# Diagnostic biomarker candidates for pulpitis revealed by bioinformatics analysis of merged microarray gene expression datasets

**Ming Chen**
Southern Medical University Nanfang Hospital
**Junkai Zeng**
Southern Medical University Nanfang Hospital
**Yeqing Yang**
Southern Medical University Nanfang Hospital
**Buling Wu** ( bulingwu@smu.edu.cn )
Southern Medical University Nanfang Hospital https://orcid.org/0000-0002-9678-6962

**Research article**

**Keywords:** pulpitis, Gene Expression Omnibus (GEO), diagnostic biomarker, bioinformatics analysis

**Posted Date:** January 13th, 2020

**DOI:** https://doi.org/10.21203/rs.2.20729/v1



**Version of Record:** A version of this preprint was published on October 12th, 2020. See the published version at https://doi.org/10.1186/s12903-020-01266-5.

# Abstract

Background Pulpitis is known as an inflammatory disease classified by the level of inflammation. The existed traditional methods of evaluating status of dental pulp tissue in clinical practice still have some shortages and limitations. Immediate and accurate diagnosis of pulpitis is essential to the choice of treatment. Through integrating different datasets from Gene Expression Omnibus (GEO) database, we analyzed the merged expression matrix of pulpitis, aiming to identified biological pathways and diagnostic biomarker of pulpitis.

Methods By integrating two datasets (GSE77459 and GSE92681) in GEO database using sva and limma packages, differentially expressed genes (DEGs) of pulpitis were identified. Then DEGs were used to analyze biological pathways of dental pulp inflammation with Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis and the Gene Set Enrichment Analysis (GSEA). Protein–protein interaction (PPI) networks and modules were constructed to identify hub genes with the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) and Cytoscape.

Results A total of 472 DEGs consisting of 396 upregulated and 76 downregulated genes were found in pulpitis tissue. DEGs in GO analysis were enriched in biological processes about inflammation and in KEGG pathway analysis were cytokine-cytokine receptor interaction, chemokine signaling pathway and NF-κB signaling pathway. GSEA results provided further functional annotations including complement system, IL6/JAK/STAT3 signaling pathway and inflammatory response pathways. According to the degrees of nodes in PPI network, 10 hub genes were obtained and 8 diagnostic biomarker candidates were screened, including PTPRC, CD86, CCL2, IL6, TLR8, MMP9, CXCL8 and ICAM1.


# Background

Dental pulp is loose connective tissue in the pulp cavity, surrounded by rigid dentin and is necessary for tooth nutrition, innervation, and immunocompetency[1]. When the dental hard tissue is broken, any kind of stimuli may bring about a pathological change in dental pulp, and it’s hard to repair itself due to the lack of collateral circulation[2]. Pulpitis is an inflammatory disease of dental pulp, and bacterial infection is considered to be the most important trigger of pulpitis[3]. Whether the pathological change occur in dental pulp or not and the degree of lesions are not only related to the bacteria virulence and amount, but also to the defensive power of host[4]. Tertiary dentin is reactively formed when dental pulp is stimulated, and there is a balance between inflammation and reparative processes. If the harmful stimuli cannot be removed in time, balance of immune-inflammatory would be destructed, and thus irreversible pulpitis could occur as a result of uncontrollable inflammation caused by invading bacteria[5]. Without appropriate treatment, pulpitis may result in pulp necrosis, periapical periodontitis and even more severe cases[6].

Different therapies of pulp disease can be selected according to the evaluation of pulp inflammation. Currently, guided by the criteria of the American Association of Endodontists (AAE), medical history and

clinical examination would be the main methods to evaluate pulp inflammation severity, including pain quality and history and responses to pulp sensitivity tests[7]. Preserving or removing vital pulp depend on the judgement about reversible or irreversible pulpitis[8]. However, histopathological examinations prove the weak correlation between clinical features and pulp status[5]. The tissues collected from the case that was diagnosed as irreversible pulpitis may not represent severe inflammation in pathologic examination[9]. On the other hand, the patients that have pulp necrosis and periapical periodontitis may not complain of pain. Hence, clinical diagnosis has limitations to access the degree of pulp inflammation[8].

At the cellular or molecular level, wide ranges of molecules are released in pulpal and periapical inflammation, including cytokines, proteases, inflammatory mediators,

growth factors, antimicrobial peptides and so on[10, 11]. The factors that can used to judge the level of the pulp inflammation are called biomarkers of pulpitis[12]. These biomarkers can be not only found in pulp tissue, but also detected in saliva, gingival crevicular fluid, dentin fluid and the other body fluids that can be collected non-invasively and analyzed without extirpating the pulpal tissue[13–15]. Combining with clinical examinations, the appropriate biomarkers may help to select precise treatment for pulp disease[16].

In this study, we focused on gene expression in pulp tissue from pulpitis patients. We selected and analyzed two microarray platform datasets in the GEO database, integrated the datasets and identified DEGs between pulpitis and normal pulp tissues. Then, GO enrichment, KEGG pathway analysis and GSEA were used to analyze the major biological functions of the DEGs. Ten hub genes related to pulpitis were identified with PPI network and Cytoscape. The aim of the present study was to identify biomarker candidates for pulpitis diagnosis and prognosis based on functional and molecular analyses by evaluating DEGs in pulpitis and normal tissue.

# Method

Two microarray datasets of pulpitis from GEO database were retrieved, with the keywords: “pulpitis”, “Homo Sapiens” and “dental pulp”. GSE77459 includes 6 samples of pulpitis and 6 samples of normal pulps, and GSE92681 includes 7 samples of pulpitis and 5 samples of normal pulps, respectively using the microarray platform GPL17692 and GPL16956. The details of both studies were shown in Table 1.

Table 1

Summary of two individual datasets of pulpitis

| GEO gene set ID | GSE77459 | GSE92681 |
|---|---|---|
| Title | Gene Expression Profile of Pulpitis | Differential Expression of LncRNAs and mRNAs in normal and inflamed human pulp |
| Platform | GPL17692: Affymetrix Human Gene 2.1 ST Array [transcript (gene) version] | GPL16956: Agilent 045997 Arraystar human lncRNA microarray V3 (Probe Name Version) |
| Number of normal samples vs. pulpitis sample | 6 vs. 6 | 5 vs. 7 |
| Clinical data | | |
| normal tissues | Picked from healthy teeth extracted for various reasons | Picked from healthy teeth extracted for various reasons |
| pulpitis tissues | Picked from teeth diagnosed with irreversible pulpitis | Picked from teeth diagnosed with irreversible pulpitis |
| PubMed ID | - | 29079059 |

# Data processing

After removing the probes of lncRNA in GSE92681, mRNAs of two datasets were merged and normalized using the “sva” package in R (https://bioconductor.org/ packages/sva/). Then the raw data was converted into the form of expression matrix and handled with the Linear Models for Microarray data (limma, https://bioconductor.org/ packages/limma/) package in Bioconductor. Up- or downregulated DEGs between samples of pulpitis and normal pulps were identified with the cut-off criteria of P-value (P) < 0.05 and |fold change (FC)| > 2.

# Functional analysis of DEGs

GO enrichment and KEGG pathway analysis were used to investigate the functional progression of pulpitis. Biological process (BP), molecular function (MF), cellular component (CC) in GO analysis and potential pathways in KEGG analysis were performed in the Database for Annotation Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/). P < 0.05 and false discovery rate (FDR) < 5% were used as the cut-off criteria.

# The Gene Set Enrichment Analysis

To further explore the function of DEGs in inflammatory progression, GSEA was performed using h.all.v7.0.symbols.gmt (http://software.broadinstitute.org/gsea/ downloads.jsp) as a reference gene set[17]. The GSEA software (version 4.0) is available on the GSEA website (http://software.broadinstitute.org/gsea/index.jsp). Gene set permutations were performed 1000 times,

and the pathway set list is sorted by the Normalized Enrichment Score (NES). P < 0.05 and FDR < 0.25 were considered statistically significant.

# PPI network analysis and hub gene identification

The PPI network of DEGs was constructed using the Search Tool for THE Retrieval of Interacting Genes (STRING) database (Version 11.0, http://string-db.org/). PPI pairs and PPI network were visualized in the Cytoscape software (Version 3.7.1), and cytohHubba and MCODE plugin in Cytoscape were used to calculate the degrees of protein nodes and select the significant modules. Top 10 genes were identified as hub genes.

## Result

# Dataset integration and identification of DEGs

According to the principal component analysis (PCA), data form two samples were removed, GSM2434473 and GSM2434475 (Fig. 1). Then expression matrix of normal pulp and pulpitis was obtained, including 12813 mRNAs and 22 groups (11 normal samples and 11 pulpitis samples, Fig. 2). 472 DEGs were screened from two merged microarray platform datasets, including 396 upregulated and 76 downregulated genes, shown as the volcano plot and heatmap (Fig. 3). The details of expression matrix and DEGs were shown in Addition File 1 and Addition File 2.

# Functional analysis of DEGs

The results were based on GO BP, CC and MF and KEGG pathway analysis. For BP terms, DEGs were enriched in inflammatory response, signal transduction, immune response and cell adhesion. For CC terms, there were integral component of membrane, plasma membrane, extracellular exosome, and for MF terms, there were protein binding, calcium ion binding, protein homodimerization activity (Fig. 4, Table 2). Significantly enriched KEGG pathways of DEGs contained cytokine-cytokine receptor interaction, osteoclast differentiation, chemokine signaling pathway, NF-κB signaling pathway, T cell receptor signaling pathway and the other pathway related to inflamed pulp (Fig. 5). The detailed results of the GO enrichment and KEGG pathway analyses are shown in Additional File 3 and 4.

Table 2

Supplementary information of GO analysis.

| Category | ID | Term | adj.pval |
|---|---|---|---|
| BP | GO:0006954 | inflammatory response | 7.13E-24 |
| BP | GO:0007165 | signal transduction | 3.73E-05 |
| BP | GO:0006955 | immune response | 3.36E-20 |
| BP | GO:0007155 | cell adhesion | 7.95E-12 |
| BP | GO:0045944 | positive regulation of transcription from RNA polymerase II promoter | 0.005548688 |
| BP | GO:0007186 | G-protein coupled receptor signaling pathway | 0.002323744 |
| BP | GO:0006915 | apoptotic process | 0.000255676 |
| BP | GO:0043066 | negative regulation of apoptotic process | 3.66E-05 |
| BP | GO:0007166 | cell surface receptor signaling pathway | 7.13E-09 |
| BP | GO:0045087 | innate immune response | 3.69E-05 |
| CC | GO:0016021 | integral component of membrane | 7.35E-06 |
| CC | GO:0005886 | plasma membrane | 1.69E-12 |
| CC | GO:0070062 | extracellular exosome | 0.00028413 |
| CC | GO:0005576 | extracellular region | 8.34E-13 |
| CC | GO:0016020 | membrane | 2.96E-05 |
| CC | GO:0005887 | integral component of plasma membrane | 1.08E-13 |
| CC | GO:0005615 | extracellular space | 1.57E-08 |
| CC | GO:0009986 | cell surface | 1.72E-07 |
| CC | GO:0009897 | external side of plasma membrane | 2.35E-12 |
| CC | GO:0005578 | proteinaceous extracellular matrix | 0.000108122 |
| MF | GO:0005515 | protein binding | 0.002283082 |
| MF | GO:0005509 | calcium ion binding | 0.003507755 |
| MF | GO:0042803 | protein homodimerization activity | 0.014495214 |
| MF | GO:0005102 | receptor binding | 0.000515236 |
| MF | GO:0004872 | receptor activity | 1.96E-06 |
| MF | GO:0030246 | carbohydrate binding | 0.00011033 |

| Category | ID | Term | adj.pval |
|---|---|---|---|
| MF | GO:0008201 | heparin binding | 0.000173777 |
| MF | GO:0004888 | transmembrane signaling receptor activity | 0.002616585 |
| MF | GO:0003779 | actin binding | 0.043764465 |
| MF | GO:0005125 | cytokine activity | 0.004406936 |

# The Gene Set Enrichment Analysis

GSEA results revealed that the enriched biological processes mainly involved IL2/STAT5 signaling pathway (NES = 1.51; P = 0.008), IL6/JAK/STAT3 signaling pathway (NES = 1.49; P = 0.006) and inflammatory response pathways (NES = 1.49; P = 0.001), shown as Fig. 6.

# PPI network and hub genes

The PPI network constructed with online STRING program consisted of 3873 edges and 465 nodes (Additional File 5 and 6). Used with MCODE plugin, two significant modules were obtained, respectively containing 22 nodes and 105 edges (module 1) and 56 nodes and 645 edges (module 2), shown as Fig. 7.

Table 3

Top 10 hub genes with higher degrees PPI network of pulpitis.

| Gene symbol | Description | Function | Degree |
|---|---|---|---|
| PTPRC | Receptor-type tyrosine-protein phosphatase C; | Protein tyrosine-protein phosphatase required for T-cell activation through the antigen receptor. Acts as a positive regulator of T-cell coactivation upon binding to DPP4. | 126 |
| TLR8 | Toll-like receptor 8 | Key component of innate and adaptive immunity | 100 |
| CD86 | T-lymphocyte activation antigen CD86 | Receptor involved in the costimulatory signal essential for T-lymphocyte proliferation and interleukin-2 production, by binding CD28 or CTLA-4. | 100 |
| IL6 | Interleukin-6 | Cytokine with a wide variety of biological functions. Plays an essential role in the final Differentiation of B-cells into Ig- secreting cells Involved in lymphocyte and monocyte differentiation. | 135 |
| CCL2 | C-C motif chemokine 2 | Chemotactic factor that attracts monocytes and basophils | 80 |
| MMP9 | Matrix metalloproteinase-9 | May play an essential role in local proteolysis of the extracellular matrix and in leukocyte migration. | 83 |
| IL8 | Interleukin-8 | A chemotactic factor that attracts neutrophils, basophils, and T-cells, major mediator of the inflammatory response | 101 |
| SPI1 | Spi-1 Proto-Oncogene | This protein is a transcriptional activator that may be specifically involved in the differentiation or activation of macrophages or B- cells. | 76 |
| ICAM1 | Intercellular adhesion molecule 1 | Encodes a cell surface glycoprotein which is typically expressed on endothelial cells and cells of the immune system | 83 |
| LCP2 | Lymphocyte cytosolic protein 2 | Involved in T-cell antigen receptor mediated signaling | 77 |

# Discussion

All the time, it is a challenge for clinicians to make an accurate diagnosis about different levels of dental pulp inflammation, which is helpful to treatment planning[4]. Since histological examinations show the extent of inflammation is not correlating with a clinical manifestation, old diagnosis approaches on account of symptoms, electrical or thermal pulp tests, should be improved[7, 18]. As new biological materials are developed, successful cases of vital pulp therapy are increasing, but district indication must be grasped[19].

During inflammation, cells in human dental pulp, including odontoblasts, macrophages, vascular endothelial cells, precursor cells and the other that can launch immune system, are secreting a lot of cytokines, chemokines and neuropeptides, of which have been reported to play critical roles in characteristics of inflammation[20, 21]. Sivakami et al found that the levels of IL-6 and cytokines interleukin-1β (IL-1β) clearly increased in saliva during pulpal and periapical inflammation[22]. Compared with caries-exposed pulps, levels of IL-8 were significantly higher in irreversible pulpitis by detecting cytokine in pulpal blood[23]. Some reported that several cytokines were expected as diagnostic markers of pulpal inflammation[12, 24]. However, up to now, to the best of our knowledge, no appropriate biomarkers with high degree of accuracy have been used in clinical examination to diagnose early pulpitis.

Researchers about microarray analysis are exploding due to the rapid development of transcriptomic studies, giving rise to more and more clarity of biological mechanisms of oral diseases[25]. Through integration of several similar datasets, we can compose more biological significant expression profiles to identify key genes as biomarkers of pulpitis diagnosis and prognosis.

In the current study, expression data of pulpitis tissue from GEO database were integrated, then were used to identify underlying characteristics of DEGs as well as biomarker candidates for diagnosing dental pulp inflammation levels. 472 DEGs were screened and enrichment results from GO BP analysis manifested on inflammatory response, signal transduction and immune system, which demonstrate that the functions of DEGs are involved in processes of dental pulp inflammation. According to KEGG pathway analysis, some signaling pathways corelated with mechanisms of inflammation were enriched, containing cytokine-cytokine receptor interaction, osteoclast differentiation, chemokine signaling pathway, NF-κB signaling pathway and T cell receptor signaling pathway. Furthermore, GSEA results provided further functional annotations that complement system, IL6/JAK/STAT3 signaling pathway and inflammatory response pathways were possible mechanism of hub genes that induced inflammation.

As PPI network suggested, we also identified PTPRC, TLR8, CD86, IL6, CCL2, MMP9, CXCL8, SPI1, ICAM1 and LCP2 as hub genes of pulpitis. Some appropriate biomarkers are expected to become new methods for diagnosis and treatment in clinical practice.

Li et al reported DEGs and enrichment results by analyze data in GSE77459[26]. Differently, we respectively extracted data from GSE77459 and GSE92681 and integrated the mRNA expression to remove batch effect and improve the quality of the data. Compared with them, common KEGG pathways were cytokine-cytokine receptor interaction and chemokine signaling pathway and common hub genes were IL6, CXCL8, PTPRC, CCL2 and ICAM1.

PTPRC encodes protein tyrosine phosphatase (PTP), known as signaling molecules that regulate various kind of cellular processes, playing a critical role in immune system. PTPRC can negatively regulate cytokine receptor signaling by suppressing JAK signaling pathway[27]. Also, PTPRC expresses lowly or barely in normal dental pulp tissue[28], but highly in pulpitis tissue as indicated above. CD86 is the receptor that commonly participates in the processes of T-lymphocyte proliferation and IL-2 production,

acting as a negative regulator for immune system[29]. Miyuki Azuma et al studied the immune responses in mouse dental pulp, and founded that expression of CD86 were enhanced in dental pulp after cusp trimming, but disappeared within 2 h and migrated into regional lymph nodes at 24 h after acid treatment[30]. As chemokines launch and boost the process of inflammation, an increased expression of CCL2 was observed in chronic periapical lesions, indicating the association between chemokines and dental pulp inflammation[31, 32] In addition, IL6, TLR8, CXCL8, MMP9, and ICAM1 are also associated with immunity and inflammation in dental pulp as reported[1, 33–37]

# Conclusion

In summary, the present study analyzed merged datasets of pulpitis tissue, aiming at providing new clues for diagnosis or treatment of different inflammatory levels of pulpitis. In addition to investigate the possible regulatory mechanisms of DEGs, we screened key genes as biomarker candidates for diagnosis of pulpitis, such as PTPRC, CD86, CCL2, IL6, TLR8, MMP9, CXCL8 and ICAM1. It's effective to use bioinformatic methods to analyze merged datasets for identifying biomarkers for diseases, but further validation needs to be performed to confirm the results obtained from analysis.

# Abbreviations

GEO: Gene Expression Omnibus; DEGs: differentially expressed genes; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PPI: Protein–protein interaction; STRING: Search Tool for the Retrieval of Interacting Genes/Proteins; AAE: American Association of Endodontists; limma: Linear Models for Microarray data; BP: biological process; MF: molecular function; CC: cellular component; DAVID: Database for Annotation Visualization and Integrated Discovery; FDR: false discovery rate; NES: Normalized Enrichment Score; PCA: principal component analysis; IL6: Interleukin-6; PTPRC: Receptor-type tyrosine-protein phosphatase C; CXCL8: Interleukin-8; CD86: lymphocyte activation antigen CD86; TLR8: Toll-like receptor 8; ICAM1: Intercellular adhesion molecule 1; MMP9: Matrix metalloproteinase-9; CCL2: C-C chemokine receptor type 2; LCP2: Lymphocyte cytosolic protein 2; SPI1:  Transcription factor PU.1; IL-1β: cytokines interleukin-1β

# Declarations


### Acknowledgements

Not applicable.


### Availability of data and materials

The datasets generated and analyzed during the current study are available in GEO DataSets repository, https://www.ncbi.nlm.nih.gov/gds.

### Funding

This work was supported by the General Program of National Natural Scientific Foundation of China (No. 8187041227), the General Program of National Natural Scientific Foundation of Guangdong Province (No. 2018A030313398), Medical Scientific Research Foundation of Guangdong Province of China (No. B2018012, No. A2018117), Youth Program of President Foundation of Nanfang Hospital, Southern Medical University (No. 2016C023), Special Program of President Foundation of Nanfang Hospital, Southern Medical University (No.2017Z004). The authors declare no potential conflict of interest with respect to the authorship and/or publication of this article.

### Authors' contributions

Ming Chen and Junkai Zeng conceived this study, participated in the design, performed the analysis and critically revised the manuscript. Buling Wu conceived this study, participated in the design and critically revised the manuscript. Yeqing Yang participated in the design and helped to draft the manuscript. All authors read and approved the final manuscript.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

## References

1. Rechenberg DK, Bostanci N, Zehnder M, Belibasakis GN. Periapical fluid RANKL and IL-8 are differentially regulated in pulpitis and apical periodontitis. Cytokine. 2014;69:116–9.
2. Hayama T, Kamio N, Okabe T, Muromachi K, Matsushima K. Kallikrein Promotes Inflammation in Human Dental Pulp Cells Via Protease-Activated Receptor-1. J Cell Biochem. 2016;117:1522–8.

3. Kim Y, Park JS, Park HJ, Kim MK, Kim Y Il, Bae SK, et al. Pentraxin 3 Modulates the Inflammatory Response in Human Dental Pulp Cells. J Endod. 2018;44:1826–31. doi:10.1016/j.joen.2018.08.003.
4. Fitzgerald M, Heys RJ. A clinical and histological evaluation of conservative pulpal therapy in human teeth. Oper Dent. 1991.
5. Zanini M, Meyer E, Simon S. Pulp Inflammation Diagnosis from Clinical to Inflammatory Mediators: A Systematic Review. J Endod. 2017;43:1033–51.
6. Seltzer S, Bender IB, Ziontz M. The dynamics of pulp inflammation: Correlations between diagnostic data and actual histologic findings in the pulp. Oral Surgery, Oral Med Oral Pathol. 1963;16:969–77.
7. Mejàre IA, Axelsson S, Davidson T, Frisk F, Hakeberg M, Kvist T, et al. Diagnosis of the condition of the dental pulp: A systematic review. Int Endod J. 2012;45:597–613.
8. Mishra S, Sharma DS, Bhusari C. Assessing inflammatory status of pulp in irreversible pulpitis cases with pulse oximeter and dental hemogram. J Clin Pediatr Dent. 2019;43:314–9.
9. Bunney PE, Zink AN, Holm AA, Billington CJ, Kotz CM. Orexin activation counteracts decreases in nonexercise activity thermogenesis (NEAT) caused by high-fat diet. Physiol Behav. 2017;176:139–48.
10. Khorasani MMY, Hassanshahi G, Brodzikowska A, Khorramdelazad H. Role(s) of cytokines in pulpitis: Latest evidence and therapeutic approaches. Cytokine. 2020;126 July 2019:154896. doi:10.1016/j.cyto.2019.154896.
11. Silva TA, Garlet GP, Lara VS, Martins W, Silva JS, Cunha FQ. Differential expression of chemokines and chemokine receptors in inflammatory periapical diseases. Oral Microbiol Immunol. 2005.
12. Emilia E, Neelakantan P. Biomarkers in the dentin-pulp complex: role in health and disease. J Clin Pediatr Dent. 2015;39:94–9.
13. Jaedicke KM, Preshaw PM, Taylor JJ. Salivary cytokines as biomarkers of periodontal diseases. Periodontol 2000. 2016;70:164–83.
14. Zehnder M, Wegehaupt FJ, Attin T. A first study on the usefulness of matrix metalloproteinase 9 from dentinal fluid to indicate pulp inflammation. J Endod. 2011;37:17–20. doi:10.1016/j.joen.2010.10.003.
15. Coffey CT, Ingram MJ, Bjorndal AM. Analysis of human dentinal fluid. Oral Surgery, Oral Med Oral Pathol. 1970;30:835–7.
16. Gatta V, Zizzari VL, Dd ' Amico V, Salini L, D'Aurora M, Franchi S, et al. Microarray evaluation of gene expression profiles in inflamed and healthy human dental pulp: the role of IL1beta and CD40 in pulp inflammation. J Biol Regul Homeost Agents. 2012;26:45–50.
17. Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular Signatures Database Hallmark Gene Set Collection. Cell Syst. 2015;1:417–25.
18. Lundy T, Stanley HR. Correlation of pulpal histopathology and clinical symptoms in human teeth subjected to experimental irritation. Oral Surgery, Oral Med Oral Pathol. 1969.

19. de Andrade CM, Galvão-Moreira LV, de Oliveira JFF, Bomfim MRQ, Monteiro SG, Figueiredo P de MS, et al. Salivary biomarkers for caries susceptibility and mental stress in individuals with facial pain. Cranio - J Craniomandib Pract. 2019;0:1–7. doi:10.1080/08869634.2019.1607445.
20. Elsalhy M, Azizieh F, Raghupathy R. Cytokines as diagnostic markers of pulpal inflammation. Int Endod J. 2013;46:573–80.
21. Eba H, Murasawa Y, Iohara K, Isogai Z, Nakamura H, Nakamura H, et al. The Anti-Inflammatory Effects of Matrix Metalloproteinase-3 on Irreversible Pulpitis of Mature Erupted Teeth. PLoS One. 2012;7:1–9.
22. Haug S, Marthinussen M. Acute Dental Pain and Salivary Biomarkers for Stress and Inflammation in Patients with Pulpal or Periapical Inflammation. J Oral Facial Pain Headache. 2019;33:227–33.
23. Liu M, Zhao Y, Wang C, Luo H, A P, Ye L. Interleukin-17 plays a role in pulp inflammation partly by WNT5A protein induction. Arch Oral Biol. 2019;103 April:33–9. doi:10.1016/j.archoralbio.2019.05.003.
24. Dong Y, Lan W, Wu W, Huang Z, Zhao J, Peng L, et al. Increased expression of epha7 in inflamed human dental pulp. J Endod. 2013;39:223–7. doi:10.1016/j.joen.2012.11.020.
25. Lee LT, Wong YK, Hsiao HY, Wang YW, Chan MY, Chang KW. Evaluation of saliva and plasma cytokine biomarkers in patients with oral squamous cell carcinoma. Int J Oral Maxillofac Surg. 2018;47:699–707. doi:10.1016/j.ijom.2017.09.016.
26. Lei F, Zhang H, Xie X. Comprehensive analysis of an lncRNA-miRNA-mRNA competing endogenous RNA network in pulpitis. PeerJ. 2019;2019:1–18.
27. Irie-Sasaki J, Sasaki T, Matsumoto W, Opavsky A, Cheng M, Welstead G, et al. CD45 is a JAK phosphatase and negatively regulates cytokine receptor signalling. Nature. 2001;409:349–54.
28. Zhou J, Shi S, Shi Y, Xie H, Chen L, He Y, et al. Role of bone marrow-derived progenitor cells in the maintenance and regeneration of dental mesenchymal tissues. J Cell Physiol. 2011;226:2081–90.
29. Nuriya S, Yagita H, Okumura K, Azuma M. The differential role of CD86 and CD80 co-stimulatory molecules in the induction and the effector phases of contact hypersensitivity. Int Immunol. 1996.
30. Zhang J, Kawashima N, Suda H, Nakano Y, Takano Y, Azuma M. The existence of CD11c+ sentinel and F4/80+ interstitial dendritic cells in dental pulp and their dynamics and functional properties. Int Immunol. 2006;18:1375–84.
31. Tavares WLF, De Brito LCN, Henriques LCF, Teles FRF, Teles RP, Vieira LQ, et al. Effects of calcium hydroxide on cytokine expression in endodontic infections. J Endod. 2012;38:1368–71.
32. Hui T, Peng A, Zhao Y, Yang J, Ye L, Wang C. EZH2 regulates dental pulp inflammation by direct effect on inflammatory factors. Arch Oral Biol. 2018;85:16–22.
33. Pääkkönen V, Rusanen P, Hagström J, Tjäderhane L. Mature human odontoblasts express virus-recognizing toll-like receptors. Int Endod J. 2014;47:934–41.
34. Mente J, Petrovic J, Gehrig H, Rampf S, Michel A, Schürz A, et al. A Prospective Clinical Pilot Study on the Level of Matrix Metalloproteinase-9 in Dental Pulpal Blood as a Marker for the State of

Inflammation in the Pulp Tissue. J Endod. 2016;42:190–7.

35. Sugiuchi A, Sano Y, Furusawa M, Abe S, Muramatsu T. Human Dental Pulp Cells Express Cellular Markers for Inflammation and Hard Tissue Formation in Response to Bacterial Information. J Endod. 2018;44:992–6. doi:10.1016/j.joen.2018.02.022.
36. Wang X, Sun H, Liu H, Ma L, Jiang C, Liao H, et al. MicroRNA-181b-5p modulates tumor necrosis factor-α-induced inflammatory responses by targeting interleukin-6 in cementoblasts. J Cell Physiol. 2019;234:22719–30.
37. De Rossi A, Lucisano M, De M, Rossi, Nelson-Filho P, Silva R, et al. Effect of intercellular adhesion molecule 1 deficiency on the development of apical periodontitis. Int Endod J. 2019;:1–12.

# Figures

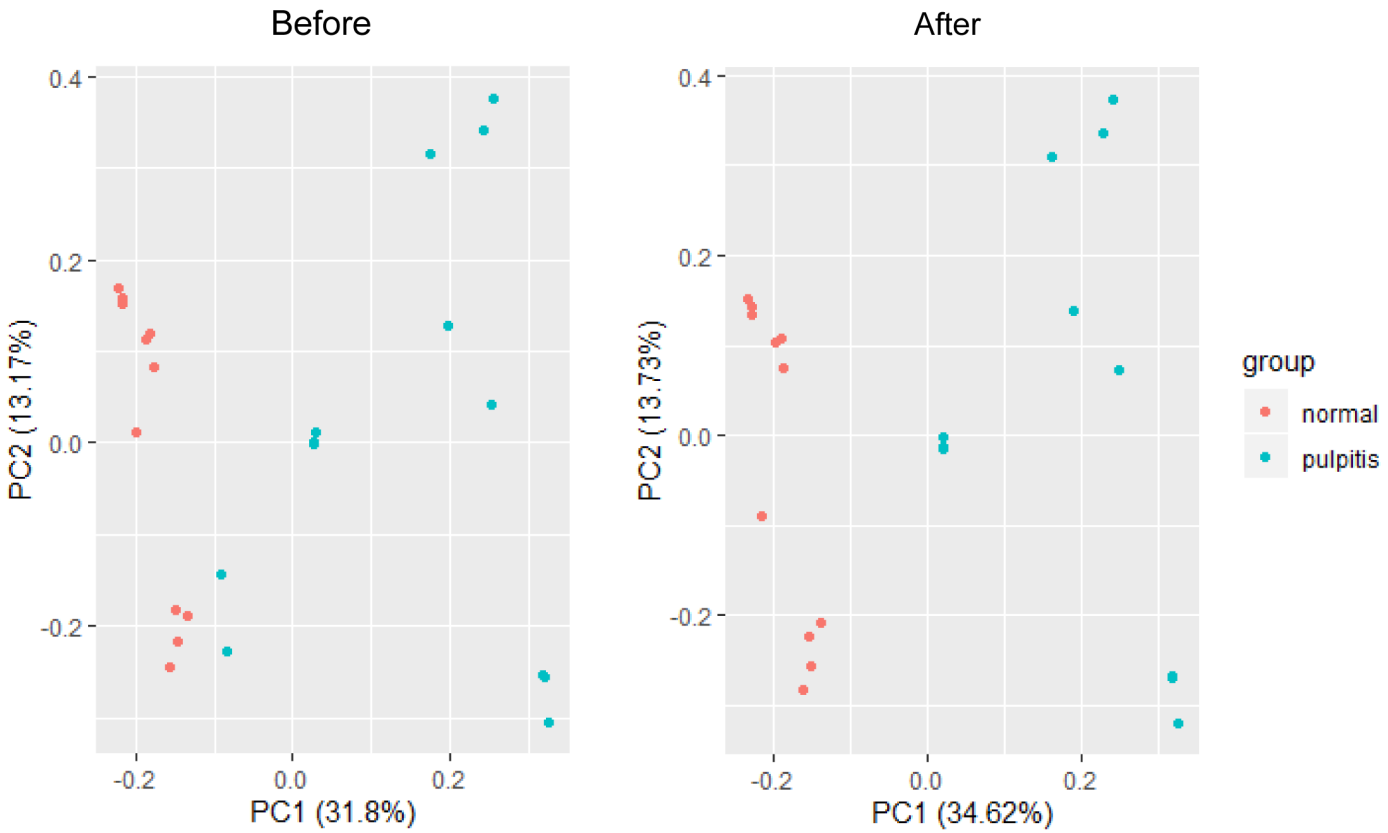
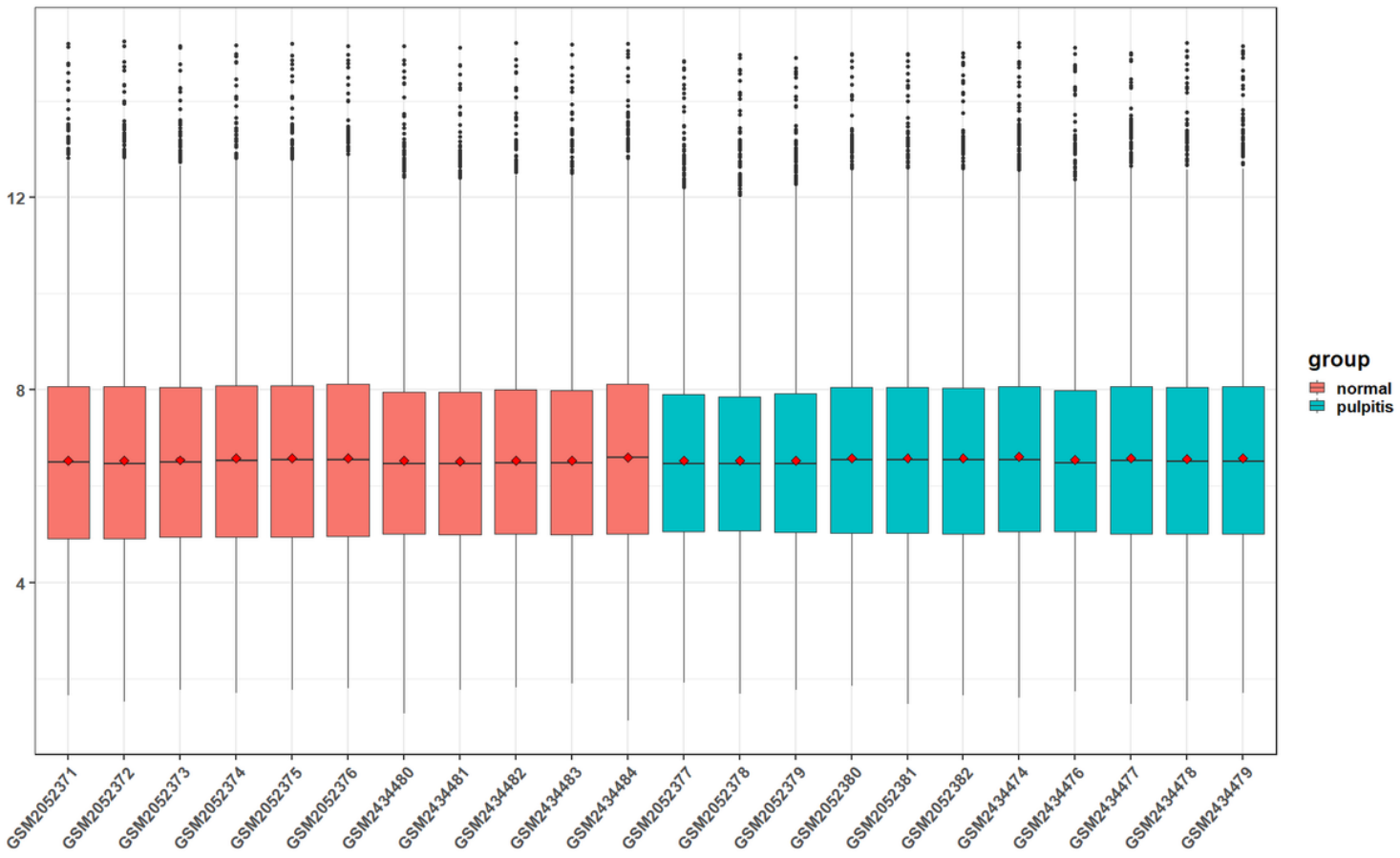


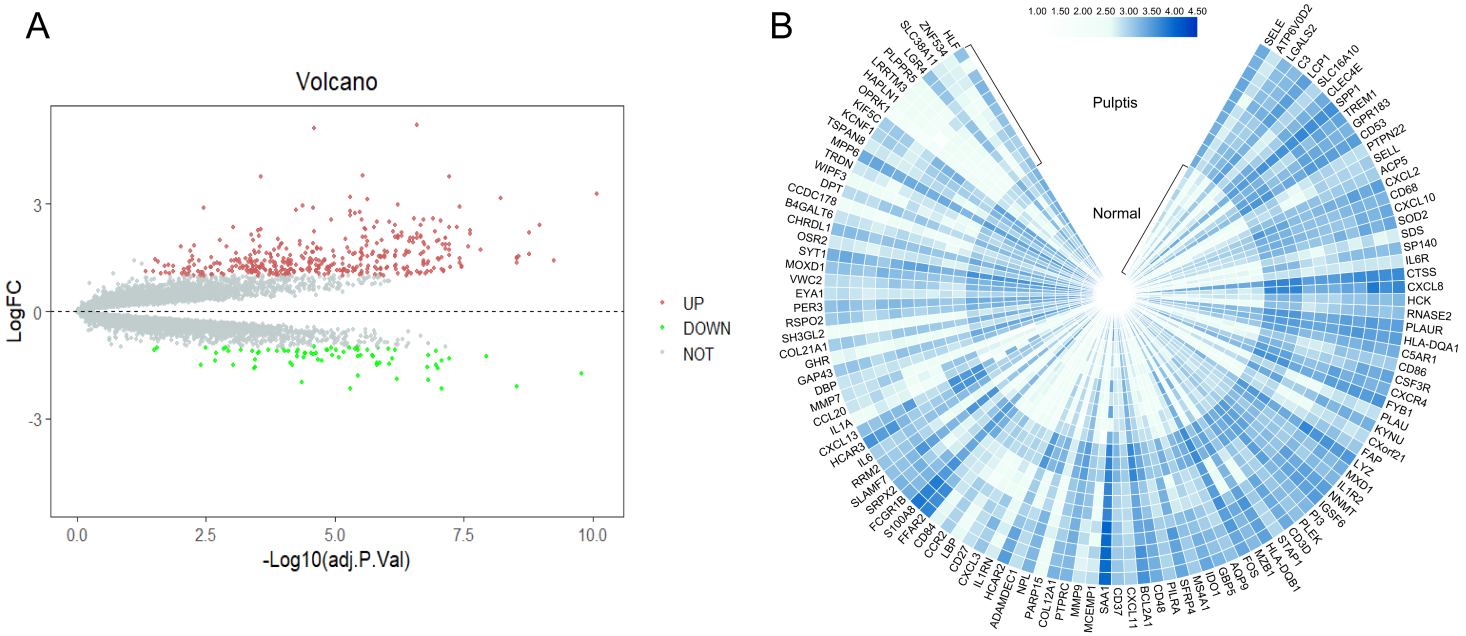

**Figure 1**

PCA before and after batch effect removal.



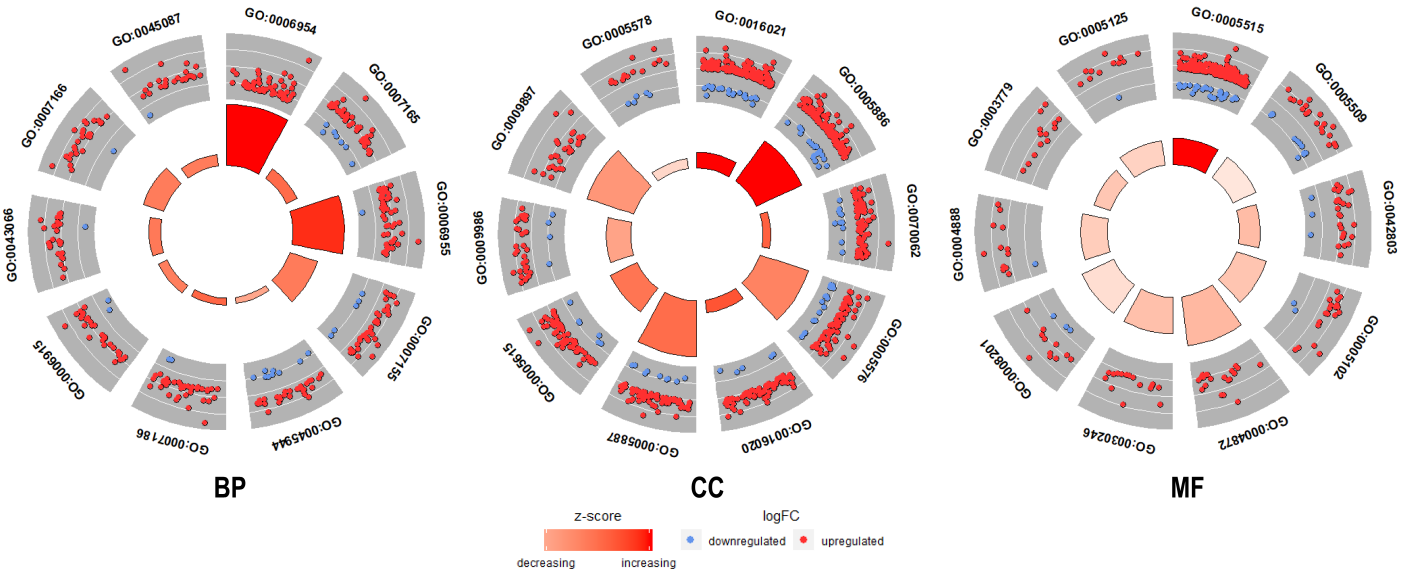

## Figure 2

Box plot of 22 samples in the pooled dataset that was normalized. X axes were samples form dataset and Y axes were normalized intensity values.




## Figure 3

Volcano plot and heatmap of DEGs. A. Volcano plot for the DEGs in merged dataset. The x-axis shows the -log10 (adjusted p-value) and y-axis shows the log FC. The DEGs were selected with the cut-off criteria of |fold change| > 1.0 and an adjusted p-value < 0.05. The red dots and green denote upregulated genes and downregulated genes. The gray dots denote genes with no significant difference. B. Heatmap of the DEGs in merged dataset. The blue and white colors respectively represent upregulation and downregulation of mRNAs.




## Figure 4

Top 10 results of GO enrichment including BP, MF and CC. The red dots represent the upregulated genes and blue dots represent downregulated genes low enrichment, and red indicates high enrichment. The sizes and colors of the sectors respectively indicated the adjusted P-Value (adj.pval) and stand score (z-score) of each GO term.

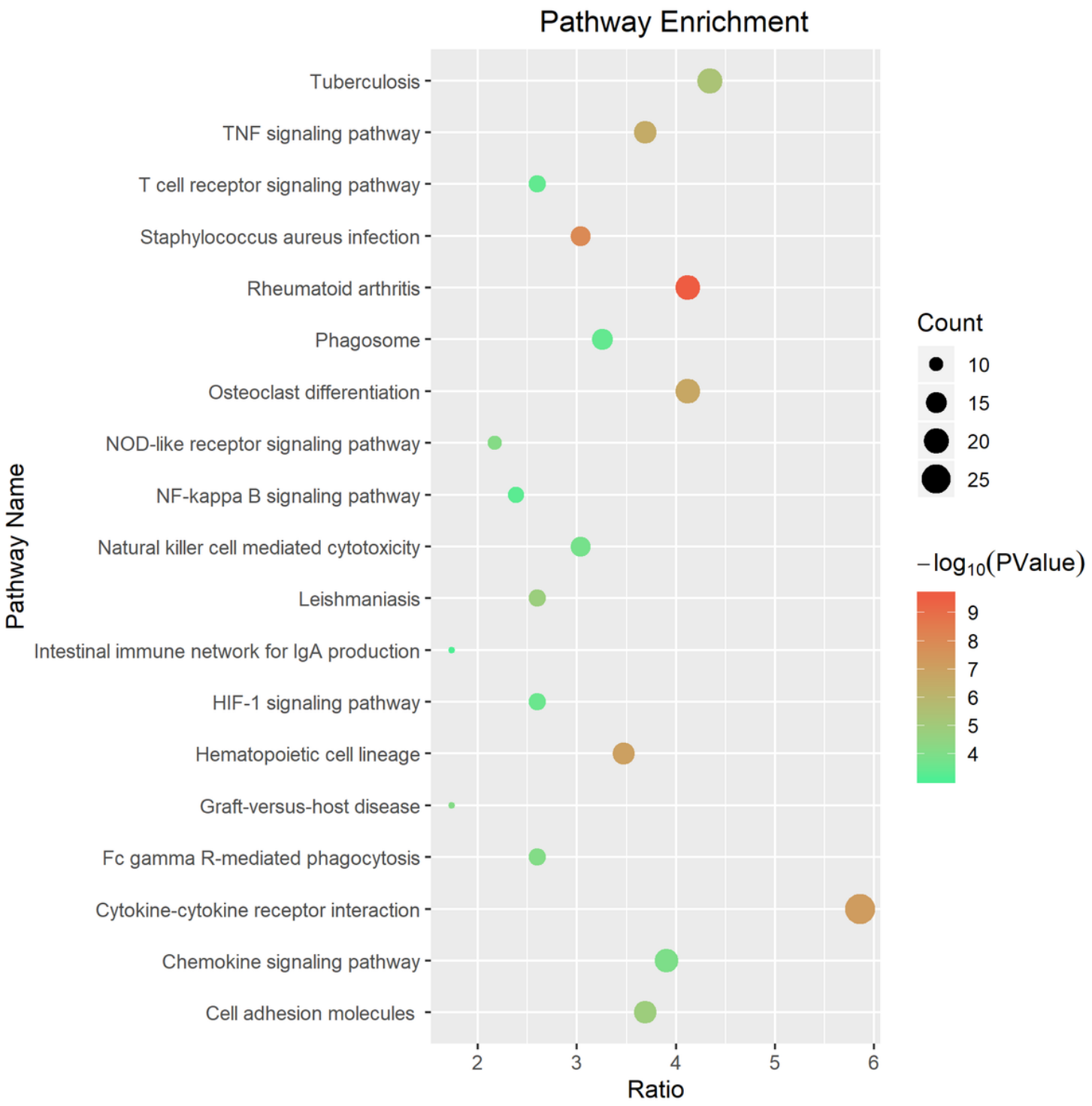
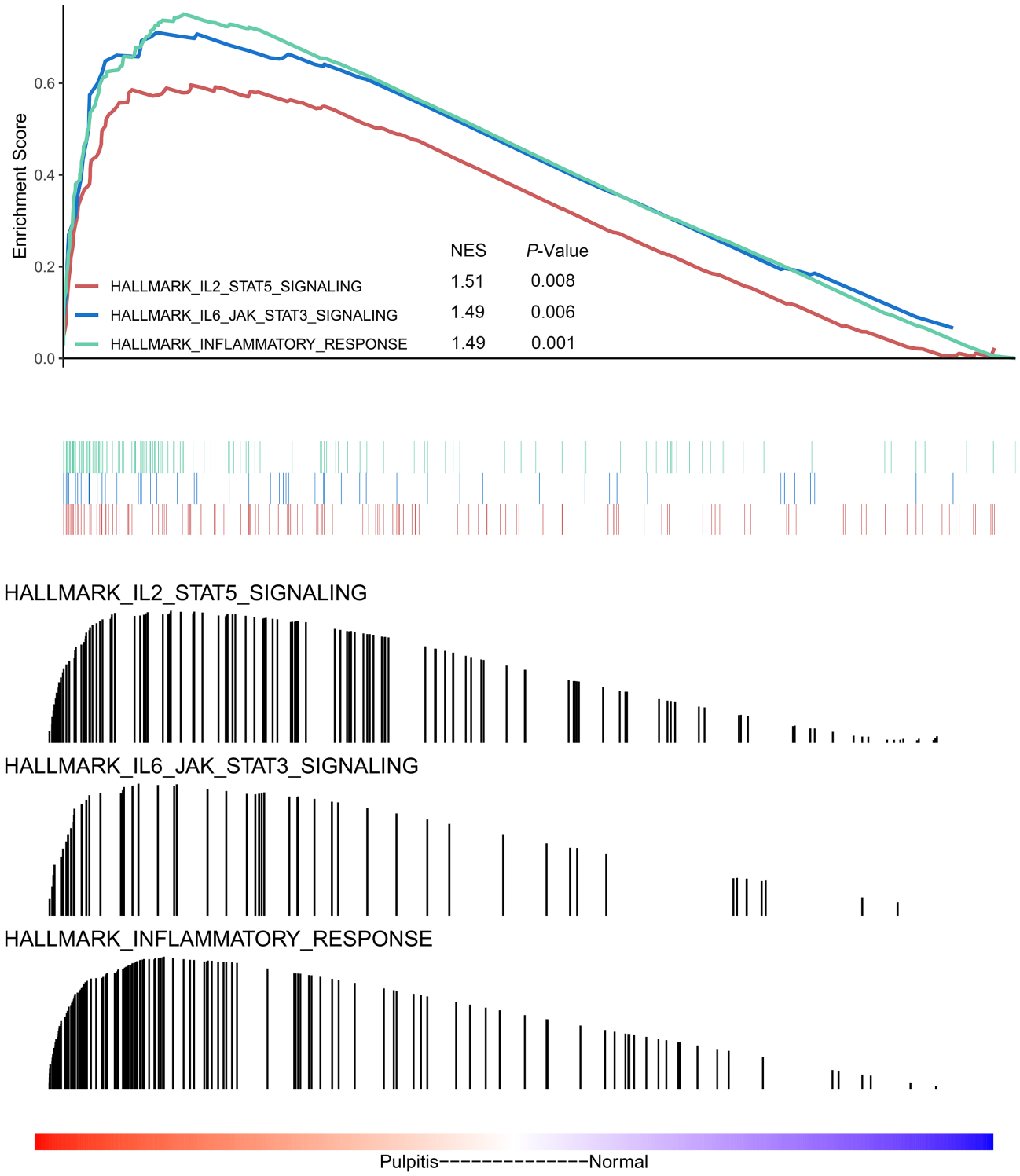



**Figure 5**

Top 20 results of KEGG pathways analysis. The X represented the ratio of enriched genes to all of DEGs, and Y axis represented the names of KEGG pathway terms. The sizes and colors of dots respectively show the count of genes and -log10(P-Value) in each term enrichment.




## Figure 6

Gene Set Enrichment Analysis results. The red, blue and green lines respectively represent the IL2/STAT5 signaling pathway, IL6/JAK/STAT3 signaling pathway and inflammatory response pathways. NES: Normalized Enrichment Score.

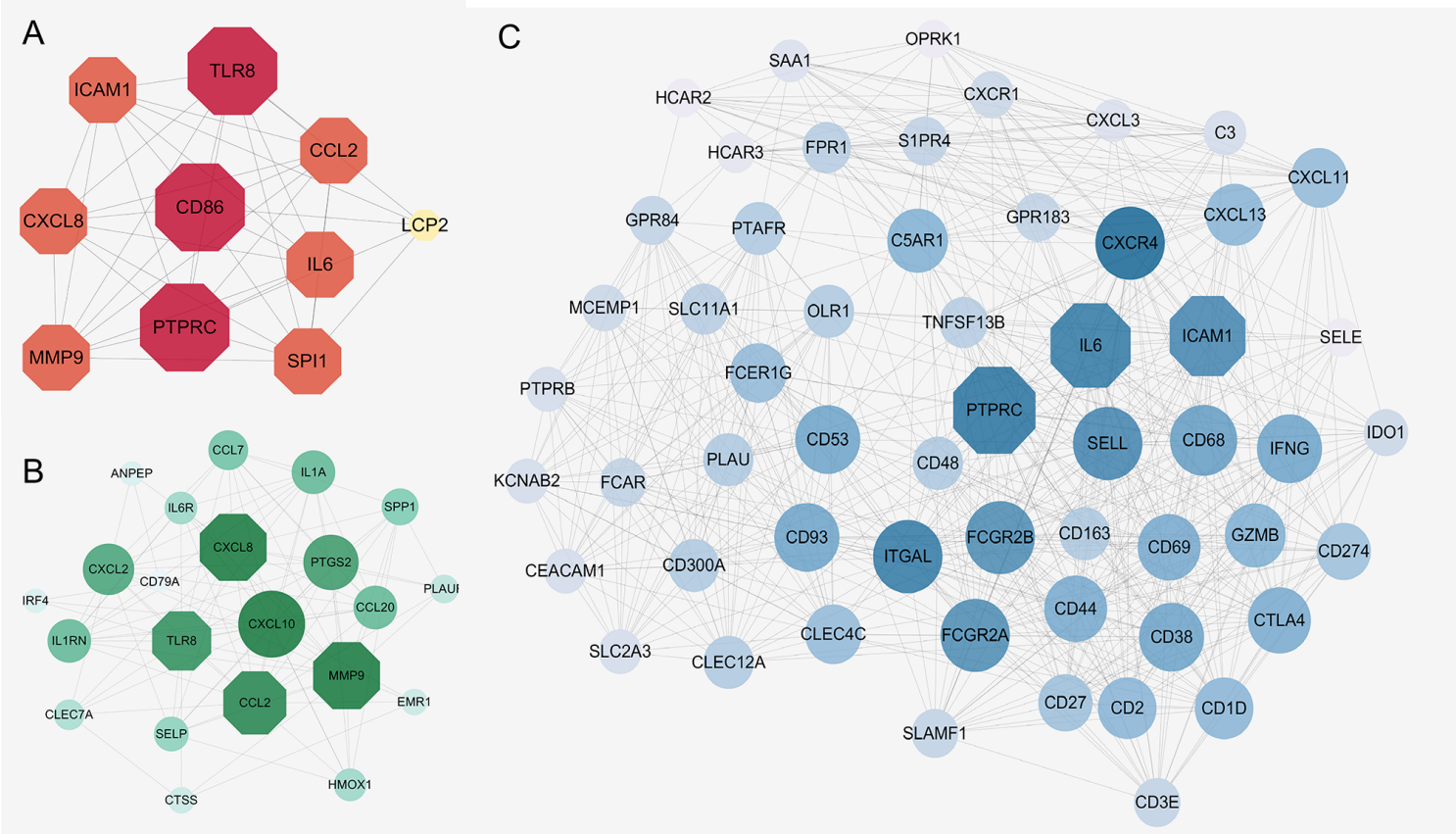



**Figure 7**

The hub genes and significant modules from PPI network. A. Top 10 hub mRNAs of pulpitis; B. Module 1 with 22 nodes and 105 edges; B. Module 2 with 56 nodes and 645 edges. The polygonal nodes represent the hub genes and round nodes are the other genes in module network. The size and color depth represent the degree of each node in each network.

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile4.xlsx
- Additionalfile1.xlsx
- Additionalfile6.jpg
- Additionalfile2.xlsx
- Additionalfile5.xlsx
- Additionalfile3.xlsx