | Annexin A8-like 2                             | -2.14           | 3.57    |
| NM_145810  | CDCA7       | cell division cycle associated 7, variant 2   | -2.13           | 4.01    |
| Accession | Gene ID | Gene Name                  | log2 Fold Change | Expression Value |
|-----------|---------|----------------------------|-----------------|------------------|
| NM_007308 | 6622    | SNCA synuclein, alpha (non A4 component of amyloid precursor), variant NACP112 | -2.12          | 4.19             |
| 23363     | OBSL1   | Obscurin-like 1             | -2.11           | 4.25             |
| NM_005669 | 7905    | REEP5 receptor accessory protein 5 | -2.09           | 3.58             |
| NM_007308 | 6622    | SNCA synuclein, alpha (non A4 component of amyloid precursor), variant NACP112 | -2.08           | 3.20             |
| NM_032812 | 84898   | PLXDC2 plexin domain containing 2 | -2.08           | 3.63             |
| NM_173061 | 831     | CAST calpastatin, variant 3 | -2.08           | 3.97             |
| NM_001333 | 1515    | CTSL2 cathepsin L2          | -2.08           | 3.51             |
| NM_181712 | 163782  | KANK4 KN motif and ankyrin repeat domains 4 | -2.07           | 4.06             |
| NM_016240 | 51435   | SCARA3 scavenger receptor class A, member 3, variant 1 | -2.06           | 3.54             |
| NM_199512 | 151887  | CCDC80 coiled-coil domain containing 80, variant 2 | -2.06           | 3.94             |
| NM_007308 | 6622    | SNCA synuclein, alpha (non A4 component of amyloid precursor), variant NACP112 | -2.06           | 6.59             |
| NM_007308 | 6622    | SNCA synuclein, alpha (non A4 component of amyloid precursor), variant NACP112 | -2.06           | 4.03             |
| NM_001111 | 019     | NAV2 neuron navigator 2, variant 4 | -2.05           | 4.17             |
| NM_001964 | 1958    | EGR1 early growth response 1 | -2.04           | 3.78             |
| NM_016731 | 2348    | FOLR1 folate receptor 1 (adult), variant 8 | -2.04           | 3.43             |
| NM_007308 | 6622    | SNCA synuclein, alpha (non A4 component of amyloid precursor), variant NACP112 | -2.04           | 3.20             |
| NM_007308 | 6622    | SNCA synuclein, alpha (non A4 component of amyloid precursor), variant NACP112 | -2.03           | 3.92             |
| 1266      | CNN3    | Calponin 3, acidic          | -2.03           | 3.02             |
| NM_007308 | 6622    | SNCA synuclein, alpha (non A4 component of amyloid precursor), variant NACP112 | -2.02           | 3.60             |
| ID    | Gene ID | Gene Symbol | Description                                              | Log2FoldChange | P-Value |
|-------|---------|-------------|----------------------------------------------------------|----------------|---------|
| NM_001127 | 464     | ZNF469      | zinc finger protein 469                                  | -2.01          | 4.08    |
| NM_001719 | 655     | BMP7        | bone morphogenetic protein 7                             | -2.00          | 4.27    |
| NM_153000 | 147495  | APCDD1      | adenomatososis polyposis coli down-regulated 1            | -4.76          | 4.25    |
| NM_002191 | 3623    | INHA        | inhibin, alpha                                           | -4.22          | 4.83    |
| NM_002048 | 2619    | GAS1        | growth arrest-specific 1                                  | -4.10          | 4.97    |
| NM_001885 | 1410    | CRYAB       | crystallin, alpha B                                      | -3.63          | 3.73    |
| NM_005218 | 1672    | DEFB1       | defensin, beta 1                                         | -3.33          | 3.26    |
|         | 9340    | GLP2R       | Glucagon-like peptide 2 receptor                          | -3.31          | 3.92    |
| NM_002272 | 3851    | KRT4        | keratin 4                                                | -3.25          | 4.11    |
| NM_001216 | 768     | CA9         | carbonic anhydrase IX                                    | -3.22          | 3.84    |
| NM_001122 | 679     | ODZ2        | odz, odd Oz/ten-m homolog 2 (Drosophila)                 | -3.18          | 4.12    |
| NM_177403 | 338382  | RAB7B       | RAB7B, member RAS oncogene family                         | -3.15          | 3.67    |
| NM_001878 | 1382    | CRABP2      | cellular retinoic acid binding protein 2                 | -3.09          | 4.39    |
| NM_001312 | 1397    | CRIP2       | cysteine-rich protein 2                                   | -3.04          | 3.97    |
| NM_175068 | 319101  | KRT73       | keratin 73                                               | -3.03          | 3.91    |
| NM_000093 | 1289    | COL5A1      | collagen, type V, alpha 1                                | -2.93          | 5.13    |
| NM_001018 | 057     | DKK3        | dickkopf homolog 3 (Xenopus laevis), variant 3           | -2.88          | 4.35    |
| NM_001018 | 057     | DKK3        | dickkopf homolog 3 (Xenopus laevis), variant 3           | -2.86          | 3.36    |
| NM_181677 | 5521    | PPP2R2B     | protein phosphatase 2 (formerly 2A), regulatory subunit B, beta isoform, variant 5 | -2.78          | 4.17    |
|         | 10451   | VAV3        | Vav 3 guanine nucleotide exchange factor                 | -2.77          | 3.26    |
| NM_001083 | 896     | SEBOX       | SEBOX homeobox, variant 2                                | -2.73          | 4.76    |
| Accession | ID     | Symbol | Description                                                                 | Fold Change | log2FC |
|-----------|--------|--------|-----------------------------------------------------------------------------|-------------|--------|
| NM_019601 | 56241  | SUSD2  | sushi domain containing 2                                                   | -2.69       | 4.74   |
| NM_001079 | 874    | VAV3   | vav 3 guanine nucleotide exchange factor, variant 2                         | -2.68       | 3.46   |
| NM_003016 | 6427   | SFRS2  | splicing factor, arginine/serine-rich 2                                     | -2.57       | 3.46   |
|           | 5087   | PBX1   | Pre-B-cell leukemia homeobox 1                                              | -2.54       | 5.20   |
| NM_001111 | 019    | NAV2   | neuron navigator 2, variant 4                                               | -2.51       | 4.32   |
| NM_007173 | 11098  | PRSS23 | protease, serine, 23                                                       | -2.48       | 4.42   |
| NM_016571 | 51557  | GLULD1 | glutamate-ammonia ligase (glutamine synthetase) domain containing 1          | -2.47       | 4.01   |
| NR_002196 | 283120 | H19    | H19, imprinted maternally expressed (non-protein coding) on chromosome 11. | -2.45       | 4.58   |
| NM_000424 | 3852   | KRT5   | keratin 5                                                                   | -2.44       | 4.34   |
| NM_000093 | 1289   | COLSA1 | collagen, type V, alpha 1                                                  | -2.37       | 3.55   |
| NM_024336 | 79191  | IRX3   | iroquois homeobox 3                                                         | -2.32       | 4.00   |
| NM_016588 | 51299  | NRN1   | neuritin 1                                                                  | -2.29       | 4.81   |
| NM_032229 | 84189  | SLITRK6| SLIT and NTRK-like family, member 6                                         | -2.28       | 3.34   |
|           |        |        | Transcribed locus                                                           | -2.28       | 4.38   |
| NM_017848 | 54954  | C      | family with sequence similarity 120C                                        | -2.27       | 4.21   |
| NM_174959 | 136306 | SVOPL  | SVOP-like                                                                   | -2.25       | 3.83   |
| NM_020686 | 18     | ABAT   | 4-aminobutyrate aminotransferase, nuclear gene encoding mitochondrial protein | -2.25       | 4.71   |
| NM_030806 | 81563  | C1orf21| chromosome 1 open reading frame 21                                          | -2.22       | 4.42   |
|           | 79883  | PODNL1 | Podocan-like 1                                                              | -2.22       | 3.77   |
| NM_199512 | 151887 | CCDC80 | coiled-coil domain containing 80, variant 2                                 | -2.22       | 3.40   |
| NM_003621 | 8495   | PPFIBP2| PTPRF interacting protein, binding protein 2 (liprin beta 2)                | -2.21       | 3.31   |
| NM_030761 | WNT4  | -2.20 | 3.60 |
| NM_001447 | FAT2  | -2.18 | 3.82 |
| NM_005581 | BCAM  | -2.18 | 4.25 |
| NM_014387 | LAT   | -2.18 | 3.66 |
| NM_000435 | NOTCH3| -2.17 | 3.53 |
| 205 AK3L1 | -2.16 | 3.84 |
| NM_016240 | SCARA3| -2.15 | 4.01 |
| NM_007308 | SNCA  | -2.18 | 4.19 |
| 244 ANXA8L| -2.14 | 3.57 |
| NM_145810 | CDC7  | -2.13 | 4.01 |
| NM_007308 | SNCA  | -2.12 | 4.19 |
| 23363 OBLS1| -2.11 | 4.25 |
| NM_005669 | REEP5 | -2.09 | 3.58 |
| NM_173061 | CAST  | -2.08 | 3.90 |
| NM_001333 | CTSL2 | -2.08 | 3.51 |
| NM_199512 | CCDC80| -2.06 | 3.94 |
| NM_000435 | SNCA  | -2.06 | 6.59 |
| Accession | Gene ID  | Gene Symbol | Description | Fold Change | Log Fold Change |
|-----------|----------|-------------|-------------|-------------|----------------|
| NM_007308 6622 | SNCA | synuclein, alpha (non A4 component of amyloid precursor), variant NACP112 | -2.06 | 4.03 |
| NM_001111 019 | NAV2 | neuron navigator 2, variant 4 | -2.05 | 4.17 |
| NM_001964 1958 | EGR1 | early growth response 1 | -2.04 | 3.78 |
| NM_016731 2348 | FOLR1 | folate receptor 1 (adult), variant 8 | -2.04 | 3.43 |
| NM_007308 6622 | SNCA | synuclein, alpha (non A4 component of amyloid precursor), variant NACP112 | -2.04 | 3.20 |
| NM_007308 6622 | SNCA | synuclein, alpha (non A4 component of amyloid precursor), variant NACP112 | -2.03 | 3.92 |
| NM_007308 6622 | SNCA | synuclein, alpha (non A4 component of amyloid precursor), variant NACP112 | -2.02 | 3.60 |
| NM_001127 464 | ZNF469 | zinc finger protein 469 | -2.01 | 4.08 |
| NM_001719 655 | BMP7 | bone morphogenetic protein 7 | -2.00 | 4.27 |
| NM_153000 147495 | APCDD1 | adenomatosis polyposis coli down-regulated 1 | -4.76 | 4.25 |
| NM_002191 3623 | INHA | inhibin, alpha | -4.22 | 4.83 |
| NM_002048 2619 | GAS1 | growth arrest-specific 1 | -4.10 | 4.97 |
| NM_001885 1410 | CRYAB | crystallin, alpha B | -3.63 | 3.73 |
| NM_005218 1672 | DEFB1 | defensin, beta 1 | -3.33 | 3.26 |
| NM_002272 3851 | KRT4 | keratin 4 | -3.25 | 4.11 |
| NM_001216 768 | CA9 | carbonic anhydrase IX | -3.22 | 3.84 |
| NM_001122 679 | ODZ2 | odz, odd Oz/ten-m homolog 2 (Drosophila) | -3.18 | 4.12 |
| NM_177403 338382 | RAB7B | RAB7B, member RAS oncogene family | -3.15 | 3.67 |
| NM_001878 1382 | CRABP2 | cellular retinoic acid binding protein 2 | -3.09 | 4.39 |
| NM_001312 | 1397 | CRIP2 | cysteine-rich protein 2 | -3.04 | 3.97 |
| NM_175068 | 319101 | KRT73 | keratin 73 | -3.03 | 3.91 |
| NM_000093 | 1289 | COL5A1 | collagen, type V, alpha 1 | -2.93 | 5.13 |
| NM_001018 | 27122 | DKK3 | dickkopf homolog 3 (Xenopus laevis), variant 3 | -2.88 | 4.35 |
| NM_001018 | 27122 | DKK3 | dickkopf homolog 3 (Xenopus laevis), variant 3 | -2.86 | 3.36 |
| NM_181677 | 5521 | PPP2R2 | protein phosphatase 2 (formerly 2A), regulatory subunit B, beta isoform, variant 5 | -2.78 | 4.17 |
| NM_001083 | 645832 | SEBOX | SEBOX homeobox, variant 2 | -2.73 | 4.76 |
| NM_019601 | 56241 | SUSD2 | sushi domain containing 2 | -2.69 | 4.74 |
| NM_001079 | 10451 | VAV3 | Vav 3 guanine nucleotide exchange factor | -2.77 | 3.26 |
| NM_003016 | 5087 | PBX1 | Pre-B-cell leukemia homeobox 1 | -2.54 | 5.20 |
| NM_001111 | 89797 | NAV2 | neuron navigator 2, variant 4 | -2.51 | 4.32 |
| NM_007173 | 11098 | PRSS23 | protease, serine, 23 | -2.48 | 4.42 |
| NM_016571 | 51557 | GLULD1 | glutamate-ammonia ligase (glutamine synthetase) domain containing 1 | -2.47 | 4.01 |
| NR_002196 | 283120 | H19 | H19, imprinted maternally expressed (non-protein coding) on chromosome 11. | -2.45 | 4.58 |
| NM_000424 | 3852 | KRT5 | keratin 5 | -2.44 | 4.34 |
| NM_000093 | 1289 | COL5A1 | collagen, type V, alpha 1 | -2.37 | 3.55 |
| NM_024336 | 79191 | IRX3 | iroquois homeobox 3 | -2.32 | 4.00 |
| NM_016588 | 51299 | NRN1 | neuritin 1 | -2.29 | 4.81 |
| Accession | Log2FoldChange | Description |
|-----------|----------------|-------------|
| NM_032229 | -2.28          | SLITR6 SLIT and NTRK-like family, member 6 |
| NM_017848 | -2.27          | FAM120C family with sequence similarity 120C |
| NM_174959 | -2.25          | SVOPL SVOP-like |
| NM_020686 | -2.25          | ABAT 4-aminobutyrate aminotransferase, nuclear gene |
| NM_030806 | -2.22          | C1orf21 chromosome 1 open reading frame 21 |
| NM_199512 | -2.22          | CCDC80 coiled-coil domain containing 80, variant 2 |
| NM_003621 | -2.21          | PPFIBP2 PTPRF interacting protein, binding protein 2 (liprin beta 2) |
| NM_030761 | -2.20          | WNT4 wingless-type MMTV integration site family, member 4 |
| NM_001447 | -2.18          | FAT2 FAT tumor suppressor homolog 2 (Drosophila) |
| NM_005581 | -2.18          | BCAM basal cell adhesion molecule (Lutheran blood group), variant 1 |
| NM_014387 | -2.18          | LAT linker for activation of T cells, variant 1 |
| NM_000435 | -2.17          | NOTCH3 Notch homolog 3 (Drosophila) |
| NM_016240 | -2.15          | SCARA3 scavenger receptor class A, member 3, variant 1 |
| NM_004772 | -2.14          | C5orf13 chromosome 5 open reading frame 13 |
| ANXA8L    | -2.14          | Annexin A8-like 2 |
| NM_145810 | -2.13          | CDCA7 cell division cycle associated 7, variant 2 |
| NM_007308 | -2.12          | SNCA synuclein, alpha (non A4 component of amyloid precursor), variant NACP112 |
| NM_004772 | -2.11          | OBSL1 Obscurin-like 1 |
| Gene Name       | Accession | Description                                           | Log2 Fold Change | p-Value |
|-----------------|-----------|-------------------------------------------------------|------------------|---------|
| NM_005669       | 7905      | REEP5 receptor accessory protein 5                    | -2.09            | 3.58    |
| NM_007308       | 6622      | SNCA synuclein, alpha (non A4 component of amyloid     | -2.08            | 3.20    |
|                 |           | precursor), variant NACP112                           |                  |         |
| NM_032812       | 84898     | PLXDC2 plexin domain containing 2                     | -2.08            | 3.63    |
| NM_173061       | 831       | CAST calpastatin, variant 3                           | -2.08            | 3.97    |
| NM_001333       | 1515      | CTSL2 cathepsin L2                                    | -2.08            | 3.51    |
| NM_181712       | 163782    | KANK4 KN motif and ankyrin repeat domains 4           | -2.07            | 4.06    |
| NM_016240       | 51435     | SCARA3 scavenger receptor class A, member 3, variant 1| -2.06            | 3.54    |
| NM_199512       | 151887    | CCDC80 coiled-coil domain containing 80, variant 2    | -2.06            | 3.94    |
| NM_007308       | 6622      | SNCA synuclein, alpha (non A4 component of amyloid     | -2.06            | 6.59    |
|                 |           | precursor), variant NACP112                           |                  |         |
| NM_007308       | 6622      | SNCA synuclein, alpha (non A4 component of amyloid     | -2.06            | 4.03    |
|                 |           | precursor), variant NACP112                           |                  |         |
| NM_001111019    | 89797     | NAV2 neuron navigator 2, variant 4                     | -2.05            | 4.17    |
| NM_001964       | 1958      | EGR1 early growth response 1                          | -2.04            | 3.78    |
| NM_016731       | 2348      | FOLR1 folate receptor 1 (adult), variant 8            | -2.04            | 3.43    |
| NM_007308       | 6622      | SNCA synuclein, alpha (non A4 component of amyloid     | -2.04            | 3.20    |
|                 |           | precursor), variant NACP112                           |                  |         |
| NM_007308       | 6622      | SNCA synuclein, alpha (non A4 component of amyloid     | -2.03            | 3.92    |
|                 |           | precursor), variant NACP112                           |                  |         |
|                 | 1266      | CNN3 Calponin 3, acidic                               | -2.03            | 3.02    |
| NM_007308       | 6622      | SNCA synuclein, alpha (non A4 component of amyloid     | -2.02            | 3.60    |
|                 |           | precursor), variant NACP112                           |                  |         |
| NM_001127       | 464       | ZNF469 zinc finger protein 469                        | -2.01            | 4.08    |
| NM_001719       | 655       | BMP7 bone morphogenetic protein 7                     | -2.00            | 4.27    |
| NM_153000       | 147495    | APCDD1 adenomatosis polyposis coli down-regulated 1    | -4.76            | 4.25    |
| NM_002191 | 3623 | INHA | inhibin, alpha | -4.22 | 4.83 |
| NM_002048 | 2619 | GAS1 | growth arrest-specific 1 | -4.10 | 4.97 |
| NM_001885 | 1410 | CRYAB | crystallin, alpha B | -3.63 | 3.73 |
| NM_005218 | 1672 | DEFB1 | defensin, beta 1 | -3.33 | 3.26 |
| 9340 | GLP2R | Glucagon-like peptide 2 receptor | -3.31 | 3.92 |
| NM_002272 | 3851 | KRT4 | keratin 4 | -3.25 | 4.11 |
| NM_001216 | 768 | CA9 | carbonic anhydrase IX | -3.22 | 3.84 |
| NM_001122 | 679 | ODZ2 | odz, odd Oz/ten-m homolog 2 (Drosophila) | -3.18 | 4.12 |
| NM_177403 | 338382 | RAB7B | RAB7B, member RAS oncogene family | -3.15 | 3.67 |
| NM_001878 | 1382 | CRABP2 | cellular retinoic acid binding protein 2 | -3.09 | 4.39 |
| NM_001312 | 1397 | CRIP2 | cysteine-rich protein 2 | -3.04 | 3.97 |
| NM_175068 | 319101 | KRT73 | keratin 73 | -3.03 | 3.91 |
| NM_000093 | 1289 | COL5A1 | collagen, type V, alpha 1 | -2.93 | 5.13 |
| NM_001018 | 057 | DKK3 | dickkopf homolog 3 (Xenopus laevis), variant 3 | -2.88 | 4.35 |
| NM_001018 | 057 | DKK3 | dickkopf homolog 3 (Xenopus laevis), variant 3 | -2.86 | 3.36 |
| NM_181677 | 5521 | B | protein phosphatase 2 (formerly 2A), regulatory subunit B, beta isoform, variant 5 | -2.78 | 4.17 |
| 10451 | VAV3 | Vav 3 guanine nucleotide exchange factor | -2.77 | 3.26 |
| NM_001083 | 896 | SEBOX | SEBOX homeobox, variant 2 | -2.73 | 4.76 |
| NM_019601 | 56241 | SUSD2 | sushi domain containing 2 | -2.69 | 4.74 |
| NM_001079 | 874 | VAV3 | Vav 3 guanine nucleotide exchange factor, variant 2 | -2.68 | 3.46 |
| NM_003016 | 6427 | SFRS2 | splicing factor, arginine/serine-rich 2 | -2.57 | 3.46 |
| Accession | Gene ID | Gene Symbol | Description | log2 Fold Change | p-value |
|-----------|---------|-------------|-------------|-----------------|---------|
| NM_001111 | 5087 PBX1 | Pre-B-cell leukemia homeobox 1 | -2.54 | 5.20 |
| NM_007173 | 89797 NAV2 | neuron navigator 2, variant 4 | -2.51 | 4.32 |
| NM_016571 | 11098 PRSS23 | protease, serine, 23 | -2.48 | 4.42 |
| NM_007173 | 51557 GLULD1 | glutamate-ammonia ligase (glutamine synthetase) domain containing 1 | -2.47 | 4.01 |
| NR_002196 | 283120 H19 | H19, imprinted maternally expressed (non-protein coding) on chromosome 11. | -2.45 | 4.58 |
| NM_000424 | 3852 KRT5 | keratin 5 | -2.44 | 4.34 |
| NM_000093 | 1289 COL5A1 | collagen, type V, alpha 1 | -2.37 | 3.55 |
| NM_024336 | 79191 IRX3 | iroquois homeobox 3 | -2.32 | 4.00 |
| NM_016588 | 51299 NRN1 | neuritin 1 | -2.29 | 4.81 |
| NM_032229 | 84189 SLITRK6 | SLIT and NTRK-like family, member 6 | -2.28 | 3.34 |
| NM_017848 | 54954 C | family with sequence similarity 120C | -2.27 | 4.21 |
Reactive oxygen species mediate inflammatory cytokine release and EGFR-dependent mucin secretion in airway epithelial cells exposed to *Pseudomonas pyocyacin*

Balázs Rada, Paul Gardina, Timothy G. Myers and Thomas L. Leto

**Online supplementary Material**

**Supplementary Table 3.** GO FAT terms associated with gene-function clusters.

**Supplementary Table 4.** List of reports documenting elevated levels of inflammatory mediators in CF patients.

**Supplementary Table 5.** Sequences of primers used in this study.
### Supplementary Table 3.

**Rada et al.**

Constituent GO FAT functions for functional clusters.

| Summary Function(s) | Constituent GO FAT Functions |
|---------------------|------------------------------|
| C3 Apoptosis        | apoptosis; cell death; death; programmed cell death |
| C7 Inflammatory/wound response, chemotaxis | behavior; chemotaxis; defense response; inflammatory response; locomotory behavior; response to wounding; taxis |
| C11 Hemostasis, wound healing | blood coagulation; coagulation; hemostasis; regulation of body fluid levels; wound healing |
| C2 Positive regulation of cell proliferation/cell communication | positive regulation of cell communication; positive regulation of cell proliferation; positive regulation of protein kinase cascade; positive regulation of signal transduction; regulation of protein kinase cascade |
| C8 Response to stimuli | response to drug/endogenous stimulus/estradiol stimulus/extracellular stimulus/hydrogen peroxide/inorganic substance/molecule of bacterial origin/nutrient/nutrient levels/organic cyclic substance/organic substance/oxidative stress/oxygen levels/reactive oxygen species/steroid hormone stimulus/vitamin |
| C6 Angiogenesis, cell migration | angiogenesis; blood vessel development; blood vessel morphogenesis; cell migration; cell motility; cell motion; localization of cell; regulation of cell migration; regulation of cell motion; regulation of locomotion; vasculature development |
| C10 Anti-apoptosis, apoptosis | anti-apoptosis; apoptosis; cell death; death; negative regulation of apoptosis; negative regulation of cell death; negative regulation of programmed cell death; programmed cell death; regulation of apoptosis; regulation of cell death; regulation of programmed cell death |
| C9 Positive regulation of apoptosis | positive regulation of apoptosis; positive regulation of cell death; positive regulation of programmed cell death |
| C5 Chromatin assembly | chromatin assembly; DNA packaging; nucleosome assembly; nucleosome organization; protein-DNA complex assembly |
| C1 Positive regulation of gene expression/macromolecule biosynthesis | positive regulation of biosynthetic process; positive regulation of cellular biosynthetic process; positive regulation of gene expression; positive regulation of macromolecule biosynthetic process; positive regulation of macromolecule metabolic process; positive regulation of nitrogen compound metabolic process |
| C4 Cation homeostasis | cation homeostasis; cellular homeostasis; cellular ion homeostasis; di-, trivalent inorganic cation homeostasis; homeostatic process; ion homeostasis |
Supplementary Table 4. Rada et al.

**List A.** List of reports documenting elevated levels of inflammatory mediators in CF patients infected with *Pseudomonas aeruginosa* compared to CF patients without *Pseudomonas* infection.

| Ref. | Sample       | Inflammatory mediator                                      |
|------|--------------|------------------------------------------------------------|
| 1    | Breath condensate | IL-6, LTB4                                                 |
| 2    | BAL          | IL-1β, IL-6, TNF-α, IL-4, IL-13, TARC (CCL17), ITAC (CXCL11) |
| 3    | Serum        | IL-8, G-CSF                                                |
| 4    | Serum        | IL-4, G-CSF                                                |

PA-associated inflammatory mediators in CF: CCL17, CXCL11, IL-1β, IL-4, IL-6, IL-8, IL-13, G-CSF (CSF3), LTB4, TNF-α.

**List B.** Collection of studies reporting increased levels of inflammatory mediators in CF patients compared to healthy controls.

| Ref. | Sample       | Inflammatory mediator                                      |
|------|--------------|------------------------------------------------------------|
| 5    | Sputum       | LTB4                                                       |
| 6    | Sputum       | IL-1β, IL-8, TNF-α, IL-1Ra, sTNFRII                       |
| 7    | Serum        | IL-6, TNF-α, CRP                                          |
| 8    | BAL          | s100A8, s100A9, s100A12                                     |
| 9    | Sputum       | s100A8, IL-8                                              |
| 10   | BAL          | s100A8, s100A9, s100A12                                     |
| 11   | Serum        | SAA                                                       |
| 12   | Serum        | SAA, CRP                                                  |
| 13   | Serum        | IL-8, LTB4                                                |
| 14   | Sputum       | IL-1β, -6, -8, -17A, -17F, IL-23p19, G-CSF, GM-CSF, GRO-α, MCP-1, MIP-1β, TNF-α |

CF-associated inflammatory mediators not listed in list A: CRP (C-reactive protein), CXCL1 (GRO-α), GM-CSF (CSF2), IL-17A, IL-17F, IL-23p19, IL-1Rα, MCP-1 (CCL2), MIP-1β (CCL4), s100A8 (calgranulin A), s100A9 (calgranulin B), s100A12 (calgranulin C), SAA (serum-amyloid A).
**Supplementary Table 5. Sequences of primers used in this study.**

| Primer pairs | Forward primer 5' - 3' | Reverse primer 5' - 3' | Amplicon (bp) | Source |
|--------------|------------------------|------------------------|---------------|--------|
| Acn-1        | CTCAGTGGACTGGCTGTGG    | AAGGAGGCTGAAGAGTGC     | 528           | 15     |
| Acn-2        | CCAAGCGCAGAGATGAGA     | CCAAGGCGTGACAGGATAG    | 97            | 15     |
| ADAM-17      | CCTCTGGCAGAAGAGGAGC    | CACCTGCGAAGATTTCAG     | 69            | 15     |
| ALD5         | AAGAGCGCTGGCGACACAGATG | CATCCTCGCGGATTGTC      | 62            | 15     |
| Catalase     | CGAGTGTGGTTCCCTCACC    | GGGTCCCACCTGTTGCA      | 60            | 15     |
| CCL20        | GCTGCTGGATGTGAGGCTT    | GCAGCTAAAGTGGTCTGCC    | 66            | 15     |
| CRYAB        | ACATAGGAGACGCAGAGA     | TCCTGATTTCTGCGGAGAC    | 62            | 15     |
| CSF2         | TCTCAAGAATTTTGGCTCCTA  | GCCCTTGGCTGTGGTGGAG    | 98            | 15     |
| CSF3         | GACGCAAGGGCAAGACGCAA   | GCAGTGCGTCGGCAGACTCTC | 78            | 15     |
| CXCL1        | TCTGCTACCCCAATGTTA    | CCTCAGAAGCCTCAGCAAT    | 105           | 15     |
| CXCL2        | CCACTGTTATAGAAATCATCG | CCTCAGAACAGGCACCAAT    | 95            | 15     |
| CXCL3        | AAAATCATGAAAAGGATGCAAAA | GGTGAGCCGAGGACAC      | 110           |       |
| CXXL11       | AGTGTAAGGAGGCTGCACT    | TGTCTTGGACATGGGAGAC    | 78            | 15     |
| D2T3         | CAGAGCTGCAAAGTGGAGAGA  | TGATACGCTGAGAACAGCA    | 95            | 15     |
| DHR22        | TGAGATCTACATCAGTCCTCAGA| CACGATGCTGGTGGTCTCTG  | 69            | 15     |
| Duox1        | CACCTCGTGAGACGGCTTTC   | GGCTCGTTGAGGTTGCA     | 60            | 15     |
| Duox2        | TACCCAGGGTGGATGAGC     | TCTCAGAACCTGAGAATCTG  | 60            | 15     |
| DURP1        | CGACGAGTATGCCTCTGATA   | TGCCAGTGAGCACAACCTCA  | 64            | 15     |
| EGF          | AAGATGCTGAGGGTTACAGCT  | TGAATGTGGTGTGGAGAC     | 69            | 15     |
| EGRF         | GCTGATCTGGAGTGACCTG    | CGGGATACGATAGAGTTGTC  | 60            | 15     |
| GDF15        | CCCAGTACATGGAACAGA     | AGAGATAAGCAGGCTGGAG    | 61            | 15     |
| HB-EGF       | TGGGCGTCTCACTGATTAGG   | TGCCACAACCTACCTTCTC   | 75            | 15     |
| HMOX1        | GGGTGATAGAAGAGGACAGA   | AGCTCCTGCAACTCCTCAA   | 67            | 15     |
| IL-1α        | GTGGATGTTACTACCTCATCA  | TGTCAGCTAGGCTTGAGTA   | 89            | 15     |
| IL-1β        | GTGCTGCTGGGCTGTAAGGA   | TGGTAAATTTTGAGGGTACCA | 70            | 15     |
| IL-6         | GATGAGCTAAAGTGCTCACTG  | TCTCAGACCTGCTCCGTCG   | 130           | 15     |
| IL-8         | CAGGATGTGAGGTGTTGGC    | AAACCAAGGCGACAGTGCA    | 180           | 15     |
| IL-11        | GGGACAGGAGGTTAGTAAAAG  | GCAGTCAGCAGCCAGAGC    | 109           | 15     |
| IL-19        | GGCCAGAGAACAGCTAGAGG   | AGCAGAAGGGAAGAACACTG  | 86            | 15     |
| IL-20        | AAGAAGAGCTCGCCTGCTG    | TGACATGGAATCTGGTGATTCA | 86          | 15     |
| IL-23A       | GCTTGCCACTATACCTCATG   | TCTTGGAGCAAGAAGTGGA    | 77            | 15     |
| IL-24        | GTCGACGAGGAGGTTCC      | CGGAGGTTGGACAGGATTA   | 65            | 15     |
| IL-1R type2  | TACGACCCACAGCTACAGAA   | AAGAAGCCGATGGAAGTTG   | 76            | 15     |
| IL-1RN       | AAGAACACTACTGCTCTAAAG  | CTGGACGCTGCTACCTTGCT  | 73            | 15     |
| MUC1         | GTCCGGCCACACTGAGAGCC   | GAGTGGCCTACTGACAGTGG  | 123           | 17     |
| MUC2-1       | CAAGAGCAAGACGGCTTCG    | CACCGTCTGTCTGTAGATGCTT | 438         | 17     |
| MUC2-2       | CTGCCAGCTCAAGGAGA      | CGACGACGCTCTAGTGGTCG  | 76            | 15     |
| MUC4         | CTCAGATGGAGGTTGCTCA    | GTTCATGCTGACGTTCTCA   | 200           | 18     |
| MUC5AC-1     | TGCCGGTCATCTCCTCCA     | ACGTGGTGGACGTGGCGT    | 683           | 19     |
| MUC5AC-2     | ACCTGCGCCAGCCGACAGA    | ACGTGGGGCGTGCTGCTG    | 164           | 19     |
| MUC5B        | GCAGGGCCGTGGTGCTC      | GTTGGGCCGTGACTTCC     | 94            | 3      |
| MUC7         | CACGCTGCTGCTTCTCGG     | AGTGGTGATTATTCAGCTGATGTTAGT  | 108    | 17     |
| MUC8         | TACCACCACTGACCTCTTC    | GGAGTGTAACCTGCTGCTC   | 240           | 20     |
| MUC11        | CTCATCTCCGGCTCTGCAA    | GGGCGGGAGGTATGTGCTCA  | 93            | 22     |
| MUC13        | CCACTGCGCAGCCTATTT    | TACTTGAGCCGAGTAGTTGCT | 145         | 22     |
| MUC15        | TCTAAGACGCTGGACTCTGCTGTACTA| AACATGTTAGGGTTCTTGTTGATTGGT | 145     | 22     |
| MUC19        | TGAGCTGGCTGTGGAAGAAAC | GGGGTCGGCAAAGCCTCTC      | 61            | 17     |
| MUC20        | TCTTGCTGTGGGAGAGGTT    | TGTTCAGCTGAGTGCTCTC   | 77            | 15     |
| Nax1         | AGATGAACAGCGGGCTGCTT   | AGATTGGAGGCGAATTAAACCAA | 66           | 11     |
| Nax2         | ACTTCAGGCTTATGATGGA    | ACAGTTTTGAAATGTCCTCCTA | 76            | 16     |
| Nax3         | GACATTGGGCGCGCTTCTTCC | CAGGGTGAGTGGATCTGCTG  | 60            | 16     |
| Nax4         | TGCTCCCTCATGAATGCTCT   | CTAGAGACACATCCACCAAA  | 103           | 16     |
| Nax5         | CGATGCACTACGCTACATGG   | AAGAAGGAGGCTACACCTAC  | 74            | 16     |
| PTGS2        | TCCTCAGGCTATGCTTCCAG   | TCACGTTAATATTTTTGATTCGCCAC  | 96           | 16     |
| S100A7       | CCAACAAACAACACATCCTCACA | TCGAGCTTGGAGGTTCTC | 89            | 22     |
| S100A8       | CAAAGCGTGGGGCCATCAT   | GAGCTGCTAGTGTGATGCAAGG | 61            | 22     |
| S100A9       | GTCCGGAAAGAACAGTCCAAA | TGTCAGCTTGGCTCTGATT   | 103           | 22     |
| S100A12      | CACGTGGCTTCTGCTTCTGAG | TACCATCCTCAATGCAAGAGAC     | 51            | 15     |
| SCAR0        | TGGGAGATGCTTCCTTCTTC   | TCCTGAGAGAAGGCTGCTCTT | 92            | 15     |
| SNAcA        | CAAAGGAGGGTGCCGAGA    | TCCTGGTGTGCTGAGCTTAC  | 72            | 15     |
| SOd1         | GACATGATCTTCTTGAGCAG   | CGACGCGCTCCAGTCTGCTT   | 80            | 15     |
| SOd2         | GTCCGAGGAGCTACGGCTCTA | TGAATGTGGCTGCGAATCTC  | 62            | 15     |
| SOd3         | GCTGGAGAGGCTTGAAGAAGAT | GAGCGAGGAGGCAAGGACATGA | 64            | 15     |
| TGp-α        | GCGAGGACGCTAGTGCTGAGA | TATGCTAGCTGGCGACAGGA  | 78            | 15     |
| TNFα         | CACCGCTCTGCTCTGCTA    | GGGCGAGGCGTGGTAGTGAAG  | 123           |       |
Supplementary references (s1-22)

1. Carpagnano, G.E., Barnes, P.J., Geddes, D.M., Hodson, M.E. & Kharitonov, S.A. Increased leukotriene B4 and interleukin-6 in exhaled breath condensate in cystic fibrosis. *Am J Respir Crit Care Med* **167**, 1109-1112 (2003).

2. Hartl, D. *et al.* Pulmonary Th2 response in Pseudomonas aeruginosa-infected patients with cystic fibrosis. *J Allergy Clin Immunol* **117**, 204-211 (2006).

3. Jensen, P.O. *et al.* Increased serum concentration of G-CSF in cystic fibrosis patients with chronic Pseudomonas aeruginosa pneumonia. *J Cyst Fibros* **5**, 145-151 (2006).

4. Moser, C. *et al.* Serum concentrations of GM-CSF and G-CSF correlate with the Th1/Th2 cytokine response in cystic fibrosis patients with chronic Pseudomonas aeruginosa lung infection. *APMIS* **113**, 400-409 (2005).

5. Zakrzewski, J.T., Barnes, N.C., Piper, P.J. & Costello, J.F. Detection of sputum eicosanoids in cystic fibrosis and in normal saliva by bioassay and radioimmunoassay. *Br J Clin Pharmacol* **23**, 19-27 (1987).

6. Osika, E. *et al.* Distinct sputum cytokine profiles in cystic fibrosis and other chronic inflammatory airway disease. *Eur Respir J* **14**, 339-346 (1999).

7. Nixon, L.S., Yung, B., Bell, S.C., Elborn, J.S. & Shale, D.J. Circulating immunoreactive interleukin-6 in cystic fibrosis. *Am J Respir Crit Care Med* **157**, 1764-1769 (1998).

8. MacGregor, G. *et al.* Biomarkers for cystic fibrosis lung disease: application of SELDI-TOF mass spectrometry to BAL fluid. *J Cyst Fibros* **7**, 352-358 (2008).

9. Gray, R.D. *et al.* Sputum proteomics in inflammatory and suppurrative respiratory diseases. *Am J Respir Crit Care Med* **178**, 444-452 (2008).

10. McMorran, B.J. *et al.* Novel neutrophil-derived proteins in bronchoalveolar lavage fluid indicate an exaggerated inflammatory response in pediatric cystic fibrosis patients. *Clin Chem* **53**, 1782-1791 (2007).

11. Smith, J.W., Colombo, J.L. & McDonald, T.L. Comparison of serum amyloid A and C-reactive protein as indicators of lung inflammation in corticosteroid treated and non-corticosteroid treated cystic fibrosis patients. *J Clin Lab Anal* **6**, 219-224 (1992).

12. Marhaug, G., Permin, H. & Husby, G. Amyloid-related serum protein (SAA) as an indicator of lung infection in cystic fibrosis. *Acta Paediatr Scand* **72**, 861-866 (1983).

13. Wyatt, H.A., Sampson, A.P., Balfour-Lynn, I.M. & Price, J.F. Production of the potent neutrophil chemokine, growth-related protein alpha (GROalpha), is not elevated in cystic fibrosis children. *Respir Med* **94**, 106-111 (2000).

14. McAllister, F. *et al.* Role of IL-17A, IL-17F, and the IL-17 receptor in regulating growth-related oncogene-alpha and granulocyte colony-stimulating factor in bronchial epithelium: implications for airway inflammation in cystic fibrosis. *J Immunol* **175**, 404-412 (2005).

15. Yan, F. *et al.* Reactive oxygen species regulate Pseudomonas aeruginosa lipopolysaccharide-induced MUC5AC mucin expression via PKC-NADPH oxidase-ROS-TGF-alpha signaling pathways in human airway epithelial cells. *Biochem Biophys Res Commun* **366**, 513-519 (2008).

16. Boudreau, H.E., Emerson, S.U., Korzeniowska, A., Jendrysik, M.A. & Leto, T.L. Hepatitis C virus (HCV) proteins induce NADPH oxidase 4 expression in a transforming growth factor beta-dependent manner: a new contributor to HCV-induced oxidative stress. *J Virol* **83**, 12934-12946 (2009).

17. Russo, C.L. *et al.* Mucin gene expression in human male urogenital tract epithelia. *Hum Reprod* **21**, 2783-2793 (2006).

18. Gersemann, M. *et al.* Differences in goblet cell differentiation between Crohn's disease and ulcerative colitis. *Differentiation* **77**, 84-94 (2009).
19. Song, J.S. et al. Nitric oxide induces MUC5AC mucin in respiratory epithelial cells through PKC and ERK dependent pathways. Respir Res 8, 28 (2007).

20. Kerschner, J.E. Mucin gene expression in human middle ear epithelium. Laryngoscope 117, 1666-1676 (2007).

21. Ebihara, T. et al. Differential gene expression of S100 protein family in leukocytes from patients with Kawasaki disease. Eur J Pediatr 164, 427-431 (2005).

22. Roche primer design web site: https://www.roche-applied-science.com/servlet/RCConfigureUser?URL=StoreFramesetView&storeId=10202&catalogId=10202&langId=-1&countryId=us
Reactive oxygen species mediate inflammatory cytokine release and EGFR-dependent mucin secretion in airway epithelial cells exposed to *Pseudomonas pyocyainin*

Balázs Rada, Paul Gardina, Timothy G. Myers and Thomas L. Leto

**Online supplementary Material**

**Supplementary Methods.** Western blot, RNA isolation, mucin dot blot assay, qualitative and quantitative RT-PCR, *Pseudomonas* strains, isolation of pyocyanin, ELISA, cell proliferation assay and additional references.
Supplementary Methods.

**Pseudomonas aeruginosa strains and purification of pyocyanin**

The following strains were used: *P. aeruginosa* ATCC 10145 (PA 10145; American Type Culture Collection); PAO1 wild-type, pilus-, flagellum- and LPS-deficient mutants (*Pseudomonas* Mutant Library, University of Washington, Seattle), PA14 wild-type and pyocyanin-deficient mutant PA14 PhzM (gift from Frederick M. Ausubel, Harvard Medical School, Boston), *P. aeruginosa* ATCC 15442 (PA 15442, American Type Culture Collection). Bacteria were grown in *Luria-Bertani* broth (KD Medical). Pyocyanin was purified from their supernatant as described \(^{22}\) and dissolved in distilled water. Pyocyanin was also purchased from a commercial source (Cayman Chemical).

**Western blotting**

Airway epithelial cells were washed three times with cold calcium- and magnesium-free PBS and lysed by NP-40 lysis buffer (Boston Biosciences) containing 150 µM PMSF (Fluka Biochemika) and 1% protease inhibitor cocktail (dissolved in DMSO). Lysates were centrifuged and protein concentrations in supernatants were determined using the BCA assay (Pierce). Equal amounts of protein were loaded and electrophoresed on SDS-polycrylamide gels (8%; Tris-Glycine Gel, Invitrogen). Gels were blotted on nitrocellulose membrane (Invitrogen) using the TE Series Transphor Electrophoresis Unit (Hoefer) wet blotting system. Blots were blocked overnight in TTBS (TBS-buffer containing 5% milk powder and 0.05% Tween-20). Blots were incubated with primary antibodies (RT, 1hr, TTBS), washed three times with TTBS and then probed with secondary HRP-linked antibodies (RT, 1hr, TTBS, GE Healthcare). After repeated washes, blots were developed by chemiluminescence using the Lumigen DS detection kit (GE Healthcare).

The primary antibodies used were: \(\alpha\)-MUC5AC (1:100, Clone 45M1, mouse, Sigma), \(\alpha\)-Hsp90 (1:1000, mouse, Santa Cruz), \(\alpha\)-ERK (1:1000, rabbit, Santa Cruz), \(\alpha\)-phospho-ERK (1:1000, rabbit, Cell Signaling), \(\alpha\)–STAT6 (1:1000, rabbit, Cell Signaling), \(\alpha\)-phospho-STAT6 (1:1000, rabbit, Cell Signaling), \(\alpha\)-EGFR (1:1000, rabbit, Cell Signaling), \(\alpha\)-Actin (1:4000, rabbit, Sigma).

To detect MUC5AC in the supernatant of epithelial cells, cells were stimulated by 8 µM pyocyanin for 2 days. The supernatant was collected and cell debris was removed by quick centrifugation (400 g 3min RT). The supernatant was concentrated using Amicon centrifugal filter units (Millipore 0.5 mL volume, 10K membrane) and mixed with 2x protein gel loading buffer (without beta-mercaptoethanol). 40 uL of each sample was run on 4% Tris-Glycine gels (Invitrogen, 100 V 3 hrs) to detect high molecular weight proteins. Further processing of the gels is as it is described above.

**RNA isolation and qualitative RT-PCR**

RNA was isolated from airway epithelial cells by Trizol/chloroform extraction followed by isopropanol precipitation and wash in 70% ethanol. RNA pellets were taken up in DEPC-treated water (Nalgene). Concentrations and purities of the preparations were determined using Nanodrop spectrophotometer. cDNA synthesis was carried out with the Thermoscript cDNA synthesis kit (Invitrogen) using 2 µg total RNA, oligodT primers and RNaseH (Invitrogen) treatment. To detect the expression of different genes, gene-specific primers of MUC2,
MUC5AC and the NOX/DUOX NADPH oxidases described in Table S5. were used in the PCR reaction (PCR Thermocycler, Biometra). Beta-actin was used as internal control. The PCR products were resolved by gel electrophoresis on 1% agarose gels containing ethidium bromide (MUC2, MUC5AC) or on 10% TBE gels stained with Gelstar DNA stain (Lonza) (NAPDH oxidases). Lack of contaminating DNA was confirmed by PCR without RT.

**Quantitative real-time PCR**

The abundance of mRNAs of genes of interest was determined by quantitative real-time PCR using SYBR Green. The template cDNAs, No RT controls and gene-specific primers were mixed with SYBR Green/ROX PCR Supermix (Invitrogen) and dispensed on a 96-well PCR plate. The source of primers is listed in Table S5. Changes in fluorescence were followed in 7500 Real-time PCR system (Applied Biosystems). Reaction parameters were 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The relative abundance of mRNAs was obtained using the comparative cycle threshold method and was normalized to beta-actin as the endogenous control. Results are also expressed as fold changes in the mRNA amount of a gene compared to the pyocyanin-treated or untreated samples. After each measurement melting curve analysis ensured the presence of only one product and the samples were also subjected to gel electrophoresis on 10% TBE gels and stained with GelStar DNA stain (Lonza) to confirm product size.

**ELISA**

The amount of released MUC5AC, IL-1β, IL-6, TNFα, TGFα and EGF was measured by ELISA. The supernatants of confluent airway epithelial cells grown on 24-well plates in the presence or absence of pyocyanin and different inhibitors, were collected and cleared from debris by low-speed centrifugation. 50 µL of each sample was incubated with carbonate-bicarbonate buffer (50 µL) at 37°C on high absorbance, flat bottom ELISA plates (Corning) until dry. Wells were washed three times with PBS, then blocked with 300 µL PBS containing 2% BSA for 1hr RT. After repeated washes with PBS again, 50 µL first antibody was applied (O/N 4°C). Wells were washed with PBS-Tween 20 again and 50 µL aliquots of the secondary antibody were added (2hrs RT). After three washes with PBS-Tween 20 color reaction was developed with 3,3',5,5'-tetramethylbenzidine (TNB, 0.16 mg/mL, 10-30 min RT) (Sigma) peroxidase solution and the reaction was stopped by adding 1M sulfuric acid. Absorbance was read at 450 nm. Serial dilution samples of bovine submaxillary gland mucin (Sigma) were treated parallel with the experimental samples to quantitate MUC5AC release in unknown samples and to ensure the linear nature of the colorimetric reaction. MUC5AC release was expressed either as mg/mL bovine mucin equivalent or as relative to the pyocyanin-untreated or –treated sample’s results because of the variable amounts of baseline MUC5AC secretion of H292 cells. Cytokine and growth factor release was also calibrated using standard curves.

**Primary antibodies used in ELISA were**: α-MUC5AC (1:100, Clone 45M1, mouse, Sigma), α-IL-1β (1:100, goat, R&D Systems), α-IL-6 (1:100, mouse, R&D Systems), α-TNFα (1:100, mouse, R&D Systems), α-TGFα (1:100, goat, R&D Systems), α-EGF (1:100, rabbit, Calbiochem). Secondary antibodies used were HRP-linked: donkey α-goat, donkey α-rabbit and sheep α-mouse (1:100, GE Healthcare). Neutralizing antibodies used were: α-IL-1β (goat, R&D Systems), α-IL-6 (goat, R&D Systems), α-TNFα (goat, R&D Systems), α-TGFα (goat, R&D Systems), all 10 µg/mL final concentration.
Cell proliferation assay

Proliferation of H292 cells in response to pyocyanin was assessed by the Click-iT Edu Imaging Kit according to the instructions of the manufacturer (Invitrogen). Cells after Edu-labeling, fixation and permeabilization were stained with Alexa Fluor 555-labelled antibody. DAPI was used to stain nuclei. Cell density was determined by counting the average number of DAPI-stained nuclei on unit surface area. Proportion of proliferating cells was calculated by dividing the number of Edu-labelled cells by the total number of DAPI-stained nuclei.

Dot blot assay of released mucin

To quantitate the amount of mucin secreted by different stimuli, the supernatant of pyocyanin-treated epithelial cells was collected, cleared and concentrated as described at the Western Blot section. 2 µL of the concentrated samples and calibration samples with known amounts of bovine submaxillary mucin were dropped on nitrocellulose membranes (0.45 µm pore size, Invitrogen) and incubated until dry. The membranes were developed by the PAS-staining kit (Sigma), the amount of mucin in each sample was determined by densitometry (UVP BioImaging Systems, Biochemi Systems, Labworks software) and calibrated using bovine mucin samples.

Sample preparation for microarrays

H292 cells were treated with or without 8 µM pyocyanin for 48 hrs in serum-free RPMI medium. RNA was isolated as described earlier. To obtain clean RNA preparations an additional clean-up step was introduced using Quiagen RNeasy RNA isolation kit. The prepared RNAs were run on RNA gel to ascertain the quality of the preparations. RNA was isolated four times in four independent experiments, collected and subjected to microarray analysis simultaneously.

Microarray Hybridization

Total RNA (40 µg) was reverse transcribed to cDNA using oligo dT primers and SuperScript II reverse transcriptase. Double-stranded cDNA was synthesized using DNA polymerase with Cy-labeled dUTP for direct dye incorporation followed by clean-up with Vivaspin column (Qiagen). Labelled samples were hybridized on the Agilent-014850 Whole Human Genome Microarray 4x44K (Product number G4112F; NCBI GEO accession GPL6480) with approximately 41,000 unique 60-mer probes targeting transcripts. Hybridization protocol was completely automated using the TECAN HS Pro 4800 hybridization station with Agilent 2x Gene expression hybridization HI-RPM buffer and 10x Blocking Reagent at 65°C for 17 hours, and washed with Agilent Gene Expression Wash Buffer 1 at room temperature and Gene Expression Wash Buffer 2 at 37°C. Slides were dried under nitrogen gas for 3 minutes at 30°C. The slides were imaged using an Agilent high-resolution DNA microarray scanner G2505C at 5 µm resolution and 100/10% PMT XDR. Agilent Feature Extraction software was used for image analysis.

References (s22-26):
22) Rada, B., Lekstrom, K., Damian, S., Dupuy, C. & Leto, T.L. The Pseudomonas toxin pyocyanin inhibits the dual oxidase-based antimicrobial system as it imposes oxidative stress on airway epithelial cells. J Immunol 181, 4883-4893 (2008).
23) Benjamini Y and Hochberg Y (1995). “Controlling the false discovery rate: a practical and powerful approach to multiple testing”. Journal of the Royal Statistical Society, Series B (Methodological) 57 (1): 289–300.
24) Yang YH, Dudoit S, Luu P, Lin DM, Peng V, Ngai J, Speed TP. Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. Nucleic Acids Res. 2002 Feb 15;30(4):e15.

25) Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources. Nature Protoc. 2009;4(1):44-57.

26) Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA. DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol. 2003;4(5):P3.]