

Research Article

# Identification of KIF4A and its effect on the progression of lung adenocarcinoma based on the bioinformatics analysis

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**Background:** Lung adenocarcinoma (LUAD) is the most frequent histological type of lung cancer, and its incidence has displayed an upward trend in recent years. Nevertheless, little is known regarding effective biomarkers for LUAD.

**Methods:** The robust rank aggregation method was used to mine differentially expressed genes (DEGs) from the gene expression omnibus (GEO) datasets. The Search Tool for the Retrieval of Interacting Genes (STRING) database was used to extract hub genes from the protein–protein interaction (PPI) network. The expression of the hub genes was validated using expression profiles from TCGA and Oncomine databases and was verified by real-time quantitative PCR (qRT-PCR). The module and survival analyses of the hub genes were determined using Cytoscape and Kaplan–Meier curves. The function of KIF4A as a hub gene was investigated in LUAD cell lines.

**Results:** The PPI analysis identified seven DEGs including BIRC5, DLGAP5, CENPF, KIF4A, TOP2A, AURKA, and CCNA2, which were significantly upregulated in Oncomine and TCGA LUAD datasets, and were verified by qRT-PCR in our clinical samples. We determined the overall and disease-free survival analysis of the seven hub genes using GEPIA. We further found that CENPF, DLGAP5, and KIF4A expressions were positively correlated with clinical stage. In LUAD cell lines, proliferation and migration were inhibited and apoptosis was promoted by knocking down KIF4A expression.

**Conclusion:** We have identified new DEGs and functional pathways involved in LUAD. KIF4A, as a hub gene, promoted the progression of LUAD and might represent a potential therapeutic target for molecular cancer therapy.

## Background

Lung cancer is one of the most frequent malignant tumors in clinical practice [1]. Its morbidity and mortality have ranked first among all types of malignant tumors, and it has been recognized as the most widespread cancer in the world [2]. More than 80% of lung cancers are non-small cell lung cancers (NSCLC), and lung adenocarcinoma (LUAD) accounts for over 70% of NSCLC [3,4]. In recent years, the treatment of LUAD has continuously improved, and a series of new diagnosis and treatment technologies have been applied in clinical practice, which can improve the treatment outcomes of LUAD patients to some extent [5–7]. Nevertheless, the 5-year survival rate is still very low, and outcomes are still unsatisfactory [8]. At present, early diagnosis and treatment can greatly improve prognosis and survival rates of LUAD patients [9,10]. Therefore, it is crucial to fully elucidate the molecular mechanism involved in LUAD.

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In recent years, with the development of microarrays and high-throughput sequencing technologies allowing large open data resources, such as The Cancer Genome Atlas (TCGA) database and Gene Expression Omnibus (GEO) datasets, large amounts of genetic data have been generated [10–12]. Researchers can effectively mine big data to identify novel cancer-related genes [13–15]. At present, how to extract valuable information from this huge database has become a new research direction, and bioinformatics has effectively solved this problem. Bioinformatics can screen for available and mine the microarray data, and ultimately applies relevant software to graph the results, so as to reveal genes potentially implicated in oncogenesis at the molecular level [16,17]. Consequently, bioinformatics can provide new diagnostic markers and novel ideas for the early diagnosis and treatment of LUAD.

The purpose of the present study was to identify the potential key genes and pathways associated with the carcinogenesis of LUAD. We first compared the gene expression profiles of LUAD and adjacent normal lung tissues utilizing the data in the GEO public database. Next, we systematically analyzed the expression profiles of microarray data and screened DEGs. Meanwhile, we determined the Gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and protein–protein interactions (PPIs) network analyses of the robust DEGs in LUAD. Further, we also obtained and analyzed the expression and prognosis of the related hub genes using the Oncomine database and the TCGA and GTEx datasets in GEPIA. We validated the mRNA expression of the seven hub genes in clinical samples of LUAD and in paired normal tissues. Furthermore, we investigated the potential functional mechanism of KIF4A in regulating LUAD cell proliferation, migration, and apoptosis.

## Materials and methods

### Data acquisition and preprocessing

Processed microarray data were searched from the websites of GEO (available: <http://www.ncbi.nlm.nih.gov/geo/>) using “LUAD” as the keyword. Of these, we selected and downloaded the GSE85716, GSE32863, and GSE116959 datasets (Supplementary Table S1). DEGs were identified by the Bioconductor limma package and the robust rank aggregation (RRA) method was used to integrate and rank all of the DEGs from three GEO datasets. In addition, the edgeR package was used to screen DEGs with thresholds of  $|\log_2\text{fold change(FC)}| > 1$  and the thresholds of the adjusted p-value false discovery rate (FDR)  $< 0.05$ .

### Hierarchical clustering analysis

The top 20 robust DEGs were determined using the robust RRA method. As in a previous report [18], the R language package was applied for hierarchical clustering analysis based on the TPM values of the genes in each group. In the analysis, TPM values represented the expression levels of genes, and the gene expression patterns were further clustered into the same or similar genes. In the clustergram, differences in subregional information were represented using different colors, and the different experimental conditions were determined using the clustering pattern control model.

### GO analysis

Direct annotation of gene function can obtain multiple and complicated functional nodes, which can result in the redundancy of results. Thus, we applied the GO analysis method to extract and integrate data and further increase the reliability of the gene analysis. GO analysis mainly includes cellular components (CC), molecular functions (MF), and biological processes (BP). In the present study, GO enrichment analysis of DEGs was also analyzed using the Bioinformatics Tool (DAVID, version 6.8, <https://david.ncifcrf.gov/>) [19,20].

### KEGG analysis

KEGG analysis, created by the Kanehisa Laboratories, is a collection of databases that can integrate genomes, biological pathways, diseases, drugs, and chemicals [21]. KOBAS 2.0 software (<http://kobas.cbi.pku.edu.cn/>) was used to carry out KEGG pathway enrichment analysis for the DEGs in LUAD [22]. A *P*-value  $< 0.05$  was used as the screening threshold to explore the biological signaling pathways involved in DEGs in LUAD.

### PPI analysis

The STRING (<http://string-db.org/>) is a database that can search for and predict the interactions between known proteins [23]. The online software string (comprehensive score  $> 0.9$ ) was adopted to analyze the PPIs of DEGs, and then a PPI diagram including the DEGs was drawn based on the analysis. Meanwhile, a number of nodes in the network was obtained to denote genetic relationships through network structure analysis, and the core genes were defined by the number of nodes in the network. Cytoscape software was used to generate the PPI network diagram.

The software includes two algorithms: MCC and DMNC, which can calculate the top 10 genes and then cross-cut their intersections. The hub genes were obtained from the PPI network and were then subjected to verification of their expression, survival analysis, and other analyses.

## Application of oncomine database

The Oncomine database (<http://www.oncomine.org>) was utilized to obtain the expression of seven hub genes in LUAD and in adjacent normal lung tissues. The screening conditions were as follows: (1) Genes: BIRC5, DLGAP5, CENPF, KIF4A, TOP2A, AURKA, or CCNA2; (2) Analysis Type: LUAD versus Normal Analysis; (3) Threshold using  $P$ -value < 0.05; FC: All; Gene Rank: All.

## Gene expression profiling interactive analysis (GEPIA) database

GEPIA is a public database established for expression profiling analysis of cancer and normal genes [24]. GEPIA analysis contains the expression analysis of RNA sequencing data from 9736 tumors and 8587 normal samples in TCGA (<http://cancergenome.nih.gov/>) [25] and Genotype-Tissue Expression (GTEx, <http://commonfund.nih.gov/GTEx/>) projects [26,27]. In our study, the expression of the seven hub genes was extracted from the TCGA database. In addition, the survival and disease-free survival analyses of the seven hub genes were also obtained based on TCGA and the GTEx data in GEPIA. Moreover, we also obtained the correlation of CENPF, DLGAP5, KIF4A, and the clinical stage of LUAD.

## Total RNA isolation and real-time quantitative PCR (qRT-PCR)

In total, samples from 10 LUAD patients (along with paired normal lung samples) that had undergone surgical treatment in our hospital, were available for examination in our study. All collected tissues were confirmed histologically by two independent pathologists. Written consent was obtained from all patients who were recruited. This research was approved by the Ethics Committee of the First Hospital of Changsha.

Total RNA from LUAD samples ( $n=10$ ) and paired normal samples ( $n=10$ ) was isolated using the RNeasy Mini Kit (Cat.74101, Qiagen, Germany) according to the manufacturer's instructions. The synthesis of cDNA used for genes was performed using the Bestar™ qPCR RT kit (DBI; #DBI-0) using 2  $\mu$ g RNA. The relative mRNA levels of BIRC5, DLGAP5, CENPF, KIF4A, TOP2A, AURKA, and CCNA2 were determined by real-time quantitative PCR (qRT-PCR) using a 20  $\mu$ l reaction system. The PCR process was performed on an ABI PRISM 7500 real-time PCR system (Applied Biosystems, Carlsbad, CA, U.S.A.) using the following settings: 95°C for 2 min, followed by 40 cycle of 94°C for 20 s, 58°C for 20 s, and 72°C for 20 s. GAPDH was used as the internal normalized reference gene. The fold change was determined as:  $2^{-\Delta\Delta Ct}$  ( $\Delta\Delta Ct = (\Delta Ct \text{ of genes of interest}) - (\Delta Ct \text{ of GAPDH})$ ). The primer sequences are listed in Supplementary Table S2.

## Cell culture and reagents

The human LUAD cell lines HCC827 and A549 were purchased from AbZyme Biotechnology Inc (Jiangsu, China). The human bronchial epithelial cells Beas2B were purchased from Zhong Qiao Xin Zhou Biotechnology Inc (Shanghai, China). Beas2B cells were grown in DMEM (Invitrogen Life Technologies, Carlsbad, CA, U.S.A.) supplemented with 5% fetal bovine serum (FBS, Gibco BRL, Gaithersburg, MD, U.S.A.). HCC827 and A549 cells were cultured in RPMI-1640 (Hyclone, Logan, UT, U.S.A.) supplemented with 10% FBS. Rabbit monoclonal antibodies against KIF4A were obtained from Abcam (#ab124903, Abcam, Cambridge, MA, U.S.A.).

## Western blot analysis

After treatment, the cells were collected, washed and lysed with ice-cold RIPA lysis buffer (Beyotime Inst. Biotech) with 1 mmol/l PMSF. Protein concentrations were calculated using BCA assay kits (Beyotime Inst. Biotech). Total cellular protein (20  $\mu$ g) was subjected to 12% SDS-PAGE and transferred to PVDF membranes (Millipore). The membranes were blocked with 5% nonfat milk powder at room temperature for 2 h, followed by immunoblotting with primary antibodies at 4°C overnight and immunoblotting with HRP-conjugated secondary antibody at room temperature for 1 h. Following each step, the membranes were washed three times with PBST for 5 min. Finally, the blots were developed using an enhanced chemiluminescence system (Pierce). Actin was used as a loading control.

## Colony formation assay

Five hundred exponentially growing cells were plated in six-well cell culture plates in a total volume of 2 ml of medium and incubated for 6 h. Next, 2 mM of KIF4A-siRNA was added to the medium, and incubation continued for 6 h.

Following replacement with fresh medium, colony formation was monitored. Ten days later, the colonies were fixed with 75% ethanol and stained with methylene blue. The optical density (OD) values were detected using a microplate reader.

### Flow cytometry for quantitative analysis of apoptosis

After treatment,  $3 \times 10^5$  cells were collected used for each sample. For apoptosis detection, cells were stained using the Annexin V-FITC Apoptosis Detection Kit I (BD Biosciences, San Diego, CA) according to the manufacturer's recommendation. The stained cells were determined by flow cytometry (BD FACS Canto) and analyzed by the FCS Express v2.0 software, as in our previous studies [28].

### Transwell assay

Cells were seeded in the chamber with  $5 \times 10^4$  cells in 100  $\mu$ l FBS-free culture medium. A 600  $\mu$ l volume of culture medium (10% FBS) was added to the 24-well plate. After a 24 h incubation, the suspended cells were washed and removed, then fixed with methylalcohol for 30 min. Subsequently, the non-migrated cells were removed and the migrated cells were stained with 1% Crystal Violet. The OD values were detected using a microplate reader as described in our previous studies [29].

### RNA interference to inhibit KIF4A expression

A mixture of 200 nM KIF4A-siRNA (KIF4A-siRNA-1: GGAACAGGGCAACAACACTCT; KIF4A-siRNA-2: TAAG-GATACCCTTCTATCT; KIF4A-siRNA-3: TGCTGTTTGAGGAACGAAA) (HonorGene, Changsha, China) and control siRNA were added to each well of a six-well plate for 6 h, after which fresh RPMI media containing 10% serum was added. Cells were harvested 48 h later.

### Statistical analysis

All statistical analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL, U.S.A.). All of the data are presented as mean  $\pm$  standard deviation (SD). Statistical significance comparing two groups was evaluated by the Student's *t* test. A *P*-value < 0.05 was considered statistically significant.

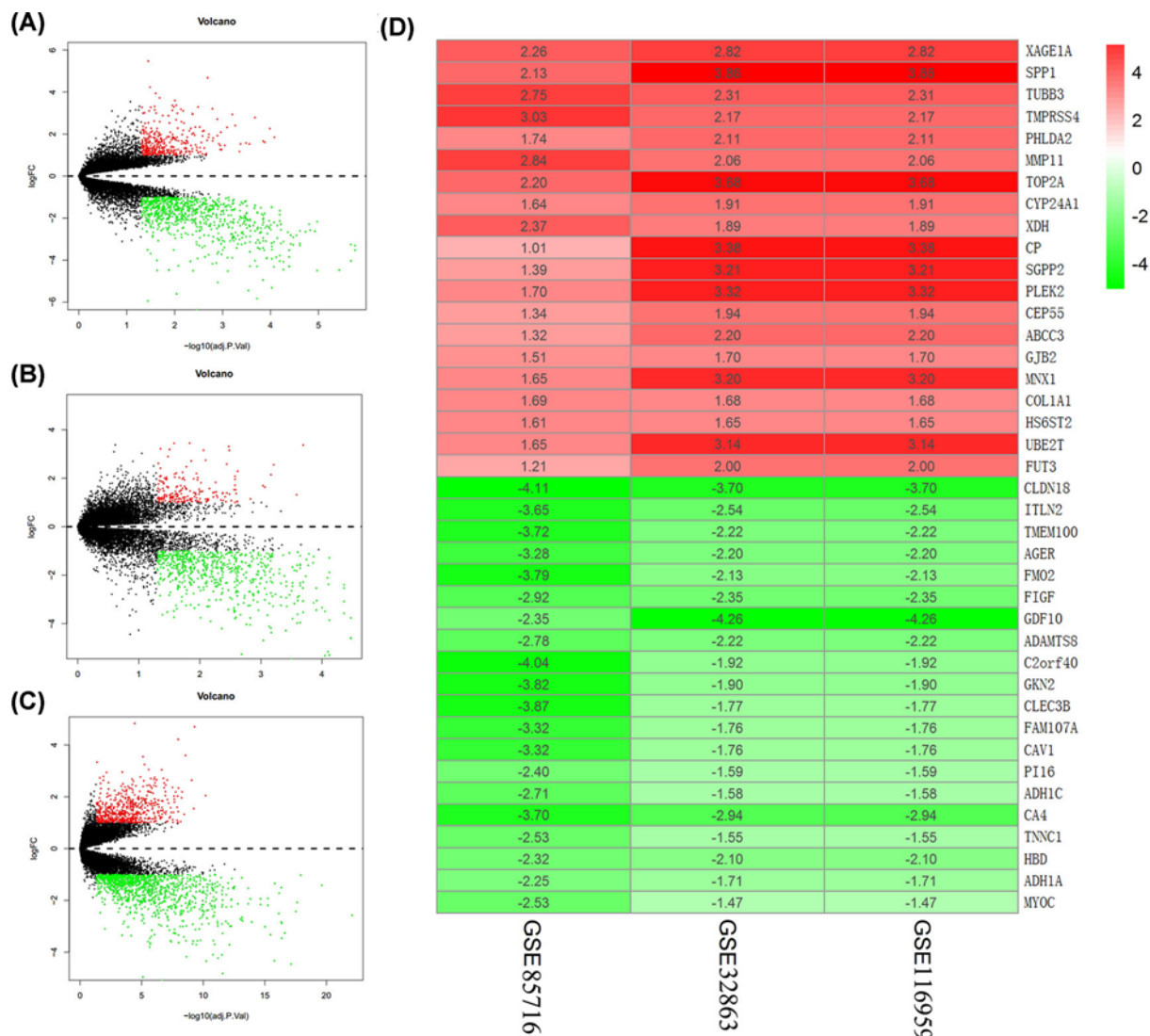
## Results

### Identification of DEGs from GSE85716, GSE32863, and GSE116959 datasets

To identify genes that might be involved in the tumorigenesis of LUAD, we analyzed the DEGs in the GSE85716, GSE32863, and GSE116959 datasets. We used a Volcano plot to present DEGs in LUAD and nontumor lung tissues based on the data from these three databases; the red dots indicated genes that were up-regulated in LUAD, and the green dots indicated genes that were down-regulated in LUAD (Figure 1A–C). Next, we screened the top 20 up-regulated and down-regulated genes in the GSE85716, GSE32863, and GSE116959 datasets, respectively, and the differential distribution of DEGs between adjacent normal lung and LUAD tissues was displayed using the hierarchical clustering (Figure 1D). The up- and downregulated DEGs are listed in Supplementary Tables S3 and 4.

### GO and KEGG analyses of robust DEGs in LUAD

Based on the expression profile of DEGs between adjacent normal lung and LUAD tissues, GO and KEGG analyses for DEGs were performed. The results of GO analysis revealed that the up-regulated DEGs were enriched in CC terms (nucleosome, centriole, spindle microtubule, nuclear chromosome, microtubule, nucleoplasm, chromosome passenger complex, and nucleus) and in BP terms (G2/M transition of mitotic cell cycle, DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest, collagen catabolic process, and chromosome segregation) (Figure 2A). The down-regulated DEGs were enriched in CC terms (plasma membrane part, integral to plasma membrane, intrinsic to plasma membrane, and extracellular region) and BP terms (blood vessel development, vasculature development, angiogenesis, cell adhesion, biological, and blood vessel morphogenesis) (Figure 2B). The results of the GO function annotation for the up-regulated and down-regulated DEGs in LUAD are shown in Tables 1 and 2. Furthermore, KEGG pathway analysis disclosed that the up-regulated DEGs mainly participated in regulating glycosaminoglycan biosynthesis-heparan sulfate/heparin, mismatch repair, and biosynthesis of amino acids (Figure 2C); the down-regulated DEGs mainly participated in regulating neuroactive ligand–receptor interactions, metabolic pathways, and the PI3K-Akt signaling pathway (Figure 2D). The detailed KEGG pathways for the up-regulated and down-regulated DEGs in LUAD are shown in Tables 3 and 4.



**Figure 1. Identification of DEGs from GSE85716, GSE32863, and GSE116959 datasets**

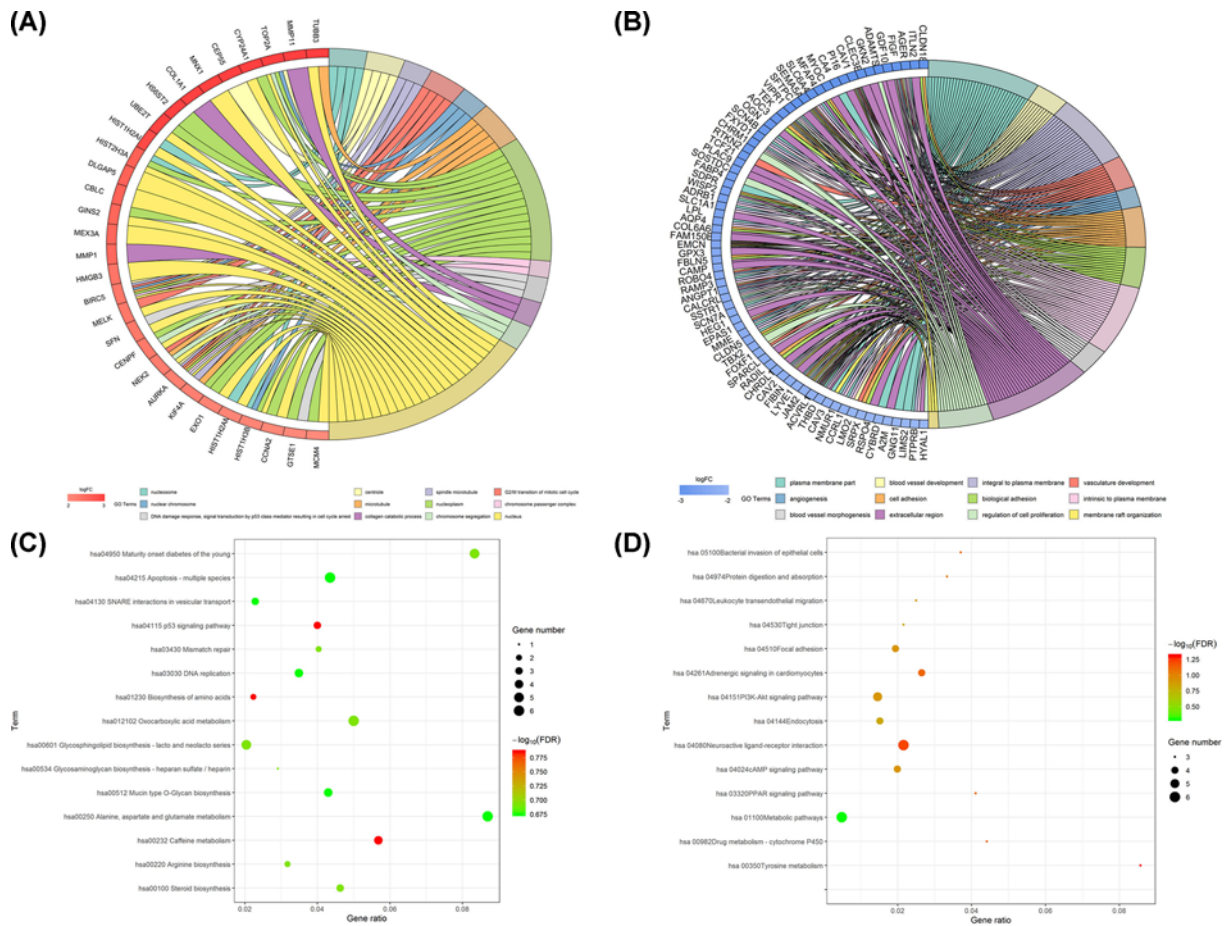
(A–C) Volcano plot showing the DEGs of the GSE85716, GSE32863, and GSE116959 datasets. (D) Hierarchical clustering comparing the top 20 up-regulated and down-regulated genes in adjacent normal lung tissues and LUAD tissues.

## Establishment of the PPI network and module analyses of genes in LUAD

To further predict the underlying functions of DEGs, we analyzed any potential co-expressed mRNAs of these DEGs and determined their associations by PPIs. As presented in Figure 3A, the entire PPI network revealed potential interrelationships between a number of genes, which might provide a foundation for the pathway research in LUAD. Moreover, we also analyzed two of the modules, and the relative networks are displayed in Figure 3B,C. Genes with the most highly connected cluster were extracted using the MCODE plug-in in Cytoscape after PPI networks were analyzed. The DMNC and MCC algorithms of Cytoscape were used to identify hub genes. The top ten hub genes based on the two methods were screened, and seven mutual hub genes were isolated, including BIRC5, DLGAP5, CENPF, KIF4A, TOP2A, AURKA, and CCNA2. These seven genes were all significantly up-regulated in LUAD samples compared with normal samples and were used for further analysis.

## Validation of seven hub genes expression in LUAD samples based on TCGA database

We utilized the Oncomine database to further reveal the expression of these seven hub genes in LUAD. The expression of BIRC5, DLGAP5, CENPF, KIF4A, TOP2A, AURKA, and CCNA2 (Figure 4) was significantly higher in LUAD



**Figure 2. Functional characteristics analyses of robust DEGs**  
 GO enrichment analyses of (A) the up-regulated DEGs and (B) the down-regulated DEGs. KEGG pathway enrichment analyses of (C) the up-regulated DEGs and (D) the down-regulated DEGs.

tissue samples than that in nontumor lung tissue samples. Meanwhile, TCGA database was used to further identify the levels of the seven hub genes in LUAD. The results from the TCGA database displayed the same expression trends as in the Oncomine database (Figure 5). Therefore, these seven hub genes were significantly highly expressed in LUAD.

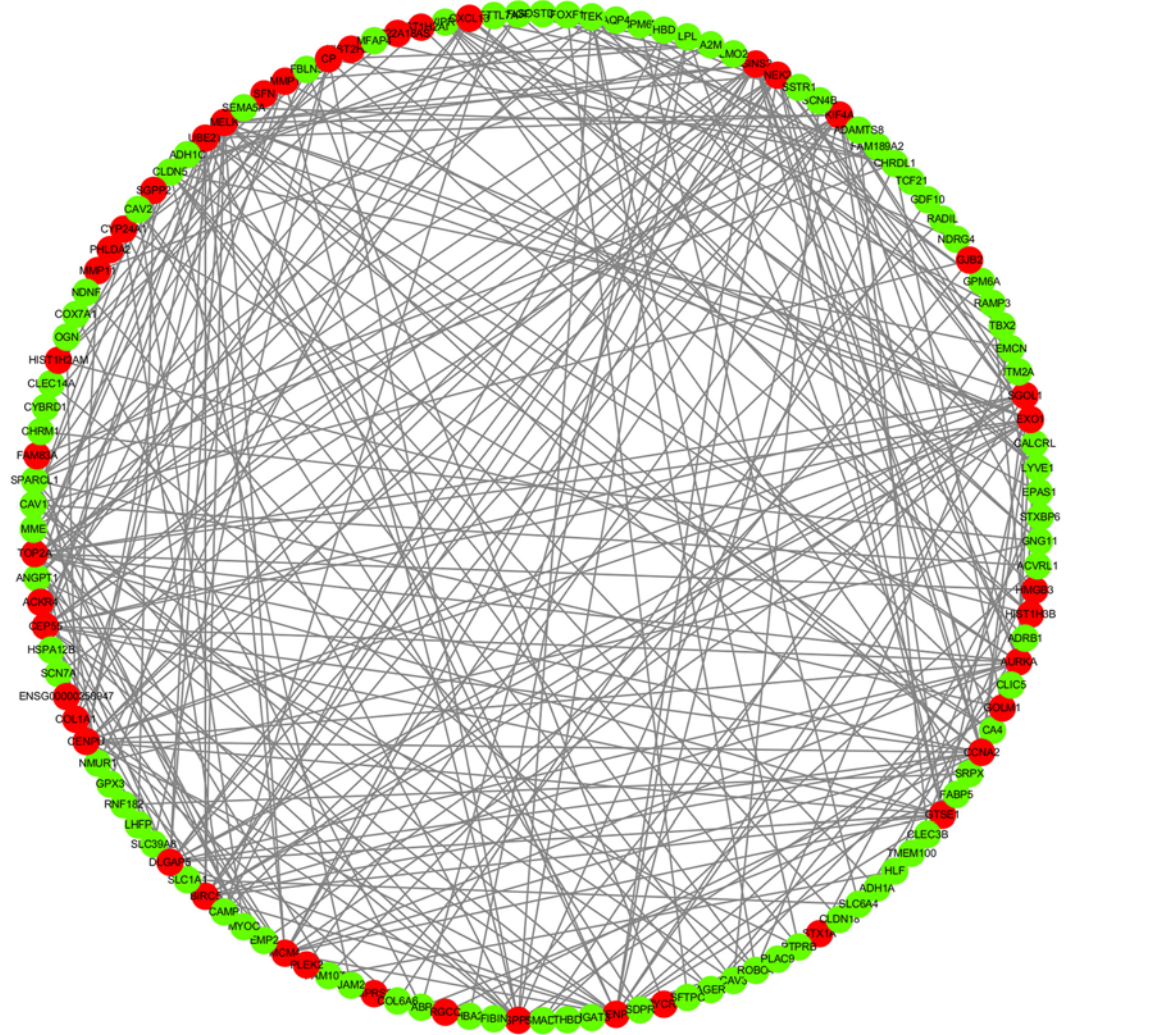
## Overall survival and disease-free survival analyses of seven hub genes in LUAD

To further assess the prognostic values of the seven hub genes in LUAD, TCGA, and GTEx datasets were obtained from GEPIA. The results of the overall survival analyses revealed that the high expression of BIRC5, DLGAP5, CENPF, KIF4A, TOP2A, AURKA, and CCNA2 (Figure 6) was associated with shorter overall survival than in patients with low expression. Simultaneously, the data also indicated that high expression of the seven hub genes could shorten the disease-free survival in LUAD (Figure 7). Therefore, the seven hub genes were associated with poor prognosis of LUAD.

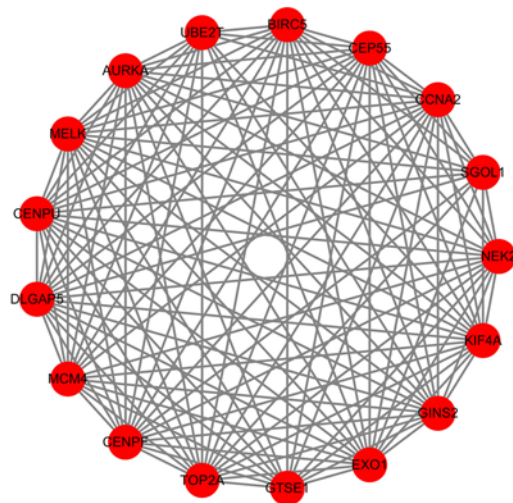
## CENPF, DLGAP5, and KIF4A expression was positively correlated with the clinical stage of LUAD

We screened the genes (CENPF, DLGAP5, and KIF4A), which were most likely to be associated with LUAD and analyzed any possible relationships with the clinical stage of LUAD. As displayed in Figure 8, the expression levels of CENPF, DLGAP5, and KIF4A were dramatically increased in the different stages of LUAD, and the expression of CENPF, DLGAP5, and KIF4A were gradually increased as the LUAD stages increased. Thus, we suggested that high

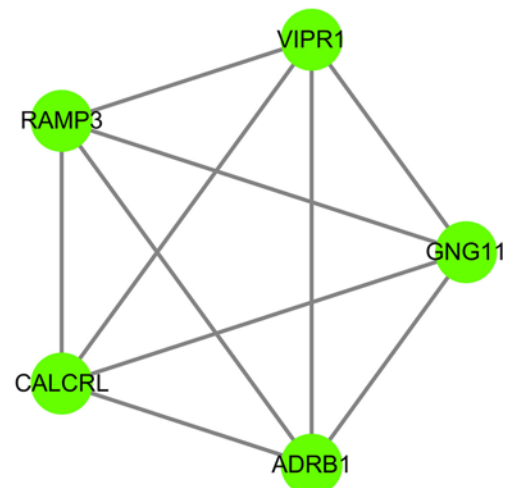
(A)



(B)



(C)

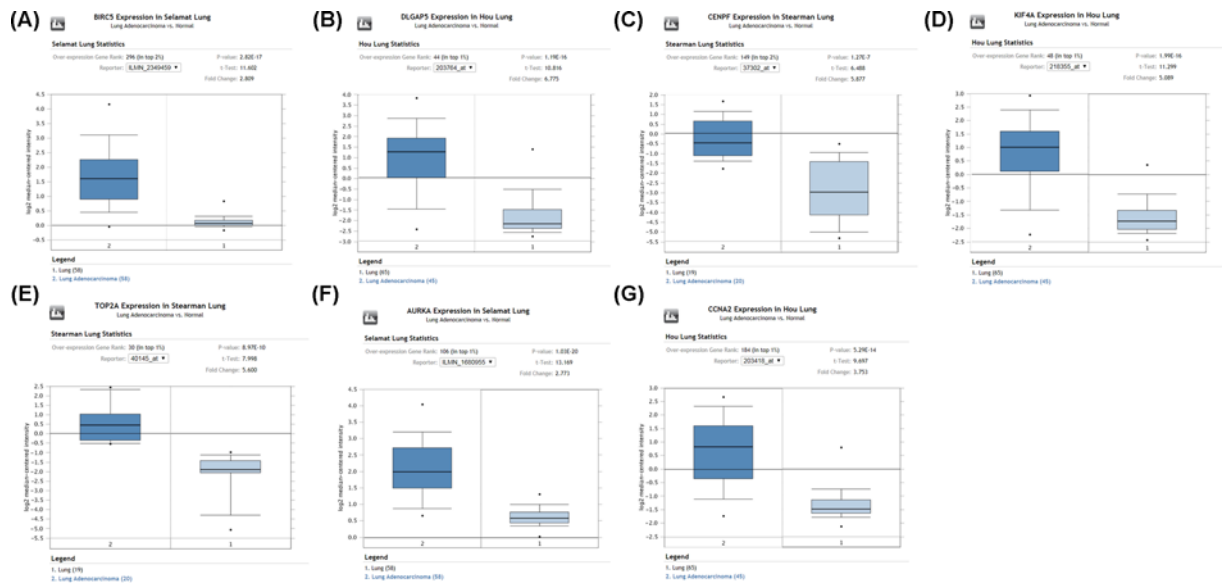


**Figure 3. Establishment of the PPI network and modules analyses of genes in LUAD**

(A) The overall PPI network in LUAD. (B) The PPI network of module 1 in LUAD. (C) The PPI network of module 2 in LUAD.

**Table 1** The GO analysis of the up-regulated DEGs in LUAD

Category	ID	Term	Genes	adj_pval
CC	GO:000786	Nucleosome	HIST2H3A, HIST1H3B, HIST1H2AI, HIST1H2AM	0.0020621
CC	GO:0005814	Centriole	BIRC5, AURKA, CEP55, TOP2A	0.00347557
CC	GO:0005876	Spindle microtubule	KIF4A, BIRC5, AURKA	0.00623428
BP	GO:0000086	G2/M transition of mitotic cell cycle	NEK2, BIRC5, AURKA, MELK	0.00702927
CC	GO:0000228	Nuclear chromosome	HIST1H3B, BIRC5, TOP2A	0.00894277
CC	GO:0005874	Microtubule	KIF4A, NEK2, BIRC5, AURKA, TUBB3	0.00964457
CC	GO:0005654	Nucleoplasm	EXO1, HIST2H3A, GINS2, CYP24A1, KIF4A, CENPF, AURKA, BIRC5, MCM4, GTSE1, HIST1H3B, HS6ST2, CCNA2, TOP2A, UBE2T	0.01268473
CC	GO:0032133	Chromosome passenger complex	BIRC5, AURKA	0.01337317
BP	GO:0006977	DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest	AURKA, SFN, GTSE1	0.01356678
BP	GO:0030574	Collagen catabolic process	COL1A1, MMP1, MMP11	0.01441139
BP	GO:0007059	Chromosome segregation	NEK2, CENPF, TOP2A	0.01616706
CC	GO:0005634	Nucleus	EXO1, HIST2H3A, CYP24A1, GINS2, HMGB3, NEK2, DLGAP5, MEX3A, CENPF, BIRC5, AURKA, SFN, MCM4, CBLC, MNX1, HIST1H2AI, HIST1H3B, HIST1H2AM, TOP2A, CCNA2, UBE2T, TUBB3, MELK	0.01726561



**Figure 4.** Validation of the expression of seven hub genes in LUAD samples based on the OncoPrint database. The expression levels of (A) BIRC5, (B) DLGAP5, (C) CENPF, (D) KIF4A, (E) TOP2A, (F) AURKA, and (G) CCNA2 in LUAD were obtained from the OncoPrint database.



**Table 2 The GO analysis of the down-regulated DEGs in LUAD**

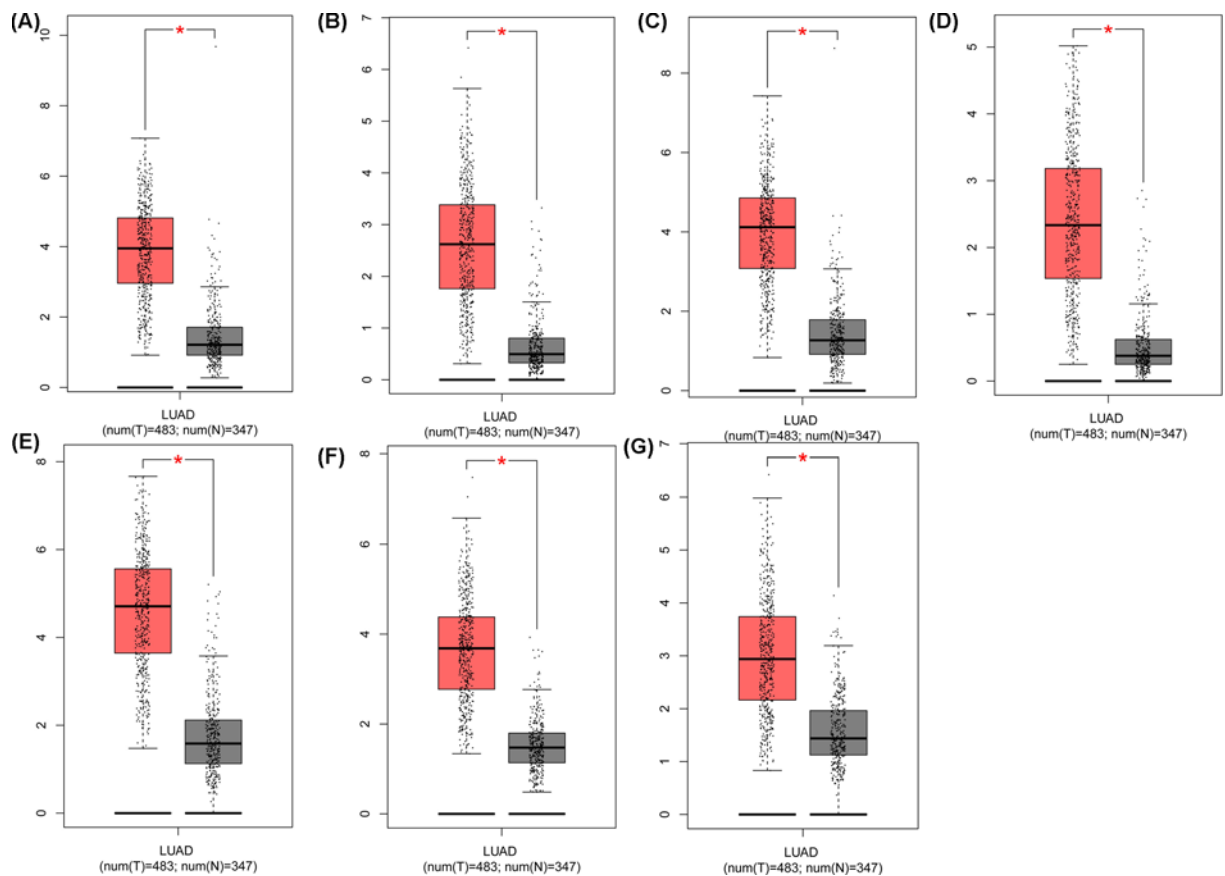
Category	ID	Term	Genes	adj_pval
CC	GO:0044459	Plasma membrane part	FXYD1, CAV3, CAV2, EMCN, CAV1, CLDN18, ACVRL1, LIMS2, CLDN5, SLC6A4, MME, AQP4, GNG11, VIPR1, NMUR1, SDPR, TEK, CALCRL, SCN7A, SLC1A1, CCRL1, PTPRB, RAMP3, AGER, LYVE1, THBD, ADRB1, SSTR1, CHRM1, CYBRD1, CA4, SCN4B, JAM2	0.00108239
BP	GO:0001568	Blood vessel development	SEMA5A, CAV1, EMCN, ACVRL1, LMO2, EPAS1, FOXF1, ROBO4, ANGPT1, FIGF	0.00230295
CC	GO:0005887	Integral to plasma membrane	FXYD1, PTPRB, RAMP3, CAV2, CAV1, ACVRL1, SLC6A4, AQP4, MME, VIPR1, AGER, LYVE1, ADRB1, THBD, SSTR1, NMUR1, CHRM1, TEK, SCN4B, SCN7A, CALCRL, JAM2, SLC1A1, CCRL1	0.00271098
BP	GO:0001944	Vasculature development	SEMA5A, TCF21, CAV1, EMCN, ACVRL1, LMO2, EPAS1, FOXF1, ROBO4, ANGPT1, FIGF	0.00271098
BP	GO:0001525	Angiogenesis	SEMA5A, EMCN, ACVRL1, EPAS1, ROBO4, ANGPT1, FIGF	0.00337538
BP	GO:0007155	Cell adhesion	CLDN18, EMCN, CLDN5, RADIL, SEMA5A, LYVE1, WISP2, SRPX, COL6A6, FBLN5, TEK, JAM2, MFAP4, AOC3	0.00362546
BP	GO:0022610	Biological adhesion	CLDN18, EMCN, CLDN5, RADIL, SEMA5A, LYVE1, WISP2, SRPX, COL6A6, FBLN5, TEK, JAM2, MFAP4, AOC3	0.00494697
CC	GO:0031226	Intrinsic to plasma membrane	FXYD1, PTPRB, RAMP3, CAV2, CAV1, ACVRL1, SLC6A4, AQP4, MME, VIPR1, AGER, LYVE1, ADRB1, THBD, SSTR1, NMUR1, CHRM1, TEK, SCN4B, SCN7A, CALCRL, JAM2, SLC1A1, CCRL1	0.00522618
BP	GO:0048514	Blood vessel morphogenesis	SEMA5A, CAV1, EMCN, ACVRL1, EPAS1, FOXF1, ROBO4, ANGPT1, FIGF	0.00561097
CC	GO:0005576	Extracellular region	A2M, EMCN, ITLN2, OGN, RSPO4, WISP2, COL6A6, SOSTDC1, GPX3, HEG1, SFTPC, ANGPT1, FIBIN, FAM150B, PH16, FIGF, MYOC, LPL, HYAL1, SPARCL1, CAMP, AGER, PLAC9, C2ORF40, GKN2, CHRDL1, ADAMTS8, THBD, CLEC3B, FBLN5, GDF10, MFAP4	0.00599981
BP	GO:0042127	Regulation of cell proliferation	HYAL1, CAV2, CAV1, ACVRL1, TBX2, VIPR1, AGER, WISP2, ADAMTS8, SSTR1, CHRM1, TEK, RTKN2, FABP4, CALCRL, FIGF	0.00656309
BP	GO:0031579	Membrane raft organization	CAV3, CAV2, CAV1	0.00679421

**Table 3 The KEGG analysis of the up-regulated DEGs in LUAD**

Term	Count	Ratio	FDR
Hsa00534 Glycosaminoglycan biosynthesis-heparan sulfate/heparin	1	0.02912621	0.20276459
Hsa03430 Mismatch repair	2	0.04032258	0.20276459
Hsa01230 Biosynthesis of amino acids	2	0.02234637	0.16376332
Hsa00100 Steroid biosynthesis	3	0.0462963	0.20276459
Hsa04115 p53 signaling pathway	3	0.04	0.16376332
Hsa04130 SNARE interactions in vesicular transport	3	0.02285714	0.21164483
Hsa00512 Mucin type O-Glycan biosynthesis	4	0.04301075	0.21164483
Hsa00601 Glycosphingolipid biosynthesis-lacto and neolacto series	5	0.02040816	0.20276459
Hsa04950 Maturity onset diabetes of the young	5	0.08333333	0.20276459
Hsa012102 Oxocarboxylic acid metabolism	6	0.05	0.20276459
Hsa00220 Arginine biosynthesis	2	0.03174603	0.20276459
Hsa04215 Apoptosis-multiple species	6	0.04347826	0.21164483
Hsa00250 Alanine, aspartate and glutamate metabolism	6	0.08695652	0.21164483
Hsa00232 Caffeine metabolism	4	0.05681818	0.16376332
Hsa03030 DNA replication	4	0.03488372	0.21164483

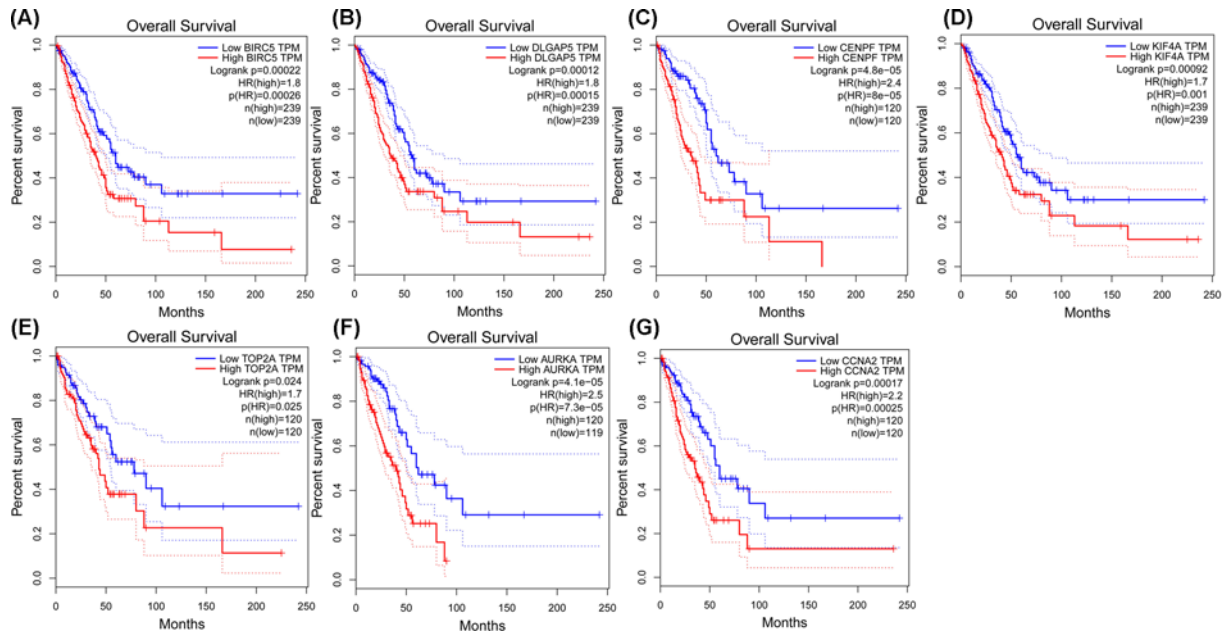
**Table 4** The KEGG analysis of the down-regulated DEGs in LUAD

Term	Count	Ratio	FDR
Hsa04080 Neuroactive ligand-receptor interaction	6	0.02158273	0.06041271
Hsa01100 Metabolic pathways	6	0.00483871	0.50410934
Hsa04151 PI3K-Akt signaling pathway	5	0.01457726	0.12284827
Hsa04261 Adrenergic signaling in cardiomyocytes	4	0.02649007	0.07636768
Hsa04024 cAMP signaling pathway	4	0.0199005	0.12086446
Hsa04510 Focal adhesion	4	0.01941748	0.12086446
Hsa04144 Endocytosis	4	0.01515152	0.1506064
Hsa00350 Tyrosine metabolism	3	0.08571429	0.04917388
Hsa00982 Drug metabolism - cytochrome P450	3	0.04411765	0.07636768
Hsa03320 PPAR signaling pathway	3	0.04109589	0.07636768
Hsa05100 Bacterial invasion of epithelial cells	3	0.03703704	0.08027977
Hsa04974 Protein digestion and absorption	3	0.03333333	0.09115089
Hsa04670 Leukocyte transendothelial migration	3	0.025	0.12284827
Hsa04530 Tight junction	3	0.02158273	0.1506064

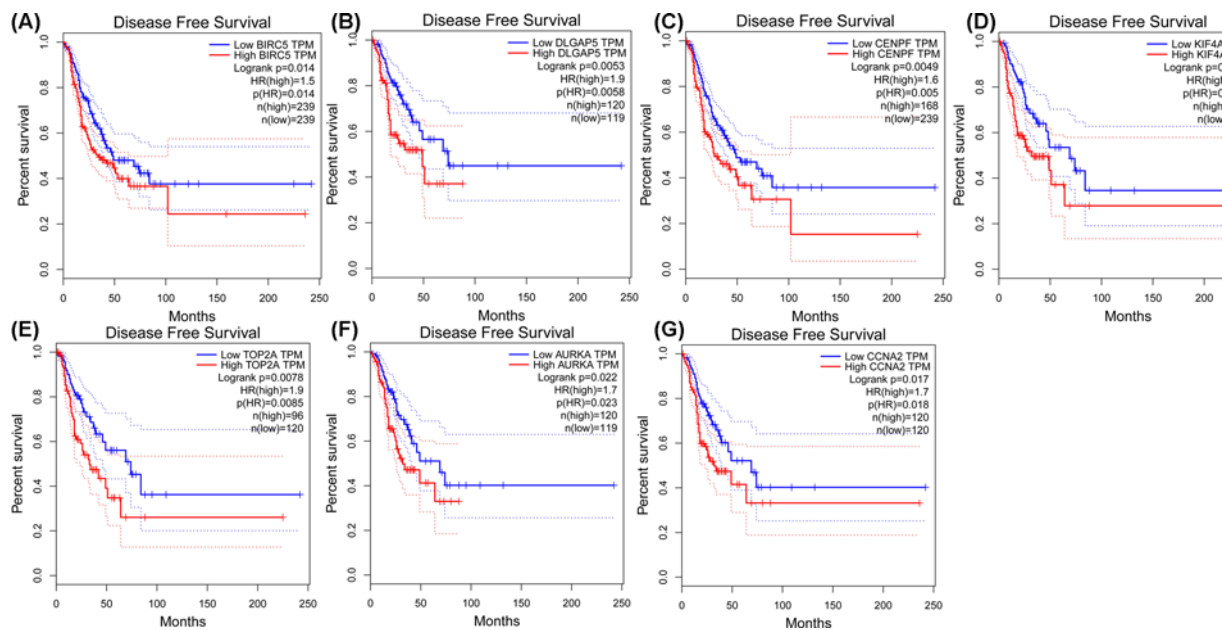


**Figure 5.** Identification of the expression of the seven hub genes in LUAD samples in the TCGA database

Box plots show the expression of (A) BIRC5, (B) DLGAP5, (C) CENPF, (D) KIF4A, (E) TOP2A, (F) AURKA, and (G) CCNA2 in LUAD from the TCGA database. \* $P < 0.05$ .

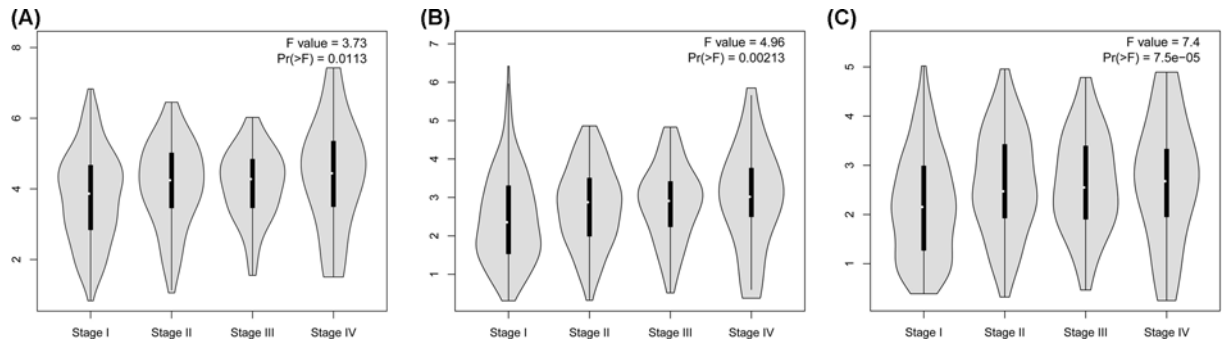


**Figure 6. Overall survival analyses of the seven hub genes in LUAD on the basis of the TCGA and GTEx data in GEPIA**  
 Statistical graphs indicate the results of overall survival analyses of (A) BIRC5, (B) DLGAP5, (C) CENPF, (D) KIF4A, (E) TOP2A, (F) AURKA, and (G) CCNA2 in LUAD. Red lines represent the high expression of genes and the blue lines represent low expression of genes.

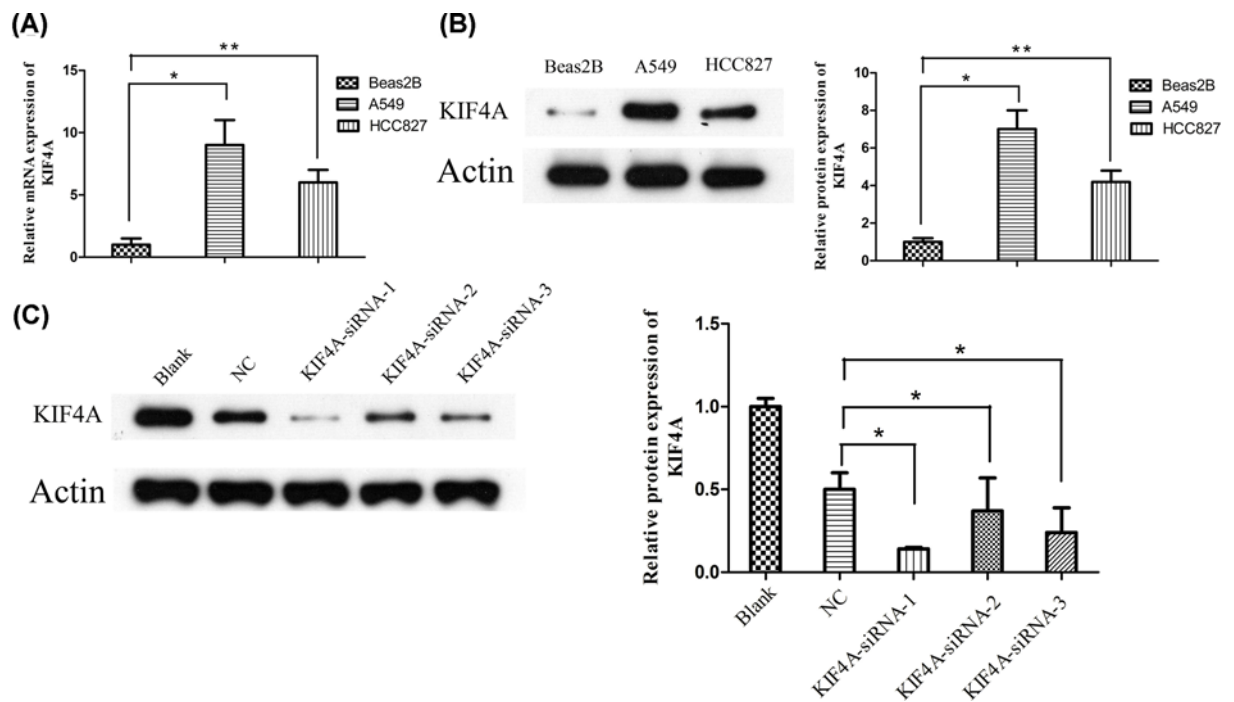


**Figure 7. Disease-free survival analyses of the seven hub genes in LUAD based on TCGA and GTEx data in GEPIA**  
 In accordance with the TCGA and GTEx data in GEPIA, the disease-free survival of the seven hub genes are shown: (A) BIRC5, (B) DLGAP5, (C) CENPF, (D) KIF4A, (E) TOP2A, (F) AURKA, and (G) CCNA2.

expression of CENPF, DLGAP5, and KIF4A might be associated with higher stages of LUAD. Meanwhile, the seven hub genes including KIF4A, CENPF, and DLGAP5 contributed mostly to the GO analysis results (Figure 2A, Table 1).



**Figure 8. CENPF, DLGAP5, and KIF4A expression was positively correlated with the clinical stage of LUAD**  
The levels of CENPF, DLGAP5, and KIF4A expression were analyzed in different stages of LUAD.



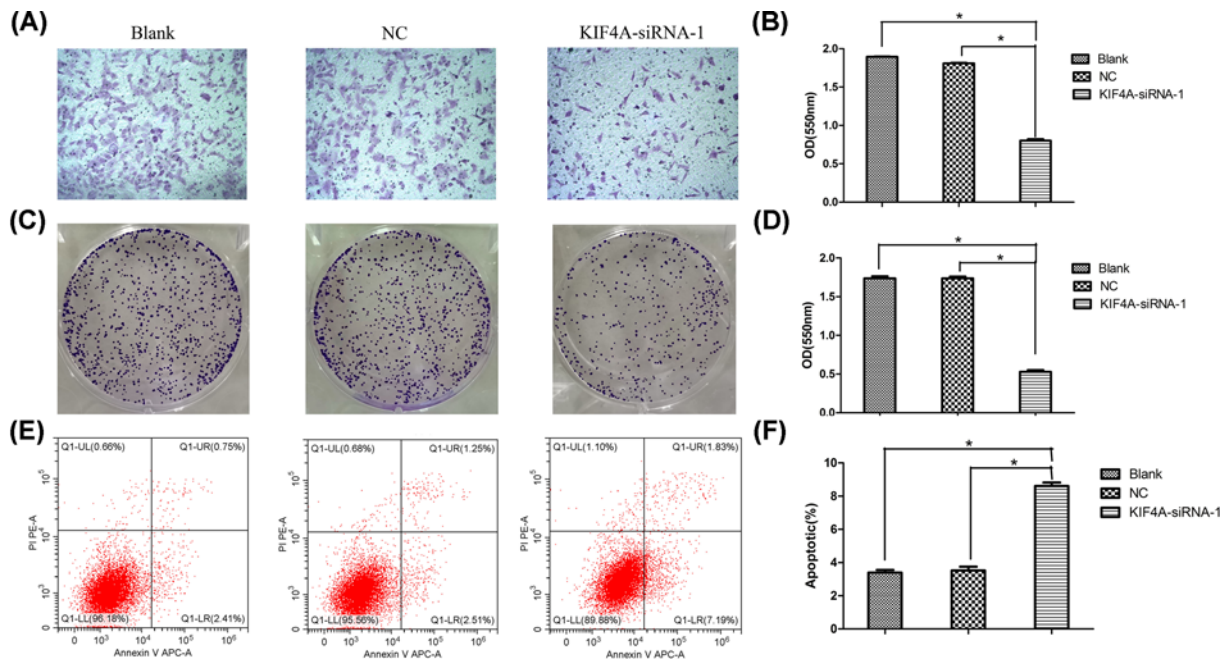
**Figure 9. KIF4A was significantly up-regulated in LUAD cells**  
(A) mRNA expression levels of KIF4A in the LUAD cells were higher than those in normal bronchial epithelial cells. (B) Expression of KIF4A protein in the LUAD cell lines was higher than in normal bronchial epithelial cells. (C) The protein expression levels of KIF4A targeted by siRNA-1, siRNA-2, and siRNA-3. \*,  $P$ -value < 0.05; \*\*,  $P$ -value < 0.01.

## Expression validation of the seven hub genes by qRT-PCR

We compared the mRNA expression levels of the seven hub genes in LUAD tissues and paired normal tissues. As shown in Supplementary Figure S1, compared with their expression in paired normal samples, the expression of BIRC5, DLGAP5, CENPF, KIF4A, TOP2A, AURKA, and CCNA2 was significantly increased in LUAD samples ( $P < 0.01$ ).

## KIF4A was highly expressed in LUAD cells

After identifying the seven hub genes and given the limited number of studies focusing on the relationship between KIF4A and the progression of LUAD, we further studied the expression and function of KIF4A *in vitro*. As shown in Figure 9A,B, the mRNA and protein expression levels of KIF4A were upregulated in LUAD cells (HCC827 and A549) compared with that in normal bronchial epithelial cells Beas2B.



**Figure 10. Silencing KIF4A inhibited proliferation, migration, and promoted apoptosis in the A549 cell line**  
 (A and B) Silencing KIF4A suppressed the migration of A549 cells. (C and D) KIF4A knockdown decreased cell proliferation capacity of A549 cells. (E and F) KIF4A knockdown promoted the apoptotic rate of A549 cells; \*,  $P$ -value < 0.05.

## Knockdown of KIF4A decreased the migration in A549 cell lines

To further confirm the role of KIF4A, siRNA experiments were performed in A549 cells. The efficiency of the knockdown of KIF4A expression was examined by Western blotting, as shown in Figure 9C. To investigate the relationship between KIF4A and migration, we conducted the Transwell assay. The results revealed that the OD value of migrated KIF4A-siRNA-1 cells was decreased significantly, compared with that in the blank and the negative control groups ( $P < 0.05$ ) (Figure 10A,B).

## Effect of KIF4A on the proliferation and apoptosis of A549 cell lines

The colony formation assay showed that the OD values of the clone numbers decreased significantly in the KIF4A-siRNA-1 cells compared with that of the blank group and the negative control group ( $P < 0.05$ ) (Figure 10C,D). In addition, the apoptosis rate of the KIF4A-siRNA-1 cells increased significantly compared with that in control group as determined by the flow cytometry assay ( $P < 0.05$ ) (Figure 10E,F). Together, these results indicated that KIF4A suppression decreased cell proliferation and increased apoptosis abilities in A549 cell lines.

## Discussion

In recent years, with the development of high-throughput sequencing and bioinformatics, a novel research model was formed [30,31]. Massive quantities of research data generated by microarrays have been uploaded to the GEO database for sharing, and scientific researchers classified and downloaded the relevant data in accordance with their area of interest [32]. Meanwhile, researchers have further integrated and compared similar experimental data carried out by different laboratories, so as to unearth potentially useful data.

LUAD is a type of lung cancer with a high degree of malignancy, poor prognosis, and characterized by the increased likelihood of developing chest, liver, bone, and even intracranial metastasis [33,34]. At present, the majority of LUAD patients are diagnosed in the advanced stages and cannot be treated with surgery due to the presence of distant metastasis [35]. Although chemoradiotherapy can improve symptoms, the adverse reactions are significant [36]. Therefore, attempts at improving the survival rate of patients have become the main purpose of LUAD treatment. High-throughput genetic sequencing and bioinformatics might provide a new approach for the study of LUAD pathogenesis.

In our study, we selected ‘LUAD’ as the keyword to search for related datasets from the GEO datasets, and then performed a detailed biological analysis of these DEGs. Subsequently, in order to better understand the DEGs and their associated gene function, related pathways, protein interaction in LUAD, we conducted bioinformatics studies on LUAD using GO functional annotation, KEGG pathway analysis, and PPIs analysis. Overall, our results may provide a theoretical basis for exploring the pathogenesis of LUAD and further studies.

The results of the GO function annotation revealed that the upregulated DEGs in LUAD were mainly concentrated in the nucleosome, centriole, and spindle microtubule, while the down-regulated DEGs in LUAD were mainly enriched in the plasma membrane, in blood vessel development, and in functions integral to the plasma membrane. Meanwhile, KEGG pathway analysis revealed that the up-regulated DEGs in LUAD were mainly enriched in alanine, aspartate, and glutamate metabolism, and oxocarboxylic acid metabolism, while the downregulated DEGs in LUAD were mainly enriched in neuroactive ligand–receptor interactions and in tyrosine metabolism. Moreover, we obtained the key DEGs in LUAD using PPI network analysis.

With the intensive study of the PPI network, the topological structure of the PPI network obtained is frequently associated with disease-related proteins [37,38]. Previous studies have documented that the proteins in the same disease are more likely to interact and be co-expressed in the network [39], while the functional changes of neighboring node proteins associated with a disease are more likely to cause the same or similar diseases [40,41]. Meanwhile, the smaller nodes that interact with numerous proteins in the protein network are called hubs [42]. Hub genes may have more crucial biological functions in disease development. In biological networks, the change in expression of hub proteins will generate serious destruction of the connected network and may seriously affect the biological functions of cells [43]. Currently, a large number of key hub genes have been identified in a variety of cancers, such as hepatocellular carcinoma [44], bladder cancer [45], renal cell carcinoma [46], and osteosarcoma [47]. However, a number of hub genes in LUAD remain undetermined. In our study, we have filtered seven key hub genes including BIRC5, DLGAP5, CENPF, KIF4A, TOP2A, AURKA, and CCNA2 in LUAD. Simultaneously, we found that these seven hub genes were significantly up-regulated in LUAD using Oncomine data and TCGA datasets, and up-regulation of these seven hub genes were associated with a poor prognosis of LUAD. In addition, we determined that the expression of CENPF, KIF4A, and DLGAP5 might be associated with the metastasis of LUAD. Moreover, we further confirmed the increased mRNA expression level of these seven key hub genes in primary LUAD samples compared with paired normal samples.

In the present study, we provided evidence that KIF4A was significantly up-regulated in LUAD cells. To date, only one study has reported that the knockdown KIF4A might suppress the growth of NSCLC cell lines [48]. As for other tumor types, KIF4A has been reported to enhance carcinoma development by promoting cell cycle progression *in vitro* and *in vivo* in colorectal cancer and hepatocellular carcinoma [49,50]. Our study revealed that silencing KIF4A expression inhibited proliferation, migration, and promoted apoptosis in A549 cell lines, which was in agreement with the previous studies indicated above.

## Conclusions

We have identified new DEGs and pathways active in LUAD, and more specifically, KIF4A as a hub gene with the capacity to promote the progression of LUAD. Our findings provide researchers with a potential therapeutic target for molecular cancer therapy.

### Data Availability

The datasets used or analyzed during the current study are available from the GEO (<https://www.ncbi.nlm.nih.gov/geo/>), Oncomine (<https://www.oncomine.org/resource/login.html>), GEPIA (<http://gepia.cancer-pku.cn/>), STRING (<https://string-db.org/>), KOBAS (<http://kobas.cbi.pku.edu.cn/>), DAVID (<https://david.ncifcrf.gov/>), and TCGA (<https://cancergenome.nih.gov/>) databases.

### Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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## Author Contribution

Yexun Song conceived the project and designed the experiments. Wenfang Tang analyzed the data and performed most of the experiments. Hui Li carried out the bioinformatics analysis, wrote the manuscript, and provided technical support.

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We acknowledge and appreciate our colleagues for their valuable efforts and comments relative to this paper.

## Abbreviations

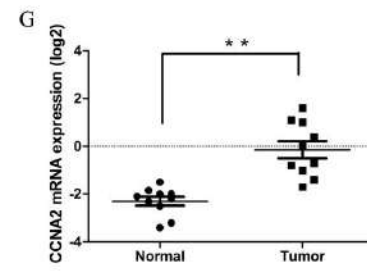
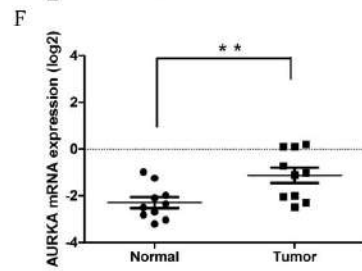
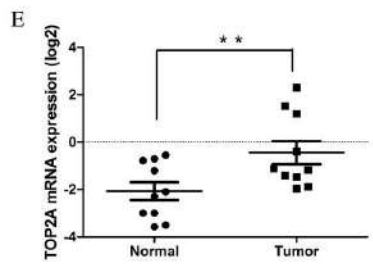
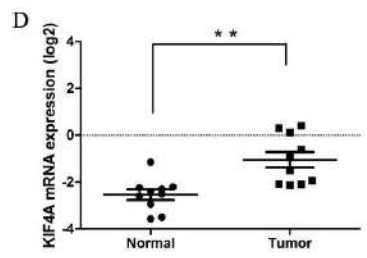
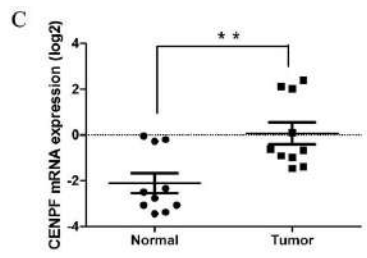
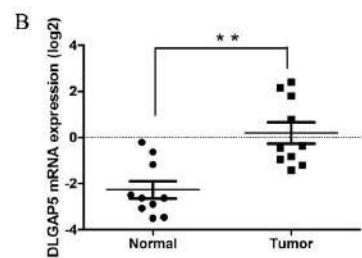
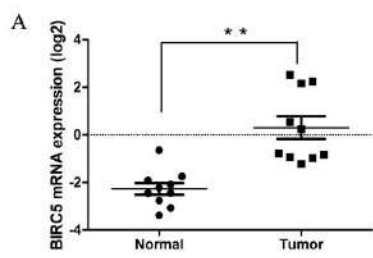
DEG, differentially expressed gene; GEO, gene expression omnibus; LUAD, lung adenocarcinoma; PPI, protein–protein interaction; qRT-PCR, real-time quantitative PCR; STRING, Search Tool for the Retrieval of Interacting Genes.

## References

- 1 Gazdar, A.F. and Zhou, C. (2018) Lung cancer in never-smokers: a different disease. *IASLC Thoracic Oncology*, pp. 23.e3–29.e3, Elsevier
- 2 Torre, L.A., Siegel, R.L. and Jemal, A. (2016) Lung cancer statistics. *Lung Cancer and Personalized Medicine*, pp. 1–19, Springer, [https://doi.org/10.1007/978-3-319-24223-1\\_1](https://doi.org/10.1007/978-3-319-24223-1_1)
- 3 Oser, M.G., Niederst, M.J., Sequist, L.V. and Engelman, J.A. (2015) Transformation from non-small-cell lung cancer to small-cell lung cancer: molecular drivers and cells of origin. *Lancet Oncol.* **16**, e165–e172, [https://doi.org/10.1016/S1470-2045\(14\)71180-5](https://doi.org/10.1016/S1470-2045(14)71180-5)
- 4 Hirsch, F.R., Suda, K., Wiens, J. and Bunn, Jr, P.A. (2016) New and emerging targeted treatments in advanced non-small-cell lung cancer. *Lancet North Am. Ed.* **388**, 1012–1024, [https://doi.org/10.1016/S0140-6736\(16\)31473-8](https://doi.org/10.1016/S0140-6736(16)31473-8)
- 5 Liu, Z., Liu, B. and Li, C. (2019) A case report of metastatic bilateral ovarian cancer due to non-small cell lung cancer with ALK gene rearrangement. *Eur. J. Gynaecol. Oncol.* **40**, 151–153
- 6 Asiedu, M.K., Reed, N.R., Aubry, M.C., Roden, A.C. and Wigle, D.A. (2019) *Genomic Alterations in Lung Adenocarcinoma Precursor Lesions*, AACR
- 7 Coroller, T.P., Grossmann, P., Hou, Y., Velazquez, E.R., Leijenaar, R.T., Hermann, G. et al. (2015) CT-based radiomic signature predicts distant metastasis in lung adenocarcinoma. *Radiother. Oncol.* **114**, 345–350, <https://doi.org/10.1016/j.radonc.2015.02.015>
- 8 Lin, J.J., Cardarella, S., Lydon, C.A., Dahlberg, S.E., Jackman, D.M., Jänne, P.A. et al. (2016) Five-year survival in EGFR-mutant metastatic lung adenocarcinoma treated with EGFR-TKIs. *J. Thoracic Oncol.* **11**, 556–565, <https://doi.org/10.1016/j.jtho.2015.12.103>
- 9 Inamura, K. and Ishikawa, Y. (2016) MicroRNA in lung cancer: novel biomarkers and potential tools for treatment. *J. Clin. Med.* **5**, 36, <https://doi.org/10.3390/jcm5030036>
- 10 Postmus, P., Kerr, K., Oudkerk, M., Senan, S., Waller, D., Vansteenkiste, J. et al. (2017) Early and locally advanced non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* **28**, iv1–iv21, <https://doi.org/10.1093/annonc/mdx222>
- 11 Vinner, L., Mourier, T., Friis-Nielsen, J., Gniadecki, R., Dybkaer, K., Rosenberg, J. et al. (2015) Investigation of human cancers for retrovirus by low-stringency target enrichment and high-throughput sequencing. *Sci. Rep.* **5**, 13201, <https://doi.org/10.1038/srep13201>
- 12 Chen, L., Sun, F., Yang, X., Jin, Y., Shi, M., Wang, L. et al. (2017) Correlation between RNA-Seq and microarrays results using TCGA data. *Gene* **628**, 200–204, <https://doi.org/10.1016/j.gene.2017.07.056>
- 13 Yuan, C., Xiang, L., Bai, R., Cao, K., Gao, Y., Jiang, X. et al. (2019) MiR-195 restrains lung adenocarcinoma by regulating CD4+ T cell activation via the CCDC88C/Wnt signaling pathway: a study based on the Cancer Genome Atlas (TCGA), Gene Expression Omnibus (GEO) and bioinformatic analysis. *Ann. Transl. Med.* **7**, 263, <https://doi.org/10.21037/atm.2019.05.54>
- 14 Zhu, Q., Sun, Y., Zhou, Q., He, Q. and Qian, H. (2018) Identification of key genes and pathways by bioinformatics analysis with TCGA RNA sequencing data in hepatocellular carcinoma. *Mol. Clin. Oncol.* **9**, 597–606, <https://doi.org/10.3892/mco.2018.1728>
- 15 Lee, E. and Moon, A. (2016) Identification of biomarkers for breast cancer using databases. *J. Cancer Prevent.* **21**, 235, <https://doi.org/10.15430/JCP.2016.21.4.235>
- 16 Gu, W., Sun, Y., Zheng, X., Ma, J., Hu, X.-Y., Gao, T. et al. (2018) Identification of gastric cancer-related circular RNA through microarray analysis and bioinformatics analysis. *BioMed Res. Int.* **2018**, <https://doi.org/10.1155/2018/2381680>
- 17 Liu, K., Fu, Q., Liu, Y. and Wang, C. (2019) An integrative bioinformatics analysis of microarray data for identifying hub genes as diagnostic biomarkers of preeclampsia. *Biosci. Rep.* **39**, <https://doi.org/10.1042/BSR20190187>
- 18 Zhang, S., Wang, L., Li, S., Zhang, W., Ma, X., Cheng, G. et al. (2018) Identification of Potential Key Genes Associated with Adipogenesis through Integrated Analysis of Five Mouse Transcriptome Datasets. *Int. J. Mol. Sci.* **19**, <https://doi.org/10.3390/ijms19113557>
- 19 Shen, S., Kong, J., Qiu, Y., Yang, X., Wang, W. and Yan, L. (2019) Identification of core genes and outcomes in hepatocellular carcinoma by bioinformatics analysis. *J. Cell. Biochem.* **120**, 10069–10081, <https://doi.org/10.1002/jcb.28290>
- 20 Klopfenstein, D., Zhang, L., Pedersen, B.S., Ramirez, F., Vesztröcy, A.W., Naldi, A. et al. (2018) GOATOOLS: A Python library for Gene Ontology analyses. *Sci. Rep.* **8**, 10872, <https://doi.org/10.1038/s41598-018-28948-z>
- 21 Kanehisa, M., Goto, S., Kawashima, S., Okuno, Y. and Hattori, M. (2004) The KEGG resource for deciphering the genome. *Nucleic Acids Res.* **32**, D277–D280, <https://doi.org/10.1093/nar/gkh063>
- 22 Wang, Z., Shang, P., Li, Q., Wang, L., Chamba, Y., Zhang, B. et al. (2017) iTRAQ-based proteomic analysis reveals key proteins affecting muscle growth and lipid deposition in pigs. *Sci. Rep.* **7**, 46717, <https://doi.org/10.1038/srep46717>
- 23 Mering, C.V., Huynen, M., Jaeggi, D., Schmidt, S., Bork, P. and Snel, B. (2003) STRING: a database of predicted functional associations between proteins. *Nucleic Acids Res.* **31**, 258–261, <https://doi.org/10.1093/nar/gkg034>

- 24 Tang, Z., Li, C., Kang, B., Gao, G., Li, C. and Zhang, Z. (2017) GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res.* **45**, W98–W102, <https://doi.org/10.1093/nar/gkx247>
- 25 Tomczak, K., Czerwińska, P. and Wiznerowicz, M. (2015) The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. *Contemporary Oncol.* **19**, A68, <https://doi.org/10.5114/wo.2014.47136>
- 26 Lonsdale, J., Thomas, J., Salvatore, M., Phillips, R., Lo, E., Shad, S. et al. (2013) The genotype-tissue expression (GTEx) project. *Nat. Genet.* **45**, 580, <https://doi.org/10.1038/ng.2653>
- 27 Consortium, G. (2015) The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science* **348**, 648–660, <https://doi.org/10.1126/science.1262110>
- 28 Song, Y.X., Li, W., Peng, X.W., Xie, J., Li, H.Q. and Tan, G.L. (2017) Inhibition of autophagy results in a reversal of taxol resistance in nasopharyngeal carcinoma by enhancing taxol-induced caspase-dependent apoptosis. *Am. J. Transl. Res.* **9**, 1934–1942
- 29 Wang, X.Y., Wang, K., Xiao, J., Song, Y.X. and Li, H.Q. (2020) Neferine sensitized Taxol-resistant nasopharyngeal carcinoma to Taxol by inhibiting EMT via downregulating miR-130b-5p. *Biochem. Biophys. Res. Co.* **531**, 573–580, <https://doi.org/10.1016/j.bbrc.2020.08.008>
- 30 Fonseca, N.A., Rung, J., Brazma, A. and Marioni, J.C. (2012) Tools for mapping high-throughput sequencing data. *Bioinformatics* **28**, 3169–3177, <https://doi.org/10.1093/bioinformatics/bts605>
- 31 Nakazato, T., Ohta, T. and Bono, H. (2013) Experimental design-based functional mining and characterization of high-throughput sequencing data in the sequence read archive. *PLoS ONE* **8**, e77910, <https://doi.org/10.1371/journal.pone.0077910>
- 32 Barrett, T., Wilhite, S.E., Ledoux, P., Evangelista, C., Kim, I.F., Tomashevsky, M. et al. (2012) NCBI GEO: archive for functional genomics data sets—update. *Nucleic Acids Res.* **41**, D991–D995, <https://doi.org/10.1093/nar/gks1193>
- 33 Network CGAR (2014) Comprehensive molecular profiling of lung adenocarcinoma. *Nature* **511**, 543, <https://doi.org/10.1038/nature13385>
- 34 Bandoh, N., Ichikawa, H., Asahi, A., Kono, M., Harabuchi, S., Sato, R. et al. (2019) Lung Adenocarcinoma with Cheek Dysesthesia as an Initial Symptom: A Case Report and Literature Review. *Case Rep. Oncol.* **12**, 650–656, <https://doi.org/10.1159/000502053>
- 35 Popper, H.H. (2016) Progression and metastasis of lung cancer. *Cancer Metastasis Rev.* **35**, 75–91, <https://doi.org/10.1007/s10555-016-9618-0>
- 36 Hsia, T.C., Liang, J.A., Li, C.C. and Chien, C.R. (2018) Comparative effectiveness of concurrent chemoradiotherapy versus EGFR-tyrosine kinase inhibitors for the treatment of clinical stage IIIb lung adenocarcinoma patients with mutant EGFR. *Thoracic Cancer* **9**, 1398–1405, <https://doi.org/10.1111/1759-7714.12847>
- 37 Francois, M., Donovan, P. and Fontaine, F. (2018) Modulating transcription factor activity: Interfering with protein-protein interaction networks. *Seminars Cell Development. Biol.* **99**, 12–19, Elsevier
- 38 Zhao, B., Wang, J., Li, M., Li, X., Li, Y., Wu, F.-X. et al. (2016) A new method for predicting protein functions from dynamic weighted interactome networks. *IEEE Trans. Nanobioscience* **15**, 131–139, <https://doi.org/10.1109/TNB.2016.2536161>
- 39 Goh, K.-I., Cusick, M.E., Valle, D., Childs, B., Vidal, M. and Barabási, A.-L. (2007) The human disease network. *Proc. Natl. Acad. Sci.* **104**, 8685–8690, <https://doi.org/10.1073/pnas.0701361104>
- 40 Oti, M., Snel, B., Huynen, M.A. and Brunner, H.G. (2006) Predicting disease genes using protein–protein interactions. *J. Med. Genet.* **43**, 691–698, <https://doi.org/10.1136/jmg.2006.041376>
- 41 Karni, S., Soreq, H. and Sharan, R. (2009) A network-based method for predicting disease-causing genes. *J. Comput. Biol.* **16**, 181–189, <https://doi.org/10.1089/cmb.2008.05TT>
- 42 Yu, D., Lim, J., Wang, X., Liang, F. and Xiao, G. (2017) Enhanced construction of gene regulatory networks using hub gene information. *BMC Bioinformatics* **18**, 186, <https://doi.org/10.1186/s12859-017-1576-1>
- 43 Batada, N.N., Hurst, L.D. and Tyers, M. (2006) Evolutionary and physiological importance of hub proteins. *PLoS Comput. Biol.* **2**, e88, <https://doi.org/10.1371/journal.pcbi.0020088>
- 44 Chen, P., Wang, F., Feng, J., Zhou, R. and Zhao, Q. (2017) Co-expression network analysis identified six hub genes in association with metastasis risk and prognosis in hepatocellular carcinoma. *Oncotarget* **8**, 48948–48958, <https://doi.org/10.18632/oncotarget.16896>
- 45 Zhang, D.Q., Zhou, C.K., Chen, S.Z., Yue, Y. and Shi, B.K. (2017) Identification of hub genes and pathways associated with bladder cancer based on co-expression network analysis. *Oncol. Lett.* **14**, 1115, <https://doi.org/10.3892/ol.2017.6267>
- 46 Wang, Y., Chen, L., Wang, G., Cheng, S. and Wang, X. (2018) Fifteen hub genes associated with progression and prognosis of clear cell renal cell carcinoma identified by coexpression analysis: WANG et al. *J. Cell. Physiol.* **234**, 10225–10237
- 47 Wu, Y.S., Liu, J.J. and Zheng, C.J. (2017) The mAP-KL algorithm combined with mutual information network used to screen hub genes in osteosarcoma. *Current Bioinformatics* **11**, 1, <https://doi.org/10.2174/1574893611666160726170147>
- 48 Taniwaki, M., Takano, A., Ishikawa, N., Yasui, W., Inai, K., Nishimura, H. et al. (2007) Activation of KIF4A as a prognostic biomarker and therapeutic target for lung cancer. *Clin. Cancer Res.* **13**, 6624–6631, <https://doi.org/10.1158/1078-0432.CCR-07-1328>
- 49 Hou, P.-F., Jiang, T., Chen, F., Shi, P.-C., Li, H.-Q., Bai, J. et al. (2018) KIF4A facilitates cell proliferation via induction of p21-mediated cell cycle progression and promotes metastasis in colorectal cancer. *Cell Death Dis.* **9**, 477, <https://doi.org/10.1038/s41419-018-0550-9>
- 50 Bai, L., Ren, Y. and Cui, T. (2020) Overexpression of CDCA5, KIF4A, TPX2, and FOXM1 Coregulated Cell Cycle and Promoted Hepatocellular Carcinoma Development. *J. Comput. Biol.* **27**, 965–974, <https://doi.org/10.1089/cmb.2019.0254>





**Supplementary Figure 1.** Validation of the mRNA expression of the seven hub genes comparing paired normal samples (n=10) and LUAD samples (n=10) by PCR analysis. (A) BIRC5, (B) DLGAP5, (C) CENPF, (D) KIF4A, (E) TOP2A, (F) AURKA, and (G) CCNA2. All of the data are presented as means  $\pm$  SD. \*\*, *P-value*<0.01.

**Supplementary Table 1.** The detailed information of the three GEO datasets.

<b>Dataset</b>	<b>Numbers of samples (Tumor / Normal)</b>	<b>Histological types</b>	<b>Experiment type</b>	<b>Origin</b>
GSE85716	12/12	Adenocarcinoma	mRNA	Sci Rep
GSE32863	58/58	Adenocarcinoma	mRNA	Genome Res
GSE116959	57/11	Adenocarcinoma	mRNA	Oncogene

Note: GEO, Gene Expression Omnibus

**Supplementary Table 2.** The primer sequences of the hub genes.

BIRC5	forward, 5'-ATTCGTCCGGTTGCGCTTCC-3'
	reverse, 5'-CACGGCGCACTTCTTCGCAG-3'
DLGAP5	forward, 5'-AAGTGGGTCGTTATAGACCTGA-3'
	reverse 5'-TGCTCGAACATCACTCTCGTTAT-3'
CENPF	forward, 5'-AAAGAAACAGACGGAACAACCTG-3'
	reverse 5'-CCAAGCAAAGACCGAGAACT-3'
KIF4A	forward, 5'-TACTGCGGTGGAGCAAGAAG-3'
	reverse 5'-CATCTGCGCTTGACGGAGAG3'
TOP2A	forward, 5'-CTAGTTAATGCTGCGGACAACA-3'
	reverse 5'-CATTTCGACCACCTGTCACTT-3'
AURKA	forward, 5'-AGTTGGAGGTCCAAAACGTG-3'
	reverse 5'-TCCAAGTGGTGCATATTCCA-3'
CCNA2	forward, 5'-CACTCTACACAGTCAGGGGA-3'
	reverse 5'-AGTGTCTCTGGTGGGTTGAG-3'
GADPH	forward 5'-CATCTCTGCCCCCTCTGCTGA-3'
	reverse 5'-GGATGACCTTGCCCACAGCCT-3'

**Supplementary Table 3.** The information of the upregulated DEGs between adjacent normal lung and LUAD tissues

<b>Gene</b>	<b>logFC</b>	<b>AveExpr</b>	<b>t</b>	<b>P.Value</b>	<b>adj.P.Val</b>	<b>B</b>
XAGE1A	2.818124	7.024731	3.59438	0.003301	0.035687	-2.03285
SPP1	3.67501	8.270667	6.117881	3.79E-05	0.002047	2.440225
TUBB3	2.527322	4.812491	3.673739	0.002838	0.032767	-1.88325
TMPRSS4	2.936224	7.172396	3.906683	0.001826	0.02494	-1.44526
PHLDA2	2.070969	6.68861	4.797153	0.000355	0.009232	0.191714
MMP11	2.36827	8.729277	13.11481	2.51E-07	0.000204	7.533217
TOP2A	3.364929	5.70608	4.614423	0.000494	0.01121	-0.13769
CYP24A1	1.814183	3.943949	3.853667	0.002018	0.026237	-1.54473
XDH	2.270497	4.378626	3.70179	0.002691	0.031642	-1.8304
CP	3.270281	6.584131	3.323473	0.005542	0.048053	-2.54374
SGPP2	3.104349	6.703745	6.3222	2.74E-05	0.001634	2.765672
PLEK2	2.177398	4.160249	4.978233	0.000258	0.007444	0.514129
CEP55	1.549506	4.897171	3.863248	0.001982	0.026053	-1.52675
ABCC3	2.004989	8.640878	5.08149	0.000215	0.006641	0.696099
GJB2	1.599979	3.840176	3.643585	0.003006	0.034034	-1.94008
MNX1	2.994084	3.579718	3.925726	0.001762	0.024347	-1.40956
COL1A1	1.690818	7.455179	3.372773	0.005042	0.045648	-2.45084
HS6ST2	1.688701	4.689706	4.838842	0.00033	0.008818	0.266306
UBE2T	2.967068	5.103812	5.459279	0.000112	0.004273	1.349591
FUT3	2.043435	5.736336	5.475034	0.000109	0.004223	1.376411
STX1A	2.935411	5.067663	7.254678	6.67E-06	0.000628	4.173654
HIST1H2AI	2.880545	5.306701	4.208568	0.001038	0.01774	-0.88221
CXCL13	2.870715	4.146376	4.44511	0.000672	0.013532	-0.44627
GPT2	2.783919	4.724283	8.466538	1.26E-06	0.000211	5.822701
FAM83A	2.707739	5.976604	6.542513	1.94E-05	0.001292	3.109719
LOC100130811	2.680684	7.72016	4.785064	0.000363	0.009328	0.170044
HIST2H3A	2.61482	8.421248	3.836221	0.002086	0.02676	-1.5775
DLGAP5	2.58805	3.474919	4.088373	0.001299	0.02047	-1.10561
CBLC	2.585204	3.886146	4.999561	0.000248	0.007272	0.55183
GINS2	2.533287	5.133182	3.601494	0.003256	0.035532	-2.01944
MEX3A	2.527057	3.452469	4.490277	0.000618	0.012874	-0.36365
MMP1	2.510838	5.588877	3.448129	0.004365	0.041578	-2.30874
PYCR1	2.406433	6.333929	7.417624	5.28E-06	0.000547	4.406941
STK32A	2.333879	6.39677	5.765152	6.72E-05	0.003005	1.863954
HMGB3	2.330866	6.033155	5.581962	9.12E-05	0.003715	1.557512
GOLM1	2.317666	8.311508	7.010564	9.55E-06	0.000807	3.817162
BIRC5	2.294236	5.50253	3.487856	0.004045	0.039984	-2.2338
MELK	2.269227	4.768067	3.718442	0.002607	0.030877	-1.79904
SFN	2.266313	9.028779	5.394662	0.000125	0.00461	1.239219
CENPF	2.264698	5.174452	3.969908	0.001621	0.023304	-1.32683
PODXL2	2.259731	5.790414	9.528018	3.33E-07	0.000104	7.114909

NEK2	2.215783	3.851914	3.383537	0.004939	0.044964	-2.43055
AURKA	2.178471	6.812019	5.083053	0.000215	0.006635	0.698841
KIF4A	2.160272	5.144072	3.417562	0.004627	0.04325	-2.36639
LOC283710	2.151973	3.818108	5.34134	0.000137	0.004939	1.1477
EXO1	2.125513	4.785475	3.954176	0.00167	0.023572	-1.35628
HIST1H2AM	2.091739	6.882594	3.705284	0.002673	0.031522	-1.82382
MLF1IP	2.088122	4.340229	4.529615	0.000576	0.012375	-0.29187
GALNT7	2.084426	5.156065	6.720718	1.48E-05	0.001086	3.382813
HIST1H3B	2.082695	10.38385	3.40007	0.004785	0.044222	-2.39938
CCNA2	2.058195	6.612935	3.869014	0.00196	0.0259	-1.51592
GTSE1	2.027084	4.658961	3.294916	0.005853	0.049899	-2.59752
IL2RA	2.02583	5.918288	4.039577	0.001423	0.021402	-1.19661
SGOL1	2.021829	3.800428	3.638276	0.003036	0.034211	-1.95009
SLC22A18AS	2.00686	4.493097	3.537846	0.003677	0.038006	-2.13949
MCM4	2.001465	7.018757	5.442296	0.000116	0.004328	1.32064

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**Supplementary Table 4.** The information of the downregulated DEGs between adjacent normal lung and LUAD tissues

<b>Gene</b>	<b>logFC</b>	<b>AveExpr</b>	<b>t</b>	<b>P.Value</b>	<b>adj.P.Val</b>	<b>B</b>
CLDN18	-3.88033	10.80772	-6.77385	3.46E-09	2.33E-07	10.76398
ITLN2	-3.51928	8.32816	-10.0317	4.25E-15	2.62E-12	24.02374
TMEM100	-3.35949	7.811716	-13.8606	1.37E-21	7.33E-18	38.49646
AGER	-3.09523	9.528863	-9.46257	4.47E-14	1.65E-11	21.73303
FMO2	-2.80928	9.846211	-10.3868	9.93E-16	7.41E-13	25.43872
FIGF	-2.70543	8.339863	-8.58609	1.74E-12	3.60E-10	18.1637
GDF10	-3.69388	8.413394	-9.57906	2.76E-14	1.16E-11	22.20396
ADAMTS8	-2.57774	8.193042	-8.38423	4.06E-12	7.24E-10	17.33719
C2orf40	-3.32916	8.144675	-7.6599	8.53E-11	9.85E-09	14.36906
GKN2	-2.58148	8.152254	-6.03009	7.30E-08	3.10E-06	7.80171
CLEC3B	-2.56803	12.07937	-8.41236	3.61E-12	6.58E-10	17.45243
FAM107A	-2.55681	8.368239	-8.62986	1.45E-12	3.02E-10	18.34278
CAV1	-2.55138	10.62217	-9.49008	3.99E-14	1.50E-11	21.84431
PI16	-2.23703	7.731624	-8.79103	7.37E-13	1.67E-10	19.00156
ADH1C	-2.46707	10.04903	-6.13795	4.71E-08	2.16E-06	8.225839
CA4	-3.45929	7.226869	-13.2789	1.18E-20	4.25E-17	36.42023
TNNC1	-2.41189	9.342043	-7.93431	2.69E-11	3.71E-09	15.49313
HBD	-2.38861	12.49211	-6.20956	3.52E-08	1.69E-06	8.508619
ADH1A	-2.03745	8.210561	-7.64582	9.05E-11	1.03E-08	14.31145
MYOC	-2.32466	6.28135	-12.5705	1.74E-19	3.48E-16	33.82893
C10orf116	-3.27965	12.82333	-7.48733	1.76E-10	1.76E-08	13.66327
MFAP4	-3.26452	12.49525	-7.38161	2.74E-10	2.54E-08	13.23151
SLC6A4	-3.25955	6.467535	-13.5003	5.19E-21	2.38E-17	37.21581
HBA2	-3.15205	12.25895	-6.23993	3.11E-08	1.51E-06	8.628815
MGAT3	-3.12558	8.290119	-8.5317	2.19E-12	4.30E-10	17.94111
SEMA5A	-3.10479	9.905222	-8.71726	1.00E-12	2.12E-10	18.70017
SFTPC	-3.09917	15.7502	-3.37863	0.001203	0.009561	-1.48333
VIPR1	-2.99041	7.810181	-10.4062	9.18E-16	7.01E-13	25.51536
DNASE1L3	-2.98458	7.885619	-8.05608	1.61E-11	2.36E-09	15.99227
TEK	-2.91956	9.15555	-8.39065	3.96E-12	7.13E-10	17.36351
AOC3	-2.91684	10.50087	-8.86689	5.36E-13	1.27E-10	19.31132
SLC39A8	-2.91019	9.620007	-9.96957	5.49E-15	3.00E-12	23.77495
OGN	-2.89869	6.90445	-9.2366	1.14E-13	3.50E-11	20.81681
SCN4B	-2.8927	7.849193	-9.74378	1.39E-14	6.77E-12	22.86814
FXYD1	-2.89169	7.51509	-10.3037	1.39E-15	9.73E-13	25.10874
CHRM1	-2.8862	6.025893	-13.2775	1.19E-20	4.25E-17	36.4151
RTKN2	-2.8333	7.255558	-13.2013	1.58E-20	4.62E-17	36.13963
EMP2	-2.80704	12.36905	-10.1903	2.22E-15	1.45E-12	24.65726
TCF21	-2.79398	7.099916	-11.1203	5.12E-17	6.32E-14	28.3203
LAMP3	-2.76558	12.05272	-7.11124	8.49E-10	6.92E-08	12.13018
PLAC9	-2.7242	7.955677	-10.9874	8.73E-17	9.34E-14	27.8024

TPPP3	-2.70132	9.710932	-5.78585	1.95E-07	7.15E-06	6.84971
SOSTDC1	-2.67723	6.454697	-7.07564	9.86E-10	7.86E-08	11.98555
C13orf15	-2.67539	11.40839	-9.12861	1.80E-13	5.14E-11	20.37784
HLF	-2.66677	8.165715	-6.61809	6.59E-09	4.04E-07	10.13715
FABP4	-2.65189	6.410904	-11.0866	5.86E-17	6.96E-14	28.18919
SDPR	-2.64536	8.993928	-8.86357	5.44E-13	1.27E-10	19.29776
CDO1	-2.63628	7.183343	-9.46852	4.36E-14	1.63E-11	21.75711
WISP2	-2.62175	10.39802	-6.43426	1.40E-08	7.70E-07	9.401333
ADRB1	-2.62134	8.288305	-6.26603	2.80E-08	1.38E-06	8.732224
SLC1A1	-2.61163	8.777875	-7.06013	1.05E-09	8.29E-08	11.92259
SMAD6	-2.58313	8.978113	-9.53131	3.36E-14	1.31E-11	22.01104
GPM6A	-2.57284	6.375643	-17.7094	2.54E-27	8.15E-23	51.06694
KIAA0408	-2.56803	7.472079	-8.92201	4.26E-13	1.06E-10	19.5362
ANXA3	-2.56381	9.819399	-7.75614	5.70E-11	6.87E-09	14.76309
LPL	-2.53862	9.809238	-5.69038	2.85E-07	9.85E-06	6.481064
AQP4	-2.51776	8.945153	-6.6101	6.81E-09	4.15E-07	10.1051
COL6A6	-2.50901	7.426245	-6.9749	1.50E-09	1.13E-07	11.57684
HIGD1B	-2.50421	8.715539	-7.84493	3.92E-11	5.07E-09	15.12682
FAM150B	-2.45985	6.972387	-9.54918	3.12E-14	1.24E-11	22.08324
EMCN	-2.44193	8.931621	-7.43292	2.21E-10	2.14E-08	13.44098
NDRG4	-2.43241	8.797698	-8.15326	1.07E-11	1.69E-09	16.39064
FLJ46446	-2.42772	7.66975	-7.76319	5.53E-11	6.74E-09	14.79199
GPX3	-2.40849	9.617297	-6.54139	9.04E-09	5.26E-07	9.829621
CLIC5	-2.39573	10.07205	-7.91113	2.97E-11	4.04E-09	15.39811
FBLN5	-2.38828	10.36003	-7.7953	4.83E-11	5.98E-09	14.92349
ARHGEF26	-2.37306	8.088031	-9.21076	1.27E-13	3.86E-11	20.71186
CAMP	-2.36179	7.088716	-6.85472	2.47E-09	1.72E-07	11.09048
ROBO4	-2.33942	8.99776	-8.75217	8.67E-13	1.89E-10	18.84284
RAMP3	-2.33174	7.34154	-9.42879	5.14E-14	1.81E-11	21.59626
MS4A7	-2.29388	10.658	-7.24186	4.92E-10	4.24E-08	12.66165
ANGPT1	-2.2899	8.331996	-8.08597	1.42E-11	2.12E-09	16.11476
CALCRL	-2.27672	7.736635	-9.5818	2.73E-14	1.16E-11	22.21502
SSTR1	-2.27103	7.010182	-7.12858	7.90E-10	6.50E-08	12.20069
SCN7A	-2.26995	7.324706	-8.85966	5.53E-13	1.28E-10	19.28179
HEG1	-2.2667	10.78066	-8.45665	3.00E-12	5.64E-10	17.63382
EPAS1	-2.26343	13.84718	-7.5337	1.45E-10	1.50E-08	13.85279
MME	-2.26028	7.445706	-7.45879	1.98E-10	1.96E-08	13.54665
CLDN5	-2.255	10.97108	-5.85337	1.49E-07	5.65E-06	7.111636
PTPN21	-2.25005	8.382917	-9.96854	5.52E-15	3.00E-12	23.77082
TBX2	-2.24897	11.12536	-7.1021	8.83E-10	7.15E-08	12.09306
STXBP6	-2.24889	7.517232	-7.50572	1.63E-10	1.67E-08	13.7384
FOXF1	-2.24084	7.643346	-10.495	6.39E-16	5.19E-13	25.86736
SPARCL1	-2.2395	11.66687	-7.16908	6.67E-10	5.57E-08	12.3654
RADIL	-2.23148	7.865908	-5.35376	1.07E-06	3.02E-05	5.199254



HSPA12B	-2.22823	7.958093	-9.40991	5.56E-14	1.90E-11	21.51983
CHRDL1	-2.2239	8.296611	-7.29723	3.90E-10	3.49E-08	12.8873
CAV2	-2.21054	7.973899	-8.14635	1.10E-11	1.73E-09	16.36231
METTL7A	-2.20995	11.73772	-7.26409	4.49E-10	3.90E-08	12.75223
FIBIN	-2.20523	6.534673	-9.56318	2.94E-14	1.20E-11	22.13983
LYVE1	-2.16628	7.032022	-10.004	4.77E-15	2.78E-12	23.91293
JAM2	-2.16477	7.706179	-8.94995	3.79E-13	9.80E-11	19.65015
BTNL9	-2.16057	6.924479	-12.0282	1.41E-18	2.23E-15	31.80034
COX7A1	-2.15655	11.56955	-8.25141	7.10E-12	1.18E-09	16.79292
ACVRL1	-2.15443	10.51495	-7.40249	2.51E-10	2.35E-08	13.31672
THBD	-2.15159	11.43407	-6.61695	6.62E-09	4.05E-07	10.13258
CAV3	-2.1331	6.517061	-11.3989	1.69E-17	2.25E-14	29.39883
NMUR1	-2.13196	6.880116	-10.8567	1.48E-16	1.53E-13	27.29144
CLEC14A	-2.13112	7.950611	-8.17879	9.64E-12	1.52E-09	16.49527
GPM6B	-2.12317	7.24714	-9.29962	8.80E-14	2.82E-11	21.07268
RNF182	-2.11516	6.528325	-8.24481	7.30E-12	1.19E-09	16.76591
LHFP	-2.1079	11.25905	-8.01901	1.89E-11	2.69E-09	15.84028
CCRL1	-2.10416	7.283198	-8.53999	2.11E-12	4.18E-10	17.97503
FABP5	-2.09151	10.64035	-6.38179	1.74E-08	9.19E-07	9.192198
FAM189A2	-2.08542	8.003897	-7.54791	1.37E-10	1.43E-08	13.91091
NDNF	-2.06641	8.50587	-5.7008	2.74E-07	9.53E-06	6.52123
LMO2	-2.06471	10.74688	-6.84159	2.61E-09	1.81E-07	11.03739
SRPX	-2.04649	7.972472	-6.35074	1.98E-08	1.02E-06	9.068613
RSPO4	-2.04349	7.008409	-6.32377	2.21E-08	1.12E-06	8.961391
ITM2A	-2.04233	9.693858	-6.91608	1.92E-09	1.38E-07	11.33864
CYBRD1	-2.03853	9.845482	-6.85508	2.47E-09	1.72E-07	11.0919
A2M	-2.03499	14.49045	-5.88357	1.32E-07	5.15E-06	7.229095
GNG11	-2.03368	11.43239	-5.78635	1.94E-07	7.15E-06	6.851624
LIMS2	-2.01763	9.253783	-9.40687	5.63E-14	1.90E-11	21.50751
CCDC85A	-2.01239	6.916163	-8.90672	4.54E-13	1.11E-10	19.47384
PTPRB	-2.00856	7.921767	-8.25679	6.94E-12	1.16E-09	16.81501
HYAL1	-2.00272	8.545659	-5.64296	3.44E-07	1.16E-05	6.298781

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