Bioinformatics Analysis of Autophagy-Related Genes in Kidney Transplantation

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Introduction. Autophagy related genes (ARGs) may play important roles in various biological processes involving kidney transplantation (KT); however, their expression characteristics are rarely used to study the relationship between autophagy and prognosis in KT patients. This study aims to construct a new autophagy related gene feature based on high-throughput sequencing datasets.
Methods. Differentially expressed ARGs (DEARGs) were identified in KT patients based on the Gene Expression Omnibus (GEO) database. Gene Ontology (GO)and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed to explore potential biological and pathological functions of *DEARGs*. Univariate and Lasso Cox regression analyses identified survival-related *DEARGs* and established a prognostic gene signature whose performance was evaluated by Kaplan-Meier curve and receiver operating characteristic (ROC). Moreover, the prognostic value of the gene signature was further validated in 48 KT patients from the GSE21374 dataset.
Results. A total of 28 common *DEARGs* were identified between rejection and non-rejection samples in 3 datasets, including GSE21374, GSE36059, and GSE48581. GO and KEGG enrichment analyses showed that *DEARGs* were mainly involved in regulating apoptotic processes. In addition, we identified and validated 7 *DEARGs* (*CASP1, CASP3, FKBP1A, RAB11A, NFKB1, RGS19, and CCL2*) as the prognostic signatures. The Kaplan-Meier (K-M) analysis showed that the survival rate of the high-risk patients was significantly lower than that of the low-risk patients.
Conclusion. The effectiveness of autophagy related features was validated by using 48 KT patients in the GSE21374 dataset, and establishing and confirming a new ARG signal with independent survival prognostic value for KT patients.

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Original Paper

INTRODUCTION

Kidney transplantation (KT) is the final treatment for end-stage kidney disease.[1,2] KT is associated with reduced mortality and improved quality of life.[3] Allograft rejection is one of the leading causes of KT inactivation.[1] Patients with end-stage kidney disease (ESKD) have a high risk of capillary inflammation, microangiopathy, necrosis, and graft failure after KT.[4]

Autophagy is an evolutionarily conserved intracellular lysosomal dependent metabolic process that is protective and harmful.[5,6] Autophagy plays a key role in a variety of diseases, including kidney diseases. Autophagic regulation of mitochondrial

metabolism may play an immuno-metabolic role in kidney diseases. Autophagy within the kidney also prevents inflammatory damage due to lysosomal rupture,[7,8] but the role of *autophagy-related genes (ARGs)* in KT rejection is not fully understood.[9]

Autophagy is a metabolic process within cells that maintains cellular homeostasis by breaking down and recovering damaged or unwanted components. Autophagy may play an important role in the process of kidney transplantation. In kidney transplantation, immune rejection is an important issue, and autophagy is believed to regulate the immune response. Autophagy regulates antigen presentation MHC molecule expression, immune cell activation, and other pathways involved in regulating immune responses, which may play a role in immune tolerance and rejection in kidney transplantation. Therefore, this study explores the role of autophagy related genes in allograft rejection after kidney transplantation.

MATERIAL AND METHODS

Data acquisition

A total of 222 *ARGs* were obtained from the Human Autophagy Database.[10] The expression data of human kidney allograft biopsies were obtained from the Gene Expression Omnibus (GEO) database. The GSE21374 dataset included 244 consenting RT patients undergoing BFCs (biopsies for cause) as the standard of care between September 2004 and October 2007 at the University of Alberta (8440 112 Street NW Edmonton, Alberta T6G 2B7) or between November 2006 and February 2007 at the University of Illinois (1102 West Hazelwood Drive Urbana, IL 61802). In addition, 48 biopsies obtained from Minnesota (state in the Upper Midwest, Great Lakes, and northern regions of the United States.) between September 2006 and September 2007 were used as an independent validation dataset. The GSE36059 dataset included 403 consenting RT patients and 8 controls (nephrectomies). The GSE48581 dataset included microarray analysis of 300 kidney transplant biopsies (non-TCMR, TCMR) (non-T cell–mediated rejection, T cell–mediated rejection) and 6 nephrectomies.

Identification of *DEARGs*

The *DEARGs* were determined between rejection and non-rejection samples in the GEO database using the 'limma' R package.[11] A *P* Value < .05 was regarded as the cut-off criteria. A volcano plot, heatmap, and box plot were created to visualize the differences in gene expression between the two groups.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

GO is a major bioinformatics tool to annotate genes and analyze gene products and sequences in underlying biological phenomena, including a biological process (BP), molecular function (MF), and cellular component (CC). The *DEARGs* functions were analyzed by the GO, which obtained the biological function of the genes. In addition, the KEGG analysis was used to enrich the differential pathway via the "Cluster Profiler" package.[12] The threshold for enrichment significance was set at a *P* value < .05.

Univariate cox and LASSO regression analysis

All of the *DEARGs* were analyzed by univariate Cox regression analysis, and $P < 0.1$ was used as a cut-off to screen out the prognosis-related genes. Next, LASSO regression analysis was conducted on the prognosis-related genes, and the penalty parameter "lambda" was selected by the cross-validation method.

Risk assessment model construction and evaluation

ROC (Receiver Operating Characteristic) curves were drawn by using the R package "survival ROC" for validation of the risk model.[12] The AUC (Area Under Curves) values of the 2- and 3-year survival rates were calculated. According to the ROC curves of the 2-year survival, samples in the TCGA (The Cancer Genome Atlas) were divided into a high-risk group and low-risk group with the cut-off. The survival curves of the patients in the high- and low-risk groups were drawn with the R-package 'Survival',[12] and the survival time of the two groups was compared by a log-rank test.

Statistical analysis

All of the analyses were performed by using R programming language. The differences between different groups were compared by Mann-Whitney-Wilcoxon test. In all of the analyses, *P*-values less than 0.05 were considered to be statistically significant.

RESULTS

DEARGs

Principal component analysis (PCA) revealed a distinguishing consequence between the rejection and non-rejection samples in (Figure S1). Among 222 *ARGs*, a total of 30 *DEARGs* were identified in the GSE21374 dataset, including 28 upregulated and two downregulated genes (Figure 1A, B; Table S1). A total of 103 *DEARGs* were identified in the GSE36059 dataset, including 63 upregulated and

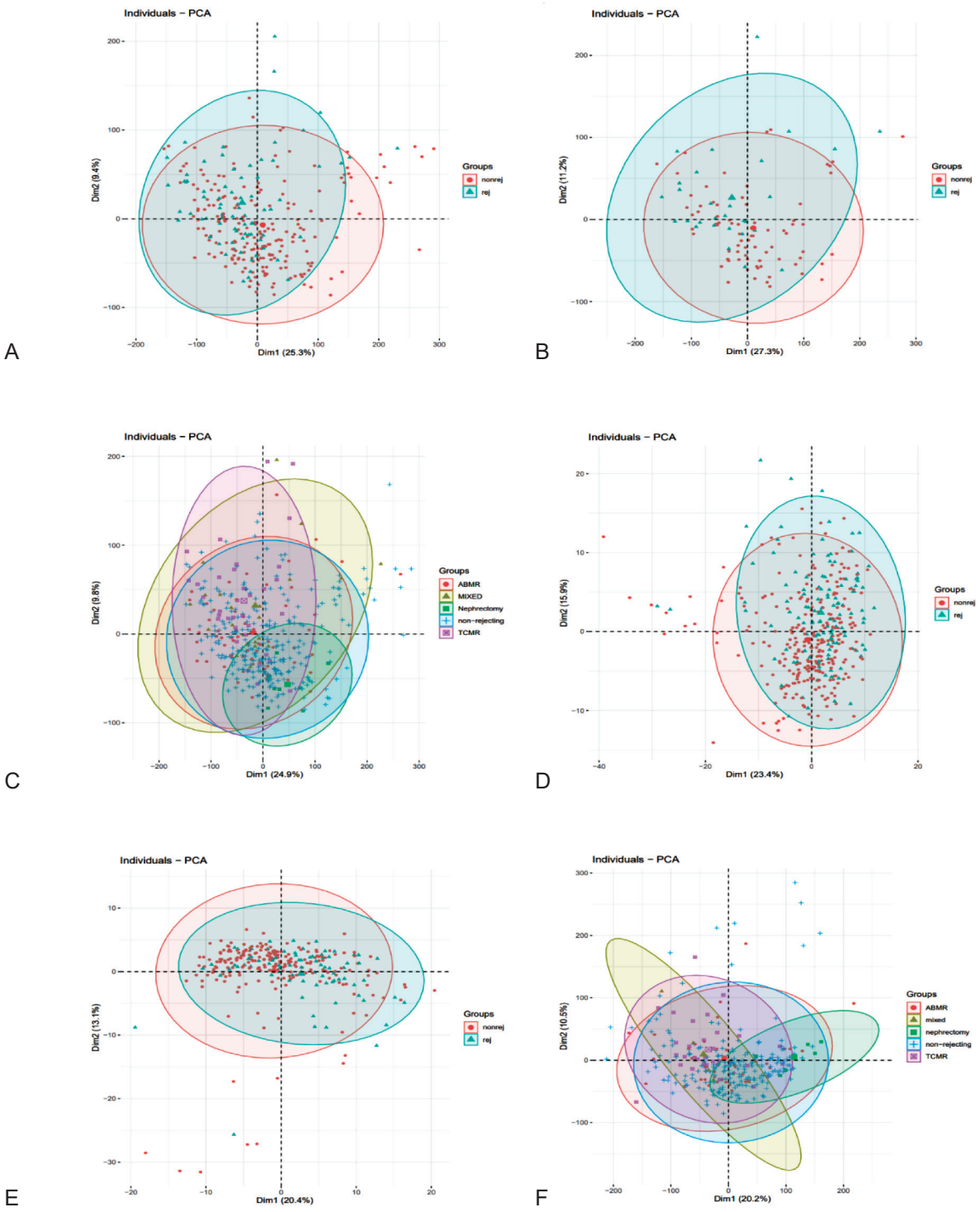


Figure S1. The results of principal component analysis (PCA) between the rejection and non-rejection samples.

Table S1. A total of 30 DEARGs identified in the GSE21374 dataset.

	logFC	AveExpr	t	*P*	adj.P.Val	B	Gene.symbol	ID	biotype
WDFY3	-0.329450099	8.714485841	-3.933733972	0.000149157	0.001773305	2.395086465	WDFY3	23001	protein_coding
ST13	-0.148856991	11.03405606	-2.796395113	0.00613118	0.045243877	-0.956610394	ST13	6767	protein_coding
RAB11A	0.142086207	10.6637208	2.842514762	0.005364585	0.041000757	-0.838963373	RAB11A	8766	protein_coding
SH3GLB1	0.16191272	9.216510513	3.085886923	0.002585718	0.023055986	-0.191253685	SH3GLB1	51100	protein_coding
PARP1	0.162370347	7.688597534	3.014130453	0.00322041	0.027566707	-0.386868709	PARP1	142	protein_coding
NFKB1	0.180185644	8.167651049	3.455869773	0.000788582	0.008437823	0.876774082	NFKB1	4790	protein_coding
ATG7	0.190996309	6.87653293	2.911930307	0.004375051	0.036010034	-0.658800076	ATG7	10533	protein_coding
RAB24	0.201491084	7.944307303	3.176981608	0.001946849	0.018114161	0.062564304	RAB24	53917	protein_coding
CALCOCO2	0.202319141	9.631870293	5.031067019	1.99E-06	4.72E-05	6.395498831	CALCOCO2	10241	protein_coding
UVRAG	0.262851684	7.039582006	4.598848551	1.17E-05	0.000251234	4.742106065	UVRAG	7405	protein_coding
FKBP1A	0.271107891	10.03731166	2.776315618	0.006495193	0.046332374	-1.007315329	FKBP1A	2280	protein_coding
CDKN1A	0.279946151	8.758400201	2.842965632	0.005357544	0.041000757	-0.837805122	CDKN1A	1026	protein_coding
IKBKB	0.301068236	8.394243965	3.431730929	0.000854486	0.008707615	0.8041354	IKBKB	3551	protein_coding
CFLAR	0.307650828	9.177605825	5.454329606	3.21E-07	3.43E-05	8.099636242	CFLAR	8837	protein_coding
NLRC4	0.32450314	4.129984065	5.092042485	1.54E-06	4.11E-05	6.636063173	NLRC4	58484	protein_coding
PEA15	0.32536937	9.77723234	3.939123011	0.000146264	0.001773305	2.413050246	PEA15	8682	protein_coding
BID	0.352295391	6.857623723	4.316770023	3.56E-05	0.000585272	3.715694773	BID	637	protein_coding
CASP8	0.353356295	8.052167639	4.051812375	9.67E-05	0.001478683	2.792800358	CASP8	841	protein_coding
KIAA0226	0.388876499	8.122572953	4.380589995	2.78E-05	0.000495298	3.944066483	KIAA0226	NA	NA
NPC1	0.406763473	7.431832881	4.47121787	1.95E-05	0.000379055	4.272280739	NPC1	4864	protein_coding
CX3CL1	0.415361574	7.73615645	3.991200271	0.000120939	0.001725402	2.587573349	CX3CL1	6376	protein_coding
CASP3	0.420919693	6.662156248	3.794325955	0.000246036	0.002771141	1.93674565	CASP3	836	protein_coding
RGS19	0.548589872	7.535186976	5.237291409	8.25E-07	3.81E-05	7.215948134	RGS19	10287	protein_coding
CASP4	0.599115601	7.182913793	5.177444875	1.07E-06	3.81E-05	6.975869774	CASP4	837	protein_coding
IFNG	0.619354322	4.541531613	5.329171222	5.55E-07	3.81E-05	7.587591109	IFNG	3458	protein_coding
CCL2	0.619535113	9.036176742	3.295678703	0.001333675	0.012973018	0.402343758	CCL2	6347	protein_coding
APOL1	0.699076549	7.786140803	6.029879763	2.40E-08	5.13E-06	10.53324744	APOL1	8542	protein_coding
CASP1	0.819188787	8.082632942	5.20999808	9.28E-07	3.81E-05	7.106261632	CASP1	834	protein_coding
CCR2	0.938547304	6.503785444	3.967205327	0.000132038	0.00176601	2.50695199	CCR2	729230	protein_coding
CXCR4	1.313751747	7.746258705	5.118462423	1.37E-06	4.11E-05	6.740830336	CXCR4	7852	protein_coding

40 downregulated genes (Figure 1C, D; Table S2). A total of 76 *DEARGs* were identified in the GSE48581 dataset, including 56 upregulated and 20 downregulated genes (Figure 1E, F; Table S3). The box plot was constructed to demonstrate the expression pattern of the *DEARGs* between rejection and non-rejection samples (Figure 1G–I). We identified 28 common *ARGs* from the GSE21374, GSE36059, and GSE48581 datasets (Figure 1J; Table S4).

Enrichment analysis of the *DEARGs*

To investigate the function of the 28 common *ARGs* from the three datasets, GO functional annotation and KEGG pathway enrichment analyses were conducted. GO analysis demonstrated that in the BP ontology, the Encyclopedia of Genes and Genomes (DEGs) were associated with the response to tumor necrosis factor, extrinsic apoptotic signaling pathway, and neuron death. In the CC ontology, the DEGs were significantly involved with the inflammasome complex, cytosolic part, and membrane raft. In the MF ontology, these genes participated in certain key functions, such as cysteine-type endopeptidase activity involved in apoptotic process, cytokine receptor binding, and cyclin-dependent protein serine/threonine kinase inhibitor activity (Figure 2A; Table S5).

KEGG pathway analysis was used to investigate the biological pathways of the DEGs; the results of the analysis are shown in Figure 2B and Table S6. The 28 common *ARGs* were mainly enriched in signaling pathways associated with the inflammatory response and apoptosis, including the TNF signaling pathway, IL-17 signaling pathway, NOD-like receptor signaling pathway, and apoptosis. In addition, the 28 common *ARGs* were also enriched in infectious diseases, including influenza A and human cytomegalovirus infection.

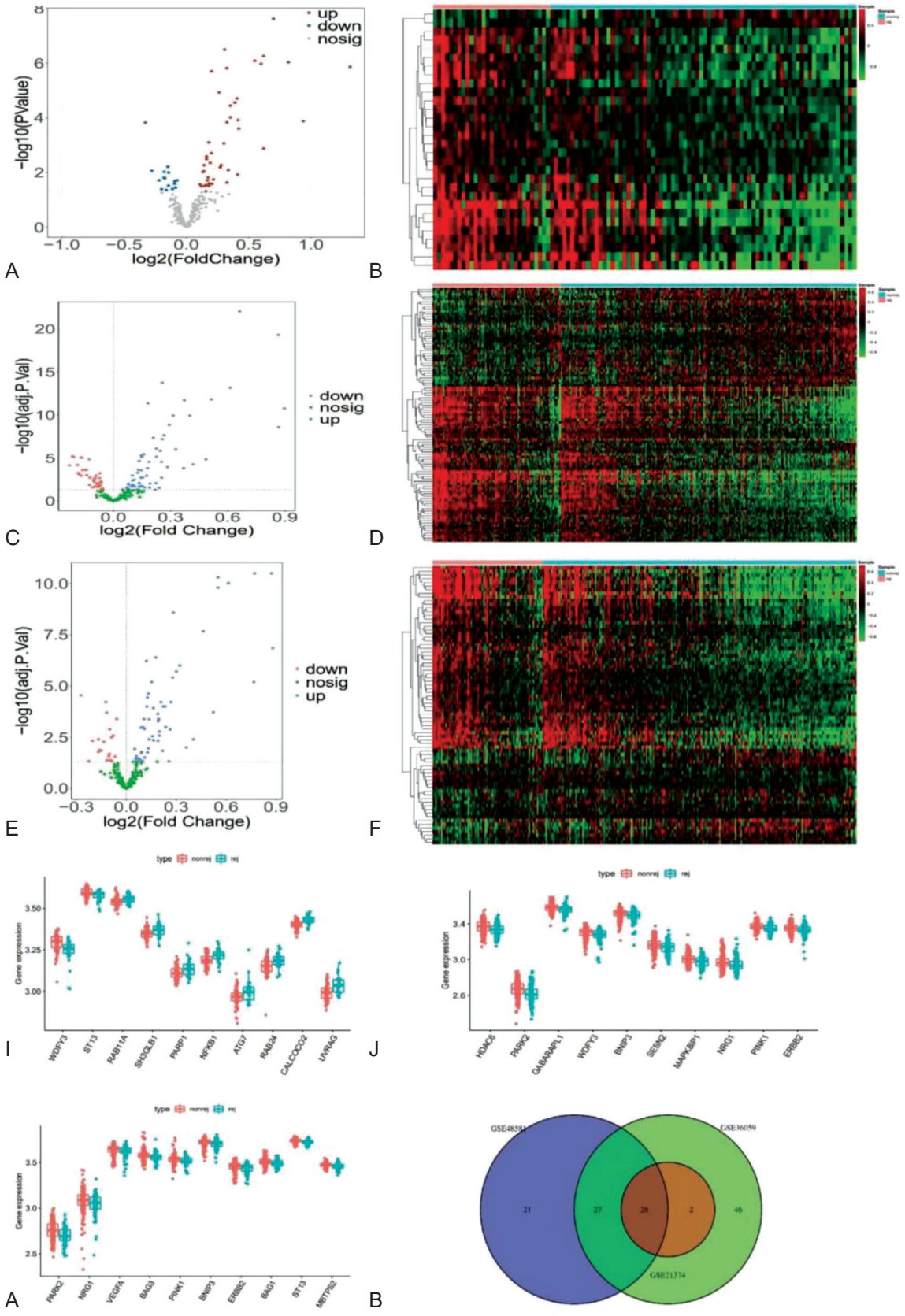


Figure 1. The identification results of DEARGs in RT patients.

Table S2. A total of 103 DEARGs identified in the GSE36059 dataset.

	logFC	AveExpr	t	*P*	adj.P.Val	B	Gene.symbol	ID	biotype
HDAC6	-0.231369363	9.252668269	-4.447322806	1.12E-05	7.08E-05	4.452765336	HDAC6	10013	protein_coding
PARK2	-0.214829874	5.305894074	-5.055845394	6.50E-07	6.63E-06	7.158160717	PARK2	NA	NA
GABARAPL1	-0.207380767	10.92542059	-5.001200212	8.50E-07	7.58E-06	6.902647436	GABARAPL1	23710	protein_coding
WDFY3	-0.188260415	8.800267378	-4.4764674	9.88E-06	6.41E-05	4.575238345	WDFY3	23001	protein_coding
BNIP3	-0.18302714	10.38774002	-3.896420415	0.000114203	0.000555443	2.275567133	BNIP3	664	protein_coding
SESN2	-0.179978292	7.90841526	-3.801263071	0.000166101	0.000772729	1.92638851	SESN2	83667	protein_coding
MAPK8IP1	-0.168775368	6.988305197	-4.957012849	1.05E-06	8.95E-06	6.697824934	MAPK8IP1	9479	protein_coding
NRG1	-0.16823468	6.816005735	-3.662743116	0.000282651	0.001186025	1.432467578	NRG1	3084	protein_coding
PINK1	-0.165189273	9.300863283	-4.950711866	1.09E-06	8.95E-06	6.66874871	PINK1	65018	protein_coding
ERBB2	-0.160484098	9.180520692	-4.118577733	4.62E-05	0.000241329	3.121806763	ERBB2	2064	protein_coding
BAG3	-0.145891627	9.463476622	-4.132675531	4.36E-05	0.000239213	3.176964783	BAG3	9531	protein_coding
BAG1	-0.142303976	8.948203303	-4.118568851	4.62E-05	0.000241329	3.121772066	BAG1	573	protein_coding
TP53INP2	-0.140041101	8.282254374	-3.73431407	0.0002152	0.000979845	1.68553371	TP53INP2	58476	protein_coding
ST13	-0.126635154	11.06067353	-4.842678279	1.83E-06	1.42E-05	6.175321689	ST13	6767	protein_coding
VEGFA	-0.125331503	10.13952307	-2.844894259	0.004668105	0.01331966	-1.129829659	VEGFA	7422	protein_coding
LAMP2	-0.123244609	10.8263206	-3.50912846	0.000500036	0.001945595	0.904797503	LAMP2	3920	protein_coding
ARSA	-0.109484586	6.647930921	-2.561763215	0.010775339	0.026203666	-1.873000797	ARSA	410	protein_coding
DNAJB1	-0.103969695	9.31448315	-3.340110114	0.000915125	0.003376496	0.348808916	DNAJB1	3337	protein_coding
MLST8	-0.102964881	7.219916507	-3.274343882	0.001150109	0.004078739	0.139480148	MLST8	64223	protein_coding
PRKAB1	-0.101443373	9.133145316	-3.170691387	0.001636446	0.005558721	-0.182420226	PRKAB1	5564	protein_coding
TP73	-0.096452927	5.96957524	-2.677905031	0.007709867	0.020120872	-1.577228664	TP73	7161	protein_coding
PEX3	-0.096207041	8.481176122	-2.642416498	0.008550821	0.021438331	-1.668949151	PEX3	8504	protein_coding
DDIT3	-0.094904364	8.24212742	-3.481240655	0.000553403	0.002114789	0.811279455	DDIT3	1649	protein_coding
GAPDH	-0.091141052	13.24942105	-3.696336769	0.000248833	0.001086738	1.550681172	GAPDH	2597	protein_coding
CLN3	-0.087930973	8.243513795	-2.992286993	0.002938418	0.009247375	-0.713366827	CLN3	1201	protein_coding
ZFYVE1	-0.082565382	8.533467972	-3.031639211	0.002588596	0.008410398	-0.598772874	ZFYVE1	53349	protein_coding
MAPK8	-0.081839247	7.607692441	-2.639822514	0.008615404	0.021438331	-1.675607001	MAPK8	5599	protein_coding
TM9SF1	-0.081802195	9.202625508	-2.964296287	0.003213041	0.009684378	-0.79400463	TM9SF1	10548	protein_coding
SIRT2	-0.078508233	8.707974412	-2.615964675	0.009230231	0.022704246	-1.73654552	SIRT2	22933	protein_coding
CANX	-0.078091513	11.75725016	-2.767591149	0.005906298	0.016630893	-1.340177854	CANX	821	protein_coding
GRID2	-0.076452222	4.449705401	-2.847332778	0.0046332	0.01331966	-1.123103668	GRID2	2895	protein_coding
NKX2-3	-0.072125807	3.60847589	-3.466682646	0.000583333	0.002190057	0.762740068	NKX2-3	159296	protein_coding
LAMP1	-0.071987085	12.52814842	-3.870111686	0.000126763	0.000602827	2.178226272	LAMP1	3916	protein_coding
ULK3	-0.065644997	7.949908795	-2.762399297	0.005999255	0.016673254	-1.354105574	ULK3	25989	protein_coding
MTOR	-0.065562676	6.137157538	-3.197505867	0.001495078	0.005160431	-0.100088743	MTOR	2475	protein_coding
NRG2	-0.064241262	5.432219948	-3.077918532	0.002226293	0.007444168	-0.462178365	NRG2	9542	protein_coding
PELP1	-0.063685292	7.383865545	-2.334762191	0.0200438	0.044220341	-2.414379809	PELP1	27043	protein_coding
TP63	-0.061721646	5.63559909	-2.356737837	0.018911648	0.042157214	-2.364101661	TP63	8626	protein_coding
EEF2	-0.059495493	12.34702097	-2.323214483	0.020662187	0.044728166	-2.440616142	EEF2	1938	protein_coding
IL24	-0.054796064	4.066191818	-2.433202497	0.015397844	0.035431599	-2.185590616	IL24	11009	protein_coding
VAMP3	0.048743151	10.52941877	2.304887786	0.021677882	0.046390668	-2.481994341	VAMP3	9341	protein_coding
MAPK1	0.070100549	9.917580996	2.51168052	0.012403998	0.029169842	-1.996628772	MAPK1	5594	protein_coding
FOXO1	0.073552642	8.205581235	2.361150654	0.018691197	0.04210438	-2.353950331	FOXO1	2308	protein_coding
RAB11A	0.079044322	10.67447107	2.965319484	0.003202603	0.009684378	-0.791069692	RAB11A	8766	protein_coding
ATF4	0.079446788	10.36130265	2.87028565	0.00431609	0.012652649	-1.059523352	ATF4	468	protein_coding
ATG7	0.0809391	6.950892798	2.401556148	0.016775343	0.038190673	-2.260142444	ATG7	10533	protein_coding
EEF2K	0.094928968	7.546861997	2.691188882	0.007414738	0.019834423	-1.542592766	EEF2K	101930123	protein_coding
PARP1	0.099942939	7.636807072	3.700564668	0.000244857	0.001086738	1.565630258	PARP1	142	protein_coding
NRG3	0.100889254	3.766383019	3.271201154	0.001162631	0.004078739	0.129575801	NRG3	10718	protein_coding
RELA	0.10167319	8.308972945	2.875905014	0.004241527	0.012606761	-1.043882855	RELA	5970	protein_coding
GNAI3	0.108499129	9.69820348	2.722057925	0.006767823	0.018542328	-1.461467693	GNAI3	2773	protein_coding
HDAC1	0.109858533	9.283747172	3.331299201	0.000943783	0.003423212	0.320535956	HDAC1	3065	protein_coding
RAB5A	0.111175475	10.11937781	2.68045018	0.007652516	0.020120872	-1.570605325	RAB5A	5868	protein_coding
CDKN1A	0.126444551	8.78743669	2.272401168	0.023585821	0.04900355	-2.554558567	CDKN1A	1026	protein_coding
TP53	0.126916842	6.498244565	2.3226654	0.020692002	0.044728166	-2.441860503	TP53	7157	protein_coding

Table S2. Continued

	logFC	AveExpr	t	*P*	adj.P.Val	B	Gene.symbol	ID	biotype
SH3GLB1	0.12817272	9.284980006	3.914198094	0.000106394	0.000529494	2.341689559	SH3GLB1	51100	protein_coding
BCL2	0.130593404	7.806060361	2.29057605	0.022501132	0.047675667	-2.51408561	BCL2	596	protein_coding
KLHL24	0.131405359	7.790519844	4.147850531	4.09E-05	0.000230413	3.236530702	KLHL24	54800	protein_coding
IKBKE	0.135493697	6.607973904	4.734294222	3.04E-06	2.25E-05	5.690030401	IKBKE	9641	protein_coding
ITGB1	0.135917879	11.64825333	3.024004953	0.002653316	0.008474772	-0.621115721	ITGB1	3688	protein_coding
ITGA6	0.136030817	8.947843708	2.667567712	0.007946798	0.020245414	-1.604067464	ITGA6	3655	protein_coding
PTEN	0.142454095	10.02155727	2.670275167	0.007884119	0.020245414	-1.597047774	PTEN	5728	protein_coding
ERO1L	0.145119082	7.809715479	3.518836307	0.000482618	0.001943071	0.937516095	ERO1L	NA	NA
ATG3	0.146429354	9.447303868	5.122170988	4.68E-07	5.01E-06	7.471567849	ATG3	64422	protein_coding
SPHK1	0.147054548	6.464343675	4.086779355	5.28E-05	0.000268807	2.998030637	SPHK1	8877	protein_coding
RAB24	0.14955245	7.946154631	5.006885836	8.27E-07	7.58E-06	6.929118576	RAB24	53917	protein_coding
EIF2AK3	0.150141037	7.588410935	2.718235646	0.006845065	0.018542328	-1.471561169	EIF2AK3	9451	protein_coding
FKBP1A	0.153174404	10.11751918	3.528905809	0.000465151	0.001914277	0.97154333	FKBP1A	2280	protein_coding
NFKB1	0.159872699	8.179543104	5.191294713	3.31E-07	3.73E-06	7.802003733	NFKB1	4790	protein_coding
TBK1	0.163357242	7.755950417	2.553272948	0.011037237	0.026538975	-1.894125156	TBK1	29110	protein_coding
CDKN1B	0.17410523	9.254585416	3.675211871	0.000269623	0.001153988	1.476226513	CDKN1B	1027	protein_coding
ERN1	0.17914753	7.110440842	4.246605927	2.69E-05	0.00015585	3.629055322	ERN1	2081	protein_coding
CALCOCO2	0.181544774	9.578316265	7.652504568	1.46E-13	4.45E-12	21.92334281	CALCOCO2	10241	protein_coding
DRAM1	0.186975694	8.625821591	4.676577121	3.98E-06	2.77E-05	5.435606558	DRAM1	55332	protein_coding
HIF1A	0.189682489	11.33861507	2.533614009	0.011665559	0.027738107	-1.942777344	HIF1A	3091	protein_coding
TNFSF10	0.191956228	11.62879875	2.985883558	0.002999276	0.009302102	-0.731878281	TNFSF10	8743	protein_coding
UVRAG	0.209575844	7.018295857	6.064034459	3.05E-09	4.66E-08	12.2994936	UVRAG	7405	protein_coding
RPS6KB1	0.219339684	7.441398433	2.277002441	0.023306994	0.048898988	-2.544341862	RPS6KB1	6198	protein_coding
HSPB8	0.223827079	9.301450529	3.031011587	0.002593861	0.008410398	-0.600611745	HSPB8	26353	protein_coding
ATG16L2	0.225451735	6.004957946	5.520905806	6.04E-08	8.07E-07	9.430463343	ATG16L2	89849	protein_coding
CASP8	0.225779852	7.973600141	5.031365786	7.34E-07	7.14E-06	7.043395108	CASP8	841	protein_coding
KIF5B	0.240990349	9.200631967	2.499133322	0.01284486	0.029878262	-2.027230848	KIF5B	3799	protein_coding
KIAA0226	0.246115175	8.122625802	5.386616487	1.22E-07	1.45E-06	8.756535229	KIAA0226	NA	NA
NPC1	0.252154353	7.555950084	4.674501699	4.02E-06	2.77E-05	5.426509902	NPC1	4864	protein_coding
FAS	0.253472072	8.349840932	3.514510716	0.000490308	0.001943071	0.922926957	FAS	355	protein_coding
CFLAR	0.256186993	9.173379337	8.549741184	2.56E-16	1.82E-14	28.09219421	CFLAR	8837	protein_coding
PEA15	0.256467151	9.852510361	5.986560636	4.73E-09	6.74E-08	11.87642539	PEA15	8682	protein_coding
NLRC4	0.266168266	4.135498517	6.199329869	1.40E-09	2.30E-08	13.04912347	NLRC4	58484	protein_coding
ATG12	0.283635379	7.165941069	4.331580898	1.87E-05	0.000114275	3.973554911	ATG12	9140	protein_coding
IKBKB	0.293267215	8.366949563	6.685317595	7.66E-11	1.49E-09	15.85282254	IKBKB	3551	protein_coding
BID	0.313829733	6.936768469	7.117359485	5.04E-12	1.20E-10	18.48628628	BID	637	protein_coding
CASP3	0.327196494	6.73192342	5.470321174	7.88E-08	9.92E-07	9.174928565	CASP3	836	protein_coding
NAMPT	0.368492942	8.011446485	4.256493055	2.58E-05	0.000153551	3.668818864	NAMPT	10135	protein_coding
CX3CL1	0.372023105	7.658516128	7.791569678	5.63E-14	2.01E-12	22.84700082	CX3CL1	6376	protein_coding
RGS19	0.400718743	7.633530266	7.097959597	5.71E-12	1.22E-10	18.365275	RGS19	10287	protein_coding
RB1	0.418232039	8.032006645	4.521521655	8.07E-06	5.40E-05	4.765990501	RB1	5925	protein_coding
CCL2	0.485569777	8.916434733	4.839039378	1.86E-06	1.42E-05	6.158869814	CCL2	6347	protein_coding
CASP4	0.514189649	6.993288667	7.853765657	3.66E-14	1.57E-12	23.26406091	CASP4	837	protein_coding
IFNG	0.612884949	4.867583512	8.320223807	1.36E-15	7.26E-14	26.46795711	IFNG	3458	protein_coding
APOL1	0.6626463	7.758922144	11.0716606	4.55E-25	9.73E-23	47.7469576	APOL1	8542	protein_coding
CASP1	0.865555249	8.173438122	10.24006714	4.88E-22	5.22E-20	40.93165216	CASP1	834	protein_coding
CXCR4	0.866912855	7.610124116	6.574040134	1.51E-10	2.70E-09	15.19577031	CXCR4	7852	protein_coding
CCR2	0.897127226	5.961716524	7.429122568	6.54E-13	1.75E-11	20.46568603	CCR2	729230	protein_coding

Construction of the prognostic gene signature

Univariate Cox analysis was used to identify potential prognostic *DEARGs* in RT patients. Twenty-two genes were screened by univariate Cox analysis with a $P < 0.1$ (Table 1). LASSO regression analysis was performed on the 21 *DEARGs*. The penalty parameter lambda was selected by a cross-validation method, and we obtained eight relatively independent gene signatures for subsequent analysis (Figure 3A, B). Next, we eliminated CDKN1 in the

Table S3. A total of 76 DEARGs identified in the GSE48581 dataset.

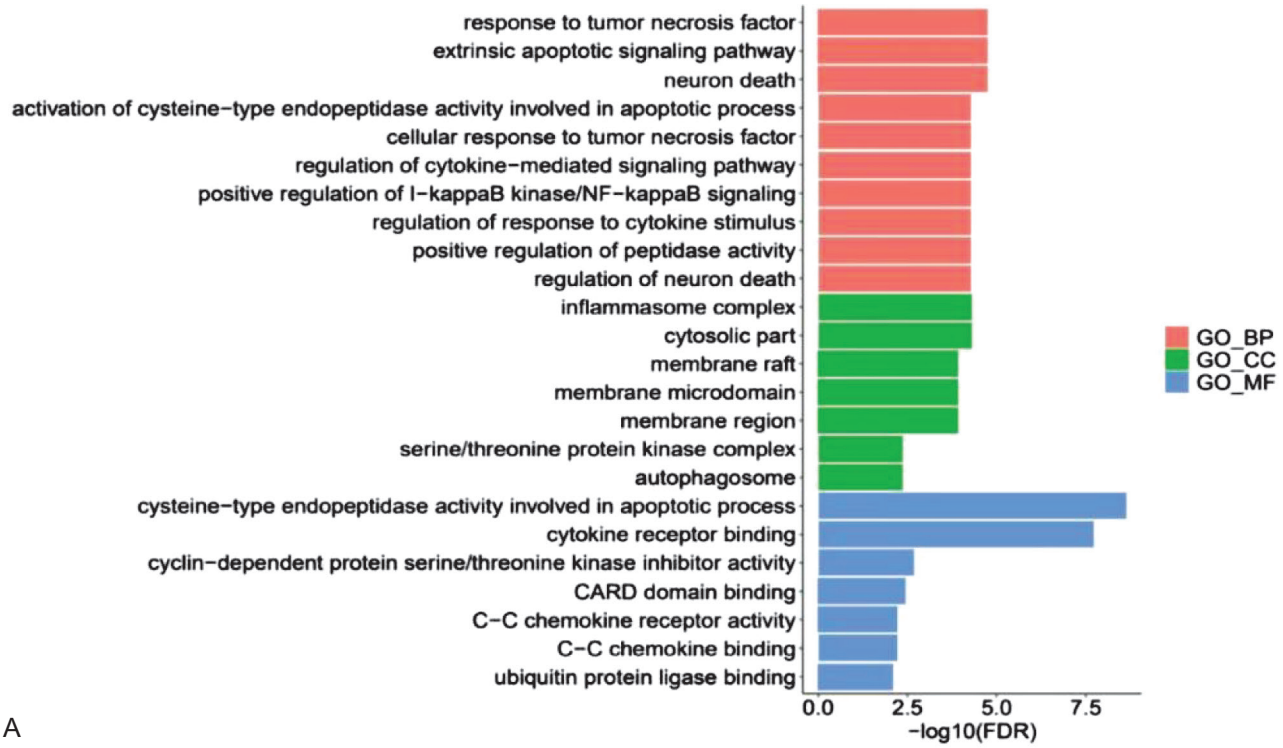
	logFC	AveExpr	t	*P*	adj.P.Val	B	Gene. symbol	ID	biotype
PARK2	-0.272904673	5.700223242	-4.820838714	2.27E-06	2.86E-05	6.07576296	PARK2	NA	NA
NRG1	-0.225608873	7.407102028	-2.417116604	0.01623748	0.046957036	-2.091442178	NRG1	3084	protein_coding
VEGFA	-0.203969788	11.40147902	-3.292275101	0.001111996	0.004759344	0.301435604	VEGFA	7422	protein_coding
BAG3	-0.167767079	10.90739456	-2.863279596	0.004486834	0.01639172	-0.958293472	BAG3	9531	protein_coding
PINK1	-0.166905221	10.5329094	-3.3655735	0.000862785	0.004013825	0.533048031	PINK1	65018	protein_coding
BNIP3	-0.161402471	12.09027724	-2.949259713	0.003434766	0.013364362	-0.719047379	BNIP3	664	protein_coding
ERBB2	-0.14955889	9.918567355	-2.922214724	0.003738437	0.014286171	-0.795022499	ERBB2	2064	protein_coding
BAG1	-0.134952724	10.33496272	-3.252269163	0.001274767	0.005349022	0.177021794	BAG1	573	protein_coding
ST13	-0.122972222	12.28419785	-4.607663168	6.02E-06	6.13E-05	5.155336727	ST13	6767	protein_coding
MBTPS2	-0.117550329	10.0712236	-4.279496582	2.52E-05	0.000199676	3.809317223	MBTPS2	51360	protein_coding
TP53INP2	-0.117205724	9.006890544	-2.476039968	0.013833938	0.041696658	-1.952290961	TP53INP2	58476	protein_coding
NCKAP1	-0.10497117	11.0797016	-2.677504783	0.007823748	0.026575906	-1.452299874	NCKAP1	10787	protein_coding
EGFR	-0.100886657	9.379567261	-2.769405248	0.005964084	0.020585709	-1.211852101	EGFR	1956	protein_coding
MAPK8	-0.099345212	8.976505883	-3.725774365	0.000232438	0.001308993	1.739360814	MAPK8	5599	protein_coding
PEX3	-0.096151981	9.608250531	-2.477551438	0.013776673	0.041696658	-1.948679213	PEX3	8504	protein_coding
HSP90AB1	-0.088720857	12.16978246	-3.448042258	0.000645056	0.003137319	0.799278427	HSP90AB1	3326	protein_coding
TUSC1	-0.08781112	10.10261273	-2.425447965	0.015876659	0.046542533	-2.07196257	TUSC1	286319	protein_coding
TSC1	-0.077485659	9.746905875	-3.508180328	0.000519937	0.002649204	0.997167097	TSC1	7248	protein_coding
ATG2B	-0.067537633	9.507635475	-2.646654317	0.008556333	0.028170081	-1.531284411	ATG2B	55102	protein_coding
EEF2	-0.057836272	13.79761593	-4.071878214	5.97E-05	0.000411808	3.003135151	EEF2	1938	protein_coding
GABARAP	0.050506803	12.78187951	2.993390598	0.002987347	0.011838745	-0.593658265	GABARAP	11337	protein_coding
SPNS1	0.057742294	8.267023341	2.540072864	0.011584856	0.036458224	-1.797429857	SPNS1	83985	protein_coding
MTMR14	0.060333611	9.569845391	2.665256295	0.008107563	0.027109663	-1.483763348	MTMR14	64419	protein_coding
CFLAR	0.075455932	10.37794812	2.552718703	0.011181359	0.035713593	-1.766398679	CFLAR	8837	protein_coding
GAA	0.079080465	9.18204496	2.387894274	0.017560958	0.04944796	-2.159257563	GAA	2548	protein_coding
FADD	0.082332316	9.438547398	2.509438552	0.012616629	0.039129836	-1.871990503	FADD	8772	protein_coding
CALCOCO2	0.087891879	10.47736085	3.791040669	0.000181197	0.001077113	1.969911216	CALCOCO2	10241	protein_coding
PARP1	0.090147152	9.333092028	2.810944249	0.005263181	0.018464275	-1.100641591	PARP1	142	protein_coding
CDKN2A	0.092325892	6.729491106	2.860937791	0.004519213	0.01639172	-0.964716124	CDKN2A	1029	protein_coding
EDEM1	0.098041746	9.288882155	2.427403949	0.015792981	0.046542533	-2.067379936	EDEM1	9695	protein_coding
RELA	0.099441181	9.058808926	3.80850486	0.000169415	0.001035855	2.032218954	RELA	5970	protein_coding
ATG16L1	0.106517395	7.454954852	4.072424427	5.95E-05	0.000411808	3.005209239	ATG16L1	55054	protein_coding
RAB11A	0.10870116	11.60658327	3.748283892	0.000213391	0.001234206	1.818463631	RAB11A	8766	protein_coding
HDAC1	0.110968905	10.63463563	2.605802692	0.009621079	0.031195619	-1.634530342	HDAC1	3065	protein_coding
RAB24	0.120335212	9.686478098	5.701779043	2.83E-08	6.05E-07	10.24689799	RAB24	53917	protein_coding
ATG7	0.123114551	8.190480418	4.475557181	1.08E-05	0.00010066	4.603040559	ATG7	10533	protein_coding
BAK1	0.12689881	7.801931582	4.756831022	3.05E-06	3.63E-05	5.795642486	BAK1	578	protein_coding
EIF2AK3	0.127396587	9.281576216	2.83499956	0.004892336	0.017449333	-1.035521815	EIF2AK3	9451	protein_coding
ATG12	0.132546737	9.669699505	3.02108791	0.002734522	0.011041277	-0.514066739	ATG12	9140	protein_coding
IKBKE	0.133602388	7.426071702	4.873688594	1.77E-06	2.37E-05	6.309461326	IKBKE	9641	protein_coding
IKBKB	0.136797056	9.530568566	3.358039989	0.000885765	0.004033056	0.509025214	IKBKB	3551	protein_coding
NFKB1	0.137638744	9.201718858	4.054480331	6.40E-05	0.000428154	2.937201881	NFKB1	4790	protein_coding
UVRAG	0.152861205	7.944158017	5.158694572	4.52E-07	6.44E-06	7.60680028	UVRAG	7405	protein_coding
TP53	0.157763455	6.637573843	3.482547926	0.000570196	0.002837721	0.912436385	TP53	7157	protein_coding
ERO1L	0.162580627	9.326327852	4.416315665	1.40E-05	0.000119936	4.359922344	ERO1L	NA	NA
NRG3	0.16266045	5.230742035	3.324014968	0.000996833	0.004444212	0.401148181	NRG3	10718	protein_coding
ATG3	0.176940004	10.53003002	5.795343791	1.72E-08	4.08E-07	10.72327378	ATG3	64422	protein_coding
BIRC5	0.189329242	6.196907527	4.237933167	3.00E-05	0.000229439	3.645080203	BIRC5	332	protein_coding
RB1	0.189630231	10.16849975	3.301870645	0.001075938	0.004698996	0.331486845	RB1	5925	protein_coding
ATG16L2	0.191346211	7.762654741	4.001867459	7.92E-05	0.000513349	2.739351785	ATG16L2	89849	protein_coding
ERN1	0.194979857	8.4758524	4.084057016	5.68E-05	0.000411808	3.049439628	ERN1	2081	protein_coding
DRAM1	0.199176786	10.34060082	3.514299538	0.000508568	0.002649204	1.017479355	DRAM1	55332	protein_coding
BAX	0.200114084	8.607569498	3.648057788	0.000311261	0.001665247	1.469588912	BAX	581	protein_coding
KIAA0226	0.208214726	9.54498711	4.616383373	5.79E-06	6.13E-05	5.19228361	KIAA0226	NA	NA

Table S3. Continued

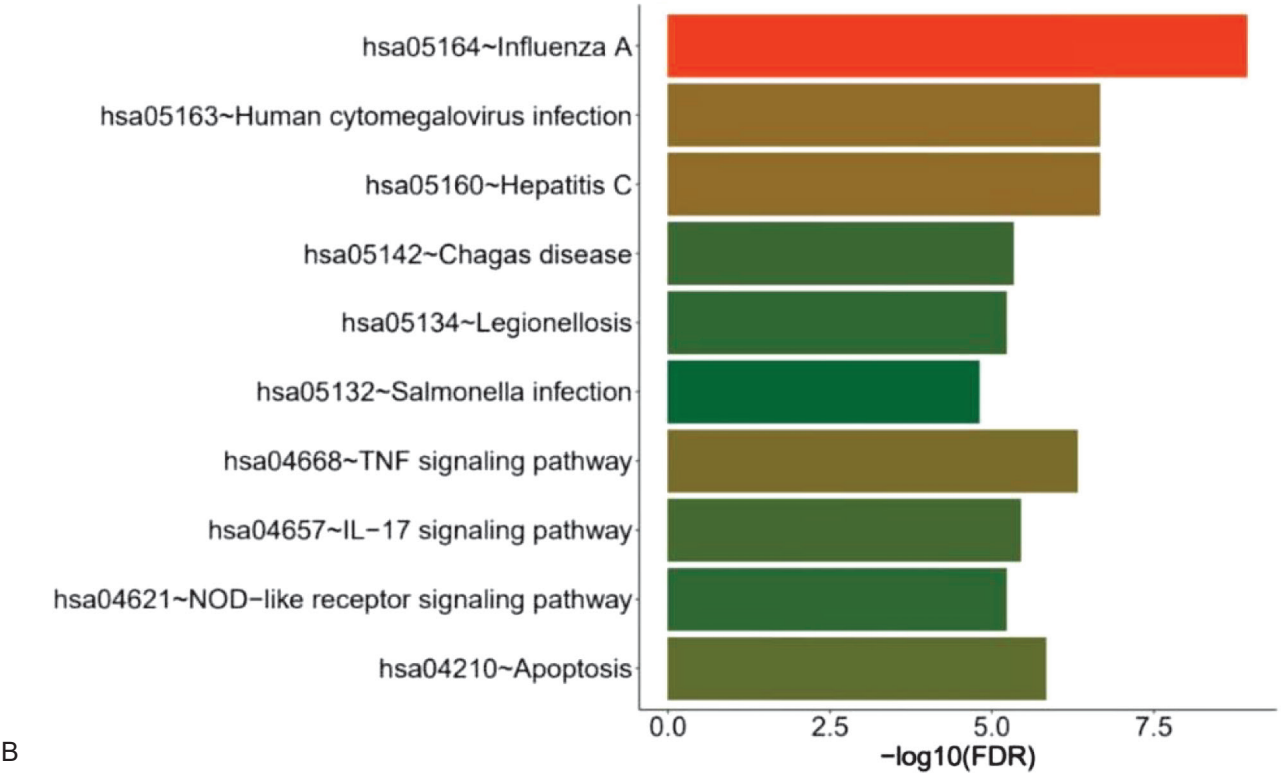
	logFC	AveExpr	t	*P*	adj.P.Val	B	Gene. symbol	ID	biotype
FAS	0.218189162	9.356948041	2.910445259	0.003878092	0.014559854	-0.82787914	FAS	355	protein_coding
PEA15	0.221476359	10.74061525	4.465447031	1.13E-05	0.000100838	4.56134967	PEA15	8682	protein_coding
CX3CL1	0.233318027	8.792024454	4.488667485	1.02E-05	9.93E-05	4.657225617	CX3CL1	6376	protein_coding
CASP3	0.236414601	8.469345203	3.818070707	0.000163273	0.001027661	2.066457454	CASP3	836	protein_coding
MYC	0.255872939	9.121710941	2.408988301	0.016596467	0.047355253	-2.110384918	MYC	4609	protein_coding
CDKN1A	0.263836359	9.807312185	3.714127361	0.000242912	0.001332903	1.698601255	CDKN1A	1026	protein_coding
NPC1	0.26844271	8.920921463	4.627742937	5.50E-06	6.13E-05	5.240503913	NPC1	4864	protein_coding
BID	0.278541456	8.27472636	5.292391405	2.33E-07	3.83E-06	8.236593841	BID	637	protein_coding
FKBP1A	0.283694652	9.882307551	6.756527201	7.30E-11	2.60E-09	15.96151672	FKBP1A	2280	protein_coding
CASP8	0.300694844	9.251866827	5.439480211	1.11E-07	1.97E-06	8.944816891	CASP8	841	protein_coding
SPHK1	0.320914519	6.403602445	5.584844453	5.23E-08	1.02E-06	9.660293453	SPHK1	8877	protein_coding
SERPINA1	0.361110952	11.56159091	3.059205946	0.002418634	0.00995361	-0.403403726	SERPINA1	5265	protein_coding
FOS	0.403133769	7.271974129	3.373108834	0.000840356	0.003996362	0.557126531	FOS	2353	protein_coding
NLRC4	0.46229955	6.160972572	6.369798312	7.06E-10	2.16E-08	13.78048227	NLRC4	58484	protein_coding
CCL2	0.523216751	9.784788288	4.298385095	2.33E-05	0.00019139	3.884422713	CCL2	6347	protein_coding
RGS19	0.550967067	9.053553538	7.501636039	7.12E-13	5.08E-11	20.42248017	RGS19	10287	protein_coding
CASP4	0.551081147	8.41757473	7.240397802	3.75E-12	1.60E-10	18.82120251	CASP4	837	protein_coding
APOL1	0.613159354	8.366863495	7.3561268	1.80E-12	9.65E-11	19.52575111	APOL1	8542	protein_coding
CCR2	0.766506169	8.099884134	5.165894141	4.36E-07	6.44E-06	7.640373006	CCR2	729230	protein_coding
CASP1	0.77112276	9.607618294	7.723018964	1.69E-13	3.20E-11	21.80949615	CASP1	834	protein_coding
IFNG	0.872435755	5.752795798	7.635925773	2.99E-13	3.20E-11	21.26061393	IFNG	3458	protein_coding
CXCR4	0.880241716	9.588015733	6.012319168	5.28E-09	1.41E-07	11.85156372	CXCR4	7852	protein_coding

Table S4. A total of 28 common ARGs in the GSE21374, GSE36059, and GSE48581 datasets.

	GSE21374 logFC	GSE21374 adj.P.Val	GSE36059 logFC	GSE36059 adj.*P*.Val	GSE48581 logFC	GSE48581 adj.*P*.Val
ST13	-0.148856991	0.045243877	-0.126635154	1.42E-05	-0.122972222	6.13E-05
RAB11A	0.142086207	0.041000757	0.079044322	0.009684378	0.10870116	0.001234206
PARP1	0.162370347	0.027566707	0.099942939	0.001086738	0.090147152	0.018464275
NFKB1	0.180185644	0.008437823	0.159872699	3.73E-06	0.137638744	0.000428154
ATG7	0.190996309	0.036010034	0.0809391	0.038190673	0.123114551	0.00010066
RAB24	0.201491084	0.018114161	0.14955245	7.58E-06	0.120335212	6.05E-07
CALCOCO2	0.202319141	4.72E-05	0.181544774	4.45E-12	0.087891879	0.001077113
UVRAG	0.262851684	0.000251234	0.209575844	4.66E-08	0.152861205	6.44E-06
FKBP1A	0.271107891	0.046332374	0.153174404	0.001914277	0.283694652	2.60E-09
CDKN1A	0.279946151	0.041000757	0.126444551	0.04900355	0.263836359	0.001332903
IKBKB	0.301068236	0.008707615	0.293267215	1.49E-09	0.136797056	0.004033056
CFLAR	0.307650828	3.43E-05	0.256186993	1.82E-14	0.075455932	0.035713593
NLRC4	0.32450314	4.11E-05	0.266168266	2.30E-08	0.46229955	2.16E-08
PEA15	0.32536937	0.001773305	0.256467151	6.74E-08	0.221476359	0.000100838
BID	0.352295391	0.000585272	0.313829733	1.20E-10	0.278541456	3.83E-06
CASP8	0.353356295	0.001478683	0.225779852	7.14E-06	0.300694844	1.97E-06
KIAA0226	0.388876499	0.000495298	0.246115175	1.45E-06	0.208214726	6.13E-05
NPC1	0.406763473	0.000379055	0.252154353	2.77E-05	0.26844271	6.13E-05
CX3CL1	0.415361574	0.001725402	0.372023105	2.01E-12	0.233318027	9.93E-05
CASP3	0.420919693	0.002771141	0.327196494	9.92E-07	0.236414601	0.001027661
RGS19	0.548589872	3.81E-05	0.400718743	1.22E-10	0.550967067	5.08E-11
CASP4	0.599115601	3.81E-05	0.514189649	1.57E-12	0.551081147	1.60E-10
IFNG	0.619354322	3.81E-05	0.612884949	7.26E-14	0.872435755	3.20E-11
CCL2	0.619535113	0.012973018	0.485569777	1.42E-05	0.523216751	0.00019139
APOL1	0.699076549	5.13E-06	0.6626463	9.73E-23	0.613159354	9.65E-11
CASP1	0.819188787	3.81E-05	0.865555249	5.22E-20	0.77112276	3.20E-11
CCR2	0.938547304	0.00176601	0.897127226	1.75E-11	0.766506169	6.44E-06
CXCR4	1.313751747	4.11E-05	0.866912855	2.70E-09	0.880241716	1.41E-07



A



B

Figure 2. The results of KEGG pathway analysis.

Table S5. The functions of 28 common ARGs in three datasets.

Terms	Counts	Gene Ratio	BgRatio	*P*	FDR	fold Enrichment	gene ID	gene Symbol	Category
GO:0034612~response to tumor necrosis factor	8	8.27	306/18012	1.06E-08	1.77E-05	17.44081336	ENSG00000109320/ENSG00000104365/ ENSG00000064012/ENSG00000006210/ ENSG00000164305/ENSG00000196954/ ENSG00000108691/ENSG00000137752	NFKB1/IKBKB/CASP8/ CX3CL1/CASP3/ CASP4/CCL2/CASP1	GO_BP
GO:0097191~extrinsic apoptotic signaling pathway	7	7.27	223/18012	2.92E-08	1.77E-05	20.94070752	ENSG00000003402/ENSG00000162734/ ENSG00000015475/ENSG00000064012/ ENSG00000006210/ENSG00000164305/ ENSG00000111537	CFLAR/PEA15/BID/ CASP8/CX3CL1/ CASP3/IFNG	GO_BP
GO:0070997~neuron death	8	8.27	349/18012	2.95E-08	1.77E-05	15.29194524	ENSG00000143799/ENSG00000197548/ ENSG00000015475/ENSG00000064012/ ENSG00000006210/ENSG00000164305/ ENSG00000111537/ENSG00000108691	PARP1/ATG7/BID/ CASP8/CX3CL1/ CASP3/IFNG/CCL2	GO_BP
GO:0006919~activation of cysteine-type endopeptidase activity involved in apoptotic process	5	5.27	85/18012	1.55E-07	5.42E-05	39.24183007	ENSG00000091106/ENSG00000015475/ ENSG00000064012/ENSG00000196954/ ENSG00000137752	NLRC4/BID/CASP8/ CASP4/CASP1	GO_BP
GO:0071356~cellular response to tumor necrosis factor	7	7.27	286/18012	1.60E-07	5.42E-05	16.32789433	ENSG00000109320/ENSG00000104365/ ENSG00000064012/ENSG00000006210/ ENSG00000196954/ENSG00000108691/ ENSG00000137752	NFKB1/IKBKB/CASP8/ CX3CL1/CASP4/CCL2/ CASP1	GO_BP
GO:0001959~regulation of cytokine-mediated signaling pathway	6	6.27	174/18012	1.86E-07	5.42E-05	23.00383142	ENSG00000104365/ENSG00000064012/ ENSG00000196954/ENSG00000111537/ ENSG00000137752/ENSG00000121966	IKBKB/CASP8/CASP4/ IFNG/CASP1/CXCR4	GO_BP
GO:0043123~positive regulation of I-kappaB kinase/NF-kappaB signaling	6	6.27	184/18012	2.59E-07	5.42E-05	21.75362319	ENSG00000088832/ENSG00000104365/ ENSG00000003402/ENSG00000064012/ ENSG00000006210/ENSG00000137752	FKBP1A/IKBKB/CFLAR/ CASP8/CX3CL1/CASP1	GO_BP
GO:0060759~regulation of response to cytokine stimulus	6	6.27	187/18012	2.85E-07	5.42E-05	21.40463458	ENSG00000104365/ENSG00000064012/ ENSG00000196954/ENSG00000111537/ ENSG00000137752/ENSG00000121966	IKBKB/CASP8/CASP4/ IFNG/CASP1/CXCR4	GO_BP
GO:0010952~positive regulation of peptidase activity	6	6.27	189/18012	3.04E-07	5.42E-05	21.17813051	ENSG00000003402/ENSG00000091106/ ENSG00000015475/ENSG00000064012/ ENSG00000196954/ENSG00000137752	CFLAR/NLRC4/BID/ CASP8/CASP4/CASP1	GO_BP
GO:1901214~regulation of neuron death	7	7.27	315/18012	3.08E-07	5.42E-05	14.82469136	ENSG00000143799/ENSG00000197548/ ENSG00000064012/ENSG00000006210/ ENSG00000164305/ENSG00000111537/ ENSG00000108691	PARP1/ATG7/CASP8/ CX3CL1/CASP3/IFNG/ CCL2	GO_BP
GO:0032611~interleukin-1 beta production	5	5.27	99/18012	3.32E-07	5.42E-05	33.69248036	ENSG00000091106/ENSG00000064012/ ENSG00000006210/ENSG00000111537/ ENSG00000137752	NLRC4/CASP8/ CX3CL1/IFNG/CASP1	GO_BP
GO:0006914~autophagy	8	8.27	496/18012	4.38E-07	6.04E-05	10.75985663	ENSG00000197548/ENSG00000169228/ ENSG00000136436/ENSG00000198382/ ENSG00000141458/ENSG00000164305/ ENSG00000171700/ENSG00000111537	ATG7/RAB24/ CALCOCO2/UVRAG/ NPC1/CASP3/RGS19/ IFNG	GO_BP
GO:0061919~process utilizing autophagic mechanism	8	8.27	496/18012	4.38E-07	6.04E-05	10.75985663	ENSG00000197548/ENSG00000169228/ ENSG00000136436/ENSG00000198382/ ENSG00000141458/ENSG00000164305/ ENSG00000171700/ENSG00000111537	ATG7/RAB24/ CALCOCO2/UVRAG/ NPC1/CASP3/RGS19/ IFNG	GO_BP

Table S5. Continued

Terms	Counts	Gene Ratio	BgRatio	*P*	FDR	fold Enrichment	gene ID	gene Symbol	Category
GO:0051092~positive regulation of NF-kappaB transcription factor activity	5	5.27	150/18012	2.61E-06	0.00018721	22.23703704	ENSG00000109320/ENSG00000104365/ ENSG00000003402/ENSG00000091106/ ENSG00000006210	NFKB1/IKBKB/CFLAR/ NLRC4/CX3CL1	GO_BP
GO:0032496~response to lipopolysaccharide	6	6.27	325/18012	7.08E-06	0.000440914	12.31589744	ENSG00000109320/ENSG00000064012/ ENSG00000006210/ENSG00000164305/ ENSG00000108691/ENSG00000137752	NFKB1/CASP8/ CX3CL1/CASP3/CCL2/ CASP1	GO_BP
GO:0010506~regulation of autophagy	6	6.27	327/18012	7.34E-06	0.000440914	12.24057085	ENSG00000197548/ENSG00000136436/ ENSG00000198382/ENSG00000141458/ ENSG00000164305/ENSG00000111537	ATG7/CALCOCO2/ UVRAG/NPC1/CASP3/ IFNG	GO_BP
GO:0071214~cellular response to abiotic stimulus	6	6.27	331/18012	7.86E-06	0.000440914	12.09264854	ENSG00000143799/ENSG00000109320/ ENSG00000124762/ENSG00000064012/ ENSG00000164305/ENSG00000137752	PARP1/NFKB1/ CDKN1A/CASP8/ CASP3/CASP1	GO_BP
GO:0104004~cellular response to environmental stimulus	6	6.27	331/18012	7.86E-06	0.000440914	12.09264854	ENSG00000143799/ENSG00000109320/ ENSG00000124762/ENSG00000064012/ ENSG00000164305/ENSG00000137752	PARP1/NFKB1/ CDKN1A/CASP8/ CASP3/CASP1	GO_BP
GO:0032651~regulation of interleukin-1 beta production	4	4.27	88/18012	8.57E-06	0.000451999	30.32323232	ENSG00000064012/ENSG00000006210/ ENSG00000111537/ENSG00000137752	CASP8/CX3CL1/IFNG/ CASP1	GO_BP
GO:0034341~response to interferon-gamma	5	5.27	195/18012	9.40E-06	0.000468293	17.10541311	ENSG00000136436/ENSG00000006210/ ENSG00000111537/ENSG00000108691/ ENSG00000137752	CALCOCO2/CX3CL1/ IFNG/CCL2/CASP1	GO_BP
GO:0051701~interaction with host	5	5.27	209/18012	1.31E-05	0.000589683	15.95959596	ENSG00000197548/ENSG00000198382/ ENSG00000064012/ENSG00000141458/ ENSG00000121966	ATG7/UVRAG/CASP8/ NPC1/CXCR4	GO_BP
GO:0014072~response to isoquinoline alkaloid	3	3.27	34/18012	1.74E-05	0.000694715	58.8627451	ENSG00000197548/ENSG00000162734/ ENSG00000121966	ATG7/PEA15/CXCR4	GO_BP
GO:0043278~response to morphine	3	3.27	34/18012	1.74E-05	0.000694715	58.8627451	ENSG00000197548/ENSG00000162734/ ENSG00000121966	ATG7/PEA15/CXCR4	GO_BP
GO:1902991~regulation of amyloid precursor protein catabolic process	3	3.27	35/18012	1.90E-05	0.000742584	57.18095238	ENSG00000088832/ENSG00000164305/ ENSG00000111537	FKBP1A/CASP3/IFNG	GO_BP
GO:2000310~regulation of NMDA receptor activity	3	3.27	36/18012	2.08E-05	0.000775562	55.59259259	ENSG00000111537/ENSG00000108691/ ENSG00000121807	IFNG/CCL2/CCR2	GO_BP
GO:1905477~positive regulation of protein localization to membrane	4	4.27	121/18012	3.02E-05	0.000984181	22.05325987	ENSG00000103769/ENSG00000015475/ ENSG00000064012/ENSG00000111537	RAB11A/BID/CASP8/ IFNG	GO_BP
GO:0042987~amyloid precursor protein catabolic process	3	3.27	45/18012	4.09E-05	0.001264179	44.47407407	ENSG00000088832/ENSG00000164305/ ENSG00000111537	FKBP1A/CASP3/IFNG	GO_BP
GO:0060359~response to ammonium ion	4	4.27	136/18012	4.77E-05	0.001449965	19.62091503	ENSG00000197548/ENSG00000162734/ ENSG00000164305/ENSG00000121966	ATG7/PEA15/CASP3/ CXCR4	GO_BP
GO:0042110~T cell activation	6	6.27	462/18012	5.16E-05	0.001517732	8.663780664	ENSG00000088832/ENSG00000064012/ ENSG00000164305/ENSG00000111537/ ENSG00000108691/ENSG00000121807	FKBP1A/CASP8/ CASP3/IFNG/CCL2/ CCR2	GO_BP
GO:1904064~positive regulation of cation transmembrane transport	4	4.27	142/18012	5.64E-05	0.001591493	18.79186228	ENSG00000006210/ENSG00000111537/ ENSG00000108691/ENSG00000121807	CX3CL1/IFNG/CCL2/ CCR2	GO_BP
GO:0050769~positive regulation of neurogenesis	6	6.27	470/18012	5.68E-05	0.001591493	8.516312057	ENSG00000103769/ENSG00000003402/ ENSG00000006210/ENSG00000111537/ ENSG00000121807/ENSG00000121966	RAB11A/CFLAR/ CX3CL1/IFNG/CCR2/ CXCR4	GO_BP

Table S5. Continued

Terms	Counts	Gene Ratio	BgRatio	*P*	FDR	fold Enrichment	gene ID	gene Symbol	Category
GO:1905517~macrophage migration	3	3.27	51/18012	5.96E-05	0.00160823	39.24183007	ENSG00000006210/ENSG00000108691/ ENSG00000121807	CX3CL1/CCL2/CCR2	GO_BP
GO:0042063~gliogenesis	5	5.27	288/18012	6.11E-05	0.001610926	11.58179012	ENSG00000006210/ENSG00000111537/ ENSG00000108691/ENSG00000121807/ ENSG00000121966	CX3CL1/IFNG/CCL2/ CCR2/CXCR4	GO_BP
GO:0031960~response to corticosteroid	4	4.27	162/18012	9.42E-05	0.002271026	16.47187929	ENSG00000143799/ENSG00000124762/ ENSG00000003402/ENSG00000164305	PARP1/CDKN1A/ CFLAR/CASP3	GO_BP
GO:0032025~response to cobalt ion	2	2.27	10/18012	9.67E-05	0.002271026	133.4222222	ENSG00000064012/ENSG00000164305	CASP8/CASP3	GO_BP
GO:0035821~modification of morphology or physiology of other organism	4	4.27	164/18012	9.87E-05	0.002271026	16.27100271	ENSG00000197548/ENSG00000064012/ ENSG00000111537/ENSG00000100342	ATG7/CASP8/IFNG/ APOL1	GO_BP
GO:0045123~cellular extravasation	3	3.27	61/18012	0.000102017	0.002316691	32.80874317	ENSG00000006210/ENSG00000108691/ ENSG00000121807	CX3CL1/CCL2/CCR2	GO_BP
GO:1903829~positive regulation of cellular protein localization	5	5.27	322/18012	0.000103411	0.002319002	10.35886818	ENSG00000103769/ENSG00000143799/ ENSG00000015475/ENSG00000064012/ ENSG00000111537	RAB11A/PARP1/BID/ CASP8/IFNG	GO_BP
GO:0016241~regulation of macroautophagy	4	4.27	171/18012	0.000116052	0.002462104	15.60493827	ENSG00000136436/ENSG00000198382/ ENSG00000141458/ENSG00000164305	CALCOCO2/UVRAG/ NPC1/CASP3	GO_BP
GO:1901096~regulation of autophagosome maturation	2	2.27	11/18012	0.000118027	0.002462104	121.2929293	ENSG00000136436/ENSG00000198382	CALCOCO2/UVRAG	GO_BP
GO:0071496~cellular response to external stimulus	5	5.27	338/18012	0.000129837	0.002559652	9.868507561	ENSG00000109320/ENSG00000197548/ ENSG00000124762/ENSG00000064012/ ENSG00000137752	NFKB1/ATG7/CDKN1A/ CASP8/CASP1	GO_BP
GO:0042368~vitamin D biosynthetic process	2	2.27	12/18012	0.000141502	0.002700576	111.1851852	ENSG00000109320/ENSG00000111537	NFKB1/IFNG	GO_BP
GO:1905475~regulation of protein localization to membrane	4	4.27	186/18012	0.000160437	0.002998164	14.34647551	ENSG00000103769/ENSG00000015475/ ENSG00000064012/ENSG00000111537	RAB11A/BID/CASP8/ IFNG	GO_BP
GO:0007183~SMAD protein complex assembly	2	2.27	13/18012	0.000167075	0.00305849	102.6324786	ENSG00000143799/ENSG00000088832	PARP1/FKBP1A	GO_BP
GO:0030656~regulation of vitamin metabolic process	2	2.27	13/18012	0.000167075	0.00305849	102.6324786	ENSG00000109320/ENSG00000111537	NFKB1/IFNG	GO_BP
GO:0042362~fat-soluble vitamin biosynthetic process	2	2.27	15/18012	0.000224493	0.003835615	88.94814815	ENSG00000109320/ENSG00000111537	NFKB1/IFNG	GO_BP
GO:0051770~positive regulation of nitric-oxide synthase biosynthetic process	2	2.27	15/18012	0.000224493	0.003835615	88.94814815	ENSG00000111537/ENSG00000108691	IFNG/CCL2	GO_BP
GO:0072347~response to anesthetic	3	3.27	80/18012	0.000228514	0.003867489	25.01666667	ENSG00000197548/ENSG00000162734/ ENSG00000121966	ATG7/PEA15/CXCR4	GO_BP
GO:0070482~response to oxygen levels	5	5.27	389/18012	0.000249784	0.00399868	8.574692945	ENSG00000197548/ENSG00000124762/ ENSG00000003402/ENSG00000164305/ ENSG00000121966	ATG7/CDKN1A/CFLAR/ CASP3/CXCR4	GO_BP
GO:0009612~response to mechanical stimulus	4	4.27	210/18012	0.000255323	0.00399868	12.70687831	ENSG00000109320/ENSG00000064012/ ENSG00000137752/ENSG00000121966	NFKB1/CASP8/CASP1/ CXCR4	GO_BP
GO:1902004~positive regulation of amyloid-beta formation	2	2.27	16/18012	0.000256326	0.00399868	83.38888889	ENSG00000164305/ENSG00000111537	CASP3/IFNG	GO_BP
GO:0022407~regulation of cell-cell adhesion	5	5.27	398/18012	0.000277638	0.004257122	8.380792853	ENSG00000006210/ENSG00000164305/ ENSG00000111537/ENSG00000108691/ ENSG00000121807	CX3CL1/CASP3/IFNG/ CCL2/CCR2	GO_BP

Table S5. Continued

Terms	Counts	Gene Ratio	BgRatio	*P*	FDR	fold Enrichment	gene ID	gene Symbol	Category
GO:0033002~muscle cell proliferation	4	4.27	223/18012	0.000320915	0.004797673	11.96611858	ENSG00000124762/ENSG00000003402/ ENSG00000006210/ENSG00000111537	CDKN1A/CFLAR/ CX3CL1/IFNG	GO_BP
GO:0007252~I-kappaB phosphorylation	2	2.27	18/18012	0.000326211	0.004836554	74.12345679	ENSG00000104365/ENSG00000006210	IKBKB/CX3CL1	GO_BP
GO:0051249~regulation of lymphocyte activation	5	5.27	415/18012	0.000336654	0.004950474	8.037483266	ENSG00000124762/ENSG00000164305/ ENSG00000111537/ENSG00000108691/ ENSG00000121807	CDKN1A/CASP3/IFNG/ CCL2/CCR2	GO_BP
GO:1990182~exosomal secretion	2	2.27	19/18012	0.000364252	0.005307307	70.22222222	ENSG00000103769/ENSG00000111537	RAB11A/IFNG	GO_BP
GO:0071216~cellular response to biotic stimulus	4	4.27	231/18012	0.000366837	0.005307307	11.55170755	ENSG00000109320/ENSG00000006210/ ENSG00000108691/ENSG00000137752	NFKB1/CX3CL1/CCL2/ CASP1	GO_BP
GO:0050663~cytokine secretion	4	4.27	234/18012	0.000385214	0.005441533	11.40360874	ENSG00000091106/ENSG00000006210/ ENSG00000111537/ENSG00000137752	NLRC4/CX3CL1/IFNG/ CASP1	GO_BP
GO:0097305~response to alcohol	4	4.27	234/18012	0.000385214	0.005441533	11.40360874	ENSG00000143799/ENSG00000124762/ ENSG00000064012/ENSG00000171700	PARP1/CDKN1A/ CASP8/RGS19	GO_BP
GO:0032042~mitochondrial DNA metabolic process	2	2.27	20/18012	0.00040435	0.005454171	66.71111111	ENSG00000143799/ENSG00000197548	PARP1/ATG7	GO_BP
GO:0042535~positive regulation of tumor necrosis factor biosynthetic process	2	2.27	20/18012	0.00040435	0.005454171	66.71111111	ENSG00000111537/ENSG00000121807	IFNG/CCR2	GO_BP
GO:0051767~nitric-oxide synthase biosynthetic process	2	2.27	20/18012	0.00040435	0.005454171	66.71111111	ENSG00000111537/ENSG00000108691	IFNG/CCL2	GO_BP
GO:0097734~extracellular exosome biogenesis	2	2.27	20/18012	0.00040435	0.005454171	66.71111111	ENSG00000103769/ENSG00000111537	RAB11A/IFNG	GO_BP
GO:0060055~angiogenesis involved in wound healing	2	2.27	21/18012	0.000446501	0.005889869	63.53439153	ENSG00000006210/ENSG00000121966	CX3CL1/CXCR4	GO_BP
GO:0045088~regulation of innate immune response	5	5.27	448/18012	0.000477979	0.00620439	7.445436508	ENSG00000109320/ENSG00000104365/ ENSG00000091106/ENSG00000064012/ ENSG00000111537	NFKB1/IKBKB/NLRC4/ CASP8/IFNG	GO_BP
GO:0042359~vitamin D metabolic process	2	2.27	22/18012	0.000490697	0.006243334	60.64646465	ENSG00000109320/ENSG00000111537	NFKB1/IFNG	GO_BP
GO:0140112~extracellular vesicle biogenesis	2	2.27	22/18012	0.000490697	0.006243334	60.64646465	ENSG00000103769/ENSG00000111537	RAB11A/IFNG	GO_BP
GO:0019233~sensory perception of pain	3	3.27	105/18012	0.000508541	0.006379879	19.06031746	ENSG00000108691/ENSG00000121807/ ENSG00000121966	CCL2/CCR2/CXCR4	GO_BP
GO:0032411~positive regulation of transporter activity	3	3.27	111/18012	0.000598148	0.007153856	18.03003003	ENSG00000111537/ENSG00000108691/ ENSG00000121807	IFNG/CCL2/CCR2	GO_BP
GO:0019054~modulation by virus of host process	2	2.27	25/18012	0.000635504	0.007218446	53.36888889	ENSG00000197548/ENSG00000064012	ATG7/CASP8	GO_BP
GO:2000679~positive regulation of transcription regulatory region DNA binding	2	2.27	25/18012	0.000635504	0.007218446	53.36888889	ENSG00000143799/ENSG00000111537	PARP1/IFNG	GO_BP
GO:0070838~divalent metal ion transport	5	5.27	477/18012	0.000635738	0.007218446	6.992778942	ENSG00000088832/ENSG00000006210/ ENSG00000111537/ENSG00000108691/ ENSG00000121966	FKBP1A/CX3CL1/IFNG/ CCL2/CXCR4	GO_BP
GO:1900739~regulation of protein insertion into mitochondrial membrane involved in apoptotic signaling pathway	2	2.27	26/18012	0.000687826	0.00747855	51.31623932	ENSG00000015475/ENSG00000064012	BID/CASP8	GO_BP
GO:1900740~positive regulation of protein insertion into mitochondrial membrane involved in apoptotic signaling pathway	2	2.27	26/18012	0.000687826	0.00747855	51.31623932	ENSG00000015475/ENSG00000064012	BID/CASP8	GO_BP

Table S5. Continued

Terms	Counts	Gene Ratio	BgRatio	*P*	FDR	fold Enrichment	gene ID	gene Symbol	Category
GO:1901623~regulation of lymphocyte chemotaxis	2	2.27	26/18012	0.000687826	0.00747855	51.31623932	ENSG00000108691/ENSG00000121807	CCL2/CCR2	GO_BP
GO:1903203~regulation of oxidative stress-induced neuron death	2	2.27	26/18012	0.000687826	0.00747855	51.31623932	ENSG00000143799/ENSG00000197548	PARP1/ATG7	GO_BP
GO:0036475~neuron death in response to oxidative stress	2	2.27	27/18012	0.000742166	0.007878381	49.41563786	ENSG00000143799/ENSG00000197548	PARP1/ATG7	GO_BP
GO:0046885~regulation of hormone biosynthetic process	2	2.27	27/18012	0.000742166	0.007878381	49.41563786	ENSG00000109320/ENSG00000111537	NFKB1/IFNG	GO_BP
GO:0046718~viral entry into host cell	3	3.27	121/18012	0.000768863	0.008019422	16.5399449	ENSG00000198382/ENSG00000141458/ ENSG00000121966	UVRAG/NPC1/CXCR4	GO_BP
GO:0035666~TRIF-dependent toll-like receptor signaling pathway	2	2.27	29/18012	0.000856875	0.008636146	46.00766284	ENSG00000104365/ENSG00000064012	IKBKB/CASP8	GO_BP
GO:0009410~response to xenobiotic stimulus	4	4.27	293/18012	0.000896121	0.008981238	9.107318923	ENSG00000197548/ENSG00000003402/ ENSG00000162734/ENSG00000121966	ATG7/CFLAR/PEA15/ CXCR4	GO_BP
GO:0000002~mitochondrial genome maintenance	2	2.27	30/18012	0.000917233	0.009091245	44.47407407	ENSG00000143799/ENSG00000197548	PARP1/ATG7	GO_BP
GO:0070661~leukocyte proliferation	4	4.27	297/18012	0.000942467	0.009290033	8.984661429	ENSG00000124762/ENSG00000006210/ ENSG00000164305/ENSG00000121807	CDKN1A/CX3CL1/ CASP3/CCR2	GO_BP
GO:0010165~response to X-ray	2	2.27	31/18012	0.000979585	0.009397726	43.03942652	ENSG00000124762/ENSG00000164305	CDKN1A/CASP3	GO_BP
GO:0045736~negative regulation of cyclin-dependent protein serine/threonine kinase activity	2	2.27	31/18012	0.000979585	0.009397726	43.03942652	ENSG00000124762/ENSG00000164305	CDKN1A/CASP3	GO_BP
GO:1904467~regulation of tumor necrosis factor secretion	2	2.27	31/18012	0.000979585	0.009397726	43.03942652	ENSG00000006210/ENSG00000111537	CX3CL1/IFNG	GO_BP
GO:0051968~positive regulation of synaptic transmission, glutamatergic	2	2.27	32/18012	0.001043925	0.009555113	41.69444444	ENSG00000108691/ENSG00000121807	CCL2/CCR2	GO_BP
GO:0006471~protein ADP-ribosylation	2	2.27	33/18012	0.001110249	0.009860333	40.43097643	ENSG00000143799/ENSG00000111537	PARP1/IFNG	GO_BP
GO:0050863~regulation of T cell activation	4	4.27	312/18012	0.001131414	0.009998804	8.552706553	ENSG00000164305/ENSG00000111537/ ENSG00000108691/ENSG00000121807	CASP3/IFNG/CCL2/ CCR2	GO_BP
GO:0061702~inflammasome complex	3	3.27	14/18950	9.29E-07	5.18E-05	150.3968254	ENSG00000091106/ENSG00000196954/ ENSG00000137752	NLRC4/CASP4/CASP1	GO_CC
GO:0044445~cytosolic part	6	6.27	243/18950	9.87E-07	5.18E-05	17.32967535	ENSG00000104365/ENSG00000003402/ ENSG00000091106/ENSG00000064012/ ENSG00000196954/ENSG00000137752	IKBKB/CFLAR/NLRC4/ CASP8/CASP4/CASP1	GO_CC
GO:0045121~membrane raft	6	6.27	316/18950	4.52E-06	0.000119556	13.32630098	ENSG00000104365/ENSG00000003402/ ENSG00000064012/ENSG00000141458/ ENSG00000164305/ENSG00000171700	IKBKB/CFLAR/CASP8/ NPC1/CASP3/RGS19	GO_CC
GO:0098857~membrane microdomain	6	6.27	317/18950	4.60E-06	0.000119556	13.28426218	ENSG00000104365/ENSG00000003402/ ENSG00000064012/ENSG00000141458/ ENSG00000164305/ENSG00000171700	IKBKB/CFLAR/CASP8/ NPC1/CASP3/RGS19	GO_CC
GO:0098589~membrane region	6	6.27	329/18950	5.69E-06	0.000119556	12.79972982	ENSG00000104365/ENSG00000003402/ ENSG00000064012/ENSG00000141458/ ENSG00000164305/ENSG00000171700	IKBKB/CFLAR/CASP8/ NPC1/CASP3/RGS19	GO_CC
GO:1902554~serine/threonine protein kinase complex	3	3.27	87/18950	0.000252442	0.004417731	24.20178799	ENSG00000198382/ENSG00000124762/ ENSG00000104365	UVRAG/CDKN1A/ IKBKB	GO_CC

Table S5. Continued

Terms	Counts	Gene Ratio	BgRatio	*P*	FDR	fold Enrichment	gene ID	gene Symbol	Category
GO:0005776~autophagosome	3	3.27	92/18950	0.000297671	0.004465063	22.88647343	ENSG00000169228/ENSG00000136436/ENSG00000198382	RAB24/CALCOCO2/UVRAG	GO_CC
GO:0097153~cysteine-type endopeptidase activity involved in apoptotic process	5	5.27	15/17293	1.86E-11	2.37E-09	213.4938272	ENSG00000003402/ENSG00000064012/ENSG00000164305/ENSG00000196954/ENSG00000137752	CFLAR/CASP8/CASP3/CASP4/CASP1	GO_MF
GO:0005126~cytokine receptor binding	9	9.27	287/17293	3.04E-10	1.93E-08	20.08478513	ENSG00000088832/ENSG00000003402/ENSG00000015475/ENSG00000064012/ENSG00000006210/ENSG00000164305/ENSG00000111537/ENSG00000108691/ENSG00000121807	FKBP1A/CFLAR/BID/CASP8/CX3CL1/CASP3/IFNG/CCL2/CCR2	GO_MF
GO:0004861~cyclin-dependent protein serine/threonine kinase inhibitor activity	2	2.27	12/17293	0.000153454	0.002165409	106.7469136	ENSG00000124762/ENSG00000164305	CDKN1A/CASP3	GO_MF
GO:0050700~CARD domain binding	2	2.27	16/17293	0.000277934	0.003529767	80.06018519	ENSG00000196954/ENSG00000137752	CASP4/CASP1	GO_MF
GO:0016493~C-C chemokine receptor activity	2	2.27	23/17293	0.000582041	0.006159937	55.69404187	ENSG00000121807/ENSG00000121966	CCR2/CXCR4	GO_MF
GO:0019957~C-C chemokine binding	2	2.27	24/17293	0.000634343	0.006197044	53.37345679	ENSG00000121807/ENSG00000121966	CCR2/CXCR4	GO_MF
GO:0031625~ubiquitin protein ligase binding	4	4.27	290/17293	0.001002801	0.007959736	8.834227331	ENSG00000124762/ENSG00000015475/ENSG00000064012/ENSG00000121966	CDKN1A/BID/CASP8/CXCR4	GO_MF

above genes by step function to optimize the model. Finally, seven genes (*CASP1, CASP3, FKBP1A, RAB11A, NFKB1, RGS19, and CCL2)* were selected as prognostic factors for RT patients.

The predictive ability of the 7-ARG risk assessment model was evaluated

We constructed a ROC curve to assess the accuracy of the model. The AUC values for 2- and 3-year survival rates were 0.845 and 0.807, respectively (Figure 3C). The samples were then divided into a high-risk group and low-risk group according to the cut-off of the 2-year ROC curves, and then the survival curves of the two groups were drawn to compare the survival time. The result showed that the survival time of the high-risk group was significantly shorter than that of the low-risk group (Figure 3D). In addition, we ranked all of the RT patients by their risk scores to analyze the survival distribution. From the scatterplot, we identified the survival status of patients with different risk scores. The mortality rate of patients increased with the increase in risk score (Figure 3E). The heat maps illustrate that as the expression of the seven *DEARGs* (*CASP1, CASP3, FKBP1A, RAB11A, NFKB1, RGS19, and CCL2*) increases, the risk scores of patients also increase (Figure 3F). The above results suggest that the 7-gene signature-based risk assessment model possess a predictive value for the prognosis of RT patients, exhibiting a higher risk score with worse prognosis.

To verify the significance of the expression of the seven feature genes in predicting the prognosis of RT patients, we conducted survival analysis on these seven feature genes. The results confirmed that the high expression level of *CASP1, CASP3, CCL2, FKBP1A, NFKB1, and RAB11A* were significantly associated with a worse survival rate in K-M curves. However, this association did not hold true of *RGS19* in K-M curves (Figure S2).

Verification of the prognostic model

To further validate the accuracy of our prognostic model, we predicted the survival probability in 48 RT patients from the GSE21374 dataset. The K-M (Kaplan-Meier) analysis indicated that the low-risk group exhibited favorable outcomes (Figure 4A). ROC curves were drawn to verify the model reliability, and the AUC values for 2- and 3-year survival rates were 0.703 (Figure 4B). We further

Table S6. Study on the results of biological pathway analysis of DEGs.

ID	Description	Counts	*P*	FDR
hsa05164	Influenza A	10	8.70E-12	1.12E-09
hsa05163	Human cytomegalovirus infection	9	3.36E-09	2.14E-07
hsa05160	Hepatitis C	8	4.98E-09	2.14E-07
hsa04668	TNF signaling pathway	7	1.45E-08	4.67E-07
hsa04210	Apoptosis	7	5.61E-08	1.45E-06
hsa04657	IL-17 signaling pathway	6	1.63E-07	3.50E-06
hsa05142	Chagas disease	6	2.50E-07	4.61E-06
hsa04621	NOD-like receptor signaling pathway	7	3.70E-07	5.87E-06
hsa05134	Legionellosis	5	4.10E-07	5.87E-06
hsa05132	Salmonella infection	7	1.20E-06	1.55E-05

Table 1. The results of univariate Cox analysis of the potential prognosis of DEARGs in RT patients.

Genes	Coefficient	HR (95% CI for HR)	wald.test	z	*P*
ST13	-1.8	0.16 (0.048-0.52)	9.2	-3	0.0024
RAB11A	4.5	86 (16-470)	26	5.1	2.70E-07
NFKB1	1.3	3.7 (0.9-15)	3.3	1.8	0.07
CALCOCO2	2	7.2 (1-49)	4	2	0.045
FKBP1A	1.6	4.7 (2-11)	13	3.6	3.00E-04
CDKN1A	1.7	5.3 (2.4-11)	18	4.2	2.20E-05
IKBKB	1.3	3.7 (1.6-8.8)	9.1	3	0.0026
PEA15	1.5	4.5 (1.8-11)	10	3.2	0.0013
BID	1.4	4.2 (1.9-9.3)	12	3.5	0.00053
CASP8	0.85	2.3 (0.95-5.7)	3.4	1.9	0.063
KIAA0226	1.3	3.7 (1.6-8.2)	10	3.2	0.0016
NPC1	1.7	5.4 (2.5-11)	19	4.3	1.40E-05
CX3CL1	0.79	2.2 (1.1-4.3)	5.4	2.3	0.021
CASP3	1.7	5.3 (2.5-11)	19	4.4	1.30E-05
RGS19	0.58	1.8 (1-3.2)	3.8	2	0.051
CASP4	1.1	2.9 (1.8-4.8)	17	4.1	3.60E-05
CCL2	1.1	3.1 (1.9-4.8)	23	4.8	1.60E-06
APOL1	1.2	3.4 (2-5.6)	23	4.8	1.90E-06
CASP1	0.98	2.7 (1.7-4.1)	21	4.5	5.80E-06
CCR2	0.41	1.5 (1.1-2)	7.3	2.7	0.0067
CXCR4	0.41	1.5 (1.1-2)	8.3	2.9	0.004

plotted a scatter diagram showing the survival of patients with different risk scores, and the result showed that with the increase of the score, the number of false events increased gradually, which was supported by previous research[13] (Figure 4C). Thus, this model has a certain reliability in evaluating prognosis.

DISCUSSION

Kidney transplantation (KT) is a common treatment for advanced kidney disease.[14,15] For many patients with end-stage kidney disease, KT offers a greater survival advantage and a better quality of life than hemodialysis or peritoneal dialysis.[16-18] Nevertheless, immune-mediated graft rejection can still occur and, if mishandled, may lead to the destruction of the donor organ.[19] Although existing evidence suggests a connection between autophagy and the pathogenesis of autoimmune diseases,[20,21] autophagy genes, which are involved in kidney transplantation have not yet been reported. This study aimed to analyze the expression profile of *ARGs* in patients experiencing allograft rejection after transplantation, explore the role *ARGs* plays in kidney rejection after transplantation, and provide a reference for the treatment of kidney transplantation.

In this study, we mined the expression profiles of *ARGs* from the GEO database and analyzed the association between *ARGs* and the prognosis of KT. First, we screened 28 differentially expressed *ARGs* between rejection and non-rejection samples. We found that several *ARGs* play a role in biological

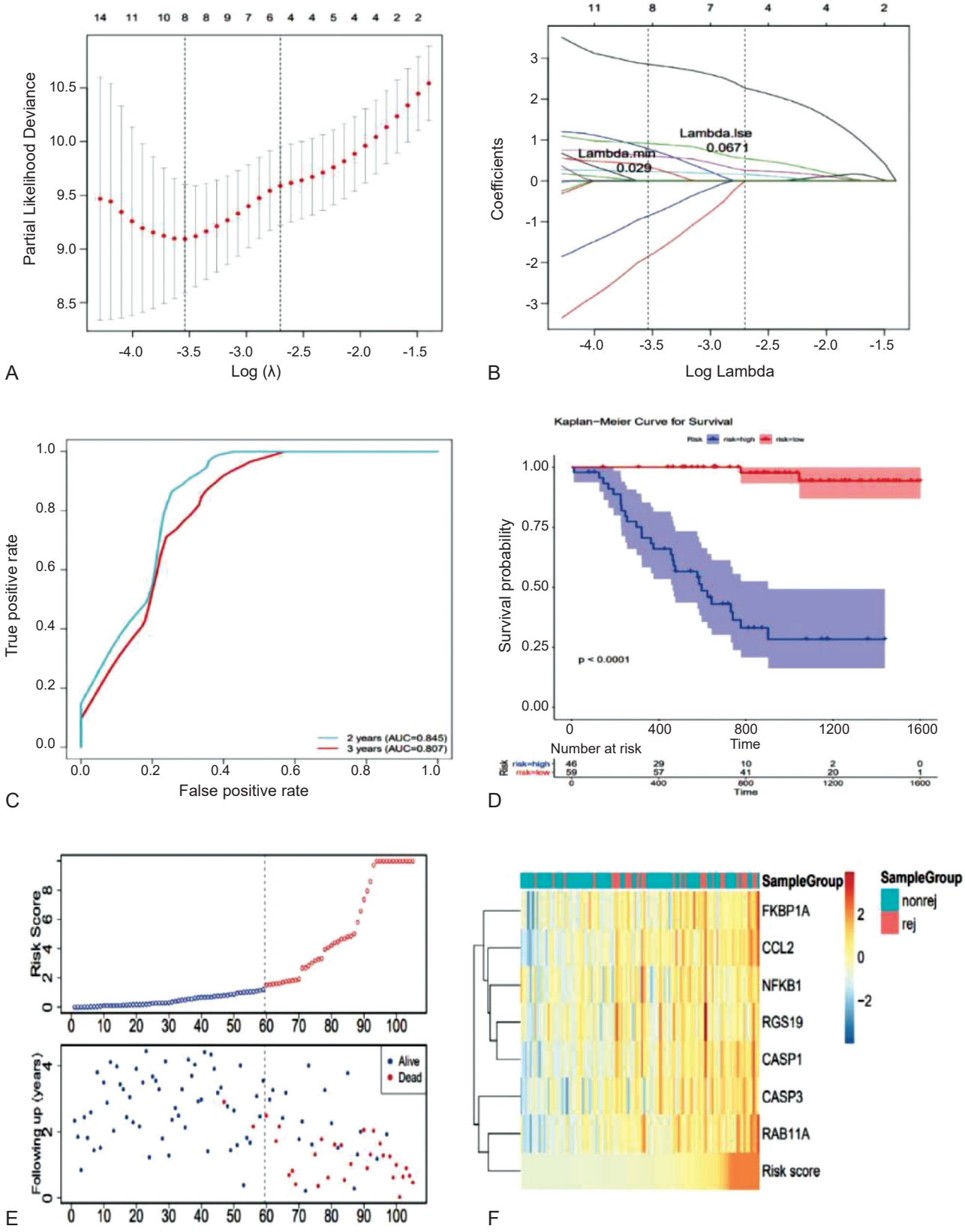


Figure 3. The predictive ability of the 7-ARG risk assessment model.

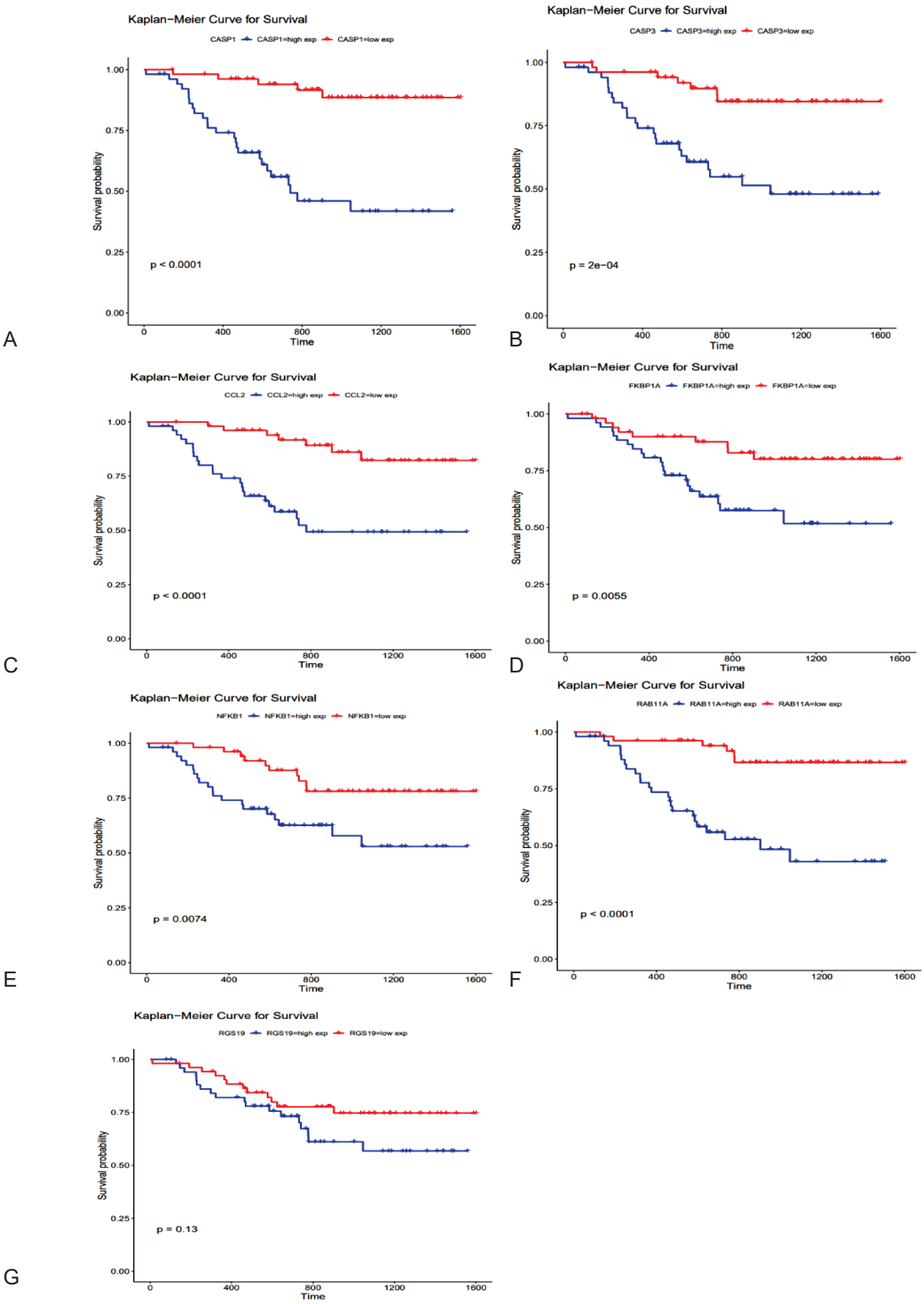


Figure S2. The results of survival analysis on the seven feature genes.

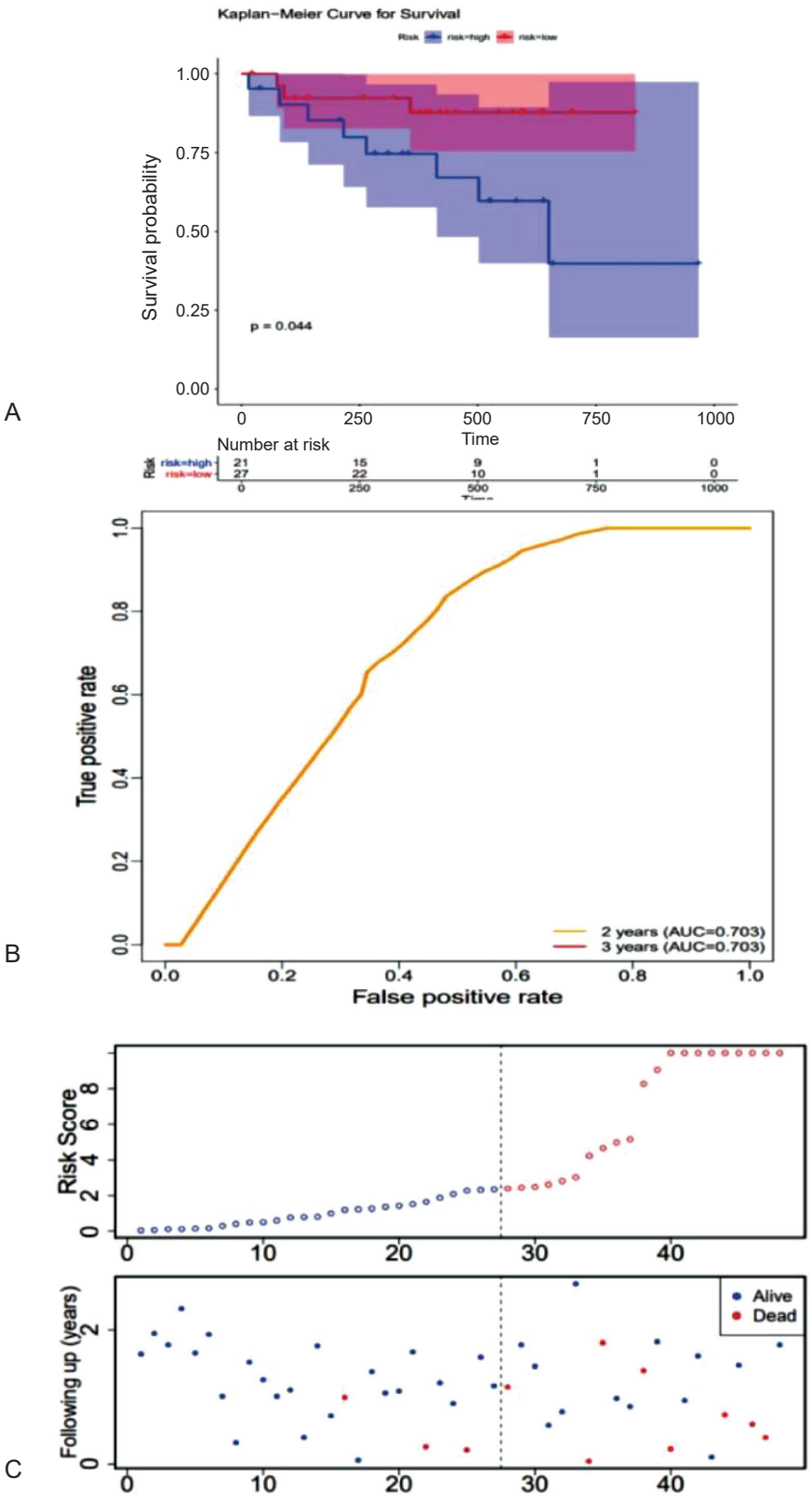


Figure 4. The verification results of the prediction model.

processes based on GO term analysis, such as the response to tumor necrosis factor, extrinsic apoptotic signaling, cysteine-type endopeptidase activity involved in apoptosis, and cytokine receptor binding. Second, a total of 28 KT-related *ARGs* were found in the single-factor Cox regression analysis. Further multivariate Cox regression analysis was performed to determine the expression level of the eight *ARGs* correlated with transplant success rate (TSR). We found that seven survival rate-related *ARGs* (*CASP1, CASP3, FKBP1A, RAB11A, NFKB1, RGS19, and CCL2)* were potential independent risk factors. We then constructed an OS (overall survival)-related prediction model, which can be used as an independent prognostic indicator for KT patients. After statistical analysis, we found that high expression of *CCL2, FKBP1A, NFKB1, RAB11A* was significantly associated with poor survival after KT. We also conducted a survival analysis on the seven feature genes. The results confirmed that high expression levels of *CASP1, CASP3, CCL2, FKBP1A, NFKB1, RAB11A* were significantly associated with a worse survival rate in K-M curves.

We searched the literature on these genes and found some of their correlations to be very informative guides to our research. According to Gniewkiewicz MS and Hirt-Minkowski P research, *CCL2* may predict BK virus (BKV) nephropathy.[22,23] This study confirmed that urinary *CCL2* is an independent predictor of long-term allograft outcomes.[24] At the same time, carriers of the *FKBP1A rs6041749 C* allele have a higher risk for estimated glomerular filtration rate *(eGFR)* deterioration, and the variant might serve as a biomarker to predict allograft function in KT patients. Wu Z *et al.* confirmed that carriers of the FKBP1A rs6041749C allele have a higher risk of eGFR deterioration. This variant may serve as a biomarker for predicting allograft function in kidney transplant patients.[25] Moreover, small-molecule inhibitors of *CASP1* can reverse this mechanism of glucocorticoid resistance, thereby improving the treatment of acute lymphoblastic leukemia (ALL) and potentially other illnesses for which glucocorticoids are used therapeutically.[26] This was demonstrated by studies of cultured *Nfkb1*$^{-/-}$ microglia that were found to express reduced levels of proinflammatory genes and increased anti - inflammatory genes.[27,28]

The functional studies of these genes may contribute to a more accurate understanding of the prognostic behavior of KT. We used a K-M curve, log rank test, and scatter plot of patient survival time to determine the risk score. The results suggested that the risk assessment model based on five gene signatures had a certain predictive value for the prognosis of KT patients. Our data show that the higher the risk score, the worse the prognosis. In the K-M curve, high expression of *CCL2, FKBP1A, NFKB1, RAB11A* was significantly associated with poor survival. To further verify the accuracy of our prognostic model, we predicted the TSR in a GEO cohort that included 48 KT patients (*GSE21374*). The results showed that the expression levels of the three *ARGs* (*RAB11A, FKBP1A, CASP1*) were correlated with TSR. This finding suggest that ARGs and TSR have different functions and mechanisms of action in the genome, but there may also be certain associations and mutual influences between them. Studying them helps to better understand the structure and function of the genome.

CONCLUSIONS

In conclusion, we established a model of ARG signatures that could be used to analyze the prognosis of patients with KT and validated this model with an independent cohort from the GEO database. Our study provided new insights into the autophagy status in RT. This study identified seven autophagy genes associated with successful kidney transplantation, which suggests that *ARGs* may be molecular markers for the prognosis of kidney transplantation. Furthermore, the results of this study may contribute to the development of targeted therapies for kidney transplantation.

LIMITATIONS

This study also has some limitations. Firstly, bioinformatics analysis often relies on existing databases and algorithms, and the completeness and accuracy of these databases and algorithms may affect the reliability of research results. Secondly, bioinformatics analysis often only provides correlated results and cannot directly prove causal relationships. Therefore, further experimental validation is needed to confirm the results of bioinformatics analysis.

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CODE OF ETHICS

This Code of Ethics serves as a guiding framework for the conduct of the research presented in this paper and will be adhered to by all members of the research team. It is our commitment to uphold these ethical principles to ensure the quality and integrity of our work.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors have declared that no Disclosure of potential competing interest exists.

RESEARCH INVOLVING HUMAN PARTICIPANTS AND/OR ANIMALS

The authors have declared that all research involving Human Participants and/or Animals are informed and allowed.

AVAILABILITY OF DATA AND MATERIAL

The authors have declared that all data and material are informed and allowed; The authors agree biostatistician review our database during the review process.

CONSENT FOR PUBLICATION

The authors have declared that this article is permitted to be published.

AUTHOR'S CONTRIBUTIONS

Cankun Xie, Wingkeung Yiu: Project development, Data Collection, Manuscript writing

Yonglu Wu, Guanjun Li: Project development, Manuscript writing, Review opinions

Jiahui Jie: Project development, Data Collection, Review opinions

CONSENT TO PARTICIPATE

The authors have declared that all patient are informed and allowed.

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