Characterisation of gene and pathway expression in stabilised blood from children with coeliac disease

Hanna Gustafsson Bragde 1,2, Ulf Jansson, 3 Mats Fredrikson, 2 Ewa Grodzinsky, 2 Jan Söderman 1,2

ABSTRACT

Introduction A coeliac disease (CD) diagnosis is likely in children with levels of tissue transglutaminase autoantibodies (anti-TG2) >10 times the upper reference value, whereas children with lower anti-TG2 levels need an intestinal biopsy to confirm or rule out CD. A blood sample is easier to obtain than an intestinal biopsy sample, and stabilised blood is suitable for routine diagnostics because transcript levels are preserved at sampling. Therefore, we investigated gene expression in stabilised whole blood to explore the possibility of gene expression-based diagnostics for the diagnosis and follow-up of CD.

Design We performed RNA sequencing of stabilised whole blood from active CD cases (n=10), non-CD cases (n=10), and treated CD cases on a gluten-free diet (n=10) to identify diagnostic CD biomarkers and pathways involved in CD pathogenesis.

Results No single gene was differentially expressed between the sample groups. However, by using gene set enrichment analysis (GSEA), significantly differentially expressed pathways were identified in active CD, and these pathways involved the inflammatory response, negative regulation of viral replication, translation, as well as cell proliferation, differentiation, migration, and survival. The results indicate that there are differences in pathway regulation in CD, which could be used for diagnostic purposes. Comparison between GSEA results based on stabilised blood with GSEA results based on small intestinal biopsies revealed that type I interferon response, defence response to virus, and negative regulation of viral replication were identified as pathways common to both tissues.

Conclusions Stabilised whole blood is not a suitable sample for clinical diagnostics of CD based on single genes. However, diagnostics based on a pathway-focused gene expression panel may be feasible, but requires further investigation.

INTRODUCTION

Small intestinal biopsies have long been an essential part of coeliac disease (CD) diagnostics, but based on the recommendations from the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition in 2012, the intestinal biopsy may be excluded from the diagnostic flow for symptomatic children with high levels (>10 times the upper reference value, URV) of tissue transglutaminase autoantibodies (anti-TG2) if additional requirements are met. 1 In symptomatic children with anti-TG2 levels that are 1–10 times the URV, an intestinal biopsy is needed to confirm or rule out CD as a diagnosis. If the biopsy sample is Marsh grade 0 or 1, the diagnosis is unclear and further investigations are recommended. 1 In symptomatic IgA-competent patients with anti-TG2 <1 times the URV, a CD diagnosis is less likely, but not ruled out. 1 Additional biomarkers would aid in diagnosing CD; we previously investigated gene expression in small intestinal biopsies by RNA sequencing and found differences in gene expression.
in active CD cases versus non-CD cases that were detectable also in CD cases with no or low-grade intestinal injuries (Marsh 0–I). However, blood is a more accessible sampling material than small intestinal biopsies that can be sampled repeatedly to both diagnose and follow-up with patients with CD. RNA sequencing of CD4+ T cells and whole genome microarray transcriptome analysis of peripheral blood mononuclear cells from mainly adult CD cases have identified genes and pathways of interest in CD. Because stabilised whole blood preserves transcript levels during collection, transport, and storage, the use of stabilised whole blood would facilitate the incorporation of gene expression-based diagnostics in clinical practice. We performed RNA sequencing of stabilised whole blood from paediatric patients with active CD, non-CD, and treated CD on a gluten-free diet (GFD) to identify potential CD diagnostic biomarkers and pathways involved in CD pathogenesis.

**METHODS**

**Study subjects**

Children and adolescents (<18 years of age) were referred to Ryhov County Hospital in Jönköping, Sweden with suspected CD or were followed-up after a period on a GFD to verify mucosal recovery. The patients included in this study were enrolled during the years 2009–2014, where biopsy samplings on GFD were still performed as a part of the standard diagnostic process for CD at the hospital. Most patients were referred for small intestinal biopsy due to elevated anti-TG2 to exclude CD. The patients provided written consent before blood and duodenal biopsy specimens for biopsy to exclude CD. The patients provided written consent before blood and duodenal biopsy specimens were collected.

**Study groups**

Two study groups (table 1) were defined based on subjects on a gluten-containing diet: (1) group CDM3 with a CD diagnosis (histopathologic assessment Marsh 3B–3C and anti-TG2 >7 U/mL), (2) group M0 cleared from CD (Marsh 0 and anti-TG2 ≤7 U/mL). A third study group (table 1) was defined based on CD subjects at follow-up on a GFD: (3) Group CDM0 (Marsh 0 and anti-TG2 ≤7 U/mL). Whole blood RNA samples were sequenced from all study subjects in all groups. Additionally, RNA samples from small intestinal biopsies from subjects in groups M0 and CDM3 were sequenced. These subjects were included as subgroups in a previous study involving RNA sequencing of small intestinal biopsies. Based on the previous analysis, one subject in group M0 was more similar to Marsh 3 subjects, and therefore, that subject was excluded from all analyses.

**Sample collection and processing**

Sera were collected using blood tubes containing polymer gel and clot activator (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA). Serum levels of anti-TG2 and IgG antibodies against deamidated gliadin were determined using EliA-kits from Thermo Fisher Scientific (Waltham, Massachusetts, USA) according to Bragde et al. A cut-off of 7 U/mL was used.

Blood for RNA isolation was collected in Tempus Blood RNA tubes (Life Technologies, Carlsbad, California, USA), kept overnight at 4°C, and then stored at −20°C until RNA isolation. Total RNA from stabilised blood was purified using the Tempus Spin RNA Isolation Reagent kit (Life Technologies) according to the manufacturer’s instructions. RNA concentrations were determined using the Qubit 2.0 Fluorometer and the Qubit RNA BR Assay Kit (Thermo Fisher Scientific). RNA integrity was assessed using the Agilent 2100 Bioanalyzer with the Agilent RNA 6000 Nano Kit (Agilent Technologies, Santa

### Table 1 Descriptive statistics of the study groups

| No. of cases | Age at biopsy (years)* | Gender M/F | Marsh grade (span) | Anti-TG2† (U/mL) | Anti-DG‡ (U/mL) | HLA-DQ2.5 cis§ |
|-------------|-----------------------|------------|-------------------|-----------------|---------------|---------------|
| M0          | 9                     | 8.4 (2.2–17) | 4/5 | M0 | 0.29 (<0.10–1.3) | 0.57 (<0.40–1.6) | 67%, 22%, 11% |
| CDM0        | 10                    | 8.8 (3.0–17) | 2/8 | M0 | 2.3 (0.10–7.0) | 2.5 (0.50–5.8) | 10%, 70%, 20% |
| CDM3        | 10                    | 8.6 (2.3–16) | 4/6 | M3B–3C | 600 (36–2858) | 151 (9.0–781) | 10%, 80%, 10% |

Subjects in the M0 and CDM3 groups consumed a gluten-containing diet. Study subjects in group M0 did not receive a coeliac disease (CD) diagnosis, whereas a CD diagnosis was confirmed in study subjects in group CDM3. Group CDM0 contained subjects with a CD diagnosis who were followed-up after a period on a gluten-free diet.

*Mean (min–max)

†Levels of IgA autoantibodies against tissue transglutaminase (anti-TG2) in sera. For two subjects in group M0 serum outcomes were not available, but plasma outcomes were within the range of the serum outcomes. Two subjects with IgA deficiency were included, one in group M0 and one in group CDM3. IgG anti-TG2 levels were measured instead, and the outcomes were within the accepted ranges for the groups (anti-TG2 ≤7 U/mL for group M0 and anti-TG2 >7 U/mL for group CDM3).

‡Levels of IgG antibodies against deamidated gliadin (anti-DG) in sera. For three subjects in group M0 and three subjects in group CDM3, no serum outcomes were available, but plasma outcomes were within range of the serum outcomes.

§The percentages of study subjects with 0, 1, or 2 human leucocyte antigen alpha chain DQA1*05 and beta chain DQB1*02 alleles (HLA-DQ2.5) in cis are accounted for in each group. HLA, human leucocyte antigen.
Clara, California, USA), according to the manufacturer’s instructions. Small intestinal biopsies were collected and RNA was isolated as described in Bragde et al. Blood was also collected in EDTA tubes (Becton, Dickinson and Company), and DNA was extracted from 350 µL of blood using the Biorobot EZ1 and the EZ1 DNA Blood 350 µL Kit according to the manufacturer’s instructions (Qiagen, Hilden, Germany).

The intestinal biopsies from the subjects were histopathologically assessed using the modified Marsh scale according to the method described in Bragde et al. Additional assessments were available for 10 subjects that were included in a previous study.10

**RNA sequencing**

Libraries for RNA sequencing of stabilised whole blood were prepared using TruSeq Stranded Total RNA with Ribo-Zero Globin (Illumina, San Diego, California, USA). RNA was sequenced as described previously in Bragde et al.2

**Genotyping**

The single nucleotide polymorphism rs2187668 was used to type the human leucocyte antigen (HLA) alpha chain DQA1*05 and beta chain DQB1*02 alleles (HLA-DQ2.5) in cis.2 11

**Statistical analysis**

RNA sequencing data were aligned and analysed using RStudio V.1.0.143.12 The analysis flow up until differential expression calculation for individual genes was based on Law et al.,13 and used functionalities from both limma (V.3.36.2) and edgeR (V.3.22.3).15 Sequencing reads were mapped and features (official gene symbols) were counted using RSubread V.1.30.416 and genome build hg38 and annotations (V.85) from Ensembl17 Entrez ID information and the name of each gene were added using the Bioco conductor package Annotationdbi.18 Genes with missing information were excluded from further analyses. Counts were converted to counts per million (cpm). Genes with expression >2 cpm in at least 10 samples were included in subsequent analyses. Multidimensional scaling with distances between samples based on the top 500 genes with the largest SD between all samples was carried out using Glimma to visualise sample differences according to the primary condition of interest (Marsh grade) or possible confounding factors. Scaling factors were calculated based on the trimmed mean of the M values. Heteroscedasticity was removed from count data using the voom function in limma. An empirical Bayes moderated t-test, with and without adjustment for multiple testing (5% false discovery rate, FDR), was used to assess significant differences from zero for each individual contrast; thus, there was no fold-change (FC) cut-off needed. P values <0.05 after adjustment for multiple testing were considered significant.

To identify potential biological processes of interest in CD, gene set enrichment analysis (GSEA) was performed using GSEA software V.3.0 from the Broad Institute,21 22 10,000 gene set permutations, and gene set versions from 1 June 2018 (‘Human.GO_bp_no.GO.iea_symbol.gmt’ and ‘Human.Reactome.June_01_2018_symbol.gmt’). The absolute value of the logarithm of the p value for each gene was multiplied by the direction (sign) of the FC, thus resulting in a ranked list of genes with upregulated genes in the top and downregulated genes in the bottom. The list was used as the input for the GSEA (GSEAPreranked), and analysis output was Gene Ontology (GO) Biological Process terms or Reactome pathways containing genes overrepresented (FDR-adjusted p value <0.075) at the top or bottom of the ranked list. Each GO term or Reactome pathway was accompanied by an enrichment score, reflecting the degree to which the gene set was overrepresented at the top or bottom of the ranked list. The enrichment score was adjusted to account for differences in gene set size and in correlations between gene sets and the expression data set, and the adjusted value was termed normalised enrichment score. The results were visualised using EnrichmentMap V.3.1.026 in Cytoscape V.3.6.127 with an edge similarity cut-off (combined Jaccard and Overlap, 50/50) of 0.375. Clusters were identified and annotated using AutoAnnotate V.1.2, and the Markov clustering algorithm (clusterMaker2 V.1.3.128), and clusters were weighted based on similarity coefficients that were calculated based on overlaps between data sets. The word clouds (WordCloud V.3.1.129) that associated with the clusters were adjusted to clarify context.

Deconvolution was performed using immunoStates within MetaIntegrator V.2.0.0 with RNA sequencing data formatted as log-transformed transcripts per million. The resulting cell ratios were investigated for differences between groups using the Kruskal-Wallis one-way analysis of variance by ranks in Statistica V.13.3 (Statsoft). To compare the GSEA results based on blood samples with the results based on duodenal biopsies, small intestinal biopsy RNA sequencing data from Bragde et al.8 were reanalysed using the same analysis pipeline as the blood data. Power calculations were performed using ssizeRNA V.1.2.9.31

**RESULTS**

All results are based on RNA sequencing of stabilised whole blood unless otherwise specified. RNA sequencing resulted in a mean of 24.5 million reads per sample (13.1–29.5 million reads), and a mean of 10.7 million reads per sample (6.4–13.2 million reads) were mapped to exons and summarised to gene counts. A total of 56,303 genes were initially included in the analysis, but after filtering and annotation, 12,290 genes were further analysed.

**Data set assessment**

Unsupervised clustering suggested that gender might influence gene expression. Twenty-two genes were

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identified as differentially expressed genes (DEGs) based on gender not considering CD status, and 21 of these associated with the X and Y chromosomes (online supplemental file 1). Five of the genes were identified as pseudogenes, including the autosomal gene. Differential expression and pathway analyses of CD produced similar results with and without controlling for gender. Therefore, to avoid unnecessary stratification, the results were not adjusted for gender.

**Differential expression on a gene level**

Even without an FC cut-off, no significant DEGs were identified in the CDM3 versus M0, CDM3 versus CDM0, and CDM0 versus M0 comparisons. Results for genes with an absolute FC difference $\geq 1.5$ are presented without FDR correction (p value <0.05; online supplemental file 2).

**Differential expression on a gene set level**

Differences in gene expression in stabilised whole blood from children with active CD (CDM3), treated CD (CDM0), and non-CD children (M0) were further investigated using GSEA with GO terms or Reactome pathways. Significant differential expression differences of genes associated with GO terms (figure 1, online supplemental file 3) and Reactome pathways (figure 2, online supplemental file 4) were identified in the CDM3 versus M0 and CDM3 versus CDM0 comparisons, but not in the CDM0 versus M0 comparison.

Eleven GO terms, related to type I interferon response and defence response to virus, were associated with higher RNA levels in the CDM3 group based on comparisons of CDM3 versus M0 and CDM3 versus CDM0. Thirteen GO terms related to ‘tube formation’, proliferation, nitric oxide biosynthesis, the p38 mitogen-activated protein kinase cascade, and lipid localisation were specific for the CDM3 versus M0 comparison and associated with higher RNA levels in CDM3. Twenty-four GO terms were specific for the CDM3 versus CDM0 comparison, and eight of these GO terms were associated with lower RNA levels in CDM3 and related to ‘protein targeting to membrane/endoplasmic reticulum (ER)’. The remaining 16 GO terms were associated with higher RNA levels in CDM3 and were related to immune response processes and monocarboxylic acid transport.

Two Reactome pathways were identified in both the CDM3 versus M0 and the CDM3 versus CDM0 comparison. Higher RNA levels in CDM3 were associated with ‘interferon alpha beta signalling’ and lower RNA levels in CDM3 associated with ‘signalling by ERBB4’. Eight Reactome pathways were identified for CDM3 versus M0, with higher RNA levels in CDM3 associated with pathway ‘formation of the beta-catenin:T cell factor (TCF)
transactivating complex’, and lower RNA levels in CDM3 associated with seven pathways in the cluster ‘fibroblast growth factor receptors (FGFR) signalling’. Three Reactome pathway clusters were identified for CDM3 versus CDM0, and higher RNA levels in CDM3 were associated with ‘activation of GABA B receptors’ and ‘interferon signalling’, and lower RNA levels in CDM3 were associated with the cluster ‘translational processes’ along with several other pathways.

**Gene expression in blood compared with small intestinal biopsies**

GO terms identified in both blood and small intestinal GSEA (CDM3 versus M0) were associated with type I interferon response, defence response to virus, and negative regulation of viral replication (online supplemental file 3), and Reactome pathways were associated with interferon signalling and formation of the beta-catenin:TCF transactivating complex (online supplemental file 4).

**Cell composition**

Abundances of blood cell types inferred from deconvolution of RNA sequencing data indicated no significant differences between the groups (online supplemental file 5; p values 0.17–0.99).

**DISCUSSION**

On a per gene level, no significant DEGs in stabilised blood were identified in this study. To identify genes suitable for use as biomarkers, we aimed for an FC >3 or FC <−3. In a previous study of gene expression in duodenal biopsies (CDM3 versus M0) using RNA sequencing, 2.5% of the analysed genes were differentially expressed at this FC level. Assuming 10 times fewer DEGs in blood, the analysed number of genes (12 290) should render approximately 25 DEGs at this FC level with group sizes corresponding to a power of 80%. However, no DEGs that withstood adjustment for multiple testing were found, indicating that even fewer DEGs are detectable at this FC level in whole blood using RNA sequencing. DEGs have previously been successfully identified in studies of gene expression based on purified cells from CD blood samples. This discrepancy indicates that RNA stabilised whole blood is not a suitable sampling material to identify differential expression in CD, and thus unsuitable for CD diagnostics based on a single or a few genes.

Based on GSEA, our study groups (table 1) differed significantly with respect to several Reactome pathways and GO terms. In this discussion, we chose to focus on the GO terms and Reactome pathways with the most positive/negative normalised enrichment scores because these would have the most diagnostic potential. The results suggest concomitant regulation by both
pro-inflammatory and anti-inflammatory processes. The GO term ‘negative regulation of innate immune response’ was enhanced in active CD when compared with treated CD, and a non-significant increase was also seen in active CD when compared with non-CD. Additionally, expression of genes associated with the Reactome pathway ‘cell surface interactions at the vascular wall’ was lower in active CD when compared with treated CD, and a non-significant decrease was also seen in active CD when compared with non-CD. This pathway describes key interactions between platelets and leukocytes and the endothelium in response to injury. Furthermore, clusters of GO terms/Reactome pathways related to translational processes and targeting of proteins to the membrane/ER were associated more with treated CD than active CD, possibly reflecting post-transcriptional processes in the initiation and regulation of inflammation.33 GO terms and Reactome pathways associated with interferon type I (alpha and beta) were enriched in active CD when compared with both non-CD and treated CD, and significant differences in Reactome pathways/GO terms related to type II interferon (interferon gamma) and the JAK-STAT cascade were observed when active CD was compared with treated CD. The JAK-STAT cascade is highly involved in immune responses, many of the JAK-STAT receptors respond to cytokines,34 and JAK-STAT is an important signal transduction pathway for interferon gamma.35 In CD lesions, there is constitutive activation of the STAT pathway,35 and the JAK-STAT pathway was identified in a transcriptome analysis of CD+ T cells from CD cases by Quinn et al.3 In this study, Reactome pathways related to the activation of GABA B receptors were identified in active CD when compared with treated CD, and GABA B receptors could be involved in the active immune response because they have been shown to be expressed in neutrophils and seem to be involved in neutrophil migration by acting as chemoattractant receptors.36 Additionally, our results indicated that negative regulation of viral replication and defence response to virus are associated with active CD. This is in agreement with the suggestion that the response to viral infection is a contributing factor to the onset of CD.37 The GO term ‘monocarboxylic acid transport’ was enriched in active CD when compared with treated CD. It is of interest to note that levels of faecal short chain fatty acids are altered in children with CD,38 and that short chain fatty acids have been proposed to have anti-inflammatory effects by influencing regulatory T cells.39 Reactome pathway cluster ‘FGFR signalling’, and GO term cluster ‘tube formation’ and the GO term ‘negative regulation of fibroblast proliferation’ were associated with active CD when compared with non-CD, indicating that processes involving cell proliferation, differentiation, migration, and survival could be altered in CD.

Biological processes identified by overlapping GSEA results from blood and small intestinal biopsies included response to type I interferon, defence response to virus, and formation of the beta-catenin:TCF transactivating complex. This indicates that, in both tissues, an immune response similar to the response found against virus infections and activation of transcription through the WNT pathway could be involved in CD.

Even if no single gene biomarker was identified, and the identified pathways were general and associated primarily with inflammation, further studies could still reveal gene expression profiles unique for CD within this biological context. In a study by Tuller et al, where gene expression and protein–protein interactions in six autoimmune diseases were analysed, pathways in common for most or all diseases were identified.40 However, there were also evidence of differential contribution of inflammation-related and more general pathways (eg, interferon and apoptosis), and also that in different diseases the same pathway (eg, apoptosis) was regulated by different mechanisms.40

Any conclusions as to whether the results from this study can be of use for diagnostic purposes cannot be drawn due to the small groups. For this purpose larger studies are needed, including patients at different stages of the disease, and should probably be based on a different sampling material.

In conclusion, our study indicates that the use of stabilised whole blood for identification of single gene expression biomarkers in patients with CD is not optimal. However, by analysing gene expression on a pathway level, it is possible to identify significant differences in expression between active CD, treated CD, and non-CD cases. Our results indicate concomitant regulation of both pro-inflammatory and anti-inflammatory processes in CD, and highlight processes such as transport, and cell proliferation, differentiation, migration, and survival. Parallel analysis of RNA sequencing results from small intestinal biopsies and stabilised blood indicate that factors involved in the defence against viral infections are activated in both tissue types in CD. With regard to CD diagnostics, results from GO term and Reactome pathway analyses could be used to develop pathway-focused gene expression panels that could be used to predict disease status in single samples, and should be investigated further. A good starting point would be to study genes involved in pathways relating to defence against viral infections.

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ORCID iD

Hanna Gustafsson Bragde http://orcid.org/0000-0001-9104-3863

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### Supplementary File 1: Differentially expressed genes based on gender.

| Gene ID | Length | Entrez ID | Name                                      | logFC | AveExpr | t    | p-value | FDR-adjusted p-value | Location    |
|---------|--------|-----------|-------------------------------------------|-------|---------|------|---------|-----------------------|-------------|
| UTY     | 9191   | 7404      | ubiquitously transcribed tetratricopeptide repeat containing, Y-linked | 11.5  | 0.474   | 40.7 | 2.0E-29 | 2.4E-25               | Yq11.221    |
| DDX3Y   | 5738   | 8653      | DEAD-box helicase 3, Y-linked              | 8.70  | 2.34    | 37.1 | 3.6E-28 | 1.0E-24               | Yq11.221    |
| RPS4Y1  | 2275   | 6192      | ribosomal protein S4, Y-linked 1           | 11.4  | 0.465   | 38.0 | 1.7E-28 | 1.0E-24               | Yp11.2      |
| EIF1AY  | 4074   | 9086      | eukaryotic translation initiation factor 1A, Y-linked | 11.4  | 0.340   | 37.5 | 2.5E-28 | 1.0E-24               | Yq11.223    |
| TTTY15  | 5284   | 64595     | testis-specific transcript, Y-linked 15 (non-protein coding) | 8.64  | -1.11   | 37.0 | 4.2E-28 | 1.0E-24               | Yq11.221    |
| TTTY10  | 1726   | 246119    | testis-specific transcript, Y-linked 10 (non-protein coding) | 6.53  | -2.00   | 36.7 | 5.1E-28 | 1.1E-24               | Yq11.223    |
| KDM5D   | 9463   | 8284      | lysine demethylase 5D                      | 10.6  | 0.386   | 35.5 | 1.4E-27 | 2.5E-24               | Yq11.223    |
| TXLNGY  | 11377  | 246126    | taxilin gamma pseudogene, Y-linked         | 11.7  | 0.200   | 32.7 | 2.1E-26 | 3.2E-23               | Yq11.222-q11.223 |
| ZFY     | 5527   | 7544      | zinc finger protein, Y-linked              | 8.18  | 0.693   | 31.6 | 5.6E-26 | 7.6E-23               | Yp11.2      |
| LINC00278 | 775  | 100873962 | long intergenic non-protein coding RNA 278 | 7.80  | -1.48   | 28.0 | 2.7E-24 | 3.3E-21               | Yp11.31     |
| XIST    | 19961  | 7503      | X inactive specific transcript (non-protein coding) | -12.1 | 5.23    | -26.2 | 2.1E-23 | 2.3E-20               | Xq13.2      |
| USP9Y   | 11237  | 8287      | ubiquitin specific peptidase S, Y-linked  | 8.37  | 1.87    | 25.1 | 8.1E-23 | 7.7E-20               | Yq11.221    |
| PRKY    | 7674   | 5616      | protein kinase, Y-linked, pseudogene       | 7.46  | 1.06    | 25.1 | 8.1E-23 | 7.7E-20               | Yp11.2      |
| DDX3P1  | 1950   | 100133180 | DEAD-box helicase 3 pseudogene 1           | 6.21  | -1.47   | 23.7 | 4.4E-22 | 3.8E-19               | Xq13.2      |
| BCORP1  | 7069   | 286554    | BCL6 corepressor pseudogene 1              | 8.21  | -1.34   | 20.2 | 5.5E-20 | 4.5E-17               | Yq11.222    |
| TMSB4Y  | 1669   | 9087      | thymosin beta 4, Y-linked                  | 5.47  | -0.923  | 19.6 | 1.5E-19 | 1.1E-16               | Yq11.221    |
| KDM6A   | 7139   | 7403      | lysine demethylase 6A                      | -0.571 | 6.76    | -8.06 | 3.0E-09 | 2.2E-06               | Xp11.3      |
| ZFX     | 9037   | 7543      | zinc finger protein, X-linked              | -0.458 | 7.38    | -5.34 | 7.0E-06 | 0.0048                | Xp22.11     |
| EIF1AX  | 4427   | 1964      | eukaryotic translation initiation factor 1A, X-linked | -0.567 | 4.96    | -5.13 | 1.3E-05 | 0.0085                | Xp22.12     |
| TCEANC  | 5231   | 170082    | transcription elongation factor A N-terminal and central domain containing | -0.600 | 5.92    | -8.80 | 3.5E-05 | 0.021                 | Xp22.2      |
| BMS1P1  | 4561   | 399761    | BMS1, ribosome biogenesis factor pseudogene 1 | -0.564 | 3.45    | -4.59 | 6.4E-05 | 0.037                 | 10q11.22    |
| DDX3X   | 8928   | 1654      | DEAD-box helicase 3, X-linked              | -0.359 | 10.0    | -4.57 | 6.7E-05 | 0.038                 | Xp11.4      |
**Supplementary File 2** Differentially expressed genes (p-value < 0.05, no adjustment for multiple testing), with FC < −1.5 or FC > 1.5 (logFC 0.58) between groups CDM3, M0, and CDM0.

| Gene ID   | Length | Entrez ID | Name                                                                 | logFC | AveExpr | t    | p-value  |
|-----------|--------|-----------|----------------------------------------------------------------------|-------|---------|------|----------|
| LRRC37A4P | 6924   | 55073     | leucine rich repeat containing 37 member A4, pseudogene             | 1.69  | 2.94    | 3.02 | 0.0052   |
| TNNT1     | 2171   | 7138      | troponin T1, slow skeletal type                                      | 1.59  | 0.69    | 2.72 | 0.011    |
| SIGLEC1   | 7886   | 6614      | sialic acid binding Ig like lectin 1                               | 1.58  | 1.86    | 2.20 | 0.035    |
| ISG15     | 942    | 9636      | ISG15 ubiquitin-like modifier                                      | 1.56  | 3.36    | 2.10 | 0.044    |
| IFI44L    | 10240  | 10964     | interferon induced protein 44 like                                  | 1.42  | 6.87    | 2.15 | 0.040    |
| OAS3      | 8251   | 4940      | 2'-5'-oligoadenylate synthetase 3                                   | 1.37  | 5.74    | 2.70 | 0.011    |
| USP18     | 2129   | 11274     | ubiquitin specific peptidase 18                                    | 1.36  | 0.97    | 2.16 | 0.039    |
| IFITM3    | 1348   | 10410     | interferon induced transmembrane protein 3                         | 1.35  | 3.98    | 2.33 | 0.027    |
| LY6E      | 2640   | 4061      | lymphocyte antigen 6 family member E                                | 1.33  | 3.09    | 2.78 | 0.0093   |
| JUP       | 4942   | 3728      | junction plakoglobin                                                | 1.25  | 1.37    | 2.43 | 0.021    |
| D2HGDH    | 5279   | 728294    | D-2-hydroxyglutarate dehydrogenase                                  | 1.12  | 1.59    | 3.27 | 0.0027   |
| CATSPERG  | 9190   | 57828     | cation channel sperm associated auxiliary subunit gamma             | 1.09  | 1.61    | 4.34 | 0.00015  |
| TCN2      | 3122   | 6948      | transcobalamin 2                                                   | 1.09  | 1.05    | 2.33 | 0.027    |
| ZDHHC8    | 5542   | 29801     | zinc finger DHHC-type containing 8                                  | 1.08  | 1.20    | 2.28 | 0.030    |
| HELZ2     | 12066  | 85441     | helicase with zinc finger 2                                         | 1.07  | 4.50    | 2.35 | 0.026    |
| RASSF7    | 2492   | 8045      | Ras association domain family member 7                              | 1.05  | 2.00    | 2.29 | 0.029    |
| PARP10    | 6033   | 84875     | poly(ADP-ribose) polymerase family member 10                       | 1.04  | 5.14    | 2.41 | 0.022    |
| NTNG2     | 7845   | 84628     | netrin G2                                                          | 1.03  | 3.26    | 3.06 | 0.0046   |
| MX1       | 8616   | 4599      | MX dynamin like GTPase 1                                           | 1.02  | 7.27    | 2.15 | 0.040    |
| FAAH       | 2776   | 2166      | fatty acid amide hydrolase                                         | 1.02  | 1.77    | 3.77 | 0.00072  |
| SCO2      | 1361   | 9997      | SCO2, cytochrome c oxidase assembly protein                         | 1.01  | 3.54    | 2.19 | 0.037    |
| AIFM3     | 5401   | 150209    | apoptosis inducing factor, mitochondria associated 3               | 1.01  | 1.00    | 2.93 | 0.0064   |
| ARHGEF10L | 7817   | 55160     | Rho guanine nucleotide exchange factor 10 like                     | 1.01  | 3.31    | 2.96 | 0.0059   |
| SPNS3     | 2684   | 201305    | sphingolipid transporter 3 (putative)                               | 1.00  | 0.95    | 2.11 | 0.043    |
| KCNC4     | 25042  | 3749      | potassium voltage-gated channel subfamily C member 4              | 0.95  | 1.23    | 3.47 | 0.0016   |
| NFRIC     | 9347   | 4782      | nuclear factor I C                                                 | 0.94  | 1.14    | 2.42 | 0.022    |
| CARD9     | 6699   | 64170     | caspase recruitment domain family member 9                         | 0.94  | 3.09    | 2.48 | 0.019    |
| THEM6     | 3055   | 51337     | thioesterase superfamily member 6                                   | 0.93  | 1.79    | 2.61 | 0.014    |
| ABCD1     | 4233   | 215       | ATP binding cassette subfamily D member 1                          | 0.93  | 1.16    | 2.36 | 0.025    |
| FAAP100   | 5354   | 80233     | Fanconi anemia core complex associated protein 100                 | 0.93  | 1.60    | 2.46 | 0.020    |
| Gene Symbol | Ensembl ID | RefSeq ID | Description | Log2 Fold Change | P Value |
|-------------|------------|-----------|-------------|-----------------|---------|
| BCL9L       | 10470      | 283149    | B cell CLL/lymphoma 9 like | 0.93 | 3.22 |
| XAF1        | 6615       | 54739     | XIAP associated factor 1 | 0.92 | 7.84 |
| TSPAN4      | 5841       | 7106      | tetraspanin 4 | 0.91 | 0.99 |
| ASPSCR1     | 12097      | 79058     | ASPSCR1, UBX domain containing tether for SLC2A4 | 0.89 | 1.68 |
| SSBP4       | 2974       | 170463    | single stranded DNA binding protein 4 | 0.88 | 1.42 |
| GPR162      | 3295       | 27239     | G protein-coupled receptor 162 | 0.88 | 1.17 |
| S1PR3       | 8754       | 1903      | sphingosine-1-phosphate receptor 3 | 0.87 | 2.40 |
| ZC3H3       | 3731       | 23144     | zinc finger CCCH-type containing 3 | 0.87 | 1.73 |
| HNRNPA1P70  | 1087       | 341333    | heterogeneous nuclear ribonucleoprotein A1 pseudogene 70 | 0.87 | 1.73 |
| ZBP1        | 5405       | 81030     | Z-DNA binding protein 1 | 0.86 | 5.28 |
| COL9A2      | 4151       | 1298      | collagen type IX alpha 2 chain | 0.86 | 1.05 |
| MTCYBP23    | 1102       | 107075131 | mitochondrially encoded cytochrome b pseudogene 23 | 0.86 | 1.24 |
| ARHGDA      | 2903       | 396       | Rho GDP dissociation inhibitor alpha | 0.86 | 2.15 |
| TEL02       | 4239       | 9894      | telomere maintenance 2 | 0.85 | 1.89 |
| NDUFB7      | 531        | 4713      | NADH-ubiquinone oxidoreductase subunit B7 | 0.85 | 2.00 |
| CAP2P1      | 1390       | 353163    | cyclase associated actin cytoskeleton regulatory protein 2 pseudogene 1 | 0.85 | 0.82 |
| SLC9A1      | 5974       | 6548      | solute carrier family 9 member A1 | 0.84 | 2.01 |
| CEP295NL    | 2758       | 100653515 | CEP295 N-terminal like | 0.84 | 1.25 |
| ECH1        | 2467       | 1891      | enoyl-CoA hydratase 1 | 0.83 | 3.79 |
| B4GALT7     | 4927       | 11285     | beta-1,4-galactosyltransferase 7 | 0.83 | 2.42 |
| PLXNB2      | 7257       | 23654     | plexin B2 | 0.81 | 4.58 |
| C7orf50     | 4961       | 84310     | chromosome 7 open reading frame 50 | 0.80 | 2.00 |
| HLA-DQB2    | 2028       | 3120      | major histocompatibility complex, class II, DQ beta 2 | 0.80 | 2.60 |
| C1QA        | 1272       | 712       | complement C1q A chain | 0.80 | 0.78 |
| C11orf24    | 3406       | 53838     | chromosome 11 open reading frame 24 | 0.80 | 1.93 |
| DPP7        | 2820       | 29952     | dipeptidyl peptidase 7 | 0.79 | 3.38 |
| LRRC45      | 3606       | 201255    | leucine rich repeat containing 45 | 0.79 | 2.25 |
| LRP1        | 20839      | 4035      | LDL receptor related protein 1 | 0.78 | 6.66 |
| NELFA       | 5935       | 7469      | negative elongation factor complex member A | 0.78 | 1.73 |
| IGSF9B      | 5753       | 22997     | immunoglobulin superfamily member 9B | 0.77 | 0.72 |
| FLYWCH1     | 8419       | 84256     | FLYWCH-type zinc finger 1 | 0.76 | 2.46 |
| STAB1       | 10588      | 23166     | stabulin 1 | 0.75 | 4.99 |
| PPP1R26     | 5276       | 9858      | protein phosphatase 1 regulatory subunit 26 | 0.75 | 1.50 |
| FAM129B     | 4629       | 64855     | family with sequence similarity 129 member B | 0.75 | 3.28 |
| DHX58       | 3843       | 79132     | DExH-box helicase 58 | 0.75 | 4.80 |
| Gene Symbol | Gene ID | Protein Name                                           | Expression 1 | Expression 2 | Expression 3 | Expression 4 |
|------------|--------|--------------------------------------------------------|--------------|--------------|--------------|--------------|
| DRAP1      | 1737   | DR1 associated protein 1                               | 0.68         | 3.82         | 2.15         | 0.040        |
| CDC42BP1   | 6112   | CDC42 binding protein kinase gamma                      | 0.68         | 0.80         | 2.08         | 0.046        |
| TNFSF13    | 2276   | TNF superfamily member 13                              | 0.68         | 1.50         | 2.12         | 0.042        |
| GADD45GIP1 | 1782   | GADD45G interacting protein 1                           | 0.68         | 2.55         | 2.07         | 0.047        |
| PARP12     | 6285   | poly(ADP-ribose) polymerase family member 12           | 0.67         | 6.44         | 2.55         | 0.016        |
| SLC25A29   | 7451   | solute carrier family 25 member 29                     | 0.67         | 2.84         | 2.37         | 0.025        |
| LLGL1      | 6638   | LLGL1, scribble cell polarity complex component        | 0.67         | 2.58         | 2.17         | 0.038        |
| BST2       | 1119   | bone marrow stromal cell antigen 2                     | 0.67         | 4.99         | 2.05         | 0.050        |
| HLA-DRB6   | 1326   | major histocompatibility complex, class II, DR beta 6 (pseudogene) | 0.66     | 4.48         | 2.17         | 0.038        |
| UNC93B1    | 3548   | unc-93 homolog B1, TLR signaling regulator             | 0.66         | 4.72         | 2.15         | 0.039        |
| C9orf139   | 4990   | chromosome 9 open reading frame 13                     | 0.66         | 2.66         | 2.21         | 0.035        |
| RFNG       | 3865   | RFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase | 0.66     | 1.23         | 2.15         | 0.040        |
| HK3        | 3290   | hexokinase 3                                           | 0.66         | 6.51         | 2.14         | 0.041        |
| SUMO2P6    | 288    | SUMO2 pseudogene 6                                     | 0.66         | 0.92         | 2.16         | 0.039        |
| GALNS      | 8402   | galactosamine (N-acetyl)-6-sulfatase                    | 0.65         | 3.34         | 2.98         | 0.0057       |
| PILRB      | 5764   | paired immunoglobulin-like type 2 receptor beta        | 0.65         | 3.81         | 2.50         | 0.018        |
| TNK2       | 11297  | tyrosine kinase non receptor 2                         | 0.65         | 4.68         | 2.09         | 0.045        |
| TP53I3     | 2312   | tumor protein p53 inducible protein 3                  | 0.65         | 1.48         | 2.25         | 0.032        |
| USP35      | 5233   | ubiquitin specific peptidase 35                        | 0.64         | 1.39         | 2.32         | 0.027        |
| KCTD17     | 2786   | potassium channel tetramerization domain containing 17 | 0.64         | 1.29         | 2.10         | 0.044        |
| MYO15B     | 12761  | myosin XVB                                             | 0.63         | 5.57         | 2.27         | 0.031        |
| TTYH2      | 5375   | tweety family member 2                                 | 0.63         | 2.54         | 3.00         | 0.0054       |
| ACAP3      | 8809   | ArfGAP with coiled-coil, ankyrin repeat and PH domains 3 | 0.63     | 3.28         | 2.17         | 0.038        |
| NRROS      | 2910   | negative regulator of reactive oxygen species         | 0.63         | 1.74         | 2.44         | 0.021        |
| ATP13A1    | 8376   | ATPase 13A1                                            | 0.63         | 3.04         | 2.54         | 0.017        |
| SLC27A5    | 8025   | solute carrier family 27 member 5                      | 0.63         | 0.92         | 2.60         | 0.014        |
| RPL7AP64   | 663    | ribosomal protein L7a pseudogene 64                    | 0.63         | 2.06         | 2.89         | 0.0070       |
| GRAMD1B    | 13946  | GRAM domain containing 1B                              | 0.62         | 3.38         | 2.43         | 0.021        |
| ARFGAP1    | 6613   | ADP ribosylation factor GTPase activating protein 1    | 0.62         | 3.12         | 2.12         | 0.042        |
| PRK2D      | 5446   | protein kinase D2                                       | 0.61         | 4.80         | 3.20         | 0.0032       |
| TRMT112P6  | 372    | tRNA methyltransferase subunit 11-2 pseudogene 6       | 0.61         | 0.92         | 2.12         | 0.043        |
| CTIF       | 8100   | cap binding complex dependent translation initiation factor | 0.61     | 0.90         | 2.21         | 0.035        |
| EEFSEC     | 2421   | eukaryotic elongation factor, selencysteine-tRNA specific | 0.61     | 1.79         | 2.76         | 0.010        |
| PSRC1      | 2855   | proline and serine rich coiled-coil 1                   | 0.60         | 2.07         | 2.39         | 0.023        |
| FAM102A    | 6100   | family with sequence similarity 102 member A           | 0.60         | 3.78         | 2.24         | 0.033        |
| Gene Symbol | Gene ID | Gene Name                                      | Log2 Fold Change | Adj P-Value |
|------------|---------|-----------------------------------------------|-----------------|-------------|
| CROCCP3    | 6813    | ciliary rootlet coiled-coil, rootletin pseudogene 3 | 0.60            | 2.21        | 3.06        | 0.0046      |
| DHRS1      | 5002    | dehydrogenase/reductase 1                      | 0.60            | 2.39        | 2.62        | 0.014       |
| HPS6       | 2649    | HPS6, biogenesis of lysosomal organelles complex 2 subunit 3 | 0.60            | 2.60        | 2.42        | 0.022       |
| ASTN2      | 8290    | astrotactin 2                                  | 0.60            | 1.02        | 2.47        | 0.019       |
| MVB12B     | 7884    | multivesicular body subunit 12B                | 0.59            | 1.52        | 2.57        | 0.015       |
| TIAF1      | 5337    | TGFβ1-induced anti-apoptotic factor 1          | 0.59            | 1.44        | 2.54        | 0.017       |
| PPARGC1B   | 12889   | PPARG coactivator 1 beta                       | 0.59            | 3.06        | 2.66        | 0.012       |
| KMT5C      | 4775    | lysine methyltransferase 5C                    | 0.59            | 0.83        | 2.33        | 0.026       |
| TPRG1      | 9535    | tumor protein p63 regulated 1                  | 0.59            | 1.65        | 2.19        | 0.036       |
| PRDX6      | 2367    | peroxiredoxin 6                               | -0.59           | 8.49        | -2.11       | 0.044       |
| CFAP53     | 1851    | cilia and flagella associated protein 53      | -0.59           | 0.77        | -2.16       | 0.038       |
| MYLK       | 22167   | myosin light chain kinase                      | -0.59           | 6.02        | -2.23       | 0.033       |
| USP12      | 5195    | ubiquitin specific peptidase 12                | -0.59           | 8.84        | -2.09       | 0.045       |
| MEST       | 4631    | mesoderm specific transcript                   | -0.59           | 2.78        | -3.19       | 0.0033      |
| F13A1      | 4690    | coagulation factor XIII A chain               | -0.63           | 9.21        | -2.55       | 0.016       |
| ITGA9      | 8277    | integrin subunit alpha 9                       | -0.63           | 1.02        | -2.31       | 0.028       |
| NCR1       | 1893    | natural cytotoxicity triggering receptor 1     | -0.63           | 2.86        | -2.50       | 0.018       |
| RNF14      | 4987    | ring finger protein 14                         | -0.64           | 7.85        | -2.51       | 0.018       |
| GRAM1D1C   | 7814    | GRAM domain containing 1C                      | -0.64           | 4.05        | -2.18       | 0.037       |
| TRAPPC3L   | 2757    | trafficking protein particle complex 3 like    | -0.64           | 3.06        | -2.20       | 0.036       |
| FAM46C     | 5751    | family with sequence similarity 46 member C    | -0.65           | 11.8        | -2.20       | 0.036       |
| PHEX       | 6384    | phosphate regulating endopeptidase homolog X-linked | -0.66          | 2.29        | -2.26       | 0.031       |
| PLEK2      | 1603    | pleckstrin 2                                   | -0.66           | 4.79        | -2.51       | 0.0177      |
| MAP7       | 5447    | microtubule associated protein 7               | -0.67           | 1.92        | -2.26       | 0.031       |
| GOLGA8N    | 7602    | golgin A8 family member N                      | -0.67           | 1.36        | -2.14       | 0.041       |
| RBM53      | 18339   | RNA binding motif single stranded interacting protein 3 | -0.67          | 0.76        | -2.13       | 0.042       |
| PCMTD1P3   | 472     | protein-L-isoaspartate (D-aspartate) O-methyltransferase domain containing 1 pseudogene 3 | -0.67          | 1.25        | -2.81       | 0.0086      |
| TRAV22     | 464     | T cell receptor alpha variable 22             | -0.69           | 0.85        | -2.53       | 0.017       |
| ZNF667-AS1 | 3629    | ZNF667 antisense RNA 1 (head to head)          | -0.69           | 0.75        | -2.17       | 0.038       |
| FKB1P18    | 1677    | FKS06 binding protein 1B                       | -0.69           | 2.44        | -2.27       | 0.030       |
| RPS4XP7    | 780     | ribosomal protein 54X pseudogene 7            | -0.69           | 0.61        | -2.28       | 0.030       |
| GZMB       | 1411    | granzyme B                                    | -0.71           | 5.26        | -2.40       | 0.023       |
| SLC6A4     | 6983    | solute carrier family 6 member 4              | -0.72           | 1.34        | -2.26       | 0.031       |
| PAGE2B     | 507     | PAGE family member 2B                         | -0.73           | 1.70        | -2.27       | 0.030       |

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| Gene     | Chr Position | Description                                      | Log2FC | p-value |
|----------|--------------|--------------------------------------------------|--------|---------|
| ELL2P1   | 1906         | elongation factor for RNA polymerase II 2 pseudogene 1 | -0.75  | 0.80    | -2.43  | 0.021 |
| ABCA13   | 19074        | ATP binding cassette subfamily A member 13       | -0.75  | 3.63    | -2.12  | 0.043 |
| SNCA     | 4014         | synuclein alpha                                  | -0.76  | 11.8    | -2.26  | 0.031 |
| GYPB     | 1566         | glycophorin B (MNS blood group)                   | -0.76  | 5.19    | -2.76  | 0.0096 |
| TCN1     | 1586         | transcobalamin 1                                 | -0.77  | 2.90    | -2.95  | 0.0061 |
| SPATA20  | 5337         | spermatogenesis associated 20                     | -0.80  | 2.57    | -2.39  | 0.023 |
| UBB      | 1621         | ubiquitin B                                      | -0.82  | 10.9    | -2.51  | 0.017 |
| IGKV3D-20| 445          | immunoglobulin kappa variable 3D-20              | -0.86  | 0.89    | -2.23  | 0.034 |
| EPB42    | 5574         | erythrocyte membrane protein band 4.2            | -0.87  | 7.14    | -2.60  | 0.014 |
| NFIIB    | 12765        | nuclear factor I B                               | -0.87  | 1.11    | -2.76  | 0.010 |
| DPCD     | 1718         | deleted in primary ciliary dysfunction homolog (mouse) | -0.87  | 2.26    | -2.13  | 0.041 |
| HAVCR1   | 2151         | hepatitis A virus cellular receptor 1            | -0.87  | 1.39    | -3.07  | 0.004 |
| EREG     | 5717         | epiregulin                                       | -0.87  | 1.69    | -2.09  | 0.045 |
| TRIM40   | 2497         | tripartite motif containing 40                   | -0.89  | 0.91    | -2.12  | 0.043 |
| SH2D1B   | 2797         | SH2 domain containing 1B                         | -0.89  | 3.35    | -2.45  | 0.020 |
| NCAM1    | 12377        | neural cell adhesion molecule 1                  | -0.89  | 3.68    | -3.01  | 0.0052 |
| PITHD1   | 2186         | PITH domain containing 1                         | -0.91  | 8.28    | -2.99  | 0.0054 |
| KLRF1    | 1260         | killer cell lectin like receptor F1              | -0.94  | 4.65    | -2.58  | 0.015 |
| AKR1C3   | 4532         | aldo-keto reductase family 1 member C3           | -0.98  | 2.23    | -2.88  | 0.0072 |
| HLA-DQB1 | 4904         | major histocompatibility complex, class II, DQ beta 1 | -1.01  | 6.17    | -2.63  | 0.013 |
| SLC8A3   | 8444         | solute carrier family 8 member A3                | -1.02  | 1.41    | -2.39  | 0.023 |
| ANO2     | 6255         | anoctamin 2                                      | -1.04  | 0.87    | -2.42  | 0.022 |
| ADAMTS1  | 7063         | ADAM metallopeptidase with thrombospondin type 1 motif 1 | -1.07  | 1.91    | -2.40  | 0.023 |
| SLPI     | 596          | secretory leukocyte peptidase inhibitor           | -1.07  | 2.16    | -2.30  | 0.029 |
| MAGI2-AS3| 11625        | MAGI2 antisense RNA 3                            | -1.14  | 2.16    | -2.21  | 0.035 |
| KANSL1-AS1| 976      | KANSL1 antisense RNA 1                           | -1.18  | 1.60    | -3.06  | 0.0046 |
| FAT4     | 16439        | FAT atypical cadherin 4                          | -1.27  | 1.48    | -3.50  | 0.0015 |
| RPS4XP22 | 780          | ribosomal protein S4X pseudogene 22              | -1.45  | 3.13    | -2.11  | 0.043 |
| CRISP3   | 2247         | cysteine rich secretory protein 3                | -1.69  | 1.96    | -3.36  | 0.0021 |
| XKR3     | 1690         | XK related 3                                     | -1.69  | -0.19   | -2.18  | 0.037 |
| DAAM2    | 12955        | dishevelled associated activator of morphogenesis 2 | -1.73  | 0.69    | -3.41  | 0.0019 |
| CEACAM8  | 2551         | carcinoembryonic antigen related cell adhesion molecule 8 | -1.74  | 1.12    | -2.84  | 0.0081 |
| Gene ID | Length | Entrez ID | Name | logFC | AveExpr | t    | p-value |
|---------|--------|-----------|------|-------|---------|------|---------|
| NACA3P  | 648    | 389240    | NACA family member 3 pseudogene | 1,53  | 3,70    | 2,68 | 0,012   |
| IFI44L  | 10240  | 10964     | interferon induced protein 44 like | 1,42  | 6,87    | 2,20 | 0,036   |
| RSAD2   | 4834   | 91543     | radical S-adenosyl methionine domain containing 2 | 1,40  | 6,70    | 2,07 | 0,047   |
| IDO1    | 3261   | 3620      | indoleamine 2,3-dioxygenase 1 | 1,36  | 3,66    | 2,91 | 0,0066  |
| VSTM1   | 1111   | 284415    | V-set and transmembrane domain containing 1 | 1,27  | 3,02    | 3,33 | 0,0023  |
| IFIT3   | 2640   | 3437      | interferon induced protein with tetratricopeptide repeats 3 | 1,18  | 8,32    | 2,11 | 0,043   |
| IFI444  | 2038   | 10561     | interferon induced protein 44 | 1,15  | 6,43    | 2,14 | 0,041   |
| HERC5   | 4766   | 51191     | HECT and RLD domain containing E3 ubiquitin protein ligase 5 | 1,13  | 6,42    | 2,06 | 0,048   |
| OAS3    | 8251   | 4940      | 2'-5'-oligoadenylate synthetase 3 | 1,08  | 5,74    | 2,20 | 0,036   |
| SLC16A14| 4946   | 151473    | solute carrier family 16 member 14 | 1,06  | 0,68    | 2,63 | 0,013   |
| RNASE2  | 755    | 15036     | ribonuclease A family member 2 | 1,00  | 3,72    | 2,43 | 0,021   |
| KIAA1024| 6800   | 23251     | KIAA1024 | 0,99  | 0,54    | 2,20 | 0,035   |
| MARCO   | 2079   | 8685      | macrophage receptor with collagenous structure | 0,96  | 2,05    | 2,37 | 0,024   |
| ZBP1    | 5405   | 80300     | Z-DNA binding protein 1 | 0,87  | 5,28    | 2,51 | 0,018   |
| NTNG2   | 7845   | 84628     | netrin G2 | 0,86  | 3,26    | 2,63 | 0,013   |
| XAF1    | 6615   | 54739     | XIAP associated factor 1 | 0,85  | 7,84    | 2,23 | 0,033   |
| FBN1    | 16057  | 2200      | fibrillin 1 | 0,85  | 1,56    | 2,33 | 0,027   |
| SAMD9L  | 7135   | 219285    | sterile alpha motif domain containing 9 like | 0,80  | 8,97    | 2,20 | 0,035   |
| DDX58   | 4353   | 23586     | DExD/H-box helicase 58 | 0,77  | 8,14    | 2,19 | 0,037   |
| DHX58   | 3843   | 79132     | DExH-box helicase 58 | 0,71  | 4,80    | 2,09 | 0,045   |
| EIF2AK2 | 10753  | 5610      | eukaryotic translation initiation factor 2 alpha kinase 2 | 0,71  | 7,19    | 2,24 | 0,033   |
| C9orf66 | 2918   | 157983    | chromosome 9 open reading frame 66 | 0,70  | 1,13    | 2,05 | 0,049   |
| CD101   | 3828   | 9398      | CD101 molecule | 0,68  | 4,64    | 3,05 | 0,0048  |
| TRIM22  | 6020   | 10346     | tripartite motif containing 22 | 0,68  | 9,19    | 2,14 | 0,041   |
| RNF213  | 28570  | 57674     | ring finger protein 213 | 0,66  | 10,6    | 2,65 | 0,013   |
| SNORA22 | 540    | 677807    | small nucleolar RNA, H/ACA box 22 | 0,66  | 1,17    | 2,72 | 0,011   |
| HERC6   | 5793   | 55008     | HECT and RLD domain containing E3 ubiquitin protein ligase family member 6 | 0,65  | 5,32    | 2,25 | 0,032   |
| CATSPERG| 9190   | 57828     | cation channel sperm associated auxiliary subunit gamma | 0,65  | 1,61    | 2,78 | 0,0093  |
| SNORA73B| 204    | 26768     | small nucleolar RNA, H/ACA box 73B | 0,65  | 6,69    | 3,12 | 0,0039  |
| HNRNPA1P70| 1087  | 341333    | heterogeneous nuclear ribonucleoprotein A1 pseudogene 70 | 0,64  | 1,73    | 2,13 | 0,042   |
| CCDC170 | 5419   | 80129     | coiled-coil domain containing 170 | 0,64  | 3,39    | 2,89 | 0,0071  |
| PARP12  | 6285   | 64761     | poly(ADP-ribose) polymerase family member 12 | 0,63  | 6,44    | 2,44 | 0,021   |
| MRPL36  | 1394   | 46979     | mitochondrial ribosomal protein L36 | 0,63  | 1,46    | 2,66 | 0,012   |
| Gene Symbol | GI Number | Entrez Gene Number | Gene Description | Fold Change |
|-------------|-----------|--------------------|------------------|-------------|
| COBW5       | 12708     | 220869             | COBW domain containing 5 | 0.62        |
| SNORA49     | 136       | 677829             | small nucleolar RNA, H/ACA box 49 | 0.62        |
| LINC00174   | 5627      | 285908             | long intergenic non-protein coding RNA 174 | 0.61        |
| KIAA1958    | 13119     | 158405             | KIAA1958         | 0.60        |
| IL15        | 7860      | 3600               | interleukin 15   | 0.60        |
| SAT1        | 2498      | 6303               | spermidine/spermine N1-acetyltransferase 1 | 0.59        |
| MAP2K6      | 6125      | 5608               | mitogen-activated protein kinase kinase 6 | 0.59        |
| VEGFA       | 14431     | 7422               | vascular endothelial growth factor A | 0.59        |
| TRAPPCC3L   | 2757      | 100128327          | trafficking protein particle complex 3 like | -0.60       |
| CLEC1B      | 3670      | 51266              | C-type lectin domain family 1 member B | -0.60       |
| GPR15       | 1252      | 2838               | G protein-coupled receptor 15 | -0.61       |
| EPB41       | 10739     | 2035               | erythrocyte membrane protein band 4.1 | -0.61       |
| ST20-AS1    | 4223      | 283687             | ST20 antisense RNA 1 | -0.61       |
| FAM46C      | 5751      | 54855              | family with sequence similarity 46 member C | -0.62       |
| RAB2B       | 3610      | 84932              | RAB2B, member RAS oncogene family | -0.64       |
| HEMGN       | 2394      | 55363              | hemogen           | -0.64       |
| MYCT1       | 3030      | 80177              | MYC target 1      | -0.65       |
| PRDX6       | 2367      | 9588               | peroxiredoxin 6   | -0.65       |
| GSPT1       | 8018      | 2935               | G1 to S phase transition 1 | -0.65       |
| FKBPB       | 1677      | 2281               | FKS06 binding protein 1B | -0.66       |
| DDX6P1      | 1436      | 442192             | DEAD-box helicase 6 pseudogene 1 | -0.66       |
| IFIT1B      | 1972      | 439996             | interferon induced protein with tetratricopeptide repeats 1B | -0.67       |
| TBC5L       | 7020      | 219899             | tubulin folding cofactor E like | -0.68       |
| GLRX5       | 2845      | 51218              | glutaredoxin 5    | -0.68       |
| MAP7        | 5447      | 9053               | microtubule associated protein 7 | -0.68       |
| TCN1        | 1586      | 6947               | transcobalamin 1   | -0.68       |
| BPGM        | 2539      | 669                | bisphosphoglycerate mutase | -0.69       |
| DYSK4       | 4218      | 8444               | dual specificity tyrosine phosphorylation regulated kinase 3 | -0.70       |
| UBXN10      | 5571      | 127733             | UBX domain protein 10 | -0.70       |
| RTCA-AS1    | 423       | 100506007          | RTCA antisense RNA 1 | -0.71       |
| DNAJA4      | 6591      | 55466              | Dnaal heat shock protein family (Hsp40) member A4 | -0.71       |
| HAVCR1      | 2151      | 26762              | hepatitis A virus cellular receptor 1 | -0.71       |
| NFIB        | 12765     | 4781               | nuclear factor I B | -0.75       |
| ARHGEF37    | 5324      | 389337             | Rho guanine nucleotide exchange factor 37 | -0.75       |
| RAB6B       | 6005      | 51560              | RAB6B, member RAS oncogene family | -0.76       |
| Symbol  | Gene Name                     | Description                                                                 | Fold Change | t-Test | p-value |
|---------|-------------------------------|-----------------------------------------------------------------------------|-------------|--------|---------|
| SPATA20 | spermatogenesis associated 20 |                                                                             | -0.78       | 2.57   | 2.33    | 0.026    |
| C9orf153| chromosome 9 open reading frame 153 |                                                                                | -0.79       | 1.59   | 2.06    | 0.048    |
| SNCA    | synuclein alpha               |                                                                             | -0.80       | 11.8   | 2.47    | 0.019    |
| UBBP4   | ubiquitin B pseudogene 4      |                                                                             | -0.80       | 2.98   | -2.07   | 0.047    |
| UBE2O   | ubiquitin conjugating enzyme E2 O |                                                                                | -0.82       | 5.69   | -2.33   | 0.027    |
| GYPB    | glycoporphin B (MNS blood group) |                                                                                | -0.82       | 5.19   | -3.02   | 0.0052   |
| ELL2P1  | elongation factor for RNA polymerase II 2 pseudogene 1 |                                                                    | -0.84       | 0.80   | -2.77   | 0.0095   |
| NINJ2   | ninjurin 2                     |                                                                             | -0.84       | 4.38   | -2.22   | 0.034    |
| NAT8B   | N-acetyltransferase 8B (putative, gene/pseudogene) |                                                | -0.85       | 0.79   | -2.73   | 0.010    |
| DPCD    | deleted in primary ciliary dyskinesia homolog (mouse) |                                                | -0.85       | 2.26   | -2.10   | 0.045    |
| UBB     | ubiquitin B                   |                                                                             | -0.86       | 10.9   | -2.74   | 0.011    |
| EPB42   | erythrocyte membrane protein band 4.2 |                                                | -0.89       | 7.14   | -2.73   | 0.011    |
| PAGE2B  | PAGE family member 2B         |                                                                             | -0.89       | 1.70   | -2.84   | 0.0080   |
| FAXDC2  | fatty acid hydroxylase domain containing 2 |                                                | -0.90       | 6.67   | -2.42   | 0.022    |
| ANO2    | anoctamin 2                   |                                                                             | -0.90       | 0.87   | -2.09   | 0.045    |
| PAQR9   | progestin and adipoQ receptor family member 9 |                                                | -0.91       | 2.95   | -2.40   | 0.023    |
| SLC8A3  | solute carrier family 8 member A3 |                                                | -0.92       | 1.41   | -2.17   | 0.038    |
| SLC6A4  | solute carrier family 6 member 4 |                                                | -0.93       | 1.34   | -3.02   | 0.0051   |
| PITHD1  | PITH domain containing 1      |                                                                             | -0.95       | 8.28   | -3.23   | 0.0029   |
| OSBP2   | oxysterol binding protein 2   |                                                                             | -0.96       | 5.78   | -2.15   | 0.040    |
| CA1     | carbonic anhydrase 1          |                                                                             | -0.98       | 8.03   | -2.23   | 0.033    |
| FADS2   | fatty acid desaturase 2       |                                                                             | -1.01       | 3.78   | -2.11   | 0.043    |
| XCL2    | X-C motif chemokine ligand 2  |                                                                             | -1.04       | 1.09   | -3.11   | 0.0040   |
| E2F2    | E2F transcription factor 2    |                                                                             | -1.06       | 5.73   | -3.35   | 0.0022   |
| MAGI2-AS3 | MAGI2 antisense RNA 3                     |                                                | -1.11       | 2.16   | -2.15   | 0.040    |
| ADAMTS1 | ADAM metallopeptidase with thrombospondin type 1 motif 1 |                                        | -1.14       | 1.91   | -2.60   | 0.014    |
| NLRP2   | NLR family pyrin domain containing 2 |                                                | -1.16       | 1.83   | -2.27   | 0.030    |
| PIGC    | phosphatidylinositol glycan anchor biosynthesis class C |                              | -1.21       | 7.03   | -2.52   | 0.017    |
| NNX3-1  | NK3 homeobox 1                |                                                                             | -1.78       | 2.48   | -2.07   | 0.047    |
| Gene ID | Length | Entrez ID | Name | logFC | AveExpr | t  | p-value |
|---------|--------|-----------|------|-------|---------|----|---------|
| TUBB2A  | 1727   | 7280      | tubulin beta 2A class IIa | 2.53 | 1.15    | 2.05 | 0.049   |
| LRRC37A4P | 6924 | 55073 | leucine rich repeat containing 37 member A4, pseudogene | 1.87 | 2.94    | 3.35 | 0.0022  |
| PF4V1   | 522    | 5197      | platelet factor 4 variant 1 | 1.60 | 1.47    | 2.46 | 0.020   |
| COL5A3  | 6750   | 50509     | collagen type V alpha 3 chain | 1.48 | 0.38    | 2.08 | 0.046   |
| LGALS2  | 828    | 3957      | galexin 2 | 1.06 | 3.29    | 2.90 | 0.0068  |
| PLA2G4C | 6613   | 8605      | phospholipase A2 group IVC | 0.94 | 1.38    | 2.35 | 0.026   |
| THEM6   | 3055   | 51337     | thioesterase superfamily member 6 | 0.88 | 1.79    | 2.43 | 0.021   |
| VWF     | 10391  | 7450      | von Willebrand factor | 0.88 | 0.99    | 2.14 | 0.040   |
| ARHGFE10L | 7817 | 55160     | Rho guanine nucleotide exchange factor 10 like | 0.82 | 3.31    | 2.36 | 0.025   |
| NECTIN3 | 6184   | 25945     | nectin cell adhesion molecule 3 | 0.81 | 0.79    | 2.07 | 0.047   |
| FAAP100 | 5354   | 80233     | Fanconi anemia core complex associated protein 100 | 0.80 | 1.60    | 2.07 | 0.047   |
| LRRC45  | 3606   | 201255    | leucine rich repeat containing 45 | 0.79 | 2.25    | 2.68 | 0.012   |
| SUMO2P6 | 288    | 100127922 | SUMO2 pseudogene 6 | 0.79 | 0.92    | 2.60 | 0.014   |
| PHLDB3  | 4553   | 653583    | pleckstrin homology like domain family B member 3 | 0.77 | 1.21    | 2.25 | 0.032   |
| G6PC3   | 4551   | 92579     | glucose-6-phosphatase catalytic subunit 3 | 0.76 | 0.84    | 2.69 | 0.012   |
| ASPSCR1 | 12097  | 79058     | ASPSCR1, UBX domain containing tether for SLC2A4 | 0.75 | 1.68    | 2.27 | 0.030   |
| FLYWCH1 | 8419   | 84256     | FLYWCH-type zinc finger 1 | 0.75 | 2.46    | 2.09 | 0.045   |
| BOP1    | 3716   | 23246     | block of proliferation 1 | 0.74 | 1.30    | 2.09 | 0.046   |
| LINCO1410 | 3038 | 103352539 | long intergenic non-protein coding RNA 1410 | 0.74 | 2.62    | 2.05 | 0.049   |
| GADD45GIP1 | 1782 | 90480     | GADD45G interacting protein 1 | 0.69 | 2.55    | 2.10 | 0.045   |
| PLA2G1S | 4464   | 23659     | phospholipase A2 group XV | 0.68 | 1.26    | 2.52 | 0.017   |
| TRMT112P6 | 372 | 391358    | tRNA methyltransferase subunit 11-2 pseudogene 6 | 0.67 | 0.92    | 2.31 | 0.028   |
| POMGNT2 | 2668   | 84892     | protein O-linked mannose N-acetylglucosaminyltransferase 2 (beta 1,4-) | 0.67 | 1.32    | 2.19 | 0.036   |
| SLC12A7 | 5984   | 10723     | solute carrier family 12 member 7 | 0.67 | 3.24    | 2.15 | 0.040   |
| SEPT10  | 4131   | 151011    | septin 10 | 0.66 | 1.34    | 2.76 | 0.010   |
| INTS5   | 3285   | 80789     | integrator complex subunit 5 | 0.65 | 2.68    | 3.16 | 0.0036  |
| C3orf18 | 3248   | 51161     | chromosome 3 open reading frame 18 | 0.63 | 2.47    | 3.31 | 0.0024  |
| B4GALT7 | 4927   | 11285     | beta-1,4-galactosyltransferase 7 | 0.62 | 2.42    | 2.14 | 0.040   |
| MORN1   | 11337  | 79906     | MORN repeat containing 1 | 0.61 | 1.30    | 2.44 | 0.021   |
| NCKIPSD | 3431   | 51517     | NCK interacting protein with SH3 domain | 0.60 | 1.62    | 2.47 | 0.019   |
| BANF1   | 1645   | 8815      | barrier to autointegration factor 1 | 0.60 | 1.34    | 2.24 | 0.033   |
| ANO8    | 4979   | 37719     | anoctamin 8 | 0.60 | 1.44    | 2.27 | 0.031   |
| DEXI    | 1722   | 28955     | Dexi homolog | 0.59 | 1.75    | 2.85 | 0.0079  |
| Gene Symbol | Chromosome | Gene Description | Enrichment Factor | FDR |
|-------------|------------|------------------|-------------------|-----|
| PDK2        | 5481       | pyruvate dehydrogenase kinase 2  | 0.59  | 2.44 |
| NEB         | 30567      | nebulin           | -0.62  | 3.65 |
| TIAM2       | 11532      | T cell lymphoma invasion and metastasis 2 | -0.62  | 4.07 |
| PLK2        | 4768       | polo like kinase 2  | -0.63  | 0.88 |
| KIAA1324L   | 8115       | KIAA1324 like     | -0.64  | 3.70 |
| C7orf25     | 3248       | chromosome 7 open reading frame 25 | -0.66  | 1.11 |
| CCDC144B    | 9762       | coiled-coil domain containing 144B (pseudogene) | -0.67  | 3.38 |
| TPST1       | 2871       | tyrosylprotein sulfotransferase 1 | -0.70  | 3.30 |
| FSIP2       | 23761      | fibrous sheath interacting protein 2 | -0.70  | 1.27 |
| DSC1        | 4271       | desmocollin 1     | -0.70  | 2.68 |
| IL18RAP     | 2966       | interleukin 18 receptor accessory protein | -0.70  | 6.90 |
| LRRC37A2    | 6756       | leucine rich repeat containing 37 member A2 | -0.74  | 2.20 |
| KLF1        | 1260       | killer cell lectin like receptor F1 | -0.76  | 4.65 |
| GZMB        | 1411       | granzyme B        | -0.77  | 5.26 |
| APOLD1      | 7243       | apolipoprotein L domain containing 1 | -0.78  | 1.72 |
| SMC1B       | 4253       | structural maintenance of chromosomes 1B | -0.79  | 1.27 |
| RGS1        | 4117       | regulator of G protein signaling 1 | -0.83  | 1.48 |
| HLA-DQB1    | 4904       | major histocompatibility complex, class II, DQ beta 1 | -0.87  | 6.17 |
| CNTNAP3     | 9521       | contactin associated protein like 3 | -0.90  | 4.00 |
| ABCA13      | 19074      | ATP binding cassette subfamily A member 13 | -0.91  | 3.63 |
| RNASE2      | 755        | ribonuclease A family member 2 | -0.92  | 3.72 |
| SLC16A14    | 4946       | solute carrier family 16 member 14 | -1.04  | 0.68 |
| EREG        | 5717       | epieregulin       | -1.11  | 1.69 |
| MYZAP       | 2561       | myocardial zonula adherens protein | -1.12  | 1.63 |
| KANSL1-AS1  | 976        | KANSL1 antisense RNA 1 | -1.12  | 1.60 |
| GSTM3       | 4750       | glutathione S-transferase mu 3 | -1.30  | 1.90 |
| DAAM2       | 12955      | dishevelled associated activator of morphogenesis 2 | -1.31  | 0.69 |
| HLA-J       | 1946       | major histocompatibility complex, class I, J (pseudogene) | -1.33  | 4.39 |

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## Gene Ontology

### Supplementary File 3

Significant (FDR-adjusted p-value < 0.075) GO terms based on GSEA with addition of modified cluster names from EnrichmentMap, AutoAnnotate, and WordCloud analysis.

| Gene Ontology ID | Gene Ontology Biological Process | Normalized enrichment score CDM3vsM0 | Normalized enrichment score CDM3vsCDM0 | FDR-adjusted p-value CDM3vsM0 | FDR-adjusted p-value CDM3vsCDM0 | Geneset size | GO term identified in small intestinal biopsy GSEA | Cluster name |
|------------------|----------------------------------|--------------------------------------|---------------------------------------|-------------------------------|-------------------------------|--------------|------------------------------------------------|--------------|
| GO:0034340       | response to type I interferon    | 2.35                                 | 2.58                                  | 0                             | 0                             | 68           | Yes                                           | type I interferon response |
| GO:0060337       | type I interferon signaling pathway | 2.31                               | 2.54                                  | 0                             | 0                             | 64           | Yes                                           | type I interferon response |
| GO:0071367       | cellular response to type I interferon | 2.30                             | 2.53                                  | 0                             | 0                             | 64           | Yes                                           | type I interferon response |
| GO:0051607       | defense response to virus         | 2.16                                 | 2.49                                  | 0.00030                       | 0                             | 92           | Yes                                           | defense response to virus |
| GO:0009615       | response to virus                 | 2.07                                 | 2.40                                  | 0.0036                        | 0                             | 186          | Yes                                           | defense response to virus |
| GO:0048147       | negative regulation of fibroblast proliferation | 2.03 | -0.82                                  | 0.0080                        | 0.97                          | 18           | No                                            | defense response to virus |
| GO:1903901       | negative regulation of viral life cycle | 2.00                             | 2.37                                  | 0.012                         | 0                             | 70           | Yes                                           | negative regulation of viral replication |
| GO:0045071       | negative regulation of viral genome replication | 1.99                          | 2.37                                  | 0.012                         | 0                             | 49           | Yes                                           | negative regulation of viral replication |
| GO:0035455       | response to interferon-alpha      | 1.99                                 | 2.08                                  | 0.012                         | 0.0019                        | 17           | Yes                                           |                      |
| GO:0048525       | negative regulation of viral process | 1.99                             | 2.35                                  | 0.011                         | 0                             | 84           | Yes                                           | negative regulation of viral replication |
| GO:0098542       | defense response to other organism | 1.95                            | 2.13                                  | 0.018                         | 0.00080                       | 309          | Yes                                           | defense response to virus |
| GO:0035148       | tube formation                    | 1.94                                 | 1.41                                  | 0.020                         | 0.62                          | 55           | No                                            | tube formation |

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### Gene Ontology

| Gene Ontology ID | Gene Ontology Biological Process                        | Normalized enrichment score CDM3vsM0 | Normalized enrichment score CDM3vsCDM0 | FDR-adjusted p-value CDM3vsM0 | FDR-adjusted p-value CDM3vsCDM0 | Geneset size | GO term identified in small intestinal biopsy GSEA | Cluster name                  |
|------------------|----------------------------------------------------------|--------------------------------------|----------------------------------------|------------------------------|------------------------------|--------------|------------------------------------------------|-----------------------------|
| GO:0060562       | epithelial tube morphogenesis                            | 1.90                                 | 0.97                                   | 0.034                        | 0.99                         | 135          | No                                            | tube formation               |
| GO:0072175       | epithelial tube formation                                 | 1.89                                 | 1.19                                   | 0.038                        | 0.97                         | 47           | No                                            | tube formation               |
| GO:0016331       | morphogenesis of embryonic epithelium                    | 1.89                                 | 1.17                                   | 0.038                        | 0.95                         | 48           | No                                            | tube formation               |
| GO:0046956       | regulation of viral entry into host cell                 | 1.87                                 | 1.72                                   | 0.046                        | 0.15                         | 26           | Yes                                           | negative regulation of viral replication |
| GO:0048732       | gland development                                         | 1.87                                 | 1.11                                   | 0.044                        | 0.98                         | 115          | No                                            |                             |
| GO:0050691       | regulation of defense response to virus by host           | 1.84                                 | 1.84                                   | 0.064                        | 0.051                        | 31           | Yes                                           | regulation of defense response to virus |
| GO:0002230       | positive regulation of defense response to virus by host  | 1.84                                 | 1.72                                   | 0.062                        | 0.15                         | 22           | Yes                                           | regulation of defense response to virus |
| GO:1905954       | positive regulation of lipid localization                | 1.82                                 | 1.17                                   | 0.074                        | 0.95                         | 56           | No                                            |                             |
| GO:0001838       | embryonic epithelial tube formation                       | 1.81                                 | 1.14                                   | 0.075                        | 0.96                         | 42           | No                                            | tube formation               |
| GO:0045428       | regulation of nitric oxide biosynthetic process           | 1.81                                 | 1.05                                   | 0.072                        | 1                            | 32           | No                                            |                             |
| GO:0014020       | primary neural tube formation                             | 1.81                                 | 1.16                                   | 0.070                        | 0.95                         | 34           | No                                            | tube formation               |
| GO:1900744       | regulation of p38MAPK cascade                             | 1.80                                 | 1.19                                   | 0.073                        | 0.96                         | 28           | No                                            |                             |
| GO:1903900       | regulation of viral life cycle                            | 1.78                                 | 2.12                                   | 0.085                        | 0.00090                      | 125          | No                                            | negative regulation of viral replication |
## Gene Ontology

| Gene Ontology ID | Gene Ontology Biological Process                                           | Normalized enrichment score CDM3vsM0 | Normalized enrichment score CDM3vsCDM0 | FDR-adjusted p-value CDM3vsM0 | FDR-adjusted p-value CDM3vsCDM0 | Geneset size | GO term identified in small intestinal biopsy GSEA | Cluster name                        |
|------------------|---------------------------------------------------------------------------|--------------------------------------|----------------------------------------|------------------------------|------------------------------|--------------|-------------------------------------------------|-------------------------------------|
| GO:0043901       | negative regulation of multi-organism process                             | 1.77                                 | 2.20                                   | 0.090                        | 0.00010                     | 136          | No                                              | negative regulation of viral replikation |
| GO:0045824       | negative regulation of innate immune response                             | 1.76                                 | 1.95                                   | 0.10                         | 0.014                        | 35           | No                                              | negative regulation of viral replikation |
| GO:0035456       | response to interferon-beta                                               | 1.74                                 | 2.07                                   | 0.11                         | 0.0021                       | 19           | No                                              | negative regulation of viral replikation |
| GO:0045069       | regulation of viral genome replication                                    | 1.72                                 | 2.08                                   | 0.13                         | 0.0016                       | 78           | No                                              | negative regulation of viral replikation |
| GO:0050792       | regulation of viral process                                                | 1.66                                 | 2.06                                   | 0.21                         | 0.0024                       | 162          | No                                              | negative regulation of viral replikation |
| GO:0032479       | regulation of type I interferon production                                | 1.64                                 | 1.91                                   | 0.24                         | 0.021                        | 98           | No                                              | regulation of type I interferon production |
| GO:0043903       | regulation of symbiosis, encompassing mutualism through parasitism        | 1.59                                 | 2.00                                   | 0.34                         | 0.0063                       | 176          | No                                              | negative regulation of viral replikation |
| GO:0015718       | monocarboxylic acid transport                                             | 1.59                                 | 2.00                                   | 0.34                         | 0.0062                       | 83           | No                                              | response to interferon-gamma           |
| GO:0034341       | response to interferon-gamma                                              | 1.54                                 | 2.10                                   | 0.39                         | 0.0013                       | 136          | No                                              | response to interferon-gamma           |
| GO:0060333       | interferon-gamma-mediated signaling pathway                                | 1.50                                 | 2.22                                   | 0.45                         | 0.00010                      | 69           | No                                              | response to interferon-gamma           |
| GO:0071346       | cellular response to interferon-gamma                                      | 1.49                                 | 2.06                                   | 0.47                         | 0.0023                       | 120          | No                                              | response to interferon-gamma           |
| GO:0032727       | positive regulation of interferon-alpha production                         | 1.41                                 | 1.87                                   | 0.61                         | 0.036                        | 19           | No                                              | regulation of type I interferon production |
| GO:0032481       | positive regulation of type I interferon production                        | 1.32                                 | 1.89                                   | 0.67                         | 0.027                        | 55           | No                                              | regulation of type I interferon production |
| Gene Ontology ID | Gene Ontology Biological Process | Normalized enrichment score CDM3vsM0 | Normalized enrichment score CDM3vsCDM0 | FDR-adjusted p-value CDM3vsM0 | FDR-adjusted p-value CDM3vsCDM0 | Geneset size | GO term identified in small intestinal biopsy GSEA | Cluster name |
|-----------------|---------------------------------|--------------------------------------|--------------------------------------|-------------------------------|-------------------------------|-------------|-------------------------------------------------|--------------|
| GO:0097696      | STAT cascade                    | 1.32                                 | 1.90                                 | 0.68                          | 0.022                         | 35          | No                                              | JAK-STAT cascade |
| GO:0007259      | JAK-STAT cascade                | 1.32                                 | 1.91                                 | 0.67                          | 0.022                         | 35          | No                                              | JAK-STAT cascade |
| GO:0045047      | protein targeting to ER         | -1.32                                | -2.00                                | 0.56                          | 0.037                         | 98          | No                                              | protein targeting to membrane/ER |
| GO:0006614      | SRP-dependent cotranslational protein targeting to membrane | -1.36                                | -2.00                                | 0.55                          | 0.042                         | 89          | No                                              | protein targeting to membrane/ER |
| GO:0072599      | establishment of protein localization to endoplasmic reticulum | -1.37                                | -1.95                                | 0.55                          | 0.049                         | 102         | No                                              | protein targeting to membrane/ER |
| GO:0070972      | protein localization to endoplasmic reticulum | -1.39                                | -1.93                                | 0.54                          | 0.049                         | 116         | No                                              | protein targeting to membrane/ER |
| GO:0006612      | protein targeting to membrane   | -1.40                                | -1.95                                | 0.53                          | 0.044                         | 127         | No                                              | protein targeting to membrane/ER |
| GO:0006613      | cotranslational protein targeting to membrane | -1.49                                | -2.14                                | 0.55                          | 0.0093                        | 95          | No                                              | protein targeting to membrane/ER |
| GO:0000184      | nuclear-transcribed mRNA catabolic process, nonsense-mediated decay | -1.52                                | -1.95                                | 0.54                          | 0.058                         | 112         | No                                              | protein targeting to membrane/ER |
| GO:0006413      | translational initiation        | -1.58                                | -2.01                                | 0.57                          | 0.054                         | 123         | No                                              | protein targeting to membrane/ER |
## Reactome

**Supplementary File 4** Significant (FDR-adjusted p-value < 0.075) Reactome pathways based on GSEA with addition of modified cluster names from EnrichmentMap, AutoAnnotate, and WordCloud analysis.

| Reactome ID     | Reactome                                                                 | Normalized enrichment score CDM3vsM0 | Normalized enrichment score CDM3vsCDM0 | FDR-adjusted p-value CDM3vsM0 | FDR-adjusted p-value CDM3vsCDM0 | Geneset size | Reactome pathway identified in small intestinal biopsy GSEA | Cluster name          |
|-----------------|--------------------------------------------------------------------------|--------------------------------------|----------------------------------------|-------------------------------|-------------------------------|--------------|-------------------------------------------------------------|-----------------------|
| R-HSA-909733    | Interferon alpha beta signaling                                         | 2.18                                 | 2.45                                   | 0.00030                       | 0                             | 66           | Yes                                                         | interferon signaling  |
| R-HSA-201722.4  | Formation of the beta-catenin:TCF transactivating complex                | 1.93                                 | 1.15                                   | 0.029                         | 1                             | 91           | Yes                                                         | interferon signaling  |
| R-HSA-913531    | Interferon Signaling                                                    | 1.62                                 | 2.27                                   | 0.33                          | 0                             | 191          | Yes                                                         | interferon signaling  |
| R-HSA-877300    | Interferon gamma signaling                                              | 1.42                                 | 2.12                                   | 0.61                          | 0.00070                       | 90           | Yes                                                         | interferon signaling  |
| R-HSA-977444.1  | GABA B receptor activation                                              | 1.40                                 | 2.03                                   | 0.66                          | 0.0030                        | 39           | No                                                          | activation of GABA B receptors |
| R-HSA-991365.1  | Activation of GABAB receptors                                            | 1.39                                 | 2.03                                   | 0.66                          | 0.0037                        | 39           | No                                                          | activation of GABA B receptors |
| R-HSA-977443.1  | GABA receptor activation                                                | 1.33                                 | 1.89                                   | 0.66                          | 0.026                         | 55           | No                                                          | activation of GABA B receptors |
| R-HSA-380108    | Chemokine receptors bind chemokines                                     | 0.90                                 | -1.77                                  | 0.79                          | 0.042                         | 48           | Yes                                                         | translational processes |
| R-HSA-8868773.2 | rRNA processing in the nucleus and cytosol                              | -0.98                                | -1.70                                  | 0.69                          | 0.075                         | 193          | No                                                          | translational processes |
| R-HSA-6791226.3 | Major pathway of rRNA processing in the nucleolus and cytosol           | -1.00                                | -1.72                                  | 0.65                          | 0.066                         | 183          | No                                                          | translational processes |
| R-HSA-72695     | Formation of the ternary complex, and subsequently, the 43S complex     | -1.21                                | -1.87                                  | 0.39                          | 0.015                         | 52           | No                                                          | translational processes |
| R-HSA-72662     | Activation of the mRNA upon binding of the cap-binding complex and eIFs, and subsequent binding to 43S | -1.89                                | 0.36                                   | 0.013                         | 60                             | No           | No                                                          | translational processes |
| Reactome ID       | Reactome                                                | Normalized enrichment score | FDR-adjusted p-value | Geneset size | Reactome pathway identified in small intestinal biopsy GSEA | Cluster name          |
|------------------|---------------------------------------------------------|-----------------------------|-----------------------|--------------|------------------------------------------------------------|----------------------|
| R-HSA-5223345.4  | Miscellaneous transport and binding events             | -1.24                       | 0.36                  | 24           | No                                                         | translational processes |
| R-HSA-168273     | Influenza Viral RNA Transcription and Replication      | -1.25                       | 0.36                  | 131          | No                                                         | translational processes |
| R-HSA-72649.3    | Translation initiation complex formation               | -1.31                       | 0.31                  | 59           | No                                                         | translational processes |
| R-HSA-2408557    | Selenocysteine synthesis                              | -1.35                       | 0.29                  | 93           | No                                                         | translational processes |
| R-HSA-2408522.2  | Selenoamino acid metabolism                           | -1.35                       | 0.29                  | 116          | No                                                         | translational processes |
| R-HSA-901042.2   | Calnexin calreticulin cycle                           | -1.36                       | 0.29                  | 26           | No                                                         | translational processes |
| R-HSA-72702.3    | Ribosomal scanning and start codon recognition        | -1.37                       | 0.28                  | 59           | No                                                         | translational processes |
| R-HSA-168255.3   | Influenza Life Cycle                                  | -1.37                       | 0.27                  | 139          | No                                                         | translational processes |
| R-HSA-140877.2   | Formation of Fibrin Clot (Clotting Cascade)           | -1.41                       | 0.25                  | 39           | No                                                         | translational processes |
| R-HSA-156842.2   | Eukaryotic Translation Elongation                      | -1.41                       | 0.25                  | 94           | No                                                         | translational processes |
| R-HSA-156902     | Peptide chain elongation                              | -1.44                       | 0.23                  | 90           | No                                                         | translational processes |
| R-HSA-2559585    | Oncogene Induced Senescence                           | -1.45                       | 0.24                  | 33           | No                                                         | translational processes |
| R-HSA-192823.3   | Viral mRNA Translation                                | -1.46                       | 0.24                  | 90           | No                                                         | translational processes |
| Reactome ID   | Reactome                                                                 | Normalized enrichment score CDM3vsM0 | Normalized enrichment score CDM3vsCDM0 | FDR-adjusted p-value CDM3vsM0 | FDR-adjusted p-value CDM3vsCDM0 | Geneset size | Reactome pathway identified in small intestinal biopsy GSEA | Cluster name          |
|--------------|---------------------------------------------------------------------------|--------------------------------------|---------------------------------------|--------------------------------|--------------------------------|--------------|------------------------------------------------|-----------------------|
| R-HSA-72764  | Eukaryotic Translation Termination                                         | -1.46                                | -2.06                                 | 0.24                          | 0.0017                        | 94           | No                                            | translational processes |
| R-HSA-975957 | Nonsense Mediated Decay (NMD) enhanced by the Exon Junction Complex (EJC) | -1.50                                | -1.99                                 | 0.21                          | 0.0038                        | 116          | No                                            | translational processes |
| R-HSA-72689.2| Formation of a pool of free 40S subunits                                  | -1.50                                | -2.21                                 | 0.21                          | 0.00080                       | 102          | No                                            | translational processes |
| R-HSA-927802.2| Nonsense-Mediated Decay (NMD)                                              | -1.51                                | -2.00                                 | 0.22                          | 0.0037                        | 116          | No                                            | translational processes |
| R-HSA-1799339.1| SRP-dependent cotranslational protein targeting to membrane              | -1.53                                | -2.14                                 | 0.21                          | 0.00050                       | 113          | No                                            | translational processes |
| R-HSA-975956 | Nonsense Mediated Decay (NMD) independent of the Exon Junction Complex (EJC) | -1.56                                | -2.03                                 | 0.20                          | 0.0022                        | 96           | No                                            | translational processes |
| R-HSA-72613  | Eukaryotic Translation Initiation                                          | -1.59                                | -2.16                                 | 0.18                          | 0.00040                       | 120          | No                                            | translational processes |
| R-HSA-72737  | Cap-dependent Translation Initiation                                       | -1.59                                | -2.17                                 | 0.18                          | 0.00050                       | 120          | No                                            | translational processes |
| R-HSA-156827 | L13a-mediated translational silencing of Ceruloplasmin expression         | -1.61                                | -2.21                                 | 0.17                          | 0.00030                       | 112          | No                                            | translational processes |
| R-HSA-72706.2| GTP hydrolysis and joining of the 60S ribosomal subunit                    | -1.63                                | -2.21                                 | 0.18                          | 0.00040                       | 113          | No                                            | translational processes |
| R-HSA-202733.3| Cell surface interactions at the vascular wall                           | -1.66                                | -1.96                                 | 0.17                          | 0.0059                        | 140          | No                                            |                      |
| R-HSA-1236394| Signaling by ERBB4                                                       | -1.90                                | -1.70                                 | 0.042                         | 0.072                         | 41           | No                                            |                      |
| R-HSA-5654733| Negative regulation of FGFR4 signaling                                    | -2.03                                | -1.62                                 | 0.0090                        | 0.12                          | 27           | No                                            | FGFR signaling         |
| Reactome ID | Reactome                      | Normalized enrichment score CDM3vsM0 | Normalized enrichment score CDM3vsCDM0 | FDR-adjusted p-value CDM3vsM0 | FDR-adjusted p-value CDM3vsCDM0 | Geneset size | Reactome pathway identified in small intestinal biopsy GSEA | Cluster name |
|------------|-------------------------------|--------------------------------------|----------------------------------------|------------------------------|------------------------------|--------------|----------------------------------------------------------|--------------|
| R-HSA-5654726 | Negative regulation of FGFR1 signaling | -2.03                                | -1.63                                  | 0.010                        | 0.12                        | 26           | No                                                       | FGFR signaling |
| R-HSA-5654727 | Negative regulation of FGFR2 signaling | -2.04                                | -1.63                                  | 0.011                        | 0.11                        | 28           | No                                                       | FGFR signaling |
| R-HSA-5654732 | Negative regulation of FGFR3 signaling | -2.05                                | -1.63                                  | 0.012                        | 0.12                        | 23           | No                                                       | FGFR signaling |
| R-HSA-5654736 | Signaling by FGFR1            | -2.05                                | -1.64                                  | 0.016                        | 0.11                        | 42           | No                                                       | FGFR signaling |
| R-HSA-5654741.2 | Signaling by FGFR3            | -2.05                                | -1.50                                  | 0.023                        | 0.23                        | 33           | No                                                       | FGFR signaling |
| R-HSA-5654743 | Signaling by FGFR4            | -2.08                                | -1.68                                  | 0.029                        | 0.085                       | 36           | No                                                       | FGFR signaling |
**Supplementary File 5** Results from deconvolution, presented as means per group. Differences in cell ratios between groups were assessed using Kruskal-Wallis one-way analysis of variance by ranks. For cell types where more than 50% of the individuals in every group had a zero result, no results are presented.

|            | monocyte | CD14 positive monocyte | natural killer cell | CD56bright natural killer cell | granulocyte | neutrophil | T cell | CD4 positive alpha beta T cell | gamma delta T cell | macrophage m0 | B cell | memory B cell | naive B cell |
|------------|----------|------------------------|---------------------|--------------------------------|--------------|------------|--------|-------------------------------|-------------------|---------------|--------|--------------|-------------|
| M0         | 0.20     | 0.20                   | 0.21                | 0.21                           | 0.20         | 0.19       | 0.18   | 0.15                          | 0.030             | 0.11          | 0.093  | 0.083        | 0.0069      |
| CDM0       | 0.19     | 0.19                   | 0.20                | 0.20                           | 0.18         | 0.18       | 0.20   | 0.17                          | 0.034             | 0.14          | 0.090  | 0.078        | 0.011       |
| CDM3       | 0.19     | 0.19                   | 0.21                | 0.21                           | 0.19         | 0.18       | 0.20   | 0.17                          | 0.023             | 0.12          | 0.077  | 0.072        | 0.0047      |
| p-value    | 0.85     | 0.85                   | 0.79                | 0.79                           | 0.95         | 0.99       | 0.17   | 0.18                          | 0.57              | 0.45          | 0.62   | 0.75         | 0.97        |