Comprehensive Analysis of Hub Genes In Prostate Cancer

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Research

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Abstract

AbstractThe present study aims to explore the involvement of hub genes in prostate cancer (PCa). Three series were used in this study: GSE46602, GSE5377, and GSE8511 from GPL570 platform in the Gene Expression Omnibus and the other resources from The Cancer Genome Atlas and UCSC Xena. A total of 47 differentially expressed genes (DEGs)from 77 patients with PCa and normal individuals were selected in this study. Cytoscape revealed 21 central nodes with 20 edges from the 47 DEGs genes. PCA3, SIM2, AMACR, HOXB6, ERG and TOP2A were overexpressed and correlated in prostate cancer cells and tumors. These findings suggest that PCA3 and TOP2A might help explore the prognosis and diagnostic markers of PCa.

Purpose:

The gene audition was completed using R software and Venn diagrams. The outcome, Gene Ontology enrichment, and Kyoto Encyclopedia of Genes and Genomes preliminary analyses of differentially expressed genes (DEGs) were performed using the R software. A string image was obtained using the Search Tool for the Retrieval of Interacting Genes. The protein–protein interaction (PPI) network was examined using Cytoscape software. The corrplot package was used to analyze the correlation of genes. Human Protein Atlas was used to confirm the protein levels. Univariate Cox regression was used to analyze whether these genes were related to survival. UALCAN was used to confirm the effect of these genes on patient survival[8].

Results: A total of 47 differentially expressed genes from 77 patients with PCa and normal individuals were selected in this study. Cytoscape revealed 21 central nodes with 20 edges from the 47 DEGs genes. The overexpressed genes as PCA3, SIM2, AMACR, HOXB6, ERG and TOP2A were selected based on the cell cycle pathway. A significant correlation was found among the 6 DEGs. The transcription levels and protein levels of these genes were verified in cells and human tissue samples. The overall survival for these genes was analyzed using univariate Cox regression, UALCAN and UCSC Xena.

Conclusion: PCA3, SIM2, AMACR, HOXB6, ERG and TOP2A were overexpressed and correlated in prostate cancer cells and tumors. PCA3 and TOP2A might help explore the prognosis and diagnostic markers of PCa.

Introduction

It's estimated that 21% cases of men suffered from cancer is prostate cancer (PCa), which is the most frequent malignancy tumor among men in the US[1]. Surgically, the treatment methods for PCa are radical prostatectomy or radiation therapy, or those two combined[2]. As the hormone-dependent gland, the androgen deprivation therapy (ADT) is the primary treatment standard of PCa. For the conservative treatment, PCa can develop from castration-sensitive to castration-resistant prostate cancer (CRPC) within 10 years. The median survival times of CRPC and neuroendocrine prostate cancer (NEPC) is less than 3 years[3]. With the consequence of high morbidity and the poor long-term survival outcome, it is

highly urgent to effectively analyze PCa with a different perspective. The bioinformation methods are involved in identifying the origin and development of PCa at the molecular level so as to achieve accurate treatment and potential mechanism pathway of PCa.

A DNA chip can detect differentially expressed genes (DEGs) quickly and accurately. After years of development, the technology has become mature and is widely used in public databases. Based on the databases, a series of processing is carried out to select the desired data. In recent years, researchers have analyzed a large amount of data on PCa. The current research situation is gratifying, and the prospect of integrated bioinformatics methods is promising.

In the present study, the DEGs in 57 pathological PCa tissue (primary prostate cancer or metastatic prostate cancer) and 20 normal tissues (benign prostatic hyperplasia or benign prostate glands) were analyzed with R software in the interest of studying the potential valuable genes in PCa. The top genes were selected to construct a protein–protein interaction (PPI) network, and Cytoscape MCODE was used to screen out more valuable genes. The protein levels were got from Human Protein Atlas (HPA). The effect of genes on the survival of patients with PCa were verified using the UALCAN-based Cancer Genome Atlas (TCGA) database. Finally, 14 genes overexpressed in PCa were obtained.

This study aimed to explore the prognosis and diagnostic markers of PCa. Three series (GSE46602, GSE5377 and GSE8511) were used in this study and the other from TCGA. The gene audition was completed using R software and Venn diagrams. A total of 47 DEGs were selected in this study. Cytoscape revealed 21 central nodes from the 20 genes. PCA3, SIM2, AMACR, HOXB6, ERG and TOP2A were selected based on the cell cycle pathway. PCA3 and AMACR had the highest correlation. The protein levels were verified from HPA. PCA3 and TOP20A were associated with a significant progress free interval (PFI) in patients with PCa.

Materials And Methods

Ethics Statement

The Institutional Review Board of the hospital approved the study in strict adherence to the Declaration of Helsinki protocol. The written informed consent form was signed by ethics committee member to approve.

Data Sources

Researchers submitted their own research data to Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/gds/) and The Cancer Genome Atlas (TCGA, https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga) to share data with other researchers freely around the world. We downloaded three series (GSE46602, GSE5377 and GSE8511) from the GEO database, which they were found in GPL570 Platforms ([HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array) and 499 cases of TCGA-PRAD from The Cancer

Genome Atlas (TCGA). Kaplan Meier gene expression of hub genes were identified from UCSC Xena(http://xena.ucsc.edu/).

Data Processing of DEGs

The quality of GSE46602, GSE5377, and GSE8511 was evaluated using R software (Version 3.6.1). At present we use the probes of Affymetrix, Affy package https://bioconductor.org/packages/release/bioc/html/affy.html and AffyPLM package http://bioconductor.org/packages/release/bioc/html/affyPLM.html to parse the original data . The log scale robust multi-array analysis was applied to background correction, quantile normalization, and median polish in the R software[4]. The first screening of genes was identified via the Linear Models for Microarray Data (http://www.bioconductor.org/packages/release/bioc/html/limma.html) package (|logFC|>2 and adjusted P value <0.05). Venn software was used to find the commonly expressed DEGs among the four series. Corrplot package was used to analyze the correlation of genes.

Gene Ontology and KEGG Pathway Analysis of DEGs:

Gene Ontology (GO) enrichment is used to define genes and their RNA or protein products so as to determine the unique biological characteristics of transcriptome and genomic data[5]. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway is a collection of databases that can deal with genomes, drugs, diseases, chemical materials, and biological pathways[6]. R software was used to analyze the DEG enrichment of biological process, molecular function, and cellular component and pathways.

PPI Network and Module Analysis

PPI information stemmed from an online tool Search Tool for the Retrieval of Interacting Genes (Version 11.0). In addition, the MCODE app in Cytoscape was used for checking cluster analysis of the PPI network (degree cutoff = 2, max. depth = 100, k-core = 2, and node score cutoff = 0.9) after analyzing the interaction between these DEGs in Cytoscape (Version 3.5.1) (maximum number of interactors = 0 and confidence score ≥0.4)[7].

Protein Level, Survival Analysis and Correlation of DEGs

The protein level was checked by immunohistochemistry from HPA http://www.proteinatlas.org/. Univariate Cox hazard analysis was used to look for genes related to survival. UALCAN is a user-friendly, comprehensive, interactive, and free web resource for analyzing cancer data from TCGA. The corrplot package was used to analyze the correlation of DEGs ($P < 0.001$).

Statistical Analysis

SPSS 24.0 software (IBM Corp, NY, USA) and GraphPad Prism 8 (GraphPad, USA) were used for statistical analysis. The Student t-test was used to analyze the difference in genes. |logFC|>2 and P <

0.05 were set as a threshold to choose the significance level.

Results

Identification of DEGs

DEGs with P < 0.05 and |logFC| > 2 were selected from 57 prostate tissues and 20 normal tissues in the present study. The R software was used to check 229, 468, and 1007 DEGs from GSE46602, GSE5377, and GSE8511 and TCGA, respectively. The |logFC| and adjusted P value were visualized using the volcano plots drawn. Venn software online was used to find the intersectional DEGs in the four series. The Venn plots showed that 47 DEGs were outputted, including 14 upregulated genes (logFC >2) and 33 downregulated genes (logFC <-2). (see Fig1-6)

GO and KEGG Pathway Analyses of DEGs in PCa

All 47 DEGs were detected using R software. The results of GO analysis demonstrated that these DEGs were for biological process, cellular component, and molecular function. The DEGs were particularly gathered in nuclear division and organelle fission for biological process; spindle and chromosomal region for cellular component; and monooxygenase activity, microtubule binding, and tubulin binding for molecular function. KEGG analysis results demonstrated that DEGs were particularly gathered in female meiotic nuclear division, meiotic cell cycle, and meiotic spindle.(see FIG7) .

Verification at the Transcriptional, Protein Level and the Effect of Genes on the Survival of Patients with HCC Using UALCAN& Xena:

The corresponding KEGG pathway was detected in 21 central nodes. Notably, the cell cycle pathway has a prominent role in tumorigenesis. which are rich in the cell cycle and divison. PCA3, SIM2, AMACR, HOXB6, ERG and TOP2A were chosen for the next study. The correlation between the genes from TCGA data had the highest correlation. The expression levels of 6 genes were detected by immunohistochemistry from HPA(supplementary materials A).Next, we searched for genes related to survival using univariate Cox hazard analysis. These six genes were high-risk genes, and the TTK hazard ratio was the highest. The overall survival for six significant genes was analyzed with UALCAN and Xena (supplementary materials B). The results showed that PCA3 and TOP2A might help explore the prognosis and diagnostic markers of PCa(see FIG8-10).

This study was based on the profile datasets. A total of 57 PCa tissues and 20 normal specimens were included in the study. Limma package was used to filter the DEGs with adjusted P value <0.05 and |logFC| >2. The results revealed 47 DEGs, including 14 upregulated genes and 33 downregulated genes. The |logFC| and adjusted P value were obtained using the volcano plots. Next, GO using R software showed biological process, cellular component, and molecular function in both upregulated and downregulated genes. KEGG analysis results demonstrated that DEGs were particularly gathered in female meiotic nuclear division, meiotic cell cycle, and meiotic spindle. Next, 47 genes were imported into the gene PPI

network with the STRING database and Cytoscape software. Six hub genes were found in the cell cycle pathway. Next, the correlation between the genes was described. PCA3 and AMACR had the highest correlation. Furthermore, the expression levels of these six genes in normal and PCa cells were verified though UALCAN and UCSC Xena. The protein levels of the six genes were verified from HPA. PCA3 and AMACR genes were associated with a significant PFI in patients with PCa.

Conclusions

The present study found six genes (PCA3, SIM2, AMACR, HOXB6, ERG and TOP2A) in TCGA and GEO databases. These genes were overexpressed in PCa. The study also explored the unreported correlation of these genes within PCa. The results of this study might help in exploring the prognosis and diagnostic markers of PCa in the future.

PCA3

Prostate cancer antigen 3 (PCA3) is long non-coding RNA (lncRNA), first described by using differential display experimental approach and named as DD3[9]. PCA3 modulates prostate cancer (PCa) cell survival through modulating androgen receptor (AR) signaling, besides controlling the expression of several androgen responsive and cancer-related genes, including epithelial–mesenchymal transition (EMT) markers. Also, PCA3 urine levels have been successfully used as a PCa diagnostic biomarker[10]. Qin Z et al [11]performed a meta-analysis with 8.139 cases and 14.116 controls to evaluate the diagnostic value of PCA3 and the summary diagnostic odds ratio (DOR) and 95% CIs for PCA3 was 5.44 (4.53-6.53). Ye LF et al [12]invented a new method, LBXexo score, to measure the urine exosomal PCA3/PRAC expression in the urine thus the LBXexo can improves diagnosis of PCa in Chinese Population. For clinical outcome, Alshalalfa M et al found PCA3 had a poor performance of predicting high grade disease in initial biopsy (GS≥8) and high false negative rates. Our results find PCA3 and AMACR are highly related and Kotova ES et al [14] detected PCA3 and AMACR mRNA in PCa patient urine samples. In this study, the PCA3 score AUC was 0.632 (95%CI: 0.511–0.752), the AMACR score AUC was 0.711 (95%CI: 0.617–0.806), which indicted a significant diagnose clinical biomarkers for prostate cancer.

SIM2

The SIM2 (single-minded 2) gene is a member of the family of transcription factors with basic helix-loop-helix/per-Arnt-Sim (bHLH/PAS) domains and has been involved in the pathogenesis of solid tumors.In breast cancer, SIM2s directly downregulates SNAI2 expression and inhibits EMT, and represses tumor growth and invasion[15]. Wyatt GL et al [16] found a cross-talk between SIM2s and NFκB which posed a inhibition effect on the breast tumor growth and metastasis.

AMACR

The isomerase α-methylacyl-CoA racemase (AMACR) is most commonly known for its physiologic role in catalyzing the stereoconversion of the α-methyl proton of branched chained fatty acids undergoing β-oxidation in the mitochondria and peroxisomes[17]. AMACR expression in needles biopsies had a 97% sensitivity and 100% specificity for PCa detection [18]. Xie H et al [19] used the miR200c to inhibits AMACR activity in prostate adenocarcinoma and they found suppression of AMACR can suppress the cell proliferation and migration.

HOXC6

There is 39 human HOX genes located at 4 chromosomal loci. HOXC6 have been previously identified as promising, easy to assay (urinary-based) biomarkers of aggressive prostate cancer that can enhance early diagnosis and predict cancer recurrence after treatment[21]-[24]. Luo Z et al[20]found that the majority of the HOXC4 binding sites overlap with the HOXC6 binding sites. Via genome-wide analysis, they identify that HOXC6 are clinically relevant biomarkers of aggressive prostate cancer.

ERG

ERG (E-26 transformation specific-related gene) family proteins belong to the larger family of ETS transcription factors, which is is one of the largest families of TFs in metazoans and is defined by a highly conserved DNA-binding ETS domain[25]. ERG family genes are implicated in oncogenic gene fusions due to translocations that typify several cancers like PCa[26]-[28]. Eerola SK et al [29]found a cooperation of PIM and the MYC and ERG oncoproteins in PCa development and progression via mRNA expression and chromatin immunoprecipitation sequencing (ChIP-Seq) datasets.

TOP2A

Topoisomerases type IIA (TOP2A), as one of the two members of topoisomerase II (TOP2) family, with another is TOP2B, is expressed only in cycling cells and responsible for enzymatic uncoupling during the replication of DNA strands[30]. In colon cancer tissues, TOP2A has been reported to be overexpressed[31]. However, the role and mechanisms of TOP2A in the development and progression still remain unknown[32]. In radical prostatectomy tissue, TOP2A was the most correlated to mCCP (r = 0.7)[33]. Cattrini C et al [34]also found TOP2A mRNA overexpression was associated with worse patients' prognosis.

Abbreviations

DEGs, differentially expressed genes; GEO, Gene Expression Omnibus; GO, Gene Ontology; PCa, prostate cancer; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein–protein interaction; STRING, Search Tool for the Retrieval of Interacting Genes; TCGA, The Cancer Genome Atlas; PFI Progress free interval; HAP, Human Protein Atlas.

Declarations

Ethics Approval and consent to participate

The study had a statement on ethics approval. The ethics committee of the Lanzhou University approved the study.

Consent for publication

Written informed consent for publication was obtained from all participants.

Competing interests

The authors have no conflicts of interest to declare that are relevant to the content of this article

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Author's Contributions

YZ contributed to the conception of the study. YZ performed the data analyses and wrote the manuscript. MJ helped to perform the analysis with constructive discussions.

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Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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Figures

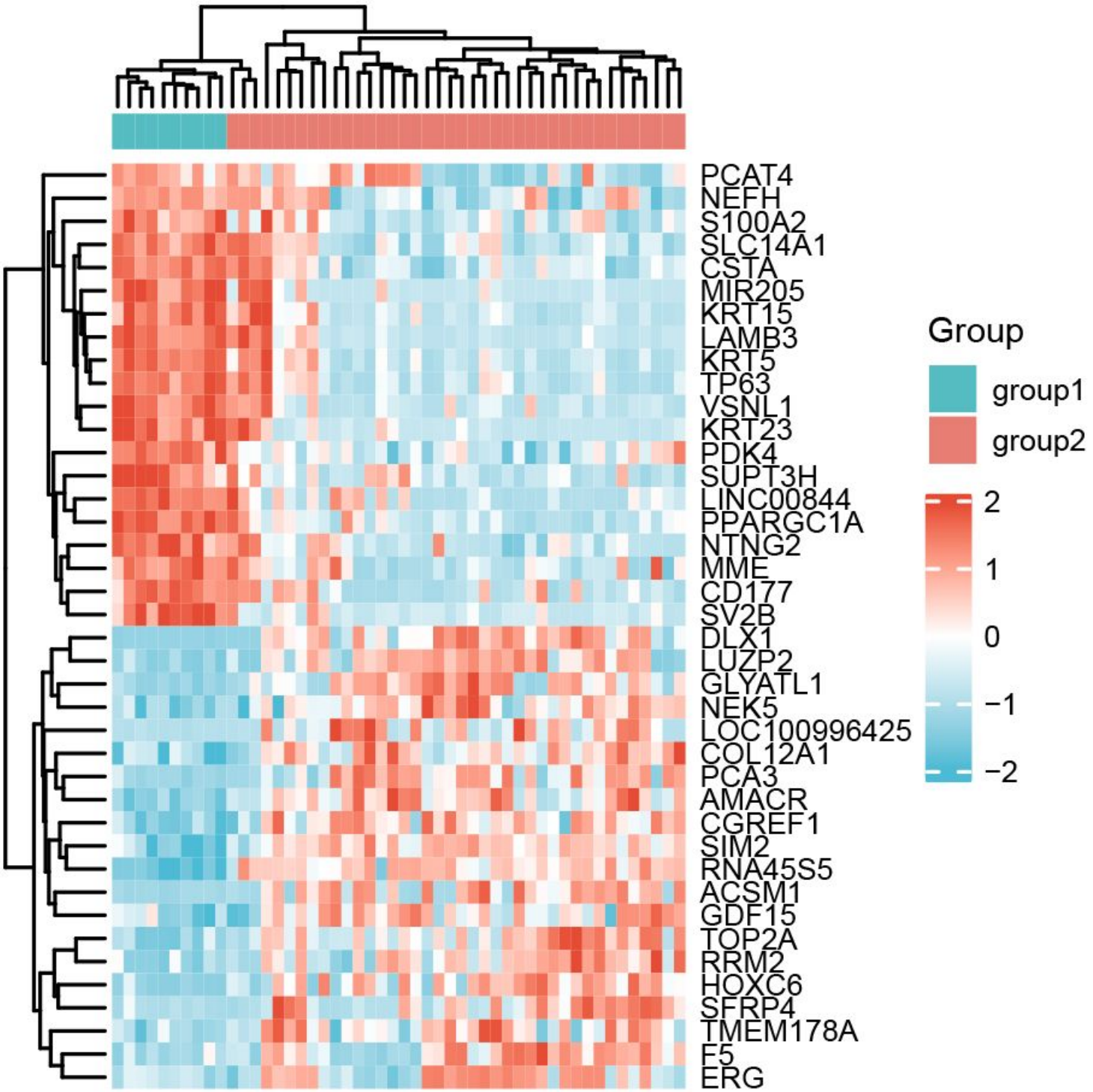

Figure 1

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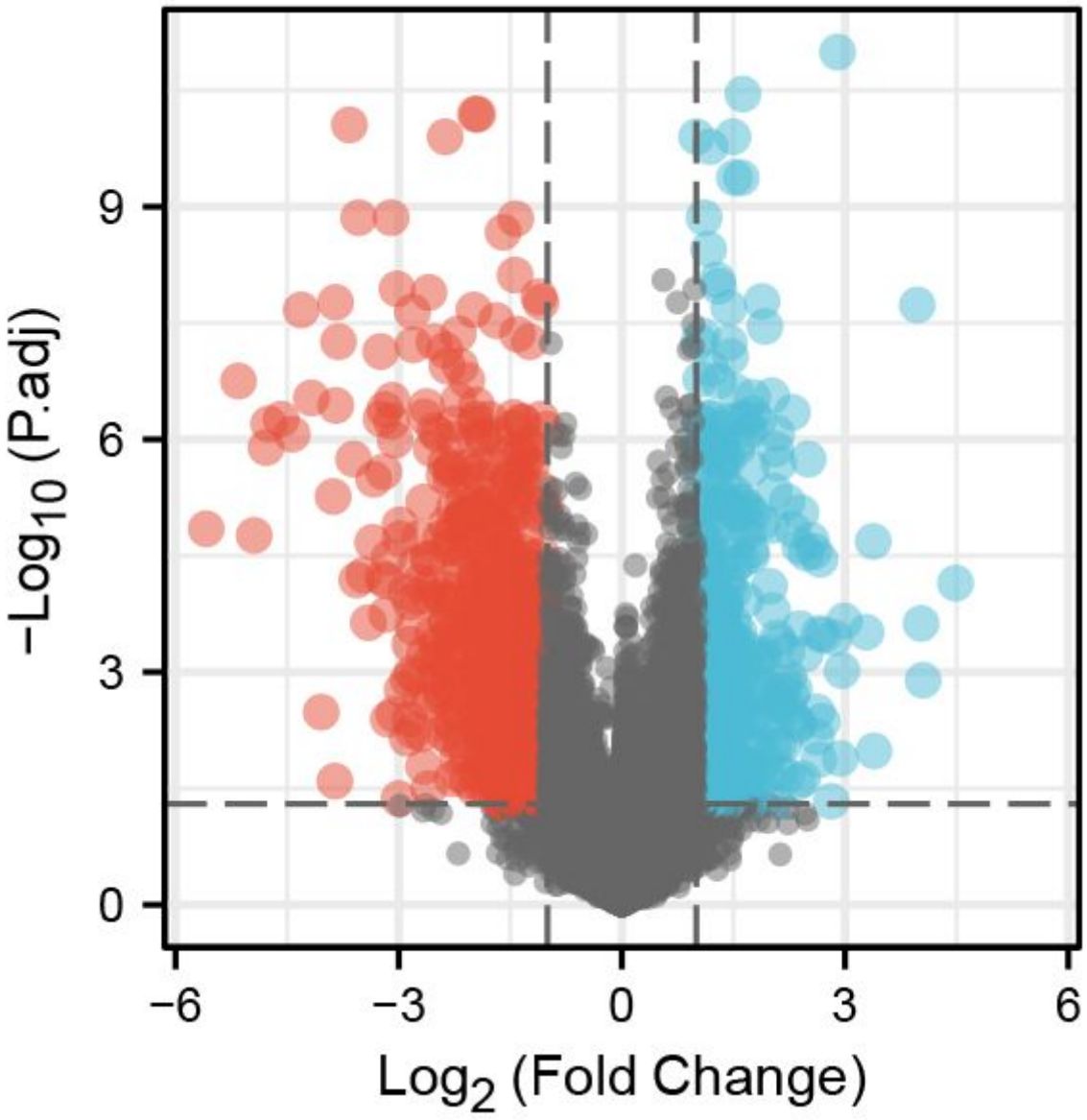

Figure 2

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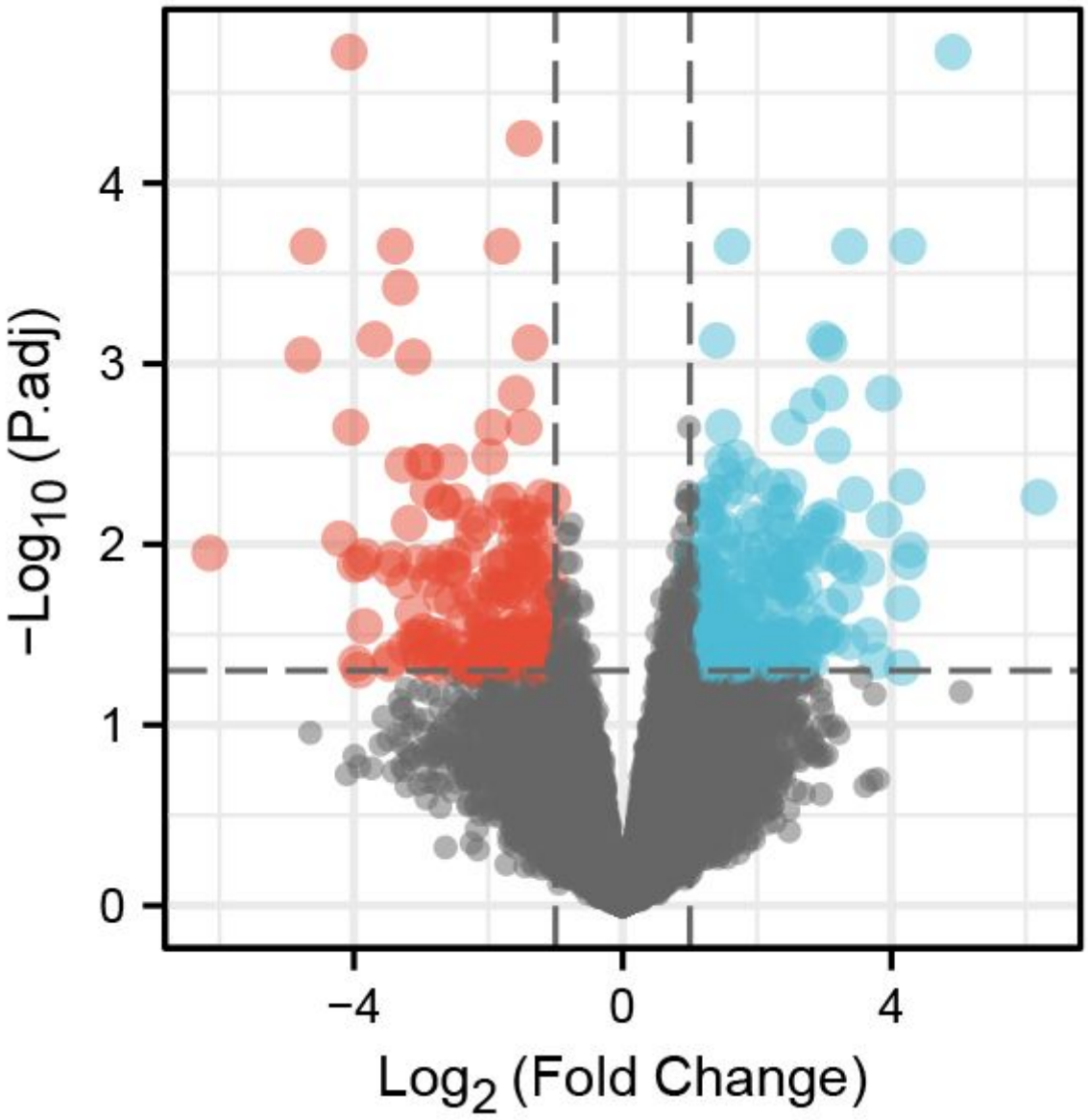

Figure 3

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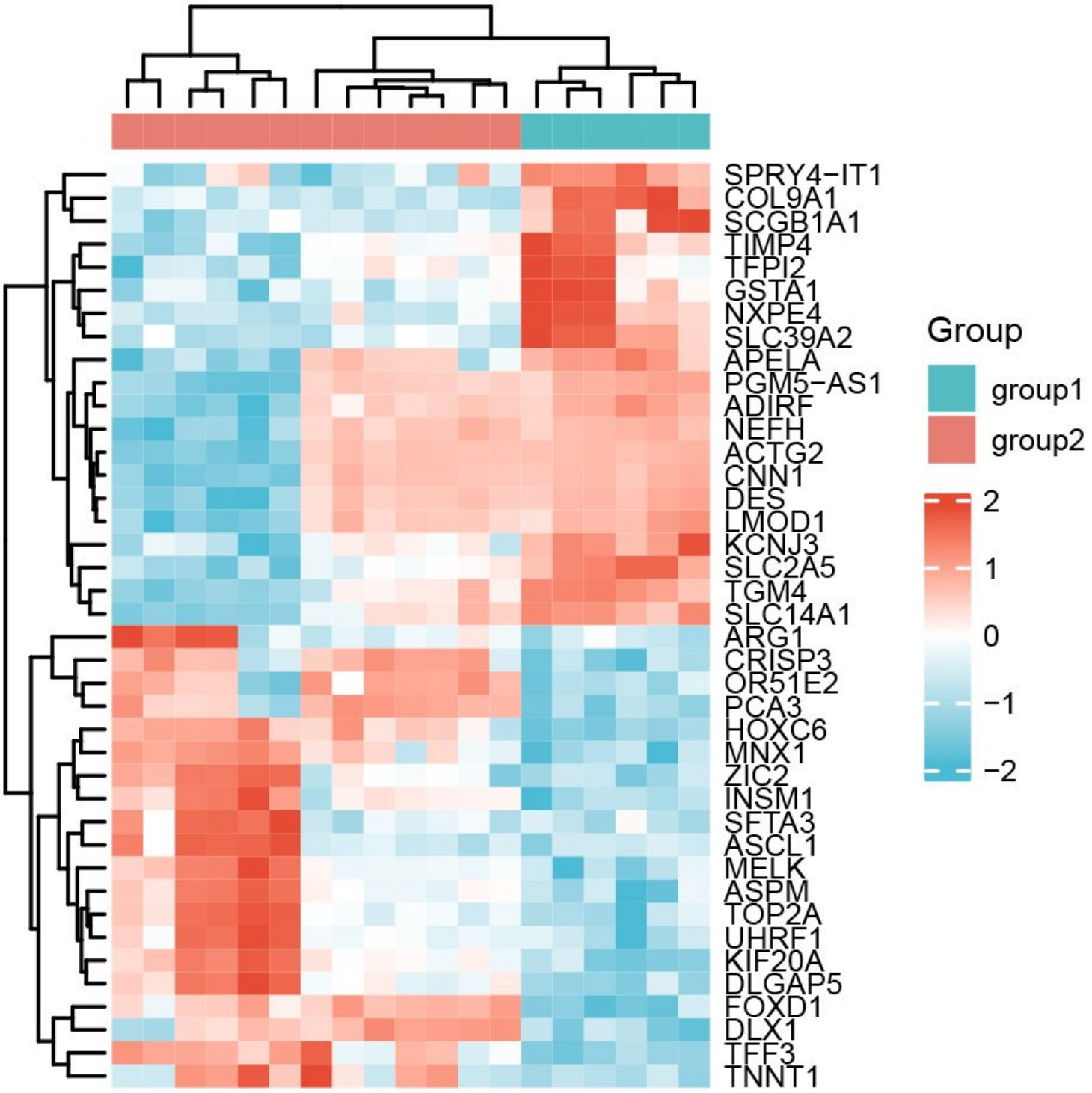

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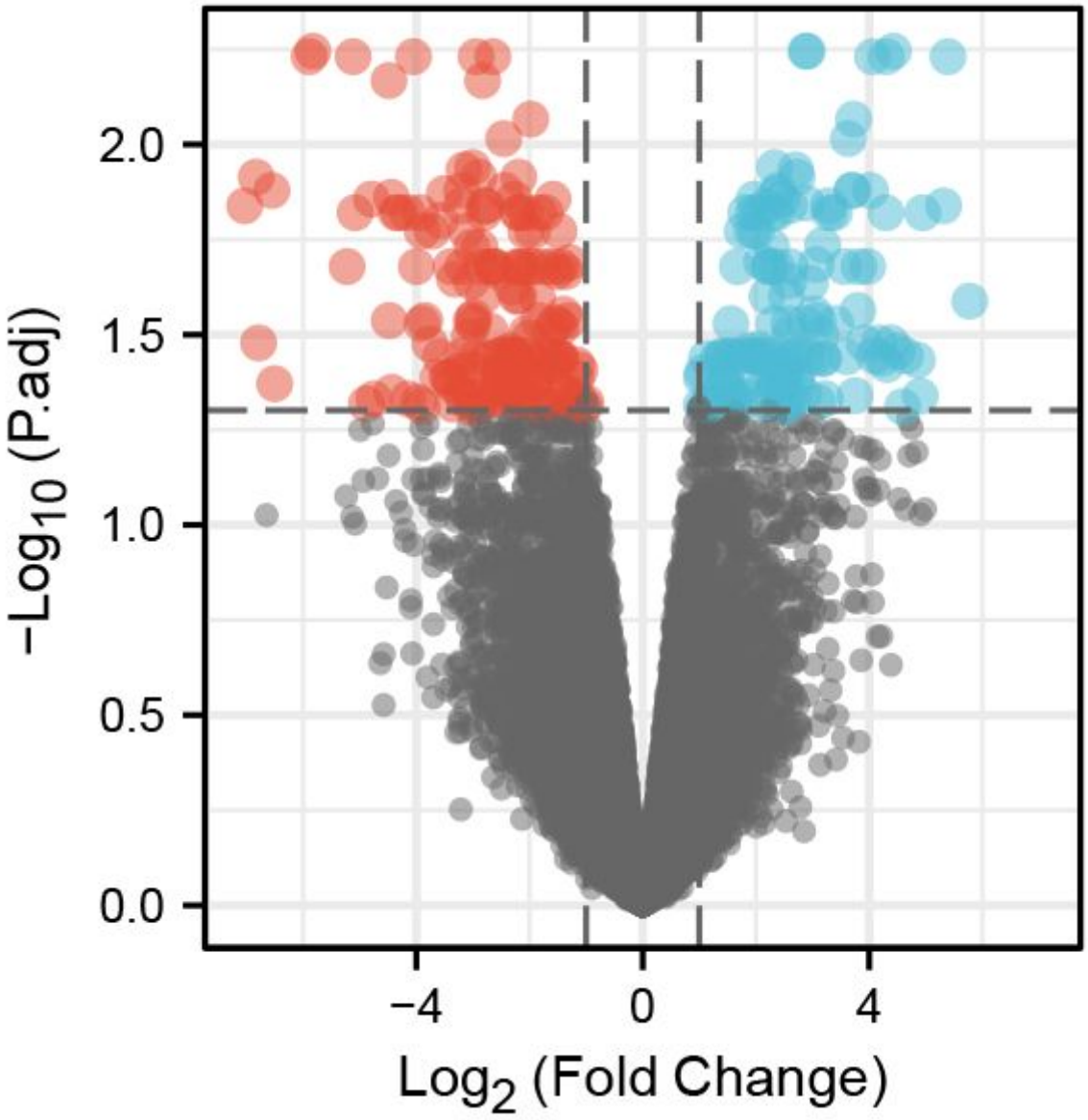

Figure 5

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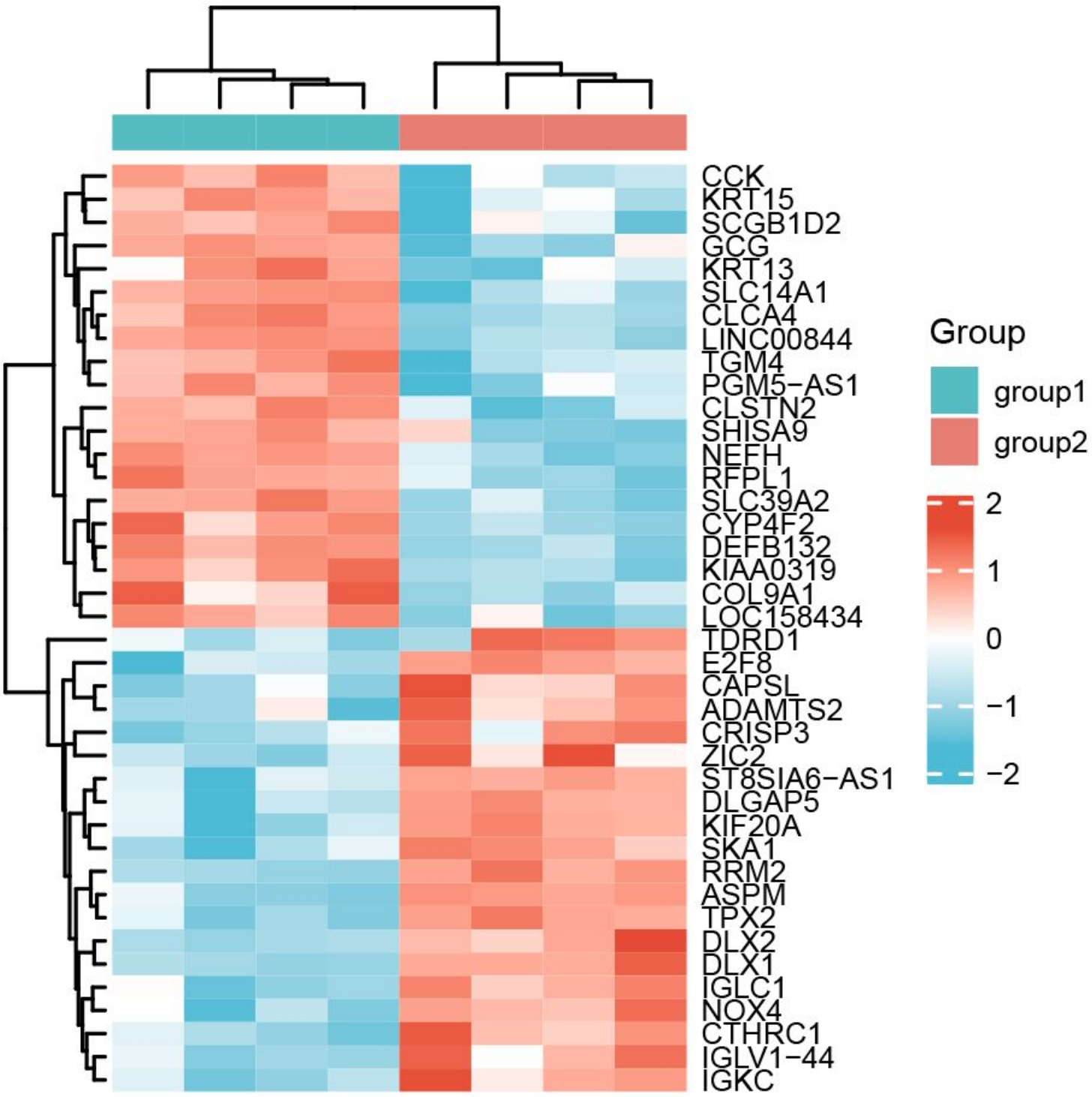

Figure 6

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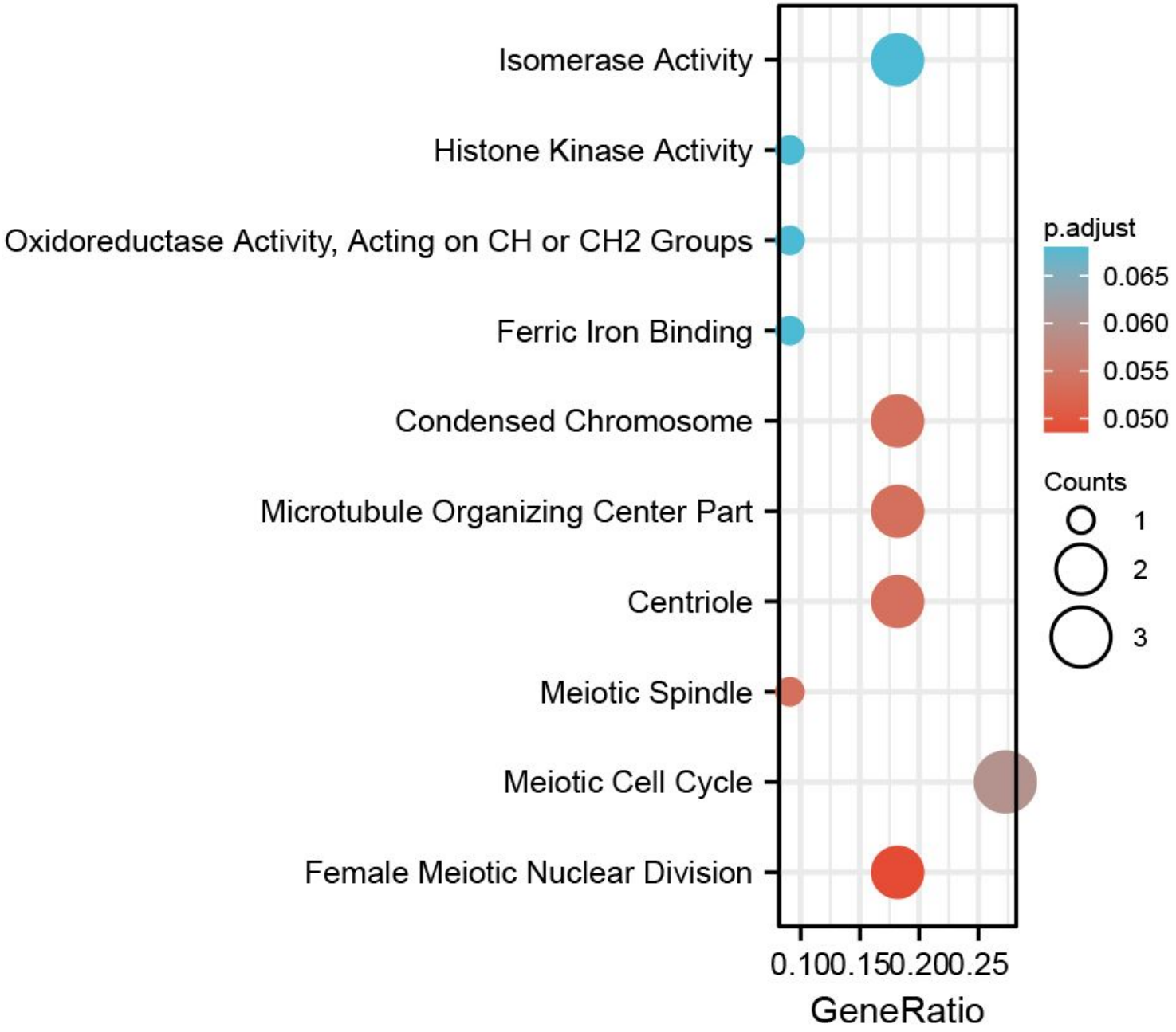

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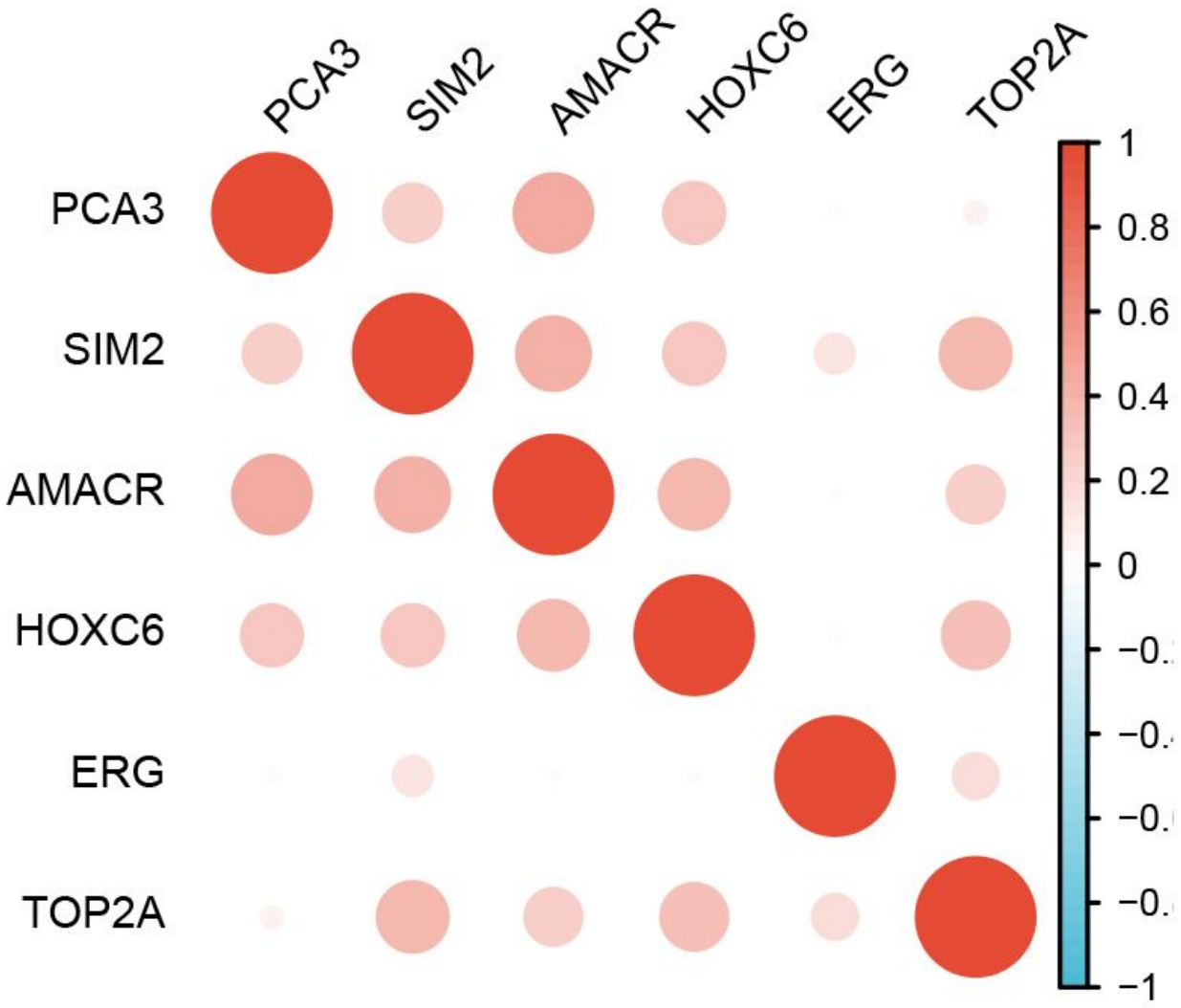

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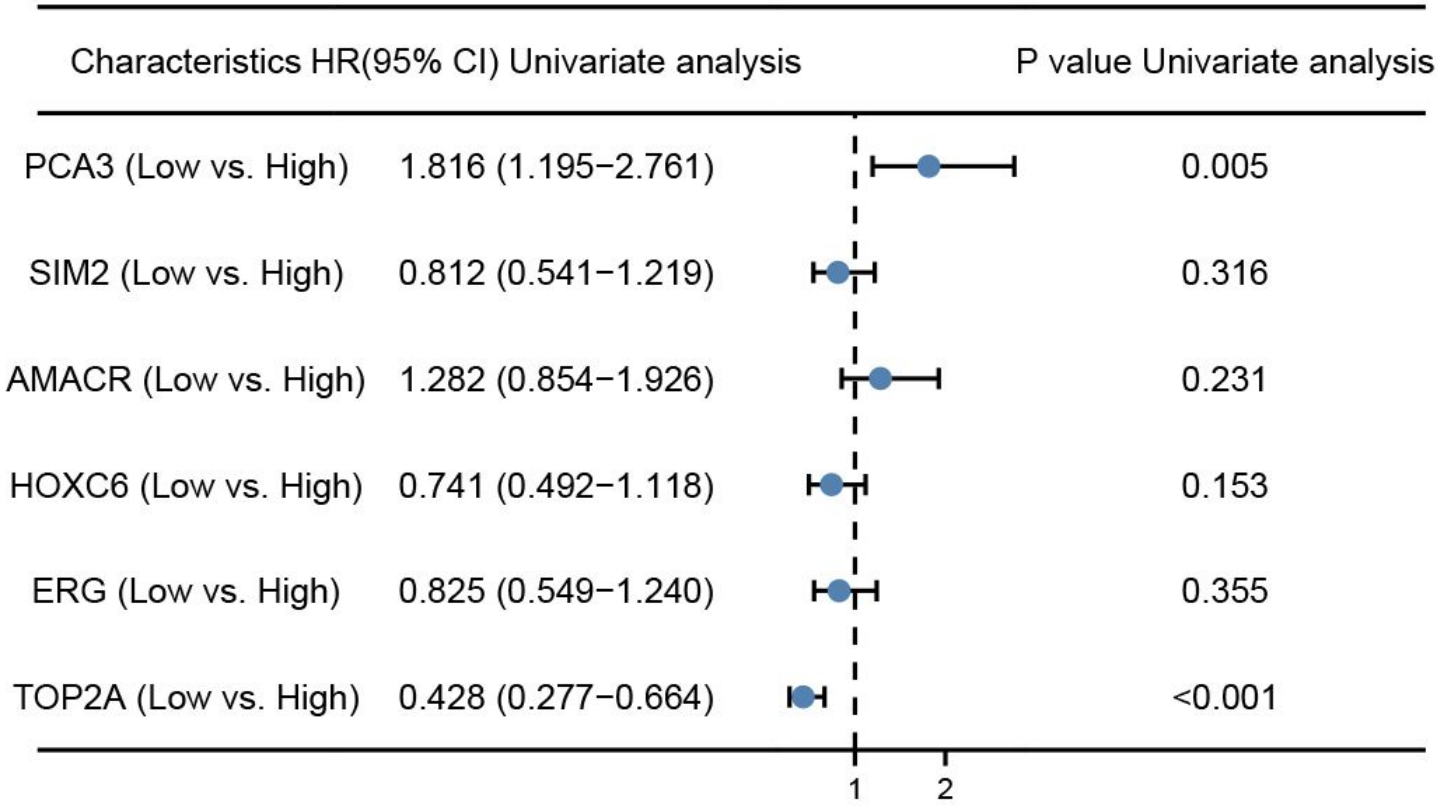

Figure 9

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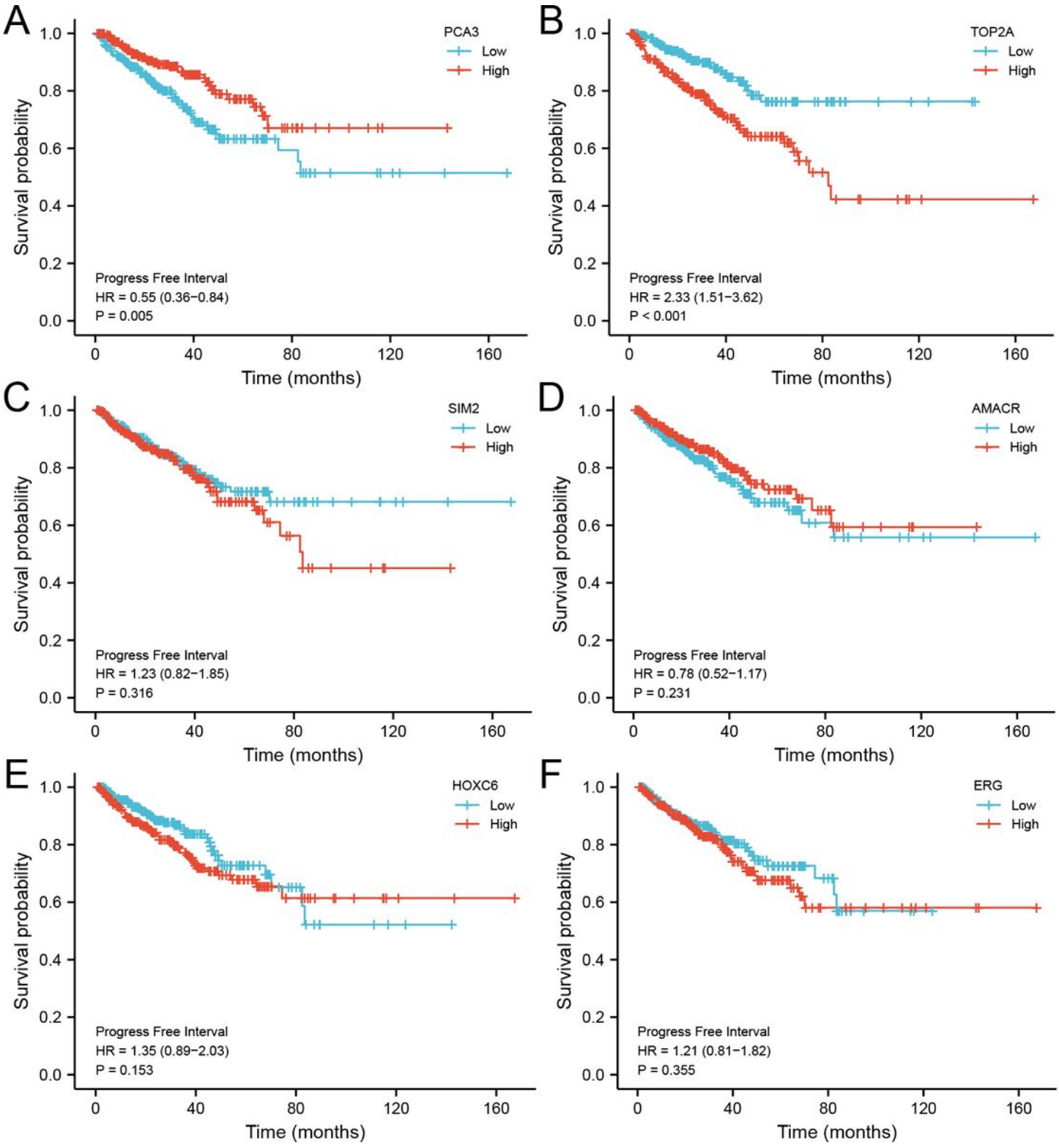

Figure 10

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