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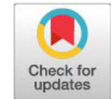
Screening and Analysis of Immune Genes Related to Disease Resistance in Tibetan Plateau Sheep



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Tibetan sheep of type plateau, Immune genes, RNA-Seq, Real-Time PCR

ABSTRACT

Total RNA was extracted from healthy spleen tissues from Tibetan sheep of type plateau and small-tailed Han sheep. After mRNA purification and segmentation, cDNA was synthesized for Illumina high-throughput sequencing. The quality of the filtered raw data was evaluated. Subsequently, the cleaned data were used for sequence alignment, KEGG enrichment analysis, differential gene cluster analysis and GO enrichment analysis to screen for disease resistance-related, immune-determinant genes. In addition, the screened differentially expressed genes were verified by Real-Time PCR. The results showed that there were 280 differentially expressed disease resistance-related and immune-related genes in the Tibetan sheep of type plateau compared with those in the small-tailed Han sheep, of which 104 genes were significantly upregulated and 176 genes were significantly downregulated. The results for the GO functional categories showed that the upregulated genes were enriched in the process of immune response, and the downregulated genes were enriched in the extracellular region. The KEGG analysis showed that genes were enriched in 153 KEGG pathways, and the upregulated genes were enriched in pathways involved in antigen processing and presentation and allogeneic rejection. Finally, the real-time PCR results showed that there were 14 differentially expressed genes, with 9 upregulated and 5 downregulated genes. The differential expression trend was consistent with the transcriptome sequencing results.

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INTRODUCTION

Tibetan sheep are one of the three original sheep breeds in China. They represent an excellent variety living in the Qinghai-Tibet Plateau, with Qinghai as the main production area (Yan *et al*., 2014). Because of the characteristics of the ecological environment, Tibetan sheep of type plateau account for the highest proportion. Tibetan sheep have a long history in China and can better adapt to cold conditions in the region after continuous breeding (Xie, 2016). Qinghai is an important animal husbandry production base in China. Due to the influence of the Qinghai Tibet Plateau, the large amount of grassland and national customs, a pattern of cultivation of herbivorous livestock has evolved. Among such livestock, sheep have become an important pillar of the animal husbandry economy of Qinghai. Qinghai Tibetan sheep are largest in number and of the best quality and have the most important cultural attributes, which allows them to have irreplaceable biological and economic characteristics. Through long-term natural selection and artificial selection, Tibetan sheep have adapted to the harsh environment of the Qinghai-Tibet Plateau and formed strong disease resistance. With the development of molecular biology and genetic e-ngineering technology, the search for disease resistance genes and genetic breeding to improve the resistance of livestock to diseases via genetic changes has been performed, and research to discover Tibetan sheep disease resi-stance genes has played an important role in the development of germplasm resources.

The immune system is generally divided into two categories, innate immunity and acquired immunity, according to the types of participating cells, the types and

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methods of identification, the forms of acquisition, and the effector mechanism (Liu *et al*., 2020). Innate immunity refers to an immune response that is mediated by innate genetic immunity. The innate immune system acts as a protective mechanism for the body to resist infection by foreign pathogens, which was gradually established during long-term germline development and evolution within the animal body in the struggle against invasive microorga-nisms (Janeway and Medzhitov, 2002). Innate immunity recognizes invading patho-gens through special pattern-recognition receptors (PRRs) and senses pathogen-associated molecular patterns (PAMPs) (McCartney and Colonna, 2009). Early genetic analysis found that higher vertebrates possess acquired immunity characterized by genes encoding RAG, MHC, TCR and BCR (Holmes and Bryceson, 2016). Eukaryotes recognize and eliminate invading pathogenic microorganisms through innate immunity and adaptive immunity. For example, dendritic cells can express the Toll-like receptors TLR7 or TLR9; among them, TLR7 can bind single-stranded RNA, and TLR9 can bind double-stranded DNA (Colonna *et al*., 2004). When stimulated by virus infection, the body can produce IFN-α, thus exerting an antiviral effect (Marshak-Rothstein and Rifikin, 2007).

Resistance is the ability of the host to tolerate pathogen infection, shorten the life cycle of the pathogen in the body, and resist the effects of the disease. Such disease resistance has long been found to be inherited stably by offspring (Hutt, 1958). The genetic variation in disease resistance exists between different species and individuals, thus forming the genetic basis for disease resistance selection (Liu, 2007). When stimulated by external factors, it can lead to resistance to disease and can produce antibodies in animals. Disease resistance can be classified as general disease resistance or special disease resistance, which have a different genetic basis. General resistance is affected by the combination of multiple genes and the environment and is not limited to a specific pathogen. Specific resistance is resistance to a specific disease or pathogen. It is mainly controlled by one gene or affected by other gene loci and environmental factors to varying degrees (Yan and Yin, 2007). Because general resistance to disease reflects the overall defence ability of the body against disease, improving the overall immune function of the body can improve general resistance to disease. However, innate immunity is particularly imp-ortant in improving general resistance (Zhao, 2013). Finding genes that help improve immunity is of great sign-ificance for studying the molecular mechanisms of disease resistance and breeding for disease resistance. Candidate genes related to immune resistance in sheep are mainly *Nramp1, MHC, TLR,* and *MBL* (Yao *et al*., 2014).

RNA-Seq has been widely used in livestock and poultry research (Wang *et al*., 2015). Meng *et al*. (2015) discovered 263 cashmere goat meat quality-related genes by RNA-Seq. Analysis showed that the candidate genes affecting the quality of cashmere goat meat were mainly *ADIPOQ, PDK*, and *CD36*. Niu (2016) used the Illumina platform to perform high-throughput sequencing of mRNA in the skin tissue of white and beaver-coloured rex rabbits, and 12,408 differentially expressed genes were found, 8 of which were involved in the cytochrome metabolism pathway. Canovas (2010) screened 33,045 SNP sites related to lactation in Holstein cattle by RNA-Seq technology. Yao (2012) scr-eened 153, 654 SNP loci in Tibetan Bangor cashmere goats and 154, 815 SNP loci in Liaoning cashmere goats by using RNA-Seq technology. Liu (2015) analysed the liver tissues of tailed and untailed Lanzhou big tail sheep by RNA-Seq technology and identified genes related to fat deposition, such as EAAT2. Geng *et al*. (2013) used RNA-seq to examine the changes in skin in the development cycle of cashmere sacs and identified 1332 differentially expressed genes. The development and growth of hair follicles may be related to the Wnt, Shh, TGF-β and Notch signalling pathways, and differentially expressed genes play an important role in these signalling pathways. Chen (2014) used RNA-Seq technology to sequence the transcriptome of the livers of normal-fed Lande goose and stuffed Lande goose, and a total of 802 differentially expressed genes were detected in the fatty liver of the stuffed geese group. Yang (2016) found six highly expressed genes related to the wolf innate immune system through RNA-seq. Therefore, this study was conducted to perform high-throughput transcriptome sequenci-ng of disease resistance-related immune genes in Tibetan sheep and the functional verification of disease resistance-related differentially expres-sed genes using real-time quantitative PCR. This study was untaken to investigate the differential expression of disease resistance-related immune genes between Tibetan sheep of type plateau and small-tailed Han sheep, so as to explore disease resistance-related immune genes. The findings described here should be highly useful for breeding highly disease-resistant Tibetan sheep.

MATERIALS AND METHODS

RNA extraction and quality inspection

Tibetan sheep of type plateau were fed in breeding field (Xining, Qinghai, China), and small-tailed Han sheep were fed in breeding field (Ledu, Qinghai, China). The spleen tissue of Tibetan sheep of type plateau and small-tailed Han sheep were collected in this experiment and stored in the spleen -80 °C until use. All the experimental

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animals were approved by the Animal Ethics Committee of Agriculture and Animal Husbandry, Qinghai University.

Total RNA from spleen tissue from Tibetan sheep of type plateau and small-tailed Han sheep was extracted using an EASY Spin Plus Tissue/Cell RNA Extraction Kit (Beijing Aidlab Biotechnologies Co., Beijing, China) as described by the manufacturer. A NanoPhotometer spectrophotometer (IMPLEN, CA, USA) and an RNA Nano 6000 Assay Kit with the Bioanalyser 2100 system (Agilent Technologies, CA, USA) were used to detect RNA integrity and purity.

RNA sequencing and data processing

Using a NEBNext® Ultra™ RNA Library Prep Kit for Illumina® to construct cDNA libraries, sequenced using an Illumina HiSeq-PE150 plat-form by Novogene Bioinformatics Technology Co. (Beijing, China). The raw reads were cleaned by removing reads with adapters, reads in which unknown bases accounted for > 10% of the sequence length, and low-quality reads. The Q20 and Q30 values were then calculated. The levels of gene expression were calculated using the reads per kb transcriptome per million mapped reads method for each sample. HT Seq software was used to analyse the differential expression in spleen tissue from different varieties and to perform the significance analysis.

Screening of DEGs

Differentially expressed genes between two samples were calculated using DEGseq software, and the P values were adjusted using the q value. The smaller the q value, the more significant the difference in gene expression was, and a q value < 0.005 was set as the threshold for significant differential expression. If the log2 fold change value was > 0, the expression of the DEG was determined to be upregulated, whereas if the log2 fold change value was < 0, the DEG was downregulated.

GO and KEGG pathway enrichment analysis of DEGs

GO enrichment analysis of the DEGs was implemented with the GOseq package, and the GO terms for which the corrected P value was < 0.05 were defined as significantly enriched. Then, KEGG pathway analysis of the DEGs was implemented by using KOBAS, and pathways with a corrected P value of ≤ 0.05 were considered to be significantly enriched among the DEGs.

Real-Time PCR analysis

A total of 14 DEGs were randomly chosen for validation by Real-Time PCR analysis. β-actin was selected as an internal control. Primer sequences are listed in (Table I). RNA was converted to first-strand cDNA by using the PrimeScript RT Reagent Kit with gDNA Eraser (Perfect Real Time) (TaKaRa, Dalian, China). The final concentration of cDNA was 35 ng. Four replicates were prepared for each gene in each Real-Time PCR assay. The reaction mixtures (25 µl) consisted of 12.5 µL SYBR Premix DimerEraser (2x), 0.75 µl of each of the forward and reverse primers, 2 µl of cDNA, and 9 µl of ddH_2O. The thermal cycler parameters consisted of an initial denaturation at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, 55 °C for 30 s, and 72 °C for 30 s, followed by melting curve analysis from 65-95 °C using a CFX96 Real Time System. The levels of relative gene expression were evaluated acc-ording to the $2^{-\triangle\triangle Ct}$ method.

Table I. Primers used for real-time PCR analysis.

Gene name	Sequence (5'-3')	Product length (bp)
S1	F 5'-AGGATCGCCCAGGTCTTCT-3' R 5'-GGGAGCCATTTCACTCATCAC-3'	93
S2	F 5'-CTCGTGGCTTCCTGTCTCATC-3' R 5'-AGTGTGAGTGTGTGTGTCTGTGTGT-3'	136
S3	F 5'-GAACAGGACAGGTGGACAGGA-3 R 5'-GCAAAGGAAAAGGGTAAATAGGATG-3'	80
S4	F 5'-TGTGCAGGGAATTAGAGTGTGG-3' R 5'-CAACAGGGCAGAGTGAAAGATG-3'	135
S5	F 5'-CTCCTCATCCTCACCATCACTTC-3' R 5'-CCTGTCTGTATCCCACCACCA-3'	136
S6	F 5'-GCAACCGAAGGCAACACA-3' R 5'-CCTCCAACAGATTCACCTCCA-3'	88
S7	F 5'-CTGTTGGGGTCTACTTCATTGCT-3' R 5'-ATTGGTCATCTTCCCGTTCCT-3	123
S8	F 5'-AAGGGCACCATCGTAAGCA-3' R 5'-GAACTCACACCCCACAGCAA-3'	254
S10	F 5'-CCACGGCATTTTCACCATC-3' R 5'-TCTCGGCATCTGGGTTTTCT-3'	131
X1	F 5'-GCCCCTACACAGCAACGACT-3' R 5'-CCACCTCCTCTGCGTTCTTC-3'	194
X2	F 5'-GTGAAGACACCGCCTACAGC-3' R 5'-AGTGGAGAAGGGGAGGGAGA-3'	101
X5	F 5'-AGCGAGGTGTCCCAGAATAGA-3' R 5'-GAAACTGAAAATGAGGAAGGTGAAG-3'	121
X6	F 5'-CAAGTGAATCCCCGTGCTCT-3' R 5'-CTTCTTGTCCTGCTGTTCCTTGT-3'	103
X7	F 5'-TTGCATCTTGAAAAGCAGAACC-3' R 5'-GATGAGGGCGAAGGAGAAGA-3'	135

RESULTS

Differential expression analysis

The quality assessment results for the sequencing data are shown in Table II, which show a low error rate and the high-quality of the sequences. RNA sequencing analysis identified 280 genes that were significantly differentially expressed in the two types of Tibetan sheep (Fig. 1). Among them, the Tibetan sheep of type plateau showed the

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upregulation of 104 genes and the downregulation of 176 genes. Partic-ularly, in the Tibetan sheep of type plateau, genes encoding protein phosphatase and tumour necrosis factor were upregulated, while genes encoding ABC (ATP-binding cassette) were downregulated.

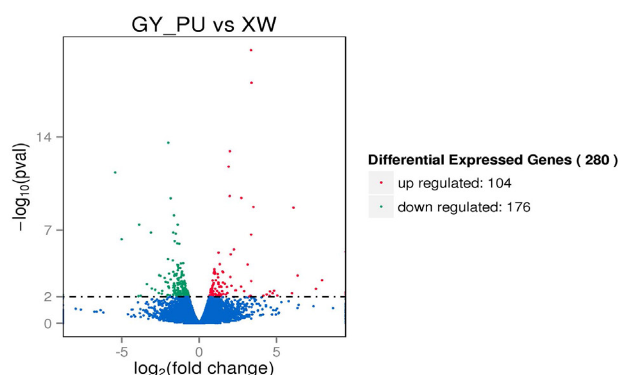

Fig. 1. Volcano plot of the differentially expressed genes. The significantly upregulated genes are marked with red dots in the figure, the significantly downregulated genes are marked with green dots in the figure, and the genes without significant differences are marked with blue dots in the figure.

Table II. Quality assessment of sample sequencing data.

Sample name	Raw reads	Clean reads	Error rate (%)	Q20 (%)	Q30 (%)	GC (%)
XW_1	50342510	45934242	0.02	96.00	89.54	52.90
XW_3	54268618	52869964	0.03	95.08	88.17	51.41
XW_5	50150542	48654756	0.03	94.87	87.76	52.95
GY_1	49675934	48298364	0.03	94.82	87.66	53.05
GY_2	45582454	41432428	0.02	96.00	89.51	51.94
GY_3	48984450	47508206	0.03	95.05	88.04	51.83

GO enrichment analysis

To better understand the functional differences in gene expression, the biological functions of the DEGs were characterized by GO enrichment analysis. The upregulated DEGs were classified according to 30 significantly enriched GO terms, including 9 involved in biological process, 19 involved in molecular function, and two involved in cellular components (Figs. 2, 3 and Supplementary Fig. 1). In the biological process category, the significantly enriched GO entries included cardiovascular system development, antigen processing and presentation and response to acid chemicals. The cellular component categories included extrac-ellular region (partial), extracellular region, extracellular space and plasma membrane (partial). No GO terms were significantly enriched for the upregulated DEGs in the molecular function category (Fig. 2). The upregulated DEGs were generally related to the "immune response" and "immune system process" categories (Fig. 3), and the downregulated DEGs were involved with the extracellular region, extrac-ellular region (partial), extracellular matrix, response to organic substance, and other categories (Supplementary Fig. 1).

KEGG pathway enrichment analysis

We also conducted KEGG pathway enrichment analysis of the DEGs to further characterize their biological functions, which were measured in terms of the enrichment, q value, and the number of genes enriched for a particular pathway. The 20 most significant pathways are displayed in the scatter diagram (Fig. 4). Among them, the pathways showing upregulated gene enrichment were involved in antigen processing and presentation (Supplementary Fig. 2), and allograft rejection (Supplementary Fig. 3), and T cell receptor signalling pathway (Supplementary Fig. 4), and B cell receptor signalling pathway (Supplementary Fig. 5), and autoimmune diseases. Ten differentially expressed genes involved in the antigen processing and presentation pathway were upregulated.

Real-Time PCR validation of RNA sequencing

To verify the RNA sequencing results, Real-Time PCR analysis was performed using 14 randomly selected DEGs. After standardizing the exp-ression level of each gene relative to that of β-actin, the gene expression ratios was calculated. The results showed that nine genes were upregulated and five genes were downregulated (Fig. 5), and the expression differences were consistent with the transcriptome sequencing results.

DISCUSSION

In this study, GO functional analysis of differentially expressed genes in Tibetan sheep of type plateau spleen and small-tailed Han sheep spleen showed that most differentially expressed genes were annotated in the cellular component category for 19 components, including the extracellular matrix, extracellular region, extracellular space, plasma membrane (partial), MHC protein complex, and T cell receptor complex. This illustrates that the spleen interacts with ligands/receptors during functioning. Macrophages perform cell migration during the functioning of the spleen and can engulf bacteria, self-aging and apoptotic substances in circulating blood. They can also respond to blood-borne antigens and synthesize and secrete bioactive substances outside of cells, such as complement, IFN, and erythropoietin, and perform phagocytosis (Zhao, 2013).

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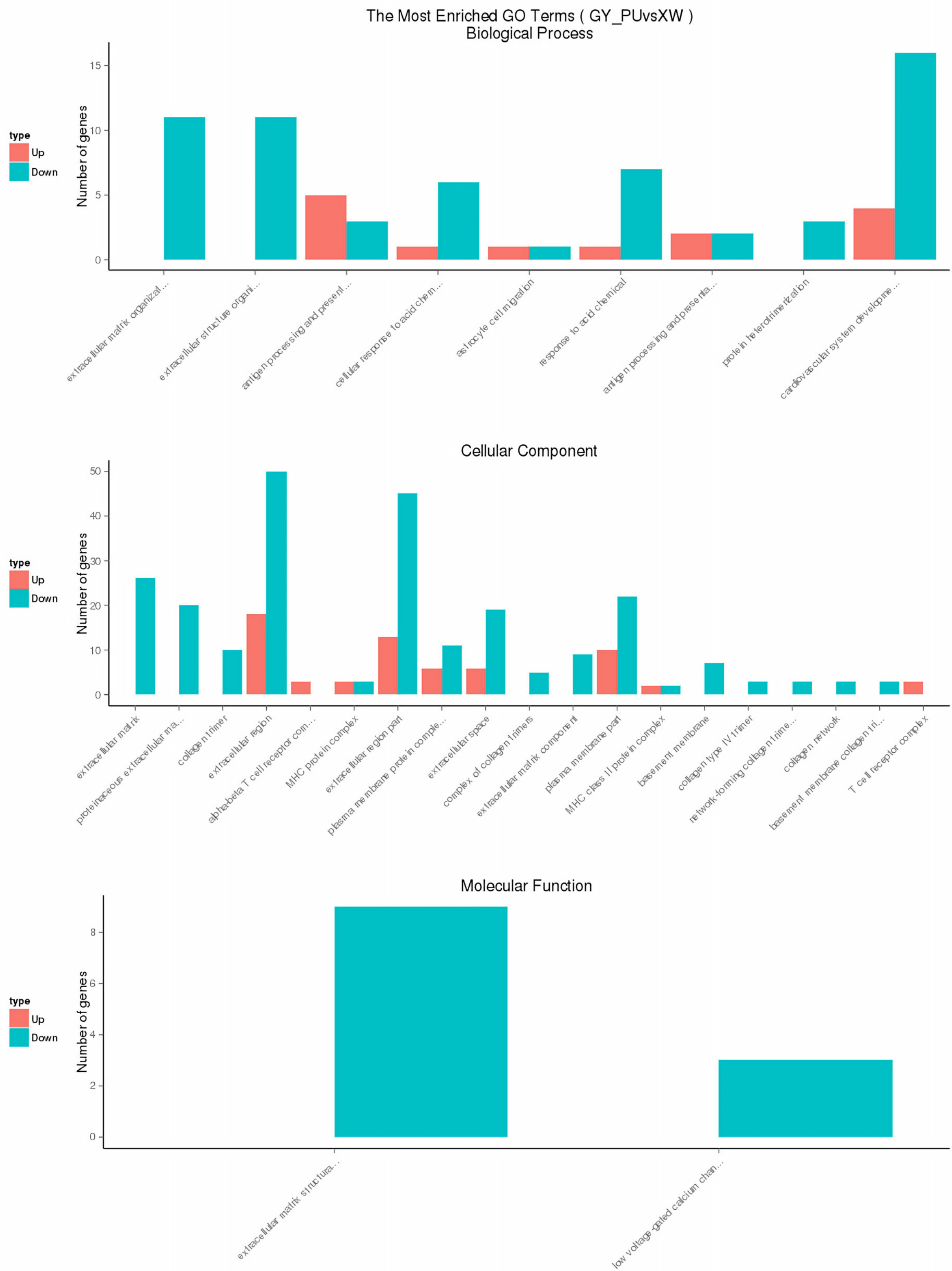

Fig. 2. Enriched GO terms of upregulated and downregulated DEGs. A represents biological processes, B represents cellular components, and C represents molecular functions.

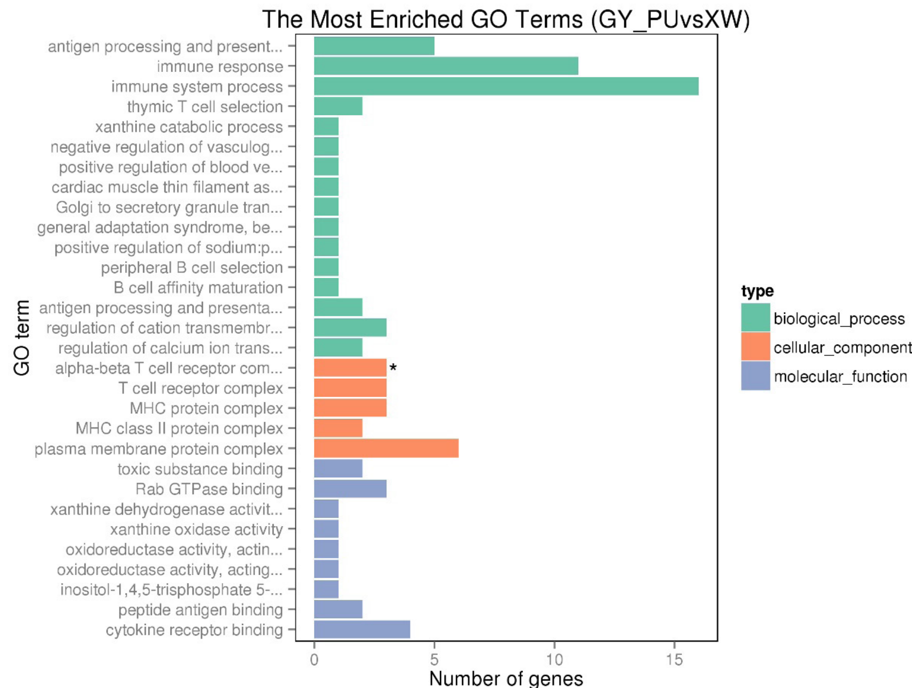

Fig. 3. Enriched GO terms of upregulated DEGs. Different colors are used to distinguish biological process, cell components and molecular functions, significant enrichment of GO term is indicated by "*".

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KEGG enrichment analysis of differentially expressed genes in Tibetan sheep of type plateau spleen and small-tailed Han sheep spleen showed that the pathways with more differentially expressed genes were involved in Graft-versus-host disease (GVHD), antigen processing and presentation, allograft rejection, viral myocarditis, TCR, and protein digestion and absorption. Most of the differentially expressed genes that were enriched in these pathways were upregulated genes, which can explain why Tibetan sheep of type plateau have higher disease resistance than small-tailed sheep.

The spleen, as an absolutely essential peripheral immune organ, is the base for the body to respond to antigens and produce immune effectors. Lymphocytes are the basic components of the immune system and are widely distributed throughout the body. T-lymphocytes and B-lymphocytes are stimulated and divide, proliferate, and undergo specific immune responses by antigen stimulation. Among them, the number of mature B lymphocytes is the largest; thus, the spleen mainly mediates a specific B lymphocyte immune response. When the pathogen enters the bloodstream and flows through the spleen, memory B cells are activated by antigen stimulation and return to the bone marrow through lymph fluid or blood to differentiate into mature plasma cells. A large number of antibodies (mainly IgG) produced in the peripheral immune organs are released into the blood circulation. Disease resistance-related differentially expressed immune genes in Tibetan sheep of type plateau and small-tailed Han sheep may be involved in B cell receptor (BCR) and T cell receptor (TCR) activity. BCR plays a key role in the development and maintenance of B cells and regulates the expression of immune genes. BCR is composed of membrane-bound imm-unoglobulin (mIg) and disulfide-linked mIgα/mIgβ (CD79a/CD79B) heterodimers and is one of the key indicators used for B cell characterization (Brezski and Monroe, 2008), The mIg of BCR can recognize antigens and bind antigens. In addition, both mIg α and mIg β intracellular regions have an immunoreceptor tyrosine-based activation motif (ITAM), which can transmit the received antigen signals downstream of the cell, so they play an important role in the immune response of B cells (Zeng, 2015). CD19 is a B cell surface marker molecule. Generally, CD19 is believed to form Cr2 with CD21, CD81 and CD225, and it is one of the positive regulators of BCR signalling (Wang, 2007). Its extracellu-lar region can bind to C3d attached to an antigen or antigen-antibody complex, thereby cross-linking BCR with the aforementioned coreceptors and transmitting signals into the cell via CD19, activating PI3K, and thus initiating the PI3K/Akt signalling pathway cascade reaction (Zhang *et al*., 2012).

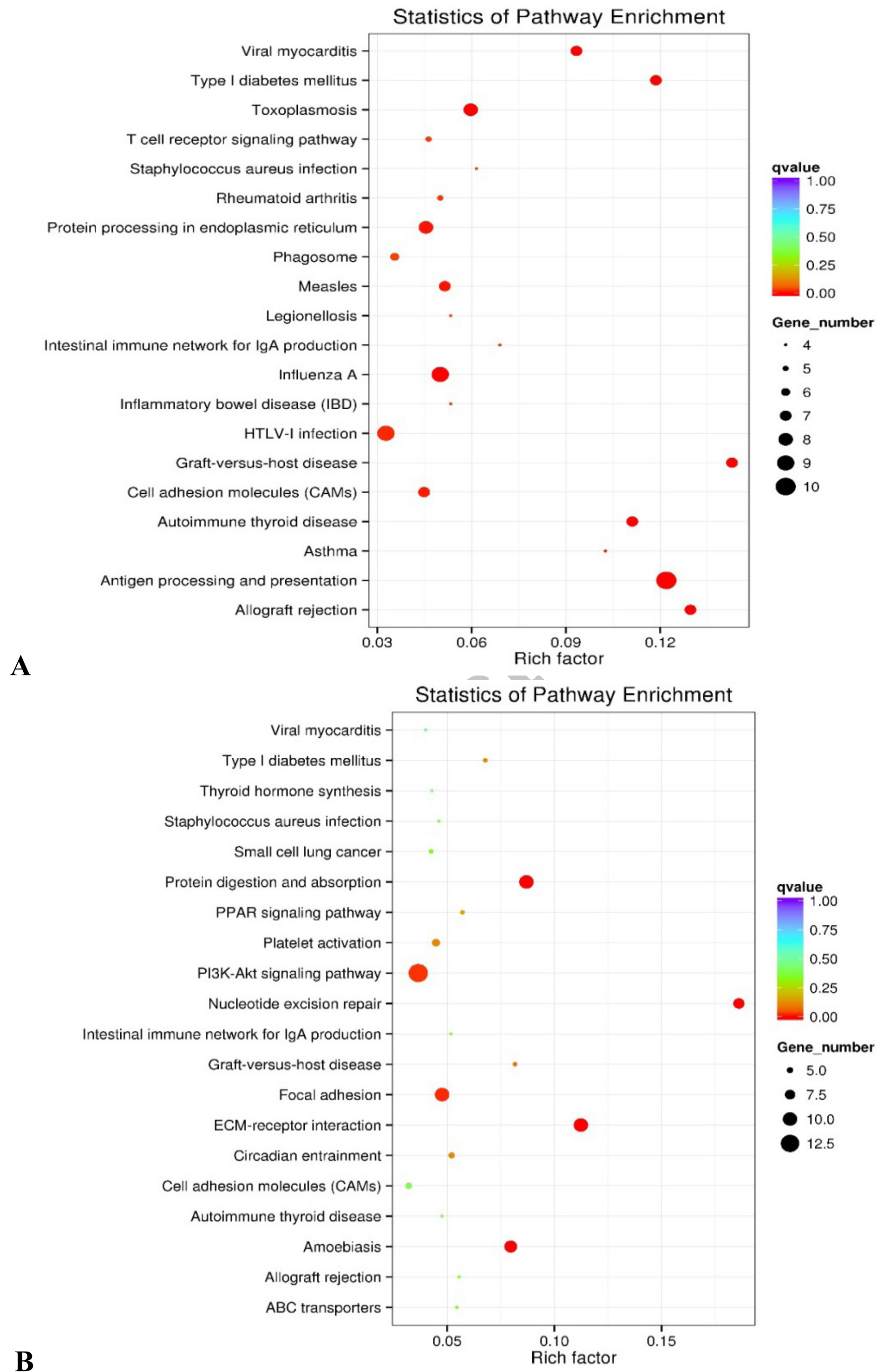

Fig. 4. Scatter diagram of upregulated (A) and downregulated (B) genes involvement pathway. The dots in the figure represent the number of differentially expressed genes in the pathway, and the colours of the dots represent various Q value boundaries. When the Q value < 0.05, the gene is significantly enriched. The enrichment factor refers to the ratio of the number of differentially expressed genes enriched in the pathway to the number of all genes. The smaller the enrichment factor, the smaller the degree of enrichment.

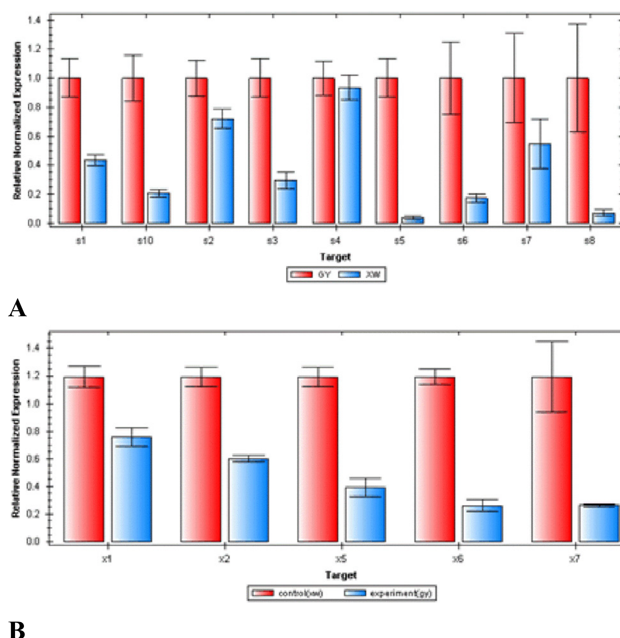

Fig. 5. Relative expression of upregulated (A) and downregulated (B) genes measured by real-time PCR.

The KEGG analysis results indicate that TCR signalling pathways are significantly enriched. T cells are the main functional cells in the acquired immune system and are responsible for recognizing antigens and assisting or inhibiting the production of antibodies by B cells. They can respond to specific antigens and mitogens and produce cytokines. TCR plays a key role in antigen recognition. The main function of TCR is to activate T cells after recognizing specific antigens (Yuan *et al*., 2006). The signalling of T cells upon activation is mediated by T cell surface antigen recognition antibodies, and foreign signals are transmitted to protein tyrosine kinases (PTKs) through receptors and related proteins. PTKs are key signal transduction molecules involved in intracellular signal transduction. The results show that the phosphorylation and dephosphorylation of proteins by CD45 are important for the process of transmembrane signalling and the activation of T cells (Mo *et al*., 2020). When the complex specifically recognizes and binds to the receptor, the receptor inputs a signal to the T cell to change the conformation of the surface receptor. This can enhance adhesion to macrophages and prolong contact between macrophages and T cells, thereby inducing antigen-specific T cell activation and proliferation. To increase the sensitivity of T cells to antigenic peptides, CD4/CD8 molecules can greatly enhance the affinity of T cells and macrophages (Yan *et al*., 2015). However, T cells can only recognize antigenic peptides processed by APC and MHC molecules (Tang, 2004). After the presentation of MHC molecules, the exogenous antigen interacts with TCR and triggers a T cell-mediated immune response, thereby eliminating invading pathogens (2016). In addition, TCR, under the action of serine/threonine kinases and their products, regulates the metabolism of T cells, thereby addressing the metabolic needs of T cells participating in the immune response (Navarro and Cantrell, 2012).

Innate immunity is the basis of the immune response, which is present in living organisms and can be passed on to offspring. Antigen presentation is a core link in the immune response process (Zhou, 2013). In the immune response, macrophages are important antigen-presenting cells that present antigens to immune-active cells and provide systemic stimulation, neutro-philic granulocytes and macrophages allow the pattern recognition receptors on the cell surface to recognize the molecules specific to pathogenic microorganisms, initiate the phagocytosis process, and mediate phagocytosis. The proteasome subunit induced by IFN-γ stimulates antigen presentation, can induce LMP2, LMP7 and MECL-1 expression, and stimulates the pres-entation of class I antigens (Fruh and Yang, 1999). LMP2, LMP7 and TAP are encoded in the MHC gene region. IFN-γ can induce the expression of various genes related to the processing and presentation of MHC-I molecules, so IFN-γ upregulates the expression of MHC-I molecules. According to the surface molecules of mature T cells, T cells can be divided into $CD4^+$ T cells and $CD8^+$ T cells (Fu *et al*., 2013). $CD8^+$ T cells have MHC-I class restriction, and $CD4^+$ T cells have MHC-II class restriction. MHC-II molecules are synth-esized in the endoplasmic reticulum and only exist in cells such as APC (Baldwin *et al*., 2004). Exogenous antigens internalized by phagocytosis are mainly presented to $CD4^+$ T cells through MHC-II molecules, while a small proportion of cytosolic antigens derived from autophagy also bind to MHC-II (Crotzer and Blum, 2009). Most of the antigens involved in the presentation of MHC-I molecules are endogenously synthesized protein molecules that exist in the cytoplasm (and nucleus). Most endogenous antigens and some exogenous antigens taken up by cells are presented by MHC-I molecules through proteasome and TAP-dependent mechanisms (Wang *et al*., 2006).

The KEGG analysis showed the upregulation of immune rejectionrelated genes in Tibetan sheep of type plateau. Billingham (Billingham, 1966) proposed that transplant immune rejection is an immune response between donor T cells and recipient tissues. The key to its occurrence is the reaction of donor T cells, and donor immunoreactive cells can recognize different histoco-mpatible antigens (Wang *et al*., 2018). Mature T cells are the main immunolo-gically active

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cells involved in the occurrence of GVHD. Transplant immune rejection is thought to be related to the imbalance of cytokines secreted by the Th1 and Th2 subpopulations (Nikolic *et al*., 2000), and GVHD is closely related to Th1 cytokines, such as IL-12, IFN-γ, and Th2 cytokines, such as IL-4 and IL-10 (Levine, 2011). When pathogenic microorganisms or other foreign antigens enter the organism, they are first phagocytized and cleared by macropha-ges; macrophages can synthesize and secrete a variety of active substances (Zheng *et al*., 2016), including IL-1, IL-6, many enzymes (lysozyme, collagenase, etc.), GM-CSF, G-CSF, IFN-α, IFN-β, and TNF-α. Therefore, the expression and activity of AGVH, IL-1, IL-2, IL-6 and TNF-α serve an important function.

In conclusion, this study revealed that the disease resistance-related immune genes of plateau Tibetan sheep were significantly enriched in the immune response pathway, which can explain the higher disease resistance of Tibetan sheep of type plateau. Antigen processing and presentation is a significantly enriched pathway. In addition, cytokines secreted by mac-rophages play an important role in anti-implantation immune rejection.

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Ethics statement and IRB approval

All the experimental animals were approved by the Animal Ethics Committee of Agriculture and Animal Husbandry, Qinghai University.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20200428000435

Statement of conflict of interest

The authors have declared no conflict of interest.

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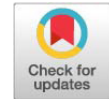

Supplementary Material

Screening and Analysis of Immune Genes Related to Disease Resistance in Tibetan Sheep of Type Plateau by RNA-Seq

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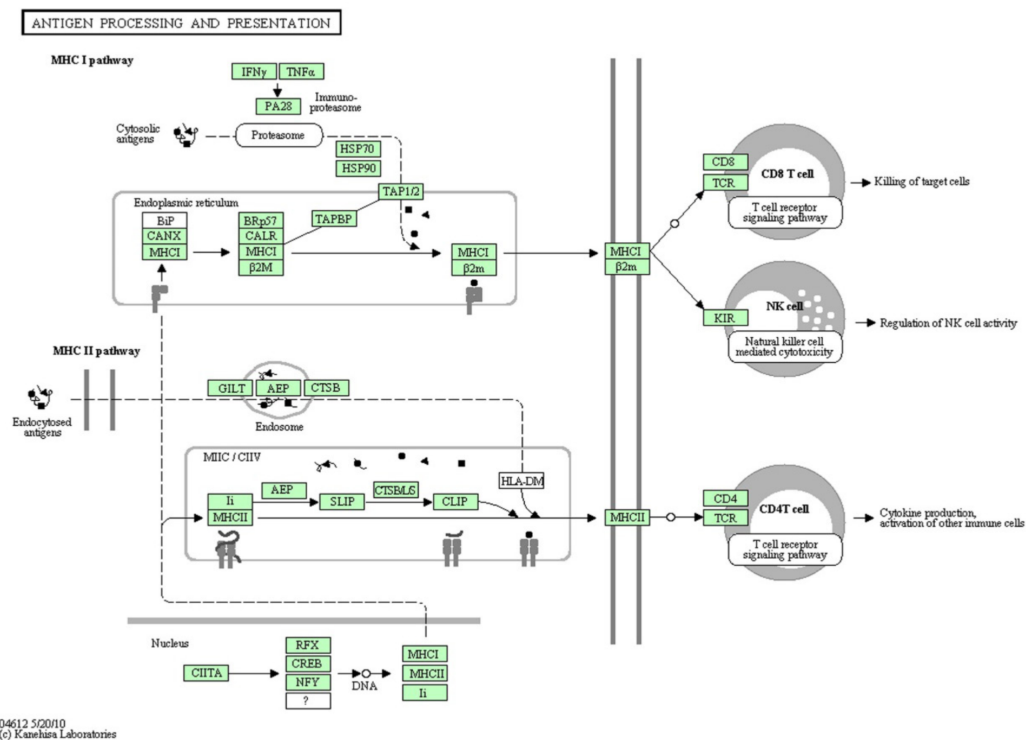

Supplementary Fig. 1. Enriched GO terms of downregulated DEGs. Different colors are used to distinguish biological process, cell components and molecular functions, significant enrichment of GOterm is indicated by "*".

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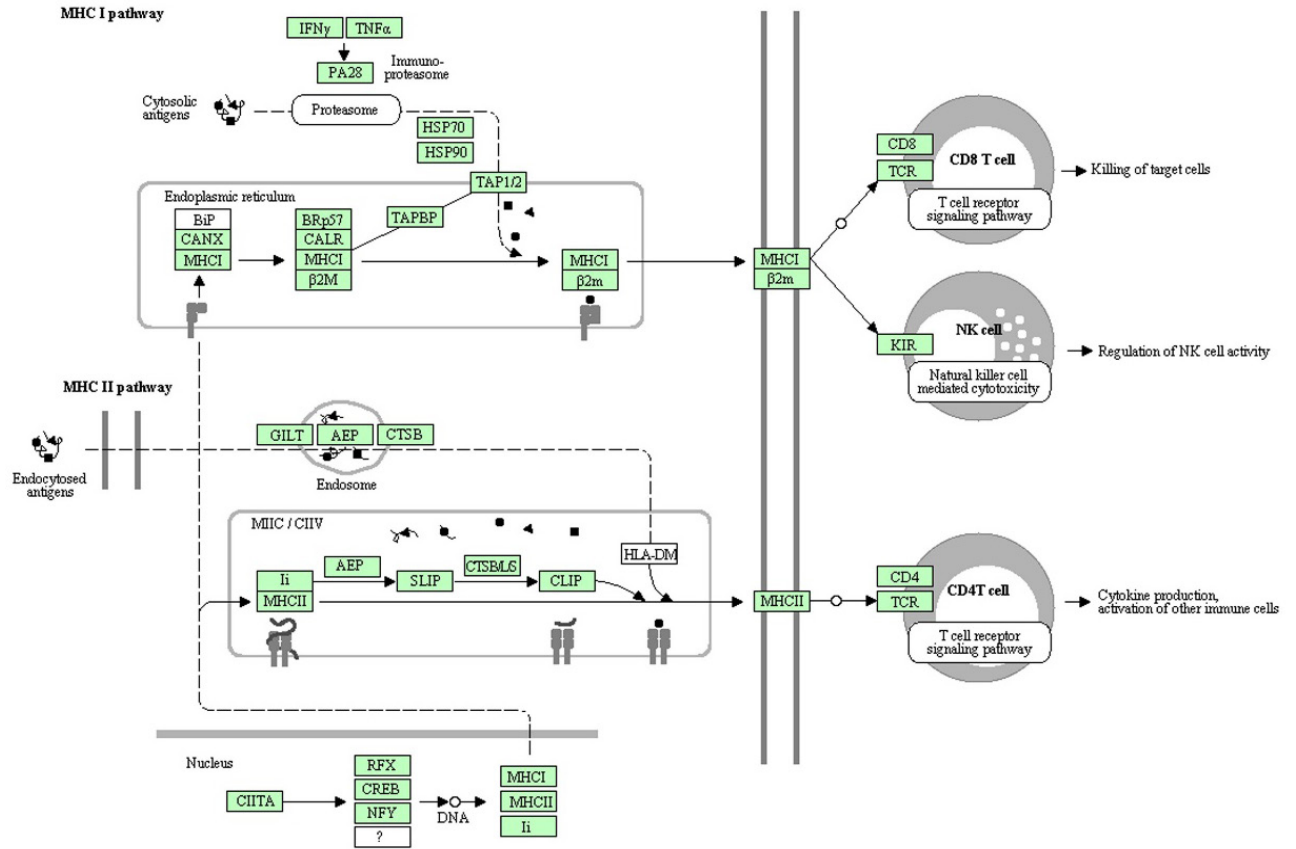
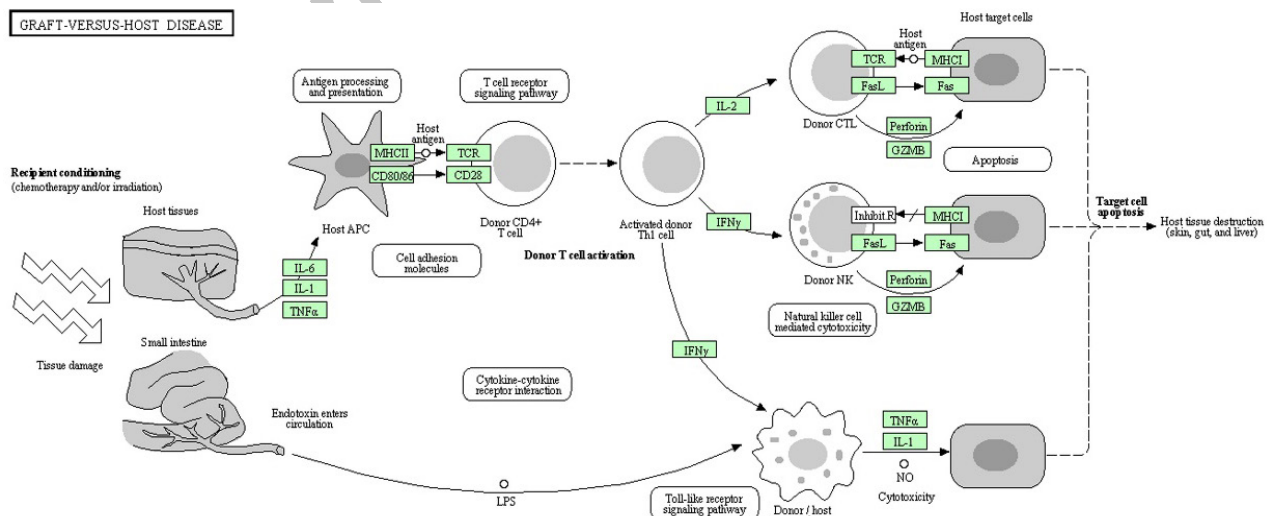
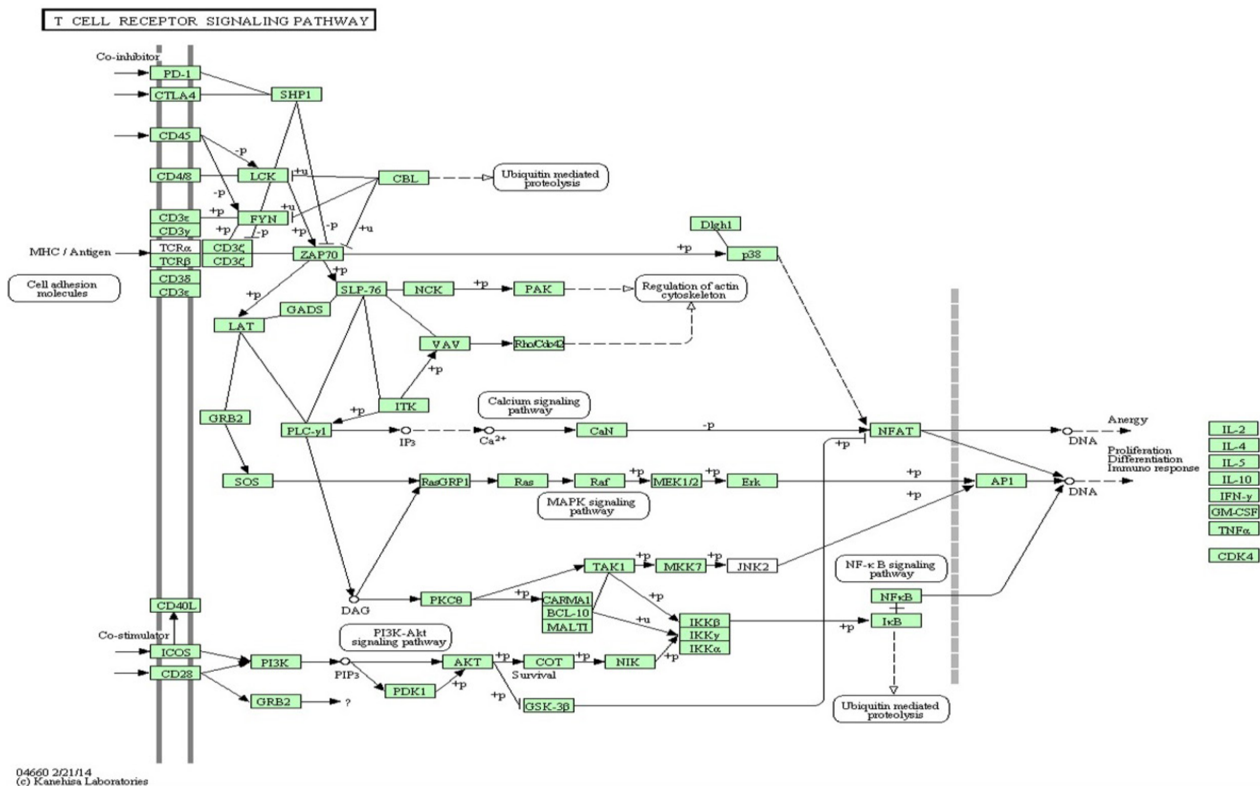


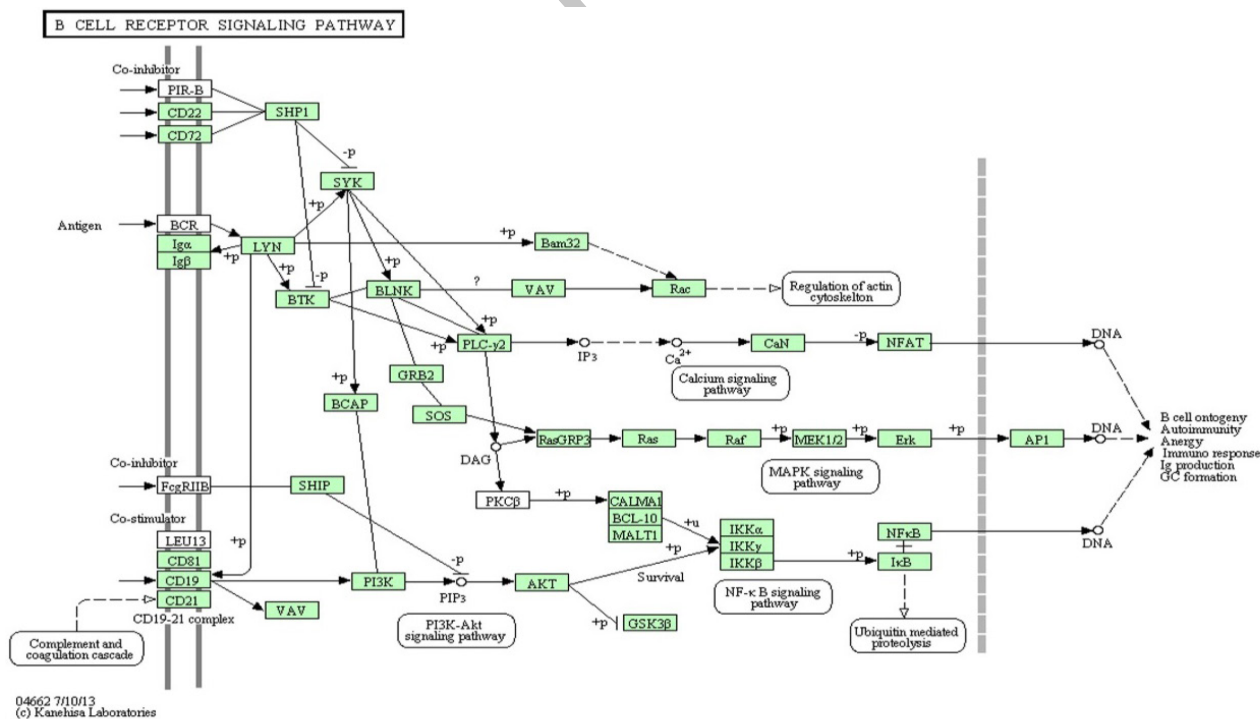
Supplementary Fig. 2. Antigen processing and presentation pathway.



Supplementary Fig. 3. Graft-versus-host-disease pathway.



Supplementary Fig. 4. T cell receptor signalling pathway.



Supplementary Fig. 5. B cell receptor signalling pathway.