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Original Article

The roles and potential mechanisms of HCST in the prognosis and immunity of KIRC via comprehensive analysis

Wei Wang[1*], Shuai Li[2*], Junhao Lin[1], Xiaobin Guo[1], Yanyan Xie[3], Wei Li[1], Yanrong Hao[3], Xudong Jiang[3]

[1]Department of Urology, The People's Hospital of Guangxi Zhuang Autonomous Region, Nanning 530021, Guangxi Zhuang Autonomous Region, China; [2]Department of Orthopaedics, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, Hubei, China; [3]Cancer Center, The People's Hospital of Guangxi Zhuang Autonomous Region, Nanning 530021, Guangxi Zhuang Autonomous Region, China. [*]Equal contributors.

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Abstract: Objectives: Hematopoietic cell signal transducer (HCST) participates in the activation of phosphatidylinositol 3 kinase-dependent signaling pathway and in the natural killer (NK) and T cell responses, which affect cell survival and proliferation. Here, the values of HCST in kidney renal clear cell carcinoma (KIRC) are analyzed. Methods: We used GEO, TCGA, GEPIA, UALCAN and TIMER databases to profile the expression of HCST in KIRC tissues, and define its clinical roles. The biological functions and signaling mechanisms modulated by HCST and its co-expressed genes were identified and analyzed via the GO and KEGG databases. On the other hand, the potential value of HCST expression in KIRC immunity was explored using the TIMER and GEPIA databases. Results: Our analysis demonstrated that HCST is significantly overexpressed in KIRC tissues. The upregulation of HCST is associated with clinical stage, tumor grade, tissue subtype and poor prognosis of KIRC patients. Increased HCST expression might be involved in signaling pathways such as antigen processing and presentation, cell adhesion molecules, cytokine-cytokine receptor, chemokine signaling pathway, T cell receptor signaling pathway, FC gammar mediated phagocytosis and B cell receptor signaling pathway. In addition, the expression of HCST was significantly correlated with the levels of KIRC purity, B cells, $CD8^+$ T Cell, $CD4^+$ T cells, macrophages, neutrophils and dendritic cells (DC). Furthermore, the HCST expression is associated with levels of immune infiltration B cells, $CD8^+$ T Cell, $CD4^+$ T cells, macrophages, neutrophils and DC. Conclusions: Our data demonstrated that HCST could be a potential prognostic biomarker, and is related to the immune infiltration in KIRC.

Keywords: HCST, KIRC, poor prognosis, immune infiltration

Introduction

Kidney renal clear cell carcinoma (KIRC) is a common tumor of urinary system, which accounts for about 80% of the renal cell carcinoma (RCC) [1]. Presently, radical renal surgery is the mainstay treatment option for patients with KIRC. However, high blood metastasis rate presents a major hurdle leading to poor prognosis despite improvement associated with the use of radiotherapy, chemotherapy or INF-α therapy. In recent years, targeted therapy has attracted major research interests, as well as for clinical applications, and thus, improves the prognosis of cancer patients [2-4]. Therefore, discovery of new and effective prognostic biomarkers and immunotherapy targets could improve the therapeutic efficacy and long-term prognosis of KIRC patients.

Hematopoietic cell signal transducer (HCST), also referred to as DNAX-activating protein 10 (DAP10), has been shown to participate in cancer progression and immune regulation [5-8]. For instance, Sakaguchi *et al*. demonstrated that RAGE-DAP10 heterodimer could activate Akt and endogenous overexpression of DAP10, which leads to cell growth and survival via the RAGE-DAP10 interaction. In contrast, interference with the expression of DAP10 could activate RAGE through S100A8/A9 to block Akt phosphorylation leading to increased cell apop-

tosis [5]. Hernández-Caselles *et al.* reported that CD33 could be an inhibitory receptor in regulating the NKG2D/DAP10 cytotoxic signaling pathway, which is involved in self-tolerance, tumor and infected cell recognition [6]. Li et al. demonstrated that PD1-DAP10/NKG2D, a new type of dual targeting chimeric receptor (DTCR), could define the damage ability in solid tumor cells through activation of NKG2D receptor. The study showed that DTCR retroviral transduction increases the expression of PD1 and NKG2D on the surface of NK92 cells, which could enhance the cytotoxicity of human gastric cells, SGC-7901. Besides, DTCR stimulation was shown to increase the expression of TNF-α and TRAIL, and then trigger apoptosis of SGC-7901 cells [8]. Qi et al. reported that CSF1R and HCST have higher predictive diagnostic value compared to PDL1 in NSCLC. CSF1R and HCST were positively correlated with PDL1 expression and $CD8^+$ T cell infiltration in the immune microenvironment and might improve the prognosis of patients with lung squamous cell carcinoma [9]. To date, the role and value of HCST in KIRC remains scanty, but the HCST was considered an immune-related factor. Here, we aimed to evaluate the prognostic value and potential mechanism of HCST in the progression of KIRC, and to investigate the potential relationship with KIRC immune cell infiltration.

Materials and methods

TCGA database

The KIRC transcriptome and clinical data were download from the Cancer Genome Atlas (TCGA) database. The transcriptome data included 72 normal kidney and 539 KIRC tissues. The 72 normal kidney tissues were matched with 72 KIRC tissues and each pair belonged to the same KIRC patient. On the other hand, the downloaded clinical data included clinicopathological characteristics and prognostic information of 537 KIRC patients.

GEPIA database

Using the Gene expression profiling interactive analysis (GEPIA) database, we analyzed and profiled the expression of HCST in both kidney and KIRC tissues with the data from the TCGA and GTEx databases. We then analyzed the relationship between the HCST expression and clinical stage, overall survival (OS) or disease-free survival (DFS) of the KIRC patients based on the KIRC data from the TCGA database. Moreover, the relationship between the expression of HCST in the KIRC tissues and KIRC immune cell markers was assessed using the correlation analysis module.

UALCAN database

The UALCAN database was used to explore the expression of HCST in the KIRC and normal tissues. Besides, we analyzed the relationship between HCST gene expression and age, race, gender, clinical stage, tumor grade, lymph node metastasis, subtype, and OS of the KIRC patients.

GEO database

The Gene Expression Omnibus (GEO) database contains sequencing data of tissues, cells and blood samples from cancer patients. The data include gene expression, DNA methylation or mutation status. The GSE781 and GSE11151 datasets in the GEO database were used to verify the expression of HCST in the KIRC tissues.

TIMER database

The expression of HCST in pan-cancer tissues were analyzed using the differential expression module in the Tumor Immune Estimation Resource (TIMER) database, while the correlation between the expression of HCST and the level of KIRC immune infiltrating cells was evaluated in the gene module. In addition, we analyzed the correlation between the HCST expression and KIRC somatic mutations in the somatic copy number alterations (SCNA) module while the relationship between the HCST expression and KIRC immune cell markers was assessed using the correlation analysis module.

The potential value of HCST in the prognosis of KIRC patients

The clinical data of 537 KIRC patients in the TCGA database were merged with the HCST gene expression and profiled against the prognostic and clinicopathological characteristics of the KIRC patients. Kaplan-Meier survival analysis was used to analyze the effect of high-

or low-expression of the HCST on the prognosis of KIRC patients. Besides, univariate Cox regression analysis was used to explore the effects of age, gender, clinical stage, tumor grade, T stage, lymph node metastasis, distant metastasis and HCST expression level on the prognosis of the KIRC patients. Thereafter, multivariate Cox regression analysis was employed to explore the effects of age, clinical stage, tumor grade, T stage, distant metastasis and HCST expression level on the prognosis of the KIRC patients.

The biological functions and signaling pathways of HCST co-expression genes

We employed the Pearson correlation analysis and R limma package to analyze the genes that co-express with HCST in the 539 KIRC tissues from the TCGA database. The genes with the P<0.001 and r>0.5 or r<-0.5 were considered as highly co-expressed genes in the HCST. Gene ontology (GO) annotation included biological process (BP), cell composition (CC) and molecular function (MF) [10, 11]. We entered the HCST co-expressed genes into the DAVID database to explore the biological functions and signaling pathways that might be mediated by the HCST co-expressed genes using the GO and Kyoto Encyclopedia of Genes and Genome (KEGG).

Gene set enrichment analysis

Gene set enrichment analysis (GSEA) is commonly used to explore the signaling pathways that are modulated by a single gene [12]. In the 539 KIRC tissues downloaded, we grouped high- and low-expression groups according to the median HCST gene expression, and then the signaling mechanism involved was analyzed in the HCST overexpression group via the GSEA (version 4.0.1). This run was executed for 1000 cycles. The screening index was set at a NOM P<0.05.

Construction of the PPI network and screening of the hub genes

Using the String database, we analyzed the relationship between proteins of the HCST co-expressed genes. A combined score >0.9 was regarded as significant statistical significance [10]. Cytoscape 3.6.1 software was used for visual analysis while CytoHubba plug-in MCC method was used to screen the top 10 genes which could be defined as the hub genes.

Statistical analysis

A t-test was used to explore the difference of HCST expression between the kidney and the KIRC tissues in the data obtained from the TCGA and GEO (GSE781 and GSE11151). Kaplan-Meier survival analysis as well as the univariate and multiple Cox regression analyses were used to analyze the relationship between the HCST expression and poor prognosis of the KIRC patients. A $P<0.05$ was considered to be statistically significant.

Results

HCST is highly expressed in the KIRC tissues

Our analysis using the TIMER database demonstrated that HCST is differentially expressed in pan-cancer tissues (Figure S1). HCST is significantly overexpressed in ESCA, HNSC, KIRC and KIRP tissues and downregulated in BLCA, COAD, KICH, LUAD, LUSC and READ tissues.

Data from the TCGA, GEO, GEPIA and ULACAN databases showed that HCST is significantly overexpressed in the KIRC tissues (**Figures 1** and S2). In the TCGA database, there was significant upregulation of the HCST expression in both the KIRC unpaired and matched tissues (**Figure 1A**). Similarly, the findings from the GSE781 and GSE11151 data sets in the GEO database showed that HCST was highly expressed in the KIRC tissues (**Figure 1B**). In the GEPIA database, HCST was highly expressed in the KIRC tissues derived from the TCGA and GTEx databases (**Figure 1C**). In addition, HCST was highly expressed in the KIRC tissues in the ULACAN database (Figure S2).

The expression of HCST is associated with the clinical stage, tumor grade and tissue subtype of the KIRC patients

The expression of HCST was correlated with the clinical stage, tumor grade and tissue subtype of the KIRC patients (**Figure 2**). In the GEPIA database, the expression of HCST was associated with the clinical stage of the KIRC patients (**Figure 2A**). Besides, subgroup analysis showed that the clinical stage (stage 1 vs stage 2, P=4.910500E-02; stage 1 vs stage 3,

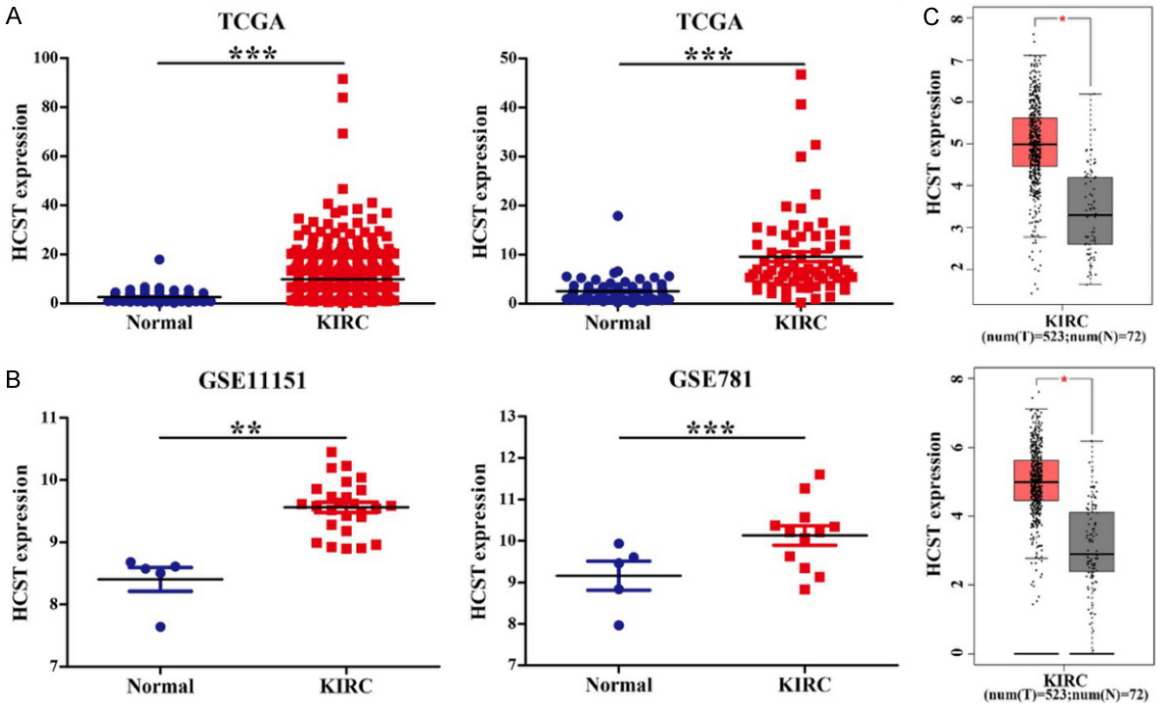


Figure 1. HCST is overexpressed in KIRC tissues in multiple databases. A. TCGA unmatched and matched tissues; B. GEO GSE11151 and GSE781 tissues; C. TCGA and GTEx tissues. Note: *P<0.05; **P<0.01; ***P<0.001; TCGA, the Cancer Genome Atlas; GEO, Gene Expression Omnibus.

P=1.967110E-04; stage 1 vs stage 4, P=1.65110000005519E-06), tumor grade (grade 1 vs grade 3, P=1.302150E-02; grade 1 vs grade 4, P=2.24739999999946E-05; grade 2 vs grade 3, P=4.350100E-04; grade 2 vs grade 4, P=3.06779999958984E-07; grade 3 vs grade 4, P=9.975900E-04) and histological subtype (ccA subtype vs ccB subtype, P=1.233850E-02) of the KIRC patients were related to the expression level of HCST (**Figure 2B**, **2C**).

HCST expression is associated with the prognosis of KIRC patients

The data from the Kaplan-Meier survival analysis showed that the KIRC patients with high HCST expression had shorter OS (**Figure 2E**). The analysis of data from the GEPIA and UALCAN databases showed that elevated HCST expression was associated with shorter OS and DFS of the KIRC patients (**Figures 2F**, **2G** and S3A). In addition, the expression of HCST was correlated with OS-related gender, race and tumor grade of the KIRC patients. However, the difference between the HCST expression level and OS-related races was not significant (Figure S3B-D). The univariate Cox regression analysis showed that age, clinical stage, tumor grade, T stage, distant metastasis as well as HCST expression level influenced the poor prognosis in the KIRC patients (**Table 1**). In contrast, the multivariate Cox regression analysis showed that age, clinical stage and tumor grade were independent factors influencing the poor prognosis of the KIRC patients (Figure S4).

The biological functions and signaling mechanisms of HCST and its co-expressed genes

Out of the 573 co-expressed genes of HCST, 480 were positively related genes while 93 were negatively related genes (**Figure 3** and Table S1). GO annotation showed that the HCST co-expressed genes were significantly enriched in signaling transduction, inflammatory response, apoptotic processes, regulation of immune response, T cell receptor signaling pathway, intracellular signal transduction, MHC class II protein complex, positive regulation of cell proliferation or cell-cell signaling (**Figure 4A-C** and Table S2). On the other hand, the KEGG pathway analysis showed that the HCST co-expressed genes were significantly enriched

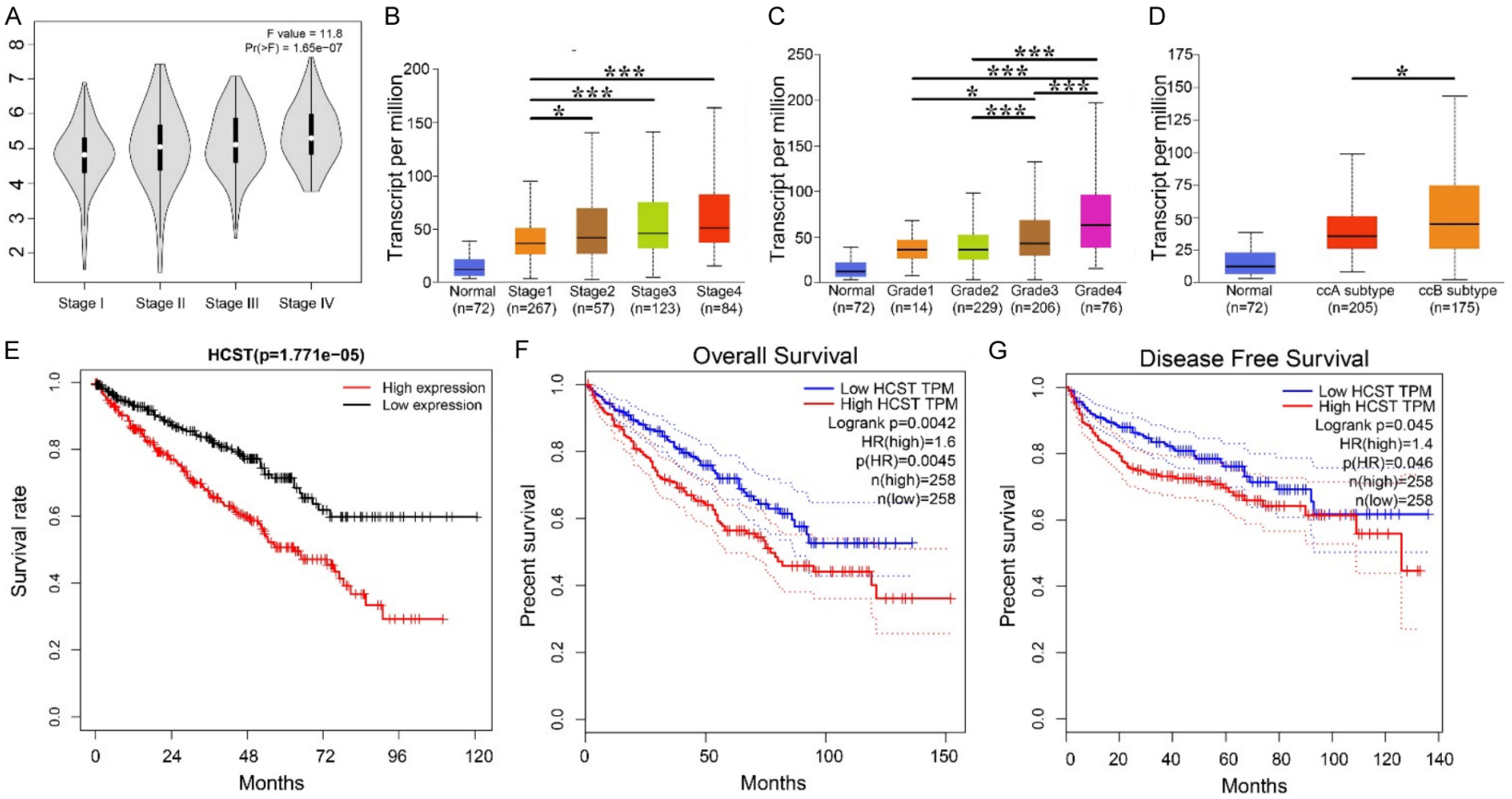


Figure 2. HCST expression is associated with clinical stage, tumor grade and histological subtype of KIRC patients. A. Clinical stage of GEPIA database; B-D. Clinical stage, tumor grade and histological subtype of UALCAN database; E, F. Overall survival in TCGA and GEPIA databases; G. Disease free survival in GEPIA database. Note: *P<0.05; **P<0.01; ***P<0.001; GEPIA, Gene expression profiling interactive analysis; GEPIA, Gene expression profiling interactive analysis; TCGA, the Cancer Genome Atlas.

Table 1. Univariate Cox regression analysis showing the risk factors affecting the prognosis of KIRC patients

Type	HR	HR.95L	HR.95H	P
Age	1.816736632	1.311104643	2.517367327	0.00033371
Gender	0.931080776	0.675353696	1.283640581	0.662936583
Grade	2.293061292	1.854092472	2.835958922	1.94E-14
Clinical stage	1.888786162	1.648774014	2.163736894	4.67E-20
T stage	1.941390125	1.639292156	2.299160404	1.50E-14
M stage	4.2835444	3.10573436	5.908023835	7.45E-19
N Stage	0.864926263	0.739457444	1.011684238	0.06957107
HCST expression	1.021210843	1.010143448	1.032399496	0.000159844

in cell adhesion molecules (CAMs), cytokine-cytokine receptor interaction, chemokine signaling pathway, natural killer cell mediated cytotoxicity, T cell receptor signaling pathway, endocytosis, autoimmune thyroid disease, JAK-STAT signaling pathway, NF-κB signaling pathway, leukocyte trans-endothelial migration, primary immunodeficiency, toll-like receptor signaling pathway, B cell receptor signaling pathway, cytosolic DNA-sensing pathway or Fc epsilon RI signaling pathway (**Figure 4D** and **Table 2**). Moreover, the GSEA analysis showed that antigen processing and presentation, CAMs, cytokine-cytokine receptor, chemokine signaling pathway, T cell receptor signaling pathway, FC gamma r mediated phagocytosis and B cell receptor signaling pathway were significantly enriched in the HCST overexpression group (Figure S5 and **Table 3**).

PPI network and hub genes

The PPI network was visualized with Cytoscope software (Figure S6A). The main component in the PPI network was the HCST-positively related genes. In addition, using plug-ins, HLA-DRA, HLA-DRB1, HLA-DPB1, HLA-DPA1, HLA-DQA1, HLA-DQB1, HLA-A, HLA-B, HLA-F and IRF7 were shown to be hub genes (Figure S6B and **Table 4**). Correlation analysis showed that the expression level of HCST expression was significantly associated with the expression of hub genes in the PPI network.

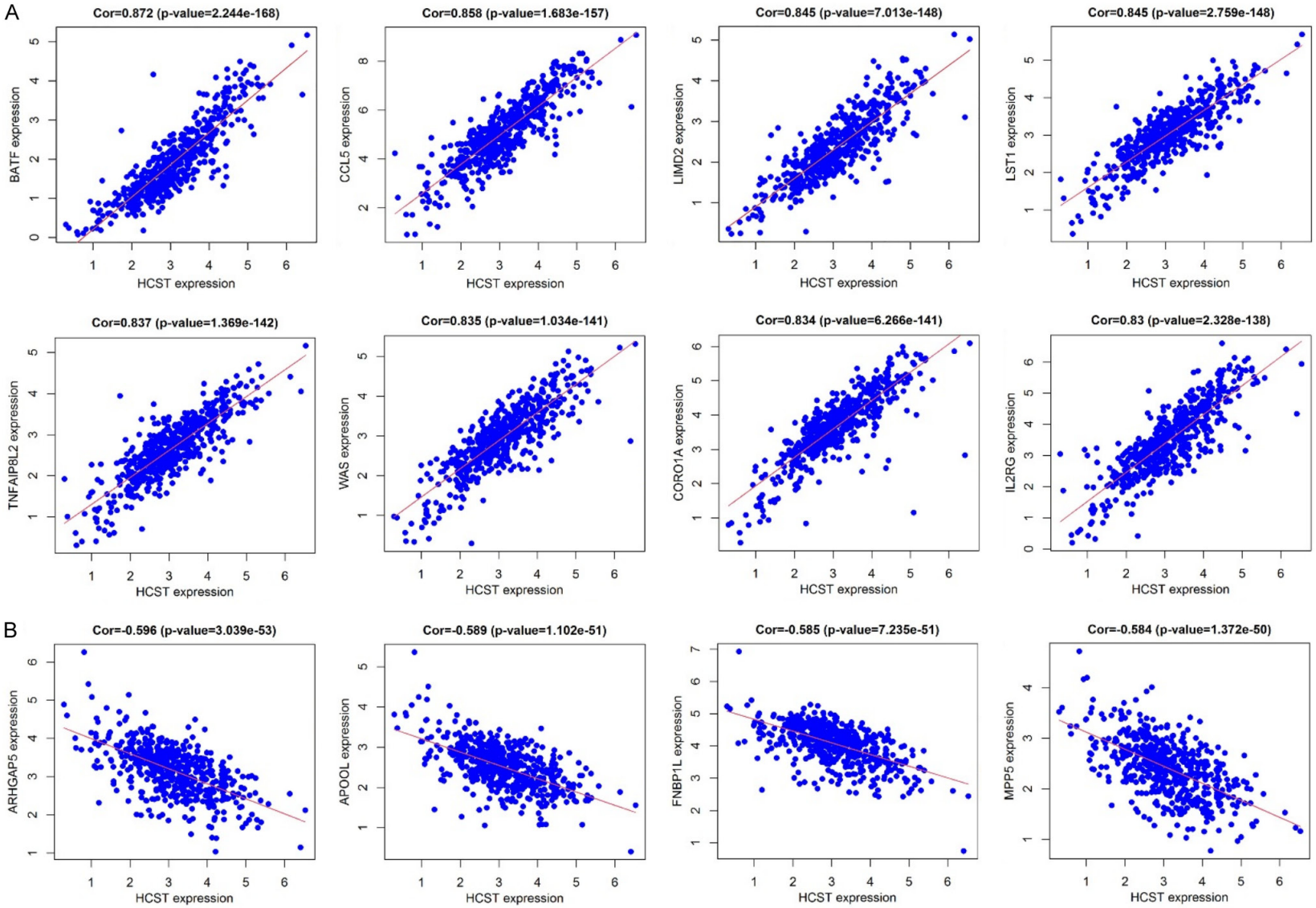
The expression of HCST correlates with the level of immune cell infiltration in the KIRC patients

Further analysis demonstrated that the expression of HCST was correlated with the level of KIRC immune cell infiltration (**Figure 5**). In details, the level of HCST was significantly associated with the KIRC purity (r=-0.386192508; P=7.00E-18), B cells (r=0.312010883; P=8.04E-12), $CD8^+$ T cells (r=0.541116885; P=1.11E-34), $CD4^+$ T cells (r=0.206255023; P=8.21E-06), macrophages (r=0.211924371; P=6.05E-06), neutrophils (r=0.392922766; P=2.33E-18) and DC (r=0.575509004; P=1.74E-41). In addition, HCST copy number was correlated with the arm-level gain of B cells, $CD8^+$ T cells, $CD4^+$ T cells, neutrophils and DCs (Figure S7).

Besides, the expression level of HCST was significantly associated with the expression of markers of KIRC immune infiltration such as B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils and DC (**Figure 6** and **Table 5**). For instance, the expression of HCST was significantly correlated with the expression of $CD8^+$ T cell markers such as CD8A and CD8B, B cell markers such as CD19 and CD79A, DC markers such as HLA-DPB1, HLA-DQB1, HLA-DRA, HLA-DPA1, Th1 cell markers such as TBX21, STAT4, STAT1 as well as IFNG (**Figure 6**). Similar results were obtained from correlation analysis based on the KIRC purity and age (**Table 6**).

The biological functions and signaling mechanisms modulated by HCST-related immune cell infiltration markers

The DAVID database was used to analyze the biological functions and signaling mechanisms that are modulated by the HCST-related immune cell infiltration markers. The data demonstrated that the HCST-related immune cell infiltration markers were involved in T cell co-stimulation, immune responses, T cell receptor signaling pathway, antigen processing and presentation, positive regulation of T cell prolifera-


A
Cor=0.872 (p-value=2.244e-168)
BATF expression
HCST expression
Cor=0.858 (p-value=1.683e-157)
CCL5 expression
HCST expression
Cor=0.845 (p-value=7.013e-148)
LIMD2 expression
HCST expression
Cor=0.845 (p-value=2.759e-148)
LST1 expression
HCST expression
Cor=0.837 (p-value=1.369e-142)
TNFAIP8L2 expression
HCST expression
Cor=0.835 (p-value=1.034e-141)
WAS expression
HCST expression
Cor=0.834 (p-value=6.266e-141)
CORO1A expression
HCST expression
Cor=0.83 (p-value=2.328e-138)
IL2RG expression
HCST expression
B
Cor=-0.596 (p-value=3.039e-53)
ARHGAP5 expression
HCST expression
Cor=-0.589 (p-value=1.102e-51)
APOOL expression
HCST expression
Cor=-0.585 (p-value=7.235e-51)
FNBP1L expression
HCST expression
Cor=-0.584 (p-value=1.372e-50)
MPP5 expression
HCST expression

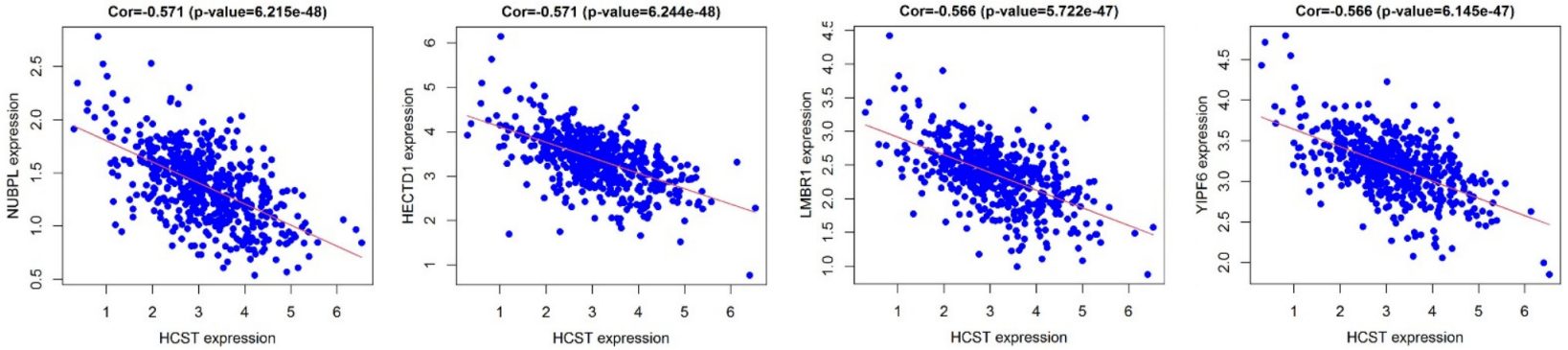


Figure 3. HCST is positively and negatively related to other genes. A. HCST was positively correlated with the expression of BATF, CCL5, LIMD2, LST1, TNFAIP8L2, WAS, CORO1A or IL2RG; B. HCST was negatively correlated with the expression of ARHGAP5, APOOL, FNBP1L, MPP5, NUBPL, HECTD1, LMBR1 or YIPF6.

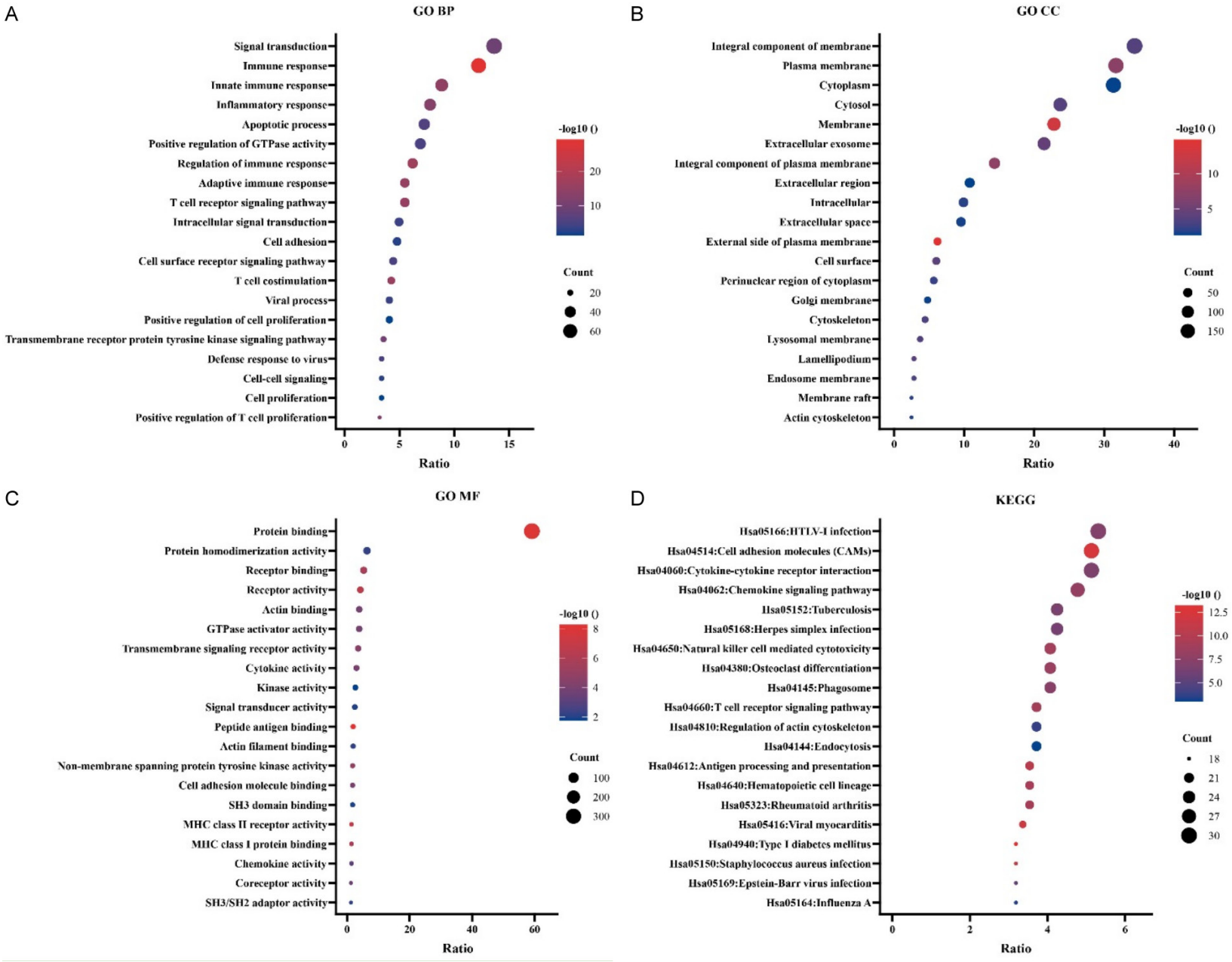

A
GO BP
Signal transduction
Immune response
Innate immune response
Inflammatory response
Apoptotic process
Positive regulation of GTPase activity
Regulation of immune response
Adaptive immune response
T cell receptor signaling pathway
Intracellular signal transduction
Cell adhesion
Cell surface receptor signaling pathway
T cell costimulation
Viral process
Positive regulation of cell proliferation
Transmembrane receptor protein tyrosine kinase signaling pathway
Defense response to virus
Cell-cell signaling
Cell proliferation
Positive regulation of T cell proliferation
0 5 10 15
Ratio
-log10 ()
20
10
Count
20
40
60
B
GO CC
Integral component of membrane
Plasma membrane
Cytoplasm
Cytosol
Membrane
Extracellular exosome
Integral component of plasma membrane
Extracellular region
Intracellular
Extracellular space
External side of plasma membrane
Cell surface
Perinuclear region of cytoplasm
Golgi membrane
Cytoskeleton
Lysosomal membrane
Lamellipodium
Endosome membrane
Membrane raft
Actin cytoskeleton
0 10 20 30 40
Ratio
-log10 ()
10
5
Count
50
100
150
C
GO MF
Protein binding
Protein homodimerization activity
Receptor binding
Receptor activity
Actin binding
GTPase activator activity
Transmembrane signaling receptor activity
Cytokine activity
Kinase activity
Signal transducer activity
Peptide antigen binding
Actin filament binding
Non-membrane spanning protein tyrosine kinase activity
Cell adhesion molecule binding
SH3 domain binding
MHC class II receptor activity
MHC class I protein binding
Chemokine activity
Coreceptor activity
SH3/SH2 adaptor activity
0 20 40 60
Ratio
-log10 ()
8
6
4
2
Count
100
200
300
D
KEGG
Hsa05166:HTLV-I infection
Hsa04514:Cell adhesion molecules (CAMs)
Hsa04060:Cytokine-cytokine receptor interaction
Hsa04062:Chemokine signaling pathway
Hsa05152:Tuberculosis
Hsa05168:Herpes simplex infection
Hsa04650:Natural killer cell mediated cytotoxicity
Hsa04380:Osteoclast differentiation
Hsa04145:Phagosome
Hsa04660:T cell receptor signaling pathway
Hsa04810:Regulation of actin cytoskeleton
Hsa04144:Endocytosis
Hsa04612:Antigen processing and presentation
Hsa04640:Hematopoietic cell lineage
Hsa05323:Rheumatoid arthritis
Hsa05416:Viral myocarditis
Hsa04940:Type I diabetes mellitus
Hsa05150:Staphylococcus aureus infection
Hsa05169:Epstein-Barr virus infection
Hsa05164:Influenza A
0 2 4 6
Ratio
-log10 ()
12.5
10.0
7.5
5.0
Count
18
21
24
27
30

Figure 4. GO and KEGG analysis showing the functions and mechanisms mediated by the HCST co-expressed genes. A. BP; B. CC; C. MF; D. KEGG. Note: GO, Gene ontology; BP, biological process; CC, cell composition; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genome.

Table 2. The KEGG analysis showing the signaling pathways of HCST co-expressed genes

Category	Count	*P* value
hsa05166: HTLV-I infection	30	1.21E-07
hsa04514: Cell adhesion molecules (CAMs)	29	4.71E-13
hsa04060: Cytokine-cytokine receptor interaction	29	1.68E-07
hsa04062: Chemokine signaling pathway	27	8.89E-09
hsa05152: Tuberculosis	24	2.62E-07
hsa05168: Herpes simplex infection	24	4.82E-07
hsa04650: Natural killer cell mediated cytotoxicity	23	1.03E-09
hsa04380: Osteoclast differentiation	23	4.18E-09
hsa04145: Phagosome	23	5.44E-08
hsa04660: T cell receptor signaling pathway	21	9.13E-10
hsa04810: Regulation of actin cytoskeleton	21	1.63E-04
hsa04144: Endocytosis	21	9.86E-04
hsa04612: Antigen processing and presentation	20	4.02E-11
hsa04640: Hematopoietic cell lineage	20	5.01E-10
hsa05323: Rheumatoid arthritis	20	6.17E-10
hsa05416: Viral myocarditis	19	1.69E-12
hsa04940: Type I diabetes mellitus	18	6.22E-14
hsa05150: Staphylococcus aureus infection	18	7.58E-12
hsa05169: Epstein-Barr virus infection	18	3.85E-06
hsa05164: Influenza A	18	3.77E-04
hsa05332: Graft-versus-host disease	17	1.04E-14
hsa05330: Allograft rejection	17	9.93E-14
hsa05320: Autoimmune thyroid disease	17	4.74E-11
hsa05321: Inflammatory bowel disease (IBD)	17	1.47E-09
hsa05140: Leishmaniasis	17	7.54E-09
hsa05322: Systemic lupus erythematosus	16	1.90E-04
hsa05162: Measles	15	5.86E-04
hsa04630: Jak-STAT signaling pathway	15	0.001377994
hsa05132: Salmonella infection	14	1.51E-05
hsa04064: NF-kappa B signaling pathway	14	2.54E-05
hsa05142: Chagas disease (American trypanosomiasis)	14	1.69E-04
hsa05145: Toxoplasmosis	14	2.98E-04
hsa04670: Leukocyte transendothelial migration	14	4.61E-04
hsa04672: Intestinal immune network for IgA production	13	1.36E-07
hsa05340: Primary immunodeficiency	12	3.05E-08
hsa04666: Fc gamma R-mediated phagocytosis	12	3.55E-04
hsa05310: Asthma	10	1.18E-06
hsa05131: Shigellosis	10	7.19E-04
hsa05133: Pertussis	10	0.002265094
hsa04620: Toll-like receptor signaling pathway	10	0.021063179
hsa05130: Pathogenic Escherichia coli infection	9	6.75E-04
hsa04662: B cell receptor signaling pathway	9	0.004867075
hsa05100: Bacterial invasion of epithelial cells	9	0.01013634
hsa03050: Proteasome	8	0.001340673
hsa04623: Cytosolic DNA-sensing pathway	8	0.011292053
hsa04664: Fc epsilon RI signaling pathway	7	0.046848111

Table 3. The GSEA analysis showing the signaling pathway associated with HCST overexpression

Name	Size	NOM p
KEGG_AUTOIMMUNE_THYROID_DISEASE	50	0
KEGG_TYPE_I_DIABETES_MELLITUS	41	0
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	80	0.001964637
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	54	0
KEGG_CELL_ADHESION_MOLECULES_CAMS	131	0
KEGG_VIRAL_MYOCARDITIS	68	0
KEGG_INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION	46	0
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	263	0
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	131	0
KEGG_ALLOGRAFT_REJECTION	35	0
KEGG_PRIMARY_IMMUNODEFICIENCY	35	0.005870841
KEGG_ASTHMA	28	0
KEGG_GRAFT_VERSUS_HOST_DISEASE	37	0.001886793
KEGG_HEMATOPOIETIC_CELL_LINEAGE	85	0
KEGG_LEISHMANIA_INFECTION	69	0.003883495
KEGG_CYTOSOLIC_DNA_SENSING_PATHWAY	54	0
KEGG_PROTEASOME	44	0.005976096
KEGG_CHEMOKINE_SIGNALING_PATHWAY	187	0.013972056
KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY	108	0.025145067
KEGG_FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS	95	0.02970297
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	75	0.036072146

Table 4. HCST expression level is related with the expression of hub genes

Name	Score	r
HLA-DRA	3.56E+14	0.561
HLA-DRB1	3.56E+14	0.641
HLA-DPB1	3.56E+14	0.677
HLA-DPA1	3.56E+14	0.518
HLA-DQA1	3.56E+14	0.563
HLA-DQB1	3.56E+14	0.511
HLA-A	3.56E+14	0.637
HLA-B	3.56E+14	0.586
HLA-F	3.56E+14	0.621
IRF7	3.56E+14	0.585

tion, negative regulation of interleukin-2 production, T cell activation and MHC class II Receptor activity (**Figure 7A-C** and Table S3). Besides, the HCST-related immune cell infiltration markers were involved in the signaling mechanisms of hematopoietic cell lineage, CAMs, antigen processing and presentation, T cell receptor signaling pathway, primary immunodeficiency, JAK-STAT signaling pathway, cytokine-cytokine receptor interaction as well as chemokine signaling pathway (**Figure 7D** and Table S4).

The expression of HCST correlates with the levels of immune cell markers in KIRC tissues

The relationship between the expression of HCST and the levels of KIRC immune cell markers was further assessed in KIRC tissues (**Figure 8** and **Table 7**). The results showed that the expression of HCST was positively correlated with the levels of CD3D (r=0.86), CD3E (r=0.85), LAG3 (r=0.79), CD2 (r=0.79), CD8A (r=0.77), CD8B (r=0.76), HLA-DPB1 (r=0.69), IFNG (r=0.67), PDCD1 (r=0.67), HLA-DRA (r=0.64), CTLA4 (r=0.62), HLA-DPA1 (r=0.6), HLA-DQB1 (r=0.59), STAT1 (r=0.56), FOXP3 (r=0.55), GZMB (r=0.53), STAT5A (r=0.52), TBX21 (r=0.47), VSIG4 (r=0.4), CCR8 (r=0.39), STAT4 (r=0.39), IL21 (r=0.36), ITGAX (r=0.35), CD163 (r=0.35), MS4A4A (r=0.33), CCR7 (r=0.3), IRF5 (r=0.29), TNF (r=0.21), CD79A (r=0.19), ITGAM (r=0.13) and CD1C (r=0.13). In contrast, the expression of HCST was negatively correlated with the levels of STAT5B (r=-0.11) and NRP1 (r=-0.17).

Discussion

In the recent years, the application of immunotherapy in disease treatment has received

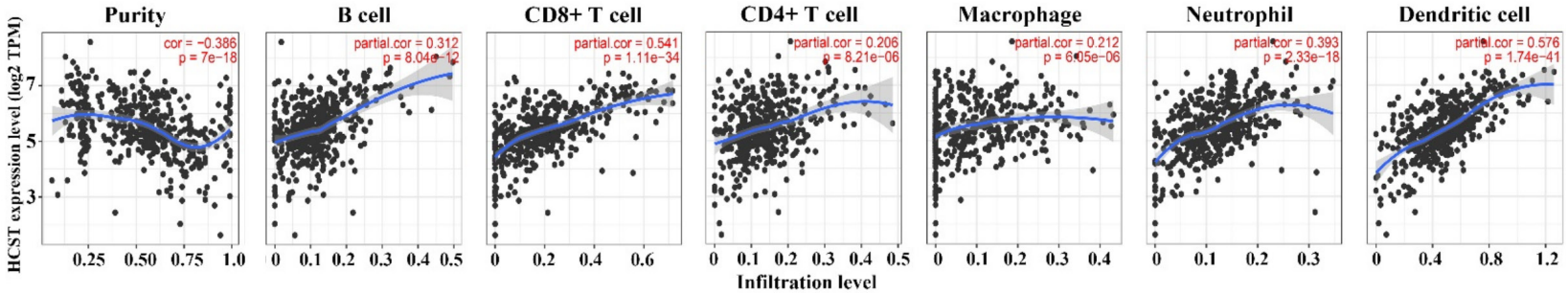
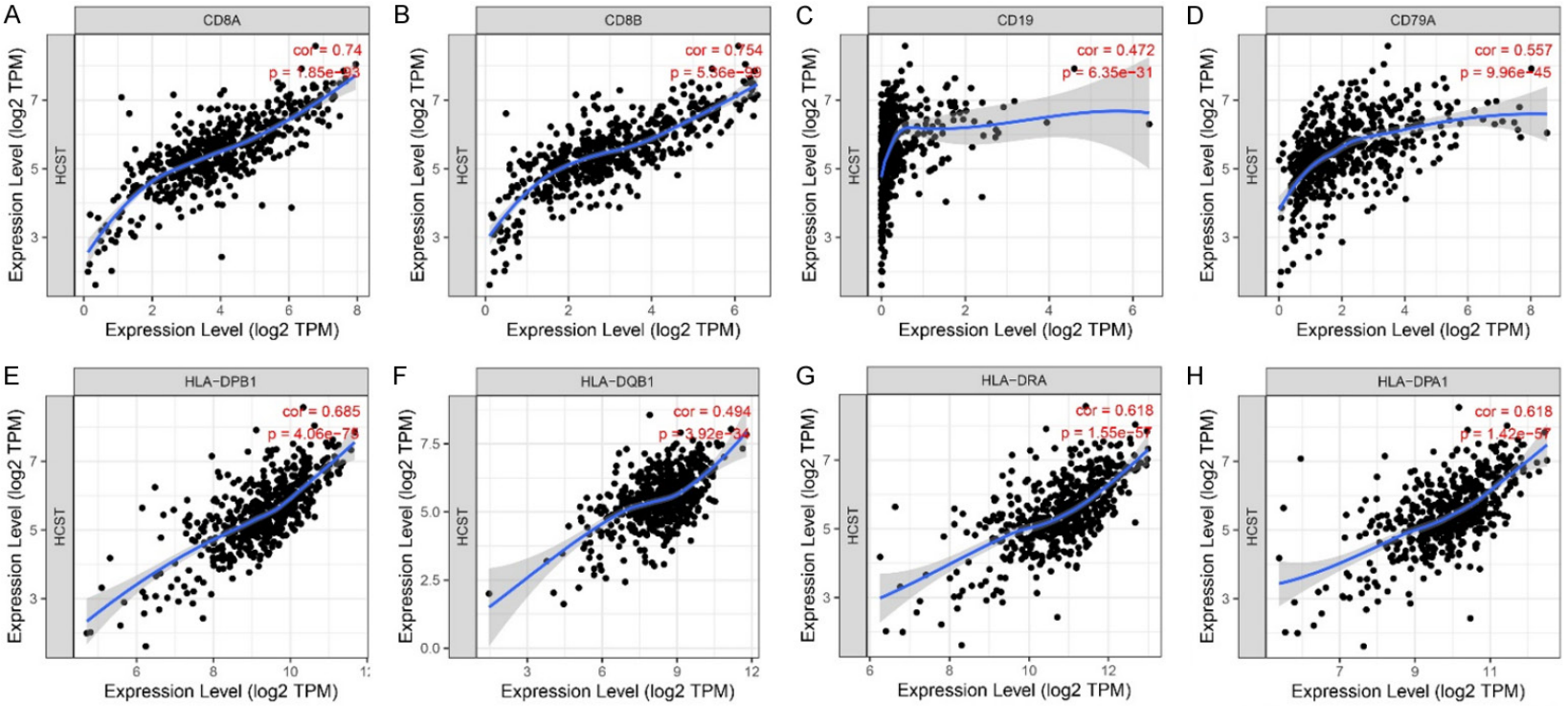


Figure 5. HCST expression is correlated with KIRC immune infiltration.



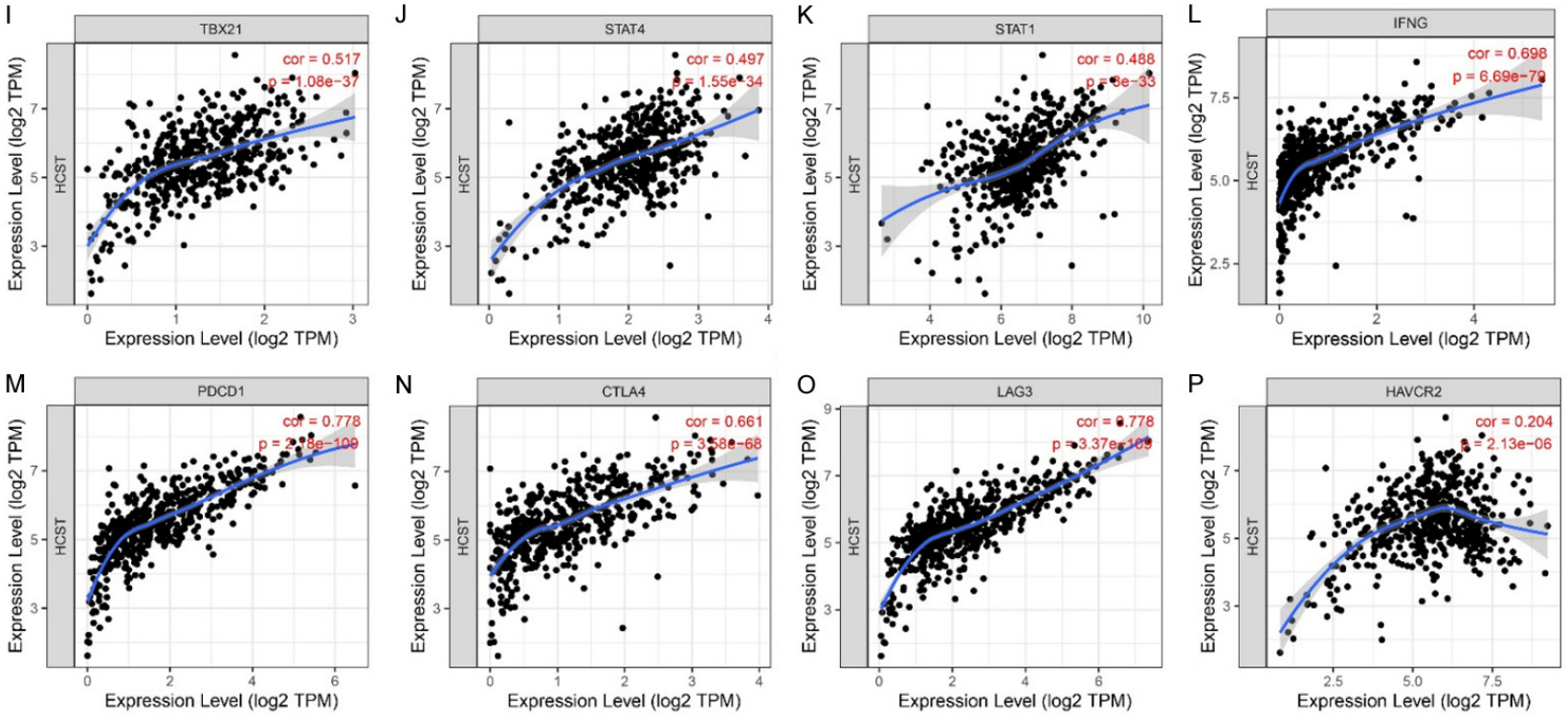


Figure 6. The expression of HCST is significantly associated with the marker levels of KIRC immune infiltration cells. A, B. $CD8^+$ T cell; C, D. B cell; E-H. DCs; I-L. Th1 cell; M-P. T cell exhaustion.

Table 5. The expression of HCST is significantly correlated with the marker levels of KIRC immune infiltration cells

Cell	Gene	Cor	P	Cell	Gene	Cor	P
$CD8^+$ T cell	CD8A	0.739852523	***	Th1	TBX21	0.516558022	***
	CD8B	0.754037027	***		STAT4	0.496597376	***
T cell (general)	CD3D	0.842206601	***		STAT1	0.488052268	***
	CD3E	0.821308286	***		IFNG	0.697553365	***
	CD2	0.793914929	***		TNF	0.339198118	***
B Cell	CD19	0.47198925	***	Th2	GATA3	0.373614771	***
	CD79A	0.556819138	***		STAT6	-0.103192588	*
M1 Macrophage	NOS2	-0.042897538	0.323		STAT5A	0.496527894	***
	IRF5	0.358277571	***		IL13	0.101097629	*
	PTGS2	-0.02422053	0.577	Tfh	BCL6	-0.056352542	0.194
M2 Macrophage	CD163	0.278394333	***		IL21	0.176278191	***
	VSIG4	0.431614549	***	Th17	STAT3	-0.028680739	0.509
	MS4A4A	0.380091981	***		IL17A	0.047318628	0.276
Neutrophils	CEACAM8	-0.035671255	0.411	Treg	FOXP3	0.623845919	***
	ITGAM	0.461521154	***		CCR8	0.489639827	***
	CCR7	0.521288218	***		STAT5B	-0.159327665	***
Dendritic cell	HLA-DPB1	0.685194433	***		TGFB1	0.125360653	**
	HLA-DQB1	0.493948463	***	T cell exhaustion	PDCD1	0.778270323	***
	HLA-DRA	0.618338129	***		CTLA4	0.660778269	***
	HLA-DPA1	0.618497621	***		LAG3	0.777854902	***
	CD1C	0.274982706	***		HAVCR2	0.203663359	***
	NRP1	-0.140691672	**		GZMB	0.603838288	***
	ITGAX	0.449222967	***				

widespread attention [13-16]. For instance, circMET (hsa_circ_0082002) was shown to be overexpressed in hepatocellular carcinoma (HCC) tissues, and the expression level of the circMET was related to the survival and tumor recurrence in HCC patients. The overexpression of circMET mediated tumor microenvironment through miR-30-5p/Snail/DPP4/CXCL10 signaling mechanism and induced epithelial-mesenchymal transition (EMT), thereby fueling the progression of HCC. A combination treatment with sitagliptin, a DPP4 inhibitor, and anti-PD1 antibody could improve the anti-tumor immunity in mice models. Besides, tissues from diabetic HCC patients under sitagliptin treatment had higher CD8 T cell infiltration [16]. Previous studies have reported that HCST could participate in tumorigenesis, development, and immune regulation [5-8]. However, there is no available data defining the effect of abnormally expressed HCST on the progression of KIRC. In this study, we used the TCGA, GEO, GEPIA and ULACAN databases to analyze the expression of in KIRC samples. The results robustly demonstrated that HCST is upregulated in KIRC. The overexpressed HCST was associated with clinical stage, tumor grade and the tissue subtype of the KIRC patients. Results from the Kaplan-Meier survival analysis showed that KIRC patients with high HCST expression had a shorter OS and DFS. In addition, elevated HCST expression was also related to the gender, race and tumor grade associated with OS in the KIRC patients. Moreover, results from the Cox regression analysis showed that HCST expression influenced poor prognosis in the KIRC patients. These results preliminarily indicated that HCST is a carcinogenic factor mediating the progression of KIRC, and it is a promising prognostic biomarker for the KIRC patients.

Other studies have reported that HCST was associated with the T cells and NK cells [17-21]. For example, DAP10-deficient mice showed antigen-specific CD8 T cell recruitment, activation and development following aerosol infection. The loss of cytotoxicity in the DAP10-deficient CD8 T cells was related to impaired

Table 6. The expression of HCST is significantly correlated with the marker levels of KIRC immune infiltration cells under the KIRC purity and age

Gene	Purity		Age	
	Cor	P	Cor	P
CD8A	0.695306707	7.70E-68	0.74088951	1.66E-93
CD8B	0.719145534	1.39E-74	0.754200334	1.07E-98
CD3D	0.81347383	4.20E-110	0.843367161	8.21E-145
CD3E	0.787803039	1.17E-98	0.82333865	3.11E-132
CD2	0.755441673	2.58E-86	0.794982591	6.41E-117
CD19	0.4226411	2.13E-21	0.473636155	4.80E-31
CD79A	0.504643406	3.71E-31	0.556985358	1.35E-44
NOS2	-0.117092532	0.011872284	-0.042138835	0.33246247
IRF5	0.334166624	1.73E-13	0.361550583	7.66E-18
PTGS2	-0.092366264	0.047474488	-0.023239748	0.59311059
CD163	0.212641781	4.10E-06	0.279456109	5.55E-11
VSIG4	0.363852574	7.07E-16	0.430994043	1.98E-25
MS4A4A	0.296812684	7.89E-11	0.382419117	6.17E-20
CEACAM8	-0.028817032	0.537118436	-0.033001033	0.447929218
ITGAM	0.404108931	1.54E-19	0.463038942	1.42E-29
CCR7	0.462420182	8.38E-26	0.530588477	6.83E-40
HLA-DPB1	0.657377131	2.24E-58	0.685992911	4.43E-75
HLA-DQB1	0.436753637	6.78E-23	0.493611562	5.83E-34
HLA-DRA	0.580324771	7.52E-43	0.619978422	1.05E-57
HLA-DPA1	0.565711099	2.36E-40	0.619889849	1.10E-57
CD1C	0.19297806	3.03E-05	0.285753517	1.95E-11
NRP1	-0.228862903	6.81E-07	-0.140706513	0.001150371
ITGAX	0.408454739	5.79E-20	0.453382281	2.80E-28
TBX21	0.477655047	1.19E-27	0.519194296	5.50E-38
STAT4	0.417558545	7.08E-21	0.498336423	1.12E-34
STAT1	0.432689804	1.86E-22	0.489048797	2.81E-33
IFNG	0.649934809	1.12E-56	0.698130456	8.61E-79
TNF	0.303601175	2.76E-11	0.340613437	6.86E-16
GATA3	0.3595671	1.62E-15	0.373169348	5.46E-19
STAT6	-0.100138359	0.031584464	-0.100963682	0.019964393
STAT5A	0.427870744	6.04E-22	0.496708327	1.98E-34
IL13	0.061309244	0.188836819	0.102466343	0.018184887
BCL6	-0.077158106	0.098000681	-0.056239829	0.195689887
IL21	0.138190885	0.002946102	0.179726187	3.10E-05
STAT3	-0.098165456	0.035111426	-0.027125543	0.532819697
IL17A	0.007023008	0.880462562	0.05223128	0.229529343
FOXP3	0.565071703	3.01E-40	0.624445993	9.45E-59
CCR8	0.406413728	9.20E-20	0.489731849	2.22E-33
STAT5B	-0.17825941	0.000119153	-0.158030612	0.000256197
TGFB1	0.073838622	0.113366736	0.126021791	0.003628928
PDCD1	0.751643436	5.43E-85	0.778437319	4.65E-109
CTLA4	0.609306971	3.42E-48	0.661240863	4.78E-68
LAG3	0.756842419	8.29E-87	0.777877907	8.34E-109
HAVCR2	0.13397112	0.003955693	0.208319431	1.28E-06
GZMB	0.560870561	1.49E-39	0.606038161	1.50E-54

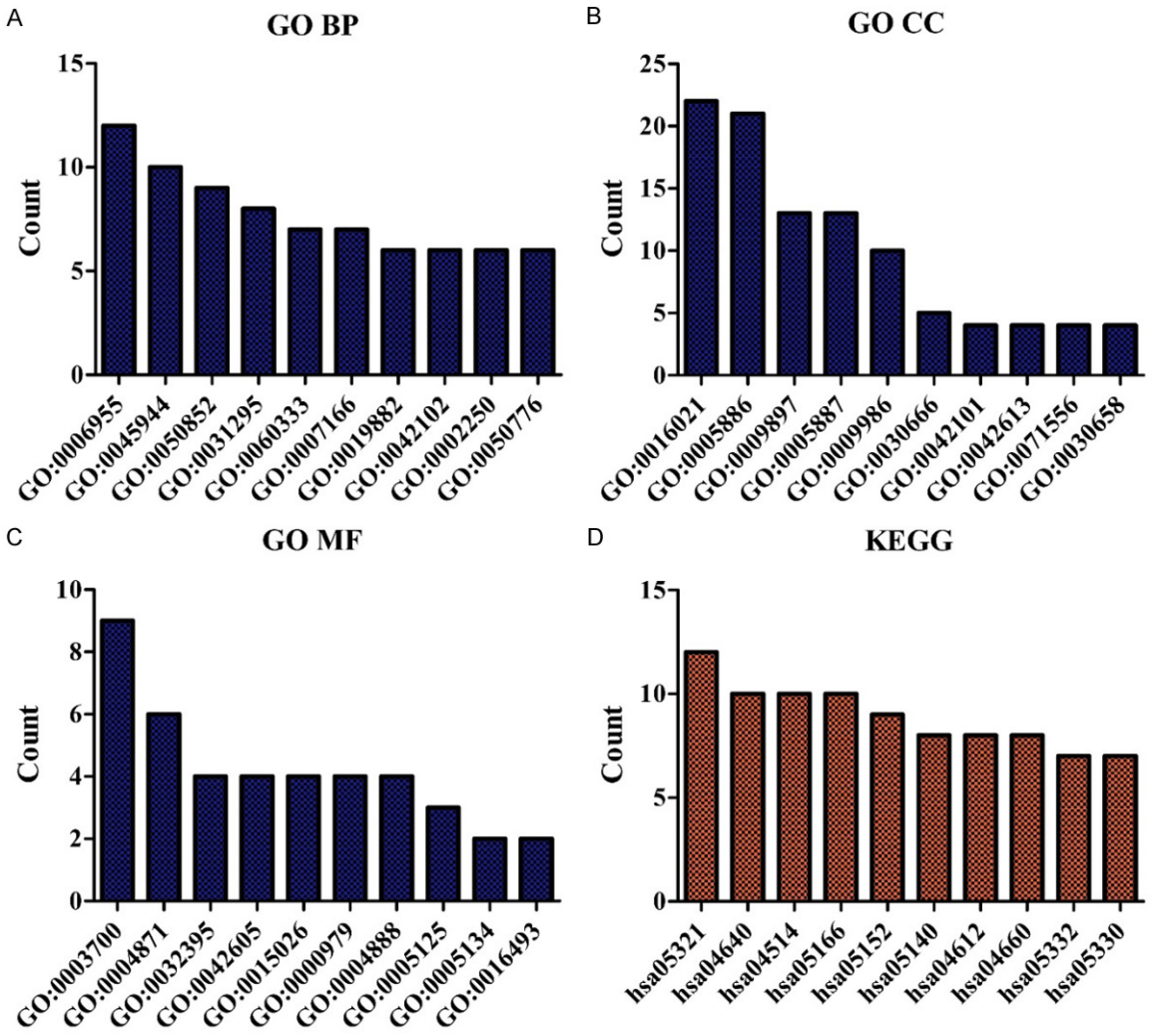
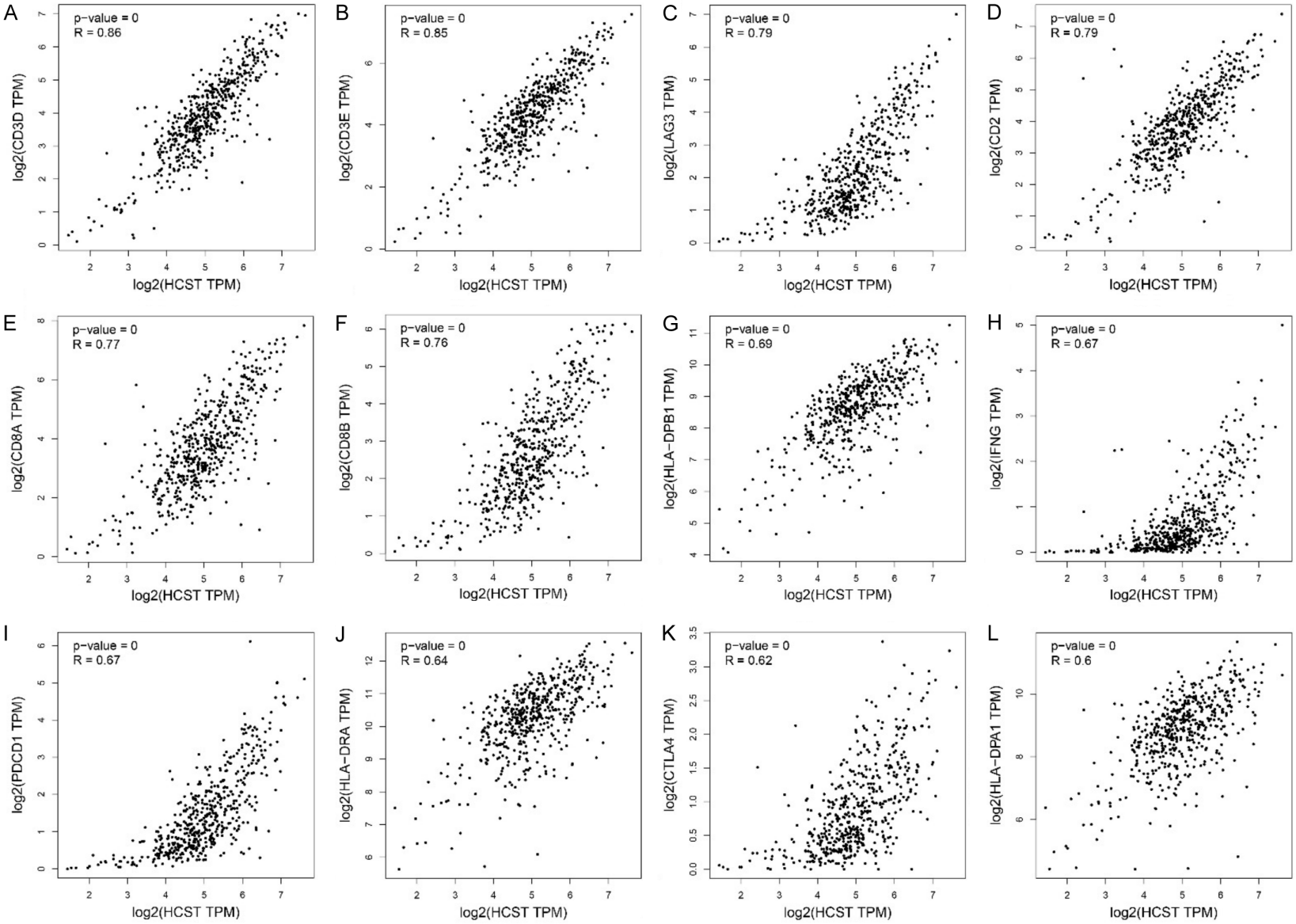


Figure 7. HCST-related immune cell infiltration markers are involved in biological functions and signaling mechanisms. A. BP; B. CC; C. MF; D. KEGG. Note: GO, Gene ontology; BP, biological process; CC, cell composition; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genome.

release of cytotoxic particles [18]. NKG2D was an important activation receptor that triggers the cytotoxic activity of the NK cells, and in conjunction with specific ligands, it could induce damage to NK cell function. Besides, the NKG2D/DAP10 receptor complex has been associated with the activation of the NK cells [20]. In our study, we showed that the HCST expression level was significantly correlated with the markers of KIRC immune infiltration such as B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils and DC. In addition, the correlation analysis showed that the HCST is significantly correlated with the levels of $CD8^+$ T cell markers including CD8A and CD8B, the levels of B cell markers such as CD19 and CD79A, the levels of DC markers such as HLA-DPB1, HLA-DQB1, HLA-DRA, HLA-DPA1, as well as the levels of Th1 cell markers such as TBX21, STAT4, STAT1 or IFNG.

The occurrence and development of tumors not only involve abnormal immune regulation, but also change in multiple signaling pathways [22-24]. For example, up-regulation of lncRNA RP11-468E2.5 has been shown to inhibit the JAK/STAT signaling pathway by targeting STAT5 and STAT6 in inhibiting colorectal cancer (CRC) cell proliferation and promotion of cell apoptosis [22]. Besides, the overexpression of chemokine receptor 7 (CCR7) was closely associated with gastric cancer (GC) metastasis, staging, differentiation and poor prognosis. CCL19 could increase the expression of p-ERK, p-AKT,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B
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R = 0.85
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log2(HCST TPM)
C
p−value = 0
R = 0.79
log2(LAG3 TPM)
log2(HCST TPM)
D
p−value = 0
R = 0.79
log2(CD2 TPM)
log2(HCST TPM)
E
p−value = 0
R = 0.77
log2(CD8A TPM)
log2(HCST TPM)
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p−value = 0
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log2(HCST TPM)
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log2(HCST TPM)
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p−value = 0
R = 0.67
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log2(HCST TPM)
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p−value = 0
R = 0.67
log2(PDCD1 TPM)
log2(HCST TPM)
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p−value = 0
R = 0.64
log2(HLA−DRA TPM)
log2(HCST TPM)
K
p−value = 0
R = 0.62
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log2(HCST TPM)
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R = 0.6
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log2(HCST TPM)

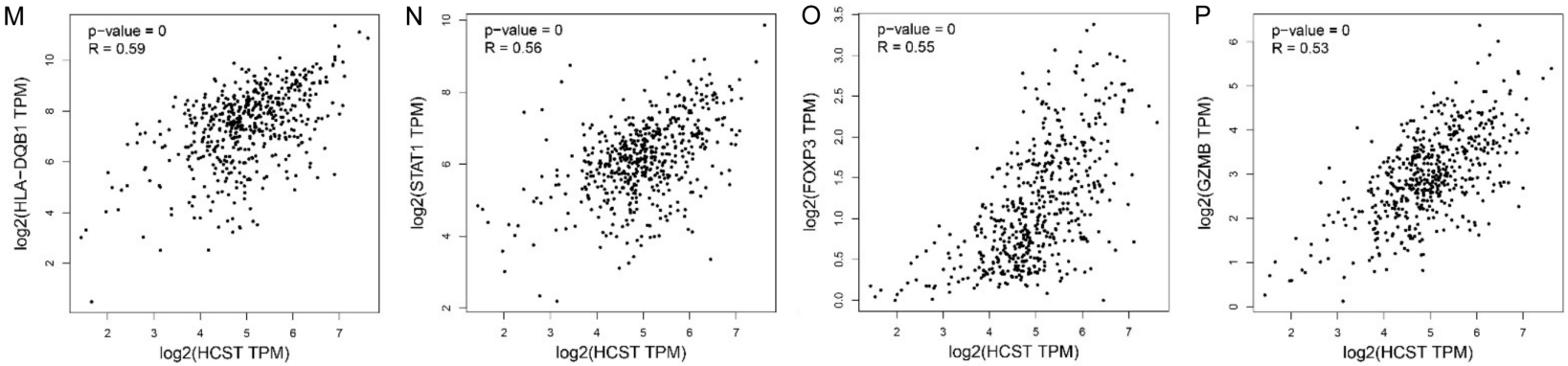

Figure 8. HCST expression is correlated with the levels of KIRC immune cell markers. A. CD3D; B. CD3E; C. LAG3; D. CD2; E. CD8A; F. CD8B; G. HLA-DPB1; H. IFNG; I. PDCD1; J. HLA-DRA; K. CTLA4; L. HLA-DPA1; M. HLA-DQB1; N. STAT1; O. FOXP3; P. GZMB.

Table 7. The relationship between the expression of HCST and the levels of KIRC immune cell markers in the GEPIA KIRC tissues

Gene	Cor	P	Gene	Cor	P	Gene	Cor	P
CD3D	0.86	0	HLA-DPA1	0.6	0	ITGAX	0.35	0
CD3E	0.85	0	HLA-DQB1	0.59	0	CD163	0.35	2.2e-16
LAG3	0.79	0	STAT1	0.56	0	MS4A4A	0.33	3.8e-15
CD2	0.79	0	FOXP3	0.55	0	CCR7	0.3	1e-12
CD8A	0.77	0	GZMB	0.53	0	IRF5	0.29	6.1e-12
CD8B	0.76	0	STAT5A	0.52	0	TNF	0.21	2.2e-06
HLA-DPB1	0.69	0	TBX21	0.47	0	CD79A	0.19	1.4e-05
IFNG	0.67	0	VSIG4	0.4	0	ITGAM	0.13	0.0032
PDCD1	0.67	0	CCR8	0.39	0	CD1C	0.13	0.0022
HLA-DRA	0.64	0	STAT4	0.39	0	NRP1	-0.17	0.00014
CTLA4	0.62	0	IL21	0.36	0	STAT5B	-0.11	0.012

Snail and MMP9 in GC cells, and decrease the expression of E-cadherin. CCR7 was shown to induce ERK and PI3K signaling pathways to regulate Snail signaling [24]. HCST-related immune cell infiltration markers involve T cell co-stimulation, immune response, T cell receptor signaling pathway, antigen processing and presentation, positive regulation of T cell proliferation, negative regulation of interleukin-2 production, T cell activation, MHC class II receptor activity, CAMs, antigen processing and presentation, T cell receptor signaling pathway, primary immunodeficiency, Jak-STAT signaling pathway, cytokine-cytokine receptor interaction as well as chemokine signaling pathway. In addition, the KEGG analysis showed that HCST is involved in signaling pathways such as antigen processing and presentation, CAMs, cytokine-cytokine receptor, chemokine signaling pathway, T cell and B cell receptor signaling pathways. This demonstrated that HCST plays an important role in tumor immune infiltration.

This study used a larger sample size with extensive data, and we showed that HCST was significantly overexpressed in KIRC tissues. Increased HCST was related to the clinical stage, tumor grade, tissue subtype and poor prognosis of KIRC patients, and it influenced poor prognosis in the KIRC patients. Increased HCST mediate signaling mechanisms such as antigen processing and presentation, CAMs, cytokine-cytokine receptor, chemokine signaling pathway, T cell receptor signaling pathway, FC gammar mediated phagocytosis as well as B cell receptor signaling pathway. In addition, the expression of HCST was significantly correlated with the levels of KIRC immune cell infiltration purity, B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils and DC. Furthermore, the expression of HCST was significantly associated with markers of KIRC immune infiltration B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils and DC. Taken together, the HCST upregulation is associated with poor prognosis and the levels of immune infiltration in KIRC. HCST might be a potential prognostic biomarker, and is related to the immune infiltration in KIRC.

Disclosure of conflict of interest

None.

Address correspondence to: Xudong Jiang and Yanrong Hao, Cancer Center, The People's Hospital of Guangxi Zhuang Autonomous Region, No. 6 Taoyuan Road, Nanning 530021, Guangxi Zhuang Autonomous Region, China. Tel: +86-0771-2186509; E-mail: jxd1984@163.com (XDJ); Tel: +86-0771-2186509; E-mail: 282174944@qq.com (YRH)

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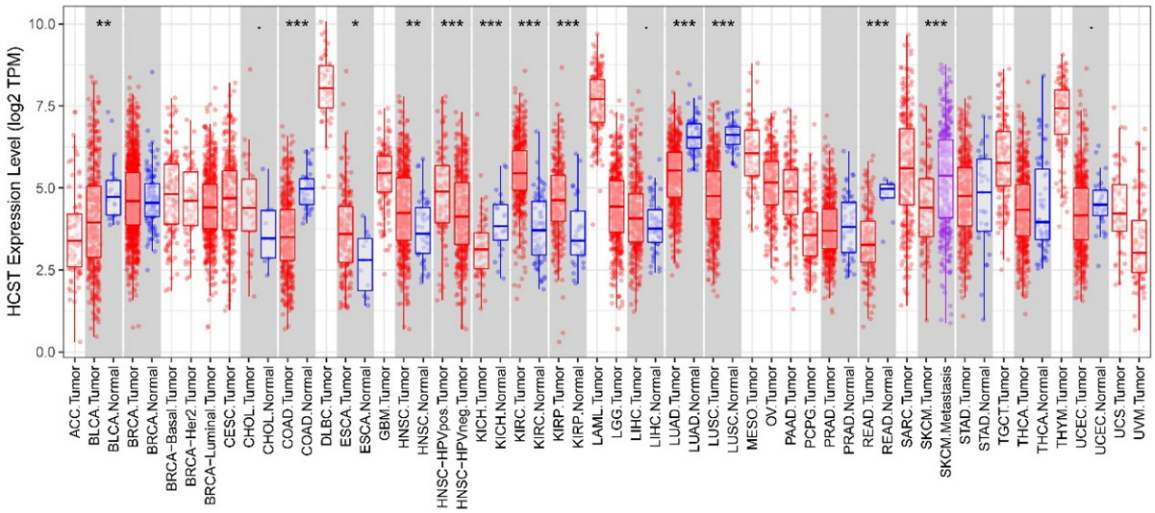


Figure S1. The expression of HCST in pan-cancer tissues. Note: *P<0.05; **P<0.01; ***P<0.001.

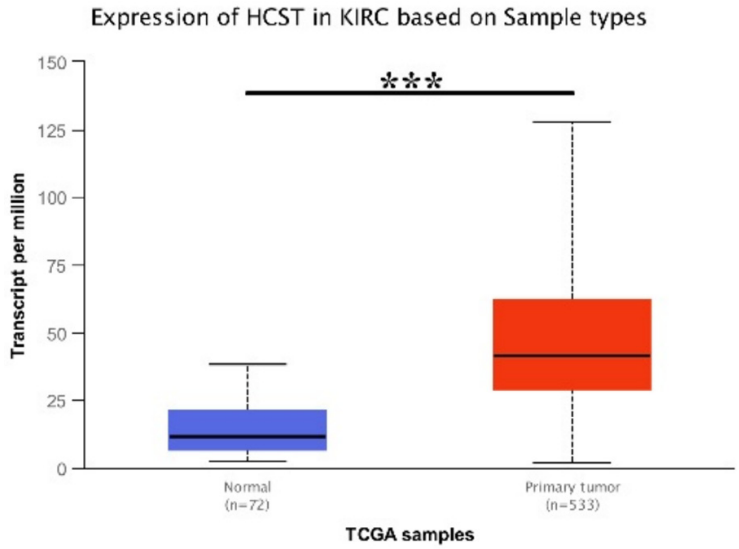


Figure S2. HCST is upregulated in KIRC tissues in the UALCAN database.

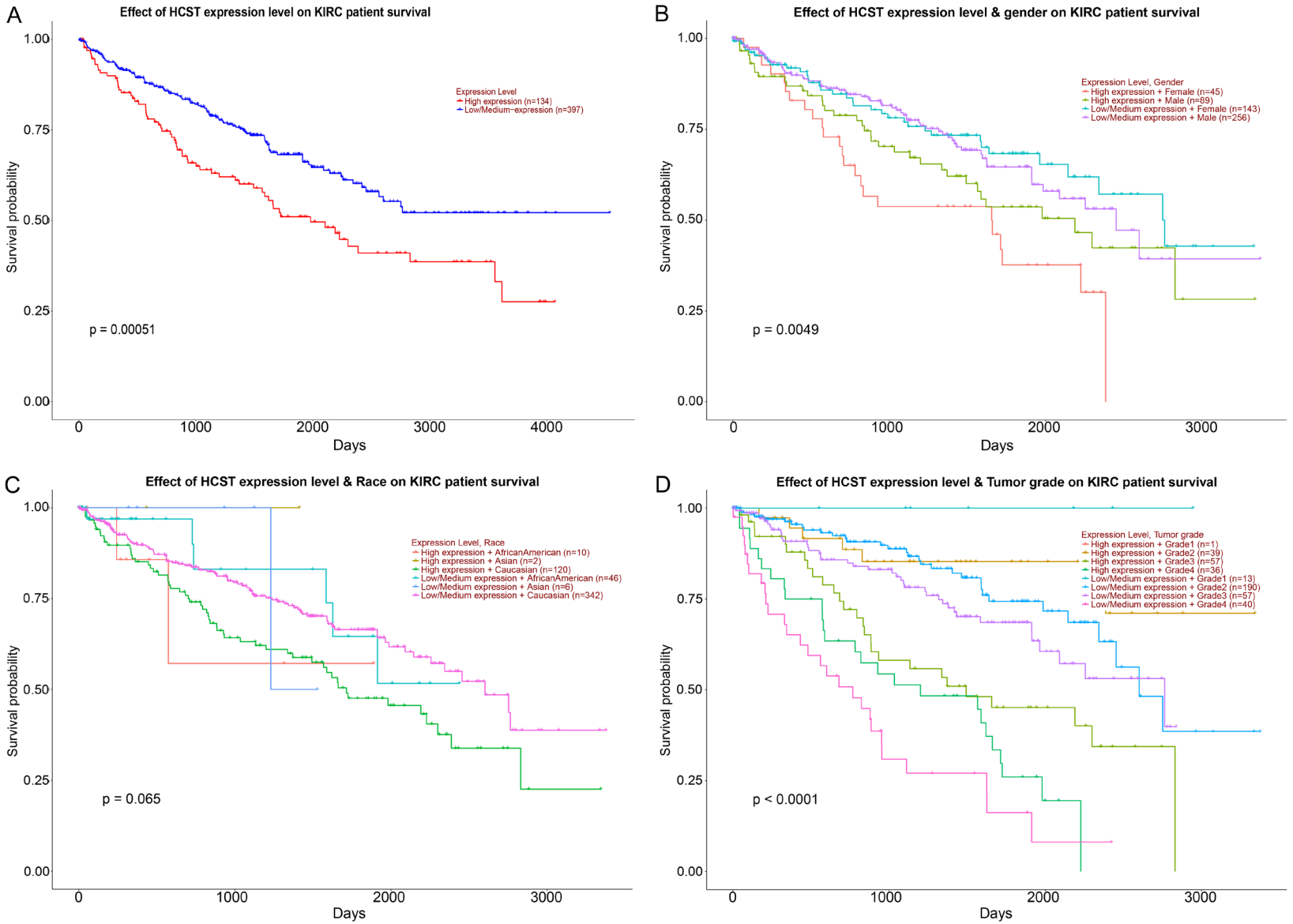


Figure S3. KIRC patients with elevated HCST expression level in the UALCAN database have poor prognosis. A. OS; B. OS-related gender; C. OS-related race; D. OS-related tumor grade. Note: OS, Overall survival.

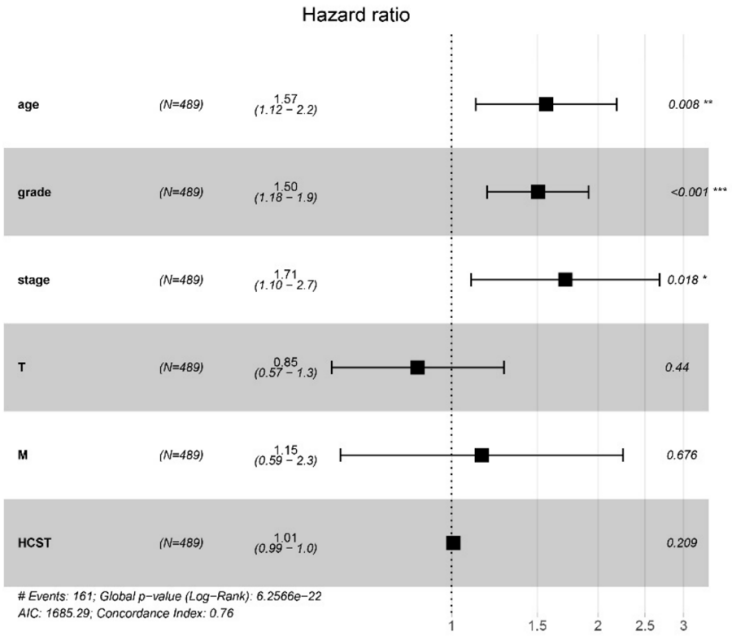


Figure S4. Age, clinical stage and tumor grade are independent factors influencing poor prognosis of KIRC patients.

Table S1. HCST co-expressed genes

Gene	cor	P	Gene	cor	P
BATF	0.872	2.24E-168	ARL11	0.575	8.59E-49
CCL5	0.858	1.68E-157	GBP2	0.575	8.35E-49
LIMD2	0.845	7.01E-148	IKBKE	0.573	2.41E-48
LST1	0.845	2.76E-148	IL32	0.573	2.03E-48
TNFAIP8L2	0.837	1.37E-142	PSMB3	0.572	3.04E-48
WAS	0.835	1.03E-141	PPP1R18	0.572	3.78E-48
CORO1A	0.834	6.27E-141	GPR171	0.572	3.25E-48
IL2RG	0.83	2.33E-138	ODF3B	0.571	5.84E-48
CD3D	0.823	4.21E-134	CD300LF	0.571	5.33E-48
NKG7	0.819	6.36E-132	IL15RA	0.571	6.79E-48
DEF6	0.814	6.15E-129	FGR	0.571	6.26E-48
LTB	0.811	6.11E-127	CORO7	0.57	7.77E-48
TYROBP	0.807	3.41E-125	IL4I1	0.57	8.95E-48
CD52	0.804	2.09E-123	ZDHHC18	0.569	1.16E-47
S1PR4	0.801	1.20E-121	AIM2	0.569	1.67E-47
RAC2	0.8	1.80E-121	CARD9	0.567	3.24E-47
CXCR3	0.797	1.46E-119	ARHGAP30	0.567	4.00E-47
GPSM3	0.796	5.30E-119	IL21R	0.567	3.80E-47
PYCARD	0.794	2.64E-118	KIAA0930	0.567	4.19E-47
SPI1	0.793	1.03E-117	NFAM1	0.566	5.83E-47
CST7	0.791	7.17E-117	CD4	0.565	1.03E-46
CD7	0.789	6.35E-116	FCMR	0.565	1.01E-46
GZMM	0.788	3.48E-115	LCP2	0.565	7.45E-47
RHOG	0.787	9.13E-115	SIGLEC10	0.565	7.57E-47
CD3E	0.787	9.34E-115	HCK	0.564	1.65E-46
SIT1	0.784	3.06E-113	ARHGAP4	0.564	1.32E-46
RGS19	0.784	3.37E-113	CXCL9	0.564	1.42E-46

PSTPIP1	0.784	4.76E-113	HLA-DQA1	0.563	1.76E-46
PLEKHO1	0.783	5.12E-113	CASP1	0.563	2.28E-46
C19orf38	0.783	5.67E-113	GSDMD	0.562	2.83E-46
GZMA	0.779	6.00E-111	CD33	0.562	3.87E-46
PTPN7	0.779	4.96E-111	PIK3CD	0.562	3.90E-46
SH3BP1	0.775	4.11E-109	APOC1	0.561	4.42E-46
MAP4K1	0.774	1.94E-108	SAC3D1	0.561	5.50E-46
EFHD2	0.772	1.04E-107	HLA-DRA	0.561	5.65E-46
FERMT3	0.77	4.69E-107	ARHGAP15	0.561	6.34E-46
CD37	0.77	7.17E-107	KLRB1	0.56	8.22E-46
LSP1	0.768	4.15E-106	CFL1	0.56	7.38E-46
TBC1D10C	0.766	2.48E-105	LSM7	0.559	1.48E-45
CD27	0.764	2.41E-104	MRPL55	0.558	1.81E-45
RGS10	0.76	1.21E-102	GPR65	0.558	2.21E-45
CTSW	0.758	8.24E-102	SSBP4	0.558	2.04E-45
SH2D2A	0.758	6.75E-102	CD38	0.557	3.68E-45
PTPN6	0.754	5.74E-100	CD86	0.556	4.45E-45
CD74	0.753	1.39E-99	BCL2A1	0.555	8.08E-45
SIRPG	0.753	1.56E-99	CSF1	0.555	6.52E-45
LAG3	0.75	1.31E-98	TMEM219	0.555	8.13E-45
GZMH	0.75	1.59E-98	IL24	0.554	1.07E-44
SNX20	0.748	1.38E-97	TMEM150B	0.554	1.07E-44
PSMB10	0.748	7.29E-98	TBX21	0.553	1.65E-44
DOK2	0.747	4.19E-97	KCNK6	0.553	1.59E-44
CD2	0.746	5.73E-97	RHEBL1	0.553	1.58E-44
SASH3	0.745	2.21E-96	GPR84	0.553	1.66E-44
LCK	0.745	1.38E-96	ARPC4	0.551	4.87E-44
CARD16	0.744	2.99E-96	S100A11	0.55	5.87E-44
ASCL2	0.743	1.33E-95	UBALD2	0.55	6.04E-44
CD247	0.741	4.01E-95	CLECL1	0.55	7.15E-44
SH3BGRL3	0.739	2.44E-94	ADAM8	0.55	6.91E-44
PDCD1	0.736	4.94E-93	SSR4	0.55	5.25E-44
IL12RB1	0.734	2.14E-92	SRM	0.55	5.29E-44
PARVG	0.732	1.70E-91	ARHGDIB	0.549	8.50E-44
CD48	0.731	3.56E-91	SDF2L1	0.547	2.17E-43
SLA2	0.731	2.48E-91	TNIP2	0.546	3.45E-43
ARHGAP9	0.731	3.76E-91	AP2S1	0.546	2.88E-43
PSME2	0.73	5.93E-91	ACTB	0.545	4.45E-43
LTA	0.73	8.53E-91	CKLF	0.543	1.03E-42
CD6	0.729	2.04E-90	EXOSC1	0.543	1.41E-42
TMC8	0.727	7.76E-90	TSTA3	0.542	1.64E-42
NCF4	0.727	8.27E-90	CORO1B	0.542	1.49E-42
CD8B	0.727	6.61E-90	PSENEN	0.542	1.47E-42
SELPLG	0.727	7.56E-90	EIF5A	0.542	1.84E-42
C1QA	0.727	1.49E-89	EVI2A	0.541	2.16E-42
CCDC88B	0.726	2.35E-89	IKZF1	0.541	2.37E-42
FMNL1	0.723	2.35E-88	ITK	0.541	2.96E-42
MYO1F	0.722	7.66E-88	BCL11B	0.541	2.51E-42
CSK	0.72	2.47E-87	LILRB4	0.541	2.49E-42
LGALS9	0.719	5.24E-87	TSPO	0.54	3.46E-42

FCER1G	0.718	1.07E-86	APOBEC3C	0.54	4.66E-42
C1orf162	0.717	2.67E-86	MATK	0.539	6.26E-42
ITGAL	0.715	2.17E-85	PLEKHF1	0.538	7.68E-42
FCGR1A	0.715	1.24E-85	IRF4	0.538	1.01E-41
AIF1	0.715	1.15E-85	SNRPA	0.537	1.15E-41
UBASH3A	0.713	1.08E-84	C4orf48	0.536	2.35E-41
TYMP	0.711	3.84E-84	FCGR3A	0.536	1.78E-41
EBI3	0.711	3.45E-84	KCNAB2	0.536	1.69E-41
RASAL3	0.711	2.91E-84	PUSL1	0.536	2.27E-41
ARHGAP45	0.711	3.31E-84	ADGRE5	0.536	1.92E-41
HLA-DMA	0.711	3.84E-84	PSMD13	0.535	3.44E-41
PSMB9	0.709	1.85E-83	APOE	0.535	2.65E-41
VMO1	0.706	1.63E-82	STAT5A	0.535	3.33E-41
C16orf54	0.704	7.43E-82	CD68	0.533	6.33E-41
CYBA	0.7	2.09E-80	PPIH	0.532	9.22E-41
SECTM1	0.699	2.48E-80	IFITM3	0.532	8.85E-41
CD72	0.698	5.29E-80	FAM89B	0.532	8.65E-41
RHOH	0.697	1.31E-79	MILR1	0.532	1.22E-40
GMIP	0.697	1.04E-79	STX10	0.532	1.05E-40
NCF1	0.696	2.65E-79	DDX39A	0.531	1.78E-40
CD8A	0.696	2.31E-79	ABI3	0.53	2.18E-40
XCL2	0.693	2.06E-78	P2RY10	0.529	3.28E-40
CMTM7	0.692	4.56E-78	ICOS	0.529	3.47E-40
APOBEC3H	0.69	1.56E-77	RHBDF2	0.529	2.82E-40
CNPY3	0.69	2.06E-77	SLC29A3	0.529	3.22E-40
FAM78A	0.688	6.23E-77	SELL	0.529	3.47E-40
PFN1	0.687	1.45E-76	TBXAS1	0.528	5.34E-40
CD5	0.686	3.03E-76	CSF3R	0.528	4.63E-40
STAC3	0.686	3.77E-76	BTK	0.527	7.36E-40
CYTH4	0.686	2.48E-76	S1PR2	0.526	9.54E-40
SLC15A3	0.686	2.87E-76	DPP9	0.526	9.55E-40
ACAP1	0.685	6.45E-76	OGFR	0.524	2.81E-39
GNA15	0.684	1.63E-75	PSMB4	0.524	2.00E-39
CALHM6	0.684	1.49E-75	TRAF1	0.523	4.03E-39
ASB2	0.682	5.22E-75	CCR2	0.523	3.40E-39
CD244	0.682	4.19E-75	BASP1	0.523	3.02E-39
LAPTM5	0.682	7.06E-75	CEACAM4	0.523	3.72E-39
GZMK	0.681	1.45E-74	TIMP1	0.523	3.80E-39
FCGR1B	0.679	5.33E-74	SLA	0.523	3.43E-39
LAT2	0.679	5.36E-74	TRPV2	0.523	3.94E-39
CD96	0.677	2.21E-73	ADRM1	0.522	4.38E-39
HLA-DPB1	0.677	1.67E-73	CEBPA	0.522	4.68E-39
PPM1M	0.677	1.81E-73	AGTRAP	0.52	1.30E-38
IGFLR1	0.677	1.64E-73	CAVIN3	0.52	1.27E-38
APOBEC3G	0.677	2.25E-73	HSD3B7	0.52	1.05E-38
FLT3LG	0.675	5.98E-73	NAGK	0.52	1.14E-38
UNC13D	0.673	2.10E-72	ZDHHC12	0.519	1.45E-38
VAV1	0.673	2.19E-72	MFSD13A	0.519	1.57E-38
GMFG	0.673	2.80E-72	NINJ1	0.518	2.20E-38

ISG15	0.672	4.86E-72	CD69	0.518	2.38E-38
SLAMF6	0.671	7.56E-72	HLA-DPA1	0.518	2.46E-38
SIPA1	0.67	1.68E-71	GUK1	0.518	2.20E-38
IFI35	0.669	3.23E-71	MICB	0.517	3.48E-38
TIGIT	0.668	4.96E-71	CLIC3	0.517	3.31E-38
SNAI3	0.668	5.60E-71	TRABD	0.516	5.42E-38
DOK3	0.666	1.87E-70	PTPN22	0.516	5.51E-38
C1QB	0.666	1.72E-70	TOX	0.516	5.00E-38
SP140	0.666	2.37E-70	NCKAP1L	0.516	6.08E-38
CSTA	0.665	4.93E-70	DGKZ	0.515	8.58E-38
SLAMF8	0.664	8.92E-70	RELB	0.515	8.54E-38
MYO1G	0.664	1.05E-69	MZB1	0.514	1.06E-37
DUSP2	0.663	1.18E-69	R3HDM4	0.514	1.06E-37
SH2D1A	0.663	1.65E-69	LGALS1	0.514	1.33E-37
SLAMF7	0.662	2.96E-69	TRAF2	0.514	1.32E-37
RAB4B	0.661	5.69E-69	LY96	0.514	9.45E-38
POU2F2	0.66	8.66E-69	JOSD2	0.514	1.01E-37
JAKMIP1	0.66	1.20E-68	STAT4	0.514	1.13E-37
FASLG	0.66	7.72E-69	HK3	0.514	1.09E-37
LSM10	0.659	1.78E-68	PARP12	0.513	1.71E-37
TMSB4X	0.658	3.07E-68	SEMA4A	0.513	1.95E-37
NCR3	0.657	6.94E-68	HAMP	0.513	1.53E-37
LYL1	0.657	7.38E-68	PLEK	0.513	1.96E-37
TWF2	0.657	6.25E-68	CFD	0.513	1.49E-37
OSCAR	0.656	1.31E-67	NFKB2	0.513	1.59E-37
ARPC1B	0.656	1.45E-67	OR2I1P	0.512	2.15E-37
XCL1	0.654	4.72E-67	CCL3	0.511	2.95E-37
GZMB	0.652	1.15E-66	HLA-DQB1	0.511	3.76E-37
PRF1	0.652	1.45E-66	ARID5A	0.511	3.39E-37
TNFRSF1B	0.649	9.77E-66	ARPC3	0.511	3.18E-37
ZNF385A	0.648	1.51E-65	PIM2	0.51	5.36E-37
HCLS1	0.647	3.62E-65	RPS6KA1	0.51	5.51E-37
MIIP	0.646	5.38E-65	DPEP2	0.509	6.45E-37
ITGB2	0.646	6.52E-65	CYP2S1	0.509	7.81E-37
ZAP70	0.645	8.75E-65	TNNI2	0.509	8.64E-37
TRPM2	0.645	8.27E-65	IFI27	0.509	6.40E-37
ICAM3	0.643	2.86E-64	ISY1	0.509	7.03E-37
CXCR6	0.643	3.05E-64	UBE2J2	0.509	6.76E-37
TNFRSF18	0.642	5.44E-64	PLA2G2D	0.509	7.03E-37
CST3	0.642	4.62E-64	COMMD5	0.509	6.82E-37
IL10RA	0.642	4.43E-64	HLA-DMB	0.508	8.83E-37
HLA-DRB1	0.641	8.80E-64	RNASE2	0.508	1.13E-36
PCED1B	0.641	8.60E-64	TOR3A	0.507	1.79E-36
GLIPR2	0.64	2.36E-63	PGLS	0.507	1.75E-36
HLA-DOB	0.639	3.45E-63	HCFC1R1	0.507	1.59E-36
ISG20	0.639	3.06E-63	TAF10	0.507	1.33E-36
CARMIL2	0.638	7.49E-63	NAA10	0.506	1.89E-36
GNGT2	0.638	7.74E-63	IGSF6	0.506	2.20E-36
CCR5	0.638	5.84E-63	MZT2B	0.506	1.88E-36
CAPZB	0.637	1.07E-62	DGUOK	0.506	2.30E-36

IL16	0.637	9.70E-63	C1orf54	0.506	2.08E-36
FBXO6	0.637	1.25E-62	ALDH16A1	0.505	3.56E-36
PYHIN1	0.637	9.30E-63	BID	0.505	3.12E-36
HLA-A	0.637	1.31E-62	LRFN1	0.504	4.99E-36
SOWAHD	0.635	4.09E-62	CASP4	0.504	5.04E-36
IL18BP	0.634	4.93E-62	CD79A	0.504	4.20E-36
JPT1	0.634	6.62E-62	SMIM29	0.504	4.03E-36
CCL4	0.633	8.51E-62	MGAT1	0.503	7.77E-36
IL2RB	0.631	3.74E-61	AUP1	0.503	7.00E-36
SLC2A6	0.631	2.81E-61	OPRL1	0.503	5.98E-36
PRAM1	0.629	8.18E-61	NBL1	0.503	7.18E-36
TREM2	0.628	1.48E-60	UBA7	0.503	5.61E-36
C1QC	0.626	6.19E-60	STX4	0.503	7.87E-36
APOBR	0.626	6.21E-60	OAZ1	0.502	1.13E-35
PILRA	0.625	8.91E-60	HGH1	0.502	1.00E-35
PSMB8	0.625	7.43E-60	FCHO1	0.501	1.18E-35
IRF1	0.625	1.04E-59	TRIR	0.501	1.36E-35
ZBP1	0.623	3.35E-59	CDKL2	-0.501	1.57E-35
CD14	0.622	4.12E-59	ZYG11B	-0.501	1.58E-35
LILRB2	0.622	5.34E-59	MEGF9	-0.501	1.31E-35
LILRB3	0.622	5.73E-59	GNPNAT1	-0.502	9.08E-36
NOD2	0.621	9.27E-59	GFM2	-0.502	8.23E-36
VAMP5	0.621	7.18E-59	OPHN1	-0.503	6.57E-36
RUNX3	0.621	9.79E-59	USP51	-0.503	5.80E-36
HLA-F	0.621	7.53E-59	NNT	-0.504	4.06E-36
COTL1	0.62	1.86E-58	VEZF1	-0.505	3.11E-36
BIN2	0.619	2.39E-58	DPY19L4	-0.505	3.80E-36
CRTAM	0.619	2.25E-58	LNX1	-0.506	1.97E-36
CYBC1	0.619	3.22E-58	PCNX4	-0.507	1.34E-36
FOXP3	0.618	4.92E-58	JADE3	-0.508	9.79E-37
GFI1	0.617	7.75E-58	PPARGC1A	-0.508	1.03E-36
DTX2	0.617	8.28E-58	KRR1	-0.509	6.84E-37
S100A4	0.615	2.29E-57	VAPB	-0.51	5.54E-37
IFNG	0.615	2.46E-57	PNPLA8	-0.51	5.40E-37
HAPLN3	0.614	4.71E-57	KTN1	-0.51	5.58E-37
TCIRG1	0.614	3.53E-57	AKAP6	-0.51	4.27E-37
AKNA	0.613	6.80E-57	ATL2	-0.51	5.00E-37
FXYD5	0.613	6.19E-57	ATRN	-0.51	4.91E-37
JAML	0.613	7.00E-57	CUL5	-0.511	3.02E-37
SCNM1	0.611	1.94E-56	FAN1	-0.511	3.23E-37
RASSF5	0.611	1.69E-56	USP53	-0.511	3.02E-37
EMP3	0.61	2.36E-56	MIB1	-0.512	2.32E-37
UNC93B1	0.61	2.35E-56	OSBPL1A	-0.512	2.76E-37
GBP5	0.609	4.68E-56	USP8	-0.513	1.75E-37
LAIR1	0.609	4.85E-56	NR3C2	-0.513	1.45E-37
SLAMF1	0.609	5.47E-56	KIAA1143	-0.513	1.76E-37
TRAF3IP3	0.608	7.45E-56	RNF141	-0.514	1.23E-37
DRAP1	0.608	8.40E-56	DLAT	-0.514	1.07E-37
LY86	0.607	1.51E-55	THRB	-0.515	6.88E-38
LPXN	0.607	1.52E-55	ZNF770	-0.515	8.10E-38

MEI1	0.607	1.84E-55	FAM160A1	-0.516	5.07E-38
CXCL13	0.607	1.64E-55	TOGARAM1	-0.516	5.69E-38
EVI2B	0.606	3.01E-55	SPIRE1	-0.518	2.37E-38
EOMES	0.604	5.52E-55	ZMYND11	-0.519	1.90E-38
TNFRSF9	0.604	7.69E-55	LGR4	-0.519	1.48E-38
GPR68	0.604	6.99E-55	CTDSPL	-0.52	1.19E-38
ZNF683	0.603	9.17E-55	SEC24B	-0.52	1.01E-38
IKZF3	0.602	2.28E-54	ARMCX3	-0.52	1.21E-38
JAK3	0.602	1.71E-54	UBR3	-0.52	1.29E-38
LAMTOR2	0.601	3.22E-54	ITGA6	-0.52	1.18E-38
LILRB1	0.601	2.75E-54	OPA1	-0.522	5.42E-39
UCP2	0.601	3.56E-54	SYPL1	-0.522	4.55E-39
RPS6KA4	0.601	3.57E-54	ZNF112	-0.522	5.95E-39
APOBEC3D	0.6	6.50E-54	SLC30A9	-0.523	4.22E-39
TNFSF13B	0.6	5.13E-54	BRMS1L	-0.523	2.96E-39
HSH2D	0.598	1.65E-53	KLHL24	-0.525	1.86E-39
GRK2	0.598	1.22E-53	SLC39A9	-0.525	1.61E-39
TACC3	0.598	1.27E-53	SBF2	-0.526	1.04E-39
CCDC167	0.595	7.93E-53	LRPPRC	-0.53	2.01E-40
CCM2	0.595	5.24E-53	MTMR12	-0.53	2.59E-40
FGD3	0.593	1.37E-52	ARHGEF12	-0.531	1.26E-40
CTLA4	0.592	2.58E-52	ZNF260	-0.532	9.45E-41
RRAS	0.59	6.03E-52	SCAMP1	-0.534	4.04E-41
REEP4	0.59	6.66E-52	MAP4K3	-0.534	4.39E-41
CEACAM21	0.59	6.28E-52	COBLL1	-0.535	2.95E-41
SPN	0.589	9.59E-52	NCKAP1	-0.536	2.09E-41
ZBED2	0.589	1.17E-51	CHM	-0.537	1.15E-41
RIN3	0.588	1.68E-51	HMGCS1	-0.537	1.38E-41
PLCB2	0.586	6.46E-51	PTPN21	-0.537	1.40E-41
HLA-B	0.586	5.02E-51	ZNF664	-0.537	1.16E-41
CD3G	0.586	5.23E-51	L2HGDH	-0.54	4.41E-42
ARRB2	0.585	7.87E-51	LRP11	-0.54	4.10E-42
LCP1	0.585	7.52E-51	PIK3C2A	-0.542	1.74E-42
IRF7	0.585	1.05E-50	TRIM2	-0.544	8.38E-43
SYNGR2	0.584	1.16E-50	SECISBP2L	-0.544	7.59E-43
ETV7	0.584	1.39E-50	ARFGEF2	-0.544	7.95E-43
CD53	0.584	1.56E-50	PDZD8	-0.546	2.92E-43
TAP1	0.584	1.07E-50	WASL	-0.546	4.01E-43
YIF1B	0.583	1.92E-50	SAV1	-0.547	1.86E-43
DOK1	0.583	1.92E-50	NAA30	-0.548	1.38E-43
NELL2	0.583	2.51E-50	PPM1A	-0.548	1.68E-43
TRAT1	0.583	2.45E-50	EPB41L5	-0.55	7.02E-44
TAPBP	0.583	2.07E-50	PRRG1	-0.553	1.81E-44
OASL	0.583	2.58E-50	SOS2	-0.553	1.82E-44
AGAP2	0.583	2.20E-50	ACADSB	-0.553	1.72E-44
KCNN4	0.582	4.24E-50	FRK	-0.554	9.78E-45
GRK6	0.582	3.13E-50	TRIP11	-0.554	1.06E-44
SCO2	0.581	5.40E-50	CMTM4	-0.556	4.45E-45
GRAP2	0.581	5.80E-50	CIPC	-0.556	4.26E-45
TOR2A	0.581	6.91E-50	SNX13	-0.563	2.20E-46

ZMYND15	0.58	7.47E-50	SYNJ2BP	-0.563	2.49E-46
RNF166	0.579	1.52E-49	TMEM184C	-0.564	1.55E-46
NFKBIE	0.579	1.22E-49	LMBR1	-0.566	5.72E-47
USF1	0.578	2.31E-49	YIPF6	-0.566	6.15E-47
TMSB10	0.578	2.82E-49	NUBPL	-0.571	6.22E-48
ACP2	0.577	3.02E-49	HECTD1	-0.571	6.24E-48
PAXX	0.577	3.72E-49	MPP5	-0.584	1.37E-50
TMC6	0.576	6.93E-49	FNBP1L	-0.585	7.24E-51
RNPEPL1	0.576	5.58E-49	APOOL	-0.589	1.10E-51
NME3	0.576	5.34E-49	ARHGAP5	-0.596	3.04E-53
BATF2	0.576	7.01E-49			

Table S2. The GO analysis showing the functions of HCST co-expressed genes

Type	Term		Count	P
BP	GO:0007165	signal transduction	77	4.21E-10
BP	GO:0006955	immune response	69	7.52E-30
BP	GO:0045087	innate immune response	50	3.06E-15
BP	GO:0006954	inflammatory response	44	1.97E-13
BP	GO:0006915	apoptotic process	41	1.25E-06
CC	GO:0009897	external side of plasma membrane	35	1.44E-15
BP	GO:0043547	positive regulation of GTPase activity	39	7.13E-06
CC	GO:0016020	membrane	129	6.92E-14
BP	GO:0050776	regulation of immune response	35	1.02E-17
BP	GO:0002250	adaptive immune response	31	1.59E-16
BP	GO:0050852	T cell receptor signaling pathway	31	1.59E-16
BP	GO:0035556	intracellular signal transduction	28	1.59E-04
BP	GO:0007155	cell adhesion	27	0.002482297
CC	GO:0042613	MHC class II protein complex	11	2.65E-10
BP	GO:0007166	cell surface receptor signaling pathway	25	5.22E-06
BP	GO:0031295	T cell costimulation	24	8.65E-17
CC	GO:0042101	T cell receptor complex	10	7.31E-10
BP	GO:0016032	viral process	23	1.76E-04
MF	GO:0042605	peptide antigen binding	11	5.52E-09
BP	GO:0008284	positive regulation of cell proliferation	23	0.034673591
CC	GO:0071556	integral component of lumenal side of endoplasmic reticulum membrane	11	6.79E-09
MF	GO:0005515	protein binding	334	9.93E-09
CC	GO:0005887	integral component of plasma membrane	81	2.98E-08
CC	GO:0001772	immunological synapse	11	3.88E-08
CC	GO:0005886	plasma membrane	179	5.48E-08
BP	GO:0007169	transmembrane receptor protein tyrosine kinase signaling pathway	20	1.01E-10
BP	GO:0051607	defense response to virus	19	3.92E-06
MF	GO:0032395	MHC class II receptor activity	8	1.30E-07
BP	GO:0007267	cell-cell signaling	19	0.001027275
BP	GO:0008283	cell proliferation	19	0.037925311
MF	GO:0004872	receptor activity	24	2.53E-07
BP	GO:0042102	positive regulation of T cell proliferation	18	2.05E-12
BP	GO:0060333	interferon-gamma-mediated signaling pathway	18	4.00E-11
MF	GO:0042288	MHC class I protein binding	8	9.11E-07
BP	GO:0033209	tumor necrosis factor-mediated signaling pathway	18	1.40E-07
BP	GO:0043065	positive regulation of apoptotic process	18	0.012925541
BP	GO:0051056	regulation of small GTPase mediated signal transduction	17	4.18E-06
BP	GO:0042981	regulation of apoptotic process	17	0.00104719
MF	GO:0005102	receptor binding	30	1.63E-06
BP	GO:0042110	T cell activation	16	6.03E-12

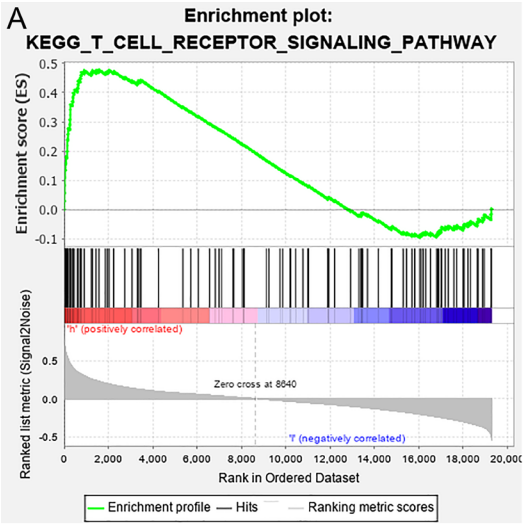
BP	GO:0006968	cellular defense response	16	4.85E-10
BP	GO:0006935	chemotaxis	16	5.78E-06
CC	GO:0030669	clathrin-coated endocytic vesicle membrane	10	2.84E-06
CC	GO:0012507	ER to Golgi transport vesicle membrane	11	2.87E-06
BP	GO:0050900	leukocyte migration	16	5.78E-06
BP	GO:0038096	Fc-gamma receptor signaling pathway involved in phagocytosis	16	9.50E-06
BP	GO:0032496	response to lipopolysaccharide	16	1.88E-04
CC	GO:0042105	alpha-beta T cell receptor complex	5	3.98E-06
BP	GO:0019882	antigen processing and presentation	15	8.94E-10
BP	GO:0002479	antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent	15	6.11E-09
BP	GO:0000165	MAPK cascade	15	0.035016057
BP	GO:0060337	type I interferon signaling pathway	14	6.72E-08
CC	GO:0031234	extrinsic component of cytoplasmic side of plasma membrane	12	5.43E-06
BP	GO:0030593	neutrophil chemotaxis	14	9.88E-08
BP	GO:0006928	movement of cell or subcellular component	14	2.38E-06
BP	GO:0019886	antigen processing and presentation of exogenous peptide antigen via MHC class II	14	5.16E-06
MF	GO:0004715	non-membrane spanning protein tyrosine kinase activity	10	9.15E-06
BP	GO:0009615	response to virus	14	3.68E-05
CC	GO:0070062	extracellular exosome	121	3.21E-05
MF	GO:0004888	transmembrane signaling receptor activity	20	3.55E-05
BP	GO:0071222	cellular response to lipopolysaccharide	14	4.90E-05
BP	GO:0070374	positive regulation of ERK1 and ERK2 cascade	14	0.003292577
BP	GO:0038095	Fc-epsilon receptor signaling pathway	14	0.003807311
BP	GO:0042127	regulation of cell proliferation	14	0.005264057
BP	GO:0007264	small GTPase mediated signal transduction	14	0.044360756
MF	GO:0023026	MHC class II protein complex binding	6	8.88E-05
CC	GO:0030027	lamellipodium	16	1.02E-04
CC	GO:0009986	cell surface	34	1.08E-04
MF	GO:0050839	cell adhesion molecule binding	10	1.09E-04
MF	GO:0005125	cytokine activity	17	1.10E-04
BP	GO:0006952	defense response	13	9.07E-07
MF	GO:0003779	actin binding	22	1.47E-04
MF	GO:0005096	GTPase activator activity	22	1.54E-04
BP	GO:0007229	integrin-mediated signaling pathway	13	5.68E-05
BP	GO:0006461	protein complex assembly	13	2.62E-04
BP	GO:0032760	positive regulation of tumor necrosis factor production	12	1.49E-07
BP	GO:0030036	actin cytoskeleton organization	12	0.002453325
BP	GO:0051092	positive regulation of NF-kappaB transcription factor activity	12	0.002931948
BP	GO:0043123	positive regulation of I-kappaB kinase/NF-kappaB signaling	12	0.011982406
CC	GO:0005829	cytosol	134	1.96E-04
BP	GO:0030335	positive regulation of cell migration	12	0.029082153
BP	GO:0032729	positive regulation of interferon-gamma production	11	1.15E-06
CC	GO:0016021	integral component of membrane	194	2.43E-04
BP	GO:0050853	B cell receptor signaling pathway	11	5.41E-06
CC	GO:0005765	lysosomal membrane	21	2.56E-04
MF	GO:0015026	coreceptor activity	7	2.60E-04
BP	GO:0070098	chemokine-mediated signaling pathway	11	6.50E-05
BP	GO:0002223	stimulatory C-type lectin receptor signaling pathway	11	0.001588159
BP	GO:0071356	cellular response to tumor necrosis factor	11	0.002250533
BP	GO:0008360	regulation of cell shape	11	0.01213597
BP	GO:0007568	aging	11	0.03360029
BP	GO:0038083	peptidyl-tyrosine autophosphorylation	10	2.93E-06
CC	GO:0005856	cytoskeleton	25	4.01E-04
BP	GO:0071346	cellular response to interferon-gamma	10	6.03E-05
CC	GO:0010008	endosome membrane	16	4.98E-04

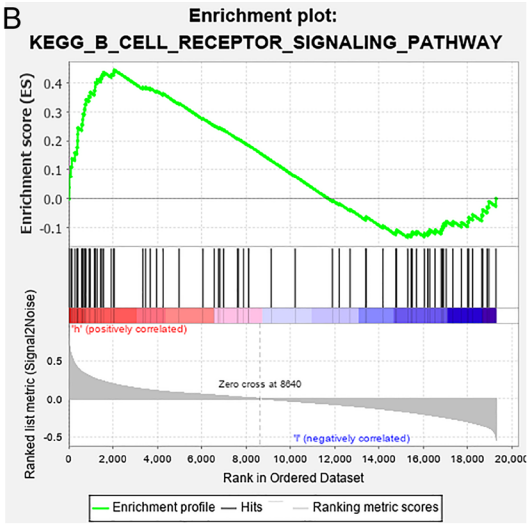
CC	GO:0097169	AIM2 inflammasome complex	4	5.08E-04
BP	GO:0038061	NIK/NF-kappaB signaling	10	1.94E-04
MF	GO:0008009	chemokine activity	8	6.99E-04
CC	GO:0030666	endocytic vesicle membrane	9	7.97E-04
CC	GO:0030658	transport vesicle membrane	7	8.84E-04
BP	GO:0097190	apoptotic signaling pathway	10	3.39E-04
BP	GO:0060071	Wnt signaling pathway, planar cell polarity pathway	10	0.002231382
BP	GO:0030168	platelet activation	10	0.009685649
MF	GO:0003785	actin monomer binding	6	0.001037634
BP	GO:0019221	cytokine-mediated signaling pathway	10	0.021182443
CC	GO:0072559	NLRP3 inflammasome complex	4	0.001359752
BP	GO:0002504	antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	9	1.57E-08
BP	GO:0030217	T cell differentiation	9	2.64E-06
BP	GO:0006959	humoral immune response	9	3.58E-04
BP	GO:0006470	protein dephosphorylation	9	0.043004976
MF	GO:0042169	SH2 domain binding	6	0.001736429
MF	GO:0005164	tumor necrosis factor receptor binding	6	0.001736429
CC	GO:0000502	proteasome complex	8	0.002101736
BP	GO:0050870	positive regulation of T cell activation	8	6.37E-07
BP	GO:0050850	positive regulation of calcium-mediated signaling	8	1.47E-06
CC	GO:0001891	phagocytic cup	5	0.00220201
BP	GO:0019835	cytolysis	8	2.15E-06
BP	GO:0050718	positive regulation of interleukin-1 beta secretion	8	3.06E-06
BP	GO:0042130	negative regulation of T cell proliferation	8	1.23E-04
BP	GO:0006909	phagocytosis	8	6.57E-04
BP	GO:0006521	regulation of cellular amino acid metabolic process	8	9.53E-04
BP	GO:0071407	cellular response to organic cyclic compound	8	0.002270252
BP	GO:0007249	I-kappaB kinase/NF-kappaB signaling	8	0.002502696
MF	GO:0046979	TAP2 binding	3	0.002772603
BP	GO:0048010	vascular endothelial growth factor receptor signaling pathway	8	0.006947348
BP	GO:0043488	regulation of mRNA stability	8	0.041549724
BP	GO:0050690	regulation of defense response to virus by virus	7	1.84E-04
BP	GO:0042113	B cell activation	7	2.26E-04
BP	GO:0002474	antigen processing and presentation of peptide antigen via MHC class I	7	2.76E-04
CC	GO:0016023	cytoplasmic, membrane-bounded vesicle	12	0.003082986
CC	GO:0005839	proteasome core complex	5	0.003241883
BP	GO:0031663	lipopolysaccharide-mediated signaling pathway	7	3.99E-04
MF	GO:0004298	threonine-type endopeptidase activity	5	0.00348582
BP	GO:0045071	negative regulation of viral genome replication	7	0.001370036
CC	GO:0042612	MHC class I protein complex	4	0.003744654
BP	GO:0051603	proteolysis involved in cellular protein catabolic process	7	0.003554002
MF	GO:0016814	hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in cyclic amidines	4	0.003964607
MF	GO:0019864	IgG binding	4	0.003964607
BP	GO:0009967	positive regulation of signal transduction	7	0.011477937
BP	GO:0051436	negative regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle	7	0.022890369
CC	GO:0048471	perinuclear region of cytoplasm	32	0.004279434
MF	GO:0004896	cytokine receptor activity	6	0.004621468
BP	GO:0051437	positive regulation of ubiquitin-protein ligase activity involved in regulation of mitotic cell cycle transition	7	0.030746748
CC	GO:0032010	phagolysosome	3	0.005220402
BP	GO:0031145	anaphase-promoting complex-dependent catabolic process	7	0.036214202
BP	GO:0006919	activation of cysteine-type endopeptidase activity involved in apoptotic process	7	0.044420379
BP	GO:0006887	exocytosis	7	0.044420379
CC	GO:0002102	podosome	5	0.005359632
MF	GO:0070891	lipoteichoic acid binding	3	0.005432264
MF	GO:0046978	TAP1 binding	3	0.005432264

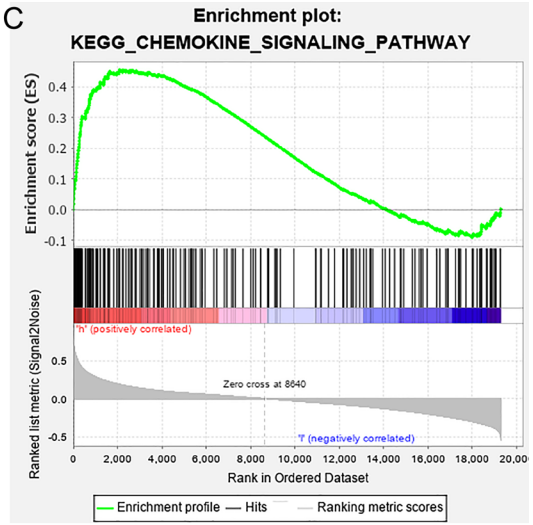
BP	GO:1902715	positive regulation of interferon-gamma secretion	6	1.48E-06
BP	GO:0045954	positive regulation of natural killer cell mediated cytotoxicity	6	1.74E-04
BP	GO:0043011	myeloid dendritic cell differentiation	6	2.31E-04
BP	GO:0002230	positive regulation of defense response to virus by host	6	4.84E-04
BP	GO:0030041	actin filament polymerization	6	0.001090125
MF	GO:0042803	protein homodimerization activity	36	0.006456933
BP	GO:0032689	negative regulation of interferon-gamma production	6	0.001547501
BP	GO:0007498	mesoderm development	6	0.002863191
BP	GO:0032720	negative regulation of tumor necrosis factor production	6	0.006127622
BP	GO:0032755	positive regulation of interleukin-6 production	6	0.012497562
BP	GO:0007157	heterophilic cell-cell adhesion via plasma membrane cell adhesion molecules	6	0.019114583
BP	GO:0032481	positive regulation of type I interferon production	6	0.020667907
MF	GO:0005070	SH3/SH2 adaptor activity	7	0.007251624
MF	GO:0051015	actin filament binding	11	0.007623415
BP	GO:0050727	regulation of inflammatory response	6	0.045871376
CC	GO:0030670	phagocytic vesicle membrane	7	0.008486008
CC	GO:1990111	spermatoproteasome complex	3	0.008527426
MF	GO:0042289	MHC class II protein binding	3	0.008869847
CC	GO:0045121	membrane raft	14	0.009852623
BP	GO:0050868	negative regulation of T cell activation	5	2.56E-04
MF	GO:0004871	signal transducer activity	14	0.01059291
CC	GO:0005622	intracellular	56	0.010618232
CC	GO:0005623	cell	9	0.010652098
MF	GO:0017124	SH3 domain binding	10	0.011190252
BP	GO:0042832	defense response to protozoan	5	0.002467041
BP	GO:0030101	natural killer cell activation	5	0.003008876
BP	GO:0032733	positive regulation of interleukin-10 production	5	0.005108873
BP	GO:0007159	leukocyte cell-cell adhesion	5	0.0069498
BP	GO:0001816	cytokine production	5	0.0069498
BP	GO:0051928	positive regulation of calcium ion transport	5	0.008015195
CC	GO:0032588	trans-Golgi network membrane	8	0.012536362
BP	GO:0043029	T cell homeostasis	5	0.009182346
MF	GO:0042608	T cell receptor binding	3	0.013035185
BP	GO:0032715	negative regulation of interleukin-6 production	5	0.01045482
BP	GO:0048873	homeostasis of number of cells within a tissue	5	0.011835954
BP	GO:0006911	phagocytosis, engulfment	5	0.02256117
BP	GO:0008625	extrinsic apoptotic signaling pathway via death domain receptors	5	0.029587109
BP	GO:0030890	positive regulation of B cell proliferation	5	0.032185299
CC	GO:0005884	actin filament	7	0.013386747
BP	GO:0002548	monocyte chemotaxis	5	0.040758546
BP	GO:0031623	receptor internalization	5	0.043876791
CC	GO:0005911	cell-cell junction	12	0.015280178
CC	GO:0015629	actin cytoskeleton	14	0.015337111
MF	GO:0016301	kinase activity	15	0.017284905
MF	GO:0031726	CCR1 chemokine receptor binding	3	0.017880455
BP	GO:2000566	positive regulation of CD8-positive, alpha-beta T cell proliferation	4	2.85E-04
BP	GO:0002503	peptide antigen assembly with MHC class II protein complex	4	2.85E-04
BP	GO:2000503	positive regulation of natural killer cell chemotaxis	4	9.52E-04
BP	GO:0010529	negative regulation of transposition	4	0.001488302
BP	GO:0045576	mast cell activation	4	0.001488302
CC	GO:0005764	lysosome	14	0.019918062
BP	GO:1900017	positive regulation of cytokine production involved in inflammatory response	4	0.002181095
BP	GO:0046641	positive regulation of alpha-beta T cell proliferation	4	0.002181095
BP	GO:0002726	positive regulation of T cell cytokine production	4	0.003044285
BP	GO:0010820	positive regulation of T cell chemotaxis	4	0.003044285
BP	GO:0016064	immunoglobulin mediated immune response	4	0.004089903

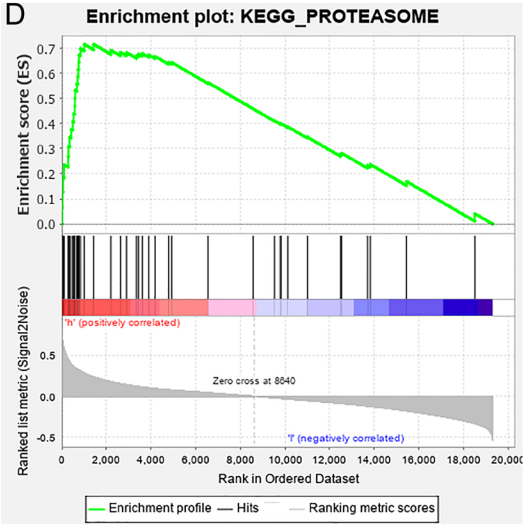
MF	GO:0031730	CCR5 chemokine receptor binding	3	0.023360075
BP	GO:0045060	negative thymic T cell selection	4	0.004089903
BP	GO:0032695	negative regulation of interleukin-12 production	4	0.005328356
BP	GO:0001779	natural killer cell differentiation	4	0.005328356
BP	GO:0045086	positive regulation of interleukin-2 biosynthetic process	4	0.005328356
BP	GO:2000484	positive regulation of interleukin-8 secretion	4	0.006768533
BP	GO:0016045	detection of bacterium	4	0.006768533
BP	GO:0001916	positive regulation of T cell mediated cytotoxicity	4	0.006768533
CC	GO:0042629	mast cell granule	4	0.024153585
CC	GO:0005615	extracellular space	54	0.027019161
MF	GO:0001530	lipopolysaccharide binding	4	0.028821224
BP	GO:0045579	positive regulation of B cell differentiation	4	0.008417914
BP	GO:0051044	positive regulation of membrane protein ectodomain proteolysis	4	0.010282668
BP	GO:0010592	positive regulation of lamellipodium assembly	4	0.012367745
BP	GO:0002407	dendritic cell chemotaxis	4	0.014676969
BP	GO:1902041	regulation of extrinsic apoptotic signaling pathway via death domain receptors	4	0.014676969
BP	GO:0042267	natural killer cell mediated cytotoxicity	4	0.01721312
BP	GO:0034142	toll-like receptor 4 signaling pathway	4	0.01721312
BP	GO:0032753	positive regulation of interleukin-4 production	4	0.022972562
MF	GO:0000982	transcription factor activity, RNA polymerase II core promoter proximal region sequence-specific binding	4	0.032407438
CC	GO:0005938	cell cortex	9	0.032655155
BP	GO:0090023	positive regulation of neutrophil chemotaxis	4	0.029650321
MF	GO:0005057	receptor signaling protein activity	5	0.033725057
BP	GO:0008037	cell recognition	4	0.029650321
BP	GO:0007202	activation of phospholipase C activity	4	0.045719326
MF	GO:0005031	tumor necrosis factor-activated receptor activity	4	0.036214972
CC	GO:0000139	Golgi membrane	27	0.036294233
CC	GO:0032587	ruffle membrane	7	0.037211815
CC	GO:0001931	uropod	3	0.041614703
CC	GO:0005737	cytoplasm	177	0.041725462
BP	GO:0071663	positive regulation of granzyme B production	3	0.002833934
BP	GO:0002283	neutrophil activation involved in immune response	3	0.005551145
BP	GO:0002725	negative regulation of T cell cytokine production	3	0.005551145
BP	GO:2000110	negative regulation of macrophage apoptotic process	3	0.005551145
BP	GO:0045953	negative regulation of natural killer cell mediated cytotoxicity	3	0.005551145
BP	GO:0030098	lymphocyte differentiation	3	0.009061874
BP	GO:0072540	T-helper 17 cell lineage commitment	3	0.009061874
BP	GO:0070269	pyroptosis	3	0.013314348
BP	GO:0035754	B cell chemotaxis	3	0.013314348
BP	GO:0045084	positive regulation of interleukin-12 biosynthetic process	3	0.013314348
BP	GO:0045577	regulation of B cell differentiation	3	0.013314348
CC	GO:0005885	Arp2/3 protein complex	3	0.048961239
CC	GO:0043020	NADPH oxidase complex	3	0.048961239
CC	GO:0005576	extracellular region	61	0.049849209
BP	GO:0002819	regulation of adaptive immune response	3	0.013314348
BP	GO:0032609	interferon-gamma production	3	0.018259237
BP	GO:0070383	DNA cytosine deamination	3	0.018259237
BP	GO:2000601	positive regulation of Arp2/3 complex-mediated actin nucleation	3	0.018259237
BP	GO:0045869	negative regulation of single stranded viral RNA replication via double stranded DNA intermediate	3	0.018259237
BP	GO:0019885	antigen processing and presentation of endogenous peptide antigen via MHC class I	3	0.018259237
BP	GO:0002467	germinal center formation	3	0.023849548
BP	GO:0010818	T cell chemotaxis	3	0.023849548
BP	GO:0050863	regulation of T cell activation	3	0.023849548
BP	GO:0050862	positive regulation of T cell receptor signaling pathway	3	0.023849548

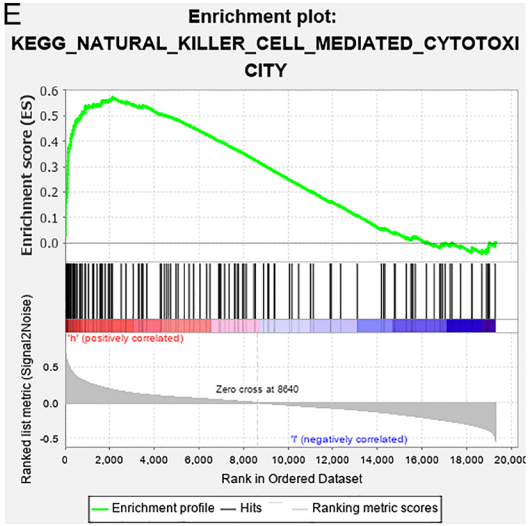
BP	GO:0045059	positive thymic T cell selection	3	0.023849548
BP	GO:0034138	toll-like receptor 3 signaling pathway	3	0.023849548
BP	GO:0002456	T cell mediated immunity	3	0.023849548
BP	GO:0009972	cytidine deamination	3	0.030040535
BP	GO:0002480	antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-independent	3	0.030040535
BP	GO:0042989	sequestering of actin monomers	3	0.036789597
BP	GO:0051639	actin filament network formation	3	0.036789597
BP	GO:0001771	immunological synapse formation	3	0.036789597
BP	GO:2000406	positive regulation of T cell migration	3	0.036789597
BP	GO:0001775	cell activation	3	0.044056196
BP	GO:0051279	regulation of release of sequestered calcium ion into cytosol	3	0.044056196
BP	GO:0043306	positive regulation of mast cell degranulation	3	0.044056196
BP	GO:0032700	negative regulation of interleukin-17 production	3	0.044056196

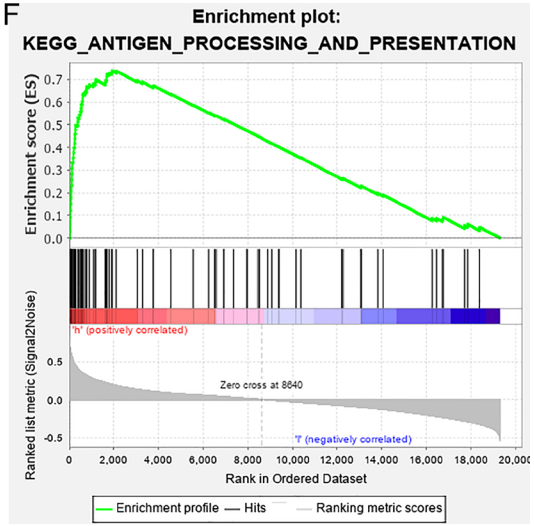

A
Enrichment plot:
KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY
Enrichment score (ES)
'h' (positively correlated)
Zero cross at 8640
't' (negatively correlated)
Ranked list metric (Signal2Noise)
Rank in Ordered Dataset
Enrichment profile — Hits — Ranking metric scores


B
Enrichment plot:
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY
Enrichment score (ES)
'h' (positively correlated)
Zero cross at 8640
't' (negatively correlated)
Ranked list metric (Signal2Noise)
Rank in Ordered Dataset
Enrichment profile — Hits — Ranking metric scores


C
Enrichment plot:
KEGG_CHEMOKINE_SIGNALING_PATHWAY
Enrichment score (ES)
'h' (positively correlated)
Zero cross at 8640
't' (negatively correlated)
Ranked list metric (Signal2Noise)
Rank in Ordered Dataset
Enrichment profile — Hits — Ranking metric scores


D
Enrichment plot: KEGG_PROTEASOME
Enrichment score (ES)
'h' (positively correlated)
Zero cross at 8640
't' (negatively correlated)
Ranked list metric (Signal2Noise)
Rank in Ordered Dataset
Enrichment profile — Hits — Ranking metric scores


E
Enrichment plot:
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY
Enrichment score (ES)
'h' (positively correlated)
Zero cross at 8640
't' (negatively correlated)
Ranked list metric (Signal2Noise)
Rank in Ordered Dataset
Enrichment profile — Hits — Ranking metric scores


F
Enrichment plot:
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION
Enrichment score (ES)
'h' (positively correlated)
Zero cross at 8640
't' (negatively correlated)
Ranked list metric (Signal2Noise)
Rank in Ordered Dataset
Enrichment profile — Hits — Ranking metric scores

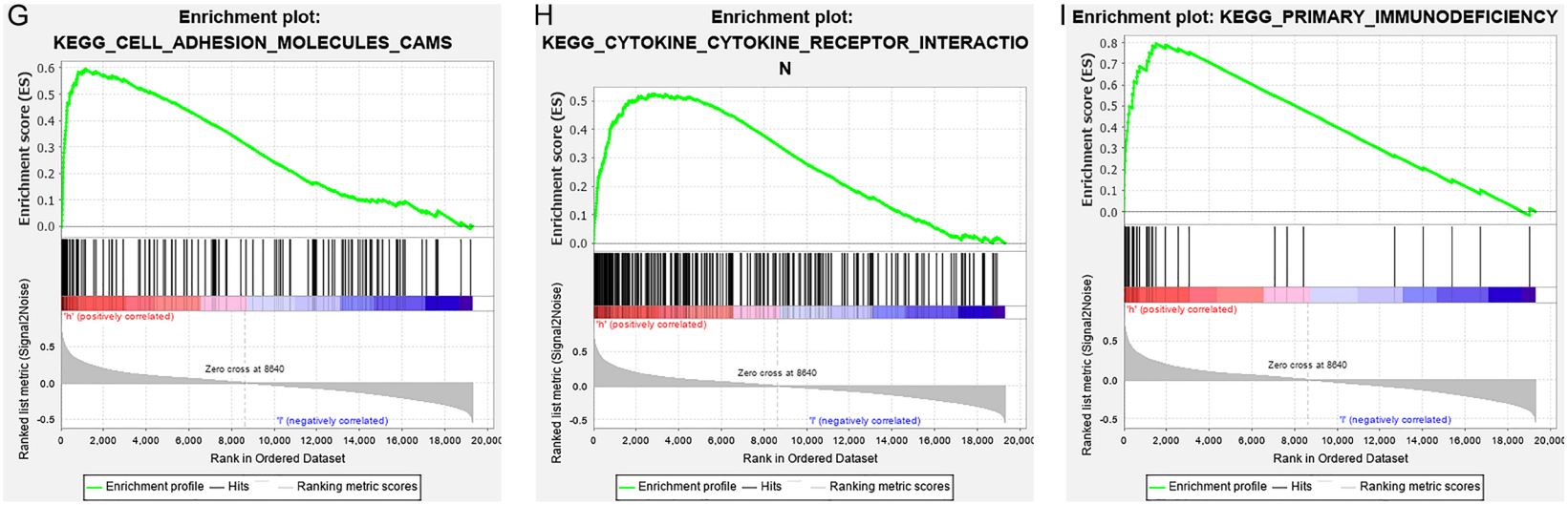


Figure S5. The GSEA showing the signaling mechanisms in the HCST overexpressed group. A. T cell receptor signaling pathway; B. B cell receptor signaling pathway; C. Chemokine signaling pathway; D. Proteasome; E. Natural killer cell mediated cytotoxicity; F. Antigen processing and presentation; G. Cell adhesion molecules CAMS; H. Cytokine-cytokine receptor interaction; I. Primary immunodeficiency.

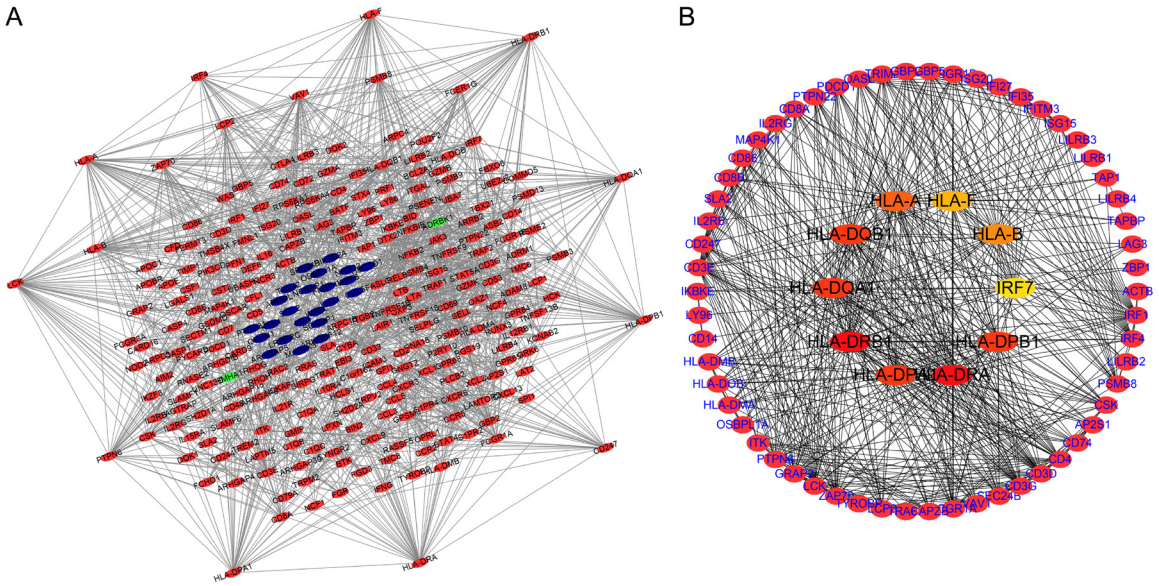


Figure S6. The PPI network of the HCST co-expressed genes. A. PPI network; B. Hub genes in the PPI network.

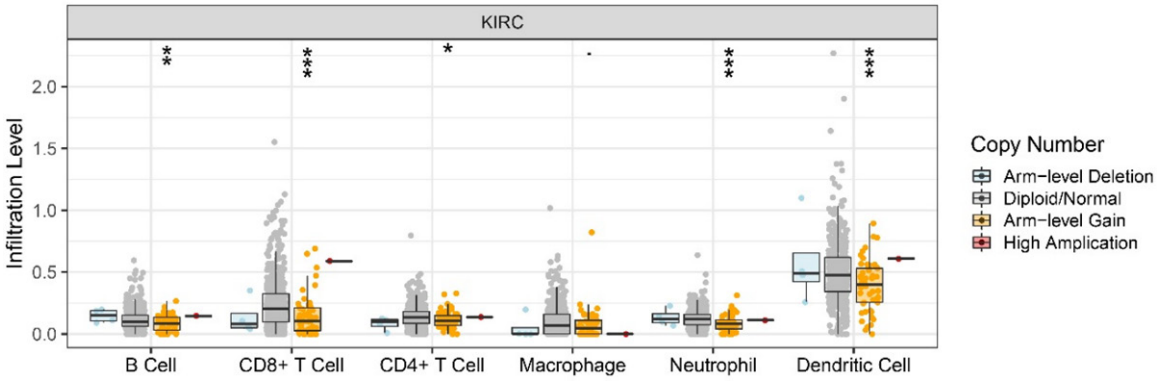


Figure S7. HCST copy number is significantly correlated with the arm-level Gain of immune infiltrating cells.

Table S3. The biological functions of HCST-related immune cell infiltration markers

Type	Term		Count	P
CC	GO:0009897	external side of plasma membrane	13	4.67E-15
BP	GO:0031295	T cell costimulation	8	2.15E-10
BP	GO:0006955	immune response	12	5.25E-10
BP	GO:0050852	T cell receptor signaling pathway	9	5.79E-10
BP	GO:0060333	interferon-gamma-mediated signaling pathway	7	6.79E-09
BP	GO:0019882	antigen processing and presentation	6	9.43E-08
BP	GO:0042102	positive regulation of T cell proliferation	6	1.47E-07
CC	GO:0009986	cell surface	10	7.83E-07
BP	GO:0032703	negative regulation of interleukin-2 production	4	1.35E-06
BP	GO:0032729	positive regulation of interferon-gamma production	5	2.42E-06
BP	GO:0042110	T cell activation	5	2.65E-06
MF	GO:0032395	MHC class II receptor activity	4	3.34E-06
BP	GO:0002504	antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	4	5.53E-06
CC	GO:0042101	T cell receptor complex	4	5.66E-06
CC	GO:0030666	endocytic vesicle membrane	5	8.47E-06
BP	GO:0032753	positive regulation of interleukin-4 production	4	9.23E-06
CC	GO:0005887	integral component of plasma membrane	13	9.89E-06
CC	GO:0042613	MHC class II protein complex	4	1.06E-05
BP	GO:0002250	adaptive immune response	6	1.30E-05
CC	GO:0005886	plasma membrane	21	1.85E-05
BP	GO:0007166	cell surface receptor signaling pathway	7	1.95E-05
MF	GO:0042605	peptide antigen binding	4	2.36E-05
CC	GO:0071556	integral component of lumenal side of endoplasmic reticulum membrane	4	2.50E-05
MF	GO:0015026	coreceptor activity	4	2.92E-05
BP	GO:0050776	regulation of immune response	6	3.17E-05
BP	GO:0007259	JAK-STAT cascade	4	3.95E-05
MF	GO:0004871	signal transducer activity	6	5.13E-05
CC	GO:0030658	transport vesicle membrane	4	5.69E-05
BP	GO:0042130	negative regulation of T cell proliferation	4	6.14E-05
CC	GO:0030669	clathrin-coated endocytic vesicle membrane	4	7.17E-05
BP	GO:0009615	response to virus	5	7.80E-05
BP	GO:0045944	positive regulation of transcription from RNA polymerase II promoter	10	1.36E-04
CC	GO:0012507	ER to Golgi transport vesicle membrane	4	1.46E-04
CC	GO:0016021	integral component of membrane	22	1.58E-04
MF	GO:0000979	RNA polymerase II core promoter sequence-specific DNA binding	4	2.03E-04
BP	GO:0006959	humoral immune response	4	2.25E-04
BP	GO:0051607	defense response to virus	5	3.71E-04
MF	GO:0003700	transcription factor activity, sequence-specific DNA binding	9	5.16E-04
CC	GO:0032588	trans-Golgi network membrane	4	5.84E-04
BP	GO:0045954	positive regulation of natural killer cell mediated cytotoxicity	3	6.32E-04
BP	GO:0019886	antigen processing and presentation of exogenous peptide antigen via MHC class II	4	9.17E-04
BP	GO:0032735	positive regulation of interleukin-12 production	3	0.001228597
BP	GO:0032689	negative regulation of interferon-gamma production	3	0.001541973
BP	GO:0032715	negative regulation of interleukin-6 production	3	0.001541973
CC	GO:0001772	immunological synapse	3	0.002045515
BP	GO:0010629	negative regulation of gene expression	4	0.002872762
BP	GO:0060557	positive regulation of vitamin D biosynthetic process	2	0.004164431
BP	GO:0002669	positive regulation of T cell anergy	2	0.004164431
BP	GO:0050853	B cell receptor signaling pathway	3	0.005642718
BP	GO:0070374	positive regulation of ERK1 and ERK2 cascade	4	0.005700556
CC	GO:0010008	endosome membrane	4	0.005745438
BP	GO:0060559	positive regulation of calcidiol 1-monooxygenase activity	2	0.006240324
BP	GO:0038161	prolactin signaling pathway	2	0.006240324
BP	GO:0000255	allantoin metabolic process	2	0.006240324

BP	GO:0006549	isoleucine metabolic process	2	0.006240324
BP	GO:0046449	creatinine metabolic process	2	0.008312013
BP	GO:0072676	lymphocyte migration	2	0.008312013
BP	GO:0006573	valine metabolic process	2	0.008312013
MF	GO:0004888	transmembrane signaling receptor activity	4	0.009001586
CC	GO:0042105	alpha-beta T cell receptor complex	2	0.009839215
MF	GO:0005134	interleukin-2 receptor binding	2	0.010031193
BP	GO:0045085	negative regulation of interleukin-2 biosynthetic process	2	0.010379507
BP	GO:0019530	taurine metabolic process	2	0.010379507
BP	GO:0051897	positive regulation of protein kinase B signaling	3	0.013220005
BP	GO:0006101	citrate metabolic process	2	0.01450194
BP	GO:0009887	organ morphogenesis	3	0.015710708
BP	GO:0000122	negative regulation of transcription from RNA polymerase II promoter	6	0.015973429
BP	GO:0002456	T cell mediated immunity	2	0.016556895
BP	GO:0048304	positive regulation of isotype switching to IgG isotypes	2	0.016556895
CC	GO:0005765	lysosomal membrane	4	0.016651093
BP	GO:0007169	transmembrane receptor protein tyrosine kinase signaling pathway	3	0.017025867
BP	GO:0045080	positive regulation of chemokine biosynthetic process	2	0.020654327
BP	GO:0006105	succinate metabolic process	2	0.020654327
BP	GO:0045893	positive regulation of transcription, DNA-templated	5	0.021591723
BP	GO:0006600	creatine metabolic process	2	0.022696818
BP	GO:0032700	negative regulation of interleukin-17 production	2	0.022696818
BP	GO:0042511	positive regulation of tyrosine phosphorylation of Stat1 protein	2	0.022696818
BP	GO:0045060	negative thymic T cell selection	2	0.022696818
BP	GO:0071222	cellular response to lipopolysaccharide	3	0.023112779
MF	GO:0016493	C-C chemokine receptor activity	2	0.023910944
BP	GO:0043065	positive regulation of apoptotic process	4	0.024244365
BP	GO:0006107	oxaloacetate metabolic process	2	0.024735172
BP	GO:0050777	negative regulation of immune response	2	0.024735172
BP	GO:0045086	positive regulation of interleukin-2 biosynthetic process	2	0.024735172
BP	GO:0050900	leukocyte migration	3	0.026647035
BP	GO:0060334	regulation of interferon-gamma-mediated signaling pathway	2	0.026769395
BP	GO:1901224	positive regulation of NIK/NF-kappaB signaling	2	0.028799497
BP	GO:0006915	apoptotic process	5	0.029433707
BP	GO:0051044	positive regulation of membrane protein ectodomain proteolysis	2	0.030825484
BP	GO:0060397	JAK-STAT cascade involved in growth hormone signaling pathway	2	0.030825484
BP	GO:0044130	negative regulation of growth of symbiont in host	2	0.032847366
BP	GO:0032722	positive regulation of chemokine production	2	0.03486515
BP	GO:0006103	2-oxoglutarate metabolic process	2	0.036878844
BP	GO:0050870	positive regulation of T cell activation	2	0.036878844
MF	GO:0042288	MHC class I protein binding	2	0.037601741
BP	GO:0045672	positive regulation of osteoclast differentiation	2	0.038888456
BP	GO:0050850	positive regulation of calcium-mediated signaling	2	0.040893995
BP	GO:0034113	heterotypic cell-cell adhesion	2	0.044892884
MF	GO:0005125	cytokine activity	3	0.048769394
BP	GO:0001819	positive regulation of cytokine production	2	0.048875575

Table S4. The signaling mechanisms of HCST-related immune cell infiltration markers

Category	Count	P
hsa05321: Inflammatory bowel disease (IBD)	12	8.64E-16
hsa04640: Hematopoietic cell lineage	10	6.25E-11
hsa05332: Graft-versus-host disease	7	4.13E-09
hsa04514: Cell adhesion molecules (CAMs)	10	5.23E-09
hsa05330: Allograft rejection	7	8.57E-09
hsa05140: Leishmaniasis	8	1.56E-08
hsa04940: Type I diabetes mellitus	7	1.90E-08
hsa04612: Antigen processing and presentation	8	2.52E-08
hsa04660: T cell receptor signaling pathway	8	1.72E-07
hsa05340: Primary immunodeficiency	6	2.83E-07
hsa05152: Tuberculosis	9	5.92E-07
hsa05166: HTLV-I infection	10	8.03E-07
hsa05323: Rheumatoid arthritis	7	1.71E-06
hsa05320: Autoimmune thyroid disease	6	2.51E-06
hsa05145: Toxoplasmosis	7	6.33E-06
hsa05310: Asthma	5	7.45E-06
hsa04630: Jak-STAT signaling pathway	7	3.09E-05
hsa05150: Staphylococcus aureus infection	5	7.99E-05
hsa05164: Influenza A	7	8.61E-05
hsa05168: Herpes simplex infection	7	1.14E-04
hsa05162: Measles	6	2.42E-04
hsa05322: Systemic lupus erythematosus	6	2.51E-04
hsa05161: Hepatitis B	6	3.62E-04
hsa04672: Intestinal immune network for IgA production	4	0.001066473
hsa05169: Epstein-Barr virus infection	5	0.00180633
hsa05416: Viral myocarditis	4	0.001868752
hsa04145: Phagosome	5	0.003828281
hsa05142: Chagas disease (American trypanosomiasis)	4	0.010134665
hsa04060: Cytokine-cytokine receptor interaction	5	0.020223895
hsa04917: Prolactin signaling pathway	3	0.03793765
hsa04062: Chemokine signaling pathway	4	0.046264684