

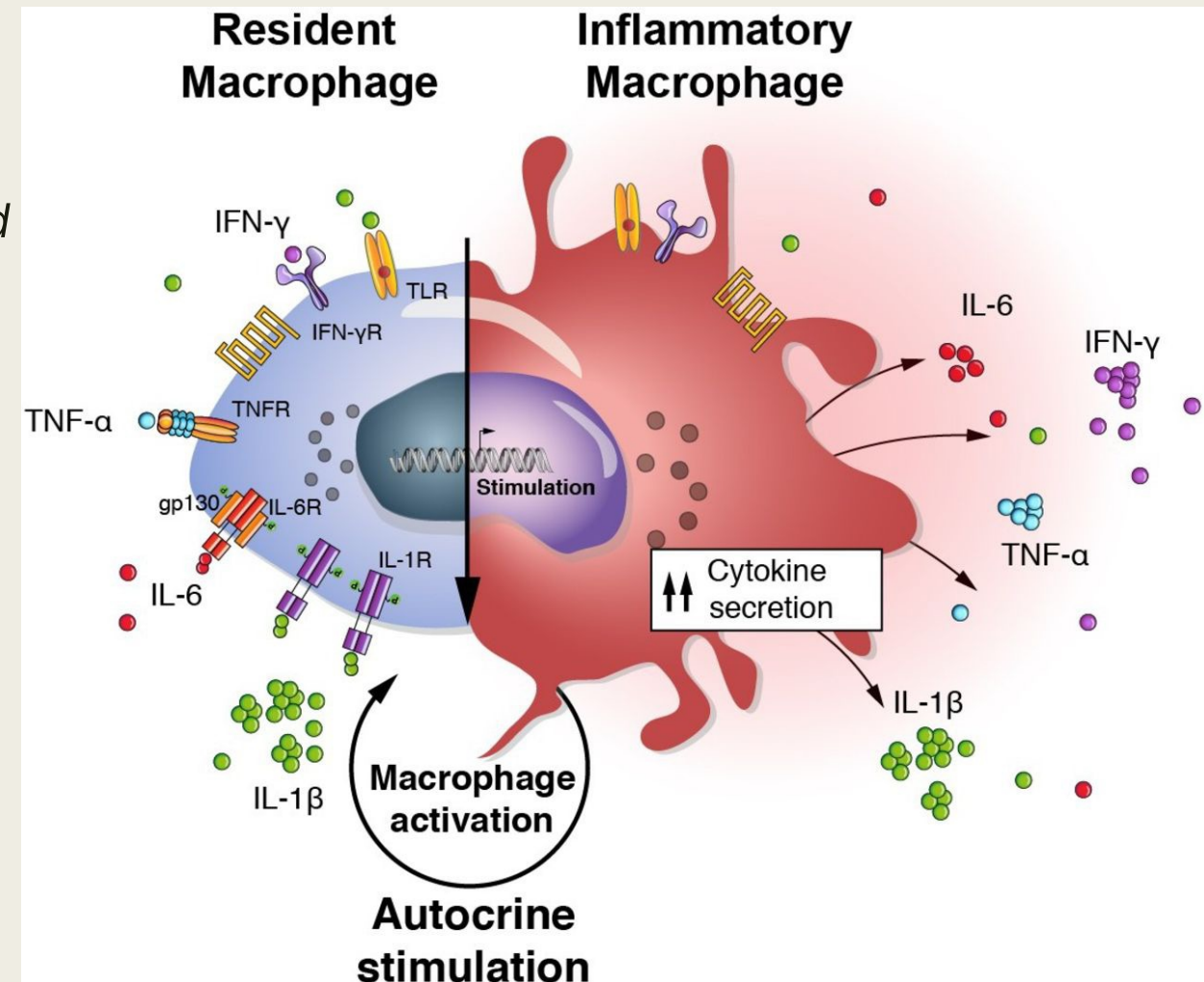
# DIFFERENTIAL GENE EXPRESSION IN HUMAN MACROPHAGES AFTER THE SARS-COV-2 VACCINE

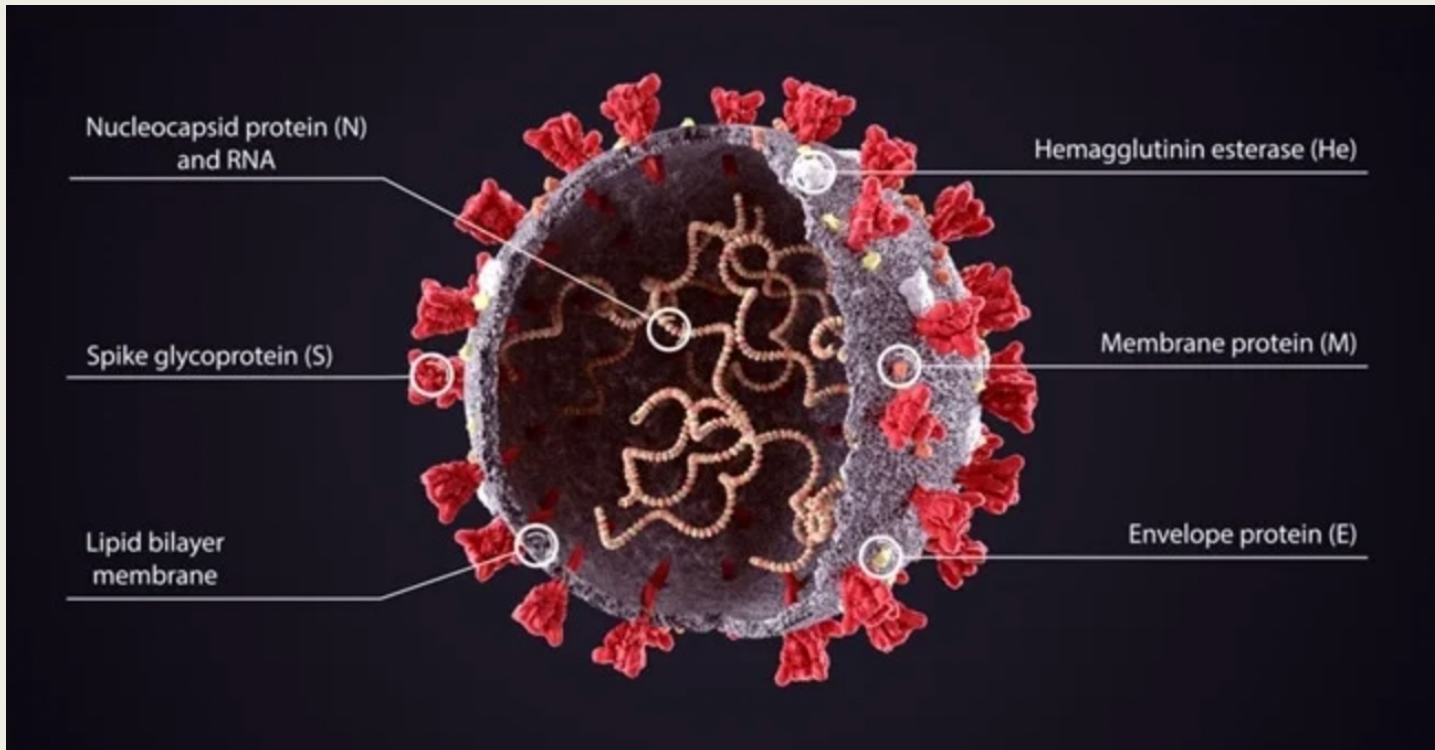
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# Macrophages & SARS-CoV-2

- Macrophages are large phagocytic cell
  - Assist with detection, phagocytosis and removal of dead cells
  - Release antigens
- Spike protein: large type I transmembrane fusion protein important in penetrating host cells
- Lipopolysaccharide (LPS): outer membrane component of Gram negative bacteria and activator of macrophages





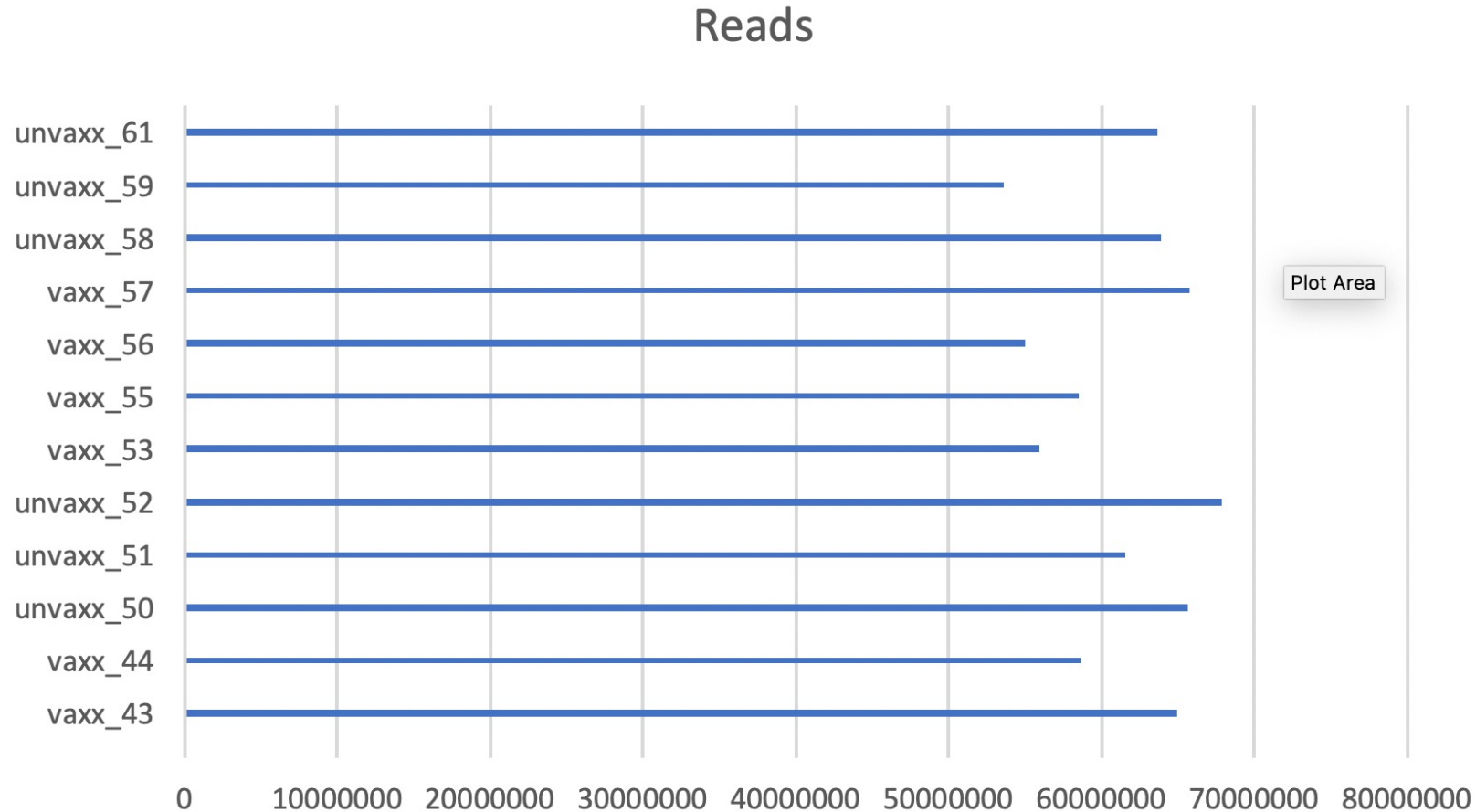
## Macrophages & SARS-CoV-2

- Previously shown that spike proteins release interleukin-1 $\beta$  in macrophages from COVID-19 patients
- SARS-CoV-2 infection causes reprogramming of macrophages that affects the SARS-CoV-2 spike protein's immunogenicity
- Due to these findings, transcriptome analysis of macrophages may show different innate immune responses between vaccinated and unvaccinated patients

# Sequencing Data

- Source: CECAD Cluster of Excellence at the University of Cologne
- Details:
  - *Downloaded from GEO (Series GSE200274)*
  - *Paired end*
  - *Data was provided as multiple SRR files that needed to be concatenated*
  - *There were 3 conditions (macrophages stimulated with S-protein, LPS, or left alone) and 12 individuals (6 vaccinated and 6 unvaccinated)*

# Pre-Alignment Sequencing Metrics

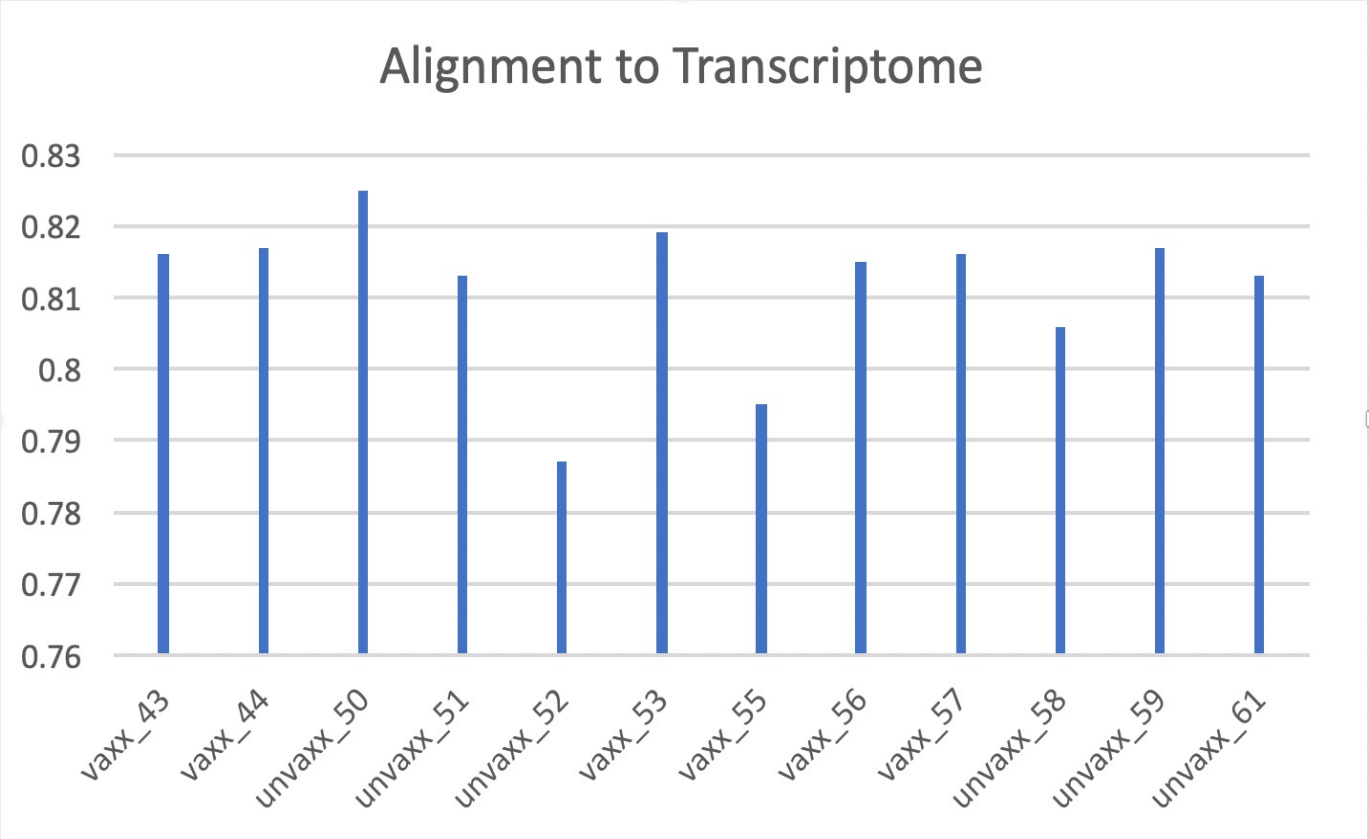


Mean: 61,260,969 Minimum: 53,609,551 Maximum: 67,855,008

# Alignment Process

1. SRA toolkit: Fasterq-dump to download files
2. Concatenate
3. <ftp.ensembl.org/pub>: Download FASTA (reference genome) and GTF files
4. Bowtie2: build index files
5. Tophat2:
  1. *Used one sample from each group (vaccinated and unvaccinated) to align to both the genome and transcriptome*
  2. *Output files: bam and statistics files*
6. Aligned remaining samples to the transcriptome using Tophat2
7. Cuffdiff
  1. *2 runs: statistics on and statistics off*
  2. *Input: BAM files created by running Tophat2*
  3. *Output:*
    1. *Statistics on: gene\_exp.diff (p-value)*
    2. *Statistics off: genes.fpkm\_tracking (FPKM values)*

MEAN: 81.15%  
MINIMUM: 78.70%  
MAXIMUM: 81.90%

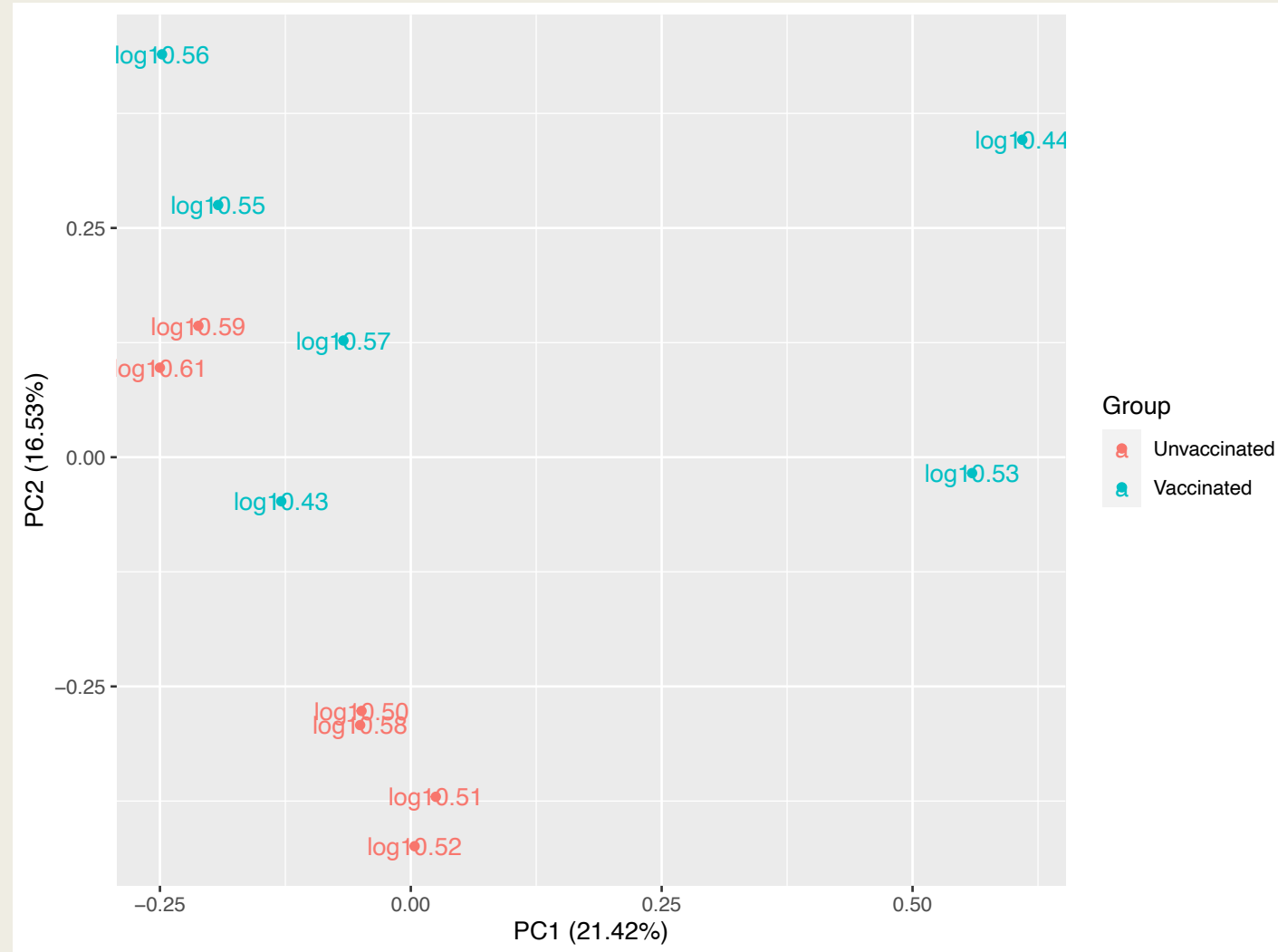


Sample	% Alignment	# of Aligned Reads
vaxx_43	81.60%	26510870
vaxx_44	81.70%	23968868
unvaxx_50	82.50%	27069622
unvaxx_51	81.30%	24998770
unvaxx_52	78.70%	26713616
vaxx_53	81.90%	22889427
vaxx_55	79.50%	23279881
vaxx_56	81.50%	22424077
vaxx_57	81.60%	26867276
unvaxx_58	80.60%	25739889
unvaxx_59	81.70%	21905165
unvaxx_61	81.30%	25889199

MEAN: 22942820  
MINIMUM: 21905165  
MAXIMUM: 26867276

# PCA Plot using FPKM values of all genes

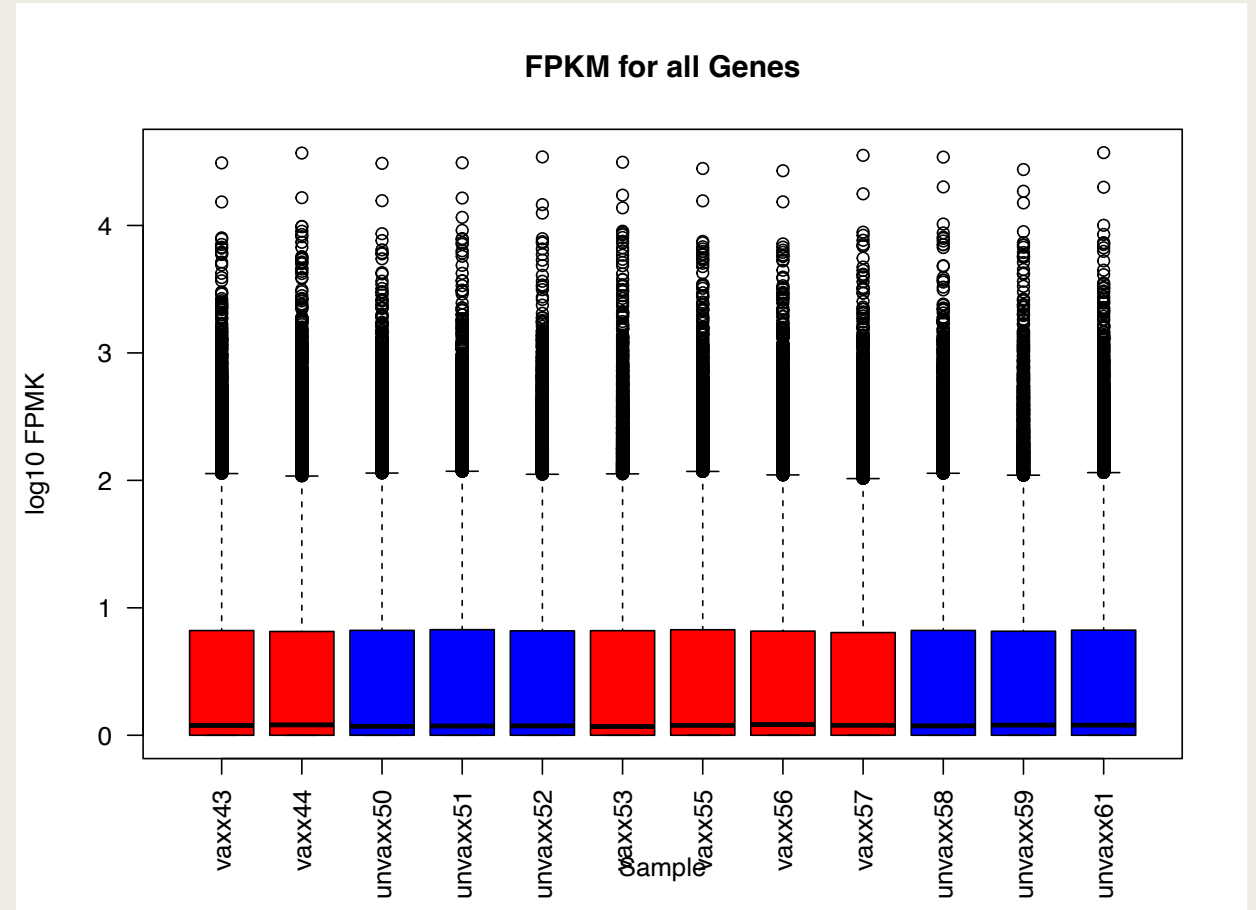
- Includes all genes except for the ones with a sum FPKM value of 0
  - Started with ~62000 genes
  - ~25000 genes had FPKM value of 0
  - Included 35760 genes
    - Took log10 and added pseudocount of 1





# Boxplot using FPKM values of all genes

- 35760 genes
- Distribution is consistent between both groups

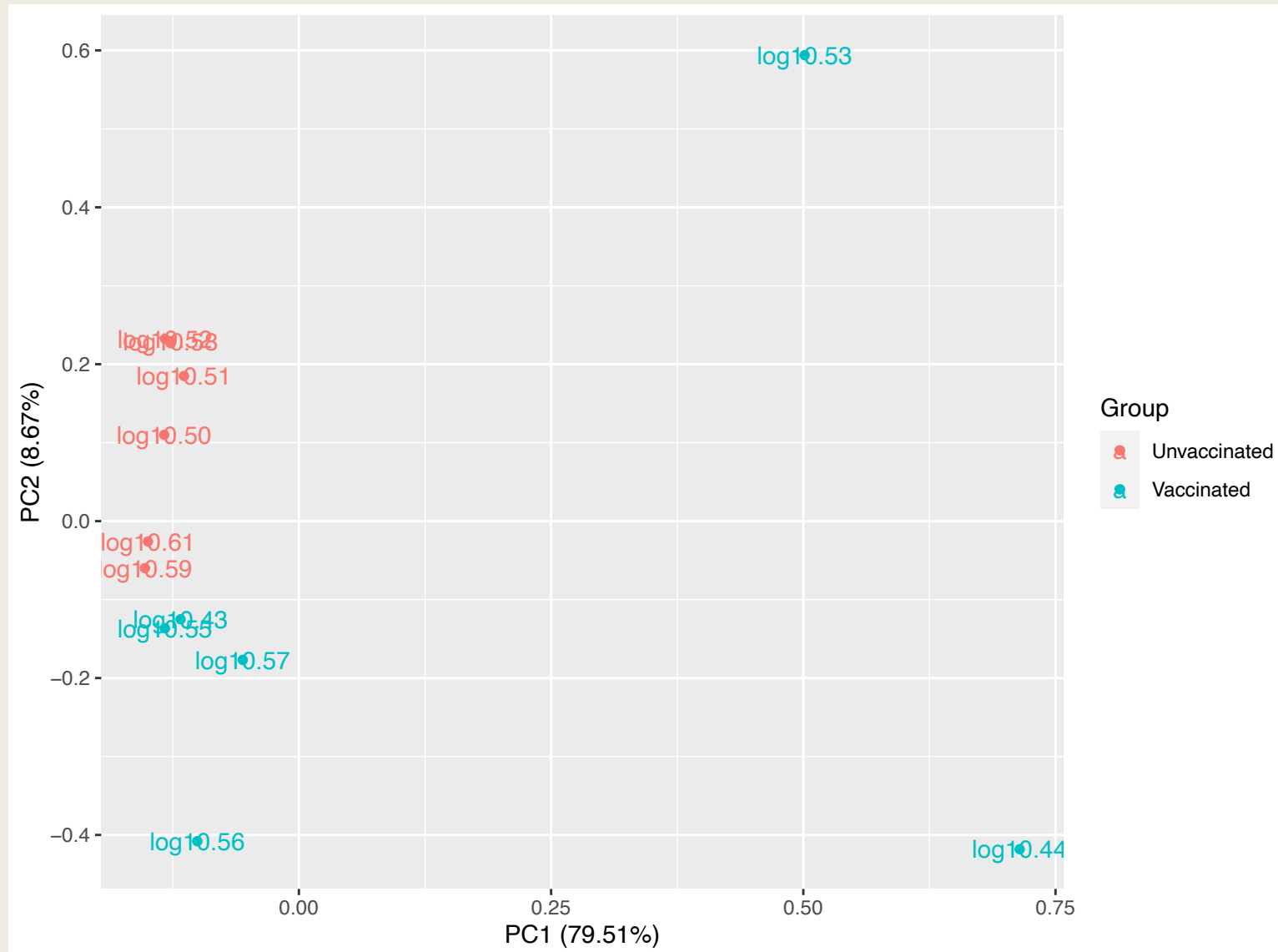


# Next plots: Used filtered genes

- For the next PCA plot, dendrogram, and heatmap:
  - *Filtered genes:*
    - Removed all genes with a sum FPKM value of 0
    - Filters:
      - *Log2*
        - Less than or equal to -2 and greater than or equal to 2
      - *P-value*
        - Less than 0.01
  - *Results:*
    - 207 genes
      - 187 upregulated genes
      - 20 downregulated genes

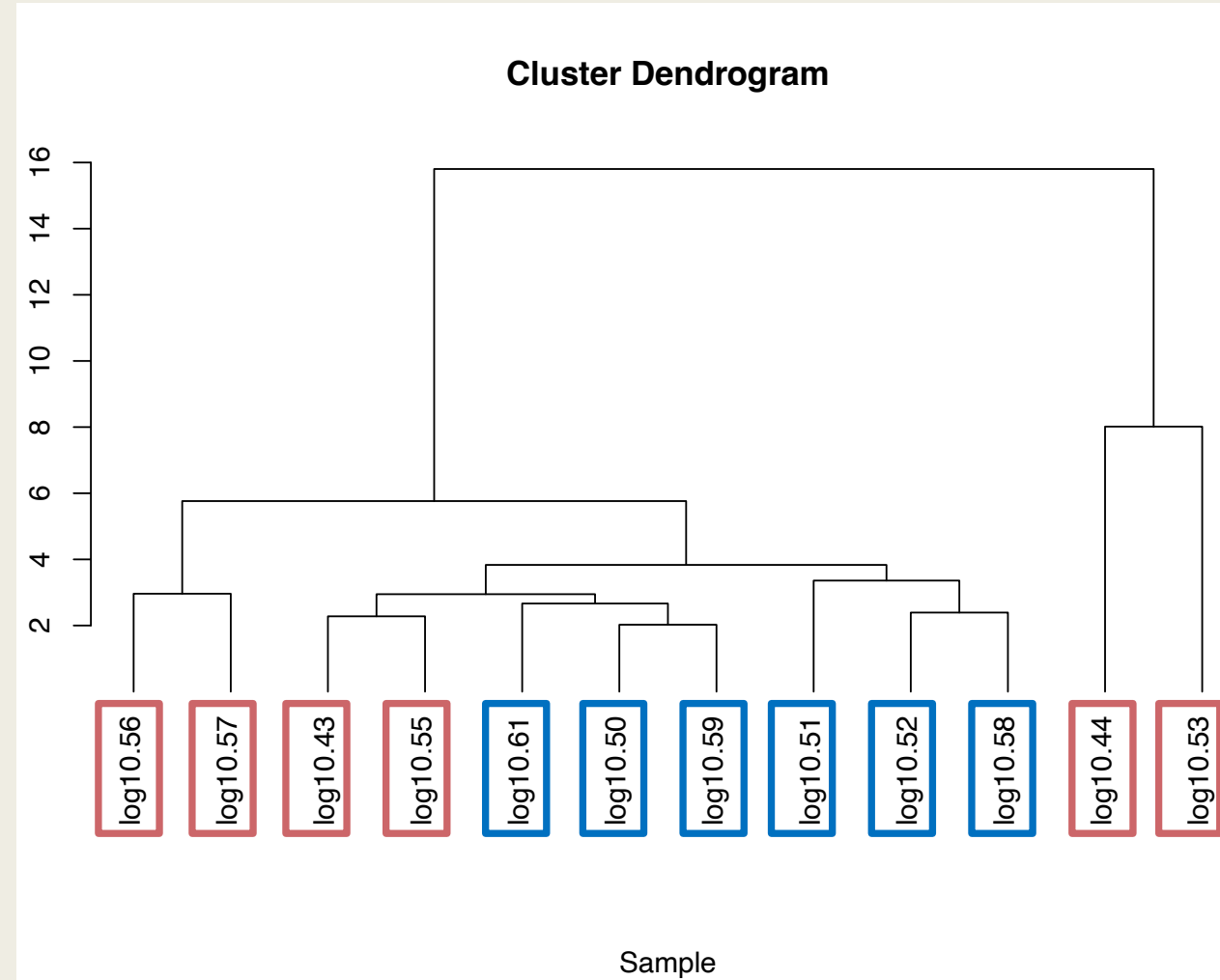
# PCA Plot using FPKM values of filtered genes

- 207 filtered genes
  - Took  $\log_{10}$  and added psuedocount of 1
- Unvaccinated clustered together
- Some of the vaccinated clustered together



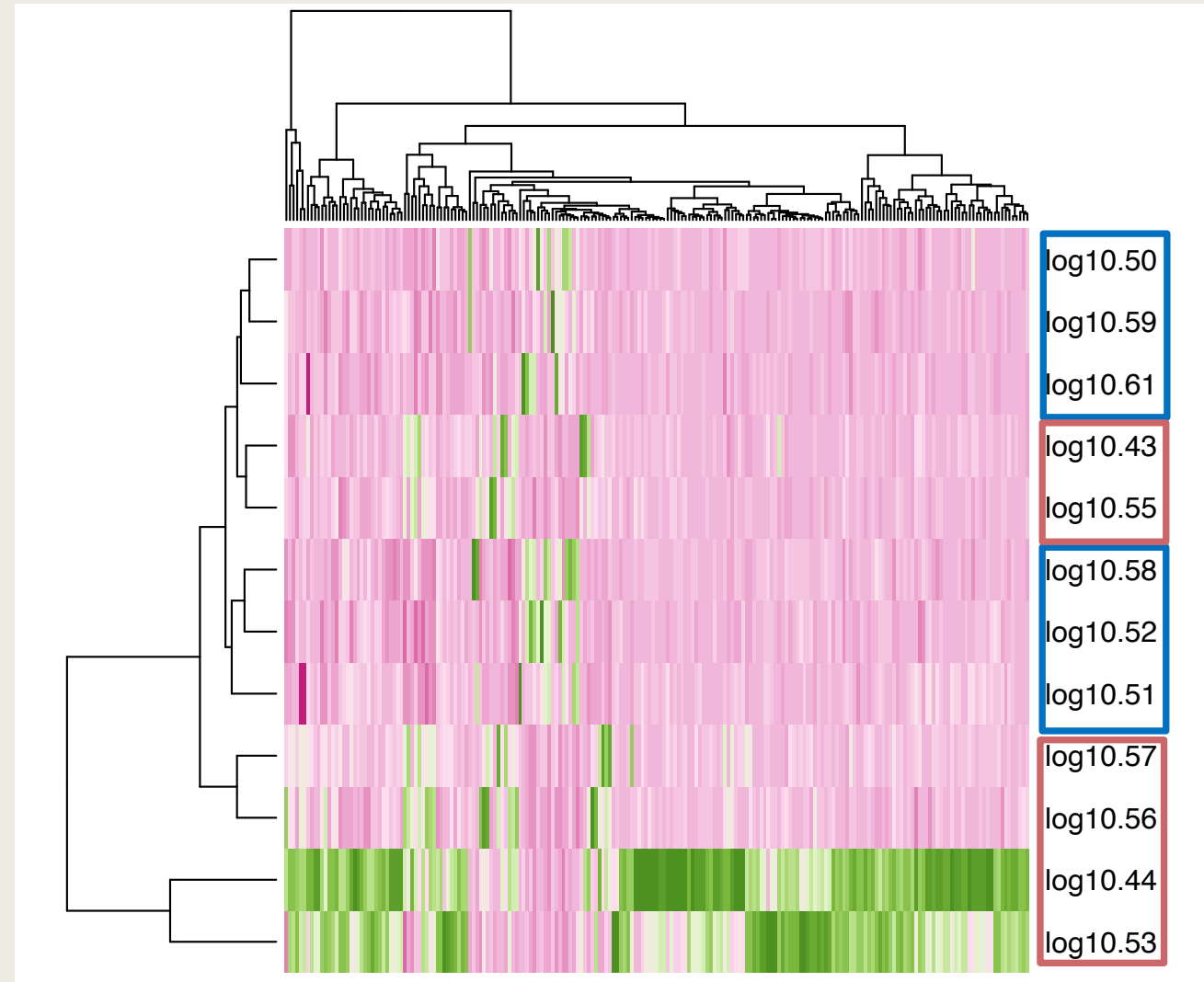
# Dendrogram using FPKM values of filtered genes

- 207 filtered genes
- Red are vaccinated individuals
- Blue are unvaccinated individuals
- Separates the 2 vaccinated individuals



# Heatmap using FPKM values of filtered genes

- 207 filtered genes
- Red are vaccinated individuals
- Blue are unvaccinated individuals
- Expression is highest in the same 2 samples
- Clustering is consistent with the clustering on the dendrogram



# Pathway Analysis

Downregulated- 20 genes

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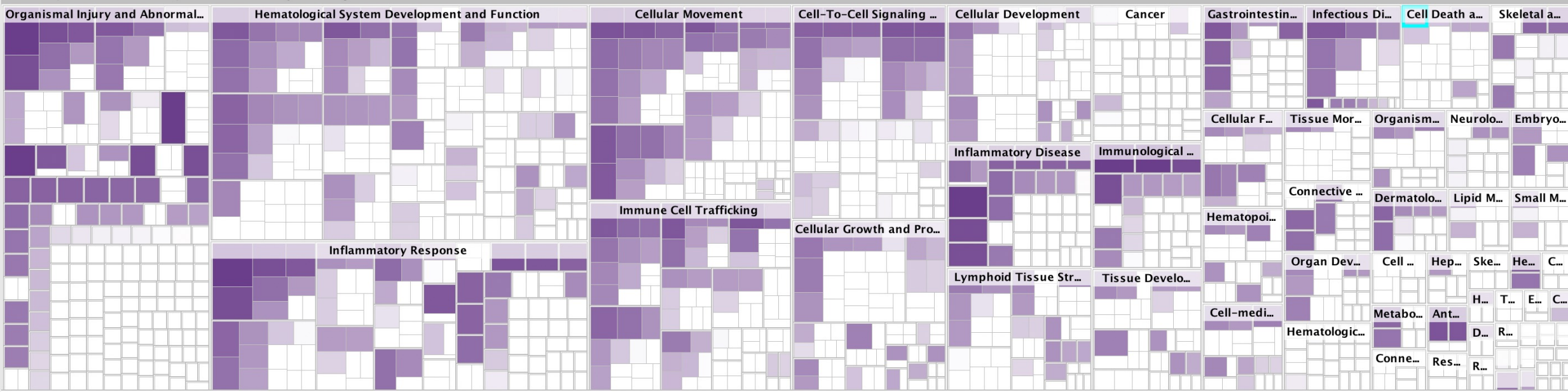
Categories	Diseases or Functions Annotation	p-value	Molecules	# Molecules
Lipid Metabolism,Molecular Transport,Nu	Quantity of 3-hydroxy-3-methylglutaryl-	5.10E-04	KLB	1
Dental Disease,Dermatological Diseases a	Growth retardation, alopecia, pseudoan	5.10E-04	ANTXR1	1
Cellular Assembly and Organization,Cellul	Receptor-mediated endocytosis of lipos	5.10E-04	ANTXR1	1
Cellular Function and Maintenance	Receptor-mediated endocytosis by gona	5.10E-04	ANTXR1	1
Connective Tissue Disorders,Developmen	Multiple epiphyseal dysplasia with myop	5.10E-04	COL9A3	1
Connective Tissue Disorders,Developmen	Holoprosencephaly-7	5.10E-04	PTCH1	1
Cell Cycle,Embryonic Development	G1 phase of neural tube cells	5.10E-04	PTCH1	1
Cancer,Connective Tissue Disorders,Dent	Keratocystic odontogenic tumor in man	5.10E-04	PTCH1	1
Connective Tissue Disorders,Developmen	Ullrich congenital muscular dystrophy	9.32E-04	COL9A3,KLB	2
Cancer,Organismal Injury and Abnormalit	Rhabdomyosarcoma in leg	1.02E-03	PTCH1	1
Cancer,Neurological Disease,Organismal	Development of medulloblastoma SHH	1.02E-03	PTCH1	1
Cancer,Gastrointestinal Disease,Organism	Sarcoma in intestine	1.02E-03	PTCH1	1
Cancer,Developmental Disorder,Embryon	Hyperplasia of granule neuron progenit	1.02E-03	PTCH1	1
Cell Death and Survival,Embryonic Develo	Survival of neural tube cells	1.02E-03	PTCH1	1
Embryonic Development,Nervous System	Area of external granular layer	1.02E-03	PTCH1	1
Cancer,Gastrointestinal Disease,Organism	Rhabdomyosarcoma in tongue	1.02E-03	PTCH1	1
Digestive System Development and Funct	Abnormal morphology of small gallblad	1.02E-03	KLB	1

Upregulated- 187 genes

Categories	Diseases or Functions Annotation	p-value	Molecules	# Molecules
Immunological Disease,Inflammatory	Immune mediated inflammatory disease	8.97E-37	ACHE,AIM2,APOBEC3A,AREG,BCL2L14,CCL1,CC	79
Inflammatory Response,Organismal In	Inflammation of organ	4.08E-36	ACOD1,AIM2,APOBEC3A,AREG,BCL2L14,CCL20,	82
Immunological Disease,Organismal Inj	Systemic autoimmune syndrome	6.75E-33	ACHE,AIM2,APOBEC3A,AREG,BCL2L14,CCL1,CC	74
Immunological Disease,Inflammatory	Lupus erythematosus	8.73E-28	AIM2,APOBEC3A,BCL2L14,CD80,CFB,CMKP2,DI	42
Inflammatory Response,Organismal In	Inflammation of body cavity	2.17E-27	ACOD1,AIM2,AREG,CCL20,CCL3,CCL3L3,CCL4,C	62
Inflammatory Response,Organismal In	Inflammation of absolute anatomical region	2.72E-27	ACOD1,AIM2,AREG,CCL20,CCL3,CCL3L3,CCL4,C	67
Immunological Disease,Inflammatory	Systemic lupus erythematosus	3.35E-27	AIM2,APOBEC3A,BCL2L14,CD80,CFB,CMKP2,DI	41
Cellular Movement	Cell movement of blood cells	2.03E-26	AIM2,AREG,C1QTNF1,CCL1,CCL20,CCL23,CCL3,	57
Antimicrobial Response,Inflammatory	Antiviral response	3.11E-26	ACOD1,AIM2,APOBEC3A,CCL4,CCL5,CCL8,CCR7	30
Cellular Movement,Immune Cell Traffi	Leukocyte migration	9.35E-26	AIM2,AREG,C1QTNF1,CCL1,CCL20,CCL23,CCL3,	56
Cell-To-Cell Signaling and Interaction,C	Recruitment of leukocytes	1.88E-25	CCL1,CCL20,CCL23,CCL3,CCL3L3,CCL4,CCL5,CCR	35
Antimicrobial Response,Inflammatory	Antimicrobial response	2.02E-25	ACOD1,AIM2,APOBEC3A,CCL20,CCL3,CCL4,CCL	35
Gastrointestinal Disease,Inflammatory	Inflammation of gastrointestinal tract	2.95E-25	ACOD1,APOBEC3A,AREG,CCL3L3,CCL5,CCR7,CD	44
Inflammatory Response	Inflammatory response	2.95E-25	ACHE,ACOD1,APOL3,CCL1,CCL20,CCL23,CCL3,C	52
Cellular Movement,Hematological Sys	Cell movement of leukocytes	3.09E-25	AIM2,AREG,C1QTNF1,CCL1,CCL20,CCL23,CCL3,	53
Infectious Diseases,Organismal Injury	COVID-19	3.97E-25	APOL4,CCL20,CCL3,CCL4,CCL5,CCL8,CFB,CXCL1,	39
Infectious Diseases,Organismal Injury	Infection by coronavirus	4.67E-25	APOL4,CCL20,CCL3,CCL4,CCL5,CCL8,CFB,CXCL1,	40
Cell-To-Cell Signaling and Interaction,C	Recruitment of cells	6.04E-25	CCL1,CCL20,CCL23,CCL3,CCL3L3,CCL4,CCL5,CCR	36
Gastrointestinal Disease,Inflammatory	Enteritis	2.31E-23	ACOD1,AREG,CCL3L3,CCL5,CCR7,CD274,CD80,C	40
Gastrointestinal Disease,Inflammatory	Inflammation of the large intestine	2.36E-23	ACOD1,AREG,CCL3L3,CCL5,CD274,CD80,CFB,CK	39
Cell-To-Cell Signaling and Interaction	Activation of cells	2.93E-23	ACHE,AIM2,AREG,C1QTNF1,CCL1,CCL20,CCL23,	55
Cell-To-Cell Signaling and Interaction,C	Activation of blood cells	4.81E-23	ACHE,AIM2,C1QTNF1,CCL1,CCL20,CCL23,CCL3,	48
Gastrointestinal Disease,Inflammatory	Colitis	5.03E-23	ACOD1,AREG,CCL3L3,CCL5,CD274,CD80,CFB,CK	38

Click squares below to explore

Currently Viewing: All



# Pathway Analysis: Insulin-dependent diabetes mellitus

- 24 upregulated genes:
  - AIM2,CCL3L3,CCL4,CCL5,CCR7,CD274,CD80,CFB,GBP2,GBP4,IFI44,IL1A,IL1B,IL2RA,IL6,IL7R,PTGS2,SAMD9L,STAT4,TNF,TNFRSF4,TNFSF10,XAF1,ZBP1
- P-value: 2.10E-11
- Commonly known as Type-1 Diabetes
  - *Usually starts before 15 years old, but can happen in adults*

gene	p-value
CCL3L3	5.00E-05
CCL4	5.00E-05
CCL5	5.00E-05
CCR7	5.00E-05
CD274	0.00025
CD80	0.00735
CFB	0.00015
GBP2	5.00E-05
GBP4	5.00E-05
IFI44	5.00E-05
IL1A	5.00E-05
IL1B	5.00E-05
IL2RA	0.0001
IL6	5.00E-05
IL7R	5.00E-05
PTGS2	5.00E-05
SAMD9L	5.00E-05
STAT4	5.00E-05
TNF	5.00E-05
TNFRSF4	5.00E-05
TNFSF10	5.00E-05
XAF1	5.00E-05
ZBP1	0.0071
AIM2	0.0071

# Pathway Analysis: Insulin-dependent diabetes mellitus

- Study with 4 cases
  - *All 4 developed type 1 diabetes a couple of weeks after receiving the Pfizer–BioNTech (BNT162b2) vaccine*
  - *Three out of four patients did not need insulin after a few follow-ups*
- Has been suggested that SARS-CoV uses ACE-2 receptors to damage the pancreas
  - *Studies have not proven this*
- Limited number of cases where COVID-19 patients get diabetes



# CD80

- Protein coding gene
- Activated protein induces T-cell proliferation and cytokine production
- Study focused on nonobese diabetic mice and was noted that by week 6 the mice had CD80 positive cells
  - *By week 12 they are shown to surround the islet of Langerhans*
- May be involved in local regulation of anti-islet immune responses

# CD80

- Study reports that CD80 transgenic mice are more susceptible to autoantigen-induced diabetes
- Injected with syngeneic fibroblastoid cell lines loaded with gp33
- Both groups led to moderate CD4-independent CTL responses, but only CD80<sup>+</sup>GP<sup>+</sup>mice got diabetes from the gp33 peptide
- Gp33 peptide activated CD8<sup>+</sup>T cells that then led to an infiltration of islet

# Conclusion

- Using a larger study sample would help identify why some of the vaccinated grouped separately from the rest
  - *Having demographic data*
- Using a larger study sample to understand why a limited number of individuals developed diabetes after receiving the vaccine
  - *Collecting more data: demographic, clinical, and time of exposure*
- Is the Insulin-dependent diabetes mellitus pathway upregulated by other vaccines?