The function of CISH in T cell differentiation and proliferation

Zhangyong Hu (Tlee1996@163.c0m)
the First Affiliated Hospital of Chengdu Medical college https://orcid.org/0000-0001-6842-2822
Yan Zhou
Chengdu Medical College
Tiane Li
Chengdu Medical College
Lei Zhang
Sichuan Academy of Medical Sciences and Sichuan People's Hospital
Min Zhou
Chengdu Medical College
Jun Yang
Chengdu Medical College
Shuang Wang
Chengdu Medical College
Xuemei Yang
Chengdu Medical College
Yanfang Li
Chengdu Medical College

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Abstract

Background: Previous studies have shown that cytokine-inducible SH2 containing protein (CISH) gene polymorphism is related to the differences in the outcomes of hepatitis B virus (HBV) infection. T cells play an important role in HBV infection. We tested the hypothesis that the CISH affects the outcome of HBV infection by influencing the T cells.

Methods: CISH gene and shRNA were cloned into pCDH and pLKO.1 plasmid, respectively. We used 293T cells to package the lentivirus and collected the lentiviral supernatant to infect CD3 T cells and compared the differences in T cell proliferation, differentiation, T-cell receptor Vβ (TCRVβ) subfamily expression, and gene expression in each group.

Results: We found that CISH had a negative effect on T cell proliferation. The expression of TCRVβ was also affected. The expression of TRVβ3.1 and TRVβ12 was up-regulated by CISH. Reducing CISH expression contributed to the formation of $CD8^{+}$T cells. Gene ontology (GO) analysis results showed that CISH mainly regulated chromosome segregation, nuclear division, and organelle fission in the biological process (BP) group; chromosomal region, centrosome, and spindle in cellular components (CC) group; ATPase activity, and carbohydrate binding in molecular function (MF) group. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis results showed that CISH primarily affected the p53 signaling pathway, PI3K-Akt signaling pathway and others.

Conclusion: We found that CISH plays an important role in T cells and this helps to further clarify the pathogenesis of HBV infections.

Introduction

Despite the availability of effective vaccines and antiviral therapies, hepatitis B virus (HBV) infection remains a significant global health threat. It is estimated that 257 million people were living with chronic HBV infection in 2015, and 1.34 million deaths are caused by viral hepatitis annually [1].

In chronic HBV infection, the natural history and course of the disease depends on the interaction of the virus with the host immune cells. Some patients develop cirrhosis, or hepatocellular carcinoma, while in others the virus remains dormant throughout their lives and does not require antiviral treatment. The different outcomes are closely related to host immunity. In HBV infection, the immune response is responsible for removing the virus and preventing its transmission within the host [2]. The T cells play an important role in the immune response and patients who spontaneously recover from HBV infection usually mount vigorous multi-epitope-specific CD4 and CD8 T cell responses [3]. Animal experiments have shown that viral specific CD8 T cells and their induced cytokines contribute to virus clearance [4]. Some studies have suggested that CD4 T cell may indirectly control HBV infection by promoting the induction of HBV specific CD8 T cell responses [5]. It is becoming clear that T cell-mediated immune responses determine the outcome of HBV.

Cytokine-inducible SH2 containing protein (CISH) is a member of the suppressor of cytokine signaling (SOCS) family. CISH polymorphism has been shown to be associated with differences in the outcomes of HBV infection [6]. CISH is expressed following the stimulation of the T-cell receptor (TCR), and is involved in the negative regulation of TCR signaling [7]. CISH can inhibit the activation of STAT3, STAT5, and STAT6 in T cells. Moreover, studies have shown that the CISH-SH2 domain is essential for PLC-γ1 regulation in TCR-stimulated CD8 T cells. Studies have also suggested that CISH may play an important role in the growth and differentiation of T cells [8, 9]. However, the effect of CISH on T cell growth and differentiation remains unclear. We sought to explore the biological effects of CISH on T cells by regulating the expression of CISH in T cells.

Materials And Methods

Isolation of CD3 T cells

Human peripheral blood mononuclear cells (PBMCs) were obtained by density-gradient centrifugation of heparinized venous blood from healthy volunteers. Informed consent was obtained from all donors. The CD3 T cells were separated from the PBMCs by magnetic cell sorting using Hu CD3 IMag Particles-DM HIT3a. The purity of the CD3 T cells was analyzed by flow cytometry.

Construction of pCDH-CISH plasmid

The CISH gene was synthesized and cloned into pCDH-puro vector by Anhui Biotechnology company. The recombinant plasmid was labeled pCDH-CISH, and the negative control was labeled pCDH.

Construction of pLKO.1-shRNA plasmid

The sequences of the CISH shRNA and negative control that were designed and synthesized by the Wuhan Qingke Biological company (Wuhan, China), are shown in Table 1. The system of the annealing oligos was followed by mixing 20 μM forward and reverse oligo in 10 μL volume each, 5 μL 10× NEB buffer 2 and ddH_2O together (total volume of 50 μL). The pLKO.1 plasmid was digested with EcoRI and BshTI. Using the Gel extraction kit, the annealed oligos were ligated into the digested pLKO.1 vector in a ligation mix that contained 20 ng PLKO.1 vector, 2 μl of annealed oligos, 1 μl 10× T4 DNA ligase buffer, 1 μl T4 DNA ligase, and ddH2O (total volume of 10 μL). The reaction was incubated at 25˚C for 2 h. A 10 μL volume of the ligation mix was transformed into 50 μL of competent DH5α cells. The transformed cells were transferred to LB agar plates containing ampicillin. These plates were placed at room temperature until the liquid was absorbed, the plates were then inverted and cultured at 37˚C overnight. The positive clones were identified by sequencing.

Lentiviral packaging and infection

The plasmids pCDH, pCDH-CISH, pLKO.1-NC, and pLKO.1-shRNA were extracted using the TIANpure Mini Plasmid Kit II. 293T cells were plated at 2.5 × 10^6 cells/mL in culture plates and incubated for 24 hours at

37˚C and 5% CO_2 before transfection. On the day of the transfection, a 450 μL aqueous mixture of DNA (15 μg plasmid, 12 μg delta 8.9 packaging plasmid, and 8 μg VSVG envelope plasmid) and $CaCl_2$ (50 μl of 2M $CaCl_2$) was added dropwise to 500 μL of 2× HBS. The medium containing the HBS/DNA mixture was then added dropwise to the cells. Eight hours post-transfection, the medium was replaced with fresh medium. The transfected 293T cells were then collected and CISH expression was analyzed by western blotting and real-time qPCR. The lentiviral supernatant was collected after 48 and 72 h.

The CD3 T cells were cultured in 6-well plates at 1 - 2 × 10^5 cells per well in X-VIVO^{TM} 15 medium supplemented with 10% fetal bovine serum (GIBCO), 0.01 μg/mL human IL-2, 1% glutamine, 5 × 10^{-5} mol/L β-mercaptoethanol, and 1% penicillin and streptomycin. The culture medium was replaced on day 2, 5, 7, and 8. Anti-human CD28 monoclonal antibody (1 g/mL) and anti-human CD3 monoclonal antibody (1 g/mL) was added on days 1 and 7. The CD3 T cells were plated 1.0 ×10^5 cells/mL in 6-well plates for the lentiviral infection. A 20 μl volume of medium containing 10× concentrated lentivirus was added to each well. After 8 h, the medium was replaced with growth medium and the transfection was repeated after 24 h.

Real-time PCR

Total RNA was extracted with TaKaRa MiniBEST Universal RNA Extraction Kit (Takara). The ratio of absorbance at 260 and 280 nm (A260/280) was used to assess the purity of the RNA. cDNA was synthesized using Primescript RT reagent kit with gDNA Eraser (Takara). Quantitative real-time PCR detection was performed using a light cycler 480 SYBR Green Master (Roche) according to the manufacturer's instructions. The primers specific for CISH, TRVβ3-1, TRVβ7, TRVβ9, TRVβ11, TRVβ12, VEGFA, PDGFR-β, DUSP1, CDKN1A, CCND2, BCL2, IM1, DM2, CRA1, and KD2 are listed in Table 2.

Western blot analysis of CISH protein expression

The T cells were lysed in RIPA buffer containing the protease inhibitor PMSF, and then centrifuged at 4˚C at 12,000 ×g for 3 - 5 min. The total protein concentration was measured using a BCA protein assay kit. Protein samples were separated by SDS-PAGE and transferred to PVDF membrane at 200 mA constant current for 1.5 h. The membrane was then blocked with 5% milk/TBST solution for 1 h. The membranes were incubated with an appropriate dilution of the primary antibody in solution at 4˚C overnight. Membranes were washed 3 times with PBST and incubated with diluted horseradish peroxidase conjugated goat anti-rabbit antibody at room temperature for 2 h. The membrane was washed with PBST 3 times for 10 min each and the ECL system was used to visualize the protein bands.

T cell colony assay

To evaluate the effect of CISH on T cell proliferation, clonogenicity was analyzed using the CFU-T assay. T cells were plated in 6-well plates at 2 ×10^5 cells per well in CFU-T medium. The CFU-T medium consisted of the VIVO 15 medium with 0.8% methylcellulose, 20% inactivated serum, 5×10^5 mol/L β-

mercaptoethanol, 1% glutamine, 1 g/mL PHA, 0.01 g/mL IL-2, 100 U/mL penicillin, and 100 g/mL streptomycin. After 7 days of incubation at 37˚C in 5% CO2, the T cell colonies in the plate were counted.

Flow cytometry

The expression of surface markers by T cells were determined using flow cytometry. The monoclonal antibodies were provided by Beckton-Dickinson (CD3 Percp Cy5.5, CD25 PE, CD45RA FITC/CD45RO ZE/CD3Percp/CD4APC), Beckman Coulter, Immunotech (CD4 APC, CD8 FITC), and DAKO (CD86 FITC). Flow cytometry was performed on a FACSCalibur, and the data were analyzed with the BD Accuri C6 software or Flowjo software.

Microarrays

Total RNA was extracted from the CD3 T cells. RNA quality was assessed using the Agilent 4200 TapeStation, and RNA quantification was performed using the Life Invitrogen Qubit® 3.0 with Qubit™ RNA HS Assay Kit. The mRNA was separated using magnetic beads with oligos. cDNA was reverse transcribed from the mRNA and sequenced by Illumina PE150 instrument. The DESeq2 was used to identify the differentially expressed genes (DEGs) in the CD3 T cells in the different groups. p-adjust < 0.05 and |log2 (FoldChange)| > 1 were set as the thresholds for significant differential expression. To further investigate and compare the functions of the DEGs, GO enrichment and KEGG pathway analyses were performed using clusterProfiler to describe the BP, CC, MF, and KEGG pathway.

Results

Isolation of CD3 T Cells

We used Hu CD3 IMag Particles-DM HIT3a to isolate CD3 T cells from the PBMCs. The results of the CD3 isolation that was performed of three times showed that the purity of the $CD3^+$ lymphocytes was 92.95%, 92.45%, and 92.61%, respectively, as measured by flow cytometry (Fig. 1a). The activity of the isolated CD3 T cells was enough to meet the needs of the subsequent experiments using T cells.

Construction of pCDH-CISH plasmid and packaging

pCDH (up-regulated control group) and pCDH-CISH (up-regulated CISH group) plasmids were provided by Anhui Biotechnology Company following verification of the ability of the inserted CISH gene to up-regulate the expression of CISH. pCDH and pCDH-CISH were transfected into 293T cells, and the transfected cells were collected to detect the CISH mRNA by RT-PCR and the CISH protein by western blotting. The results demonstrated that the expression of CISH mRNA in the pCDH-CISH group was significantly higher when compared to the control group (Fig. 1b1). In addition, western blot analysis revealed that the expression of CISH protein in the pCDH-CISH group was significantly higher compared to the pCDH group (Fig. 1b2).

Construction of pLKO.1-shCISH plasmid and packaging

The CISH shRNA1, CISH shRNA2, CISH shRNA3 and the scrambled shRNA were provided by the Wuhan Qingke Biological company (Wuhan, China). The CISH shRNA was amplified by the PCR and cloned into the PLKO.1 vector. *Escherichia coli* were transformed with pLKO.1-shCISH. pLKO.1-shRNA1 and pLKO.1-shRNA3 were successfully confirmed by DNA sequencing. pLKO.1-shRNA1 and pLKO.1-shRNA3 were transfected into 293T cell lines and the cells were collected. PCR and western blotting were used to analyze the expression of CISH. The results revealed that the expression of CISH in the 293T-shRNA1 and 293T-shRNA3 group was significantly lower compared to the 293T and 293T pLKO.1-NC groups (Fig. 1c1, c2).

Transfection of the recombinant lentiviral plasmid in CD3 T cells and expression of CISH

Lentiviral supernatant was collected from 293T cells that were transfected with the recombinant plasmids for 48 and 72 h. The lentiviral supernatant was then used to infect CD3 T cells and CISH expression in the CD3 T cells was analyzed using RT-PCR and Western blotting (Fig. 1d1, d2). We found that the expression of CISH in the up-regulated group (pCDH-CISH) was significantly higher than the down-regulated group (pLKO.1-shRNA) and the control group.

Effect of CISH on T cell proliferation

The ability of the T cell to proliferate was studied using the T cell colonies (CFU-T) assays. Methylcellulose medium was prepared to cultivate the blank, pCDH, pCDH-CISH, pLKO.1-NC, and pLKO.1-shRNA. CFU-T were evaluated following 7 days of culture. The colonies were used to evaluated proliferation ability. pCDH-CISH had lower colony-forming ability (Fig 2). The pCDH-CISH group formed no colonies compared to 10 colonies in the pLKO.1-shRNA group. This indicates that CISH up-regulation inhibits T cell proliferation.

The expression of the TCRVβ subfamily members

TCR is a molecule found on the surface of T cells. It is responsible for recognizing antigens bound to major histocompatibility complex (MHC) molecules. The interaction of TCR with an antigen and MHC activates T cells. Gene expression and cloning of TCRVβ subfamily members is a sensitive and reliable method to detect lineage changes and antigen-specific responses of T cells. The analysis of TCRVβ gene repertoire may provide more information about the effect of CISH gene on T cell. We used RT-PCR to detect the gene expression of TRVβ3-1, TRVβ7, TRVβ9, TRVβ11, and TRVβ12. There were significant differences in the expression of TRVβ3-1 and TRVβ12 between the groups. The expression of TRVβ3-1 and TRVβ12 were up-regulated in the pCDH-CISH group ($p < 0.01$, $p < 0.001$, respectively), but down-regulated in the pLKO.1-CISH group ($p < 0.01$, $p < 0.05$, respectively). The expression of TRVβ7, TRVβ9, and TRVβ11 however, did not differ among the groups (Fig. 3).

Effect of CISH on T cell differentiation

To determine the effect of CISH on T cell differentiation, flow cytometry was used to analyze the changes of T cell subsets in each group. Seven different subgroups were detected: CD3, CD4, CD8, CD45RA,

CD45RO, CD25, and CD86. CD3 is the surface marker of T cells, while CD4, CD8, CD45RA, and CD45RO are surface markers of Th cells, CTL cells, initial cells, and memory cells, respectively, and CD25 and CD86 are related to cell growth and activation. Flow cytometry results showed that the CD8 population was significantly up-regulated in the pCDH-shRNA group compared to the other groups, while CD25 and CD86 were significantly down-regulated. CD3, CD4, CD45RA, and CD45RO markers showed no significant change in the CISH overexpression group (Fig. 4).

Differentially expressed genes (DEGs) and functional enrichment analyses

A total of 33531 DEGs were identified in the CD3 T cells across the blank and up-regulated group. There were significant differences in the expression of 2124 genes between the blank and the up-regulated group (p-adjust < 0.05 and |log2 (FoldChange)| > 1). Of these, 940 genes that were up-regulated and 1184 genes that were downregulated in the up-regulated group compared to the blank group, which were described as a volcano plot and heatmaps (Fig. 5a and b)

The GO and KEGG enrichment analyses was used to understand the functions of the DEGs. A GO analysis of the DEGs in the up-regulated group and the blank group identified clusters enriched for 6648 GO terms, among them, for 154 GO terms the p-adjust values were less than 0.005. The top 10 DEGs involved in the GO terms are shown in Fig. 5c. GO terms were classified as biological process (BP), cellular component (CC), and molecular function (MF). GO analysis results showed that the DEGs were mainly involved in chromosome segregation, nuclear division, organelle fission in the BP group; chromosomal region, centrosome, and spindle in the CC group; and ATPase activity, and carbohydrate binding in the MF group.

Based on the KEGG pathway enrichment analysis, 296 KEGG pathways were enriched. A total of 14 KEGG pathways were significantly enriched (p-adjust < 0.05). The top DEGs involved in the KEGG pathways are shown in Fig. 5d, and these include the cell cycle, DNA replication, p53 signaling pathway, and PI3K-Akt signaling pathway and others.

Validation of analyses using RT-PCR

Ten significant DEGs, identified from the RNA-seq data, were randomly selected for RT-PCR validation. These included VEGFA, PDGFR-β, DUSP1, CDKN1A, CCND2, BCL2, PIM1, MDM2, BCRA1, and CKD2. RT-PCR confirmed that the DEGs had the same pattern of expression as observed in the RNA-seq (Fig 6). Therefore, the gene expression observed in the CD3 T cell was highly credible.

Discussion

In our study, we aimed to explore the effect of CISH in T cells. CISH gene and CISH shRNA were cloned into the pCDH and pLKO.1 plasmid, respectively. We used 293T cells to package the lentivirus and then used the lentiviral supernatant to infect CD3 T cells and generate CD3 T cells with up-regulated or down-regulated CISH gene expression. We analyzed the expression of the CISH gene by quantitating CISH

mRNA and CISH protein levels. The results revealed that the expression of CISH in the pCDH-CISH was significantly higher than in pCDH, while that in pLKO.1-shCISH was lower than in pLKO.1-NC. We compared the differences in T cell proliferation, differentiation, TCRVβ subfamily expression, and gene expression between each group. We used high-throughput sequencing technologies to generated CD3 T cell RNA information, and investigated the DEGs in pCDH-CISH and blank group. The GO and KEGG pathway analysis were used to investigate the molecular mechanism of CISH effect on T cell.

Previous studies indicated that CISH has a negative effect on the proliferation of erythroid progenitor cells and dendritic cells [10, 11], but some studies have suggested that CISH was important for T-cell proliferation and survival via activation of the T cell [8]. In our study, the number of colonies in the pCDH-CISH group were lower than in the pLKO.1-CISH group. We have demonstrated that CISH cells inhibit T cell proliferation. This indicates that CISH is likely involved in the inhibition of cell proliferation and/or the activation of cell apoptosis.

TCRs are a class of T cell surface molecules that recognize antigen-derived peptides. Many studies have determined that the expression of the TCRV subfamilies can be different in various diseases [12, 13]. TRVβ gene rearrangement is essential for T cell antigen recognition. CISH is induced by TCR stimulation in $CD8^+$ T cells and is involved in the negative regulation of TCR signaling [7]. However, to our knowledge, there is no direct experimental evidence that establishes a causal relationship between CISH and TRVβ thus far. In our study, we found that CISH increased the expression of TRVβ3.1 and TRVβ12. Multiple biases have been reported in the expression of TRVβ family members in subjects who have recovered from acute hepatitis B virus infection [14]. Our data indicates that HBV may alter the expression of the TCR subfamily through CISH while simultaneously, CISH can influence the T cell differentiation. The levels of CISH were higher in Th2 cells compared to Th1 cells [15]. CISH induces development of Tregs from CCR41 cells [16]. We believe that CISH plays an important role in T cell differentiation, and this was confirmed through our study. Reducing CISH expression helps CD8 T cell formation. Previous studies have shown that CD8 T cells and their induced cytokines contribute to HBV clearance [4]. This may explain why the polymorphism of the CISH gene is related to the outcome of HBV infection.

CISH is a regulatory molecule in cytokine signal transduction [17], and plays an important role in the growth and differentiation of T cells. CISH interact directly with CD127 and directs the receptor to the proteasome for degradation [18]. SOCS1, SOCS3, and CISH control the balance of IL-7-mediated JAK-STAT signaling and TCR-mediated ERK/AKT signaling to control the T cell homeostasis [19]. CISH was also shown to contribute to the expansion of Tregs in response to a microbial pathogen [16]. CISH is involved in a variety of biological processes in T cells. In our study, we screened out the DEGs regulated by CISH. The GO analysis results showed that DEGs were mainly involved in chromosome segregation, nuclear division, organelle fission in BP; chromosomal region, centrosome, and spindle in CC; and ATPase activity, and carbohydrate binding in MF. The KEGG pathway analysis showed that DEGs were mainly response to the Cell cycle, DNA replication, p53 signaling pathway, PI3K-Akt signaling pathway.

In this study, we explored the effect of CISH on T cells in T cell proliferation, differentiation, TCRVβ subfamily expression and expressed genes. CISH is involved in a variety of biological processes and plays an important role in the development and differentiation of T cells. Further study and analysis of these biological processes in T cells will help our understanding of the role of CISH in the pathogenesis of HBV infection.

Declarations

Ethics approval and consent to participate: This study was approved by the Research Subjects Review Board at The First Affiliated Hospital of Chengdu Medical College. All participants were informed with a written description of the aims of the study and provided an oral consent to participate.

Consent for publication: Not applicable

Availability of data and materials: The reagents are available publicly, as referenced in the “Methods” section. The datasets generated and/or analysed during the current study are not publicly available due to format issue but are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests

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Authors' contributions: ZYH and YZ conceived and designed the study. ZYH, YZ, TEL, LZ, MZ, JY, SW, XMY and YFL performed the experiments. TEL and LZ wrote the paper. ZYH, YZ and JY reviewed and edited the manuscript. All authors read and approved the manuscript.

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Abbreviations

CISH: cytokine-inducible SH2 containing protein

HBV: hepatitis B virus

TCRVβ: T-cell receptor Vβ

GO: Gene ontology

KEGG: Kyoto Encyclopedia of Genes and Genomes

BP: biological process

CC: cellular components

MF: molecular function

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Tables

Table1. The sequences of the CISH shRNA and scrambled shRNA.

Primer name	Sequence 5’———3’
CISH shRNA1F	CCGGCCTTCGGGAATCTGGCTGGTACTCGAGTACCAGCCAGATTCCCGAAGGTTTTTG
CISH shRNA1R	CCGGCCTTCGGGAATCTGGCTGGTACTCGAGTACCAGCCAGATTCCCGAAGGTTTTTG
CISH shRNA2F	CCGGCCTTCGGGAATCTGGCTGGTACTCGAGTACCAGCCAGATTCCCGAAGGTTTTTG
CISH shRNA2R	AATTCAAAAAGCTGTGCATAGCCAAGACCTTCTCGAGAAGGTCTTGGCTATGCACAGC
CISH shRNA3F	CCGGGCACGTTCTTAGTACGTGACACTCGAGTACCAGCCAGATTCCCGAAGGTTTTTG
CISH shRNA3R	AATTCAAAAAGCACGTTCTTAGTACGTGACACTCGAGTGTCACGTACTAAGAACGTGC
ScrambleF	AATTCAAAAAGCACGTTCTTAGTACGTGACACTCGAGTGTCACGTACTAAGAACGTGC
ScrambleR	AATTCAAAAACCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG

Table 2. Primer sequences for real-time RT-PCR.

Gene	Forward primer (5’ to 3’)	Forward primer (5’ to 3’)
GAPDH	TCAAGAAGGTGGTGAAGCAGG	TCAAAGGTGGAGGAGTGGGT
CISH	GATGACACAGCCATGGACAC	TTGGCTCACTCTCTGTCTGG
TRVB3-1	GTGGGTGAATGGGAAGGA	TTGACAGCGGAAGTGGTT
TRBV7	CACAGAGGTCTGAGGGATC	CACAGAGGTCTGAGGGATC
TRBV9	GAGTATGGGTAGGAATGG	ACAATGTCTGCTGGTAAT
TRBV11	GGAATTACCCAGACACCA	TCCAGGGATTTCTTAGCT
TRBV12	GAGATGGGACAAGAAGTGA	GAGATGGGACAAGAAGTGA
VEGFA	GGCCAGCACATAGGAGAGAT	GGCCAGCACATAGGAGAGAT
PDGFRβ	AGACTGTTGGGCGAAGGTTA	CGGCAGTATAGAGGACGGAG
DUSP1	TCCTGCCCTTTCTGTACCTG	AACACCCTTCCTCCAGCATT
CDKN1A	GGATGTCCGTCAGAACCCAT	GTGGGAAGGTAGAGCTTGGG
CCND2	ACTTGTGATGCCCTGACTGA	ATATCCCGCACGTCTGTAGG
BCL2	TTCTTTGAGTTCGGTGGGGT	TTCTTTGAGTTCGGTGGGGT
PIM1	GACCCGAGTGTATAGCCCTC	AAGAAAACCTGGCCCCTGAT
MDM2	ACGACAAAGAAAACGCCACA	CTCTCCCCTGCCTGATACAC
BRCA1	TGAAGAAAGAGGAACGGGCT	TGGCTCCCATGCTGTTCTAA
CDK2	TTCTGCCATTCTCATCGGGT	GTCCCCAGAGTCCGAAAGAT

Figures

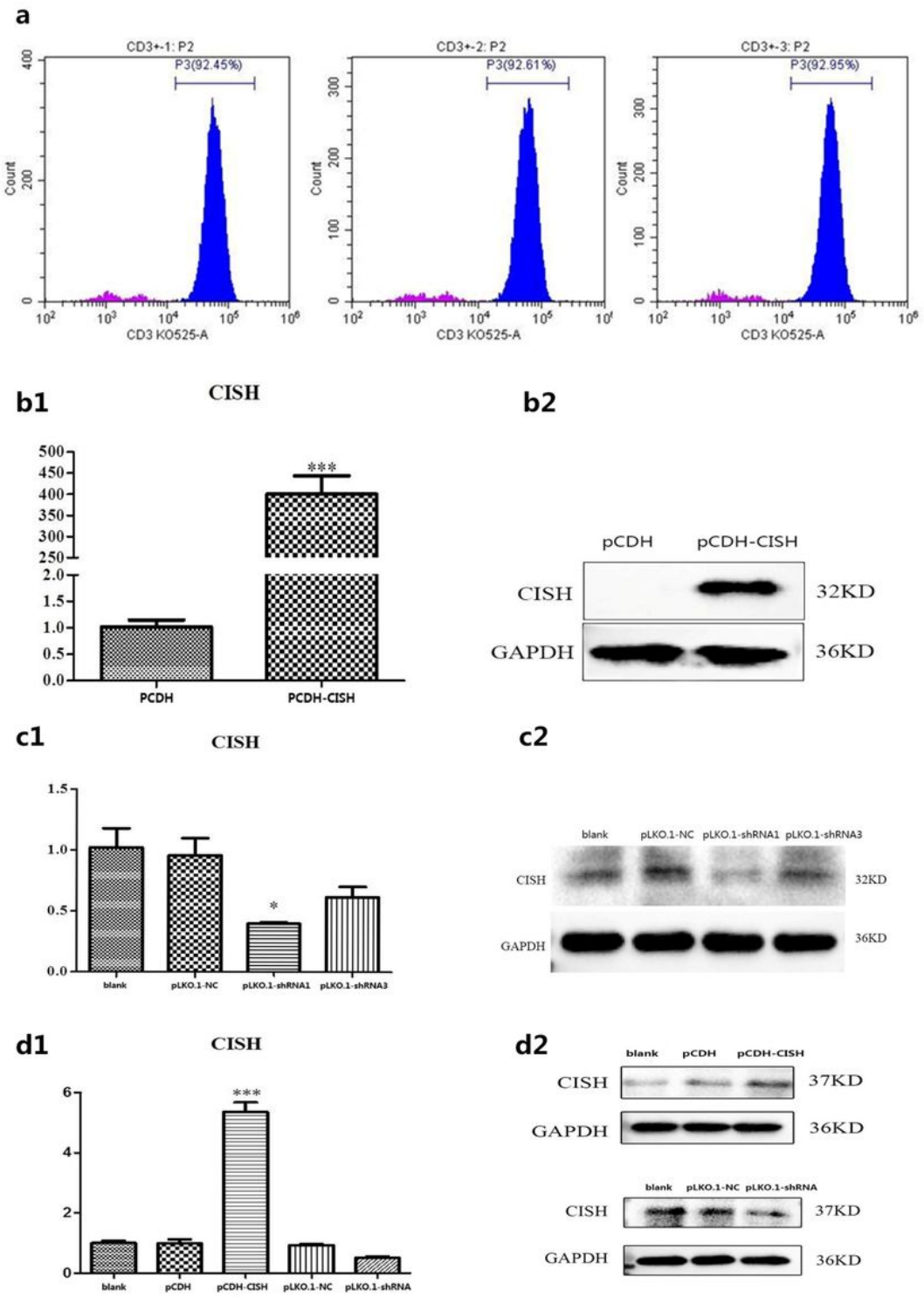

Figure 1

Up-regulated and down-regulated expression of CISH gene in T cells. a Detection of CD3 positive cells by FACS. b1 RT-PCR analysis of CISH mRNA expression in pCDH and pCDH-CISH 293T cells. b2 Western blot analysis of CISH protein expression in pCDH and pCDH–CISH 293T cells. c1 mRNA expression in 293T,

pLKO.1-NC, pLKO.1-shRNA1, and pLKO.1-shRNA2 293T cells. c2 CISH protein expression in 293T (blank), pLKO.1-NC, pLKO.1-shRNA1, and pLKO.1-shRNA2 293T cells. d1, d2 Detection of the expression of CISH in CD3 T cell using RT-PCR and western blotting.

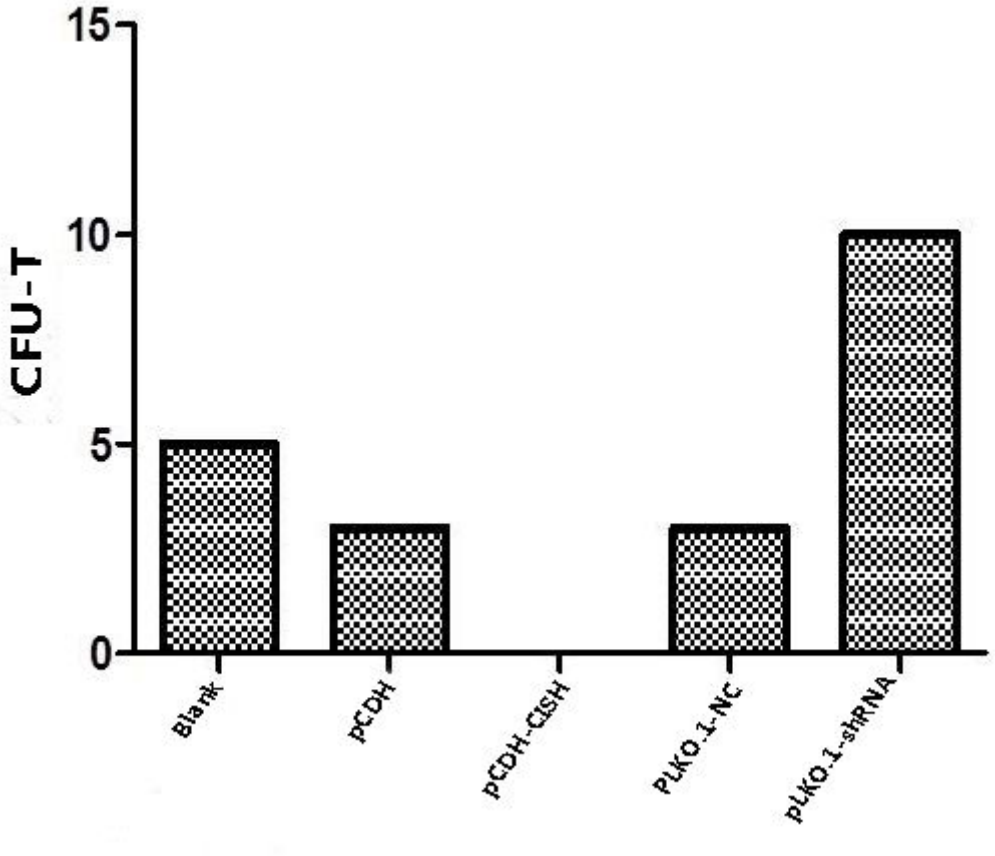

Figure 2

Evaluation of T cell proliferation ability using T cell colonies (CFU-T).

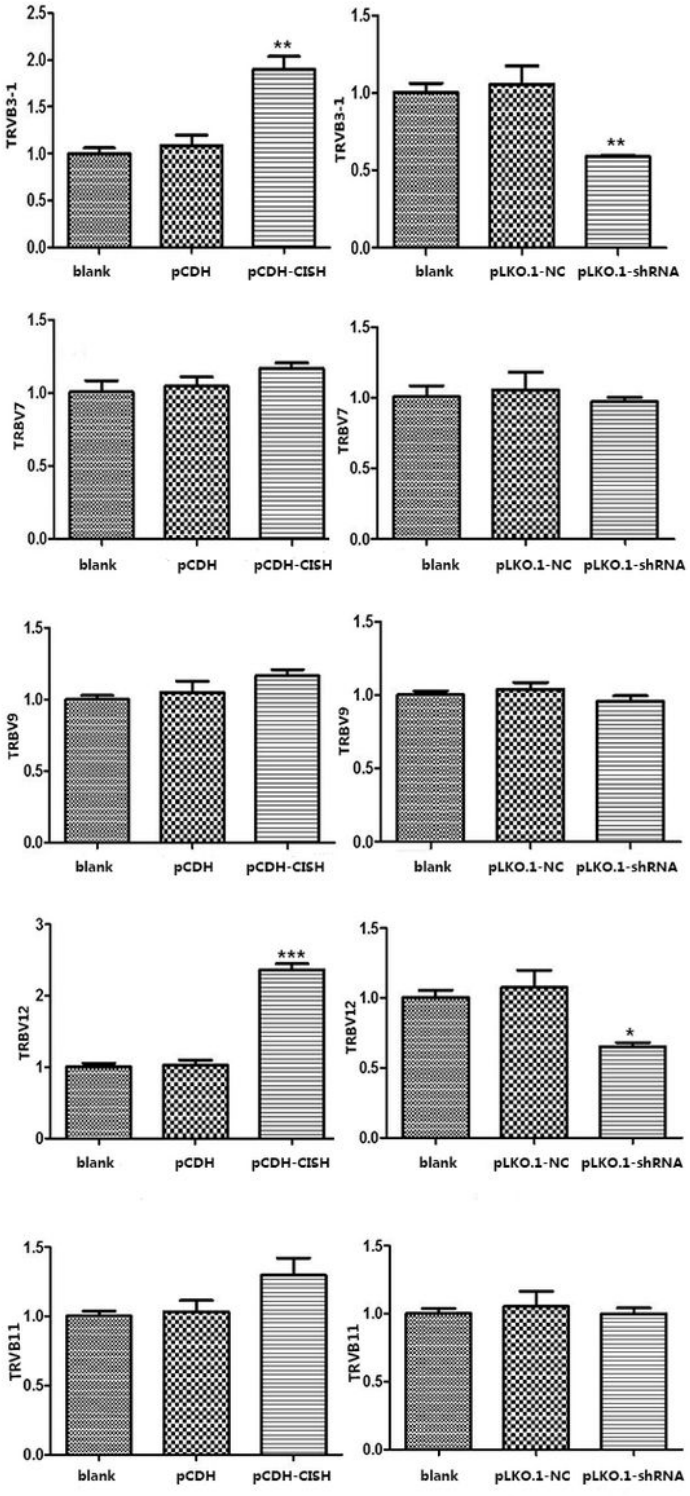

Figure 3

RT-PCR analysis of gene expression of TCRV subfamilies. TRVβ3-1 and TRVβ12 were up-regulated in the pCDH-CISH, but down-regulated in the pLKO.1-shRNA. The expression of TRVβ7, TRVβ9, and TRVβ11 did not differ among the groups.

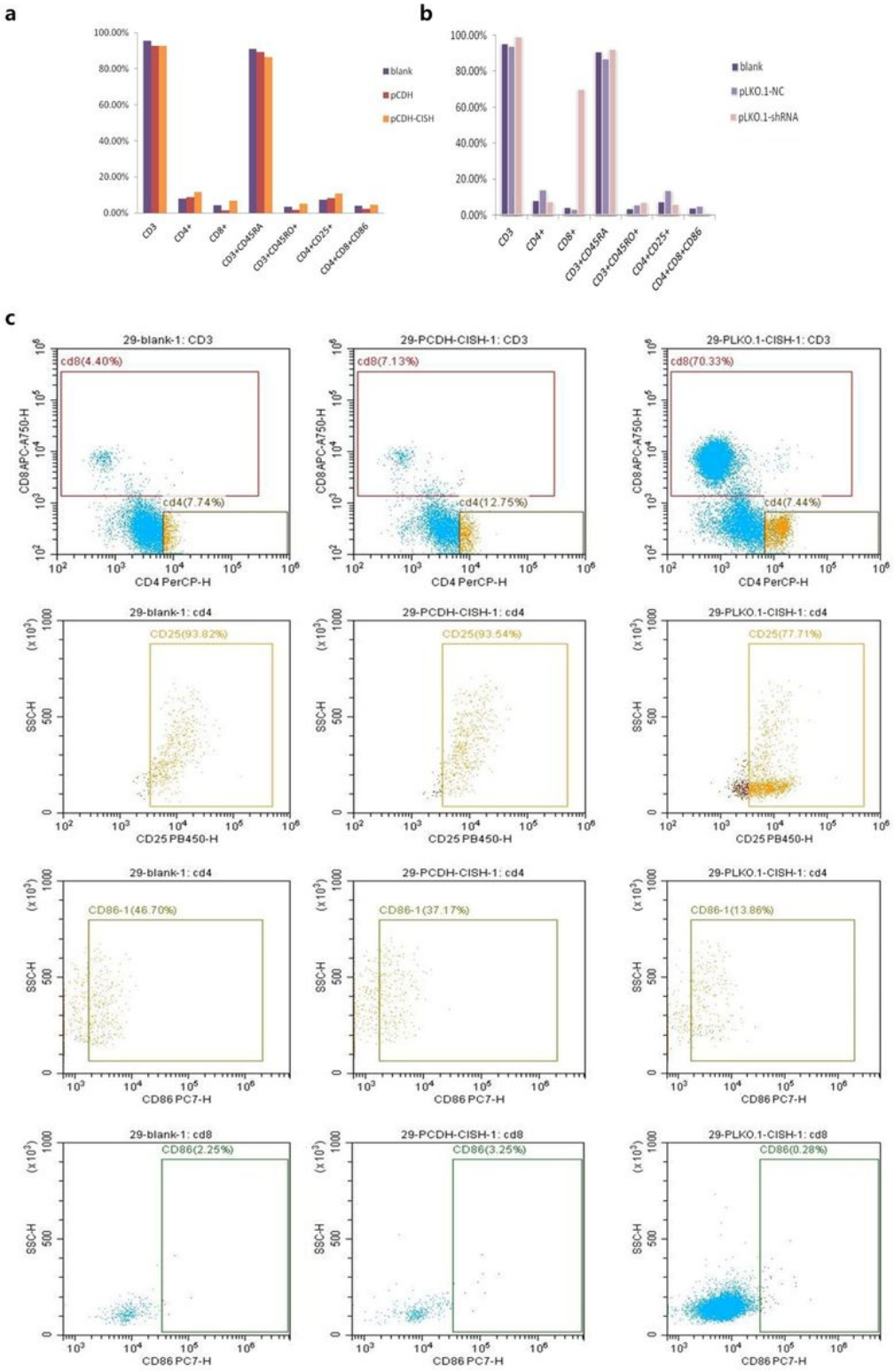

Figure 4

Detection of the changes in T cell subsets using flow cytometry. CD8 was significantly up-regulated in pLKO.1-shRNA, while CD25 and CD86 were significantly down-regulated.

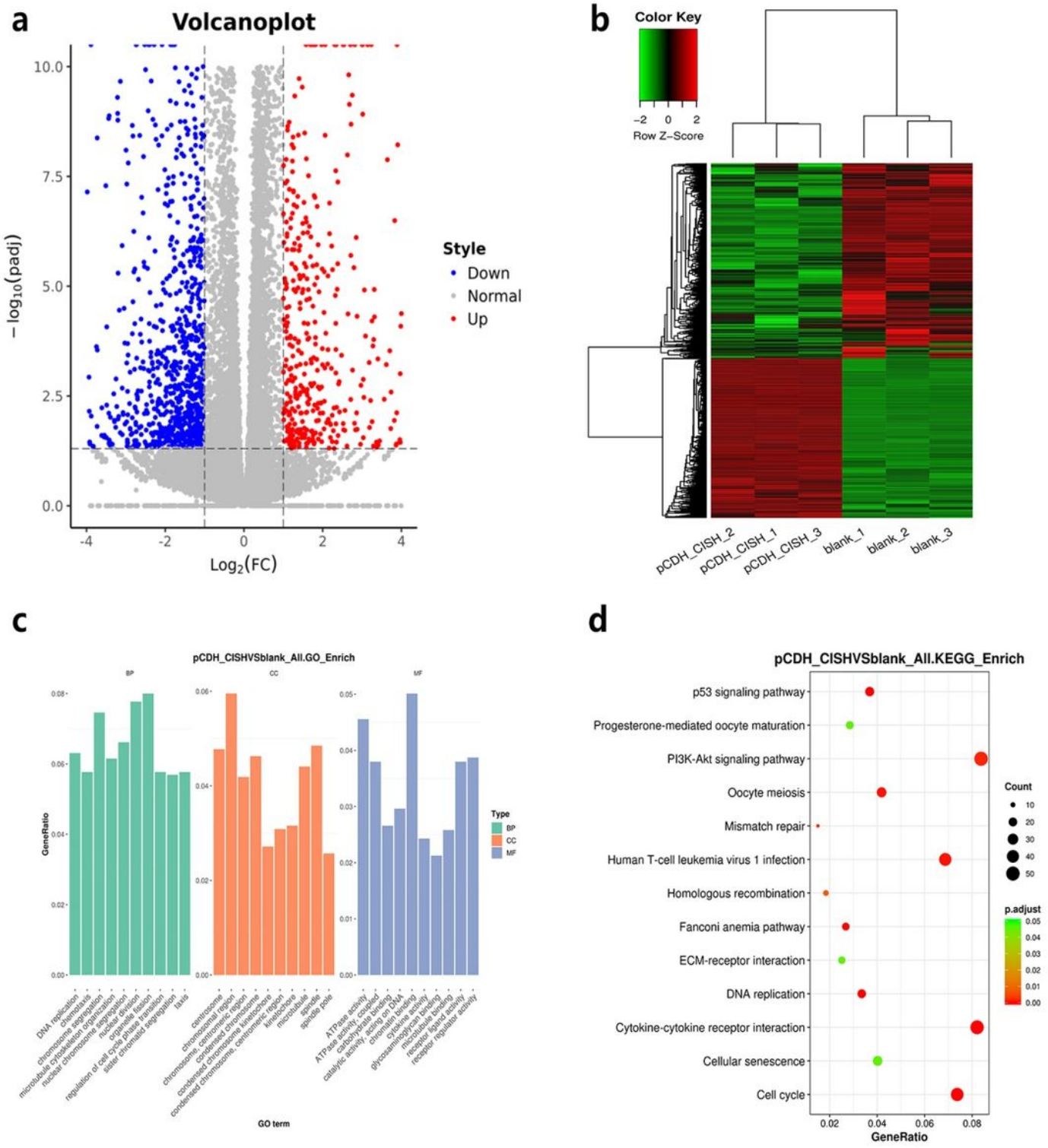

Figure 5

Global gene expression profile analysis after the expression patterns of CISH were up-regulated or down-regulated respectively. a Volcano plot displaying the differentially expressed genes in the PCDH-CISH. The y-axis corresponds to the mean expression value of -log10 (p-adjust), and the x-axis displays the log2 fold-change value. The red and green dots circled in the frame represent the significant DEGs ($p < 0.05$); the blue and grey dots represent the transcripts whose expression levels did not reach statistical

significance. b Hierarchical clustering analysis of the expression level of the DEGs in the PCDH –CISH. Red and blue indicate higher and lower expression values, respectively. c GO enrichment analysis of the DEGs in the PCDH-CISH. The GO terms belonging to biological processes (BP), cellular components (CC), and molecular functions (MF) are shown in green, red, and blue, respectively. d Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the DEGs in the PCDH-CISH.

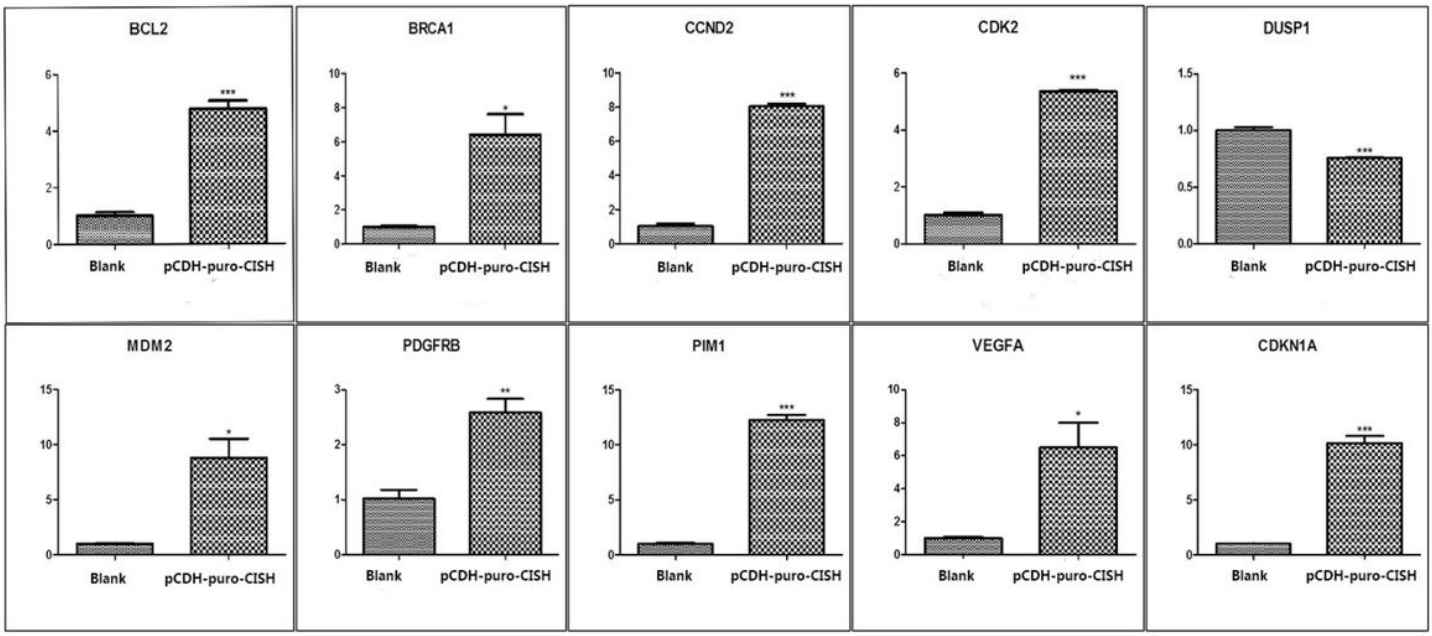

Figure 6

Quantitative reverse-transcription polymerase chain reaction (RT-qPCR) analysis of differentially expressed genes in the blank group and pCDH-CISH.