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Identifying novel druggable inhibitors for Chronic Obstructive Pulmonary Disease: an *in-silico* approach

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Project Report

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Identifying novel druggable inhibitors for Chronic Obstructive Pulmonary Disease: an *in-silico* approach

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Resumo

Este projeto aborda a doença pulmonar obstrutiva crónica (DPOC), que é uma doença pulmonar progressiva com uma elevada taxa de incidência e um impacto significativo na mortalidade. Este estudo focou-se na identificação, network funcional e de interação, e enriquecimento funcional de genes diferencialmente expressos em pacientes com COPD. Utilizando a base de dados NCBI, foram retirados dois conjuntos de dados de expressão génica, a partir dos estudos GSE38974_GLP4133 e GSE106986. Os genes diferencialmente expressos em ambos os estudos foram analisados, obtendo-se quatro genes comuns - PPP6R1, FGFBP1, CD86 e LRRC2. Foi ainda realizada uma análise funcional com o objetivo de identificar genes com interaçoes físicas, co-localizados ou que se encontram na mesma via de sinalização, obtendo-se três networks, aos que se aplicou uma análise de enriquecimento funcional. O network do FGFBP1 revelou estar principalmente associado à via de sinalização dos fatores de crescimento dos fibroblastos e respetivos recetores; o network do CD86 mostrou uma forte relação com mecanismos de resposta inflamatória; e o network PPP6R1 destacou a associação com a desfosforilação de proteínas. O gene LRRC2 não apresentou nenhum network. Estes resultaram permitiram destacar três genes sobre-expressos em pacientes com COPD, que podem sustentar mais estudos focando no seu potencial enquanto alvos terapêuticos.

Palavras-chave

DPOC; Potenciais alvos; Genes diferencialmente expressos; Processos biológicos; Tecido pulmonar.

Abstract

This project addresses chronic obstructive pulmonary disease (COPD), which is a progressive lung disease with a high incidence rate and a significant impact on mortality. This study focused on the identification, functional and interaction network, and functional enrichment of differentially expressed genes in COPD patients. Using the NCBI database, two gene expression datasets were retrieved from the GSE38974_GLP4133 and GSE106986 studies. The differentially expressed genes in both studies were analyzed and four common genes were obtained - PPP6R1, FGFBP1, CD86 and LRRC2. A functional analysis was also carried out with the aim of identifying genes with physical interactions, co-located or found in the same signaling pathway, resulting in three networks to which a functional enrichment analysis was applied. The FGFBP1 network proved to be mainly associated with the signaling pathway of fibroblast growth factors and their receptors; the CD86 network showed a strong relationship with inflammatory response mechanisms; and the PPP6R1 network highlighted its association with protein dephosphorylation. The LRRC2 gene had no network. These results allowed us to highlight three genes over-expressed in COPD patients, which may support further studies focusing on their potential as therapeutic targets.

Key words

COPD; Potential targets; Genes diferencialmente expressos; Biological processes; Lung tissue.

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List of abbreviations

- (COPD) – Chronic obstructive pulmonary disease.
- (DALYs) – Disability-adjusted life years.
- (EU) – Europe.
- (DNA) – Deoxyribonucleic acid.
- (AAT) – Alpha-1-antitrypsin.
- (Vitamin D) – Vitamin D-binding protein.
- (TLRs) – Toll-type receivers.
- (MMP9) – Metalloproteinase 9.
- (AI) – Artificial intelligence.
- (CYP17A1) – Cytochrome P450.
- (GSE) – Gene Expression Omnibus Series.
- Genes in common:
    - (*PPP6R1A*) – Protein phosphatase 6 regulatory subunit 1.
    - (*FGFBP1*) – Fibroblast growth factor binding protein 1.
    - (*CD86*) – Cluster of Differentiation 86.
    - (*LRRC2*) – Leucine rich repeat containing 2.
- Another Genes:
    - (*MAPK*) – Mitogen-activated protein kinases.
    - (TLR signaling) – Toll-Like Receptor Signaling Pathways.

1-Introduction

Chronic obstructive pulmonary disease (COPD) is a progressive respiratory condition, with a high incidence rate and significant impact on mortality and social burden (1). In 2019, there were 212.3 million reported cases of COPD, resulting in 3.3 million deaths and 74.4 million disability-adjusted life years (DALY) lost, with the majority occurring in low- and middle-income countries (2). The prevalence of COPD is expected to increase with an ageing global population, maintaining the high number of cases (2). From 2001 and 2019, COPD prevalence in the EU has generally risen among females while decreased among males, with some countries reporting higher prevalence in females than in males (3).

COPD is characterized by persistent inflammation in the lung parenchyma and peripheral airways (4, 5). This inflammation leads to the narrowing of small airways and the gradual destruction of lung tissue, resulting in gas trapping, reduced expiratory flow, and impaired gas exchange (4). Increased infiltration of immune cells, including alveolar macrophages, neutrophils, specific T-cell subtypes (Th1, Th17), and innate lymphoid cells, contribute to the ongoing inflammatory process (5).

This pulmonary disease is heavily influenced by both exogenous and endogenous sources of oxidative stress. External factors, like cigarette smoke and air pollution, along with internal oxidative stress generated by inflammatory cells, significantly contribute to the development and progression of COPD (6, 7, 8). This heightened oxidative stress triggers intracellular signaling pathways associated with inflammatory mediators, leading to corticosteroid resistance, the accumulation of senescent cells, increased mucus secretion and DNA damage (9, 10). The increased oxidative stress and DNA damage are also linked to cancer, suggesting that COPD may be a driving factor in lung cancer, as both conditions exhibit shared gene expression and epigenetic alterations (11). Moreover, single-cell omics analysis has revealed transcriptional plasticity in macrophages, altered lipid metabolism, and mitochondrial dysfunction in COPD patients (12). These changes are potentially associated with alterations in gene expression related to cell adhesion, inflammatory response, and mitochondrial functions. Large-scale exome array meta-analyses have identified potential functional coding variants in genes involved in DNA methylation, cell-matrix interactions, cell proliferation, and cell death in COPD cases (13). Additionally, epigenetic alterations in lung cells of COPD patients affect pathways related to proliferation, inflammation, and viral immunity, with dysregulation occurring from the early stages of the disease (14).

COPD presents a range of respiratory symptoms, including shortness of breath, dyspnea, cough, sputum production, wheezing, and chest tightness (15, 16). Acute exacerbations, often triggered by bacterial infections with pathogens like *H. influenzae, M. catarrhalis, S. pneumoniae, S. aureus, S. aeruginosa, and C. pneumoniae*, are characterized by increased sputum volume, purulence, and worsening dyspnea. The severity of these exacerbations is determined by the extent of these symptoms (17). Several other factors heighten the risk of COPD exacerbations, such as smoking, exposure to environmental pollutants, advanced age, male gender, comorbidities, family history of respiratory disease, lower education level, and overweight/obesity (18, 19, 20). Moreover, genetic polymorphisms

in genes like alpha-1-antitrypsin (AAT) (21), Vitamin D binding protein (VDBP) (22), Toll-like receptors (TLRs) (23) and metalloproteinase 9 (MMP9) (24) are linked to both the susceptibility to and progression of COPD.

The diagnosis of COPD is typically conducted using spirometry, a lung function test that measures airflow limitation and oxygen transfer (25). Other diagnostic tests may include chest X-rays, CT scans, blood tests, and arterial blood gas analysis (26). Current treatments for COPD include bronchodilators, antimuscarinic drugs, corticosteroids, pulmonary rehabilitation, and supplemental oxygen (26, 27, 25). These therapies aim to manage symptoms, improve quality of life, prevent exacerbations, and slow disease progression. However, further research is needed to better understand COPD´s molecular mechanisms, enhance early diagnosis and develop alternative therapies that can inhibit disease progression without causing side effects, such as infections and immunosuppression, which are common with current therapeutic responses (28).

While COPD diagnosis relies on pulmonary function tests, the accuracy can vary due to patient cooperation (29). Artificial intelligence (AI) techniques offer promising alternatives for more effective COPD diagnosis. For example, a study by Topalovic et al. demonstrated that AI software could interpret pulmonary function tests more accurately than senior pulmonologists (30). Additionally, the use of deep residual networks in thoracic computed tomography scans has shown high performance in COPD detection and diagnosis (31). To reduce reliance on lung function tests and imaging for early diagnosis, machine learning can also analyse transcriptomic data. For instance, a transcriptomic machine learning method identified 15 genes with differential expression between smokers and non-smokers (31).

Machine learning methods can also help identify new therapeutic targets and drugs that may influence COPD progression. For example, Sun et al. identified a key biomarker associated with inflammatory immune response in both COPD and atrial fibrillation using a combination of bioinformatics and machine learning algorithms (32). Furthermore, training machine learning algorithms can predict the chemical information and bioactivity of a large number of compounds for a specific target (33). For instance, cytochrome P450 17A1 (CYP17A1), a key enzyme in steroidogenesis, can contribute to the progression of several tumours. Using chemoinformatics and machine learning analysis, Yu et al. identified potential CYP17A1 inhibitors with high accuracy (34).

Identifying effective therapeutic options for COPD remains a significant challenge due to the limited number of specific treatments. Bioinformatics can play a crucial role in this context, characterizing genes and proteins related to COPD by processing high-dimensional data. These tools support personalized medicine approaches and have the potential to improve therapeutic options by identifying new targets.

2-Methods

Next, below is figure 1, with include the *in-silico* diagram of the methods used during this study.

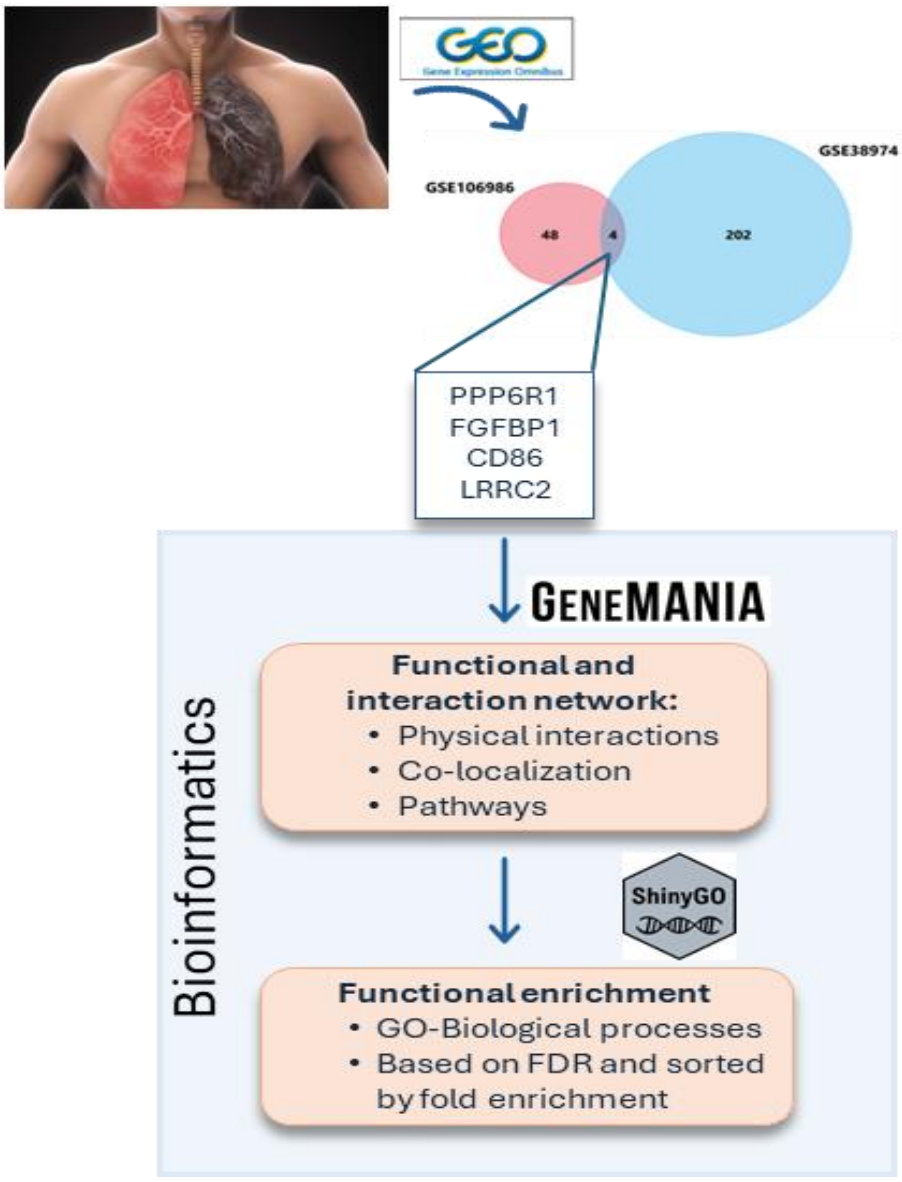


Figure 1: Methodology diagram.

2.1-Data collection

COPD gene expression data was obtained from the GEO database (https://www.ncbi.nlm.nih.gov/geo/), using GSE38974-GPL4133 and GSE106986 datasets. The GSE38974 dataset includes samples from 23 COPD patients and 9 controls, all smokers, and derived from the Agilent-014850 Whole Human Genome Microarray 4x44K G4112F platform. The GSE106986 dataset includes 14 COPD patients who are smokers and 5 non-smoker controls, derived from the Agilent-026652 Whole Human Genome Microarray 4x44K v2 platform. All samples were collected from human lung tissue.

2.2-Differentially Expressed Genes (DEGs) screening

To identify the differentially expressed genes in each dataset, the GEO2R online tool (https://www.ncbi.nlm.nih.gov/geo/geo2r/), was utilized, adopting $|\log2(FC)| > 2$ and $p < 0.05$ as criteria, with adjustment of p-values using the Benjamini & Hochberg (False discovery rate) method. Volcano plot-generated tables were utilized to identify both under- and overexpressed genes. Only protein-coding genes were selected for analysis.

2.3-DEGs interactions analysis and Functional Enrichment

To explore the functional and interaction networks of known DEGs and identify additional genes potentially relevant to COPD, the GeneMANIA tool (https://genemania.org/). was used. Networks were constructed integrating physical interactions, co-localized genes, and common pathways, setting a maximum of 20 genes and 15 attributes. The network weights were determined using a machine learning algorithm that optimizes the relevance of each data source (e.g., Gene Expression Omnibus, BioGRID, I2D) to the query genes, assigning higher weights to networks that better predict the functions of the query genes (35).

3-Results and Discussion

To identify potential biological processes related to these networks, a functional enrichment analysis was performed using ShinyGO v0.80 (http://bioinformatics.sdstate.edu/go/). The analysis was based on Gene Ontology biological processes (GO-BP), with criteria of pathways containing a minimum of ten genes and a False Discovery Rate (FDR) < 0.05. The ten most significant pathways were selected based on FDR and sorted by fold enrichment. This approach prioritized the top pathways likely to be enriched, highlighting the ratio of genes in each overlapping set that belongs to a certain pathway, as well as the total number of genes annotated to each pathway (44).

3.1-Differentially Expressed Genes (DEGs) in COPD

Differentially expressed genes (DEGs) in COPD were identified by comparing gene expression profiles from COPD patients to those of control groups. In the GSE38974 dataset (derived from the GPL4133 platform), 206 protein-coding genes were identified, with 194 upregulated and 12 downregulated. In the GSE106986 dataset, 52 genes were identified, with 35 upregulated and 17 downregulated (Supplementary 1). Volcano plots illustrate the screened DEGs in both datasets (Figure 2A).

The intersection of DEGs between the two datasets revealed four common genes – Protein phosphatase 6 regulatory subunit 1 *(PPP6R1A),* Fibroblast growth factor binding protein 1 *(FGFBP1),* Cluster of Differentiation 86 *(CD86),* and Leucine rich repeat containing 2 *(LRRC2)* (Figure 2B).

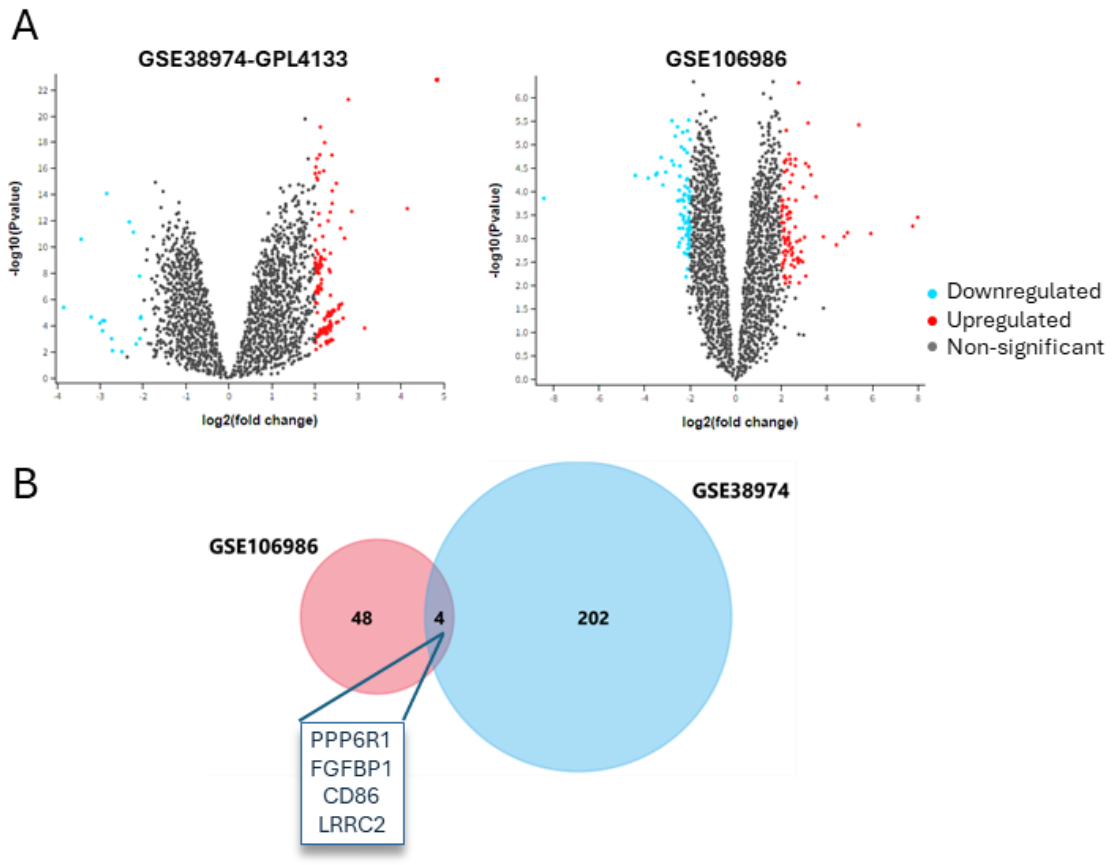


Figure 2: Vulcan graphs of both data sets and their common genes.

Referring to figure 2, differentially expressed genes (DEGs) between COPD lung tissues and normal samples. A) Volcano plots of the GSE38974-GPL4133 and GSE106986 datasets highlighting the DEGs, with red dots representing up-regulated genes, blue dots representing down-regulated genes and gray dots representing genes not significant according to the cut-off criteria. B) The Venn diagram shows the intersection of DEGs between the two data sets, revealing four common genes.

3.2-Funtional and Interaction network of the common genes

To explore the potential joint genetic effects of the four genes (FGFBP1, CD86, PPP6R1 and LRRC2) and identify physical interactions, co-localized genes, and common pathways, the GeneMANIA tool was used. Interestingly, these genes are not part of the same network; however, FGFBP1, CD86, and PPP6R1 each interact within their respective network (Fig 3A-C), while LRRC2 is not integrated into any network (Fig.3D).

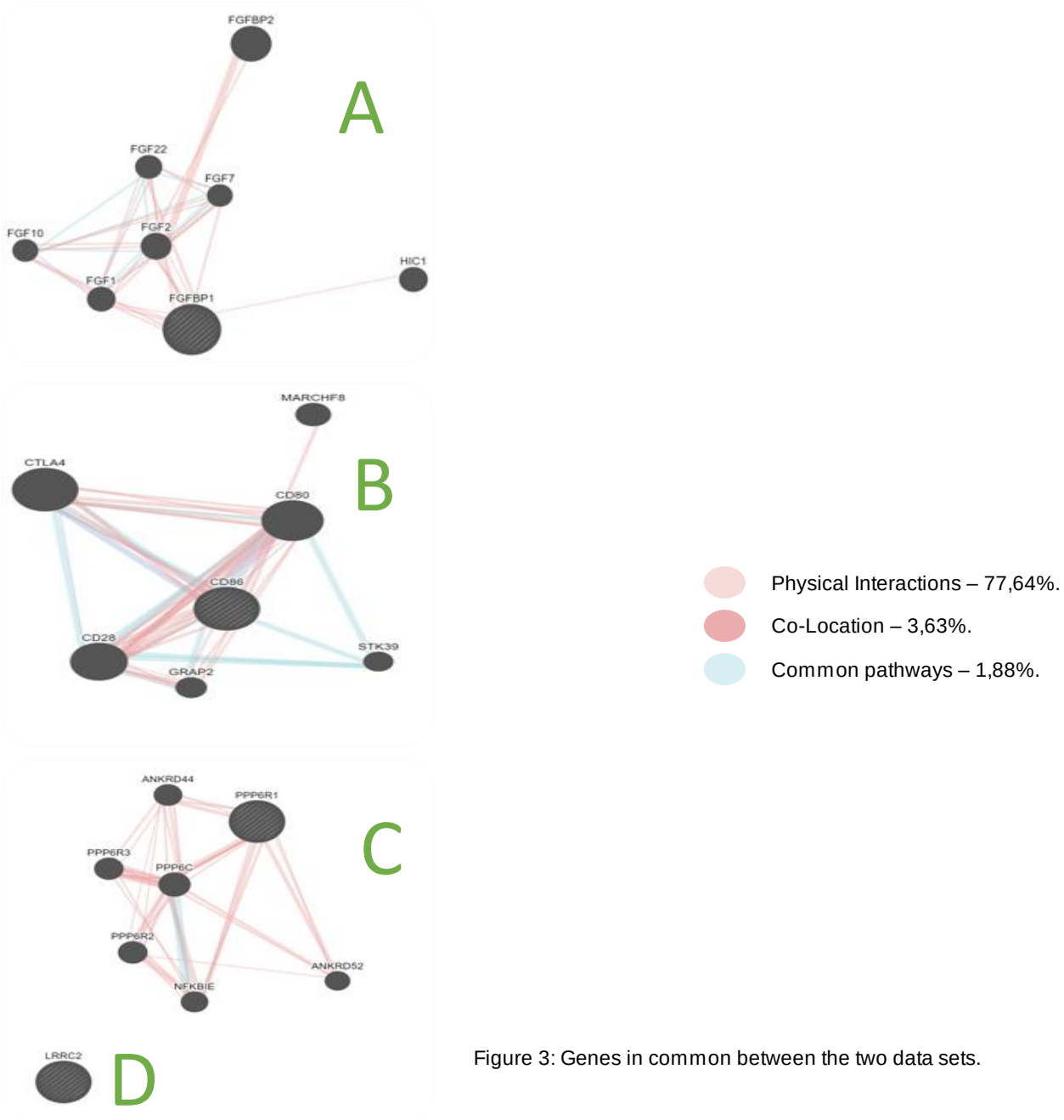
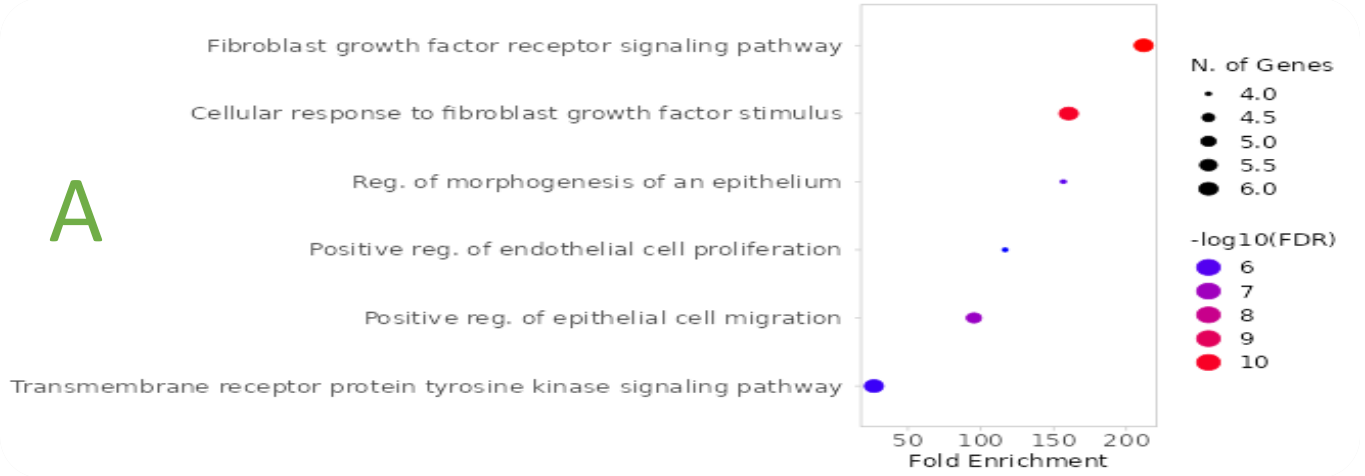


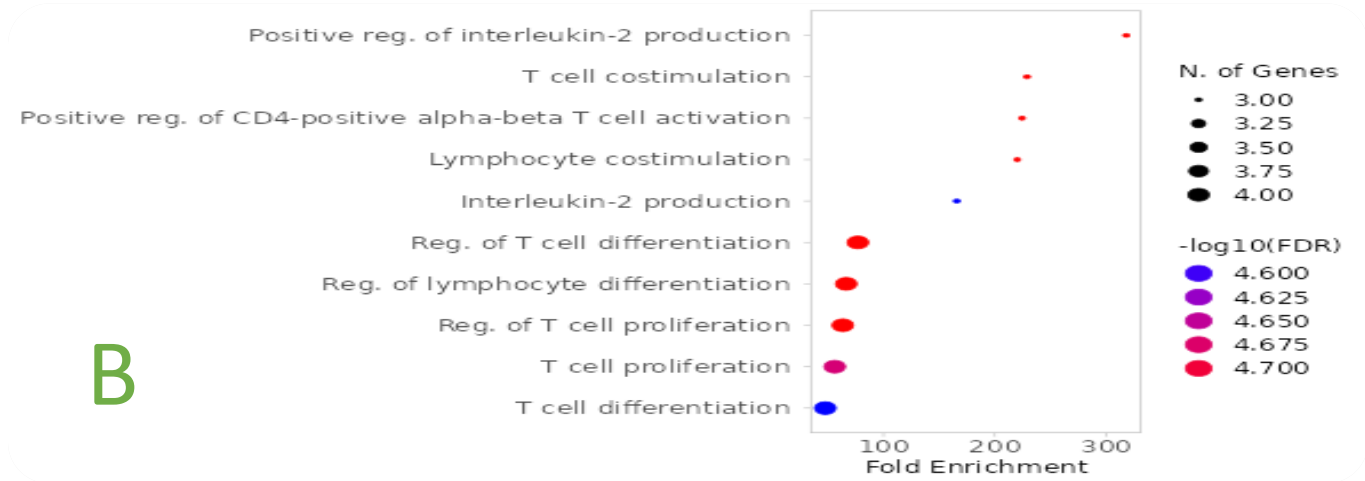
Figure 3: Genes in common between the two data sets.

FGFBP1 showed moderate physical interactions with FGF2, FGF7, FGF1, FGF10, and HIC1. Notably, FGF2 exhibited a strong interaction with FGFBP2. FGFBP1 plays a crucial role in neovascularization, a process that increases the recruitment of infiltrating inflammatory cells to the inflamed microenvironment of bronchial tissue, thereby impairing lung function (36). FGFBP1 acts as an extracellular chaperone for FGF-2, facilitating its release from the extracellular matrix and enabling it to bind to FGF receptors, which subsequently activates angiogenesis and tissue repair (37, 38, 39). Furthermore, FGFBP1 was upregulated in both studied datasets, as well as in human asthma samples and mouse models (40). In these models, the hyperactivation of the mTOR/STAT3/FGFBP1 pathway in the airway epithelium increases angiogenesis (40).

3.3-Funtional Enrichment of the common genes

To explore the Fold Enrichment of the four genes (FGFBP1, CD86, PPP6R1 and LRRC2), the Shiny Go tool was used, as we can see on figure 4 below.





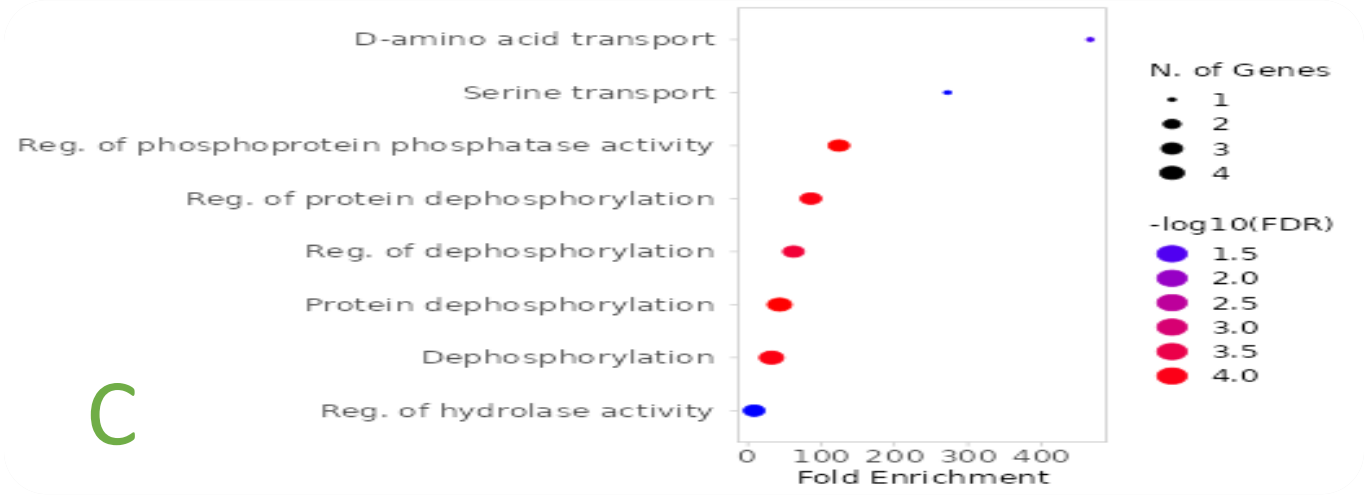


Figure 4: Graph of the genes in relation Fold Enrichment.

Functional enrichment analysis of this network (Fig.4A) revealed that "fibroblast growth factor receptor signaling pathway" and "cellular response to fibroblast growth factor stimulus" were the most enriched biological processes (Fig. 4C). Fibroblast growth factors (FGFs) bind to different FGF receptors (FGFRs) on the cell surface, initiating diverse intracellular pathways that lead to specific biological responses (41). FGFs and FGFRs play significant roles in airway wall remodeling in COPD patients (42). For instance, FGF1 levels are increased in the bronchial epithelium, FGF2 levels are elevated in both the bronchial epithelium and smooth muscle nucleus, and FGFR1 levels are raised in bronchial epithelial and smooth muscle cells (42). Additionally, depending on the type of FGF and FGFR, cellular responses can initiate various intracellular pathways, such as proliferation, differentiation, inflammation, and cell survival (41, 43). All this can be seen of detail on Tables 3 to 5 on the Attachments.

In the second network, CD86 revealed strong physical interactions with CTLA4, CD80, and CD28, and moderate interactions with MARCHF8 and GRAP2. CD86 is also co-localized with CD80 and CTLA4 with high confidence. All genes in the network (CD86, CD80, CTLA4, CD28, STK39, and GRAP2) share pathways, except for MARCHF8 (Fig. 4B). CD86 is involved in COPD development and its recruitment to pulmonary tissues by dendritic cells support its participation in the pathogenesis of COPD (45). Upregulation of CD80 and CD86 is common in dendritic cells from COPD patients (PMC4503082). Furthermore, CD80 and CD86 are the ligands for CD28 and CTLA-4, and this inflammatory pathway is frequently dysregulated in COPD patients (46). Conversely, MARCH8 is associated with poor prognosis (47) and GRAP2 is a prognostic biomarker (48) in lung cancer patients; however, their relationship with COPD remains unclear.

Functional enrichment analysis also supports these findings, showing a strong enrichment in inflammatory biological processes, particularly related to IL-2 production, TCD4+ activation, T cell stimulation, production, differentiation, and lymphocyte stimulation and differentiation (Fig. 4B).

CD86, along with CD80, are co-stimulatory molecules expressed on antigen-presenting cells that bind to CD28 on T cells, enhancing their activation and proliferation (49, 50). Conversely, CTLA-4 is an inhibitory receptor upregulated on activated T cells and competes with CD28 for binding to CD80/CD86

due to its higher affinity for these ligands (49, 51). CD28-mediated co-stimulation promotes T cell activation and inflammation by increasing the production of pro-inflammatory cytokines, such as IL-2, and IFN-γ. On the contrary, CTLA-4 provides a crucial inhibitory signal to maintain immune balance and prevents excessive inflammation (51).

In the last network, PPP6R1 shared a pathway with NFKBIE and exhibits moderate physical interactions with PPP6C, ANKRD44, NFKBIE, and ANKRD52 (Fig. 4C). PPP6R1, ANKRD44 and ANKRD52 are regulatory subunits that modulates the activity of the PP6 holoenzyme, particularly the catalytic subunit PPP6C (52, 53). Expression of both PPP6C and PPP6R1 is especially high in hematopoietic cells and lymphoid tissues, suggesting significant roles for the PP6 complex in immune cell function (52, 54). Moreover, PPP6R1 plays a role in the PP6-mediated dephosphorylation of NFKBIE, which inhibits NF-kB transcriptional activity thus containing inflammatory pathways promoted by NF-kB (55).

These findings are also supported by functional enrichment analysis, with the regulation of phosphoprotein phosphatase activity and protein dephosphorylation being the most enriched biological processes. Although these outcomes highlight the potential regulatory role of the inflammatory process of COPD, further research is needed to elucidate the proteins and pathways involved.

LRRK2 does not integrate into any network, however, it is a pivotal regulator of inflammatory pathways in immune cells. Its expression and activity are influenced by pro-inflammatory signals, such as interferon-γ (IFN-γ), in various immune cell types including B cells, T cells, macrophages, and non-classical monocytes (56). The involvement of LRRK2 in modulating inflammatory cytokine production occurs through pathways such as MAPK and TLR signaling (56, 57). While the precise mechanisms remain to be fully elucidated, further research is necessary to comprehend its role in the pathogenesis of COPD.

In conclusion, although this study highlights the identified genes of common interest in both sets of data, it also delimits the limitations of the study because smoking modifies genetic expressions, the sample size and the possible variability in the data collected show that future studies should look for larger and more representative samples. On the other hand, this study provides a set of genes that exist between a COPD patient and an ordinary person, whether a smoker or not, and also serves as valuable information for possible future studies as a guide and as existing limitations (58,59).

4-Conclusion

Through this study, we understand the importance of this disease (COPD) as it is one of the leading causes of death worldwide, as it is an irreversible inflammatory disease of the airways, caused predominantly by prolonged exposure to toxins and tobacco. In addition, with this study we were able to find common genes between differentially expressed genes in COPD patients and healthy individuals, in this study we associated 4 common genes between both data sets and then between the data samples (PPP6R1, FGFBP1, CD86 and LRRC2). Furthermore, we use these genes to explore an in-silico approach, we predict the level of enrichment of each biological process, as well as the number of gene sets and certainty for each level of enrichment of each gene. That being said, the FGFBP1 network is associated with the signaling pathway of fibroblast growth factors and their receptors; the CD86 network showed a strong relationship with inflammatory response mechanisms; and the PPP6R1 network highlighted its association with protein dephosphorylation. These results allowed us to highlight three genes over-expressed in COPD patients. Nevertheless, exist two limitations: first do develop are the data with few samples and the second is controls are not consistent, one is smoker and other not. Finally, continued research into COPD developing mechanims will address the gaps identified, and help improve clinical outcomes, leading to a reduction in the socio-economic burden of the disease. Progress in this area and the development of new therapeutics areis essential to providing a better quality of life for the millions of people affected by COPD around the world.

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6-Attachments

Table 1: GSE38974_GLP4133 dataset.

ID	Gene.symbol	log2(fold change)	-log10(Pvalue)
3017	2BC5	4.847	22.77
25287	ACADL	4.847	22.77
29650	ADAM10	4.847	22.77
8751	ADAM15	4.847	22.77
13524	ADAM18	4.847	22.77
8757	ADAM5	2.31	5.046
21793	ADAMTS4	3.156	3.818
11549	ADRA1A	2.015	8.619
43982	ADRA1A	4.847	22.77
209	AKU	4.847	22.77
5881	APOL6	4.148	12.943
33086	ARHGAP26	2.153	7.526
18643	ARNTL2	2.054	5.001
438	ASMT	4.847	22.77
445	ASS1	4.847	22.77
7973	AUT	2.079	7.007
17637	BOLA3-AS1	-2.073	3.005
6149	CABP7	-2.733	3.004
25194	CACNA1E	2.012	2.712
12424	CCK	4.847	22.77
12500	CD3D	4.847	22.77
16290	CD86	-2.94	4.4
982	CDB2	4.847	22.77
12630	CFI	2.346	4.754
10036	CHAF1A	2.054	8.575
12660	CHKA	4.847	22.77
11889	CHMP4B	2.061	15.125
9486	CHST10	4.847	22.77
29393	COL1A1	2.087	12.564
12836	COL7A1	4.847	22.77
10920	COPS8	4.847	22.77
1401	CRP	2.104	3.346
7990	CRS1C	4.847	22.77
1429	CRYZ	2.057	6.782
1465	CSRP1	2.006	8.827
12409	CTSS	2.143	7.211
6372	CXCL6	-2.846	14.095
7644	CXCL8	2.255	3.379
1638	DCT	2.226	4.556

13350	DGAT1	4.847	22.77
26125	DKFZP434A014	2.357	12.672
25889	DKFZP434B105	2.363	3.636
25982	DKFZP566D213	4.847	22.77
26067	DKFZP586P2421	2.02	8.112
1742	DLG4	2.012	8.705
7629	DLST	2.125	19.165
1767	DNAH5	4.847	22.77
25562	ELOA	2.295	4.81
6785	ELOVL4	4.847	22.77
8909	ENDOU	4.847	22.77
2045	EPHA7	4.847	22.77
2060	EPS15	2.105	8.349
14067	F5	4.847	22.77
14114	FBLN1	4.847	22.77
14127	FCER1G	2.177	3.512
30938	FGD3	2.199	3.79
14181	FGFBP1	2.062	3.291
18009	FOXE1	2.655	4.574
8456	FOXN1	4.847	22.77
2389	FRA4B	4.847	22.77
8087	FXR1	2.002	6.512
14431	GAMT	2.068	8.574
14472	GBX2	4.847	22.77
11308	GDF10	-2.051	4.682
14673	GNA12	2.497	14.866
33842	GNL3L	2.098	8.007
2811	GP1BA	4.847	22.77
2841	GPR18	4.847	22.77
27200	GPR79	4.847	22.77
17714	GRPEL2	2.288	3.452
25260	GSTT1	4.847	22.77
2963	GTF2F2	2.361	4.981
3008	H1-4	4.847	22.77
3019	H3F2	-2.224	11.137
25433	HBEGF	4.847	22.77
7909	HEMC	4.847	22.77
3139	HLA-L	4.847	22.77
34381	HNRNPA0	2.778	21.268
30438	HOXA13	4.847	22.77
5997	HRASLS5	2.396	17.029
30811	HUNK	4.847	22.77
3402	IDDM3	4.847	22.77

3403	IDDM4	-2.896	4.381
28420	IGHV3-53	4.847	22.77
28357	IGHVII-74-1	2.352	4.119
28350	IGHVIII-16-1	4.847	22.77
28869	IGKV6D-41	2.141	3.463
28836	IGLC5	4.847	22.77
28785	IGLV4-60	4.847	22.77
16176	IL1B	2.07	9.502
14530	IL6	2.027	2.187
32080	IL6	2.13	2.462
10592	IL6	2.256	2.767
42277	IL6	2.279	2.618
39120	IL6	2.28	2.803
22295	IL6	2.327	2.802
10073	IL6	2.358	2.842
28841	IL6	2.359	2.899
20756	IL6	2.374	2.937
36638	IL6	2.413	2.91
9118	INA	2.427	5.189
16431	ITM2A	4.847	22.77
3943	LDHAL4	4.847	22.77
16847	LEPR	4.847	22.77
40595	LMNB1	2.596	11.432
9170	LPAR2	2.008	9.163
26020	LRP10	2.326	3.796
45160	LRRC2	-3.209	4.655
21873	MACC1	2.332	8.297
9223	MAGI1	2.053	8.686
10982	MAPRE2	4.847	22.77
2011	MARK2	4.847	22.77
10150	MBNL2	2.226	3.662
8143	MDB	4.847	22.77
14494	METTL7A	2.688	10.68
4524	MTHFR	2.156	3.558
30644	MTMR2	4.847	22.77
25132	MYO5B	2.053	6.622
29104	N6AMT1	4.847	22.77
27163	NAAA	4.847	22.77
3071	NCKAP1L	4.847	22.77
4752	NEK3	2.132	9.076
4810	NHS	2.055	3.216
18094	NKX2-8	4.847	22.77
4830	NME1	2.271	3.713

4851	NOTCH1	2.564	5.518
5098	NR4A2	2.036	3.147
10718	NRG3	4.847	22.77
18245	OAZ1	4.847	22.77
24610	ODF1	4.847	22.77
26218	OR1N1	4.847	22.77
8393	OR20A1P	2.314	3.949
26215	OR2A8	2.402	14.296
26349	OR5D1P	2.191	3.471
26595	OR8B2	4.847	22.77
18599	PADI1	2.154	8.554
25849	PARM1	2.234	9.062
5132	PDC	4.847	22.77
24627	PDE4D	4.847	22.77
5147	PDE6D	2.118	8.957
5181	PEPA	4.847	22.77
5824	PEX19	4.847	22.77
6906	PFKFB3	-3.441	10.599
17497	PHLDA2	2.047	5.712
30955	PIK3CG	2.352	8.099
18720	PIP5K1A	4.847	22.77
25692	PLAT	4.847	22.77
25031	PLCB4	4.847	22.77
25953	PNKD	2.15	6.759
18988	POUF2F	4.847	22.77
10105	PPIF	2.108	3.313
37734	PPP1R3C	-2.059	4.564
13572	PPP6R1	-2.159	2.601
30440	PRIM1	2.354	4.918
29037	PRO0195	4.847	22.77
28933	PRO0461	2.368	4.937
30259	PRPH	4.847	22.77
5804	PRPRZ2	4.847	22.77
19172	PSMB4	4.847	22.77
5706	PSMC6	2.154	3.592
19200	PSTPIP1	4.847	22.77
29222	PTMA	2.163	3.468
23475	QPRT	4.847	22.77
6035	RNASE1	4.847	22.77
40524	RNF125	2.216	9.777
6093	ROCK1	4.847	22.77
6167	RPL37	4.847	22.77
6194	RPS6	2.559	5.553

20111	RPS6KA1	4.847	22.77
23200	S100A9	2.361	6.318
10698	SALL1P	4.847	22.77
20394	SCG5	2.39	4.925
6393	SDU	2.139	9.273
6412	SEN6A	4.847	22.77
26135	SERBP1	2.175	3.664
9869	SETDB1	4.847	22.77
29309	SFTPA1	-2.08	7.783
6583	SLC22A4	4.847	22.77
20526	SLC2A2	4.847	22.77
6569	SLC34A1	4.847	22.77
40565	SLC39A7	2.106	11.666
7823	SLC43A2	2.111	17.053
20535	SLC4A2	4.847	22.77
38649	SMG1P5	2.033	9.743
8822	SMG1P5	2.183	10.81
6654	SOS1	4.847	22.77
35814	SPP1	2.014	2.831
21320	SPP1	2.059	3.152
23976	SPP1	2.082	3.231
3830	SPP1	2.144	3.441
1083	SPP1	2.156	3.635
15407	SPP1	2.216	3.845
17188	SPP1	2.219	3.739
19546	SPP1	2.352	4.347
6741	SSB	2.064	7
20856	STC2	2.402	13.334
8801	SUCLG2	4.847	22.77
25830	SULT4A1	4.847	22.77
29643	SV2C	2.112	8.688
25650	TAF5L	2.307	12.003
6895	TARBP2	4.847	22.77
21385	TBX2	2.101	15.686
22180	TDRD10	-2.321	11.924
6086	TEP1	2.857	12.719
7046	TGFBR1	4.847	22.77
26521	TIMM8B	4.847	22.77
10430	TMEM147	2.366	4.912
14679	TMEM201	2.205	15.818
9087	TMSB4Y	4.847	22.77
37785	TNFRSF1A	2.006	15.623
6075	TNFRSF1A	2.021	16.114

37998	TNFRSF1A	2.042	15.314
18427	TNFRSF1A	2.049	16.754
13434	TRDMT1	4.847	22.77
4556	TRNE	2.419	5.089
7250	TSC3	4.847	22.77
7254	TSHRL1	4.847	22.77
7295	TXN	4.847	22.77
7343	UBTF	2.051	8.458
7346	UCHL2	2.138	9.183
22236	UGT1A2P	2.032	8.224
23032	USP33	4.847	22.77
27042	UTP25	4.847	22.77
29461	VGF	2.121	3.455
27377	YME1L1	4.847	22.77
7705	ZNF146	2.078	8.556
6203	ZNF652	2.36	9.527
35404		2.003	10.556
14754		2.011	8.39
31748		2.013	5.755
38362		2.018	6.586
12872		2.023	8.61
41538		2.024	8.086
34745		2.028	8.515
30487		2.036	8.176
44697		2.041	8.188
8977		2.059	6.694
35749		2.059	8.278
32581		2.07	8.009
43026		2.075	6.477
43334		2.101	6.762
34724		2.106	3.481
38292		2.118	3.515
41139		2.119	3.719
43142		2.122	8.738
27162		2.125	3.549
14817		2.129	8.67
40990		2.13	3.438
19984		2.133	3.512
35023		2.138	6.901
21545		2.146	3.515
39176		2.147	8.703
14574		2.148	3.522
43116		2.162	9.133

15246		2.191	3.559
33715		2.226	17.977
43729		2.23	4.43
43257		2.235	3.702
16550		2.253	3.611
33668		2.264	3.674
38403		2.266	3.682
44807		2.267	3.834
24463		2.285	4.71
18528		2.295	3.809
14072		2.296	3.896
21720		2.299	4.734
35717		2.301	3.849
37758		2.338	4.821
30311		2.347	4.786
21032		2.349	4.843
31734		2.351	3.925
27661		2.356	4.815
40513		2.369	4.809
21904		2.379	3.916
20077		2.38	4.104
16158		2.399	5.118
15031		2.43	5.198
14993		2.444	5.143
37812		2.45	5.14
25030		2.494	4.233
33615		-2.495	2.009
15346		2.54	4.29
34716		2.549	5.274
34905		2.565	5.578
27889		2.625	5.664
17554		-2.712	2.098
40820		-2.944	3.608
36120		-3.007	4.195
32385		-3.849	5.398
9222		4.847	22.77
11868		4.847	22.77
12230		4.847	22.77
12699		4.847	22.77
13978		4.847	22.77
14091		4.847	22.77
14487		4.847	22.77
14746		4.847	22.77

14831		4.847	22.77
14861		4.847	22.77
14956		4.847	22.77
14957		4.847	22.77
14972		4.847	22.77
15198		4.847	22.77
16644		4.847	22.77
16674		4.847	22.77
16940		4.847	22.77
17226		4.847	22.77
17280		4.847	22.77
17725		4.847	22.77
19100		4.847	22.77
20152		4.847	22.77
21083		4.847	22.77
21113		4.847	22.77
21257		4.847	22.77
21378		4.847	22.77
22305		4.847	22.77
24177		4.847	22.77
24228		4.847	22.77
24648		4.847	22.77
24963		4.847	22.77
25221		4.847	22.77
27637		4.847	22.77
27724		4.847	22.77
27968		4.847	22.77
28047		4.847	22.77
28168		4.847	22.77
29552		4.847	22.77
30024		4.847	22.77
30472		4.847	22.77
30577		4.847	22.77
30682		4.847	22.77
30697		4.847	22.77
31309		4.847	22.77
31416		4.847	22.77
31620		4.847	22.77
32165		4.847	22.77
32340		4.847	22.77
32467		4.847	22.77
32994		4.847	22.77
33004		4.847	22.77

33314		4.847	22.77
33321		4.847	22.77
33420		4.847	22.77
33807		4.847	22.77
34186		4.847	22.77
34375		4.847	22.77
34455		4.847	22.77
34603		4.847	22.77
35283		4.847	22.77
35562		4.847	22.77
35896		4.847	22.77
35954		4.847	22.77
36030		4.847	22.77
36148		4.847	22.77
36303		4.847	22.77
36349		4.847	22.77
36696		4.847	22.77
36771		4.847	22.77
36861		4.847	22.77
37116		4.847	22.77
37281		4.847	22.77
37304		4.847	22.77
37311		4.847	22.77
37556		4.847	22.77
38111		4.847	22.77
38139		4.847	22.77
38331		4.847	22.77
38633		4.847	22.77
38738		4.847	22.77
38881		4.847	22.77
39246		4.847	22.77
39307		4.847	22.77
39405		4.847	22.77
39483		4.847	22.77
39527		4.847	22.77
39570		4.847	22.77
39897		4.847	22.77
39898		4.847	22.77
40003		4.847	22.77
40221		4.847	22.77
40402		4.847	22.77
40853		4.847	22.77
41209		4.847	22.77

42198		4.847	22.77
42363		4.847	22.77
42412		4.847	22.77
42538		4.847	22.77
42563		4.847	22.77
42995		4.847	22.77
43268		4.847	22.77
43337		4.847	22.77
43770		4.847	22.77
43786		4.847	22.77
44083		4.847	22.77
44125		4.847	22.77
44597		4.847	22.77

Table 2: GSE106986 dataset.

ID	Genes	log2(fold change)	-log10(Pvalue)
A_23_P112482	AQP3	2.061	4.386
A_23_P117157	SUCLA2	2.022	3.219
A_23_P118158	HS3ST2	2.015	4.107
A_23_P119448	PPP6R1	-2.743	4.019
A_23_P126278	CHIT1	2.741	3.574
A_23_P137238	KDM5D	3.463	3.601
A_23_P148088	FGG	4.275	5.029
A_23_P155463	LRRC2	-2.927	3.539
A_23_P165624	TNFAIP6	2.119	4.089
A_23_P206077	AEN	2.492	5.668
A_23_P259314	RPS4Y1	6.1	4.013
A_23_P30126	FGFBP1	2.651	3.609
A_23_P324384	RPS4Y2	5.461	3.438
A_23_P369899	TMEM158	2.416	4.18
A_23_P375372	FGA	2.173	3.09
A_23_P39550	TMEM163	2.967	5.201
A_23_P6119	SEC23B	2.431	4.003
A_23_P73848	NCRNA00185	3.906	3.578
A_23_P81507	FAT2	-3.423	4.123
A_23_P92202	GMPPB	2.134	4.045
A_24_P12397	TREM2	2.166	3.353
A_24_P131589	CD86	-3.863	3.481
A_24_P148450	UBE2E3	2.042	3.251
A_24_P153324	DEFB123	2.276	3.169

A_24_P158089	SERPINE1	2.887	3.607
A_24_P18802	VPS18	-2.059	4.405
A_24_P227141	ELF5	2.123	3.664
A_24_P271696	XAGE1A	3.006	3.955
A_24_P297078	C20orf3	2.535	3.492
A_24_P413669	PFKFB2	2.023	4.883
A_24_P57367	AHCY	2.096	2.906
A_24_P932736	HMBOX1	-4.317	3.972
A_24_P941736	ACSBG1	-2.487	4.929
A_32_P107029	NAPSA	2.132	3.913
A_32_P190049	LRRC58	2.234	3.858
A_32_P62963	KRT16P2	2.194	3.701
A_32_P75902	C16orf73	-2.131	5.036
A_33_P3213832		-2.48	4.826
A_33_P3217700	USP9Y	3.872	3.57
A_33_P3224331	DDX3Y	4.637	3.634
A_33_P3236416	GPR179	-3.211	4.092
A_33_P3241511	SERPIND1	3.42	3.693
A_33_P3260223	TXLNG2P	3.935	3.272
A_33_P3283201		-2.12	3.363
A_33_P3284253	GYG2	2.753	2.873
A_33_P3290403	IMPA2	2.354	3.214
A_33_P3291976	TERF1	-2.14	3.6
A_33_P3316770	SLCO1A2	-2.085	3.999
A_33_P3320808	NDUFS7	-3.03	4.65
A_33_P3323934		-2.057	3.362
A_33_P3336822		2.021	2.884
A_33_P3337609		2.066	2.931
A_33_P3341686	XIST	-7.122	3.241
A_33_P3347417	SPEN	-2.36	4.292
A_33_P3347503		-2.065	3.384
A_33_P3366296	C14orf23	-2.156	3.679
A_33_P3382276	ST6GAL1	-2.106	3.171
A_33_P3424754		2.407	3.129
A_33_P3576317	LOC340178	-2.285	4.61

Table 3: Fold Enrichment table of figure 4A.

Enrichment FDR	nGenes	Pathway Genes	Fold Enrichment	Pathways
4.1E-11	6	81	211.9	Fibroblast growth factor receptor signaling pathway GO:0008543
1.0E-10	6	107	160.4	Cellular response to fibroblast growth factor stimulus GO:0044344
8.7E-07	4	73	156.7	Reg. of morphogenesis of an epithelium GO:1905330
2.0E-06	4	98	116.7	Positive reg. of endothelial cell proliferation GO:0001938
1.4E-07	5	150	95.3	Positive reg. of epithelial cell migration GO:0010634
1.4E-06	6	642	26.7	Transmembrane receptor protein tyrosine kinase signaling pathway GO:0007169

Table 4: Fold Enrichment table of figure 4B.

Enrichment FDR	nGenes	Pathway Genes	Fold Enrichment	Pathways
1.9E-05	3	36	317.8	Positive reg. of interleukin-2 production GO:0032743

1.9E-05	3	50	228.8	T cell costimulation GO:0031295
1.9E-05	3	51	224.3	Positive reg. of CD4-positive alpha-beta T cell activation GO:2000516
1.9E-05	3	52	220	Lymphocyte costimulation GO:0031294
2.5E-05	3	69	165.8	Interleukin-2 production GO:0032623
1.9E-05	4	198	77	Reg. of T cell differentiation GO:0045580
1.9E-05	4	229	66.6	Reg. of lymphocyte differentiation GO:0045619
1.9E-05	4	240	63.6	Reg. of T cell proliferation GO:0042129
2.1E-05	4	271	56.3	T cell proliferation GO:0042098
2.5E-05	4	319	47.8	T cell differentiation GO:0030217

Table 5: Fold Enrichment table of figure 4C.

Enrichment FDR	nGenes	Pathway Genes	Fold Enrichment	Pathways
3.4E-02	1	7	467	D-amino acid transport GO:0042940
4.6E-02	1	12	272.4	Serine transport GO:0032329

7.7E-05	3	79	124.1	Reg. of phosphoprotein phosphatase activity GO:0043666
9.3E-05	3	114	86	Reg. of protein dephosphorylation GO:0035304
2.0E-04	3	157	62.5	Reg. of dephosphorylation GO:0035303
7.7E-05	4	301	43.4	Protein dephosphorylation GO:0006470
9.3E-05	4	403	32.4	Dephosphorylation GO:0016311
4.6E-02	3	1128	8.7	Reg. of hydrolase activity GO:0051336