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# Mitophagy and immune infiltration in vitiligo: evidence from bioinformatics analysis

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**Background:** Vitiligo is an acquired, autoimmune, depigmented skin disease with unclear pathogenesis. Mitochondrial dysfunction contributes significantly to vitiligo, and mitophagy is vital for removing damaged mitochondria. Herein, using bioinformatic analysis, we sought to determine the possible role of mitophagy-associated genes in vitiligo and immune infiltration.

**Methods:** Microarrays GSE53146 and GSE75819 were used to identify differentially expressed genes (DEGs) in vitiligo. By crossing vitiligo DEGs with mitophagy-related genes, the mitophagy-related DEGs were identified. Functional enrichment and protein-protein intersection (PPI) analyses were conducted. Then, the hub genes were identified using two machine algorithms, and receiver operating characteristic (ROC) curves were generated. Next, the immune infiltration and its connection with hub genes in vitiligo were investigated. Finally, the Regnetwork database and NetworkAnalyst were used to predict the upstream transcriptional factors (TFs), microRNAs (miRNAs), and the protein-compound network.

**Results:** A total of 24 mitophagy-related genes were screened. Then, five mitophagy hub genes (*GABARAPL2*, *SP1*, *USP8*, *RELA*, and *TBC1D17*) were identified using two machine learning algorithms, and these genes showed high diagnostic specificity for vitiligo. The PPI network showed that hub genes interacted with each other. The mRNA expression levels of five hub genes were validated in vitiligo lesions by qRT-PCR and were compatible with the bioinformatic results. Compared with controls, the abundance of activated CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, immature dendritic cells and B cells, myeloid-derived suppressor cells (MDSCs), gamma delta T cells, mast cells, regulatory T cells (Tregs), and T helper 2 (Th2) cells was higher. However, the abundance of CD56 bright natural killer (NK) cells, monocytes, and NK cells was lower. Correlation analysis revealed a link between hub genes and immune infiltration. Meanwhile, we predicted the upstream TFs and miRNAs and the target compounds of hub genes.

**Conclusion:** Five hub mitophagy-related genes were identified and correlated with immune infiltration in vitiligo. These findings suggested that mitophagy may promote the development of vitiligo by activating immune infiltration. Our study might enhance our comprehension of the pathogenic mechanism of vitiligo and offer a treatment option for vitiligo.

#### KEYWORDS

immune infiltration, vitiligo, biomarkers, bioinformatics analysis, LASSO, random forest, mitophagy

## 1 Introduction

Vitiligo is a common, acquired, autoimmune depigmented skin disorder that manifests as skin patches of depigmentation that can be distributed in every body part (1). It is estimated to affect 0.5–2% of the global population, with no difference in prevalence observed between childhood, adolescence, and adulthood (2, 3). Vitiligo is characterized by a consistent and selective loss of epidermal melanocytes (MCs) as the disease progresses (4, 5). Several factors have been proposed to cause MCs' mortality, including genetic inheritance, environmental variables, oxidative stress, inflammation, immunological responses, and intrinsic abnormalities of MCs and keratinocytes (KCs) (6, 7). Despite emerging evidence providing considerable mechanistic insight into this condition, the exact mechanism of MCs' destruction is still being debated. As vitiligo has unclear pathogenesis and an unsatisfactory response to treatment, it is necessary to explore the mechanism of vitiligo to develop effective target treatments.

Reactive oxygen species (ROS) may be produced in response to both external and endogenous stimuli, and the mitochondria are the primary locations for the production of cellular ROS (8). Mitochondrial damage may result in the overproduction of ROS, which contributes to oxidative stress, cell injury, and cell death (9, 10). Oxidative stress-induced immunological responses were considered a primary contributor to vitiligo (2, 10). And mitochondrial damage driven by oxidative stress is a significant factor in the death of MCs (11, 12). Previous studies have reported that ultrastructural and functional mitochondrial aberrations and increased oxidative byproducts were also found in MCs and KCs from perilesional vitiligo skin or lesions (9).

Mitophagy is a biochemical mechanism that protects cells. After the damage overwhelms the mitochondria's quality control mechanisms, mitophagy will preferentially remove the damaged mitochondria (13). A previous study reported the link between mitophagy and vitiligo. Gao-Zhong Ding et al. discovered that, compared to healthy controls and stable vitiligo, mitochondrial autophagosomes vanished in active vitiligo, implying that impaired mitophagy may contribute to the progression of vitiligo (14). In addition, mitophagy is also involved in the regulation of the immunological reaction. Mitophagy may exert anti-inflammatory effects by inhibiting excessive interleukin (IL)-1 $\beta$  and IL-18 production (15). Impaired mitophagy activates inflammation by activating the pyrin domain-containing protein 3 (NLRP3) inflammasome to oversecrete IL-1 $\beta$  and IL-18 (16, 17).

Furthermore, released mitochondrial DNA may increase the transcription of multiple inflammatory cytokines, such as tumor necrosis factor (TNF- $\alpha$ ) and IL-6 (18). When blocking autophagy, the released IL-1 $\beta$  could stimulate the secretion of IL-17 and IL-23 (15). And these inflammatory factors, such as IL-1 $\beta$ , NLRP3, TNF- $\alpha$ , IL-6, IL-23, and IL-17, play a vital role in the progression of vitiligo (19). Taken together, we aimed to reveal the correlation between mitophagy, vitiligo, and immune infiltration by analyzing the transcriptomics of mitophagy-associated genes in vitiligo.

Herein, we used GSE53146 and GSE75819 from the Gene Expression Omnibus (GEO) database to create a new microarray dataset to search for differentially expressed genes (DEGs) in vitiligo. The DEGs in vitiligo were then crossed with mitophagy-related genes to find mitophagy-related DEGs. To uncover the possible functions and enriched pathways, the DEGs in vitiligo and mitophagy-related DEGs were subjected to gene ontology (GO), the Kyoto Encyclopedia of Genes and Genomes (KEGG), and protein-protein interaction (PPI) network analyses. After that, machine learning methods were used to scan for mitophagy-related hub genes in vitiligo. Furthermore, the expression levels of mitophagy-related hub genes in vitiligo patients and healthy individuals were confirmed. In addition, we examined the connection between mitophagy-related hub genes and immune infiltration for a better understanding of the vitiligo immunity process. Finally, we constructed a transcriptional factor (TF)-micro-RNA (miRNA) regulatory network and a protein-compound network of hub genes, which will be helpful in future research on the regulatory mechanisms of these hub genes.

## 2 Materials and methods

### 2.1 Microarray data and mitophagy-related genes dataset

The raw gene expression data in vitiligo and controls were gathered from datasets GSE53146 and GSE75819 of the GEO database. The dataset GSE53146 included five samples from vitiligo patients and five from healthy individuals. The microarray GSE75819, detected by GPL6884, included 30 skin samples from 15 vitiligo patients' lesional and non-lesional skin samples. Mitophagy-related genes were obtained from the KEGG database, hsa04137, which contains 72 genes (<https://www.kegg.jp/pathway/hsa04137>).

## 2.2 Collection of skin samples

The ethical approval of this study was achieved by the Ethics Committee of the Hospital for Skin Disease, Institute of Dermatology, Chinese Academy of Medical Sciences, and Peking Union Medical College (2022-KY-006). Skin tissue samples from lesions of six vitiligo patients and skin tissue samples from six healthy controls were obtained from the Hospital for Skin Disease, Institute of Dermatology, Chinese Academy of Medical Sciences, and Peking Union Medical College. And the skin samples from participants were obtained after written informed consent was signed. Skin tissue from the upper limbs was taken from controls, and the lesions from upper limbs were taken from vitiligo patients. The skin tissue samples were then preserved in liquid nitrogen for subsequent investigation.

## 2.3 RNA extraction and real-time polymerase chain reaction

Total RNA was extracted from skin tissue samples using the TRIZOL reagent (15596026, Invitrogen). The cDNA was transcribed using the HiScript III RT SuperMix for qPCR kit (+gDNA wiper) procedure (R323-01, Vazyme, Nanjing, China). The SYBR qPCR kit procedure was used for the PCR system (Q711, Vazyme, Nanjing, China). Primer sequences used in this study are listed in [Supplementary Table S1](#). The LightCycler 480 equipment was utilized to examine the mRNA transcription of mitophagy-related hub genes (Roche, Basel, Switzerland). The  $2^{-\Delta\Delta C_t}$  approach was adopted to investigate differences in the relative transcriptional level of mitophagy-related hub genes compared to  $\beta$ -actin.

## 2.4 Screening of DEGs in vitiligo

The expression profiling of GSE53146 and GSE75819 was merged into a new microarray set, the batch effects were removed using the R packages “limma” and “sva,” and homogenization was implemented using the packages “preprocessCore”. Principal component analysis (PCA) plots were drawn employing the programs “FactoMineR” and “Factoextra.” After that, DEGs of skin tissue samples from vitiligo lesions and controls were screened using R software’s “limma” package. Genes in lines with an adjusted  $P < 0.05$  and  $|\log_{2}FC| > 0.5$  were considered DEGs. After that, the programs “heatmap” and “ggplot2” of the R language were applied to create heat maps, volcano maps and box line plots.

## 2.5 Analyses of DEGs’ function

To look into the DEGs function in vitiligo and mitophagy, GO and KEGG analyses were done by the program “clusterProfiler”. A  $P$  value below 0.05 was of statistical significance.

## 2.6 PPI analysis of mitophagy-related DEGs

The PPI network was established using the String and Genemania databases for evaluating the interactions between the 24 differentially expressed mitophagy-related genes.

## 2.7 Identification and ROC curves of hub genes

Two machine learning algorithms were applied for screening the hub genes: the least absolute shrinkage and selection operator (LASSO) and random forest. The LASSO algorithm was carried out *via* the program “glmnet.” The random forest algorithm was conducted by the software package “randomForest,” and the top ten genes were listed. Mitophagy-related hub genes were confirmed by intersecting the genes screened by two methods. Correlation analyses of five mitophagy-related hub genes were conducted by the package “Circle”. The ROC curve analysis of hub genes conducted by the package “pROC” was employed to analyze the specificity and diagnostic value of hub genes to vitiligo. The AUC was quantified, with AUCs  $> 0.6$  having statistical significance.

## 2.8 Immune infiltration analysis

The infiltration of immune cells in each skin sample in microarray datasets was quantified using single-sample gene set enrichment analysis (ssGSEA). The link between hub genes and the abundance of 23 immune cells was detected and then visualized using the package “ggplot2,” with a  $P < 0.05$  level of statistical significance.

## 2.9 Gene set enrichment analysis based on reactome

To investigate the enriched biological signaling of five mitophagy-related hub genes, GSEA was performed. The normalized enrichment score (NES) was employed to assess the magnitude of the enrichment, with a value greater than 0 indicating that this gene is positively linked with the pathway.

## 2.10 Prediction of miRNAs and TFs of hub genes

Upstream miRNAs and TFs of five mitophagy-related hub genes were predicted using the RegNetwork database (<https://regnetworkweb.org/>) and visualized by Cytoscape.

## 2.11 Prediction of target compounds

NetworkAnalyst, a web-based comprehensive application, may be utilized to design customized networks for pharmacogenomics

investigations (20). In this study, NetworkAnalyst was used to establish a protein-compound network.

## 2.12 Statistical analysis

R software (version 4.1.1) and GraphPad Prism 5 have been used for statistical analysis. The qRT-PCR tests were done in triplicate or more. GraphPad Prism (CA, USA) was employed for data analysis and for transforming from value data into bar graphs. For the identification of group differences, a statistical method called the student's t-test was utilized, with a result of  $P < 0.05$  for statistical significance.

## 3 Results

### 3.1 Identification and functional enrichment analysis of DEGs in vitiligo

To investigate DEGs in vitiligo lesions, we downloaded the raw gene expression data in the GSE53146 and GSE75819 databases from GEO datasets. After data preprocessing and cleaning, we adjusted the batch effect (Figure 1A, B) and normalized the expression matrices of the two datasets. The box plot's trend was almost a straight line (Figure 1C, D). Next, a threshold of adjusted  $P < 0.05$ ,  $|\log_{2}FC| > 0.5$  was utilized to screen DEGs in the combined datasets of GSE53146 and GSE75819. Then, 3950 DEGs were identified, including 2065 elevated-expressed DEGs and 1885 negatively regulated DEGs. DEGs were presented in Figure 1E in the volcano plots, and the top 20 DEGs were shown in the heat map (Figure 1F).

Then, GO and KEGG enrichment analyses were implemented with the "clusterProfiler" package of R to explore the potential biofunction of DEGs. The findings revealed that the most enriched GO keywords were associated with the catabolic process of proteasomal protein, RNA and mRNA, mitochondrial translation, protein-containing complex disassembly, termination and elongation of mitochondrial translation (biological process, BP); ribosome, ribosome and matrix of mitochondria and mitochondrial protein-containing complex (cellular component, CC); transcription coregulator activity, ATPase activity, cadherin activity, ubiquitin-like protein ligase binding, and structural constituent of ribosome (molecular function, MF) (Figure 2A). According to the KEGG enrichment study, DEGs have a significant role in neurodegeneration and multiple disease pathways, Alzheimer's disease, amyotrophic lateral sclerosis, human papillomavirus infection, and Huntington's disease (Figure 2B).

### 3.2 Identification and PPI network analysis of mitophagy-related DEGs

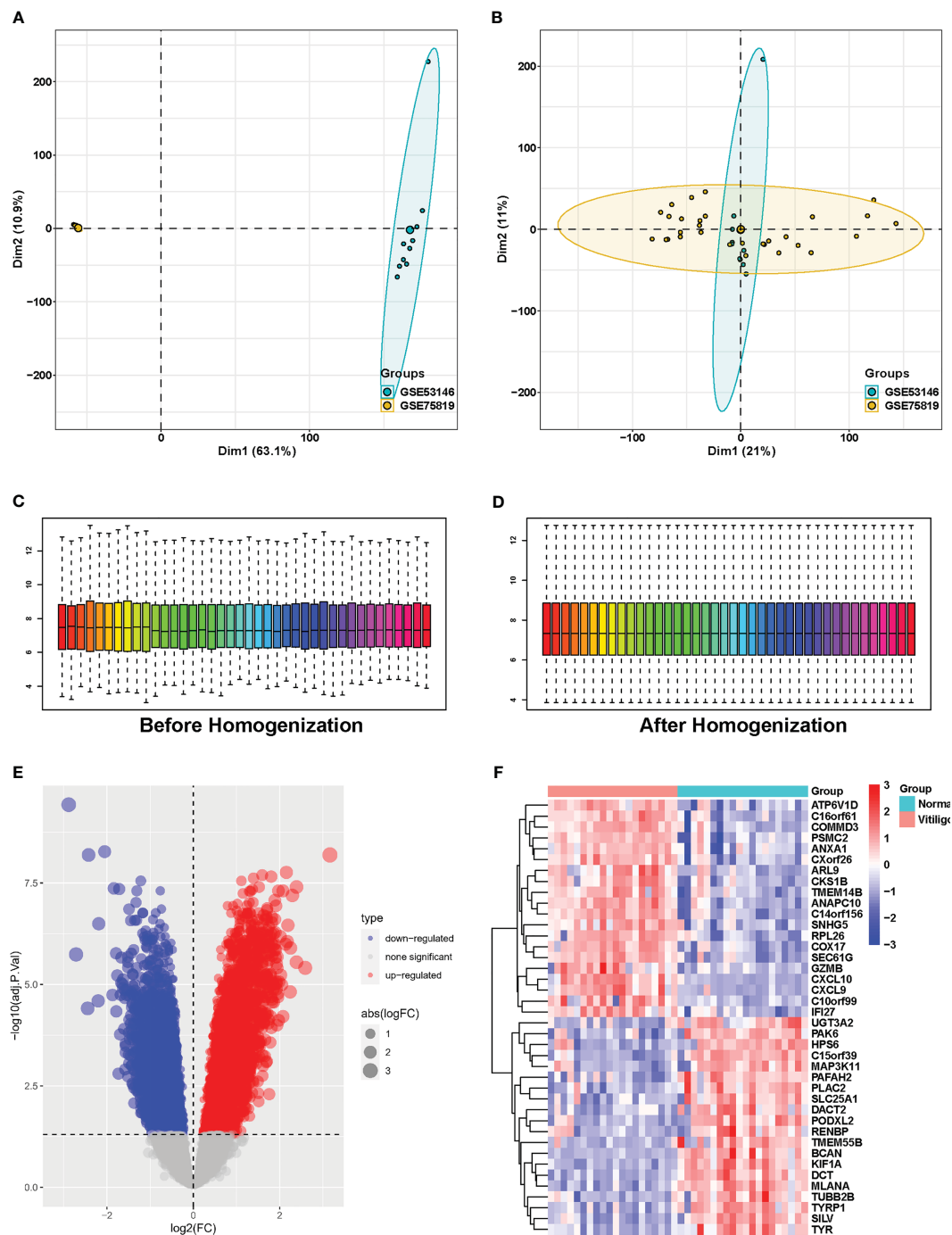
To screen mitophagy-related genes that are differentially expressed in vitiligo lesions, we intersected the DEGs in vitiligo with 72 genes encoding mitophagy-related proteins listed in the

KEGG database (hsa04137). A total of 24 mitophagy-related DEGs were detected, with 12 highly expressed genes (Figure 3A) and 12 less expressed genes (Figure 3B). Next, the GO and KEGG enrichment analyses on 24 mitophagy-related genes were performed to reveal the function of mitophagy-related DEGs. Mitophagy-related DEGs were primarily enriched in macroautophagy and autophagy of mitochondrion (BP), the outer membrane of organelle and mitochondria (CC), and histone deacetylase binding and ubiquitin protein ligase binding (MF) (Figure 3C). In KEGG enrichment analysis, these 24 mitophagy-related DEGs were involved in mitophagy and autophagy in animals, NOD-like receptor signaling pathways, pathways of neurodegeneration diseases, and Shigellosis (Figure 3D). The circle graph showed the top five enriched signal pathways involved in the 24 mitophagy-related DEGs from the KEGG analysis (Figure 3E).

The volcano map (Figure 4A) and heat map (Figure 4B) displayed mitophagy-related genes from microarrays GSE53146 and GSE75819. Figure 4C shows the differential expression of mitophagy-related DEGs in vitiligo lesions and the controls. The genes with elevated expression included *GABARAPL2*, *USP8*, *TAX1BP1*, *FUNDC1*, *TOMM7*, *TBK1*, *TBC1D15*, *MAPK9*, *OPTN*, *RHOT1*, *BNIP3L*, and *USP15*. And the down-regulated genes included *RELA*, *ATG9A*, *TBC1D17*, *MFN2*, *SPI*, *SRC*, *BVL2L1*, *GABARAP*, *PINK1*, *TP53*, *TFE3*, and *SQSTM1* (Figure 4C). Subsequently, we constructed a PPI network of 24 mitophagy-related DEGs using a STRING database and the Genemania database (Figure 5A, B), demonstrating the interaction between these differentially expressed mitophagy-related genes.

### 3.3 Screening and the diagnostic value of hub genes

LASSO regression and random forest techniques were applied to investigate mitophagy-related hub genes in vitiligo. Seven genes were identified using the LASSO regression algorithm (Figure 6A). And the top 10 genes identified by the random forest algorithm are shown in Figure 6B, namely *GABARAPL2*, *SPI*, *USP8*, *RELA*, *MFN2*, *TBC1D17*, *ATG9A*, *SRC*, *TAX1BP1*, and *BCL2L1*. After the intersection, five hub genes shared by the LASSO regression algorithm and the random forest algorithm were finally confirmed [ $\gamma$ -aminobutyric acid receptor-associated protein-like 2 (*GABARAPL2*), specificity Protein 1 (*SPI*), ubiquitin specific peptidase 8 (*USP8*), *RELA* proto-oncogene, NF- $\kappa$ B subunit (*RELA*), and TBC1 domain family member 17 (*TBC1D17*)] (Figure 6C). The chord diagram analyzed and drawn by the R package "circlize" exhibited an interaction of five hub genes with each other (Figure 6D). Then, to access the potential prediction function of hub genes in vitiligo, we conducted ROC curve analyses of five mitophagy-related hub genes using the "pROC" package in the combined GSE53146 and GSE75819 cohorts. The AUC values were as follows: *GABARAPL2* (AUC = 0.988), *SPI* (AUC = 0.894), *USP8* (AUC = 0.94), *RELA* (AUC = 0.927), and *TBC1D17* (AUC = 0.927) (Figure 6E). These AUC values implied that all five mitophagy-related hub genes have high specificity for vitiligo.



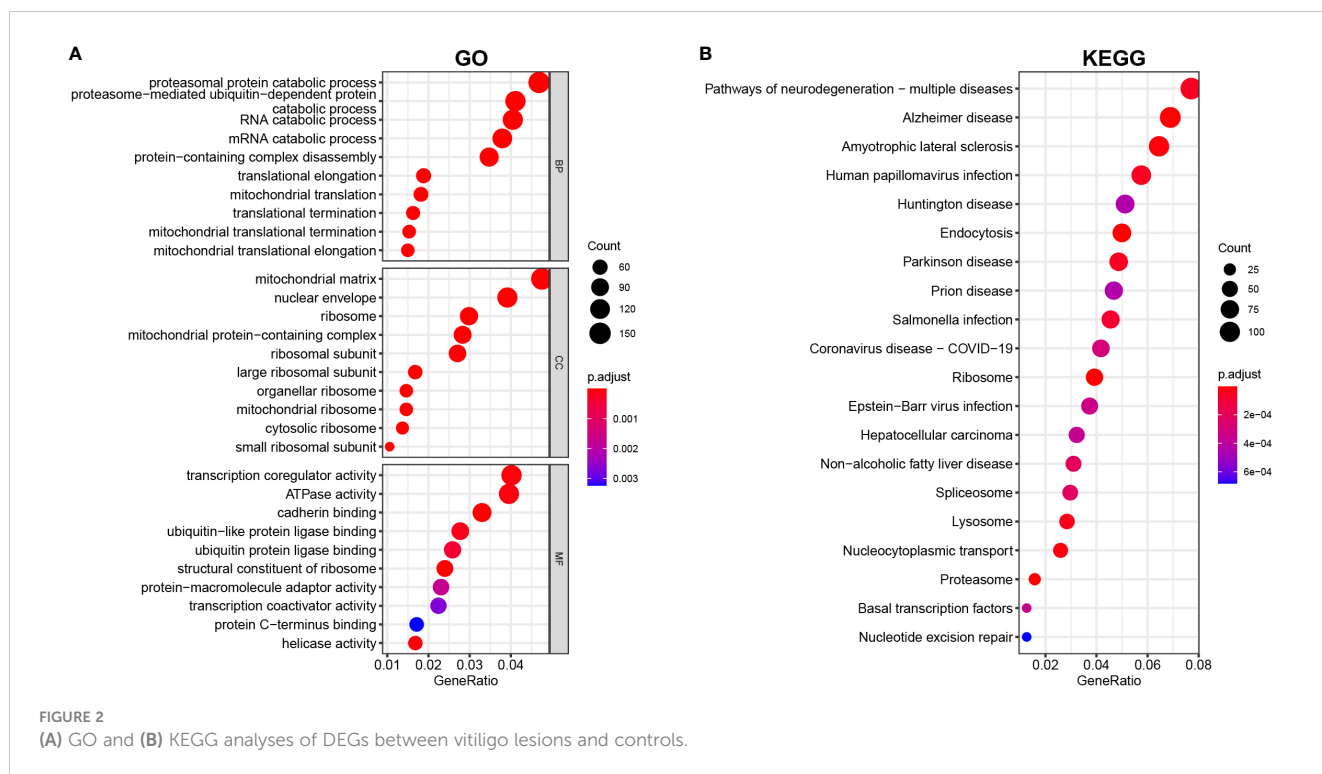
**FIGURE 1** Identification of DEGs between vitiligo lesions and controls in the combined microarray set of GSE53146 and GSE75819. **(A)** PCA plots showing the expression data of GSE53146 and GSE75819. **(B)** PCA plots showing the combined microarray set of GSE53146 and GSE75819 with the removal of batch effects. **(C)** box line blots before homogenization. **(D)** box line blots after homogenization. **(E)** volcano map of DEGs. **(F)** heatmap of the top 20 significantly upregulated or downregulated DEGs.

### 3.4 Validation of mitophagy-related hub genes in clinical skin tissues and immune cell infiltration.

To validate the reliability of data analysis results, we verified the mRNA profiling of five mitophagy-related hub genes in skin tissues from vitiligo patients and healthy individuals. Consistent with the

bioinformatic analysis results, GABARAPL2 and USP8 mRNA levels were raised in vitiligo lesions, whereas the mRNA levels of RELA, TBC1D17, and SP1 were decreased (Figure 7).

Vitiligo is now classified as an autoimmune, inflammatory skin condition (21). The humoral and cell-mediated immune mechanisms are likely the main contributors to MC's death (22). So, our research studied the immune infiltration in vitiligo using the



“GSVA” package. A total of 23 subtypes of infiltrated immune cells were identified in vitiligo lesions and skin tissues from healthy controls. And in vitiligo lesions, interactions between different immune cell subsets were observed (Figure 8A). Compared with controls, the abundance of activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells, immature dendritic cells and B cells, myeloid-derived suppressor cells (MDSCs), gamma delta T cells, mast cells, regulatory T cells (Tregs), and T helper 2 (Th2) cells in vitiligo was substantially higher than in controls, while monocytes, CD56-positive natural killer (NK) cells, and NK cells were considerably less abundant (Figure 8B). In addition, we looked into the connection between immune infiltration and five mitophagy-related genes. Our findings revealed that five hub genes were linked to the abundance of activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells, CD56-bright NK cells, immature dendritic cells, mast cells, Tregs, Th2 cells, monocytes, and NK cells (Figure 8C), all of which contribute to vitiligo pathogenesis (1). These results suggested that hub genes may affect immunological characteristics in vitiligo.

### 3.5 Identification of hub genes-related Signal pathways

A correlation study of five hub genes with all genes was performed, and the heatmap exhibited the top 50 positively associated genes (Figure 9). Based on the results shown in Figure 9, we analyzed the signal pathways implicated in hub genes using single gene-based GSEA. The top 20 signal pathways involved in different hub genes were displayed in Figure 10. Our findings demonstrated that all five hub genes were linked to cell

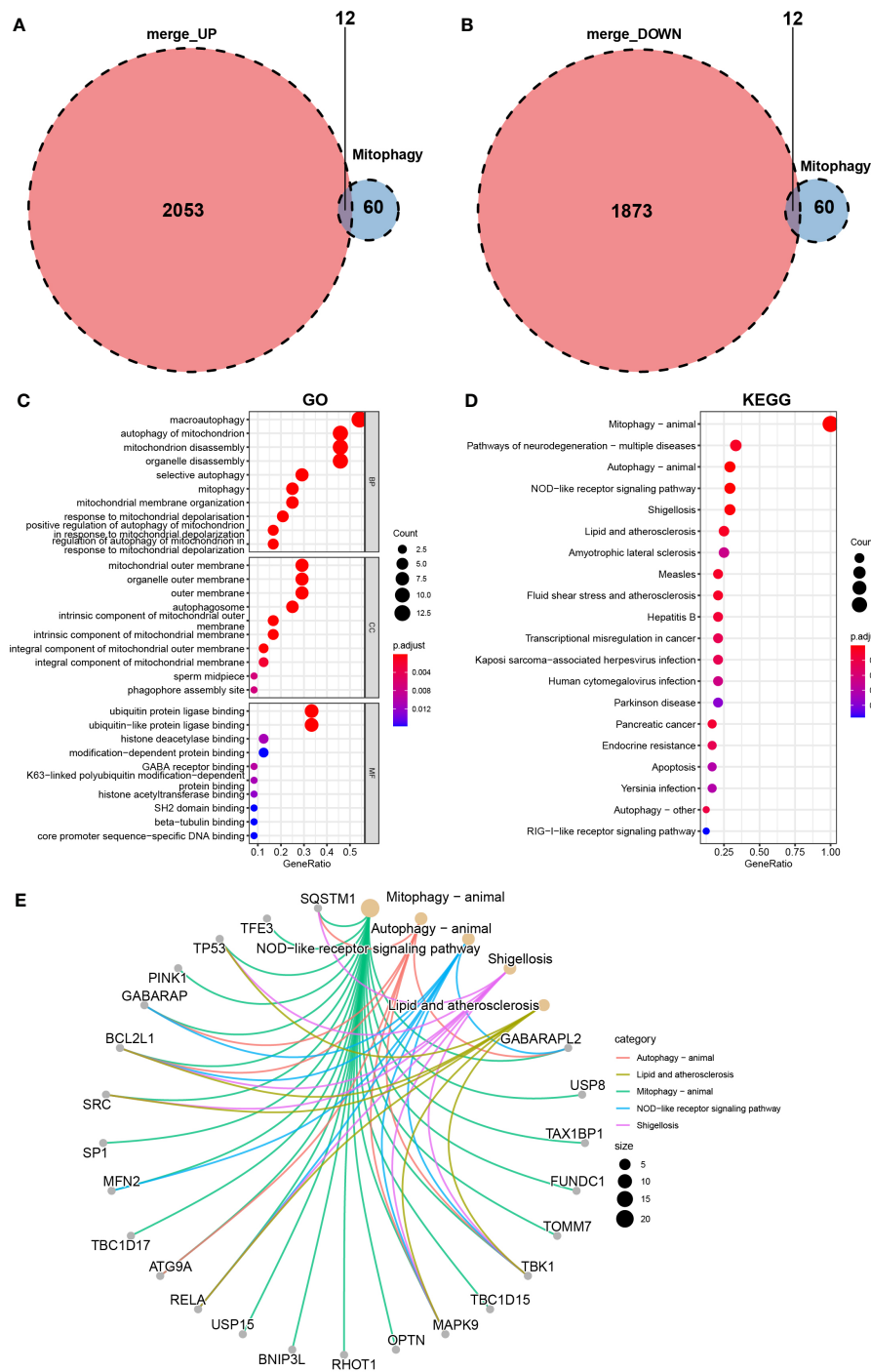
proliferation (cell cycles, cell cycle checkpoints, death receptor signals, et al.). Furthermore, *GABARAPL2*, *RELA*, *TBC1D17*, and *USP8*, except of *SPI1*, are implicated in cellular responses to stress and external stimuli; External stimuli, such as stress and oxidative stress, are vital factors in the etiology of vitiligo (10). As a result, it is reasonable to infer that hub genes may regulate cell cycle and death signals caused by oxidative stress in vitiligo.

### 3.6 Prediction of upstream miRNA and TF and construction of the protein-compound network

The upstream regulatory miRNAs and TFs were predicted using the RegNetwork database and are shown in Supplementary Figure 1. Then, we forecasted the hub gene-related compounds to identify compounds with potential vitiligo therapeutic effects (Supplementary Figure 2). A total of 607 target compounds were screened, and these compounds and their corresponding target genes are given in Supplementary Table 1 based on their degree and betweenness.

## 4 Discussion

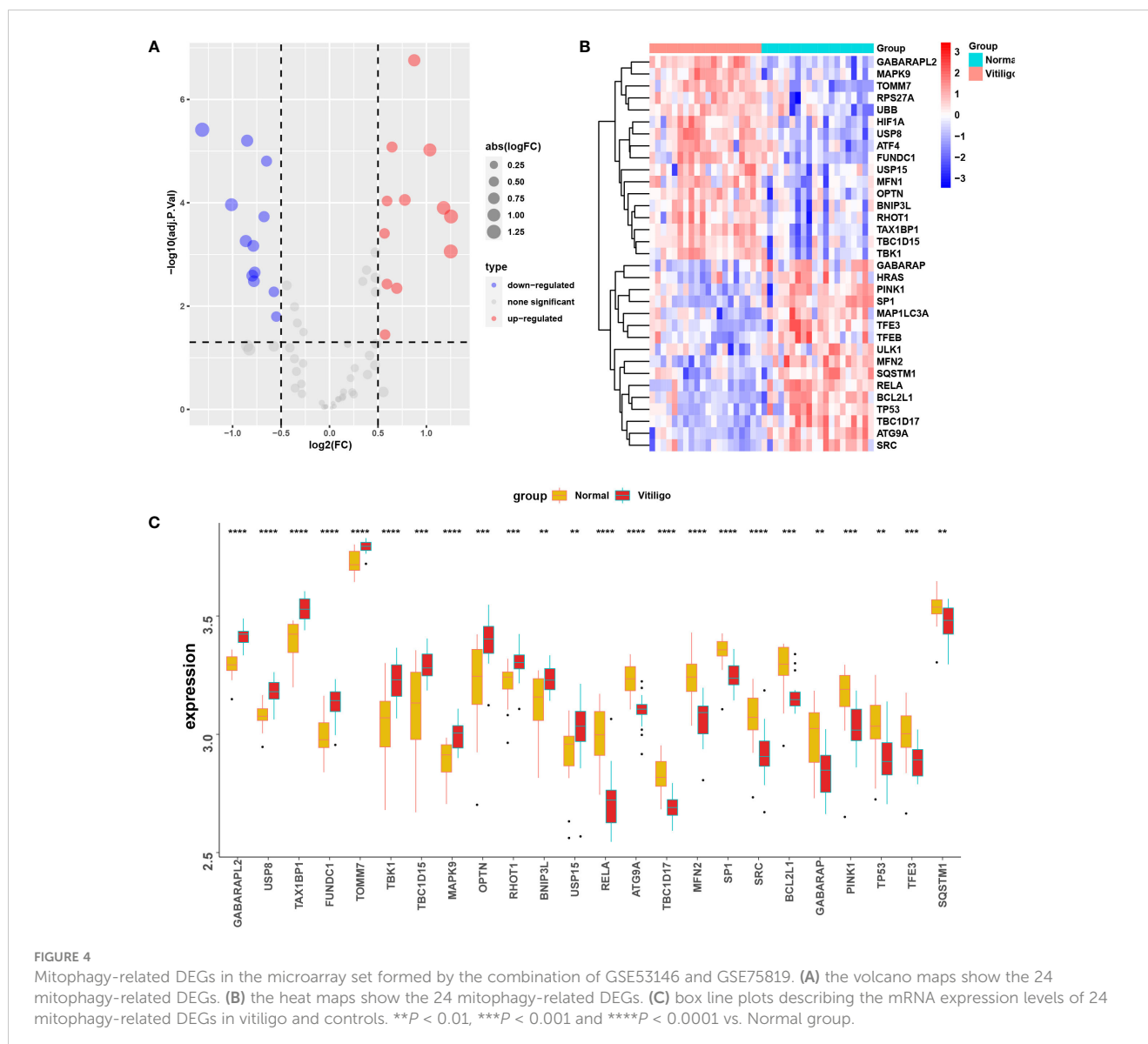
Mitochondria are essential organelles that provide energy for cell metabolism and other bodily functions. During the process of ATP production, ROS may be released by the mitochondria (23). Although ROS has physiological roles at normal levels, excess ROS and stressors such as cell aging could impair mitochondrial function



**FIGURE 3** Identification of mitophagy-related DEGs in the combined microarray set of GSE53146 and GSE75819. **(A)** Venn diagram showing 12 overlapping genes associated with mitophagy and upregulated DEGs. **(B)** Venn diagram showing 12 overlapping genes associated with mitophagy and downregulated DEGs. **(C)** GO analysis of 24 mitophagy-related DEGs. **(D)** KEGG analysis of 24 mitophagy-related DEGs. **(E)** correlation between 24 mitophagy-related DEGs and the top five pathways of KEGG.

and organization, leading to an overproduction of cellular ROS and oxidative stress (23, 24). Mounting data suggests that elevated ROS, dysfunctional mitochondria, and oxidative stress are involved in aging, vitiligo, and several illnesses (24–27). Mitophagy is a kind of selective cellular autophagy that is responsible for the removal of damaged or redundant mitochondria produced by external stimuli

such as ROS and cell aging (27). The elimination of dysfunctional mitochondria by mitophagy is imperative to cell survival. A research study found that autophagosomes can be observed in MCs from controls, stable vitiligo lesions, and halo nevi lesions but are absent in MCs of active vitiligo lesions (14). Qingli Gong discovered that calcipotriol might rescue MCs and mitochondria

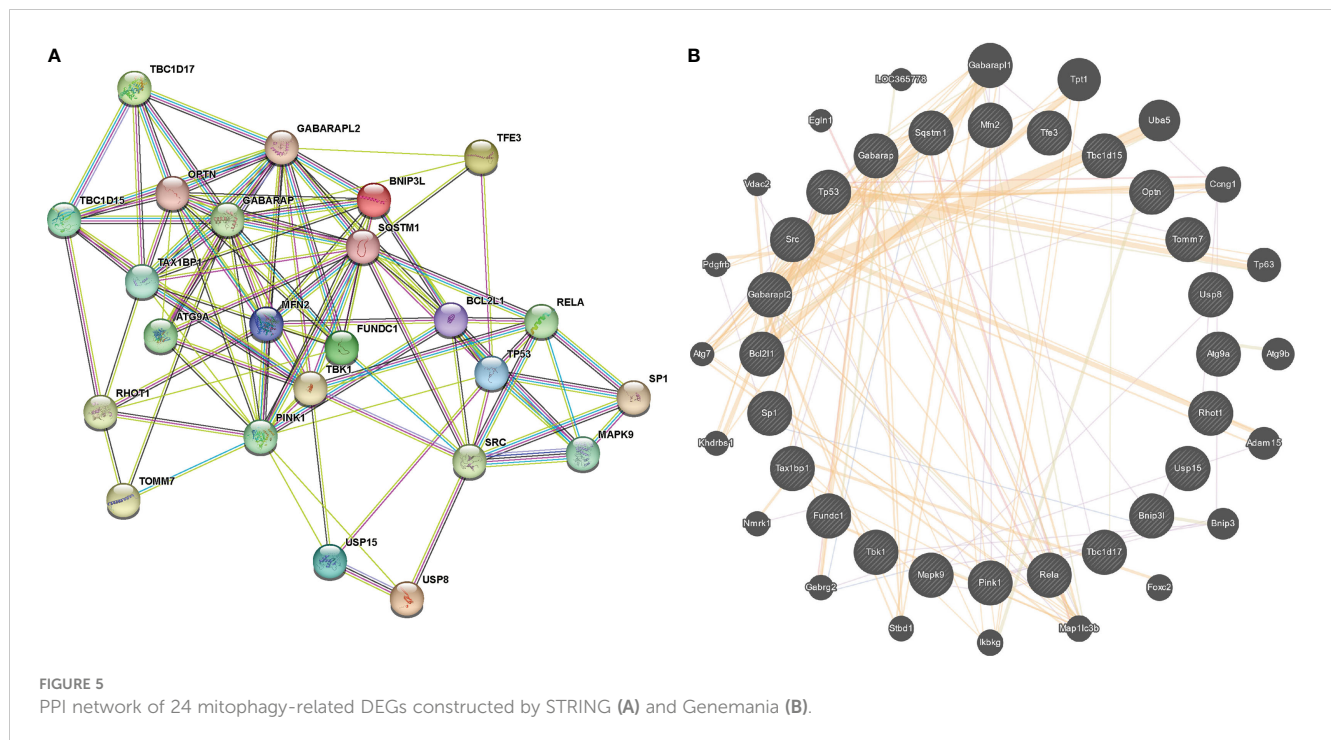


from oxidative damage by inducing autophagy or mitophagy (28). Hence, the dysfunction of mitophagy in MCs in vitiligo lesions may impair their resistance to mitochondrial damage and oxidative stress, making them more susceptible to death and depigmented patches. Nevertheless, investigations on mitophagy in other cells, such as KCs, in the context of vitiligo have yet to be documented. Current research on mitophagy in vitiligo has been restricted to morphological observations of autophagosomes and mitochondrial alterations. Bioinformatics analysis of mitophagy-related genes in vitiligo has not been conducted. Herein, in the combined expression array of GSE53146 and GSE75819, we screened 3950 DEGs in vitiligo. Then, we obtained a total of 24 mitophagy-related DEGs in vitiligo by intersecting mitophagy-related genes with DEGs in vitiligo, some of which had been verified to be involved in vitiligo. Yiwen Yang et al. found that sequestosome 1 (SQSTM1) expression increased in vitiligo lesions (29), contradictory to our research. Another bioinformatics investigation found that TANK

binding kinase 1 (TBK1) is overexpressed in vitiligo lesions (29), consistent with our findings. And a transcriptome and differential methylation integration analysis has demonstrated that B-cell lymphoma-2-like 1 (BCL2L1) was hypermethylated and downregulated, further confirming the results of BCL2L1 in our research (30). Downregulation of PTEN-induced kinase 1 (PINK1) in MCs may increase intracellular ROS, cellular sensitivity to oxidative stress, and degradation of mitochondrial shape and function (31).

Through LASSO regression analysis and the random forest algorithm, we screened five mitophagy-related hub DEGs (*GABARAPL2*, *USP8*, *RELA*, *SP1*, and *TBC1D17*) in vitiligo. The AUC values of hub genes were high in the combined dataset (*GABARAPL2*, AUC=0.988; *USP8*, AUC=0.94; *RELA*, AUC=0.927; *SP1*, AUC=0.894; and *TBC1D17*, AUC=0.927), implying that the five hub genes have high specificity for vitiligo and could be used as vitiligo diagnostic biomarkers. To validate the





results of the bioinformatic analysis, a qRT-PCR experiment was employed to access the expression of mitophagy-related hub genes in vitiligo patients and healthy individuals. The results showed elevated GABARAPL2 and USP8 and decreased RELA, SP1, and TBC1D17 in vitiligo compared to healthy controls, which were consistent with bioinformatic analysis results.

Vitiligo is an autoimmune skin condition characterized by an overactive immune response. Previous research studies have proven that CD8<sup>+</sup> T cells are necessary for the occurrence of vitiligo; and immune responses in vitiligo are mainly driven by autoreactive CD8<sup>+</sup> T cells (32). Similarly, CD4<sup>+</sup> T lymphocytes infiltrate vitiligo lesions and may accelerate vitiligo development by releasing interferon (IFN)- $\gamma$  (7, 33). Although vitiligo is thought to be a skin condition driven by T helper 1 (Th1) cells (34), type 2 cytokine signatures have recently been proposed (35). And Rong Jin et al. discovered that IFN- $\gamma$ , C-C motif chemokine ligand (CCL)-2, and CCL8 could promote Th2 cell differentiation and chemotaxis (36). Furthermore, B cells, NK cells, dendritic cells, and mast cells were shown to be higher in vitiligo lesions and might contribute to vitiligo development (37–40). Hence, we employed the ssGSEA function of the “GSVA” package to assess immune infiltration in vitiligo. Compared with skin samples from the control group, the numbers of activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells, immature dendritic cells and B cells, MDSCs, gamma delta T cells, mast cells, Tregs, and Th2 cells were significantly higher in vitiligo lesions, while those of CD56 bright NK cells, monocytes, and NK cells were significantly lower in vitiligo lesions. To sum up, our results were generally consistent with previous studies, emphasizing that these immune cells are crucial for the etiology of vitiligo. Then, we explored the link between these hub genes and immunological infiltration. Notably, GABARAPL2 and USP8 are positively related to

activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells and Th2 cells, while RELA, SP1, and TBC1D17 are negatively linked to those immune cells.

GABARAPL2, a member of the GABARAP family, is one of the Atg8 (yeast) orthologs found in mammals. Its increased expression was also discovered in another vitiligo investigation (29). The GABARAPL/GABARAPL2 subfamily participates in the later stage of autophagosome maturation, but its specific mechanism remains unclear (41). In addition, GABARAPL2 may recruit guanylate-binding protein (GBP), an IFN- $\gamma$ -inducible GTPase, in response to microbial infection (42). And autophagy regulates TNF receptors through GABARAPL and GABARAPL2 (41). Considering the central role of IFN- $\gamma$  in vitiligo, it is speculated that GABARAPL2 engages in vitiligo by regulating mitophagosome maturation and the IFN- $\gamma$ -driven immune response. USP8 is a pleiotropic deubiquitinating enzyme. Lacking USP8, K6-linked ubiquitin conjugates persist in Parkinson’s disease protein 2 (PARK2), which delays PARK2 translocation to damaged mitochondria and the completion of mitophagy (43, 44). Furthermore, USP8 engages in the development and hemostasis of T cells and regulates the expression of IFN- $\gamma$  by forkhead box protein O1 (Foxo1) (45). SP1 is a vital TF that modulates the transcription of various genes that engage in diverse cellular bioprocesses. Consistent with our results, SP1 was downregulated in vitiligo (46). SP1 interacts with E2F transcription factor 1 (E2F1) to activate the expression of mitofusin2 (MFN2) which has been confirmed to regulate mitophagy by regulating mitochondrial fusion (47). In addition, SP1 can bind to the promoter of TBET to regulate the release of IFN- $\gamma$  from NK cells, T cells, B cells, and dendritic cells (48).

RELA, also called NF- $\kappa$ B p65, is vital for the NF- $\kappa$ B transcriptional machinery. A high level of RELA was observed in

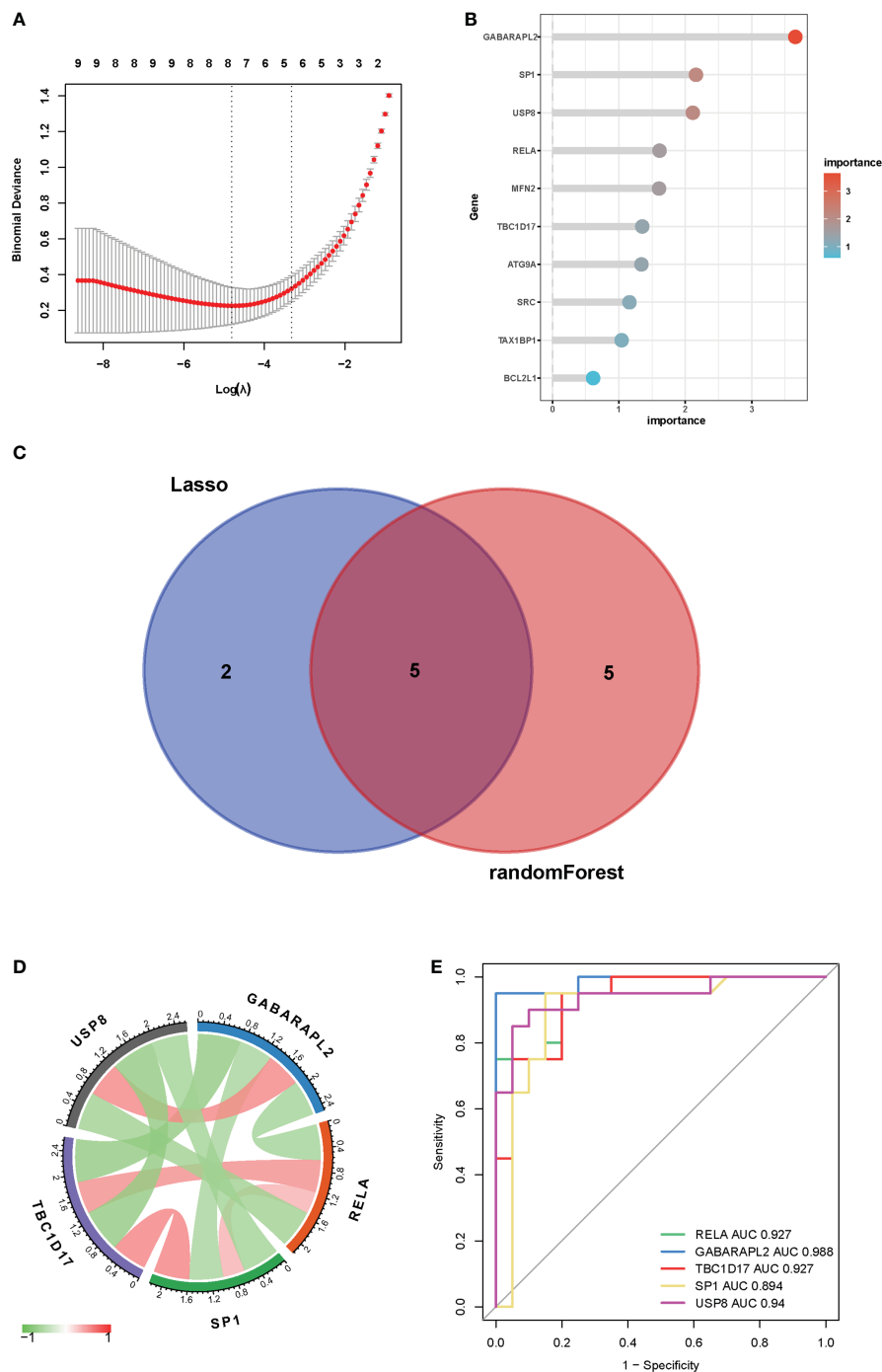
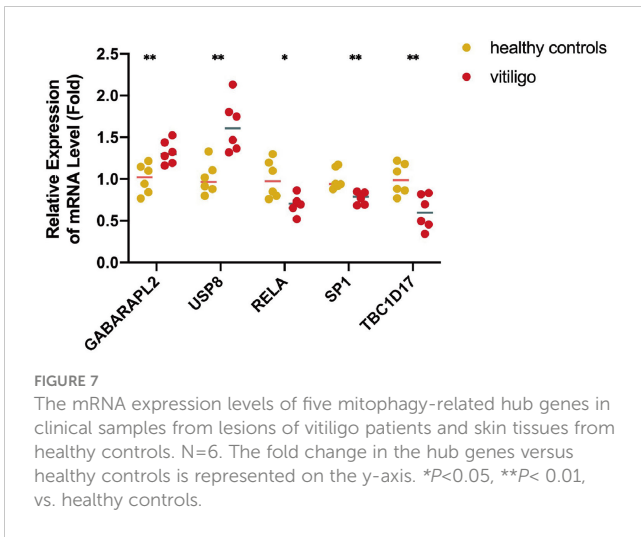


FIGURE 6

Identification of key genes by machine learning algorithms and ROC curves. (A) LASSO logistic regression algorithm to identify key genes. (B) the top 10 key genes screened by the random-forest algorithm. (C) Venn diagram displayed the overlap of genes screened by two machine learning algorithms. (D) Interactions between five mitophagy-related hub genes. (E) ROC curves of five mitophagy-related hub genes in vitiligo and controls.

KCs from perilesional skin tissues in vitiligo (49). Oxidative stress increased IL-15 expression to activate CD8<sup>+</sup> T cells by p65 signaling in vitiligo (50). However, another study found that increased translocation of p65 into the nucleus may protect melanocytes from death (51). P65 accumulation may lead to mitophagy defects, further contributing to senescence (52). However, whether p65 plays a role in mitophagy in vitiligo remains unclear.

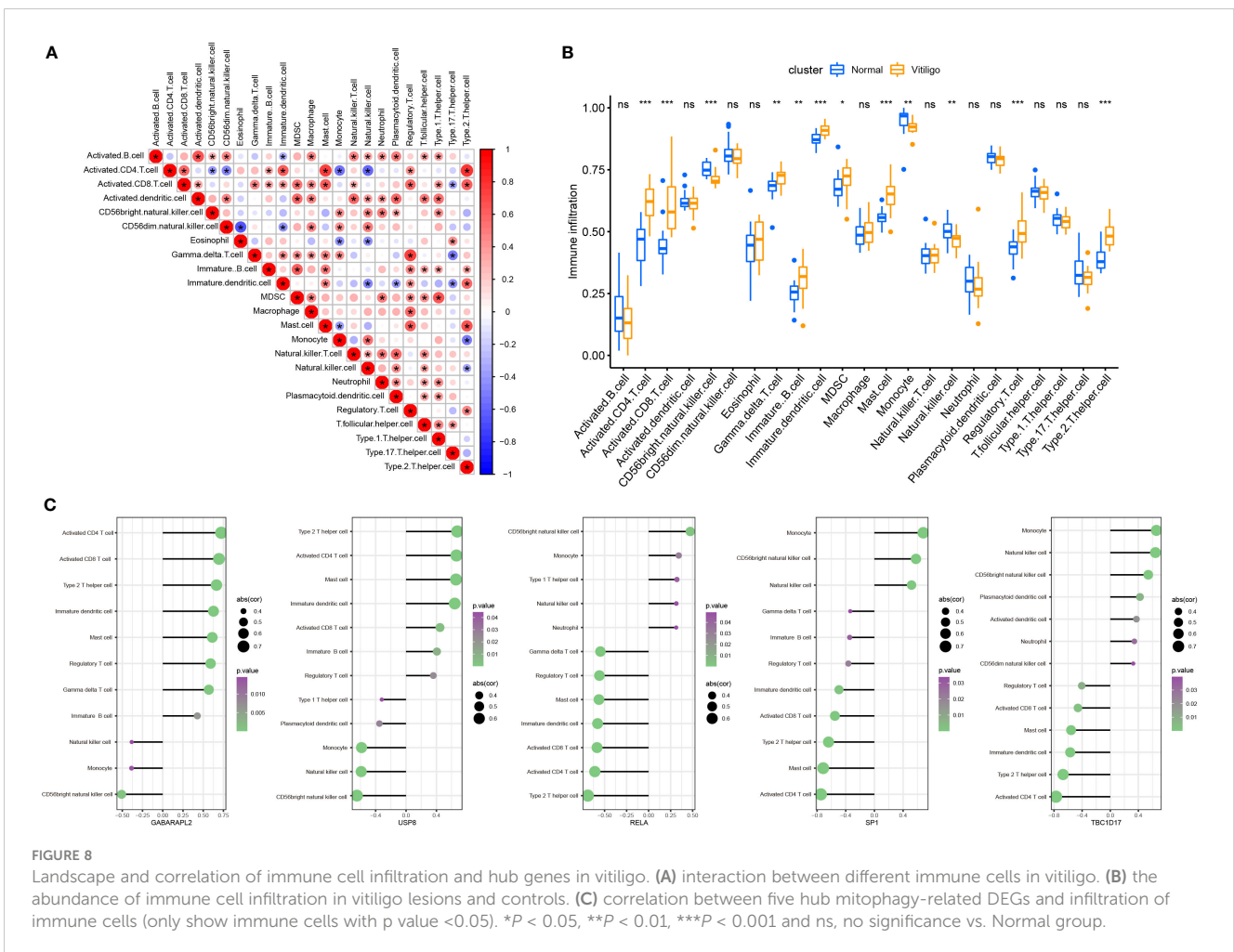
By interacting with TBC1D15 to regulate Rab7 activity, TBC1D17 facilitates appropriate autophagic encapsulation of mitochondria; Deletion of TBC1D17 and TBC1D15 causes Rab7 to no longer localize to damaged mitochondria, leading to impaired mitophagy (53, 54). Accordingly, we hypothesize that these hub genes may regulate mitophagy, and further activate immune cell infiltration and immune response to accelerate the



examine hub genes' expression is limited. More clinical samples are desired to support the outcomes of our work. Third, we did not conduct experiments on other cell and mouse models to investigate the expression of these hub genes and probable mechanisms. Further research studies on mechanisms are needed. Finally, we only explored the link between mitophagy-related hub genes and immune infiltration using bioinformatic analysis. More research studies are needed to explore the function of these hub genes in regulating the immune response and inflammation in vitiligo.

## 5 Conclusion

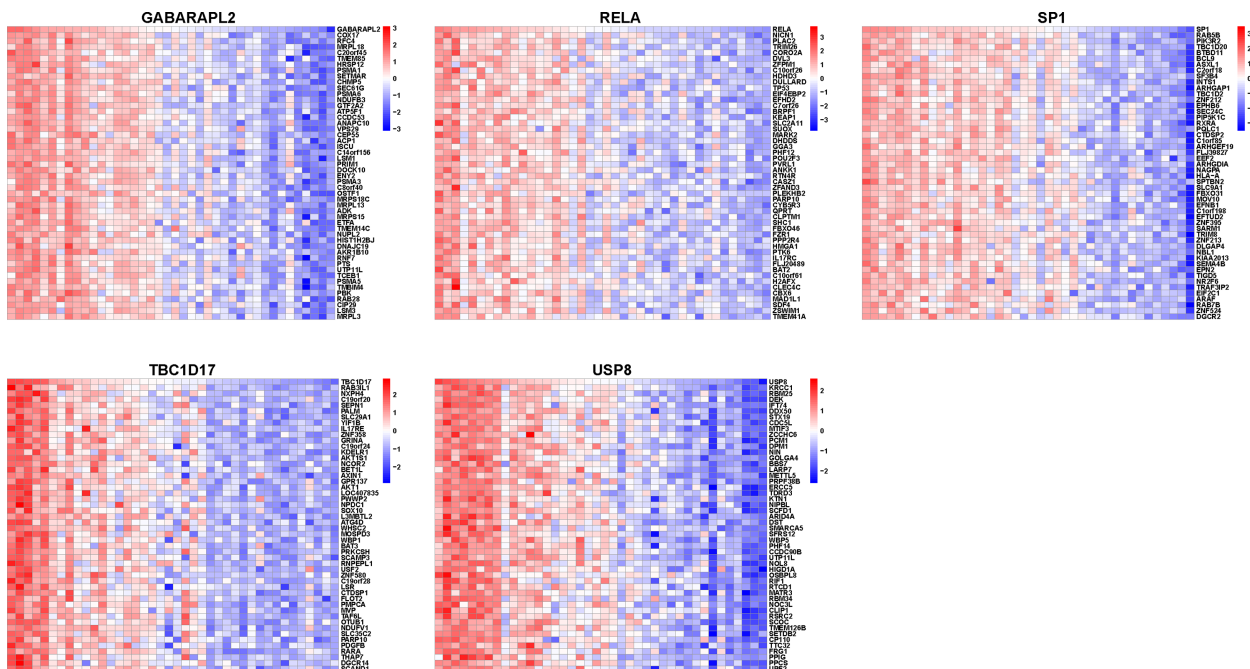
In conclusion, we identified five mitophagy-related hub genes with high diagnostic specificity for vitiligo. In clinical samples of vitiligo patients and healthy individuals, the expression levels of



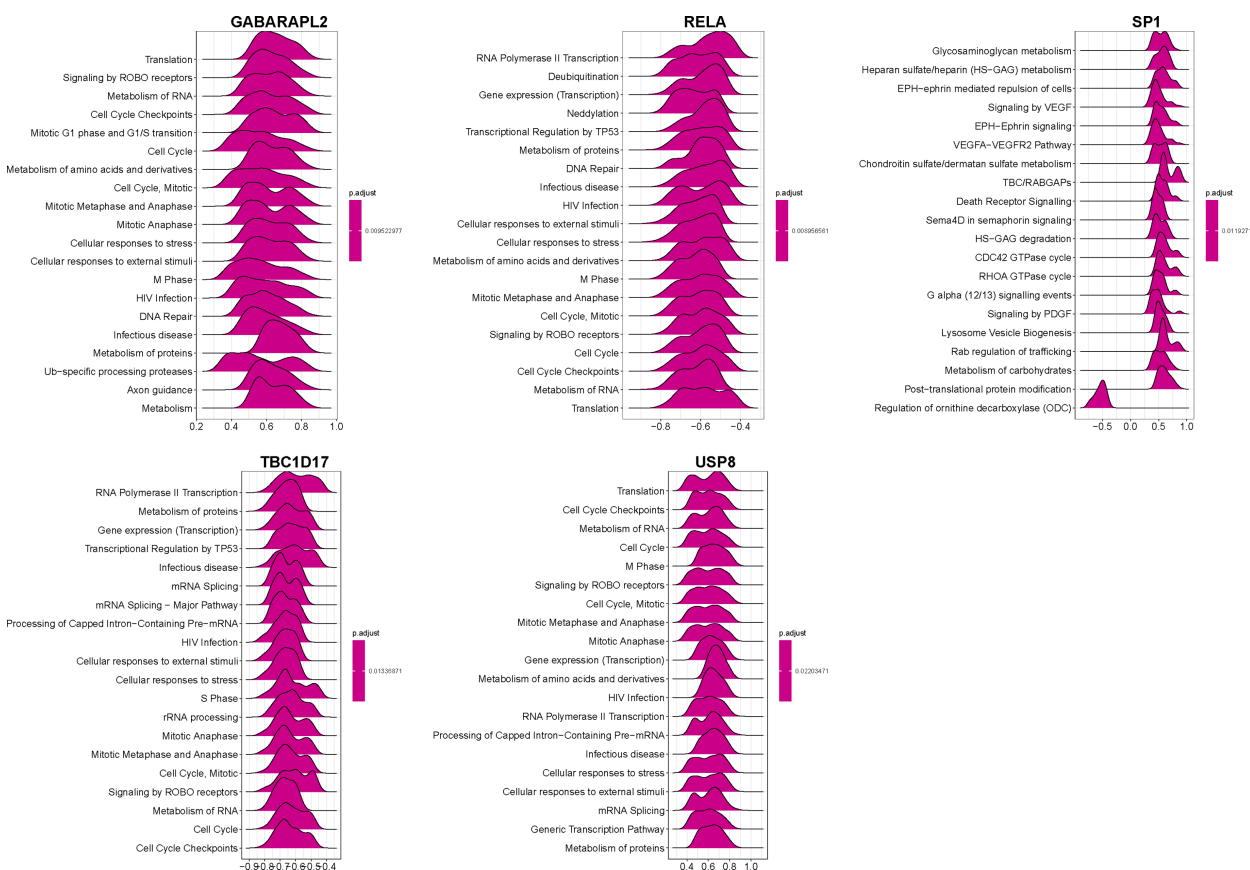
progress of vitiligo. Nevertheless, the mechanisms of the five hub genes in vitiligo remained unexplored and warrant additional research.

There are still some limitations in our research. First, the number of individuals included in the databases for further study is restricted. Second, the number of clinical samples utilized to

mitophagy hub genes were confirmed. Furthermore, correlation analysis revealed that mitophagy-related hub genes were involved in immune cell infiltration, indicating that hub genes may influence the onset and progression of vitiligo by modulating mitophagy and the immune response. The current findings add to our understanding of vitiligo and may be helpful in vitiligo treatment.



**FIGURE 9** Heatmap of mitophagy-related hub genes-associated genes. Heatmap showing the top 50 genes positively correlating with five mitophagy-related hub genes.



**FIGURE 10** GSEA identifies signaling pathways involved in the hub genes. Top20 enriched signaling pathways of five mitophagy-related hub genes identified by GSEA.

## Data availability statement

The datasets (GSE53146 and GSE75819) for this study can be found in the GEO database (GSE53146: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE53146>; GSE75819: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE75819>).

## Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the Hospital for Skin Disease, Institute of Dermatology, Chinese Academy of Medical Sciences, and Peking Union Medical College. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

Conceptualization, LL, and C-RL. Data curation, LL. Formal analysis, LL. Funding acquisition, C-RL. Investigation, LL. Methodology, LL, and JZ. Project administration, C-RL. Resources, JZ, YG, and C-RL. Software, LL. Supervision, JZ, YG, and C-RL. Validation, LL, and JZ. Visualization, LL. Writing—original draft, LL. Writing—review and editing, JZ, YG and C-RL. All participating authors gave their consent for this work to be published. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fimmu.2023.1164124/full#supplementary-material>

### SUPPLEMENTARY FIGURE 1

TFs and miRNAs regulatory network of hub genes. Upstream regulatory TFs and miRNAs of five mitophagy-related hub genes identified by RegNetwork database

### SUPPLEMENTARY FIGURE 2

Predicted target compounds of hub genes. Protein-compound network of five mitophagy-related hub genes was constructed.

### SUPPLEMENTARY TABLE 2

Predicted target compounds.

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